

# **Approaches for the Discovery of Inhibitors of Protein-Protein Interactions**

Jack Herbert

Submitted in accordance with the requirements for the degree  
of Doctor of Philosophy

The University of Leeds  
School of Chemistry

December 2022

The candidate confirms that the work submitted is his own and that the appropriate credit has been given where references has been made to the work of others.

This copy has been supplied on the understand that it is copyright material and that no quotation from the thesis may be published without prior acknowledgement.

The right of Jack Herbert to be identified as Author of this work has been asserted by him in accordance with the Copyright, Designs and Patents Act 1988.

© 2022 The University of Leeds and Jack Herbert

## Acknowledgements

Having spent the last eight years of my life in Leeds, it will be sad to say goodbye, but I believe I am not ready to move on to whatever the future may bring!

I would first like to thank both of my supervisors, Adam Nelson and Stuart Warriner, for accepting me into their research groups and for all of their support over the past few years. You both provided me with the perfect PhD project I was looking for! Although it was challenging at times it has been a very rewarding experience and has allowed me to grow both as a chemist and as a person.

I have had the pleasure of working with some fantastic people over the years in G56. I would like to thank all the past and present members of the Nelson group I have met.

I would like to thank George, Chloe and Adam for helping me so much when I first begun my PhD. Chris, Luke and Liam – thank you for the laughs over the years and all your help with biology. Abbie and Scott – when the lab group shrunk after the lockdown your friendship and the comradery between us became even more pronounced, thank you for keeping up lab morale. Sam and Dan, your sense of humours never failed to brighten up my day and your advice has been invaluable! A special thanks to Dan for helping me with protein expression and purification. Julian – although I've only known you for a year it's been a pleasure and I can't wait to see what you do next, even if you complain a lot. Karan and Muneeba – although you've only just started your PhDs I know you're going to smash it. Best of luck!

I'd also like to thank members of the Marsden group for your help and laughs over the past few years, in particular Alex, Harrison and Callum, and of course Steve for the mechanism questions.

Finally, I would like to give a special thanks to my parents, family and friends for their support throughout my PhD.

# Table of Contents

<b>Acknowledgements</b> .....	<b>i</b>
<b>Table of Contents</b> .....	<b>ii</b>
<b>Abstract</b> .....	<b>vi</b>
<b>List of Abbreviations</b> .....	<b>vii</b>
<b>1 Introduction</b> .....	<b>1</b>
1.1 Protein-Protein Interactions .....	1
1.2 Overview of the Drug Discovery Process .....	4
1.3 Exploration of Biologically Relevant Chemical Space .....	6
1.4 Lead Discovery Technologies .....	8
1.4.1 High-Throughput Screening .....	9
1.4.2 Fragment-Based Drug Discovery .....	10
1.4.3 DNA Encoded Libraries .....	15
1.4.4 Activity-Directed Synthesis .....	17
1.4 Thesis Aims .....	20
<b>2 Activity-Directed Synthesis of Mcl-1 Inhibitors</b> .....	<b>22</b>
2.1 Introduction to Mcl-1 .....	22
2.1.1 Biology of Mcl-1 .....	22
2.1.2 Structural Biology of Mcl-1 .....	24
2.1.3 Discovery of Mcl-1 Inhibitors .....	25
2.2 Aims and Objectives .....	30
2.3 Results and Discussion .....	32
2.3.1 Round One of Activity-Directed Synthesis .....	32
2.3.1.1 Design of Fragments for Activity-Directed Synthesis .....	32
2.3.1.2 Synthesis of Diazos and Co-Substrates .....	36
2.3.1.3 Optimisation of In Situ Deprotection .....	40
2.3.1.4 Configuration of Fluorescence Anisotropy Assay .....	42
2.3.1.5 Determination of the Inherent Activity of Round One Substrates ..	45
2.3.1.6 Implementation of Reaction Array .....	46
2.3.1.7 Scale-up of Round One Hit Combinations .....	51
2.3.2 Round Two of Activity-Directed Synthesis .....	55
2.3.2.1 Design of Fragments for Activity-Directed Synthesis .....	55
2.3.2.2 Synthesis of Diazos .....	56
2.3.2.3 Inherent Activity of Round Two Substrates .....	59
2.3.2.4 Implementation of Reaction Array .....	60

2.3.2.5 Scale-up of Round Two Hit Combinations.....	64
2.4 Summary and Conclusion .....	67
<b>3 Activity-Directed Synthesis of PEX5 Inhibitors .....</b>	<b>68</b>
3.1 Introduction to PEX5.....	68
3.1.1 PEX5 Biology .....	68
3.1.2 Small-Molecule PEX5 Inhibitors .....	74
3.2 Aims and Objectives.....	76
3.3 Results and discussion .....	78
3.3.1 Synthesis of Fluorescence Anisotropy Tracer: Lissamine-Labelled YQSKL.....	78
3.3.2 Expression and Purification of PEX5 .....	79
3.3.3 Configuration of Fluorescence Anisotropy Assay .....	80
3.3.4 Synthesis and Evaluation of N-Acetylated Peptides .....	81
3.3.5 Determination of DMSO Tolerance of the Assay.....	84
3.3.6 Optimisation of In Situ Deprotection .....	85
3.3.7 Design of Fragments for Activity-Directed Synthesis .....	86
3.3.8 Synthesis of Diazos .....	87
3.3.9 Inherent Activity of Diazos and Substrates .....	89
3.3.10 Implementation of Reaction Arrays .....	90
3.4 Summary and Conclusion .....	96
<b>4 Design, Synthesis and Evaluation of PEX5 Inhibitors.....</b>	<b>97</b>
4.1 Aims and Objectives.....	97
4.2 Design of a First Round of PEX5-PTS1 Inhibitors .....	98
4.3 Synthesis of Designed PEX5-PTS1 Inhibitors .....	100
4.4 Screening of PEX5 Inhibitors .....	103
4.5 SAR Studies of Hit Compounds.....	105
4.5.1 Attempts to Discover a Growth Vector .....	105
4.5.2 Optimisation of Sulfonamide Hit <b>121</b> .....	106
4.5.3 Optimisation of Amide Hit <b>119</b> .....	108
4.5.3.1 Variation of C-Terminal Amino Acid .....	108
4.5.3.2 Effects of Amine Position and Stereochemistry .....	110
4.5.3.3 Effects of Ring Size and Methylation.....	112
4.5.3.4 Effects of Substitution and Conformation Restriction .....	114
4.5.3.5 Combining Positive Elements of Hit Compounds .....	116
4.6 Docking Studies .....	116
4.6.1 Docking of Previously Assessed Compounds .....	116
4.6.2 Design of Docking Library.....	119

4.6.3 Synthesis of Docking Hits .....	124
4.6.4 Screening of Docking Hits.....	130
4.7 Summary and Conclusion .....	131
4.8 Thesis Conclusion .....	135
<b>5 Experimental .....</b>	<b>136</b>
5.1 General Information.....	136
5.2 Implementation of Reaction Arrays for Mcl-1 .....	137
5.2.1 Round One .....	138
5.2.2 Round Two .....	139
5.3 Implementation of Reaction Arrays for PEX5.....	141
5.3.1 Round One .....	141
5.4 General Procedures .....	143
5.5 Experimental Data .....	146
5.6 Biology .....	250
5.6.1 FAM-Bid Tracer .....	250
5.6.2 Mcl-1 Protein Sequence.....	250
5.6.3 At-His-PEX5C Plasmid Map .....	251
5.6.4 At-His-PEX5C DNA Sequence .....	251
5.6.5 At-His-PEX5C Protein Sequence .....	252
5.6.6 Protein Expression.....	253
5.6.6.1 Cell lines, media and buffers .....	253
5.6.6.2 Transformation of Plasmids.....	255
5.6.6.3 Protein Expression .....	255
5.6.6.4 Cell Lysis .....	255
5.6.6.5 Purification of At-His <sub>6</sub> -PEX5C.....	256
5.6.7 Fluorescence Anisotropy Inhibition Assay .....	258
5.6.7.1 Measurement of Anisotropy .....	258
5.6.7.2 Determination of Limits of Anisotropy .....	259
5.6.7.2.1 Mcl-1.....	259
5.6.7.2.2 PEX5 .....	260
5.6.7.3 Determination of the Dissociation Constant of the Fluorescent Tracers.....	260
5.6.7.4 Determination of IC <sub>50</sub> of Unlabelled Ligands.....	261
5.6.7.4.1 Mcl-1 .....	261
5.6.7.4.2 PEX5 .....	262
5.6.7.5 Screening of Crude Reaction Array Mixtures.....	262
5.6.7.5.1 Mcl-1.....	262

5.6.7.5.2 PEX5 .....	263
5.6.7.6 Determination of the Inhibitory Constant of Unlabelled Ligands...	263
5.7 Docking .....	264
5.7.1 Protocol for Docking of PEX5 Ligands .....	264
5.7.2 SMILES String for Docked Library.....	265
<b>6 References</b> .....	<b>278</b>
<b>7 Appendix</b> .....	<b>289</b>
7.1 Dose-Response for PEX5 Inhibitors.....	289

## Abstract

Activity-directed synthesis (ADS) is a concept that aims to expand the exploration of chemical space by performing diverse reaction arrays in which multiple products can be performed. The crude products are then screened and active combinations, validated, scaled-up and compounds purified, characterised and biologically assessed. Reaction arrays targeting protein-protein interactions (PPIs), Mcl-1/BID and PEX5/PTS1, were performed. This approach to the discovery of inhibitors of PPIs was then compared to a more conventional approach to discovery with one of the PPI targets, utilising rounds of design-synthesis-purification-evaluation.

Chapter one discusses the nature of PPIs, reviewing their structure and their importance within biology and drug discovery. Some examples of PPI inhibitors are also discussed. Medicinal chemistry will then be discussed, covering the pharmaceutical industry and the drug development process, as well as techniques used for lead discovery.

Chapter two describes the ADS of inhibitors of the Mcl-1/BID PPI. A fluorescence anisotropy assay was re-established for screening crude reaction mixtures. A workflow for the execution of reaction arrays, and the screening of the resulting crude products, was established. An array of diazos and co-substrates was designed and executed. Active compounds were discovered which were then used to inform the design a second array.

Chapter three describes the ADS of inhibitors of the PEX5/PTS1 PPI. PEX5 was expressed and purified and a fluorescent tracer synthesised and purified to be used in assays. A fluorescence anisotropy assay was re-established for screening crude reaction mixtures. An array of diazos and co-substrates was designed and executed. Crude reaction mixtures were screened and active combinations scaled-up, although no intermolecular bioactive products were discovered.

Chapter four describes the synthesis and evaluation of inhibitors of PEX5/PTS1 in which a structure-guided medicinal chemistry approach. The structure of the PTS1 peptide was used as a basis to design compounds. Several rounds of synthesis and screening were performed resulting in compounds that are more ligand efficient than the starting point. Molecular docking was used to rationalise the choice of which compounds were to be synthesised.



## List of Abbreviations

ADS	Activity-Directed Synthesis
Ac	Acetyl
Ac <sub>2</sub> O	Acetic Anhydride
Ahx	6-Aminohexanoic Acid
AIM	Auto-Induction Media
app.	Apparent
Ar	Aromatic
ATP	Adenosine Triphosphate
b.p.	Boiling Point
BAD	Bcl-2 Associated Agonist of Cell Death
BAK	Bcl-2 homologous killer
BAX	Bcl-2-associated X protein
BB	Building block
Bcl-2	B-cell lymphoma-2
BID	BH3 interacting-domain death agonist
BIM	Bcl-2-like protein 11
Boc	<i>tert</i> -Butyloxycarbonyl
br.	Broad
cap	Caprolactam
CAS	Chemical Abstracts Service
Cbz	Benzyl Chloroformate
CbzCl	Benzyloxycarbonyl
COSY	Correlation Spectroscopy
d	Doublet
Da	Daltons
DBA	Dibenzylideneacetone

DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DEL	DNA-Encoded Libraries
DEPT	Distortionless Enhancement by Polarization Transfer
dia	Diastereomer
DIAD	Diisopropyl Azodicarboxylate
DIC	<i>N,N'</i> -Diisopropylcarbodiimide
DIPEA	<i>N,N</i> -diisopropylethylamine
DMA	Dimethylacetamide
DMF	<i>N,N'</i> -dimethylformamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribose Nucleic Acid
<i>e.g.</i>	Example gratia; for example
EC <sub>50</sub>	Half Maximal Effective Concentration
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic Acid
ELSD	Evaporative Light Scattering Detector
ERK1/2	Extracellular Signal-Regulated Kinase 1/2
ES	Electrospray Ionisation
Et	Ethyl
<i>etc.</i>	Extracellular Signal-Regulated Kinases
EtOH	Ethanol
FA	Fluorescence Anisotropy
FAM	6-Carboxyfluorescein
FBDD	Fragment-Based Drug Discovery
FITC	Fluorescein Isothiocyanate
FP	Fluorescence Polarisation
GKAP	Guanylate Kinase-Associated <i>Protein</i>
HA	Heavy Atoms

<i>hDM2</i>	Human Double Minute 2 <i>Protein</i>
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
HTS	High-Throughput Screening
i.e.	<i>id est</i> ; that is
IC <sub>50</sub>	Half-Maximal Inhibitory Concentration
ICAM-1	Intercellular Adhesion Molecule 1
<i>in vitro</i>	Outside of the living
<i>in vivo</i>	Within the living
IR	Infrared
<i>J</i>	Spin-Spin Coupling Constant
K <sub>d</sub>	Dissociation Constant
K <sub>i</sub>	Inhibitory Constant
LB	Luria-Bertani
LCMS	Liquid Chromatography-Mass Spectrometry
LE	Ligand Efficiency
LFA-1	Lymphocyte Function-Associated Antigen 1
logP	Octanol-Water Partition Coefficient
m	Multiplet
Mcl-1	Induced Myeloid Leukemia Cell Differentiation Protein
MDAP	Mass-Directed Autopurification
MDM2	Mouse Double Minute 2 Homolog
Me	Methyl
M <sub>w</sub>	Molecular weight
NME	New Molecular Entity
NMR	Nuclear Magnetic Resonance

NOE	Nuclear Overhauser Effect
NOESY	nuclear Overhauser Effect Spectroscopy
NOXA	Phorbol-12-myristate-13-acetate-induced <i>protein 1</i>
<i>p</i>	Para
p	Pentet
p53	Tumor Protein P53
<i>p</i> -ABSA	4-Acetamidobenzenesulfonyl azide
PDB	Protein Data Bank
PEX14	Peroxisomal Membrane Protein 14
PEX5	Peroxisomal Targeting Signal 1 Receptor
pfb	Perfluorobutyrate
PHAs	Polyhydroxyalkanoates
piv	Pivaloyl
PMI	Principle Moment of Inertia
PMT	Photomultiplier Tube
PPIs	Protein-Protein Interactions
ppm	Parts Per Million
PTS1	Peroxisomal Targeting Signal 1
q	Quarter
r.t	Room Temperature
R <sub>f</sub>	Retention Factor
ROS	Reactive Oxygen Species
rot	Rotamer
s	Single
SAR	Structure-Activity Relationship
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SHANK1	SH3 And Multiple Ankyrin Repeat Domains 1
SMILES	Simplified Molecular Input Line Entry System
S <sub>N</sub> Ar	Nucleophilic Aromatic Substitution

t	Triplet
TEBAC	Benzyl Triethyl Ammonium Chloride
<i>tert</i>	Tertiary
TES	Triethylsilane
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin-Layer Chromatography
TPRs	Tetratricopeptide Repeats
Ts	Tosyl
XP	Extra-Precision
$\delta$	Chemical shift

# 1 Introduction

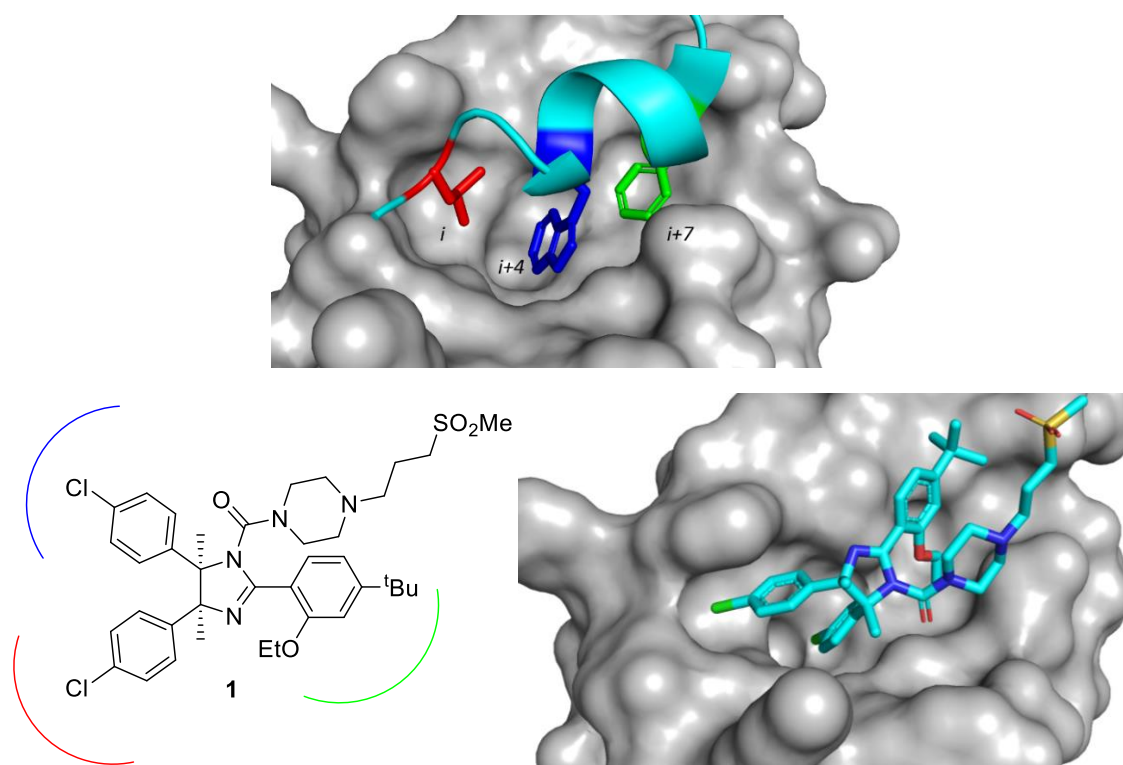
Biologically active small molecules are of the utmost importance in medicinal chemistry and chemical biology.<sup>1</sup> The dominance of small molecules in drug discovery can be ascribed to their small size and simple structures, often leading to predictable pharmacological properties, allowing diffusion into cells and ease of synthesis on a large scale.<sup>2</sup> In addition to their application to drug discovery, small molecules can be used as chemical probes to enable research into biological function. However, the discovery of bioactive small molecules is very challenging. Some of the difficulties in their discovery are as result of the techniques that are currently employed to aid in their development. The workflow used in conventional medicinal chemistry has resulted in a reliance on a small yet robust set of reactions, which in turn has limited chemical diversity in bioactive molecules.<sup>3</sup> A lack of productivity within the pharmaceutical industry, largely due to high attrition rates, has increase interest in alternative molecular discovery processes, such as DNA encoded libraries.<sup>4</sup> Herein, an overview of current approaches for the discovery of bioactive molecules is given (Section 1.4). Function driven approaches, which aim to disrupt the workflow used in current molecular discovery techniques are also discussed.

## 1.1 Protein-Protein Interactions

Protein-protein interactions (PPIs) are fundamental within biology and disease, with an estimated 130,000 – 6000,000 pairwise PPIs in the human body.<sup>5,6</sup> Despite their importance, there are relatively few drugs on the market that target PPIs, with most drug targets being enzymes, ion channels and receptors.<sup>7</sup> These classic drug targets usually contain small, well-defined binding sites for ligands to interact with.<sup>8</sup> On the other hand, PPIs are characterised by exceedingly large binding sites, usually reaching 1500-3000Å, containing few grooves or pockets for compounds to bind.<sup>9</sup> In addition, most classic drug targets have endogenous small molecule ligands, which may be used as a starting point for the development of a drug, whereas PPIs lack these ligands for reference.<sup>10</sup>

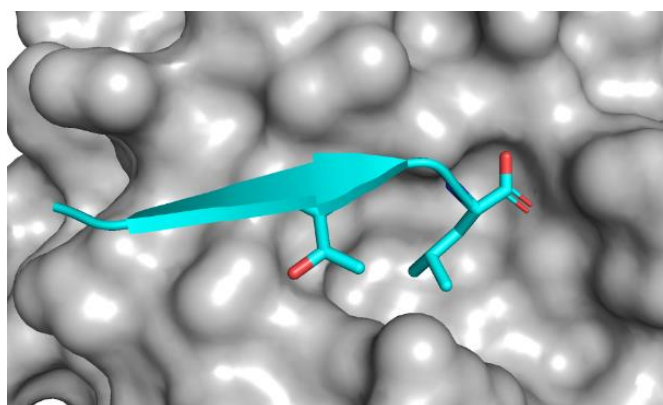
With the binding site for PPIs being so large they are not considered tractable drug targets. However, areas on the proteins surface known as 'hot-spots' make inhibition possible.<sup>11</sup> These are areas on the surface that form key interactions with a binding peptide, responsible for much of the binding.<sup>12</sup> Hot spot residues are commonly identified through alanine scanning experiments. Each amino acid in the sequence of the binding peptide is swapped for alanine and the change in binding free energy observed to identify residues which are important for binding.<sup>13</sup>

Protein-protein interactions can be mediated by proteins with differing secondary structure, for example  $\alpha$ -helices. An example of a PPI mediated by  $\alpha$ -helices is MDM2/p53. The binding of p53 is dominated by three key hot-spot residues targeting three sub-pockets on the surface of MDM2 (Figure 1).<sup>14</sup> Inhibitors of the MDM2/p53 PPI can be designed by mimicking these three key residues, for example RG7112 **1**.<sup>15</sup> The *p*-chlorophenyl groups mimic the *i* and *i*+4 residues of p53, whereas the *p*-*tert*-butyl phenyl mimics the *i*+7 residue.



**Figure 1:** The MDM2/p53 PPI highlighting the hot-spot residues responsible for binding (above). PDB Accession Code: 4HFZ. RG7112 **1**, a known inhibitor of MDM2/p53 mimics the p53 hot-spot residues, *i* (red), *i*+4 (blue), *i*+7 (green)(above). PDB Accession Code: 4IPF.

Protein-protein interactions may also be mediated by  $\beta$ -strands, for example in the case of GKAP/SHANK1-PDZ. GKAP consists of six amino acids (Ac-Glu-Ala-Gln-Thr-Arg-Leu-CO<sub>2</sub>H), with the C-terminal carboxylate forming key hydrogen bonds with the backbone of SHANK1.<sup>16,17</sup> Through computational and experimental analysis, it was revealed that the Leu and Thr residues were crucial to binding, along with the C-terminal carboxylate (Figure 2).<sup>18,19</sup>

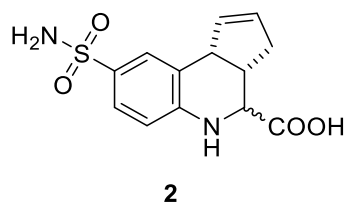


**Figure 2:** The GKAP/SHANK1-PDZ PPI, highlighting the key Leu and Thr residues responsible for binding. PDB Accession Code: 1Q3P.

Inhibition of the stand-mediated PPIs, such as GKAP/SHANK1-PDZ, by small-molecule is challenging, because binding of the native peptide relies on both side chain recognition and an extensive hydrogen bonding network formed with the peptide backbone, which is not easily mimicked by small-molecule inhibitors.<sup>20</sup>

The query-guided discovery of GKAP/SHANK1-PDZ inhibitors has recently been reported, in which key parts of the peptide were used to guide molecular design.<sup>20</sup> In an attempt to discover inhibitors of the PPI, the hotspots were incorporated into queries, which libraries of small-molecule were shape matched against and promising compounds docked, synthesised and screened for activity. When the workflow was implemented, only flexible peptide-based inhibitors were discovered, possibly due to the high degrees of freedom in the Leu sidechain. After switching to phenylalanine and conforming the change was tolerated, the workflow was employed against. This resulted in the discovery of a range of small-molecule candidate inhibitors, including compound **2**, which displayed an IC<sub>50</sub> ~ 300  $\mu$ M.



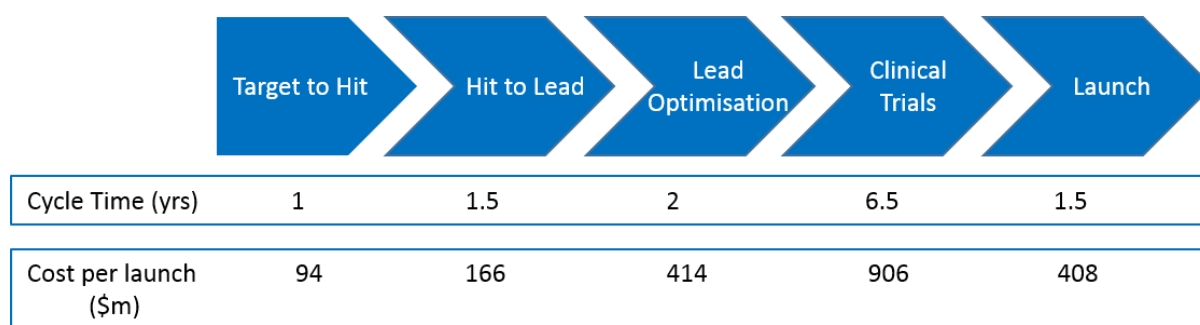


**Figure 3:** Structure of compound **2**, an inhibitor of the GKAP/SHANK1-PDZ PPI.

## 1.2 Overview of the Drug Discovery Process

The process of developing a new molecular entity (NME) takes approximately 13 years and costs *c.a.* \$2 billion, with only around 8% of NMEs making it to launch from preclinical selection.<sup>4</sup> Rates of attrition in clinical trials are extremely high, estimated at 66% and 30% in phase II and III respectively, meaning new NMEs carry large costs incurred from failed campaigns. Early-stage drug discovery is very expensive because many programmes are needed in order to develop a new drug. The clinical development is the costliest part of the process, accounting for up to 63% of the total costs involved in the discovery and development of a new NME. Advancing knowledge in the lead discovery area will result in lower failure rates in the clinical development stage. The costs associated with the development of a new NME and the high rates of attrition show the need for more high quality and cost-effective drug candidates and discovery processes.

The drug discovery process (Figure 4) starts with target identification and target validation. The aim is to synthesise molecules which affect the biological pathway of the associated disease.<sup>21</sup> A review of clinical candidates published in the *Journal of Medicinal Chemistry* between 2016 and 2017 revealed the most common lead generation strategies, including; known starting point from a previous campaign (44%), high-throughput screening (HTS) of libraries (29%) and structure-based design (14%).<sup>22</sup>



**Figure 4:** Overview of the drug discovery process from target identification to launch. The general timescales and costs associated with each step are given, with the cost per launch including the cost of any failed programmes.<sup>4</sup>

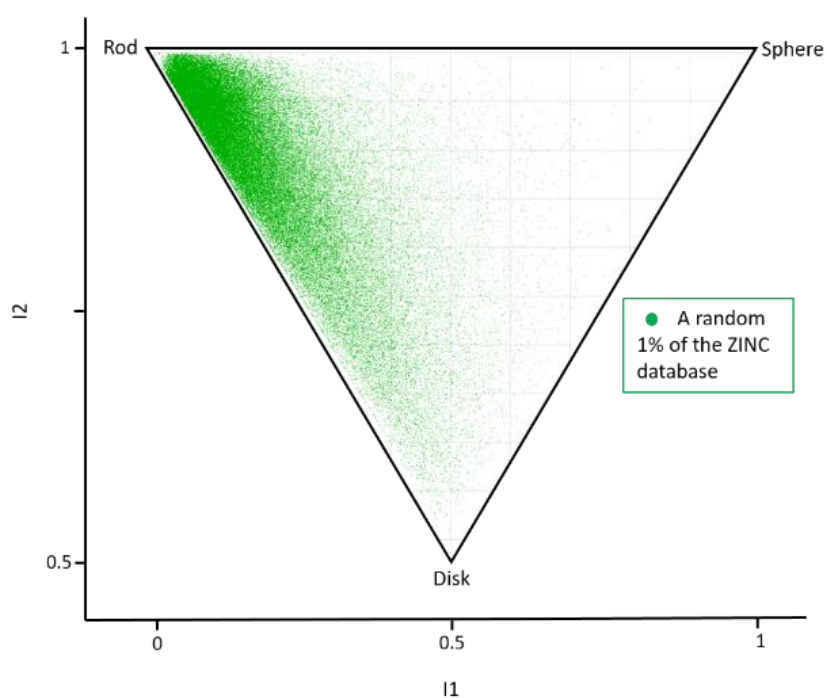
Following the identification of a hit compound, structure-activity relationships (SAR) can be performed to improve potency and generate a lead compound. This involves variation of chemical groups in order to determine which part of the molecule is responsible for biological activity and increase affinity to the target in design-make-purify-test cycles.

Strategies for parallel synthesis of analogues are also investigated to quickly enumerate a library of related molecules. The physical properties of the molecules are also investigated and altered by chemical modification, for example lipophilicity. Lead optimisation involves the discovery of an optimal structure that demonstrates efficacy in disease models, giving confidence that it would be safe and effective in man. Pharmacokinetics are also considered (the study of the movement of drugs within the body) and optimised with chemical modification. Initial safety tests can be performed following the discovery a molecule of interest which include pharmacokinetics and how it affects the organism's physiology (pharmacodynamics).<sup>21</sup> The second stage aims to determine whether the compound is suitable for testing in humans through *in-vitro* and *in-vivo* assays, as well as animal studies. If successful, it proceeds to stage three in which the efficacy and side effects are characterized. Stage four involves submission of all collected data to determine whether the drug is approved to be sold on the marketplace.<sup>21</sup>

### 1.3 Exploration of Biologically Relevant Chemical Space

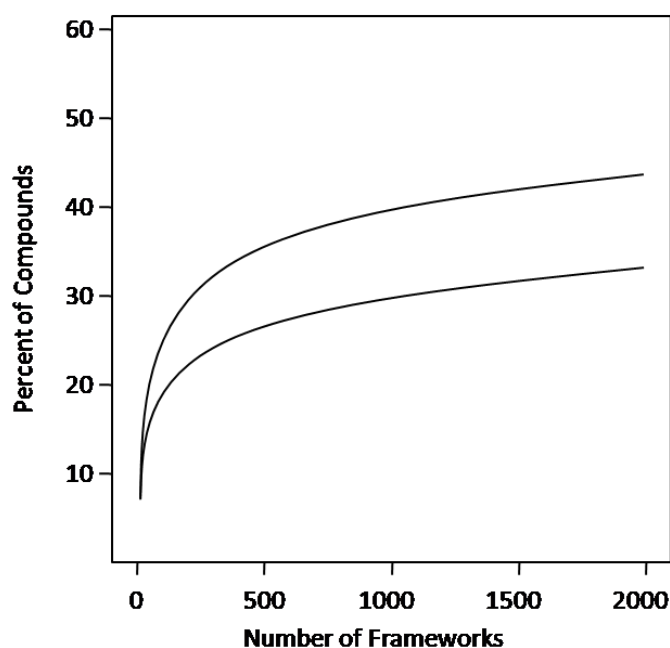
To enable thorough exploration of challenging targets (e.g. protein-protein interactions), new classes of bioactive small molecules need to be developed. This is a challenging endeavour as chemical space is vast, with the number of possible molecules increasing exponentially with molecular weight. Estimates of the vastness of chemical space vary, one estimate based on extrapolation of GDB-17, an exhaustively enumerated set of fragments, has led to the estimation that there are  $10^{33}$  possible molecules with molecular weight  $<500$ .<sup>23</sup> Chemical space can be described by the properties of molecules, such as charge, surface area, hydrogen bonding acceptors/donors as well as its shape (Figure 5).

Historically, the exploration of chemical space has been incredibly uneven. The lack of efficient exploration of chemical space can largely be attributed to the workflows used in conventional medicinal chemistry and the chemistries utilised which has led to interest in alternative methods of bioactive molecule discovery.<sup>3</sup>



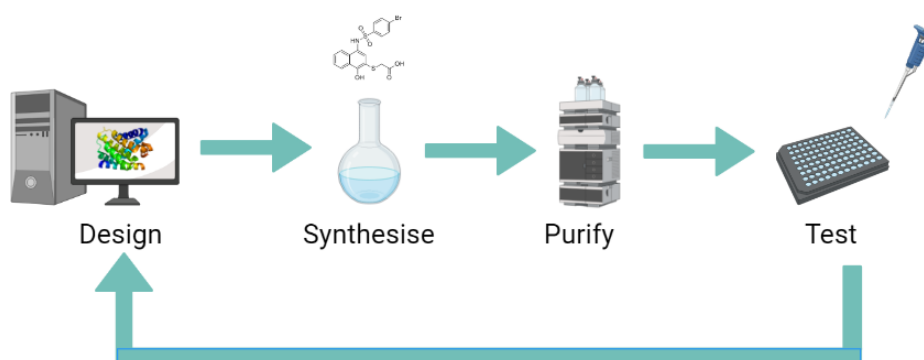
**Figure 5:** PMI plot showing the shape distribution of a random 1% of commercially available molecules in the ZINC database.<sup>24</sup>

The inefficient exploration of chemical space can be exemplified by the lack of scaffold diversity present in bioactive molecules. In 2008, half of all molecules in the CAS registry were based on just 0.25% of frameworks.<sup>25</sup> It has been suggested that this is the result of a “rich-get-richer” process, in which the more a particular framework is used in compounds, the more likely it will be used again in the future. In the last ten years, the diversity of frameworks has increased due to introduction of a large range of new scaffolds (Figure 6).<sup>26</sup> Despite an increase in scaffold diversity, the steep rise shown by the bottom curve suggests the distribution is still very top-heavy. For example, 31.7% of compounds before 2009, and 23.8% reported in 2009-2018, are based on just 250 frameworks. The top-heavy nature of both curves suggests that the more a scaffold was used pre-2009, the more likely it was to be used in 2009-2018. The large influx in new scaffolds outweighs the negative impact of the reuse of popular scaffolds on diversity. Although the recent increase in scaffold diversity is positive, more needs to be done to bolster this effort.



**Figure 6:** Two curves showing the percentage of compounds containing specific scaffolds. Upper curve refers to pre 2009 compounds, whereas the lower curve refers to compounds reported in 2009-2018.<sup>26</sup>

The lack of diversity in bioactive molecules can be attributed to the workflow that dominates traditional medicinal chemistry. Large libraries of similar molecules are produced through iterative design-synthesis-purification-test cycles (Figure 7). The design and synthesis of these compounds all takes place in isolation from assessment of biological function, meaning roughly equal time is spent on compounds regardless of their biological activity. Most compounds will not be taken forward in the drug development process. This workflow requires robust and reliable chemistries that give predictable outcomes, such as amide and Suzuki couplings.<sup>3</sup> The medicinal chemistry toolkit has been largely unchanged in the last 30 years, with the most recent addition being the Suzuki coupling that was discovered in 1984.<sup>27</sup> The reliance of medicinal chemists on this narrow set of reactions has contributed to uneven exploration of chemical space, as they tend to result in larger, flatter and more lipophilic molecules.<sup>28,29</sup>



**Figure 7:** Overview of the current workflow used in bioactive molecule discovery.

## 1.4 Lead Discovery Technologies

Following the identification of validation of a protein target implicated in a particular disease, screening of libraries of compounds can be performed to determine which types of compounds interact with the target, proving a starting point for lead discovery.

To speed up the drug discovery process, high-throughput approaches to lead discovery are utilised. These can include high-throughput screening, which utilised automation and robotics to analyse libraries of compounds of 400-600 Da and

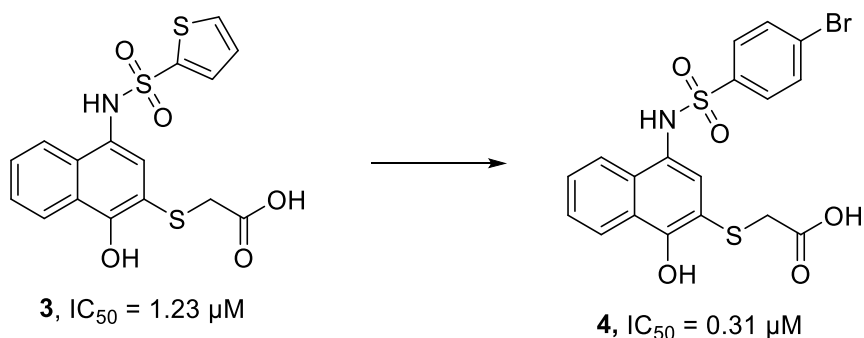
detect compounds which may be suitable as drug candidates.<sup>30,31</sup> DNA-encoded library technology involves conjugation of compounds to DNA fragments, allowing a far greater number of molecules to be examined for drug-like activity as compared to HTS.<sup>32</sup> Fragment-based drug discovery (FBDD) involves the screening of small compounds <250 Da, which often form weak interactions to the target protein, meaning the interactions must be of a high-quality to be detected, proving a good starting point for lead development.<sup>33,34</sup>

### 1.4.1 High-Throughput Screening

A range of high-throughput techniques can be utilised in lead discovery. High-throughput screening has become a popular method of identifying lead-like bioactive compounds in the pharmaceutical industry.<sup>30,31</sup> HTS is unbiased by expectations and so is a powerful tool in the discovery of novel starting points or modes of action and is also a very useful technique if there are no known ligands of the target. Large libraries of around  $10^6$  molecules are screened against the biological target of interest. However, for some libraries, many of these molecules may have poor drug-like properties, such as a high lipophilicity or low solubility, and therefore make for poor starting points for elaboration and drug discovery. They also tend to lack diversity due to the narrow toolkit of chemical reactions utilised in medicinal chemistry. Careful consideration of the physical and chemical properties hits is required before elaboration, as this influences the success of the drug discovery project.

HTS only covers a tiny area of chemical space and so it may be impossible to find a good starting point for further development. In addition, if a hit is obtained, it can be unclear which parts of the molecule are responsible for the observed affinity to the biological target, which can make deciding on a direction for elaboration difficult.

An example of HTS is given in below. Initial hit **3** was discovered for inhibition of Mcl-1 through a high-throughput screening campaign with an  $IC_{50} = 1.23 \mu\text{M}$ . Subsequent expansion of SAR led to the discovery of compound **4** UMI-77 with  $IC_{50} = 0.31 \mu\text{M}$  (Scheme 1).<sup>35</sup> A detailed description of its discovery is given in Section 2.1.3.



**Scheme 1:** Growth from initial HTS hit compound **3**.

### 1.4.2 Fragment-Based Drug Discovery

Another approach generated for the discovery of small bioactive molecules is fragment-based drug discovery (FBDD), which involves the identification and elaboration of weakly binding molecules with molecular weight < 250 Da. It usually takes a structural and molecular view of biological targets of interest alongside traditional medicinal chemistry.<sup>33,34</sup> Fragments are small, typically aromatic, organic molecules which are highly soluble and stable. Astex initially introduced the rule of three to parallel the Lipinski rule of five: fragments should have up to three hydrogen bond acceptors, up to three hydrogen bond donors and a  $\text{clogP} < 3$ .<sup>36</sup>

There are multiple advantages to using a FBDD approach. There are an estimated  $10^{33}$  compounds which are suitable for a HTS (300-500 Da)<sup>23</sup> but only  $10^7$  molecules comprised of C, H, N, O and F of up to eleven atoms which obey the rule of three,<sup>31</sup> so a much larger proportion of chemical space can be sampled from a smaller library size compared to HTS. Secondly, fragments bind very weakly to the target, which means the interactions it forms with the target must be of high quality to allow for detection. Ligand efficiency (LE) is a metric used to determine the success of a fragment and may be used to monitor how a modification to a fragment alters its efficiency as a binder. LE is defined as the free energy of binding of a ligand divided by the number of heavy atoms (Equation 1 and 2).<sup>37,38</sup> Fragment hits can be very ligand efficient and so have great potential for elaboration.<sup>39</sup>

$$LE = \frac{\Delta G}{N} \quad \text{Equation 1}$$

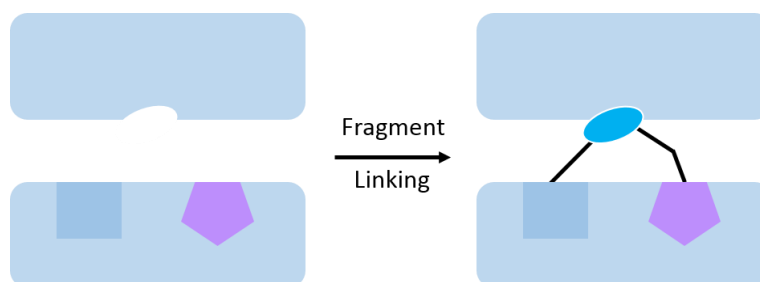
$$\Delta G^\circ = -RT \ln K_i$$

and N is the number of non – hydrogen atoms

$$LE = \frac{1.4(pK_i)}{N} \quad \text{Equation 2}$$

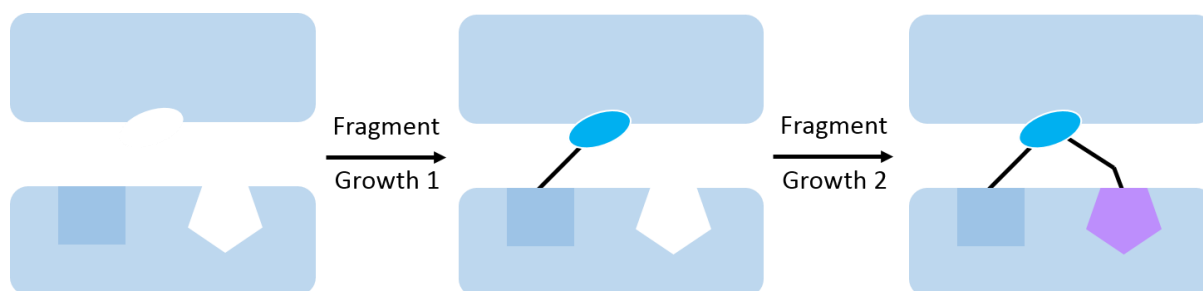
Following identification of a hit, the fragment will undergo iterative cycles of optimisation in order to develop it into a lead compound. The orientation the fragment assumes in the binding pocket is also determined to give guidance for growth strategies.<sup>36</sup> Development of a lead compound can occur *via* three main methods – fragment growth, fragment merging and fragment linking.<sup>30</sup> Fragment growth involves growing a fragment along a vector with the aim of gaining additional interactions with the target. Fragment merging takes two fragment that overlap in their binding to the target and combining them to increase affinity. Fragment linking requires effective linking of two fragments with non-overlapping binding to the target, this is a challenging process as it is difficult to introduce a linker which does not interfere with the binding orientation of the fragments. However, this approach can be very powerful as effective fragment linking can lead to superadditivity of binding affinities, meaning the binding affinity of the linked compound is significantly greater than the sum of the individual binding affinities of the fragments.

A.)

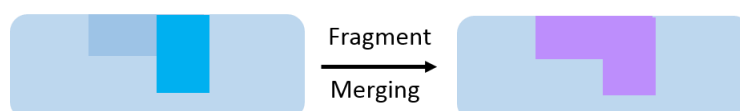




B.)

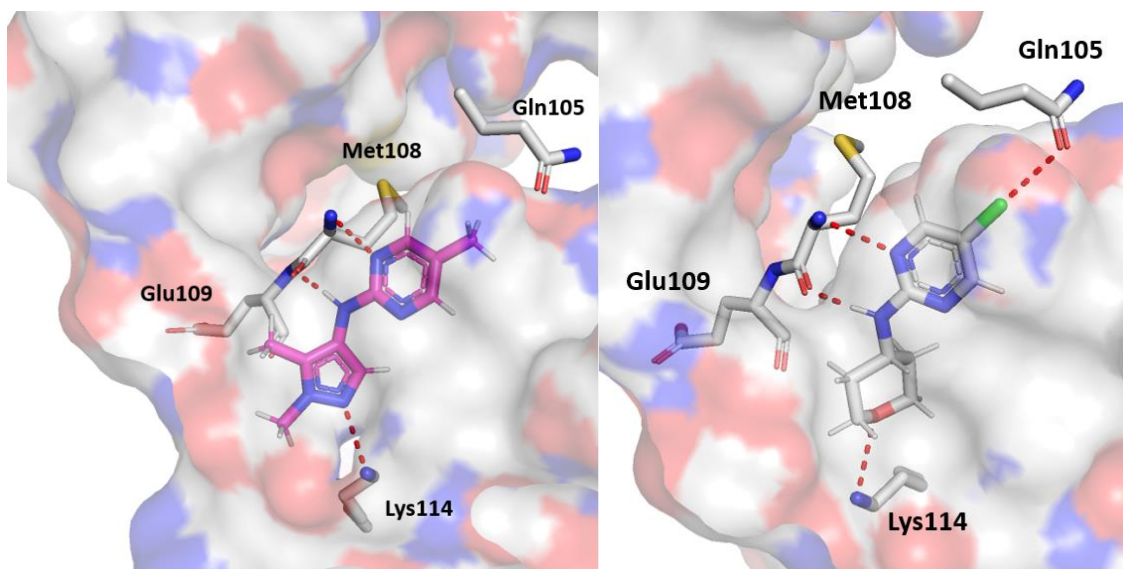
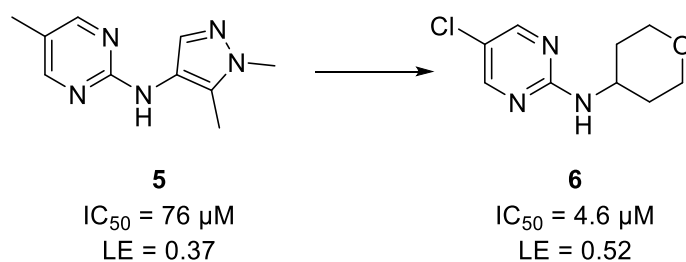


C.)



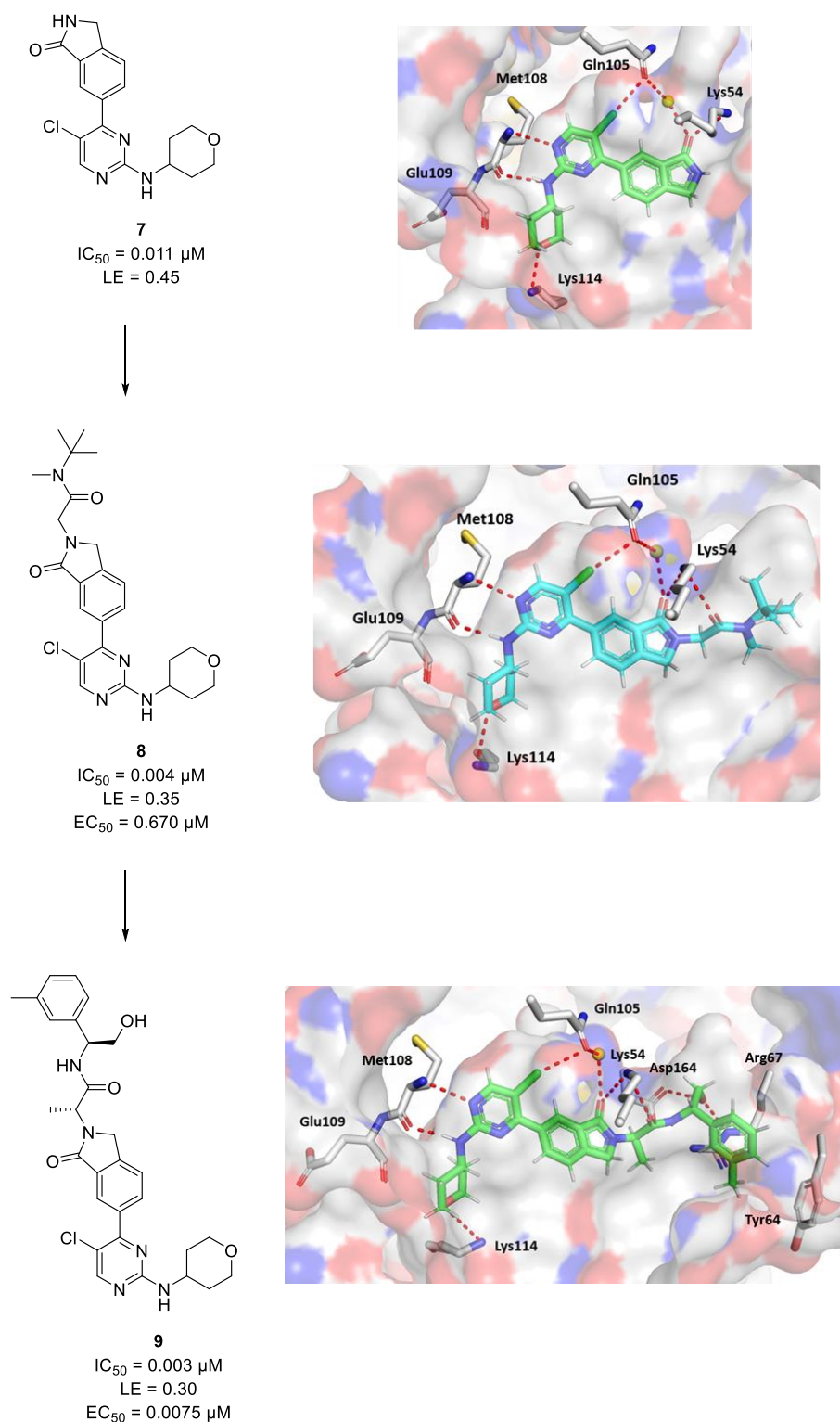
**Figure 8:** Overview of fragment elaboration. A.) Fragment linking joining two fragments in adjacent binding pockets. B.) Fragment growth into adjacent binding pockets. C.) Fragment merging of two fragments in the same binding pocket.<sup>30</sup>

A fragment growing strategy was employed in the discovery of inhibitors of ERK1/2.<sup>40</sup> A combination of virtual, crystallographic and thermal-shift fragment screens was performed, resulting in the discovery of several promising fragments, the best performing being compound **5**. Most kinase inhibitors bind to the so called 'hinge region' of the protein, where the adenine moiety of ATP binds, as is the case with compound **5**. Although there appeared to be good vectors for growth, it was recognised that forming interactions with a second pocket would require a significantly larger molecule, with compound **5** containing a lot of sp<sup>2</sup> character, it was decided to swap the pyrazole for a tetrahydropyran, giving compound **6** with increased solubility as well as activity.



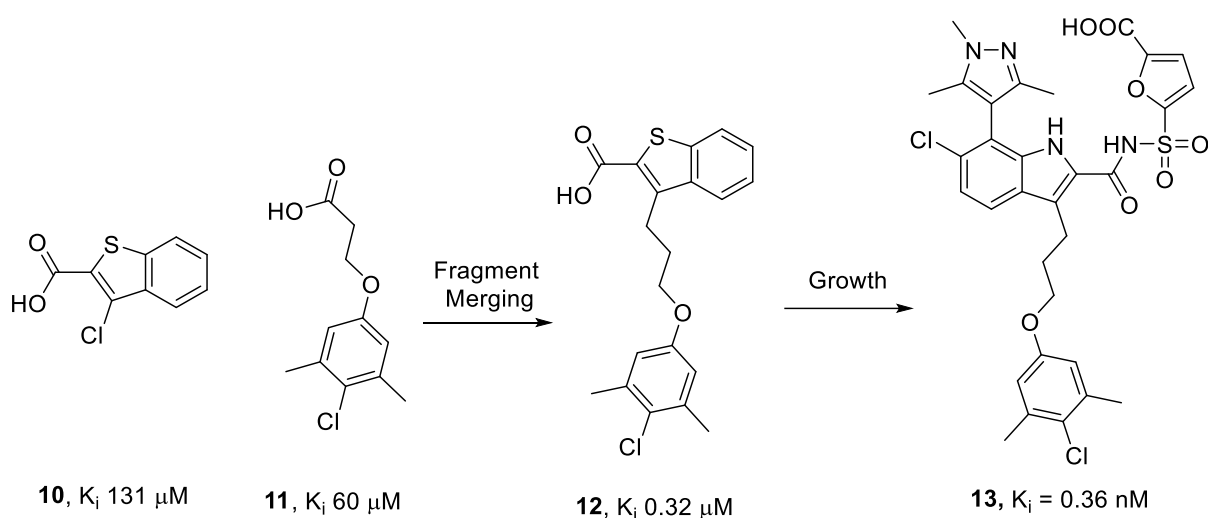
**Figure 9:** Initial screening hit **5** along with the crystal structure of **5** bound to ERK1, hydrogen bonds are formed between the Met108 and Glu109 backbone with the hit compound. PDB Accession Code: 6G92. Result of initial SAR **6** along with the crystal structure of **6** bound to ERK1, an additional hydrogen bond is formed between the chlorine and Gln105 sidechain. PDB Accession Code: 6G91.

Growth towards a second pocket was begun, guided by docking, resulting in the discovery of compound **7** in which the phthalimidine forms hydrogen bonding interactions with Gln105 via a water mediated hydrogen bond and a hydrogen bond with Lys54. Further growth to compound **8**, which forms an additional hydrogen bonding interaction between the amide backbone and Lys54, resulted in improved biochemical potency, but showed poor results in cells. Further design, guided by modelling and crystallography, led to the discovery of compound **9**, with significantly improved cell activity. The alcohol is forms hydrogen bonds with Asp164 and Arg67 side chains, with Tyr forming a  $\pi$ - $\pi$  stacking interaction. Compound **9** was shown to be orally bioavailable, have good pharmacokinetics and lead to tumour regression in mouse models along with good selectivity for ERK1/2 in a panel of 429 kinases. Compound **9** is currently in phase II clinical trials.



**Figure 10:** Compound **7** discovered through fragment growth along with the crystal structure of **7** bound to ERK1. Additional hydrogen bonds are formed between Gln105 and Lys54 with the phthalimidine moiety. PDB Accession Code: 6G93. Compound **8**, shown alongside its crystal structure bound to ERK1. An additional hydrogen bond is formed between Lys54 and the amide backbone in compound **8**. PDB Accession Code: 6G9H. Compound **9** shown alongside its crystal structure bound to ERK1. Additional hydrogen bonds are formed between the alcohol in **9** and Asp164 and Arg67. A  $\pi$ - $\pi$  interaction is formed between the phenyl ring and Tyr64. PDB Accession Code: 6G9N.

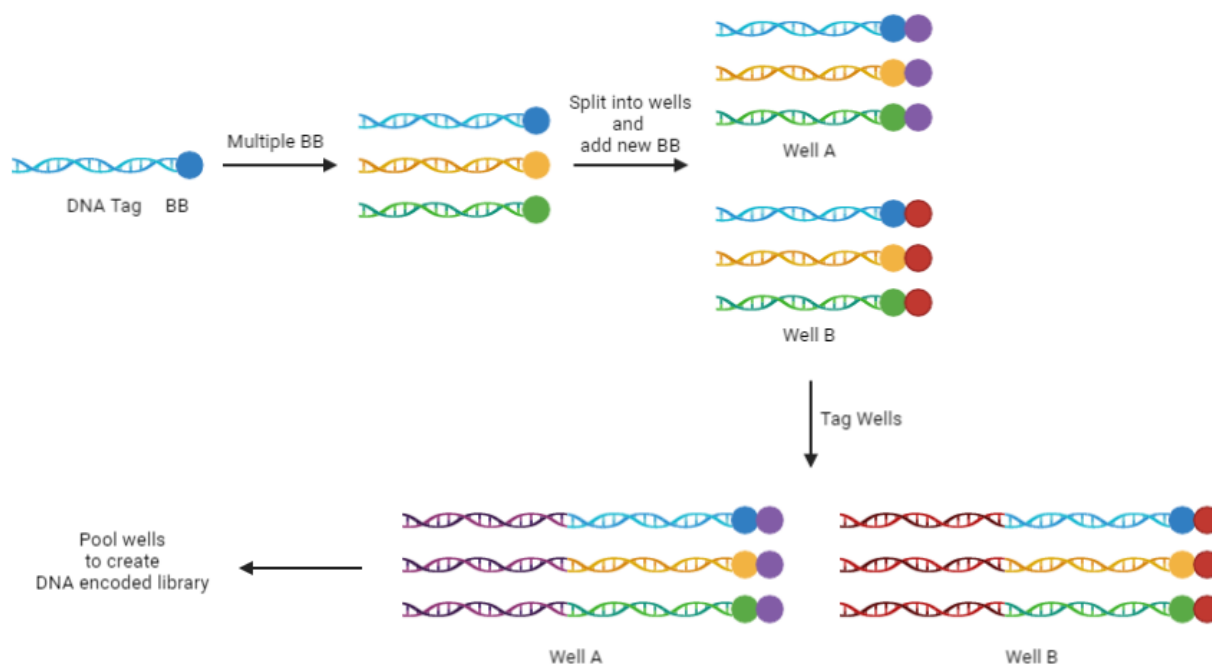
An example of fragment merging is given in Scheme 2. A fragment screen was performed against Mcl-1. Initial fragment hits **10** and **11** were found to bind to different areas of the binding pocket and combined to form compound **12** with significantly increased activity. Subsequent optimisation and growth guided by crystal structures resulted in the discovery of compound **13** with  $K_i = 0.36$  nM. A detailed description of its discovery is given in Section 2.1.3.



**Scheme 2:** Fragment hit **10** and **11** were combined to give merged compound **12** with greatly increased affinity. Optimisation and growth of compound **12** lead to the discovery of compound **13**.

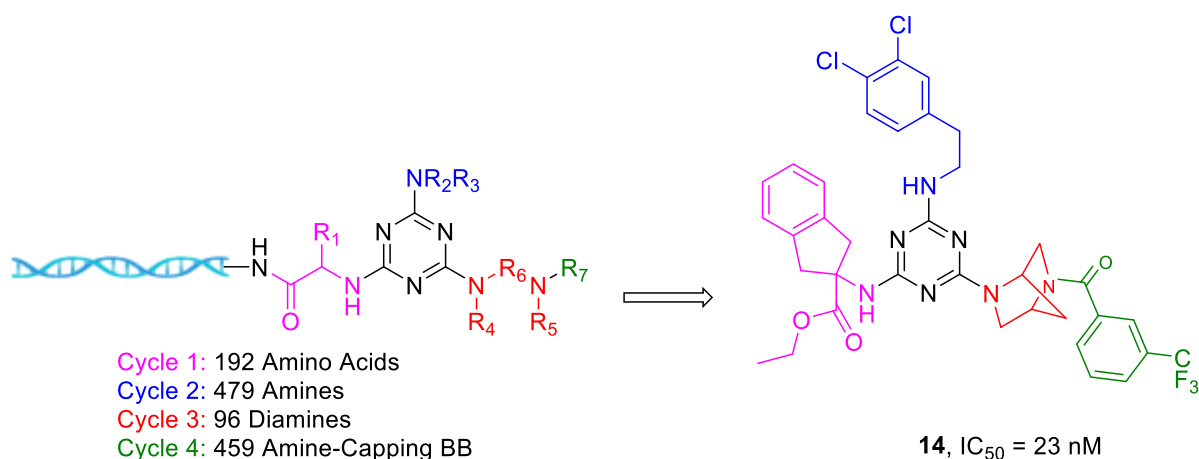
### 1.4.3 DNA Encoded Libraries

Alternative high-throughput technologies such as DNA Encoded Libraries (DELs) have emerged in recent years, which allow a deeper sampling of chemical space compared to traditional HTS approaches (Figure 11).<sup>41</sup> DELs allow for the assessment of huge libraries of billions of compounds to be assessed for activity.<sup>32</sup> DELs suffer from a lack of chemistry applicable to the technology, as chemistries have to be compatible with DNA and must be performed using buffer as a solvent.



**Figure 11:** The process of building a DNA encoded library. BB = Building Block.

DELs have been used to target challenging targets, such as the LFA-1/ICAM-1 protein-protein interaction.<sup>42</sup> A library of 4.1 billion compounds was generated through four cycles of reactions (Scheme 3). The library is based on a 1,3,5-triazine core, cycle one involves  $S_NAr$  of an amino acid, which is coupled to the DNA tag by the C-terminal, with cyanuric chloride. Two further  $S_NAr$  reactions were performed using 479 amines, followed by 96 diamines to give the trisubstituted triazine. Finally, a capping group was added to the other amine of the diamine (carboxylic acid, aldehyde, sulfonyl chloride or isocyanate). Following screening and resynthesizing hit compounds, compound **14** was discovered with  $IC_{50} = 23$  nM, demonstrating that DELs can be used in the discovery of inhibitors of PPIs.

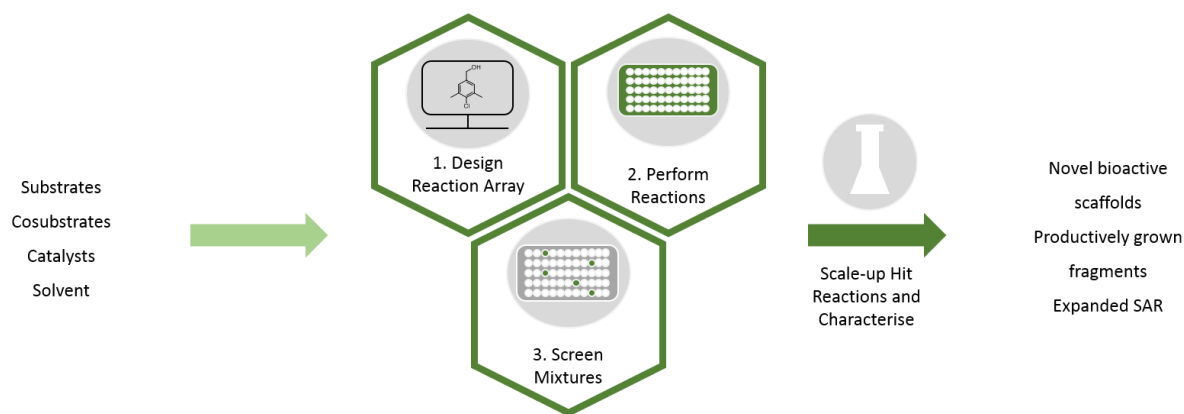


**Scheme 3:** Summary of the DEL utilised in the discovery of inhibitors of LFA-1/ICAM-1 protein-protein interaction.<sup>42</sup>

#### 1.4.4 Activity-Directed Synthesis

Activity-Directed Synthesis is a method of bioactive small molecule discovery in which bioactive molecules appear in parallel with associated synthesis.<sup>43–45</sup> It aims to overcome some of the issues with conventional drug discovery as described previously.<sup>3,25,28</sup> ADS utilises promiscuous chemistries in which multiple outcomes are possible, resulting in more efficient exploration of chemical space. An iterative approach is used, each round at the set of components used is varied widely and resources are focused on those sets of components which yield significant bioactivity. The hits that are generated are then used to inform the choices of components used in the next round; this approach leads to the optimisation of the structure of the active compound as well as a route for its synthesis.

An overview of activity-directed synthesis is shown in Figure 12. A diverse reaction array is performed in plate-based format, followed by scavenging of the catalyst, evaporation of solvent, dissolved in buffer and screened, highlighting reaction mixtures which yield bioactive molecules.

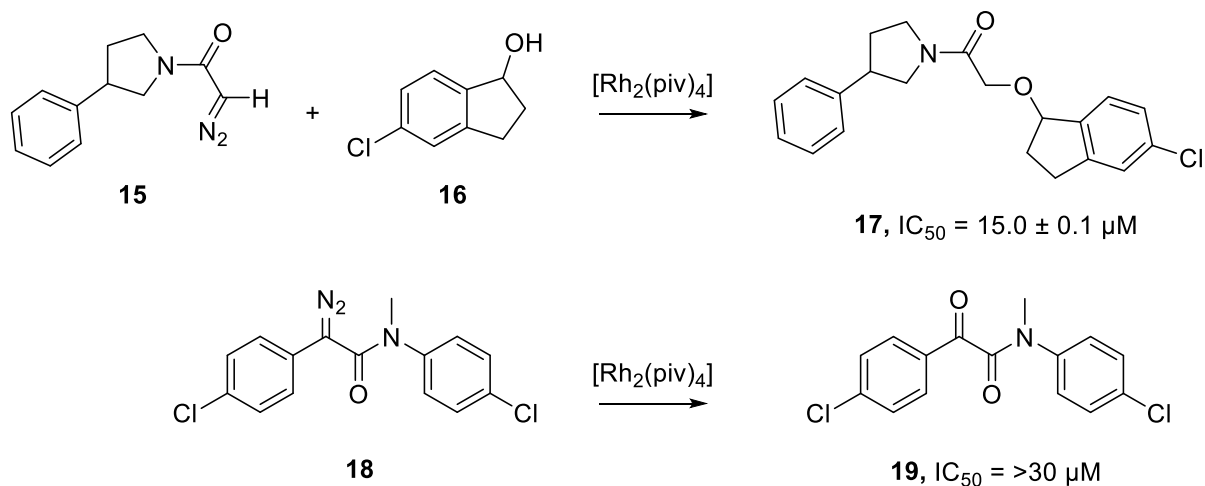


**Figure 12:** An overview of the ADS approach, combining all the areas of the discovery of bioactive molecules.

The active mixtures would then be scaled up, purified and compounds isolated to assess biological activity. The hits that are generated are then used to inform the choices of components used in the next round; this approach leads to the optimisation of the structure of the active compound as well as a route for its synthesis. Subsequent arrays can then be screened at a lower concentration, which enforces a selection pressure upon the system to identify only the most active compounds. ADS has been successfully harnessed in the discovery of inhibitors of the androgen receptor and in the discovery of antibiotics.<sup>43,46</sup>

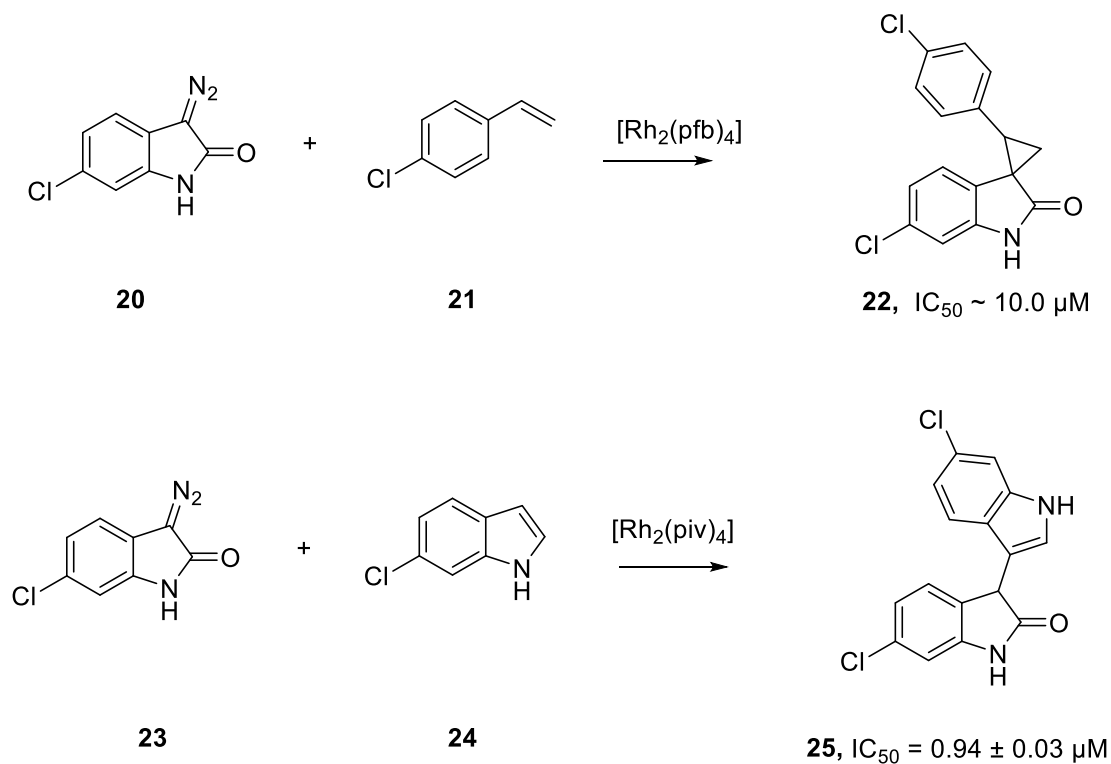
ADS has also been used to target PPIs, as in the case of p53/hDM2.<sup>45</sup> The chosen diazos and co-substrates utilised in the reaction array contain groups which may, or have been demonstrated to, mimic p53 hotspot residues. They also contain multiple functionalities reactive towards rhodium carbenoid chemistry, e.g. alkene, hydroxyl, indole and nitrile. A total of seven diazos, ten co-substrates and two catalysts was chosen for the first array.

In the first array, 154 reactions were performed, testing all possible combinations of diazo, co-substrate and catalyst. Crucially, the diazos, co-substrates and were shown to be inactive at relevant screening concentrations. The catalysts were scavenged from the reaction mixtures prior to testing. The reactions were then assembled in 96-well plate format, executed and screened for activity. Scale-up of active combinations, purification and validation revealed active compounds **17** and **19** (Scheme 4).



**Scheme 4:** Active compounds discovered in round one of ADS of p53/hMD2 inhibitors.

A second round of ADS was then designed based on the hit reactions discovered in round one. A total of six diazos, 16 co-substrates and two catalysts were chosen giving a total of 196 reactions. Following screening, scale-up, purification and characterisation, additional active compounds **22** and **25** were discovered (Scheme 5).



**Scheme 5:** Active compounds discovered in round two of ADS of p53/hMD2 inhibitors.



The hit compounds were assessed *via*  $^1\text{H}$ - $^{15}\text{N}$  HSQC NMR spectroscopy and the chemical shift perturbations induced by **17**, **19**, **22** and **25** were consistent with binding to the p53 cleft. Docking studies were then performed which indicated that the aromatic groups across the series may be targeting the same pair of *hDM2* sub pockets. This study indicates that ADS may enable scaffold hopping as inhibitors have been discovered with a common pharmacophore is displayed across a range of scaffolds.

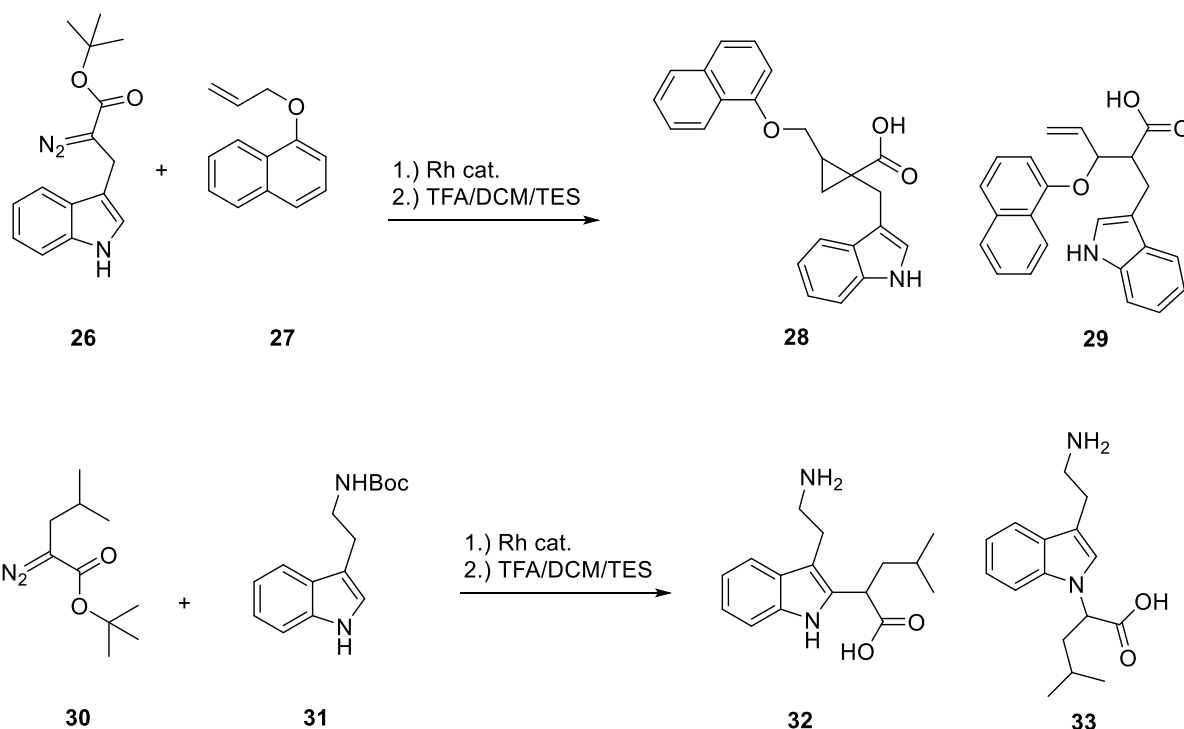
Function driven approaches, such as activity-directed synthesis, have the potential to overcome many of the issues facing the pharmaceutical industry as they ensure resources are spent on only the discovery of bioactive molecules. In order to expand the use of function driven approaches, both the scope of its application and the applicable chemistries need to be broadened.

## **1.4 Thesis Aims**

### **Aim 1: Activity-Directed Synthesis of Inhibitors of PPIs**

It was planned to investigate the discovery of inhibitors of two contrasting PPIs using ADS. The PPIs chosen were Mcl-1/BID and PEX5/PTS1. Mcl-1/BID is a helix mediated-PPI, whereas PEX5/PTS1 is strand-mediated. A detailed description of the targets are given in Sections 2.1 and 3.1, with the detailed objectives for each target provided in Sections 2.2 and 3.2.

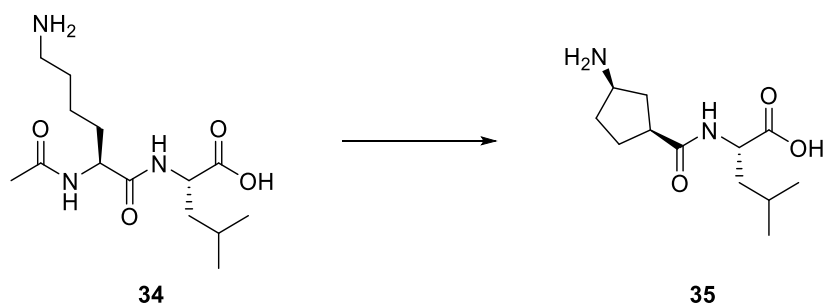
Reaction arrays consisting of diazos, co-substrates and catalysts (Section 2.3.1.1 and Section 3.3.7) will be designed and executed. Examples of possible reactions from the arrays are given in Scheme 6. Active combinations of diazos and co-substrates will be scaled-up and purified, with products characterised and assessed for activity. Information from the screening will be used to design subsequent reaction arrays in an attempt to increase affinity towards the target.



**Scheme 6:** Example reaction from Mcl-1/BID ADS array (above). Example reaction from PEX5/PTS1 ADS array (below).

## Aim 2: Conventional Molecular Discovery Approach to Inhibitors of PPIs

Following the use of ADS to discover inhibitors of PPIs, a more conventional approach will be employed and the two techniques compared. Detailed objectives are given in Section 4.1. Peptide-based ligands for PEX5 will be used as a starting point to inhibitor design (Scheme 7) with the aim of discovering more ligand efficient inhibitors (Section 4.2). The active compounds will then be grown with the aim of the increasing activity towards PEX5, using docking as a guide to aid in the design and synthesis of further compounds (Section 4.5 onwards).



**Scheme 7:** An example of a designed compound based on the native peptide sequence.

## 2 Activity-Directed Synthesis of Mcl-1 Inhibitors

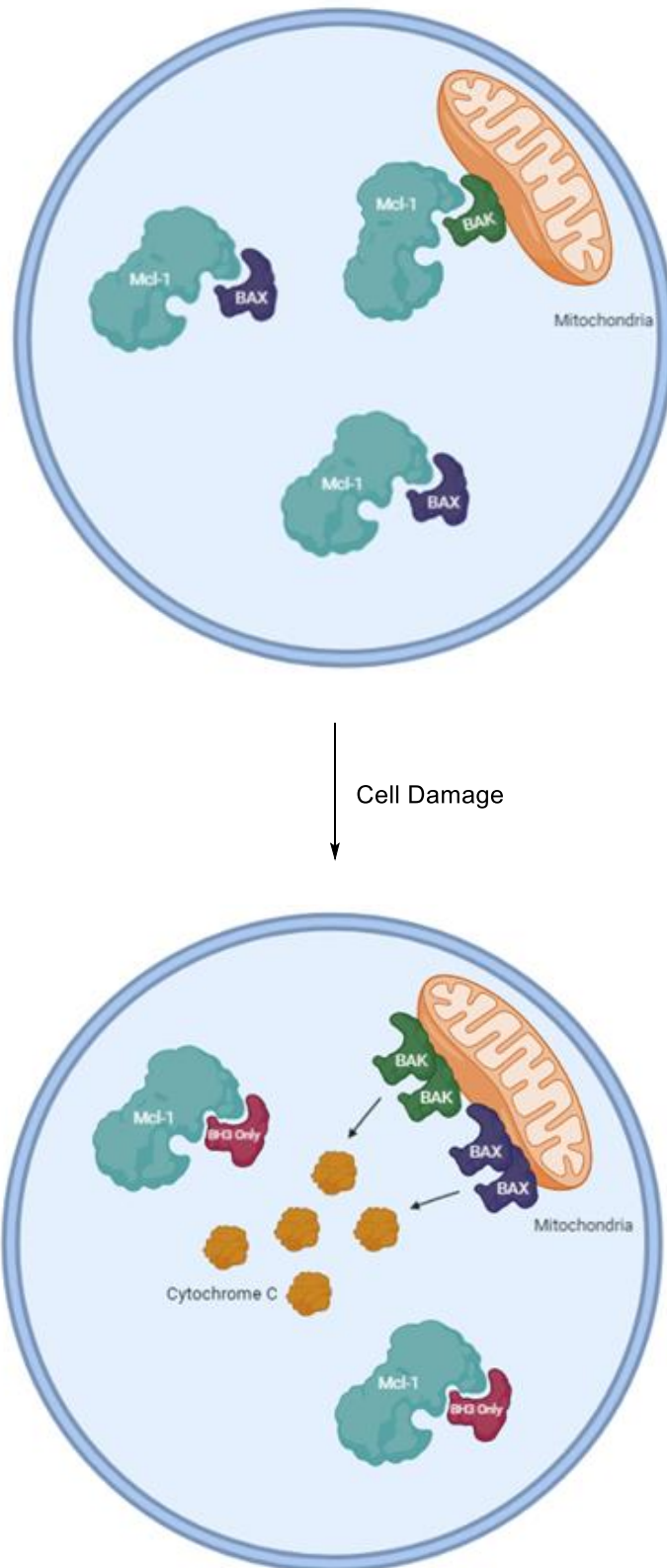
### 2.1 Introduction to Mcl-1

#### 2.1.1 Biology of Mcl-1

Mcl-1 is a Bcl-2 family protein which promotes cell survival and has been linked to the development of a wide array of different cancers, with up to 36% of breast and 54% lung cancers showing elevated levels of Mcl-1 expression.<sup>47</sup>

The Bcl-2 family of proteins comprises of three different groups classified by their function: apoptotic, anti-apoptotic and BH3-only proteins.<sup>48</sup> The fate of the cell is determined by the interactions between these groups of proteins. Mcl-1 is an example of an anti-apoptotic member of the Bcl-2 family.<sup>49</sup> It contains a hydrophobic tail which allows it to embed itself within the membrane of mitochondria, where it performs its anti-apoptotic function, which is essential to cell homeostasis.<sup>50</sup>

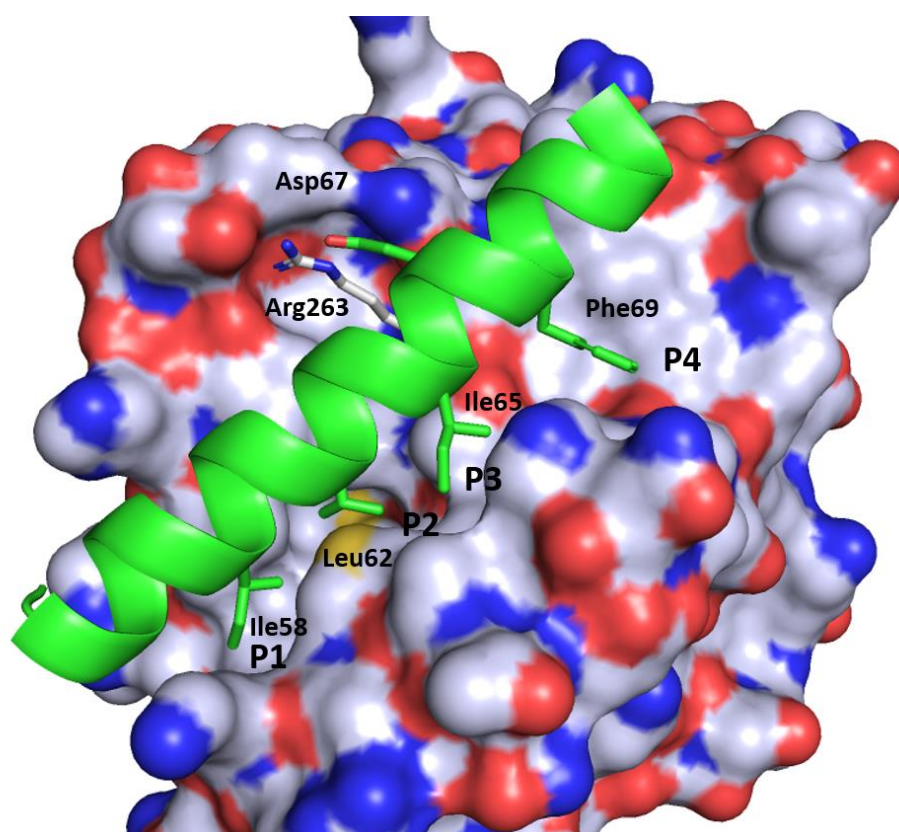
Mcl-1 performs its anti-apoptotic functions by binding to BAX/BAK, members of the apoptotic family of Bcl-2 proteins. BAX is in the cytosol, whereas BAK is anchored to the mitochondrial membrane *via* a hydrophobic tail. During cell stress, BAX/BAK may be activated by signals which act upon BH3-only proteins.<sup>51</sup> BH3-only proteins (e.g. NOXA, BID, BIM, BAD) are able to bind to Mcl-1, releasing BAX/BAK, allowing them to carrying out their apoptotic function. Once released, BAX is translocated to the mitochondrial membrane.<sup>52</sup> The now exposed BH3 domains of BAX/BAK allow the formation of oligomers which can rupture the mitochondrial membrane, resulting in the release of cytochrome C and ultimately apoptosis.<sup>53</sup> Restoring the balance between apoptotic and anti-apoptotic proteins *via* small-molecule inhibition of Mcl-1 is an attractive therapeutic goal, as elevated levels of Mcl-1 are associated with many cancers.<sup>47</sup>



**Figure 12:** The balance of pro and anti-apoptotic Bcl-2 proteins is crucial to cell survival.<sup>8</sup> Survival mode (above) and apoptotic mode (below). In survival mode, Mcl-1 is bound to BAK/BAX. When the cell is stressed, BH3-only proteins are activated and displace BAX/BAK. BAX is then translocated to do the mitochondrial membrane, where it forms oligomers which rupture the mitochondrial membrane and release cytochrome C, leading to apoptosis.

### 2.1.2 Structural Biology of Mcl-1

Mcl-1 binds to the  $\alpha$ -helical BH3 domains of BAX/BAK. The binding groove of Mcl-1 is different to other anti-apoptotic members as it is more positively charged<sup>54</sup> and more rigid, making the design of selective inhibitors more challenging.<sup>55</sup> Crystallography of the complex between Mcl-1 and BH3-only proteins has shown a salt bridge between an aspartic acid residue of BH-3 only proteins and Arg-263 on Mcl-1 is required for binding (Figure 13).<sup>56,57</sup>

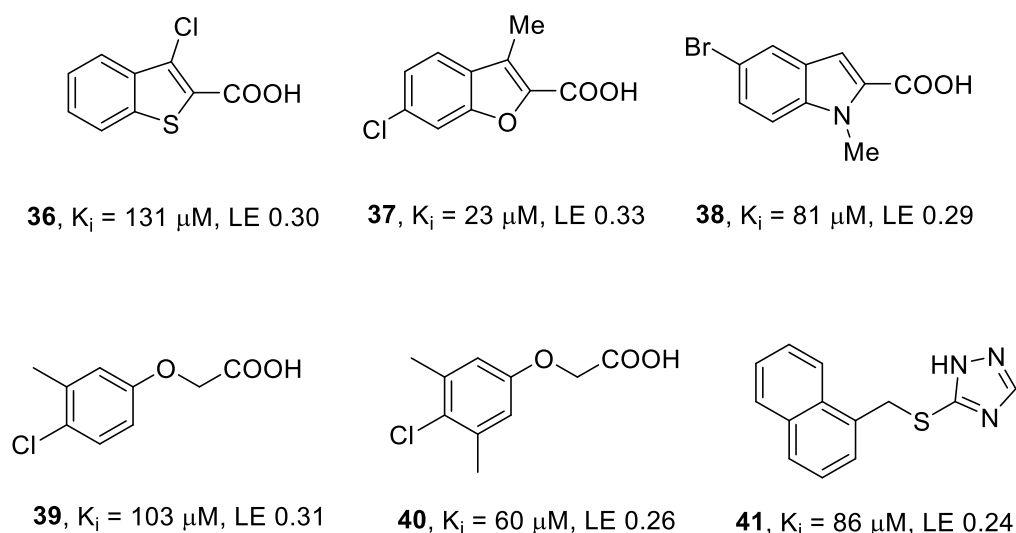


**Figure 13:** Crystal structure of Mcl-1 bound to a BIM peptide highlighting key binding pockets and amino acids. PDB Accession Code: 2PQK.

The surface of Mcl-1 contains several hydrophobic binding pockets which form interactions with the hydrophobic side chains of binding peptides. The P2 pocket is deep and contains hydrophobic residues, most notably Ph270. P3 is shallower than P2 and contains some polar residues (Thr266 and His224). The P4 pocket is not well defined and is largely solvent exposed. Potent inhibitors tend to contain an acidic group to form a salt bridge with Arg263 as well as be large and hydrophobic to occupy the various hydrophobic pockets (P1-P4), which is an unattractive feature in a drug-like molecule.<sup>58</sup>

### 2.1.3 Discovery of Mcl-1 Inhibitors

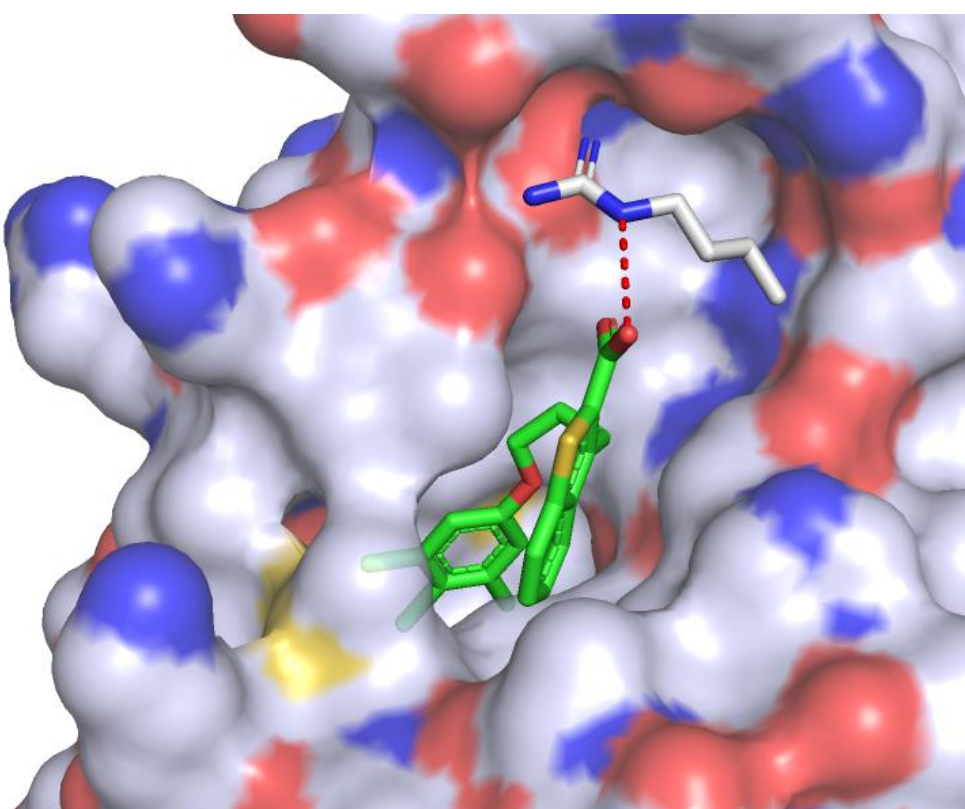
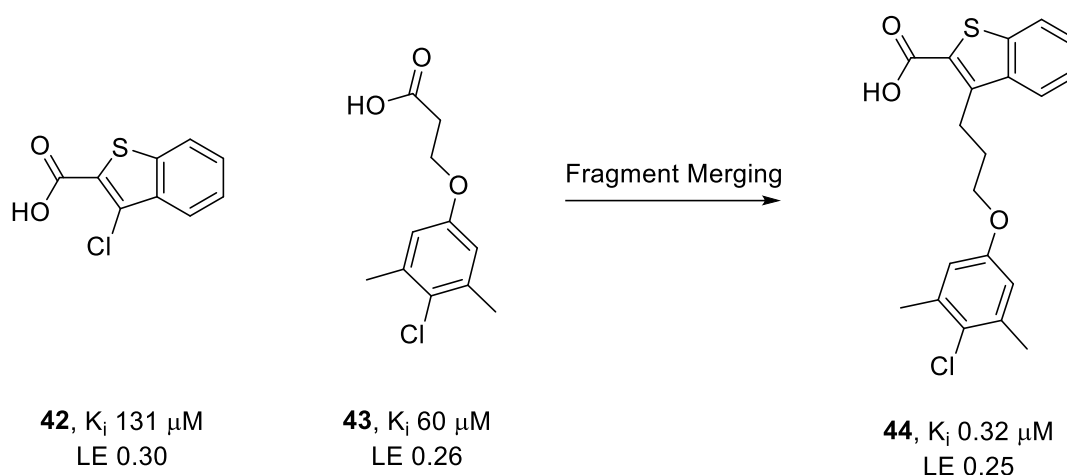
In 2013, the fragment-based discovery of a series of small molecule inhibitors of Mcl-1 ( $K_i < 100$  nM) was described.<sup>59</sup> Initially, an NMR-based screen was used to identify two distinct classes of hits which bind to different regions of Mcl-1 (Figure 14).



**Figure 14:** Class I (top) and II (bottom) fragment hits for binding to Mcl-1.

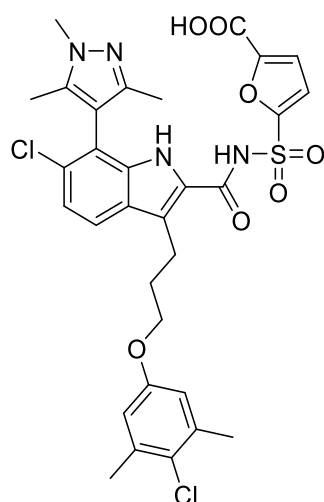
Class I hits contain 6,5-fused heterocyclic carboxylic acids (**36-38**), unsubstituted rings are generally inactive, with one or two Cl and/or Me substitutions resulting in greatly increased activity. Class II hits are hydrophobic aromatic groups tethered to a polar functionality (**39-41**), most commonly a carboxylic acid. NOE guided fragment docking was used to determine how class I and II hits bind to Mcl-1. NMR derived models show that the hydrophobic portions of class I and II hits bind at adjacent hydrophobic sites and the carboxylic acid groups of both series point towards Arg-263, which explains why class I and II hits are not able to bind simultaneously to Mcl-1. NOE-guided molecular modelling suggests class I hits sit above the hydrophobic pocket that is occupied by class 2 hits.

Based on this information, hits **42** and **43** were merged to create compound **44** with >100-fold enhanced activity over the individual components (Figure 15). Analogues were generated using SAR and the crystal structures of inhibitor bound complexes of Mcl-1 were used to guide their design.

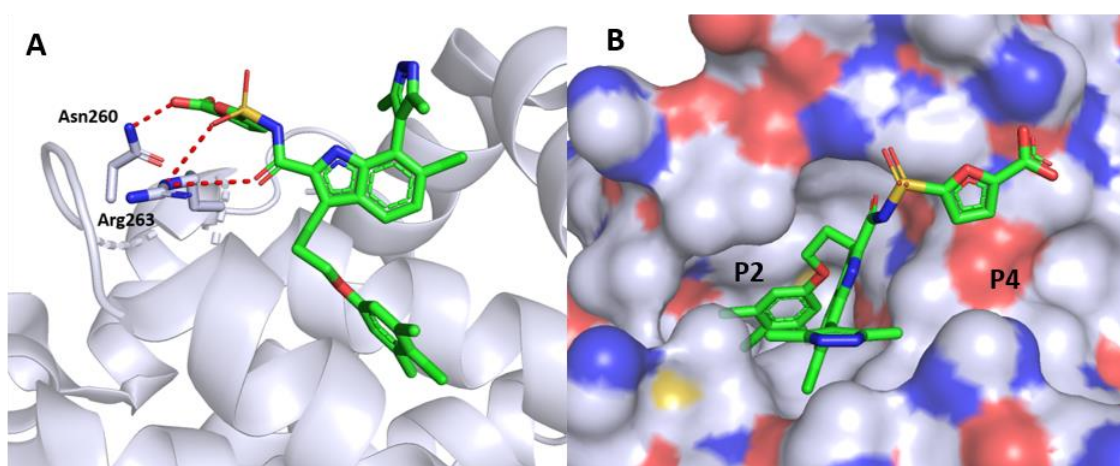


**Figure 15:** Fragment hits **42** and **43** underwent fragment merging to form compound **44** with greatly increased affinity for Mcl-1. The co-crystal structure of **44** bound to Mcl-1 is shown, the substituted phenyl ring sits in the hydrophobic P2 pocket with the carboxylic acid of the benzothiopehene oriented towards Arg263. PDB Accession Code: 4HW2.

Following the discovery of compound **44**, an additional fragment screen was performed in the presence of compound **44**, in order to identify fragments which could be used to pick up additional interactions with Mcl-1.<sup>60</sup> Several fragments showing millimolar binding were discovered. Fragment linking with hit **44** along with SAR and structure-based design resulted in the discovery of a series of potent Mcl-1 inhibitors, for example **45**, which show picomolar or low nanomolar activity (Figure 16).



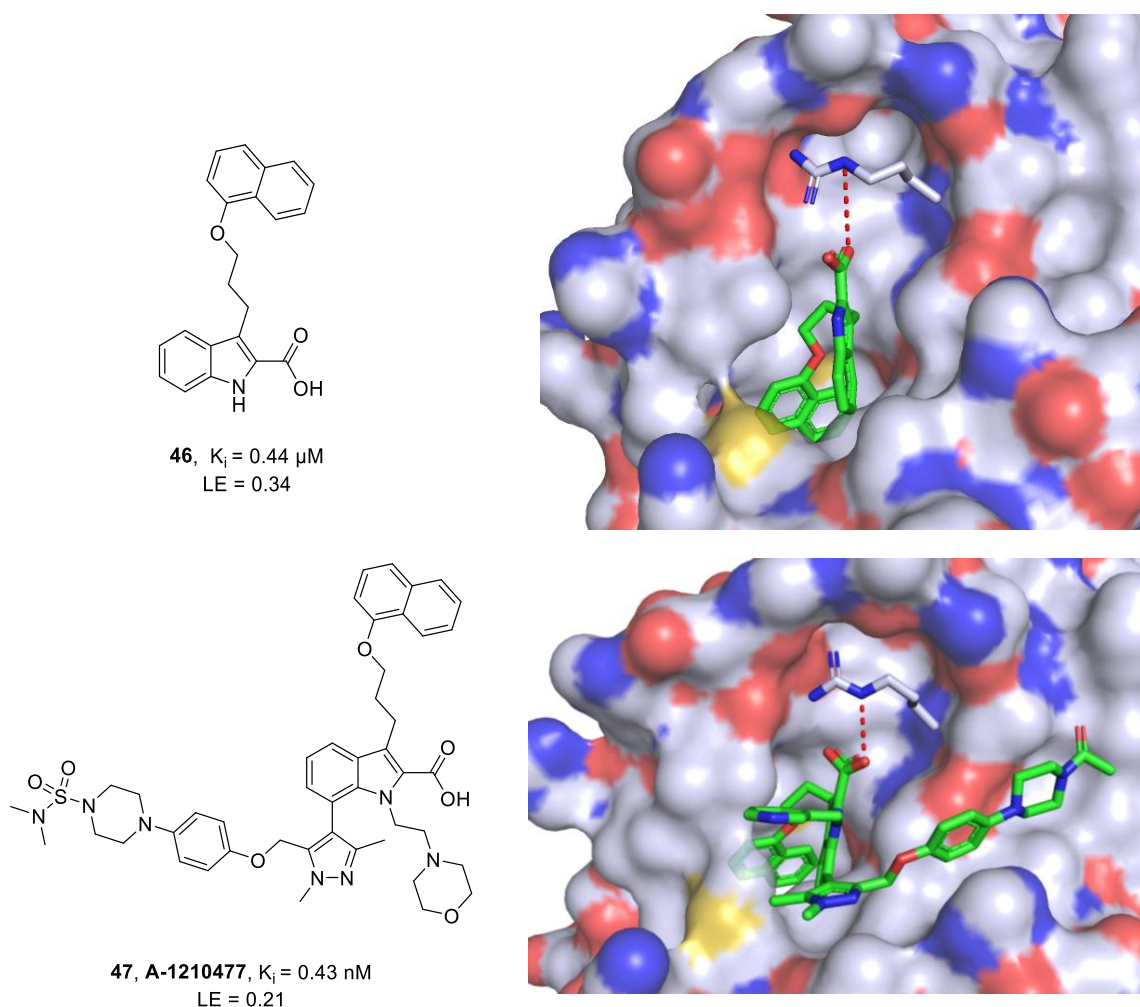
**45**,  $K_i = 0.36$  nM  
LE = 0.29



**Figure 16:** Example of a compound identified by development of merged fragment hit **3** with the co-crystal structure of **45** bound to Mcl-1. A) Compound **45** makes polar contacts to Arg263 and Asp260. B) Compound **45** binds to the Mcl-1 BH3 groove. PDB Accession Code: 5FDR.

Hit **46** was identified through high-throughput screening as a small molecule inhibitor of Mcl-1 with  $K_i = 0.44$   $\mu$ M.<sup>61,62</sup> The length of the linker was investigated but both a shorter and longer tether resulted in no activity against Mcl-1. The co-crystal structure of the naphthyl substituted species **46** was used to determine rational growth vectors in order to increase potency towards Mcl-1. Structure-activity relationship and crystal structures were used to inform decisions for substitution on the 1 and 7 position on the indole ring. The extended *N*-acetylpiperazine moiety results in occupation of P3 and P4 sites (Figure 17). A-1210477 **47** has a  $K_i = 0.43$  nM and shows selectivity towards Mcl-1 over other members of the Bcl-2 family of proteins. Its mode of action was shown to be through the disruption of the Mcl-1:BIM PPI in live cells.<sup>63</sup>

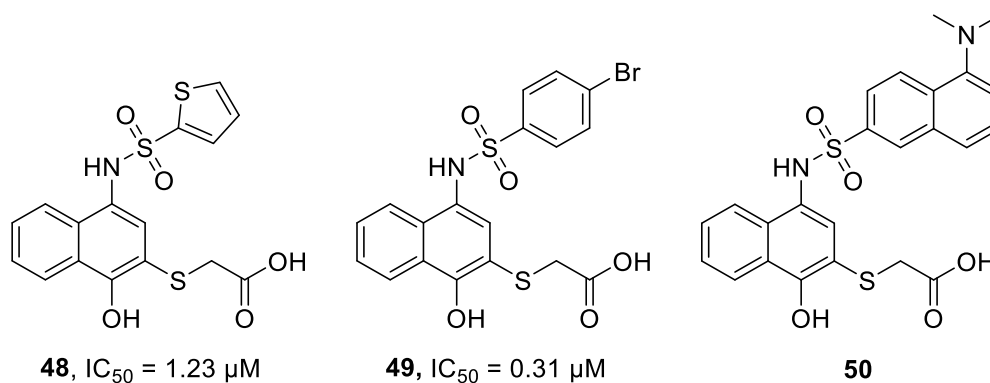




**Figure 17:** The structure of initial hit **46** and the crystal structure bound to Mcl-1 (above). The naphthyl group occupies a deep hydrophobic groove and the carboxylic acid orientates towards Arg263, PDB Accession Code: 6B4L. The structure of A-1210477 and the crystal structure of a closely related compound bound to Mcl-1 (below). PDB Accession Code: 5VKC.

Compound **47** highlights some key issues with many Mcl-1 inhibitors, the first issue is their size, with **47** having  $M_w = 849.4 \text{ Da}$ , making it more likely for these compounds to exhibit issues with permeability and solubility. In addition, compound **47** has an LE of 0.21 (Equation 2) making it an inefficient binder.

An additional example of a Mcl-1 inhibitor discovered using a high-throughput screening approach is compound **49** (UMI-77, Figure 6). Lead compound **48** was discovered through a high-throughput screen with 53,000 compounds using a fluorescence anisotropy inhibition assay. Compound **48** was then resynthesised and its binding to Mcl-1 confirmed. UMI-77 was discovered through structured-based chemical modification of lead compound **48**.<sup>35</sup> UMI-77 has an  $\text{IC}_{50} = 0.31 \mu\text{M}$  and has been shown to induce apoptosis in cancerous pancreatic cells in a dose-dependent manner.



**Figure 18:** Structure of lead compound **48** and optimised compound **49**, further highlighting the importance of an acidic group. Structure of compound **50**, a derivative of UMI-77 used as a fluorescent probe.

Analogues of UMI-77 have been utilised as chemical tools, for example compound **50** (Figure 6) is used as a fluorescence probe to image overexpressed Mcl-1 protein in living cells.<sup>64</sup> Compound **50** contains a dansyl chloride fluorophore, the fluorescence of which displays sensitivity to solvent as well as the surrounded environment, making it an ideal candidate to image Mcl-1 protein, as its fluorescence changes upon binding.

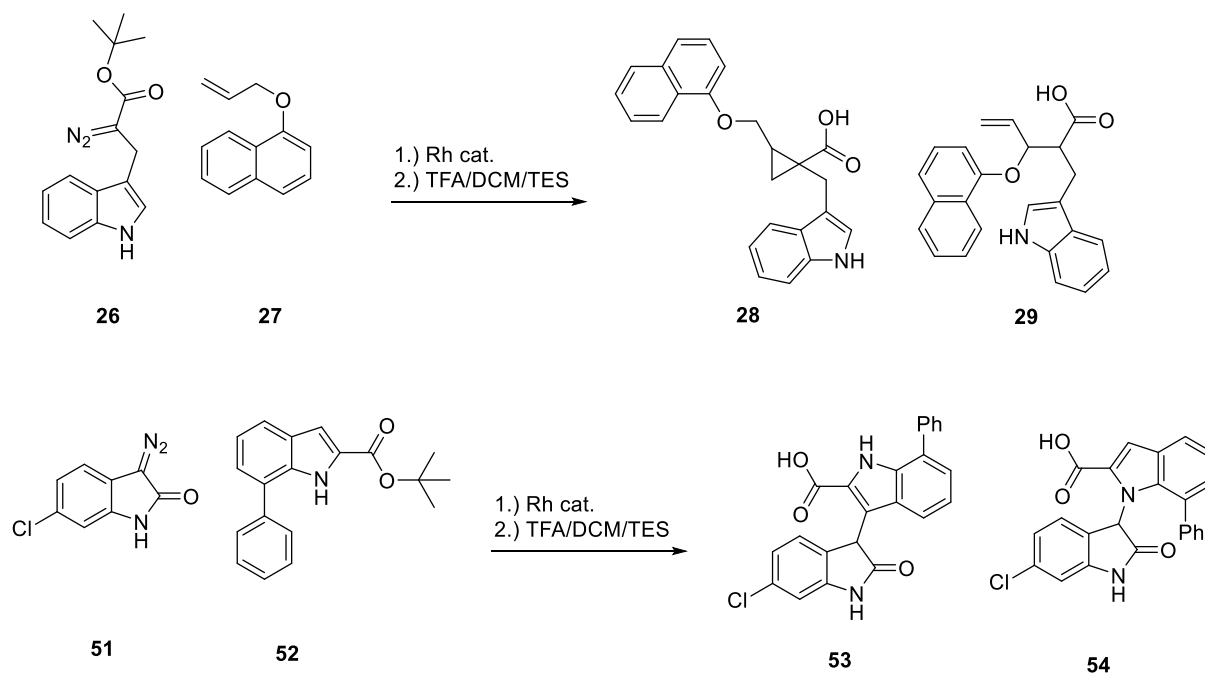
## 2.2 Aims and Objectives

Fragment linking is a challenging process as it can be difficult to introduce a linker which does not interfere with the binding mode of the fragments. It was envisaged that activity-directed synthesis could be used in order to identify effective linkers to connect Mcl-1 fragments. For activity-directed synthesis to be employed for fragment-linking, chemistries must be utilised which enables synthetic elaboration at multiple reaction sites of the fragment. It was proposed that rhodium carbenoid chemistry established in the group previously has the potential to elaborate along one or many vector(s) in order to produce a range of linked fragments for biological evaluation. Active combinations of substrates of substrates will be scaled up and active molecules isolated, before using that information to design subsequent arrays.

A series of short-term and long-term project objectives was outlined to evaluate the use of activity-directed synthesis for fragment linking. Mcl-1 was chosen as the biological target to exemplify the fragment linking strategy with activity-directed synthesis as a wide array of inhibitors have been reported using a fragment-based drug discovery approach.

### **Objective 1.1: Design and Synthesis of Fragments for Mcl-1.**

It was envisaged that exemplar fragments for Mcl-1 would be well suited to direct elaboration using rhodium carbenoid chemistry. Diazos would be reacted with co-substrates containing at least one functionality reactive towards rhodium carbenoid chemistry. Many of the fragments utilised will contain a *tert*-butyl ester which following the execution of the rhodium-carbenoid reaction, will be deprotected under suitable conditions to reveal the free carboxylic acid, seen in many Mcl-1 inhibitors. Examples of reactions which could occur are given in Scheme 8. Diazo **26** could react with naphthalene **27** *via* cyclopropanation to give **28** or C-H insertion to give **29**. Diazo **51** could react with **52** *via* C-H insertion at the C-3 position to give **53** or *via* N-H insertion to give **54**. The products of these reactions would then be assessed for their biological activity. Suitable ADS compatible conditions for this deprotection will be investigated. The designed diazos and co-substrates will then be synthesised or acquired ready to perform the reaction arrays.



**Scheme 8:** Example of diazos and co-substrates that could be utilised to give a range of products.

### Objective 1.2: Configuration of High-throughput Assay

For the biological assessment of the prepared fragments and screening of crude reaction mixtures, a high-throughput robust assay was required. The proposed assay for the project was a biophysical fluorescence anisotropy assay. A similar assay was used for ADS of p53/*h*DM2 inhibitors.<sup>45</sup>

It would be critical to ensure the reaction array components do not interfere in the assay. Here, a robust assay would be established which was suitable for identifying reaction mixtures containing biologically active products from ADS arrays. It was envisaged the biologically active reaction mixtures would be a result of effective fragment-linking (Scheme 8).

### Objective 1.3: Design and Execute Rounds of ADS

Following the synthesis and acquisition of the desired diazos and substrates, an ADS array was designed. The array will be performed using the diazos, substrates and catalysts, performing all possible combinations. DCM will be used as a solvent in all combinations. The catalyst will then be scavenged, and the solvent left to evaporate. Many of the diazos and substrates designed will contain a *tert*-butyl ester group which will be deprotected to reveal the free carboxylic acid group.

Ideally, productive fragment-linking will occur and hits with significantly higher activity than the individual components will emerge.

Results from round one of the ADS array will be analysed in order to identify the most potent combinations of substrates and substrates. Active combinations will be scaled-up and hits purified and characterised. Hits will be validated using LCMS and dose-response curves. Active mixtures will then be scaled up and the resultant products purified, characterised and their affinity for Mcl-1 measured. The components and catalysts that show activity in round one will be taken forward to round two alongside a range of structurally related compounds and related catalysts. Round two will be screened at half the concentration of round one in order to force a selection pressure upon the system, which will result in the optimization of inhibitors.

## **2.3 Results and Discussion**

### **2.3.1 Round One of Activity-Directed Synthesis**

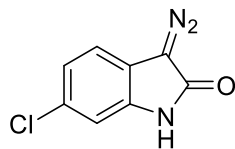
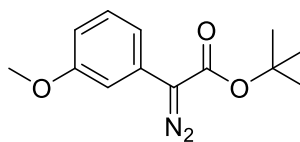
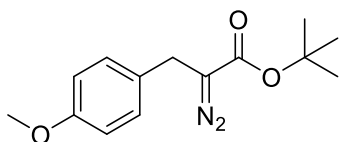
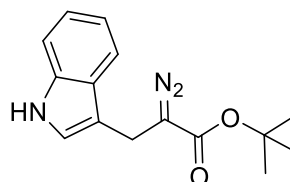
#### **2.3.1.1 Design of Fragments for Activity-Directed Synthesis**

Mcl-1 is well-studied yet challenging target, with many ligands reported >500 molecular weight. Many of these ligands have been designed using a fragment-based approach and so it was proposed that Mcl-1 would make an ideal target for exploring the potential of activity-directed fragment-based ligand discovery. The fragments were designed to include at least one functionality that could react with a rhodium carbenoid compound in order to increase promiscuity, resulting in increased efficiency in the exploration of chemical space.

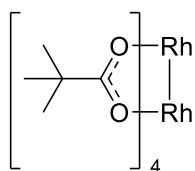
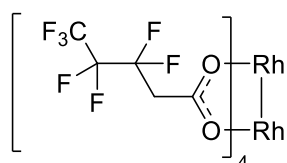
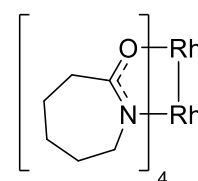
For evaluation of metal carbenoid chemistry as fragment-linking strategy in activity-directed synthesis, two sets of fragments were designed, based on fragment-size substructures contained in reported Mcl-1 inhibitors. It was aimed for one set of fragments to contain the diazo functionality which enable metal carbenoid formation and subsequent transformation. The second set of fragments would contain multiple functionalities amenable towards metal carbenoid chemistry. Reacting the fragments together and treating them with an appropriate catalyst would result in their linkage, through multiple different potential linkage vectors.

The selected diazo subset is shown in Figure 19. Diazo **D1** (=51) was designed through deconstruction of known Mcl-1 ligands, for example A-1210477.<sup>61,62</sup> Many known Mcl-1 ligands contain an indole core and chlorine atoms which are known to increase affinity through interaction with Ala227.<sup>65</sup> Fragment growth from the 3-position is known to enable interaction with the hydrophobic pocket P2 pocket and increase affinity,<sup>66</sup> so the reactive diazo group is placed on the 3-position. Diazos **D2**, **D3** and **D4**(=26) are homologated version of fragments found in Mcl-1 inhibitors, hydrophobic aromatic groups are known to occupy the pockets of Mcl-1.<sup>66</sup> The inclusion and movement of the methoxy group increases diversity along with variation in the length of linkage between the aromatic and diazo components. All three contain a *tert*-butyl ester which, upon deprotection, will reveal a free carboxylic acid that may interact with Arg263.<sup>56,57</sup>

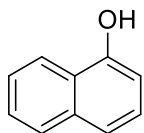
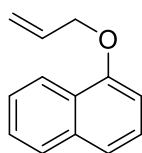
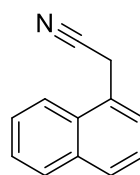
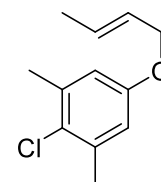
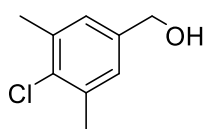
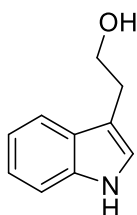
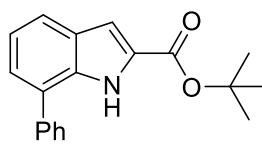
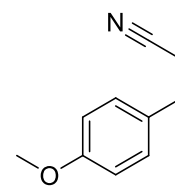
## a.) Diazos

**D1****D2****D3****D4**

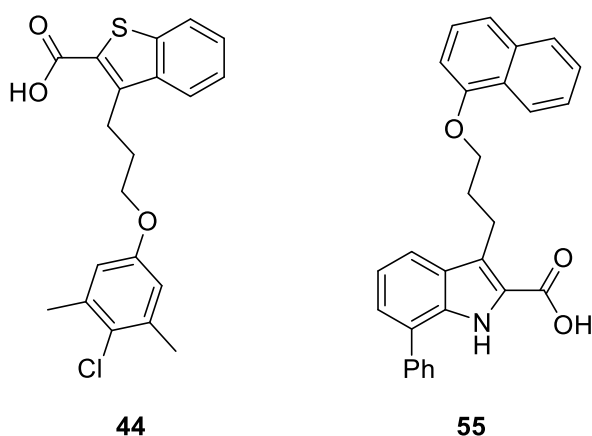
## b.) Catalysts

 $[\text{Rh}_2(\text{piv})_4]$ **C1** $[\text{Rh}_2(\text{pfb})_4]$ **C2** $[\text{Rh}_2(\text{cap})_4]$ **C3**

## c.) Substrates

**Co1****Co2****Co3****Co4****Co5****Co6****Co7****Co8****Figure 19:** Diazos, catalysts and co-substrates chosen for the reaction array.

The selected co-substrates subset are shown in Figure 19. **Co1**, **Co2(=27)** and **Co3** were designed through deconstruction of known inhibitor A-1210477 which contains a naphthyl group.<sup>61,62</sup> The three naphthalene compounds contain different functional groups at the 1-position which may react with a rhodium carbenoid species through different pathways, leading to different linkages between the diazo bearing fragment and the substrate. Co-substrates **Co4** and **Co5** were designed through deconstruction of compounds designed by Fesik (**44**).<sup>59</sup> Many of these contain a 3,5-dimethyl-4-chlorophenyl substituent tethered to an indole by a linker (Figure 20). Co-substrates **Co6** and **Co7(=52)** were designed through deconstruction of known binder **55**.<sup>62</sup>



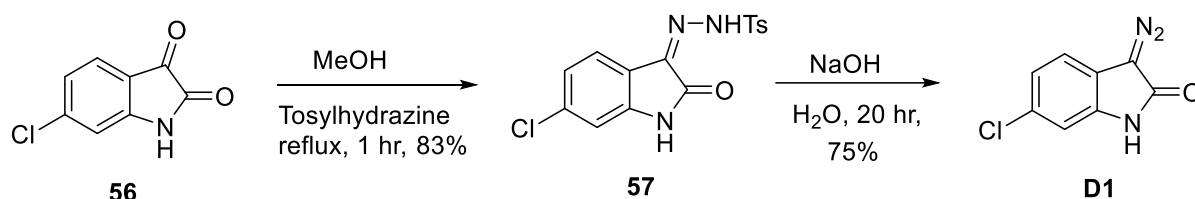
**Figure 20:** Compound **44** and **55**, both known binders which were deconstructed to generate diazos and co-substrates.

Substitution from the 7-position of indole-based inhibitors is known to increase affinity to Mcl-1 through additional interactions with the P3 site.<sup>66</sup> **Co7** contains a *tert*-butyl ester which upon deprotection will reveal a free carboxylic acid, known to increase affinity for Mcl-1.<sup>56,57</sup> **Co8** contains a hydrophobic phenyl group which can occupy the hydrophobic pockets of Mcl-1. The methoxy group and methylenes can undergo C-H insertions, increasing the promiscuity of the reaction, as well as a nitrile which can react with a rhodium carbenoid to form an oxazole or undergo C-H insertions.<sup>67</sup> The chosen catalysts have historically been used in the group for performing arrays of rhodium carbenoid chemistry.



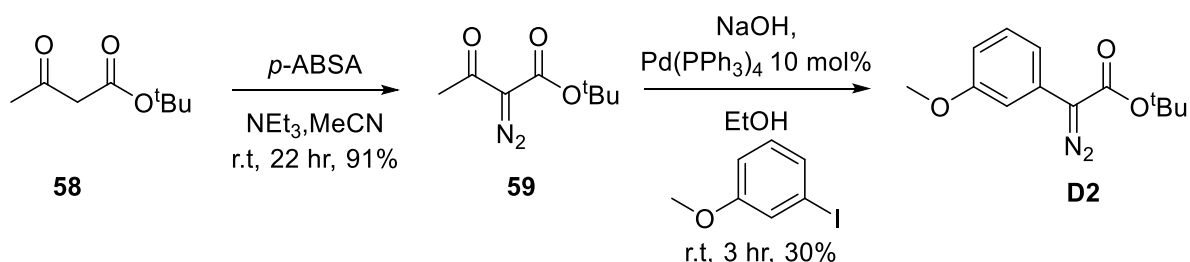
### 2.3.1.2 Synthesis of Diazos and Co-Substrates

Diazo **D1** was prepared following a known literature procedure.<sup>68</sup> 6-Chloroisatin **56** was treated with tosylhydrazine and refluxed in methanol for 1 hr to form intermediate **57** in a yield of 83%, which was carried forward without further purification. Intermediate **57** was then treated with aq. NaOH and stirred at r.t for 20 hr to give the diazo **D1** in a yield of 75%.



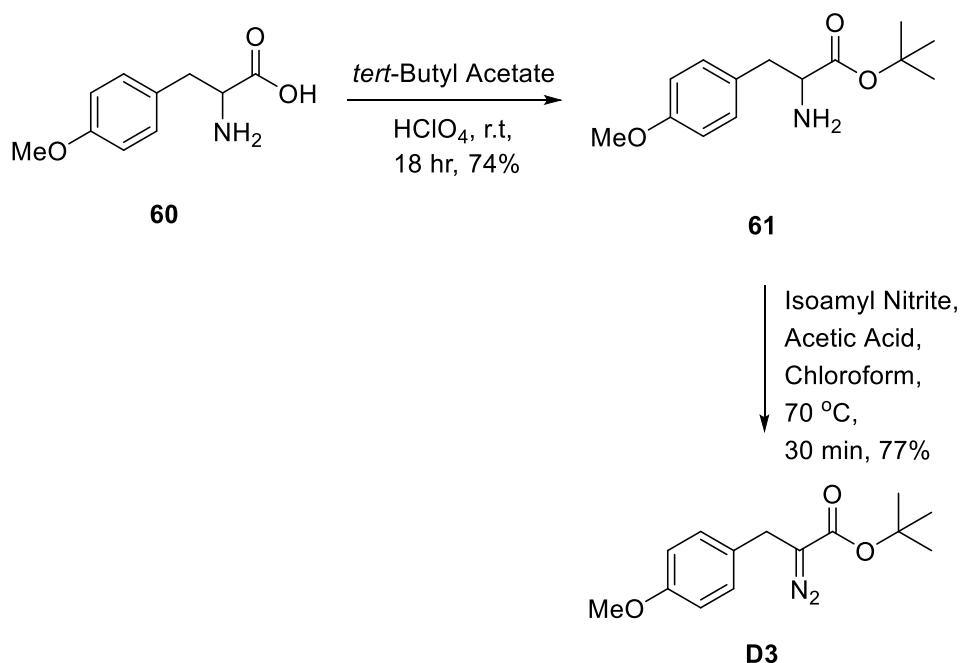
**Scheme 9:** Preparation of diazo **D1** through the base-aided decomposition of tosylhydrazone **57**.

Diazo **D2** was prepared using a known literature procedure.<sup>69</sup> *tert*-Butyl acetoacetate **58** was treated with diazo transfer reagent *p*-ABSA and triethylamine then stirred at r.t for 22 hr in acetonitrile to give **59** in a yield of 91%. Compound **59** was taken forward and was treated with NaOH, Pd(PPh<sub>3</sub>)<sub>4</sub> and 3-iodoanisole in EtOH and stirred at r.t for 3 hr to give diazo **D2** in a yield of 30%.



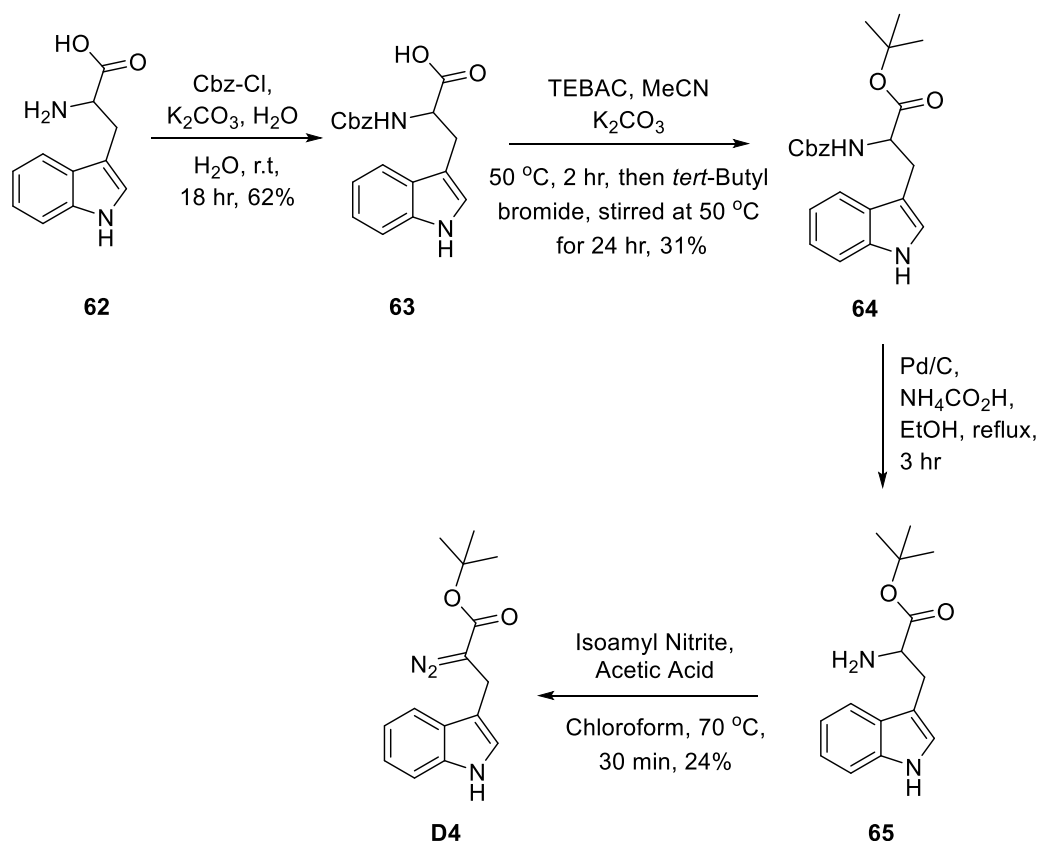
**Scheme 10:** Preparation of diazo **D2** using diazo transfer followed by deacylation and palladium catalysed cross coupling with 3-iodoanisole.

The synthesis of diazo **D3** required a third approach to diazo synthesis. The first step involved treatment of **60** with *tert*-butyl acetate and perchloric acid then stirred at r.t for 18 hr to give **61** in a 74% yield.<sup>70</sup> Amino ester **61** was then treated with isoamyl nitrite and a catalytic amount of acetic acid and refluxed in chloroform for 30 min to give diazo **D3** in a yield of 77%.<sup>71</sup>



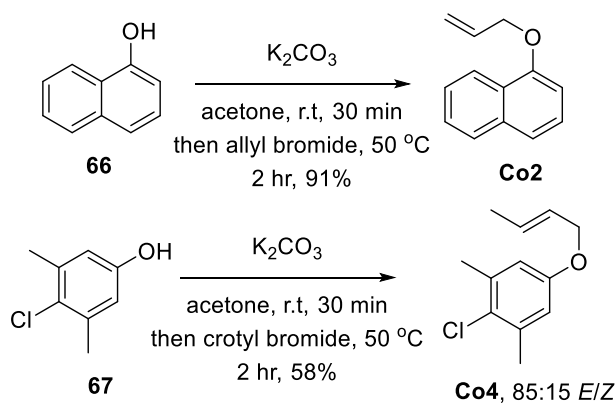
**Scheme 11:** Preparation of diazo **D3** through *tert*-butyl protection of **60** followed by diazotisation.

The synthesis of diazo **D4** was designed using tryptophan as a starting point. Firstly, L-Tryptophan **62** was treated with CbzCl and NaHCO<sub>3</sub> in H<sub>2</sub>O then stirred at r.t for 18 hr to give **63** in a 62% yield.<sup>72</sup> The carboxylic acid **63** was then treated with TEBAAC, K<sub>2</sub>CO<sub>3</sub> and *tert*-butylbromide then stirred at 50 °C for 24 hr to give **64** in 31% yield.<sup>73</sup> Next, compound **64** was treated with Pd/C (10 mol%) and NH<sub>4</sub>CO<sub>2</sub>H in EtOH under reflux for 3 hr to give primary amine **65** in 64% yield.<sup>74</sup> Finally, compound **65** was treated with isoamyl nitrite and a catalytic amount of acetic acid (8 mol%) in chloroform and stirred at 70 °C for 30 min to give diazo **D4** in 24% yield.<sup>71</sup>



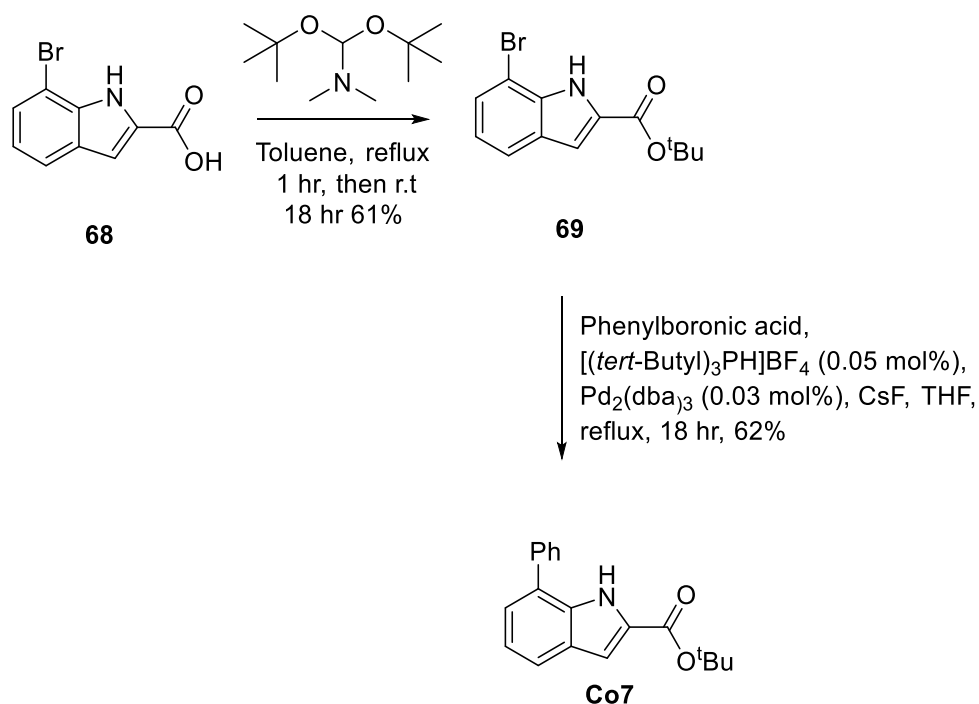
**Scheme 12:** Preparation of diazo **D4** from tryptophan **62**.

Co-substrate **Co2** and **Co4** were both synthesised using a Williamson ether synthesis. **66** and **67** were stirred at room temperature for 30 min with potassium carbonate in acetone followed by the addition of the corresponding bromoalkene and heating to 50 °C for 2 hr to give the desired products in 91% and 58% yield respectively.<sup>75</sup>



**Scheme 13:** Preparation of co-substrates **Co2** and **Co4** via Williamson ether synthesis.

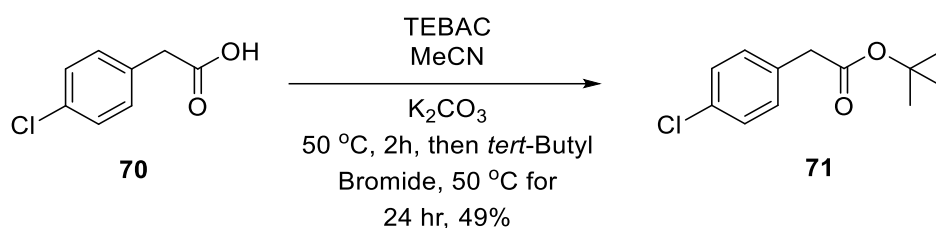
Co-substrate **Co7** was the final co-substrate which required synthesis to perform the array. 7-bromoindole-2-carboxylic acid **68** was treated with *N,N*-Dimethylformamide di-*tert*-butyl acetal and refluxed in toluene to give **69** in 61% yield. Compound **69** was then treated with phenylboronic acid, Pd<sub>2</sub>(dba)<sub>3</sub> (0.03 mol%), [(*tert*-Butyl)<sub>3</sub>PH]BF<sub>4</sub> (0.06 mol%) and CsF and refluxed in THF to give **Co7** in 62% yield.



**Scheme 14:** Preparation of co-substrate **Co7** via *tert*-Butyl protection and Suzuki coupling.

### 2.3.1.3 Optimisation of *In Situ* Deprotection

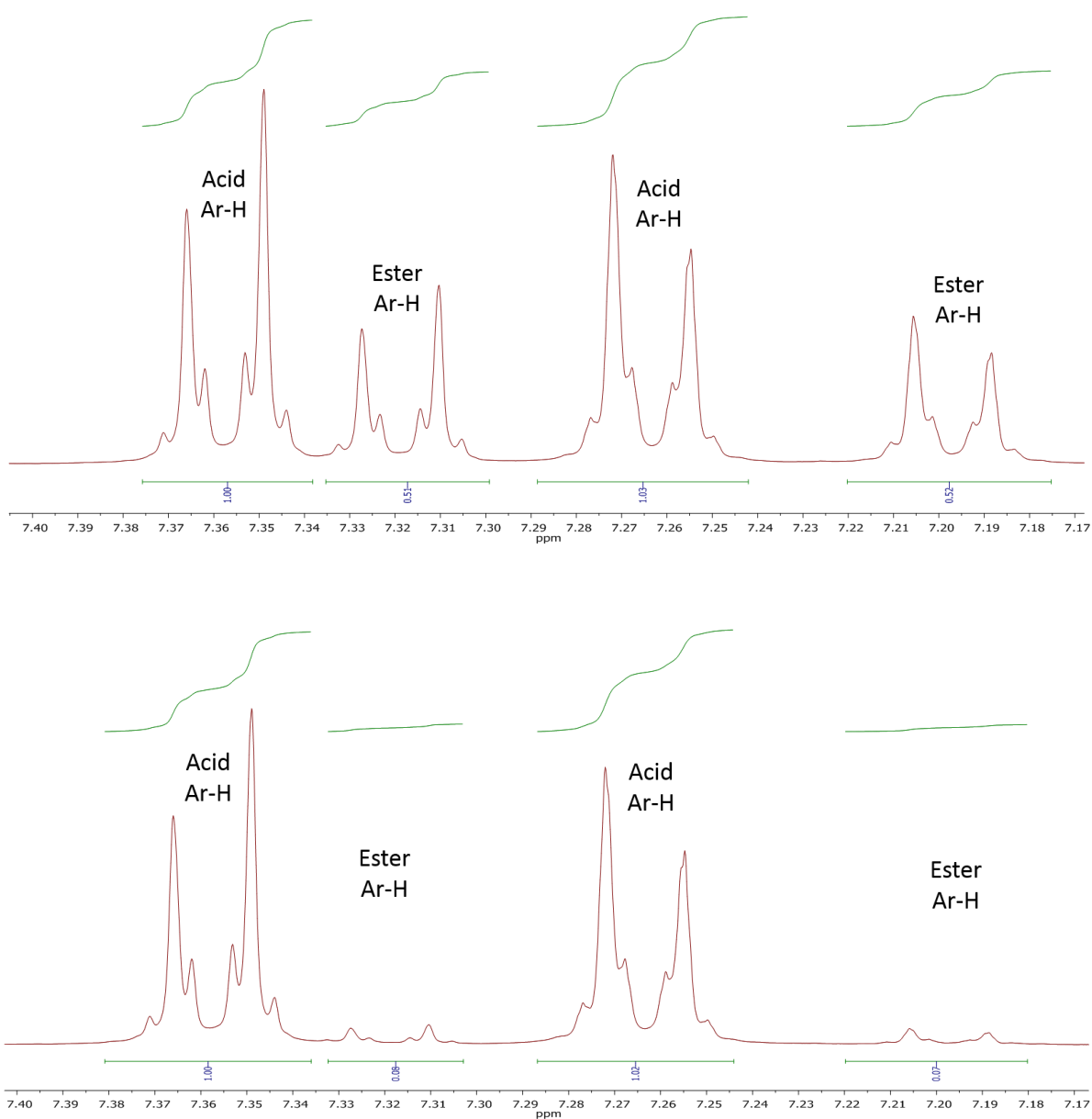
A *tert*-butyl ester deprotection step was a crucial part of the envisaged ADS workflow. Robust deprotection conditions will result in liberation of the carboxylic acid in product mixtures and must be achievable at room temperature. Any reagents used in the deprotection must also either be removable or ensured that they will not interfere with the assay. A model *tert*-butyl ester was designed for the deprotection study and synthesised.



**Scheme 15:** The synthesis of a model ester **71** to be used in TFA deprotection studies.

The deprotection study involved varying the volume of TFA in deuterated DCM. Triethylsilane was used as a scavenger to capture the released *tert*-butyl cation. The reaction was monitored using  $^1\text{H}$  NMR (500 MHz) and a reference compound (1,2,4,5-tetrachlorobenzene) was included in the NMR tubes as an internal standard, so percentage of deprotection could be calculated relative to the amount of internal standard. Four different volumes of TFA in DCM were used and the time taken to achieve deprotection is given below (Figure 21). The diagnostic signals used to determine whether deprotection had taken place were the aromatic protons, as they appear at a different chemical shift in the  $^1\text{H}$  NMR of the acid and ester. The disappearance of one set of aromatic peaks and the appearance of another was used to quantify the deprotection.

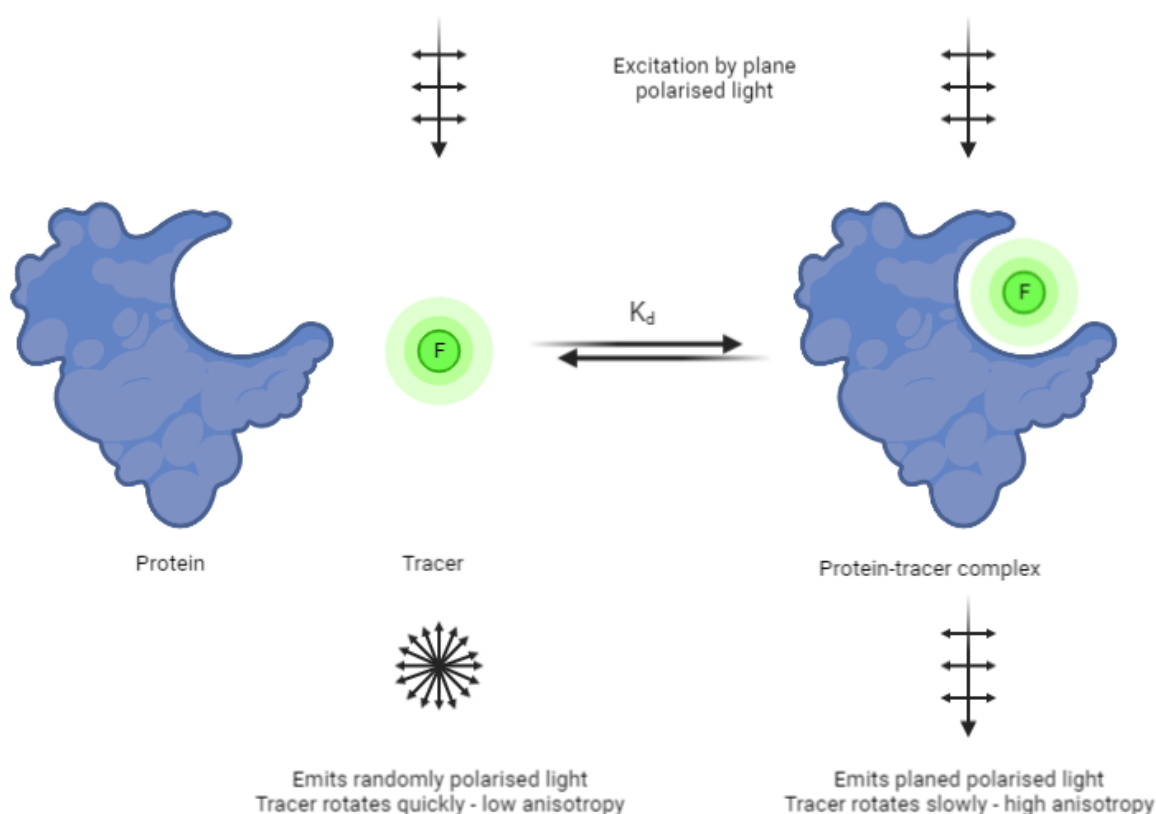
% TFA in DCM (by volume)	Time taken to achieve >98% deprotection
5	48 hr
10	2 hr
20	<30 min
30	<30 min



**Figure 21:** Approximate time required for different volumes of TFA to achieve complete deprotection of model *tert*-butyl ester **71** and <sup>1</sup>H NMR (500 MHz) of 10% TFA in DCM after 0.5 hr (above) and 1 hr (below).

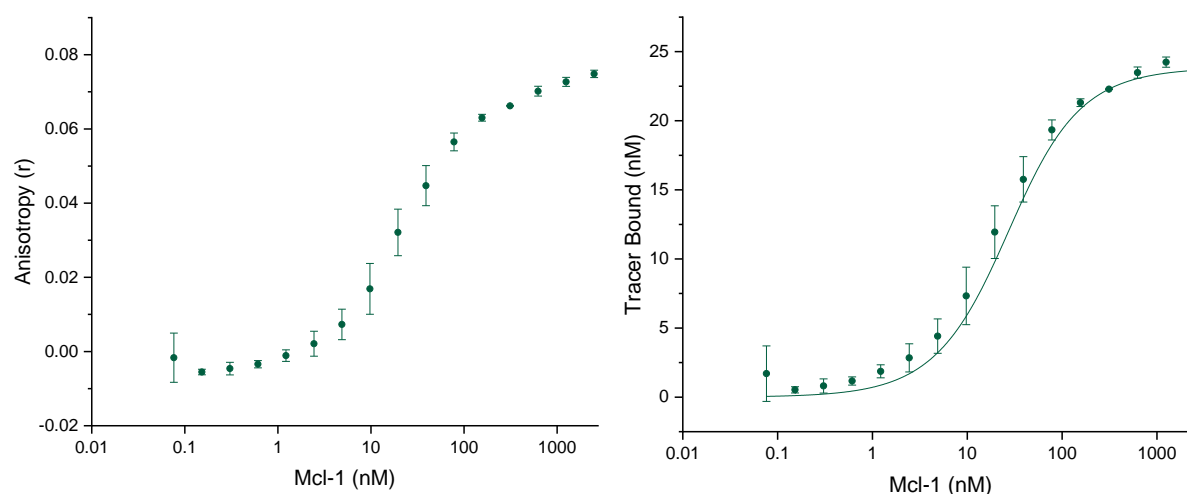
### 2.3.1.4 Configuration of Fluorescence Anisotropy Assay

A fluorescence anisotropy inhibition assay depends on the interaction of a target protein, and a tracer protein labelled with a fluorescent tag.<sup>76</sup> The tracer is excited by plane polarised light and the fluorescence emitted is measured parallel and perpendicular to the plane of polarisation of the incident light (Figure 22). The basis of the assay is that the free tracer tumbles quickly relative its fluorescence lifetime and so upon emission of fluorescence the orientation of polarisation is random. When the tracer is bound to the protein it tumbles slower and so the fluorescence emitted is also polarised. The difference of the measurements of the fluorescence emitted parallel and perpendicular to the plane of polarisation of the light can be used to determine the dissociation constant ( $K_d$ ) and determine affinity to the protein of compounds of interest (e.g. inhibitors).



**Figure 22:** The concept of fluorescence anisotropy inhibition assays. The free tracer rotates quickly, resulting in low polarisation. The tracer rotates slowly when complexed with the protein target, resulting in high polarisation.<sup>76</sup>

Mcl-1 protein and fluorescent tracer (fluorescein labelled BID, see Section 5.6.1) were obtained in-house from Dr Fruzsina Hobor. Experiments were performed to assess the limits of anisotropy of the assay, which is the largest assay window resulting from free and complexed tracer. Limits of anisotropy were determined by titrating protein into a fixed concentration of tracer (25 nM) at 0% DMSO. The polarisation of emitted light was measured parallel and perpendicular to the plane of polarisation of the excited light and an anisotropy value for each concentration of protein calculated. The plate was incubated for 2 hr at room temperature before being read. The anisotropy was plotted against protein concentration and the data fitted using a logistical fit. The limits of anisotropy were determined to be  $r_{\max} = 0.08$  and  $r_{\min} = -0.007$ . Tracer bound was then plotted against protein concentration to give  $K_d$  of tracer:  $11.9 \pm 0.2$  nM.



**Figure 23:** The calculated anisotropy plotted against protein concentration in the presence of fixed amount of tracer (25 nM) and buffer: Tris (50 mM), NaCl (150 mM). Buffer: Triton X-100: 0.01%, pH 7.4 at 0% DMSO. The anisotropy was calculated at 2 hr min and the upper and lower anisotropy limits were determined to be  $r_{\max} = 0.08$  and  $r_{\min} = -0.007$  respectively.

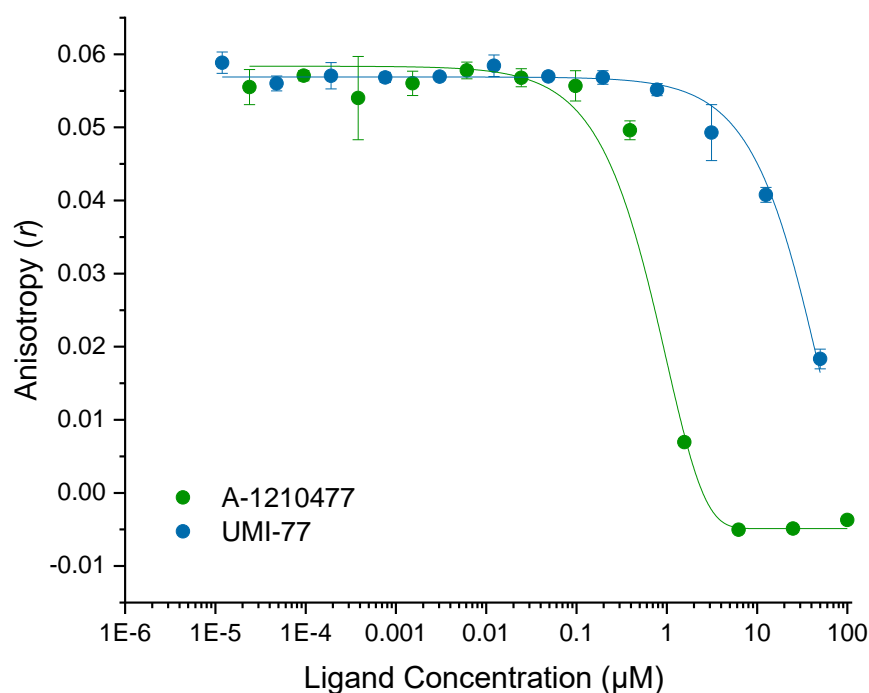
To evaluate the ability of the assay to determine affinities of unlabelled compounds, experiments were performed using known inhibitors of Mcl-1. A 12-point 4-fold dose-response was performed on A-1210477 (initial concentration 100  $\mu$ M) and UMI-77 (initial concentration 50  $\mu$ M) with tracer (25 nM) and protein (150 nM) at 1% final DMSO concentration. The anisotropy was calculated after 2 hr and plotted against concentrated. The data was plotted using an logistical fit to determine  $IC_{50}$  values.



The  $IC_{50}$  values were then converted into  $K_i$  of the competitor ligand by combination with  $K_d$  of the protein using the method of Nicolovska-Coleska (see Section 5.6.7.6).<sup>77</sup>

A  $K_i$  of  $20.8 \pm 2.9$  nM was obtained for A-1210477 and a  $K_i$  of  $2.5 \pm 0.1$   $\mu$ M was obtained for UMI-77. Literature values for A-1210477 are  $K_i = 0.44$  nM<sup>61,62</sup> and for UMI-77 are  $K_i = 0.49 \pm 0.06$   $\mu$ M. The discrepancy between the measured and literature values may be to differences in assay conditions.

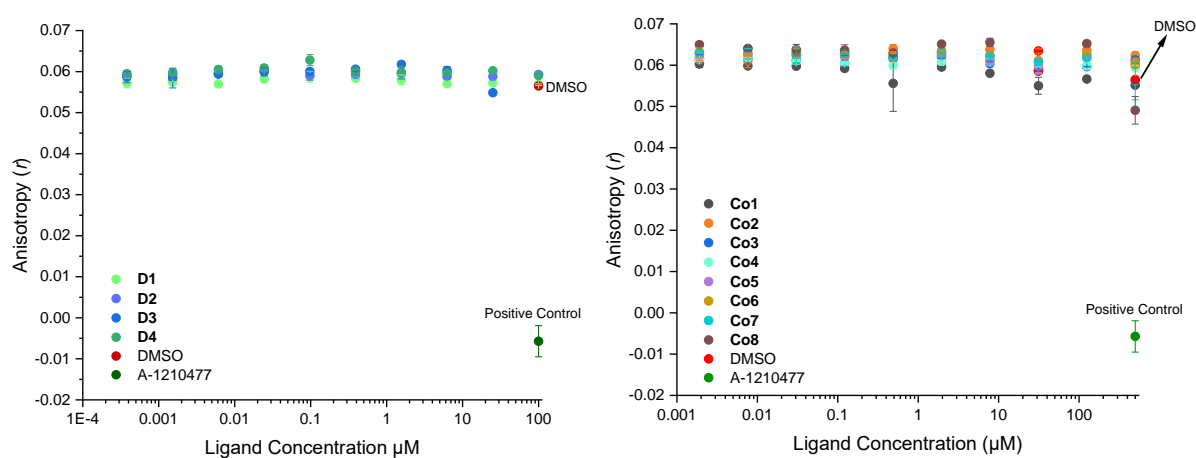
The positives controls will be in used to ensure that assay is working as expected during screening of crude reaction mixtures and isolated products.



**Figure 24:** Dose-response of positive controls. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr. The value of the bottom asymptote was fixed to determine an  $IC_{50}$  for UMI-77. A-1210477 (above)  $IC_{50}$ :  $0.91 \pm 0.06$   $\mu$ M, UMI-77 (below)  $IC_{50} \sim 80.9$   $\mu$ M.

### 2.3.1.5 Determination of the Inherent Activity of Round One Substrates

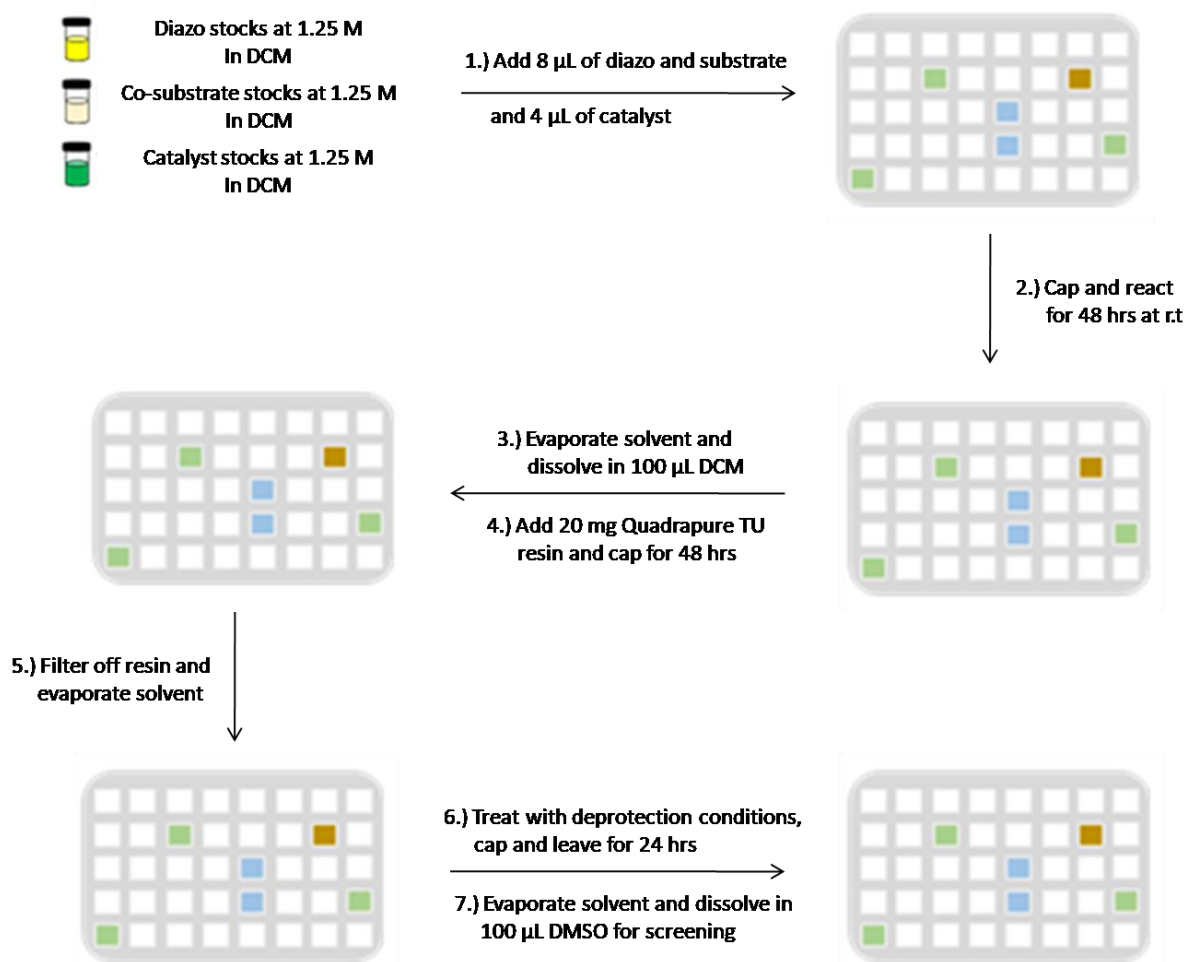
The inherent activity of the diazos and co-substrates to be used in arrays was assessed in order to be certain that any activity observed in screening the array is due to the formation of products rather than the presence of starting materials. This would also inform the total product concentration used for screening. Diazos and co-substrates were first treated with the TFA deprotection conditions, 10% TFA in DCM with 2 eq. triethylsilane overnight, established in section 2.3.1.3. A 4-fold 10-point dilution series was performed on the crude products derived from the diazos (initial concentration 100  $\mu\text{M}$ ) and co-substrates (initial concentration 500  $\mu\text{M}$ ) with tracer (25 nM) and protein (150 nM) at 1% final DMSO concentration. The anisotropy was calculated after 2 hr and plotted against concentrated. All the diazos and co-substrates are essentially inactive below 100  $\mu\text{M}$ , with one co-substrate showing slight activity at the highest concentration. Therefore, 100  $\mu\text{M}$  crude product concentration would serve as an initial starting point for screening of reaction mixture.



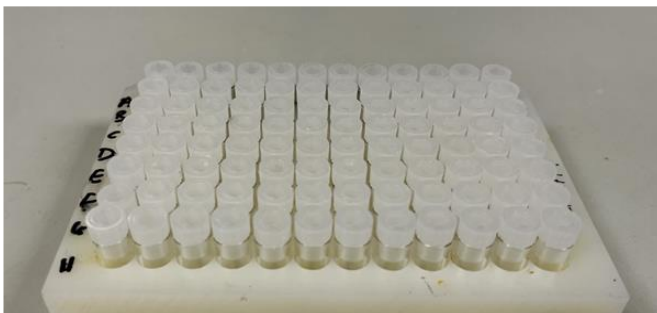
**Figure 25:** Inherent activity of round one diazos and co-substrates. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr. Diazos (above) and co-substrates (below)

### 2.3.1.6 Implementation of Reaction Array

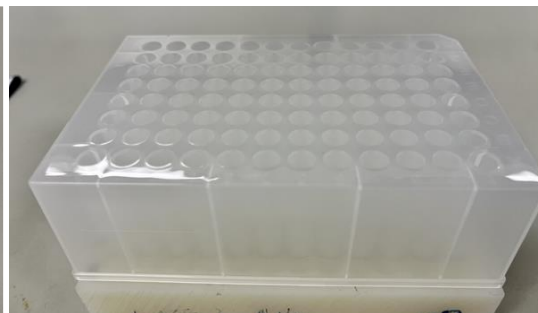
A reaction array was executed that exploited all combinations of four diazos (**D1-D4**), 8 co-substrates (**Co1-Co8**) and three catalysts (**C1-C3**) (Figure 19). Reaction arrays were performed in 0.75 mL borosilicate glass vials (8 x 30 mm) in 96-vial (12 x 8) array format and sealed with PTFE snap plugs (Figure 26). Diazos (1.25 M), substrates (6.25 M) and catalyst (25 mM) stock solutions were prepared. To each vial, one substrate (8  $\mu$ L), followed by one catalyst (4  $\mu$ L) and then one diazo (8  $\mu$ L) was added, then made up to 100  $\mu$ L with  $\text{CH}_2\text{Cl}_2$  and capped, to give a final reaction concentration of 0.1 M diazo, 0.5 M substrate and 1 mM catalyst. The reactions were left at r.t for 48 hr upon which QuadraPure<sup>®</sup> TU resin (20.0 mg) was added along with 100  $\mu$ L  $\text{CH}_2\text{Cl}_2$ . The reaction mixtures were capped and left to scavenge for 24 h. Reaction mixtures were filtered, washed with  $\text{CH}_2\text{Cl}_2$  (3 x 100  $\mu$ L),  $\text{CH}_2\text{Cl}_2$  evaporated open to air followed by drying under vacuum. To each vial, 450  $\mu$ L of  $\text{CH}_2\text{Cl}_2$  along with 50  $\mu$ L TFA and 2.4  $\mu$ L triethylsilane was added, the reaction mixtures capped and left overnight, then dried open to air followed by drying under vacuum. Finally, reaction mixtures were dissolved in DMSO (100)  $\mu$ L to give a final total product concentration (with respect to diazo, the limiting reactant) of 100 mM. Control reactions of diazo or co-substrate with catalyst were included to give confidence that observed activity was coming from the formation of intermolecular products.



b.)



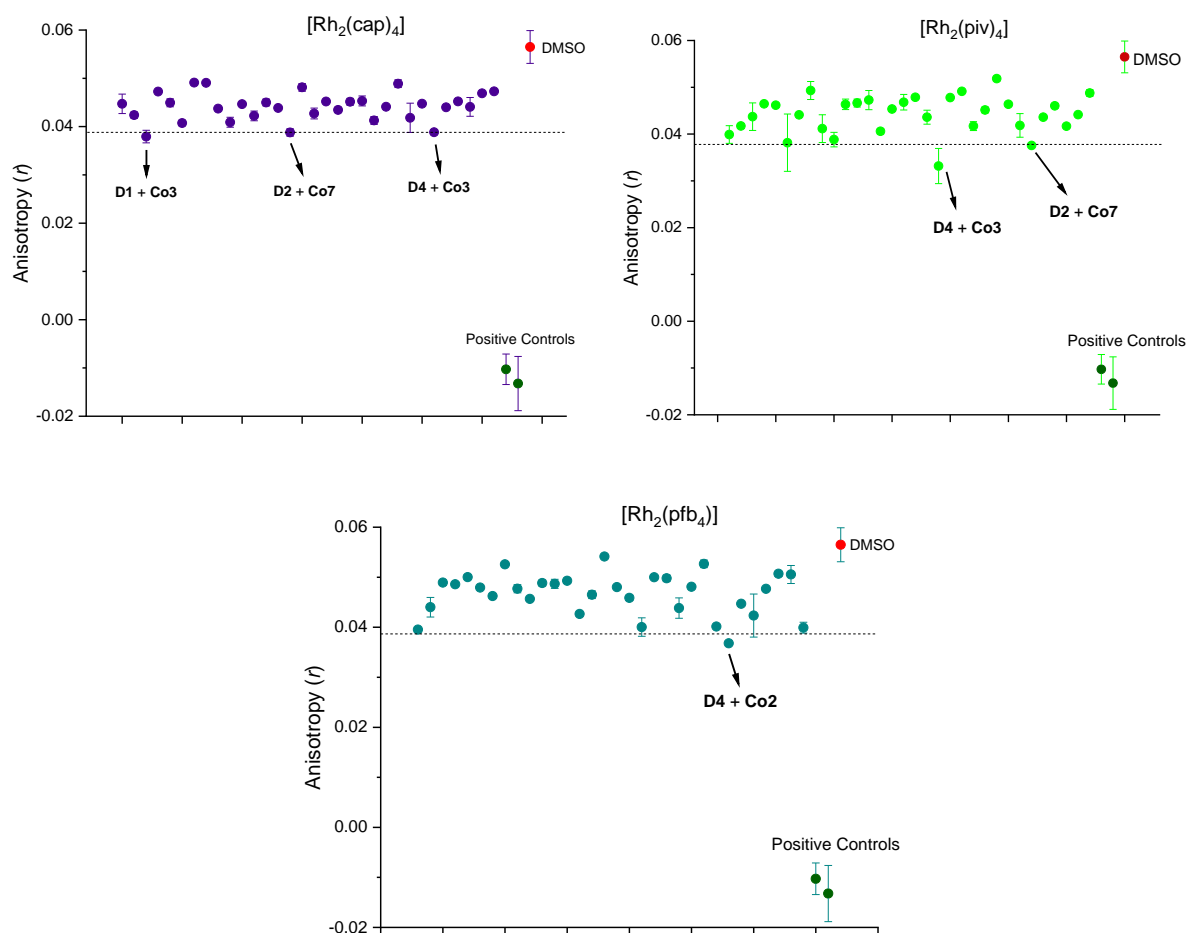
c.)



**Figure 26:** a) Overview of ADS workflow b) Room temperature reaction set up c) Filter plate for removing resin after scavenging.

Following the completion of reaction arrays, the crude product mixtures were screened against Mcl-1 in the fluorescence anisotropy inhibition assay. A-1210477 and UMI-77 (100  $\mu\text{M}$ ) were included as a positive control with DMSO negative control. Crude reaction mixtures were dissolved in DMSO (100  $\mu\text{L}$ ). Reaction array DMSO stocks (100 mM total product concentration) were diluted into DMSO and

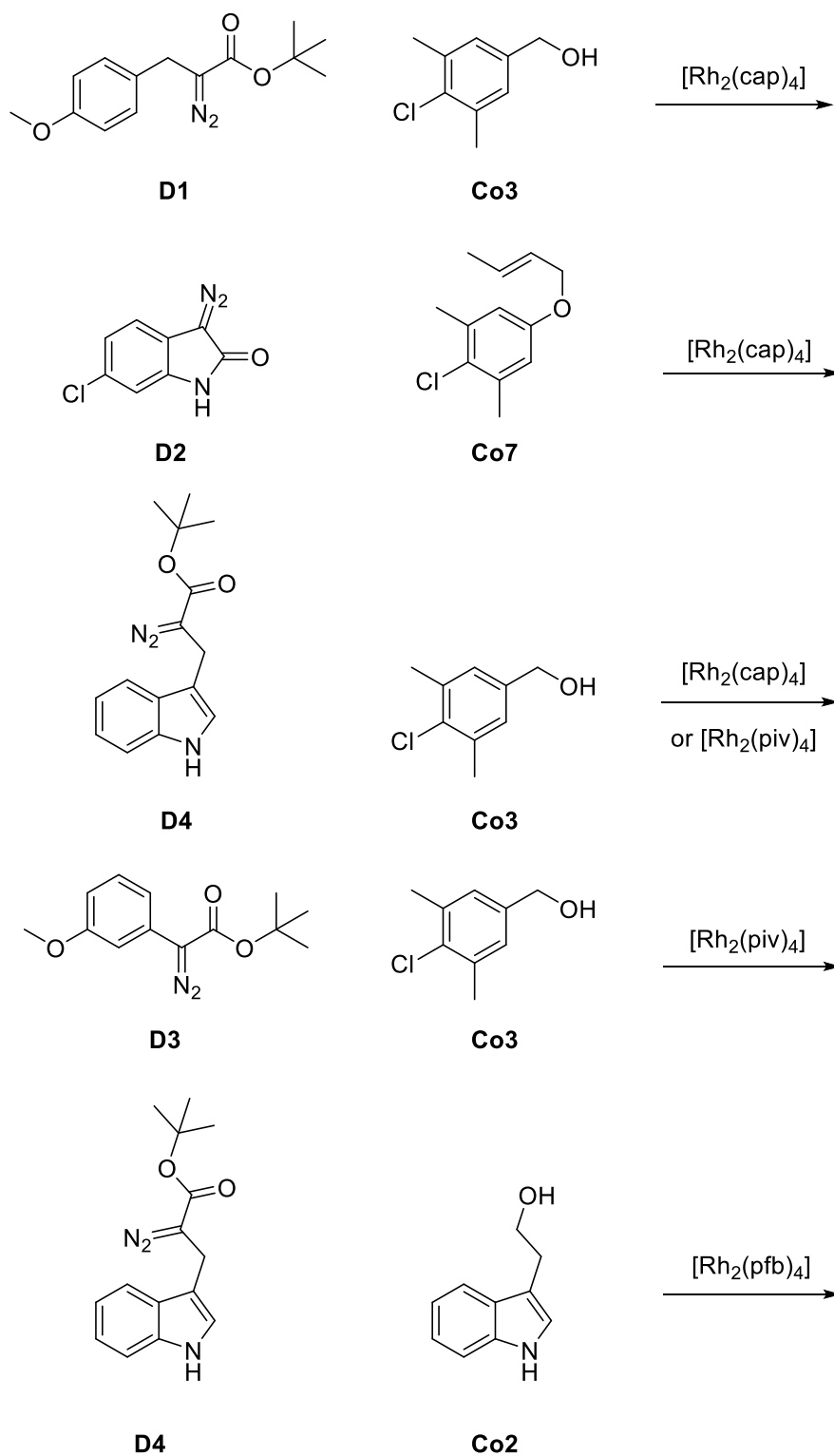
buffer to give stock solutions at 300  $\mu\text{M}$  in 3:97 DMSO:Buffer (three times the final assay concentration). 20  $\mu\text{L}$  of the DMSO:Buffer (3:97) crude product stock solution was added to the assay plate followed by 20  $\mu\text{L}$  of a 75 nM tracer stock solution and 20  $\mu\text{L}$  of 450 nM protein stock solution to give final concentration of tracer and protein of 25 nM and 150 nM respectively. The crude products of the reaction array were screened at a total product concentration of 100  $\mu\text{M}$  in duplicate. The anisotropy was calculated after two hr (Figure 27)



**Figure 27:** Results from round one of ADS. The most potent combinations of diazos, co-substrates and catalysts are highlighted. The mean anisotropy + 2 standard deviations (dashed line) was used to assess their significance. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr.

The most potent potential hit combinations are listed in Scheme 16. Combinations that were two or more standard deviations higher than the average anisotropy of the screened reaction mixtures were investigated. These mixtures require dose-

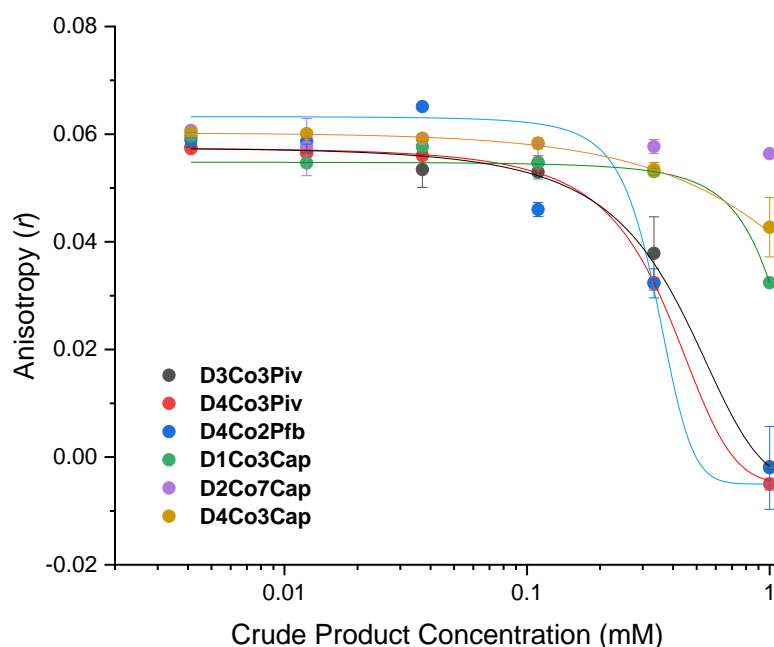
response on the crude reaction mixtures. Validated mixtures will be scaled up and the products purified, characterised and a dose-response recorded to measure the affinity.



**Scheme 16:** Potential hit combinations from ADS array one.

Following the identification of active combinations from the Mcl-1 rhodium carbenoid array, validation was performed on the promising reactions. First, a 3-fold 6-point dose-response was performed on the crude products screening in duplicate with initial product concentration of 1 mM. Dose-response revealed activity in five combinations out of the six, with **D2Co7Cap** being a false positive result. Based on the dose-response, four combinations were taken forward to scale-up, **D1Co3Cap**, **D3Co3Piv**, **D4Co3Piv** and **D4Co2Pfb**. It was decided not to take **D4Co3Cap** forward to scale-up as the dose-response for **D4Co3Piv** was better and so likely a higher yield of intermolecular product.

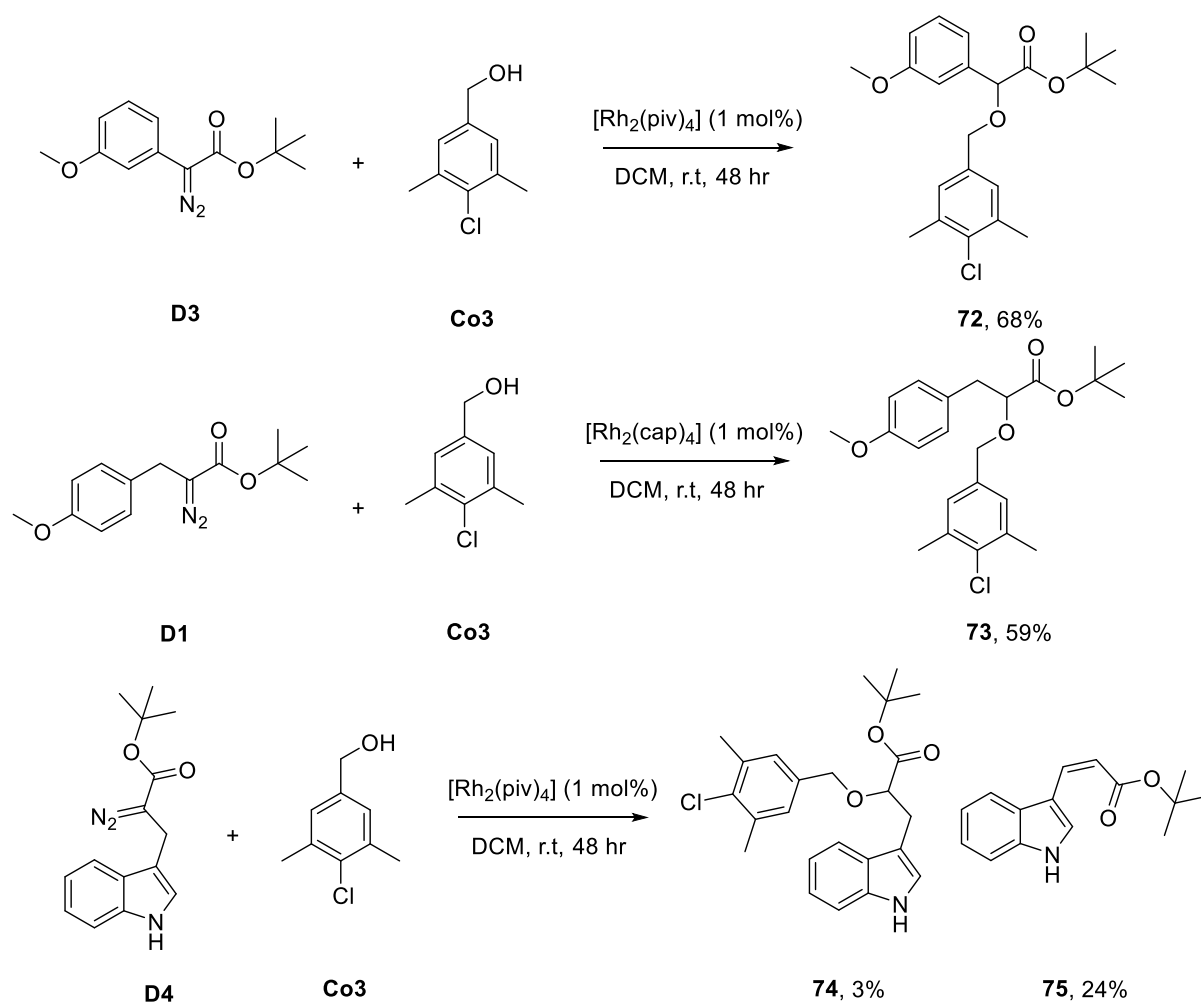
Combination	IC <sub>50</sub> (mM)	Scale-up
D1Co3Cap	1.3 ± 0.2	✓
D2Co7Cap	Inactive	X
D4Co3Cap	3.7 ± 1.0	X
D3Co3Piv	0.4 ± 0.05	✓
D4Co3Piv	0.4 ± 0.03	✓
D4Co2Pfb	0.3 ± 0.2	✓



**Figure 28:** Summary of the dose-response performed on crude active combinations. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr.

### 2.3.1.7 Scale-up of Round One Hit Combinations

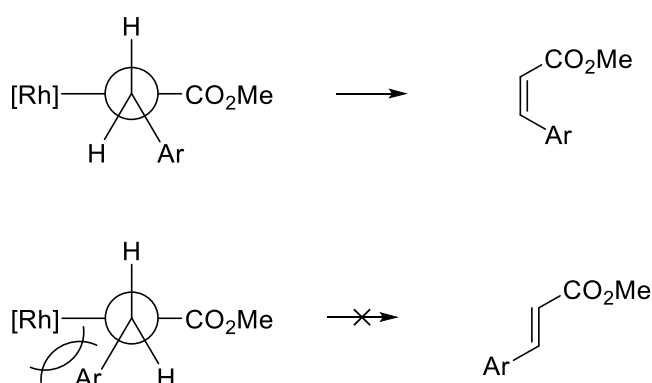
Following validation of hit combinations discovered from screening the round one ADS reaction array, several combinations were chosen for scale-up. These reactions were performed on a 50-fold increased scale. The reactions were performed under a nitrogen atmosphere in a crimped vial. The diazo was added dropwise to a solution of the co-substrate and catalyst in DCM over 4 hr, this technique has been used in the group previously to increase the yield of intermolecular products. The reaction was then left to stir for 48 hr. The reaction mixtures were purified using column chromatography or HPLC.



**Scheme 17:** Scale-up of hit reactions identified using activity-directed synthesis.

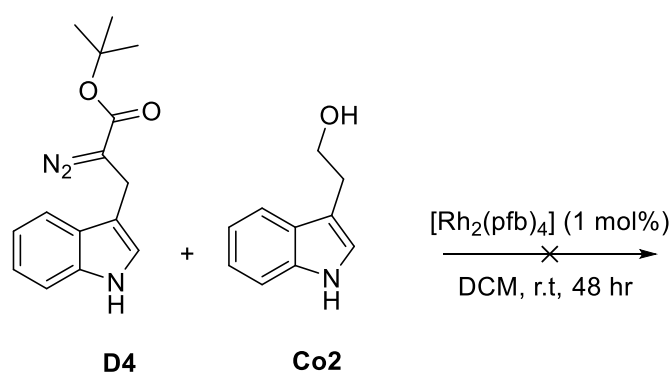


The anticipated O-H insertion products were isolated from the crude reaction mixtures (**72**, **73** and **74**). Interestingly, the scale-up of **D4** and **Co3** revealed  $\alpha,\beta$ -unsaturated ester **75** as the major product and only the thermodynamically less stable *Z*-isomer was isolated (1H, d, *J* 12.6 Hz). The mechanism of its formation involves conversion of the diazo to a rhodium carbenoid followed by  $\beta$ -hydride elimination to give the alkene.<sup>78</sup> The selective formation of the *Z*-isomer is believed to be due to steric constraints of the aryl group in the rhodium carbenoid intermediate (Figure 29).<sup>79</sup>



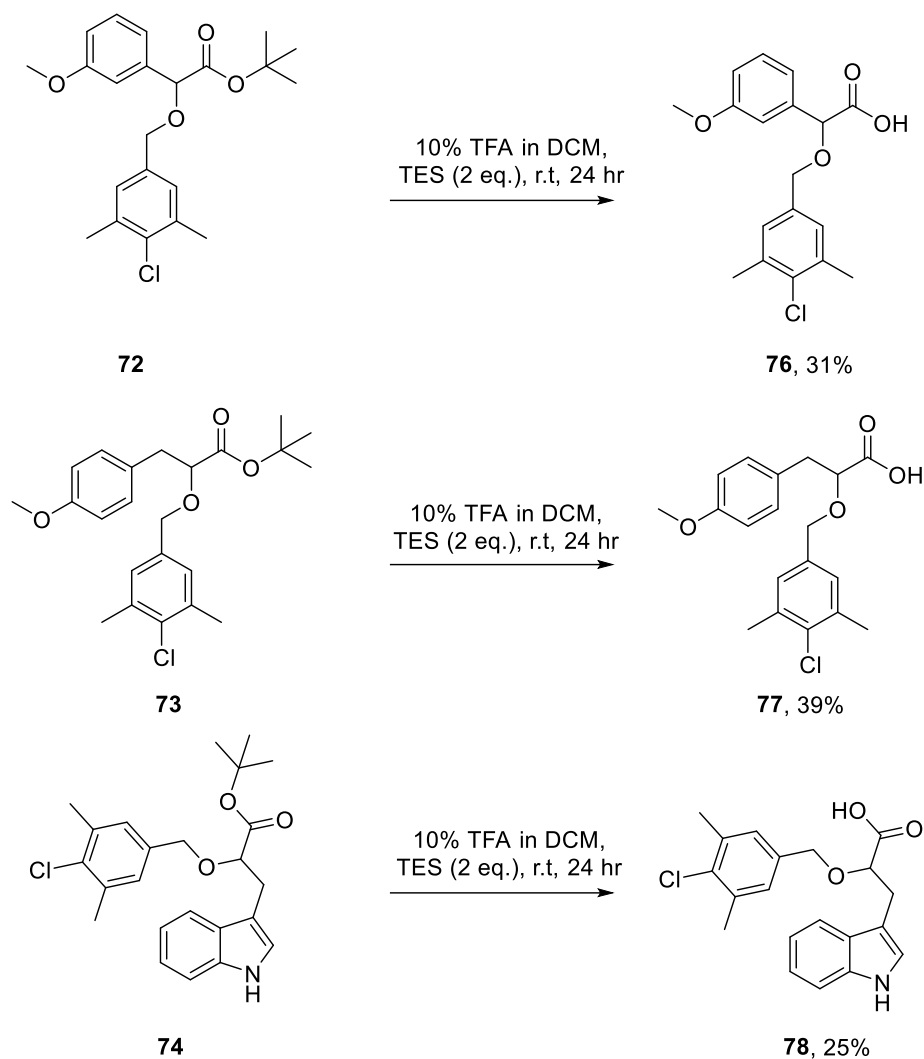
**Figure 29:** Formation of the *E*-isomer is unfavourable due to the steric constraints imposed by the aryl group in the rhodium carbenoid intermediate.

No intermolecular products could be isolated from the scale-up of diazo **D4** and co-substrate **Co2**, suggesting the activity observed when screening the crude mixture may be a false positive. It is likely the major product from the reaction is also  $\alpha,\beta$ -unsaturated ester **75** as it involves the same diazo substrate.



**Scheme 18:** Unsuccessful round one ADS scale-up.

Following purification and characterisation of products isolated from the round one ADS scale-ups, each product was *tert*-butyl deprotected using the conditions established in Section 2.3.1.3 (Scheme 19).

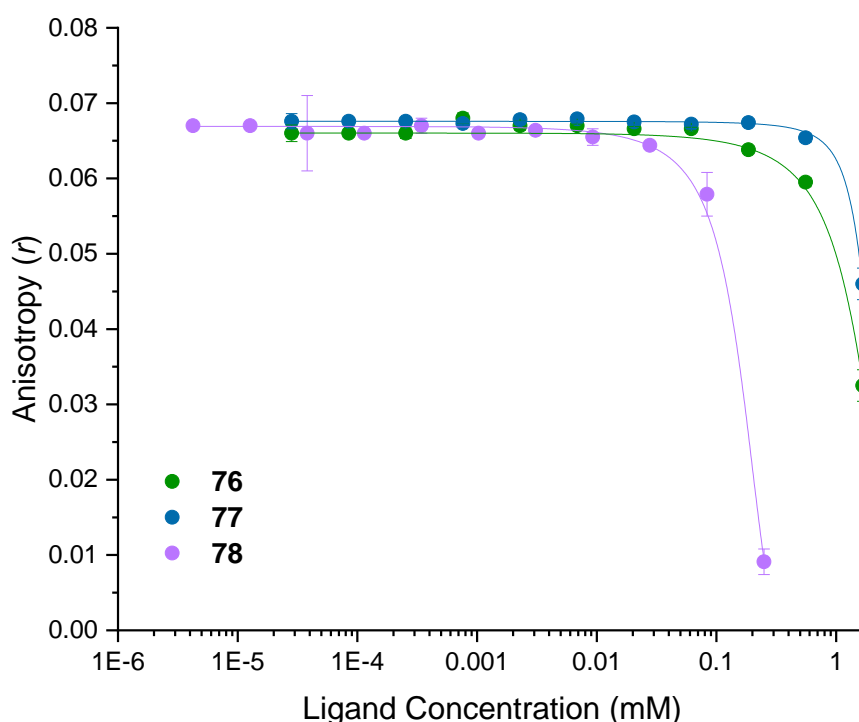


**Scheme 19:** *tert*-Butyl deprotection of intermolecular products isolated.

Following deprotection, each crude carboxylic was assessed *via* dose-response to ensure it possessed activity before it was purified using reverse-phase HPLC. The corresponding crude carboxylic of **77** was not assessed prior to purification due to the small amount of material obtained.

Following purification of the carboxylic acids, each product was evaluated in a three-fold twelve-point dose-response against Mcl-1/BID to obtain an  $IC_{50}$  value. Compound **76** and **77** were screened with initial concentration 1.66 mM and compound **78** at 0.25 mM, due to the small amount of material obtained. **76** showed  $IC_{50}$   $2.1 \pm 0.5$  and, **77**  $2.4 \pm 0.3$  mM and **78** showed roughly 10-fold better activity with an  $IC_{50}$  of around  $0.23 \pm 0.02$  mM.

Compound	$IC_{50}$ (mM)
<b>76</b>	$2.1 \pm 0.5$
<b>77</b>	$2.4 \pm 0.3$
<b>78</b>	$0.23 \pm 0.02$



**Figure 30:** Dose-response of purified carboxylic acids from round one of ADS targeting Mcl-1. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr. The value of the bottom asymptote was fixed against the positive control to determine an  $IC_{50}$ .

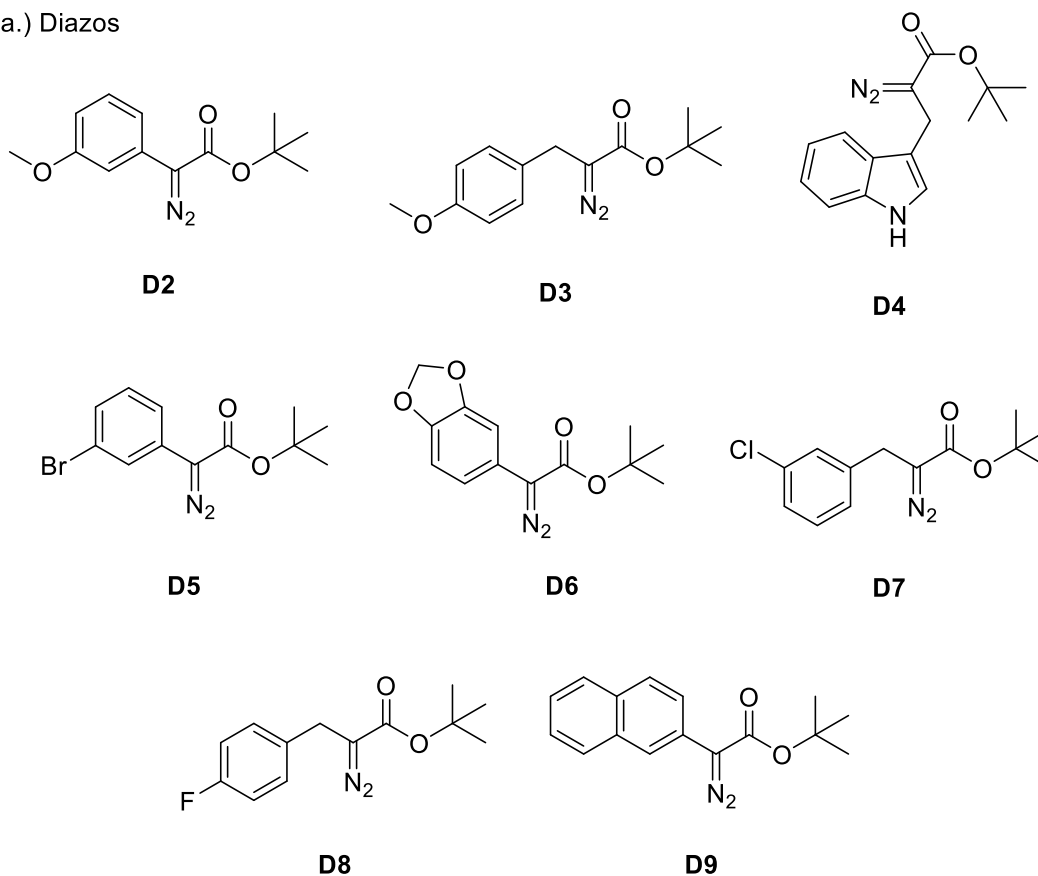
Deprotection of **75** yielded a complex reaction mixture which was determined to have an  $IC_{50} = 0.1$  mM. Although this is more active than the intermolecular products, no products could be identified or purified. The crude reaction mixture also showed poor solubility in DMSO, which may be responsible for the activity. With the discovery of three active intermolecular products, it was decided to execute a second round of ADS based on the active compounds.

## 2.3.2 Round Two of Activity-Directed Synthesis

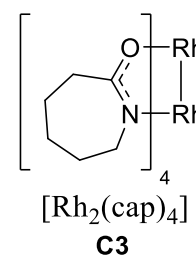
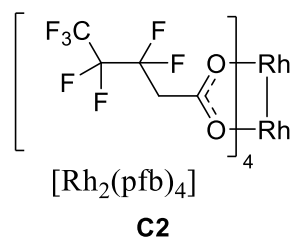
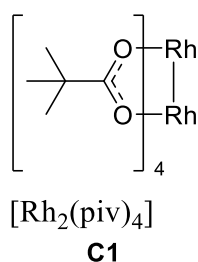
### 2.3.2.1 Design of Fragments for Activity-Directed Synthesis

Following the discovery of several mM inhibitors of Mcl-1/BID in round one, a second round of ADS was designed. It was envisaged that the diazos and co-substrates utilised in round two would bear resemblance to those that yielded hits in round one. The diazos and co-substrates which yielded hits in round one would also be included in round two. The set of diazos and co-substrates chosen is given in Figure 31.

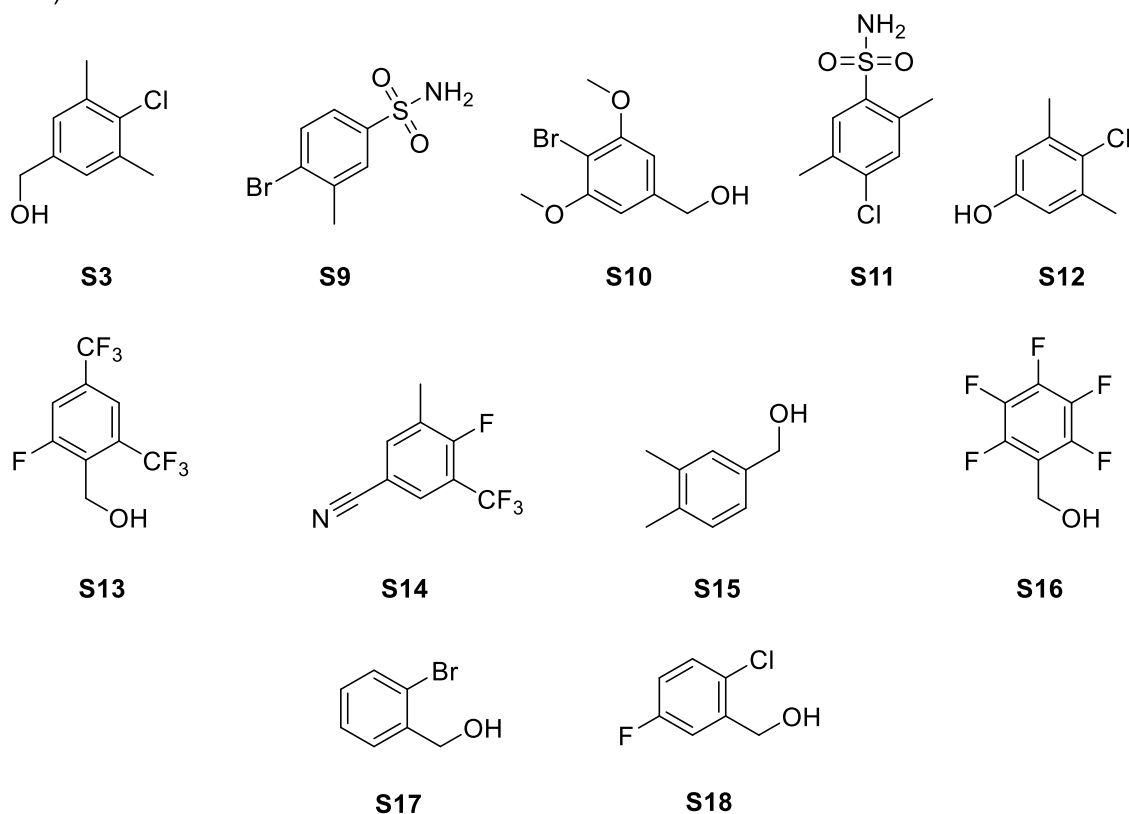
a.) Diazos



## b.) Catalysts



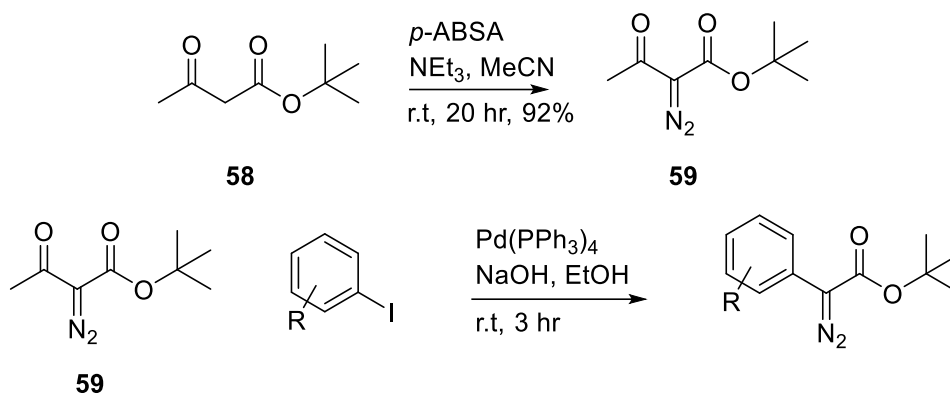
## c.) Substrates

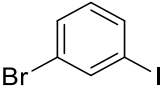
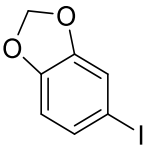


**Figure 31:** Diazos, catalysts and co-substrates chosen for the reaction array.

### 2.3.2.2 Synthesis of Diazos

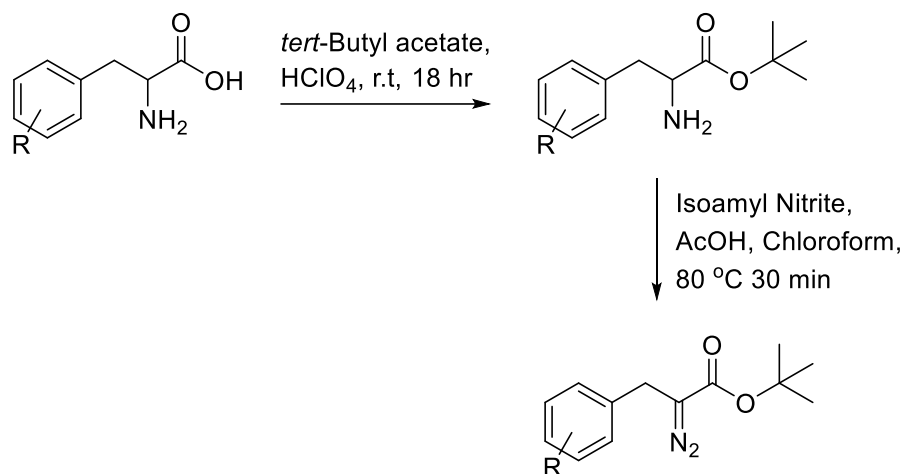
Diazos **D5** and **D6** were synthesised from *tert*-butyl acetoacetate **58** with *p*-ABSA and triethylamine and stirred at room temperature for 22 hr in acetonitrile to give **59** in 92% yield. These materials were taken forward and underwent deacylation and palladium-catalysed cross-coupling with corresponding aryl iodides to yield diazos **D5** and **D6** in 22% and 33% yield respectively.



Diazo	Aryl Iodide	Yield (%)
<b>D5</b>		22
<b>D6</b>		33

**Figure 32:** Preparation of diazo **D5** and **D6** using diazo transfer following by deacylation and palladium catalysed cross coupling with aryl iodides.

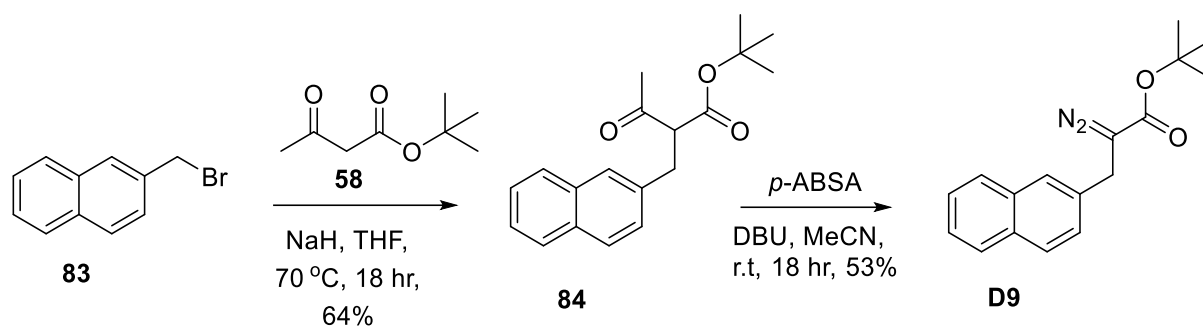
Diazos **D7** and **D8** were synthesized through *tert*-butyl protection of the corresponding amino acids **79** and **81** with *tert*-butyl acetate, perchloric acid (8 mol%) and stirred at r.t for 18 hr, giving **80** and **82** in 41% and 14% yield respectively. Compounds **80** and **82** were then treated with isoamyl nitrite and a catalytic amount of acetic acid in chloroform under reflux for 30 min to give diazos **D7** and **D8** in 71% and 73% yield respectively.



Diazo	Amino Acid	Step 1 Yield (%)	<i>tert</i> -Butyl ester	Step 2 Yield (%)
<b>D7</b>	 79	41	 80	71
<b>D8</b>	 81	14	 82	73

**Figure 33:** Preparation of diazo **D7** and **D8** through *tert*-butyl protection of corresponding amino acids followed by diazotisation.

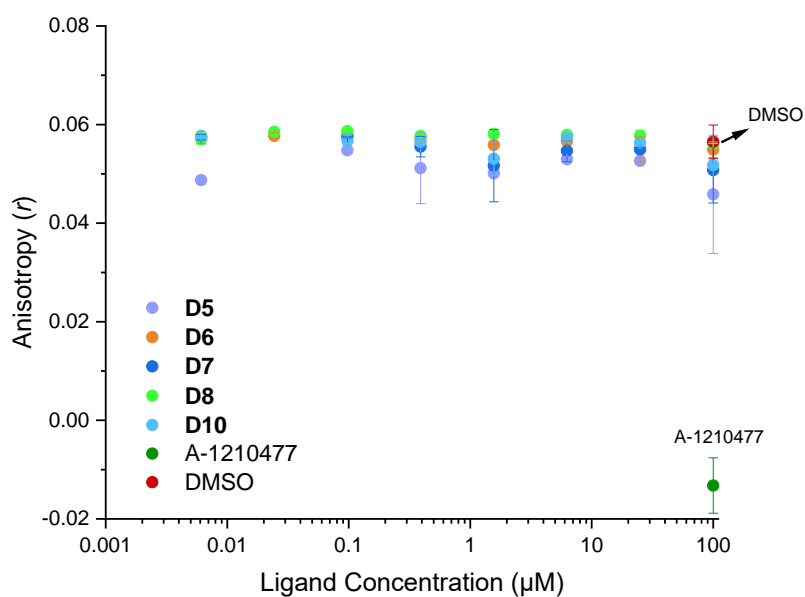
Finally, diazo **D9** was synthesised from 2-(bromoethyl)naphthalene **83** which was treated with *tert*-butyl acetoacetate and sodium hydride and refluxed in THF overnight to give **84** in 64% yield. The synthesised 1,3-dicarbonyl **84** then underwent deacylation and diazotisation with *p*-ABSA and DBU in MeCN overnight to yield the diazo **D9** in 53% yield.



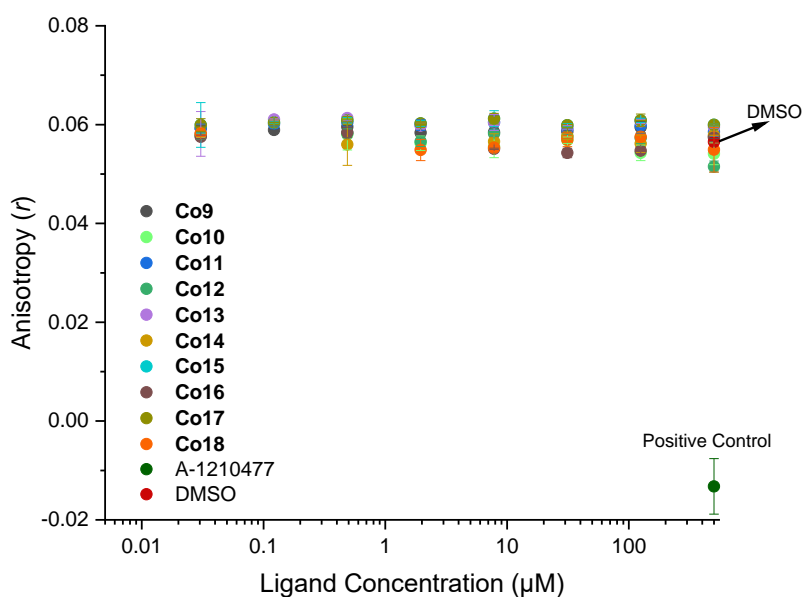
**Scheme 20:** Preparation of diazo **D9** from 2-(bromoethyl)naphthalene **69**.

### 2.3.2.3 Inherent Activity of Round Two Substrates

As in round one, the inherent activity of round two diazos and co-substrates were evaluated. These were first treated with the TFA deprotection conditions established in section 2.3.1.3. A 4-fold 8-point dilution series was performed on the diazos (initial concentration 100  $\mu\text{M}$ ) and co-substrates (initial concentration (500  $\mu\text{M}$ ) with tracer (25 nM) and protein (150 nM) at 1% final DMSO concentration. The anisotropy was calculated after 2 hr and plotted against concentrated (Figure 34). All the diazos and co-substrates are essentially inactive below 100  $\mu\text{M}$ . This concentration would serve as an initial starting point for screening of reaction mixture.



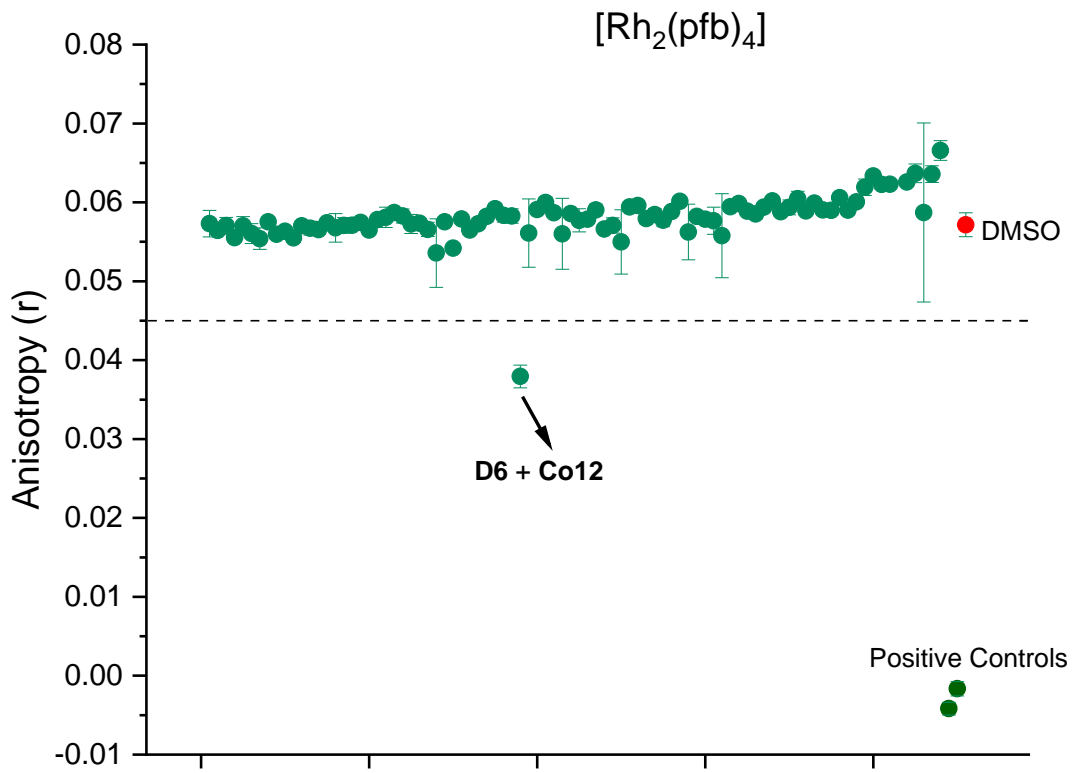
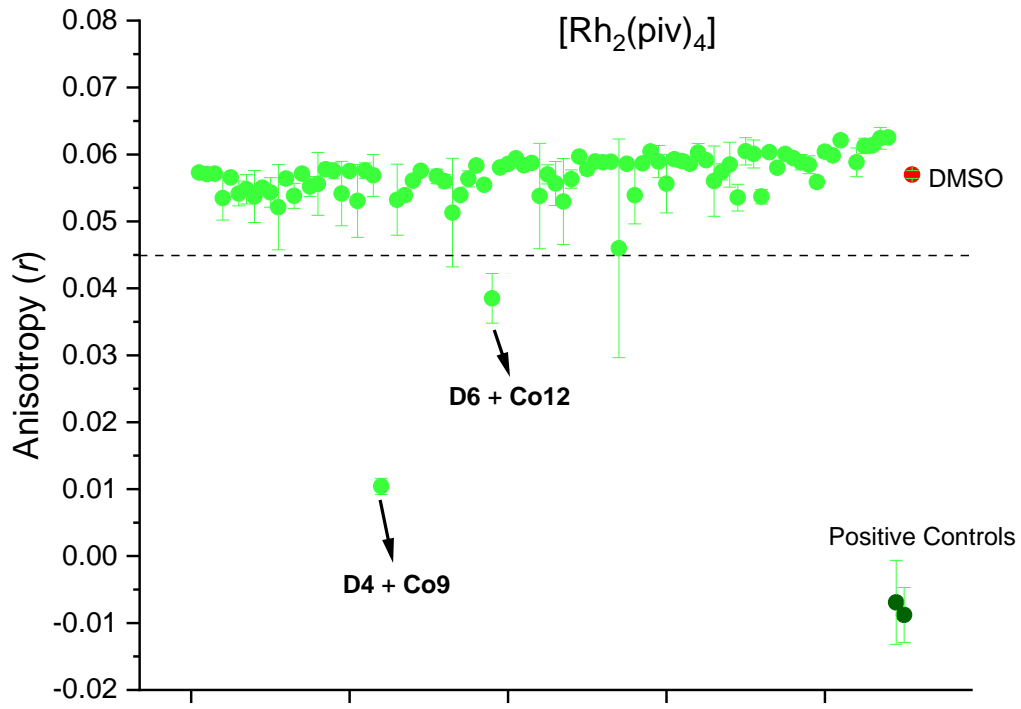


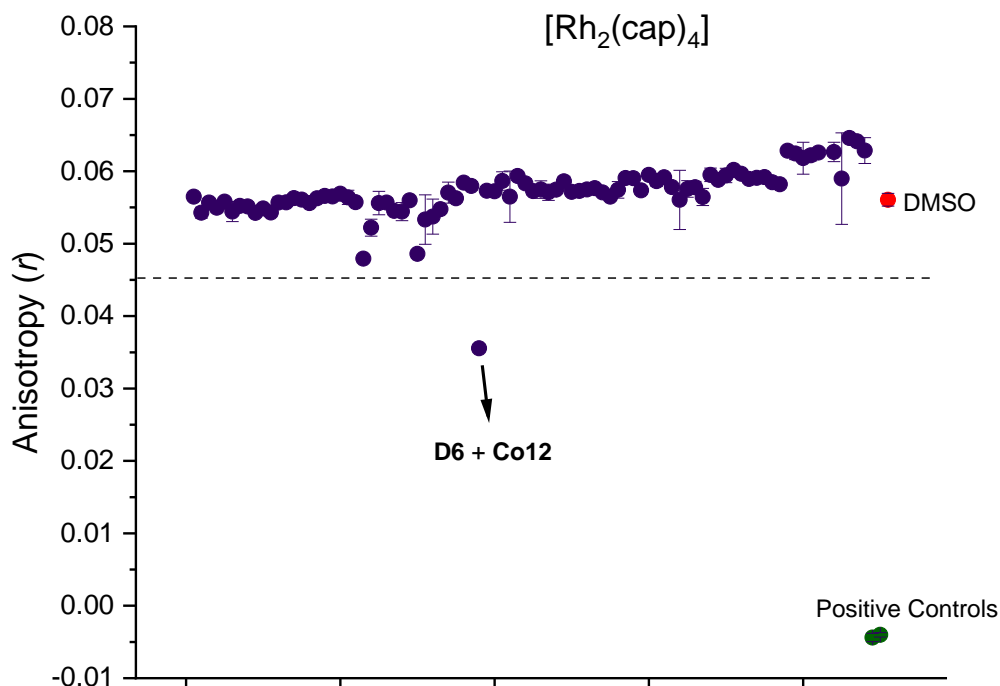


**Figure 34:** Inherent activity of round two diazos and co-substrates. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr. Diazos (above) and co-substrates (below).

### 2.3.2.4 Implementation of Reaction Array

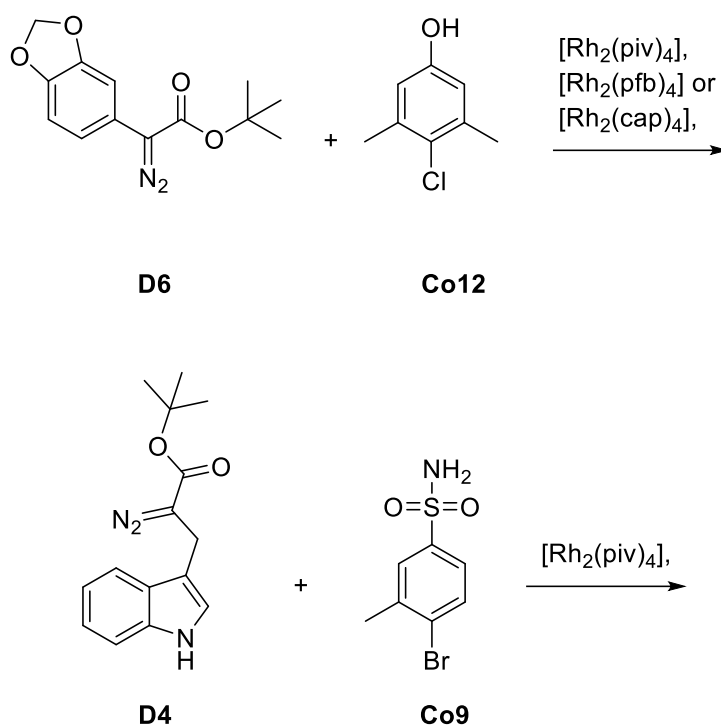
The array was implemented and screened in the same way as with round one (see Section 2.3.1.6). Every possible combination of diazo, co-substrates and catalysts were explored, giving a total of 288 reactions. Following the completion of reaction arrays, the crude product mixtures were screened against Mcl-1 in the fluorescence anisotropy inhibition assay. Controls wells of the individual reaction components with catalyst were included. A-1210477 and UMI-77 (100  $\mu\text{M}$ ) was included as a positive control with DMSO negative control. Crude reaction mixtures were dissolved in DMSO (100  $\mu\text{L}$ ). Reaction array DMSO stocks (100 mM total product concentration) were diluted into DMSO and buffer to give stock solutions at 300  $\mu\text{M}$  in 3:97 DMSO:Buffer (three times the final assay concentration). 20  $\mu\text{L}$  of the DMSO:Buffer (3:97) crude product stock solution was added to the assay plate followed by 20  $\mu\text{L}$  of a 75 nM tracer stock solution and 20  $\mu\text{L}$  of 450 nM protein stock solution to give final concentration of tracer and protein of 25 nM and 150 nM respectively. The crude products of the reaction array were screened at a total product concentration of 100  $\mu\text{M}$  in duplicate. The anisotropy was calculated after 2 hr (Figure 35).





**Figure 35:** Results from round two ADS Array. The most potent combinations of diazos, co-substrates and catalysts are highlighted. The mean anisotropy + 2 standard deviations (dashed line) was used to assess their significance. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr.

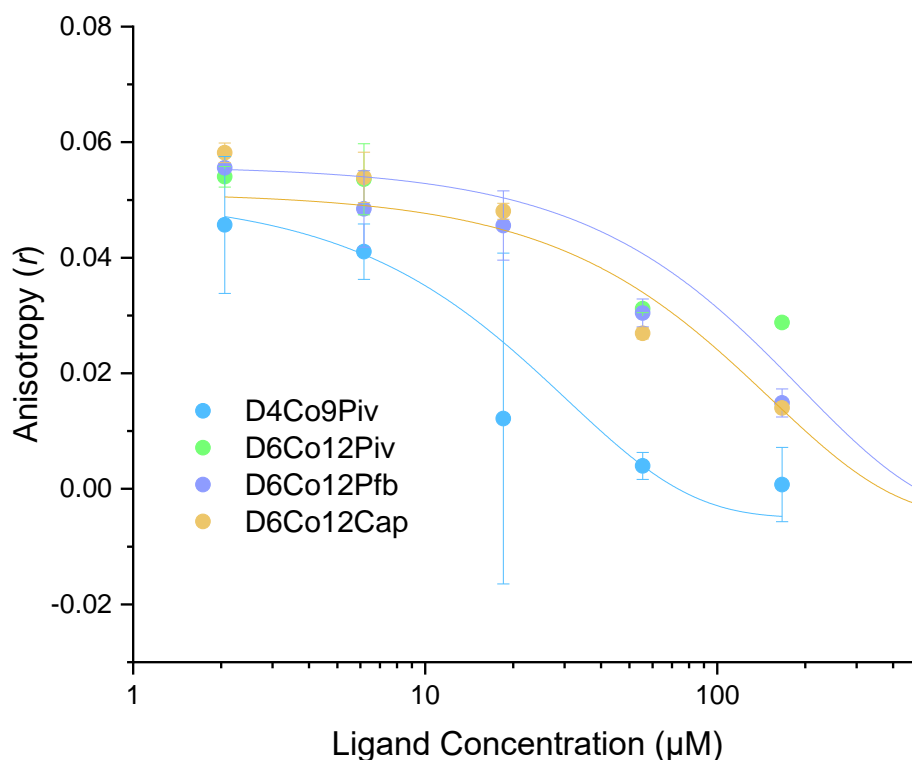
The most potent potential hit combinations are listed in Scheme 21. Combinations that were two or more standard deviations higher than the average anisotropy of the screened reaction mixtures will be investigated. These mixtures require dose-response on the crude reaction mixtures. Validated mixtures will be scaled up and the products purified, characterised and a dose-response recorded to measure the affinity.



**Scheme 21:** Potential hit combinations from ADS array two.

Following the identification of active combinations from the Mcl-1 rhodium carbenoid array, validation was performed on the promising reactions. First, a 3-fold 6-point dose-response was performed on the crude products screening in duplicate with initial product concentration of 500  $\mu\text{M}$  with tracer (25 nM) and protein (150 nM) at 1% final DMSO concentration. Dose-response revealed a lack of activity in combinations **D6Co12Piv** and **D6Co12Pfb**, suggesting the activity seen in the screening of the array could be a false positive. **D6Co12Cap** and **D4Co9Piv** show activity of  $90 \pm 30 \mu\text{M}$  and  $14 \pm 3 \mu\text{M}$  respectively, which is more active than any compound discovered in round 1, and so these two combinations were chosen for scale-up.

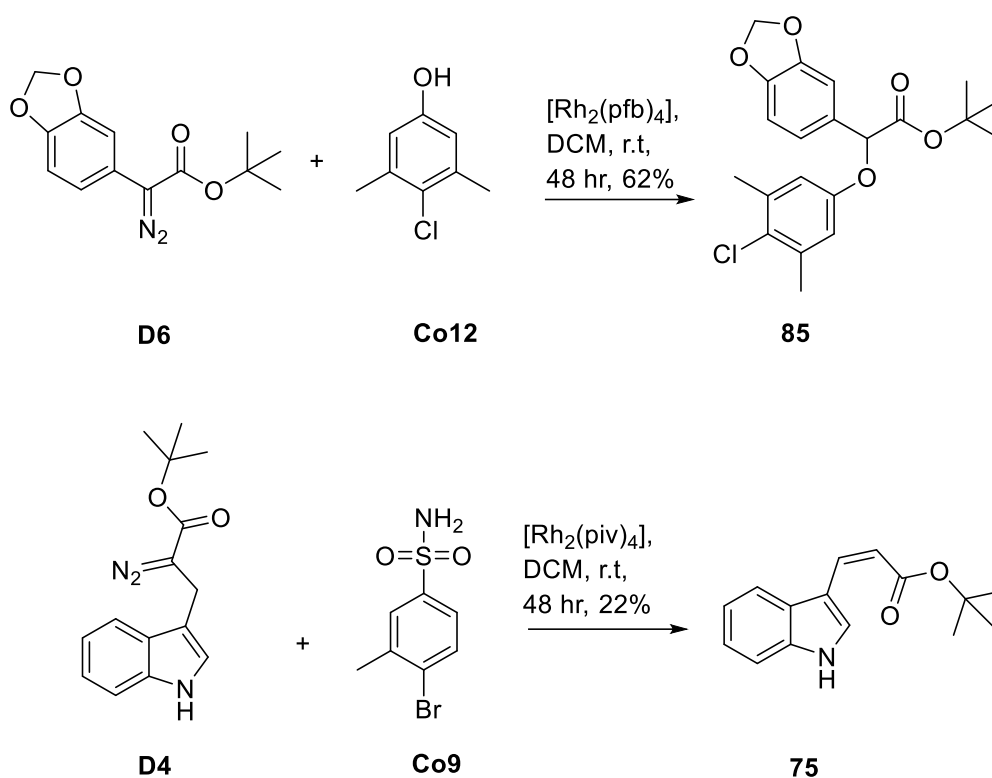
Combination	IC <sub>50</sub> ( $\mu\text{M}$ )	Scale-up
D6Co12Piv	Could not be determined	X
D6Co12Pfb	$76 \pm 8$	X
D6Co12Cap	$90 \pm 30$	✓
D4Co9Piv	$14 \pm 3$	✓



**Figure 36:** Summary of the dose-response performed on active combinations. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr.

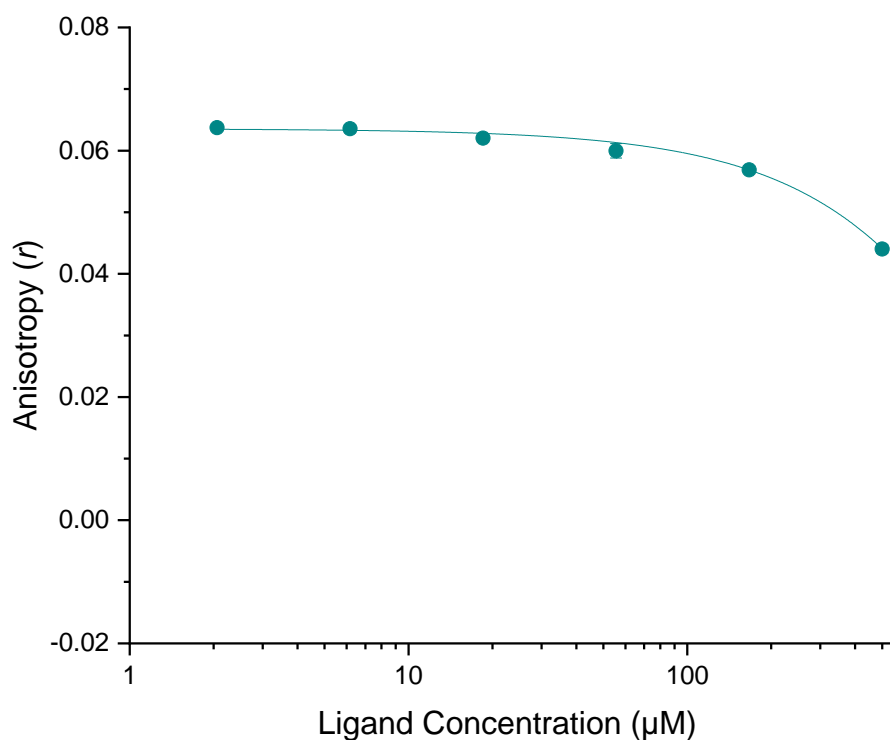
### 2.3.2.5 Scale-up of Round Two Hit Combinations

Following validation of hit combinations discovered from screening the round two ADS reaction array, two combinations were chosen for scale-up. These reactions were performed on a 50-fold increased scale. The reactions were performed under a nitrogen atmosphere in a crimped vial. The diazo was added dropwise to a solution of the co-substrate and catalyst in DCM over 4 hr. The reaction was then left to stir for 48 hr. The reaction mixtures were purified using column chromatography or reverse-phase HPLC.



**Scheme 22:** Scale-up of hit reactions identified using activity-directed synthesis.

The anticipated O-H insertion product was isolated from the crude reaction mixtures (**85**) in a 62% yield. Similar to round one, the scale-up of utilising **D4** yielded the  $\alpha,\beta$ -unsaturated ester **75** as the major product, no intermolecular product could be isolated from the scale up of **D4** + **Co9**. Following purification and characterisation, **85** was *tert*-butyl deprotected using the conditions established in section 2.3.1.3. The crude carboxylic acid was assessed using a 3-fold 6-point dose-response with initial crude product concentration of 500  $\mu\text{M}$  with tracer (25 nM) and protein (150 nM) at 1% final DMSO concentration (Figure 37).



**Figure 37:** Dose-response of crude deprotected **85**. The calculated anisotropy is plotted against ligand concentration in the presence of fixed concentration of tracer (25 nM) and protein (150 nM). Buffer: Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4 at 1% DMSO. The anisotropy was calculated after 2 hr.

The dose-response shows that crude deprotected **85** shows some activity, estimated at  $IC_{50} = 1.1 \pm 0.1$  mM, however the activity observed in the crude reaction mixture is likely not coming from this product, as the crude reaction mixture had >10 fold higher activity. The crude carboxylic acid was not purified and re-evaluated as a significant jump in activity would not be expected.

## 2.4 Summary and Conclusion

A range of fragments was designed using knowledge of known Mcl-1 inhibitors. The designed fragments were then synthesised or purchased ready to execute a round of ADS. A fluorescence anisotropy assay was re-established to assess the activity of crude reaction mixtures and purified products. An *in-situ tert*-butyl ester deprotection step was optimised to allow liberation of a free carboxylic acid, which is present in most Mcl-1 inhibitors. The inherent activity of the fragments was first assessed after exposure to the *in-situ* deprotection conditions previously established. The array was then executed performing all possible combinations of reactions, giving a total of 96 reactions. Upon screening the array and performing validation in the form of dose-response, several active compounds were discovered in which a second array could be designed.

The second round of ADS targeting Mcl-1 involved taking information from the hits identified in the first round, all of which contained a benzylic alcohol. The designed fragments were synthesised and assessed as in round one. The array was then executed performing all possible combinations of reactions, giving a total of 264 reactions. Validation resulted in two combinations to be investigated in scale-up. One intermolecular compound was discovered, but was not more active than most active compound discovered in the first round.

Due to the moderate activity of the compounds discovered and the lack of success in the second round it was decided that a third round would not be pursued.

The low levels of activity observed in the products isolated from the arrays speaks to the challenging nature of inhibiting protein-protein interactions. The binding site of a protein-protein interaction is often large and poorly defined, and the compounds discovered may be too small to pick up significant interactions with the target.

If a further round of ADS were to be performed targeting Mcl-1, more careful consideration for the design of fragments should be undertaken. For example, taking fragments which are known to bind to the P2 site of Mcl-1 and reacting those with fragments which are known to bind to the P1 or P3 sites, with hopes of finding effective fragment linkage. The size of fragments could also be reconsidered, using larger fragments should result in higher activity, which would also allow screening at lower concentrations.



## 3 Activity-Directed Synthesis of PEX5 Inhibitors

### 3.1 Introduction to PEX5

#### 3.1.1 PEX5 Biology

Plants have been used for millennia for a wide range of purposes, including as food, colourants, pharmaceuticals, fuels and flavours.<sup>80</sup> The compounds utilised in these applications are often difficult to synthesise efficiently. Thus, the use of metabolic engineering to increase the yield of plant products as well as to incorporate novel pathways into the peroxisome is an attractive goal.

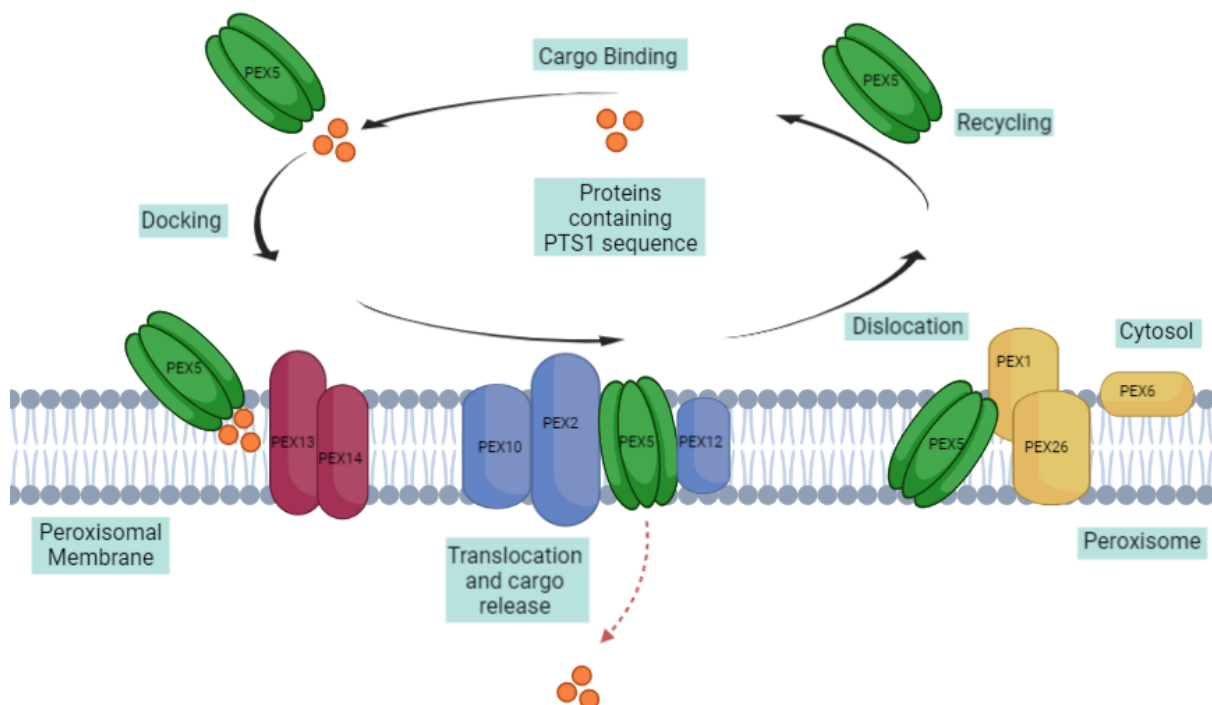
Compartmentalisation of cells is a fundamental aspect of cell biology which allows isolation of potentially incompatible biological pathways, concentration of key pathway components, and separation of harmful products or the generation of a specific micro-environment. Peroxisomes are subcellular organelles, approximately 1  $\mu\text{M}$  in diameter, and are ubiquitous within eukaryotic cells.<sup>81</sup> They are responsible for a wide range of processes, which are required for a cell or organism to function. They participate in many steps of significant metabolic reactions, including  $\beta$ -oxidation of fatty acids, photorespiration, synthesis of glucosinolates, synthesis of plant hormones and pathogen defence.<sup>82</sup>

Peroxisomes have certain unique properties which allow for the creation of a highly customizable compartment within the cell, these include:

- i.) Peroxisomes are highly dynamic organelles which can multiply by proliferation, triggered by a number of metabolic and environmental cues.<sup>82,83</sup> Increasing the number of peroxisomes within the cell leads to an increase in the production of plant metabolites.<sup>81</sup>
- ii.) Peroxisomes are surrounded by a lipid bilayer which allows isolation of metabolic reactions from the rest of the cell.<sup>82</sup> Peroxisomes contain a reaction oxygen species (ROS) removal system and so pathways which produce ROS may be utilised within peroxisomes without issue.<sup>84</sup>
- iii.) Peroxisomes do not contain a genome, so any proteins utilised within must be exported from the cytosol. Protein targeting signals for peroxisomes are well established. The majority of proteins use peroxisome targeting signal 1 (PTS1), consisting of a tripeptide amino acid sequence at the C-terminus (SKL).<sup>85</sup> Fusion of a protein with a

peroxisomal targeting sequence leads to peroxisomal targeting. This property allows the protein contents of a peroxisome to be controlled.<sup>82</sup>

PEX5 (Peroxisomal Biogenesis Factor 5) is the receptor for PTS1 sequences. PEX5 recognises and binds to PTS1, then transports it to the peroxisomal membrane, creating a dynamic pore with the aid of various membrane proteins to deliver the cargo across the peroxisomal membrane into the peroxisome (Figure 38).<sup>86</sup> PEX5 is then recycled back into the cytosol allowing it to bind more PTS1 and continue the cycle.



**Figure 38:** Peroxisomal protein import pathway.<sup>86</sup> PEX5 binds to peptides containing a PTS1 sequence which is transported across the peroxisome membrane with the aid of membrane proteins.

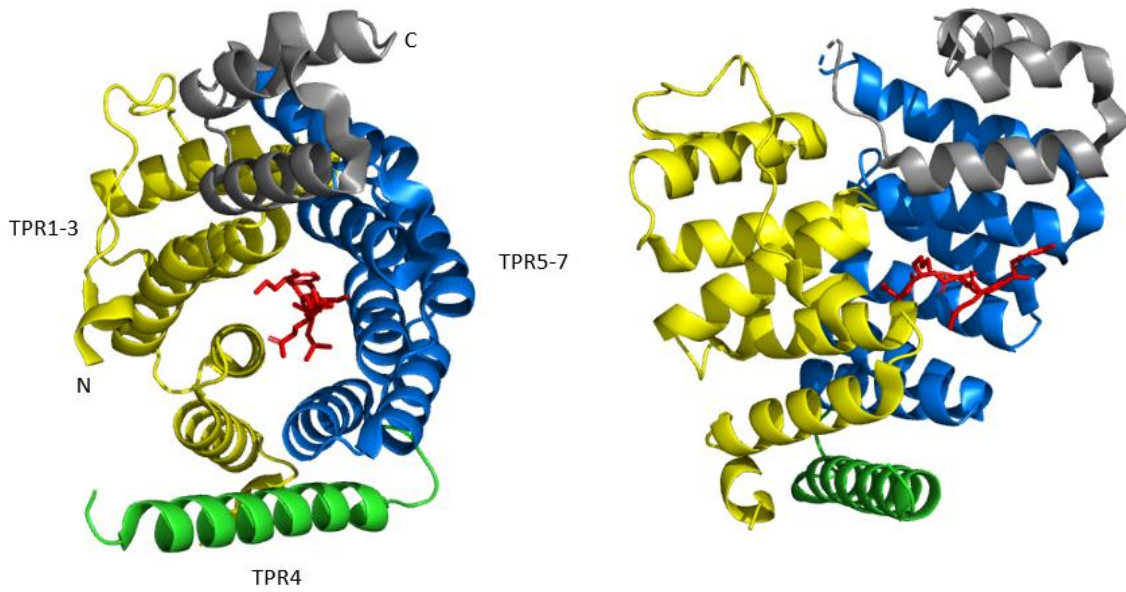
PEX5C is a 41 kDa fragment of human PEX5 which encompasses several tetratricopeptide repeats (TPRs), a 34-amino acid repeat, which is responsible for PTS1 recognition in the C-terminal half.<sup>87</sup> The structure of TPRs consist of two individual  $\alpha$ -helices separated by a turn.<sup>88</sup> Clusters of TPRs are common in biological proteins and appear to mediate protein-protein interaction.<sup>89,90</sup>

YQSKL is a pentapeptide commonly used as a model PTS1 sequence. His<sub>6</sub>AtPEX5C is able to bind to N-terminally modified YQSKL with a  $K_d$  of  $4.0 \pm 0.5$  nM, determined *via* fluorescence anisotropy.<sup>91</sup> The crystal structure of YQSKL bound to PEX5 has been determined (Figure 39).<sup>92</sup> The structure of PEX5 includes two separate clusters of TPRs separated by a hinge region. TPRs 1-3 and 5-7 sit in a conformation that is expected of protein domains containing three TPRs.<sup>93</sup> However, TPR 4 does not adopt a typical conformation consisting of two  $\alpha$ -helices separate by a fold, instead it forms a continuous  $\alpha$ -helix of 21 residues.<sup>92</sup>

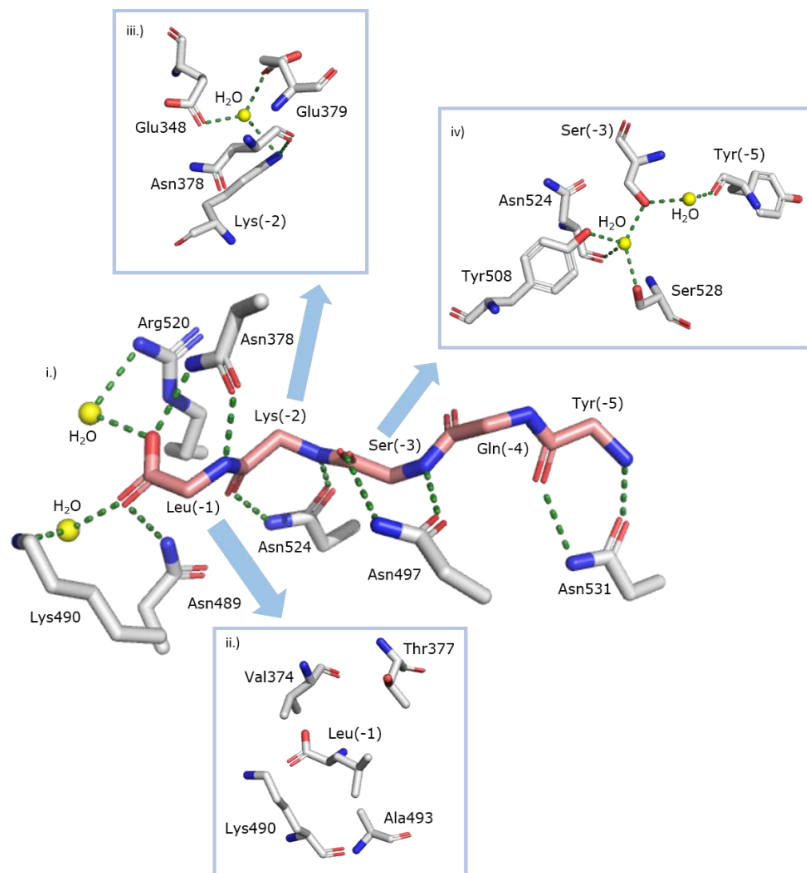
The PTS1 peptide lies in the groove between the two clusters of TPRs (Figure 2). Asn residues located in TPR6 and TPR7 bind the peptide backbone of PTS1, Asn378 binds with the backbone of Leu(-1), Asn524 with Lys(-2), Asn497 with Ser(-3) and Asn531 with Tyr(-5). Water mediated interactions between Arg520 and Lys490 with the carboxylate anion of the C-terminal are present. Additional hydrogen bonds between the amide side chains of Asn378 and Asn489 and the carboxylate anion are also present.<sup>92</sup>

Side chains of PTS1 are recognised by a series of pockets along the binding site. Leu (-1) occupies a hydrophobic pocket formed from Ala493, Thr377, Val374 and Lys490 residues. Lys(-2) occupies a pocket lined with acid side chains of Glu348 and Glu379 which contact Lys(-2) *via* the same water molecule. The Ser(-3) side chain interacts with Tyr508, Ser528 and the backbone carbonyl oxygen of Asn524 *via* a water mediated hydrogen bonding interaction.<sup>92</sup>

A)

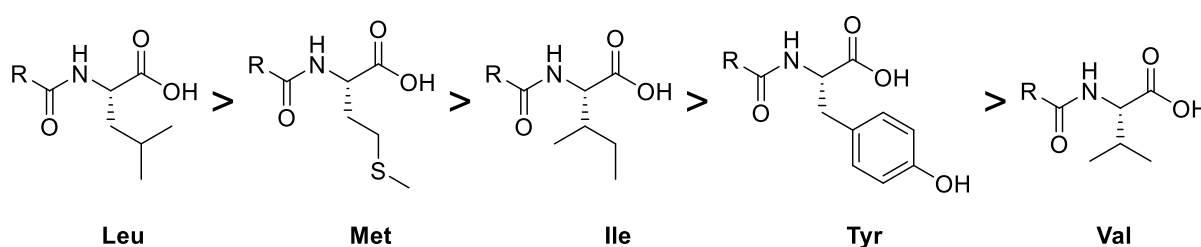


B)



**Figure 39:** A) Crystal structure of PEX5C bound to YQSKL (ball-and-stick, red). TPRs 1-3 are shown in yellow, the TPR 4 hinge region in green and TPRs 5-7 in blue. Other regions are shown in white. Another view rotated 90 ° is also presented. B) Region i.) shows the pentapeptide backbone contacts involving various bidentate interactions as well as contacts with the terminal carboxylate (side chains are omitted for clarity). Key interactions responsible for side chain recognition of ii.) Leu(-1), iii.) Lys(-2) and iv.) Ser(-3) are shown.<sup>92</sup> PDB accession code: 1FCH.

Although the SKL is the consensus C-terminal PTS1 tripeptide sequence found in nature,<sup>94</sup> work has been done to investigate the effects of single point mutations on binding to PEX5.<sup>95</sup> A model peptide of VAKTTRPSRV was selected and positions along the peptide mutated. The activity of the model peptide was assessed *via* fluorescence anisotropy using fluorescently labelled YQSKL as a tracer. Mutations in position -1 determined a hierarchy of Leu>Met>Ile>Tyr>Val (Figure 40). Mutation in the shorter peptide YQSKV to YQSKL also resulted in a significant increase in activity. Mutations in the -2 position were also investigated and showed equivalency of Lys and Arg residues, with Asn resulting in a significant 20-fold drop in activity. Mutation further upstream are known to also affect activity.<sup>95</sup>



**Figure 40:** Hierarchy of C-terminal amino acids for the PTS1 sequence.

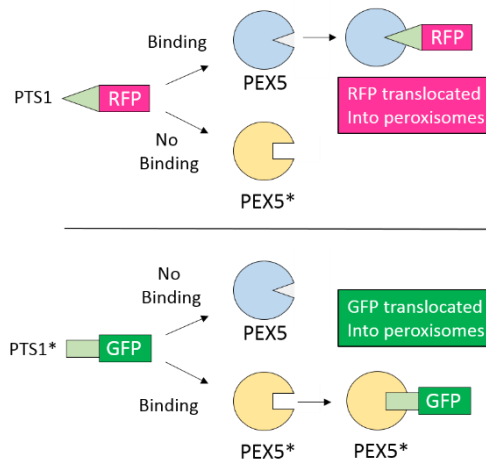
Metabolic engineering has been used to optimize existing peroxisome function as well to introduce novel peroxisomal function. An example of introducing novel peroxisomal function is in the use of peroxisomes as factories to produce biodegradable polymers, such as polyhydroxyalkanoates (PHAs).<sup>96</sup>

Peroxisomes have gained interest in biotechnology for their unique characteristics which could be used to create a 'designer organelle'.<sup>97</sup> One component of such a system is a bespoke protein import pathway that can deliver specified cargo to the peroxisome in the presence of pre-existing protein import pathways.<sup>97</sup>

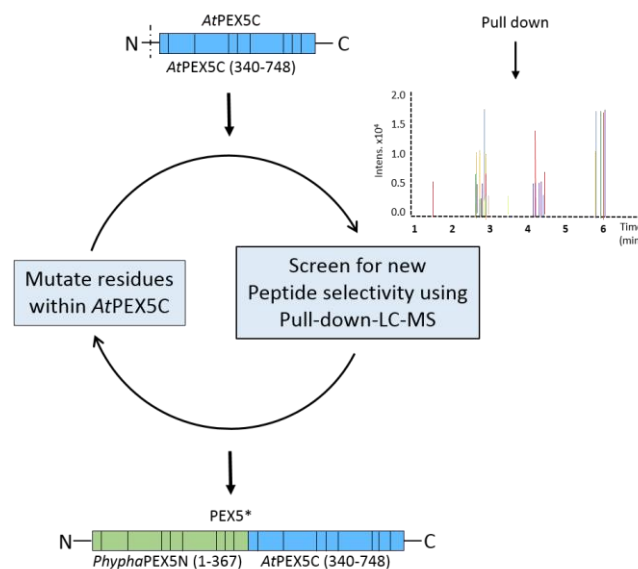
The *N*-terminal of PEX5 is natively unstructured and is responsible for docking and cargo delivery,<sup>98</sup> while the C-terminal is responsible for binding to PTS1.<sup>92</sup> It was envisaged that the C-terminal could be mutated to produce a variant with orthogonal target sequence recognition (PEX5\*) that could recognise a mutant PTS1 sequence (PTS1\*).<sup>97</sup> A PEX5\*-PTS1\* pair was discovered through assessing the activity of a range of mutated *Arabidopsis thaliana* PEX5 (*AtPEX5C*) with a library of pentapeptides in pull-down experiments (Figure 41). The pair was optimised and the mutated C-terminal PEX5\* sequence was combined with the *N*-

terminal domain of PEX5 from *Physcomitrella patens* (*Phypa*PEX5N) to create a functional PEX5\* receptor. Expression of PEX5\* in the presence of cargo protein PTS1\* bearing a fluorescent reporter protein (red fluorescent protein – RFP or green fluorescent protein – GFP) resulted in import into the peroxisome at the expense of naturally occurring peroxisomal components.

A)



B)



**Figure 41:** A) PTS1 or PTS1\* sequences appended with fluorescent proteins used to determine orthogonality of protein import. Strategy developed to identify a pair of orthogonal PEX5-PTS1 like interactions.<sup>97</sup> B) *AtPex5C* was mutated and screened against a library of non-PTS1 sequences. Once a PEX5\*-PTS1\* pair was discovered, the mutated *AtPEX5C\** was fused to *Phypa*PEX5N to create a full-length hybrid receptor

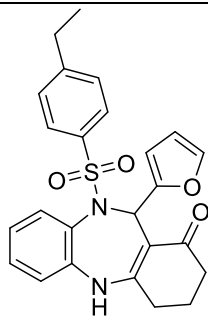
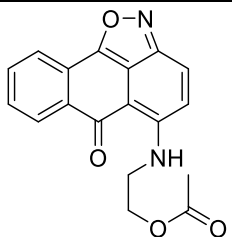
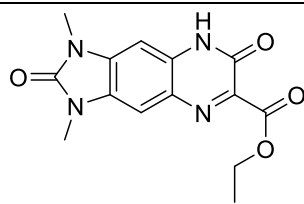
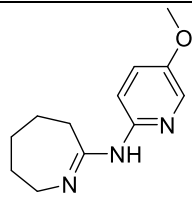
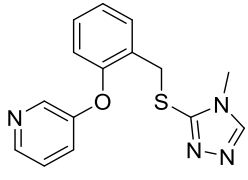
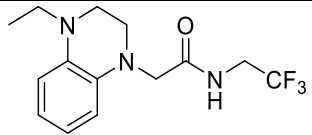
These results suggest that peroxisomal function can be switched, allowing the import of proteins specified by the user with competition of endogenous proteins.<sup>97</sup> The discovery of the PEX5\*-PTS1\* pair can also be used to measure rates of import and the half-life of cytosolic and peroxisome proteins, since they outcompete the import of endogenous PTS1 proteins. These parameters are useful for systems biology and modelling *in vivo* peroxisome protein trafficking.

### 3.1.2 Small-Molecule PEX5 Inhibitors

*Trypanosoma* are a genus of parasitic protozoa able to infect humans and domestic animals, causing severe mortality. The most threatening trypanosomiasis is Chagas disease, affecting up to twelve million people in the Americas. It has been shown that inhibition of the PEX5/PEX14 can kill *Trypanosoma* parasites in cell-based assays.<sup>99,100</sup>

It was believed that inhibition of PEX5/PTS1 may also be lethal to *Trypanosoma*, some small-molecule inhibitors of the PEX5/PTS1 PPI were recently reported.<sup>101</sup> A library of 30,000 compounds was screened against PEX5 in an FA based assay, resulting in 48 compounds that were then validated. The validation was performed through <sup>1</sup>H-<sup>15</sup>N HSQC to monitor amide chemical shifts, with significant spectral shifts observed for six compounds **86-91** (Table 1) at a 1:1 ratio of PEX5 to compound. These six compounds were assessed by an orthogonal AlphaScreen assay in order to obtain EC<sub>50</sub> values. Computational prediction of binding to PEX5 suggested that they occupied the PTS1 binding sites..

**Table 1:** Hit compounds with their EC<sub>50</sub> values.

Compound	TcPEX5-PTS1 EC <sub>50</sub> (μM)
 <p style="text-align: center;"><b>86</b></p>	190 ± 37.6
 <p style="text-align: center;"><b>87</b></p>	33.0 ± 3.1
 <p style="text-align: center;"><b>88</b></p>	705 ± 85.2
 <p style="text-align: center;"><b>89</b></p>	78.2 ± 7.4
 <p style="text-align: center;"><b>90</b></p>	411 ± 98.2
 <p style="text-align: center;"><b>91</b></p>	43.4 ± 6.3



### 3.2 Aims and Objectives

It was envisaged that activity-directed synthesis could be used to identify a potent small-molecule PEX5 binder for use in chemical biology or potentially as a strategy against *Trypanosoma*. This is of particular interest as there are few small-molecule inhibitors of PEX5. It was proposed that the diazo chemistry used previously in the group could also be used in this project. A short peptide core bearing a diazo group at the *N*-terminal could be exposed to a broad range of substrates to explore additional upstream interactions (Scheme 23).

A series of short-term project objectives has been outlined to evaluate the feasibility of the use of activity-directed synthesis for the development of chemical biology tools. There are few small-molecule inhibitors of PEX5 and so ADS could be a powerful tool to aid in the exploration of chemical space and ultimately the discovery a potent small-molecule inhibitor of PEX5.

#### **Objective 1: Re-establish PEX5 Fluorescence Anisotropy Assay.**

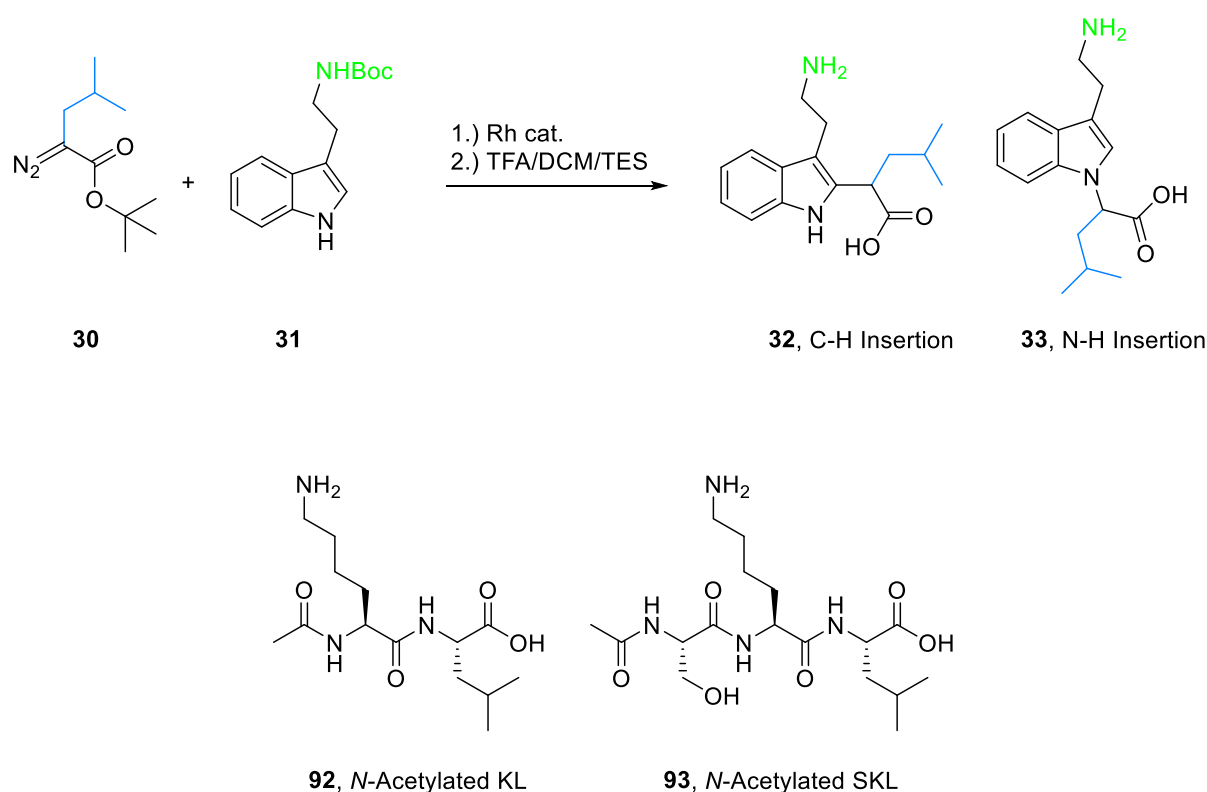
In order to assess the activity of crude ADS mixtures, a robust assay needed to be established. A fluorescence anisotropy assay used previously was modified for this purpose.<sup>91</sup> For this to be achieved, *AtHis<sub>6</sub>PEX5C*, the His tagged C-terminal construct of PEX5 from *Arabidopsis thaliana* needed to be expressed. A fluorescent tracer also needed to be synthesised for the purpose of the assay, YQSKL *N*-terminally modified with Lissamine was chosen. The DMSO tolerance of the assay also needed to be evaluated.

#### **Objective 2: Synthesis of Assessment of N-Acetylated Peptides**

Once inhibitors of PEX5/PTS1 were identified, a way to compare the activity to the native peptide was needed. It was planned that the *N*-Acetylated QSKL, SKL and KL would be synthesised *via* solid-phase peptide synthesis and assessed in dose-response in the fluorescence anisotropy assay. The activity of the synthesised inhibitors would ideally have an activity greater than the *N*-Acetylated KL that is to be mimicked. These peptides would also serve as positive controls to ensure the assays are working as intended.

### Objective 3: Design and synthesis of substrates for ADS

Rhodium carbenoid chemistry was chosen for the project based on the success within the group previously with this type of chemistry. A set of diazo substrates based on Leu were designed and synthesised. A set of substrates with at least one functionality reactive towards a rhodium carbenoid were designed and purchased or synthesised. The substrates contained a basic group which may be able to mimic the Lys residue in the PTS1 sequence (Ser/Lys/Leu). It was envisaged that every combination of diazo, co-substrate and catalyst would be investigated. An example reaction is given in Scheme 23. A *tert*-butyl ester is included in the diazos which would undergo *in situ* deprotection following reaction completion to reveal the free carboxylic acid.



**Scheme 23:** Example reaction of a diazo **30** containing a group to mimic Leu (blue) and a co-substrate **31** containing a protected basic nitrogen (green) which may mimic Lys to form C-H insertion product **32** and N-H insertion product **33**. The structure of *N*-Acetylated KL **92** and SKL **93** is also given.

#### **Objective 4: Execution and evaluation of an ADS array**

Once the substrates and substrates were obtained an ADS array was performed. The array was planned to be exhaustive, testing every possible combination of diazo, substrate and rhodium catalyst. The crude products were then deprotected using TFA and TES in DCM and prepared for screening in the fluorescence anisotropy assay at a single concentration. Combinations which showed activity at the chosen concentration were then selected for analysis *via* dose-response and LCMS. Any promising combinations which passed analysis were scaled-up and the products purified, characterised, and screened for biological activity. The results of screening would be used to design subsequent arrays.

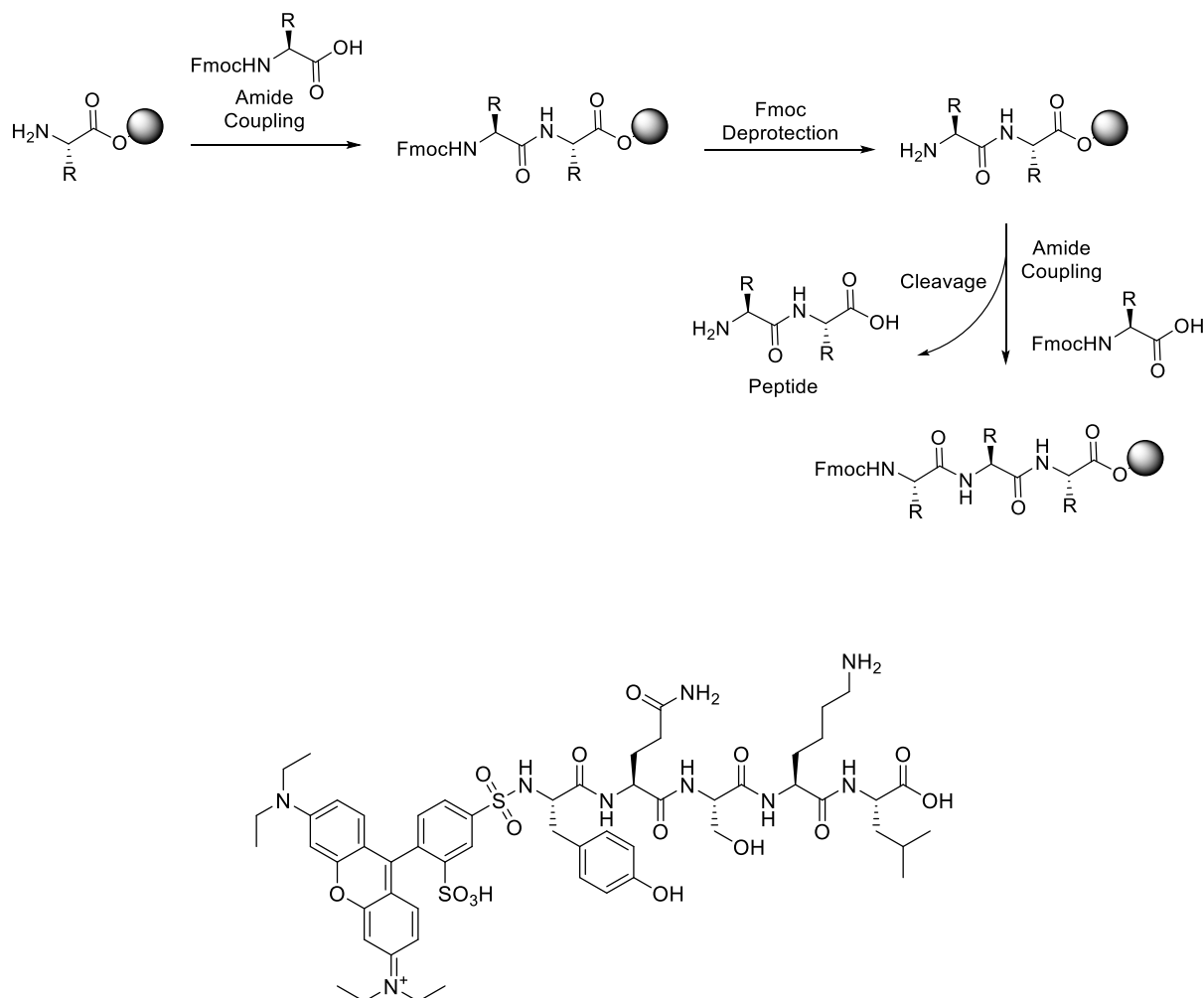
### **3.3 Results and discussion**

#### **3.3.1 Synthesis of Fluorescence Anisotropy Tracer: Lissamine-Labelled YQSKL**

A fluorescence anisotropy assay will be used to determine the activity of crude products in the ADS array. A fluorescent tracer is needed for the assay to obtain  $IC_{50}$  and  $K_i$  values for unlabelled compounds that interact with PEX5. Lissamine-labelled YQSKL **94** was chosen as it is commonly used for fluorescence anisotropy assays of PEX5.<sup>91,97</sup>

The tracer was prepared *via* solid-phase peptide synthesis (Figure 42), the technique uses a 2-chlorotrityl resin which is loaded with Leucine at the C-terminal, leaving the N-terminal free to perform modifications. An amide coupling with an Fmoc protected amino acid (5 eq.) with protected side chains (Boc, *tert*-butyl, trityl) is performed using DIC (5 eq.) and OxymaPure (5 eq.) in DMF with agitation for 1 hr at r.t. The reaction solution is then drained from the resin which is washed with 20% piperidine in DMF to remove the Fmoc group, revealing the free amine to perform subsequent amide couplings, resulting in the growth of a peptide. Once the desired peptide was synthesised, the N-terminal was functionalised with a fluorescent tag *via* a sulfonamide formation. Finally, a global deprotecting using a cocktail of TFA/TIPS/H<sub>2</sub>O (95:2.5:2.5) with agitation for 1 hr at r.t to remove any protecting groups and cleave the peptide from the resin. The

crude tracer was then purified *via* reverse-phase biotage to give the desired tracer (1.0 mg).<sup>97</sup>

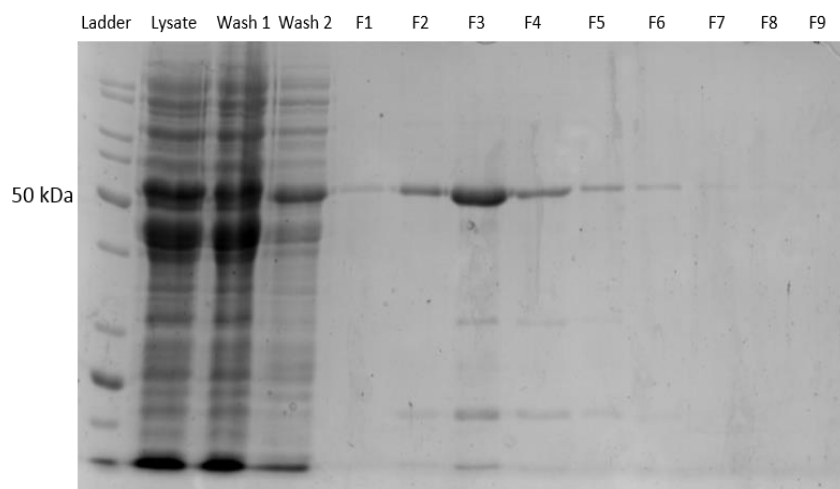


**Figure 42:** The process of solid-phase peptide synthesis and Lissamine-labelled YQSKL, commonly used in PEX5 fluorescence anisotropy assays. HRMS found  $MH^+$  1179.4903.  $C_{56}H_{77}N_9O_{15}S_2$  requires  $MH$ , 1179.4970.

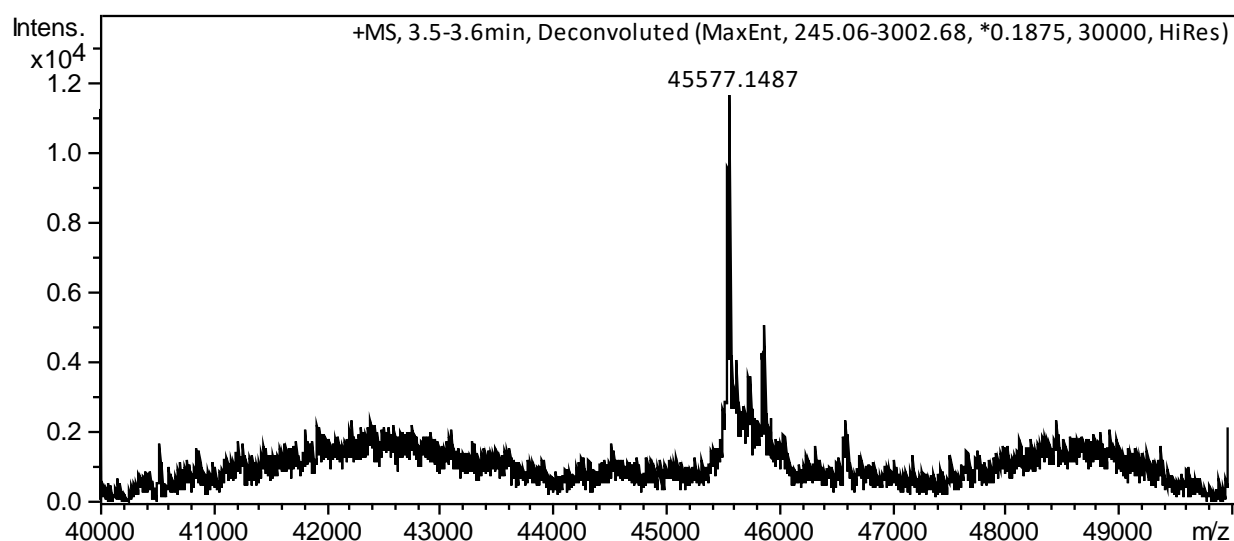
### 3.3.2 Expression and Purification of PEX5

The plasmid containing the gene for *AtHis<sub>6</sub>PEX5C* was transformed in *E. coli* (BL21 D3) *via* heatshock treatment. The protein was expressed in auto-induction media. Following cell lysis *via* sonication in the presence of lysozymes, the protein was purified on a cobalt affinity column (see Section 5.6.6 for full procedure). The fractions obtained from the column were analysed *via* SDS-PAGE and HRMS to confirm protein expression and purity (Figure 43).

A)



B)

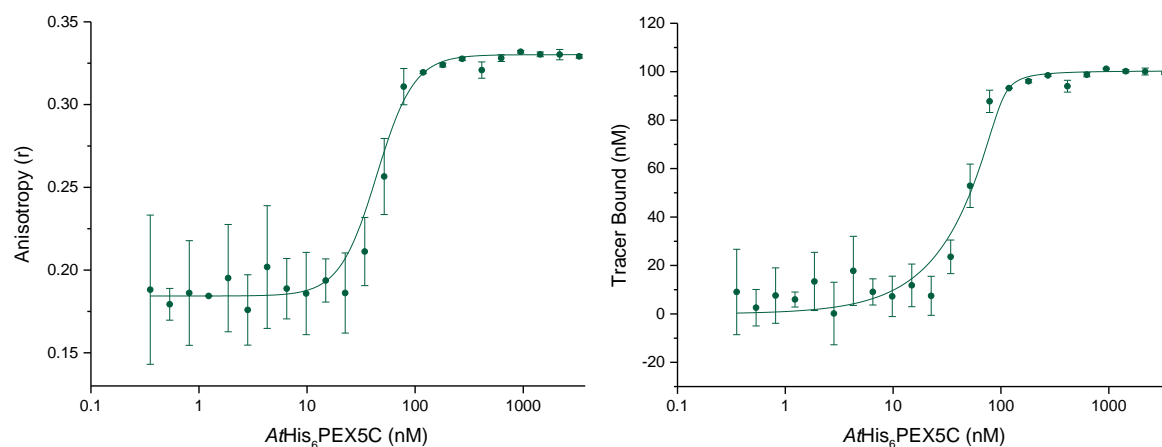


**Figure 43:** Analysis of *AtHis<sub>6</sub>PEX5C* protein express and purity. A) SDS PAGE analysis for purification of *AtHis<sub>6</sub>PEX5C* by Co-chromatography. Aliquots of lysate and column washes followed by column fractions were assessed. B) HRMS for purified and isolated *AtHis<sub>6</sub>PEX5C*. HRMS found mass 45577.1487  $C_{2016}H_{3038}N_{554}O_{635}S_{12}$  requires  $M_r$  45580.35.

### 3.3.3 Configuration of Fluorescence Anisotropy Assay

Following successful protein and tracer synthesis, experiments were performed to assess the limits of anisotropy of the assay, which is the largest assay window resulting from free and complexed tracer (Figure 44). The assay protocol was modified from a known procedure.<sup>91</sup> Limits of anisotropy were determined by titrating protein into a fixed concentration of tracer (100 nM). The polarisation of

emitted light was measured parallel and perpendicular to the plane of polarisation of the excited light and an anisotropy value for each concentration of protein calculated. The plate was incubated for 20 min at room temperature before being read. The anisotropy was plotted against protein concentration and the data fitted using a logistical fit. The limits of anisotropy were determined to be  $r_{\max} = 0.33$  and  $r_{\min} = 0.18$ .



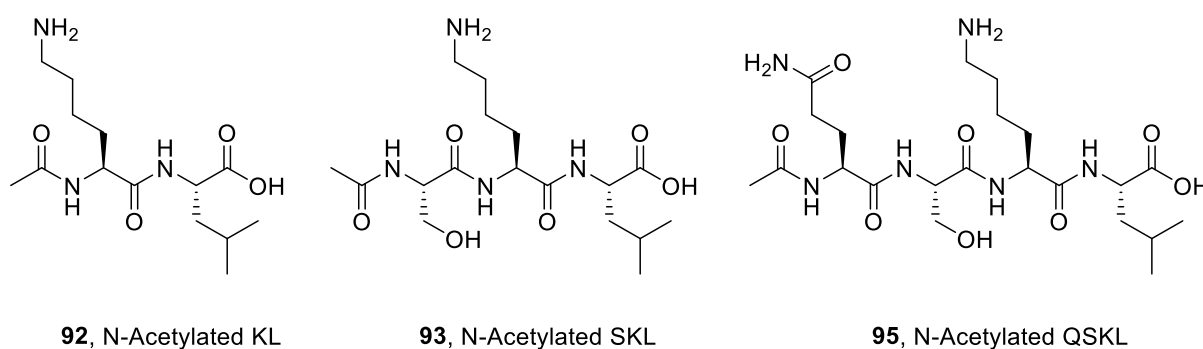
**Figure 44:** Left: The calculated anisotropy plotted against protein concentration in the presence of fixed amount of tracer (100 nM), buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 0% DMSO. The anisotropy was calculated at 20 min and the upper and lower anisotropy limits were determined to be  $r_{\max} = 0.33$  and  $r_{\min} = 0.18$  respectively. Right: Determination of  $K_d$  from plotting the amount of tracer bound vs protein concentration,  $K_d$  of tracer:  $1.9 \pm 0.1$  nM.

The  $K_d$  of Lissamine-labelled YQSKL was determined by converting the measured anisotropy into fraction of tracer bound and then multiplying fraction bound by the concentration of tracer (100 nM). The tracer bound was plotted against the concentration of protein and the data was then fitted to a non-linear least squares fitting algorithm. The dissociation constant was determined to be  $K_d = 1.9 \pm 0.1$  nM. This value matches well with the literature values of  $1.1 \pm 0.6$  nM<sup>91</sup> and  $4.0 \pm 0.5$  nM.<sup>97</sup>

### 3.3.4 Synthesis and Evaluation of *N*-Acetylated Peptides

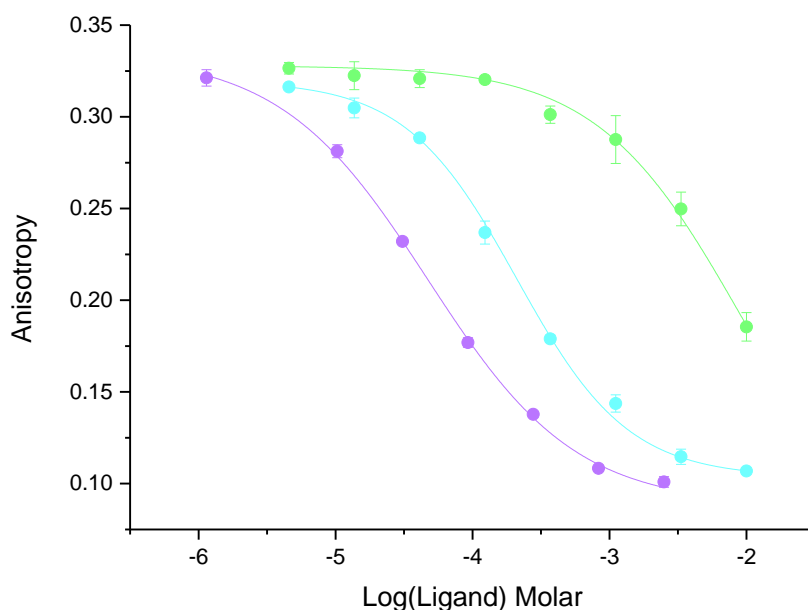
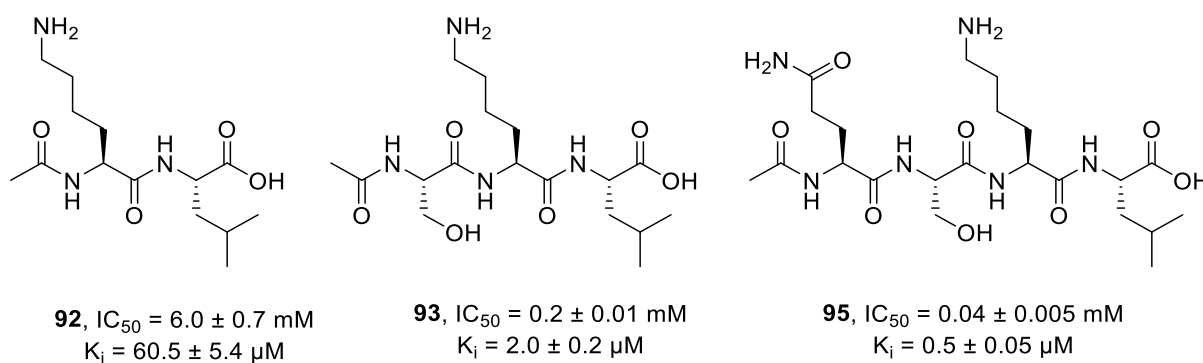
In order to assess the activity of the synthesised mimics of KL it would be useful to know the activity of various lengths of the PTS1 sequences in order to make comparisons. For this purpose, *N*-acetylated KL, SKL and QSKL were synthesised *via* solid-phase peptide synthesis. The peptides were synthesised beginning with Leucine loaded onto 2-chlorotrityl resin. Each Fmoc protected amino acid (5 eq.) was coupled onto the resin with DIC (5 eq.) and OxymaPure (5 eq.) in DMF and

agitated for an hr at r.t. After each amino acid was coupled, the resin was washed with 20% piperidine in DMF to remove the Fmoc protecting group and reveal the free amine for the next coupling. Following synthesis of the desired peptide, an N-acetylation was performed with acetic anhydride (5 eq.),  $\text{NEt}_3$  (10 eq.) in DCM for an hr at r.t. The resin was then washed with DMF, DCM and MeOH and a cleavage cocktail of TFA/TIPS/ $\text{H}_2\text{O}$  (95:2.5:2.5) added to the resin and agitated for an hr at r.t. The eluent was then collected and concentrated under vacuum to give the crude peptide. The crude peptide was purified *via* mass-directed HPLC (see Section 5.5) to give the pure N-Acetylated Peptides (Figure 45). The purity of the peptides was assessed *via* 95:5→5:95 over 10 min.



**Figure 45:** N-acetylated peptides prepared *via* solid-phase peptide synthesis for assessment using a fluorescence anisotropy.

Following the synthesis, the peptides were assessed *via* a 3-fold 8-point dose-response in a fluorescence anisotropy assay at 0% DMSO in duplicate with initial concentration of 10 mM for KL and SKL and 2.5 mM for QSKL (Figure 46). Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 0% DMSO. The assay protocol was modified from a known procedure.<sup>91</sup> The anisotropy was plotted against the ligand concentration to obtain a curve, which was fitted to a single site competition model to obtain  $\text{IC}_{50}$  values.  $K_i$  values were calculated using the method as in Section 2.3.1.4.



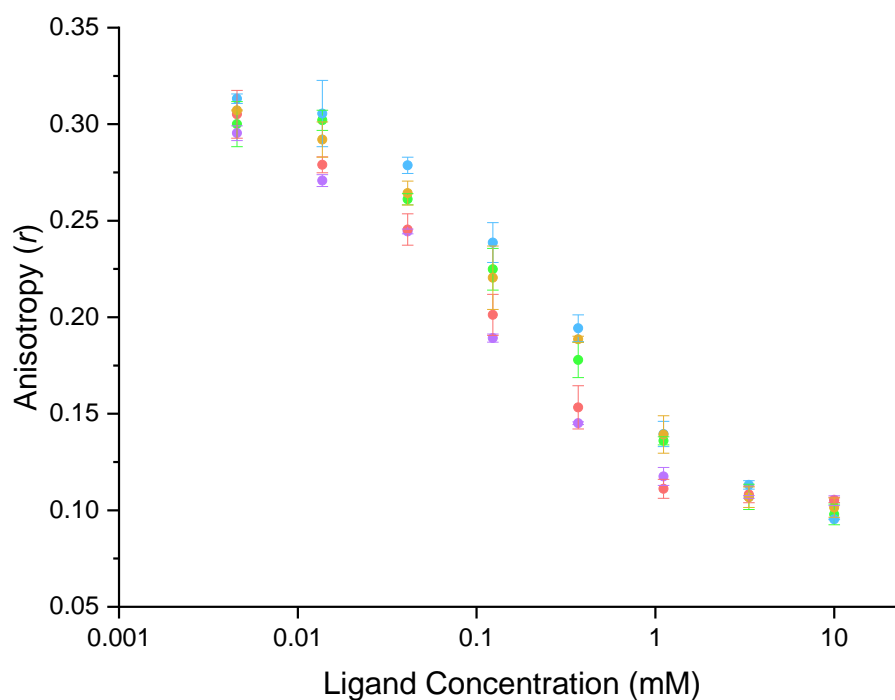
**Figure 46:** Dose-response of *N*-acetylated peptides. The calculated anisotropy is plotted against the ligand concentration. Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 0% DMSO. The anisotropy was calculated at 20 min and the  $IC_{50}$  determined. KL (green,  $IC_{50} = 6.0 \pm 0.7$  mM), SKL (blue,  $IC_{50} = 0.2 \pm 0.01$  mM) QSKL (purple,  $IC_{50} = 0.04 \pm 0.005$  mM).

The activity of the *N*-acetylated peptides increases with each amino acid added to the peptide. A 30-fold increase in activity is seen from KL to SKL, and a 5-fold increase in activity is seen from SKL to QSKL. The dose-response suggests that the Ser residue is far more important to the activity of the PTS1 sequence than the Gln residue. Comparison of synthesised ligands with the activity of *N*-acetylated KL will inform success of the approach. The goal is mimic the activity of *N*-acetylated KL and so an  $IC_{50} = 6.0 \pm 0.7$  mM or lower is ideal.



### 3.3.5 Determination of DMSO Tolerance of the Assay

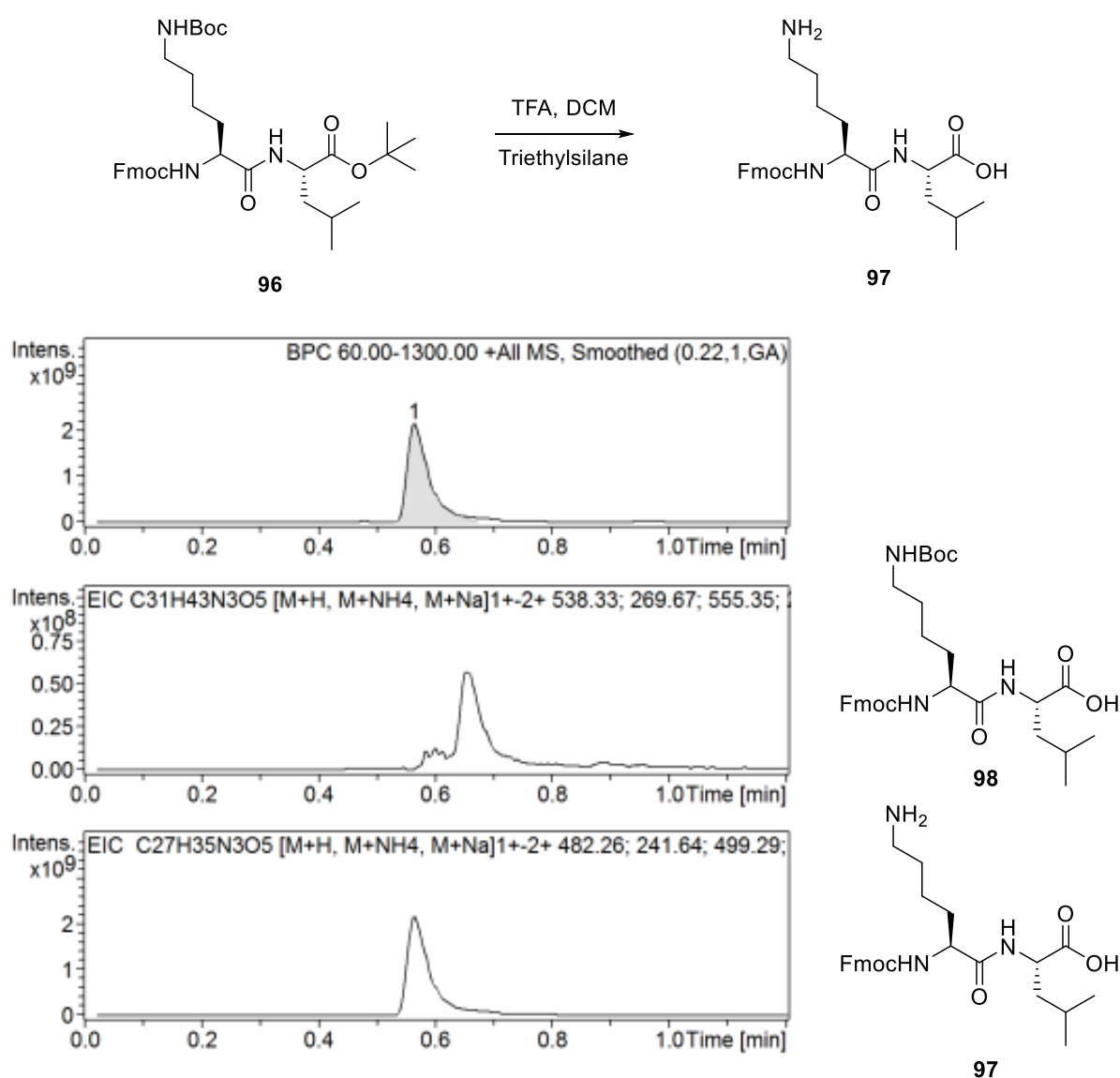
The final step before the ADS array could be planned was to determine the DMSO tolerance of the assay. Following the execution of an ADS array, the crude products will be dissolved in DMSO for screening. Determining how much DMSO the assay can tolerate will determine the concentrations at which the products can be screened at. A 3-fold 8-point dose-response on the previously assessed *N*-acetylated SKL at various concentrations of DMSO (0, 2, 4, 6 and 8%) and examining the curves obtained (Figure 47). Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5. The plate was incubated for 20 min at room temperature before being read. No large deviation was observed, showing that the assay is tolerant of high DMSO concentrations (up to 8% DMSO).



**Figure 47:** Dose-response of *N*-acetylated SKL at varying concentrations of DMSO. The calculated anisotropy is plotted against the concentration of ligand. Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5. The anisotropy was calculated at 20 min and the dose-response curves compared. Green – 0% DMSO, blue – 2% DMSO, purple – 4% DMSO, pink – 6% DMSO, orange – 8% DMSO.

### 3.3.6 Optimisation of *In Situ* Deprotection

An *in situ* deprotection step is a key part of the planned workflow. The products from the chemistry will contain a *tert*-butyl ester and a Boc-protected amine which upon deprotection will reveal the free carboxylic acid and amine. An investigation was performed looking into the conditions required for the deprotection. Varying concentrations of TFA (10% or 20%) in DCM with triethylsilane (2.0 eq.) were assessed. 10% TFA in DCM with triethylsilane (2.0 eq.) resulted in incomplete deprotection. Conditions of 20% TFA in DCM with triethylsilane (2.0 eq.) gave almost complete deprotection overnight, as assessed by LCMS, and so these conditions were chosen deprotection method to be used in the ADS array.



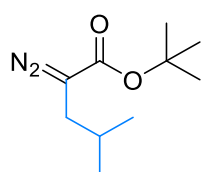
**Figure 48:** The deprotection of dipeptide **96** to deprotected dipeptide **97** was monitored by LCMS. The top chromatogram is of the reaction mixture, the middle chromatogram shows compound **98** and the bottom chromatogram shows compound **97**. After 18 hr, LCMS showed almost complete deprotection to **97** with a small amount of **98** present.

### 3.3.7 Design of Fragments for Activity-Directed Synthesis

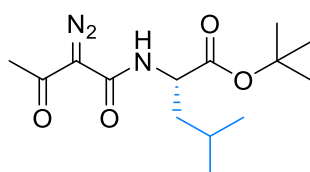
A set of diazos and co-substrates were designed to be utilized in the Activity-Directed Synthesis of PEX5 ligands. A set of diazo substrates was designed using knowledge of the PTS1 recognition sequence which binds to PEX5 (Figure 49). The ideal amino acid for the C-terminus of the PTS1 recognition sequence is a leucine. A free acid is also required for activity, therefore the diazos were designed to be based on leucine and include a *tert*-butyl ester which could be deprotected to reveal the free carboxylic acid.

A set of co-substrates was designed to include one or more points of reactivity towards rhodium-carbenoid chemistry for examples alkenes, alcohols, aromatic CH and NH indole (Figure 49). They were designed to mimic the second amino acid in the PTS1 recognition sequence, lysine. Many of the substrates contain a Boc-protected amine which will reveal a free amine upon deprotection. Since this position in the PTS1 recognition sequence also tolerates a less basic histidine residue, nitrogen containing structures of various basicity were also included to assess how basic a nitrogen is required, such as primary amine, secondary amine, quinoline and aminopyridine.

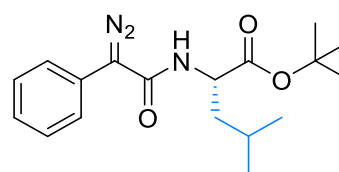
#### a.) Diazos



**D10**

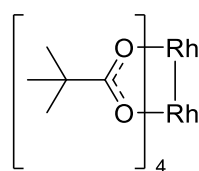


**D11**



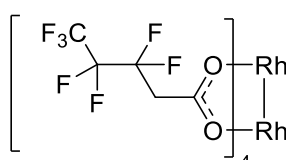
**D12**

#### b.) Catalysts



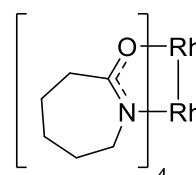
$[\text{Rh}_2(\text{piv})_4]$

**C1**



$[\text{Rh}_2(\text{pfb})_4]$

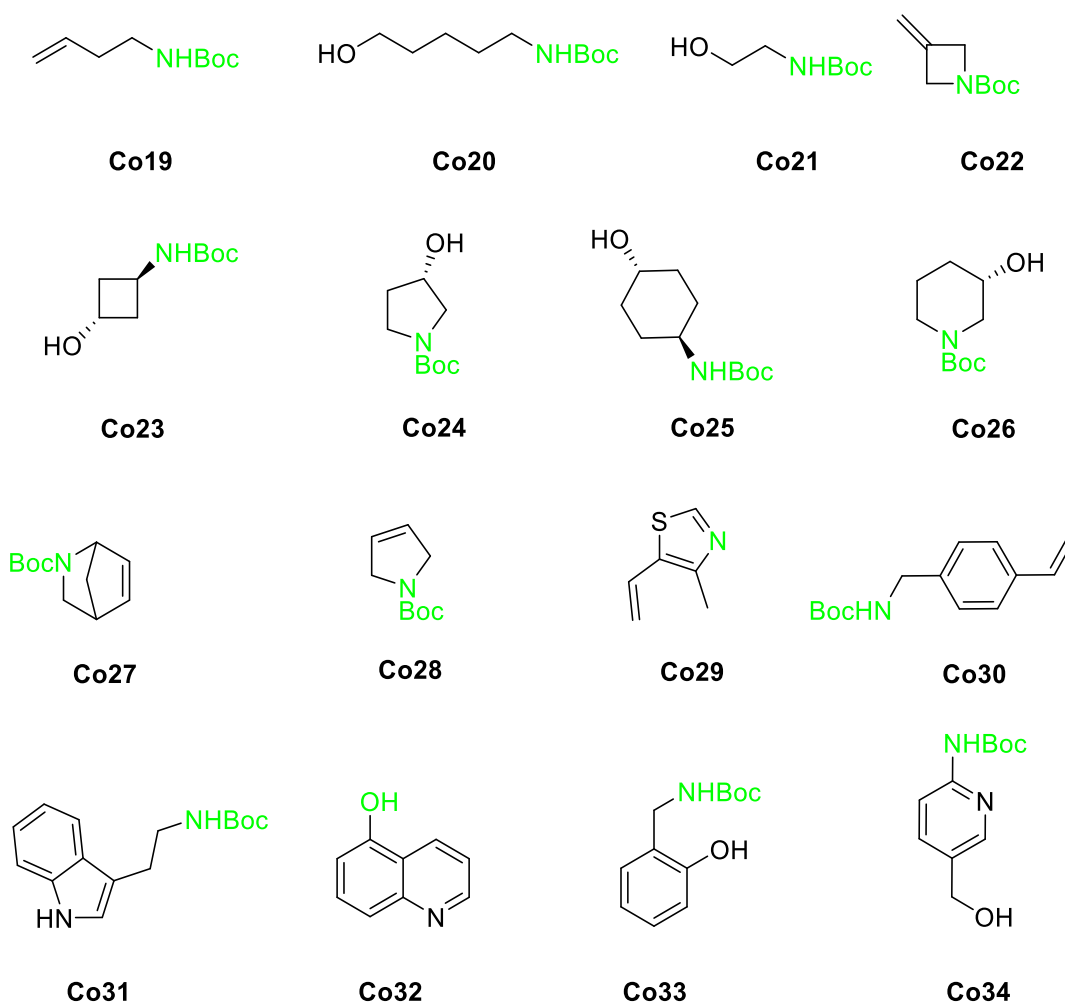
**C2**



$[\text{Rh}_2(\text{cap})_4]$

**C3**

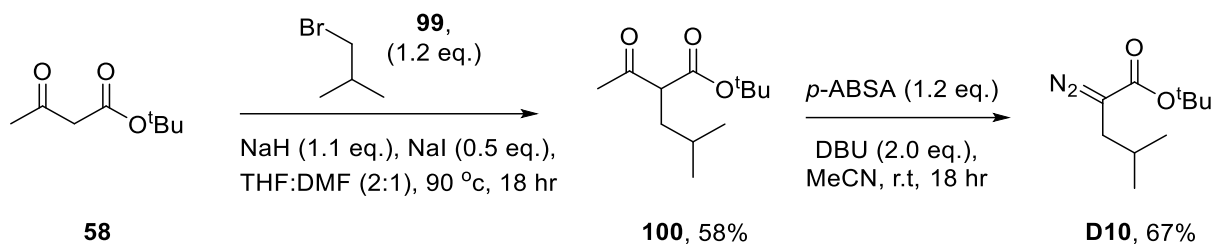
c.) Substrates



**Figure 49:** Diazos, catalysts and co-substrates chosen for the array. Diazos were designed to include a group to mimic the C-terminal Leu (blue). A *tert*-Butyl ester is included in the design to reveal a free carboxylic acid upon deprotection. The co-substrates were designed to include a group that may mimic the Lys side chain (green). Many of these compounds contain Boc-protected amines which will reveal the free amine upon deprotection.

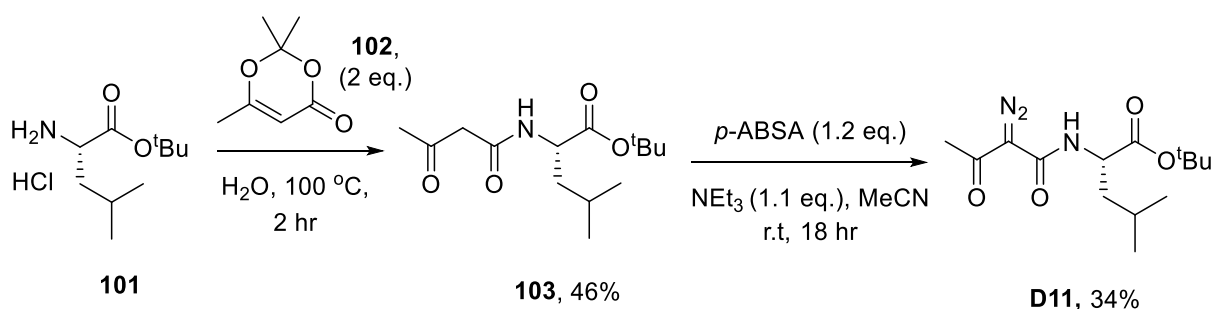
### 3.3.8 Synthesis of Diazos

**D10** (=30) was prepared following a known procedure.<sup>103</sup> *tert*-Butyl acetoacetate **58** was treated with sodium hydride in THF before a solution of 1-bromo-2-methylpropane **99** and sodium iodide in DMF was added and left to stir at 90 °C for 18 hr to give alkylated product **100** in 58% yield. Alkylated product **100** was then treated with DBU and *p*-ABSA in acetonitrile at r.t for 18 hr to give **D10** in 67% yield.



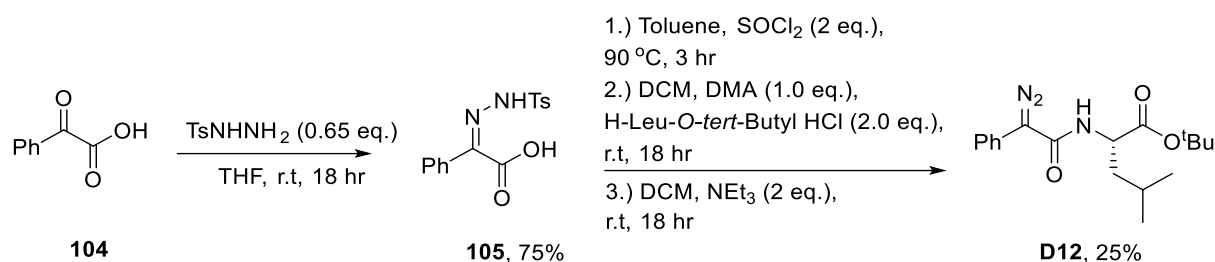
**Scheme 24:** Synthesis of diazo **D10** through alkylation of *tert*-butyl acetoacetate **58** followed by deacetylation and diazotisation and the alkylated product **100**.

**D11** was prepared following a procedure to make similar compounds.<sup>104,105</sup> H-Leu-*O*-*tert*-butyl HCl **101** was treated with 2,2,6-trimethyl-1,3-dioxin-4-one **102** in H<sub>2</sub>O and left to stir at 100 °C for 2 hr to give the 1,3-dicarbonyl **103** in 46% yield. The 1,3-dicarbonyl **103** was then treated with NEt<sub>3</sub> and *p*-ABSA in acetonitrile and left to stir at r.t for 18 hr to give **D11** in 34% yield.



**Scheme 25:** Synthesis of **D11** through reaction of H-Leu-*O*-*tert*-butyl HCl **101** with a dioxinone to form **103** followed by diazotisation.

**D12** was prepared following a procedure to make similar compounds.<sup>105</sup> Phenylglyoxylic acid **104** was treated with *p*-toluenesulfonylhydrazine in THF and left to stir at r.t. for 18 hr to give the hydrazone **105** in 75% yield. The hydrazone was then treated with SOCl<sub>2</sub> in toluene and left to stir at 90 °C for 3 hr to give the sulfonyl chloride. The sulfonyl chloride was then treated with H-Leu-*O*-*tert*-butyl HCl **101** and DMA in DCM and left to stir at r.t. for 18 hr. Following coupling, the amide was treated with NEt<sub>3</sub> in DCM and left to stir at r.t for 18 hr to give **D12**.

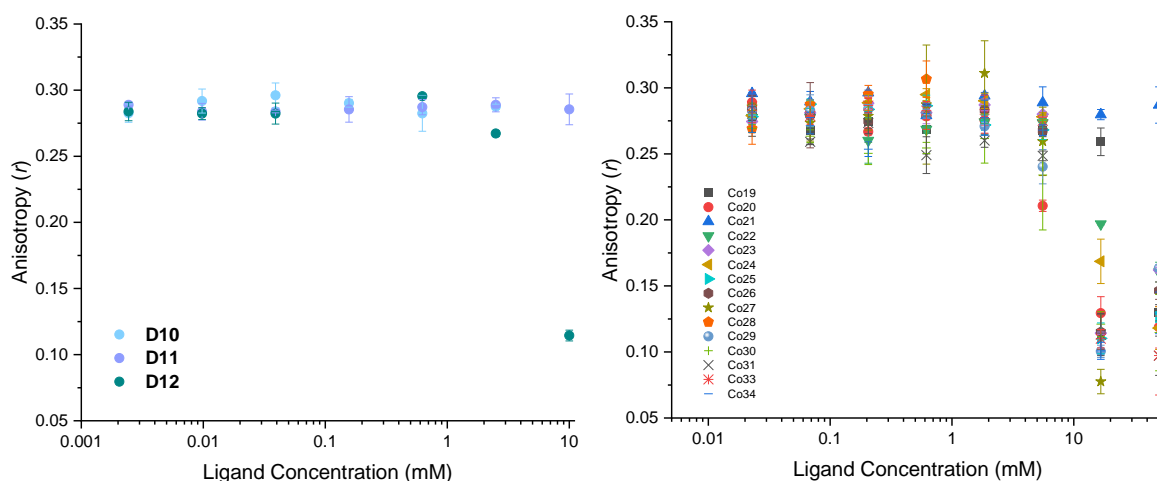


**Scheme 26:** Synthesis of **D12** through reaction of phenylglyoxylic acid **104** with *p*-toluenesulfonylhydrazine to form **105** followed by conversion to sulfonyl chloride using  $\text{SOCl}_2$ , coupling to H-Leu-*O-tert*-butyl **101** and base aided decomposition to give **D12**.

### 3.3.9 Inherent Activity of Diazos and Substrates

In order to determine the screening concentration for the ADS array the inherent activity of the diazos and substrates needed to be determined. A screening concentration will then be chosen below that of which activity for diazos and substrates is observed to ensure activity is coming from the formation of products rather than starting materials.

Diazos and substrates were treated with the TFA deprotection conditions established in Section 3.3.6 and dissolved in DMSO for screening. An 4-fold 8-point dose-response was then performed on the crude deprotected starting materials. Products of diazos were screened with initial concentration of 10 mM, whereas co-substrates were screened at 50 mM, as they are used in 5 eq. in the array (Figure 50). Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 5% DMSO. The plate was incubated for 20 min at room temperature before being read. These results informed the choice of a screening concentration for the array. A crude product concentration of 1 mM was chosen for screening the ADS array as the diazos and the co-substrates display little activity at 1 mM and 5 mM respectively.



**Figure 50:** Dose-response of deprotected diazos (left) and co-substrates (right). Diazos were tested with initial concentration of 10 mM and substrates with an initial concentration of 50 mM. Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 5% DMSO. The anisotropy was calculated at 20 min. A crude product screening concentration of 1 mM was chosen for the array as there is no significant activity coming from starting materials at this concentration.

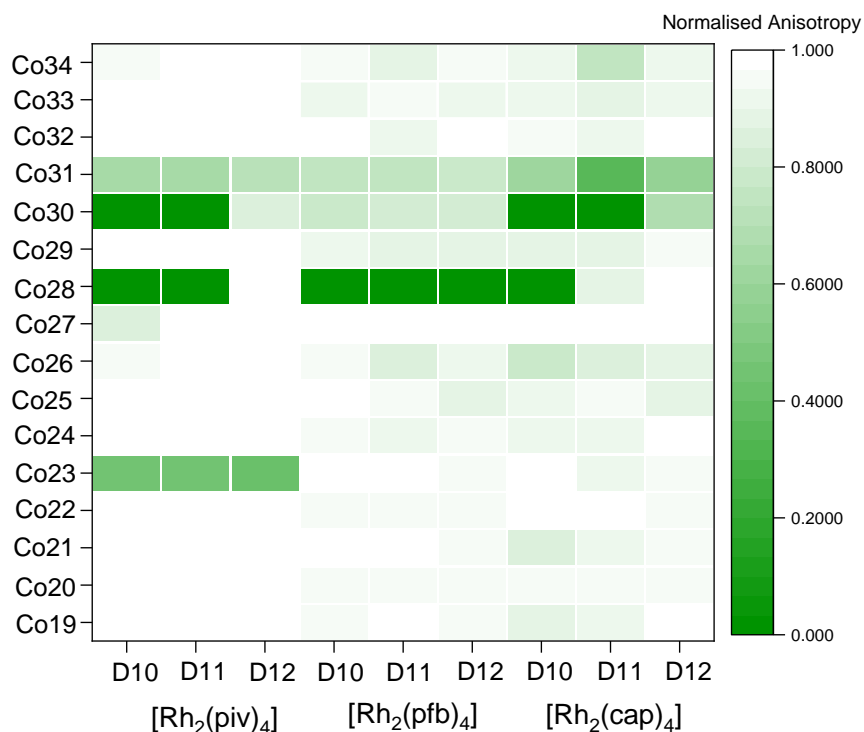
### 3.3.10 Implementation of Reaction Arrays

With the three diazos, three catalysts and 16 substrates selected, a total of 144 reactions were performed, enabling more efficient exploration of chemical space. The array was explored exhaustively, performing every possible combination of diazo, substrate and catalyst.

Reaction arrays were performed in 0.75 mL borosilicate glass vials (8 x 30 mm) in 96-vial (12 x 8) array format and sealed with PTFE snap plugs. Diazos, substrates and catalyst stock solutions were prepared. To each vial, one diazo (8  $\mu$ L of 1.25 M solution), one substrate (8  $\mu$ L of 6.25 M solution), one catalyst (4  $\mu$ L of 25 mM solution) was added, made up to 100  $\mu$ L with  $\text{CH}_2\text{Cl}_2$  and capped, to give a final reaction concentration of 0.1 M diazo, 0.5 M substrate and 1 mM catalyst. The reactions were left at r.t for 48 hr upon which QuadraPure<sup>®</sup> TU resin (20.0 mg) was added along with 100  $\mu$ L  $\text{CH}_2\text{Cl}_2$ . The reaction mixtures were capped and left to scavenge for 24 h. Reaction mixtures were filtered, washed with  $\text{CH}_2\text{Cl}_2$  (3 x 100  $\mu$ L),  $\text{CH}_2\text{Cl}_2$  evaporated open to air followed by desiccation. To each vial, 400  $\mu$ L of  $\text{CH}_2\text{Cl}_2$  along with 100  $\mu$ L TFA and 2.4  $\mu$ L triethylsilane was added, the reaction mixtures capped and left overnight, then dried open to air followed by desiccation. Finally, reaction mixtures were dissolved in DMSO (100)  $\mu$ L to give a final total product concentration (with respect to the limiting diazo substrate) of

100 mM. The diazos, substrates and catalyst stock solutions were prepared as described and then implemented in the reaction array.

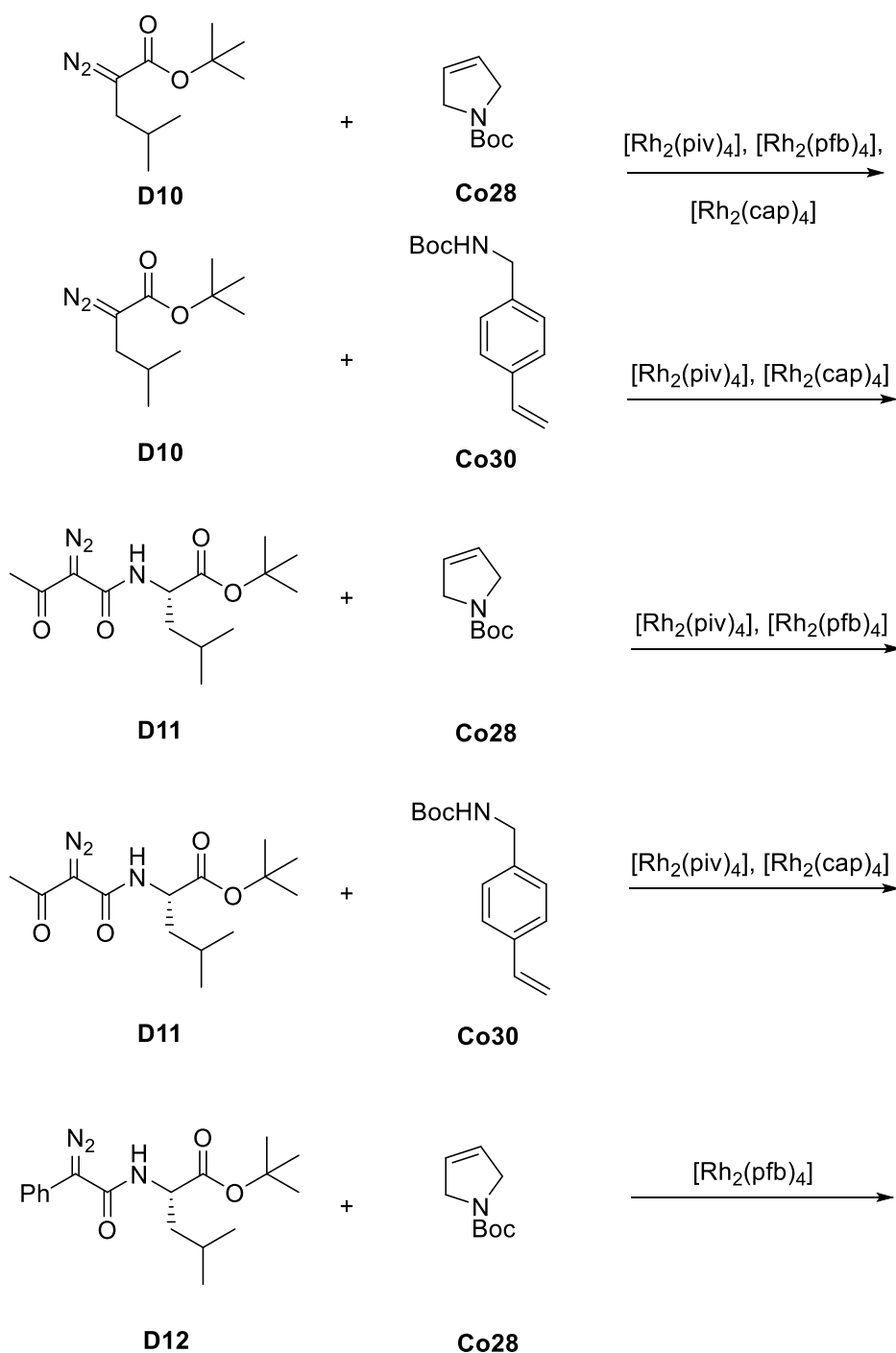
Following array completion, reaction mixtures were diluted in DMSO (100  $\mu$ L) and were screened in the fluorescence anisotropy assay against PEX5 in duplicate at a total product concentration of 1 mM. Positive controls of SKL (10 mM) and negative control of DMSO (5%) were included. Reaction array DMSO stocks were first diluted into DMSO and then FA assay buffer to give 4 mM stock solutions in 20:80 DMSO:buffer (four times the final assay concentration). 10  $\mu$ L of buffer was added to every well to be tested, followed by 10  $\mu$ L of the DMSO:Buffer (20:80) reaction stocks and 10  $\mu$ L of 120 nM tracer stock solution. Finally, 10  $\mu$ L of 800 nM PEX5 stock solution was added to give a final concentration of tracer and protein of 30 nM and 200 nM respectively. The anisotropy data was normalised relative to 10 mM SKL (100% activity) and 5% DMSO - blank (0% activity) and plotted as a heat map (Figure 51).



**Figure 51:** Normalised anisotropy data against PEX5 for the 144 rhodium carbenoid reactions screened at 1 mM. Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 5% DMSO. The anisotropy was calculated at 20 min. The data was normalised relative to 10 mM SKL (green) and 5% DMSO (white).



Of the 144 rhodium carbenoid reactions performed, a total of ten reactions showed significant biological activity with complete displacement of the tracer when screened at 1 mM product concentration. The hit combinations are given (Scheme 27).

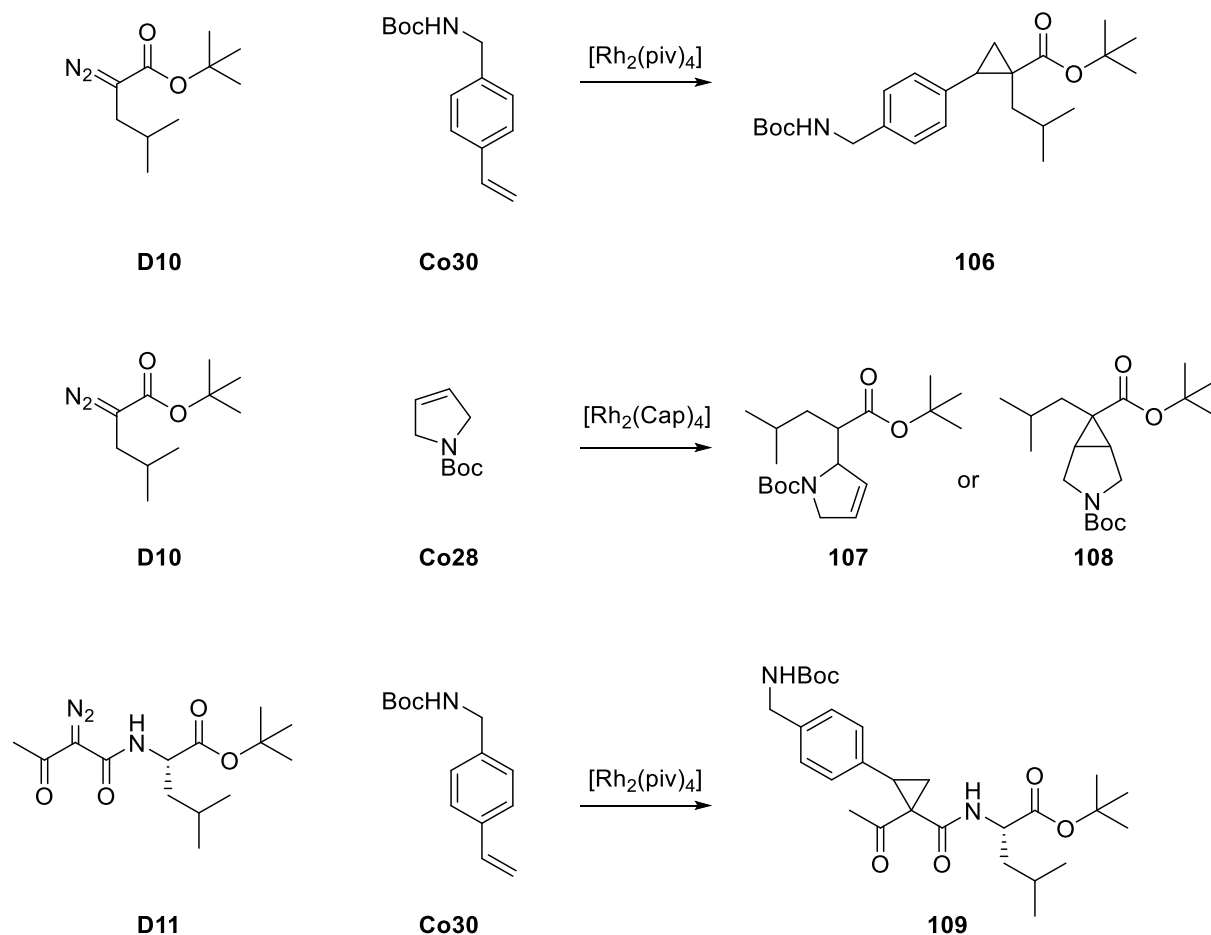


**Scheme 27:** The ten hit combinations obtained from the rhodium carbenoid PEX5 array.

Following the identification of active combinations from the PEX5 rhodium carbenoid array, validation was performed on the promising reactions. First, 3-fold 8-point dose-response was performed on the crude products screening in duplicate with initial total product concentration of 1 mM. Protein (30 nM), tracer (200 nM) and buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 5% DMSO. The anisotropy was calculated at 20 min.

<b>Combination</b>	<b>IC<sub>50</sub> (mM)</b>	<b>Scale-up</b>
D10Co28Piv	Could not be determined	X
D10Co28Pfb	Inactive	X
D10Co28Cap	3.0 ± 0.6 mM	✓
D10Co30Piv	3.5 ± 2.5 mM	✓
D10Co30Cap	3.0 ± 2.3 mM	X
D11Co28Piv	Could not be determined	X
D11Co28Pfb	Inactive	X
D11Co30Piv	1.6 ± 0.2 mM	✓
D11Co30Cap	1.6 ± 0.6 mM	X
D12Co28Pfb	Inactive	X

After a dose-response was performed on the hit combinations, three reactions were chosen for a 50-fold scale-up (Scheme 28). The structures of the expected products are also given. The scale-up was performed by having the catalyst and co-substrate dissolved in dry DCM in a crimped vial under nitrogen. The diazo was then dissolved in dry DCM and added to the reaction mixture dropwise over 3-4 hr *via* syringe pump. After 48 hr, QuadraPure TU Resin (1.0 g) was added to remove the catalyst. The solvent was then evaporated under reduced pressure to give the crude product which was assessed by <sup>1</sup>H NMR and LCMS.



**Scheme 28:** The three combinations selected for a 50-fold scale-up. The expected products are given. The crude products were assessed *via* LCMS and  $^1\text{H}$  NMR.

LCMS and  $^1\text{H}$  NMR analysis of the crude products showed no sign of intermolecular product formation. The activity observed in the fluorescence anisotropy assay was likely a false positive – a result of protein aggregation, solubility issues or tracer crashing out of solution. After this discovery, it was decided to analyse the array using ELSD-LCMS which can be used for product quantification, this would allow determination of the amount of product present in each combination. The results of the ELSD-LCMS analysis for the  $[\text{Rh}_2(\text{piv})_4]$  catalyst is shown (Table 2).

**Table 2:** Results of ELSD-LCMS analysis of the PEX5 rhodium carbenoid array for  $\text{Rh}_2(\text{piv})_4$ . No intermolecular product detected (red), less than 1 mg intermolecular product detected (yellow), more than 1 mg intermolecular product detected (green). 1 mg of products corresponds to ~40% yield.

D10Co19	D11Co19	D12Co19
D10Co20	D11Co20	D12Co20
D10Co21	D11Co21	D12Co21
D10Co22	D11Co22	D12Co22
D10Co23	D11Co23	D12Co23
D10Co24	D11Co24	D12Co24
D10Co25	D11Co25	D12Co25
D10Co26	D11Co26	D12Co26
D10Co27	D11Co27	D12Co27
D10Co28	D11Co28	D12Co28
D10Co29	D11Co29	D12Co29
D10Co30	D11Co30	D12Co30
D10Co31	D11Co31	D12Co31
D10Co32	D11Co32	D12Co32
D10Co33	D11Co33	D12Co33
D10Co34	D11Co34	D12Co34

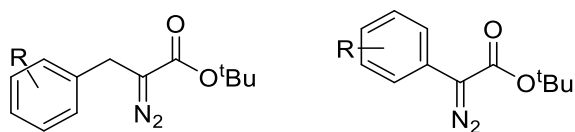
ELSD-LCMS analysis of the PEX5 rhodium carbenoid array for  $[\text{Rh}_2(\text{piv})_4]$  shows that the chemistry is unproductive. Most reactions do not form intermolecular products, with only 6/48 reactions (12.5%) giving intermolecular products. The lack of productivity of the chemistry led to the decision not to pursue another round of ADS.

### 3.4 Summary and Conclusion

To summarise, a fluorescent tracer to be used in FA assays was synthesised and purified. AtHis<sub>6</sub>PEX5C was expressed and purified. An FA assay was re-established by performing a titration of protein into tracer and a  $K_d$  obtained, which was similar to literature values.

In order to mimic the activity of *N*-acetylated KL, it was important to know what the activity of KL itself would be. *N*-acetylated KL, SKL and QSKL were synthesised, purified and evaluated in dose-response. If the activity of KL is to be mimicked, inhibitors would ideally have an  $IC_{50}$  of KL or better. The DMSO tolerance of the assay was then assessed by performing a dose-response on *N*-acetylated SKL at varying concentrations of DMSO, finding the assay is tolerant to concentrations of DMSO of up to 8%. This informed the concentrations at which crude mixtures from the reaction array could be screened at. An *in situ* deprotection step was optimised in a similar was optimised to give deprotected products overnight. A concentration of 20% TFA in DCM with 2 eq. triethylsilane was chosen.

An ADS array targeting PEX5 was then designed and diazos synthesised. Following execution and screening of the array, several combinations were chosen for scale-up but no intermolecular product could be identified. Analysis of a portion of the ADS array *via* ELSD-LCMS revealed the chemistry to be generally unproductive, with only 12.5% of reactions giving intermolecular products in detectable amounts. As can be seen from the results of the ELSD-LCMS analysis, the lack of productivity of the chemistry hindered the project. This might be overcome through re-design of the diazos used in the project. It is known that the C-terminal position of the PTS1 sequence tolerates Phe. Phe based diazos were utilised in round one and two of the activity-directed synthesis of Mcl-1 inhibitors, and so productive chemistry would be expected. Phenylglycine based diazos could also be investigated.



**Figure 52:** Diazos which could be utilised in another PEX5 ADS project.

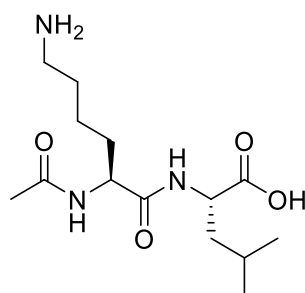
## 4 Design, Synthesis and Evaluation of PEX5 Inhibitors

### 4.1 Aims and Objectives

It was envisaged that inhibitors of PEX5-PTS1 could be discovered through employing a more conventional medicinal chemistry style workflow, involving rounds of design, synthesis and evaluation. A series of short-term project goals have been outlined to evaluate the feasibility of this approach for the discovery of small-molecule inhibitors of PEX5-PTS1 that are both more potent and more ligand efficient than the native PTS1 sequence of comparable size.

#### Objective C1: Design of PEX5-PTS1 inhibitors

PEX5 inhibitors were designed to be mimics of *N*-acetylated KL ( $IC_{50} = 6.0 \pm 0.7$  mM), the first two amino acids in the PTS1 sequence. The first round of molecules were designed by mimicking the Lys residue. The compounds contain nitrogens of varying basicity in order to evaluate the level of basicity needed to pick up interactions formed by the Lys residue. These groups containing basic nitrogens were then linked to Leu *via* several reactions, such as amide coupling, sulfonamide formation and reductive amination. Assuming that the binding of Leu is conserved, this should allow us to properly evaluate the impact of the changes to the Lys mimic. The Leu component was then varied in an attempt to further increase potency and ligand efficiency.



92

**Figure 53:** Structure of *N*-Acetylated KL.

## **Objective C2: Synthesis and Screening of KL Mimics**

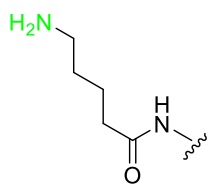
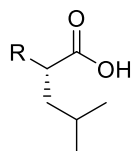
In order to synthesise a large number of compounds quickly, solid-phase synthesis was chosen as the preferred method of analogue synthesis. This would allow us to take a leucine bound to a resin by the C-terminal and add on various groups *via* the N-terminal to rapidly assess the effects of varying the left-hand side of the molecule on activity. Once the left-hand side of the molecule was optimised, the right-hand side would then be varied using solution-phase synthesis. The synthesised compounds were assessed using the PEX5-PTS1 fluorescence anisotropy assay established previously (Section 3.3.4).

## **Objective C3: SAR Studies of KL Mimics**

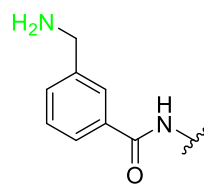
In order to further increase potency and ligand efficiency, hit compounds discovered through initial rounds of synthesis underwent structure-activity relationship studies through variation of ring sizes, chain lengths, stereochemistry and position of heteroatoms. Docking was used to rationalise results and inspire molecular design.

### **4.2 Design of a First Round of PEX5-PTS1 Inhibitors**

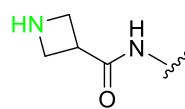
A total of 34 compounds was initially designed to be synthesised and assessed for activity (Figure 54). The C-terminal amino acid (Leu) remained constant, while the left-hand side of the compound (Lys mimetic) is varied. The Lys mimetic contains nitrogens of varying basicity (shown in green). The chosen compounds contain varying chain length, ring sizes, aliphatic and aromatic rings, and linked ring systems. The Lys mimetic is fused to Leu in three different ways, amide formation, sulphonamide formation and reductive amination. The products of the reductive amination will be screened both as the free secondary amine and the acetylated derivative to generate further diversity in the compound set. The products were also designed for ease of synthesis, using commercially available compounds including the required protecting groups for coupling to leucine, increasing the efficiency of synthesis and ultimately allowing more compounds to be generated.



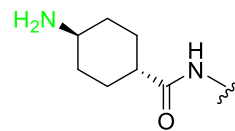
110



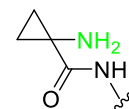
111



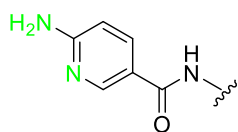
112



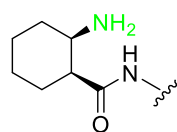
113



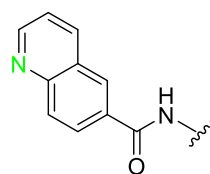
114



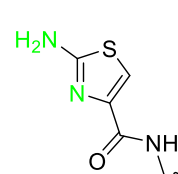
115



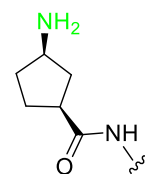
116



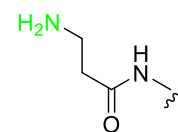
117



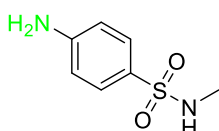
118



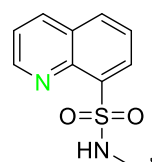
119



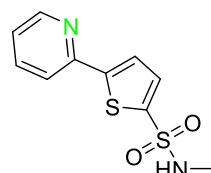
120



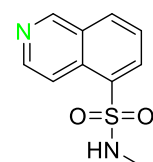
121



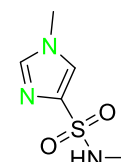
122



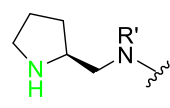
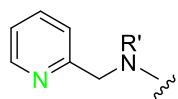
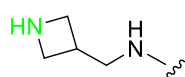
123



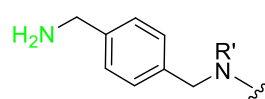
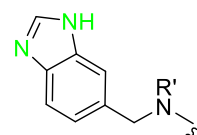
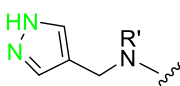
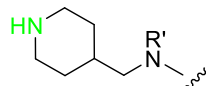
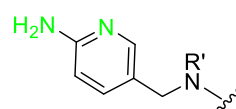
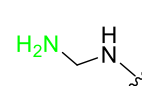
124



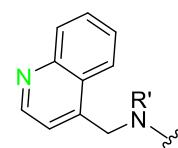
125

126, R' = H  
127, R' = Ac128, R' = H  
129, R' = Ac

130

131, R' = H  
132, R' = Ac133, R' = H  
134, R' = Ac135, R' = H  
136, R' = Ac137, R' = H  
138, R' = Ac139, R' = H  
140, R' = Ac

141

142, R' = H  
143, R' = Ac

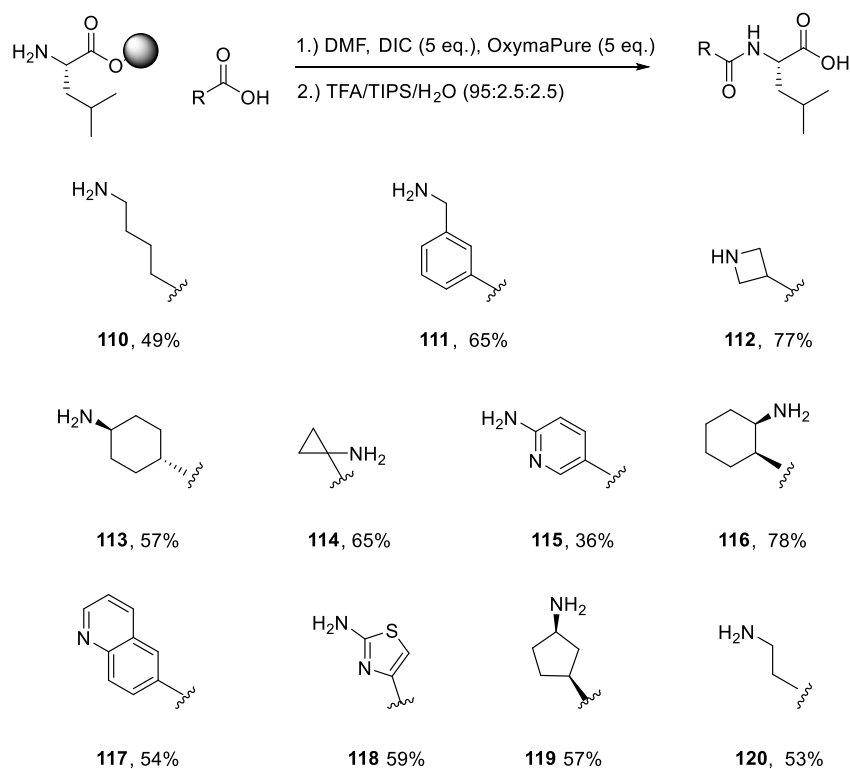
**Figure 54:** Designed mimics of *N*-Acetylated KL. Groups which may mimic the basic Lys side chain are highlighted in green.



### 4.3 Synthesis of Designed PEX5-PTS1 Inhibitors

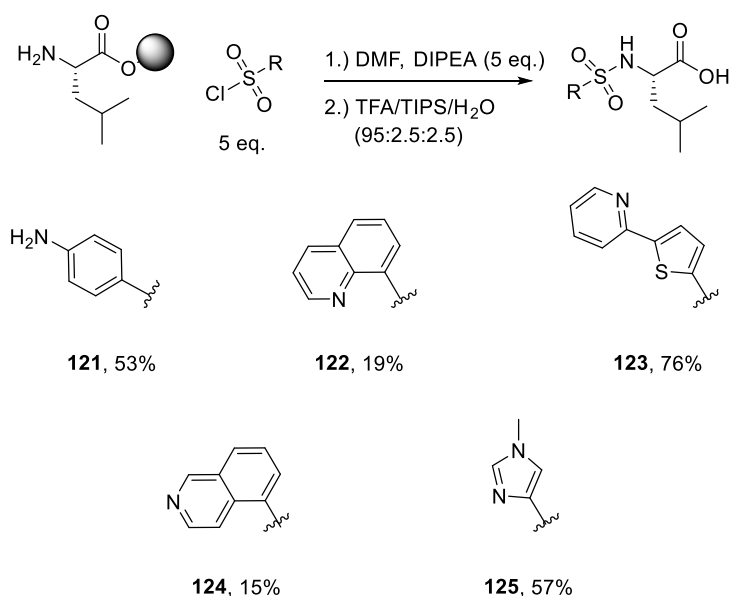
The preferred method chosen for the synthesis of the designed inhibitors was solid-phase synthesis. This involves taking Leu bound to an acid-labile 2-chlorotrityl resin bound by the C-terminal, with the N-terminal free for functionalisation (amide coupling, sulfonamide formation, reductive amination). Following reaction completion, the product is cleaved from the resin using TFA, which also removes any acid labile protecting groups (Boc, *tert*-butyl, trityl).

Amide coupling were performed by taking the required carboxylic acid (5 eq.) dissolved in DMF (1 mL), along with DIC (5 eq.) and OxymaPure (5 eq.) and adding it to the resin (100 mg, 0.079 mmol/mg loading). This was agitated for 1 hr and the reaction mixture drained from the resin. A cleavage cocktail of TFA/TIPS/H<sub>2</sub>O (1 mL, 95:2.5:2.5) was added and agitated for 1 hr (see Section 5.1 for a full protocol). The eluent was then collected and concentrated under reduced pressure to give the crude product which was then purified *via* mass-directed autopurification (MDAP) prior to screening (Scheme 29).



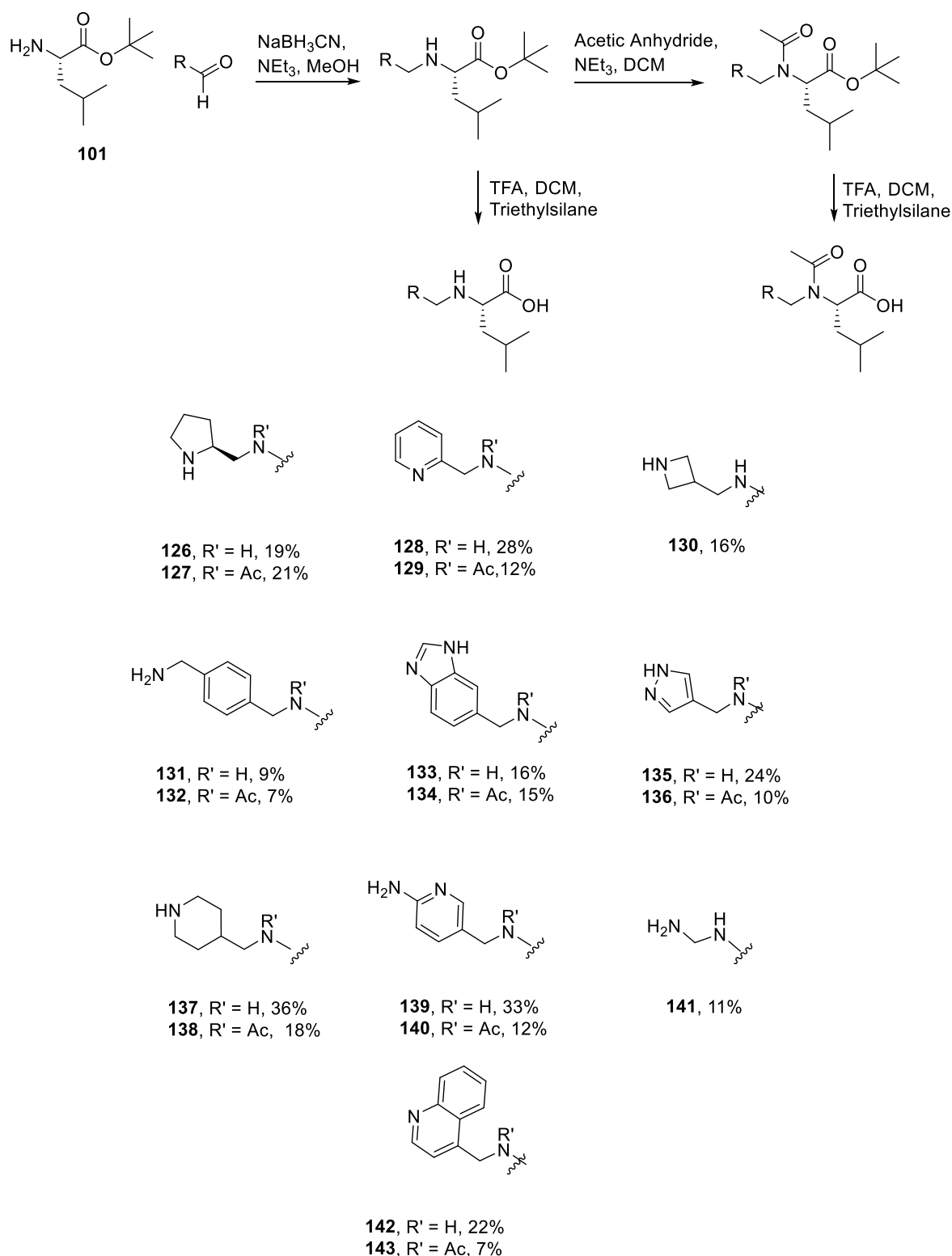
**Scheme 29:** Synthesised amide-containing compounds in which the Lys mimic was varied. In all compounds but **117**, the corresponding Boc amine protected carboxylic acid was used in the synthesis.

Sulfonamide formations were performed by taking the required sulfonyl chloride dissolved in DMF with DIPEA and adding it to the resin. This was agitated for 1 hr and the reaction mixture drained from the resin. A cleavage cocktail of TFA/TIPS/H<sub>2</sub>O (1 mL, 95:2.5:2.5) was added and agitated for 1 hr (see Section 5.1 for a full protocol). The eluent was then collected and concentrated under reduced pressure to give the crude product which was then purified *via* mass-directed autopurification (MDAP) prior to screening (Scheme 30).



**Scheme 30:** Synthesised sulfonamide compounds in with the Lys mimic was varied. Compound **121** used the Boc-protected aniline sulfonyl chloride in the synthesis.

Reductive aminations were performed in solution phase using *O*-*tert*-butyl Leucine with the required aldehyde (1.5 eq.), NaBH<sub>3</sub>CN (2.0 eq.) and NEt<sub>3</sub> (1 eq.) in DMF with stirring at r.t overnight. This procedure was attempted on solid-phase using the same protocol but gave little success. The crude products were then deprotected using TFA or acetylated with acetic anhydride (1.5 eq.) and DIPEA (1.5 eq.) then deprotected using TFA to give the acetylated derivative. Crude products were then purified *via* mass-directed autopurification (MDAP) prior to screening (Scheme 31).

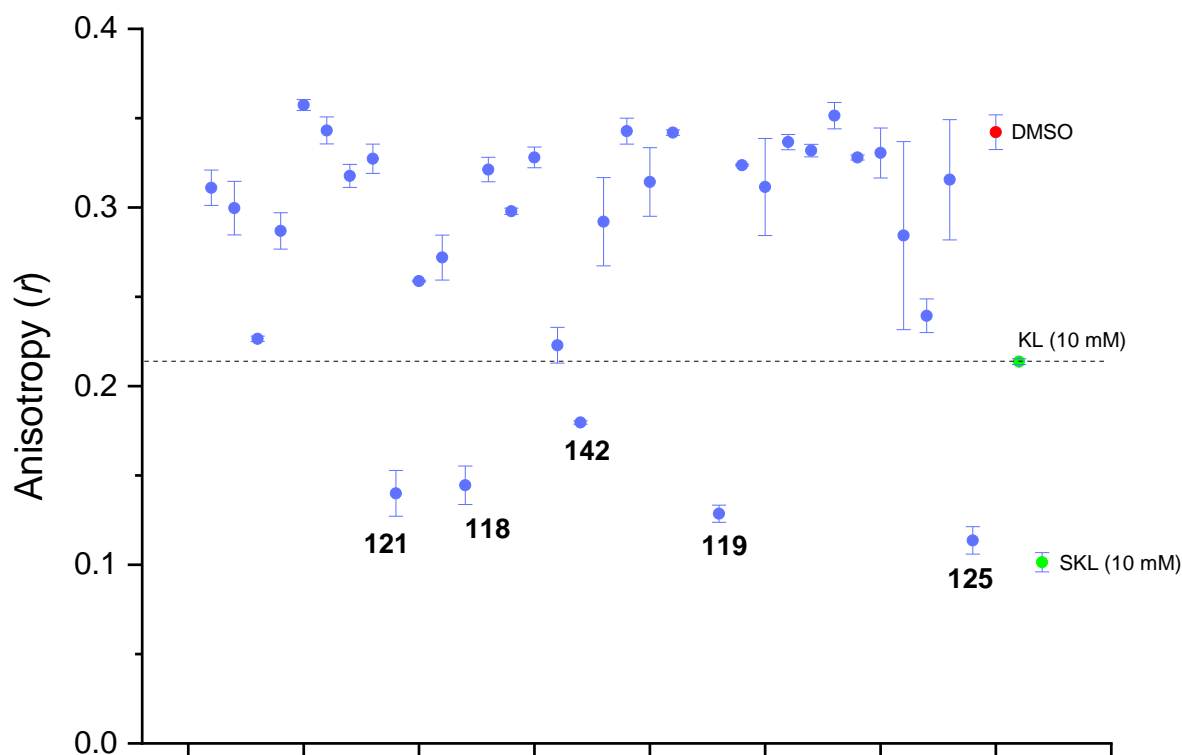


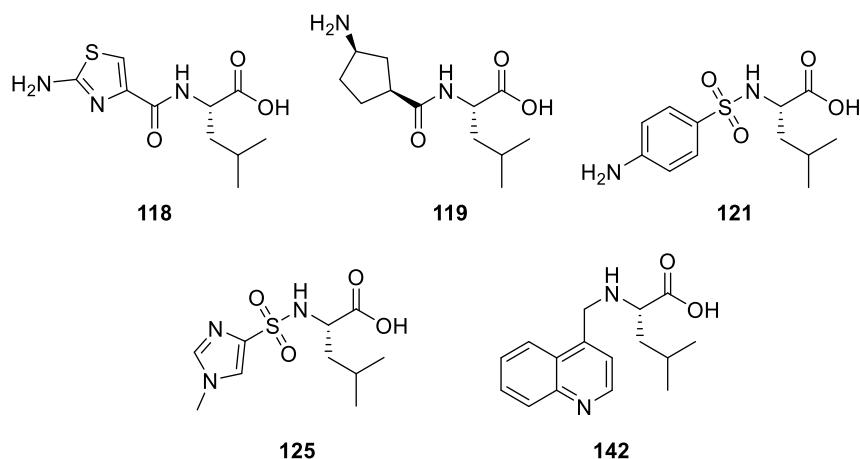
**Scheme 31:** Synthesised compound *via* reductive amination in which the Lys mimic was varied. Yields were calculated over two or three steps and reactions were telescoped. In compounds **126**, **127**, **130**, **131**, **132**, **137**, **138**, **139**, **140**, and **141** aldehydes with Boc-protected amines were used in the synthesis.

A total of 34 compounds was prepared for screening against PEX5.

#### 4.4 Screening of PEX5 Inhibitors

The activity of the synthesised *N*-acetylated KL mimics were assessed in duplicate in the PEX5-PTS1 fluorescence anisotropy assay established previously. The compounds were first dissolved in DMSO (100 mM). The stock solutions were then diluted into FA assay buffer to give 40 mM stock solutions in 20:80 DMSO:buffer (four times the final assay concentration of 10 mM). 10  $\mu$ L of buffer was added to every well to be tested, followed by 10  $\mu$ L of the DMSO:Buffer (20:80) reaction stocks and 10  $\mu$ L of 120 nM tracer stock solution. Finally, 10  $\mu$ L of 800 nM PEX5 stock solution was added to give a final concentration of compound, tracer and protein of 10 mM, 30 nM and 200 nM respectively at 5% final DMSO concentration. A screening concentration of 10 mM was chosen as we are looking for compounds which are at least as active as *N*-acetylated KL, whose activity is detectable at this concentration. Compounds were designated as hits if they were more active than *N*-acetylated KL at the same concentration, leading to five hit compounds: **118**, **119**, **121**, **125** and **142**.

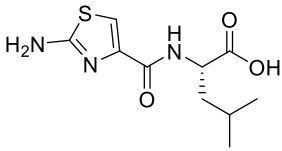
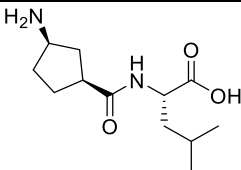


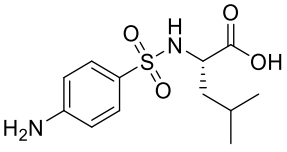
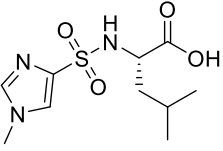
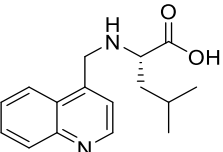


**Figure 55:** Compounds more active than *N*-acetylated KL are labelled. PEX5 (200 nM) and tracer (30 nM). Buffer: HEPES (20 mM) and NaCl (150 mM), pH 7.5 at 5% DMSO. Anisotropy was calculated after 20 min.

In order to validate the hit compounds identified and determine an  $IC_{50}$  for their binding, an 8-point 2-fold dose-response was performed on the five highlighted compounds with initial concentration of 10 mM, tracer 30 mM, protein 200 mM and a final DMSO concentration of 5% (Table 3). The anisotropy was plotted against ligand concentration and the data fitted using a logistical fit and the  $IC_{50}$  obtained from fitting. The  $IC_{50}$  values were then converted into  $K_i$  of the competitor ligand by combination with  $K_d$  of the protein using the method of Nicolovska-Coleska (as in Section 2.3.1.4).<sup>77</sup>

**Table 3:** Summary of screening results for round one of inhibitor synthesis.  $IC_{50}$  (mM) and  $K_i$  ( $\mu$ M) for each compound is shown. For compound **118** and **125**, an error could not be calculated by the fitting.

Compound	$IC_{50}$ (mM)	$K_i$ ( $\mu$ M)
 <b>118</b>	~ 9	~ 73
 <b>119</b>	$4.2 \pm 0.2$	$42 \pm 2$

 <p><b>121</b></p>	4.9 ± 1.1	50 ± 6
 <p><b>125</b></p>	~ 7.2	~ 86
 <p><b>142</b></p>	Not determined	Not determined

An IC<sub>50</sub> value could not be determined for compound **142**. Poor behaviour and solubility may have led to initial identification as a hit compound.

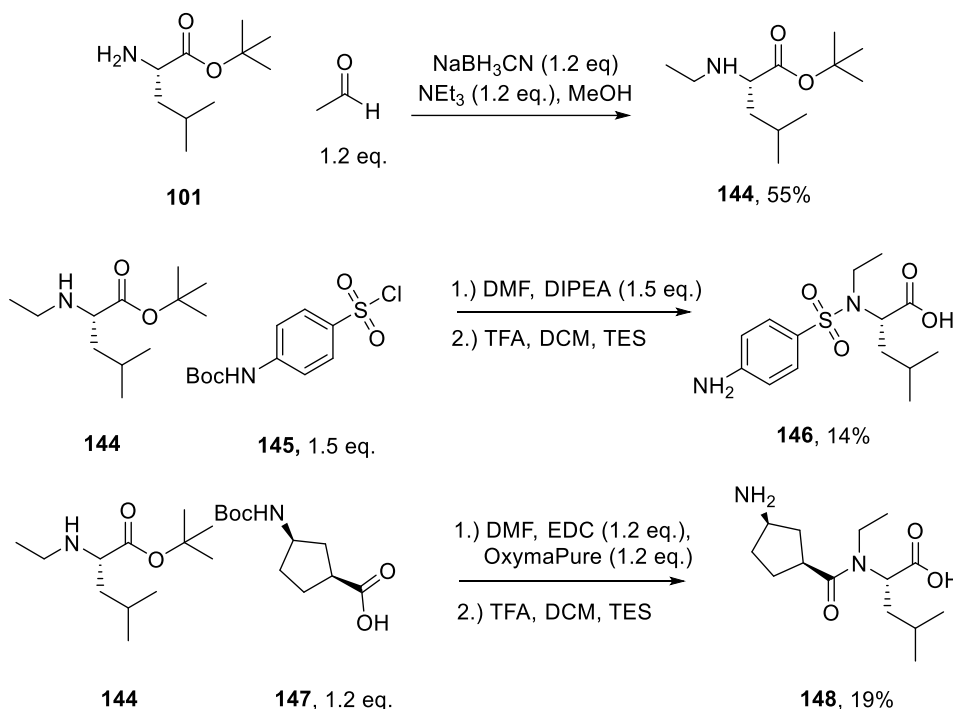
#### 4.5 SAR Studies of Hit Compounds

With validated hit compounds **119** and **121** in hand a series of experiments were designed to examine the effects of substitution, homologation, swapping out the leucine for other amino acids and searching for growth vectors. The first experiment performed was ethylating at the amide and sulfonamide in an attempt to find a potential growth vector, a direction in which to grow the molecule to increase activity. If activity is improved or retained it may indicate that this position is amenable to substitution.

##### 4.5.1 Attempts to Discover a Growth Vector

It was envisaged that growing from hit compounds **119** and **121** would result in increased activity. In order to assess where the compounds could be grown, the original hits **119** and **121** were first ethylated at the amide or sulfonamide nitrogen. Compound **144** was first prepared through reductive amination using *O*-*tert*-butyl leucine (**35**) with acetaldehyde, NaBH<sub>3</sub>CN and NEt<sub>3</sub> in MeOH and left to stir at r.t overnight to give **144** in 55% yield. Compound **144** was then treated with sulfonyl chloride **145**, DMF and DIPEA and left to stir at r.t overnight, then

treated with TFA, DCM and TES and left to stir at r.t overnight to give compound **146**. Compound **144** was also treated with carboxylic acid **147**, EDC, OxymaPure in DMF and left to stir at r.t overnight, then treated with TFA, DCM and TES and left to stir at r.t overnight to give compound **148**.

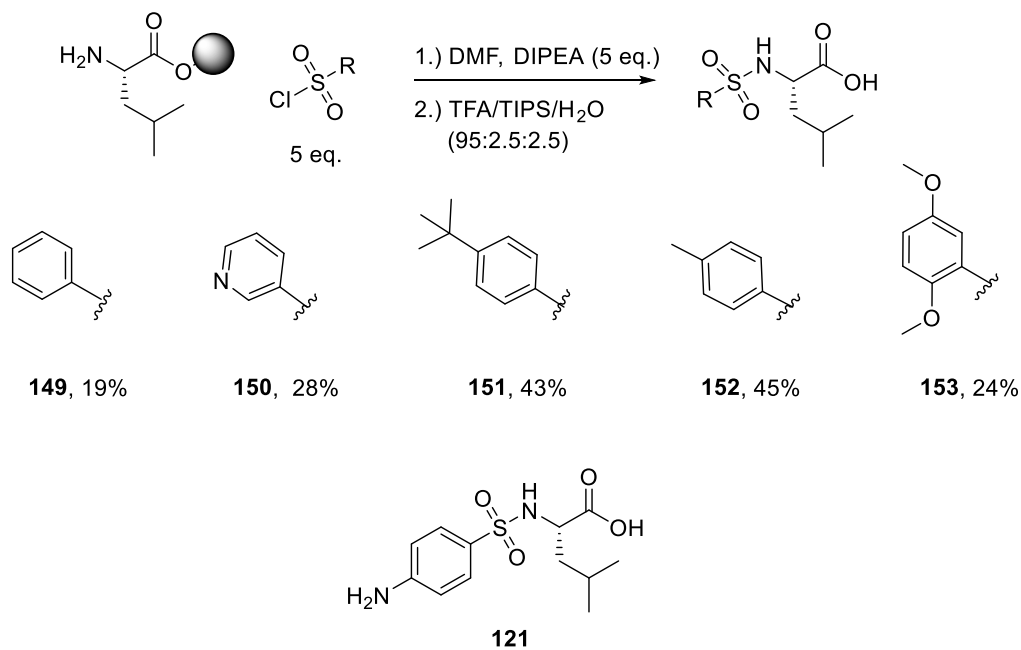


**Scheme 32:** Synthesis of ethylated analogues, **146** and **148**, of hit compounds **119** and **121**.

The synthesised ethylated analogues were then screened in dose-response against PEX5 in the same way as in Section 4.4 at 10 mM initial product concentration. The dose-response shows little to no activity in the compounds ( $\text{IC}_{50} > 15 \text{ mM}$ ), compared to  $\text{IC}_{50} = 4.2 \pm 0.2 \text{ mM}$  for **119** and  $\text{IC}_{50} = 4.9 \pm 1.1 \text{ mM}$  for **121**, indicating that substitution at the amide or sulfonamide nitrogen has a deleterious effect on activity, possibly due to the change in conformation.

#### 4.5.2 Optimisation of Sulfonamide Hit **121**

The effects of substitution on the aromatic ring in compound **121** was then investigated. A range of sulfonamide formations were performed on solid-phase using available sulfonyl chlorides as in section 4.3. The sulfonyl chlorides chosen will answer whether the amine is needed and if any other substitutions are tolerated.



**Scheme 33:** Synthesised analogues of compound **121**.

The synthesised analogues of compound **121** were then assessed in dose-response mode, as in Section 4.4, with a top concentration of 10 mM.

**Table 4:** Summary of screening results for analogues of compound **121**.  $IC_{50}$  (mM) and  $K_i$  ( $\mu\text{M}$ ) for each compound is shown.

Compound	$IC_{50}$ (mM)	$K_i$ ( $\mu\text{M}$ )
 <b>149</b>	> 15	> 150
 <b>150</b>	> 15	> 150
 <b>151</b>	$7.4 \pm 0.6$	$75 \pm 6$



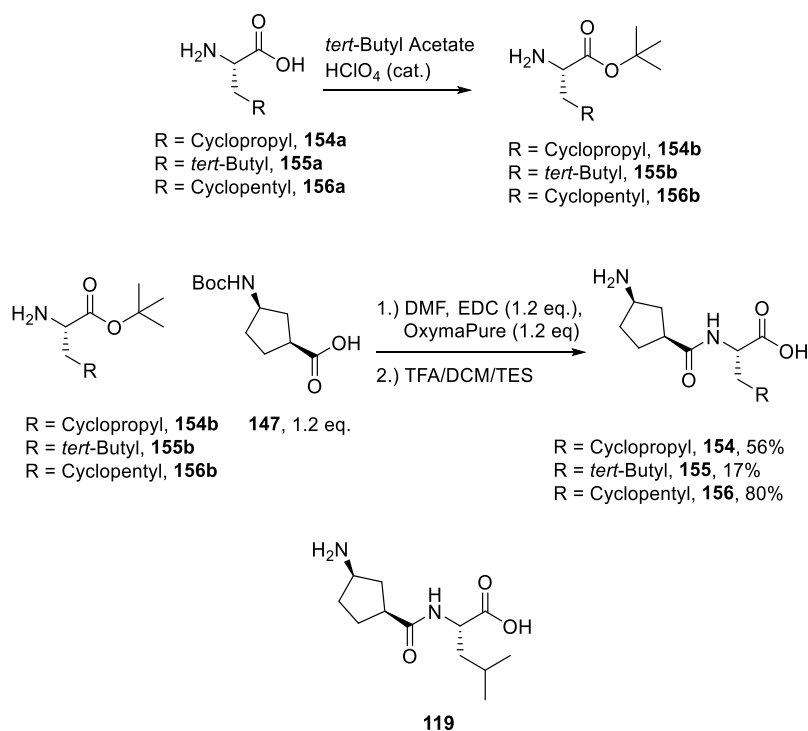
 <p style="text-align: center;"><b>152</b></p>	>15	>150
 <p style="text-align: center;"><b>153</b></p>	>15	>150

The dose-response shows a reduction in activity across the range of compounds, including those with substitution at the same position as the amine, which may indicate its presence is required for activity.  $IC_{50}$  values could only be determined for compounds **151** and **153**. Compound **153** also appears to have a very steep Hill slope as seen with previous compounds. Due to the lack of increase in activity and the observed steep Hill slopes, further optimisation efforts were focused on amide hit **119**.

### 4.5.3 Optimisation of Amide Hit 119

#### 4.5.3.1 Variation of C-Terminal Amino Acid

Prior to variation of the left-hand side of hit compound **119**, the right-hand side amino acid was varied. It is known that leucine is the optimal natural amino acid at the C-terminal, but other natural amino acids with hydrophobic side chains are tolerated, such as Met and Phe. To test the tolerance of unnatural amino acids, a small series of compounds was designed based on compound **119**, synthesised and screened for activity.

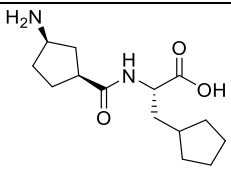


**Scheme 33:** Synthesised analogues of hit compound **119**, aiming to investigate the effects of switching out the C-terminal amino acid.

The synthesised analogues of compound **119** were assessed in dose-response mode, as in Section 4.4, with a top concentration of 10 mM.

**Table 5:** Summary of screening results for analogues of compound **119** generated through varying the C-terminal amino acid. IC<sub>50</sub> (mM) and K<sub>i</sub> (μM) for each compound is shown.

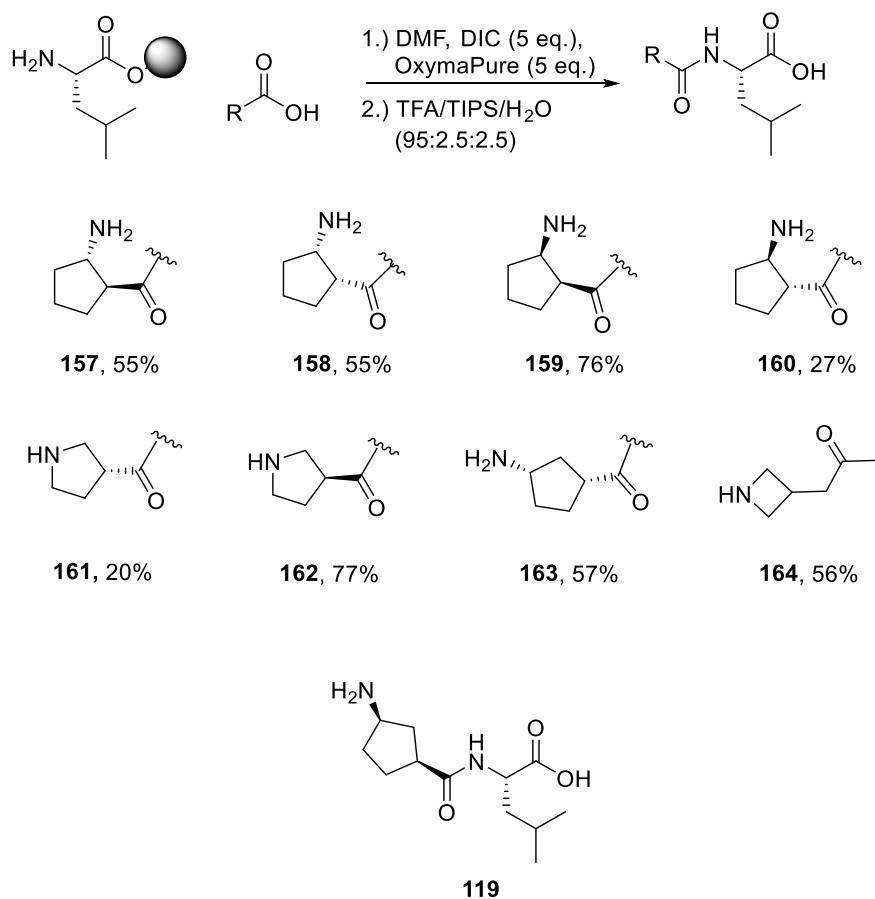
Compound	IC <sub>50</sub> (mM)	K <sub>i</sub> (μM)
<p><b>154</b></p>	8.4 ± 0.3	86 ± 5
<p><b>155</b></p>	9.8 ± 0.1	99 ± 9

 <b>156</b>	$1.3 \pm 0.3$	$13 \pm 2$
---	---------------	------------

The dose-response shows a reduction in activity for cyclopropylalanine and *tert*-butyl alanine analogues **154** and **155**, but a 3-fold increase in activity is observed for compound **156** with cyclopropylalanine, suggesting it is forming better hydrophobic contacts than with leucine.

#### 4.5.3.2 Effects of Amine Position and Stereochemistry

Further attempts at optimisation were then performed by varying the left-hand side of hit compound **119**. This optimisation was performed on the weaker binding compound **119** rather than **156** due to ease of synthesis, since derivatives containing Leu can be synthesised using solid-phase synthesis. A set of carboxylic acids was chosen for coupling based on hit **119**. The position of the primary amine on the ring was varied, as well as stereochemistry and switching the aminocyclopentyl for a pyrrolidine (Scheme 34). Synthesis was performed as in Section 4.4.

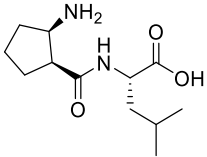
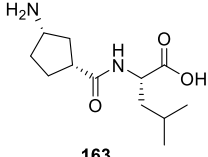
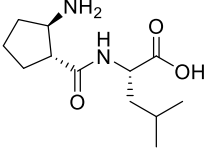
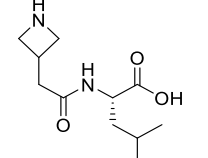


**Scheme 34:** Synthesised analogues of compound **119**, aiming to investigate the effects of amino position and stereochemistry.

The synthesised analogues of compound **119** were assessed in dose-response mode, as in Section 4.4, with a top concentration of 10 mM.

**Table 6:** Summary of screening results for analogues of compound **119** generated through varying the left-hand side of the molecule. IC<sub>50</sub> (mM) and K<sub>i</sub> (μM) for each compound is shown.

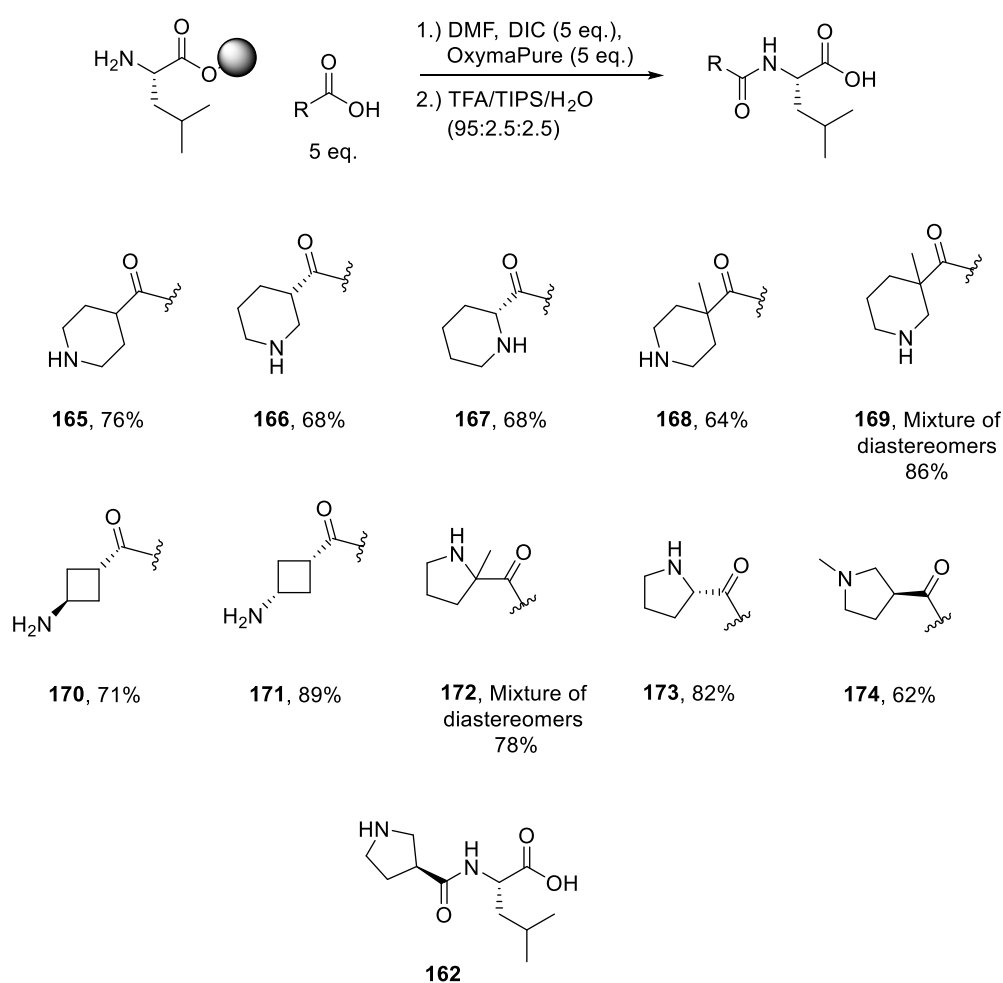
Compound	IC <sub>50</sub> (mM)	K <sub>i</sub> (μM)	Compound	IC <sub>50</sub> (mM)	K <sub>i</sub> (μM)
 157	> 15	> 150	 161	> 15	> 150
 158	> 15	> 150	 162	~ 1.6	~ 16

 159	>15	>150	 163	6.1 ± 0.3	54 ± 4
 160	>15	>150	 164	>15	>105

The dose-response of the synthesised compounds indicate that the position of the amine on the ring in compound **119** was already optimal as compounds **157**, **158**, **159** and **160** all appeared inactive. The stereochemistry of the amine relative to the amide is also optimal, as compound **119** has almost two-fold increase in activity over compound **163**. The cyclopentylamine in **119** may be switched for a pyrrolidine in **162** resulting in increased activity. The amine in the pyrrolidine is **162** in the same position as the primary amine in **119** and the amide has the same stereochemistry. No activity was observed with the enantiomeric compound **161**.

#### 4.5.3.3 Effects of Ring Size and Methylation

The ring size in compound **162** was then varied to expand to six-membered rings or contract to four membered rings. The position of the secondary amine was then walked around the ring to investigate the effects of position in the six-membered ring series. Methylated versions of some of the series were also tested alongside in an attempt to find a growth vector (Scheme 35). Synthesis was performed as in Section 4.4.



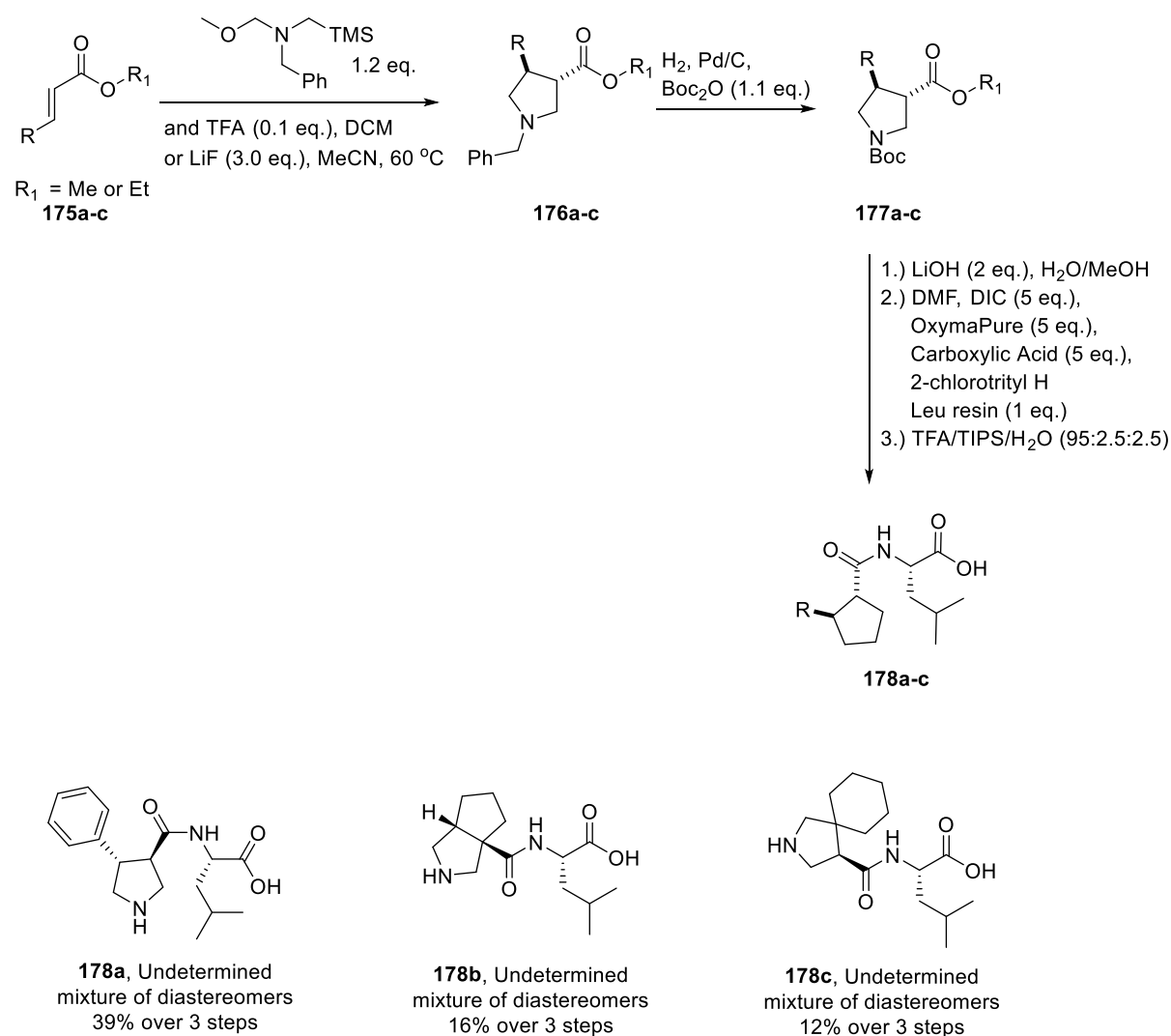
**Scheme 35:** Synthesis of analogues of compound **162**, aiming to investigate the effects of ring size and methylation.

The synthesised analogues of compound **162** were assessed in dose-response as in Section 4.4 at 10 mM initial concentration. The dose-responses showed a loss of activity across the range of compounds. An  $IC_{50} < 10$  mM was obtained **165** and an  $IC_{50} \sim 9$  mM for **171**, the data for **165** could not be fit to a curve and so an exact value cannot be obtained. This suggests that ring size is critical to activity, and a 5-membered ring is the preferred size. Moving the position of the amine in **162** to the prolines **172** and **173** also resulted in a complete loss of activity. Methylation on the nitrogen as in compound **174** also resulted in loss of activity,

possibly because the change in conformation means the amine in the pyrrolidine can no longer make contacts with Glu346 and Glu379

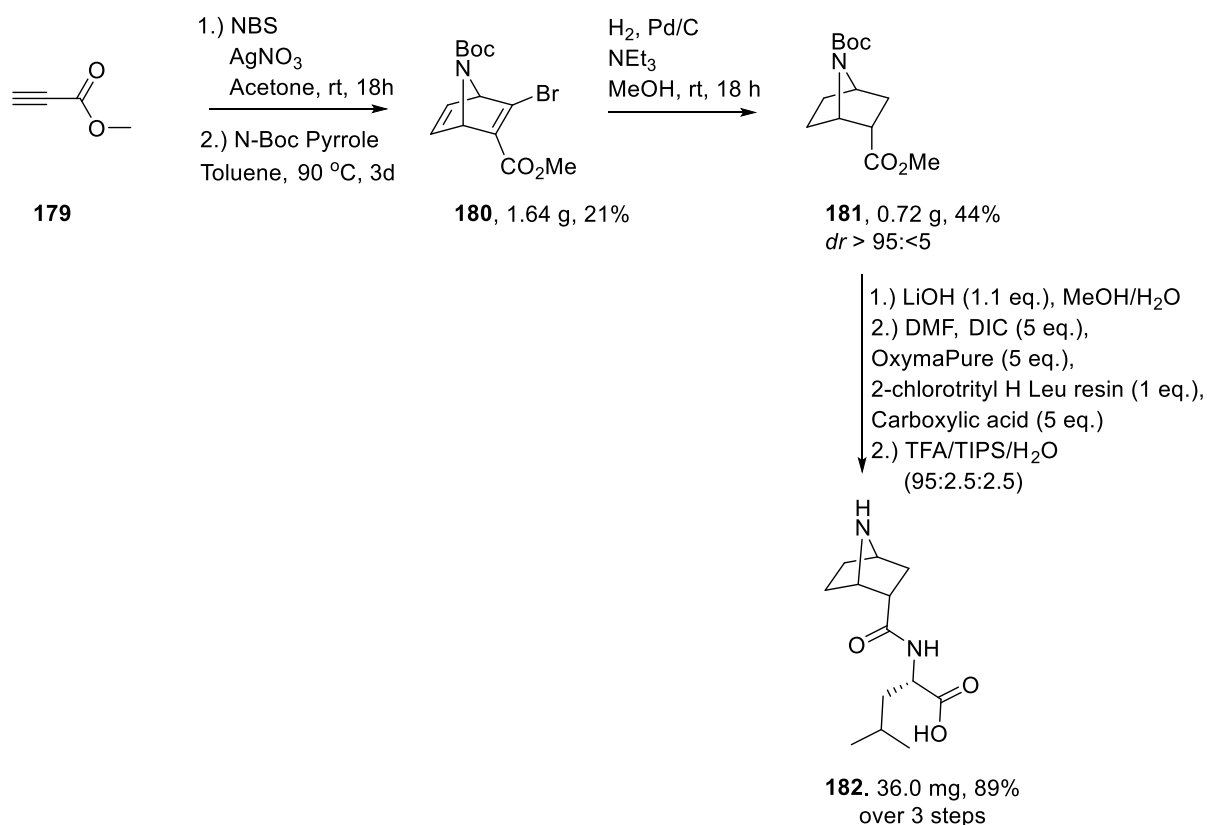
#### 4.5.3.4 Effects of Substitution and Conformation Restriction

Substitution from the pyrrolidine in compound **162** was examined through the use of dipolar cycloaddition chemistry to install groups on the pyrrolidine ring (Scheme 36).<sup>107,108</sup>  $\alpha,\beta$ -unsaturated esters **175a-c** were treated with *N*-(methoxy)-*N*-(trimethylsilyl-methyl)benzylamine and TFA in DCM at r.t or LiF in MeCN at 60 °C to yield the pyrrolidines **176a-c**. These were then treated with hydrogenation conditions in the presence of Boc<sub>2</sub>O to give the Boc derivatives **177a-c**. These then underwent ester hydrolysis to yield the crude carboxylic acids, followed by amide coupling with 2-chlorotrityl Leucine resin to give the substituted analogues **178a-c** as undetermined mixtures of diastereomers.



**Scheme 36:** Dipolar cycloaddition chemistry used to give substituted pyrrolidine analogues of **162**.

The diastereoselective synthesis of compound **181** has been reported in the literature and has been synthesised *via* a Diels-Alder reaction between *N*-Boc Pyrrole and methyl-3-bromopropiolate.<sup>109</sup> Therefore, the synthesis began with cycloaddition between *N*-Boc Pyrrole and methyl-3-bromopropiolate to give the cycloaddition intermediate **180** in 21% yield. This intermediate was subjected to hydrogenation conditions in the presence of trimethylamine to give the reduced intermediate **181** as a single diastereoisomer (*dr* > 95:<5) in 44% yield. Ester hydrolysis followed by amide coupling to 2-chlorotrityl H Leu resin and cleavage gave the conformationally restricted analogue **182**.



**Scheme 37:** Synthetic pathway outline the preparation of analogue **182**, *via* Diels-Alder reaction of *N*-Boc Pyrrole.

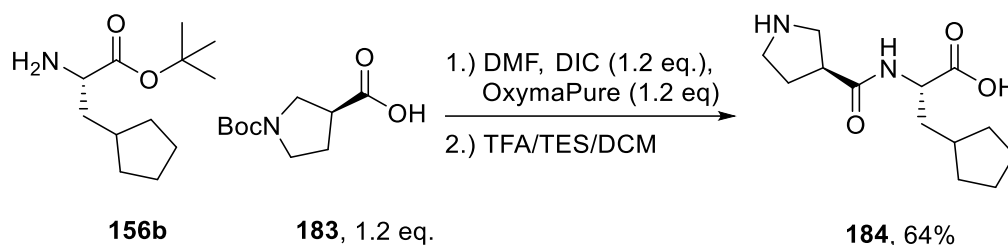
Upon screening of the synthesised compounds, dose-response revealed no increases in activity. This suggests that conformational restriction in compound **182** is not tolerated. The chosen substitutions from the pyrrolidine ring did not



lead to any increases in activity, compound **178a** exhibited an  $IC_{50} = 11 \pm 0.2$  mM and **178c** had an  $IC_{50} \sim 9$  mM. Compound **178b** had no activity at the screening concentration.

#### 4.5.3.5 Combining Positive Elements of Hit Compounds

Increases in activity were seen when swapping out the cyclopentylamine in compound **119** for the pyrrolidine in compound **162**, and swapping out the leucine in compound **119** for the cyclopentylalanine in compound **156**, leading to a 2-fold and 3-fold increase in activity respectively. These positive changes were combined to give compound **184**, synthesised *via* an amide coupling between **156b** and **183** in a 64% yield.



**Scheme 38:** Combining changes which led to increases in activity seen in compounds **156** and **162** to give compound **183**.

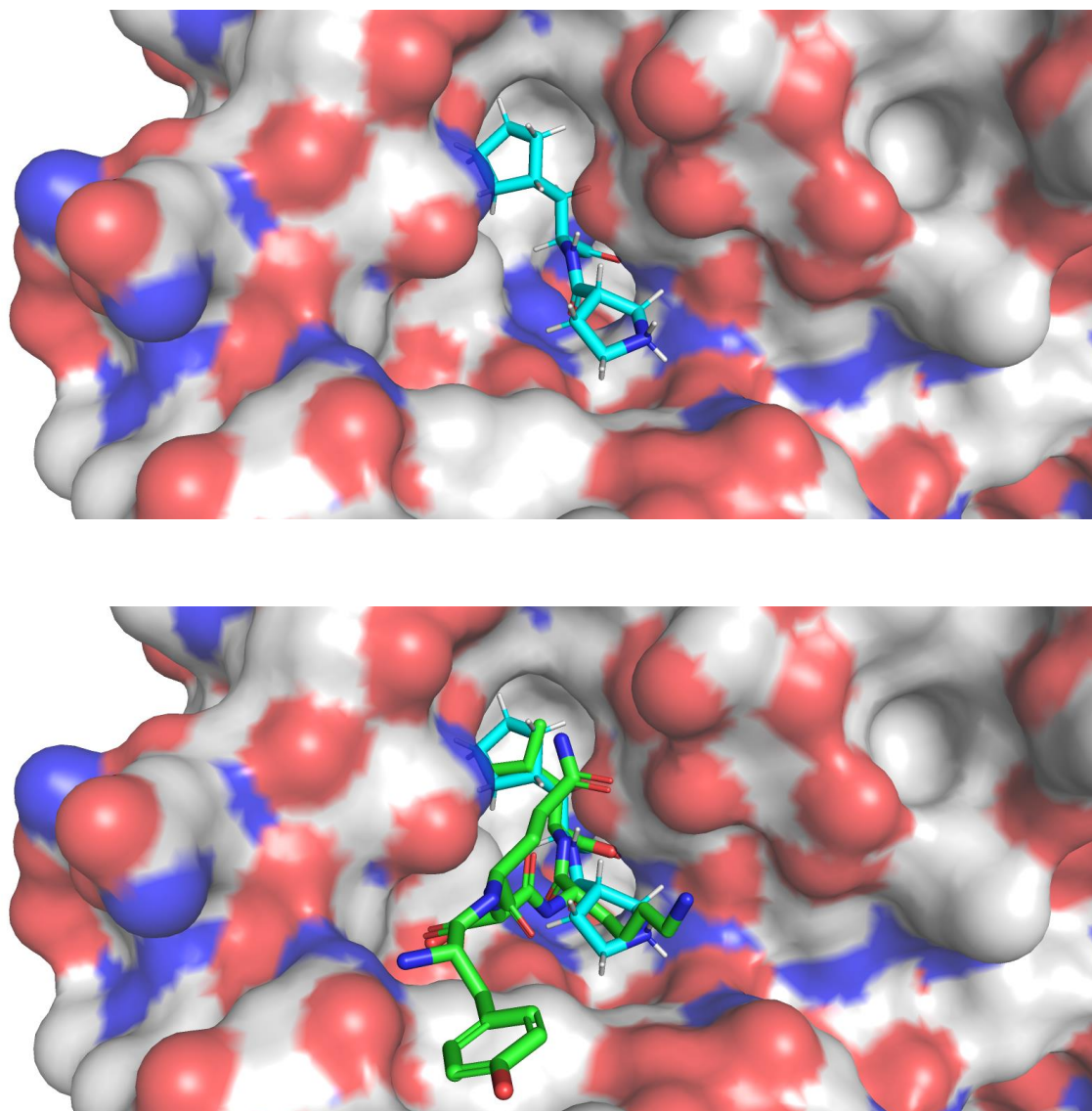
Compound **184** was assessed in dose-response as in Section 4.4 at 10 mM initial concentration. Screening revealed an  $IC_{50}$  of  $1.5 \pm 0.1$  mM and a  $K_i$  of  $15 \pm 1$   $\mu$ M. The activity observed in compound **184** is comparable to that of compound **162**, suggesting that swapping out the leucine for cyclopentylalanine will not always give a significant increase in activity as it did in compound **119** to **156**.

## 4.6 Docking Studies

### 4.6.1 Docking of Previously Assessed Compounds

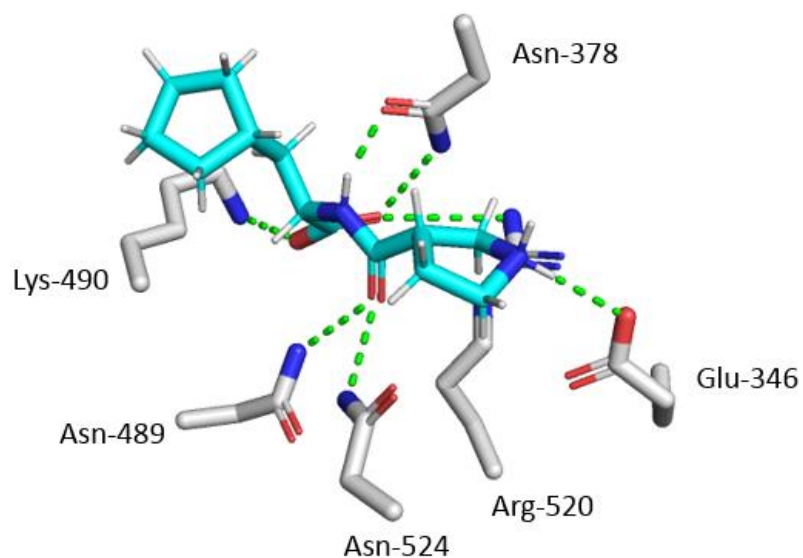
Due to the difficulties in achieving gains in activity from the original hit compound **119** and the number of compounds that had to be synthesised, it was decided to generate analogues computationally and dock them into the crystal structure of PEX5 to guide which compounds should be made. To begin with, hit compound **184** was docked into the crystal structure of human PEX5 co-crystallised with

YQSKL and examined by visual inspection (Figure 56, see Section 5.7.1 for full docking protocol).



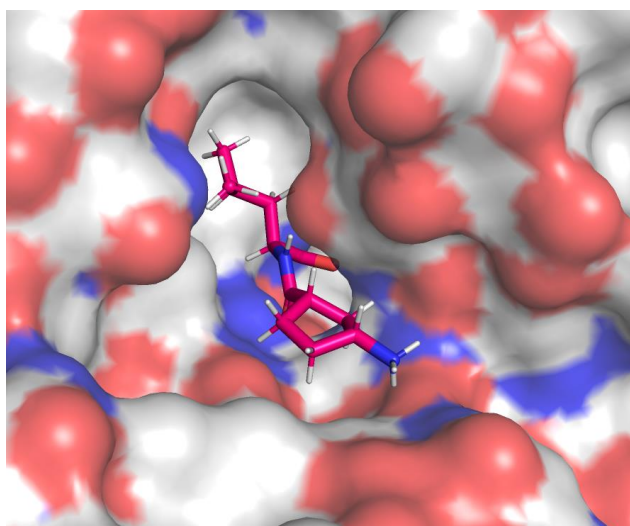
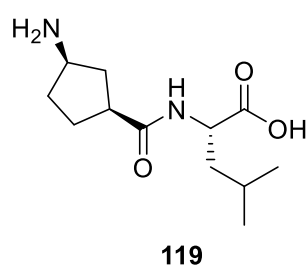
**Figure 56:** Docking of hit compound **184** into PEX5 (top). Docking of hit compound **184** overlaid with YQSKL (below). Docked into - PDB Accession Code: 1FCH.

Docking of hit compound **184** predicts that many of the contacts made by YQSKL are maintained. The C-terminal leucine in YQSKL overlays well with the cyclopentylalanine in compound **184**, with the carboxylic acid in the same orientation. The amine in compound **184** is orientated towards the lysine side chain of YQSKL and is predicted to interact with Glu-346 as opposed to Glu-348 and Glu-379 as in the lysine (Figure 57).



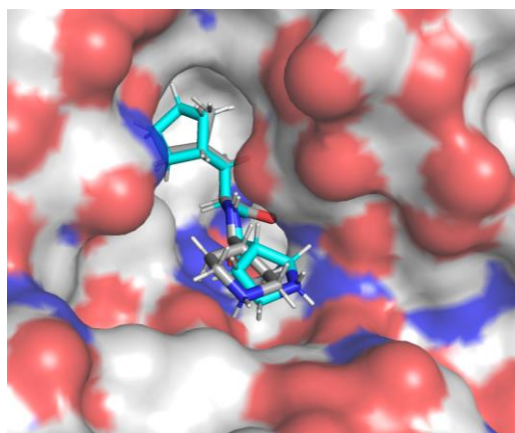
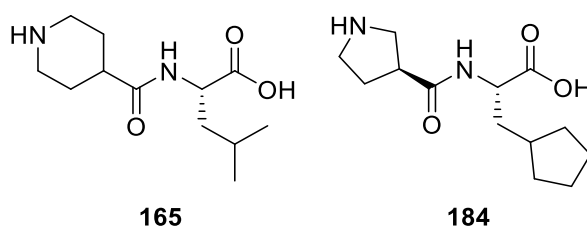
**Figure 57:** Ligand interaction diagram highlighting key interactions formed by compound **184**.

The docking poses of some of the previously assessed compounds were assessed to give structural insight. Original hit compound **119** was first assessed. Compound **119** is predicted to bind in a similar fashion to compound **184** with the amino group orientated towards the Glu346.



**Figure 58:** Compound **119** along with its predicted binding pose. Docked into - PDB Accession Code: 1FCH.

The docking pose of a 6-membered ring analogue (**165**) was then evaluated to assess the impact ring size has on the predicted binding. The overlay of **165** with **184** reveals that the cyclohexyl ring is now too large to orientate the amine into the groove which contains Glu346, Glu348 and Glu379 residues, resulting in loss of activity.

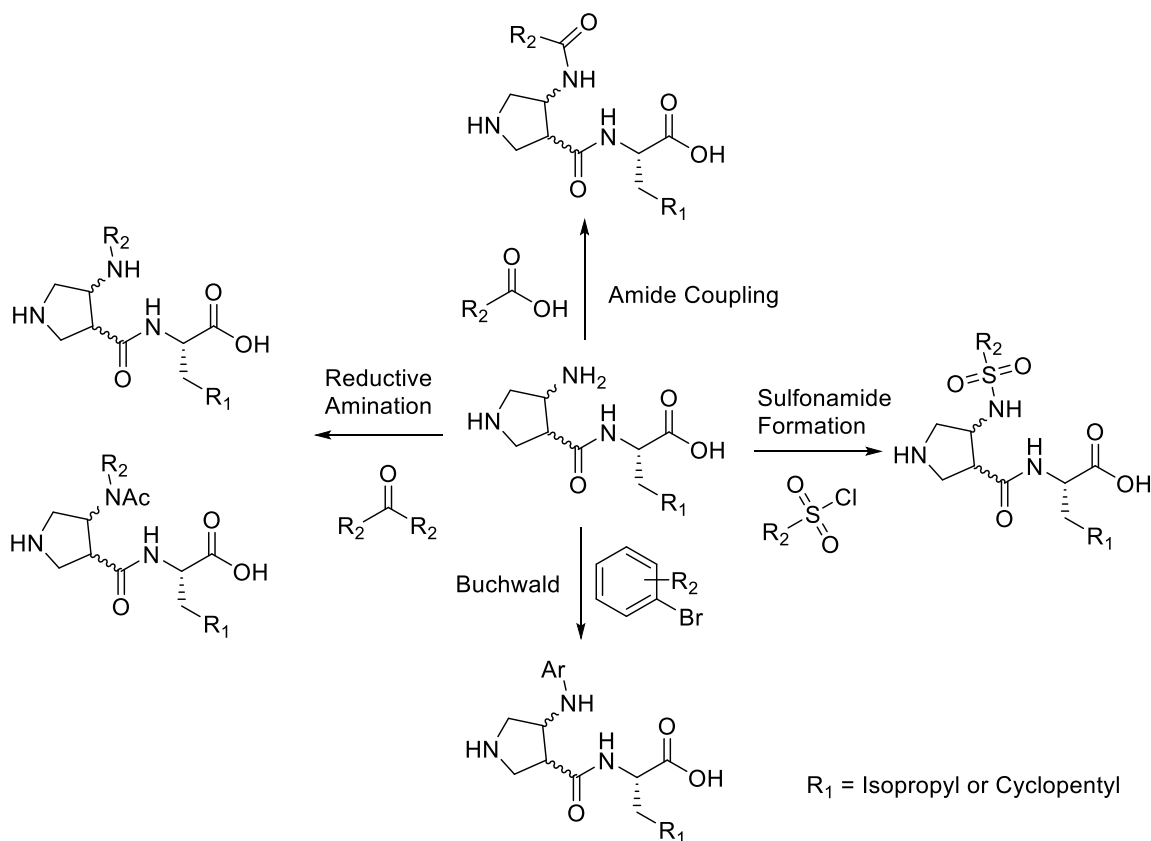


**Figure 59:** Docking pose of compound **165** overlaid with the docking pose of **184**. PDB Accession Code: 1FCH.

#### 4.6.2 Design of Docking Library

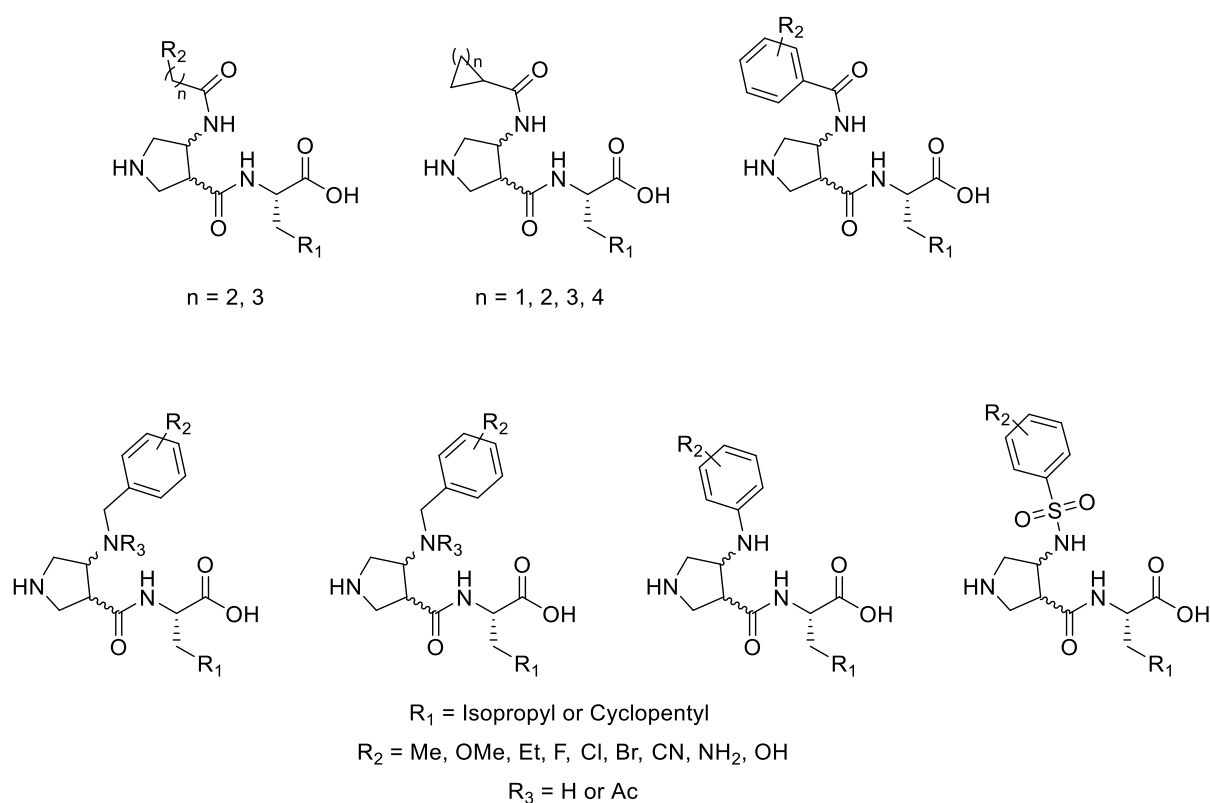
From visual inspection, it appears that substitution at the 4-position of the pyrrolidine in **184** could result in contact with the pocket occupied by the serine of YQSKL (Figure 56). As a result of this observation, a set of ligands was designed including substitution at the 4-position (Scheme 39). A small library of compounds was enumerated manually in ChemDraw. The chemistries used to enumerate the

library include amide coupling, sulfonamide formation, Buchwald and reductive amination.



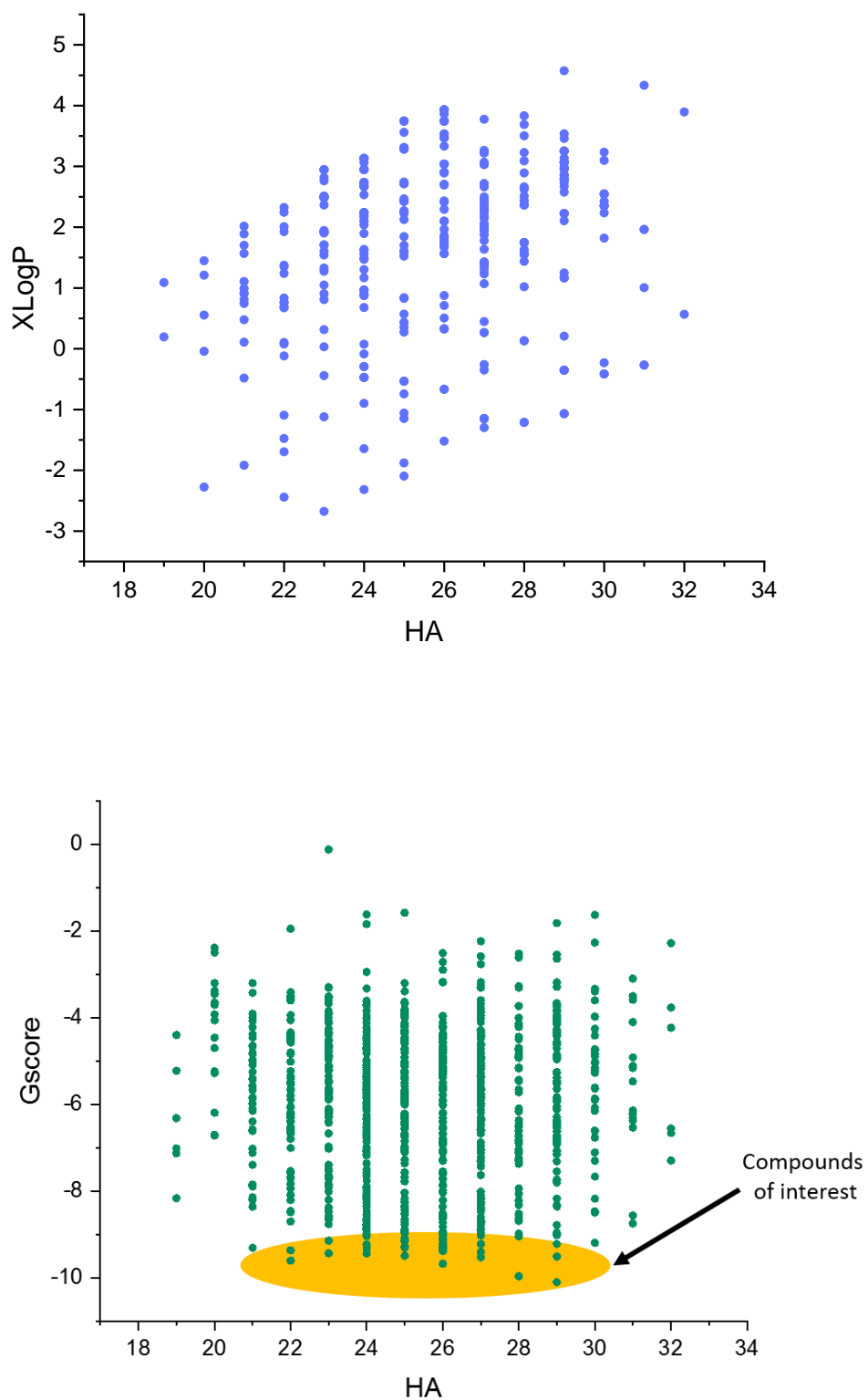
**Scheme 39:** Summary of the chemistries used to generate the library for docking.

Products of reductive amination were assessed with both the free secondary amine and the acetylated derivative. All possible stereochemistries were assessed around the pyrrolidine ring and both leucine and cyclopentylalanine analogues for each compound were assessed. This gave a total of 1506 compounds to be docked into the crystal structure of YQSKL (Section 5.7.2 for the SMILES string for the compound library). A summary of the compounds include is given in Figure 60. The docking was performed using a HTVS (high-throughput screening method). See Section 5.7.1 for docking protocol.



**Figure 60:** Summary of compounds included in the library for docking.

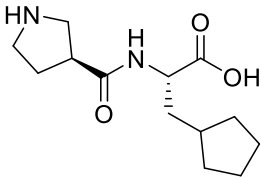
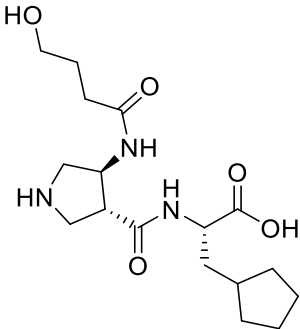
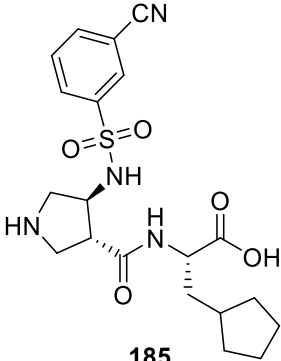
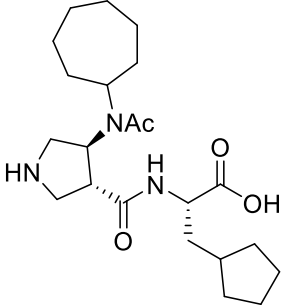
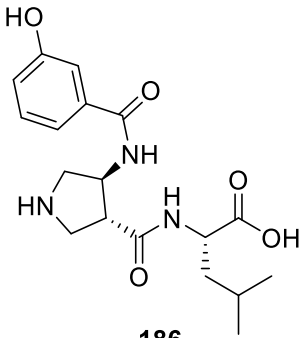
Glide gscore is a term which approximates the ligand binding free energy for each compound, this metric was used to assess how well each compound was predicted to bind to PEX5. The gscore distribution for the designed library is given in Figure 61. Compounds with the lowest gscore were assessed again using the XP (extra-precision) method and the docking poses inspected. The XLogP and HA count for the library was also plotted (Figure 61). The results indicate that much of the library occupy fragment-like chemical space with  $X\text{LogP} < 3$  and  $\text{HA} < 25$ .



**Figure 61:** Distribution of gscore values vs. HA for the docked library (above). The average gscore was determined to be gscore = -5.9. Plot of XLogP vs. HA count (below).

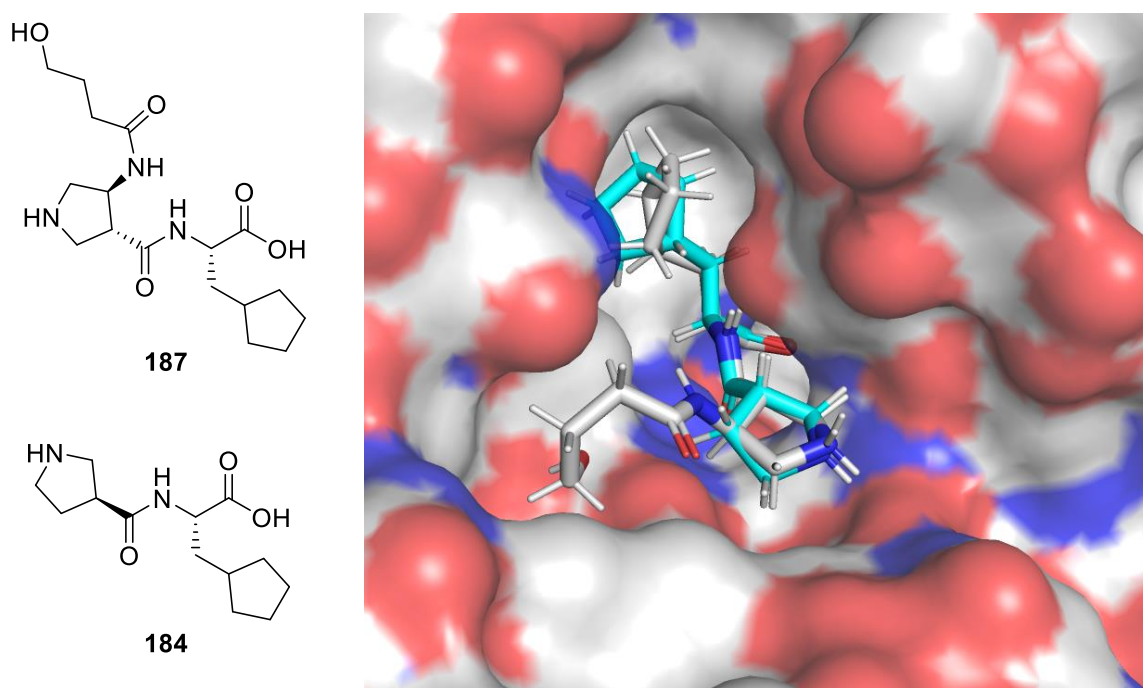
The binding poses of the top performing compounds was assessed to check that they were sensible. A set of compounds were then chosen for synthesis based on ease of synthesis and availability of starting materials. The compounds chosen for synthesis along with compound **184** and their gscores are given in Table 7.

**Table 7:** Hit compound **184** and top performing compounds **185-188** along with their gscores.

Compound	gscore	Compound	gscore
 <p><b>184</b></p>	-10.065	 <p><b>187</b></p>	-11.774
 <p><b>185</b></p>	-10.803	 <p><b>188</b></p>	-12.367
 <p><b>186</b></p>	-10.982		



An example docking pose of compound **187** overlaid with the original hit compound **184** is given in Figure 62. The binding pose of the pyrrolidine ring is well maintained, with the side chain of the cyclopropylalanine slightly twisted. The position of the carboxylic acid is conserved. An additional interaction is predicted to be formed between the newly introduced alcohol and Tyr508 which is known to interact with the serine side-chain of YQSKL.

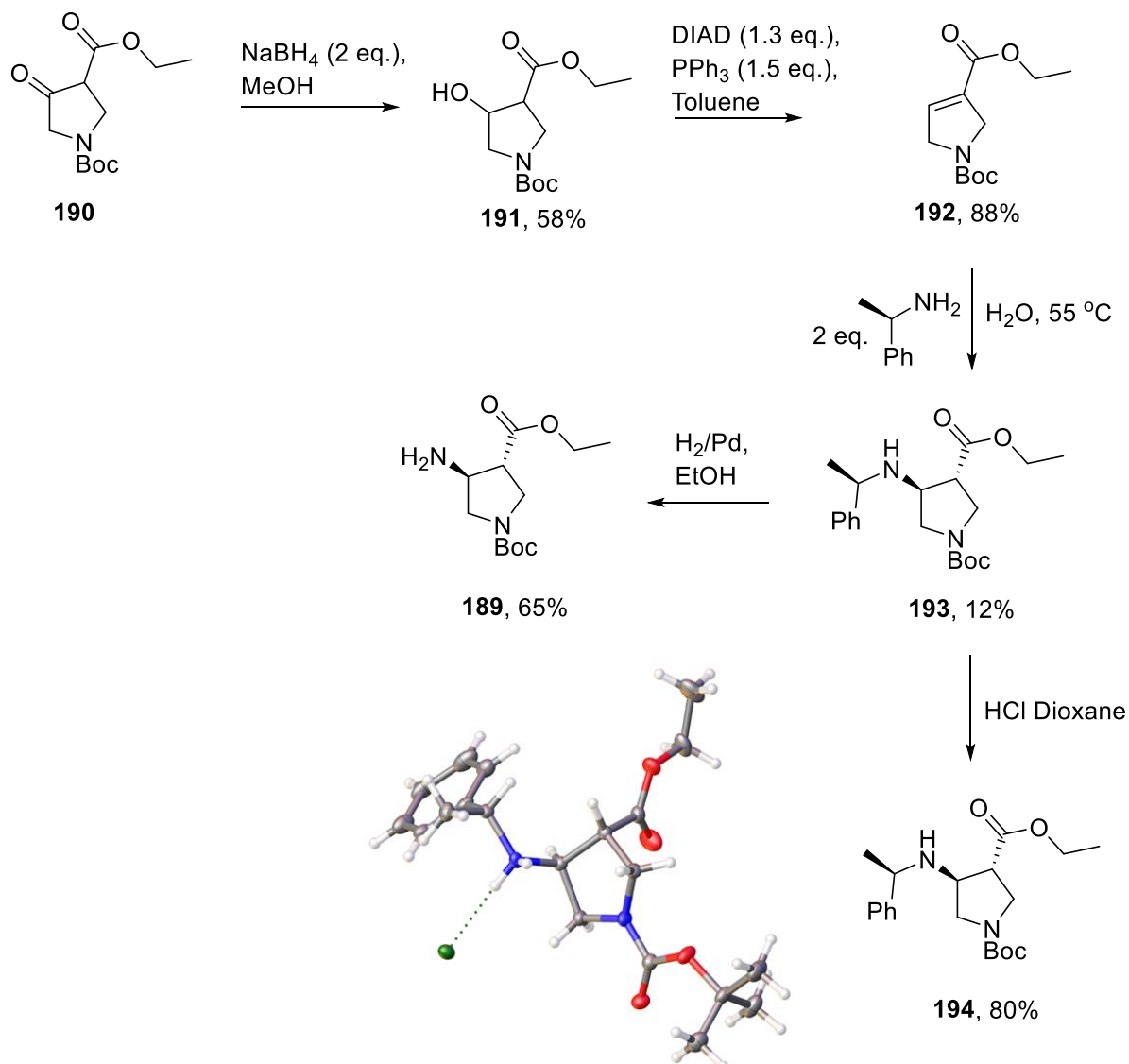


**Figure 62:** Docking pose of compound **187** overlaid with compound **184**. PDB Accession Code: 1FCH.

#### 4.6.3 Synthesis of Docking Hits

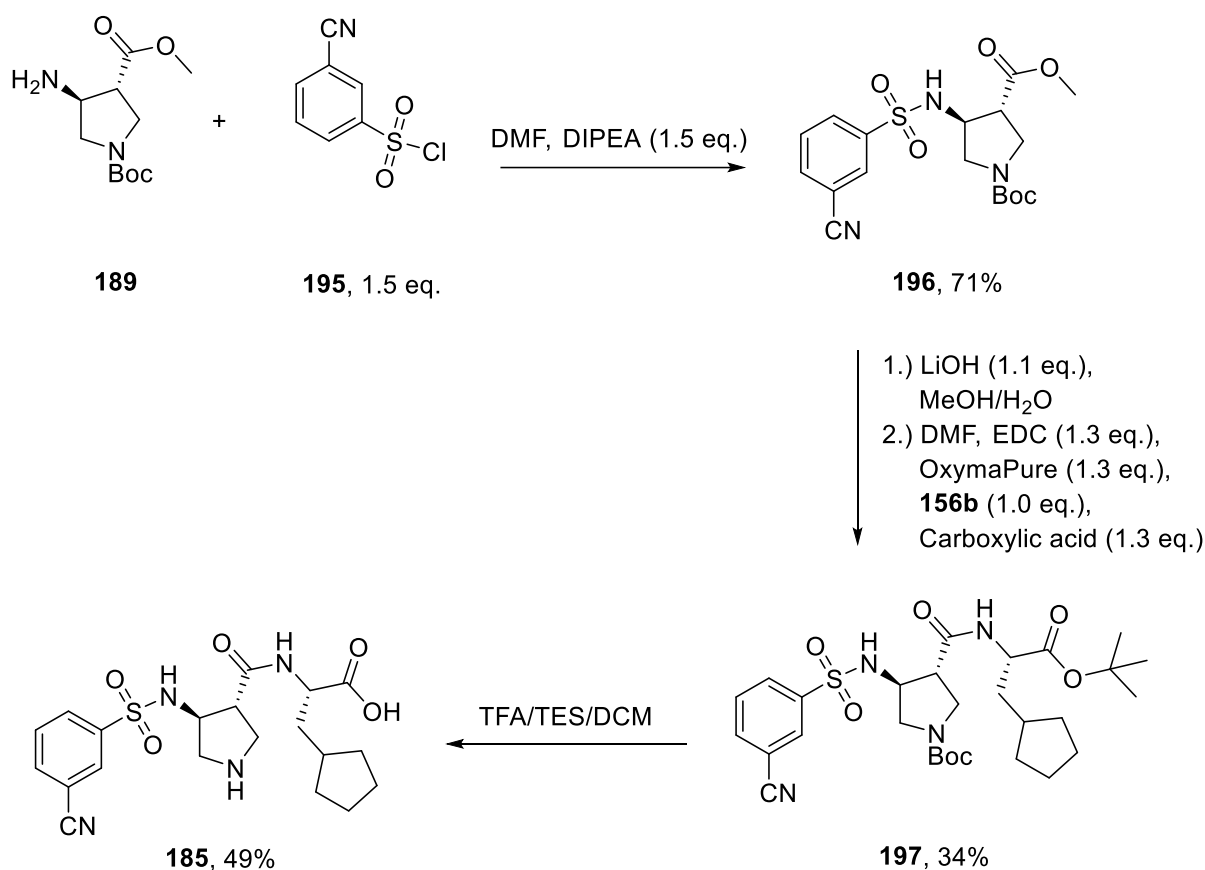
To synthesise compounds **185-188**, a chiral aminopyrrolidine building block was required. Chiral aminopyrrolidine **189** (Scheme 40) was prepared according to a known procedure.<sup>110</sup> To begin, compound **190** underwent reduction with NaBH<sub>4</sub> to give compound **191** in 58% yield. The alcohol **191** was then treated with DIAD and PPh<sub>3</sub> to give the alkene **192** in 88% yield. Conjugate addition with the chiral amine gave compound **193** in 12% yield, which was separated from the other three diastereomers. Conversion to the HCl salt (**194**) with HCl/Dioxane was achieved in 80% yield and allowed a crystal structure to be determined to ensure

the correct diastereomer was isolated. This then underwent hydrogenation with  $\text{H}_2$ , Pd/C to give the chiral aminopyrrolidine **189** in 65% yield.



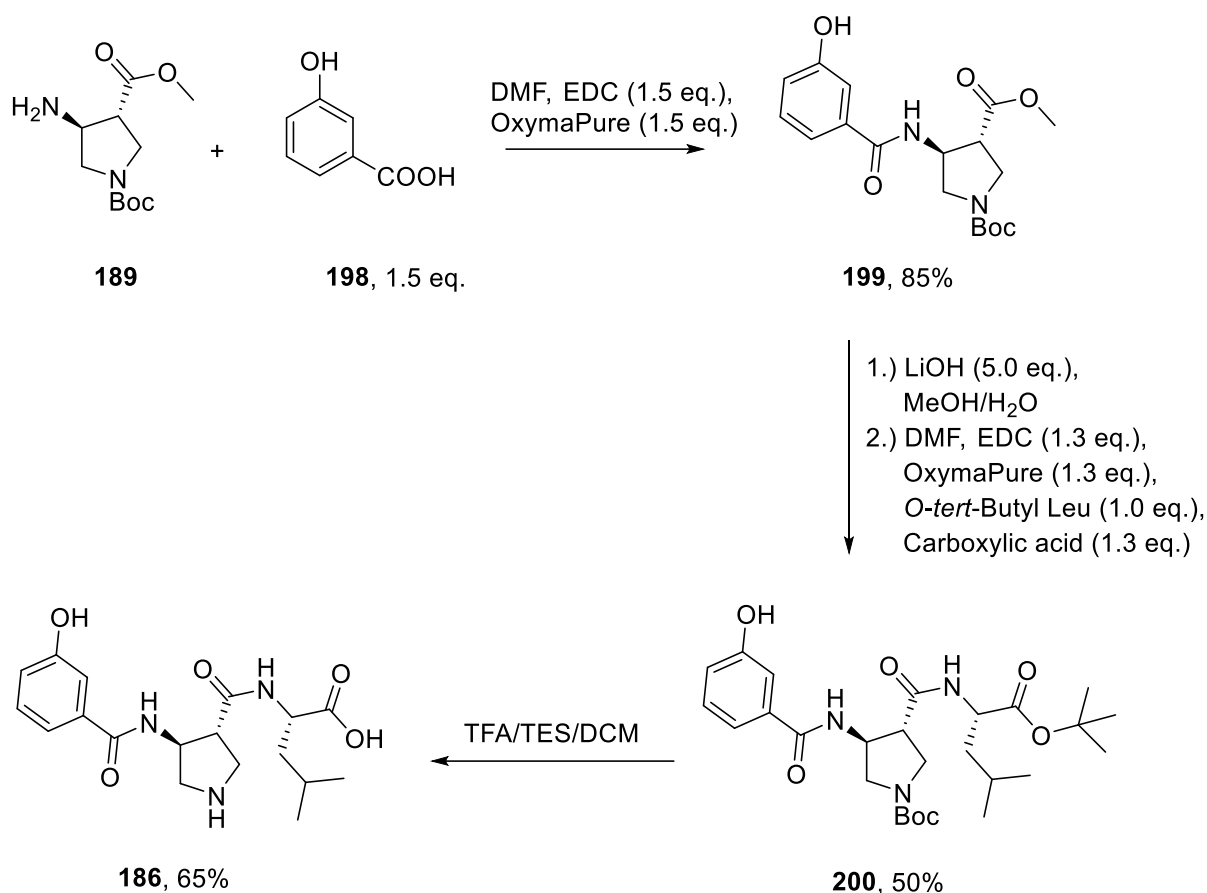
**Scheme 40:** Synthesis of chiral aminopyrrolidine building block **189** to be utilised in the synthesis of docking hits **185-188** and the X-ray crystal structure of **194**.

With chiral aminopyrrolidine building block **189**, docking hits **185-188** could then be synthesised. Beginning with compound **185**, sulfonamide formation was performed with compound **195** to give compound **196** in 71% yield (Scheme 41). **196** underwent ester hydrolysis with LiOH to give the crude carboxylic acid, which was used to perform an amide coupling with **156b**, EDC and OxymaPure to give **197** in 34% yield. Finally, **197** was treated with TFA deprotection conditions to give compound **185** in 49% yield.



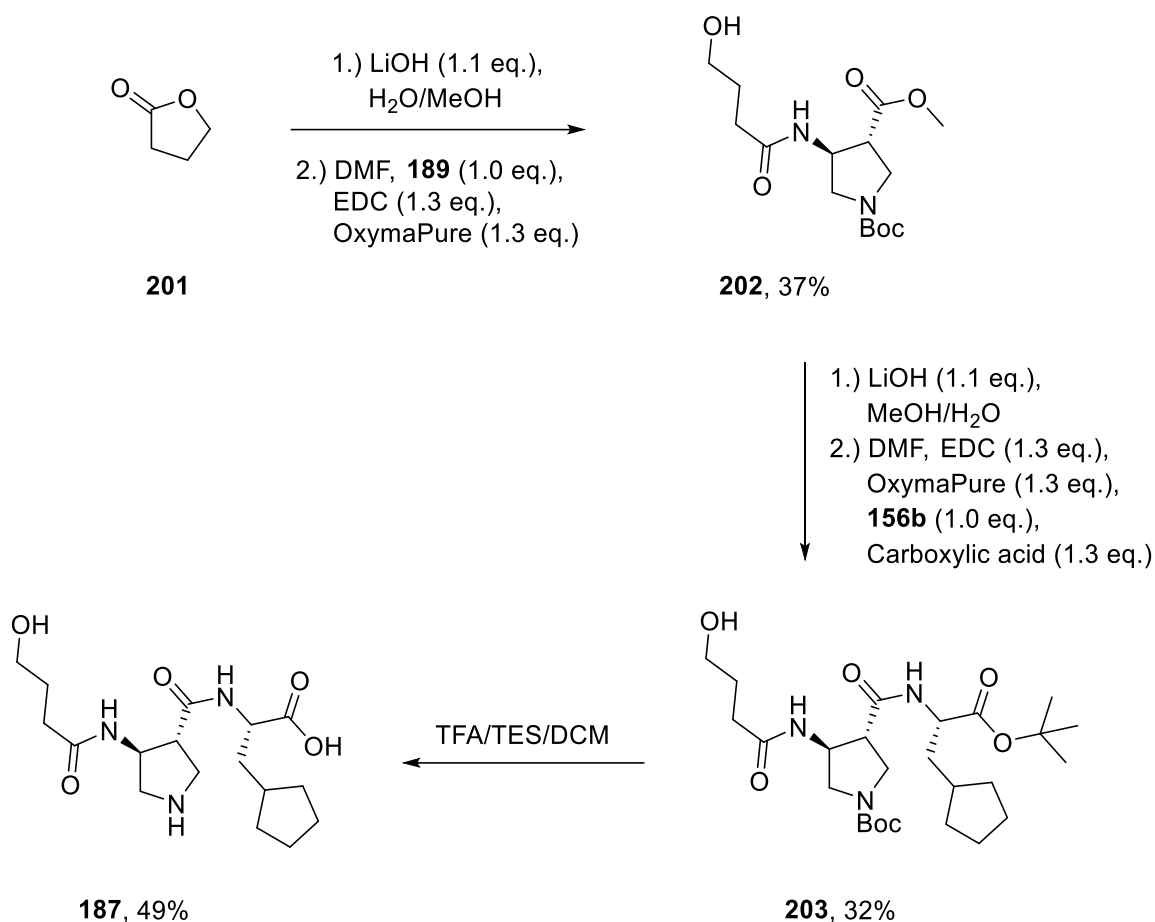
**Scheme 41:** Synthesis of docking hit **185** from chiral aminopyrrolidine **189**.

Compound **186** was synthesised beginning with an amide coupling with **189**, **198**, EDC, OxymaPure to give **199** in 85% yield. **199** underwent ester hydrolysis with LiOH to give the crude carboxylic acid, which was used to perform an amide coupling with *O*-*tert*-butyl Leu, EDC and OxymaPure to give compound **200** in 50% yield. Finally, **200** was treated with TFA deprotection conditions to give compound **186** in 65% yield.



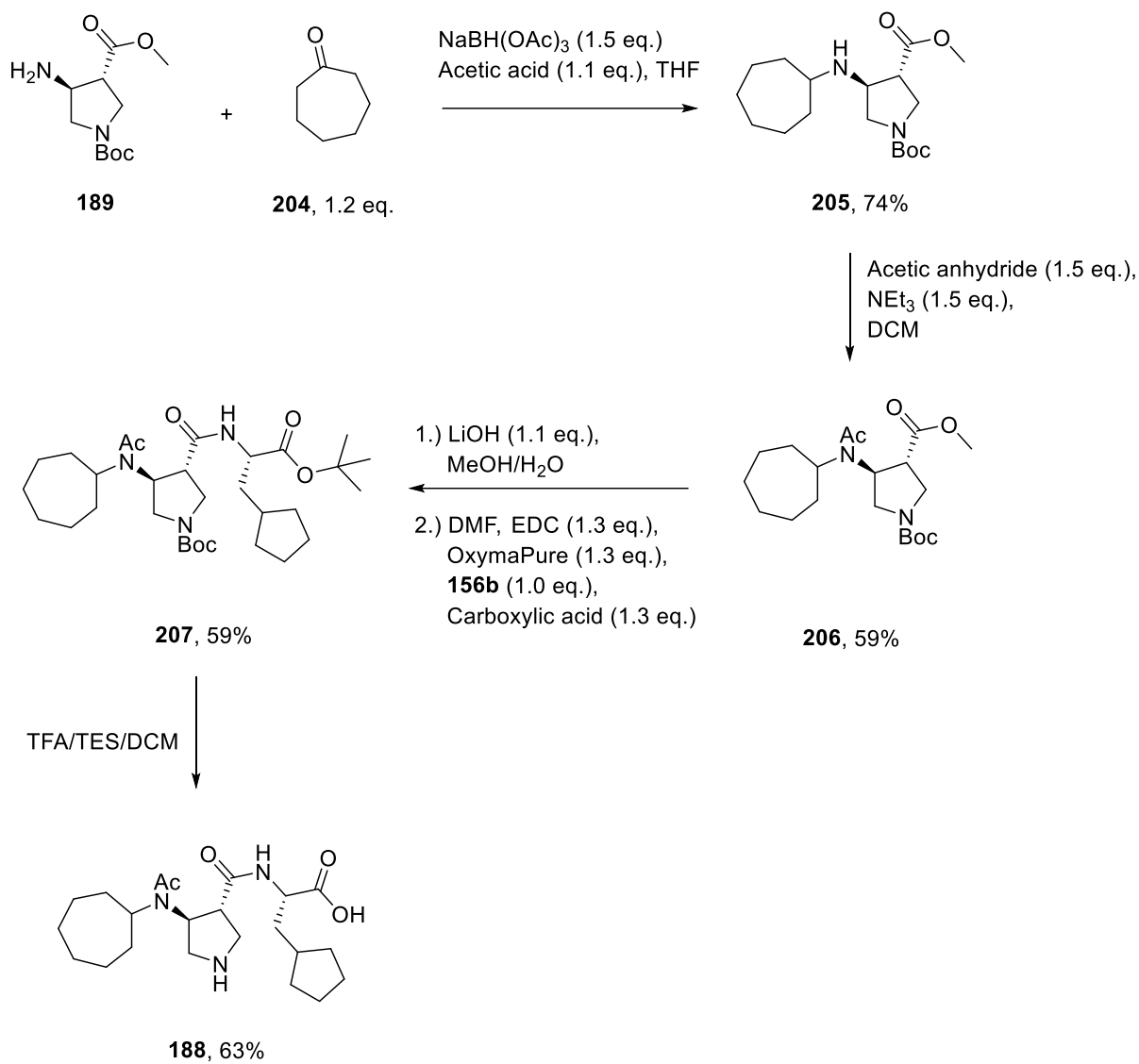
**Scheme 42:** Synthesis of docking hit **186** from chiral aminopyrrolidine **184**.

Compound **187** was synthesised starting with ring opening of  $\gamma$ -Butyrolactone **201** under basic conditions to give the crude carboxylic acid. An amide coupling was then performed with the crude carboxylic acid, EDC, OxymaPure and chiral aminopyrrolidine **189** to give **202** in 37% yield. **202** then underwent ester hydrolysis with LiOH to give the crude carboxylic acid, which was then used to perform an amide coupling with **156b**, EDC and OxymaPure to give **203** in 32% yield. Finally, **203** was treated with TFA deprotection conditions to give **187** in 49% yield.



**Scheme 43:** Synthesis of docking hit **187** from chiral aminopyrrolidine **189**.

Compound **188** was synthesised starting with reductive amination of compound **91** with cycloheptanone **204** and NaBH(OAc)<sub>3</sub> with acetic acid to give **205** in 74% yield. Compound **205** was then acetylated with acetic anhydride and triethylamine to give **206** in 59% yield. **206** then underwent ester hydrolysis with LiOH to give the crude carboxylic acid, which was used to perform an amide coupling with **156b**, EDC and OxymaPure to give **207** in 59% yield. Finally, **207** was treated with TFA deprotection conditions to give **188** in 63% yield.

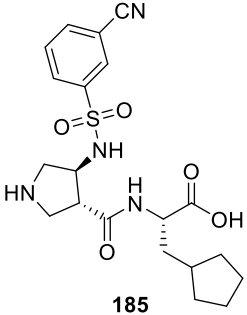
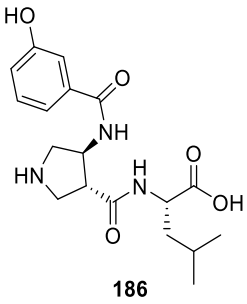
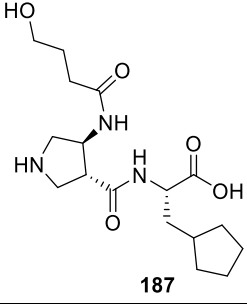
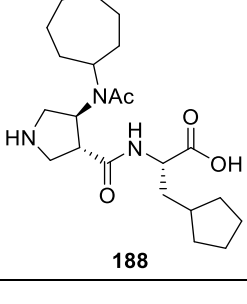


**Scheme 44:** Synthesis of docking hit **188** from chiral aminopyrrolidine **189**.

#### 4.6.4 Screening of Docking Hits

Compounds **185-188** were assessed in dose-response as in Section 4.4. Compound **185** was screened at 1 mM initial concentration, **187** at 5 mM initial concentration and **186** and **188** at 10 mM initial concentration.

**Table 8:** Summary of screening results for docking hits **185-188**.

Compound	IC <sub>50</sub> (mM)	K <sub>i</sub> (μM)
 <p><b>185</b></p>	No Activity	No Activity
 <p><b>186</b></p>	1.8 ± 0.2	17.8 ± 1.5
 <p><b>187</b></p>	1.3 ± 0.2	13.5 ± 1.4
 <p><b>188</b></p>	6.0 ± 1.9	62.2 ± 13.0

Compound **185** appears to be inactive at the chosen screening concentration, this could be due to poor solubility. Compounds **186** has an  $IC_{50} = 1.50 \pm 0.1$  mM and  $K_i = 17.8 \pm 1.5$   $\mu$ M and compound **187** has an  $IC_{50} = 1.47 \pm 0.2$  mM and a  $K_i = 13.5 \pm 1.4$   $\mu$ M which are very similar to hit compound **185**, whose  $IC_{50} = 1.5 \pm 0.1$  mM and  $K_i = 14.8 \pm 1.3$   $\mu$ M, this suggests that substitution at the chosen position is tolerated, but the changes made have not gained any significant additional interactions. Inclusion of the cycloheptyl ring in compound **188** resulted in a deleterious effect, retaining some activity, but yielding a 4-fold decrease in activity over hit compound **185**.

#### 4.7 Summary and Conclusion

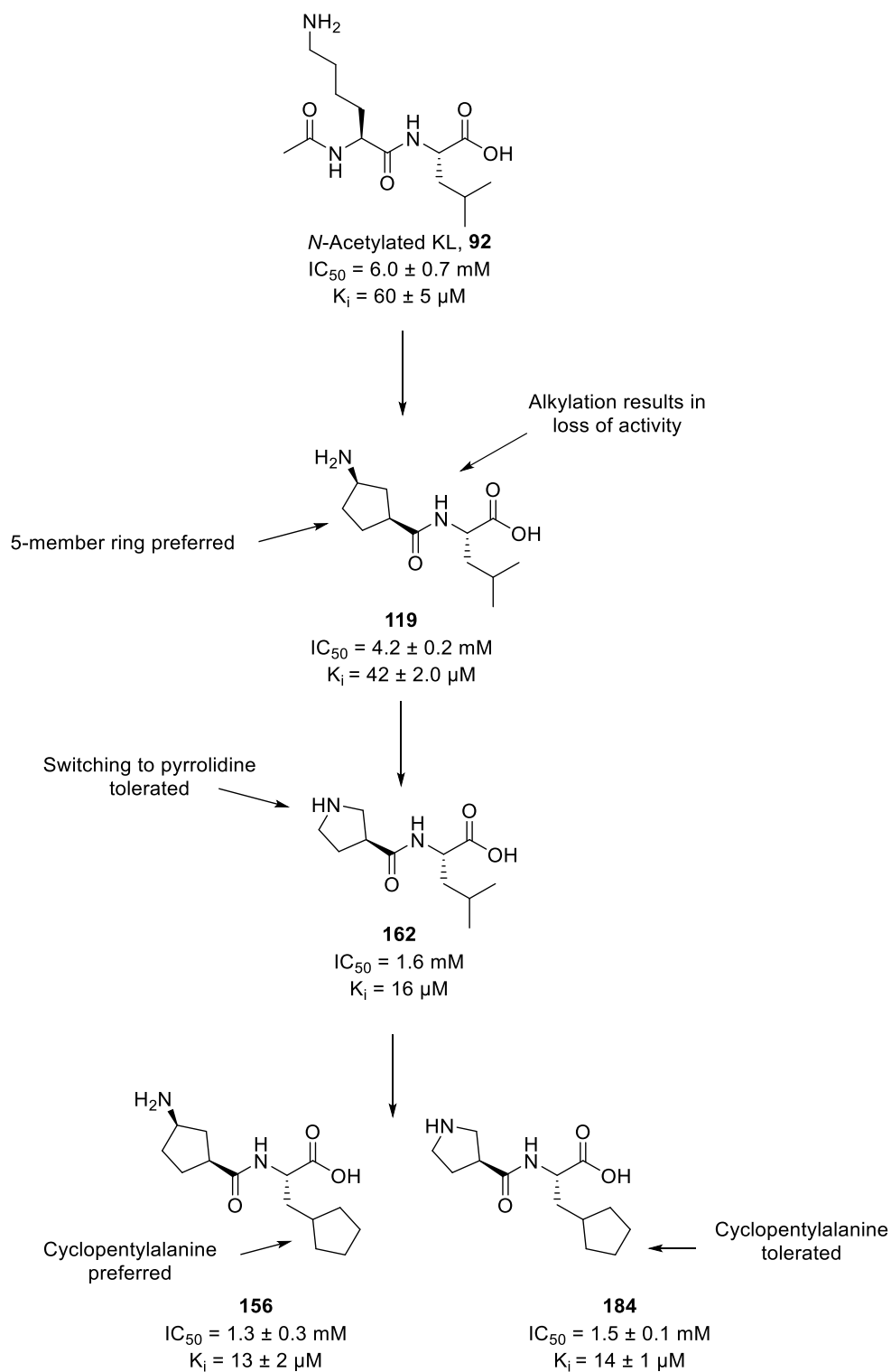
Initially, a set of compounds aiming to mimic *N*-Acetylated KL were synthesised via a combination of solid-phase and solution-phase synthesis, giving 34 compounds for testing against PEX5. The screening results revealed compound **119** and **121** as hit compounds, both demonstrating more activity than KL.

SAR studies on the sulfonamide **121** proved fruitless, with no gains in activity observed. As a result, the sulfonamide line of compounds was halted at this stage. SAR was then performed on the *C*-terminal amino acid of compound **119**, revealing an increase in activity when cyclopentylalanine is used in place of Leu as in compound **156**. SAR of the left-hand side of **119** led to the discovery of compound **162** with 2-fold increased activity over **119**. Further extensive SAR on the left-hand side of compound **119** investigating ring size, position of amine, substitution, conformational restriction and stereochemistry proved fruitless. Combining the features which led to increases in activity in compounds **156** and **162** led to the discovery of **184** with comparable activity to that of **162** (Scheme 45).

In an attempt to increase activity, docking studies were performed on compound **184** which showed it may be amenable to substitution at the 4-position on the pyrrolidine ring. A library of compounds were generated based on conventional chemistries and docked into the crystal structure of PEX5 bound to YQSKL. Several promising compounds (**185-188**) were chosen for synthesis. Screening revealed no increase in activity, however the changes employed in compounds **186** and **187** led to no significant changes in activity, which may indicate that substitution



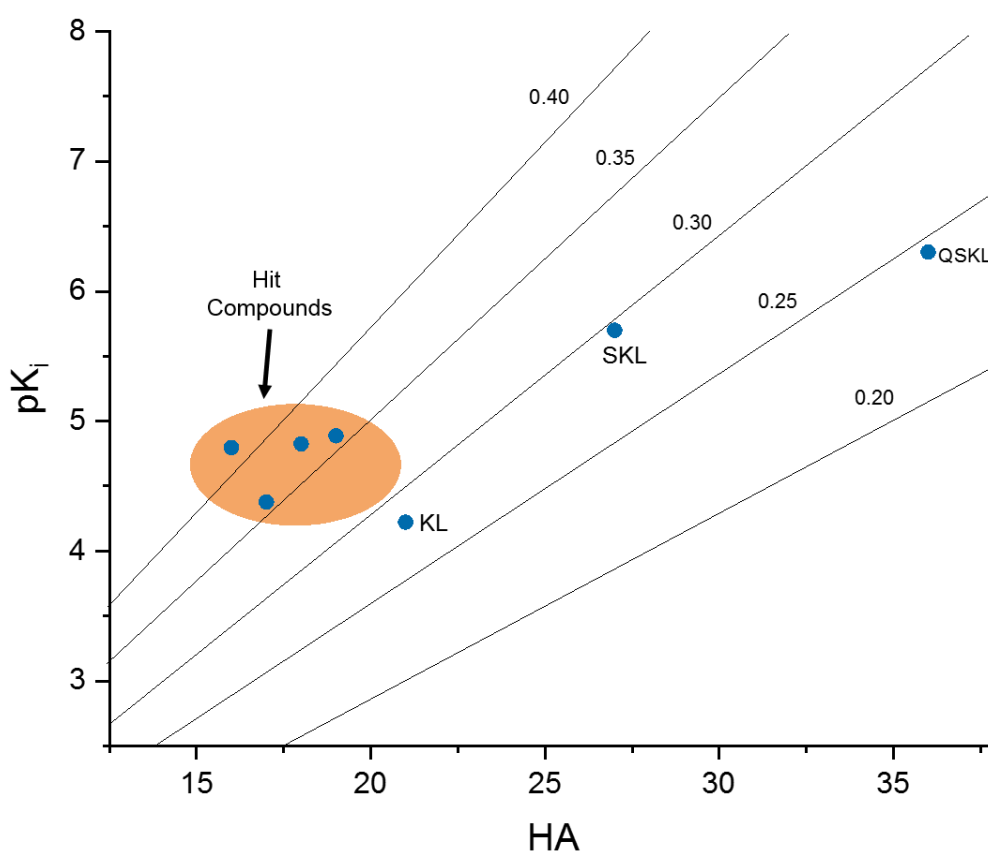
at the position is tolerated, but that not new interactions are being formed, whereas **188** had a deleterious effect on activity.



**Scheme 45:** Summary of initial SAR performed on hit compounds.

A useful metric in analysing the success of the approach taken is ligand efficiency. The ligand efficiency of a compound expresses the binding energy of a compound normalised against its size, giving a measurement of efficiency. The ligand efficiency for small *N*-acetylated peptides KL, SKL and QSKL as well as key compounds **119**, **156**, **162** and **184** were calculated (Equation 2) where LE = Ligand Efficiency and HA = Heavy Atoms.

Compound	$K_i$ ( $\mu\text{M}$ )	HA	LE
<b>119</b>	42	17	0.36
<b>156</b>	13	19	0.36
<b>162</b>	16	16	0.42
<b>184</b>	15	18	0.38
KL	60	21	0.28
SKL	2	27	0.30
QSKL	0.5	36	0.25



**Figure 63:** Table of  $K_i$ , HA and LE for small peptides and key compounds (above). Plot of HA vs.  $pK_i$  (below). The ligand efficiencies are marked on the graph, the hit compounds are all more ligand efficient than the peptides.

Through analysis of the calculated ligand efficiencies, it can be seen that synthesised compounds **119**, **156**, **162** and **184** are all more ligand efficient than the starting point, KL, as well as both SKL and QSKL. This suggests that the hits discovered are more efficient ligands relative to their mass than the peptides we are aiming to mimic. Ligand efficiency is also being maintained or improved on from initial hit **119** to subsequent hits **156**, **162**, **184**, as relatively subtle changes are being performed which are not leading to a significant change in size, while increasing the activity towards PEX5.

The compounds discovered are good fragment hits, with the heaviest being **162** with  $M_w = 268$  Da. In order to increase activity further, another round of design and synthesis would be required. To design further compounds, it would be useful to obtain a crystal structure of compound **184** bound to PEX5, to confirm position and orientation of ligand binding, which would give greater confidence that the designed compounds would show increased activity.

Validation of hit compound binding could be executed *via*  $^1\text{H}$ - $^{15}\text{N}$  HSQC NMR studies. Chemical shift perturbations in the PTS1 binding site can be used to validate binding.

## 4.8 Thesis Conclusion

Protein-protein interactions are fundamental to biology and the development of disease. Despite their importance, there are relatively few drugs on the market that target PPIs. This lack of representation of PPI inhibitors can be attributed to the challenging nature of PPI inhibition. Their binding sites are often large and, poorly defined and are featureless. The lack of distinct binding pockets means that inhibitors have to be very well designed to be sufficiently potent, and often results in compounds that are particularly large and have low ligand efficiency, leading to issues with solubility and bioavailability.

For Mcl-1, ADS enabled the discovery of compounds that were more active than the starting diazos and co-substrates. The viability of the approach against Mcl-1 was demonstrated. However, the approach probably did not enable optimal fragment linking. For PEX5, the underpinning chemistry was not productive. In both of these cases, ADS might be more successful with different underpinning chemistry, enabling fragment-linking in the case of Mcl-1 and productive intermolecular reactions for PEX5.

A conventional medicinal chemistry approach was more successful for PEX5. It was possible to identify ligand-efficient PPI inhibitors. The discovered hit compounds are currently good fragment starting points, and the identification of productive vector(s) for growth would be necessary to identify high-quality inhibitors of this PPI.

## 5 Experimental

### 5.1 General Information

Commercially available starting materials were obtained from Sigma–Aldrich, Fluorochem and Alfa Aesar. All non-aqueous reactions were performed under nitrogen atmosphere unless otherwise stated. Water-sensitive reactions were performed in anhydrous solvents in oven-dried glassware cooled under nitrogen before use. Anhydrous dichloromethane (DCM), anhydrous tetrahydrofuran (THF), anhydrous toluene, anhydrous diethyl ether, anhydrous ethanol, anhydrous methanol and anhydrous acetonitrile were obtained from a PureSolv MD5 Purification System. All other solvents used were of chromatography or analytical grade. An IKA RV 10 rotary evaporator was used to remove the solvents under reduced pressure.

Thin layer chromatography (TLC) was performed using aluminium backed silica (Merck silica gel 60 F254) plates obtained from Merck. Ultraviolet lamp ( $\lambda_{\text{max}} = 254 \text{ nm}$ ) and  $\text{KMnO}_4$  were used for visualisation. Flash column chromatography was performed using silica gel 60 (35-70  $\mu\text{m}$  particles) supplied by Merck.

Analytical LC-MS was performed using an Ultimate3000 HPLC instrument with a UV diode array detector and an MS detector Bruker Amazon Speeds with electrospray ionisation run positive and negative switching mode. The system used a Phenomenex Kinetex C18 2.1  $\times$  50 mm 2.6 micron column and two solvent systems: MeCN/ $\text{H}_2\text{O}$  + 0.1% Formic acid or MeCN/ $\text{H}_2\text{O}$ . Preparative HPLC was performed using a Water (2767) instrument with a Water SQ detector 2. The system used an XBridge C18 19.0  $\times$  100 mm 5 micron OBD column. The general preparation method used a solvent system of MeCN/ $\text{H}_2\text{O}$  (5–95%) + 0.1% Formic acid.

A Bruker MaXis Impact spectrometer with electrospray (ES) ionisation source was used for high-resolution mass spectrometry (HRMS). A Bruker Alpha-P ATR FR-IR spectrometer was used to analyse the infrared spectra.

Proton ( $^1\text{H}$ ) and carbon ( $^{13}\text{C}$ ) NMR data was collected on a Bruker 300 (AV3 NMR spectrometer operating at 7.05 T and equipped with a 5 mm BBO probe), 400 (AV3HD NMR spectrometer operating at 9.4 T and equipped with a 5 mm BBO probe) and 500 (AV-NEO NMR spectrometer operating at 11.7 T and equipped

with a 5 mm DCH cryoprobe) MHz spectrometer. Data was collected at 298 K unless otherwise stated. Chemical shifts ( $\delta$ ) are given in parts per million (ppm) and they are referenced to the residual solvent peak. Coupling constants ( $J$ ) are reported in Hertz (Hz) and splitting patterns are reported in an abbreviated manner: app. (apparent), s (singlet), d (doublet), t (triplet), q (quartet), pent (pentet), sept (septet), m (multiplet), br (broad). Assignments were made using COSY, DEPT, HSQC, HMBC and NOESY experiments.

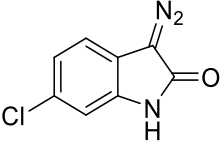
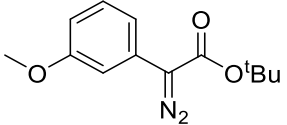
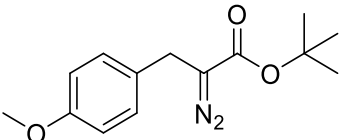
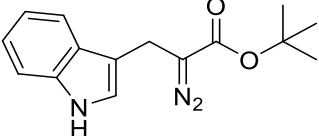
Mass directed auto-purification (MDAP) was performed using an Agilent 1290 Infinity II Preparative HPLC system with mass spectrometer (LC/MSD XT) and fraction collector. The system ran in positive mode with an Agilent Technologies PLRP-S, 300Å, 8  $\mu$ M particle size, 150x25 mM column at ambient temperature with a binary solvent system: MeCN and H<sub>2</sub>O with 0.1% formic acid.

## 5.2 Implementation of Reaction Arrays for Mcl-1

Reaction arrays were performed in 0.75 mL borosilicate glass vials (8 x 30 mm) in 96-vial (12 x 8) array format and sealed with PTFE snap plugs. Diazos, co-substrates and catalyst stock solutions were prepared as shown in Tables 9-13. To each vial, one diazo (8  $\mu$ L of a 1.25 M solution), one co-substrate (8  $\mu$ L of a 6.25 M solution) and one catalyst (4  $\mu$ L of a 25 mM solution) was added Gilson pipettes, made up to 100  $\mu$ L with CH<sub>2</sub>Cl<sub>2</sub> and capped, to give a final reaction concentration of 0.1 M diazo, 0.5 M co-substrate and 1 mM catalyst. The reactions were left at r.t for 48 hr upon which QuadraPure® TU resin (20.0 mg) was added along with 100  $\mu$ L CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixtures were capped and left for 24 hr. Reaction mixtures were filtered, washed with CH<sub>2</sub>Cl<sub>2</sub> (3 x 100  $\mu$ L), CH<sub>2</sub>Cl<sub>2</sub> evaporated open to air followed drying in a dessicator under vacuum for an hr. To each vial, 450  $\mu$ L of CH<sub>2</sub>Cl<sub>2</sub> along with 50  $\mu$ L TFA and 2.4  $\mu$ L triethylsilane was added, the reaction mixtures capped and left overnight, then dried open to air followed by desiccation. Finally, reaction mixtures were dissolved in DMSO (100  $\mu$ L to give a final total product concentration (with respect to diazo) of 100  $\mu$ M. The diazos, co-substrates and catalyst stock solutions were prepared as described and then implemented in the reaction array. Combinations were investigated exhaustively, that is every possible combination of diazo, co-substrate and catalyst was investigated.

### 5.2.1 Round One

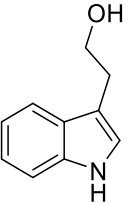
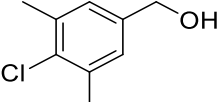
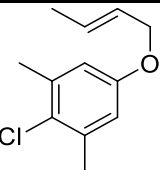
**Table 9:** Diazo stock solutions prepared in CH<sub>2</sub>Cl<sub>2</sub> for round one of ADS targeting Mcl-1.

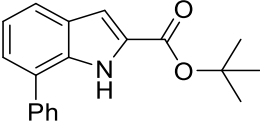
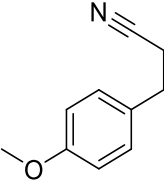
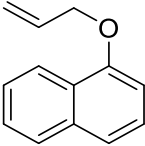
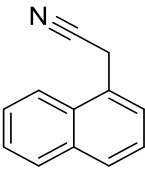
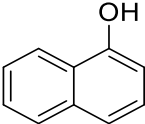
Compound	M <sub>w</sub>	Conc. (M)
	193.0	0.1
	248.1	0.1
	262.1	0.1
	271.1	0.1

**Table 10:** Catalysts stock solutions prepared in THF for round one of ADS targeting Mcl-1.

Catalyst	M <sub>w</sub>	Conc. (mM)
Rh <sub>2</sub> (piv) <sub>4</sub>	610.3	25
Rh <sub>2</sub> (pfb) <sub>4</sub>	1057.9	25
Rh <sub>2</sub> (cap) <sub>4</sub>	654.4	25

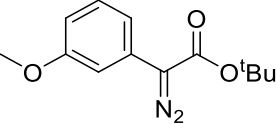
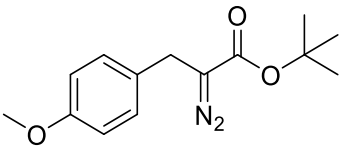
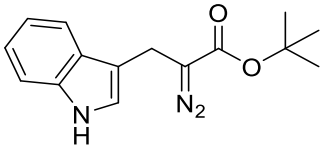
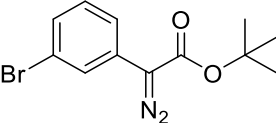
**Table 11:** Co-substrates stock solutions prepared in CH<sub>2</sub>Cl<sub>2</sub> for round one of ADS targeting Mcl-1.

Compound	M <sub>w</sub>	Conc. (M)
	161.1	0.5
	170.1	0.5
	210.1	0.5

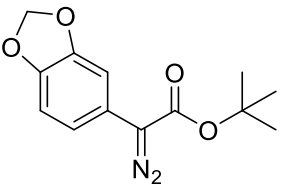
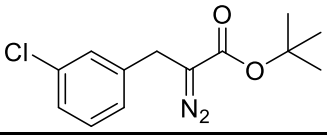
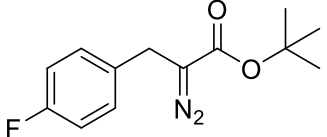
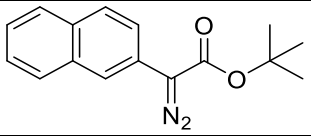
	293.1	0.5
	161.1	0.5
	184.1	0.5
	167.1	0.5
	144.1	0.5

## 5.2.2 Round Two

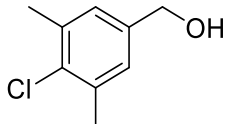
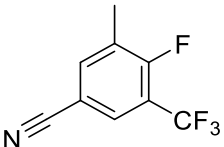
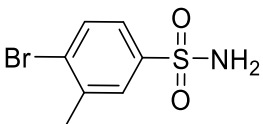
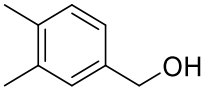
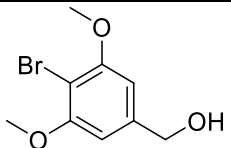
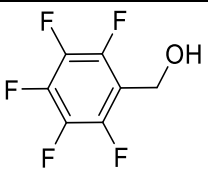
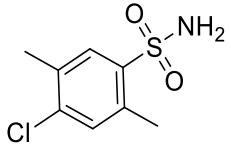
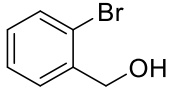
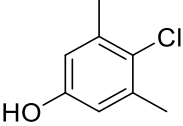
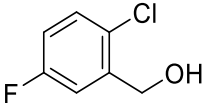
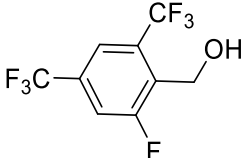
**Table 12:** Diazo stock solutions prepared in CH<sub>2</sub>Cl<sub>2</sub> for round two of ADS targeting Mcl-1.

Compound	M <sub>w</sub>	Conc. (M)
	248.1	0.1
	262.1	0.1
	271.1	0.1
	296.0	0.1



	262.1	0.1
	266.1	0.1
	250.1	0.1
	268.1	0.1

**Table 13:** Co-substrates stock solutions prepared in CH<sub>2</sub>Cl<sub>2</sub> for round two of ADS targeting Mcl-1.

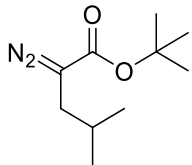
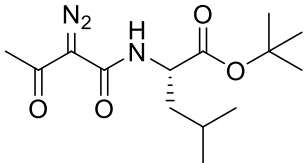
Compound	M <sub>w</sub>	Conc. (M)	Compound	M <sub>w</sub>	Conc. (M)
	170.0	0.5		203.0	0.5
	248.9	0.5		136.0	0.5
	246.0	0.5		198.0	0.5
	219.0	0.5		186.0	0.5
	156.0	0.5		160.0	0.5
	262.0	0.5	-	-	-

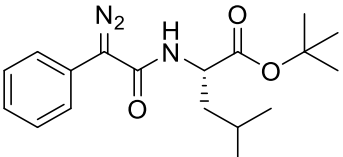
### 5.3 Implementation of Reaction Arrays for PEX5

Reaction arrays were performed in 0.75 mL borosilicate glass vials (8 x 30 mm)) in 96-vial (12 x 8) array format and sealed with PTFE snap plugs. Diazos, co-substrates and catalyst stock solutions were prepared as shown in Tables 14 and 15. To each vial, one diazo (8  $\mu$ L of a 1.25 M solution), one co-substrate (8  $\mu$ L of a 6.25 M solution) and one catalyst (4  $\mu$ L of a 25 mM solution) was added using Gilson pipettes, made up to 100  $\mu$ L with  $\text{CH}_2\text{Cl}_2$  and capped, to give a final reaction concentration of 0.1 M diazo, 0.5 M co-substrate and 1 mM catalyst. The reactions were left at r.t for 48 hr upon which QuadraPure<sup>®</sup> TU resin (20.0 mg) was added along with 100  $\mu$ L  $\text{CH}_2\text{Cl}_2$ . The reaction mixtures were capped and left to scavenge for 24 hr. Reaction mixtures were filtered, washed with  $\text{CH}_2\text{Cl}_2$  (3 x 100  $\mu$ L),  $\text{CH}_2\text{Cl}_2$  evaporated open to air and dried in a dessicator under vacuum for an hr. To each vial, 400  $\mu$ L of  $\text{CH}_2\text{Cl}_2$  along with 100  $\mu$ L TFA and 2.4  $\mu$ L triethylsilane was added, the reaction mixtures capped and left overnight, then dried open to air followed by desiccation. Finally, reaction mixtures were dissolved in DMSO (100)  $\mu$ L to give a final total product concentration (with respect to diazo) of 100  $\mu$ M. The diazos, co-substrates and catalyst stock solutions were prepared as described and then implemented in the reaction array. Combinations were investigated exhaustively, that is every possible combination of diazo, co-substrate and catalyst was investigated.

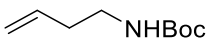
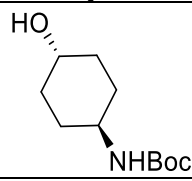
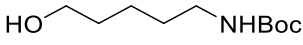
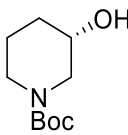
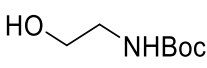
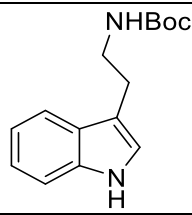
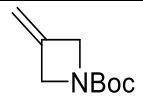
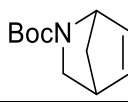
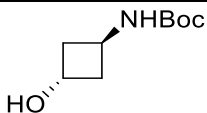
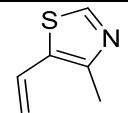
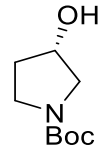
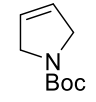
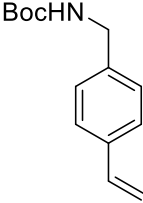
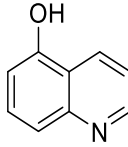
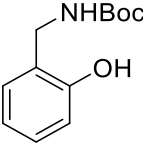
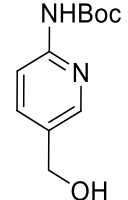
#### 5.3.1 Round One

**Table 14:** Diazo stock solutions prepared in  $\text{CH}_2\text{Cl}_2$  for round one of ADS targeting PEX5.

Compound	M <sub>w</sub>	Conc. (M)
	198.1	0.1
	297.2	0.1

	331.2	0.1
---	-------	-----

**Table 15:** Co-substrate stock solutions prepared in CH<sub>2</sub>Cl<sub>2</sub> for round one of ADS targeting PEX5.

Compound	M <sub>w</sub>	Conc. (M)	Compound	M <sub>w</sub>	Conc. (M)
	171.1	0.5		215.2	0.5
	203.2	0.5		201.1	0.5
	161.1	0.5		260.2	0.5
	161.1	0.5		195.1	0.5
	187.1	0.5		125.0	0.5
	187.1	0.5		169.1	0.5
	233.1	0.5		145.0	0.5
	223.1	0.5		224.1	0.5

Catalyst stock solutions were prepared as in section 5.2.1.

## 5.4 General Procedures

### General Procedure A – Scale-up of Array Reactions

Diazo (400  $\mu$ L, 1.25 M in DCM) was added dropwise over 4 hr to a stirred solution containing co-substrate and catalyst in DCM to give a final diazo concentration (0.1 M), final co-substrate concentration (0.5 M) and final rhodium catalyst concentration (1 mM) with a final volume of 5 mL. After 48 hr, the rhodium catalyst was treated with QuadraPure TU Resin (1.0 g) overnight. The reaction mixture was then filtered and the filtrate concentrated under reduced pressure to give the crude product.

### General Procedure B – Solid-Phase Amide Coupling

DMF (2 mL) was added to H-Leu-2-CITr resin (100 mg, 0.079 mmol/g loading) and left to swell for 1 hr. DMF was drained from the resin which was then washed with 20% piperidine in DMF (2 mL x 2 min x 5) following by DMF (2 mL x 2 min x 5). A solution of carboxylic acid (5.0 eq.), DIC (5.0 eq.) and OxymaPure (5.0 eq.) in DMF (1 mL) was added to the resin which was then agitated overnight. The solution was drained from the resin which was then washed with DMF (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL). A cleavage cocktail of TFA/TIPS/H<sub>2</sub>O (95:5:5, 2 mL) was then added to the resin and agitated for 1 hr. The eluent was collected and concentrated to give a crude product.

### General Procedure C – Solid-Phase Sulfonamide Formation

DMF (2 mL) was added to H-Leu-2-CITr resin (100 mg, 0.079 mmol/g loading) and left to swell for 1 hr. DMF was drained from the resin which was then washed with 20% piperidine in DMF (2 mL x 2 min x 5) following by DMF (2 mL x 2 min x 5). A solution of acid chloride (5.0 eq.) and DIPEA (10.0 eq.) in DMF (1 mL) was added to the resin which was then agitated overnight. The solution was drained from the resin which was then washed with DMF (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL). A cleavage cocktail of TFA/TIPS/H<sub>2</sub>O (95:5:5, 2 mL) was then added to the resin and agitated for 1 hr. The eluent was collected and concentrated to give a crude product.

**General Procedure D** – Reductive Amination followed by Deprotection

Aldehyde (1.5 eq.) was added to a solution of H-Leu-O-*tert*-Butyl HCl (1.0 eq. substrate concentration 0.450 M), NaBH<sub>3</sub>CN (2.0 eq.) and NEt<sub>3</sub> (1.0 eq.) in methanol and left to stir overnight at r.t. The reaction mixture was diluted with ethyl acetate and washed with sat. NaHCO<sub>3</sub> solution, H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. 20% TFA in DCM with triethylsilane (2 eq.) was added to the crude product and left to stir overnight at r.t. The reaction mixture was then concentrated under reduced pressure to give a crude product.

**General Procedure E** - Reductive Amination followed by Acetylation and Deprotection

Aldehyde (1.5 eq.) was added to a solution of H-Leu-O-*tert*-Butyl HCl (1.0 eq. substrate concentration 0.450 M), NaBH<sub>3</sub>CN (2.0 eq.) and NEt<sub>3</sub> (1.0 eq.) in methanol and left to stir overnight at r.t. The reaction mixture was diluted with ethyl acetate and washed with sat. NaHCO<sub>3</sub> solution, H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was dissolved in DCM (0.450 M) with acetic anhydride (1.5 eq.) and NEt<sub>3</sub> (1.5 eq.). The reaction mixture was concentrated under reduced pressure and 20% TFA in DCM with triethylsilane (2 eq.) was added to the crude product and left to stir overnight at r.t. The reaction mixture was then concentrated under reduced pressure to give a crude product.

**General Procedure F** – Solution-phase Amide Coupling

DMF was added to carboxylic acid (1.3 eq.), EDC HCl (1.3 eq.) and OxymaPure (1.3 eq.) and stirred for 10 min at r.t. This solution was then added to amine (1 eq. to give a 0.450M solution) and left to stir overnight at r.t. The reaction mixture was diluted with ethyl acetate and washed with sat. NaHCO<sub>3</sub> solution, H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. 20% TFA in DCM with triethylsilane (2 eq.) was added to the crude product and left to stir overnight at r.t. The reaction mixture was then concentrated under reduced pressure to give a crude product.

**General Procedure G** – Solution-Phase Sulfonamide Formation

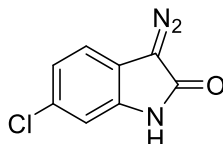
DIPEA (1.5 eq.) was added to a solution of amine (1.0 eq. substrate concentration 0.450 M) and sulfonyl chloride (1.5 eq.) in DMF and left to stir overnight at r.t. The reaction mixture was diluted with ethyl acetate and washed with sat.  $\text{NaHCO}_3$  solution,  $\text{H}_2\text{O}$  and brine, dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give a crude product. 20% TFA in DCM with triethylsilane (2 eq.) was added to the crude product and left to stir overnight at r.t. The reaction mixture was then concentrated under reduced pressure to give a crude product.

**General Procedure H** – Solid-Phase Peptide Synthesis

DMF (2 mL) was added to H-Leu-2-CITr resin (100 mg, 0.079 mmol/g loading) and left to swell for 1 hr. DMF was drained from the resin which was then washed with 20% piperidine in DMF (2 mL x 2 min x 5) following by DMF (2 mL x 2 min x 5). A solution of Fmoc-protected amino acid (5.0 eq.), DIC (5.0 eq.) and OxymaPure (5.0 eq.) in DMF (1 mL) was added to the resin which was then agitated for an hr. The solution was drained from the resin which was then washed with DMF (3 x 2 mL x 2 min), 20% piperidine in DMF (5 x 2 mL x 2 min) and DMF (5 x 2 mL x 2 min). Further amino acid were added onto the peptide using the same procedure. Following the Fmoc removal of the final amino acid residue, a solution of acetic anhydride (5.0 eq.) and DIPEA (10.0 eq.) in DMF (1 mL) was added to the resin which was then agitated for an hr. The solution was drained and washed with DMF (3 x 2 mL), DCM (3 x 2 mL) and MeOH (3 x 2 mL). A cleavage cocktail of TFA/TIPS/ $\text{H}_2\text{O}$  (95:5:5, 2 mL) was then added to the resin and agitated for 1 hr. The eluent was collected and concentrated to give a crude product.

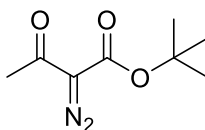
## 5.5 Experimental Data

### 6-Chloro-2-methylidene-3-diazo-1H-indole (**D1**)<sup>111</sup>



To a solution of tosylhydrazine (379 mg, 2.04 mmol) in methanol (9 mL) was added 6-chloroisatin (363 mg, 2.00 mmol) and left to stir at reflux for 1 hr. The precipitate was filtered, washed with methanol (10 mL) and dried in a desiccator under vacuum overnight. The resulting solid was stirred in water (15 mL) with NaOH (127 mg, 3.18 mmol) and left to stir overnight at 50 °C. The reaction mixture was neutralised with dry ice and the precipitate filtered and dried in a desiccator to yield the diazo **D1** (240 mg, 75%) as an orange solid which did not require further purification.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3096, 2083, 1686;  $\delta_{\text{H}}$  (400 MHz, DMSO- $d_6$ ) 10.82 (1H, s, NH), 7.45 (1H, d,  $J$  8.2 Hz, 4-H), 7.05 (1H, dd,  $J$  8.2 and 1.9 Hz, 5-H), 6.92 (1H, d,  $J$  8.2 Hz, 7-H);  $\delta_{\text{C}}$  (125 MHz, MeOD) 168.8 (C-2), 132.5 (C-7a), 131.3 (C-6), 122.4 (C-4), 119.0 (C-5), 115.6 (C-3a), 111.2 (C-7), 88.5 (C-3); HRMS found  $M\text{Na}^+$  215.9929.  $\text{C}_8\text{H}_4\text{ClN}_3\text{O}$  requires  $M\text{Na}$ , 215.9935. Spectroscopic data are consistent with those reported in the literature.<sup>111</sup>

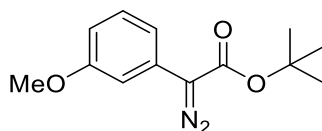
### *tert*-Butyl 2-diazo-3-oxobutanoate (**59**)<sup>69</sup>



To a solution of *tert*-butyl-acetoacetate and trimethylamine (2.52 mL, 18.0 mmol) in acetonitrile (25 mL) was added *p*-ABSA (3.96 g, 16.5 mmol) and left to stir at r.t for 22 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 chloroform–diethyl ether to yield diazo **59** (2.53 g, 92%) as a yellow oil.  $R_f$  0.79 (90:10 chloroform–diethyl ether).  $\nu_{\max}/\text{cm}^{-1}$  (film) 3055, 2984, 2135, 1711, 1685;  $\delta_{\text{H}}$  (400 MHz, MeOD) 2.46 (3H, s, 4- $\text{H}_3$ ), 1.54 (9H, s, *tert*-Butyl);  $\delta_{\text{C}}$  (125 MHz, MeOD) 190.6 (C-3), 160.6 (C-1), 83.2 (*tert*-Butyl C-1), 77.2

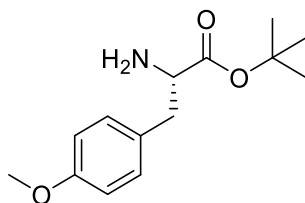
(C-2), 28.3 (*tert*-Butyl C-2), 28.2 (C-4); HRMS found  $MNa^+$  207.0741.  $C_8H_{12}N_2O_3$  requires  $MNa$ , 207.0740. Spectroscopic data are consistent with those reported in the literature.<sup>69</sup>

***tert*-Butyl 2-diazo-2-(3-methoxyphenyl)acetate (D2)**<sup>69</sup>



To a solution of  $Pd(PPh_3)_4$  (43.0 mg, 0.075 mmol, 1 mol%), NaOH (300 mg, 7.5 mmol) and 3-iodoanisole (290  $\mu$ L, 2.50 mmol) in EtOH (10 mL), was added *tert*-butyl 2-diazo-3-oxobutanoate (470 mg, 3.00 mmol) and left to stir at r.t. for 3 hr. The reaction mixture was then passed through a short plug of silica and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography, eluting with 99:1 hexane–EtOAc to yield the diazo **D2** (224 mg, 30%) as an orange oil.  $R_f$  0.37 (99:1 hexane–EtOAc).  $\nu_{max}/cm^{-1}$  (film) 2978, 2936, 2837, 2078, 1695;  $\delta_H$  (400 MHz,  $CDCl_3$ ) 7.23 (1H, app. t,  $J$  8.1 Hz, Phenyl 5-H), 7.12 (1H, app. t,  $J$  2.1 Hz, Phenyl 2-H), 6.94 (1H, ddd,  $J$  7.8, 1.7 and 0.9 Hz, Phenyl 4-H), 6.68 (1H, ddd,  $J$  8.3, 2.5 and 0.7 Hz, Phenyl 6-H), 3.79 (3H, s, OMe), 1.52 (9H, s, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 164.4 (C-1), 160.0 (Phenyl C-3), 129.7 (Phenyl C-5), 127.6 (Phenyl C-6), 116.0 (Phenyl C-1), 111.3 (Phenyl C-4), 109.6 (Phenyl C-2), 82.1 (*tert*-Butyl C-1) 55.3 (OMe), 28.4 (*tert*-Butyl C-2)(Diazo carbon not observed); HRMS found  $MNa^+$  271.1053.  $C_{13}H_{16}N_2O_3$  requires  $MNa$ , 271.1053. Spectroscopic data are consistent with those reported in the literature.<sup>112</sup>

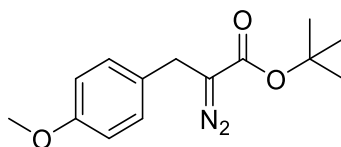
***tert*-Butyl (2S)-2-amino-3-(4-methoxyphenyl)propanoate (61)**<sup>70</sup>





To a solution of 4-methoxy-L-phenylalanine (1.85 g, 10.0 mmol) in *tert*-butyl acetate (25 mL) was added HClO<sub>4</sub> (170 μL, 2.0 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-butyl ester **61** (2.51 g, 74%) as a colourless oil. *R*<sub>f</sub> 0.35 (90:10 DCM–MeOH). δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 7.12 (2H, d, *J* 8.2 Hz, Phenyl 2-H), 6.82 (2H, d, *J* 8.2 Hz, Phenyl 3-H), 3.77 (3H, s, OMe), 3.55 (1H, dd, *J* 7.6 and 5.6 Hz, 2-H), 2.97 (1H, dd, *J* 13.7 and 5.6 Hz, 3-H<sub>a</sub>), 2.77 (1H, dd, *J* 13.7 and 7.6 Hz, 3-H<sub>b</sub>), 1.44 (9H, s, *tert*-Butyl); δ<sub>C</sub> (125 MHz, CDCl<sub>3</sub>) 174.4 (C-1), 158.4 (Phenyl C-4), 130.4 (Phenyl C-3), 129.5 (Phenyl C-2), 113.9 (Phenyl C-1), 81.1 (*tert*-Butyl C-1), 56.4 (C-2), 55.2 (OMe), 40.3 (C-3), 28.0 (*tert*-Butyl C-2); HRMS found MH<sup>+</sup> 252.1594. C<sub>14</sub>H<sub>20</sub>NO<sub>3</sub> requires *MH*, 252.1594. Spectroscopic data are consistent with those reported in the literature.<sup>114</sup>

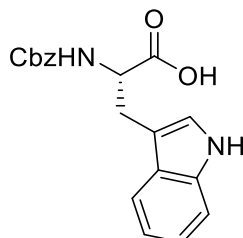
### ***tert*-Butyl 2-diazo-3-(4-methoxyphenyl)propanoate (D3)**<sup>71</sup>



To a solution of *tert*-butyl 2-amino-3-(4-methoxyphenyl)propanoate (502 mg, 2.00 mmol) and acetic acid (11.0 μL, 0.20 mmol) in chloroform (13 mL) was added isoamyl nitrite (320 μL mL, 2.40 mmol) and left to stir at 70 °C for 30 min. The reaction mixture was diluted with ethyl acetate and washed with H<sub>2</sub>O (100 mL), sat. NaHCO<sub>3</sub> solution (100 mL) and brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography, eluting with 99:1 hexane–EtOAc to yield the diazo **D3** (402 mg, 77%) as an orange oil. *R*<sub>f</sub> (50:50 petroleum ether–EtOAc) 0.80. δ<sub>H</sub> (400 MHz, Acetone-d<sub>6</sub>) 7.11 (2H, d, *J* 6.6 Hz, Phenyl 2-H), 6.79 (2H, d, *J* 6.6 Hz, Phenyl 3-H), 3.82 (3H, s, OMe), 3.68 (2H, s, 3-H<sub>2</sub>), 1.40 (9H, s, *tert*-Butyl); δ<sub>C</sub> (125 MHz, CDCl<sub>3</sub>) 158.6 (C-1), 131.8 (Phenyl C-4), 129.5 (Phenyl C-1), 114.1 (Phenyl C-2), 113.3 (Phenyl C-3), 81.3 (*tert*-Butyl C-1), 55.2 (OMe), 28.4 (*tert*-Butyl C-2), 28.1 (C-3)(Diazo carbon not observed); HRMS found MNa<sup>+</sup>

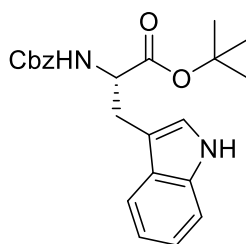
285.1209.  $C_{14}H_{18}N_2O_3$  requires *MNa*, 285.1210. Spectroscopic data are consistent with those reported in the literature.<sup>71</sup>

**(2S)-2-[[ (Benzyloxy)carbonyl]amino]-3-(1H-indol-3-yl)propanoic acid (63)**<sup>72</sup>



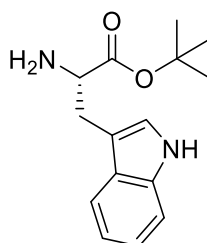
To a solution of L-tryptophan (1.02 g, 5.00 mmol) and  $K_2CO_3$  (2.08 g, 15.0 mmol) in water (10 mL) was added benzyl chloroformate (1.06 mL, 7.50 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was acidified to pH 1 with HCl (1M) and extracted with EtOAc (3 × 50 mL), washed with brine (3 × 50 mL), dried over  $MgSO_4$  and concentrated under reduced pressure to give a crude product. The crude product was triturated with petroleum ether (10 mL) and DCM (2 mL), then cooled to  $-30\text{ }^\circ\text{C}$ , filtered and dried in a desiccator to yield the Cbz protected derivative **63** (1.04 g, 62%) as a colourless solid.  $R_f$  0.45 (95:4:1 DCM–MeOH–AcOH).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 8.05 (1H, s, Indolyl 1-H), 7.61-7.56 (1H, m, Ar-H), 7.43-7.28 (6H, m, Ar-H), 7.23-7.18 (1H, m, Ar-H), 7.13-7.07 (1H, m, Ar-H), 6.98-6.94 (1H, m, Ar-H), 5.35-5.30 (1H, m, 2-H), 5.19-5.03 (2H, m,  $CH_2$  Benzyl), 3.42-3.30 (2H, m, 3- $H_a$  and 3- $H_b$ );  $\delta_C$  (125 MHz,  $CDCl_3$ ) 175.3 (C=O), 156.0 (C=O), 136.1 (Ar-C), 128.6 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 127.7 (Ar-C), 127.0 (Ar-C), 123.0 (Ar-C), 122.2 (Ar-C), 119.9 (Ar-C), 118.6 (Ar-C), 111.3 (Ar-C), 109.6 (Ar-C), 54.4 (C-2), 27.6 (C-3); HRMS found  $MNa^+$  361.1159.  $C_{19}H_{18}N_2O_4$  requires *MNa*, 361.1159. Spectroscopic data are consistent with those reported in the literature.<sup>115</sup>

***tert*-Butyl (2S)-2-[[[(benzyloxy)carbonyl]amino]-3-(1H-indol-3-yl)propanoate (64)<sup>116</sup>**



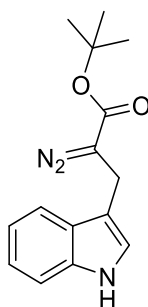
To a solution of  $K_2CO_3$  (1.33 g, 9.59 mmol) and (2S)-2-[[[(benzyloxy)carbonyl]amino]-3-(1H-indol-3-yl)propanoic acid (500 mg, 1.48 mmol) in acetonitrile (5 mL) was added tetraethylammonium chloride (337 mg, 1.48 mmol) and stirred at 50 °C for 2 hr. *tert*-Butyl bromide (1.69 mL, 15.1 mmol) was then added and the reaction left to stir for at 50 °C for 18 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 50:50 hexane–EtOAc to yield the *tert*-butyl ester **64** (183 mg, 31%) as a yellow oil.  $R_f$  (50:50 hexane–EtOAc) 0.47.  $\delta_H$  (500 MHz,  $CDCl_3$ ) 8.08 (1H, s, 1-H Indolyl), 7.62–7.58 (1H, m, Ar-H), 7.40–7.23 (6H, m, Ar-H), 7.21–7.16 (1H, m, Ar-H), 7.13–7.07 (1H, m, Ar-H), 7.00–6.96 (1H, m, Ar-H), 5.45–5.40 (1H, m, 3- $H_a$ ), 5.15–5.05 (1H, m, 3- $H_b$ ), 4.65–4.59 (1H, m, 2-H), 3.35–3.20 (2H, m,  $CH_2$  Benzylic), 1.40 (9H, s, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 171.1 (C-1), 155.8 (C=O), 136.1 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 128.1 (Ar-C), 127.7 (Ar-C), 127.0 (Ar-C), 122.7 (Ar-C), 122.2 (Ar-C), 119.6 (Ar-C), 118.9 (Ar-C), 111.1 (Ar-C), 110.4 (Ar-C), 82.1 (*tert*-Butyl C-1), 55.0 (C-2), 28.0 (C-3), 27.9 (*tert*-Butyl C-2); HRMS found  $MH^+$  395.1965.  $C_{23}H_{26}N_2O_4$  requires  $MH$ , 395.1965.

***tert*-Butyl (2S)-2-amino-3-(1H-indol-3-yl)propanoate (65)<sup>117</sup>**



To a solution of of *tert*-butyl 2-[[[(benzoyloxy)carbonyl]amino]-3-(1H-indol-3-yl)propanoate, Pd/C (185 mg, 0.25 mmol, 10 mol%) in ethanol (25 mL) was added ammonium formate (1.59 g, 25.0 mmol) and stirred at reflux for 3 hr. The reaction mixture was filtered through celite and washed with methanol (2 x 50 mL). The organic extract was washed with a sat. Na<sub>2</sub>CO<sub>3</sub> solution (50 mL), brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 99:1 DCM–methanol (sat. with NH<sub>3</sub>) to yield the amino ester **65** as a colourless oil. *R*<sub>f</sub> 0.20 (99:1 DCM–methanol (sat. with NH<sub>3</sub>)). δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 8.22 (1H, s, Indolyl 1-H), 7.65 (1H, br. d, *J* 7.8 Hz, Indolyl 7-H), 7.38-7.33 (1H, m, Indolyl 4-H), 7.24-7.17 (1H, m, Indolyl 5-H), 7.13 (1H, ddd, *J* 8.0, 7.1 and 1.0 Hz, Indolyl 6-H), 7.06 (1H, d, *J* 2.2 Hz, Indolyl 2-H), 3.73 (1H, dd, *J* 8.0 and 5.0 Hz, 3-H<sub>a</sub>), 3.26 (1H, ddd, *J* 14.5, 5.0 and 0.9 Hz, 2-H), 2.99 (1H, dd, *J* 14.5 and 8.0 Hz, 3-H<sub>b</sub>), 1.44 (9H, s, *tert*-Butyl); δ<sub>C</sub> (125 MHz, MeOD) 174.6 (C-1), 136.3 (Indolyl C-7a), 127.6 (Indolyl C-3a), 122.8 (Indolyl C-2), 122.1 (Indolyl C-6), 119.4 (Indolyl C-5), 119.0 (Indolyl C-4), 111.8 (Indolyl C-3), 111.1 (Indolyl C-7), 81.0 (*tert*-Butyl C-1), 55.6 (C-1), 30.9 (C-2), 28.0 (*tert*-Butyl C-2); HRMS found MH<sup>+</sup> 261.1594. C<sub>15</sub>H<sub>19</sub>N<sub>2</sub>O<sub>2</sub> requires *MH*, 261.1598.

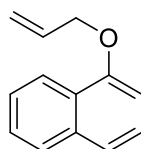
***tert*-Butyl 2-diazo-3-(1H-indol-3-yl)propanoate (D4)**<sup>71</sup>



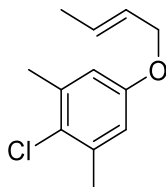
To a solution of *tert*-butyl 2-amino-3-(1H-indol-3-yl)propanoate (790 mg, 3.24 mmol) and acetic acid (19 μL, 0.32 mmol) in chloroform (15 mL) was added isoamyl nitrite (0.52 mL, 3.89 mmol) and left to stir at 70 °C for 30 min. The reaction mixture was diluted with ethyl acetate and washed with H<sub>2</sub>O (100 mL), sat. NaHCO<sub>3</sub> solution (100 mL) and brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 70:30 hexane–EtOAc to

yield the *diazo* **D4** (210 mg, 24%) as a yellow oil.  $R_f$  (70:30 hexane–EtOAc) 0.80.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3408, 3206, 3122, 3059, 2977, 2932, 2078, 1662;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 8.03 (1H, s, Indolyl 1-H), 7.61 (1H, app. d,  $J$  7.9 Hz, Indolyl 7-H), 7.37 (1H, app. d,  $J$  7.9 Hz, Indolyl 4-H), 7.22 (1H, app. t,  $J$  7.9 Hz, Indolyl 5-H), 7.08 (1H, app. t,  $J$  7.9 Hz, Indolyl 6-H), 7.08 (1H, d,  $J$  2.4 Hz, Indolyl 2-H), 3.75 (2H, s, 3-H<sub>2</sub>), 1.51 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz, MeOD) 171.2 (C-1), 136.4 (Indolyl C-7a), 126.9 (Indolyl C-7a), 122.5 (Indolyl C-2), 122.4 (Indolyl C-6), 119.7 (Indolyl C-5), 118.9 (Indolyl C-4), 111.6 (Indolyl C-3), 111.2 (Indolyl C-7), 81.2 (*tert*-Butyl C-1), 60.4 (C-3), 28.4 (*tert*-Butyl C-1) (Diazo carbon not observed) HRMS found  $\text{MNa}^+$  294.1205.  $\text{C}_{15}\text{H}_{17}\text{N}_3\text{O}_2$  requires  $\text{MNa}$ , 294.1213.

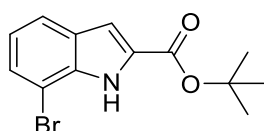
### 1-(Prop-2-en-1-yloxy)naphthalene (**Co2**)<sup>75</sup>



To a solution of  $\text{K}_2\text{CO}_3$  (575 mg, 4.16 mmol) in acetone (6 mL) was added 1-naphthol (300 mg, 2.08 mmol) and left to stir at r.t for 30 min. Allyl bromide (22.0  $\mu\text{L}$ , 2.50 mmol) was then added left to stir at 60 °C for 18 hr. The reaction mixture was concentrated under reduced pressure to give a crude material. The crude material was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the ether **Co2** (347 mg, 91%) as a colourless oil.  $R_f$  0.66 (90:10 hexane–EtOAc).  $\nu_{\max}/\text{cm}^{-1}$  (film) 3503, 1595;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 8.42–8.34 (1H, m, Ar-H), 7.89–7.82 (1H, m, Ar-H), 7.56–7.52 (2H, m, Ar-H), 7.51–7.46 (1H, m, Ar-H), 7.44–7.39 (1H, m, Ar-H), 6.88–6.84 (1H, m, Ar-H), 6.19 (1H, ddt,  $J$  17.3, 10.6 and 5.1 Hz, Propenyl 2-H), 5.54 (1H, dd,  $J$  17.3 and 1.7 Hz, Propenyl 3-H<sub>trans</sub>), 5.35 (1H, dd,  $J$  10.6 and 1.7 Hz, Propenyl 3-H<sub>cis</sub>), 4.73 (2H, d,  $J$  5.1 Hz, Propenyl 1-H<sub>2</sub>);  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 154.3 (Ar-C), 134.6 (Ar-C), 133.6 (Propenyl C-2), 127.5 (Ar-C), 126.4 (Ar-C), 125.8 (Ar-C), 125.8 (Ar-C), 125.2 (Ar-C), 122.1 (Ar-C), 120.4 (Ar-C), 117.4 (Propenyl C-3), 105.1 (Ar-C), 70.3 (Propenyl C-1); HRMS found  $\text{MH}^+$  185.0934.  $\text{C}_{13}\text{H}_{12}\text{O}$  requires,  $\text{MH}$  185.0934. Spectroscopic data are consistent with those reported in the literature.<sup>75</sup>

**5-[(2E)-But-2-en-1-yloxy]-2-chloro-1,3-dimethylbenzene (Co4)**<sup>75</sup>

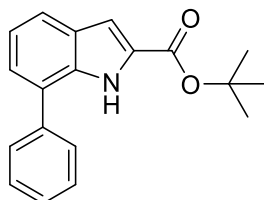
To a solution of  $K_2CO_3$  (554 mg, 4.00 mmol) in acetone (6 mL) was added 4-chloro-3,5-dimethyl phenol (313 mg, 2.00 mmol) and left to stir for at r.t for 30 min. Crotyl bromide (250  $\mu$ L, 2.40 mmol) was then added and left to stir at 60  $^{\circ}C$  for 18 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the *ether* **Co4** (241 mg, 58%, 85:15 *E/Z*) as a colourless oil.  $R_f$  0.50 (90:10 hexane–EtOAc).  $\nu_{max}/cm^{-1}$  (film) 2917, 2858, 1676;  $\delta_H$  (400 MHz,  $CDCl_3$ , Major isomer reported) 6.64 (2H, s, 4-H and 6-H), 5.90-5.80 (1H, m, Butenyl 3- $H_{trans}$ ) 5.78-5.65 (1H, m, Butenyl 2-H), 4.39 (2H, d,  $J$  6.0 Hz, Butenyl 1- $H_2$ ), 2.34 (6H, s, Me) 1.75 (3H, dd,  $J$  6.4 and 1.2 Hz, Butenyl 4- $H_3$ );  $\delta_C$  (125 MHz,  $CDCl_3$ ) 156.5 (C-5), 137.0 (C-1), 130.6 (Butenyl C-2), 126.2 (Butenyl C-3), 125.9 (C-2), 114.7 (C-4), 68.8 (Butenyl C-1), 21.0 (Me), 17.8 (Butenyl C-3); HRMS found  $MH^+$  211.0881.  $C_{12}H_{15}ClO$  requires  $MH$ , 211.0884.

***tert*-Butyl 7-bromo-1H-indole-2-carboxylate (69)**<sup>118</sup>

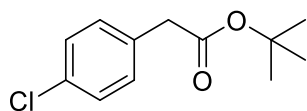
To a solution of 7-bromoindole-2-carboxylic acid (481 mg, 2.00 mmol) in toluene (10 mL) was added *N,N*-Dimethylformamide di-*tert*-butylacetal (1.92 mL, 8.00 mmol) dropwise and stirred at reflux overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield the *tert-butyl ester* **69** (360 mg, 61%) as a colourless oil.  $R_f$  0.63 (99:1 hexane–EtOAc).  $\nu_{max}/cm^{-1}$  (film) 3462, 2977, 2931, 1694;  $\delta_H$  (400 MHz,  $CDCl_3$ ) 8.87 (1H, s, 1-H), 7.61 (1H, app. d,  $J$  8.0 Hz, 6-H), 7.46 (1H, dd,  $J$  7.6 and 0.9 Hz, 4-H), 7.19 (1H, d,  $J$  2.2 Hz, 3-H), 7.03 (1H, app. t,  $J$  7.8 Hz, 5-H), 1.63 (1H,

s, *tert*-Butyl);  $\delta_c$  (125 MHz,  $\text{CDCl}_3$ ) 160.8 (C=O), 135.3 (C-7a), 129.6 (C-6), 128.5 (C-5), 127.2 (C-3a), 121.7 (C-2), 121.6 (C-4), 108.8 (C-3), 105.1 (C-7), 82.2 (*tert*-Butyl C-1), 28.3 (*tert*-Butyl C-2); HRMS found  $\text{MNa}^+$  318.0095.  $\text{C}_{13}\text{H}_{14}\text{BrNO}_2$  requires *MNa*, 318.0100.

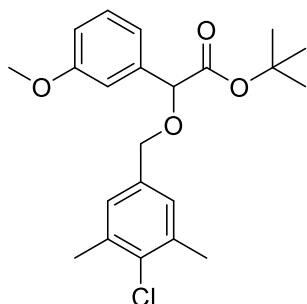
***tert*-Butyl 7-phenyl-1H-indole-2-carboxylate (Co7)**<sup>113</sup>



To a solution of tri-*tert*-butyl-phosphonium tetrafluoroborate (16.0 mg, 0.06 mmol), phenylboronic acid (116 mg, 0.95 mmol),  $\text{Pd}_2[\text{dba}]_3$  (35.0 mg, 0.04 mmol, 0.03 mol%), CsF (280 mg, 1.90 mmol) in THF (6 mL) was added *tert*-butyl 7-bromo-1H-indole-2-carboxylate (330 mg, 1.11 mmol) and left to stir at r.t for 5 hr. The reaction mixture was diluted with ethyl acetate (50 mL), washed with  $\text{H}_2\text{O}$  (50 mL) and brine (50 mL), dried over  $\text{MgSO}_4$  and concentrated to give a crude product. The crude product was purified *via* column chromatography eluting with 99:1 hexane–diethyl ether to yield the *indole* **Co7** (201 mg, 62%) as a colourless solid.  $R_f$  0.08 (99:1 hexane–diethyl ether).  $\nu_{\text{max}}/\text{cm}^{-1}$  (film) 3408, 3226, 3059, 3028, 3001, 2981, 2930, 1685, 1368;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 8.87 (1H, s, 1-H), 7.61-7.58 (1H, m, 6-H), 7.58-7.54 (2H, m, Ar-H) 7.58-7.53 (2H, m, Ar-H), 7.37-7.33 (1H, m, Ar-H), 7.32 (1H, dd,  $J$  7.8 and 0.9 Hz, 4-H), 7.24 (1H, app. t,  $J$  7.8 Hz, 5-H), 7.19 (1H, d,  $J$  2.1 Hz, 3-H), 1.60 (9H, s, *tert*-Butyl);  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 161.3 (C=O), 138.4 (Ar-C), 134.7 (Ar-C), 129.3 (Ar-C), 129.2 (Ar-C), 128.1 (Ar-C), 128.0 (Ar-C), 127.7 (Ar-C), 126.4 (Ar-C), 124.7 (Ar-C), 121.6 (Ar-C), 121.2 (Ar-C), 108.8 (C-3), 81.3 (*tert*-Butyl C-1), 28.3 (*tert*-Butyl C-2); HRMS found  $\text{MNa}^+$  316.1308.  $\text{C}_{19}\text{H}_{19}\text{NO}_2$  requires *MNa*, 316.1313.

***tert*-Butyl 2-(4-chlorophenyl)acetate (71)**<sup>116</sup>

To a solution of 4-chlorophenylacetic acid (340 mg, 2.00 mmol) and  $K_2CO_3$  (1.80 g, 13.0 mmol) in acetonitrile (6.6 mL) was added tetraethylammonium chloride (456 mg, 2.00 mmol) and stirred at 50 °C for 2 hr. *tert*-Butyl bromide (2.20 mL, 20.4 mmol) was then added and the reaction left to stir at 50 °C for 19 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 80:20 petroleum ether–EtOAc to yield the *tert*-butyl ester **71** (453 mg, 49%) as a colourless oil.  $R_f$  (80:20 petroleum ether–EtOAc).  $\delta_H$  (400 MHz,  $CDCl_3$ ) 7.28 (2H, d,  $J$  8.0 Hz, Phenyl 3-H), 7.20 (2H, d,  $J$  8.0 Hz, Phenyl 2-H), 3.48 (2H, s, 2-H<sub>2</sub>), 1.43 (9H, s, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 170.5 (C=O), 133.2 (Phenyl C-2), 132.8 (Phenyl C-1), 130.6 (Phenyl C-2), 128.6 (Phenyl C-3), 81.1 (*tert*-Butyl C-1), 42.0 (C-2), 28.0 (*tert*-Butyl C-2); HRMS found  $MNa^+$  249.0747.  $C_{12}H_{15}ClO_2$  requires  $MNa$ , 249.0761. Spectroscopic data are consistent with those reported in the literature.<sup>119</sup>

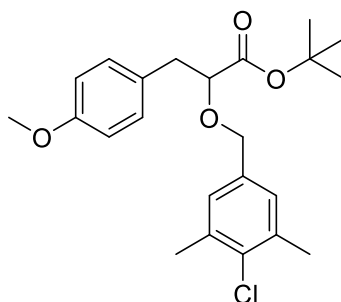
***tert*-Butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-2-(3-methoxyphenyl)acetate (72)**

According to general procedure A, *tert*-butyl 2-diazo-2-(3-methoxyphenyl)acetate (124 mg, 0.50 mmol) and 4-chloro-3,5-dimethyl benzyl alcohol (425 mg, 2.50 mmol) with  $[Rh_2(Piv)_4]$  (3.0 mg, 0.005 mmol) gave a crude material. The crude material was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield the *tert*-butyl acetate **72** (132 mg, 68%) as a colourless oil.  $R_f$  (99:1 hexane–EtOAc) 0.05.  $\delta_H$  (500 MHz,  $CDCl_3$ ) 7.26 (1H, app. t,  $J$  8.0 Hz, Methoxyphenyl 5-H), 7.07 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 7.05–7.00



(2H, m, Methoxyphenyl 2-H and Methoxyphenyl 4-H), 6.87 (1H, ddd,  $J$  8.3, 2.6 and 1.0 Hz, Methoxyphenyl 6-H), 4.75 (1H, s, 2-H), 4.50 (1H, d,  $J$  11.8 Hz, OCH<sub>a</sub>), 4.47 (1H, d,  $J$  11.8 Hz, OCH<sub>b</sub>), 3.80 (3H, s, OMe), 2.36 (6H, s, Me), 1.40 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz, CDCl<sub>3</sub>) 169.9 (C=O), 159.8 (Methoxyphenyl C-3), 138.4 (Methoxyphenyl C-1), 136.4 (4-chloro-3,5-dimethylphenyl C-3), 135.3 (4-chloro-3,5-dimethylphenyl C-1), 134.2 (4-chloro-3,5-dimethylphenyl C-4), 129.6 (Methoxyphenyl C-2), 128.3 (4-chloro-3,5-dimethylphenyl C-2), 119.9 (Methoxyphenyl C-5), 114.5 (Methoxyphenyl C-6), 112.5 (Methoxyphenyl C-4), 82.0 (*tert*-Butyl C-2), 80.2 (C-2), 70.6 (OCH<sub>2</sub>), 55.4 (OMe), 28.1 (*tert*-Butyl C-2), 20.8 (Me). HRMS found  $MNa^+$  413.1495. C<sub>22</sub>H<sub>27</sub>ClO<sub>4</sub> requires  $MNa$ , 413.1490.

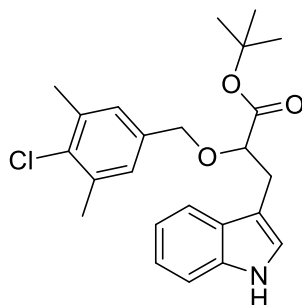
***tert*-Butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-3-(4-methoxyphenyl)propanoate (73)**



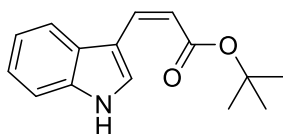
According to general procedure A, *tert*-butyl 2-diazo-2-(3-methoxyphenyl)acetate (131 mg, 0.50 mmol) and 4-chloro-3,5-dimethyl benzyl alcohol (425 mg, 2.50 mmol) with [Rh<sub>2</sub>(Cap)<sub>4</sub>] (3.3 mg, 0.005 mmol) gave a crude material. The crude material was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield *tert*-butyl propanoate **73** (120 mg, 59%) as a colourless oil.  $R_f$  (90:10 hexane–EtOAc) 0.26.  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 7.15 (2H, d,  $J$  8.7 Hz, Methoxyphenyl 2-H), 6.84 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 6.82 (2H, d,  $J$  8.7 Hz, Methoxyphenyl 3-H), 4.58 (1H, d,  $J$  12.0 Hz, OCH<sub>a</sub>), 4.25 (1H, d,  $J$  12.0 Hz, OCH<sub>b</sub>), 3.92 (1H, dd,  $J$  8.6 and 4.8 Hz, 2-H), 3.80 (3H, s, OMe), 2.98 (1H, dd,  $J$  14.0 and 4.8 Hz, 3-H<sub>a</sub>), 2.92 (1H, dd,  $J$  14.0 and 8.6 Hz, 3-H<sub>b</sub>), 2.30 (6H, s, Me), 1.44 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz, CDCl<sub>3</sub>) 171.5 (C=O), 158.5 (Methoxyphenyl C-4), 136.2 (4-chloro-3,5-dimethylphenyl C-4), 135.5 (4-chloro-3,5-dimethylphenyl C-1), 133.9 (4-chloro-3,5-dimethylphenyl C-3), 130.7 (Methoxyphenyl C-2), 129.6 (Methoxyphenyl C-1), 127.9 (4-chloro-3,5-dimethylphenyl C-2), 113.7 (Methoxyphenyl C-3), 81.7 (*tert*-Butyl C-2), 79.9 (C-2), 71.6 (OCH<sub>2</sub>), 55.4 (OMe),

38.6 (C-3), 28.2 (*tert*-Butyl C-2), 20.77 (Me). HRMS found  $MNa^+$  427.1667.  $C_{23}H_{29}ClO_4$  requires  $MNa$ , 427.1647.

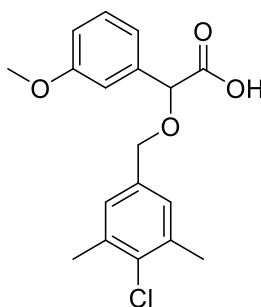
***tert*-Butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-3-(1H-indol-3-yl)propanoate (74)**



According to general procedure A, *tert*-butyl 2-diazo-3-(1H-indol-3-yl)propanoate (136 mg, 0.50 mmol) and 4-chloro-3,5-dimethyl benzyl alcohol (425 mg, 2.50 mmol) with  $[Rh_2(Piv)_4]$  (3.0 mg, 0.005 mmol) gave a crude product. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5→5:95  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *tert*-butyl propanoate **74** (14.0 mg, 3%) as a colourless oil.  $\delta_H$  (500 MHz,  $CDCl_3$ ) 8.02 (1H, s, NH), 7.60 (1H, app. d,  $J$  6.9 Hz, Indolyl 7-H), 7.35 (1H, app. d,  $J$  8.1 Hz, Indolyl 4-H), 7.19 (1H, ddd,  $J$  8.1, 7.1 and 1.1 Hz, Indolyl 6-H), 7.10-7.08 (2H, m, Indolyl 2-H and Indolyl 5-H), 6.89 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 4.59 (1H, d,  $J$  11.8 Hz,  $OCH_a$ ), 4.30 (1H, d,  $J$  11.8 Hz,  $OCH_b$ ), 4.11 (1H, dd,  $J$  8.0 and 5.0 Hz, 2-H), 3.24 (1H, ddd,  $J$  14.8, 5.0 and 0.8 Hz, 3- $H_a$ ), 3.16 (1H, ddd,  $J$  14.8, 8.0 and 0.8 Hz, 3- $H_b$ ), 2.27 (6H, s, Me), 1.40 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz,  $CDCl_3$ ) 171.7 (C=O), 136.1 (Indolyl C-3a), 136.0 (4-chloro-3,5-dimethylphenyl C-4), 135.5 (4-chloro-3,5-dimethylphenyl C-1), 133.8 (4-chloro-3,5-dimethylphenyl C-3), 127.9 (4-chloro-3,5-dimethylphenyl C-2), 127.6 (Indolyl C-7a), 123.1 (Indolyl C-2 or Indolyl C-5), 121.9 (Indolyl C-7), 119.3 (Indolyl C-2 or Indolyl C-5), 119.1 (Indolyl C-6), 111.6 (Indolyl C-3), 111.0 (Indolyl C-4), 81.4 (*tert*-Butyl C-1), 79.1 (C-2), 71.5 ( $OCH_2$ ), 29.0 (C-3), 28.0 (*tert*-Butyl C-2), 20.6 (Me); HRMS found  $MNa^+$  436.1654.  $C_{24}H_{28}ClNO_3$  requires  $MNa$ , 436.1650.

***tert*-Butyl (2Z)-3-(1H-indol-3-yl)prop-2-enoate (75)**

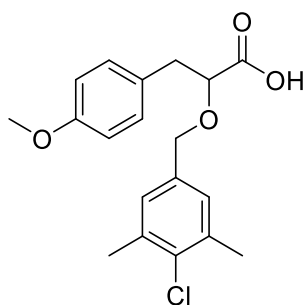
According to general procedure A, *tert*-butyl 2-diazo-3-(1H-indol-3-yl)propanoate (136 mg, 0.5 mmol) and 4-chloro-3,5-dimethyl benzyl alcohol (425 mg, 2.5 mmol) with  $[\text{Rh}_2(\text{Piv})_4]$  (3.0 mg, 0.005 mmol) gave a crude product. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5→5:95 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *alkene* **75** (14.0 mg, 24%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 8.73 (1H, d, *J* 2.8 Hz, Indolyl 2-H), 8.66 (1H, s, NH), 7.66-7.61 (1H, m, Indolyl 4-H), 7.29-7.25 (1H, m, Indolyl 7-H), 7.16-7.09 (3H, m, 2-H, Indolyl 5-H and Indolyl 6-H), 5.69 (1H, d, *J* 12.6 Hz, 2-H), 1.47 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz, CDCl<sub>3</sub>) 167.2 (C=O), 135.4 (Indolyl C-3a), 134.0 (C-3), 130.6 (Indolyl C-2), 128.4 (Indolyl 7a), 122.7 (Indolyl C-6), 120.8 (Indolyl C-5), 118.1 (Indolyl C-4), 114.4 (C-2), 111.6 (C-3), 111.5 (Indolyl C-7), 79.7 (*tert*-Butyl C-1), 28.5 (*tert*-Butyl C-2); HRMS found  $\text{MNa}^+$  266.1164. C<sub>15</sub>H<sub>17</sub>NO<sub>2</sub> requires *MNa*, 266.1151. Geometry was assigned on the basis of *J* value.

**2-[(4-Chloro-3,5-dimethylphenyl)methoxy]-2-(3-methoxyphenyl)acetic acid (76)**

To a solution of *tert*-butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-3-(4-methoxyphenyl)propanoate (69.0 mg, 0.20 mmol) and triethylsilane (100  $\mu\text{L}$ , 0.40 mmol) in DCM (3.60 mL) was added TFA (400  $\mu\text{L}$ ) and left to stir at r.t. overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5→5:95 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the

*carboxylic acid 76* (26.0 mg, 31%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2800 (broad), 1926, 2836, 1717;  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.32 (1H, app. t,  $J$  7.9 Hz, Methoxyphenyl 5-H), 7.03 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 7.03-7.00 (1H, m, Methoxyphenyl 4-H), 6.98-6.95 (1H, m, Methoxyphenyl 2-H), 6.92 (1H, ddd,  $J$  7.9, 2.5 and 0.9 Hz, Methoxyphenyl 6-H), 4.90 (1H, s, 2-H), 4.53 (1H, d,  $J$  11.6 Hz,  $\text{OCH}_a$ ), 4.43 (1H, d,  $J$  11.6 Hz,  $\text{OCH}_b$ ), 3.81 (3H, s, OMe), 2.37 (6H, s, Me);  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 170.0 (C=O), 160.0 (Methoxyphenyl C-3), 136.6 (Methoxyphenyl C-1 or 4-chloro-3,5-dimethylphenyl C-3), 136.6 (Methoxyphenyl C-1 or 4-chloro-3,5-dimethylphenyl C-3), 134.7 (4-chloro-3,5-dimethylphenyl C-4), 133.9 (4-chloro-3,5-dimethylphenyl C-1), 130.0 (Methoxyphenyl C-5), 128.3 (4-chloro-3,5-dimethylphenyl C-2), 119.8 (Methoxyphenyl C-4), 114.9 (Methoxyphenyl C-6), 112.3 (Methoxyphenyl C-2), 79.0 (C-2), 70.7 ( $\text{OCH}_2$ ), 55.3 (OMe), 20.7 (Me); HRMS found  $\text{MNa}^+$  357.0866.  $\text{C}_{18}\text{H}_{19}\text{ClO}_4$  requires  $\text{MNa}$ , 357.0864.

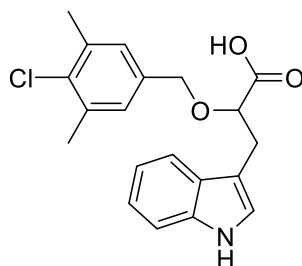
**2-[(4-Chloro-3,5-dimethylphenyl)methoxy]-3-(4-methoxyphenyl)propanoic acid (77)**



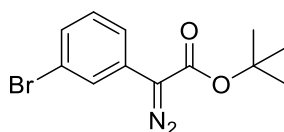
To a solution of *tert*-butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-3-(4-methoxyphenyl)propanoate (20.0 mg, 0.05 mmol) and triethylsilane (20  $\mu\text{L}$ , 0.10 mmol) in DCM (900  $\mu\text{L}$ ) was added TFA (100  $\mu\text{L}$ ) and left to stir at r.t. overnight. The reaction mixture was concentrated under reduced pressure to give the crude product. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5 $\rightarrow$ 5:95  $\text{H}_2\text{O}$  (0.1% formic acid)/MeCN over 12 min to yield the *carboxylic acid 77* (8.0 mg, 39%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.61 (2H, d,  $J$  8.6 Hz, Methoxyphenyl 2-H), 6.84 (2H, d,  $J$  8.6 Hz, Methoxyphenyl 3-H), 6.82 (2H, s, 2-H chloro-3,5-dimethylphenyl), 4.58 (1H, d,  $J$  11.9 Hz,  $\text{OCH}_a$ ), 4.31 (1H, d,  $J$  11.9 Hz,  $\text{OCH}_b$ ), 4.11 (1H, dd,  $J$  8.7 and 3.9 Hz, 2-H), 3.81 (3H, s, OMe), 3.11 (1H, dd,  $J$  14.2 and 3.9 Hz, 3- $\text{H}_a$ ), 2.97 (1H, dd,  $J$  14.2 and 3.9 Hz, 3- $\text{H}_b$ ), 2.30

(6H, s, Me);  $\delta_D$  (125 MHz,  $CDCl_3$ ) 176.4 (C=O), 159.0 (Methoxyphenyl C-4), 136.7 (4-chloro-3,5-dimethyl phenyl C-4), 134.8 (Methoxyphenyl C-2), 134.7 (4-chloro-3,5-dimethylphenyl C-1), 131.0 (4-chloro-3,5-dimethylphenyl C-3), 129.0 (Methoxyphenyl C-1), 128.3 (4-chloro-3,5-dimethylphenyl C-2), 114.1 (Methoxyphenyl C-3) 79.1 (C-2), 72.6 ( $OCH_2$ ), 55.6 (OMe), 38.5 (C-3), 21.0 (Me); HRMS found  $MNa^+$  371.1038.  $C_{19}H_{21}ClO_4$  requires  $MNa$ , 371.1021.

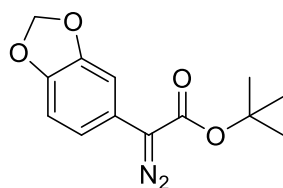
## 2-[(4-Chloro-3,5-dimethylphenyl)methoxy]-3-(1H-indol-3-yl)propanoic acid (**78**)



To a solution of *tert*-butyl 2-[(4-chloro-3,5-dimethylphenyl)methoxy]-3-(1H-indol-3-yl)propanoate (5.0 mg, 0.015 mmol) and triethylsilane (5  $\mu$ L, 0.03 mmol) in DCM (900  $\mu$ L) was added TFA (100  $\mu$ L) and left to stir at r.t. overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5 $\rightarrow$ 5:95  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *carboxylic acid* **78** (1.0 mg, 25%) as a brown solid.  $\delta_H$  (500 MHz,  $CDCl_3$ ) 8.04 (1H, s, NH), 7.61 (1H, app. d,  $J$  7.9 Hz, Indolyl 7-H), 7.37 (1H, app. d,  $J$  7.9 Hz, Indolyl 4-H), 7.23-7.17 (1H, m, Indolyl 6-H), 7.14-7.06 (2H, m, Indolyl 2-H and Indolyl 5-H), 6.83 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 4.51 (1H, d,  $J$  11.6 Hz,  $OCH_a$ ), 4.38 (1H, d,  $J$  11.6 Hz,  $OCH_b$ ), 4.30 (1H, dd,  $J$  7.5 and 4.2 Hz, 3-H), 3.36 (1H, dd,  $J$  15.0 and 4.2 Hz, 3- $H_a$ ), 3.22 (1H, dd,  $J$  15.0 and 7.5 Hz, 3- $H_b$ ), 2.28 (6H, s, Me);  $\delta_D$  (125 MHz,  $CDCl_3$ ) 173.2 (C=O), 136.4 (Indolyl C-3a), 136.1 (4-chloro-3,5-dimethylphenyl C-4), 134.2 (4-chloro-3,5-dimethylphenyl C-1), 128.1 (4-chloro-3,5-dimethylphenyl C-2), 127.5 (Indolyl C-7a), 123.2 (Indolyl C-2 or Indolyl C-5), 122.2 (Indolyl C-7), 119.6 (Indolyl C-2 or Indolyl C-5), 119.0 (Indolyl C-6), 111.2 (Indolyl C-3), 110.7 (Indolyl C-4), 78.3 (C-2), 72.4 ( $OCH_2$ ), 29.7 (C-3), 20.6 (Me); HRMS found  $MNa^+$  380.1023.  $C_{20}H_{20}ClNO_3$  requires  $MNa$ , 380.1024.

***tert*-Butyl 2-(3-bromophenyl)-2-diazoacetate (D5)**<sup>69</sup>

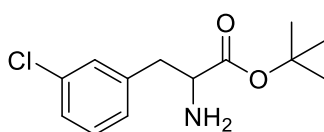
To a solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (208 mg, 0.18 mmol, 1 mol%), NaOH (870 mg, 21.6 mmol) and 1-bromo-3-iodobenzene (880 μL, 6.87 mmol) in EtOH (43 mL) was added *tert*-butyl 2-diazo-3-oxobutanoate (2.00 g, 10.8 mmol) and left to stir at r.t. for 3 hr. The reaction mixture was then passed through a short plug of silica and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield the *diazo* **D5** (675 mg, 33%) as a yellow oil. *R*<sub>f</sub> 0.46 (99:1 hexane–EtOAc).  $\nu_{\max}/\text{cm}^{-1}$  (film) 2978, 2933, 2082, 1696;  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 7.71 (1H, app. t, *J* 1.7 Hz, Phenyl 2-H), 7.35 (1H, ddd, *J* 7.9, 1.9 and 1.1 Hz, Phenyl 4-H or Phenyl 6-H), 7.28 (1H, m, Phenyl 4-H or Phenyl 6-H), 7.20 (1H, app. t, *J* 7.9 Hz, Phenyl 5-H); 1.55 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz, CDCl<sub>3</sub>) 163.9 (C=O), 130.2 (Phenyl C-5), 128.6 (Phenyl C-1), 128.4 (C-4 or C-6 Phenyl), 126.6 (Phenyl C-2), 123.1 (Phenyl C-3), 112.0 (Phenyl C-4 or C-6), 82.5 (*tert*-Butyl C-1), 28.4 (*tert*-Butyl C-2); HRMS found MNa<sup>+</sup> 319.0047. C<sub>12</sub>H<sub>13</sub>BrN<sub>2</sub>O<sub>2</sub> requires *MNa*, 319.0053.

***tert*-Butyl 2-(2H-1,3-benzodioxol-5-yl)-2-diazoacetate (D6)**<sup>69</sup>

To a solution of Pd(PPh<sub>3</sub>)<sub>4</sub> (104 mg, 0.09 mmol, 1 mol%), NaOH (0.43 g, 10.8 mmol) and 5-iodo-1,3-benzodioxole (450 mg, 3.56 mmol) in EtOH (22 mL) was added *tert*-butyl 2-diazo-3-oxobutanoate (1.00 g, 5.40 mmol) and left to stir at r.t. for 3 hr. The reaction mixture was then passed through a short plug of silica and concentrated under reduced pressure to give a crude product. The crude product was then purified *via* flash column chromatography eluting with 99:1 hexane–EtOAc to yield the *diazo* **D6** (343 mg, 37%) as an orange solid. *R*<sub>f</sub> 0.24 (99:1 hexane–EtOAc).  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 7.05 (1H, d, *J* 1.4 Hz, 4-H Benzodioxolyl), 6.87–6.80 (2H, m, Benzodioxolyl 6-H and Benzodioxolyl 7-H), 5.95

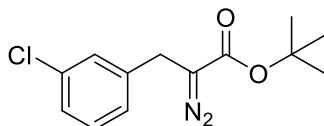
(2H, s, Benzodioxolyl 2-H<sub>2</sub>), 1.54 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz, CDCl<sub>3</sub>) 164.9 (C=O), 148.3 (Benzodioxolyl C-7a or Benzodioxolyl 3a), 145.8 (Benzodioxolyl C-6 or Benzodioxolyl C-7), 119.3 (Benzodioxolyl C-5), 117.8 (Benzodioxolyl C-6 or Benzodioxolyl C-7), 108.8 (Benzodioxolyl C-3a or Benzodioxolyl C-7a), 105.7 (Benzodioxolyl C-4), 101.2 (Benzodioxolyl C-2), 82.0 (*tert*-Butyl C-1), 28.4 (*tert*-Butyl C-1); Diazo carbon not observed; HRMS found MNa<sup>+</sup> 285.0846. C<sub>13</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub> requires *MNa*, 285.0846. Spectroscopic data are consistent with those reported in the literature.<sup>120</sup>

***tert*-Butyl 2-amino-3-(3-chlorophenyl)propanoate (80)**<sup>70</sup>



To a solution of 3-chlorophenylalanine (2.00 g, 10.0 mmol) in *tert*-butyl acetate (25 mL) was added HClO<sub>4</sub> (170  $\mu$ L, 2.0 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-butyl ester **80** (360 mg, 14%) as a colourless oil. *R<sub>f</sub>* 0.42 (90:10 DCM–MeOH).  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 7.19–7.10 (3H, m, Phenyl 3-H, Phenyl 4-H and Phenyl 6-H), 7.06–7.03 (1H, m, Phenyl 2-H), 3.52 (1H, dd, *J* 7.5 and 5.8 Hz, 2-H), 2.92 (1H, dd, *J* 13.6 and 5.8 Hz, 3-H<sub>a</sub>), 2.76 (1H, dd, *J* 13.6 and 7.5 Hz, 3-H<sub>b</sub>), 1.43 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz, CDCl<sub>3</sub>) 174.0 (C=O), 139.7 (Phenyl C-1), 134.2 (Phenyl C-3), 129.7 (Ar-C), 129.6 (Ar-C), 129.5 (Ar-C), 127.6 (Ar-C), 81.5 (*tert*-Butyl C-1), 56.2 (C-2), 40.9 (C-3), 28.0 (*tert*-Butyl C-2); HRMS found MNa<sup>+</sup> 278.0918. C<sub>13</sub>H<sub>18</sub>ClNO<sub>2</sub> requires *MNa*, 278.0918.

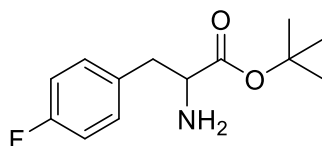
***tert*-Butyl 3-(3-chlorophenyl)-2-diazopropanoate D7**<sup>71</sup>



To a solution of *tert*-butyl 2-amino-3-(3-chlorophenyl)propanoate (307 mg, 1.45 mmol) and acetic acid (9  $\mu$ L, 0.15 mmol) in chloroform (9 mL) was added isoamyl nitrite (350  $\mu$ L, 1.74 mmol) and left to stir at 70 °C for 30 min. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with H<sub>2</sub>O (100 mL),

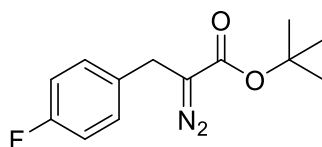
sat.  $\text{NaHCO}_3$  solution (100 mL) and brine (100 mL), dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography, eluting with 99:1 hexane–EtOAc to yield the *dialo* **D7** (409 mg, 73%) as a yellow oil.  $R_f$  (99:1 hexane–EtOAc) 0.28.  $\delta_H$  (500 MHz,  $\text{CDCl}_3$ ) 7.27–7.20 (3H, m, Phenyl 3-H, Phenyl 4-H and Phenyl 6-H), 7.12–7.09 (1H, m, Phenyl 2-H), 3.56 (2H, s, 3-H<sub>2</sub>), 1.48 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz,  $\text{CDCl}_3$ ) 166.3 (C=O), 139.7 (Phenyl C-1), 134.5 (Phenyl C-3), 130.0 (Ar-C), 128.5 (Ar-C), 127.3 (Ar-C), 126.5 (Ar-C), 81.7 (*tert*-Butyl C-1), 28.4 (*tert*-Butyl C-2 and C-3) (Diazo carbon not observed); HRMS found  $\text{MNa}^+$  289.0719.  $\text{C}_{13}\text{H}_{15}\text{ClN}_2\text{O}_2$  requires *MNa*, 289.0714.

***tert*-Butyl 2-amino-3-(4-fluorophenyl)propanoate (82)**<sup>70</sup>

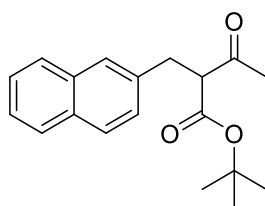


To a solution of 3-chlorophenylalanine 4-fluorophenylalanine (2.00 g, 10.10 mmol) in *tert*-butyl acetate (25 mL) was added  $\text{HClO}_4$  (170  $\mu\text{L}$ , 2.00 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-butyl ester **82** (1.00 g, 41%) as a colourless oil.  $R_f$  0.40 (90:10 DCM–MeOH).  $\delta_H$  (500 MHz,  $\text{CDCl}_3$ ) 7.20–7.13 (2H, m, Phenyl 2-H), 7.02–6.97 (2H, m, Phenyl 3-H), 3.57 (1H, dd,  $J$  7.5 and 5.8 Hz, 2-H), 2.99 (1H, dd,  $J$  13.7 and 5.8 Hz, 3-H<sub>a</sub>), 2.82 (1H, dd,  $J$  13.7 and 7.5 Hz, 3-H<sub>b</sub>), 1.43 (9H, s, *tert*-Butyl);  $\delta_D$  (125 MHz,  $\text{CDCl}_3$ ) 174.2 (C=O), 161.8 (d,  $J$  244.6 Hz, Phenyl C-4), 133.2 (d,  $J$  3.2 Hz, Phenyl C-1), 130.8 (d,  $J$  7.9 Hz, Phenyl C-2), 115.2 (d,  $J$  21.2 Hz, Phenyl C-3), 81.3 (*tert*-Butyl C-1), 56.3 (C-2), 40.3 (C-3), 28.0 (*tert*-Butyl C-2); HRMS found  $\text{MNa}^+$  262.1214.  $\text{C}_{13}\text{H}_{18}\text{FNO}_2$  requires *MNa*, 262.1214.



***tert*-Butyl 2-diazo-3-(4-fluorophenyl)propanoate (**D8**)**<sup>71</sup>

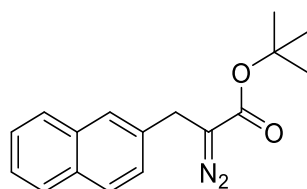
To a solution of *tert*-butyl 2-amino-3-(4-fluorophenyl)propanoate (1.00 g, 4.10 mmol) and acetic acid (23  $\mu$ L, 0.41 mmol) in chloroform (26 mL) was added isoamyl nitrite (1.00 mL, 4.92 mmol) and left to stir at 70 °C for 30 min. The reaction mixture was diluted with ethyl acetate and washed with H<sub>2</sub>O (100 mL), sat. NaHCO<sub>3</sub> solution (100 mL) and brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography, eluting with 99:1 hexane–EtOAc to yield the *diazo* **D8** (735 mg, 72%) as a yellow oil.  $R_f$  (99:1 hexane–EtOAc) 0.26.  $\nu_{\max}/\text{cm}^{-1}$  (film) 2980, 2934, 2080, 1684;  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 7.23–7.18 (2H, m, Phenyl 2-H), 7.03–6.99 (2H, m, Phenyl 3-H), 3.55 (2H, s, 3-H<sub>2</sub>), 1.48 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz, CDCl<sub>3</sub>) 166.4 (C=O), 161.9 (d,  $J$  245.1 Hz, Phenyl C-4), 133.3 (d,  $J$  3.2 Hz, Phenyl C-1), 129.9 (d,  $J$  8.0 Hz, Phenyl C-2), 115.6 (d,  $J$  21.4 Hz, Phenyl C-3), 81.5 (*tert*-Butyl C-1), 28.7 (*tert*-Butyl C-2 and C-3) (Diazo carbon not observed); HRMS found  $M\text{Na}^+$  273.1014. C<sub>13</sub>H<sub>15</sub>FN<sub>2</sub>O<sub>2</sub> requires  $M\text{Na}$ , 273.1010.

***tert*-Butyl 2-[(naphthalen-2-yl)methyl]-3-oxobutanoate (**84**)**<sup>121</sup>

To a solution of NaH (60% in mineral oil, 360 mg, 11.00 mmol) in THF (25 mL) was added *tert*-butyl acetoacetate (1.83 mL, 11.00 mmol) dropwise at 0 °C and stirred for 1 hr. 2-bromoethyl naphthalene (2.43 g, 10.00 mmol) in THF (5 mL) was added dropwise to the reaction over 5 min and left to stir at reflux for 18 hr. The reaction mixture was diluted in water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic extract was washed with brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield *tert*-butyl oxobutanoate **84** (2.09 g, 64%) as a colourless oil.  $R_f$  0.10 (99:1

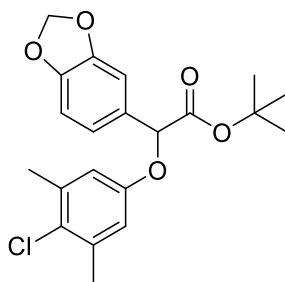
hexane–EtOAc).  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.79–7.70 (3H, m, Naphthyl 4-H, Naphthyl 6-H and Naphthyl 8-H), 7.63 (1H, br. s, Naphthyl 1-H), 7.48–7.40 (2H, m, Naphthyl 5-H and Naphthyl 7-H), 7.32 (1H, dd,  $J$  8.4 and 1.8 Hz, Naphthyl 3-H), 3.79 (1H, m, 2-H), 3.29–3.26 (2H, m,  $\text{CH}_2$ ), 2.20 (3H, s, 4- $\text{H}_3$ ), 1.38 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 168.3 (C=O), 136.0 (Naphthyl C-2), 133.5 (Naphthyl C-8), 132.3 (Naphthyl C-4a), 128.2 (Ar-C), 127.6 (Naphthyl C-1), 127.5 (Ar-C), 127.4 (Ar-C), 127.2 (Ar-C), 126.1 (Ar-C), 125.5 (Ar-C), 82.2 (*tert*-Butyl C-1), 62.3 (C-2), 34.0 ( $\text{CH}_2$ ), 29.5 (Me), 27.8 (*tert*-Butyl C-2); HRMS found  $\text{MNa}^+$  321.1477.  $\text{C}_{19}\text{H}_{22}\text{O}_3$  requires  $\text{MNa}$ , 321.1461.

***tert*-Butyl 2-diazo-3-(naphthalen-2-yl)propanoate (D9)**<sup>121</sup>

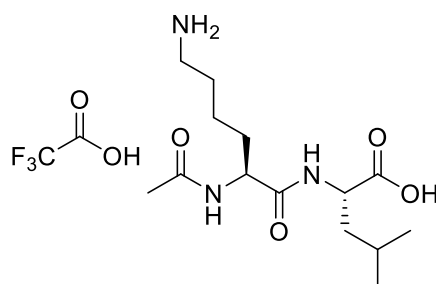


To a solution of *tert*-butyl 2-[(naphthalen-2-yl)methyl]-3-oxobutanoate (2.09 g, 7.00 mmol) and DBU (1.50 mL, 9.80 mmol) in acetonitrile (15 mL) was added *p*-ABSA (2.10 g, 8.40 mmol) and left to stir at r.t for 22 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude material was purified *via* column chromatography eluting with 99:1 hexane–EtOAc to yield the diazo **D9** (1.05 g, 53%) as a yellow oil.  $R_f$  0.45 (99:1 hexane–EtOAc).  $\nu_{\text{max}}/\text{cm}^{-1}$  (film) 3054, 2977, 2931, 2075, 1679;  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.83–7.76 (3H, m, Naphthyl 4-H, Naphthyl 6-H and Naphthyl 8-H), 7.67 (1H, br. s, Naphthyl 1-H), 7.50–7.45 (2H, m, Naphthyl 5-H and Naphthyl 7-H), 7.38 (1H, dd,  $J$  8.4 and 1.8 Hz, Naphthyl 3-H), 3.75 (2H, s, 3- $\text{H}_2$ ), 1.50 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 168.2 (C=O), 135.0 (Naphthyl C-2), 133.5 (Naphthyl C-8a), 132.5 (Naphthyl C-4a), 128.6 (Ar-C), 127.7 (Naphthyl C-1), 127.6 (Ar-C), 126.8 (Ar-C), 126.7 (Ar-C), 126.2 (Ar-C), 125.8 (Ar-C), 81.5 (*tert*-Butyl C-1), 29.6 (C-3), 28.4 (*tert*-Butyl C-2) (Diazo carbon not observed); HRMS found  $\text{MNa}^+$  305.1265.  $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_2$  requires  $\text{MNa}$ , 305.1260. Spectroscopic data are consistent with those reported in the literature.<sup>121</sup>

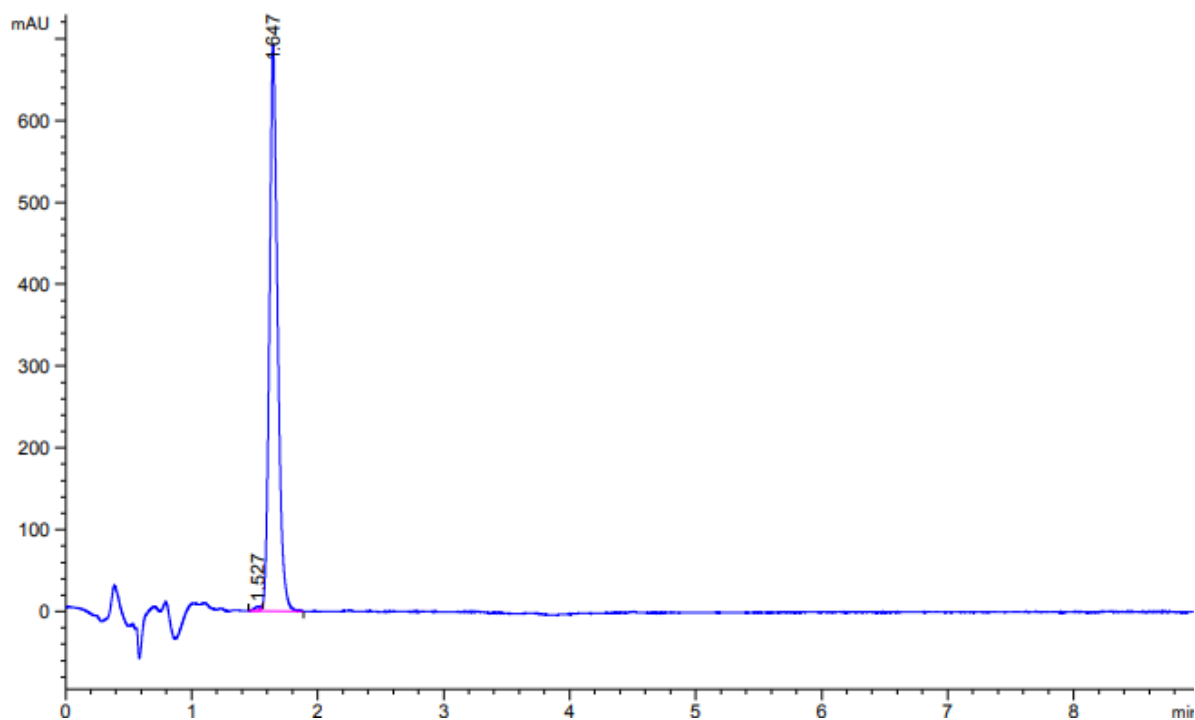
***tert*-Butyl 2-(2H-1,3-benzodioxol-5-yl)-2-(4-chloro-3,5-dimethylphenoxy)acetate (85)**

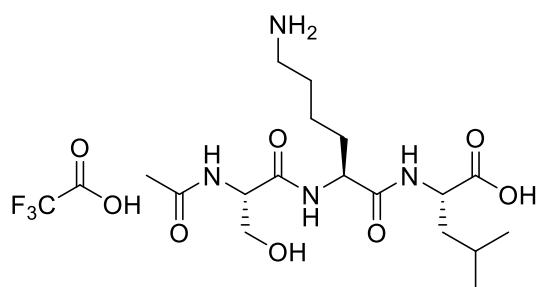


According to general procedure A, *tert*-butyl 2-(2H-1,3-benzodioxol-5-yl)-2-diazoacetate (131.0 mg, 0.50 mmol) and 4-chloro-3,5-dimethylphenol (390.0 mg, 2.50 mmol) with  $[\text{Rh}_2(\text{pfb})_4]$  (4.00 mg, 0.005 mmol) gave a crude material. The crude product was purified *via* reverse-phase HPLC, with a gradient of 95:5→5:95  $\text{H}_2\text{O}$  (0.1% formic acid)/MeCN over 12 min to yield the *tert*-butyl acetate **85** (128 mg, 68%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.06 (1H, d,  $J$  1.7 Hz, Benzodioxolyl 4-H), 7.02-6.99 (1H, m, Benzodioxolyl 6-H), 6.81 (1H, d,  $J$  8.0 Hz, Benzodioxolyl 7-H), 6.68 (2H, s, 4-chloro-3,5-dimethylphenyl 2-H), 5.97 (2H, s, Benzodioxolyl 2-H), 5.36 (1H, s, 2-H), 2.32 (6H, s, Me), 1.41 (9H, s, *tert*-Butyl);  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 168.9 (C=O), 155.1 (Benzodioxolyl C-3a or Benzodioxolyl C-7a), 148.1 (Benzodioxolyl C-6 or Benzodioxolyl C-7), 147.9 (Benzodioxolyl C-5), 137.2 (4-chloro-3,5-dimethylphenyl C-4), 129.5 (Benzodioxolyl C-6 or Benzodioxolyl C-7), 127.1 (4-chloro-3,5-dimethylphenyl C-3), 121.1 (Benzodioxolyl C-3a or Benzodioxolyl C-7a), 115.3 (4-chloro-3,5-dimethylphenyl C-2), 108.4 (Benzodioxolyl C-4), 107.5 (Benzodioxolyl C-2), 101.3 (4-chloro-3,5-dimethylphenyl C-1), 82.5 (*tert*-Butyl C-1), 78.7 (C-2), 27.9 (*tert*-Butyl C-2), 21.0 (Me); HRMS found  $\text{MNa}^+$  413.1131.  $\text{C}_{21}\text{H}_{23}\text{ClO}_5$  requires  $\text{MNa}$ , 413.1126.

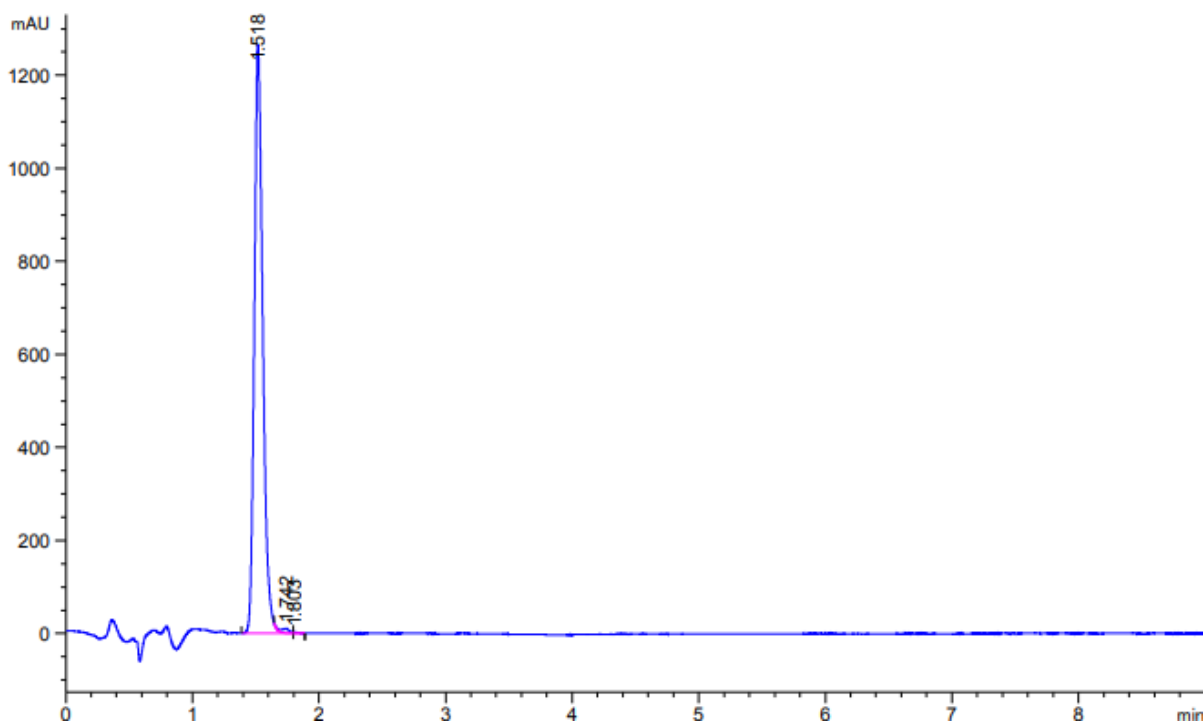
**N-Acetylated KL TFA Salt (92)**

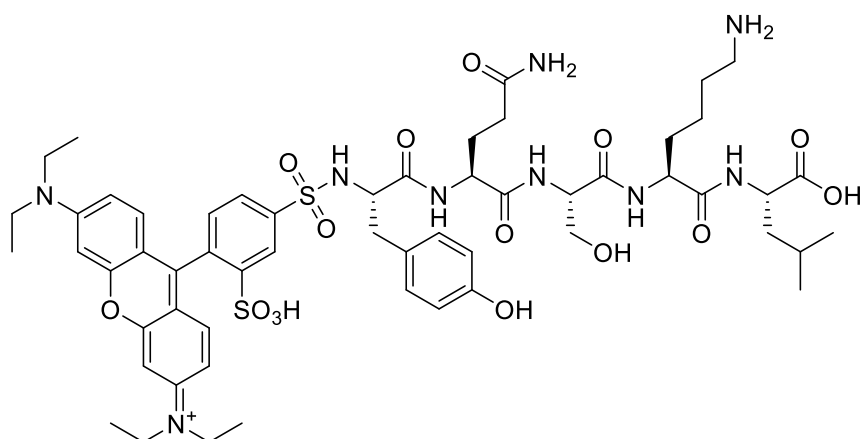
According to general procedure H, Fmoc-Lys(Boc)-OH (185.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the dipeptide **92** (17.4 mg, 53%) as a white solid. HRMS found MH<sup>+</sup> 302.2102. C<sub>14</sub>H<sub>28</sub>N<sub>3</sub>O<sub>4</sub> requires *MH*, 302.2074; purity 99.45% (determined *via* HPLC 95:5→5:95 over 10 min).



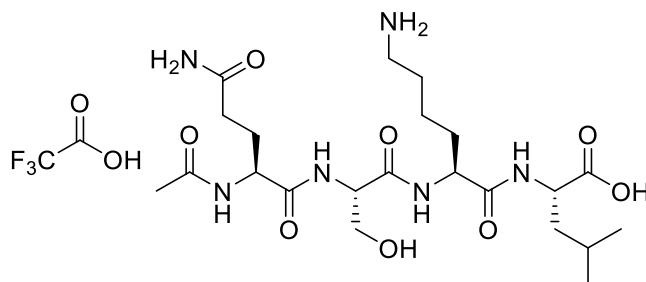
**N-Acetylated SKL TFA Salt (93)**

According to general procedure H, Fmoc-Lys(Boc)-OH (185 mg, 0.395 mmol) and Fmoc-Ser(*tert*-Butyl)-OH (151 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the peptide **93** (16.3 mg, 41% mg) as a white solid. HRMS found MH<sup>+</sup> 389.2416. C<sub>17</sub>H<sub>33</sub>N<sub>4</sub>O<sub>6</sub> requires *MH*, 389.2395; purity 99.15% (determined *via* HPLC 95:5→5:95 over 10 min).

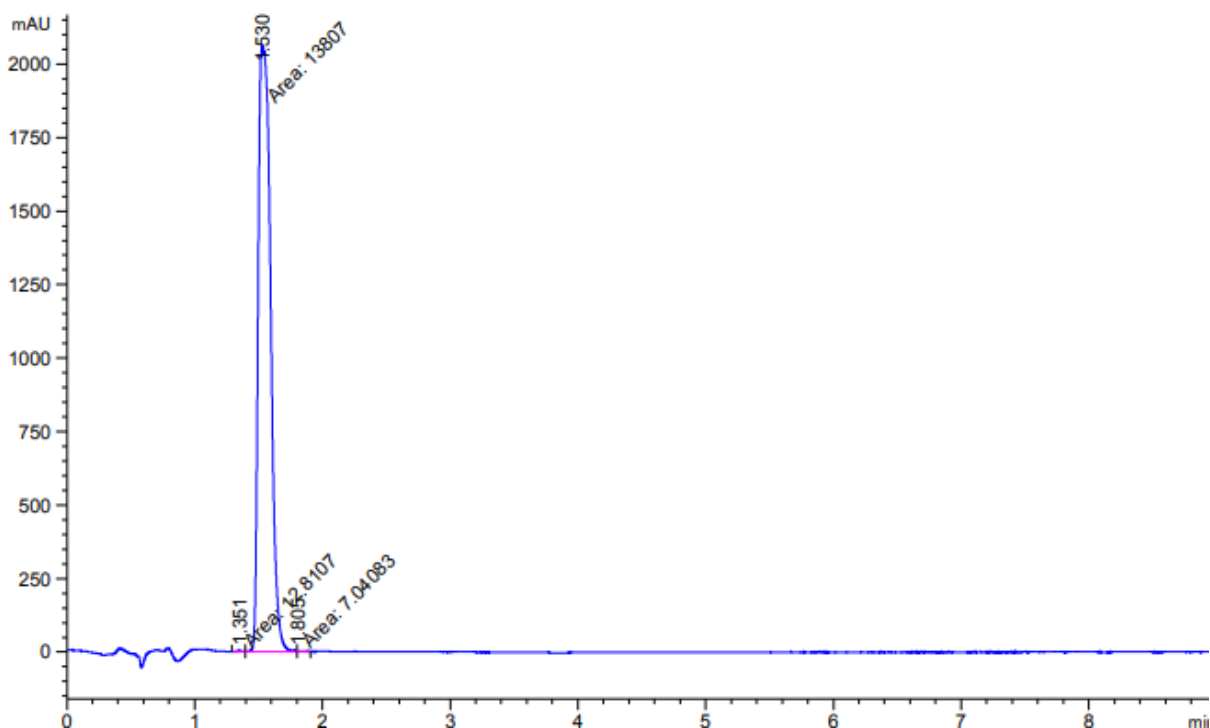


**Lissamine-labelled YQSKL (94)**

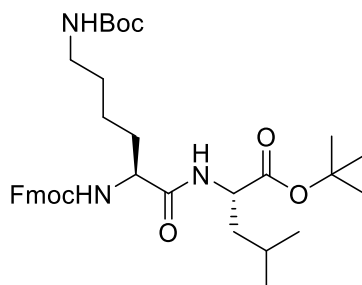
Lissamine labelled YQSKL was prepared using Fmoc solid-phase peptide synthesis: 200 mg of leucine loaded 2-chlorotrityl chloride resin was added to a fritted reaction vessel. The resin was swelled in DMF (2 ml) for 1 hr, and then the reaction vessel was drained. DMF washes were performed (3 × 2 ml × 2 min), followed by washes with 20% piperidine in DMF (5 × 2 ml × 2 min), and DMF (5 × 2 ml × 2 min). FmocBoc Lys (5 eq.) in DMF (1 ml) was added, along with N, N'-Diisopropylcarbodiimide (5 eq.) and OxymaPure (5 eq.). The reaction was agitated for 1 hr at RT then washed with DMF, deprotected (20% piperidine in DMF) and washed (DMF). The remaining couplings, of the S, Q, and Y residues, were performed using similar procedures. The N-terminal position was capped by coupling with lissamine rhodamine B sulfonyl chloride Lissamine (5 eq.) was added to the resin-bound peptide (1 eq.) along with DIC (5 eq.) in DMF (1 mL) before stirring the solution overnight in the absence of light. The solution was filtered from the resin, which was then washed with DMF (3 × 2 ml), dichloromethane (DCM) (3 × 2 ml), and MeOH (3 × 2 ml) and dried under reduced pressure. The peptide was cleaved from the solid resin support using a cleavage cocktail of trifluoroacetic acid (TFA), H<sub>2</sub>O, and triisopropylsilane (95:2.5:2.5), which was added to dried resin (500 µl per 25 mg resin) and agitated for 1 hr at room temperature. The cleavage mixture was filtered drop-wise into cold diethyl ether (1:100). The peptide was extracted with water and the aqueous layer collected, lyophilized and purified by reverse-phase biotage column. YQSKL-Lissamine was obtained as a pink solid (1.0 mg, 0.5%). HRMS found MH<sup>+</sup> 1179.4903. C<sub>56</sub>H<sub>77</sub>N<sub>9</sub>O<sub>15</sub>S<sub>2</sub> requires *MH*, 1179.4970.

**N-Acetylated QSKL TFA Salt (95)**

According to general procedure H, Fmoc-Lys(Boc)-OH (185 mg, 0.395 mmol), Fmoc-Ser(*tert*-Butyl)-OH (151 mg, 0.395 mmol) and Fmoc-Gln(Trt)-OH (241 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the peptide **95** (35.0 mg, 70%) as a white solid. HRMS found MH<sup>+</sup> 517.3005. C<sub>22</sub>H<sub>41</sub>N<sub>6</sub>O<sub>8</sub> requires *MH*, 517.2980; purity 99.86% (determined *via* HPLC 95:5→5:95 over 10 min).

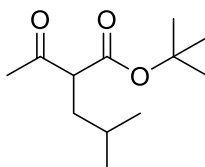


***tert*-Butyl (2*S*)-2-[(2*S*)-5-{[(*tert*-Butoxy)carbonyl]amino}-2-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)pentanamido]-4-methylpentanoate (**96**)**

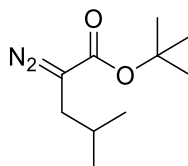


According to general procedure F, H-Leu-O-*tert*-butyl HCl (108 mg, 0.48 mmol) with Fmoc-Lys(Boc)-OH (217 mg, 0.62 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 1:1 hexane–EtOAc to yield the dipeptide **96** (202 mg, 36%) as an undetermined mixture of rotamers as a colourless oil.  $R_f$  0.40 (50:50 hexane–ethyl acetate).  $\nu_{\max}/\text{cm}^{-1}$  (film) 2961, 1709 (br), 1509, 1451, 1367, 1264, 1150;  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.78 (2H, d,  $J$  7.6 Hz, Fluorenyl 4-H), 7.66-7.63 (2H, m, Fluorenyl 1-H), 7.39 (2H, t,  $J$  7.5 Hz, Fluorenyl 3-H), 7.31 (2H, t,  $J$  7.5 Hz, Fluorenyl 2-H), 4.42-4.34 (3H, m, 2-H and  $\text{CH}_2$  Fmoc), 4.24-4.16 (3H, m, 1-H Pentanamido and 9- $\text{H}_2$  Fluorenyl), 3.14-3.02 (2H, m, 5- $\text{H}_2$  Pentanamido), 1.87-1.79 (2H, m,  $\text{CH}_2$  Pentanamido), 1.56-1.39 (22H, m, *tert*-Butyl ester, *tert*-Butyl Boc and 2 x  $\text{CH}_2$  Pentanamido), 0.95 (3H, d,  $J$  6.6 Hz, 4-Me), 0.91 (3H, d,  $J$  6.6 Hz, 5- $\text{H}_3$ );  $\delta_{\text{D}}$  (125 MHz, MeOD) 173.1 (C=O), 171.9 (C=O), 157.1 (C=O), 156.0 (C=O), 144.0 (Fluorenyl C-8a<sup>rot(min)</sup>), 143.7 (Fluorenyl C-8a<sup>rot(maj)</sup>), 127.4 (Fluorenyl C-3<sup>rot(maj)</sup>), 127.4 (Fluorenyl C-3<sup>rot(min)</sup>), 126.8 (Fluorenyl C-2<sup>rot(maj)</sup>), 126.8 (Fluorenyl C-2<sup>rot(min)</sup>), 124.9 (Fluorenyl C-1<sup>rot(min)</sup>), 124.9 (Fluorenyl C-1<sup>rot(maj)</sup>), 119.6 (Fluorenyl C-4), 81.3 (*tert*-Butyl C-1), 78.5 (*tert*-Butyl C-1), 54.7 (Pentanamido C-1), 51.6 (C-2), 47.0 (Fluorenyl C-9), 40.3 (C-3), 39.7 (Pentanamido C-5), 31.7 (Pentanamido-C), 29.2 (Pentanamido-C), 27.5 (*tert*-Butyl C-2), 27.0 (*tert*-Butyl C-2), 24.6 (C-4), 22.8 (Pentanamido-C), 22.0 (C-4 Me), 20.7 (C-5)(31/52 signals present); HRMS found  $\text{MNa}^+$  660.3630.  $\text{C}_{36}\text{H}_{51}\text{N}_3\text{O}_7$  requires  $\text{MNa}$ , 660.3619.



***tert*-Butyl 2-acetyl-4-methylpentanoate (**100**)**<sup>102</sup>

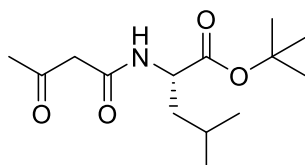
To a solution of NaH (1.10 g, 26.0 mmol) in THF (50 mL) was added *tert*-butyl acetoacetate (4.50 mL, 25.0 mmol) and left to stir at r.t for 1 hr. To a separate solution of NaI (1.88 g, 12.5 mmol) in DMF (25 mL) was added 1-bromo-2-methylpropane (4.00 mL, 27.0 mmol) and left to stir at r.t for 1 hr. The solutions were combined and left to stir at reflux for 18 hr. The reaction mixture was quenched with sat. NH<sub>4</sub>Cl (15 mL) then diluted in sat. NH<sub>4</sub>Cl (500 mL) and extracted with diethyl ether (3 x 300 mL). The organic extract was washed with brine (300 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with hexane–EtOAc to yield the *alkylated product* **100** (3.08 g, 58%) as a colourless oil. *R*<sub>f</sub> 0.15 (99:1 hexane–EtOAc). δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 4.48 (1H, dd, *J* 14.4 and 7.7 Hz, 2-H), 2.21 (3H, s, Acetyl), 1.69–1.66 (1H, m, 4-H), 1.60–1.55 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.39 (9H, s, *tert*-Butyl), 0.90 (3H, d, *J* 6.5 Hz, 4-Me), 0.87 (3H, d, *J* 6.5 Hz, 5-H<sub>3</sub>); δ<sub>D</sub> (125 MHz, CDCl<sub>3</sub>) 171.9 (C=O), 164.6 (C=O), 81.3 (*tert*-Butyl C-1), 51.9 (C-2), 41.2 (C-3), 28.7 (*tert*-Butyl C-2), 22.7 (C-4 Me), 22.3 (C-4), 22.0 (C-4 Me and C-5), 19.4 (Acetyl Me); HRMS found MNa<sup>+</sup> 237.1459. C<sub>12</sub>H<sub>22</sub>O<sub>3</sub> requires *MNa*, 237.1461.

***tert*-Butyl 2-diazo-4-methylpentanoate (**D10**)**<sup>102</sup>

To a solution of *tert*-butyl 2-acetyl-4-methylpentanoate (3.08 g, 14.4 mmol), *p*-ABSA (4.15 g, 17.3 mmol) in acetonitrile (100 mL) was added DBU (4.30g, 28.8 mmol) at 0 °C. The reaction mixture was left to warm to room temperature overnight while stirring. The reaction mixture was diluted in H<sub>2</sub>O (100 mL) and extracted with diethyl ether (3 x 100 mL). The organic extract was washed with brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to

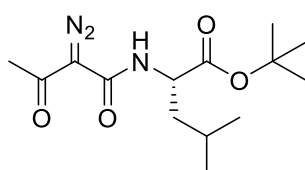
give a crude product. The crude product was purified *via* column chromatography eluting with hexane–EtOAc to yield the *diazo* **D10** (1.91 g, 67%) as a yellow oil.  $R_f$  0.15 (99:1 hexane–EtOAc).  $\nu_{\max}/\text{cm}^{-1}$  (film) 2961, 2933, 2872, 2078, 1682;  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 2.05 (2H, d,  $J$  7.1 Hz, 3- $\text{H}_2$ ), 1.75–1.72 (1H, m, 4-H), 1.40 (9H, s, *tert*-Butyl), 0.88 (6H, d,  $J$  6.7 Hz, 4-Me and 5- $\text{H}_3$ );  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 167.3 (C=O), 80.9 (*tert*-Butyl C-1), 32.2 (C-4), 28.4 (*tert*-Butyl C-2), 28.0 (C-3), 22.0 (C-4 Me and C-5); Diazo carbon not observed.

***tert*-Butyl (2S)-4-methyl-2-(3-oxobutanamido)pentanoate (103)**<sup>104</sup>



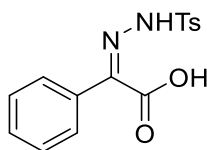
To a solution of  $\text{K}_2\text{CO}_3$  (550 mg, 4.00 mmol) and 2,2,6-trimethyl-1,3-dioxin-4-one (1.3 mL, 10.00 mmol) in  $\text{H}_2\text{O}$  (10 mL) was added H-Leu-O-*tert*-butyl HCl (660.0 mg, 5.00 mmol) and left to stir at reflux for 2 hr. The reaction mixture was then acidified to pH 1 using HCl (1M) and extracted with ethyl acetate (50 mL). The organic extract was washed with brine (50 mL), dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give a crude product. The crude product was filtered through a short plug of silica eluting with ethyl acetate and concentrated under reduced pressure to yield *tert*-butyl ester **103** as a brown oil.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 7.32 (1H, d,  $J$  8.1 Hz, NH), 4.27–4.23 (1H, m, 2-H), 1.48 (3H, s, Me), 1.36–1.31 (3H, m, 3- $\text{H}_a$  3- $\text{H}_b$  and 4-H), 1.25 (9H, s, *tert*-Butyl), 0.72 (6H, app. t,  $J$  6.5 Hz, 4-Me and 5- $\text{H}_3$ );  $\delta_{\text{D}}$  (125 MHz,  $\text{CDCl}_3$ ) 203.3 (C=O), 168.8 (C=O), 161.0 (C=O), 81.4 (*tert*-Butyl C-1), 51.4 (C-2), 50.2 (C-3), 41.0 (Oxobutanamido C-2), 27.7 (*tert*-Butyl C-2), 24.8 (Oxobutanamido C-4), 24.6 (C-4), 22.6 (C-4 Me), 21.8 (C-5). HRMS found  $\text{MNa}^+$  272.1848.  $\text{C}_{14}\text{H}_{26}\text{NO}_4$  requires *MH*, 272.1856.

***tert*-Butyl (2S)-2-(2-diazo-3-oxobutanamido)-4-methylpentanoate (D11)**<sup>69</sup>



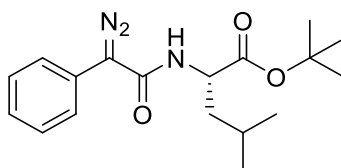
To a solution of *tert*-butyl 4-methyl-2-(3-oxobutanamido)pentanoate (2.71 g, 10.00 mmol) and  $\text{NEt}_3$  (1.40 ml, 11.00 mmol) in acetonitrile (78 mL) was added *p*-ABSA (2.88 g, 12.00 mmol) and left to stir at r.t for 22 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 80:20 hexane–ethyl acetate to yield the *dialzo* **D11** (1.02 g, 34%) as a yellow oil.  $R_f$  0.20 (80:20 hexane–ethyl acetate).  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 8.45 (1H, d,  $J$  7.8 Hz, NH), 4.47-4.44 (1H, m, 2-H), 2.28 (3H, s, Oxobutanamido 4- $\text{H}_3$ ), 1.56-1.51 (3H, m, 3- $\text{H}_a$ , 3- $\text{H}_b$  and 4-H), 1.39 (9H, s, *tert*-Butyl), 0.88 (3H, d,  $J$  3.8 Hz, 4-Me), 0.87 (3H, d,  $J$  3.8 Hz, 5- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 189.4 (C=O), 171.4 (C=O), 160.2 (C=O), 81.8 (*tert*-Butyl C-1), 51.9 (C-2), 41.2 (C-3), 27.9 (*tert*-Butyl C-2), 26.6 (Oxobutanamido C-4), 25.0 (C-4), 22.9 (C-4 Me), 21.9 (C-5)(Dialzo carbon not observed); HRMS found  $\text{MNa}^+$  320.1581.  $\text{C}_{14}\text{H}_{23}\text{N}_3\text{O}_4$  requires  $\text{MNa}$ , 320.1525.

## 2-[(4-Methylbenzenesulfonamido)imino]-2-phenylacetic acid (**105**)<sup>105</sup>

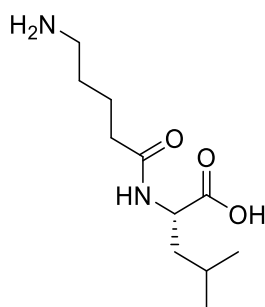


To a solution of phenylglyoxylic acid (10.00 g, 66.6 mmol) in THF (60 mL) was added *p*-toluenesulfonylhydrazine (7.75 g, 41.6 mmol) and left to stir at r.t for 24 hr. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was triturated using  $\text{H}_2\text{O}$  and dried overnight in a desiccator. The crude material was then recrystallized in ethyl acetate, collected by filtration and dried overnight in a desiccator under vacuum to yield the phenylacetic acid **105** (9.84 g, 74%) as a colourless solid.  $\nu_{\text{max}}/\text{cm}^{-1}$  (film) 3274, 3200-2800 (broad), 1688;  $\delta_{\text{H}}$  (500 MHz, Acetone- $\text{d}_6$ ) 10.1 (1H, s, NH), 7.83 (2H, d,  $J$  7.8 Hz, 4-Methylbenzenesulfonamido 2-H), 7.44-7.34 (5H, m, 4-Methylbenzenesulfonamido 3-H, Phenyl 4-H and Phenyl 2-H), 7.32-7.28 (2H, m, Phenyl 3-H), 2.42 (3H, s, Me);  $\delta_{\text{C}}$  (125 MHz, Acetone- $\text{d}_6$ ) 163.3 (C=O), 144.4 (Ar-C), 144.3 (Ar-C), 136.1 (C-2), 130.0 (Ar-C), 129.6 (Ar-C), 129.5 (Ar-C), 128.8 (Ar-C), 128.7 (Ar-C), 127.9 (Ar-C), 20.6 (Me); HRMS found  $\text{MNa}^+$  341.0577.  $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_4$  requires  $\text{MNa}$ , 341.0566. Spectroscopic data are consistent with those reported in the literature.<sup>122</sup>

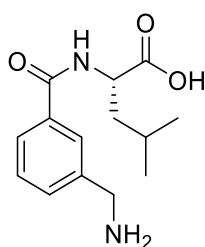
***tert*-Butyl (2*S*)-2-(2-diazo-2-phenylacetamido)-4-methylpentanoate (D12)<sup>105</sup>**



To a solution of 2-[(4-methylbenzenesulfonamido)imino]-2-phenylacetic acid (3.63 g, 15.00 mmol) in toluene (50 mL) was added thionyl chloride (2.2 mL, 30 mmol) and left to stir at 90 °C for 3 hr. The reaction mixture was concentrated under reduced pressure to give a crude material. The crude material was then dissolved in DCM (25 mL) and cooled to 0 °C. A solution of H-Leu-O-*tert*-butyl HCl (6.72 g, 30.00 mmol) in DCM (25 mL) and a separate solution of dimethylaniline (1.89 mL, 15.00 mmol) was added dropwise over 10 min. The reaction mixture was stirred at 0 °C overnight before NEt<sub>3</sub> (10.4 mL, 75.00 mmol) was added over 10 min. The reaction mixture was allowed to return to r.t gradually and the solution stirred overnight. The reaction mixture was washed with sat. Na<sub>2</sub>CO<sub>3</sub> solution (100 mL) and brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–ethyl acetate to yield the *diazo* **D12** (1.24 g, 25%) as an orange yellow. *R<sub>f</sub>* 0.35 (90:10 hexane–ethyl acetate). δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 7.39-7.30 (4H, m, Phenyl 3-H and Phenyl 2-H), 7.22-7.19 (1H, m, Phenyl 4-H), 5.79 (1H, d, *J* 8.4 Hz, NH), 4.60 (1H, td, *J* 8.4 and 5.3 Hz, 2-H), 1.60-1.52 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.44-1.41 (1H, m, 4-H), 1.39 (9H, s, *tert*-Butyl), 0.90 (3H, d, *J* 6.5 Hz, 4-Me), 0.87 (3H, d, *J* 6.5 Hz, 5-H<sub>3</sub>); δ<sub>C</sub> (125 MHz, CDCl<sub>3</sub>) 172.4 (C=O), 164.3 (C=O), 129.7 (Phenyl C-3), 127.6 (Phenyl C-1), 127.3 (Phenyl C-2), 126.3 (Phenyl C-4), 82.0 (*tert*-Butyl C-1), 51.8 (C-2), 42.2 (C-2), 28.0 (*tert*-Butyl C-2), 25.0 (C-4), 22.8 (C-4 Me), 22.1 (C-5)(Diazo carbon not observed); HRMS found MNa<sup>+</sup> 354.1788. C<sub>18</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub> requires *MNa*, 354.1788.

**(2S)-2-(5-Aminopentanamido)-4-methylpentanoic acid (110)**

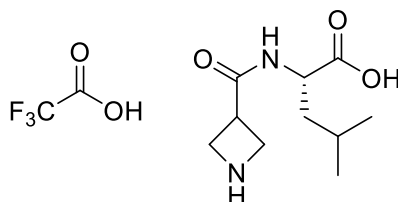
According to general procedure B, 5-((*tert*-butoxycarbonyl)amino)pentanoic acid (86.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **110** (8.9 mg, 49%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.26 (1H, dd, *J* 8.6 and 5.9 Hz, 2-H), 3.04-2.95 (2H, m, Aminopentanamido 5-H<sub>2</sub>), 2.40-2.32 (2H, m, Aminopentanamido 2-H<sub>2</sub>), 1.75-1.57 (7H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Aminopentanamido 3-H<sub>2</sub> and Aminopentanamido 4-H<sub>2</sub>), 0.93 (3H, d, *J* 6.2 Hz, 4-Me), 0.89 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.7 (C=O), 175.8 (C=O), 52.9 (C-2), 39.9 (C-3), 39.0 (Aminopentanamido C-5), 34.7 (Aminopentanamido C-2), 26.1 (Aminopentanamido C-3 or Aminopentanamido C-4), 24.5 (C-4), 22.3 (C-4 Me), 22.1 (Aminopentanamido C-3 or Aminopentanamido C-4), 20.5 (C-5); HRMS found MH<sup>+</sup> 231.1702. C<sub>11</sub>H<sub>23</sub>N<sub>2</sub>O<sub>3</sub> requires *MNa*, 231.1703.

**(2S)-2-{[3-(Aminomethyl)phenyl]formamido}-4-methylpentanoic acid (111)**

According to general procedure B, 3-(((*tert*-butoxycarbonyl)amino)methyl)benzoic acid (99.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **111** (13.6 mg, 65%) as a colorless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 7.82-7.78 (2H, m, Phenyl 2-H

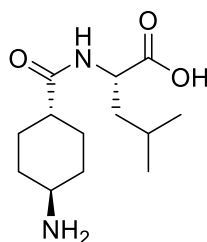
and Phenyl 6-H), 7.65-7.60 (1H, m, Phenyl 4-H), 7.58 (1H, app. t,  $J$  8.0 Hz, Phenyl 5-H), 4.46 (1H, dd,  $J$  11.5 and 7.5 Hz, 2-H), 4.26 (2H, s, Benzyl CH<sub>2</sub>), 1.78-1.70 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.96 (3H, d,  $J$  6.2 Hz, 4-Me), 0.94 (3H, d,  $J$  6.2 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 179.6 (C=O), 169.9 (C=O), 162.9 (q,  $J$  291.6 Hz, TFA C=O), 134.6 (Phenyl C-3), 133.1 (Phenyl C-1), 132.3 (Phenyl C-4), 129.5 (Phenyl C-5), 127.8 (Phenyl C-2 or Phenyl C-6), 127.6 (Phenyl C-2 or Phenyl C-6), 116.3 (q,  $J$  35.4, TFA CF<sub>3</sub>), 54.2 (C-2), 42.8 (Benzyl CH<sub>2</sub>), 40.3 (C-3), 24.8 (C-4), 22.4 (C-4 Me), 20.7 (C-5); HRMS found MH<sup>+</sup> 265.1560. C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires  $MH$ , 265.1547.

**(2S)-2-[(Azetidin-3-yl)formamido]-4-methylpentanoic acid TFA salt (112)**



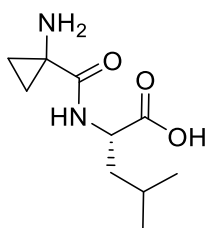
According to general procedure B, 1-boc-azetidine-3-carboxylic acid (80.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **112** (13.0 mg, 50%) as a colourless solid.  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.30-4.24 (5H, m, Azetidiny 2-H<sub>2</sub>, Azetidiny 4-H<sub>2</sub> and 2-H), 3.79 (1H, tt,  $J$  9.0 and 7.3 Hz, Azetidiny 1-H), 1.64-1.59 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.92 (3H, d,  $J$  6.4 Hz, 4-Me), 0.89 (3H, d,  $J$  6.4 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 178.1 (C=O), 171.9 (C=O), 52.9 (C-2), 48.1 (Azetidiny C-2 or Azetidiny C-4), 47.9 (Azetidiny C-2 or Azetidiny C-4), 39.8 (C-3), 35.4 (Azetidiny C-1), 24.4 (C-4), 22.1 (C-4 Me), 20.5 (C-5); HRMS found MH<sup>+</sup> 215.1390. C<sub>10</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> requires  $MH$ , 215.1390.

**(2S)-4-Methyl-2-{-[(1*r*,4*r*)-4-aminocyclohexyl]formamido}pentanoic acid (113)**



According to general procedure B, *trans*-4-(*boc*-amino)cyclohexanecarboxylic acid (96.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **113** as a colourless solid (11.5 mg, 57%).  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.32-4.28 (1H, m, 2-H), 3.19 (1H, tt, *J* 11.2 and 3.8 Hz, Aminocyclohexyl 4-H), 2.34 (1H, tt, *J* 11.9 and 3.4 Hz, Aminocyclohexyl 1-H), 2.15-2.10 (2H, m, Aminocyclohexyl 3-H<sub>a</sub> and Aminocyclohexyl 5-H<sub>a</sub>), 1.99-1.94 (2H, m, Aminocyclohexyl 2-H<sub>a</sub> and Aminocyclohexyl 6-H<sub>a</sub>), 1.70-1.60 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 1.59-1.40 (4H, m, Aminocyclohexyl 2-H<sub>b</sub>, Aminocyclohexyl 3-H<sub>b</sub>, Aminocyclohexyl 5-H<sub>b</sub> and Aminocyclohexyl 6-H<sub>b</sub>), 0.93 (3H, d, *J* 6.3 Hz, 4-Me), 0.88 (3H, d, *J* 6.3 Hz, 5-H<sub>3</sub>); 178.3 (C=O), 167.6 (C=O), 52.2 (C-1), 49.4 (Aminocyclohexyl C-4), 42.8 (Aminocyclohexyl C-1), 39.9 (C-3), 29.2 (Aminocyclohexyl C-3 or Aminocyclohexyl C-5), 29.1 (Aminocyclohexyl C-3 or Aminocyclohexyl C-5), 27.8 (Aminocyclohexyl C-2 or Aminocyclohexyl C-6), 26.5 (Aminocyclohexyl C-2 or Aminocyclohexyl C-6), 24.5 (C-4), 22.3 (C-5), 20.5 (C-5); HRMS found MNa<sup>+</sup> 279.1680. C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires *MNa*, 279.1679

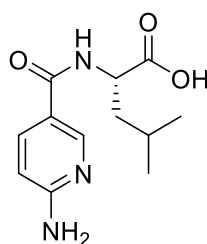
**(2S)-2-[(1-Aminocyclopropyl)formamido]-4-methylpentanoic acid (114)**



According to general procedure B, 1-(*boc*-amino)cyclopropanecarboxylic acid (80.0 mg, 0.395 mmol) gave a crude material. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **114** as a colourless solid (11.0 mg, 65%).  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.32-4.28 (1H, m, 2-H), 1.65-1.60 (4H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and Aminocyclopropyl 2-H<sub>a</sub> and Aminocyclopropyl 3-H<sub>a</sub>), 1.53-1.45 (3H, 4-H and Aminocyclopropyl 2-H<sub>b</sub> and Aminocyclopropyl 3-H<sub>b</sub>), 0.91 (3H, d, *J* 6.2 Hz, 4-Me), 0.88 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.5 (C=O), 170.4 (C=O), 53.2 (C-2), 39.6 (C-3), 35.0 (Aminocyclopropyl C-1), 24.5 (C-4), 22.2 (C-4 Me), 20.5

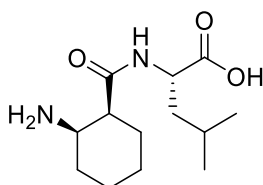
(C-5), 12.9 (Aminocyclopropyl C-2 or Aminocyclopropyl C-3), 12.6 (Aminocyclopropyl C-2 or Aminocyclopropyl C-3); HRMS found  $MH^+$  215.1404.  $C_{10}H_{18}N_2O_3$  requires  $MH$ , 215.1390.

**(2S)-2-[(6-Aminopyridin-3-yl)formamido]-4-methylpentanoic acid (115)**



According to general procedure B, 6-(*boc*-amino)nicotinic acid (94.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **115** (7.1 mg, 36%) as a colourless solid.  $\delta_H$  (500 MHz, MeOD) 8.32 (1H, dd,  $J$  2.2 and 0.7 Hz, Aminopyridinyl 2-H), 8.19 (1H, dd,  $J$  9.4 and 2.2 Hz, Aminopyridinyl 5-H), 7.04 (1H, dd,  $J$  9.4 and 0.7 Hz, Aminopyridinyl 6-H), 4.43-4.38 (1H, m, 2-H), 1.74-1.66 (3H, m, 3- $H_a$ , 3- $H_b$  and 4-H), 0.95 (3H, d,  $J$  6.1 Hz, 4-Me), 0.92 (3H, d,  $J$  6.1 Hz, 5- $H_3$ );  $\delta_C$  (125 MHz, MeOD) 179.4 (C=O), 165.5 (C=O), 155.2 (Aminopyridinyl C-6), 141.5 (Aminopyridinyl C-4), 137.2 (Aminopyridinyl C-2), 119.3 (Aminopyridinyl C-3), 113.3 (Aminopyridinyl C-5), 54.2 (C-2), 40.3 (C-3), 24.7 (C-4), 22.3 (C-4 Me), 20.6 (C-5); HRMS found  $MH^+$  252.1352.  $C_{12}H_{17}N_3O_3$  requires  $MH$ , 252.1343.

**(2S)-2-{[(1R,2S)-2-Aminocyclohexyl]formamido}-4-methylpentanoic acid (116)**

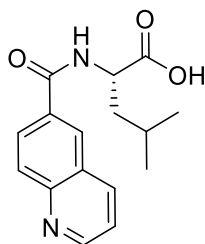


According to general procedure B, *cis*-2-((*tert*-bututoxycarbonyl)amino)cyclohexanecarboxylic acid (96.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient



of 96.5:3.5→76.5:23.5 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **116** (15.7 mg, 78%) as a 60:40 mixture of rotamers as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.26-4.18 (1H, m, 2-H) 3.56 (1H, app. br. s, Aminocyclohexyl 2-H), 2.91-2.85 (0.4H, m, Aminocyclohexyl 1-H<sup>rot(min)</sup>), 2.87-2.78 (0.60H, m, Aminocyclohexyl 1-H<sup>rot(maj)</sup>), 2.01-1.91 (1H, m, Aminocyclohexyl 3-H<sub>a</sub>) 1.88-1.74 (3H, m, Aminocyclohexyl 3-H<sub>b</sub>, Aminocyclohexyl 6-H<sub>2</sub>), 1.71-1.53 (6H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Aminocyclohexyl 4-H<sub>2</sub>, Aminocyclohexyl 5-H<sub>a</sub>), 1.50-1.41 (1H, m, Aminocyclohexyl 5-H<sub>b</sub>), 0.96-0.85 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 179.6 (C=O), 178.8 (C=O), 175.4 (C=O), 53.0 (C-2), 49.3 (Aminocyclohexyl C-2<sup>rot(min)</sup>), 49.2 (Aminocyclohexyl C-2<sup>rot(maj)</sup>), 42.4 (Aminocyclohexyl C-1<sup>rot(maj)</sup>), 42.1 (Aminocyclohexyl C-1<sup>rot(min)</sup>), 39.9 (C-3<sup>rot(min)</sup>), 39.8 (C-3<sup>rot(maj)</sup>), 27.4 (Aminocyclohexyl C-3<sup>rot(maj)</sup>), 27.2 (Aminocyclohexyl C-3<sup>rot(min)</sup>), 25.6 (Aminocyclohexyl C-6), 24.6 (C-4<sup>rot(min)</sup>), 24.5 (C-4<sup>rot(maj)</sup>), 22.8 (Aminocyclohexyl C-4 or Aminocyclohexyl C-5), 22.4 (Aminocyclohexyl C-4 or Aminocyclohexyl C-5), 22.3 (C-4 Me<sup>rot(min)</sup>), 22.2 (C-4 Me<sup>rot(maj)</sup>), 20.7 (C-5<sup>rot(maj)</sup>), 20.4 (C-5<sup>rot(min)</sup>), 20.2 (Aminocyclohexyl C-4 or Aminocyclohexyl C-5), 19.6 (Aminocyclohexyl C-4 or Aminocyclohexyl C-5) (23 out of 26 signals present); HRMS detected MH<sup>+</sup> 257.1869. C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 257.1860.

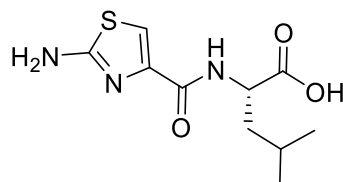
### (2S)-4-Methyl-2-[(quinolin-6-yl)formamido]pentanoic acid (**117**)



According to general procedure B, 6-quinolinecarboxylic acid (68.0 mg, 0.395 mmol) gave a crude material. The crude product was purified *via* MDAP, with a gradient of 82:18→62:38 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **117** (12.2 mg, 54%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 8.93 (1H, dd, *J* 4.3 and 1.5 Hz, Quinolinylyl 2-H), 8.50-8.43 (2H, m, Quinolinylyl 3-H and Quinolinylyl 5-H), 8.19 (1H, dd, *J* 8.8 and 1.9 Hz, Quinolinylyl 7-H), 8.08 (1H, d, *J* 8.8 Hz, Quinolinylyl 8-H), 7.61 (1H, dd, *J* 8.3 and 4.3 Hz, Quinolinylyl 4-H), 4.76-4.65 (1H, m, 2-H), 1.89-1.73 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 1.01 (3H, d, *J* 6.2 Hz, 4-Me), 1.00 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.8 (C-1), 167.9

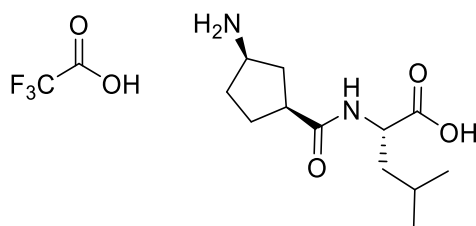
(Formamido C-1), 151.4 (Quinoliny C-2), 148.5 (Ar-C), 137.8 (Quinoliny C-3 or Quinoliny C-5), 132.4 (Ar-C), 128.1 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 122.0 (Quinoliny C-4), 51.5 (C-2), 39.9 (C-3), 24.9 (C-4), 22.1 (C-4 Me), 20.4 (C-5); HRMS found  $MH^+$  287.1454.  $C_{16}H_{18}N_2O_3$  requires  $MH$ , 287.1390.

**(2S)-2-[(2-Amino-1,3-thiazol-4-yl)formamido]-4-methylpentanoic acid (118)**



According to general procedure B, 2-boc-aminothiazole-4-carboxylic acid (96.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 81.5:18.5→61.5:38.5  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **118** (12.0 mg, 59%) as a colourless solid.  $\delta_H$  (500 MHz, MeOD) 7.26 (1H, s, Thioazolyl 5-H), 4.62-4.55 (1H, m, 2-H), 1.76-1.68 (3H, m, 3-H<sub>a</sub> 3-H<sub>b</sub> and 4-H), 0.98-0.94 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, MeOD) 174.3 (C=O), 169.0 (Thiazolyl C-2), 162.2 (C=O), 144.9 (Thiazolyl C-4), 112.8 (Thiazole C-5), 50.4 (C-2), 40.9 (C-3), 24.8 (C-4), 22.0 (C-4 Me), 20.6 (C-5); HRMS found  $MH^+$  258.0919.  $C_{10}H_{15}N_3O_3S$  requires  $MH$ , 258.0907.

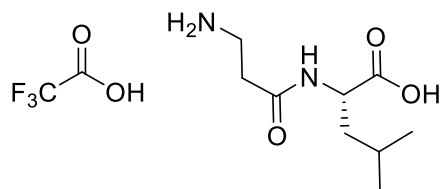
**(2S)-2-{[(1S,3R)-3-Aminocyclopentyl]formamido}-4-methylpentanoic acid TFA salt (119)**



According to general procedure B, (1S,3R)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (96.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 96.4:3.6→76.4:23.6  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **119** (15.2 mg, 57%) as a colourless solid.  $\delta_H$  (500 MHz,  $D_2O$ ) 4.38

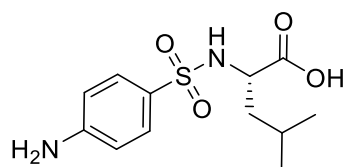
(1H, dd, *J* 9.9 and 4.7 Hz, 2-H), 3.81-3.72 (1H, m, Aminocyclopentyl 3-H), 3.04-2.97 (1H, m, Aminocyclopentyl 1-H), 2.35-2.27 (1H, m, Aminocyclopentyl 2-H<sub>a</sub>), 2.18-2.04 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>a</sub>), 1.95-1.77 (3H, m, Aminocyclopentyl 2-H<sub>b</sub>, Aminocyclopentyl 4-H<sub>b</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.73-1.63 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.94 (3H, d, *J* 6.2 Hz, 4-Me), 0.89 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.4 (C=O), 176.7 (C=O), 163.0 (q, *J* 35.6 Hz, TFA C=O), 116.3 (q, *J* 291.7 Hz, TFA CF<sub>3</sub>) 51.8 (Aminocyclopentyl C-3), 51.5 (C-2), 43.1 (Aminocyclopentyl C-1), 39.2 (C-3), 34.0 (Aminocyclopentyl C-4), 30.1 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 28.3 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 24.4 (C-4), 22.1 (C-4 Me), 20.4 (C-5); HRMS found MH<sup>+</sup> 243.1717. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

**(2S)-2-(3-Aminopropanamido)-4-methylpentanoic acid TFA salt (120)**



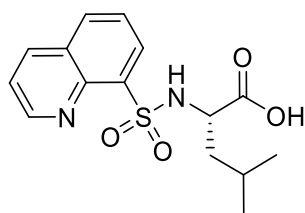
According to general procedure B, boc- $\beta$ -Ala-OH (75.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2 $\rightarrow$ 90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **120** (12.5 mg, 53%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.28 (1H, app. t, *J* 7.0 Hz, 2-H), 3.33-3.21 (2H, m, Aminopropanamido 3-H<sub>2</sub>), 2.74-2.70 (2H, m, Aminopropanamido 2-H<sub>2</sub>), 1.70-1.57 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.93 (3H, d, *J* 5.9 Hz, 4-Me), 0.89 (3H, d, *J* 5.9 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.5 (C=O), 171.9 (C=O), 162.9 (q, *J* 291.6 Hz, TFA C=O), 116.3 (q, *J* 35.4, TFA CF<sub>3</sub>), 52.8 (C-2), 39.8 (C-3), 35.6 (Aminopropanamido C-3), 31.8 (Aminopropanamido C-2), 24.5 (C-4), 22.2 (C-4 Me), 20.5 (C-5); HRMS found MH<sup>+</sup> 203.1405. C<sub>9</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 203.1390.

**(2S)-2-(4-Aminobenzenesulfonamido)-4-methylpentanoic acid (121)**



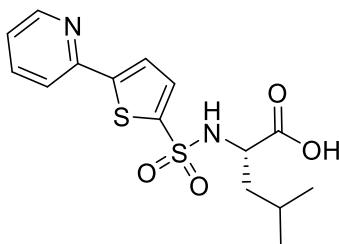
According to general procedure C, *tert*-butyl (4-(chlorosulfonyl)phenyl)carbamate (115.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 77.9:22.1→57.9:42.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **121** (12.0 mg, 53%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.51 (2H, d, *J* 8.3 Hz, Aminobenzenesulfonamido 2-H), 6.66 (2H, d, *J* 8.3 Hz, Aminobenzenesulfonamido 3-H), 3.72 (1H, app. br. s, 2-H), 1.78-1.65 (1H, m, 4-H), 1.58-1.39 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.88 (3H, d, *J* 6.5 Hz, 4-Me), 0.80 (3H, d, *J* 6.5 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.9 (C=O), 152.7 (Aminobenzenesulfonamido C-4), 128.8 (Aminobenzenesulfonamido C-2), 126.3 (Aminobenzenesulfonamido C-1), 112.9 (Aminobenzenesulfonamido C-3), 54.3 (C-2), 41.8 (C-3), 24.1 (C-4), 21.8 (C-4 Me), 20.4 (C-5); HRMS found  $\text{MNa}^+$  309.0880. C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S requires *MNa*, 309.0874.

### (2S)-4-Methyl-2-(quinoline-8-sulfonamido)pentanoic acid (**122**)



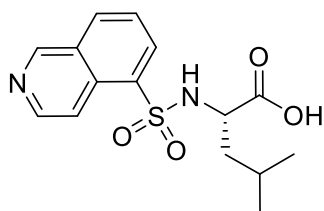
According to general procedure C, 8-quinolinesulfonyl chloride (89.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 78:22→58:42 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **122** (4.8 mg, 19%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 9.04 (1H, dd, *J* 4.2 and 1.7 Hz, Quinoline 2-H), 8.43 (1H, dd, *J* 8.3 and 1.7 Hz, Quinoline 4-H), 8.37 (1H, dd, *J* 7.3 and 1.2 Hz, Quinoline 5-H), 8.20 (1H, dd, *J* 8.2 and 1.2 Hz, Quinoline 7-H), 7.71-7.68 (1H, m, Quinoline 6-H), 7.64 (1H, dd, *J* 8.3 and 4.2 Hz, Quinoline 3-H), 4.19-4.02 (1H, m, 2-H), 1.82-1.65 (1H, m, 4-H), 1.59-1.45 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.87 (3H, d, *J* 6.7 Hz, 4-Me), 0.81 (3H, d, *J* 6.7 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.0 (C=O), 150.9 (Quinoline C-2), 143.3 (Quinoline C-8a), 137.0 (Quinoline C-8), 136.7 (Quinoline C-4), 133.4 (Quinoline C-7), 130.3 (Quinoline C-5), 129.0 (Quinoline C-4a), 125.1 (Quinoline C-6), 122.1 (Quinoline C-3), 55.2 (C-2), 42.1 (C-3), 24.1 (C-4), 21.8 (C-4 Me), 20.4 (C-5); HRMS found  $\text{MH}^+$  323.1071. C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S requires *MH*, 323.1060.

**(2S)-4-Methyl-2-[5-(pyridin-2-yl)thiophene-2-sulfonamido]pentanoic acid (123)**



According to general procedure C, 5-(pyridyl)thiophene-2-sulfonyl chloride (107.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 63.1:36.9→43.1:56.9 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **123** (21.4 mg, 76%) as a brown solid.  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 8.56 (1H, app. br. d, *J* 4.6 Hz, Pyridinyl 6-H), 7.72 (1H, td, *J* 7.8 and 1.5 Hz, Pyridinyl 4-H), 7.59 (1H, app. br. d, *J* 8.0 Hz, Pyridinyl 3-H), 7.56 (1H, d, *J* 3.9 Hz, Thiophene 3-H), 7.40 (1H, d, *J* 3.9 Hz, Thiophene 4-H), 7.25-7.21 (1H, m, Pyridinyl 5-H), 4.05 (1H, dd, *J* 8.5 and 5.9 Hz, 2-H), 3.38 (1H, br.s, NH), 1.94-1.82 (1H, m, 4-H), 1.66-1.47 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.93 (6H, br. d, *J* 6.6 Hz, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, CDCl<sub>3</sub>) 174.3 (C=O), 150.8 (Pyridinyl C-2 or Thiophene C-2), 150.5 (Pyridinyl C-2 or Thiophene C-2), 149.5 (Pyridinyl C-6), 141.6 (Thiophene C-5), 137.4 (Pyridinyl C-4), 133.4 (Thiophene C-3), 124.3 (Thiophene C-4), 123.5 (Pyridinyl C-5), 119.9 (Pyridinyl C-3), 54.6 (C-2), 42.2 (C-3), 24.4 (C-4), 22.8 (C-4 Me), 21.4 (C-5); HRMS found MH<sup>+</sup> 355.0802. C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub> requires *MH*, 355.0781.

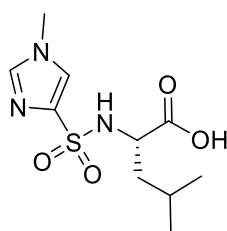
**(2S)-2-(Isoquinoline-5-sulfonamido)-4-methylpentanoic acid (124)**



According to general procedure C, isoquinoline-5-sulfonyl chloride HCl (104.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 78:22→58:42 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **124** as a colourless solid (3.9 mg, 15%).  $\delta_{\text{H}}$  (500 MHz, MeOD) 9.36 (1H, app. br. s, Isoquinoline 1-H), 8.61 (2H, app. br. s, Isoquinoline 3-H and Isoquinoline 4-H), 8.47 (1H, dd, *J* 7.4 and 1.2 Hz, Isoquinoline 6-H), 8.37

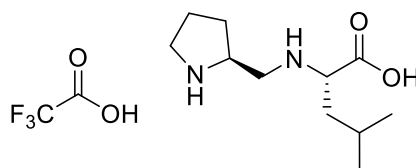
(1H, dd, *J* 8.2 and 0.8 Hz, Isoquinoline 8-H), 7.79 (1H, dd, *J* 8.2 and 7.4 Hz, Isoquinoline 7-H), 3.78 (1H, dd, *J* 10.1 and 4.8 Hz, 2-H), 1.62-1.54 (1H, m, 4-H), 1.49 (1H, ddd, *J* 14.8, 10.1 and 4.8 Hz, 3-H<sub>a</sub>), 1.41 (1H, ddd, *J* 13.8, 9.2 and 4.8 Hz, 3-H<sub>b</sub>), 0.82 (3H, d, *J* 6.7 Hz, 4-Me), 0.63 (3H, d, *J* 6.7 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, MeOD) 174.2 (C=O), 152.5 (Isoquinoline C-1), 143.0 (Isoquinoline C-3), 135.3 (Isoquinoline C-5), 133.4 (Isoquinoline C-6 or Isoquinoline C-8), 133.3 (Isoquinoline C-6 or Isoquinoline C-8), 131.7 (Isoquinoline C-4a or Isoquinoline C-8a), 129.2 (Isoquinoline C-4a or Isoquinoline C-8a), 126.2 (Isoquinoline C-7), 118.3 (Isoquinoline C-4), 54.6 (C-2), 41.4 (C-3), 24.0 C-4), 21.8 (C-4 Me), 19.8 (C-5); HRMS found  $MH^+$  323.1071.  $C_{15}H_{18}N_2O_4S$  requires *MH*, 323.1060. 323.1071.  $C_{15}H_{19}N_2O_4S$  requires *MH*, 323.1060.

**(2S)-4-Methyl-2-(1-methyl-1H-imidazole-4-sulfonamido)pentanoic acid (125)**



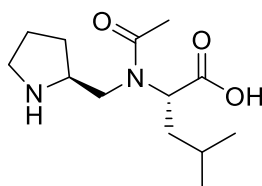
According to general procedure C, 1-methyl-1H-imidazole-4-sulfonyl chloride (71.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 85.7:14.3→65.7:34.3 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **125** (12.4 mg, 57%) as a colourless solid.  $\delta_H$  (500 MHz, MeOD) 7.70 (1H, d, *J* 1.0 Hz, Imidazole 2-H), 7.63 (1H, d, *J* 1.3 Hz, Imidazole 5-H), 3.90 (1H, dd, *J* 8.9 and 5.9 Hz, 2-H), 3.76 (3H, s, Me), 1.75 (1H, dddd, *J* 13.2, 12.2, 7.0 and 4.1 Hz, 4-H), 1.56-1.45 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.90 (3H, d, *J* 6.6 Hz, 4-Me), 0.84 (3H, d, *J* 6.6 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, MeOD) 174.4 (C=O), 139.8 (Imidazole C-4), 139.5 (Imidazole C-2), 124.3 (Imidazole C-5), 54.3 (C-2), 41.7 (C-3), 32.8 (Me), 24.1 (C-4), 21.8 (C-4 Me), 20.2 (C-5); HRMS found  $MNa^+$  298.0842.  $C_{10}H_{17}N_3O_4S$  requires *MNa*, 298.0832.

**(2S)-4-Methyl-2-({[(2S)-pyrrolidin-2-yl]methyl}amino)pentanoate  
TFA salt (**126**)**



According to general procedure D, boc-L-propinal (65.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **126** (7.2 mg, 11%) as a white solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.04 (1H, dd, *J* 8.3 and 4.7 Hz, 2-H), 3.99-3.85 (1H, m, Pyrrolidinyl 2-H), 3.77 (1H, dd, *J* 12.7 and 4.0 Hz, Pyrrolidinyl 5-H<sub>a</sub>), 3.59-3.54 (2H, m, NHCH<sub>2</sub>), 3.16 (1H, app. t, *J* 11.9 Hz, Pyrrolidinyl 5-H<sub>b</sub>), 2.27 (1H, dt, *J* 12.4 and 6.2 Hz, Pyrrolidinyl 3-H<sub>a</sub>), 2.16-2.03 (1H, m, Pyrrolidinyl 4-H<sub>a</sub>), 2.01-1.85 (2H, m, Pyrrolidinyl 3-H<sub>b</sub> and Pyrrolidinyl 4-H<sub>b</sub>), 1.89-1.77 (1H, m, 4-H), 1.79-1.67 (1H, m, 3-H<sub>a</sub>), 1.69-1.59 (1H, m, 3-H<sub>b</sub>), 0.98 (6H, br. d, *J* 5.9 Hz, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 165.7 (C=O), 163.0 (q, *J* 35.5 Hz, TFA C=O), 116.3 (q, *J* 291.6 Hz, TFA CF<sub>3</sub>), 54.2 (Pyrrolidinyl C-2), 53.3 (C-2), 45.9 (NHCH<sub>2</sub>), 42.6 (Pyrrolidinyl C-5), 37.9 (C-3), 29.8 (Pyrrolidinyl C-3), 24.3 (C-4), 21.8 (Pyrrolidinyl C-4), 21.7 (C-4 Me), 20.6 (C-5); HRMS found MNa<sup>+</sup> 237.1515. C<sub>11</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub> requires *MNa*, 237.1573.

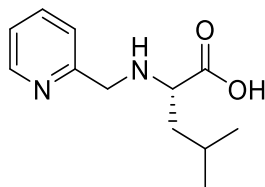
**(2S)-4-Methyl-2-(N-{{[(2S)-pyrrolidin-2-yl]methyl}acetamido}pentanoic acid (**127**)**



According to general procedure E, boc-L-propinal (65.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.4:6.6→73.4:26.6 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **127** (12.1 mg, 21%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 4.47-4.40 (0.4H, m, Pyrrolidinyl 2-H<sup>rot(min)</sup>), 4.34 (0.4H, dd, *J* 10.5 and 4.5 Hz, 2-H<sup>rot(min)</sup>), 4.24

(0.6H, dd,  $J$  8.8 and 6.2 Hz, 2-H<sup>rot(maj)</sup>), 3.90 (0.4H, dd,  $J$  14.9 and 9.7 Hz, NCH<sub>a</sub><sup>rot(min)</sup>), 3.84-3.75 (0.6H, m, 2-H Pyrrolidinyl<sup>rot(maj)</sup>), 3.60 (0.4H, dd,  $J$  14.3 and 3.8 Hz, NCH<sub>b</sub><sup>rot(min)</sup>), 3.49-3.42 (0.6H, m, NCH<sub>a</sub><sup>rot(maj)</sup>), 3.29-3.13 (2.6H, m, Pyrrolidinyl 5-H<sub>2</sub> and NCH<sub>b</sub><sup>rot(maj)</sup>), 2.15-1.53 (10H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Pyrrolidinyl 3-H<sub>2</sub>, Pyrrolidinyl 4-H<sub>2</sub>, Me), 1.01-0.96 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, MeOD) 174.7 (C=O), 173.9 (C=O), 62.5 (C-2), 60.7 (Pyrrolidinyl C-2<sup>rot(min)</sup>), 55.9 (Pyrrolidinyl C-2<sup>rot(maj)</sup>), 45.9 (NCH<sub>2</sub> or Pyrrolidinyl C-5), 44.8 (NCH<sub>2</sub> or Pyrrolidinyl C-5), 44.4 (NCH<sub>2</sub> or Pyrrolidinyl C-5), 39.9 (C-3<sup>rot(maj)</sup>), 38.9 (C-3<sup>rot(maj)</sup>), 28.3 (Pyrrolidinyl C-3 or Pyrrolidinyl C-4), 27.5 (Pyrrolidinyl C-3 or Pyrrolidinyl C-4), 25.0 (C-4<sup>rot(maj)</sup>), 25.0 (C-4<sup>rot(min)</sup>), 23.9 (Pyrrolidinyl C-3 or Pyrrolidinyl C-4), 22.3 (Pyrrolidinyl C-3 or Pyrrolidinyl C-4 or C-5), 22.2 (Pyrrolidinyl C-3 or Pyrrolidinyl C-4 or C-4 Me), 21.9 (C-4 Me), 21.1 (C-5<sup>rot(maj)</sup>), 21.1 (C-5<sup>rot(min)</sup>), 20.8 (Me<sup>rot(maj)</sup>), 20.8 (Me<sup>rot(min)</sup>)(22/26 signals present); HRMS found MH<sup>+</sup>, 261.1582. C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires  $MH$  257.1860.

**((2S)-4-Methyl-2-[[pyridin-2-yl]methyl]amino}pentanoic acid (128)**

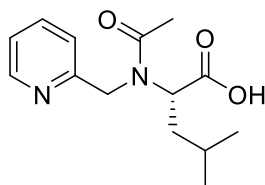


According to general procedure D, 2-pyridinecarboxaldehyde (35  $\mu$ L, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2 $\rightarrow$ 90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **128** (13.6 mg, 28%) as a colourless solid.  $\delta_H$  (500 MHz, D<sub>2</sub>O) 8.60 (1H, ddd,  $J$  5.0, 1.7 and 0.9 Hz, Pyridinyl 3-H), 7.91 (1H, td,  $J$  7.8 and 1.7 Hz, Pyridinyl 5-H), 7.51 (1H, app. br. d,  $J$  7.8 Hz, Pyridinyl 6-H), 7.48 (1H, ddd,  $J$  7.8, 5.0 and 1.1 Hz, Pyridinyl 4-H), 4.39 (1H, d,  $J$  14.1 Hz, NHCH<sub>a</sub>), 4.33 (1H, d,  $J$  14.1 Hz, NHCH<sub>b</sub>), 3.57 (1H, app. t,  $J$  6.8 Hz, 2-H), 1.80-1.64 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.84 (3H, d,  $J$  6.0 Hz, 4-Me), 0.81 (3H, d,  $J$  6.0 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 174.0 (C=O), 150.0 (Pyridinyl C-1), 149.4 (Pyridinyl C-6), 138.5 (Pyridinyl C-4), 124.5 (Pyridinyl C-2 or Pyridinyl C-5), 124.3 (Pyridinyl C-2 or Pyridinyl C-5), 61.1 (C-2), 50.3 (NHCH<sub>2</sub>), 39.1 (C-3), 24.4 (C-4), 21.7 (C-4 Me), 21.3 (C-5); HRMS



found  $MNa^+$  245.1272.  $C_{12}H_{18}N_2O_2$  requires  $MNa$  245.1260. 323.1071.  $C_{15}H_{19}N_2O_4S$  requires  $MH$ , 323.1060.

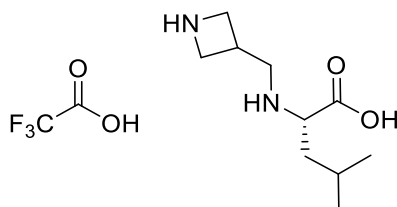
**(2S)-4-Methyl-2-{N-[(pyridin-2-yl)methyl]acetamido}pentanoic acid (129)**



According to general procedure E, 2-pyridinecarboxaldehyde (35  $\mu$ L, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2 $\rightarrow$ 90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **129** (6.7 mg, 12%) as a 70:30 mixture of rotamers as a colourless oil.  $\delta_H$  (500 MHz, MeOD) 8.44 (0.7H, ddd,  $J$  5.0, 1.7 and 0.9 Hz, Pyridiny 6-H<sup>rot(maj)</sup>), 8.37 (0.3, ddd,  $J$  5.2, 1.7 and 0.9 Hz, Pyridiny 6-H<sup>rot(min)</sup>), 7.96 (0.3H, td,  $J$  7.8 and 1.7 Hz, Pyridiny 4-H<sup>rot(min)</sup>), 7.92 (0.7H, td,  $J$  7.7 and 1.7 Hz, Pyridiny 4-H<sup>rot(maj)</sup>), 7.56 (0.3H, app. br. d,  $J$  7.9 Hz, Pyridiny 5-H<sup>rot(min)</sup>), 7.52 (0.7H, app. br. d,  $J$  7.9 Hz, Pyridiny 5-H<sup>rot(maj)</sup>), 7.46-7.40 (1H, m, Pyridiny 2-H), 4.94 (0.7H, d,  $J$  18.2 Hz, NHCH<sub>a</sub><sup>rot(maj)</sup>), 4.88 (0.3H, d,  $J$  16.4 Hz, NHCH<sub>a</sub><sup>rot(min)</sup>), 4.62 (0.7H, d,  $J$  18.2 Hz, NHCH<sub>b</sub><sup>rot(maj)</sup>), 4.46 (0.3H, dd,  $J$  9.3 and 5.2 Hz, 2-H<sup>rot(min)</sup>), 4.41 (0.7H, dd,  $J$  8.7 and 5.4 Hz, 2-H<sup>rot(maj)</sup>), 4.35 (0.3H, d,  $J$  18.2 Hz, NHCH<sub>b</sub><sup>rot(min)</sup>), 2.20 (0.9H, s, Me<sup>rot(min)</sup>), 2.03 (2.1H, s, Me<sup>rot(maj)</sup>), 1.91-1.83 (1H, m, 3-H<sub>a</sub><sup>rot(min)</sup>), 1.79-1.70 (1H, m, 3-H<sub>b</sub>), 1.64-1.58 (0.7H, m, 4-H<sup>rot(maj)</sup>), 1.40 (0.3H, m, 4-H<sup>rot(min)</sup>), 0.85 (0.9H, d,  $J$  6.6 Hz, 4-Me<sup>rot(min)</sup>), 0.82 (2.1H, d,  $J$  6.6 Hz, 4-Me<sup>rot(maj)</sup>), 0.72 (2.1H, d,  $J$  6.6 Hz, 5-H<sub>3</sub><sup>rot(maj)</sup>), 0.71 (0.9H, d,  $J$  6.6 Hz, 5-H<sub>3</sub><sup>rot(min)</sup>);  $\delta_C$  (125 MHz, MeOD) 173.7 (C=O), 173.3 (C=O), 173.1 (C=O), 157.5 (Pyridinyl C-1<sup>rot(min)</sup>), 156.7 (Pyridinyl C-1<sup>rot(maj)</sup>), 147.9 (Pyridinyl C-6<sup>rot(maj)</sup>), 145.9 (Pyridinyl C-6<sup>rot(min)</sup>), 139.5 (Pyridinyl C-4<sup>rot(min)</sup>) 138.3 (Pyridinyl C-4<sup>rot(maj)</sup>), 123.2 (Pyridinyl C-2<sup>rot(min)</sup> or Pyridinyl C-5<sup>rot(min)</sup>), 123.1 (Pyridinyl C-2<sup>rot(maj)</sup> or Pyridinyl C-5<sup>rot(maj)</sup>), 122.8 (Pyridinyl C-2<sup>rot(min)</sup> or Pyridinyl C-5<sup>rot(min)</sup>), 122.1 (Pyridinyl C-2<sup>rot(maj)</sup> or Pyridinyl C-5<sup>rot(maj)</sup>), 53.0 (NCH<sub>2</sub><sup>rot(maj)</sup>), 50.7 (NCH<sub>2</sub><sup>rot(min)</sup>), 40.2 (C-2), 39.0 (C-3<sup>rot(min)</sup>), 38.2 (C-3<sup>rot(maj)</sup>), 24.7 (C-4<sup>rot(min)</sup>), 24.6 (C-4<sup>rot(maj)</sup>), 21.9 (C-4 Me<sup>rot(maj)</sup>), 21.8 (C-4 Me<sup>rot(min)</sup>), 21.0 (C-5<sup>rot(maj)</sup>), 20.7 (C-5<sup>rot(min)</sup>), 20.6 (Me<sup>rot(maj)</sup>), 20.5

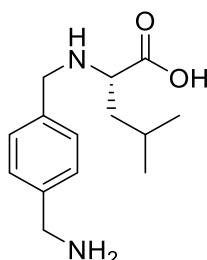
(Me<sup>rot(min)</sup>)(26/28 signals present); HRMS found MH<sup>+</sup> 265.1543. C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires MH, 265.1547.

**(2S)-2-[[4-(Azetidin-3-yl)methyl]amino]-4-methylpentanoic acid TFA salt (130)**



According to general procedure D, *tert*-butyl 3-formylazetidine-1-carboxylate (122.0 mg, 0.33 mmol) gave a crude material. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **130** (10.5 mg, 16%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.31-4.20 (2H, m, Azetidiny 2-H<sub>a</sub> and Azetidiny 4-H<sub>a</sub>), 4.09-3.93 (2H, m, Azetidiny 2-H<sub>b</sub> or Azetidiny 4-H<sub>b</sub>), 3.69 (1H, dd, *J* 7.9 and 5.9 Hz, 2-H), 3.47-3.30 (3H, m, Azetidiny 3-H and NHCH<sub>2</sub>), 1.84-1.61 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.96 (3H, d, *J* 6.1 Hz, 4-Me), 0.94 (3H, d, *J* 6.1 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 172.9 (C=O), 162.9 (q, *J* 35.5 C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 60.8 (C-2), 49.3 (Azetidiny C-2 or Azetidiny C-4), 49.1 (Azetidiny C-2 or Azetidiny C-4), 48.3 (NHCH<sub>2</sub>), C-3, 28.6 (Azetidiny C-3), 24.3 (C-4), 21.9 (C-4 Me), 21.0 (C-5); HRMS found MH<sup>+</sup> 201.1604. C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> requires MH, 201.1598.

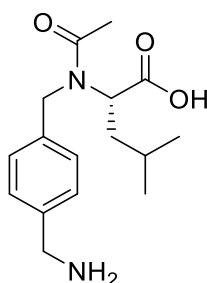
**(2S)-2-([4-(Aminomethyl)phenyl]methyl)amino)-4-methylpentanoic acid (131)**



According to general procedure D, *tert*-butyl N-(4-formylbenzyl)carbamate (155.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield

the *pentanoic acid* **131** (4.8 mg, 9%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz,  $\text{D}_2\text{O}$ ) 7.54-7.51 (4H, m, Phenyl 2-H and Phenyl 3-H), 4.29 (1H, d,  $J$  13.2 Hz,  $\text{NHCH}_a$ ), 4.25-4.21 (3H, m,  $\text{NHCH}_b$  and  $\text{NH}_2\text{CH}_2$ ), 3.54 (1H, app. t,  $J$  6.8 Hz, 2-H), 1.74-1.61 (3H, m, 3- $\text{H}_a$ , 3- $\text{H}_b$  and 4-H), 0.91 (3H, d,  $J$  6.2 Hz, 4-Me), 0.85 (3H, d,  $J$  6.2 Hz, 5- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{D}_2\text{O}$ ) 174.0 (C=O), 134.1 (Phenyl C-1), 131.3 (Phenyl C-4), 130.9 (Phenyl C-3), 129.5 (Phenyl C-2), 60.4 (C-2), 49.6 ( $\text{NHCH}_2$ ), 42.6 ( $\text{NH}_2\text{CH}_2$ ), 39.2 (C-3), 24.4 (C-4), 21.7 (C-4 Me), 21.2 (C-5); HRMS found  $\text{MH}^+$  251.1760.  $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_2$  requires  $\text{MH}$ , 251.1754.

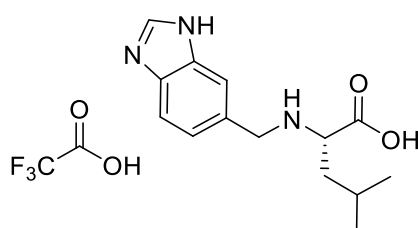
**(2S)-2-(N-{[4-(Aminomethyl)phenyl]methyl}acetamido)-4-methylpentanoic acid (132)**



According to general procedure E, *tert*-butyl *N*-(4-formylbenzyl)carbamate (155.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 89.7:10.3→69.7:30.3  $\text{H}_2\text{O}$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **132** (4.4 mg, 7%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.47-7.40 (2H, m, Phenyl 3-H), 7.37-7.30 (2H, m, Phenyl 2-H), 4.98 (0.6H, d,  $J$  15.9 Hz,  $\text{NCH}_a^{\text{rot(maj)}}$ ), 4.90-4.85 (0.4H, m, 2- $\text{H}^{\text{rot(min)}}$  under MeOD), 4.84-4.78 (0.4H, d,  $J$  14.4 Hz,  $\text{NCH}_a^{\text{rot(min)}}$ ), 4.57 (0.4H, d,  $J$  14.4 Hz,  $\text{NCH}_b^{\text{rot(min)}}$ ), 4.38 (0.6H, app. t,  $J$  6.8 Hz, 2- $\text{H}^{\text{rot(maj)}}$ ), 4.24 (0.6H, d,  $J$  15.9 Hz,  $\text{NCH}_b^{\text{rot(maj)}}$ ), 4.11 (0.8H, app. br. s,  $\text{NH}_2\text{CH}_2^{\text{rot(min)}}$ ), 4.06 (1.2H, app. br. s,  $\text{NH}_2\text{CH}_2^{\text{rot(maj)}}$ ), 2.25 (1.8H, s,  $\text{Me}^{\text{rot(maj)}}$ ), 2.05 (1.2H, s,  $\text{Me}^{\text{rot(min)}}$ ), 1.86-1.72 (1H, m, 3- $\text{H}_a$ ), 1.61-1.54 (0.6H, m, 4- $\text{H}^{\text{rot(maj)}}$ ), 1.54-1.44 (1H, m, 3- $\text{H}_b$ ), 1.44-1.37 (0.4H, m, 4- $\text{H}^{\text{rot(min)}}$ ), 0.89 (1.8H, d,  $J$  6.6 Hz, 4- $\text{Me}^{\text{rot(maj)}}$ ), 0.99 (1.8H, d,  $J$  6.6 Hz, 5- $\text{H}_3^{\text{rot(maj)}}$ ), 0.77 (1.2H, d,  $J$  6.6 Hz, 4- $\text{Me}^{\text{rot(min)}}$ ), 0.65 (1.2 H, d,  $J$  6.6 Hz, 5- $\text{H}_3^{\text{rot(min)}}$ );  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.0 (C=O $^{\text{rot(maj)}}$ ), 174.5 (C=O $^{\text{rot(min)}}$ ), 173.5 (C=O $^{\text{rot(maj)}}$ ), 173.4 (C=O $^{\text{rot(min)}}$ ), 140.5 (Phenyl C-1 $^{\text{rot(maj)}}$ ), 139.3 (Phenyl C-1 $^{\text{rot(min)}}$ ), 132.1 (Phenyl C-4 $^{\text{rot(min)}}$ ), 131.3 (Phenyl C-4 $^{\text{rot(maj)}}$ ), 128.9 (Phenyl C-2 $^{\text{rot(min)}}$  or Phenyl C-3 $^{\text{rot(min)}}$ ), 128.4 (Phenyl C-2 $^{\text{rot(maj)}}$  or Phenyl C-3 $^{\text{rot(maj)}}$ ), 128.0

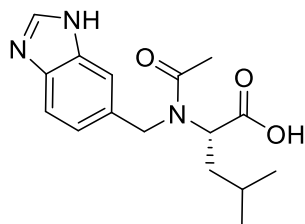
(Phenyl C-2<sup>rot(maj)</sup> or Phenyl C-3<sup>rot(maj)</sup>), 127.2 (Phenyl C-2<sup>rot(min)</sup> or Phenyl C-3<sup>rot(min)</sup>), 62.0 (C-2<sup>rot(maj)</sup>), 57.6 (C-2<sup>rot(min)</sup>), 50.2 (NCH<sub>2</sub><sup>rot(maj)</sup>), 47.6 (NCH<sub>2</sub><sup>rot(min)</sup>), 42.6 (NH<sub>2</sub>CH<sub>2</sub><sup>rot(maj)</sup>), 42.5 (NH<sub>2</sub>CH<sub>2</sub><sup>rot(min)</sup>), 39.4 (C-3<sup>rot(maj)</sup>), 38.8 (C-3<sup>rot(min)</sup>), 25.2 (C-4<sup>rot(min)</sup>), 24.8 (C-4<sup>rot(maj)</sup>), 21.6 (C-4 Me<sup>rot(min)</sup>), 21.5 (C-4 Me<sup>rot(maj)</sup>), 21.2 (C-5<sup>rot(min)</sup>), 21.0 (C-5<sup>rot(maj)</sup>), 20.9 (Me<sup>rot(min)</sup>), 20.8 (Me<sup>rot(maj)</sup>) (28/28 signals present); HRMS found MH<sup>+</sup>, 293.1861. C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 293.1860.

**(2S)-2-{[(1H-1,3-Benzodiazol-6-yl)methyl]amino}-4-methylpentanoic acid TFA salt (133)**



According to general procedure D, 1-H benzimidazole-5-carbaldehyde (96.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **133** (13.5 mg, 16%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 9.19 (1H, s, Benzodiazolyl 2-H), 8.00 (1H, app. br. s, Benzodiazolyl 5-H), 7.97 (1H, d, *J* 8.6 Hz, Benzodiazolyl 8-H), 7.73 (1H, app. d, *J* 8.6 Hz, Benzodiazolyl 7-H), 4.52 (1H, d, *J* 13.2 Hz, NHCH<sub>a</sub>), 4.46 (1H, d, *J* 13.2 Hz, NHCH<sub>b</sub>), 3.66 (1H, dd, *J* 7.4 and 6.1 Hz, 2-H), 1.85-1.65 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.97 (3H, d, *J* 6.0 Hz, 4-Me), 0.92 (3H, d, *J* 6.0 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 173.9 (C=O), 163.0 (q, *J* 35.4, TFA C=O), 140.7 (Benzodiazolyl C-2), 131.6 (Benzodiazolyl C-9), 131.2 (Benzodiazolyl C-4), 128.6 (Benzodiazolyl C-6), 127.8 (Benzodiazolyl C-7), 116.6 (Benzodiazolyl C-5), 116.3 (q, *J* 291.7, C=O CF<sub>3</sub>), 115.3 (Benzodiazolyl C-8), 60.6 (C-2), 49.9 (NHCH<sub>2</sub>), 39.2 (C-3), 24.4 (C-4), 21.7 (C-4 Me), 21.2 (C-5); HRMS found MH<sup>+</sup> 262.1547. C<sub>14</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> requires *MH*, 262.1550.

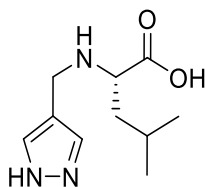
**(2S)-2-{N-[(1H-1,3-Benzodiazol-6-yl)methyl]acetamido}-4-methylpentanoic acid (**134**)**



According to general procedure E, 1-H benzimidazole-5-carbaldehyde (96.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 92.9:7.1→72.9:27.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **134** (10.0 mg, 15%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 8.26-8.20 (1H, m, Benzodiazolyl 2-H), 7.63-7.59 (1.4H, m, Benzodiazolyl 5-H and Benzodiazolyl 7-H<sup>rot(min)</sup>), 7.57-7.51 (0.6H, m, Benzodiazolyl 7-H<sup>rot(maj)</sup>), 7.27 (1H, d, *J* 8.5 Hz, Benzodiazolyl 8-H), 5.07 (0.4H, d, *J* 15.5 Hz, NCH<sub>a</sub><sup>rot(min)</sup>), 4.84 (0.6H, d, *J* 17.0 Hz, NCH<sub>a</sub><sup>rot(maj)</sup>), 4.72 (0.6H, d, *J* 17.0 Hz, NCH<sub>b</sub><sup>rot(maj)</sup>), 4.68 (0.6H, app. t, *J* 6.5 Hz, 2-H<sup>rot(maj)</sup>), 4.51 (0.4H, dd, *J* 8.9 and 5.3 Hz, 2-H<sup>rot(min)</sup>), 4.34 (0.4H, d, *J* 15.5 Hz, NCH<sub>b</sub><sup>rot(min)</sup>), 2.26 (1.2H, s, Me<sup>rot(min)</sup>), 2.15 (1.8H, s, Me<sup>rot(maj)</sup>), 1.89-1.81 (0.6H, m, 3-H<sub>a</sub><sup>rot(maj)</sup>), 1.74 (0.4H, ddd, *J* 14.2, 8.8 and 5.4 Hz, 3-H<sub>a</sub><sup>rot(min)</sup>), 1.64 (0.4H, ddd, *J* 14.1, 9.1 and 4.7 Hz, 3-H<sub>b</sub><sup>rot(min)</sup>), 1.58-1.49 (1.2H, m, 3-H<sub>b</sub><sup>rot(maj)</sup> and 4-H<sup>rot(maj)</sup>), 1.34-1.26 (0.4H, m, 4-H<sup>rot(min)</sup>), 0.89 (1.2H, d, *J* 6.6 Hz, 4-Me<sup>rot(min)</sup>), 0.81 (1.8H, d, *J* 6.6 Hz, 4-Me<sup>rot(maj)</sup>), 0.65 (1.8H, d, *J* 6.6 Hz, 5-H<sub>3</sub><sup>rot(maj)</sup>), 0.49 (1.2H, d, *J* 6.6 Hz, 5-H<sub>3</sub><sup>rot(min)</sup>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 173.6 (C=O), 173.4 (C=O), 173.3 (C=O), 141.7 (Benzodiazolyl C-2<sup>rot(maj)</sup>), 141.2 (Benzodiazolyl C-2<sup>rot(min)</sup>), 137.3 (Benzodiazolyl C-5<sup>rot(maj)</sup> or Benzodiazolyl C-5<sup>rot(maj)</sup>), 136.7 (Benzodiazolyl C-5<sup>rot(maj)</sup> or Benzodiazolyl C-7<sup>rot(maj)</sup>), 136.4 (Benzodiazolyl C-5<sup>rot(min)</sup> or Benzodiazolyl C-7<sup>rot(min)</sup>), 136.0 (Benzodiazolyl C-5<sup>rot(min)</sup> or Benzodiazolyl C-7<sup>rot(min)</sup>), 134.1 (Benzodiazolyl C-9<sup>rot(min)</sup>), 132.2 (Benzodiazolyl C-9<sup>rot(maj)</sup>), 123.2 (Benzodiazolyl C-8<sup>rot(min)</sup>), 121.9 (Benzodiazolyl C-8<sup>rot(maj)</sup>), 115.1 (Benzodiazolyl C-4<sup>rot(maj)</sup> or Benzodiazolyl C-6<sup>rot(maj)</sup>), 114.5 (Benzodiazolyl C-4<sup>rot(min)</sup> or Benzodiazolyl C-6<sup>rot(min)</sup>), 113.9 (Benzodiazolyl C-4<sup>rot(min)</sup> or Benzodiazolyl C-6<sup>rot(min)</sup>), 113.3 (Benzodiazolyl C-4<sup>rot(maj)</sup> or Benzodiazolyl C-6<sup>rot(maj)</sup>), 60.3 (C-2<sup>rot(min)</sup>), 56.9 (C-2<sup>rot(maj)</sup>), 51.6 (NCH<sub>2</sub><sup>rot(maj)</sup>), 48.6 (NCH<sub>2</sub><sup>rot(min)</sup>), 39.0 (C-3<sup>rot(maj)</sup>), 38.4 (C-3<sup>rot(min)</sup>), 25.1 (C-4<sup>rot(maj)</sup>), 24.5 (C-4<sup>rot(min)</sup>), 21.4 (C-4 Me<sup>rot(maj)</sup>), 21.3 (C-4 Me<sup>rot(min)</sup>), 21.2

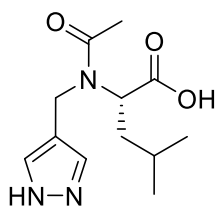
(C-5<sup>rot(maj)</sup>), 21.0 (C-5<sup>rot(min)</sup>), 20.9 (Me<sup>rot(maj)</sup>), 20.7 (Me<sup>rot(min)</sup>)(31/32 signals present); HRMS found MH<sup>+</sup> 304.1669. C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub> requires *MH*, 304.1656.

**(2S)-4-Methyl-2-{[(1H-pyrazol-4-yl)methyl]amino}pentanoic acid (135)**



According to general procedure D, *tert*-butyl 4-formyl-1H-pyrazole-1-carboxylate (125.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **135** (11.0 mg, 24%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.67 (2H, s, Pyrazolyl 3-H and Pyrazolyl 5-H), 4.10 (1H, d, *J* 13.6 Hz, NHCH<sub>a</sub>), 4.05 (1H, d, *J* 13.6 Hz, NHCH<sub>b</sub>), 3.57 (1H, app. t, *J* 6.8 Hz, 2-H), 1.87-1.74 (2H, m, 3-H<sub>a</sub> and 4-H), 1.68-1.59 (1H, m, 3-H<sub>b</sub>), 0.89 (3H, d, *J* 6.3, 4-Me), 0.84 (3H, d, *J* 6.3, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 171.3 (C=O), 134.8 (Pyrazolyl C-3 or Pyrazolyl C-5), 134.7 (Pyrazolyl C-3 or Pyrazolyl C-5), 110.4 (Pyrazolyl C-4), 58.9 (C-2), 40.0 (NHCH<sub>2</sub>), 39.1 (C-3), 24.6 (C-4), 21.7 (C-4 Me), 20.9 (C-5); HRMS found MNa<sup>+</sup> 234.1213. C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub> requires *MNa*, 234.1213.

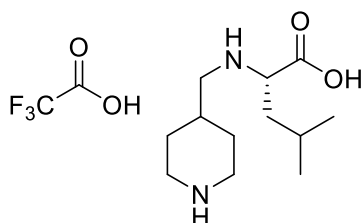
**(2S)-4-Methyl-2-{N-[(1H-pyrazol-4-yl)methyl]acetamido}pentanoic acid (136)**



According to general procedure E, *tert*-butyl 4-formyl-1H-pyrazole-1-carboxylate (125.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 92.9:7.1→72.9:27.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **136** (5.8 mg, 10%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.63 (1.2H, br. s, Pyrazolyl 3-H<sup>rot(maj)</sup> and Pyrazolyl 5-H<sup>rot(maj)</sup>), 7.54 (0.8H, br. s, Pyrazolyl 3-

H<sup>rot(min)</sup> and Pyrazolyl 5-H<sup>rot(min)</sup>), 4.71 (0.6H, dd, *J* 8.8 and 5.6 Hz, 2-H<sup>rot(maj)</sup>), 4.62 (1H, d, *J* 16.3 Hz, NHCH<sub>a</sub>), 4.52-4.44 (1H, m, NHCH<sub>b</sub><sup>rot(maj)</sup> and 2-H<sup>rot(min)</sup>), 4.15 (0.4H, d, *J* 15.2 Hz, NHCH<sub>b</sub><sup>rot(min)</sup>), 2.19 (1.2H, s, Me<sup>rot(min)</sup>), 2.15 (1.8H, s, Me<sup>rot(maj)</sup>), 1.87-1.76 (1.4H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub><sup>rot(min)</sup>), 1.65 (0.6H, ddd, *J* 14.1, 8.8 and 5.3 Hz, 3-H<sub>b</sub><sup>rot(maj)</sup>), 1.54-1.43 (0.6H, m, 4-H<sup>rot(maj)</sup>), 1.37-1.29 (0.4H, m, 4-H<sup>rot(min)</sup>), 0.90 (1.2H, d, *J* 6.7 Hz, 4-Me<sup>rot(min)</sup>), 0.87 (1.8H, d, *J* 6.7 Hz, 4-Me<sup>rot(maj)</sup>), 0.79 (1.8H, d, *J* 6.7 Hz, 5-H<sub>3</sub><sup>rot(maj)</sup>), 0.75 (1.2H, d, *J* 6.7 Hz, 5-H<sub>3</sub><sup>rot(min)</sup>); δ<sub>c</sub> (125 MHz, MeOD) 173.3 (C=O<sup>rot(min)</sup>), 172.9 (C=O<sup>rot(maj)</sup>), 172.8 (C=O<sup>rot(min)</sup>), 172.7 (C=O<sup>rot(maj)</sup>), 133.6 (br. s, Pyrazolyl C-3<sup>rot(min)</sup> and Pyrazolyl C-5<sup>rot(min)</sup>), 132.8 (br. s, Pyrazolyl C-3<sup>rot(maj)</sup> and Pyrazolyl C-5<sup>rot(maj)</sup>), 117.8 (Pyrazolyl C-4<sup>rot(min)</sup>), 117.1 (Pyrazolyl C-4<sup>rot(maj)</sup>), 59.4 (C-2<sup>rot(min)</sup>), 56.5 (C-2<sup>rot(maj)</sup>), 42.3 (NCH<sub>2</sub><sup>rot(min)</sup>), 39.0 (C-3<sup>rot(min)</sup>), 38.1 (C-3<sup>rot(maj)</sup>), 37.5 (NCH<sub>2</sub><sup>rot(maj)</sup>), 24.9 (C-4<sup>rot(maj)</sup>), 24.3 (C-4<sup>rot(min)</sup>), 21.7 (C-4 Me<sup>rot(maj)</sup>), 21.6 (C-4 Me<sup>rot(min)</sup>), 20.9 (C-5<sup>rot(min)</sup>), 20.8 (C-5<sup>rot(maj)</sup>), 20.6 (Me<sup>rot(maj)</sup>), 20.5 (Me<sup>rot(min)</sup>) (22/24 signals present); HRMS found MNa<sup>+</sup> 276.1321. C<sub>12</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub> requires *MNa*, 276.1319.

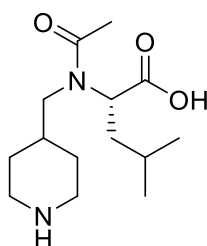
**(2S)-4-Methyl-2-{[(piperidin-4-yl)methyl]amino}pentanoic acid TFA salt (137)**



According to general procedure D, 1-boc-piperidine-4-carboxaldehyde (140.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **137** (28.3 mg, 36%) as a colourless solid. δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 3.85 (1H, dd, *J* 8.5 and 5.4 Hz, 2-H), 3.48 (1H, app. br. s, NHCH<sub>a</sub>), 3.46 (1H, app. br. s, NHCH<sub>b</sub>), 3.12-2.92 (4H, m, Piperidiny 2-H<sub>2</sub> and Piperidiny 6-H<sub>2</sub>), 2.15-1.97 (3H, m, Piperidiny 4-H and Piperidiny 5-H<sub>2</sub>), 1.87-1.78 (1H, m, Piperidiny 3-H<sub>a</sub>), 1.77-1.64 (2H, Piperidiny 3-H<sub>b</sub> and 4-H), 1.56-1.43 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.95 (6H, app. t, *J* 5.5 Hz, 4-Me and 5-H<sub>3</sub>); δ<sub>c</sub> (125 MHz, D<sub>2</sub>O) 172.3 (C=O), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 60.1 (C-2), 50.9 (Piperidiny C-6), 43.1 (NHCH<sub>2</sub> or Piperidiny C-2), 43.0 (NHCH<sub>2</sub> or Piperidiny C-2), 38.2 (Piperidiny

C-3), 30.9 (Piperidinyl C-4), 25.9 (C-3 or Piperidinyl C-5), 25.8 (C-3 or Piperidinyl C-5), 24.4 (C-4), 21.9 (C-4 Me), 20.8 (C-5); HRMS found  $MH^+$  229.1916.  $C_{12}H_{24}N_2O_2$  requires  $MH$ , 229.1911.

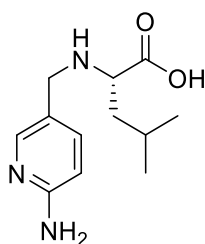
**(2S)-4-Methyl-2-{N-[(piperidin-4-yl)methyl]acetamido}pentanoic acid (138)**



According to general procedure E, 1-boc-piperidine-4-carboxylate (70.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 95.7:4.3→75.7:24.3 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **138** (10.5 mg, 18%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_H$  (500 MHz, MeOD) 4.20 (0.6H, app. br. s, 2-H<sup>rot(maj)</sup>), 4.06 (0.4H, app. br. s, 2-H<sup>rot(min)</sup>), 3.48-3.22 (4H, m, NCH<sub>2</sub>, Piperidinyl 2-H<sub>a</sub>, Piperidinyl 6-H<sub>a</sub>) 2.17 (1.8H, s, Me<sup>rot(maj)</sup>), 2.11 (1.2H, s, Me<sup>rot(min)</sup>), 2.08-1.79 (4H, m, Piperidinyl 3-H<sub>2</sub>, Piperidinyl 4-H, Piperidinyl 5-H<sub>a</sub>), 1.82-1.68 (0.4H, m, Piperidinyl 3-H<sub>b</sub><sup>rot(min)</sup>), 1.66-1.52 (2.6H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 1.50-1.33 (1.6H, 3-H<sub>b</sub> Piperidinyl<sup>rot(maj)</sup>, 5-H<sub>b</sub> Piperidinyl<sup>rot(min)</sup> and 5-H<sub>b</sub> Piperidinyl<sup>rot(maj)</sup>), 1.05-0.90 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, MeOD) 176.3 (C=O), 175.1 (C-1), 173.4 (C=O), 62.7 (C-2<sup>rot(min)</sup>), 60.1 (C-2<sup>rot(maj)</sup>), 55.6 (NCH<sub>2</sub>), 45.2 (Piperidinyl C-2 and Piperidinyl C-6), 45.1 (Piperidinyl C-2 and Piperidinyl C-6), 40.7 (C-3<sup>rot(maj)</sup>), 39.6 (C-3<sup>rot(min)</sup>), 35.0 (Piperidinyl C-4<sup>rot(min)</sup>), 34.7 (Piperidinyl C-4<sup>rot(maj)</sup>), 28.0 (Piperidinyl C-3<sup>rot(maj)</sup> and Piperidinyl C-5<sup>rot(maj)</sup>), 28.0 (Piperidinyl C-3<sup>rot(min)</sup> and Piperidinyl C-5<sup>rot(maj)</sup>), 26.5 (C-4<sup>rot(min)</sup>), 26.4 (C-4<sup>rot(maj)</sup>), 23.4 (C-4 Me<sup>rot(min)</sup>), 23.1 (C-4 Me<sup>rot(maj)</sup>), 22.8 (C-5<sup>rot(maj)</sup>), 22.7 (C-5<sup>rot(min)</sup>), 22.5 (Me<sup>rot(maj)</sup>), 22.1 (Me<sup>rot(min)</sup>); HRMS found  $MH^+$ , 271.2029.  $C_{14}H_{26}N_2O_3$  requires  $MH$  271.2016.

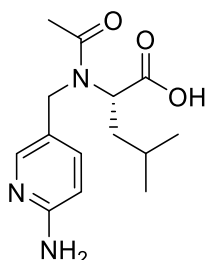


**(2S)-2-[[ (6-Aminopyridin-3-yl)methyl]amino]-4-methylpentanoic acid (139)**



According to general procedure D, 5-formylpyridin-2-yl carbamic acid *tert*-butyl ester (146.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **139** (25.3 mg, 33%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.90 (1H, d, *J* 1.7 Hz, Aminopyridinyl 2-H), 7.87 (1H, dd, *J* 9.2 and 1.7 Hz, Aminopyridinyl 4-H), 6.90 (1H, d, *J* 9.2 Hz, Aminopyridinyl 5-H), 4.11 (1H, d, *J* 13.4 Hz, NHCH<sub>a</sub>), 4.06 (1H, d, *J* 13.4 Hz, NHCH<sub>b</sub>), 3.68 (1H, dd, *J* 8.3 and 5.8 Hz, 2-H), 1.87-1.74 (2H, m, 3-H<sub>a</sub> and 4-H), 1.71-1.60 (1H, m, 3-H<sub>b</sub>), 0.94 (3H, d, *J* 6.4 Hz, 4-Me), 0.91 (3H, d, *J* 6.4 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 172.0 (C=O), 161.8 (q, *J* 34.6, TFA C=O), 155.6 (Aminopyridinyl C-6), 144.5 (Aminopyridinyl C-4), 138.6 (Aminopyridinyl C-2), 116.8 (q, *J* 290.5, TFA CF<sub>3</sub>), 116.1 (Aminopyridinyl C-3), 113.1 (Aminopyridinyl C-5), 60.0 (C-2), 46.4 (NHCH<sub>2</sub>), 39.4 (C-3), 21.9 (C-4 Me), 20.8 (C-5); HRMS found MH<sup>+</sup> 283.1563. C<sub>12</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub> requires *MH*, 283.1550.

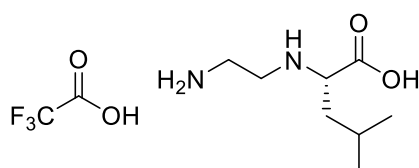
**(2S)-2-{N-[(6-Aminopyridin-3-yl)methyl]acetamido}-4-methylpentanoic acid (140)**



According to general procedure E, 5-formylpyridin-2-yl carbamic acid *tert*-butyl ester (146.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.7:6.3→73.7:26.3 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **140** (25.3 mg, 33%) as a colourless solid.

acid)/MeCN over 12 min to yield the *pentanoic acid* **140** (7.5 mg, 12%) as a 70:30 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.94 (0.3H, br. s, Aminopyridinyl 2-H<sup>rot(min)</sup>), 7.81-7.71 (1.4 H, m, Aminopyridinyl 2-H<sup>rot(maj)</sup> and Aminopyridinyl 5-H<sup>rot(maj)</sup>), 7.65 (0.3H, d, *J* 8.5 Hz, Aminopyridinyl 5-H<sup>rot(min)</sup>), 6.75-6.68 (1H, m, Aminopyridinyl 4-H), 4.74 (0.3, app. t, *J* 6.6 Hz, 2-H<sup>rot(min)</sup>), 4.68 (0.7H, d, *J* 15.3 Hz, NHCH<sub>a</sub><sup>rot(maj)</sup>), 4.57 (0.3H, d, *J* 17.2 Hz, NHCH<sub>a</sub><sup>rot(min)</sup>), 4.52 (0.3H, d, *J* 17.2 Hz, NHCH<sub>b</sub><sup>rot(min)</sup>), 4.34 (0.7H, app. t, *J* 7.3 Hz, 2-H<sup>rot(maj)</sup>), 4.27 (0.7H, d, *J* 15.3 Hz, NHCH<sub>b</sub><sup>rot(maj)</sup>), 2.27 (2.1H, s, Me<sup>rot(maj)</sup>), 2.15 (0.9H, s, Me<sup>rot(min)</sup>), 1.92 (0.7H, dt, *J* 14.1 and 7.2 Hz, 3-H<sub>a</sub><sup>rot(maj)</sup>), 1.82 (0.3H, m, 3-H<sub>a</sub><sup>rot(min)</sup>), 1.65-1.57 (1H, m, 3-H<sub>b</sub>), 1.56-1.47 (1H, m, 4-H), 0.97 (2.1H, d, *J* 6.5 Hz, 4-Me<sup>rot(maj)</sup>), 0.93 (2.1H, d, *J* 6.5 Hz, 5-H<sub>3</sub><sup>rot(maj)</sup>), 0.91 (0.9H, d, *J* 6.5 Hz, 4-H<sup>rot(min)</sup>), 0.88 (0.9H, d, *J* 6.5 Hz, 5-H<sub>3</sub><sup>rot(min)</sup>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.9 (C=O<sup>rot(maj)</sup>), 175.5 (C=O<sup>rot(min)</sup>), 173.4 (C=O<sup>rot(maj)</sup>), 172.5 (C=O<sup>rot(min)</sup>), 156.4 (Aminopyridinyl C-6<sup>rot(min)</sup>), 154.7 (Aminopyridinyl C-6<sup>rot(maj)</sup>), 143.7 (Aminopyridinyl C-4<sup>rot(maj)</sup>), 140.8 (Aminopyridinyl C-4<sup>rot(min)</sup>), 138.7 (Aminopyridinyl C-2<sup>rot(min)</sup>), 135.8 (Aminopyridinyl C-2<sup>rot(maj)</sup>), 123.0 (Aminopyridinyl C-3<sup>rot(maj)</sup>), 121.7 (Aminopyridinyl C-3<sup>rot(min)</sup>), 111.6 (Aminopyridinyl C-5<sup>rot(maj)</sup>), 111.1 (Aminopyridinyl C-5<sup>rot(min)</sup>), 61.1 (C-2<sup>rot(maj)</sup>), 57.3 (C-2<sup>rot(min)</sup>), 42.3 (NHCH<sub>2</sub>), 38.7 (C-3<sup>rot(maj)</sup>), 38.4 (C-3<sup>rot(min)</sup>), 25.0 (C-4<sup>rot(min)</sup>), 24.8 (C-4<sup>rot(maj)</sup>), 21.9 (C-4 Me<sup>rot(min)</sup>), 21.7 (C-4 Me<sup>rot(maj)</sup>), 21.4 (C-5<sup>rot(maj)</sup>), 21.3 (C-5<sup>rot(min)</sup>), 20.8 (Me<sup>rot(min)</sup>), 20.8 (Me<sup>rot(maj)</sup>) (27/28 signals present); HRMS found MH<sup>+</sup> 280.1668. C<sub>14</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub> requires *MH*, 280.1656.

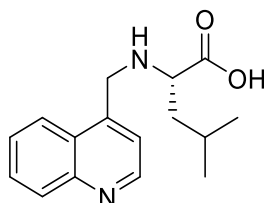
**(2S)-2-[(2-Aminoethyl)amino]-4-methylpentanoic acid TFA salt (141)**



According to general procedure D, N-boc-aminoacetaldehyde (105.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **141** (6.4 mg, 11%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.08 (1H, dd, *J* 9.2 and 4.4 Hz, 2-H) 3.66-3.58 (2H, m, Aminoethyl 1-H<sub>2</sub>), 3.49-3.37 (2H, m, Aminoethyl 2-H<sub>2</sub>), 1.99-1.92 (1H, m, 3-H<sub>a</sub>), 1.81-1.74 (2H, m, 3-H<sub>b</sub> and

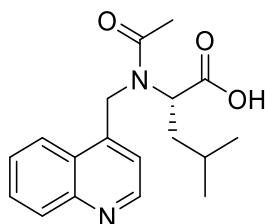
4-H), 0.99 (3H, d, *J* 6.4 Hz, 4-Me), 0.96 (3H, d, *J* 6.4 Hz, 5-H<sub>3</sub>);  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 168.6 (C=O), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 54.4 (C-2), 39.0 (Aminoethyl C-2) 38.0 (C-3), 37.4 (Aminoethyl C-1), 23.8 (C-4), 22.0 (C-4 Me), 20.2 (C-5); HRMS found MH<sup>+</sup> 175.1436. C<sub>8</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub> requires *MH*, 175.1441.

**(2S)-4-Methyl-2-[[[(quinolin-4-yl)methyl]amino]pentanoic acid (142)**

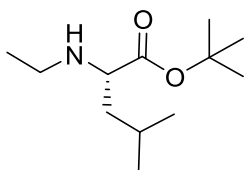


According to general procedure D, 4-quinolinecarboxaldehyde (94.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:2 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **142** (13.1 mg, 22%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 8.94-8.82 (1H, m, Quinoliny 2-H), 8.41-8.31 (1H, m, Ar-H), 8.13-8.03 (1H, m, Ar-H), 7.88-7.78 (1H, m, Ar-H), 7.78-7.62 (1H, m, Ar-H), 7.68 (1H, m, Quinoliny 3-H), 4.74 (1H, d, *J* 13.6 Hz, NHCH<sub>a</sub>), 4.68 (1H, d, *J* 13.6 Hz, NHCH<sub>b</sub>), 3.72 (1H, app. t, *J* 7.2 Hz, 2-H), 1.94-1.85 (1H, m, 3-H<sub>a</sub>), 1.85-1.74 (1H, m, 3-H<sub>b</sub>), 1.68-1.60 (1H, m, 4-H), 1.00 (3H, d, *J* 6.2 Hz, 4-Me), 0.94 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 172.7 (C=O), 149.6 (Quinoliny C-2), 147.6 (Quinoliny C-8a), 139.3 (C-4 Quinoliny), 130.2 (Ar-C), 128.7 (Ar-C), 127.7 (Ar-C), 126.6 (Quinoliny C-4a), 123.6 (Ar-C), 122.4 (Quinoliny C-3), 61.2 (C-2), 46.2 (NHCH<sub>2</sub>), 39.9 (C-3), 24.8 (C-4), 21.9 (C-4 Me), 21.1 (C-5); HRMS found MH<sup>+</sup> 273.1602. C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> requires *MH*, 273.1598.

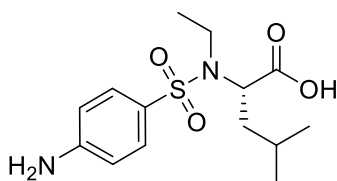
**(2S)-4-Methyl-2-{N-[(quinolin-4-yl)methyl]acetamido}pentanoic acid  
(143)**



According to general procedure E, 4-quinolinecarboxaldehyde (94.0 mg, 0.33 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 85.7:14.3→65.7:34.3 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **143** (4.5 mg, 7%) as a 60:40 mixture of rotamers as a colourless oil.  $\delta_{\text{H}}$  (500 MHz, MeOD) 8.86 (0.6H, d, *J* 4.6 Hz, Quinolinylyl 2-H<sup>rot(maj)</sup>), 8.76 (0.4H, d, *J* 4.6 Hz, Quinolinylyl 2-H<sup>rot(min)</sup>), 8.16 (1H, br. d, *J* 8.6 Hz, Ar-H), 8.10 (0.6H, app. d, *J* 8.4 Hz, Ar-H<sup>rot(maj)</sup>), 8.06 (0.4H, app. d, *J* 8.4 Hz, Ar-H<sup>rot(min)</sup>), 7.88-7.77 (1H, m, Ar-H), 7.76-7.66 (1H, m, Ar-H), 7.65 (0.6H, app. d, *J* 4.5 Hz, Quinolinylyl 3-H<sup>rot(maj)</sup>), 7.37 (0.4H, app. d, *J* 4.5 Hz, Quinolinylyl 3-H<sup>rot(min)</sup>), 4.99-4.92 (0.6H, m, 2-H<sup>rot(maj)</sup>), 4.90-4.80 (2H, m, NCH<sub>2</sub> under MeOD) 4.74-7.62 (0.4H, m, 2-H<sup>rot(min)</sup>), 2.36 (1.3H, s, Me<sup>rot(min)</sup>), 2.08 (1.7H, s, Me<sup>rot(maj)</sup>), 1.96-1.82 (1H, m, 3-H<sub>a</sub>), 1.74-1.49 (2H, m, 3-H<sub>b</sub>, 4-H), 0.93 (3.6H, app. t, *J* 6.2 Hz, 4-Me<sup>rot(maj)</sup> and 5-H<sub>3</sub><sup>rot(maj)</sup>), 0.83 (1.2H, d, *J* 6.2 Hz, 5-H<sub>3</sub><sup>rot(min)</sup>), 0.78-0.74 (1.2H, m, 4-Me<sup>rot(min)</sup>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 173.8 (C=O<sup>rot(min)</sup>), 173.5 (C=O<sup>rot(maj)</sup>), 173.1 (C=O<sup>rot(maj)</sup>), 172.7 (C=O<sup>rot(min)</sup>), 149.6 (Quinolinylyl C-2<sup>rot(maj)</sup>), 149.1 (Quinolinylyl C-2<sup>rot(min)</sup>), 146.9 (Ar-C<sup>rot(maj)</sup>), 146.7 (Ar-C<sup>rot(min)</sup>), 145.2 (Ar-C<sup>rot(min)</sup>), 144.5 (Ar-C<sup>rot(maj)</sup>), 129.9 (Ar-C<sup>rot(maj)</sup>), 129.7 (Ar-C<sup>rot(min)</sup>), 128.5 (Ar-C<sup>rot(maj)</sup>), 128.1 (Ar-C<sup>rot(min)</sup>), 127.2 (Ar-C<sup>rot(maj)</sup>), 126.9 (Ar-C<sup>rot(min)</sup>), 126.1 (Ar-C<sup>rot(min)</sup>), 125.8 (Ar-C<sup>rot(maj)</sup>), 122.8 (Ar-C<sup>rot(min)</sup>), 122.6 (Ar-C<sup>rot(maj)</sup>), 118.7 (Quinolinylyl C-3<sup>rot(min)</sup>), 118.3 (Quinolinylyl C-3<sup>rot(maj)</sup>), 59.9 (C-2<sup>rot(min)</sup>), 56.4 (C-2<sup>rot(maj)</sup>), 47.6 (NHCH<sub>2</sub>), 38.7 (C-3<sup>rot(min)</sup>), 38.4 (C-3<sup>rot(maj)</sup>), 25.3 (C-4<sup>rot(maj)</sup>), 25.0 (C-4<sup>rot(min)</sup>), 21.4 (C-4 Me<sup>rot(maj)</sup>), 21.3 (C-4 Me<sup>rot(min)</sup>), 21.2 (C-5<sup>rot(min)</sup>), 21.1 (C-5<sup>rot(maj)</sup>), 20.8 (Me<sup>rot(min)</sup>), 20.5 (Me<sup>rot(maj)</sup>) (35/36 signals present); HRMS found MH<sup>+</sup> 315.1715. C<sub>18</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 315.1703.

***tert*-Butyl (2*S*)-2-(ethylamino)-4-methylpentanoate (144)**

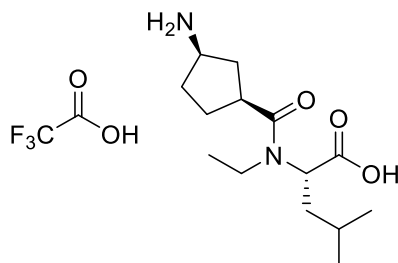
To a solution of H-Leu-O-*tert*-butyl HCl (1.00 g, 4.40 mmol), NaBH<sub>3</sub>CN (300 mg, 4.80 mmol) and NEt<sub>3</sub> (560  $\mu$ L, 4.40 mmol) in methanol (10 mL) was added acetaldehyde (270  $\mu$ L, 4.80 mmol) and stirred at r.t for 18 hr. The reaction mixture was diluted in ethyl acetate (50 mL) and washed with a sat. Na<sub>2</sub>CO<sub>3</sub> (50 mL), brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to yield the *pentanoate* **144** (520 mg, 55%) as a colourless oil which was used without further purification.  $\delta_{\text{H}}$  (500 MHz, MeOD) 3.21 (1H, dd, *J* 8.5 and 6.2 Hz, 2-H), 2.66 (1H, dq, *J* 11.3 and 7.2 Hz, Ethylamino 1-H<sub>a</sub>), 2.56 (1H, dq, *J* 11.3 and 7.2 Hz, Ethylamino 1-H<sub>b</sub>), 1.79-1.69 (1H, m, 4-H), 1.61-1.40 (11H, m, *tert*-Butyl, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.15 (3H, t, *J* 7.2 Hz, Ethylamino 2-H<sub>3</sub>), 1.01 (3H, d, *J* 6.6 Hz, 4-Me), 0.98 (3H, d, *J* 6.6 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.9 (C=O), 82.2 (*tert*-Butyl C-1), 61.4 (C-2), 43.6 (C-3), 42.9 (Ethylamino C-1), 28.5 (*tert*-Butyl C-2), 26.2 (C-4), 23.3 (C-4 Me), 22.6 (C-5), 14.9 (Ethylamino C-2); HRMS found MH<sup>+</sup> 216.1961. C<sub>12</sub>H<sub>25</sub>NO<sub>2</sub> requires *MH*, 216.1958.

**(2*S*)-2-(*N*-Ethyl-4-aminobenzenesulfonamido)-4-methylpentanoic acid (146)**

According to general procedure G, *tert*-butyl (2*S*)-2-(ethylamino)-4-methylpentanoate (100.0 mg, 0.46 mmol) and *tert*-butyl (4-(chlorosulfonyl)phenyl)carbamate (204.0 mg, 0.70 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 61:39→41:59 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **146** (23.6 mg, 16%) as a colourless solid.  $\nu_{\text{max}}/\text{cm}^{-1}$  (film) 3478, 3380, 3326, 3100-2600 (carboxylic acid), 2958, 2871, 1718, 1628, 1310;  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.50 (2H, d, *J* 7.8 Hz, Aminobenzene 2-H), 6.66 (2H, d, *J* 7.8 Hz, Aminobenzene 3-H), 4.42

(1H, dd,  $J$  9.3 and 5.7 Hz, 2-H), 3.3-3.31 (1H, m, Ethylamino 1-H<sub>a</sub>), 3.28-3.18 (1H, m, Ethylamino 1-H<sub>b</sub>), 1.73-1.65 (1H, m, 4-H), 1.65-1.54 (2H, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.19 (3H, t,  $J$  7.1 Hz, Ethylamino 2-H<sub>3</sub>), 0.93 (3H, d,  $J$  6.6 Hz, 4-Me), 0.92 (3H, d,  $J$  6.6 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, MeOD) 175.1 (C=O), 154.2 (Aminobenzene C-4), 130.5 (Aminobenzene C-2), 127.1 (Aminobenzene C-1), 114.3 (Aminobenzene C-3), 59.0 (C-2), 41.2 (C-3), 40.7 (Ethylamino C-1), 25.7 (C-4), 23.2 (C-4 Me), 21.8 (C-5), 17.2 (Ethylamino C-2); HRMS found MH<sup>+</sup> 315.1387. C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>S requires  $MH$ , 315.1373.

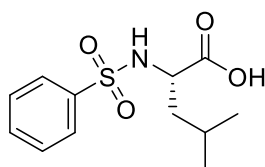
**(2S)-2-{1-[(1S,3R)-3-Aminocyclopentyl]-N-ethylformamido}-4-methylpentanoic acid TFA salt (148)**



According to general procedure G, *tert*-butyl (2S)-2-(ethylamino)-4-methylpentanoate (100.0 mg, 0.46 mmol) and (1R,3S)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (137.0 mg, 0.60 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 90.9:9.1→70.9:29.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **148** (24.1 mg, 19%) as a 75:25 mixture of rotamers as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 2957, 2872, 1678, 1616, 1321, 1252;  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.44 (0.75H, dd,  $J$  9.3 and 5.4 Hz, 2-H<sup>rot(maj)</sup>), 4.40 (0.25H, dd,  $J$  8.9 and 5.8 Hz, 2-H<sup>rot(min)</sup>), 3.73-3.66 (1H, m, Aminocyclopentyl 3-H), 3.61-3.50 (0.75H, m, Ethyl 1-H<sub>a</sub><sup>rot(maj)</sup>), 3.49-3.38 (0.25H, m, Ethyl 1-H<sub>a</sub><sup>rot(min)</sup>), 3.32-3.23 (1.75H, m, Aminocyclopentyl 1-H<sup>rot(maj)</sup> and Ethyl 1-H<sub>b</sub><sup>rot(min)</sup>), 3.14-3.05 (0.25H, m, Aminocyclopentyl 1-H<sup>rot(min)</sup>), 2.26-2.17 (1H, m, Aminocyclopentyl 2-H<sub>a</sub>), 2.11-1.98 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>a</sub>), 1.87-1.58 (5H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Aminocyclopentyl 2-H<sub>b</sub>, Aminocyclopentyl 4-H<sub>b</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.54-1.45 (1H, m, 4-H<sup>rot(min)</sup>), 1.15 (2.25H, t,  $J$  7.1 Hz, Ethyl 2-H<sub>3</sub><sup>rot(maj)</sup>), 1.02 (0.75H, t,  $J$  7.1 Hz, Ethyl 2-H<sub>3</sub><sup>rot(min)</sup>), 0.87 (1.5H, d,  $J$  6.6 Hz, 4-Me<sup>rot(min)</sup>), 0.84 (4.5H, d,  $J$  6.6 Hz, 4-Me<sup>rot(maj)</sup> and 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, MeOD) 178.4 (C=O<sup>rot(min)</sup>), 178.1 (C=O<sup>rot(maj)</sup>), 177.1

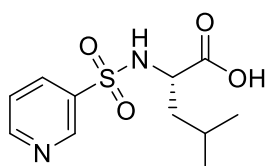
(C=O<sup>rot(min)</sup>), 176.3 (C=O<sup>rot(maj)</sup>), 163.0 (q, *J* 35.4, TFA C=O), 116.3 (q, *J* 291.7, TFA CF<sub>3</sub>), 61.2 (C-2<sup>rot(min)</sup>), 58.4 (C-2<sup>rot(maj)</sup>), 52.0 (Aminocyclopentyl C-3<sup>rot(min)</sup>), 52.0 (Aminocyclopentyl C-3<sup>rot(maj)</sup>), 43.2 (Ethyl C-1), 39.8 (Aminocyclopentyl C-1<sup>rot(min)</sup>), 39.7 (Aminocyclopentyl C-1<sup>rot(maj)</sup>), 37.8 (C-3), 34.8 (Aminocyclopentyl C-2<sup>rot(min)</sup>), 34.5 (Aminocyclopentyl C-2<sup>rot(maj)</sup>), 30.3 (Aminocyclopentyl C-4<sup>rot(min)</sup> or Aminocyclopentyl C-5<sup>rot(min)</sup>), 30.2 (Aminocyclopentyl C-4<sup>rot(maj)</sup> or Aminocyclopentyl C-5<sup>rot(maj)</sup>), 28.3 (Aminocyclopentyl C-4<sup>rot(maj)</sup> or Aminocyclopentyl C-5<sup>rot(maj)</sup>), 28.2 (Aminocyclopentyl C-4<sup>rot(min)</sup> or Aminocyclopentyl C-5<sup>rot(min)</sup>), 24.6 (C-4), 22.3 (C-4 Me<sup>rot(min)</sup>), 22.1 (C-4 Me<sup>rot(maj)</sup>), 21.4 (C-5<sup>rot(maj)</sup>), 21.2 (C-5<sup>rot(min)</sup>), 14.3 (Ethyl C-2<sup>rot(maj)</sup>), 12.9 (Ethyl C-2<sup>rot(min)</sup>) (27/29 signals present); HRMS found MNa<sup>+</sup> 293.1829. C<sub>14</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub> requires *MNa*, 293.1836.

### (2S)-2-Benzenesulfonamido-4-methylpentanoic acid (149)



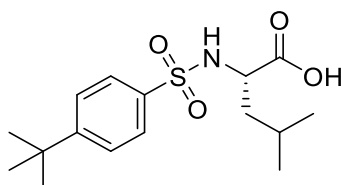
According to general procedure C, benzenesulfonyl chloride (50.0  $\mu$ L, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient 93.9:36.1 $\rightarrow$ 43.9:56.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **149** (4.0 mg, 19%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.88-7.81 (2H, m, Benzyl 2-H), 7.62-7.58 (1H, m, Benzyl 4-H), 7.56-7.51 (2H, m, Benzyl 3-H), 3.83 (1H, dd, *J* 8.9 and 6.0 Hz, 2-H), 1.77-1.62 (1H, m, 4-H), 1.54-1.43 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.90 (3H, d, *J* 6.7 Hz, 4-Me), 0.81 (3H, d, *J* 6.7 Hz, 5-H<sub>3</sub>),  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.5 (C=O), 142.3 (Benzyl C-1), 133.6 (Benzyl C-4), 130.0 (Benzyl C-3), 128.1 (Benzyl C-2), 56.0 (C-2), 43.1 (C-3), 25.5 (C-4), 23.2 (C-4 Me), 21.6 (C-5); HRMS found MNa<sup>+</sup> 294.0777. C<sub>12</sub>H<sub>17</sub>NO<sub>4</sub>S requires *MH*, 294.0770.

### (2S)-4-Methyl-2-(pyridine-3-sulfonamido)pentanoic acid (150)



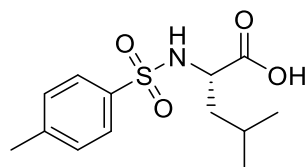
According to general procedure C, pyridine-3-sulfonyl chloride (50.0  $\mu\text{L}$ , 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.9:36.1 $\rightarrow$ 43.9:56.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **150** (6.1 mg, 28%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 8.97 (1H, app. br. s, Pyridine 2-H), 8.74 (1H, app. br. d, *J* 4.2 Hz, Pyridine 4-H), 8.24 (1H, ddd, *J* 8.1, 2.3 and 1.6 Hz, Pyridine 6-H), 7.60 (1H, ddd, *J* 8.1, 4.9 and 0.7 Hz, Pyridine 5-H), 3.93 (1H, dd, *J* 8.5 and 6.4 Hz, 2-H), 1.84-1.72 (1H, m, 4-H), 1.60-1.47 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.93 (3H, d, *J* 6.6 Hz, 4-Me), 0.89 (3H, d, *J* 6.6 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 173.6 (C=O), 152.1 (Pyridine C-4), 147.2 (Pyridine C-2), 138.2 (Pyridine C-1), 135.3 (Pyridine C-6), 123.9 (Pyridine C-5), 54.3 (C-2), 41.4 (C-3), 24.2 (C-4), 21.8 (C-4 Me), 20.1 (C-5); HRMS found  $\text{MNa}^+$  295.0720. C<sub>11</sub>H<sub>16</sub>N<sub>2</sub>O<sub>4</sub>S requires *MNa*, 295.0723.

**(2S)-2-(4-*tert*-Butylbenzenesulfonamido)-4-methylpentanoic acid (151)**

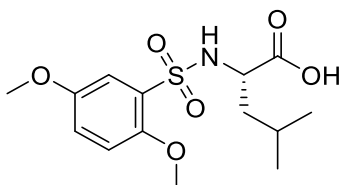


According to general procedure C, 4-*tert*-butyl-benzenesulfonyl chloride (92.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.9:6.1 $\rightarrow$ 43.9:56.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **151** (11.2 mg, 43%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.79-7.73 (2H, m, Benzene 2-H), 7.60-7.54 (2H, m, Benzene 3-H), 3.79 (1H, dd, *J* 8.7 and 6.1 Hz, 2-H), 1.80-1.60 (1H, m, 4-H), 1.55-1.39 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.35 (9H, s, *tert*-Butyl), 0.88 (3H, d, *J* 6.8 Hz, 4-Me), 0.77 (3H, d, *J* 6.8 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.2 (C=O), 156.1 (Benzene C-4), 137.8 (Benzene C-1), 126.7 (Benzene C-2), 125.6 (Benzene C-3), 54.2 (C-2), 41.6 (C-3), 34.6 (*tert*-Butyl C-1), 30.1 (*tert*-Butyl C-2), 24.1 (C-4), 21.8 (C-4 Me), 20.2 (C-5); HRMS found  $\text{MNa}^+$  350.1405. C<sub>16</sub>H<sub>25</sub>NO<sub>4</sub>S requires *MNa*, 350.1397.



**(2S)-4-Methyl-2-(4-methylbenzenesulfonamido)pentanoic acid (152)**

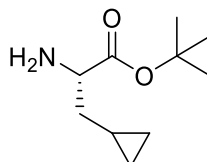
According to general procedure C, *p*-toluenesulfonyl chloride (75.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.9:6.1→43.9:56.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **152** (10.2 mg, 45%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.75-7.65 (2H, m, Benzene 2-H), 7.40-7.30 (2H, m, Benzene 3-H), 3.80 (1H, dd, *J* 9.0 and 5.8 Hz, 2-H), 2.41 (3H, s, Me), 1.77-1.62 (1H, m, 4-H), 1.53-1.38 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.89 (3H, d, *J* 6.6 Hz, 4-Me), 0.81 (3H, d, *J* 6.6 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.2 (C=O), 143.2 (Benzene C-4), 137.8 (Benzene C-1), 129.1 (Benzene C-2), 126.8 (Benzene C-3), 54.2 (C-2), 41.6 (C-3), 24.2 (C-4), 21.9 (C-4 Me), 20.1 (C-5), 20.0 (Me); HRMS found  $\text{MNa}^+$  308.0934.  $\text{C}_{13}\text{H}_{19}\text{NO}_4\text{S}$  requires *MNa*, 308.0927.

**(2S)-2-(2,5-Dimethoxybenzenesulfonamido)-4-methylpentanoic acid (153)**

According to general procedure C, 2,5-dimethoxybenzenesulfonyl chloride (81.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.9:6.1→43.9:56.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **153** (6.4 mg, 24%) as a white solid.  $\delta_{\text{H}}$  (500 MHz, MeOD) 7.36 (1H, d, *J* 3.1 Hz, Benzene 6-H), 7.13 (1H, dd, *J* 9.0 and 3.1 Hz, Benzene 4-H), 7.08 (1H, d, *J* 9.0 Hz, Benzene 3-H), 3.91 (1H, dd, *J* 9.7 and 5.1 Hz, 2-H), 3.88 (3H, s, Benzene 5-OMe), 3.79 (3H, s, Benzene 2-OMe), 1.85-1.72 (1H, m, 4-H), 1.61-1.42 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.92 (3H, d, *J* 6.6 Hz, 4-Me), 0.86 (3H, d, *J* 6.6 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 174.1 (C=O), 152.9 (Benzene C-2), 150.9 (Benzene C-5), 128.4 (Benzene C-1), 119.4 (Benzene C-4), 114.3 (Benzene C-6), 113.2 (Benzene C-3), 55.4 (C-2 or Benzene 2-OMe or Benzene 5-

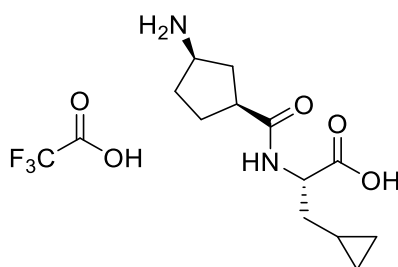
OMe), 55.0 (C-2 or Benzene 2-OMe or Benzene 5-OMe), 54.6 (C-2 or Benzene 2-OMe or Benzene 5-OMe), 41.9 (C-3), 24.1 (C-4), 21.9 (C-4 Me), 20.3 (C-5); HRMS found  $MNa^+$  354.0996.  $C_{14}H_{21}NO_6S$  requires  $MNa$ , 354.0982.

***tert*-Butyl (2*S*)-2-amino-3-cyclopropylpropanoate (154b)<sup>70</sup>**



To a solution of L-3-cyclopropylalanine (500 mg, 3.87 mmol) in *tert*-butyl acetate (53 mL) was added  $HClO_4$  (46.0  $\mu$ L, 0.77 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-butyl ester **154b** (316 mg, 44%) as a colourless oil.  $R_f$  0.60 (90:10 DCM–MeOH).  $\delta_H$  (500 MHz, MeOD) 3.65 (1H, app. t,  $J$  6.3 Hz, 2-H), 1.89–1.76 (2H, m, 3- $H_a$  and 3- $H_b$ ), 1.74 (9H, s, *tert*-Butyl), 1.09–0.98 (1H, m, Cyclopropyl 1-H), 0.79–0.65 (2H, m, Cyclopropyl 2- $H_a$  and Cyclopropyl 3- $H_a$ ), 0.40–0.29 (2H, m, Cyclopropyl 2- $H_b$  and Cyclopropyl 3- $H_b$ );  $\delta_C$  (125 MHz, MeOD) 175.9 (C=O), 82.0 (*tert*-Butyl C-1), 56.4 (C-2), 40.7 (C-3), 28.4 (*tert*-Butyl C-2), 7.2 (Cyclopropyl C-1), 5.1 (Cyclopropyl C-2), 5.0 (Cyclopropyl C-3); HRMS found  $MH^+$  186.1482.  $C_{10}H_{19}NO_2$  requires  $MH$ , 186.1489.

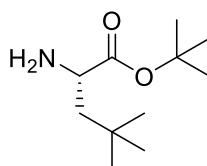
**(2*S*)-2-{[(1*S*,3*R*)-3-Aminocyclopentyl]formamido}-3-cyclopropylpropanoic acid TFA salt (154)**



According to general procedure G, *tert*-butyl (2*S*)-2-amino-3-cyclopropylpropanoate (85.0 mg, 0.46 mmol) and (1*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (137.0 mg, 0.60 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *propanoic*

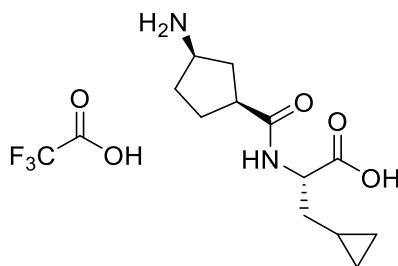
*acid 154* (61.5 mg, 52%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz,  $\text{D}_2\text{O}$ ) 4.23 (1H, dd,  $J$  7.9 and 5.7 Hz, 2-H), 3.68-3.59 (1H, m, Aminocyclopentyl 3-H), 2.93-2.79 (1H, m, Aminocyclopentyl 1-H), 2.23-2.13 (1H, m, Aminocyclopentyl 2- $\text{H}_a$ ), 2.05-1.89 (2H, m, Aminocyclopentyl 4- $\text{H}_a$  and Aminocyclopentyl 5- $\text{H}_a$ ), 1.83-1.74 (3H, m, Aminocyclopentyl 2- $\text{H}_b$ , Aminocyclopentyl 4-H, Aminocyclopentyl 5- $\text{H}_b$ ), 1.65-1.46 (2H, m, 3- $\text{H}_a$  and 3- $\text{H}_b$ ), 0.71-0.51 (1H, m, Cyclopropyl 1-H), 0.41-0.26 (2H, m, Cyclopropyl 2- $\text{H}_a$  and Cyclopropyl 3- $\text{H}_a$ ), 0.03--0.02 (1H, m, Cyclopropyl 2- $\text{H}_b$  or Cyclopropyl 3- $\text{H}_b$ ), 0.00--0.09 (1H, m, Cyclopropyl 2- $\text{H}_b$  or Cyclopropyl 3- $\text{H}_b$ );  $\delta_{\text{C}}$  (125 MHz, MeOD) 178.1 (C=O), 176.5 (C=O), 163.0 (q,  $J$  35.4, TFA C=O), 116.3 (q,  $J$  291.7, TFA  $\text{CF}_3$ ), 54.1 (C-2), 51.8 (C-3 Aminocyclopentyl), 43.1 (C-1 Aminocyclopentyl), 35.6 (C-3), 34.2 (C-2 Aminocyclopentyl), 30.1 (C-4 Aminocyclopentyl or C-5 Aminocyclopentyl), 28.0 (C-4 Aminocyclopentyl or C-5 Aminocyclopentyl), 7.0 (C-1 Cyclopropyl), 3.8 (C-2 Cyclopropyl or C-3 Cyclopropyl), 3.2 (C-2 Cyclopropyl or C-3 Cyclopropyl); HRMS found  $\text{MH}^+$  241.1550.  $\text{C}_{12}\text{H}_{20}\text{N}_2\text{O}_3$  requires  $\text{MH}$ , 241.1547

***tert*-Butyl (2*S*)-2-amino-4,4-dimethylpentanoate (155b)<sup>70</sup>**



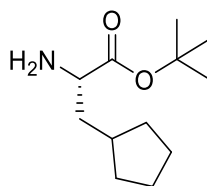
To a solution of 3-*tert*-butyl-L-alanine (561.0 mg, 3.87 mmol) in *tert*-butyl acetate (53 mL) was added  $\text{HClO}_4$  (46.0  $\mu\text{L}$ , 0.77 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-Butyl ester **155b** (290 mg, 37%) as a colourless oil.  $R_f$  0.60 (90:10 DCM–MeOH).  $\delta_{\text{H}}$  (500 MHz, MeOD) 3.57 (1H, dd,  $J$  6.5 and 5.2 Hz, 2-H), 2.03 (1H, dd,  $J$  14.0 and 6.5 Hz, 3- $\text{H}_a$ ), 1.72 (9H, s, *tert*-Butyl), 1.61 (1H, dd,  $J$  14.0 and 5.2 Hz, 3- $\text{H}_b$ ), 1.21 (9H, s, 2  $\times$  4-Me and 5- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.2 (C-1), 80.7 (*tert*-Butyl C-1), 52.2 (C-2), 48.5 (C-3), 29.9 (C-4 Me), 29.4 (C-4 Me) 28.9 (C-5), 27.0 (*tert*-Butyl C-2); HRMS found  $\text{MH}^+$  202.1800.  $\text{C}_{11}\text{H}_{23}\text{NO}_2$  requires  $\text{MH}$ , 202.1802.

**(2S)-2-{[(1S,3R)-3-Aminocyclopentyl]formamido}-3-cyclopropylpropanoic acid TFA salt (154)**



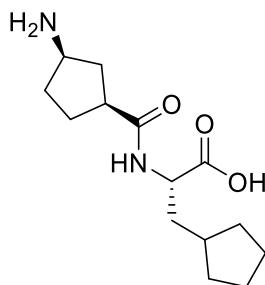
According to general procedure G, *tert*-butyl (2S)-2-amino-3-cyclopropylpropanoate (85.0 mg, 0.46 mmol) and (1R,3S)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (137.0 mg, 0.60 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **154** (61.5 mg, 52%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.23 (1H, dd, *J* 7.9 and 5.7 Hz, 2-H), 3.68-3.59 (1H, m, Aminocyclopentyl 3-H), 2.93-2.79 (1H, m, Aminocyclopentyl 1-H), 2.23-2.13 (1H, m, Aminocyclopentyl 2-H<sub>a</sub>), 2.05-1.89 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>a</sub>), 1.83-1.74 (3H, m, Aminocyclopentyl 2-H<sub>b</sub>, Aminocyclopentyl 4-H, Aminocyclopentyl 5-H<sub>b</sub>), 1.65-1.46 (2H, m, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 0.71-0.51 (1H, m, Cyclopropyl 1-H), 0.41-0.26 (2H, m, Cyclopropyl 2-H<sub>a</sub> and Cyclopropyl 3-H<sub>a</sub>), 0.03--0.02 (1H, m, Cyclopropyl 2-H<sub>b</sub> or Cyclopropyl 3-H<sub>b</sub>), 0.00--0.09 (1H, m, Cyclopropyl 2-H<sub>b</sub> or Cyclopropyl 3-H<sub>b</sub>);  $\delta_{\text{C}}$  (125 MHz, MeOD) 178.1 (C=O), 176.5 (C=O), 163.0 (q, *J* 35.4, TFA C=O), 116.3 (q, *J* 291.7, TFA CF<sub>3</sub>), 54.1 (C-2), 51.8 (C-3 Aminocyclopentyl), 43.1 (C-1 Aminocyclopentyl), 35.6 (C-3), 34.2 (C-2 Aminocyclopentyl), 30.1 (C-4 Aminocyclopentyl or C-5 Aminocyclopentyl), 28.0 (C-4 Aminocyclopentyl or C-5 Aminocyclopentyl), 7.0 (C-1 Cyclopropyl), 3.8 (C-2 Cyclopropyl or C-3 Cyclopropyl), 3.2 (C-2 Cyclopropyl or C-3 Cyclopropyl); HRMS found MH<sup>+</sup> 241.1550. C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 241.1547

***tert*-Butyl (2S)-2-amino-3-cyclopentylpropanoate (156b)**<sup>70</sup>



To a solution of L-3-cyclopentylalanine (608.0 mg, 3.87 mmol) in *tert*-butyl acetate (53 mL) was added HClO<sub>4</sub> (46.0 μL, 0.77 mmol) dropwise and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *tert*-butyl ester **156b** (324 mg, 39%) as a colourless oil. *R*<sub>f</sub> 0.60 (90:10 DCM–MeOH). δ<sub>H</sub> (500 MHz, MeOD) 3.61–3.57 (1H, m, 2-H), 2.29–2.20 (1H, m, Cyclopentyl 1-H), 2.19–2.08 (2H, m, Cyclopentyl 2-H<sub>a</sub> and Cyclopentyl 5-H<sub>a</sub>), 2.03–1.94 (3H, m, 3-H<sub>a</sub>, Cyclopentyl 3-H<sub>a</sub> and Cyclopentyl 4-H<sub>a</sub>), 1.92–1.83 (3H, m, 3-H<sub>b</sub>, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>), 1.78 (9H, s, *tert*-Butyl), 1.50–1.33 (2H, m, Cyclopentyl 2-H<sub>b</sub> and Cyclopentyl 5-H<sub>b</sub>); δ<sub>C</sub> (125 MHz, MeOD) 175.0 (C=O), 80.6 (*tert*-Butyl C-1), 54.1 (C-2), 41.2 (C-3), 36.7 (Cyclopentyl C-1), 32.5 (Cyclopentyl C-2 or Cyclopentyl C-5), 32.3 (Cyclopentyl C-2 or Cyclopentyl C-5), 27.1 (*tert*-Butyl), 24.8 (Cyclopentyl C-3 or Cyclopentyl C-4), 24.7 (Cyclopentyl C-3 or Cyclopentyl C-4); HRMS found MH<sup>+</sup> 214.1809. C<sub>12</sub>H<sub>23</sub>NO<sub>2</sub> requires *MH*, 214.1802

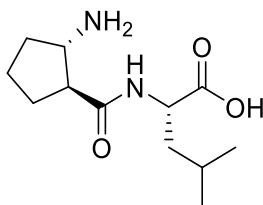
**(2S)-2-{[(1S,3R)-3-Aminocyclopentyl]formamido}-3-cyclopentylpropanoic acid (156)**



According to general procedure G, *tert*-butyl (2S)-2-amino-3-cyclopentylpropanoate (194.0 mg, 0.92 mmol) and (1R,3S)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (274.0 mg, 1.20 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 88.9:11.1→68.9:31.1 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **156** (98.2 mg, 60%) as a colourless solid. δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 4.18 (1H, d, *J* 8.5 and 6.1 Hz, 2-H), 3.71–3.66 (1H, m, Aminocyclopentyl 3-H), 2.94–2.84 (1H, m, Aminocyclopentyl 1-H), 2.26–2.15 (1H, m, Aminocyclopentyl 2-H<sub>a</sub>), 2.09–1.92 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>a</sub>), 1.90–1.71 (8H, m, Aminocyclopentyl 2-H<sub>b</sub>, Aminocyclopentyl 4-H<sub>b</sub>, Aminocyclopentyl 5-H<sub>b</sub>,

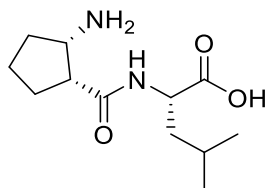
Cyclopentyl 1-H, Cyclopentyl 3-H<sub>a</sub>, Cyclopentyl 4-H<sub>a</sub>, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.56-1.50 (2H, m, Cyclopentyl 2-H<sub>a</sub> and Cyclopentyl 5-H<sub>a</sub>), 1.47-1.38 (2H, m, Cyclopentyl 2-H<sub>b</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.10-1.01 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_c$  (125 MHz, MeOD) 178.1 (C=O), 177.5 (C=O), 53.6 (C-2), 51.9 (C-3 Aminocyclopentyl), 43.1 (Aminocyclopentyl C-1), 36.8 (C-3), 36.4 (Cyclopentyl C-1), 34.2 (Aminocyclopentyl C-2), 32.3 (Cyclopentyl C-3 or Cyclopentyl C-4), 31.5 (Cyclopentyl C-3 or Cyclopentyl C-4), 30.1 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 28.0 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 24.6 (Cyclopentyl C-2 or Cyclopentyl C-5), 24.5 (Cyclopentyl C-2 or Cyclopentyl C-5); HRMS found  $MH^+$  269.1873.  $C_{14}H_{24}N_2O_3$  requires  $MH$ , 269.1860.

**(2S)-2-([(1S,2S)-2-Aminocyclopentyl]formamido)-4-methylpentanoic acid (157)**



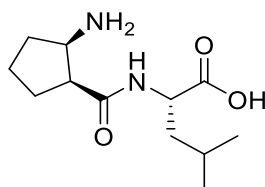
According to general procedure B, (1S,2S)-2-aminocyclopentanecarboxylic acid (91.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **157** (10.5 mg, 55%) as a colourless solid.  $\nu_{max}/cm^{-1}$  (film) 3300-2500, 3055, 2952, 1655, 1630, 1535, 1295;  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.09 (1H, m, 2-H), 3.72 (1H, app. q,  $J$  7.6 Hz, Aminocyclopentyl 2-H), 2.73 (1H, m, Aminocyclopentyl 1-H), 2.06-2.01 (2H, m, Aminocyclopentyl 3-H<sub>a</sub> and Aminocyclopentyl 3-H<sub>b</sub>), 1.72-1.63 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 4-H<sub>b</sub>), 1.61-1.54 (2H, m, Aminocyclopentyl 5-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.48-1.44 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.77 (3H, d,  $J$  6.5 Hz, 4-Me), 0.72 (3H, d,  $J$  6.2 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 179.9 (C=O), 174.9 (C=O), 53.8 (C-2), 53.6 (Aminocyclopentyl C-1), 49.5 (Aminocyclopentyl C-2), 40.3 (C-3), 30.6 (Aminocyclopentyl 3-4 or Aminocyclopentyl C-5), 30.5 (Aminocyclopentyl C-3 or Aminocyclopentyl C-5), 24.6 (C-4), 23.4 (Aminocyclopentyl C-4), 22.4 (C-4 Me), 20.4 (C-5); HRMS found  $MH^+$  243.1710.  $C_{12}H_{22}N_2O_3$  requires  $MH$ , 243.1713.

**(2S)-2-{-[(1R,2S)-2-Aminocyclopentyl]formamido}-4-methylpentanoic acid (158)**



According to general procedure B, (1R,2S)-2-Aminocyclopentanecarboxylic acid (91.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **158** (13.9 mg, 77%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 2955, 2872, 1646(br), 1541(br), 1384;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.13 (1H, dd, *J* 9.2 and 5.3 Hz, 2-H), 3.76 (1H, td, *J* 6.5 and 4.1 Hz, Aminocyclopropyl 2-H), 2.93 (1H, ddd, *J* 9.2, 8.3 and 6.5 Hz, Aminocyclopropyl 1-H), 2.15-2.00 (2H, m, Aminocyclopropyl 3-H<sub>a</sub> and Aminocyclopropyl 5-H<sub>a</sub>), 1.87-1.79 (2H, m, Aminocyclopropyl 4-H<sub>a</sub> and Aminocyclopropyl 5-H<sub>a</sub>), 1.79-1.64 (2H, m, Aminocyclopropyl 3-H<sub>b</sub> and Aminocyclopropyl 4-H<sub>b</sub>) 1.61-1.49 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.85 (3H, d, *J* 6.1 Hz, 4-Me), 0.82 (3H, d, *J* 6.1 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.7 (C=O), 174.4 (C=O), 53.6 (Aminocyclopentyl C-2), 53.2 (C-2), 45.6 (C-3), 39.8 (Aminocyclopentyl C-1), 30.1 (Aminocyclopentyl C-3), 27.8 (Aminocyclopentyl C-5), 24.5 (C-4), 22.2 (C-4 Me), 21.4 (Aminocyclopentyl C-4), 20.7 (C-5); HRMS found MH<sup>+</sup> 243.1716. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1713.

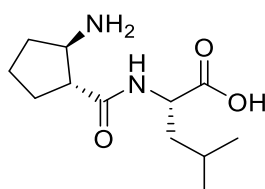
**(2S)-2-{-[(1S,2R)-2-Aminocyclopentyl]formamido}-4-methylpentanoic acid (159)**



According to general procedure B, (1S,2R)-2-aminocyclopentanecarboxylic acid (91.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **159** (14.6 mg, 76%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.14 (1H, dd, *J* 8.8 and 6.1, 2-H), 3.81 (1H, td, *J* 7.0 and 5.0,

Aminocyclopentyl 2-H), 3.00 (1H, td,  $J$  8.4 and 7.0 Hz, Aminocyclopentyl 1-H), 2.09 (1H, m, Aminocyclopentyl 3-H<sub>a</sub>), 1.99 (1H, m, Aminocyclopentyl 5-H<sub>a</sub>), 1.82 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.68 (Aminocyclopentyl 3-H<sub>b</sub> and Aminocyclopentyl 4-H<sub>b</sub>), 1.52 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.84 (3H, d,  $J$  6.0 Hz, 4-Me), 0.80 (3H, d,  $J$  6.0 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 179.7 (C=O), 174.3 (C=O), 53.8 (Aminocyclopentyl C-2), 53.8 (C-2), 45.8 (C-3), 39.9 (Aminocyclopentyl C-1), 30.8 (Aminocyclopentyl C-3), 27.9 (Aminocyclopentyl C-5), 24.6 (C-4), 22.4 (C-4 Me), 21.0 (Aminocyclopentyl C-4), 20.4 (C-5); HRMS found MH<sup>+</sup> 243.1718. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires  $MH$ , 243.1713.

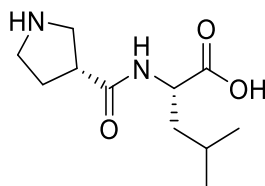
**(2S)-2-{[(1R,2R)-2-Aminocyclopentyl]formamido}-4-methylpentanoic acid (160)**



According to general procedure B, (1R,2R)-2-aminocyclopentanecarboxylic acid (91.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **160** (24.0 mg, 63%) as a colourless solid.  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.01 (1H, dd,  $J$  9.4 and 5.2 Hz, 2-H), 3.72 (1H, app. dd,  $J$  14.8 and 7.1 Hz, Aminocyclopentyl 2-H), 2.74-2.68 (1H, m, Aminocyclopentyl 1-H), 2.10-2.00 (Aminocyclopentyl 3-H), 1.72-1.54 (4H, m, Aminocyclopentyl 4-H<sub>a</sub>, Aminocyclopentyl 4-H<sub>b</sub>, Aminocyclopentyl 5-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.54-1.38 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.77 (3H, d,  $J$  6.2 Hz, 5-Me), 0.74 (3H, dd,  $J$  6.2 Hz, 5-H<sub>3</sub>);  $\delta_c$  (125 MHz, D<sub>2</sub>O) 179.7 (C=O), 174.7 (C=O), 54.2 (C-2), 53.8 (Aminocyclopentyl C-2), 49.3 (Aminocyclopentyl C-1), 40.3 (C-3), 30.6 (Aminocyclopentyl C-3 or Aminocyclopentyl C-5), 30.0 (Aminocyclopentyl C-3 or Aminocyclopentyl C-5), 24.6 (C-4), 23.1 (Aminocyclopentyl C-4), 22.3 (C-4 Me), 20.8 (C-5); HRMS found MH<sup>+</sup> 243.1711. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires  $MH$ , 243.1713.

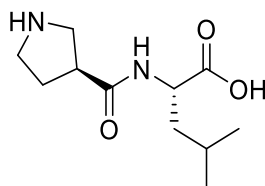


**(2S)-4-Methyl-2-{[(3R)-pyrrolidin-3-yl]formamido}pentanoic acid (161)**



According to general procedure B, (*R*)-1-*N*-*boc*- $\beta$ -proline (85.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **161** (10.6 mg, 55%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.11 (1H, app. t, *J* 7.0 Hz, 2-H), 3.37-3.30 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 2-H<sub>b</sub>) 3.30-3.20 (2H, m, Pyrrolidinyl 5-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>b</sub>), 3.20-3.12 (1H, m, Pyrrolidinyl 3-H), 2.22 (1H, dtd, *J* 13.5, 8.0 and 6.7 Hz, Pyrrolidinyl 4-H<sub>a</sub>), 2.03 (1H, dtd, *J* 13.5, 8.0 and 6.7 Hz, Pyrrolidinyl 4-H<sub>b</sub>), 1.53-1.43 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.78 (3H, d, *J* 6.0 Hz, 4-Me), 0.74 (3H, d, *J* 6.0 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.3 (C=O), 173.9 (C=O) 53.0 (C-2), 47.3 (Pyrrolidinyl C-2), 45.3 (Pyrrolidinyl C-5), 42.2 (Pyrrolidinyl C-3), 39.8 (C-3), 28.5 (Pyrrolidinyl C-4), 24.5 (C-4), 22.2 (C-4 Me), 20.6 (C-5); HRMS found MH<sup>+</sup> 229.1558. C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 229.1547.

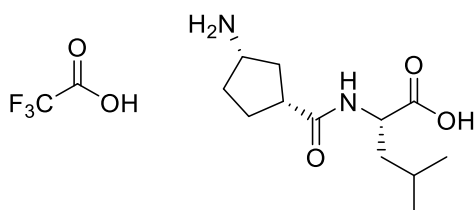
**(2S)-4-Methyl-2-{[(3S)-pyrrolidin-3-yl]formamido}pentanoic acid (162)**



According to general procedure B, (*S*)-1-*N*-*boc*- $\beta$ -proline (85.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **162** (12.0 mg, 67%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.06-4.01 (1H, m, 2-H), 3.41-3.31 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 2-H<sub>b</sub>) 3.30-3.18 (2H, m, Pyrrolidinyl 5-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>b</sub>), 3.18-3.12 (1H, m, Pyrrolidinyl 3-H), 2.21 (1H, dtd, *J* 13.4, 8.0 and 6.6 Hz, Pyrrolidinyl 4-H<sub>a</sub>), 1.95 (1H, dddd, *J* 13.4,

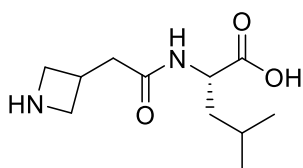
8.0, 7.1 and 6.3 Hz, Pyrrolidinyl 4-H<sub>b</sub>), 1.51-1.33 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.77 (3H, d, *J* 6.0 Hz, 4-Me), 0.73 (3H, d, *J* 6.0 Hz, 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, D<sub>2</sub>O) 178.0 (C=O), 173.7 (C=O) 54.0 (C-2), 47.4 (Pyrrolidinyl C-2), 45.4 (Pyrrolidinyl C-5), 42.4 (Pyrrolidinyl C-3), 40.4 (C-3), 28.9 (Pyrrolidinyl C-4), 24.7 (C-4), 22.4 (C-4 Me), 20.6 (C-5); HRMS found MH<sup>+</sup> 229.1558. C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 229.1560.

**(2S)-2-{[(1R,3S)-3-Aminocyclopentyl]formamido}-4-methylpentanoic acid TFA salt (163)**



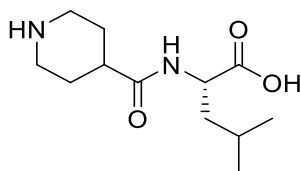
According to general procedure B, (1R,3S)-3-((*tert*-butoxycarbonyl)amino)cyclopentanecarboxylic acid (96.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the pentanoic acid **163** (10.9 mg, 57%) as a colourless solid.  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.12-4.04 (1H, m, 2-H), 3.67-3.56 (1H, m, Aminocyclopentyl 3-H), 2.85 (1H, app. tt, *J* 8.4 and 6.3 Hz, Aminocyclopentyl 1-H), 2.21-2.09 (1H, m, Aminocyclopentyl 2-H<sub>a</sub>), 2.04-1.85 (2H, m, Aminocyclopentyl 4-H<sub>a</sub> and Aminocyclopentyl 5-H<sub>a</sub>), 1.70-1.64 (3H, m, Aminocyclopentyl 2-H<sub>b</sub>, Aminocyclopentyl 4-H<sub>b</sub> and Aminocyclopentyl 5-H<sub>b</sub>), 1.54-1.34 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.77 (3H, d, *J* 6.1 Hz, 4-Me), 0.73 (3H, d, *J* 6.1 Hz, 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, D<sub>2</sub>O) 179.1 (C=O), 178.1 (C=O), 53.3 (C-2), 51.9 (Aminocyclopentyl C-3), 43.3 (Aminocyclopentyl C-1), 40.1 (C-3), 34.0 (Aminocyclopentyl C-2), 30.2 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 28.4 (Aminocyclopentyl C-4 or Aminocyclopentyl C-5), 24.6 (C-4), 22.3 (C-4 Me), 20.5 (C-5); HRMS found MH<sup>+</sup> 243.1711. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

**(2S)-2-(2-(Azetidin-3-yl)acetyl)-4-methylpentanoic acid (164)**



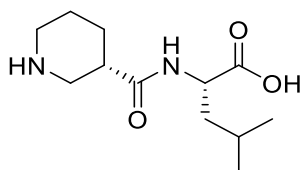
According to general procedure B, 2-(1-boc-azetidine 3-yl)acetic acid (85.0 mg, 0.395 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **164** (10.1 mg, 56%) as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.11-4.01 (3H, m, 2-H, Azetidiny 2-H<sub>a</sub> and Azetidiny 4-H<sub>a</sub>), 3.82 (2H, dd, *J* 19.1 and 9.7 Hz, Azetidiny 2-H<sub>b</sub> and Azetidiny 4-H<sub>b</sub>), 3.19-3.09 (1H, m, Azetidiny 3-H), 2.54 (2H, dd, *J* 7.6 and 1.2 Hz, Acetyl CH<sub>2</sub>), 1.50-1.39 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.76 (3H, d, *J* 6.5 Hz, 4-Me), 0.73 (3H, d, *J* 6.5 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.7 (C=O), 172 (C=O), 52.9 (C-2), 51.0 (Azetidiny C-2 and Azetidiny C-4), 39.9 (C-3), 37.7 (Acetyl CH<sub>2</sub>), 28.6 (Azetidiny C-3), 24.6 (C-4), 22.3 (C-4 Me), 20.5 (C-5); HRMS found MH<sup>+</sup> 229.1556. C<sub>11</sub>H<sub>20</sub>N<sub>3</sub>O<sub>4</sub> requires *MH*, 229.1547.

### (2S)-4-Methyl-2-[(piperidin-4-yl)formamido]pentanoic acid (**165**)



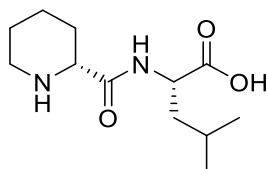
According to general procedure B, *N-tert*-butylpiperidine-4-carboxamide (181.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **165** (29.0 mg, 76%) as a colourless solid.  $\nu_{\text{max}}$ /cm<sup>-1</sup> (film) 3300-2500, 3281, 2961, 2869, 1685, 1656;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.36-4.32 (1H, m, 2-H), 3.51-3.46 (2H, m, Piperidiny 2-H<sub>a</sub> and Piperidiny 6-H<sub>a</sub>), 3.07 (2H, td, *J* 12.9 and 3.1 Hz, Piperidiny 2-H<sub>b</sub> and Piperidiny 6-H<sub>b</sub>), 2.70 (1H, tt, *J* 11.5 and 3.8 Hz, Piperidiny 4-H), 2.10-2.00 (2H, m, Piperidiny 3-H<sub>a</sub> and Piperidiny 5-H<sub>a</sub>), 1.91-1.82 (2H, m, Piperidiny 3-H<sub>a</sub> and Piperidiny 5-H<sub>a</sub>) 1.69-1.62 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.93 (3H, d, *J* 6.4 Hz, 4-Me), 0.89 (3H, d, *J* 6.4 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 177.5 (C=O), 176.2 (C=O), 52.0 (C-2), 43.0 (Piperidiny C-2 or Piperidiny C-6), 43.0 (Piperidiny C-2 or Piperidiny C-6), 39.5 (C-3 or Piperidiny C-4), 39.4 (C-3 or Piperidiny C-4), 25.1 (C-4), 24.6 (Piperidiny C-3 and Piperidiny C-5), 22.2 (Piperidiny C-3 and Piperidiny C-5), 22.2 (C-4 Me), 20.4 (C-5); HRMS found MH<sup>+</sup> 243.1723. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

**(2S)-4-Methyl-2-{[(3S)-piperidin-3-yl]formamido}pentanoic acid (166)**



According to general procedure B, (3S)-N-*tert*-butylpiperidine-3-carboxamide (181.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **166** (26.0 mg, 68%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3256, 3080, 2945, 1684, 1600, 1438;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.32 (1H, m, 2-H), 3.40 (1H, dd, *J* 12.8 and 3.9 Hz, Piperidinyll 2-H<sub>a</sub>), 3.30 (1H, ddd, *J* 13.0, 5.6 and 3.7 Hz, Piperidinyll 6-H<sub>a</sub>), 3.21 (1H, dd, *J* 12.8 and 9.2 Hz, Piperidinyll 2-H<sub>b</sub>), 3.09 (1H, ddd, *J* 12.8, 9.7 and 3.5 Hz, Piperidinyll 6-H<sub>b</sub>), 2.88 (1H, tt, *J* 9.4 and 4.1 Hz, Piperidinyll 3-H), 2.09-2.01 (1H, m, Piperidinyll 4-H<sub>a</sub>), 1.99-1.90 (1H, m, Piperidinyll 5-H<sub>a</sub>), 1.84-1.74 (2H, m, Piperidinyll 4-H<sub>b</sub> and Piperidinyll 5-H<sub>b</sub>), 1.69-1.61 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.93 (3H, d, *J* 6.3 Hz, 4-Me), 0.89 (3H, d, *J* 6.3 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 177.9 (C-1), 174.5 (Formamido C-1), 52.1 (C-2), 44.5 (Piperidinyll C-2), 43.8 (Piperidinyll C-6), 39.7 (C-4), 38.6 (Piperidinyll C-3), 25.9 (Piperidinyll C-4), 24.5 (C-4), 22.2 (C-4 Me), 20.6 (Piperidinyll C-5), 20.4 (C-5); HRMS found MH<sup>+</sup> 243.1713. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

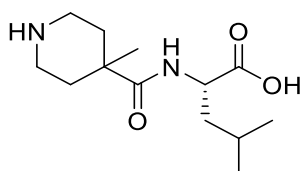
**(2S)-4-Methyl-2-{[(2S)-piperidin-2-yl]formamido}pentanoic acid (167)**



According to general procedure B, (2S)-N-*tert*-butylpiperidine-3-carboxamide (181.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **167** (26.0 mg, 68%) as a colourless solid.  $\delta_{\text{H}}$  (500

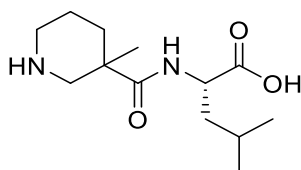
MHz, D<sub>2</sub>O) 4.32-4.27 (1H, m, 2-H), 3.95-3.89 (1H, dd, *J* 12.2 and 3.3 Hz, Piperidiny 2-H), 3.51-3.43 (1H, m, Piperidiny 6-H<sub>a</sub>), 3.07 (1H, td, *J* 12.6 and 3.1 Hz, Piperidiny 6-H<sub>b</sub>), 2.23-2.15 (1H, m, Piperidiny 3-H<sub>a</sub>), 1.97-1.86 (2H, m, Piperidiny 4-H<sub>a</sub> and Piperidiny 5-H<sub>a</sub>), 1.78-1.58 (6H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Piperidiny 3-H<sub>b</sub>, Piperidiny 4-H<sub>b</sub> and Piperidiny 5-H<sub>b</sub>), 0.93 (3H, d, *J* 6.2 Hz, 4-Me), 0.88 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>); δ<sub>c</sub> (125 MHz, D<sub>2</sub>O) 178.1 (C=O), 169.6 (C=O), 57.8 (C-2), 43.8 (Piperidiny C-6), 39.8 (C-2), 27.3 (Piperidiny C-3), 24.6 (C-4), 22.4 (C-4 Me), 21.3 (Piperidiny C-4 or Piperidiny C-5), 21.2 (Piperidiny C-4 or Piperidiny C-5), 20.4 (C-5); HRMS found MH<sup>+</sup> 243.1721. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

**(2S)-4-Methyl-2-[(4-methylpiperidin-4-yl)formamido]pentanoic acid (168)**



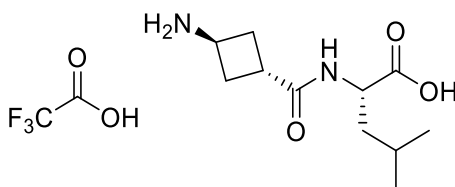
According to general procedure B, 1-boc-4-methylpiperidine-4-carboxylic acid (192.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **168** (26.0 mg, 64%) as white solid. δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 4.43-4.37 (1H, m, 2-H), 3.37-3.31 (2H, m, Piperidiny 2-H<sub>a</sub> and Piperidiny 6-H<sub>a</sub>), 3.13-3.00 (2H, m, Piperidiny 2-H<sub>b</sub> and Piperidiny 6-H<sub>b</sub>), 2.36-2.23 (2H, m, Piperidiny 3-H<sub>a</sub> and Piperidiny 5-H<sub>a</sub>), 1.81-1.59 (5H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Piperidiny 3-H<sub>b</sub>, Piperidiny 5-H<sub>b</sub> and 4-H), 0.94 (3H, d, *J* 6.2 Hz, 4-Me), 0.90 (3H, d, *J* 6.5 Hz, 5-H<sub>3</sub>); δ<sub>c</sub> (125 MHz, D<sub>2</sub>O) 177.9 (C=O), 177.0 (C=O), 55.3 (C-2), 41.3 (Piperidiny C-2 or Piperidiny C-6), 41.2 (Piperidiny C-2 or Piperidiny C-6), 39.8 (Piperidiny C-4), 39.3 (C-3), 31.0 (Piperidiny C-3 or Piperidiny C-5), 30.9 (Piperidiny C-3 or Piperidiny C-5), 24.8 (Me or C-4), 24.6 (Me or C-4), 22.3 (C-4 Me), 20.4 (C-5); HRMS found MH<sup>+</sup> 257.1867. C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 257.1860.

**(2S)-4-Methyl-2-[(3-methylpiperidin-3-yl)formamido]pentanoic acid (169)**



According to general procedure B, 1-boc-3-methylpiperidine-3-carboxylic acid (192.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **169** (35.0 mg, 86%) as a 50:50 mixture of diastereomers as colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.37-4.29 (1H, m, 2-H), 3.62-3.56 (1H, m, Piperidinyll 2-H<sub>a</sub>), 3.35-3.27 (1H, m, Piperidinyll 6-H<sub>a</sub>), 3.00-2.90 (1H, m, Piperidinyll 6-H<sub>b</sub>), 2.84-2.74 (1H, m, Piperidinyll 2-H<sub>b</sub>), 2.28-2.14 (1H, m, Piperidinyll 5-H<sub>a</sub>), 1.94-1.83 (1H, m, Piperidinyll 4-H<sub>a</sub>), 1.77-1.55 (5H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Piperidinyll 4-H<sub>b</sub>, Piperidinyll 5-H<sub>b</sub>), 1.33-1.28 (1.5H, s, Me), 1.28-1.24 (1.5H, s, Me), 1.00-0.91 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.4 (C=O), 178.3 (C=O), 177.1 (C=O), 177.0 (C=O), 52.7 (C-2), 52.6 (C-2), 50.1 (Piperidinyll C-2), 50.0 (Piperidinyll C-2), 43.5 (Piperidinyll C-6), 43.4 (Piperidinyll C-6), 40.5 (Piperidinyll C-3), 39.4 (C-3), 39.4 (C-3), 32.4 (Piperidinyll C-4), 32.2 (Piperidinyll C-4), 24.6 (C-4), 24.6 (C-4), 23.0 (Me), 22.8 (Me), 22.3 (C-5), 22.3 (C-5), 20.5 (C-4 Me), 20.4 (C-5), 19.4 (Piperidinyll C-5), 19.3 (Piperidinyll C-5); HRMS found  $M\text{Na}^+$  279.1674. C<sub>13</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires  $M\text{Na}$ , 279.1679.

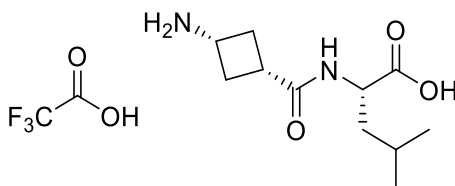
**(2S)-4-Methyl-2-{[(1*r*,3*r*)-3-aminocyclobutyl]formamido}pentanoic acid TFA salt (170)**



According to general procedure B, *trans*-3-((*tert*-butoxycarbonyl)amino)cyclobutanecarboxylic acid (170.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic*

*acid 170* (27.0 mg, 71%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3269, 3085, 2953, 1685, 1635, 1548;  $\delta_{\text{H}}$  (500 MHz,  $\text{D}_2\text{O}$ ) 4.37-4.30 (1H, m, 2-H), 3.98 (1H, app. p,  $J$  7.5 Hz, Aminocyclobutyl 3-H), 3.30 (1H, dddd,  $J$  14.6, 9.9, 4.7 and 1.1 Hz, Aminocyclobutyl 1-H), 2.65-2.53 (2H, m, Aminocyclobutyl 2- $\text{H}_a$  and Aminocyclobutyl 4- $\text{H}_a$ ), 2.52-2.42 (2H, m, Aminocyclobutyl 2- $\text{H}_b$  and Aminocyclobutyl 4- $\text{H}_b$ ), 1.65-1.62 (3H, m, 3- $\text{H}_a$ , 3- $\text{H}_b$  and 4-H), 0.92 (3H, d,  $J$  6.3 Hz, 4-Me), 0.89 (3H, d,  $J$  6.3 Hz, 5- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{D}_2\text{O}$ ) 177.9 (C=O), 176.9 (C=O), 162.9 (q,  $J$  35.5, C=O TFA), 116.3 (q,  $J$  291.6,  $\text{CF}_3$  TFA), 52.3 (C-2), 43.7 (Aminocyclobutyl C-1), 39.7 (C-3), 33.4 (Aminocyclobutyl C-3), 29.3 (Aminocyclobutyl C-2 or Aminocyclobutyl C-4), 29.1 (Aminocyclobutyl C-2 or Aminocyclobutyl C-4), 24.5 (C-4), 22.2 (C-4 Me), 20.5 (C-5); HRMS found  $\text{MNa}^+$  251.1359.  $\text{C}_{11}\text{H}_{21}\text{N}_2\text{O}_3$  requires  $MH$ , 251.1365.

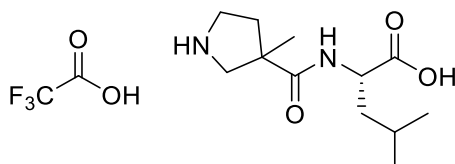
**(2S)-4-Methyl-2-{[(1s,3s)-3-aminocyclobutyl]formamido}pentanoic acid TFA salt (171)**



According to general procedure B, *cis*-3-((*tert*-butoxycarbonyl)amino)cyclobutanecarboxylic acid (170.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10  $\text{H}_2\text{O}$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid 171* (36.0 mg, 89%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3309, 3300-2500, 1662, 1535, 1328;  $\delta_{\text{H}}$  (500 MHz,  $\text{D}_2\text{O}$ ) 4.35-4.30 (1H, m, 2-H), 3.86-3.78 (1H, m, Aminocyclobutyl 3-H), 3.12-3.04 (1H, m, Aminocyclobutyl 1-H), 2.64-2.57 (2H, m, Aminocyclobutyl 2- $\text{H}_a$  and Aminocyclobutyl 4- $\text{H}_a$ ), 2.36-2.27 (2H, m, Aminocyclobutyl 2- $\text{H}_b$  and Aminocyclobutyl 4- $\text{H}_b$ ), 1.66-1.61 (3H, m, 3- $\text{H}_a$ , 3- $\text{H}_b$  and 4-H), 0.92 (3H, d,  $J$  6.4 Hz, 4-Me), 0.89 (3H, d,  $J$  6.4 Hz, 5- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{D}_2\text{O}$ ) 177.6 (C=O), 176.7 (C=O), 162.9 (q,  $J$  35.5, C=O TFA), 116.3 (q,  $J$  291.6,  $\text{CF}_3$  TFA), 52.2 (C-2), 41.3 (Aminocyclobutyl C-1), 39.5 (C-3), 32.2 (Aminocyclobutyl C-3), 30.0 (Aminocyclobutyl C-2 or Aminocyclobutyl C-4), 29.9

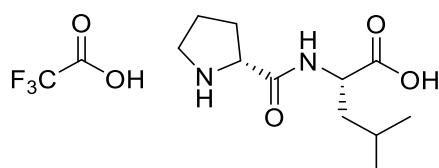
(Aminocyclobutyl C-2 or Aminocyclobutyl C-4), 24.4 (C-4), 22.2 (C-4 Me), 20.5 (C-5); HRMS found  $MH^+$  229.1549.  $C_{11}H_{20}N_2O_3$  requires  $MH$ , 229.1547.

**(2S)-4-Methyl-2-[(3-methylpyrrolidin-3-yl)formamido]pentanoic acid TFA salt (172)**



According to general procedure B, 1-Boc-3-methylpyrrolidine-3-carboxylic acid (181mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10  $H_2O$  (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **172** (29.0 mg, 78%) as a 50:50 mixture of diastereomers as colourless solid.  $\delta_H$  (500 MHz,  $D_2O$ ) 4.40-4.31 (1H, m, 2-H), 3.80 (0.5H, d,  $J$  12.0 Hz, Pyrrolidinyl 2- $H_a$ ), 3.79 (0.5H, d,  $J$  12.0 Hz, Pyrrolidinyl 2- $H_a$ ), 3.54-3.45 (1H, m, Pyrrolidinyl 5- $H_a$ ), 3.41-3.32 (1H, m, Pyrrolidinyl 5- $H_b$ ), 3.13 (0.5H,  $J$  12.0 Hz, Pyrrolidinyl 2- $H_b$ ), 3.09 (0.5H, d,  $J$  12.0 Hz, Pyrrolidinyl 2- $H_b$ ), 2.50-2.40 (1H, m, Pyrrolidinyl 4- $H_a$ ), 2.13-2.02 (1H, m, Pyrrolidinyl 4- $H_b$ ), 1.75-1.59 (3H, m, 3- $H_a$ , 3- $H_b$ , 4-H), 1.47 (1.5H, s, Me), 1.47 (1.5H, s, Me), 0.94 (3H, d,  $J$  5.9 Hz, 4-Me), 0.89 (3H, d,  $J$  5.9 Hz, 5- $H_3$ );  $\delta_C$  (125 MHz,  $D_2O$ ) 177.6 (C=O), 176.7 (C=O), 176.7 (C=O), 162.9 (q,  $J$  35.5, C=O TFA), 116.3 (q,  $J$  291.6,  $CF_3$  TFA), 53.1 (Pyrrolidinyl C-2), 53.0 (Pyrrolidinyl C-2), 52.5 (C-2), 49.0 (Pyrrolidinyl C-3), 49.0 (Pyrrolidinyl C-3), 44.9 (Pyrrolidinyl C-5), 44.9 (Pyrrolidinyl C-5), 39.3 (C-3), 39.2 (C-3), 35.1 (Pyrrolidinyl C-4), 35.1 (Pyrrolidinyl C-4), 24.6 (C-4), 24.5 (C-4), 22.0 (C-4 Me), 21.4 (Me), 21.3 (Me), 20.5 (C-5), 20.4 (C-5); HRMS found  $MNa^+$  265.1519.  $C_{12}H_{23}N_2O_3$  requires  $MH$ , 265.1523.

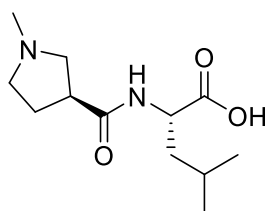
**(2S)-4-Methyl-2-{[(2R)-pyrrolidin-2-yl]formamido}pentanoic acid TFA salt (173)**





According to general procedure B, N-boc-D-proline (170.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **173** (32.0 mg, 88%) as colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.44-4.40 (1H, m, Pyrrolidinyl 2-H), 4.37-4.33 (1H, m, 2-H), 3.48-3.36 (2H, m, Pyrrolidinyl 5-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>b</sub>), 2.53-2.42 (1H, m, Pyrrolidinyl 3-H<sub>a</sub>), 2.13-2.00 (3H, m, Pyrrolidinyl 3-H<sub>b</sub>, Pyrrolidinyl 4-H<sub>a</sub> and Pyrrolidinyl 4-H<sub>b</sub>), 1.72-1.58 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.94 (3H, d, *J* 6.2 Hz, 4-Me), 0.89 (3H, d, *J* 6.2 Hz, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 177.6 (C=O), 169.4 (C=O), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 59.8 (Pyrrolidinyl C-2), 52.7 (C-2), 46.5 (Pyrrolidinyl C-5), 39.7 (C-3), 29.9 (Pyrrolidinyl C-3), 24.6 (C-4), 23.8 (Pyrrolidinyl C-4), 22.2 (C-4 Me), 20.4 (C-5); HRMS found 2MH<sup>+</sup> 457.3026. C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> requires 2MH, 457.3021.

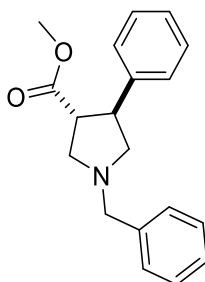
**(2S)-4-Methyl-2-{[(3S)-1-methylpyrrolidin-3-yl]formamido}pentanoic acid (174)**



According to general procedure B, (S)-Methylpyrrolidine-3-carboxylic acid (102.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **174** (23.6 mg, 62%) as a 55:45 mixture of rotamers as a colourless solid.  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.33-2.25 (1H, m, 2-H<sup>rot(min)</sup>), 3.93-3.86 (1H, m, Pyrrolidinyl 2-H<sub>a</sub>), 3.80-3.70 (1H, m, Pyrrolidinyl 5-H<sub>a</sub>), 3.50-3.42 (0.55H, m, Pyrrolidinyl 3-H<sup>rot(maj)</sup>), 3.41-3.26 (1.45H, m, Pyrrolidinyl 2-H<sub>b</sub> and Pyrrolidinyl 3-H<sup>rot(min)</sup>), 3.25-3.14 (1H, m, Pyrrolidinyl 5-H<sub>b</sub>), 2.99 (1.35H, s, Me<sup>rot(min)</sup>), 2.96 (1.65H, s, Me<sup>rot(maj)</sup>), 2.59-2.52 (0.55H, m, Pyrrolidinyl 4-H<sub>a</sub><sup>rot(maj)</sup>), 2.44-2.34 (0.45H, m, Pyrrolidinyl 4-H<sub>a</sub><sup>rot(min)</sup>), 2.32-2.19 (0.45H, m, Pyrrolidinyl 4-H<sub>b</sub><sup>rot(min)</sup>), 2.17-2.06 (0.55H, m, Pyrrolidinyl 4-H<sub>b</sub><sup>rot(maj)</sup>), 1.72-1.62 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H), 0.96-0.92 (3H, m, 4-Me), 0.91-0.87 (3H, m, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 178.3 (C=O<sup>rot(maj)</sup>), 178.1 (C=O<sup>rot(min)</sup>), 174.3 (C=O<sup>rot(maj)</sup>), 173.7 (C=O<sup>rot(min)</sup>), 57.4 (Pyrrolidinyl C-2<sup>rot(maj)</sup>), 57.2 (Pyrrolidinyl C-2<sup>rot(min)</sup>), 55.6 (Pyrrolidinyl C-5<sup>rot(maj)</sup>), 55.4 (Pyrrolidinyl C-5<sup>rot(min)</sup>), 52.8 (C-2<sup>rot(min)</sup>), 52.8 (C-2<sup>rot(maj)</sup>), 42.2 (Pyrrolidinyl

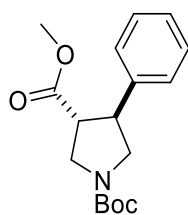
C-3<sup>rot(min)</sup>), 41.9 (Pyrrolidiny C-3<sup>rot(maj)</sup>), 40.8 (Me<sup>rot(maj)</sup>), 40.7 (Me<sup>rot(min)</sup>), 39.8 (C-3), 28.4 (Pyrrolidiny C-4), 24.6 (C-4<sup>rot(min)</sup>), 24.5 (C-4<sup>rot(maj)</sup>), 22.3 (C-4 Me), 20.5 (C-5<sup>rot(maj)</sup>), 20.5 (C-5<sup>rot(min)</sup>)(21/24 signals present); HRMS found MH<sup>+</sup> 243.1703. C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 243.1703.

**Methyl (3R\*,4S\*)-1-benzyl-4-phenylpyrrolidine-3-carboxylate (176a)**<sup>107</sup>



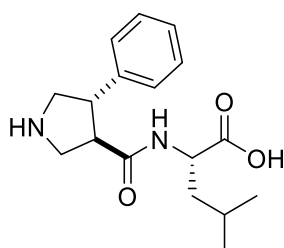
To DCM (10 mL) was added methyl cinnamate (1.49 mL g, 10 mmol) and stirred at 0 °C for 5 min. *N*-(Methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (3.07 mL, 12 mmol) was added dropwise and stirred for 10 min. TFA (80 uL, 1 mmol in 1 mL DCM) was added dropwise and left to stir while warming to room temperature overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the phenylpyrrolidine **176a** (1.73 g, 59%) as a colourless oil. *R*<sub>f</sub> 0.12 (90:10 hexane–EtOAc). δ<sub>H</sub> (500 MHz, CDCl<sub>3</sub>) 7.32-7.11 (10H, m, Ar-H), 3.65-3.56 (6H, m, 4-H, Benzyl CH<sub>2</sub>, Me), 3.07-2.92 (3H, m, Pyrrolidine 2-H<sub>a</sub>, Pyrrolidine 3-H, Pyrrolidine 5-H<sub>a</sub>), 2.81 (1H, dd, *J* 8.9 and 6.5 Hz, Pyrrolidine 2-H<sub>b</sub>), 2.69 (1H, dd, *J* 9.4 and 6.3 Hz, Pyrrolidine 5-H<sub>b</sub>); δ<sub>C</sub> (125 MHz, CDCl<sub>3</sub>) 174.7 (C=O), 144.2 (Ar-C), 138.8 (Ar-C), 128.7 (Ar-C), 128.6 (Ar-C), 128.3 (Ar-C), 127.5 (Ar-C), 127.1 (Ar-C), 126.6 (Ar-C), 61.8 (Pyrrolidine C-5), 60.0 (Benzylic CH<sub>2</sub>), 57.5 (Pyrrolidine C-2), 52.0 (Pyrrolidine C-3 or Pyrrolidine C-4), 51.7 (Pyrrolidine C-3 or Pyrrolidine C-4), 47.0 (Me); HRMS found MH<sup>+</sup> 296.1649. C<sub>19</sub>H<sub>21</sub>NO<sub>2</sub> requires *MH*, 296.1645. Spectroscopic data are consistent with those reported in the literature.<sup>124</sup>

**1-*tert*-Butyl 3-methyl (3R\*,4S\*)-4-phenylpyrrolidine-1,3-dicarboxylate (177a)**



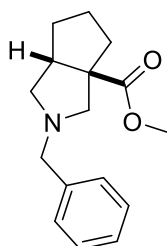
To a solution of methyl (3*R*\*,4*S*\*)-1-benzyl-4-phenylpyrrolidine-3-carboxylate (1.73 g, 5.86 mmol) and Pd/C (65.0 mg, 10 wt%) in MeOH (100 ml) was added Boc anhydride (1.41 g, 6.45 mmol) and left to stir for at r.t for 18 hr under H<sub>2</sub> atmosphere. The reaction mixture was then filtered through celite and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to give the *Boc derivative* **177a** (1.68 g, 94%) as a mixture of rotamers as a pale-yellow oil. *R<sub>f</sub>* 0.10 (90:10 hexane–EtOAc).  $\nu_{\max}/\text{cm}^{-1}$  (film) 2975, 2887, 1736, 1690, 1401, 1365, 1161, 1125;  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 7.34-7.29 (2H, m, Phenyl 2-H and Phenyl 6-H), 7.26-7.21 (3H, m, Phenyl 3-H, Phenyl 4-H, Phenyl 5-H), 3.96-3.75 (2H, m, Pyrrolidine 2-H<sub>a</sub> and Pyrrolidine 5-H<sub>a</sub>), 3.70-3.53 (5H, m, Me, Pyrrolidine 2-H<sub>b</sub>, Pyrrolidine 4-H), 3.49-3.33 (1H, m, Pyrrolidine 5-H<sub>b</sub>), 3.23-3.13 (1H, m, Pyrrolidine 3-H), 1.49-1.43 (9H, m, *tert*-Butyl);  $\delta_{\text{C}}$  (125 MHz, CDCl<sub>3</sub>) 172.8 (C=O), 172.8 (C=O), 154.2 (C=O), 139.4 (Phenyl C-1), 139.0 (Phenyl C-1), 128.9 (Phenyl C-2 and Phenyl C-6), 127.5 (Phenyl C-4 or Phenyl C-3 and Phenyl C-5), 127.4 (Phenyl C-4 or Phenyl C-3 and Phenyl C-5), 79.9 (*tert*-Butyl C-1), 52.8 (Pyrrolidine C-5), 52.2 (Me), 50.6 (Pyrrolidine C-3), 49.6 (Pyrrolidine C-3), 49.1 (Pyrrolidine C-2), 48.8 (Pyrrolidine C-2), 47.9 (Pyrrolidine C-4), 47.1 (Pyrrolidine C-4), 28.6 (*tert*-Butyl C-2)(18/26 signals present); HRMS found MNa<sup>+</sup> 328.1525. C<sub>17</sub>H<sub>23</sub>NO<sub>4</sub> requires MNa<sup>+</sup>, 328.1519.

**(2*S*)-4-Methyl-2-{[(3*R*,4*S*)-4-phenylpyrrolidin-3-yl]formamido}pentanoic acid and (2*S*)-4-methyl-2-{[(3*S*,4*R*)-4-phenylpyrrolidin-3-yl]formamido}pentanoic acid and (178a)**



To a solution of Boc derivative **177a** (1.68g, 5.50 mmol) in MeOH-H<sub>2</sub>O (30 mL: 30 mL) was added LiOH.H<sub>2</sub>O (256 mg, 6.10 mmol) and left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (250 mL), washed with brine (250 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure B, the crude carboxylic acid (233 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 87.3:12.7→67.3:32.7 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **178a** (44.0 mg, 91%) as a mixture of diastereomers as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3249, 3064, 3002, 2958, 2868, 1703, 1671, 1584;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 7.41-7.28 (5H, m, Ar-H), 4.24-4.16 (1H, m, 2-H), 3.83-3.76 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>a</sub>), 3.59-3.38 (3H, m, Pyrrolidinyl 2-H<sub>b</sub>, Pyrrolidinyl 4-H, Pyrrolidinyl 5-H<sub>b</sub>), 3.34-3.25 (1H, m, Pyrrolidinyl 3-H), 1.42-1.28 (2H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>), 0.57 (7H, m, 4-H, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 176.4 (C=O), 176.3 (C=O), 171.8 (C=O), 171.7 (C=O), 134.8 (Phenyl C-1), 129.3 (Phenyl C-2 and Phenyl C-6 or Phenyl C-3 and Phenyl C-5), 128.5 (Phenyl C-4), 127.6 (Phenyl C-2 and Phenyl C-6 or Phenyl C-3 and Phenyl C-5), 51.3 (C-2 or Pyrrolidinyl C-3), 51.2 (C-2 or Pyrrolidinyl C-3), 51.2 (C-2 or Pyrrolidinyl C-3), 50.9 (Pyrrolidinyl C-5), 49.2 (Pyrrolidinyl C-4), 47.5 (Pyrrolidinyl C-2), 38.8 (C-3), 38.8 (C-3), 23.5 (C-4), 22.2 (C-4 Me), 19.8 (C-5); HRMS found MNa<sup>+</sup> 327.1673. C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> requires MNa, 327.1679.

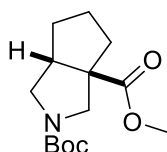
**Methyl (3aR\*,6aR\*)-2-benzyl-octahydrocyclopenta[c]pyrrole-3a-carboxylate (176b)**<sup>107</sup>



To DCM (10 mL) was added methyl-1-cyclopentene-1-carboxylate (1.22 mL g, 10 mmol) and stirred at 0 °C for 5 min. *N*-(Methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (3.07 mL, 12 mmol) was added dropwise and stirred for 10 min. TFA (80 uL, 1 mmol in 1 mL DCM) was added dropwise and left to stir while warming to room temperature overnight. The reaction

mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the phenylpyrrolidine **176b** (780 mg, 30%) as a colourless oil.  $R_f$  0.18 (90:10 hexane–EtOAc).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 7.34–7.10 (5H, m, Ar-H), 3.60 (3H, s, Me), 3.51 (1H, d,  $J$  13.2 Hz, Benzyl  $CH_a$ ), 3.45 (1H, d,  $J$  13.2 Hz, Benzyl  $CH_b$ ), 2.91–2.74 (2H, m, 3- $H_a$  and 6a-H), 2.66–2.56 (1H, m, 1- $H_a$ ), 2.41–2.33 (1H, m, 3- $H_b$ ), 2.29–2.19 (1H, m, 1- $H_b$ ), 2.01–1.90 (1H, m, 4- $H_a$ ), 1.86–1.74 (1H, m, 6- $H_a$ ), 1.74–1.61 (2H, m, 5- $H_a$  and 5- $H_b$ ), 1.61–1.52 (1H, m, 4- $H_b$ ), 1.50–1.39 (1H, m, 6- $H_b$ );  $\delta_C$  (125 MHz,  $CDCl_3$ ) 179.1 (C=O), 140.5 (Phenyl C-1), 129.8 (Phenyl C-2 and Phenyl C-6 or Phenyl C-3 and Phenyl C-5), 129.5 (Phenyl C-2 and Phenyl C-6 or Phenyl C-3 and Phenyl C-5), 128.1 (Phenyl C-4), 64.9 (C-3), 62.5 (C-1), 60.9 (Benzylic  $CH_2$ ), 60.8 (C-3a), 53.3 (Me), 49.0 (C-6a), 39.6 (C-4), 34.8 (C-6), 28.0 (C-5); HRMS found  $MH^+$  260.1650.  $C_{16}H_{21}NO_2$  requires  $MH$ , 260.1645. Spectroscopic data are consistent with those reported in the literature.<sup>125</sup>

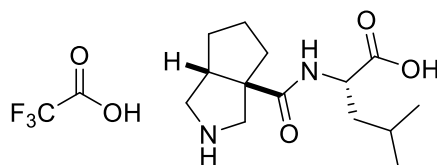
### 2-*tert*-Butyl 3a-methyl (3a*R*\*,6a*R*\*)-octahydrocyclopenta[*c*]pyrrole-2,3a-dicarboxylate (**177b**)



To a solution of ethyl (3a*R*\*,6a*R*\*)-2-benzyl-octahydrocyclopenta[*c*]pyrrole-3a-carboxylate (780 mg, 3.00 mmol) and Pd/C (78.0 mg, 10 wt%) in MeOH (50 ml) was added Boc anhydride (720 mg, 3.30 mmol) and left to stir at r.t. for 18 hr under  $H_2$  atmosphere. The reaction mixture was then filtered through celite and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to give the Boc derivative **177b** (718 mg, 77%) as a mixture of rotamers as a pale-yellow oil.  $R_f$  0.20 (90:10 hexane–EtOAc).  $\nu_{max}/cm^{-1}$  (film) 2954, 2874, 1730, 1694, 1393, 1366, 1360, 1130;  $\delta_H$  (500 MHz,  $CDCl_3$ ) 3.91–3.81 (1H, m, 3- $H_a$ ), 3.72–3.66 (3H, m, Me), 3.61–3.52 (1H, m, 1- $H_a$ ), 3.36–3.27 (1H, m, 3- $H_b$ ), 3.26–3.16 (1H, m, 1- $H_b$ ), 2.91–2.83 (1H, m, 6a-H), 2.21–2.10 (1H, m, 4- $H_a$ ), 2.02–1.89 (1H, m, 6- $H_a$ ), 1.84 (3H, m, 4- $H_a$ , 5- $H_a$  and 5- $H_b$ ), 1.51 (1H, m, 6- $H_b$ ), 1.46–1.41 (9H, m, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 176.5 (C=O), 154.4 (C=O), 79.4 (*tert*-Butyl C-

1), 59.9 (C-3a), 59.0 (C-3a), 54.8 (C-3), 52.3 (Me), 52.1 (C-1), 51.9 (C-1), 48.1 (C-6a), 47.3 (C-6a), 36.5 (C-4), 32.6 (C-6), 28.5 (*tert*-Butyl C-2), 25.4 (C-5)(15/24 signals present); HRMS found  $2MH^+$  539.3335.  $C_{14}H_{23}NO_4$  requires  $2MH$ , 539.3327.

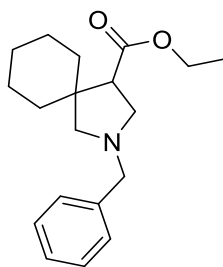
**(2S)-2-{\[(3aR,6aR)-octahydrocyclopenta[c]pyrrol-3a-yl]formamido}-4-methylpentanoic acid TFA salt and (2S)-2-{\[(3aS,6aS)-octahydrocyclopenta[c]pyrrol-3a-yl]formamido}-4-methylpentanoic acid TFA salt and (178b)**



To a solution of boc derivative **177b** (718 mg, 2.67 mmol) in MeOH-H<sub>2</sub>O (15 mL: 15 mL) was added LiOH.H<sub>2</sub>O (123 mg, 2.93 mmol) and left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (100 mL), washed with brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure B, the crude carboxylic acid (199 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 87.3:12.3→67.8:32.2 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **178b** (35.0 mg, 83%) a mixture of diastereomers as a colourless solid.  $\nu_{max}/cm^{-1}$  (film) 3300-2500, 3295, 2958, 2872, 1670 (br);  $\delta_H$  (500 MHz, D<sub>2</sub>O) 4.41-4.34 (1H, m, 2-H), 3.91-3.84 (1H, m, Octahydrocyclopenta[c]pyrrolyl 1-H<sub>a</sub>), 3.65-3.58 (1H, m, Octahydrocyclopenta[c]pyrrolyl 3-H<sub>a</sub>), 3.22-3.14 (2H, m, Octahydrocyclopenta[c]pyrrolyl 6a-H and Octahydrocyclopenta[c]pyrrolyl 1-H<sub>b</sub>), 3.09-3.02 (1H, m, Octahydrocyclopenta[c]pyrrolyl 3-H<sub>b</sub>), 2.06-1.92 (3H, Octahydrocyclopenta[c]pyrrolyl 4-H<sub>a</sub>, Octahydrocyclopenta[c]pyrrolyl 4-H<sub>b</sub> and Octahydrocyclopenta[c]pyrrolyl 6-H<sub>a</sub>), 1.89-1.76 (2H, m, Octahydrocyclopenta[c]pyrrolyl 5-H<sub>a</sub> and Octahydrocyclopenta[c]pyrrolyl 5-H<sub>b</sub>), 1.76-1.57 (4H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H and Octahydrocyclopenta[c]pyrrolyl 6-H<sub>b</sub>), 0.95-0.87 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, D<sub>2</sub>O) 177.7 (C=O), 177.6 (C=O), 176.9 (C=O), 176.9 (C=O), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 60.1 (Octahydrocyclopenta[c]pyrrolyl C-3a), 60.0

(Octahydrocyclopenta[c]pyrrolyl C-3a), 53.7 (Octahydrocyclopenta[c]pyrrolyl C-1), 53.5 (Octahydrocyclopenta[c]pyrrolyl C-1), 52.6 (C-2), 51.3 (Octahydrocyclopenta[c]pyrrolyl C-3), 51.2 (Octahydrocyclopenta[c]pyrrolyl C-3), 46.5 (Octahydrocyclopenta[c]pyrrolyl C-6a), 46.1 (Octahydrocyclopenta[c]pyrrolyl C-6a), 39.3 (C-3), 39.2 (C-3), 36.6 (Octahydrocyclopenta[c]pyrrolyl C-4), 36.1 (Octahydrocyclopenta[c]pyrrolyl C-4), 31.0 (Octahydrocyclopenta[c]pyrrolyl C-6), 30.9 (Octahydrocyclopenta[c]pyrrolyl C-6), 24.9 (C-4 or Octahydrocyclopenta[c]pyrrolyl C-5), 24.8 (C-4 or Octahydrocyclopenta[c]pyrrolyl C-5), 24.6 (C-4 or Octahydrocyclopenta[c]pyrrolyl C-5), 24.6 (C-4 or Octahydrocyclopenta[c]pyrrolyl C-5), 22.2 (C-4 Me), 22.2 (C-4 Me), 20.4 (C-5), 20.4 (C-5); HRMS found  $MH^+$  269.1867.  $C_{14}H_{24}N_2O_3$  requires  $MH$ , 269.1867.

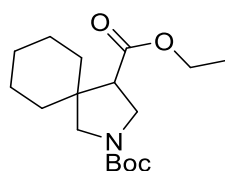
### **Ethyl 2-benzyl-2-azaspiro[4.5]decane-4-carboxylate (176c)**<sup>108</sup>



To a solution of ethyl cyclohexylideneacetate (1.60 mL g, 10 mmol), LiF (777 mg, 30 mmol) and in acetonitrile (17 mL) was added *N*-(Methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine (2.85 mL, 12 mmol) and left to stir at 60 °C overnight. The reaction mixture was concentrated under reduced pressure and the residue redissolved in ethyl acetate (250 mL), washed with sat.  $K_2CO_3$  solution (100 mL), sat.  $CuSO_4$  (100 mL) and brine (100 mL), dried over  $MgSO_4$  and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the spirocycle **176c** (1.14 g, 38%) as a colourless oil.  $R_f$  0.10 (90:10 hexane–EtOAc).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 7.28–7.13 (5H, m, Ar-H), 4.11–4.00 (2H, m, 1- $H_2$  Ethyl), 3.58 (1H, d,  $J$  13.2 Hz,  $CH_a$  Benzylic), 3.53 (1H, d,  $J$  13.2 Hz,  $CH_b$  Benzylic), 2.85 (1H, dd,  $J$  9.3 and 5.7 Hz, 3- $H_a$ ), 2.73–2.65 (2H, m, 1- $H_a$  and 3- $H_b$ ), 2.56 (1H, dd,  $J$  8.7 and 7.5 Hz, 4-H), 2.19 (1H, d,  $J$  9.1 Hz, 1- $H_b$ ), 1.62–1.44 (6H, m, 6- $H_a$ , 6- $H_b$ , 10- $H_a$ , 10- $H_b$  and Cyclohexyl-H), 1.25–1.17 (4H, m, Ethyl 2-

H<sub>3</sub> and Cyclohexyl-H), 1.15-1.06 (3H, m, Cyclohexyl-H);  $\delta_{\text{C}}$  (125 MHz, CDCl<sub>3</sub>) 173.5 (C=O), 139.6 (Phenyl C-1), 128.6 (Phenyl C-2 and Phenyl C-6 or Phenyl C-3 and Phenyl C-5), 128.3 (Phenyl C-2 and Phenyl C-6 or Phenyl C-2 and Phenyl C-5), 126.9 (Phenyl C-4), 63.4 (C-1), 60.4 (Ethyl C-1 or Benzylic CH<sub>2</sub>), 60.3 (Ethyl C-1 or Benzylic CH<sub>2</sub>), 55.6 (C-3), 54.5 (C-4), 45.8 (C-5), 38.2 (C-6), 33.4 (C-7 or C-8 or C-9 or C-10), 25.9 (C-7 or C-8 or C-9 or C-10), 24.2 (C-7 or C-8 or C-9 or C-10), 23.3 (C-7 or C-8 or C-9 or C-10), 14.5 (Ethyl C-2); HRMS found MH<sup>+</sup> 302.2121. C<sub>19</sub>H<sub>28</sub>NO<sub>2</sub> requires *MH*, 302.2115. Spectroscopic data are consistent with those reported in the literature.<sup>108</sup>

### 2-*tert*-Butyl 4-ethyl 2-azaspiro[4.5]decane-2,4-dicarboxylate (**177c**)

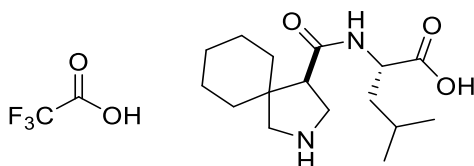


To a solution of ethyl 2-benzyl-2-azaspiro[4.5]decane-4-carboxylate **176c** (1.14 g, 3.78 mmol) and Pd/C (114.0 mg, 10 wt%) in MeOH (65 ml) was added Boc anhydride (908 mg, 4.16 mmol) and left to stir for 18 hr at r.t under H<sub>2</sub> atmosphere. The reaction mixture was then filtered through celite and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting 80:20 hexane–EtOAc to give the *Boc derivative* **177c** (756 mg, 77%) as a 60:40 mixture of rotamers as a colourless oil. *R<sub>f</sub>* 0.32 (80:20 hexane–EtOAc).  $\nu_{\text{max}}/\text{cm}^{-1}$  (film) 2976, 2930, 2860, 1731, 1696, 1401, 1178, 1157;  $\delta_{\text{H}}$  (500 MHz, CDCl<sub>3</sub>) 4.25-4.07 (2H, m, Ethyl 1-H<sub>2</sub>), 3.70-3.48 (2.6H, m, 1-H<sub>a</sub>, 1-H<sub>b</sub>, 3-H<sub>a</sub><sup>rot(maj)</sup>), 3.37 (0.4H, d, *J* 10.7 Hz, 3-H<sub>a</sub><sup>rot(min)</sup>), 3.26-3.11 (1H, m, 3-H<sub>b</sub>), 2.75-2.66 (1H, m, 4-H), 1.70-1.14 (22H, m, Cyclohexyl-H, *tert*-Butyl, Ethyl 2-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, CDCl<sub>3</sub>) 172.4 (C=O<sup>rot(min)</sup>), 172.1 (C=O<sup>rot(maj)</sup>), 162.9 (q, *J* 35.5, C=O TFA), 154.7 (C=O<sup>rot(min)</sup>), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA) 154.6 (C=O<sup>rot(maj)</sup>), 79.3 (*tert*-Butyl C-1), 60.5 (Ethyl C-1<sup>rot(maj)</sup>), 60.5 (Ethyl C-1<sup>rot(min)</sup>), 54.5 (C-3<sup>rot(min)</sup>), 54.1 (C-3<sup>rot(maj)</sup>), 52.9 (C-4<sup>rot(maj)</sup>), 51.8 (C-4<sup>rot(min)</sup>), 46.7 (C-1<sup>rot(maj)</sup>), 46.6 (C-1<sup>rot(min)</sup>), 45.4 (C-5<sup>rot(min)</sup>), 44.6 (C-5<sup>rot(maj)</sup>), 36.0 (C-6<sup>rot(maj)</sup> or C-7<sup>rot(maj)</sup> or C-8<sup>rot(maj)</sup> or C-9<sup>rot(maj)</sup> or C-10<sup>rot(maj)</sup>), 35.8 (C-6<sup>rot(min)</sup> or C-7<sup>rot(min)</sup> or C-8<sup>rot(min)</sup> or C-9<sup>rot(min)</sup> or C-10<sup>rot(min)</sup>), 30.8 (C-6<sup>rot(min)</sup> or C-7<sup>rot(min)</sup> or C-8<sup>rot(min)</sup> or C-9<sup>rot(min)</sup> or C-10<sup>rot(min)</sup>), 30.5 (C-6<sup>rot(maj)</sup> or C-7<sup>rot(maj)</sup> or C-8<sup>rot(maj)</sup>



or C-9<sup>rot(maj)</sup> or C-10<sup>rot(maj)</sup>), 28.6 (*tert*-Butyl C-2<sup>min</sup>), 28.6 (*tert*-Butyl C-2<sup>maj</sup>), 25.9 (C-6<sup>rot(min)</sup> or C-7<sup>rot(min)</sup> or C-8<sup>rot(min)</sup> or C-9<sup>rot(min)</sup> or C-10<sup>rot(min)</sup>), 25.9 (C-6<sup>rot(maj)</sup> or C-7<sup>rot(maj)</sup> or C-8<sup>rot(maj)</sup> or C-9<sup>rot(maj)</sup> or C-10<sup>rot(maj)</sup>), 23.5 (C-6<sup>rot(maj)</sup> or C-7<sup>rot(maj)</sup> or C-8<sup>rot(maj)</sup> or C-9<sup>rot(maj)</sup> or C-10<sup>rot(maj)</sup>), 23.3 (C-6<sup>rot(min)</sup> or C-7<sup>rot(min)</sup> or C-8<sup>rot(min)</sup> or C-9<sup>rot(min)</sup> or C-10<sup>rot(min)</sup>) 23.0 (C-6<sup>rot(min)</sup> or C-7<sup>rot(min)</sup> or C-8<sup>rot(min)</sup> or C-9<sup>rot(min)</sup> or C-10<sup>rot(min)</sup>), 22.9 (C-6<sup>rot(maj)</sup> or C-7<sup>rot(maj)</sup> or C-8<sup>rot(maj)</sup> or C-9<sup>rot(maj)</sup> or C-10<sup>rot(maj)</sup>), 14.4 (Ethyl C-2<sup>maj</sup>), 14.3 (Ethyl C-2<sup>min</sup>) (29/30 signals present); HRMS found MNa<sup>+</sup> 334.1988. C<sub>17</sub>H<sub>29</sub>NO<sub>4</sub> requires MNa, 334.1989.

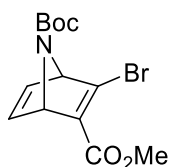
**(2S)-2-{[(4R)-2-azaspiro[4.5]decan-4-yl]formamido}-4-methylpentanoic acid TFA salt and (2S)-2-{[(4S)-2-azaspiro[4.5]decan-4-yl]formamido}-4-methylpentanoic acid TFA salt (178c)**



To a solution of boc derivative **177c** (756 mg, 2.43 mmol) in MeOH-H<sub>2</sub>O (10 mL: 10 mL) was added LiOH.H<sub>2</sub>O (204 mg, 4.86 mmol) and left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (100 mL), washed with brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure B, the crude carboxylic acid (221.0 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 85.2:14.8→65.2:34.8 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **178c** (49.0 mg, 77%) as a 60:40 mixture of rotamers as a mixture of diastereomers as a white solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3197, 2926, 2859, 1657, 1644, 1544;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.43-4.31 (1H, m, 2-H), 3.64-3.51 (2H, m, Azaspirodecanyl 3-H), 3.40-3.24 (2H, m, Azaspirodecanyl 1-H and Azaspirodecanyl 3-H), 3.09-2.95 (1H, m, Azaspirodecanyl 4-H), 1.80-1.27 (13H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, 4-H, Cyclohexyl-H), 0.97-0.87 (6H, m, 4-Me and 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 176.7 (C=O<sup>rot(min),dia(min)</sup>), 176.6 (C=O<sup>rot(min),dia(maj)</sup>), 172.2 (C=O<sup>rot(maj),dia(min)</sup>), 176.2 (C=O<sup>rot(maj),dia(maj)</sup>), 173.6 (C=O<sup>rot(min),dia(min)</sup>), 173.5 (C=O<sup>rot(maj),dia(min)</sup>), 173.2 (C=O<sup>rot(min),dia(maj)</sup>), 173.1 (C=O<sup>rot(maj),dia(maj)</sup>), 52.9 (Azaspirodecanyl C-1), 51.9 (Azaspirodecanyl C-1), 51.6 (C-2 or Azaspirodecanyl C-4), 51.5 (C-2 or Azaspirodecanyl C-4), 51.5 (C-2 or

Azaspirodecanyl C-4), 51.4 (C-2 or Azaspirodecanyl C-4), 46.5 (Azaspirodecanyl C-3<sup>rot(maj),dia(maj)</sup>), 46.5 (Azaspirodecanyl C-3<sup>rot(min),dia(min)</sup>), 46.4 (Azaspirodecanyl C-3<sup>rot(maj),dia(min)</sup>), 46.2 (Azaspirodecanyl C-3<sup>rot(min),dia(maj)</sup>), 39.3 (C-3<sup>rot(maj),dia(min)</sup>), 39.2 (C-3<sup>rot(maj),dia(maj)</sup>), 39.0 (C-3<sup>rot(min),dia(min)</sup>), 39.0 (C-3<sup>rot(min),dia(maj)</sup>), 35.0 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 34.9 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 30.8 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 30.4 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 25.0 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 24.9 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 24.4 (C-4), 24.4 (C-4), 22.8 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 22.8 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 22.4 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 22.3 (Azaspirodecanyl C-6 or Azaspirodecanyl C-7, Azaspirodecanyl C-8, Azaspirodecanyl C-9, Azaspirodecanyl C-10), 22.3 (C-4 Me), 22.0 (C-4 Me), 20.0 (C-5), 19.9 (C-5)(38/64 signals present); HRMS found MNa<sup>+</sup> 319.1981. C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>3</sub> requires *MNa*, 319.1992.

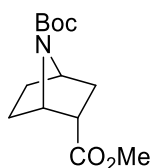
***tert*-Butyl 2-methyl (1*R*\*,4*S*\*)-3-bromo-7-azabicyclo[2.2.1]hepta-2,5-diene-2,7- dicarboxylate (180)**<sup>109</sup>



To a solution of NBS (4.89 g, 27.40 mmol) and AgNO<sub>3</sub> (0.40 g, 2.38 mmol) in acetone (100 mL) was added methyl propiolate (2.00 g, 23.80 mmol) left to stir for at r.t for 2 hr. Water (100 ml) was added and the mixture extracted with DCM (3 x 80 ml). The organic layers were combined, washed with sat. NaHCO<sub>3</sub> (20 ml), brine (20 ml), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give

the crude intermediate. This was then dissolved in toluene (10 ml) and N-Boc pyrrole (8.00 g, 48.0 mmol) was added and the resulting reaction mixture stirred at 90 °C for 3 days. The mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography, eluting with 90:10 hexane–EtOAc to yield the bicyclic alkene derivative **180** (1.64 g, 21%) as an indetermined mixture of rotamers as a bright yellow oil.  $R_f$  0.31 (hexane–EtOAc 90:10).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 7.17-7.03 (2H, m, 5-H, 6-H), 5.48 (1H, app. br. s, 1-H), 5.20-5.05 (1H, app. br. s, 4-H), 3.78 (3H, s, OMe), 1.40 (9H, s, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 162.8 (C=O), 154.2 (C=O), 148.2 (C-3<sup>rot(min)</sup>), 147.6 (C-3<sup>rot(maj)</sup>), 144.2 (C-2<sup>rot(min)</sup>), 143.5 (C-2<sup>rot(maj)</sup>), 141.6 (C-6<sup>rot(min)</sup>), 140.6 (C-5<sup>rot(maj)</sup>), 81.7 (*tert*-Butyl C-1), 75.1 (C-4<sup>rot(maj)</sup>), 74.7 (C-4<sup>rot(min)</sup>), 69.0 (C-1<sup>rot(min)</sup>), 68.5 (C-1<sup>rot(maj)</sup>), 52.0 (OMe), 28.2 (*tert*-Butyl C-2) (20/30 signals present). HRMS found  $MNa^+$  352.0157.  $C_{13}H_{16}BrNO_4$  requires  $MNa$ , 352.0152. All data is consistent with known literature values.<sup>109</sup>

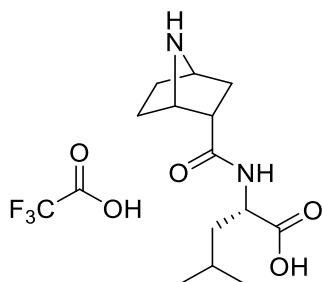
**7-*tert*-Butyl 2-methyl (1*R*,2*S*,4*R*)-7-azabicyclo[2.2.1]heptane-2,7-dicarboxylate (181)**<sup>123</sup>



To a solution of bicyclic alkene derivative **180** (1.64 g, 6.43 mmol) and Pd/C (65.0 mg, 10 wt%) in MeOH (45 ml) was added  $NEt_3$  (1.80 ml, 12.86 mmol) and left to stir at r.t for 18 hr under  $H_2$  atmosphere. The reaction mixture was then filtered through celite and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting 95:5 hexane–EtOAc to yield methyl ester derivative **181** (720 mg, 44%, dr>95:>5 by  $^1H$  NMR) as a pale-yellow oil.  $R_f$  0.35 (EtOAc–hexane 10:90).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 4.19 (1H, app. br. s, 1-H), 4.00 (1H, app. br. s, 4-H), 3.51 (3H, s, OMe), 2.91-2.81 (1H, m, 2-H), 1.82-1.74 (1H, m, 3- $H_a$ ), 1.70 (1H, dd,  $J$  12.4 and 4.9 Hz, 3- $H_b$ ), 1.63-1.55 (1H, m, 5- $H_a$ ), 1.55-1.47 (1H, m, 6- $H_a$ ), 1.33-1.28 (2H, m, 5- $H_b$  and 6- $H_b$ ), 1.26 (9H, s, *tert*-Butyl);  $\delta_C$  (125 MHz,  $CDCl_3$ ) 172.7 (C=O), 155.1 (C=O), 79.5 (*tert*-Butyl C-1), 58.1 (C-1), 56.9 (C-4), 51.5 (OMe), 46.1 (C-2), 32.2 (C-3), 29.0 (C-5), 28.0 (*tert*-Butyl C-2), 25.2 (C-6). HRMS found  $MNa^+$  278.1365.

$C_{13}H_{21}NO_4$  requires *MH*, 278.1363. All data is consistent with known literature values.<sup>123</sup>

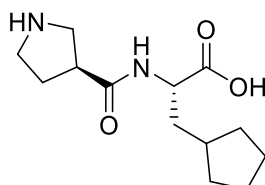
**(2S)-2-({7-Azabicyclo[2.2.1]heptan-2-yl}formamido)-4-methylpentanoic acid TFA salt (**182**)**



To a solution of methyl ester derivative **181** (164 mg, 6.43 mmol) in MeOH-H<sub>2</sub>O (18 mL: 18 mL) was added LiOH.H<sub>2</sub>O (131 mg, 3.11 mmol) and left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (50 mL), washed with brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure B, the crude carboxylic acid (190 mg, 0.79 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 93.7:6.3→73.7:26.3 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *pentanoic acid* **182** (36.0 mg, 56%) as a 60:40 mixture of rotamers as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 2959, 1655, 1648, 1561, 1187;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.49 (0.4H, app. *t* *J* 4.6 Hz, 2-H<sup>rot(min)</sup>), 4.44 (0.6H, app. *t*, *J* 4.6 Hz, 2-H<sup>rot(maj)</sup>), 4.40-4.36 (1H, m, Azabicyclo 4-H), 4.32-4.29 (1H, m, Azabicyclo 1-H), 3.38-3.33 (1H, m, Azabicyclo 2-H), 2.15-2.05 (2H, m, Azabicyclo 3-H<sub>a</sub> and Azabicyclo 3-H<sub>b</sub>), 2.02-1.95 (1H, m, Azabicyclo 5-H<sub>a</sub>), 1.94-1.84 (1H, m, Azabicyclo 6-H<sub>a</sub>), 1.83-1.74 (2H, m, Azabicyclo 5-H<sub>b</sub> and Azabicyclo 6-H<sub>b</sub>), 1.70-1.60 (3H, m, 2-H, 3-H<sub>a</sub>, 3-H<sub>b</sub>), 0.94 (3H, d, *J* 6.2 Hz, 4-Me), 0.90 (3H, m, 5-H<sub>3</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 177.2 (C=O<sup>rot(maj)</sup>), 177.1 (C=O<sup>rot(min)</sup>), 171.3 (C=O<sup>rot(min)</sup>), 171.1 (C=O<sup>rot(maj)</sup>), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 60.5 (C-2<sup>rot(min)</sup>), 60.4 (C-2<sup>rot(maj)</sup>), 59.7 (Azabicyclo C-1<sup>rot(min)</sup>), 59.6 (Azabicyclo C-1<sup>rot(maj)</sup>), 55.2 (Azabicyclo C-4<sup>rot(min)</sup>), 55.2 (C-4 Azabicyclo<sup>rot(maj)</sup>), 44.2 (Azabicyclo C-2<sup>rot(maj)</sup>), 43.9 (Azabicyclo C-2<sup>rot(min)</sup>), 39.3 (C-3<sup>rot(maj)</sup>), 39.2 (C-3<sup>rot(min)</sup>), 29.0 (Azabicyclo C-3<sup>rot(min)</sup>), 28.9 (Azabicyclo C-3<sup>rot(maj)</sup>), 26.4 (Azabicyclo C-5<sup>rot(maj)</sup>), 26.3 (Azabicyclo C-5<sup>rot(min)</sup>), 24.5 (C-4<sup>rot(maj)</sup>), 24.5 (C-4<sup>rot(min)</sup>), 22.4 (Azabicyclo C-

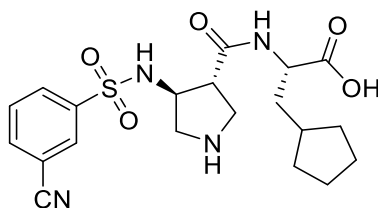
6<sup>rot(maj)</sup>), 22.3 (Azabicyclo C-6<sup>rot(min)</sup>), 22.2 (C-4 Me<sup>rot(maj)</sup>), 22.1 (C-4 Me<sup>rot(min)</sup>), 20.5 (C-5<sup>rot(min)</sup>), 20.3 (C-5<sup>rot(maj)</sup>)(26/26 signals present); HRMS found MH<sup>+</sup> 255.1697. C<sub>13</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 255.1703.

**(2S)-3-Cyclopentyl-2-{[(3S)-pyrrolidin-3-yl]formamido}propanoic acid (184)**



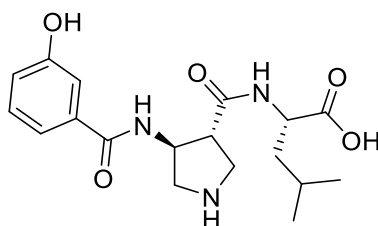
According to general procedure G, *tert*-butyl (2S)-2-amino-3-cyclopentylpropanoate (130.0 mg, 0.65 mmol) and (S)-1-N-boc- $\beta$ -proline (183.0 mg, 0.85 mmol) gave a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2→90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **X** (106.0 mg, 64%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 2950, 2989, 1655 (br), 1182, 1133;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.32-4.28 (1H, m, 2-H), 3.51 (2H, app. br. d, *J* 7.1 Hz, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 2-H<sub>b</sub>), 3.47-3.29 (3H, m, Pyrrolidinyl 3-H, Pyrrolidinyl 5-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>b</sub>), 2.38 (1H, dtd, *J* 13.6, 8.0 and 6.6 Hz, Pyrrolidinyl 4-H<sub>a</sub>), 2.12 (1H, dddd, *J* 13.6, 8.0, 7.1 and 6.6 Hz, Pyrrolidinyl 4-H<sub>b</sub>), 1.90-1.67 (5H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Cyclopentyl 1-H, Cyclopentyl 3-H<sub>a</sub> and Cyclopentyl 4-H<sub>a</sub>), 1.66-1.56 (2H, m, Cyclopentyl 2-H<sub>a</sub> and Cyclopentyl 5-H<sub>a</sub>), 1.56-1.47 (2H, m, Cyclopentyl 2-H<sub>b</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.21-1.08 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 177.4 (C=O), 174.0 (C=O), 162.9 (q, *J* 35.5, C=O TFA), 116.3 (q, *J* 291.6, CF<sub>3</sub> TFA), 53.5 (C-2), 47.3 (Pyrrolidinyl C-2), 45.4 (Pyrrolidinyl C-5), 42.3 (Pyrrolidinyl C-3), 36.9 (C-2 or C-3), 36.5 (C-2 or C-3), 32.3 (Cyclopentyl C-3 or Cyclopentyl C-4), 31.5 (Cyclopentyl C-3 or Cyclopentyl C-4), 28.8 (Pyrrolidinyl C-4), 24.6 (Cyclopentyl C-2 or Cyclopentyl C-5), 24.5 (Cyclopentyl C-2 or Cyclopentyl C-5); HRMS found 2MH<sup>+</sup> 509.3353. C<sub>13</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> requires *MH*, 509.3334.

**(S)-2-((3R,4S)-4-((3-cyanophenyl)sulfonamido)pyrrolidine-3-carboxamido)-3-cyclopentylpropanoic acid (**185**)**



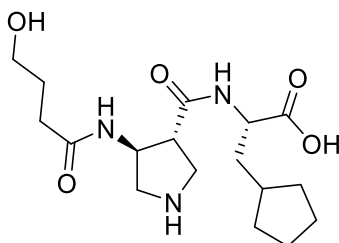
To a solution of *tert*-butyl (4S)-3-{[(2S)-1-(*tert*-butoxy)-3-cyclopentyl-1-oxopropan-2-yl]carbamoyl}-4-(3-cyanobenzenesulfonamido)pyrrolidine-1-carboxylate (76.0 mg, 0.13 mmol) in DCM DCM-TFA (5 mL : 5 mL) was added triethylsilane (41.0  $\mu$ L, 0.26 mmol) and left to stir overnight at r.t. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2 $\rightarrow$ 90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **185** (27.4 mg, 49%) as a colourless solid.  $[\alpha]_D = -8.6$ ;  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3255, 3006, 2942, 1655, 1648, 1561;  $\delta_{\text{H}}$  (500 MHz, DMSO-*d*<sup>6</sup>) 8.14 (1H, td, *J* 1.8 and 0.6 Hz, Phenyl 2-H), 8.11 (1H, ddd, *J* 7.7, 1.6 and 1.1 Hz, Phenyl 6-H), 8.06 (1H, ddd, *J* 8.0, 1.9 and 1.1 Hz, Phenyl 4-H), 7.96 (1H, d, *J* 7,8 Hz, Amide NH), 7.79 (1H, td, *J* 7.9 and 0.6 Hz, Phenyl 5-H), 4.02 (1H, ddd, *J* 9.2, 7.8 and 5.4 Hz, 2-H), 3.90-3.81 (1H, m, Pyrrolidinyl 4-H), 3.11-3.04 (1H, m, Pyrrolidinyl 2-H<sub>a</sub>), 2.83 (1H, dd, *J* 11.6 and 6.9 Hz, Pyrrolidinyl 5-H<sub>a</sub>), 2.72-2.62 (2H, m, Pyrrolidinyl 2-H<sub>b</sub> and Pyrrolidinyl 3-H), 2.44 (1H, dd, *J* 11.6 and 6.9 Hz, Pyrrolidinyl 5-H<sub>b</sub>), 1.84-1.74 (1H, m, Cyclopentyl 1-H), 1.75-1.65 (2H, m, Cyclopentyl 3-H<sub>a</sub> and Cyclopentyl 4-H<sub>a</sub>), 1.62-1.42 (6H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Cyclopentyl 2-H<sub>a</sub>, Cyclopentyl 2-H<sub>b</sub>, Cyclopentyl 5-H<sub>a</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.12-0.98 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_{\text{C}}$  (125 MHz, DMSO-*d*<sup>6</sup>) 174.6 (C-1), 172.5 (C=O), 142.9 (Phenyl C-1), 136.4 (Phenyl C-6), 131.4 (Phenyl C-4), 131.2 (Phenyl C-5), 130.5 (Phenyl C-2), 118.1 (Phenyl C-3), 112.9 (C $\equiv$ N), 57.5 (Pyrrolidinyl C-4), 53.9 (Pyrrolidinyl C-5), 52.3 (C-2), 51.4 (Pyrrolidinyl C-3), 51.0 (Pyrrolidinyl C-2), 37.9 (C-3), 36.7 (Cyclopentyl C-1), 32.7 (Cyclopentyl C-3 or Cyclopentyl C-4), 32.2 (Cyclopentyl C-3 or Cyclopentyl C-4), 25.2 (Cyclopentyl C-2 or Cyclopentyl C-5), 25.0 (Cyclopentyl C-2 or Cyclopentyl C-5); HRMS found MH<sup>+</sup> 435.1713. C<sub>20</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub>S requires *MH*, 435.1697.

**(2S)-2-{[(3S,4S)-4-(3-hydroxybenzoyl)pyrrolidin-3-yl]formamido}-4-methylpentanoic acid (**186**)**



To a solution of *tert*-butyl (3*R*,4*S*)-3-{[(2*S*)-1-(*tert*-butoxy)-4-methyl-1-oxopentan-2-yl]carbamoyl}-4-(3-hydroxybenzamido)pyrrolidine-1-carboxylate (125.0 mg, 0.41 mmol) in DCM DCM-TFA (5 mL : 5 mL) was added triethylsilane (131.0  $\mu$ L, 0.81 mmol) and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* MDAP, with a gradient of 98:2 $\rightarrow$ 90:10 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **186** (56.5 mg, 65%) as a colourless solid.  $[\alpha]_D = -0.1$ ;  $\nu_{\max}/\text{cm}^{-1}$  (film) 330–2500, 3267, 3066, 2956, 2870, 1651(br), 1549 (br), 1308;  $\delta_{\text{H}}$  (500 MHz, DMSO-*d*<sup>6</sup>) 10.25 (1H, br. s, OH), 8.62 (1H, d, *J* 6.6 Hz, NH), 8.51 (1H, d, *J* 8.6 Hz, NH), 7.93 (1H, app. br. s, Phenyl 2-H), 7.27 (1H, dt, *J* 7.1 and 1.3 Hz, Phenyl 6-H), 7.21 (1H, t, *J* 7.8 Hz, Phenyl 5-H), 6.86 (1H, ddd, *J* 10.3, 8.6 and 3.8 Hz, Phenyl 4-H), 4.39-4.33 (1H, m, Pyrrolidinyl 4-H), 4.19 (1H, ddd, *J* 10.3, 8.6 and 3.8 Hz, 2-H), 3.41-3.31 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>a</sub>), 3.22 (1H, dd, *J* 11.5 and 8.2 Hz, Pyrrolidinyl 2-H<sub>b</sub>), 3.16-3.09 (1H, m, Pyrrolidinyl 3-H), 2.87 (1H, dd, *J* 11.5 and 8.2 Hz, Pyrrolidinyl 5-H<sub>b</sub>), 1.52-1.37 (3H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub> and 4-H), 0.71 (3H, d, *J* 5.9 Hz, 4-Me), 0.61 (3H, d, *J* 5.9 Hz, 5-H);  $\delta_{\text{C}}$  (125 MHz, DMSO-*d*<sup>6</sup>) 175.8 (C=O), 169.8 (C=O), 166.4 (C=O), 157.8 (Phenyl C-3), 134.7 (Phenyl C-1), 129.1 (Phenyl C-5), 118.1 (Phenyl C-4), 117.6 (Phenyl C-6), 114.8 (Phenyl C-2), 54.2 (Pyrrolidinyl C-4), 51.6 (C-2), 51.0 (Pyrrolidinyl C-5), 48.4 (Pyrrolidinyl C-3), 47.2 (Pyrrolidinyl C-2), 41.1 (C-3), 24.4 (C-4), 22.9 (C-5), 21.2 (C-5); HRMS found  $M\text{H}^+$  364.1879. C<sub>18</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub> requires *MH*, 364.1867.

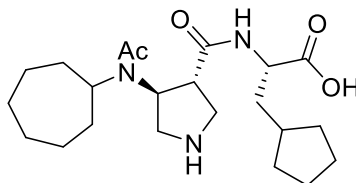
**(2S)-3-cyclopentyl-2-{-[(3R,4S)-4-(4-hydroxybutanamido)pyrrolidin-3-yl]formamido}propanoic acid **(187)****



To a solution of *tert*-butyl (3R,4S)-3-(((S)-1-(*tert*-butoxy)-3-cyclopentyl-1-oxopropan-2-yl)carbamoyl)-4-(4-hydroxybutanamido)pyrrolidine-1-carboxylate (115.0 mg, 0.23 mmol) in DCM DCM-TFA (5 mL : 5 mL) was added triethylsilane (41.0  $\mu$ L, 0.46 mmol) and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* MDAP, with a gradient of 95:5 $\rightarrow$ 75:25 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **187** (27.4 mg, 49%) as a colourless solid.  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500, 3268, 3081. 2940, 2866, 1667, 1654, 1558;  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 4.68-4.57 (1H, m, Pyrrolidinyl 4-H), 4.22-4.11 (1H, m, 2-H), 3.81-3.67 (2H, m, Pyrrolidinyl 3-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>a</sub>), 3.64-3.53 (3H, m, Hydroxybutanamido 4-H and Pyrrolidinyl 5-H<sub>b</sub>), 3.28-3.18 (2H, m, Pyrrolidinyl 3-H and Pyrrolidinyl 5-H<sub>b</sub>), 2.39-2.28 (2H, m, Hydroxybutanamido 2-H), 1.86-1.78 (2H, m, Hydroxybutanamido 3-H), 1.77-1.56 (7H, m, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Cyclopentyl 1-H<sub>a</sub>, Cyclopentyl 2-H<sub>a</sub>, Cyclopentyl 3-H<sub>a</sub>, Cyclopentyl 4-H<sub>a</sub>, Cyclopentyl 5-H<sub>a</sub>), 1.56-1.44 (2H, m, Cyclopentyl 2-H<sub>b</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.18-1.08 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 179.5 (C=O), 176.2 (C=O), 170.6 (C=O), 60.8 (Hydroxybutanamido C-4), 55.2 (C-2), 52.1 (Pyrrolidinyl C-4), 48.2 (Pyrrolidinyl C-2 or Pyrrolidinyl C-3), 48.1 (Pyrrolidinyl C-2 or Pyrrolidinyl C-3), 46.7 (Pyrrolidinyl C-5), 37.7 (C-3), 36.6 (Cyclopentyl C-1), 32.5 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 32.2 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 31.3 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 27.6 (Hydroxybutanamido C-3), 24.7 (Cyclopentyl C-2 or Cyclopentyl C-5), 24.6 (Cyclopentyl C-2 or Cyclopentyl C-5); HRMS found MH<sup>+</sup> 356.2182. C<sub>17</sub>H<sub>29</sub>N<sub>3</sub>O<sub>5</sub> requires *MH*, 356.2180.

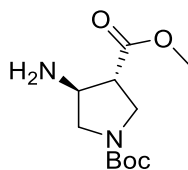


**(2S)-2-[[[(3R,4S)-4-(N-cycloheptylacetylamido)pyrrolidin-3-yl]formamido]-3-cyclopentylpropanoic acid (**188**)**



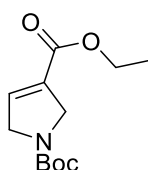
To a solution of amide **207** (125.0 mg, 0.22 mmol) in DCM DCM-TFA (2 mL : 2 mL) was added triethylsilane (70.0  $\mu$ L, 0.44 mmol) and left to stir at r.t overnight. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* MDAP, with a gradient of 85:15 $\rightarrow$ 65:35 H<sub>2</sub>O (0.1% formic acid)/MeCN over 12 min to yield the *propanoic acid* **188** (56.0 mg, 63%) as a colourless solid.  $[\alpha]_D = -37.7$ ;  $\nu_{\max}/\text{cm}^{-1}$  (film) 3300-2500 (carboxylic acid), 3234, 2929, 2860, 1649, 1606, 1544, 1492, 1199, 1166;  $\delta_{\text{H}}$  (500 MHz, DMSO- $d_6$ ) 8.24 (1H, d,  $J$  8.1 Hz, NH), 4.12-4.06 (1H, m, 2-H), 4.00-3.93 (1H, m, Pyrrolidinyl 4-H), 3.66-3.59 (1H, m, Cycloheptyl 1-H), 3.55-3.49 (1H, m, Pyrrolidinyl 5-H<sub>a</sub>), 3.35-3.29 (1H, m, Pyrrolidinyl 3-H), 3.19-3.09 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 2-H<sub>b</sub>), 2.89-2.83 (1H, m, Pyrrolidinyl 5-H<sub>b</sub>), 2.05 (3H, s, Me), 1.85-1.75 (1H, m, Cycloheptyl 1-H), 1.84-1.34 (20H, m, Cycloheptyl-H, Cyclopentyl-H, 3-H<sub>a</sub> and 3-H<sub>b</sub>), 1.21-1.01 (2H, m, Cycloheptyl-H or Cyclopentyl-H);  $\delta_{\text{C}}$  (125 MHz, DMSO- $d_6$ ) 174.8 (C=O), 172.6 (C=O), 170.0 (C=O), 60.3 (Cycloheptyl C-1), 57.6 (Pyrrolidinyl C-4), 53.1 (C-2), 51.4 (Pyrrolidinyl C-5), 50.8 (Pyrrolidinyl C-2), 48.7 (Pyrrolidinyl C-3), 38.4 (C-3), 36.9 (Cyclopentyl C-1), 33.9 (Cycloheptyl-C or Cyclopentyl-C), 32.9 (Cycloheptyl-C or Cyclopentyl-C), 32.0 (Cycloheptyl-C or Cyclopentyl-C), 27.4 (Cycloheptyl-C or Cyclopentyl-C), 27.2 (Cycloheptyl-C or Cyclopentyl-C), 25.3 (Cycloheptyl-C or Cyclopentyl-C), 25.1 (Cycloheptyl-C or Cyclopentyl-C), 25.0 (Cycloheptyl-C or Cyclopentyl-C), 24.8 (Cycloheptyl-C or Cyclopentyl-C), 23.4 (Me); HRMS found  $\text{MH}^+$  408.2865. C<sub>22</sub>H<sub>37</sub>N<sub>3</sub>O<sub>4</sub> requires  $\text{MH}$ , 408.2857.

**1-*tert*-Butyl 3-methyl (3*R*,4*S*)-4-aminopyrrolidine-1,3-dicarboxylate (189)**<sup>110</sup>



To a solution of 1-*tert*-butyl 3-ethyl (3*R*,4*S*)-4-[(1*R*)-1-phenylethyl]amino}pyrrolidine-1,3-dicarboxylate (1.67g, 4.60 mmol) in MeOH (100 ml) was added Pd/C (114.0 mg, 10 wt%) and left to stir for at r.t for 18 hr under a H<sub>2</sub> atmosphere. The reaction mixture was then filtered through celite and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the primary amine **189** (726 mg, 65%) as a mixture of rotamers as a colourless oil. *R<sub>f</sub>* 0.50 (90:10 DCM–MeOH).  $\delta_{\text{H}}$  (500 MHz, MeOD) 3.09-2.96 (6H, m, Me, 2-H<sub>a</sub>, 4-H, 5-H<sub>a</sub>), 2.88-2.80 (1H, m, 2-H<sub>b</sub>), 2.45-2.38 (1H, m, 5-H<sub>b</sub>), 2.29-2.20 (1H, m, 3-H), 0.80 (9H, s, *tert*-Butyl);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 172.6 (C=O), 172.5 (C=O), 154.5 (C=O), 154.4 (C=O), 79.6 (*tert*-Butyl C-1), 54.1 (C-4), 53.4 (C-4), 52.5 (C-5), 52.1 (C-5), 51.5 (Me), 50.5 (C-3), 50.2 (C-3), 46.9 (C-2), 46.4 (C-2), 27.6 (*tert*-Butyl C-2)(15/18 signals present); HRMS found 2MH<sup>+</sup> 489.2918. C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> requires *MH*, 489.2919. Spectroscopic data are consistent with those reported in the literature.<sup>110</sup>

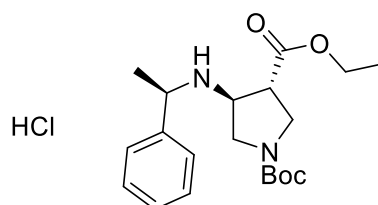
**1-*tert*-Butyl 3-ethyl 2,5-dihydro-1H-pyrrole-1,3-dicarboxylate (192)**<sup>110</sup>



To a solution of ethyl N-boc-4-oxopyrrolidine-3-carboxylate (20.0 g, 77.7 mmol) in MeOH (120 mL) was added NaBH<sub>4</sub> (3.52g, 93.2 mmol) in portions over at 0 °C 10 min, the reaction mixture was then allowed to warm to r.t and stir for 3 hr. The reaction mixture was concentrated under reduced pressure, the residue was then redissolved in diethyl ether (250 mL), washed with sat. NaHCO<sub>3</sub> (250 mL), brine (250 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give a crude product. The reduced product (13.21 g, 51.00 mmol) and PPh<sub>3</sub> (20.05 g, 76.50 mmol) was dissolved in toluene (100 mL) at r.t. DIAD (12.6 mL, 66.30

mmol, in 50 mL toluene) was added slowly over 10 min then left to stir overnight at r.t. The reaction mixture was concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 70:30 hexane–EtOAc to yield the alkene **192** (6.19 g, 33%) as a colourless oil.  $R_f$  0.45 (70:30 hexane–EtOAc).  $\nu_{\max}/\text{cm}^{-1}$  (film) 2980, 2934, 1754, 1698, 1477, 1277, 1088;  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 6.65–6.62 (0.6H, m, 4- $\text{H}^{\text{rot(maj)}}$ ), 6.61–6.58 (0.4H, m, 4- $\text{H}^{\text{rot(min)}}$ ), 4.23–4.09 (6H, m, 1- $\text{H}_2$  Ethyl $^{\text{rot(min)}}$ , 1- $\text{H}_2$  Ethyl $^{\text{rot(maj)}}$ , 2- $\text{H}^{\text{rot(min)}}$ , 2- $\text{H}^{\text{rot(maj)}}$ , 5- $\text{H}^{\text{rot(min)}}$  and 5- $\text{H}^{\text{rot(maj)}}$ ), 1.37 (9H, s, *tert*-Butyl $^{\text{rot(min)}}$  and *tert*-Butyl $^{\text{rot(maj)}}$ ), 1.23–1.17 (3H, m, 2- $\text{H}_3$  Ethyl $^{\text{rot(min)}}$  and 2- $\text{H}_3$  Ethyl $^{\text{rot(maj)}}$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{CDCl}_3$ ) 162.6 ( $\text{C}=\text{O}^{\text{rot(maj)}}$ ), 162.6 ( $\text{C}=\text{O}^{\text{rot(min)}}$ ), 153.9 ( $\text{C}=\text{O}^{\text{rot(maj)}}$ ), 153.7 ( $\text{C}=\text{O}^{\text{rot(min)}}$ ), 136.5 (C-4 $^{\text{rot(maj)}}$ ), 136.4 (C-4 $^{\text{rot(min)}}$ ), 132.2 (C-3 $^{\text{rot(min)}}$ ), 132.2 (C-3 $^{\text{rot(maj)}}$ ), 79.7 (*tert*-Butyl C-1 $^{\text{rot(maj)}}$ ), 79.6 (*tert*-Butyl C-1 $^{\text{rot(min)}}$ ), 60.6 (Ethyl C-1 $^{\text{rot(maj)}}$ ), 60.6 (Ethyl C-1 $^{\text{rot(min)}}$ ), 53.8 (C-5 $^{\text{rot(min)}}$ ), 53.6 (C-5 $^{\text{rot(maj)}}$ ), 14.1 (Ethyl C-2 $^{\text{rot(min)}}$ ), 14.1 (Ethyl C-2 $^{\text{rot(maj)}}$ ); HRMS found  $\text{MNa}^+$  264.1199.  $\text{C}_{12}\text{H}_{19}\text{NO}_4$  requires *MH*, 264.1206. Spectroscopic data are consistent with those reported in the literature.<sup>110</sup>

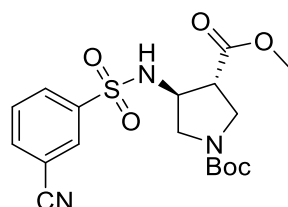
**1-*tert*-Butyl 3-ethyl (3*R*,4*S*)-4-{[(1*R*)-1-phenylethyl]amino}pyrrolidine-1,3-dicarboxylate HCl salt (194)**<sup>110</sup>



To a solution of 1-*tert*-butyl 3-ethyl 2,5-dihydro-1H-pyrrole-1,3-dicarboxylate (2.35 g, 9.75 mmol) in  $\text{H}_2\text{O}$  (35 mL) was added *R*-(+)- $\alpha$ -methylbenzylamine (2.5 mL, 19.5 mmol) and left to stir at 55 °C for 2 days. The reaction mixture was extracted with ethyl acetate (100 mL), washed with brine (100 mL), dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting 70:30 hexane–EtOAc to give the pyrrolidine derivative (343 mg, 13%) as a colourless oil.  $R_f$  0.21 (70:30 hexane–EtOAc). To a solution of pyrrolidine derivative (343 mg, 0.95 mmol) in ethyl acetate (16 mL) was added HCl in dioxane (0.35 mL) and stirred at r.t until a white precipitate formed. The precipitate was collected by vacuum filtration and dried in a dessicator to yield the HCl salt **194** (298 mg,

80%) as a 60:40 mixture of rotamers as a colourless solid.  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 10.61-10.41 (2H, m,  $\text{NH}_2$ ), 7.69-7.64 (2H, m, Ar-H), 7.48-7.38 (3H, m, Ar-H), 4.43-4.32 (1H, m, Pyrrolidiny 1-H), 4.26-4.15 (2H, m, Ethyl 1- $\text{H}_2$ ), 3.89 (1H, app. br. s, Pyrrolidiny 4-H); 3.82-3.70 (2.4H, m, Pyrrolidiny 3-H, Pyrrolidiny 4-H and Pyrrolidiny 2-H or Pyrrolidiny 5-H), 3.61 (0.6H, br. s, Pyrrolidiny 2-H or Pyrrolidiny 5-H), 3.48-3.36 (1.4H, m, Pyrrolidiny 2-H or Pyrrolidiny 5-H), 3.32 (0.4 H, br. s, Pyrrolidiny 2- $\text{H}^{\text{rot}(\text{min})}$  or Pyrrolidiny 5- $\text{H}^{\text{rot}(\text{min})}$ ), 1.94 (3H, d,  $J$  6.9 Hz, Me), 1.35 (9H, br. s, *tert*-Butyl), 1.26 (3H, t,  $J$  7.2 Hz, Ethyl 2- $\text{H}_3$ );  $\delta_{\text{C}}$  (125 MHz,  $\text{D}_2\text{O}$ ) 171.0 (C=O), 153.4 (C=O), 135.9 (Ar-C), 129.8 (Ar-C), 129.6 (Ar-C), 128.3 (Ar-C), 80.3 (*tert*-Butyl C-1), 61.2 (Ethyl C-1), 60.0 (Phenylethyl C-1 $^{\text{rot}(\text{min})}$ ), 59.7 (Phenylethyl C-1 $^{\text{rot}(\text{maj})}$ ), 56.9 (Pyrrolidiny C-4 $^{\text{rot}(\text{min})}$ ), 56.5 (Pyrrolidiny C-4 $^{\text{rot}(\text{maj})}$ ), 48.2 (Pyrrolidiny C-2 $^{\text{rot}(\text{min})}$ ), 47.2 (Pyrrolidiny C-2 $^{\text{rot}(\text{maj})}$ ), 46.4 (Pyrrolidiny C-3 $^{\text{rot}(\text{min})}$ ), 45.7 (Pyrrolidiny C-3 $^{\text{rot}(\text{maj})}$ ), 28.4 (*tert*-Butyl C-2), 20.8 (Me $^{\text{rot}(\text{min})}$ ), 14.2 (Ethyl C-2)(20/32 signals present); HRMS found  $\text{MNa}^+$  385.2094.  $\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_4$  requires  $\text{MNa}$ , 385.2098. Spectroscopic data are consistent with those reported in the literature.<sup>110</sup>

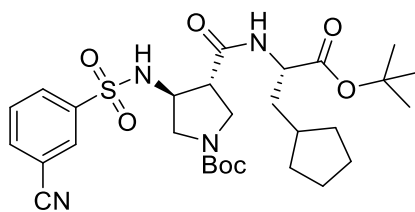
**1-*tert*-Butyl 3-methyl (3*R*,4*S*)-4-(3-cyanobenzenesulfonamido)pyrrolidine-1,3-dicarboxylate (196)**



To a solution of 1-*tert*-butyl 3-methyl (3*R*,4*S*)-4-aminopyrrolidine-1,3-dicarboxylate (200.0 mg, 0.82 mmol), 3-cyanobenzenesulfonyl chloride (248.0 mg, 1.23 mmol) in DMF (5 mL) was added DIPEA (215.0  $\mu\text{L}$ , 1.23 mmol) and left to stir overnight at r.t. The reaction mixture was diluted in ethyl acetate (100 mL), washed with water (100 mL), brine (100 mL), dried over  $\text{MgSO}_4$  and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 90:10 hexane–EtOAc to yield the *sulfonamide* **196** (181 mg, 71%) as a 60:40 mixture of rotamers as a colourless oil.  $R_f$  0.40 (90:10 hexane–EtOAc).  $\delta_{\text{H}}$  (500 MHz,  $\text{CDCl}_3$ ) 8.15 (1H, app. br. s, Phenyl 2-H), 8.11-8.07 (1H, app. d,  $J$  7.9 Hz, Phenyl 6-H), 7.84 (1H, app. d,  $J$  7.9 Hz, Phenyl 5-H), 7.67-7.62 (1H, m, Phenyl 4-H), 4.03-3.95 (1H, m, 4-H),

3.70-3.55 (5H, m, Me, 2-H<sub>a</sub>, 5-H<sub>a</sub>), 3.45 (1H, dd, *J* 11.3 and 7.2 Hz, 2-H<sub>b</sub>), 3.23-3.13 (1H, m, 5-H<sub>b</sub>), 3.14-3.03 (0.6H, m, 3-H<sup>rot(maj)</sup>), 3.01-2.92 (0.4H, m, 3-H<sup>rot(min)</sup>), 1.38 (9H, s, *tert*-Butyl);  $\delta_c$  (125 MHz, CDCl<sub>3</sub>) 171.3 (C=O<sup>rot(maj)</sup>), 170.8 (C=O<sup>rot(min)</sup>), 154.1 (C=O<sup>rot(min)</sup>), 154.0 (C=O<sup>rot(maj)</sup>), 142.9 (Phenyl C-1), 135.9 (Phenyl C-5), 131.2 (Phenyl C-6), 130.8 (Phenyl C-2<sup>rot(maj)</sup>), 130.8 (Phenyl C-2<sup>rot(min)</sup>), 130.3 (Phenyl C-4), 117.2 (Phenyl C-3), 113.6 (C≡N), 80.5 (*tert*-Butyl C-1), 55.1 (C-4<sup>rot(min)</sup>), 54.6 (C-4<sup>rot(maj)</sup>), 52.6 (Me), 51.3 (C-5<sup>rot(min)</sup>), 50.4 (C-5<sup>rot(maj)</sup>), 49.2 (C-3<sup>rot(maj)</sup>), 47.9 (C-3<sup>rot(min)</sup>), 46.7 (C-2<sup>rot(maj)</sup>), 46.4 (C-4<sup>rot(min)</sup>), 28.4 (*tert*-Butyl C-2<sup>rot(min)</sup>)(23/32 signals present); HRMS found MNa<sup>+</sup> 432.1183. C<sub>18</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>S requires *MNa*, 432.1200.

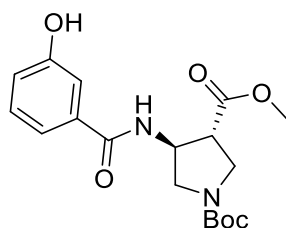
***tert*-Butyl (4*S*)-3-{[(2*S*)-1-(*tert*-butoxy)-3-cyclopentyl-1-oxopropan-2-yl]carbamoyl}-4-(3-cyanobenzenesulfonamido)pyrrolidine-1-carboxylate (**197**)**



To a solution of methyl ester **196** (181.0 mg, 0.44 mmol) in MeOH-H<sub>2</sub>O (3 mL: 3 mL) was added LiOH.H<sub>2</sub>O (21.0 mg, 0.49 mmol) and left to stir at r.t 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (50 mL), washed with brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure F, *tert*-butyl (2*S*)-2-amino-3-cyclopentylpropanoate (62.0 mg, 0.29 mg) with crude carboxylic acid (151.0 mg, 0.38 mmol) gave a crude product. The crude product was purified *via* recrystallisation in 90:10 hexane-EtOAc to yield the *sulfonamide* **197** (76.0 mg, 34%) as a mixture of rotamers as a colourless solid.  $\delta_H$  (500 MHz, MeOD) 8.21 (1H, app. br. s, 1H, Phenyl 2-H), 8.14 (1H, dt, *J* 8.0 and 1.4 Hz, Phenyl 6-H), 8.00 (1H, d, *J* 7.7 Hz, Phenyl 4-H), 7.77 (1H, t, *J* 8.0 Hz, Phenyl 5-H), 4.19-4.12 (1H, m, Oxopropanyl 2-H), 4.12-4.05 (1H, m, 4-H), 3.71-3.63 (1H, m, 2-H<sub>a</sub>), 3.50 (1H, dd, *J* 11.0 and 7.5 Hz, 5-H<sub>a</sub>), 3.37-3.32 (1H, m, 2-H<sub>b</sub>), 3.03-2.92 (2H, m, 3-H and 5-H<sub>b</sub>), 1.99-1.90 (1H, m, Cyclopentyl 1-H), 1.88-1.78 (2H, m, Cyclopentyl 3-H<sub>a</sub> and Cyclopentyl 4-H<sub>a</sub>), 1.77-1.51 (6H, m, Oxopropanyl 3-H<sub>a</sub>, Oxopropanyl 3-H<sub>b</sub>, Cyclopentyl 2-H<sub>a</sub>, Cyclopentyl 2-H<sub>b</sub>,

Cyclopentyl 5-H<sub>a</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.49-1.35 (18H, m, *tert*-Butyl ester and *tert*-Butyl Boc), 1.20-1.06 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_c$  (125 MHz, MeOD) 179.1 (Oxopentyl C-1), 171.7 (C=O), 171.2 (C=O), 154.3 (C=O), 142.9 (Phenyl C-1), 142.8 (Phenyl C-1), 130.9 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 130.8 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 139.3 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 130.2 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 117.0 (Phenyl C-3), 113.3 (C $\equiv$ N), 81.3 (*tert*-Butyl C-1), 80.1 (*tert*-Butyl C-1), 80.1 (*tert*-Butyl C-1), 54.8 (C-4), 54.1 (C-4), 53.2 (Oxopropanyl C-2), 50.6 (C-5), 50.0 (C-5), 49.9 (C-3), 49.0 (C-3), 47.6 (C-2 (under MeOD)), 37.2 (Oxopentyl C-3), 36.7 (Cyclopentyl C-1), 32.4 (Cyclopentyl C-3 and Cyclopentyl C-4), 31.8 (Cyclopentyl C-3 and Cyclopentyl C-4), 27.2 (*tert*-Butyl C-2), 26.8 (*tert*-Butyl C-2), 24.7 (Cyclopentyl C-2 and Cyclopentyl C-5), 24.5 (Cyclopentyl C-2 and Cyclopentyl C-5)(33/46 signals present); HRMS found MH<sup>+</sup> 591.2830. C<sub>29</sub>H<sub>42</sub>N<sub>4</sub>O<sub>7</sub>S requires MH, 591.2847.

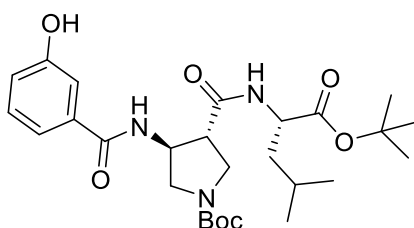
**1-*tert*-Butyl 3-methyl (3*R*,4*S*)-4-(3-hydroxybenzamido)pyrrolidine-1,3-dicarboxylate (199)**



According to general procedure F, 1-*tert*-butyl 3-methyl (3*R*,4*S*)-4-aminopyrrolidine-1,3-dicarboxylate (200.0 mg, 0.82 mmol) with 3-hydroxybenzoic acid (148.0 mg, 1.07 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 50:50 hexane–EtOAc to yield the *amide* **199** (254.0 mg, 85%) as a mixture of rotamers as a colourless oil. *R*<sub>f</sub> 0.15 (50:50 hexane–EtOAc).  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 8.51 (1H, br. s, OH), 7.41-7.35 (1H, m, NH), 7.24-7.21 (1H, m, Phenyl 2-H), 7.19-7.10 (2H, m, Phenyl 4-H or Phenyl 5-H and Phenyl 6-H), 6.98-6.79 (1H, m, Phenyl 4-H or Phenyl 5-H), 4.78-4.68 (1H, m, Pyrrolidinyl 4-H), 3.85-3.74 (1H, m, Pyrrolidinyl 5-H<sub>a</sub>), 3.72-3.52 (5H, m, Me, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 2-H<sub>b</sub>), 3.37-3.24 (1H, m, Pyrrolidinyl 5-H<sub>b</sub>), 3.19-3.04 (1H, m, Pyrrolidinyl 3-H), 1.41-1.36 (9H, m, *tert*-Butyl);  $\delta_c$  (125 MHz, CDCl<sub>3</sub>) 172.1 (C=O), 171.5 (C=O), 168.5 (C=O), 157.1 (Phenyl C-3), 154.5 (C=O), 135.0 (Phenyl C-1), 129.7 (Phenyl C-2), 119.3 (Phenyl C-4 or Phenyl C-5 or Phenyl

C-6), 118.5 (Phenyl C-4 or Phenyl C-5 or Phenyl C-6), 114.3 (Phenyl C-4 or Phenyl C-5 or Phenyl C-6), 80.5 (*tert*-Butyl C-1), 52.6 (Pyrrolidinyl C-4), 52.5 (Me), 52.1 (Pyrrolidinyl C-4), 50.5 (Pyrrolidinyl C-5), 49.8 (Pyrrolidinyl C-5), 48.7 (Pyrrolidinyl C-3), 47.7 (Pyrrolidinyl C-3), 47.1 (Pyrrolidinyl C-2), 46.4 (Pyrrolidinyl C-2), 28.4 (*tert*-Butyl C-2)(21/30 signals present); HRMS found  $MNa^+$  387.1518.  $C_{18}H_{24}N_2O_6$  requires  $MNa$ , 387.1527.

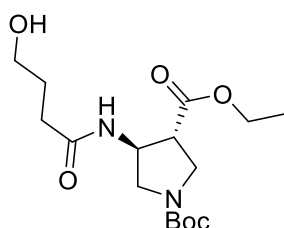
***tert*-Butyl (3*R*,4*S*)-3-[(2*S*)-1-(*tert*-butoxy)-4-methyl-1-oxopentan-2-yl]carbamoyl}-4-(3-hydroxybenzamido)pyrrolidine-1-carboxylate (200)**



To a solution of methyl ester **199** (254.0 mg, 0.70 mmol) in MeOH-H<sub>2</sub>O (5 mL: 5 mL) was added LiOH.H<sub>2</sub>O (147 mg, 3.50 mmol) and left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (100 mL), washed with brine (100 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure F, *tert*-Butyl (2*S*)-2-amino-4-methylpentanoate (108.0 mg, 0.48 mg) with crude carboxylic acid (217.0 mg, 0.62 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 50:50 hexane–EtOAc to yield the *amide* **200** (125 mg, 50%) as a mixture of rotamers as a colourless solid.  $R_f$  0.25 (50:50 hexane–EtOAc).  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 7.27-7.23 (3H, m, Ar-H), 6.96-6.93 (1H, m, Phenyl 4-H), 4.82-4.76 (1H, m, 4-H), 4.31 (1H, dd,  $J$  6.0 and 6.1 Hz, Oxopentanyl 2-H), 3.88-3.80 (1H, m, 5-H<sub>a</sub>), 3.77-3.69 (1H, m, 2-H<sub>a</sub>), 3.58-3.53 (1H, m, 2-H<sub>b</sub>), 3.37-3.29 (1H, m, 5-H<sub>b</sub>), 3.22-3.12 (1H, m, 3-H), 1.51-1.40 (21H, m, *tert*-Butyl ester, *tert*-Butyl Boc, Oxopentanyl 3-H<sub>a</sub>, Oxopentanyl 3-H<sub>b</sub> and Oxopentanyl 4-H), 0.97-0.77 (6H, m, Oxopentanyl 4-Me and Oxopentanyl 5-H<sub>3</sub>);  $\delta_C$  (125 MHz, CDCl<sub>3</sub>) 176.3 (C=O), 173.4 (C=O), 173.3 (C=O), 173.1 (C=O), 173.0 (C=O), 170.1 (C=O), 158.9 (Phenyl C-3), 156.0 (C=O), 155.9 (C=O), 136.5 (Phenyl C-1), 130.5 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 119.8 (Phenyl C-4), 119.2 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 115.6 (Phenyl C-2 or Phenyl C-5 or Phenyl C-6), 82.7 (*tert*-Butyl C-1), 82.2 (*tert*-Butyl C-1), 81.3

(*tert*-Butyl C-1), 54.1 (C-4), 53.1 (C-4), 51.2 (Oxopentanyl C-2), 50.2 (Oxopentanyl C-2), 41.4 (Oxopentanyl C-3), 28.7 (*tert*-Butyl C-2), 28.3 (*tert*-Butyl C-2), 28.2 (*tert*-Butyl C-2), 25.9 (Oxopentanyl C-4), 25.9 (Oxopentanyl C-4), 23.3 (Oxopentanyl C-4 Me), 23.1 (Oxopentanyl C-4 Me), 22.6 (Oxopentanyl C-5), 21.7 (Oxopentanyl C-5)(31/46 signals present); HRMS found  $MH^+$  520.3004.  $C_{27}H_{41}N_3O_7$  requires  $MH$ , 520.3017.

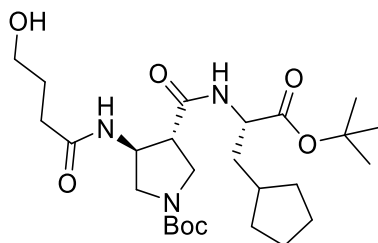
**1-(*tert*-Butyl) 3-ethyl (3*R*,4*S*)-4-(4-hydroxybutanamido)pyrrolidine-1,3-dicarboxylate (**202**)**



To a solution of  $\gamma$ -butyrolactone (200  $\mu$ L, 2.62 mmol) in MeOH-H<sub>2</sub>O (5 mL : 5 mL) was added LiOH.H<sub>2</sub>O (165 mg, 3.94 mmol) and left to stir until the reaction was determined to be complete by TLC and concentrated under reduced pressure to give the crude carboxylate. According to general procedure F, 1-*tert*-butyl 3-methyl (3*R*,4*S*)-4-aminopyrrolidine-1,3-dicarboxylate (100.0 mg, 0.41 mmol) with crude carboxylic acid (58.0 mg, 0.53 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 99:1 DCM–MeOH to yield the *amide* **202** (37 mg, 37%) as a mixture of rotamers as a colourless oil.  $R_f$  0.12 (99:1 DCM–MeOH).  $\delta_H$  (500 MHz, MeOD) 4.63–4.58 (1H, m, 4-H), 4.20 (2H, q,  $J$  7.1 Hz, Ethyl 1-H<sub>2</sub>), 3.78–3.66 (2H, m, 3-H<sub>a</sub> and 5-H<sub>a</sub>), 3.65–3.56 (3H, m, 3-H<sub>b</sub> and Hydroxybutanamido 4-H), 3.25–3.19 (1H, m, 5-H<sub>b</sub>), 3.11–3.04 (1H, m, 3-H), 2.34–2.29 (2H, m, Hydroxybutanamido 2-H), 1.88–1.81 (2H, m, Hydroxybutanamido 3-H), 1.50 (9H, s, *tert*-Butyl), 1.30 (3H, t,  $J$  7.1 Hz, Ethyl 2-H<sub>3</sub>);  $\delta_C$  (125 MHz, MeOD) 174.4 (C=O), 171.5 (C=O), 171.5 (C=O), 154.6 (C=O), 154.5 (C=O), 80.0 (*tert*-Butyl C-1), 61.0 (Ethyl C-1 or Hydroxybutanamido C-4), 60.8 (Ethyl C-1 or Hydroxybutanamido C-4), 51.7 (C-4), 51.0 (C-4), 50.1 (C-5), 49.6 (C-5), 46.8 (C-2), 46.4 (C-2), 32.1 (Hydroxybutanamido C-2), 22.3 (Hydroxybutanamido C-3), 27.4 (*tert*-Butyl C-2), 27.3 (*tert*-Butyl C-2), 13.1 (Ethyl C-2), 13.1 (Ethyl C-2)(21/28 signals present); HRMS found  $MH^+$  345.2005.  $C_{16}H_{28}N_2O_6$  requires  $MH$ , 345.2020.



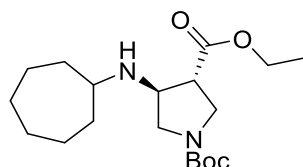
***tert*-Butyl (3*R*,4*S*)-3-(((*S*)-1-(*tert*-butoxy)-3-cyclopentyl-1-oxopropan-2-yl)carbamoyl)-4-(4-hydroxybutanamido)pyrrolidine-1-carboxylate (203)**



To a solution of ethyl ester **202** (74.0 mg, 0.21 mmol) in MeOH-H<sub>2</sub>O (3 mL: 3 mL) was added LiOH.H<sub>2</sub>O (45.0 mg, 1.07 mmol) and left to stir for at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (50 mL), washed with brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure F, *tert*-butyl (2*S*)-2-amino-3-cyclopentylpropanoate (35.0 mg, 0.16 mg) with crude carboxylic acid (66.0 mg, 0.21 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 90:10 DCM–MeOH to yield the *amide* **203** (26.2 mg, 32%) as a colourless oil. *R*<sub>f</sub> 0.35 (90:10 DCM–MeOH).  $\delta_{\text{H}}$  (500 MHz, MeOD) 4.59-4.48 (1H, m, Pyrrolidinyl 4-H), 4.25 (1H, dd, *J* 8.6 and 6.0 Hz, 2-H), 3.80-3.63 (2H, m, Pyrrolidinyl 2-H<sub>a</sub> and Pyrrolidinyl 5-H<sub>a</sub>), 3.61-3.50 (3H, m, Pyrrolidinyl 2-H<sub>b</sub> and Hydroxybutanamido 4-H), 3.21 (1H, dd, *J* 10.7 and 6.1 Hz, Pyrrolidinyl 5-H<sub>b</sub>), 3.09-3.00 (1H, m, Pyrrolidinyl 3-H), 2.31-2.27 (2H, m, Hydroxybutanamido 2-H), 1.90-1.68 (5H, m, Cyclopentyl 1-H, 3-H<sub>a</sub>, 3-H<sub>b</sub>, Hydroxybutanamido 3-H), 1.68-1.64 (2H, m, Cyclopentyl 2-H<sub>a</sub> and Cyclopentyl 5-H<sub>a</sub>), 1.61-1.55 (2H, m, Cyclopentyl 2-H<sub>b</sub> and Cyclopentyl 5-H<sub>b</sub>), 1.50-1.44 (18H, m, *tert*-Butyl Ester and *tert*-Butyl Boc), 1.22-1.11 (2H, m, Cyclopentyl 3-H<sub>b</sub> and Cyclopentyl 4-H<sub>b</sub>);  $\delta_{\text{C}}$  (125 MHz, D<sub>2</sub>O) 175.8 (C=O), 174.7 (C=O), 173.3 (C=O), 82.7 (*tert*-Butyl C-1), 81.3 (*tert*-Butyl C-1), 60.8 (Hydroxybutanamido C-4), 53.0 (C-2), 51.7 (Pyrrolidinyl C-4), 50.6 (Pyrrolidinyl C-5), 48.4 (Pyrrolidinyl C-3 under MeOD), 47.0 (Pyrrolidinyl C-2 under MeOD), 38.7 (C-3), 38.1 (Cyclopentyl C-1), 33.8 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 33.5 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 33.1 (Hydroxybutanamido C-2 or Cyclopentyl C-3 or Cyclopentyl C-4), 29.6 (Hydroxybutanamido C-3), 28.7 (*tert*-Butyl C-2), 28.3 (*tert*-Butyl C-

2), 26.1 (Cyclopentyl C-2 or Cyclopentyl C-5), 25.9 (Cyclopentyl C-2 or Cyclopentyl C-5); HRMS found  $MH^+$  512.3349.  $C_{26}H_{45}N_3O_7$  requires  $MH$ , 512.3330.

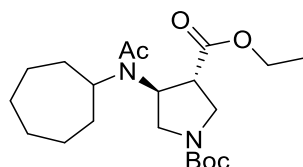
**1-(*tert*-Butyl) 3-ethyl (3R,4S)-4-(cycloheptylamino)pyrrolidine-1,3-dicarboxylate (205)**



To a solution of 1-*tert*-butyl 3-methyl (3R,4S)-4-aminopyrrolidine-1,3-dicarboxylate (200.0 mg, 0.82 mmol), cycloheptanone (115.0  $\mu$ L, 0.98 mmol) and sodium triacetoxyborohydride (260.0 mg, 1.23 mmol) in THF (5 mL) was added AcOH (52.0  $\mu$ L, 0.90 mmol) and left to stir at r.t overnight. The reaction mixture was then concentrated under reduced pressure and redissolved in ethyl acetate (50 mL), washed with  $H_2O$  (50 mL), brine (50 mL), dried over  $MgSO_4$  and concentrated under reduced pressure to give a crude product. The crude material was purified *via* column chromatography eluting with 99:1 DCM–MeOH to yield the *secondary amine* **205** (215.0 mg, 74%) as a 60:40 mixture of rotamers as a colourless oil.  $R_f$  0.15 (99:1 DCM–MeOH).  $\delta_H$  (500 MHz,  $CDCl_3$ ) 4.16-4.07 (2H, m, Ethyl 1- $H_2$ ), 3.77-3.70 (0.4H, m, 5- $H_a^{rot(min)}$ ), 3.69-3.56 (1.6H, m, 5- $H_a^{rot(maj)}$  and 2- $H_a$ ), 3.55-3.41 (2H, m, 2- $H_b$  and 4- $H$ ), 3.02-2.92 (1H, m, 5- $H_b$ ), 2.83-2.72 (1H, m, 3- $H$ ), 2.61-2.52 (1H, m, Cycloheptyl 1- $H$ ), 1.80-1.72 (2H, m, Cycloheptyl 2- $H_a$  and Cycloheptyl 7- $H_a$ ), 1.62-1.53 (2H, m, Cycloheptyl 3- $H_a$  and Cycloheptyl 6- $H_a$ ), 1.53-1.43 (4H, m, Cycloheptyl 4- $H_a$ , Cycloheptyl 4- $H_b$ , Cycloheptyl 5- $H_a$ , Cycloheptyl 5- $H_b$ ), 1.40 (9H, s, *tert*-Butyl), 1.34-1.27 (4H, m, Cycloheptyl 2- $H_b$ , Cycloheptyl 3- $H_b$ , Cycloheptyl 6- $H_b$ , Cycloheptyl 7- $H_b$ ), 1.25-1.19 (3H, m, Ethyl 2- $H_3$ );  $\delta_C$  (125 MHz,  $CDCl_3$ ) 172.5 ( $C=O^{rot(min)}$ ), 172.4 ( $C=O^{rot(maj)}$ ), 154.3 ( $C=O^{rot(min)}$ ), 154.2 ( $C=O^{rot(maj)}$ ), 79.6 (*tert*-Butyl C-1), 61.0 (Ethyl C-1), 58.0 (C-4 $^{rot(min)}$ ), 57.4 (C-4 $^{rot(maj)}$ ), 57.2 (Cycloheptyl C-1 $^{rot(maj)}$ ), 56.9 (Cycloheptyl C-1 $^{rot(min)}$ ), 52.4 (C-5 $^{rot(min)}$ ), 52.0 (C-5 $^{rot(maj)}$ ), 49.8 (C-3 $^{rot(maj)}$ ), 48.9 (C-3 $^{rot(min)}$ ), 47.1 (C-2 $^{rot(maj)}$ ), 46.9 (C-2 $^{rot(min)}$ ), 35.6 (Cycloheptyl C-2 $^{rot(maj)}$  or Cycloheptyl C-7 $^{rot(maj)}$ ), 35.4 (Cycloheptyl C-2 $^{rot(min)}$  or Cycloheptyl C-7 $^{rot(min)}$ ), 34.8 (Cycloheptyl C-2 $^{rot(min)}$  or Cycloheptyl C-7 $^{rot(min)}$ ), 34.6 (Cycloheptyl C-2 $^{rot(maj)}$  or Cycloheptyl C-7 $^{rot(maj)}$ ), 28.5 (*tert*-Butyl C-2), 28.3 (Cycloheptyl C-4 or Cycloheptyl C-5), 28.2 (Cycloheptyl C-4 or Cycloheptyl C-5), 24.2 (Cycloheptyl C-3 or Cycloheptyl C-6),

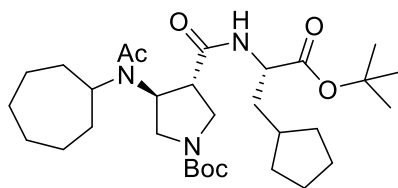
24.1 (Cycloheptyl C-3 or Cycloheptyl C-6), 14.2 (C-2 Ethyl<sup>rot(min)</sup>)(26/34 signals present); HRMS found  $MNa^+$  377.2406.  $C_{19}H_{34}N_2O_4$  requires  $MNa$ , 377.2411.

**1-(*tert*-Butyl) 3-ethyl (3*R*,4*S*)-4-(*N*-cycloheptylacetylamido)pyrrolidine-1,3-dicarboxylate (206)**



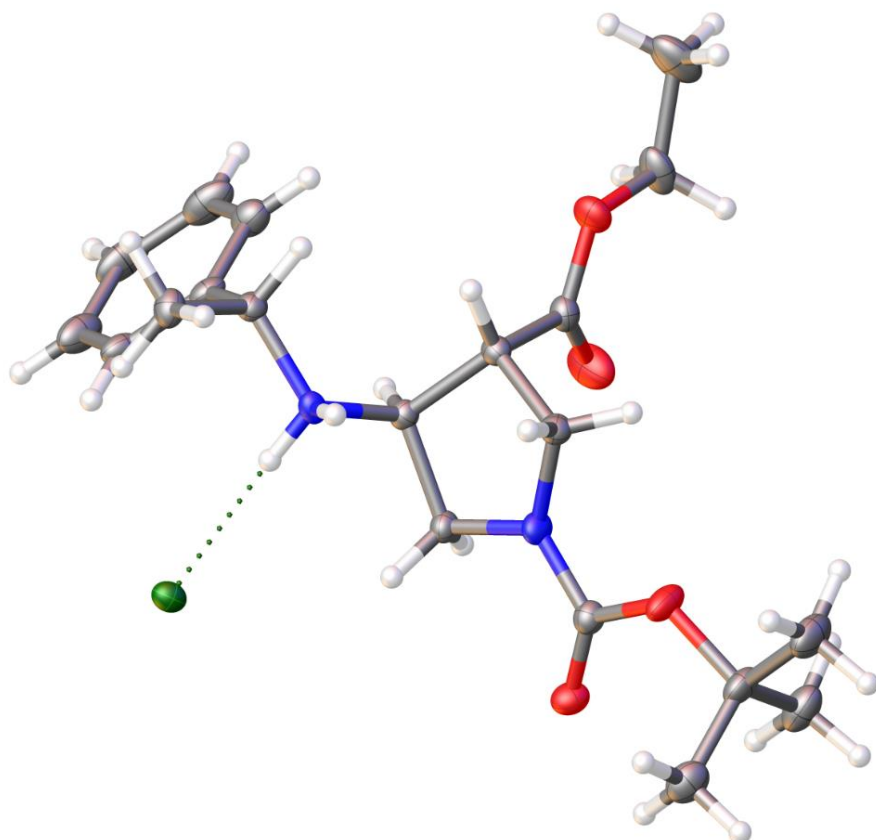
To a solution of ethyl ester **205** (215.0 mg, 0.61 mmol) and  $NEt_3$  (127.0  $\mu$ L, 0.91) in DCM (5 mL) was added acetic anhydride (86.0  $\mu$ L, 0.91 mmol) and left to stir overnight at r.t. The reaction mixture was diluted in DCM (50 mL), washed with sat.  $NaHCO_3$  (50 mL), brine (50 mL), dried over  $MgSO_4$  and concentrated under reduced pressure to give a crude product. The crude product was purified *via* column chromatography eluting with 50:50 hexane–EtOAc to yield the *acetylated derivative* **206** (149 mg, 59%) as a 66:33 mixture of rotamers as a colourless oil.  $R_f$  0.35 (50:50 hexane–EtOAc).  $\nu_{max}/cm^{-1}$  (film) 2934, 1729, 1683, 1633, 1264, 1156;  $\delta_H$  (500 MHz, MeOD) 4.78 (0.33H, m), 4.25–4.06 (2.67H, m), 3.89–3.49 (4.67H, m), 3.47–3.38 (0.33H, m), 3.36–3.30 (0.33H, m), 3.28–3.22 (0.33H, m), 3.20–3.11 (0.33H, m), 2.45–2.33 (0.67H, m), 2.16–2.13 (3H, m), 1.81–1.50 (11.33H, m), 1.49–1.44 (9H, m), 1.30–1.23 (3H, m);  $\delta_C$  (125 MHz, MeOD) 175.0, 174.7, 172.8, 172.7, 155.7, 155.7, 81.2, 62.4, 62.3, 61.4, 60.8, 58.8, 58.4, 57.6, 49.7, 48.0, 47.9, 47.7, 47.3, 46.8, 46.4, 46.1, 35.0, 34.9, 34.0, 33.5, 29.1, 28.7, 28.7, 28.4, 28.3, 27.4, 27.3, 26.1, 26.0, 23.4, 23.3, 14.5 (38/42 signals present); HRMS found  $MH^+$  397.2702.  $C_{21}H_{37}N_2O_5$  requires  $MH$ , 397.2697.

***tert*-Butyl (3*R*,4*S*)-3-(((*S*)-1-(*tert*-Butoxy)-3-cyclopentyl-1-oxopropan-2-yl)carbamoyl)-4-(*N*-cycloheptylacetylamido)pyrrolidine-1-carboxylate (207)**



To a solution of acetylated derivative **206** (210.0 mg, 0.59 mmol) in MeOH-H<sub>2</sub>O (5 mL: 5 mL) was added LiOH.H<sub>2</sub>O (124.0 mg, 2.96 mmol) left to stir at r.t for 3 hr. The reaction mixture was then acidified to pH 3 with HCl (1M), extracted with DCM (50 mL), washed with brine (50 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure to give the crude carboxylic acid. According to general procedure F, *tert*-butyl (2*S*)-2-amino-3-cyclopentylpropanoate (96.0 mg, 0.45 mmol) with crude carboxylic acid (217.0 mg, 0.59 mmol) gave a crude product. The crude product was purified *via* column chromatography eluting with 50:50 hexane–EtOAc to yield the *amide* **207** (149 mg, 59%) as a 70:30 mixture of rotamers as a colourless oil. *R<sub>f</sub>* 0.35 (50:50 hexane–EtOAc).  $\delta_{\text{H}}$  (500 MHz, MeOD) 4.33-4.27 (0.3H, m), 4.26-4.20 (0.7H, m), 4.19-4.15 (0.7H, m), 3.93-3.57 (4.3H, m), 3.48-3.36 (0.7H, m), 3.30-3.20 (0.3H, m), 3.14-3.06 (0.3H, m), 2.45-2.31 (0.7H, m), 2.18 (0.8H, s), 2.14 (2.2H, s), 1.90-1.41 (40.3H, m), 1.22-1.09 (2H, m);  $\delta_{\text{C}}$  (125 MHz, MeOD) 175.5, 175.5, 173.2, 173.1, 172.7, 172.2, 155.8, 155.7, 82.7, 81.1, 62.2, 58.9, 58.1, 57.2, 54.5, 54.5, 54.2, 51.0, 50.5, 47.6, 38.3, 38.3, 35.1, 33.9, 33.9, 33.0, 28.7, 28.7, 28.3, 28.3, 28.2, 26.1, 26.0, 25.9, 25.9, 23.2 (38/54 signals present); HRMS found  $\text{MH}^+$  564.4030. C<sub>31</sub>H<sub>54</sub>N<sub>3</sub>O<sub>6</sub> requires *MH*, 564.4007.

## X-Ray structures



**Figure 64:** ORTEP diagram of compound **194** (prepared through conjugate addition of 1-*tert*-butyl 3-ethyl 2,5-dihydro-1H-pyrrole-1,3-dicarboxylate with R-(+)- $\alpha$ -methylbenzylamine, followed by separation of diastereomers and conversion to HCl salt)

<b>Crystal data and structure refinement for compound 194</b>	
Empirical formula	C <sub>20</sub> H <sub>31</sub> ClN <sub>2</sub> O <sub>4</sub>
Formula weight	398.92
Temperature/K	100.01(10)
Crystal system	orthorhombic
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
a/Å	6.1628(2)
b/Å	16.2980(4)
c/Å	21.5041(5)
$\alpha$ /°	90
$\beta$ /°	90
$\gamma$ /°	90
Volume/Å <sup>3</sup>	2159.90(10)
Z	4
$\rho_{\text{calc}}$ /cm <sup>3</sup>	1.227
$\mu$ /mm <sup>-1</sup>	1.782
F(000)	856.0
Crystal size/mm <sup>3</sup>	0.49 × 0.04 × 0.03
Radiation	Cu K $\alpha$ ( $\lambda$ = 1.54184)

2 $\theta$ range for data collection/ $^{\circ}$	6.806 to 146.256
Index ranges	$-7 \leq h \leq 7, -20 \leq k \leq 20, -26 \leq l \leq 26$
Reflections collected	20137
Independent reflections	4268 [ $R_{\text{int}} = 0.0661, R_{\text{sigma}} = 0.0448$ ]
Data/restraints/parameters	4268/0/257
Goodness-of-fit on $F^2$	1.045
Final R indexes [ $I \geq 2\sigma(I)$ ]	$R_1 = 0.0365, wR_2 = 0.0877$
Final R indexes [all data]	$R_1 = 0.0406, wR_2 = 0.0903$
Largest diff. peak/hole / $e \text{ \AA}^{-3}$	0.25/-0.33

## 5.6 Biology

### 5.6.1 FAM-Bid Tracer

Binding domain of the Bid protein synthesised by Dr Fruzsina Hobor at the University of Leeds.

Tracer sequence: FAM-Ahx-EDIIRNIARHLAQVGDSDRSIW

Molecular weight: 3163 Da

### 5.6.2 Mcl-1 Protein Sequence

Mcl-1 (residues 172-327) expressed and purified by Dr Fruzsina Hobor at the University of Leeds.

SELYRQSLEIISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGM  
LRKLDIKNEDDVKSLSRVMIHVFS DGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAES  
ITDVLVRTKRDWLVKQRGWDGFVEFFHVEDLEGG

Number of amino acids: 156

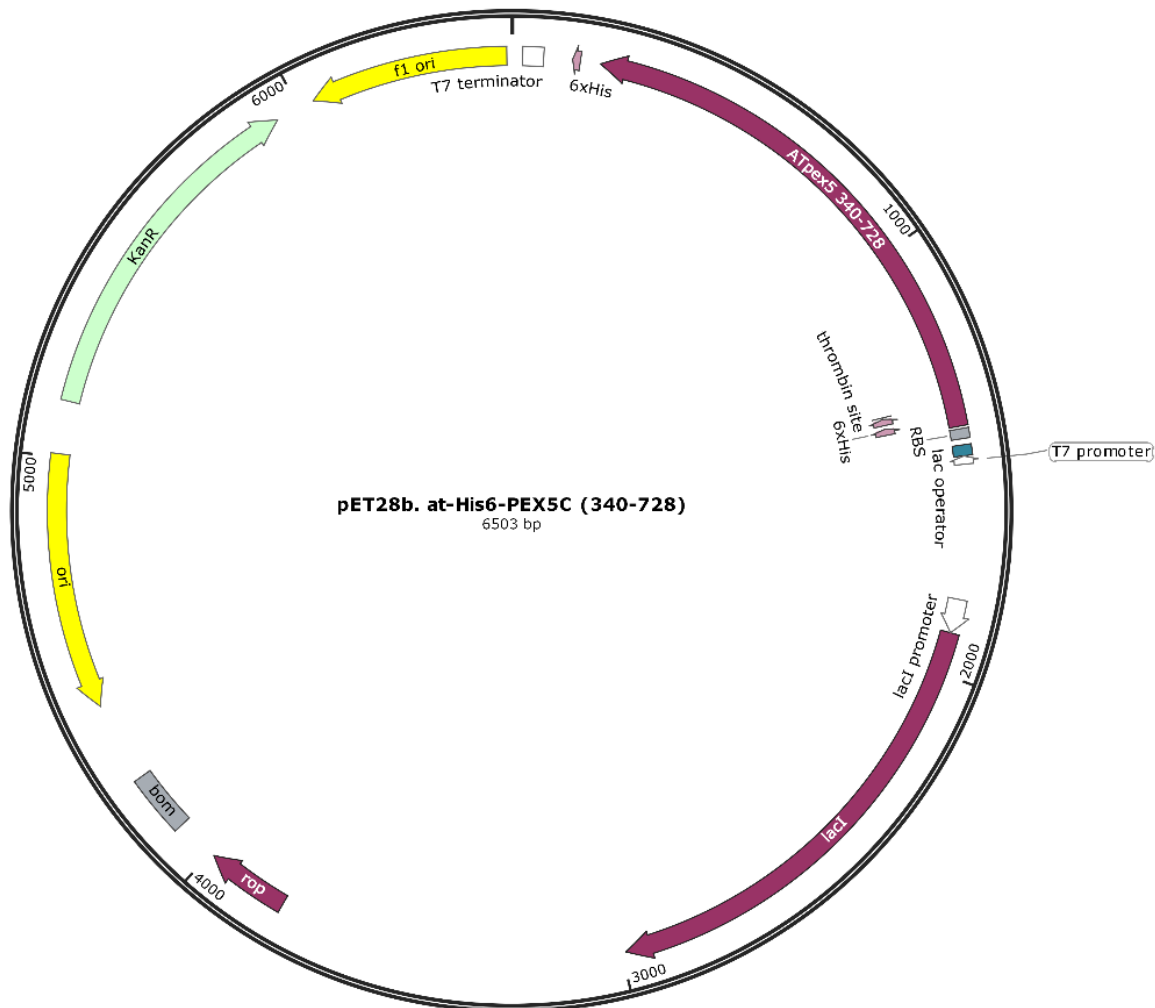
Molecular weight: 17737.20 Da

Formula:  $C_{782}H_{1255}N_{229}O_{234}S_4$

Extinction coefficient:  $19480 \text{ M}^{-1}\text{cm}^{-1}$

### 5.6.3 *At*-His-PEX5C Plasmid Map

Created with SnapGene®



### 5.6.4 *At*-His-PEX5C DNA Sequence

```

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCC
ATATGCAAGCTTCAGCCCCGGGAATGGGCTACTGAATATGAACAGCAGTATCTGGG
GCCACCAAGTTGGGCTGATCAATTTGCAAATGAGAACTTTCACATGGACCAGAACAGT
GGGCTGATGAGTTTGCTCCGGGAGAGGACAGCAAGAAACAGCTGAGGACCAATGGG
TTAATGAGTTTTCAAAGTTGAATGTTGATGACTGGATAGATGAATTTGCTGAAGGTCCC
GTGGGTGATAGTTCAGCTGATGCATGGGCAAATGCTTACGATGAGTTTCTGAATGAGA
AAAATGCTGGAAAACAAACCAGTGGTGTCTACGTCTTCTCTGACATGAATCCTTATGTG
GGTCACCCTGAACCTATGAAAGAAGGGCAAGAATTGTTTCGAAAAGGACTTCTGAGTG
AAGCAGCGCTTGCTCTAGAAGCTGAGGTTATGAAAACCCTGAGAATGCTGAAGGTTG

```



GAGATTACTTGGGGTCACACACGCAGAGAACGATGATGATCAACAGGCAATAGCTGCA  
 ATGATGCGTGCACAGGAGGCTGATCCCACAAATCTAGAGGTGCTTCTTGCGCTTGGTG  
 TGAGTCATACCAACGAGTTAGAGCAAGCAACTGCTTTGAAATATCTATATGGATGGCTG  
 CGAAATCACCCAAAGTATGGAGCAATTGCGCCTCCGGAGCTAGCGGATTCTTTGTACCA  
 TGCTGATATTGCTAGATTATTCAATGAAGCTTCTCAGTTGAATCCTGAGGACGCCGATG  
 TGCATATAGTGTTGGGCGTGCTCTACAATCTGTGAGAGAGTTTCGATAGAGCAATCACA  
 TCCTTCCAAACAGCATTACAATAAAACCAAACGATTATTCTCTGTGGAATAAGCTAGGT  
 GCAACGCAAGCCAAACAGTGTCCAGAGTGCTGATGCCATATCTGCTTATCAACAGGCTCT  
 AGATTTAAAACCAAATTATGTTTCGTGCTTGGGCAAACATGGGAATCAGTTACGCAAACC  
 AGGGGATGTACAAAGAATCAATCCCGTATTATGTCCGTGCCCTTGCATGAATCCTAAA  
 GCTGATAACGCATGGCAATACTTGAGACTCTCGTTAAGTTGTGCATCAAGGCAAGACAT  
 GATAGAAGCTTGTGAGTCAAGGAATCTCGATCTCTTGCAGAAAGAATTCCCGCTGTGA

### 5.6.5 *At*-His-PEX5C Protein Sequence

MGSSHHHHHSSGLVPRGSHMQASAPGEWATEYEQQYLGPPSWADQFANEKLSHGPEQ  
 WADEFASGRGQQETAEDQWVNEFSKLNVDWIDEFAEGPVGDSADAWANAYDEFLNE  
 KNAGKQTSGVYVFSDMNPYVGHPEPMKEGQELFRKGLLSEAALALEAEVMKNPENAEW  
 RLLGVTHAENDDDDQQAIAAMMRAQEADPTNLEVLLALGVSHNELEQATALKYLYGWLRN  
 HPKYGAIAPPELADSLYHADIARLFNEASQLNPEDADVHIVLGVLYNLSREFDRAITSFQTA  
 LQLKPNDSLWNKLGATQANSVQSADAISAYQQALDLKPNYVRAWANMGISYANQGMKY  
 ESIPYYVRALAMNPKADNAWQYLRLSLSCASRQDMIEACESRNLDLLQKEFPL

Number of amino acids: 408

Molecular weight: 45580.35 Da

Formula: C<sub>2016</sub>H<sub>3038</sub>N<sub>554</sub>O<sub>635</sub>S<sub>12</sub>

Extinction coefficient: 87445 M<sup>-1</sup>cm<sup>-1</sup>

Species: *Arabidopsis thaliana*

## 5.6.6 Protein Expression

### 5.6.6.1 Cell lines, media and buffers

#### **Bacterial Strains and Plasmids**

***E. coli* (BL21 D3):** - For plasmid cloning and protein production.

**pET28b (Kan) plasmid:** - Contains the *At*-His<sub>6</sub>-PEX5C (340-728) gene.

#### **Media:**

##### **Luria-Bertani Broth – 25 g/L**

For 100 mL of Luria-Bertani broth (25 g/L) 2.50g of Luria-Bertani medium was added to dH<sub>2</sub>O (100 mL) and autoclaved (20 min, 121 °C) and stored at r.t.

##### **Kanamycin 1000x stock – 25 mg/mL**

For 5 mL 1000X Kanamycin, 125.0 mg of Kanamycin was added to dH<sub>2</sub>O (5 mL), sterile filtered (0.22 µM syringe filter) and stored at -20 °C (1 mL aliquots).

##### **Luria-Bertani Agar with Kanamycin (25 µg/mL) Plates**

For 400 mL Luria Bertani/Agar solution containing 25.0 g/L LB and 15.0 g/L Agar, 10.0 g Luria Bertani medium and 6.0 g Agar was added to dH<sub>2</sub>O (400 mL) and autoclaved (20 min, 121 °C). Once the solution had reached 50 °C, 1000x Kanamycin (400 µL) was added and the solution poured into plates (25 mL/plate). The plates were left to harden at rt and stored at 4 °C.

#### **Buffers:**

##### **Buffer A** Lysis Buffer

NaH<sub>2</sub>PO<sub>4</sub> (50 mM), NaCl (400 mM), pH 8.0

For 500 mL buffer, NaH<sub>2</sub>PO<sub>4</sub> (3.00 g) and NaCl (11.69 g) was added to dH<sub>2</sub>O (500 mL) and stirred till dissolved, adjusted to pH 8.0 and filter sterilised.

##### **Buffer B** Wash Buffer

NaH<sub>2</sub>PO<sub>4</sub> (50 mM), NaCl (400 mM), imidazole (20 mM), pH 7.4

For 500 mL buffer,  $\text{NaH}_2\text{PO}_4$  (3.00 g), NaCl (11.69 g) and imidazole (0.68 g) was added to distilled  $\text{dH}_2\text{O}$  (500 mL) and stirred till dissolved, adjusted to pH 7.4 and filter sterilised.

#### **Buffer C** Elution Buffer

$\text{NaH}_2\text{PO}_4$  (50 mM), NaCl (400 mM), imidazole (250 mM), pH 7.4

For 500 mL buffer,  $\text{NaH}_2\text{PO}_4$  (3.00 g), NaCl (11.69 g) and imidazole (8.51 g) was added to distilled  $\text{dH}_2\text{O}$  (500 mL) and stirred till dissolved, adjusted to pH 7.4 and filter sterilised.

#### **Buffer D (SDS-Gel)**

Tris-HCl (1.5 M), pH 8.8

For 100 mL buffer Tris (18.17 g) was added to distilled  $\text{dH}_2\text{O}$  (100 mL) and stirred till dissolved, adjusted to pH 8.8 and filter sterilised.

#### **Buffer E (SDS-Gel)**

Tris-HCl (0.5 M), pH 6.8

For 100 mL buffer Tris (6.06 g) was added to distilled  $\text{dH}_2\text{O}$  (100 mL) and stirred till dissolved, adjusted to pH 8.8 and filter sterilised.

#### **Buffer F** Mcl-1 FA Assay Buffer

Tris (50 mM), NaCl (150 mM), Triton X-100: 0.01%, pH 7.4

For 500 mL buffer, Tris (3.03 g), NaCl (4.38 g) and Triton X-100 (0.05 mL) was added to distilled  $\text{dH}_2\text{O}$  (500 mL) and stirred till dissolved, adjusted to pH 7.4 and filter sterilised.

#### **Buffer G** PEX5 FA Assay Buffer

HEPES (20 mM) and NaCl (150 mM), pH 7.5

For 500 mL buffer, HEPES (2.83 g) and NaCl (4.38 g) was added to distilled  $\text{dH}_2\text{O}$  (500 mL) and stirred till dissolved, adjusted to pH 7.5 and filter sterilised.

#### **5.6.6.2 Transformation of Plasmids**

Stock solution containing the desired plasmid (1  $\mu$ L) was added into an aliquot of *E. coli* (BL21 D3). The solution was then incubated for 10 min on ice, heatshocked at 42 °C for 45 seconds, then incubated on ice for a further 10 min. LB solution (1 mL) was then added and incubated at 38 °C for an hr. Aliquots of the solution were taken (100  $\mu$ L, 400  $\mu$ L and 500  $\mu$ L) and added to the agar plates, a cell spreader was used to give an even distribution of *E. coli*. The plates were allowed to dry for 5 min before being placed in an incubator overnight at 38 °C. Single cultures were selected and placed into 50 mL falcon tubes containing 10 mL of LB solution and left to incubate overnight at 38 °C.

#### **5.6.6.3 Protein Expression**

Conical flasks containing AIM media (14 g) in water (400 mL) were placed in an autoclave at 120 °C for 90 min. Kanamycin solution (400  $\mu$ L) was added to each flask. The flasks were then inoculated with 1 mL of culture in LB solution and incubated overnight at 28 °C. The incubated solution was placed in centrifuge vessels and centrifuged at 5000 g x 20 min. The supernatant was discarded and the pellets stored at -80 °C.

#### **5.6.6.4 Cell Lysis**

The bacterial pellet was removed from the -80 °C and allowed to thaw to r.t. Lysozyme (20 mg/mL) and one protease inhibitor P8849 tablet (Roche complete EDTA free) dissolved in buffer A (10 mL) was added to the thawed pellet. The pellet was then sonicated (6 sec. on/6 sec. off x 10 cycles) at 4 °C using a Bandelin SONPULS ultrasonic homogeniser with a MS 73 micro tip probe. The sonicated pellet was centrifuged (25,000 rpm, 30 min, 4 °C). The supernatant was sterile filtered (0.45  $\mu$ m syringe filter) and kept on ice ready for purification.

### 5.6.6.5 Purification of At-His<sub>6</sub>-PEX5C

A column was packed with 1 mL cobalt resin and the storage buffer was drained from the resin by gravity. The column was then equilibrated with two resin-bed volumes of wash buffer (buffer B). The buffer was allowed to drain from the resin.

A small amount of DNase was added to the cell lysate. The prepared protein extract was added to the resin and the flow-through collected in a tube. The flow-through was reapplied to the resin in order to maximise binding of the protein. The resin was washed with wash buffer and the flow-through collected. The washing step was repeated until the A280 value on the nanodrop was <0.009 to ensure removal of unbound and non-specific binding proteins. Each fraction was collected using a new collection tube. The His-tagged PEX5 was eluted from the resin using elution buffer (buffer C) and repeated until the A280 value on the nanodrop was <0.009. Each fraction was collected using a new collection tube.

The purity of the collected fractions was assessed *via* SDS-PAGE gel. The following samples were tested:

- Soluble Protein Supernatant (5 µL)
- Supernatant after incubation on resin (unbound proteins) (20 µL)
- Resin washes (50 µL)
- Eluted Protein (50 µL)

Sample loading dye (1:1) was added to each sample and boiled for 5 min in a heat block. The samples were then incubated on ice for 5 min before loading onto the gel.

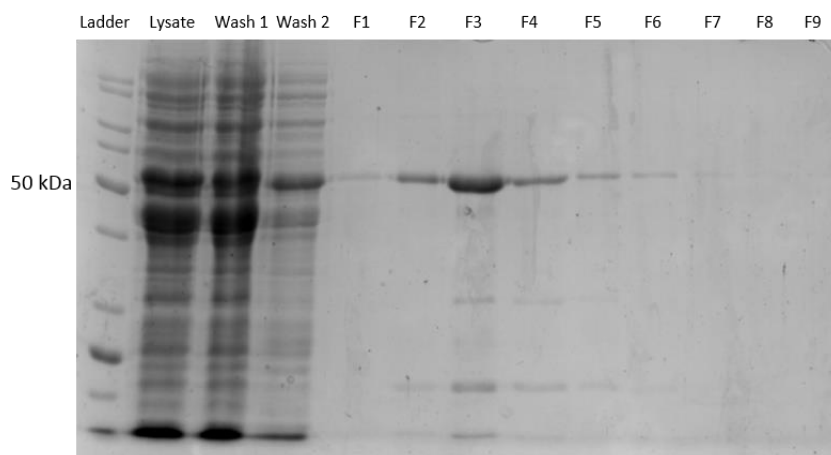
The separation gel (12%) was prepared through addition of the following reagents in order:

H <sub>2</sub> O	4.257 mL
Acrylamide (40%)	3.000 mL
Tris-HCl (1.5 M, pH 8.8)	2.533 mL
SDS, 10%	100 µL
<i>N,N,N',N'</i> -tetramethylethylene-diamine	10 µL
Ammonium persulfate (10%)	100 µL

The gel was then poured, leaving ~2 cm below the bottom of the comb for the stacking gel. Bubbles were removed by gentle agitation. The top of the gel was layered with isopropanol to help remove bubbles at the top of the gel and prevent drying out. After 30 min, the isopropanol was poured off the gel and then washed with distilled water. The stacking gel (4%) was then prepared through addition of the following reagents, in order:

H <sub>2</sub> O	3.320 mL
Acrylamide (40%)	0.625 mL
Tris-HCl (0.5 M, pH 6.8)	0.925 mL
SDS, 10%	50 µL
TEMED	10 µL
Ammonium persulfate (APS), 10%	50 µL

The gel was then poured on top of the separate gel. The combs were added to make wells and left for 30 min to completely polymerise. The gel was clamped into the apparatus and both buffer chambers filled with gel running buffer. Once the gel was run, it was stained with Coomassie blue for visualisation.



**Figure 65:** SDS-Page gel for *At*-His<sub>6</sub>-PEX5C purification. SDS PAGE analysis for purification of *At*-His<sub>6</sub>-PEX5C *via* Co-chromatography.

Fractions containing *At*-His<sub>6</sub>-PEX5C were combined and the buffer swapped to buffer F for use in screening *via* spin concentration. The concentration of *At*-His<sub>6</sub>-PEX5C was assessed *via* nanodrop. The sample was tested three times and an average value taken to give the concentration.

## 5.6.7 Fluorescence Anisotropy Inhibition Assay

### 5.6.7.1 Measurement of Anisotropy

Fluorescence Anisotropy measurements were carried using black 384-well plates and read on an Envision™ 2013 multilabel plate reader (PerkinElmer) using the following setups:

**Table 16:** Plate reader setup used for fluorescence anisotropy assays with Mcl-1.

	<b>Manufacturer's Description</b>	<b>Wavelength (nm)</b>	<b>Bandwidth (nm)</b>
Dichroic Mirror	FITC FP D505 Single Mirror	505	
Excitation Filter	FITC FP 480	480	30
Emission Filter 1:	FITC FP P-pol 535	535	40
Emission Filter 2:	FITC FP S-pol 535	535	40

**Table 16:** Plate reader setup used for fluorescence anisotropy assays with PEX5.

	<b>Manufacturer's Description</b>	<b>Wavelength (nm)</b>	<b>Bandwidth (nm)</b>
Dichroic Mirror	BODIPY TMR FP	555	
Excitation Filter	BODIPY TMR FP 531	531	25
Emission Filter 1:	BODIPY TMR FP P-pol 595	595	60
Emission Filter 2:	BODIPY TMR FP S-pol 595	595	60

The reading protocol was optimised using the instrument software to ensure that the detector was not saturated.

**Table 17:** Optimisation results for Mcl-1 and PEX5 fluorescence anisotropy assays.

<b>Property</b>	<b>Mcl-1</b>	<b>PEX5</b>
Measurement height	5.0 mm	7.4 mm
Number of flashes	60	30
PMT gain	155	300
G-factor	1	1

Anisotropy ( $r$ ) was calculated using equation 3.

$$r = 1000 \times \frac{s - gp}{s + g2p} \quad \text{Equation 3}$$

Total fluorescence intensity ( $I$ ) was calculated using equation 4.

$$I = s + g2p \quad \text{Equation 4}$$

Where  $s$  is the intensity of the light emitted with the same polarisation as the excitation light,  $p$  is the light emitted with perpendicular polarisation to the excitation light,  $g$  is the grating factor which corrects for how responsive the machine is to light with different polarisations. In these assays this was set to 1.

Blank corrections were performed by subtracting the average of the appropriate blank in the  $s$  and  $p$  channels prior to calculations of the anisotropy.

The FAM-labelled Bid and Lissamine-labelled YQSKL was stored as stock solutions in buffer F and buffer G respectively at  $-20\text{ }^{\circ}\text{C}$ . All stock solutions were kept on ice at all times.

### **5.6.7.2 Determination of Limits of Anisotropy**

#### **5.6.7.2.1 Mcl-1**

To rows A, C, E, G, I and K, wells 1-16 was added 20  $\mu\text{L}$  of buffer F. 20  $\mu\text{L}$  of a protein stock solution (60  $\mu\text{M}$ ) was added to well 1 in each of the rows A, C, E, G, I and K, and the contents of the well agitated with a pipette. Following addition and agitation 20  $\mu\text{L}$  of the contents of well 1 in rows A, C, E, G, I and K was transferred to well 2, agitated, then 20  $\mu\text{L}$  transferred to well 3. The process was repeated across the entire series to well 16, where after agitation 20  $\mu\text{L}$  was removed and discarded. 20  $\mu\text{L}$  of 50 nM FAM-Bid (final concentration 25 nM) was added to all well in rows A, C and E and 20  $\mu\text{L}$  of FA buffer added to all wells in rows G, I, and K to serve as blanks. Plates were incubated for 2 hr and the anisotropy of each well was measured and the average intensities measured in the wells containing no tracer (rows G, I and K) were used to provide a blank correction for each protein concentration. The anisotropy was plotted against protein concentration with the standard deviation of the replicates used as the error. The data was fitted to a logistic function (Equation 5) to find the lower and



upper limits of the anisotropy  $r_{max}$ ,  $r_{min}$  which reflect the anisotropy of the tracer when in the unbound and bound state state respectively.

$$r = \frac{r_{min} - r_{max}}{1 + \left(\frac{x}{x_0}\right)^p} + r_{max} \quad \text{Equation 5}$$

Average values were calculated to be  $r_{max} = 0.08$  and  $r_{min} = -0.007$  and these values were used throughout subsequent assays.

#### 5.6.7.2.2 PEX5

To rows A, C, E, G, I and K, wells 1-24 was added 20  $\mu$ L of buffer G. 40  $\mu$ L of a protein stock solution (15  $\mu$ M) was added to well 1 in each of the rows A, C, E, G, I and K, and the contents of the well agitated with a pipette. Following addition and agitation 20  $\mu$ L of the contents of well 1 in rows A, C, E, G, I and K was transferred to well 2, agitated, then 20  $\mu$ L transferred to well 3. The process was repeated across the entire series to well 24, where after agitation 20  $\mu$ L was removed and discarded. 20  $\mu$ L of 200 nM Lissamine-labelled YQSKL (final concentration 100 nM) was added to all well in rows A, C and E and 20  $\mu$ L of buffer G added to all wells in rows G, I, and K to serve as blanks. Plates were incubated for 20 min and the anisotropy of each well was measured and the average intensities measured in the wells containing no tracer (rows G, I and K) were used to provide a blank correction for each protein concentration. The limits of anisotropy were calculated as previously and determined to be  $r_{max} = 0.33$  and  $r_{min} = 0.18$  and these values were used throughout subsequent assays.

#### 5.6.7.3 Determination of the Dissociation Constant of the Fluorescent Tracers

The anisotropy data for each well in Sections 5.6.7.2.1 and 5.6.7.2.2 was converted to the fraction of tracer bound using equation 6 and then into the amount of tracer bound by multiplying by the total concentration of tracer in the well of interest (25 nM for Mcl-1 or 100 nM PEX5).

$$\frac{L_B}{L_T} = \left[ \frac{r_{max} - r}{r - r_{min}} + 1 \right]^{-1} \quad \text{Equation 6}$$

The data was plotted and fitted to Equation 7 using a non-linear least squares fitting algorithm in Originpro to determine the  $K_d$  of the tracer for the protein. The error in  $K_d$  was obtained from the fitting error within the procedure.

$$L_B = \frac{(L_T + P_T + K_d) - \sqrt{(L_T + P_T + K_d)^2 - 4L_T P_T}}{2} \quad \text{Equation 7}$$

$L_B$  is the concentration of fluorescent tracer bound to protein,  $L_T$  is the total tracer concentration,  $P_T$  is the total concentration of protein.

#### **5.6.7.4 Determination of $IC_{50}$ of Unlabelled Ligands**

##### **5.6.7.4.1 Mcl-1**

Compounds to be assessed in dose-response were dissolved into DMSO at the appropriate concentration. To wells 1 and subsequent wells was added 6  $\mu$ L of DMSO. 2  $\mu$ L of compound stock solution was added to well 1 and the contents of the well agitated with a pipette. Following addition and agitation 2  $\mu$ L of the contents of well 1 was transferred to well 2, agitated, then 2  $\mu$ L transferred to well 3. The process was repeated across the entire series to the final well, where after agitation 2  $\mu$ L was removed and discarded. 5  $\mu$ L of each dilution series was then further diluted into 160  $\mu$ L of buffer F to give DMSO/Buffer G (3:97) at three times the final assay concentration prior to addition into the assay plate. Compounds were screened in duplicate and with blanks (no tracer) as a control. 20  $\mu$ L of the serial dilution were added to the wells of interest followed by 20  $\mu$ L of 75 nM tracer solution (20  $\mu$ L of buffer F for the blank). Finally, 20  $\mu$ L of 450 nM protein stock solution was added to all wells to give a final concentration of 25 nM tracer and 150 nM protein. The final DMSO concentration was 1%. Assay plates were read after 20 min. Positive controls (A-1210477 100  $\mu$ M) and DMSO (1%) negative control was included.

#### **5.6.7.4.2 PEX5**

Compounds to be assessed in dose-response were dissolved into DMSO at the appropriate concentration. To well 1 was added 40  $\mu\text{L}$  of stock solution and to the subsequent wells was added 20  $\mu\text{L}$  of DMSO. To well 2 was added 20  $\mu\text{L}$  of the stock solution from well 1, and the contents agitated with a pipette. Following addition and agitation 20  $\mu\text{L}$  of the contents of well 2 was transferred to well 3, agitated, then 20  $\mu\text{L}$  transferred to well 4. The process was repeated across the entire series to the final well, where after agitation 20  $\mu\text{L}$  was removed and discarded. 15  $\mu\text{L}$  of each dilution series was then further diluted into 60  $\mu\text{L}$  of buffer G to give DMSO/Buffer G (20:80) at four times the final assay concentration prior to addition into the assay plate. Compounds were screened in duplicate and with blanks (no tracer) as a control. 10  $\mu\text{L}$  of buffer G was added to all wells to be tested. 10  $\mu\text{L}$  of the serial dilution were added to the wells of interest followed by 10  $\mu\text{L}$  of 120 nM tracer solution (10  $\mu\text{L}$  of buffer G for the blank). Finally, 10  $\mu\text{L}$  of 800 nM protein stock solution was added to all wells to give a final concentration of 30 nM tracer and 200 nM protein. The final DMSO concentration was 5%. Assay plates were read after 20 min. Positive controls (SKL 10 mM) and DMSO (5%) negative control was included.

#### **5.6.7.5 Screening of Crude Reaction Array Mixtures**

##### **5.6.7.5.1 Mcl-1**

Crude reaction mixtures were stored as 100 mM DMSO stocks and were diluted accordingly into DMSO and then DMSO:Buffer F (3:97) at three times the final assay concentration prior to addition into the assay plate. Reaction array mixtures were screened in duplicate and with blanks (no tracer) as a control. 20  $\mu\text{L}$  of the reaction array mixtures in DMSO:Buffer F (3:97) were added to the wells of interest followed by 20  $\mu\text{L}$  of 75 nM tracer solution (20  $\mu\text{L}$  of buffer F for the blank). Finally, 20  $\mu\text{L}$  of 450 nM protein stock solution was added to all wells to give a final concentration of 25 nM tracer and 150 nM protein. The final DMSO concentration was 1%. Assay plates were read after 2 hr. Positive controls (A-1210477, 100  $\mu\text{M}$ ) and DMSO (1%) negative control was included.

### 5.6.7.5.2 PEX5

Crude reaction mixtures were stored as 100 mM DMSO stocks and were diluted accordingly into DMSO and then DMSO:Buffer G (20:80) at four times the final assay concentration prior to addition into the assay plate. Reaction array mixtures were screened in duplicate and with blanks (no tracer) as a control. 10  $\mu$ L of buffer G was added to all wells to be tested. 10  $\mu$ L of the reaction array mixtures in DMSO:Buffer G (20:80) were added to the wells of interest followed by 10  $\mu$ L of 120 nM tracer solution (10  $\mu$ L of buffer G for the blank). Finally, 10  $\mu$ L of 800 nM protein stock solution was added to all wells to give a final concentration of 30 nM tracer and 200 nM protein. The final DMSO concentration was 5%. Assay plates were read after 20 min. Positive controls (SKL 10 mM) and DMSO (5%) negative control was included.

### 5.6.7.6 Determination of the Inhibitory Constant of Unlabelled Ligands

IC<sub>50</sub> were converted into K<sub>i</sub> by combination with K<sub>d</sub> using the method of Nicolovska-Coleska using Equations 8-11.<sup>77</sup>

$$K_i = \frac{I_{50}}{\frac{L_{50}}{K_d} + \frac{P_0}{K_d} + 1} \quad \text{Equation 8}$$

Equation 8, where K<sub>i</sub> is the dissociation constant of the unlabelled peptide and K<sub>d</sub> is the dissociation constant of the fluorescent tracer. The remaining factors were calculated using Equations 9-11.

P<sub>0</sub> is the unbound concentration of protein at zero inhibitor concentration is calculated from:

$$P_0 = -\frac{\left((K_d + L_T - P_T) + \sqrt{((K_d + L_T - P_T)^2 - 4K_dP_T)}\right)}{2} \quad \text{Equation 9}$$

Equation 9 where K<sub>d</sub> is the dissociation constant of the fluorescent tracer, L<sub>T</sub> is the total tracer concentration. P<sub>T</sub> is the total concentration of PEX5.

L<sub>50</sub> is the concentration of the unbound fluorescent tracer at the IC<sub>50</sub> point is calculated from:

$$L_{50} = L_T - \frac{(P_T - P_0)}{2} \quad \text{Equation 10}$$

Equation 10, where  $L_T$  is the total concentration of tracer.  $P_T$  is the total concentration of PEX5 and  $P_0$  is the unbound concentration of protein at zero inhibitor concentration, as calculated in Equation 9.

$I_{50}$  is the unbound inhibitor concentration at the  $IC_{50}$  point is calculated from:

$$I_{50} = IC_{50} - P_T + \left( \frac{(P_T - P_0)}{2} \right) \times \left( 1 + \frac{K_d}{L_{50}} \right) \quad \text{Equation 11}$$

Equation 11, where  $IC_{50}$  is determined from the curve fitted from tracer titration.  $P_T$  is the total concentration of PEX5 and  $K_d$  is the dissociation constant of fluorescent tracer.  $P_0$  is calculated from Equation 9 and  $L_{50}$  is calculated from Equation 10.

The error in  $K_i$  was determined by the simulation through the given errors of  $IC_{50}$  and  $K_d$ . A series of simulations were run with a simulated value of  $K_d$  and  $IC_{50}$  generated around the observed mean, the probability of the value occurring was based upon a normal distribution with standard deviation equal to the fitting error of each value. The simulated value was then propagated to give a simulated  $K_i$ . 2000 simulations were performed with the final error estimated from the standard deviation of the simulated results.

## 5.7 Docking

### 5.7.1 Protocol for Docking of PEX5 Ligands

All docking experiments were performed using Maestro by Schrödinger. The crystal structure of the PTS1 complexed to the TPR region of human PEX5 was used to perform docking (PDB accession code: 1FCH). Following the import of the protein structure, protein preparation was performed using the 'proten preparation wizard'. First, the 'preprocess' workflow was executed to add hydrogens to the protein. On the refine tab, the 'optimize' workflow was executed to optimize the hydrogen-bonding network within the protein. Finally, the optimized structure was minimised to alleviate any strain generated through the preprocessing.

Docking experiments were then performed on designed ligands. These were drawn in ChemDraw and imported to Maestro in .sdf format. The ligands then underwent ligand preparation using the 'lig prep' function with default settings. Specified chiralities were retained, with diastereomers being generated in compounds which contained unspecified chiralities.

The area of the protein to be used for docking was specified using 'grid generation'. The ligand in the protein was selected using 'pick to identify the ligand', the default size of the grid was used to perform the docking experiments.

Docking of ligands was performed using the default settings. First, all ligands were docked against the protein using the generated receptor grid using the HTVS method (high throughput virtual screening). The ligands which performed in the top 10% (evaluated *via* gscore) were then docked against using the XP (extra precision) method.

### 5.7.2 SMILES String for Docked Library

**Table 18:** SMILES for docked library.

<chem>O=C(O)[C@@H](NC(C1CNCC1C2=CC=CC=C2)=O)CC3CCCC3</chem>
<chem>O=C(O)[C@@H](NC(C4CNCC4C5=CC=CN=C5)=O)CC6CCCC6</chem>
<chem>O=C(O)[C@@H](NC(C7CNCC7C8=CC=C(O)C=C8)=O)CC9CCCC9</chem>
<chem>O=C(O)[C@@H](NC(C%10CNCC%10C%11=CC=C(OC)C=C%11)=O)CC%12CCCC%12</chem>
<chem>O=C(O)[C@@H](NC(C%13CNCC%13C%14=CC=CC(O)=C%14)=O)CC%15CCCC%15</chem>
<chem>O=C(O)[C@@H](NC(C%16CNCC%16C%17=CC=CC(OC)=C%17)=O)CC%18CCCC%18</chem>
<chem>O=C(O)[C@@H](NC(C%19CNCC%19C%20=C(O)C=CC=C%20)=O)CC%21CCCC%21</chem>
<chem>O=C(O)[C@@H](NC(C%22CNCC%22C%23=C(OC)C=CC=C%23)=O)CC%24CCCC%24</chem>
<chem>O=C(O)[C@@H](NC(C%25CNCC%25C%26=C(Cl)C=CC=C%26)=O)CC%27CCCC%27</chem>
<chem>O=C(O)[C@@H](NC(C%28CNCC%28C%29=C(F)C=CC=C%29)=O)CC%30CCCC%30</chem>
<chem>O=C(O)[C@@H](NC(C%31CNCC%31C%32=CC(Cl)=CC=C%32)=O)CC%33CCCC%33</chem>
<chem>O=C(O)[C@@H](NC(C%34CNCC%34C%35=CC(F)=CC=C%35)=O)CC%36CCCC%36</chem>
<chem>O=C(O)[C@@H](NC(C%37CNCC%37C%38=CC=C(Cl)C=C%38)=O)CC%39CCCC%39</chem>
<chem>O=C(O)[C@@H](NC(C%40CNCC%40C%41=CC=C(F)C=C%41)=O)CC%42CCCC%42</chem>
<chem>O=C(O)[C@@H](NC(C%43CNCC%43%44CCC%45(OCCO%45)CC%44)=O)CC%46CCCC%46</chem>

<chem>O=C(O)[C@@H](NC(C%47CNCC%47%48CCNCC%48)=O)CC%49CCCC%49</chem>
<chem>O=C(O)[C@@H](NC(C%50CNCC%50%51CCOCC%51)=O)CC%52CCCC%52</chem>
<chem>O=C(O)[C@@H](NC(C%53CNCC%53NCCSC)=O)CC%54CCCC%54</chem>
<chem>O=C(O)[C@@H](NC(C%55CNCC%55N(C(C)=O)CCSC)=O)CC%56CCCC%56</chem>
<chem>O=C(O)[C@@H](NC(C%57CNCC%57N%58CCSCC%58)=O)CC%59CCCC%59</chem>
<chem>O=C(O)[C@@H](NC(C%60CNCC%60N(C(C)=O)CCO)=O)CC%61CCCC%61</chem>
<chem>O=C(O)[C@@H](NC(C%62CNCC%62NCCO)=O)CC%63CCCC%63</chem>
<chem>O=C(O)[C@@H](NC(C%64CNCC%64N(C(C)=O)CCOC)=O)CC%65CCCC%65</chem>
<chem>O=C(O)[C@@H](NC(C%66CNCC%66NCCOC)=O)CC%67CCCC%67</chem>
<chem>O=C(O)[C@@H](NC(C%68CNCC%68N(C(C)=O)CCCO)=O)CC%69CCCC%69</chem>
<chem>O=C(O)[C@@H](NC(C%70CNCC%70NCCCO)=O)CC%71CCCC%71</chem>
<chem>O=C(O)[C@@H](NC(C%72CNCC%72N(C(C)=O)CCCOC)=O)CC%73CCCC%73</chem>
<chem>O=C(O)[C@@H](NC(C%74CNCC%74NCCCOC)=O)CC%75CCCC%75</chem>
<chem>O=C(O)[C@@H](NC(C%76CNCC%76N(C(C)=O)CCC)=O)CC%77CCCC%77</chem>
<chem>O=C(O)[C@@H](NC(C%78CNCC%78NCCC)=O)CC%79CCCC%79</chem>
<chem>O=C(O)[C@@H](NC(C%80CNCC%80N(C(C)=O)CC)=O)CC%81CCCC%81</chem>
<chem>O=C(O)[C@@H](NC(C%82CNCC%82NCC)=O)CC%83CCCC%83</chem>
<chem>O=C(O)[C@@H](NC(C%84CNCC%84N(C(C)=O)CCCC)=O)CC%85CCCC%85</chem>
<chem>O=C(O)[C@@H](NC(C%86CNCC%86NCCCC)=O)CC%87CCCC%87</chem>
<chem>O=C(O)[C@@H](NC(C%88C(NC%89CNC%89)CNC%88)=O)CC%90CCCC%90</chem>
<chem>O=C(O)[C@@H](NC(C%91C(N(C%92CNC%92)C(C)=O)CNC%91)=O)CC%93CCCC%93</chem>
<chem>O=C(O)[C@@H](NC(C%94C(NC%95CC(N)C%95)CNC%94)=O)CC%96CCCC%96</chem>
<chem>O=C(O)[C@@H](NC(C%97C(N(C%98CC(N)C%98)C(C)=O)CNC%97)=O)CC%99CCCC%99</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCN)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCN)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCN)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCN)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N2CCNCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N2CC(CN)C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C2(CCNCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C2(CNC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC(CN)=CC=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>

<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC(CN)=CC=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC=C(CN)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC=C(CN)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(CN)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(CN)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(CN)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(CN)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCCO)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCCO)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2F)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2F)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(F)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(F)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(F)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(F)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2O)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(O)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(O)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2Cl)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2Cl)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(Cl)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(Cl)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(O)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(O)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(Cl)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(Cl)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2OC)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2OC)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(OC)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(OC)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(OC)C=C2)CNC1)=O)CC1CCCC1</chem>



<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(OC)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2N)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2N)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(N)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(N)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(N)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(N)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC(CO)=CC=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC(CO)=CC=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC=C(CO)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC=C(CO)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(CO)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(CO)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC3=C2C=CC=C3)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC3=C2C=CC=C3)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=NC3=C2C=CC=C3)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=NC3=C2C=CC=C3)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2C)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2C)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(C)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(C)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(C)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2C#N)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2C#N)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(C#N)=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(C#N)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(C#N)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(C#N)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2C)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2C)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(C)=C2)CNC1)=O)CC1CCCC1</chem>

<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(C)=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(C)C=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(C)C=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=C(C)C=CC=C1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC(C)=CC=C1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=C(C)C=C1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N1CCOCC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CCC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CCCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CCCC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CCCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CCCC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CCCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CCCC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CCCC2)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CCCC2)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C(NC(CO)C(C)=O)=O)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=CN=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=C(O)C=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=C(OC)C=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=CC(O)=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=CC(OC)=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=C(O)C=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=C(OC)C=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=C(Cl)C=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=C(F)C=CC=C1)=O)CC(C)C</chem>

<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC(Cl)=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC(F)=CC=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=C(Cl)C=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1C1=CC=C(F)C=C1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC11CCC2(OCCO2)CC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC11CCNCC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC11CCOCC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCSC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCSC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N1CCSCC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCOC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCOC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCOC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCC)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CNC2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CNC2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2CC(N)C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2CC(N)C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCN)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCN)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCN)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCN)=O)CC(C)C</chem>

<chem>O=C(O)[C@@H](NC(C1C(N2CCNCC2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N2CC(CN)C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C2(CCNCC2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C2(CNC2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC(CN)=CC=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC(CN)=CC=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC=C(CN)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC=C(CN)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(CN)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(CN)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(CN)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(CN)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1N(C(C)=O)CCCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1CNCC1NCCCCO)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2F)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2F)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(F)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(F)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(F)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(F)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2O)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(O)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(O)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2Cl)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2Cl)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(Cl)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(C)[C@@H](NC(C1C(N(C2=CC=CC(Cl)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(O)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(O)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(Cl)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(Cl)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>

<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2OC)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2OC)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(OC)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(OC)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(OC)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(OC)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2N)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2N)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(N)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(N)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(N)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(N)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC(CO)=CC=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC(CO)=CC=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(CC2=CC=C(CO)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NCC2=CC=C(CO)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(CO)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(CO)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC3=C2C=CC=C3)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC3=C2C=CC=C3)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=NC3=C2C=CC=C3)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=NC3=C2C=CC=C3)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2C)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2C)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(C)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(C)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=C(C)C=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=C(C)C=C2)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC=C2C#N)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC=C2C#N)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2=CC=CC(C#N)=C2)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2=CC=CC(C#N)=C2)C(C)=O)CNC1)=O)CC(C)C</chem>



OC([C@@H](NC(C1C(NS(=O)(C2=CC=C(O)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=C(O)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC(OC)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC=C(OC)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=C(OC)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC(Cl)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC=C(Cl)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=C(Cl)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC(F)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC=C(F)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=C(F)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC(C#N)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=CC=C(C#N)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NS(=O)(C2=C(C#N)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(C)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(C)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(C)C=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(O)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(O)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(O)C=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(OC)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(OC)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(OC)C=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(Cl)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(Cl)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(Cl)C=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(F)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(F)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(F)C=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC(C#N)=CC=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=CC=C(C#N)C=C2)=O)CNC1)=O
CC(C)C[C@@H](C(O)=O)NC(C1C(NS(=O)(C2=C(C#N)C=CC=C2)=O)CNC1)=O

OC([C@@H](NC(C1C(NC(C)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CC)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CCC)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CCCC)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CO)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CCO)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(CCCO)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(C)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC(C)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(C)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(O)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC(O)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(O)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(OC)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC(OC)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(OC)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(Cl)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC(Cl)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(Cl)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(F)C=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC(F)=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(F)C=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CCC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CCCC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CCCCC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CCCCC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2CCOCC2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=CC=C(C=CC=C3)C3=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(C=CC=C3)C3=CC=C2)=O)CNC1)=O)CC1CCCC1)=O
OC([C@@H](NC(C1C(NC(C2=C(C=CC=C3)C3=NC=C2)=O)CNC1)=O)CC1CCCC1)=O



<chem>OC([C@@H](NC(C1C(NC(C2=CC=CC=N2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CC=CN=C2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CC=NC=C2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CC(C=CC=C3)=C3N2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CNC=N2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CN(C)C=N2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=COC=C2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>OC([C@@H](NC(C1C(NC(C2=CC=CO2)=O)CNC1)=O)CC1CCCC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CC)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CCC)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CCCC)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CO)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CCO)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(CCCO)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(C)C=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(C)=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(C)C=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(O)C=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(O)=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(O)C=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(OC)C=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(OC)=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(OC)C=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(Cl)C=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(Cl)=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(Cl)C=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(F)C=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(F)=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(F)C=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CC2)=O)CNC1)=O</chem>

<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CCC2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CCCC2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CCCCC2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CCCCC2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2CCOCC2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=C(C=CC=C3)C3=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(C=CC=C3)C3=CC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=C(C=CC=C3)C3=NC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=CC=N2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=CN=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=NC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC(C=CC=C3)=C3N2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CNC=N2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CN(C)C=N2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=COC=C2)=O)CNC1)=O</chem>
<chem>CC(C)C[C@@H](C(O)=O)NC(C1C(NC(C2=CC=CO2)=O)CNC1)=O</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2C3CCC2CC3)C(C)=O)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2C3CCC2CC3)CNC1)=O)CC(C)C</chem>
<chem>O=C(O)[C@@H](NC(C1C(N(C2C3CCC2CC3)C(C)=O)CNC1)=O)CC1CCCC1</chem>
<chem>O=C(O)[C@@H](NC(C1C(NC2C3CCC2CC3)CNC1)=O)CC1CCCC1</chem>

## 6 References

- 1 U. Rix and G. Superti-Furga, *Nat. Chem. Biol.*, 2009, **5**, 616–624.
- 2 H. X. Ngo and S. Garneau-Tsodikova, *Medchemcomm*, 2018, **9**, 757.
- 3 N. Schneider, D. M. Lowe, R. A. Sayle, M. A. Tarselli and G. A. Landrum, *J. Med. Chem.*, 2016, **59**, 4385–4402.
- 4 S. M. Paul, D. S. Mytelka, C. T. Dunwiddie, C. C. Persinger, B. H. Munos, S. R. Lindborg and A. L. Schacht, *Nat. Rev. Drug Discov.*, 2010, **9**, 203–214.
- 5 L. Bonetta, *Nature*, 2010, **468**, 851–854.
- 6 K. Venkatesan, J. F. Rual, A. Vazquez, U. Stelzl, I. Lemmens, T. Hirozane-Kishikawa, T. Hao, M. Zenkner, X. Xin, K. Il Goh, M. A. Yildirim, N. Simonis, K. Heinzmann, F. Gebreab, J. M. Sahalie, S. Cevik, C. Simon, A. S. de Smet, E. Dann, A. Smolyar, A. Vinayagam, H. Yu, D. Szeto, H. Borick, A. Dricot, N. Klitgord, R. R. Murray, C. Lin, M. Lalowski, J. Timm, K. Rau, C. Boone, P. Braun, M. E. Cusick, F. P. Roth, D. E. Hill, J. Tavernier, E. E. Wanker, A. L. Barabási and M. Vidal, *Nat. Methods.*, 2009, **6**, 83–90.
- 7 L.-G. Milroy, T. N. Grossmann, S. Hennig, L. Brunsveld and C. Ottmann, *Chem. Rev.*, 2014, **9**, 4695–4748.
- 8 R. Santos, O. Ursu, A. Gaulton, A. P. Bento, R. S. Donadi, C. G. Bologa, A. Karlsson, B. Al-Lazikani, A. Hersey, T. I. Oprea and J. P. Overington, *Nat. Rev. Drug Discov.*, 2016, **16**, 19–34.
- 9 M. C. Smith and J. E. Gestwicki, *Expert Rev. Mol. Med.*, 2012, **14**, e16.
- 10 A. A. Ivanov, F. R. Khuri and H. Fu, *Trends Pharmacol. Sci.*, 2013, **34**, 393–400.
- 11 S. Shangary and S. Wang, *Annu. Rev. Pharmacol. Toxicol.*, 2009, **49**, 223–241.
- 12 T. Geppert, B. Hoy, S. Wessler and G. Schneider, *Chem. Biol.*, 2011, **18**, 344–353.
- 13 K. S. Thorn and A. A. Bogan, *Bioinformatics*, 2001, **17**, 284–285.

- 14 K. Khoury and A. Dömling, *Curr. Pharm. Des.*, 2012, **18**, 4668-4678.
- 15 C. Klein and L. T. Vassilev, *Br. J. Cancer*, 2004, **91**, 1415-1419.
- 16 Y. J. Im, J. H. Lee, S. H. Park, S. J. Park, S. H. Rho, G. B. Kang, E. Kim and S. H. Eom, *J. Biol. Chem.*, 2003, **278**, 48099-48104.
- 17 F. Ye and M. Zhang, *Biochem. J.*, 2013, **455**, 1-14.
- 18 A. A. Ibarra, G. J. Bartlett, Z. Hegedüs, S. Dutt, F. Hobor, K. A. Horner, K. Hetherington, K. Spence, A. Nelson, T. A. Edwards, D. N. Woolfson, R. B. Sessions and A. J. Wilson, *ACS Chem. Biol.*, 2019, **14**, 2252-2263.
- 19 C. W. Wood, A. A. Ibarra, G. J. Bartlett, A. J. Wilson, D. N. Woolfson, R. B. Sessions, *Bioinformatics*, 2020, **36**, 2917-2919.
- 20 S. Celis, F. Hobor, T. James, G. J. Bartlett, A. A. Ibarra, D. K. Shoemark, Z. Hegedüs, K. Hetherington, D. N. Woolfson, R. B. Sessions, T. A. Edwards, D. M. Andrews, A. Nelson and A. J. Wilson, *Chem. Sci.*, 2021, **12**, 4753-4762.
- 21 C. Horien and P. Yuan, *Yale J Bio Med* 2017, **90**, 1-3.
- 22 D. G. Brown and J. Boström, *J. Med. Chem.*, 2018, **61**, 9442-9468.
- 23 L. Ruddigkeit, R. Van Deursen, L. C. Blum and J. L. Reymond, *J. Chem. Inf. Model.*, 2012, **52**, 2864-2875.
- 24 W. H. B. Sauer and M. K. Schwarz, *J. Chem. Inf. Comput. Sci.*, 2003, **43**, 987-1003.
- 25 A. H. Lipkus, Q. Yuan, K. A. Lucas, S. A. Funk, W. F. Bartelt, R. J. Schenck and A. J. Trippe, *J. Org. Chem.*, 2008, **73**, 4443-4451.
- 26 A. H. Lipkus, S. P. Watkins, K. Gengras, M. J. McBride and T. J. Wills, *J. Org. Chem.*, 2019, **84**, 13948-13956.
- 27 N. Miyaura, K. Yamada and A. Suzuki, *Tetrahedron Lett.*, 1979, **20**, 3437-3440.
- 28 W. P. Walters, J. Green, J. R. Weiss and M. A. Murcko, *J. Med. Chem.*, 2011, **54**, 6405-6416.

- 29 F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752–6756.
- 30 D. E. Scott, A. G. Coyne, S. A. Hudson and C. Abell, *Biochemistry*, 2012, **51**, 4990–5003.
- 31 T. Fink and J. L. Raymond, *J. Chem. Inf. Model.*, 2007, **47**, 342–353.
- 32 H. Deng, H. O’Keefe, C. P. Davie, K. E. Lind, R. A. Acharya, G. J. Franklin, J. Larkin, R. Matico, M. Neeb, M. M. Thompson, T. Lohr, J. W. Gross, P. A. Centrella, G. K. O’Donovan, K. L. Bedard, K. Van Vloten, S. Mataruse, S. R. Skinner, S. L. Belyanskaya, T. Y. Carpenter, T. W. Shearer, M. A. Clark, J. W. Cuozzo, C. C. Arico-Muendel and B. A. Morgan, *J. Med. Chem.*, 2012, **55**, 7061–7079.
- 33 J. Tsai, J. T. Lee, W. Wang, J. Zhang, H. Cho, S. Mamo, R. Bremer, S. Gillette, J. Kong, N. K. Haass, K. Sproesser, L. Li, K. S. M. Smalley, D. Fong, Y. L. Zhu, A. Marimuthu, H. Nguyen, B. Lam, J. Liu, I. Cheung, J. Rice, Y. Suzuki, C. Luu, C. Settachatgul, R. Shellooe, J. Cantwell, S. H. Kim, J. Schlessinger, K. Y. J. Zhang, B. L. West, B. Powell, G. Habets, C. Zhang, P. N. Ibrahim, P. Hirth, D. R. Artis, M. Herlyn and G. Bollag, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3041–3046.
- 34 G. Bollag, P. Hirth, J. Tsai, J. Zhang, P. N. Ibrahim, H. Cho, W. Spevak, C. Zhang, Y. Zhang, G. Habets, E. A. Burton, B. Wong, G. Tsang, B. L. West, B. Powell, R. Shellooe, A. Marimuthu, H. Nguyen, K. Y. J. Zhang, D. R. Artis, J. Schlessinger, F. Su, B. Higgins, R. Iyer, K. Dandrea, A. Koehler, M. Stumm, P. S. Lin, R. J. Lee, J. Grippo, I. Puzanov, K. B. Kim, A. Ribas, G. A. McArthur, J. A. Sosman, P. B. Chapman, K. T. Flaherty, X. Xu, K. L. Nathanson and K. Nolop, *Nature*, 2010, **467**, 596–599.
- 35 F. Abulwerdi, C. Liao, M. Liu, A. S. Azmi, A. Aboukameel, A. S. A. Mady, T. Gulappa, T. Cierpicki, S. Owens, T. Zhang, D. Sun, J. A. Stuckey, R. M. Mohammad and Z. Nikolovska-Coleska, *Mol. Cancer. Ther.*, 2014, **3**, 565–575.
- ,36 M. Congreve, R. Carr, C. Murray and H. Jhoti, *Drug Discov. Today*, 2003, **8**, 876–877.
- 37 A. L. Hopkins, C. R. Groom and A. Alex, *Drug Discov. Today*, 2004, **9**, 430–

- 431.
- 38 M. D. Shultz, *Bioorganic Med. Chem. Lett.*, 2013, **23**, 5980–5991.
- 39 I. D. Kuntz, K. Chen, K. A. Sharp and P. A. Kollman, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 9997–10002.
- 40 T. D. Heightman, V. Berdini, H. Braithwaite, I. M. Buck, M. Cassidy, J. Castro, A. Courtin, J. E. H. Day, C. East, L. Fazal, B. Graham, C. M. Griffiths-Jones, J. F. Lyons, V. Martins, S. Muench, J. M. Munck, D. Norton, M. O'Reilly, N. Palmer, P. Pathuri, M. Reader, D. C. Rees, S. J. Rich, C. Richardson, H. Saini, N. T. Thompson, N. G. Wallis, H. Walton, N. E. Wilsher, A. J. A. Woolford, M. Cooke, D. Cousin, S. Onions, J. Shannon, J. Watts and C. W. Murray, *J. Med. Chem.*, 2018, **61**, 4978–4992.
- 41 R. A. Goodnow, C. E. Dumelin and A. D. Keefe, *Nat. Rev. Drug Discov.*, 2017, **16**, 131–147.
- 42 C. S. Kollmann, X. Bai, C. H. Tsai, H. Yang, K. E. Lind, S. R. Skinner, Z. Zhu, D. I. Israel, J. W. Cuzzo, B. A. Morgan, K. Yuki, C. Xie, T. A. Springer, M. Shimaoka and G. Evindar, *Bioorg. Med. Chem.*, 2014, **22**, 2353–2365.
- 43 G. Karageorgis, S. Warriner and A. Nelson, *Nat. Chem.*, 2014, **6**, 872–876.
- 44 A. Leggott, J. E. Clarke, S. Chow, S. L. Warriner, A. J. O'Neill and A. Nelson, *Chem. Commun.*, 2020, **56**, 8047.
- 45 A. Green, F. Hobor, C. Tinworth, S. Warriner, A. Wilson and A. Nelson, *Eur. J. Chem.*, 2020, **26**, 10682–10689.
- 46 D. Francis, S. Farooque, A. Meager, D. Derks, A. Leggott, S. Warriner, A. J. O'Neill and A. Nelson, *Org. Biomol. Chem.*, 2022, **20**, 9672–9678.
- 47 R. Beroukhim, C. H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J. S. Boehm, J. Dobson, M. Urashima, K. T. McHenry, R. M. Pinchback, A. H. Ligon, Y. J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M. S. Lawrence, B. A. Weir, K. E. Tanaka, D. Y. Chiang, A. J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F. J. Kaye, H. Sasaki, J. E. Tepper, J. A. Fletcher, J. Taberero, J. Baselga, M. S. Tsao, F. Demichelis, M. A. Rubin, P. A. Janne, M. J. Daly, C.

- Nucera, R. L. Levine, B. L. Ebert, S. Gabriele, A. K. Rustgi, C. R. Antonescu, M. Ladanyi, A. Letai, L. A. Garraway, M. Loda, D. G. Beer, L. D. True, A. Okamoto, S. L. Pomeroy, S. Singer, T. R. Golub, E. S. Lander, G. Getz, W. R. Sellers and M. Meyerson, *Nature*, 2010, **463**, 899–905.
- 48 A. Shamas-Din, J. Kale, B. Leber and D. W. Andrews, *Cold Spring Harb. Perspect. Biol.*, 2013, **5**.
- 49 R. M. Perciavalle and J. T. Opferman, *Trends Cell Biol.*, 2013, **23**, 22–29.
- 50 V. Peperzak, I. Vikström, J. Walker, S. P. Glaser, M. Lepage, C. M. Coquery, L. D. Erickson, K. Fairfax, F. MacKay, A. Strasser, S. L. Nutt and D. M. Tarlinton, *Nat. Immunol.*, 2013, **14**, 290–297.
- 51 M. Anand, R. B. Sunoj, F. Anson, B. M. S. Hw, F. Anson, B. M. S. Hw, C. B-factors, C. Refinement, M. Chen, S. Choe, L. Jolla, S. Galdiero, M. Galdiero, C. Pedone, W. F. D. E. Grado, H. Gratkowski, J. D. Lear, A. V Grizel, G. S. Glukhov, O. S. Sokolova, M. E. O. Leary, J. C. Hancox, M. Leunissen, S. W. Muchmore, M. Sattler, H. Liang, R. P. Meadows, J. Harlan, H. S. Yoon, D. Nettesheim, B. S. Chang, C. B. Thompson, S.-L. Wong, S.-C. Ng, S. W. Fesik, P. S. Performance, S. Sack, J. Mu, A. Marx, M. Thorma, E. Mandelkow, S. T. Brady, A. Shamas-Din, H. Brahmhatt, B. Leber, D. W. Andrews, P. M. Vadhadiya, C. V Ramana, R. D. Vale, R. A. Milligan, S. N. Willis, J. I. Fletcher, T. Kaufmann, M. F. van Delft, L. Chen, P. E. Czabotar, H. Ierino, E. F. Lee, W. D. Fairlie, P. Bouillet, A. Strasser, R. M. Kluck, J. M. Adams, D. C. S. Huang, A. Wlodawer, W. Minor, Z. Dauter, M. Jaskolski and G. Yellen, *Biochim. Biophys. Acta - Mol. Cell Res.*, 2016, **1813**, 335–341.
- 52 J. Haneef, P. M, R. S. K. Thankayyan, H. Sithul and S. Sreeharshan, *PLoS One*, 2012, **7**.
- 53 K. McArthur, L. W. Whitehead, J. M. Heddleston, L. Li, B. S. Padman, V. Oorschot, N. D. Geoghegan, S. Chappaz, S. Davidson, H. S. Chin, R. M. Lane, M. Dramicanin, T. L. Saunders, C. Sugiana, R. Lessene, L. D. Osellame, T. L. Chew, G. Dewson, M. Lazarou, G. Ramm, G. Lessene, M. T. Ryan, K. L. Rogers, M. F. Van Delft and B. T. Kile, *Science*, 2018, **359**.
- 54 B. A. Quinn, R. Dash, B. Azab, S. Sarkar, S. K. Das, S. Kumar, R. A.

- Oyesanya, S. Dasgupta, P. Dent, S. Grant, M. Rahmani, D. T. Curiel, I. Dmitriev, M. Hedvat, J. Wei, B. Wu, J. L. Stebbins, J. C. Reed, M. Pellecchia, D. Sarkar and P. B. Fisher, *Expert Opin. Investig. Drugs*, 2011, **20**, 1397–1411.
- 55 C. Billard, *Leukemia*, 2012, **26**, 2032–2038.
- 56 S. Dutta, S. Gullá, T. S. Chen, E. Fire, R. A. Grant and A. E. Keating, *J. Mol. Biol.*, 2010, **398**, 747–762.
- 57 M. L. Stewart, E. Fire, A. E. Keating and L. D. Walensky, *Nat. Chem. Biol.*, 2010, **6**, 595–601.
- 58 A. W. Hird and A. E. Tron, *Pharmacol. Ther.*, 2019, **198**, 59–67.
- 59 A. Friberg, D. Vigil, B. Zhao, R. N. Daniels, J. P. Burke, P. M. Garcia-Barrantes, D. Camper, B. A. Chauder, T. Lee, E. T. Olejniczak and S. W. Fesik, *J. Med. Chem.*, 2013, **56**, 15–30.
- 60 N. F. Pelz, Z. Bian, B. Zhao, S. Shaw, J. C. Tarr, J. Belmar, C. Gregg, D. M. V. Camper, C. M. Goodwin, A. L. Arnold, J. L. Sensintaffar, A. Friberg, O. W. Rossanese, T. Lee, E. T. Olejniczak and S. W. Fesik, *J. Med. Chem.*, 2016, **59**, 2054–2066.
- 61 J. D. Levenson, H. Zhang, J. Chen, S. K. Tahir, D. C. Phillips, J. Xue, P. Nimmer, S. Jin, M. Smith, Y. Xiao, P. Kovar, A. Tanaka, M. Bruncko, G. S. Sheppard, L. Wang, S. Gierke, L. Kategaya, D. J. Anderson, C. Wong, J. Eastham-Anderson, M. C. Ludlam, D. Sampath, W. J. Fairbrother, I. Wertz, S. H. Rosenberg, C. Tse, S. W. Elmore and A. J. Souers, *Cell Death Dis.*, 2015, **6**, e1590.
- 62 M. Bruncko, L. Wang, G. S. Sheppard, D. C. Phillips, S. K. Tahir, J. Xue, S. Erickson, S. Fidanze, E. Fry, L. Hasvold, G. J. Jenkins, S. Jin, R. A. Judge, P. J. Kovar, D. Madar, P. Nimmer, C. Park, A. M. Petros, S. H. Rosenberg, M. L. Smith, X. Song, C. Sun, Z. F. Tao, X. Wang, Y. Xiao, H. Zhang, C. Tse, J. D. Levenson, S. W. Elmore and A. J. Souers, *J. Med. Chem.*, 2015, **58**, 2180–2194.
- 63 J. Lin, D. Fu, Y. Dai, J. Lin and T. Xu, *Oncotarget*, 2017, **8**, 114457–114462.



- 64 J. Li, X. Hou, J. Bai, Y. Zhou, C. Chen, X. Yang and H. Fang, *Bioorg. Med. Chem.*, 2021, **29**, 115850.
- 65 H. E. Ramsey, M. A. Fischer, T. Lee, A. E. Gorska, M. P. Arrate, L. Fuller, K. L. Boyd, S. A. Strickland, J. Sensintaffar, L. J. Hogdal, G. D. Ayers, E. T. Olejniczak, S. W. Fesik and M. R, *Cancer Discov.*, 2018, **8**, 1566-1581.
- 66 M. C. Denis, J. Sopková S De, O. Santos, R. Bureau and A.S. Voisin-Chiret, *J. Med. Chem.*, 2020, **63**, 928-943.
- 67 B. Shi, A. J. Blake, W. Lewis, I. B. Campbell, B. D. Judkins and C. J. Moody, *J. Org. Chem*, 2010, **75**, 152–161.
- 68 H. Song, J. Yang, W. Chen and Y. Qin, *Org. Lett.*, 2006, **8**, 6011–6014.
- 69 F. Ye, C. Wang, Y. Zhang and J. Wang, *Angew. Chemie Int. Ed.*, 2014, **53**, 11625–11628.
- 70 N. Leygue, M. Enel, A. Diallo, B. Mestre-Voegtlié, C. Galaup and C. Picard, *European J. Org. Chem.*, 2019, **2019**, 2899–2913.
- 71 C. Matheis, T. Krause, V. Bragoni and L. J. Goossen, *Eur. J.*, 2016, **22**, 12270–12273.
- 72 T. Yamamoto, T. Iwasaki, T. Morita and Y. Yoshimi, *J. Org. Chem.*, 2018, **83**, 3702–3709.
- 73 J. T. Anderson, P. L. Toogood and E. N. G. Marsh, *Org. Lett.*, 2002, **4**, 4281–4283.
- 74 G. Karageorgis, E. S. Reckzeh, J. Ceballos, M. Schwalfenberg, S. Sievers, C. Ostermann, A. Pahl, S. Ziegler and H. Waldmann, *Nat. Chem.*, 2018, **10**, 1103–1111.
- 75 M. Choi, H. Jo, D. Kim, J. Yun, J. S. Kang, Y. Kim, J. K. Jung, J. T. Hong, J. Cho, J. H. Kwak and H. Lee, *Arch. Pharm. Res.*, 2016, **39**, 618–630.
- 76 J. R. Lakowicz, *Principles of fluorescence spectroscopy*, 2006.
- 77 Z. Nikolovska-Coleska, R. Wang, X. Fang, H. Pan, Y. Tomita, P. Li, P. P. Roller, K. Krajewski, N. G. Saito, J. A. Stuckey and S. Wang, *Anal. Biochem.*,

- 2004, **332**, 261–273.
- 78 A. DeAngelis, R. Panish and J. M. Fox, *Acc. Chem. Res.*, 2016, **49**, 115.
- 79 A. Rosales, I. Rodríguez-García, C. López-Sánchez, M. Álvarez-Corral and M. Muñoz-Dorado, *Tetrahedron*, 2011, **67**, 3071–3075.
- 80 J. M. Hibberd and A. P. M. Weber, *Curr. Opin. Plant Biol.*, 2012, **15**, 225–227.
- 81 M. Hayashi and M. Nishimura, *Curr. Opin. Plant Biol.*, 2003, **6**, 577–582.
- 82 J. Hu, A. Baker, B. Bartel, N. Linka, R. T. Mullen, S. Reumann and B. K. *Plant. Cell.*, 2012, **24**, 2279–2303.
- 83 M. Schrader, N. A. Bonekamp and M. Islinger, *Biochim. Biophys. Acta - Mol. Basis Dis.*, 2012, **1822**, 1343–1357.
- 84 J. M. Palma, A. Nez, L. M. Sandalio, F. J. Corpas, M. Lundqvist, M. Mez, F. Sevilla and L. A. *Plant. Physiol.*, 1998, **116**, 1195–2000.
- 85 S. J. Gould, . Keller, N. Hosken, J. Wilkinson and S. Subramani. *Plant Cell. Bio.*, 1989, **108**, 1657–1664.
- 86 P. Aubourg and R. Wanders, *Handb. Clin. Neurol.*, 2013, **113**, 1593–1609.
- 87 G. Dodt, N. Braverman, C. Wong, A. Moser, H. W. Moser, P. Watkins, D. Valle and S. J. Gould, *Nat. Genet.* 1995, **9**, 115–125.
- 88 R. S. Sikorski, M. S. Boguski, M. Goebel and P. Hieter, *Cell*, 1990, **60**, 307–317.
- 89 J. R. Lamb, S. Tugendreich and P. Hieter, *Trends Biochem. Sci.*, 1995, **20**, 257–259.
- 90 G. L. Blatch and M. Läsle, *BioEssays*, 1999, **21**, 932–939.
- 91 N. S. Skoulding, G. Chowdhary, M. J. Deus, A. Baker, S. Reumann and S. L. Warriner, *J. Mol. Biol.*, 2015, **427**, 1085–1101.
- 92 G. J. Gatto, B. V. Geisbrecht, S. J. Gould and J. M. Berg, *Nat. Struct. Biol* 2000, **7**, 1091–1095.

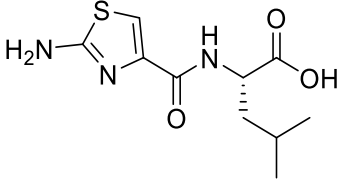
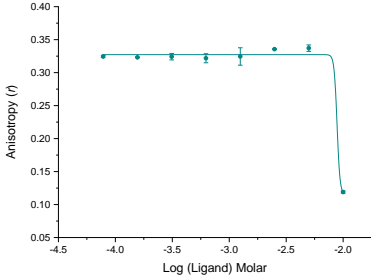
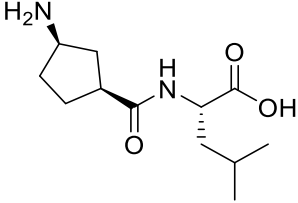
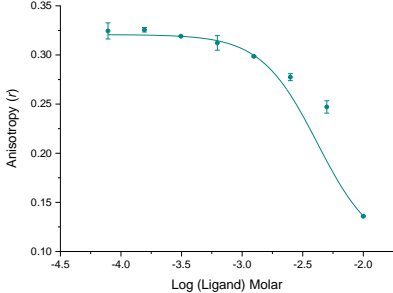
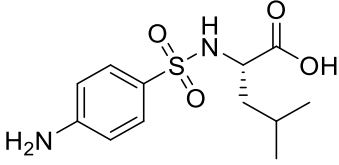
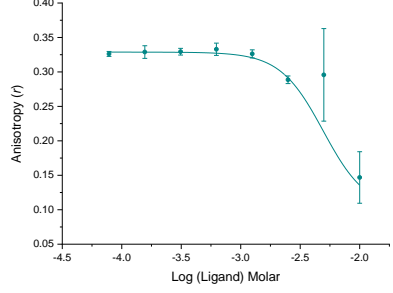
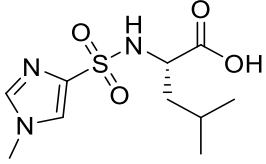
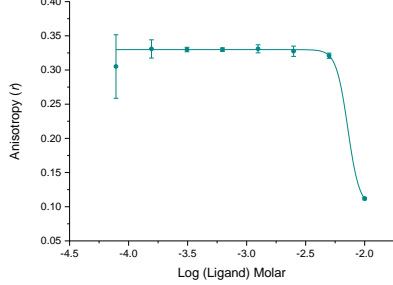
- 93 C. Scheufler, A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F. U. Hartl and I. Moarefi, *Cell*, 2000, **101**, 199–210.
- 94 S. J. Gould, G. A. Keller, N. Hosken, J. Wilkinson and S. Subramani, *J. Cell Biol.*, 1989, **108**, 1657–1664.
- 95 E. L. Maynard, G. J. Gatto and J. M. Berg, *Proteins Struct. Funct. Bioinforma.*, 2004, **55**, 856–861.
- 96 V. Mittendorf, E. J. Robertson, R. M. Leech, N. Krüger, A. Steinbüchel and Y. Poirier, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 13397–13402.
- 97 L. L. Cross, R. Paudyal, Y. Kamisugi, A. Berry, A. C. Cuming, A. Baker and S. L. Warriner, *Nat. Commun* 2017, **8**, 1–9.
- 98 A. F. Carvalho, J. Costa-Rodrigues, I. Correia, J. Costa Pessoa, T. Q. Faria, C. L. Martins, M. Fransen, C. Sá-Miranda and J. E. Azevedo, *J. Mol. Biol.*, 2006, **356**, 864–875.
- 99 M. Dawidowski, L. Emmanouilidis, V. C. Kalel, K. Tripsianes, K. Schorpp, K. Hadian, M. Kaiser, P. Mäser, M. Kolonko, S. Tanghe, A. Rodriguez, W. Schliebs, R. Erdmann, M. Sattler and G. M. Popowicz, *Science*, 2017, **355**, 1416–1420.
- 100 V. Napolitano, P. Mróz, M. Marciniak, V. C. Kalel, C. A. Softley, J. D. Janna Olmos, B. G. Tippler, K. Schorpp, S. Rioton, T. Fröhlich, O. Plettenburg, K. Hadian, R. Erdmann, M. Sattler, G. M. Popowicz, M. Dawidowski and G. Dubin, *Eur. J. Med. Chem.*, 2022, **243**, 114778.
- 101 V. Napolitano, C. A. Softley, A. Blat, V. C. Kalel, K. Schorpp, K. Hadian, R. Erdmann, M. Sattler, G. M. Popowicz and G. Dubin, *Sci.*, **12**, 14705.
- 102 B. Ye and N. Cramer, *Angew. Chem., Int. Ed. Engl.*, 2014, **53**, 7896–7899.
- 103 T. Hashimoto, Y. Naganawa and K. Maruoka, *ChemComm*, 2007, **48**, 5143–5145.
- 104 F. H. S. Gama, R. O. M. A. De Souza and S. J. Garden, *RSC Adv.*, 2015, **5**, 70915–70928.
- 105 S. Chow, A. I. Green, C. Arter, S. Liver, A. Leggott, L. Trask, G. Karageorgis,

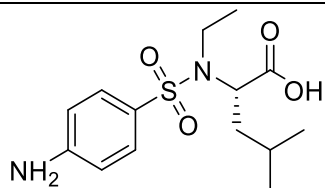
- S. Warriner and A. Nelson, *Synthesis* 2020, **52**, 1695–1706.
- 107 G. L. Hamilton and B. J. Backes, *Tetrahedron Lett.*, 2006, **47**, 2229–2231.
- 108 B. C. Halyk, M. V Butko, O. O. Yanshyna, K. S. Gavrilenko, Te, T. V Druzhenko and P.K Mykhailiuk, *Eur. J. Chem.*, 2017, **23**, 16782-16786.
- 109 A. T. Murray, E. Packard, A. Nortcliffe, W. Lewis, D. Hamza, G. Jones and C. J. Moody, *Eur. J. Org. Chem.*, 2017, **1**, 138-148.
- 110 X. Wang, J. F. Espinosa and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 4821–4822.
- 111 F. Varano, D. Catarzi, V. Colotta, F. R. Calabri, O. Lenzi, G. Filacchioni, A. Galli, C. Costagli and F. Deflorian, *J. Med. Chem.*, 2004, **47**, 262-272.
- 112 T. Hashimoto, Y. Naganawa, T. Kano and K. Maruoka, *Chem Comm*, **2007**, 5143–5145.
- 113 L. Mayer and T. J. J. Müller, *J. Org. Chem.*, 2021, **10**, 3516–3527.
- 114 K. Cheng, X. Wang and H. Yin, *J. Am. Chem. Soc.*, 2011, **133**, 3764-3767.
- 115 L. H. J. Kleijn, F. M. Müskens, S. F. Oppedijk, G. De Bruin and N. I. Martin, *Tetrahedron Lett.*, 2012, **53**, 6430-6432.
- 116 Lin, H. Lam, W. Han, N. Cotroneo, B. A. Pandya and X. Li, *ACS Med. Chem. Lett.*, 2020, **11**, 1442-1449.
- 117 M. Behforouz, W. Cai, M. G. Stocksdale, J. S. Lucas, J. Y. Jung, D. Briere, A. Wang, K. S. Katen and N. C. Behforouz, *J. Med. Chem.*, 2003, **46**, 5773–5780.
- 118 J. Ludwig and M. Lehr, *Synth. Commun.*, 2004, **34**, 3691–3695.
- 119 P. Chevallet, P. Garrouste, B. Malawska, J. Martinez, *Tetrahedron Lett.*, 1993, **34**, 7409-7412.
- 120 E. C. Lee and G. C. Fu, *J. Am. Chem. Soc.*, 2007, **129**, 12066–12067.
- 121 T. Hashimoto, Y. Naganawa and K. Maruoka, *J. Am. Chem. Soc.*, 2011, **133**, 8834–8837.

- 122 H. E. Zimmerman and R. A. Bunce, *J. Org. Chem*, 1982, **47**, 3377–3396.
- 123 T. D. Ashton, K. M. Aumann, S. P. Baker, C. H. Schiesser and P. J. Scammells, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6779–6784.
- 124 J. J. Hale, R. J. Budhu, S. G. Mills, M. Maccoss, L. Malkowitz, S. Siciliano, S. L. Gould, J. A. Demartino and M. S. Springer, *Biorg. Med. Chem. Lett.*, 2001, **11**, 1437-1440.
- 125 V. I. Savych, V. L. Mykhalchuk, P. V Melnychuk, A. O. Isakov, T. Savchuk, V. M. Timoshenko, S. A. Siry, S. O. Pavlenko, D. V Kovalenko, O. V Hryshchuk, V. A. Reznik, B. A. Chalyk, V. S. Yarmolchuk, E. B. Rusanov and P. K. Mykhailiuk, *Chem*, 2021, **86**, 13289–13309.

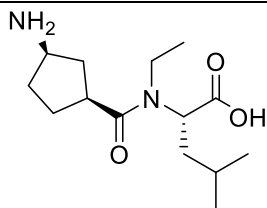
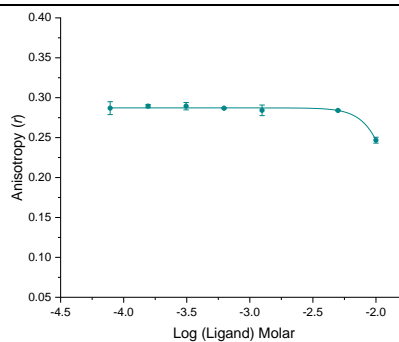
## 7 Appendix

### 7.1 Dose-Response for PEX5 Inhibitors

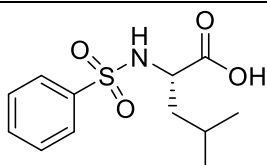
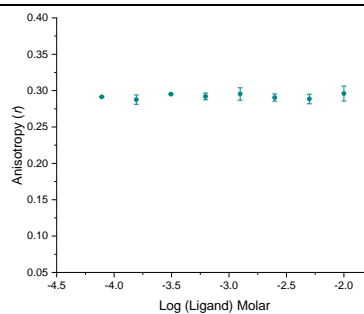
Compound	Dose-response curves
 <p><b>118</b>, <math>IC_{50} \sim 9</math> mM</p>	
 <p><b>119</b>, <math>IC_{50} 4.2 \pm 0.2</math> mM</p>	
 <p><b>121</b>, <math>IC_{50} 4.9 \pm 1.1</math> mM</p>	
 <p><b>125</b>, <math>IC_{50} \sim 7.2</math> mM</p>	



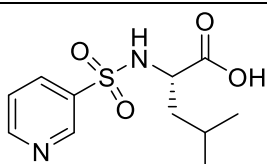
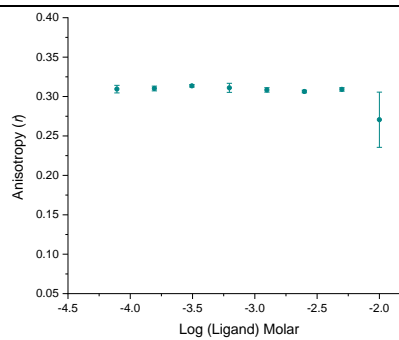
**146**,  $IC_{50} > 15$  mM



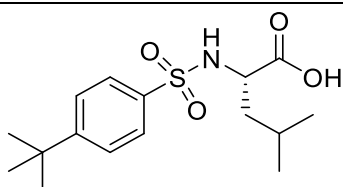
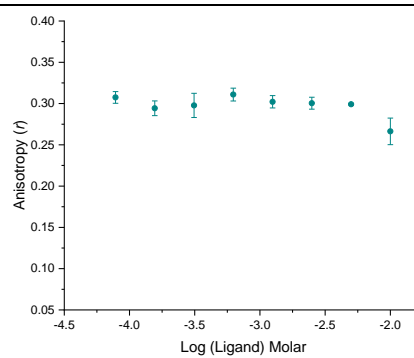
**148**,  $IC_{50} > 15$  mM



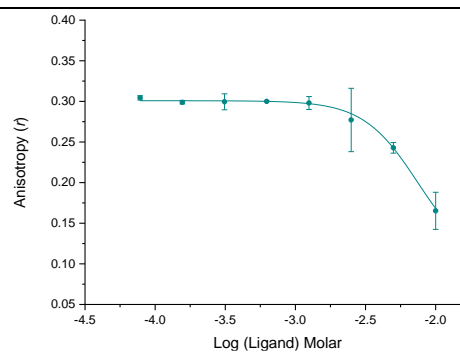
**149**,  $IC_{50} > 15$  mM

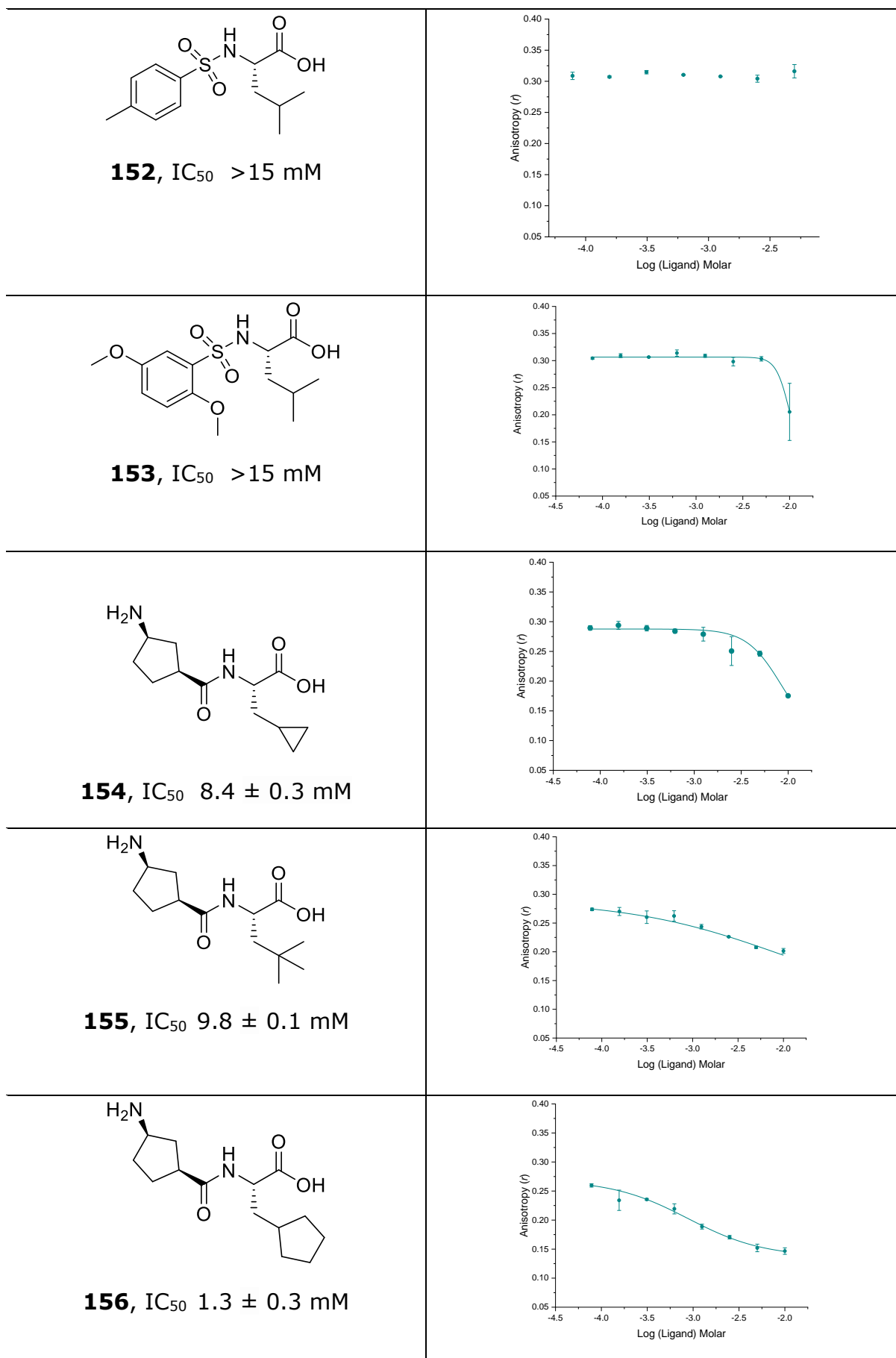


**150**,  $IC_{50} > 15$  mM

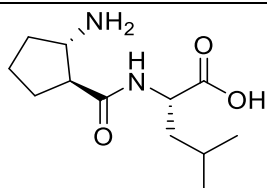
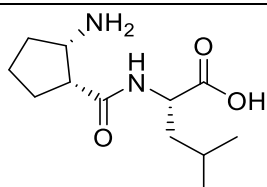
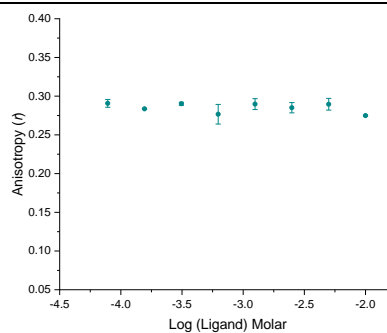
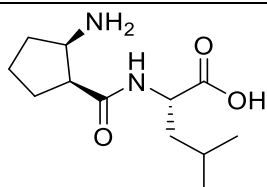
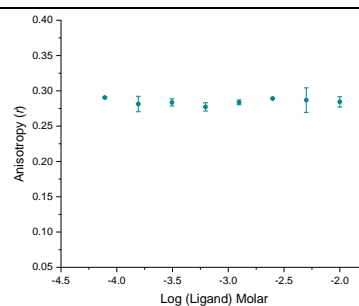
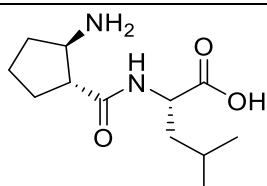
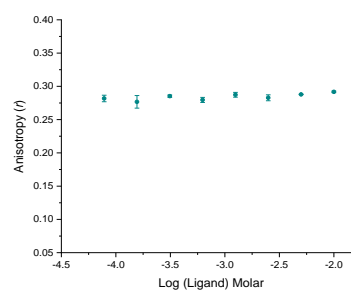
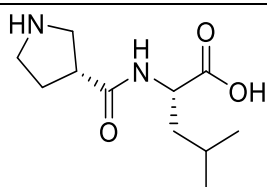
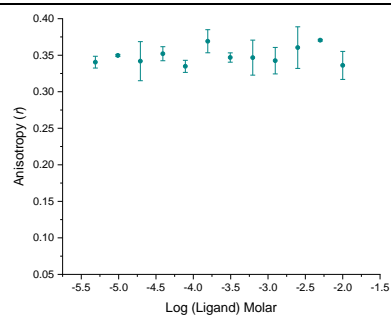
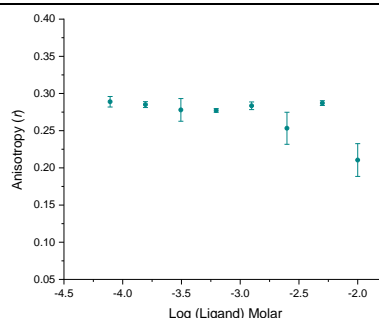


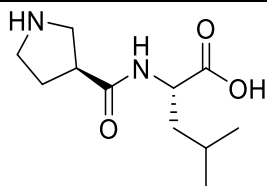
**151**,  $IC_{50} 7.4 \pm 0.6$



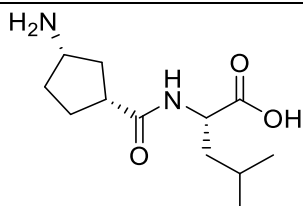
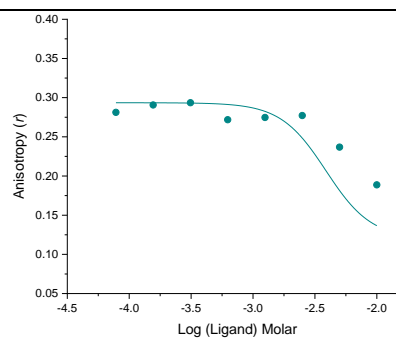




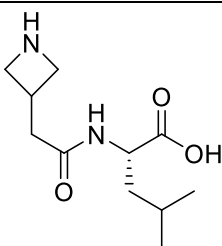
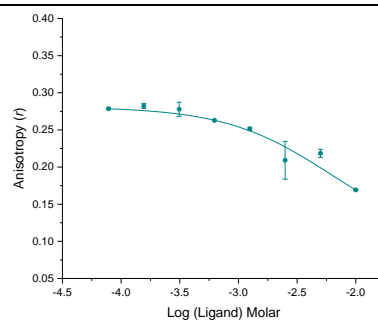
**157**,  $IC_{50} > 15$  mM**158**,  $IC_{50} > 15$  mM**159**,  $IC_{50} > 15$  mM**160**,  $IC_{50} > 15$  mM**161**,  $IC_{50} > 15$  mM



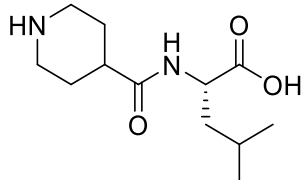
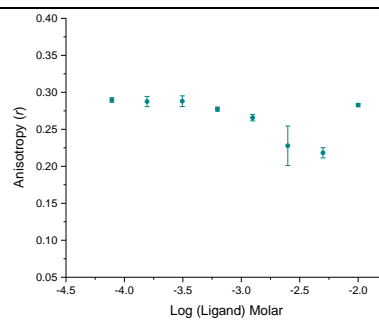
**162**,  $IC_{50} \sim 1.6$  mM



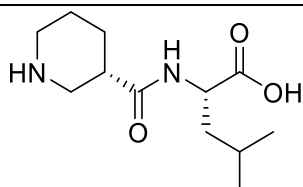
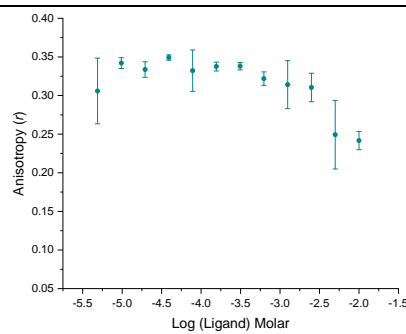
**163**,  $IC_{50} = 6.1 \pm 0.3$  mM



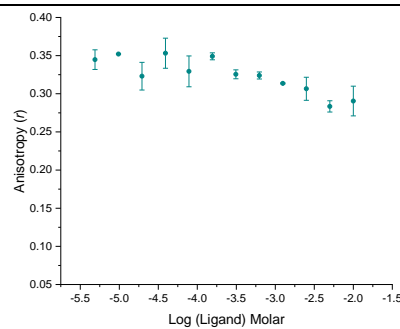
**164**,  $IC_{50} > 15$  mM

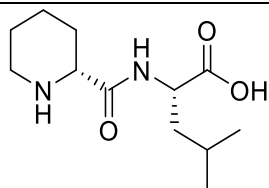
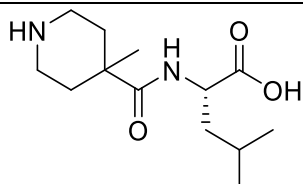
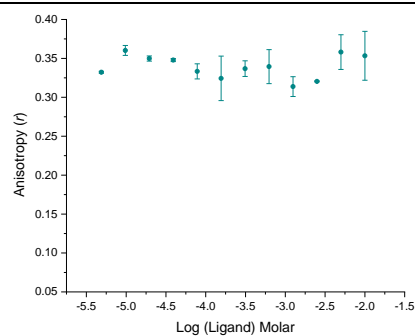
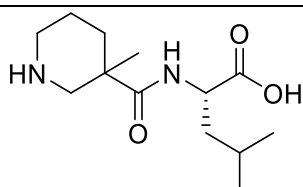
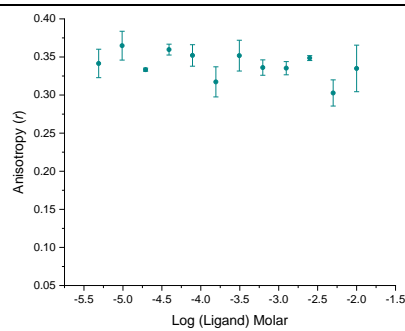
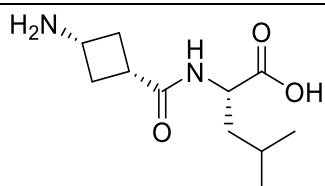
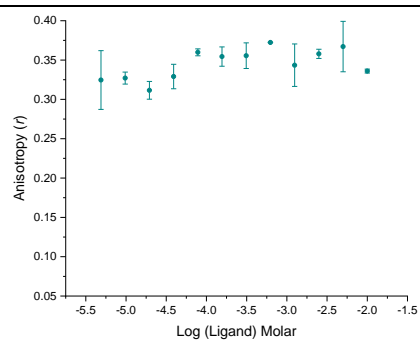
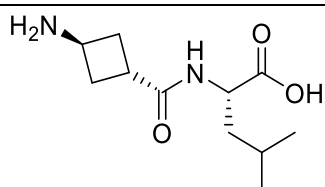
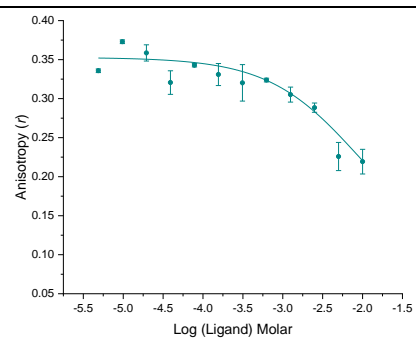
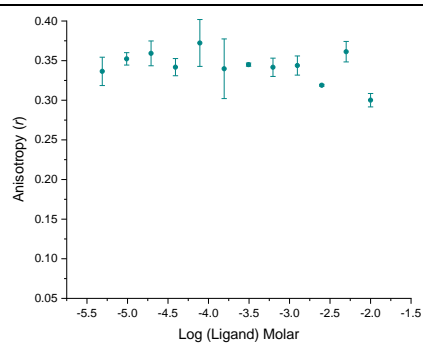


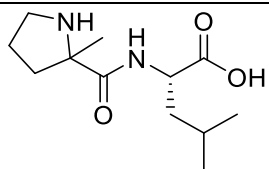
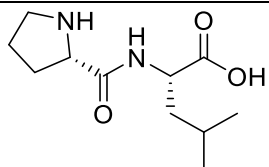
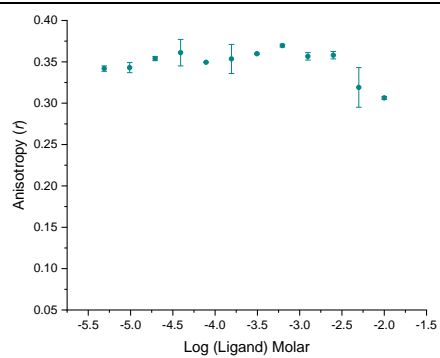
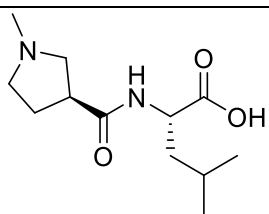
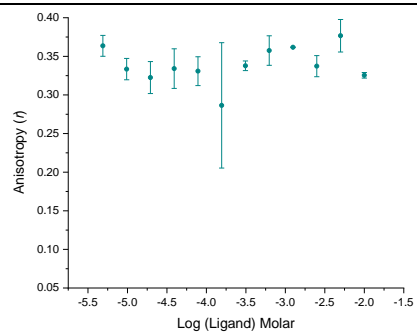
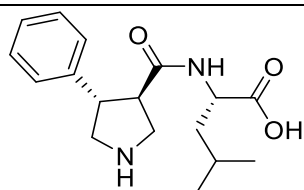
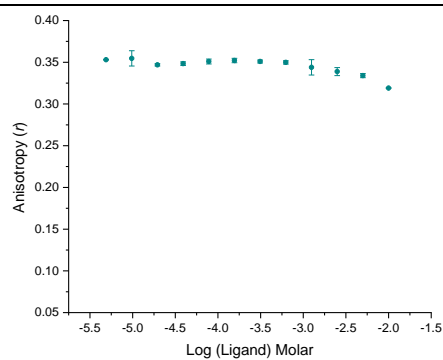
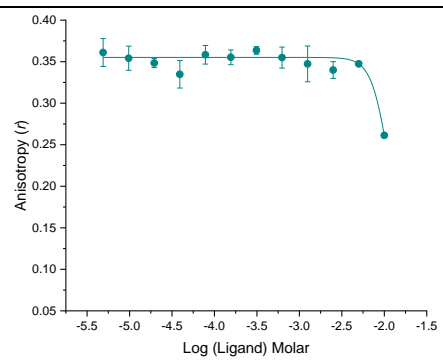
**165**,  $IC_{50} > 10$  mM

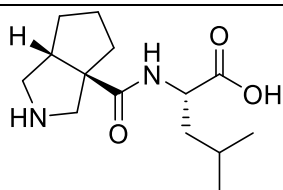
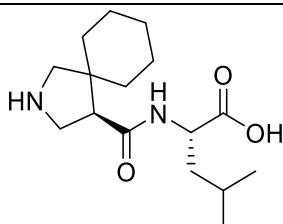
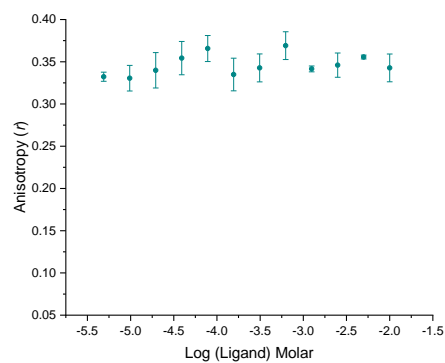
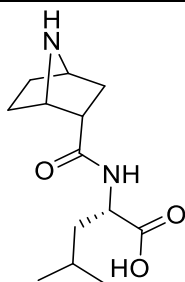
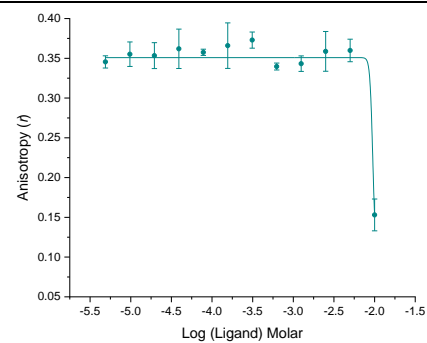
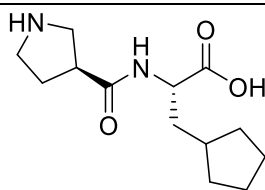
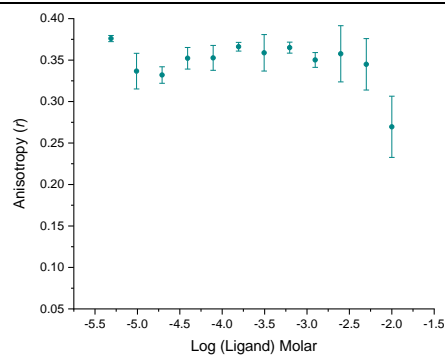
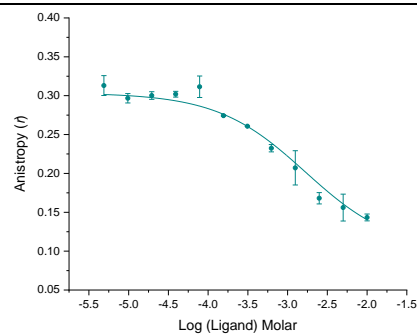


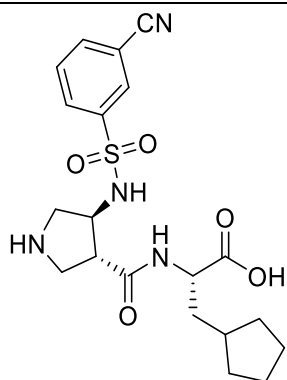
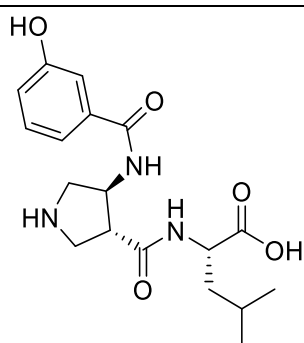
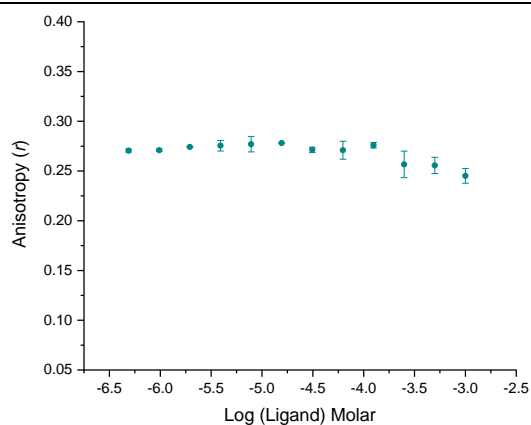
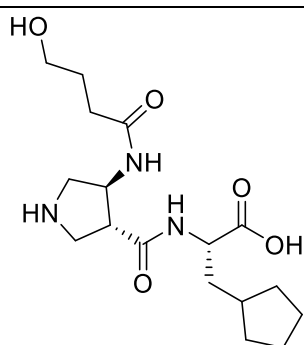
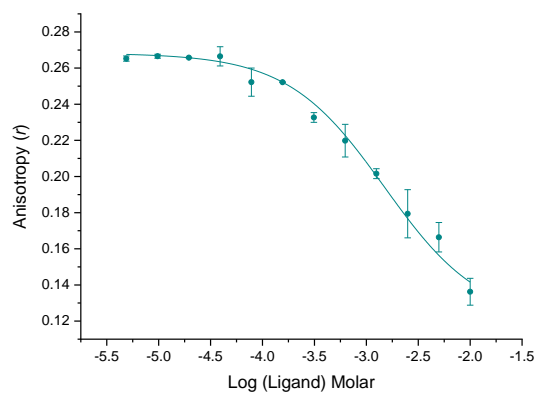
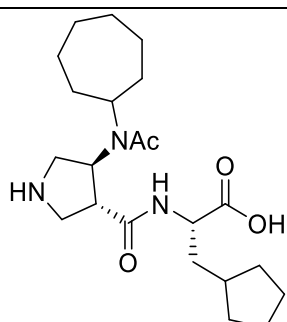
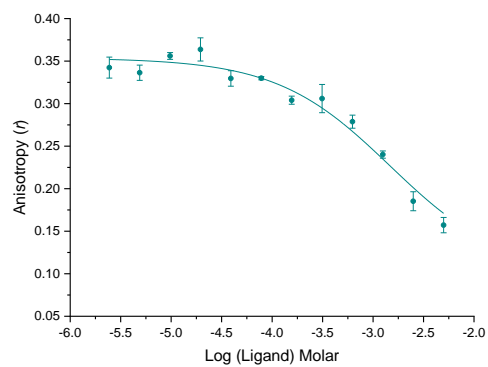
**166**,  $IC_{50} > 15$  mM



**167**,  $IC_{50} > 15$  mM**168**,  $IC_{50} > 15$  mM**169**,  $IC_{50} > 15$  mM**171**,  $IC_{50} \sim 9$  mM**170**,  $IC_{50} > 15$  mM

**172**,  $IC_{50} > 15$  mM**173**,  $IC_{50} > 15$  mM**174**,  $IC_{50} > 15$  mM**178a**,  $IC_{50} = 11 \pm 0.2$  mM

**178b**,  $IC_{50} > 15$  mM**178c**,  $IC_{50} \sim 9$  mM**182**,  $IC_{50} > 15$  mM**184**,  $IC_{50} = 1.5 \pm 0.1$  mM

**185**,  $IC_{50} > 15$  mM**186**,  $IC_{50} = 1.8 \pm 0.2$  mM**187**,  $IC_{50} = 1.3 \pm 0.2$  mM**188**,  $IC_{50} = 6.0 \pm 1.9$  mM