A Thesis Submitted for the Degree of PhD at the University of Warwick

Permanent WRAP URL:

http://wrap.warwick.ac.uk/158174

Copyright and reuse:
This thesis is made available online and is protected by original copyright.
Please scroll down to view the document itself.
Please refer to the repository record for this item for information to help you to cite it.
Our policy information is available from the repository home page.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk
Synthesis of oxetane and azetidine containing cyclic peptides

by

George Joseph Saunders

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry

Department of Chemistry, University of Warwick

September 2020
# Table of contents

Acknowledgements 6
Declaration 7
Abstract 8
Abbreviations 9

## Chapter 1: Introduction

1.1 Peptides and Peptidomimetics 13
  1.1.1 Peptide based therapeutics 13

1.2 Oxetane modified peptides 14
  1.2.1 Oxetanes in medicinal chemistry 14
  1.2.2 Synthesis of oxetane modified peptides and proteins 16

1.3 Importance of cyclic peptides 24
  1.3.1 Types of cyclic peptide 24
  1.3.2 Applications of cyclic peptides 25
  1.3.3 Challenges to cyclic peptide development 30

1.4 Synthesis of cyclic peptides 32
  1.4.1 General experimental considerations 32
  1.4.2 Natural turn-inducing amino acids 36
  1.4.3 Incorporation of D-amino acids 37
  1.4.4 N-alkylated peptides 38
  1.4.5 Pseudoprolines 38
  1.4.6 Dehydro amino acids 40
  1.4.7 Extending the length and flexibility of the precursor 41
  1.4.8 Ring contraction strategies 41
  1.4.9 Macrocyclisation of N-terminal thioamides 43
  1.4.10 Side chain ligation 45
  1.4.11 Concluding remarks 46

## Chapter 2: Synthesis of Oxetane Containing Cyclic Peptides

2.1 Previous Work and Project Aims 48

2.2 Results and Discussion 49
  2.2.1 Initial study to form an oxetane modified pentapeptide 49
  2.2.2 Expanding the scope of the macrocyclisation 53
2.2.3 Synthesis of oxetane modified alanine containing cyclic peptides 60
2.2.4 Investigation of the kinetics of the macrocyclisation 61
2.2.5 Comparing oxetanes with other peptide modifications 63
2.2.6 Deprotection of the side chain protecting groups 67

2.3 Conclusions 70

Chapter 3: Synthesis of Azetidine Containing Cyclic Peptides 72
3.1 Previous Work and Project Aims 72
3.2 Results and Discussion 75
  3.2.1 Synthesis of nitroalkenes with carbamate protecting groups 75
  3.2.2 Synthesis of nitroalkenes with non-carbamate protecting groups 76
3.3 Solution phase synthesis of azetidine modified cyclic peptides 77
  3.3.1 Proof of concept study with a pentapeptide 77
  3.3.2 Exploring macrocyclisation of other ring sizes 81
3.4 SPPS of precursors followed by cyclisation 84
  3.4.1 Synthesis of solid-phase building blocks 84
  3.4.2 Utilisation of building blocks for SPPS and cyclisation 86
3.5 Deprotection of azetidine modified cyclic peptides 88
  3.5.1 Comparative study between oxetane and azetidine modified peptides 88
  3.5.2 Deprotection of other substrates 90
3.6 Synthesis of cyclic peptides containing alanine analogues 94
  3.6.1 Additions to the trisubstituted nitroalkene 260 94
  3.6.2 Synthesis of an AAz(Boc)-Pro based cyclic peptide 96
  3.6.3 Cyclisation of a mixture of diastereomers 99
3.7 Functionalisation of the azetidine nitrogen 103
  3.7.1 Optimisation of conditions for the Cbz deprotection and functionalisation 103
  3.7.2 Scope of Cbz deprotection and functionalisation chemistry 107
  3.7.3 Click reactions of 2-propynyl carbamate azetidines 109
3.8 Investigating the structure and properties of functionalised cyclic peptides 112
  3.8.1 Structure of azetidine modified cyclic peptides 112
  3.8.2 Fluorescent properties of azetidine modified cyclic peptides 114
  3.8.3 Cellular imaging using functionalised azetidine modified cyclic peptides 115
3.9 Summary and Future Work 117
  3.9.1 Conclusions 117
3.9.2 Future work

Chapter 4: Experimental Section

4.1 General experimental considerations 126
4.2 General procedures 128
4.3 Experimental procedures and characterisation 135
4.4 Kinetic measurement of the cyclisation reaction 276
4.5 UV/Vis and fluorescence spectroscopy of azetidine functionalised peptides 282
4.6 XRD analysis of 398 284
4.7 Confocal microscopy of 413 285

References 286

Appendix I Overlaid $^1$H spectra of 175 and 343 after acidic treatment 295
Appendix II NOESY analysis of 378 296
Appendix III Comparative data for 215, 381-A and 381-B 297
Appendix IV $^1$H NMR analysis to determine d.r of building block 383 299
Appendix V Analytical HPLC and $^1$H NMR analysis of crude cyclisation mixture of precursor 385 and cyclic peptide 386 299
Acknowledgements

Firstly I would like to thank Professor Mike Shipman for giving me the opportunity to undertake a PhD at the University of Warwick on such an exciting and interesting project. Your guidance and advice was always excellent, and I wouldn’t have been able to write this thesis without your help and support.

I would like to thank various people for their collaborative contributions to this research project. Firstly, I am grateful to Dr Stefan Roesner and Dr Ina Wilkening for their expertise in synthetic and analytical aspects of the work, for fruitful discussions during office hours and group meetings, and for proofreading this thesis. I would also like to thank Eleanor Jayawant for her computational and analytical work towards this project, as well as Dr Ann Dixon and Dr Rebecca Notman for guidance and expertise during group meetings. I am thankful to Dr Guy Clarkson for invaluable help towards crystal growth and X-ray crystallography, as well as Dr Alexia Hapeshi for microscopy work. Special thanks also to Dr Ivan Prokes, Dr Lijiang Song, Robert Perry and various other technical staff during my time at the University of Warwick for their analytical expertise.

My PhD work would not have been possible without the fantastic members of the Shipman group throughout the last four years, who were always there to listen to my ramblings and provide support. Memorable mentions go to Conor, Jon, Stefan and Ina for putting up with me for the longest, but not forgetting Stuart, Alpa, Dave, Raj, Martin, Nastja, Vince, Shain, Raja, Leo and Bonnie amongst various others. I would also like to thank the members of the Chaplin group over the years for Thursday morning distractions as well as many other memorable moments. Special thanks to Georgia and Tom for providing me with a fantastic place to come home to at Apple Way and for many, many hours of fun and laughter over the years.

Finally, I would not be where I am today without the continued support of my friends and family. In particular, I would like to thank my mother and sister for their invaluable encouragement, belief and faith in me, this thesis would not be possible without them.
Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented in this thesis is an account of my own work carried out at the University of Warwick between October 2016 and August 2020, except in the cases outlined below:

**Chapter 2**: Peptides \textbf{161, 162, 219, 234} and \textbf{235} (Table 5) and their requisite precursors were synthesised and characterised by Dr Stefan Roesner at the University of Warwick. Analytical HPLC and LC-MS measurements for kinetic analysis of the cyclisations of \textbf{178} and \textbf{217} were conducted by Dr Ina Wilkening at the University of Warwick.

**Chapter 3**: NOESY spectroscopy of \textbf{371} was collected and analysed by Eleanor Jayawant, Dixon/Notman groups at the University of Warwick. Confocal microscopy study of \textbf{415} was carried out by Dr Alexia Hapeshi, Perrier group at the University of Warwick.

At this time of submission, elements of this work have appeared in the scientific literature:


*authors contributed equally.
Abstract

This thesis describes work towards the synthesis of small cyclic peptides by incorporation of turn-promoting 3-aminooxetane and 3-aminooazetidine units into the backbone. Functionalisation of the 3-aminooazetidine after macrocyclisation is also described.

Chapter 1 provides an introduction to cyclic peptides including their importance in medicinal chemistry, current synthetic methodologies towards the synthesis of head-to-tail cyclic peptides and an overview of work on the incorporation of oxetanes into peptides and proteins.

Chapter 2 describes the synthesis of oxetane modified cyclic peptides. The 3-aminooxetane unit was introduced into a series of tetra-, penta-, and hexapeptides using solution-phase coupling, and the macrocyclisation efficiency of these precursors assessed. Comparative studies with the parent peptides using isolated yield, product distribution and kinetic studies, including against other commonly used peptide modifications, demonstrate the value of the 3-aminooxetane as a motif to enhance the macrocyclisation of small peptides.

Chapter 3 explores the synthesis of azetidine modified cyclic peptides including late-stage functionalisation of the azetidine nitrogen after macrocyclisation. An approach exploiting conjugate addition and nitro group reduction was used to synthesise short linear peptides in solution, which showed improved cyclisation compared to the parent peptides. In contrast to the work with oxetanes, these cyclic peptides tolerated strongly acidic conditions required for side chain deprotection. A highly practical and general route towards azetidine modified cyclic peptides was demonstrated utilising Fmoc/tBu SPPS from pre-formed dipeptide building blocks. Using Cbz for selective deprotection of the azetidine nitrogen by hydrogenation allowed for N-functionalisation by acylation, sulfonation or S$_N$Ar chemistries. In this way, biotin and fluorescent dyes could be conjugated to the cyclic peptide, as well as different azide functionalities. Insights into the structural impact of azetidine modification were gained by analysis of a crystal structure obtained from an azide functionalised derivative. Alternatively, a 2-propynyl carbamate could be installed early into the synthetic route and functionalised by CuAAC click chemistry post-cyclisation, enabling the synthesis of fluorescently conjugated cyclic peptides which could be visualised by confocal microscopy.

Chapter 4 provides detailed experimental procedures for the work carried out in Chapters 2 and 3.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>[α]D</td>
<td>Optical rotation</td>
</tr>
<tr>
<td>1D</td>
<td>1 Dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>2 Dimensional</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>AA</td>
<td>Amino Acid</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>AICAR</td>
<td>5-Aminoimidazole-4-carboxamide ribonucleotide</td>
</tr>
<tr>
<td>Alloc</td>
<td>Allyloxy carbonyl</td>
</tr>
<tr>
<td>AAz</td>
<td>Azetidine-modified alanine</td>
</tr>
<tr>
<td>AOX</td>
<td>Oxetane-modified alanine</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>b</td>
<td>Broad</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxy carbonyl</td>
</tr>
<tr>
<td>Bt</td>
<td>Ben佐triazole</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>BOP</td>
<td>Ben佐triazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>c</td>
<td>Concentration</td>
</tr>
<tr>
<td>Calcd.</td>
<td>Calculated</td>
</tr>
<tr>
<td>Cbz</td>
<td>Carboxybenzyl</td>
</tr>
<tr>
<td>CD</td>
<td>Circular Dichroism</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper(I)-catalyzed azide alkyne cycloaddition</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCE</td>
<td>1,2-Dichloroethane</td>
</tr>
<tr>
<td>d.e</td>
<td>Diastereomeric excess</td>
</tr>
<tr>
<td>DEPBT</td>
<td>3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one</td>
</tr>
<tr>
<td>DIC</td>
<td>N,N'-Diisopropylcarbodiimide</td>
</tr>
<tr>
<td>Abbr.</td>
<td>Full Name</td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>DIPEA</td>
<td><em>N,N</em>-Diisopropylethylamine</td>
</tr>
<tr>
<td>DKP</td>
<td>Diketopiperazine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMB</td>
<td>Dimethoxybenzyl</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DMTMM</td>
<td>(4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium)</td>
</tr>
<tr>
<td>d.r</td>
<td>Diastereomeric ratio</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EDC</td>
<td><em>N</em>-(3-Dimethylaminopropyl)-<em>N</em>'-ethylcarbodiimide</td>
</tr>
<tr>
<td>eq</td>
<td>Equation</td>
</tr>
<tr>
<td>equiv</td>
<td>Equivalent</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray Ionisation</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>FDPP</td>
<td>Pentafluorophenyl diphenylphosphinate</td>
</tr>
<tr>
<td>Fmoc</td>
<td>Fluorenylmethyloxycarbonyl</td>
</tr>
<tr>
<td>GAz</td>
<td>Azetidine-modified glycine</td>
</tr>
<tr>
<td>GOx</td>
<td>Oxetane-modified glycine</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HATU</td>
<td>1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate</td>
</tr>
<tr>
<td>HAPyU</td>
<td>1-(pyrrolidin-1-ium-1-ylidene(pyrrrolidin-1-yl)methyl)-1H benzo[d][1,2,3]triazole 3-oxide hexafluorophosphate</td>
</tr>
<tr>
<td>HBTU</td>
<td>(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrogen chloride</td>
</tr>
<tr>
<td>HCTU</td>
<td>O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HFIP</td>
<td>Hexafluoroisopropanol</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency viruses</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectroscopy</td>
</tr>
<tr>
<td>Hsp</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>HOAt</td>
<td>1-Hydroxy-7-azabenzotriazole</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-Hydroxy-7-benzotriazole</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>‘Pr</td>
<td>iso-Propyl</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>K</td>
<td>Kelvin</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography Mass Spectrometry</td>
</tr>
<tr>
<td>lit.</td>
<td>Literature</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular Dynamics</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting Point</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>n</td>
<td>Normal</td>
</tr>
<tr>
<td>NMM</td>
<td>4-Methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>′Pr</td>
<td>Neopropyl</td>
</tr>
<tr>
<td>Nu</td>
<td>Nucleophile</td>
</tr>
<tr>
<td>oNB</td>
<td>Ortho-nitrobenzyl</td>
</tr>
<tr>
<td>Ox</td>
<td>Oxetane</td>
</tr>
<tr>
<td>Pbf</td>
<td>Pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl</td>
</tr>
<tr>
<td>Pg</td>
<td>Protecting group</td>
</tr>
<tr>
<td>Ph</td>
<td>Phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PyBOP</td>
<td>(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>quint</td>
<td>Quintet</td>
</tr>
<tr>
<td>Ref.</td>
<td>Reference</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Rf</td>
<td>Retention factor</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reverse phase high performance liquid chromatography</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Second, Singlet</td>
</tr>
<tr>
<td>SICLOPPS</td>
<td>Split-intein circular ligation of peptides and proteins</td>
</tr>
<tr>
<td>sep</td>
<td>Septet</td>
</tr>
<tr>
<td>solv.</td>
<td>Solvent</td>
</tr>
<tr>
<td>SPPS</td>
<td>Solid-phase peptide synthesis</td>
</tr>
<tr>
<td>Su</td>
<td>Succinimide</td>
</tr>
<tr>
<td>t</td>
<td>Temperature</td>
</tr>
<tr>
<td>t</td>
<td>Time, Triplet</td>
</tr>
<tr>
<td>T3P</td>
<td>Propylphosphonic anhydride</td>
</tr>
<tr>
<td>TBTU</td>
<td>2-((1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylylaminium tetrafluoroborate</td>
</tr>
<tr>
<td>TCEP</td>
<td>(tris(2-carboxyethyl)phosphine</td>
</tr>
<tr>
<td>‘Bu</td>
<td>tert-Butyl</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFMSA</td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIS</td>
<td>Triisopropylsilane</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>Tris</td>
<td>2-Amino-2-(hydroxymethyl)-1,3-propanediol</td>
</tr>
<tr>
<td>Trt</td>
<td>Triphenylmethyl</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl</td>
</tr>
<tr>
<td>UPLC-MS</td>
<td>Ultra-Performance Liquid Chromatography Mass Spectrometry</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>v</td>
<td>Volume</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>ε</td>
<td>Extinction coefficient</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
</tr>
<tr>
<td>µW</td>
<td>Microwave</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

My doctoral work has focused on developing new methods for the synthesis of small cyclic peptides, using the 3-aminooxetane and 3-aminoazetidine subunit within the peptide backbone as turn inducers. Before reporting on my own results, this chapter introduces the field of peptides and peptidomimetics; early work in the field of oxetane modified peptides from our laboratory and beyond, followed by the importance of small cyclic peptides in medicinal chemistry and the current state of the art with respect to their chemical synthesis.

1.1 Peptides and Peptidomimetics

1.1.1 Peptide based therapeutics

Peptides have garnered significant interest as therapeutics. They are known to mediate many biological functions such as regulation of the immune system, signal transduction and growth amongst many others.¹ As a consequence, they have been used for the treatment of many different diseases, notably for autoimmune disorders, cancer and diabetes.¹ There are over 60 peptide based therapeutics on the market, with more than 500 additional derivatives currently undergoing clinical trials globally.²

The degradation products from the breakdown of peptides are amino acids, making them far less toxic compared to other small molecule drugs.³ However, owing to their ubiquity in biological systems, natural peptides often suffer from poor chemical and physical stability due to the widespread action of proteases.⁴ They also often suffer from poor oral bioavailability and are rapidly cleared by the body.⁵ Moreover, linear peptides often have poor selectivity due to their conformational flexibility.⁶

To combat these weaknesses, a range of new therapeutics called peptidomimetics have been developed which aim to mimic the structure and function of the parent peptide, but with improved biochemical and physicochemical properties. The most common peptidomimetic backbone modifications are summarised in Figure 1, but these are not exhaustive and many others have been reported in a number of excellent reviews.⁶–¹⁰
1.2 Oxetane modified peptides

1.2.1 Oxetanes in medicinal chemistry

The 4-membered oxetane ring has been used widely in medicinal chemistry as a bioisostere of the carbonyl group (or in replacement of gem-dimethyl groups) to impart enhanced physicochemical properties, such as membrane permeability.\(^\text{11}\) The oxetane ring has a similar lone pair arrangement and can act as a hydrogen bond acceptor, although overall the C—O bond distance is longer than in a C=O bond and occupies a larger spatial volume. Furthermore, increase in pKa of the α-position renders this position less susceptible to epimerisation in aldehydes, ketones or carboxylic acids. The ring provides added lipophilicity, which can influence the effective permeability of some scaffolds (Figure 2).\(^\text{12}\)
Specifically, 3,3-disubstituted oxetanes 4–7 in replacement for successive methylene units in 3 have been reported to modulate the aqueous solubility and the pKa of adjacent amines (Figure 3). Compounds containing oxetanes can also be less susceptible to metabolic degradation and show slower clearance rates in human liver microsomes compared to their carbonyl equivalents.\textsuperscript{13}

An excellent review by Bull and coworkers details the synthesis of oxetane containing molecules and provides fuller details of the impact of oxetane substitution on molecules.\textsuperscript{11}
1.2.2 Synthesis of oxetane modified peptides and proteins

By replacement of the amide bond C=O with the 3-aminooxetane unit, Shipman and coworkers reported the synthesis of a new type of peptidomimetic. The modification was expected to offer reduced vulnerability to proteases whilst still participating in hydrogen bonding to maintain secondary structures. Furthermore, the 3-aminooxetane unit was anticipated to enable the backbone to explore greater conformational space by removing one of the rigid peptide bonds.\(^{14}\)

Tripeptide motifs containing the 3-aminooxetane were generated by a three step sequence from 3-oxetanone 8. The process utilised a Henry reaction, with elimination of the corresponding mesylate to generate nitroalkene 9. This can undergo a conjugate addition with an α-amino ester 11 (or the N-terminus of a larger peptide) to produce oxetane modified peptide building blocks 12–23. The scope of this process includes a variety of hydrophobic and polar amino acid residues. Moreover, a trisubstituted nitroalkene 10 can be used as an acceptor in the conjugate addition to generate oxetane modified phenylalanine residues 22 and 23 as racemates (Scheme 1).\(^{14}\)

Scheme 1: Conjugate addition of amino acid ester 11 to nitroolefins 9 – 10, generating oxetane modified di- and tripeptide building blocks 12–23.\(^{14}\)

The resulting nitroalkane could be reduced to form a primary amine, followed by peptide coupling to afford tripeptide motifs 24–29 with an oxetane modified glycine in the middle of the chain (Scheme 2).\(^{14}\) By further application of this chemistry, N- and C-terminal oxetane containing tripeptides could also be made.\(^{15}\)
Scheme 2: Indium mediated reduction of nitroalkanes 12–17, followed by peptide coupling to form oxetane modified tripeptides 24–29.\(^{14}\)

Computational experiments were carried out comparing the structures Leu-Gly-Ile 30 and Leu-GOx-Ile 25 to gain insight into their conformational preferences. 30 was dominated by conformations where the chain is linear, with the C- and N- termini separated by \(>7\) Å. However, the most populated conformation of 25 displayed a more folded arrangement where the C- and N-termini were in close proximity between 3–4 Å apart. This demonstrated the ability for oxetane modified peptides to adopt turn-like features (Figure 4).

Shipman and co-workers also showed that dipeptide analogues could be cyclised to form oxetane containing spirocyclic 2,5-diketopiperazines. The cyclisation step utilised a Raney nickel
reduction of the nitro group of \( \text{31} \), with the amine formed undergoing an intramolecular cyclisation with the C-terminal ester group to form oxetane modified 2,5-diketopiperazines \( \text{32–37} \) (Scheme 3). Furthermore, the synthetic procedure was also applied to generate Boc protected azetidine variants \( \text{38–39} \), demonstrating that this heterocycle can also be incorporated into the peptide backbone.

![Scheme 3: Formation of oxetane modified 2,5-diketopiperazines from dipeptide building blocks.](image)

Alongside the work published by Shipman, Carreira has also investigated the synthesis of oxetane modified peptides. Their approach included a conjugate addition of \( \alpha \)-amino \( n \)-propyl esters \( \text{45} \) to oxetanyl nitroolefins \( \text{9, 40–44} \) which were isolated rather than used \textit{in-situ}. They also found that Raney Ni reduction of the nitroalkane was efficient, and the amine formed could be protected \textit{in-situ} during the reaction. Similarly to the results published by Shipman, additions to trisubstituted nitroalkanes (\( R^1 \neq H \)) were also found to proceed with poor selectivity and were isolated as inseparable mixtures of diastereomers or racemates (Scheme 4).
Although the synthesis of non-glycine modified residues was possible by conjugate addition of amino acids towards trisubstituted nitroalkenes 40–44 followed by nitro group reduction, a major limitation of the work was the poor stereocontrol of the conjugate addition step, leading to isolation of a mixture of enantiomers or diastereoisomers.

Carreira and coworkers went on to develop a method to produce oxetane modified variants of twelve naturally occurring amino acids with a high level of diastereocontrol. Protected amino alcohol 58 could be prepared in 26% yield in multigram quantities over 5 steps from Tris•HCl 57. Oxidation to 59 followed by imine formation with chiral sulfoxide 60 (Ellman auxiliary) generated building block 61 (Scheme 5).
With intermediate 61 in hand, enantiopure 3-aminooxetane building blocks could be prepared by nucleophilic addition of organolithium or Grignard reagents to the imine followed by functional group interconversion. Excellent diastereoselectivity was observed, enabling the synthesis of a range of different oxetane modified analogues as depicted below for phenylalanine (Scheme 6).  

To generate dipeptide building blocks, the 3-aminooxetanes 65 were coupled with α-amino acid electrophiles 66 in an S$_2$2 manner. D-amino acids were converted to the hydroxyester, which was activated as the triflate for coupling. For glycine, the commercially available bromoacetate was applied. Inversion of stereochemistry produced the all-L oxetanyl dipeptides 67–71 (Scheme 7).
The authors of this work utilised these dipeptide building blocks in the synthesis of different oxetane modified analogues of Leu enkephalin. By sequential replacement of each of the amide bonds with the relevant 3-aminooxetane unit, it was found that each derivative had improved hydrolytic stability in human serum compared to the parent peptide. Moreover, two of the analogues retained their affinity for the rat δ-opioid receptor, demonstrating that the oxetane acts as a good isostere for the carbonyl group it replaces (Figure 5).18

To study the impact of the oxetane modification in larger peptides, a strategy for the synthesis of oxetane modified peptides via Fmoc SPPS was required. To accomplish this, a dipeptide building block strategy was devised by Shipman. N-Fmoc oxetane modified dipeptides 76 were
synthesised from amino cumyl ester 74 via conjugate addition followed by nitro group reduction of 75 using Raney Ni. The cumyl ester was identified as the optimal C-terminus protecting group, which could be deprotected under mildly acidic conditions without deprotection of the acid-sensitive side chain protecting groups.$^{19}$

Scheme 8: Synthesis of oxetane modified glycine containing dipeptides for use in solid phase synthesis.

Using these building blocks, several linear peptides were synthesised with an oxetane modified glycine: met-enkephalin and leu-enkephalin, which bind to opioid receptors, and the vasodilator bradykinin (Scheme 9).$^{19}$

Scheme 9: Solid phase peptide synthesis of oxetane modified peptides from dipeptide building blocks.

Oxetane modified dipeptide building blocks were also found to be effective hydrogels. The modified peptides were unsurprisingly more stable towards exoproteases than the parent peptide, owing to the replacement of the amide bond from 77 to 78 (Figure 6).$^{20}$
Shipman and coworkers developed a streamlined method for the synthesis of oxetane modified alanine SPPS building blocks, involving conjugate addition of \((R)\)-\(\alpha\)-methylbenzylamine 80 to trisubstituted nitroalkene 79. Although modest diastereoselectivity is achieved, the diastereomer 81 was readily separable by column chromatography and could be converted to 3-aminooxetane 82, achieving dipeptide building block 84 after applying the conditions developed by Carreira and coworkers with triflate 83 (Scheme 10). Additionally, preliminary results suggested that this synthetic methodology could also be applied to make oxetane modified valine residues.\(^{21,22}\)

Oxetanes have also found use in peptide grafts used to stabilise peptide structures. Bernardes and coworkers reported the bis-alkylation of cysteine residues in peptides and in native proteins and antibodies using 86 to generate an oxetane staple. These compounds contain enhanced biological activity and immunogenicity \textit{in vivo}, and an oxetane stapled variant of octreotide 87 demonstrated slightly improved binding to the SSR2 receptor (Scheme 11).\(^{23}\) The site-selective incorporation
of oxetanes into proteins and antibodies by a similar strategy has also been reported by Bernardes. In the selected examples, no loss of biological activity was observed.\textsuperscript{24}

Scheme 11: Oxetane stapling of octreotide leads to improved binding to the SSR2 receptor.\textsuperscript{24}

1.3 Importance of cyclic peptides

1.3.1 Types of cyclic peptide

In addition to the development of peptidomimetics to improve the properties of peptides, constraining the linear peptide into a macrocyclic ring can be beneficial. Of the >60 peptides that are approved for clinical use in the USA, over 40 are cyclic.\textsuperscript{25,26} There are different types of macrocyclic peptides depending on the method of cyclisation and the parts of the backbone which form the ring. Amide bond formation between the C- and N- termini forms a head-to-tail cyclic peptide. Cyclisation can occur between two of the side chains on the backbone, for example via disulfide formation, shown in Octreotide 85. Nucleophilic side chains can also form esters, thioesters or amides with the side chain Glu/Asp residues or the C-terminus (Figure 7).\textsuperscript{27}
Another classification of cyclic peptides relates to the nature of the amino acids and side chains within the backbone. Homodetic cyclic peptides contain all-amide bonds, whereas heterodetic cyclic peptides contain other linkages such as ester or disulfide bonds. Cyclic peptides which contain more than one macrocycle in their structure also exist. Romidepsin 88 is a cyclic tetradepsipeptide containing both a 16-membered macrocycle and a 17-membered heterodetic macrocycle joined by a disulfide bridge (Figure 8). It is a selective and potent inhibitor of histone deacetylases and among the first-in-class to gain FDA approval as a treatment for cutaneous T-cell lymphomas.

1.3.2 Applications of cyclic peptides

Linear peptides are highly disordered in aqueous environments, meaning that they can sample a range of conformations in a short period of time. By joining two elements of the backbone together, a conformational restraint is introduced which produces a rigid structure with preorganised functional groups. This often leads to increased potency and specificity against many targets.
The ability to synthesise cyclic peptides or peptide mimics in an iterative manner from simple building blocks in a stereocontrolled fashion, with the ability to access diverse yet rigid conformational space means that they are highly attractive molecules for drug discovery.\textsuperscript{33}

Protein-protein interactions (PPIs) have been implicated in a variety of different cellular processes. They are very important in controlling cell signalling and development, enzyme catalysis and biological networks which control cell survival. This means that they offer potential as targets for therapeutic intervention.\textsuperscript{34} However, PPIs commonly have large, flat binding sites which means that classical small molecule drugs are not suitable as inhibitors. Although monoclonal antibodies can serve as useful binding partners to protein surfaces, they struggle to cross the cell membrane in order to act on intracellular targets.\textsuperscript{35} Owing to their high polar surface area, cyclic peptides can also serve as binding partners for these particularly challenging interactions.\textsuperscript{36}

Macrocyclisation often leads to increased stability to proteases. Generally, endopeptidases act by recognising β-sheet type arrangements between polypeptide sequences, which cannot be easily achieved in the case of small cyclic peptides.\textsuperscript{37} Due to the lack of $C_\text{-}$ and $N_\text{-}$termini, cyclic peptides also often have enhanced resistance to exopeptidases.\textsuperscript{38} Additionally, the lack of charged ends can lead to improved cell permeability compared to linear analogues, but this of course varies depending on many other factors including the nature of the side chains (\textit{vide infra}).\textsuperscript{39}

Cyclic peptides are highly ubiquitous in nature, and a number of important drugs have been extracted from natural sources or derived from natural products.\textsuperscript{40} Cyclosporin A \textbf{89} is a head-to-tail cyclic undecapeptide containing seven $N$-methylated amino acids as well as two unnatural amino acid side chains, isolated in 1971 from the fungus \textit{Tolypocladium inflatum} (Figure 9).\textsuperscript{41,42} It was found to have potent immunosuppressant properties and is used in the clinic to treat autoimmune diseases and in the prevention of organ transplant rejection.\textsuperscript{33}
The tripeptide sequence RGD has been implicated as a highly selective motif for binding to integrins, which are key receptors vital to many physiological processes such as apoptosis, cell survival, proliferation and migration. Each integrin is a heterodimer of an α and β subunit, of which there are a total of 18 α and 8 β subunits respectively. Therefore, it is challenging to find potent and selective inhibitors for specific integrins which are implicated in the progression of many diseases.

Kessler and coworkers have studied the incorporation of the RGD sequence into a variety of pentapeptides and hexapeptides to serve as targets for integrin binding. By head-to-tail macrocyclisation, the peptides were improved more than 100-fold as inhibitors of cell adhesion compared to linear analogues. The gradual development of these cyclic variants led to the discovery of Cilengitide 90 (Figure 10), which is highly selective towards the αvβ3 receptor, and entered phase III clinical trials for the treatment of glioblastomas. The backbone conformation and the conformational rigidity of the cyclic peptide is crucial for the selectivity and activity observed.
Head-to-tail cyclic peptides have found a variety of other applications. For example, radiolabel tagged RGD-containing cyclic peptides have found use as imaging agents for cancer. Multimeric peptides such as 91 lead to higher tumour uptake of the radiolabel, and with appropriately sized linkers, can bind two integrins at once (Figure 11).

![Figure 11: Structure of a dimeric, cyclic RGD derivative for tumour imaging.](image)

Drug development often utilises high-throughput screening as a method to generate libraries of compounds to identify lead compounds which bind to biological targets. Towards this aim, the generation of cyclic peptide libraries containing $>10^6 – 10^9$ compounds have been developed using methods such as phage display, mRNA display and split-intein circular ligation of peptides and proteins (SICLOPPS).

For the synthesis of libraries containing small head-to-tail cyclic peptides, SICLOPPS is the most efficient method and is used to target intracellular PPIs which are otherwise difficult to access. Using this approach hexapeptide 92 was identified from a library of $10^7$ compounds and found to inhibit AICAR transformylase homodimerisation, a PPI involved in purine nucleotide biosynthesis. Structure-activity relationships determined by alanine scanning showed that only the dipeptide motif Arg-Tyr was important for biological activity, leading to the development of potent, cell permeable small molecule dipeptide inhibitor 93 of this PPI interaction (Figure 12).
Figure 12: Development of small molecule inhibitor 93 from an initial cyclic peptide 92 discovered from a SICLOPPS library.\textsuperscript{53}

Highly cationic cyclic peptides containing multiple Arg and Trp residues can display antimicrobial properties by targeting negatively charged membrane lipids.\textsuperscript{54} Parang and coworkers reported the synthesis of highly charged cyclic peptides which showed antibacterial activity against multi-drug resistant pathogens, all with higher activity than the respective linear analogues.\textsuperscript{55}

Amongst other reported examples, cyclo(R\textsubscript{4}W\textsubscript{4}) 94 (Figure 13) was highly cell-penetrating. When 94 was used in conjunction with the antibiotic tetracycline, the antibiotic activity of the drug increased suggesting that the presence of the cyclic peptide led to improved uptake of the antibiotic.\textsuperscript{55} In follow up work, similar cyclic peptides were used to enable the intracellular delivery of a fluorescently labelled derivative of the HIV drug emtricitabine, which was otherwise cell-impermeable.\textsuperscript{56}

Figure 13: Structure of cell-penetrating cyclic peptide 94.
Head-to-tail macrocycles which contain alternating D- and L- amino acids can self assemble into hollow nanotubes due to the macrocycle adopting a flat conformation where the backbone amide bonds are perpendicular to the adjacent side chain, allowing a hydrogen bonding network to develop. These structures have found use in gene delivery, as antivirals and as antibacterials amongst other applications, and are well reviewed in the literature.

1.3.3 Challenges to cyclic peptide development

Despite the multiple benefits of macrocyclic peptides compared to their linear counterparts, there are some limitations to the development of cyclic peptides. Although macrocyclic peptides are generally more cell permeable than linear peptides, a key challenge to their development relates to their poor cellular uptake, despite the discovery of many cell penetrating cyclic peptides. This means that many cyclic peptides have been developed towards extracellular targets, and few have been able to be designed to act within the cell.

Several features are known to improve the cell permeability of cyclic peptides. Lokey and coworkers have studied the factors which affect the high membrane permeability in head-to-tail cyclic hexapeptide scaffold 95. They determined that it was governed by three key factors: intramolecular hydrogen bonding of the macrocycle, steric factors which protect the amide N-H groups from solvation, and the stability of impermeable conformers in aqueous solution.

![Figure 14: Structure of scaffold 95, which has high membrane permeability.](image)

To achieve the desired network of hydrogen bonding, partial backbone N-methylation 96 has been shown to be a useful tool (Figure 15A), as well as adding lipophilic groups to the amino acid side chains or incorporation of D-amino acids which stabilise membrane permeable conformations 97 (Figure 15B). Installation of exocyclic amide bonds 98 have also been shown to improve passive permeability by promoting the formation of intermolecular hydrogen bonding networks.
(Figure 15C). However, none of these methods provide general solutions as membrane permeability is often sequence specific.

![Figure 15: Different approaches to improve the membrane permeability of cyclic peptides. a) Per N-methylation and D-amino acids by Lokey et al; b) Alkylation of side chains by McAlpine et al; c) Incorporation of exocyclic amide bonds, N-methylated and D-amino acids by Yudin et al.](image)

Another property of cyclic peptides which is difficult to control is oral bioavailability, which directly correlates with the poor membrane permeability previously described. Due to the presence of multiple amide bonds, cyclic peptides commonly have high polar surface areas and many hydrogen bond donors and acceptors, two factors which are known to cause poor oral bioavailability. Cyclic peptides such as CJ15208 99 (Figure 16) are orally bioavailable and comply with Lipinski’s rule of five, but larger cyclic penta- and hexapeptides require other modifications to enhance their bioavailability.

![Figure 16: Structure of CJ15208 99, which has high oral bioavailability.](image)
1.4 Synthesis of cyclic peptides

The greatest challenge in the development of macrocyclic peptides relates to difficulties in their synthesis. The conventional approach towards the synthesis of cyclic peptides and peptidomimetics is to undergo macrolactamisation of a linear precursor, for which there have been a number of activating agents and strategies developed.

However, if a linear macrocyclisation precursor is to undergo an intramolecular ring closure, it needs to adopt a conformation with the reactive groups in close proximity. Due to the natural propensity of amide bonds to favour the \textit{trans} configuration, the required conformation is often at a high energy which makes the synthesis of small cyclic peptides (4 – 6 AA’s) particularly difficult. By-products relating to dimerisation and oligomerisation often predominate, and slower reaction rates can lead to unwanted C-terminal epimerisation if bond formation is occurring at this position (Scheme 12).

\begin{center}
\textbf{Scheme 12: Limitations to macrocyclisation of short peptide chains.}
\end{center}

This section will highlight important strategies towards the synthesis of homodetic head-to-tail cyclic peptides between 4- and 6- amino acids in length. There have been many other approaches which install backbone modifications or use chemical methods to generate heterodetic cyclic peptides or cyclic peptide mimics which successfully overcome the limitations of the conventional peptide macrocyclisation. There are excellent reviews which detail these innovations and they will not be discussed in detail in this thesis.

1.4.1 General experimental considerations

A general method which is commonly used to prevent dimerisation and oligomerisation is to conduct the macrocyclisation reaction at low concentration. This favours the intramolecular process, but can lead to slower reactions which promote the formation of epimerisation at the C-
The choice of C-terminal activating reagent and additives are also important in controlling the amount of epimerisation, dimerisation and oligomerisation formed, especially under these dilute conditions.

The activating agent DEPBT 103 has been shown to be superior to some coupling agents for peptide macrocyclisation, and is highly efficient at controlling the amount of epimerisation. Although commonly used in peptide synthesis, tetramethyluronium derivatives such as HBTU 104 are prone to hydrolysis over extended periods of time in solution and can lead to unproductive guanidinylation 105 at the N-terminus (Figure 17).

As well as high dilution, pseudodilution has also been shown to favour intramolecular cyclisation over dimerisation, oligomerisation and epimerisation. By slow addition of the linear precursor and the coupling agent in parallel using a syringe pump, the concentration of the substrates can remain low without requiring a large excess of solvent to achieve high dilution. This approach has been used to synthesise a range of cyclic pentapeptides and hexapeptides (Table 1, Method B).

Another way to achieve a pseudodilution effect is to cyclise the peptides on-resin, which requires the loading of the resin to be low. By attachment of the peptide to the backbone via side chain functionality, the linear macrocyclisation precursor can be assembled iteratively by SPPS followed by cyclisation and cleavage to yield the desired cyclic peptide. A comparison of this on-resin cyclisation approach where the peptide was anchored via the Asp residue with cyclisation in solution under pseudodilution conditions can be found in Table 1. It is apparent that solution phase cyclisation is superior, but notably the ease of synthesis and potential for automation makes on-resin synthesis particularly attractive.
Pei and coworkers synthesised a peptide library on-resin to assess the effect of ring size and peptide sequence on the macrocyclisation efficiency. Over two million sequences could be synthesised using a split and pool method and cyclised. A colorimetric assay was used to identify sequences which had a cyclization efficiency of ≤99.9%, and the peptide sequence was established by partial Edman degradation-mass spectrometry (PED-MS).

The results of this study showed that for medium to large sized rings (>6 amino acids in length), the cyclisation efficiency was very high, but poor for tetrapeptides and pentapeptides, even when the extent of resin loading was low (~0.2 mmol/g). Poorly cyclising sequences often contained many Lys(Boc) and Arg(Pbf), attributed to hydrogen bonding networks established between the side chain and the C-terminus lowering its reactivity. Bulky residues towards the N-terminus such as Thr(tBu) also slowed down the cyclisations.

The addition of metal salts to the reaction mixture has been shown to enhance head-to-tail macrocyclisation. In a study by Ye and coworkers, it was found that the addition of 5 equivalents of NaCl improved the isolated yield in the cyclisation of pentapeptide 106 (Scheme 13). CD spectroscopy confirmed that a change in conformation occurred when monovalent metal ions were present. The authors hypothesised that oxophilic monovalent alkali metal ions coordinate to the amide C=O oxygen atoms to preorganise the linear peptide into a conformation ready for macrocyclisation. Interestingly, the addition of bivalent and trivalent metal ions had an inhibitory effect due to complexation at the C-terminus, preventing attack of the N-terminal amine.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sequence</th>
<th>Ring size</th>
<th>Method A (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Method B (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Asp-Ser-Pro-Leu-Asn</td>
<td>15</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Gln-Ile-Asp-Ser-Pro</td>
<td>15</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Ile-Asp-Ser-Pro-Leu-Asn</td>
<td>18</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>Thr-Gln-Ile-Asp-Ser-Pro</td>
<td>18</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>Thr-Gln-Val-Asp-Ser-Pro</td>
<td>18</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Selected examples in a comparative study of cyclic peptide synthesis via on-resin cyclisation (Method A) and pseudodilution (Method B).<sup>a</sup> Yield over all steps from preloaded resin. <sup>b</sup> Yield for macrocyclisation step.
Tomišić and coworkers reinvestigated this effect and concluded that it was the presence of the chloride anion which led to preorganisation of the backbone. They found that upon addition of sodium salts of different anions (e.g. perchlorate, tetraphenylboron) to the reaction mixture, the cyclic peptides could not be obtained, and no binding event was detected by microcalorimetry studies.

Alternatively, good results could be achieved using salts containing chloride anions with a weakly ion paired cation (Table 2), and the binding of chloride to the backbone could be studied using microcalorimetry and MD simulations. The study includes cyclic peptides with multiple lysines, which are known to be difficult to cyclise.

The use of microwave heating has also been shown to improve the macrocyclisation in some tetrapeptide substrates. However, this study is limited to amino acids which contain one D-amino acid in the backbone, which are known to be easier to cyclise than all-L peptides (vide infra).
1.4.2 Natural turn inducing amino acids

The inclusion of one or more amino acids which are turn inducing such as glycine or proline can facilitate cyclisation as they favour a cis amide bond within the linear peptide. However, this is not a general solution, and cyclisation of sequences which contain proline or glycine are not always high yielding. In the synthesis of all-L pentapeptide containing both proline and glycine, ring closure between each of the different possible sequences was assessed under identical reaction conditions (Table 3).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Peptide</th>
<th>Sequence</th>
<th>Yield (5-mer)(%)</th>
<th>Yield (10-mer)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>Gly-Pro-Tyr-Leu-Ala</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>109</td>
<td>Pro-Tyr-Leu-Ala-Gly</td>
<td>54</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>Tyr-Leu-Ala-Gly-Pro</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>111</td>
<td>Leu-Ala-Gly-Pro-Tyr</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>112</td>
<td>Ala-Gly-Pro-Tyr-Leu</td>
<td>45</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

Table 3: Yield of pentapeptide by ring closure between different precursors.

Despite the presence of proline and glycine in the sequence, the isolated yield across all ring closures was modest. Analysis of the CD spectrum of the precursor showed that the linear peptide adopts a type II β turn structure which is favourable for cyclisation. However, this is the poorest yielding example in the study and the formation of the cyclic dimer predominates.

These results also demonstrate that if the sequence contains a glycine, it can be productive to cyclise with this residue at the C-terminus (i.e. 109) as it cannot undergo epimerisation, which excludes the formation of this by-product and can lead to higher yield.
1.4.3 Incorporation of D-amino acids

The replacement of an L-amino acid with a D-amino acid has been shown to stabilise turn structures in short peptide sequences, and are well known to lead to a more favourable macrocyclisation irrespective of their position in the sequence. Indeed, some all-L peptide sequences fail to cyclise until epimerisation of the C-terminal residue has occurred. Additionally, sequences which contain alternating L- and D-amino acids are less challenging to cyclise.

Carpino and coworkers reported that switching the C-terminal Tyr in the all-L sequence to D-changed the conformation of the linear peptide 113, leading to an 82% yield for the pentapeptide 114. The cyclisation of the all-L sequence was particularly poor, yielding only 35% of the cyclic decapeptide as well as 7% of the epimerised product (Scheme 14).

Scheme 14: Incorporation of a D-amino acid by Carpino et al to facilitate cyclisation.

1.4.4 N-alkylated peptides

Peptide bonds generally predominate in the trans geometry to minimise unfavourable steric interactions between the adjacent side chain groups. By introducing an N-alkyl substituent to the amide nitrogen, the barrier to interconversion between the alternative cis conformation is lowered. Consequently, N-alkyl substituents have been introduced to peptide backbones to induce turn-like structures.

This phenomenon has been used to improve the head-to-tail macrocyclisation of small peptides. Tetraglycine is known to cyclise in a meagre 5% yield, whereas the N-methylated tetrapeptide tetrasarcosine can be isolated in 43% yield. Significant efforts have also been made to install reversible alkylation or acylation derivatives to induce beneficial conformations for cyclisation, with groups that can be removed to generate the unmodified cyclic peptide. Towards the synthesis
of 116, N-methylation as well as alkylation with the acid-removable Hmb unit led to improved macrocyclisation (Scheme 15). The ortho-nitrobenzyl unit, which can be cleaved under photochemical conditions, has also been demonstrated as a useful turn-inducing alkyl substituent in other systems.

Scheme 15: N-alkylation approaches to access cyclic pentapeptide 116.

1.4.5 Pseudoprolines

Originally developed for their ability to prevent aggregation and diketopiperazine formation during SPPS, pseudoprolines have become important building blocks in peptide chemistry. They are five membered ring derivatives of proline which are formed by condensation of Thr, Ser or Cys residues with aldehydes or ketones (Scheme 16). These modifications are easily incorporated by solution phase synthesis or SPPS from dipeptide building blocks 120 and some are commercially available. They can be cleaved from the peptide backbone to give the native amino acid using strongly acidic conditions.

Scheme 16: Synthesis of pseudoproline building blocks for SPPS.

Pseudoprolines have been utilised as a tool to enhance the macrocyclisation of small peptides owing to their ability to easily access cisoid conformations in linear peptides. 2,2-Dimethyl substituted pseudoproline derivatives (ψ\(^{Me,Me}\)Pro) are known to adopt this conformation most commonly. Mutter and coworkers reported that inclusion of a ψ\(^{Me,Me}\)Pro led to the formation of a
type VI β turn structure, indicating these proline mimics were good candidates for the head-to-tail cyclisation of small peptides.\textsuperscript{86,87}

Jolliffe and coworkers investigated the use of Thr $\psi^{\text{Me,Me}} \text{Pro}$ as a turn inducing element towards the synthesis of cyclic peptide cyclo(Val-Thr)$_3$ \textbf{122}. The parent peptide containing TBS protected Thr did not cyclise under a range of different conditions.\textsuperscript{88} It was found that multiple pseudoprolines in the backbone were required for efficient cyclisation of this sequence in up to 91% yield. Installation of the Thr $\psi^{\text{Me,Me}} \text{Pro}$ at the C-terminus led to no epimerisation during the cyclisation. Linear precursors with only one or two Thr $\psi^{\text{Me,Me}} \text{Pro}$ units cyclised in moderate yield, and with significant levels of C-terminal epimerisation. Importantly, the pseudoproline could be removed after cyclisation with acid to yield the all-L cyclic peptide \textbf{122} (Scheme 17).\textsuperscript{88}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme17.png}
\end{center}

\textbf{Scheme 17:} Synthesis of \textbf{122} using multiple Thr $\psi^{\text{Me,Me}} \text{Pro}$ modifications.\textsuperscript{88}

This study was extended further to evaluate the positional dependence of the pseudoproline in the backbone. In hexapeptides, $\psi^{\text{Me,Me}} \text{Pro}$ residues at every second amino acid in the sequence led to optimal macrocyclisations including one at the C-terminus to prevent epimerisation.\textsuperscript{89} The use of pseudoprolines to synthesise all-L cyclic head-to-tail tetrapeptides has also been reported.\textsuperscript{90}

One limitation of this methodology is that it cannot be applied to target peptides that do not contain Thr, Ser or Cys. To expand the scope of the methodology, a deprotection and desulfurisation method was developed which allows for the synthesis of Ala or Val containing cyclic peptides with Cys $\psi^{\text{Me,Me}} \text{Pro}$. This strategy has been used for the synthesis of several naturally occurring cyclic peptides such as segetalin B \textbf{124} (Scheme 18), segetalin G, cyclogossine B and dichotomin A.\textsuperscript{91-93}
1.4.6 Dehydro amino acids

Dong and co-workers used the unsaturated amino acid dehydrophenylalanine as a turn inducing residue towards the synthesis of dichotomin E 128, a pentapeptide which has shown activity against leukemia cells.94

Macrocyclisation was improved from 15% to 81% when dehydrophenylalanine was included in the sequence instead of phenylalanine, with high selectivity for pentapeptide 126 over the cyclodimer, improved from 1.5:1 to 39:1 (Scheme 19). Furthermore, inclusion of a second unsaturated residue – dehydrotyrosine rather than tyrosine – increased the isolated yield to 84%. Asymmetric hydrogenation was applied to generate the desired all-L natural product.95

Scheme 18: Synthesis of segetalin B 124 using a Cys pseudoproline approach by Jolliffe et al.91

Scheme 19: Dong’s synthesis of dichotomin E 128 using dehydrophenylalanine as a turn inducing residue.95
1.4.7 Extending the length and flexibility of the macrocyclic precursor

By increasing the flexibility of the linear precursor, the backbone can preorganise itself to access the conformation required for macrocyclisation more easily. Hunter and co-workers replaced the central leucine side chain in a pentapeptide sequence with additional methylene units. The isolated yield could be increased four-fold by simply replacing the leucine with glycine, but further improvement was observed when the linker length was increased to two or three methylene units (Scheme 20).96

![Scheme 20: Increasing the length and flexibility of the backbone increases macrocyclisation efficiency.](image)

1.4.8 Ring contraction strategies

Smythe and coworkers developed a ring contraction strategy for the synthesis of head-to-tail cyclic peptides by introduction of a photocleavable auxiliary. Linear peptides such as 136 could be synthesised using Fmoc-SPPS including reductive amination to install the HnB auxiliary 135 at the N-terminus (Scheme 21).97

![Scheme 21: Synthesis of N-terminal HnB macrocyclisation precursors.](image)

The auxiliary extends the length of the sequence, adding flexibility and allowing ring closure to occur between the activated C-terminus and the phenol 137. This puts the N-terminal amine in close proximity with the C-terminal ester, leading to formation of the amide bond by ring
contraction 138. The auxiliary 139 can be subsequently removed by photolysis to form 140 (Scheme 22).^{97}

![Scheme 22: Ring contraction strategy towards the synthesis of pentapeptide 140 by Smythe et al.]{fig1}

This methodology has been used to synthesise a library of head-to-tail cyclic tetrapeptides. Each of the library members contained glycine at the C-terminus to avoid epimerisation. Of the forty four sequences attempted, thirty four could be successfully isolated after on-resin linear precursor synthesis, ring contraction, auxiliary removal and final purification by RP-HPLC.^{98} For targets that were difficult to cyclise, installation of the HnB auxiliary in the middle of the sequence on one of the amide nitrogens as well as at the N-terminus was shown to be favourable, by promoting formation of a cis-amide bond to facilitate the cyclisation.^{99}

In a similar approach, the auxiliary can be added to the C-terminus. Van Maarseveen and coworkers installed the (E)-2-(2-nitrovinyl)phenol 142 via esterification in good yield, which acted as a Michael acceptor for the N-terminal amine of 143. Subsequent ring contraction gave the desired tetrapeptide 144 in 28% yield (Scheme 23).^{100} The HnB auxiliary approach by Smythe et al produced the same peptide in only 11% yield.^{98}
1.4.9 Macrocyclisation of N-terminal thioamides

By incorporation of a thioamide at the N-terminus, Hutton and coworkers showed that head-to-tail macrocyclisation could be achieved using Ag(I) activation without the need for additional peptide coupling agents. The mechanism includes extrusion of the thioamide via $\text{Ag}_2\text{S}$, generating an isoimide intermediate 147 that can rearrange into an imide 148 which hydrolyses to form the amide bond of cyclic peptide 149 (Scheme 24).
Scheme 24: Synthesis of head-to-tail cyclic peptides promoted by Ag(I) macrocyclisation of N-terminal thioamides.101

The Ag(I) salt has two roles in the macrocyclisation: to enable chemoselective activation of the thioamide, and to bring the C- and N-termini into close proximity by coordination. This chemistry also bypasses problems associated with dimerisation and epimerisation and yields the all-L macrocycle. This methodology has been applied to the synthesis of several naturally occurring cyclic peptides including mahafacyclin B and gramicidin S.101
1.4.10 Side chain ligation

In 1994, Kent and coworkers developed native chemical ligation (NCL) for the efficient synthesis of peptides and proteins. This method requires two components: (i) an N-terminal cysteine, and (ii) an acid activated as a thioester. Spontaneous transthioesterification between the free cysteine and the thioester is followed by an irreversible S-to-N acyl transfer to generate the amide bond.\textsuperscript{102}

This technique has been employed for the synthesis of head-to-tail cyclic peptides. Tam and coworkers described the synthesis of several cyclic enkephalin analogues between five and eight residues in length (Scheme 25), as well as other larger systems. The cyclisation could be conducted at high concentration without the formation of dimers or higher oligomers and importantly, without side reactions on unprotected residues meaning that the cyclisations could be carried out without protecting groups and in aqueous solution.\textsuperscript{103}

![Scheme 25: NCL as an approach to synthesise head-to-tail cyclic peptides containing cysteine.\textsuperscript{103}](image)

The free Cys in the resulting cyclic peptide has been utilised as a handle for further transformations. Tam also used this Cys to make a disulfide bridged bicyclic peptide.\textsuperscript{103} Desulfurisation has been used to convert the Cys into an Ala residue, expanding the scope of this methodology.\textsuperscript{104,105} The use of other thiol containing amino acids for NCL followed by desulfurization to give natural amino acids has been explored.\textsuperscript{106}

The side chain alcohol of Thr and Ser can also be used as a handle for facile amide bond formation via O-to-N acyl transfer and has been employed in the synthesis of head-to-tail cyclic peptides. Amblard and coworkers demonstrated the synthesis of cyclic pentapeptide 156 by O-to-N acyl
migration from depsipeptide 154 (Scheme 26). This chemistry also worked for hexa- and heptapeptides, although the ring strain in the tetrapeptide sequence was too high for the O-to-N acyl transfer, leading to hydrolysis.\textsuperscript{107} The use of Ser/Thr ligation to generate head-to-tail cyclic peptides, including tetrapeptides, \textit{via} C-terminal salicylaldehyde esters has also been reported.\textsuperscript{108,109}

\textbf{Scheme 26:} Synthesis of head-to-tail cyclic pentapeptide 156 by O-to-N acyl transfer of depsipeptide 154 by Amblard and coworkers.\textsuperscript{107}

\textit{1.4.11 Concluding remarks}

Cyclic peptides are privileged scaffolds which have great potential in medicinal chemistry, but several challenges hinder the development of these molecules in drug discovery programs.\textsuperscript{25} They can suffer from poor oral bioavailability and membrane permeability, which reduces their ability to act on intracellular targets.\textsuperscript{31} By understanding the factors which can affect the stabilisation of membrane permeable conformations such as hydrogen bonding, and adding functional groups to enhance permeability can help to optimise the bioavailability of some structures.\textsuperscript{39}

One of the biggest challenges to the development of cyclic peptides is their synthesis.\textsuperscript{28} Some sequences may cyclise well due to conformational preorganisation, but the underlying preference for \textit{trans} amide bonds leads to unproductive dimerisation, oligomerisation and epimerisation in many cases.

Notable methods towards the synthesis of cyclic peptides include the addition of a turn-inducing element such as a pseudoproline, but these also require the presence of certain amino acids in the
sequence and their conversion or deprotection to form native amino acids requires harsh conditions and can be poorly yielding.\textsuperscript{93} The addition of turn-inducing, removable $N$-alkylated substituents are also important advances, but the removal of these groups by photochemical or acidic methods can be problematic.\textsuperscript{97} Ligation strategies offer a useful method to synthesise cyclic peptides limited to Ser, Thr or Cys (and Ala via further chemistry), and can be applied to aqueous conditions.\textsuperscript{68} Despite these advances, there is currently no general method for the macrocyclisation of small cyclic peptides.
Chapter 2: Synthesis of Oxetane Modified Head-to-Tail Cyclic Peptides

2.1 Previous Work and Project Aims

Building on previous work in the Shipman group on oxetane containing peptidomimetics, this project will focus on the incorporation of the 3-aminooxetane unit into small head-to-tail cyclic peptides (Scheme 27). Following on from these investigations, we want to understand the impact of this bioisosteric modification on the conformation and properties of the macrocycle compared to the parent peptides, with a specific focus on medicinally and biologically relevant examples. We hope that oxetane modification will become a useful tool for the synthesis of head-to-tail cyclic peptides, expanding chemical space and enabling the discovery of new active molecules for medicinal chemistry.

In previous work, molecular dynamics simulations of tripeptide sequences showed that replacement of the central C=O of an amide bond with an oxetane ring leads to a higher population of clusters where the C- and N- termini are in close proximity (Figure 4, Chapter 1). It was hypothesised that this motif could be used to induce turns in short peptide sequences, which could preorganise the linear precursor for macrocyclisation to form small cyclic peptides.

Prior to the start of my PhD investigations, Dr Joanna Geden had successfully synthesised a linear oxetane modified pentapeptide 159 and successfully cyclised it to 161 in 51% yield. This peptide was chosen because the parent linear peptide 160 has been studied and is reported to undergo macrocyclisation to 162 in 31% yield. Encouragingly, the isolated yield was significantly improved compared to the literature conditions (Scheme 28).
Following this early result, we initiated work to see if this improvement was general over a range of other ring sizes and sequences. We were also interested in examining the extent of epimerisation, dimerisation and rates of macrocyclisation compared to conventional substrates. Furthermore, we wanted to establish how oxetane modified peptides compare to other literature-known peptide modifications that enhance macrocyclisations (e.g. N-methylation).

In parallel, my colleagues sought to understand why the macrocyclisation is improved, how the structure of the macrocycle differs and if the biological activity of a known inhibitor is changed by the installation of an oxetane as an isostere of the carbonyl group.

2.2 Results and Discussion

2.2.1 Initial study to form an oxetane modified pentapeptide

We decided to synthesise a similar substrate to the initial example 161 with an isolated yield whose macrocyclisation is again reported in the literature, to see if oxetane incorporation improved the yield in a different substrate. The pentapeptide LAGPY 107 was chosen as it is known to cyclise poorly in 13% yield (Table 3, Chapter 1),\textsuperscript{71,75} whilst the tyrosine residue, with a strong UV chromophore would enable facile analysis of the products by LC-MS.

The synthetic route to precursor 169 is outlined in Scheme 29. We started with O-benzyl protected tyrosine 163, which was chosen because it would permit for a global deprotection \textit{via} hydrogenation at the end of the synthesis to provide a salt-free precursor ready for cyclisation. We chose a solution phase coupling approach, which enabled accurate comparisons to be made with different substrates without having to correct for the impact of salts on the cyclisation.
3-oxetanone 8 was converted to oxetanyl nitroalkene 9, which underwent conjugate addition with the free N-terminus of dipeptide 165. This generated nitroalkane 166, which could be reduced with Raney nickel in the presence of Boc-Ala-OSu to form tetrapeptide building block 167. The final coupling to 168 followed by global deprotection gave linear precursor 169 in good yield. In parallel, the parent compound 111 was synthesised using conventional solution phase peptide synthesis for use as a comparison.

Scheme 29: Synthetic sequence for the oxetane modified pentapeptide 169.

In order to identify the optimal conditions for the macrocyclisation, the linear substrates 111 and 169 were subjected to head-to-tail coupling using a range of different peptide coupling reagents and reaction conditions (Table 4).
Table 4: Macrocyclisation yields for pentapeptide 111 and 167. All reactions completed on 0.1 mmol scale. Yield in parenthesis relates to larger scale reaction (0.5 mmol) run at higher concentration (5 mM). a DIPEA omitted. b Isolated as an 18:1 mixture of diastereomers determined by 1H NMR. c Yield from ref:71,75.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Coupling reagent</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>169</td>
<td>DEPBT</td>
<td>170</td>
<td>48 (50)</td>
</tr>
<tr>
<td>2</td>
<td>169</td>
<td>DEPBT</td>
<td>170</td>
<td>33a</td>
</tr>
<tr>
<td>3</td>
<td>169</td>
<td>PyBOP</td>
<td>170</td>
<td>60b</td>
</tr>
<tr>
<td>4</td>
<td>169</td>
<td>HATU</td>
<td>170</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>169</td>
<td>T3P</td>
<td>170</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>111</td>
<td>DEPBT</td>
<td>107</td>
<td>13c</td>
</tr>
<tr>
<td>7</td>
<td>111</td>
<td>PyBOP</td>
<td>107</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>111</td>
<td>HATU</td>
<td>107</td>
<td>15</td>
</tr>
</tbody>
</table>

In general, a three-fold improvement in isolated yield was seen upon oxetane incorporation. The macrocyclisation of the parent peptide performed poorly with a variety of different coupling agents. PyBOP was the most effective coupling agent for this ring closure for both systems (entries 3 and 7), although 170 was formed as a mixture of diastereomers which were difficult to separate by column chromatography. DEPBT and HATU were also effective (entries 1 and 4) with only trace amounts of the epimerised product. Adding base to the reaction was important as the isolated yield was lowered without DIPEA (entry 2). The reaction could also be run on a larger scale at higher concentration with no loss in yield (entry 1).

Encouraged by the improvement that oxetane modification had on the isolated yield, we investigated the relative amounts of dimers and epimers which were formed under the reaction conditions. The crude reaction mixtures of the macrocyclisation of 111 and 169 from entry 1 and 6 in Table 4 were analysed by LC-MS in order to visualise the product distribution (Figure 18).
Figure 18: EIC traces of the crude reaction mixtures of the macrocyclisations of 169 (a) and 111 (b). Analysis performed in collaboration with Dr Ina Wilkening.

The macrocyclisation of 111 is particularly poor. Significant amounts of starting material are observed even after 48 h. Alongside this, the linear and cyclised dimers are present as well as the epimerised product. In comparison, macrocyclisation of 169 is a much cleaner reaction. Essentially complete conversion is observed on the same time scale, with only trace amounts of the epimerised and dimerised products visible.
2.2.2 Expanding the scope of the macrocyclisation

With confidence that oxetane modification had a beneficial impact on the product distribution and isolated yield for pentapeptides $161$ and $170$, we wanted to explore if this methodology would also be suitable for smaller and larger ring sizes.

It is reported that head-to-tail macrocyclisation of the sequence PLGG proceeds with an isolated yield of 23%.\textsuperscript{110} This substrate was chosen to investigate the macrocyclisation of tetrapeptides as the C-terminal glycine cannot epimerise, eliminating a potential by-product which could form. Dipeptide $12$ was prepared following a literature procedure in 84% yield.\textsuperscript{14} Reduction of the nitro group and \textit{in-situ} coupling with Boc-Leu-OSu gave tripeptide $171$ in 68% yield. Following removal of the N-terminal Boc group, EDC-mediated coupling gave the protected linear precursor which could be deprotected by hydrogenation in quantitative yield to provide the salt-free tetrapeptide $172$ ready for macrocyclisation (Scheme 30).

The cyclisation of the tetrapeptide $172$ was attempted using DEPBT conditions (Scheme 31). Purification by repeat flash column chromatography allowed for the isolation of material with 18% mass recovery. This material has the correct $m/z$ for $174$, but the $^1$H NMR was inconclusive due to the presence of impurities or its existence as a mixture of conformers. Attempts to simplify and sharpen the spectrum by heating in $d_6$-DMSO to 373 K failed to improve the spectrum.

![Scheme 30: Synthetic route to tetrapeptides 172 and 173.](image)

![Scheme 31: Initial cyclisation of 172.](image)
The inclusion of D-amino acids can lead to improved macrocyclisations in small cyclic peptides (Chapter 1, Scheme 14). We reasoned that changing the stereochemistry of the proline residue from L- to D- may resolve the problem with mixtures of conformations and could be higher yielding, enabling for easy isolation of the cyclic peptide. Coupling of Cbz-D-Pro-OH to 171 followed by hydrogenation gave access to the linear precursor 173 in 70% yield over 3 steps (Scheme 30). Cyclisation was successful using DEPBT as the coupling agent to give 175 in 54% yield (Scheme 32). The isolated cyclic peptide 175 gave a single set of sharp signals in the 1H NMR and the structure could be fully assigned.

In order to compare this result with the corresponding parent system, linear peptide 176 was synthesised using standard peptide couplings. Interestingly, under identical macrocyclisation conditions, the dimeric octapeptide 177 was the only peptide which could be isolated in 20% yield, and no evidence for the desired tetrapeptide could be detected by LC-MS analysis (Scheme 33). The presence of the dimer was confirmed by observation of the isotopic distributions in the HRMS, which clearly showed a difference in m/z of 0.5 between each isotopic peak. This result amply demonstrates the utility of our new methodology, as oxetane modified cyclic peptides can be prepared in cases where the parent system cannot be formed by macrocyclisation.

Following from this result, cyclic tetrapeptides 181–183 were synthesised to expand the scope, prepared from common building block 171 (Scheme 34). The cyclisation step was carried out using DEPBT and DIPEA as these reactions were easier to purify compared to macrocyclisations carried out with PyBOP and HATU. Alongside these peptides, the corresponding parent cyclic peptides 184 and 185 containing glycine instead of Gox were prepared using conventional solution phase coupling (see Chapter 4) to compare against 181 and 182. In both cases, three- to
four-fold improvements in yields were witnessed compared to the conventional peptides (Figure 19).

Figure 19: Oxetane modified cyclic tetrapeptides. Yield in parenthesis for the parent system macrocyclisation. Reaction conditions: 2 eq. DEPBT, 2 eq. DIPEA, 1 mM, DMF, 48 h, rt. a Using PyBOP as activator.

Jolliffe and coworkers reported a poorly yielding macrocyclisation from 186 to form the cyclic hexapeptide Dichotomin A 188 from an all-L amino acid backbone. Their approach to access this macrocyclic natural product was to incorporate a pseudoproline motif of valine 187, which improved the macrocyclisation step to 88% yield. However, the overall yield was hampered by poor yields for the conversion of the pseudoproline back to the valine residue by reductive desulfination (Scheme 35).
We were curious to see if oxetane incorporation would improve the yield for macrocyclisation, and this was an ideal substrate to probe the macrocyclisation of a larger ring size as it contains a glycine residue which we could modify without changing the sequence of the peptide. Our strategy was to place the oxetane modified glycine in the middle of the sequence to get the maximum benefit from the turn inducing unit. The yield for the macrocyclisation of the parent sequence reported by Jolliffe was 33% (Scheme 35).\(^{93}\)

The initial synthesis of the hexapeptide precursor employed dipeptide 190, which was used in a conjugate addition to form tripeptide 191. However, the yield for the conjugate addition step was very low and variable (Scheme 36).
During deprotection of the Fmoc group of 189, a white precipitate formed which was insoluble in a variety of polar and non-polar solvents and could not be characterised. We speculate this by-product to be either diketopiperazine 192, which are known to have poor solubility, or a polymerised oligomer of the dipeptide 190 (Scheme 37).

Scheme 37: Proposed intramolecular cyclisation of 190 to form diketopiperazine 192.

The formation of an insoluble precipitate was not observed during the synthesis of 166 (Scheme 29), where the dipeptide Boc group of 164 was removed under acidic conditions prior to the conjugate addition. In this case, the TFA salt would render the amine much less nucleophilic and unable to undergo intramolecular cyclisation during the deprotection (Scheme 37). In contrast, removal of the Fmoc group in 189 provided a free amine which could undergo further side reactions. Simply changing the N-terminal protecting group to Boc was not possible, because the side chain Thr contained a tert-butyl ether; highly concentrated acid would cleave this protecting group.

In order to avoid this, the cumyl ester of Thr was synthesised using methodology developed previously in the group (Scheme 8). Although the cumyl ester requires 2% TFA/CH₂Cl₂ to be removed, it would be mild enough to ensure that the tert-butyl ether is unscathed. Furthermore, extension of the C-terminus of similar building blocks has been reported, so we would be able to add the Phe residue at a later stage in the synthesis.

Cumyl ester 194 was synthesised using literature conditions in 95% yield and employed in the conjugate addition forming dipeptide 196 in 75% yield. Reduction of the nitro group with concomitant coupling to Fmoc-Val-OSu gave tripeptide 197 in 53% yield. Cumyl ester deprotection using 2% TFA/CH₂Cl₂ allowed for C-terminus coupling, with no side chain deprotection on Thr detected, obtaining tetrapeptide 198 in 83% yield (Scheme 38).
Scheme 38: Synthesis of tetrapeptide 198 from cumyl ester 194.

Further extension at the N-terminus with Fmoc-Tyr(Bu)-OH and Cbz-Leu-OH gave access to fully protected hexapeptide which was hydrogenated to provide the desired linear precursor 200 in good overall yield (Scheme 39).

Scheme 39: Final steps to access linear precursor 200.
The initial cyclisation test for the hexapeptide 200 followed identical conditions reported by Jolliffe and co-workers (Scheme 35).\textsuperscript{93} LC-MS analysis of the crude reaction mixture after 3 days showed full consumption of the starting material 200, with no linear or cyclic dodecapeptide observed. Furthermore, no epimerisation was detected. Unfortunately upon purification by flash column chromatography, by-products derived from the coupling reagent co-eluted with the cyclic hexapeptide, making purification very difficult. The Jolliffe group report that parent peptide 202 was purified by reverse phase flash column chromatography. However, changing the macrocyclisation conditions to DEPBT and DIPEA (Table 4) led to isolation of the cyclic hexapeptide 201 in 58% yield (Scheme 40). This is approximately a two-fold improvement compared to the reported yield by Jolliffe of 33%, although using a different coupling agent and at higher concentration.

\textbf{Scheme 40}: Cyclisation to form oxetane modified cyclic hexapeptide 201.\textsuperscript{a} Using 3 equiv. DMTMM•BF\textsubscript{4}, DIPEA, DMF, 5 mM, 72 h.\textsuperscript{93}

Whilst this hexapeptide example nicely illustrated the usefulness of our methodology, the solution phase synthesis of larger substrates is rather impractical. To expedite the process, the synthesis of a pentapeptide substrate was explored using a SPPS approach to synthesise the linear precursor utilising oxetane modified dipeptide building blocks. This methodology was developed by my colleague Dr Ina Wilkening, with further examples reported in our publication.\textsuperscript{112}

Following the known procedure, glycine dipeptide building block 203 was made.\textsuperscript{19} Using preloaded glycine 2-chlorotrityl resin 205, linear peptide 208 was synthesised in excellent overall yield, and this peptde was cyclised to furnish the macrocyclic peptide 209 in moderate yield (Scheme 41). This result confirms the utility of the SPPS methodology and could potentially enable automation which would allow for the synthesis of peptide libraries containing the oxetane subunit.
2.2.3 Synthesis of oxetane modified alanine containing cyclic peptides

Until this point, only oxetane modified glycine residues had been incorporated into the backbone of the cyclic peptides. We were interested if other oxetane modified amino acids would also improve the macrocyclisation of peptides. Although Carreira had reported the diastereoselective synthesis of oxetane modified dipeptide building blocks, their route has several drawbacks requiring many steps and is not especially practicable.\textsuperscript{18}

Synthesis of the oxetane modified alanine precursor 214 was completed using a modified method initially developed in the group to generate dipeptide building block 84 (Scheme 10).\textsuperscript{21} Conjugate addition of (R)-\(\alpha\)-methylbenzylamine 80 to nitroalkene 79 produced a 60:40 mixture of diastereomers which were separable by column chromatography. The previous work had established the stereochemistry of the major, (S)-diastereomer by XRD. Raney nickel reduction of this (S)-diastereomer 81 with \textit{in situ} Boc protection generated 210. Subsequent hydrogenolysis followed by \(S_N2\) displacement with benzyl bromoacetate 211 gave dipeptide 212. This building
block could then be elongated in a routine fashion to generate linear precursor 214. Alongside this, the corresponding parent peptide was made using conventional solution phase coupling to serve as a comparison. The cyclisation of oxetane modified 214 proceeded in 50% isolated yield. In comparison, the parent peptide 216 could only be isolated in 20% yield demonstrating that the oxetane modified alanine residue also enhances the macrocyclisation.

Scheme 42: Synthesis of cyclic tetrapeptide 215 containing an alanine-oxetane modified residue.

2.2.4 Investigation of the kinetics of the macrocyclisation

In order to learn about the rate of macrocyclisation, time-course studies were undertaken (Figure 20). The macrocyclisation of 178 forming oxetane modified 181 and the corresponding parent cyclic peptide 184 were compared, as the tryptophan moiety allowed for quantitative analysis by analytical HPLC at 280 nm. Macrocyclisations of each precursor were run side by side, aliquots were taken over 74 h and directly analysed by analytical HPLC in order to quantify the formation of cyclic peptides 180 and 214. LC-MS confirmed the retention times of these compounds by their mass.
A sample was taken before the coupling agent was added to determine the initial value by HPLC. Integration of the corresponding HPLC peaks allowed for a measure of conversion from the linear precursors 178 and 217 to the desired tetrapeptides 180 and 184.

Figure 20: Time-course kinetic plot for the macrocyclisation of WLGG 217 and oxetane modified WLGG 178. Work conducted in collaboration with Dr Ina Wilkening.
The consumption of \textbf{178} and \textbf{217} occurred at a very similar rate. However, the initial rate of formation of oxetane modified cyclic peptide \textbf{181} occurs much faster than the corresponding parent system. Furthermore, formation of cyclic octapeptide \textbf{218} is evident in the parent system cyclisation but is not observed in the oxetane modified system. After 34 h, there is no apparent increase in the conversion to cyclic peptides \textbf{180} or \textbf{184} despite traces of the linear precursors \textbf{178} and \textbf{217} remaining in the reaction mixture. Overall, oxetane modification leads to a maximum conversion of 83\% whilst the unmodified system only reaches 50\% due to the formation of unwanted dimer \textbf{218}.

2.2.5 \textit{Comparing oxetanes with other peptide modifications}

Although oxetane modification improves the cyclisation across a variety of different sequences, it was unclear how significant these improvements are compared to other common peptide modifications. To explore this issue, cyclisation of the sequence LAGAY \textbf{160} was studied, with different modifications made to the central glycine residue (Table 5). Five additional modifications were explored, with the work undertaken in partnership with Dr Stefan Roesner.

The modifications chosen were designed to encourage the peptide to undergo macrocyclisation by increasing the conformational flexibility of the peptide (\textbf{222}, \textbf{225}),\textsuperscript{96} by changing the \textit{cis}/\textit{trans} amide bond geometry (\textbf{222}),\textsuperscript{113} or by adding substituents which might induce a beneficial Thorpe-Ingold effect (\textbf{228}, \textbf{229}).\textsuperscript{114} Reduced amides (\textbf{230}) have also been shown to promote \(\beta\)-turns in pentapeptides by protonation of these residues at the secondary amine, leading to hydrogen bonding with the neighbouring carbonyl oxygen.\textsuperscript{115}

Linear peptides \textbf{222} containing an \(N\)-methylated amino acid in the middle of the sequence and \textbf{225} containing an additional methylene unit were produced using conventional peptide coupling from \textbf{219} with commercially available building blocks (Scheme 43 and 44).
Scheme 43: Synthesis of linear precursor 222.

Scheme 44: Synthesis of linear precursor 225.
Dimethyl variant 228 was accessed employing a conjugate addition of commercial 2-methyl-1-nitroprop-1-ene 226, with following nitro group reduction, chain extension and global deprotection chemistry analogous to that used for oxetane modified peptides (Scheme 45).

Scheme 45: Synthesis of linear precursor 228.
Table 5: Isolated yields of macrocyclic peptides 161, 162, 231–235. * Yield is an average of two runs. # Isolated as TFA salt after RP-HPLC. † Compound prepared by Dr Stefan Roesner.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>AA</th>
<th>Cyclic peptide</th>
<th>Yield (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>160</td>
<td></td>
<td>162</td>
<td>31%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>159</td>
<td></td>
<td>161</td>
<td>51%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+20%</td>
</tr>
<tr>
<td>222</td>
<td></td>
<td>231</td>
<td>36%</td>
<td>+5%</td>
</tr>
<tr>
<td>225</td>
<td></td>
<td>232</td>
<td>39%</td>
<td>+8%</td>
</tr>
<tr>
<td>228</td>
<td></td>
<td>233</td>
<td>32%</td>
<td>+1%</td>
</tr>
<tr>
<td>229</td>
<td></td>
<td>234</td>
<td>29%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-2%</td>
</tr>
<tr>
<td>230</td>
<td></td>
<td>235</td>
<td>28%&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>-3%</td>
</tr>
</tbody>
</table>

Compared with the parent peptide 160, only the oxetane modification in 159 led to an appreciable improvement in the isolated yield. Substrates 228 and 229 indicated there was no significant Thorpe-Ingold effect taking place, with yields comparable to the parent system. Sarcosine derivative 222, which introduces a cis-amide bond in the middle of the sequence led to a small improvement. Increasing the size of the macrocycle by a single methylene unit 225 added extra flexibility to the linear precursor and led to some improvement.
2.2.6 Deprotection of the side chain protecting groups

Our attention turned to the deprotection of the acid sensitive protecting groups on the side chains of the oxetane modified cyclic peptide. Previous work on linear oxetane modified peptides had suggested that the ring is stable to highly acidic conditions. Indeed, preparation of some of the linear precursors in this work involved the use of Boc deprotection with 50% TFA in CH₂Cl₂ (Scheme 29, 30, 34). However, oxetanes are known to be liable to ring opening under mildly acidic conditions.¹¹ Cyclic tetrapeptide 183 which contains the aryl tBu ether was subjected to standard deprotection conditions of 50% TFA in CH₂Cl₂ for 1 h. Unfortunately, under these conditions the cyclic peptide 236 was not observed. In the ESI-MS, a + 18 Da peak relative to the desired product was observed suggesting that ring opening of the oxetane of the product by water 237 had occurred during the reaction. However, purification by flash column chromatography did not lead to the successful isolation of this by-product.

Reducing the amount of acid was expected to reduce the extent of ring opening. Gratifyingly, using 15% TFA, tetrapeptide 236 could be isolated in 72% yield. The reaction was complete after 15 minutes, monitored by TLC and ESI-MS (Scheme 46). If the reaction was driven by ring opening by water under acidic conditions, then rigorous drying or addition of a drying agent might further improve the deprotection yield. The previous reaction with 15% TFA was repeated, this time adding freshly vacuum dried MgSO₄, using anhydrous dichloromethane dried over 4Å molecular sieves, including anhydrous TFA stored in an ampule purchased from Sigma Aldrich. However, no improvement was observed under these conditions (Scheme 46).

![Scheme 46: Deprotection of 183.](image)

A: 50% TFA/CH₂Cl₂, RT, 1 h
B: 15% TFA, CH₂Cl₂, RT, 15 min
C: 15% anhyd. TFA, anhyd. CH₂Cl₂, RT, 15 min

**Conds.** A: 0%
**Conds.** B: 72%
**Conds.** C: 70%

m/z = 419 [M+H]⁺
m/z = 437 [M+H]⁺
Next, we sought to explore the deprotection of a more challenging substrate, 201, containing both an aryl ether and an alkyl ether. Aryl tBu ethers are generally easy to remove under mildly acidic conditions, but alkyl ethers require higher TFA concentrations.\textsuperscript{116} We treated cyclic hexapeptide 201 to the initial deprotection conditions, and a mixture of products formed, including a species with additional 18 Da relative to the desired product, indicative of ring opening. Purification by flash column chromatography led to isolation of the fully deprotected cyclic peptide 238 in low yield, but the ring opened product 239 was not isolated (Scheme 47).

The milder conditions were also applied to hexapeptide 201 to see if the lower acid concentration would minimise ring opening. After 15 minutes, ESI-MS showed only starting material alongside the mono-deprotected compound 240. This was proposed to be due to deprotection of Tyr rather than Thr due to their relative labilities under acidic conditions.\textsuperscript{116} Allowing the reaction to continue for a further 45 mins led to full consumption of starting material, at which point the reaction was stopped. The mono deprotected 240 and the ring opened product 239 were also observed alongside 238. After purification by column chromatography, 238 could be obtained in a disappointing 21\% yield (Scheme 48). This suggested that under these conditions, cleavage of the tert-butyl ester of Thr occurs at a similar rate to oxetane ring opening, and longer reaction times would certainly lead to further ring opening of the product, reducing the yield.

Scheme 47: Deprotection of cyclic hexapeptide 201.
An alternative strategy was briefly explored in changing the protecting groups on the side chains to remove them under milder conditions. The use of peptides with O-trityl protected side chains is well known, the amino acid building blocks are commercially available, and can be removed with as mild as 1% TFA in CH$_2$Cl$_2$. Deprotection of the cyclic peptide 209 containing Ser(Trt) could be achieved in 95% yield after column chromatography (Scheme 48). This methodology could allow for the deprotection of cyclic peptides containing Tyr, Thr and Ser residues.

![Scheme 48: Deprotection of cyclic peptide 206.](image)

As shown in earlier work, peptides made in solution phase can be cyclised with unprotected Tyr (Table 4), and this may be possible for other alcohol side chains. However, other protecting groups which require harshly acidic conditions such as Arg(Pbf) are unlikely to be generally compatible with this chemistry. However, we observe that issues with ring opening appear to be highly substrate dependent. My colleague, Dr Ina Wilkening was able to successfully deprotect 243 in moderate yield containing such a protecting group. A more radical solution to addressing this limitation is developed in Chapter 3.

![Scheme 49: Deprotection of 242 under strongly acidic conditions without extensive ring opening.](image)
2.3 Conclusions

This work has demonstrated that incorporation of an oxetane ring into the backbone of a short linear peptide leads to improvement in the macrocyclisation to cyclic peptides, as measured by isolated yield, product distribution and reaction rate. The linear precursors for these macrocyclisations can be generated by solution phase or SPPS.

The methodology is applicable over a range of different ring sizes, including tetrapeptides which are known to be very difficult to synthesise. In one example the parent peptide could not be synthesised at all, but upon incorporation of the oxetane to the backbone gave \textbf{175} in good yield. The macrocyclisation was also extended to oxetane modified alanine residues (Scheme 42). In Table 6, we summarise the successes achieved with a total of 8 macrocycles made. Through a detailed investigation using one substrate, oxetane modification appears superior to other common peptide modifications (Table 5).

![Chemical structures](image1)

\textbf{Table 6}: Overall scope of the macrocyclisation of oxetane modified peptides. \(^a\) Yield of the dimeric octapeptide. \(^b\) Yield for macrocyclisation and deprotection over 2 steps. \(^c\) Yield reported by Jolliffe and co-workers.\(^3\)
The main limitation of this work relates to the poor stability of the oxetane ring to harshly acidic conditions. This problem can be overcome by altering the protecting groups employed and using mildly acidic conditions, although the synthesis of oxetane modified cyclic peptides containing residues with protecting groups which require harshly acidic conditions is still a significant challenge.

A further limitation of this work relates to the incorporation of oxetane modified residues other than glycine or alanine into the backbone. Arguably this could be achieved using work published by Carreira\textsuperscript{18}, but the approach is lengthy and rather impractical.

Additional biological and modelling work has been conducted on these oxetane modified cyclic peptides and this is detailed in our publication in Chemical Science.\textsuperscript{112}
Chapter 3: Synthesis and Functionalisation of Azetidine Modified Cyclic Peptides

3.1 Previous Work and Project Aims

The work detailed in Chapter 2 had established that replacement of one of the backbone C=O bonds with an oxetane ring had a beneficial impact on the macrocyclisation of small peptides. However, the oxetane ring is unstable towards the acidic conditions required for side chain deprotection. In order to solve this problem, we decided to extend our studies to other heterocyclic systems which might also induce turns to provide enhanced macrocyclisation but would also be more acid stable. Azetidine, in which the ring oxygen is replaced by a nitrogen atom, was considered an obvious place to begin. Moreover, the azetidine nitrogen could be used as a handle for further functionalisation.

![Diagram](image1)

Figure 21: The focus for the work described in Chapter 3.

The chemistry to make azetidine modified glycine residues has been achieved previously. It was established that chemistry applied to oxetanes to make 2,5-diketopiperazine mimics could also be applied to N-Boc azetidinone 245 (in two examples) (Scheme 50).16

![Diagram](image2)

Scheme 50: Previous work by Shipman towards azetidine modified 2,5-diketopiperazine frameworks.16

3-aminoazetidine-3-carboxylic acid (Azt) based residues have also been incorporated into the backbone of peptide chains. Tripeptide 249 could be synthesised from 3-azidoazetidine-3-carboxylic acid 248 in good yield over four steps. Interestingly, IR and NMR analysis indicated
that the azetidine ring was turn-inducing, and the nitrogen of the azetidine ring could act as a hydrogen bond acceptor with the amide NH of the adjacent amino acid (Scheme 51).\textsuperscript{118}

We wanted to explore the use of the 3-aminoazetidine modification in the macrocyclisation of small peptides and discover if the azetidine would also be able to enhance the cyclisation in a similar way to the oxetane. Other derivatives of azetidinone \textsuperscript{245} including N-Cbz and N-benzhydryl azetidinones are commercially available and would allow us to extend the scope of the substituent on the nitrogen atom.

One of the key reasons for pursuing the azetidine modification was because we expected that it may be less prone to ring opening under acidic conditions compared to oxetanes. Azetidines are more difficult to ring open, and many of the reactions reported for azetidine ring opening require the use of an electron withdrawing group such as a sulfonamide on the nitrogen atom to facilitate nucleophilic attack.\textsuperscript{119} Lewis acid catalysis is commonly needed for activation.\textsuperscript{120}

The broad aims of the planned work were to: (i) undertake the synthesis of a range of azetidine modified precursors \textsuperscript{251} both in solution and by SPPS; (ii) determine their effectiveness as substrates for macrocyclisation across a range of ring sizes compared with the oxetane modified and native peptides; and (iii) confirm their stability towards acidic conditions during removal of the side chain protecting groups. The synthesis of azetidine modified peptides other than glycine would also be explored, to determine if more useful levels of selectivity would be achieved in the conjugate addition step (Scheme 52).

Importantly, incorporation of an azetidine modified fragment into the cyclic peptide backbone may not only enable use of the heterocycle to enhance macrocyclisation, but also as a handle for
further late stage functionalisation. This might allow us to tune the properties of the macrocycle such as cell membrane permeability or biological activity. As well as this, conjugated derivatives could be synthesised which contain biological motifs for study (e.g. biotin); fluorescent tags (for cellular imaging), radionuclotides (for PET imaging), drug conjugates (for cargo delivery into cells) or peptide sequences (for cell penetration).

Two principal methods of azetidine functionalisation were to be explored. The first, by incorporation of an orthogonal protecting group which could be removed after the cyclisation to enable $N$-functionalisation by a range of different chemistries to install a variety of substituents of interest (Scheme 53). This approach brings some challenges in finding a suitable orthogonal protecting group, finding conditions for deprotection and functionalisation which are chemoselective for the azetidine over the secondary amine in the backbone.

The second approach involves incorporation of a handle on the azetidine early into the sequence, which can be functionalised after cyclisation that does not interfere with the macrocycle synthesis. Copper(I) azide-alkyne cycloaddition (CuAAC) chemistry has been widely used for bioconjugation, including cyclic peptides (Scheme 54). This complementary strategy for functionalisation of azetidine modified peptides could allow for functionalisation under aqueous conditions after macrocyclisation and deprotection of the side chains.
3.2 Synthesis of azetidinones and nitroalkenes

3.2.1 Synthesis of nitroalkenes with carbamate protecting groups

Initial studies began by synthesising Michael acceptors for the conjugate addition step. Previous work had demonstrated that Boc azetidinone 245 could be converted into the corresponding nitroalkene in-situ.\textsuperscript{16} We wanted to explore if azetidine based nitroalkenes were stable and could be isolated after Henry reaction and elimination. Purifying them at this stage might circumvent the need for purification after the conjugate addition step.

Commercially available azetidinones 245 and 257 could both be converted to their nitroalkenes 258 and 259 in good to excellent yields on up to 30 mmol scale (Scheme 55). Notably, these materials are much more stable than the corresponding oxetanyl nitroalkene 9, which generally needs to be made and used in-situ.

Scheme 55: Synthesis of nitroalkenes 258 and 259.

Additionally, trisubstituted alkene 260 could be made from nitroethane in excellent yield on 50 mmol scale (Scheme 56).

Scheme 56: Synthesis of trisubstituted alkene 260.

Next, we wanted to explore the synthesis of functionalised derivatives. Initial studies focussed on the 2-propynyl carbamate (2-PC), which could be used in CuAAC chemistry. This alkyne was chosen because the starting chloroformate 261 was cheap and commercially available. Boc azetidinone 245 was converted to 2-PC azetidinone 263 using 261 over two steps in low yield following a modified literature procedure (Scheme 57).\textsuperscript{127} However, converting the chloroformate into known succinimide ester 262 for the acylation led to much higher yields of 263 and this reaction could be applied on gram scale.\textsuperscript{128} Furthermore, 263 could be used in the Henry reaction and elimination procedure to generate nitroalkene 264 in good yield.
3.2.2 Synthesis of nitroalkenes with non-carbamate protecting groups

In order to expand the scope of the azetidine protecting group further, we wanted to see if it would be possible to make other alkenes which were not carbamate protected on the azetidine nitrogen. Initially, the tosyl group was examined as it was expected to be robust and tolerate the acidic and basic conditions which were needed for peptide synthesis. Removal of this group could be achieved orthogonally using magnesium in methanol.  

Reaction of Ts-protected azetidinone with nitromethane and catalytic NEt₃ provided crude alcohol in full conversion as determined by ¹H NMR. This material was used without further purification for the elimination to form nitroalkene, followed by direct addition of H-Gly-OBn to form dipeptide. However, after work-up and analysis of the crude ¹H NMR, no product could be observed and the ESI-MS showed formation of Ms-Gly-OBn (Scheme 58).

This suggested that the intermediate mesylation of prior to elimination was slow, leaving unreacted MsCl available to react with the amino ester. Leaving the elimination for longer and attempting to isolate nitroalkene was not productive. Changing the electrophile for the elimination to the more reactive triflic anhydride was also unsuccessful.
Next, we considered the use of the benzhydryl group, as the azetidinone is commercially available and this protecting group is well known to be removed using hydrogenation to provide the free amine. It has been used routinely for azetidine protection. Unfortunately, attempts to form nitroalkene 272 using MsCl as well as changing the electrophile to Ms₂O were not successful (Scheme 59). At this juncture, further efforts to make and use nitroalkenes 267 and 272 in this chemistry were abandoned.

![Scheme 59: Attempted elimination to form nitroalkene 272.](image)

3.3 Solution phase synthesis of azetidine modified cyclic peptides

3.3.1 Proof of concept study with a pentapeptide

By analogy with our work on oxetane modified peptides and other common peptide modifications (Chapter 2, Table 5), we chose the cyclisation of LAGAY as a test substrate to determine if azetidines could also be used as cyclisation precursors.

In solution, the macrocyclisation precursor 276 was synthesised from dipeptide 219 using a similar synthetic route that was used towards the oxetane modified pentapeptide 169 (Scheme 29), except with Fmoc for chain extension at the N-terminus to provide orthogonality to the azetidine Boc group (Scheme 60).
We then explored the cyclisation of this substrate to form cyclic pentapeptide 277 (Scheme 61). The desired product could be isolated in 46% yield and extending the reaction time allowed for an isolated yield of 98% for this ring closure. However, in both cases, the desired product was isolated as an 8:1 mixture of diastereomers, confirmed by $^1$H NMR, LC-MS and analytical HPLC. Under identical conditions to those used for the oxetane modified 161 and parent peptide 162 described in Chapter 2, the isolated yield of 277 is similar to the oxetane modified substrate and higher than the parent peptide. The oxetane modified and parent peptides were isolated as single diastereomers without the epimerised product.
These results suggest that the cyclisation is very selective for pentapeptide 277 over the dimers or other higher oligomers, as an excellent yield is obtained after 72 h reaction time. However, the reaction appears more sluggish, allowing for the activated ester to epimerise prior to cyclisation. LC-MS analysis of the crude reaction mixture for cyclisation to oxetane modified 161 after 24 hours showed only small quantities of epimerisation alongside traces of starting material, indicating that no further improvement in isolated yield could be achieved for this macrocyclisation by increasing the reaction time. The reduced formation of the epimerised product means that oxetane modified 161 could be isolated as a single diastereomer. In contrast, LC-MS analysis of the reaction mixture for the macrocyclisation of 276 after 24 hours shows significant amounts of starting material alongside larger quantities of the undesired epi-277.

We were concerned that although the azetidine modification was facilitating cyclisation, epimerisation may be a general problem. We reasoned that changing the active ester to a more reactive species may lead to faster reaction times. Seven different coupling agents were screened and the amount of epimer formed after 48 hours was measured by analytical HPLC (Table 7). Alongside DEPBT 103 which is known in the literature to reduce the amount of epimerisation in peptide macrocyclisation, we chose coupling reagents which would provide different activated esters from each other: PyBOP 278 would generate an OBt ester, HATU 279 and PyAOP 280 would generate OAt esters, DMTMM 281 generates a dimethoxytriazole based ester, and COMU 282 which contains the additive Oxyma Pure 283. Oxyma Pure derivatives are reported as safer, less explosive additives with similar or lower rates of epimerisation compared to HOAt and HOBt derivatives.
The results show that DEPBT and DMTMM are most efficient at suppressing epimerisation of this substrate. Interestingly, addition of Oxyma Pure led to elevated amounts of epimer and the coupling agent COMU, which contains this additive in its structure was very poor at controlling the amount of epimerisation. Although DMTMM is marginally better at reducing epimerisation compared to DEPBT, the HPLC trace showed more impurities. Thus, it was concluded that DEPBT remained the best choice for these macrocyclisations. Next, we decided to make a different cyclisation substrate to see if the problem of epimerisation was only limited to this pentapeptide, or if it was a general problem for azetidine modified peptides.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Coupling reagent</th>
<th>Epimerisation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DEPBT</td>
<td>10.2</td>
</tr>
<tr>
<td>2</td>
<td>HATU</td>
<td>11.5</td>
</tr>
<tr>
<td>3</td>
<td>PyBOP</td>
<td>16.0</td>
</tr>
<tr>
<td>4</td>
<td>DMTMM•BF₄</td>
<td>8.4</td>
</tr>
<tr>
<td>5</td>
<td>HATU/2 eq. Oxyma Pure</td>
<td>12.2</td>
</tr>
<tr>
<td>6</td>
<td>PyAOP</td>
<td>16.2</td>
</tr>
<tr>
<td>7</td>
<td>COMU</td>
<td>30.4</td>
</tr>
</tbody>
</table>

Table 7: Effect of coupling agent on epimerisation levels in the cyclisation of 276.
3.3.2 Exploring macrocyclisation of other ring sizes

Linear hexapeptide 288 was synthesised using the same route as reported for the oxetane modified peptide (Scheme 38, 39). This contains Phe at the C-terminus and would be an ideal target to probe the amount of epimerisation in azetidine modified substrates, whilst continuing to explore the scope of the macrocyclisation over a range of ring sizes (Scheme 62).

Cyclisation of 288 proceeded in 57% yield and the product could be isolated as a single diastereomer, indicating that the epimerisation seen for pentapeptide 277 may be sequence dependent (Scheme 63). This yield is identical to that achieved for the oxetane modified substrate, suggesting that the enhancement to macrocyclisation is similar for both modifications and approximately a two-fold improvement over the parent system.
With good results for a pentapeptide and hexapeptide in hand, we wanted to show that azetidine modification was also beneficial for tetrapeptides. Linear precursors 292–294 were synthesised from common building block 291 in good yields. Interestingly, the isolated yield for macrocyclisation of these tetrapeptides is not only improved dramatically compared to the parent peptide but also shows further improvement in isolated yield compared to the oxetane modified substrates. Additionally, increasing the concentration from 1 mM to 5 mM provided 295 and 296 in 82% and 80% yield respectively (Scheme 64).

Scheme 64: Synthesis of cyclic tetrapeptides 295–297. * 20% yield of the dimeric octapeptide. Yield in square parenthesis relates to reaction at 5 mM concentration.
An explanation for the increase in isolated yield for these tetrapeptides compared to the oxetane series could be due to the ease of purification. The azetidine modified peptides are less polar making it easier to remove the impurities from the DEPBT coupling agent. Typically, it is possible to isolate the product cleanly after a single column chromatography. In contrast, two columns are often required to isolate the oxetane modified peptides.

Next, we explored if other N-protecting groups for the azetidine nitrogen would be tolerated. Linear tetrapeptide 299 containing a Cbz protected azetidine, and tBu and Boc terminal protecting groups was synthesised using isolated nitroalkene 259. The acid labile termini were cleaved from 299 with TFA to give the linear macrocyclisation precursor 300 as the TFA salt. The stoichiometry of this compound was determined by $^{19}$F NMR using TFE as an internal standard. Cyclisation of this substrate proceeded in good yield and could be performed on scales up to 1.83 mmol, providing the Cbz protected azetidine 301 (Scheme 65). The yield is 8% lower than 295 containing Boc on the azetidine, but still demonstrates that other carbamates can be used on the azetidine nitrogen with positive impact on the macrocyclisation.

![Scheme 65: Synthesis of the Cbz protected tetrapeptide 301.](image-url)
3.4 SPPS of precursors followed by cyclisation

3.4.1 Synthesis of solid phase building blocks

A major limitation with the chemistry remained the laborious synthesis of substrates by multi-step solution phase synthesis. To further advance the chemistry, methods for the solid-phase synthesis of substrates were required. Similarly to the oxetane work, a dipeptide building block strategy was envisioned (Scheme 66). Here, the additional complexity of the azetidine nitrogen protecting group had to be considered. We hoped to use the cumyl ester protection for the C-terminus, anticipating that 2% TFA required to cleave this ester would not cleave the azetidine Boc group.

![Scheme 66: Overall strategy for the SPPS synthesis of azetidine modified peptides, depicted for a tetrapeptide.](image)

We devised a one-pot process to make azetidine modified dipeptide building blocks from isolated nitroalkenes 258–259. The crude nitroalkane product from the conjugate addition was clean enough to be used directly in the Raney nickel reduction. In this way, the Fmoc protected cumyl esters 306 could be converted into dipeptide building blocks 307 – 316 over three steps with only one chromatographic purification (Scheme 67). This chemistry worked well for both Boc and Cbz protected azetidines. A total of ten different building blocks were produced using this methodology, with a variety of amino acids at the C-terminus.
We were also interested in making azetidine modified building blocks containing amino acids which would not tolerate the Raney nickel reduction, such as Cys and Met. It was suspected that this methodology would also not work with 2-propynyl carbamates due to the propensity of the alkyne to reduce.

Nitroalkanes 317–319 could be generated in excellent yields (Scheme 68). Previous experience with oxetane modified Cys and Met derivatives had shown that a zinc mediated reduction can be used to reduce the nitro group. Gratifyingly, under these conditions with coupling of the free amine to FmocOSu, building blocks 320–322 could be generated (Scheme 69). Although the yield for these reductions was low in some cases, they provide access to further novel building blocks for use in SPPS.
Scheme 68: Synthesis of azetidine based nitroalkanes containing Met or 2-propynyl carbamates.

Scheme 69: Synthesis of azetidine based dipeptide building blocks containing Met or 2-propynyl carbamates.

3.4.2 Utilisation of building blocks for SPPS and cyclisation

With orthogonally protected dipeptide building blocks in hand, they were used to generate a small library of cyclic peptides by SPPS of precursors, followed by solution phase cyclisation (Table 8). Building blocks containing Boc, Cbz and the 2-propynyl carbamate were incorporated.
Table 8: Synthesis of azetidine modified cyclic peptides via SPPS with solution phase cyclisation. CP = cyclic peptide. GAZ = azetidine modified glycine residue. a Yield based on initial loading of commercially pre-loaded 2-chlorotrityl resins. b Isolated as 55:45 mixture of epimerised product to desired tetrapeptide.

A total of eighteen azetidine modified macrocycles were prepared in this way, incorporating nineteen of the twenty naturally occurring amino acids. The remaining one, Pro, had been included in earlier examples (Scheme 64). The precursors were generated in good yields without
the need for purification before cyclisation. Yields are based on the initial loading of the commercially available preloaded 2-CITrt resins. The purity of the linear peptides was established by $^1$H NMR and ESI-MS.

Macrocyclisation of the linear precursors was accomplished in all cases using DEPBT. A variety of different C-terminal residues including Leu, Trp(Boc), Phe and Ala could be incorporated without appreciable epimerisation and all the compounds were isolated as single diastereomers as judged by $^1$H NMR. The cyclisation worked well with various N-terminal groups including bulky, protected residues such as Asn(Trt), Ser(Bu) and Trp(Boc). Sequences were randomly selected to demonstrate broad scope.

Entries 5, 6 and 7 demonstrate the utility of the azetidine modification compared with native peptides. Without any modifications, cyclic peptide 329 could not be isolated as a single diastereomer when cyclised with Trp(Boc) at the C-terminus. The desired tetrapeptide is the minor product alongside its C-terminal epimerised product in a 55:45 ratio in 63% yield, determined by ESI-MS and $^1$H NMR (entry 6). To isolate 329, the sequence had to be cyclised with Gly at the C-terminus which cannot epimerise, but still cyclised poorly in 13% yield (entry 7). Upon azetidine incorporation, the desired cyclic peptide 328 can be isolated in 55% yield as a single diastereomer (entry 5).

### 3.5 Deprotection of azetidine modified cyclic peptides

#### 3.5.1 Comparative study between oxetane and azetidine modified cyclic peptides

In order to investigate the stability of azetidine modified peptides to acidic conditions, we first attempted to deprotect cyclic tetrapeptide 295 using 50% TFA in CH$_2$Cl$_2$. Free azetidine 343 was isolated as the TFA salt in quantitative yield without the need for further purification (Scheme 70). We used $^1$H and $^{19}$F NMR with TFE as an internal standard to determine the TFA content of the peptide. Despite the presence of 1 equiv. TFA, the mass recovery was higher than expected, which was assumed to be due to the presence of traces of water.
Scheme 70: Deprotection of azetidine modified cyclic peptide 343.

To compare the stability with an oxetane modified peptide, both 175 and 343 were subjected to 70% TFA in CH$_2$Cl$_2$ for 24 hours. Although some of the oxetane modified 175 remained, a product with mass 18 Da higher than expected by LC-MS was clearly observed in the extracted ion chromatogram (Figure 22). In the $^1$H NMR, new peaks appeared in the region between 4.5 – 3.2 ppm, with distinct changes in the doublets usually observed for the oxetane ring (Appendix I). We suggest that this is ring opened product 344, but this could not be isolated after purification by column chromatography.

Conversely, the $^1$H NMR spectrum of the azetidine modified peptide (Appendix I) did not change after 24 hours under identical reaction conditions, and only traces of a ring opened product 345 were identified by LC-MS analysis (Figure 23). Overall, it is clear that the azetidine modified peptides show superior stability under acidic conditions and should be stable to the strongly acidic conditions required for side chain deprotection in other peptides.

Figure 22: LC-MS EIC traces for the oxetane modified peptide after 70% TFA/CH$_2$Cl$_2$ for 24 hours.
3.5.2 Deprotection of other substrates

Next, we attempted to deprotect a wide range of azetidine modified cyclic peptides. For the peptides which only contained ‘Bu based protecting groups, we could achieve deprotection to the free azetidines 346–354 in quantitative yield without the need for purification using the same method with TFA/CH₂Cl₂ (Table 9). In the case of 352, it was isolated with the azetidine N-substituent intact. Alternatively, 4M HCl in dioxane could be applied successfully to generate the HCl salt if required.
Table 9: Deprotection of cyclic peptides containing tBu protecting groups. Reaction conditions: 70% TFA/CH₂Cl₂, RT, 1 – 2 h.  
   a Also prepared using 4M HCl in dioxane to generate the HCl salt.  
   b Isolated as a 9:1 mixture of diastereomers.

For peptides which contained Pbf or Trt groups, and for peptides which had more sensitive or highly polar side chains, we had to modify the deprotection conditions (Table 10). For peptides containing Cys, DTT was added to prevent oxidation or disulfide bridge formation. In all cases, TIS and H₂O were included as scavengers. To fully characterise the compounds after deprotection, purification by preparative HPLC was undertaken to remove Trt/Pbf by-products. No evidence of ring opening by-products were seen, as demonstrated by the traces shown in Figure 24 for 355.
Figure 24: (a) Preparative HPLC trace of the deprotection mixture of **332** at 220 nm. (b) Preparative HPLC trace for the deprotection mixture of **332** at 280 nm. (c) Analytical HPLC trace at 280 nm of **355** after purification.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>356&lt;sup&gt;a&lt;/sup&gt; (from 326)</td>
<td><img src="image1" alt="Structure 356" /></td>
</tr>
<tr>
<td>357&lt;sup&gt;a&lt;/sup&gt; (from 327)</td>
<td><img src="image2" alt="Structure 357" /></td>
</tr>
<tr>
<td>358&lt;sup&gt;a&lt;/sup&gt; (from 331)</td>
<td><img src="image3" alt="Structure 358" /></td>
</tr>
<tr>
<td>359&lt;sup&gt;h&lt;/sup&gt; (from 333)</td>
<td><img src="image4" alt="Structure 359" /></td>
</tr>
<tr>
<td>360&lt;sup&gt;a&lt;/sup&gt; (from 340)</td>
<td><img src="image5" alt="Structure 360" /></td>
</tr>
<tr>
<td>361&lt;sup&gt;a&lt;/sup&gt; (from 339)</td>
<td><img src="image6" alt="Structure 361" /></td>
</tr>
<tr>
<td>362&lt;sup&gt;a&lt;/sup&gt; (from 338)</td>
<td><img src="image7" alt="Structure 362" /></td>
</tr>
</tbody>
</table>

**Table 10**: Scope of deprotection of azetidine modified cyclic peptides with side chain protecting groups other than ’Bu.

Deprotection conditions: a) 90% TFA/5% CH<sub>2</sub>Cl<sub>2</sub>/5% TIS, RT, 1.5 h; b) 90% TFA/5% DTT/2.5% CH<sub>2</sub>Cl<sub>2</sub>/2.5% TIS, RT, 1 – 2 h.
3.6 Synthesis of cyclic peptides containing alanine analogues

3.6.1 Additions to the trisubstituted nitroalkene 260

To date, all cyclic peptides had been prepared with an azetidine modified glycine (GAz) residue. However, we felt it would be of interest to explore more complex derivatives containing other amino acid side chains. Our first approach was to target alanine-like compounds (AAz) possessing a methyl group (Figure 25), the synthesis of which required the imposition of stereocontrol in the conjugate addition. Related work for oxetanes had previously proven challenging (Scheme 1 & 4).14,17,21

![Glycine and Alanine Modified Azetidine Variants](image)

Figure 25: Glycine and alanine modified azetidine variants.

We first investigated the addition of simple amino acid building blocks to the trisubstituted nitroalkene 260, to see if the natural chirality of the amino acid could impart control during the conjugate addition. We screened a range of different amino esters, measuring the conversion and the diastereoselectivity by $^1$H NMR where possible (Table 11). TLC analysis was also carried out to see if the diastereomers formed could be separated by column chromatography.
Although additions to the Michael acceptor were quantitative, essentially no stereocontrol could be achieved. However, the diastereomers generated by addition of proline benzyl ester 372 (entry 9) could be separated by column chromatography and the major diastereomer 371 isolated (Scheme 71).

Table 11: Conjugate addition of amino esters to nitroalkene 260. * Determined by $^1$H NMR.

<table>
<thead>
<tr>
<th>Entry</th>
<th>AA</th>
<th>R’</th>
<th>Product</th>
<th>Conversion (%)</th>
<th>d.r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ala</td>
<td>OMe</td>
<td>363</td>
<td>100</td>
<td>55:45</td>
</tr>
<tr>
<td>2</td>
<td>Val</td>
<td>OMe</td>
<td>364</td>
<td>100</td>
<td>53:47</td>
</tr>
<tr>
<td>3</td>
<td>Leu</td>
<td>OBn</td>
<td>365</td>
<td>100</td>
<td>50:50</td>
</tr>
<tr>
<td>4</td>
<td>Ile</td>
<td>OBn</td>
<td>366</td>
<td>100</td>
<td>N.D</td>
</tr>
<tr>
<td>5</td>
<td>Phe</td>
<td>OBn</td>
<td>367</td>
<td>100</td>
<td>51:49</td>
</tr>
<tr>
<td>6</td>
<td>Ser</td>
<td>OMe</td>
<td>368</td>
<td>100</td>
<td>N.D</td>
</tr>
<tr>
<td>7</td>
<td>Tyr</td>
<td>OMe</td>
<td>369</td>
<td>100</td>
<td>N.D</td>
</tr>
<tr>
<td>8</td>
<td>Trp</td>
<td>OMe</td>
<td>370</td>
<td>100</td>
<td>53:47</td>
</tr>
<tr>
<td>9</td>
<td>Pro</td>
<td>OBn</td>
<td>371</td>
<td>100</td>
<td>57:43</td>
</tr>
</tbody>
</table>

Scheme 71: Synthesis of 371 isolated as a single diastereomer after column chromatography.
3.6.2 Synthesis of an AAz(Boc)-Pro based cyclic peptide

At this stage, we did not know the stereochemistry of 371 at the newly created centre. We hoped that either the final cyclic peptide or one of the intermediates would be crystalline enabling the stereochemistry to be resolved by XRD. However, this building block was used to synthesise a cyclic peptide via solution phase methods.

Raney nickel reduction and coupling of 371 with Fmoc-Val-OSu was attempted, but the building block was isolated in low yield. Reduction of this building block required much longer for full consumption of the starting material. Alongside the product, dipeptide 374 was also isolated in low yield (Scheme 72).

![Scheme 72: Raney Ni reduction of building block 371.](image)

The presence of dipeptide 374 suggests that a retro-addition pathway is taking place, leading to formation of H-Pro-OBn 372 which is trapped by excess Fmoc-Val-OSu in the reaction mixture (Scheme 73).

![Scheme 73: Formation of dipeptide 374 via retro-addition pathway.](image)

By scaling up the process to 15 mmol, enough material could be generated to continue the sequence to the linear precursor 376 without further optimisation. Pleasingly, cyclisation afforded cyclic peptide 377 in good yield which could be readily deprotected using 4M HCl in dioxane (Scheme 74).
Unfortunately, despite many attempts to crystallise the protected cyclic peptide or the free azetidine as a TFA or HCl salt, no crystals suitable for XRD could be obtained. In order to determine the stereochemistry of the azetidine building block, we turned to NOESY NMR in collaboration with Eleanor Jayawant in the Notman/Dixon groups at the University of Warwick.

To estimate the relative distances between key atoms in the macrocycle, a structure of each macrocycle containing the L- and D- alanine were generated using the Avogadro 1.1 molecular editor and energy minimised using the Universal Force Field. Since NOE’s are ∝ $1/r^6$ distance, estimates can be used to predict the magnitude of the NOE’s for each diastereomer (Table 12).
Table 12: Calculated distances obtained from PyMOL. Description in parenthesis classifies each NOE interaction (strong (1.8 – 2.7 Å), medium (1.8-3.3 Å), weak (1.8 – 5.0 Å) or very weak (1.8 – 6.0 Å)). Data obtained by Eleanor Jayawant.

From the calculated distances, it was apparent that the important correlations to compare would be between the alanine AlaHα/AlaHβ and ProCH₂δ atoms, other NOE’s would be too similar in both compounds and would not be diagnostic. With the expected data for each diastereomer in hand, NOESY spectra were collected for 378 at a variety of different mixing times. Strong NOE’s could be clearly observed between the AlaHα and the ProCH₂δ atoms of the cyclic peptide, at short, medium and long mixing times measured (See Appendix II). Furthermore, even at the longest mixing time measured, no NOE correlations were observed between the AlaCHβ and the ProCHδ atoms. These indicated that the cyclic peptide most likely is the D-configuration at the alanine centre. From this analysis we also extrapolate the D-configuration for the building block 371 (Scheme 73).

The reliability of this method to determine the stereochemistry relies on the accuracy of the atomic distances extrapolated from the initial computed structures generated. Moreover, full confidence with the assignment is hampered by the fact that the NOE’s have not been recorded for the
alternative L-diastereomer. However, in absence of other methods, it does provide a tentative stereochemical assignment for 378.

3.6.3 Cyclisation of a mixture of diastereomers

As control of the diastereoselectivity of the conjugate addition was difficult, we wanted to see if it would be productive to cyclise a mixture of diastereomers at the azetidine stereocentre. We hypothesised that differences in polarity of the macrocycles containing the D- or L- residues may make it possible to separate both cyclic peptides post-macrocyclisation. In this way, two macrocycles would be produced from a single cyclisation. Of course, one diastereomer may cyclise faster than the other, which would reveal how this stereocentre controls the conformation prior to cyclisation.

To investigate this hypothesis, we synthesised tetrapeptide 380 as a 1:1 mixture of diastereomers, determined by ¹H NMR (Scheme 75).

Upon macrocyclisation of 380, two separate diastereomers 381–A and 381–B could be cleanly isolated after column chromatography (Scheme 76). This proof of concept shows that it is not necessary to control the stereochemistry, rather we can use the differences in properties of the two macrocycles to separately isolate the cyclic peptides. The diastereomeric ratio changes little in this transformation, which indicates that the diastereomers both cyclise at similar rates.
In order to determine the configuration of the stereocentre of the alanine in each compound, we compared their $^1$H and $^{13}$C NMR spectra with the analogous oxetane modified cyclic peptide 215 which was synthesised in Chapter 2, and known to have the L-configuration.

Firstly, the $^1$H NMR spectrum of 381–A appears as an 83:17 mixture of conformational isomers, determined by integration of the alanine $\beta$-CH$_3$ signals. However, the oxetane modified compound and 381–B are present as single conformational isomers at RT. It is possible that the conformation of the ring in 381–A could be flexible due to the presence of the D-alanine stereocentre.

Interestingly, overlaid $^1$H NMR of 381–B and 215 show a very common set of signals, but in comparison 381–A is very different to the spectrum of all-L, oxetane modified 215 (Figure 26). For example, the CH, $\alpha$-Trp signals are at similar shifts of 4.97 ppm (381–B) and 4.93 ppm (215) in contrast to 381–A at 4.60 ppm.
Figure 26: Stacked $^1$H NMR spectra of oxetane modified 215 (red), 381–A (green) and 381–B (blue) between 5.5 ppm and 3.0 ppm.

In the $^{13}$C spectra, when comparing all carbon atoms except those of the azetidine and oxetane rings, all of the carbon atoms in 215 and 381–B are within 0.1 ppm of each other, with the exception of the methylene of the glycine and alpha carbon of alanine. In contrast, the major conformer of 381–A shows large differences in chemical shift of up to ±1.6 ppm (Appendix III). The mean difference in $^{13}$C chemical shift for 381–A is 0.56 ppm, but only 0.09 for 381–B.

Together, this data allows us to assign 381–A tentatively as the D-stereoisomer, and 381–B as the L-stereoisomer. ROESY NMR were recorded in an attempt to support the stereochemical assignments, however these did not prove fruitful.

To explore if this strategy was general and could be extended to other cyclic peptides, we turned to our more practical SPPS approach. Firstly, trisubstituted nitroalkene 260 was used with the one-pot method for synthesis of building blocks to generate 383, which was isolated as a 2:1 mixture of diastereomers after column chromatography (Scheme 77, Appendix IV). Longer reaction times were required to achieve full consumption of starting material, which led to lower isolated yields of the building block.
Next, this building block was used to synthesise pentapeptide precursor 385, which had a d.r. of 61:39 as judged by analytical HPLC and $^1$H NMR. This precursor was then cyclised using DEPBT to give a crude 55:45 mixture of diastereomers confirmed by analytical HPLC. Despite an overall yield of 60% for the cyclisation, the two cyclic peptides could not be separated by column chromatography leading to isolation of a 56:44 mixture of cyclic peptides, confirmed by $^1$H NMR and analytical HPLC (Scheme 78, Appendix V). Preparative HPLC may be possible as a route to the isolation of single diastereomers as the two protected cyclic peptides are separated by a retention time of 1 minute, although this was not undertaken.

Scheme 78: Synthesis of cyclic pentapeptide 387 by cyclisation of a mixture of diastereomers.
3.7 Functionalisation of the azetidine nitrogen

3.7.1 Optimisation of conditions for Cbz deprotection and functionalisation

Having established the feasibility of making a variety of azetidine modified cyclic peptides, we turned our attention to exploring methods for the post-cyclisation functionalisation of the azetidine nitrogen. Our studies began by exploring the deprotection of Cbz protected azetidine 301 using hydrogen, Pd/C in ethanol for 16 hours. Analysis of the $^1$H NMR of the crude material showed a 1:1 mixture of the free azetidine 388 alongside a second compound, 389. Additionally, in the ESI-MS, a peak for 388 of 352 Da was accompanied by a second peak of 396 Da (Scheme 79).

![Scheme 79: Initial deprotection of 301 to give free azetidine 388.](image)

LC-MS analysis confirmed the presence of both unknown compound 389 ($m/z$ 396, [M+H]$^+$) with an increase in 44 Da relative to the free azetidine 388 ($m/z$ 352, [M+H]$^+$). In order to purify the free azetidine we attempted a silica-catch technology reported by Aggarwal & coworkers. This involves treating the mixture with silica gel to adsorb the azetidine, followed by flushing the silica with low polarity solvent to remove impurities, with a final high polarity flush to collect the compound. Disappointingly, the free azetidine could not be recovered from the silica gel, but by-product 389 eluted cleanly from the silica gel using 20% MeOH/CH$_2$Cl$_2$. Analysis of the $^1$H and $^{13}$C NMR spectra could not conclusively identify the structure of 389. Attempts to grow a crystal of this compound for XRD were also not fruitful.

Conditions were sought to optimise the hydrogenation in favour of the free amine 388. By careful monitoring of the reaction time, the free azetidine 388 was isolated after 2 hours as a single compound without detection of 389 by ESI-MS or $^1$H NMR. Moreover, the crude amine could be acylated on the azetidine nitrogen with a variety of acyl chlorides to generate functionalised azetidines 390–392, in good yield over two steps (Scheme 80).
Importantly, double acylation from reaction of the acyl chloride with the secondary amine in the backbone was not observed, suggesting that chemoselectivity could be achieved without the need for optimisation of the functionalisation conditions.

Interestingly, the acylation had introduced a rotatable amide bond around the azetidine nitrogen which led to the observation of a mixture of cis/trans rotamers in the $^1$H and $^{13}$C NMR spectra. Upon heating to 373 K in D$_6$-DMSO, these conformers converge into one set of signals.

With promising results for acylation in hand, we decided to explore the reaction of 301 with other electrophiles to install different functional groups 393–395 (Scheme 81). We chose mild chemistries which would be simple to carry out and would allow for a range of different functional groups to be installed on the azetidine nitrogen.
The free amine is reactive with a variety of other electrophiles. We wanted to show that succinimide esters could be used for the acylation step as they are a milder alternative to acyl chlorides, are commonly used in bioconjugation, such reagents are often more commercially available. Using a protected amino acid activated as its succinimide ester led to isolation of 393 in good yield, which again exhibited rotamers in the NMR.

Following a literature procedure for SNAr of a 3,3-substituted azetidine with 2-chloropyrimidine, the nitrogen could also be functionalised with a pyrimidine ring, isolated as 394. This deprotection and functionalisation approach also provides a route to sulfonamide 395 by reaction with tosyl...
chloride in good yield, which could not be introduced directly using the conjugate addition strategy described in Section 3.2.

However, under the conditions attempted for reductive amination, no product could be isolated despite full consumption of starting material and observation of the expected m/z for the product in the ESI-MS of the reaction mixture after 16 hours. Disappointingly, 396 could not be isolated after column chromatography (Scheme 82).

These results showed that a variety of different functionalisation chemistries could be used to install different groups onto the azetidine nitrogen, in good yields and in a chemoselective manner. These chemistries enable modification of the azetidine nitrogen to potentially modulate the properties of the peptide and install useful functional groups.

We also attempted to use the corresponding TFA salt 343 arising from Boc deprotection of 295, but these were much less successful despite the literature precedence of functionalisation of azetidine salts. Fortuitously, this is a less useful approach because it could only be applied to substrates which do not contain acid-labile side chain protecting groups such as this model system.

Scheme 82: Failed reductive amination towards 396.
3.7.2 Scope and application of Cbz deprotection and functionalisation chemistry

Next, we sought to verify if this chemistry was applicable to a range of different substrates and ring sizes, and if the functionalised cyclic peptide could be deprotected cleanly. Cbz protected cyclic peptides synthesised in Section 3.4.2 were used to explore these ideas.

Firstly, succinimide ester chemistry was used to install a biotin unit. Biotin has a strong binding interaction with avidin and has been widely used for peptide immobilisation. Tetrapeptide 328 could be selectively deprotected and acylated with D-biotin-OSu in good yield (Scheme 83). We were also interested in producing azetidine functionalised azides for use in CuAAC chemistry. 328 could also be acylated with azidoglycine-OSu to give azetidine functionalised azide 398 in good yield (Scheme 83). Both acyl azetidines appeared as a mixture of rotamers around the amide bond by NMR.

The presence of rotamers in the $^1$H NMR made it difficult to study further reactions with azide 398 as the products were expected to retain their conformational flexibility around the amide bond which made the NMR difficult to analyse. To avoid this, carbamate based azide 400 was generated following a modified literature procedure. Indeed, deprotection and functionalisation of 336 with 400 led to isolation of 401 which showed a single set of signals at RT (Scheme 84).
We were also interested in installing fluorescent dyes onto the azetidine which could potentially be used to visualise cyclic peptides inside cells. The dansyl group has been used commonly to tag amino acids and proteins for sequencing analysis,\textsuperscript{137} and has been incorporated onto the Lys side chain of macrocyclic peptides for imaging.\textsuperscript{138} Using dansyl chloride, cyclic pentapeptide 402 was synthesised in good yield (Scheme 85).

Additionally, pentapeptide 335 could also be synthesised by reaction with 2-fluoropyrazine to give 403 in 73% yield (Scheme 86).
Furthermore, three of the five azetidine functionalised cyclic peptides were deprotected in quantitative yield as the TFA salt, with no need for further purification (Figure 27). In 404, the mixture of rotamers observed in the $^1$H NMR remained the same after deprotection.

![Scheme 86](image)

**Scheme 86:** Synthesis of cyclic peptide 403 containing a pyrazine functionalised azetidine 403.

**Figure 27:** Deprotection of the functionalised cyclic peptides 404–406. Reaction conditions: 70% TFA, CH$_2$Cl$_2$, RT, 1.5 h.

### 3.7.3 Click reactions of 2-propynyl carbamate azetidines

We were interested in using click chemistry as an alternative method of functionalisation which could be used on fully side-chain deprotected peptides, under aqueous conditions without the need for deprotection of the azetidine nitrogen. Again, we sought to functionalise the cyclic peptide with a dye to enable imaging. We chose azide 409, which was synthesised over 3 steps following a literature procedure and has been used for cellular imaging in cyclic peptides (Scheme 87).
Using 2-PC derivatised azetidines, we tested the click reaction on protected peptide 337 and deprotected TFA salt 352 to see if we could perform click reactions before the deprotection of the side chains, or after on the fully deprotected macrocycle. Treatment of cyclic peptide 337 with azide 409 in the presence of copper sulfate and sodium ascorbate in semi-aqueous conditions furnished 410 after flash column chromatography. This peptide could be deprotected using TFA to yield the fully deprotected triazole functionalised cyclic peptide 411 in good overall yield (Scheme 87).

Scheme 87: Click reaction of coumarin azide 409 with cyclic peptide alkyne 337. a Compound isolated as the TFA salt.

Disappointingly, reordering the reactions and attempting the click reaction on the fully deprotected TFA salt 352 was not productive, and the desired click functionalised peptide could not be observed by ESI-MS despite consumption of the starting material (Scheme 88).
The CuAAC click chemistry was also applied to 341 to generate 412 with the fluorescent triazole aminocoumarin in excellent yield, which could then be deprotected to give cyclic peptide 413 as a HCl salt. In this case, we chose to deprotect the peptide with 4M HCl/dioxane to generate a more crystalline compound in an attempt to grow a crystal for XRD analysis, although this was unsuccessful.
3.8 Investigating the structure and properties of functionalised cyclic peptides

3.8.1 Structure of azetidine modified cyclic peptides

Cyclic tetrapeptide 398, derived from acylation of the azetidine nitrogen with azidoglycine-OSu, was a white crystalline solid from which suitable crystals for XRD could be obtained by slow evaporation from methanol. The structure contains three amide bonds which all exist in the trans configuration, with no hydrogen bonds between amide C=O and neighbouring amide NH atoms. The dihedral angles of each residue are reported in Table 13. Each of the amide bonds are non-planar, deviating by 12–22° from the ideal angle of 180° within a peptide bond due to the strain around the tetrapeptide ring.

<table>
<thead>
<tr>
<th>Residue</th>
<th>Dihedral angle</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ</td>
<td>ψ</td>
<td>Ω</td>
</tr>
<tr>
<td>Val</td>
<td>-74.35</td>
<td>-29.6</td>
<td>167.83</td>
</tr>
<tr>
<td>GAz(COCH₂N₃)</td>
<td>-99.66</td>
<td>-65.4</td>
<td>158.72</td>
</tr>
<tr>
<td>Leu</td>
<td>-89.56</td>
<td>-30.9</td>
<td>166.85</td>
</tr>
<tr>
<td>Trp(Boc)</td>
<td>-98.87</td>
<td>-81.81</td>
<td>158.73</td>
</tr>
</tbody>
</table>

*Table 13: Dihedral angles for each residue in 398.*

![Figure 28: Mercury plot of the structure of 398.](image)
Due to the strain around cyclic tetrapeptide structures, they are often found to interconvert between multiple conformations of varying energies. In order to compare against other cyclic tetrapeptide conformations, the notation described by André and co-workers can be used. \textbf{398} is found in the tttt-uuuu orientation, with each of the amide C=O atoms up relative to the adjacent Cα atom of the same residue and all of the amide bonds in the trans configuration, treating the azetidine ring as if it were an amide bond.\textsuperscript{140} Computational measurements of simple all-\textit{L} cyclic tetrapeptides have shown that in the tttt-uuuu conformation, the global energy minimum generally does not contain any hydrogen bonds as this would introduce significant strain into the backbone.\textsuperscript{141}

To understand more about the impact of the azetidine modification on the structure, it was compared with MD generated structures of oxetane modified cyclic pentapeptide \textbf{161} in the proximity of the heterocyclic ring. \textbf{161} has a rigid structure and spends $>99.1\%$ of the frames of the trajectory in a single cluster, therefore it is reasonable to use one of the structures to compare the conformation of the oxetane modified residue with the azetidine modified residue in the XRD structure of \textbf{398}.

In oxetane-modified \textbf{161}, the $\Omega$ angle is 69.49°, deviating away from the normal 180° seen in a \textit{trans} amide. In contrast, this angle is 158.72° in azetidine-modified \textbf{398}, which is much closer to a regular \textit{trans} amide bond. Pentapeptides are less strained, and this means that more conformational flexibility can be imparted around the oxetane ring (Figure 29A). However, the 12-membered tetrapeptide backbone is much more strained, so the azetidine ring in \textbf{398} adopts a conformation which is similar to a conventional \textit{trans} amide bond (Figure 29B).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure29.png}
\caption{Mercury plots of cyclic peptides \textbf{161} and \textbf{398} around the modification, generated by Mercury. a) Representation of oxetane-modified \textbf{161}, continued peptide chain and oxetane H’s omitted for clarity. b) Representation of azetidine-modified \textbf{398}, side chains, acyl azetidine functionality, azetidine H’s and continued peptide chain omitted for clarity.}
\end{figure}
We also attempted to obtain a suitable crystal of parent peptide 329 to make direct comparisons, but this was not fruitful.

3.8.2 Fluorescent properties of azetidine modified cyclic peptides

The incorporation of azetidines and aziridines in replacement for the dialkylamino groups in fluorescent dyes has been shown to improve their photophysical properties such as brightness and photostability, as well as physicochemical properties such as membrane permeability and water solubility.142–144 We were interested to see if any such effects may be present in the azetidine modified cyclic peptides containing fluorescent groups.

The fluorescent properties of the functionalised cyclic peptides were explored by UV-Vis and fluorescence spectroscopy. In 402 prior to deprotection, the excitation (341 nm), emission (534 nm) and Stokes’ shift of 193 nm are consistent with other dansylated secondary amines in the literature (c.f dansyl proline 414, Figure 30). However, upon deprotection of the compound forming the TFA salt in 405, there is a red shift of the emission value to 680 nm (Figure 30). This large and increased Stokes’ shift of 339 nm could be advantageous for fluorescence imaging. Many typical dyes such as Fluorescein ($\Delta \lambda = 24$ nm), Cy2 ($\Delta \lambda = 13$ nm) and BODIPY 505 ($\Delta \lambda = 10$ nm) have low Stokes’ shifts, which can lead to background interference due to reabsorption of the emitted photons.145

A possible explanation for this is the presence of a zwitterion between the carboxylate of Glu and the backbone amine. A similar effect has been reported for a squaraine-dansyl dye 416 (Figure 30), and the large Stokes’ shift was attributed to its zwitterionic structure.146 The deprotected variants of the coumarin functionalised cyclic peptides 411 and 413 both showed similar excitation, emission and Stokes’ shift values to similar derivatives in the literature.139,144
3.8.3 Cellular imaging using functionalised azetidine modified cyclic peptides

Triazole aminocoumarin functionalised peptide 413 is a derivative of dichotomin A 188, which has shown toxicity towards p-388 lymphocytic leukemia cancer cells. We were interested in screening the parent peptide, along with oxetane and azetidine modified variants to see if the presence of the modification had any impact on the biological activity.

188 was synthesised using a literature procedure,93 and screened against several cancer cell lines in collaboration with Dr Alexia Hapeshi in the Perrier group at the University of Warwick. The cell lines chosen were routinely used within their laboratory and include prostate cancer cells (PC3), mouse fibroblast cells (3T3) and lung cancer cells (A549). Disappointingly, neither the parent peptide 188 or the fluorescent variant 413 were toxic against the cells tested. At this juncture, the other oxetane modified derivative 238 and azetidine modified derivative 354 were not screened.
Nevertheless, we decided to explore the possibility of using 413 for imaging within cells using confocal microscopy. Human alveolar epithelial (A549) cells were treated with the 413 for 4 hours and visualised by confocal microscopy (Figure 31). The cells were stained with Phalloidin-iFluor™ to visualise the actin cytoskeleton (red), and DAPI to visualise the nucleic acids (blue). Although these images do not conclusively prove the compound has entered the cell, it provides a proof of principle that these fluorescently modified cyclic peptides can be clearly visualised using confocal microscopy and may enable study in other biologically active cyclic peptide sequences.

Figure 31: Confocal microscopy images of 413 (left) with human alveolar epithelial (A549) cells and control image without compound (right). Image collected by Dr Alexia Hapeshi.
3.9 Summary and Future Work

3.9.1 Conclusions

The experimental findings in this chapter have covered the incorporation of the 3-aminoazetidine subunit into short peptide sequences, building on previous work by Beadle et al.,\textsuperscript{16} and investigated their ability to undergo macrocyclisation to form small head-to-tail cyclic peptides.

Similar to oxetane modified peptides, the azetidine can be introduced into the backbone of a peptide \textit{via} conjugate addition of the \textit{N}-terminus of an amino ester or peptide chain towards a nitroalkene, followed by nitro group reduction with Raney nickel and \textit{in-situ} coupling of the amine with a succinimide ester of another amino acid or FmocOSu. We have shown that nitroalkene derivatives of protected azetidines containing Boc, Cbz and 2-propynyl carbamates can be readily synthesised from commercially available materials. These nitroalkenes can be isolated as bench stable solids or used \textit{in-situ}. This methodology can be used to make linear peptide precursors between four- and six- amino acids in length by solution phase coupling (Scheme 90, Route A).

Alternatively, dipeptide building blocks for incorporation into Fmoc SPPS using 2-chlorotrityl resins can be made (Scheme 90, Route B). For substrates which contained side chains or protecting groups that were sensitive to Raney nickel hydrogenation, a zinc metal reduction was applied. A total of thirteen building blocks were made, incorporating Asp, Ile, Glu, Tyr, Met, Lys, Leu, Val, Thr and Gly (Scheme 67, 69). An alanine-modified azetidine building block could also be synthesised, although longer reaction times were required, leading to lower yields (Scheme 77).

\begin{center}
\textbf{Scheme 90:} Summary of synthesis of linear precursors by solution phase or SPPS.
\end{center}
By macrocyclisation with DEPBT, we confirmed that the 3-aminooazetidine subunit leads to enhanced macrocyclisation compared with conventional systems to form head-to-tail cyclic peptides in markedly improved yields (Table 14). This indicates that the azetidine has analogous turn-inducing properties to the oxetane modification. For tetrapeptides, the azetidine modification is superior to first-generation oxetane based systems. In one instance, we observed a small amount of C-terminal epimerisation during cyclisation.

Table 14: Scope of macrocyclisation of linear precursors synthesised by solution phase. Reaction conditions: 2 equiv DEPBT, DIPEA, DMSO, 1 mM, RT, 48 h. a Reaction concentration 5 mM. b Isolated as an 8:1 mixture of diastereomers. c Reaction time 24 h. d Reaction time 72 h. e Yield from ref 93 using DMTMM•BF₄.

In order to make the chemistry more practical, we synthesised linear precursors by Fmoc SPPS using building blocks 307–316, 320–322 and then cyclised them in solution. Using this methodology, we were able to synthesise nineteen examples of azetidine modified cyclic peptides which included each of the three protecting groups on the azetidine, in ring sizes ranging from tetra- to hexapeptides (Scheme 91). This work demonstrates the practicability of the chemistry, enabling us to synthesise a diverse set of sequences and isolate them in good yields after cyclisation. We are yet to find a cyclic peptide that cannot be made using this method.
Importantly, the azetidine modification is robust under the acidic conditions required to deprotect the side chains of the amino acids in the macrocycle. A total of twenty cyclic peptides could be deprotected without any ring opening or side reactions observed. Peptides with side chains which only contained ‘Bu based protecting groups could be deprotected using TFA or HCl without the need for further purification. For peptides containing other protecting groups, purification by preparative HPLC was carried out to remove Pbf or Trt based by-products yielding the peptides as TFA salts.

We also investigated the synthesis of cyclic peptides containing azetidine modified alanine residues. Incorporation of the azetidine modified alanine unit without stereocontrol to produce tetrapeptide linear precursor 380 as a 1:1 mixture of diastereomers (Scheme 75), followed by cyclisation, led to the formation of two cyclic peptides 381–A and 381–B which were readily separable by column chromatography after cyclisation (Scheme 76). However, after cyclisation of linear pentapeptide mixture 386 synthesised using the SPPS approach from building block 383, the products were inseparable (Scheme 78). In both cases the isolated yield matched the diastereomeric ratio of the linear precursor, which suggests that the relative rate of cyclisation is the same for both the L- and D- alanine modified azetidine diastereomers.

Another advantage of azetidine based systems is the ability to functionalise on the nitrogen atom post-cyclisation. Cbz protected azetidine modified cyclic peptides could be hydrogenated to the free azetidine 421, then chemoselectively functionalised. A second method was introduced that utilises a CuAAC reaction of the 2-propynyl carbamate protected azetidine (Scheme 92).
A range of different $N$-functionalisation chemistries worked well on tetra- and pentapeptides, including acylation, sulfonylation and $S_N$Ar reactions. Functionalisation of the azetidine using reductive amination or using the TFA salt were unsuccessful. Using this strategy, we were able to synthesise azetidine modified cyclic peptides containing a range of functional groups. These chemistries demonstrate that we can synthesise functional derivatives which contain additional peptide residues (393); azides for further conjugation (398, 401), useful biological molecules (404), fluorescent handles (405) or other groups which may alter the properties of the peptide (406). Additionally, we were able to install a fluorescent coumarin group in a penta- and hexapeptide using the CuAAC method. These peptides could also be deprotected without side reactions on the azetidine or coumarin group (Table 15).

Scheme 92: Summary of the functionalisation of azetidine modified cyclic peptides.
Table 15: Summary of functionalisation of the azetidine nitrogen of cyclic peptides.\(^a\) Yield over two steps after side-chain deprotection.

3.9.2 Future work

The chemistry developed in this chapter has shown that azetidine modified cyclic peptides such as 398 and 401 containing an azide can be readily made. It would be interesting to investigate whether CuAAC or the catalyst-free SPAAC click reactions can be performed with carbamate

\(^a\) Yield over two steps after side-chain deprotection.
based azide 401. This would provide a third method of functionalisation that may be applicable to the final, side-chain deprotected cyclic peptide (Scheme 93).

Scheme 93: An example of functionalisation of 401 via SPAAC click chemistry with strained alkyne DBCO 423.

Owing to the ability for azetidines to enhance the fluorescent properties of some dyes in the literature, it would also be interesting to perform a more in-depth study on the fluorescent properties of the azetidine modified cyclic peptide compared with conventional peptides containing fluorescently tagged amino acids or side chains. This may provide an additional benefit of using the azetidine modified residue within the cyclic peptide backbone as opposed to more conventional strategies of attaching dyes to amino acid side chains.

Furthermore, peptide 404 containing a biotin unit was synthesised. By immobilisation of the cyclic peptide onto a surface containing avidin/streptavidin, molecular interactions between the cyclic peptide and another biomolecule could be measured. For example, this could enable the study of binding interactions between azetidine modified cyclic peptides and protein surfaces for interruption of antibody-antigen interactions.

One of the key applications of this work relates to discovering the impact of the azetidine modified residue on biological activity. McAlpine and co-workers have reported the development of the cyclic peptide LB51 425, which inhibits the therapeutic target Hsp90 and is involved in a number of cellular processes such as protein folding, degradation and signal transduction. Several derivatives have been synthesised with the aim of improving the passive membrane permeability and oral bioavailability of this cyclic peptide, such as N-methylated, hydrophobic and asparagine-containing variants. However, balancing the selective binding and cell permeability of these peptides has been elusive.

By substitution of an amino acid residue with an azetidine modified residue, an “alanine-scanning” approach could be used to investigate the effect of the modification on the conformation, properties and biological activity of the peptide. This may lead to enhanced understanding of the binding to Hsp90, as well as the individual factors which may influence the
properties of these compounds. Furthermore, when the SAR of different analogues has been determined, second generation functionalised analogues with varied groups on the azetidine nitrogen could be synthesised (Figure 32). The passive membrane permeability of these compounds relative to the parent compound could be established using PAMPA or CACO-2 assays to establish the relationship between azetidine modification and functionalisation on the penetration of the compound.

Figure 32: Amino acid scanning approach to identify new azetidine modified inhibitors of Hsp90.

One limitation of the method for deprotection and functionalisation of Cbz protected azetidines is that the Pd catalyst used may not be suitable for sulfur containing peptides containing Cys, Met or Pbf. A cyclic peptide containing one of these groups could be easily added to a peptide sequence via SPPS followed by cyclisation, from any of the Cbz protected building blocks synthesised in Table 8, and the deprotection chemistry tested. If this methodology is not suitable, it would be advantageous to identify an alternative protecting group or deprotection conditions which are suitable for these peptides.

A discovery which warrants more detailed investigation is the macrocyclisation of linear peptides containing azetidine modified alanine as mixtures of diastereomers; separation of these diastereomers by column chromatography can provide two cyclic peptides from one reaction (Scheme 76 and 78). The use of preparative HPLC can also be explored to isolate each individual peptide.

To explore the generality of this chemistry, a suitable dipeptide building block 428 could be synthesised and incorporated with SPPS as shown for 383 (Scheme 78) to investigate the effect of ring size and sequence on the cyclisation rate of both diastereomers and their relative ease of chromatographic separation. There is literature precedence for the synthesis of oxetane based trisubstituted nitroalkenes (where R ≠ Me) by Carreira117 and Shipman11,21 which could be applied
to azetidinone 245 in order to diversify the methodology to azetidine modified residues of other
natural amino acids (Scheme 94).

\[
\begin{align*}
\text{Scheme 94: Synthesis of diastereomeric azetidine modified dipeptide building blocks.}^{14,17,21}
\end{align*}
\]

If the synthesis of azetidine modified cyclic peptides using this method cannot be realised, it may be possible to use the nitrogen of the azetidine to display the side chain functionality, in an analogous way to peptoids (Figure 33).\textsuperscript{150}

\[
\begin{align*}
\text{Figure 33: Applying the idea of peptoids to azetidine modified peptides to display the amino acid side chain functional groups.}
\end{align*}
\]

To achieve this, conditions for N-alkylation and Buchwald-Hartwig cross-coupling of the azetidine nitrogen would be useful. This would give access to alkylated azetidine derivatives 434 which could not be made via reductive amination as well as other more electron rich aryl functionalised azetidines 435 which cannot be made using the S\textsubscript{N}Ar approach (Scheme 95).
Scheme 95: Functionalisation of the azetidine nitrogen by Buchwald-Hartwig coupling and N-alkylation.
Chapter 4: Experimental Section

4.1 General experimental considerations

Anhydrous solvents were purchased from Sigma-Aldrich or Acros Organics in SureSeal™ bottles for use as reaction solvents. All other solvents were reagent grade and used as received. Petroleum ether refers to the fraction that boils in the range 40-60 °C. Commercially available starting materials were used without purification unless otherwise stated. All amino acids are of L-configuration unless otherwise stated.

NO$_2$-GOx-Gly-OBn 12, 14 NO$_2$-AOx-(R)-CH(Me)Ph 81, 22 Boc-Tyr(Bn)-OBn 163, 151 Benzyl 3-(nitromethylene)azetidine-1-carboxylate 259, 152 2,5-Dioxopyrrolidin-1-yl prop-2-yn-1-yl carbonate 262, 128 3-Azido-7-(diethylamino)chroman-2-one 409, 139 TsOH-H-Gly-Gly-OBn 443, 153 Boc-Ala-Gly-OBn 452, 154 D-Biotin-OSu 155 and azidoglycine-OSu 156, as well as all cumyl esters 22 were prepared following previously described literature procedures.

$^1$H Nuclear Magnetic Resonance (NMR) spectra were recorded in CDCl$_3$, CD$_2$Cl$_2$, CD$_3$OD, DMSO-d$_6$, CD$_3$CN or D$_2$O on a Bruker HD400 (400 MHz), AV500 (500 MHz) or AV600 (600 MHz) Fourier transform spectrometer. Chemical shifts ($\delta$H) are quoted in parts per million (ppm) and referred to the residual protic solvent signals of CDCl$_3$ (7.26 ppm), CD$_2$Cl$_2$ (5.32 ppm), CD$_3$OD (3.31 ppm), DMSO-d$_6$ (2.50 ppm), CD$_3$CN (1.94 ppm), toluene-d$_8$ (2.09 ppm) or D$_2$O (4.79 ppm). $^1$H NMR coupling constants are reported in hertz and refer to apparent multiplicities. Data are reported as follows: chemical shift, multiplicity (s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, dd = doublet of doublet, etc.), coupling constant, integration, and assignment. $^{13}$C NMR spectra were recorded at 101, 126 or 151 MHz. Chemical shifts ($\delta$C) are quoted in ppm referenced to CHCl$_3$ (77.16 ppm), CH$_2$Cl$_2$ (54.00 ppm), CD$_3$OD (49.00 ppm), DMSO-d$_6$ (39.52 ppm), or CD$_3$CN (1.32 ppm). NMR assignments were deduced using 2D experiments (COSY, HSQC and HMBC). NH and OH are not visible in protic solvents (CD$_3$OD, D$_2$O). Oxetanyl and azetidinyl NH in the backbone is reported if visible, but in most cases is not observed.

Low-resolution mass spectra were recorded on an Agilent 6130B single Quad (ESI) instrument. High-resolution mass spectra were recorded using a Bruker MaXis Impact. All infrared spectra were recorded on the neat compounds using a Bruker ALPHA-Platinum FTIR spectrometer, irradiating between 4000 cm$^{-1}$ and 600 cm$^{-1}$. Only strong and selected absorbances ($\nu_{\text{max}}$) are reported. Analytical TLC was performed on aluminium backed silica plates (Merck, Silica Gel 60 F$_{254}$, 0.25 mm). Compounds were visualised by fluorescence quenching or by staining the plates with 5% solution of phosphomolybdic acid (H$_3$PMO$_{12}$O$_{40}$) in EtOH or 1% solution of
potassium permanganate (KMnO₄) in water followed by heating. Flash column chromatography was performed on silica gel (Aldrich, Silica Gel 60, 40–63 µm). All mixed solvent eluents are reported as v/v solutions. Optical rotations were obtained using an AA-1000 polarimeter at 589 nm (Na D-line) in a cell with a path length of 2 dm. Specific rotation values are given in (deg mL)/(g dm). Melting points were measured with a Gallenkamp melting point apparatus.

LC-MS analysis were conducted on a Bruker Amazon X or Bruker HCT Ultra ETD instrument with a PLRP-S column from Agilent (100 Å, 8 µm, 150 × 4.6 mm) and UV detection at 210, 254 or 280 nm. A binary gradient of acetonitrile (0.1% formic acid) and water (0.1% formic acid) was used at a flow rate of 1 mL/min.

Analytical HPLC measurements were conducted on an Agilent 1260 Infinity analytical HPLC system on an Agilent Eclipse Plus C18 column (5.0 µm, 4.6 × 150 mm) or an Agilent PLRP-S column (100 Å, 8 µm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN).

Peptides were purified on a Agilent 1260 preparative HPLC (solvent A: H₂O (0.1% TFA), solvent B: acetonitrile (0.1% TFA)) on a PLRP-S column (Agilent, 100 Å, 8 µM 150 × 25 mm) at 20 °C.
4.2 General procedures

4.2.1 General procedure 1: Solution phase Boc peptide coupling

To a solution of Boc-protected peptide (1.0 equiv) in CH₂Cl₂ (1 mL/mmol) was added TFA (1 mL/mmol) and the mixture was stirred at room temperature for 1 h (Caution – gas evolution!). The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 10 mL/mmol) and concentrated under reduced pressure to give the crude amine. The residue was dissolved in CH₂Cl₂ (10 mL/mmol), N-protected amino acid (1.1 equiv), EDC·HCl (1.1 equiv), HOBt·H₂O (1.1 equiv) and NMM (4.0 equiv) were added subsequently, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with EtOAc (10 mL/mmol) and washed with brine (3 × 50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂).

4.2.2 General procedure 2: Solution phase Fmoc peptide coupling

To a solution of Fmoc-protected peptide (1.0 equiv) in CH₂Cl₂ (1 mL/mmol) was added diethylamine (1 mL/mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 10 mL/mmol) and concentrated under reduced pressure to give the crude amine. The residue was dissolved in CH₂Cl₂ (10 mL/mmol), N-protected amino acid (1.0 equiv), EDC·HCl (1.0 equiv), HOBt·H₂O (1.0 equiv) and NMM (4.0 equiv) were added subsequently, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with brine (3 × 50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The residue was purified by column chromatography (SiO₂).
4.2.3 General procedure 3: Synthesis of nitroalkanes by in-situ conjugate addition

Oxetane-3-one or N-protected azetidinone (2.0 equiv), nitromethane (for oxetanone: 2.8 equiv, for azetidinones: 1 mL/mmol) and triethylamine (0.4 equiv) were combined at 0 °C and stirred for 1 h at room temperature. In the case of the azetidinone, the excess nitromethane was removed under reduced pressure. The mixture was dissolved in CH$_2$Cl$_2$ (10 mL/mmol), cooled to −78 °C, and triethylamine (4.0 equiv) was added followed by dropwise addition of methanesulfonyl chloride (2.0 equiv). The reaction mixture was stirred at −78 °C for 1.5 h followed by addition of the N-terminal amine in CH$_2$Cl$_2$ (2.5 mL/mmol). The reaction mixture was allowed to warm to room temperature and stirred for 16 h. A saturated solution of NH$_4$Cl (10 mL/mmol) was added and stirred for 10 min. The layers were separated and the aqueous one extracted with CH$_2$Cl$_2$ (2 × 10 mL/mmol) and EtOAc (2 × 10 mL/mmol). The combined organic phases were washed with saturated aqueous NaHCO$_3$ solution (10 mL/mmol), brine (100 mL/mmol), dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography.

4.2.4 General procedure 4: Raney-Ni reduction and in-situ coupling

To a solution of nitroalkane (1.0 equiv) in THF (10 mL/mmol) was added succinimide ester (2.0 equiv), NaHCO$_3$ (4.0 equiv) and Raney Ni (1.5 mL/mmol). The solution was placed under an atmosphere of nitrogen, evacuated and filled with hydrogen (balloon). The reaction mixture was stirred vigorously for 4.0 h at room temperature. Then, the mixture was filtered through a plug of Celite eluting with EtOAc, concentrated under reduced pressure, the filtrate was suspended in
EtOAc (10 mL/mmol), washed with saturated Na$_2$CO$_3$ (3 × 10 mL/mmol), brine (10 mL/mmol), dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude residue was purified by column chromatography.

4.2.5 General procedure 5: Benzyl deprotection

To a solution of protected peptide (1.0 equiv) in MeOH (1 mL/mmol) was added 10 wt% Pd/C (10 wt%) and the reaction flask was evacuated, filled with nitrogen, evacuated, and placed under an atmosphere of hydrogen (balloon). The mixture was stirred at room temperature until full consumption of the starting material was observed (TLC/MS monitoring), placed under nitrogen and filtered through a plug of Celite, which was washed with MeOH (3×). The filtrate was concentrated in vacuo to provide the deprotected peptide, which required no further purification.

4.2.6 General procedure 6: Peptide macrocyclisation

To a solution of linear peptide precursor (1.0 equiv) in DMF (1–5 mM) was added DEPBT (2.0 equiv) and DIPEA (2.0 equiv) and the mixture was stirred for 24–72 h at room temperature. The solvent was removed in vacuo and the residue was purified by column chromatography (SiO$_2$).
4.2.7 General procedure 7: One-pot synthesis of azetidine modified dipeptide building blocks

To a solution of Fmoc-protected peptide (1.0 equiv) in CH₂Cl₂ (1 mL/mmol) was added diethylamine (1 mL/mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 10 mL/mmol) and concentrated under reduced pressure to give the crude amine. The residue was dissolved in CH₂Cl₂ (10 mL/mmol), and to the solution was added the nitroalkene (1.0 equiv) and left to stir for 16 h. At this time, the solution was reduced in vacuo and resuspended in THF (10 mL/mmol). To the solution was added succinimide ester (2.0 equiv), NaHCO₃ (4.0 equiv) and Raney Ni (1.5 mL/mmol). The solution was placed under an atmosphere of nitrogen, evacuated and filled with hydrogen (balloon). The reaction mixture was stirred vigorously for 4.0 h at room temperature. Then, the mixture was filtered through a plug of Celite eluting with EtOAc, concentrated under reduced pressure, the filtrate was suspended in EtOAc (10 mL/mmol), washed with saturated Na₂CO₃ (3 × 10 mL/mmol), brine (10 mL/mmol), dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified by column chromatography.

4.2.8 General procedure 8: Zinc reduction

To a solution of nitroalkane (1.0 equiv) in THF (0.05 M) was added freshly activated, ground zinc powder (3.0 equiv) and acetic acid (8.0 equiv) and the reaction mixture was vigorously stirred with a glass-coated magnetic stir bar at room temperature for 1 h. Additional zinc powder (3.0 equiv) and acetic acid (8.0 equiv) were added and the mixture was stirred at ambient temperature for 1 h (repeat until all starting material had been consumed, ESI-MS monitoring). The reaction
mixture was concentrated \textit{in vacuo} and resuspended in EtOAc (20 mL/mmol), filtered through a pad of Celite® eluting with EtOAc, and concentrated \textit{in vacuo}. The resulting residue was dissolved in CH$_2$Cl$_2$ (10 mL/mmol), and triethylamine (3.0 equiv) followed by Fmoc-OSu (2.0 equiv) was added and the solution was stirred at room temperature for 16 h. The solution was quenched with saturated Na$_2$CO$_3$ (10 mL/mmol), and the organic layer washed with saturated Na$_2$CO$_3$ (2 × 10 mL/mmol), brine (10 mL/mmol), dried (Na$_2$SO$_4$) and concentrated \textit{in vacuo} and the crude products purified by column chromatography (SiO$_2$).

4.2.9 General procedure 9 for synthesis of cyclic peptides via SPPS and cyclisation

Dipeptide building block (0.6 mmol, 4.0 equiv) was stirred at room temperature in 2% TFA in CH$_2$Cl$_2$ (0.05M) for 3 – 4 h until complete deprotection of the cumyl ester was observed by TLC and ESI-MS. The solvent was removed under reduced pressure and the resulting residue was repeatedly dissolved in CH$_2$Cl$_2$ (3 × 10 mL) and concentrated under reduced pressure. The crude acid was used for coupling without further purification.

H-AA-2-chlorotrityl resin (1.0 equiv) was placed in a 10 mL reaction vessel and the resin was pre-swollen in DMF (20 mL/mmol) for 30 min. For coupling of the dipeptide building block, the crude acid generated previously was dissolved in DMF (20 mL/mmol). HATU (4.0 equiv) and DIPEA (12.0 equiv) were added to the solution of the building block and the coupling solution was added to the resin. The coupling reaction was allowed to proceed for 2 h at room temperature under slight agitation. The resin was filtered, washed with DMF (5 × 25 mL/mmol). In case of a positive TNBS test, the coupling step was repeated with additional 2.0 equiv of building block. The Fmoc group was removed with 20% piperidine in DMF (20 mL/mmol) for 20 min at room temperature. After washing the resin with DMF (5 × 4.0 mL), Coupling of Fmoc-AA-OH to the preloaded resin or to the free N-terminus was completed as described previously for the building
block, using Fmoc-AA-OH (5.0 equiv), HATU (5 equiv), and DIPEA (10 equiv) in DMF (20 mL/mmol) for 1 h at room temperature. In case of a positive TNBS test, the coupling step was repeated. The resin was washed with DMF (5 × 25 mL/mmol) before the Fmoc group was removed and coupling of amino acids were repeated as before. After the final deprotection of the Fmoc group, the resin was washed vigorously with CH$_2$Cl$_2$ (8 – 10 × 30 mL/mmol) and the peptide was then cleaved from the resin with TFE in CH$_2$Cl$_2$ (1:4, 30 mL/mmol) for 1 h at room temperature. This was repeated twice and the combined cleavage solutions were evaporated to dryness under reduced pressure. Success of the synthesis was confirmed by mass spectrometry and NMR. Based on the crude weight, the peptide was dissolved in DMF (5 mM) and DEPB (2.0 equiv) and DIPEA (2.0 equiv) were added. The reaction mixture was stirred at room temperature for 72 h, the solvent was then removed under reduced pressure and the residue purified by column chromatography (2.5 – 10% MeOH in CH$_2$Cl$_2$).

4.2.10 General procedure 10 for acidic deprotection of azetidine modified compounds containing ‘Bu or Boc protecting groups only

Cyclic peptide was suspended in 70% TFA/CH$_2$Cl$_2$ and stirred at room temperature for 1 – 2 h. Upon completion of the deprotection monitored by TLC and ESI-MS, the reaction mixture was removed under reduced pressure. The residue was repeatedly resuspended in CH$_2$Cl$_2$ (50 mL/mmol) and the solvent removed under reduced pressure (3×) to reveal the deprotected peptide which required no further purification.

4.2.11 General procedure 11 for acidic deprotection of azetidine modified compounds containing Pbf or Trt protecting groups

Cyclic peptide was suspended in 90% TFA/5% CH$_2$Cl$_2$/5% TIS and stirred at room temperature for 1 – 2 h. For compounds containing Cys, 5% DTT was also added to prevent formation of disulfides or oxidation. Upon completion of the deprotection monitored by TLC and ESI-MS, the reaction mixture was removed under reduced pressure. The residue was repeatedly resuspended in CH$_2$Cl$_2$ (50 mL/mmol) and the solvent removed under reduced pressure (3×) to reveal the crude residue, a portion of which was purified by reverse phase HPLC for full analytical data.
Cbz-protected azetidine modified peptide (1.0 equiv) was deprotected following general procedure 5 using ethanol (10 mg/mL) as the solvent. The crude azetidine was suspended in CH₂Cl₂ (10 mg/mL), and to the solution was added NEt₃ (2.5 equiv) followed by the acyl chloride/succinimide ester/sulfonyl chloride (1.5 equiv). For reactions containing acyl chlorides, DMAP (0.2 equiv) was also added. The reaction was stirred under an atmosphere of N₂ for 16 h at RT. The reaction mixture was quenched with brine (10 mg/mL) and the organic layer extracted with CH₂Cl₂ (10 mg/mL) and washed with brine (10 mg/mL). The organic layer was dried (Na₂SO₄), filtered and reduced in vacuo and the crude products purified by column chromatography (SiO₂).
4.3 Experimental procedures and characterisation

Preparation of cyclic pentapeptide 170

Boc-Pro-Tyr(Bn)-OBn (164)

Following general procedure 1 from Boc-Tyr(Bn)-OBn 163 (11.4 g, 24.8 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, PE/EtOAc 4:1) to give dipeptide Boc-Pro-Tyr(Bn)-OBn (164) (10.8 g, 19.3 mmol, 78%) as a white foam. \( R_f \) (PET ether/EtOAc 4:1) 0.30; m.p 105‒108 °C; \(^1\)H NMR (600 MHz, DMSO-d₆) \( \delta \)H 7.70 (d, \( J = 7.5 \) Hz, 1H, NH), 7.44–7.27 (m, 10H, ArH), 7.11 (d, \( J = 8.4 \) Hz, 2H, ArH), 6.89 (d, \( J = 8.4 \) Hz, 2H, ArH), 5.10 (s, 2H, CH₂Ph), 5.07 (s, 2H, CH₂Ph), 4.61–4.56 (m, 1H, CHβ-Tyr), 4.13 (dd, \( J = 8.5, 3.0 \) Hz, 1H, CHα-Pro), 3.35–3.26 (m, 2H, CH₂δ-Pro), 3.04 (dd, \( J = 14.1, 6.0 \) Hz, 1H, CHHβ-Tyr), 2.97–2.92 (dd, \( J = 18.0, 8.0 \) Hz, 1H, CHHβ-Tyr), 2.00 (dq, \( J = 16.3, 8.3 \) Hz, 1H, CHHγ-Pro or CH₂β-Pro or CH₂γ-Pro), 1.78–1.66 (m, 3H, CHHβ-Pro or CH₂β-Pro or CH₂γ-Pro, CH₂β-Pro or CH₂γ-Pro), 1.32 (s, 9H, 3 × CH₃, Boc); \(^1\)C NMR (101 MHz, DMSO-d₆) \( \delta \)C 172.7 (C=O), 171.5 (C=O), 157.1 (C=O, Boc), 153.3 (C), 137.2 (C), 135.7 (C), 130.0 (CH), 129.2 (C), 128.41 (CH), 128.35 (CH), 128.1 (CH), 128.0 (CH), 127.8 (CH), 127.5 (CH), 114.6 (CH), 78.3 (C, Boc), 69.1 (CH₂, Bn), 66.0 (CH₂, Bn), 59.3 (CH, α-Pro), 53.9 (CH, α-Tyr), 46.4 (CH₂, δ-Pro), 35.7 (CH₂, β-Tyr), 30.7 (CH₂, β-Pro or CH₂γ-Pro), 28.1 (CH₃, Boc, minor rotamer), 27.8 (CH₃, Boc, major rotamer), 22.9 (CH₂, β-Pro or CH₂γ-Pro); \( \nu _{\text{max}} \) (neat) = 2977, 1734, 1686, 1669, 1508, 1160, 734 cm⁻¹; MS (ESI⁺) m/z 581 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₃₃H₃₈N₂O₆ [M+Na⁺] 581.2622, found 581.2624; [\( \alpha \)ₜ]D²⁷ = -50.0 (c 0.10, CHCl₃).

NO₂-GOx-Pro-Tyr(Bn)-OBn (166)

To a solution of Boc-Pro-Tyr(Bn)-OBn (164) (6.72 g, 12.0 mmol, 1.0 equiv) in CH₂Cl₂ (12.0 mL) was added TFA (12.0 mL) and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 50 mL) and concentrated under reduced pressure to give the crude amine. This was added to the nitroalkene formed in-situ following general procedure 3. The crude residue was purified by column chromatography (SiO₂, EtOAc/PE 4:1) to yield NO₂-GOx-Pro-Tyr(Bn)-OBn (166) (6.10 g, 10.6 mmol, 89%) as an orange oil. \( R_f \) (EtOAc/PE 4:1) 0.31; \(^1\)H NMR (500 MHz, CDCl₃) \( \delta \)H 7.44–7.28 (m, 10H, ArH), 7.25 (m, 1H, NH), 7.04 (d, \( J = 8.5 \) Hz, 2H, ArH), 6.85 (d, \( J = 8.5 \) Hz, 2H, ArH), 5.21–5.12 (m, 2H, CH₂Ph), 5.04 (s, 2H, CH₂Ph), 4.94 (d, \( J = 13.1 \) Hz, 1H, OCHH-Ox), 4.73 (d, \( J = 13.1 \) Hz, 1H, OCHH-Ox), 4.70–4.63 (m, 2H, OCHH-Ox, CHα-Tyr), 4.62 (d, \( J = 7.4 \) Hz, 1H, CHHGOx), 4.56 (d, \( J = 8.5 \) Hz, 2H, OCHH-Ox, CHα-Tyr).
Following general procedure 4 from NO2-GOx-Pro-Tyr(Bn)-OBn (166) (7.80 g, 13.6 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, CH2Cl2/EtOAc 7:3) to yield Boc-Ala-GOx-Pro-Tyr(Bn)-OBn (167) as an off-white solid (3.99 g, 5.59 mmol, 41%). Rf (CH2Cl2/EtOAc 7:3) 0.22; m.p 72–74 °C; 1H NMR (500 MHz, CDCl3) δH 7.73 (d, J = 9.1 Hz, 1H, NH), 7.44–7.29 (m, 10H, ArH), 7.02 (d, J = 7.8 Hz, 2H, ArH), 6.86 (s, 1H, NH), 6.82 (d, J = 8.4 Hz, 2H, ArH), 5.26 (d, J = 12.1 Hz, 1H, CHHPh), 5.19–5.10 (m, 1H, CHHPh), 5.01 (s, 2H, CH2Ph), 4.92 (td, J = 8.8, 5.3 Hz, 1H, CHα-Tyr), 4.63 (d, J = 6.9 Hz, 1H, OCHH-Ox), 4.39 (d, J = 7.2 Hz, 1H, OCHH-Ox), 4.34 (d, J = 6.9 Hz, 1H, OCHH-Ox), 4.30 (d, J = 7.2 Hz, 1H, OCHH-Ox), 4.11 (quint, J = 6.9 Hz, 1H, CHα-Ala), 3.94 (dd, J = 14.0, 6.8 Hz, 1H, CHHGOx), 3.85–3.80 (m, 1H, CHα-Pro), 3.56 (dd, J = 14.1, 3.8 Hz, 1H, CHHGOx), 3.19 (dd, J = 14.0, 5.0 Hz, 1H, CHHβ-Tyr), 3.05 (t, J = 7.6 Hz, 1H, CHHβ-Pro), 2.99 (dd, J = 14.0, 8.6 Hz, 1H, CHHβ-Tyr), 2.47 (ddd, J = 10.8, 8.8, 5.9 Hz, 1H, CHHβ-Pro), 2.08–1.97 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.77–1.64 (m, 2H, CHHβ-Pro or CHHγ-Pro), 1.59–1.48 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.42 (s, 9H, 3 × CH3, Boc), 1.26 (d, J = 8.1 Hz, 3H, CHβ-Ala). Note: Peak at 1.77–1.64 overlaps with residual H2O peak, Boc NH not observed; 13C NMR (126 MHz, CDCl3) δC 175.0 (C=O), 173.9 (C=O), 173.4 (C=O), 157.8 (C), 155.7 (C=O, Boc), 137.1 (C), 135.0 (C), 130.4 (CH), 128.72 (CH), 128.68 (CH), 128.7 (CH), 128.6 (CH), 128.1 (CH), 127.6 (CH), 114.9 (CH), 79.8 (C, Boc), 78.3 (OCH2), 76.5 (OCH2), 70.1 (CH2, Bn), 67.8 (CH2, Bn), 62.8 (C, Ox), 61.7 (CH, α-Pro), 52.7 (CH, α-Tyr), 50.0 (CH, α-Ala), 48.8 (CH2, δ-Pro), 45.1 (CH2, GOx), 37.1 (CH2, β-Tyr), 31.6 (CH2, β-Pro or CH2, γ-Pro), 28.5 (CH3, Boc), 24.5
(CH₂, β-Pro or CH₂, γ-Pro), 18.4 (CH₃, β-Ala). Note: One aromatic CH signal not observed; νmax (neat) = 3323, 2970, 2935, 1736, 1661, 1510, 1163, 734 cm⁻¹; MS (ESI⁺) m/z 715 [M+H]⁺, 737 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₆H₅N₂NaO₈ [M+Na]⁺ 737.3521, found 737.3517; [α]D²⁷ –10.0 (c 0.10, CHCl₃).

Cbz-Leu-Ala-GOx-Pro-Tyr(Bn)-OBn (168)

Following general procedure 1 from Boc-Ala-GOx-Pro-Tyr(Bn)-OBn (167) (3.99 g, 5.58 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) to give Cbz-Leu-Ala-GOx-Pro-Tyr(Bn)-OBn (168) (2.93 g, 3.40 mmol, 61%) as a white solid. Rf (CH₂Cl₂/MeOH 19:1) 0.32; m.p 62–64 °C; ¹H NMR (400 MHz, CDCl₃) δH 7.82 (d, J = 9.3 Hz, 1H, NH), 7.45–7.27 (m, 16H, ArH, NH), 7.04 (d, J = 8.5 Hz, 2H, ArH), 6.81 (d, J = 8.5 Hz, 2H, ArH), 6.66 (d, J = 7.0 Hz, 1H, NH), 5.30–5.18 (d, J = 12.2 Hz, 1H, CHHPh), 5.14–5.05 (m, 3H, CHHPh, CH₂Ph), 5.03–4.91 (m, 4H, CH₂Ph, CHα-Tyr, NH), 4.62 (d, J = 6.8 Hz, 1H, OCH₃-Ox), 4.36 (m, 4H, OCH₂-Ox, OCH₂-Ox, CHα-Ala), 4.16 (m, 1H, CHα-Leu), 3.86 (m, 2H, CHHGOx, CHα-Pro), 3.59 (dd, J = 14.1, 4.2 Hz, 1H, CHHβ-Tyr), 3.08–2.92 (m, 2H, CHHβ-Tyr, CHHδ-Pro), 2.43 (m, 1H, CHHδ-Pro), 2.09–1.91 (m, 1H, CH₂β-Pro or CH₂γ-Pro), 1.78–1.41 (m, 5H, CHγ-Leu, CHββ-Leu, CHββ-Pro or CHβγ-Pro), 1.28 (d, J = 7.0 Hz, 3H, CH₃β-Ala), 0.92 (d, J = 5.6 Hz, 6H, 2 × CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δC 171.5 (C=O), 173.3 (C=O), 173.0 (C=O), 172.2 (C=O), 157.9 (C), 156.3 (C=O, Cbz), 137.1 (C), 136.0 (C), 135.0 (C), 130.4 (CH), 128.84 (CH), 128.76 (CH), 128.72 (CH), 128.70 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.1 (CH), 114.9 (CH), 78.4 (OCH₂), 76.5 (OCH₂), 70.0 (CH₂, Bn), 67.7 (CH₂, Bn), 67.4 (CH₂, Bn), 62.7 (C, Ox), 61.8 (CH, α-Pro), 53.8 (CH, α-Leu), 52.6 (CH, α-Tyr), 49.1 (CH, α-Ala), 48.8 (CH₂ δ-Pro), 45.2 (CH₂, GOx), 41.6 (CH₂, β-Leu), 36.9 (CH₂, β-Tyr), 31.6 (CH₂, β-Pro or CH₂ γ-Pro), 24.8 (CH, γ-Leu), 24.5 (CH₂, β-Pro or CH₂ γ-Pro), 23.1 (CH₂δ-Leu), 21.9 (CH₃δ-Leu), 17.6 (CH₃β-Ala). Note: One aromatic CH signal not observed; νmax (neat) = 2955, 2879, 1720, 1649, 1509, 1347, 1119 cm⁻¹; MS (ESI⁺) m/z 860 [M–H]⁻; HRMS (ESI⁻) calcd. for C₂₉H₃₈N₅O₈ [M–H]⁻ 860.4240, found 860.4222; [α]D²⁷ −18.5 (c 0.10, CHCl₃).
H-Leu-Ala-GOx-Pro-Tyr-OH (169)

Following general procedure 5 from Cbz-Leu-Ala-GOx-Pro-Tyr(Bn)-OBn (168) (0.48 g, 0.55 mmol, 1.0 equiv), H-Leu-Ala-GOx-Pro-Tyr-OH (169) was obtained as a white solid (30 mg, 0.55 mmol, quant. yield) which required no further purification; m.p 161–163 °C; \(^1\)H NMR (500 MHz, CD\(_2\)OD) \(\delta_H 7.06\) (d, \(J = 8.3\) Hz, 2H, ArH), 6.70 (d, \(J = 8.3\) Hz, 2H, ArH), 4.70 (d, \(J = 7.0\) Hz, 1H, OCHH-Ox), 4.61 (dd, \(J = 8.0, 4.8\) Hz, 1H, CHO-Tyr), 4.48–4.38 (m, 4H, OCH\(_2\)-Ox, OCHH-Ox, CH\(_\alpha\)-Ala), 3.95 (dd, \(J = 8.1, 6.2\) Hz, 1H, CH\(\alpha\)-Leu), 3.79 (d, \(J = 9.0\) Hz, 1H, CH\(\alpha\)-Pro), 3.70 (d, \(J = 14.2\) Hz, 1H, CHHGOx), 3.60 (d, \(J = 14.2\) Hz, 1H, CHHGOx), 3.18 (dd, \(J = 13.9, 4.7\) Hz, 1H, CHH\(\beta\)-Tyr), 3.11 (t, \(J = 7.4\) Hz, 1H, CHH\(\delta\)-Pro), 3.00 (dd, \(J = 13.9, 8.4\) Hz, 1H, CHH\(\beta\)-Tyr), 2.64 (dt, \(J = 8.7, 5.9\) Hz, 1H, CHH\(\delta\)-Pro), 2.18–2.06 (m, 1H, CHH\(\beta\)-Pro or CHH\(\gamma\)-Pro), 1.83–1.54 (m, 5H, CH\(\beta\)-Leu, CH\(\gamma\)-Leu, CH\(\gamma\)-Pro or CH\(\gamma\)-Pro), 1.36 (d, \(J = 7.1\) Hz, 3H, CH\(\beta\)-Ala), 0.99 (d, \(J = 4.0\) Hz, 3H, CH\(\delta\)-Leu), 0.98 (d, \(J = 4.0\) Hz, 3H, CH\(\delta\)-Leu); \(^13\)C NMR (126 MHz, CD\(_2\)OD) \(\delta_C 177.5\) (C=O), 175.9 (C=O), 175.4 (C=O), 170.3 (C=O), 157.4 (C), 131.5 (CH), 129.2 (C), 116.2 (CH), 79.0 (OCH\(_2\)), 77.6 (OCH\(_2\)), 64.4 (C, Ox), 62.9 (CH, \(\alpha\)-Pro), 54.9 (CH, \(\alpha\)-Tyr), 52.9 (CH, \(\alpha\)-Leu), 50.9 (CH, \(\alpha\)-Ala), 49.7 (CH, \(\delta\)-Pro), 45.1 (CH, GOx), 41.6 (CH, \(\beta\)-Tyr), 37.8 (CH, \(\beta\)-Leu), 32.4 (CH, \(\beta\)-Pro or CH\(_\gamma\)-Pro), 25.4 (CH, \(\beta\)-Pro or CH\(_\gamma\)-Pro), 25.3 (CH, \(\gamma\)-Leu), 23.1 (CH\(_\delta\), \(\delta\)-Leu), 22.2 (CH\(_\delta\), \(\delta\)-Leu), 18.2 (CH\(_\delta\), \(\beta\)-Ala); \(v_{\text{max}}\) (neat) = 3267, 2989, 2900, 1644, 1513, 1234 cm\(^{-1}\); MS (ESI) \(m/z\) 546 [M–H]+; HRMS (ESI) calcd. for C\(_27\)H\(_{40}\)N\(_3\)O\(_7\) [M–H]– 546.2933, found 546.2933; [\(\alpha\)]\(_D\)^27 = 1.00 (c 0.20, MeOH).

Cyclo(Leu-Ala-GOx-Pro-Tyr) (170)

Following general procedure 6, H-Leu-Ala-GOx-Pro-Tyr-OH (169) (55 mg, 0.10 mmol, 1.0 equiv) was cyclised using PyBOP (104 mg, 0.20 mmol, 2.0 equiv) at 1 mM for 24 h. The crude residue was purified twice by column chromatography (SiO\(_2\), CH\(_3\)Cl/MeOH 19:1→9:1) to give the cyclic pentapeptide (170) as a colourless glassy solid (32 mg, 60 \(\mu\)mol, 60%). R\(_f\) (CH\(_3\)Cl/MeOH 19:1) 0.15; m.p 174–176 °C; \(^1\)H NMR (500 MHz, CD\(_2\)OD) \(\delta_H 7.10\) (d, \(J = 8.4\) Hz, 2H, ArH), 6.73 (d, \(J = 8.4\) Hz, 2H, ArH), 4.69 (d, \(J = 6.9\) Hz, 1H, OCHH-Ox), 4.54–4.45 (m, 2H, CHO-Tyr, CH\(\alpha\)-Ala), 4.43 (d, \(J = 7.5\) Hz, 1H, OCHH-Ox), 4.35 (d, \(J = 6.8\) Hz, 1H, OCHH-Ox), 4.27 (d, \(J = 7.5\) Hz, 1H, OCHH-Ox), 4.13 (dd, \(J = 10.8, 4.9\) Hz, 1H, CH\(\alpha\)-Leu), 3.93 (dd, \(J = 9.8, 3.2\) Hz, 1H, CH\(\alpha\)-Pro), 3.79 (d, \(J = 14.0\) Hz, 1H, CHHGOx), 3.58 (d, \(J = 14.0\) Hz,
1H, CHHGOx), 3.28–3.21 (m, 1H, CHHδ-Pro), 3.16–3.04 (m, 2H, CHβ-Tyr), 2.58 (q, J = 8.3 Hz, 1H, CHHβ-Pro), 2.19–2.09 (m, 1H CHHβ-Pro or CHHγ-Pro), 1.94–1.80 (m, 3H, CHHβ-Leu, CHβ-Pro or CHγ-Pro), 1.75–1.68 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.63–1.55 (m, 1H, CHHβ-Leu), 1.49–1.40 (m, 4H, CHγ-Leu, CHβ-Ala), 0.94 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.85 (d, J = 6.5 Hz, 3H, CHδ-Leu); 13C NMR (126 MHz, CD3OD) δC 178.4 (C=O), 176.0 (C=O), 174.3 (C=O), 173.8 (C=O), 157.4 (C), 131.3 (CH), 128.8 (C), 116.3 (CH), 78.8 (OCH2), 77.9 (OCH2), 63.8 (C, Ox), 62.9 (CH, α-Pro), 57.0 (CH, α-Tyr), 56.1 (CH, α-Leu), 51.5 (CH, α-Ala), 49.3 (CHδ, δ-Pro), 46.2 (CH2, GOx), 40.6 (CH2, β-Leu), 36.5 (CH2, β-Tyr), 32.7 (CH2, β-Pro or CH2, γ-Pro), 25.9 (CH2, β-Pro or CH2, γ-Pro), 25.7 (CH, γ-Leu), 23.4 (CHδ, δ-Leu), 21.5 (CH3, δ-Leu), 18.5 (CH3, β-Ala); νmax (neat) = 3271, 2956, 2872, 1642, 1512, 1241 cm⁻¹; MS (ESI⁺) m/z 552 [M+Na⁺]; HRMS (ESI⁺) calcd. for C27H30N4NaO6 [M+Na⁺] 552.2793, found 552.2796; [α]D 77 – 76.5 (c 0.10, MeOH).

Preparation of cyclic pentapeptide 107

Boc-Gly-Pro-Tyr(Bn)-OBn (436)

Following general procedure 1 from dipeptide 164 (5.06 g, 9.08 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, PET ether/EtOAc 1:1) to give tripeptide Boc-Gly-Pro-Tyr(Bn)-OBn (436) (4.36 g, 7.09 mmol, 78%) as a white solid. Rf (PET ether/EtOAc 1:1) 0.23; m.p 52–55 °C; 1H NMR (500 MHz, CDCl3) δH 7.46–7.29 (m, 10H, ArH), 7.23 (d, J = 7.8 Hz, 1H, NH), 6.94 (d, J = 8.4 Hz, 2H, ArH), 6.81 (d, J = 8.4 Hz, 2H, ArH), 5.39 (s, 1H, NH), 5.15 (d, J = 12.2 Hz, 2H, CH2Ph), 5.02 (s, 2H, CH2Ph), 4.82 (dd, J = 13.4, 7.0 Hz, 1H, CHα-Tyr), 4.54 (d, J = 7.0 Hz, 1H, CHα-Pro), 3.94 (dd, J = 17.4, 4.9 Hz, 1H, CHHγ-Gly), 3.72 (dd, J = 17.4, 3.8 Hz, 1H, CHHβ-Gly), 3.26 (t, J = 6.9 Hz, 2H, CH2δ-Pro), 3.11 (dd, J = 14.1, 5.6 Hz, 1H, CHHβ-Pro or CHHγ-Pro), 2.94 (dd, J = 14.1, 7.0 Hz, 1H, CHHβ-Leu), 2.35–2.29 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.96–1.89 (m, 2H, CHβ-Pro or CHγ-Pro), 1.82–1.73 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.45 (s, 3H, 3 × CH3, Boc); 13C NMR (126 MHz, CDCl3) δC 171.4 (C=O), 170.4 (C=O), 168.7 (C=O), 157.9 (C), 155.9 (C=O, Boc), 137.2 (C), 135.4 (C), 130.5 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.3 (C), 128.1 (CH), 127.7 (CH), 127.6 (CH), 114.8 (CH), 79.9 (C, Boc), 70.0 (CH2Ph), 67.3 (CH2Ph), 60.0 (CH, α-Pro), 53.5 (CH, α-Tyr), 46.2 (CH2, δ-Pro), 43.2 (CH2, Gly), 37.1 (CH2, β-Tyr), 28.5 (CH3, Boc), 27.1 (CH2, β-Pro or CH2, γ-Pro), 24.9 (CH2, β-Pro or CH2, γ-Pro); νmax (neat) = 3307, 2974, 1739, 1709, 1646, 1509, 1238, 1161, 695 cm⁻¹; MS (ESI⁺) m/z 638 [M+Na⁺]; HRMS (ESI⁺) calcd. for C35H38N4NaO7 [M+Na⁺] 638.2837, found 638.2833; [α]D 77 – 66.0 (c 0.27, CHCl3).
Boc-Ala-Gly-Pro-Tyr(Bn)-OBn (437)

Following general procedure 1 from tripeptide **436** (4.21 g, 6.84 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, EtOAc/PE 8:2) to give tetrapeptide Boc-Ala-Gly-Pro-Tyr(Bn)-OBn (437) (3.58 g, 4.71 mmol, 76%) as a white solid. **Rₜ** (EtOAc/PE 8:2) 0.26; **m.p** 62–64 °C; **¹H NMR** (500 MHz, CDCl₃) δₜ 7.44–7.30 (m, 11H, ArH, NH), 7.11 (d, J = 7.2 Hz, 1H, NH), 6.94 (d, J = 8.5 Hz, 2H, ArH), 6.81 (d, J = 8.5 Hz, 2H, ArH), 5.22–5.09 (m, 3H, CH₂Ph, NH), 5.04–4.99 (m, 2H, CH₃Ph), 4.84 (dd, J = 13.6, 7.1 Hz, 1H, CHα-Tyr), 4.52 (d, J = 6.8 Hz, 1H, CHα-Pro), 4.28–4.19 (m, 1H, CHα-Ala), 3.98 (dd, J = 17.6, 4.3 Hz, 1H, CHH-Gly), 3.86 (dd, J = 17.6, 3.9 Hz, 1H, CHH-Gly), 3.34–3.24 (m, 2H, CH₂-S-Pro), 3.11 (dd, J = 14.1, 5.7 Hz, 1H, CHHβ-Tyr), 2.96 (dd, J = 14.1, 7.0 Hz, 1H, CHHβ-Tyr), 2.31–2.25 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.96–1.88 (m, 2H, CHβ-Pro or CHγ-Pro), 1.85–1.76 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.44 (s, 9H, 3 × CH₃, Boc), 1.33 (d, J = 7.1 Hz, 3H, CHβ-Ala); **¹³C NMR** (126 MHz, CDCl₃) δ₂ 72.9 (C=O), 171.5 (C=O), 170.3 (C=O), 167.9 (C=O), 157.8 (C), 155.5 (C=O, Boc), 137.1 (C), 135.4 (C), 130.5 (CH), 128.75 (CH), 128.73 (CH), 128.70 (CH), 128.66 (CH), 128.64 (CH), 128.3 (C), 128.1 (CH), 127.7 (CH), 114.9 (CH), 80.2 (C, Boc), 70.1 (CH₂Ph), 67.3 (CH₂Ph), 60.1 (CH, α-Pro), 53.3 (CH, α-Tyr), 50.3 (CH, α-Ala), 46.4 (CH₂, δ-Pro), 42.2 (CH₂, Gly), 37.0 (CH₂, β-Tyr), 28.5 (CH₃, Boc), 27.5 (CH₂, β-Pro or CH₂, γ-Pro), 24.8 (CH₂, β-Pro or CH₂, γ-Pro), 18.7 (CH₃, β-Ala); **νmax (neat) = 3293, 2974, 1739, 1639, 1509, 1239, 1163, 1023, 696 cm⁻¹; MS (ESI⁺) m/z 709 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₈H₆₆Na₅O₈ [M+Na]⁺ 709.3208, found 709.3206; [α]₀²⁵ = −46.1 (c 0.27, CHCl₃).

Cbz-Leu-Ala-Gly-Pro-Tyr(Bn)-OBn (438)

Following general procedure 1 from tetrapeptide **437** (3.23 g, 4.71 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 97:3) to give pentapeptide Cbz-Leu-Ala-Gly-Pro-Tyr(Bn)-OBn (438) (2.63 g, 3.16 mmol, 67%) as a white solid. **Rₜ** (CH₂Cl₂/MeOH 97:3) 0.18; **m.p** 69–72 °C; NMR data reported for the major rotamer: **¹H NMR** (600 MHz, DMSO-d₆ @ 373 K) δₜ 7.65 (d, J = 7.2 Hz, 1H, NH), 7.57 (s, 1H, NH), 7.46–7.25 (m, 15H, ArH), 7.11 (d, J = 8.4 Hz, 2H, ArH), 6.90 (d, J = 8.4 Hz, 2H, ArH), 5.12–5.04 (m, 6H, 3 × CH₂Ph), 4.61–4.53 (m, 1H, CHα-Tyr), 4.42 (dd, J = 8.5, 2.6 Hz, 1H, CHα-Pro), 4.40–4.35 (m, 1H, CHα-Ala), 4.08 (td, J = 8.9, 5.3 Hz, 1H, CHα-Leu), 3.85 (br. s, 2H, CH₂Gly), 3.44 (m,
2H, CH₂δ-Pro), 3.08–3.00 (m, 2H, CH₃β-Tyr), 1.86–1.47 (m, 7H, CH₃β-Leu, CHy-Leu, CH₃β-Pro, CH₂γ-Pro), 1.26 (d, J = 7.0 Hz, 3H, CH₃β-Ala), 0.89 (d, J = 6.6 Hz, 3H, CH₂δ-Leu), 0.87 (d, J = 6.6 Hz, 3H, CH₃δ-Leu). Note: Two NH signals not observed; ¹³C NMR (151 MHz, DMSO-d₆ @ 373 K) δc 171.5 (C=O), 171.3 (C=O), 170.8 (C=O), 170.5 (C=O), 166.6 (C=O), 156.9 (C=O, Cbz), 155.2 (C),136.9 (C), 136.6 (C), 135.3 (C), 129.5 (CH), 128.9 (C), 127.6 (CH), 127.7 (CH), 127.4 (CH), 127.3 (CH), 127.1 (CH), 126.9 (CH), 126.8 (CH), 114.5 (CH), 69.2 (CH₂Ph), 65.6 (CH₂Ph), 65.1 (CH₂Ph), 58.9 (CH, α-Pro), 53.2 (CH α-Leu or CH, α-Tyr), 53.1 (CH α-Leu or CH, α-Tyr), 47.8 (CH, α-Ala), 45.3 (CH₂, δ-Pro), 42.2 (CH₂, Gly), 40.9 (CH₂, β-Leu), 35.6 (CH₂, β-Tyr), 32.5 (CH₂, β-Pro or CH₂, γ-Pro), 28.5 (CH₂, β-Pro or CH₂, γ-Pro), 23.8 (CH, γ-Leu), 22.3 (CH₃, δ-Leu), 21.1 (CH₃, δ-Leu), 17.6 (CH₃, β-Ala). Note: CH₃, β-Tyr, β-Pro and γ-Pro peaks assigned by HSQC correlations; two aromatic CH signals not observed; vₘₐₓ (neat) = 3285, 3064, 2955, 1717, 1268, 1509, 1174, 695 cm⁻¹; MS (ESI⁺) m/z 856 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₄₇H₃₈N₅O₇ [M+Na⁺] 856.3892, found 856.3903; [α]D²²  –52.6 (c 0.30, CHCl₃).

H-Leu-Ala-Gly-Pro-Tyr-OH (111)

Following general procedure 5 from Cbz-Leu-Ala-Gly-Pro-Tyr(Bn)-OBn (439) (1.00 g, 1.19 mmol, 1.0 equiv), H-Leu-Ala-Gly-Pro-Tyr-OH (111) was obtained as a pale pink solid (622 mg, 1.19 mmol, quant. yield) which required no further purification; m.p 172–175 °C; ¹H NMR (600 MHz, DMSO-d₆ @ 373 K) δH 7.02 (d, J = 8.2 Hz, 2H, ArH), 6.70 (d, J = 8.1 Hz, 2H, ArH), 4.51–4.35 (m, 4H, CH α-Tyr, CHα-Pro, CHα-Ala, CHHα-Gly), 3.87 (dd, J = 16.0, 4.3 Hz, 1H, CHHα-Gly), 3.77 (t, J = 6.9 Hz, 1H, CHα-Leu), 3.52–3.41 (m, 2H, CH₂δ-Pro), 2.99 (dd, J = 13.0, 3.8 Hz, 1H, CHHβ-Tyr), 2.91–2.83 (m, 1H, CHHβ-Tyr), 1.96–1.73 (m, 5H, CHy-Leu, CHβ-Pro, CH₂γ-Pro), 1.72–1.64 (m, 1H, CHHβ-Leu), 1.64–1.56 (m, 1H, CHHβ-Leu), 1.34 (d, J = 7.0 Hz, 3H, CH₃β-Ala), 0.96–0.90 (d, J = 6.6 Hz, 6H, 2 × δ-CH₃ Leu); ¹³C NMR (151 MHz, DMSO-d₆ @ 373 K) δc 171.8 (C=O), 171.0 (C=O), 170.5 (C=O), 168.0 (C=O), 166.6 (C=O), 155.5 (C), 129.3 (CH), 127.0 (C), 114.7 (CH), 58.9 (CH, α-Pro), 53.2 (CH, α-Tyr), 51.0 (CH, α-Leu), 48.2 (CH, α-Ala), 45.6 (CH₂, δ-Pro) 40.9 (CH₂, Gly), 39.6 (CH₂, β-Leu), 35.7 (CH₂, β-Tyr), 23.1 (CH, γ-Leu), 21.9 (CH₃, δ-Leu), 21.5 (CH₃, δ-Leu), 17.3 (CH₃, β-Ala). Note: β-CH₂, Pro and γ-CH₂, Pro signals are not observed; vₘₐₓ (neat) = 3217, 2961, 1639, 1513, 1445, 1336, 1224 cm⁻¹; MS (ESI⁺) m/z 520 [M+H⁺], 542 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₂₅H₃₈N₅O₇ [M+H⁺] 520.2766, found 520.2768; [α]D²²  –49.5 (c 0.09, MeOH).
Cyclo(Leu-Ala-Gly-Pro-Tyr) (107)

Following general procedure 6, H-Leu-Ala-Gly-Pro-Tyr-OH (111) (52 mg, 0.10 mmol, 1.0 equiv) was cyclised using PyBOP (104 mg, 0.20 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO2, CH2Cl2/MeOH 9:1→4:1) to give the cyclic pentapeptide (107) as a colourless glassy solid (12 mg, 23 μmol, 23%). Rf (CH2Cl2/MeOH 9:1) 0.28; m.p 178–181 °C; 1H NMR (500 MHz, CD3OD) δH 7.09 (d, J = 8.4 Hz, 2H, ArH), 6.72 (d, J = 8.4 Hz, 2H, ArH), 4.65 (t, J = 8.1 Hz, 1H, CHα-Tyr), 4.46 (q, J = 7.0 Hz, 1H, CHα-Ala), 4.30 (dt, J = 8.7, 4.3 Hz, 1H, CHα-Pro), 4.16 (d, J = 14.6 Hz, 1H, CHH Gly), 4.09–4.03 (m, 1H, CHHδ-Pro), 3.90 (dd, J = 10.2, 5.9 Hz, 1H, CHα-Leu), 3.69–3.62 (m, 1H, CHHδ-Pro), 3.57 (d, J = 14.6 Hz, 1H, CHH Gly), 3.07–2.97 (m, 2H, CHβ-Tyr), 2.22–2.13 (m, 1H, CHHγ-Pro), 2.02–1.88 (m, 3H, CHHβ-Leu, CHβ-Pro), 1.79–1.71 (m, 1H, CHHγ-Pro), 1.68–1.64 (m, 1H, CHγ-Leu), 1.54–1.51 (m, 1H, CHHβ-Leu), 1.37 (d, J = 7.1 Hz, 3H, CHβ-Ala), 0.92 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.83 (d, J = 6.5 Hz, 3H, CHδ-Leu); 13C NMR (126 MHz, CD3OD) δc 176.1 (C=O), 175.0 (C=O), 174.1 (C=O), 173.4 (C=O), 170.7 (C=O), 175.4 (C), 131.3 (CH), 128.8 (C), 116.2 (CH), 62.9 (CH, α-Pro), 57.5 (CH, α-Leu), 56.5 (CH, α-Tyr), 50.1 (CH, α-Ala), 48.5 (CH2, δ-Pro), 43.0 (CH2, Gly), 39.7 (CH2, β-Leu), 37.3 (CH2, β-Tyr), 30.7 (CH2, β-Pro or CH2, γ-Pro), 25.8 (CH, γ-Leu), 25.4 (CH2, β-Pro or CH2, γ-Pro), 23.3 (CH3, δ-Leu), 21.7 (CH3, δ-Leu), 18.3 (CH3, β-Ala); νmax (neat) = 3268, 2956, 2930, 1641, 1514, 1233 cm−1; MS (ESI+) m/z 524 [M+Na]+; HRMS (ESI+) calcd. for C25H33N3NaO6[M+Na]+ 524.2480, found 524.2479; [α]D22 −88.1 (c 0.08, MeOH).

Preparation of cyclic tetrapeptides 175, 181–183

Boc-Leu-GOx-Gly-OBn (171)

Following general procedure 4 from NO2-GOx-Gly-OBn (12) (1.70 g, 6.06 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, EtOAc/PE 3:2) to give tripeptide Boc-Leu-GOx-Gly-OBn (171) as a colourless viscous oil (1.91 g, 4.13 mmol, 68%); Rf (EtOAc/PE 3:2) 0.28; 1H NMR (500 MHz, CDCl3) δH 7.40–7.32 (m, 5H, ArH), 6.73 (br. t, J = 5.0 Hz, 1H, NH), 5.17 (s, 2H, CH2Ph), 4.93 (br. s, 1H, NH), 4.44 (m, 2H, OCH2-Ox), 4.36 (d, J = 7.0 Hz, 2H, OCH2-Ox), 4.07 (br. m, 1H, CHα-Leu), 3.65–3.57 (m, 2H, CH2GOx), 3.54–3.48 (m, 2H, CH2Gly), 2.00 (br. s, 1H, NH), 1.70–1.61 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.48–1.44 (1H, m, CHHβ-Leu), 1.42 (s, 9H, 3 × CH3, Boc), 0.94 (d, J = 1.7 Hz, 3H, CH3δ-Leu), 0.93 (d, J = 1.7 Hz, 3H, CH3δ-Leu); 13C NMR (125 MHz, CDCl3) δc
173.4 (C=O), 172.5 (C=O), 155.7 (C=O, Boc), 135.2 (C), 128.7 (CH), 128.6 (CH), 128.5 (CH), 79.3 (2 × OCH₂), 67.1 (CH₂, Bn), 59.6 (C, Ox), 53.3 (CH, α-Leu), 44.0 (CH₂, Gly), 43.2 (CH₂, GOx), 41.3 (CH₂, β-Leu), 28.3 (CH₃, Boc), 24.9 (CH, γ-Leu), 20.8 (2 × CH₃, δ-Leu); νmax (neat) = 2956, 2871, 1739, 1656, 1520, 1164 cm⁻¹; MS (ESI⁺) m/z 464 [M+H]⁺, 486 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₃₇N₃NaO₆ [M+Na]⁺ 486.2575, found 486.2572; [α]D⁰ +4.4 (c 0.14, CHCl₃).

**Chz-D-Pro-Leu-GOx-Gly-OBn (439)**

Following general procedure 1 from tripeptide 171 (1.15 g, 2.48 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH, 40:1) to give tetrapeptide Cbz-D-Pro-Leu-GOx-Gly-OBn (439) (1.03 g, 1.73 mmol, 70%) as a colourless viscous oil. Rf (CH₂Cl₂/MeOH 49:1) 0.22; ¹H NMR (600 MHz, DMSO-d₆ @ 373 K) δH 7.69 (s, 1H, NH), 7.48 (s, 1H, NH), 7.39–7.27 (m, 10H, ArH), 5.15 (s, 2H, CH₂Ph), 5.11–5.02 (m, 2H, CH₂Ph), 4.35 (d, J = 6.2 Hz, 2H, OCH₂-Ox), 4.30 (td, J = 8.9, 5.3 Hz, 1H, CH₃-Leu), 4.28–4.24 (m, 3H, OCH₂-Ox, CHα-Pro), 3.51–3.42 (m, 6H, CH₂δ-Pro, CH₂GOx, CH₂Gly), 2.16–2.08 (m, 1H, CHHβ-Pro or CHHγ-Pro), 1.92–1.78 (m, 3H, CHHβ-Pro or CHHγ-Pro, CHβ-Pro or CHβγ-Pro), 1.65–1.54 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.52–1.47 (m, 1H, CHHβ-Leu), 0.88 (d, J = 6.4 Hz, 3H, CH₃δ-Leu), 0.84 (d, J = 6.4 Hz, 3H, CH₃δ-Leu). Note: Secondary amine NH not observed; ¹³C NMR (151 MHz, DMSO-d₆ @ 373 K) δC 172.0 (C=O), 171.5 (C=O), 171.3 (C=O, Cbz), 136.5 (C), 135.6 (C), 127.8 (CH), 127.7 (CH), 127.4 (CH), 127.3 (CH), 127.0 (CH), 126.7 (CH), 77.7 (2 × OCH₂), 65.6 (CH₃, Bn), 65.2 (CH₂, Bn), 59.7 (CH, α-Pro), 59.3 (C, Ox), 51.1 (CH, α-Leu), 46.4 (CH₂, δ-Pro or CH₂, Gly), 44.2 (CH₂, δ-Pro or CH₂, Gly), 42.5 (CH₂, GOx), 40.3 (CH₂, β-Leu), 29.8 (CH₂, β-Pro or CH₂, γ-Pro), 23.9 (CH, γ-Leu), 23.0 (CH₂, β-Pro or CH₂, γ-Pro), 22.3 (CH₂, δ-Leu), 21.1 (CH₃, δ-Leu); νmax (neat) = 3307, 2954, 1738, 1655, 1529, 1171, 737 cm⁻¹; MS (ESI⁺) m/z 595 [M+H]⁺, 617 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₄₂NaNO₇ [M+Na]⁺ 617.2946, found 617.2955; [α]D⁰ +6.7 (c 0.11, CHCl₃).

**H-D-Pro-Leu-GOx-Gly-OH (173)**

Following general procedure 5 from Cbz-D-Pro-Leu-GOx-Gly-OBn (439) (440 mg, 0.74 mmol, 1.0 equiv), H-D-Pro-Leu-GOx-Gly-OH (173) was obtained as a white solid (276 mg, 0.74 mmol, quant. yield) which required no further purification; m.p 67–70 °C; ¹H NMR (500 MHz, D₂O) δH 4.68 (d, J = 7.6 Hz, 1H, OCHH-Ox), 4.67 (d, J = 7.6 Hz, 1H, OCHH-Ox), 4.59 (d, J = 7.7 Hz, 1H, OCHH-Ox), 4.56 (d, J = 7.7 Hz, 1H, OCHH-Ox), 4.46–4.42 (m, 1H,
CHα-Pro), 4.39–4.35 (m, 1H, CHα-Leu), 3.78 (d, J = 14.7 Hz, 1H, CHH Gly or CHHGOx), 3.69 (d, J = 14.7 Hz, 1H, CHH Gly or CHH GOx), 3.47–3.38 (m, 4H, CH2 Gly or CH2 GOx, CH2δ-Pro), 2.52–2.43 (m, 1H, CHHβ-Pro or CHHγ-Pro), 2.12–2.00 (m, 3H, CH3β-Pro or CH3γ-Pro, CHHβ-Pro or CHHγ-Pro), 1.72–1.61 (m, 3H, CH3β-Leu, CH3γ-Leu), 0.94 (d, J = 5.5 Hz, 3H, CH3δ-Leu), 0.90 (d, J = 5.5 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, D2O) δC 175.6 (C=O), 175.3 (C=O), 169.9 (C=O), 77.4 (OCH2), 77.2 (OCH2), 60.1 (C, Ox), 59.7 (CH, α-Pro), 53.0 (CH, α-Leu), 46.5 (CH2, GOx or CH2, Gly), 45.5 (CH2, GOx or CH2, Gly), 41.6 (CH2, δ-Pro), 39.6 (CH2, β-Leu), 29.8 (CH2, β-Pro or CH2, γ-Pro), 24.4 (CH, γ-Leu), 23.8 (CH2, β-Pro or CH2, γ-Pro), 22.1 (CH3, δ-Leu), 20.4 (CH3, δ-Leu); \( \nu_{\text{max}} \) (neat) = 3258, 2956, 1655, 1561, 1174, 719 cm\(^{-1} \); MS (ESI\(^+\)) \( m/z \) 371 [M+H]\(^+\), 393 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C17H31N4O5 [M+H]\(^+\) 371.2289, found 371.2283; [α]\(^D\)\(^{25} \) +162 (c 0.03, MeOH).

**Cyclo(D-Pro-Leu-GOx-Gly) (175)**

Following general procedure 6, H-D-Pro-Leu-GOx-Gly-OH (173) (74 mg, 0.20 mmol, 1.0 equiv) was cyclised using DEPBT (120 mg, 0.40 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 19:1→9:1) to give the cyclic tetrapeptide 175 as a white solid (38 mg, 108 μmol, 54%). \( \text{Rf} \) (CH\(_2\)Cl\(_2\)/MeOH 19:1) 0.28; m.p 71–73 °C; \(^1\)H NMR (500 MHz, CD\(_3\)OD) δH 4.72 (d, J = 6.7 Hz, 1H, OCHH-Ox), 4.57 (d, J = 6.9 Hz, 1H, OCHH-Ox), 4.46–4.37 (m, 3H, OCHH-Ox, CHα-Pro, CHα-Leu), 4.33 (d, J = 6.7 Hz, 1H, OCHH-Ox), 3.78 (d, J = 13.9 Hz, 1H, CHHGOx), 3.66–3.52 (m, 2H, CH2δ-Pro), 3.50–3.38 (m, 3H, CHHGOx, CH2 Gly), 2.35–2.24 (m, 1H, CHHβ-Pro or CHHγ-Pro), 2.05–1.86 (m, 3H, CH3β-Pro or CH3γ-Pro, CHHβ-Pro or CHHγ-Pro), 1.72–1.63 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.60–1.51 (m, 1H, CHHβ-Leu), 0.98 (d, J = 6.2 Hz, 3H, CH3δ-Leu), 0.93 (d, J = 6.1 Hz, 3H, CH3δ-Leu); \(^13\)C NMR (126 MHz, CD\(_3\)OD) δC 176.3 (C=O), 175.8 (C=O), 173.1 (C=O), 80.3 (OCH2), 79.2 (OCH2), 62.1 (CH, α-Pro), 62.0 (C, Ox), 53.9 (CH, α-Leu), 48.8 (CH2, δ-Pro), 48.5 (CH2, Gly), 47.2 (CH2, GOx), 38.3 (CH2, β-Leu), 33.0 (CH2, γ-Pro), 26.1 (CH, γ-Leu), 23.4 (CH2, β-Pro), 23.0 (CH3, δ-Leu), 22.6 (CH3, δ-Leu); \( \nu_{\text{max}} \) (neat) = 3274, 2954, 1657, 1539, 1173, 970 cm\(^{-1} \); MS (ESI\(^+\)) \( m/z \) 353 [M+H]\(^+\), 375 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{17}\)H\(_{28}\)N\(_4\)NaO\(_4\) [M+Na]\(^+\) 375.2003, found 375.2006; [α]\(^D\)\(^{25} \) –132 (c 0.06, MeOH).
Cbz-Trp-Leu-GOx-Gly-OBn (440)

Following general procedure 1 from tripeptide 171 (1.29 g, 2.79 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\(_2\), 49:1 CH\(_2\)Cl\(_2\)/MeOH) to give tetrapeptide Cbz-Trp-Leu-GOx-Gly-OBn (440) (834 mg, 1.22 mmol, 44%) as a colourless viscous oil. \(R_t\) (CH\(_2\)Cl\(_2\)/MeOH 49:1) 0.31; \(^1\text{H NMR}\) (500 MHz, CDCl\(_3\)) \(\delta_H\) ppm 8.45 (s, 1H, NH), 7.62 (d, \(J = 7.3\) Hz, 1H, ArH), 7.41–7.27 (m, 11H, ArH), 7.17 (t, \(J = 7.5\) Hz, 1H, ArH), 7.08 (t, \(J = 7.1\) Hz, 1H, ArH), 7.02 (s, 1H, ArH), 5.13 (s, 2H, CH\(_2\)Ph), 5.06 (s, 2H, CH\(_2\)Ph), 4.53 (q, \(J = 6.5\) Hz, 1H, CH\(\alpha\)-Trp), 4.40–4.32 (m, 3H, CH\(\alpha\)-Leu, OCH\(_2\)-Ox), 4.31–4.25 (m, 2H, OCH\(_2\)-Ox), 3.54–3.41 (m, 4H, CH\(_2\)Gly, CH\(_2\)GOx), 3.30–3.16 (m, 2H, CH\(_2\)β-Trp), 1.64–1.52 (m, 1H, CH\(\beta\)-Leu), 1.47–1.29 (m, 2H, CH\(\gamma\)-Leu), 0.82 (d, \(J = 6.3\) Hz, 3H, CH\(_3\)δ-Leu), 0.81 (d, \(J = 6.3\) Hz, 3H, CH\(_3\)δ-Leu); \(^{13}\text{C NMR}\) (126 MHz, CDCl\(_3\)) \(\delta_C\) ppm 172.7 (C=O), 172.5 (C=O), 171.5 (C=O), 156.3 (C=O, Cbz), 136.3 (C), 136.1 (C), 135.3 (C), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.1 (CH), 127.3 (C), 123.5 (CH), 122.3 (CH), 119.8 (CH), 118.8 (CH), 111.4 (CH), 110.0 (C), 79.1 (OCH\(_2\)), 79.0 (OCH\(_2\)), 67.2 (CH\(_2\), Bn), 67.1 (CH\(_2\), Bn), 59.6 (C, OMe), 55.6 (CH, α-Trp), 52.2 (CH, α-Leu), 44.7 (CH\(_2\), GOx or CH\(_2\), Gly), 43.4 (CH\(_2\), GOx or CH\(_2\), Gly), 40.7 (CH\(_2\), β-Leu), 28.1 (CH\(_2\), β-Trp), 24.7 (CH, γ-Leu), 22.8 (CH\(_3\), δ-Leu), 21.9 (CH\(_3\), δ-Leu); \(\nu_{\text{max}}\) (neat) = 3316, 2952, 1707, 1645, 1510, 1173, 738 cm\(^{-1}\); MS (ESI\(^+\)) \text{m/z} 684 [M+H]\(^+\), 706 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{38}\)H\(_{45}\)N\(_5\)NaO\(_7\) [M+Na]\(^+\) 706.3211, found 706.3211; \([\alpha]_D^{26}\) –23.6 (c 0.14, CHCl\(_3\)).

H-Trp-Leu-GOx-Gly-OH (178)

Following general procedure 5 from Cbz-Trp-Leu-GOx-Gly-OBn (440) (683 mg, 1.00 mmol, 1.0 equiv), H-Trp-Leu-GOx-Gly-OH (178) was obtained as a yellow solid (458 mg, 1.00 mmol, quant. yield) which required no further purification; m.p 260–263 °C; \(^1\text{H NMR}\) (500 MHz, D\(_2\)O) \(\delta_H\) ppm 7.64 (d, \(J = 7.9\) Hz, 1H, ArH), 7.53 (d, \(J = 8.2\) Hz, 1H, ArH), 7.32 (s, 1H, ArH), 7.28 (t, \(J = 7.6\) Hz, 1H, ArH), 7.18 (t, \(J = 7.5\) Hz, 1H, ArH), 4.72–4.67 (m, 2H, OCH\(_2\)-Ox), 4.56 (d, \(J = 8.2\) Hz, 1H, OCH\(_2\)-Ox), 4.52 (d, \(J = 8.2\) Hz, 1H, OCH\(_2\)-Ox), 4.32 (t, \(J = 7.3\) Hz, 1H, CHα-Trp), 4.27 (dd, \(J = 9.0\), 5.8 Hz, 1H, CHα-Leu), 3.63–3.53 (m, 4H, CH\(_2\)Gly, CH\(_2\)GOx), 3.43 (dd, \(J = 14.7\), 7.9 Hz, 1H, CH\(\beta\)-Trp), 3.36 (dd, \(J = 15.0\), 6.5 Hz,
Following general procedure 6, H-Trp-Leu-GOx-Gly-OH (178) (46 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.10 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→9:1) to give cyclic tetrapeptide (181) as a yellow solid (29 mg, 65 µmol, 65%); Rᵣ (CH₂Cl₂/MeOH 9:1) 0.41; m.p 200–203 °C; [α]DB +9.7 (c 0.06, MeOH).

Cyclo(Trp-Leu-GOx-Gly) (181)

1H, CHHβ-Trp), 1.61–1.34 (m, 3H, CH₃β-Leu, CH₇-Leu), 0.87 (d, J = 6.4 Hz, 3H, CH₃, δ-Leu), 0.83 (d, J = 6.4 Hz, 3H, CH₃, δ-Leu); ¹³C NMR (126 MHz, D₂O) δc 174.5 (C=O), 169.3 (C=O), 136.2 (C), 126.5 (C), 125.2 (CH), 122.1 (CH), 119.5 (CH), 118.0 (CH), 112.0 (CH), 106.3 (C), 75.9 (2 × OCH₃), 60.6 (C, Ox), 53.5 (CH, α-Trp), 52.6 (CH, α-Leu), 45.1 (CH₂, GOx or CH₂, Gly), 40.6 (CH₂, GOx or CH₂, Gly), 40.0 (CH₂, β-Leu), 26.7 (CH₂, β-Trp), 24.1 (CH, γ-Leu), 21.9 (CH₃, δ-Leu), 20.8 (CH₃, δ-Leu). Note: One carbonyl signal not observed; νmax (neat) = 3231, 2951, 1653, 1522, 1167, 741 cm⁻¹; MS (ESI⁺) m/z 460 [M+H], 482 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₃₅N₅O₅ [M+H]⁺ 460.2554, found 460.2557; [α]DB +0.01, MeOH).
Chz-Asp(Bu)-Leu-GOx-Gly-OBn (441)

Following general procedure 1 from tripeptide 171 (487 mg, 1.05 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH, 25:1) to give tetrapeptide Chz-Asp(Bu)-Leu-GOx-Gly-OBn (441) (413 mg, 0.62 mmol, 59%) as a colourless oil; Rf (CH₂Cl₂/MeOH 25:1) 0.41; ¹H NMR (500 MHz, CDCl₃) δ nuclei ppm 7.39–7.31 (m, 10H, ArH), 6.86 (br. t, 1H, NH), 6.79 (d, J = 8.0 Hz, 1H, NH), 5.93 (d, J = 8.0 Hz, 1H, NH), 5.16 (s, 2H, CH₂Ph), 5.11 (s, 2H, CH₂Ph), 4.50 (dd, J = 13.0, 6.1 Hz, 1H, CHα-Asp), 4.46–4.33 (m, 5H, CH α-Leu, 2 × OCH₂-Ox), 3.68–3.59 (m, 1H, CHHGOx), 3.51 (m, 3H, CHHGOx, CH₂Gly), 2.86 (dd, J = 17.0, 4.5 Hz, 1H, CHHβ-Asp), 2.67 (dd, J = 17.0, 6.5 Hz, 1H, CHHβ-Asp), 1.77–1.69 (m, 1H, CHHβ-Asp), 1.65–1.58 (m, 1H, CHγ-Leu), 1.56–1.49 (m, 1H, CHHβ-Asp), 1.42 (s, 9H, 3 × CH₃), 0.91 (d, J = 6.5 Hz, 3H, CH₃δ-Leu), 0.88 (d, J = 6.4 Hz, 3H, CH₃δ-Leu). Note: Amine NH not observed; ¹³C NMR (126 MHz, CDCl₃) δ nuclei ppm 172.8 (C=O), 172.4 (C=O), 171.2 (C=O), 170.7 (C=O), 156.2 (C=O, Cbz), 135.9 (C), 135.3 (C), 128.7 (CH), 128.64 (CH), 128.57 (CH), 128.5 (CH), 128.4 (CH), 128.2 (CH), 82.1 (C, tBu), 79.2 (2 × OCH₂), 67.4 (CH₂, Bn), 67.1 (CH₂, Bn), 59.6 (C, Ox), 52.1 (CH, α-Asp), 51.5 (CH, α-Leu), 44.8 (CH₂, Gly), 43.3 (CH₂, GOx), 40.6 (CH₂, β-Leu), 36.9 (CH₂, β-Asp), 28.0 (CH, tBu), 24.7 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 21.7 (CH₃, δ-Leu); νmax (neat) = 3310, 2955, 1716, 1526, 1150, 696 cm⁻¹; MS (ESI⁺) m/z 669 [M+H]⁺, 691 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₅₀H₄₆N₄NaO₉ [M+Na]⁺ 691.3313, found 691.3301; [α]D²⁶ −23.0 (c 0.12, MeOH).

H-Asp(Bu)-Leu-GOx-Gly-OH (179)

Following general procedure 5 from Chz-Asp(Bu)-Leu-GOx-Gly-OBn (441) (384 mg, 0.57 mmol, 1.0 equiv), H-Asp(Bu)-Leu-GOx-Gly-OH (179) was obtained as a white solid (255 mg, 0.57 mmol, quant. yield); m.p 92–94 °C; ¹H NMR (500 MHz, CD₃OD) δ nuclei ppm 4.56–4.51 (m, 2H, OCH₂-Ox), 4.47–4.42 (m, 2H, OCH₂-Ox), 4.32 (dd, J = 9.2, 5.2 Hz, 1H, CHα-Leu), 4.10–4.03 (m, 1H, CHα-Asp), 3.70 (d, J = 14.2 Hz, 1H, CHHGOx), 3.60 (d, J = 14.2 Hz, 1H, CHHGOx), 3.34–3.31 (m, 2H, CH₂Gly), 2.99 (dd, J = 17.6, 4.3 Hz, 1H, CHHβ-Asp), 2.78 (dd, J = 17.6, 7.6 Hz, 1H, CHHβ-Asp), 1.71–1.63 (m, 3H, CHββ-Leu, CHγ-Leu), 1.49 (s, 9H, 3 × CH₃, tBu), 0.97 (d, J = 5.9 Hz, 3H, CH₃δ-Leu), 0.93 (d, J = 5.9 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, CD₃OD) δ nuclei ppm 177.1 (C=O), 175.0 (C=O), 171.4 (C=O), 171.3 (C=O), 83.4 (C, tBu), 79.6 (OCH₂), 79.5 (OCH₂), 61.7 (C, Ox), 54.1 (CH, α-Leu), 51.6 (CH, α-Asp), 47.0
(CH₂, Gly), 43.4 (CH₂, GOx), 41.3 (CH₂, β-Leu), 37.9 (CH₂, β-Asp), 28.3 (CH₃, tBu), 25.9 (CH, γ-Leu), 23.5 (CH₃, δ-Leu), 21.7 (CH₂, δ-Leu); vₘₐₓ (neat) = 3302, 1721, 1648, 1533, 1367, 1152, 975 cm⁻¹; MS (ESI⁺) m/z 445 [M+H]⁺, 467 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₀H₃₇Na₃O₇ [M+H]⁺ 445.2657, found 445.2657; [α]D³⁶ +6.8 (c 0.11, MeOH).

**Cyclo(Asp(Bu)-Leu-GOx-Gly) (182)**

Following general procedure 6, H-Asp(Bu)-Leu-GOx-Gly-OH (179) (89 mg, 0.20 mmol, 1.0 equiv) was cyclised using DEPBT (120 mg, 0.40 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→9:1) to give the cyclic tetrapeptide (182) as a white solid (1st run: 41.5 mg, 97 μmol, 49%; 2nd run: 42.7 mg, 100 μmol, 50%). Rₜ (CH₂Cl₂/MeOH 12:1) 0.33; m.p 134–137 °C; ¹H and ¹³C NMR data reported for the major conformer: ¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.02 (d, J = 10.6 Hz, 1H, NH), 7.57 (d, J = 8.7 Hz, 1H, NH), 7.37–7.33 (m, 1H, NH), 4.54 (dd, J = 18.7, 8.7 Hz, 1H, CHα-Asp), 4.39–4.37 (m, 1H, OCHH-Ox), 4.16–4.12 (m, 2H, OCHH-Ox, OCHH-Ox), 4.00 (td, J = 10.1, 5.1 Hz, 1H, CHα-Leu), 3.88 (d, J = 7.0 Hz, 1H, OCHH-Ox), 3.82 (dd, J = 13.5, 8.0 Hz, 1H, CHHGOx), 3.37–3.34 (m, 1H, CHHgly), 3.24 (d, J = 14.4 Hz, 1H, CHHgly), 2.94 (d, J = 13.5 Hz, 1H, CHHGOx), 2.78 (dd, J = 15.8, 8.9 Hz, 1H, CHHβ-Asp), 2.62 (dd, J = 15.8, 8.2 Hz, 1H, CHHβ-Asp), 1.65–1.59 (m, 1H, CHγ-Leu), 1.56–1.50 (m, 1H, CHHβ-Leu), 1.49–1.43 (m, 1H, CHHβ-Asp), 1.36 (s, 9H, 3 × CH₃, tBu), 0.91 (d, J = 6.5 Hz, 3H, CH3δ-Leu), 0.84–0.81 (m, 3H, CH3δ-Leu). Note: Amine NH not observed; ¹³C NMR (126 MHz, DMSO-d₆) δ ppm 173.0 (C=O), 172.8 (C=O), 170.0 (C=O), 168.6 (C=O), 80.5 (C, tBu), 78.0 (OCH₂), 76.0 (OCH₂), 60.3 (C, Ox), 54.1 (CH, α-Asp), 51.8 (CH, α-Leu), 44.2 (CH₂, GOx), 39.2 (CH₂, β-Leu), 36.7 (CH₂, β-Asp), 27.6 (CH₃, tBu), 24.6 (CH, γ-Leu), 22.8 (CH₃, δ-Leu), 21.3 (CH₃, δ-Leu). Note: CH₂, β-Leu overlaps with solvent peak; vₘₐₓ (neat) = 3308, 2956, 1726, 1656, 1520, 1153, 971 cm⁻¹; MS (ESI⁺) m/z 449 [M+Na]⁺, 875 [2M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₀H₃₇Na₃O₇ [M+Na]⁺ 449.2371, found 449.2369; [α]D³⁶ –74.5 (c 0.07, MeOH).

**Cbz-Tyr(Bu)-Leu-GOx-Gly-OBn (442)**

Following general procedure 1 from tripeptide 171 (1.01 g, 2.18 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH, 25:1) to give tetrapeptide Cbz-Tyr(Bu)-Leu-GOx-Gly-OBn (442) (1.17 g, 1.63 mmol, 75%) as a white foam; Rₜ
(CH₂Cl₂/MeOH 25:1) 0.31; m.p 64 – 67 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.36 – 7.28 (m, 10H, ArH), 7.05 (d, J = 8.1 Hz, 2H, ArH), 6.89 (d, J = 8.3 Hz, 2H, ArH), 6.78 (s, 1H, NH), 6.41 (d, J = 7.8 Hz, 1H, NH), 5.26 (d, J = 7.0 Hz, 1H, NH), 5.15 (s, 2H, CH₂Ph), 5.05 (s, 2H, CH₂Ph), 4.47 – 4.32 (m, 6H, 2 × CH₂-Ox, CHα-Tyr, CHα-Leu), 3.58 (d, J = 4.6 Hz, 2H, CH₂GOx), 3.49 (s, 2H, CH₂Gly), 3.03 (d, J = 6.4 Hz, 2H, CH₂β-Tyr), 1.66 (m, 1H, CHHβ-Leu), 1.54 – 1.39 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.31 (s, 9H, CH₃-Bu), 0.88 (m, 6H, 2 × CH₃-δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δ 172.7 (C=O), 172.4 (C=O), 171.2 (C=O), 156.4 (C=O, Cbz), 154.7 (C), 136.0 (C), 135.4 (C), 130.8 (C), 129.9 (CH), 128.8 (CH), 128.7 (CH), 128.5 (CH), 128.5 (CH), 128.2 (CH), 124.5 (CH), 79.33 (CH₂, Ox), 79.26 (CH₂, Ox), 78.6 (C, 'Bu), 67.5 (CH₂Ph Bn), 67.2 (CH₂Ph Bn), 59.7 (C, Ox), 56.5 (CH, α-Tyr), 52.2 (CH, α-Leu), 44.8 (CH₃, Gly), 43.4 (CH₃, GOx), 40.9 (CH₂, β-Leu), 37.2 (CH₂, β-Tyr), 29.0 (CH₃, 'Bu), 24.9 (CH, γ-Leu), 23.0 (CH₃, δ-Leu), 21.9 (CH₃, δ-Leu); νmax (neat) = 3309, 2959, 1736, 1647, 1505, 1160 cm⁻¹; MS (ESI⁺) m/z 717 [M+H]+, 739 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₆H₆₅Na₂O₈ [M+Na]⁺ 739.3677, found 739.3681; [α]D³₀ –9.4 (c 0.31, MeOH)

**H-Tyr('Bu)-Leu-GOx-Gly-OH (180)**

![Structure of H-Tyr('Bu)-Leu-GOx-Gly-OH (180)](image)

Following general procedure 5 from Cbz-Tyr('Bu)-Leu-GOx-Gly-OBn (442) (885 mg, 1.24 mmol, 1.0 equiv), H-Tyr('Bu)-Leu-GOx-Gly-OH (180) was obtained as a white solid (609 mg, 1.24 mmol, quant. yield); m.p 101 – 103 °C; ¹H NMR (500 MHz, D₂O) δ 7.23 (d, J = 8.4 Hz, 2H, ArH), 7.07 (d, J = 8.4 Hz, 2H, ArH), 4.61 (d, J = 7.3 Hz, 2H, CH₂-Ox), 4.50 (d, J = 7.3 Hz, 2H, CH₂-Ox), 4.31 (dd, J = 8.9, 6.2 Hz, 1H, CHα-Leu), 4.17 (t, J = 7.0 Hz, 1H, CHα-Tyr), 3.66 (d, J = 14.4 Hz, 1H, CHHGOx), 3.58 (d, J = 14.4 Hz, 1H, CHHGOx), 3.31 (s, 2H, CH₂Gly), 3.18 (dd, J = 14.2, 7.2 Hz, 1H), 3.12 (dd, J = 14.2, 7.2 Hz, 1H), 1.63 – 1.45 (m, 3H, CH₂β-Leu, CHγ-Leu), 1.34 (s, 9H, CH₃-Bu), 0.90 (d, J = 6.5 Hz, 3H, CH₃-δ-Leu), 0.86 (d, J = 6.5 Hz, 3H, CH₃-δ-Leu); ¹³C NMR (126 MHz, D₂O) δ 177.9 (C=O), 174.3 (C=O), 169.9 (C=O), 153.2 (C), 130.2 (CH), 130.1 (C), 125.0 (CH), 81.0 (C, 'Bu), 78.4 (2 × CH₂, GOx), 59.4 (C, Ox), 54.4 (CH, α-Tyr), 52.8 (CH, α-Leu), 45.9 (CH₂, Gly), 42.3 (CH₂, Ox), 39.6 (CH₂, β-Leu), 36.5 (CH₂, β-Tyr), 27.8 (CH₃, 'Bu), 24.2 (CH, γ-Leu), 21.9 (CH₃, δ-Leu), 20.9 (CH₃, δ-Leu); νmax (neat) = 3282, 2975, 1651, 1566, 1159 cm⁻¹; MS (ESI⁺) m/z 493 [M+H]+, 515 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₅H₄₁NaO₆ [M+H]+ 493.3021, found 493.3024; [α]D³₀ –15.8 (c 0.19, MeOH).
Cyclo(Tyr(Bu)-Leu-GOx-Gly) (183)

Following general procedure 6, H-Tyr(Bu)-Leu-GOx-Gly-OH (180) (89 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.20 mmol, 2.0 equiv) at 5 mM for 24 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→9:1) to give the cyclic tetrapeptide (183) as a white solid (33 mg, 70 μmol, 70%); Rₜ (SiO₂, CH₂Cl₂/MeOH 19:1) 0.28; m.p 162 – 164 °C; ¹H NMR (500 MHz, DMSO) δ 8.16 (d, J = 10.3 Hz, 1H, NH), 7.93 (d, J = 9.1 Hz, 1H, NH), 7.42 (d, J = 3.3 Hz, 1H, NH), 7.12 (d, J = 8.1 Hz, 2H, ArH), 6.86 (d, J = 8.1 Hz, 2H, ArH), 4.46 (dd, J = 18.3, 8.9 Hz, 1H, CHα-Tyr), 4.38 (t, J = 9.0 Hz, 1H, CHH-Ox), 4.17 (dd, J = 6.8 Hz, 1H, CHH-Ox), 4.14 (dd, J = 6.8 Hz, 1H, CHH-Ox), 4.05 – 3.96 (m, 1H, CHα-Leu), 3.90 (d, J = 6.8 Hz, 1H, CHH-Ox), 3.74 (dd, J = 13.2, 7.1 Hz, 1H, CHHGOx), 3.19 (d, J = 14.3 Hz, 1H, CHH Gly), 3.02 (dd, J = 13.8, 8.1 Hz, 2H, CHHβ-Tyr, CHHGOx), 2.91 (dd, J = 14.1, 8.2 Hz, 1H, CHHβ-Tyr), 1.61 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.51 – 1.41 (m, 1H, CHHβ-Leu), 1.26 (s, 9H, CH₃Bu), 0.93 (d, J = 6.2 Hz, 3H, CH₃δ-Leu), 0.79 (d, J = 6.2 Hz, 3H, CH₃δ-Leu). Note: CHH Gly overlaps with solvent signal; ¹³C NMR (126 MHz, DMSO) δ 173.1 (C=O), 172.9 (C=O), 170.9 (C=O), 153.6 (C), 131.6 (C), 129.3 (CH), 123.5 (CH), 78.1 (CH₂Ox), 77.7 (C, Bu), 76.5 (CH₂Ox), 60.2 (C, Ox), 56.5 (CH, α-Tyr), 53.9 (CH, α-Leu), 47.2 (CH₂Gly), 44.2 (CH₂GOx), 39.1 (CH₂β-Leu), 35.5 (CH₂β-Tyr), 28.5 (CH₃, Bu), 24.7 (CH, γ-Leu), 22.9 (CH₃, δ-Leu), 21.1 (CH₃, δ-Leu); νmax (neat) = 3306, 2956, 1660, 1505, 1160 cm⁻¹; MS (ESI⁺) m/z 497 [M+Na]+, 971 [2M+Na]+; HRMS (ESI⁺) calcd. for C₂₅H₂₅N₂O₇ [M+H]+ 475.2915, found 475.2919; [α]D 30° – 69.5 (c 0.31, MeOH).

Preparation of cyclic tetrapeptides 184 and 185, and cyclic octapeptide 177 and 218

Boc-Leu-Gly-Gly-OBn (444)

Following general procedure 1 from dipeptide 443¹⁵³ (3.94 g, 10.0 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/EtOAc 1:1→EtOAc) to give Boc-Leu-Gly-Gly-OBn (444) (2.98 g, 6.84 mmol, 68%) as a white solid; Rₜ (CH₂Cl₂/EtOAc 1:1) 0.26; m.p 53–57 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.38–7.30 (m, 5H, ArH), 7.24 (s, 1H, NH), 7.06 (s, 1H, NH), 5.16 (s, 2H, CH₂Ph), 5.09 (d, J = 5.7 Hz, 1H, NH), 4.15–4.04 (m, 3H, CH₂Gly, CHα-Leu), 4.00 (dd, J = 18.1, 5.0 Hz, 1H, CHH Gly), 3.94 (dd, J = 16.8, 5.4 Hz, 1H, CHH Gly), 1.72–1.62 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.55–1.46 (m, 1H, CHHβ-Leu), 1.40 (s, 9H, 3 × CH₃, Boc), 0.94 (d, J = 6.4 Hz, 3H, CH₃δ-Leu), 0.92 (d, J = 6.4 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δC 173.5 (C=O), 169.7 (C=O), 162.0, 150.5, 116.0 cm⁻¹; MS (ESI⁺) m/z 475.2915, found 475.2919; [α]D 30° – 69.5 (c 0.31, MeOH).
169.6 (C=O), 156.3 (C=O, Boc), 135.3 (C), 128.8 (CH), 128.6 (CH), 128.5 (CH), 80.6 (C, Boc), 67.3 (CH₂, Bn), 53.8 (CH, α-Leu), 43.1 (CH₂, Gly), 41.4 (CH₂, Gly), 41.0 (CH₂, β-Leu), 28.4 (CH₃, Boc), 24.9 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 22.0 (CH₃, δ-Leu); ν max (neat) = 3306, 2957, 1746, 1654, 1518, 1163, 736 cm⁻¹; MS (ESI⁺) m/z 436 [M+H]⁺, 458 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₂H₃₄N₅NaO₆ [M+Na]⁺ 458.2268, found 458.2264; [α]₂⁵ D +2.4 (c 0.12, CHCl₃).

**Chz-D-Pro-Leu-Gly-Gly-OBn (445)**

Following general procedure 5 from tripeptide 444 (1.23 g, 2.84 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 38:1) to give Chz-D-Pro-Leu-Gly-Gly-OBn (445) (1.25 g, 2.21 mmol, 78%) as a white solid; Rf (CH₂Cl₂/MeOH 38:1) 0.20; m.p 146–149 °C; ¹H NMR (500 MHz, DMSO-d₆) δ₀ ppm 7.84 (s, 1H, NH), 7.79–7.65 (m, 2H, 2 × NH), 5.16 (s, 2H, CH₂Ph), 5.10 (d, J = 12.8 Hz, 1H, CHHPh), 5.05 (d, J = 12.8 Hz, 1H, CHHPh), 4.38–4.25 (m, 2H, CH₃-Pro, CH₃-Leu), 4.00–3.88 (m, 2H, CH₂Gly), 3.83–3.72 (m, 2H, CH₂Gly), 3.54–3.43 (m, 2H, CH₃δ-Pro), 2.21–2.11 (m, 1H, CHHγ-Pro), 1.96–1.87 (m, 2H, CHHβ-Pro, CHHβ-Pro), 1.87–1.78 (m, 1H, CHHβ-Pro), 1.70–1.50 (m, 3H, CH₃β-Leu, CH₃γ-Leu), 0.94–0.83 (d, 6H, J = 6.3 Hz, 2 × CH₃δ-Leu); ¹³C NMR (126 MHz, DMSO-d₆) δ₁ ppm 172.96 (C=O), 172.94 (C=O), 170.2 (C=O), 170.0 (C=O), 155.3 (C=O, Cbz), 137.9 (C), 136.9 (C), 129.2 (CH), 129.1 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 128.1 (CH), 67.0 (CH₂, Bn), 66.8 (CH₂, Bn), 61.1 (CH, α-Pro), 52.6 (CH₂, α-Leu), 47.8 (CH₂, δ-Pro), 43.1 (CH₂, Gly), 41.8 (CH₂, Gly), 41.5 (CH₂, β-Leu), 31.2 (CH₂, γ-Pro), 25.2 (CH, γ-Leu), 24.4 (CH₂, β-Pro), 23.7 (CH₃, δ-Leu), 22.5 (CH₃, δ-Leu); ν max (neat) = 3066, 3010, 1745, 1706, 1649, 1420, 1171, 694 cm⁻¹; MS (ESI⁺) m/z 589 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₀H₄₂N₅NaO₇ [M+Na]⁺ 589.2633, found 589.2637; [α]₂⁵ D +7.3 (c 0.16, MeOH).

**H-D-Pro-Leu-Gly-Gly-OH (448)**

Following general procedure 5 from Chz-D-Pro-Leu-Gly-Gly-OBn (445) (1.00 g, 1.77 mmol, 1.0 equiv), H-D-Pro-Leu-Gly-Gly-OH (448) was obtained as a white solid (604 mg, 1.77 mmol, quant. yield), which required no further purification; m.p 137–140 °C; ¹H NMR (500 MHz, D₂O) δ₀ ppm 4.47–4.38 (m, 2H, CHα-Pro, CHα-Leu), 4.00 (d, J = 17.0 Hz, 1H, CHHγ-Gly), 3.94 (d, J = 17.0 Hz, 1H, CHHγ-Gly), 3.83–3.75 (m, 2H, CH₂Gly), 3.49–3.37 (m, 2H, CH₂δ-Pro), 2.54–2.43 (m, 1H, CHHγ-Pro), 2.11–2.03 (m, 3H, CHHγ-Pro, CH₂β-Pro), 1.72–1.63 (m, 3H, CH₂β-Leu, CHγ-Leu), 0.95 (d, J = 5.6 Hz, 3H, CH₃δ-Leu), 0.90 (d, J = 5.6 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, D₂O) δ₁ ppm 176.3 (C=O),
175.0 (C=O), 170.9 (C=O), 170.0 (C=O), 59.7 (CH, α-Pro), 52.9 (CH, α-Leu), 46.5 (CH, δ-Pro), 43.1 (CH₂, Gly), 42.4 (CH₂, Gly), 39.5 (CH₂, β-Leu), 29.8 (CH₂, γ-Pro), 24.4 (CH, γ-Leu), 23.7 (CH₂, β-Pro), 22.1 (CH₃, δ-Leu), 20.5 (CH₃, δ-Leu); ν₁max (neat) = 3637, 3256, 1654, 1523, 1383, 1249, 668 cm⁻¹; MS (ESI⁺) m/z 365 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₁₅H₂₆Na₂O₅ [M+Na⁺] 365.1795, found 365.1797; [α]β⁺ = 9.3 (c 0.20, MeOH).

**Cyclo(D-Pro-Leu-Gly-Gly-D-Pro-Leu-Gly-Gly) (177)**

Following general procedure 6, H-D-Pro-Leu-Gly-Gly-OH (448) (68 mg, 0.20 mmol, 1.0 equiv) was cyclised using DEPBT (120 mg, 0.40 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 6:1→4:1) to give the cyclic octapeptide 177 as a white solid (1st run: 26.2 mg, 40 μmol, 20%; 2nd run (0.10 mmol scale): 12.9 mg, 20 μmol, 20%). Rₚ (CH₂Cl₂/MeOH 4:1) 0.31; m.p 206–207 °C (decomposition); ¹H NMR (500 MHz, DMSO-d₆) δH ppm 8.60 (d, J = 8.0 Hz, 2H, NH), 8.18 (t, J = 6.3 Hz, 2H, NH), 7.59 (t, J = 6.1 Hz, 2H, NH), 4.22 (t, J = 6.8 Hz, 2H, CHα-Pro), 4.06 (ddd, J = 11.4, 8.0, 3.2 Hz, 2H, CHα-Leu), 3.98 (dd, J = 17.0, 6.6 Hz, 2H, CHHGly), 3.92 (ddd, J = 16.6, 7.5 Hz, 2H, CHHGly), 3.78 (dd, J = 17.0, 5.5 Hz, 2H, CHHGly), 3.60–3.54 (m, 2H, CHHδ-Pro), 3.52–3.46 (m, 4H, CHHδ-Pro, CHHGly), 2.11–2.01 (m, 4H, CHHβ-Pro, CHHγ-Pro), 1.92–1.84 (m, 2H, CHHγ-Pro), 1.82–1.74 (m, 2H, CHHβ-Pro), 1.68–1.52 (m, 6H, CHβ-Leu, CHγ-Leu), 0.89 (d, J = 6.0 Hz, 6H, CHδ-Leu), 0.81 (d, J = 5.9 Hz, 6H, CHδ-Leu); ¹³C NMR (126 MHz, DMSO-d₆) δC ppm 172.4 (C=O), 172.2 (C=O), 169.9 (C=O), 167.4 (C=O), 60.3 (CH, α-Pro), 51.6 (CH, α-Leu), 46.0 (CH₂, δ-Pro), 41.9 (CH₂, Gly), 41.1 (CH₂, Gly), 28.5 (CH₂, γ-Pro), 25.1 (CH₂, β-Pro), 24.4 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 20.9 (CH₃, δ-Leu). Note: CH₂, β-Leu overlaps with DMSO solvent peak; ν₁max (neat) = 2955, 1738, 1681, 1630, 1544, 1241, 1160, 650 cm⁻¹; MS (ESI⁺) m/z 671 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₃₀H₄₆Na₂O₈ [M+Na⁺] 671.3487, found 671.3490.

**Cbz-Trp-Leu-Gly-Gly-OBn (446)**

Following general procedure 1 from tripeptide 444 (0.93 g, 2.15 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 40:1) to give Cbz-Trp-Leu-Gly-Gly-OBn (446) (1.21 g, 1.89 mmol, 88%) as a white solid; Rₚ (CH₂Cl₂/MeOH 40:1) 0.13; m.p 170–173 °C; ¹H NMR (500 MHz, CD₃CN) δH ppm 9.28 (s, 1H, NH), 7.60 (d, J = 7.8 Hz, 1H, ArH), 7.40–7.27 (m, 3.46 (m, 4H, CHα-Leu), 1.68–1.52 (m, 6H, CHβ-Leu, CHγ-Leu),
11H, ArH), 7.21 (t, J = 5.5 Hz, 1H, NH), 7.13–7.09 (m, 2H, ArH), 7.06–7.03 (m, 1H, ArH), 6.97 (s, 1H, NH), 6.01 (d, J = 6.8 Hz, 1H, NH), 5.12 (s, 2H, CH3Ph), 5.06–4.97 (m, 2H, CH3Ph), 4.42 (q, J = 7.0 Hz, 1H, CHα-Trp), 4.18 (m, 1H, CHα-Leu), 3.92 (dd, J = 5.8, 1.4 Hz, 2H, CH2Gly), 3.74 (dd, J = 17.0, 6.3 Hz, 1H, CHH Gly), 3.68 (dd, J = 17.0, 5.9 Hz, 1H, CHH Gly), 3.24 (dd, J = 14.7, 6.0 Hz, 1H, CHH β-Trp), 3.08 (dd, J = 14.6, 7.7 Hz, 1H, CHH β-Trp), 1.56–1.45 (m, 3H, CH2 β-Leu, CHγ-Leu), 0.87 (d, J = 6.0 Hz, 3H, CH3 δ-Leu), 0.82 (d, J = 6.0 Hz, 3H, CH3 δ-Leu);

13C NMR (126 MHz, CD3CN) δC 173.7 (C=O), 173.4 (C=O), 170.8 (C=O), 170.7 (C=O), 157.4 (C=O, Cbz), 138.0 (C), 137.4 (C), 137.1 (C), 129.52 (CH), 129.46 (CH), 129.2 (CH), 129.1 (CH), 128.9 (CH), 128.7 (CH), 128.4 (C), 124.9 (CH), 122.6 (CH), 120.1 (CH), 119.4 (CH), 112.4 (CH), 110.8 (C), 67.5 (CH2, Bn), 67.3 (CH2, Bn), 56.9 (CH, α-Trp), 53.5 (CH, α-Leu), 43.3 (CH2 Gly), 41.9 (CH2 Gly), 40.7 (CH2 β-Leu), 28.4 (CH2 β-Trp), 25.3 (CH γ-Leu), 23.3 (CH3 δ-Leu), 21.8 (CH3 δ-Leu); νmax (neat) = 3285, 2954, 1667, 1226, 740 cm−1; MS (ESI+) m/z 678 [M+Na]+; HRMS (ESI+) calcd. for C36H41N5NaO7 [M+Na]+ 678.2898, found 678.2901; [α]D26 +14.3 (c 0.7, MeOH).

H-Trp-Leu-Gly-Gly-OH (449)

Following general procedure 5 from Cbz-Trp-Leu-Gly-Gly-OBn (446) (900 mg, 1.37 mmol, 1.0 equiv), H-Trp-Leu-Gly-Gly-OH (449) was obtained as a white solid (590 mg, 1.37 mmol, quant. yield), which required no further purification; mp 210–213 °C; 1H NMR (500 MHz, D2O) δH 7.63 (d, J = 8.0 Hz, 1H, ArH), 7.52 (d, J = 8.2 Hz, 1H, ArH), 7.29 (s, J = 7.3 Hz, 1H, ArH), 7.26 (t, J = 7.6 Hz, 1H, ArH), 7.16 (t, J = 7.5 Hz, 1H, ArH), 4.34 (t, J = 7.1 Hz, 1H, CHα-Leu or CHα-Trp), 4.30 (t, J = 7.3 Hz, 1H, CHα-Leu or CHα-Trp), 3.89–3.70 (m, 4H, 2 × CH2 Gly), 3.44–3.35 (m, 2H, CH2 β-Leu), 1.57–1.47 (m, 3H, CH3 β-Leu, CHγ-Leu), 0.88 (d, J = 6.2 Hz, 3H, CH3 δ-Leu), 0.84 (d, J = 6.2 Hz, 3H, CH3 δ-Leu); 13C NMR (126 MHz, D2O) δC 174.9 (C=O), 173.8 (C=O), 170.9 (C=O), 169.4 (C=O), 136.1 (C), 126.5 (C), 125.2 (CH), 122.2 (CH), 119.6 (CH), 118.0 (CH), 112.0 (CH), 106.1 (C), 53.4 (CH, α-Leu or CH, α-Trp), 52.6 (CH, α-Leu or CH, α-Trp), 42.23 (CH2 Gly), 42.16 (CH2 Gly), 39.8 (CH2 β-Leu), 26.7 (CH2 β-Trp), 24.1 (CH γ-Leu), 21.9 (CH3 δ-Leu), 20.9 (CH3 δ-Leu); νmax (neat) = 3276, 2956, 1630, 1529, 741 cm−1; MS (ESI+) m/z 432 [M+H]+, 454 [M+Na]+; HRMS (ESI+) calcd. for C23H29N3NaO7 [M+Na]+ 454.2061, found 454.2054; [α]D27 +14.3 (c 0.07, MeOH).
Cyclo(Trp-Leu-Gly-Gly) (184)

Following general procedure 6, H-Trp-Leu-Gly-Gly-OH (449) (86 mg, 0.20 mmol, 1.0 equiv) was cyclised using DEPBT (120 mg, 0.40 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 92.5:7.5→4:1) to give cyclic tetrapeptide 184 as a yellow solid (1ˢᵗ run: 10.3 mg, 25 μmol, 13%; 2ⁿᵈ run: 13.0 mg, 31 μmol, 16%) and cyclic octapeptide 218 as a yellow solid (11.3 mg, 14 μmol, 7%).

Analytical data for cyclic tetrapeptide 184: Rᵣ (CH₂Cl₂/MeOH 9:1) 0.24; m.p 255–257 °C; NMR data reported for the major conformational isomer at 298 K.

$^1$H NMR (500 MHz, DMSO-δ6) δH
10.89 (s, 1H, NH), 8.53 (dd, $J = 7.9, 4.3$ Hz, 1H, NH), 8.38 (t, $J = 9.8$ Hz, 2H, 2 × NH), 8.18 (s, 1H, NH), 7.56 (d, $J = 7.8$ Hz, 1H, ArH), 7.33 (d, $J = 8.0$ Hz, 1H, ArH), 7.12 (s, 1H, ArH), 7.07 (t, $J = 7.5$ Hz, 1H, ArH), 6.98 (t, $J = 7.4$ Hz, 1H, ArH), 4.43 (dd, $J = 17.2, 8.6$ Hz, 1H, CHα-Trp), 4.21 (dd, $J = 17.1, 8.4$ Hz, 1H, CHα-Leu), 3.77 (dd, $J = 14.2, 8.8$ Hz, 1H, CHHGly), 3.58 (dd, $J = 14.0, 4.0$ Hz, 1H, CHβ-Trp), 3.07 (dd, $J = 14.6, 8.8$ Hz, 1H, CHHGly), 1.62‒1.54 (m, 3H, CHγ-Leu, CHβ-Leu), 0.90 (d, $J = 6.2$ Hz, 3H, CHδ-Leu), 0.83 (d, $J = 6.1$ Hz, 3H, CHδ-Leu). Note: CHγ-Leu overlaps with DMSO solvent peak.

$^{13}$C NMR (126 MHz, DMSO-δ6) δC 172.7 (C=O), 172.0 (C=O), 169.9 (C=O), 169.4 (C=O), 136.1 (C), 127.1 (C), 123.3 (CH), 120.9 (CH), 118.3 (CH), 118.1 (CH), 111.4 (CH), 109.7 (C), 56.8 (CH, α-Trp), 5.37 (CH, α-Leu), 43.8 (CH₂, Gly), 43.5 (CH₂, Gly), 39.6 (CH₂, β-Leu), 26.8 (CH₂, β-Trp), 24.6 (CH, γ-Leu), 22.4 (CH₃, δ-Leu), 22.0 (CH₃, δ-Leu); $ν_{max}$ (neat) = 3286, 3057, 1650, 1530, 739 cm⁻¹; MS (ESI⁺) m/z 436 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₁H₂₇N₅O₄ [M+Na]⁺ 436.1955, found 436.1954; $[^{\alpha}]D_{30}$ −207 (c 0.05, DMF).

Cyclo(Trp-Leu-Gly-Gly-Trp-Leu-Gly-Gly) (218)

Analytical data for cyclic octapeptide 218: Rᵣ (CH₂Cl₂/MeOH 9:1) 0.20; m.p 216–219 °C; $^1$H NMR (500 MHz, CD₃OD) δH 7.59 (d, $J = 7.9$ Hz, 2H, ArH), 7.36 (d, $J = 8.1$ Hz, 2H, ArH), 7.20 (s, 2H, ArH), 7.11 (t, $J = 7.5$ Hz, 2H, ArH), 7.02 (t, $J = 7.5$ Hz, 2H, ArH), 4.55 (dd, $J = 8.2, 5.2$ Hz, 2H, CHα-Trp), 4.20 (dd, $J = 10.6, 4.4$ Hz, 2H, CHα-Leu), 4.01 (d, $J = 16.8$ Hz, 2H, CHHGly), 3.86 (d, $J = 16.1$ Hz, 2H, CHHGly), 3.74 (dd, $J = 16.1$ Hz, 2H, CHHGly).
CHH(Gly), 3.39–3.34 (m, 2H, CHHβ-Trp), 3.23 (dd, J = 14.9, 8.3 Hz, 2H, CHHβ-Trp), 1.73–1.65 (m, 2H, CHHβ-Leu), 1.54 (dd, J = 13.9, 9.6, 4.5 Hz, 2H, CHHβ-Leu), 1.36–1.26 (m, 2H, CHγ-Leu), 0.84 (d, J = 6.6 Hz, 6H, CH3δ-Leu), 0.78 (d, J = 6.6 Hz, 6H, CH3δ-Leu); 13C NMR (126 MHz, CD3OD) δc 175.7 (C=O), 174.9 (C=O), 173.0 (C=O), 172.6 (C=O), 138.1 (C), 128.6 (C), 124.7 (CH), 122.6 (CH), 120.0 (CH), 119.2 (CH), 112.4 (CH), 110.5 (C), 56.8 (CH, α-Trp), 54.0 (CH, α-Leu), 44.1 (CH2, Gly), 43.4 (CH2, Gly), 40.6 (CH2, β-Leu), 27.9 (CH2, β-Trp), 25.7 (CH, γ-Leu), 23.7 (CH3, δ-Leu), 21.6 (CH3, δ-Leu); νmax (neat) = 3298, 2869, 1645, 1522, 1234, 742 cm⁻¹; MS (ESI⁺) m/z 849 [M+Na]⁺; HRMS (ESI⁺) calcd. for C35H40N10NaO6 [M+Na]⁺ 849.4018, found 849.4020; [α]D27 −16.6 (c 0.04, MeOH).

Cbz-Asp(Bu)-Leu-Gly-Gly-OBn (447) Following general procedure 1 from tripeptide 444 (1.09 g, 2.50 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, CH2Cl2/MeOH 32:1) to give tetrapeptide 447 (1.49 g, 2.33 mmol, 93%) as a white solid; Rf (CH2Cl2/MeOH 32:1) 0.35; m.p 136–139 °C; 1H NMR (500 MHz, CDCl3) δ1H ppm 7.39–7.28 (m, 11H, ArH, NH), 7.08 (t, J = 5.3 Hz, 1H, NH), 6.92 (d, J = 6.7 Hz, 1H, NH), 5.92 (d, J = 8.1 Hz, 1H, NH), 5.13 (s, 2H, CH2Ph), 5.11 (s, 2H, CH2Ph), 4.49 (dd, J = 12.3, 7.1 Hz, 1H, CHα-Asp), 4.35–4.28 (m, 1H, CHα-Leu), 4.09 (dd, J = 18.0, 5.6 Hz, 1H, CHHGly), 4.01 (dd, J = 18.0, 5.3 Hz, 1H, CHHGly), 3.98–3.91 (m, 2H, CH2Gly), 2.80 (dd, J = 17.0, 4.4 Hz, 1H, CHHβ-Asp), 2.74 (dd, J = 17.0, 6.9 Hz, 1H, CHHβ-Leu), 1.77–1.53 (m, 3H, CH2β-Leu, CHγ-Leu), 1.40 (s, 9H, 3 × CH3, tBu), 0.93 (d, J = 6.4 Hz, 3H, CH3δ-Leu), 0.88 (d, J = 6.4 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, CDCl3) δc ppm 172.3 (C=O), 171.7 (C=O), 171.4 (C=O), 169.8 (C=O), 169.5 (C=O), 156.4 (C=O, Cbz), 136.0 (C), 135.4 (C), 128.74 (CH), 128.73 (CH), 128.60 (CH), 128.58 (CH), 128.5 (CH), 128.3 (CH), 82.4 (C, tBu), 67.6 (CH2, Bn), 67.2 (CH2, Bn), 53.0 (CH, α-Asp), 51.6 (CH, α-Leu), 43.1 (CH2, Gly), 41.3 (CH2, Gly), 40.2 (CH2, β-Leu), 37.1 (CH2, β-Asp), 28.1 (CH3, tBu), 24.9 (CH, γ-Leu), 23.1 (CH3, δ-Leu), 21.7 (CH3, δ-Leu); νmax (neat) = 3283, 2955, 1736, 1714, 1631, 1522, 1204, 697 cm⁻¹; MS (ESI⁺) m/z 663 [M+Na]⁺; HRMS (ESI⁺) calcd. for C33H36N6NaO6 [M+Na]⁺ 663.3000, found 663.3006; [α]D56 −16.3 (c 0.16, MeOH).

H-Asp(Bu)-Leu-Gly-Gly-OH (450) Following general procedure 5 from Cbz-Asp(Bu)-Leu-Gly-Gly-OBn (447) (1.07 g, 1.67 mmol, 1.0 equiv), H-Asp(Bu)-Leu-GoX-Gly-OH (450) was obtained as a white solid (692 mg, 1.67 mmol, quant.
yield), which required no further purification; \textbf{m.p} 172–175 °C; \textit{\textsuperscript{1}H NMR} (500 MHz, CD\textsubscript{3}OD) \(\delta\) ppm 4.32 (t, \(J = 7.4\) Hz, 1H, Ch\alpha-Leu), 4.15 (dd, \(J = 8.3, 4.1\) Hz, 1H, Ch\alpha-Asp), 3.99 (d, \(J = 16.9\) Hz, 1H, CHH\textsuperscript{Gly}), 3.83–3.75 (m, 3H, CH\textsubscript{2}Gly, CHH\textsuperscript{Gly}), 3.01 (dd, \(J = 18.0, 4.1\) Hz, 1H, CHH\beta-Asp), 2.84 (dd, \(J = 18.0, 8.4\) Hz, 1H, CHH\beta-Asp), 1.77–1.59 (m, 3H, CH\beta-Leu, CH\gamma-Leu), 1.48 (s, 9H, 3 \times CH\textsubscript{3}, tBu), 0.98 (d, \(J = 6.3\) Hz, 3H, CH\delta-Leu), 0.95 (d, \(J = 6.2\) Hz, 3H, CH\delta-Leu); \textit{\textsuperscript{13}C NMR} (126 MHz, CD\textsubscript{3}OD) \(\delta\) ppm 175.6 (C=O), 174.7 (C=O), 171.3 (C=O), 171.1 (C=O), 170.9 (C=O), 83.6 (C, tBu), 54.3 (CH, \alpha-Leu), 51.1 (CH, \alpha-Asp), 44.0 (CH\textsubscript{2} Gly), 43.5 (CH\textsubscript{2} Gly), 41.1 (CH\textsubscript{2}, \beta-Leu), 37.5 (CH\textsubscript{2}, \beta-Asp), 28.3 (CH\textsubscript{3}, tBu), 25.9 (CH, \gamma-Leu), 23.3 (CH\textsubscript{3}, \delta-Leu), 22.0 (CH\textsubscript{3}, \delta-Leu); \(\nu\text{max} \text{(neat)} = 3279, 2957, 1724, 1630, 1520, 1153, 674 \text{ cm}^{-1};\) \textbf{MS} (ESI\textsuperscript{+}) \(m/z\) 417 [M+H\textsuperscript{+}], 439 [M+Na\textsuperscript{+}]; \textbf{HRMS} (ESI\textsuperscript{+}) calcd. for C\textsubscript{18}H\textsubscript{32}N\textsubscript{4}NaO\textsubscript{7} [M+Na\textsuperscript{+}] 439.2163, found 439.2167; [\(\alpha\)]\textsubscript{D}\textsuperscript{56} +15.9 (c 0.07, MeOH).

\textbf{Cyclo(Asp(Bu)-Leu-Gly-Gly) (185)}

Following general procedure 6, H-Asp(Bu)-Leu-Gly-Gly-OH (450) (208 mg, 0.50 mmol, 1.0 equiv) was cyclised using DEPBT (299 mg, 1.00 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/MeOH 9:1 → 4:1) to give the cyclic tetrapeptide 185 as a white solid (1\textsuperscript{st} run: 27.2 mg, 68 \mu mol, 14%; 2\textsuperscript{nd} run (0.20 mmol scale): 10.5 mg, 26 \mu mol, 13%); \textbf{R\textsubscript{f}} (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 9:1) 0.24; \textbf{m.p} 202–205 °C; \textit{\textsuperscript{1}H NMR} (500 MHz, CD\textsubscript{3}OD) \(\delta\) ppm 4.36 (dd, \(J = 8.4, 4.4\) Hz, 1H, Ch\alpha-Leu), 4.36 (dd, \(J = 11.5, 3.6\) Hz, 1H, Ch\alpha-Asp), 4.04 (d, \(J = 16.7\) Hz, 1H, CHH\textsuperscript{Gly}), 3.90 (d, \(J = 15.8\) Hz, 1H, CHH\textsuperscript{Gly}), 3.73 (d, \(J = 15.8\) Hz, 1H, CHH\textsuperscript{Gly}), 3.69 (d, \(J = 16.7\) Hz, 1H, CHH\textsuperscript{Gly}), 2.85 (dd, \(J = 16.7, 4.4\) Hz, 1H, CHH\beta-Asp), 2.72 (dd, \(J = 16.8, 8.4\) Hz, 1H, CHH\beta-Asp), 1.96–1.87 (m, 1H, CHH\beta-Leu), 1.81–1.70 (m, 1H, CHH\gamma-Leu), 1.60–1.51 (m, 1H, CHH\delta-Leu), 1.45 (s, 9H, 3 \times CH\textsubscript{3}, tBu), 0.95 (d, \(J = 6.6\) Hz, 3H, CH\delta-Leu), 0.89 (d, \(J = 6.5\) Hz, 3H, CH\delta-Leu); \textit{\textsuperscript{13}C NMR} (126 MHz, CD\textsubscript{3}OD) \(\delta\) ppm 175.4 (C=O), 171.9 (C=O), 171.3 (C=O), 169.9 (C=O), 81.2 (C, tBu), 52.7 (CH, \alpha-Asp), 50.7 (CH, \alpha-Leu), 43.1 (CH\textsubscript{2} Gly), 41.8 (CH\textsubscript{2} Gly), 40.3 (CH\textsubscript{2}, \beta-Leu), 36.1 (CH\textsubscript{2}, \beta-Asp), 26.9 (CH\textsubscript{3}, tBu), 24.5 (CH, \gamma-Leu), 22.6 (CH\textsubscript{3}, \delta-Leu), 20.1 (CH\textsubscript{3}, \delta-Leu); \(\nu\text{max} \text{(neat)} = 3292, 2956, 2930, 1647, 1526, 1151 \text{ cm}^{-1};\) \textbf{MS} (ESI\textsuperscript{+}) \(m/z\) 421 [M+Na\textsuperscript{+}]; \textbf{HRMS} (ESI\textsuperscript{+}) calcd. for C\textsubscript{18}H\textsubscript{30}N\textsubscript{4}NaO\textsubscript{6} [M+Na\textsuperscript{+}] 421.2058, found 421.2061; [\(\alpha\)]\textsubscript{D}\textsuperscript{56} +15.9 (c 0.09, MeOH).
Preparation of cyclic hexapeptide 201

Fmoc-Thr(tBu)-OCumyl (194)

To sodium hydride (60% dispersion in mineral oil, 84 mg, 2.50 mmol, 0.5 equiv) in Et₂O (5.0 mL) at 0 °C was added freshly distilled 2-phenyl-2-propanol 193 (1.50 g, 11.0 mmol, 2.2 equiv) in Et₂O (5.5 mL) and stirred at room temperature for 1 h. The mixture was cooled to 0 °C, trichloroacetonitrile (1.44 mL, 10.0 mmol, 2.0 equiv) was added, the reaction mixture stirred at room temperature for 3 h and then concentrated in vacuo. Petroleum ether (2.25 mL) and MeOH (100 μL, 0.5 equiv) were added and stirred at room temperature for 10 min. The mixture was filtered through a plug of Celite eluting with PE. The eluent was concentrated under reduced pressure to give the crude imidate. To the crude imidate in CH₂Cl₂ (10 mL) was added Fmoc-Thr(tBu)-OH (1.99 g, 5.00 mmol, 1.0 equiv) and the reaction mixture stirred at room temperature for 16 h. The mixture was filtered through a plug of Celite, the crude product was concentrated in vacuo and purified by column chromatography (PET ether/EtOAc 4:1) to yield 194 as a waxy solid (2.45 g, 4.75 mmol, 95%).

**Rf** (PET ether/EtOAc 4:1) 0.28; **1H NMR** (500 MHz, CDCl₃) δH 7.77 (d, J = 7.5 Hz, 2H, ArH), 7.63 (d, J = 7.1 Hz, 2H, ArH), 7.45–7.38 (m, 4H, ArH), 7.36–7.27 (m, 4H, ArH), 7.28–7.22 (m, 1H, ArH), 5.56 (d, J = 9.2 Hz, 1H, NH), 4.45–4.36 (m, 2H, CH₂-Fmoc), 4.29–4.22 (m, 3H, CH-Fmoc, CHα-Thr, CHβ-Thr), 1.82 (d, J = 4.4 Hz, 6H, 2 × CH₃, cumyl), 1.24–1.17 (m, 12H, CH₃γ-Thr, 3 × CH₃, tBu); **13C NMR** (126 MHz, CDCl₃) δC 169.9 (C=O), 156.7 (C=O, Fmoc), 145.5 (C), 144.2 (C), 144.0 (C), 128.4 (CH), 127.8 (CH), 127.29 (CH), 127.20 (CH), 127.18 (CH), 125.3 (CH), 124.7 (CH), 120.1 (CH), 83.4 (C, cumyl), 74.2 (C, tBu), 67.4 (CH, α-Thr), 67.2 (CH₂, Fmoc), 60.4 (CH, β-Thr), 47.4 (CH, Fmoc), 28.9 (CH₃, tBu), 28.7 (CH₃, cumyl), 28.4 (CH₃, cumyl), 20.6 (CH₃, γ-Thr). Note: One aromatic C and one aromatic CH signal not observed. **νmax** (neat) = 2975, 1720, 1497, 1196, 1031, 738 cm⁻¹; **MS** (ESI⁺) m/z 516 [M+H]+, 538 [M+Na]+; **HRMS** (ESI⁺) calcd. for C₃₂H₃₇NNaO₅ [M+Na]+ 538.2564, found 538.2569; [α]D²⁷ +20.0 (c 0.10, CHCl₃).

NO₂-GOx-Thr'(Bu)-OCumyl (196)

To a solution of Fmoc-Thr(Bu)-OCumyl (194) (2.22 g, 4.30 mmol, 1.0 equiv) in CH₂Cl₂ (4.3 mL) was added diethylamine (4.3 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 50 mL) and concentrated under reduced pressure to give the crude amine. This was added to the nitroalkene formed in-situ following general procedure 3. The crude residue was purified by column chromatography (SiO₂, PET ether/EtOAc...
to yield 196 (1.35 g, 3.31 mmol, 77%) as a highly viscous yellow oil. Rf (PET ether/EtOAc 4:1) 0.20; 1H NMR (500 MHz, CDCl3) δH 7.47 (d, J = 7.7 Hz, 1H, ArH), 7.36 (t, J = 7.7 Hz, 1H, ArH), 7.31–7.25 (m, 3H, ArH), 4.77 (s, 2H, CH2GOx), 4.60–4.55 (m, 2H, OCH2Ox), 4.51 (d, J = 7.2 Hz, 1H, OCHH-Ox), 4.32 (d, J = 7.2 Hz, 1H, OCHH-Ox), 3.89 (quint, J = 6.2 Hz, 1H, CHβ-Thr), 3.36 (dd, J = 9.8, 4.6 Hz, 1H, CHβ-Thr), 2.50 (d, J = 9.9 Hz, 1H, NH), 1.86 (s, 3H, CH3, cumyl), 1.80 (s, 3H, CH3, cumyl), 1.22 (s, 9H, 3 × CH2Bu), 1.13 (d, J = 6.2 Hz, 3H, CH3γ-Thr); 13C NMR (126 MHz, CDCl3) δC 172.6 (C=O), 145.0 (C), 128.2 (CH), 127.4 (CH), 124.7 (CH), 83.0 (C, cumyl), 79.0 (OCH2), 78.9 (OCH2), 78.6 (CH2, GOx), 74.0 (C, Bu), 68.9 (CH, α-Thr), 61.5 (CH, β-Thr), 59.5 (C, Ox), 28.8 (CH3, cumyl), 28.5 (CH3, Bu), 27.6 (CH3, cumyl), 19.4 (CH3, γ-Thr); νmax (neat) = 2975, 2934, 1724, 1554, 1271, 1194, 980, 763 cm⁻¹; MS (ESI⁺) m/z 431 [M+Na⁺]; HRMS (ESI⁺) calcd. for C31H32N6NaO6 [M+Na⁺] 431.2153, found 431.2144; [α]D27 +33.0 (c 0.10, CHCl3).

Following general procedure 4 from NO2-GOx-Thr(Bu)-OCumyl (196) (1.24 g, 3.00 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, CH2Cl2/EtOAc 4:1) to give tripeptide Fmoc-Val-GOx-Thr(Bu)-OCumyl (197) as a white solid (1.11 g, 1.59 mmol, 53%); m.p 74–76 °C; Rf (CH2Cl2/EtOAc 4:1) 0.34; 1H NMR (500 MHz, CDCl3) δH 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.59 (d, J = 7.4 Hz, 2H, ArH), 7.40 (m, 4H, ArH), 7.31 (m, 4H, ArH), 7.23 (m, 1H, ArH), 6.54–6.43 (t, J = 4.8 Hz, 1H, NH), 5.47 (d, J = 8.7 Hz, 1H, NH), 4.42 (dd, J = 10.3, 7.4 Hz, 1H, CHH-Fmoc), 4.36–4.28 (m, 3H, OCH2-Ox, CHH-Fmoc), 4.20 (m, 3H, OCH2-Ox, CHFmoc), 4.00–3.95 (dd, J = 7.9, 6.4 Hz, 1H, CHα-Val), 3.94–3.88 (m, 1H, CHα-Thr), 3.80 (dd, J = 13.8, 5.8 Hz, 1H, CHHGOx), 3.32 (dd, J = 13.8, 4.1 Hz, 1H, CHHGOx), 3.19 (d, J = 4.0 Hz, 1H, CHβ-Thr), 2.00 (m, 1H, CHβ-Val), 1.84 (s, 3H, CH3, cumyl), 1.74 (s, 3H, CH3, cumyl), 1.22–1.16 (m, 12H, CH3γ-Thr, 3 × CH3, Bu), 0.90 (d, J = 6.6 Hz, 3H, CH3γ-Val), 0.86 (d, J = 6.6 Hz, 3H, CH3γ-Val); 13C NMR (126 MHz, CDCl3) δC 173.9 (C=O), 171.8 (C=O, Fmoc), 145.0 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 124.8 (CH), 120.11 (CH), 83.2 (C, cumyl), 80.5 (OCH2), 79.4 (OCH2), 74.2 (C, Bu), 68.9 (CH, α-Thr), 67.1 (CH2, Fmoc), 61.9 (CH, β-Thr), 60.4 (CH, α-Val), 59.2 (C, Ox), 47.3 (CH, Fmoc), 44.1 (CH2, GOx), 31.5 (CH, β-Val), 29.4 (CH3, cumyl), 28.8 (CH3, Bu), 27.2 (CH3, cumyl), 20.4 (CH3, γ-Val), 19.3 (CH3, γ-Val), 17.9 (CH3, γ-Val).

Note: One aromatic C and two aromatic CH signals not observed; νmax (neat) = 2971, 2873, 1721, 1658, 1466, 1237, 759 cm⁻¹; MS (ESI⁺) m/z 700 [M+H⁺], 722 [M+Na⁺]; HRMS (ESI⁺) calcd. for C41H35N7NaO7 [M+Na⁺] 722.3776, found 722.3779; [α]D27 +25.0 (c 0.10, CHCl3).
at room temperature for 85 min. The mixture was concentrated under reduced pressure, the resulting residue was repeatedly re-dissolved in CH₂Cl₂ (50 mL) and the solvent was removed under reduced pressure. To the crude acid in CH₂Cl₂ (34 mL) was added H-Ph-OBn-HCl (1.10 g, 3.70 mmol, 1.1 equiv), NMM (1.51 mL, 13.7 mmol, 5.0 equiv), HOBr·H₂O (0.51 g, 3.70 mmol, 1.1 equiv) and EDC·HCl (0.72 g, 3.70 mmol, 1.1 equiv). The reaction mixture was allowed to stir for 18 h at room temperature under an atmosphere of nitrogen. The mixture was diluted with EtOAc (250 mL) and washed with brine (3 × 150 mL), dried (Na₂SO₄) and concentrated in vacuo to afford a yellow oil which was purified by flash column chromatography (CH₂Cl₂/MeOH 49:1).

Fmoc-Val-GOx-Thr(tBu)-Phe-OBn (198) was obtained as a white solid in 73% yield (2.03 g, 2.48 mmol). Rᵣ(CH₂Cl₂/MeOH 49:1) 0.31; m.p 68–70 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.81–7.74 (m, 3H, NH, 2 × ArH), 7.63–7.56 (m, 2H, ArH), 7.43–7.27 (m, 10H, ArH), 7.24–7.17 (m, 2H, ArH), 7.11–7.01 (m, 3H, NH, 2 × ArH), 5.51 (d, J = 8.9 Hz, 1H, NH), 5.22–5.09 (m, 2H, CH₂Ph), 4.91 (dt, J = 8.1, 5.9 Hz, 1H, CHα-Phe), 4.47–4.38 (m, 2H, OCH₂-Ox, CHF-Fmoc), 4.36–4.20 (m, 5H, CHF-Fmoc, CH-Fmoc, OCH₂-Ox, OCH₂-Ox), 4.08–4.02 (m, 1H, CHα-Val), 3.92 (dd, J = 13.9, 7.3 Hz, 1H CHHGOx), 3.57–3.50 (m, 1H, CHβ-Thr), 3.21–3.07 (m, 2H, CHHGOx, CHHβ-Phe), 2.95–2.89 (m, 2H, CHβ-Val, CHβ-Thr, CHβ-Phe), 2.41 (br. s, 1H, NH), 2.11 (dq, J = 13.2, 6.4 Hz, 1H, CHβ-Val), 1.08 (s, 9H, 3 × CH₃, tBu), 1.00–0.96 (m, 6H, CH₃γ-Thr, CH₃γ-Val), 0.93 (d, J = 6.6 Hz, 3H, CH₃δ-Val); ¹³C NMR (126 MHz, CDCl₃) δC 173.3 (C=O), 172.3 (C=O), 172.1 (C=O), 156.5 (C=O, Fmoc), 144.0 (C), 141.4 (C), 136.2 (C), 135.1 (C), 129.3 (CH), 128.8 (CH), 128.73 (CH), 128.70 (CH), 128.6 (CH), 127.9 (CH), 127.24 (CH), 127.23 (CH), 125.3 (CH), 125.2 (CH), 120.2 (CH), 120.1 (CH), 80.1 (OCH₃), 79.0 (OCH₃), 74.8 (C, tBu), 69.8 (CH, α-Thr), 67.6 (CH₂, Bn), 67.2 (CH₂, Fmoc), 61.8 (CH, β-Thr), 60.6 (CH, α-Val), 60.3 (C, Ox), 53.3 (CH, α-Phe), 47.3 (CH, Fmoc), 44.1 (CH₂, GOx), 38.2 (CH₂, β-Phe), 31.2 (CH, β-Val), 28.4 (CH₃, tBu), 19.5 (CH₃, γ-Val), 18.7 (CH₃, γ-Thr), 18.1 (CH₃, γ-Val). Note: Two aromatic C and two aromatic CH signals not observed; νmax (neat) = 2968, 1722, 1659, 1514, 1449, 1187, 1076, 738 cm⁻¹; MS (ESI⁺) m/z 819 [M+H]⁺, 841 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₉H₸₆Na₃O₈ [M+Na]⁺ 841.4147, found 841.4134; [α]D²⁷ +21.5 (c 0.10, CHCl₃).
Fmoc-Tyr(tBu)-Val-GOx-Thr(tBu)-Phe-OBn (199)

Following general procedure 2 from tetrapeptide Fmoc-Val-GOx-Thr(tBu)-Phe-OBn (198) (1.40 g, 1.71 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 49:1) to give pentapeptide Fmoc-Tyr(tBu)-Val-GOx-Thr(tBu)-Phe-OBn (199) (1.65 g, 1.59 mmol, 94%) as a white solid. 

**Rᶠ** (CH₂Cl₂/MeOH 49:1) 0.23; **m.p** 89–91 °C; **¹H NMR** (400 MHz, CDCl₃) δ H 7.80 (d, J = 8.1 Hz, 1H, NH), 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.56–7.49 (m, 2H, ArH), 7.44–7.20 (m, 12H, ArH), 7.13–6.96 (m, 5H, 4 × ArH, NH), 6.90 (d, J = 8.2 Hz, 2H, ArH), 6.55 (d, J = 7.8 Hz, 1H, NH), 5.29 (d, J = 6.5 Hz, 1H, NH), 5.22–5.08 (m, 2H, CH₃Ph), 4.97–4.86 (m, 1H, CHα-Phe), 4.49–4.18 (m, 9H, CH-Fmoc, CH₂-Fmoc, 2 × OCH₂-Ox, CHα-Tyr, CHα-Val), 3.90–3.79 (m, 1H, C HGOx), 3.67–3.58 (m, 1H, CHα-Thr), 3.22–2.98 (m, 6H, CHHGOx, CHβ-Thr, CHβ-Tyr, CHβ-Phe), 2.47 (s, 1H, NH), 2.15–2.02 (m, 1H, CHβ-Val), 1.31 (s, 9H, 3 × CH₃, tBu), 1.13 (s, 9H, 3 × CH₃, tBu), 1.02 (d, J = 6.2 Hz, 3H, CH₃γ-Thr), 0.89 (d, J = 6.7 Hz, 3H, CH₃γ-Val), 0.81 (d, J = 6.7 Hz, 3H, CH₃γ-Val); **¹³C NMR** (126 MHz, CDCl₃) δC 173.3 (C=O), 172.0 (C=O), 171.5 (C=O), 171.1 (C=O), 156.1 (C=O, Fmoc), 154.6 (C), 143.9 (C), 143.8 (C), 141.4 (C), 136.1 (C), 135.1 (C), 129.3 (CH), 128.78 (CH), 128.76 (CH), 128.7 (CH), 128.6 (CH), 127.9 (CH), 127.3 (CH), 127.2 (CH), 125.20 (CH), 125.15 (CH), 124.5 (CH), 123.0 (CH), 120.1 (CH), 79.9 (OCH₂), 79.0 (OCH₂), 78.6 (C, tBu), 74.9 (C, tBu), 69.8 (CH, α-Thr), 67.5 (CH₂, Bn), 67.2 (CH₂, Fmoc), 61.6 (CH, β-Thr), 60.2 (C, Ox), 58.9 (CH, α-Val), 56.3 (CH, α-Tyr), 53.4 (CH, α-Phe), 47.2 (CH, Fmoc), 44.3 (CH₂, GOx), 38.2 (CH₂, β-Tyr), 37.5 (CH₂, β-Phe), 30.9 (CH, β-Val), 29.0 (CH₃, tBu), 28.5 (CH₃, tBu), 19.4 (CH₃, γ-Val), 18.7 (CH₃, γ-Thr), 18.1 (CH₃, γ-Val). Note: Two aromatic C and three aromatic CH signals not observed; νmax (neat) = 2971, 2927, 1733, 1644, 1505, 1232, 1160, 757 cm⁻¹; **MS** (ESI⁺) m/z 1038 [M+H]⁺, 1060 [M+Na]⁺; **HRMS** (ESI⁺) calcd. for C₆₁H₇₃N₅NaO₁₀ [M+Na]⁺ 1060.5406, found 1060.5381; [α]D²⁷ +19.5 (c 0.10, CHCl₃).
Following general procedure 2 from tetrapeptide Fmoc-Tyr(tBu)-Val-GOX-Thr(tBu)-Phe-OBn (199) (1.80 g, 1.73 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 49:1) to give hexapeptide Cbz-Leu-Tyr(tBu)-Val-GOX-Thr(tBu)-Phe-OBn (451) (1.37 g, 1.29 mmol, 74%) as a white solid. 

$R_f$ (CH$_2$Cl$_2$/MeOH 49:1) 0.16; m.p 146–148 ºC; 

$^1$H NMR (500 MHz, CDCl$_3$) δH 7.80 (d, 1H, $J$ = 8.1 Hz, NH), 7.39–7.25 (m, 10H, ArH), 7.25–7.21 (m, 3H, ArH), 7.14–7.04 (m, 5H, 4 × ArH, NH), 6.88 (d, $J$ = 8.3 Hz, 2H, ArH), 6.75 (d, $J$ = 7.6 Hz, 1H, NH), 6.68 (d, $J$ = 6.5 Hz, 1H, NH), 5.16 (d, $J$ = 12.2 Hz, 1H, CHPh), 5.11–5.04 (m, 3H, CH$_2$Ph, NH), 4.98 (d, $J$ = 12.2 Hz, 1H, CHPh), 4.89 (dd, $J$ = 13.8, 7.5 Hz, 1H, CH$\alpha$-Tyr), 4.60–4.52 (m, 1H, CH$\alpha$-Phe), 4.42 (d, $J$ = 6.4 Hz, 1H, OCH$\alpha$-OX), 4.34 (d, $J$ = 6.4 Hz, 1H, OCH$\alpha$-OX), 4.32–4.25 (m, 3H, OCH$_2$-OX, CH$\alpha$-Val), 4.08–4.02 (m, 1H, CH$\alpha$-Leu), 3.81 (dd, $J$ = 13.9, 7.0 Hz, 1H, CH$\alpha$-Thr), 3.22–3.00 (m, 6H, CH$\beta$-GOx, CH$\beta$-Thr, CH$\gamma$-Val, 2 × CH$\gamma$-Leu); $^1$C NMR (126 MHz, CDCl$_3$) δC 173.4 (C=O), 172.7 (C=O), 171.8 (C=O), 171.6 (C=O), 171.1 (C=O), 156.4 (C=O, Cbz), 154.6 (C), 154.6 (C), 154.6 (C), 136.1 (C), 136.0 (C), 135.2 (C), 131.3 (C), 129.8 (CH), 129.3 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 127.3 (CH), 124.5 (CH), 79.8 (OCH$_2$), 79.1 (OCH$_2$), 78.5 (C, tBu), 74.8 (C, tBu), 70.0 (CH, $\alpha$-Thr), 67.5 (CH$_2$, Bn), 67.4 (CH$_2$, Bn), 61.4 (CH, $\beta$-Thr), 60.2 (C, Ox), 59.1 (CH, $\alpha$-Val), 55.4 (CH, $\alpha$-Phe), 54.1 (CH, $\alpha$-Leu), 53.4 (CH, $\alpha$-Tyr), 44.3 (CH$_2$, GOx), 41.0 (CH$_2$, $\beta$-Leu), 38.2 (CH$_2$, $\beta$-Phe), 36.6 (CH$_2$, $\beta$-Tyr), 30.3 (CH, $\beta$-Val), 29.0 (CH$_3$, tBu), 28.5 (CH$_3$, tBu), 24.8 (CH, $\gamma$-Leu), 23.0 (CH$_3$, $\delta$-Leu), 21.9 (CH$_3$, $\delta$-Leu), 19.5 (CH$_3$, $\gamma$-Val), 18.4 (CH$_3$, $\gamma$-Thr), 17.8 (CH$_3$, $\gamma$-Val).

Note: Two aromatic CH signals not observed; $\nu_{max}$ (neat) = 2969, 2932, 1739, 1694, 1532, 1505, 1232, 1161, 696 cm$^{-1}$; MS (ESI$^+$) $m/z$ 1063 [M+H]$^+$, 1085 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{60}$H$_{82}$N$_6$NaO$_{11}$ [M+Na]$^+$ 1085.5934, found 1085.5936; $[\alpha]_D^{27}$ +13.5 (c 0.10, CHCl$_3$).
H-Leu-Tyr('Bu)-Val-GOx-Thr('Bu)-Phe-OH (200)

Following general procedure 5 from Cbz-Leu-Tyr('Bu)-Val-GOx-Thr('Bu)-Phe-OBn (451) (1.33 g, 1.25 mmol, 1.0 equiv), H-Leu-Tyr('Bu)-Val-GOx-Thr('Bu)-Phe-OH (200) was obtained as a white solid (1.04 g, 1.25 mmol, quant. yield) which required no further purification; m.p 143–145 °C; \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta_H\) 7.28–7.16 (m, 7H, ArH), 6.91 (d, \(J = 8.4 \text{ Hz}\), 2H, ArH), 4.69 (dd, \(J = 9.8, 5.2 \text{ Hz}\), 1H, CH\(\alpha\)-Tyr or CH\(\alpha\)-Phe), 4.65 (dd, \(J = 8.3, 4.9 \text{ Hz}\), 1H, CH\(\alpha\)-Tyr or CH\(\alpha\)-Phe), 4.39 (d, \(J = 6.5 \text{ Hz}\), 1H, OCH\(\text{H-Ox}\)), 4.35 (d, \(J = 6.6 \text{ Hz}\), 1H, OCH\(\text{H-Ox}\)), 4.32 (d, \(J = 6.5 \text{ Hz}\), 1H, OCH\(\text{H-Ox}\)), 4.28 (d, \(J = 6.6 \text{ Hz}\), 1H, OCH\(\text{H-Ox}\)), 4.16 (d, \(J = 7.6 \text{ Hz}\), 1H, CH\(\alpha\)-Val), 3.87–3.80 (m, 2H, CH\(\beta\)-Tyr or CH\(\beta\)-Val), 3.68–3.60 (m, 1H, CH\(\beta\)-Tyr or CH\(\beta\)-Val), 3.68–3.60 (m, 1H, CH\(\beta\)-Tyr or CH\(\beta\)-Val), 3.06–2.99 (m, 3H, CH\(\beta\)-Thr, CH\(\beta\)-GOx, CH\(\beta\)-Val), 2.96 (dd, \(J = 14.1, 9.9 \text{ Hz}\), 1H, CH\(\beta\)-Tyr or CH\(\beta\)-Phe), 2.07–1.96 (m, 1H, CH\(\beta\)-Val), 1.69–1.56 (m, 3H, CH\(\beta\)-Leu, CH\(\gamma\)-Leu), 1.32 (s, 9H, 3 × CH\(\beta\)-tBu), 1.18 (s, 9H, 3 × CH\(\beta\)-tBu), 1.06 (d, \(J = 6.1 \text{ Hz}\), 3H, CH\(\gamma\)-Thr), 0.99–0.87 (m, 12H, 2 × CH\(\beta\)-Leu, 2 × CH\(\gamma\)-Val); \(^1\)C NMR (126 MHz, CD\(_3\)OD) \(\delta_c\) 176.6 (C=O), 175.4 (C=O), 174.1 (C=O), 173.5 (C=O), 170.8 (C=O), 155.4 (C), 139.0 (C), 133.5 (C), 130.9 (CH), 130.6 (CH), 129.5 (CH), 127.4 (CH), 125.2 (CH), 80.8 (OCH\(\text{O}\)), 80.5 (OCH\(\text{O}\)), 79.5 (C, tBu), 75.7 (C, tBu), 70.5 (CH, \(\alpha\)-Thr), 64.4 (CH, \(\beta\)-Thr), 62.0 (C, Ox), 61.5 (CH, \(\alpha\)-Val), 57.2 (CH, \(\alpha\)-Tyr or CH, \(\alpha\)-Phe), 55.9 (CH, \(\alpha\)-Tyr or CH, \(\alpha\)-Phe), 52.9 (CH, \(\alpha\)-Leu), 45.1 (CH\(_2\), GOx), 41.7 (CH\(_2\), \(\beta\)-Leu), 39.3 (CH\(_3\), \(\beta\)-Tyr or CH\(_2\), \(\beta\)-Phe), 37.9 (CH\(_3\), \(\beta\)-Tyr or CH\(_2\), \(\beta\)-Phe), 32.3 (CH, \(\beta\)-Val), 29.2 (CH\(_3\), tBu), 29.1 (CH\(_3\), tBu), 25.2 (CH, \(\gamma\)-Leu), 23.3 (CH\(_3\), \(\delta\)-Leu), 21.9 (CH\(_3\), \(\delta\)-Leu), 21.0 (CH\(_3\), \(\gamma\)-Thr), 19.8 (CH\(_3\), \(\gamma\)-Val), 19.2 (\(\gamma\)-CH\(_3\), Val); \(\nu_{\text{max}}\) (neat) = 3064, 2968, 2873, 1643, 1505, 1160, 698 cm\(^{-1}\); MS (ESI\(^+\)) \(m/z\) 839 [M+H]\(^+\), 861 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{45}\)H\(_{71}\)N\(_6\)O\(_9\) [M+H]\(^+\) 839.5277, found 839.5273; [\(\alpha\)]\(_D\)\(^{27}\) = –9.6 (c 0.16, MeOH).
Cyclo(Leu-Tyr(Bu)-Val-GOx-Thr(Bu)-Phe) (201)

Following general procedure 6, H-Leu-Tyr(Bu)-Val-GOx-Thr(Bu)-Phe-OH (200) (416 mg, 0.50 mmol, 1.0 equiv) was cyclised using DEPBT (297 mg, 0.99 mmol, 2.0 equiv) at 5 mM for 48 h. The crude residue was purified twice by column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/MeOH 49:1→19:1) to give the cyclic hexapeptide (201) as a white solid (235 mg, 0.29 mmol, 58%). R\textsubscript{f} (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 49:1) 0.22; m.p 127–129 °C; \textsuperscript{1}H NMR (500 MHz, CD\textsubscript{3}OD) δ\textsubscript{H} 7.36–7.31 (m, 2H, ArH), 7.30–7.23 (m, 3H, ArH), 7.17 (d, J = 8.3 Hz, 2H, ArH), 6.94 (d, J = 8.4 Hz, 2H, ArH), 4.63 (dd, J = 9.6, 6.2 Hz, 1H, CHα-Phe), 4.50–4.45 (m, 3H, OCH\textsubscript{2}-Ox, OCH\textsubscript{2}-Ox), 4.41–4.35 (m, 2H, OCH\textsubscript{2}-Ox, CHα-Val), 4.14 (dd, J = 11.4, 3.6 Hz, 1H, CHα-Tyr), 4.03 (d, J = 14.9 Hz, 1H, CHHGOx), 3.79–3.71 (m, 1H, CHβ-Phe), 3.59–3.45 (m, 2H, CHβ-Tyr), 3.37 (dd, J = 13.9, 3.9 Hz, 1H, CHHβ-Tyr), 3.29–3.18 (m, 2H, C\textsubscript{H}Hβ-Tyr), 3.07 (d, J = 4.3 Hz, 1H, CHβ-Thr), 2.75 (dd, J = 13.8, 9.8 Hz, 1H, CHHβ-Phe), 2.13–2.03 (m, 1H, CHβ-Val), 1.58–1.50 (m, 1H, CHHβ-Leu), 1.36–1.28 (m, 10H, CHHβ-Leu, 3 × CH\textsubscript{3}, tBu), 1.20–1.15 (m, 1H, CHβ-Leu), 1.10–1.03 (m, 18H, 2 × CH\textsubscript{3}γ-Val, CH\textsubscript{2}γ-Thr, 3 × CH\textsubscript{3}, tBu), 0.81 (d, J = 6.5 Hz, 3H, CH3δ-Leu), 0.77 (d, J = 6.5 Hz, 3H, CH3δ-Leu); \textsuperscript{13}C NMR (126 MHz, CD\textsubscript{3}OD) δ\textsubscript{C} 176.4 (C=O), 174.9 (C=O), 174.5 (C=O), 174.4 (C=O), 173.0 (C=O), 155.3 (C), 137.7 (C), 134.7 (C), 130.6 (CH), 130.2 (CH), 129.9 (CH), 128.3 (CH), 125.3 (CH), 80.3 (OCH\textsubscript{2}), 80.2 (OCH\textsubscript{2}), 79.4 (C, tBu), 75.3 (C, tBu), 69.2 (CH, α-Thr), 64.8 (CH, β-Thr), 62.4 (CH, β-Val), 62.1 (C, Ox), 59.3 (CH, α-Tyr), 55.7 (CH, α-Leu), 54.7 (CH, α-Phe), 45.6 (CH\textsubscript{2}, GOx), 40.2 (CH\textsubscript{2}, β-Leu), 39.5 (CH\textsubscript{2}, β-Phe), 35.3 (CH\textsubscript{2}, β-Tyr), 33.2 (CH, β-Val), 29.2 (CH\textsubscript{3}, tBu), 28.9 (CH\textsubscript{3}, tBu), 25.2 (CH, γ-Leu), 23.2 (CH\textsubscript{3}, δ-Leu), 22.1 (CH\textsubscript{3}, δ-Leu), 22.0 (CH\textsubscript{3}, γ-Val), 19.9 (CH\textsubscript{3}, γ-Val), 19.5 (CH\textsubscript{3}, γ-Thr); ν\textsubscript{max} (neat) = 2964, 2930, 1641, 1504, 1389, 1161 cm\textsuperscript{-1}; MS (ESI\textsuperscript{+}) m/z 843 [M+Na]\textsuperscript{+}; HRMS (ESI\textsuperscript{+}) calcd. for C\textsubscript{45}H\textsubscript{68}N\textsubscript{6}NaO\textsubscript{8} [M+Na]\textsuperscript{+} 843.4991, found 843.4995; [α]\textsubscript{D}\textsuperscript{27} −74.3 (c 0.13, MeOH).
Preparation of cyclic tetrapeptides 215 and 216

**Boc-AOx-(R)-CH(Me)Ph (210)**

![Structure of Boc-AOx-(R)-CH(Me)Ph (210)]

To a solution of NO$_2$-AOx-(R)-CH(Me)Ph $^{81,22}$ (1.23 g, 3.83 mmol, 1.0 equiv) in THF (40 mL) was added Boc$_2$O (1.67 g, 7.65 mmol, 2.0 equiv) and Raney Ni (slurry in H$_2$O, 8.0 mL). The solution was placed under an atmosphere of nitrogen, evacuated and filled with hydrogen (balloon). The reaction mixture was stirred vigorously for 6 h at room temperature. Then, the mixture was filtered through a plug of Celite eluting with EtOAc, the filtrate was concentrated under reduced pressure, EtOAc (40 mL) was added, the mixture was washed with saturated Na$_2$CO$_3$ (3 × 40 mL), dried over Na$_2$SO$_4$ and concentrated under reduced pressure. Boc-AOx-(R)-CH(Me)Ph (210) was afforded after purification by column chromatography (SiO$_2$, EtOAc/PE 3:7) as a colourless oil (1.06 g, 3.30 mmol, 86%). $^\text{R}$ ($\text{EtOAc/PE 3:7}$) 0.44; $^\text{m.p}$ 100–103 °C; $^\text{1H NMR}$ (500 MHz, CDCl$_3$) δ $^\text{H}$ 7.36‒7.29 (m, 4H, ArH), 7.23 (t, $^\text{J}$ = 7.0 Hz, 1H, ArH), 4.82 (s, 1H, NH Boc), 4.51–4.56 (m, 1H, OC$_2$H$_4$-Ox), 4.41–4.37 (m, 2H, OC$_2$H$_4$-Ox, OCH$_2$-Ox), 4.33–4.28 (m, 1H, OCH$_2$-Ox), 4.14 (q, $^\text{J}$ = 6.6 Hz, 1H, CH$_3$), 4.10–4.01 (m, 1H, CH$_2$-AOx), 1.47 (s, 3 × 9H, CH$_3$, Boc), 1.37 (d, $^\text{J}$ = 6.7 Hz, 3H, CH$_3$), 1.11 (d, $^\text{J}$ = 6.6 Hz, 3H, CH$_3$-β-AOx); $^\text{13C NMR}$ (126 MHz, CDCl$_3$) δ $^\text{C}$ 156.0 (C=O, Boc), 147.3 (C), 128.8 (CH), 127.1 (CH), 126.3 (CH), 78.9 (OCH$_2$), 78.5 (C, Boc), 63.3 (C, Ox), 52.9 (CH$_2$), 49.7 (CH, α-AOx), 28.5 (CH$_3$, Boc), 26.5 (CH$_3$), 15.3 (CH$_3$, β-AOx). $^\text{ν}_{\text{max}}$ (neat) = 3301, 2970, 2879, 1708, 1541, 1167 cm$^{-1}$; $^\text{MS}$ (ESI$^+$) $m/z$ 321 [M+H]$^+$, 343 [M+Na]$^+$; $^\text{HRMS}$ (ESI$^+$) calcd. for C$_{18}$H$_{25}$N$_2$O$_3$ [M+H]$^+$ 321.2173, found 321.2175; $[\alpha]_D^{25}$ +23.6 (c 0.18, CHCl$_3$).

**Boc-AOx-Gly-OBn (212)**

![Structure of Boc-AOx-Gly-OBn (212)]

To a solution of Boc-AOx-(R)-CH(Me)Ph (210) (1.62 g, 5.08 mmol, 1.0 equiv) in MeOH (51 mL) was added 31 wt% Pd(OH)$_2$/C (508 mg, 10 wt%) and the reaction flask was evacuated, filled with nitrogen, evacuated, and placed under an atmosphere of hydrogen (balloon). The reaction mixture was stirred at room temperature for 16 h, placed under nitrogen, and filtered through a plug of Celite, which was washed with MeOH (3×). The filtrate was concentrated in vacuo to a pale yellow solid, which was re-dissolved in anhydrous CH$_3$CN (100 mL) and BrCH$_2$CO$_2$Bn $^{211}$ (1.68 mL, 10.2 mmol, 2.0 equiv) and DIPEA (1.85 mL, 10.2 mmol, 2.0 equiv) were added. The reaction mixture was stirred at 35 °C under an atmosphere of nitrogen for 3 d, at which time the solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO$_2$, EtOAc/PE 4:6) to give Boc-AOx-Gly-OBn (212) as a yellow solid.
(1.27 g, 3.50 mmol, 69%). \( R_t \) (EtOAc/PE 4:6) 0.22; \( m.p \) 79–81 °C; \(^1\text{H NMR} \) (500 MHz, CDCl\(_3\)) \( \delta_{H} 7.40–7.32 \) (m, 5H, ArH), 5.19 (s, 2H, CH\(_3\)Ph), 4.93 (s, 1H, NH Boc), 4.51–4.37 (m, 4H, 2 × OCH\(_2\)-Ox), 4.12 (d, \( J = 6.9 \) Hz, 1H, CH\( _{\alpha}-\)Aox), 3.73 (d, \( J = 17.6 \) Hz, 1H, CHHGly), 3.65 (d, \( J = 17.6 \) Hz, 1H, CHHGly), 1.94 (s, 1H, NH), 1.44 (s, 9H, 3 × CH\(_3\), Boc), 1.14 (d, \( J = 6.8 \) Hz, 3H, CH\( _{\beta}-\)Aox); \(^{13}\text{C NMR} \) (126 MHz, CDCl\(_3\)) \( \delta_{C} 172.7 \) (C=O), 155.9 (C=O, Boc), 135.4 (C), 128.8 (CH), 128.7 (CH), 78.2 (C, Boc), 77.3 (OCH\(_3\)), 67.2 (CH\(_2\), Bn), 62.8 (C, Ox), 50.6 (CH, \( \alpha\)-Aox), 45.0 (CH\(_2\), Gly), 28.5 (CH\(_3\), Boc), 14.5 (CH\(_3\), \( \beta\)-Aox); \( v_{\text{max}} \) (neat) = 3342, 2884, 1746, 1716, 1248 cm\(^{-1}\); MS (ESI\(^{+}\)) \( m/z \) 365 [M+H]\(^{+}\), 387 [M+Na]\(^{+}\); HRMS (ESI\(^{+}\)) calcd. for C\(_{19}\)H\(_{28}\)N\(_2\)O\(_5\) [M+Na]\(^{+}\) 387.1890, found 387.1894; \([\alpha]_{D}^{29}\) –6.0 (c 0.12, MeOH).

**Boc-Leu-Aox-Gly-OBn (213)**

Following general procedure 1 from dipeptide 212 (574 mg, 1.58 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/EtOAc 6:4) to give Boc-Leu-Aox-Gly-OBn (213) (328 mg, 0.69 mmol, 44%) as a colourless oil; \( R_t \) (CH\(_2\)Cl\(_2\)/EtOAc 6:4) 0.29; \(^1\text{H NMR} \) (500 MHz, CDCl\(_3\)) \( \delta_{H} 7.41–7.30 \) (m, 5H, ArH), 6.64 (d, \( J = 8.3 \) Hz, 1H, NH), 5.19 (s, 2H, CH\(_2\)Ph), 4.91 (s, 1H, Boc NH), 4.52–4.30 (m, 5H, 2 × OCH\(_2\)-Ox, CH\(_{\alpha}\)-Leu), 4.07–3.97 (m, 1H, CH\( _{\alpha}\)-Aox), 3.72 (d, \( J = 17.7 \) Hz, 1H, CHHGly), 3.65 (d, \( J = 17.7 \) Hz, 1H, CHHGly), 1.92 (s, 1H, NH), 1.68–1.55 (m, 2H, CH\(_{\gamma}\)-Leu, CHHGly\(_{\beta}\)-Leu), 1.49–1.38 (m, 10H, CHHGly\(_{\beta}\)-Leu, 3 × CH\(_3\), Boc), 1.14 (d, \( J = 6.8 \) Hz, 3H, CH\(_{\beta}\)-Aox), 0.92 (d, \( J = 4.8 \) Hz, 6H, 2 × CH\(_3\)\( _{\delta}\)-Leu); \(^{13}\text{C NMR} \) (126 MHz, CDCl\(_3\)) \( \delta_{C} 172.8 \) (C=O), 172.7 (C=O), 155.8 (C=O, Boc) 135.4 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 78.2 (C, Boc), 77.4 (OCH\(_3\)), 67.3 (CH\(_2\), Bn), 62.5 (C, Ox), 53.5 (CH, \( \alpha\)-Leu), 49.7 (CH, \( \alpha\)-Aox), 45.0 (CH\(_2\), Gly), 41.4 (CH\(_2\), \( \beta\)-Leu), 28.4 (CH\(_3\), Boc), 25.0 (CH, \( \gamma\)-Leu), 23.0 (CH\(_3\), \( \delta\)-Leu), 22.2 (CH\(_3\), \( \delta\)-Leu), 14.5 (CH\(_3\), \( \beta\)-Aox); \( v_{\text{max}} \) (neat) = 3329, 2975, 1737, 1682, 1649, 1117 cm\(^{-1}\); MS (ESI\(^{+}\)) \( m/z \) 478 [M+H]\(^{+}\), 500 [M+Na]\(^{+}\); HRMS (ESI\(^{+}\)) calcd. for C\(_{25}\)H\(_{36}\)N\(_4\)O\(_5\) [M+H]\(^{+}\) 478.2912, found 478.2915; \([\alpha]_{D}^{29}\) –25.0 (c 0.20, MeOH).

**Cbz-Trp-Leu-Aox-Gly-OBn (454)**

Following general procedure 1 from tripeptide 213 (460 mg, 0.97 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 19:1) to give Cbz-Trp-Leu-Aox-Gly-OBn (454) (456 mg, 0.65 mmol, 68%) as a white solid; \( R_t \) (CH\(_2\)Cl\(_2\)/MeOH 19:1) 0.20; \( m.p \) 81–84 °C; \(^1\text{H NMR} \) (500 MHz, CDCl\(_3\)) \( \delta_{H} 8.30 \) (s, 1H, NH), 7.65 (d, \( J = 7.1 \) Hz, 1H, ArH), 7.39–7.27 (m,
11H, ArH), 7.19 (t, J = 7.5 Hz, 1H, ArH), 7.11 (t, J = 6.8 Hz, 1H, ArH), 7.04 (s, 1H, ArH), 6.72 (d, J = 8.0 Hz, 1H, NH), 6.25 (d, J = 6.7 Hz, 1H, NH), 5.48 (d, J = 6.9 Hz, 1H, NH), 5.17 (s, J = 5.8 Hz, 2H, CH₂Ph), 5.12–5.04 (m, 2H, CH₂Ph), 4.56–4.42 (m, 7H, 2 × OCH₂-Ox, CHα-Leu, CHα-Ala, CHα-Trp), 3.67 (s, 2H, CH₂Gly), 3.28 (dd, J = 14.6, 5.7 Hz, 1H, CHHβ-Trp), 3.20 (dd, J = 14.6, 6.7 Hz, 1H, CHHβ-Trp), 1.97 (s, 1H, NH), 1.61–1.51 (m, 1H, CHHβ-Leu), 1.42–1.28 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.09 (d, J = 6.6 Hz, 3H, CHβ-AOx), 0.81 (d, J = 6.4 Hz, 6H, 2 × CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δc 172.9 (C=O), 171.7 (C=O), 171.5 (C=O), 156.4 (C=O, Cbz), 136.4 (C), 136.1 (C), 135.4 (C), 128.8 (CH), 128.72 (CH), 128.67 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 127.4 (C), 123.5 (CH), 122.5 (CH), 120.0 (CH), 118.9 (CH), 111.5 (CH), 110.2 (C), 78.1 (OCH₂), 77.5 (OCH₂), 67.3 (CH₂, Bn), 67.2 (CH₂, Bn), 62.6 (C, Ox), 55.6 (CH, α-Trp), 52.5 (CH, α-Leu), 49.5 (CH, α-AOx), 45.0 (CH₂, Gly), 40.8 (CH₂, β-Leu), 28.1 (CH₂, β-Trp), 24.9 (CH, γ-Leu), 22.9 (CH₃, δ-Leu), 22.1 (CH₃, δ-Leu), 14.5 (CH₃, β-AOx); vₘₐₓ (neat) = 3282, 2952, 1703, 1643, 1342 cm⁻¹; MS (ESI⁺) m/z 698 [M+H]⁺, 720 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₉H₄₅N₅NaO₇ [M+Na]⁺ 720.3368, found 720.3369; [α]D²⁹ = -29.0 (c 0.05, MeOH).

**H-Trp-Leu-AOx-Gly-OH (214)**

Following general procedure 5 from Cbz-Trp-Leu-AOx-Gly-OBn (454) (404 mg, 0.58 mmol, 1.0 equiv), H-Trp-Leu-AOx-Gly-OH (214) was obtained as a yellow solid (273 mg, 0.58 mmol, quant. yield), which required no further purification; m.p 170–173 °C; ¹³C NMR (500 MHz, CD₃OD) δc 7.73 (d, J = 7.9 Hz, 1H, ArH), 7.41 (d, J = 8.1 Hz, 1H, ArH), 7.26 (s, 1H, ArH), 7.16 (t, J = 7.5 Hz, 1H, ArH), 7.09 (t, J = 7.4 Hz, 1H, ArH), 4.62–4.57 (m, 2H, OCH₂-Ox), 4.56–4.50 (m, 2H, OCH₂-Ox), 4.46–4.37 (m, 2H, CHα-Leu, CHα-AOx), 4.24 (dd, J = 8.3, 5.6 Hz, 1H, CHα-Trp), 3.63 (d, J = 16.9 Hz, 1H, CHH Gly), 3.58 (d, J = 16.9 Hz, 1H, CHH Gly), 3.51 (dd, J = 15.0, 5.3 Hz, 1H, CHHβ-Trp), 3.23 (dd, J = 15.0, 8.5 Hz, 1H, CHHβ-Trp), 1.71–1.60 (m, 3H, CH₂β-Leu, CHγ-Leu), 1.28 (d, J = 6.8 Hz, 3H, CHβ-AOx), 0.97 (d, J = 5.7 Hz, 3H, CHδ-Leu), 0.95 (d, J = 5.7 Hz, 3H, CHδ-Leu); ¹³C NMR (126 MHz, CD₃OD) δc 174.9 (C=O), 174.7 (C=O), 170.3 (C=O), 138.3 (C), 128.4 (C), 125.8 (CH), 123.0 (CH), 120.3 (CH), 119.2 (CH), 112.6 (CH), 107.9 (C), 77.5 (OCH₂), 77.4 (OCH₂), 64.6 (C, Ox), 54.8 (CH, α-Trp), 54.0 (CH, α-Leu), 49.9 (CHα-AOx), 46.0 (CH₂, Gly), 42.0 (CH₂, β-Leu), 28.7 (CH₂, β-Trp), 25.9 (CH, γ-Leu), 23.4 (CH₃, δ-Leu), 22.0 (CH₃, δ-Leu), 14.7 (CH₃, β-AOx); vₘₐₓ (neat) = 3228, 2954, 1643, 1537, 1237 cm⁻¹; MS (ESI⁺) m/z 474 [M+H]⁺, 496 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₉H₃₅N₅NaO₇ [M+Na]⁺ 496.2530, found 496.2533; [α]D²⁹ = -37.0 (c 0.05, MeOH).
Cyclo(Trp-Leu-AOx-Gly) (215)

Following general procedure 6, H-Trp-Leu-AOx-Gly-OH (214) (47 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.2 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 9:1→85:15) to give cyclic tetrapeptide 215 as a white solid (1st run: 22.9 mg, 50 μmol, 50%; 2nd run (289 μmol scale): 64.4 mg, 142 μmol, 49%). Rf (CH₂Cl₂/MeOH 9:1) 0.19; m.p. 220–223 °C; ¹H NMR (500 MHz, CD₃OD) δH 7.62 (d, J = 7.9 Hz, 1H, ArH), 7.32 (d, J = 8.1 Hz, 1H, ArH), 7.12 (s, 1H, ArH), 7.10 (t, J = 7.7 Hz, 1H, ArH), 7.03 (t, J = 7.4 Hz, 1H, ArH), 4.93 (dd, J = 10.2, 7.2 Hz, 1H, CHα-Trp), 4.57 (q, J = 7.2 Hz, 1H, CHα-AOx), 4.50 (d, J = 6.8 Hz, 1H, OCHH-Ox), 4.40 (d, J = 6.8 Hz, 1H, OCHH-Ox), 4.38 (d, J = 7.8 Hz, 1H, OCHH-Ox), 4.20 (dd, J = 10.9, 4.2 Hz, 1H, CHβ-Leu), 4.04 (d, J = 7.8 Hz, 1H, OCHH-Ox), 3.67 (d, J = 16.6 Hz, 1H, CHβ-Gly), 3.57 (d, J = 16.6 Hz, 1H, CHβ-Gly), 3.37–3.27 (m, 1H, CHββ-Trp), 3.21 (dd, J = 15.1, 7.2 Hz, 1H, CHββ-Trp), 1.72–1.63 (m, 1H, CHββ-Trp), 1.59–1.48 (m, 2H, CHββ-Leu, CHγ-Leu), 1.12 (d, J = 6.9 Hz, 3H, CHβ-β-AOx), 0.90 (d, J = 6.0 Hz, 3H, CHβ-β-Leu), 0.73 (d, J = 6.0 Hz, 3H, CHβ-β-Leu). Note: CHββ-Trp overlaps with solvent peak; ¹³C NMR (126 MHz, CD₃OD) δc 176.4 (C=O), 175.3 (C=O), 173.7 (C=O), 138.0 (C), 128.5 (C), 123.8 (CH), 122.6 (CH), 119.9 (CH), 119.1 (CH), 112.3 (CH), 110.3 (C), 79.2 (OCH₂), 77.3 (OCH₂), 64.7 (C, Ox), 57.7 (CH, α-Trp), 55.3 (CH, α-Leu), 51.6 (CH, α-AOx), 48.8 (CH₂, Gly), 40.6 (CH₂, β-Leu), 27.6 (CH₂, β-Trp), 26.2 (CH, γ-Leu), 23.3 (CH₂, δ-Leu), 21.2 (CH₃, δ-Leu), 13.5 (CH₃, AOx). Note: CH₂, Gly signal overlaps with solvent peak; νmax (neat) = 3256, 2956, 1659, 1532, 740 cm⁻¹; MS (ESI⁺) m/z: 478 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₂₁H₂₁NO₄Na [M+Na⁺] 478.2425, found 478.2423; [α]D⁰ − 108 (c 0.06, MeOH).

Boc-Leu-Ala-Gly-OBn (453)

Following general procedure 1 from dipeptide 452 pregnancy (885 mg, 2.63 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, EtOAc/CH₂Cl₂ 4:6) to give Boc-Leu-Ala-Gly-OBn (453) as a white foam (863 mg, 1.92 mmol, 73%); Rf (EtOAc/CH₂Cl₂ 4:6) 0.29; m.p. 60–63 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.38–7.30 (m, 5H, ArH), 6.97 (s, 1H, NH), 6.71 (d, J = 7.4 Hz, 1H, NH), 5.16 (s, 2H, CH₂Ph), 4.96 (d, J = 6.7 Hz, 1H, Boc NH), 4.54 (quint, J = 7.1 Hz, 1H, CHα-Ala), 4.15–3.98 (m, 3H, CHα-Leu, CH₂Gly), 1.71–1.59 (m, 2H, CHγ-Leu, CHββ-Leu), 1.52–1.44 (m, 1H, CHββ-Leu), 1.42 (s, 9H, 3 × CH₃, Boc), 1.39 (d, J = 7.1 Hz, 3H, CHβ-β-Ala), 0.93 (d, J = 6.5 Hz, 6H, 2
x CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δC 172.8 (C=O), 172.5 (C=O), 169.6 (C=O), 156.1 (C=O, Boc), 135.3 (C), 128.8 (CH), 128.7 (CH), 128.5 (CH), 80.6 (C, Boc), 67.3 (CH₂, Bn), 53.5 (CH, α-Leu), 48.8 (CH, α-Ala), 41.5 (CH₂, Gly), 41.1 (CH₂, β-Leu), 28.4 (CH₃, Boc), 24.9 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 21.9 (CH₃, δ-Leu), 18.0 (CH₃, β-Ala); νmax (neat) = 3297, 2932, 1748, 1645, 1453, 1162 cm⁻¹; MS (ESI⁺) m/z 472 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₃₅N₃NaO₆ [M+Na]+ 472.2418, found 472.2420; [α]D²⁹ −45.0 (c 0.15, MeOH).

Cbz-Trp-Leu-Ala-Gly-OBn (455)

Following general procedure 1 from tripeptide 453 (760 mg, 1.69 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 24:1) to give Cbz-Trp-Leu-Ala-Gly-OBn (455) (945 mg, 1.41 mmol, 84%) as a white solid; Rf (CH₂Cl₂/MeOH 24:1) 0.41; m.p 93–96 ºC; ¹H NMR (500 MHz, CD₃OD) δH 7.59 (d, J = 7.9 Hz, 1H, ArH), 7.37–7.23 (m, 11H, ArH), 7.13–7.05 (m, 2H, ArH), 6.99 (t, J = 7.4 Hz, 1H, ArH), 5.13 (s, 2H, CH₂Ph), 5.02 (d, J = 5.3 Hz, 2H, CH₂Ph), 4.49–4.43 (m, 1H, CHα-Trp), 4.37–4.30 (m, 2H, CHα-Ala, CHα-Leu), 4.01 (d, J = 17.5 Hz, 1H, CHHβ-Trp), 3.94 (d, J = 17.5 Hz, 1H, CHHβ-Trp), 0.87 (d, J = 6.0 Hz, 3H, CH₃δ-Leu), 0.82 (d, J = 6.0 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, CD₂OD) δC 175.3 (C=O), 175.0 (C=O), 174.4 (C=O), 170.9 (C=O), 158.5 (C=O, Cbz), 138.1 (C), 138.0 (C), 137.1 (C), 129.53 (CH), 129.45 (CH), 129.3 (CH), 129.0 (CH), 128.8 (CH), 124.7 (CH), 122.5 (CH), 119.9 (CH), 119.4 (CH), 112.3 (CH), 110.7 (C), 67.9 (CH₂, Bn), 67.7 (CH₂, Bn), 57.5 (CH, α-Trp), 53.4 (CH, α-Leu), 50.3 (CH, α-Ala), 42.1 (CH₂, Gly), 41.4 (CH₂, β-Leu), 28.7 (CH₂, β-Trp), 25.6 (CH, γ-Leu), 23.5 (CH₃, δ-Leu), 21.9 (CH₃, δ-Leu), 17.9 (CH₃, β-Ala). Note: One quaternary aromatic C and one CH not observed; νmax (neat) = 3270, 2952, 1703, 1634, 1191 cm⁻¹; MS (ESI⁺) m/z 692 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₅₇H₄₃N₅NaO₇ [M+Na]+ 692.3055, found 692.3061; [α]D²⁹ −37.0 (c 0.17, MeOH).

H-Trp-Leu-Ala-Gly-OH (456)

Following general procedure 5 from Cbz-Trp-Leu-Ala-Gly-OBn (455) (854 mg, 1.28 mmol, 1.0 equiv), H-Trp-Leu-Ala-Gly-OH (456) was obtained as a DMF adduct as a white solid (426 mg, 0.96 mmol, 75%), which required no further purification; m.p 198–201 ºC; ¹H NMR (500 MHz, D₂O) δH 7.59
(d, J = 8.0 Hz, 1H, ArH), 7.52 (d, J = 8.2 Hz, 1H, ArH), 7.31–7.25 (m, 2H, ArH), 7.16 (t, J = 7.4 Hz, 1H, ArH), 4.32–4.26 (m, 2H, CHα-Trp, CHα-Leu), 4.15 (q, J = 7.2 Hz, 1H, CHα-Ala), 3.75 (s, 2H, CH2Gly), 3.44–3.33 (m, 2H, CH2β-Trp), 1.53–1.47 (m, 3H, CHγ-Leu, CHβ-Leu), 1.37 (d, J = 7.2 Hz, 3H, CHβ-Ala), 0.87 (t, J = 5.8 Hz, 3H, CH3δ-Leu), 0.85 (d, J = 5.8 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, D2O) δc 176.0 (C=O), 174.2 (C=O), 172.8 (C=O), 169.0 (C=O), 169.0 (C=O), 136.2 (C), 126.6 (C), 125.1 (CH), 122.1 (CH), 119.4 (CH), 117.9 (CH), 111.9 (CH), 106.2 (C), 53.5 (CH, α-Trp), 52.0 (CH, α-Leu), 49.6 (CH, α-Ala), 43.1 (CH2, Gly), 40.5 (CH2, β-Leu), 26.7 (CH3, β-Trp), 24.0 (CH, γ-Leu), 21.1 (CH3, δ-Leu), 16.5 (CH3, β-Ala); v max (neat) = 3270, 2956, 1626, 1525, 1438 cm⁻¹; MS (ESI⁺) m/z 468 [M+Na⁺]; HRMS (ESI⁺) calcd. for C22H29N5NaO5 [M+Na⁺] 468.2217, found 468.2220; [α]D 29° –23.0 (c 0.04, DMF).

Cyclo(Trp-Leu-Ala-Gly) (216)

Following general procedure 6, H-Trp-Leu-Ala-Gly-OH (456) (89 mg, 0.20 mmol, 1.0 equiv) was cyclised using DEPBT (120 mg, 0.4 mmol, 2.0 equiv) at 1 mM for 48 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→9:1) to give cyclic tetrapeptide 216 as a white solid (1st run: 17.4 mg, 40 μmol, 20%; 2nd run: 18.4 mg, 43 μmol, 22%). Rf (CH₂Cl₂/MeOH 92.5:7.5) 0.34; m.p 296–297 °C (decomposition); The data reported for this compound is for the major conformational isomer at 298 K; ¹H NMR (500 MHz, DMSO-d₆) δH 10.88 (s, 1H, NH), 8.29 (t, J = 5.6 Hz, 1H, NH), 7.80 (d, J = 9.4 Hz, 1H, NH), 7.73 (d, J = 9.1 Hz, 1H, NH), 7.54 (d, J = 7.9 Hz, 1H, ArH), 7.40 (d, J = 9.3 Hz, 1H, NH), 7.34 (d, J = 8.0 Hz, 1H, ArH), 7.13 (s, 1H, ArH), 7.08 (t, J = 7.4 Hz, 1H, ArH), 7.00 (t, J = 7.4 Hz, 1H, ArH), 4.52 (m, 1H, CHα-Trp), 4.32–4.25 (m, 1H, CHα-Ala), 4.12 (dd, J = 15.6, 9.4 Hz, 1H, CHα-Leu), 3.85 (dd, J = 14.0, 6.0 Hz, 1H, CHHGly), 3.43 (dd, J = 14.0, 6.0 Hz, 1H, CHHGly), 3.13 (dd, J = 14.8, 8.2 Hz, 1H, CHHGβ-Trp), 3.03 (dd, J = 14.8, 8.2 Hz, 1H, CHHGβ-Trp), 1.63–1.44 (m, 3H, CHγ-Leu, CHβ-Leu), 1.26 (d, J = 7.1 Hz, 3H, CH3δ-Leu), 0.89 (d, J = 6.1 Hz, 3H, CH3δ-Leu), 0.80 (d, J = 6.1 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, DMSO-d₆) δc 172.8 (C=O), 172.5 (C=O), 171.6 (C=O), 169.7 (C=O), 169.0 (C=O), 136.1 (C), 127.0 (C), 123.1 (CH), 121.0 (CH), 118.4 (CH), 118.1 (CH), 111.4 (CH), 109.4 (C), 56.2 (CH, α-Trp), 54.0 (CH, α-Leu), 49.6 (CH, α-Ala), 43.6 (CH₂, Gly), 39.0 (CH₂, β-Leu) 26.5 (CH3, β-Trp), 24.6 (CH, γ-Leu), 22.6 (CH3, δ-Leu), 21.6 (CH3, δ-Leu), 16.2 (CH3, β-Ala); v max (neat) = 3291, 2956, 1649, 1529, 737 cm⁻¹; MS (ESI⁺) m/z 450 [M+Na⁺], 877 [2M+Na⁺]; HRMS (ESI⁺) calcd. for C22H29N5NaO5 [M+Na⁺] 450.2112, found 450.2115; [α]D 29° –112 (c 0.04, DMF).
Preparation of cyclic pentapeptide 231

Boc-Sar-Ala-Tyr(Bn)-OBn (220)

Following general procedure 1 from dipeptide \textsuperscript{219} (3.19 g, 6.00 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\textsubscript{2}, EtOAc/PE 1:1) to give Boc-Sar-Ala-Tyr(Bn)-OBn (220) (2.64 g, 3.91 mmol, 65%) as a white foam; \textit{R}_{f} (EtOAc/PE 1:1) 0.17; \textit{m.p} 118–121 °C; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) H 7.44–7.27 (m, 10H, ArH), 6.92 (d, \(J = 8.4\) Hz, 2H, ArH), 6.70–6.38 (m, 2H, 2 × NH), 5.17 (d, \(J = 12.1\) Hz, 1H, CH\textsubscript{2}Ph), 5.09 (d, \(J = 12.1\) Hz, 1H, CH\textsubscript{2}Ph), 5.00 (s, 2H, CH\textsubscript{2}Ph), 4.82 (dd, \(J = 13.2, 6.1\) Hz, 1H, CH\textsubscript{2}Ytr), 4.47 (s, 1H CH\textsubscript{2}A), 3.90–3.70 (m, 2H, CH\textsubscript{2}Sar), 3.09–3.00 (m, 2H, CH\textsubscript{2}\beta T), 2.90 (s, 3H, NCH\textsubscript{3}), 1.46 (s, 9H, 3 × CH\textsubscript{3}, Boc), 1.32 (d, \(J = 6.9\) Hz, 3H, CH\textsubscript{3}βA); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) C 171.6 (C=O), 171.2 (C=O), 158.1 (C), 156.5 (C=O, Boc), 137.1 (C), 135.2 (C), 130.4 (CH), 128.73 (CH), 128.70 (CH), 128.66 (CH), 128.1 (CH), 127.8 (C), 127.6 (CH), 115.0 (CH), 80.9 (C, Boc), 70.1 (CH\textsubscript{2}, Bn), 67.4 (CH\textsubscript{2}, Bn), 53.5 (CH, α-Tyr), 53.0 (CH\textsubscript{2}, Sar), 48.7 (CH, α-A), 37.0 (CH\textsubscript{2}, β-Tyr), 35.9 (NCH\textsubscript{3}), 28.4 (CH, Boc), 18.1 (CH, β-A). Note: C=O Boc, CH\textsubscript{2}, Sar and CH\textsubscript{3}, β-A are broad and not seen clearly, assignment by HSQC and HMBC correlations; one aromatic CH not observed; \(\nu_{\text{max}}\) (neat) = 3282, 2974, 1731, 1699, 1638, 1117 cm\(^{-1}\); MS (ESI\textsuperscript{+}) \(m/z\) 626 [M+Na]\textsuperscript{+}; HRMS (ESI\textsuperscript{+}) calcd. for C\textsubscript{34}H\textsubscript{41}N\textsubscript{3}NaO\textsubscript{7} [M+Na]\textsuperscript{+} 626.2837, found 626.2835; [\(\alpha\)]\textsubscript{D}\textsuperscript{29} = –38.0 (c 0.12, MeOH).

Boc-Ala-Sar-Ala-Tyr(Bn)-OBn (221)

Following general procedure 1 from tripeptide \textsuperscript{220} (2.05 g, 3.40 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/MeOH 97:3) to give Boc-Ala-Sar-Ala-Tyr(Bn)-OBn (221) (1.42 g, 2.11 mmol, 62%) as a white foam. \textit{R}_{f} (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 97:3) 0.32; \textit{m.p} 69–72 °C; \textsuperscript{1}H NMR (500 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) H 7.86 (d, \(J = 5.7\) Hz, 1H, NH), 7.47–7.24 (m, 10H, ArH), 7.10 (d, \(J = 8.5\) Hz, 2H, ArH), 6.90 (d, \(J = 8.5\) Hz, 2H, ArH), 6.23 (s, 1H, NH), 5.11–5.04 (m, 4H, 2 × CH\textsubscript{2}Ph), 4.56 (q, \(J = 7.2\) Hz, 1H, CH\textsubscript{2}A), 4.46–4.30 (m, 2H, 2 × CH\textsubscript{2}A), 4.07–3.78 (m, 2H, CH\textsubscript{2}Sar), 3.03 (dd, \(J = 14.1, 6.3\) Hz, 1H, CH\textsubscript{2}βTyr), 2.98–2.94 (m, 1H, CH\textsubscript{2}βTyr), 1.39 (s, 9H, 3 × CH\textsubscript{3}, Boc), 1.23–1.17 (m, 6H, 2 × CH\textsubscript{3}β-A). Note: NCH\textsubscript{3} and one NH signal not observed; \textsuperscript{13}C NMR (126 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) C 172.3 (C=O), 171.4 (C=O), 170.3 (C=O), 167.1 (C=O), 156.9 (C), 154.2 (C=O, Boc), 136.9 (C), 135.3 (C), 129.5 (CH), 128.8 (C), 127.7 (CH), 127.6.
(CH), 127.3 (CH), 127.2 (CH), 127.0 (CH), 126.8 (CH), 114.4 (CH), 77.7 (C, Boc), 69.2 (CH$_3$, Bn), 65.5 (CH$_2$, Bn), 53.3 (CH, α-Tyr), 47.6 (CH, α-Ala), 45.7 (CH, α-Ala), 35.6 (CH$_2$, β-Tyr), 27.6 (CH$_3$, Boc), 17.3 (CH$_3$β-Ala), 17.1 (CH$_3$β-Ala). Note: NCH$_3$ and CH$_2$, Sar not observed, assignment by HSQC and HMBC correlations; $\nu_{\text{max}}$ (neat) = 2986, 1744, 1714, 1654, 1164, 1013 cm$^{-1}$; MS (ESI$^+$) $m/z$ 697 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{37}$H$_{56}$Na$_2$O$_9$ [M+Na]$^+$ 697.3208, found 697.3211; $[\alpha]_D^{20}$ = −43.0 (c 0.11, MeOH).

**Cbz-Leu-Ala-Sar-Ala-Tyr(Bn)-OBn (457)**

Following general procedure 1 from tetrapeptide 221 (1.33 g, 2.06 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 19:1) to give pentapeptide 457 (1.43 g, 1.73 mm mol, 84%) as a white foam; $R_f$ (CH$_2$Cl$_2$/MeOH 19:1) 0.35; $m.p.$ 77–80 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.79 (s, 1H, NH), 7.55 (d, $J = 6.4$ Hz, 1H, NH), 7.38–7.19 (m, 16H, ArH, NH), 7.03 (d, $J = 8.3$ Hz, 2H, ArH), 6.82 (d, $J = 8.3$ Hz, 3H, ArH, Cbz NH), 5.12–4.87 (m, 6H, 3 × CH$_2$Ph), 4.68–4.59 (m, 1H, CH$_3$-Ala), 4.50 (q, $J = 7.3$ Hz, 1H, CH$_3$-Tyr), 4.29 (quint, $J = 6.9$ Hz, 1H, CH$_3$-Ala), 4.13–3.65 (m, 3H, CH$_3$-Leu, CH$_3$Sar), 2.95 (dd, $J = 14.1$, 6.3 Hz, 1H, CH$_3$βTyr), 2.91–2.87 (m, 1H, CH$_3$βTyr), 1.60 (m, 1H, CH$_3$γ-Leu), 1.49–1.37 (m, 2H, CH$_2$β-Leu), 1.14 (d, $J = 6.9$ Hz, 6H, 2 × CH$_3$β-Ala), 0.82 (d, $J = 6.8$ Hz, 3H, CH$_3$δ-Leu), 0.80 (d, $J = 6.6$ Hz, 3H, CH$_3$δ-Leu). Note: NCH$_3$ signal not observed; $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$c. 171.8 (C=O), 171.4 (C=O), 170.9 (C=O), 170.4 (C=O), 167.0 (C=O), 156.9 (C), 155.2 (C=O, Cbz), 136.9 (C), 136.6 (C), 135.3 (C), 129.5 (CH), 128.8 (C), 127.69 (CH), 127.66 (CH), 127.6 (CH), 127.3 (CH), 127.2 (CH), 127.0 (CH), 126.83 (CH), 126.77 (CH), 114.5 (CH), 69.2 (CH$_2$, Bn), 65.5 (CH$_2$, Bn), 65.1 (CH$_2$, Bn), 53.3 (CH, α-Tyr), 53.1 (CH, α-Leu), 50.7 (CH$_2$, Sar), 47.7 (CH, α-Ala), 44.2 (CH, α-Ala), 40.4 (CH$_2$, β-Leu), 35.6 (CH$_2$, β-Tyr), 23.8 (CH, γ-Leu), 22.2 (CH$_3$, δ-Leu), 21.0 (CH$_3$, δ-Leu), 17.3 (CH$_3$β-Ala), 17.0 (CH$_3$β-Ala). Note: NCH$_3$ and one aromatic CH not observed; $\nu_{\text{max}}$ (neat) = 3277, 2955, 1719, 1632, 1452, 1174 cm$^{-1}$; MS (ESI$^+$) $m/z$ 844 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{36}$H$_{53}$Na$_2$O$_9$ [M+Na]$^+$ 844.3892, found 844.3894; $[\alpha]_D^{20}$ = −39.0 (c 0.12, MeOH).

**H-Leu-Ala-Sar-Ala-Tyr-OH (222)**

Following general procedure 5 from Cbz-Leu-Ala-Sar-Ala-Tyr(Bn)-OBn (457) (821 mg, 1.0 mmol, 1.0 equiv), H-Leu-Ala-Sar-Ala-Tyr-OH (222) was obtained as a white solid (506 mg, 0.99 mmol, 99%), which required no further purification; $m.p.$ 179–182 °C; $^1$H...
NMR (600 MHz, DMSO-d6 @ 373 K) δH 7.00 (d, J = 8.2 Hz, 2H, ArH), 6.67 (d, J = 8.2 Hz, 2H, ArH), 4.80–4.66 (m, 1H, CHα-Ala), 4.40 (d, J = 5.7 Hz, 1H, CHα-Tyr), 4.36–4.29 (m, 1H, CHα-Ala), 4.05–3.97 (m, 1H, CHHSar), 3.57–3.46 (m, 1H, CHα-Leu), 2.97 (dd, J = 14.0, 5.5 Hz, 1H, CHHβ-Tyr), 2.85 (dd, J = 14.0, 7.7 Hz, 1H, CHHβ-Tyr), 1.79–1.69 (m, 1H, CHγ-Leu), 1.61–1.51 (m, 1H CHHβ-Leu), 1.47–1.39 (m, 1H, CHHβ-Leu), 1.24 (d, J = 6.5 Hz, 3H, CHβ-Ala), 1.22 (d, J = 7.0 Hz, 3H, CHβ-Ala), 0.91 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.89 (d, J = 6.6 Hz, 3H, CHδ-Leu). Note: CH3β-Tyr peaks overlap with water signal; NH protons, NCH3 and CHHSar broad and not visible; Data reported are for the major conformational isomer: 1H NMR (151 MHz, DMSO-d6 @ 373 K) δC 171.9 (C=O), 171.7 (C=O), 171.2 (2 × C=O), 167.0 (C=O), 155.5 (C), 129.4 (CH), 127.1 (C), 114.7 (CH), 53.3 (CH, α-Tyr), 51.6 (CH, α-Leu), 50.3 (CH3 Sar) 47.9 (CH, α-Ala), 44.3 (CH, α-Ala), 41.5 (CH2, β-Leu), 35.8 (CH2, β-Tyr), 23.4 (CH, γ-Leu), 22.2 (CH3, δ-Leu), 21.5 (CH3, δ-Leu), 17.4 (CH3β-Ala). Note: Both CH3β-Ala peaks overlap, NCH3 not observed; νmax (neat) = 3223, 2955, 1637, 1512, 1226 cm⁻¹; MS (ESI⁺) m/z 508 [M+H]⁺, 530 [M+Na]⁺; HRMS (ESI⁺) calcd. for C24H33NaO7 [M+Na]⁺ 530.2585, found 530.2587; [α]D²⁹ = 14.0 (c 0.07, MeOH).

Cyclo(Leu-Ala-Sar-Ala-Tyr) (231) Following general procedure 6, H-Leu-Ala-Sar-Ala-Tyr-OH (222) (51 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.2 mmol, 2.0 equiv) at 1 mM for 24 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/ MeOH 92.5:7.5→9:1) to give cyclic pentapeptide 231 as a white solid (1st run: 19.0 mg, 39 μmol, 39%; 2nd run: 15.8 mg, 33 μmol, 33%). Rf (CH₂Cl₂/MeOH 9:1) 0.36; m.p 179–182 °C; 1H NMR (500 MHz, CD₂OD) δH 7.07 (d, J = 8.3 Hz, 2H, ArH), 6.71 (d, J = 8.3 Hz, 2H, ArH), 4.85–4.83 (m, 1H, CHα-Ala), 4.49 (d, J = 14.0 Hz, 1H, CHHSar), 4.35 (t, J = 8.0 Hz, 1H, CHα-Tyr), 4.23 (q, J = 7.3 Hz, 1H, CHα-Ala), 3.83 (dd, J = 11.3, 4.9 Hz, 1H, CHα-Leu), 3.29–3.27 (m, 1H, CHHSar), 3.16 (s, 3H, NCH3), 3.03 (dd, J = 13.3, 7.8 Hz, 1H, CHHβ-Tyr), 2.97 (dd, J = 13.3, 8.3 Hz, 1H, CHHβ-Tyr), 2.00 (ddd, J = 13.8, 11.5, 4.5 Hz, 1H, CHHβ-Leu), 1.50 (ddd, J = 14.5, 10.0, 5.0 Hz, 1H, CHHβ-Leu), 1.32 (d, J = 6.7 Hz, 3H, CHβ-Ala), 1.30 (d, J = 7.4 Hz, 3H, CHβ-Ala), 1.28–1.22 (m, 1H, CHγ-Leu), 0.88 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.77 (d, J = 6.6 Hz, 3H, CHδ-Leu). Note: CHHSar and one CHα-Ala signal overlaps with MeOH and H₂O solvent signal respectively, assignment by HMBC & HSQC; 13C NMR (126 MHz, CD₂OD) δC 175.0 (C=O), 174.91 (C=O), 174.89 (C=O), 173.3 (C=O), 171.9 (C=O), 157.4 (C), 131.4 (CH), 128.9 (C), 116.2 (CH), 57.7 (CH, α-Tyr), 57.5 (CH, α-Leu), 54.4 (CH2, Sar), 51.0 (CH, α-Ala), 46.8 (CH, α-Ala), 39.7 (CH2, β-Leu), 37.9 (NCH3), 36.6 (CH2, β-Tyr), 25.7
(CH, γ-Leu), 23.5 (CH₃, δ-Leu), 21.2 (CH₃, δ-Leu), 18.3 (CH₃, β-Ala), 17.6 (CH₃, β-Ala); νₘₐₓ (neat) = 3275, 2957, 1631, 1513, 1170 cm⁻¹; **MS** (ESI⁺) m/z 512 [M+Na⁺]; **HRMS** (ESI⁺) calcd. for C₄₂H₃₅N₅O₆ [M+Na⁺]⁺ 512.2480, found 512.2484; [α]°D₂⁹ −119 (c 0.06, MeOH).

### Preparation of cyclic pentapeptide 232

Following general procedure 1 from dipeptide 219 (3.19 g, 6.00 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, EtOAc/PE 7:3) to give Boc-βAla-Ala-Tyr(Bn)-OBn 223 (2.47 g, 4.10 mmol, 68%) as a white solid. Rₜ (EtOAc/PE 7:3) 0.20; m.p 154–157 ºC; **1H NMR** (500 MHz, DMSO-d₆) δH 8.28 (d, J = 7.4 Hz, 1H, NH), 7.99 (d, J = 7.7 Hz, 1H, NH), 7.46–7.23 (m, 10H, ArH), 7.11 (d, J = 8.5 Hz, 2H, ArH), 6.89 (d, J = 8.5 Hz, 2H, ArH), 6.71 (t, J = 5.3 Hz, 1H, NH), 5.05 (s, 4H, 2 × CH₂Ph), 4.44 (dd, J = 14.5, 7.4 Hz, 1H, CHβ-Tyr), 4.30 (quint, J = 7.7 Hz, 1H, CHα-Ala), 3.10 (dd, J = 13.4, 6.8 Hz, 2H, CH₂NHoc), 2.97 (dd, J = 13.8, 8.4 Hz, 1H, CHFβ-Tyr), 2.24 (t, J = 7.3 Hz, 2H, CH₂CONH), 1.36 (s, 9H, 3 × CH₃, Boc), 1.11 (d, J = 7.1 Hz, 3H, CHβ-Ala); **13C NMR** (126 MHz, DMSO-d₆) δC 173.0 (C=O), 171.7 (C=O), 170.5 (C=O), 157.6 (C), 155.9 (C=O, Boc), 137.6 (C), 136.2 (C), 130.7 (CH), 129.5 (C), 128.9 (CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.1 (CH), 115.0 (CH), 78.0 (C, Boc), 69.6 (CH₂, Bn), 66.4 (CH₂, Bn), 54.4 (CH, α-Tyr), 48.1 (CH, α-Ala), 37.1 (CH₂NHoc), 36.2 (CH₂, β-Tyr), 36.0 (CH₂CONH), 28.7 (CH₃, Boc), 18.6 (CH₃, β-Ala); νₘₐₓ (neat) = 3342, 3300, 1728, 1687, 1632, 1176 cm⁻¹; **MS** (ESI⁺) m/z 626 [M+Na⁺⁺]; **HRMS** (ESI⁺) calcd. for C₃₄H₄₁N₃O₇ [M+Na⁺⁺] 626.2837, found 626.2841; [α]°D₂⁹ −10.0 (c 0.08, DMF).

### Boc-βAla-Ala-Tyr(Bn)-OBn (224)

Following general procedure 1 from tripeptide 223 (3.22 g, 3.86 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 24:1) to give Boc-βAla-Ala-Tyr(Bn)-OBn (224) (1.84 g, 2.72 mmol, 73%) as a white solid. Rₜ (CH₂Cl₂/MeOH 24:1) 0.16; m.p 158–161 ºC; **1H NMR** (500 MHz, CD₂Cl₂) δH 7.45–7.31 (m, 11H, ArH, NH), 6.97–6.90 (m, 3H, ArH, NH), 6.84–6.78 (m, 3H, ArH, NH), 5.27 (d, J = 6.7 Hz, 1H, Boc NH), 5.20 (d, J = 12.1 Hz, 1H, CHHPh), 5.11 (d, J = 12.1 Hz, 1H, CHHPh), 5.04–4.97 (m, 2H, CH₂Ph), 4.88 (dd, J = 12.8, 5.9 Hz, 1H, CHα-Tyr), 4.38–4.29 (m, 1H, CHα-Ala), 3.80–3.72 (m, 1H, CHα-Ala), 3.72–3.62
\[
\text{Cbz-Leu-Ala-βAla-Ala-Tyr(Bn)-OBn (458)}
\]

Following general procedure 1 from tetrapeptide 224 (1.54 g, 2.28 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) to give Cbz-Leu-Ala-βAla-Ala-Tyr(Bn)-OBn (458) (1.27 g, 1.55 mmol, 68%) as a white solid. Rf (CH₂Cl₂/MeOH 19:1) 0.46; m.p 176–179 °C; \(^1\)H NMR (500 MHz, DMSO-d6) δH 8.31 (d, J = 7.4 Hz, 1H, NH), 8.03 (d, J = 7.7 Hz, 1H, NH), 7.91 (d, J = 7.4 Hz, 1H, NH), 7.86 (t, J = 5.5 Hz, 1H, NH), 7.48–7.24 (m, 16H, NH, ArH), 7.12 (d, J = 8.4 Hz, 2H, ArH), 6.90 (d, J = 8.5 Hz, 2H, ArH), 5.11–5.01 (m, 6H, CH₂Ph), 4.45 (dd, J = 14.6, 7.4 Hz, 1H, CHβ-Tyr), 4.33 (quint, J = 7.1 Hz, 1H, CHα-Ala), 4.21 (quint, J = 7.0 Hz, 1H, CHα-Ala), 4.04 (dd, J = 14.8, 8.5 Hz, 1H, CHα-Leu), 3.31–3.17 (m, 2H, CH₂NH), 2.97 (dd, J = 13.8, 6.3 Hz, 1H, CHHβ-Tyr), 2.91 (dd, J = 13.8, 8.4 Hz, 1H, CHHβ-Tyr), 2.32–2.20 (m, 2H, CH₂CONH), 1.67–1.56 (m, 1H, CHγ-Leu), 1.48–1.38 (m, 2H, CHβ-Leu), 1.17 (d, J = 7.0 Hz, 3H, CHβ-Ala), 1.13 (d, J = 7.0 Hz, 3H, CHβ-Ala), 0.87 (d, J = 7.4 Hz, 3H, CHδ-Leu), 0.85 (d, J = 7.4 Hz, 3H, CHδ-Leu); \(^1\)^1^C NMR (126 MHz, DMSO-d6) δC 172.6 (C=O), 171.9 (C=O), 171.9 (C=O), 171.3 (C=O), 170.0 (C=O), 157.2 (C=O, Cbz), 156.0 (C), 137.2 (C), 137.1 (C), 135.7 (C), 130.2 (CH), 129.0 (C), 128.4 (CH), 128.3 (CH), 128.0 (CH), 127.9 (CH), 127.8 (CH), 127.8 (CH), 127.6 (CH), 127.6 (CH), 114.5 (CH), 69.1 (CH₂, Bn), 66.0 (CH₂, Bn), 65.4 (CH₂, Bn), 54.0 (CH, α-Tyr), 53.1 (CH, α-Leu), 48.1 (CH, α-Ala), 47.7 (CH, α-Ala), 40.6 (CH₂, β-Leu), 35.7 (CH₂, β-Tyr), 35.3 (CH₂NH), 35.0 (CH₂CONH), 24.2 (CH, γ-Leu), 23.1 (CH₂, δ-Leu), 21.3 (CH₃, δ-Leu), 18.5 (CH₃, β-Ala), 18.2 (CH₃, β-Ala). Note: One aromatic CH signal not observed due to overlapping; \(v_{\text{max}}\) (neat) = 3279, 3065, 1635, 1385, 693 cm\(^{-1}\); MS (ESI\(^+\)) \(m/z\) 844 [M+Na\(^+\)]; HRMS (ESI\(^+\)) calcd. for C\(_{49}\)H\(_{59}\)N\(_{2}\)NaO\(_{8}\) [M+Na\(^+\)] 844.3892, found 844.3899; [\(\alpha\)]\(_D\)\(^{29}\) +3.0 (c 0.13, DMF).
H-Leu-Ala-βAla-Ala-Tyr-OH (225)

Following general procedure 5 from Cbz-Leu-Ala-βAla-Ala-Tyr(Bn)-OBn (458) (1.07 g, 1.31 mmol, 1.0 equiv) in MeOH/DMF (1:1), H-Leu-Ala-βAla-Ala-Ala-Tyr-OH (225) was obtained as a DMF adduct as a white solid (647 mg, 1.28 mmol, 98%), which required no further purification; m.p 89–92 °C; 1H NMR (500 MHz, CD3OD) δH 7.02 (d, J = 8.3 Hz, 2H, ArH), 6.67 (d, J = 8.3 Hz, 2H, ArH), 4.43 (t, J = 5.5 Hz, 1H, CHα-Tyr), 4.29 (q, J = 7.2 Hz, 1H, CHα-Ala), 4.26 (q, J = 7.3 Hz, 1H, CHα-Ala), 3.97 (dd, J = 8.4, 6.0 Hz, 1H, CHα-Leu), 3.56 (ddd, J = 13.4, 6.1, 4.7 Hz, 1H, CHHNH), 3.38–3.34 (1H, m, CHHNH), 3.10 (dd, J = 13.6, 5.0 Hz, 1H, CHHCONH), 3.00 (dd, J = 13.6, 6.0 Hz, 1H, CHHbCONH), 2.45 (ddd, J = 13.4, 8.9, 4.3 Hz, 1H, CHHβ-Tyr), 2.36 (ddd, J = 14.8, 5.9, 4.3 Hz, 1H, CHHβ-Tyr), 1.84–1.63 (m, 3H, CH2-Leu, CH2β-Leu), 1.35 (d, J = 7.2 Hz, 3H, CHββ-Leu), 1.31 (d, J = 7.2 Hz, 3H, CHββ-Ala), 1.02 (d, J = 6.4 Hz, 3H, CHδδ-Leu), 1.00 (d, J = 6.4 Hz, 3H, CHδδ-Leu); 13C NMR (126 MHz, CD3OD) δC 177.0 (C=O), 174.6 (C=O), 174.0 (C=O), 173.9 (C=O), 170.6 (C=O), 157.0 (C), 131.7 (CH), 129.5 (C), 115.9 (CH), 56.4 (CH, α-Tyr), 53.2 (CH, α-Leu), 51.2 (CH, α-Ala), 50.9 (CH, α-Ala), 41.6 (CH2, β-Leu), 37.9 (CH2NH), 36.8 (CH2CONH), 36.4 (CH2, β-Tyr), 25.5 (CH, γ-Leu), 23.1 (CH3, δ-Leu), 22.1 (CH3, δ-Leu), 18.1 (CH3, β-Ala), 17.7 (CH3, β-Ala); γmax (neat) = 3263, 2932, 1644, 1512, 1189 cm⁻¹; MS (ESI⁺) m/z 508 [M+H]⁺, 530 [M+Na]⁺; HRMS (ESI⁺) calcd. for C25H33NsNaO7 [M+Na]⁺ 530.2585, found 530.2589; [α]D⁹ +3.0 (c 0.07, DMF).

Cyclo(Leu-Ala-βAla-Ala-Tyr) (232)

Following general procedure 6, H-Leu-Ala-βAla-Ala-Ala-Tyr-OH (225) (51 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.2 mmol, 2.0 equiv) at 1 mM for 24 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 9:1) to give cyclic pentapeptide 232 as a white solid (1st run: 20 mg, 41 μmol, 41%; 2nd run: 18 mg, 37 μmol, 37%). Rf (CH₂Cl₂/MeOH 9:1) 0.28; m.p 288–290 °C (decomposition); 1H NMR (500 MHz, CD3OD) δH 7.05 (d, J = 8.4 Hz, 2H, ArH), 6.75 (d, J = 8.4 Hz, 2H, ArH), 4.41 (q, J = 7.3 Hz, 1H, CHα-Ala), 4.23 (q, J = 7.2 Hz, 1H, CHα-Ala), 4.16 (ddd, J = 11.4, 3.9 Hz, 1H, CHα-Leu), 4.00 (ddd, J = 10.2, 5.7 Hz, 1H, CHα-Tyr), 3.73 (dt, J = 13.3, 3.9 Hz, 1H, CHHNH), 3.28–3.09 (m, 3H, CHHNH, CHβ-Tyr), 2.51 (ddd, J = 13.6, 11.7, 4.3 Hz, 1H, CHHCONH), 2.19 (dt, J = 13.6, 3.4 Hz, 1H, CHHCONH), 1.88 (ddd, J = 14.4, 11.7, 4.2 Hz,
1H, CHβ-Leu), 1.71 (ddd, J = 13.9, 10.2, 3.9 Hz, 1H, CHβ-Leu), 1.59–1.46 (m, 4H, CHβ-Ala, CHγ-Leu), 1.27 (d, J = 7.2 Hz, 3H, CHβ-Ala), 0.96 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.90 (d, J = 6.6 Hz, 3H, CHδ-Leu); 13C NMR (126 MHz, CD3OD) δC 175.7 (C=O), 174.8 (C=O), 174.4 (C=O), 174.3 (2 × C=O), 157.4 (C), 131.3 (CH), 129.5 (C), 116.4 (CH), 58.0 (CH, α-Tyr), 50.6 (CH, α-Leu), 50.4 (CH, α-Ala), 39.7 (CH2, β-Leu), 37.6 (CH2NH), 36.1 (CH2CONH), 35.2 (CH2, β-Tyr), 26.0 (CH, γ-Leu), 23.7 (CH3, δ-Leu), 21.2 (CH3, δ-Leu), 17.8 (CH3, β-Ala), 17.1 (CH3, β-Ala); νmax (neat) = 3281, 2956, 1642, 1513, 1237 cm−1; MS (ESI+) m/z 512 [M+Na]+, HRMS (ESI+) calcd. for C23H35N5NaO6 [M+Na]+ 512.2480, found 512.2483; [α]D29 −102 (c 0.05, MeOH).

Preparation of cyclic pentapeptide 233

Boc-Ala-NHCH2C(Me)-Ala-Tyr(Bn)-OBn (227)

To a solution of dipeptide 21912 (1.39 g, 2.60 mmol, 1.0 equiv) in CH2Cl2 (2.0 mL) was added TFA (2.0 mL) and the mixture was stirred for 40 min (Caution – gas evolution!). The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH2Cl2 (3 × 25 mL) and concentrated under reduced pressure to give the crude amine. The residue was dissolved in CH2Cl2 (26 mL) and NEt3 (1.09 mL, 7.80 mmol, 3.0 equiv) and 2-methyl-1-nitroprop-1-ene 226 (252 μL, 2.60 mmol, 1.0 equiv) were added subsequently, and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure to give a yellow oil which was dissolved in EtOAc/PE 1:1 and filtered through a plug of silica gel, eluting with EtOAc/PE 1:1. The eluent was concentrated under reduced pressure to reveal a colourless oil, which was suspended in THF (15 mL). Boc-Ala-OSu (842 mg, 3.00 mmol, 1.2 equiv) and Raney-Ni (slurry in H2O, 3.0 mL) were added subsequently, the solution was placed under an atmosphere of nitrogen, evacuated and filled with hydrogen (balloon). The reaction mixture was stirred vigorously for 5 h at room temperature. Then, the mixture was filtered through a plug of Celite eluting with EtOAc, the filtrate was concentrated under reduced pressure, EtOAc (30 mL) was added, the mixture was washed with saturated Na2CO3 (3 × 30 mL), dried over Na2SO4 and concentrated under reduced pressure. Boc-Ala-NHCH2C(Me)-Ala-Tyr(Bn)-OBn (227) was afforded after purification by column chromatography (SiO2, EtOAc/CH2Cl2 1:1) as a colourless oil (502 mg, 0.75 mmol, 29%). Rf (EtOAc/CH2Cl2 1:1) 0.19; 1H NMR (500 MHz, CDCl3) δH 8.01 (d, J = 8.0 Hz, 1H, NH), 7.45–7.28 (m, 10H, ArH), 6.92 (d, J = 7.7 Hz, 2H, ArH), 6.80 (d, J = 8.4 Hz, 2H, ArH), 6.68 (s, 1H, NH), 5.31–5.24 (m, 2H, CHHPh, NH Boc), 5.12 (d, J = 12.1
Hz, 1H, CH/HPh), 5.00 (s, 2H, CH$_2$Ph), 4.88 (dd, $J = 14.5, 6.3$ Hz, 1H, CH$_\alpha$-Tyr), 4.24–4.15 (m, 1H, CH$_\alpha$-Ala), 3.28 (dd, $J = 13.9, 7.3$ Hz, 1H, CHHGly(Me$_2$)), 3.22 (q, $J = 7.0$ Hz, 1H, CH$_\alpha$-Ala), 3.12 (dd, $J = 14.0, 5.5$ Hz, 1H, CHH$\beta$-Tyr), 3.04 (dd, $J = 14.0, 6.7$ Hz, 1H, CHH$\beta$-Tyr), 2.87 (dd, $J = 13.9, 5.1$ Hz, 1H, CHH(Gly(Me)$_2$)), 1.44 (s, 9H, 3 × CH$_3$, Boc), 1.25 (d, $J = 6.9$ Hz, 3H, CH$_\beta$-Ala), 1.16 (d, $J = 6.9$ Hz, 3H, CH$_\beta$-Ala), 1.00 (s, 3H, CH$_3$Gly(Me$_2$)), 0.94 (s, 3H, CH$_3$Gly(Me$_2$)). Note: Amine NH signal not observed; $^{13}$C NMR (126 MHz, CDCl$_3$) δ$_C$ 176.1 (C=O), 173.4 (C=O), 172.6 (C=O), 158.0 (C), 155.6 (C=O, Boc), 137.1 (C), 135.1 (C), 130.5 (CH), 128.83 (CH), 128.79 (CH), 128.76 (CH), 128.7 (CH), 128.3 (C), 128.1 (CH), 127.6 (CH), 114.9 (CH), 79.9 (C, Boc), 70.1 (CH$_2$, Bn), 67.6 (CH$_2$, Bn), 54.5 (C, Gly(Me$_2$)), 52.6 (CH, $\alpha$-Tyr), 52.0 (CH, $\alpha$-Ala), 50.3 (CH, $\alpha$-Ala), 48.2 (CH$_2$, Gly(Me$_2$)), 37.2 (CH$_2$, $\beta$-Tyr), 28.5 (CH$_3$, Boc), 25.9 (CH$_3$, Gly(Me$_2$)), 23.9 (CH$_3$, Gly(Me$_2$)), 21.9 (CH$_3$, $\beta$-Ala), 18.5 (CH$_3$, $\beta$-Ala); vmax (neat) = 3314, 2972, 1737, 1656, 1509, 1165 cm$^{-1}$; MS (ESI$^+$) $m/z$ 675 [M+H]$^+$, 697 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{38}$H$_{51}$N$_3$O$_7$ [M+H]$^+$ 675.3752, found 675.3756; [α]$_D$ $^0$ = −26.0 (c 0.07, MeOH).

**Chz-Leu-Ala-NHCH$_2$C(Me)$_2$-Ala-Tyr(Bn)-OBn (459)**

Following general procedure 1 from tetrapeptide 227 (472 mg, 0.70 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 96:5:3.5) to give Chz-Leu-Ala-NHCH$_2$C(Me)$_2$-Ala-Tyr(Bn)-OBn (459) (373 mg, 0.45 mmol, 65%) as a white foam. $R_f$ (CH$_2$Cl$_2$/MeOH 19:1) 0.45; m.p 67–70 °C; $^1$H NMR (500 MHz, CDCl$_3$) δ$_H$ 8.02 (d, $J = 8.4$ Hz, 1H, NH), 7.43–7.28 (m, 15H, ArH), 6.93 (d, $J = 8.4$ Hz, 2H, ArH), 6.86–6.78 (m, 4H, ArH, 2 × NH), 5.24 (d, $J = 12.1$ Hz, 1H, CH/HPh), 5.20–4.98 (m, 6H, NH Cbz, CH/HPh, 2 × CH$_2$Ph), 4.92–4.85 (m, 1H, CH$_\alpha$-Tyr), 4.49 (quint, $J = 7.1$ Hz, 1H, CH$_\alpha$-Ala), 4.18 (m, 1H, CH$_\alpha$-Leu), 3.28–3.17 (m, 2H, CH$_\alpha$-Ala, CHHGly(Me$_2$)), 3.12 (dd, $J = 14.0, 5.6$ Hz, 1H, CHH$\beta$-Tyr), 3.03 (dd, $J = 14.0, 6.8$ Hz, 1H, CHH$\beta$-Tyr), 2.91 (dd, $J = 13.9, 5.3$ Hz, 1H, CHHGly(Me$_2$)), 1.71–1.58 (m, 2H, CH$_\gamma$-Leu, CHH$\beta$-Leu), 1.55–1.46 (m, 1H, CHH$\beta$-Leu), 1.25 (d, $J = 7.0$ Hz, 3H, CH$_3$-Ala), 1.16 (d, $J = 7.0$ Hz, 3H, CH$_3$-Ala), 1.00 (s, 3H, CH$_3$Gly(Me$_2$)), 0.97–0.86 (m, 9H, CH$_3$Gly(Me$_2$), 2 × CH$_3$δ-Leu). Note: Amine signal NH not observed; $^{13}$C NMR (126 MHz, CDCl$_3$) δ$_C$ 176.2 (C=O), 172.8 (C=O), 172.6 (C=O), 172.0 (C=O), 158.0 (C), 156.4 (C=O, Boc), 137.1 (C), 136.1 (C), 135.0 (CH), 128.81 (CH), 128.75 (CH), 128.73 (CH), 128.71 (CH), 128.69 (CH), 128.5 (CH), 128.3 (C), 128.2 (CH), 128.1 (CH), 127.6 (CH), 114.9 (CH), 70.1 (CH$_2$, Bn), 67.7 (CH$_2$, Bn), 67.3 (CH$_2$, Bn), 54.5 (C, Gly(Me$_2$)), 54.0 (CH, α-Leu), 52.6 (CH, α-Tyr), 52.0 (CH, α-Ala), 49.1 (CH, α-Ala), 48.7 (CH$_2$, Gly(Me$_2$)), 41.7 (CH$_2$, β-Leu), 37.2 (CH$_2$, β-Tyr), 25.9 (CH,
γ-Leu), 24.9 (CH₃, Gly(Me)₂), 23.7 (CH₃, Gly(Me)₂), 23.1 (CH₃, δ-Leu), 21.9 (CH₃, δ-Leu), 21.8 (CH₃, β-Ala), 18.1 (CH₃, β-Ala); νmax (neat) = 3293, 2961, 1705, 1645, 1509, 1236 cm⁻¹; MS (ESI⁺) m/z 822 [M+H]⁺, 844 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₉₇H₆₀N₃O₈ [M+H]⁺ 822.4436, found 822.4428; [α]D²⁰ +37.0 (c 0.11, MeOH).

**H-Leu-Ala-NHCH₂(C(Me)₂)-Ala-Tyr-OH (228)**

Following general procedure 5 from Cbz-Leu-Ala-NHCH₂(C(Me)₂)-Ala-Tyr-OBn (459) (329 mg, 0.40 mmol, 1.0 equiv), H-Leu-Ala-NHCH₂(C(Me)₂)-Ala-Tyr-OH (228) was obtained as a white solid (203 mg, 0.40 mmol, quant. yield), which required no further purification; m.p 144–147 °C; ¹H NMR (500 MHz, CD₂OD) δH 7.03 (d, J = 8.3 Hz, 2H, ArH), 6.65 (d, J = 8.3 Hz, 2H, ArH), 4.46 (dd, J = 8.0, 4.7 Hz, 1H, CHα-Tyr), 4.38 (q, J = 7.1 Hz, 1H, CHα-Ala), 3.99–3.93 (m, 1H, CHα-Leu), 3.55 (q, J = 6.6 Hz, 1H, CHα-Ala), 3.31–3.28 (m, 1H, CHHδGly(Me)₂), 3.12 (dd, J = 13.9, 4.6 Hz, 1H, CHHβ-Tyr), 2.90 (dd, J = 13.9, 8.2 Hz, 1H, CHHβ-Tyr), 2.74 (d, J = 14.0 Hz, 1H, CHHδGly(Me)₂), 1.81–1.62 (m, 3H, CHβ-Leu, CHβ-Ala), 1.34 (d, J = 7.2 Hz, 3H, CHβ-Ala), 1.31 (d, J = 7.0 Hz, 3H, CHβ-Ala), 1.05 (s, 3H, CH₃Gly(Me)₂), 1.01 (s, 3H, CH₃Gly(Me)₂), 0.99 (d, J = 5.8 Hz, 3H, CH₃δ-Leu), 0.97 (d, J = 5.8 Hz, 3H, CH₃δ-Leu). Note: CHHδGly(Me)₂ signal partially overlaps with solvent peak; ¹³C NMR (126 MHz, CD₂OD) δC 177.0 (C=O), 175.3 (2 x C=O), 170.8 (C=O), 157.2 (C), 131.5 (CH), 129.8 (C), 116.1 (CH), 58.1 (C, Gly(Me)₂), 56.7 (CH, α-Tyr), 53.1 (CH, α-Leu), 53.0 (CH, α-Ala), 51.2 (CH, α-Ala), 48.3 (CH₂, Gly(Me)₂), 41.7 (CH₂, β-Leu), 38.4 (CH₂, β-Tyr), 25.4 (CH, γ-Leu), 24.4 (CH₂, Gly(Me)₂), 23.3 (CH₂, Gly(Me)₂), 23.2 (CH₂, δ-Leu), 22.2 (CH₂, δ-Leu), 20.4 (CH₃, β-Ala), 18.0 (CH₃, β-Ala); νmax (neat) = 3226, 1655, 1512, 1449, 1234 cm⁻¹; MS (ESI⁺) m/z 508 [M+H]⁺, 530 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₉H₂₀N₅O₈ [M+H]⁺ 508.3130, found 508.3133; [α]D²⁰ +4.0 (c 0.07, MeOH).

**Cyclo(Leu-Ala-NHCH₂C(Me)₂)-Ala-Tyr** (233)

Following general procedure 6, H-Leu-Ala-NHCH₂C(Me)₂-Ala-Tyr-OH (228) (50.7 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPB (60 mg, 0.2 mmol, 2.0 equiv) at 1 mM for 24 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→9:1) to give the cyclic pentapeptide (233) as a white solid (1st run: 15.9 mg, 33 μmol, 33%; 2nd run: 15.4 mg, 31 μmol, 31%). Rf (CH₂Cl₂/MeOH 9:1) 0.38; m.p 178–180 °C; ¹H NMR (500 MHz, CD₂OD) δH 7.08 (d, J = 8.4 Hz,
2H, ArH), 6.71 (d, J = 8.4 Hz, 2H, ArH), 4.41 (q, J = 7.2 Hz, 1H, CHα-Ala), 4.35 (t, J = 7.9 Hz, 1H, CHα-Tyr), 4.14 (dd, J = 10.5, 4.9 Hz, 1H, CHα-Leu), 3.28 (q, J = 7.0 Hz, 1H, CHα-Ala), 3.16 (d, J = 13.9 Hz, 1H, CHHGOx(Me)2), 3.12 (dd, J = 13.6, 9.3 Hz, 1H, CHHβ-Tyr), 3.06 (dd, J = 13.6, 7.5 Hz, 1H, CHHβ-Tyr), 2.97 (d, J = 13.9 Hz, 1H, CHHGOx(Me)2), 1.85 (ddd, J = 14.0, 10.9, 4.9 Hz, 1H, CHHβ-Leu), 1.61–1.52 (m, 1H, CHHβ-Leu), 1.43 (d, J = 7.2 Hz, 3H, CHβ-Ala), 1.41–1.33 (m, 1H, CHγ-Leu), 1.17 (d, J = 7.0 Hz, 3H, CHβ-Ala), 1.06 (s, 3H, CH3Gly(Me)2), 0.95–0.89 (m, 6H, CH3Gly(Me)2), CHδ-Leu), 0.83 (d, J = 6.5 Hz, 3H, CHδ-Leu).

Note: CHα-Ala signal partially overlaps with solvent signal; 13C NMR (126 MHz, CD3OD) δc 179.3 (C=O), 175.3 (C=O), 174.6 (C=O), 173.7 (C=O), 157.4 (C), 131.3 (C), 128.9 (CH), 116.3 (CH), 57.6 (CH, α-Tyr), 56.2 (CH, α-Leu), 55.9 (C, Gly(Me)2), 52.8 (CH, α-Ala), 51.4 (CH, α-Ala), 49.2 (CH2, Gly(Me)2), 40.8 (CH2, β-Leu), 36.3 (CH2, β-Tyr), 26.6 (CH3, Gly(Me)2), 25.8 (CH3, γ-Leu), 23.4 (CH2, δ-Leu), 22.8 (CH3, Gly(Me)2), 21.9 (CH3, β-Ala), 21.6 (CH3, δ-Leu), 18.3 (CH3, β-Ala); νmax (neat) = 3259, 2960, 1649, 1513, 1448, 1302 cm−1; MS (ESI+) m/z 490 [M+H]+, 512 [M+Na]+; HRMS (ESI+) calcd. for C23H40N6O5 [M+H]+ 490.3024, found 490.3027; [α]D29 ≈ −71.0 (c 0.04, MeOH).

Deprotection of 183

Cyclo(Tyr-Leu-GOx-Gly) (236)

Cyclo-(Tyr(Bu)-Leu-GOx-Gly) (183) (24 mg, 50 μmol, 1.0 equiv) was suspended in CH2Cl2 (0.8 mL) and to the solution was added TIS (0.05 mL) and TFA (0.15 mL). The solution was stirred under an atmosphere of N2 for 15 min, then reduced in vacuo. The crude residue was purified by column chromatography (CH2Cl2/MeOH 9:1→6:1) to give the cyclic tetrapeptide (236) as a glassy white solid (15 mg, 36 μmol, 72%); Rf (CH2Cl2/MeOH 6:1) 0.49; m.p 182 – 185 °C; 1H NMR (500 MHz, DMSO) δ 9.24 (s, 1H, OH), 8.22 (d, J = 10.4 Hz, 1H, NH), 8.07 (d, J = 9.2 Hz, 1H, NH), 7.55 (d, J = 4.3 Hz, 1H, NH), 7.01 (d, J = 8.3 Hz, 2H, ArH), 6.65 (d, J = 8.3 Hz, 2H, ArH), 4.44 – 4.36 (m, 2H, CHα-Tyr, CHH-Ox), 4.18 (d, J = 6.9 Hz, 1H, CHH-Ox), 4.13 (d, J = 6.3 Hz, 1H, CHH-Ox), 4.02 (td, J = 10.0, 5.4 Hz, 1H, CHα-Leu), 3.89 (d, J = 6.8 Hz, 1H, CHH-Ox), 3.78 (dd, J = 13.4, 7.7 Hz, 1H, CHHGOx), 3.19 (d, J = 15.3 Hz, 1H, CHHGOx), 3.02 – 2.93 (m, 2H, CHHGOx, CHHβ-Tyr), 2.85 (dd, J = 14.0, 8.2 Hz, 1H, CHHβ-Tyr), 1.72 – 1.60 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.49 (m, 1H, CHHβ-Leu), 0.94 (d, J = 6.3 Hz, 3H, CHδ-Leu), 0.83 (t, J = 8.3 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, DMSO) δ 173.2 (C=O), 172.9 (C=O), 171.2 (C=O), 155.9 (C), 129.8 (CH), 127.1 (C), 115.1 (CH), 78.2 (CH3, Ox), 76.5 (CH2, Ox), 60.3 (C, Ox), 56.9 (CH, α-Tyr), 54.0 (CH, α-Leu), 47.2 (CH2, Gly), 179
44.2 (CH$_3$, GOx), 39.1 (CH$_3$, β-Leu), 35.4 (CH$_3$, β-Tyr), 24.7 (CH, γ-Leu), 22.9 (CH, δ-Leu), 21.2 (CH$_3$, δ-Leu); ν$_{\text{max}}$ (neat) = 3262, 2923, 1656, 1514, 696 cm$^{-1}$; MS (ESI$^+$) m/z 441 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{21}$H$_{30}$N$_4$NaO$_5$ [M+Na]$^+$ 441.2095, found 441.2096; [α]$_D^{30}$ –60.5 (c 0.08, MeOH).

**Deprotection of 201**

Cyclo(Leu-Tyr-Val-GOx-Thr-Phe) (238)

Cyclo(Leu-Tyr('Bu)-Val-GOx-Thr('Bu)-Phe) (201) (30 mg, 37 µmol, 1.0 equiv) was suspended in CH$_2$Cl$_2$ (0.5 mL) and to the solution was added TFA (0.5 mL). The solution was stirred under an atmosphere of N$_2$ for 1 h min, then reduced in vacuo. The crude residue was purified by column chromatography (CH$_2$Cl$_2$/MeOH 9:1→6:1) to give the cyclic hexapeptide (238) as a glassy white solid (5 mg, 7 µmol, 18%); m.p 176 – 179 ºC; $^1$H NMR (600 MHz, MeOD) δ 7.20 (m, 2H, ArH), 7.13 (m, 3H, ArH), 6.94 (d, $J$ = 8.3 Hz, 2H, ArH), 6.61 (d, $J$ = 8.4 Hz, 2H, ArH), 4.58 (d, $J$ = 6.6 Hz, 1H, CHα-Tyr), 4.31 – 4.23 (m, 3H, CH$_2$-Ox, CHα-Phe), 3.99 (dd, $J$ = 11.8, 4.2 Hz, 1H, CHβ-Phe), 3.89 (d, $J$ = 14.2 Hz, 1H, CHHGOx), 3.62 (t, $J$ = 7.5 Hz, 1H, CHα-Leu), 3.35 (m, 1H, CHHβ-Thr), 3.06 – 3.00 (m, 1H, CHβ-Leu), 2.96 (d, $J$ = 6.8 Hz, 1H, CHβ-Thr), 2.60 (dd, $J$ = 14.0, 10.3 Hz, 1H, CHHβ-Thr), 1.95 – 1.86 (m, 1H, CHβ-Val), 1.40 – 1.31 (m, 1H, CHHβ-Leu), 1.28 – 1.22 (m, 1H, CHHβ-Leu), 0.96 – 0.89 (m, 7H, CH$_3$γ-Val, CH$_3$γ-Thr, CHγ-Leu), 0.84 (dd, $J$ = 6.3 Hz, 3H, CH$_3$γ-Val), 0.69 (d, $J$ = 6.5 Hz, 3H, CH$_3$δ-Leu), 0.65 (d, $J$ = 6.5 Hz, 3H, CH$_3$δ-Leu). Note: CHHβ-Tyr, CHHβ-Phe overlaps with solvent peak; $^{13}$C NMR (151 MHz, MeOD) δ 175.9 (C=O), 174.9 (C=O), 174.3 (C=O), 174.4 (C=O), 173.1 (C=O), 157.3 (C), 137.7 (C), 131.0 (CH), 130.2 (C), 129.7 (CH), 128.1 (CH), 116.3 (CH), 80.0 (CH$_2$, Ox), 79.5 (CH$_2$, Ox), 69.4 (CH, α-Thr), 66.2 (CH, β-Thr), 62.5 (CH, α-Phe), 62.0 (C, Ox), 59.5 (CH, α-Val), 55.8 (CH, α-Leu), 54.1 (CH, α-Tyr), 46.0 (CH$_2$, GOx), 40.4 (CH$_2$, β-Leu), 39.6 (CH$_2$, β-Phe), 35.1 (CH$_2$, β-Tyr), 33.3 (CH, β-Val), 25.0 (CH, γ-Leu), 22.8 (CH$_3$, γ-Val), 22.7 (CH$_3$, γ-Val), 20.7 (CH$_3$, γ-Thr), 19.8 (CH$_3$, δ-Leu), 19.6 (CH$_3$, δ-Leu); ν$_{\text{max}}$ (neat) = 3272, 2993, 1634, 1515, 699 cm$^{-1}$; MS (ESI$^+$) m/z 709 [M+H]$^+$, 731 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{37}$H$_{52}$N$_6$O$_8$ [M+Na]$^+$ 731.3739, found 731.3737; [α]$_D^{30}$ = –44.4 (c 0.04, MeOH).
Preparation of cyclic pentapeptide 241

Cyclo(Trp-GOx-Gly-Ser-Gly)

Following general procedure 9 from H-Gly-2CITrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GOx-Gly-OCumyl (203) (300 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (105 mg, 144 µmol, 96%). This material was subjected to cyclisation to yield 209 compound after repeat column chromatography (SiO2, CH2Cl2:MeOH 39:1 → 19:1) as a glassy colourless solid (47 mg, 66 µmol, 46%). A portion of this material (20 mg, 28 µmol, 1.0 equiv) was suspended in CH2Cl2 (0.8 mL) and to the solution was added TIS (50 µL) and TFA (0.15 mL). The solution was stirred at RT for 15 min and reduced in vacuo, resuspended in CH2Cl2 and reduced in vacuo (3 ×). The crude material was purified by flash column chromatography (CH2Cl2/MeOH 9:1→4:1) to yield a white glassy solid (12.6 mg, 27 µmol, 95%); Rf (CH2Cl2/MeOH 9:1) 0.22; m.p 240 – 242 ºC (decomp.); 1H NMR (500 MHz, MeOD) δ H 7.59 (d, J = 7.9 Hz, 1H, ArH), 7.32 (d, J = 8.0 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 7.08 (t, J = 7.4 Hz, 1H, ArH), 7.01 (t, J = 7.4 Hz, 1H, ArH), 4.74 (dd, J = 7.7, 5.4 Hz, 1H, CHα-Trp), 4.46 (d, J = 6.9 Hz, 1H, CHH-Ox), 4.37 (d, J = 6.1 Hz, 1H, CHH-Ox), 4.33 – 4.26 (m, 2H, CHα-Ser, CHH-Ox), 4.20 (d, J = 6.6 Hz, 1H, CHH-Ox), 4.02 (d, J = 15.6 Hz, 1H, CHH-Gly), 3.91 (dd, J = 11.1, 6.0 Hz, 1H, CHHβ-Ser), 3.86 (dd, J = 11.2, 5.8 Hz, 1H, CHHβ-Ser), 3.71 (d, J = 14.0 Hz, 1H, CHH-GOx), 3.56 (d, J = 15.6 Hz, 1H, CHH-Gly), 3.49 – 3.39 (m, 2H, CHHβ-Trp, CHH-Gly), 3.20 (dd, J = 14.6, 8.0 Hz, 1H, CHHβ-Trp), Note: CHH-GOx and CHH-Gly overlaps with solvent signal; 13C NMR (126 MHz, MeOD) δ C 175.7 (C=O), 174.6 (C=O), 173.3 (C=O), 171.8 (C=O), 138.1 (C), 128.8 (C), 124.8 (CH), 122.4 (CH), 119.8 (CH), 119.4 (CH), 112.3 (CH), 110.7 (C), 80.9 (CH2, Ox), 80.1 (CH2, Ox), 61.4 (CH2, β-Ser), 61.2 (C, OX), 56.6 (CH, α-Ser), 56.0 (CH, β-Trp), 46.5 (CH2, Gly), 45.4 (CH2, Gly), 44.8 (CH2, GOx), 28.7 (CH2, β-Trp); νmax (neat) = 3068, 1655, 1535, 1183, 536 cm⁻¹; MS (ESI⁺) m/z 495 [M+Na]+; HRMS (ESI⁺) calcd. for C22H28N6NaO6 [M+Na]+ 495.1963, found 495.1956; [α]20B –11.0 (c 0.09, MeOH).
Preparation of azetidinones & nitroalkenes

\textit{tert}-Butyl 3-(nitromethylene)azetidine-1-carboxylate (258)

\textit{tert}-Butyl 3-oxoazetidine-1-carboxylate (5.14 g, 30.0 mmol, 1.0 equiv) was suspended in nitromethane (30 mL). Triethylamine (0.84 mL, 6.0 mmol, 0.2 equiv) was added and the reaction mixture was stirred at room temperature for 90 min. THF (300 mL) was added and the reaction cooled to −78 °C. Triethylamine (8.36 mL, 60.0 mmol, 2.0 equiv) was added followed by the dropwise addition of methanesulfonyl chloride (2.79 mL, 36.0 mmol, 1.2 equiv). The reaction mixture was left to stir at −78 °C for 90 min, after which the dry-ice bath was removed, and the reaction mixture stirred for 60 min. The reaction mixture was filtered through a plug of silica gel eluting with 25% EtOAc in PET ether. The eluent was concentrated under reduced pressure and purified by column chromatography (SiO\textsubscript{2}; 85:15 PET ether/EtOAc) to yield the title compound 258 as a yellow solid (5.14 g, 24.0 mmol, 80%); \textit{R}\textsubscript{f} (85:15 PET ether/EtOAc) 0.22; m.p. 64 – 67 °C; \textit{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) δ 7.06 – 7.01 (m, 1H, CH), 5.05 – 4.97 (m, 2H, CH\textsubscript{2}, Az), 4.73 – 4.66 (m, 2H, CH\textsubscript{2}, Az), 1.45 (s, 9H, CH\textsubscript{3}, Boc); \textit{\textsuperscript{13}C NMR} (126 MHz, CDCl\textsubscript{3}) δC 156.0 (C=O, Boc), 149.2 (C, Az), 132.5 (CH), 81.0 (C, Boc), 59.8 (CH\textsubscript{2}, Az), 28.4 (CH\textsubscript{3}, Boc); \textit{\nu}_{\text{max}} \text{ (neat)} = 1689, 1523, 1364, 1149 cm\textsuperscript{-1}; MS (ESI\textsuperscript{+}) \textit{m/z} 451 \textit{[2M+Na]}\textsuperscript{+}; HRMS (ESI\textsuperscript{+}) calcd. for C\textsubscript{9}H\textsubscript{14}N\textsubscript{2}NaO\textsubscript{4} [M+Na]\textsuperscript{+} 237.0846, found 237.0849.

\textit{tert}-Butyl 3-(1-nitroethylidene)azetidine-1-carboxylate (260)

\textit{tert}-Butyl 3-oxoazetidine-1-carboxylate (8.56 g, 50.0 mmol, 1.0 equiv) was suspended in nitroethane (10 mL). Triethylamine (1.39 mL, 10.0 mmol, 0.2 equiv) was added and the reaction mixture was stirred at room temperature for 90 min. THF (250 mL) was added and the reaction was cooled to −78 °C. Triethylamine (14.0 mL, 100.0 mmol, 2.0 equiv) was added followed by the dropwise addition of methanesulfonyl chloride (4.64 mL, 60.0 mmol, 1.2 equiv). The reaction mixture was left to stir at −78 °C for 90 min, after which the dry-ice bath was removed, and the reaction mixture stirred for 60 min. The reaction mixture was filtered through a plug of silica gel eluting with 25% EtOAc in PET ether. The eluent was concentrated under reduced pressure and purified by column chromatography (SiO\textsubscript{2}; 85:15 PET ether/EtOAc) to yield the title compound 260 as a colourless oil (9.51 g, 41.7 mmol, 84%), which became a pale white solid upon storage at 0 °C; \textit{R}\textsubscript{f} (PET ether/EtOAc 85:15) 0.25; m.p. 56 – 59 °C; \textit{\textsuperscript{1}H NMR} (500 MHz, CDCl\textsubscript{3}) δ\textit{H} 4.92 (d, \textit{J} = 1.6 Hz, 2H, CH\textsubscript{2}-Az), 4.62 (s, 2H, CH\textsubscript{2}-Az), 2.07 (m, 3H, CH\textsubscript{3}), 1.46 (s, 9H, CH\textsubscript{3}, Boc); \textit{\textsuperscript{13}C NMR} (126 MHz, CDCl\textsubscript{3}) δC 156.1 (C=O, Boc), 142.6 (C, Az), 140.0 (C=CCH\textsubscript{3}), 80.6 (C, Boc), 28.4 (CH\textsubscript{3}, Boc), 13.5 (CH\textsubscript{3}); \textit{\nu}_{\text{max}} \text{ (neat)} =
1731, 1691, 1474, 1333, 1156 cm⁻¹; MS (ESI⁺) m/z 479 [2M+Na]⁺; HRMS (ESI⁺) calcd. for C₁₀H₁₆N₂NaO₄ [M+Na]⁺ 251.1002, found 251.1002.

**Prop-2-yn-1-yl 3-oxoazetidine-1-carboxylate (263)**

To a solution of tert-butyl 3-oxoazetidine-1-carboxylate (583 mg, 3.41 mmol, 1.0 equiv) in CH₂Cl₂ (25 mL) was added TFA (6.25 mL, 20% v/v) and left to stir until full consumption of the starting material was observed (TLC monitoring). The solution was reduced in vacuo and resuspended in CH₂Cl₂, then reduced in vacuo (3×). The crude residue was suspended in THF (25 mL) and triethylamine (1.21 mL, 8.87 mmol, 2.6 equiv) and 2,5-dioxopyrrolidin-1-yl prop-2-yn-1-yl carbonate 262 (873 mg, 4.40 mmol, 1.3 equiv) were added subsequently. The solution was left to stir for 16 h at RT, reduced in vacuo, resuspended in EtOAc (25 mL) then quenched with sat. aq. Na₂CO₃ (25 mL). The organic layer was separated and washed with sat. aq. Na₂CO₃ (2 × 25 mL), brine (25 mL), dried (Na₂SO₄), filtered and concentrated in vacuo to give a white solid which was purified by column chromatography (SiO₂, PET ether/EtOAc 85:15) to give the title compound 263 as a white solid (386 mg, 2.52 mmol, 74%); Rf (PET ether/EtOAc 85:15) 0.30; m.p. 68 – 70 °C; ¹H NMR (400 MHz, CDCl₃) δH 4.81 (s, 4H, 2 × CH₂-Az), 4.75 (d, J = 2.4 Hz, 2H, OCH₂), 2.51 (s, 1H, C≡CH); ¹³C NMR (101 MHz, CDCl₃) δC 195.2 (C=O), 155.4 (C=O), 77.9 (C=C), 75.3 (C≡CH), 71.6 (2 × CH₂-Az), 53.6 (OCH₂); νmax (neat) = 3235, 2123, 1817, 1778, 1697 cm⁻¹; MS unable to be detected; HRMS (ESI⁺) calcd. for C₇H₇NNaO₃ [M+Na]⁺ 176.0318, found 176.0317.

**Prop-2-yn-1-yl 3-(nitromethylene)azetidine-1-carboxylate (264)**

Azetidinone 263 (1.27 g, 8.30 mmol, 1.0 equiv) was suspended in nitromethane (8 mL). Triethylamine (0.23 mL, 1.66 mmol, 0.2 equiv) was added and the reaction mixture was stirred at room temperature for 90 min. CH₂Cl₂ (83 mL) was added and the reaction was cooled to −78 °C. Triethylamine (3.45 mL, 24.8 mmol, 3.0 equiv) was added followed by the dropwise addition of methanesulfonyl chloride (1.60 mL, 20.7 mmol, 2.5 equiv). The reaction mixture was left to stir for 90 min, after which the dry-ice bath was removed, and the reaction mixture stirred for 60 min. The reaction mixture was filtered through a plug of silica gel eluting with 25 % EtOAc in PET ether. The eluent was concentrated under reduced pressure and purified by column chromatography (SiO₂, PET ether/EtOAc 7:3) to yield the title compound 264 as an orange oil (1.29 g, 6.60 mmol, 80%); Rf (PET ether/EtOAc 7:3) 0.33; ¹H NMR (500 MHz, CDCl₃) δH 7.09 – 7.04 (m, 1H, C=CH), 5.16 – 5.11 (m, 2H, CH₂-Az), 4.83 – 4.80 (m, 2H, CH₂-Az), 4.72 (d, J = 2.4 Hz, 2H, OCH₂), 2.50 (t, J = 2.4 Hz, 1H, C≡CH); ¹³C NMR (126 MHz, CDCl₃) δC 155.2 (C=O), 148.0 (C, Az), 132.8 (C=CH), 75.3 (C≡CH), 60.1 (CH₂-Az), 56.0 (CH₂, Az), 53.3 (OCH₂), Note: C≡CH not observed;
$\nu_{\text{max}}$(neat) = 3288, 1715, 1556, 1349 cm$^{-1}$; MS unable to be detected; HRMS (ESI$^+$) calcd. for C$_8$H$_8$N$_2$NaO$_4$ [M+Na]$^+$ 219.0376, found 219.0382.

Solution-phase synthesis of linear precursors & cyclic peptides

Preparation of pentapeptide 277

NO$_2$-GAz(Boc)-Ala-Tyr(OBn)-OBn (274)

To a solution of dipeptide 219$^{112}$ (5.3 g, 10.0 mmol, 1.0 equiv) in CH$_2$Cl$_2$ (5.0 mL) was added TFA (5 mL) and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH$_2$Cl$_2$ (3 × 50 mL) and concentrated under reduced pressure to give the crude amine. This was added to the nitroalkene formed $in$-situ following general procedure 3. The crude residue was purified by column chromatography (SiO$_2$, PET ether/EtOAc 1:1) to yield the title compound 274 (3.8 g, 5.9 mmol, 59%) as an orange oil; $R_f$ (PET ether/EtOAc 1:1) 0.31; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.46 – 7.30 (m, 11H, ArH, NH), 6.92 (d, $J = 8.5$ Hz, 2H, ArH), 6.83 (d, $J = 8.5$ Hz, 2H, ArH), 5.17 (d, $J = 12.1$ Hz, 1H, C$_H$HPh), 5.12 (d, $J = 12.0$ Hz, 1H, C$_H$HPh), 5.03 (s, 2H, CH$_2$Ph), 4.79 (dd, $J = 14.2$, 6.4 Hz, 1H, CH$_2$Tyr), 4.54 (d, $J = 13.7$ Hz, 1H, CHHNO$_2$), 4.47 (d, $J = 13.7$ Hz, 1H, CHHNO$_2$), 3.93 – 3.63 (m, 4H, 2 × CH$_2$-Az), 3.34 (q, $J = 6.6$ Hz, 1H, CH$_H$-Ala), 3.10 (dd, $J = 14.1$, 5.7 Hz, 1H, CH$_H$-Tyr), 3.00 (dd, $J = 14.1$, 6.7 Hz, 1H, CH$_H$-β-Tyr), 1.91 (s, $J = 13.9$ Hz, 1H, NH), 1.43 (s, 9H, CH$_3$Boc), 1.26 (d, $J = 7.0$ Hz, 3H, CH$_3$-β-Ala); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ C 174.2 (C=O), 171.2 (C=O), 158.0 (C=O Boc), 156.0 (C), 137.1 (C), 135.3 (C), 130.4 (CH), 128.81 (CH), 128.76 (CH), 128.69 (2 × CH), 128.1 (CH), 128.0 (C), 127.6 (CH), 115.0 (CH), 80.6 (C, Boc), 79.6 (CH$_2$NO$_2$), 70.0 (CH$_2$Ph), 67.4 (CH$_2$Ph), 56.7 (2 × CH$_2$, Az), 54.0 (C, Az), 52.90 (CH, α-Tyr), 52.87 (CH, α-Ala), 36.9 (CH$_2$, β-Tyr), 28.4 (CH$_3$, Boc), 20.9 (CH$_3$, β-Ala); $\nu_{\text{max}}$(neat) = 2977, 1735, 1692, 1555, 1371, 756 cm$^{-1}$; MS (ESI$^+$) $m/z$ 647 [M+H]$^+$, 669 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{35}$H$_{42}$N$_4$NaO$_8$ [M+Na]$^+$ 669.2895, found 669.2876; [α]$^D_{257}$ –5.3 (c 0.29, CHCl$_3$).

Fmoc-Ala-GAz(Boc)-Ala-Tyr(OBn)-OBn (275)

Following general procedure 4 from NO$_2$-GAz(Boc)-Ala-Tyr(OBn)-OBn (274) (3.4 g, 5.3 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/EtOAc 7:3) to yield (SiO$_2$, CH$_2$Cl$_2$/EtOAc 4:1$\rightarrow$ 1:1) to give the title compound 275 as a white foam (2.6 g, 2.90 mmol,
55%). \( \text{R}_{1} (\text{CH}_{2}\text{Cl}_{2}/\text{EtOAc} \ 1:1) \ 0.27; \ \text{m.p} \ 94 – 97 ^\circ \text{C}; \ \text{\textsuperscript{1}H NMR} \ (500 \text{MHz, CDCl}_{3}) \ \delta_{\text{H}} \ 7.75 \ (d, \ J = 7.5 \text{ Hz}, 2\text{H, ArH}), 7.63 – 7.56 \ (m, 3\text{H, NH, ArH}), 7.40 – 7.29 \ (m, 14\text{H, ArH}), 7.00 \ (s, 1\text{H, NH}), 6.88 \ (d, \ J = 7.9 \text{ Hz}, 2\text{H, ArH}), 6.77 \ (d, \ J = 8.5 \text{ Hz}, 2\text{H, ArH}), 5.57 \ (d, \ J = 7.5 \text{ Hz}, 1\text{H, NH Fmoc}), 5.23 \ (d, \ J = 12.1 \text{ Hz}, 1\text{H, CHHPh}), 5.08 \ (d, \ J = 12.0 \text{ Hz}, 1\text{H, CHHPh}), 4.98 \ (s, 2\text{H, CH}_{2}\text{Ph}), 4.90 \ (dt, \ J = 7.6, 5.9 \text{ Hz}, 1\text{H, CH}\alpha-\text{Tyr}), 4.43 – 3.47 \ (m, 2\text{H, CH}_{2}\text{Fmoc}), 4.32 – 4.24 \ (m, 1\text{H, CH}\beta-\text{Ala}), 4.21 \ (t, \ J = 6.9 \text{ Hz}, 1\text{H, CH Fmoc}), 3.85 – 3.63 \ (m, 3\text{H, CHHGAz, 2} \times \text{CHH-Az}), 3.57 – 3.50 \ (m, 2\text{H, 2} \times \text{CHH-Az}), 3.18 – 2.94 \ (m, 4\text{H, CHHGAz, CH}\beta-\text{Tyr, CH}\alpha-\text{Ala}), 1.43 \ (s, 9\text{H, CH}_{3}\text{ Boc}), 1.28 \ (d, \ J = 6.8 \text{ Hz}, 3\text{H, CH}_{3}\beta-\text{Ala}), 1.15 \ (d, \ J = 6.7 \text{ Hz}, 3\text{H, CH}_{3}\beta-\text{Ala}); \ \text{\textsuperscript{13}C NMR} \ (126 \text{MHz, CDCl}_{3}) \ \delta \ 175.0 \ (\text{C}=\text{O}), 173.7 \ (\text{C}=\text{O}), 173.2 \ (\text{C}=\text{O}), 158.0 \ (\text{C}), 156.3 \ (\text{C}=\text{O} \text{Fmoc}), 156.0 \ (\text{C}=\text{O} \text{Boc}), 143.9 \ (\text{C}), 141.5 \ (\text{C}), 137.0 \ (\text{C}), 134.9 \ (\text{C}), 130.4 \ (\text{CH}), 128.9 \ (\text{CH}), 128.8 \ (\text{CH}), 128.7 \ (\text{CH}), 128.1 \ (\text{CH}), 127.9 \ (\text{CH}), 127.6 \ (\text{CH}), 127.24 \ (\text{CH}), 125.23 \ (\text{CH}), 125.17 \ (\text{CH}), 120.2 \ (\text{CH}), 115.0 \ (\text{CH}), 80.1 \ (\text{C, Boc}), 70.0 \ (\text{CH}_{2}\text{Ph}), 68.0 \ (\text{CH}_{2}\text{Ph}), 67.0 \ (\text{CH}_{2}\text{Ph Fmoc}), 56.4 \ (\text{CH}_{2}, \text{Az}), 55.4 \ (\text{CH}_{2}, \text{Az}), 53.5 \ (\text{CH, CH}\beta-\text{Ala}), 52.6 \ (\text{CH, CH}\alpha-\text{Tyr}), 50.5 \ (\text{CH, CH}\alpha-\text{Ala}), 47.3 \ (\text{CH, Fmoc}), 37.0 \ (\text{CH}_{2}, \text{CH}\beta-\text{Tyr}), 28.5 \ (\text{CH}_{3}, \text{Boc}), 21.5 \ (\text{CH}_{3}, \text{CH}\beta-\text{Ala}), 18.9 \ (\text{CH}_{2}, \text{CH}\beta-\text{Ala}). \ \text{Note: CH}_{2}, \text{GAz and C, Az not observed; } \nu_{\max} \ (\text{neat}) = 2955, 1690, 1655, 1121, 753 \ \text{cm}^{-1}; \ \text{MS (ESI\textsuperscript{+})} \ m/z \ 910 \ [\text{M+H}]^{+}, 932 \ [\text{M+Na}]^{+}; \ \text{HRMS (ESI\textsuperscript{+})} \ \text{calcd. for } \text{C}_{53}\text{H}_{66}\text{N}_{3}\text{O}_{5} \ [\text{M+H}]^{+} \ 910.4386, \ \text{found} \ 910.4388; \ [\alpha]_{D}^{\text{27}} = -10.1 \ (\text{c} 0.23, \text{CHCl}_{3}).

\text{Cbz-Leu-Ala-GAza(Boc)-Ala-Tyr(Obn)-Obn (460)}$

Following general procedure 2 from \text{Fmoc-Ala-GAza(Boc)-Ala-Tyr(Obn)-Obn (275)} \ (2.17 \ \text{g, 2.39 mmol, 1.0 equiv}), the crude residue was purified by column chromatography (SiO\textsubscript{2}, \text{CH}_{2}\text{Cl}_{2}/\text{MeOH} \ 97:3) to give the title compound \textbf{460} \ (1.6 \ \text{g, 1.68 mmol, 70%}) as a colourless oil; \text{R}_{1} \ (\text{CH}_{2}\text{Cl}_{2}/\text{MeOH} \ 97:3) \ 0.18; \ \text{m.p} \ 85 – 88 \ \text{C}; \ \text{\textsuperscript{1}H NMR} \ (500 \text{MHz, CDCl}_{3}) \ \delta \ 7.77 \ (s, 1\text{H, NH}), 7.42 – 7.28 \ (m, 16\text{H, ArH, NH}), 7.01 – 6.90 \ (m, 3\text{H, NH, ArH}), 6.80 \ (d, \ J = 8.5 \text{ Hz}, 2\text{H, ArH}), 5.37 \ (d, \ J = 6.9 \text{ Hz}, 1\text{H, NH Fmoc}), 5.23 \ (d, \ J = 12.1 \text{ Hz}, 1\text{H, CHHPh}), 5.12 – 5.06 \ (m, 2\text{H, CHHPh, CHHPh}), 5.04 – 4.98 \ (m, 3\text{H, CHHPh, CH}_{2}\text{Ph}), 4.88 \ (dd, \ J = 13.8, 7.7 \text{ Hz}, 1\text{H, CH}\alpha-\text{Tyr}), 4.45 \ (p, \ J = 7.0 \text{ Hz}, 1\text{H, CH}\alpha-\text{Ala}), 4.21 – 4.13 \ (m, 1\text{H, CH}\alpha-\text{Leu}), 3.78 – 3.53 \ (m, 5\text{H, 2} \times \text{CH}_{2}-\text{Az, CHHGAz}), 3.25 – 2.97 \ (m, 4\text{H, CHHGAz, CH}\alpha-\text{Ala, CH}\beta-\text{Tyr}), 2.29 \ (s, 1\text{H, NH}), 1.69 – 1.60 \ (m, 2\text{H, CH} \gamma-\text{Leu, CHH}\beta-\text{Leu}), 1.53 – 1.49 \ (m, 1\text{H, CHH}\beta-\text{Leu}), 1.40 \ (s, 9\text{H, CH}_{3}\text{ Boc}), 1.23 \ (d, \ J = 6.9 \text{ Hz}, 3\text{H, CH}_{3}\beta-\text{Ala}), 1.18 \ (d, \ J = 6.6 \text{ Hz}, 3\text{H, CH}_{3}\beta-\text{Ala}), 0.92 \ (d, \ J = 5.9 \text{ Hz}, 6\text{H, 2} \times \text{CH}_{2}\delta-\text{Leu}); \ \text{\textsuperscript{13}C NMR} \ (126 \text{MHz, CDCl}_{3}) \ \delta \ 175.2 \ (\text{C}=\text{O}), 173.2 \ (\text{C}=\text{O}), 172.7 \ (\text{C}=\text{O}), 172.2 \ (\text{C}=\text{O}), 157.9 \ (\text{C}), 156.5 \ (\text{C}=\text{O} \text{Cbz}), 156.2 \ (\text{C}=\text{O} \text{Boc}), 137.40 \ (\text{C}), 136.0 \ (\text{C}), 134.9 \ (\text{C}), 130.3 \ (\text{CH}), 128.8 \ (\text{CH}), 128.72 \ (\text{CH}), 128.65 \ (\text{CH}), 128.62 \ (\text{CH}), 128.58 \ (\text{CH}), 128.4 \ (\text{CH}), 128.1
(CH), 128.0 (CH), 127.5 (CH), 114.9 (C, Boc), 70.0 (CH₂Ph), 67.7 (CH₂Ph), 67.3 (CH₃Ph), 56.0 (2 × CH₂-Az), 54.0 (CH, α-Leu), 53.1 (CH, α-Ala), 52.8 (CH, α-Tyr), 49.0 (CH, α-Ala), 41.5 (CH₂, β-Leu), 36.8 (CH₂, β-Tyr), 28.4 (CH₃, Boc), 24.8 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 21.8 (CH₃, δ-Leu), 21.3 (CH₃, β-Ala), 17.7 (CH₃, β-Ala), Note: 1 × C and CH₂, Az not observed; ν max (neat) = 3308, 2956, 1704, 1683, 1647, 1360, 695 cm⁻¹; MS (ESI⁺) m/z 935 [M+H]⁺, 957 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₅₂H₆₇N₆O₁₀ [M+H]⁺ 935.4913, found 935.4913; [α]D²⁷ +38 (c 0.23, CHCl₃).

H-Leu-Ala-GAz(Boc)-Ala-Tyr-OH (276)

Following general procedure 5 from Cbz-Leu-Ala-GAz(Boc)-Ala-Tyr(Obn)-Obn (460) (1.0 g, 1.07 mmol, 1.0 equiv), H-Leu-Ala-GAz(Boc)-Ala-Tyr-OH (276) was obtained as a white solid (0.65 g, 1.07 mmol, quant. yield) which required no further purification; m.p 123 – 126 °C; ¹H NMR (500 MHz, D₂O) δH 7.15 (d, J = 8.3 Hz, 2H, ArH), 6.82 (d, J = 8.3 Hz, 2H, ArH), 4.42 (dd, J = 9.7, 4.1 Hz, 1H, CHα-Tyr), 4.34 (q, J = 7.1 Hz, 1H, CHα-Ala), 4.03 (t, J = 7.2 Hz, 1H, CHα-Leu), 3.67 – 3.60 (m, 3H, CH₂-Az, CHH-Az), 3.44 (d, J = 9.3 Hz, 1H, CHH-Az), 3.33 – 3.28 (m, 2H, CHα-Ala, CHHGaz), 3.18 (dd, J = 14.0, 3.9 Hz, 1H, CHHβ-Tyr), 2.81 (dd, J = 14.0, 10.0 Hz, 1H, CHHβ-Tyr), 2.76 (d, J = 14.6 Hz, 1H, CHHGaz), 1.80 – 1.69 (m, 3H, CH2β-Leu, CHγ-Leu), 1.42 (s, 9H, CH₃ Boc), 1.38 (d, J = 7.2 Hz, 3H, CHβ-Ala), 1.25 (d, J = 6.8 Hz, 3H, CHβ-Ala), 0.99 – 0.95 (m, 6H, 2 × CHδ-Leu); ¹³C NMR (126 MHz, D₂O) δC 177.7 (C=O), 176.4 (C=O), 174.5 (C=O), 170.0 (C=O), 157.7 (C=O Boc), 154.3 (C), 130.5 (CH), 129.6 (C), 115.4 (CH), 82.0 (C, Boc), 56.2 (CH, α-Tyr), 55.0 (2 × CH₂, Az), 52.2 (CH, α-Leu), 51.7 (CH, α-Ala), 50.0 (CH, α-Ala), 42.3 (CH₂, GAz), 39.8 (CH₂, β-Leu), 36.8 (CH₂, β-Tyr), 27.6 (CH₃, Boc), 23.8 (CH, γ-Leu), 21.7 (CH₃, δ-Leu), 21.1 (CH₃, δ-Leu), 19.5 (CH₃, β-Ala), 16.7 (CH₃, β-Ala); ν max (neat) = 3270, 2969, 1611, 1529, 1456, 864 cm⁻¹; MS (ESI⁺) m/z 621 [M+H]⁺, 643 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₀H₄₀N₆O₈ [M+H]⁺ 621.3606, found 621.3608; [α]D²⁷ +3.8 (c 0.11, MeOH).
Cyclo-(Leu-Ala-GAz(Boc)-Ala-Tyr) (277)

Following general procedure 6, H-Leu-Ala-GAz(Boc)-Ala-Tyr-OH (276) (62.0 mg, 0.10 mmol, 1.0 equiv) was cyclised using DEPBT (60 mg, 0.20 mmol, 2.0 equiv) at 1 mM for 24 h. The crude residue was purified twice by column chromatography (SiO₂, CH₂Cl₂/MeOH 40:1→19:1) to give the cyclic tetrapeptide 277 as a glassy colourless solid (28.1 mg, 47 μmol, 47%). Product isolated as an inseparable 8:1 mixture of diastereomers by ¹H NMR and analytical HPLC, data reported for the major diastereomer; Rᵣ(CH₂Cl₂/MeOH 19:1) 0.28; ¹H NMR (500 MHz, CD₃OD) δₜ 7.07 (d, J = 8.5 Hz, 2H, ArH), 6.74 (d, J = 8.5 Hz, 2H, ArH), 4.44 (q, J = 7.0 Hz, 1H, CHα-Ala), 4.23–4.17 (m, 1H, CHα-Tyr), 4.12 (dd, J = 10.5, 5.0 Hz, 1H, CHα-Leu), 3.82–3.62 (m, 5H, CH₂HGAz, 2 × CH₂-Az), 3.26 (q, J = 7.0 Hz, 1H, CHα-Ala), 3.16 (dd, J = 13.5, 11.0, 5.0 Hz, 1H, CHHβ-Tyr), 1.67 – 1.59 (m, 1H, CHHβ-Leu), 1.44 (s, 9H, CH₃Boc), 1.42 (d, J = 7.0 Hz, 3H, CH₃β-Ala), 1.22 (d, J = 7.0 Hz, 3H, CH₃β-Ala), 0.95 (d, J = 6.5 Hz, 3H, CH₃δ-Leu), 0.87 (d, J = 6.5 Hz, 3H, CH₃δ-Leu); ¹³C NMR (126 MHz, CD₃OD) δC 178.7 (C=O), 175.6 (C=O), 174.5 (C=O), 174.4 (C=O), 158.2 (C=O Boc), 157.5 (C), 131.3 (CH), 129.0 (C), 116.4 (CH), 81.2 (C, Boc), 57.7 (CH, α-Tyr), 57.3 (2 × BocNCH₂), 56.2 (CH, α-Leu), 53.7 (CH, α-Ala), 50.8 (CH, α-Ala), 46.0 (CH₂, GAZ(Boc)), 40.2 (CH₂, β-Leu), 35.6 (CH₂, β-Tyr), 28.6 (CH₃, Boc), 25.9 (CH, γ-Leu), 23.4 (CH₃, δ-Leu), 21.6 (CH₃, δ-Leu), 21.5 (CH₃, β-Ala), 17.8 (CH₃, β-Ala). Note: C, Az not observed; ν max (neat) = 3304, 2969, 1668, 1535, 736 cm⁻¹; MS (ESI⁺) m/z 625 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₀H₅₆N₆O₇ [M+Na]⁺ 625.3320, found 625.3324.

Analysis of the product mixture by analytical HPLC at 280 nm shows epi-277, Rᵣ = 24.9 min, and the desired product 277, Rᵣ = 24.9 min. Integration of the two peaks shows a d.r 89:11. Agilent PLRP-S column (100 Å, 8 μm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.

187
Preparation of cyclic hexapeptide 289

NO₂-GAz(Boc)-Thr(tBu)-OCumyl (284)

To a solution of Fmoc-Thr(tBu)-OCumyl (194) (1.91 g, 3.71 mmol, 1.0 equiv) in CH₂Cl₂ (3.7 mL) was added diethylamine (3.7 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 15 mL) and concentrated under reduced pressure to give the crude amine. This was added to the nitroalkene formed in-situ following general procedure 3. The residue was purified by column chromatography (SiO₂, CH₂Cl₂/EtOAc 4:1) to give the title compound 284 (1.62 g, 3.18 mmol, 86%) as a pale yellow oil. Rᵣ (CH₂Cl₂/EtOAc 4:1) 0.30; ¹H NMR (500 MHz, CDCl₃) δH 7.46 – 7.42 (m, 2H, ArH), 7.36 – 7.31 (m, 3H, ArH), 4.62 (d, J = 13.1 Hz, 1H, CHHNO₂), 4.53 (d, J = 13.1 Hz, 1H, CHHNO₂), 3.93 (d, J = 9.4 Hz, 1H, CHH-Az), 3.82 – 3.72 (m, 4H, CHH-Az, CH₂-Az, CHα-Thr), 3.20 (d, J = 2.9 Hz, 1H, CHβ-Thr), 2.61 – 2.25 (m, 1H, NH), 1.83 (s, 3H, CH₃-Cumyl), 1.80 (s, 3H, CH₃-Cumyl), 1.43 (s, 9H, 3 × CH₃, Boc), 1.20 (s, 9H, 3 × CH₃, tBu), 1.08 (d, J = 6.2 Hz, 3H, CHγ-Thr); ¹³C NMR (126 MHz, CDCl₃) δC 172.7 (C=O), 156.2 (C=O, Boc), 145.2 (C), 128.4 (CH), 127.5 (CH), 124.8 (CH), 83.3 (C, Cumyl), 80.3 (C, Boc), 79.0 (CH₂NO₂), 74.2 (C, tBu), 69.2 (α-CH, Thr), 61.8 (β-CH, Thr), 54.2 (2 × CH₂, Az), 28.6 (CH₃, tBu), 28.4 (CH₃, Boc), 27.9 (2 × CH₃, Cumyl), 19.4 (CH₃, γ-Thr); νmax (neat) = 2975, 1700, 1555, 1377, 1194, 699 cm⁻¹; MS (ESI⁺) m/z 530 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₆H₄₁N₅NaO₇ 530.2837 [M+Na]⁺, found 530.2833; [α]D²⁴ –9.5 (c 0.27, CHCl₃).
Fmoc-Val-GAz(Boc)-Thr(\textit{t}Bu)-O\textit{Cumyl} (285)

Following general procedure 4 from NO\textsubscript{2}-GAz(Boc)-Thr(\textit{t}Bu)-O\textit{Cumyl} (284) (1.61 g, 3.18 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/EtOAc 19:1) to give the title compound (285) as a white foam (1.53 g, 1.91 mmol, 60%); m.p. 73 – 75 °C; \textit{R}\textsubscript{f} (CH\textsubscript{2}Cl\textsubscript{2}/EtOAc 19:1) 0.19; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ H 7.76 (d, \textit{J} = 7.5 Hz, 2H, ArH), 7.59 (d, \textit{J} = 7.2 Hz, 2H, ArH), 7.43 – 7.37 (m, 6H, ArH), 7.34 – 7.28 (m, 2H, ArH), 7.25 – 7.20 (m, 1H, ArH), 6.50 (s, 1H, NH), 5.44 (d, \textit{J} = 8.6 Hz, 1H, NH, Fmoc), 4.42 (dd, \textit{J} = 10.3, 7.5 Hz, 1H, CH\textsubscript{H}Fmoc), 4.34 – 4.28 (m, 1H, CH\textsubscript{α}Fmoc), 4.21 (t, \textit{J} = 7.0 Hz, 1H, CHFmoc), 4.00 – 3.92 (m, 1H, CH\textsubscript{α}-Thr), 3.90 – 3.82 (m, 1H, CH\textsubscript{α}-Val), 3.81 – 3.70 (m, 1H, CH\textsubscript{H}GAz), 3.62 (d, \textit{J} = 8.7 Hz, 1H, CHH-Az), 3.60 – 3.45 (m, 3H, CHH-Az, CH\textsubscript{2}-Az), 3.23 – 2.70 (m, 2H, CHHGAz, CHβ-Thr), 2.00 – 1.92 (m, 1H, CHβ-Val) 1.83 (s, 3H, CH\textsubscript{3}-Cumyl), 1.75 (s, 3H, CH\textsubscript{3}, Boc), 1.19 (s, 9H, CH\textsubscript{3}, \textit{t}Bu), 1.17 (d, \textit{J} = 6.1 Hz, 3H, CH\textsubscript{3}-γ-Thr), 0.89 (d, \textit{J} = 6.5 Hz, 3H, CH\textsubscript{3}-γ-Val), 0.85 (d, \textit{J} = 6.6 Hz, 3H, CH\textsubscript{3}-γ-Val); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ 174.0 (C=O), 171.6 (C=O), 156.4 (C=O Boc and C=O Fmoc), 145.0 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.7 (CH), 120.1 (CH), 83.4 (C, Cumyl), 79.8 (C, Boc), 74.2 (C, \textit{t}Bu), 69.2 (CH, α-Thr), 67.1 (CH\textsubscript{2}, Fmoc), 61.8 (CH, β-Thr), 60.4 (CH\textsubscript{α}-Val), 54.7 (2 × CH\textsubscript{2}, Az), 47.3 (CH, Fmoc), 44.0 (CH\textsubscript{2}, GAz), 31.5 (CH, β-Val), 28.8 (CH\textsubscript{3}, Cumyl), 28.7 (CH\textsubscript{3}, Boc), 28.5 (CH\textsubscript{3}, \textit{t}Bu), 27.3 (2 × CH\textsubscript{3}, Cumyl), 20.2 (CH\textsubscript{3}, γ-Thr), 19.3 (CH\textsubscript{3}, γ-Val), 17.8 (CH\textsubscript{3}, γ-Val), Note: one Ar C not observed; \textit{ν}_{\text{max}} (neat) = 2968, 1702, 1658, 1399, 1365 cm\textsuperscript{-1}; MS (ESI\textsuperscript{+}) \textit{m/z} 799 [M+H]\textsuperscript{+}, 821 [M+Na]\textsuperscript{+}; HRMS (ESI\textsuperscript{+}) calcd. for C\textsubscript{46}H\textsubscript{62}N\textsubscript{4}NaO\textsubscript{8} 821.4460 [M+Na]\textsuperscript{+}, found 821.4467; [\textsuperscript{2}H\textsubscript{24}\textalpha\textsubscript{D}] = 3.9 (c 0.25, CHCl\textsubscript{3}).

Fmoc-Val-GAz(Boc)-Thr(\textit{t}Bu)-Phe-OBn (286)

Fmoc-Val-GAz(Boc)-Thr(\textit{t}Bu)-O\textit{Cumyl} (285) (1.47 g, 1.84 mmol, 1.0 equiv) was suspended in 2% TFA/CH\textsubscript{2}Cl\textsubscript{2} (37 mL) and stirred at room temperature full consumption of starting material, monitored by ESI-MS. The mixture was concentrated under reduced pressure, the resulting residue was repeatedly re-dissolved in CH\textsubscript{2}Cl\textsubscript{2} (20 mL) and the solvent was removed under reduced pressure. To the crude acid in CH\textsubscript{2}Cl\textsubscript{2} (20 mL) was added H-Phe-OBn-HCl (0.65 g, 2.22 mmol, 1.2 equiv), NMM (1.01 mL, 9.22 mmol, 5.0 equiv), HOBt-H\textsubscript{2}O (0.25 g, 1.84 mmol, 1.0 equiv) and EDC-HCl (0.35 g, 1.84 mmol, 1.0 equiv).
The reaction mixture was allowed to stir for 18 h at room temperature under an atmosphere of nitrogen. The mixture was diluted with EtOAc (50 mL) and washed with brine (3 × 50 mL), dried (Na2SO4) and concentrated in vacuo to afford a yellow oil which was purified by column chromatography (SiO2, CH2Cl2/MeOH 49:1) to give the title compound 286 as a yellow solid (1.26 g, 1.37 mmol, 74%). Rf (CH2Cl2/MeOH 49:1) 0.44; mp 56 – 58 °C; 1H NMR (500 MHz, CDCl3) δH 7.80 (d, J = 8.1 Hz, 1H, NH), 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.63 – 7.57 (m, 2H, ArH), 7.42 – 7.27 (m, 10H, ArH), 7.22 – 7.18 (m, 3H, ArH), 7.08 – 7.02 (m, 2H, 1 × NH, 1 × ArH), 5.49 (d, J = 8.8 Hz, 1H, NH Fmoc), 5.18 (d, J = 12.1 Hz, 1H, CHHPh), 5.10 (d, J = 12.1 Hz, 1H, CHHPh), 4.90 (dt, J = 7.9, 5.9 Hz, 1H, CHα-Phe), 4.43 (dd, J = 10.2, 7.5 Hz, 1H, CH Fmoc), 4.33 (dd, J = 10.1, 7.5 Hz, 1H, CHH Fmoc), 4.23 (t, J = 7.1 Hz, 1H, CH Fmoc), 4.12 – 4.03 (m, 1H, CHα-Val), 3.78 (d, J = 8.6 Hz, 2H, CHH-Az, CHHGAz), 3.59 – 3.48 (m, 4H, CHα-Thr, CHH-Az, CH2-Az), 3.17 (dd, J = 13.9, 5.5 Hz, 1H, CHHβ-Phe), 3.00 (dd, J = 13.8, 7.9 Hz, 1H, CHHβ-Phe), 2.95 – 2.70 (m, 2H, CHβ-Thr, CHHGAz), 2.40 (s, 1H, NH), 2.15 – 2.08 (m, 1H, CHβ-Val), 1.42 (s, 9H, CH3, Boc), 1.08 (s, 9H, CH3, t-Bu), 1.01 – 0.95 (m, 6H, CHγ-Thr, CHγ-Val), 0.93 (d, J = 6.6 Hz, 3H, CH3γ-Val); 13C NMR (126 MHz, CDCl3) δC 173.1 (C=O), 172.1 (C=O), 156.5 (C=O, Fmoc), 156.4 (C=O, Boc), 144.04 (C), 141.4 (C), 136.1 (C), 135.1 (C), 129.3 (CH), 128.8 (CH), 128.74 (CH), 128.69 (CH), 128.66 (CH), 127.9 (CH), 127.2 (CH), 127.2 (CH), 125.3 (CH), 120.1 (CH), 79.8 (C, Boc), 74.9 (C, t-Bu), 67.5 (CH2Ph), 67.2 (CH2, Fmoc), 60.6 (CH, α-Val, CH, β-Thr), 55.6 (2 × CH2, Az), 53.3 (CH, γ-Phe), 47.3 (CH, Fmoc), 38.2 (CH2, β-Phe, 31.3 (CH, β-Val), 28.5 (CH3, Boc or t-Bu), 28.4 (CH3, Boc or t-Bu), 19.4 (CH3, γ-Thr), 18.0 (2 × CH3, γ-Val), Note: one C=O, CH2, GAz, CHα-Thr not observed; νmax (neat) = 2970, 1734, 1698, 1667, 1390, 1106 cm⁻¹; MS (ESI⁺) m/z 918 [M+H]+, 940 [M+Na]+; HRMS (ESI⁺) calcd. for C35H36N4O9, 918.5012 [M+H]+, found 918.5010; [α]D 24° = 5.1 (c 0.16, CHCl3).

Fmoc-Tyr(t-Bu)-Val-GAzm(Boc)-Thr(t-Bu)-Phe-OBn (287)

Following general procedure 2 from Fmoc-Val-GAzm(Boc)-Thr(t-Bu)-Phe-OBn (286) (994 mg, 1.08 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO2, CH2Cl2/MeOH 49:1) to give the title compound 287 (898 mg, 0.79 mmol, 73%) as a white solid. Rf (CH2Cl2/MeOH 97:3) 0.27; mp 103 – 105 °C; 1H NMR (500 MHz, CDCl3) δH 7.82 (d, J = 8.0 Hz, 1H, NH), 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.54 (dd, J = 7.3, 2.9 Hz, 2H, ArH), 7.44 – 7.27 (m, 9H, ArH), 7.25 – 7.19 (m, 3H, ArH), 7.11 – 7.04 (m, 4H, ArH), 6.90 (d, J = 8.1 Hz, 2H, ArH), 6.53 (d, J = 6.6 Hz, 1H, NH), 5.35 – 5.28 (m, 1H, NH, Fmoc), 5.18 (d, J = 12.1 Hz, 1H, CHHPh), 5.12 (d, J = 12.2 Hz, 1H, CHHPh), 4.91 (dd, J = 13.8, 7.6 Hz, 1H, CHα-Phe), 4.55 – 4.36 (m, 2H,
Following general procedure 2 from Fmoc-Tyr(Bu)-Val-GAz(Boc)-Thr(Bu)-Phe-OBn (287) (845 mg, 0.74 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 97:3) to give the title compound 461 (708 mg, 0.61 mmol, 82%) as a white foam. Rf (CH₂Cl₂/MeOH 97:3) 0.23; m.p. 128 – 128 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.79 (d, J = 8.1 Hz, 1H, NH), 7.43 – 7.15 (m, 14H, ArH, NH), 7.10 – 7.04 (m, 4H, ArH), 6.88 (d, J = 8.1 Hz, 2H, ArH), 6.77 – 6.60 (m, 2H, 2 × NH), 5.18 – 4.96 (m, 5H, 2 × CH₂Ph, NH Cbz), 4.88 (dd, J = 13.9, 7.3 Hz, 1H, CHα-Phe), 4.58 – 4.49 (m, 1H, CHα-Tyr), 4.34 – 4.25 (m, 1H, CHα-Val), 4.07 – 4.00 (m, 1H, CHα-Leu), 3.87 – 3.48 (m, 6H, CHHGAz, 2 × NCH₂-Az, CHα-Thr), 3.21 – 2.80 (m, 6H, CHHGAz, CHβ-Phe, CHβ-Tyr, CHβ-Thr), 2.57 (s, 1H, NH), 2.28 – 2.14 (m, 1H, CHβ-Val), 1.59 – 1.48 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.39 (s, 10H, CHHβ-Leu, CH₃, 'Bu), 1.30 (s, 9H, CH₃, 'Bu), 1.11 (s, 9H, CH₃, 'Bu), 1.00 (d, J = 6.2 Hz, 3H, CH₂γ-Thr), 0.91 (d, J = 6.4 Hz, 3H, CH₂γ-Val), 0.87 – 0.81 (m, 9H, CH₂γ-Val, 2 × CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δC 173.3 (C=O), 172.9 (C=O), 171.8 (C=O), 171.4 (C=O), 156.4 (C=O Boc), 154.6 (C), 143.9 (C), 141.4 (C), 136.1 (C), 135.1 (C), 131.2 (C), 129.3 (CH), 128.8 (CH), 128.74 (CH), 128.67 (CH), 128.65 (CH), 127.9 (CH), 127.3 (CH), 127.2 (CH), 125.2 (CH), 124.5 (CH), 120.1 (CH), 79.8 (C, Boc), 78.6 (C, 'Bu), 74.9 (C, 'Bu), 67.5 (CH₂Ph), 67.2 (CH₂Fmoc), 58.9 (CH, α-Val), 56.3 (CH, α-Tyr), 55.6 (2 × CH₂, Az), 53.4 (CH, α-Phe), 47.2 (CH, Fmoc), 38.2 (CH₂, β-Tyr), 37.4 (CH₂, β-Phe), 30.9 (CH, β-Val), 29.0 (CH₃, 'Bu), 28.5 (CH₃, 'Bu), 28.45 (CH₃, Boc), 19.4 (CH₃, γ-Val), 18.0 (CH₃, γ-Thr, CH₃, γ-Val), Note: CH₂ GAZ, CH β-Thr, CH α-Thr and 1 × Ar CH not observed; vmax (neat) = 2970, 1698, 1643, 1529, 1232 cm⁻¹; MS (ESI⁺) m/z 1137 [M+H]^+, 1159 [M+Na]^⁺; HRMS (ESI⁺) calcd. for C₆₀H₄₄Na₂NaO₁₁ 1159.6090 [M+Na]^⁺, found 1159.6101; [α]D²⁴ 7.9 (c 0.25, CHCl₃).
(CH), 128.64 (CH), 128.63 (CH), 128.4 (CH), 128.3 (CH), 127.3 (CH), 124.5 (CH), 79.7 (C, tBu), 78.5 (C, tBu), 74.9 (C, Boc), 67.40 (CH2Ph), 67.37 (CH3Ph), 61.1 (CH, β-Thr), 59.0 (CH, α-Val), 55.8 (CH, α-Tyr), 55.5 (2 × CH2, Az), 54.1 (CH, α-Leu), 53.5 (CH, α-Phe), 41.0 (CH2, β-Leu), 38.2 (CH3, β-Phe), 36.4 (CH2, β-Tyr), 30.3 (CH, β-Val), 29.0 (CH3, tBu), 28.5 (CH3, tBu), 28.4 (CH3, Boc), 24.8 (CH, γ-Leu), 23.0 (CH3, δ-Leu), 21.9 (CH3, γ-Val), 19.5 (CH3, δ-Leu), 17.7 (CH3, γ-Val, CH3, γ-Thr). Note: CH2GAz and CH α-Thr missing; νmax (neat) = 2971, 1697, 1637, 1365, 696 cm⁻¹; MS (ESI⁺) m/z 1184 [M+Na]+; HRMS (ESI⁺) calcd. for C₆₅H₉₀N₇NaO₁₂ 1184.6618 [M+Na]+, found 1184.6623; [α]D²⁴ −15.2 (c 0.33, CHCl₃).

H-Leu-Tyr(tBu)-Val-GAz(Boc)-Thr(tBu)-Phe-OH (288)

Following general procedure 5 from Cbz-Leu-Tyr(tBu)-Val-GAz(Boc)-Thr(tBu)-Phe-OBn (461) (590 mg, 0.52 mmol, 1.0 equiv), H-Leu-Tyr(tBu)-Val-GAz(Boc)-Thr(tBu)-Phe-OH (288) was isolated as a white solid (491 mg, 0.52 mmol, quant. yield), which required no further purification;

m.p. 126 – 128 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 7.31 – 7.21 (m, 7H, ArH), 6.91 (d, J = 8.3 Hz, 2H, ArH), 4.76 – 4.70 (m, 2H, CHα-Tyr, CHα-Phe), 4.21 (d, J = 6.9 Hz, 1H, CHα-Val), 3.87 – 3.81 (m, 1H, CHα-Leu), 3.71 – 3.44 (m, 6H, CHHGAz, 2 × CH2-Az, CHα-Thr), 3.26 – 3.21 (m, 2H, CHββ-Phe, CHββ-Tyr), 3.07 – 2.82 (m, 4H, CHHGAz, CHHβ-Phe, CHHβ-Tyr, CHβ-Thr), 2.10 (m, 1H, CHβ-Val), 1.72 – 1.62 (m, 3H, CHββ-Val, CHγ-Leu), 1.42 (s, 9H, CH3, Boc), 1.32 (s, 9H, CH3, tBu), 1.14 (s, 9H, CH3, tBu), 1.06 (d, J = 5.8 Hz, 3H, CHγγ-Thr), 0.97 (d, J = 3.2 Hz, 3H, CH3δ-Leu), 0.96 (d, J = 3.0 Hz, 3H, CH3δ-Leu), 0.94 (d, J = 3.3 Hz, 3H, CH3γ-Val), 0.93 (d, J = 3.2 Hz, 3H, CH3γ-Val); ¹³C NMR (126 MHz, MeOD-d₄) δC 175.4 (C=O), 174.9 (C=O), 173.8 (C=O), 173.4 (C=O), 170.8 (C=O), 158.1 (C=O Boc), 155.4 (C), 138.4 (C), 133.4 (C), 130.8 (CH), 130.4 (CH), 129.7 (CH), 128.0 (CH), 125.3 (CH), 81.2 (C, tBu), 79.5 (C, tBu), 75.6 (C, Boc), 70.7 (CH, α-Thr), 63.7 (CH, β-Thr), 60.5 (CH, α-Val), 57.1 (2 × CH2, Az), 56.6 (CH, α-Tyr), 54.9 (CH, α-Phe), 52.8 (CH, α-Leu), 44.9 (CH2, GAz), 41.8 (CH2, β-Leu), 38.7 (CH2, β-Tyr), 37.7 (CH2, β-Phe), 32.0 (CH, β-Val), 29.2 (CH3, tBu), 28.9 (CH3, tBu), 28.6 (CH3, Boc), 25.3 (CH, γ-Leu), 23.3 (CH3, δ-Leu), 21.8 (CH3, δ-Leu), 20.3 (CH3, γ-Thr), 19.9 (CH3, γ-Val), 18.6 (CH3, γ-Val); νmax (neat) = 3287, 2967, 1641, 1608, 1506, 1160 cm⁻¹; MS (ESI⁺) m/z 938 [M+H]+, 960 [M+Na]+; HRMS (ESI⁺) calcd. for C₆₅H₈₀N₇NaO₁₀ 938.5961 [M+H]+, found 938.5966; [α]D²⁴ −1.2 (c 0.17, MeOH).
Cyclo(Leu-Tyr(tBu)-Val-GAzh(Boc)-Thr(tBu)-Phe) (289)

This compound was prepared following general procedure 6 from H-Leu-Tyr(tBu)-Val-GAzh(Boc)-Thr(tBu)-Phe-OH (288) (94 mg, 0.10 mmol, 1.0 equiv) in DMF (20 mL, 0.005 M) for 72 h to give a crude residue which was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 49:1→19:1) to give the cyclic tetrapeptide 288 as a glassy colourless solid (52.1 mg, 57 μmol, 57%); R$_f$ (CH$_2$Cl$_2$/MeOH 19:1) 0.35; m.p. 156 – 158 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) δH 7.38 – 7.31 (m, 2H, ArH), 7.29 – 7.24 (m, 3H, ArH), 7.15 (d, J = 8.3 Hz, 2H, ArH), 6.93 (d, J = 8.3 Hz, 2H, ArH), 4.62 (dd, J = 9.9, 5.6 Hz, 1H, CHα-Phe), 4.36 (d, J = 9.0 Hz, 1H, CHα-Val), 4.08 (dd, J = 11.5, 3.3 Hz, 1H, CHH-Az), 3.98 – 3.83 (m, 2H, CHH-Az, CHHGAz), 3.81 – 3.71 (m, 2H, CHH-GAzh, CHα-Leu), 3.66 – 3.49 (m, 3H, CHH-Az, CHα-Tyr, CHHβ-Tyr), 3.40 – 3.35 (m, 2H, CHβ-Thr), 2.74 (dd, J = 13.4, 10.8 Hz, 1H, CHHβ-Phe), 2.18 – 1.88 (m, 1H, CHβ-Val), 1.58 – 1.51 (m, 1H, CHHβ-Leu), 1.42 (s, 9H, CH$_3$, Boc), 1.35 – 1.30 (m, 10H, CHHβ-Leu, CH$_3$, tBu), 1.26 – 1.22 (m, 1H, CHγ-Leu), 1.09 – 0.97 (m, 18H, CH$_3$, tBu, 2 × CH$_3$γ-Val, CH$_3$γ-Thr), 0.81 (d, J = 6.5 Hz, 3H, CH$_3$δ-Leu), 0.77 (d, J = 6.4 Hz, 3H, CH$_3$δ-Leu), Note: CHHβ-Phe overlaps with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) δC 176.2 (C=O), 174.9 (C=O), 174.7 (C=O), 174.5 (C=O), 172.8 (C=O), 158.2 (C=O, Boc), 155.3 (C), 137.7 (C), 134.9 (C), 130.6 (CH), 130.2 (CH), 128.3 (CH), 125.3 (CH), 81.3 (C, tBu), 79.4 (C, tBu), 75.5 (C, Boc), 69.5 (CH, α-Thr), 65.1 (CH, β-Thr), 62.6 (CH, α-Val), 59.5 (CH, α-Tyr), 57.7 (2 × NCH$_2$), 55.8 (CH, α-Leu), 54.7 (CH, α-Phe), 40.3 (CH$_2$, β-Leu), 39.3 (CH$_2$, β-Phe), 35.1 (CH$_3$, β-Tyr), 33.6 (CH$_3$, β-Val), 29.2 (CH$_3$, tBu), 28.9 (CH$_3$, tBu), 28.6 (CH$_3$, Boc), 25.2 (CH, γ-Leu), 23.1 (CH$_3$, δ-Leu), 22.3 (CH$_3$, δ-Leu), 21.8 (CH$_3$, γ-Thr), 19.9 (CH$_3$, γ-Val), 19.5 (CH$_3$, γ-Val). Note: GAzh, CH$_2$ not observed; ν$_{max}$ (neat) = 2973, 1642, 1505, 1161 cm$^{-1}$; MS (ESI$^+$) m/z 920 [M+H]$^+$, 942 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{52}$H$_{76}$N$_7$NaO$_9$ 942.5699 [M+Na]$^+$, found 942.5692; [α]$_D^{24}$ –34.3 (c 0.11, MeOH).
Preparation of cyclic tetrapeptides 295–297

NO$_2$-GAz(Boc)-Gly-OBn (290)

H-Gly-OBn•TsOH (3.49 g, 10.4 mmol, 2.0 equiv) was suspended in CH$_2$Cl$_2$ (10.4 mL) and to the solution was added triethylamine (1.44 mL, 10.4 mmol, 2.0 equiv) and stirred at room temperature for 15 min. This was added to the nitroalkene formed in-situ following general procedure 3. The crude residue was purified by column chromatography (SiO$_2$, PET ether/EtOAc 7:3) to yield NO$_2$-GAz(Boc)-Gly-OBn (290) (1.66 g, 4.37 mmol, 84%) as a yellow oil. $R_f$ (PET ether/EtOAc 7:3) 0.34; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ H 7.41 – 7.30 (m, 5H, ArH), 5.18 (s, 2H, CH$_2$Ph), 4.67 (s, 2H, CH$_2$NO$_2$), 4.02 – 3.83 (m, 4H, 2 × CH$_2$-Az), 3.51 (d, $J$ = 4.5 Hz, 2H, CH$_2$Gly), 2.33 (s, 1H, NH), 1.44 (s, 9H, 3 × CH$_3$, Boc); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$C 171.4 (C=O), 156.2 (C=O, Boc), 135.2 (C), 128.9 (CH), 128.8 (CH), 80.6 (C, Boc); $\nu_{\text{max}}$ (neat) = 2976, 1739, 1692, 1552, 1378, 1163 cm$^{-1}$; MS (ESI$^+$) m/z 402 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{18}$H$_{25}$N$_3$O$_6$[M+Na]$^+$ 402.1636, found 402.1636.

Fmoc-Leu-GAza(Boc)-Gly-OBn (291)

Following general procedure 4 from NO$_2$-GAz(Boc)-Gly-OBn (290) (1.66 g, 4.4 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, 9:1 → 3:2 CH$_2$Cl$_2$/EtOAc) to give the title compound 291 as a white foam (1.84 g, 2.70 mmol, 62%). $R_f$ (CH$_2$Cl$_2$/EtOAc 9:1) 0.26; mp 77 – 79 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ H 7.76 (d, $J$ = 7.5 Hz, 2H, ArH), 7.59 (d, $J$ = 7.2 Hz, 2H, ArH), 7.42 – 7.29 (m, 9H, ArH), 6.69 (s, 1H, NH, Fmoc), 5.11 (s, 2H, CH$_2$Ph), 4.61 (d, $J$ = 7.1 Hz, 2H, CH$_2$, Fmoc), 4.25 – 4.15 (m, 2H, CH, Fmoc, CH$_\alpha$-Leu), 3.69 – 3.60 (m, 4H, 2 × CH$_2$-Az), 3.52 – 3.35 (m, 4H, CH$_2$Gly, CH$_2$GAz), 1.64 – 1.60 (m, 3H, CH$_\gamma$-Leu, CH$_\delta$-Leu), 1.42 (s, 9H, CH$_3$, Boc), 0.98 – 0.91 (m, 6H, 2 × CH$_3$δ-Leu); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$C 172.9 (C=O), 172.6 (C=O), 156.3 (C=O Boc), 144.0 (C), 143.9 (C), 141.5 (C), 135.2 (C), 132.1 (C), 128.81 (CH), 128.76 (CH), 128.6 (CH), 127.9 (CH), 127.2 (CH), 125.2 (CH), 120.2 (CH), 80.1 (C, Boc), 67.4 (CH$_2$Ph), 67.1 (CH$_2$, Fmoc), 55.0 (2 × CH$_2$, Az), 53.9 (CH, $\alpha$-Leu), 47.3 (CH, Fmoc), 44.8 (CH$_2$, GAz), 43.5 (CH$_2$, Gly), 41.8 (CH$_2$, $\beta$-Leu), 28.5 (CH$_3$, Boc), 24.9 (CH, $\gamma$-Leu), 23.1 (CH$_3$, $\delta$-Leu), 22.1 (CH$_3$, $\delta$-Leu). Note: Fmoc C=O overlaps with Boc C=O; $\nu_{\text{max}}$ (neat) = 2955, 1696, 1657, 1120, 757 cm$^{-1}$; MS (ESI$^+$) m/z 685 [M+H]$^+$, 707 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{39}$H$_{49}$N$_4$O$_7$ [M+H]$^+$ 685.3596, found 685.3604; $[\alpha]_{D}^{24}$ –13.3 (c 0.12, CHCl$_3$).
Cbz-D-Pro-Leu-GA\(z\)(Boc)-Gly-OBn (462)

Following general procedure 2 from Fmoc-Leu-GA\(z\)(Boc)-Gly-OBn (291) (752 mg, 1.10 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 97:3) to give Cbz-D-Pro-Leu-GA\(z\)(Boc)-Gly-OBn (462) (471 mg, 0.68 mmol, 62%) as a white foam. \(R_f\) (CH\(_2\)Cl\(_2\)/MeOH 97:3) 0.18; m.p. 65 – 67 °C; \(^1\)H NMR (500 MHz, DMSO-d\(_6\) @ 373 K) \(\delta\) H 7.65 (s, 1H, NH), 7.46 (s, 1H, NH), 7.42 – 7.26 (m, 10H, ArH), 5.15 (s, 2H, CH\(_2\)Ph), 5.12 – 5.02 (m, 2H, CH\(_2\)Ph), 4.33 – 4.23 (m, 2H, CH\(_2\)-Pro, CH\(_2\)-Leu), 3.66 – 3.57 (m, 4H, 2 × CH\(_2\)-Az), 3.50 – 3.42 (m, 4H, CH\(_2\)GA\(z\), CH\(_2\)δ-Pro), 3.38 – 3.28 (m, 2H, CH\(_2\)Gly), 2.17 – 2.09 (m, 1H, CH\(_H\gamma\)-Pro), 1.93 – 1.78 (m, 3H, CH\(_H\gamma\)-Pro, CH\(_2\)β-Pro), 1.64 – 1.47 (m, 3H, CH\(_\gamma\)-Leu, CH\(_β\)-Leu), 1.39 (s, 9H, 3 × CH\(_3\), Boc), 0.88 (d, \(J\) = 6.4 Hz, 3H, CH\(_3\)δ-Leu), 0.85 (d, \(J\) = 6.3 Hz, 3H, CH\(_3\)-δ-Leu); \(^{13}\)C NMR (126 MHz, DMSO @ 373 K) \(\delta\) C 171.9 (C=O), 171.3 (C=O), 171.2 (C=O), 155.2 (C=O, Cbz), 153.8 (C=O, Boc), 136.4 (C), 135.5 (C), 127.7 (CH), 127.6 (CH), 127.3 (CH), 127.2 (CH), 127.0 (CH), 126.6 (CH), 77.9 (C, Boc), 65.6 (CH\(_2\)Ph, Bn), 65.2 (CH\(_2\)Ph), 59.7 (CH, α-Pro), 56.7 (2 × CH\(_2\)-Az), 54.6 (C, Az), 51.1 (CH, α-Leu), 46.3 (CH\(_2\)GA\(z\)), 44.1 (CH\(_2\), δ-Pro), 42.7 (CH\(_2\), Gly), 40.3 (CH\(_2\), β-Leu), 29.7 (CH\(_2\), γ-Pro), 27.6 (CH\(_3\), Boc), 23.8 (CH, γ-Leu), 23.0 (CH\(_2\), δ-Leu), 22.2 (CH\(_3\), δ-Leu); \(\nu\)\(_{\text{max}}\) (neat) = 2955, 1740, 1667, 1407, 1120 cm\(^{-1}\); MS (ESI\(^+\)) m/z 694 [M+H]\(^+\), 716 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{37}\)H\(_{52}\)N\(_5\)O\(_8\) [M+H]\(^+\) 694.3810, found 694.3803; \([\alpha]\)\(_D\)\(^{24}\) −3.0 (c 0.32, CHCl\(_3\)).

Cbz-Trp-Leu-GA\(z\)(Boc)-Gly-OBn (463)

Following general procedure 2 from Fmoc-Leu-GA\(z\)(Boc)-Gly-OBn (291) (752 mg, 1.10 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/MeOH 97:3) to give Cbz-Trp-Leu-GA\(z\)(Boc)-Gly-OBn (463) (568 mg, 0.73 mmol, 66%) as a white foam. \(R_f\) (CH\(_2\)Cl\(_2\)/MeOH 97:3) 0.18; m.p. 90 – 92 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) H 7.66 (d, \(J\) = 7.3 Hz, 1H, ArH), 7.48 – 7.27 (m, 11H, ArH), 7.18 (t, \(J\) = 7.5 Hz, 1H, ArH), 7.13 – 7.07 (m, 1H, ArH), 7.03 (s, 1H, ArH), 6.57 (s, 1H, NH), 6.18 (s, 1H, NH), 5.52 (d, \(J\) = 6.7 Hz, 1H, NH Cbz), 5.15 (s, 2H, CH\(_2\)Ph), 5.10 (s, 2H, CH\(_2\)Ph), 4.60 – 4.45 (m, 1H, CHα-Leu), 4.33 (dd, \(J\) = 14.0, 8.2 Hz, 1H, CHα-Trp), 3.72 – 3.13 (m, 10H, 2 × CH\(_2\)-Az, CH\(_2\) Gly, CH\(_2\) GA\(z\), CH\(_2\)-β-Trp), 1.95 (s, 1H, NH), 1.60 – 1.50 (m, 1H, CH\(_Hβ\)-Leu), 1.50 – 1.38 (m, 10H, 3 × CH\(_3\), Boc, CH\(_Hβ\)-Leu), 1.38
$– 1.29 \text{ (m, 1H, CH}_2\text{Hβ-Leu)}$, 0.84 (d, $J = 6.1 \text{ Hz, 6H, 2 × CH}_3\text{δ-Leu})$; $^{13}$C NMR (126 MHz, CDCl$_3$) δc 172.7 (C=O), 172.3 (C=O), 171.3 (C=O), 156.6 (C=O Cbz), 156.3 (C=O Boc), 136.5 (C), 136.3 (C), 135.3 (C), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.6 (CH), 128.4 (CH), 128.3 (CH), 127.4 (C), 123.6 (CH), 122.3 (CH), 119.9 (CH), 118.9 (CH), 111.6 (CH), 80.3 (C, Boc), 67.31 (CH$_3$Ph), 67.26 (CH$_3$Ph), 55.7 (2 × CH$_2$Az), 55.2 (CH, α-Leu), 52.2 (CH, α-Trp), 44.8 (CH$_2$ Gly), 43.5 (CH$_2$ GAza), 41.1 (CH$_2$β-Leu), 28.5 (CH$_3$ Boc, CH$_2$β-Trp), 24.8 (CH, γ-Leu), 22.9 (CH$_3$δ-Leu), 22.1 (CH$_3$δ-Leu). Note: One Ar C not observed; ν$_{max}$ (neat) = 3293, 2955, 1645, 1408, 1124, 739 cm$^{-1}$; MS (ESI$^+$) m/z 783 [M+H]$^+$, 805 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_43$H$_{54}$N$_6$NaO$_8$ [M+Na]$^+$ 805.3895, found 805.3899; $[^\alpha]_D^{26} – 12.7$ (c 0.41, MeOH). 

Cbz-Asp(Bu)-Leu-GAza(Boc)-Gly-OBn (464) Following general procedure 2 from Fmoc-Leu-GAza(Boc)-Gly-OBn (291) (285 mg, 0.42 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 97:3) to give Cbz-Asp(Bu)-Leu-GAza(Boc)-Gly-OBn (464) (230 mg, 0.30 mmol, 72%) as a colourless oil; $R_f$ (CH$_2$Cl$_2$/MeOH 97.5/2.5) 0.20; $^1$H NMR (500 MHz, CDCl$_3$) δ$_H$ 7.38 – 7.32 (m, 10H, ArH), 6.85 (s, 1H, NH), 6.80 (d, $J = 7.9 \text{ Hz, 1H, NH}$), 5.94 (d, $J = 8.0 \text{ Hz, 1H, NH Cbz}$), 5.16 (s, 2H, CH$_2$Ph), 5.12 (s, 2H, CH$_2$Ph), 4.53 – 4.47 (m, 1H, CHα-Asp), 4.38 (td, $J = 9.0, 5.3 \text{ Hz, 1H, CHα-Leu}$), 3.72 – 3.61 (m, 4H, 2 × CH$_2$Az), 3.55 – 3.35 (m, 4H, CH$_2$ Gly, CH$_2$GAza), 2.88 (dd, $J = 17.0, 4.5 \text{ Hz, 1H, CHHβ-Asp}$), 2.66 (dd, $J = 17.0, 6.3 \text{ Hz, 1H, CHHβ-Leu}$), 1.71 – 1.66 (m, 1H, CHHβ-Leu), 1.65 – 1.59 (m, 1H, CHγ-Leu), 1.56 – 1.50 (m, 1H, CHHβ-Leu), 1.42 (s, 18H, 3 × CH$_3$CO$_2$Bu, 3 × CH$_3$ Boc), 0.92 (d, $J = 6.5 \text{ Hz, 3H, CH}_3$δ-Leu), 0.88 (d, $J = 6.4 \text{ Hz, 3H, CH}_3$δ-Leu); $^{13}$C NMR (126 MHz, CDCl$_3$) δc 172.7 (C=O), 172.4 (C=O), 171.3 (C=O), 170.8 (C=O), 156.4 (C=O, Boc), 136.1 (C), 135.3 (C), 128.81 (CH), 128.76 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.4 (CH), 82.2 (C, Bu-Asp), 79.9 (C, Boc), 67.5 (CH$_2$Ph), 67.3 (CH$_2$Ph), 55.0 (2 × CH$_2$, Az), 52.3 (CH, α-Leu), 51.6 (CH, α-Asp), 44.8 (CH$_2$ Gly), 43.6 (CH$_2$ GAza), 40.8 (CH$_2$, β-Leu), 37.0 (CH$_2$, β-Asp), 28.5 (CH$_3$, Boc/Bu), 28.2 (CH$_3$, Boc/Bu), 24.9 (CH, γ-Leu), 23.2 (CH$_3$, δ-Leu), 21.8 (CH$_3$, δ-Leu). Note: one C=O not observed; ν$_{max}$ (neat) = 3306, 2954, 1700, 1684, 1647, 1365, 1125, 695 cm$^{-1}$; MS (ESI$^+$) m/z 768 [M+H]$^+$, 790 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{49}$H$_{57}$N$_5$NaO$_{10}$ [M+Na]$^+$ 790.3998, found 790.3989; $[^\alpha]_D^{26} – 13.9$ (c 0.23, MeOH).
H-D-Pro-Leu-GAz(Boc)-Gly-OH (292)

Following general procedure 5 from Cbz-D-Pro-Leu-GAz(Boc)-Gly-OBn (462) (450 mg, 0.65 mmol, 1.0 equiv), H-(D)Pro-Leu-GAz(Boc)-Gly-OH (292) was isolated as a white solid (304 mg, 0.647 mmol, quant. yield), which required no further purification; \( \text{m.p.} 89 \sim 91 \, ^{\circ} \mathrm{C} \); \( ^{1} \text{H NMR} \) (500 MHz, MeOD-d4) \( \delta \) 4.41 – 4.30 (m, 2H, CHα-Leu, CHα-Pro), 3.76 – 3.57 (m, 5H, 2 × CH2-Az, CHHGAz), 3.44 – 3.38 (m, 1H, CHHδ-Pro), 3.36 – 3.31 (m, 1H, CHHδ-Pro), 3.30 – 3.26 (m, 1H, CHHGAz), 3.17 (d, \( J = 17.4 \, \text{Hz} \), 1H, CHHGly), 3.06 (d, \( J = 17.4 \, \text{Hz} \), 1H, CHHGly), 2.39 (td, \( J = 13.3 \), 7.8 Hz, 1H, CHHγ-Pro), 2.16 – 2.01 (m, 2H, CHβ-Pro), 1.96 (dq, \( J = 15.6 \), 7.8 Hz, 1H, CHHγ-Pro), 1.79 – 1.62 (m, 2H, CHγ-Leu, CHHβ-Leu), 1.61 – 1.54 (m, 1H, CHHβ-Leu), 1.42 (s, 9H, CH3, Boc), 0.98 (d, \( J = 6.5 \, \text{Hz} \), 3H, CHδ-Leu), 0.92 (d, \( J = 6.4 \, \text{Hz} \), 3H, CHδ-Leu); \( ^{13} \text{C NMR} \) (126 MHz, MeOD-d4) \( \delta \) C 178.5, 173.0, 169.2 (C=O), 169.2 (C=O), 156.9 (C=O, Boc), 79.8 (C, Boc), 60.1 (CH, α-Pro), 55.5 (2 × CH2, Az), 52.5 (CH, α-Leu), 46.2 (CH2, Gly), 45.6 (CH2, β-Pro), 42.0 (CH2, GAz), 39.8 (CH2, β-Leu), 29.0 (CH2, γ-Pro), 27.2 (CH3, Boc), 24.8 (CH, γ-Leu), 23.8 (CH2, β-Pro), 22.2 (CH3, δ-Leu), 20.0 (CH3, δ-Leu); \( \nu_{\text{max}} \) (neat) = 3262, 3052, 1651, 1556, 1387, 1118 cm\(^{-1}\); MS (ESI\(^{+}\)) \( m/z \) 470 [M+H]\(^{+}\), 342 [M+Na]\(^{+}\); HRMS (ESI\(^{+}\)) calcd. for C22H30N3O6 [M+H]\(^{+}\) 470.2973, found 470.2971; [\( \alpha \)]\(^{24}\)D –14.4 (c 0.10, MeOH).

H-Trp-Leu-GAz(Boc)-Gly-OH (293)

Following general procedure 5 from Cbz-Trp-Leu-GAz(Boc)-Gly-OBn (463) (538 mg, 0.69 mmol, 1.0 equiv), H-Trp-Leu-GAz(Boc)-Gly-OH (293) was isolated as a white solid (304 mg, 0.68 mmol, 97%), which required no further purification; \( \text{m.p.} 161 \sim 163 \, ^{\circ} \mathrm{C} \) (decomp.); \( ^{1} \text{H NMR} \) (500 MHz, MeOD-d4) \( \delta \) 7.68 (d, \( J = 7.9 \, \text{Hz} \), 1H, ArH), 7.38 (d, \( J = 8.1 \, \text{Hz} \), 1H, ArH), 7.21 (s, 1H, ArH), 7.14 (t, \( J = 7.4 \, \text{Hz} \), 1H, ArH), 7.07 (t, \( J = 7.4 \, \text{Hz} \), 1H, ArH), 4.24 – 4.09 (m, 2H, CHα-Leu, CHα-Sc), 3.77 – 3.64 (m, 4H, 2 × CH2-Az), 3.51 – 3.41 (m, 3H, CH2GAz, CHHβ-Trp), 3.26 – 3.16 (m, 3H, CH2Gly, CHHβ-Trp), 1.67 – 1.56 (m, 2H, CH2β-Leu), 1.43 (s, 9H, 3 × CH3, Boc), 1.35 – 1.29 (m, 1H, CH, γ-Leu), 0.86 (d, \( J = 6.6 \, \text{Hz} \), 3H, CHδ-Leu), 0.81 (d, \( J = 6.5 \, \text{Hz} \), 3H, CHδ-Leu); \( ^{13} \text{C NMR} \) (126 MHz, MeOD-d4) \( \delta \) C 184.7, 174.4, 170.8 (C=O), 158.2 (C=O Boc), 138.3 (C), 128.3 (C), 125.5 (CH), 122.9 (CH), 120.3 (CH), 119.2 (CH), 112.6 (CH), 108.3 (C), 81.2 (C, Boc), 56.9 (2 × CH2-Az), 55.2 (CH, α-Trp), 54.4 (CH, α-Leu), 47.5 (CH2, Gly), 43.6.
Following general procedure 5 from Chz-Asp(Bu)-Leu-GAz(Boc)-Gly-OBn (464) (221 mg, 0.29 mmol, 1.0 equiv), H-Asp(Bu)-Leu-GAz(Boc)-Gly-OH (294) was isolated as a white solid (155 mg, 0.29 mmol, quant. yield), which required no further purification; m.p. 121 – 124 °C; 1H NMR (500 MHz, MeOD-d$_4$) δ$_H$ 4.32 (dd, $J = 9.7, 5.3$ Hz, 1H, CH$_\alpha$-Leu), 4.19 (dd, $J = 8.8, 4.0$ Hz, 1H, CH$_\alpha$-Asp), 3.92 – 3.76 (m, 4H, 2 × CH$_2$-Az), 3.65 (d, $J = 14.6$ Hz, 1H, CHHGly), 3.56 – 3.38 (m, 3H, CHHGly, CH$_2$Gaz), 3.06 (dd, $J = 18.1, 4.0$ Hz, 1H, CHH$_\beta$-Asp), 2.83 (dd, $J = 18.0, 8.9$ Hz, 1H, CHH$_\beta$-Asp), 1.70 – 1.60 (m, 3H, CH$_\gamma$-Leu, CH$_\beta$-Leu), 1.50 (s, 9H, 3 × CH$_3$-Bu or 3 × CH$_3$Boc), 1.45 (s, 9H, 3 × CH$_3$-Bu or 3 × CH$_3$Boc), 0.98 (d, $J = 6.4$ Hz, 3H, CH$_3$-Leu), 0.95 (d, $J = 6.4$ Hz, 3H, CH$_3$-Leu); 13C NMR (126 MHz, MeOD-d$_4$) δ$_C$ 173.7 (C=O), 169.6 (C=O), 168.6 (C=O), 166.3 (C=O, Boc), 82.3 (C, Boc or CO$_2$-Bu), 80.1 (C, Boc or CO$_2$-Bu), 55.7 (2 × CH$_2$-Az), 52.8 (CH, α-Leu), 49.6 (CH, α-Asp), 44.7 (CH$_2$-Gaz), 41.8 (CH$_2$, Gly), 39.9 (CH$_2$, β-Leu), 35.8 (CH$_3$, β-Asp), 27.2 (CH$_3$, Boc or CO$_2$-Bu), 26.9 (CH$_3$, Boc or CO$_2$-Bu), 24.5 (CH, γ-Leu), 22.1 (CH$_3$, β-Leu), 20.4 (CH$_3$, δ-Leu); ν$_{max}$ (neat) = 3331, 2958, 2930, 1654, 1546, 1366, 1157 cm$^{-1}$; MS (ESI$^+$) $m/z$ 544 [M+H]$^+$, 566 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{29}$H$_{46}$N$_5$O$_8$ [M+H]$^+$ 544.3341, found 544.3339; [α]$_D^{26}$ +7.0 (c 0.31, MeOH).

Cyclo(D-Pro-Leu-GAz(Boc)-Gly) (295)

This compound was prepared following general procedure 6 from H-D-Pro-Leu-GAz(Boc)-Gly-OH (292) (47 mg, 0.10 mmol, 1.0 equiv) in DMF (100 mL, 1 mM) for 48 h to give a crude residue which was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 40:1→19:1) to give the cyclic tetrapeptide 295 as a glassy colourless solid (35.8 mg, 79 μmol, 79%). This procedure was repeated on 0.44 mmol scale at 5 mM concentration to yield the cyclic peptide (163 mg, 362 μmol, 82%); R$_f$ (CH$_2$Cl$_2$/MeOH 19:1) 0.40; m.p. 129 – 131 °C; 1H NMR (500 MHz, MeOD-d$_4$) δ$_H$ 4.49 – 4.40 (m, 2H, CHα-Pro, CHα-Leu), 3.95 (s, 1H, CHH-Az), 3.84 – 3.78 (m, 1H, CHH-Az), 3.74 – 3.55 (m, 5H, 2 × CHH-Az, CHHδ-Pro, CH$_2$Gly), 3.37 – 3.35 (m, 3H, CHHδ-Pro, CH$_2$Gaz), 2.38 –
2.29 (m, 1H, CHHγ-Pro), 2.05 – 1.89 (m, 3H, CHHγ-Pro, CH2β-Pro), 1.73 – 1.65 (m, 2H, CHγ-Leu, CHHβ-Lee), 1.61 – 1.56 (m, 1H, CHHβ-Leu), 1.45 (s, 9H, 3 × CH3 Boc), 1.00 (d, J = 6.1 Hz, 3H, CH3δ-Leu), 0.95 (d, J = 6.0 Hz, 3H, CH3δ-Leu). Note: CHHδ-Pro, CH2GAz overlaps with solvent signal; 13C NMR (126 MHz, MeOD-d4) δc 176.3 (C=O), 157.5 (C=O), 173.0 (C=O), 158.2 (C=O, Boc), 81.2 (C, Boc), 62.1 (CH, α-Leu), 57.0 (2 × CH2 Az), 54.0 (CH, α-Pro), 48.4 (CH2, GAz), 47.8 (CH2, Gly), 38.3 (CH2, β-Leu), 33.0 (CH2, γ-Pro), 28.6 (CH3, Boc), 26.1 (CH, γ-Leu), 23.5 (CH2, β-Pro), 23.0 (CH3, δ-Leu), 22.5 (CH3, δ-Leu). Note: CH2, δ-Pro overlaps with solvent signal; νmax (neat) = 2954, 1662, 1623, 1404, 1164 cm⁻¹; MS (ESI⁺) m/z 474 [M+Na]+, 925 [2M+Na]+; HRMS (ESI⁺) calcd. for C23H37N3NaO5 [M+Na]+ 474.2687, found 474.2684; [α]D24 –54.4 (c 0.12, MeOH).

Cyclo(Trp-Leu-GAz(Boc)-Gly) (296)

This compound was prepared following general procedure 6 from H-Trp-Leu-GAz(Boc)-Gly-OH (293) (56 mg, 0.10 mmol, 1.0 equiv) in DMF (100 mL, 0.001 M) for 48 h to give a crude residue which was purified by column chromatography (SiO2, CH2Cl2/MeOH 40:1→19:1) to give the cyclic tetrapeptide as a glassy colourless solid (39 mg, 72 µmol, 72%). This procedure was repeated on 0.49 mmol scale at 5 mM concentration to yield the cyclic peptide (210 mg, 388 µmol, 80%); Rr (CH2Cl2/MeOH 19:1) 0.31; m.p. 200 – 202 °C (decomp.); 1H NMR (500 MHz, DMSO-d6) δh 10.87 (s, 1H, NH), 8.21 (d, J = 10.4 Hz, 1H, NH), 7.90 (d, J = 9.1 Hz, 1H, NH), 7.53 (d, J = 7.9 Hz, 1H, ArH), 7.42 (s, 1H, NH), 7.33 (d, J = 8.1 Hz, 1H, ArH), 7.11 (s, 1H, ArH), 7.07 (t, J = 7.5 Hz, 1H, ArH), 6.99 (t, J = 7.4 Hz, 1H, ArH), 4.61 (dd, J = 17.7, 9.4 Hz, 1H, CHα-Trp), 4.00 (dd, J = 9.5, 5.5 Hz, 1H, CHβ-Leu), 3.63 (d, J = 8.3 Hz, 1H, CHH-Az), 3.55 – 3.49 (m, 2H, CHH-Az, CHHGAz), 3.20 (dd, J = 14.9, 9.4 Hz, 1H, CHHβ-Trp), 3.06 – 3.00 (m, 2H, CHHβ-Trp, CHHGly), 2.95 (dd, J = 13.3, 2.4 Hz, 1H, CHHGAz), 1.61 – 1.54 (m, 2H, CH2β-Leu), 1.49 – 1.45 (m, 1H, CHγ-Leu), 1.37 (s, 9H, 3 × CH3 Boc), 0.91 (d, J = 6.2 Hz, 3H, CH3δ-Leu), 0.78 (d, J = 6.3 Hz, 3H, CH3δ-Leu), Note: CH2-Az & CHHGly overlap with solvent signal; 13C NMR (126 MHz, DMSO-d6) δc 173.1 (C=O), 172.9 (C=O), 171.5 (C=O), 155.6 (C=O, Boc), 136.1 (C), 127.1 (C), 123.0 (CH), 121.1 (CH), 118.4 (CH), 118.1 (CH), 111.5 (CH), 109.5 (C), 78.8 (C, Boc), 56.1 (CH, α-Trp), 55.5 (2 × CH2 Az), 54.0 (CH, α-Leu), 47.1 (CH2, Gly), 44.7 (CH2, GAz), 39.3 (CH2, β-Leu) 28.1 (CH3, Boc), 26.4 (CH2, β-Trp), 24.6 (CH, γ-Leu), 22.8 (CH3, δ-Leu), 21.3 (CH3, δ-Leu), Note: CH2, β-Leu overlaps with solvent signal; νmax (neat) = 3278, 2969, 1665, 1524, 1456, 879 cm⁻¹; MS (ESI⁺) m/z 563 [M+Na]+; HRMS (ESI⁺) calcd. for C23H40N3NaO5 [M+Na]+ 563.2952, found 563.2953; [α]D26 –74.6 (c 0.14, MeOH).
**Cyclo(Asp(Bu)-Leu-GAzm(Boc)-Gly) (297)**

This compound was prepared following general procedure 6 from H-Asp(Bu)-Leu-GAzm(Boc)-Gly-OH (294) (76 mg, 0.14 mmol, 1.0 equiv) in DMF (140 mL, 0.001 M) for 48 h to give a crude residue which was purified by column chromatography (SiO2, CH2Cl2/MeOH 40:1→19:1) to give the cyclic tetrapeptide 297 as a glassy colourless solid (49 mg, 93 μmol, 66%); **Rt** (CH3Cl2/MeOH 19:1) 0.41; **mp** 154 – 157 °C; **1H NMR** (500 MHz, DMSO-d6) δH 8.49 (d, J = 8.5 Hz, 1H, NH), 7.87 (d, J = 8.7 Hz, 1H, NH), 7.38 – 7.32 (m, 1H, NH), 4.49 – 4.40 (m, 1H, CHα-Asp), 4.27 – 4.22 (m, 1H, CHα-Leu), 3.66 – 3.51 (m, 5H, 2 × CH2-Az, CHHGm), 3.21 (d, J = 18.2 Hz, 1H, CHHGly), 3.13 (dd, J = 13.9, 4.7 Hz, 1H, CHHGm), 3.01 (d, J = 17.1 Hz, 1H, CHHGly), 2.73 (dd, J = 14.4, 4.3 Hz, 1H, CHHβ-Asp), 2.61 (dd, J = 14.1, 6.0 Hz, 1H, CHHβ-Leu), 1.56 – 1.47 (m, 3H, CH2β-Leu, CHβ-Leu), 1.37 (s, 9H, CH3, Boc), 1.11 (s, 9H, CH3, Bu), 0.87 (d, J = 6.1 Hz, 3H, CH3δ-Leu), 0.82 (d, J = 6.1 Hz, 3H, CH3δ-Leu); **13C NMR** (126 MHz, DMSO) δC 172.3 (C=O), 172.0 (C=O), 169.6 (C=O), 155.6 (C=O Boc), 78.7 (C, Boc), 67.0 (C, CO2Bu), 55.3 (CH2-Az), 51.1 (CH, α-Leu), 48.4 (CH, α-Asp), 45.4 (CH2, Gly), 44.0 (CH2, GAzm), 38.1 (CH2, β-Leu), 37.0 (CH3, β-Asp), 31.3 (CH3, CO2Bu), 28.1 (CH3, Boc), 24.3 (CH, γ-Leu), 22.9 (CH3, δ-Leu), 21.7 (CH3, δ-Leu); vmax (neat) = 3292, 2958, 1672, 1526, 1366, 1154 cm⁻¹; **MS** (ESI⁺) m/z 548 [M+Na]+'; **HRMS** (ESI¹') calcd. for C25H38N7O7Na; [M+Na]+' 548.3055, found 548.3050; [α]D²⁶ = 57.2 (c 0.09, MeOH).

**Preparation of cyclic pentapeptide 301**

**Fmoc-Leu-GAzm(Cbz)-Gly-O'Bu (298)**

Following general procedure 7 from H-Gly-O'Bu•HCl (1.00 g, 6.00 mmol, 1.0 equiv) and Fmoc-Leu-OSu (4.05 g, 9.00 mmol, 1.5 equiv), the crude product was purified by column chromatography (SiO2, CH2Cl2:EtOAc 9:1 → CH2Cl2:EtOAc 1:1) to yield the title compound 298 as a white foam (2.95 g, 4.32 mmol, 72% yield); **Rt** (CH3Cl2:EtOAc 9:1) 0.19; **mp** 80 – 82 °C; **1H NMR** (500 MHz, CDCl3) δH 7.75 (d, J = 7.4 Hz, 2H, ArH), 7.61 – 7.56 (m, 2H, ArH), 7.41 – 7.28 (m, 9H, ArH), 6.86 (s, 1H, NH), 5.33 (d, J = 7.2 Hz, 1H, NH Fmoc), 5.08 (s, 2H, CH2Ph), 4.45 – 4.33 (m, 2H, CH2Fmoc), 4.26 – 4.15 (m, 2H, CHα-Leu, CH Fmoc), 3.92 – 3.40 (m, 6H, 2 × CH2-Az, CH2GAzm), 3.39 – 3.20 (m, 2H, CH2Gly), 1.68 – 1.53 (m, 3H, CH2β-Leu, CHγ-Leu), 1.44 (s, 9H, CO2Bu), 0.94 (d, J = 5.0 Hz, 6H, 2 × CH3δ-Leu); **13C NMR** (126 MHz, CDCl3) δC 173.1 (C=O), 156.5 (C=O Fmoc or Cbz), 156.3 (C=O Fmoc or Cbz), 144.0 (C), 143.9 (C), 141.4 (C), 133.3 (C), 132.1 (C), 129.1 (C), 128.1 (C), 127.4 (C), 123.1 (C), 121.1 (C), 119.4 (C), 115.4 (C), 107.5 (C), 101.5 (C), 98.5 (C), 90.5 (C), 88.5 (C), 79.5 (C), 81.0 (C), 77.0 (C), 72.5 (C), 70.5 (C), 60.5 (C), 54.5 (C), 44.5 (C), 43.5 (C), 42.5 (C), 41.5 (C), 40.5 (C), 39.5 (C), 38.5 (C), 37.5 (C), 36.5 (C), 35.5 (C), 34.5 (C), 33.5 (C), 32.5 (C), 31.5 (C), 30.5 (C), 29.5 (C), 28.5 (C), 27.5 (C), 26.5 (C), 25.5 (C), 24.5 (C), 23.5 (C), 22.5 (C), 21.5 (C), 20.5 (C), 19.5 (C), 18.5 (C), 17.5 (C), 16.5 (C), 15.5 (C), 14.5 (C), 13.5 (C), 12.5 (C), 11.5 (C), 10.5 (C), 9.5 (C), 8.5 (C), 7.5 (C), 6.5 (C), 5.5 (C), 4.5 (C), 3.5 (C), 2.5 (C), 1.5 (C), 0.5 (C).
Following general procedure 2 from Fmoc-Leu-GAz(Cbz)-Gly-OtBu (298) (2.74 g, 4.00 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 98.5:2.5) to give the title compound 299 (1.48 g, 2.25 mmol, 56%) as a white foam; \( R_f \) (CH₂Cl₂/MeOH 98.5:2.5) 0.17; m.p. 77–80 °C; \(^1^H\) NMR (500 MHz, DMSO-d₆, 373 K) \( \delta \)H 7.54 (s, 1H, NH), 7.45 (d, \( J = 7.5 \) Hz, 1H, NH), 7.38 – 7.27 (m, 5H, ArH), 5.04 (s, \( J = 4.0 \) Hz, 2H, CH₂Ph), 4.32 (td, \( J = 8.7, 5.5 \) Hz, 1H, CHα-Leu), 4.14 (dd, \( J = 8.4, 3.7 \) Hz, 1H, CHα-Pro), 3.77 – 3.73 (m, 2H, CH₂-Az), 3.70 (d, \( J = 8.8 \) Hz, 2H, CH₂-Az), 3.42 – 3.33 (m, 4H, CH₂Gly, CH₂δ-Pro), 3.27 (s, 2H, CH₂Gly), 2.12 – 2.06 (m, 1H, CHHβ-Pro), 1.87 – 1.75 (m, 3H, CHHβ-Pro, CH₂γ-Pro), 1.68 – 1.62 (m, 1H, CHγ-Leu), 1.57 – 1.49 (m, 2H, CH₂β-Leu), 1.44 (s, 9H, CH₃, CO₂Bu), 1.39 (s, 9H, CH₃, Boc), 0.89 (d, \( J = 6.6 \) Hz, 3H, CH₃δ-Leu), 0.87 (d, \( J = 6.5 \) Hz, 3H, CH₃δ-Leu); \(^1^C\) NMR (126 MHz, DMSO-d₆) \( \delta \)C 172.0 (C=O), 171.4 (C=O), 155.4 (C=O, Cbz), 153.3 (C=O, Boc), 136.5 (C), 127.7 (CH), 127.1 (CH), 126.8 (CH), 79.9 (C, CO₂Bu), 78.3 (C, Boc), 65.2 (CH₂, Cbz), 59.5 (CH, α-Pro), 56.7 (2 × CH₂, Az), 55.1 (C, Az), 50.9 (CH, α-Leu), 46.2 (CH₂, GAz), 44.9 (CH₂, Gly), 42.8 (CH₂, δ-Pro), 40.6 (CH₂, β-Leu), 29.7 (CH₂, β-Pro), 27.6 (CH₃, CO₂Bu), 27.3 (CH₃, Boc), 23.8 (CH, γ-Leu), 22.9 (CH₂, γ-Pro), 22.2 (CH₃, δ-Leu), 21.1 (CH₃, δ-Leu), Note: one C=O not observed; \( \nu_{\text{max}} \) (neat) = 3299, 2957, 1694, 1659, 1155, 765 cm\(^{-1}\); MS (ESI⁺) \( m/z \) 660 [M+H]+, 682 [M+Na]+; HRMS (ESI⁺) calcd. for C₃₉H₄₈N₄NaO₇ [M+Na]+ 707.3415, found 707.3418; [α] \(_D\) \(^{26}\) –9.1 (c 0.29, MeOH).
H-(D)Pro-Leu-GA(Cbz)-Gly-OH (300)

To a solution of Boc-(D)Pro-Leu-GA(Cbz)-Gly-O'Bu (299) (1.45 g, 2.20 mmol, 1.0 equiv) in CH₂Cl₂ (4 mL) was added TFA (4 mL) dropwise and allowed to stir at RT for 1.5 h. Upon complete consumption of the starting material monitored by TLC and ESI-MS, the solvent was removed in vacuo, resuspended in CH₂Cl₂ and the solvent removed in vacuo (3 ×) to give the title compound 300 as a white foam (1.74 g, 1.88 mmol, 86%) as a TFA salt, which required no further purification. ¹H and ¹⁹F NMR using TFE as an internal standard confirmed 3.7 eq. of TFA salt; m.p. 98 – 101 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 7.43 – 7.24 (m, 5H, ArH), 5.12 (s, 2H, CH₂Ph), 4.40 – 4.26 (m, 2H, CHα-Pro, CHα-Leu), 4.25 – 3.94 (m, 6H, CH₂Gly, 2 × CH₂-Az), 3.86 (d, J = 14.9 Hz, 1H, CH₂HGAz), 3.65 (d, J = 13.3 Hz, 1H, CHHGAz), 2.51 – 2.38 (m, 1H, CHβ-Pro), 2.09 – 1.93 (m, 3H, CHβ-γ-Pro), 1.73 – 1.59 (m, 3H, CHβ-Leu, CHγ-Leu), 0.98 (d, J = 6.2 Hz, 3H, CHδ-Leu), 0.93 (d, J = 6.2 Hz, 3H, CHδ-Leu), Note: CH₂-Pro overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δC 176.1 (C=O), 170.6 (C=O), 169.8 (C=O), 157.9 (C=O Cbz), 137.6 (C), 129.6 (CH), 129.4 (CH), 129.2 (CH), 68.5 (CH₂, Cbz), 61.2 (CH, α-Pro), 58.5 (C, Az), 54.1 (CH, α-Leu), 47.5 (CHβ-γ-Pro), 44.4 (CH₂, Gly), 41.4 (CH₂, β-Leu), 41.3 (CH₂, GAz), 31.2 (CH₂, β-Pro), 26.1 (CH, γ-Leu), 25.2 (CH₂, γ-Pro), 23.4 (CH₃, δ-Leu), 21.7 (CH₃, δ-Leu), Note: 2 × CH₂, Az not observed/broad signal; νmax (neat) = 3229, 2962, 1666, 1638, 1173, 1130 cm⁻¹; MS (ESI⁺) m/z 504 [M+H]⁺, 526 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₅H₃₈N₅O₆ [M+H]⁺ 504.2817, found 504.2814; [α]D²⁶ +6.5 (c 0.31, MeOH).

Cyclo-(D-Pro-Leu-GA(Cbz)-Gly) (301)

To a solution of H-D-Pro-Leu-GA(Cbz)-Gly-OH•3.7 TFA (300) (93 mg, 0.1 mmol, 1.0 equiv) in DMF (2 mL) was added DIPEA (70 µL, 0.40 mmol, 4.0 equiv) and stirred at RT for 10 min. To this solution was added DMF (18 mL, total concentration 5 mM), DIPEA (35 µL, 0.20 mmol, 2.0 equiv) and DEPBT (60 mg, 0.20 mmol, 2.0 equiv) and the solution was stirred at RT for 48 h. The solvent was removed in vacuo at 60 °C over 30 min and the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 39:1 → 19:1) to give the title compound 301 as a glassy white solid (35 mg, 73 µmol, 73%); Rf (CH₂Cl₂/MeOH 19:1) 0.22; m.p. 125 – 128 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 7.39 – 7.26 (m, 5H, ArH), 5.07 (s, 2H, CH₂Ph), 4.47 – 4.25 (m, 2H, CHα-Pro, CHα-Leu), 4.11 – 3.97 (m, 1H, CHH-Az), 3.89 – 3.52 (m, 6H, CHH-Az, CH₂-Az, CH₂δ-Pro, CHHGAz), 2.37 – 2.18 (m, 1H,
**Preparation of dipeptide building blocks for SPPS via one-pot method**

**Fmoc-GAz(Boc)-Thr(tBu)-OCumyl (307)**

Following general procedure 4 from NO$_2$-GAz(Boc)-Thr(tBu)-OCumyl (284) (2.00 g, 3.94 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/EtOAc 19:1) to give the title compound as a white foam (1.43 g, 2.04 mmol, 52%). Following the one-pot method using general procedure 7 from Fmoc-Thr(tBu)-OCumyl (194) could be applied on 5 mmol scale in 63% yield; R$_f$ (CH$_2$Cl$_2$/EtOAc 19:1) to give the title compound as a white foam (1.43 g, 2.04 mmol, 52%). Following the one-pot method using general procedure 7 from Fmoc-Thr(tBu)-OCumyl (194) could be applied on 5 mmol scale in 63% yield; R$_f$ (CH$_2$Cl$_2$/EtOAc 19:1) to give the title compound as a white foam (1.43 g, 2.04 mmol, 52%).
Fmoc-GAz(Chzm)-Thr(Bu)-OCumyl (308)

Following the general procedure 7 for building block synthesis, Fmoc-Thr(Bu)-OCumyl (194) (5.51 g, 10.7 mmol, 1.0 equiv) and benzyl 3-(nitromethylene)azetidine-1-carboxylate (259) (2.65 g, 10.7 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO2, CH2Cl2/EtOAc 19:1), the title compound 308 (3.46 g, 4.72 mmol, 44%) as a white foam; Rf (CH2Cl2/EtOAc 19:1) 0.37; m.p. 73 – 76 °C; 1H NMR (500 MHz, CDCl3) δH 7.77 (d, J = 7.5 Hz, 2H, ArH), 7.61 – 7.56 (m, 2H, ArH), 7.43 – 7.28 (m, 14H, ArH), 5.43 (s, 1H, NH, Fmoc), 5.10 (s, 2H, CH2Ph), 4.42 – 4.33 (m, 2H, CH2, Fmoc), 4.19 (t, J = 6.9 Hz, 1H, CH, Fmoc), 3.82 – 3.54 (m, 6H, 2 × CH2-Az, CHα-Thr, CHHGAz, 3.27 – 2.99 (m, 2H, CHHGAz, CHβ-Thr), 1.85 (s, 3H, CH3, Cumyl), 1.79 (s, 3H, CH3, Cumyl), 1.22 – 1.15 (m, 12H, CH3, ’Bu, CH3γ-Thr); 13C NMR (126 MHz, CDCl3) δC 173.5 (C=O), 156.9 (C=O), 156.6 (C=O), 144.7 (C), 144.03 (C), 144.01 (C), 141.41 (C), 141.40 (C), 136.6 (CH), 128.6 (CH), 128.4 (CH), 128.2 (CH), 128.1 (CH), 127.8 (CH), 127.6 (CH), 127.1 (CH), 125.3 (CH), 124.8 (CH), 120.1 (CH), 83.3 (C, Cumyl), 74.4 (C, ’Bu), 69.1 (CH, α-Thr), 66.9 (CH2 Fmoc and CH2Ph), 61.9 (CH, β-Thr), 55.3 (CH3, Az), 47.3 (CH, Fmoc), 45.6 (CH2, GAz), 28.9 (CH3, Cumyl), 28.7 (CH3, ’Bu), 27.5 (CH3, Cumyl), 20.1 (CH3, γ-Thr), Note: one Ar C missing; vmax (neat) = 2957, 1699, 1513, 1182, 745 cm⁻¹; MS (ESI⁺) m/z 734 [M+H]⁺, 756 [M+Na]⁺; HRMS (ESI⁺) calcd. for C44H33N3NaO7 [M+Na]⁺ 756.3619, found 756.3618; [α]D30 +2.5 (c 0.20, MeOH).

Fmoc-GAz(Boc)-Asp(Bu)-OCumyl (309)

Following the general procedure 7 for building block synthesis, Fmoc-Asp(Bu)-OCumyl (465) (2.28 g, 4.30 mmol, 1.0 equiv) and tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (922 mg, 4.30 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO2, CH2Cl2/EtOAc 98:2), the title compound 309 (2.25 g, 3.15 mmol, 73%) as a white foam; Rf (CH2Cl2/EtOAc 19:1) 0.27; m.p. 83 – 86 °C; 1H NMR (500 MHz, CDCl3) δH 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.63 (d, J = 7.5 Hz, 1H, ArH), 7.61 (d, J = 7.6 Hz, 1H, ArH), 7.40 (t, J = 7.5 Hz, 2H, ArH), 7.35 – 7.26 (m, 7H, ArH), 5.74 (s, 1H, NH, Fmoc), 4.41 – 4.32 (m, 2H, CH2, Fmoc), 4.19 (t, J = 7.2 Hz, 1H, CH, Fmoc), 3.87 – 3.32 (m, 7H, CH2GAz, CHα-Asp, 2 × CH2-Az), 2.66 (dd, J = 15.7, 3.4 Hz, 1H, CHHβ-Asp), 2.46 (dd, J = 15.8, 9.4 Hz, 1H, CHHβ-Asp), 2.11 (s, 1H, NH), 1.80 (s, 3H, CH3, Cumyl), 1.80 (s, 3H, CH3, Cumyl), 1.48 (s, 9H, CH3, Boc), 1.42 (s, 9H, CH3, ’Bu); 13C NMR (126 MHz, CDCl3) δC 173.5 (C=O), 170.5 (C=O), 157.1 (C=O, Boc), 156.3 (C=O, Fmoc), 144.8 (C), 144.2 (C), 144.1 (C), 141.43 (C), 204
141.40 (C), 128.6 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.4 (CH), 125.3 (CH), 124.4 (CH), 120.1 (CH), 83.6 (C, Cumyl), 82.0 (C, Boc), 79.7 (C, CO₂Bu), 67.0 (CH₂, Fmoc), 55.0 (CH₂, Az), 53.1 (CH, α-Asp), 47.4 (CH, Fmoc), 45.3 (CH₂, GAz), 40.4 (CH₂, β-Asp), 28.6 (CH₃, Boc), 28.5 (CH₃, Cumyl), 28.4 (CH₃, Cumyl), 28.2 (CH₃, CO₂Bu); νₘₘₚ (neat) = 2975, 1700, 1132, 1100, 698 cm⁻¹; MS (ESI⁺) m/z 714 [M+H]⁺, 736 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₁H₅₁N₃NaO₈ 736.3568 [M+Na]⁺, found 736.3570; [α]D²⁶ +6.7 (c 0.22, CHCl₃)

**Fmoc-GA(Z-Boc)-Ile-OCumyl (310)**

Following the general procedure 7 for building block synthesis, Fmoc-Ile-OCumyl (466) (1.35 g, 2.87 mmol, 1.0 equiv) and tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (615 mg, 2.87 mmol) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 19:1), the title compound 310 (1.54 g, 2.34 mmol, 82%) as a white foam; Rₐ (CH₂Cl₂/EtOAc 19:1) 0.22; m.p. 120–122 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.77 (d, J = 7.5 Hz, 2H, ArH), 7.60–7.54 (m, 2H, ArH), 7.42–7.30 (m, 8H, ArH), 7.26–7.22 (m, 1H, ArH), 5.28 (s, 1H, NH, Fmoc), 4.35 (d, J = 7.0 Hz, 2H, CH₂, Fmoc), 4.20 (t, J = 7.0 Hz, 1H, CH, Fmoc), 3.67–3.52 (m, 5H, 2 × CH₂-Az, CHHGaZ), 3.16–3.00 (m, 2H, CHHGaZ, CHα-Ile), 1.85–1.77 (m, 7H, 2 × CH₃, Cumyl, CHβ-Ile), 1.47–1.39 (m, 10H, CH₃, Boc, CCHγ-Ile), 1.17–1.09 (m, 1H, CHHγ-Ile), 0.99 (d, J = 6.6 Hz, 3H, CH₃-δ-Ile), 0.93 (t, J = 7.3 Hz, 3H, CH₃-δ-Ile); ¹³C NMR (126 MHz, CDCl₃) δC 175.0 (C=O), 156.8 (C=O, Boc), 156.4 (C=O, Fmoc), 144.9 (C), 144.1 (C), 144.0 (C), 141.42 (C), 141.41 (C), 128.4 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.6 (CH), 120.1 (CH), 83.0 (C, Cumyl), 79.8 (C, Boc), 67.0 (CH₂, Fmoc), 60.6 (CH, α-Ile), 55.0 (2 × CH₂, Az), 47.3 (CH, Fmoc), 45.9 (CH₂, GAz), 39.4 (CH, β-Ile), 28.5 (CH₃, Boc, CH₃, Cumyl), 27.9 (CH₃, Cumyl), 24.5 (CH₂, γ-Ile), 16.2 (CH₃, ε-Ile), 12.0 (CH₃, δ-Ile); νₘₚ (neat) = 2970, 1726, 1702, 1435, 1131, 739 cm⁻¹; MS (ESI⁺) m/z 656 [M+H]⁺, 678 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₉H₄₉N₃NaO₆ 678.3514 [M+Na]⁺, found 678.3514; [α]D²⁶ +13.5 (c 0.15, CHCl₃).

**Fmoc-GAz(Boc)-Tyr(tBu)-OCumyl (311)**

Following the general procedure 7 for building block synthesis, Fmoc-Tyr(tBu)-OCumyl (467) (1.50 g, 2.56 mmol, 1.0 equiv) and tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (548 mg, 2.56 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 98:2), the title compound 311 (1.29 g, 1.70 mmol, 66%) as a white foam; Rₐ (CH₂Cl₂/EtOAc 98:2) 0.17; m.p. 86–89 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.77 (d, J = 7.5 Hz,
Following the general procedure 7 for building block synthesis, Fmoc-Lys(Boc)-OCumyl (468) (0.58 g, 1.04 mmol, 1.0 equiv) and tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (0.22 g, 1.04 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 9:1), the title compound 312 (0.52 g, 0.68 mmol, 65%) as a white foam; Rf (CH₂Cl₂/EtOAc 9:1) 0.17; m.p. 87 – 90 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.60 – 7.54 (m, 2H, ArH), 7.42 – 7.38 (m, 2H, ArH), 7.37 – 7.29 (m, 6H, ArH), 7.26 – 7.22 (m, 1H, ArH), 5.29 (s, 1H, NH, Fmoc), 4.54 (s, 1H, NH, Boc), 4.42 – 4.30 (m, 2H, CH₂, Fmoc), 4.19 (t, J = 6.8 Hz, 1H, CH, Fmoc), 3.66 (d, J = 8.7 Hz, 1H, CHH-Az), 3.63 (d, J = 8.9 Hz, 1H, CHH-Az), 3.59 – 3.51 (m, 3H, CH₂-Az, CHHGAz), 3.21 – 3.03 (m, 4H, CHHGAz, CHα-Lys, CH₂-E-Lys), 1.93 (s, 1H, NH), 1.80 (s, 3H, CH₃, Cumyl), 1.79 (s, 3H, CH₃, Cumyl), 1.73 – 1.67 (m, 1H, CHHβ-Lys), 1.54 – 1.39 (m, 23H, CHHβ-Lys, CH₂γ-Lys, CH₂δ-Lys, 6 × CH₃, Boc); ¹³C NMR (126 MHz, CDCl₃) δC 175.2 (C=O), 156.9 (C=O, Boc), 156.4 (C=O, Boc), 156.1 (C=O, Fmoc), 144.8 (C), 144.1 (C), 144.0 (C), 141.4 (C), 141.3 (C), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.5 (CH), 124.5 (CH), 120.1 (CH), 83.1 (C, Cumyl), 79.9 (C, Boc), 79.3 (C, Boc), 67.0 (CH₂, Fmoc), 56.1 (CH₂, Az), 55.0 (CH, α-Lys), 47.4 (CH, Fmoc), 45.9 (CH₂, GAz), 40.4 (CH₂, ε-Lys), 34.5 (CH₂, δ-Lys), 30.1 (CH₂, γ-Lys), 28.6 (CH₃, Boc), 28.5 (CH₃, Boc, CH₃, Cumyl), 28.1 (CH₃, Cumyl), 23.0 (CH₂, β-Lys); v max (neat) = 2974, 1688, 1512, 1132, 860 cm⁻¹; MS (ESI⁺) m/z 771 [M+H]⁺, 793
[M+Na]+; HRMS (ESI+) calcd. for C₄₇H₅₈N₄NaO₈ 793.4147 [M+Na]+, found 793.4145; [α]D²⁶ +5.0 (c 0.24, CHCl₃).

**Fmoc-GAz(Cbz)-Lys(Boc)-OCumyl (313)**

Following the general procedure 7 for building block synthesis, Fmoc-Lys(Boc)-OCumyl (468) (0.90 g, 1.50 mmol, 1.0 equiv) and benzyl-3-(nitromethylene)azetidine-1-carboxylate (259) (0.37 g, 1.50 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 85:15), the title compound (313) (0.70 g, 0.86 mmol, 58%) as a white foam; Rf (CH₂Cl₂/EtOAc 85:15) 0.27; m.p. 70 – 73 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.56 (t, J = 7.0 Hz, 2H, ArH), 7.41 – 7.28 (m, 14H, ArH), 5.33 – 5.24 (m, 1H, NH), 5.09 (s, 2H, CH₂Ph), 4.58 – 4.51 (m, 1H, NH), 4.40 – 4.32 (m, 2H, CH₂Fmoc), 4.18 (t, J = 6.9 Hz, 1H, CH Fmoc), 3.77 – 3.56 (m, 5H, 2 × CH₂-Az, C₃H₅GAz), 3.21 – 3.05 (m, 4H, CH₃GAz, CH₂ε-Lys, CHα-Lys), 1.80 (s, 3H, CH₃Cumyl), 1.78 (s, 3H, CH₃Cumyl), 1.72 – 1.68 (m, 1H, CHβ-Lys), 1.55 – 1.39 (m, 14H, CHβ-Lys, CH₂δ-Lys, CH₃Boc); ¹³C NMR (126 MHz, CDCl₃) δ 175.2 (C=O), 156.9 (C=O), 156.6 (C=O), 156.1 (C=O), 144.7 (C), 144.03 (C), 143.99 (C), 141.4 (C), 136.6 (C), 128.6 (CH), 128.5 (CH), 128.22 (CH), 128.19 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 83.2 (C, Cumyl), 79.3 (C, Boc), 67.0 (2 × CH₂, Fmoc and Cbz), 56.0 (2 × CH₂, Az), 55.6 (CH, α-Lys), 47.3 (CH, Fmoc), 45.8 (CH₂, GAz), 40.4 (CH₂, δ-Lys), 34.4 (CH₂, β-Lys), 30.0 (CH₂, γ-Lys), 28.6 (CH₃, Boc), 28.0 (CH₃, Cumyl), 23.0 (CH₂, δ-Lys), Note: C, Az and 1 × C not observed; νmax (neat) = 3322, 2972, 1688, 1130, 1099, 561 cm⁻¹; MS (ESI+) m/z 805 [M+H]+, 827 [M+Na]+; HRMS (ESI+) calcd. for C₄₇H₅₈N₄NaO₈ [M+Na]+ 827.3990, found 827.3986; [α]D²⁸ +4.5 (c 0.20, CHCl₃).

**Fmoc-GAz(Boc)-Glu(tBu)-OCumyl (314)**

Following the general procedure 7 for building block synthesis, Fmoc-Glu(tBu)-OCumyl (469) (2.99 g, 5.51 mmol, 1.0 equiv) and tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (1.18 g, 5.51 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 98:2), the title compound 314 (2.49 g, 3.42 mmol, 62%) as a white foam; Rf (CH₂Cl₂/EtOAc 98:2) 0.20; m.p. 79 – 82 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.56 (t, J = 7.0 Hz, 2H, ArH), 7.42 – 7.28 (m, 8H, ArH), 7.26 – 7.23 (m, 1H, ArH), 5.49 (s, 1H, NH, Fmoc), 4.45 – 4.27 (m, 2H, CH₂, Fmoc), 4.20 (t, J = 7.2 Hz, 1H, CH, Fmoc), 3.71 – 3.40 (m, 5H, 2 × CH₂-Az, CHHGAz), 3.34 – 3.04 (m, 2H, CHHGAz, CHα-Glu), 2.47 – 2.28 (m, 2H, γ-CH₂Glu), 2.10 – 2.00 (m, 1H, β-CHHGlu), 1.94 (s,
1H, NH), 1.85 – 1.71 (m, 7H, β-CH/HGlu, 2 × CH₃, Cumyl), 1.47 (s, 9H, CH₃, Boc), 1.43 (s, 9H, CH₃, CO₂tBu); ¹³C NMR (126 MHz, CDCl₃) δc 174.9 (C=O), 172.8 (C=O), 157.0 (C=O, Boc), 156.3 (C=O, Fmoc), 144.8 (C), 144.11 (C), 144.06 (C), 141.42 (C), 141.40 (C), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.4 (CH), 124.4 (CH), 120.1 (CH), 83.3 (C, Cumyl), 80.9 (C, Boc), 79.8 (C, tBu), 67.1 (CH₂, Fmoc), 55.4 (CH₂, Az), 55.0 (CH, α-Glu), 47.4 (CH, Fmoc), 45.8 (CH₂, GAz), 32.0 (CH₂, γ-Glu), 29.7 (CH₂, β-Glu), 28.5 (CH₃, Boc), 28.4 (CH₃, Cumyl), 28.3 (CH₃, tBu, CH₃, Cumyl); νmax (neat) = 2975, 1722, 1699, 1134, 1101, 843 cm⁻¹; MS (ESI⁺) m/z 728 [M+H⁺], 750 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₄₃H₅₄N₃O₇ 728.3905 [M+H⁺], found 728.3904; [α]D²⁶ –2.4 (c 0.13, CHCl₃).

Fmoc-GAz(Cbz)-Leu-OCumyl (315)

Following the general procedure 7 for building block synthesis, Fmoc-Leu-OCumyl (382) (2.92 g, 6.20 mmol, 1.0 equiv) and benzyl 3-(nitromethylene)azetidine-1-carboxylate (259) (1.54 g, 6.20 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 19:1), the title compound 315 (2.22 g, 3.22 mmol, 52%) as a white foam; Rf(CH₂Cl₂/EtOAc 19:1) 0.35; m.p. 64 – 67 °C; ¹H NMR (600 MHz, CDCl₃) δH 7.76 (d, J = 7.4 Hz, 2H, ArH), 7.58 – 7.54 (m, 2H, ArH), 7.41 – 7.28 (m, 13H, ArH), 7.21 (t, J = 7.2 Hz, 1H, ArH), 5.29 (s, 1H, NH, Fmoc), 5.09 (s, 2H, CH₂Ph), 4.35 (d, J = 7.1 Hz, 2H, CH₂, Fmoc), 4.18 (t, J = 7.1 Hz, 1H, CH, Fmoc), 3.77 – 3.56 (m, 5H, 2 × CH₂-Az, CHHGAz), 3.21 (s, 2H, CHHGAz, CHα-Leu), 1.83 – 1.76 (m, 7H, 2 × CH₃, Cumyl, CHHβ-Leu), 1.54 – 1.48 (m, 1H, CHHβ-Leu), 1.44 – 1.35 (m, 1H, CHγ-Leu), 0.97 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.95 (d, J = 6.5 Hz, 3H, CHδ-Leu); ¹³C NMR (151 MHz, CDCl₃) δc 175.5 (C=O), 156.9 (C=O), 156.6 (C=O), 144.7 (C), 144.1 (C), 144.0 (C), 141.4 (C), 136.6 (C), 128.6 (CH), 128.5 (CH), 128.22 (CH), 128.18 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 83.2 (C, Cumyl), 67.0 (CH₂Ph Fmoc and CH₂Ph Cbz), 55.5 (CH₂, Az), 54.7 (CH, α-Leu), 47.3 (CH, Fmoc), 43.8 (CH₂, GAz), 39.2 (CH₂, β-Leu), 28.8 (CH₃, Cumyl), 27.8 (CH₃, Cumyl), 24.9 (CH, γ-Leu), 23.2 (CH₃, δ-Leu), 22.2 (CH₂, δ-Leu), Note: C, Az and one Ar C not observed; νmax (neat) = 3320, 2954, 1702, 1131, 739 cm⁻¹; MS (ESI⁺) m/z 690 [M+H⁺], 712 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₄₂H₅₃N₃O₇ [M+Na⁺] 712.3357, found 712.3359; [α]D³⁰ –17.0 (c 0.62, MeOH).
Fmoc-GAz(Chz)-Val-OCumyl (316)

Following the general procedure 7 for building block synthesis, Fmoc-Val-OCumyl (470) (4.25 g, 9.29 mmol, 1.0 equiv) and benzyl 3-(nitromethylene)azetidine-1-carboxylate (259) (2.31 g, 9.29 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO₂, CH₂Cl₂/EtOAc 19:1), the title compound 316 (3.14 g, 4.65 mmol, 50%) as a white foam; Rf (CH₂Cl₂/EtOAc 19:1) 0.37; m.p. 72 – 75 °C; 1H NMR (500 MHz, CDCl₃) δ H 7.76 (d, J = 7.3 Hz, 2H, ArH), 7.59 – 7.53 (m, 2H, ArH), 7.42 – 7.29 (m, 13H, ArH), 7.21 (t, J = 7.3 Hz, 1H, ArH), 5.28 (s, 1H, NH, Fmoc), 5.09 (s, 2H, CH₂Ph), 4.34 (d, J = 7.0 Hz, 2H, CH₂Fmoc), 4.19 (t, J = 7.0 Hz, 1H, CH₂Fmoc), 3.93 – 3.39 (m, 5H, 2 × CH₂-Az, C₆H₄GAz), 3.22 – 2.91 (m, 2H, CHα-Val, CHβGAz), 2.16 – 2.05 (m, 1H, CHβ-Val), 1.83 (s, 3H, CH₃, Cumyl), 1.78 (s, 3H, CH₃, Cumyl), 1.03 (d, J = 6.6 Hz, 3H, CHγ-Val), 0.85 (d, J = 6.7 Hz, 3H, CHγ-Val); 13C NMR (126 MHz, CDCl₃) δ C 174.7 (C=O), 156.9 (C=O, Fmoc), 156.7 (C=O, Cbz), 144.8 (C), 144.1 (C), 144.03 (C), 143.97 (C), 141.4 (C), 136.6 (C), 128.6 (CH), 128.5 (CH), 128.2 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 124.6 (CH), 120.1 (CH), 83.2 (C, Cumyl), 67.1 (CH₂, Cbz), 67.0 (CH₂, Fmoc), 61.0 (CH, α-Val), 55.6 (CH₂, Az), 47.3 (CH, Fmoc), 46.0 (CH₂, GAz), 32.0 (CH, β-Val), 28.8 (CH₃, Cumyl), 27.7 (CH₃, Cumyl), 19.9 (CH₃, γ-Val), 17.3 (CH₃, γ-Val); νmax (neat) = 2960, 1701, 1517, 1131, 792 cm⁻¹; MS (ESI⁺) m/z 698 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₁H₄₅N₃O₆ [M+Na]⁺ 698.3201, found 698.3204; [α]D³⁰ -15.6 (c 0.24, MeOH).

Preparation of nitroalkanes 317 – 319

NO₂-GAz(Boc)-Met-OCumyl (317)

To a solution of Fmoc-Met-OCumyl (471) (1.21 g, 2.48 mmol, 1.0 equiv) in CH₂Cl₂ (2.5 mL) was added diethylamine (2.5 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (25 mL) and concentrated under reduced pressure to give the crude amine. The crude amine was resuspended in CH₂Cl₂ (25 mL) and to the solution was added tert-butyl 3-(nitromethylene)azetidine-1-carboxylate (258) (0.53 g, 2.48 mmol, 1.0 equiv) and stirred at room temperature for 16 h. The solvent was removed in vacuo to reveal an orange oil which was purified by column chromatography (SiO₂, PET ether:EtOAc 7:3) to give the title compound 317 (1.23 g, 2.35 mmol, 95%) as a yellow oil; Rf (PET ether:EtOAc 85:15) 0.22; 1H NMR (500 MHz, CDCl₃) δ H 7.31 – 7.27 (m, 1H, ArH), 4.64 (d, J = 13.2 Hz, 1H, CH₂NO₂), 4.52 (d, J = 13.2 Hz, 1H,
CHHNO₂), 3.94 – 3.78 (m, 3H, CHH-Az, CH₂-Az), 3.72 (d, J = 9.6 Hz, 1H, CHH-Az), 3.51 (dd, J = 8.8, 4.3 Hz, 1H, CHα-Met), 2.57 (t, J = 7.2 Hz, 2H, CH₂γ-Met), 2.11 (s, 3H, SCH₃), 2.04 – 1.92 (m, 1H, CHHβ-Met), 1.80 (s, 6H, 2 × CH₃, Cumyl), 1.72 (dd, J = 8.5, 6.5 Hz, 1H, CHHβ-Met), 1.45 (s, 9H, 3 × CH₃, Boc); ¹³C NMR (126 MHz, CDCl₃) δ C=O 174.1 (C=O), 156.2 (C=O, Boc), 144.8 (C), 128.6 (CH), 127.7 (CH), 124.4 (CH), 83.4 (C, Boc), 80.4 (C, Cumyl), 79.3 (CH₂NO₂), 54.9 (2 × CH₂, Az), 54.4 (CH, α-Met), 33.5 (CH₂, β-Met), 30.3 (CH₂, γ-Met), 28.5 (CH₃, Boc), 28.34 (CH₃, Cumyl), 28.3 (CH₃, Cumyl), 15.3 (CH₃, Met); ν max (neat) = 2977, 1728, 1696, 1554, 1366, 1133, 699 cm⁻¹; MS (ESI⁺) m/z 504 [M+Na]⁺, 985 [2M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₃₅N₃NaO₆ [M+Na]⁺ 504.2139, found 504.2142; [α]D²⁶ −14.6 (c 0.09, CHCl₃).

NO₂-GAzm(2-PC)-Gly-OCumyl (318)

To a solution of Fmoc-Gly-OCumyl (472) (660 mg, 1.59 mmol, 1.0 equiv) in CH₂Cl₂ (2 mL) was added diethylamine (2 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 20 mL) and concentrated under reduced pressure to give the crude amine. The crude amine was resuspended in CH₂Cl₂ (16 mL) and to the solution was added nitroalkene 264 (311 mg, 1.59 mmol, 1.0 equiv) and stirred at room temperature for 16 h. The solvent was removed in vacuo to reveal an orange oil which was purified by column chromatography (SiO₂, PET ether:EtOAc 7:3) to give the title compound 318 (1.23 g, 2.35 mmol, 95%) as a colourless oil; Rf (PET ether:EtOAc 7:3) 0.21; ¹H NMR (500 MHz, CDCl₃) δ 7.37 – 7.26 (m, 5H, ArH), 4.68 (d, J = 2.3 Hz, 2H, OCH₂), 4.67 (s, 2H, CH₂NO₂), 4.10 – 3.86 (m, 4H, 2 × CH₂-Az), 3.48 (s, 2H, CH₂Gly), 2.49 (t, J = 2.4 Hz, 1H, C≡CH), 1.80 (s, 6H, 2 × CH₃, Cumyl); ¹³C NMR (126 MHz, CDCl₃) δ 169.9 (C=O), 155.4 (C=O), 154.9 (C=O), 144.9 (C), 128.6 (CH), 127.6 (CH), 124.4 (CH), 83.7 (C, Cumyl), 78.7 (CH₂NO₂), 75.1 (C≡CH), 54.9 (C, Az), 53.1 (OCH₂), 45.4 (CH₂, Gly), 28.6 (2 × CH₃, Cumyl). Note: 2 × CH₂, Az and C≡CH not observed; ν max (neat) = 3288, 2979, 1707, 1553, 1135, 701 cm⁻¹; MS (ESI⁺) m/z 412 [M+H]⁺; HRMS (ESI⁺) calcd. for C₁₉H₂₃N₃O₆ [M+H]⁺ 412.1479, found 412.1476.

NO₂-GAzm(2-PC)-Thr(tBu)-OCumyl (319)

To a solution of Fmoc-Thr(tBu)-OCumyl (194) (3.40 g, 6.60 mmol, 1.0 equiv) in CH₂Cl₂ (6 mL) was added diethylamine (6 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting residue repeatedly dissolved in CH₂Cl₂ (3 × 40 mL) and concentrated under reduced pressure to give the crude amine. The crude amine was resuspended in CH₂Cl₂ (66 mL) and to
the solution was added nitroalkene 264 (1.29 g, 6.60 mmol, 1.0 equiv) and stirred at room temperature for 16 h. The solvent was removed in vacuo to reveal an orange oil which was purified by column chromatography (SiO₂, PET ether:EtOAc 85:15) to give the title compound 319 (2.58 g, 5.28 mmol, 80%) as a yellow oil; Rf (PET ether:EtOAc 85:15) 0.30; ¹H NMR (500 MHz, CDCl₃) δH 7.44 (d, J = 7.6 Hz, 2H, ArH), 7.35 (t, J = 7.7 Hz, 2H, ArH), 7.27 (d, J = 7.4 Hz, 1H, ArH), 4.66 (s, 2H, OCH₂), 4.61 (d, J = 13.1 Hz, 1H, CHHNO₂), 4.53 (d, J = 13.1 Hz, 1H, CHHNO₂), 4.01 (d, J = 9.5 Hz, 1H, CHH-Az), 3.91 (d, J = 9.6 Hz, 1H, CHH-Az), 3.86 – 3.80 (m, 3H, 2 × CHH-Az, CHα-Thr), 3.19 (d, J = 4.9 Hz, 1H, CHβ-Thr), 2.47 (t, J = 2.4 Hz, 1H, C=CH), 1.84 (s, 3H, CH₃, Cumyl), 1.79 (s, 3H, CH₃, Cumyl), 1.19 (s, 9H, CH₃, tBu), 1.09 (d, J = 6.2 Hz, 3H, CH₃γ-Thr); ¹³C NMR (126 MHz, CDCl₃) δc 172.6 (C=O), 155.4 (C=O), 145.0 (C), 128.4 (CH), 127.6 (CH), 124.8 (CH), 83.4 (C, Cumyl), 78.9 (CH₃NO₂), 78.2 (C=CH), 75.0 (C=CH), 74.2 (C, tBu), 69.1 (CH, α-Thr), 61.8 (CH, β-Thr), 54.7 (C, Az), 53.0 (OCH₂), 28.9 (CH₃, Cumyl), 28.7 (CH₃, tBu), 27.7 (CH₃, Cumyl), 19.5 (CH₃, γ-Thr); v max (neat) = 2974, 1712, 1555, 1133, 700 cm⁻¹; MS (ESI⁺) m/z 512 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₂₅H₄₃N₃NaO₂ [M+Na⁺] 512.2367, found 512.2370; [α]D²⁶ +9.2 (c 0.09, MeOH).

Preparation of dipeptide building blocks 320–322 via Zn reduction

Fmoc-GAz(Boc)-Met-OCumyl (320)

Following general procedure 8, NO₂-GAz(Boc)-Met-OCumyl 317 (481 mg, 1.00 mmol, 1.0 equiv) gave the title compound 320, after column chromatography (SiO₂, 19:1 → 4:1 CH₂Cl₂/EtOAc) as a white foam (252 mg, 0.37 mmol, 37%); Rf (CH₂Cl₂/EtOAc 19:1) 0.20; m.p. 72 – 75 °C; ¹H NMR (500 MHz, CDCl₃) δH 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.57 (dd, J = 7.3, 4.3 Hz, 2H, ArH), 7.40 (t, J = 7.5 Hz, 2H, ArH), 7.38 – 7.26 (m, 7H, ArH), 5.46 (s, 1H, NH, Fmoc), 4.46 – 4.31 (m, 2H, CH₂, Fmoc), 4.24 – 4.14 (m, 1H, CH, Fmoc), 3.86 – 3.05 (m, 7H, Met, CHHβ-Met, 2 × CH₃, Cumyl), 1.86 – 1.77 (m, 7H, CHHβ-Met, 2 × CH₃, Cumyl), 1.44 (s, 9H, CH₃, Boc); ¹³C NMR (126 MHz, CDCl₃) δc 157.0 (C=O, Boc or Fmoc), 156.4 (C=O, Boc or Fmoc), 144.1 (C), 144.0 (C), 141.5 (C), 128.6 (CH), 127.8 (CH), 127.7 (CH), 127.2 (CH), 125.3 (CH), 124.5 (CH), 120.1 (CH), 80.0 (C, Boc), 67.1 (CH₂, Fmoc), 54.9 (CH, α-Met), 47.3 (CH, Fmoc), 45.8 (CH₃, GaZ), 30.5 (CH₃γ-Met), 33.1 (CH₂β-Met), 28.5 (CH₃, Boc), 28.4 (CH₃, Cumyl), 28.3 (CH₃, Cumyl), 15.4 (CH₃, Met), Note: C=O Met, one C, Ar, 2 × CH₂, Az and C, Cumyl not observed; v max (neat) = 2975, 1696, 1240, 1132, 698 cm⁻¹; MS (ESI⁺) m/z 674 [M+H⁺], 696 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₃₃H₄₅N₃NaO₆S [M+Na⁺] 696.3078, found 696.3079; [α]D²⁶ −5.1 (c 0.32, CHCl₃).
Fmoc-GAz(2-PC)-Gly-OCumyl (321)

Following general procedure 8, NO$_2$-GAz(2-PC)-Gly-OCumyl 318 (509 mg, 1.31 mmol, 1.0 equiv) gave the title compound 321, after column chromatography (SiO$_2$, CH$_2$Cl$_2$/EtOAc 9:1) as a white foam (477 mg, 0.82 mmol, 62%); $R_f$ (CH$_2$Cl$_2$/EtOAc 9:1) 0.16; m.p. 77 – 80 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.76 (d, $J$ = 7.5 Hz, 2H, ArH), 7.57 (d, $J$ = 7.5 Hz, 2H, ArH), 7.42 – 7.31 (m, 9H, ArH), 5.35 (s, 1H, NH, Fmoc), 4.66 (s, 2H, OCH$_2$), 4.40 (d, $J$ = 6.8 Hz, 2H, CH$_2$, Fmoc), 4.19 (t, $J$ = 6.6 Hz, 1H, CH, Fmoc), 3.85 – 3.67 (m, 4H, 2 × CH$_2$-Az), 3.39 (s, 4H, CH$_2$GAz, CH$_2$Gly), 2.47 (t, $J$ = 2.4 Hz, 1H, C=CH), 1.81 (s, 6H, 2 × CH$_3$, Cumyl); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 171.1 (C=O), 156.9 (C=O Fmoc), 155.5 (C=O), 145.0 (C), 144.0 (C), 141.5 (C), 128.6 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.2 (CH), 124.4 (CH), 120.1 (CH), 83.5 (C, Cumyl), 74.9 (C=CH), 67.0 (CH$_2$Ph Fmoc), 55.7 (2 × CH$_2$, Az), 52.9 (OCH$_3$, CH, Fmoc), 45.5 (CH$_2$, Gly or GAz), 45.4 (CH$_2$, Gly or GAz), 28.6 (2 × CH$_3$, Cumyl); Note: C, Az not observed; $\nu_{\text{max}}$ (neat) = 3290, 1699, 1434, 1418, 1133, 740 cm$^{-1}$; MS (ESI$^+$) m/z 582 [M+H]$^+$, 604 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{34}$H$_{35}$N$_3$O$_6$ [M+Na]$^+$ 604.2418, found 604.2416.

Fmoc-GAz(2-PC)-Thr(tBu)-OCumyl (322)

Following general procedure 8, NO$_2$-GAz(2-PC)-Thr(tBu)-OCumyl 319 (2.51 g, 5.12 mmol, 1.0 equiv) gave the title compound 322, after column chromatography (SiO$_2$, CH$_2$Cl$_2$/EtOAc 9:1) as a white foam (1.14 g, 1.67 mmol, 33%); $R_f$ (CH$_2$Cl$_2$/EtOAc 9:1) 0.54; m.p. 81 – 83 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.76 (d, $J$ = 7.5 Hz, 2H, ArH), 7.58 (dd, $J$ = 7.3, 3.7 Hz, 2H, ArH), 7.44 – 7.39 (m, 4H, ArH), 7.34 – 7.29 (m, 4H, ArH), 7.25 – 7.21 (m, 1H, ArH), 5.51 – 5.35 (m, 1H, NH, Fmoc), 4.66 (s, 2H, OCH$_2$), 4.43 – 4.31 (m, 2H, CH$_2$, Fmoc), 4.19 (t, $J$ = 6.9 Hz, 1H, CH, Fmoc), 3.85 – 3.56 (m, 5H, 2 × CH$_2$, Az, CH$_2$-Thr), 3.31 – 3.00 (m, 2H, CH/HGAz, CH$_2$-Thr), 2.46 (t, $J$ = 2.4 Hz, 1H, C=CH), 1.85 (s, 3H, CH$_3$, Cumyl), 1.79 (s, 3H, CH$_3$, Cumyl), 1.21 – 1.15 (m, 12H, CH$_3$, tBu, CH$_3$-Thr); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 173.3 (C=O), 156.9 (C=O), 155.5 (C=O), 144.7 (C), 144.1 (C), 144.0 (C), 141.4 (C), 128.5 (CH), 127.8 (CH), 127.6 (CH), 127.2 (CH), 125.3 (CH), 124.8 (CH), 120.1 (CH), 83.5 (C, Cumyl), 79.4 (C=CH), 74.8 (C=CH), 74.4 (C, tBu), 69.0 (CH, $\alpha$-Thr), 67.0 (CH$_2$, Fmoc), 61.9 (CH$_2$-Thr), 55.5 (CH$_3$, Az), 52.8 (OCH$_2$), 47.4 (CH, Fmoc), 45.6 (CH$_2$, GAz), 28.9 (CH$_3$, Cumyl), 28.8 (CH$_3$, tBu), 27.5 (CH$_3$, Cumyl), 20.2 (CH$_3$, $\gamma$-Thr), Note: C, Az not observed; $\nu_{\text{max}}$ (neat) = 2974, 1710, 1098, 826, 754 cm$^{-1}$; MS (ESI$^+$) m/z 682 [M+H]$^+$, 704 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{33}$H$_{37}$N$_3$O$_7$ [M+Na]$^+$ 704.3306, found 704.3308; $[\alpha]_D^{26}$ = 2.0 (c 0.46, MeOH).
Preparation of cyclic peptides by SPPS followed by cyclisation

**Cyclo(Tyr(Bu)-GAz(Boc)-Thr(Bu)-Leu) (324)**

Following general procedure 9 from H-Leu-2CITrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Thr(Bu)-OCumyl (307) (420 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (60.4 mg, 87 µmol, 58%). A portion of this material was subjected to cyclisation (47.3 mg, 68 µmol, 1.0 equiv) to yield the title compound 324 after repeat column chromatography (SiO₂, CH₃Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (19.4 mg, 29 µmol, 42%, 24% over all steps); Rₕ (SiO₂, CH₃Cl₂:MeOH 19:1) 0.31; m.p. 149 – 152 ºC; ¹H NMR (500 MHz, MeOD-d₄) δ 7.17 (d, J = 8.3 Hz, 2H, ArH), 6.94 (d, J = 8.3 Hz, 2H, ArH), 4.53 – 4.46 (m, 2H, CHα-Tyr, CHα-Leu), 3.92 – 3.88 (m, 1H, CHα-Thr), 3.76 (d, J = 9.5 Hz, 2H, CHH-Az, CHH-Az), 3.69 (d, J = 8.9 Hz, 1H, CHH-Az), 3.62 – 3.55 (m, 1H, CHHGaZ), 3.50 (d, J = 9.7 Hz, 1H, CHH-Az), 3.22 (dd, J = 14.2, 4.4 Hz, 1H, CHHβ-Tyr), 3.15 (d, J = 5.5 Hz, 1H, CHβ-Thr), 2.82 (dd, J = 13.9, 11.9 Hz, 1H, CHHβ-Tyr), 1.65 – 1.59 (m, 1H, CHHβ-Leu), 1.53 – 1.48 (m, 1H, CHHβ-Leu), 1.44 (s, 9H, CH₃, Boc), 1.40 – 1.36 (m, 1H, CHγ-Leu), 1.31 (s, 9H, CH₃, ‘Bu), 1.27 (d, J = 6.1 Hz, 3H, CHγ-Thr), 1.24 (s, 9H, CH₃, ‘Bu), 0.88 (d, J = 6.6 Hz, 3H, CHδ-Leu), 0.84 (d, J = 6.5 Hz, 3H, CHδ-Leu). Note: CHHGaZ overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δ 177.0 (C=O), 174.6 (C=O), 173.8 (C=O), 158.1 (C=O Boc), 155.6 (C), 133.5 (C), 130.4 (CH), 125.4 (CH), 81.4 (C, Boc), 79.6 (C, ‘Bu), 75.6 (C, ‘Bu), 68.9 (CH, α-Thr), 67.0 (CH, β-Thr), 58.1 (CH, α-Tyr), 56.9 (2 × CH₂, Az), 56.0 (CH, α-Leu), 46.3 (CH, GaZ) 40.1 (CH₂, β-Leu), 36.8 (CH₂, β-Thr), 29.2 (CH₂, Boc), 29.1 (CH₃, ‘Bu), 28.6 (CH₃, ‘Bu), 26.5 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 22.2 (CH₃, δ-Leu), 21.4 (CH₂, γ-Thr);

**Cyclo(Val-GAz(Boc)-Asp(Bu)-Trp(Boc)) (325)**

Following general procedure 9 from H-Trp(Boc)-2CITrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Asp(Bu)-OCumyl (309) (428 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (74.9 mg, 99 µmol, 66%). A portion of this material was subjected to cyclisation (65.8 mg, 87 µmol, 1.0 equiv) to yield the title compound 325 after column chromatography (SiO₂, CH₃Cl₂:MeOH 39:1 → 19:1) as a glassy
colourless solid (34.0 mg, 46 µmol, 52%, 34% over all steps); \( R_t (\text{CH}_2\text{Cl}_2; \text{MeOH} 19:1) 0.30; \text{m.p.} 202 – 205°C; \) \(^1\text{H} \text{NMR} (500 \text{ MHz, DMSO-}\text{d}_6) \delta_{\text{H}} 8.36 (d, J = 10.4 \text{ Hz, 1H, NH}), 8.05 (d, J = 8.1 \text{ Hz, 1H, ArH}), 7.71 (d, J = 9.3 \text{ Hz, 1H, NH}), 7.65 (d, J = 7.7 \text{ Hz, 1H, ArH}), 7.53 (s, 1H, NH), 7.44 (s, 1H, ArH), 7.34 (t, J = 7.6 \text{ Hz, 1H, ArH}), 7.27 (t, J = 7.4 \text{ Hz, 1H, ArH}), 4.52 (dd, J = 18.6, 8.7 \text{ Hz, 1H, CH}_\alpha\text{-Trp}), 3.82 – 3.41 (m, 7H, CHHGAz, 2 × CH$_2$-Az, CH$_\alpha$-Asp, CH$_\gamma$-Val), 3.22 (dd, J = 14.9, 7.9 \text{ Hz, 1H, CHH}_\beta\text{-Trp}), 3.08 – 2.96 (m, 2H, CHH$\beta$-Trp, CHHGAz), 2.42 (dd, J = 16.2, 2.9 Hz, 1H, CHH$\beta$-Asp), 2.29 (dd, J = 16.2, 9.9 Hz, 1H, CHH$\beta$-Asp), 2.01 – 1.93 (m, 1H, CH$\beta$-Val), 1.61 (s, 9H, CH$_3$ 'Bu), 1.41 (s, 9H, CH$_3$, Boc), 1.36 (s, 9H, CH$_3$, Boc), 0.97 (d, J = 6.6 \text{ Hz, 3H, CH$_3\gamma$-Val}), 0.91 (d, J = 6.7 \text{ Hz, 3H, CH$_3\gamma$-Val}), Note: Val NH not observed; \(^{13}\text{C} \text{NMR} (126 \text{ MHz, DMSO-}\text{d}_6) \delta_{\text{C}} 174.2 (\text{C}=\text{O}), 172.0 (\text{C}=\text{O}), 170.9 (\text{C}=\text{O}), 169.3 (\text{C}=\text{O}), 155.6 (\text{C}=\text{O}, \text{Boc}), 143.6 (\text{C}), 137.8 (\text{C}), 131.0 (\text{CH}), 130.9 (\text{CH}), 129.5 (\text{CH}), 129.3 (\text{CH}), 120.9 (\text{CH}), 81.3 (\text{C}, \text{Boc}), 77.0 (\text{C}, \text{Trt}), 65.5 (\text{CH}, \alpha\text{-Ile}), 57.4

Cyclo(His(Trt)-GAz(Boc)-Ile-Leu) (326)

Following general procedure 9 from H-Leu-2ClTrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GA@(Boc)-Ile-
OCumyl (310) (393 mg, 0.60 mmol, 4.0 equiv) linear precursor
was synthesised (72.3 mg, 90 µmol, 60%). A portion of this
material was subjected to cyclisation (62.8 mg, 77 µmol, 1.0
equiv) to yield the title compound 326 after column
chromatography (SiO$_2$, Cl$_2$C$_2$:MeOH 39:1 → 19:1) as a glassy
colourless solid (30.8 mg, 40 µmol, 51%, 31% over all steps); \( R_t (\text{CH}_2\text{Cl}_2; \text{MeOH} 19:1) 0.30; \text{m.p.} 164 – 167°C; \) \(^1\text{H} \text{NMR} (500 \text{ MHz, MeOD-}\text{d}_4) \delta_{\text{H}} 7.43 (s, 1H, ArH), 7.41 – 7.39 (m, 9H, ArH), 7.16 – 7.13 (m, 6H, ArH), 6.83 (s, 1H, ArH), 4.62 – 4.50 (m, 1H, CH$_\alpha$-Leu), 4.47 (dd, J = 10.4, 4.7 Hz, 1H, CH$_\alpha$-His), 3.79 – 3.66 (m, 4H, CH$_2$-Az, CHH-Az, CHHGAZ), 3.46 (d, J = 9.9 Hz, 1H, CHH-Az), 3.23 (d, J = 7.1 Hz, 1H, CH$_\alpha$-Ile), 3.15 – 3.05 (m, 2H, CHHGAz, CHH$\beta$-His), 2.87 (dd, J = 14.8, 10.5 Hz, 1H, CHH$\beta$-His), 1.75 – 1.55 (m, 5H, CHH$\delta$-Ile, CH$_\beta$-Leu, CH$_\beta$-Ile, CH$\gamma$-Leu), 1.44 (s, 9H, CH$_3$ Boc), 1.29 – 1.23 (m, 1H, CHH$\delta$-Ile), 1.00 – 0.94 (m, 9H, 2 × CH$_\delta$-
Leu, CH$_\gamma$-Ile), 0.90 (d, J = 5.6 Hz, 3H, CH$_\text{36}$-
Ile); \(^{13}\text{C} \text{NMR} (126 \text{ MHz, MeOD-}\text{d}_4) \delta_{\text{C}} 178.3 (\text{C}=\text{O}), 174.1 (\text{C}=\text{O}), 173.7 (\text{C}=\text{O}), 158.0 (\text{C}=\text{O} \text{ Boc}), 143.6 (\text{C}), 137.8 (\text{C}), 131.0 (\text{CH}), 130.9 (\text{CH}), 129.5 (\text{CH}), 129.3 (\text{CH}), 120.9 (\text{CH}), 81.3 (\text{C}, \text{Boc}), 77.0 (\text{C}, \text{Trt}), 65.5 (\text{CH}, \alpha\text{-Ile}), 57.4
(CH, α-His), 57.2 (2 × CH₂, Az), 55.5 (CH, α-Leu), 46.8 (CH₂, GAz), 39.6 (CH₂, β-Leu), 39.1 
(CH, β-Ile), 30.0 (CH₂, β-His), 28.6 (CH₃, Boc), 27.3 (CH₂, δ-Ile), 26.4 (CH, γ-Leu), 22.9 (CH₃, 
δ-Leu), 22.4 (CH₃, δ-Leu), 15.9 (CH₃, γ-Ile), 11.7 (CH₃, ε-Ile), Note: C, Az not observed; νₘₐₓ 
(neat) = 3317, 2960, 1669, 1365, 659 cm⁻¹; MS (ESI⁺) m/z 790 [M+H⁺]; HRMS (ESI⁺) calcd. 
for C₄₆H₆₀N₇O₅ [M+H⁺] 790.4650, found 790.4658; [α]D²⁴ – 48.6 (c 0.12, MeOH).

**Cyclo(Asn(Trt)-GAz(Boc)-Lys(Boc)-Gly)**

Following general procedure 9 from H-Gly-2ClTrt 
(288 mg, 0.15 mmol, 0.52 mmol/g, 1.0 equiv) and 
Fmoc-GAz(Boc)-Lys(Boc)-OCumyl (312) (462 mg, 
0.60 mmol, 4.0 equiv), linear precursor was 
synthesised (91.7 mg, 109 µmol, 72%). A portion of 
this material was subjected to cyclisation (63.4 mg, 75 
µmol, 1.0 equiv) to yield the title compound after 
column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (33.1 
mg, 40 µmol, 54%, 37% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.32; m.p. 240 – 242 °C; H NMR 
(500 MHz, MeOD-d₄) δH 7.33 – 7.19 (m, 15H, ArH), 4.62 (dd, J = 7.0, 4.8 Hz, 1H, CHα-
Lys), 4.10 – 3.56 (m, 6H, CH₂Gly, CHH-Az, CH₂-Az, CHHGAz), 3.44 (d, J = 9.7 Hz, 1H, CHH-Az), 3.18 – 3.00 (m, 3H, CHHGAz, CH₂ε-Lys), 2.94 – 2.75 (m, 2H, CH₂β-Asn), 1.66 – 1.26 (m, 
24H, 6 × CH₃, Boc, CH₂β-Lys, CH₂γ-Lys, CH₂δ-Lys), Note: CHα-Lys overlaps with solvent 
signal; C NMR (126 MHz, MeOD-d₄) δC 180.1 (C=O), 173.9 (C=O), 171.6 (C=O), 171.2 
(C=O), 158.6 (C=O, Boc), 158.2 (C=O, Boc), 145.8 (C), 130.0 (CH), 128.8 (CH), 127.9 (CH), 
81.3 (C, Boc), 79.9 (C, Boc), 71.8 (C, Trt), 60.3 (CH, α-Lys), 56.5 (2 × CH₃, Az), 53.7 (CH, α-
Asn), 47.6 (CH₂, GAz), 45.2 (CH₂, Gly), 40.9 (CH₂, ε-Lys), 37.6 (CH₂, β-Asn), 34.4 (CH₂, β-
Lys), 30.8 (CH₃, δ-Lys), 28.8 (CH₃, βBu), 28.7 (CH₃, βBu), 24.5 (CH₂, γ-Lys), Note: C, Az not 
observed; νₘₐₓ (neat) = 3304, 2920, 1666, 1519, 1165 cm⁻¹; MS (ESI⁺) m/z 848 [M+Na⁺]; HRMS 
(ESI⁺) calcd. for C₄₅H₅₉N₇NaO₆ [M+Na⁺] 848.4317, found 848.4314; [α]D²⁴ – 27.7 (c 0.09, 
MeOH).
Cyclo(Val-GAζ(Chz)-Leu-Trp(Boc)) (328)

Following general procedure 9 from H-Trp(Boc)-2ClTrt (424 mg, 0.25 mmol, 0.59 mmol/g, 1.0 equiv) and Fmoc-GAζ(Chz)-Leu-OCumyl (315) (689 mg, 1.00 mmol, 4.0 equiv), linear precursor was synthesised (154 mg, 155 µmol, 76%). This material was subjected to cyclisation to yield the title compound 328 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (78 mg, 109 µmol, 55%, 42% over all steps); RF (CH₂Cl₂:MeOH 19:1) 0.34; m.p. 157 – 160 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 8.08 (d, J = 7.7 Hz, 1H, ArH), 7.61 (d, J = 7.6 Hz, 1H, ArH), 7.52 (s, 1H, ArH), 7.33 – 7.23 (m, 7H, ArH), 5.06 (s, 2H, CH₂Ph), 3.98 (d, J = 8.9 Hz, 1H, CHα-Val), 3.82 – 3.69 (m, 4H, CH₂-Az, C₃H₃H₂Az, C₃H₃HGAζ), 3.60 (d, J = 9.8 Hz, 1H, CHH-Az), 3.45 – 3.36 (m, 1H, CHα-Leu), 3.21 – 3.16 (m, 1H, CHβ-Trp), 3.13 – 3.04 (m, 2H, CHβ-Trp, CHβ-GAζ), 2.08 – 2.00 (m, 1H, CHβ-Val), 1.63 (s, 9H, CH₃Boc), 1.52 – 1.38 (m, 3H, CH₂β-Leu, CHγ-Leu), 1.04 (d, J = 6.6 Hz, 3H, CH₃δ-Leu), 0.99 (d, J = 6.7 Hz, 3H, CH₃δ-Leu), 0.89 (d, J = 6.3 Hz, 3H, CH₃γ-Val), 0.81 (d, J = 6.4 Hz, 3H, CH₃γ-Val), Note: CHα-Trp overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δC 179.1 (C=O), 174.6 (C=O), 173.6 (C=O), 158.2 (C=O, Boc), 150.8 (C), 137.9 (C), 136.8 (C), 131.3 (C), 129.5 (CH), 129.2 (CH), 129.0 (CH), 125.7 (CH), 124.8 (CH), 123.8 (CH), 119.9 (CH), 116.6 (CH), 116.2 (CH), 84.9 (C, 'Bu), 68.1 (CH₂Ph), 63.7 (CH, α-Val), 59.0 (CH, α-Leu), 57.6 (CH₂, Az), 56.8 (CH, α-Trp), 56.5 (CH₂, Az), 46.5 (CH₂, GAζ), 44.2 (CH₂, β-Leu), 30.6 (CH, β-Val), 28.4 (CH₃, Boc), 26.8 (CH₂, β-Trp), 25.9 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 22.9 (CH₃, δ-Leu), 20.3 (CH₃, γ-Val), 19.9 (CH₃, γ-Val), Note: C=O Cbz not observed; νmax (neat) = 3323, 2959, 1732, 1671, 1525 cm⁻¹; MS (ESI⁺) m/z 739 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₉H₅₂N₆O₇ [M+Na]⁺ 739.3790, found 739.3785; [α]D²⁰ = -68.7 (c 0.05, MeOH).

Cyclo-(Val-Leu-Gly-Trp(Boc)) (329)

Following general procedure 9 from H-Gly-2ClTrt (435 mg, 0.30 mmol, 0.69 mmol/g, 1.0 equiv), linear precursor was synthesised (122 mg, 210 µmol, 71%). This material was subjected to cyclisation using DEPBT to yield the title compound 329 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (23.5 mg, 42 µmol, 20%, 14% over all steps); RF (CH₂Cl₂:MeOH 19:1) 0.24; m.p. 220 °C (decomp.); ¹H NMR (500 MHz, DMSO) δH 8.60 – 8.53 (m, 1H, NH), 8.04 (d, J = 8.0 Hz, 1H, ArH), 7.72 (d, J = 9.2 Hz, 1H, ArH), 7.32 (d, J = 7.9 Hz, 1H, ArH), 7.00 – 6.89 (m, 5H, ArH), 4.85 (s, 2H, CH₂Ph), 3.72 – 3.48 (m, 4H, CH₂-Az, C₃H₃H₂Az, C₃H₃HGAζ), 3.48 – 3.29 (m, 1H, CHα-Trp), 3.21 – 3.12 (m, 1H, CHβ-Val), 2.08 – 2.00 (m, 1H, CHβ-Val), 1.62 (s, 9H, CH₃Boc), 1.51 – 1.37 (m, 3H, CH₂β-Leu, CHγ-Leu), 1.05 (d, J = 6.6 Hz, 3H, CH₃δ-Leu), 0.87 (d, J = 6.7 Hz, 3H, CH₃δ-Leu), 0.84 (d, J = 6.2 Hz, 3H, CH₃γ-Val), 0.75 (d, J = 6.3 Hz, 3H, CH₃γ-Val), Note: C=O Cbz not observed; νmax (neat) = 3323, 2959, 1731, 1672, 1525 cm⁻¹; MS (ESI⁺) m/z 739 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₉H₅₂N₆O₇Na⁺ [M+NaH⁺]⁺ 760.2146, found 760.2145; [α]D²⁰ = -48.7 (c 0.05, MeOH).
1H, NH), 7.65 (d, J = 7.7 Hz, 1H, ArH), 7.57 (d, J = 9.0 Hz, 1H, NH), 7.47 (s, 1H, ArH), 7.33 (t, J = 7.6 Hz, 1H, ArH), 7.26 (t, J = 7.4 Hz, 1H, ArH), 6.92 (d, J = 7.9 Hz, 1H, NH), 4.42 (dd, J = 16.5, 9.4 Hz, 1H, CHα-Trp), 4.18 (dd, J = 17.3, 8.3 Hz, 1H, CHα-Leu), 4.07 (dd, J = 13.8, 7.2 Hz, 1H, CHHGly), 3.82 (t, J = 10.1 Hz, 1H, CHα-Val), 3.16 (dd, J = 14.7, 6.9 Hz, 1H, CHHβ-Trp), 3.08 (dd, J = 14.6, 9.6 Hz, 1H, CHHβ-Trp), 2.02 – 1.96 (m, 1H, CHβ-Val), 1.61 (s, 9H, CH3 Boc), 1.46 – 1.37 (m, 3H, CH3β-Leu, CH3γ-Leu), 0.90 (d, J = 6.6 Hz, 3H, CH3γ-Val), 0.84 (d, J = 6.6 Hz, 3H, CH3δ-Leu), 0.82 (d, J = 6.4 Hz, 3H, CH3δ-Leu), 0.75 (d, J = 6.3 Hz, 3H, CH3δ-Leu), Note: CHHGly overlapped with solvent signal; 13C NMR (126 MHz, DMSO) δc 172.7 (C=O), 172.5 (C=O), 171.7 (C=O), 170.0 (C=O), 148.9 (C=O Boc), 134.7 (C), 129.9 (C), 124.5 (CH), 123.8 (CH), 122.6 (CH), 119.2 (CH), 116.1 (CH), 114.7 (CH), 83.6 (C, Boc), 60.9 (CH, α-Val), 55.8 (CH, α-Trp), 53.9 (CH, α-Leu), 43.4 (CH2 Gly), 28.6 (CH, β-Val), 27.7 (CH3, Boc), 25.5 (CH2, β-Trp), 24.5 (CH, γ-Leu), 22.2 (CH3, γ-Val), 22.0 (CH3, γ-Val), 19.2 (CH3, δ-Leu), 18.9 (CH3, δ-Leu), Note: CH2β-Leu overlapped with solvent signal; νmax (neat) = 3284, 2958, 1649, 1452, 1155 cm⁻¹; MS (ESI+) m/z 578 [M+Na]+; HRMS (ESI+) calcd. for C20H14N6O6 [M+Na]+ 578.2949, found 578.2943; [α]D28 = -194.6 (c 0.04, DMF).

Cyclo(Phe-Glu(Bu)-GAGz(Boc)-Thr(Bu)-Gly) (330)

Following general procedure 9 from H-Gly-2CITrt (217 mg, 0.15 mmol, 0.69 mmol/g, 1.0 equiv) and Fmoc-GAGz(Boc)-Thr(Bu)-OCumyl (307) (420 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (114 mg, 155 µmol, 62%). A portion of this material was subjected to cyclisation (61 mg, 81 µmol, 1.0 equiv) to yield the title compound 330 after column chromatography (SiO2, CH2Cl2:MeOH 39:1 → 19:1) as a glassy colourless solid (43 mg, 59 µmol, 73%, 45% over all steps); Rf (CH2Cl2/MeOH 19:1) 0.29; m.p. 125 – 127 ºC; 1H NMR (500 MHz, MeOD-d4) δH 7.36 – 7.20 (m, 5H, ArH), 4.49 (dd, J = 8.9, 5.2 Hz, 1H, CHα-Glu), 4.45 (dd, J = 10.0, 3.9 Hz, 1H, CHα-Phe), 4.00 – 3.90 (m, 2H, CHHGly, CHH-Az), 3.83 – 3.74 (m, 3H, CHH-Az, CHHGAz, CHβ-Thr), 3.66 (d, J = 8.3 Hz, 1H, CHH-Az), 3.60 (d, J = 8.9 Hz, 1H, CHH-Az), 3.52 (d, J = 14.6 Hz, 1H, CHHφ), 2.97 (dd, J = 14.4, 4.1 Hz, 1H, CHHβ-Phe), 3.15 (d, J = 4.3 Hz, 1H, CHα-Thr), 3.02 – 2.94 (m, 2H, CHHGAz, CHHβ-Phe), 2.34 (t, J = 7.5 Hz, 2H, CH2γ-Glu), 2.25 – 2.14 (m, 1H, CHHβ-Glu), 2.00 – 1.91 (m, 1H, CHHβ-Glu), 1.51 – 1.44 (m, 18H, CH3, Boc, CH3, 'Bu), 1.23 (s, 9H, CH3, 'Bu), 1.10 (d, J = 6.2 Hz, 3H, CH3γ-Thr); 13C NMR (126 MHz, MeOD-d4) δc 177.0 (C=O), 174.1 (C=O), 173.82 (C=O), 173.79 (C=O), 158.3 (C=O Boc), 138.4 (C), 130.0 (CH), 129.7 (CH), 128.0 (CH), 81.8 (C, 'Bu), 81.1 (C, 'Bu), 75.4 (C, Boc), 71.2 (CH, β-Thr), 62.5 (CH, α-Thr), 58.5 (2 × CH2, Az), 57.1 (CH, α-Phe), 54.0 (CH, α-Glu), 46.3 (CH2,
GAz), 44.8 (CH₂, Gly), 37.7 (CH₂, β-Phe), 32.8 (CH₂, γ-Glu), 28.64 (CH₂, CO₂tBu), 28.58 (CH₃, Boc), 28.4 (CH₃, 'Bu), 28.2 (CH₂, β-Glu), 19.1 (CH₃, γ-Thr), Note: One C=O overlapping; ν∞ (neat) = 2974, 1649, 1532, 1366, 1152 cm⁻¹; MS (ESI⁺) m/z 731 [M+H]⁺, 753 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₇H₅₈N₆NaO₉ 753.4157 [M+Na]⁺, found 753.4162; [α]D²⁴ +7.1 (c 0.11, MeOH)

Cyclo(Ala-Met-GAz(Boc)-Asp(Bu)-Phe) (331)

Following general procedure 9 from H-Phe-2ClTrt (195 mg, 0.15 mmol, 0.77 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Asp(Bu)-OCumyl (309) (428 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (70.2 mg, 97 µmol, 65%). A portion of this material was subjected to cyclisation (50 mg, 69 µmol, 1.0 equiv) to yield the title compound 331 after repeat column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (24.4 mg, 34 µmol, 50%, 33% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.21; m.p. 134 – 137 ºC; ¹H NMR (500 MHz, MeOD-d₄) δH 7.33 – 7.29 (m, 2H, ArH), 7.26 – 7.24 (m, 1H, ArH), 7.20 (d, J = 7.3 Hz, 2H, ArH), 4.59 (dd, J = 8.5, 5.2 Hz, 1H, CHα-Met), 4.22 (q, J = 7.4 Hz, 1H, CHα-Ala), 3.97 (dd, J = 11.3, 3.2 Hz, 1H, C-HAz), 3.86 (d, J = 9.3 Hz, 1H, C-HAz), 3.73 (d, J = 14.3 Hz, 1H, C-HGAz), 3.60 – 3.55 (m, 2H, 2 × CH₂-Az), 3.47 (dd, J = 10.6, 3.2 Hz, 1H, CHα-Asp), 3.41 – 3.35 (m, 1H, C-Hβ-Phe), 2.92 (d, J = 14.3 Hz, 1H, C-HGAz), 2.61 (dd, J = 7.9, 5.6 Hz, 1H, C-Hβ-Met), 2.56 – 2.52 (m, 1H, C-Hβ-Met), 2.28 – 2.18 (m, 2H, C-Hβ-Asp, C-Hγ-Met), 2.16 – 2.11 (m, 4H, SCH₃, CHβ-Asp), 2.05 – 1.99 (m, 1H, C-Hγ-Met), 1.50 (d, J = 7.4 Hz, 3H, CHβ-Ala), 1.46 (s, 9H, CH₃, Boc), 1.41 (s, 9H, CH₃, CO₂tBu); ¹³C NMR (126 MHz, MeOD-d₄) δC 177.0 (C=O), 175.3 (C=O), 174.9 (C=O), 174.3 (C=O), 171.5 (C=O), 158.1 (C=O, Boc), 139.1 (C), 130.3 (CH), 129.7 (CH), 127.9 (CH), 82.4 (C, Boc), 80.9 (C, 'Bu), 58.2 (CH, α-Phe), 56.6 (CH₂, Az), 54.5 (CH, α-Asp), 53.6 (CH, α-Met), 53.5 (CH, α-Ala), 46.7 (CH₂, GAZ), 42.5 (CH₂, β-Asp), 35.1 (CH₂, β-Phe), 32.3 (CH₂, β-Met), 31.4 (CH₂, γ-Met), 28.6 (CH₃, Boc), 28.4 (CH₃, 'Bu), 16.9 (CH₃, β-Ala), 15.3 (SMe), Note: C, Az and 1 × CH₂, Az not observed; ν∞ (neat) = 3298, 2975, 1650, 1524, 1153 cm⁻¹; MS (ESI⁺) m/z 727 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₃H₅₈N₆NaO₉S [M+Na]⁺ 727.3460, found 727.3462; [α]D²⁴ −79.8 (c 0.06, MeOH).
Cyclo(Val-Gln(Trt)-GAz(Boc)-Tyr(Bu)-Leu) (332)

Following general procedure 9 from H-Leu-2ClTrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Tyr(Bu)-OCumyl (311) (462 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (110 mg, 110 µmol, 73%). A portion of this material was subjected to cyclisation (84.7 mg, 84 µmol, 1.0 equiv) to yield the title compound 332 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (41.1 mg, 46 µmol, 49%, 36% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.32; m.p. 160–162 ºC; ¹H NMR (500 MHz, MeOD-d₄) δ 7.30–7.20 (m, 17H, ArH), 6.96 (d, J = 8.1 Hz, 2H, ArH), 4.24 (dd, J = 8.3, 5.8 Hz, 2H, CHα-Leu, CHα-Gln), 3.73–3.58 (m, 3H, CHH-Az, CHHGAz, CHα-Val), 3.45 (d, J = 9.0 Hz, 1H, CHH-Az), 3.36 (d, J = 8.7 Hz, 1H, CHH-Az), 3.15–2.95 (m, 3H, CHβ-Tyr, CHHGAz, CHH-Az), 2.78–2.67 (m, 1H, CHβ-Tyr), 2.52–2.39 (m, 2H, CHγ-Gln), 2.30–2.20 (m, 1H, CHβ-Val), 2.14–2.05 (m, 1H, CHHβ-Gln), 1.99–1.91 (m, 1H, CHHβ-Gln), 1.78–1.69 (m, 1H, CHHβ-Leu), 1.60–1.50 (m, 2H, CHγ-Leu, CHHβ-Leu), 1.40 (s, 9H, CH₃, Boc), 1.33 (s, 9H, CH₃, Bu), 0.99–0.90 (m, 12H, 2 × CHγ-Val, 2 × CHδ-Leu), Note: CHα-Tyr overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δ 177.3 (C=O), 175.0 (C=O), 174.6 (C=O), 174.3 (C=O), 173.8 (C=O), 158.0 (C=O, Boc), 155.8 (C), 145.9 (C), 133.6 (C), 131.1 (CH), 130.03 (CH), 129.96 (CH), 128.7 (CH), 128.6 (CH), 127.8 (CH), 127.6 (CH), 125.5 (CH), 81.2 (C, Boc), 79.5 (C, Bu), 71.6 (C, Trt), 65.1 (CH, α-Val), 61.1 (CH, α-Tyr), 57.1 (CH₂, Az), 55.3 (CH, α-Leu), 54.2 (CH, α-Gln), 45.3 (CH₂, GAz), 41.1 (CH₂, β-Tyr), 40.2 (CH₂, β-Leu), 34.3 (CH₂, γ-Gln), 29.8 (CH, β-Val), 29.3 (CH₃, Boc), 28.7 (CH₂, β-Gln), 28.6 (CH₃, βBu), 26.1 (CH, γ-Leu), 23.3 (CH₃, δ-Leu), 22.1 (CH₃, δ-Leu), 20.1 (CH₃, γ-Val), 19.9 (CH₃, γ-Val), Note: C, Az and CH₂, Az not observed; νmax (neat) = 3293, 2965, 1658, 1505, 1160, 635 cm⁻¹; MS (ESI⁺) m/z 1008 [M+H]⁺; HRMS (ESI⁺) calcd. for C₅₇H₇₅N₇O₈ [M+H]⁺ 1008.5569, found 1008.5575; [α]D³⁴ = 45.2 (c 0.08, MeOH).
Cyclo(Tyr(Bu)-Cys(Trt)-GAz(Boc)-Lys(Boc)-Leu) (333)

Following general procedure 9 from H-Leu-2CITrt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Lys(Boc)-OCumyl (312) (462 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (93.2 mg, 84 µmol, 56%) and a portion of this material was subjected to cyclisation (83.1 mg, 75 µmol, 1.0 equiv) to yield the title compound 333 after column chromatography (SiO$_2$, CH$_2$Cl$_2$:MeOH 39:1 → 19:1) as a glassy colourless solid (50.3 mg, 46 µmol, 62%, 35% over all steps); $R_f$ (CH$_2$Cl$_2$:MeOH 19:1) 0.34; m.p. 129 – 131 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) $\delta$H 7.42 (d, $J$ = 7.6 Hz, 6H, ArH), 7.32 (t, $J$ = 7.7 Hz, 6H, ArH), 7.25 (d, $J$ = 7.3 Hz, 3H, ArH), 7.13 (d, $J$ = 8.4 Hz, 2H, ArH), 6.86 (d, $J$ = 8.3 Hz, 2H, ArH), 4.30 (m, 1H, CH$_\alpha$-Tyr), 4.23 (t, $J$ = 6.7 Hz, 1H, CH$_\alpha$-Cys), 3.91 (dd, $J$ = 9.6, 5.1 Hz, 1H, CH$_\alpha$-Leu), 3.78 – 3.64 (m, 4H, CH$_2$-Az, CH$_H$-Az, CH$_H$GAz), 3.61 (d, $J$ = 9.0 Hz, 1H, CHH-Az), 3.16 – 3.10 (m, 3H, CH$_\alpha$-Lys, CH$_2$-β-Cys), 3.05 – 3.00 (m, 3H, CH$_2$-ε-Lys, CH$_H$GAz), 2.79 – 2.69 (m, 1H, CHHβ-Tyr), 2.64 – 2.55 (m, 1H, CHHβ-Tyr), 1.75 (dd, $J$ = 15.7, 7.2 Hz, 1H, CHHβ-Lys), 1.48 – 1.41 (m, 26H, CH$_2$-β-Leu, 6 $\times$ CH$_3$, Boc, CH$_3$-β-Lys, CH$_2$γ-Lys, CH$_2$δ-Lys), 1.30 (s, 9H, CH$_3$ tBu), 0.90 (d, $J$ = 6.6 Hz, 3H, CH$_3$δ-Leu), 0.88 (d, $J$ = 6.5 Hz, 3H, CH$_3$δ-Leu); $^{13}$C NMR (126 MHz, MeOD-d$_4$) $\delta$C 178.1 (C=O), 175.2 (C=O), 173.3 (C=O), 172.7 (C=O), 158.6 (C=O, Boc), 158.2 (C=O, Boc), 155.6 (C), 146.0 (C), 133.3 (C), 130.81 (CH), 130.75 (CH), 129.1 (CH), 128.0 (CH), 125.4 (CH), 81.2 (C, tBu), 79.9 (C, tBu), 79.6 (C, tBu), 68.1 (C, Trt), 59.2 (CH, α-Lys), 58.7 (CH, α-Cys), 57.6 (2 $\times$ CH$_2$, Az), 54.5 (CH, α-Tyr), 54.3 (CH, α-Leu), 41.0 (CH$_2$, ε-Lys), 39.8 (CH$_2$, β-Leu), 36.4 (CH$_2$, β-Cys), 35.8 (CH$_2$, β-Lys), 34.6 (CH$_2$, β-Tyr), 30.8 (CH$_2$, δ-Lys), 29.2 (CH$_3$, Boc), 28.8 (CH$_3$, Boc), 28.6 (CH$_3$, tBu), 26.1 (CH, γ-Leu), 24.3 (CH$_2$, γ-Lys), 23.4 (CH$_3$, δ-Leu), 21.9 (CH$_3$, δ-Leu), Note: CH$_2$, GAz and C, Az not observed; $\nu$$_{max}$ (neat) = 3302, 2971, 1654, 1505, 1364, 1160 cm$^{-1}$; MS (ESI$^+$) m/z 1090 [M+H]$^+$, 1112 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{61}$H$_{83}$N$_7$O$_9$S [M+Na]$^+$ 1112.5865, found 1112.5863; $[\alpha]_D^{24}$ = –23.5 (c 0.13, MeOH).
Cyclo(Ser(tBu)-Ile-GAzm(Boc)-Met-Ala) (334)

Following general procedure 9 from H-Ala-2CITrt (205 mg, 0.15 mmol, 0.73 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Met-OCumyl (320) (404 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (64.2 mg, 97 µmol, 65%). This material was subjected to cyclisation to yield the title compound 334 after repeat column chromatography (SiO2, CH2Cl2:MeOH 39:1 → 19:1) as a glassy colourless solid (34.6 mg, 54 µmol, 56%, 36% over all steps); Rf (CH2Cl2:MeOH 19:1) 0.33; m.p. 138 – 140 °C; 1H NMR (500 MHz, MeOD-d4) δ H 4.41 (d, J = 6.7 Hz, 1H, CHα-Ile), 4.26 (t, J = 3.3 Hz, 1H, CHα-Ser), 3.95 – 3.79 (m, 4H, 2 × CHH-Az, CβH-Ser, CαAla), 3.73 – 3.61 (m, 4H, 2 × CHH-Az, CβH-Ser, CHα-Ala), 3.43 (dd, J = 8.8, 4.3 Hz, 1H, CHα-Met), 3.07 (d, J = 13.9 Hz, 1H, CHGAz), 2.70 – 2.61 (m, 2H, CHγ-Met), 2.11 (s, 3H, SCH3), 2.04 – 1.97 (m, 1H, CHβ-Ile), 1.87 – 1.79 (m, 1H, CHβ-Met), 1.78 – 1.69 (m, 1H, CHβ-Met), 1.64 – 1.53 (m, 4H, CHβ-Ala, CHHδ-Ile), 1.43 (s, 9H, CH3, Boc), 1.25 – 1.18 (m, 10H, CH3, tBu, CHδ-Ile), 0.96 (d, J = 6.8 Hz, 3H, CHγ-Ile), 0.93 (t, J = 7.4 Hz, 3H, CHε-Ile); 13C NMR (126 MHz, MeOD-d4) δ C 178.5 (C=O), 174.7 (C=O), 173.8 (C=O), 172.4 (C=O), 158.3 (C=O, Boc), 81.1 (C, Boc), 62.0 (CH2, β-Ser), 59.9 (CH, α-Ile), 57.6 (CH, α-Ser), 56.9 (CH2, Az), 56.2 (CH, α-Met), 52.3 (CH, α-Ala), 46.5 (CH2, GAz), 37.9 (CH, β-Ile), 35.4 (CH2, β-Met), 31.2 (CH2, γ-Met), 28.6 (CH3, Boc), 27.7 (CH3, tBu), 25.8 (CH3, δ-Ile), 16.2 (CH3, γ-Ile), 15.4 (CH3, β-Ala), 15.1 (SCH3), 11.8 (CH3, ε-Ile), Note: 1 × CH2, Az and C, Az not observed; νmax (neat) = 3305, 2933, 1649, 1524 cm⁻¹; MS (ESI⁺) m/z 665 [M+Na]+; HRMS (ESI⁺) calcd. for C30H54N6O7S [M+Na]+ 665.3667, found 665.3663; [α]D²⁴ = −41.4 (c 0.06, MeOH).

Cyclo(Tyr(tBu)-Thr(tBu)-GAz(Cbz)-Val-Leu) (335)

Following general procedure 9 from H-Leu-2CITrt (177 mg, 0.125 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Cbz)-Val-OCumyl (316) (420 mg, 0.50 mmol, 4.0 equiv), linear precursor was synthesised (55 mg, 67 µmol, 54%). This material was subjected to cyclisation to yield the title compound 335 after column chromatography (SiO2, CH2Cl2:MeOH 39:1 → 19:1) as a glassy colourless solid (24.2 mg, 30 µmol, 45%, 24% over all steps); Rf (CH2Cl2:MeOH 19:1) 0.29; m.p. 126 – 130 °C; 1H NMR (500 MHz, MeOD-d4) δ H 7.35 – 7.29 (m, 5H, ArH), 7.21 (d, J = 8.5 Hz, 2H, ArH), 6.95 (d, J = 8.5 Hz, 2H, ArH), 5.07 (s, 2H, CH2Ph),
Following general procedure 9 from H-Gly-2CITrt (362 mg, 0.25 mmol, 0.69 mmol/g, 1.0 equiv) and Fmoc-GAz(Cbz)-Leu-OCumyl (315) (689 mg, 1.00 mmol, 4.0 equiv), linear precursor was synthesised (114 mg, 155 µmol, 62%). This material was subjected to cyclisation to yield the title compound 336 after column chromatography (SiO2, CH2Cl2:MeOH 39:1 → 19:1) as a glassy colourless solid (80 mg, 112 µmol, 72%, 45% over all steps); \( \text{Rf} \) (CH2Cl2:MeOH 19:1) 0.32; m.p. 120 – 123 °C; \(^1\)H NMR (500 MHz, MeOD-d4) \( \delta_1 \) 7.37 – 7.28 (m, 10H, ArH), 5.10 – 5.04 (m, 2H, CH2Ph), 4.42 (dd, \( J = 8.8, 5.3 \) Hz, 1H, CHα-Glu), 4.48 (dd, \( J = 10.4, 3.7 \) Hz, 1H, CHα-Phe), 4.09 – 3.71 (m, 5H, \( \text{CHH-Az, CH}_2\text{-Az, CHHGly, CHHGaz} \)), 3.67 (d, \( J = 8.8 \) Hz, 1H, \( \text{CHH}-\text{Az} \)), 3.44 (d, \( J = 14.5 \) Hz, 1H, \( \text{CHHGly} \)), 3.38 – 3.31 (m, 2H, CHα-Leu, \( \text{CHHβ-Phe} \)), 3.02 (d, \( J = 14.1 \) Hz, 1H, \( \text{CHHGaz} \)), 2.93 (dd, \( J = 14.3, 10.6 \) Hz, 1H, \( \text{CHHβ-Phe} \)), 2.32 – 2.28 (m, 2H, CHβ-Glu), 2.22 – 2.16 (m, 1H, \( \text{CHHGlu} \)), 1.91 – 1.86 (m, 1H, \( \text{CHHGlu} \)), 1.80 – 1.75 (m, 1H, \( \text{CHHβ-Leu} \)), 1.47 (s, 9H, CH3, 'Bu), 1.38 – 1.34 (m, 1H, \( \text{CHHβ-Leu} \)), 1.28 – 1.23 (m, 1H, CHγ-Leu), 0.93 (d, \( J = 4.5 \) Hz, 3H, CHδ-Leu), 0.92 (d, \( J = 4.4 \) Hz, 3H, CHδ-Leu); \(^13\)C NMR (126 MHz, MeOD-d4) \( \delta_{c} \) 180.2 (C=O), 174.1 (C=O), 173.9 (C=O), 173.7 (C=O), 173.6 (C=O), 158.4 (C=O, Cbz), 138.5 (C), 138.0 (C), 130.0 (CH), 129.7 (CH), 129.5 (CH), 129.1 (CH), 128.9 (CH), 128.0 (CH), 81.7 (C, 'Bu), 67.9 (CH2Ph), 62.2 (CH2, Az), 58.0 (CH, α-Phe), 57.8 (CH2, Az), 56.0 (CH, α-Leu), 53.6 (CH, α-Glu), 47.0 (CH3, Ga), 45.7 (CH3, β-Leu), 44.8 (CH2, Gly), 37.7 (CH2, β-Phe), 32.7 (CH2, β-Glu), 28.4 (CH3, 'Bu), 28.1 (CH2, γ-Glu), 25.6 (CH,
γ-Leu), 23.5 (CH₃, δ-Leu), 22.5 (CH₃, δ-Leu); νₓ max (neat) = 3304, 2948, 1645, 1531, 1186 cm⁻¹; 
MS (ESI⁺) m/z 743 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₈H₅₂N₆NaO₈ [M+Na]⁺ 743.3739, found 743.3738; [α]D³⁰ -27.4 (c 0.12, MeOH)

Cyclo(Tyr(tBu)-Val-GAaz(2-PC)-Gly-Phe) (337)

Following general procedure 9 from H-Phe-2CITrt (0.78 g, 0.60 mmol, 0.77 mmol/g, 1.0 equiv) and Fmoc-GAaz(2-PC)-Gly-OCumyl (321) (1.24 g, 2.40 mmol, 4.0 equiv), linear precursor was synthesised (0.26 g, 0.37 mmol, 61%) and subjected to cyclisation to yield the title compound 337 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (109 mg, 0.16 mmol, 45%, 27% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.20; m.p. 151 – 153 °C; 
¹H NMR (500 MHz, MeOD-d₄) δH 7.28 – 7.19 (m, 5H, ArH), 7.05 (d, J = 8.3 Hz, 2H, ArH), 6.87 (d, J = 8.4 Hz, 2H, ArH), 4.66 (s, 2H, OCH₂), 4.29 (t, J = 7.7 Hz, 1H, CHα-Phe), 4.23 (d, J = 5.9 Hz, 1H, CHα-Val), 4.08 (dd, J = 10.3, 5.5 Hz, 1H, CHα-Tyr), 3.91 – 3.72 (m, 5H, CHHGaz, 2 × CH₂-Az), 3.22 (d, J = 14.3 Hz, 1H, CHHGaz), 3.18 – 3.13 (dd, J = 13.9, 5.5 Hz, 1H, CHβ-Tyr), 3.08 (d, J = 16.3 Hz, 1H, CHH/Gly), 2.99 (m, 2H, CHβ-Phe), 2.91 (d, J = 2.3 Hz, 1H, C=CH), 2.33 (dq, J = 13.2, 6.6 Hz, 1H, CHβ-Val), 1.30 (s, 9H, CH₃, tBu), 1.00 – 0.95 (m, 6H, 2 × CH₃γ-Val), Note: CHHgly and CHHβ-Tyr overlap with solvent signal; 
¹³C NMR (126 MHz, MeOD-d₄) δC 174.8 (C=O), 174.3 (C=O), 173.9 (C=O), 157.4 (C=O), 155.5 (C), 138.2 (C), 133.7 (C), 130.6 (CH), 130.3 (CH), 129.7 (CH), 127.9 (CH), 125.4 (CH), 79.6 (C, tBu), 79.1 (C=CH), 76.2 (C=CH), 61.4 (CH, α-Tyr), 60.7 (CH, α-Val), 57.63 (2 × CH₂, Az), 57.62 (CH, α-Phe), 53.7 (OCH₃), 46.7 (CH₂, Gly), 44.6 (CH₂, GAz), 37.5 (CH₂, β-Phe), 36.7 (CH₂, β-Tyr), 31.7 (CH, β-Val), 29.2 (CH₂, βBu), 20.0 (CH₃, γ-Val), 18.2 (CH₃, γ-Val), Note: C, Az not observed; νₓ max (neat) = 3285, 2970, 1645, 1505, 1159 cm⁻¹; MS (ESI⁺) m/z 711 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₇H₄₈N₆NaO₇ [M+Na]⁺ 711.3477, found 711.3481; [α]D²⁶ -37.5 (c 0.13, MeOH).
Cyclo(Ile-Glu(Bu)-GAz(Boc)-Met-Ala-Trp(Boc)) (338)

Following general procedure 9 from H-Trp(Boc)-2CI-Trt (254 mg, 0.15 mmol, 0.59 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Met-OCumyl (320) (404 mg, 0.6 mmol, 4.0 equiv), linear precursor was synthesised (117 mg, 138 µmol, 79%). This material was subjected to cyclisation to yield the title compound 338 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (58 mg, 60 µmol, 51%, 40% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.48; m.p. 150 – 153 ºC; ¹H NMR (500 MHz, MeOD-d₄) δH 8.14 (d, J = 8.2 Hz, 1H, ArH), 7.64 (d, J = 7.7 Hz, 1H, ArH), 7.54 (s, 1H, ArH), 7.33 (t, J = 7.6 Hz, 1H, ArH), 7.27 (t, J = 7.5 Hz, 1H, ArH), 4.35 – 4.25 (m, 3H, CHα-Trp, CHα-Ala, CHα-Glu), 4.03 (d, J = 2.6 Hz, 1H, CHα-Ile), 3.93 – 3.84 (m, 3H, CHHGAz, 2 × CHH-Az), 3.65 (d, J = 9.0 Hz, 1H, CHH-Az), 3.55 (d, J = 9.6 Hz, 1H, CHH-Az), 3.50 – 3.44 (m, 2H, CHα-Met, CCHβ-Trp), 3.39 (dd, J = 14.3, 6.9 Hz, 1H, CHHβ-Trp), 3.09 (d, J = 14.2 Hz, 1H, CHHGAz), 2.66 – 2.59 (m, 2H, CHβ-Glu), 2.47 – 2.36 (m, 2H, CHβ-Met), 2.33 – 2.27 (m, 1H, CHHγ-Met), 2.17 – 2.00 (m, 6H, CHβ-Ile, CHHγ-Met, SCH₃, CCHγ-Glu), 1.82 – 1.76 (m, 1H, CHHγ-Glu), 1.67 (s, 9H, CH₃, CO₂tBu), 1.46 (s, 9H, CH₃, Boc), 1.42 (m, 10H, CH₃, Boc, CHHδ-Ile), 1.28 (d, J = 6.8 Hz, 3H, CHβ-Ala), 1.06 – 0.99 (m, 1H, CHHδ-Ile), 0.87 – 0.82 (m, 6H, CHγ-Ile, CHε-Ile); ¹³C NMR (126 MHz, MeOD-d₄) δC 177.7 (C=O), 174.6 (C=O), 174.6 (C=O), 173.9 (C=O), 173.8 (C=O), 173.3 (C=O), 158.2 (C=O, Boc), 150.9 (C=O, Boc), 137.0 (C), 131.4 (C), 125.8 (CH), 125.5 (CH), 123.9 (CH), 120.1 (CH), 117.3 (C), 116.3 (CH), 85.0 (C, CO₂Bu), 81.8 (C, Boc), 81.3 (C, Boc), 61.4 (CH, α-Ile), 57.2 (CH, α-Trp), 57.1 (2 × CH₂, Az), 56.9 (CH, α-Met), 55.2 (CH, α-Glu), 50.0 (CH, α-Ala), 46.6 (CH₂, GAz), 36.7 (CH, β-Ile), 34.1 (CH₂, γ-Glu), 32.9 (CH₂, β-Met), 31.1 (CH₂, β-Glu), 28.6 (CH₃, CO₂Bu), 28.4 (2 × CH₃, Boc), 27.8 (CH₂, γ-Met), 26.4 (CH₂, δ-Ile), 26.2 (CH₂, β-Trp), 18.7 (CH₃, β-Ala), 16.6 (CH₃, γ-Ile), 15.0 (SCH₃), 12.0 (CH₃, ε-Ile), Note: C, Az not observed; νmax (neat) = 3308, 3056, 1729, 1646, 1152 cm⁻¹; MS (ESI⁺) m/z 993 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₈H₇₄N₉NaO₁₁S [M+Na]⁺ 993.5090, found 993.5083; [α]D²⁴ 60.7 (c 0.12, MeOH).
Cyclo(Trp(Boc)-Arg(Pbf)-GAz(Boc)-Asp(Bu)-Arg(Pbf)-Leu) (339)

Following general procedure 9 from H-Leu-2CI Trt (211 mg, 0.15 mmol, 0.71 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Asp(Bu)-OCumyl (309) (428 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (129 mg, 81 µmol, 54%). A portion of this material was subjected to cyclisation (113 mg, 71 µmol, 1.0 equiv) to yield the title compound 339 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 9:1) as a glassy colourless solid (41.2 mg, 26 µmol, 37%, 33% over all steps); R₄(CH₂Cl₂:MeOH 19:1) 0.15; m.p. 202 – 205 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 8.11 (d, J = 8.0 Hz, 1H, ArH), 7.62 (d, J = 7.8 Hz, 1H, ArH), 7.45 (s, 1H, ArH), 7.31 (t, J = 7.7 Hz, 1H, ArH), 7.24 (t, J = 7.5 Hz, 1H, ArH), 4.60 – 4.35 (m, 3H, CHα-Trp, CHα-Leu, CHα-Arg), 4.04 – 3.58 (m, 8H, 2 × CH₂-Az, CHα-Asp, CHα-Arg, CHHGAz, CHHβ-Trp), 3.49 – 3.44 (m, 1H, CHHβ-Trp), 3.26 – 3.05 (m, 4H, 2 × CH₂δ-Arg), 3.01 – 2.96 (m, 4H, 2 × CH₂Pbf), 2.72 (dd, J = 16.1, 3.8 Hz, 1H, CHHβ-Asp), 2.69 – 2.62 (m, 1H, CHHβ-Asp), 2.58 (s, 6H, 2 × CH₃, Pbf), 2.52 (s, 3H, CH₃, Pbf), 2.11 – 1.84 (m, 8H, 2 × CH₂, Pbf, CH₂β-Arg), 1.73 – 1.54 (m, 15H, CH₃, CO₂tBu, CH₂β-Arg, 2 × CH₂γ-Arg), 1.48 – 1.42 (m, 32H, 6 × CH₃ Boc, 4 × CH₃, Pbf, CH₂β-Leu), 1.11 (m, 1H, CH, γ-Leu), 0.70 (d, J = 6.2 Hz, 3H, CH₃δ-Leu), 0.66 (d, J = 6.2 Hz, 3H, CH₃δ-Leu), Note: CHHGAz overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δC = 176.8 (C=O), 175.0 (C=O), 174.6 (C=O), 174.3 (C=O), 173.0 (C=O), 171.7 (C=O Boc), 159.9 (C=O Boc), 150.1 (C), 149.4 (C), 139.4 (C), 136.9 (C), 134.33 (C), 134.28 (C), 133.6 (C), 133.5 (C), 131.6 (C), 126.08 (C), 126.05 (C), 125.6 (CH), 125.2 (CH), 123.8 (CH), 120.2 (CH), 118.53 (C), 118.49 (C), 118.45 (C), 116.2 (CH), 87.7 (2 × C-O Pbf), 84.9 (C, 'Bu), 82.8 (C, 'Bu), 81.3 (C, 'Bu), 57.4 (2 × CH₂, Az), 57.1 (CH, α-Trp), 55.7 (CH, α-Asp), 55.5 (CH, α-Leu), 53.01 (CH, α-Arg), 52.96 (CH, α-Arg), 46.2 (CH₂, GAz) 43.98 (CH₂, Pbf), 43.97 (CH₂, Pbf), 41.6 (2 × CH₂, δ-Arg), 40.4 (CH₂, β-Leu & CH₂, β-Asp), 31.6 (CH₂, β-Arg) 31.4 (CH₂, β-Arg), 28.7 (4 × CH₃, Pbf), 28.6 (CH₃, 'Bu), 28.5 (CH₃, 'Bu), 28.4 (CH₃, 'Bu), 26.88 (CH₂, γ-Arg) 26.85 (CH₂, γ-Arg), 26.1 (CH₂, β-Trp), 25.3 (CH, γ-Leu), 22.8 (CH₃, δ-Leu), 22.5 (CH₃, δ-Leu), 19.71 (CH₃, Pbf), 19.69 (CH₃, Pbf), 18.51 (CH₃, Pbf), 18.50 (CH₃, Pbf), 12.6 (2 × CH₃, Pbf), Note: C, Arg and C, Az not observed; νmax (neat) = 3312, 2969, 1729, 1641, 1452, 1152 cm⁻¹; MS (ESI⁺) m/z 1593 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₇₇H₁₁₄N₁₄NaO₁₇S₂ [M+Na⁺] 1593.7820, found 1593.7831; [α]D²⁰ = –20.8 (c 0.08, MeOH).
Cyclo(Glu(tBu)-Arg(Pbf)-GAz(Boc)-Ile-Ala-Gly) (340)

Following general procedure 9 from H-Gly-2CITrt (267 mg, 0.14 mmol, 0.52 mmol/g, 1.0 equiv) and Fmoc-GAz(Boc)-Ile-OCumyl (310) (393 mg, 0.60 mmol, 4.0 equiv), linear precursor was synthesised (100 mg, 97 µmol, 69%). A portion of this material was subjected to cyclisation (87 mg, 84 µmol, 1.0 equiv) to yield the title compound 340 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (40.9 mg, 40 µmol, 48%, 33% over all steps); Rf (CH₂Cl₂:MeOH 19:1) 0.37; m.p. 176 – 178 °C; ¹H NMR (500 MHz, MeOD-d₄) δH 4.42 – 4.25 (m, 3H, CHα-Arg, CHα-Ala, CHα-Glu), 4.03 – 3.91 (m, 3H, CHHGly, CHHGAz, CHH-Arg), 3.84 (d, J = 8.9 Hz, 1H, CHH-Az), 3.60 (d, J = 15.2 Hz, 1H, CHHGly), 3.54 (d, J = 9.5 Hz, 2H, CH2-Az), 3.30 – 3.27 (m, 1H, CHα-Ile), 3.21 – 3.15 (m, 2H, CH2δ-Arg), 3.08 (d, J = 14.3 Hz, 1H, CHHGAz), 2.99 (s, 2H, CH2, Pbf), 2.57 (s, 3H, CH3, Pbf), 2.51 (s, 3H, CH3, Pbf), 2.42 – 2.30 (m, 3H, CH2β-Glu, CHHγ-Glu), 2.12 – 2.06 (m, 4H, CH2δ-Arg, CHHβ-Arg), 2.00 – 1.90 (m, 2H, CH/β-Arg, CHHγ-Glu), 1.82 – 1.72 (m, 2H, CHβ-Ile, CHHγ-Arg), 1.60 – 1.55 (m, 1H, CHHγ-Arg), 1.46 – 1.42 (m, 25H, CH3, 'Bu, CH3, Boc, 2 × CH3, Pbf, CHHδ-Ile), 1.28 (d, J = 6.7 Hz, 3H, CH3β-Ala), 0.96 (d, J = 6.9 Hz, 3H, CH3γ-Ile), 0.91 (t, J = 7.0 Hz, 3H, CH3ε-Ile), Note: CHα-Ile overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δC 176.9 (C=O), 175.1 (C=O), 173.9 (C=O), 173.7 (C=O), 173.0 (C=O), 159.9 (C), 158.13 (C=O, Boc), 158.07 (C=NH), 134.4 (C), 133.5 (C), 126.0 (C), 118.4 (C), 87.7 (C-O, Pbf), 81.8 (C, Boc), 81.3 (C, CO₂tBu), 62.1 (CH, α-Ile), 57.2 (CH2, Az), 55.4 (CH, α-Arg), 54.8 (CH, α-Glu), 50.1 (CH, α-Ala), 46.7 (CH2, GAz), 45.1 (CH2, Gly), 44.0 (CH2, δ-Arg), 41.5 (CH, β-Ile), 39.9 (CH2, Pbf), 32.8 (CH3, β-Glu), 28.9 (CH3, β-Arg), 28.7 (2 × CH3, Pbf), 28.6 (CH3, CO₂tBu), 28.3 (CH3, Boc), 27.4 (CH2, γ-Arg), 27.1 (CH2, γ-Glu), 25.9 (CH2, δ-Ile), 19.6 (CH3, Pbf), 19.1 (CH3, β-Ala), 18.5 (CH3, Pbf), 16.2 (CH3, γ-Ile), 12.5 (CH3, ε-Ile), 12.4 (CH3, Pbf), Note: C, Az and 1 × CH3, Az not observed; νmax (neat) = 3305, 2967, 1643, 1544, 1151 cm⁻¹; MS (ESI⁺) m/z 1041 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₈H₇₈N₁₀O₁₂ [M+Na]⁺ 1041.5414, found 1041.5402; [α]D²⁴ – 20.3 (c 0.05, MeOH).
Cyclo(Leu-Tyr(Bu)-Val-GAz(2-PC)-Thr(Bu)-Phe) (341)

Following general procedure 9 from H-Phe-2ClTrt (390 mg, 0.30 mmol, 0.77 mmol/g, 1.0 equiv) and Fmoc-GAz(2-PC)-Thr(Bu)-OCumyl (322) (818 mg, 1.20 mmol, 4.0 equiv), linear precursor was synthesised (189 mg, 0.20 mmol, 68%) and subjected to cyclisation to yield the title compound 341 after column chromatography (CH$_2$Cl$_2$:MeOH 39:1 → 19:1) as a glassy colourless solid (52 mg, 57 µmol, 46%, 31% over all steps); RF (CH$_2$Cl$_2$:MeOH 19:1) 0.45; m.p. 157 – 160 °C; $^1$H NMR (500 MHz, MeOD-d$_4$) δ$_H$ 7.37 – 7.31 (m, 2H, ArH), 7.28 – 7.24 (m, 3H, ArH), 7.15 (d, $J = 8.4$ Hz, 2H, ArH), 6.93 (d, $J = 8.4$ Hz, 2H, ArH), 4.66 – 4.59 (m, 3H, OCH$_3$, CHα-Phe), 4.37 (d, $J = 9.0$ Hz, 1H, CHα-Val), 4.09 (dd, $J = 11.6$, 3.7 Hz, 1H, CHα-Tyr), 3.99 (d, $J = 7.1$ Hz, 1H, CHβ-Az), 3.95 – 3.57 (m, 5H, CH$_2$-Az, CHH-Az, CHHGAz, CHα-Leu), 3.37 (dd, $J = 14.5$, 4.5 Hz, 1H, CHHβ-Tyr), 3.01 – 2.89 (m, 3H, CHHGAz, CHβ-Thr, C≡CH), 2.75 (dd, $J = 13.7$, 10.4 Hz, 1H, CHβ-Phe), 2.09 – 1.98 (m, 1H, CHβ-Val), 1.56 – 1.51 (m, 1H, CHHβ-Leu), 1.36 – 1.31 (m, 10H, CHHβ-Leu, CH$_3$ tBu), 1.19 – 1.16 (m, 1H, CHγ-Leu), 1.09 – 1.00 (m, 18H, 3 × CH$_3$, tBu, CHγ-Thr, 2 × CH$_3$γ-Val), 0.80 (d, $J = 6.5$ Hz, 3H, CHδ-Leu), 0.77 (d, $J = 6.5$ Hz, 3H, CHδ-Leu), Note: CHHβ-Phe overlaps with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) δ$_C$ 176.1 (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 172.8 (C=O), 157.4 (C=O), 155.3 (C), 137.7 (C), 134.8 (C), 130.6 (CH), 130.2 (CH), 130.0 (CH), 128.3 (CH), 125.3 (CH), 79.4 (C, tBu), 79.1 (C=CH), 76.3 (C=CH), 75.4 (C, tBu), 69.4 (CH, α-Thr), 65.0 (CH, β-Thr), 62.6 (CH, α-Val), 59.4 (CH, α-Tyr), 58.2 (CH$_2$-Az), 55.8 (CH, α-Leu), 54.8 (CH, α-Phe), 53.7 (OCH$_3$), 45.7 (CH$_2$, GAz), 40.3 (CH$_2$, β-Leu), 39.3 (CH$_2$, β-Phe), 35.2 (CH$_2$, β-Tyr), 33.5 (CH, β-Val), 29.2 (CH$_3$, tBu), 28.9 (CH$_3$, tBu), 25.2 (CH, γ-Leu), 23.1 (CH$_3$, γ-Leu), 22.3 (CH$_3$, δ-Leu), 21.8 (CH$_3$, δ-Leu), 19.9 (CH$_3$, γ-Val), 19.4 (CH$_3$, γ-Val); v$_\text{max}$ (neat) = 2969, 1635, 1418, 1235, 1160; MS (ESI$^+$) m/z 924 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{49}$H$_{71}$N$_7$NaO$_9$ [M+Na]$^+$ 924.5205, found 924.5211; [$\alpha$]$_D^{26}$ – 62.4 (c 0.06, MeOH).
Cyclo-(Trp(Boc)-Ile-Ser(tBu)-GAz(Cbz)-Lys(Boc)-Ala) (342)

Following general procedure 9 from H-Ala-2ClTrt (342 mg, 0.25 mmol, 0.73 mmol/g, 1.0 equiv) and Fmoc-GAz(Cbz)-Lys(Boc)-OCumyl (313) (804 mg, 1.00 mmol, 4.0 equiv), linear precursor was synthesised (153 mg, 142 µmol, 57%). A portion of this material was subjected to cyclisation (136 mg, 126 µmol, 1.0 equiv) to yield the title compound 342 after column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) as a glassy colourless solid (60.0 mg, 57 µmol, 45%, 26% over all steps); Rf (CH₂Cl₂/MeOH 19:1) 0.34; m.p. 110 – 113 °C; ¹H NMR (400 MHz, MeOD) δ 8.13 (d, J = 8.2 Hz, 1H, ArH), 7.62 (d, J = 7.7 Hz, 1H, ArH), 7.51 (s, 1H, ArH), 7.35 – 7.23 (m, 7H, ArH), 5.06 (d, J = 1.0 Hz, 2H, CH₂Ph Cbz), 4.44 (dd, J = 6.7, 4.3 Hz, 1H, CHα-Ser), 4.21 (dd, J = 9.5, 6.1 Hz, 1H, CHα-Trp), 4.18 – 4.08 (m, 2H, CHα-Lys, CHα-Ala), 4.01 – 3.71 (m, 6H, CH₂Az, CHHβ-Az, CHHGaz, CHβ-Ser), 3.63 (d, J = 9.6 Hz, 1H, CHHβ-Trp), 3.40 – 3.09 (m, 3H, CHHGaz, CH₂β-Lys), 2.19 – 2.10 (m, 1H, CHβ-Val), 1.76 – 1.40 (m, 27H, CH₃ Boc, CH₃ tBu, CH₂γ-Lys, CH₂δ-Lys, CHβ-Lys, CHβ-Ile, CH₂δ-Ile), 1.28 – 1.21 (m, 12H, CH₃ Boc, CHβ-Ala), 0.91 – 0.84 (m, 6H, CH₂γ-Ile, CH₂δ-Ile); ¹³C NMR (101 MHz, MeOD) δ 178.0 (C=O), 174.8 (C=O), 173.6 (C=O), 173.4 (C=O), 173.2 (C=O), 158.6 (C=O), 158.3 (C=O), 150.8 (C=O), 137.9 (C) 131.4 (C), 129.5 (CH), 129.2 (CH), 129.0 (CH), 127.3 (C), 125.8 (CH), 125.5 (CH), 123.9 (CH), 120.0 (CH), 117.5 (C), 116.3 (CH), 85.0 (C, O'Bu), 79.9 (C, Boc), 74.9 (C, Boc), 68.0 (CH₂Ph Cbz), 62.3 (CH₂, β-Ser), 61.1 (CH, α-Ala), 58.0 (CH, α-Ile), 57.6 (CH₂ Az), 57.1 (CH, α-Trp), 56.8 (CH, α-Ser), 45.5 (CH₂, GAZ), 40.8 (CH₂, ε-Lys), 36.6 (CH, β-Val), 35.1 (CH₂, β-Ile), 30.8 (CH₂, β-Lys), 28.8 (CH₃, tBu), 28.4 (CH₃, Boc), 28.0 (CH₃, Boc), 26.1 (CH₂, β-Trp), 23.9 (CH₂, δ-Lys), 18.6 (CH₃, β-Ala), 16.5 (CH₃, γ-Ile), 12.0 (CH₃, ε-Ile). Note: CH, α-Ile overlaps with solvent signal, 1 × CH₂, Az, C, Az, CH, β-Ile and CH₂, γ-Lys not observed; νmax (neat) = 2971, 1641, 1420, 1364, 1154 cm⁻¹; MS (ESI⁺) m/z 1082 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₅₅H₈₁N₉O₁₂ [M+Na]⁺ 1082.5891, found 1082.5891; [α]₀D²⁸ = -36.6 (c 0.04, MeOH).
Deprotection of azetidine modified cyclic peptides

Cyclo(D-Pro-Leu-GAz-Gly) (343)

Following general procedure 10, Cyclo((D)Pro-Leu-GAz(Boc)-Gly) (295) (17.5 mg, 38.7 μmol, 1.0 equiv) was deprotected to reveal the title compound 343 as a pale yellow glassy solid (17.9 mg) as the TFA salt in quantitative yield, which required no further purification. \(^1\)H and \(^{19}\)F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 123 – 125 °C (decomp.); \(^1\)H NMR (500 MHz, MeOD-d\(_4\)) δ\(_H\) 4.49 (dd, \(J = 8.1, 4.3\) Hz, 1H, CH\(_\alpha\)-Pro), 4.27 (t, \(J = 7.7\) Hz, 1H, CH\(_\alpha\)-Leu), 4.01 (d, \(J = 11.2\) Hz, 1H, CHH-Az), 3.98 (d, \(J = 11.4\) Hz, 1H, CHH-Az), 3.81 (d, \(J = 10.9\) Hz, 1H, CHH-Az), 3.75 (d, \(J = 11.1\) Hz, 1H, CHH-Az), 3.70 (d, \(J = 14.4\) Hz, 1H, CHHGAz), 3.65 – 3.60 (m, 1H, CHH\(_\delta\)-Pro), 3.59 – 3.54 (m, 1H, CHH\(_\delta\)-Pro), 3.41 (d, \(J = 8.5\) Hz, 1H, CHHGly), 3.38 (d, \(J = 8.3\) Hz, 1H, CHHgly), 3.23 (d, \(J = 14.2\) Hz, 1H, CHHGaz), 2.36 (dt, \(J = 19.7, 7.6\) Hz, 1H, CHHF-Pro), 2.04 – 1.88 (m, 3H, CH\(_2\)-β-Pro, CH\(_2\)-γ-Pro), 1.71 – 1.60 (m, 3H, CH\(_3\)-β-Leu, CH\(_3\)-γ-Leu), 0.99 (d, \(J = 6.4\) Hz, 3H, CH\(_3\)-δ-Leu), 0.94 (d, \(J = 6.4\) Hz, 3H, CH\(_3\)-δ-Leu); \(^{13}\)C NMR (126 MHz, MeOD-d\(_4\)) δ\(_C\) 176.2 (C=O), 176.0 (C=O), 173.1 (C=O), 62.0 (CH, \(\alpha\)-Pro), 59.8 (C, Az), 57.2 (CH\(_2\), Az), 55.9 (CH, \(\alpha\)-Leu), 54.2 (CH\(_2\), Az), 47.5 (CH\(_2\), GAz), 46.5 (CH\(_2\), Gly), 39.2 (CH, β-Leu), 33.0 (CH\(_2\), γ-Pro), 26.1 (CH, γ-Leu), 23.7 (CH\(_3\), β-Pro), 22.9 (CH\(_3\), δ-Leu), 22.5 (CH\(_3\), δ-Leu), Note: CH\(_3\), δ-Pro overlaps with solvent signal; ν\(_{\text{max}}\) (neat) = 2956, 1665, 1199, 1177, 1128 cm\(^{-1}\); MS (ESI\(^+\)) m/z 352 [M+H\(^+\)], 374 [M+Na\(^+\)]; HRMS (ESI\(^+\)) calcd. for C\(_{17}\)H\(_{30}\)N\(_5\)O\(_3\) [M+H\(^+\)] 352.2343, found 352.2338; [α\(_D\)]\(_{24}^\circ\) –25.3 (c 0.05, MeOH).

Cyclo(Trp-Leu-GAz-Gly) (346)

Following general procedure 10 from Cyclo(Trp-Leu-GAz(Boc)-Gly) (296) (18.6 mg, 34 μmol, 1.0 equiv) was deprotected reveal the title compound 346 as a pale yellow glassy solid (23.2 mg) as the TFA salt in quantitative yield, which required no further purification. \(^1\)H and \(^{19}\)F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 190 – 192 °C; \(^1\)H NMR (600 MHz, DMSO-d\(_6\)) δ\(_H\) 10.88 (s, 1H, NH), 8.94 – 8.81 (m, 1H, NH), 8.46 – 8.35 (m, 1H, NH), 8.15 (d, \(J = 10.2\) Hz, 1H, NH), 7.81 (d, \(J = 8.9\) Hz, 1H, NH), 7.59 (dd, \(J = 8.2, 3.2\) Hz, 1H, NH), 7.52 (d, \(J = 7.9\) Hz, 1H, ArH), 7.34 (d, \(J = 8.1\) Hz, 1H, ArH), 7.12 (d, \(J = 1.8\) Hz, 1H, ArH), 7.08 (t, \(J = 7.5\) Hz, 1H, ArH), 7.00 (t, \(J = 7.4\) Hz, 1H, ArH), 4.59 (dd, \(J = 17.8, 9.2\) Hz, 1H, CHα-Trp), 4.05 – 3.98 (m, 1H, CHα-Leu), 3.90 (dd, \(J = 13.7, 8.4\) Hz, 1H, CHHGAz), 3.87 – 3.80 (m, 1H, CHH-Az), 3.33 – 3.29 (m, 2H, CHHGly, CHH-Az), 3.18 (dd, \(J = 14.8, 9.2\) Hz, 1H, CHβ-Trp),
3.05 – 2.98 (m, 2H, CHHβ-Trp, CHHGly), 1.65 – 1.56 (m, 2H, CHγ-Leu, CHHβ-Leu), 1.48 (m, 1H, CHHβ-Leu), 0.91 (d, J = 6.2 Hz, 3H, CHδ-Leu), 0.79 (d, J = 6.3 Hz, 3H, CHδ-Leu)

Note:

2 × CHH-Az, CHHGly overlaps with H2O signal; 13C NMR (151 MHz, DMSO-d6) δC 173.4 (C=O), 172.9 (C=O), 171.5 (C=O), 136.1 (C), 127.1 (CH), 121.1 (CH), 118.4 (CH), 118.0 (CH), 111.5 (CH), 109.4 (C), 58.1 (C, Az), 56.1 (CH, α-Trp), 54.0 (CH, α-Leu), 52.9 (CH2, Az), 51.7 (CH2, Az), 47.0 (CH2, Gly), 43.6 (CH2, GAc), 26.4 (CH2, β-Trp), 24.6 (CH, γ-Leu), 22.9 (CH3, δ-Leu), 21.2 (CH3, δ-Leu); Note: CH2β-Leu overlaps with DMSO signal;

νmax (neat) = 3264, 2957, 2922, 1659, 1535, 1296, 740 cm−1; MS (ESI+) m/z 441 [M+H]+, 463 [M+Na]+; HRMS (ESI+) calcd. for C23H33N6O3 [M+H]+ 441.2609, found 441.2625; [α]D26 –38.5 (c 0.05, MeOH).

Cyclo(Asp-Leu-GAc-Gly) (347)

Following general procedure 10, Cyclo(Asp(tBu)-Leu-GAc(Boc)-Gly) (297) (31.1 mg, 59 μmol, 1.0 equiv) was deprotected to reveal the title compound 347 as a maroon solid (50.8 mg) as the TFA salt in quantitative yield, which required no further purification. 1H and 19F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 87 – 90 °C; Note: NMR data reported for the major conformational isomer at 25 ºC: 1H NMR (500 MHz, D2O) δH 4.63 (t, J = 7.0 Hz, 1H, CHα-Asp), 4.38 – 4.14 (m, 4H, CH2-Az, CHH-Az, CHα-Leu), 4.10 (dd, J = 10.5, 3.9 Hz, 1H, CHH-Az), 3.90 (d, J = 15.1 Hz, 1H, CHH-Gly), 3.80 (d, J = 15.4 Hz, 1H, CHH-Gly), 3.59 (d, J = 17.7 Hz, 1H, CHH-GAc), 3.48 (d, J = 17.7 Hz, 1H, CHH-GAc), 3.08 (dd, J = 16.8, 8.2 Hz, 1H, CHHβ-Asp), 2.70 (dd, J = 16.8, 6.1 Hz, 1H, CHHβ-Asp), 1.77 – 1.59 (m, 3H, CH2β-Leu, CHγ-Leu), 0.93 (d, J = 6.7 Hz, 3H, CH3δ-Leu), 0.91 (d, J = 6.7 Hz, 3H, CH3δ-Leu); 13C NMR (126 MHz, D2O) δC 174.8 (C=O), 174.7 (C=O), 172.7 (C=O), 57.3 (C, GAc), 55.8 (CH, α-Leu), 55.6 (2 × CH2, Az), 48.4 (CH, α-Asp), 45.0 (CH2, GAc or Gly), 41.9 (CH2, GAc or Gly), 40.5 (CH2, β-Leu), 34.2 (CH2, β-Asp), 24.1 (CH, γ-Leu), 22.1 (CH3, δ-Leu), 20.1 (CH3, δ-Leu); Note: one C=O not observed; νmax (neat) = 3262, 2921, 1662, 1177, 1130, 720 cm−1; MS (ESI+) m/z 370 [M+H]+, 392 [M+Na]+; HRMS (ESI+) calcd. for C16H26N6O5 [M+H]+ 370.2079, found 370.2085; [α]D26 –54.2 (c 0.15, MeOH).
Cyclo(Tyr-GAz-Thr-Leu) (348)

Following general procedure 10, Cyclo(Tyr(Bu)-GAz(Boc)-Thr(Bu)-Leu) (324) (12.7 mg, 19 µmol, 1.0 equiv) was deprotected to give the fully deprotected cyclic peptide 348 as a glassy colourless solid (10.6 mg) which required no further purification. 

$^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 168 – 170 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) $\delta$H 7.08 (d, $J$ = 8.4 Hz, 2H, ArH), 6.72 (d, $J$ = 8.4 Hz, 2H, ArH), 4.48 (t, $J$ = 8.3 Hz, 1H, CH$_\alpha$-Leu), 4.38 (dd, $J$ = 10.6, 5.3 Hz, 1H, CH$_\alpha$-Tyr), 4.04 – 3.96 (m, 3H, CH$_H$H$_2$-Az, CH$_H$H$_2$-Az, C$_H$H$_3$Gaz), 3.79 – 3.72 (m, 2H, CH$_H$H$_2$-Az, CH$_\alpha$-Thr), 3.53 (d, $J$ = 11.6 Hz, 1H, CHH-Az), 3.19 (d, $J$ = 13.8 Hz, 1H, CHHGaz), 3.08 (dd, $J$ = 14.1, 5.3 Hz, 1H, CHH$\beta$-Tyr), 2.88 (dd, $J$ = 14.0, 10.7 Hz, 1H, CHH$\beta$-Tyr), 1.57 (t, $J$ = 7.7 Hz, 2H, CH$_2$-Thr), 1.45 – 1.39 (m, 1H, CH$\gamma$-Leu), 1.24 (d, $J$ = 6.2 Hz, 3H, CH$_3$-Thr), 0.89 (d, $J$ = 6.6 Hz, 3H, CH$_3$-Leu), 0.86 (d, $J$ = 6.6 Hz, 3H, CH$_3$-Thr). Note: CH$\beta$-Thr overlaps with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) $\delta$C 176.9 (C=O), 175.3 (C=O), 174.0 (C=O), 157.6 (C), 131.0 (C), 128.9 (CH), 116.4 (CH), 68.2 (CH, $\alpha$-Thr), 68.0 (CH, $\beta$-Thr), 59.8 (C, Az), 58.9 (CH, $\alpha$-Tyr), 55.6 (CH, $\alpha$-Leu), 54.9 (CH$_2$, Az), 53.5 (CH$_2$, Az ), 45.5 (CH$_2$, GAz), 39.5 (CH$_2$, $\beta$-Leu), 36.6 (CH$_2$, $\beta$-Tyr), 26.6 (CH, $\gamma$-Leu), 22.6 (CH$_3$, $\delta$-Leu), 22.5 (CH$_3$, $\delta$-Leu), 20.7 (CH$_3$, $\gamma$-Thr); $\nu$$_{max}$ (neat) = 3290, 1659, 1515, 1132 cm$^{-1}$; MS (ESI$^+$) m/z 462 [M+H]$^+$; HRMS (ESI$^+$) calcd. for C$_{23}$H$_{36}$N$_5$NaO$_5$ [M+H]$^+$ 462.2711, found 462.2702; [$\alpha$]$_D^{24}$ = -54.5 (c 0.03, MeOH).

Analytical HPLC, 280 nm: $R_f$ = 0.14 min. Agilent PLRP-S column (100 Å, 8 µm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.
Cyclo(Val-GAz-Asp-Trp) (349)

Following general procedure 10, Cyclo(Val-GAz(Boc)-Asp(Bu)-Trp(Boc)) (325) (15.5 mg, 21 µmol, 1.0 equiv) was deprotected to give the fully deprotected cyclic peptide 349 as a glassy pale yellow solid (16.7 mg) which required no further purification. $^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 184 – 186 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) δ$_H$ 7.63 (d, $J$ = 7.9 Hz, 1H, ArH), 7.32 (d, $J$ = 8.1 Hz, 1H, ArH), 7.13 (s, 1H, ArH), 7.09 (t, $J$ = 7.5 Hz, 1H, ArH), 7.02 (t, $J$ = 7.3 Hz, 1H, ArH), 4.84 – 4.80 (m, 1H, CHα-Trp), 4.30 (d, $J$ = 11.2 Hz, 1H, CHH-Az), 4.17 (d, $J$ = 14.0 Hz, 1H, CHHGaz), 3.97 – 3.88 (m, 2H, CHα-Asp, CHH-Az), 3.83 (d, $J$ = 9.8 Hz, 1H, CHα-Val), 3.73 – 3.69 (m, 1H, CHH-Az), 3.62 (d, $J$ = 11.9 Hz, 1H, CHH-Az), 3.38 – 3.33 (m, 1H, CHHβ-Trp), 3.20 (dd, $J$ = 14.8, 7.6 Hz, 1H, CHHβ-Trp), 3.08 (d, $J$ = 15.3 Hz, 1H, CHHGaz), 2.67 (dd, $J$ = 17.4, 2.7 Hz, 1H, CHHβ-Asp), 2.58 (dd, $J$ = 17.4, 10.6 Hz, 1H, CHHβ-Asp), 2.07 – 2.01 (m, 1H, CHβ-Val), 1.01 (d, $J$ = 6.5 Hz, 3H, CH$_3$-Val), 0.97 (d, $J$ = 6.7 Hz, 3H, CH$_3$-Val); $^{13}$C NMR (126 MHz, MeOD-d$_4$) δ$_C$ 177.3 (C=O), 175.6 (C=O), 174.1 (C=O), 173.6 (C=O), 138.1 (C), 128.5 (C), 124.0 (CH), 122.6 (CH), 119.9 (CH), 119.2 (CH), 112.4 (CH), 110.5 (C), 64.4 (CH, α-Val), 60.2 (C, Az), 58.2 (CH, α-Val), 56.8 (CH, α-Asp), 54.4 (CH$_2$, Az), 53.1 (CH$_2$, Az), 45.2 (CH$_2$, GAZ), 37.4 (CH$_2$, β-Asp), 30.4 (CH, β-Val), 27.2 (CH$_2$, β-Trp), 20.5 (CH$_3$, γ-Val), 19.7 (CH$_3$, γ-Val); $\nu_{max}$ (neat) = 3292, 2962, 1658, 1181 cm$^{-1}$; MS (ESI$^+$) m/z 485 [M+H]$^+$; HRMS (ESI$^+$) calcd. for C$_{24}$H$_{33}$N$_6$O$_5$ [M+H]$^+$ 485.2507, found 485.2510; [α]$_D^{24}$ = 71.4 (c 0.08, MeOH).

Analytical HPLC, 280 nm: $R_t$ = 23.2 min. Agilent PLRP-S column (100 Å, 8 µm, 150 x 4.6 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.
Cyclo(Phe-Glu-GAz-Thr-Gly) (350)

Following general procedure 10 from Cyclo(Phe-Glu(Bu)-GAz(Boc)-Thr(Bu)-Gly) (330) (29 mg, 40 μmol, 1.0 equiv), Cyclo(Phe-Glu-GAz-Thr-Gly) (350) was isolated as an off-white glassy solid (39.7 mg) as the TFA salt in quantitative yield, which required no further purification. 1H and 19F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; mp 81 – 83 °C; 1H NMR (500 MHz, MeOD-d4) δ 7.37 – 7.29 (m, 5H, ArH), 4.50 (dd, J = 9.7, 4.3 Hz, 1H, CHα-Glu), 4.39 (dd, J = 10.0, 4.4 Hz, 1H, CHα-Phe), 4.26 (d, J = 10.9 Hz, 1H, CHH-Az), 4.03 (d, J = 14.9 Hz, 1H, CHH-Gly), 3.91 (d, J = 11.0 Hz, 1H, CHH-Az), 3.78 (d, J = 10.3 Hz, 1H, CHH-Az), 3.74 – 3.63 (m, 3H, CHH-Glu, CHH-Glu, CHβ-Thr), 3.54 (d, J = 14.9 Hz, 1H, CHH-Gly), 3.25 (dd, J = 14.4, 4.4 Hz, 1H, CHβ-Phe), 3.10 – 2.98 (m, 3H, CHH-Glu, CHβ-Phe, CHα-Thr), 2.43 – 2.26 (m, 3H, CH2γ-Glu, CHHβ-Glu), 2.05 – 1.92 (m, 1H, CHHβ-Glu), 1.20 (d, J = 6.3 Hz, 3H, CH3γ-Thr); 13C NMR (126 MHz, MeOD-d4) δ 176.5 (C=O), 176.4 (C=O), 175.1 (C=O), 174.4 (C=O), 173.9 (C=O), 138.1 (C), 130.0 (CH), 129.7 (CH), 128.1 (CH), 70.4 (CH, β-Thr), 63.3 (CH, α-Thr), 60.3 (C, Az), 60.0 (CH2, Az), 59.1 (CH, α-Phe), 53.5 (CH2, Az), 53.5 (CH, α-Glu), 45.9 (CH2, GAz), 44.4 (CH2, Gly), 37.4 (CH2, β-Phe), 31.3 (CH2, γ-Glu), 27.8 (CH2, β-Glu), 19.2 (CH2, γ-Thr); νmax (neat) = 3273, 3033, 2922, 1649, 1538, 1177 cm–1; MS (ESI+) m/z 519 [M+H]+, 541 [M+Na]+; HRMS (ESI+) calcd. for C32H35N6O7 519.2562 [M+H]+, found 519.2555; [α]D24 +6.0 (c 0.07, MeOH).

Cyclo(Leu-Ala-GAz-Ala-Tyr) (351)

Following general procedure 10 from Cyclo(Leu-Ala-GAz(Boc)-Ala-Tyr) (277) (32 mg, 53 μmol, 1.0 equiv), Cyclo(Leu-Ala-GAz-Ala-Tyr) (351) was isolated as a pale yellow glassy solid (44 mg) as the TFA salt in quantitative yield, which required no further purification. 1H and 19F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt, product isolated as an inseparable 8:1 mixture of diastereomers, data reported for the major diastereomer; 1H NMR (500 MHz, MeOD) δ 7.05 (d, J = 8.3 Hz, 2H, ArH), 6.73 (d, J = 8.3 Hz, 2H, ArH), 4.48 (q, J = 7.1 Hz, 1H, CHα-Ala), 4.23 (dd, J = 10.8, 4.4 Hz, 1H, CHα-Leu), 4.16 (dd, J = 10.5, 5.8 Hz, 1H, CHα-Tyr), 3.95 (d, J = 10.6 Hz, 1H, CHH-Az), 3.86 – 3.71 (m, 3H, CHH-Az, CH2-Az), 3.59 (d, J = 14.4 Hz, 1H, CHH-Glu), 3.25 – 3.19 (m, 1H, CHHβ-Tyr), 3.10 (dd, J = 13.5, 5.7 Hz, 1H, CHHβ-Leu), 1.83 – 1.73 (m, 1H, CHHβ-Leu), 1.67 – 1.56 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.41 (d, J = 7.2 Hz, 3H, CH3β-Ala), 1.20 (d, J = 6.9 Hz, 3H, CH3β-Ala),
0.96 (d, J = 6.3 Hz, 3H, CH₃δ-Leu), 0.88 (d, J = 6.2 Hz, 3H, CH₃δ-Leu), Note: CHHGAz and CHα-Ala overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD) δ 178.4 (C=O), 175.9 (C=O), 174.8 (C=O), 174.5 (C=O), 157.5 (C), 131.3 (CH), 129.1 (C), 116.4 (CH), 59.9 (C, Az), 57.9 (CH, α-Tyr), 57.8 (CH₃, Az), 55.7 (CH, α-Leu), 53.9 (CH₂, Az), 53.1 (CH, α-Ala), 50.5 (CH, α-Ala), 45.5 (CH₂, GAz), 40.5 (CH₂, β-Leu), 35.1 (CH₂, β-Leu), 26.0 (CH, γ-Leu), 23.4 (CH₃, β-Ala), 21.8 (CH₃, β-Ala), 21.5 (CH₃, δ-Leu), 18.0 (CH₃, δ-Leu); νmax (neat) = 3300, 2966, 1660, 1535, 735 cm⁻¹; MS (ESI⁺) m/z 503 [M+H]⁺; HRMS (ESI⁺) calcd. for C₂₅H₃₉N₆O₅ [M+H]⁺ 503.2976, found 503.2982.

Cyclo(Tyr-Val-GAz(2-PC)-Gly-Phe) (352)

Following general procedure 10 from Cyclo(Tyr(Bu)-Val-GAz(2-PC)-Gly-Phe) (337) (19.8 mg, 29 µmol, 1.0 equiv), Cyclo(Tyr-Val-GAz(2-PC)-Gly-Phe) (352) was isolated as a glassy colourless solid (22.5 mg) which required no further purification. ¹H and ¹⁹F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 193 – 196 °C; ¹H NMR (500 MHz, MeOD-d₄) δ 7.30 – 7.13 (m, 5H, ArH), 7.01 (d, J = 8.3 Hz, 2H, ArH), 6.69 (d, J = 8.3 Hz, 2H, ArH), 4.69 (d, J = 1.8 Hz, 2H, OCH₂), 4.28 (m, J = 7.8 Hz, 1H, CHα-Tyr), 4.22 – 4.14 (m, 2H, CHHGAz, CHα-Phe), 4.10 – 3.97 (m, 5H, 2 × CH₂-Az, CH₃-Val), 3.88 (d, J = 15.8 Hz, 1H, CHHNY), 3.70 (d, J = 15.6 Hz, 1H, CHHgly), 3.50 (d, J = 15.2 Hz, 1H, CHHGAz), 3.23 – 3.15 (m, 2H, CH₂β-Phe), 2.99 (d, J = 7.8 Hz, 2H, CH₂β-Tyr), 2.95 (t, J = 2.3 Hz, 1H, C=CH), 2.16 (dq, J = 13.1, 6.5 Hz, 1H, CHβ-Val), 0.99 – 0.93 (m, 6H, 2 × CH₃γ-Val); ¹³C NMR (126 MHz, MeOD-d₄) δC 174.5 (C=O), 174.4 (C=O), 157.5 (C=O), 157.0 (C), 137.7 (C), 131.1 (CH), 130.1 (CH), 129.7 (CH), 129.2 (C), 128.0 (CH), 116.4 (CH), 78.8 (C=CH), 76.5 (C=CH), 61.4 (CH, α-Val), 60.2 (CH, α-Phe), 58.5 (CH, α-Tyr), 58.3 (CH₂-Az), 54.0 (OCH₂), 45.4 (CH₂, Gly), 42.7 (CH₂, GAz), 37.1 (CH₂, β-Tyr), 36.7 (CH₂, β-Phe), 32.3 (CH, β-Val), 19.9 (CH₃, γ-Val), 18.4 (CH₃, γ-Val), Note: 1 × CH₂, Az, C, Az not observed, 2 × C=O overlapping with other C=O signals; νmax (neat) = 3270, 1659, 1515, 1438, 1264 cm⁻¹; MS (ESI⁺) m/z 655 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₅H₄₀N₆NaO₇ [M+Na]⁺ 655.2851, found 655.2851; [α]D²⁶ –29.0 (c 0.07, MeOH).
Cyclo(Ser-Ile-GAzm-Ala) (353)

Following general procedure 10, Cyclo(Ser(Bu)-Ile-GAzm(Boc)-Met-Ala) (334) (16.1 mg, 25 µmol, 1.0 equiv) was deprotected to give the fully deprotected cyclic peptide 353 as a glassy colourless solid (19.6 mg) which required no further purification. \( ^1 \text{H} \) and \( ^{19} \text{F} \) NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 144 – 147 °C; \( ^1 \text{H} \) NMR (500 MHz, MeOD-d4) \( \delta \) 4.55 – 4.44 (m, 1H, CHα-Ile), 4.20 (dd, \( J = 4.6, 3.7 \) Hz, 1H, CHα-Ser), 4.11 (d, \( J = 10.9 \) Hz, 1H, CHH-Az), 3.97 (dd, \( J = 11.3, 5.3 \) Hz, 1H, CHHβ-Ser), 3.94 – 3.86 (m, 3H, CHH-Az, CHHβ-Ser, CHα-Ala), 3.80 (d, \( J = 11.3 \) Hz, 1H, CHH-Az), 3.74 (d, \( J = 10.9 \) Hz, 1H, CHH-Az), 3.58 (dd, \( J = 14.3, 3.1 \) Hz, 1H, CHHGAz), 3.44 (dd, \( J = 9.0, 3.8 \) Hz, 1H, CHα-Met), 2.79 – 2.66 (m, 2H, CHγ-Met), 2.12 (s, 3H, SCh3), 2.07 – 2.01 (m, 1H, CHβ-Ile), 1.90 – 1.83 (m, 1H, CHHβ-Met), 1.81 – 1.71 (m, 1H, CHHβ-Met), 1.61 – 1.51 (m, 4H, CHβ-Ala, CHHδ-Ile), 1.23 – 1.14 (m, 1H, CHHδ-Ile), 0.96 – 0.91 (m, 6H, CHγ-Ile, CHε-Ile), Note: CHHGaz overlaps with solvent signal; \( ^{13} \text{C} \) NMR (126 MHz, MeOD-d4) \( \delta \) C 177.8 (C=O), 176.0 (C=O), 174.2 (C=O), 172.4 (C=O), 61.8 (CH, β-Ser), 60.1 (CH, α-Ser), 59.9 (C, Az), 59.6 (CH, α-Ile), 59.1 (CH2, Az), 55.4 (CH, α-Met), 53.8 (CH2, Az), 52.3 (CH, α-Ala), 46.0 (CH2, GAz), 37.5 (CH, β-Ile), 35.2 (CH2, β-Met), 30.9 (CH2, γ-Met), 25.8 (CH2, δ-Ile), 16.0 (CH3, γ-Ile), 15.2 (CH3, β-Ala), 14.9 (SCh3), 11.9 (CH3, ε-Ile); νmax (neat) = 3289, 2964, 1648, 1524, 1130 cm\(^{-1}\); MS (ESI\(^+\)) \( m/z \) 487 [M+H]\(^+\), 973 [2M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C23H37N3O4S [M+Na]\(^+\) 487.2697, found 487.2699; [α]D\(^{24}\) = –49.1 (c 0.08, MeOD-d4).

Cyclo(Leu-Tyr-Val-GAzm-Thr-Phe) (354)

Following general procedure 10 from Cyclo(Leu-Tyr(Bu)-Val-GAzm(Boc)-Thr(Bu)-Phe) (289) (15.9 mg, 16.3 µmol, 1.0 equiv) in CHCl3 (1 mL) was added TFA (1 mL), Cyclo(Leu-Tyr-Val-GAzm-Thr-Phe) (354) was isolated as a white glassy solid (16.7 mg) as the TFA salt, which required no further purification. \( ^1 \text{H} \) and \( ^{19} \text{F} \) NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 186 – 187 °C (decomp.); \( ^1 \text{H} \) NMR (500 MHz, MeOD-d4) \( \delta \) 7.37 – 7.25 (m, 5H, ArH), 7.05 (d, \( J = 7.8 \) Hz, 2H, ArH), 6.73 (d, \( J = 7.6 \) Hz, 2H, ArH), 4.64 (dd, \( J = 9.8, 4.9 \) Hz, 1H, CHα-Phe), 4.33 (d, \( J = 9.2 \) Hz, 1H, CHα-Val), 4.18 (d, \( J = 11.0 \) Hz, 1H, CHH-Az), 4.10 (dd, \( J = 11.3, 3.5 \) Hz, 1H, CHα-Tyr), 4.05 – 3.98 (m, 2H, CHHGaz, CHH-Az), 3.98 – 3.88 (m, 2H, CH2-Az), 3.75 (t, \( J = 7.2 \) Hz, 1H, CHα-Leu), 3.46 (m, 1H, CHHβ-Tyr), 3.03 (d, \( J = 16.2 \) Hz, 1H, CHHGaz), 2.88 – 2.76 (m, 2H, CHHβ-Phe, CHβ-Thr), 2.05 – 1.92 (m, 1H, CHβ-Val), 1.54 – 1.47 (m, 1H, CHHβ-Leu), 1.43 – 1.32 (m, 2H, CHHβ-Leu, 235
CHγ-Leu), 1.06 (d, J = 6.5 Hz, 3H, CH2γ-Thr), 1.03 (d, J = 6.9 Hz, 3H, CH2γ-Val), 1.00 (d, J = 5.8 Hz, 3H, CH2δ-Leu), 0.82 (d, J = 6.0 Hz, 3H, CH2δ-Leu), 0.79 (d, J = 6.1 Hz, 3H, CH2δ-Leu),

Note: CHHβ-Tyr, CHα-Thr and CHHβ-Phe overlap with solvent signal; 13C NMR (126 MHz, MeOD-d4) δ 175.4 (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 173.0 (C=O), 157.3 (C), 137.6 (C), 131.0 (CH), 130.2 (C), 130.1 (CH), 129.8 (CH), 128.2 (CH), 116.3 (CH), 69.2 (CH, α-Thr), 66.0 (CH, β-Thr), 62.8 (CH, α-Val), 60.6 (C, GAz), 59.4 (CH, α-Tyr), 55.9 (2 × CH2 Az), 55.8 (CH, α-Leu), 54.8 (CH, α-Phe), 44.2 (CH2, GAz), 40.3 (CH2, β-Leu), 38.9 (CH2, β-Phe), 35.1 (CH2, β-Tyr), 33.4 (CH, β-Val), 25.1 (CH, γ-Leu), 22.8 (CH3, δ-Leu), 22.6 (CH3, δ-Leu), 20.8 (CH3, γ-Thr), 19.9 (CH3, γ-Val), 19.5 (CH3, γ-Val); νmax (neat) = 3302, 3029, 1639, 1515, 1436, 1198, 1132 cm⁻¹; MS (ESI⁺) m/z 708 [M+H⁺], 730 [M+Na⁺]; HRMS (ESI⁺) calcd. for C17H24N4O7 708.4079 [M+H⁺], found 708.4082; [α]D²⁴ +24.0 (c 0.06, MeOH).

Cyclo(Val-Gln-GAaz-Tyr-Leu) (355)

Following general procedure 11, Cyclo(Val-Gln-GAaz(Trt)-Gaz(Boc)-Tyr(Bu)-Leu) 332 (16.0 mg, 16 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 355 after freeze drying as a foamy white solid. 1H and 19F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 170 – 173 °C; 1H NMR (500 MHz, MeOD-d4) δH 7.13 (d, J = 7.8 Hz, 2H, ArH), 6.76 (d, J = 7.8 Hz, 2H, ArH), 4.31 (dd, J = 9.5, 4.3 Hz, 1H, CHα-Gln), 4.13 (dd, J = 10.1, 5.3 Hz, 1H, CHα-Leu), 3.83 – 3.78 (m, 2H, CHα-Val, CHH-Az), 3.66 – 3.58 (m, 3H, CHH-Az, CHH-Az, CHHGAz), 3.45 – 3.41 (m, 1H, CHα-Tyr), 3.39 – 3.32 (m, 2H, CHH-Az, CHHGAz), 2.92 (dd, J = 14.1, 5.1 Hz, 1H, CHHβ-Tyr), 2.72 (dd, J = 13.2, 8.4 Hz, 1H, CHHβ-Tyr), 2.33 (t, J = 7.2 Hz, 2H, CH2γ-Gln), 2.27 – 2.16 (m, 2H, CHγ-Val, CHHβ-Gln), 2.06 – 1.99 (m, 1H, CHHβ-Gln), 1.94 – 1.86 (m, 1H, CHHβ-Leu), 1.59 – 1.53 (m, 1H, CHHβ-Leu), 1.46 – 1.38 (m, 1H, CHγ-Leu), 1.02 (d, J = 6.6 Hz, 3H, CH3γ-Val), 0.99 (d, J = 7.0 Hz, 3H, CH3γ-Val), 0.97 (d, J = 6.6 Hz, 3H, CH3δ-Leu), 0.90 (d, J = 6.6 Hz, 3H, CH3δ-Leu), Note: CHH-Az and CHHGAz overlapped with solvent signal; 13C NMR (126 MHz, MeOD-d4) δC 177.6 (C=O), 177.0 (C=O), 175.3 (C=O), 174.9 (C=O), 173.8 (C=O), 157.7 (C), 131.5 (CH), 129.2 (C), 116.6 (CH), 64.1 (CH, α-Val), 60.2 (CH, α-Tyr), 59.6 (C, Az), 57.0 (CH2, Az), 55.3 (CH, α-Gln), 54.7 (CH, α-Leu), 54.5 (CH2 Az), 45.1 (CH2, GAZ), 41.2 (CH2, β-Tyr), 39.6 (CH2, β-Leu), 32.9 (CH2, γ-Gln), 30.5 (CH, γ-Val), 28.3 (CH2, β-Gln), 25.9 (CH, γ-Leu), 23.6 (CH3, γ-Val), 21.6 (CH3, β-Val), 20.0 (CH3, δ-Leu), 19.8 (CH3, δ-Leu); νmax (neat) = 3271, 1654, 1514, 1238, 1179, 1135
cm⁻¹; **MS** (ESI⁺) *m/z* 588 [M+H]⁺, 610 [M+Na]⁺; **HRMS** (ESI⁺) calcd. for C₂₉H₄₈N₇O₆ [M+H]⁺ 588.3504, found 588.3502; [α]D²¹ − 42.2 (c 0.03, MeOD).

Preparative HPLC trace:

Rᵣ = 26.6 min, 280 nm; Method: 0 – 3 min 5% B; 3 – 25 min, 5 – 50% B; 25 – 40 min 100% B; 40 – 50 min, 100% B.

Analytical HPLC, 280 nm: Rᵣ = 21.5 min. Agilent PLRP-S column (100 Å, 8 µm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.

Cyclo(His-GAz-Ile-Leu) (356)

Following general procedure 11, Cyclo(His(Trt)-GAz(Boc)-Ile-Leu) 326 (16.0 mg, 20 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 356 after freeze drying as a foamy white solid. 

Following general procedure 11, Cyclo(His(Trt)-GAz(Boc)-Ile-Leu) 326 (16.0 mg, 20 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 356 after freeze drying as a foamy white solid. 

H and ¹⁹F NMR using TFE as an internal standard confirmed 4 eq. of TFA salt; m.p. 198 – 201 ºC; ¹H NMR (500 MHz, MeOD-d₄) δ 8.84 (s, 1H, ArH), 7.45 (s, 1H, ArH), 4.57 – 4.51 (m, 2H, CHα-His, CHα-Leu), 4.04 (d, J = 14.0 Hz, 1H, CHHGAz), 3.95 (d, J = 11.8 Hz, 1H, CHH-Az), 3.89 (d, J = 11.2 Hz, 1H, CHH-Az), 3.79 (dd, J
= 11.1, 1.6 Hz, 1H, CHH-Az), 3.53 (dd, J = 11.7, 1.7 Hz, 1H, CHH-Az), 3.20 (d, J = 14.1 Hz, 1H, CHHGaz), 1.80 – 1.48 (m, 5H, CHHδ-Ile, CHβ-Ile, CHγ-Leu, CHβ-Leu), 1.25 – 1.19 (m, 1H, CHHδ-Ile), 1.00 – 0.96 (m, 6H, CHε-Ile, CHδ-Ile), 0.93 (d, J = 6.4 Hz, 3H, CHδ-Leu), 0.90 (d, J = 6.3 Hz, 3H, CHδ-Leu), Note: CHα-Ile and CHβ-His overlap with solvent peak; 13C NMR (126 MHz, MeOD-d4) δ C 178.5 (C=O), 174.7 (C=O), 174.0 (C=O), 131.2 (CH), 118.6 (CH), 65.8 (CH, α-Ile), 60.1 (C, Az), 56.5 (CH, α-His), 55.6 (CH2, Az), 55.5 (CH, α-Leu), 53.7 (CH2, Az), 45.4 (CH2, GAZ), 39.3 (CH2, β-Leu), 38.7 (CH, β-Ile), 27.2 (CH2, δ-Ile), 26.5 (CH2, β-His), 26.4 (CH, γ-Leu), 23.0 (CH3, δ-Leu), 22.2 (CH3, δ-Leu), 15.8 (CH3, γ-Ile), 11.6 (CH3, ε-Ile); νmax (neat) = 3291, 2964, 1661, 1129 cm⁻¹; MS (ESI⁺) m/z 448 [M+H]⁺, 470 [M+Na]⁺, 917 [2M+Na]⁺; HRMS (ESI⁺) calcd. for C22H38N7O3 [M+H]⁺ 448.3031, found 448.3031; [α]D24 = −56.3 (c 0.05, MeOH).

Preparative HPLC trace:

![HPLC trace image]

Rf = 26.6 min, 220 nm; Method: 0 – 3 min 5% B; 3 – 25 min, 5 – 50% B; 25 – 40 min 100% B; 40 – 50 min, 100% B.

**Cyclo(Asn-GAZ-Lys-Gly) (357)**

Following general procedure 11, cyclo(Asn(Trt)-GAGz(Boc)-Lys(Boc)-Gly) 327 (15.8 mg, 19 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 357 after freeze drying as a foamy white solid. 1H and 19F NMR using TFE as an internal standard confirmed 2 eq. TFA salt; m.p. 97 – 100 ºC; This compound appears as a 1:1 mixture of conformational isomers at RT. Data reported as a mixture of isomers at 298 K, 1H NMR (500 MHz, MeOD-d4) δ 4.58 (t, J = 6.5 Hz, 0.5H, CHα-Asn), 4.27 – 4.22 (m, 0.5H, CHβ-Asn), 4.09 (d, J = 11.7 Hz, 0.5H, CH2-Az), 4.06 – 3.87 (m, 4H, CH2Gaz, CH2Gly, CH2-Az), 3.81 – 3.76 (m, 2×0.5H, CH2Gly, CH2-Az), 3.69 (d, J = 14.4 Hz, 0.5H, CH2Gaz), 3.62 – 3.47 (m, 3×0.5H, CH2Gaz, CH2-Az, CHα-Lys), 3.38 (t, J = 6.4 Hz, 0.5H, CHα-Lys), 3.16 (d, J = 13.9 Hz, 0.5H, CH2Gaz), 2.96 – 2.90 (m, 2.5H, CHβ-Asn,
239

CH₂β-Lys), 2.85 (dd, \(J = 16.8, 7.1\) Hz, CH₂β-Asn), 2.73 – 2.68 (m, 1H, CH₂β-Asn), 1.72 – 1.40 (m, 6H, CH₂β-Lys, CH₂γ-Lys, CH₂δ-Lys); \(^{13}C\) NMR (126 MHz, MeOD-d₄) δ 179.6 (C=O Lys), 178.0 (C=O Lys), 174.7 (C=O Asn), 174.2 (C=O), 173.33 (C=O), 173.29 (C=O), 171.6 (C=O), 170.7 (C=O), 59.88 (C, Az), 59.85 (CH, α-Lys), 59.3 (C, Az), 57.7 (CH, α-Lys), 57.1 (CH₂, Az), 55.4 (CH₂, Az), 55.2 (CH₂, Az), 54.2 (CH, α-Asn), 52.9 (CH₂, Az), 51.4 (CH, α-Asn), 46.1 (CH₂, Gαz), 45.1 (CH₂, Gly), 44.5 (CH₂, Gαz), 41.7 (CH₂, Gly), 40.5 (CH₂, ε-Lys), 36.3 (CH₂, β-Asn), 36.2 (CH₂, β-Asn), 35.1 (CH₂, δ-Lys), 33.9 (CH₂, δ-Lys), 28.4 (CH₂, β-Lys), 28.3 (CH₂, β-Lys), 24.1 (CH₂, γ-Lys), 23.6 (CH₂, γ-Lys); ν\text{max} (neat) = 3375, 1666, 1187, 1132 cm\(^{-1}\);

MS (ESI\(^+\)) \(m/z\) 384 [M+H]\(^+\), 406 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{16}\)H\(_{29}\)N\(_7\)NaO\(_4\) [M+Na]\(^+\) 406.2173, found 406.2169; \(\alpha\)D\(_{24}\) – 19.7 (c 0.04, MeOH).

Preparative HPLC trace:

\(R_f = 14.7\) min, 280 nm; Method: 0 – 3 min 5% B; 3 – 25 min, 5 – 50% B; 25 – 40 min 100% B; 40 – 50 min, 100% B.

Cyclo(Ala-Met-Gαz-Asp-Phe) (358)

Following general procedure 11, Cyclo(Ala-Met-Gαz(Boc)-Asp(‘Bu)-Phe) (331) (8.4 mg, 12 \(\mu\)mol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 358 after freeze drying as a foamy white solid. \(^1H\) and \(^19\)F NMR using TFE as an internal standard confirmed 2 eq. of TFA salt; m.p. 178 – 180 °C; \(^1\)H NMR (700 MHz, MeOD-d₄) δ\(\text{H}\) 7.35 – 7.32 (m, 2H, ArH), 7.27 – 7.25 (m, 1H, ArH), 7.22 – 7.20 (m, 2H, ArH), 4.63 (dd, \(J = 9.7, 4.2\) Hz, 1H, CHα-Phe), 4.21 (q, \(J = 7.5\) Hz, 1H, CHα-Ala), 4.18 (d, \(J = 11.0\) Hz, 1H, CHH-Az), 4.11 (dd, \(J = 10.8, 1.8\) Hz, 1H, CHH-Az), 3.97 (dd, \(J = 12.0, 4.4\) Hz, 1H, CHα-Met), 3.77 (d, \(J = 10.8\) Hz, 1H, CHH-Az), 3.65 (m, 2H, CHHGαz, CHH-Az), 3.47 – 3.43 (m, 2H, CHα-Asp, CHHβ-Phe), 2.98 (d, \(J = 14.7\) Hz, 1H, CHHGαz), 2.64 (ddd, \(J = 13.3, 7.9, 5.4\) Hz, 1H, CHHβ-Met), 2.54 (dt, \(J = 13.4, 7.7\) Hz, 1H, CHHβ-Met), 2.40 (dd, \(J = 16.5, 10.7\) Hz, 1H, CHHβ-Asp), 2.28 (ddd, \(J = 12.1, 7.8, 4.2\) Hz, 1H CHHγ-Met), 2.20 (dd, \(J = 16.5, 2.7\) Hz, 1H, CHHβ-Asp), 2.12 (s, 3H, SMe), 2.06 – 2.00 (m, 1H, CHHγ-Met), 1.52 (d, \(J = 7.5\) Hz, 3H,
CHβ-Ala), Note: CHβ-Phe overlaps with solvent signal; $^{13}$C NMR (176 MHz, MeOD-d$_4$) δC 176.6 (C=O), 175.7 (C=O), 175.4 (C=O), 174.6 (C=O), 139.1 (CH), 130.3 (CH), 129.8 (CH), 128.0 (CH), 60.1 (C, Az), 59.7 (C, Az), 58.2 (CH, δ-Met), 53.8 (CH, δ-Asp), 53.5 (CH, α-Ala), 53.3 (CH, δ-Met), 51.4 (CH, γ-Met), 16.8 (CH, β-Ala), 15.4 (SMe); ν$_{max}$(neat) = 3292, 2921, 1656, 1526, 1129 cm$^{-1}$; MS (ESI$^+$) m/z 549 [M+H]$^+$; HRMS (ESI$^+$) calcd. for C$_{20}$H$_{34}$N$_{10}$O$_7$[M+H]$^+$ 549.2504, found 549.2493; $[^\alpha]$D$_{24}$ – 75.5 (c 0.05, MeOD-d$_4$).

Preparative HPLC trace:

\[ R_t = 32.8 \text{ min, 280 nm; Method: 0 – 3 min 5% B; 3 – 33 min, 5 – 50% B; 33 – 38 min 100% B; 38 – 45 min, 100% B, 45 – 55 min 100% – 5% B.} \]

Cyclo(Tyr-Cys-GAza-Lys-Leu) (359)

Following general procedure 11, Cyclo(Tyr(tBu)-Cys(Trt)-GAza-Lys(Boc)-Leu) (333) (16.4 mg, 15 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 359 after freeze drying as a foamy white solid. $^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 4 eq. of TFA salt; m.p. 151 – 154 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) δH 7.10 (d, J = 8.3 Hz, 2H, ArH), 6.74 (d, J = 8.3 Hz, 2H, ArH), 4.62 (t, J = 6.1 Hz, 1H, CHα-Cys), 4.41 (dd, J = 9.8, 4.4 Hz, 1H, CHα-Tyr), 3.95 (d, J = 10.7 Hz, 1H, CHH-Az), 3.89 – 3.79 (m, 4H, CHH-Az, CH$_2$-Az, CHα-Leu), 3.72 (d, J = 14.7 Hz, 1H, CHHGAza), 3.20 (dd, J = 14.7, 5.3 Hz, 1H, CHHβ-Tyr), 3.02 (dd, J = 14.3, 10.0 Hz, 1H, CHHβ-Tyr), 2.94 – 2.89 (m, 3H, CH$_2$δ-Lys, CHHβ-Cys), 2.82 (dd, J = 13.8, 6.5 Hz, 1H, CHHβ-Cys), 1.88 – 1.77 (m, 1H, CHHβ-Leu), 1.72 – 1.53 (m, 5H, CHβ-Lys, CH$_2$δ-Lys, CHγ-Leu), 1.49 – 1.39 (m, 3H, CHHβ-Leu, CH$_2$γ-Lys), 0.91 (d, J = 5.8 Hz, 3H, CHδ-Leu), 0.91, 0.88 (d, J = 5.7 Hz, 3H, CHδ-Leu), Note: CHα-Lys and CHHGAza overlap with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) δC 177.5 (C=O), 174.8 (C=O), 173.7 (C=O), 173.1 (C=O), 157.7 (C), 131.1 (CH), 128.8 (C), 116.5 (CH), 60.4 (C, Az), 58.4 (CH, α-
(Tyr), 57.7 (CH, α-Lys), 57.4 (CH₂, Az), 57.0 (CH, α-Cys), 54.8 (CH, α-Leu), 54.0 (CH₂, Az), 45.6 (CH₂, GAZ), 40.5 (CH₂, ε-Lys), 39.2 (CH₂, β-Leu), 36.7 (CH₂, β-Tyr), 35.6 (CH₂, β-Lys), 28.5 (CH₂, δ-Lys), 26.5 (CH₂, β-Cys), 26.1 (CH, γ-Leu), 23.9 (CH₂, γ-Lys), 23.3 (CH₃, δ-Leu), 21.9 (CH₃, δ-Leu); ν_max (neat) = 3272, 2957, 1659, 1514, 1180, 1129 cm⁻¹; MS (ESI⁺) m/z 592 [M+H]⁺, 614 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₉H₄₆N₇O₅S [M+H]⁺ 592.3272, found 592.3276; [α]_D²⁴ = -49.1 (c 0.04, MeOH).

Preparative HPLC trace:

\[ R_f = 25.1 \text{ min, 280 nm; Method: 0 – 3 min 5\% B; 3 – 25 \text{ min, 5 – 50\% B; 25 – 40 \text{ min 100\% B; 40 – 50 \text{ min, 100\% B.}} \]

Analytical HPLC, 280 nm: \[ R_f = 20.1 \text{ min. Agilent PLRP-S column (100 Å, 8 μm, 150 × 4.6 mm)} \] with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3\% B; 3-33 min, 3-50\% B; 33-38 min, 50-100\% B, 38-40 min, 100\%-3\% B; 40-45 min, 3\% B.
Cyclo(Glu-Arg-GAz-Ile-Ala-Gly) (360)

Following general procedure 11, Cyclo(Glu(Bu)-Arg(Pbf)-GAz(Boc)-Ile-Ala-Gly) 340 (16.0 mg, 16 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 360 after freeze drying as a foamy white solid. ¹H and ¹³F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 105 – 107 ºC; ¹H NMR (500 MHz, MeOD-d₄) δ H 4.40 – 4.31 (m, 2H, CHα-Ala, CHα-Glu), 4.26 (d, J = 8.2 Hz, 1H, CHα-Arg), 4.20 (d, J = 10.9 Hz, 1H, CHH-Az), 4.12 – 4.03 (m, 2H, CHH-Gly, CHH-Az), 4.00 (d, J = 15.6 Hz, 1H, CHH-GAz), 3.78 – 3.68 (m, 2H, CHH-Az, CHH-Az), 3.62 (d, J = 15.5 Hz, 1H, CHH-Gly), 2.48 – 2.37 (m, 2H, CH2γ-Arg), 2.34 – 2.26 (m, 1H, CHHγ-Glu), 2.22 – 2.12 (m, 1H, CHHβ-Glu), 1.79 – 1.72 (m, 1H, CHHβ-Arg), 1.71 – 1.62 (m, 1H, CHHβ-Arg), 1.46 – 1.40 (m, 1H, CHHδ-Ile), 1.30 (d, J = 6.7 Hz, 3H, CH3β-Ala), 1.17 (ddd, J = 13.5, 9.4, 7.3 Hz, 1H, CHHδ-Ile), 1.00 (d, J = 6.8 Hz, 3H, CH3γ-Ile), 0.93 (t, J = 7.4 Hz, 3H, CH3ε-Ile). Note: CHH-GAz, CH2ε-Arg and CHα-Ile overlap with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δ C 177.4 (C=O), 176.6 (C=O), 175.1 (C=O), 174.8 (C=O), 174.0 (C=O), 173.1 (C = O), 158.7 (C, Arg), 61.8 (CH, α-Ile), 60.0 (C, Az), 55.2 (CH, α-Arg, and CH, α-Glu), 54.5 (CH2, Az), 53.6 (CH2, Az), 50.5 (CH, α-Ala), 45.2 (CH2, Gly), 44.9 (CH2, GAz), 42.0 (CH2, δ-Arg), 40.0 (CH, β-Ile), 32.0 (CH2, γ-Arg), 28.3 (CH2, β-Glu), 27.2 (CH2, γ-Glu), 26.9 (CH2, β-Arg), 25.7 (CH2, δ-Ile), 18.3 (CH3, β-Ala), 16.1 (CH3, γ-Ile), 12.3 (CH3, ε-Ile); νmax (neat) = 3283, 1640, 1545, 1177, 1127 cm⁻¹; MS (ESI⁺) m/z 611 [M+H]+; HRMS (ESI⁺) calcd. for C26H47N10O7 [M+H]+ 611.3624, found 611.3622; [α]D²¹ = −12.5 (c 0.03, MeOH).

Preparative HPLC trace:

\[
R_f = 23.4 \text{ min, } 220 \text{ nm; Method: } 0 - 3 \text{ min } 5\% \text{ B; } 3 - 38 \text{ min, } 5 - 50\% \text{ B; } 38 - 44 \text{ min } 50 - 100\% \text{ B; } 44 - 50 \text{ min, } 100\% \text{ B; } 50 - 58 \text{ min } 100\% - 5\% \text{ B.}
\]
Cyclo(Trp-Arg-GAz-Asp-Arg-Leu) (361)

Following general procedure 11, Cyclo(Trp(Boc)-Arg(Pbf)-GAz(Boc)-Asp(t-Bu)-Arg(Pbf)-Leu) 339 (16.0 mg, 10 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 361 after freeze drying as a foamy white solid. ¹H and ¹³F NMR using TFE as an internal standard confirmed 4 eq. of TFA salt; m.p. 150 – 152 °C; ¹H NMR (500 MHz, MeOD-d₄) δ HJ 7.61 (d, J = 7.9 Hz, 1H, ArH), 7.35 (d, J = 8.1 Hz, 1H, ArH), 7.10 (t, J = 7.7 Hz, 1H, ArH), 7.08 (s, 1H, ArH), 7.02 (t, J = 7.4 Hz, 1H, ArH), 4.42 (dd, J = 8.1, 6.2 Hz, 1H, CHα-Trp), 4.35 – 4.27 (m, 2H, CHα-Leu, CHα-Arg), 4.16 (d, J = 10.9 Hz, 1H, CHH-Az), 4.03 (d, J = 11.0 Hz, 1H, CHH-Az), 3.97 (d, J = 10.9 Hz, 1H, CHH-Az), 3.92 (d, J = 14.5 Hz, 1H, CHHGAz), 3.85 – 3.80 (m, 2H, CHH-Az, CHα-Arg), 3.71 (dd, J = 6.1, 4.3 Hz, 1H, CHα-Asp), 3.53 (dd, J = 14.0, 9.5 Hz, 1H, CHHβ-Trp), 3.45 (dd, J = 14.4, 5.8 Hz, 1H, CHHβ-Trp), 3.24 – 3.16 (m, 2H, CH₂δ-Arg), 3.13 – 3.02 (m, 2H, CH₂δ-Arg), 2.84 (dd, J = 17.1, 6.8 Hz, 1H, CHHβ-Asp), 2.78 (dd, J = 16.7, 3.8 Hz, 1H, CHHβ-Asp), 2.03 – 1.98 (m, 2H, CHHβ-Arg), 1.85 – 1.80 (m, 1H, CHHβ-Arg), 1.68 – 1.56 (m, 5H, 2 × CHHβ-Arg, CHHβ-Leu, CHγ-Leu, CHHγ-Arg), 1.48 – 1.34 (m, 4H, CHHβ-Leu, CHHγ-Arg, CHγ-Arg), 0.81 (d, J = 6.5 Hz, 3H, CH3δ-Leu), 0.77 (d, J = 6.5 Hz, 3H, CH3δ-Leu), Note: CHHGAz overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δc 177.2 (C=O), 176.1 (C=O), 174.8 (C=O), 174.6 (C=O), 174.2 (C=O), 173.7 (C=O), 158.7 (2 × C, Arg), 138.2 (C), 128.7 (C), 124.5 (CH), 122.6 (CH), 119.9 (CH), 119.5 (CH), 112.4 (CH), 111.7 (C), 60.4 (C, Az), 57.7 (CH, α-Arg), 55.9 (CH₂, Az), 55.2 (CH, α-Leu), 55.1 (CH, α-Asp), 54.9 (CH, α-Arg), 54.5 (CH₂, Az), 53.9 (CH, α-Arg), 44.7 (CH₂, GAz), 41.9 (CH₂, δ-Arg), 41.8 (CH₂, δ-Arg), 40.3 (CH₂, β-Asp), 40.1 (CH₂, β-Leu), 31.0 (CH₂, β-Arg), 30.0 (CH₂, β-Arg), 26.6 (CH₂, β-Trp), 26.2 (CH₂, γ-Arg), 26.1 (CH₂, γ-Arg), 25.6 (CH, γ-Leu), 23.1 (CH₃, δ-Leu), 22.0 (CH₃, δ-Leu); νmax (neat) = 3291, 1643, 1525, 1179, 1128 cm⁻¹; MS (ESI⁺) m/z 811 [M+H]⁺; HRMS (ESI⁺) calcd. for C₃₇H₅₉N₁₄O₇ [M+H]⁺ 811.4678, found 811.4686; [α]D²¹ − 42.9 (c 0.04, MeOH).
Preparative HPLC trace

\[ R_f = 24.98 \text{ min, } 280 \text{ nm; Method: } 0 - 3 \text{ min } 5\% \text{ B; } 3 - 38 \text{ min, } 5 - 50\% \text{ B; } 38 - 44 \text{ min } 50 - 100\% \text{ B; } 44 - 50 \text{ min, } 100\% \text{ B; } 50 - 58 \text{ min } 100\% - 5\% \text{ B.} \]

Analytical HPLC, 280 nm: \( R_f = 20.9 \) min. Agilent PLRP-S column (100 Å, 8 μm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.

Cyclo(Ile-Glu-GAz-Met-Ala-Trp) (362)

Following general procedure 11, Cyclo(Ile-Glu(Bu)-GAz(Boc)-Met-Ala-Trp(Boc)) 338 (16.1 mg, 17 µmol, 1.0 equiv) was deprotected to give a crude residue. A portion of this residue was purified by preparative HPLC for analytical data to give the title compound 362 after freeze drying as a foamy white solid. \(^1\)H and \(^19\)F NMR using TFE as an internal standard confirmed 1 eq. of TFA salt; m.p. 168 – 170 °C; \(^1\)H NMR (700 MHz, MeOD-d₄) \( \delta \)H 7.60 (d, \( J = 8.3 \text{ Hz, } 1\text{H, ArH} \)), 7.22 (s, 1H, ArH), 7.14 (t, \( J = 7.9 \text{ Hz, } 1\text{H, ArH} \)), 7.06 (t, \( J = 7.7 \text{ Hz, } 1\text{H, ArH} \)), 4.28 – 4.24 (m, 3H, CHα-Trp, CHα-Ala, CHα-Met), 4.13 (dd, \( J = 19.8, 12.8 \text{ Hz, } 2\text{H, CH₂-Az} \)),
4.05 (d, J = 4.8 Hz, 1H, CHα-Ile), 3.98 (d, J = 15.7 Hz, 1H, CHHAz), 3.84 (d, J = 12.5 Hz, 1H, CHH-Az), 3.77 (d, J = 12.3 Hz, 1H, CHH-Az), 3.56 (dd, J = 9.3, 4.3 Hz, 1H, CHα-Glu), 3.52 (dd, J = 14.7, 8.0 Hz, 1H, CHHβ-Trp), 3.44 (dd, J = 14.7, 8.0 Hz, 1H, CHHβ-Trp), 3.42 (dd, J = 14.7, 8.6 Hz, 1H, CHβ-Glu), 3.20 (d, J = 15.6 Hz, 1H, CHHGAz), 2.69 – 2.64 (m, 2H, CHβ-Glu), 2.52 – 2.45 (m, 2H, CHγ-Glu), 2.42 – 2.32 (m, 1H, CHHβ-Met), 2.19 – 2.15 (m, 1H, CHHβ-Met), 2.12 (s, 3H, SCH3), 2.08 – 1.93 (m, 2H, CHβ-Ile, CHHγ-Met), 1.90 – 1.76 (1H, CHHγ-Met), 1.31 (d, J = 7.1 Hz, 3H, CHβ-Ala), 1.28 – 1.20 (m, 1H, CHHδ-Ile), 0.85 – 0.75 (m, 4H, CH3γ-Ile, CH3δ-Ile), 0.69 (d, J = 7.2 Hz, 3H, CH3ε-Ile); 13C NMR (176 MHz, MeOD-d4) δC 177.3 (C=O), 176.4 (C=O), 175.0 (C=O), 174.6 (C=O), 174.3 (C=O), 173.5 (C=O), 128.4 (C), 124.6 (CH), 122.9 (CH), 120.3 (CH), 119.2 (CH), 112.6 (CH), 110.7 (C), 61.0 (CH, α-Ile), 60.0 (C, Az), 57.5 (CH, α-Met), 56.5 (CH, α-Glu), 55.6 (CH, α-Trp), 55.4 (CH2, Az), 54.1 (CH2, Az), 50.2 (CH, α-Ala), 45.2 (CH2, GAz), 36.6 (CH, β-Ile), 33.6 (CH2, β-Met), 31.6 (CH2, β-Glu), 30.8 (CH2, γ-Glu), 27.1 (CH2, γ-Met), 26.9 (CH3, β-Trp), 25.8 (CH3δ-Ile), 18.3 (CH3, β-Ala), 16.1 (CH3, γ-Ile), 14.8 (SMe), 12.0 (CH3, ε-Ile); νmax (neat) = 3279, 1643, 1523, 1198, 1179 cm⁻¹; MS (ESI+) m/z 715 [M+H]+, 737 [M+Na]+; HRMS (ESI+) calcd. for C34H51N8O7S [M+H]+ 715.3596, found 715.3589; [α]D24 − 57.4 (c 0.07, MeOD-d4).

Preparative HPLC traces:

\[ R_f = 36.03 \text{ min, 280 nm; Method: 0 – 3 min 5\% B; 3 – 25 min, 5 – 50\% B; 25 – 40 min 100\% B; 40 – 50 min, 100\% B.} \]

Analytical HPLC, 280 nm: \[ R_f = 26.6 \text{ min. Agilent PLRP-S column (100 Å, 8 µm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3\% B; 3-33 min, 3-50\% B; 33-38 min, 50-100\% B, 38-40 min, 100%-3\% B; 40-45 min, 3\% B. Peak at 24.3 min relates to Met oxidation.} \]
Synthesis of alanine-azetidine (AAz) cyclic peptides

Preparation of tetrapeptide 378

\[
\text{NO}_2\text{-D-AAz(Boc)-Pro-OBn (371)}
\]

To a solution of H-Pro-OBn•HCl (97 mg, 0.40 mmol, 2.0 equiv) in CH$_2$Cl$_2$ (4 mL) was added triethylamine (56 µL, 0.40 mmol, 2.0 equiv) and stirred for 15 min at RT. The solvent was removed in vacuo and resuspended in CH$_2$Cl$_2$ (4 mL). To this solution was added trisubstituted nitroalkene \textbf{260} (46 mg, 0.20 mmol, 1.0 equiv) and left to stir at RT for 24 h. Complete consumption of the alkene was monitored by TLC. The solvent was removed in vacuo and the crude products analysed by $^1$H NMR to confirm a 57:43 mixture of diastereomers. The crude products were purified by repeat column chromatography (PET ether:EtOAc 85:15 → 4:1) to give the major diastereomer \textbf{371} (40 mg, 92 µmol, 46%) as a colourless oil; $R_f$ (PET ether:EtOAc 4:1) 0.40; $^1$H NMR (500 MHz, CDCl$_3$) δ$_H$ 7.39 – 7.31 (m, 5H, ArH), 5.10 (s, 2H, CH$_2$Ph), 4.90 (q, $J = 6.4$ Hz, 1H, CH$\alpha$-AAz), 4.30 (d, $J = 10.6$ Hz, 1H, CHH-Az), 4.01 (d, $J = 10.9$ Hz, 1H, CHH-Az), 3.95 (dd, $J = 7.7$, 3.0 Hz, 1H, CH$\alpha$-Pro), 3.85 (d, $J = 9.8$ Hz, 1H, CHH-Az), 3.72 (d, $J = 9.8$ Hz, 1H, CHH-Az), 3.32 – 3.23 (m, 1H, CHHδ-Pro), 2.68 – 2.62 (m, 1H, CHHδ-Pro), 2.08 – 1.91 (m, 3H, CH$_2$$\gamma$-Pro, CHHβ-Pro), 1.89 – 1.79 (m, 1H, CHHβ-Pro), 1.63 (d, $J = 6.4$ Hz, 3H, CH$_3$β-AAz), 1.43 (s, 9H, CH$_3$ Boc); $^{13}$C NMR (126 MHz, CDCl$_3$) δ$_C$ 174.2 (C=O), 155.9 (C=O, Boc), 135.7 (C), 128.7 (CH), 128.50 (CH), 128.48 (CH), 84.9 (CH, α-AAz), 80.4 (C, Boc), 66.9 (CH$_2$Ph), 61.1 (CH, α-Pro), 60.4 (C, Az), 48.3 (CH$_2$, δ-Pro), 30.5 (CH$_2$, γ-Pro), 28.5 (CH$_3$, Boc), 24.5 (CH$_3$, β-Pro), 14.6 (CH$_3$, β-AAz). Note: 2 × CH$_2$, Az not observed; $\nu_{\text{max}}$(neat) = 2975, 1698,
1552, 1152, 697 cm⁻¹; MS (ESI⁺) m/z 434 [M+H]⁺, 456 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₂H₃₁N₃NaO₆ [M+H]⁺ 456.2105, found 456.2091; [α]D²⁸ −24.4 (c 0.17, MeOH).

**Fmoc-Val-(D)AAz(Boc)-Pro-OBn (375)**

Following general procedure 4 from NO₂-(D)AAz(Boc)-Pro-OBn (371) (1.33 g, 3.07 mmol, 1.0 equiv), the crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/EtOAc 9:1 → 4:1) to give the title compound 375 as a white foam (0.42 g, 0.58 mmol, 19%); Rf (CH₂Cl₂/EtOAc 4:1) 0.29; m.p. 99 – 102 °C; ¹H NMR (500 MHz, CDCl₃) δ H 7.76 (d, J = 7.5 Hz, 2H, ArH), 7.65 – 7.57 (m, 2H, ArH), 7.42 – 7.27 (m, 9H, ArH), 7.17 (s, 1H, NH), 5.68 (d, J = 8.4 Hz, 1H, NH, Fmoc), 5.13 (s, 2H, CH₂Ph), 4.49 – 4.33 (m, 2H, CH₂, Fmoc), 4.28 – 4.20 (m, 1H, CH, Fmoc), 4.14 (dd, J = 8.2, 5.3 Hz, 1H, CHα-Val), 3.84 – 3.76 (m, 2H, CH₂-Az), 3.71 (d, J = 9.3 Hz, 1H, CHH-Az), 3.62 (d, J = 9.4 Hz, 1H, CHH-Az), 3.06 – 2.96 (m, 1H, CHHδ-Pro), 2.61 – 2.49 (m, 1H, CHHδ-Pro), 2.25 – 2.08 (m, 2H, CHβ-Val, CHHβ-Pro), 1.43 (s, 9H, Boc), 1.30 (d, J = 6.5 Hz, 3H, CHβ-Pro), 0.96 (d, J = 6.7 Hz, 3H, CHγ-Val), 0.86 (d, J = 6.8 Hz, 3H, CHγ-Val); ¹³C NMR (126 MHz, CDCl₃) δ C 176.3 (C=O), 171.8 (C=O), 156.5 (C=O, Boc), 155.9 (C=O, Fmoc), 144.1 (C), 141.4 (C), 135.6 (C), 128.8 (CH), 128.6 (CH), 128.5 (CH), 127.83 (CH), 127.82 (CH), 127.2 (CH), 125.3 (CH), 125.2 (CH), 120.12 (CH), 120.10 (CH), 80.2 (C, Boc), 67.3 (CH₂, Fmoc), 67.0 (CH₂Ph), 60.3 (CH, α-Pro and CH, α-Val), 60.1 (C, Aa), 50.0 (CH, α-AAa), 47.8 (CH₂, δ-Pro), 47.4 (CH, Fmoc), 31.5 (CH, β-Val), 31.1 (CH₂, β-Pro), 28.5 (CH₃, Boc), 24.4 (CH₂, γ-Pro), 19.3 (CH₃, γ-Val), 17.5 (CH₃, γ-Val), 14.6 (CH₃, β-AAa), Note: 2 × CH₂, Az not observed; νmax (neat) = 3304, 1699, 1672, 1152, 757 cm⁻¹; MS (ESI⁺) m/z 725 [M+H]⁺, 747 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₂H₅₂N₄NaO₇ [M+Na]⁺ 747.3728, found 747.3732; [α]D²⁶ +36.1 (c 0.22, MeOH).

**Cbz-Trp-Val-D-AAz(Boc)-Pro-OBn (472)**

This compound was prepared following general procedure 2 from 375 (389 mg, 0.54 mmol, 1.0 equiv) and Cbz-Trp-OH (182 mg, 0.54 mmol, 1.0 equiv). The crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 49:1) to give the title compound 472 (321 mg, 0.33 mmol, 73%) as a white foam; Rf (CH₂Cl₂/MeOH 49:1) 0.21; m.p. 107 – 110 °C; ¹H NMR (500 MHz, CDCl₃) δ H 8.91 (s, 1H, NH), 7.68 (d, J = 7.7 Hz,
1H, ArH), 7.39 – 7.28 (m, 1H, ArH), 7.15 (t, J = 7.4 Hz, 1H, ArH), 7.10 – 7.03 (m, 1H, ArH), 6.99 – 6.89 (m, 1H, ArH), 6.73 (m, 1H, NH), 6.59 – 6.23 (m, 1H, NH), 5.52 (s, 1H, NH Cbz), 5.25 – 4.93 (m, 4H, 2 × CH2Ph), 4.68 – 4.52 (m, 1H, CHα-Trp), 4.37 – 4.15 (m, 1H, CHα-Val), 4.14 – 3.91 (m, 1H, CHα-AAz), 3.87 – 3.60 (m, 4H, CHα, CHβ-Az), 3.54 – 2.96 (m, 4H, CHH-Az, CHβ-Trp, CHHδ-Pro), 2.79 – 2.43 (m, 1H, CHHδ-Pro), 2.20 – 1.90 (m, 3H, CHβ-Pro, CHβ-Val), 1.85 – 1.73 (m, 2H, CH2γ-Pro), 1.46 (s, 9H, CH3, Boc), 1.13 – 1.04 (m, 3H, CHβ-AAz), 0.83 – 0.70 (m, 6H, 2 × CH2γ-Val); 1H NMR (126 MHz, CDCl3) δH 7.28 (s, 1H, ArH), 7.39 (d, J = 6.6 Hz, 3H, CHβ-Val), 14.7 (CHβ-Pro), 2.26 – 2.17 (m, 1H, CHHβ-Pro), 2.12 – 2.03 (m, 1H, CHβ-Val), 2.03 – 1.93 (m, 1H, CHHγ-Pro), 1.93 – 1.79 (m, 1H, CHHδ-Pro), 1.45 (s, 9H, CH3, Boc), 1.38 (d, J = 6.6 Hz, 3H, CHβ-AAz), 1.00 (d, J = 6.2 Hz, 3H, CH2γ-Val), 0.99 (d, J = 6.1 Hz, 3H, CH2γ-Val); 13C NMR (126 MHz, MeOD-d4) δC 175.8 (C=O), 174.3 (C=O), 170.2 (C=O), 157.3 (C=O, Boc), 138.4 (C), 128.3 (C), 125.8 (CH), 123.0 (CH), 120.4 (CH), 119.2 (CH), 112.6 (CH) 108.1 (C), 82.2 (C, Boc), 65.8 (CH, α-Pro), 65.6 (C, Az), 61.4 (CH, α-Val), 54.7 (CH, α-Trp), 52.4 (CH2, δ-Pro), 48.3 (CH, α-AAz), 31.6 (CH2, β-Pro), 31.5 (CH, β-Val), 28.5 (CH3, Boc), 28.5

H-Trp-Val-D-AAz(Boc)-Pro-OH (376)

This compound was prepared following general procedure 5 from 472 (282 mg, 0.34 mmol, 1.0 equiv) to give the title compound 376 as a white solid (197 mg, 0.33 mmol, quant. yield), which required no further purification; m.p. 190 – 193 °C; 1H NMR (500 MHz, MeOD-d4) δH 7.73 (d, J = 7.9 Hz, 1H, ArH), 7.39 (d, J = 8.1 Hz, 1H, ArH), 7.28 (s, 1H, ArH), 7.15 (t, J = 7.5 Hz, 1H, ArH), 7.08 (t, J = 7.4 Hz, 1H, ArH), 4.50 – 4.40 (m, 1H, CHα-AAz), 4.25 (dd, J = 8.8, 5.5 Hz, 1H, CHα-Trp), 4.18 (m, 1H, CHα-Pro), 4.11 – 4.00 (m, 4H, CHα-Val, CH2-Az, CHH-Az), 3.81 (d, J = 9.4 Hz, 2H, CHH-Az, CHHδ-Pro), 3.47 (dd, J = 15.1, 5.4 Hz, 1H, CHHβ-Trp), 3.22 (dd, J = 15.1, 8.8 Hz, 1H, CHHβ-Trp), 3.08 – 2.95 (m, 1H, CHHδ-Pro), 2.42 – 2.30 (m, 1H, CHHβ-Pro), 2.26 – 2.17 (m, 1H, CHHβ-Pro), 2.12 – 2.03 (m, 1H, CHβ-Val), 2.03 – 1.93 (m, 1H, CHHγ-Pro), 1.93 – 1.79 (m, 1H, CHHδ-Pro), 1.45 (s, 9H, CH3, Boc), 1.38 (d, J = 6.6 Hz, 3H, CHβ-AAz), 1.00 (d, J = 6.2 Hz, 3H, CH2γ-Val), 0.99 (d, J = 6.1 Hz, 3H, CH2γ-Val); 13C NMR (126 MHz, MeOD-d4) δC 175.8 (C=O), 174.3 (C=O), 170.2 (C=O), 157.3 (C=O, Boc), 138.4 (C), 128.3 (C), 125.8 (CH), 123.0 (CH), 120.4 (CH), 119.2 (CH), 112.6 (CH) 108.1 (C), 82.2 (C, Boc), 65.8 (CH, α-Pro), 65.6 (C, Az), 61.4 (CH, α-Val), 54.7 (CH, α-Trp), 52.4 (CH2, δ-Pro), 48.3 (CH, α-AAz), 31.6 (CH2, β-Pro), 31.5 (CH, β-Val), 28.5 (CH3, Boc), 28.5
This compound was prepared following general procedure 6 from 376 (60 mg, 0.10 mmol, 1.0 equiv) in DMF (20 mL, 0.005 M) for 48 h to give a crude residue which was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 40:1→19:1) to give the cyclic tetrapeptide as a glassy colourless solid (41.2 mg, 71 μmol, 71%); Rf (CH₂Cl₂/MeOH 19:1) 0.28; m.p. 182 – 184 °C; ¹H NMR (500 MHz, MeOD-d₄) δ H 7.65 (d, J = 7.9 Hz, 1H, ArH), 7.31 (d, J = 8.1 Hz, 1H, ArH), 7.11 (s, J = 13.7, 5.9 Hz, 1H, ArH), 7.09 (t, J = 7.6 Hz, 1H, ArH), 7.02 (t, J = 7.4 Hz, 1H, ArH), 4.65 (dd, J = 9.7, 6.7 Hz, 1H, CHα-Trp), 4.43 (q, J = 6.8 Hz, 1H, CHα-AAz), 3.98 – 3.84 (m, 3H, CHα-Val, CH₂-Az), 3.83 – 3.67 (m, 2H, CHH-Az, CHα-Pro), 3.59 – 3.49 (m, 2H, CHHβ-Trp), 2.52 – 2.43 (m, 1H, CHHδ-Pro), 2.26 – 2.10 (m, 2H, CHHβ-Pro, CHβ-Val), 1.97 – 1.81 (m, 3H, CHHβ-Pro, CH₂γ-Pro), 1.43 (s, 9H, CH₃ Boc), 1.29 (d, J = 6.9 Hz, 3H, CH₃β-AAz), 0.92 (d, J = 6.7 Hz, 3H, CH₃γ-Val), 0.90 (d, J = 6.7 Hz, 3H, CH₃γ-Val); ¹³C NMR (126 MHz, MeOD-d₄) δ C 178.9 (C=O), 174.9 (C=O), 173.4 (C=O), 138.0 (C), 128.6 (C), 124.0 (CH), 122.5 (CH), 119.9 (CH), 119.3 (CH), 112.3 (CH), 110.5 (C), 81.6 (C, Boc), 64.0 (C, Az), 63.8 (CH, α-Pro), 63.1 (CH, α-Val), 59.2 (CH, α-Trp), 48.2 (CH₃, α-AAz), 32.0 (CH₂, β-Pro), 28.7 (CH, β-Val), 28.6 (CH₃, Boc), 27.7 (CH₂, β-Trp), 25.7 (CH₂, γ-Pro), 19.8 (CH₃, γ-Val), 19.2 (CH₂, γ-Val), 16.3 (CH₃, β-AAz).

Note: CH₂, δ-Pro overlaps with solvent signal, C=O Boc and 2 × CH₂, Az not observed; νmax (neat) = 3284, 2961, 1649, 1520, 739; MS (ESI⁺) m/z 603 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃₁H₄₄N₆O₅Na[M+Na]⁺ 603.3265, found 603.3271; [α]D²⁶ −80.2 (c 0.22, MeOH)

Preparation of cyclic tetrapeptides 381–A and 381–B

Fmoc-Leu-(±)AAz(Boc)-Gly-OBn (379)

Preparation of cyclic tetrapeptides 381–A and 381–B
resuspended in CH\textsubscript{2}Cl\textsubscript{2} (50 mL). To this solution was added trisubstituted nitroalkene 260 (1.14 g, 5.00 mmol, 1.0 equiv) and stirred at RT for 48 h. The solvent was removed \textit{in vacuo} and resuspended in THF (50 mL). To this solution was added Fmoc-Leu-OSu (3.37 g, 7.50 mmol, 1.5 equiv), NaHCO\textsubscript{3} (1.68 g, 20.00 mmol, 4.0 equiv) and Raney Ni (slurry in H\textsubscript{2}O, 10 mL). The solution was placed under an atmosphere of nitrogen, evacuated and filled with hydrogen (balloon). The reaction mixture was stirred vigorously for 4.0 h at room temperature. Then, the mixture was filtered through a plug of Celite eluting with EtOAc, concentrated under reduced pressure, the filtrate was suspended in EtOAc (100 mL), washed with saturated Na\textsubscript{2}CO\textsubscript{3} (3 × 100 mL), dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated \textit{in vacuo}. The crude products were purified by repeat column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}:EtOAc 4:1 then CH\textsubscript{2}Cl\textsubscript{2}:MeOH 98:2) to yield the title compound 379 as a white foam (1.46 g, 2.09 mmol, 42% yield) as a 1:1 mixture of diastereomers determined by \textsuperscript{1}H NMR. R\textsubscript{f} (CH\textsubscript{2}Cl\textsubscript{2}:MeOH 98:2) 0.24; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ\textsubscript{H} 7.75 (d, J = 7.5 Hz, 4H, ArH), 7.59 (d, J = 7.4 Hz, 4H, ArH), 7.43 – 7.27 (m, 18H, ArH), 6.59 (d, J = 8.4 Hz, 1H, NH), 6.54 (d, J = 8.0 Hz, 1H, NH), 5.22 (d, J = 7.1 Hz, 2H, NH, Fmoc), 5.15 (s, 2H, CH\textsubscript{2}Ph), 5.14 (s, 2H, CH\textsubscript{2}Ph), 4.48 – 4.35 (m, 4H, 2 × CH\textsubscript{2}, Fmoc), 4.31 – 4.10 (m, 6H, 2 × CH, Fmoc, 2 × CH\textalpha-Leu, 2 × CH\textalpha-AAz), 3.76 – 3.62 (m, 8H, 4 × CH\textsubscript{2}, Az), 3.60 – 3.51 (m, 4H, 2 × CH\textsubscript{2}Gly), 1.68 – 1.59 (m, 4H, 2 × CHH\textbeta-Leu, 2 × CH\textgamma-Leu), 1.56 – 1.48 (m, 2H, 2 × CHH\textbeta-Leu), 1.42 (s, 9H, CH\textsubscript{3}, Boc), 1.40 (s, 9H, CH\textsubscript{3}, Boc), 1.13 (d, J = 6.4 Hz, 6H, 2 × CH\textbeta-AAz), 0.96 – 0.91 (m, 12H, 4 × CH\textdelta-Leu); \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) δ\textsubscript{C} 172.76 (C=O), 172.24 (C=O), 172.20 (C=O), 156.3 (C=O, Fmoc), 155.1 (C=O, Fmoc), 144.0 (C), 143.8 (C), 141.5 (C), 135.3 (C), 128.82 (CH), 128.81 (CH), 128.74 (CH), 128.72 (CH), 128.58 (CH), 128.55 (CH), 127.9 (CH), 127.2 (CH), 125.2 (CH), 120.15 (CH), 120.12 (CH), 80.09 (C, Boc), 80.07 (C, Boc), 67.3 (CH\textsubscript{2}, Bn/Fmoc), 67.13 (CH\textsubscript{2}, Bn/Fmoc), 67.05 (CH\textsubscript{2}, Bn/Fmoc), 67.0 (CH\textsubscript{2}, Bn/Fmoc), 57.6 (4 × CH\textsubscript{2}, Az), 53.9 (CH, α-Leu), 53.8 (CH, α-Leu), 50.2 (CH, α-AAz), 47.32 (CH, Fmoc), 47.29 (CH, Fmoc), 44.9 (CH\textsubscript{2}, Gly), 44.8 (CH\textsubscript{2}, Gly), 42.0 (CH\textsubscript{2}, β-Leu), 41.9 (CH\textsubscript{2}, β-Leu), 28.5 (CH\textsubscript{3}, Boc), 24.91 (CH, γ-Leu), 24.88 (CH, γ-Leu), 23.1 (CH\textsubscript{3}, δ-Leu), 23.0 (CH\textsubscript{3}, δ-Leu), 22.2 (CH\textsubscript{3}, δ-Leu), 22.1 (CH\textsubscript{3}, δ-Leu), 14.54 (CH\textsubscript{3}, β-AAz), 14.47 (CH\textsubscript{3}, β-AAz), Note: C=O Boc and 3 × CH not observed or overlapping with other peaks; ν\textsubscript{max} (neat) = 3307, 2956, 1694, 1651, 858 cm\textsuperscript{-1}; MS (ESI\textsuperscript{*}) m/z 699 [M+H]\textsuperscript{+}, 721 [M+Na]\textsuperscript{+}; HRMS (ESI\textsuperscript{*}) calcd. for C\textsubscript{40}H\textsubscript{50}N\textsubscript{4}NaO\textsubscript{7} [M+Na]\textsuperscript{+} 721.3572, found 721.3566;
This compound was prepared following general procedure 2 from 379 (1.35 g, 1.93 mmol, 1.0 equiv) and Cbz-Trp-OH (653 mg, 1.93 mmol, 1.0 equiv). The crude residue was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 98.5:2.5) to give the title compound 473 (1:1 mixture of diastereomers determined by ¹H NMR) (1.51 g, 1.44 mmol, 75%) as a white foam; Rf (CH₂Cl₂/MeOH 98.5:2.5) 0.19; ¹H NMR (500 MHz, CDCl₃) δ 9.57 (s, 1H, NH), 8.54 (s, 1H, NH), 7.63 (d, J = 7.5 Hz, 2H, ArH), 7.36 – 7.29 (m, 22H, ArH), 7.20 – 7.13 (m, 2H, ArH), 7.11 – 7.05 (m, 2H, ArH), 7.03 (s, 1H, ArH), 6.97 (s, 1H, ArH), 6.70 (d, J = 8.4 Hz, 1H, NH), 6.56 (d, J = 8.2 Hz, 1H, NH), 6.42 – 6.18 (m, 2H, 2 × NH), 5.62 (d, J = 6.6 Hz, 1H, NH Cbz), 5.55 (d, J = 5.4 Hz, 1H, NH Cbz), 5.19 – 5.03 (m, 8H, 4 × CH₂Ph), 4.62 – 4.49 (m, 2H, 2 × CHα-Trp), 4.35 – 4.15 (m, 4H, 2 × CHα-AAz, 2 × CHα-Leu), 3.74 – 3.45 (m, 12H, 4 × CH₂Az, 2 × CH₂Gly), 3.26 – 3.19 (m, 4H, 2 × CHβ-Trp), 1.52 – 1.39 (m, 24H, 2 × CHγ-Leu, 2 × CH₃, Boc), 1.08 (d, J = 6.8 Hz, 3H, CHβ-AAz), 1.05 (d, J = 6.7 Hz, 3H, CHβ-AAz), 0.85 – 0.80 (m, 12H, 4 × CH₃δ-Leu); ¹³C NMR (126 MHz, CDCl₃) δ 172.8 (C=O), 172.6 (C=O), 171.5 (C=O), 156.5 (2 × C=O, Boc), 156.4 (C=O, Cbz), 156.2 (C=O, Cbz), 136.6 (C), 136.4 (C), 136.3 (C), 136.1 (C), 135.4 (C), 135.3 (C), 128.77 (CH), 128.75 (CH), 128.7 (CH), 128.67 (CH), 128.63 (CH), 128.62 (CH), 128.53 (CH), 128.51 (CH), 128.4 (CH), 128.3 (CH), 128.22 (CH), 128.2 (CH), 127.4 (C), 126.3 (CH), 122.4 (CH), 122.0 (CH), 119.6 (CH), 118.8 (CH), 111.6 (CH), 111.5 (CH), 110.1 (C), 80.4 (C, Boc), 80.0 (C, Boc), 67.3 (2 × CH₂Ph), 67.2 (2 × CH₂Ph), 57.8 (2 × CH₂, Az), 57.7 (2 × CH₂, Az), 55.61 (CH, α-Trp), 55.58 (CH, α-Trp), 52.5 (CH, α-Leu), 52.2 (CH, α-Leu), 49.6 (CH, α-AAz), 49.3 (CH, α-AAz), 44.8 (2 × CH₂, Gly), 40.8 (2 × CH₂, β-Leu), 28.49 (CH₃, Boc), 28.48 (CH₃, Boc), 28.0 (2 × CH₂, β-Trp), 24.83 (CH, γ-Leu), 24.77 (CH, γ-Leu), 22.9 (CH₃, δ-Leu), 22.8 (CH₃, δ-Leu), 22.2 (CH₃, δ-Leu), 22.1 (CH₃, δ-Leu), 14.5 (CH₃, β-AAz), 14.3 (CH₃, β-AAz), Note: 2 × C=O, 2 × C, 2 CH not observed or overlapping with other peaks; νmax (neat) = 3302, 2956, 1644, 1536, 1143 cm⁻¹; MS (ESI⁺) m/z 797 [M+H]⁺, 819 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₄H₅₆N₆O₈ [M+Na]⁺ 819.4052, found 819.4037.
This compound was prepared following general procedure 5 from 473 (1.00 g, 1.26 mmol, 1.0 equiv) to give the title compound 380 (1:1 mixture of diastereomers determined by $^1$H NMR) as a white solid (714 mg, 1.24 mmol, quant. yield), which required no further purification: $^1$H NMR (500 MHz, MeOD-d$_4$) $\delta$H 7.77 (d, $J = 7.9$ Hz, 1H, ArH), 7.71 (d, $J = 7.9$ Hz, 1H, ArH), 7.38 (d, $J = 3.4$ Hz, 1H, ArH), 7.31 (s, 1H, ArH), 7.18 – 7.12 (m, 2H, ArH), 7.11 – 7.05 (m, 2H, ArH), 4.48 – 4.41 (m, 2H, CH$_\alpha$-Leu, CH$_\alpha$-AAz), 4.34 – 4.26 (m, 2H, CH$_\alpha$-Leu, CH$_\alpha$-AAz), 3.56 – 3.47 (m, 4H, 2 × CH$_\alpha$-Trp), 3.24 – 3.15 (m, 2H, 2 × CH$_\alpha$-Trp), 1.70 – 1.56 (m, 6H, 2 × CH$_\beta$-Leu, 2 × CH$_\gamma$-Leu), 1.46 – 1.43 (m, 18H, 2 × CH$_3$-Boc), 1.31 (d, $J = 6.8$ Hz, 3H, CH$_3$-AAz), 1.22 (d, $J = 6.8$ Hz, 3H, CH$_3$-AAz), 1.02 – 0.93 (m, 12H, 4 × CH$_3$-Leu), $^1$C NMR (126 MHz, MeOD-d$_4$) $\delta$C 174.6 (C=O), 174.5 (C=O), 174.1 (C=O), 172.9 (C=O), 170.8 (C=O), 170.1 (C=O), 170.0 (C=O), 157.9 (2 × C=O Boc), 138.4 (C), 128.4 (C), 128.3 (C), 126.2 (CH), 125.8 (CH), 122.9 (2 × CH), 120.3 (2 × CH), 119.24 (CH), 119.20 (CH), 112.64 (CH), 112.60 (CH), 107.9 (C), 107.8 (C), 81.8 (C, Boc), 81.5 (C, Boc), 60.2 (C, Az), 59.7 (C, Az), 54.7 (CH, α-Trp), 54.6 (CH, α-Trp), 54.5 (CH, α-AAz), 53.7 (CH, α-Leu), 45.7 (CH$_2$, Gly), 45.2 (CH$_2$, Gly), 42.2 (CH$_2$, β-Leu), 41.3 (CH$_2$, β-Leu), 28.8 (2 × CH$_3$-β-Trp), 28.64 (CH$_3$, Boc), 28.57 (CH$_3$, Boc), 25.9 (2 × CH, γ-Leu) 23.4 (CH$_3$, δ-Leu), 23.3 (CH$_3$, δ-Leu), 22.12 (CH$_3$, δ-Leu), 22.08 (CH$_3$, δ-Leu), 15.0 (CH$_3$, β-AAz), 14.7 (CH$_3$, β-AAz), Note: 1 × CH, α-Leu, 1 × CH, α-AAz and 4 × CH$_2$, Az not observed; $\nu_{\text{max}}$ (neat) = 3029, 1664, 1562, 1367, 1162 cm$^{-1}$; MS (ESI$^+$) m/z 573 [M+H]$^+$, 595 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{29}$H$_{45}$N$_6$O$_6$ [M+H]$^+$ 573.3395, found 573.3393.

**Cyclisation of 380**

Following general procedure 6, H-Trp-Leu-(±)AAz(Boc)-Gly-OH (380) (114 mg, 0.20 mmol, 1.0 equiv) in DMF (200 mL, 0.001 M) was stirred for 48 h. The residue was purified by column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 49:1→19:1) to give two cyclic pentapeptides as described below.
Cyclo(Trp-Leu-D-AAz(Boc)-Gly) (381-A)

This cyclic peptide was isolated by column chromatography (33 mg, 59 μmol, 30%) as a pale yellow glassy solid; Rf (CH2Cl2/MeOH 9:1) 0.44; m.p. 200 – 203 °C; Note: This compound appears as an 83:17 mixture of conformational isomers at RT; 1H NMR (500 MHz, MeOD-d4) δH 7.58 (d, J = 7.9 Hz, 0.8H, ArH major), 7.52 (d, J = 7.9 Hz, 0.2H, ArH minor), 7.35 – 7.30 (m, 1H, ArH), 7.15 – 7.06 (m, 2H, ArH), 7.04 – 7.01 (m, 1H, ArH), 4.60 (t, J = 7.9 Hz, 1H, CHα-Trp), 4.20 – 3.72 (m, 4H, CHα-AAz, CH2-Az, CHH-Az), 3.59 – 3.34 (m, 4H, CHH-Az, CHα-Leu, CH2Gly), 3.13 (dd, J = 14.3, 7.5 Hz, 1H, CHHβ-Trp), 1.74 – 1.58 (m, 2H, CHβ-Leu), 1.45 (s, 7.5H, CH3 Boc major), 1.42 (s, 1.5H, CH3 Boc minor), 1.40 – 1.33 (m, 1H, CHγ-Leu), 1.20 (d, J = 6.7 Hz, 0.5H, CH3β-AAz minor), 1.16 (d, J = 6.8 Hz, 2.5H, CH3β-AAz major), 0.92 (d, J = 6.4 Hz, 0.5H, CH3δ-Leu minor), 0.87 (d, J = 6.6 Hz, 2.5H, CH3δ-Leu major), 0.83 (d, J = 6.5 Hz, 0.5H, CH3δ-Leu minor), 0.79 (d, J = 6.3 Hz, 2.5H, CH3δ-Leu major). Note: CHHβ-Trp overlaps with solvent signal; 13C NMR (126 MHz, MeOD-d4) δC 177.9 (C=O), 174.2 (C=O), 173.7 (C=O), 158.5 (C=O Boc), 138.1 (C), 124.4 (CH), 122.6 (CH), 119.9 (CH), 119.1 (CH), 112.3 (CH), 110.2 (C), 81.3 (C, Boc), 59.1 (C, Az), 58.7 (CH, α-Trp), 53.7 (CH, α-Leu), 52.5 (CH, α-AAz), 48.2 (CH2, Gly), 39.3 (CH2, β-Leu), 28.6 (CH3, Boc), 26.76 (CH3, β-Trp), 25.8 (CH, γ-Leu), 23.9 (CH3, δ-Leu), 21.5 (CH3, δ-Leu), 14.3 (CH3, β-AAz), Note: CH2, Az not observed; νmax (neat) = 3315, 2955, 1651, 1520, 1147 cm⁻¹; MS (ESI⁺) m/z 577 [M+Na⁺]; HRMS (ESI⁺) calcd. for C29H42NaNO5 [M+Na⁺] 577.3109, found 577.3108; [α]D30 −18.1 (c 0.04, MeOH).

Cyclo(Trp-Leu-L-AAz(Boc)-Gly) (381-B)

This cyclic peptide was isolated by column chromatography (33 mg, 60 μmol, 30%) as a pale yellow glassy solid; Rf (CH2Cl2/MeOH 9:1) 0.19; m.p. 212 – 215 °C; 1H NMR (500 MHz, MeOD-d4) δH 7.62 (d, J = 7.9 Hz, 1H, ArH), 7.35 (d, J = 8.1 Hz, 1H, ArH), 7.13 (s, 1H, ArH), 7.10 (t, J = 7.5 Hz, 1H, ArH), 7.03 (t, J = 7.4 Hz, 1H, ArH), 4.97 (dd, J = 10.2, 7.2 Hz, 1H, CHα-Trp), 4.27 – 4.20 (m, 2H, CHα-Leu, CHα-AAz), 3.78 (d, J = 9.1 Hz, 1H, CHH-Az), 3.69 (d, J = 9.0 Hz, 1H, CHH-Az), 3.63 (d, J = 8.8 Hz, 1H, CHH-Az), 3.52 (d, J = 16.7 Hz, 1H, CHHHGly), 3.44 – 3.34 (m, 2H, CHH-Az, CHHHGly), 3.21 (dd, J = 15.1, 7.1 Hz, 1H, CHHβ-Trp), 1.68 – 1.60 (m, 1H, CHHβ-Leu), 1.58 – 1.45 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.42 (s, 9H, CH3, Boc), 1.14 (d, J = 6.9 Hz, 3H, CH3β-AAz), 0.89 (d, J = 6.3 Hz, 3H, CH3δ-Leu), 0.72 (d, J = 6.3 Hz, 3H, CH3δ-Leu), Note: CHHβ-Trp overlaps with solvent signal; 13C NMR (126 MHz, MeOD-d4) δC 176.3 (C=O),
175.2 (C=O), 173.6 (C=O), 138.0 (C), 128.5 (CH), 122.6 (CH), 119.9 (CH), 119.0 (CH), 112.4 (CH), 110.3 (C), 60.0 (C, Az), 57.6 (CH, α-Leu), 55.2 (CH, α-Leu), 52.0 (CH, α-AAz), 48.2 (CH₂, Gly), 40.6 (CH₃, β-Leu), 28.6 (CH₃, Boc), 27.6 (CH₃, β-Trp), 26.2 (CH, γ-Leu), 23.3 (CH₃, δ-Leu), 21.2 (CH₃, δ-Leu), 13.6 (CH₃, β-AAz), Note: C=O, Boc and 2 × CH₂, Az not observed; \( \nu_{\text{max}} \) (neat) = 3288, 1685, 1671, 1648, 1502, 739 cm\(^{-1}\); MS (ESI\(^+\)) \( m/z \) 577 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{29}\)H\(_{42}\)N\(_6\)NaO\(_5\) [M+Na]\(^+\) 577.3109, found 577.3108; [\( \alpha \)]\(_D\) \( ^{30} \) = 102.9 (c 0.05, MeOH).

**Preparation of pentapeptide mixture 387**

Following the general procedure 7 for building block synthesis, Fmoc-Leu-OCumyl 382 (3.77 g, 8.00 mmol, 2.0 equiv) and tert-butyl-3-(1-nitroethyldene)azetidine-1-carboxylate (258) (0.91 g, 4.00 mmol, 1.0 equiv) were combined to give, after column chromatography (SiO\(_2\), CH\(_2\)Cl\(_2\)/EtOAc 85:15), the title compound 383 (1.02 g, 1.52 mmol, 38%) as a mixture of diastereomers as a white foam. Analysis of the product by \(^1\)H NMR showed a 2:1 mixture of diastereomers (see appendix); \( R_f \) (CH\(_2\)Cl\(_2\)/EtOAc 90:10) 0.28; \(^1\)H NMR (500 MHz, DMSO) \( \delta \) 7.89 (d, \( J = 7.5 \) Hz, 4H, ArH), 7.73 – 7.62 (m, 5H, ArH, NH), 7.51 – 7.28 (m, 21H, ArH, 3 × NH), 4.39 – 4.21 (m, 6H, 2 × CH₂ Fmoc, 2 × CH Fmoc), 3.93 – 3.45 (m, 10H, 4 × CH₂ Az, 2 × CHα-AAz), 3.28 – 3.20 (m, 2H, CHα-Leu), 1.75 – 1.66 (m, 14H, 4 × CH₃ Cumyl, 2 × CH₇-Leu), 1.46 – 1.32 (m, 22H, 6 × CH₃ Boc, 2 × CH₂β-Leu), 1.04 – 0.96 (m, 6H, 2 × CH₂β-AAz), 0.93 – 0.86 (m, 12H, 4 × CH₃δ-Leu); \(^1\)C NMR (126 MHz, DMSO) \( \delta \) 174.8 (C=O), 156.6 (C=O Boc), 156.3 (C=O Boc), 156.2 (C=O Fmoc), 155.7 (C=O Fmoc), 145.4 (2 × C), 144.0 (C), 143.9 (C), 143.82 (C), 143.76 (C), 143.75 (C), 140.75 (C), 140.73 (C), 140.71 (C), 128.09 (CH), 128.07 (CH), 127.62 (CH), 127.59 (CH), 127.05 (CH), 127.02 (CH), 126.9 (CH), 125.18 (CH), 125.15 (CH), 125.07 (CH), 124.3 (CH), 124.2 (CH), 120.14 (CH), 120.12 (CH), 81.6 (C, Cumyl), 81.5 (C, Cumyl), 78.5 (C, Boc), 78.4 (C, Boc), 65.44 (CH₂, Fmoc), 65.35 (CH₂, Fmoc), 58.5 (CH₂, Az), 58.2 (CH₂, Az), 54.3 (CH, α-Leu), 54.1 (CH, α-Leu), 50.7 (CH, α-AAz), 50.0 (CH, α-AAz), 46.76 (CH, Fmoc), 46.73 (CH, Fmoc), 43.4 (CH₂, β-Leu), 43.3 (CH₂, β-Leu), 28.2 (CH₃, Cumyl), 28.1 (2 × CH₃, Boc), 27.8 (CH₃, Cumyl), 24.17 (CH, γ-Leu), 24.15 (CH, γ-Leu), 22.8 (CH₂, δ-Leu), 22.6 (CH₃, δ-Leu), 22.3 (CH₃, δ-Leu), 22.0 (CH₂, δ-Leu), 14.7 (CH₃, β-AAz), 14.6 (CH₃, β-AAz), Note: 2 × C, Az and 2 × CH₂, Az not observed; \( \nu_{\text{max}} \) (neat) = 3327, 3066, 1698, 1327, 1075 cm\(^{-1}\); MS (ESI\(^+\)) \( m/z \) 670 [M+H]\(^+\), 692 [M+Na]\(^+\); HRMS (ESI\(^+\)) calcd. for C\(_{36}\)H\(_{52}\)N\(_6\)O\(_6\) 670.3824 [M+H]\(^+\), found 670.3829.
Cyclo-(Trp(Boc)-Val-AAz(Boc)-Leu-Gly) (386)

Following general procedure 9 from H-Gly-2ClTrt (362 mg, 0.25 mmol, 0.69 mmol/g, 1.0 equiv) and Fmoc-AAz(Boc)-Leu-OCumyl (383) (669 mg, 1.00 mmol, 4.0 equiv), linear precursor was synthesised (141 mg, 182 µmol, 73%). Analytical HPLC analysis (see below) identified this material as a 61:39 mixture of two diastereomers. A portion of this material was subjected to cyclisation (116 mg, 150 µmol, 1.0 equiv) for 96 h. The d.r of the crude mixture was determined by analytical HPLC (see appendix) and the crude mixture was purified by column chromatography (SiO₂, CH₂Cl₂:MeOH 39:1 → 19:1) to yield 386 as an inseparable mixture of diastereomers as a glassy colourless solid (67.2 mg, 89 µmol, 60%) as an inseparable mixture of diastereomers. Analytical HPLC analysis (see below) and ¹H NMR analysis (see appendix) both identified the purified mixture as a 56:44 mixture of diastereomers; Rf (CH₂Cl₂/MeOH 49:1) 0.27; ¹H NMR (500 MHz, CD₂OD) δ 8.09 (t, J = 7.0 Hz, 1H, ArH), 7.69 – 7.63 (m, 1H, ArH), 7.57 (s, 0.6H, ArH major), 7.52 (s, 0.4H, ArH minor), 7.33 – 7.22 (m, 2H, ArH), 4.73 (dd, J = 10.4, 1.6 Hz, 0.4H, CHα-Trp minor), 4.63 (dd, J = 10.7, 1.9 Hz, 0.6H, CHα-Trp major), 4.52 (d, J = 5.0 Hz, 0.6H, CHα-Val major), 4.25 (d, J = 9.2 Hz, 0.4H, CHα-Val minor), 4.09 – 3.67 (m, 5.4H, CHα-AAz, CHα-Leu, CHHGly, 2 × CHH-Az, CHH-Az minor), 3.57 (d, J = 9.1 Hz, 1H, CHH-Az), 3.48 – 3.44 (m, 2H, CHHβ-Trp, CHHGly), 3.38 (d, J = 10.5 Hz, 1H, CHH-Az major), 3.04 (dd, J = 15.0, 11.1 Hz, 1H, CHHβ-Trp), 2.46 – 2.38 (m, 0.6H, CHβ-Val major), 2.08 – 2.03 (m, 0.6H, CHβ-Val minor), 1.84 – 1.78 (m, 1H, CHγ-Leu), 1.69 – 1.66 (m, 9H, CH₂ Boc), 1.45 – 1.37 (m, 11H, CH₃ Boc, CHβ-Leu), 1.23 (d, J = 7.0 Hz, 1.6H, CHβ-AAz major), 1.18 (d, J = 6.6 Hz, 1.4H, CHβ-AAz minor), 1.00 – 0.93 (m, 12H, 2 × CH3γ-Val, 2 × CH3δ-Leu); ¹³C NMR (126 MHz, CD₂OD) δ 180.9 (C=O minor), 180.0 (C=O major), 173.9 (C=O major), 173.8 (C=O minor), 173.4 (C=O), 173.1 (C=O), 173.0 (2 × C=O), 158.2 (C=O Boc minor), 157.9 (C=O Boc major), 151.0 (2 × C=O Boc), 136.9 (2 × C), 131.4 (C, minor), 131.3 (C, major), 125.7 (CH, major), 125.6 (CH, minor), 125.1 (CH, major), 125.0 (CH, minor), 123.80 (CH, major), 123.79 (CH, minor), 120.1 (CH, minor), 120.0 (CH, major), 117.8 (C, minor), 117.6 (C, major), 116.23 (CH, major), 116.15 (CH, minor), 85.0 (C, Boc, major), 84.9 (C, Boc, minor), 81.1 (2 × C, Boc), 62.7 (CH, α-Val minor), 60.0 (CH₂, Az), 59.3 (CH, α-Val major), 58.2 (CH₂, Az), 56.3 (CH, α-Trp major), 56.2 (CH, α-Leu minor), 56.0 (CH, α-Leu major), 55.3 (CH, α-Trp minor), 54.4 (CH, α-AAz minor), 51.5 (CH, α-AAz major), 46.1 (CH₂, β-Leu major), 45.2 (CH₂, Gly), 45.19 (CH₂, β-Leu minor), 32.6 (CH, β-Val minor), 31.0 (CH, β-Val major), 28.63 (CH₂, Boc major), 28.60 (CH₂, Boc minor), 28.4 (2 × CH₃, Boc), 28.1 (CH₂, β-Trp major), 27.8 (CH₂, β-Trp minor), 25.7 (CH, γ-Leu minor), 25.5 (CH, γ-Leu major), 23.7
(CH₃, γ-Val minor), 23.6 (CH₃, γ-Val major), 22.5 (CH₃, γ-Val major), 22.1 (CH₃, γ-Val minor), 20.0 (CH₃, δ-Leu major), 19.5 (CH₃, δ-Leu minor), 18.0 (2 × CH₃, δ-Leu minor), 15.3 (CH₃, β-AAz major), 13.6 (CH₃, β-AAz minor), Note: 1 × CH₃, Gly, C, Az and 2 × CH₂, Az not observed; νmax (neat) = 3299, 3058, 1642, 1367, 1154 cm⁻¹; MS (ESI⁺) m/z 547 [M+H]⁺, 776 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₃⁹H₅₉N₇NaO₈ [M+Na]⁺ 776.4304, found 776.4298.

Analytical HPLC for linear precursor:

Analytical HPLC, 280 nm: Rf = 32.7 min (major) and 34.00 (minor). Agilent PLRP-S column (100 Å, 8 μm, 150 × 4.6 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.

Analytical HPLC for purified cyclic peptides:

Analytical HPLC, 280 nm: Rf = 38.5 min (major) and 39.2 (minor). Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.
Cyclo-(Trp-Val-AAz-Leu-Gly) (387)

Following general procedure 12 from Cyclo(Trp(Boc)-Val-GAza(Boc)-Leu-Gly) (386) (18 mg, 24 μmol, 1.0 equiv), Cyclo(Trp-Val-GAza-Leu-Gly) (387) was isolated as a pale yellow glassy solid (18.5 mg) in quantitative yield as the TFA salt, which required no further purification. $^1$H NMR also confirmed 55:45 d.r. $^1$H NMR (500 MHz, MeOD) δ 7.63 (d, J = 7.9 Hz, 0.6H, ArH major), 7.61 (d, J = 7.9 Hz, 0.4H, ArH minor), 7.34 (t, J = 7.5 Hz, 1H, ArH), 7.23 (s, 0.6H, ArH major), 7.14 (s, 0.4H, ArH minor), 7.12 – 7.07 (m, 1H, ArH), 7.04 – 6.99 (m, 1H, ArH), 4.30 – 3.57 (m, 5H, CHH-Gly, CHα-AAz, CH$_2$-Az, CHH-Az), 3.53 – 3.43 (m, 3H, CHH-Gly, CHHβ-Trp, CHH-GAza), 3.13 (dd, J = 15.0, 10.8 Hz, 1H, CHHβ-Trp major), 3.07 (dd, J = 14.8, 10.5 Hz, 1H, CHHβ-Trp minor), 2.51 – 2.35 (m, 1H, CHβ-Val major), 2.03 (m, 0.4H, CHβ-Val minor), 1.86 (m, 1H, CHγ-Leu), 1.55 – 1.35 (m, 2H, CHβ-Leu), 1.26 (d, J = 7.4 Hz, 3H, CHβ-AAz), 1.03 – 0.83 (m, 12H, 2 × CH$_3$δ-Leu, 2 × CH$_7$γ-Val), Note: CHα-Val, CHα-Trp and CHα-Leu overlaps with solvent/water signal; $^{13}$C NMR (126 MHz, MeOD) δ 180.2 (C=O minor), 179.4 (C=O major), 174.9 (C=O minor), 174.51 (C=O minor), 174.47 (C=O major), 173.7 (C=O minor), 173.2 (C=O major), 173.0 (C=O minor), 138.24 (C, major), 138.20 (C, minor), 128.4 (C, minor), 128.3 (C, major), 124.5 (CH, major), 124.4 (CH, minor), 122.64 (CH, major), 122.58 (CH, minor), 120.0 (CH, major), 119.9 (CH, minor), 119.2 (CH, minor), 119.1 (CH, major), 112.41 (CH, major), 112.37 (CH, minor), 111.1 (C, minor), 110.9 (C, major), 63.4 (2 × C, Az), 61.3 (CH$_3$, Az major), 58.4 (2 × CH, α-Trp), 57.9 (2 × CH, α-Val), 56.8 (CH$_3$, Az minor), 55.9 (CH, α-Leu minor), 55.6 (CH, α-Leu major), 52.7 (CH, α-AAz major), 52.4 (CH, α-AAz minor), 52.4 (CH$_3$, Az major), 47.8 (CH$_3$, β-Leu major), 45.1 (CH$_3$, β-Leu minor), 44.9 (2 × CH$_2$, Gly), 30.9 (CH, β-Val minor), 30.6 (CH, β-Val major), 28.4 (CH$_2$, β-Trp major), 28.3 (CH$_2$, β-Trp minor), 25.7 (CH, γ-Leu minor), 25.2 (CH, γ-Leu major), 23.7 (CH$_3$, γ-Val major), 23.6 (CH$_3$, γ-Val minor), 22.5 (CH$_3$, γ-Val major), 22.0 (CH$_3$, γ-Val minor), 19.8 (CH$_3$, δ-Leu major), 19.5 (CH$_3$, δ-Leu minor), 17.8 (2 × CH$_3$, δ-Leu), 15.0 (CH$_3$, β-AAz major), Note: CH$_3$, β-AAz minor and 1 × CH$_2$, Az minor not observed; $ν_{max}$ (neat) = 3260, 3051, 1650, 1537, 1135 cm$^{-1}$; MS (ESI$^+$) m/z 554 [M+H]$^+$; HRMS (ESI$^+$) calcd. for C$_{29}$H$_{34}$N$_7$O$_4$: 554.3449 [M+H]$^+$, found 554.3442.
Functionalisation of the azetidine nitrogen

Cbz deprotection and functionalisation of 301

Cyclo(D-Pro-Leu-GAzm(4-MeOBenzyl)-Gly) (390)

Following general procedure 12 from Cyclo(D-Pro-Leu-GAzm(Cbz)Gly) 301 (34 mg, 70 µmol, 1.0 equiv) and 4-methoxybenzyl chloride (12 µL, 84 µmol, 1.2 equiv), the title compound 390 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) as a white glassy solid (20 mg, 40 µmol, 57%); Rₛ (CH₂Cl₂/MeOH 19:1) 0.39; m.p. 147 – 150 °C; NMR data reported as a 1:1 mixture of conformers; 1H NMR (500 MHz, MeOD-d₄) δ H 7.68 – 7.59 (m, 2H, ArH), 6.98 (d, J = 8.8 Hz, 2H, ArH), 4.50 – 4.32 (m, 2.5H, CHα-Leu, CHα-Pro, 0.5CH₂Az major), 4.23 – 4.10 (m, 2H, 0.5CH₂Az minor, 0.5CHH-Az major, 0.5CHH-Az, CHH-Az), 3.99 (dd, J = 18.3, 11.4 Hz, 1H, CHH-Az), 3.89 (d, J = 10.7 Hz, 0.5H, CHH-Az minor), 3.84 (s, 3H, OCH₃), 3.76 (d, J = 14.1 Hz, 0.5H, CHHG Az major), 3.69 (d, J = 14.0 Hz, 0.5H, CHHG Az minor), 3.65 – 3.51 (m, 2H, CHβ-Pro), 3.50 – 3.36 (m, 1.5H, CH₂Az, CHHGly major), 2.36 – 2.24 (m, 1H, CHHβ-Pro), 2.04 – 1.86 (m, 3H, CH₂γ-Pro, CHHβ-Pro), 1.70 – 1.54 (m, 3H, CHβ-Leu, CHγ-Leu), 0.97 (d, J = 6.1 Hz, 3H, CHβδ-Leu), 0.93 (d, J = 6.1 Hz, 3H, CHβδ-Leu), Note: CHHGly minor overlaps with solvent signal; 13C NMR (126 MHz, MeOD-d₄) δ C 176.3 (C=O, major), 176.2 (C=O, minor), 175.8 (C=O, major), 175.7 (C=O, minor), 173.2 (C=O, major), 172.9 (C=O, minor), 172.3 (C=O, major), 172.1 (C=O, minor), 163.7 (C), 131.0 (CH), 126.2 (C), 114.8 (CH), 62.4 (CH₂, Az, major), 62.2 (CH, α-Pro minor) 62.0 (CH, α-Pro major), 61.5 (CH₂, Az, minor), 58.4 (CH₂, Az, major), 57.8 (C, Az, major), 57.4 (CH₂-Az, minor), 57.2 (C, Az, minor), 55.9 (OCH₃), 54.4 (CH, α-Leu major) 53.8 (CH, α-Leu minor), 48.3 (CH₂, Gly, minor), 47.7 (CH₂, GAz, major), 47.6 (CH₂, GAz, minor), 38.5 (CH₂, β-Leu major) 38.2 (CH₂, β-Leu minor), 33.03 (CH₂, β-Pro major) 32.98 (CH₂, β-Pro major), 26.1 (CH, γ-Leu), 23.5 (CH₂, γ-Pro minor) 23.4 (CH₂, γ-Pro major), 23.0 (CH₂, δ-Leu), 22.6 (CH₂, δ-Leu), Note: CH₂, δ-Pro and CH₂, Gly, major overlap with solvent signal; ν max (neat) = 3274, 2954, 1603, 1428, 1251 cm⁻¹; MS (ESI⁺) m/z 508 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₅H₂₅N₅NaO₆ [M+Na]⁺ 508.2530, found 508.2533; [α]D²⁰ – 63.8 (c 0.11, MeOH)
Cyclo(D-Pro-Leu-GAz(C=OQuinoline)-Gly) (391)

Following general procedure 12 from Cyclo(D-Pro-Leu-GAz(Cbz)-Gly) 301 (34 mg, 71 µmol, 1.0 equiv) and quinaldoyl chloride (17 mg, 89 µmol, 1.3 equiv), the title compound 391 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) as a white glassy solid (22 mg, 42 µmol, 60%); Rₜ (CH₂Cl₂/MeOH 19:1) 0.30; m.p. 141 – 143 °C; ¹H NMR (400 MHz, MeOD-d₄) δH 8.41 (dd, J = 8.6, 2.0 Hz, 1H, ArH), 8.16 – 8.09 (m, 1H, ArH), 8.06 (dd, J = 8.5, 0.8 Hz, 1H, ArH), 7.96 (d, J = 8.2 Hz, 1H, ArH), 7.83 – 7.78 (m, 1H, ArH), 7.70 – 7.65 (m, 1H, ArH), 4.74 – 4.65 (m, 1.5H, CHH-Az, CHH-Az minor), 4.52 – 4.41 (m, 2H, CHα-Leu, CHα-Pro), 4.27 (d, J = 11.1 Hz, 0.5H, CHH-Az major), 4.15 – 4.06 (m, 1H, CHH-Az), 4.03 (d, J = 11.1 Hz, 0.5H, CHH-Az minor), 3.81 (dd, J = 14.0, 5.5 Hz, 1H, CHHGaz), 3.65 – 3.54 (m, 2H, CH₂β-Pro), 3.46 – 3.35 (m, 3H, CH₂Gly, CHHGaz), 2.37 – 2.27 (m, 1H, CHHβ-Pro), 2.05 – 1.86 (m, 3H, CHHβ-Pro, CH₂γ-Pro), 1.72 – 1.55 (m, 3H, CH₂β-Leu, CHγ-Leu), 0.98 (d, J = 6.2 Hz, 3H, CH₂δ-Leu), 0.94 (d, J = 6.1 Hz, 3H, CH₂δ-Leu), Note: CHH-Az major pairs with CHH-Az minor at 4.74 – 4.65 Hz and overlaps with solvent peak at 4.8 ppm; ¹³C NMR (126 MHz, DMSO-D₆) δC 174.1 (C=O major) 174.0 (C=O minor), 173.0 (C=O major) 172.7 (C=O minor), 170.2 (C=O major) 169.7 (C=O minor), 164.80 (C=O major) 164.75 (C=O minor), 151.6 (C, major) 151.5 (C, minor), 146.00 (C, major) 145.97 (C, minor), 137.34 (CH, major) 137.28 (CH, minor), 130.30 (CH, major) 130.29 (CH, minor), 129.48 (CH, major) 129.47 (CH, minor), 128.13 (CH), 128.09 (C), 128.01 (CH, major) 127.99 (CH, minor), 119.9 (CH, major) 119.8 (CH, minor), 63.0 (CH₂-Az, major) 62.6 (CH₂-Az, minor), 59.8 (CH, α-Pro, major) 59.6 (CH, α-Pro, minor), 57.4 (C, Az, major) 56.4 (C, Az, minor), 55.9 (CH₂-Az, major) 55.8 (CH₂-Az, minor), 52.9 (CH, α-Leu, major) 52.0 (CH, α-Leu, minor), 47.1 (CH₂, Gly, major) 47.0 (CH₂, Gly, minor), 46.92 (CH₂, δ-Pro, major), 46.88 (CH₂, δ-Pro, minor) 46.5 (CH₂, GAZ, major) 46.3 (CH₂, GAZ, minor), 37.4 (CH₂, β-Leu, major) 37.1 (CH₂, β-Leu, minor), 31.63 (CH₂, δ-Pro, major) 31.55 (CH₂, β-Pro, minor), 24.5 (CH, γ-Leu), 22.54 (CH₂, δ-Leu, major) 22.51 (CH₂, δ-Leu, minor), 22.3 (CH₂, γ-Pro), 22.10 (CH₂, δ-Leu, major), 22.07 (CH₂, δ-Leu, minor); νmax (neat) = 3288, 2953, 1613, 1420, 770 cm⁻¹; MS (ESI⁺) m/z 529 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₂₃H₂₃N₆O₅Na⁺ [M+Na⁺] 529.2534, found 529.2535; [α]D²⁰ +59.6 (c 0.13, MeOH).
Cyclo(D-Pro-Leu-GAz(COC₄H₄)Gly) (392)

Following general procedure 12 from Cyclo(D-Pro-Leu-GAz(Cbz)Gly) 301 (32 mg, 66 µmol, 1.0 equiv) and valeryl chloride (10 µL, 82 µmol, 1.3 equiv), the title compound 392 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) as a white glassy solid (16 mg, 37 µmol, 57%); Rᵣ (CH₂Cl₂/MeOH 19:1) 0.15; m.p. 102 – 105 °C; ¹H NMR (500 MHz, DMSO-d₆) δH 8.39 (dd, J = 14.5, 8.7 Hz, 1H, NH), 7.16 – 7.09 (m, 1H, NH), 4.38 (dd, J = 8.2, 3.1 Hz, 1H, CH₂-Pro), 4.21 (m, 1H, CH₂-Leu), 3.99 (d, J = 8.9 Hz, 0.5H, CHH-Az minor), 3.84 (d, J = 8.9 Hz, 0.5H, CHH-Az major), 3.79 (d, J = 8.9 Hz, 1H, CHH-Az), 3.74 (d, J = 10.1 Hz, 0.5H, CHH-Az minor), 3.57 – 3.48 (m, 1.5H, CHH-Az major, CHH-Az), 3.47 – 3.38 (m, 3H, CH₂β-Pro, CHHGLy), 3.24 – 3.14 (m, 2H, CHHGLy, CHHGAz), 3.09 (dd, J = 14.0, 2.3 Hz, 1H, CHHGAz), 2.20 – 2.14 (m, 1H, CHHβ-Pro), 1.99 (dt, J = 12.2, 7.5 Hz, 2H, CH₂β-valeryl), 1.86 – 1.75 (m, 3H, CHHβ-Pro, CH₂γ-Pro), 1.61 – 1.41 (m, 5H, CH₂β-Leu, CHγ-Leu, CH₂δ-valeryl), 1.31 – 1.22 (m, 2H, CH₂γ-valeryl), 0.90 (d, J = 6.4 Hz, 3H, CH₃δ-Leu), 0.87 – 0.83 (m, 6H, CH₃δ-Leu, CH₂e-valeryl); ¹³C NMR (126 MHz, DMSO) δC 174.2 (C=O, major) 174.0 (C=O, minor), 173.0 (C=O, major) 172.8 (C=O, minor), 172.4 (C=O, major) 172.3 (C=O, minor), 169.9 (C=O), 59.63 (CH, α-Pro, major) 59.58 (CH, α-Pro, minor), 57.7 (CH₂-Az, major) 57.5 (CH₂-Az, minor), 55.7 (CH₂-Az, minor) 55.1 (CH₂-Az, major), 55.0 (C, Az, major), 54.8 (C, Az, minor), 52.4 (CH, α-Leu, major) 52.2 (CH, α-Leu, minor), 47.0 (CH₂, δ-Pro), 46.88 (CH₂, Gly, minor) 46.87 (CH₂, Gly, major), 46.54 (CH₂, GAZ, minor) 46.51 (CH₂, GAZ, major), 37.2 (CH₂, β-Leu, minor) 37.1 (CH₂, β-Leu, major), 31.6 (CH₂, β-Pro), 30.5 (CH₂, β-valeryl), 26.4 (CH₂, γ-valeryl), 24.4 (CH, γ-Leu), 22.5 (CH₂, δ-Leu), 22.2 (CH₂, γ-Pro), 22.1 (CH₂, δ-Leu), 21.89 (CH₂, δ-valeryl, minor), 21.86 (CH₂, δ-valeryl, major), 13.8 (CH₃, ε-valeryl); νmax (neat) = 3287, 3035, 1618, 1537 cm⁻¹; MS (ESI⁺) m/z 458 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₂₃H₂₇N₅NaO₄ [M+Na]⁺ 458.2738, found 458.2739; [α]D²⁰ –76.2 (c 0.04, MeOH).

Cyclo((D)Pro-Leu-GAz(Ala-NHCBz)-Gly) (393)

Following general procedure 12 from cyclo(D-Pro-Leu-GAz(Cbz)-Gly) 301 (29 mg, 59 µmol, 1.0 equiv) and Z-Ala-OSu (38 mg, 117 µmol, 2.0 equiv), the title compound 393 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→ 9:1) as a white glassy solid (19 mg, 34 µmol, 57%); Rᵣ(SiO₂, CH₂Cl₂/MeOH 12:1) 0.26; m.p. 135 – 138 °C; ¹H NMR (500 MHz, DMSO-d₆) δH 8.46 (d, J = 8.7 Hz, 1H, NH), 7.47 (dd, J = 10.2, 7.6 Hz, 1H, NH), 7.38 – 7.30 (m, 5H,
Cyclo((D)Pro-Leu-GAz(pyrimidine)-Gly) (394)

Cyclo((D)Pro-Leu-GAz(Cbz)-Gly) (301) (23 mg, 60 µmol, 1.0 equiv) was deprotected following general procedure 5 using ethanol as the solvent for 2 h. The crude azetidine was suspended in DMF (2.5 mL) and to this solution was added 2-chloropyrimidine (23 mg, 180 µmol, 3.0 equiv) and NEt₃ (36 µL, 240 µmol, 4.0 equiv). The reaction flask was fitted with a reflux condenser and heated to 80 °C for 48 h, at which time full consumption of starting material was observed by ESI-MS. The reaction mixture was reduced in vacuo at 60 °C over 30 min and the crude products purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1 → 9:1) to yield the title compound 394 as a yellow glassy solid (16 mg, 37 µmol, 62%); Rₚ (CH₂Cl₂/MeOH 19:1) 0.19; m.p. 92 – 95 °C; ¹H NMR (600 MHz, MeOD-d₄) δH 8.31 (d, J = 4.9 Hz, 2H, ArH), 6.67 (t, J = 4.8 Hz, 1H, ArH), 4.50 – 4.44 (m, 2H, CHα-Pro, CHα-Leu), 4.13 (d, J = 9.3 Hz, 1H, CHH-Az), 4.00 (d, J = 9.6 Hz, 1H, CHH-Az), 3.92 (d, J = 9.6 Hz, 1H, CHH-Az), 3.88 (d, J = 9.3 Hz, 1H, CHH-Az), 3.81 (d, J = 14.0 Hz, 1H, CHHGaz), 3.67 – 3.60 (m, 1H,
CHHδ-Pro), 3.58 – 3.54 (m, 1H, CHHδ-Pro), 3.42 (d, J = 14.0 Hz, 1H, CHHGly), 3.37 (d, J = 14.1 Hz, 1H, CHHGly), 2.31 (dq, J = 11.7, 8.4 Hz, 1H, CHHβ-Pro), 2.05 – 1.96 (m, 2H, CHHβ-Pro, CHHγ-Pro), 1.93 – 1.88 (m, 1H, CHHγ-Pro), 1.72 – 1.66 (m, 2H, CHγ-Leu, CHHβ-Leu), 1.61 – 1.56 (m, 1H, CHHβ-Leu), 0.99 (d, J = 6.1 Hz, 3H, CHδ-Leu), 0.94 (d, J = 6.0 Hz, 3H, CHδ-Leu), Note: CHHG Az overlaps with solvent signal; 13C NMR (151 MHz, MeOD-d4) δ C 176.3 (C=O), 175.8 (C=O), 173.0 (C=O), 163.6 (C), 159.2 (CH), 111.6 (CH), 62.2 (CH α-Pro), 59.1 (C, Az), 58.1 (CH2, Az), 57.7 (CH2, Az), 53.7 (CH, α-Leu), 48.5 (CH3, Gly), 48.0 (CH3, G Gaz), 38.3 (CH2, β-Leu) 33.1 (CH2, β-Pro), 26.2 (CH, γ-Leu), 23.4 (CH2, γ-Pro), 23.0 (CH3, δ-Leu), 22.5 (CH3, δ-Leu), Note: CHδ-Pro overlaps with solvent signal; νmax ( neat) = 3269, 3034, 1631, 1581, 1466 cm−1; MS (ESI+) m/z 452 [M+Na]+, 881 [2M+Na]+; HRMS (ESI+) calcd. for C22H22N3NaO3 [M+Na]+ 452.2381, found 452.2385; [α]D 30 62.2 (c 0.06, MeOH).

Cyclo((D)Pro-Leu-GA(z(Ts))-Gly) (395)

Following general procedure 12 from Cyclo(D-Pro-Leu-GA(z(Cbz))-Gly) 301 (29 mg, 59 µmol, 1.0 equiv) and tosy l chloride (17 mg, 88 µmol, 1.5 equiv), the title compound 395 was isolated after column chromatography (SiO2, CH2Cl2/MeOH 19:1 → 9:1) as a white glassy solid (19 mg, 33 µmol, 56%); Rf (SiO2, CH2Cl2/MeOH 19:1) 0.33; m.p. 137 – 140 °C; 1H NMR (500 MHz, MeOD-d4) δ 7.74 (d, J = 8.2 Hz, 2H, ArH), 7.49 (d, J = 8.1 Hz, 2H, ArH), 4.39 (dd, J = 8.4, 6.4 Hz, 1H, CH α-Pro), 4.34 (dd, J = 8.3, 3.1 Hz, 1H, CHα-Leu), 3.74 (d, J = 8.8 Hz, 1H, CHH-Az), 3.68 (d, J = 8.9 Hz, 1H, CHH-Az), 3.61 – 3.47 (m, 5H, CH2-Az, CHHG Az, CHδ-Pro), 3.19 (d, J = 13.8 Hz, 1H, CHHGly), 3.13 (d, J = 13.8 Hz, 1H, CHHGly), 2.93 (d, J = 14.0 Hz, 1H, CHHG Az), 2.47 (s, 3H, ArCH3), 2.32 – 2.24 (m, 1H, CHHβ-Pro), 2.01 – 1.86 (m, 3H, CHHβ-Pro, CHγ-Pro), 1.67 – 1.60 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.56 – 1.50 (m, 1H, CHHβ-Leu), 0.95 (d, J = 6.2 Hz, 3H, CHδ-Leu), 0.91 (d, J = 6.1 Hz, 3H, CHδ-Leu); 13C NMR (126 MHz, MeOD-d4) δ C 176.2 (C=O), 175.6 (C=O), 172.4 (C=O), 146.0 (C), 132.7 (C), 131.1 (CH), 129.6 (CH), 62.0 (CH, α-Pro), 59.2 (C, Az), 58.8 (CH2, Az), 56.5 (CH2, Az), 53.5 (CH, α-Leu), 48.7 (CH2, δ-Pro), 48.3 (CH2, Gly), 47.3 (CH3, G Az), 38.2 (CH2, β-Leu), 33.0 (CH2, β-Pro), 26.1 (CH, γ-Leu), 23.4 (CH2, γ-Pro), 23.0 (CH3, δ-Leu), 22.5 (CH3, δ-Leu), 21.6 (ArCH3); νmax ( neat) = 3307, 2954, 1661, 1651, 1248 cm−1; MS (ESI+) m/z 528 [M+Na]+; HRMS (ESI+) calcd. for C22H26N3NaO3S [M+Na]+ 528.2251, found 528.2247; [α]D 30 78.6 (c 0.11, MeOH).
Cbz deprotection and functionalisation of other azetidine modified cyclic peptides

Cyclo(Val-GAzb(biotin)-Leu-Trp(Boc)) (397)

Following general procedure 12 from Cyclo(Val-GAzb(Cbz)-Leu-Trp(Boc)) 328 (30 mg, 42 µmol, 1.0 equiv) and (D)biotin-OSu\textsuperscript{159} (21 mg, 63 µmol, 1.5 equiv), the title compound 397 was isolated after column chromatography (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/MeOH 19:1→ 9:1) as a pale orange glassy solid (23 mg, 29 µmol, 69%); Ru (SiO\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}/MeOH 12:1) 0.25; m.p. 242 – 245 °C; This compound appears as a 66:34 mixture of conformational isomers at RT. \textsuperscript{1}H NMR (500 MHz, MeOD-d\textsubscript{4}) \delta_H 8.12 – 8.07 (m, 1H, ArH), 7.73 – 7.69 (m, 1H, ArH), 7.59 – 7.55 (m, 1H, ArH), 7.31 – 7.24 (m, 2H, ArH), 4.51 (dd, \textit{J} = 7.8, 4.9 Hz, 0.3H, CH-biotin minor), 4.46 (dd, \textit{J} = 7.7, 4.8 Hz, 0.7H, CH-biotin major), 4.35 (dd, \textit{J} = 7.8, 4.4 Hz, 0.7H, CH-biotin major), 4.31 (dd, \textit{J} = 7.8, 4.4 Hz, 1H, 0.3H, CH-biotin minor), 4.03 – 3.98 (m, 0.7H, CHH-Az major), 3.98 – 3.90 (m, 2.3H, CHHGaz major, CH\textalpha-Val, CHH-Az major), 3.80 – 3.63 (m, 3H, CHHGaz minor, CH\textgamma-Az, 2 x CHH-Az minor), 3.45 – 3.42 (m, 0.7H, CH\textalpha-Leu major), 3.28 – 3.16 (m, 2.3H, CHHGaz minor, CH\textepsilon-biotin, CHH\textbeta-Trp), 3.01 (d, \textit{J} = 13.5 Hz, 0.7H, CHHGaz major), 2.93 (dd, \textit{J} = 12.7, 4.9 Hz, 1H, SCHH biotin), 2.71 – 2.66 (m, 1H, SCHH biotin), 2.21 – 2.08 (m, 3H, CH\textbeta-Val, CH\textbeta\textalpha-biotin), 1.80 – 1.36 (m, 18H, CH\textsubscript{3} Boc, CH\textbeta-biotin, CH\textgamma-biotin, CH\textepsilon-biotin, CH\textgamma-Leu, CH\textbeta-Leu), 1.09 – 1.06 (m, 3H, CH\textepsilon\textgamma-Val), 1.02 – 0.99 (m, 3H, CH\textgamma\textgamma-Val), 0.93 – 0.90 (m, 3H, CH\textepsilon\textdelta-Leu), 0.85 – 0.82 (m, 3H, CH\textdelta\textdelta-Leu), Note: CH\textepsilon-Trp, CHH\textbeta-Trp and CH\textepsilon-Leu minor overlaps with solvent signal, C, Az not observed; \textsuperscript{13}C NMR (126 MHz, MeOD-d\textsubscript{4}) \delta_C 179.6 (C=O major) 179.1 (C=O minor), 176.0 (C=O major) 175.7 (C=O minor), 175.3 (C=O major) 175.1 (C=O minor), 174.1 (C=O minor) 173.7 (C=O major), 166.1 (C=O), 150.9 (C), 136.9 (C), 131.5 (C), 125.6 (CH), 124.9 (CH), 123.8 (CH), 120.2 (CH), 117.0 (C), 116.1 (CH), 84.8 (C, Boc), 64.3 (CH, \textalpha-Val major) 64.2 (CH, \textalpha-Val minor), 63.3 (CH, biotin minor) 63.3 (CH, biotin major), 61.6 (CH, biotin), 59.1 (CH, \textalpha-Leu major) 59.0 (CH, \textalpha-Leu minor), 57.7 (CH\textbeta, Az major) 57.6 (CH\textbeta, Az minor), 57.3 (CH, \textalpha-Trp major) 57.2 (CH, \textalpha-Trp minor), 57.0 (CH\textbeta, Az major), 56.9 (CH, \textepsilon-biotin minor) 56.7 (CH, \textepsilon-biotin major), 56.6 (CH\textgamma, Az minor), 46.5 (CH\textgamma, GAzb major) 46.2 (CH\textgamma, GAzb minor), 44.6 (CH\textbeta, \textbeta-Leu minor) 44.0 (CH\textbeta, \textbeta-Leu major), 41.2 (SCH\textgamma, biotin minor) 41.1 (SCH\textgamma, biotin major), 32.1 (CH\textbeta, \textalpha-biotin major) 31.6 (CH\textbeta, \textalpha-biotin minor), 30.6 (CH, \textbeta-Val major) 30.5 (CH, \textbeta-Val minor), 29.5 (CH\textgamma, \textbeta-biotin minor) 29.4 (CH\textgamma, \textbeta-biotin major), 29.1 (CH\textgamma, \textgamma-biotin), 28.4 (CH\textgamma, Boc), 27.0 (CH\textbeta, \textbeta-Trp major) 26.8 (CH\textbeta, \textbeta-Trp minor), 26.0 (CH, \textgamma-Leu minor) 25.9 (CH, \textgamma-Leu major), 25.7 (CH\textbeta, \textdelta-biotin), 23.2 (CH\textgamma, \textgamma-Leu major) 23.1 (CH\textgamma, \textgamma-Val

263
minor), 23.0 (CH₃, γ-Val minor) 22.9 (CH₃, γ-Val major), 20.7 (CH₃, δ-Leu major) 20.6 (CH₃, δ-Leu minor), 19.9 (CH₃, δ-Leu minor) 19.8 (CH₃, δ-Leu major). Note: C=O Boc and C, Az not observed; νmax (neat) = 3238, 2923, 1670, 1648, 1631, 1529 cm⁻¹; MS (ESI⁺) m/z 831 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₀H₆₈N₆NaO₇S [M+Na]⁺ 831.4198, found 831.4198; [α]D²⁰ −15.8 (c 0.07, MeOH)

Cyclo-(Val-GAz(COCH₂N₃)-Leu-Trp(Boc)) (398)

Following general procedure 12 from Cyclo(Val-GAz(Cbz)-Leu-Trp(Boc)) 328 (24 mg, 34 µmol, 1.0 equiv) and azidoglycine-OSu¹⁵⁶ (13 mg, 67 µmol, 2.0 equiv), the title compound 398 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→ 9:1) as a white glassy solid (17.9 mg, 27 µmol, 79%). Crystals suitable for analysis by XRD were obtained by slow evaporation from methanol. Rf (CH₂Cl₂/MeOH 19:1) 0.34; m.p 158 – 161 °C; Note: Data reported as a 65:35 mixture of rotamers at RT. ¹H NMR (500 MHz, DMSO-d₆) δH 8.24 (m, 1H, NH), 8.04 (d, J = 8.0 Hz, 1H, ArH), 7.88 (d, J = 7.5 Hz, 0.4H, ArH minor), 7.80 – 7.71 (m, 1H, NH), 7.67 (d, J = 7.7 Hz, 1H, ArH major), 7.58 (d, J = 7.9 Hz, 1H, NH), 7.51 – 7.46 (m, 1H, ArH), 7.34 – 7.25 (m, 3H, NH, 2 × ArH), 4.64 – 4.53 (m, 1H, CHα-Trp), 3.89 – 3.60 (m, 7H, CHα-Val, CH₂N₃, CH₂-Az, CHH-Az, CHHGAz), 3.58 – 3.52 (m, 0.7H, CHH-Az major), 3.24 – 3.14 (m, 2.4H, CHHβ-Trp, CHH-Az minor), 3.11 – 2.99 (m, 2H, CHHβ-Trp), 2.90 – 2.86 (m, 1H, CHHGAz), 2.04 – 1.94 (m, 1H, CH₂Val), 1.61 (s, 9H, CH₃ Boc), 1.50 (m, 1H, CHβ-Leu), 1.36 – 1.28 (m, 1H, CHHβ-Leu), 1.22 – 1.17 (m, 1H, CHHβ-Leu), 1.01 – 0.96 (m, 3H, CHγ-Val), 0.95 – 0.90 (m, 3H, CH₂γ-Val), 0.87 – 0.82 (m, 3H, CH₂δ-Leu), 0.76 (d, J = 6.5 Hz, 2H, CH₂δ-Leu minor), 0.73 (d, J = 6.5 Hz, 1H, CH₂δ-Leu minor), Note: CHα-Leu overlaps with solvent peak; ¹³C NMR (126 MHz, DMSO-d₆) δC 176.0 (C=O major), 175.6 (C=O minor), 172.04 (C=O minor), 171.95 (C=O major), 171.4 (C=O minor), 171.1 (C=O major), 167.4 (C=O major), 167.1 (C=O minor), 148.9 (C=O, Boc), 140.7 (C, major), 134.7 (C, minor), 130.0 (C), 128.4 (CH), 125.2 (CH, minor), 124.4 (CH, major), 123.4 (CH, minor), 122.5 (CH, major), 120.1 (CH, minor), 119.2 (CH, major), 116.1 (C), 114.7 (CH), 83.5 (C, Boc), 62.0 (CH, α-Val minor), 61.7 (CH, α-Val major), 57.4 (CH₂, Az major), 57.2 (CH, α-Leu), 56.59 (CH₂, Az minor), 56.55 (CH₂, Az major), 54.8 (CH, α-Trp minor), 54.6 (CH, α-Trp major), 53.1 (CH₂, Az minor), 48.1 (CH₂N₃ major), 48.0 (CH₂N₃ minor), 45.0 (CH₂, GAz), 42.9 (CH₂, β-Leu major), 42.8 (CH₂, β-Leu minor), 29.0 (CH, γ-Val, 27.7 (CH₃, Boc), 25.7 (CH₂, β-Trp), 24.1 (CH, γ-Leu), 22.9 (CH₂, δ-Leu major), 22.7 (CH₃, δ-Leu minor), 22.3 (CH₃, δ-Leu major), 22.2 (CH₂, δ-Leu minor), 19.7 (CH₂, γ-Val), Note: C, Az not observed; νmax (neat) = 3320, 2959, 2106, 1732, 1668, 1634, 1505,
1153 cm⁻¹; MS (ESI⁺) m/z 688 [M+Na]+; HRMS (ESI⁺) calcd. for C_{33}H_{47}N_{9}NaO_{6} [M+Na]+ 688.3542, found 688.3541; [α]_{D}^{28} −30.0 (c 0.07, DMF).

**Cyclo-(Phe-Glu(Bu)-GAz(CO(CH_{2})_{3}N_{3})-Leu-Gly)** (401) Following general procedure 12 from Cyclo(Phe-Glu(Bu)-GAz(Cbz)-Leu-Gly) 336 (23.6 mg, 33 µmol, 1.0 equiv) and succinimide ester 400 336 (12 mg, 50 µmol, 1.5 equiv), the title compound 401 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→ 9:1) as a white glassy solid (15.2 mg, 21 µmol, 65%); Rₚ (CH₂Cl₂/MeOH 19:1) 0.14; m.p. 124 – 127 °C; ¹H NMR (400 MHz, MeOD) δ_H 7.33 – 7.22 (m, 5H, ArH), 4.54 – 4.47 (m, 2H, CHα-Phe, CHα-Glu), 4.15 – 4.07 (m, 2H, CH₂α-azide), 3.99 – 3.75 (m, 5H, CH₂-Az, CHH-Az, CHHGAz, CHHGly), 3.66 (d, J = 9.1 Hz, 1H, CHH-Az), 3.47 (d, J = 14.5 Hz, 1H, CHHGAz), 3.40 (t, J = 6.6 Hz, 2H, CH₂γ-azide), 3.36 – 3.33 (m, 1H, CHα-Leu), 3.04 (d, J = 14.1 Hz, 1H, CHHGly), 2.94 (dd, J = 14.3, 10.6 Hz, 1H, CHHβ-Phe), 2.30 (t, J = 7.5 Hz, 2H, CHβ-azide), 2.19 (dt, J = 14.1, 7.4 Hz, 1H, CHHβ-Glu), 1.94 – 1.83 (m, 3H, CH₂γ-Glu, CHHβ-Glu), 1.79 (dt, J = 13.2, 6.7 Hz, 1H, CHγ-Leu), 1.48 (s, 9H, CO₂Bu), 1.32 – 1.25 (m, 2H, CHβ-Leu), 0.94 (2 × d, J = 6.6 Hz, 6H, 2 × CH₃δ-Leu), Note: CHHβ-Phe overlaps with solvent signal; ¹³C NMR (101 MHz, MeOD) δ_C 180.3 (C=O), 174.2 (C=O), 174.0 (C=O), 173.6 (C=O azide), 138.5 (C), 130.0 (CH), 129.7 (CH), 128.0 (CH), 81.8 (C, 'Bu), 63.5 (CH₂, α-azide), 58.1 (CH, α-Phe), 57.7 (CH₂, Az), 56.1 (CH, α-Leu), 53.7 (CH, α-Glu), 47.0 (CH₂, Gly), 45.7 (CH₂, β-Leu), 44.8 (CH₂, GAZ), 37.7 (CH₂, β-Phe), 32.7 (CH₂, β-azide), 29.5 (CH₂, γ-Glu), 28.4 (CH₃, CO₂Bu), 28.1 (CH₂, β-Glu), 25.6 (CH, γ-Leu), 23.5 (CH₃, δ-Leu), 22.5 (CH₃, δ-Leu), Note: One C=O, CH₂-Az and C, Az missing, CH₂, γ-azide overlaps with solvent signal; ν_max (neat) = 3303, 2956, 2096, 1643, 1425, 1151 cm⁻¹; MS (ESI⁺) m/z 736 [M+Na]+; HRMS (ESI⁺) calcd. for C_{34}H_{51}N_{9}NaO_{8} [M+Na]+ 736.8571, found 736.8576; [α]_{D}^{28} −16.7 (c 0.03, MeOH).
Cyclo(Phe-Glu(Bu)-GAz(dansyl)-Leu-Gly) (402)

Following general procedure 12 from cyclo(Phe-Glu(Bu)-GAz(Cbz)-Leu-Gly) (336) (40 mg, 55 µmol, 1.0 equiv) and dansyl chloride (22 mg, 83 µmol, 1.5 equiv), the title compound 402 was isolated after column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1→ 9:1) as a yellow glassy solid (30 mg, 36 µmol, 66%); R_f (CH₂Cl₂/MeOH 9:1) 0.61; m.p. 150 – 153 °C; 

¹H NMR (500 MHz, MeOD-d₄) δ H 8.64 (d, J = 8.5 Hz, 1H, ArH), 8.39 (d, J = 8.7 Hz, 1H, ArH), 8.25 (dd, J = 7.3, 0.7 Hz, 1H, ArH), 7.65 – 7.58 (m, 2H, ArH), 7.33 – 7.21 (m, 6H, ArH), 4.56 – 4.45 (m, 2H, CHα-Glu, CHα-Phe), 3.95 (d, J = 9.0 Hz, 1H, CHH-Az), 3.80 (d, J = 14.4 Hz, 1H, CHH-Gly), 3.72 (d, J = 14.0 Hz, 1H, CHHGAz), 3.65 – 3.58 (m, 3H, CHH-Az, CH₂-Az), 3.42 (d, J = 14.4 Hz, 1H, CHH-Gly), 3.25 (dd, J = 9.3, 4.9 Hz, 1H, CHα-Leu), 2.98 (d, J = 14.0 Hz, 1H, CHHGAz), 2.93 – 2.87 (m, 7H, 2 × NCH₃, CHHβ-Phe), 2.33 – 2.25 (m, 2H, CHβ-Glu), 2.20 – 2.13 (m, 1H, CHHγ-Glu), 1.89 – 1.82 (m, 1H, CHHγ-Glu), 1.65 – 1.60 (m, 1H, CHγ-Leu), 1.47 (s, 9H, CH₃, t-Bu), 1.28 – 1.23 (m, 1H, CHHβ-Leu), 1.17 – 1.12 (m, 1H, CHHβ-Leu), 0.86 (d, J = 6.7 Hz, 3H, CHδ-Leu), 0.80 (d, J = 6.6 Hz, 3H, CHδ-Leu), Note: CHHβ-Phe overlaps with solvent signal; ¹³C NMR (126 MHz, MeOD-d₄) δC 179.9 (C=O), 174.1 (C=O), 174.0 (C=O), 173.5 (C=O), 173.4 (C=O), 153.1 (C), 138.7 (C), 133.3 (C), 132.3 (CH), 132.0 (C), 131.8 (CH), 131.3 (C), 130.0 (CH), 129.6 (CH), 129.3 (CH), 127.9 (CH), 124.4 (CH), 121.2 (CH), 116.6 (CH), 81.7 (C, t-Bu), 62.0 (CH₂, Az), 57.7 (CH₂, Az), 57.6 (CH, α-Phe), 56.4 (C, Az), 55.9 (CH, α-Leu), 53.3 (CH, α-Glu), 46.6 (CH₂, GAz), 45.8 (2 × NCH₃), 45.2 (CH₂, β-Leu), 45.0 (CH₂, Gly), 37.7 (CH₂, β-Phe), 32.6 (CH₂, β-Glu), 28.4 (CH₃, 'Bu), 28.1 (CH₂, γ-Glu), 25.4 (CH, γ-Leu), 23.6 (CH₃, δ-Leu), 22.2 (CH₃, δ-Leu); ν_max (neat) = 3325, 2926, 1723, 1651, 1530, 1047 cm⁻¹; MS (ESI⁺) m/z 842 [M+Na⁺]; HRMS (ESI⁺) calcd. for C₄₂H₅₇N₇O₈S [M+Na⁺] 842.3882, found 842.3875; [α]D²⁰ –23.5 (c 0.05, MeOH)
Cyclo(Tyr(Bu)-Thr(Bu)-GAz(Pyrazine)-Val-Leu) (403)

Cyclo(Tyr(Bu)-Thr(Bu)-GAz(Cbz)-Val-Leu) 335 (16.0 mg, 20 µmol, 1.0 equiv) was deprotected following general procedure 5 using ethanol as the solvent for 3 h. The crude azetidine was suspended in DMF (1.5 mL) and to this solution was added 2-fluoropyrazine (5 µL, 60 µmol, 3.0 equiv) and NEt₃ (11 µL, 80 µmol, 4.0 equiv). The reaction flask was fitted with a reflux condenser and heated to 80 °C for 24 h, at which time full consumption of starting material was observed by ESI-MS. The reaction mixture was reduced in vacuo at 60 °C over 30 min and the crude products purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1 → 9:1) to yield the title compound 403 as a yellow glassy solid (10.8 mg, 14 µmol, 72%); RF (CH₂Cl₂/MeOH 19:1) 0.23; m.p. 100 – 103 °C; ¹H NMR (500 MHz, MeOD-d₄) δ H 8.01 (s, 1H, ArH), 7.82 (s, 1H, ArH), 7.79 (d, J = 2.3 Hz, 1H, ArH), 7.22 (d, J = 8.3 Hz, 2H, ArH), 6.96 (d, J = 8.4 Hz, 2H, ArH), 4.44 – 4.32 (m, 3H, CHα-Thr, CHβ-Thr, CHα-Tyr), 4.05 (d, J = 8.6 Hz, 1H, CHH-Az), 3.91 (d, J = 9.3 Hz, 1H, CHH-Az), 3.86 – 3.78 (m, 3H, CHH-Az), 3.76 (s, 1H, ArH), 3.64 – 3.62 (m, 1H, CHHG-Az), 3.42 – 3.34 (m, 1H, CHHG-Az), 3.28 – 3.19 (m, 2H, CH₂β-Tyr), 3.17 (d, J = 5.0 Hz, 1H, CHα-Leu), 1.96 – 1.77 (m, 2H, CHHβ-Leu, CHβ-Val), 1.54 – 1.43 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.33 (s, 9H, CH₃-tBu), 1.24 – 1.21 (m, 12H, CH₃-tBu, CH₃γ-Val), 0.88 (d, J = 6.3 Hz, 3H, CHδ-Leu); ¹³C NMR (126 MHz, MeOD-d₄) δ C 177.6 (C=O), 175.3 (C=O), 174.3 (C=O), 173.8 (C=O), 155.6 (C), 143.2 (CH), 133.7 (CH), 131.7 (CH), 130.6 (CH), 125.4 (CH), 79.6 (C, 'Bu), 75.4 (C, 'Bu), 68.3 (CH, β-Thr), 63.6 (CH, α-Leu), 62.3 (CH₂, Az), 61.1 (CH, α-Thr), 59.9 (CH, α-Tyr), 58.4 (CH₂, Az), 57.9 (C, Az), 54.7 (CH, α-Val), 47.9 (CH₂, GAZ), 40.0 (CH₂, β-Leu), 36.8 (CH₂, β-Tyr), 33.7 (CH, β-Val), 29.2 (CH₃, 'Bu), 29.2 (CH₃, 'Bu), 26.1 (CH, γ-Leu), 23.5 (CH₃, γ-Val), 21.8 (CH₃, γ-Val), 21.7 (CH₃, γ-Thr), 20.0 (CH₃, δ-Leu), 18.5 (CH₃, δ-Leu), Note: C, pyrazine not observed; νmax (neat) = 3305, 2969, 1651, 1578, 1505, 1120 cm⁻¹; MS (ESI⁺) m/z 751 [M+H]⁺, 773 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₀H₆₅N₈O₆ [M+H]⁺ 751.4865, found 751.4854; [α]D³⁰ = –38.8 (c 0.05, MeOH)
Cyclo(Val-GAz(biotin)-Leu-Trp) (404)

Following general procedure 12 from cyclo(Val-GAz(biotin)-Leu-Trp(Boc)) (397) (14.0 mg, 17 μmol, 1.0 equiv), Cyclo(Val-GAz(biotin)-Leu-Trp) (404) was isolated as an orange glassy solid (18.4 mg) in quantitative yield as the TFA salt, which required no further purification. $^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 2 eq. of TFA salt; m.p. 141 – 144 °C; This compound appears as a 66:34 mixture of conformational isomers at RT. $^1$H NMR (500 MHz, MeOD-d$_4$) δ$_{H}$ 7.67 – 7.58 (m, 1H, ArH), 7.32 (d, $J$ = 8.1 Hz, 1H, ArH), 7.14 (s, 1H, ArH), 7.11 – 7.06 (m, 1H, ArH), 7.06 – 6.99 (m, 1H, ArH), 4.50 – 4.41 (m, 1H, CH-biotin), 4.34 – 4.27 (m, 1H, CH-biotin), 4.04 – 3.88 (m, 4H, CH/HGAz major, CHα-Val, CHH-Az major, CHH-Az), 3.78 – 3.62 (m, 3H, CHHGAz minor, CH$_2$-Az, $CHH$-Az minor), 3.42 (t, $J$ = 7.3 Hz, 1H, CHα-Leu major), 3.27 – 3.17 (m, 3H, CHHβ-Trp, CHHGAz minor, CHε-biotin), 3.02 (d, $J$ = 13.5 Hz, 1H, CHHGAz major), 2.91 (dd, $J$ = 12.7, 5.0 Hz, 1H, SCHH biotin), 2.72 – 2.64 (m, 1H, SCHH biotin), 2.20 – 2.04 (m, 3H, CHα-biotin, CHβ-Val), 1.73 – 1.43 (m, 9H, CH$_3$β-Leu, CH$_7$-Leu, CH$_3$β-biotin, CH$_2$-biotin, CH$_3$δ-biotin), 1.05 – 1.00 (m, 3H, CH$_3$γ-Val), 1.00 – 0.97 (m, 3H, CH$_3$γ-Val), 0.94 – 0.90 (m, 3H, CH$_3$δ-Leu), 0.89 – 0.83 (m, 3H, CH$_3$δ-Leu), Note: CHα-Leu minor, CHHβ-Trp and CHα-Trp overlaps with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) δ$_{C}$ 179.4 (C=O major) 179.0 (C=O minor), 176.0 (C=O major) 175.7 (C=O minor), 175.3 (C=O major) 175.2 (C=O minor), 174.6 (C=O minor), 174.3 (C=O major), 166.1 (C=O), 161.8 (C major), 161.4 (C minor), 138.0 (C), 128.6 (C), 124.14 (CH major), 124.07 (CH minor), 122.5 (CH), 119.9 (CH), 119.3 (CH), 112.4 (CH), 110.6 (C major), 110.5 (C minor), 64.2 (CH, α-Val major) 64.1 (CH, α-Val minor), 63.4 (CH, biotin minor), 63.3 (CH, biotin major), 61.6 (CH, biotin), 59.1 (CH, α-Leu major) 59.0 (CH, α-Leu minor), 58.1 (CH, α-Trp major), 57.9 (CH, α-Trp minor), 57.63 (CH$_2$, Az major), 57.55 (CH$_2$, Az minor), 57.3 (CH, ε-biotin major), 57.1 (CH$_2$, Az major), 56.7 (CH, ε-biotin minor), 56.6 (CH$_2$, Az minor), 46.4 (CH$_2$, GAz major) 46.2 (CH$_2$, GAz minor), 44.5 (CH$_2$, β-Leu minor) 43.9 (CH$_2$, β-Leu major), 41.2 (SCH$_2$, biotin minor) 41.1 (SCH$_2$, biotin major), 32.1 (CH$_2$, α-biotin major), 31.6 (CH$_2$, α-biotin minor), 30.6 (CH, β-Val minor), 30.5 (CH, β-Val major), 29.5 (CH$_2$, β-biotin major) 29.4 (CH$_2$, β-biotin minor), 29.13 (CH$_2$, δ-biotin major) 29.09 (CH$_2$, δ-biotin minor), 27.4 (CH$_2$, β-Trp minor) 27.2 (CH$_2$, β-Trp major), 25.93 (CH, γ-Leu minor) 25.89 (CH, γ-Leu major), 25.7 (CH$_2$, γ-biotin), 23.2 (CH$_3$, γ-Val major) 23.1 (CH$_3$, γ-Val minor), 22.9 (CH$_3$, γ-Val minor) 22.8 (CH$_3$, γ-Val major), 20.5 (CH$_3$, δ-Leu major) 20.4 (CH$_3$, δ-Leu minor), 19.9 (CH$_3$, δ-Leu minor) 19.8 (CH$_3$, δ-Leu major);
$v_{\text{max}}$ (neat) = 3374, 1670, 1457, 1202 cm$^{-1}$; MS (ESI$^+$) $m/z$ 731 [M+Na]$^+$; HRMS (ESI$^+$) calcd. for C$_{36}$H$_{52}$N$_8$NaO$_5$S [M+Na]$^+$ 731.3674, found 731.3673; $[\alpha]_D^{20} -29.8$ (c 0.06, MeOH).

Analytical HPLC, 280 nm: $R_f = 20.2$ min. Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-23 min, 3-50% B; 23-30 min, 50-100% B, 30-33 min, 100%-3% B; 33-35 min, 3% B.

Cyclo(Phe-Glu-GA\text{z}(dansyl)-Leu-Gly) (405)

Following general procedure 10 from Cyclo(Phe-Glu(Bu)-GA\text{z}(dansyl)-Leu-Gly) (402) (14.2 mg, 17 μmol, 1.0 equiv), Cyclo(Phe-Glu-GA\text{z}(dansyl)-Leu-Gly) (405) was isolated as a pale yellow glassy solid (20.1 mg) in quantitative yield as the TFA salt, which required no further purification. $^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 2 eq. of TFA salt; m.p. 135 – 137 °C; $^1$H NMR (500 MHz, MeOD-d$_4$) δ $H$ 8.61 (d, $J = 8.6$ Hz, 1H, ArH), 8.46 (d, $J = 8.7$ Hz, 1H, ArH), 8.30 (d, $J = 7.3$ Hz, 1H, ArH), 7.70 – 7.65 (m, 2H, ArH), 7.48 (d, $J = 7.6$ Hz, 1H, ArH), 7.43 – 7.05 (m, 5H, ArH), 4.51 (dd, $J = 8.6, 5.7$ Hz, 1H, CH$\alpha$-Glu), 4.46 (dd, $J = 10.8, 4.0$ Hz, 1H, CH$\alpha$-Phe), 4.07 (d, $J = 10.1$ Hz, 1H, CHH-Az), 3.89 – 3.72 (m, 6H, CHH-Az, CH$_2$-Az, CHHGAz, CHHGly, CH$\alpha$-Leu), 3.50 (d, $J = 14.5$ Hz, 1H, CHH$\beta$-Glu), 3.07 (d, $J = 14.7$ Hz, 1H, CHHGAz), 3.03 (s, 6H, 2 × NCH$_3$), 2.88 (dd, $J = 14.4, 10.9$ Hz, 1H, CHH$\beta$-Phe), 2.37 – 2.33 (m, 2H, CH$_3$$\beta$-Phe), 2.16 – 2.09 (m, 1H, CHH$\gamma$-Glu), 1.95 – 1.88 (m, 1H, CHH$\gamma$-Glu), 1.69 – 1.63 (m, 1H, CH$\gamma$-Leu), 1.54 – 1.49 (m, 1H, CHH$\beta$-Leu), 1.45 – 1.40 (m, 1H, CHH$\beta$-Leu), 0.93 (d, $J = 6.6$ Hz, 3H, CH$_3$$\delta$-Leu), 0.89 (d, $J = 6.5$ Hz, 3H, CH$_3$$\delta$-Leu), Note: CHH$\beta$-Phe overlaps with solvent signal; $^{13}$C NMR (126 MHz, MeOD-d$_4$) δ C: 176.5 (C=O), 175.9 (C=O), 174.8 (C=O), 173.4 (C=O), 172.8 (C=O), 150.5 (C), 138.6 (C), 133.4 (C), 132.2 (CH), 131.7 (C), 131.5 (CH), 130.4 (C), 129.9 (CH), 129.7 (CH), 129.4 (CH), 127.9 (CH), 125.2 (CH), 122.6 (CH), 117.6 (CH), 59.6 (CH$_2$, Az), 58.2 (C, Az), 57.8 (CH, α-Phe), 56.9 (CH, α-Leu), 56.3 (CH$_2$, Az), 53.4 (CH, α-Glu), 46.2 (2 × NCH$_3$), 45.6 (CH$_2$, GAz), 45.1 (CH$_2$,...
Gly), 43.5 (CH$_3$, β-Leu), 37.7 (CH$_3$, β-Phe), 31.1 (CH$_3$, γ-Glu), 27.7 (CH$_3$, γ-Glu), 25.6 (CH, γ-Leu), 23.1 (CH$_3$, δ-Leu), 22.4 (CH$_3$, δ-Leu); ν$_{max}$ (neat) = 3303, 2963, 1660, 1651, 1176 cm$^{-1}$; MS (ESI$^+$) m/z 786 [M+Na$^+$]; HRMS (ESI$^+$) calcd. for C$_{38}$H$_{49}$N$_7$O$_8$S [M+Na$^+$] 786.3256, found 786.3252; [α]$^D_{30}$ = 7.4 (c 0.10, MeOH).

Analytical HPLC, 280 nm: R$_f$ = 19.8 min. Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-23 min, 3-50% B; 23-30 min, 50-100% B, 30-33 min, 100%-3% B; 33-35 min, 3% B.

Cyclo(Tyr-Thr-GAz(Pyrazine)-Val-Leu) (406)

Following general procedure 10 from Cyclo(Tyr(Bu)-Thr(Bu)-GAz(Pyrazine)-Val-Leu) (403) (8.0 mg, 11 μmol, 1.0 equiv), Cyclo(Tyr-Thr-GAz(Pyrazine)-Val-Leu) 406 was isolated as a white glassy solid (6.8 mg) in quantitative yield as the TFA salt, which required no further purification. $^1$H and $^{19}$F NMR using TFE as an internal standard confirmed 2 eq. of TFA salt; m.p. 108 – 110 ºC; $^1$H NMR (500 MHz, MeOD-d$_4$) δ$_H$ 8.12 – 7.77 (m, 3H, ArH), 7.12 (d, $J$ = 8.4 Hz, 2H, ArH), 6.75 (d, $J$ = 8.4 Hz, 2H, ArH), 4.44 – 4.38 (m, 2H, CH$\alpha$-Tyr, CH$\alpha$-Thr), 4.35 – 4.31 (m, 1H, CH$\beta$-Thr), 4.24 (d, $J$ = 9.5 Hz, 1H, CHH-Az), 4.12 – 3.96 (m, 4H, CHH-Az, CH$_2$-Az, CHHGAz), 3.87 – 3.80 (m, 1H, CH$\alpha$-Leu), 3.70 – 3.65 (m, 1H, CH$\alpha$-Val), 3.35 (d, $J$ = 14.7 Hz, 1H, CHHGAz), 3.24 (d, $J$ = 14.1, 4.7 Hz, 1H, CHH$\beta$-Tyr), 3.07 (dd, $J$ = 13.5, 11.4 Hz, 1H, CHH$\beta$-Tyr), 2.16 – 2.06 (m, 1H, CH$\beta$-Val), 2.03 – 1.95 (m, 1H, CHH$\beta$-Leu), 1.51 – 1.45 (m, 2H, CHH$\beta$-Val, CH$\gamma$-Leu), 1.23 (d, $J$ = 6.4 Hz, 3H, CH$\gamma$Thr), 1.06 (d, $J$ = 6.9 Hz, 3H, CH$\gamma$Val), 1.02 (d, $J$ = 6.8 Hz, 3H, CH$_3$Val), 0.93 (d, $J$ = 6.1 Hz, 3H, CH$_3$δ-Leu), 0.89 (d, $J$ = 5.9 Hz, 3H, CH$_3$δ-Leu); $^{13}$C NMR (126 MHz, MeOD-d$_4$) δ$_C$ 174.7 (C=O), 174.5 (C=O), 174.2 (2 × C=O), 157.6 (C), 142.9 (CH), 133.7 (CH), 132.0 (CH), 131.0 (CH), 129.1 (C), 116.5 (CH), 68.1 (CH, β-Thr), 63.8 (CH, α-Val), 60.1 (CH$_2$, Az), 59.5 (CH, α-Thr), 59.4 (CH, α-Tyr), 57.5 (CH$_2$, Az), 55.3 (CH, α-Leu), 45.5 (CH$_2$, GAZ), 39.7 (CH$_3$, β-Leu), 36.8 (CH$_3$, β-Tyr), 32.9 (CH, β-Val), 26.2...
(CH, γ-Leu), 23.4 (CH₃, δ-Leu), 21.7 (CH₃, δ-Leu), 20.3 (CH₃, γ-Thr), 18.9 (CH₃, γ-Val), 18.7 (CH₃, γ-Val). Note: C, pyrazine and C, Az not observed; ν_{max} (neat) = 3322, 2962, 1660, 1651, 1645, 1134 cm⁻¹; MS (ESI⁺) m/z 661 [M+Na]⁺; HRMS (ESI⁺) calcd. for C_{32}H_{46}N_{8}NaO_{6} [M+Na]⁺ 661.3433, found 661.3431; [α]_D^{30} = -22.7 (c 0.06, MeOH)

Analytical HPLC, 280 nm: R_f = 22.7 min. Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-23 min, 3-50% B; 23-30 min, 50-100% B, 30-33 min, 100%-3% B; 33-35 min, 3% B.

Click reaction of cyclic peptides 337 and 341

Cyclo(Tyr('Bu)-Val-GAz(triazole-coumarin)-Gly-Phe) (410)

To a solution of cyclo(Tyr('Bu)-Val-GAz(2-PC)-Gly-Phe) (337) (20.0 mg, 29 µmol, 1.0 equiv) and coumarin azide 409 (7.5 mg, 29 µmol, 1.0 equiv) in EtOH/H₂O (2:1, 1.5 mL) was added aq. CuSO₄ (5.8 µL, 0.5 M, 10 mol%) and aq. sodium ascorbate (5.8 µL, 1 M, 20 mol%). The reaction was stirred for 16 h at RT under an atmosphere of N₂. Analysis of the reaction mixture by ESI-MS showed full consumption of starting material. The reaction was cooled to 0 ºC and cold H₂O was added, forming a brown precipitate. The solution was filtered and the solid precipitate was suspended in CH₂Cl₂, dried (Na₂SO₄), filtered and reduced in vacuo to give a brown solid which was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) to give the title compound 410 as a yellow glassy solid (17.5 mg, 19 µmol, 64%); R_f (SiO₂, CH₂Cl₂/MeOH 19:1) 0.39; m.p. 184 – 187 ºC; ¹H NMR (500 MHz, MeOD-d₄) δ_H 8.54 (s, 1H, CH-triazole), 8.36 (s, 1H, C=CH), 7.54 (d, J = 8.9 Hz, 1H, ArH), 7.28 – 7.16 (m, 5H, ArH), 7.05 (d, J = 8.3 Hz, 2H, ArH), 6.87 (d, J = 8.3 Hz, 2H, ArH), 6.83 (dd, J = 9.0, 2.1 Hz, 1H, ArH), 6.63 (d, J = 2.0 Hz, 1H, ArH), 5.24 (s, 2H, OCH₂), 4.29 (t, J = 7.5 Hz, 1H, CHα-Phe), 4.22 (d, J = 5.9 Hz, 1H, CHα-Val), 4.10 (dd, J = 10.2, 5.4 Hz, 1H, CHα-
Tyr), 3.92 – 3.84 (m, 2H, CHH-Az, CHHGAz), 3.79 – 3.71 (m, 3H, CHH-Az, CH2-Az), 3.52 (q, J = 7.1 Hz, 4H, 2 × NCH2CH3), 3.43 (s, 1H, CHHGly), 3.28 – 3.22 (m, 2H, CHHGAz, CHHβ-Tyr), 3.18 – 3.10 (m, 2H, CHHβ-Tyr, CHHGly), 3.01 – 2.96 (m, 2H, CH2β-Phe), 2.34 – 2.27 (m, 1H, CHβ-Val), 1.29 (s, 9H, CH3 tBu), 1.24 (t, J = 7.1 Hz, 6H, 2 × NCH2CH3), 0.98 – 0.94 (m, 6H, 2 × CH3γ-Val); 13C NMR (126 MHz, MeOD-d4) δC 174.7 (C=O), 174.3 (C=O), 174.3 (C=O), 173.9 (C=O), 158.9 (C=O), 158.0 (C=O), 157.6 (C), 155.4 (C), 153.6 (C), 151.3 (CH), 138.13 (CH), 138.08 (C), 133.6 (C), 131.7 (CH), 130.6 (CH), 130.3 (CH), 129.6 (CH), 127.9 (CH), 125.4 (CH), 117.6 (c), 111.5 (CH), 108.2 (C), 97.8 (CH), 79.5 (C, tBu), 61.2 (CH, α-Tyr), 60.7 (CH, α-Val), 59.1 (OCH3), 57.7 (CH, α-Phe), 46.6 (CH2 Gly) 45.9 (CH2, GAz) 37.5 (CH2, β-Phe), 36.7 (CH2, β-Tyr), 31.7 (CH, β-Val), 29.2 (CH3, tBu), 20.0 (CH3, γ-Val), 18.3 (CH3, γ-Val), 12.7 (NCH2CH3), Note: 2 × CH2, Az, C, Az, 1 × C and 1 × CH not observed; νmax (neat) = 3290, 2966, 1715, 1659, 1622, 1601; MS (ESI+) m/z 969 [M+Na]+; HRMS (ESI+) calcd. for C50H62N10O9Na [M+Na]+ 969.4593, found 969.4590; Optical rotation could not be measured due to high fluorescence of the compound.

Cyclo(Tyr-Val-GAztetrazolate-coumarin)-Gly-Phe (411)

Following general procedure 10, Cyclo(Tyr(tBu)-Val-GAZ(triazole-coumarin)-Gly-Phe) 410 (8.9 mg, 9 µmol, 1.0 equiv) was deprotected to give the title compound 411 as a glassy yellow solid (12.0 mg) which required no further purification. 1H and 19F NMR using TFE as an internal standard confirmed 2 eq. of TFA salt; m.p. 182 – 185 °C; 1H NMR (500 MHz, MeOD-d4) δH 8.54 (s, 1H, CH-triazole), 8.37 (s, 1H, C=CH), 7.55 (d, J = 9.0 Hz, 1H, ArH), 7.26 – 7.21 (m, 3H, ArH), 7.15 (d, J = 7.3 Hz, 2H, ArH), 6.98 (d, J = 8.4 Hz, 2H, ArH), 6.84 (dd, J = 9.0, 2.3 Hz, 1H, ArH), 6.69 – 6.63 (m, 3H, ArH), 5.26 (s, 2H, OCH2), 4.27 (t, J = 7.6 Hz, 1H, CHα-Tyr), 4.20 (d, J = 6.1 Hz, 1H, CHα-Val), 4.08 – 3.87 (m, 7H, CHα-Phe, 2 × CH2-Az, CHHGly, CHHGAz), 3.53 (q, J = 7.1 Hz, 4H, 2 × NCH2CH3), 3.21 – 3.10 (m, 3H, CHβ-Tyr, CHHGaz), 3.04 – 2.96 (m, 2H, CH2β-Phe), 2.26 – 2.17 (m, 1H, CHβ-Val), 1.24 (t, J = 7.1 Hz, 6H, 2 × NCH2CH3), 0.95 (t, J = 6.8 Hz, 6H, 2 × CH3γ-Val), Note: CHHGly overlaps with solvent signal; 13C NMR (126 MHz, MeOD-d4) δC 174.3 (C=O), 174.25 (C=O), 174.23 (C=O), 158.9 (C=O), 157.8 (C=O), 157.6 (C), 157.5 (C), 153.6 (C), 138.1 (CH), 137.9 (C), 131.7 (CH), 131.1 (CH), 130.2 (CH), 129.7 (CH), 129.1 (C), 128.0 (C), 117.6 (CH), 116.4 (CH), 111.6 (CH), 108.2 (C), 97.8 (CH), 61.4 (CH, α-Tyr), 60.4 (CH, α-Val), 59.2 (OCH2), 58.1 (CH, α-Phe), 57.9
(2 × CH₂, Az) 45.9 (NCH₂CH₃), 42.7 (CH₂, Gly), 37.2 (CH₂, β-Phe), 36.7 (CH₂, β-Tyr), 32.1 (CH₂, β-Val), 19.9 (CH₃, γ-Val), 18.4 (CH₃, γ-Val), 12.7 (NCH₂CH₃). Note: 1 × C, 1 × CH, C, Az and CH₂, GAZ not observed; νmax (neat) = 3289, 2966, 1666, 1621, 1598, 1181; MS (ESI⁺) m/z 913 [M+Na]⁺; HRMS (ESI⁺) calcd. for C₄₆H₄₅N₁₀O₉ [M+Na]⁺ 913.3967, found 913.3965; Optical rotation could not be measured due to high fluorescence of the compound.

Analytical HPLC, 280 nm: Rf = 24.0 min. Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-23 min, 3-50% B; 23-30 min, 50-100% B, 30-33 min, 100%-3% B; 33-35 min, 3% B.

Cyclo(Leu-Tyr(Bu)-Val-GAZ(triazole-coumarin)-Thr(Bu)-Phe) (412)

To a solution of Cyclo(Leu-Tyr(Bu)-Val-GAZ(2-PC)-Thr(Bu)-Phe) 341 (29 mg, 32 µmol, 1.0 equiv) and coumarin azide 409 (10 mg, 32 µmol, 1.0 equiv) in EtOH/H₂O (2:1, 1.5 mL) was added aq. CuSO₄ (6.6 µL, 0.5 M, 10 mol%) and aq. sodium ascorbate (6.6 µL, 1 M, 20 mol%). The reaction was stirred for 24 h at RT under an atmosphere of N₂. At this time, further aq. CuSO₄ (6.6 µL, 0.5 M, 10 mol%) and aq. sodium ascorbate (6.6 µL, 1 M, 20 mol%) were added and the reaction was stirred for 24 h. Analysis of the reaction mixture by ESI-MS showed full consumption of starting material. The reaction was cooled to 0 °C and cold H₂O was added, forming a brown precipitate. The solution was filtered and the solid precipitate was suspended in CH₂Cl₂, dried (Na₂SO₄), filtered and reduced in vacuo to give a brown solid which was purified by column chromatography (SiO₂, CH₂Cl₂/MeOH 19:1) to give the title compound 412 as a brown glassy solid (30 mg, 26 µmol, 81%); Rf (SiO₂, CH₂Cl₂/MeOH 19:1) 0.38; m.p. 178 – 181 °C; ¹H NMR (600 MHz, MeOD-d₄) δ_H 8.54 (s, 1H, CH-triazole), 8.37 (s, 1H, C=CH), 7.54 (d, J = 8.8 Hz, 1H, ArH), 7.34 – 7.30 (m, 2H, ArH), 7.28 – 7.23 (m, 4H, ArH), 7.14 (d, J = 8.2 Hz, 2H, ArH), 6.92 (d, J = 8.2 Hz, 2H, ArH), 6.83 (d, J =
8.2 Hz, 1H, ArH), 6.63 (s, 1H, ArH), 5.27 – 5.19 (m, 2H, OCH2), 4.61 (dd, J = 9.5, 6.2 Hz, 1H, CHα-Tyr), 4.37 (d, J = 8.7 Hz, 1H, CHα-Leu), 4.12 – 4.07 (m, 1H, CHα-Phe), 3.97 – 3.83 (m, 3H, CHHGAz, CHH-Az, CHH-Az), 3.80 – 3.60 (m, 3H, CHH-Az, CHH-Az, CHα-Val), 3.56 – 3.44 (m, 6H, CHHβ-Phe, CHα-Thr, 2 × CH2CH3), 3.38 – 3.34 (m, 1H, CHHβ-Phe), 3.29 – 3.23 (m, 1H, CHHβ-Tyr), 3.06 – 3.00 (m, 1H, CHHGAz), 2.93 (d, J = 3.8 Hz, 1H, CHβ-Thr), 2.74 (dd, J = 13.3, 10.6 Hz, 1H, CHHβ-Tyr), 2.10 – 2.02 (m, 1H, CHβ-Val), 1.54 – 1.49 (m, 1H, CHHβ-Leu), 1.32 (s, 9H, CHt-Bu), 1.25 – 1.22 (m, 7H, CHHβ-Leu, 2 × NCH2CH3), 1.15 (m, 1H, CHγ-Leu), 1.06 – 1.04 (m, 6H, 2 × CH2γ-Val), 1.03 – 1.02 (m, 3H, CHγ-γ-Thr), 1.00 (s, 9H, CH3, t’Bu), 0.79 – 0.72 (m, 6H, 2 × CH3δ-Leu); 13C NMR (151 MHz, MeOD-d4) δC 178.4 (C=O), 176.2 (C=O), 175.6 (C=O), 175.4 (C=O), 174.5 (C=O), 159.5 (C=O), 158.8 (C=O), 157.6 (C), 155.3 (C), 153.6 (C), 138.0 (C), 137.7 (CH), 134.6 (C), 131.7 (CH), 130.6 (CH), 130.2 (CH), 129.9 (CH), 128.3 (CH), 125.3 (CH), 122.7 (C), 122.1 (CH), 117.6 (C), 111.5 (CH), 108.2 (C), 97.8 (CH), 79.4 (C, t’Bu), 75.4 (C, t’Bu), 69.3 (CH, α-Thr), 64.9 (CH, β-Thr), 62.5 (CH, α-Leu), 59.1 (OCH2), 55.7 (CH, α-Val), 54.7 (CH, α-Phe and CH, α-Tyr), 46.9 (CH2, GAz), 45.9 (NCH2CH3), 40.2 (CH2, β-Leu), 37.9 (CH2, β-Tyr), 35.2 (CH2, β-Phe), 33.3 (CH, β-Val), 29.3 (CH3, t’Bu) 28.9 (CH3, t’Bu), 25.2 (CH, γ-Leu), 22.2 (CH3, γ-Val), 21.9 (CH3, γ-Thr), 21.8 (CH3, γ-Val), 19.9 (CH3, δ-Leu), 19.5 (CH3, δ-Leu), 12.7 (NCH2CH3). Note: C, Az and CH2, Az not observed; υmax (neat) = 3305, 2969, 1715, 1643, 1622, 1601; MS (ESI+) m/z 1182 [M+Na]+; HRMS (ESI+) calcd. for C62H53N11NaO11 [M+Na]+ 1182.6322, found 1182.6313; Optical rotation could not be measured due to high fluorescence of the compound.

Cyclo(Leu-Tyr-Val-GAz(click)-Thr-Phe) (413)

Cyclo(Leu-Tyr/(Bu)-Val-GAz( triazole-coumarin)-Thr/(Bu)-Phe) (412) (10.0 mg, 9.5 µmol, 1.0 equiv) was suspended in 4M HCl in dioxane (1.5 mL). MeOH (50 µL) was added to aid solubility and the reaction was stirred at RT for 1.5 h under N2. The reaction was monitored by TLC and ESI-MS until the starting material was completely consumed. At this time, the reaction mixture was reduced in vacuo and the material was resuspended in CH2Cl2 (10 mL) and reduced in vacuo (3×) to give the title compound 413 (11.8 mg) as a dark yellow solid without further purification required; m.p. 186 – 189 °C; 1H NMR (500 MHz, MeOD-d4) δH 8.57 (s, 1H, CH-triazole), 8.35 (s, 1H, C=CH), 7.55 (d, J = 8.9 Hz, 1H, ArH), 7.30 – 7.22 (m, 5H, ArH), 7.04 (d, J = 8.3 Hz, 2H, ArH), 6.86 (d, J = 8.3 Hz, 1H, ArH), 6.73 – 6.65 (m, 3H, ArH), 5.24 (s, 2H, OCH2), 4.63 – 4.51 (m, 1H, CHα-Tyr), 4.31 – 4.24
(m, 1H, CHα-Val), 4.21 – 4.14 (m, 1H, CHH-Az), 4.09 – 3.77 (m, 7H, CHH-Az, CH2-Az, CHHGAz, CHβ-Thr, CHα-Leu, CHα-Phe), 3.56 – 3.43 (m, 5H, CHα-Thr, 2 × NCH2CH3), 3.28 – 3.16 (m, 3H, CHHβ-Tyr, CHHβ-Phe, CHHGAz), 2.97 – 2.87 (m, 1H, CHHβ-Tyr), 2.11 – 2.01 (m, 1H, CHβ-Val), 1.52 – 1.47 (m, 1H, CHHβ-Leu), 1.38 – 1.31 (m, 2H, CHHβ-Leu, CHγ-Leu), 1.23 (t, J = 7.1 Hz, 6H, NCH2CH3), 1.04 – 0.99 (m, 6H, CHγ-Val, CHγ-Thr), 0.94 – 0.91 (m, 3H, CHγ-Val), 0.80 – 0.71 (m, 6H, 2 × CHδ-Leu), Note: CHHβ-Phe overlaps with solvent peak; 13C NMR (126 MHz, MeOD-d4) δC 174.93 (C=O), 174.85 (C=O), 174.8 (C=O), 173.32 (C=O), 173.25 (C=O), 158.8 (C=O), 157.7 (C=O), 157.4 (C), 157.3 (C), 155.4 (C), 137.92 (C), 137.86 (C), 131.7 (CH), 131.1 (CH), 130.2 (CH), 129.7 (CH), 128.1 (CH), 116.3 (CH), 108.9 (C), 68.8 (CH, β-Thr), 65.7 (CH, α-Thr), 62.2 (CH, α-Val), 62.2 (C, Az), 59.2 (OCH2), 55.8 (CH, α-Tyr, CH, α-Leu), 55.4 (CH, α-Leu), 46.3 (NCH2CH3), 43.7 (CH2, GAz), 40.5 (CH2, β-Leu), 38.8 (CH2, β-Tyr), 36.0 (CH2, β-Phe), 30.9 (CH, β-Val), 25.2 (CH, γ-Leu), 22.9 (CH3, γ-Thr), 22.5 (CH3, γ-Val), 20.4 (CH3, γ-Val), 19.9 (CH3, δ-Leu), 19.4 (CH3, δ-Leu), 12.6 (NCH2CH3), Note: 3 × C, 3 × CH, CH2, Az not observed; νmax (neat) = 3258, 2962, 1714, 1665, 1650, 1621, 1598 cm⁻¹; MS (ESI⁺) m/z 1070 [M+Na⁺]; HRMS (ESI⁺) calcd. for C54H69N11NaO11 [M+Na⁺] 1070.5070, found 1070.5077; Optical rotation could not be measured due to high fluorescence of the compound.

Analytical HPLC, 280 nm: Rf = 37.9 min. Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 ºC (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.
4.4 Kinetic measurement of the cyclisation reaction

HPLC measurements were conducted on an Agilent 1260 Infinity analytical HPLC system on an Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN; gradient: 0‒3 min, 3% B; 3‒14 min, 3‒20% B; 14‒20 min, 20% B; 20‒41 min, 20‒50% B; 41‒43 min, 50‒100% B; 43‒45 min, 100% B).

To separate 20 mL vials were added linear precursor 178 or 217 (10 μmol, 1.0 equiv) and anhydrous DMF (10 mL). At this time a 500 μL sample was withdrawn to determine the initial value by analytical HPLC before DEPBT (20 μmol, 2.0 equiv) and DIPEA (20 μmol, 2.0 equiv) were added to the solution. At designated time points, 500 μL of reaction mixture was taken and diluted with 200 μL distilled water. 10 μL of these samples were directly injected into the analytical HPLC. Further samples were taken after 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 26, 30, 34, 50 and 74 h and treated in the same manner. Signals for linear precursors 178 or 217 and cyclic peptides 181 or 184 as well as dimer 218 were integrated at 280 nm.

To check the accuracy of the integration at 280 nm, calibration curves for the linear and cyclic oxetane modified peptide 178 and 181 were measured by injecting 10 μL of stock solutions of known concentration. UV signals at 280 nm were integrated and the resulting areas plotted against the amount of injected compound in nmol. Linear fitting gave equations shown below for each compound. The obtained data show that conversions obtained from sole integration of the peaks at 280 nm are in accordance with yields obtained from the calibration curves. The same was assumed for the parent system (compound 184 and 217). Conversions were calculated from initial integral of linear precursor determined before addition of coupling reagent and led to 49% for cyclic peptide 184, 11% for the dimer 218 and 83% for cyclic oxetane modified peptide 181. Conversion obtained for the dimer was divided by two due to two Trp-residues in the structure. Retention times of linear precursors and formed products was confirmed by LC-MS (Bruker Amazon X) under the same HPLC conditions and injection of purified compounds.
4.4.1 Calibration curve for linear WLGOxG (178)

\[ y = 220.7x \]

4.4.2 Calibration curve for cyclic WLGOxG (181)

\[ y = 219.8x \]
4.4.3 UV and LC-MS traces (280 nm) of the cyclization toward oxetane modified peptide 181 over 74 h
4.4.4 LC-MS trace (280 nm) of the cyclization reaction toward cyclic peptide 181 after 8 h
4.4.4 UV and LC-MS traces (280 nm) of the cyclization reaction toward cyclic peptide 184 and cyclic dimer 218 over 74 h
4.4.5 LC-MS trace (280 nm) of the cyclization reaction toward cyclic peptide 184 and cyclic dimer 218 after 8 h
4.5 UV/vis and fluorescence spectroscopy of azetidine functionalised compounds

A solution of the fluorescent compound was prepared in methanol to a given concentration (nM-mM). Prior to UV/Vis spectroscopy, a blank was measured in methanol. All measurements were carried out using a quartz cuvette (10 mm × 10 mm) filled to a total volume of 2 mL.

Fluorescence excitation spectra were measured using UV/Vis absorption spectroscopy on an Agilent Cary 60 UV-vis spectrometer. Measurements were taken in the range of 200-800 nm.

Fluorescence emission spectra were measured using fluorescence spectroscopy on an Agilent Technologies Cary Eclipse Fluorescence spectrometer. Emission spectra were collected by excitation at the maximal value obtained from the UV/Vis measurement. Slit widths are reported below for each compound and are either 2.5 mm or 5 mm.

The data was normalised by dividing the UV absorption/fluorescence values at each wavelength by the maximal UV absorption/fluorescence value for each set of data.

Compound **402**: 0.250 mM in MeOH. Fluorescence slit width: 5 mm.

\[ \lambda_{\text{max, ex}} = 341 \text{ nm}, \lambda_{\text{max, em}} = 534 \text{ nm}, \Delta \lambda = 193 \text{ nm} \]
Compound 405: 0.378 mM in MeOH. Fluorescence slit width = 5 mm.

$\lambda_{\text{max, ex}} = 341$ nm, $\lambda_{\text{max, em}} = 680$ nm, $\Delta\lambda = 339$ nm

![Graph of Cyclo(FEGAzLG) dansyl •TFA](image)

Compound 411: 0.105 mM in MeOH. Fluorescence slit width: 2.5 mm.

$\lambda_{\text{max}} = 409$ nm, $\lambda_{\text{max}} = 483$ nm, $\Delta\lambda = 74$ nm

![Graph of Cyclo(YVGAzGF) coumarin conjugate](image)
Compound 413: 0.100 mM in methanol. Fluorescence slit width = 5 mm.

\[ \lambda_{\text{max}} = 407 \text{ nm}, \lambda_{\text{max}} = 482 \text{ nm}, \Delta \lambda = 75 \text{ nm} \]

### 4.6 XRD analysis of 398

Single crystals of C_{33}H_{47}N_{9}O_{6.33333} were grown from methanol. A suitable crystal was selected and mounted on a glass fibre with Fomblin oil and placed on a Rigaku Oxford Diffraction Synergy-S diffractometer with a duel source equipped with a Hybrid pixel array detector. The crystal was kept at 100(2) K during data collection. Using Olex2, the structure was solved with the ShelXT structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimisation.

Crystal Data for C_{33}H_{47}N_{9}O_{6.33333} (M =671.13 g/mol): monoclinic, space group P2_1 (no. 4), \( a = 22.4901(8) \text{ Å}, b = 11.3207(2) \text{ Å}, c = 24.6000(11) \text{ Å}, \beta = 116.322(5)^\circ, V = 5613.9(4) \text{ Å}^3, Z = 6, T = 100(2) \text{ K}, \mu(\text{CuK}\alpha) = 0.693 \text{ mm}^{-1}, D_{\text{calc}} = 1.191 \text{ g/cm}^3, 110723 \text{ reflections measured} (7.134^\circ \leq 2\theta \leq 160.59^\circ), 23865 \text{ unique} (R_{\text{int}} = 0.0629, R_{\text{sigma}} = 0.0398) \text{ which were used in all calculations. The final} R_1 \text{ was 0.0766 (I > 2}\sigma(1) \text{ and} wR_2 \text{ was 0.2493 (all data).}
4.7 Confocal microscopy of compound 413

The human alveolar epithelial cell line, A549, was maintained in DMEM medium supplemented with 10 % (v/v) fetal bovine serum and 1 % (v/v) penicillin/streptomycin at 37°C in an atmosphere containing 5 % CO2. For microscopy, cells were seeded in a detachable 8-well chamber tissue culture-treated glass slide (Falcon, Corning) at a density of 3.75 x 103/well and incubated overnight. The following day the media was removed and fresh media were added containing dimethyl sulfoxide (0.5 % v/v) or coumarine-conjugated dichotomin at a concentration of 25 μg/ml. For this purpose, a stock solution of the compound was freshly prepared in DMSO at 5 mg/ml and diluted down to the working concentration in cell culture media directly. After 4 h of incubation, the media was removed from the wells and the cells were washed twice in PBS and fixed using 4 % paraformaldehyde solution. The cells were washed in PBS and permeabilised using 0.25 % Triton-X100 in PBS (v/v) for 10 min, followed by another two washes in PBS. The cells were then stained using Phalloidin-iFluorTM 555 (AAT Bioquest) to visualise the actin cytoskeleton (at a 1:500 dilution of the stock solution) and DAPI (1 μg/ml) to visualise nucleic acids for 30 min. The cells were again washed twice with PBS and the slide was allowed to air-dry following removal of the polystyrene chamber. To prevent photobleaching, SlowFade Gold antifade mountant was added and a coverslip was mounted for viewing.

Confocal microscopy was performed using a Zeiss LSM 880 instrument using an EC Plan-Neofluar 40x/1.30 Oil objective. The pinhole size was set to 1 AiryUnit. Z-sectioning was performed to allow three-dimensional reconstruction.
References


[21] J. D. Beadle, Oxetane-Based Peptidomimetics, University of Warwick, **2018**.


1548.


Appendix I - Overlaid $^1$H NMR spectra of oxetane modified 175, before (black) and after (red) treatment with 70% TFA/CH$_2$Cl$_2$ for 24 hours.

Overlaid $^1$H NMR spectra of azetidine modified 343, before (black) and after (red) treatment with 70% TFA/CH$_2$Cl$_2$ for 24 hours.
Appendix II - NOESY analysis of 378 to determine stereochemistry over a range of mixing times, collected by Eleanor Jayawant. NOE’s observed between the alanine CHα and the proline CH₂δ atoms:
<table>
<thead>
<tr>
<th>Signal</th>
<th>$\delta_H$ (ppm), multiplicity, $J$ value</th>
<th>$381$–A</th>
<th>$381$–B</th>
<th>$215$</th>
<th>$\Delta \delta_H$ ($381$–A and $215$)</th>
<th>$\Delta \delta_H$ ($381$–B and $215$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ar-H$_{a1}$, Trp</td>
<td>7.58, d, 7.9 Hz</td>
<td>7.62, d, 7.9 Hz</td>
<td>7.62, d, 7.9 Hz</td>
<td>-0.04</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Ar-H$_{a2}$, Trp</td>
<td>7.35 - 7.30, m</td>
<td>7.33, d, 8.1 Hz</td>
<td>7.32, d, 8.1 Hz</td>
<td>n/a</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ar-H$_c$, Trp</td>
<td>7.15 - 7.06, m</td>
<td>7.13, s</td>
<td>7.12, s</td>
<td>n/a</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ar-H$_{b1}$, Trp</td>
<td>7.15 - 7.06, m</td>
<td>7.10, t, 7.5 Hz</td>
<td>7.10, t, 7.7 Hz</td>
<td>n/a</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Ar-H$_{b2}$, Trp</td>
<td>7.04 - 7.01, m</td>
<td>7.03, t, 7.4 Hz</td>
<td>7.03, t, 7.4 Hz</td>
<td>n/a</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>CH, α-Trp</td>
<td>4.60, t, 7.9 Hz</td>
<td>4.97, dd, 10.2, 7.2 Hz</td>
<td>4.93, dd, 10.2, 7.2 Hz</td>
<td>0.37</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>CH, α-Leu</td>
<td>3.51 - 3.47, m</td>
<td>4.27 - 4.20, m</td>
<td>4.20, dd, 10.9, 4.2 Hz</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>CH, α-Ala</td>
<td>4.18, q, 6.6 Hz</td>
<td>4.27 - 4.20, m</td>
<td>4.57, q, 7.2 Hz</td>
<td>-0.39</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>CHH Gly</td>
<td>3.57, d, 17.0 Hz</td>
<td>3.52, d, 16.7 Hz</td>
<td>3.67, d, 16.6 Hz</td>
<td>-0.10</td>
<td>-0.15</td>
<td></td>
</tr>
<tr>
<td>CHH Gly</td>
<td>3.40, d, 17.0 Hz</td>
<td>3.40, d, 16.8 Hz</td>
<td>3.57, d, 16.6 Hz</td>
<td>-0.17</td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>CHH $\beta$-Trp</td>
<td>3.13, dd, 14.3, 7.5 Hz</td>
<td>3.21, dd, 15.1, 7.1 Hz</td>
<td>3.21, dd, 15.1, 7.2 Hz</td>
<td>-0.08</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>CHH, β-Leu</td>
<td>1.74 - 1.58, m</td>
<td>1.68 - 1.60, m</td>
<td>1.72 - 1.63, m</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>CHH, β-Leu</td>
<td>1.74 - 1.58, m</td>
<td>1.58 - 1.45, m</td>
<td>1.59 - 1.48, m</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>CH$_3$, β-Ala</td>
<td>1.18, d, 6.8 Hz</td>
<td>1.14, d, 6.9 Hz</td>
<td>1.12, d, 6.9 Hz</td>
<td>0.06</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>CH, γ-Leu</td>
<td>1.40 - 1.36, m</td>
<td>1.58 - 1.45, m</td>
<td>1.59 - 1.48, m</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>CH$_3$, δ-Leu1</td>
<td>0.87, d, 6.6 Hz</td>
<td>0.89, d, 6.3 Hz</td>
<td>0.90, d, 6.0 Hz</td>
<td>-0.01</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>CH$_3$, δ-Leu2</td>
<td>0.79, d, 6.3 Hz</td>
<td>0.72, d, 6.3 Hz</td>
<td>0.73, d, 6.0 Hz</td>
<td>-0.01</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

**Appendix IIIa:** Comparison of the $^1$H NMR spectra of 215, major conformer of 381–A and 381–B. (CHH$\beta$-Trp not included due to overlap with solvent signal).
<table>
<thead>
<tr>
<th>Signal</th>
<th>δ&lt;sub&gt;C&lt;/sub&gt; 381–A</th>
<th>δ&lt;sub&gt;C&lt;/sub&gt; 381–B</th>
<th>δ&lt;sub&gt;C&lt;/sub&gt; 215</th>
<th>Δ δ&lt;sub&gt;C&lt;/sub&gt; (381–A and 215)</th>
<th>Δ δ&lt;sub&gt;C&lt;/sub&gt; (381–B and 215)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=O, Gly</td>
<td>177.9</td>
<td>176.3</td>
<td>176.4</td>
<td>1.50</td>
<td>−0.10</td>
</tr>
<tr>
<td>C=O, Leu</td>
<td>174.2</td>
<td>175.2</td>
<td>175.3</td>
<td>1.10</td>
<td>−0.10</td>
</tr>
<tr>
<td>C=O, Trp</td>
<td>173.7</td>
<td>173.6</td>
<td>173.7</td>
<td>0.00</td>
<td>−0.10</td>
</tr>
<tr>
<td>Ar-H&lt;sub&gt;a1&lt;/sub&gt;, Trp</td>
<td>119.1</td>
<td>119</td>
<td>119.1</td>
<td>0.00</td>
<td>−0.10</td>
</tr>
<tr>
<td>Ar-H&lt;sub&gt;a2&lt;/sub&gt;, Trp</td>
<td>112.3</td>
<td>112.4</td>
<td>112.3</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>Ar-H&lt;sub&gt;c&lt;/sub&gt;, Trp</td>
<td>124.4</td>
<td>123.8</td>
<td>123.8</td>
<td>0.60</td>
<td>0.00</td>
</tr>
<tr>
<td>Ar-H&lt;sub&gt;b1&lt;/sub&gt;, Trp</td>
<td>122.6</td>
<td>122.6</td>
<td>122.6</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ar-H&lt;sub&gt;b2&lt;/sub&gt;, Trp</td>
<td>119.9</td>
<td>119.9</td>
<td>119.9</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Ar-C</td>
<td>138.1</td>
<td>138</td>
<td>138</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Ar-Cγ</td>
<td>110.2</td>
<td>110.3</td>
<td>110.3</td>
<td>−0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>CH, α-Trp</td>
<td>58.7</td>
<td>57.6</td>
<td>57.7</td>
<td>1.00</td>
<td>−0.10</td>
</tr>
<tr>
<td>CH, α-Leu</td>
<td>53.7</td>
<td>55.2</td>
<td>55.3</td>
<td>−1.60</td>
<td>−0.10</td>
</tr>
<tr>
<td>CH, α-Ala</td>
<td>52.5</td>
<td>52</td>
<td>51.6</td>
<td>0.90</td>
<td>0.40</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt; Gly</td>
<td>48.2</td>
<td>48.2</td>
<td>48.8</td>
<td>−0.60</td>
<td>−0.60</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;, β-Trp</td>
<td>39.3</td>
<td>40.6</td>
<td>40.6</td>
<td>−1.30</td>
<td>0.00</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;, β-Leu</td>
<td>26.8</td>
<td>27.6</td>
<td>27.6</td>
<td>−0.80</td>
<td>0.00</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;, β-Ala</td>
<td>14.3</td>
<td>13.6</td>
<td>13.5</td>
<td>0.80</td>
<td>0.10</td>
</tr>
<tr>
<td>CH, γ-Leu</td>
<td>25.8</td>
<td>26.2</td>
<td>26.2</td>
<td>−0.40</td>
<td>0.00</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;, δ-Leu1</td>
<td>23.9</td>
<td>23.2</td>
<td>23.3</td>
<td>0.60</td>
<td>−0.10</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt;, δ-Leu2</td>
<td>21.5</td>
<td>21.2</td>
<td>21.2</td>
<td>0.30</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Appendix IIIb:** Comparison of the $^{13}$C NMR spectra of 215, major conformer of 381-A and 381-B.
Appendix IV: $^1$H NMR analysis to determine d.r of building block 383

$^1$H NMR, DMSO-$d_6$, 500 MHz: integration of CH$_3$, β-AAz:

[Image of H NMR spectrum]

Appendix V: Analytical HPLC and $^1$H NMR analysis of crude cyclisation mixture of precursor 385 and cyclic peptide 386.

Analytical HPLC, 280 nm: $R_f = 38.4$ min (major) and 39.1 (minor). Agilent Eclipse Plus C18 column (5.0 μm, 4.6 × 150 mm) with a flow rate of 1.0 mL/min at 20 °C (solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Gradient: 0-3 min, 3% B; 3-33 min, 3-50% B; 33-38 min, 50-100% B, 38-40 min, 100%-3% B; 40-45 min, 3% B.

[Image of HPLC chromatogram]

$^1$H NMR, CD$_3$OD, 500 MHz: Integrated peaks relate to the indole aromatic CH protons.