The Design and Synthesis of Novel Selective Cannabinoid Receptor Type 2 Ligands

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

by

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School of Chemistry
The University of Sydney
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Declaration

This thesis is a summary of research performed by the author in the School of Chemistry, The University of Sydney, under the supervision of Professor Michael Kassiou between January 2016 and October 2019. This thesis contains no material, which has been accepted for the award of any degree at any university. No other individual’s work has been used without recognition and every effort has been made to acknowledge previously published material and prior art. This thesis contains less than 100,000 words in accordance with The University of Sydney guidelines and with approval from the Associate Dean. Sections of original research described in this thesis have been published in peer-reviewed scientific journals:


Michael Moir

As the supervisor for the candidate upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Professor Michael Kassiou
Acknowledgements

Professor Allyn Howlett (pre-eminent cannabinoid pharmacologist) speculated that the human cannabinoid system evolved to help us endure (and selectively forget) the routine slings and arrows of life “so that we can get up in the morning and do it all over again”

—Excerpt From: Michael Pollan’s “The Botany of Desire”

The pertinence of this statement to the completion of a PhD is obvious. While I am grateful for my endocannabinoid system, I am even more indebted to all the people who have supported and helped me over the past few years.

First and foremost, I would like to acknowledge the guidance of my supervisor Professor Michael Kassiou who first introduced me to the world of chemistry research as a third year undergraduate student and has been a fantastic mentor ever since. You have always been willing to take time out of your very busy schedule to provide advice, offer words of wisdom and listen to, mould and refine my research ideas. Your sense of humour and “life advice” have never failed to put a smile on my face, even when things weren’t going my way in the lab.

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It has been a pleasure to have collaborated with a number of brilliant researchers from fields outside chemistry. Pharmacology is an essential component of medicinal chemistry and without the hard work of Mark Connor and Sam Lane this project would not have been possible. Collaborators from the Sydney Pharmacy School, Professor Dai Hibbs and Felicia Lai, introduced me to a whole host of concepts in the field of molecular modeling and assisted in rationalising sometimes seemingly irrational biological data.

Last but not least, I would like to express my genuine gratitude to all my friends and family, for their endless support and for being there for me at every step of this journey. My parents have always encouraged me to find and follow my passions. Without their guidance, care and the many sacrifices they have made, I would not have been able to pursue a postgraduate degree. To my loving girlfriend Carolyn, thank you for always being by my side, to celebrate achievements and to pick me back up during challenging times. Thank you for your always considerate, empathetic and kindhearted nature. I am looking forward to our next adventure together.
Abstract

This thesis describes the design, synthesis and pharmacological evaluation of cannabinoid type 2 receptor (CB₂R) ligands.

The endocannabinoid system plays an important role in a variety of physiological processes including appetite, pain-sensation, mood and memory. A key player in this system, the CB₂R, is involved in the pathophysiology of numerous diseases. It is believed that in response to injury or damage, activation of CB₂Rs triggers protective mechanisms for the resolution of inflammation and its associated symptoms. It follows that the CB₂R has become a potential therapeutic target to treat inflammatory diseases. A prerequisite for the development of CB₂R-based therapeutics is the avoidance of unwanted CB₁R mediated central effects, namely psychoactivity. Medicinal chemistry efforts by many pharmaceutical companies and academic groups have resulted in the development of compounds that selectively bind to or activate the CB₂R. While many of these ligands have demonstrated promising results in pre-clinical animal models, no CB₂R-based therapeutics are currently used in the clinic. Therefore, there is a grave need to develop novel, more efficacious and safer drugs that target the CB₂R. We devised three approaches to facilitate the development of novel CB₂R-based therapeutics. A common theme of the three approaches is a desire to determine the structural requirements for selective ligands of the CB₂R.

Computer-aided pharmacophore modelling, based on reported potent and selective CB₂R agonists, has highlighted some of the structural features pertinent to activation of the receptor. Using a known pyrazolylidene benzamide agonist as a lead compound we systematically investigated the validity of our preliminary pharmacophore model. A library of heteroaromatic benzonamides was prepared to explore how the heteroaromatic core influences functional activity. It was found that the –ylidene amide functionality of the lead compound is imperative for functional activity. To further investigate this, we prepared a library of analogues with varied linkage moieties between the heteroaromatic core and substituted phenyl group. This study culminated in the discovery of a novel pyrazolotriazine agonist with low nanomolar potency and complete selectivity over the CB₁R.
In recent years, synthetic cannabinoid ingestion has become a popular alternative to recreational cannabis use. Such synthetic cannabinoids are often potent, high efficacy agonists at both the CB₁ and CB₂ receptors. We decided to use these simple scaffolds to design selective CB₂R agonists as potential therapeutic drugs. Movement of the amide substituent of non-selective indole 3-carboxamide synthetic cannabinoids to the 2-position was demonstrated to be a simple and general strategy to abolish CB₁R activity. Likewise, the use of a 7-azaindole scaffold in conjunction with judicious choice of substituents was shown to be a reliable way to design CB₂R selective agonists.

Allosteric modulation of the CB₂R is thought to pose a number of advantages over orthosteric activation, which may atone for some of the shortcomings of CB₂R agonists that have fared poorly in clinical trials. A recent report of the first synthetic allosteric modulator of the CB₂R inspired our efforts to develop novel allosteric modulators. Scaffold hopping from the 2-pyridone core of the lead compound led to a small library of potential modulators. The in vitro evaluation of these compounds is currently in progress. Identification of novel allosteric modulators is required to further characterise the pharmacological profile, structure-activity relationships and allosteric binding site of these promising therapeutic agents.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>μ</td>
<td>Micro</td>
</tr>
<tr>
<td>Δ⁹-THF</td>
<td>Δ⁹-Tetrahydrocannabinol</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Proton nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>¹³C NMR</td>
<td>Carbon nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>¹⁹F NMR</td>
<td>Fluorine nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>ADMET</td>
<td>Absorption, distribution, metabolism, excretion and toxicity</td>
</tr>
<tr>
<td>AEA</td>
<td>Anandamide</td>
</tr>
<tr>
<td>2-AG</td>
<td>2-Arachidonylglycerol</td>
</tr>
<tr>
<td>2-AGE</td>
<td>2-Arachidonyl glyceryl ether</td>
</tr>
<tr>
<td>Akt</td>
<td>Protein kinase B</td>
</tr>
<tr>
<td>APICA</td>
<td>(N-(1\text{-Adamantyl})-1\text{-penty}-1\text{H}-\text{indole-3-carboxamide})</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Ar</td>
<td>Aryl</td>
</tr>
<tr>
<td>ArT20</td>
<td>Immortalised mouse pituitary adenoma cells</td>
</tr>
<tr>
<td>B.C.</td>
<td>Before Christ</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>\textit{tert}-Butyloxy carbonyl group</td>
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<tr>
<td>Bpy</td>
<td>2,2'-Bipyridine</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CAN</td>
<td>Ceric ammonium nitrate</td>
</tr>
<tr>
<td>CB</td>
<td>Cannabinoid</td>
</tr>
<tr>
<td>CB₁R</td>
<td>Cannabinoid type 1 receptor</td>
</tr>
<tr>
<td>CB₂R</td>
<td>Cannabinoid type 2 receptor</td>
</tr>
<tr>
<td>CDI</td>
<td>Carbonyldiimidazole</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Celite®</td>
<td>Diatomaceous earth</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
</tr>
<tr>
<td>cLogP</td>
<td>Calculated partition coefficient</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>Wavenumbers</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COSY</td>
<td>Homonuclear correlation spectroscopy</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DAST</td>
<td>Diethylaminosulfur trifluoride</td>
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<tr>
<td>DBAD</td>
<td>Di-tert-butyl azodicarboxylate</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-Diazabicyclo[5.4.0]undec-7-ene</td>
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<td>DDQ</td>
<td>2,3-Dichloro-5,6-dicyano-1,4-benzoquinone</td>
</tr>
<tr>
<td>Decompo.</td>
<td>Decomposition</td>
</tr>
<tr>
<td>DHP</td>
<td>3,4-Dihydropyran</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N,N-Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess–Martin periodinane</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNC</td>
<td>Did not converge</td>
</tr>
<tr>
<td>DPPA</td>
<td>Diphenylphosphoryl azide</td>
</tr>
<tr>
<td>dppf</td>
<td>1,1'-Bis(diphenylphosphino)ferrocene</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>Half maximal effective concentration</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride</td>
</tr>
<tr>
<td>ECS</td>
<td>Endocannabinoid system</td>
</tr>
<tr>
<td>EI</td>
<td>Electron ionisation</td>
</tr>
<tr>
<td>Eₘₐₓ</td>
<td>Maximum efficacy</td>
</tr>
<tr>
<td>Eq.</td>
<td>Equivalent(s)</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular receptor kinase</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionisation</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>eV</td>
<td>Electron volts</td>
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### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>FAAH</td>
<td>Fatty acid amide hydrolase</td>
</tr>
<tr>
<td>FLIPR</td>
<td>Fluorescent imaging plate reader</td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
</tr>
<tr>
<td>GDP</td>
<td>Guanosine diphosphate</td>
</tr>
<tr>
<td>GIRK</td>
<td>G protein-gated inwardly rectifying potassium channel</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>GRK</td>
<td>G protein-coupled receptor kinase</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s balanced salt solution</td>
</tr>
<tr>
<td>hCB₁R</td>
<td>Human cannabinoid type 1 receptor</td>
</tr>
<tr>
<td>hCB₂R</td>
<td>Human cannabinoid type 2 receptor</td>
</tr>
<tr>
<td>HOBt</td>
<td>Hydroxybenzotriazole</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane or hexamethyldisilazide</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High-resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>iPr</td>
<td>iso-propyl</td>
</tr>
<tr>
<td>J</td>
<td>J-Coupling</td>
</tr>
<tr>
<td>Kᵢ</td>
<td>Inhibitory constant</td>
</tr>
<tr>
<td>L</td>
<td>Litre(s)</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LipE</td>
<td>Lipophilic efficiency</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low-resolution mass spectrometry</td>
</tr>
<tr>
<td>m</td>
<td>meta</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MAGL</td>
<td>Monoacylglycerol lipase</td>
</tr>
<tr>
<td>m-CPBA</td>
<td>meta-Chloroperbenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>mol</td>
<td>Mole(s)</td>
</tr>
<tr>
<td>mp</td>
<td>Melting point</td>
</tr>
<tr>
<td>Ms</td>
<td>Methanesulfonyl</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>MAP</td>
<td>Mitogen-activated protein</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>MM-GBSA</td>
<td>Molecular mechanics/generalized Born surface area</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>NA</td>
<td>Not Active</td>
</tr>
<tr>
<td>NADA</td>
<td>N-Arachidonoyl dopamine</td>
</tr>
<tr>
<td>NAM</td>
<td>Negative allosteric modulator</td>
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<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
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<tr>
<td>ND</td>
<td>Not determined</td>
</tr>
<tr>
<td>NIS</td>
<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>NPS</td>
<td>Novel psychoactive substance</td>
</tr>
<tr>
<td>°</td>
<td>Degree</td>
</tr>
<tr>
<td>o</td>
<td>ortho</td>
</tr>
<tr>
<td>O-AEA</td>
<td>O-Arachidonoyl ethanolamine</td>
</tr>
<tr>
<td>p</td>
<td>para</td>
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<tr>
<td>PAM</td>
<td>Positive allosteric modulator</td>
</tr>
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<td>PCC</td>
<td>Pyridinium chlorochromate</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>Phenyl</td>
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<td>pin</td>
<td>Pinacol</td>
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<td>Protein kinase A</td>
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<td>ppm</td>
<td>Parts per million</td>
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<tr>
<td>PMB</td>
<td>para-Methoxy benzyl</td>
</tr>
<tr>
<td>PSA</td>
<td>Polar surface area</td>
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</table>
Abbreviations

PyBOP® = Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
q = Quartet
QPlogBB = Predicted brain/blood Partition coefficient
QPPCaco = Predicted apparent Caco-2 permeability
QPPMDCK = Predicted apparent MDCK cell permeability
quat. = Quaternary
quin. = Quintet
R = Unspecified functional group
$R_f$ = Retention factor
rt = Room temperature
s = Singlet
SAM = Silent allosteric modulator
SAR = Structure activity relationship
sat. = Saturated
SC = Synthetic cannabinoid
SD = Standard deviation
SEM = Standard error of the mean
sept = Septet
$S_N\text{Ar}$ = Nucleophilic aromatic substitution
t = Triplet
TBAB = Tetrabutylammonium bromide
TBAI = Tetrabutylammonium iodide
TBAF = Tetrabutylammonium fluoride
TBC = trans-β-Caryophyllene
Tf = Trifluoromethanesulfonyl
TM = Transmembrane
TMEDA = Tetramethylethylenediamine
$tBu$ = $tert$-Butyl
TFA = Trifluoroacetic acid
THF = Tetrahydrofuran
TLC = Thin layer chromatography
TMS = Trimethylsilyl group
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP</td>
<td>Transient receptor potential</td>
</tr>
<tr>
<td>Ts</td>
<td>Tosyl</td>
</tr>
<tr>
<td>Xantphos</td>
<td>4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene</td>
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</table>
Chapter 1: Introduction
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1.1 The Endocannabinoid System

1.1.1 Overview and Historical Context

_Cannabis sativa_ has long been used by mankind both as a therapeutic and recreational drug, with evidence of its cultivation dating back 12,000 years and reports of its consumption as early as 3000 B.C.\(^1\)\(^2\) Throughout history, different civilizations have exploited the therapeutic potential of cannabis for the treatment of numerous ailments including pain, nausea, fever and infections.\(^3\) Despite its perennial use as a therapeutic and psychoactive agent, the molecular basis for the medicinal actions of the cannabis plant remained enigmatic up until only very recent times.

In 1964 Gaoni and Mechoulam identified (−)-\(\Delta^9\)-6a,10a-trans-tetrahydrocannabinol (\(\Delta^9\)-THC, 1, **Figure 1**) as the principle bioactive component of marijuana.\(^4\) Subsequent research into the pharmacology of the active constituents of the cannabis plant (phytocannabinoids) focused mainly the psychoactive effects these compounds elicited. Animal and human studies identified a number of effects attributable to \(\Delta^9\)-THC, including the ability of cannabis to elevate mood, cause dysphoria, precipitate feelings of anxiety, panic or paranoia, produce a sense of time dilation, change auditory and visual perception, impair memory and induce drowsiness.\(^5\) This early research however, did little to explain the mechanisms by which these effects were produced.

Prior to the 1980s it was believed that nonspecific interactions with cell membranes produced the well-documented effects elicited by lipophilic cannabinoids. There was however some evidence, namely the significant influence of chemical structure and stereochemistry on pharmacological activity, which pointed to the existence of a receptor for cannabinoid ligands. More concrete evidence soon came from two major findings by the Howlett laboratory. First, it was demonstrated that cannabinoids inhibit adenylyl cyclase by acting through \(G_{a_{i/o}}\) proteins (effect was blocked with \(G_{a_{i/o}}\) binding pertussis toxin),\(^6\) and following this a receptor binding site was identified through a competitive radioligand binding assay using the tritiated cannabinoid CP 55,940 (2) developed by Pfizer.\(^7\) The ability of unlabelled cannabinoids to displace the radioligand and also to
induce G\textsubscript{i/o}-mediated adenylyl cyclase inhibition was found to correlate well with \textit{in vivo} effects, thus confirming the existence of a cannabinoid G protein-coupled receptor (GPCR).

In 1990 a receptor later termed the cannabinoid type 1 receptor (CB\textsubscript{1}R) was cloned from rat brain\textsuperscript{8} and shortly after the human CB\textsubscript{1} receptor was also cloned.\textsuperscript{9} Through sequence homology studies another cannabinoid receptor, expressed peripherally, known as the cannabinoid type 2 receptor (CB\textsubscript{2}R), was cloned by Munro and co-workers in 1993.\textsuperscript{10} While only the CB\textsubscript{1}R and the CB\textsubscript{2}R are widely acknowledged as cannabinoid receptors, there are a number of other receptors, including GPCRs and ion channel receptors, that have been shown to interact with cannabinoid ligands.\textsuperscript{11}

Once the cannabinoid receptors were discovered, the search for their endogenous ligands began. Devane, working in Mechoulam’s laboratory, discovered a lipophilic molecule derived from arachidonic acid, named anandamide (\textit{N}-arachidonoylethanolamide, 3), that bound to the CB\textsubscript{1}R and acted as a partial agonist.\textsuperscript{12} This was followed by reports of other fatty acid derivatives that behave as endogenous cannabinoids (endocannabinoids), the most studied of which is 2-arachidonoyl glycerol (2-AG, 4). Others include noladin ether (2-arachidonyl glyceryl ether, 2-AGE, 5), virodhamine (\textit{O}-arachidonoyl ethanolamine, O-AEA, 6) and \textit{N}-arachidonoyldopamine (NADA, 7).\textsuperscript{13} Endocannabinoids have been shown to be synthesised on demand and following their release are removed from their sites of action by cellular uptake processes.\textsuperscript{14} They are then metabolised intracellularly: anandamide by fatty acid amide hydrolase (FAAH)\textsuperscript{15} and 2-arachidonoyl glycerol mainly by monoacylglycerol lipase.\textsuperscript{16}

The endocannabinoid system (ECS) comprises endogenous lipid-based ligands, GPCRs that are engaged by cannabinoid ligands and enzymes that synthesise and degrade endocannabinoids. The ECS is thought to modulate diverse physiological processes including motor function, memory, motivation, pain and emotion.\textsuperscript{17} A greater understanding of the physiological and pathological events that trigger the release of endocannabinoids, their cellular uptake and metabolism as well as the roles that endocannabinoids and their receptors play in health and disease has meant that the ECS has become an attractive therapeutic target.
Figure 1: Key compounds that led to the discovery of the endocannabinoid system: Δ⁹-THC (1), CP 55,940 (2) and the known endocannabinoids: anandamide (3), 2-AG (4), 2-AGE (5), O-AEA (6) and NADA (7).

1.1.2 Cannabinoid Receptors

1.1.2.1 G Protein-Coupled Receptors

The effects produced by endogenous, plant-based and synthetic cannabinoids are mediated by two receptors, CB₁ and CB₂. In addition, two other orphan receptors, GPR18 and GPR55, engage endocannabinoids and have emerged as possible members of the endocannabinoid family.¹⁸ The cannabinoid receptors belong to a large superfamily of membrane-bound receptors known as G protein-coupled receptors (CPCRs) that function to detect a wide range of signalling molecules such as hormones, neurotransmitters and odour compounds outside the cell, and activate internal signal transduction leading to cellular responses. GPCRs represent the largest family of cell surface receptors with around 800 different human GPCRs known.¹⁹ Structurally and functionally diverse GPCRs are associated with a broad range of physiological processes, and conversely are implicated in the pathophysiology of various diseases.²⁰ On account of their extensive involvement in human disease states, GPCRs are the most intensively studied drug targets with around a
third of clinically approved drugs having their effects mediated by modulating GPCR signalling pathways.\textsuperscript{21}

Despite their varied functions, GPCRs all share a common structure consisting of a single polypeptide folded into seven transmembrane-spanning helices connected by alternating extracellular and intracellular loops. Within the GPCR superfamily there are five classes, of which Class A (the Rhodopsin-like family) is the largest. Class A receptors are comprised of four groups (α, β, δ and λ), which are further divided into subfamilies (for example cannabinoid receptors) and then individual subtypes (for example cannabinoid type 1 and type 2 receptors).\textsuperscript{22}

Upon binding an external signalling molecule, a GPCR undergoes a conformational change, which triggers an interaction between the receptor and a G protein (Figure 2). The G protein complex (made up of alpha (α), beta (β), and gamma (γ) subunits) associated with the active state receptor, catalyses the release of guanosine diphosphate (GDP) bound to the Gα subunit. Following this, binding of guanosine triphosphate (GTP) to the Gα subunit results in dissociation of both the G protein from the receptor and the Gα subunit from the Gβγ subunit.\textsuperscript{23} The dissociated subunits are released into the cell where they can continue the signalling cascade by interacting with effector molecules such as adenylyl cyclase. When the ligand dissociates from the receptor, a conformational change occurs, reverting the receptor back to its inactive state. There are multiple isoforms of Gα proteins, and a single GPCR can elicit diverse biological responses by interacting with different G proteins.\textsuperscript{24}
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1.1.2.2 The CB₁ Receptor

In humans the CB₁R is encoded by the gene *CNR1* and consists of 472 amino acids. In recent years there have been three reports of the crystal structure of the CB₁R. The first is with the antagonist AM6538 (8) bound, another with inverse agonist tarrababant bound and in 2017 the structure of the active state receptor bound to agonists AM11542 (9) or AM841 was solved (Figure 3). Due to its high expression in the central nervous system (CNS), it was initially thought that the CB₁R was only localised in the brain, however the receptor has been found in many tissues and organs throughout the body. Within the brain, CB₁Rs are mainly found on axons and nerve terminals, but are also thought to be expressed in lower density on somata and glia.
In addition to inhibiting adenyl cyclase and activating mitogen-activated protein (MAP) kinase, CB₁Rs also inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels, playing a key role in the suppression of neuronal excitability and neurotransmitter release. Further signalling mechanisms involve focal adhesion kinase, phosphatidylinositol-3-kinase, sphingomyelinase and nitric oxide synthase.

Activation of the CB₁R leads to both central and peripheral effects. CB₁R-mediated regulatory mechanisms in the central nervous, cardiovascular, gastrointestinal and reproductive systems are of paramount importance in human physiology and pathophysiology. The CB₁R is thought to be involved in the maintenance of homeostasis in health and disease. CB₁R antagonists have been developed as anti-obesity drugs, while agonists have been shown to exert analgesic effects, stimulate appetite and decrease nausea. Despite some clinical progress, centrally-mediated psychoactive side effects associated with activation of the CB₁R have hampered CB₁R-based drug discovery efforts.

**Figure 3:** Side view of the inactive state CB₁R crystal structure with antagonist AM6538 (8) bound (blue), overlaid with the active state structure with agonist AM11542 (9) bound (orange) (Reprinted with permission from Hua, T.; et al. *Nature* 2017, 547, 468. Copyright (2019) Nature Publishing).
1.1.2.3 The CB₂ Receptor

Structure and Expression

In humans the CB₂R is encoded by the gene CNR2 and consists of 360 amino acids, sharing 44% sequence homology with the CB₁R. When only the transmembrane regions are considered, the similarity increases to 68%.³⁷ In comparison to the CB₁R, the CB₂R is poorly conserved across species.³⁸ For instance, the human CB₂R differs from its murine counterpart by 60 amino acids (82% sequence homology, compared to 97% homology for the CB₁R).³⁹ Very recently the crystal structure of the CB₂R with antagonist AM10257 (10) bound was solved (Figure 4).⁴⁰

![Figure 4: Side view of the active state CB₂R crystal structure with antagonist AM10257 (10) bound (Reprinted with permission from Li, X.; et al. Cell 2019, 176 (3), 459-467. Copyright (2019) Elsevier Inc.).](image)

Despite structural and functional similarities, the two cannabinoid receptor subtypes differ quite markedly in distribution throughout the body. At the time of its discovery, it was thought that CB₂Rs were not expressed in the CNS.¹⁰ Further research has found that CB₂Rs are primarily found in the periphery on immune cells, but are also found centrally on microglia and neurons.⁴¹-⁴² CB₂Rs are expressed in almost all human peripheral blood immune cells. They are found in highest levels in B lymphocytes followed by natural killer cells, monocytes, neutrophils, T8 lymphocytes and T4 lymphocytes.⁴³ It follows that CB₂Rs are highly expressed in organs of the immune system such as tonsils, spleen, lymph nodes and thymus.⁴⁴ Studies to determine CB₂R expression throughout the body have been...
complicated by the low expression levels of CB$_2$R in healthy individuals, as well as the
difficulties associated with efforts to detect the receptor using immunocytochemical
techniques.$^{45}$ Despite this, systematic examination has found CB$_2$R expression in specific
regions of the brain,$^{46-47}$ dorsal root ganglia,$^{48}$ gastrointestinal tract,$^{49}$ liver,$^{50}$ bone,$^{51}$
reproductive system,$^{52}$ pancreas,$^{53}$ muscle$^{54}$ and cardiovascular system$^{55}$ (Figure 5).

**Figure 5:** Distribution of the CB$_2$ receptor throughout the human body. (Adapted from

**Signalling Pathways**

Analogous to all GPCRs, binding of an agonist inside the crevice formed by the
transmembrane helical bundle induces a conformational change of the CB$_2$R that uncovers
previously masked G protein binding sites, leading to G protein coupling.$^{11,56}$ The
signalling pathways of the CB$_2$R are dependent on cell and tissue type, the specific G
protein involved and the kinases present in the cell.$^{57}$ Figure 6 outlines the various CB$_2$R
signalling pathways involved in modulation of adenyl cyclase, cyclic adenosine
monophosphate (cAMP), several mitogen-activated protein (MAP) kinases, ion channels,
phospholipase C and small G proteins (such as Rho, Rac, and cdc42).$^{58}$
1.2 Therapeutic Potential of Targeting the CB2R

1.2.1 Role of the CB2R in Pathophysiology

The discovery of selective CB2R antagonists such as SR144528,66 and CB2R gene-deficient mice,67 has facilitated several studies that have resulted in a greater understanding of the role of the CB2R in health and disease. While the CB2R is expressed throughout the body, it is thought that it plays a very minimal role in normal human physiology.68 This is supported by the absence of obvious pathological indications in normal CB2R knockout mice (only subtle changes in immune system function are observed).69
The CB2R however is thought to play an important role in pathophysiology. Changes in endocannabinoid levels and or CB2R expression have been reported in almost all diseases that affect humans.70 It has been proposed that CB2Rs represent a protective or inhibitory system for the resolution of inflammation and its associated symptoms. Receptor expression is highly inducible and under various pathological conditions, CB2Rs are upregulated in affected tissues or cells as part of an inflammatory response. Increased expression of the CB2R and local endocannabinoid levels regulates signalling responses to modulate critical cell functions. Strong data from multiple researchers using diverse preclinical models suggests that modulation of the CB2R to promote protective mechanisms against injury or inflammation is a promising therapeutic strategy for a range of pathologies which include an inflammatory state and where there is immune activation and dysfunction.

It is well accepted that the psychoactive effects of cannabinoids are mediated by the CB1R, while anti-inflammatory and immunomodulatory actions are associated with the CB2R. The undesirable psychotropic effects mediated by the CB1R are a major practical and administrative consideration for the development of cannabinoid-based therapeutics. One approach to mitigate this issue is to develop non-selective peripherally restricted ligands, which would not enter the CNS. However, another issue regarding the development of safe CB1R-based therapeutics is the high expression level of CB1Rs throughout the body in healthy individuals and its involvement in multiple physiological processes. We believe modulation of the CB2R is a more attractive strategy for the treatment of a wide range of conditions without CB1R-mediated psychotropic effects.71-73 Tabrizi and co-workers have comprehensively reviewed the pharmacological role of CB2Rs in various diseases including immune disorders, inflammatory and neuropathic pain, cardiovascular disorders, gastrointestinal diseases, neuroinflammation and neurodegenerative disorders (Figure 7).74 Many of these diseases do not have current viable treatments. Depending on the disease and progression, selective CB2R agonists or inverse agonists/antagonists could be used to modulate CB2R activity.
Figure 7: Functional physiological and pathological role of the CB2R in the human body. Adapted with permission from Tabrizi, M. A.; et al. Chem. Rev. 2016, 116 (2), 519-560. Copyright (2019) American Chemical Society.

1.2.2 Preclinical and Clinical Progress

The two main therapeutic approaches that target the endocannabinoid system either aim to manipulate the activity of cannabinoid receptors or to inhibit the enzymes that degrade endocannabinoids. Questions regarding the safety of the latter approach were raised in 2016 when a number of serious adverse events, including a death, resulted from a phase I study involving FAAH inhibitor BIA 10-2474.75

While numerous CB2R ligands have displayed efficacy in preclinical studies, only a small number of compounds have progressed to human clinical trials and no CB2R ligands are on the market as therapeutic drugs. The vast majority of clinical candidates that target the CB2R have been developed for the treatment of pain. There are numerous reports describing the analgesic and anti-inflammatory effects of CB2R agonists and inverse agonists in various animal models.76-80 It is generally accepted that CB2R activation results in antinociceptive effects, however the exact mechanism by which this occurs is not well understood. It is believed that pain relief is facilitated by the inhibition of pro-inflammatory cytokine release, as well as the prevention of cellular oxidative and nitrosative damage.81
In addition to pain, CB2R ligands have also been developed for the treatment of neuroinflammation and neurodegenerative diseases, as well as autoimmune diseases, osteoporosis and cancers.\textsuperscript{82} The development of CB2R ligands as potential therapeutics is an active area of research for many academic laboratories and pharmaceutical companies. A summary of the compounds that have been progressed into clinical trials by pharmaceutical companies is shown in Table 1.

Table 1: CB2R agonists that have progressed to clinical trials. Progress of the candidates was determined using the ClinicalTrials.gov database.\textsuperscript{83}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Therapeutic Implication</th>
<th>Pharmaceutical Company</th>
<th>Progress</th>
</tr>
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<tbody>
<tr>
<td>PRS-211375</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Chronic pain</td>
<td>Pharmos Corp.</td>
<td>Unknown</td>
</tr>
<tr>
<td>GW842166</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Osteoarthritis, pain</td>
<td>GlaxoSmithKline</td>
<td>Phase 2 completed</td>
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<tr>
<td>GRC10693</td>
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<td>Glenmark Pharmaceuticals</td>
<td>Unknown</td>
</tr>
<tr>
<td>JBT101</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>Diffuse cutaneous systemic sclerosis</td>
<td>JB Therapeutics, Corbus Pharmaceuticals</td>
<td>Phase 3 active</td>
</tr>
<tr>
<td>KHK6188</td>
<td>Not disclosed</td>
<td>Postherpetic neuralgia</td>
<td>Kyowa Hakko Kirin</td>
<td>Completed Phase 2</td>
</tr>
<tr>
<td>ABT521</td>
<td>Not disclosed</td>
<td>Pain</td>
<td>Abbott Laboratories</td>
<td>Unknown</td>
</tr>
<tr>
<td>LY 2828360</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>Knee pain, osteoarthritis</td>
<td>Eli Lilli</td>
<td>Completed Phase 2</td>
</tr>
</tbody>
</table>
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In the cases where the results of the clinical trial have been published, it appears that safe drugs have been developed, but with insufficient efficacy to progress to subsequent stages. Structural modifications of failed candidates to improve efficacy, physicochemical properties, metabolic stability and oral bioavailability will offer more CB2R ligands for clinical testing.

Conventionally, preclinical models are used to evaluate the efficacy of a particular therapeutic agent towards its intended target. Across the board in drug discovery, there is a large disparity between preclinical and clinical success.\textsuperscript{84} In recent reviews by Dhopeshwakar and Mackie as well as Atwood, Straiker and Mackie, the authors postulate the possible reasons for this lack of preclinical to clinical translation for CB2R agonists.\textsuperscript{45, 65} Some of these include: species differences in receptors and signalling pathways, differences between the preclinical model and the clinical condition being treated, lack of receptor subtype selectivity and functional selectivity and issues surrounding drug tolerance.

As previously mentioned, CB2Rs exhibit significant divergence between species. While human and mouse CB2Rs share 82% homology, the amino terminus and carboxy terminus are more divergent, with only 56% and 57% similarity, respectively.\textsuperscript{85} Since these regions are important in many aspects of GPCR regulation, it may be the case that there are significant species differences in CB2R signaling. As the consequences of species
differences are not well understood, it is unreliable to extrapolate the effects of CB₂R modulation from one species to another. This has obvious implications for the validation of preclinical data and may explain the lack of translation from preclinical efficacy in animal models to efficacy in human clinical trials.

Most CB₂R ligands that have progressed to clinical trials are high efficacy agonists. Full agonists globally activate receptors irrespective of the level of endogenous tone. This translates to an enhancement of both constitutively activated CB₂R pathways, as well as constitutively inactive pathways. This can often lead to on-target side effects, that is, undesirable effects mediated by the target receptor. Another complication relates to the low expression of CB₂Rs in healthy individuals. This could explain poor clinical results of older CB₂R agonists, which are not completely selective for the CB₂R and hence could bind to the more abundant CB₁R at high concentrations. Therefore, the putative CB₂R-mediated effects could potentially be confused with CB₁R functional responses.

Functional selectivity, or biased agonism, refers to the ability of an agonist to activate distinct signalling pathways with differing rank order potencies. In the most extreme case, an agonist will maximally activate a particular signalling pathway and fail to activate others. If an agonist stabilises a certain receptor conformation whereby interactions with particular G proteins are favoured, then preferential activation of the corresponding signalling pathway will occur. This has obvious implications in drug design, in theory, allowing one to fine-tune receptor stimulation to elicit a particular therapeutic effect in the absence of undesirable side effects. However, it is currently unknown which particular signalling pathways elicit therapeutic effects and which ones cause undesirable effects for a given disease state. CB₂R ligands have been shown to exhibit significant functional selectivity, whereby different classes of ligands demonstrate functional selectivity over receptor internalisation, inhibition of adenylyl cyclase and arrestin recruitment.

In developing GPCR agonists there are often issues pertaining to tolerance (the requirement to administer more drug to elicit the desired effect after repeated dosage), and dependency (appearance of physical symptoms after ending drug administration). For CB₂R-targeted drug development, this is of particular importance as the vast majority of ligands that have been developed thus far are high-efficacy agonists.
There is limited data pertaining to the consequences of long-term CB2R agonist administration. For cannabinoid receptors, prolonged exposure to an agonist can result in desensitisation or down-regulation of the receptor. After activation of a GPCR by an agonist, signalling is attenuated by G protein-coupled receptor kinase (GRK) mediated phosphorylation, which results in β-arrestin recruitment and internalisation. After internalisation, the receptor may be trafficked to endosomes and dephosphorylated and reinserted into the membrane where it is available for further interactions with an agonist. Alternatively, the receptor may be directed towards lysosomes where it is degraded, thus resulting in down-regulation of signalling. It is also possible for arrestin-bound, internalised receptors to continue to activate signalling pathways distinct to those present on the membrane. While CB1R activation has proven to result in tolerance and dependency, certain studies show that CB2R activation does not precipitate tolerance or withdrawal symptoms.

In addition to CB2R full agonists, there are a number of alternative strategies to develop safer and more efficacious drugs. Partial agonists may have a lower propensity to cause adverse effects and precipitate withdrawal and tolerance. Another option is exploiting allostery at the CB2R, which may account for some of the shortcomings of full agonists. This idea will be discussed in more detail in Chapter 6. With a greater understanding of biased agonism, compounds that selectively target certain therapeutically relevant signalling pathways could be designed.

1.3 Cannabinoid Ligands

Ligands of the cannabinoid receptors can be grouped into the following broad classes: endogenous cannabinoids (endocannabinoids), plant-derived cannabinoids (phytocannabinoids) and chemically synthesised cannabinoids (synthetic cannabinoids, SCs). Synthetic cannabinoids can be further divided into compounds that are defined by the tricyclic terpenophenolic moiety of Δ9-THC (classical cannabinoids) and those that are structurally distinct (non-classical cannabinoids).

In light of the proscription of Δ9-THC, cannabinoid research has focused on the development of cannabimimetics for therapeutic use, without psychotropic side effects
associated with the use of Δ⁹-THC. Structure-activity relationship (SAR) studies of phytocannabinoids have revealed many compounds, which are more potent and efficacious than Δ⁹-THC. These early synthetic cannabinoids showed minimal selectivity between the CB₁ and CB₂ receptors. In more recent times, ligands with good selectivity for a particular receptor subtype have been developed. In addition, compared to lipophilic phyto- and endocannabinoids and early synthetic cannabinoids, there has been a wide range of scaffolds developed with improved pharmacokinetic properties (for example oral bioavailability) from a drug development perspective.

GPCRs exist in an equilibrium between inactive and activated conformational states. Depending on how they affect the position of this equilibrium, ligands are classified as agonists, neutral antagonists, or inverse agonists. Selective agonists, antagonists, inverse agonists and allosteric modulators have been developed that interact with CB₁ or CB₂ receptors to modify their basal tone.

1.3.1 CB₁R Ligands

In light of the therapeutic potential of the CB₁R-mediated effects of phytocannabinoids, there has been extensive research into the development of drugs that target the CB₁R. Despite this, very few candidates have made it into the clinic. Dronabinol, the trade name for Δ⁹-THC is sold in the US, Canada and New Zealand as an antiemetic for chemotherapy patients. Over 500 compounds have been identified from the cannabis plant, of which 105 have been defined as cannabinoid ligands. The pharmacological profiling of these compounds is an active area of research and may uncover more potential therapeutics. Rimonabant, a CB₁R inverse-agonist, was approved in Europe in 2006 as an anti-obesity drug, though it was soon withdrawn due to depression-related side effects. Four major series of CB₁R ligands have been developed, the CP series (developed by Pfizer), the AM series (developed by Alexandros Makriyannis from Northeastern University), the JWH series (developed by John W. Huffman from Clemson University) and the HU series (developed by Raphael Mechoulam from the Hebrew University).
1.3.2 CB₂R Ligands

The prospect of selectively targeting CB₂Rs as an approach for developing therapeutic agents devoid of psychotropic effects means there is a need for CB₂R selective ligands. Medicinal chemistry efforts by many academic groups and pharmaceutical companies have resulted in the discovery of many structurally diverse CB₂R ligands (mainly agonists) with excellent receptor subtype selectivity. There are a number of reviews that comprehensively catalogue CB₂R ligands reported in the literature and patent literature.⁷⁴, ⁷⁸, ⁹⁷-⁹⁸

1.3.2.1 Agonists

An agonist is defined as a drug that binds to and activates a receptor. A full agonist has high efficacy, producing the maximal response while occupying a relatively low proportion of the receptors. A partial agonist produces sub-maximal activation even when occupying the total receptor population. In terms of receptor conformation, binding of an agonist pushes the equilibrium towards the active receptor state. The first reported compound to exhibit selectivity for the CB₂R was WIN-55,212-2 (22, Figure 8), which exhibited approximately 19-fold selectivity for the CB₂R over the CB₁R.⁹⁹ WIN-55,212-2 is one of the best characterised synthetic cannabinoids and has been used (along with its radiolabelled analogue) as a pharmacological tool for cannabinoid research. Other early CB₂R agonists with some selectivity over the CB₁R include the tetrahydrocannabinol mimetic JWH-133 (23, $K_\text{CB}_1 = 680 \text{ nM}, K_\text{CB}_2 = 3.4 \text{ nM}$),¹⁰⁰ bicyclic phytocannabinoid analogue HU-308 (24, $K_\text{CB}_1 > 10 \text{ μM}, K_\text{CB}_2 = 23 \text{ nM}$),¹⁰¹ and aminoalkylindole AM-1241 (25, $K_\text{CB}_1 = 280 \text{ nM}, K_\text{CB}_2 = 3.4 \text{ nM}$).¹⁰² In recent times numerous research articles and patent applications have disclosed highly potent CB₂R agonists that exhibit no activity at the CB₁R in in vitro assays up to the highest test concentrations. Furthermore, compared to early examples, recent agonists generally possess superior physicochemical properties with clogP values between 0 and 3. The decrease in lipophilicity may reflect attempts to develop orally available drugs, but also may be a general strategy to afford receptor subtype selectivity. Most CB₂R agonists reported in the literature are based on numerous heterocyclic scaffolds. In many cases, scaffold hopping and retention of the relative spatial orientation of key pharmacophoric groups results in the identification of novel chemotypes as selective CB₂R agonists.
Figure 8: Representative examples of some of the first synthetic cannabinoids that exhibited higher affinity for the CB$_2$R over the CB$_1$R. First generation compounds were based on the structures of phytocannabinoids; JWH-133 (23) and HU-308 (24). These were followed by aminoalkylindoles such as WIN-55,212-2 (22) and AM-1241 (25).

**Indoles**

Among the first non-classical synthetic cannabinoids discovered, indole-based ligands have been extensively studied and detailed structure-activity relationships (SARs) have been developed within large series of compounds. In order to explore the structural requirements for binding at the cannabinoid receptors, the laboratory of John Huffman at Clemson University synthesised a huge number of indole, indazole and pyrrole derivatives. For the indole scaffold, Huffman and co-workers illustrated the importance of the N1-substituent for receptor subtype selectivity leading to many 3-naphthoyl and 3-aryloxy indoles with good selectivity (for example JWH-015, 26, Figure 9, $K_i$ CB$_1$ = 383 nM, $K_i$ CB$_2$ = 14 nM). In addition to the aforementioned indoles, AM1241 (25) developed by the Makriyannis group, and WIN-55,212-2 (22), a diverse chemical series bearing a tetramethyl cycloalkyl ketone at the 3-position of the indole were found to be potent ligands. Representative compound A-796260 (27) demonstrated single digit nanomolar affinity at the CB$_2$R compared to near micromolar affinity at the CB$_1$R ($K_i$ CB$_1$ = 945 nM, $K_i$ CB$_2$ = 4.6 nM) and has displayed analgesic and anti-inflammatory effects in models of neuropathic pain. Bristol-Myers Squibb have developed a series of indole derivatives bearing a bulky amide residue at the 3-position. Initial compounds with amino acid derived amides such as 28 ($K_i$ CB$_1$ = 4 μM, $K_i$ CB$_2$ = 8 nM) suffered from poor metabolic stability. Replacement of the amino acid derived amide with a ($S$)-fenchyl moiety (for example 29, $K_i$ CB$_1$ = 245 nM, $K_i$ CB$_2$ = 11 nM) lead to improved metabolic stability with similar binding affinity, although with diminished CB$_2$R selectivity. Although SAR data has been reported for several indole-based series of compounds, such trends rarely translate across series.
Figure 9: Representative examples of potent and selective CB₂R ligands based on the indole scaffold.

Benzimidazoles

A number of pharmaceutical companies have developed CB₂R selective agonists based on the benzimidazole scaffold. AstraZeneca identified 1,2,5-trisubstituted benzimidazole 30 (Figure 10) as part of a high throughput screening program ($K_i$ CB₁ > 5 μM, $K_i$ CB₂ = 36 nM, $EC_{50}$ CB₂ = 38 nM).\(^{105}\) SAR studies revealed tolerance to modifications of the secondary amide and N₁-substituent leading to a large number of selective agonists. Similarly, substituted benzimidazoles have also been examined by Pfizer. Over the course of two patent applications, a number of sulfonyl derivatives such as 31 ($EC_{50}$ CB₁ NA, $EC_{50}$ CB₂ = 0.15 nM), which incorporated a neopentyl group at the 2-position were described.\(^{106-107}\) Similar structures were reported by RaQualia Pharma containing a saturated heterocyclic sulfone at the 5-position. Representative compound RQ-00202730 (32, $EC_{50}$ CB₁ >25 μM, $EC_{50}$ CB₂ = 7.4 nM) is currently being developed for the treatment of irritable bowel syndrome and inflammatory bowel disease.\(^{108}\) 2-tert-Butylbenzimidazoles bearing an aryl sulfone at the 5-position have been reported by Janssen Pharmaceuticals as potent sub nanomolar agonists at the CB₂R with no activity observed at the CB₁R (for example 33, $EC_{50}$ CB₁ >10 μM, $EC_{50}$ CB₂ = 0.26 nM).\(^{109-110}\) Other efforts by Pfizer have aimed to improve the pharmacokinetic properties of such benzimidazole compounds by considering lipophilic efficiency ($LiPE = pEC_{50} - clogP$). This study has led to the development of 34 ($EC_{50}$ CB₁ = 15 μM, $EC_{50}$ CB₂ = 11 nM) and imidazotetrahydropyridines such as 35 ($EC_{50}$ CB₁ = 18 μM, $EC_{50}$ CB₂ = 55 nM), which retain the selectivity and potency of the previously developed benzimidazole compounds, but with improved water solubility.\(^{111-112}\)
Chapter 1: Introduction

Figure 10: Representative examples of potent and selective CB₂R agonists based on the benzimidazole scaffold.

**Imidazo Bicycles**

Several pharmaceutical companies have developed CB₂R selective ligands based on bicyclic imidazole scaffolds. In particular, imidazo[1,5-a]pyridine compounds have been explored by Merck for the treatment of inflammatory and osteoarthritic pain.¹¹³⁻¹¹⁴ A large optimisation study involving the substituents at the two carbon positions of the imidazole ring led to the discovery of selective agonists such as 36 (Figure 11, EC₅₀ CB₁ > 17 μM, EC₅₀ CB₂ = 5.4 nM) and 37 (EC₅₀ CB₁ > 17 μM, EC₅₀ CB₂ = 33 nM). In addition to aromatic bicyclic systems, imidazodiazepine 38 (EC₅₀ CB₁ > 10 μM, EC₅₀ CB₂ = 10-100 nM) as well as tetrahydropyrazine and other saturated heterocyclic rings of different sizes have been developed by Cara Therapeutics.¹¹⁵⁻¹¹⁶

Figure 11: Representative examples of imidazopyridine CB₂R agonists developed by Merck and Cara Therapeutics.
Bicyclic Pyrazoles

Resembling the bicyclic imidazole compounds developed by Cara Therapeutics, bicyclic pyrazole compounds have been a focus for a number of pharmaceutical companies including Sanofi-Aventis, Glenmark and Janssen Pharmaceuticals. Sanofi-Aventis originally identified 39 (Figure 12), which exhibited potent activity at the CB2R but had poor receptor subtype selectivity (EC50 CB1 37 nM, EC50 CB2 = 2.5 nM).117 Following this, Glenmark developed a series of fused norbornane derivatives with improved CB2R selectivity (for example GRC-10693, 13, EC50 CB1 854 nM, EC50 CB2 = 0.61 nM).118 GRC-10693 was selected by Glenmark for clinical development and has successfully completed phase I trials in Europe for both efficacy and safety for the treatment of osteoarthritis and neuropathic pain.119 While studying a series of selective CB2R antagonists, Janssen Pharmaceuticals were able to change the functional profile to develop selective CB2R agonist activity (for example 40, EC50 CB1 > 5 μM, EC50 CB2 = 9 nM).120

![Figure 12: Representative examples of bicyclic pyrazole CB2R agonists.](image)

Other 5,6-Fused Systems

In addition to the aforementioned bicyclic systems, GlaxoSmithKline have released a series of patents exploring several fused aromatic bicyclic scaffolds, including imidopyridines, pyrrolopyridines and azaindoles.121-123 For azaindole compounds it was found that a morpholino amide substituent conferred good potency and selectivity. Compound 41 (Figure 13) was found to be a potent and selective agonist (EC50 CB1 = 6.3 μM, EC50 CB2 = 5 nM), with good CNS penetrability and efficacy in animal models for pain.124 A series of patents granted to Eli Lilly describe the development of purine-based agonists.125-127 LY 2828360 (17, EC50 CB1 >100 μM, EC50 CB2 = 20 nM), a tetrasubstituted purine has undergone Phase II clinical trials for osteoarthritic knee pain.128 Despite failing to demonstrate efficacy in the trial, the drug has recently been repurposed to treat neuropathic
In a slightly different approach to what is conventionally observed for CB$_2$R agonists, highly polar triazolopyrimidines have been developed by Roche as peripherally restricted agonists for the treatment of inflammatory kidney diseases. Tetrazole derivative 42 displayed potent and selective activity in a cAMP functional assay and demonstrated efficacy in a model for inflammatory kidney damage.

Figure 13: Examples of potent and selective CB$_2$R agonists based on miscellaneous 5,6-fused scaffolds.

6,6-Fused Systems

Unsurprisingly, bicyclic agonists are not restricted to 5,6-fused systems. There have been numerous reports of CB$_2$R selective agonists comprising 6,6-fused heterocyclic systems. Manera and co-workers have disclosed a series of naphthyridine and quinoline compounds. Compound 43 (Figure 14, $K_i$ CB$_1$ = 560 nM, $K_i$ CB$_2$ = 11 nM) was found to have selectivity for the CB$_2$R and exhibited antinociceptive effects in mice. A subsequent publication revealed that migration of the carbonyl group to the 2-position of the naphthyridine yielded compounds with improved receptor subtype selectivity (for example 44, $K_i$ CB$_1$ = 1.52 μM, $K_i$ CB$_2$ = 5.8 nM). A comprehensive SAR study of the 4-quinolone-3-carboxamide scaffold undertaken by Pasquini and co-workers revealed adamantyl amide compounds such as 45 had improved affinity for the CB$_2$R and impressive selectivity over the CB$_1$R ($K_i$ CB$_1$ > 10 μM, $K_i$ CB$_2$ = 0.6 nM). A series of quinoline compounds with varying substituents at the 8-position were reported in a patent application by GSK (for example 46, EC$_{50}$ CB$_1$ NA, EC$_{50}$ CB$_2$ < 1 μM). In a similar fashion to what has been observed for the development of 5,6-fused analogues, saturated or partially saturated bicyclic systems have also been reported. Tetrahydro-1,6-naphthyridinone 47 (EC$_{50}$ CB$_1$ > 10 μM, EC$_{50}$ CB$_2$ < 100 nM) and related compounds were developed by Cara Therapeutics, while Merck have studied decahydroquinoline amides such as 48 (EC$_{50}$ CB$_1$ = 5.02 μM, EC$_{50}$ CB$_2$ = 7.3 nM).
Six-Membered Aromatics

CB₂R agonists with six-membered (hetero)aromatic cores have been developed by a number of pharmaceutical companies and academic laboratories. GSK have filed a number of patents for pyridine and pyrimidine CB₂R agonists. Pyrimidine compound GW842166X (12, Figure 15, EC₅₀ CB₁ > 30 μM, EC₅₀ CB₂ = 63 nM) has completed phase II clinical trials for third molar tooth extraction dental pain and osteoarthritis pain. Pyridine-3-carboxamide analogues were later released (for example 49, EC₅₀ CB₁ > 30 μM, EC₅₀ CB₂ = 79 nM) as alternatives with improved aqueous solubility. GSK has also investigated biaryl scaffolds such as those appearing in pyridine 50 (EC₅₀ CB₁ = 10 μM, EC₅₀ CB₂ = 39 nM) and pyridazine 51 (EC₅₀ CB₁ = 7.9 μM, EC₅₀ CB₂ = 10 nM). Boehringer Ingelheim have also disclosed similar aryl compounds bearing a chiral morpholine group. One enantiomer was found to be a potent and selective agonist (52, EC₅₀ CB₁ = 5.76 μM, EC₅₀ CB₂ = 6 nM) while the other functions as an inverse agonist of the CB₂R. Simple benzamides have been considered by various groups such as 53 (EC₅₀ CB₁ > 9.3 μM, EC₅₀ CB₂ = 4 nM) and reversed amide analogues such as 54 (Kᵢ CB₁ = 3.4 μM, Kᵢ CB₂ = 23 nM) and pyridine 55 (Kᵢ CB₁ = 3.8 μM, Kᵢ CB₂ = 24 nM).
Five Membered Heterocycles

Five membered heterocycles represent the most common scaffold used for the development of CB2R agonists. A plethora of heterocycles have appeared with varied substitution and structural features. Taisho Pharmaceuticals originally developed a series of thiazolylidene agonists with excellent receptor subtype selectivity.\(^\text{148-149}\) Sulfonamide compounds, while exhibiting favourable potency and good selectivity (for example 56, Figure 16, \(K_i\) CB\(_1\) = 1.7 \(\mu\)M, \(K_i\) CB\(_2\) = 16 nM) were found to possess poor metabolic stability. Therefore, amide derivatives (for example 57, \(K_i\) CB\(_1\) = 3.5 \(\mu\)M, \(K_i\) CB\(_2\) = 13 nM) were developed with improved selectivity for the CB2R as well as superior pharmacokinetic properties. Another publication followed, reporting analogous pyrazole compounds (for example 58, \(K_i\) CB\(_1\) = 4.0 \(\mu\)M, \(K_i\) CB\(_2\) = 2.9 nM), which displayed even better aqueous solubility, affinity and selectivity.\(^\text{150}\) Extensive SAR studies have been reported by Abbott Laboratories for a diverse range of five-membered heterocycles including pyrazoylidene, triazoylidene, thiazolylidene and thiadaizoylidene arylcarboxamide derivatives.\(^\text{151-153}\) The comprehensive study yielded a large number of very potent agonists with no reported activity at the CB1R (for example 59 EC\(_{50}\) CB\(_1\) > 27 \(\mu\)M, EC\(_{50}\) CB\(_2\) = 0.47 nM). A high-throughput screening effort by Amgen afforded novel oxadiazole CB2R agonists (for example 60, EC\(_{50}\) CB\(_1\) > 2 \(\mu\)M, EC\(_{50}\) CB\(_2\) = 11 nM).\(^\text{154}\) The 2,4-disubstituted aryl group at the 3-position was found to be important for CB2R potency. Diphenyl imidazole derivatives were developed by Merck for the treatment of chronic pain. It was found that the piperidine group of 61 (EC\(_{50}\) CB\(_1\) =
12.5 μM, EC50 CB2 = 8 nM) was imperative for potency and selectivity over the CB1R.\textsuperscript{155} Boehringer Ingelheim have released a large number of publications and patents surrounding the development of α-sulfonyl carboxamide and α-amino carboxamide five-membered heterocyclic CB2R agonists.\textsuperscript{156-160} Isoxazole 62 (EC50 CB1 > 50 μM, EC50 CB2 = 11 nM), 1,2,4-triazole 63 (EC50 CB1 > 20 μM, EC50 CB2 = 0.66 nM) and isoxazole α-amino carboxamide 64 (EC50 CB1 > 50 μM, EC50 CB2 = 56 nM) were amongst the most potent and selective compounds described. 1,4-Diazepane derivatives were also investigated including a range of isoxazole, pyrazole, pyridine, phenyl and bicyclic compounds.\textsuperscript{161} The thiazole and in particular benzoxazole derivatives are shown to be very potent CB2R agonists (for example 65 EC50 CB2 = 0.017 nM). Finally, Boehringer Ingelheim have further explored the diazepane series by examining the effect of ring size. Chiral saturated heterocyclic amides including azetidines,\textsuperscript{162} pyrrolidines,\textsuperscript{163} piperidines\textsuperscript{164} and azapines\textsuperscript{164} were prepared. Apart from the azapine derivative, all analogues were potent agonists at the CB2R, with the most potent being pyrrolidine 66 (EC50 CB1 = NA, EC50 CB2 = 0.067 nM).

![Figure 16: Representative examples of 5-membered aromatic CB2R agonists.](image-url)
Tricyclic Structures

A number of compounds with tricyclic cores have been described as potent and selective CB₂R agonists. Pyrazole 67 (Figure 17) was discovered serendipitously from a SAR study of CB₁R antagonist SR141716A. The rigid tricyclic system reduced CB₁R affinity and resulted in a selective CB₂R ligand ($K_{i}; CB₁ = 363 \text{ nM}, K_{i}; CB₂ = 0.037 \text{ nM}$). Similar tricyclic pyrazoles were also described by Arena in a patent application. Representative structure 68 ($EC_{50}; CB₁ > 50 \mu\text{M}, EC_{50}; CB₂ = 32 \text{ nM}$) displayed potent activity at the CB₂R with complete selectivity over the CB₁R. Cara Therapeutics have also developed octahydrophenanthridine-based CB₂R agonists. Representative compound 69 ($EC_{50}; CB₁ > 10 \mu\text{M}, EC_{50}; CB₂ = 0.56 \text{ nM}$) exhibited potent and selective functional activity and also demonstrated efficacy in a mouse model for pain.

![Figure 17: Examples of potent and selective CB₂R agonists based on miscellaneous tricyclic scaffolds.](image)

1.3.2.2 Antagonists and Inverse Agonists

An antagonist is a drug that attenuates the effect of an agonist. An inverse agonist is defined as a drug that binds to the same receptor binding site as an agonist yet produces an opposite effect. A prerequisite for an inverse agonist response is that the receptor must have constitutive activity in the absence of any ligand. An inverse agonist decreases receptor activity below the basal level shifting the equilibrium position of the receptor conformation towards the inactive state, while a neutral antagonist does not affect the equilibrium position.

Compared to agonists, there are very few known CB₂R selective antagonists and inverse agonists. Additionally, the use of CB₂R antagonists for drug development is much less explored than CB₁R antagonists. Despite a paucity of in-depth studies exploring the
therapeutic potential of CB₂R antagonists, the patent literature suggests these compounds can be developed as immunomodulators, anti-inflammatory and anti-allergic agents. Sanofi have made a number of important contributions to cannabinoid research, namely, the discovery of CB₁R inverse agonist rimonabant and also reference CB₂R antagonist (later described as an inverse agonist) SR144528 (70, Figure 18, $K_i$ CB₁ = 400 nM, $K_i$ CB₂ = 0.6 nM).⁶⁶ In 1999, Ross and co-workers determined that selective CB₂R ligand AM630 (71) acted as an inverse agonist at the CB₂R and a weak partial agonist at the CB₁R ($K_i$ CB₁ = 5150 nM, $K_i$ CB₂ = 31 nM).⁶⁸ A series of 2-oxoquinoline derivatives exemplified by JTE907 (72) were found to be potent and selective CB₂R inverse agonists.⁷⁹, ¹⁶⁹ Other quinoline carboxamide structures have also been developed as selective CB₂R inverse agonists.¹⁷⁰ Lunn and co-workers have demonstrated the immunomodulatory effects of CB₂R antagonists/inverse agonists by showing the ability of triaryl bis-sulphone, Sch.336 (73) to block leukocyte recruitment in vivo.¹⁷¹ Such positive results may motivate further development of more compounds and preclinical studies.

Figure 18: Representative examples of selective CB₂R antagonists.

1.3.2.3 Allosteric Modulators

All GPCRs are thought to contain an allosteric binding site that is independent of the orthosteric site. An allosteric modulator binds to a receptor at a site distinct to the orthosteric binding site. This induces a conformational change in the receptor that alters the affinity of the receptor for an orthosteric ligand. As illustrated in Figure 19, positive allosteric modulators (PAMs) increase the affinity while negative allosteric modulators (NAMs) decrease the affinity. A third type of allosteric modulator are silent allosteric modulators (SAMs) which occupy the allosteric binding site but are functionally neutral. An allosteric modulator does not cause any down-stream activation within the cell by itself but alters the activity of endogenous ligands. Compared to orthosteric agonists, allosteric
modulators have a number of favourable characteristics that make them desirable drug candidates. Since allosteric modulators do not trigger a response unless the endogenous ligand is present, long lasting effects such as dependence and desensitisation can be avoided. In general, allosteric sites are poorly conserved and so subtype selectivity is generally more achievable. Because of the proposed uniqueness of allosteric sites, allosteric modulators generally result in fewer adverse side effects compared to the inherent side effects caused by orthosteric ligands. This also results in tissue selectivity, as allosteric modulators only exert effects where the endogenous ligand is present in response to tissue damage or other stimuli.

Despite these desirable properties, there has been minimal progress in developing novel CB2R allosteric modulators. It was recently reported that the CB1R has an allosteric binding site that is topologically distinct from the orthosteric site. Since then, a variety of CB1R allosteric modulators have been reported, of which the most studied are indole-2-carboxamides such as Org27569 (74, Figure 20), ureas such as PSNBAM-1 (75) and indole ZCZ-011 (76).

**Figure 19:** General classification of allosteric modulators as PAMs, NAMs or SAMs. (Adapted with permission from Gado, F.; et al. *J. Med. Chem.* 2019, 62 (1), 276-287. Copyright (2019). American Chemical Society).

**Figure 20:** Examples of known allosteric modulators of the CB1 receptor.
Up until recently there were only two natural products, dihydro-gambogic acid (77, Figure 21) and trans-β-caryophyllene (TBC, 78)\textsuperscript{175} and an endogenous peptide, Pepcan-12 (80),\textsuperscript{176} that had been proposed as CB\textsubscript{2}R allosteric modulators. From a medicinal chemistry point of view these compounds are poor leads for developing potential drugs. Fortuitously, Gado and co-workers reported the first synthetic allosteric modulator of the CB\textsubscript{2}R in 2019.\textsuperscript{177} The authors demonstrate 79 displays antinociceptive activity \textit{in vivo} in an experimental mouse model of neuropathic pain. More details regarding the development of novel CB\textsubscript{2}R allosteric modulators are provided in Chapter 6.

![Figure 21: Known allosteric modulators of the CB\textsubscript{2}R. Compound 79 is the first reported synthetic CB\textsubscript{2}R allosteric modulator.](image)

**1.4 Pharmacological Evaluation of Ligands**

The discovery and pharmacological evaluation of new cannabinoid receptor ligands is facilitated by suitable bioassays.\textsuperscript{178} Established \textit{in vitro} assays for the cannabinoid receptors all involve the use of membrane or cell preparations that contain naturally expressed or transfected CB\textsubscript{1} and/or CB\textsubscript{2} receptors. To measure affinity, binding assays assess the ability of a test compound to displace a radiolabelled receptor ligand such as \textsuperscript{3}H]CP-55,940 from cannabinoid receptor expressing cells or tissues. A determination of functional activity is
usually achieved by measuring the effects of test compounds on receptor signalling. Some examples include stimulation of binding to G proteins of the hydrolysis-resistant GTP analogue $[^{35}\text{S}]{\text{G}}\text{TP}_{\gamma}\text{S}$, $G_{\text{i/o}}$-mediated inhibition of basal or drug-induced cyclic AMP production and changes in ion levels, such as elevation of intracellular free calcium ions, which is presumably a $G_{\text{s}}$-mediated effect.\textsuperscript{44}

1.4.1 Cell Lines

Studies conducted in the Kassiou research group utilise immortalised mouse pituitary adenoma (AtT20) cells, which have been transfected with either human CB\textsubscript{1} or CB\textsubscript{2} receptors. These cells express native G protein-coupled inwardly rectifying potassium channels and two GIRK channels, which are vital for signalling pathways associated with GCPR activation.

1.4.2 FLIPR Membrane Potential Assay

The Fluorescence Imaging Plate Reader (FLIPR) membrane potential assay is a non-invasive, automated process allowing for rapid analysis. The cells are equilibrated with a membrane permeable fluorescent dye, in which the extracellular fluid contains a fluorescence masking dye. AtT20 cells express native G protein-coupled inwardly rectifying potassium (GIRK) channels. Upon receptor activation the interaction of G protein subunits and GIRK channels leads to the opening of the channels. This results in an efflux of potassium ions and the hyperpolarisation of the cell. Hyperpolarisation allows the fluorescent dye to permeate through the cell membrane to the extracellular fluid, which contains the masking dye and hence a reduction in fluorescence is observed. (Figure 22).
Figure 22: Depiction of the FLIPR membrane potential assay; (a) in the resting state a membrane permeable fluorescent dye equilibrates across the cell membrane; (b) cell hyperpolarisation allows the dye to permeate through the cell membrane to the extracellular fluid which contains a masking dye hence causing a reduction in fluorescence; (c) cell depolarisation causes an influx of potassium ions and an increase in permeation of the dye through the cell membrane into the cell, hence causing an increase in fluorescence.

1.5 Project Aims

The overall aim of this project is to develop novel CB₂R ligands. A particular focus of the project is to investigate structure-activity relationships in order to better understand the general structural features of ligands that are required for modulation of the receptor. In order to achieve this, the project has three main approaches as outlined in Figure 23:

Figure 23: Schematic summarising the overall aims of the research project. Three different approaches were taken to develop drugs targeting the CB₂R.

Explore the SAR of potent and selective agonists reported in the literature

There are numerous reports in the literature (and particularly the patent literature) of extremely potent CB₂R agonists. Despite these reports and promising preclinical data, no
ligands that target the CB$_2$R have progressed to the clinic. Strong preclinical evidence suggests that activation of CB$_2$Rs can ameliorate a number of disease states that currently do not have cures or viable treatment options. Therefore, there is an unmet need to develop new, safer and more efficacious drugs that target the CB$_2$R. While there are many reports of potent CB$_2$R agonists, a unified pharmacophore has not been developed. Generally, comprehensive SAR studies are performed for a particular chemotype. However, studies that compare different chemotypes are sparse. If the particular structural requirements for functional activity can be determined, novel agonists can potentially be developed without the shortcomings of previous ligands that have failed in clinical trials.

*Develop selective CB$_2$R agonists from non-selective synthetic cannabinoids*

In recent years the Kassiou research group has sought to synthesise and pharmacologically characterise next generation synthetic cannabinoids identified by collaborators in the forensic field. These studies have determined that such SCs are generally highly potent full agonists at both the CB$_1$ and CB$_2$ receptors. Furthermore, *in vivo* studies suggest these compounds elicit potent cannabimimetic effects. With this in mind, we thought if activity at the CB$_1$R could be suppressed, then CB$_2$R receptor agonists with favourable properties for *in vivo* activity could be developed. General strategies to impart CB$_2$R selectivity will be described for some of the most potent synthetic cannabinoids known.

*Develop novel CB$_2$R positive allosteric modulators*

Recently, the first synthetic allosteric modulator of the CB$_2$R (79) was reported by Gado and co-workers. While the *in vitro* results were positive and the authors were able to demonstrate efficacy in an experimental mouse model for neuropathic pain, very little SAR analysis of the identified PAM was conducted. It therefore follows that the reported modulator would serve as a good lead compound to assess the structural requirements for CB$_2$R allosteric binding and activation. A small library of analogues has been synthesised to identify novel CB$_2$R PAMs.
Chapter 2: Proposal of a Common Pharmacophore for CB₂ Receptor Agonists
2.1 Introductory Remarks

There have been many reports of potent CB$_2$R agonists that exhibit good to excellent selectivity over the CB$_1$R. Whilst these agonists are derived from structurally diverse chemotypes, certain structural similarities exist. Each individual chemotype has generally undergone comprehensive SAR analysis, however there are few, if any studies that compare different chemotypes for the purpose of pharmacophore exploration. The information gained from such an investigation would be invaluable for future drug discovery efforts.

A pharmacophore is defined by the International Union of Pure and Applied Chemistry as: the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response. Pharmacophore modelling is a common technique used in computer-aided drug design to represent and identify key elements of molecular recognition in three-dimensions. Atoms or groups involved in molecular recognition can be categorised as a collection of pharmacophore features including hydrogen bond donating or accepting, cationic or anionic, aromatic, hydrophobic or combinations of these as demonstrated in Figure 24. Pharmacophore modelling can be used to analyse the common elements of active molecules to identify the key structural features contributing to the biological function.

Figure 24: Example of a pharmacophore model, comprising of different features, which represent molecular recognition motifs. The radius of the spheres represents geometric constraints for the recognition. Image was reprinted with permission from Quin, X.; et al. J. Receptor Ligand Channel Res. 2014, 7, 81-92. Copyright, Dovepress (2019).
While some studies have used computer-aided drug design to develop a pharmacophore model for CB₂R agonists, these have typically involved classical cannabinoids or older generation agonists as training ligands.\textsuperscript{180-181} In the years since these initial studies, a plethora of potent and selective agonists have been reported. It would be expected that this new data would confer a more reliable and general pharmacophore model.

\subsection{2.2 Development of a Pharmacophore Model}

\textbf{Figure 25} outlines the workflow used to determine a pharmacophore for CB₂R agonists. An \textit{in-silico} pharmacophore model was formulated based on reported potent and selective CB₂R agonists. To refine this crude model we performed SAR studies around a reported scaffold.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure25.png}
\caption{Proposed workflow to develop a general pharmacophore for CB₂R agonists.}
\end{figure}

To help determine the structural features that are important for functional activity at the CB₂R we aimed to develop a pharmacophore model using two complementary programs—Pharmacophore Alignment and Scoring Engine (Phase) running on Maestro 12.0 (Schrödinger\textsuperscript{®})\textsuperscript{182-183} and Forge\textsuperscript{TM} developed by Cresset\textsuperscript{®}.\textsuperscript{184-185} Phase determines the spatial arrangement of certain chemical features such hydrogen-bond donors and acceptors. A common pharmacophore hypothesis is generated, which conveys characteristics of the three-dimensional chemical structures that are likely to be critical for binding by utilising fine-grained conformational sampling and a range of scoring techniques.\textsuperscript{186} The FieldTemplater\textsuperscript{TM} software\textsuperscript{187} in Forge\textsuperscript{TM} generates pharmacophore models by aligning field points of active compounds. The programs developed by the Cresset group aim to look at ligands in the same way they are seen or experienced by protein receptors. To do this they consider a ligand as a three-dimensional distribution of shape, charge and hydrophobicity. Rather than considering full molecular electrostatic potential surfaces, which would be very computationally demanding, this information is condensed into field points or areas of local maxima in the surfaces.\textsuperscript{185} The field points within a ligand correspond to areas that participate in favourable interactions with a receptor. Three-
dimensional field point patterns can be used to compare structurally distinct ligands in a biologically relevant way.

To develop a pharmacophore model using Phase we selected 27 ligands of differing chemotypes that were discussed in Chapter 1. Despite the structural diversity of this set of ligands, a pharmacophore model was generated that was consistent with 22 of the 27 agonists. Further details of the study are provided in Chapter 8.

Due to the computational intensity of pharmacophore modelling using FieldTemplater™ a large number of ligands cannot be sampled. The program developers suggest using fewer than five ligands in order to avoid lengthy computation times. In order to compare the pharmacophore models generated by the two programs, five ligands were selected at random from the 22 compounds that adhered to the model generated using Phase. The program screened for conformations of the five ligands that had high molecular field similarity. Clustering of the field points of the aligned ligands confers areas that participate in interactions with the receptor. Further details of the study are provided in Chapter 8.

**Figure 26** highlights the similarities between the two models. Hydrophobic and aromatic features identified using Phase correlate well with clustering of hydrophobic field points shown in the Cresset® generated field map. Likewise, the hydrogen bond accepting motif highlighted in the Phase model appears to overlay well with a clustering of negative field points in the Cresset® model. The high fidelity of agonists considered in this study to this pharmacophore model is suggestive that the pharmacophore features identified are important for functional activity, and that most active ligands adopt a common binding conformation.
Chapter 2: Proposal of a Common Pharmacophore for CB2 Receptor Agonists

Figure 26: Pharmacophore models based on reported potent and selective CB2R agonists, generated using (A) Phase by Schrödinger® and (B) Forge™ by Cresset®. Reported CB2R agonist 66 is shown in purple in both models for clarity of the three-dimensional arrangement of the chemical features and field points.

2.3 Selection and Synthesis of Lead Compound Candidates

We sought to synthesise some of the ‘best-in-class’ CB2R agonists in order to evaluate their suitability as lead scaffolds for further pharmacophore studies. Although the functional activity data is already reported in the literature, different assay types and cell lines generally provide variable data. In order to compare the functional activity of a selection of known agonists, the compounds needed to be screened using the same assay under the same experimental conditions.

A comprehensive review of CB2R agonists reported in the academic and patent literature was conducted. Agonists were assessed based on their reported functional activity at the CB2R, selectivity over the CB1R, physicochemical properties (adherence to Lipinski’s rule
of 5)\textsuperscript{188} and ease of synthesis. Based on these factors, six compounds (33, 63, 81-84) were classified as ‘best-in-class’ agonists (Figure 27). It was noted that these CB\textsubscript{2}R agonists shared structural similarities in accordance with the pharmacophore models discussed above (Figure 26). Figure 27 highlights these structures are generally composed of a heterocyclic core with a bulky aliphatic group (such as tert-butyl), a saturated heterocycle or alkyl group (such as tetrahydropyran or tetrahydrofuran), a hydrogen bond donating moiety or region of negative electrostatic potential and a large hydrophobic region.

![Figure 27](image)

**Figure 27:** Some of the most potent and selective CB\textsubscript{2}R agonists reported in the literature were selected as ‘best-in-class’ ligands. These ligands were aligned according to the pharmacophore model presented in Figure 26. Colour coding highlights the structural similarities between the reported selective CB\textsubscript{2}R agonists, which generally comprise a heteroaromatic core (red), a hydrogen bond accepting group or area of negative electrostatic potential (green), a bulky aliphatic group (blue), a alkyl or cycloalkyl group (pink) and an hydrophobic group (purple).

In order to reduce the number of candidates to be synthesised, purine 81 was not considered for further investigation based on its inferior potency. Furthermore, the synthesis of benzimidazole 33 is not included in this thesis as it is currently being investigated within the Kassiou research group as a lead compound for the development of CB\textsubscript{2}R positron emission tomography (PET) radioligands. The in-house functional activity data for this compound will however be included for the purpose of comparison.

\[
\begin{align*}
\text{EC}_{50} \text{ CB2} &= 22.4 \text{ nM} \\
\text{CB2/CB1} &> 4400 \\
\text{EC}_{50} \text{ CB2} &= 0.26 \text{ nM} \\
\text{CB2/CB1} &> 38000 \\
\text{EC}_{50} \text{ CB2} &= 0.22 \text{ nM} \\
\text{CB2/CB1} &> 44000 \\
\text{EC}_{50} \text{ CB2} &= 1 \text{ nM} \\
\text{CB2/CB1} &> 57000 \\
\text{EC}_{50} \text{ CB2} &= 0.66 \text{ nM} \\
\text{CB2/CB1} &> 30300 \\
\text{EC}_{50} \text{ CB2} &= 1.4 \text{ nM} \\
\text{CB2/CB1} &> 35700
\end{align*}
\]
Heteroarylidene-benzamide derivatives developed originally by Taisho Pharmaceuticals Co. Ltd. and then Abbott Laboratories are potent and selective CB$_2$R agonists, as discussed in Chapter 1. Pyrazole derivative 82 and thiazole derivative 83 are amongst the most potent analogues reported and both possess favourable drug-like properties. The synthesis of lead compounds 82 and 83 followed modified procedures reported by Carroll and co-workers from Abbott Laboratories (Schemes 1–4).

Both compounds contain a chiral tetrahydrofuran moiety. SAR studies by Abbott Laboratories revealed the (R)-enantiomer exhibits improved potency, compared to the (S)-enantiomer. To access the requisite optically pure tetrahydrofuran moiety we used commercially available and inexpensive (R)-tetrahydrofuran-2-carboxylic acid. For the synthesis of 5-aminopyrazole 89, we required a mono-substituted hydrazine. Due to the presence of two nucleophilic nitrogens, the synthesis of mono-substituted hydrazines is often not achievable using simple substitution chemistry. The reaction of hydrazine hydrate with alkyl halides often results in a mixture of products. To circumvent this problem, we employed Mitsunobu chemistry using di-Boc-protected hydrazine to afford the masked mono-substituted hydrazine 86. Access to the primary alcohol pronucleophile 85 was achieved by lithium aluminium hydride mediated reduction of optically pure (R)-tetrahydrofuran-2-carboxylic acid. Standard Mitsunobu conditions using di-tert-butyl azodicarboxylate (DBAD) afforded the protected hydrazine, with the starting di-tert-butyl hydrazine-1,2-dicarboxylate generated as a reaction by-product. We found that once the Boc groups were removed under acidic conditions, the hydrazine salt (87) that formed was very hygroscopic and so the deprotection was performed just prior to the condensation reaction with $\alpha$-cyano ketone 88. $\alpha$-Cyano ketone 88 was formed from commercially available pivaloyl chloride by reaction with the anion of acetonitrile. After deprotection of 86 under acidic conditions, the hydrazine hydrochloride salt was heated at reflux in ethanol in the presence of $\alpha$-cyano ketone 88 to afford 89.
Scheme 1: Synthesis of 5-aminopyrazole portion of lead compound candidate 82. Reagents and conditions: (a) LiAlH₄, THF, 0 °C–reflux, 16 h, 97%; (b) Di-tert-butyl hydrazine-1,2-dicarboxylate, PPh₃, di-tert-butyl azodicarboxylate, THF, 0 °C–rt, 16 h, 72%; (c) HCl, dioxane, rt, 16 h, 100%; (d) MeCN, n-BuLi, THF, -78 °C–rt, 41%; (e) EtOH, reflux, 4 h, 75%.

Following this, an amide coupling using 2-fluoro-5-(trifluoromethyl)benzoic acid, via the corresponding acid chloride afforded amide 90 (Scheme 2). To install the requisite azolyidene-benzamide moiety, a regioselective methylation was performed, using methyl triflate as the electrophile. Under neutral conditions the lone pair of the nitrogen at the 2-position of the pyrazole is the most nucleophilic group and hence reacts with the methylating agent resulting in a shift of π electrons and the formation of 91. Nucleophilic aromatic substitution (SₐNAr) using 2-methylpropane-1,2-diol, which was prepared in a two-step procedure from 2-hydroxyisobutyric acid (Scheme 3), and bulky non-nucleophilic base potassium tert-butoxide afforded final product 82.

Scheme 2: Synthesis of lead compound candidate 82. Reagents and conditions: (a) i) Oxalyl chloride, DMF, CH₂Cl₂, rt, 3 h, ii) 89, (iPr)₂EtN, CH₂Cl₂, rt, 4 h, 67%; (b) MeOTf, MePh, 100 °C, 16 h, 72%; (c) 2-Methylpropane-1,2-diol (93), KOrBu, 0 °C–rt, 2 h, 60%.

Scheme 3: Synthesis of diol 93. Reagents and conditions: (a) H₂SO₄, EtOH, reflux, 16 h, 71%; (b) LiAlH₄, THF, 0 °C–rt, 16 h, 86%.
A slightly different approach was taken to access thiazole analogue 83 (Scheme 4). Again the chiral primary alcohol 85 was used as a starting point. From here, the requisite primary amine 96 was synthesised in a three step procedure using Staudinger chemistry to avoid disubstitution. The alcohol was first converted to a mesylate, which underwent a substitution reaction with sodium azide and then a Staudinger reaction to provide the desired amine 96. Reaction with commercially available, 3,3-dimethylbutanal, in the presence of a desiccant afforded a compound with a mass to charge ratio (m/z) as observed by ESI mass spectrometry consistent with that of the imine. The imine intermediate was used immediately in the following reaction where it was treated with potassium thiocyanate and iodine to afford the thiazole nucleus as the imine hydroiodide salt. Formation of 2-fluoro-5-(trifluoromethyl)benzoyl chloride from the corresponding carboxylic acid and treatment with 97 furnished thiazoylidene-benzamide 98. Finally, a nucleophilic aromatic substitution with 2-pyridinyl methanol gave the final product 83.

![Scheme 4: Synthesis of lead compound candidate 83. Reagents and conditions: (a) MsCl, Et₃N, CH₂Cl₂, rt, 12 h, 97%; (b) NaN₃, DMF, 70 °C, 16 h, 85%; (c) PPh₃, THF, H₂O, 50 °C, 16 h, 68%; d) 3,3-Dimethylbutanal, 4 Å molecular sieves, MeCN, rt, 16 h; (e) Potassium thiocyanate, I₂, MeCN, 50 °C, 16 h, 51%; (f) i) 2-Fluoro-5-(trifluoromethyl)benzoic acid, oxaly chloride, DMF, CH₂Cl₂, rt, 1.5 h, ii) 97, CH₂Cl₂, rt, 2 h, 48%; (g) 2-Pyridinyl methanol, KOtBu, THF, rt, 5 h, 56%.](image)

Triazole derivative 63 was reported by Boehringer Ingelheim as part of a series of patent disclosures describing numerous heterocyclic CB₂R agonists. The synthesis of triazole 63 followed modified procedures reported by Bartolozzi and co-workers (Schemes 5–7). The final amide coupling between aminotriazole 101 and carboxylic acid 107 however required much optimisation to obtain the desired product. Reaction of commercially available 4-methoxy benzoic acid methyl ester with hydrazine hydrate in methanol at reflux
afforded the desired hydrazide 99 in excellent yield. The synthesis of the aminotriazole building block 101, followed procedures reported by Dolzhenko and co-workers.\textsuperscript{189} Arylamidoguanidine 100 was synthesised from hydrazide 99 by reaction with S-methyl isothiourea hemisulfate. At elevated temperatures in water, intramolecular condensation of arylamidoguanidine 100 formed aminotriazole 101. Dolzhenko and co-workers have studied the tautomerism of 3(5)-amino-1,2,4-triazoles. The product is found to exist exclusively as the electronically favourable 5-amino-1,2,4-triazole tautomer and spectroscopic data matched reported values.\textsuperscript{189}

\begin{center}
\textbf{Scheme 5:} Synthesis of amino triazole portion of lead compound candidate 63. \textit{Reagents and conditions:} (a) Hydrazine hydrate, MeOH, reflux, 16 h, 92%; (b) S-Methyl isothiourea hemisulfate, NaOH (aq.), rt–50 °C, 76 h, 43%; (c) H2O, reflux, 16 h, 100%; (d) (Boc)2O, Et3N, THF, rt, 5 h, 52%.
\end{center}

Sulfone 107 required a multistep synthesis from commercially available tetrahydro-2H-pyran-4-ol (Scheme 6). First, formation of the tosylate allowed for a substitution reaction with potassium thioacetate, which installed the requisite protected thiol. A one-pot thioacetate hydrolysis and substitution with ethyl bromoisobutyrate afforded the desired tertiary thioether 105. Oxidation to the sulfone using Oxone\textsuperscript{®} and ethyl ester hydrolysis gave the carboxylic acid 107, equipped for amide coupling with aminotriazole 101.

\begin{center}
\textbf{Scheme 6:} Synthesis of sulfone portion of lead compound candidate 63. \textit{Reagents and conditions:} (a) Tosyl chloride, pyridine, CH2Cl2, rt, 5 h, 87%; (b) Potassium thioacetate, sodium iodide, DMF, 50 °C, 20 h, 32%; (c) KOH (aq.), ethyl bromoisobutyrate, EtOH, rt, 16 h, 76%; (d) Oxone\textsuperscript{®}, dioxane, H2O, rt, 16 h, 69%; (e) NaOH, THF, H2O, rt, 48 h, 85%.
\end{center}

Initially a PyBOP\textsuperscript{®} mediated amide coupling was attempted, however only starting material was recovered, most likely due to the poor nucleophilicity of the aminotriazole. Therefore, the acid chloride was formed by treatment of 107 with oxalyl chloride and catalytic DMF.
Reaction with 101 produced a number of products, primarily from reaction of the triazole nitrogens, rather than the amino group. It was therefore decided to first protect the nucleophilic triazole nitrogen with a Boc group to form 102. The reaction of the protected species (102) and the acid chloride formed from 107 was found to be slow in the presence of a weak base such as triethylamine. Similarly, coupling attempts with a strong base at room temperature failed to produce appreciable quantities of the desired compound. Only in the presence of sodium hydride in toluene at reflux was the desired amide formed and conveniently Boc cleavage occurred simultaneously and hence the final product 63 was obtained in moderate yield after chromatography and recrystallisation from a mixture of ethyl acetate and hexane.

In a series of publications, Boehringer Ingelheim outlined their discovery process for several novel CB₂R chemotypes. The initial publication addressed the identification of novel ligands through computer-aided drug design approaches. The following publications disclosed further SAR studies and improvement of drug-like properties. Isoxazole 84 was one of the most potent compounds discovered. The synthesis of isoxazole 84 using the procedures reported by Riether and co-workers required only 3 steps in total (Scheme 8). 5-Aminoisoxazole 108 was synthesised from α-cyano ketone 88 and hydroxylamine, while the carboxylic acid 109 was synthesised from L-pyroglutamic acid via a Chan-Lam type coupling using 4-trifluoromethylphenyl boronic acid and a copper-tetramethylethylenediamine (TMEDA) catalyst. The catalyst [Cu(TMEDA)(OH)Cl₂]₂ was easily prepared by stirring copper chloride and TMEDA in 95% ethanol under an atmosphere of air, and recovering the resulting precipitate by filtration. The final amide 84 was formed by a one-pot amide coupling reaction using phosphorus oxychloride in pyridine.
Scheme 8: Synthesis of lead compound candidate 84. Reagents and conditions: (a) NH$_2$OH.HCl, NaOH (aq.), 50 °C, 4 h, 80%; (b) DBU, Cu-TMEDA catalyst, 4-(trifluoromethyl)phenylboronic acid, MeCN, rt, 20 h, 71%; (c) POCl$_3$, pyridine, 0 °C–rt, 30 min, 81%.

2.4 In vitro Evaluation of Lead Compound Candidates

The lead compound candidates 33, 63, 82-84 were assessed for their ability to activate the CB$_1$ and CB$_2$ receptors using a Fluorescence Imaging Plate Reader (FLIPR) membrane potential assay in AtT-20 cells expressing human CB$_1$ or CB$_2$ receptors. Compounds displaying more than 50% activation at 10 μM in the assay were evaluated further in dose-response studies. Their half maximal effective concentrations (EC$_{50}$) and maximal effect relative to CP 55,940 (2) (E$_{\text{max}}$) were calculated as listed in Table 2. Further experimental details are provided in Chapter 8.

Table 2: Agonist activities of lead compound candidates (33, 63, 82-84) in AtT-20 cells expressing human CB$_1$ or CB$_2$ receptors by a FLIPR membrane potential assay$^a$

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB$_1$</th>
<th></th>
<th>CB$_2$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC$_{50}$ ± SEM</td>
<td>E$_{\text{max}}$ (%)</td>
<td>pEC$_{50}$ ± SEM</td>
<td>E$_{\text{max}}$ (%)</td>
</tr>
<tr>
<td>33</td>
<td>6.07 ± 0.66 (843)</td>
<td>48</td>
<td>8.46 ± 0.30 (3.5)</td>
<td>89</td>
</tr>
<tr>
<td>63</td>
<td>NA</td>
<td>ND</td>
<td>7.42 ± 0.07 (38)</td>
<td>120</td>
</tr>
<tr>
<td>82</td>
<td>NA</td>
<td>ND</td>
<td>7.72 ± 0.08 (19)</td>
<td>93</td>
</tr>
<tr>
<td>83</td>
<td>NA</td>
<td>ND</td>
<td>7.15 ± 0.11 (71)</td>
<td>88</td>
</tr>
<tr>
<td>84</td>
<td>4.76 ± 0.23</td>
<td>170</td>
<td>5.63 ± 0.53</td>
<td>231</td>
</tr>
<tr>
<td></td>
<td>(17400)</td>
<td></td>
<td>(2370)</td>
<td></td>
</tr>
<tr>
<td>CP 55,940 (2)</td>
<td>7.66 ± 0.05 (22)</td>
<td>105</td>
<td>7.40 ± 0.04 (40)</td>
<td>102</td>
</tr>
</tbody>
</table>

$^a$See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. $^b$NA: Not active, defined as <50% activation at 10 μM in the assay. $^c$ND: Not determined; for compounds defined as not active, their maximal effects were not determined.
In line with literature reports, lead compounds \textit{33, 63, 82-84} were generally found to be potent and selective agonists in our assay. Benzimidazole \textit{33} was evaluated as a racemic mixture, which may explain its reduced receptor subtype selectivity in our hands. Pyrazole derivative \textit{82} was found to be the most potent compound at the CB$_2$R, with no functional activity observed at the CB$_1$R. Interestingly, isoxazole \textit{84} exhibited drastically different results in this assay compared to literature reports. Riether and co-workers used a cAMP assay to determine the functional activity of \textit{84} and so differences in data may have arisen from strong functional selectivity. The very high efficacy (E$_{max}$ over 200\%) suggests there are issues with selectivity. Further investigation is planned to look at G protein dependence.

\section*{2.5 Concluding Remarks}

Despite numerous reports of potent and selective CB$_2$R agonists, a unified pharmacophore has not been reported in the literature. We therefore aimed to determine structural similarities between best-in-class agonists, in order to identify features important for functional activity. Two complementary pharmacophore modelling programs were used to develop a preliminary model.

In order to validate the pharmacophore model generated in this chapter, additional agonists not included in the study as well as inactive compounds would need to be screened to determine if the model is predictive of functional activity. Once this is done, chemical libraries could be screened to find potential ligands that fit the model. Evaluation of the functional activities of a truncated library inclusive of compounds or fragments that contain the requisite pharmacophoric features, could potentially identify a number of ‘hits’ and novel leads for the development of CB$_2$R agonists. Such strategies are under continuing investigation in the Kassiou research group.

Pyrazole derivative \textit{82} was found to be the most suitable lead compound candidate based on its potent and selective functional activity at the CB$_2$R. It was therefore chosen as a representative compound to further our pharmacophore studies. Analogues of the lead compound will determine the importance of various structural features for potent and selective functional activity. This information will therefore aid the refinement of a general pharmacophore of CB$_2$R agonists and hence facilitate the rational design of future leads.
Chapter 3: Synthesis and Evaluation of Various Heteroaromatic Benzamides as Potential CB₂ Receptor Agonists
3.1 Introductory Remarks

Having confirmed the reported activity of several agonists, our efforts turned towards systematically exploring the chemical features identified in our pharmacophore model. Considering the potent functional activity and selectivity of azolylidene benzamide 82, it was selected as our lead compound for further investigation. Like many small molecule drugs, CB2R agonists generally contain a central aromatic or heteroaromatic core. Unsurprisingly, our pharmacophore model identified an aromatic group as an important chemical feature. We therefore sought to explore how the identity of the aromatic core influences functional activity.

Whilst the pendent functionalities surrounding the azolylidene benzamide scaffold have been thoroughly investigated by Abbott Laboratories (Figure 28), the influence of the heteroaromatic core is unclear.

![Figure 28: Reported SAR data for CB2R selective agonist 82.](image)

We aimed to expand on the minimal SAR studies focused on the core heterocyclic unit. A library of varying aromatic analogues of 82 were synthesised (110-117) to provide further information pertaining to the size, electronics and other features of the aromatic moiety (Figure 29). As the –ylidene benzamide moiety is only applicable to certain heterocycles, we decided to explore isosteric amides.
Analysis of the lowest energy conformations of the lead compound 82 and amide analogue 118, suggested that substitution of the –ylidene benzamide moiety with an amide would confer a similar arrangement of the two aromatic rings (Figure 30). It was envisioned that the increased conformational freedom of the amide functionality compared to the restricted –ylidene benzamide would result in a reduction in functional activity. However, as this study aims only to compare the substitution of the aromatic core, a reduction in potency was not of concern. In light of results from previous SAR studies, simplified pendent groups (achiral tetrahydropyran and 4-trifluoromethylbenzamide) were also chosen to expedite synthesis and structural characterisation (Figure 29). The study aimed to investigate the number, position and identity of heteroatoms as well as the size of the (hetero)aromatic ring. Synthetic strategies were developed to access these compounds, many of which did not have reliable established syntheses, and their in vitro activity at the cannabinoid receptors was evaluated.
Chapter 3: Synthesis and Evaluation of Various Heteroaromatic Benzamides as Potential CB2 Receptor Agonists

Figure 30: Lowest energy conformations of lead compound 82 (left) and amide analogue 118 (right). Calculated using Avogadro AutoOptimisation, UFF force field.

3.2 Synthesis of Heteroaromatic Benzamide Derivatives

The synthesis of the heteroaromatic amide derivatives (110-117) was achieved via two main approaches, either: 1) direct synthesis of the requisite amino-heterocycle followed by amide coupling, or 2) synthesis of the corresponding carboxylic acid followed by a Curtius rearrangement and treatment of the resulting isocyanate with an aryl Grignard reagent to afford the desired heteroaromatic benzamides.

Pyrroles are notoriously capricious heterocycles and are prone to undergo degradation under a variety of conditions. Initial attempts to synthesise 110 involved synthesis of an appropriately substituted 2-aminopyrrole via a multicomponent heterocycle formation reaction. Frolova and co-workers have reported the synthesis of tetrasubstituted 2-aminopyrroles via a one-pot multicomponent reaction of an aldehyde, N-(alkylsulfonamido)acetophenone and activated methylene compound (Scheme 9).\textsuperscript{193}
Chapter 3: Synthesis and Evaluation of Various Heteroaromatic Benzamides as Potential CB2 Receptor Agonists

Scheme 9: Multicomponent synthesis of tetr substi tuted 2-aminopyrroles.

We wondered if the scope of this reaction could be extended to N-(alkylsulfonamido) acetates. It was envisioned that the two ester groups could be removed after formation of the 2-aminopyrrole scaffold via decarboxylation to afford the appropriately substituted pyrrole. Treatment of commercially available ethyl glycinate hydrochloride with mesyl chloride afforded ethyl 2-(methylsulfonamido) acetate (119, Scheme 10).

Scheme 10: Synthesis of 119. Reagents and conditions: (a) MsCl, Et3N, MeCN, 0 °C-rt, 16 h, 34%.

Under the mild reported conditions, the reaction of 119, commercially available pivalaldehyde and ethyl cyanoacetate afforded exclusively the Knoevenagel condensation intermediate 120 as determined by NMR spectroscopy (Scheme 11). In light of this, multiple attempts were made to promote the subsequent reaction of the Knoevenagel condensation product with 119 to form the 2-aminopyrrole scaffold. The use of a Lewis acid (TiCl4) or various bases (DBU and NaOEt) failed to provide the desired product.

Scheme 11: Proposed synthetic route to access the suitably substituted 2-aminopyrrole. Reagents and conditions: (a) K2CO3, EtOH, reflux, 16 h, 88%.
It is assumed that the low acidity of the protons adjacent to the ester in 119 (predicted $pK_a = 23.7$) compared to acetophenone derivatives (predicted $pK_a = 15.4$) is the reason for the poor outcome of the reaction (Figure 31). Another approach was taken where deprotonation of a di-Boc-protected species 121 using a strong base and reaction with Knoevenagel condensation product 120 was anticipated to form an adduct poised for intramolecular condensation after protecting group removal to form the desired aminopyrrole (Scheme 12).

![Figure 31: Predicted $pK_a$'s of $N$-(alkylsulfonamido)acetophenones and $N$-(alkylsulfonamido or Boc)acetates 119 and 121. $pK_a$'s were predicted using MarvinSketch 14.4.3.](image)

Initial screens of weak bases ($K_2CO_3$, DBU and $Et_3N$) at elevated temperatures failed to promote the Michael addition, likewise, trialling strong, non-nucleophilic bases (LiHMDS, LDA and NaH) failed to produce the desired compound, with no reaction observed.

![Scheme 12: Proposed synthetic route to 2-aminopyrroles using di-Boc protected glycine. Reagents and conditions: (a) (Boc)$_2$O, NaHCO$_3$, MeCN, rt, 16 h, 83%; (b) (Boc)$_2$O, $Et_3N$, DMAP, CH$_2$Cl$_2$, rt, 72 h, 71%.](image)
Due to the problems associated with formation of the pyrrole scaffold, it was decided to employ synthetic approaches involving the substitution of readily available pyrrole. Initial attempts to install the 2-amino group entailed nitrination of pyrrole followed by reduction of the nitro group. There are a number of reports for the nitration of pyrrole using ceric ammonium nitrate (CAN) or triphenylphosphine, bromine and silver nitrite.\(^{194}\) Yields of 2-nitropyrrrole using these methods were poor and the obtained nitro product was not stable. The presence of an electron-donating group on an already electron-rich nucleus makes 2-aminopyrroles unstable unless the ring is substituted with electron withdrawing groups.\(^{195}\) The known instability of electron-rich pyrroles meant that access to the requisite 2-aminopyrrole was not possible. We therefore sought to bypass any 2-aminopyrrole precursors and access the desired 2-amidopyrrole directly from a stable starting material. Installation of the requisite tert-butyl group at the 3-position of the pyrrole was found to be possible after benzyl sulfonyl protection and Friedel-Crafts alkylation with tert-butyl chloride and aluminium chloride to afford 124 (Scheme 13).\(^{196}\) From here, benzene sulfonyl deprotection using magnesium under ultrasonic irradiation,\(^{197}\) and N-alkylation with tetrahydro-2H-pyran-4-yl)methyl tosylate (125), which was formed by tosylation of the commercially available alcohol under standard conditions, afforded the desired disubstituted pyrrole 126. The moderate yield of the product can be attributed to both elimination of the tosyl group to form the undesired alkene and also reaction at the 2- and 5-position of the pyrrole.

![Scheme 13: Synthesis of pyrrole derivative 110. Reagents and conditions: a) Bu₄NHSO₄, benzene sulfonyl chloride, NaOH (aq.), CH₂Cl₂, rt, 24 h, 99%; (b) t-BuCl, AlCl₃, CH₂Cl₂, rt, 2 h, 81%; (c) i) Mg, NH₄Cl, MeOH, 1 h, ii) NaH, Bu₄NBr, tetrahydro-2H-pyran-4-yl)methyl tosylate (125), DMF, 0–50 °C, 16 h, 52%.]

Recently, Brachet and co-workers reported the visible light C–H amidation of heteroarenes with benzoyl azides.\(^{198}\) We envisioned that this reaction could be applicable to our particular system based on the substrate scope reported by the authors. The reaction is assumed to proceed through a reactive nitrene species. Upon sensitisation, the benzoyl
azide loses dinitrogen to afford the nitrene and in the presence of hydrogen donors, benzamides are produced (Scheme 14).

Scheme 14: Proposed mechanism for the visible light mediated amidation of heteroarenes with benzoyl azides.

The requisite ruthenium catalyst (tris(bipyridine)ruthenium(II) chloride) was prepared from ruthenium chloride using the procedures reported by Broomhead and co-workers. Under the conditions reported by Brachet and co-workers, using 4-(trifluoromethyl)benzoyl azide (formed from the corresponding carboxylic acid by reaction with diphenyolphosphoryl azide (DPPA)) and pyrrole 126, no desired reaction occurred (Scheme 15). The only product that was observed was a dimeric urea product, which is proposed to form by thermal decomposition of the acyl azide via a Curtius rearrangement.

Scheme 15: Failed synthesis of pyrrole derivative 110 using photoredox catalysis.

Scheme 16 shows an alternative approach, whereby a Curtius rearrangement of a stable pyrrole-2-carboxylic acid precursor could afford the challenging molecular synthon. Reaction of 126 with trichloroacetyl chloride followed by hydrolysis under basic conditions gave the requisite 2-substituted carboxylic acid 128. The amide was formed via a Curtius rearrangement using a three-step procedure. Formation of the acyl azide with DPPA
followed by heating at 90 °C for 1.5 hours gave clean conversion to the corresponding isocyanate. The isocyanate was treated with 4-trifluoromethylphenylmagnesium chloride to afford the desired amide 110 in moderate yield (45% over 3 steps).

**Scheme 16:** Synthesis of pyrrole derivative 110. Reagents and conditions: (a) Trichloroacetyl chloride, pyridine, THF, rt, 6 h, 97%; (b) NaOH (aq.), MeOH, reflux, 16 h, 92%; (c) i) DPPA, Et3N, CH2Cl2, rt, 16 h, ii) MePh, 90 °C, 1.5 h, iii) (4-(Trifluoromethyl)phenyl)magnesium chloride, 0 °C–rt, 1 h, 45%.

Pyrazole derivative 111 was synthesised via 5-aminopyrazole 132 using standard procedures (Scheme 17). Mono-substituted hydrazine 131 was synthesised via hydrazone 129 by reaction of tetrahydro-2H-pyran-4-carbaldehyde and tert-butyl carbazate followed by sodium cyanoborohydride reduction and Boc group deprotection. Reaction of the hydrazine hydrochloride salt 131 and 4,4-dimethyl-3-oxopentanenitrile (88) afforded 5-aminopyrazole 132. Finally, amide coupling with 4-trifluoromethylbenzoyl chloride yielded the desired amide 111.

**Scheme 17:** Synthesis of pyrazole derivative 11. Reagents and conditions: (a) t-Butyl carbazate, MgSO4, MeOH, rt, 16 h, 63%; (b) NaCNBH3, TsOH.H2O, THF, rt, 16 h, 87%; (c) HCl, dioxane, MeOH rt, 16 h, 100%; (d) 4,4-Dimethyl-3-oxopentanenitrile (88), EtOH, reflux, 16 h, 52%; (e) 4-Trifluoromethylbenzoic acid, oxalyl chloride, DMF, CH2Cl2, rt, 4 h, ii) (iPr)2EtN, CH2Cl2, rt, 16 h, 72%.

Imidazole derivative 112 was synthesised via 2-aminoimidazole 134 (Scheme 18). Bromination of pinacolone gave α-bromoketone 133 which was added slowly to an excess
of 4-methanamine tetrahydropyran at -78 °C to afford the secondary amine 134 in moderate yield. Condensation with cyanamide gave the requisite 2-aminoimidazole (135), which was converted to amide 112 via an acid chloride amide coupling.

Scheme 18: Synthesis of imidazole derivative 112. Reagents and conditions: (a) Br₂, MeOH, -40 °C–rt, 30 min, 83%; (b) 4-Methanamine tetrahydropyran, diethyl ether, -78 °C–rt, 16 h, 43%; (c) Cyanamide, EtOH, reflux, 16 h, 94%; (d) 4-Trifluoromethylbenzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 4 h, ii) iPr₂EtN, CH₂Cl₂, rt, 16 h, 65%.

In a similar fashion, triazole 113 was synthesised via amino species 138 (Scheme 19). Several attempts were made to access the desired 5-aminotriazole. The procedures reported by Yin and co-workers for the cyanoimidation of aldehydes using cyanamide as a nitrogen source and NBS as an oxidant were trialled, however the substrate scope for their reaction appears to be restricted to aromatic aldehydes. Under the reported conditions only a dimerized tetrazine product was obtained as confirmed by mass spectrometry and NMR spectroscopy. We therefore sought to synthesise a cyanoimidate species, which are common precursors to aminotriazoles. A Pinner reaction with pivalonitrile (hydrogen chloride was formed in situ by reaction of ethanol with acetyl chloride) afforded the imidate salt (136), which reacted with cyanamide to form cyanoimidate 137 under buffered conditions. Reaction with hydrazine 131 afforded the poorly nucleophilic 5-aminotriazole 138, which required deprotonation with sodium hydride and elevated temperatures to react with 4-trifluoromethylbenzoyl chloride to yield amide 113.

Scheme 19: Synthesis of triazole derivative 113. Reagents and conditions: (a) EtOH, AcCl, 0 °C–rt, 16 h, 79%; (b) Cyanamide, Na(OH)₂PO₄, H₂O, Na₂HPO₄, 7H₂O, MeCN, rt, 72 h, 51%; (c) Hydrazine 131, DBU, MeOH, reflux, 16 h, 78%; (d) 4-Trifluoromethylbenzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 4 h, ii) NaH, MePh, reflux, 3 h, 75%.
It was envisioned that oxazole 114 and thiazole 115 could be formed from a common \( \beta \)-carbonyl amide intermediate (142) (Scheme 20). To access this intermediate several routes were investigated, however it was found that \( \beta \)-carbonyl amines were generally unstable and difficult to synthesise. To avoid this instability, we aimed to access the requisite amide in a one-pot fashion from a stable amine precursor. Due to their ease of synthesis from \( \beta \)-ketoesters and stability, we chose to synthesise an oxime precursor. A Horner-Wadsworth-Emmons olefination of dihydro-2H-pyran-4(3H)-one with 2-oxopropyl dimethyl ester phosphonic acid followed by reaction of the enolate with Mander’s reagent afforded \( \beta \)-ketoester 141 after selective hydrogenation of the alkene under hydrogen transfer conditions.\(^{204}\) Oxime formation using sodium nitrite followed by hydrogenation under acidic conditions afforded the stable amine hydrochloride salt, which reacted cleanly with pivalic anhydride (formed from pivalic acid according to the procedures reported by Bartoli and co-workers)\(^{205}\) to yield the amide common intermediate 142. Cyclodehydration of the \( \beta \)-ketoamide using triphenylphosphine, iodine and triethylamine\(^{206}\) afforded substituted oxazole 143 while thiazole 144 was formed by reaction with Lawesson’s reagent under elevated temperatures.\(^{207}\) Ester hydrolysis under basic conditions, followed by reaction with diphenylphosphoryl azide afforded the requisite acyl azides, which underwent Curtius rearrangements at elevated temperatures. Addition of an appropriate Grignard reagent to the intermediate isocyanates afforded amides 114 and 115.
Scheme 20: Synthesis of oxazole and thiazole derivatives 114 and 115. Reagents and conditions: (a) KOH, 2-oxopropyl dimethyl ester phosphonic acid, EtOH, 0 °C–rt, 5 h, 95%; (b) LDA, methyl cyanoformate, THF, -78 to -40 °C, 1 h, 58%; (c) NH$_4$HCO$_2$, Pd/C, MeOH, rt, 3 h, 95%; (d) i) NaNO$_2$, AcOH, H$_2$O, -5 °C–rt, 2 h, ii) HCl, Pd/C, H$_2$, EtOH, rt, 16 h, iii) Pivalic anhydride, Et$_3$N, CH$_2$Cl$_2$, rt, 16 h; (e) PPh$_3$, I$_2$, Et$_3$N, CH$_2$Cl$_2$, rt, 3 h, to give 143, 60% over 4 steps; (f) Lawesson’s reagent, THF, reflux, 3 h, to give 144, 40% over 4 steps; (g) NaOH (aq.), THF, MeOH, rt, 4 h, 78-95%; (h) i) DPPA, Et$_3$N, CH$_2$Cl$_2$, rt, 16 h, ii) MePh, 90 °C, 1.5 h, iii) 4-(Trifluoromethyl)phenyl)magnesium chloride, 0 °C–rt, 1 h, 22-30%.

For the synthesis of phenyl and pyridyl derivatives 116 and 117, it was envisioned that the tetrahydropyran group could be incorporated using cross-coupling chemistry. Reliable methods to achieve sp$^2$-sp$^3$ cross-couplings are sparsely reported. The stability of sp$^3$ organotin reagents means that the Stille reaction is a potential candidate to achieve the desired bond formation, however, the high toxicity of organotin reagents is undesirable. We therefore decided to utilise sp$^2$-sp$^2$ Suzuki cross coupling to install the requisite tetrahydropyran group. Bromination of commercially available 4-(tert-butyl)aniline, amide coupling and finally a Miyaura borylation afforded cross-coupling partner 149 (Scheme 21). It was found that for the Miyaura coupling, strictly anhydrous conditions were required to avoid homocoupling or dehalogenation. A Suzuki cross-coupling with 4-(bromomethylene)tetrahydro-2H-pyran (150), which was formed by a Wittig-type reaction of dihydro-2H-pyran-4(3H)-one with (bromomethyl)triphenylphosphonium bromide, achieved the key carbon-carbon bond formation. Hydrogenation of the resulting alkene (151) gave the final product 116.
Scheme 21: Synthesis of phenyl derivative 116. Reagents and conditions: (a) NBS, DMF, 0 °C–rt, 16 h, 78%; (b) 4-Trifluoromethylbenzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 4 h; ii) (iPr)₂EtN, CH₂Cl₂, rt, 16 h, 75%; (c) KOAc, Pd(dppf)Cl₂, (Bpin)₂, MePh, 90 °C, 16 h, 79%; (d) (bromomethyl)triphenylphosphonium bromide, KOtBu, THF, -78 °C to -40 °C, 3 h, 37%; (e) K₂CO₃, Pd(PPh₃)₄, DMF, H₂O, 80 °C, 4 h, 92%; (f) Pd/C, H₂, EtOAc, 16 h, 86%.

tert-Butyl-substituted aminopyridine 153 is not readily available and hence required synthesis in order to access pyridine derivative 117 (Scheme 22). Reaction of Boc-protected 2-amino-5-bromopyridine with an excess of tert-butyl magnesium chloride and copper cyanide afforded the desired alkylated product 152 in low, but sufficient yield. After trialling numerous reaction conditions it was found that the Suzuki cross-coupling reaction was only successful when the coupling partners were reversed compared to the phenyl derivative 116 (coupling of heteroaryl bromide 154 and vinyl boronate ester 155) and performed before the amide was formed. Optimised conditions for the reaction were adopted from a report by Hanazawa and co-workers using Pd(dppf)Cl₂ and aqueous potassium hydroxide as the base. Interestingly, amide coupling via the requisite acid chloride yielded exclusively the imide product. Therefore, a one-pot procedure was developed to form the imide, but also hydrolyse the imide to form the desired amide 157. A Schotten-Baumann reaction was performed using aqueous sodium hydroxide and once the imide had been observed by thin-layer chromatography, tetrahydrofuran and tetrabutylammonium bromide were added to form a homogeneous mixture and facilitate imide hydrolysis. Finally, hydrogenation of the alkene yielded the final product 117.
Scheme 22: Synthesis of pyridine derivative 117. Reagents and conditions: (a) t-BuMgCl, CuCN, THF, -78 °C–rt, 16 h, 19%; (b) TFA, CH₂Cl₂, rt, 2 h, 100%; (c) NaOAc, Br₂, AcOH, rt, 3 h, 69%; (d) t-BuLi, isopropyl pinacol borate, THF, -78 °C–rt, 2.5 h, 63%; (e) KOH (aq.), Pd(dppf)Cl₂, THF, 60 °C, 16 h, 69%; (f) (i) 4-Trifluoromethylbenzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 4 h, ii) NaOH (aq.), CH₂Cl₂, 0 °C–rt, 2 h, iii) Bu₄NBr, THF, rt, 16 h, 87%; (g) Pd/C, H₂, EtOAc, 16 h, 91%.

3.3 In vitro Evaluation of Heteroaromatic Benzamides

The heteroaromatic amides (110-117) were assessed for their ability to activate the CB₁ and CB₂ receptors using a Fluorescence Imaging Plate Reader (FLIPR) membrane potential assay in AtT-20 cells expressing human CB₁ or CB₂ receptors. Compounds displaying more than 40% activation at 10 μM in the assay were evaluated further in dose-response studies. Their half maximal effective concentrations (EC₅₀) and maximal effect relative to CP 55,940 (2) (Eₘₐₓ) were calculated as listed in Table 3. Further experimental details are provided in Chapter 8.

Unfortunately, most of the (hetero)aromatic amide derivatives displayed no activity at the either of the CBRs. Pyrrole derivative 110 was the only exception, possessing micromolar activity at the CB₂R. We consequently decided to investigate the structural basis for the observed loss of activity. We assumed the dramatic loss of activity was due to either substitution of the –ylidene-benzamide functionality for a benzamide or the specific combination of pendent groups used.
Table 3: Agonist activities of lead compound (82) and heteroaromatic amide derivatives (110-117) in AtT-20 cells expressing human CB1 or CB2 receptors by a FLIPR membrane potential assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB1</th>
<th></th>
<th></th>
<th></th>
<th>CB2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC50 ± SEM (EC50 nM)</td>
<td>Emax (%)</td>
<td>pEC50 ± SEM (EC50 nM)</td>
<td>Emax (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>NA</td>
<td>ND</td>
<td>7.72 ± 0.08 (19)</td>
<td>93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>NA</td>
<td>ND</td>
<td>5.61 ± 0.24 (2470)</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>111</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>112</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP 55,940 (2)</td>
<td>7.67 ± 0.04 (21)</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.50 ± 0.05 (32)</td>
<td>101</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. **NA: Not active, defined as <40% activation at 10 μM in the assay. **ND: Not determined; for compounds defined as not active, their maximal effects were not determined.

3.4 Further SAR Studies to Investigate the Inactivity of Heteroaromatic Benzamide Analogues

Considering the structural similarities of the lead compound 82 and heteroaromatic benzamides 110-117 it was surprising that no functional activity was observed for the amide analogues. In order to explore the potential reasons for this disparity, we proposed a small library of compounds (Figure 32) to determine the structural features of the lead compound that are necessary for functional activity.
In order to explore the importance of the –ylidene benzamide functional group, a direct analogue bearing an amide moiety (118) was synthesised. To explore the importance of the chiral tetrahydrofuran group, the corresponding tetrahydropyran derivative was synthesised (158). –Ylidene benzamide (159) and amide (160) analogues that do not contain the polar tertiary alcohol group were also synthesised to explore the importance of this group.

### 3.4.1 Synthesis of Lead Compound Analogues

The synthesis of direct amide analogue 118 could be easily achieved from the previously synthesised amide 90 by a nucleophilic aromatic substitution reaction with diol 93 (Scheme 23).

![Scheme 23: Synthesis of amide analogue 118. Reagents and conditions: (a) 2-Methylpropane-1,2-diol (93), KOtBu, 0 °C–reflux, 4 h, 72%.

Tetrahydropyran derivative 158 followed procedures analogous to what was reported for the tetrahydrofuran analogue 118 (Scheme 24).
Chapter 3: Synthesis and Evaluation of Various Heteroaromatic Benzamides as Potential CB2 Receptor Agonists

Scheme 24: Synthesis of tetrahydropyran analogue 158. Reagents and conditions: (a) i) 2-Fluoro-5-(trifluoromethyl)benzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 2 h, ii) iPr₂EtN, CH₂Cl₂, rt, 16 h, 85%; (b) 2-Methylpropane-1,2-diol (93), KOtBu, 0 °C–reflux, 3 h, 81%.

Analogues not bearing the polar aryl ether group were synthesised in a similar fashion from 5-aminopyrazole 89 (Scheme 25).

Scheme 25: Synthesis of analogues 159 and 160. Reagents and conditions: (a) i) 3-(Trifluoromethyl)benzoic acid, oxalyl chloride, DMF, CH₂Cl₂, rt, 2 h, ii) iPr₂EtN, CH₂Cl₂, rt, 16 h, 78%; (b) Methyl triflate, PhMe, reflux, 16 h, 69%.

3.4.2 In vitro Evaluation of Lead Compound Analogue

The four analogues 118, 158-160 were assessed for their ability to activate the CB₁ and CB₂ receptors and the data is summarised in Table 4.
Table 4: Agonist activities of lead compound 82 and analogues 118, 158-160 in AtT-20 cells expressing human CB1 or CB2 receptors by a FLIPR membrane potential assay\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB1 pEC\textsubscript{50} ± SEM (EC\textsubscript{50} nM)</th>
<th>E\textsubscript{max} (%)</th>
<th>CB2 pEC\textsubscript{50} ± SEM (EC\textsubscript{50} nM)</th>
<th>E\textsubscript{max} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
<td>NA 7.72 ± 0.08 (19)</td>
<td>ND</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>118</td>
<td>NA 7.64 ± 0.06 (23)</td>
<td>ND</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>NA 5.12 ± 0.16 (7490)</td>
<td>ND</td>
<td>7.64 ± 0.06 (23)</td>
<td>ND</td>
</tr>
<tr>
<td>159</td>
<td>5.12 ± 0.16 (7490)</td>
<td>ND</td>
<td>7.64 ± 0.06 (23)</td>
<td>ND</td>
</tr>
<tr>
<td>160</td>
<td>NA 7.67 ± 0.04 (21)</td>
<td>ND</td>
<td>7.50 ± 0.05 (32)</td>
<td>101</td>
</tr>
</tbody>
</table>

\textsuperscript{a}See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. \textsuperscript{b}NA: Not active, defined as <50% activation at 10 μM in the assay. 'ND: Not determined; for compounds defined as not active, their maximal effects were not determined.

Analysis of the functional activity data for this small library of compounds clearly shows the importance of the –ylidene benzamide moiety for activity at the CB\textsubscript{2}R (Table 4). Amide 118, which possesses a substitution pattern identical to lead compound 82 failed to exhibit any activity at the cannabinoid receptors. This is quite surprising considering the near identical structure and expected lowest energy three-dimensional conformations these compounds would expect to adopt. Considering the importance of this functionality for activity it was not surprising that amide-linked tetrahydropyran 158 or 160, which lacks both the ether group and –ylidene amide functionality were inactive. Interestingly, lead compound 82 and 159 were near equipotent at the CB\textsubscript{2}R, highlighting that the ether group at the 2-position of the benzamide can tolerate substitution without affecting activity. Some functional activity however was also observed at the CB\textsubscript{1}R. This suggests that the ether group functions to modulate physicochemical properties (particularly lipophilicity) and improve the receptor subtype selectivity within this class of compounds.

3.5 Docking Studies

To investigate the molecular basis for their differing functional activity, lead compound 82 and direct amide analogue 118 were subjected to docking simulations. For the docking
study we used the first and only reported crystal structure of the CB₂R, recently published by Li and co-workers.⁴⁰ Similar docking scores and near identical binding poses were observed for both –ylidene benzamide 82 and amide 118 (Figure 33). A caveat to the simulations performed is that the inactive cannabinoid receptor structure, solved with an antagonist bound, is likely unsuitable for predicting agonist interactions. Different binding modes and interactions with key residues may have been observed if the active state structure was modelled. Unfortunately, at the time of writing this thesis the active state crystal structure of the CB₂R has not been solved. In light of the recent report of the active state crystal structure of the CB₁R,²⁷ it is expected the structure of the CB₂R will be reported shortly. Another major general limitation of docking studies is that the ligand and receptor are treated as static objects. In reality, binding to and activation of a receptor is a dynamic process and there are many factors to consider in addition to the enthalpy of binding to the receptor. While the predicted lowest energy conformations of the two compounds may overlap, consideration of the entropy of binding may shed some light on the differences in functional activity. As 82 is a more rigid structure on account of the –ylidiene amide moiety, a lower entropy penalty would be expected upon binding. More in depth molecular dynamics and mutagenesis studies would be required to elucidate the molecular basis for the lack of functional activity of amide 118 despite its structural similarity to the lead compound 82.²¹⁰

Figure 33: Docking mode of lead compound 82 (yellow) and amide analogue 118 (green) in the CB₂R active site. The two structurally similar compounds exhibit almost identical binding poses and docking scores (82: -10.7 kcal mol⁻¹; 118: -10.8 kcal mol⁻¹).
The substrate scope reported by Abbott Laboratories included a few heterocycles, such as thiazoles (for example 83), which are amenable to the –ylidene benzamide moiety. In cases such as thiazole derivatives, methylation of the heterocycle is not necessary to obtain the desired –ylidene benzamide. This suggests that the methyl group at the 2-position of lead compound 82 is not imperative for activity, but rather functions to ensure the formation of the –ylidene benzamide functionality. However, to investigate this claim, we attempted to synthesise 5-methylimidazole derivative 162, which would be a direct analogue of pyrazole 82 but with the addition of a methyl group akin to lead compound 82 (Figure 34).

The proposed synthesis of this analogue involved condensation of commercially available 1-acetyl guanidine with the appropriate α-bromoketone 164 to afford the protected 2-amino-5-methylimidazole (Scheme 26). Synthesis of α-bromoketone 164 involved a two-step process from readily available trimethyl acetamide. Reaction of trimethyl acetamide with 4 equivalents of ethyl magnesium bromide afforded the desired ketone 163 after fractional distillation. Bromination using copper(II) bromide in a mixture of refluxing ethyl acetate and chloroform (1:1) provided the α-bromoketone 164 in good yield. Reaction of 164 with 1-acetyl guanidine at elevated temperatures afforded 165 in low, but workable yield. Unfortunately, all attempts to further this synthetic route by N-alkylation or amidation after acetamide deprotection were largely unsuccessful resulting only in the formation a complex mixture of products in each case. A new synthetic route to access this analogue is yet to be developed.
Scheme 26: Attempted synthesis of 2-amido-5-methylimidazole analogue 162. Reagents and conditions: (a) EtMgBr, Et₂O, reflux, 16 h, 66%; (b) CuBr₂, EtOAc, CH₃Cl, reflux, 16 h, 74%; (c) 1-Acetyl guanidine, DMF, 80 °C, 16 h, 26%.

3.6 Concluding Remarks

In order to explore the functional significance of the heteroaromatic core of lead compound 82 we prepared a series of heteroaromatic benzamide analogues 110-117. Unfortunately, all the amide analogues, apart from pyrrole derivative 110, were inactive at the cannabinoid receptors. To further explore this we synthesised a small library of analogues 118, 158-160. The inactivity of direct amide analogue 118 suggests the –ylidene amide moiety is imperative for functional activity (Figure 35). The reason for the disparity in functional activity of –ylidene benzamide derivatives and amides is not immediately obvious and warrants further investigation.

Figure 35: The –ylidene amide functionality was shown to be imperative for functional activity.

Our further investigations into the structural features of lead compound 82 that are important for functional activity have focused on the linkage between the pyrazole and substituted phenyl group. Factors such as planarity, position and identity of heteroatoms and hydrogen bonding were investigated. The results of this investigation are detailed in the following chapter.
Chapter 4: Exploration of Variable Linkage Analogues of Pyrazoylidene Benzamides
4.1 Introductory Remarks

In Chapter 3 it was demonstrated that the –ylidene benzamide moiety of lead compound 82 is essential for functional activity. Preliminary docking studies were unsuccessful in determining the molecular basis for the paucity of functional activity of amide analogue 118. To investigate this interesting result, we aimed to conduct a structure-activity relationship study focusing on the –ylidene benzamide motif (Figure 36).

Several analogues (166–184) were proposed to explore the linkage between the pyrazole and substituted phenyl group as outlined in Figure 37. The rigidity, number, position and identity of heteroatoms and to some extent the length of linker was explored. The suitability of these replacements as bioisoteres of the –ylidene benzamide motif depends on the role the moiety plays in eliciting the biological activity of 82 and the availability of the bioisostere to mimic this role. It is possible that the hydrogen bonding atoms of the –ylidene amide are necessary for functional activity, or rather, the group may only function as a spacer between the two aromatic groups whilst simultaneously controlling the three dimensional conformation of the molecule. In the latter case, the electronic properties or hydrogen bonding ability of the linker moiety would not be important. Consideration of the pharmacophore model developed in Chapter 2 suggests that a hydrogen bond donating moiety or region of negative electrostatic potential between the aromatic pyrazole and hydrophobic substituted phenyl group is favourable for functional activity.

The absence of a signal in the carbonyl stretching region of the infrared spectrum (1650-1750 cm⁻¹) of -ylidene benzamide analogues, such as lead compound 82, indicates that these compounds may exist in a zwitterionic resonance form. For inactive amide analogues such as 118 the neutral carbonyl species would exist as the major resonance contributor as...
indicated by the stretching frequency at 1665 cm\(^{-1}\). This may explain the stark difference in functional activity at the CB\(_2\)R and warrants further investigation.

![Proposed analogues](image)

**Figure 37:** Proposed analogues (166-184) to explore the linkage between the pyrazole and substituted phenyl group.
The linker unit of the azoylidene benzamide lead compound 82 does not contain a hydrogen bond donor, while amide analogue 118 does. It is possible that the NH hydrogen bond donor is not tolerated in the binding site of the CB2R. Methylation of the NH group of the amide to afford 166 would prevent hydrogen bond donation, however it would be expected to also change the three-dimensional shape of the molecule. A better way to explore this potential hydrogen bonding interaction is bioisosteric replacement of the amide with an ester (167). Evidently esters are not ideal functional groups for drug molecules as they are generally rapidly cleaved in vivo, however in this case exploration of this analogue is merely to explore structure-activity relationships. The ester, like the –ylidene benzamide, does not contain a hydrogen bond donor. The ester does differ however in the planarity of the functional group. Unlike an amide where the lone pair of electrons on the nitrogen are delocalised into the carbonyl group, thus restricting rotation around the carbon-nitrogen bond, the corresponding carbon-oxygen bond of an ester has less double bond character and therefore has a lower rotational energy barrier.

Amide analogue 168 aims to investigate the positioning of the heteroatoms and hydrogen bond donors and acceptors by the reversal of the amide direction. As the bonds that possess some planarity due to restricted rotation (carbonyl carbon-nitrogen bond) have been shifted in this structure, the three-dimensional shape of the molecule would also be somewhat dissimilar to amide 118. The strategy to reverse the amide direction has been shown in some cases to drastically change the pharmacological profile of a drug as discussed in Chapter 6.

Exploration of sulfur containing bioisosteres was also conducted. Thioamide derivative 169 would be expected to adopt a similar conformation to amide 118, however due to the different properties of sulfur and oxygen, the hydrogen bonding interactions are expected to be different. Sulfur is not as electronegative as oxygen (2.58 vs 3.44), has a larger covalent radius (1.02 vs 0.73 Å) and forms longer, weaker bonds with carbon (C=S 140 kcal mol⁻¹ vs C=O 180 kcal mol⁻¹). The small electronegativity difference between sulfur and carbon and the large size of sulfur means that charge transfer from nitrogen to sulfur in thioamides is greater than the corresponding nitrogen to oxygen charge transfer in amides. The rotational barrier for thioamides is therefore larger than for amides. The
hydrogen bonding abilities of sulfur differs from oxygen in terms of both strength and directionality. Hydrogen bonds to sulfur are generally weaker and also show a marked preference for a more “perpendicular” direction of approach to the donor atom.\textsuperscript{213} Lee and co-workers have calculated the hydrogen bonding abilities of thioamides and have demonstrated that thioamides are better hydrogen bond donors than amides, while conversely they are poorer hydrogen bond acceptors.\textsuperscript{214} Sulfonamide derivative 170 would also be expected to probe the topology of heteroatoms and hydrogen bonding trajectories. The linker length of \textit{N}-acyl sulfonamide 171 is increased and differs in the number and arrangement of heteroatoms. In addition, the NH proton is quite acidic (pK\textsubscript{a} approximately 5)\textsuperscript{215} and would be expected to exist as the anion at physiological pH. Similarly, urea analogue 172 extends the length of the linker and increases the number of heteroatoms available for hydrogen bonding. Ureas are comparable to amides in that the lone pair of electrons on the nitrogen are delocalised into the carbonyl group. Therefore, the carbonyl carbon-nitrogen bonds exhibit some double bond character and rotation is restricted around this bond.

Amine derivatives 173 and 174 explore the removal of the carbonyl oxygen and the planarity of the linker group. It was envisioned that benzyl amine 173 could be synthesised from amide 118 in a single step via reduction of the amide moiety. The methylene linker has rotational freedom and so it would be expected that a large entropy penalty would occur upon binding to the receptor. Direct nitrogen linked aniline analogue 174 also shortens the distance between the pyrazole and substituted phenyl group. It is likely that the two aromatic rings would not lie in the same plane, but rather would skew to avoid steric interactions.

The application of 5-membered heterocycles as amide bioisosteres is a common strategy in medicinal chemistry.\textsuperscript{211} The introduction of the heterocyclic unit often increases structural rigidity, leading to improved potency, selectivity and metabolic stability. The use of cross coupling and “click” chemistry for the synthesis and functionalisation of 5-membered heterocycles is favourable for the generation of large libraries of compounds for further development. Two bioisosteric amide analogues, furan 175 and 1,2,3-triazole 176 were chosen to explore the identity and positioning of the heteroatoms around the 5-membered aromatic linker unit. The oxygen atom of furan derivative 175 could possibly mimic the
carbonyl oxygen of the lead compound 82, however the exact positioning of these two atoms would be slightly different in the two derivatives. Triazole 176, which could be accessed by convenient “click” chemistry, contains three heteroatoms available for hydrogen bonding interactions and further probes the electronic requirements of the heterocyclic group.

Hydrocarbon analogues 177-180 were proposed to explore the importance of heteroatoms within the linker unit. Alkane 180, alkene 179 and alkyne 178 explore rigidity and planarity without the incorporation of heteroatoms. While the alkene and alkyne groups would be expected to afford a relatively planar structure, alkane 180 would possess high conformational flexibility. While this would allow the structure to access the requisite conformation for binding to the CB₂R, the significant entropy penalty upon binding would likely have a negative impact on potency. The requirement for a linker unit was explored by synthesis of directly linked bi-aryl analogue 177. It is envisioned that this analogue could be conveniently accessed by cross-coupling of the two aromatic groups.

5,6-Bicyclic analogues 181-184 were proposed to investigate the effect of enclosing the linker functionality into a six-membered ring. Indazole 181 represents the replacement of the amide with a planar phenyl group containing no heteroatoms. Pyrazolopyrimidine analogue 182 maintains the planarity of the indazole moiety but contains heteroatoms at positions similar to lead compound 82. In a similar way, lactam 183 is a more rigid derivative of reverse amide 168 and fixes the carbonyl oxygen in a particular orientation. Indazole sulfone 184 represents a hybrid structure containing elements of lead compound 82 and benzimidazole sulfone compounds such as 33 discovered by AstraZeneca¹⁰⁵ and studied within the Kassiou research group as potential CB₂R PET-radioligands for imaging neuroinflammation.²¹⁶

4.2 Synthesis of Variable Linkage Analogues

The synthesis of a number of the proposed analogues required orthogonal protection of the tertiary alcohol fragment. For most purposes it was envisioned that a benzyl ether group could be used and removed under hydrogenolysis conditions, however in cases where functionality present was not stable to hydrogenolysis conditions, a p-methoxy benzyl
group was used instead and removed under oxidative conditions. Protected diols 187 and 188 were synthesised using a two-step procedure (Scheme 27). First, reaction of ester 92 with the appropriate benzyl halide afforded benzyl ether derivatives 185 and 186. Reduction of the ester functional group to the primary alcohol using lithium aluminium hydride afforded the requisite mono-protected diols 187 and 188.

**Scheme 27:** Synthesis protected diols. *Reagents and conditions:* (a) NaH, BnBr or PMBCl, DMF, 0 °C–rt, 16 h, 58–70%; (b) LiAlH₄, THF, 0 °C–rt, 2 h, 90–100%.

Methylated amide derivative 166 was obtained from benzyl ether protected amide 189 using methyl iodide and the strong base sodium hydride (Scheme 28). Hydrogenolysis of the protecting group afforded the final product 166.

**Scheme 28:** Synthesis of methylated amide. *Reagents and conditions:* (a) KOtBu, 187, THF, reflux, 16 h, 90%; (b) NaH, Mel, THF, rt, 6 h, 65%; (c) H₂, Pd/C, EtOAc, rt, 16 h, 91%.

Synthesis of ester derivative 167 was achieved by a coupling reaction between hydroxypyrazole derivative 192 and carboxylic acid 193 (Scheme 29). Hydroxypyrazole 192 was synthesised from pinacolone by a Claisen condensation with dimethyl carbonate, followed by condensation with hydrazine 87. Carboxylic acid 193 was obtained by a nucleophilic aromatic substitution of commercially available 2-fluoro-5-
(trifluoromethyl)benzoic acid with protected diol 187. Formation of the acid chloride of 193 using oxalyl chloride and catalytic DMF and reaction with hydroxypyrazole 192 formed the ester linkage. Hydrogenolysis of the benzyl ether protecting group afforded ester analogue 167.

The synthesis of reverse amide 168 required access to carboxylic acid portion 198 and aniline 200 (Scheme 30 and 31). Claisen condensation of pinacolone with diethyl oxalate followed by condensation with hydrazine afforded the requisite 3,5-disubstituted pyrazole 196. N-Alkylation of this group was found to be low yielding using typical substitution chemistry. Therefore, a Mitsunobu reaction using alcohol 85 was attempted. Adopting conditions developed by Seinreiber and co-workers, the desired N-alkylated product 197 was formed cleanly under microwave irradiation within 10 minutes. It was found that dropwise addition of the azo compound at 0 °C was required for successful Mitsunobu reactions. After the slow addition, rapid heating afforded the desired products in high yield. From here, ethyl ester hydrolysis gave the requisite carboxylic acid 198.
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Scheme 30: Synthesis of carboxylic acid fragment 198. Reagents and conditions: (a) Pinacolone, NaOEt, EtOH, reflux, 2 h, 79%; (b) Hydrazine hydrate, EtOH, AcOH, reflux, 2 h, 95%; (c) Alcohol, 85, DIAD, PPh3, 0–100 °C, 10 min, 84%; (d) LiOH, THF, H2O, rt, 16 h, 100%.

Aniline fragment 200 was synthesised from commercially available 1-fluoro-2-nitro-4-(trifluoromethyl)benzene via a nucleophilic aromatic substitution with protected diol 187 and selective nitro reduction using iron and acetic acid. The acid chloride of 198 was formed by treatment with oxalyl chloride and coupled to the aniline 200 to form amide 201. Finally, benzyl ether deprotection afforded reverse amide analogue 168.

Scheme 31: Synthesis of reverse amide analogue 168. Reagents and conditions: (a) Alcohol 187, Cs2CO3, DMF, 60 °C, 16 h, 73%; (b) Fe, AcOH, EtOH, H2O, ), 1 h, 91%; (c) i) 198, oxalyl chloride, DMF, CH2Cl2, rt, 2 h, ii) iPr2EtN, CH2Cl2, rt, 16 h, 52%; (d) H2, Pd/C, EtOAc, rt, 16 h, 90%.

It was envisioned that thioamide derivative 169 could be obtained from amide 118 by treatment with Lawesson’s reagent. However, under standard reaction conditions a number of products were formed. It is assumed that the free tertiary alcohol was the reason for the poor reaction outcome. Therefore, the reaction was attempted with benzyl ether protected derivative 189. Despite clean formation of the thioamide, any attempts to remove the protecting group were unsuccessful. Sulfur is known to poison palladium catalysts, so it is not surprising that palladium catalysed hydrogenolysis was unsuccessful. Removal of a p-methoxy benzyl ether blocking group using DDQ was also troublesome. Under the
oxidative conditions, the thioamide was converted back to the corresponding amide. A different protecting group strategy was therefore required. Protection of tertiary alcohols is notoriously difficult, and any attempts to install a bulky silyl protecting group were largely unsuccessful. It was found that a tetrahydropyran (THP) group could be installed using triphenylphosphine hydrobromide as a catalyst after extended reaction time (Scheme 32). The reaction of protected species 202 and Lawesson’s reagent at elevated temperature fortuitously afforded the thioamide and also removal of the protecting group to give 169.

Scheme 32: Synthesis of thioamide analogue 169. Reagents and conditions: (a) DHP, PPh₃.HBr, CH₂Cl₂, rt, 72 h, 61%; (b) Lawesson’s reagent, PhMe, reflux, 16 h, 43%.

Sulfonamide derivative 170 was synthesised in a simple two-step procedure (Scheme 33). Coupling of aminopyrazole 89 and commercially available 2-fluoro-5-(trifluoromethyl)benzene-1-sulfonyl chloride afforded the requisite sulfonamide. Nucleophilic aromatic substitution with diol 93 provided the final product 170.

Scheme 33: Synthesis of sulfonamide 170. Reagents and conditions: (a) Pyridine, DMAP, CH₂Cl₂, reflux, 96 h, 50%; (b) Diol 93, KOtBu, THF, reflux, 16 h, 67%.

N-Acyl sulfonamide derivative 171 was accessed through a coupling reaction between primary amide 204 and 2-fluoro-5-(trifluoromethyl)benzene-1-sulfonyl chloride (Scheme 34). The primary amide 204 was synthesised from carboxylic acid 198 via the corresponding acid chloride. Deprotonation of the primary amide with sodium hydride,
followed by treatment with the sulfonyl chloride at elevated temperature afforded the \( N \)-acyl sulfonamide linkage. From here, nucleophilic aromatic substitution with protected diol \( 187 \) and benzyl ether hydrogenolysis provided the final product \( 171 \).

![Scheme 34: Synthesis of N-acyl sulfonamide 171. Reagents and conditions: (a) i) Oxalyl chloride, DMF, \( \text{CH}_2\text{Cl}_2 \), rt, 2 h, ii) \( \text{NH}_3 \) (aq.), rt, 4 h, 69%; (b) NaH, 2-fluoro-5-(trifluoromethyl)benzene-1-sulfonyl chloride, THF, reflux, 5 h, 53%; (c) Alcohol 187, KOtBu, THF, reflux, 2 h, 55%; (d) \( \text{H}_2 \), Pd/C, EtOAc, rt, 16 h, 93%.]

Initial attempts to synthesise urea derivative \( 172 \) utilised carbonyldiimidazole (CDI) as a coupling reagent. However, the desired product was not obtained, assumedly due to the poor nucleophilicity of aniline \( 200 \). An alternative urea synthesis was attempted involving the addition of aminopyrazole \( 89 \) to an isocyanate (Scheme 35). The requisite isocyanate was accessed via a Curtius rearrangement of the corresponding acyl azide formed from carboxylic acid \( 193 \). At elevated temperature, thin-layer chromatography indicated clean conversion of the acyl azide to the corresponding isocyanate. Addition of aminopyrazole \( 89 \) afforded urea \( 207 \) in moderate yield. Finally, removal of the benzyl ether protecting group provided the urea analogue \( 172 \).
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Scheme 35: Synthesis of urea derivative 172. Reagents and conditions: (a) DPPA, Et$_3$N, PhMe, rt, 30 min; (b) PhMe, 100 °C, 45%; (c) H$_2$, Pd/C, EtOAc, AcOH, rt, 24 h, 88%.

Benzyl amine derivative 173 was easily synthesised from amide 189 by reduction of the amide functional group with lithium aluminium hydride (Scheme 36). Interestingly, the reaction produced an inseparable by-product, which was determined to be a benzyl amine derivative without the phenyl ether substituent. The mechanism for this transformation is unknown, however, it is assumed it occurs via a nucleophilic aromatic substitution pathway. Removal of the benzyl ether protecting group afforded the final product 173, which could be purified by chromatography to remove the by-product from the previous reaction.

Scheme 36: Synthesis of benzyl amine derivative 173. Reagents and conditions: (a) LiAlH$_4$, THF, 0 °C–reflux, 16 h; (b) H$_2$, Pd/C, EtOAc, AcOH, rt, 30 h, 69% over 2 steps.

Aniline derivative 174 was accessed via a Buchwald-Hartwig coupling reaction (Scheme 37). The aryl bromide coupling partner 208 was synthesised from commercially available 2-bromo-4-(trifluoromethyl)phenol by a Mitsunobu reaction with protected diol 187. To synthesise the desired diarylamine we surveyed the literature to determine optimal reaction conditions for the Buchwald-Hartwig reaction. Because of its broad scope and high reactivity Xantphos was used as the phosphine ligand and cesium carbonate as the base,
again for its high reactivity, but also functional group tolerance. Under these reaction conditions the desired N-C bond formation occurred in reasonable yield. Removal of the benzyl ether protecting group afforded the final product 174.

Scheme 37: Synthesis of aniline derivative 174. Reagents and conditions: (a) Alcohol 187, DIAD, PPh₃, THF, 0–100 °C, 25 min, 80%; (b) Pd(OAc)₂, Xantphos, Cs₂CO₃, PhMe, 100 °C, 16 h, 66%; (c) H₂, Pd/C, EtOAc, AcOH, rt, 24 h, 95%.

Cross-coupling chemistry was also utilised to synthesise furan derivative 175 (Scheme 38). It was envisioned that a halide functional handle could be installed at an early step in the synthesis and carried through until a late-stage cross-coupling reaction. Bromination of commercially available furan-2-carboxylic acid with bromine in acetic acid afforded a mixture of mono- and dibrominated products as determined by NMR spectroscopy and mass spectrometry. Due to the difficulty in separating the carboxylic acid products, the mixture was carried through to the next step in the synthetic sequence. A Fischer esterification afforded methyl ester 210, which was isolated by chromatography in low, but workable yield. A Claisen condensation with pinacolone using potassium tert-butoxide as base yielded the requisite diketone 211, which reacted cleanly with hydrazine to form pyrazole 212. N-Alkylation of the pyrazole afforded a mixture of regioisomeric products, which were separated by chromatography with the N1-regioisomer obtained as the major product, assumedly due to the steric influence of the large tert-butyl group. The position of alkylation was confirmed by 2D HMBC (Heteronuclear Multiple Bond Correlation) NMR spectroscopy (see Appendix 1). A correlation between the methylene protons of the N1-substituent and the carbon at the C5-position of the pyrazole was observed. Conversion of aryl bromide 208 to the corresponding pinacol boronate ester (215) via a Miyaura reaction
allowed for Suzuki cross-coupling with bromo-furan 214 using potassium hydroxide as the base. Finally, removal of the benzyl ether protecting group afforded furan derivative 175.

Scheme 38: Synthesis of furan derivative 175. Reagents and conditions: (a) Br₂, AcOH, 60 °C, 16 h; (b) MeOH, H₂SO₄, reflux, 16 h, 16% over 2 steps; (c) Pinacolone, KOtBu, THF, rt, 1 h, 68%; (d) Hydrazine hydrate, AcOH, EtOH, reflux, 16 h, 98%; (e) NaH, tosylate 213, DMF, 0-60 °C, 5 h, 49%; (f) KOAc, B₂Pin₂, Pd(dppf)Cl₂, PhMe, 90 °C, 16 h, 70%; (g) Pd(dppf)Cl₂, KOH (aq.), THF, 60 °C, 4 h, 60%; (h) H₂, Pd/C, EtOAc, rt, 48 h, 67%.

Click chemistry is a convenient and reliable way to join fragments via an amide bioisosteric 1,2,3-triazole unit. This allows for the rapid formation of chemical libraries from diverse azide and alkyne fragments. Azide 217 was synthesised from aminopyrazole 89 by treatment with sodium nitrite under acidic conditions to form the corresponding diazonium salt, followed by reaction with sodium azide (Scheme 39). Alkyne coupling partner 219 was synthesised from commercially available 2-iodo-4-(trifluoromethyl)phenol via a Mitsunobu reaction to install the ether group and Sonogashira reaction with TMS acetylene followed by TBAF mediated TMS removal. Under standard click chemistry conditions using sodium ascorbate and copper sulfate, the desired Huisgen azide-alkyne 1,3-dipolar cycloaddition occurred in good yield to obtain triazole 220. Subsequent PMB ether hydrogenolysis provided the desired compound 176 in excellent yield.
Bi-aryl linked derivative 177 was formed by a Suzuki cross-coupling reaction between triflate 221 and pinacol boronate ester 215 (Scheme 40). Triflate 221 could be accessed from hydroxy pyrazole 192 by treatment with the mild triflating agent phenyl triflimide and weak tertiary amine base N,N-diisopropylethylamine. Suzuki reaction using potassium carbonate as the base, followed by benzyl ether deprotection afforded the desired product 177.

It was envisioned that hydrocarbon analogues 178-180 could be accessed from a common alkyne intermediate, which can be formed from reliable Sonogashira cross-coupling.
chemistry. Sonogashira cross-coupling of aforementioned triflate 221 and alkyne 219 provided PMB ether protected intermediate 223 (Scheme 41). Removal of the protecting group using an oxidising agent such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded alkyne derivative 178.

Scheme 41: Synthesis of alkyne derivative 178. Reagents and conditions: (a) PdCl₂(PPh₃)₂, CuI, Et₃N, 70 °C, 2 h, 48%; (b) DDQ, CH₂Cl₂, H₂O, rt, 2 h, 85%.

To access the desired alkene derivative 179 a partial reduction of alkyne 223 was required. Under standard hydrogenation conditions using palladium or platinum catalysts it is not possible to isolate the alkene intermediate. The use of Lindlar’s catalyst (a combination of palladium-calcium carbonate, lead acetate and quinoline) prevents the conversion of the intermediate alkene to an alkane. This reaction forms exclusively the cis-alkene product. To better emulate the expected configuration of the lead compound 82, the trans-alkene was synthesised. Conditions developed by Luo and co-workers were used to obtain the desired alkene 224 (Scheme 42).²¹⁹ The method utilises Pd(dpff)Cl₂ as a catalyst and triethylsilane as a reductant to partially reduce the alkyne. The addition of copper sulfate results in cis/trans-isomerisation to the more stable trans-product. Under these reaction conditions some PMB ether deprotection was also observed. The mixture of products was carried through to the final step, where the PMB group was removed under oxidative conditions to afford trans-alkene 179.

Scheme 42: Synthesis of alkene derivative 179. Reagents and conditions: (a) Pd(dpff)Cl₂, dpff, HSiEt₃, CuSO₄, PhMe, H₂O, reflux, 48 h; (b) DDQ, CH₂Cl₂, H₂O, rt, 2 h, 61% over 2 steps.
Under standard hydrogenation conditions both the PMB ether group of 223 was removed and the alkyne was reduced to the corresponding ethyl linker to provide 180 (Scheme 43).

![Scheme 43: Synthesis of alkane derivative 180. Reagents and conditions: (a) H₂, Pd/C, EtOAc, rt, 16 h, 71%.](image)

Indazole derivative 181 was synthesised via a key Suzuki cross-coupling reaction of aryl bromide 229 and previously synthesised pinacol boronate ester 215 (Scheme 44). The appropriately substituted indazole was accessed from commercially available 4-bromo-2-fluorobenzaldehyde. The requisite tert-butyl ketone 226 was synthesised in a two-step procedure by first reaction of the aldehyde with tert-butyl magnesium chloride followed by oxidation of the alcohol to ketone 226 using pyridinium chlorochromate (PCC). Reaction of the sterically crowded ketone with hydrazine hydrate in refluxing tetrahydrofuran and catalytic acetic acid, afforded exclusively hydrazone 227 with no further reaction to the desired indazole. In order to overcome the steric requirement for this reaction, the hydrazone intermediate was heated at 165 °C in ethylene glycol. At the elevated temperature the intramolecular nucleophilic aromatic substitution proceeded in high yield. N-Alkylation of indazole 228 with tosylate 213 afforded mainly the desired N1 regioisomer due to the steric influence of the large tert-butyl group at the 3-position. A Suzuki cross-coupling reaction using aqueous potassium hydroxide as the base followed by removal of the benzyl ether protecting group afforded indazole 181.
Scheme 44: Synthesis of indazole derivative 181. Reagents and conditions: (a) t-BuMgCl, THF, 0 °C–rt, 16 h, 66%; (b) PCC, Celite®, CH₂Cl₂, rt, 16 h, 80%; (c) Hydrazine hydrate, AcOH, THF, reflux, 16 h, 79%; (d) Ethylene glycol, 165 °C, 16 h, 80%; (e) Tosylate 213, NaH, DMF, 0–60 °C, 5 h, 77%; (f) Pd(dppf)Cl₂, KOH (aq.), THF, 60 °C, 4 h, 59%; (g) H₂, Pd/C, EtOAc, rt, 48 h, 90%.

Formation of pyrazolopyrimidine analogue 182 followed a similar synthetic strategy (Scheme 45). Starting with widely available uracil, bromination at elevated temperature, followed by treatment with phosphorus oxychloride afforded 2,4,5-trisubstituted pyrimidine 232. It was found that judicious choice of base (Hünig’s base) was imperative for the success of the chlorination reaction. When the corresponding reaction was attempted using pyridine or N,N-dimethylaniline as a base, a complex mixture of products was obtained. Selective magnesium-bromine exchange with isopropylmagnesium chloride followed by quenching with pivalaldehyde afforded the requisite ketone 234 after oxidation of the alcohol with PCC. In this case, treatment of 234 with hydrazine in refluxing tetrahydrofuran and acetic acid formed directly the bicyclic heterocyclic core (235). A Mitsunobu reaction was used to regioselectively alkylate the N1-position to afford 236. The position of alkylation was confirmed by 2D HMBC NMR spectroscopy, which showed a correlation between the methylene protons of the N1-substituent and the quaternary carbon at the 7α-position (see Appendix 1). Suzuki cross-coupling with pinacol boronate ester 215 and benzyl ether deprotection provided pyrazolopyrimidine analogue 182.
Scheme 45: Synthesis of pyrazolopyrimidine derivative 182. Reagents and conditions: (a) Br₂, DMF, 120 °C, 4 h, 65%; (b) POCl₃, iPr₂EtN, 100 °C, 16 h, 81%; (c) Pivalaldehyde, iPrMgCl, THF, -40 °C–rt, 16 h, 47%; (d) PCC, Celite®, CH₂Cl₂, rt, 16 h, 88%; (e) Hydrazine hydrate, AcOH, THF, reflux, 2 h, 42%; (f) Alcohol 85, DIAD, PPh₃, THF, 0–100 °C, 15 min, 91%; (g) Pd(dppf)Cl₂, CsCO₃, dioxane, H₂O, 100 °C, 4 h, 79%; (h) H₂, Pd/C, EtOAc, rt, 16 h, 75%.

Synthesis of lactam derivative 183 required considerable method development and reaction optimisation. While the final synthetic route is quite lengthy, each step was operationally simple, reliable and high yielding. Initial attempts to synthesise key intermediate 239 involved ring opening lactam 238 to form the γ-aminoketone (Scheme 46). Pyrrolidone derivative 238 was formed by an Ullman-type reaction of 2-pyrrolidone with aryl iodide 218 using the conditions developed by Chen and co-workers. The use of tripod ligand 1,1,1-tris(hydroxyethyl)ethane facilitates the reaction to obtain the desired product in moderate yield. However, attempts to open the lactam with bulky Grignard reagent tert-butyl magnesium chloride were unsuccessful, even at elevated temperatures. Another proposed synthetic route to access the desired γ-aminoketone intermediate was via a reductive amination of aniline 200 (Scheme 46). To synthesise the required γ-keto aldehyde a three-step procedure from allyl alcohol developed by Murai and co-workers was employed. Formation of TMS protected allyl alcohol 240 using TMSCl in hexamethyldisilazane, followed by a silylation of the allyloxy carbanion, afforded α-siloxyallylsilane 241. Hosomi and co-workers found that allyloxy carbanions exist in rapid equilibrium with the corresponding silyl alkoxide and reaction with chlorosilanes occurs...
exclusively at oxygen. Murai and co-workers report that α-siloxyallylsilanes react with acid chlorides in the presence of Lewis acids such as titanium tetrachloride to form γ-keto aldehydes. Under the reported reaction conditions the desired product was obtained, but as a complex mixture. Any attempts to purify the desired product by distillation or chromatography were unsuccessful. Subjecting the crude product mixture to reductive amination conditions with aniline were ultimately unsuccessful.

**Scheme 46:** Attempted synthetic routes to lactam derivative 183. Reagents and conditions: (a) 2-Pyrrolidone, CuI, 1,1,1-tris(hydroxyethyl)ethane, Cs₂CO₃, DMF, 110 °C, 24 h, 55%; (b) t-BuMgCl, THF, 0 °C–reflux; (c) TMSCl, HMDS, 100 °C, 16 h, 100%; (d) i) t-BuLi, TMEDA, THF, -78 °C, 1.5 h, ii) TBSCl, -78 °C, 1 h, -78 °C–rt, 16 h, 61%; (e) Pivaloyl chloride, TiCl₄, CH₂Cl₂, -78 °C, 3 h.

In light of the difficulties associated with obtaining the desired γ-aminoketone, a more step-intensive route via amide 244 was taken (Scheme 47). The requisite carboxylic acid 243 was formed from α-bromoketone 133 by alkylation of methyl malonate, ester hydrolysis and decarboxylation. Formation of amide 244 proved to be rather troublesome with amide coupling reagents and amidation via the acid chloride producing low yields of the desired product. A one-pot procedure for acid chloride formation and amidation using phosphorus oxychloride in pyridine however was successful. Treatment of 244 with lithium aluminium hydride resulted in reduction of both the ketone and amide to form 245. Oxidation of the alcohol back to the ketone was unsuccessful using a range of oxidising agents, with a
complex mixture of products formed in each case. It was presumed that the nucleophilic aniline results in the poor reaction outcome. Rather than choosing to protect the aniline, the next step in the sequence, reaction with methyl chloroglyoxylate was performed. Some diacylated product was obtained from this reaction, however the ester could be selectively converted back to the secondary alcohol by treatment with sodium methoxide. The alcohol could now be cleanly oxidised to ketone using PCC. Intramolecular Claisen condensation using potassium tert-butoxide as a base formed key intermediate 246. From here, pyrazole formation by treatment with hydrazine, $N$-alkylation using tosylate 213 and benzyl ether deprotection afforded lactam derivative 183.
Scheme 47: Synthesis of lactam derivative 183. Reagents and conditions: (a) Methyl malonate, NaOMe, MeOH, rt–reflux, 2 h, 52%; (b) i) KOH (aq.) MeOH, rt, 2 h, ii) HCl (aq.), iii) 145 °C, 2 h, 65%; (c) POCl₃, pyridine, aniline 200, 0 °C–rt, 2 h, 40%; (d) LiAlH₄, THF, reflux, 16 h, 85%; (e) Monomethyl oxalyl chloride, Et₃N, CH₂Cl₂, 0 °C–rt, 2 h; (f) PCC, Celite®, CH₂Cl₂, rt, 16 h; (g) KOrBu, THF, reflux, 2 h, 75% over 3 steps; (h) Hydrazine hydrate, EtOH, reflux, 4 h, 100%; (i) Tosylate 213, NaH, DMF, 60 °C, 5 h, 71%; (j) H₂, Pd/C, EtOAc, rt, 16 h, 92%.

The reported chemistry to synthesise benzimidazole sulfones such as 33 requires multiple steps to install the aryl sulfone moiety.₂²³ We envisioned that since we had access to commercially available 2-fluoro-5-(trifluoromethyl)benzene-1-sulfonyl chloride, it might be possible to convert the corresponding sulfonyl fluoride to the desired sulfone using chemistry developed by Sharpless.₂²⁴ Sulfonyl fluoride 249 was synthesised from the
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sulfonyl chloride by treatment with aqueous potassium hydrogen difluoride (Scheme 48). Organolithiums do not react with the most commonly used SVI electrophiles, sulfonyl chlorides, to afford sulfones. The high energy barrier to attack at the sulfur centre and the high polarizability of the chlorine centre means that it is vulnerable to reductive attack leading to mainly chlorinated derivatives of the carbon nucleophile. Sulfonyl fluorides however react cleanly at the sulfur centre with nucleophiles to give the desired sulfone product. Lithium-halogen exchange of 229 with n-butyllithium and reaction of the aryl lithium species with sulfonyl fluoride 249 afforded sulfone 250 in good yield. A nucleophilic aromatic substitution with diol 93 afforded hybrid structure 184.

Scheme 48: Synthesis of sulfone derivative 184. Reagents and conditions: (a) KHF$_2$ (aq.), MeCN, rt, 16 h, 88%; (b) n-BuLi, THF, -78 °C, 3 h, 50%; (c) Diol 93, KOrBu, THF, reflux, 6 h, 79%.

4.3 In vitro Evaluation of Variable Linkage Analogues

The variable linkage analogues 166-184 were evaluated for their functional activity at the cannabinoid receptors using a FLIPR membrane potential assay. The functional activity data for the analogues at the CB$_1$ and CB$_2$ receptors is summarised in Table 5. As expected, all analogues were inactive at the CB$_1$R. The compounds synthesised can be classed into three broad categories based on their functional activity at the CB$_2$R: 1) Inactive, as defined by <50% activation at 10 μM; 2) Functionally active (>50% activation at 10 μM), but the potency could not be calculated as the data did not converge and 3) functionally active with defined potencies. In the second case where the data did not converge, efficacy was observed at high concentrations (10 μM) but activation declined rapidly to basal levels at even slightly lower concentrations. This is indicative of the ligands having intrinsic efficacy, but poor functional affinity and so significant interaction with the receptor is only observed when the cells are flooded with high concentrations of the ligand.
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Table 5: Agonist activities of analogues 166-184 in AtT-20 cells expressing human CB₁ or CB₂ receptors by a FLIPR membrane potential assay

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<th>Compound</th>
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<td>pEC₅₀ ± SEM</td>
<td>Eₘₐₓ (%)</td>
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<td>184</td>
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<tr>
<td>CP 55,940 (2)</td>
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*See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. *NA: Not active, defined as <50% activation at 10 μM in the assay. *ND: Not determined; for compounds defined as not active, their maximal effects were not determined. *DNC: Did not converge (average activation at 10 μM).

In all cases, except pyrazolopyrimidine analogue 182, the variable linkage analogues exhibited significantly reduced or abolished activity at the CB₂R. Even still, the data provides an indication of the strict structural requirements of the linkage moiety for functional activity.

Figure 38 groups the variable linkage analogues based on their functional activity at the CB₂R. Analysis of the lowest energy conformations of 166-184 (using ChemDraw 3D®) has provided some rationale for the varying functional activities within this series (Figure 39). The grouping appears to correlate with the planarity of the linkage moiety and the particular arrangement of heteroatoms. Planar linkage groups tend to confer functional activity. Comparison of alkyl linker derivatives highlights this trend. Alkane derivative 180 exhibited no functional activity, while rigid alkene 179 and alkyne 178 exhibited functional
activity at high concentrations. Likewise, indazole derivatives 181 and 184 containing a bicyclic, planar system were functionally active. Structures that adopt conformations unlike lead compound 82 such as methylated amide 166, benzylamine 173, aniline derivative 174 and bi-aryl analogue 177 were inactive. For analogues that adopt similar lowest energy conformations to lead compound 82, functional activity tends to correlate with the exact positioning of heteroatoms within the linkage functionality. In their lowest energy conformations, active compounds such as ester 167, reverse amide 168, thioamide 169, urea 172 and pyrazolopyrimidine 182 contain a hydrogen bond accepting group lying roughly in the plane of the pyrazole ring and pointing up in the direction of the tert-butyl group. Conversely, inactive compounds such as sulfonamide 170, N-acyl sulfonamide 171, furan 175, triazole 176 and lactam 183 contain a hydrogen bond accepting moiety that points away from the tert-butyl group, towards the tetrahydrofuran group. The positioning of the hydrogen bond acceptor or region of negative electrostatic potential appears to agree with the pharmacophore model developed in Chapter 2. To confirm this, we assessed how well these analogues fit the model generated by Phase. Unfortunately, all analogues synthesised were able to adopt conformations that satisfied the requirements of the pharmacophore model. In the absence of a crystal structure of the active state receptor, it cannot be determined whether such conformations are suitable for binding. Further work is therefore required to validate and improve the model to allow it to be a useful tool for rationalising and even predicting the functional activity of ligands.
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Figure 38: Summary of functional activity data for analogues 166-184.
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Figure 39: Adherence of lead compound 82 (A) to the pharmacophore model generated using phase (B). It was noticed that the orientation of the hydrogen bonding accepting moiety correlated with functional activity. For amide analogue 118 (C) the carbonyl hydrogen bond acceptor points down (shown in red box), while for reverse amide 168 (D) the carbonyl group points upwards (shown in red box), similar to lead compound 82. All three-dimensional structures were minimised using ChemDraw 3D.

4.4 Synthesis and Evaluation of a Pyrazolotriazine Analogue

While compiling this thesis Ghonim and co-workers published results for a similar series of compounds (251-253), which corroborated some of the findings from this chapter.225 Working on a series of thiazole and benzothiazole compounds, the authors found that amide analogues possessed some selective binding affinity for the CB2R, but functionally were either very weak agonists or inactive (Figure 40). It was only when the benzothiazole core was substituted with an N-alkyl group that a significant boost in affinity and potent functional activity was gained (253). The authors suggest that this marked increase in affinity and functional activity was due to the installation of the hydrophobic alkyl group. However, in conjunction with our findings it is more likely that the concomitant formation of the –ylidene amide functional group that occurs upon alkylation of the benzothiazole, is the reason for the significant shift in pharmacological profile.
Chapter 4: Exploration of Variable Linkage Analogues of Pyrazolylidene Benzamides

Figure 40: Summary of SAR data of thiazole and benzothiazole derivatives (251-253) developed by Ghonim and co-workers. The azaolylidene benzamide functionality is shown to be imperative for functional activity.

In 2013 and 2014 Hoffman La-Roche released a series of patents covering four structurally related heterocyclic series, [1,2,3]-triazolo-[4,5-d]-pyrimidines, pyrrolo-[2,3-d]-pyrimidines, pyrazolo-[3,4-d]-pyrimidines and purines. Figure 41 summarises SAR data surrounding the identity of the heteroaromatic moiety (254-260).

Figure 41: A summary of the data reported by Hoffman La-Roche comparing the identity of the heteroaromatic core (254-260).

These compounds and the pyrazolopyrimidine agonist developed in this chapter (182) share obvious structural similarities. When the heteroaromatic cores are aligned, the substitution
patterns of the Hoffman-La Roche compounds and 182 appear to be dissimilar. Alternatively, if the pendent groups are aligned, the structural similarities become clearer. The pendent groups considered in the SAR studies by Hoffman-La Roche and Abbott Laboratories (when developing 82) can be classed into three broad categories. As depicted in Figure 42, the heterocyclic cores are decorated with: 1) a bulky hydrophobic group such as a tert-butyl group, 2) an alkyl or saturated heterocycle such as a tetrahydrofuran group and 3) a substituted phenyl, heteroaromatic or cycloalkyl group. This alignment of the pendent groups rather than the heteroaromatic core, allows for the formation of a simplified pharmacophore as summarised in Figure 42 based on the Abbott Laboratories lead compound 82, the benzothiazole derivatives reported by Ghonim and co-workers (253), the Hoffman La-Roche compounds (258), and 182. This simplified model shares certain similarities with the pharmacophore model discussed in Chapter 2.

Figure 42: Comparison of four CB2R agonists highlights structural features that are important for functional activity. The compounds share structural similarities comprising: a core heteroaromatic ring shown in red; a bulky hydrophobic group such as a phenyl or tert-butyl group coloured blue; a substituted phenyl or aromatic group shown in purple; an aliphatic or cycloalkyl group shown in pink; and a hydrogen bond acceptor or region of negative electrostatic potential shown in green.

The SAR data reported by Hoffman La-Roche shows a clear relationship between the number of nitrogens within the aromatic core and potency. Triazolopyrimidine compounds (259 and 260) were the most potent analogues discovered, exhibiting sub-nanomolar potency in a cAMP assay. Comparison of analogues that contain the same number but different distribution of nitrogens around the heteroaromatic ring suggests a preference for
Chapter 4: Exploration of Variable Linkage Analogues of Pyrazoylidene Benzamides

certain arrangements of heteroatoms. The data appears to coincide with the predicted strength of potential \( \pi-\pi \) interactions with the heteroaromatic ring as suggested by Bootsma and co-workers. Their recent study aimed to predict the strength of \( \pi-\pi \) stacking interactions between different heterocycles and aromatic amino acid side chains. They concluded that stacking interactions with phenylalanine, tryptophan and tryptamine were enhanced by: 1) increasing the number of S, O, imino N, and carbonyl groups, with the size of this effect increasing across this series; 2) grouping like heteroatoms on opposing sides of the heterocycle. The congruence of these trends with the data reported by Hoffman La-Roche suggests the heteroaromatic ring is involved in \( \pi-\pi \) stacking interactions with one or more aromatic amino acid side chains and perhaps this interaction locks the ligand in a certain conformation to position the pendent groups into hydrophobic pockets within the receptor binding site. The strength of this interaction would therefore correlate with the binding affinity and potency of the ligand.

Based on the SAR data reported by Hoffman La-Roche and the pharmacophore models we have developed in this thesis, we decided to synthesise pyrazolotriazine derivative 261 containing another nitrogen within the heteroaromatic ring (Figure 43). The additional nitrogen should sit in a favourable region based on our pharmacophore modelling and potentially increase the strength of \( \pi-\pi \) interactions with the heteroaromatic ring.

![Figure 43: Rationale for the investigation of pyrazolotriazine 261. Based on our SAR data and literature reports, installation of another nitrogen in the heteroaromatic core may confer an increase in potency.]

There are no reported synthesises for pyrazolotriazines with the desired substitution pattern and so a novel synthetic route was designed to access 261 (Scheme 49). 1,2,4-Triazines are typically synthesised from the reaction of a 1,2-diketone species and an amidrazone (hydrazide imide) derivative. Synthesis of the requisite amidrazone however would require
multiple steps from carboxylic acid 193. Instead we envisioned that reaction of pyrazole dione 262 and a readily available semithiocarbazide reagent would form a thioether substituted pyrazolotriazine equipped for a Liebeskind–Srogl cross-coupling reaction. Synthesis of the pyrazole dione 262 required a two-step procedure from previously synthesised pyrazolone 192. A base-catalysed condensation reaction of the pyrazolone with nitrosobenzene afforded the intermediate phenyl imine, which could be hydrolysed with aqueous acid to afford dione 262 in low, but reproducible yield. Reaction of the dione 262 with thiosemicarbazide under basic conditions in refluxing water afforded the triazine scaffold and the thiol could be easily alkylated with methyl iodide to provide the requisite thioether 263. The Liebeskind–Srogl reaction is a palladium catalysed, copper(I) carboxylate-mediated cross-coupling of organosulfur compounds with nucleophilic organometallic reagents. Since the seminal work of Liebeskind and Srogl in 2000,232 for the coupling of thioesters and boronic acids to form ketones, the reaction has received much attention as an attractive procedure for carbon-carbon bond formation under neutral conditions using readily available and stable starting materials. In 2002 Liebeskind and Srogl reported the use of heteroaryl thioethers as substrates for their cross-coupling reaction.233 It was found that π-deficient heteroaryl thioethers could undergo efficient cross-coupling with boronic acids or organostannanes.234 Under standard conditions using an excess of boronic acid 264, an excess of copper(I) carboxylate reagent copper(I)-thiophene-2-carboxylate (CuTC) and a palladium(0) catalyst, the reaction appeared to stall after 16 hours and only a small amount of the desired product (<20%) was obtained. Liebeskind and Srogl report the addition of stoichiometric amounts of zinc acetate to shield basic nitrogens from interfering with the reaction system, may prevent poisoning of the palladium catalyst. Under these modified conditions, the starting thioether 263 was consumed and a moderate yield of the desired product (265) was obtained. Removal of the benzyl ether protecting group to furnish the final product 261 proved to be rather troublesome. Under standard hydrogenolysis conditions using palladium on carbon, no reaction was observed. Likewise, the addition of acetic acid did not facilitate the reaction. When a large amount of palladium on carbon and palladium hydroxide (total 0.5 eq. of Pd) was used, the starting material was consumed, however mass spectrometry and proton NMR analysis suggested that rather than hydrogenolysis of the benzyl ether group, the heteroaromatic ring was reduced. Other reaction conditions were therefore trialled for the deprotection. Okano and co-workers report the use of boron trichloride in the presence of
a cation scavenger as a mild and chemoselective method for the debenzylation of benzyl ethers.\textsuperscript{235} However, under the reported reaction conditions, only starting material was returned. Therefore, a stronger Lewis acid, boron tribromide, was trialled. At -78 °C using a slight excess of the Lewis acid a mixture of the starting material, desired product, alkene and tertiary alkyl bromide was obtained. A Brønsted acid, trifluoroacetic acid, was therefore used in order to potentially suppress carbocation formation from the desired tertiary alcohol product. At room temperature, no reaction was observed, however delightedly at elevated temperatures the desired product 261 was formed, with only minimal elimination product observed.

Scheme 49: Synthesis of pyrazolotriazine derivative 261. Reagents and conditions: (a) Nitrosobenzene, K$_2$CO$_3$, MeOH, reflux, 3 h; (b) HCl (aq.), THF, rt, 1.5 h, 35% over 2 steps; (c) i) Thiosemicarbazide, K$_2$CO$_3$, H$_2$O, rt–reflux, 16 h, ii) MeI, K$_2$CO$_3$, THF, rt, 4 h, 47% over 2 steps; (d) i) n-BuLi, THF, -78 °C, 1 h, ii) (iPrO)$_3$B, -78–rt °C, 1 h, iii) NH$_4$Cl (aq.), rt, 1 h, 61%; (e) CuTC, Zn(OAc)$_2$, Pd(dppf)Cl$_2$, THF, 90 °C, 16 h, 56%; (f) TFA, MePh, 90 °C, 16 h, 40%.

Pyrazolotriazine analogue 261 was evaluated for its functional activity at the cannabinoid receptors using a FLIPR membrane potential assay. The functional activity data for the analogue at the CB$_1$ and CB$_2$ receptors is summarised in Table 6.
Table 6: Agonist activity of 261 in AtT-20 cells expressing human CB₁ or CB₂ receptors by a FLIPR membrane potential assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB₁</th>
<th>CB₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pEC₅₀ ± SEM</td>
<td>E_max (%)</td>
</tr>
<tr>
<td>261</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>CP 55,940 (2)</td>
<td>7.64 ± 0.04 (23)</td>
<td>100</td>
</tr>
</tbody>
</table>

*See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. *NA: Not active, defined as <50% activation at 10 μM in the assay. *ND: Not determined; for compounds defined as not active, their maximal effects were not determined. *DNC: Did not converge (average activation at 10 μM).

Pyrazolotriazine 261 was found to have equal potency to lead compound 82. This is impressive considering the pendent groups used were not optimised for this scaffold. The increase in potency is consistent with our hypothesis that the heteroaromatic ring is involved in aromatic stacking interactions. The compound represents an attractive lead for further optimisation studies.

4.5 Concluding Remarks

In light of the inactivity of amide 118 we sought to explore the SAR surrounding the interesting –ylidene amide functional group of lead compound 82. Several analogues were synthesised with varying linkage moieties between the pyrazole and substituted phenyl group. The functional activity data of these analogues highlights a correlation between the planarity of the linkage and the specific arrangement of heteroatoms within the group. Pyrazolopyrimidine analogue 182 was identified as a potent and selective CB₂R agonist (EC₅₀ = 41 nM). Based on data for other reported compounds and pharmacophore models we have developed, we decided to synthesise pyrazolotriazine derivative 261. This analogue exhibited improved potency (EC₅₀ = 19 nM) and offers an attractive lead scaffold for further studies.

Future SAR studies, focusing on the identity of the pendent groups could further improve the potency of this compound and its physicochemical properties. These efforts would aim to reduce molecular weight, decrease lipophilicity and replace metabolically liable functionalities such as the tert-butyl group.
Chapter 5: Strategies to Develop Selective CB₂ Receptor Agonists from Indole Carboxamide Synthetic Cannabinoids
5.1 Introductory Remarks

Marijuana, the collective term for the resin, flowers and leaves of *Cannabis sativa*, is the most widely produced and consumed illicit substance worldwide. In the early 2000’s, smokable herbal products, adulterated with known synthetic cannabinoids, appeared as legal alternatives to recreational marijuana use. Many of these synthetic cannabinoids were based on the aminoalkylindole scaffold developed and extensively studied by Huffman and co-workers. Over the past two decades synthetic cannabinoid receptor agonists have proliferated as novel psychoactive substances (NPS). More than 200 synthetic cannabinoids have been reported in countries all over the world. A number of factors have led to the vast popularity of synthetic cannabinoid use, including ease of access, novelty, promise of a stronger high than cannabis and the difficulty involved in detecting these compounds in standard urine toxicology tests. A number of countries have thus moved to control synthetic cannabinoids, in response to reports of the health risks associated with their use. As a result of these measures, new analogues of the controlled substances, which are not covered by the scheduling legislation, and had not been previously reported in the scientific literature, have subsequently appeared. Unlike cannabis, synthetic cannabinoid ingestion has been associated with numerous serious adverse effects.

In their recent book chapter, Banister and Connor comprehensively review the evolution of synthetic cannabinoid designer drugs. SAR studies around the aminoalkylindole scaffold by academic laboratories and pharmaceutical companies have resulted in the discovery of many potent 3-acylindole cannabinoid receptor agonists. These compounds and ‘designed’ analogues, incorporating features of different reported cannabinoids, have subsequently appeared as NPS. This has led to an exponential increase in synthetic cannabinoids identified by forensic scientists. These novel synthetic cannabinoids often have not been assessed for their pharmacological effects. In recent years the Kassiou research group have synthesised and characterised the pharmacological activity of many high-concern synthetic cannabinoids, particularly 3-substituted indoles and indazoles. Common medicinal chemistry techniques such as molecular hybridization of pharmacophoric subunits, scaffold hopping and bioisosteric replacements implemented by clandestine chemists, although seemingly random, have trended towards the discovery of increasingly more potent compounds. Figure 44 highlights the evolution of structurally diverse synthetic
cannabinoids in the past decade. Varied aromatic cores (shown in red) have appeared as a result of scaffold hopping from originally reported indoles to pyrroles, indazoles, benzimidazoles and azaindoles. The N1 alkyl group (shown in blue) appears to be quite tolerant to substitution with alkyl, cycloalkyl, heterocyclic, aromatic, and heteroaromatic moieties reported. The appearance of a wide variety of heteroatoms within the N1 alkyl group has proven a common strategy to modify potency at the cannabinoid receptors. For instance, bioisosteric fluorination generally results in improved potency and metabolic stability. 3-Acylindole agonists reported by Huffman and co-workers have also evolved to include other linker groups such as esters and amides (shown in pink). A large diversity of ketone, ester and amide substituents have been observed (shown in green). Bulky aromatic, cycloalkyl and amino acid derived pendent groups have all appeared.

Research in the Kassiou group has established simple and reliable methods to access synthetic cannabinoids and their analogues for pharmacological evaluation. These compounds are generally potent, high efficacy agonists at both the CB1 and CB2 receptors, and have been shown to exhibit potent *in vitro* activity, cannabimimetic phenotypes *in vivo*, and anecdotal cannabimimetic activity in humans. 

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**Figure 44:** Evolution of synthetic cannabinoids. Synthetic cannabinoids (general structure shown) are commonly potent, full agonists at both the CB1 and CB2 receptors. (Adapted with permission from Banister, S. D.; Connor, M. *New Psychoactive Substances: Pharmacology, Clinical, Forensic and Analytical Toxicology*, Maurer, H. H.; Brandt, S. D., Eds. Springer International Publishing: Cham, 2018; pp 191-226. Copyright (2019) Springer Publishing).
Recently it was demonstrated that CB$_2$R selectivity could be realised for non-selective 3-adamantylamidoalkyldoindoles (for example APICA, 266) by simply moving the amide from the 3-position to the 2-position of the indole.\textsuperscript{247} The 2-substituted adamantyl amide compounds discovered (for example 267) exhibited low to moderate potencies and are generally quite lipophilic, owing to the non-polar adamantyl group. Previous research has also demonstrated that 3-substituted bulky amino acid derived amides (from L-valinamide, methyl-L-valinate, tert-leucinamide and tert-leucinate) as well as cumyl amides, are amongst the most potent synthetic cannabinoids known.\textsuperscript{241, 243, 245} We have therefore applied the amide shift strategy to such derivatives in an effort to obtain more potent and selective compounds (268, 270, 273, 275 and 277) with favorable physicochemical properties, namely reduced lipophilicity (Figure 45). In addition, it is also reported that incorporation of a polar group, particularly a hydroxyl group, at the terminal position of the $N$-pentyl chain can improve CB$_2$R selectivity.\textsuperscript{244} With this in mind we prepared the 5-hydroxyl analogues (269, 271, 274, 276 and 278).

![Chemical structures showing the amide shift strategy](image)

**Figure 45:** A strategy to develop selective CB$_2$R agonists from the non-selective synthetic cannabinoid APICA (266) by moving the amide from the 3-position to the 2-position of the indole.

A recent study by the Kassiou group involving the pharmacological evaluation of azaindole derived synthetic cannabinoids revealed that the 7-azaindole scaffold may impart some CB$_2$R selectivity (for example 279).\textsuperscript{248} Again, we sought to determine if this was a general strategy to improve the CB$_2$R selectivity of potent synthetic cannabinoids (279-289, Figure
46). In addition, bioisosteric replacement of the indole core with the 7-azaindole moiety may also improve physicochemical properties, namely a reduction in lipophilicity.

**Figure 46:** A strategy to develop selective CB2R agonists from the non-selective synthetic cannabinoid APICA (266) via bioisosteric replacement of the indole nucleus with a 7-azaindole moiety.

A hybrid strategy was also explored by moving the amide group from the 3-position to the 2-position of the 7-azaindole scaffold (290, Figure 47). Previous research has found that indazole-based synthetic cannabinoids are generally more potent than their indole analogues.\(^{245}\) We envisioned that a similar trend may be observed for pyrazolo[3,4-b]pyridine analogues (291) compared to azaindole-based compounds. The Kassiou group has also recently published the pharmacological evaluation of 2-alkyl-2H-indazole regioisomers of synthetic cannabinoids commonly observed in forensic samples as possible manufacturing impurities.\(^{249}\) These regioisomers demonstrated low potency (micromolar) functional activity at both receptor subtypes. In a similar fashion we aimed to investigate the position of N-alkylation of the 7-azaindole scaffold by the synthesis of regioisomeric 7-substituted azaindole 292.
Figure 47: Further analogues of the 7-azaindole scaffold. Movement of the amide from the 3- to the 2-position of the 7-azaindole nucleus represents a hybrid of the strategies outlined in Figures 45 and 46. Pyrazolo[3,4-b]pyridine 291 probes the effect of additional nitrogens within the azaindole scaffold. Generally indazole synthetic cannabinoids are more potent than their indole congeners, therefore 291 was synthesised to determine if the same trend is observed for azaindole analogues. The position of the N-alkyl substituent has not been explored for 7-azaindoles and hence 292 will determine the effect of substitution at the 7-position of the azaindole.

5.2 Synthesis of Synthetic Cannabinoid Analougues

5.2.1 Synthesis of 2-Amidoalkylindoles

The chemistry to access indole carboxamide synthetic cannabinoids is well established and reliable synthetic methods have been developed within the Kassiou research group.\(^\text{241-242}\). 2-Amidoalkylindole analogues 267-278 were prepared from commercially available ethyl indole-2-carboxylate. Sodium hydride mediated deprotonation followed by reaction with alkyl bromides, 1-bromopentane or 294 afforded the N1 alkylated products 298 and 299 in high yield. In the case of 298 quantitative yield of the desired product was obtained without the need for chromatography as excess alkyl bromide could be removed \textit{in vacuo}. Alkyl bromide 294 was not commercially available and hence required synthesis (Scheme 50). A convenient and scalable two-step procedure was used for the synthesis of 294, which provided high yields of the desired product without chromatography. Mono-benzyl protection of commercially available 1,5-pentanediol was achieved using an excess of the
diol (5 equivalents) and slow addition of benzyl bromide. Excess diol was easily removed by successive aqueous washes. An Appel reaction using bromine and pyridine furnished the desired alkyl bromide 294. The use of bromine instead of carbon tetrabromide as a bromine source negated the need for chromatography to obtain pure sample. An aqueous workup and trituration with a mixture of diether ether and hexane was sufficient to remove reaction by-products, namely triphenylphosphine oxide. Base mediated ester hydrolysis afforded the requisite carboxylic acid intermediates 300 and 301 (Scheme 51).

Operationally simple and high yielding EDCI-HOBt amide coupling using the appropriate amines gave the 11 amides 267, 268, 270, 273, 275, 277, 302-306. In all cases simple aqueous workup removed the amide coupling reagent by-products while usually trivial chromatography and/or recrystallisation was sufficient to obtain pure products. While a number of the amines were commercially available, 296 was synthesised from Boc-l-tert-leucine via an EDCI-HOBt mediated amide coupling and subsequent Boc group deprotection under acidic conditions (Scheme 50). Likewise, amine 297 was prepared from commercially available l-tert-leucine via the corresponding acid chloride (Scheme 50). The amino acid-derived amines used were optically pure or obtained from optically pure starting materials. Measurement of the optical rotation of the final amide products suggested that racemisation had not occurred for any of the analogues synthesised.

Hydrogenolysis of the benzyl protecting groups from 302-306 afforded the 5-hydroxyl analogues 269, 271, 274, 276 and 278 in high yields (Scheme 51). Difluoromethyl analogue 272 was prepared in a two-step procedure from alcohol 276 via a Dess-Martin periodinane (DMP) mediated oxidation followed by treatment of the aldehyde with nucleophilic fluorinating reagent diethylaminosulfur trifluoride (DAST).
Scheme 50: Synthesis of building blocks 293-297. **Reagents and conditions:** (a) NaH, BnBr, DMF, 0 °C–rt, 16 h, 85%; (b) PPh₃, Br₂, pyridine, CH₂Cl₂, 0 °C–rt, 4 h, 88%; (c) NH₄Cl, EDCI, HOBt, Et₃N, DMF, rt, 16 h, 100%; (d) HCl, dioxane, EtOAc, rt, 16 h, 95%; (e) SOCl₂, MeOH, reflux, 16 h, 100%.

Scheme 51: Synthesis of 2-amidoalkylindoles (267-278). **Reagents and conditions:** (a) NaH, appropriate alkyl bromide (1-bromopentane or 294), DMF, 0 °C–rt, 16 h, 76–100%; (b) NaOH (aq.), EtOH, reflux, 2 h, 94–100%; (c) Appropriate amine (1-adamantylamine, 2-phenylpropan-2-amine, L-valinamide hydrochloride, methyl-L-valinate hydrochloride, 296 or 297), EDCI, HOBt, iPr₂EtN, DMF, rt, 16 h, 40–100%; (d) Pd/C, H₂, EtOAc, rt, 16 h, 67–87%; (e) i) DMP, CH₂Cl₂, THF, rt, 16 h, ii) DAST, CH₂Cl₂, rt, 24 h, 40%.
5.2.2 Synthesis of 3-Amidoalkyl-7-azaindole and Analogues

3-Amidoalkyl-7-azaindole derivatives 279-289 were prepared from commercially available 7-azaindole (Scheme 52). Sodium hydride mediated deprotonation followed by reaction with 1-bromopentane or 294 gave the N1-alkyl intermediates 307 and 308. Aluminium chloride mediated Friedel-Crafts acylation with trifluoroacetic anhydride furnished the 3-substituted trifluoromethyl ketones 309 and 310. The hydrolysis of the trifluoromethyl ketones required somewhat forcing conditions. The use of aqueous sodium hydroxide returned only starting material even after long reaction times at elevated temperature. It was found that an excess of lithium hydroxide in refluxing methanol over the course of 2 days was required to obtain good yields of the desired carboxylic acids 311 and 312. EDCI-HOBt mediated amide coupling gave the requisite amides 279, 282, 283, 285, 286, 288, 313-316 and hydrogenolysis to remove the benzyl protecting groups from 313-316 furnished the 5-hydroxyl analogues 280, 284, 287 and 289. It was found that the hydrogenolysis of the benzyl protecting groups was sluggish. Addition of acetic acid was generally required to promote the reactions, and even then, reactions were slow. Li and co-workers have reported the use of a combination of palladium hydroxide and palladium on carbon as a more efficient hydrogenolysis catalytic system than either catalyst alone. Improved reaction rates were observed using this catalyst system allowing for complete deprotection within two days.
Scheme 52: Synthesis of 3-amidoalkyl-7-azaindoles (279-289). Reagents and conditions: (a) NaH, appropriate alkyl bromide (1-bromopentane or 294), DMF, 0 °C–rt, 16 h, 85–100%; (b) AlCl₃, (CF₃CO)₂O, DMF, 0 °C–rt, 3 h, 78–88%; (c) LiOH (aq.), MeOH, reflux, 48 h, 74–84%; (d) Appropriate amine (1-adamantylamine, 2-phenylpropan-2-amine, L-valinamide hydrochloride, methyl-L-valinate hydrochloride, 296 or 297), EDCI, HOBt, iPr₂EtN, DMF, rt, 16 h, 45–91%; (e) Pd/C, Pd(OH)₃/C, H₂, EtOAc, rt, 16–48 h, 68–86%.

2-Amidoalkyl-7-azaindoles derivative 290 was prepared using a similar procedure, from ethyl 7-azaindoles-2-carboxylate 319 (Scheme 53). The ester was synthesised using a procedure reported by Cai and co-workers from 2-bromonicotinaldehyde (317) with ethyl isocyanocacetate (318). 2-Bromonicotinaldehyde (317) was synthesised from 2-bromopyridine using the conditions reported by Numata and co-workers. Lithiation of 2-bromopyridine using lithium diisopropyl amide, followed by electrophilic substitution with DMF afforded 317 in low but acceptable yield. Compared to fluorine and other heteroatoms, the ortho-directing ability of bromine in metalation reactions is relatively
weak.\textsuperscript{253} If alkyllithium reagents are employed for this reaction, competitive bromine-lithium exchange occurs. The use of non-carbon-based strong bases such as LDA are more effective deprotonating agents in this case.\textsuperscript{254} Ortho-metalated aryl bromides have low thermal stability and may decompose to form arynes or isomerise via the Bunnett’s “halogen dance” process.\textsuperscript{255} These factors and potentially others explain the low yield for 317.

The synthesis of ethyl isocyanoacetate (318) employed a two-step procedure starting from widely available glycine ethyl ester hydrochloride. Reaction with ethyl formate under basic conditions afforded the crude formamide intermediate with a mixture of other products and triethylamine hydrochloride. The hydrochloride salt was removed in the most part by filtration, and the crude product was further purified by vacuum distillation (140-145 °C, 10 mmHg). NMR analysis suggested the product was still contaminated with some triethylamine hydrochloride. Despite this, the crude product was taken on to the next reaction. Treatment of the formamide intermediate with phosphorus oxychloride and triethylamine resulted in dehydration to produce malodorous, toxic and water sensitive ethyl isocyanoacetate (318).

Using procedures developed by Cai and co-workers, 7-azaindole-2-carboxylic acid ester 319 was obtained in moderate yield (55%). The reaction involves a ligand-free copper-catalysed condensation/coupling/deformylation cascade process. Although the authors do not propose an explicit mechanism for the complex transformation, we suggest that the initial portion of the mechanism would be similar to the Van Leusen reaction (Figure 48).\textsuperscript{256} The electron withdrawing ester and isocyanide groups dictate that the methylene protons of 318 are quite acidic and can be deprotonated with a weak base such as cesium carbonate. Reaction of the anion with aldehyde 317 followed by a 5\textendash endo\textendash dig cyclisation of the resulting alkoxide onto the electrophilic carbon of the isocyanide moiety forms a five-membered ring. In the Van Leusen reaction, elimination of a tosyl leaving group results in formation of an oxazole. In this case the ethyl ester is not lost and rather tautomerisation and ring opening occurs to generate a formamide intermediate. Oxidation of the isocyanide carbon atom is the driving force for this reaction. An intramolecular copper-mediated Ullman-type coupling forms the azaindole nucleus and finally deformylation affords the final product 319.
N1-Alkylation using potassium carbonate and 1-bromopentane and base-mediated ester hydrolysis gave the carboxylic acid 321. Finally an EDCI-HOBt mediated amide coupling provided the desired amide 290.

Scheme 53: Synthesis of 2-amidoalkyl-7-azaindole derivative 290. Reagents and conditions: (a) LDA, DMF, THF, -78 °C–rt, 2 h, 37%; (b) i) Ethyl formate, Et3N, 60 °C, 16 h; ii) POCl3, Et3N, CH2Cl2, 0 °C–rt, 1 h; (c) CuI, Cs2CO3, DMSO, 80 °C, 16 h, 55% over 2 steps; (d) K2CO3, 1-bromopentane, 50 °C, 16 h, 63%; (e) NaOH (aq.), EtOH, reflux, 2 h, 91%; (f) 1-Adamantylamine, EDCI, HOBt, iPr2EtN, DMF, rt, 16 h, 59%.

Figure 48: Proposed mechanism for Cai and co-workers’ condensation/coupling/deformylation cascade for the synthesis of indole-2-carboxylic acid esters.

3-Amidoalkylpyrazolo[3,4-b]pyridine derivative 291 was prepared from commercially available 3-acetylpyridine (Scheme 54). N-Oxide 322 was obtained in high yield by reaction with a slight excess of meta-chloroperbenzoic acid. Addition of triphenylphosphine quenched any remaining oxidant and the triphenylphosphine oxide formed was easily removed by chromatography. The N-oxide allowed for a regioselective chlorination at the 2-position of the pyridine using phosphorus oxychloride. The reaction
is presumed to proceed through an ionic addition-elimination mechanism (Figure 49). Reaction of the N-oxide oxygen with phosphorus oxychloride forms a phosphorus-oxygen bond and expels a chloride anion. 1,2-Addition adjacent to both electron withdrawing groups forms a non-aromatic intermediate and finally deprotonation with elimination of the good leaving group forms the 2-substituted pyridine product.257 The low isolated yield (36%) can be accounted for by some reaction at the 4- and 6-positions and some decomposition under the relatively harsh reaction conditions.

![Figure 49: Proposed mechanism for the regioselective chlorination of 3-acetyl pyridine N-oxide (322) using phosphorus oxychloride.](image)

Subsequent condensation with methyl hydrazine formed the pyrazolo[3,4-b]pyridine nucleus 324. Heating at reflux in ethanol resulted in a mixture of the intermediate hydrazone and desired product 324. The reaction was repeated in ethylene glycol and heated to 165 °C resulting in improved conversion to 324. Deprotonation with sodium hydride and reaction with 1-bromopentane afforded the N1 alkylated product 325. Oxidation of the 3-methyl group according to the procedures reported by Lynch and co-workers using potassium permanganate in the presence of sodium hydroxide gave carboxylic acid 326 after acidic workup.258 Finally an EDCI-HOBt mediated amide coupling with adamantyl amine afforded the desired amide 291.
Scheme 54: Synthesis of 3-amidoalkylpyrazolo[3,4-b]pyridine derivative 291. Reagents and conditions: (a) m-CPBA, CH₂Cl₂, rt, 16 h, 58%; (b) POCl₃, 100 °C, 2 h, 50%; (c) Hydrazine hydrate, ethylene glycol, 165 °C, 16 h, 81%; (d) NaH, 1-bromopentane, 0 °C–rt, 16 h, 46%; (e) KMnO₄, NaOH (aq.), H₂O, 90 °C, 2 h, 36%; (f) 1-Adamantylamine, EDCI, HOBT, iPr₂EtN, DMF, rt, 16 h, 52%.

Pyrrolo[2,3-b]pyridines or 7-azaindoles share some chemical similarities with imidazoles since they contain both a pyridine- and pyrrole-like nitrogen atom within an aromatic system. Akin to substituted imidazoles, isomeric products can be obtained upon alkylation depending on whether reaction occurs on the pyridine-like nitrogen or pyrrole-like nitrogen.²⁵⁹ The site of reaction can be controlled based on the reaction conditions used. In the absence of base, the lone pair of electrons on the N1 nitrogen are involved in the aromaticity of the heterocycle and hence are not reactive towards electrophilic reagents. Conversely, at the N7-position, the lone pair of electrons sit perpendicular to the plane of the ring system and are available for reaction with suitable electrophiles, akin to the reaction of pyridines with acids, alkyl halides and electrophilic oxidising agents. Therefore, under neutral conditions N7-alkylated products can be obtained.

N7-Alkyl derivative 292 was formed from 3-amido-7-azaindole 329. Starting from 7-azaindole, an aluminium chloride-mediated Friedel-Crafts acylation with trichloroacetyl chloride afforded trichloromethyl ketone 327. Hydrolysis under basic conditions and EDCI-HOBT amide coupling afforded the desired amide 329. Initial attempts to access 292 aimed to perform the N7 alkylation first, followed by formation of the amide at the 3-position. While regioselective N7 alkylation of the methyl ester of carboxylic acid 328 proceeded smoothly, the hydrolysis of the ester was low yielding due to the formation of a range of side products. Alternatively, regioselective alkylation of amide 329 under neutral
conditions provided pseudooazulene derivative 292 after basic workup (Scheme 55). The regioselectivity of the alkylation was confirmed by an nOe correlation between the C6 proton of the azaindole nucleus and the methylene protons adjacent to the nitrogen of the pentyl chain (see Appendix 1).

It was found that the pseudooazulene products had seemingly interesting photophysical properties. Compared to the N1 substituted regioisomers, which are generally colourless solids, the N7 product was a yellow solid. Further, solutions of the product appear to fluoresce under ultra-violet light. If the N7 regioisomers prove to be active at the cannabinoid receptors, they could potentially be useful tool compounds for fluorescence imaging studies. Naturally, further optimisation of the photophysical properties of the system would be required for such applications, however intrinsic fluorophores that do not require conjugation of a known fluorescent probe would be of high value.

Scheme 55: Synthesis of 3-aminoalkyl-7-azaindole derivative substituted at the 7-position 292. Reagents and conditions: (a) AlCl3, Cl3COCl, CH2Cl2, 0 °C–rt, 4 h, 91%; (b) NaOH (aq.), rt, 6 h, 100%; (c) 1-Adamantylamine, EDCI, HOBt, iPr2EtN, DMF, rt, 16 h, 61%; (d) 1-Bromopentane, TBAI, MeCN, PhMe, 150 °C, 16 h, 60%.

5.3 In vitro Evaluation of Synthetic Cannabinoid Analogues

5.3.1 2-Aminoalkylindoles

The synthetic cannabinoid analogues 267-278 and lead compound 266 were assessed for their ability to activate the CB1 and CB2 receptors. The functional activities of 266-278 were assessed in AtT-20 neuroblastoma cells stably transfected with human CB1 or CB2 receptors using a FLIPR membrane potential assay. Compounds displaying more than 50% activation at 10 μM in the assay were evaluated further in dose-response studies. The data is listed in Table 7. Further experimental details are provided in Chapter 8.
In agreement with previously reported data, 3-amidoalkylindole APICA (266) exhibited potent functional activity at both the CB₁ and CB₂ receptors (Table 7). The simple structural modification of moving the amide from the 3-position to the 2-position to afford 2-amidoindole 267 was found to completely suppress activity at the CB₁ receptor. Due to its high lipophilicity, we were unable to solubilise the compound in the assay medium at high concentrations using dimethyl sulfoxide (DMSO). Even when higher concentrations of DMSO were used, solubility in the assay medium was problematic. The use of an alcoholic solvent was more successful in solubilising the compound, however due to the volatility of the solvent, reliable data could not be obtained. It appeared from the limited data that the compound is a partial agonist with low micromolar activity, similar to what has previously been reported. The data obtained appeared to corroborate the results obtained by Shi and co-workers that 2-adamantyl amidoalkylindoles such as 267 exhibit selective functional activity at the CB₂R. The poor aqueous solubility of 267 highlights the need to develop more drug-like compounds using this strategy. It was envisioned that amino acid derived amides and cumyl amides 268-278 would retain not only the selectivity observed for adamantyl derivative 267, but also the high potency of their respective 3-amidoalkylindole analogues. Furthermore, the polar amide substituents would favourably decrease the lipophilicity, thus generating more drug-like compounds. As suspected, the receptor subtype selectivity was retained for analogues 268-278 and improved sub-micromolar potency was observed. In particular, tert-leucinamide analogue 275 (CB₂R EC₅₀ = 189 nM) was significantly more potent than the adamantyl lead compound 267. Unfortunately, for 270 and cumyl amide 277, EC₅₀ values could not be generated for activity at the CB₂R due to poor solubility at high concentrations. From the limited data it appeared that they would have similar potencies at the CB₂R to the other analogues. The tolerance of amide substitution suggests this is a general strategy for imparting CB₂R selectivity. Further optimisation of the amide and N-alkyl chain could further improve potency and physicochemical properties.

Interestingly, 5-hydroxypentyl derivatives 269, 271, 274 and 276 exhibited no activity at either the CB₁ or CB₂ receptors, while cumyl amide derivative 278 retained selectivity and exhibited improved potency (CB₂R EC₅₀ = 217 nM) compared to the pentyl derivative 277. This finding for the cumyl amide derivative is consistent with what was observed for the adamantyl derivatives developed by Shi and co-workers. The addition of a polar group at
the terminal position of the alkyl chain substantially increased potency. As illustrated in Figure 50, it is possible that intramolecular hydrogen bonding between the terminal hydroxyl group and polar amino acid derived amide substituents (but not the non-polar cumyl or adamantyl amides) is unfavourable for CB$_2$R activation.

![Figure 50: Proposed intramolecular hydrogen bonding (shown with red dotted lines) between the terminal alcohol and polar amino acid derived amides of analogues such as 269 that may result in the stabilisation of a conformation not suitable for functional activity at the CB$_2$R.](image)

Further exploration of other polar alkyl derivatives that would not participate in such hydrogen bonding interactions could yield more potent compounds. As fluorine is a weak hydrogen bond acceptor$^{260}$ and based on data for similar compounds, difluoromethyl analogue 272 was synthesised.$^{247}$ Introduction of the polar group reduced activity at the CB$_2$R, but not completely, as was the case for the 5-hydroxyl analogue 271. It is possible that interactions between the difluoromethyl group and polar amino acid derived amide still affords an unfavourable conformation for CB$_2$R activation. These findings warrant further exploration of the N-alkyl moiety to determine the optimal functionality for potent CB$_2$R activity. This could involve assessing the suitability of a range of polar and non-polar functionalities, but also determining the effect of the length of the alkyl chain. Shorter, longer or conformationally restricted alkyl chains with polar substituents may afford compounds with improved functional activity at the CB$_2$R.
Table 7: Agonist activities of 2-amidoalkylindoles (267-278) in AtT-20 cells expressing human CB₁ or CB₂ receptors by a FLIPR membrane potential assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB₁ ( \text{pEC}_{50} \pm \text{SEM} ) (EC₅₀ μM)</th>
<th>( E_{\text{max}} ) (%)</th>
<th>CB₂ ( \text{pEC}_{50} \pm \text{SEM} ) (EC₅₀ μM)</th>
<th>( E_{\text{max}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>266</td>
<td>7.13 ± 0.09 (0.074)</td>
<td>108</td>
<td>7.02 ± 0.08 (0.096)</td>
<td>85</td>
</tr>
<tr>
<td>267</td>
<td>NA</td>
<td>ND</td>
<td>DNC</td>
<td>ND</td>
</tr>
<tr>
<td>268</td>
<td>NA</td>
<td>ND</td>
<td>6.15 ± 0.11 (0.713)</td>
<td>74</td>
</tr>
<tr>
<td>269</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>270</td>
<td>NA</td>
<td>ND</td>
<td>DNC</td>
<td>ND</td>
</tr>
<tr>
<td>271</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>272</td>
<td>NA</td>
<td>ND</td>
<td>5.26 ± 0.73 (5.46)</td>
<td>128</td>
</tr>
<tr>
<td>273</td>
<td>NA</td>
<td>ND</td>
<td>6.13 ± 0.16 (0.741)</td>
<td>74</td>
</tr>
<tr>
<td>274</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>275</td>
<td>NA</td>
<td>ND</td>
<td>6.72 ± 0.12 (0.189)</td>
<td>73</td>
</tr>
<tr>
<td>276</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>277</td>
<td>NA</td>
<td>ND</td>
<td>DNC</td>
<td>ND</td>
</tr>
<tr>
<td>278</td>
<td>NA</td>
<td>ND</td>
<td>6.66 ± 0.09 (0.217)</td>
<td>88</td>
</tr>
<tr>
<td>CP 55,940 (2)</td>
<td>7.66 ± 0.04 (0.022)</td>
<td>101</td>
<td>7.20 ± 0.04 (0.063)</td>
<td>106</td>
</tr>
</tbody>
</table>

*See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 (2) used as a positive control. NA: Not active, defined as <50% activation at 10 μM in the assay. ND: Not determined; for compounds defined as not active, their maximal effects were not determined. DNC: Did not converge; data does not fit a reliable curve to generate EC₅₀ values (due to solubility issues at high concentration).

5.3.2 3-Amidoalkyl-7-azaindole and Analogues

A recent report by the Kassiou group suggests 3-amidoalkyl-7-azaindole 279 is a potent, CB₂R selective agonist. In our current study, receptor subtype selectivity (roughly 3.5-fold) was also observed for 279, but not to the same extent as the previous report (Table 8). As the assay conditions were seemingly identical, the reason for this discrepancy is unknown. Any significant level of selectivity however was not observed for amino acid-derived amides 282, 283, 285 and 286 and cumyl amide 288, which all exhibited potent functional activity at both receptor subtypes. Compared to the azaindole lead compound 279, 3-amidoalkylpyrazolo[3,4-b]pyridine derivative 291 exhibited improved receptor subtype selectivity with a 7-fold preference for CB₂R activation. 2-Amidoalkyl-7-azaindole 290, which represents a hybrid strategy of the use of the 7-azaindole scaffold, and also movement of the amide from the 3- to the 2-position, exhibited no activity at either receptor subtype. As the 2-adamantyl amidoalkylindole derivatives were not overly potent agonists,
further investigation of the 2-amidoalkyl-7-azaindole scaffold with different amide and N1 alkyl substituents may yield active and selective compounds. Pseudo-azulene 292 was inactive at both receptor subtypes suggesting it is not a suitable scaffold for further investigation. Fortuitously, 5-hydroxypentyl derivative 280 exhibited complete receptor subtype selectivity. In addition, potency at the CB2R was essentially retained. With this positive result in hand we investigated whether other amide substitutions would exhibit similar selectivity. We synthesised the 5-hydroxy derivatives of the two most potent 3-amidoalkyl-7-azaindole compounds 284 and 287 and also cumyl amide derivative 289 with the expectation that their potency at the CB2R would be retained with improved receptor subtype selectivity. The 5-hydroxypentyl analogues 284, 287 and 289 exhibited potent activity at the CB2R with vastly improved subtype selectivity, however some activity at the CB1R was also observed. It appears this is a suitable approach for generating potent CB2R agonists, however the receptor subtype selectivities are not sufficient for the development of selective CB2R agonists. Adopting this approach for the corresponding 3-amidoalkylpyrazolo[3,4-b]pyridine derivatives may yield even more selective compounds.
Table 8: Agonist activities of azaindole derivatives (279-292) in AtT-20 cells expressing human CB1 or CB2 receptors by a FLIPR membrane potential assay.\(^a\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB1 pEC(<em>{50}) ± SEM (EC(</em>{50}) μM)</th>
<th>E(_{\text{max}}) (%)</th>
<th>CB2 pEC(<em>{50}) ± SEM (EC(</em>{50}) μM)</th>
<th>E(_{\text{max}}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>279</td>
<td>6.82 ± 0.14 (0.152)</td>
<td>75</td>
<td>7.37 ± 0.08 (0.043)</td>
<td>101</td>
</tr>
<tr>
<td>280</td>
<td>NA</td>
<td>ND</td>
<td>7.31 ± 0.10 (0.049)</td>
<td>93</td>
</tr>
<tr>
<td>281</td>
<td>6.78 ± 0.13 (0.164)</td>
<td>98</td>
<td>7.41 ± 0.05 (0.038)</td>
<td>86</td>
</tr>
<tr>
<td>282</td>
<td>7.25 ± 0.10 (0.056)</td>
<td>97</td>
<td>7.84 ± 0.07 (0.015)</td>
<td>98</td>
</tr>
<tr>
<td>283</td>
<td>8.77 ± 0.06 (0.0017)</td>
<td>103</td>
<td>8.37 ± 0.09 (0.0043)</td>
<td>102</td>
</tr>
<tr>
<td>284</td>
<td>6.63 ± 0.12 (0.234)</td>
<td>99</td>
<td>7.72 ± 0.12 (0.019)</td>
<td>96</td>
</tr>
<tr>
<td>285</td>
<td>6.92 ± 0.14 (0.121)</td>
<td>117</td>
<td>7.23 ± 0.12 (0.058)</td>
<td>103</td>
</tr>
<tr>
<td>286</td>
<td>8.40 ± 0.06 (0.004)</td>
<td>110</td>
<td>8.29 ± 0.08 (0.005)</td>
<td>94</td>
</tr>
<tr>
<td>287</td>
<td>6.31 ± 0.08 (0.491)</td>
<td>98</td>
<td>7.30 ± 0.11 (0.050)</td>
<td>96</td>
</tr>
<tr>
<td>288</td>
<td>7.45 ± 0.09 (0.035)</td>
<td>106</td>
<td>6.98 ± 0.13 (0.104)</td>
<td>96</td>
</tr>
<tr>
<td>289</td>
<td>6.33 ± 0.12 (0.466)</td>
<td>110</td>
<td>6.82 ± 0.18 (0.152)</td>
<td>95</td>
</tr>
<tr>
<td>290</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>291</td>
<td>6.08 ± 0.10 (0.824)</td>
<td>97</td>
<td>6.92 ± 0.10 (0.122)</td>
<td>104</td>
</tr>
<tr>
<td>292</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>CP 55,940</td>
<td>7.66 ± 0.04 (0.022)</td>
<td>101</td>
<td>7.20 ± 0.04 (0.063)</td>
<td>106</td>
</tr>
</tbody>
</table>

\(^a\)See Chapter 8 for more details. Data represent mean values ± SEM from at least three independent experiments each performed in duplicate, with CP 55,940 used as a positive control. \(^b\)NA: Not active, defined as <50% activation at 10 μM in the assay. \(^c\)ND: Not determined; for compounds defined as not active, their maximal effects were not determined.

5.4 In Silico Studies

5.4.1 Docking Studies

Relatively small structural modifications can have profound effects on the binding affinity and functional profile of GPCR ligands. To investigate the molecular basis for the varied functional activity of the investigated compounds at the CBRs, we performed docking simulations. As only the structure of the inactive state of the CB\(_2\)R with an antagonist bound is currently known,\(^{40}\) the corresponding inactive state of the CB\(_1\)R was also used for this study.\(^{25}\) A caveat to these calculations is that the inactive cannabinoid receptor structures,
solved with an antagonist bound, are not ideal for predicting agonist interactions.\textsuperscript{25, 40} The manner in which agonists bind to the orthosteric site of the CB\(_1\)R has been investigated by integrating docking studies, mutagenesis studies and SAR data.\textsuperscript{261-263} Hua and co-workers have docked known CB\(_1\)R agonists, including indole JWH-018, into the inactive state crystal structure and in combination with mutation studies suggest that interactions with Phe268 and Phe379 are important for functional activity.\textsuperscript{25} Similar studies were conducted based on the active state agonist-bound crystal structure of the CB\(_1\)R and found that \(\pi-\pi\) interactions with Phe177, Phe189, Phe268 and Phe379 and hydrogen bonding with Ser383 were conserved for the agonist-bound complexes.\textsuperscript{27}

It has been extensively reported that the CB\(_1\)R seems to use an extended molecular toggle switch involving a synergistic conformational change between Phe200 and Trp356 for receptor activation.\textsuperscript{27} It has been suggested that agonists disrupt the \(\pi-\pi\) stacking of the side chains of Phe200 and Trp356 leading to conformational change. Similar molecular toggle switches appear to be conserved among numerous GPCRs. For the CB\(_2\)R, agonist binding promotes a conformational change involving TMH6 flexible hinge residue Trp258 and Phe117.\textsuperscript{264} It is difficult, however, to probe the interaction of an agonist with these residues using the inactive state of the receptor.

Compounds \textbf{266, 267, 279} and \textbf{280} were chosen as representative compounds based on their differing receptor subtype selectivities. Docking and \(\Delta G\) binding scores obtained by combining molecular mechanics (MM) terms with a generalised Born and surface area (GBSA) solvent mode (MM-GBSA)\textsuperscript{265} are summarised in Table \textbf{9}. 

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Table 9: Docking and MM-GBSA ΔG binding scores and key interacting residues for compounds docked into the chosen receptors via extra precision (XP) docking.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Protein</th>
<th>Docking score(^a) (kcal mol(^{-1}))</th>
<th>MM-GBSA ΔG(^b) Bind (kcal mol(^{-1}))</th>
<th>Receptor Strain (kcal mol(^{-1}))</th>
<th>Ligand strain (kcal mol(^{-1}))</th>
<th>EC(_{50})(^c) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>266</td>
<td>CB(_1)</td>
<td>-10.40</td>
<td>-73.35</td>
<td>18.26</td>
<td>4.97</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>CB(_2)</td>
<td>-10.31</td>
<td>-67.78</td>
<td>23.00</td>
<td>2.50</td>
<td>74</td>
</tr>
<tr>
<td>267</td>
<td>CB(_1)</td>
<td>-10.43</td>
<td>-55.00</td>
<td>20.92</td>
<td>7.54</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CB(_2)</td>
<td>-9.24</td>
<td>-77.73</td>
<td>7.39</td>
<td>4.11</td>
<td>720(^d)</td>
</tr>
<tr>
<td>279</td>
<td>CB(_1)</td>
<td>-10.71</td>
<td>-71.16</td>
<td>13.65</td>
<td>2.51</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>CB(_2)</td>
<td>-10.14</td>
<td>-77.97</td>
<td>16.28</td>
<td>4.31</td>
<td>43</td>
</tr>
<tr>
<td>280</td>
<td>CB(_1)</td>
<td>-11.44</td>
<td>-60.23</td>
<td>30.51</td>
<td>2.59</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CB(_2)</td>
<td>-11.86</td>
<td>-71.63</td>
<td>21.84</td>
<td>9.15</td>
<td>49</td>
</tr>
</tbody>
</table>

\(^{a}\)Docking scores obtained from extra precision Glide docking are an estimate of protein-ligand binding energies. More negative scores indicate more favourable binding interactions; \(^{b}\)MM-GBSA binding energies are approximate free energies of binding. More negative scores indicate more favourable binding interactions; \(^{c}\)EC\(_{50}\) values are included here for clarity; \(^{d}\)Data obtained from Shi et al.\(^{247}\) due to solubility issues.

APICA (266) is a non-selective agonist and this is well reflected in the docking score (difference of 0.09 kcal mol\(^{-1}\)) and MM-GBSA ΔG\(_{\text{Bind}}\) values (difference of 5.57 kcal mol\(^{-1}\)). At the CB\(_1\)R the compound has π–π interactions with Phe379 and a hydrogen bonding interaction with Ser383, which as previously mentioned are believed to be important interactions for functional activity (Figure 51). At the CB\(_2\)R, the agonist has π–π interactions with Phe91 and Phe183. The importance of these residues for binding has been confirmed by Feng and co-workers via site-directed mutation studies.\(^{266}\)
Figure 51: Docking mode of 3-amidoalkylindole 266 in the: (a) CB$_1$R active site, and (b) CB$_2$R active site. The π-π interactions are shown with blue dashed lines and hydrogen bonding is shown with yellow dashed lines; 2D representation of 266 in the: (c) CB$_1$R active site, and (d) CB$_2$R active site. The π-π interactions are shown with solid green lines and hydrogen bonding is shown with purple arrows.

Movement of the amide from the 3-position to the 2-position of the indole moiety has been shown to eradicate functional activity at the CB$_1$R. This is consistent with this docking study where a more positive MM-GBSA $\Delta$G$_{\text{bind}}$ value (-55 kcal mol$^{-1}$) is observed for 267 compared to 266 (-73.35 kcal mol$^{-1}$). Higher receptor and ligand strain are also observed for this regioisomer at the CB$_1$R. Importantly, the hydrogen bonding interaction with key
residue Ser383 was not observed, potentially explaining the eradication of functional activity (Figure 52). At the CB$_2$R similar docking scores and MM-GBSA $\Delta G_{\text{Bind}}$ values (differences of 1.07 kcal mol$^{-1}$ and 9.95 kcal mol$^{-1}$ respectively) were observed for the two regioisomeric compounds. Furthermore, for the indole nucleus exhibits $\pi-\pi$ interactions with Phe94 and the carbonyl oxygen of the amide has a hydrogen bonding interaction with Ser285 (Figure 52). Li and co-workers suggest mutations of Phe94 greatly reduce the potency of CB$_2$R agonists, while Feng and co-workers also suggest Ser285 is an important residue for ligand binding.
Figure 52: Docking mode of 2-amidoalkylindole 267 in the: (a) CB₁R active site, and (b) CB₂R active site. The π–π interactions are shown with blue dashed lines and hydrogen bonding is shown with yellow dashed lines; 2D representation of 267 in the: (c) CB₁R active site, and (d) CB₂R active site. The π–π interactions are shown with solid green lines and hydrogen bonding is shown with purple arrows.

It was found that for the azaindole scaffold, terminal hydroxylation of the N₁ pentyl chain precluded CB₁R functional activity. Non-selective agonist 279, had a similar docking score and MM-GBSA ΔG_{Bind} value to 266 at both the CB₁ and CB₂ receptors, consistent with their similar functional activity data. Analogous to 266, π–π interactions with Phe91 and Phe183 were observed at the CB₂R (Figure 53).
Chapter 5: Strategies to Develop Selective CB2 Receptor Agonists from Indole Carboxamide Synthetic Cannabinoids

Figure 53: Docking mode of 3-amido-7-azaindole 279 in the: (a) CB1R active site, and (b) CB2R active site. The π-π interactions are shown with blue dashed lines and hydrogen bonding is shown with yellow dashed lines; 2D representation of 279 in the: (c) CB1R active site, and (d) CB2R active site. The π-π interactions are shown with solid green lines and hydrogen bonding is shown with purple arrows.

By comparison, 280 displayed a similar docking score and MM-GBSA ΔG_{bind} value at the CB2R to 279. However, the preference for the CB2R becomes quite clear when examining the MM-GBSA ΔG_{bind} value (CB1R: -60.23 kcal mol^{-1}, CB2R -71.63 kcal mol^{-1}) and particularly the receptor strain (30.51 kcal mol^{-1}) at the CB1R. Although this binding mode interacts with a number of important residues at CB1R (Phe102, Phe379, Ser128 and Ser383), the high receptor strain suggests this is not a favourable pose (Figure 54). For the
CB₂R, 280 shares π–π interactions with Phe91 and Phe183 as well as hydrogen bonding interactions with His95 and Lys278.

**Figure 54:** Docking mode of 3-amido-7-azaindole 280 in the: (a) CB₁ receptor active site, and (b) CB₂ receptor active site. The π-π interactions are shown with blue dashed lines and hydrogen bonding is shown with yellow dashed lines; 2D representation of 280 in the: (c) CB₁ receptor active site, and (d) CB₂ receptor active site. The π-π interactions are shown with solid green lines and hydrogen bonding is shown with purple arrows.
5.4.2 Physicochemical and Pharmacokinetic Properties

An important consideration for this study was to develop more drug-like compounds compared to the previously reported adamantyl compounds published by Shi and co-workers.\textsuperscript{247} A rapid way to determine this is to perform \textit{in silico} predictions of physiochemical and pharmacokinetic properties using QikProp by Schrödinger.\textsuperscript{267} The calculated properties of 275 and 280 were predominantly within the recommended values as suggested by QikProp, and with similar values to 95\% of known drugs (Table 10). Solubility issues are widely recognised in cannabinoid pharmacology because of the lipophilic nature of cannabinoid ligands.\textsuperscript{268} Of particular note, substitution of the adamantyl group for a polar amino acid derived amide resulted in a significant drop in the calculated log P value (6.25 for 266 and 3.66 for 275). Furthermore, the 7-azaindole scaffold with 5-hydroxypentyl group resulted in a significant reduction in calculated log P (6.25 for 266 and 4.38 for 280). While the calculated physicochemical and pharmacokinetic properties of these compounds are promising, likely issues relating to the metabolic stability of the amino acid-derived amides, adamantyl amide and N-pentyl chain would have to be considered before the compounds are advanced further.\textsuperscript{248, 269} In addition, synthetic cannabinoids have been shown to have off-target effects, which can result in major adverse side effects. The main off-target receptors of concern are transient receptor potential (TRP) channels TRPA1 and TRPV1.\textsuperscript{270} These channels function to modulate ion entry, thus mediating a variety of neural signalling processes implicated in the sensation of temperature, pressure, and pH, as well as smell, taste, vision and pain perception. TRP channel dysfunction has been implicated in the pathophysiology of several diseases including neuropathic pain, inflammation, and respiratory disorders. Activation of these receptors by synthetic cannabinoids has been suggested as a potential reason for inflammatory and vasodilatory side effects reported with APICA (266) ingestion. In light of the reported negative side effects of synthetic cannabinoid ingestion, compounds derived from these scaffolds would need to be assessed for their potential off-target effects.
Chapter 5: Strategies to Develop Selective CB2 Receptor Agonists from Indole Carboxamide Synthetic Cannabinoids

Table 10: Calculated physicochemical and pharmacokinetic properties of lead compound 266 and analogues 275 and 280.

<table>
<thead>
<tr>
<th>Calculated properties</th>
<th>MW (g/mol)</th>
<th>log P</th>
<th>PSA (Å²)</th>
<th>Oral Absorption (%)</th>
<th>QPPCaco (nm/sec)</th>
<th>QPPMDCK K (nm/sec)</th>
<th>QPlogBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended Values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;450</td>
<td>&lt;5</td>
<td>&lt;70</td>
<td>&lt;25% poor</td>
<td>&gt;80% high</td>
<td>&gt;500 great</td>
<td>&gt;500 great</td>
<td>-3.0 to -1.2</td>
</tr>
<tr>
<td>266</td>
<td>364.53</td>
<td>6.25</td>
<td>34.80</td>
<td>100</td>
<td>4948</td>
<td>2786</td>
<td>-0.13</td>
</tr>
<tr>
<td>275</td>
<td>343.47</td>
<td>3.66</td>
<td>80.86</td>
<td>100</td>
<td>628</td>
<td>568</td>
<td>-0.88</td>
</tr>
<tr>
<td>280</td>
<td>381.52</td>
<td>4.38</td>
<td>66.97</td>
<td>100</td>
<td>1325</td>
<td>671</td>
<td>-0.82</td>
</tr>
</tbody>
</table>

*Physicochemical properties (molecular weight, MW; lipophilicity, log P; polar surface area, PSA) and pharmacokinetic properties (Oral Absorption; Predicted Apparent Caco-2 Permeability, QPPCaco; Predicted apparent MDCK cell permeability, QPPMDCK; Predicted Brain/Blood Partition Coefficient, QPlogBB) were calculated with QikProp, Schrödinger v5.9 software.

5.5 Concluding Remarks

The work in Chapter 5 represents the development of selective CB2R agonists from non-selective synthetic cannabinoid designer drugs. A small library of 2-amidoalkylindoles and 7-azaindole derivatives were synthesised, characterised and their activity at the cannabinoid receptors evaluated. It has been demonstrated that simple structural modifications can impart receptor subtype selectivity and here we have increased the scope of these strategies to include analogues of some of the most potent synthetic cannabinoids known. In addition, the compounds developed have improved physicochemical properties, more suitable for drug development. Substitution of the amide of 2-amidoalkylindoles has been shown to be a general and effective strategy to improve potency and optimise physicochemical properties. Bioisosteric replacement of the indole core for the 7-azaindole scaffold, in conjunction with the addition of a polar hydroxyl group at the terminal position of the pentyl chain is another example of a general strategy to significantly suppress CB1R activity. Molecular modelling results corroborated the selectivity and potency data gained from activity measurements, and this analysis recapitulated previous literature implicating significant residues at both receptors. The structure-activity relationships developed will facilitate the design of novel CB2 agonists. Future work will focus on the optimisation of the N-alkyl group and amide functionality to further improve potency, selectivity and ADMET properties.
Chapter 6: Development of Novel CB$_2$ Receptor Positive Allosteric Modulators
6.1 Introductory Remarks

The development of allosteric modulators of the cannabinoid receptors offers a promising therapeutic approach with minimal side effects. Like most GPCRs it has been reported that cannabinoid receptors possess allosteric sites that can be targeted by endogenous or exogenous ligands to modulate the receptor’s functional state. As drugs, allosteric modulators possess a number of advantageous properties compared to orthosteric ligands. Compared to the orthosteric site, allosteric sites are generally less conserved, and hence greater subtype selectivity would be expected. Because an allosteric modulator only exerts effects when the endogenous ligand is present, tissue selectivity would also be expected. For these reasons we believe allosteric modulation offers a practical therapeutic approach for targeting the endocannabinoid system.

As discussed in Chapter 1, there have been few reports of allosteric modulators of the cannabinoid receptors. To date, there has been more progress in the development of allosteric modulators of the CB₁R. Until recently only natural products and the endogenous peptide, Pepcan-12, have been reported as CB₂R allosteric modulators. Gado and co-workers recently disclosed a pyridone based compound (79) as the first synthetic allosteric modulator of the CB₂R. The compound was shown to act as a positive allosteric modulator (PAM) and its efficacy in a mouse model for neuropathic pain relief was demonstrated.

The discovery of the PAM (79) came about from a SAR study of known CB₂R orthosteric ligands. In a previous publication, the Manera research group described the polypharmacological profile of 2-oxopyridine-3-carboxamides with the general structure shown in Figure 55. The derivatives synthesised display a broad spectrum of affinity and functional activity towards both cannabinoid receptors, but also exert other effects on different components of the endocannabinoid system. The SAR study determined that the combination of a methyl group at the 4-position, a bulky substituent at the 5-position and a 4-fluorobenzyl group at the N1-position of the 2-pyridone nucleus afforded high affinity for both cannabinoid receptors and also high potency to inhibit the main AEA hydrolytic enzyme, fatty acid amide hydrolase (FAAH). Further studies around the developed scaffold aimed to generate more derivatives to expedite this interesting multi-target approach. In their recent publication detailing the discovery of 79, Gado and co-workers describe the
exploration of structure-activity relationships around the amide substituent at the 3-position of the 2-pyridone ring. In particular, a series of compounds where the direction of the amide is reversed was synthesised and their affinity and functional activity at the cannabinoid receptors assessed (Figure 55). Only a small subset of derivatives was synthesised, with substituents chosen based on the best results reported in previous publications.

Figure 55: SAR study by Gado and co-workers that led to the discovery of the first synthetic CB$_2$R allosteric modulator (79). The key modification of known 2-pyridone orthosteric ligands was reversal of the amide direction at the 3-position.

The binding affinity of the synthesised compounds were assessed in a $[^{3}H]$CP 55,940 binding assay. The ligands were incubated in the presence of membrane preparations obtained from Chinese hamster ovary (CHO) cells overexpressing human CB$_1$ or CB$_2$ receptors and the high affinity orthosteric radioligand $[^{3}H]$CP 55,940. An initial screen at 100 nM showed that a number of the compounds surprisingly enhanced the binding of the radioligand at the CB$_1$R. Compound 79 was shown to induce approximately a 40% increase in the binding of the radioligand up to a concentration of 1 μM (Figure 56), suggesting it may be a CB$_1$R PAM. At the CB$_2$R the majority of compounds were either weak orthosteric ligands or had no binding affinity. Compound 79 however, strongly increased the binding of $[^{3}H]$CP 55,940 at low concentrations suggesting that it is also a PAM of the CB$_2$R.

Functional activity at the cannabinoid receptors was evaluated using a $[^{35}S]$GTP$\gamma$S assay. The assays were carried out with CHO cell membranes overexpressing human CB$_1$ or CB$_2$ receptors in the presence of $[^{35}S]$GTP$\gamma$S, GDP and GTP$\gamma$S and also in the presence of CP 55,940. The effects of 79 on receptor signalling were measured by $[^{35}S]$GTP$\gamma$S receptor binding. At the CB$_1$R 79 had no measurable effect on stimulation of $[^{35}S]$GTP$\gamma$S receptor binding. This suggests that while 79 increases the binding of an orthosteric ligand, this enhancement does not translate to an increased activation of the CB$_1$R. The authors however were hesitant to label 79 as a silent allosteric modulator of the CB$_1$R with only the
current data available. In the case of the CB₂R, the ability of CP 55,940 to stimulate \(^{[35}S\)GTP\(_γ\)S binding was significantly enhanced. It was also demonstrated that in the absence of an orthosteric ligand, 79 did not display any functional activity. This data suggests that 79 is a PAM of the CB₂R. Receptor binding assays were also performed in the presence of the two major endocannabinoids, anandamide and 2-arachidonoylglycerol. 79 was shown to enhance the stimulation of \(^{[35}S\)GTP\(_γ\)S binding to the CB₂R induced by 2-AG, but not AEA. The authors demonstrated that 79 did not induce this enhancement by inhibiting monoacylglycerol lipase (MAGL). Dissociation kinetic assays were also performed to better characterise the allosteric modulatory actions of 79. Dissociation of orthosteric radioligand \(^{[3}H\)CP 55,940 was monitored over the course of 2 hours in the absence or presence of 79. Dissociation of the radioligand was completely prevented by 79 over the period studied, thus supporting the hypothesis that 79 acts at an allosteric binding site.

**Figure 56:** *In vitro* data to characterise the pharmacological profile of 79. Binding assays and functional activity data suggest that 79 is a positive allosteric modulator of the CB₂R. (A) Effects of 79 (C2) on \(^{[3}H\)CP 55,940 binding to CB₁R (left) and CB₂R (right). (B) \(^{[35}S\)GTP\(_γ\)S assay at the CB₁R (left) and CB₂R (right) performed with CP 55,940 and in the presence of 79 (C2). (C) CB₂R \(^{[35}S\)GTP\(_γ\)S binding assay performed in the presence of 79 (C2) with 2-AG (left) and AEA (right). (D) Dissociation of \(^{[3}H\)CP 55,940 from CB₂Rs in the presence or absence of 79 (C2). Asterisks indicate mean values significantly different from zero (*\(P < 0.05\); **\(P < 0.01\); ***\(P < 0.001\); ****\(P < 0.0001\)) (Student’s unpaired t-test). Data are expressed as the mean ± SEM of 6 and 12 independent experiments for CB₁R and CB₂R, respectively, each performed in duplicate. (Adapted

The antinociceptive effects of 79 were assessed in a mouse model of neuropathic pain (Figure 57). Neuropathic pain was induced by chemotherapeutic agent oxaliplatin, whereby successive dosage progressively decreased the pain threshold of mice. The effect of a single dose of 79 on oxaliplatin treated mice showed antinociceptive effects from a 5 mg kg\(^{-1}\) dose. Additional experiments explored the effects of higher endocannabinoid concentrations by the co-administration of a MAGL inhibitor. The effect of both 79 and MAGL inhibitor was higher than that produced by either compound alone. To show that the antinociceptive effects were mediated by the CB\(_2\)R, mice were pre-treated with selective CB\(_1\)R antagonist SR141716A or selective CB\(_2\)R antagonist SR144528 before administration of 79. The efficacy of 79 was significantly decreased by SR144528, while SR141716A only reduced the duration of antihyperalgesic effects.

Figure 57: Data for the antinociceptive effects of 79 (C2) assessed in a mouse model of neuropathic pain. Hypersensitivity was induced by repeated treatment with oxaliplatin and the response to a thermal stimulus was evaluated by the cold plate test measuring the latency (s) to pain-related behaviours (lifting or licking of the paw). (A) 79 (C2) or vehicle were administered and measurements taken every 15 minutes until 75 minutes. (B) The effect of co-administration of CB\(_1\)R and CB\(_2\)R antagonists on the efficacy of 79 (C2). Each point on the graph represents a mean of 16 individual values obtained from 16 mice analysed in 2 different experimental sets. ∧∧\(P < 0.01\) vs vehicle + vehicle treated mice. *\(P < 0.05\) and **\(P < 0.01\) vs oxaliplatin + vehicle treated mice. ANOVA followed by Bonferroni test was performed. (Adapted with permission from Gado et al. J. Med. Chem. 2019, 62 (1), 276-287. Copyright (2019) American Chemical Society).

Figure 58 summarises the minimal SAR data developed from this initial study. Of note, all derivatives in the study conserved the cycloheptyl amide at the 3-position and 4-fluorobenzyl group at the N1-position. The methyl group at the 4-position was briefly
investigated by the synthesis of a 4-hydroxyl analogue and movement of the methyl group to the 6-position. The polar hydroxyl group at the 4-position reduces allosteric activity, while the 6-methyl derivative was inactive. The most in-depth investigation surrounded the substituent at the 5-position. Of the analogues investigated, only the 5-bromo derivative 79 was shown to be a CB$_2$R allosteric modulator. The lack of a substituent at the 5-position, a phenyl group and even a chlorine abolished allosteric activity.

![Figure 58](image_url)

**Figure 58:** A summary of the minimal structure-activity relationship studies performed by Gado and co-workers. The preliminary data suggests that the bromo and methyl substituents at the 4- and 5-positions of the pyridone core as well as the direction of the amide at the 3-position are important for allosteric activity.

Obvious points for further exploration include SAR studies of the amide and N1 substituents. It is assumed that by the time Gado and co-workers had reported 79, efforts towards the synthesis and evaluation of such analogues would already be in progress or perhaps already performed. With the aim of developing novel CB$_2$R allosteric modulators we decided to explore bioisoteric replacement of the 2-pyridone scaffold. Scaffold hopping is a common approach in medicinal chemistry to modify affinities or functional activity, improve physicochemical and ADMET (absorption, distribution, metabolism, excretion and toxicity) properties and to arrive at patentable analogues. Scaffold hopping also allows one to explore structure-activity relationships that are often not possible by conventional analogue preparation. When substituents are appended to a different scaffold then they invariably will adopt slightly different conformations and occupy slightly different areas of space within a receptor binding pocket. In combination with *in silico* docking studies, this allows one to fine-tune the binding requirements for a particular series of compounds. We envisioned that bicyclic aromatic systems where a nitrogen is positioned in a similar spatial arrangement to the carbonyl oxygen of the 2-pyridone moiety would be suitable bioisosteric scaffolds (**Figure 59**). It was also envisioned that the hydrogen bond
accepting carbonyl of the pyridone could potentially be replaced by a nitrogen within a pyridine system.

**Figure 59:** Proposed bioisosteric replacement of the 2-pyridone core of lead compound 79 with nitrogen containing bicyclic systems or a pyridine. Potential lone pairs of electrons available for hydrogen bonding that are not involved in the aromaticity of the heterocycle are shown.

With a focus on synthetic viability we proposed a small library of analogues 330-339 (Figure 60). 7-Azaindoles (330-335) were chosen as a suitable bicyclic candidate for scaffold hopping from the lead 2-pyridone core. As discussed in Chapter 5, 7-azaindoles possess two potentially nucleophilic nitrogens. Based on the reaction conditions used, reaction at either position with suitable electrophiles is possible. Therefore, a series of compounds where the 4-fluorobenzyl group is attached at the N1-position were proposed as well the 7-substituted regioisomers. As the chemistry to access these compounds was anticipated to be non-trivial and since substituted azaindole starting materials are either more expensive than unsubstituted 7-azaindole or require synthesis, we aimed to first develop the chemistry on the unsubstituted 7-azaindole scaffold. Once the chemistry had been refined we would work up to analogues with the same substitution pattern as the lead compound 79. The use of the 7-azaindole scaffold changes the spatial orientation of the amide substituent. Compared to lead compound 79, the amide nitrogen at the 3-position of the azaindole analogues are an additional two bonds from the 6-membered pyridine ring system. For lead compound 79 it is presumed the cycloheptyl moiety sits in a hydrophobic pocket within the binding site. Evaluation of the azaindole analogues may therefore probe the size and tolerance of this binding pocket.

Imidazo[4,5-b]pyridine derivative 336 was designed to better replicate the spatial orientation of the cycloheptyl group. While the structure overlays quite well with the lead compound in two dimensions, the three-dimensional conformations would be expected to be slightly different. In addition, this compound also probes the requirement for a hydrogen bond donating group.

Imidazo[1,2-α]pyridine derivatives 337 and 338 reproduce the spatial orientation of substituents at the 3-, 4- and 5-positions of lead compound 79. However, compared to the
lead compound these structures differ in the arrangement of the 4-fluorobenzyl group. Phenyl derivative 337 prevents free bond rotation of the 4-fluorophenyl group and so the substituent is locked in a particular orientation. The benzyl derivative 338 allows for rotation, however the extra methylene group places the 4-fluorophenyl moiety further from the heterocyclic core.

Attempts to develop a retrosynthetic strategy to access a pyridine analogue of 79 were fruitless. We realised however that a pyrimidine derivative would be more synthetically viable. Pyrimidine analogue 339 would be expected to adopt a very similar three-dimensional conformation as the lead compound. This analogue investigates the placement of heteroatoms within the heteroaromatic core. It is unknown whether the pyridone nitrogen of the lead compound is important for functional activity or whether it is just a convenient handle for the 4-fluorobenzyl group.
6.2 Synthesis of CB₂R PAM Analogues

The synthesis of lead compound 79 followed the procedures reported by Gado and co-workers (Scheme 56). Starting with commercially available 2-amino-4-methyl-3-nitropyridine, treatment with sodium nitrite in aqueous acid afforded nitro pyridone 340. Reduction of the nitro group under hydrogenation conditions followed by reaction with cycloheptanecarbonyl chloride formed amide 342. Bromination and alkylation using 4-fluorobenzyl bromide and cesium fluoride gave the final product 79. 2-Pyridones are weak acids that form mesomeric anions that react readily with electrophilic reagents at the nitrogen, oxygen or carbon atom depending on the reaction conditions. The position of alkylation depends on the solvent, counterion and reagent. It has been reported that the regioselectivity of pyridone alkylation can be tuned toward selective N-alkylation using...
cesium fluoride as a base. While some of the regioisomeric product $O$-alkylated product was obtained, separation of the desired regioisomer was possible by chromatography. The position of alkylation was confirmed by an nOe correlation between the methylene protons of the benzyl substituent and the proton at the 6-position of the 2-pyridone nucleus (see Appendix 1). We thought it would be interesting to evaluate the pharmacological profile of the $O$-alkylated regioisomer 344. In order to promote $O$-alkylation, a Koenigs-Knorr-type reaction was performed whereby the use of a silver salt results in the formation of a benzylic carbocation intermediate. Wang and Barnes have performed computational studies to explain the $O$-regioselective alkylation of pyridone and uracil systems. They conclude the transition state energy barrier for $O$-alkylation is much lower than for the $N$-reaction barrier. While this is consistent with hard-soft acid base (HSAB) theory, in that there is a favourable ionic interaction between the hard carbocation and the negatively charged oxygen of the pyridone, and also a favourable covalent interaction between the soft silver ion and nitrogen, Mayr and co-workers advise against the use of HSAB theory to explain the reactivity of ambidentate nucleophiles. Rather, they suggest the regioselectivity can be simply explained by the blockage of $N$-attack by silver coordination. The use of silver carbonate resulted in high yields of the $O$-alkylated product 344 and the regioselectivity of the reaction was confirmed using 2D HMBC NMR spectroscopy (see Appendix 1).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme56.png}
\caption{Synthesis of lead compound 79 regioisomer 344. Reagents and conditions: (a) NaNO$_2$, H$_2$SO$_4$, H$_2$O, 0 °C-rt, 16 h, 90%; (b) H$_2$, Pd/C, MeOH, rt, 16 h, 88%; (c) i) Cycloheptanecarboxylic acid, oxalyl chloride, DMF, CH$_2$Cl$_2$, rt, 2 h, ii) 341, Et$_3$N, DMF, MePh, rt, 72 h, 67%; (d) Br$_2$, CHCl$_3$, rt, 16 h, 53%; (e) 4-Fluorobenzyl bromide, Ag$_2$CO$_3$, MePh, THF, 66 °C, 16 h, 55%; (f) 4-Fluorobenzyl bromide, CsF, DMF, 50 °C, 16 h, 60%.
\end{figure}
Chapter 6: Development of Novel CB2 Receptor Positive Allosteric Modulators

$N$-(Indol-3-yl)amides and $N$-(azaindol-3-yl)amides are notoriously difficult motifs to prepare on account of the poor stability of the corresponding 3-aminopic precursors. The lack of commercially available 3-aminindoles or 3-aminooazaindoles infers their instability and the difficulties associated with their synthesis. Recently, the Kassiou group developed a convenient synthetic method to access $N$-(indol-3-yl)amides from readily available indole-3-carboxylic acids.\textsuperscript{278} The method involves formation of the corresponding acyl azide followed by a Curtius rearrangement to afford an isocyanate, which is reacted with a carboxylic acid to form a mixed anhydride (Scheme 57). Catalytic 4-dimethylaminopyridine (DMAP) facilitates the decomposition of the anhydride leading to the desired amide with concomitant loss of carbon dioxide. While the reaction works well for a large range of carboxylic acids, it is heavily influenced by steric and electronic, with tert-butyl, adamantyl and 4-nitrophenyl carboxylic acids yielding only trace amounts of product.

Scheme 57: Proposed mechanism for formation of $N$-(indol-3-yl)amides via the Curtius rearrangement.

Preliminary test reactions with cycloheptane carboxylic acid and 7-azaindole-3-carboxylic acid afforded poor yields of the desired amide, with a urea by-product the main isolated species. Since the amide substituent is an obvious point of interest for future studies, a more general synthetic route that allows access to any amide was pursued.

3-Nitro-7-azaindoles are easily accessible from the regioselective nitration of 7-azaindoles. It was envisioned that a one-pot reduction of the nitro group and protection of the resulting amino group would avoid isolating the unstable 3-aminooazaindole intermediate. In the case of a Boc-protected amine, deprotection under acidic conditions would afford a quaternary
amine salt, which would presumably afford stability. Finally, amidation under basic conditions would provide the desired amide products.

A number of conditions were trialled for the nitration of 7-azaindole. The use of a mixture of silver nitrate and N-bromosuccinimide is reported as a convenient reagent system for the nitration of aromatic compounds.\textsuperscript{279} However, under the reported reaction conditions a low yield (about 30%) of 345 was obtained. Improved results were obtained using a mixture of nitric and sulfuric acid (48-82%, Scheme 58). Some attempts were made to reduce the nitro group to probe the stability of the 3-amino-7-azaindole structure. Under a range of common reaction conditions (Pd catalysed hydrogenation, iron-acetic acid and tin chloride-acid) obvious decomposition rapidly occurred.

N-Alkylation of 345 under basic conditions afforded intermediate 347. The position of alkylation (N1-position) was confirmed by an nOe correlation between the methylene protons of the benzyl substituent and the proton at the 2-position of the azaindole nucleus (see Appendix 1). For the proposed one-pot nitro reduction and Boc protection, a suitable reducing agent was required with high chemoselectivity for the nitro group over aryl bromides, Boc anhydride and the carbamate product. Several common nitro reducing agents were tested in the presence of Boc anhydride. It was found that nickel borohydride produced the best results with other reagents producing a mixture of mainly decomposition products. Nickel borohydride, which is generally formed \textit{in situ} by reaction of sodium borohydride and nickel chloride, is often used for the chemoselective reduction of nitriles. If the nitrile reduction is performed in the presence of Boc anhydride, protected amines are obtained.\textsuperscript{280} The reduction of aryl nitro groups using nickel borohydride has been reported.\textsuperscript{281} The portionwise addition of sodium borohydride to a mixture of the 3-nitroazaindole 347, Boc anhydride, 4-dimethylaminopyridine and catalytic nickel chloride afforded the Boc protected intermediate in low but workable yield (Scheme 58). Removal of the Boc group using trifluoroacetic acid afforded the amino trifluoroacetate salt, which was used immediately in the following reaction. Amidation with cycloheptylcarbonyl chloride under basic conditions afforded the desired amide 330 in reasonable yield over 3 steps. Following the same procedures, 5-bromo derivative 331 was synthesised from commercially available 5-bromo-7-azaindole.
Scheme 58: Synthesis of 1-substituted azaindole derivatives 330 and 331. Reagents and conditions:
(a) H$_2$SO$_4$, HNO$_3$, 0 °C 30–60 min, 56–82%; (b) 4-Fluorobenzyl bromide, NaH or K$_2$CO$_3$, DMF, 0 °C–rt, 4 h, 61–69%; (c) (Boc)$_2$O, DMAP, NiCl$_2$.6H$_2$O, NaBH$_4$, EtOH, THF, 0°C–rt, 1 h; (d) TFA, CH$_2$Cl$_2$, rt, 2 h; (e) i) Cycloheptyl carboxylic acid, DMF, CH$_2$Cl$_2$, rt, 2 h, ii) iPr$_2$EtN, CH$_2$Cl$_2$, rt, 16 h, 30% over 3 steps, for 330 cycloheptanecarboxylic anhydride, DMAP, iPr$_2$EtN, CH$_2$Cl$_2$, rt, 2 h, 13% over 3 steps.

In the case of derivative 332, requisite 5-bromo-4-methyl-7-azaindole 351 required synthesis (Scheme 59). Regioselective iodination of commercially available 5-bromo-4-methylpyridin-2-amine and Sonogashira cross coupling with TMS acetylene gave alkyne 350. A base mediated ring closure afforded the azaindole scaffold. Using similar conditions to those reported above, nitration and $N$-alkylation afforded key intermediate 353. Using the nickel borohydride reduction conditions developed, a complex mixture of products was obtained. Despite attempts to optimise the reaction conditions for this substrate, the desired Boc protected amino species was not obtained. In light of this poor result we required a new methodology to access the desired amide. After a review of the literature for mild and chemoselective reducing agents we came across metallic indium. Indium is non-toxic, unreactive towards air and water and has a useful first ionisation potential (5.8 eV) for the reduction of imines, the heterocyclic ring in benzo-fused nitrogen heterocycles, oximes, nitro compounds and conjugated alkenes. A major shortcoming of the use of indium is its high cost, which is similar to other rare elements such as silver. As the nitro reduction is the final step in the synthetic sequence, the reactions were carried out on a rather small scale and hence the cost was not too exorbitant. Reported conditions for the indium-mediated reduction of nitro groups use elemental indium in acetic acid. It was envisioned that in the presence of an anhydride the desired amide would be formed. The requisite
anhydride was prepared from the carboxylic acid according to the procedures described by Bartoli and co-workers.\textsuperscript{205} Reaction of nitro derivative \textit{353} with indium, acetic acid and cycloheptanecarboxylic anhydride unfortunately afforded the undesired acetamide derivative, presumably by reaction of the anhydride with acetic acid to form a mixed anhydride and then reaction of the amino-azaindole at the more sterically accessible carbonyl carbon of the mixed anhydride. Therefore, a two-step procedure was implicated, whereby the nitro reduction was performed in the presence of ethanolic hydrochloric acid rather than acetic acid, to form the quaternary hydrochloride salt and subsequently reacted with cycloheptanecarboxylic anhydride under basic conditions to afford the desired amide \textit{332}.

\begin{center}
\textbf{Scheme 59:} Synthesis of 7- azaindole \textit{332}. \textit{Reagents and conditions:} (a) NIS, TFA, AcOH, rt, 16 h, 49%; (b) TMS acetylene, CuI, PdCl$_2$(PPh$_3$)$_2$, Et$_3$N, THF, rt, 16 h, 71%; (c) KOtBu, DMF, 80 °C, 30 min, 73%; (d) H$_2$SO$_4$, HNO$_3$, 0 °C, 1 h, 48%; (e) 4-Fluorobenzyl bromide, K$_2$CO$_3$, rt, 2 h, 58%; (f) i) Indium, ethanolic HCl, MeOH, 55 °C, 1.5 h, ii) Cycloheptanecarboxylic anhydride, DMAP, iPr$_2$EtN, CH$_2$Cl$_2$, 0 °C – rt, 24 h, 27%.
\end{center}

Similar methods were used to access regioisomeric products \textit{333-335} (\textbf{Scheme 60}). Under basic conditions 7-azaindoles react with suitable electrophiles at the N1-position. However, in the absence of a base, the lone pair of electrons on the nitrogen at the N1-position are tied up in the aromaticity of the azaindole scaffold, and hence are not reactive towards electrophiles. In the presence of a suitable base, deprotonation of the NH, provides an additional orthogonal lone pair of electrons, available for reaction with electrophiles. The lone pair on the nitrogen at the N7-position however sits perpendicular to the plain of the ring and is not involved in the aromaticity of the system and therefore is reactive towards electrophilic reagents. Because of the strong electron withdrawing capabilities of the nitro group, rather harsh conditions are required for reaction of the poorly nucleophilic pyridine-
like nitrogen with alkyl halides. Attempts were made to reverse the order of the steps, that is, perform the regioselective alkylation and then install the nitro group. While alkylation at the $N7$-position occurred in high yield, subsequent nitration was unsuccessful with a number of nitrating reagents. It was found that at $150 \, ^\circ \mathrm{C}$ in the presence of sodium iodide, the desired alkylated nitro derivatives 354-356 were obtained after basic workup. The position of alkylation was confirmed by 2D HMBC NMR spectroscopy (see Appendix 1). A correlation between the methylene protons of the benzyl substituent and the carbon at the 6-position of the azaindole nucleus was observed. As in the case of analogue 332, the nickel borohydride reduction method was unsuccessful. Mass spectrometry analysis indicated that the nitro group was rapidly reduced, but also, further reduction of the heteroaromatic ring was occurring. Using the indium mediated reduction procedure discussed above, the final amides 333-335 were obtained in moderate yield.

![Scheme 60: Synthesis of 7-substituted azaindoles 333-335. Reagents and conditions:](image)

**Scheme 60:** Synthesis of 7-substituted azaindoles 333-335. *Reagents and conditions:* (a) 4-Fluorobenzyl bromide, NaI, MeCN, $150 \, ^\circ \mathrm{C}$, 24 h, 50–72%; (b) i) Indium, ethanolic HCl, MeOH, $55 \, ^\circ \mathrm{C}$, 1 h, ii) Cycloheptanecarboxylic anhydride, DMAP, iPr$_2$EtN, CH$_2$Cl$_2$, 0–rt, 2 h, 36–43%.

It was envisioned that the synthesis of imidazopyridine derivative 336 could be achieved by the retrosynthetic strategy outlined in **Scheme 61**. Disconnection of the $N$-benzyl group at the $N7$-position of 336 affords imidazopyridine 357. This could be synthesised from a condensation of $\alpha$-hydroxy carboxylic acid 360 and diaminopyridine 359 followed by oxidation of the alcohol.

![Scheme 61: Retrosexual strategy for the synthesis of 336.](image)
Diaminopyridine 359 was synthesised from commercially available 5-bromo-4-methylpyridin-2-amine by nitration followed by chemoselective iron-acetic acid mediated reduction (Scheme 62). The mechanism to form 3-nitro species 361 is not as straightforward as it first seems. Treatment of 5-bromo-4-methylpyridin-2-amine with a mixture of nitric acid in sulfuric acid forms the 2-nitraminopyridine. Treatment with concentrated sulfuric acid results in a nitramine rearrangement to the 3-nitro product 361.283

The synthesis of α-hydroxy carboxylic acid 360 required a multi-step synthesis. Initially a one-step homologation reaction reported by Mizuno and co-workers was attempted.284 Reaction of a carboxylic acid with diethyl phosphorocyanidate provides dicyanophosphates, which can be hydrolysed under acidic conditions to provide homologated α-hydroxy carboxylic acids. While both the intermediate dicyanophosphate and desired α-hydroxy carboxylic acid were observed under the reported reaction conditions, a number of products were formed and the purification was not trivial. An alternative procedure was therefore adopted. A Darzens reaction of cycloheptanone with ethyl chloroacetate using sodium hydride as a base afforded α,β-epoxide ester (glycidic ester) 362.285 There are a number of reported reagents for the isomerisation of glycidic esters to α-hydroxy-β,γ-unsaturated esters. The use of strong protic acids such as sulfuric acid and strong Lewis acids such as boron trifluoride, can lead to the formation of multiple products. Kumareswaran and co-workers have reported the use of mild Lewis acid trimethylsilyl triflate for the clean conversion of glycidic esters to α-hydroxy-β,γ-unsaturated esters.286 Under the reported conditions the desired α-hydroxy ester 363 was obtained after hydrogenation of the intermediate alkene in good yield. Attempts to condense ester 363 or α-hydroxy carboxylic acid 360 with diaminopyridine 359 under a variety of conditions to form the imidazopyridine core were ultimately unsuccessful. Likewise, attempts to mono-amidate 359 followed by intramolecular condensation also failed. It was decided that the hydroxy group should be protected as it was presumed that the free nucleophilic alcohol group was the cause of the poor reaction outcome. Protection of the alcohol with an acetate group to form 364, acid chloride formation and reaction with diaminopyridine 359 formed a mixture of the two regioisomeric amides which were taken on to the next step without rigorous purification as both products converge to the same imidazopyridine intermediate. Removal of the acetate group and oxidation of the alcohol to the ketone afforded imidazopyridine 357. A variety of oxidising agents were tried for the oxidation to ketone 357, however only the mild oxidant manganese dioxide resulted in
clean conversion. Finally, regioselective alkylation at the 7-position under neutral conditions as discussed above, afforded 336 in good yield. The regioselectivity of the reaction was confirmed by 2D NOESY and HMBC NMR spectroscopy, which showed correlations between the methylene protons of the benzyl substituent and the proton and carbon at the 6-position of the imidazopyridine core (see Appendix 1).

![Scheme 62: Synthesis of imidazo[4,5-b]pyridine derivative 336. Reagents and conditions: (a) i) H₂SO₄, HNO₃, -10 °C–rt, 30 min, ii) H₂SO₄, rt, 2 h, 63%; (b) Fe, AcOH, EtOH, H₂O, ), 1 h, 82%; (c) Ethyl chloroacetate, NaH, MeCN, 60 °C, 2.5 h, 59%; (d) i) TMSOTf, CH₂Cl₂, -40 °C, 1 h, ii) H₂, Pd/C, EtOAc, rt, 16 h, 80% over 2 steps; (e) LiOH, H₂O, THF, H₂O, rt, 16 h, 84%; (f) AcCl, AcOH, 0 °C–rt, 16 h, 100%; (g) i) 364, oxalyl chloride, DMF, CH₂Cl₂, 0 °C–rt, 3 h, ii) 359 jPrEtN, CH₂Cl₂, rt, 16 h, iii) AcOH, reflux, 16 h, iv) NaOH (aq.), MeOH, rt, 1 h; (h) MnO₂, CHCl₃, reflux, 48 h; 10% over 4 steps (i) 4-Fluorobenzyl bromide, NaI, MeCN, 150 °C, 24 h, 86%.]

Imidazo[1,2-a]pyridine derivative 337 was synthesised from diaminopyridine 359 (Scheme 63). Reaction of 359 with chloroacetaldehyde resulted in formation of the imidazo[1,2-a]pyridine core. From here, amide coupling afforded intermediate 366. Significant reaction optimisation was required to promote the desired amidation. Coupling via the acid chloride, formed either by reaction of the carboxylic acid with oxalyl chloride, or in situ by reaction with phosphorus oxychloride in pyridine, resulted only in decomposition of 365. Likewise, the use of coupling reagents such as PyBOP® or HOBT-EDCI in the presence of tertiary amine bases also led to decomposition. Interestingly, Kang and co-workers report poor yields for coupling of a similar amino imidazo[1,2-a]pyridine scaffold using standard peptide coupling conditions.²⁸⁷ They found that the omission of an
auxiliary base resulted in markedly improved conversion. Optimal results were obtained using two equivalents of EDCI without added base. Under the reported reaction conditions the desired product was not observed, however, it did appear that the EDCI mediated the formation of cycloheptanecarboxylic acid anhydride. Catalytic DMAP was therefore added and the reaction mixture was heated at reflux in toluene. Under these conditions the desired amide 366 was obtained in moderate yield. The 4-fluorophenyl group was installed via a Suzuki cross-coupling reaction. Iodination at the 3-position using N-iodosuccinimide of 366 afforded the desired cross coupling partner, which was taken on immediately to the Suzuki reaction due to its perceived instability. Using one equivalent of 4-fluorophenyl boronic acid, the desired product 337 was obtained in good yield. A small amount of the di-aryl species was formed by reaction at the 6-position, however these products could be separated by chromatography.

![Scheme 63](image)

**Scheme 63**: Synthesis of imidazo[1,2-a]pyridine derivative 337. Reagents and conditions: (a) Chloroacetaldehyde (aq.), iPrOH, reflux, 24 h, 82%; (b) i) Cycloheptanecarboxylic acid, EDCI, MePh, rt, 16 h, ii) DMAP, reflux, 16 h, 41%; (c) NIS, DMF, 0 °C–rt, 16 h; (d) 4-Fluorophenyl boronic acid, Pd(dppf)Cl₂, K₂CO₃, dioxane, H₂O, 100 °C, 5 h, 69% over 2 steps.

Initial attempts to synthesise imidazo[1,2-a]pyridine derivative 338 utilised cross-coupling chemistry using the previously synthesised 3-iodo derivative of 366. Cross-coupling reactions using sp³ hybridised coupling partners are notoriously more capricious than their sp² hybridised counterparts. Attempts to form Negishi reagents or solid zinc pivalate reagents as reported by Knochel and co-workers were ultimately unproductive. Efforts were therefore refocused on installing the requisite benzyl group in the same step the imidazo[1,2-a]pyridine core is formed (Scheme 64). Tan and co-workers report the
synthesis of imidazo[1,2-a]pyridines through elemental sulfur-mediated oxidative annulation of 2-aminopyridines and aldehydes. Under the reported reaction conditions using commercially available 3-(4-fluorophenyl)propanal, perhaps unsurprisingly, an undesired imidazo[4,5-b]pyridine formed as determined by NMR analysis. Therefore, we sought to synthesise the α-chloro aldehyde 367 in the hope that it will react in a similar fashion to chloroacetaldehyde with diaminopyridine 359. α-Chloro aldehyde 367 was synthesised from 3-(4-fluorophenyl)propanal using iminium organocatalysis. Reaction of the aldehyde with catalytic proline facilitates facile reaction with N-chlorosuccinimide via the corresponding enamine. Using the same conditions as above, reaction of α-chloro aldehyde 367 and diaminopyridine 359 afforded the desired imidazo[1,2-a]pyridine derivative 368. Finally, amide coupling, this time via the requisite acid chloride, furnished the final analogue 338.

![Scheme 64: Synthesis of imidazo[1,2-a]pyridine derivative 338](image)

The synthesis of pyrimidine derivative 339 took advantage of the use of symmetrical dibromo intermediate 371 (Scheme 65). Starting with commercially available 4-fluorobenzyl cyanide, treatment with trimethylaluminium and ammonium chloride followed by hydrochloric acid afforded the amidine hydrochloride salt 369. Condensation with dimethyl methyl malonate and bromination with neat phosphorus oxybromide at 180 °C gave the desired dibromo-pyrimidine 371. Milder conditions for the bromination reaction did not result in the formation of the desired product. A nucleophilic aromatic substitution with ammonia afforded mono-amino product 372. Amidation using cycloheptanecarbonyl chloride afforded mainly the imide product and so the mixture was treated with lithium hydroxide to furnish the desired amide 339.
Scheme 65: Synthesis of pyrimidine derivative 339. Reagents and conditions: (a) i) AlMe$_3$, NH$_4$Cl, MePh, 0 °C–rt, 2 h, ii) 4-Fluorobenzylcyanide, 80 °C, 16 h, 66%; (b) NaOEt, Diethyl methyl malonate, reflux, 16 h, 97%; (c) POBr$_3$, 180 °C, 1 h, 62%; (d) Methanolic NH$_3$, 100 °C, 72 h, 82%; (e) i) Cycloheptane carboxylic acid, oxalyl chloride, DMF, CH$_2$Cl$_2$, rt, 3 h, ii) 372, iPr$_2$EtN, THF, 60 °C, 2 h, iii) LiOH (aq.), MeOH, rt, 30 min, 42%.

6.3 In vitro Evaluation of PAM Analogues

The in vitro pharmacological evaluation of allosteric modulators is notoriously fastidious. Small differences in the cell cultures used or assay medium can have drastic effects on the success of the assay. Initial attempts to reproduce the results reported by Gado and co-workers using lead compound 79 were met with minimal success. Preliminary investigations focused on replicating the ability of 79 to increase the potency of CP 55,940 in a functional activity assay. Consistent with the data reported in the previous chapters of this thesis, we utilised a FLIPR membrane potential assay to assess modulation of functional activity. However, initial attempts showed no modulation of the functional activity of CP 55,940. Conversely, Gado and co-workers used a [$^{35}$S]GTP$_7$S assay to determine the functional activity of 79 on the cannabinoid receptors. It was suspected that the limited success using the FLIPR membrane potential assay might be caused by strong functional selectivity (that is, 79 may not modulate GIRK activation). To investigate this we performed [$^3$H]CP55940 binding assays analogous to that reported by Gado and co-workers. Again 79 appeared to have no effect on the binding of the radiolabelled CP55940 ligand. At the time of compiling this thesis, 79 and its analogues were undergoing continuing biological evaluation.
6.4 Concluding Remarks

The work in Chapter 6 comprises the synthesis of a small library of potential CB₂R PAMs based on 79. Bioisosteric replacement of the 2-pyridone core with a range of nitrogen containing heterocycles aims to identify novel PAMs of the CB₂R and potentially enhance functional activity compared to lead compound 79. With *in vitro* analysis of the analogues currently underway, indications of the structural requirements of the core motif for allosteric activity will be obtained in the near future.
Chapter 7: Summary and Future Directions
7.1 A Pharmacophore Model for the Design of CB₂R Agonists

The work reported in Chapter 2 encompasses the development of a preliminary pharmacophore model for CB₂R agonists. A comprehensive survey of the literature found reports of potent and selective CB₂R agonists based on various different chemotypes. Despite this, it was noted that most of the agonists shared certain structural similarities. To refine this postulate, we analysed 27 agonists based on different chemotypes using two programs: Phase by Schrodinger® and Forge™ by Cresset®, in the hope of identifying common structural or pharmacophoric features. The congruent models produced by the two programs identified a three-dimensional arrangement of certain chemical features and electrostatic properties that is common for the majority of agonists considered. We aimed to systematically investigate this model by performing an SAR study on known CB₂R agonists. To select a suitable lead compound we considered a number of potent and selective agonists reported in the literature. Of the compounds synthesised, pyrazoylidene benzamide 82 (EC₅₀ CB₁ > 10 μM, EC₅₀ CB₂ = 19 nM) was chosen as a suitable lead compound based on its potent functional activity and excellent receptor subtype selectivity.

Further computational work is required to validate and refine our preliminary pharmacophore model. Only a small subset of potent and selective agonists was chosen for this initial study. Consideration of a greater number of agonists would refine the model and may identify more chemical features that are required for functional activity. Furthermore, consideration of agonists with a range of potencies as well as compounds that are inactive will likely improve the ability of the model to predict functional activity. Compound libraries could then be pre-screened for ligands or fragments that fit the model. If the model is valid, novel CB₂R agonists could be reliably identified.

Chapter 3 details an SAR study of lead compound 82, focused on the heteroaromatic core. A small library of heteroaromatic benzamides were synthesised to investigate the influence of the core aromatic moiety on functional activity. It was shown that while –ylidene benzamide lead compound 82 exhibited potent and selective functional activity at the CB₂R, the corresponding amide derivatives were inactive. It was proposed that the –ylidene amide moiety was imperative for functional activity. While docking studies failed to explain the inactivity of the amide derivatives, more in-depth molecular dynamics studies could potentially rationalise the results.
While unlikely, it is possible that the methyl group at the N2-position of the pyrazole plays an important role in the functional activity of pyrazolylidene benzamide derivatives such as 82. To investigate this, 2-amido-5-methylimidazole analogue 162 was proposed. However, all efforts to access this compound were ultimately unsuccessful. An alternative synthetic route has been proposed (Figure 61). Starting with readily available S-methylthiopseudourea sulfate, reaction with 2-fluoro-5-(trifluoromethyl)benzoyl chloride followed by displacement of methyl sulfide with amine 96 would afford a disubstituted guanidine, primed for cyclisation with α-bromo ketone 164 to form the imidazole core. Finally, a nucleophilic aromatic substitution would afford the 5-methylimidazole analogue.

Figure 61: Proposed synthetic route to access 2-amido-5-methylimidazole analogue 162.

To further investigate the finding that the –ylidene amide moiety is imperative for functional activity, we synthesised a series of analogues of 82 with varying linkage moieties between the pyrazole and substituted phenyl group (Chapter 4). The functional activity of the analogues 166-184 appeared to correlate with the planarity of the linkage moiety and the particular arrangement of heteroatoms within the group. These findings allowed for the rational design of potent and selective pyrazolotriazine agonist 261 (CB1R EC50 > 10 μM, CB2R EC50 = 19 nM). The pyrazolotriazine scaffold represents an attractive lead for further optimisation of potency and physicochemical properties. Figure 62 summarises potential avenues of further investigation based on SAR studies on the three pendent groups.

It is presumed that the tert-butyl group (Figure 62, highlighted in pink) fits into a hydrophobic pocket in the CB2R binding site. However, tert-butyl groups are not ideal
substituents from a drug discovery point of view as they increase lipophilicity and are not metabolically stable. Barnes-Seeman and co-workers have demonstrated that trifluoromethycyclopropyl groups are suitable tert-butyl bioisosteres and demonstrate increased metabolic stability. Incorporation of a trifluoromethycyclopropyl group or similar tert-butyl bioisostere would not only improve stability, but also favourably reduce lipophilicity.

The design of a divergent synthesis of the pyrazolotriazine scaffold that allows for incorporation of a range of different substituents at the $N_1$-position of the heterocycle (Figure 62, shown in blue) would allow for rapid SAR analysis. Reported studies for similar chemotypes suggest that varied substitution at this position would be tolerated. Exploration of other saturated heterocycles of varying size and heteroatom content would aim to further improve potency, reduce lipophilicity and improve metabolic stability.

The substituted phenyl group (Figure 62, shown in red) represents a large portion of the molecular mass of 261. A truncation study on this group would aim to determine the minimum fragment required for potent and selective functional activity. Furthermore, other aromatic heterocycles or non-aromatic moieties could be investigated to probe structural requirements for this region of the ligand.

Figure 62: Further SAR studies of the pendent groups around the newly discovered pyrazolotriazine scaffold.
7.2 Selective CB₂R Agonists from Synthetic Cannabinoid Designer Drugs

The work detailed in Chapter 5 identified a number of general strategies to develop novel CB₂R agonists from indole carboxamide synthetic cannabinoid designer drugs. Shifting the amide substituent of indole 3-carboxamide non-selective CBR agonists to the 2-position was shown to be an effective and general strategy to eradicate CB₁R functional activity. A library of 2-amidoalkylindoles were synthesised and their functional activity at the CB receptors evaluated. Certain compounds, particularly 275 (CB₁R EC₅₀ > 10 μM, CB₂R EC₅₀ = 189 nM) exhibited improved potencies and physicochemical properties compared to the adamantyl amide compound 266 reported by Shi and co-workers.²⁴⁷

Optimisation of the N-alkyl group would be expected to yield compounds with improved potencies (Figure 63). Shi and co-workers demonstrated that for adamantyl indole-2-carboxamides varying functional activity was observed based on the substitution at the N₁-position of the indole. Pentyl chain derivatives containing terminal heteroatom substitution or functional groups much as a methoxy group or nitrile moiety would be screened to evaluate how these polar groups influence functional activity. Cyclic groups including 4-fluorobenzyl, tetrahydropyran and tetrahydrofuran are also commonly reported for synthetic cannabinoid agonists and may afford improved functional activity. Finally, the length of the alkyl group could also be investigated as longer or shorter alcohol chains may not eradicate functional activity, as was the case for the 5-hydroxy pentyl derivatives reported in Chapter 5.

Figure 63: Proposed analogues of 2-amidoindole 275 with varying substitution at the N₁-position of the indole.
The work presented in Chapter 5 also demonstrated that 3-amido-7-azaindoles containing a 5-hydroxypentyl chain at the N1-position are potent and selective CB2R agonists. Adamantyl amide 280 (CB1 R EC$_{50}$ > 10 μM, CB2 R EC$_{50}$ = 49 nM) displayed complete selectivity for the CB2R while the amino acid derived amides 284 (CB1 R EC$_{50}$ = 234 nM, CB2 R EC$_{50}$ = 19 nM) and 287 (CB1 R EC$_{50}$ = 491 nM, CB2 R EC$_{50}$ = 50 nM) retained some activity at the CB1 R. 3-Amidoalkylpyrazolo[3,4-b]pyridine 291 (CB1 R EC$_{50}$ = 824 nM, CB2 R EC$_{50}$ = 122 nM) exhibited improved receptor subtype selectivity compared to the corresponding 7-azaindole 279 (CB1 R EC$_{50}$ = 152 nM, CB2 R EC$_{50}$ = 43 nM). It follows that for the pyrazolo[3,4-b]pyridine scaffold, substitution of the adamantyl amide for an amino acid derived amide, in conjunction with a 5-hydroxypentyl moiety at the N1-position would confer more potent and selective compounds (Figure 64).

![Figure 64: Strategy to develop potent and selective CB2R agonists based on the 3-amidoalkylpyrazolo[3,4-b]pyridine scaffold.](image)

Further exploration of the core aromatic heterocycle could also be conducted. 2-Amidobenzimidazoles are easily accessed from commercially available 1H-benzimidazole-2-carboxylic acid. Due to the positioning of the amide substituent at the 2-position of the heterocycle, it would be expected that benzimidazole-based analogues are CB2R selective agonists like 2-amidoindoles (Figure 65). Further exploration of the amide and N-alkyl group would aim to optimise potency and physicochemical properties.
Another consideration for the compounds developed in Chapter 5 is the metabolic liability of the amino acid derived amides and the $N_1$ alkyl chains. The strategies developed in Chapter 5 appear to be quite general and tolerant of different amide substituents. Elaboration of these preliminary results to include a larger number of amide substitutions would potentially result in more drug-like compounds in terms of potency, selectivity but also metabolic stability. The Kassiou group has recently demonstrated the bioisoteric replacement of an adamantyl group with a trifluoroadamantyl moiety improved physicochemical properties and metabolic stability for a P2X$_7$ antagonist. A similar strategy could be employed here to reduce lipophilicity and improve the metabolic stability of the adamantyl lead compounds 267 and 279. In another recent publication, the Kassiou group has shown that metabolism of the terminal position of the $N$-pentyl group of synthetic cannabinoid ADB-PINACA to the carboxylic acid precludes functional activity at the cannabinoid receptors. With this result in mind, judicious choice of the $N$-alkyl group is required for the development of a potential clinical candidate.

7.3 Development of Novel CB$_2$R PAMs

As outlined in Chapter 6, the development of PAMs is an attractive therapeutic strategy for targeting the CB$_2$R. Following Gado and co-workers’ recent report of the first synthetic PAM of the CB$_2$R (79) we aimed to develop novel analogues via bioisosteric replacement of the 2-pyridone scaffold. A series of nitrogen containing heterocycles were chosen as suitable replacements. With biological results still pending, it is unknown whether the novel scaffolds retain binding or functional activity at the CB$_2$R allosteric site.

If any of the analogues demonstrate allosteric modulation then future efforts would focus on SAR studies pertaining to the substituents decorating the heteroaromatic core. Figure 66 summarises potential future directions if a novel scaffold is discovered. The amide substituent is an obvious point of potential diversification (Figure 66, shown in blue). A
Chapter 7: Summary and Future Directions

divergent synthesis of the amide analogues would allow for a rapid survey of the steric and electronic requirements for this group. Based on the functional activity of the cycloheptyl amide derivative, it is likely that the amide substituent sits in a hydrophobic pocket within the allosteric binding site. Exploration of other cycloalkyl amides or bulky polycyclic groups such as adamantane could enhance PAM activity. The cycloheptyl group drastically influences the lipophilicity of the molecule and represents a metabolic liability. Exploration of saturated heterocycles or aromatic amides could favourably reduce lipophilicity and increase metabolic stability. The 4-fluorobenzyl group also offers an obvious point of diversification for further study (Figure 66, shown in red). Design of a divergent synthesis to allow for the incorporation of different substituents at this position would aim to enhance PAM activity and modulate physicochemical properties. Systematic investigation of the benzyl substituent would follow the Topliss scheme to rapidly arrive at the most potent compound.292 Investigation of alkyl, cycloalkyl and heterocyclic groups could also improve the functional activity. Gado and co-workers reported limited SAR data for the bromine substituent at the 5-position of the 2-pyridone core (Figure 66, shown in green). From the limited data, it appears that this position is very sensitive to substitution. Further studies would aim to probe the steric and electronic requirements of this group. Common bromine bioisosteres such methyl, isopropyl and trifluoromethyl groups could be explored as well as larger groups such as iodo and tert-butyl substituents to investigate steric requirements. Other potential substituents to explore would include hydrogen bond accepting groups such as a carbonyl or methoxy group or conversely a hydrogen bond donor such as a hydroxyl group. Finally, it should be determined whether the methyl group (Figure 66, shown in pink) is necessary for activity. Further studies could also investigate whether other substituents at this position could enhance PAM activity.

Figure 66: Further SAR studies to be conducted once a novel heterocyclic scaffold (Het.) is discovered.
7.4 Conclusions

This body of work demonstrates the successful application of three distinct approaches to the discovery and development of CB₂R-based therapeutics. In each case, the structural requirements of ligands for modulation of the CB₂R have been explored and definitive structure-activity relationships have been determined.

In the absence of a crystal structure of the active state CB₂R, a ligand-based drug design approach was taken. Efforts towards the development of a pharmacophore model have elucidated key structural characteristics necessary for functional activity and have assisted in the development of a novel lead (261), suitable for further development.

In contrast to the rational design of selective CB₂R agonists, we also considered strategies to improve the receptor subtype selectivity of known non-selective cannabinoid receptor agonists. Synthetic cannabinoids have recently emerged as increasingly popular psychoactive substances, originally designed to mimic or produce similar effects to cannabis. These typically simple, indole-based compounds are highly potent agonists at both cannabinoid receptor subtypes. Using synthetic cannabinoids as lead compounds we have demonstrated the efficacy of a number of simple structural modifications that can eradicate functional activity at the CB₁R. Using these strategies novel leads have been identified for further optimisation and development.

Allosteric modulation of the CB₂R offers an appealing strategy for the development of cannabinoid-based therapeutics. We have explored structure-activity relationships of the first reported synthetic PAM of the CB₂R. Identification of novel scaffolds will assist in the development of suitable drug-like leads and the evolution of the emerging field.
Chapter 8: Experimental
8.1 *In Vitro* Pharmacological Assay Details

Mouse AtT-20 neuroblastoma cells stably transfected with human CB₁ or human CB₂ receptors were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U penicillin/streptomycin, and 80 μg/mL Hygromycin B. Cells were passaged at 80% confluency, as required. Cells for assays were grown in 75 cm² flasks and used at 90% confluency. The day before the assay, cells were detached from the flask with trypsin/EDTA (Sigma) and resuspended in 10 mL of Leibovitz’s L-15 media supplemented with 1% FBS, 100 U penicillin/streptomycin, and 15 mM glucose. The cells were plated in a volume of 90 μL in black-walled, clear-bottomed 96-well microplates (Corning) that had been precoated with poly-L-lysine (Sigma, Australia). Cells were incubated overnight at 37 °C in ambient CO₂.

Membrane potential was measured using a FLIPR membrane potential assay kit (blue) from Molecular Devices, as described previously. The dye was reconstituted with assay buffer of the following composition (mM): NaCl 145, HEPES 22, Na₂HPO₄ 0.338, NaHCO₃ 4.17, KH₂PO₄ 0.441, MgSO₄ 0.407, MgCl₂ 0.493, CaCl₂ 1.26, and glucose 5.56 (pH 7.4, osmolarity 315 ± 5 mOsm). Prior to the assay, cells were loaded with 90 μL/well of the dye solution without removal of the L-15, giving an initial assay volume of 180 μL/well. Plates were then incubated at 37 °C at ambient CO₂ for 45 minutes. Fluorescence was measured using a FlexStation 3 (Molecular Devices) microplate reader, with cells excited at a wavelength of 530 nm and emission measured at 565 nm. Baseline readings were taken every 2 seconds for at least 2 minutes, at which time either drug or vehicle was added in a volume of 20 μL. The background fluorescence of cells without dye or dye without cells was negligible. Changes in fluorescence were expressed as a percentage of baseline fluorescence after subtraction of the changes produced by vehicle addition, which was negligible for drugs dissolved in assay buffer or DMSO. The final concentration of DMSO was not more than 0.1%. Data were analysed with PRISM (GraphPad Software Inc., SanDiego, CA), using four-parameter non-linear regression to fit concentration–response curves. In all plates, a maximally effective concentration of CP55940 was added to allow for normalization between assays.
8.2 In Silico Study Details

8.2.1 Pharmacophore Modelling from Chapter 2

Phase

Phase v4.9\textsuperscript{183, 295} implemented in the Schrödinger\textsuperscript{®} 2019-2\textsuperscript{182} software package was used for the generation of a pharmacophore model for CB\textsubscript{2}R agonists. Twenty-seven representative CB\textsubscript{2}R selective agonists (shown in Figure 67) were collected from the literature. When multiple structures were reported in the original publication or patent, one representative compound (usually the most potent) from each structural class was selected.

The three-dimensional structures of the 27 agonists were generated with ChemDraw 3D 16.0. All ligands were prepared using the LigPrep v4.9\textsuperscript{296} module implemented within Maestro v12.0 to generate possible low energy conformations of the ligands as well as generating all potential ionisation states at pH 7±2.

Pharmacophore features used for hypothesis generation were: hydrophobic group (H), hydrogen bond donor (D), hydrogen bond acceptor (A), aromatic ring (R), positively ionisable (P) and negatively ionisable (N) defined by a set of chemical structure patterns. For the current dataset, default settings were used with a maximum of 50 conformers generated. Four to five features were chosen for model construction. The pharmacophore features were used to create pharmacophore sites for all the compounds. The alignment was measured using a survival score and the default values were used for the hypothesis generation. The best-ranked pharmacophore model obtained by Phase (Chapter 2), consisted of four features: one aromatic ring (R), one hydrogen-bond acceptor (A) and two hydrophobic sites (H).
Figure 67: List of 27 CB₂R agonists used for the pharmacophore modelling study. The reported functional activity data at the CB₂R are reported for reference. See Chapter 1 for the associated literature references.
FieldTemplater™

FieldTemplater™ (Forge™, Cresset®)\textsuperscript{184-185} was used to determine a hypothesis for the three-dimensional conformation adopted by agonists in binding to the CB\textsubscript{2}R. Field and 3D-shape information was used to create a template using five random agonists (38, 48, 65, 66 and 67 see Figure 67). The five compounds were conformationally populated using Cresset’s Xedex conformational search method (embedded within Forge™).\textsuperscript{297} The 3D field point pattern for each conformation was calculated and used to cross-compare to each conformer of the other ligands, with no dependence on chemical structure. Using 3D field point patterns, the conformations of the five agonists were compared in a pair-wise fashion until field point patterns common to all five molecules were identified.

8.2.2 Docking Studies from Chapter 3

Lead compound 82 and amide analogue 118 were docked into the binding site of the only reported CB\textsubscript{2}R crystal structure\textsuperscript{40} (PDB id: 5ZTY) with AutoDock Vina version 1.1.2.\textsuperscript{298} The structure of the CB\textsubscript{2}R was prepared for docking with AutoDockTools 1.5.6 (ADT).\textsuperscript{299} The three-dimensional structures of the compounds studied were prepared with ChemDraw 16.0 and Avogadro v1.2.0.,\textsuperscript{300} with ADT used to assign both rigid and rotatable bonds and to remove non-polar hydrogen atoms. PROPKA 3.1\textsuperscript{301} was used to predict the protonation state of active site residues and ionisable ligand groups at pH 7.0. Docking was performed in a 30×30×30 Å box centred at the active site highlighted in the CB\textsubscript{2}R crystal structure (PDB id: 5ZTY). The dimension was chosen to ensure it was big enough to accommodate key residues within the CB\textsubscript{2}R binding site. In all the docking calculations, the receptor was kept rigid and no explicit waters have been included. The docking procedure was validated by re-docking of antagonist AM10257 to replicate the crystallographically determined CB\textsubscript{2}R–AM10257 complex.

8.2.3 Docking Studies from Chapter 4

Target protein Preparation

The X-ray crystal structures of the CB\textsubscript{1} and CB\textsubscript{2} proteins (PDB ID: 5TGZ and 5ZTY)\textsuperscript{25,40} were obtained from the RCSB Protein Data Bank (\url{https://www.rcsb.org/}). The protein structures were prepared using preparation and refinement protocols, directed by the Protein Preparation Wizard\textsuperscript{302} embedded in Maestro v11.9 (Schrödinger, LLC, New York, NY).
York, USA). This process includes assigning bond orders, adding hydrogen atoms, creating zero-order bonds to metals and disulphide bonds, deleting water molecules beyond 5 Å from heteroatoms and filling in missing side chains using Prime v5.5.303 Missing side chains and atoms were added using Prime for the both systems. The hydrogen bond network within the protein was also optimised with all het groups within the receptor grid bounding box previously removed and the protein structure minimised to a root mean square deviation (RMSD) of 0.3 Å using the OPLS3e force field.302, 304

### Ligand Preparation

All ligands were prepared using the LigPrep v4.9296 module to generate possible low energy conformations of the ligands as well as generating all potential ionisation states at pH 7±2.

### Docking Studies

The ligands were docked into the receptor with Induced Fit Docking305 with default settings. Standard protocol and OPLS3e force field304 was used for the calculations. Box centre was set with the ligand bound to the reported protein structure. Receptor van der Waals scaling and Ligand van der Waals scaling was set to 0.50. Residues within 5 Å of ligand poses were refined, with optimisation of side chains. Ligands were re-docked into the protein structure with Extra Precision (XP)306 to refine binding energy estimates. All ligands were docked with flexible states to allow sampling of the effect of nitrogen inversion, changing ring conformations and non-planar amide functional groups were penalised. Prime MM-GBSA calculations307 which combines molecular mechanics (MM) terms, and a generalised Born and surface area (GBSA) solvent mode,265 was utilised to calculate the free energy of binding for the ligands. The output poses from Induced Fit Docking were used as the basis for these calculations. The calculations were performed using the variable-dielectric generalised Born (VSBG)308 solvation model and OPLS3 force field.304 Residues within 20 Å from the ligand were set flexible for the calculation.

### 8.3 General Experimental

All reactions were performed under an atmosphere of nitrogen unless otherwise specified. Pyrrole was purified by fractional distillation before use. Molecular sieves were activated
in a microwave before use. Anhydrous N,N-dimethylformamide, dichloromethane, tetrahydrofuran, acetonitrile, diethyl ether and toluene were obtained from a PureSolv MD 7 solvent purification system (Innovative Technology, Inc.). All other solvents and reagents were used as received from commercial sources. Analytical thin-layer chromatography (TLC) was performed using Merck aluminium backed silica gel 60 F254 (0.2 mm) plates, which were visualised with shortwave (254 nm) ultraviolet light. Products were also visualised with potassium permanganate, vanillin, cerium molybdate, bromocresol green or ninhydrin stains. Flash column chromatography was performed using Merck Kieselgel 60 (230-400 mesh) silica gel, with the eluent mixture reported as the volume:volume ratio. Melting points were measured in open capillaries using a Stanford Research System Optimelt Automated melting point apparatus. Infrared absorption spectra were recorded on a Bruker ALPHA FT-IR spectrometer as a solid or a thin film from ethanol, and the data are reported as vibrational frequencies (cm\(^{-1}\)). Nuclear magnetic resonance spectra were recorded at 300 K or 350 K using either a Bruker AVANCE DRX300 (300 MHz), AVANCE DRX400 (400 MHz) or AVANCE DRX500 (500 MHz) spectrometer. \(^1\)H chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26), methanol (δ 3.31) and dimethyl sulfoxide (δ 2.50) as reference and are reported as chemical shift (δ); relative integral; multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, dtd = doublet of triplets of doublets, t = triplet, td = triplet of doublets, tt = triplet of triplets,qtt = triplet of triplet of triplets, q = quartet, qd = quartet of doublets, quin = quintet, sept = septet, m = multiplet, bm = broad multiplet); coupling constants (J) reported in Hz. \(^{13}\)C chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.16), methanol (δ 49.00) and dimethyl sulfoxide (δ 39.52) as reference and reported as chemical shift (δ); multiplicity, coupling constants (J) reported in Hz. Proton decoupled \(^{19}\)F chemical shifts are reported as parts per million (ppm). Low-resolution mass spectra (LRMS) were recorded using electrospray ionisation (ESI) recorded on a Bruker AmaZon SL ion trap spectrometer. High-resolution mass spectrometry was performed on a Bruker Apex Qe 7T Fourier Transform Ion Cyclotron Resonance mass spectrometer equipped with an Apollo II ESI/MALDI dual source. Samples were run with syringe infusion at 150 μL/hr on a Cole Palmer syringe pump into the electrospray ionisation (ESI) source. Optical rotation was performed on an Optical Activity AA-65 Automatic Polarimeter. Specific rotations based on the equation [\(\alpha\)] = (100α)/(lc) are reported as unitless numbers and are in the form: [\(\alpha\)]\(D\)\(^{20}\) ±XX (c, solvent), where c is the concentration.
in g/l00 mL and the path length, l, is in decimetres. High performance liquid chromatography (HPLC) analysis of organic purity was conducted on a Waters Alliance 2695 instrument using a SunFire™ C18 column (5 μm, 2.1 x 150 mm) and detected using a Waters 2996 photodiode array (PDA) detector set at either 230 or 254 nm. Separation was achieved using water + 0.1% trifluoroacetic acid (solvent A) and acetonitrile + 0.1% trifluoroacetic acid (solvent B) at a flow rate of 0.2 mL/min and a gradient of 0% B to 100% B over 30 minutes (Method A) or over 1 hour (Method B). HPLC data is reported as percentage purity and retention time (RT) in minutes.

8.4 General Procedures

General Procedure A: 4-Toluenesulfonyl protection of alcohols

To a solution of the alcohol in anhydrous dichloromethane (1 mL/mmol) at 0 °C was added pyridine (2 eq.) followed by 4-toluenesulfonyl chloride (2 eq.), portionwise. The reaction mixture was stirred at room temperature for 5 hours before dichloromethane (10 mL/mmol) and water (10 mL/mmol) were added and the layers separated. The organic phase was washed with aqueous hydrochloric acid (2 M, 10 mL/mmol), saturated aqueous sodium bicarbonate (10 mL/mmol) and water (10 mL/mmol), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure.

General Procedure B: Ester, trichloromethylketone and trifluoromethylketone hydrolysis

To a solution of the ester/trichloromethylketone/trifluoromethylketone in a mixture of tetrahydrofuran and methanol (1:1, 2 mL/mmol) was added aqueous alkali metal hydroxide (3 M, 10 eq.) and the mixture was stirred at room temperature or reflux until the starting material was consumed, as determined by thin-layer chromatography. The volatiles were removed under a stream of nitrogen and the aqueous residue washed with dichloromethane (10 mL/mmol) and acidified with aqueous hydrochloric acid (6 M). The aqueous suspension was extracted with dichloromethane (3 x 10 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.
General Procedure C: Acid chloride formation and reaction with amines or alcohols to form amides or esters

The carboxylic acid was dissolved in anhydrous dichloromethane (3 mL/mmol) and to this was added oxalyl chloride (2 eq.) and \(N, N\)-dimethylformamide (1-2 drops) and the mixture stirred at room temperature for 4 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in dichloromethane (1-2 mL) and added to a solution of amine (1.2 eq.) and \(N, N\)-diisopropylethylamine (1.5 eq.) in dichloromethane (5 mL/mmol) at 0 °C. The mixture was warmed to room temperature and stirred for 16 hours, diluted with dichloromethane (15 mL/mmol), washed with saturated aqueous sodium hydrogen carbonate (10 mL/mmol) and saturated aqueous ammonium chloride (10 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure D: Lithium aluminium hydride reduction of esters, carboxylic acids and amides

To a suspension of lithium aluminium hydride (1.2-3 eq. depending on electrophile) in anhydrous tetrahydrofuran (3 mL/mmol) at 0 °C was added a solution of the amide, carboxylic acid or ester in tetrahydrofuran (1-3 mL), dropwise. The reaction mixture was stirred at room temperature or reflux until the consumption of the starting material as determined by thin-layer chromatography. The mixture was cooled to 0 °C and quenched with sodium sulfate decahydrate, portionwise, until gas evolution had ceased. The white suspension was filtered through Celite® and the filtrate concentrated under reduced pressure.

General Procedure E: Nucleophilic aromatic substitution using potassium tert-butoxide as a base

A solution of the nucleophile (1.5 eq.) in tetrahydrofuran (2 mL/mmol) was treated with potassium tert-butoxide (1.8 eq.) and stirred for 15 minutes. To this mixture was added a solution of the aryl halide in tetrahydrofuran (1-3 mL) and the mixture stirred at room temperature or reflux until the starting material was consumed, as determined by thin-layer chromatography. The reaction mixture was quenched with saturated aqueous ammonium chloride (10 mL/mmol) and extracted with ethyl acetate (3 x 10 mL/mmol). The combined
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organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

**General Procedure F: Amide formation by addition of a Grignard reagent to an isocyanate formed by a Curtius rearrangement**

The carboxylic acid was dissolved in dichloromethane (2 mL/mmol) and triethylamine (2 eq.) and stirred for 5 minutes before diphenylphosphoryl azide (1 eq.) was added and the mixture stirred for 16 hours. The solvent was removed under a stream of nitrogen and the crude product purified by flash column chromatography to obtain the acyl azide intermediate.

The aryl halide (0.8 eq.) was dissolved in anhydrous tetrahydrofuran (1 mL/mmol) and to this was added isopropylmagnesium chloride lithium chloride complex (0.85 eq.) at 0 °C. The reaction mixture was stirred for 2 hours, then warmed to room temperature and allowed to stand for 16 hours to obtain Grignard reagent.

A solution of the acyl azide in anhydrous toluene (2 mL/mmol) was heated at 90 °C for 1.5 hours. The mixture was cooled to 0 °C and the freshly prepared Grignard reagent was added slowly. The reaction mixture was warmed to room temperature and stirred for 1 hour, quenched with saturated aqueous ammonium chloride (10 mL/mmol), extracted with ethyl acetate (3 x 10 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

**General Procedure G: Suzuki cross coupling**

A mixture of the aryl halide or pseudohalide, boronate ester (1 eq.), [1,1’-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.08 eq.) and base (3 eq.) in a mixture of degassed dioxane and water (6:1, 2 mL/mmol) was heated at 100 °C for 4-16 hours. After cooling to room temperature, water (10 mL/mmol) was added and the mixture extracted with ethyl acetate (3 x 10 mL/mmol). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure.
**General Procedure H: Hydrogenation of alkenes, alkynes and nitro groups**

To a solution of alkene, alkyne or nitro compound in ethyl acetate (3 mL/mmol) was added palladium on carbon (10% w/w, 0.1 eq.) under an atmosphere of nitrogen. The reaction vessel was evacuated and flushed with hydrogen three times before being stirred at room temperature until the consumption of starting material, as determined by thin-layer chromatography. The mixture was filtered through a pad of Celite® and the filtrate concentrated under reduced pressure.

**General Procedure I: Mitsunobu reaction**

To a solution of triphenylphosphine (1.5 eq.) and the pronucleophile in tetrahydrofuran (3 mL/mmol) at 0 °C was added the alcohol (1 eq.) followed by the dropwise addition of diisopropyl azodicarboxylate (1.5 eq.). The mixture was stirred for 15 minutes before being stirred at 100 °C in a sealed vessel for 30 minutes. The volatiles were removed under a stream of nitrogen.

**General Procedure J: Hydrogenolysis of benzyl ether protected alcohols**

To a solution of the benzyl ether in ethyl acetate (3 mL/mmol) was added palladium on carbon (10% w/w, 0.1 eq.) under an atmosphere of nitrogen. The reaction vessel was evacuated and flushed with hydrogen three times and the mixture stirred for 16-48 hours. The suspension was filtered through a pad of Celite® and the filtrate concentrated under reduced pressure.

**General Procedure K: Sonogashira cross coupling**

The aryl halide or pseudohalide, alkyne (1 eq.), bis(triphenylphosphine)palladium(II) dichloride (0.1 eq.) and copper iodide (0.2 eq.) were dissolved in degassed triethylamine (10 mL/mmol) and heated at 70 °C for 2 hours. After cooling to room temperature, water (50 mL/mmol) was added and the mixture extracted with ethyl acetate (3 x 30 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.
General Procedure L: *N*-Alkylation of aromatic heterocycles under basic conditions

To a solution of the *NH*-aromatic heterocycle in anhydrous *N*,*N*-dimethylformamide (3 mL/mmol) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 1.5 eq.) and the mixture stirred for 5 minutes before the alkyl halide or pseudohalide (1.2 eq.) in *N*,*N*-dimethylformamide (1-3 mL) was added dropwise. The mixture was stirred until the starting material was consumed, as determined by thin-layer chromatography. The reaction mixture was quenched with saturated aqueous ammonium chloride (20 mL/mmol) and extracted with ethyl acetate (3 x 20 mL/mmol). The combined organic extracts were washed with water (3 x 20 mL/mmol) and aqueous lithium chloride (1 M, 20 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure M: EDCI/HOBt mediated amide coupling

The carboxylic acid, amine (1.2 eq.), hydroxybenzotriazole (1.5 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.5 eq.) and *N*,*N*-diisopropylethylamine (5 eq.) were dissolved in *N*,*N*-dimethylformamide (3 mL/mmol) and the mixture stirred for 24 hours. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate (30 mL/mmol), extracted with ethyl acetate (3 x 20 mL/mmol), washed with saturated aqueous sodium hydrogen carbonate (30 mL), saturated aqueous ammonium chloride (30 mL/mmol) and aqueous lithium chloride (1 M, 30 mL/mmol), dried over anhydrous magnesium sulfate and concentrated under reduced pressure.

General Procedure N: Nitration of aromatic heterocycles

To concentrated sulfuric acid (6.5 eq.) at 0 °C was added the aromatic heterocycle, portionwise. A mixture of concentrated nitric acid (1.1 eq.) and concentrated sulfuric acid (2.5 eq.) was slowly added and the mixture stirred for 30 minutes before cold water (3 mL/mmol) was added. The resulting precipitate was collected by filtration, washed with water (3 x 10 mL/mmol) and diethyl ether (10 mL/mmol) and dried *in vacuo*. 
General Procedure O: Alkylation of 7-azaindoles at the 7-position

To a solution of the 7-azaindole in anhydrous acetonitrile (2 mL/mmol) was added the alkyl halide (10 eq.) and sodium iodide (1 eq.) and the mixture was stirred at 150 °C in a sealed vessel for 24 hours. The volatiles were removed under a stream of nitrogen and the residue suspended in hexane (5 mL/mmol). The precipitate was collected by filtration, dissolved in a mixture of saturated methanolic ammonia (5 mL/mmol) and tetrahydrofuran (5 mL/mmol), adsorbed onto Celite® and purified by flash column chromatography.

General Procedure P: Iridium mediated nitro reduction and amidation

To a solution of the nitro compound (120 mg, 330 μmol) in methanol (5 mL/mmol) was added indium (5 eq.) followed by ethanolic hydrochloric acid (2 M, 10 eq.) and the mixture was heated at 55 °C for 1.5 hours. The volatiles were removed under a stream of nitrogen and the residue suspended in anhydrous dichloromethane (5 mL/mmol) at 0 °C and treated with cycloheptanecarboxylic anhydride (2 eq.), 4-dimethylaminopyridine (0.1 eq.) and N,N-diisopropylethylamine (5 eq.). The mixture was warmed to room temperature and stirred for 24 hours. The volatiles were removed under a stream of nitrogen.

8.5 Synthetic Procedures

8.5.1 Synthetic Procedures from Chapter 2

(R)-(Tetrahydrofuran-2-yl)methanol 85

Prepared according to General Procedure D using (R)-tetrahydrofuroic acid (2.0 g, 17.2 mmol) and lithium aluminium hydride (1.3 g, 34.4 mmol) at reflux for 16 hours to obtain the title compound (2.6 g, 97%) as a colourless oil, the characterisation data of which corresponded to that previously described.\textsuperscript{309} \textit{Rf}: 0.24 (3:2 ethyl acetate, hexane); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 26.1, 27.2, 65.0, 68.3, 79.6 ppm; LRMS (+ESI) \textit{m/z}: 125.1([M+Na]\textsuperscript{+} 100%).
(R)-di-tert-Butyl 1-((tetrahydrofuran-2-yl)methyl)hydrazine-1,2-dicarboxylate 86

Prepared according to a modified version of General Procedure I using triphenylphosphine (7.2 g, 27.4 mmol), di-tert-butyl hydrazine-1,2-dicarboxylate (4.6 g, 19.6 mmol), alcohol 85 (2.0 g, 19.6 mmol) and di-tert-butyl azodicarboxylate (6.3 g, 27.4 mmol) at room temperature for 16 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:4) as an eluent to obtain the title compound (4.5 g, 72%), as a white solid, mp: 78.8-82.3 °C; Rf: 0.19 (1:4 ethyl acetate, hexane); IR (v max (neat)): 3314, 3254, 2831, 1737, 1661, 1229, 1144 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.45 (18H, s), 1.78-1.99 (4H, m), 3.29-3.66 (2H, m), 3.67-3.91 (2H, m), 4.00-4.16 (1H, m), 6.58 (1H, bs) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 25.6, 28.3, 29.1, 31.3, 68.0, 74.1, 81.1, 155.4 ppm; LRMS (+ESI) m/z: 339.2 ([M+Na]⁺ 100%).

4,4-Dimethyl-3-oxopentanenitrile 88

To a solution of anhydrous acetonitrile (4.6 mL, 87.2 mmol) in anhydrous tetrahydrofuran (50 mL) at −78 °C was added n-butyl lithium (2.3 M, 36.1 mL, 83.0 mmol), dropwise. The mixture was stirred for 1 hour and then pivaloyl chloride (5.1 mL, 51.5 mmol) was added dropwise and stirred at −78 °C for a further 1 hour. The reaction mixture was warmed to room temperature and quenched with aqueous hydrochloric acid (1 M, 10 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:17) as an eluent to obtain the title compound (2.1 g, 41%) as a light yellow solid, the characterisation data of which corresponded to that previously described. Rf: 0.35 (3:17 ethyl acetate, hexane); LRMS (EI) m/z: 126 (18), 82 (15), 68 (14), 57 (100).
The diprotected hydrazine 86 (2.5 g, 7.9 mmol) was stirred in hydrochloric acid in 1,4-dioxane (4 M, 9.9 mL, 39.5 mmol) for 16 hours. The solvent was removed under a stream of nitrogen and the residue taken up in ethyl acetate. The solid was collected by filtration and dried in vacuo to obtain 87 as a hygroscopic white solid that was used immediately without further purification or characterisation.

The hydrazine hydrochloride salt (87) was suspended in ethanol (40 mL) and to this was added α-cyanoketone 88 (1.2 g, 9.5 mmol) and the mixture heated at reflux for 4 hours. The mixture was cooled, concentrated under reduced pressure and the residue taken up in ethyl acetate (50 mL). The organic phase was washed with saturated aqueous sodium hydrogen carbonate (2 x 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain a crude residue that was purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:99) as an eluent to obtain the title compound (1.4 g, 75% over 2 steps) as an off-white solid, mp: 95.4-98.8 °C; Rf: 0.48 (1:49 saturated methanolic methanol, dichloromethane); IR (vmax (neat)): 3382, 3308, 3152, 2959, 2868, 1649, 1158, 1218, 1070, 1015 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.23 (9H, s), 1.60-1.89 (3H, m), 1.90- 2.06 (1H, m), 3.62-3.86 (2H, m), 3.94 (1H, dd, J = 15.2, 6.3 Hz), 4.09 (2H, bs), 4.13-4.26 (2H, m), 5.33 (1H, s) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 25.8, 28.3, 30.6, 32.1, 51.8, 68.5, 79.9, 87.2, 146, 160.6 ppm; LRMS (+ESI) m/z: 224.2 ([M+H]⁺ 100%).

(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-2-fluoro-5-(trifluoromethyl)benzamide 90

Prepared according to General Procedure C using 2-fluoro-5-trifluoromethylbenzoic acid (500 mg, 2.4 mmol) and aminopyrazole 89. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 3:7) as an eluent to obtain the title compound (660 mg, 67%) as an off-white waxy solid, Rf: 0.45 (2:3 ethyl acetate, hexane);
IR (v<sub>max</sub> (neat)): 3294, 2962, 1680, 1326, 1129, 1092 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.31 (9H, s), 1.62-1.76 (2H, m), 1.80-2.94 (1H, m), 2.00-2.08 (1H, m), 3.68-3.89 (2H, m), 4.11 (1H, dd, J = 15.1, 5.8 Hz), 4.18-4.29 (1H, m), 4.41 (1H, dd, J = 15.4, 1.9 Hz), 6.55 (1H, s), 7.28-7.36 (1H, m), 7.74-7.84 (1H, m), 8.39 (1H, dd, J = 6.9, 2.5 Hz), 10.08 (1H, d, J = 8.1 Hz) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 25.9, 28.1, 30.6, 32.4, 53.0, 68.8, 79.7, 95.6, 117.3 (d, J = 25.3 Hz), 122.4 (d, J = 13.8 Hz), 122.9 (q, J = 272.1 Hz), 128.0 (q, J = 30.3 Hz), 130.1-130.2 (m), 130.7-140.0 (m), 136.4, 159.0 (d, J = 2.8 Hz), 161.0, 161.9 (d, J = 255.2 Hz) ppm; <sup>19</sup>F NMR (282 MHz, DMSO-d<sub>6</sub>): δ −62.3, -108.4 ppm; LRMS (+ESI) m/z: 436.2 ([M+Na]<sup>+</sup> 100%), 849.2 ([2M+Na]<sup>+</sup> 43%).

(R)-N-(5-(tert-Butyl)-1-methyl-2-(((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-3(2H)-ylidene)-2-fluoro-5-(trifluoromethyl)benzamide 91

To a solution of amide 90 (300 mg, 730 μmol) in anhydrous toluene (15 mL) was added methyl trifluoromethanesulfonate (120 μL, 1.1 mmol) and the mixture heated at 100 °C for 16 hours. The reaction mixture was cooled to room temperature and water (1 mL) and acetone (3 mL) were added and the mixture stirred for 30 minutes before aqueous saturated ammonia (500 μL) was added and the mixture stirred for additional 30 minutes. Brine (50 mL) was added and the aqueous mixture extracted with ethyl acetate (3 x 40 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using methanol, ethyl acetate and hexane (0:1:1 to 1:9:0) as an eluent to obtain the title compound (230 mg, 72%) as an off-white waxy solid, Rf: 0.54 (1:9 methanol, ethyl acetate); IR (v<sub>max</sub> (neat)): 2973, 1529, 1312, 1118, 1070, 1031 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.43 (9H, s), 1.68-1.97 (3H, m), 1.97-2.16 (1H, m), 3.68-3.84 (2H, m), 3.92 (3H, s), 4.21 (1H, qd, J = 6.7, 2.8 Hz), 4.33 (1H, dd, J = 15.3, 6.1 Hz), 4.61 (1H, dd, J = 15.3, 2.9 Hz), 7.06 (1H, s), 7.15 (1H, dd, J = 10.1, 8.6 Hz), 7.53-7.62 (1H, m), 8.32 (1H, dd, J = 6.8, 2.5 Hz) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 25.7, 28.8, 29.3, 32.3, 35.4, 47.6, 68.6, 77.9, 97.9, 117.2 (d, J = 25.1 Hz), 124.1 (q, J = 272.0 Hz), 126.0 (q, J = 33.9 Hz), 127.8-128.2 (m), 129.0-129.2 (m), 129.5-129.9 (m), 155.3, 157.8, 163.2 (d, J = 257.9 Hz), 169.1 ppm; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>): δ −62.0, -107.5 ppm; LRMS (+ESI) m/z: 428.2 ([M+H]<sup>+</sup> 100%).
(R)-N-(5-(tert-Butyl)-1-methyl-2-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-3(2H)-ylidene)-2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)benzamide 82

Prepared according to General Procedure E using 91 (100 mg, 230 μmol) and diol 93 at room temperature for 2 hours. The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (70 mg, 60%) as a white solid, mp: 175.9-178.3 °C; Rf: 0.19 (1:19 methanol, dichloromethane); IR (v_max (neat)): 3266, 2973, 1529, 1284, 1110 cm⁻¹; ¹H NMR (400 MHz, MeOD): δ 1.28 (6H, s), 1.47 (9H, s), 1.64-1.78 (1H, m), 1.77-1.93 (2H, m), 1.97-2.09 (1H, m), 3.67-3.89 (2H, m), 3.96 (2H, s), 4.02 (3H, s), 4.20 (1H, qd, J = 7.0, 3.0 Hz), 4.37 (1H, dd, J = 15.5, 7.2 Hz), 4.53 (1H, dd, J = 15.5, 3.0 Hz), 6.82 (1H, s), 7.18 (1H, d, J = 8.7 Hz), 7.61 (1H, dd, J = 8.8, 2.4 Hz), 7.79 (1H, d, J = 2.4 Hz) ppm; ¹³C NMR (100 MHz, MeOD): δ 26.3, 26.5, 29.4, 29.7, 33.2, 36.2, 48.5, 69.3, 71.0, 78.7, 79.0, 98.4, 115.1, 123.4 (q, J = 32.9 Hz), 125.9 (q, J = 271.5 Hz), 127.5 (q, J = 3.7 Hz), 128.0 (q, J = 3.5 Hz), 158.6, 161.0 (three carbon signals were not observed) ppm; ¹⁹F NMR (376 MHz, MeOD): δ –63.1 ppm; LRMS (+ESI) m/z: 498.3 ([M+H]^+ 100%); HRMS (ESI)^+ Calcd for C₂₅H₃₄F₃N₃O₄ [M+H]^+: 498.25797, found 498.25701; [α]D^22: -6.8 ° (1.48, CHCl₃); HPLC: 97.1%, RT: 20.8 mins. (Method A, 254 nm).

Ethyl 2-hydroxy-2-methylpropanoate 92

To a solution of 2-hydroxyisobutyric acid (5.0 g, 48 mmol) in ethanol (100 mL) was added concentrated sulfuric acid (130 μL, 2.4 mmol) and the mixture was heated at reflux for 24 hours. The solvent was removed under reduced pressure and the residue taken up in water (100 mL). The aqueous solution was extracted with dichloromethane (3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated to obtain the ester 92 (4.5 g, 71%) as a colourless, volatile liquid, Rf: 0.12 (1:9 ethyl acetate, hexane); IR (v_max (neat)): 3056, 2984, 1725, 1264, 1181, 732 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.26 (3H, t, J = 7.1 Hz),
1.39 (6H, s), 3.16 (1H, bs), 4.19 (2H, q, $J = 7.1$ Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): 14.2, 27.2, 61.8, 72.0, 177.5 ppm; LRMS (+ESI) $m/z$: 155.1 ([M+Na]$^+$ 100%).

2-Methylpropane-1,2-diol 93

Prepared according to General Procedure D using ester 92 (4.2 g, 31.8 mmol) at room temperature to obtain the title compound (2.5 g, 86%) as a light yellow oil, $R_f$: 0.08 (1:1 ethyl acetate, hexane); IR (v$_{max}$ (neat)): 3330, 2971, 1470, 1160, 1048, 907 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.21 (6H, s), 2.39 (2H, s), 3.42 (2H, s) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.9, 71.1, 71.2 ppm; LRMS (+ESI) $m/z$: 113.1 ([M+Na]$^+$ 100%).

(R)-(Tetrahydrofuran-2-yl)methyl methanesulfonate 94

The alcohol 85 (1.5 g, 14.9 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. To this was added methanesulfonyl chloride (1.3 mL, 16.3 mmol), dropwise and the mixture was warmed to room temperature and stirred for 3 hours. The mixture was washed with water (3 x 20 mL) and saturated aqueous sodium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated to yield the title compound (2.6 g, 97%) as a light yellow oil, $R_f$: 0.48 (3:2 ethyl acetate, hexane); IR (v$_{max}$ (film)): 2956, 2877, 1347, 1169, 951, 819 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.11-1.77 (1H, m), 1.83-2.10 (3H, m), 3.05 (3H, s), 3.70-3.95 (2H, m), 4.07-4.37 (3H, m) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.8, 27.7, 37.8, 68.8, 71.5, 76.4 ppm; LRMS (+ESI) $m/z$: 203.0([M+Na]$^+$ 100%), 383.0 ([2M+Na]$^+$ 14%).

(R)-2-(Azidomethyl)tetrahydrofuran 95

To a solution of mesylate 94 (2.5 g, 13.9 mmol) in N,N-dimethylformamide (15 mL) was added sodium azide (2.7 g, 41.6 mmol) and the mixture was heated at 70 °C for 16 hours. The mixture was diluted with water (60 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were washed with water (3 x 30 mL) and aqueous lithium chloride (1 M, 2 x 20 mL), dried over anhydrous magnesium sulfate and concentrated to
obtain the desired compound (1.5 g, 85%) as a yellow oil, the characterisation data of which corresponded to that previously described. $^{311}$ RF: 0.46 (1:4 ethyl acetate, hexane); IR (v$_{\text{max}}$ (film)): 2976, 2870, 2091, 1680, 1268, 1075 cm$^{-1}$.

(R)-(Tetrahydrofuran-2-yl)methanamine 96

![Chemical Structure]

To a solution of the azide 95 (600 mg, 4.7 mmol) in tetrahydrofuran (10 mL) was added triphenylphosphine (1.5 g, 5.6 mmol) and water (3.4 mL, 188 mmol) and the mixture was heated at 50 °C for 16 hours. The organic solvent was removed under a stream of nitrogen and the residue diluted with water (30 mL) and filtered. The aqueous filtrate was saturated with sodium chloride and extracted with a mixture of chloroform and methanol (9:1, 3 x 30 mL), dried over magnesium sulfate and concentrated to obtain the title compound (320 mg, 68%) as a light yellow oil, RF: 0.40 (1:9 methanol saturated with ammonia, dichloromethane); IR (v$_{\text{max}}$ (film)): 3372, 2929, 2862, 1675, 1054, 747 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.46-1.58 (1H, m), 1.50 (2H, s), 1.80-1.97 (3H, m), 2.59-2.84 (2H, m), 3.67-3.91 (3H, m) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 26.0, 28.7, 46.6, 68.1, 80.8 ppm; LRMS (+ESI) m/z: 102.2 ([M+H]$^+$ 100%).

(R)-5-(tert-Butyl)-3-((tetrahydrofuran-2-yl)methyl)thiazol-2(3H)-imine hydroiodide 97

![Chemical Structure]

To a solution of the amine 96 (150 mg, 1.5 mmol) and 3,3-dimethylbutanal (230 μL, 1.8 mmol) in acetonitrile was added activated 4 Å molecular sieves (0.2 g) and the reaction mixture was stirred at room temperature for 16 hours then filtered through a pad of Celite®. To the filtrate was added potassium thiocyanate (190 mg, 2.0 mmol) and the temperature was increased to 50 °C and stirred until the complete dissolution of solids. Iodine (380 mg, 1.49 mmol) was added and the mixture was stirred for a further 16 hours at 50 °C. The solution was cooled to room temperature and treated with aqueous sodium metabisulfite (20% w/v, 5 mL) and stirred for 30 minutes. The organic phase was separated and the aqueous phase extracted with dichloromethane (3 x 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated to obtain the title compound (280 mg, 51%) as a yellow oil, the characterisation data of which corresponded to that
previously described.\textsuperscript{152} IR (\(v_{\text{max}}\) (film)): 3421, 3202, 2961, 2054, 1626, 1562, 1405, 1326, 1023, 999, 821 cm\(^{-1}\); \textsuperscript{13}C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 25.0, 28.3, 30.6, 32.7, 51.5, 67.6, 75.6, 124.6, 134.4, 166.8 ppm; LRMS (+ESI) \textit{m/z}: 241.1 ([M+H]\(^+\) 100%).

\textbf{(R)-N-(5-(tert-Butyl)-3-\(((\text{tetrahydrofuran-2-yl)methyl})\text{thiazol-2(3H)-ylidene})-2\text{-fluoro-5-}\text{(trifluoromethyl)benzamide 98}}

\begin{center}
\includegraphics[width=0.2\textwidth]{98.png}
\end{center}

Prepared according to General Procedure C using 2-fluoro-5-(trifluoromethyl)benzoic acid (160 mg, 780 \(\mu\)mol) and 97 (230 mg, 630 \(\mu\)mol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (70 mg, 48\%) as a yellow oil, the characterisation data of which corresponded to that previously described.\textsuperscript{152} \(R_f\): 0.52 (3:7 ethyl acetate, hexane); IR (\(v_{\text{max}}\) (film)): 2963, 2871, 1622, 1491, 1365, 1123, 829 cm\(^{-1}\); \textsuperscript{13}C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 24.9, 28.3, 30.9, 32.7, 51.6, 67.3, 75.9, 118.4 (d, \(J = 24.1\) Hz), 122.3, 123.7 (q, \(J = 271.8\) Hz), 124.9 (q, \(J = 29.6\) Hz), 126.5 (d, \(J = 8.7\) Hz), 128.6, 129.8 (q, \(J = 2.9\) Hz), 136.6, 162.9 (d, \(J = 261.7\) Hz), 166.0, 168.4 (d, \(J = 3.6\) Hz) ppm; \textsuperscript{19}F NMR (282 MHz, DMSO-\(d_6\)): \(\delta\) –60.7, -105.9 ppm; LRMS (+ESI) \textit{m/z}: 431.0 ([M+H]\(^+\) 16\%), 453.1 ([M+Na]\(^+\) 100\%), 883.2 ([2M+Na]\(^+\) 30\%).

\textbf{(R)-N-(5-(tert-Butyl)-3-\(((\text{tetrahydrofuran-2-yl)methyl})\text{thiazol-2(3H)-ylidene})-2-(pyridin-2-ylmethoxy)-5-\text{(trifluoromethyl)benzamide 83}}

\begin{center}
\includegraphics[width=0.2\textwidth]{83.png}
\end{center}

Prepared according to General Procedure E using 2-pyridinyl methanol (15 mg, 140 \(\mu\)mol) and 98 (60 mg, 140 \(\mu\)mol) at room temperature for 5 hours. The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:1) as an eluent to obtain the desired product (40 mg, 56\%) as a colourless solid, the characterisation data of which corresponded to that previously described.\textsuperscript{152} \textit{mp}: 125.2-127.2 °C; \(R_f\): 0.31 (3:2 ethyl acetate, hexane); IR (\(v_{\text{max}}\) (film)): 2963, 2870, 1619, 1491, 1363, 1272, 1252, 1116, 1093,
820 cm\(^{-1}\); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 24.9, 28.2, 30.9, 32.7, 51.4, 67.2, 70.7, 75.9, 114.3, 120.8 (q, \(J = 29.5\) Hz), 121.0, 121.9, 122.9, 124.3 (q, \(J = 272.0\) Hz), 127.4 (q, \(J = 3.2\) Hz), 122.2 (q, \(J = 3.2\) Hz), 128.8, 135.9, 137.0, 149.0, 156.4, 159.2, 165.8, 171.5 ppm; \(^{19}\)F NMR (282 MHz, DMSO-\(d_6\)): \(\delta\) -60.7 ppm; LRMS (+ESI) \(m/z\): 520.0 ([M+H]\(^+\) 33%), 542.2 ([M+Na]\(^+\) 100%), 1061.3 ([2M+Na]\(^+\) 19%); HRMS (ESI)\(^+\) Cald for C\(_{26}\)H\(_{28}\)F\(_3\)N\(_3\)O\(_3\)S [M+Na]\(^+\): 542.16957, found 542.16951; HPLC: 98.6%, RT: 23.6 mins (Method A, 254 nm).

4-Methoxybenzohydrazide 99

\[ \text{Methyl 4-methoxybenzoate (3.0 g, 18.1 mmol) and hydrazine hydrate (4.4 mL, 90.3 mmol) were dissolved in methanol (10 mL) and heated at reflux for 16 hours. The reaction mixture was cooled to room temperature and the resulting precipitate collected by filtration. The liquor was concentrated under a stream of nitrogen and taken up in ethyl acetate (15 mL). The organic phase was washed with water (2 x 20 mL) and saturated aqueous sodium chloride (20 mL), dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to obtain a white solid. The two batches were combined and dried in vacuo to obtain the title compound (2.7 g, 92%) as a white solid, the characterisation data of which corresponded to that previously described.} \]

\[^{31}\]R\(_f\): 0.14 (7:3 ethyl acetate, hexane); IR (v\(\text{max (neat)}\)): 3322, 1603, 1434, 1254, 1035, 926, 842, 791, 764 cm\(^{-1}\); LRMS (+ESI) \(m/z\): 189.1 ([M+Na]\(^+\) 39%), 355.2 ([2M+Na]\(^+\) 100%).

4-Methoxy-2-(aminoiminomethyl)hydrazide benzoic acid 100

\[ \text{A suspension of 99 (2.6 g, 15.6 mmol) and S-methyl isothiourea hemisulfate (2.2 g, 7.8 mmol) in aqueous sodium hydroxide (1\% w/v, 62 mL, 15.6 mmol) was stirred at room temperature for 72 hours. The reaction mixture was warmed to 50 °C and stirred for a further 4 hours. The mixture was slowly cooled and the resulting precipitate collected by filtration and dried in vacuo to obtain the title compound (1.4 g, 43%) as a white solid, mp: 223.1-224.5 °C; IR (v\(\text{max (neat)}\)): 3411, 3121, 1604, 1429, 1351, 1240, 1167, 756 cm\(^{-1}\); \(^{1}\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 3.76 (3H, s), 6.84 (2H, d, \(J = 8.3\) Hz), 7.02 (bs, 4H), 7.87


(2H, d, J = 8.3 Hz), 10.84 (1H, s) ppm; $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 55.0, 112.6, 128.0, 131.2, 152.8, 159.4, 160.7 ppm; LRMS (+ESI) m/z: 209.1 ([M+H]$^+$ 100%).

5-(4-Methoxyphenyl)-4H-1,2,4-triazol-3-amine 101

A suspension of 100 (1.0 g, 4.8 mmol) in water (40 mL) was heated at reflux for 16 hours. The reaction mixture was cooled to 0 °C and the resulting precipitate collected by filtration and dried in vacuo to obtain a tautomeric mixture the triazole (0.91 g, 100%) as a white crystalline solid, the characterisation data of which corresponded to that previously described. $^{189}$ $R_f$: 0.11 (3:2 ethyl acetate, hexane); IR ($v_{max}$ (neat)): 3439, 3328, 1613, 1539, 1505, 1452, 1393, 1249, 1177, 1133, 1021, 982, 836, 758 cm$^{-1}$; LRMS (+ESI) m/z: 191.0 ([M+H]$^+$ 100%).

tert-Butyl 3-amino-5-(4-methoxyphenyl)-4H-1,2,4-triazole-4-carboxylate 102

A mixture of 101 (200 mg, 1.1 mmol), di-tert-butyl dicarbonate (240 mg, 1.1 mmol) and triethylamine (290 μL, 2.10 mmol) in tetrahydrofuran (20 mL) was stirred at room temperature for 5 hours. The solvent was removed under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (3:2) as an eluent, to obtain the title compound (160 mg, 52%) as a white solid, mp: 146 °C (decomposition); $R_f$: 0.55 (3:2 ethyl acetate, hexane); IR ($v_{max}$ (neat)): 3447, 3303, 2980, 2935, 1730, 1647, 1612, 1500, 1459, 1366, 133, 1250, 1135, 802, 767 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.68 (9H, s), 3.84 (3H, s), 6.49 (2H, s), 6.92 (2H, d, $J = 8.7$ Hz), 7.98 (2H, d, $J = 8.7$ Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 28.1, 55.4, 86.4, 113.9, 122.8, 128.6, 157.9, 159.9, 161.2 ppm; LRMS (+ESI) m/z: 313.1 ([M+Na]$^+$ 9%), 603.2 ([2M+Na]$^+$ 100%).
Chapter 8: Experimental

Tetrahydro-2H-pyran-4-yl 4-methylbenzenesulfonate 103

Prepared according to General Procedure A using tetrahydro-4-pyranyl (2.8 mL, 29.4 mmol). The crude product was purified by flash column chromatography, using ethyl acetate, hexane (0:100 to 3:7) as an eluent to obtain the title compound (6.5 g, 87%) as a light yellow oil, the characterisation data of which corresponded to that previously described.\textsuperscript{158} \( R_f \) 0.31 (1:3 ethyl acetate, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (film)}) \): 3323, 2961, 1599, 1342, 1187, 1171, 1092, 913, 876, 814 cm\(^{-1}\); \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): \( \delta \) 21.7, 32.5, 64.9, 77.4, 127.7, 130.0, 134.5, 144.8 ppm; \( \text{LRMS} \) (+ESI) \( m/z \): 279.0 ([M+Na]\(^+\) 100%), 535.1 ([2M+Na]\(^+\) 50%).

S-(Tetrahydro-2H-pyran-4-yl) ethanethioate 104

Tosylate 103 (6.1 g, 23.8 mmol) was dissolved in \( N,N \)-dimethylformamide (20 mL) and to this was added potassium thioacetate (5.4 g, 47.6 mmol), followed by sodium iodide (360 mg, 2.38 mmol). The reaction mixture was warmed to 50 °C and stirred for 20 hours. The mixture was cooled to room temperature, diluted with ethyl acetate (80 mL) and washed with water (2 x 50 mL). The aqueous phase was extracted with ethyl acetate (30 mL) and the organic components combined and washed with water (3 x 50 mL) and brine (30 mL), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude oil was purified by flash column chromatography using diethyl ether, hexane (1:9) as an eluent to obtain the title compound (1.2 g, 32%) as an orange oil, the characterisation data of which corresponded to that previously described.\textsuperscript{158} \( R_f \) 0.34 (1:4 diethyl ether, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (film)}) \): 3322, 2958, 2847, 1687, 1599, 1353, 1175, 1114, 1087, 946, 877 cm\(^{-1}\); \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): \( \delta \) 30.8, 32.8, 39.2, 67.5, 195.3 ppm; \( \text{LRMS} \) (EI) \( m/z \): 160 (18), 117 (70), 84 (28), 83 (100), 69 (36), 55 (35).
**Ethyl 2-methyl-2-((tetrahydro-2H-pyran-4-yl)thio)propanoate 105**

A solution of 104 (1.0 g, 6.2 mmol) in ethanol (30 mL) was degassed by sparging nitrogen for 30 minutes, before potassium hydroxide (810 mg, 14.4 mmol) and a solution of ethyl bromoisobutyrate (1.6 mL) in ethanol (5 mL) was added portionwise. The reaction mixture was stirred for 16 hours, filtered and the filtrate concentrated under reduced pressure. The crude product was adsorbed to silica gel and purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent, to obtain the sulfide 105 (1.1 g, 76%) as an orange oil, the characterisation data of which corresponded to that previously described.\(^\text{158}\)

\[ R_f: 0.29 \] (1:9 ethyl acetate, hexane); \[ \text{IR} (v_{\text{max}} \text{ (film))}: 2957, 2842, 1717, 1466, 1300, 1234, 1124, 1086, 1008 \text{ cm}^{-1}; \]
13C NMR (75 MHz, CDCl\(_3\)): \[ \delta \] 14.2, 26.4, 35.2, 39.1, 47.5, 61.4, 67.6, 174.8 ppm; LRMS (+ESI) \text{m/z}: 255.0 ([M+Na]\(^+\) 100%).

**Ethyl 2-methyl-2-((tetrahydro-2H-pyran-4-yl)sulfonyl)propanoate 106**

To a solution of sulfide 105 (1.0 g, 4.30 mmol) in a mixture of dioxane and water (4:1, 10 mL) was added Oxone®, portionwise over 30 minutes. The reaction mixture was then stirred at room temperature for 16 hours. The precipitate was removed by filtration and washed with dioxane (3 x 15 mL). The combined filtrates were concentrated under reduced pressure and the residue taken up in ethyl acetate (50 mL) and washed with water (3 x 25 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3) as an eluent to obtain the title compound (790 mg, 69%) as a crystalline solid, the characterisation data of which corresponded to that previously described.\(^\text{158}\)

\[ \text{mp}: 44.1-46.8 \text{ °C}; \]
\[ R_f: 0.31 \] (2:3 ethyl acetate, hexane); \[ \text{IR} (v_{\text{max}} \text{ (film))}: 2968, 2851, 1723, 2569, 1300, 1265, 1156, 1127, 1108, 885 \text{ cm}^{-1}; \]
13C NMR (75 MHz, DMSO-\text{d}_6): \[ \delta \] 13.7, 19.6, 26.6, 55.3, 62.1, 65.6, 68.1, 168.8 ppm; LRMS (+ESI) \text{m/z}: 287.1 ([M+Na]\(^+\) 84%), 551.1 ([2M+Na]\(^+\) 100%).
2-Methyl-2-((tetrahydro-2H-pyran-4-yl)sulfonyl)propanoic acid 107

![Structure of compound 107]

Prepared according to General Procedure B using ethyl ester 106 (750 mg, 2.84 mmol) and sodium hydroxide at room temperature for 48 hours to obtain the carboxylic acid 107 (570 mg, 85%) as a white crystalline solid, the characterisation data of which corresponded to that previously described.\(^{158}\) mp: 166.3-168.4 °C; IR (\(\nu_{\text{max}}\) film): 2929, 2770, 2637, 1724, 1465, 1284, 1253, 1162, 1117, 1072, 1010, 885 cm\(^{-1}\); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 19.8, 26.7, 55.2, 65.6, 68.0 ppm; LRMS (−ESI) \(m/z\): 235.1 ([M-H]\(^-\) 17%), 471.2 ([2M-H]\(^-\) 100%).

\[-(5-(4-Methoxyphenyl)-4H-1,2,4-triazol-3-yl)-2-methyl-2-((tetrahydro-2H-pyran-4-yl)sulfonyl)propanamide\] 63

![Structure of compound 63]

Prepared according to a modified version of General Procedure C using carboxylic acid 107 (25 mg, 110 \(\mu\)mol). The acid chloride was dissolved in toluene (2 mL) and added to a suspension of 102 (32 mg, 110 \(\mu\)mol) and sodium hydride (60% suspension in mineral oil, 2.8 mg, 120 \(\mu\)mol) in toluene (2 mL) at 0 °C. The reaction mixture was heated at reflux for 4 hours, cooled to room temperature, poured into water (15 mL) and extracted with ethyl acetate (3 x 15 mL). The organic phase was washed with saturated aqueous sodium chloride (15 mL), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3 to 3:2) as an eluent and recrystallisation from dichloromethane, hexane to afford the title compound (30 mg, 70%) as a white fluffy solid, the characterisation data of which corresponded to that previously described.\(^{158}\) mp: 163.1-164.6 °C; \(R_f\): 0.21 (3:2 ethyl acetate, hexane); IR (\(\nu_{\text{max}}\) film): 3336, 3294, 2959, 1669, 1589, 1573, 1442, 1372, 1351, 1295, 1246, 1145, 1124, 1082, 944, 885 cm\(^{-1}\); \(^{13}\)C NMR (75 MHz, DMSO): \(\delta\) 19.5, 26.0, 54.8, 55.2, 65.6, 68.0, 113.6, 127.0, 159.9, 167.4 (three carbon signals were not observed) ppm; LRMS (+ESI) \(m/z\): 431.2 ([M+Na]\(^+\) 100%), 453 (46%), 839.2 ([2M+Na]\(^+\)
33%). **HRMS** (ESI)\(^+\) Calcd for C\(_{18}\)H\(_{24}\)NaO\(_5\)S [M+Na]\(^+\): 431.13596, found 431.13601; **HPLC**: 99.4%, RT: 18.5 mins. (Method A, 254 nm).

**3-(tert-Butyl)isoazol-5-amine 108**

![Chemical structure](image)

To an aqueous solution of sodium hydroxide (5.1 M, 9 mL, 45.9 mmol) was added hydroxylamine hydrochloride (1.1 g, 16.4 mmol) and 88 (1.9 g, 15.2 mmol). The resulting solution was stirred at 50 °C for 4 hours, cooled to room temperature and extracted with chloroform (3 x 15 mL). The organic phase was washed with brine (15 mL), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure to obtain the title compound (1.7 g, 80%), as a light yellow solid, the characterisation data of which corresponded to that previously described\(^{313}\) mp: 86.6-89.3 °C; **Rf**: 0.53 (1:1 ethyl acetate, hexane); **IR** (v\(_\text{max}\) (film)): 3442, 3291, 3237, 3150, 2961, 1633, 1584, 1473, 1434, 1363, 1240, 986, 883 cm\(^{-1}\); **\(^{13}\)C NMR** (75 MHz, DMSO-\(d_6\)): δ 29.3, 31.6, 75.0, 169.9, 172.4 ppm; **LRMS** (EI) m/z: 140 (100), 126 (26), 110 (54), 68 (55).

**(S)-5-Oxo-1-(4-(trifluoromethyl)phenyl)pyrrolidine-2-carboxylic acid 109**

![Chemical structure](image)

To a stirred suspension of L-pyroglutamic acid (700 mg, 5.4 mmol) in acetonitrile (10 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (1.6 mL, 10.8 mmol) and the mixture stirred for 10 minutes, before di-μ-hydroxo-bis[(N,N,N',N'-tetramethylethylenediamine)copper(II)] chloride (280 mg, 590 μmol) was added. The blue solution was stirred for 10 minutes before 4-(trifluoromethyl)phenylboronic acid (1.1 g, 5.80 mmol) was added and the mixture stirred at room temperature for 20 hours. The volatiles were removed under reduced pressure and the residue taken up in saturated aqueous sodium hydrogen carbonate (15 mL) and washed with ethyl acetate (2 x 20 mL). The aqueous layer was cooled to 0 °C, acidified (pH = 3) with aqueous sulfuric acid (1 M) and extracted with ethyl acetate (3 x 20 mL). The combined extracts were washed with saturated aqueous sodium chloride (20 mL), dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using acetic acid, methanol and dichloromethane (1:9:90) as an eluent to
obtain carboxylic acid 109 (1.0 g, 71%) as a gummy mass, the characterisation data of which corresponded to that previously described.\textsuperscript{314} \textit{Rt}: 0.21 (1:9:90 acetic acid, methanol, dichloromethane); LRMS (−ESI) \textit{m/z}: 272.1 ([M-H]\textsuperscript{−} 50%), 545.2 ([2M-H]\textsuperscript{−} 100%), 567.0 ([2M+Na-2H]\textsuperscript{−} 38%).

\textit{(S)}-N-(3-(tert-Butyl)isoxazol-5-yl)-5-oxo-1-(4-(trifluoromethyl)phenyl)pyrrolidine-2-carboxamide 84

\begin{center}
\includegraphics[width=0.4\textwidth]{image}
\end{center}

To a suspension of carboxylic acid 109 (1.0 g, 3.8 mmol) and 108 (540 mg, 3.8 mmol) in pyridine (5 mL) was added phosphorus oxychloride (590 mg, 3.8 mmol) at 0 °C. The mixture was stirred at this temperature for 30 minutes before being poured into water (30 mL) and extracted with ethyl acetate (3 x 40 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (1.2 g, 81%) as a white solid, \textit{mp}: 182.1-184.6 °C; \textit{Rf}: 0.36 (1:1 ethyl acetate, hexane); IR (\textit{v}_{\text{max}} (film)): 2966, 1732, 1696, 1659, 1607, 1355, 1322, 1286, 1152, 1110, 883 cm\textsuperscript{−1}; \textit{\textsuperscript{1}H NMR} (300 MHz, CDCl\textsubscript{3}): \delta 1.29 (9H, s), 2.16-2.34 (1H, m), 2.46-2.73 (2H, m), 2.73-2.93 (1H, m), 4.79-4.97 (1H, m), 6.30 (1H, s), 7.45-7.69 (4H, m), 9.79 (1H, s) ppm; \textit{\textsuperscript{13}C NMR} (75 MHz, CDCl\textsubscript{3}): \delta 23.9, 29.4, 31.0, 32.6, 63.0, 88.3, 121.2, 123.8 (q, \textit{J} = 272.1 Hz), 126.6 (q, \textit{J} = 3.9 Hz), 127.9 (q, \textit{J} = 32.7 Hz), 140.7, 159.1, 167.5, 174.0, 175.6 ppm; \textit{\textsuperscript{19}F NMR} (282 MHz, CDCl\textsubscript{3}): \delta -62.5 ppm; LRMS (+ESI) \textit{m/z}: 396.1 ([M+H]\textsuperscript{+} 15%), 418.1 ([M+Na]\textsuperscript{+} 100%), 813.1 ([2M+Na]\textsuperscript{+} 14%); HRMS (ESI): Calcd for C\textsubscript{19}H\textsubscript{2}O\textsubscript{1}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} [M+Na]\textsuperscript{+}: 418.13940, found 418.13491; HPLC: 99.6%, RT: 25.3 mins (Method A, 254 nm).
8.5.2 Synthetic Procedures from Chapter 3

**Ethyl (methylsulfonyl)glycinate 119**

![Structure of Ethyl (methylsulfonyl)glycinate](image)

To a stirred suspension of ethyl glycinate hydrochloride (3.0 g, 21.5 mmol) in anhydrous acetonitrile (25 mL) was added triethylamine (7.5 mL, 53.8 mmol), dropwise at 0 °C and the mixture stirred for 5 minutes. Methanesulfonyl chloride (2.0 mL, 25.8 mmol) was added dropwise and the reaction mixture warmed to room temperature and stirred for 16 hours. The solvent was removed under reduced pressure and the residue taken up in dichloromethane (25 mL), washed with aqueous hydrochloric acid (1 M, 15 mL), saturated aqueous sodium bicarbonate (15 mL) and water (15 mL), dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3) as an eluent, to obtain the title compound (1.3 g, 34%) as light yellow oil, \( R_f: 0.40 \) (1:1 ethyl acetate, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (film)}): 3221, 2930, 1738, 1413, 1315, 1200, 1142, 1110, 930, 754 \text{ cm}^{-1} \); \( ^1\text{H NMR} \) (300 MHz, CDCl\(_3\)): \( \delta 1.29 \) (3H, t, \( J = 7.3 \text{ Hz} \)), 3.02 (3H, s), 3.94 (2H, d, \( J = 5.6 \text{ Hz} \)), 4.22 (2H, q, \( J = 7.3 \text{ Hz} \)), 5.12 (1H, s) ppm; \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): \( \delta 14.2, 41.5, 44.4, 62.1, 169.9 \) ppm; \( \text{LRMS (+ESI)} \) \( m/z: 203.9 \) ([M+Na]\(^+\) 58%), 385.0 ([2M+Na]\(^+\) 100%).

**Ethyl 2-cyano-4,4-dimethylpent-2-enoate 120**

![Structure of Ethyl 2-cyano-4,4-dimethylpent-2-enoate](image)

To a stirred solution of 119 (100 mg, 550 μmol), pivalaldehyde (78 μL, 720 μmol) and ethyl cyanoacetate (81 mg, 720 μmol) in ethanol (10 mL) was added potassium carbonate (38 mg, 280 μmol). The mixture was heated at reflux overnight, cooled and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to exclusively obtain the Knoevenagel condensation intermediate 120 (115 mg, 88%) as a colourless oil, \( R_f: 0.39 \) (1:9 ethyl acetate, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (film)}): 2967, 2231, 1730, 1619, 1466, 1250, 1204, 1091, 1032, 762 \text{ cm}^{-1} \); \( ^1\text{H NMR} \) (300 MHz, CDCl\(_3\)): \( \delta 1.28 \) (9H, t, \( J = 7.3 \text{ Hz} \)), 1.32 (3H, s), 3.94 (2H, d, \( J = 5.6 \text{ Hz} \)), 4.22 (2H, q, \( J = 7.3 \text{ Hz} \)), 7.61 (1H, s) ppm; \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): \( \delta 14.2, 28.9, 35.1, 44.4, 62.7, 106.0, 114.3, 162.3, 171.9 \) ppm; \( \text{LRMS (+ESI)} \) \( m/z: 204.1 \) ([M+Na]\(^+\) 100%).
Ethyl 2-((tert-butoxycarbonyl)amino)acetate 122

To a cooled suspension of glycine ethyl ester hydrochloride (1.0 g, 7.2 mmol) and sodium hydrogen carbonate (1.8 g, 21.6 mmol) in acetonitrile (20 mL) was added di-tert-butyl dicarbonate (1.6 g, 7.2 mmol) and the mixture was warmed to room temperature and stirred for 16 hours. The solvent was removed under reduced pressure and the residue was taken up in dichloromethane (50 mL), washed with water (3 x 20 mL), dried over magnesium sulfate and concentrated. The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3) as an eluent to obtain the desired compound (1.2 g, 83%) as a colourless oil, the characterisation data of which corresponded to that previously described.\textsuperscript{315} \textit{Rf}: 0.52 (1:1 ethyl acetate, hexane); LRMS (+ESI) \textit{m/z}: 226.1 ([M+Na]\textsuperscript{+} 100%), 429.2 ([2M+Na]\textsuperscript{+} 91%).

Ethyl N, N-bis[(1, 1-dimethylethoxy) carbonyl] glycinate 121

To a solution of \textit{122} (400 mg, 1.97 mmol) and 4-(dimethylamino)pyridine (24 mg, 200 \textmu mol) in dichloromethane (10 mL) was added triethylamine (410 \textmu L, 3.0 mmol) and di-tert-butyl dicarbonate (860 mg, 3.94 mmol) and the mixture was stirred at room temperature for 72 hours. The reaction mixture was concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (420 mg, 71%) as a colourless oil, \textit{Rf}: 0.71 (3:7 ethyl acetate, hexane); IR (\textit{v}_{\text{max}} (film)): 2980, 1736, 1697, 1366, 1336, 1143, 1027, 963, 852 cm\textsuperscript{-1}; \textit{\textsuperscript{1}H NMR} (300 MHz, CDCl\textsubscript{3}): \textit{\delta} 1.27 (3H, t, \textit{J} = 7.1 Hz), 1.51 (18H, s), 4.20 (2H, q, \textit{J} = 7.1 Hz), 4.31 (1H, s) ppm; \textit{\textsuperscript{13}C NMR} (75 MHz, CDCl\textsubscript{3}): \textit{δ} 14.3, 28.1, 47.5, 61.3, 83.2, 152.1, 169.3 ppm; LRMS (+ESI) \textit{m/z}: 326.2 ([M+Na]\textsuperscript{+} 31%), 629.3 ([2M+Na]\textsuperscript{+} 100%).
To a cooled solution of aqueous sodium hydroxide (50% w/v, 20 mL) and dichloromethane (20 mL) was added tetrabutylammonium hydrogen sulfate (250 mg, 750 μmol) and freshly distilled pyrrole (1.0 g, 14.9 mmol) followed by benzenesulfonyl chloride (2.1 mL, 16.4 mmol). The mixture was stirred at room temperature for 3 hours, quenched with saturated aqueous ammonium chloride (200 mL) and extracted with dichloromethane (3 x 40 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (2 x 50 mL) and saturated aqueous sodium chloride (50 mL), dried over magnesium sulfate and concentrated to afford the desired product (3.1 g, 99%) as an off-white solid, the characterisation data of which corresponded to that previously described.\(^{316}\) \(R_f\): 0.47 (1:4 diethyl ether, hexane) \(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\)): δ 113.9, 121.1, 127.0, 129.6, 134.1, 139.4 ppm.

To a suspension of aluminium chloride (3.9 g, 29.0 mmol) in dichloromethane (15 mL) at 0 °C was added 123 (2.0 g, 9.65 mmol) followed by a solution of tert-butyl chloride (1.3 mL, 12.1 mmol) in dichloromethane (10 mL). The mixture was warmed to room temperature and stirred for 2 hours, then poured into ice-cold water (100 mL) and extracted with dichloromethane (3 x 40 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (2 x 50 mL) and saturated aqueous sodium chloride (50 mL), dried over anhydrous magnesium sulfate and concentrated. The crude residue was purified by flash column chromatography using diethyl ether, hexane (1:9) as an eluent to obtain the title compound (2.1 g, 81%) as a waxy solid, the characterisation data of which corresponded to that previously described.\(^{317}\) \(R_f\): 0.52 (1:9 diethyl ether, hexane); \(\text{IR } (v_{\text{max}}\text{ (film))}\): 2961, 1364, 1167, 1061, 726 cm\(^{-1}\); \(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\)): δ 30.9, 31.0, 112.9, 115.2, 121.0, 126.8, 129.4, 133.7, 139.5, 140.8 ppm; \(\text{LRMS (ESI) } m/z\): 286.0 ([M+Na]\(^+\) 14%), 302.1 ([M+K]\(^+\) 100%).
3-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrole 126

![Chemical Structure](image)

To a solution of the protected pyrrole 124 (6.0 g, 22.8 mmol) and ammonium chloride (390 mg, 7.3 mmol) in methanol (15 mL) was added magnesium powder (4.4 g, 182 mmol) and the mixture was sonicated for 1 hour. The reaction mixture was slowly quenched with saturated aqueous ammonium chloride (200 mL), extracted with diethyl ether (3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude pyrrole intermediate was used immediately without further purification. To mixture of the pyrrole (2.3 g, 18.7 mmol), tosylate 125 (6.1 g, 22.4 mmol) and tetrabutylammonium bromide (603 mg, 1.9 mmol) in N,N-dimethylformamide (100 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 972 mg, 24.3 mmol) and the mixture was immediately warmed to 50 °C and stirred for 16 hours. The mixture was cooled to 0 °C and quenched with saturated aqueous ammonium chloride (30 mL). The solution was diluted with water (200 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 2 x 50 mL), dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (2.2 g, 52%) as an orange oil, $R_f$: 0.36 (1:9 ethyl acetate, hexane); IR ($v_{\text{max}}$ (film)): 2955, 2863, 2842, 1200, 1093, 770 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.24 (9H, s), 1.21-1.42 (2H, m), 1.42-1.58 (2H, m), 1.82-2.02 (1H, m), 3.36 (2H, td, $J = 11.8, 2.1$ Hz), 3.68 (2H, d, $J = 7.2$ Hz), 3.89-4.04 (2H, m), 6.06 (1H, s), 6.39 (1H, s), 6.52 (1H, s) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 30.7, 30.9, 32.0, 37.5, 55.8, 67.7, 105.8, 116.2, 120.6, 135.7 ppm; LRMS (-ESI) $m/z$: 220.4 ([M-H]$^-$ 100%).
1-(4-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrole-2-yl)-2,2,2-trichloroethanone 127

To a solution of pyrrole 126 (2.0 g, 9.0 mmol) in tetrahydrofuran (40 mL) was added pyridine (1.3 mL, 16.2 mmol) and the mixture was stirred for 10 minutes before being cooled to 0 °C. To the solution was added trichloroacetyl chloride (1.8 mL, 16.2 mmol) and the mixture stirred at room temperature for 6 hours. The organic solvent was removed under a stream of nitrogen and the residue taken up in diethyl ether (50 mL), washed with saturated aqueous sodium hydrogen carbonate (2 x 30 mL) and aqueous hydrochloric acid (1 M, 30 mL), dried over magnesium sulfate and concentrated under reduced pressure to obtain the title compound (3.2 g, 97%) as a brown gummy solid, Rf: 0.44 (1:4 ethyl acetate, hexane); IR (v_max (film)): 2957, 2846, 1707, 1665, 1345, 1093, 735 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 1.25 (9H, s), 1.21-1.42 (2H, m), 1.47-1.59 (2H, m), 1.82-2.10 (1H, m), 3.35 (2H, t, J = 11.6), 3.96 (2H, dd, J = 11.5, 4.4 Hz), 4.12 (2H, d, J = 7.0 Hz), 6.80 (1H, s), 7.38 (1H, s) ppm; \(^13\)C NMR (75 MHz, CDCl\(_3\)): δ 30.5, 31.6, 36.2, 56.4, 67.6, 96.8, 120.6, 121.6, 130.8, 135.8, 172.6 ppm; LRMS (+ESI) m/z: 388.1/390.1 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{16}\)H\(_{22}\)Cl\(_3\)NO\(_2\) [M+Na]\(^+\): 388.06083, 390.05788 found 388.06066, 390.5769.

4-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrole-2-carboxylic acid 128

Prepared according to General Procedure B using 127 (3.2 g, 8.7 mmol) in methanol (60 mL) and sodium hydroxide at reflux for 16 hours to obtain the desired compound 128 (2.1 g, 92%) as a brown oil, IR (v_max (film)): 2955, 2864, 1700, 1660, 1447, 1265, 1085 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 1.24 (9H, s), 1.21-1.42 (2H, m), 1.42-1.54 (2H, m), 1.93-2.10 (1H, m), 3.35 (2H, t, J = 11.9 Hz), 3.97 (2H, dd, J = 11.8, 3.6 Hz), 4.11 (2H, d, J = 6.5 Hz), 6.66 (1H, s), 7.02 (1H, s) ppm; \(^13\)C NMR (75 MHz, CDCl\(_3\)): δ 30.5, 30.6, 31.7, 36.9, 55.0, 67.7, 117.8, 120.2, 127.0, 135.4, 166.0 ppm; LRMS (+ESI) m/z: 288.2
([M+Na]$^+$ 100%), 553.3 ([2M+Na]$^+$ 43%); HRMS (ESI)$^+$ Calcd for C$_{15}$H$_{23}$NO$_3$ [M+Na]$^+$: 288.15701, found 288.15685.

$N$-(4-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrol-2-yl)-4-(trifluoromethyl)benzamide 110

Prepared according to General Procedure F using the acid 128 (100 mg, 380 μmol) and 4-bromobenzotrifluoride (68 mg, 304 μmol). The crude residue was purified by flash column chromatography (neutral alumina) using ethyl acetate, hexane (1:19 to 1:9) as an eluent and recrystallisation from tetrahydrofuran, water to obtain the title compound (68 mg, 45%) as a white solid, mp: 65.9-66.8 °C; $R_f$: 0.18 (1:4 ethyl acetate, hexane); IR ($v_{\text{max}}$ (film)): 3258, 2959, 2865, 1660, 1573, 1532, 1128, 1065, 1015, 905 cm$^{-1}$; $^1$H NMR (300 MHz, MeOD): δ 1.23 (9H, s), 1.21-1.37 (2H, m), 1.38-1.56 (2H, m), 1.85-2.06 (1H, m), 3.29-3.43 (2H, m), 3.66 (2H, d, $J = 7.3$ Hz), 3.91 (2H, dd, $J = 11.4$, 3.9 Hz), 5.95 (1H, s), 6.45 (1H, s), 7.84 (2H, d, $J = 8.1$ Hz), 8.09 (2H, d, $J = 8.1$ Hz) ppm; $^{13}$C NMR (75 MHz, MeOD): δ 31.5, 31.8, 32.2, 52.8, 68.6, 103.3, 115.7, 125.2, 125.3 (q, $J = 271.0$ Hz), 126.7 (q, $J = 3.9$ Hz), 129.5, 133.5 (q, $J = 30.0$ Hz), 134.7, 139.0, 169.0 ppm; $^{19}$F NMR (282 MHz, MeOD): δ −64.5 ppm; LRMS (-ESI) $m/z$: 407.4 ([M-H]$^-100$%); HRMS (ESI)$^+$ Calcd for C$_{22}$H$_{27}$F$_3$N$_2$O$_2$ [M+Na]$^+$: 431.19223, found 431.19249; HPLC: 96.9%, RT: 43.9 mins. (Method B, 230 nm).

**tert-Butyl 2-((tetrahydro-2H-pyran-4-yl)methylene)hydrazinecarboxylate 129**

A solution of tetrahydro-2H-pyran-4-carbaldehyde (1.2 g, 10.5 mmol), tert-butyl carbazate (1.4 g, 10.5 mmol) and anhydrous magnesium sulfate (3 g) in methanol (10 mL) was stirred at room temperature for 16 h. The solids were removed by filtration and the filtrate concentrated to obtain the title compound (1.5 g, 63%) as a white solid, mp: 152.7-154.4 °C; $R_f$: 0.68 (1:19 methanol, dichloromethane); IR ($v_{\text{max}}$ (neat)): 3284, 2842, 1703, 1529,
1367, 1246, 1153, 1083, 966 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.48 (9H, s), 1.43-1.70 (4H, m), 2.41-2.63 (1H, m), 3.30-3.50 (2H, m), 3.81-4.03 (2H, m), 7.05 (1H, d, \(J = 5.6\) Hz), 7.73 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 28.4, 30.0, 38.1, 67.3, 81.2, 149.3, 152.6 ppm; LRMS (+ESI) \(m/z\): 251.1 ([M+Na]\(^+\) 100%), 479.2 ([2M+Na]\(^+\) 94%).

tert-Butyl 2-((tetrahydro-2H-pyran-4-yl)methyl)hydrazinecarboxylate 130

![Structural formula](image)

To a solution of the hydrazone 129 (800 mg, 3.5 mmol) in anhydrous tetrahydrofuran (5 mL) was added, dropwise, a solution of sodium cyanoborohydride (220 mg, 3.5 mmol) in tetrahydrofuran (5 mL), followed by a solution of \(p\)-toluene sulfonic acid monohydrate (670 mg, 3.5 mmol) in tetrahydrofuran (5 mL) and the mixture was stirred at room temperature for 16 hours. Aqueous sodium hydroxide (1 M, 14 mL) was added slowly and the mixture was extracted with dichloromethane (3 x 20 mL), washed with saturated aqueous sodium bicarbonate (30 mL) and saturated aqueous sodium chloride (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (700 mg, 87%) as a colourless oil, \(R_f\): 0.30 (1:19 methanol, dichloromethane); IR (\(\nu_{\text{max}}\) (neat)): 3350, 3276, 2918, 1687, 1479, 1288, 1145, 1087 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.14-1.47 (2H, m), 1.44 (9H, s), 1.60-1.83 (3H, m), 2.72 (2H, d, \(J = 6.4\) Hz), 3.36 (2H, td, \(J = 11.7, 1.9\) Hz), 3.94 (2H, ddd, \(J = 11.8, 4.9, 1.7\) Hz), 6.11 (1H, bs) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 28.5, 31.3, 33.9, 58.2, 67.9, 80.6, 157.0 ppm; LRMS (+ESI) \(m/z\): 253.1 ([M+Na]\(^+\) 100%).

\((\text{Tetrahydro-2H-pyran-4-yl})\text{methyl)}\text{hydrazine dihydrochloride 131}

![Structural formula](image)

The protected hydrazine 130 (150 mg, 650 \(\mu\)mol) was dissolved in a mixture of dioxane and methanol (1:1, 2 mL) and to this was added hydrochloric acid in dioxane (4 M, 650 \(\mu\)L, 2.6 mmol) and the mixture was stirred for 16 hours. The solvent was removed under reduced pressure to obtain the title compound (130 mg, 100%), as a white solid, mp: 110.0-
113.2 °C; Rf: 0.17 (1:19 saturated methanolic ammonia, dichloromethane); IR (vmax (neat)): 3313, 2921, 2409, 1466, 1076, 843 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 1.17 (2H, qd, J = 11.9, 4.4 Hz), 1.54-1.74 (2H, m), 2.78 (2H, d, J = 6.8 Hz), 3.25 (2H, td, J = 11.7, 2.1 Hz), 3.81 (2H, d, J = 4.5, 1.9 Hz), 6.92 (3H, bs) ppm; ¹³C NMR (75 MHz, DMSO-d₆): δ 30.4, 31.4, 55.6, 66.6 ppm; LRMS (+ESI) m/z: 131.1 ([M+H]+ 100%).

3-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazol-5-amine 132

A solution of the hydrazine hydrochloride salt 131 (350 mg, 1.7 mmol) and 88 (258 mg, 2.1 mmol) in ethanol (10 mL) was heated at reflux for 16 hours. The ethanol was removed under reduced pressure and the residue purified by flash column chromatography using saturated ammonia in methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (210 mg, 52%) as a white solid, mp: 108.1-111.3 °C; Rf: 0.73 (1:9 saturated ammonia in methanol, dichloromethane); IR (vmax (film)): 3326, 3223, 2957, 2861, 1635, 1559, 1239, 1088, 980 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.24 (9H, s), 1.27-1.43 (2H, m), 1.43-1.55 (2H, m), 2.06-2.23 (1H, m), 3.35 (2H, td, J = 11.7, 2.3 Hz), 3.76 (2H, d, J = 7.3 Hz), 3.95 (2H, ddd, J = 11.4, 4.5, 1.8 Hz), 5.41 (1H, s) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 30.6, 30.7, 32.2, 36.1, 52.6, 67.7, 88.3, 144.2, 160.7 ppm; LRMS (+ESI) m/z: 238.2 ([M+H]+ 100%); HRMS (ESI)+ Calcd for C₁₅H₂₃N₃O [M+Na]+: 260.17333, found 260.17316.

N-(3-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazol-5-yl)-4-(trifluoromethyl)benzamide 11

Prepared according to General Procedure C using 4-trifluoromethylbenzoic acid (50 mg, 260 μmol) and aminopyrazole 132 (77 mg, 330 μmol). The crude solid was purified by flash column chromatography using ethyl acetate, hexane (2:3) as an eluent to obtain the desired compound (77 mg, 72%) as a colourless solid, mp: 62.9-64.4 °C; Rf: 0.44 (2:3 ethyl
acetate, hexane); IR (ν max (film)): 3270, 2962, 2864, 1665, 1559, 1324, 1129, 1048 cm⁻¹; ¹H NMR (300 MHz, MeOD): δ 1.17-1.40 (2H, m), 1.31 (9H, s), 1.40-1.77 (2H, m), 2.00-2.23 (1H, m), 3.25-3.45 (2H, m), 3.80-4.02 (4H, m), 6.19 (1H, s), 7.86 (2H, d, J = 8.0 Hz), 8.11 (2H, d, J = 8.0 Hz) ppm; ¹³C NMR (75 MHz, MeOD): δ 30.8, 31.6, 33.2, 37.3, 54.6, 68.5, 90.0, 125.2 (q, J = 271.9 Hz), 126.8 (q, J = 3.9 Hz), 129.6, 134.8 (q, J = 32.7 Hz), 137.2, 138.5, 162.2, 167.8 ppm; ¹⁹F NMR (282 MHz, MeOD): δ −64.5 ppm; LRMS (-ESI) m/z: 408.3 ([M-H]⁻ 100%); HRMS (ESI)+ Calcd for C₂₁H₂₆F₃N₃O₂ [M+Na]⁺: 432.18693, found 432.18648; HPLC: 99.4%, RT: 38.7 mins. (Method B, 230 nm).

1-Bromo-3,3-dimethylbutan-2-one 133

To a solution of pinacolone (1.25 mL, 10.0 mmol) in methanol (5 mL) at -40 °C was added bromine (510 μL, 10.0 mmol) dropwise over 30 minutes. The reaction mixture was warmed to room temperature and quenched with cold saturated aqueous sodium hydrogen carbonate (1 mL). After stirring for 5 minutes, water (10 mL) was added and the mixture extracted with hexane (3 x 15 mL). The combined organic extracts were dried over magnesium sulfate and concentrated to obtain the desired product (1.5 g, 83%) as a colourless liquid, the characterisation data of which corresponded to that previously described.³¹⁸ Rf: 0.40 (1:9 ethyl acetate, hexane); IR (ν max (film)): 2969, 1717, 1477, 1367, 1056, 1002 cm⁻¹.

3,3-Dimethyl-1-(((tetrahydro-2H-pyran-4-yl)methyl)amino)butan-2-one 134

To a solution of 4-methanamine tetrahydropyran (1.0 mL, 8.4 mmol) in diethyl ether (5 mL) was added 133 (500 mg, 2.8 mmol) in diethyl ether (2 mL) dropwise over 15 minutes at -78 °C and the mixture was stirred for 16 hours over which time the mixture warmed to room temperature. The suspension was washed with saturated aqueous sodium hydrogen carbonate (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated to obtain the title compound (350 mg, 43%) as an unstable yellow oil, Rf: 0.61 (1:9 saturated methanolic ammonia, dichloromethane); IR (ν max (film)): 3292, 2955, 2846, 1667, 1591, 1143, 1091, 852 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.15 (9H, s), 1.13-1.44 (2H, m), 1.46-1.80 (3H, m), 2.23 (1H, bs), 2.43 (2H, d, J = 5.7 Hz), 3.37 (2H, t, J = 11.6 Hz), 3.60 (2H, s), 3.94 (2H, dd, J = 7.8, 4.5 Hz) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 26.3, 31.2,
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35.7, 43.0, 54.1, 56.0, 67.8, 214.0 ppm; LRMS (+ESI) m/z: 214.2 ([M+H]+ 100%), 236.0 ([M+Na]+ 42%).

4-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-imidazol-2-amine 135

A solution of freshly synthesised 134 (300 mg, 1.41 mmol) and cyanamide (590 mg, 14.1 mmol) in ethanol (95%, 10 mL) was heated at reflux for 16 hours. The ethanol was removed under reduced pressure and the crude product purified by flash column chromatography using saturated ammonia in methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (315 mg, 94%) as an off-white solid, mp: 172.6-175.1 °C; Rf: 0.58 (1:9 saturated ammonia in methanol, dichloromethane); IR (v max (film)): 3338, 3233, 2952, 2862, 1629, 1541, 1501, 1226, 1088, 938 cm⁻¹; ¹H NMR (300 MHz, MeOD): δ 1.19 (9H, s), 1.34 (2H, qd, J = 11.5, 4.1 Hz), 1.46-1.60 (2H, m), 1.85-2.11 (1H, m), 3.32-3.45 (2H, m), 3.56 (2H, d, J = 7.7 Hz), 3.86-4.01 (2H, m), 6.23 (1H, s) ppm; ¹³C NMR (75 MHz, MeOD): δ 30.3 31.5, 32.2, 36.7, 50.9, 68.5, 109.8, 147.0, 149.8 ppm; LRMS (+ESI) m/z: 238.2 ([M+H]+ 100%).

N-(4-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-imidazol-2-yl)-4-(trifluoromethyl)benzamide 112

Prepared according to General Procedure C using 4-trifluoromethylbenzoic acid (53 mg, 280 μmol) and aminimidazole 135 (80 mg, 390 μmol). The crude solid was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (75 mg, 65%) as a white solid, mp: 156.7-157.8 °C; Rf: 0.37 (2:3 ethyl acetate, hexane); IR (v max (film)): 3402, 2964, 2847, 1608, 1561, 1510, 1317, 1122, 1094, 1062, 781 cm⁻¹; ¹H NMR (300 MHz, MeOD): δ 1.34 (9H, s), 1.33-1.51 (2H, m), 1.51-1.65 (2H, m), 2.03-2.28 (1H, m), 3.39 (2H, td, J = 11.7, 2.2 Hz), 3.85-4.03 (4H, m), 6.74 (1H, s), 7.72 (2H, d, J = 8.1 Hz), 8.31 (2H, d, J = 8.1 Hz) ppm; ¹³C NMR (75 MHz, CDCl₃): δ
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29.3, 30.5, 30.6, 35.7, 50.5, 67.5, 108.3, 124.4 (q, $J = 271.8$ Hz), 124.9 (q, $J = 3.8$ Hz), 129.1, 130.6 (q, $J = 32.3$ Hz), 134.9, 142.1, 151.1, 173.3 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ –62.6 ppm; LRMS (+ESI) m/z: 410.2 ([M+H]$^+$ 100%), 432.2 ([M+Na]$^+$ 63%); HRMS (ESI)$^+$ Cald for C$_{21}$H$_{26}$F$_3$N$_3$O$_2$ [M+Na]$^+$: 432.18693, found 432.18644; HPLC: 98.4%, RT: 28.3 mins. (Method B, 230 nm).

*Ethyl pivalimidate hydrochloride 136*

![NH.HCl]

To a solution of trimethylacetonitrile (6.6 mL, 60.1 mmol) in ethanol (42 mL) at 0 °C was added acetyl chloride (34 mL, 481 mmol), dropwise. Once the addition was complete the mixture was warmed to room temperature and stirred for 16 hours. The volatiles were removed under a stream of nitrogen and the resulting solid dried *in vacuo* to obtain the title compound (7.9 g, 79%) as a white solid, the characterisation data of which corresponded to that previously described.$^{319}$ IR ($\nu_{\text{max}}$ (film)): 3370, 2979, 1638, 1553, 1358, 1123, 886 cm$^{-1}$; $^{13}$C NMR (75 MHz, MeOD): $\delta$ 13.7, 27.1, 39.6, 71.1, 187.5 ppm; LRMS (+ESI) m/z: 130.4 ([M+H]$^+$ 100%).

*Ethyl N-cyanopivalimidate 137*

![N≡N]

To a solution of 136 (1.0 g, 6.0 mmol) in acetonitrile (5 mL) was added a solution of sodium phosphate monobasic dihydrate (3.7 g, 24 mmol), sodium phosphate dibasic (1.7 g, 12 mmol) and cyanamide (510 mg, 12.0 mmol) in water (45 mL). The reaction mixture was stirred at room temperature for 72 hours, extracted with dichloromethane (3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated to obtain the title compound (470 mg, 51%) as a white solid, mp: 55.5-57.4 °C; $R_f$: 0.50 (3:7 ethyl acetate, hexane); IR ($\nu_{\text{max}}$ (film)): 3355, 2973, 2879, 2194, 1599, 1312, 1047, 880 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.32 (3H, t, $J = 7.0$ Hz), 1.38 (9H, s), 4.23 (2H, q, $J = 7.1$ Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 13.7, 26.9, 41.6, 66.0, 112.8, 186.9 ppm; LRMS (+ESI) m/z: 155.1 ([M+H]$^+$ 11%), 177.1 ([M+Na]$^+$ 100%).
3-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-1,2,4-triazol-5-amine 138

137 (280 mg, 1.8 mmol) and the hydrazine hydrochloride 131 (440 mg, 2.2 mmol) were dissolved in anhydrous methanol (15 mL) and to this was added 1,8-diazabicyclo[5.4.0]undec-7-ene (600 μL, 4.0 mmol) and the mixture was heated at reflux for 16 hours. The solvent was removed under reduced pressure and the residue purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (340 mg, 78%) as a white solid, mp: 179.9-182.3 °C; Rf: 0.38 (1:19 saturated methanolic ammonia, dichloromethane); IR (νmax (neat)): 3281, 3097, 2958, 2933, 1656, 1569, 1526, 1207, 1092, 845 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.27 (9H, s), 1.24-1.44 (2H, m), 1.45-1.62 (2H, m), 2.01-2.26 (1H, m), 3.35 (2H, td, J = 11.8, 2.1 Hz), 3.65 (2H, d, J = 7.2 Hz), 3.83-4.02 (2H, m), 4.93 (2H, bs) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 29.5, 30.6, 32.7, 35.5, 52.3, 67.5, 154.3, 166.4 ppm; LRMS (+ESI) m/z: 239.1 ([M+H]+ 100%); HRMS (ESI)⁺ Calcd for C₁₂H₂₂N₄O [M+H]⁺: 239.18664, found 239.18673.

N-(3-(tert-Butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-1,2,4-triazol-5-yl)-4-(trifluoromethyl)benzamide 113

To a solution of 138 (35 mg, 150 μmol) in anhydrous toluene (2 mL) at 0 °C was added sodium hydride (60% in mineral oil, 6.6 mg, 170 μmol), followed by 4-trifluoromethyl benzoyl chloride (31 mg, 150 μmol) and the mixture was heated at reflux for 3 hours. After cooling to room temperature, the reaction mixture was quenched with saturated aqueous ammonium chloride (10 mL), extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent and recrystallisation from hexane to afford the title compound (45 mg, 75%), as a colourless crystalline solid, mp: 111.3-112.7 °C; Rf: 0.48 (1:4 ethyl acetate, hexane); IR (νmax (neat)):
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2961, 2852, 1663, 1539, 1284, 1129, 1092, 866 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 1.38 (9H, s), 1.36-1.66 (4H, m), 2.11-2.34 (1H, m), 3.39 (2H, td, $J = 11.6, 2.4$ Hz), 3.91-4.03 (2H, d, $J = 7.0$ Hz), 7.68 (2H, d, $J = 8.1$ Hz), 8.31 (2H, d, $J = 8.1$ Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 28.6, 30.5, 32.3, 35.1, 52.4, 67.5, 124.2 (q, $J = 272.2$ Hz), 125.2 (q, $J = 3.9$ Hz), 129.3, 130.4, 133.0 (q, $J = 32.3$ Hz), 140.2, 153.4, 172.9 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): δ −62.8 ppm; LRMS (+ESI) m/z: 433.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{20}$H$_{25}$F$_3$N$_4$O$_2$ [M+Na]$^+$: 433.18218, found 433.18208; HPLC: 99.8%, RT: 41.4 mins. (Method B, 230 nm).

1-(Dihydro-2H-pyran-4(3H)-ylidene)propan-2-one 139

![ structure ]

To a solution of potassium hydroxide (760 mg, 13.3 mmol) in a mixture of ethanol and water (4:1, 10 mL) at 0 °C was added tetrahydropyranone (1.1 mL, 12.1 mmol) and 2-oxopropyl dimethyl ester phosphonic acid (1.9 mL, 13.3 mmol). The mixture was warmed to room temperature and stirred for 5 hours before the solvent was removed and the residue taken up in ethyl acetate, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to yield the title compound (1.6 g, 95%), as a light yellow oil, $R_f$: 0.31 (1:9 ethyl acetate, hexane); IR ($v_{max}$ (film)): 2961, 2846, 1685, 1619, 1355, 1095, 964, 883 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 2.17 (3H, s), 2.28 (2H, t, $J = 5.6$ Hz), 2.96 (2H, t, $J = 5.6$ Hz), 3.70 (2H, t, $J = 5.6$ Hz), 3.77 (2H, t, $J = 5.5$ Hz), 6.04 (1H, s) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 31.2, 32.0, 37.7, 68.7, 69.3, 122.5, 155.6, 199.1 ppm; LRMS (+ESI) m/z: 163.1 ([M+Na]$^+$ 100%).

Methyl 4-(dihydro-2H-pyran-4(3H)-ylidene)-3-oxobutanoate 140

![ structure ]

To a solution of diisopropylamine (2.3 mL, 16.6 mmol) in anhydrous tetrahydrofuran (80 mL) at -78 °C was added n-butyl lithium (2.5 M in hexanes, 5.0 mL, 12.5 mmol). The mixture was warmed to -60 °C and 139 (1.6 g, 11.4 mmol) was added slowly. After 15 minutes methyl cyanoformate (1.5 mL, 19.8 mmol) was added and the mixture stirred at -40 °C for 1 hour. The reaction mixture was poured into saturated aqueous ammonium chloride (30 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic fractions were dried over anhydrous magnesium sulfate, concentrated under reduced
pressure and purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (1.3 g, 58%) as a light yellow oil, \( R_f \): 0.21 (3:17 ethyl acetate, hexane); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 2.32 (2H, t, \( J = 5.5 \) Hz), 3.00 (2H, t, \( J = 5.7 \) Hz), 2.12 (2H, s), 3.67-3.76 (5H, m), 3.79 (2H, t, \( J = 5.6 \) Hz), 6.09 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 31.5, 37.8, 50.7, 52.5, 68.6, 69.2, 120.9, 159.1, 168.1, 199.1 ppm; LRMS (+ESI) \( m/z \): 221.0 ([M+Na]\(^+\) 100%), HRMS (ESI)\(^+\) Cald for C\(_{10}\)H\(_{14}\)N\(_2\)O\(_4\) [M+Na]\(^+\): 221.07843, found 221.07856.

**Methyl 3-oxo-4-(tetrahydro-2H-pyran-4-yl)butanoate 141**

![chemical structure](image)

To a solution of 140 (1.0 g, 5.0 mmol) in methanol (15 mL) was added ammonium formate (950 mg, 15.1 mmol) and palladium on charcoal (10% w/w, 540 mg, 500 μmol) and the mixture was stirred at room temperature for 3 hours. The mixture was filtered through a pad of Celite\(^\circledR\) and the filtrate concentrated under reduced pressure, taken up in dichloromethane (50 mL) and washed with water (2 x 20 mL). The organic phase was dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (960 mg, 95%) as a light yellow oil, \( R_f \): 0.10 (2:3 ethyl acetate, hexane); IR (\( \nu_{max} \) (film)): 3426, 3323, 2946, 2843, 1746, 1716, 1664, 1616, 1558, 1443, 1156, 1087 cm\(^{-1}\); \(^1\)H NMR (300 MHz, MeOD): \( \delta \) 1.28 (2H, dtd, \( J = 13.3, 11.7, 1.0 \) Hz), 1.64 (2H, ddd, \( J = 13.1, 4.1, 2.0 \) Hz), 1.84 (1H, tt, \( J = 11.2, 7.4, 3.8 \) Hz), 2.06 (2H, d, \( J = 7.4 \) Hz), 3.26-3.47 (2H, m), 3.58 (3H, s), 3.84-3.98 (2H, m), 4.41 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 32.9, 34.5, 44.2, 50.3, 67.9, 84.3, 161.5, 170.7 ppm; LRMS (+ESI) \( m/z \): 223.1 ([M+Na]\(^+\) 100%) HRMS (ESI)\(^+\) Cald for C\(_{10}\)H\(_{16}\)O\(_4\) [M+Na]\(^+\): 223.09408, found 223.09430.

**Methyl 2-(tert-butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)oxazole-4-carboxylate 143**

![chemical structure](image)

\( \beta \)-Keto ester 141 (600 mg, 3.0 mmol) was dissolved in glacial acetic acid (15 mL) and cooled to -5 °C. To the mixture was added a solution of sodium nitrite (310 mg, 4.5 mmol) in water (5 mL), dropwise and the solution stirred for 2 hours at 0 °C and another 2 h at
room temperature. The reaction mixture was quenched with saturated aqueous sodium chloride (30 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic fractions were washed with water (3 x 30 mL), dried over anhydrous magnesium sulfate and concentrated to obtain an oxime intermediate, which was taken on to the next step without further purification or characterization.

The oxime was taken up ethanol (10 mL) and to this was added ethanolic hydrochloric acid (2 M, 3.5 mL) and palladium on carbon (10% w/w, 810 mg, 760 μmol) under an atmosphere of nitrogen. The reaction vessel was evacuated and flushed with hydrogen three times before the mixture was stirred at room temperature for 16 hours. The palladium catalyst was removed by filtration through Celite® and the solvent removed to obtain the crude unstable amine hydrochloride salt which was taken on directly to the next step without further purification or characterisation.

The amine hydrochloride salt was suspended in anhydrous dichloromethane (20 mL) and cooled to 0 °C. To the suspension was added pivalic anhydride (930 μL, 4.6 mmol) and triethylamine (640 μL, 4.6 mmol) and the mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into water (15 mL), extracted with dichloromethane (3 x 15 mL), washed with saturated aqueous ammonium chloride (15 mL) and saturated aqueous sodium hydrogen carbonate (15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain amide intermediate 142 which was taken on to the next step without further purification or characterisation.

To a solution of triphenylphosphine (1.2 g, 4.6 mmol) and iodine (1.2 g, 4.6 mmol) in anhydrous dichloromethane (120 mL) was added triethylamine (1.3 mL, 9.2 mmol) and a solution of the amide in dichloromethane (10 mL). The mixture was stirred at room temperature for 3 hours before the solvent was removed under reduced pressure and the crude residue purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (500 mg, 60% over 4 steps) as a colourless oil, \( R_f: 0.48 \) (2:3 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 2962, 2876, 1710, 1617, 1457, 1354, 1141, 1052 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 1.37 (9H, s), 1.31-1.48 (2H, m), 1.49-1.62 (2H, m), 1.84-2.01 (1H, m), 2.95 (2H, d, \( J = 7.1 \) Hz), 3.34 (2H, td, \( J = 11.7, 2.3 \) Hz), 3.87 (3H, s), 3.88-3.99 (2H, m) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 29.5, 32.8, 32.9, 33.8, 34.8, 52.0, 67.8, 127.5, 158.1, 163.1, 169.7 ppm;LRMS (+ESI) \( m/z \): 304.2 ([M+Na\(^+\) 88%]), 585.2 ([2M+Na\(^+\) 100%]); HRMS (ESI)\(^+\) Cald for C\(_{15}\)H\(_{23}\)NO\(_4\) [M+Na\(^+\)]: 304.15193, found 304.15200.
Methyl 2-(tert-butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)thiazole-4-carboxylate 144

![Chemical Structure Image]

The common amide intermediate was prepared using the same procedures as detailed for 142.

The crude amide and Lawesson’s reagent (2.4 g, 6.0 mmol) were dissolved in anhydrous tetrahydrofuran (15 mL) and heated at reflux for 3 hours. The reaction mixture was cooled to room temperature, poured into saturated aqueous sodium hydrogen carbonate (30 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic fractions were washed with water (20 mL) and brine (20 mL), dried over anhydrous magnesium sulfate, concentrated and the crude residue purified by flash column chromatography to obtain the title compound (0.36 g, 40% over 4 steps) as a colourless oil, 

\[ R_f : 0.52 \text{ (2:3 ethyl acetate, hexane); } \text{IR} (\nu_{\text{max}} \text{ (neat)}): 2955, 2841, 1702, 1650, 1495, 1321, 1169, 1064 \text{ cm}^{-1}; \text{H NMR} (300 MHz, CDCl}_3): \delta 1.29-1.42 (2H, m), 1.42 (9H, s), 1.54-1.67 (2H, m), 1.85 (1H, tt, \( J = 11.0, 7.2, 3.8 \text{ Hz}), 3.12 (2H, d, J = 7.1 \text{ Hz}), 3.33 (2H, td, J = 11.8, 2.1 \text{ Hz}), 3.89 (3H, s), 3.88-3.99 (2H, m) \text{ ppm; C NMR} (75 MHz, CDCl}_3): \delta 30.9, 32.9, 34.2, 37.2, 37.9, 52.2, 67.9, 140.5, 146.9, 163.2, 177.7 \text{ ppm; LRMS (+ESI) m/z: 320.1 ([M+Na]^+ 100%), 617.2 ([2M+Na]^+ 60%); HRMS (ESI)^+ } \text{Cald for C}_{15}H_{23}NO_3S [M+Na]^+: 320.12909, found 320.12919.}

2-(tert-Butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)oxazole-4-carboxylic acid 145

![Chemical Structure Image]

Prepared according to General Procedure B using ester 143 (200 mg, 710 μmol) and aqueous sodium hydroxide to obtain the carboxylic acid 145 (180 mg, 95%) as a colourless oil, 

\[ \text{IR} (\nu_{\text{max}} \text{ (neat)}): 3434, 3368, 2914, 1699, 1626, 1199 \text{ cm}^{-1}; \text{H NMR} (300 MHz, CDCl}_3): \delta 1.38 (9H, s), 1.38-1.50 (2H, m), 1.50-1.61 (2H, m), 1.87-2.07 (1H, m), 2.97 (2H, d, J = 7.1 \text{ Hz}), 3.36 (2H, td, J = 11.6, 2.4 \text{ Hz}), 3.96 (2H, ddd, J = 11.6, 4.2, 1.9 \text{ Hz}), 6.41 (1H, bs) \text{ ppm; C NMR} (75 MHz, CDCl}_3): \delta 28.4, 32.7, 33.0, 33.9, 34.8, 67.8, 127.2, ...]
\[
\begin{align*}
158.1, 165.8, 169.9 \text{ ppm; LRMS (+ESI) } m/z: & \ 290.1 ([M+Na]^+ 100\%), 557.2 ([2M+Na]^+ 86\%); \ HRMS (ESI)^+ \text{ Cald for C}_{14}H_{21}NO_4 [M+Na]^+: 290.13628, \text{ found 290.13639.} \\
\end{align*}
\]

2-(tert-Butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)thiazole-4-carboxylic acid 146

Prepared according to General Procedure B using methyl ester 144 (270 mg, 910 μmol) and aqueous sodium hydroxide to obtain the title compound (200 mg, 78%) as a colourless oil, IR (\(v_{\text{max}}\) (neat)): 3262, 2958, 2841, 1706, 1177, 1090, 1064, 982 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.42 (9H, s), 1.34-1.52 (2H, m), 1.55-1.70 (2H, m), 1.88 (1H, ttt, \(J = 11.1, 7.3, 3.8\) Hz), 3.19 (2H, d, \(J = 7.0\) Hz), 3.36 (2H, td, \(J = 11.7, 2.1\) Hz), 3.97 (2H, ddd, \(J = 11.6, 5.2, 1.9\) Hz), 6.53 (1H, bs ppm); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 30.8, 32.7, 33.8, 37.2, 37.8, 67.9, 139.7, 146.8, 161.9, 178.0 ppm; LRMS (-ESI) \(m/z\): 282.1 ([M-H]^- 100%); HRMS (ESI)^+ Cald for C\(_{14}\)H\(_{21}\)NO\(_3\)S [M+Na]^+: 306.11344, found 306.11352.

\[\begin{align*}
\text{N-(2-(tert-Butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)oxazol-4-yl)-4-}
\text{(trifluoromethyl)benzamide 113} \\
\end{align*}\]

Prepared according to General Procedure F using carboxylic acid 145 (150 mg, 560 μmol) and 1-bromo-4-(trifluoromethyl)benzene. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (40 mg, 22%) as a colourless foam, \(R_f\): 0.48 (1:4 ethyl acetate, hexane); IR (\(v_{\text{max}}\) (neat)): 2962, 2835, 1710, 1654, 1140, 1053 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.31 (9H, s), 1.24-1.47 (2H, m), 1.47-1.68 (2H, m), 1.84-1.99 (1H, m), 2.79 (2H, d, \(J = 6.9\) Hz), 3.36 (2H, m), 3.93 (2H, m), 7.71 (2H, d, \(J = 8.1\) Hz), 8.00 (2H, d, \(J = 8.1\) Hz), 8.56 (1H, s) ppm; \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 28.5, 32.9, 32.9, 33.8, 34.6, 67.9, 123.7 (q, \(J = 272.1\) Hz), 125.9 (q, \(J = 3.1\) Hz), 128.0, 129.9, 133.9 (q, \(J = 33.1\) Hz), 137.1, 141.5, 164.0, 168.6 ppm; \(^19\)F NMR (282 MHz, CDCl\(_3\)): \(\delta\) −63.0 ppm; LRMS (+ESI) \(m/z\): 433.2 ([M+Na]^+}
HRMS (ESI) \( ^{+} \) Calcd for \( \text{C}_{21}\text{H}_{25}\text{F}_{3}\text{N}_{2}\text{O}_{3} \) [M+Na] \( ^{+} \): 433.17095, found 433.17095; 

**HPLC**: 98.8%, RT: 40.2 mins. (Method B, 254 nm).

\[
N-(2-\text{(tert-Butyl)-5-((tetrahydro-2H-pyran-4-yl)methyl)thiazol-4-yl)-4-(trifluoromethyl)benzamide 115}
\]

Prepared according to General Procedure F using carboxylic acid 146 (200 mg, 710 \( \mu \)mol) and 1-bromo-4-(trifluoromethyl)benzene. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (91 mg, 30%) as a colourless solid, \( mp \): 61.7-62.5 \( ^{\circ} \)C; \( R_f \): 0.37 (1:4 ethyl acetate, hexane); 

**IR** \( (v_{\text{max}} \text{ (neat))} \): 3260, 2960, 1661, 1559, 1324, 1126, 1065, 1016 \( \text{cm}^{-1} \); **\( ^{1}H \) NMR** (300 MHz, CDCl\(_3\)): \( \delta \) 1.22-1.37 (2H, m), 1.38 (9H, s), 1.51-1.73 (2H, m), 1.73-1.92 (1H, m), 2.72 (2H, \( d, J = 7.0 \) Hz), 3.35 (2H, \( \text{td}, J = 11.8, 2.0 \) Hz), 3.94 (2H, \( \text{dt}, J = 11.8, 2.0 \) Hz), 7.72 (2H, \( d, J = 8.0 \) Hz), 7.99 (2H, \( d, J = 8.0 \) Hz), 8.36 (1H, s) ppm; **\( ^{13}C \) NMR** (75 MHz, CDCl\(_3\)): \( \delta \) 30.8, 33.1, 34.0, 36.7, 37.8, 68.0, 123.8 (q, \( J = 274.5 \) Hz), 125.9 (q, \( J = 3.1 \) Hz), 128.0, 129.8, 133.8 (q, \( J = 33.1 \) Hz), 137.3, 140.6, 164.4, 177.0 ppm; **\( ^{19}F \) NMR** (282 MHz, CDCl\(_3\)): \( \delta \) -63.0 ppm; **LRMS (+ESI) \( m/z \)**: 449.2 ([M+Na] \( ^{+} \) 100%); **HRMS** (ESI) \( ^{+} \) Calcd for \( \text{C}_{21}\text{H}_{25}\text{F}_{3}\text{N}_{2}\text{S} \) [M+Na] \( ^{+} \): 449.14810, found 449.14795; **HPLC**: 100%, RT: 41.4 mins. (Method B, 1 hour, 230 nm).

**2-Bromo-4-(tert-butyl)aniline 147**

To a solution of 4-\( \text{tert} \)-butylaniline (1.0 g, 6.7 mmol) in \( \text{N,N} \)-dimethylformamide (15 mL) at 0 \( ^{\circ} \)C was added \( \text{N} \)-bromosuccinimide (1.2 g, 6.7 mmol) and the mixture was stirred for 16 hours at room temperature. The reaction mixture was poured into water (30 mL) and extracted with dichloromethane (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (1.2 g, 78%) as a brown oil, the characterisation data of which corresponded to that
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previously described.\textsuperscript{320} R\textsubscript{f}: 0.46 (3:17 ethyl acetate, hexane); IR (v\textsubscript{max} (film)): 3437, 3347, 2962, 1617, 1502, 1255, 824 cm\textsuperscript{-1}; \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 31.5, 34.1, 109.4, 115.7, 125.5, 129.5, 141.6, 143.0 ppm; LRMS (+ESI) m/z: 228.0/230.0 ([M+H]\textsuperscript{+} 100%).

\textit{N-(2-Bromo-4-(tert-butyl)phenyl)-4-(trifluoromethyl)benzamide 148}

\[\text{O} \quad \text{H} \quad \text{Br} \]
\[\text{F} \quad \text{C} \quad \text{F} \quad \text{C} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{B} \quad \text{O} \quad \text{O}\]

Prepared according to General Procedure C using 4-trifluoromethylbenzoic acid (420 mg, 2.2 mmol) and aniline 147 (500 mg, 2.2 mmol). The crude solid was purified by flash column chromatography using ethyl acetate, hexane (1:24) as an eluent and recrystallisation from isopropyl alcohol, water to obtain the title compound (660 mg, 75%) as a white solid, mp: 151.7-152.7 °C; R\textsubscript{f}: 0.63 (1:9 ethyl acetate, hexane); IR (v\textsubscript{max} (film)): 3343, 2966, 1660, 1509, 1318, 1111, 1065 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 1.31 (9H, s), 7.41 (1H, s), 7.79 (2H, d, J = 8.0 Hz), 8.04 (2H, d, J = 8.0 Hz), 8.31-8.45 ppm; \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): δ 31.4, 34.8, 114.1, 121.7, 123.6 (q, J = 270.0 Hz), 125.8 (CH), 126.2 (q, J = 3.6 Hz), 127.7, 129.4, 132.9, 133.1 (q, J = 31.2 Hz), 138.1, 149.6, 164.0 ppm; \textsuperscript{19}F NMR (282 MHz, CDCl\textsubscript{3}): δ −63.0 ppm; LRMS (+ESI) m/z: 422.0/424.0 ([M+Na]\textsuperscript{+} 100%); HRMS (ESI)\textsuperscript{+} Cald for C\textsubscript{18}H\textsubscript{17}BrF\textsubscript{3}NO [M+Na]\textsuperscript{+}: 422.03378/424.03174, found 422.03396/424.03190.

\textit{N-(4-(tert-Butyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-4-(trifluoromethyl)benzamide 149}

\[\text{O} \quad \text{H} \quad \text{Br} \]
\[\text{F} \quad \text{C} \quad \text{F} \quad \text{C} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{B} \quad \text{O} \quad \text{O}\]

The aryl bromide 148 (200 mg, 500 μmol), anhydrous potassium acetate (147 mg, 1.5 mmol), [1,1’-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11.0 mg, 15.0 μmol) and bis(pinacolato)diboron (250 mg, 1.0 mmol) were suspended in dry, degassed toluene (8 mL) and heated at 90 °C for 16 hours. The mixture was cooled to room temperature, diluted with ethyl acetate (20 mL) and filtered through a pad of Celite\textsuperscript{®}. The filtrate was concentrated and purified by flash column chromatography using ethyl acetate, hexane (1:24) as an eluent to obtain the desired compound (180 mg, 79%) as a white solid, mp:
203.8-204.9 °C; Rf: 0.40 (1:9 ethyl acetate, hexane); IR (ν_{max} (film)): 3362, 2968, 1676, 1590, 1365, 1068, 851 cm^{-1}; \textbf{^1H NMR} (300 MHz, CDCl3): δ 1.34 (9H, s), 1.41 (12H, s), 7.57 (1H, d, J = 8.8 Hz), 7.75 (2H, d, J = 8.0 Hz), 7.83 (1H, s), 8.14 (2H, d, J = 8.0 Hz), 8.64 (1H, d, J = 8.8 Hz) ppm; \textbf{^{13}C NMR} (75 MHz, CDCl3): δ 25.2, 31.1, 34.6, 84.7, 119.2, 123.9 (q, J = 270.1 Hz), 125.7 (q, J = 3.9 Hz), 127.8, 130.4, 133.1, 133.3 (q, J = 32.0 Hz), 139.0, 142.4, 146.4, 163.7, (one carbon signal was not observed) ppm; \textbf{^{19}F NMR} (282 MHz, CDCl3): δ −62.9 ppm; LRMS (-ESI) m/z: 446.1 ([M-H]^{-} 100%); HRMS (ESI)^+ Calcd for C_{24}H_{29}BF_{3}NO_{3} [M+Na]^+: 470.20848, found 470.20856.

4-(Bromomethylene)tetrahydro-2H-pyran 150

\[
\text{\includegraphics[width=0.5\textwidth]{image.png}}
\]

To a solution of triphenylphosphine (3.3 g, 12.6 mmol) in toluene (10 mL) was added methylene bromide (1.1 mL, 16.4 mmol), dropwise and the resulting solution was heated to reflux and stirred for 16 hours. The precipitate that formed upon cooling was filtered, washed with toluene and dried in vacuo to obtain the phosphonium salt intermediate (4.5 g, 82%) as a white solid, which was used immediately without further purification or characterisation.

To a suspension of the phosphonium salt in tetrahydrofuran (50 mL) at -78 °C was added potassium tert-butoxide (1.3 g, 11.9 mmol). The mixture was stirred for 1.5 hours before tetrahydro-4H-pyran-4-one (1.0 g, 10.3 mmol) in tetrahydrofuran (3 mL) was added slowly. The mixture was warmed to -40 °C over 1 hour and then stirred at room temperature for a further 2 hours. Water was added (50 mL) and the mixture was extracted with diethyl ether (3 x 50 mL). The combined organic extracts were concentrated under reduced pressure and the resulting suspension filtered and washed with diethyl ether (10 mL). The filtrate was concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (3:97) as an eluent to obtain the title compound (670 mg, 37%) as a colourless oil, Rf: 0.46 (1:19 ethyl acetate, hexane); IR (ν_{max} (film)): 2960, 2847, 1234.1, 1149, 1097, 996, 790, 778 cm^{-1}; \textbf{^1H NMR} (300 MHz, CDCl3): δ 2.20-2.52 (4H, m), 3.58-3.78 (4H, m), 5.96 (1H, s) ppm; \textbf{^{13}C NMR} (75 MHz, CDCl3): δ 32.0, 35.9, 68.0, 68.7, 99.7, 140.2 ppm.
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*N-(4-(tert-Butyl)-2-((dihydro-2H-pyran-4(3H)-ylidene)methyl)phenyl)-4-(trifluoromethyl)benzamide* 151

![Structure of N-(4-(tert-Butyl)-2-((dihydro-2H-pyran-4(3H)-ylidene)methyl)phenyl)-4-(trifluoromethyl)benzamide](image)

The boronate 149 (150 mg, 340 μmol), vinyl bromide 150 (60 mg, 340 μmol), potassium carbonate (93 mg, 680 μmol) and tetrakis(triphenylphosphine)palladium(0) (39 mg, 34 μmol) were dissolved in a mixture of degassed N,N-dimethylformamide and water (10:1, 5 mL) and the mixture was stirred at 80 °C for 4 hours. The reaction mixture was cooled to room temperature, diluted with water (10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were washed with water (3 x 15 mL) and aqueous lithium chloride (1 M, 15 mL), dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (130 mg, 92%) as a white solid, mp: 98.7-99.9 °C; *Rf*: 0.24 (1:9 ethyl acetate, hexane); **IR** (*v*<sub>max</sub> (film)): 3394, 2961, 1667, 1325, 1164, 1126, 1064 cm<sup>−1</sup>; **1H NMR** (300 MHz, CDCl<sub>3</sub>): δ 1.32 (9H, s), 2.25 (2H, t, *J* = 5.5 Hz), 2.47 (2H, t, *J* = 5.2 Hz), 3.61 (2H, t, *J* = 5.5 Hz), 3.79 (2H, t, *J* = 5.4 Hz), 6.3 (1H, s), 7.16 (1H, d, *J* = 2.3 Hz), 7.36 (1H, dd, *J* = 8.6, 2.4 Hz), 7.76 (2H, d, *J* = 8.1 Hz), 7.85-8.03 (3H, m), 8.21 (1H, d, *J* = 8.6 Hz) ppm; **13C NMR** (75 MHz, CDCl<sub>3</sub>): δ 31.3, 31.5, 34.6, 37.0, 68.8, 69.7, 119.2, 121.0, 124.2 (q, *J* = 272.0 Hz), 125.2, 126.1 (q, *J* = 3.8 Hz), 126.9, 127.5, 132.7, 133.7 (q, *J* = 34.0 Hz), 138.5, 142.8, 147.7, 163.8, (one carbon signal was not observed) ppm; **19F NMR** (282 MHz, CDCl<sub>3</sub>): δ −63.0 ppm; **LRMS** (-ESI) *m/z*: 416.1 ([M-H]<sup>−</sup> 100%); **HRMS** (ESI)<sup>+</sup> Cald for C<sub>24</sub>H<sub>26</sub>F<sub>3</sub>NO<sub>2</sub> [M+Na]<sup>+</sup>: 440.18078, found 440.18090.

*N-(4-(tert-Butyl)-2-((tetrahydro-2H-pyran-4-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide* 116

![Structure of N-(4-(tert-Butyl)-2-((tetrahydro-2H-pyran-4-yl)methyl)phenyl)-4-(trifluoromethyl)benzamide](image)

Prepared according to General Procedure H using alkene 151 (90 mg, 220 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (77 mg, 86%) as a white solid, mp: 139.2-139.9 °C; *Rf*: 0.36 (1:4 ethyl acetate, hexane); **IR** (*v*<sub>max</sub> (neat)): 3291, 2951, 1650, 1323,
1165, 1064 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 1.33 (9H, s), 1.31-1.46 (2H, m), 1.51-1.63 (2H, m), 1.66-1.84 (1H, m), 2.58 (2H, d, \(J = 7.1\) Hz), 3.31 (2H, td, \(J = 11.8, 3.1\) Hz), 3.86-4.03 (2H, m), 7.20 (1H, d, \(J = 2.3\) Hz), 7.31 (1H, dd, \(J = 8.4, 2.3\) Hz), 7.64 (1H, bs), 7.66-7.74 (1H, m), 7.77 (2H, d, \(J = 8.1\) Hz), 7.97 (2H, d, \(J = 8.1\) Hz) ppm; \(^1\)C NMR (75 MHz, CDCl\(_3\)): δ 31.5, 33.3, 34.6, 36.7, 39.6, 68.1, 123.8 (q, \(J = 273.2\) Hz), 124.4, 124.7, 126.1 (q, \(J = 3.1\) Hz), 127.6, 127.9, 132.2, 132.4, 133.8 (q, \(J = 33.6\) Hz), 138.4, 139.4, 164.8 ppm; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)): δ −63.0 ppm; LRMS (+ESI) \(m/z\): 442.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{24}\)H\(_{28}\)F\(_3\)NO\(_2\) [M+Na]\(^+\): 442.19643, found 442.19703; HPLC: 99.6%, RT: 46.5 mins. (Method B, 254 nm).

**tert-Butyl (5-(tert-butyl)pyridin-2-yl)carbamate 152**

To a solution of copper cyanide (4.9 g, 55.2 mmol) in anhydrous tetrahydrofuran (150 mL) at -78 °C was added tert-butylmagnesium chloride (freshly prepared from tert-butyl chloride and magnesium turnings in tetrahydrofuran, 0.69 M, 160 mL, 110 mmol), slowly and the mixture was allowed to stir for 30 minutes. A solution of 2-(Boc-amino)-5-bromopyridine (3.8 g, 13.8 mmol) in tetrahydrofuran (15 mL) was added and the mixture stirred at -78 °C for 1 hour and then at room temperature for a further 16 hours. The reaction mixture was quenched with aqueous ammonia (28% w/v, 30 mL) and the resulting solid removed by filtration. The solid was washed with ethyl acetate (3 x 50 mL) and the combined organic components were washed with saturated aqueous ammonium chloride (2 x 100 mL) and water (100 mL) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the crude product purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound (660 mg, 19%) as a white solid, mp: 160.5-163.3 °C; \(R_f\): 0.15 (1:19 ethyl acetate, hexane); IR (\(v_{max}\) (film)): 2962, 2871, 1716, 1528, 1269, 1154, 1055, 773 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 1.31 (9H, s), 1.53 (9H, s), 7.62-7.71 (1H, m), 7.86 (1H, d, \(J = 8.8\) Hz), 8.23-8.35 (1H, m), 8.62 (1H, s) ppm; \(^1\)C NMR (75 MHz, CDCl\(_3\)): δ 28.6, 31.3, 33.3, 80.7, 112.1, 135.7, 140.8, 144.7, 150.2, 153.0 ppm; LRMS (+ESI) \(m/z\): 273.1 ([M+Na]\(^+\) 100%), 523.2 ([2M+Na]\(^+\) 58%).
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5-(tert-Butyl)pyridin-2-amine 153

To a solution of Boc protected aminopyridine 152 (250 mg, 1.0 mmol) in anhydrous dichloromethane (2 mL) was added trifluoroacetic acid (1 mL) at 0 °C and the mixture warmed to room temperature and stirred for 2 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in water (10 mL) and neutralised by the addition of saturated aqueous sodium hydrogen carbonate (15 mL). The mixture was extracted with ethyl acetate (3 x 15 mL), washed with brine (15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (150 mg, 100%) as an off-white solid, mp: 128.0-130.2 °C; \( R_f \): 0.15 (2:3 ethyl acetate, hexane); IR \( (\nu_{\text{max}} \text{ (neat)}) \): 3445, 3304, 3141, 2952, 1644, 1506, 1392, 1267, 829 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 1.27 (9H, s), 4.27 (2H, bs), 6.45 (1H, dd, \( J = 8.5, 0.5 \) Hz), 7.45 (1H, dd, \( J = 8.6, 2.6 \) Hz), 8.04-8.11 (1H, m) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 31.3, 32.9, 108.3, 135.5, 136.4, 144.9, 156.4 ppm; LRMS (+ESI) \( m/z \): 151.1 ([M+H]\(^+\) 100%).

3-Bromo-5-(tert-butyl)pyridin-2-amine 154

Bromine (49 μL, 950 μmol) was added to a solution of 153 (130 mg, 870 μmol) and sodium acetate (130 mg, 1.57 mmol) in acetic acid (1 mL) at room temperature. The mixture was stirred for 3 hours before it was diluted with water (10 mL) and aqueous sodium sulfite (1 M, 10 mL) and basified to pH 9 with saturated aqueous ammonia. The aqueous mixture was extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (140 mg, 69%) as a brown oil, \( R_f \): 0.50 (3:7 ethyl acetate, hexane); IR \( (\nu_{\text{max}} \text{ (neat)}) \): 3471, 3304, 3136, 2952, 1635, 1482, 1245, 1040, 751 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 1.28 (9H, s), 4.79 (2H, bs), 7.66 (1H, d, \( J = 2.2 \) Hz), 8.03 (1H, d, \( J = 2.2 \) Hz) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 31.3, 33.1, 104.5, 138.1, 138.4, 144.0, 153.5 ppm; LRMS (+ESI) \( m/z \): 229.0/231.0 ([M+H]\(^+\) 100%).
2-((Dihydro-2H-pyran-4(3H)-ylidene)methyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 155

To a solution of 150 (100 mg, 560 μmol) in anhydrous tetrahydrofuran (5 mL) at -98 °C was added tert-butyllithium (1.2 M in pentane, 900 μL, 1.1 mmol) dropwise. The reaction mixture was stirred for 15 minutes before isopropyl pinacol borate (160 μL, 780 μmol) was added and the mixture stirred for 1 hour then warmed to room temperature and stirred for a further 2.5 hours. Saturated aqueous ammonium chloride (10 mL) was added and mixture extracted with diethyl ether (3 x 15 mL), washed with saturated aqueous sodium chloride (10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (81 mg, 63%) as a colourless gummy mass, Rf: 0.25 (1:19 ethyl acetate, hexane); IR (νmax (neat)): 2962, 2843, 1643, 1138, 1097, 854 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.25 (12H, s), 2.26-2.36 (2H, m), 2.62-2.72 (2H, m), 3.65-3.76 (4H, m), 5.11 (1H, s) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 25.0, 34.3, 40.0, 69.4, 69.5, 82.9, 160.8 (one carbon signal was not observed) ppm; LRMS (+ESI) m/z: 247.1 ([M+Na]⁺ 100%).

5-((tert-Butyl)-3-((dihydro-2H-pyran-4(3H)-ylidene)methyl)pyridin-2-amine 156

To a mixture of the boronate 155 (49 mg, 220 μmol) in degassed tetrahydrofuran (200 μL) and aqueous potassium hydroxide (3 M, 72 μL) was added, dropwise, a solution of aryl bromide 154 (30 mg, 130 μmol) in tetrahydrofuran (200 μL) followed by [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (7.6 mg, 10 μmol) and the resulting mixture stirred at 60 °C for 16 hours. The mixture was diluted with water (15 mL), extracted with ethyl acetate (3 x 10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate as an eluent to obtain the title compound (22 mg, 69%) as a light brown solid, mp: 136.1-137.9 °C; Rf: 0.30 (ethyl acetate); IR (νmax (neat)): 3457,
3289, 3140, 2957, 2828, 1637, 1465, 1093, 996 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.28 (9H, s), 2.33 (2H, t, J = 5.5 Hz), 2.42 (2H, t, J = 5.5 Hz), 3.66 (2H, t, J = 5.5 Hz), 3.79 (2H, t, J = 5.5 Hz), 6.05 (1H, s), 7.23 (1H, s), 7.98 (1H, s) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 31.1, 31.3, 32.9, 37.1, 68.9, 69.6, 116.7, 118.7, 135.7, 136.4, 141.2, 143.2, 154.3 ppm; LRMS (+ESI) m/z: 247.2 ([M+H]⁺ 100%); HRMS (ESI)⁺ Cald for C₁₃H₂₂N₂O [M+H]⁺: 247.18049, found 247.18064.

N-(5-(tert-Butyl)-3-((dihydro-2H-pyran-4(3H)-ylidene)methyl)pyridin-2-yl)-4-(trifluoromethyl)benzamide 157

Aminopyridine 156 (21 mg, 85 μmol) was dissolved in a biphasic mixture of dichloromethane and aqueous sodium hydroxide (1 M) (2:1, 1.5 mL). To this was added 4-trifluoromethyl benzyol chloride (25 μL, 170 μmol) in dichloromethane (0.5 mL), dropwise, at 0 °C. The mixture was stirred at room temperature for 2 hours before it was diluted with tetrahydrofuran (5 mL) and treated with tetra-n-butylammonium bromide (2.7 mg, 8.5 μmol) and stirred for 16 hours. The reaction mixture was diluted with water (10 mL), extracted with dichloromethane (3 x 10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (31 mg, 87%) as a white solid, mp: 161.4-162.7 °C; Rf: 0.31 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 3230, 2954, 1677, 1488, 1325, 1127, 1064 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.34 (9H, s), 2.29 (2H, t, J = 5.5 Hz), 2.38 (2H, t, J = 5.5 Hz), 3.65 (2H, t, J = 5.5 Hz), 3.72 (2H, t, J = 5.5 Hz), 6.27 (1H, s), 7.56 (1H, s), 7.72 (2H, d, J = 8.0 Hz), 8.02 (2H, d, J = 8.0 Hz), 8.28 (1H, s), 8.66 (1H, bs) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 31.1, 31.2, 33.6, 37.1, 68.7, 69.6, 119.3, 123.8 (q, J = 270.6 Hz), 125.9 (q, J = 3.1 Hz), 128.2, 133.8 (q, J = 33.8 Hz), 137.1, 140.9 (four carbon signals were not observed due to peak broadening) ppm; ¹⁹F NMR (282 MHz, CDCl₃): δ −63.0 ppm; LRMS (+ESI) m/z: 441.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Cald for C₂₃H₂₃F₃N₂O₂ [M+Na]⁺: 441.17603, found 441.17617.
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**N-(5-(tert-Butyl)-3-((tetrahydro-2H-pyran-4-yl)methyl)pyridin-2-yl)-4-(trifluoromethyl)benzamide 116**

![Chemical structure](image)

Prepared according to General Procedure H using 157 (30 mg, 72 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to afford the title compound (27 mg, 91%) as a colourless gummy mass, \( R_f \): 0.27 (3:7 ethyl acetate, hexane); **IR** (\( \nu_{\text{max}} \) (neat)): 3228, 2957, 1676, 1643, 1325, 1128, 1095, 854 cm\(^{-1}\); **\(^1\)H NMR** (300 MHz, CDCl\(_3\)): \( \delta \) 1.35 (9H, s), 1.21-1.43 (2H, m), 1.45-1.60 (2H, m), 1.76-2.01 (1H, m), 2.70 (2H, d, \( J = 7.0 \) Hz), 3.32 (2H, td, \( J = 11.7, 2.1 \) Hz), 3.82-4.02 (2H, m), 7.62 (1H, d, \( J = 2.3 \) Hz), 7.72 (2H, d, \( J = 8.0 \) Hz), 7.94-8.27 (3H, m), 8.66 (1H, bs) ppm; **\(^13\)C NMR** (75 MHz, CDCl\(_3\)): 31.1, 33.2, 33.5, 35.7, 39.2, 68.1, 122.1, 125.7, 128.4, 138.1 (aromatic peaks not well resolved due to broadening even after long acquisition) ppm; **\(^19\)F NMR** (282 MHz, CDCl\(_3\)): \( \delta \) -62.9 ppm; **LRMS** (+ESI) \( m/z \): 421.1 ([M+H]\(^+\) 29%), 443.2 ([M+Na]\(^+\) 100%); **HRMS** (ESI)\(^+\) Cald for C\(_{23}\)H\(_{27}\)F\(_3\)N\(_2\)O\(_2\) [M+H]\(^+\): 421.20974, found 421.20974; **HPLC**: 99.1%, RT: 31.4 mins (Method B, 254 nm).

**\( (R)\)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)benzamide 118**

![Chemical structure](image)

Prepared according to General Procedure E using amide 90 (50 mg, 120 μmol) and diol 93 at reflux for 4 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 1:1) as an eluent to obtain the title compound (35 mg, 61%) as a colourless foam, \( R_f \): 0.19 (1:1 ethyl acetate, hexane); **IR** (\( \nu_{\text{max}} \) (neat)): 3301, 2964, 1670, 1553, 1332, 1273, 1119, 1092 cm\(^{-1}\); **\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \( \delta \) 1.31 (9H, s), 1.36 (3H, s), 1.38 (3H, s), 1.58-1.65 (1H, m), 1.76-1.88 (2H, m), 1.97-2.08 (1H, m), 3.56-3.75 (2H, m), 4.02 (1H, d, \( J = 9.4 \) Hz), 4.10-4.23 (3H, m, H6), 4.25-4.33 (1H, m), 4.40 (1H, dd, \( J = 15.2, 2.6 \) Hz), 6.56 (1H, s), 7.13 (1H, d, \( J = 8.7 \) Hz), 7.72 (1H, d, 8.7 Hz), 8.30 (1H, s), 9.97 (1H, s) ppm; **\(^13\)C NMR** (100 MHz, CDCl\(_3\)): \( \delta \) 25.7, 26.4, 26.5, 28.0, 30.6, 32.4, 52.4,
Chapter 8: Experimental

68.7, 69.8, 79.0, 79.4, 95.7, 114.1, 123.7, 123.9 (q, \(J = 272.1\) Hz), 124.2 (q, \(J = 32.7\) Hz), 129.5 (q, \(J = 3.5\) Hz), 130.1 (q, \(J = 3.5\) Hz), 136.7, 159.9, 161.1, 162.1 ppm; \(^{19}\)F NMR (376 MHz, DMSO-\(d_6\)): \(\delta -62.0\) ppm; LRMS (+ESI) \(m/z\): 506.2 ([M+Na]\(^+\) 100%); HRMS (ESI\(^+\) Calcd for C\(_{24}\)H\(_{32}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 506.22426, found 506.22332; \([\alpha]_D\)\(^{22}\): -70.3 ° (0.64, CHCl\(_3\)); HPLC: 98.1%, RT: 25.4 mins. (Method A, 254 nm).

\(N\)-(3-(tert-butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazol-5-yl)-2-fluoro-5-(trifluoromethyl)benzamide 158

\[
\begin{align*}
\text{O} & \\
\text{F} & \\
\text{N} & \\
\text{O} & \\
\text{F} & \\
\text{C} & \\
\text{N} & \\
\text{H} & \\
\text{O} & \\
\end{align*}
\]

Prepared according to General Procedure C using aminopyrazole 129 (190 mg, 800 \(\mu\)mol) and 2-fluoro-5-(trifluoromethyl)benzoic acid (170 mg, 800 \(\mu\)mol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 2:3) as an eluent to obtain the title compound (290 mg, 85%) as a colourless oil, \(R_f\): 0.38 (2:3 ethyl acetate, hexane); IR (\(\nu_{\text{max}}\) (neat)): 2958, 2924, 1673, 1556, 1332, 1272, 1127 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 1.21-1.41\) (2H, m), \(1.30\) (9H, s), \(1.48-1.56\) (2H, m), 2.10-2.26 (1H, m), 3.35 (2H, td, \(J = 11.8, 2.1\) Hz), 3.88 (2H, d, \(J = 7.3\) Hz), 3.95 (2H, dd, \(J = 11.7, 4.5, 1.7\) Hz), 6.43 (1H, s), 7.37 (1H, dd, \(J = 11.5, 8.7\) Hz), 7.84 (1H, ddd, \(J = 7.9, 4.5, 2.5\) Hz), 8.25 (1H, d, \(J = 15.4\) Hz), 8.49 (1H, dd, \(J = 7.1, 2.4\) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta 30.5, 30.7, 32.4, 36.2, 53.9, 67.6, 96.4, 117.5\) (d, \(J = 26.7\) Hz), 120.9 (d, \(J = 12.6\) Hz), 123.2 (q, \(J = 272.3\) Hz), 128.6 (q, \(J = 30.1\) Hz), 130.5-130.8 (m), 131.4-131.7 (m), 134.4, 159.3 (d, \(J = 2.8\) Hz), 160.9, 162.0 (d, \(J = 255.2\) Hz) ppm; \(^{19}\)F NMR (376 MHz, DMSO-\(d_6\)): \(\delta -62.5, -108.8\) ppm; LRMS (+ESI) \(m/z\): 450.2 ([M+Na]\(^+\) 100%); HRMS (ESI\(^+\) Calcd for C\(_{21}\)H\(_{25}\)F\(_4\)N\(_3\)O\(_2\) [M+Na]\(^+\): 450.17806, found 450.17720.
N-(3-(tert-butyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrazol-5-yl)-2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)benzamide 158

Prepared according to General Procedure E using 161 (100 mg, 230 μmol) and diol 93 (32 mg, 350 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:1 to 3:2) as a eluent to obtain the title compound (93 mg, 81%) as a colourless gummy mass, Rf: 0.17 (3:2 ethyl acetate, hexane); IR (v max (neat)): 3427, 3267, 2956, 1681, 1561, 1363, 1314, 1166 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.22-1.31 (2H, m), 1.28 (9H, s), 1.40 (6H, s), 1.40-1.45 (2H, m), 2.06-2.22 (1H, m), 3.28 (2H, td, J = 11.8, 2.1 Hz), 3.85 (2H, ddd, J = 11.6, 4.6, 1.8 Hz), 3.92 (2H, d, J = 7.3 Hz), 4.08 (2H, s), 6.16 (1H, s), 7.12 (1H, d, J = 8.6 Hz), 7.74 (1H, dd, J = 8.8, 2.5 Hz), 8.25 (1H, d, J = 15.4 Hz), 8.51 (1H, d, J = 2.4 Hz), 9.84 (1 H, s) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 27.2, 30.6, 30.7, 32.4, 36.0, 53.7, 67.7, 70.3, 77.8, 96.9, 113.8, 121.9, 123.8 (q, J = 272.3 Hz), 124.7 (q, J = 32.5 Hz), 130.6 (q, J = 3.5 Hz), 130.7 (q, J = 3.5 Hz), 135.7, 159.5, 161.1, 162.7 ppm; ¹⁹F NMR (376 MHz, DMSO-d₆): δ −62.0, -108.8 ppm; LRMS (+ESI) m/z: 520.2 ([M+Na]+ 100%); HRMS (ESI)⁺ Cald for C₂₅H₃₄F₃N₃O₄ [M+Na]⁺: 520.23991, found 520.24021; HPLC: 95.4%, RT: 26.4 mins. (Method A, 254 nm).

(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-3-(trifluoromethyl)benzamide 160

Prepared according to General Procedure C using 3-(trifluoromethyl)benzoic acid (100 mg, 530 μmol) and amine 89. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (160 mg, 78%) as a colourless oil, Rf: 0.48 (2:3 ethyl acetate, hexane); IR (v max (neat)): 3290, 2960, 1667, 1555, 1331, 1125, 1049 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.32 (9H, s), 1.59-1.72 (1H, m), 1.80-2.02 (2H, m), 2.06-2.18 (1H, m), 3.82-4.00 (2H, m), 4.17 (1H, dd, J = 15.4, 6.2 Hz), 4.29 (1H, q, J =7.2 Hz), 4.50 (1H, d, J = 15.4 Hz), 6.60 (1H, s), 7.65 (1H, t, J = 7.8 Hz).
Hz), 7.82 (1H, d, J = 7.8 Hz), 8.11-8.25 (2H, m), 10.45 (1H, s) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 25.9, 28.2, 30.6, 32.4, 53.4, 68.9, 80.4, 94.5, 123.9 (q, J = 3.8 Hz), 128.7 (q, J = 3.7 Hz), 129.7, 131.1, 131.4 (q, J = 32.7 Hz), 134.9, 137.1, 161.2, 162.3 (two carbon signals were not observed) ppm; $^{19}$F NMR (282 MHz, DMSO-d$_6$): δ −60.7 ppm; LRMS (-ESI) m/z: 394.3 ([M-H] $-$ 100%); HRMS (ESI)$^+$: Calcd for C$_{20}$H$_{24}$F$_3$N$_3$O$_2$ [M-H]: 394.17424, found 394.17498; [α]$_D$ $^{22}$: -44.6 ° (1.01, CHCl$_3$); HPLC: 96.3%, RT: 26.6 mins. (Method A, 254 nm).

(R)-N-(5-(tert-Butyl)-1-methyl-2-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-3(2H)-ylidene)-3-(trifluoromethyl)benzamide 159

To a solution of the amide 160 (50 mg, 130 μmol) in anhydrous toluene (3 mL) was added methyl trifluoromethanesulfonate (21 μL, 190 μmol) and the mixture was stirred at reflux for 16 hours. The reaction mixture was cooled and water (100 μL) and acetone (200 μL) were added and the mixture stirred for 30 minutes before aqueous saturated ammonia (100 μL) was added and the mixture stirred for additional 30 minutes. Brine (20 mL) was added and the aqueous mixture extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using methanol, ethyl acetate and hexane (0:1:1 to 1:9:0) as an eluent to obtain the title compound (230 mg, 72%) as an off-white waxy solid, $R_f$: 0.33 (1:19 methanol, dichloromethane); IR (v$_{max}$ (neat)): 2975, 1529, 1320, 1238, 1162, 1121, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 1.46 (9H, s), 1.68-2.00 (3H, m), 2.09-2.22 (1H, m), 3.73-3.88 (2H, m), 3.99 (3H, s), 4.26 (1H, qd, J = 6.8, 2.7 Hz), 4.46 (1H, dd, J = 15.7, 6.4 Hz), 4.74 (1H, dd, J = 15.6, 2.7 Hz), 7.05 (1H, s), 7.56 (1H, t, J = 7.8 Hz), 7.71 (1H, d, J = 7.8 Hz), 8.36 (1H, d, J = 7.8 Hz), 8.45 (1H, s) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 25.7, 28.9, 29.3, 32.5, 35.8, 48.5, 68.8, 78.0, 98.1, 124.3 (q, J = 271.1 Hz), 125.7 (q, J = 3.9 Hz), 127.6 (q, J = 3.8 Hz), 128.8, 130.6 (q, J = 32.9 Hz), 131.9, 138.4, 158.3, 168.8, 169.2 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): δ −62.6 ppm; LRMS (+ESI) m/z: 410.2 ([M+H]$^+$ 100%); HRMS (ESI)$^+$: Calcd for C$_{21}$H$_{26}$F$_3$N$_3$O$_2$ [M+Na]$^+$: 410.20554, found 410.20489; [α]$_D$ $^{22}$: -47.2 ° (0.53, CHCl$_3$); HPLC: 98.1%, RT: 20.3 mins. (Method A, 254 nm).
2,2-Dimethylpentan-3-one 163

![Structure](image)

The ketone 163 was prepared according to the procedures reported by Whitmore and coworkers\(^{321}\) using trimethylacetamide (10 g, 99 mmol) and freshly prepared ethyl magnesium bromide (4 eq.). The crude residue was purified by fractional distillation (125-128 °C, 760 mmHg) to obtain the title compound (7.5 g, 66%) as a colourless liquid, the characterisation data of which corresponded to that previously described.\(^{322}\)

4-Bromo-2,2-dimethylpentan-3-one 164

![Structure](image)

To a suspension of copper(II) bromide (7.8 g, 35 mmol) in ethyl acetate (20 mL) at reflux was added a solution of 163 (2.0 g, 17.5 mmol) in chloroform (20 mL) and the mixture was heated for 16 hours. The reaction mixture was cooled to room temperature and filtered through a pad of Celite\(^{®}\) and the filtrate concentrated under reduced pressure to yield the title compound (2.5 g, 74%) as a dark yellow oil, the characterisation data of which corresponded to that previously described.\(^{323}\)

N-(4-(tert-Butyl)-5-methyl-1H-imidazol-2-yl)acetamide 165

![Structure](image)

To a solution of 164 (1.5 g, 7.8 mmol) in anhydrous dimethylformamide (25 mL) was added acetyl guanidine (2.4 g, 23 mmol) and the mixture was stirred at 80 °C for 16 hours. The solvent was removed under a stream of nitrogen and the residue purified by flash column chromatography using methanol, dichloromethane (1:99 to 1:49) as an eluent to obtain the title compound (0.4 g, 26%) as a light yellow solid, \(R_f\): 0.30 (1:19 methanol, dichloromethane); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 1.23 (9H, s), 1.99 (3H, s), 2.19 (3H, s), 10.87 (2H, bs) ppm; LRMS (+ESI) \(m/z\): 218.3 ([M+Na]\(^+\) 100%).
8.5.3 Synthetic Procedures from Chapter 4

2-(Benzyloxy)-2-methylpropan-1-ol 187

![Chemical Structure](image)

To a solution of ester 92 (3.4 g, 25.7 mmol) in anhydrous N,N-dimethylformamide (50 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 1.2 g, 30.9 mmol) followed by benzyl bromide (3.7 mL, 30.9 mmol) and the mixture was stirred at room temperature for 16 hours. Saturated aqueous ammonium chloride was added (100 mL) and the mixture extracted with ethyl acetate (3 x 60 mL). The combined organic extracts were washed with water (3 x 50 mL) and aqueous lithium chloride (1 M, 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the benzyl protected alcohol 185 (5.0 g, 88%) as a colourless oil, which was taken on to the next step without characterisation.

The intermediate ester 185 (4.8 g, 21.6 mmol) was reduced according to General Procedure D at room temperature for 2 hours to obtain the title compound (3.7 g, 90%) as a colourless oil, the characterisation data of which corresponded to that previously described.\(^{324}\) \(R_f\): 0.20 (1:9 ethyl acetate, hexane); \(\text{IR} (\nu_{\text{max}} \text{ (neat)})\): 3419, 2971, 1364, 1155, 1054, 733 cm\(^{-1}\); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 21.1, 64.1, 70.1, 75.9, 127.6, 128.5, 128.7, 139.3 ppm; LRMS (+ESI) \(m/z\): 203.1 ([M+Na]\(^+\) 100%).

2-((4-Methoxybenzyl)oxy)-2-methylpropan-1-ol 188

![Chemical Structure](image)

To a solution of sodium hydride (60% dispersion in mineral oil, 430 mg, 18.1 mmol) in N,N-dimethylformamide (50 mL) at 0 °C was added 92 (2.0 g, 15.1 mmol) followed by 4-methoxybenzyl chloride (2.5 mL, 18.1 mmol). The reaction mixture was warmed to room temperature and stirred for 16 hours. Saturated aqueous ammonium chloride (100 mL) was added and the mixture extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane
(1:19) as an eluent to obtain the protected ester (2.2 g, 58%) as a colourless oil, which was used immediately without characterisation.

The intermediate ester (186) (2.0 g, 7.9 mmol) was reduced according to General Procedure D at room temperature for 2 hours to obtain the title compound (1.7 g, 100%) as a colourless liquid, the characterisation data of which corresponded to that previously described. \( R_f \): 0.07 (1:9 ethyl acetate, hexane); IR \((\nu_{\text{max}} \text{ (neat)})\): 3425, 2970, 1613, 1512, 1301, 1155, 1032, 820 cm\(^{-1}\); \( ^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 22.2, 55.4, 63.9, 69.9, 75.7, 114.0, 129.1, 131.4, 159.2 ppm; LRMS (+ESI) \(m/z\): 233.1 ([M+Na]\(^+\) 100%), 443.2 ([2M+Na]\(^+\) 100%).

\((R)-2-(2-(Benzyloxy)-2-methylpropoxy)-N-(3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-5-(trifluoromethyl)benzamide 189\)

Prepared according to General Procedure E using 90 (600 mg, 1.5 mmol) and 187 at reflux for 3 hours. The crude residue was purified by flash column chromatography using ethyl acetate, dichloromethane (0:1 to 1:9) as an eluent to obtain the title compound (750 mg, 90%) as a colourless oil, \( R_f \): 0.10 (1:4 hexane, dichloromethane); IR \((\nu_{\text{max}} \text{ (neat)})\): 2961, 1680, 1572, 1327, 1233, 1117, 1030 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.31 (9H, s), 1.42 (3H, s), 1.44 (3H, s), 1.60-1.78 (3H, m), 1.81-1.93 (1H, m), 3.51-3.65 (2H, m), 3.95-4.09 (1H, m), 4.13-4.28 (4H, m), 4.48 (2H, s), 6.37 (1H, s), 7.13-7.25 (6H, m), 7.76 (1H, dd, \(J = 8.6, 2.5\) Hz), 8.32 (1H, d, \(J = 2.4\) Hz), 9.91 (1H, s) ppm; \( ^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 22.9, 23.0, 25.7, 28.3, 30.6, 32.3, 52.4, 64.7, 68.5, 75.2, 76.3, 78.7, 95.9, 113.9, 123.7 (q, \(J = 37.7\) Hz), 124.0 (q, \(J = 272.4\) Hz), 127.4, 127.6, 127.6, 128.5, 129.9 (q, \(J = 3.9\) Hz), 129.9 (q, \(J = 3.5\) Hz), 136.5, 138.7, 159.4, 160.7, 162.2 ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) -61.6 ppm; LRMS (+ESI) \(m/z\): 596.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{31}\)H\(_{38}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 596.27121, found 596.27033.
(R)-2-(2-(Benzyloxy)-2-methylpropoxy)-N-(3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-N-methyl-5-(trifluoromethyl)benzamide 190

To a solution of 189 (150 mg, 260 μmol) in anhydrous tetrahydrofuran (5 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 13 mg, 310 μmol) and the mixture stirred for 30 minutes before methyl iodide (18 μL, 290 μmol) was added slowly. The reaction mixture was warmed to room temperature and stirred for 6 hours, quenched with saturated aqueous ammonium chloride (15 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (100 mg, 65%) as a colourless oil, \( R_f \): 0.17 (1:4 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 2959, 1670, 1324, 1084 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.06 (9H, s), 1.44 (3H, s), 1.44 (3H, s), 1.75-1.89 (3H, m), 1.92-2.08 (1H, m), 3.39 (3H, s), 3.64-3.86 (3H, m), 3.94 (2H, s), 4.11-4.27 (2H, m), 4.59 (2H, s), 5.92 (1H, s), 6.81 (1H, d, \( J = 8.6 \) Hz), 7.27-7.53 (7H, m) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 23.0, 25.8, 29.3, 30.2, 32.2, 36.9, 52.0, 64.8, 68.2, 75.1, 76.0, 77.4, 99.5, 112.1, 127.3 (q, \( J = 3.8 \) Hz), 127.5, 127.6, 128.3 (q, \( J = 3.9 \) Hz), 128.9, 139.4, 141.0, 157.8, 161.0, 168.1 (three carbon signals were not observed) ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \( \delta \) -61.9 ppm; LRMS (+ESI) \( m/z \): 610.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{32}\)H\(_{40}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 610.28686, found 610.28581.
(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-2-(2-hydroxy-2-methylpropoxy)-N-methyl-5-(trifluoromethyl)benzamide 166

Prepared according to General Procedure J using 190 (65 mg, 110 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (50 mg, 91%) as a colourless oil, \( R_f \): 0.08 (3:7 ethyl acetate, hexane); **IR** (\( \nu_{\text{max}} \) (neat)): 3420, 2962, 1655, 1557, 1324, 1276, 1120, 1083 cm\(^{-1} \); **\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \( \delta \) (rotameric mixture observed, major peaks reported) 1.13 (9H, s), 1.32 (3H, s), 1.35 (3H, s), 1.49-2.04 (4H, m), 3.42 (3H, s), 3.62-3.86 (4H, m), 3.87-4.02 (2H, m), 4.09-4.33 (1H, m), 5.94 (1H, d, \( J = 8.7 \) Hz), 7.21-7.32 (1H, m), 7.43 (1H, dd, \( J = 8.8, 2.2 \) Hz) ppm; **\(^{13}\)C NMR** (100 MHz, CDCl\(_3\)): \( \delta \) 25.7, 26.2, 29.4, 30.2, 32.3, 37.3, 52.3, 68.2, 70.3, 77.7, 79.0, 99.4, 114.0, 122.8 (q, \( J = 33.3 \) Hz), 123.8 (q, \( J = 271.6 \) Hz), 125.6 (q, \( J = 3.3 \) Hz), 126.0, 128.0 (q, \( J = 3.8 \) Hz), 141.0, 158.9, 161.3, 168.0 ppm; **\(^{19}\)F NMR** (376 MHz, CDCl\(_3\)): \( \delta \) −62.1 ppm; **LRMS** (+ESI) \( m/z \): 520.2 ([M+Na]\(^+\) 100%); **HRMS** (ESI)\(^+\) Calcd for C\(_{25}\)H\(_{34}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 520.23991, found 520.23913; [\( \alpha \)]\(_D\): -57.7 ° (0.52, CHCl\(_3\)); **HPLC**: 99.5%, RT: 27.8 mins. (Method A, 230 nm).

Methyl 4,4-dimethyl-3-oxopentanoate 191

To a solution of pinacolone (2.0 g, 20.0 mmol) in anhydrous toluene (20 mL) was added dimethyl carbonate (5.1 mL, 60.0 mmol) followed by sodium hydride (60% dispersion in mineral oil, 2.7 g, 40.0 mmol) and the mixture heated at reflux for 5 hours. After cooling to room temperature, the mixture was poured into ice-water (100 mL), acidified with aqueous hydrochloric acid (1 M) and extracted with ethyl acetate (3 x 50 mL). The combined organic fractions were dried over anhydrous magnesium sulfate and concentrated to obtain the title compound (2.7 g, 87%) as a colourless liquid, \( R_f \): 0.42 (1:9 ethyl acetate, hexane); **IR** (\( \nu_{\text{max}} \) (neat)): 2962, 1747, 1707, 1680, 1551, 1324, 1230, 1167, 1042 cm\(^{-1} \); **\(^1\)H NMR** (300 MHz, CDCl\(_3\)): \( \delta \) 1.14 (9H, s), 3.53 (2H, s), 3.69 (3H, s) ppm; **\(^{13}\)C NMR** (75 MHz, CDCl\(_3\)): \( \delta \) 26.0, 43.7, 44.8, 52.2, 168.3, 207.9 ppm; **LRMS** (+ESI) \( m/z \): 181.1 ([M+Na]\(^+\) 100%).
To a solution of freshly prepared hydrazine hydrochloride 87 (1.2 g, 6.3 mmol) and 191 (1.0 g, 6.3 mmol) in ethanol (20 mL) at 0 °C was added triethylamine (1.8 mL, 12.6 mmol) and the reaction mixture heated at 70 °C for 16 hours. The solvent was removed under reduced pressure and the residue purified by flash column chromatography using methanol, dichloromethane (1:19 to 1:9) as an eluent to obtain the title compound (680 mg, 48%) as an orange solid, mp: 116.9-119.5 °C; R<sub>f</sub>: 0.12 (1:1 ethyl acetate, hexane); IR (v<sub>max</sub> (neat)): 2961, 2602, 1602, 1538, 1366, 1076, 758 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.05 (9H, s), 1.43-1.64 (1H, m), 1.64-1.87 (3H, m), 3.10 (2H, s), 3.51-3.80 (4H, m), 4.02-4.17 (1H, m) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 25.2, 28.0, 34.4, 37.1, 47.7, 60.1, 67.8, 76.1, 165.7, 172.5 ppm; LRMS (+ESI) m/z: 225.2 ([M+H]<sup>+</sup> 18%), 247.2 ([M+Na]<sup>+</sup> 92%), 471.3 ([2M+Na]<sup>+</sup> 100%); HRMS (ESI)<sup>+</sup> Cald for C<sub>12</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> [M+Na]<sup>+</sup>: 247.14225, found 247.14161.

To a solution of 2-fluoro-5-(trifluoromethyl)benzoic acid (250 mg, 1.2 mmol) in anhydrous N,N-dimethylformamide (2 mL) was added a solution of 187 (430 mg, 2.4 mmol) in N,N-dimethylformamide (2 mL) and the mixture was stirred at room temperature for 30 minutes. A solution of 2-fluoro-5-(trifluoromethyl)benzoic acid (250 mg, 1.2 mmol) in N,N-dimethylformamide (2 mL) was added and the mixture was stirred at 80 °C for 2 hours. After cooling to room temperature, the reaction mixture was quenched with water (30 mL) and washed with hexane (3 x 20 mL). The aqueous phase was acidified with aqueous hydrochloride acid (6 M), extracted with ethyl acetate (3 x 30 mL), washed with aqueous hydrochloride acid (1 M, 3 x 20 mL) dried over anhydrous magnesium sulfate and
concentrated under reduced pressure. The crude product was purified by flash column chromatography using acetic acid, ethyl acetate and hexane (1:19:80) as an eluent to obtain the title compound (360 mg, 81%), as a white solid, mp: 77.7-80.2 °C; Rf: 0.13 (1:19:80 acetic acid, ethyl acetate, hexane); IR (v max (neat)): 3037, 2988, 1702, 1680, 1616, 1506, 1210, 1119, 1092, 1054, 826, 770 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.39 (6H, s), 4.05 (2H, s), 4.46 (2H, s), 7.01 (1H, d, J = 8.7 Hz), 7.14-7.31 (5H, m), 7.67 (1H, dd, J = 8.7, 2.5 Hz), 8.31 (1H, d, J = 2.5 Hz) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 22.7, 64.9, 74.7, 76.7, 113.6, 119.0, 123.7 (q, J = 270.9 Hz), 124.5 (q, J = 34.1 Hz), 127.8, 127.9, 128.6, 131.2 (q, J = 3.8 Hz), 131.7 (q, J = 3.6 Hz), 138.3, 160.2, 165.7 ppm; ¹⁹F NMR (282 MHz, CDCl₃): δ −62.2 ppm; LRMS (+ESI) m/z: 391.1 ([M+Na]+ 100%); HRMS (ESI)+ Cald for C₁₉H₁₉F₃O₄[M+Na]+: 391.1133, found 391.11259.

(R)-3-((tert-Butyl)-1-((tetrahydrofuran-2-y1)methyl)-1H-pyrazol-5-yl 2-(2-(benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)benzoate 194

Prepared according to General Procedure C using acid 193 (120 mg, 330 μmol) and 192 (73 mg, 330 μmol) at room temperature for 4 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (110 mg, 58%) as a yellow oil, Rf: 0.59 (3:7 ethyl acetate, hexane); IR (v max (neat)): 2969, 1709, 1618, 1330, 1281, 1120, 1089 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.19 (6H, s), 1.47 (9H, s), 1.59-1.77 (1H, m), 1.84-2.05 (3H, m), 3.52-3.94 (4H, m), 4.15 (2H, s), 4.18-4.32 (1H, m), 4.52 (2H, s), 6.09 (1H, d, J = 8.5 Hz), 7.09 (1H, d, J = 6.0 Hz), 7.25-7.36 (5H, m), 7.75 (1H, dd, J = 8.5, 2.3 Hz), 8.42 (1H, d, J = 2.3 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 22.6, 25.6, 28.4, 29.3, 32.5, 48.2, 65.0, 68.2, 74.6, 76.5, 77.4, 91.1, 113.5, 119.1, 124.9 (q, J = 33.8 Hz), 125.4 (q, J = 271.1 Hz), 127.9, 127.9, 128.4, 128.6, 131.3 (q, J = 3.8 Hz), 131.6 (q, J = 3.7 Hz), 138.1, 164.6, 166.1, 172.9 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ −62.1 ppm; LRMS (+ESI) m/z: 575.3 ([M+H]+ 43%), 597.3 ([M+Na]+ 100%); HRMS (ESI)+ Cald for C₃₁H₃₇F₃N₂O₅ [M+Na]⁺: 597.25523, found 597.25450.
Prepared according to General Procedure J using ester 194 (68 mg, 120 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (52 mg, 92%) as a colourless oil, \( R_f: 0.23 \) (3:7 ethyl acetate, hexane); IR (\( \nu_{max} \) (neat)): 3429, 2962, 1762, 1617, 1460, 1337, 1286, 1203, 1118, 1024 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.31 (9H, s), 1.37 (6H, s), 1.65-1.87 (3H, m), 1.93-2.02 (1H, m), 3.23 (1H, bs), 3.60-3.77 (2H, m), 3.98 (2H, s), 4.07 (1H, dd, \( J = 14.4, 6.6 \) Hz), 4.14 (1H, dd, \( J = 14.4, 4.0 \) Hz), 4.18-4.27 (1H, m), 7.10 (1H, d, \( J = 8.8 \) Hz), 7.80 (1H, dd \( J = 8.8, 2.4 \) Hz), 8.38 (1H, d, \( J = 2.4 \) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 25.6, 26.3, 28.8, 30.5, 32.7, 52.0, 68.4, 70.0, 77.9, 78.1, 91.1, 114.2, 118.2, 123.3 (q, \( J = 33.7 \) Hz), 123.8 (q, \( J = 271.7 \) Hz), 130.1 (q, \( J = 3.9 \) Hz), 132.0 (q, \( J = 3.4 \) Hz), 144.6, 159.4, 160.6, 162.3 ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \( \delta \) -62.0 ppm; LRMS (+ESI) \( m/z: \) 507.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{24}\)H\(_{31}\)F\(_3\)N\(_2\)O\(_5\) [M+Na]\(^+\): 507.20828, found 507.20740; \([\alpha]_D^{22}\): -10.4 \( ^\circ \) (0.48, CHCl\(_3\)); HPLC: 95.2%, RT: 29.6 mins. (Method A, 230 nm).

**Ethyl 5,5-dimethyl-2,4-dioxohexanoate 195**

Metallic sodium (480 mg, 21.0 mmol) was added portionwise to ethanol (12.5 mL) and the mixture stirred until hydrogen evolution had ceased. To this solution was added pinacolone (2.5 mL, 20.0 mmol) and diethyl oxalate (3.0 mL, 22.0 mmol) dropwise and the mixture was heated at reflux for 2 hours. The solvent was removed and the residue taken up in ethyl acetate (100 mL), washed with water (3 x 50 mL), dried over magnesium sulfate and concentrated under reduced pressure to obtain the title compound (3.2 g, 79%) as a colourless liquid, the characterisation data of which corresponded to that previously described.\(^{326}\) \( R_f: 0.53 \) (1:4 ethyl acetate, hexane); IR (\( \nu_{max} \) (neat)): 3294, 2964, 1734, 1681, 1551, 1234 cm\(^{-1}\); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta \) 14.1, 26.8, 41.8, 62.6, 98.0, 162.5, 167.6, 209.3 ppm; LRMS (+ESI) \( m/z: \) 223.1 ([M+Na]\(^+\) 100%).
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*Ethyl 3-(tert-butyl)-1H-pyrazole-5-carboxylate 196*

A solution of 195 (500 mg, 2.5 mmol), hydrazine hydrate (120 μL, 2.5 mmol) and acetic acid (1 drop) in ethanol (10 mL) was heated at reflux for 16 hours. The solvent was removed under reduced pressure and the residue taken up in ethyl acetate (25 mL), washed with water (20 mL) and brine (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (470 mg, 95%) as a white solid, *mp*: 125.3-131.1 °C; *Rf*: 0.50 (3:7 ethyl acetate, hexane); *IR* (*ν*<sub>max</sub> (neat)): 3143, 3080, 2963, 1732, 1720, 1148, 1094, 1010, 777 cm<sup>-1</sup>; *<sup>1</sup>H NMR* (300 MHz, CDCl<sub>3</sub>): δ 1.34 (9H, s), 1.36 (3H, t, *J* = 7.1 Hz), 4.36 (2H, q, *J* = 7.1 Hz), 6.64 (1H, s), 9.14 (1H, bs) ppm; *<sup>13</sup>C NMR* (75 MHz, CDCl<sub>3</sub>): δ 14.4, 30.3, 31.6, 61.1, 104.7, 140.4, 158.0, 161.5 ppm; *LRMS (+ESI) m/z*: 219.1 ([M+Na]<sup>+</sup> 100%), 415.2 ([2M+Na]<sup>+</sup> 63%).

*(R)-Ethyl 3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-carboxylate 197*

Prepared according to General Procedure I using ester 196 (220 mg, 1.1 mmol) and alcohol 85. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (260 mg, 84%) as a colourless oil, *Rf*: 0.62 (1:4 ethyl acetate, hexane); *IR* (*ν*<sub>max</sub> (neat)): 2960, 1719, 1254, 1088, 767 cm<sup>-1</sup>; *<sup>1</sup>H NMR* (300 MHz, CDCl<sub>3</sub>): δ 1.29 (9H, s), 1.36 (3H, t, *J* = 7.0 Hz), 1.60-1.99 (4H, m), 3.62-3.87 (2H, m), 4.20-4.37 (3H, m), 4.43 (1H, dd, *J* = 13.7, 5.1 Hz), 4.71 (1H, dd, *J* = 13.6, 6.7 Hz), 6.65 (1H, s) ppm; *<sup>13</sup>C NMR* (75 MHz, CDCl<sub>3</sub>): δ 14.4, 25.5, 28.9, 30.6, 32.1, 54.5, 60.9, 68.3, 78.1, 107.5, 133.1, 160.3, 160.7 ppm; *LRMS (+ESI) m/z*: 303.2 ([M+Na]<sup>+</sup> 100%).
Prepared according to General Procedure B using ester 197 (150 mg, 540 μmol) and lithium hydroxide at room temperature for 16 hours to obtain the carboxylic acid 198 (130 mg, 100%) as a colourless oil, IR (v<sub>max</sub> (neat)): 2957, 2870, 1715, 1484, 1460, 1230, 1045, 724 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.32 (9H, s), 1.65-2.04 (4H, m), 3.68-3.91 (2H, m), 4.38 (1H, ddd, J = 12.0, 6.8, 5.0 Hz), 4.52 (1H, dd, J = 13.6, 4.9 Hz), 4.76 (1H, dd, J = 13.6, 6.8 Hz), 6.76 (1H, s), 10.34 (1H, s) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 25.5, 28.8, 30.5, 32.1, 54.4, 68.3, 78.1, 108.7, 133.0, 161.0, 163.1 ppm; LRMS (+ESI) m/z: 275.2 ([M+Na]<sup>+</sup>) 100%.

To a solution of 4-fluoro-3-nitrobenzotrifluoride (1.6 mL, 11.6 mmol) in anhydrous N,N-dimethylformamide (80 mL) was added alcohol 187 (2.5 g, 13.9 mmol) and cesium carbonate (11.3 g, 34.8 mmol) and the mixture was stirred at 60 °C for 16 hours. Water (150 mL) was added and the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:24 to 1:19) as an eluent to obtain the title compound (3.1 g, 73%) as an orange oil, R<sub>f</sub>: 0.43 (1:9 ethyl acetate, hexane); IR (v<sub>max</sub> (neat)): 2979, 1626, 1538, 1325, 1290, 1156, 1123, 1092, 1061 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.43 (6H, s), 4.05 (2H, s), 4.56 (2H, s), 7.13 (1H, d, J = 8.3 Hz), 7.16-7.45 (5H, m), 7.70 (1H, d, 8.4 Hz), 8.10 (1H, s) ppm; <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 22.8, 64.7, 74.9, 76.6, 115.1, 122.7 (q, J = 33.5 Hz), 123.2 (q, J = 271.8 Hz), 123.3 (q, J = 3.6 Hz), 127.4, 128.6, 131.0 (q, J = 3.6 Hz), 139.2, 139.3, 154.7 ppm; <sup>19</sup>F NMR (282 MHz, CDCl<sub>3</sub>): δ −62.0 ppm; LRMS (+ESI)
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$m/z$: 392.1 ([M+Na]$^+$ 100%); **HRMS** (ESI)$^+$ Calcd for C$_{18}$H$_{18}$F$_3$NO$_4$ [M+Na]$^+$: 392.10856, found 392.10777.

2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)aniline 200

![Chemical Structure](image)

To a solution of 199 (400 mg, 1.1 mmol) in a mixture of ethanol (2 mL), acetic acid (2 mL) and water (1 mL) was added iron powder (450 mg, 8.1 mmol) and the suspension sonicated for 1 hour. The mixture was filtered to remove the iron and the filtrate diluted with saturated aqueous sodium hydrogen carbonate (20 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with water (20 mL) and saturated aqueous sodium hydrogen carbonate (2 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:19) as an eluent to obtain the aniline 200 (310 mg, 91%) as a yellow oil, **$R_f$:** 0.35 (1:1 dichloromethane, hexane); **IR** ($\nu_{\text{max}}$ (neat)): 3369, 2976, 1620, 1448, 1331, 1218, 1110, 1057, 735 cm$^{-1}$; **$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 1.44 (6H, s), 3.98 (2H, s), 4.55 (2H, s), 6.82 (1H, d, $J = 8.2$ Hz), 6.92 (1H, s), 6.96 (1H, d, $J = 8.2$ Hz), 7.24-7.37 (5H, m) ppm; **$^{13}$C NMR** (100 MHz, CDCl$_3$): $\delta$ 23.4, 64.4, 70.0, 74.7, 111.4 (q, $J = 3.7$ Hz), 111.5, 115.5 (q, $J = 4.2$ Hz), 123.7 (q, $J = 32.3$ Hz), 124.6 (q, $J = 270.6$ Hz), 127.5, 127.6, 128.5, 137.0, 139.3, 148.8 ppm; **$^{19}$F NMR** (376 MHz, CDCl$_3$): $\delta$ $-61.7$ ppm; **LRMS** (+ESI) $m/z$: 340.1 ([M+H]$^+$ 38%), 362.2 ([M+Na]$^+$ 100%), 701.0 ([2M+Na]$^+$ 41%); **HRMS** (ESI)$^+$ Calcd for C$_{18}$H$_{20}$F$_3$NO$_2$ [M+H]$^+$: 340.15244, found 340.15169.
Prepared according to General Procedure C using carboxylic acid 198 (100 mg, 400 μmol) and aniline 200. The resulting crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:7) as an eluent to obtain the amide 201 (120 mg, 52%) as a colourless oil, \( R_f \): 0.38 (1:4 ethyl acetate, hexane); IR (ν\(_{\text{max}}\) (neat)): 2960, 1684, 1540, 1443, 1272, 1118 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 1.24 (9H, s), 1.47 (6H, s), 1.69-1.88 (3H, m), 1.90-2.04 (1H, m), 3.59-3.87 (2H, m), 4.07 (2H, s), 4.29-4.55 (2H, m), 4.48 (2H, s), 4.77 (1H, dd, J = 13.5, 7.1 Hz), 6.44 (1H, s), 6.69 (1H, d, J = 8.5 Hz), 7.10-7.42 (6H, m), 8.64 (1H, s, NH), 8.76 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): δ 23.2, 25.5, 28.9, 30.3, 32.0, 54.4, 64.6, 68.2, 74.6, 74.7, 78.1, 103.3, 111.4, 116.7 (q, J = 3.4 Hz), 121.1 (q, J = 3.3 Hz), 123.9 (q, J = 32.4 Hz), 124.3 (q, J = 270.5 Hz), 127.4, 127.5, 128.4, 128.5, 136.0, 138.6, 149.8, 158.1, 160.7 ppm; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)): δ −61.8 ppm; LRMS (+ESI) m/z: 574.3 ([M+H]\(^+\) 24%), 596.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{31}\)H\(_{38}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 596.2712, found 596.27006.

Prepared according to General Procedure J using 201 (80 mg, 140 μmol). The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 1:1) as an eluent to obtain the title compound (61 mg, 90%) as colourless crystals, mp: 98.8-101.1 °C; \( R_f \): 0.11 (1:4 ethyl acetate, hexane); IR (ν\(_{\text{max}}\) (neat)): 3390, 2961, 1673, 1543, 1439, 1272, 1116 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 1.31 (9H, s), 1.39 (3H, s), 1.40 (3H, s), 1.64-2.08 (4H, m), 3.65-3.75 (2H, m), 3.93 (1H, d, J = 8.9 Hz), 7.41 (1H, d,
$J = 8.9 \text{ Hz}$, 4.36-4.45 (1H, m), 4.51 (1H, dd, $J = 14.0, 4.0 \text{ Hz})$, 4.68 (1H, dd, $J = 14.0, 6.4 \text{ Hz}$), 6.57 (1H, s), 6.98 (1H, m), 7.31-7.39 (1H, m), 8.67 (1H, d, $J = 2.2 \text{ Hz}$), 8.94 (1H, s) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 25.6, 26.5, 26.6, 28.7, 30.6, 32.2, 54.4, 68.6, 70.0, 77.6, 78.2, 104.5, 111.8, 118.0 (q, $J = 3.8 \text{ Hz}$), 121.7 (q, $J = 4.1 \text{ Hz}$), 124.1 (q, $J = 33.0 \text{ Hz}$), 124.2 (q, $J = 272.4 \text{ Hz}$), 128.2, 137.3, 150.3, 158.4, 161.4 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ −61.7 ppm; LRMS (+ESI) $m/z$: 506.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{24}$H$_{32}$F$_3$N$_3$O$_4$ [M+Na]$^+$: 506.22426, found 506.22332; $[\alpha]_D^{22}$: −13.2 ° (1.90, CHCl$_3$); HPLC: 95.2%, RT: 29.7 mins. (Method A, 254 nm).

(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)benzothioamide 169

A solution of 118 (75 mg, 160 μmol), 3,4-dihydropyran (71 μL, 780 μmol) and triphenylphosphine hydrobromide (6 mg, 16 μmol) in anhydrous dichloromethane (5 mL) was stirred at room temperature for 72 hours. The mixture was diluted with dichloromethane (20 mL), washed with brine (20 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the resulting residue purified by flash column chromatography using ethyl acetate, hexane (2:3 to 3:2) as an eluent to obtain the protected alcohol (202) (54 mg, 61%) as a colourless oil. $^1$H NMR analysis as well as low resolution mass spectrometry indicated the compound was isolated as an inseparable mixture of diastereomers and therefore the mixture was taken on to the following step without further purification or characterisation.

To a solution of protected alcohol 202 in anhydrous toluene (5 mL) was added Lawesson’s reagent (39 mg, 95 μmol) and the mixture was heated at reflux for 16 hours. The solvent was removed under a stream of nitrogen and the crude residue purified by flash column chromatography using ethyl acetate, hexane (1:1 to 3:2) as an eluent to obtain the title compound (20 mg, 43%) as a yellow foam, mp: 59.8-63.6 °C; $R_F$: 0.38 (3:7 ethyl acetate, dichloromethane); IR ($\nu_{\max}$ (neat)): 3240, 2960, 1551, 1331, 1305, 1168, 1119, 1007 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.27 (3H, s), 1.28 (3H, s), 1.33 (9H, s), 1.57-1.69 (1H, m), 1.71-1.93 (2H, m), 2.01-2.13 (1H, m), 2.96 (1H, bs), 3.55 (1H, dt, $J = 8.3, 6.9 \text{ Hz}$), 3.65
(1H, td, J = 7.9, 5.8 Hz), 3.88 (1H, d, J = 8.9 Hz), 4.00 (1H, d, J = 8.9 Hz), 4.09 (1H, dd, J = 15.3, 7.3 Hz), 4.22-4.31 (1H, m), 4.42 (1H, dd, J = 15.3, 1.8 Hz), 6.98-7.07 (2H, m), 7.62 (1H, dd, J = 8.7, 2.1 Hz), 7.94 (1H, d, J = 2.1 Hz), 11.24 (1H, s) ppm; 13C NMR (100 MHz, CDCl3): δ 25.8, 26.2, 28.4, 30.5, 32.5, 53.7, 68.5, 70.0, 78.0, 80.0, 96.3, 113.3, 123.6 (q, J = 32.0 Hz), 124.0 (q, J = 272.6 Hz), 127.8 (q, J = 3.6 Hz), 132.2, 138.4, 156.6, 160.8, 192.3 ppm; 19F NMR (376 MHz, CDCl3): δ −61.7 ppm; LRMS (+ESI) m/z: 522.2 ([M+Na]+ 100%); HRMS (ESI)⁺ Cald for C24H32F3N3O3S [M+Na]+: 522.20142, found 522.20059; [α]D22: −35.8 ° (0.42, CHCl3); HPLC: 95.3%, RT: 29.9 mins. (Method A, 230 nm).

(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-2-fluoro-5-(trifluoromethyl)benzenesulfonamide 203

To a solution of 89 (100 mg, 450 μmol) in anhydrous dichloromethane (5 mL) at 0 °C was added pyridine (76 μL, 950 μmol) and 4-dimethylaminopyridine (5 mg, 45 μmol) followed by 2-fluoro-5-(trifluoromethyl)benzene-1-sulfonyl chloride (120 mg, 470 μmol). The reaction mixture was heated at reflux for 72 hours, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (100 mg, 50%) as an orange oil, Rf: 0.21 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 2963, 1323, 1169, 1127, 1081 cm⁻¹; 1H NMR (400 MHz, CDCl3): δ 1.19 (9H, s), 1.48-1.61 (1H, m), 1.67-1.79 (1H, m), 1.81-1.94 (1H, m), 1.95-2.06 (1H, m), 3.80 (1H, ddd, J = 8.3, 7.5, 6.2 Hz), 3.90-3.95 (1H, dd, J = 15.0, 2.0 Hz), 4.22 (1H, dd, J = 15.0, 2.0 Hz), 5.90 (1H, s), 7.35 (1H, t, J = 9.0 Hz), 7.81-7.90 (1H, m), 8.17 (1H, dd, J = 2.4, 6.5 Hz), 9.18 (1H, bs) ppm; 13C NMR (100 MHz, CDCl3): δ 25.8, 28.1, 30.3, 32.3, 52.5, 68.5, 79.9, 95.4, 118.2 (d, J = 22.3 Hz), 122.9 (q, J = 272.6 Hz), 127.2 (q, J = 38.6 Hz), 128.8 (q, J = 3.6 Hz), 132.5-132.8 (m), 160.7 (d, J = 261.9 Hz), 161.1 (two carbon signals were not observed) ppm; 19F NMR (376 MHz, CDCl3): δ −62.4 (CF3), 104.0 (CF) ppm; LRMS (+ESI) m/z: 472.1 ([M+Na]+ 100%); HRMS (ESI)⁺ Cald for C19H23F4N3O3S [M+Na]+: 472.12940, found 472.12848.
(R)-N-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-yl)-2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)benzenesulfonamide 170

Prepared according to General Procedure E using 203 (70 mg, 160 μmol) and diol 93 at reflux for 16 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (2:3 to 3:2) as an eluent to obtain the title compound (54 mg, 67%) as a colourless foam, mp: 48.1-50.4 °C; Rf: 0.08 (2:3 ethyl acetate, hexane); IR (v_{max} (neat)): 3270, 2963, 1613, 1327, 1282, 1166, 1124, 1082, 914 cm^{-1}; ¹H NMR (400 MHz, CDCl₃): δ 1.19 (9H, s), 1.37 (3H, s), 1.47 (3H, s), 1.50-1.65 (2H, m), 1.78-1.98 (2H, m), 3.71-3.91 (3H, m), 3.95-4.08 (2H, m), 6.95 (1H, s), 7.13 (1H, d, J = 8.7 Hz), 7.77 (1H, dd, J = 8.7, 2.3 Hz), 8.10 (1H, d, J = 2.3 Hz), 8.89 (1H, bs) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 25.8, 26.0, 26.2, 27.3, 30.3, 32.3, 51.5, 68.8, 69.8, 79.1, 80.0, 96.5, 114.2, 123.2 (q, J = 34.2 Hz), 123.4 (q, J = 272.4 Hz), 128.3, 128.7 (q, J = 3.3 Hz), 132.2 (q, J = 3.5 Hz), 134.9, 158.6, 161.2 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ −62.0 ppm; LRMS (+ESI) m/z: 542.2 ([M+Na]^+) 100%; HRMS (ESI)⁺ Calcd for C_{23}H_{32}F_{3}N_{3}O_{5}S [M+Na]^+: 542.19125, found 542.19055; [α]_D^{22}: +50.0 ° (0.9, CHCl₃); HPLC: 98.6%, RT: 28.1 mins. (Method A, 230 nm).

(R)-3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-carboxamide 204

Prepared according to a modified version of General Procedure C using acid 198 (110 mg, 440 μmol). The acid chloride was taken up in dichloromethane (2 mL) and slowly added to saturated aqueous ammonia (900 μL) at 0 °C. The mixture was warmed to room temperature and stirred for 4 hours. The crude product was purified by flash column chromatography using methanol, dichloromethane (1:19) as an eluent to obtain the title compound (75 mg, 69%) as a white solid, mp: 159.1-162.9 °C; Rf: 0.58 (1:9 methanol, dichloromethane); IR (v_{max} (neat)): 3334, 3133, 2961, 1678, 1531, 1308, 1059 cm^{-1}; ¹H NMR (300 MHz, CDCl₃): δ 1.26 (9H, s), 1.68-1.89 (3H, m), 1.91-2.08 (1H, m), 3.63-3.87
(2H, m), 4.24-4.39 (1H, m), 4.43 (1H, dd, J = 14.0, 3.8 Hz), 4.56 (1H, dd, J = 14.0, 6.9 Hz), 6.22 (1H, bs), 6.45 (1H, s), 7.06 (1H, bs) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.5, 28.7, 30.5, 32.0, 54.2, 68.3, 78.5, 104.8, 136.7, 160.9, 162.6 ppm; LRMS (+ESI) m/z: 274.1 ([M+Na]$^+$ 100%), 525.2 ([2M+Na]$^+$ 60%); HRMS (ESI)$^+$ Calcd for C$_{13}$H$_{21}$N$_3$O$_2$ [M+Na]$^+$: 274.15315, found 274.15251.

(R)-3-(tert-Butyl)-N-((2-fluoro-5-(trifluoromethyl)phenyl)sulfonyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-carboxamide 205

To a solution of the primary amide 204 (250 mg, 1.0 mmol) in anhydrous tetrahydrofuran (15 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, (52 mg, 1.3 mmol) and the mixture stirred for 15 minutes before 2-fluoro-5-(trifluoromethyl)benzenesulfonyl chloride (170 mg, 1.0 mmol) was added. The mixture was heated to reflux and stirred for 5 hours, cooled to room temperature and quenched with saturated aqueous ammonium chloride (20 mL). The mixture was extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (250 mg, 53%) as a gummy solid, $R_f$: 0.22 (1:9 methanol, dichloromethane); IR (v$_{max}$ (neat)): 2926, 1694, 1269, 1133, 1080 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.23 (9H, s), 1.42-1.79 (4H, m), 3.23-3.39 (2H, m), 3.87-3.97 (1H, m), 4.19 (1H, dd, J = 13.6, 4.6 Hz), 4.41 (1H, dd, J = 13.6, 7.3 Hz), 6.95 (1H, s), 7.74 (1H, t, J = 9.3 Hz), 8.16-8.25 (2H, m) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 24.7, 27.9, 30.2, 31.6, 53.6, 66.8, 77.1, 107.0, 119.0 (d, J = 22.9 Hz), 128.4-128.6 (m), 133.2-133.6 (m), 159.0, 159.3, 160.4 (d, J = 261.7 Hz) (four carbon signals were not observed) ppm; $^{19}$F NMR (376 MHz, DMSO-$d_6$): δ -60.8 (CF$_3$), -103.8 (CF) ppm; LRMS (-ESI) m/z: 476.0 ([M-H]$^-$ 100%).
(R)-N-((2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)sulfonyl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-carboxamide 206

Prepared according to General Procedure E using 205 (50 mg, 105 μmol) and alcohol 187 (38 mg, 210 μmol) at reflux for 2 hours. The crude residue was purified by flash column chromatography using acetic acid, ethyl acetate and hexane (1:38:161) as an eluent to obtain the title compound (37 mg, 55%) as a colourless oil, \( R_f \): 0.54 (1:9 methanol, dichloromethane); IR (\( \nu_{\text{max}} \) (neat)): 2963, 1698, 1614, 1427, 1328, 1280, 1244, 1162, 1126, 1083, 736 cm\(^{-1}\); \(^{1}\text{H} \text{ NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \) 1.23 (9H, s), 1.33 (3H, s), 1.42 (3H, s), 1.58-1.70 (1H, m), 1.76-1.86 (2H, m), 1.93-2.04 (1H, m), 3.52 (1H, s), 3.55-3.63 (1H, m), 3.71-3.79 (1H, m), 4.10 (1H, d, \( J = 9.4 \) Hz), 4.13 (1H, d, \( J = 9.4 \) Hz), 4.22-4.34 (2H, m), 4.44 (3H, m), 6.47 (1H, s), 7.18 (1H, d, \( J = 8.8 \) Hz), 7.24-7.38 (5H, m), 7.80 (1H, dd, \( J = 8.8, 2.4 \) Hz), 8.45 (1H, d, \( J = 2.4 \) Hz) ppm; \(^{13}\text{C} \text{ NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \) 22.9, 25.5, 28.8, 30.4, 32.1, 54.8, 64.8, 68.3, 75.2, 75.5, 78.5, 106.9, 114.0, 123.4 (q, \( J = 33.6 \) Hz), 123.5 (q, \( J = 272.0 \) Hz), 127.6, 127.8, 127.9, 128.6, 129.8 (q, \( J = 3.8 \) Hz), 132.5 (q, \( J = 3.6 \) Hz), 134.6, 138.6, 157.4, 158.6, 161.2 ppm; \(^{19}\text{F} \text{ NMR} \) (376 MHz, CDCl\(_3\)): \( \delta \) -61.7 ppm; LRMS (+ESI) \( m/z \): 660.2 ([M+Na]\(^+\) 61%), 676.3 ([M+K]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{31}\)H\(_{38}\)F\(_3\)N\(_3\)O\(_6\)S [M+Na]\(^+\): 660.23311, found 660.23298.

(R)-3-(tert-Butyl)-N-((2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)phenyl)sulfonyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-5-carboxamide 171

Prepared according to General Procedure J using 206 (100 mg, 160 μmol) in the presence of acetic acid (10 drops). The crude product was purified by flash column chromatography using methanol, dichloromethane (1:19) as an eluent to obtain the title compound (80 mg, 93%) as a colourless foam, \( \text{mp} \): 68.3-74.1 °C; \( R_f \): 0.28 (1:9 methanol, dichloromethane);
To a solution of carboxylic acid 193 (95 mg, 260 μmol) in anhydrous toluene (3 mL) was added diphenylphosphoryl azide (84 μL, 390 μmol) followed by triethylamine (110 μL, 780 μmol) and the mixture was stirred for 30 minutes. The amine 89 (58 mg, 260 μmol) was added and the mixture heated at 100 °C for 1 hour. Saturated aqueous sodium chloride (15 mL) was added and the mixture was extracted with ethyl acetate (3 x 15 mL), washed with saturated aqueous sodium hydrogen carbonate (20 mL) and saturated aqueous ammonium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 2:3) as an eluent to obtain the title compound (68 mg, 45%) as a colourless oil, \( R_r: 0.31 \) (1:1 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ (neat)}): 3337, 2959, 1713, 1538, 1441, 1339, 1210, 1115, 1042 \text{ cm}^{-1} \); \( \text{H NMR} \) (400 MHz, CDCl\(_3\)): δ 1.29 (9H, s), 1.40 (6H, s), 1.45-1.55 (1H, m), 1.61-1.79 (2H, m), 1.88-1.99 (1H, m), 3.52-3.71 (2H, m), 3.92-4.05 (3H, m), 4.06-4.12 (1H, m), 4.16 (1H, dd, \( J = 15.0, 2.3 \text{ Hz} \)), 4.46 (2H, s), 6.26 (1H, s), 6.93 (1H, d, \( J = 8.5 \text{ Hz} \)), 7.19-7.34 (6H, m), 7.85 (1H, bs), 8.21 (1H, bs), 8.62 (1H, d, \( J = 2.2 \text{ Hz} \)) ppm; \( \text{C NMR} \) (100 MHz, CDCl\(_3\)): δ 23.4, 23.6, 25.6, 28.0, 30.6, 32.3,
51.9, 64.7, 68.7, 73.8, 75.0, 79.6, 94.2, 111.0, 116.6 (q, \( J = 3.9 \) Hz), 119.8 (q, \( J = 4.0 \) Hz), 124.0 (q, \( J = 33.9 \) Hz), 124.4 (q, \( J = 271.4 \) Hz), 127.8, 127.8, 128.6, 129.2, 137.3, 138.5, 149.4, 151.5, 161.1 ppm; \( ^{19}F \) NMR (376 MHz, CDCl\(_3\)): \( \delta -61.6 \) ppm; LRMS (+ESI) \( m/z: 611.3 \) ([M+Na]\(^{+}\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{31}\)H\(_{39}\)F\(_3\)N\(_4\)O\(_4\) [M+Na]\(^{+}\): 611.28211, found 611.28115.

\((R)-1-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-3-(2-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)phenyl)urea 172

Prepared according to General Procedure J using urea 207 (65 mg, 110 μmol) in the presence of acetic acid (10 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3 to 3:2) as an eluent to obtain the title compound (48 mg, 88%) as a white solid, \( \text{mp}: 72.4-75.8 \) °C; \( R_f: 0.11 \) (3:2 ethyl acetate, hexane); \( \text{IR (\( \nu_{\text{max}} \) (neat)): 3285, 2959, 1546, 1442, 1114 cm}^{-1} \); \( ^{1}H \) NMR (400 MHz, CDCl\(_3\)): \( \delta 1.17 \) (3H, s), 1.27 (12H, s), 1.63-1.88 (3H, m), 1.98-2.11 (1H, m), 2.34 (1H, bs), 3.55 (1H, bs), 3.66 (2H, t, \( J = 6.4 \) Hz), 3.75 (1H, d, \( J = 9.0 \) Hz), 3.85 (1H, d, \( J = 9.0 \) Hz), 4.04-4.15 (2H, m), 4.33-4.42 (1H, m), 6.17 (1H, s), 6.87 (1H, d, \( J = 8.5 \) Hz), 7.23 (1H, dd, 8.5, 2.2 Hz), 7.96 (1H, bs), 8.58 (1H, d, \( J = 2.2 \) Hz) ppm; \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)): \( \delta 25.6, 26.1, 26.5, 28.2, 30.5, 32.4, 51.6, 68.1, 69.8, 77.4, 78.8, 96.9, 111.4, 116.6 \) (q, \( J = 3.5 \) Hz), 120.1 (q, \( J = 3.4 \) Hz), 124.0 (q, \( J = 33.4 \) Hz), 124.4 (q, \( J = 272.1 \) Hz), 129.0, 136.4, 149.5, 153.0, 161.6 ppm; \( ^{19}F \) NMR (376 MHz, CDCl\(_3\)): \( \delta -61.7 \) ppm; LRMS (+ESI) \( m/z: 521.2 \) ([M+Na]\(^{+}\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{24}\)H\(_{33}\)F\(_3\)N\(_4\)O\(_4\) [M+Na]\(^{+}\): 521.23516, found 521.23435; [\( \alpha \)]\(_{D}^{22}\): -50.8 ° (1.28, CHCl\(_3\)); \( HPLC: 99.7\% \), RT: 26.8 mins. (Method A, 230 nm).
(R)-1-(2-((3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)amino)methyl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 173

Amide 189 (300 mg, 520 μmol) was reduced according to General Procedure D. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) to obtain an inseparable mixture of products, which was taken on to the next step without further purification or characterisation.

The benzyl ether protecting group was removed according to General Procedure J in the presence of acetic acid (10 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 3:2) as an eluent to obtain the title compound (170 mg, 69%) as a colourless oil, \( R_f \): 0.11 (3:7 ethyl acetate, hexane); \( IR (\nu_{\text{max}}) \): 3333, 2963, 1566, 1329, 1161, 1114, 1037 cm\(^{-1}\); \( ^{1}H\ NMR \) (400 MHz, CDCl\(_3\)): \( \delta \) 1.25 (9H, s), 1.36 (6H, s), 1.68-1.83 (2H, m), 1.90-2.02 (2H, m), 3.11 (1H, bs), 3.52-3.69 (2H, m), 3.86-4.00 (3H, s), 5.19-5.30 (1H, m), 5.35 (1H, s), 6.94 (1H, d, \( J = 8.5 \) Hz), 7.52 (1H, dd, \( J = 8.5, 2.3 \) Hz), 7.56 (1H, d, \( J = 2.3 \) Hz) ppm; \( ^{13}C\ NMR \) (100 MHz, CDCl\(_3\)): \( \delta \) 25.7, 26.3, 26.4, 28.2, 30.6, 32.2, 45.6, 51.7, 68.6, 70.0, 77.0, 79.9, 84.5, 111.4, 121.1 (q, \( J = 33.1 \) Hz), 124.4 (q, \( J = 271.6 \) Hz), 126.2 (q, \( J = 3.9 \) Hz), 126.6 (q, \( J = 3.8 \) Hz), 128.5, 149.0, 159.4, 160.6 ppm; \( ^{19}F\ NMR \) (376 MHz, CDCl\(_3\)): \( \delta \) -61.6 ppm; \( LRMS \) (+ESI) \( m/z \): 470.3 ([M+H]\(^+\) 100%), 493.3 ([M+Na]\(^+\) 40%); \( HRMS \) (ESI)\(^+\) Calcd for C\(_{24}\)H\(_{34}\)F\(_3\)N\(_3\)O\(_3\) [M+H]\(^+\): 470.26305, found 470.26236; \( [\alpha]D \): -5.8 ° (0.86, CHCl\(_3\)); \( HPLC \): 98.7%, RT: 23.9 mins. (Method A, 230 nm).

1-(2-(Benzyloxy)-2-methylpropoxy)-2-bromo-4-(trifluoromethyl)benzene 208

Prepared according to General Procedure I using 2-bromo-4-(trifluoromethyl)phenol (1.5 g, 6.2 mmol) and alcohol 187. The crude residue was purified by flash column
chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (2.0 g, 80%) as a colourless oil, $R_f$: 0.57 (1:9 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 2978, 1607, 1324, 1274, 1118, 1027 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.39 (6H, s), 3.89 (2H, s), 4.53 (2H, s), 6.81 (1H, d, $J$ = 8.6 Hz), 7.10-7.31 (5H, m), 7.41 (1H, dd, $J$ = 8.7, 2.2 Hz), 7.72 (1H, d, $J$ = 2.2 Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 23.3, 64.9, 75.1, 76.0, 112.5, 123.7 (q, $J$ = 270.7 Hz), 124.1 (q, $J$ = 33.2 Hz), 126.0 (q, $J$ = 3.4 Hz), 127.5, 128.4, 130.6 (q, $J$ = 3.6 Hz), 139.4, 158.0 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ −61.6 ppm; LRMS (+ESI) $m/z$: 425.0/427.0 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{18}$H$_{18}$BrF$_3$O$_2$ [M+Na]$^+$: 425.03400, found 425.03317.

(R)-N-(2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-amine 209

A solution of aminopyrazole 89 (66 mg, 300 μmol), aryl bromide 208 (120 mg, 300 μmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (26 mg, 45 μmol), palladium acetate (7 mg, 30 μmol) and cesium carbonate (290 mg, 900 μmol) in anhydrous, degassed toluene (4 mL) was heated at 100 °C in a sealed vessel for 16 hours. The mixture was filtered through a pad of Celite®, concentrated under reduced pressure and the resulting residue purified by flash column chromatography using ethyl acetate, hexane (1:19 to 2:3) as an eluent to obtain the title compound (110 mg, 66%) as a light yellow oil, $R_f$: 0.25 (1:9 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 3342, 2959, 1561, 1446, 1320, 1209, 1164, 1114, 1061 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.32 (9H, s), 1.49 (6H, s), 1.61-1.81 (3H, m), 1.91-2.03 (1H, m), 3.56-3.64 (1H, m), 3.74-3.81 (1H, m), 3.99 (1H, d, $J$ = 9.3 Hz), 4.03-4.10 (2H, m), 4.12-4.19 (1H, m), 4.28 (1H, dd, $J$ = 14.7, 2.5 Hz), 4.55 (2H, s), 6.00 (1H, s), 6.89 (1H, d, $J$ = 8.4 Hz), 7.03 (1H, dd, $J$ = 8.4, 2.3 Hz), 7.22-7.41 (6H, m) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 23.1, 23.5, 25.6, 28.1, 30.6, 32.4, 51.2, 64.6, 68.8, 74.9, 74.9, 79.5, 92.7, 109.8 (q, $J$ = 3.8 Hz), 110.8, 116.2 (q, $J$ = 3.9 Hz), 123.4 (q, $J$ = 33.4 Hz), 124.6 (q, $J$ = 271.6 Hz), 127.5, 127.6, 128.4, 134.1, 139.2, 140.7, 148.7, 160.9 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ −61.8 ppm; LRMS (+ESI) $m/z$: 546.3 ([M+H]$^+$ 51%), 568.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{30}$H$_{38}$F$_3$N$_3$O$_3$ [M+Na]$^+$: 568.27630, found 568.27552.
(R)-1-(2-((3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)amino)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 174

Prepared according to General Procedure J using 209 (74 mg, 140 μmol) in the presence of acetic acid (10 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (59 mg, 95%) as a colourless waxy solid, Rf: 0.12 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 3345, 2962, 1562, 1448, 1264, 1114, 1037 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.32 (9H, s), 1.37 (3H, s), 1.40 (3H, s), 1.72-1.86 (1H, m), 1.90-2.06 (3H, m), 3.72-3.81 (3H, m), 3.84 (1H, d, 8.7 Hz), 3.99 (1H, d, J = 8.7 Hz), 4.14 (1H, dd, J = 15.5, 2.7 Hz), 4.29-4.37 (2H, m), 6.03 (1H, s), 6.85 (1H, d, J = 8.3 Hz), 7.04 (1H, dd, J = 15.5, 2.7 Hz), 7.33 (1H, d, J = 2.1 Hz), 7.95 (1H, s) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 25.6, 26.1, 26.3, 27.7, 30.6, 32.4, 51.0, 69.2, 69.7, 76.7, 80.1, 91.8, 108.8 (q, J = 3.8 Hz), 110.6, 116.1 (q, J = 4.3 Hz), 123.8 (q, J = 32.2 Hz), 124.6 (q, J = 271.1 Hz), 133.6, 140.3, 148.3, 161.2 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -61.6 ppm; LRMS (+ESI) m/z: 478.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Calcd for C₂₄H₃₅F₃N₄O₄ [M+Na]⁺: 521.23516, found 521.23435; [α]D²²: -31.3 ° (0.48, CHCl₃); HPLC: 98.0%, RT: 27.5 mins. (Method A, 230 nm).

Methyl 5-bromofuran-2-carboxylate 210

To a solution of furan-2-carboxylic acid (10 g, 89.2 mmol) in acetic acid (120 mL) was added bromine (4.6 mL, 89.2 mmol), dropwise at room temperature. The mixture was warmed to 60 °C and stirred for 16 hours. After cooling to room temperature the reaction mixture was quenched with sodium bisulfite (100 mL) and extracted with dichloromethane (3 x 100 mL). The combine organic extracts were washed with water (2 x 100 mL) and saturated aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. NMR analysis revealed the residue obtained was an inseparable mixture of mono- and dibrominated products and so the crude product was taken on to the next step without further purification or characterisation.
The crude carboxylic acid (15.6 g) was dissolved in methanol (250 mL) and to this was added concentrated sulfuric acid (5 mL) and the mixture heated at reflux for 16 hours. The methanol was removed under reduced pressure and the residue taken up in ethyl acetate (200 mL) and washed with saturated aqueous sodium hydrogen carbonate, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:39) as an eluent to obtain the title compound (3 g, 16% over 2 steps) as a yellow solid, the characterisation data of which corresponded to that previously described.\(^{327}\) mp: 54.1-57.4 °C; \(R_f\): 0.44 (1:9 ethyl acetate, hexane); IR \((\nu_{\text{max}} \text{ (neat)})\): 2956, 1734, 1708, 1580, 1466, 1297, 1153, 1018, 921, 755 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.88 (3H, s), 6.45 (1H, d, \(J = 3.5\) Hz), 7.12 (1H, d, \(J = 3.5\) Hz) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 52.2, 114.1, 120.2, 127.6, 146.4, 158.2 ppm; LRMS (+ESI) \(m/z\): 226.7/228.7 ([M+Na]\(^+\) 25%), 432.8 ([2M+Na]\(^+\) 100%).

\(1-(5\text{-Bromofuran-2-yl})\)-4,4-dimethylpentane-1,3-dione 211

To a solution of methyl ester 210 (2.0 g, 9.8 mmol) in anhydrous tetrahydrofuran (40 mL) at 0 °C was added potassium tert-butoxide (2.2 g, 19.6 mmol), followed by a solution of pinacolone (1.5 mL, 11.7 mmol) in tetrahydrofuran (10 mL), slowly. The mixture was warmed to room temperature and stirred for 1 hour. Saturated aqueous ammonium chloride (50 mL) was carefully added and the mixture extracted with ethyl acetate (3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (1.8 g, 68%) as a gummy solid, \(R_f\): 0.62 (1:9 ethyl acetate, hexane); IR \((\nu_{\text{max}} \text{ (neat)})\): 3145, 3109, 2968, 1673, 1573, 1464, 1445, 1289, 1014, 919, 779 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 1.23 (9H, s), 6.13 (1H, s), 6.42-6.53 (1H, m), 7.04-7.14 (1H, m), 15.81 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 27.5, 39.2, 91.5, 114.6, 117.4, 127.0, 152.7, 175.7, 200.1 ppm; LRMS (+ESI) \(m/z\): 295.0/297.0 ([M+Na]\(^+\) 100%).
5-(5-Bromofuran-2-yl)-3-(tert-butyl)-1H-pyrazole 212

To a solution of diketone 211 (1.0 g, 3.7 mmol) in ethanol (30 mL) was added hydrazine hydrate (220 μL, 4.4 mmol) followed by acetic acid (5 drops) and the mixture heated at reflux for 16 hours. The solvent was removed under a stream of nitrogen and the residue taken up in ethyl acetate (50 mL), washed with water (2 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (970 mg, 98%) as a tan solid, mp: 125.4-128.9 °C; Rf: 0.14 (1:9 ethyl acetate, hexane); IR (v_max (neat)): 3141, 3065, 2956, 1517, 1288, 1129, 1071, 773 cm⁻¹; 1H NMR (300 MHz, CDCl₃): δ 1.29 (9H, s), 6.22-6.35 (2H, s), 6.48-6.54 (1H, m), 10.67 (1H, s) ppm; 13C NMR (75 MHz, CDCl₃): δ 30.3, 31.3, 98.6, 108.0, 113.0, 121.0, 141.8, 150.8, 155.7 ppm; LRMS (+ESI) m/z: 261.0/271.0 ([M+H]^+ 27%), 291.0/293.0 ([M+Na]^+ 100%); HRMS (ESI)^+ Cald for C₁₁H₁₃BrN₂O [M+H]^+: 269.02895, found 269.02832/271.02626.

(R)-(Tetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate 213

Prepared according to General Procedure A using alcohol 85 (2.0 g, 19.6 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (2.1 g, 41%) as a colourless oil, Rf: 0.57 (1:1 ethyl acetate, hexane); IR (v_max (neat)): 2954, 2874, 1598, 1355, 1173, 956 cm⁻¹; 1H NMR (300 MHz, CDCl₃): δ 1.54-1.75 (1H, m), 1.75-2.07 (2H, m), 2.43 (3H, s), 3.62-3.85 (2H, m), 3.89-4.13 (3H, m), 7.33 (2H, d, J = 7.8 Hz), 7.78 (2H, d, J = 7.0 Hz) ppm; 13C NMR (75 MHz, CDCl₃): δ 21.7, 25.7, 27.9, 68.9, 71.6, 76.0, 128.0, 129.1, 133.1, 144.9 ppm; LRMS (+ESI) m/z: 279.1 ([M+Na]^+ 100%), 353.1 ([2M+Na]^+ 43%).
Prepared according to General Procedure L using \textit{212} (130 mg, 490 μmol) and tosylate \textit{213} at 60 °C for 3 hours. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound (85 mg, 50%) as a light pink oil, \( R_f: 0.37 \) (1:4 ethyl acetate, hexane); \textbf{IR} (\( \nu_{\text{max}} \) (neat)): 2958, 1530, 1495, 1361, 1201, 1067, 1011, 928, 782 cm\(^{-1}\); \textbf{\(^{1}H\) NMR} (400 MHz, CDCl\(_3\)): \( \delta \) 1.31 (9H, s), 1.70-2.02 (4H, m), 3.66-3.82 (2H, m), 4.23-4.39 (3H, m), 6.31 (1H, s), 6.39 (1H, d, \( J = 3.4 \) Hz), 6.60 (1H, d, \( J = 3.4 \) Hz) ppm; \textbf{\(^{13}C\) NMR} (100 MHz, CDCl\(_3\)): 25.6, 29.0, 30.6, 32.1, 54.2, 68.4, 73.8, 79.1, 102.2, 111.3, 113.2, 122.2, 133.7, 147.1, 161.4 ppm; \textbf{LRMS (+ESI) \( m/z \)}: 375.1/377.1 ([M+Na]\(^+\) 100%); \textbf{HRMS} (ESI)\(^+\) Calcd for C\(_{16}\)H\(_{21}\)BrN\(_2\)O\(_2\) [M+Na]\(^+\): 375.06841, found 375.06782/377.06578.

The aryl bromide \textit{208} (200 mg, 500 μmol), anhydrous potassium acetate (150 mg, 1.5 mmol), [1,1' bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11 mg, 15 μmol) and bis(pinacolato)diboron (250 mg, 1.0 mmol) were suspended in anhydrous, degassed toluene (3 mL) and heated at 90 °C under an atmosphere of nitrogen for 16 hours. The mixture was cooled to room temperature, filtered through a pad of Celite\(^\circledR\) and the filtrate concentrated under reduced pressure. The residue was purified by flash column chromatography using ethyl acetate, hexane (1:49 to 1:24) as an eluent to obtain the title compound (150 mg, 70%) as a colourless solid, \textbf{mp}: 65.1-68.4 °C; \( R_f: 0.24 \) (1:19 ethyl acetate, hexane); \textbf{IR} (\( \nu_{\text{max}} \) (neat)): 2975, 1611, 1315, 1257, 1164, 1141, 1111 cm\(^{-1}\); \textbf{\(^{1}H\) NMR} (300 MHz, CDCl\(_3\)): \( \delta \) 1.20 (12H, s), 1.36 (6H, s), 3.81 (2H, s), 4.52 (2H, s), 6.70
(1H, d, J = 8.6 Hz), 7.03-7.27 (5H, m), 7.48 (1H, dd, J = 8.8, 2.5 Hz), 7.85 (1H, d, J = 2.4 Hz) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 23.2, 25.0, 64.9, 75.2, 83.8, 110.8, 114.7, 122.4 (q, \(J = 33.2\) Hz), 124.7 (q, \(J = 271.4\) Hz), 127.3, 127.5, 128.3, 129.8 (q, \(J = 3.6\) Hz), 134.0 (q, \(J = 3.9\) Hz), 139.7, 165.9 ppm; \(^{19}\)F NMR (282 MHz, CDCl\(_3\)): \(\delta\) −61.2 ppm; LRMS (+ESI) \(m/z\): 473.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{24}\)H\(_{30}\)BF\(_3\)O\(_4\) [M+Na]\(^+\): 473.20869, found 473.20779.

\((R)\)-5-(5-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)furan-2-yl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole 216

Prepared according to a modified version of General Procedure G using aryl bromide 214 (50 mg, 140 \(\mu\)mol), boronate ester 215 and aqueous potassium hydroxide (3 M, 93 \(\mu\)L, 280 \(\mu\)mol) as base in degassed tetrahydrofuran (1 mL) at 60 °C for 3 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (51 mg, 60%) as an inseparable mixture of products. The impurities were carried through to the next step where purification of the final compound was possible by chromatography. Partial characterisation was obtained, \(R_f\): 0.42 (1:4 ethyl acetate, hexane); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.35 (9H, s), 1.50 (6H, s), 1.75-2.03 (4H, m), 2.90-3.02 (2H, m), 3.61-3.86 (2H, m), 4.10 (2H, s), 4.23-4.42 (3H, m), 4.52 (2H, s), 6.39 (1H, s), 6.64 (1H, d, \(J = 3.5\) Hz), 7.03 (1H, d, \(J = 8.6\) Hz), 7.23-7.36 (6H, m), 7.48 (1H, dd, \(J = 8.6, 2.4\) Hz), 8.15 (1H, d, \(J = 2.4\) Hz) ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) −61.7 ppm; LRMS (+ESI) \(m/z\): 619.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{34}\)H\(_{39}\)F\(_3\)N\(_2\)O\(_4\) [M+Na]\(^+\): 619.27596, found 619.27454.
(R)-1-(2-(5-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)furan-2-yl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 175

Prepared according to General Procedure J using 216 (65 mg, 110 μmol) in the presence of acetic acid (5 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (37 mg, 67%) as a colourless waxy solid, \( R_f \): 0.08 (1:4 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ (neat))}: 3376, 2963, 1563, 1509, 1331, 1242, 1116, 1020, 791 \text{ cm}^{-1} \); \( \text{1H NMR} (400 \text{ MHz, CDCl}_3): \delta 1.35 \) (9H, s), 1.45 (6H, s), 1.76-1.90 (3H, m), 1.93-2.07 (1H, m), 3.68-3.86 (2H, m), 4.01 (2H, s), 4.30-4.42 (2H, m), 4.45-4.53 (1H, m), 6.40 (1H, d, \( J = 3.5 \text{ Hz} \)), 7.04 (1H, d, \( J = 8.7 \text{ Hz} \)), 7.12 (1H, d, \( J = 3.5 \text{ Hz} \)), 7.50 (1H, dd, \( J = 8.3, 1.9 \text{ Hz} \)), 8.13 (1H, d, \( J = 2.3 \text{ Hz} \)) ppm; \( \text{13C NMR} (100 \text{ MHz, CDCl}_3): \delta 25.6, 26.9, 29.3, 30.7, 32.2, 54.6, 68.5, 70.3, 77.3, 78.3, 102.2, 110.9, 112.2, 112.8, 120.2, 123.6 (q, \( J = 3.8 \text{ Hz} \)), 123.7 (q, \( J = 33.2 \text{ Hz} \)), 124.4 (q, \( J = 271.8 \text{ Hz} \)), 125.4 (q, \( J = 3.7 \text{ Hz} \)), 134.1, 144.8, 148.9, 156.9, 161.6 ppm; \( \text{19F NMR} (376 \text{ MHz, CDCl}_3): \delta -61.7 \text{ ppm} \); \( \text{LRMS (ESI)} \ m/z: 529.2 ([M+Na]^+ 100%); \( \text{HRMS (ESI)}^+ \) Calcd for C$_{27}$H$_{33}$F$_3$N$_2$O$_4$ [M+Na]$^+$: 529.22901, found 529.22807; \( [\alpha]_D^{22}: +33.3^\circ \) (0.60, CHCl$_3$); \( \text{HPLC:} 98.1\%, \text{RT:} 32.0 \text{ mins. (Method A, 230 nm).} \)

(R)-5-Azido-3-(tert-buty)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole 217

To a solution of 89 (200 mg, 900 μmol) in trifluoroacetic acid (2.1 mL) at 0 °C was added a solution of sodium nitrite (280 mg, 4.0 mmol) in water (500 μL). After stirring for 10 minutes a solution of sodium azide (590 mg, 9.0 mmol) in water (2 mL) was added and the mixture stirred at room temperature for 30 minutes. Water (20 mL) was added and the mixture extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (140 mg, 65%) as a red oil, \( R_f \): 0.61 (2:3 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ (neat))}: 2966, 2140, 1508, 1144, 1068 \)
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\( ^1H \) NMR (300 MHz, CDCl\(_3\)): δ 1.32 (9H, s), 1.56-1.74 (1H, m), 1.78-2.11 (3H, m), 3.69-3.94 (2H, m), 3.96-4.20 (2H, m), 4.20-4.34 (1H, m), 5.97 (1H, s) ppm; \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)): δ 25.5, 28.9, 30.0, 32.6, 51.4, 68.5, 78.5, 92.2, 140.5, 161.5 ppm; LRMS (+ESI) \( m/z \): 250.2 ([M+H]\(^+\) 34%), 272.1 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{12}\)H\(_{19}\)N\(_3\)O [M+Na]\(^+\): 272.14873, found 272.14807.

2-Iodo-1-(2-((4-methoxybenzyl)oxy)-2-methylpropoxy)-4-(trifluoromethyl)benzene 218

Prepared according to General Procedure I using 2-iodo-4-trifluoromethyl phenol (1.5 g, 5.2 mmol) and alcohol 188 (1.1 g, 5.2 mmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound (2.3 g, 92%) as a yellow oil, \( R_f \): 0.52 (1:9 ethyl acetate, hexane); IR (\( v_{\text{max}} \) (neat)): 2976, 1602, 1321, 1244, 1117, 1038, 813 cm\(^{-1}\); \( ^1H \) NMR (400 MHz, CDCl\(_3\)): δ 1.50 (6H, s), 3.79 (3H, s), 3.97 (2H, s), 4.56 (2H, s), 6.81 (1H, d, \( J = 8.5 \) Hz), 6.86 (2H, d, \( J = 8.6 \) Hz), 7.29 (2H, d, \( J = 8.6 \) Hz), 7.55 (1H, dd, \( J = 8.6, 2.3 \) Hz), 8.03 (1H, d, \( J = 2.2 \) Hz) ppm; \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)): δ 23.5, 55.5, 64.6, 74.9, 76.1, 86.3, 111.3, 113.9 123.4 (q, \( J = 272.4 \)), 124.7 (q, \( J = 33.0 \) Hz), 127.0 (q, \( J = 3.9 \) Hz), 129.1, 131.5, 136.6 (q, \( J = 3.5 \) Hz), 159.2, 160.1 ppm; \( ^{19}F \) NMR (376 MHz, CDCl\(_3\)): δ -61.6 ppm; LRMS (+ESI) \( m/z \): 503.1 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{19}\)H\(_{20}\)F\(_3\)O\(_3\) [M+Na]\(^+\): 503.03070, found 503.02996.
2-Ethynyl-1-(2-((4-methoxybenzyl)oxy)-2-methylpropoxy)-4-(trifluoromethyl)benzene 219

The TMS protected alkyne was prepared according to a modified version of General Procedure K using 218 (300 mg, 670 μmol) and trimethylsilylacetylene (280 μL, 2.0 mmol) at 70 °C for 16 hours. The residue was taken up in anhydrous tetrahydrofuran (10 mL) and cooled to 0 °C. To this was added tetra-n-butylammonium fluoride (1 M in tetrahydrofuran, 1.3 mL, 1.3 mmol) and the mixture stirred for 30 minutes before water (30 mL) was added. The aqueous mixture was extracted with diethyl ether (3 x 25 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (200 mg, 86%) as an orange oil, $R_f$: 0.22 (1:19 ethyl acetate, hexane); $\text{IR}(\nu_{\text{max}} \text{ (neat)})$: 3284, 2958, 2866, 1514, 1331, 1243, 1125, 1013 cm$^{-1}$; $^1\text{H NMR}$ (300 MHz, CDCl$_3$): $\delta$ 1.33 (6H, s), 3.20 (1H, s), 3.65 (3H, s), 3.86 (2H, s), 4.43 (2H, s), 6.73 (2H, d, $J = 8.6$ Hz), 6.79 (1H, d, $J = 8.8$ Hz), 7.14 (2H, d, $J = 8.5$ Hz), 7.39 (1H, dd, $J = 8.8$, 2.3 Hz), 7.60 (1H, d, $J = 2.3$ Hz) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl$_3$): $\delta$ 23.2, 55.3, 64.6, 74.9, 75.6, 78.7, 82.5, 111.8, 112.6, 113.9, 122.9 (q, $J = 33.2$ Hz), 124.0 (q, $J = 271.4$ Hz), 127.3 (q, $J = 3.8$ Hz), 129.0, 131.1 (q, $J = 3.5$ Hz), 131.6, 159.1, 162.5 ppm; $^{19}\text{F NMR}$ (282 MHz, CDCl$_3$): $\delta$ −61.8 ppm; $\text{LRMS}$ (+ESI) $m/z$: 401.2 ([M+Na]$^+$ 100%); $\text{HRMS}$ (ESI)$^+$ Cald for C$_{21}$H$_{21}$F$_3$O$_3$ [M+Na]$^+$: 401.13405, found 401.13326.
Azide 217 (100 mg, 400 μmol) and alkyne 219 (140 mg, 400 μmol) were suspended in a mixture of water and tert-butyl alcohol (1:1, 2 mL). Sodium ascorbate (40 mg, 200 μmol) was added followed by copper sulfate pentahydrate (10 mg, 40 μmol) and the mixture was stirred at room temperature for 16 hours. The mixture was diluted with water (20 mL) and extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (3:17 to 1:4) as an eluent to obtain the title compound (160 mg, 64%) as yellow oil, \( R_f: 0.47 \) (1:4 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ (neat)}): 2960, 1575, 1512, 1323, 1118, 1077, 1032, 818 \text{ cm}^{-1}; \) \( ^1\text{H NMR} (400 \text{ MHz}, \text{CDCl}_3): \delta 1.29 (9H, s), 1.43 (3H, s), 1.44 (3H, s), 1.53-1.78 (3H, m), 1.82-1.93 (1H, m), 3.43-3.57 (2H, m), 3.73 (3H, s), 4.12 (2H, s), 4.13-4.23 (3H, m), 4.36 (1H, d, \( J = 10.3 \text{ Hz} \)), 4.39 (1H, d, \( J = 10.3 \text{ Hz} \)), 5.97 (1H, s), 6.68 (2H, m), 7.02-7.08 (3H, m), 7.57-7.62 (1H, m), 7.68 (1H, s), 8.37 (1H, dd, \( J = 2.4, 0.8 \text{ Hz} \)) ppm; \( ^{13}\text{C NMR} (100 \text{ MHz}, \text{CDCl}_3): \delta 23.5, 23.7, 25.5, 28.8, 30.4, 32.5, 53.2, 55.3, 64.1, 68.1, 73.4, 74.5, 77.5, 98.1, 111.7, 113.9, 119.9, 123.8 (q, \( J = 33.4 \text{ Hz} \)), 124.4 (q, \( J = 271.4 \text{ Hz} \)), 125.4 (q, \( J = 3.6 \text{ Hz} \)), 126.3 (q, \( J = 3.8 \text{ Hz} \)), 126.5, 129.2, 130.6, 136.5, 141.9, 157.3, 159.2, 161.2 ppm; \( ^{19}\text{F NMR} (376 \text{ MHz}, \text{CDCl}_3): \delta -61.6 \text{ ppm} \); \( \text{LRMS (ESI) m/z: 650.3 ([M+Na]^+) 100%}; \) \( \text{HRMS (ESI)^+} \text{ Cald for C}_{33}\text{H}_{40}\text{F}_3\text{N}_5\text{O}_4 [\text{M+Na}^+]: 650.29301, \text{ found 650.29210}. \)
Prepared according to General Procedure J using 220 (130 mg, 210 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (100 mg, 95%) as an off-white foam, mp: 52.8-55.3 °C; \( R_f \): 0.07 (3:7 ethyl acetate, hexane); IR (\( v_{\text{max}} \) (neat)): 3410, 2963, 1575, 1342, 1269, 1232, 1077, 1026 cm\(^{-1}\); \( ^1H \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.34 (9H, s), 1.38 (6H, s), 1.58-1.83 (2H, m), 1.86-2.05 (2H, m), 2.98 (1H, bs), 3.41 (1H, dt, \( J = 8.1, 6.7 \) Hz), 3.61 (1H, ddd, \( J = 8.3, 7.3, 6.2 \) Hz), 3.99 (1H, d, \( J = 9.2 \) Hz), 4.03 (1H, d, \( J = 9.2 \) Hz), 4.14 (2H, d, \( J = 4.9 \) Hz), 4.23-4.32 (1H, m), 6.35 (1H, s), 7.07 (1H, d, \( J = 8.7 \) Hz), 7.57 (1H, dd, \( J = 8.7, 2.3 \) Hz), 8.56 (1H, d, \( J = 2.3 \) Hz), 8.64 (1H, s) ppm; \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 25.6, 26.8, 28.7, 30.5, 32.5, 52.7, 68.4, 70.0, 77.4, 77.5, 99.1, 112.4, 119.8, 123.9 (q, \( J = 33.3 \) Hz), 124.4 (q, \( J = 271.2 \) Hz), 125.8 (q, \( J = 3.9 \) Hz), 126.0, 126.6 (q, \( J = 3.8 \) Hz), 135.7, 142.3, 157.7, 161.4 ppm; \( ^{19}F \) NMR (376 MHz, CDCl\(_3\)): \( \delta \) −61.7 ppm; LRMS (+ESI) \( m/z \): 530.3 ([M+Na\(^{+}\)] 100%); HRMS (ESI) \( ^{+} \) Calcd for C\(_{28}\)H\(_{32}\)F\(_3\)NO\(_5\) [M+Na\(^{+}\)]: 542.2131, found 542.21211; \([\alpha]_D^{22}\): -38.6 ° (2.3, CHCl\(_3\)); HPLC: 99.5%, RT: 34.7 mins. (Method A, 230 nm).

\( (R)-3-(\text{tert-Butyl})-1-((\text{tetrahydrofuran-2-yl})\text{methyl})-1H-\text{pyrazol-5-yl} \)

trifluoromethanesulfonate 221

Pyrazolone 192 (170 mg, 760 µmol) was dissolved in anhydrous dichloromethane (15 mL) and treated with \( N \)-phenylbis(trifluoromethanesulfonamide) (330 mg, 910 µmol) and \( N,N \)-diisopropylethylamine (400 µL, 2.3 mmol) and the mixture was heated at reflux for 3 hours. After cooling to room temperature water (10 mL) was added and the layers separated. The organic phase was washed with water (15 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column
chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (200 mg, 74%) as a colourless oil, \( R_f \): 0.45 (1:9 ethyl acetate, hexane); \( \text{IR} \) (\( \nu_{\text{max}} \) (neat)): 2962, 1541, 1432, 1210, 1135, 875, 827 cm\(^{-1}\); \( ^1\text{H NMR} \) (300 MHz, CDCl\(_3\)): \( \delta \) 1.19 (9H, s), 1.59-1.83 (3H, m), 1.87-1.99 (1H, m), 3.59-3.82 (2H, m), 3.89-4.06 (2H, m), 4.13-4.26 (1H, m), 5.87 (1H, s) ppm; \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): \( \delta \) 25.6, 28.8, 30.1, 32.7, 52.0, 68.3, 77.6, 90.9, 118.7 (q, \( J = 321.0 \) Hz), 141.6, 161.0 ppm; \( ^{19}\text{F NMR} \) (282 MHz, CDCl\(_3\)): \( \delta \) -73.1 ppm; \( \text{LRMS (ESI)} \) \( m/z \): 379.0 ([M+Na]\(^+\) 100%); \( \text{HRMS (ESI)}^+ \) Calcd for C\(_{13}\)H\(_{19}\)F\(_3\)N\(_2\)O\(_4\)S [M+Na]\(^+\): 379.09153, found 379.09097.

(R)-5-(2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole 222

Prepared according to General Procedure G using triflate 221 (100 mg, 280 \( \mu \)mol), boronate ester 215 and cesium carbonate (180 mg, 560 \( \mu \)mol). The resulting crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (61 mg, 41%) as a colourless oil, \( R_f \): 0.39 (1:4 ethyl acetate, hexane); \( \text{IR} \) (\( \nu_{\text{max}} \) (neat)): 2959, 1327, 1304, 1163, 1118, 1064 cm\(^{-1}\); \( ^1\text{H NMR} \) (300 MHz, CDCl\(_3\)): \( \delta \) 1.18 (6H, s), 1.23 (9H, s), 1.37-1.84 (4H, m), 3.39-3.57 (2H, m), 3.80-3.95 (4H, m), 4.04-4.17 (1H, m), 4.33 (2H, s), 6.05 (1H, s), 6.92 (1H, d, \( J = 8.7 \) Hz), 7.11-7.27 (5H, m), 7.53 (1H, d, \( J = 8.7 \) Hz), 7.62 (1H, s) ppm; \( ^{13}\text{C NMR} \) (75 MHz, CDCl\(_3\)): A complex mixture of what is suspected to be rotamers was observed; \( ^{19}\text{F NMR} \) (282 MHz, CDCl\(_3\)): \( \delta \) -61.6 ppm; \( \text{LRMS (ESI)} \) \( m/z \): 553.2 ([M+Na]\(^+\) 100%); \( \text{HRMS (ESI)}^+ \) Calcd for C\(_{30}\)H\(_{37}\)F\(_3\)N\(_2\)O\(_3\) [M+Na]\(^+\): 553.26540, found 553.26176.
(R)-1-(2-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 177

Prepared according to General Procedure J using 222 (35 mg, 66 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 1:1) as an eluent to obtain the title compound (23 mg, 78%) as a colourless oil, \( R_f: 0.23 \) (1:1 ethyl acetate, hexane); \( \text{IR (v}_{\text{max}} \text{ (neat))}: 3398, 2961, 1327, 1272, 1118 \text{ cm}^{-1}; \) \( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)): \( \delta 1.17 \) (3H, s), 1.19 (3H, s), 1.33 (9H, s), 1.51-1.62 (1H, m), 1.63-1.83 (2H, m), 1.86-1.97 (1H, m), 2.46 (1H, bs), 3.49 (1H, dt, \( J = 8.4, 6.7 \text{ Hz} \)), 3.62 (1H, ddd, \( J = 8.4, 4.5 \text{ Hz} \)), 4.23-4.31 (1H, m), 6.12 (1H, s), 6.99 (1H, d, \( J = 8.6 \text{ Hz} \)), 7.62 (1H, dd, \( J = 8.6, 2.3 \text{ Hz} \)), 7.65 (1H, d, \( J = 2.3 \text{ Hz} \)) ppm; \( ^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)): \( \delta 25.5, 25.9, 26.0, 30.8, 32.3, 53.3, 67.9, 69.9, 77.3, 78.0, 104.0, 121.9, 123.2 \) (q, \( J = 32.3 \text{ Hz} \)), 124.3 (q, \( J = 272.6 \text{ Hz} \)), 127.5 (q, \( J = 3.6 \text{ Hz} \)), 129.1 (q, \( J = 3.8 \text{ Hz} \)), 138.7, 159.0, 161.4 ppm; \( ^{19}\text{F NMR} \) (376 MHz, CDCl\(_3\)): \( \delta -61.6 \text{ ppm} \); \( \text{LRMS (+ESI) m/z}: 463.2 ([M+Na]^+ 100\%); \) \( \text{HRMS (ESI)^+} \) Cald for C\(_{23}\)H\(_{31}\)F\(_3\)N\(_2\)O\(_3\) [M+Na]^+: 463.21845, found 463.21758; \([\alpha]_D^{22}\): -55.6 ° (0.54, CHCl\(_3\)); \( \text{HPLC}: 98.2\%, \text{RT}: 28.6 \text{ mins.} \) (Method A, 230 nm).

(R)-3-(tert-Butyl)-5-((2-((4-methoxybenzyl)oxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)ethynyl)1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole 223

Prepared according to General Procedure K using triflate 221 (510 mg, 1.4 mmol) and alkyne 219. The crude residue was purified by flash chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (400 mg, 48%) as a yellow oil, \( R_f: 0.27 \) (1:4 ethyl acetate, hexane); \( \text{IR (v}_{\text{max}} \text{ (neat))}: 2960, 1539, 1364, 1245, 1119, 1031, 816 \)
(R)-1-(2-((3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)ethynyl)-4-
(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 178

To a solution of 223 (55 mg, 94 μmol) in dichloromethane (3 mL) at 0 °C was added water (150 μL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (32 mg, 140 μmol) and the mixture warmed to room temperature and stirred for 2 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (15 mL) and extracted with dichloromethane (3 x 10 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the resulting crude residue purified by flash column chromatography using ethyl acetate, hexane (1:4 to 2:3) as an eluent to obtain the title compound (37 mg, 85%) as a colourless oil, Rf: 0.45 (1:1 ethyl acetate, hexane); IR (v max (neat)): 3427, 2961, 1330, 1120 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.31 (9H, s), 1.38 (3H, s), 1.40 (3H, s), 1.55-1.70 (1H, m), 1.76-1.88 (1H, m), 1.89-2.05 (2H, m), 3.63 (1H, bs), 3.67-3.84 (2H, m), 3.91 (2H, s), 4.24-4.33 (1H, m), 4.33-4.42 (2H, m), 6.36 (1H, s), 6.95 (1H, d, J = 8.7 Hz), 7.54 (1H, dd, J = 8.7, 2.3 Hz), 7.72 (1H, d, J = 2.3 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 25.4, 26.2, 26.4, 28.8, 30.6, 32.2, 54.0, 68.6, 69.6, 77.3, 77.9, 83.7, 90.8, 107.2, 111.9, 112.9, 123.4 (q, J = 32.3 Hz), 124.0 (q, J = 271.6 Hz), 125.6, 127.2 (q, J = 3.9 Hz), 130.6 (q, J = 3.8 Hz), 161.4, 161.6 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -61.9 ppm; LRMS (+ESI) m/z: 487.2 ([M+Na]+ 100%); HRMS (ESI)⁺ Cald for C₂₅H₃₁F₃N₂O₃ [M+Na]⁺: 487.2076; found 487.2075.
487.21845, found 487.21760; $[\alpha]_D^{22} = -22.7^\circ$ (0.66, CHCl$_3$); HPLC: 99.8%, RT: 32.1 mins. (Method A, 230 nm).

(R,E)-1-(2-(2-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)vinyl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 179

The alkyne 223 (40 mg, 68 μmol), triethylsilane (22 μL, 136 μmol), [1,1’-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (1.0 mg, 1.0 μmol), 1,1’-ferrocenediyli-bis(diphenylphosphine) (2.0 mg, 3.4 μmol) and copper sulfate (1.6 mg, 10 μmol) were suspended in a mixture of water (50 μL) and toluene (500 μL) in a sealed vessel under an atmosphere of air. The mixture was heated at reflux for 48 hours, cooled to room temperature, poured into water (15 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain a mixture of the protected tertiary alcohol 223 and deprotected species 179 as indicated by low-resolution mass spectrometry and $^1$H NMR analysis. The mixture was taken on to the next step without further purification or characterisation.

The crude residue was taken up in anhydrous dichloromethane (3 mL) cooled to 0 °C. To the solution was added water (100 μL) followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (12 mg, 51 μmol) and the mixture was stirred at room temperature for 2 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (10 mL), extracted with dichloromethane (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:7) as an eluent to obtain the alkene 179 (19 mg, 61% over 2 steps) as a colourless waxy solid, $R_f$: 0.56 (1:1 ethyl acetate, hexane); IR ($v_{max}$ (neat)): 3240, 2960, 1551, 1331, 1305, 1188, 1119, 1007 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 1.32 (9H, s), 1.40 (3H, s), 1.46 (3H, s), 1.41-1.52 (1H, m), 1.70-1.81 (1H, m), 1.91-2.00 (2H, m), 3.61 (1H, bs), 3.64-3.75 (2H, m), 3.93 (1H, d, $J = 8.9$ Hz), 3.96 (1H, d, $J = 8.9$ Hz), 4.23-4.40 (3H, m), 6.36 (1H, s), 6.97 (1H, d, $J = 8.6$ Hz), 7.12 (1H, d, $J = 16.4$ Hz), 7.46 (1H, dd, $J = 8.6, 2.3$ Hz), 7.55 (1H, d,
$J = 16.4 \text{ Hz}$, 7.64 (1H, d, $J = 2.3 \text{ Hz}$) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 25.5, 26.6, 26.7, 28.5, 30.7, 32.1, 53.2, 68.6, 69.7, 77.1, 78.4, 99.4, 112.0, 120.0, 123.3 (q, $J = 33.5 \text{ Hz}$), 124.4 (q, $J = 270.6 \text{ Hz}$), 125.6 (q, $J = 3.5 \text{ Hz}$), 126.1, 126.4, 126.7 (q, $J = 3.8 \text{ Hz}$), 142.1, 159.2, 161.4 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ −61.7 ppm; LRMS (+ESI) $m/z$: 522.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{25}$H$_{33}$F$_3$N$_2$O$_3$ [M+Na]$^+$: 489.2341, found 489.23320; $[\alpha]_D^{22}$: −34.1 ° (0.44, CHCl$_3$); HPLC: 95.5%, RT: 29.1 mins. (Method A, 230 nm).

(R)-1-((tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazol-5-yl)ethyl)4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 180

Prepared according to General Procedure H using 223 (75 mg, 130 μmol). The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:7) as an eluent to obtain the title compound (43 mg, 71%) as a colourless oil, $R_f$: 0.26 (3:7 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 3426, 3195, 2967, 1329, 1119, 814 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.27 (9H, s), 1.37 (6H, s), 1.51-1.65 (1H, m), 1.71-2.01 (3H, m), 2.89-3.10 (4H, m), 3.67 (2H, t, $J = 6.7 \text{ Hz}$), 3.85 (2H, s), 4.05 (1H, dd, $J = 14.3, 4.9 \text{ Hz}$), 4.10-4.23 (2H, m), 5.86 (1H, s), 6.90 (1H, d, $J = 8.6 \text{ Hz}$), 7.39 (1H, d, $J = 2.3 \text{ Hz}$), 7.46 (1H, dd, $J = 8.6, 2.3 \text{ Hz}$) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 25.6, 26.1, 26.5, 28.8, 30.7, 30.7, 32.0, 52.6, 68.5, 69.9, 76.6, 78.5, 100.8, 111.1, 123.0 (q, $J = 32.0 \text{ Hz}$), 124.5 (q, $J = 271.0 \text{ Hz}$), 125.2 (q, $J = 3.9 \text{ Hz}$), 127.5 (q, $J = 3.6 \text{ Hz}$), 130.4, 143.2, 159.3, 161.0 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ −61.5 ppm; LRMS (+ESI) $m/z$: 419.1 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{25}$H$_{35}$F$_3$N$_2$O$_3$ [M+Na]$^+$: 491.24975, found 491.24902; $[\alpha]_D^{22}$: −16.1 ° (0.62, CHCl$_3$); HPLC: 99.8%, RT: 26.4 mins. (Method A, 230 nm).

1-(4-Bromo-2-fluorophenyl)-2,2-dimethylpropan-1-ol 225

To a solution of 4-bromo-2-fluorobenzaldehyde (1.5 g, 7.4 mmol) in anhydrous tetrahydrofuran (40 mL) at 0 °C was added tert-butylmagnesium chloride (freshly prepared
from tert-butylchloride and magnesium, 540 mM, 20.5 mL, 11.1 mmol) and the mixture was warmed to room temperature and stirred for 16 hours. The reaction mixture was carefully quenched with saturated aqueous ammonium chloride (50 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (2:3 to 1:1) as an eluent to obtain the title compound (1.3 g, 66%) as a colourless oil, $R_f$: 0.31 (1:1 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 3432, 2958, 1603, 1479, 1211, 1047, 1008, 871 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.86 (9H, s), 1.38 (1H, s), 4.68 (1H, s), 7.02-7.48 (3H, m) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 25.6, 36.4, 74.5, 118.6 (d, $J = 26.6$ Hz), 121.2, 127.1, 128.8 (d, $J = 20.2$ Hz), 130.6 (d, $J = 5.3$ Hz), 169.6 (d, $J = 251.6$ Hz) ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ −112.4 ppm.

$1$-(4-Bromo-2-fluorophenyl)-2,2-dimethylpropan-1-one 226

To a solution of alcohol 225 (840 mg, 3.2 mmol) in anhydrous dichloromethane (20 mL) was added Celite® (1.0 g), followed by pyridinium chlorochromate (1.6 g, 7.4 mmol) and the mixture was stirred at room temperature for 16 hours. The mixture was diluted with diethyl ether (20 mL) and filtered through a pad of Celite® and the filtrate concentrated under reduced pressure. The crude residue was purified by flash column chromatography using hexane as an eluent to obtain the title compound (670 mg, 80%) as a colourless oil, $R_f$: 0.32 (hexane); IR ($v_{\text{max}}$ (neat)): 2969, 1696, 1599, 1476, 1395, 1215, 1068, 963, 871, 821 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.23 (9H, s), 7.04-7.10 (1H, m), 7.27-7.34 (2H, m) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 29.6, 45.2, 119.8 (d, $J = 25.5$ Hz), 123.7 (d, $J = 9.1$ Hz), 127.4 (d, $J = 3.5$ Hz), 128.1 (d, $J = 20.5$ Hz), 129.0 (d, $J = 5.4$ Hz), 157.9 (d, $J = 251.6$ Hz), 209.1 ppm; $^{19}$F NMR (282 MHz, CDCl$_3$): $\delta$ −109.7 ppm; LRMS (+ESI) $m/z$: 259.1 ([M+H]$^+$ 100%).
6-Bromo-3-(tert-butyl)-1H-indazole 228

To a solution of ketone 226 (510 mg, 2.0 mmol) in tetrahydrofuran (10 mL) was added hydrazine (1 M in tetrahydrofuran, 3.9 mL, 3.9 mmol) and acetic acid (110 μL, 2.0 mmol) and the mixture was heated at reflux for 16 hours. Low-resolution mass spectrometry and $^1$H NMR analysis revealed the intermediate hydrazone had formed exclusively. The solvent was removed under a stream of nitrogen, taken up in ethylene glycol and heated at 165 °C for 16 hours. After cooling to room temperature the reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate (40 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were washed with water (40 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the resulting residue purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (400 mg, 80%) as a white solid, mp: 170.9-174.4 °C; $R_f$: 0.32 (1:4 ethyl acetate, hexane); IR ($\nu_{max}$ (neat)): 3134, 3094, 3074, 2962, 1615, 1329, 1046, 1046, 1020, 916, 802, 758 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.49 (9H, s), 7.18 (1H, dd, $J = 8.7, 1.6$ Hz), 7.59 (1H, d, $J = 1.6$ Hz), 7.72 (1H, d, $J = 8.7$ Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 30.1, 33.9, 113.2, 119.4, 120.8, 123.3, 123.5, 142.8, 154.6 ppm; LRMS (-ESI) m/z: 251.1/253.0 ([M-H]- 100%).

(R)-6-Bromo-3-(tert-butyl)-1-(((tetrahydrofuran-2-yl)methyl)-1H-indazole 229

Prepared according to General Procedure L using 228 (210 mg, 830 μmol) and tosylate 213 (260 mg, 1.0 mmol) at 60 °C for 5 hours. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (220 mg, 77%) as a colourless oil, $R_f$: 0.45 (1:4 ethyl acetate, hexane); IR ($\nu_{max}$ (neat)): 2964, 1601, 1499, 1461, 1052, 994, 845, 800, 758 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.49 (9H, s), 1.65-2.03 (4H, m), 3.66-3.83 (2H, m), 4.26-4.40 (3H, m), 7.15 (1H, dd, $J = 8.7, 1.6$ Hz), 7.65 (1H, dd, $J = 1.7, 0.6$ Hz), 7.68 (1H, dd, $J = 8.7, 0.6$ Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 25.7, 29.1, 30.3, 33.8, 53.1, 68.5, 78.3, 113.0, 120.2, 120.2, 122.9, 123.3 142.7, 153.3 ppm; LRMS (+ESI) m/z: 359.1/361.1 ([M+Na$^+$] 100%), 697.1

(R)-6-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-indazole 230

Prepared according to a modified version of General Procedure G using aryl bromide 229 (90 mg, 270 μmol), boronate ester 215 (120 mg, 270 μmol) and aqueous potassium hydroxide (3 M, 180 μL, 540 μmol) in degassed tetrahydrofuran (2 mL) and at 60 °C for 4 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (91 mg, 59%) as a colourless oil, R_f: 0.16 (1:4 ethyl acetate, hexane); IR (v_max (neat)): 2968, 1331, 1272, 1238, 1116, 1061, 776 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.30 (6H, s), 1.54 (9H, s), 1.65-1.85 (3H, m), 2.20-2.98 (1H, m), 3.63-3.80 (2H, m), 3.99 (2H, s), 4.28-4.45 (3H, m), 4.37 (2H, s), 7.05 (1H, d, J = 8.7 Hz), 7.15-7.26 (5H, m), 7.25-7.30 (1H, m), 7.55-7.61 (2H, m), 7.63-7.66 (1H, m), 7.81-7.85 (1H, m) ppm; ¹³C NMR (100 MHz, CDCl₃): 23.4, 25.7, 29.2, 30.5, 33.9, 52.9, 64.7, 68.4, 74.9, 75.5, 78.1, 110.7, 112.1 120.6, 121.4, 121.8, 127.3, 127.3, 128.3, 128.3, 132.0, 134.9, 139.4, 142.0, 152.9 (four carbon signals were not observed) ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ −61.5 ppm; LRMS (+ESI) m/z: 603.3 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Calcd for C₃₄H₃₉F₃N₂O₃ [M+Na]⁺: 603.28105, found 603.27975.
(R)-1-(2-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-indazol-6-yl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol \text{181}

Prepared according to General Procedure J using \text{230} (48 mg, 83 μmol) in the presence of acetic acid (5 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (36 mg, 90%) as a colourless waxy solid, \( R_f \): 0.14 (3:7 ethyl acetate, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (neat)}) \): 3434, 2968, 1612, 1331, 1115, 1031, 816 cm\(^{-1}\); \( ^1\text{H NMR} \) (400 MHz, CDCl\(_3\)): δ 1.25 (6H, s), 1.55 (9H, s), 1.60-2.03 (4H, m), 3.63-3.71 (2H, m), 3.88 (2H, s), 4.33-4.48 (3H, m), 7.05 (1H, d, \( J = 8.6 \) Hz), 7.21 (1H, dd, \( J = 8.5, 1.4 \) Hz), 7.56-7.60 (1H, m), 7.66 (1H, dd, \( J = 2.3, 0.8 \) Hz), 7.72 (1H, dd, \( J = 1.5, 0.8 \) Hz), 7.88 (1H, dd, \( J = 8.5, 0.8 \) Hz) ppm; \( ^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)): δ 25.6, 26.4, 29.1, 30.4, 33.9, 52.7, 68.5, 70.0, 77.1, 78.5, 110.9, 112.4, 120.6, 121.2, 121.7, 123.6 (q, \( J = 33.8 \) Hz), 124.5 (q, \( J = 271.8 \) Hz), 126.0 (q, \( J = 3.9 \) Hz), 128.5 (q, \( J = 3.8 \) Hz), 131.7, 134.5, 142.1, 153.0, 158.4 ppm; \( ^{19}\text{F NMR} \) (376 MHz, CDCl\(_3\)): δ -61.7 ppm; \( LRMS \) (+ESI) \( m/z \): 513.2 ([M+Na]\(^+ \) 100%); \( HRMS \) (ESI)\(^+ \) Calcd for C\(_{27}\)H\(_{33}\)F\(_3\)N\(_2\)O\(_3\) [M+Na]\(^+ \): 513.23410, found 513.23327; [\( \alpha \)]\(_D\)\(^{22} \): -10.0° (0.50, CHCl\(_3\)); \( HPLC \): 99.8%, RT: 32.5 mins. (Method A, 230 nm).

5-Bromopyrimidine-2,4(1H,3H)-dione \text{231}

To a stirred solution of uracil (3.0 g, 26.8 mmol) in \( N,N \)-dimethylformamide (15 mL) at 120 °C was added bromine (1.5 mL, 29.4 mmol), dropwise. The reaction mixture was stirred for a further 4 hours, cooled to room temperature and triturated with dichloromethane. The resulting precipitate was collected by filtration and recrystallised from water to obtain the title compound (3.3 g, 65%) as an off-white solid, \( \text{mp} \): 250 °C (decomposition); \( R_f \): 0.07 (1:19 methanol, dichloromethane); \( \text{IR} (\nu_{\text{max}} \text{ (neat)}) \): 3054, 2894, 2810, 1697, 1663, 1620, 1437, 1226, 843 cm\(^{-1}\); \( ^1\text{H NMR} \) (300 MHz, DMSO-d\(_6\)): δ 7.89 (1H, s), 11.2 (1H, bs), 11.5 (1H, bs) ppm; \( ^{13}\text{C NMR} \) (75 MHz, DMSO-d\(_6\)): δ 78.9, 142.2,
150.9, 160.1 ppm; LRMS (+ESI) m/z: 212.9/214.9 ([M+Na]⁺ 58%), 402.9/404.9 ([2M+Na]⁺ 100%).

5-Bromo-2,4-dichloropyrimidine 232

To a solution of 231 (1.0 g, 5.2 mmol) in phosphorus oxychloride (1.5 mL, 15.7 mmol) at 0 °C was added N,N-diisopropylethylamine (1.8 mL, 10.5 mmol), slowly. The mixture was heated at 100 °C for 16 hours. After cooling to room temperature, ice-water (100 mL) was added and the aqueous mixture extracted with diethyl ether (3 x 40 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (960 mg, 81%) as a colourless oil, $R_f$: 0.52 (1:9 ethyl acetate, hexane); IR (v$_{max}$ (neat)): 1527, 1509, 1385, 1361, 1291, 1195, 1181, 1019, 833 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 8.69 (1H, s) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 119.0, 159.0, 161.6, 161.8 ppm.

1-(2,4-Dichloropyrimidin-5-yl)-2,2-dimethylpropan-1-ol 233

To a solution of 232 (600 mg, 2.6 mmol) in anhydrous tetrahydrofuran (15 mL) at -40 °C was added isopropylmagnesium chloride (2 M, 1.3 mL, 2.6 mmol), dropwise and the mixture stirred for 20 minutes. Pivalaldehyde (290 μL, 2.6 mmol) was added, dropwise and the mixture warmed to 0 °C and stirred for 16 hours. Saturated aqueous ammonium chloride (20 mL) was added and the mixture extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the alcohol 233 (290 mg, 47%) as a white solid, mp: 121.0-122.9 °C; $R_f$: 0.22 (1:9 ethyl acetate, hexane); IR (v$_{max}$ (neat)): 3352, 3041, 2963, 1555, 1521, 1379, 1179, 1057, 902, 863, 779 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): δ 0.99 (9H, s), 2.11 (1H, bs), 4.89 (1H, s), 8.72 (1H, s) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): δ 25.6, 37.4, 75.4, 133.1, 159.1, 160.9, 161.1 ppm; LRMS (-ESI) m/z: 469.0 ([2M-H]⁻ 100%).
1-(2,4-Dichloropyrimidin-5-yl)-2,2-dimethylpropan-1-one 234

To solution of Celite® (500 mg) and pyridinium chlorochromate (630 mg, 2.9 mmol) in anhydrous dichloromethane (10 mL) was added alcohol 233 (300 mg, 1.3 mmol) and the mixture was stirred for 16 hours. The mixture was filtered through a pad of Celite® and the filtrate concentrated to obtain a crude residue that was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (260 mg, 88%) as a colourless oil, $R_f$: 0.40 (1:9 ethyl acetate, hexane); **IR** ($\nu_{\text{max}}$ (neat)): 2960, 1715, 1529, 1230, 1045, 724 cm$^{-1}$; **$^1$H NMR** (300 MHz, CDCl$_3$): $\delta$ 1.28 (9H, s), 8.39 (1H, s) ppm; **$^{13}$C NMR** (75 MHz, CDCl$_3$): $\delta$ 26.5, 46.0, 132.8, 156.1, 158.6, 160.5, 205.9 ppm.

3-(tert-Butyl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidine 235

The ketone 234 (270 mg, 1.2 mmol) was dissolved in tetrahydrofuran (5 mL) and to this was added acetic acid (2 drops) and hydrazine (1 M in tetrahydrofuran, 1.2 mL, 1.2 mmol) and the mixture was heated at reflux for 2 hours. The volatiles were removed under a stream of nitrogen, the residue taken up in saturated aqueous sodium hydrogen carbonate (20 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with water (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced to pressure to afford a crude residue that was purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (1:99 to 1:49) as an eluent to obtain the title compound (100 mg, 42%) as a yellow solid, $mp$: 278.3-283.4 °C; $R_f$: 0.65 (7:13 ethyl acetate, hexane); **IR** ($\nu_{\text{max}}$ (neat)): 3097, 2959, 1592, 1569, 1324, 1157, 1041, 1015, 756 cm$^{-1}$; **$^1$H NMR** (300 MHz, DMSO-$d_6$): $\delta$ 1.44 (9H, s), 9.41 (1H, s) ppm; **$^{13}$C NMR** (75 MHz, DMSO-$d_6$): $\delta$ 29.7, 33.8, 110.4, 154.5, 155.2, 156.0, 156.4 ppm; **LRMS** (-ESI) $m/z$: 209.0/211.0 ([M-H]$^-$ 100%); **HRMS** (ESI)$^+$ Cald for C$_9$H$_{11}$ClN$_4$ [M-H]$^-$: 209.05940, found 209.05996/211.05702.
Chapter 8: Experimental

(R)-3-(tert-Butyl)-6-chloro-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidine 236

Prepared according to General Procedure I using 235 (80 mg, 380 μmol) and alcohol 85. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound (100 mg, 91%) as a yellow oil, Rf: 0.40 (1:4 ethyl acetate, hexane); IR (v max (neat)): 2970, 1707, 1549, 1356, 1133, 1090 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.45 (9H, s), 1.64-1.79 (1H, m), 1.81-2.07 (3H, m), 3.63-3.92 (2H, m), 4.16-4.55 (3H, m), 9.01 (1H, s) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 25.5, 29.2, 30.2, 34.4, 50.6, 68.2, 76.9, 111.4, 154.1, 154.5, 155.4, 157.5 ppm; LRMS (+ESI) m/z: 317.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Calcd for C₁₄H₁₉ClN₄O [M+Na]⁺: 317.11451, found 317.11381/319.11083.

(R)-6-(2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidine 237

Prepared according to General Procedure G using 236 (50 mg, 170 μmol), boronate ester 215 and cesium carbonate. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (78 mg, 79%) as a colourless oil, Rf: 0.38 (1:4 ethyl acetate, hexane); IR (v max (neat)): 2970, 1582, 1334, 1248, 1117 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.34 (6H, s), 1.53 (9H, s), 1.70-1.82 (1H, m), 1.82-2.04 (3H, m), 3.70-3.93 (2H, m), 4.04 (2H, s), 4.43 (1H, dd, J = 13.7, 5.3 Hz), 4.43 (2H, s), 4.45-4.53 (1H, m), 4.61 (1H, dd, J = 13.6, 6.9 Hz), 7.09 (1H, d, J = 8.7 Hz), 7.12-7.24 (5H, m), 7.63-7.69 (1H, m), 8.03 (1H, d, J = 2.4 Hz), 9.29 (1H, s) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 23.4, 23.5, 25.6, 29.5, 30.5, 34.5, 50.5, 64.8, 68.3, 75.1, 75.6, 110.5, 113.0, 123.1 (q, J = 32.6 Hz), 124.4 (q, J = 271.4 Hz), 127.3, 128.0 (q, J = 3.0 Hz), 128.3,
129.5 (q, 3.4 Hz), 139.5, 152.3, 154.0, 154.6, 159.7, 160.7 (one carbon signal was not observed) ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): δ –61.5 ppm; LRMS (+ESI) m/z: 583.3 ([M+H]$^+$ 90%), 605.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{32}$H$_{37}$F$_3$N$_4$O$_3$ [M+Na]$^+$: 605.27155, found 605.27036.

(R)-1-(2-(3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 182

Prepared according to General Procedure J using 237 (70 mg, 120 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (44 mg, 75%) as a colourless waxy solid, $R_f$: 0.10 (1:4 ethyl acetate, hexane); IR (v$_{\text{max}}$ (neat)): 3317, 2969, 1583, 1333, 1274, 1117, 1079 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 1.31 (6H, s), 1.53 (9H, s), 1.77-2.10 (4H, m), 3.73-3.97 (2H, m), 4.12 (2H, s), 4.39 (1H, dd, J = 13.8, 4.9 Hz), 4.48-4.57 (1H, m), 4.67 (1H, dd, J = 13.7, 7.2 Hz), 6.30 (1H, bs), 7.11 (1H, d, J = 8.6 Hz), 7.68 (1H, dd, J = 8.7, 2.4 Hz), 8.23 (1H, d, J = 2.3 Hz), 9.29 (1H, s) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 25.6, 26.2, 29.4, 30.4, 34.5, 50.6, 68.3, 70.1, 77.3, 80.1, 110.5, 115.9, 123.8 (q, J = 33.1 Hz), 124.4 (q, J = 271.7 Hz), 128.4 (q, J = 3.9 Hz), 128.6, 130.2 (q, J = 3.8 Hz), 151.9 154.3, 154.7, 160.1, 161.2 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): δ –61.7 ppm; LRMS (+ESI) m/z: 515.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{23}$H$_{31}$F$_3$N$_4$O$_3$ [M+Na]$^+$: 515.22459, found 515.22361; $[^a]_{b=22}^D$: -90.0 ° (0.5, CHCl$_3$); HPLC: 99.0%, RT: 28.1 mins. (Method A, 230 nm).
1-(2-(2-((4-Methoxybenzyl)oxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)pyrrolidin-2-one 238

A solution of cesium carbonate (150 mg, 450 μmol), 1,1,1-tris(hydroxyethyl)ethane (2.6 mg, 22 μmol), copper iodide (4 mg, 22 μmol), aryl iodide 218 (100 mg, 220 μmol) and 2-pyrrolidone (20 μL, 260 μmol) in dry, degassed dimethylformamide (1 mL) was heated under an atmosphere of nitrogen at 110 °C for 24 hours. The mixture was cooled, filtered through a pad of Celite®, washed with ethyl acetate (3 x 15 mL), concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (1:4 to 1:1) as an eluent to obtain the title compound (53 mg, 55%) as a light yellow oil, \( R_f: 0.32 \) (1:1 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ neat}) \): 2973, 1692, 1614, 1514, 1333, 1247, 1110, 1031, 817 cm\(^{-1}\); \( \text{^1H NMR} \) (300 MHz, CDCl\(_3\)): δ 1.39 (6H, s), 2.13 (2H, p, \( J = 7.5 \) Hz), 2.51 (2H, t, \( J = 8.0 \) Hz), 3.75 (2H, t, \( J = 7.0 \) Hz), 3.78 (3H, s), 3.95 (2H, s), 4.44 (2H, s), 6.85 (2H, d, \( J = 8.4 \) Hz), 7.00 (1H, d, \( J = 8.6 \) Hz), 7.22 (2H, d, \( J = 8.2 \) Hz), 7.47-7.57 (2H, m) ppm; \( \text{^{13C NMR} } \) (75 MHz, CDCl\(_3\)): δ 19.4, 23.3, 31.2, 50.1, 55.4, 64.2, 74.6, 74.7, 112.8, 113.9, 123.4 (q, \( J = 33.7 \) Hz), 126.1 (q, \( J = 3.9 \) Hz), 126.4 (q, \( J = 3.9 \) Hz), 127.9, 129.0, 131.2, 156.7, 159.2, 175.4 (one carbon signal was not observed) ppm; \( \text{^{19F NMR} } \) (282 MHz, CDCl\(_3\)): δ −61.5 ppm; \( \text{LRMS (+ESI) m/z: 460.2 ([M+Na]^+ 100%); HRMS (ESI)^+ Cald for C}_{23}H_{28}F_{3}NO_{4}[M+Na]^+: 460.17116, found 460.17027.} \)

(Allyloxy)trimethylsilane 240

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Allyl alcohol (11.7 mL, 170 mmol) was treated with trimethylsilyl chloride (1.1 mL, 8.5 mmol) followed by hexamethyldisilazane (24.9 mL, 120 mmol), slowly. The mixture was stirred at 100 °C for 16 hours and the product isolated by distillation (100-102 °C, 760 mmHg), to obtain the title compound (20 g, 90%) as a colourless liquid, the spectroscopic data of which corresponded to that previously described.328
**tert-Butyldimethyl((1-(trimethylsilyl)allyl)oxy)silane 241**

To a solution of 240 (500 mg, 3.8 mmol) in anhydrous tetrahydrofuran (10 mL) at -78 °C was added freshly distilled tetramethylethylenediamine (630 μL, 4.2 mmol) followed by **tert**-butyl lithium (1.28 M, 3.3 mL, 4.2 mmol), dropwise and the mixture stirred for 45 minutes. **tert**-Butyldimethylsilyl chloride (570 mg, 3.8 mmol) was added and the mixture stirred for a further 1 hour at -78 °C and then slowly warmed to room temperature and stirred for 16 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (20 mL), extracted with diethyl ether (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography (basic alumina) using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound (570 mg, 61%) as a colourless oil, the characterisation data of which corresponded to that previously described.222

**Dimethyl 2-(3,3-dimethyl-2-oxobutyl)malonate 242**

Sodium (290 mg, 12.7 mmol) was added portionwise to methanol (5.5 mL) and allowed to stir until hydrogen evolution had ceased. To the mixture was added a solution of dimethyl malonate (1.5 mL, 12.2 mmol) in methanol (50 mL), slowly. After stirring for 30 minutes, 133 (2.5 g, 12.7 mmol) was added slowly and the mixture was heated at reflux for 2 hours. After cooling to room temperature, water (50 mL) was added and the mixture extracted with diethyl ether (3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (1.7 g, 52%) as a colourless oil, the characterisation data of which corresponded to that previously described.329 **Rf**: 0.26 (1:4 ethyl acetate, hexane); **IR** (ν<sub>max</sub> (neat)): 2958, 1736, 1706, 1681, 1435, 1281, 1228, 1144, 1114 cm<sup>-1</sup>. 


**5,5-Dimethyl-4-oxohexanoic acid 243**

A solution of 242 (1.6 g, 6.9 mmol) and freshly ground potassium hydroxide (1.4 g, 24.3 mmol) in aqueous methanol (50% v/v, 20 mL) was heated at reflux for 2 hours. The reaction mixture was acidified with aqueous hydrochloric acid (1 M), extracted with diethyl ether (3 x 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the intermediate diacid species as a white solid.

The intermediate diacid was heated, neat, at 145 °C for 2 hours, cooled to room temperature and the crude solid recrystallised from hexane to obtain the title compound (710 mg, 65%) as an off-white crystalline solid, the characterisation data of which corresponded to that previously described.329 **IR (ν<sub>max</sub> (neat)): 2968, 2952, 1696, 1250, 1083, 942 cm⁻¹.**

**N-(2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxohexanamide 244**

To a solution of aniline 200 (1.5 g, 4.4 mmol) and carboxylic acid 244 (700 mg, 4.4 mmol) in anhydrous pyridine (15 mL) at 0 °C was added phosphorus oxychloride (500 μL, 5.3 mmol) and the mixture was warmed to room temperature and stirred for 2 hours. The reaction mixture was poured into aqueous hydrochloric acid (1 M, 100 mL), extracted with ethyl acetate (3 x 50 mL), washed with aqueous hydrochloric acid (1 M, 3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (850 mg, 40%) as an off-white waxy solid, **R<sub>r</sub>: 0.52 (3:7 ethyl acetate, hexane); IR (ν<sub>max</sub> (neat)): 3181, 2972, 1704, 1660, 1365, 1115, 1037, 896, 736 cm⁻¹; **<sup>1</sup>H NMR (300 MHz, CDCl₃): δ 1.09 (9H, s), 1.39 (6H, s), 2.40 (2H, t, J = 6.4 Hz), 2.79 (2H, t, J = 6.4 Hz), 3.93 (2H, s), 4.46 (2H, s), 6.87 (1H, d, J = 8.5 Hz), 7.10-7.31 (6H, m), 8.13 (1H, s), 8.60 (1H, s) ppm; **<sup>13</sup>C NMR (75 MHz, CDCl₃): δ 23.1, 26.7, 31.4, 32.0, 44.1, 64.6, 75.0, 75.6, 112.0, 117.1 (q, J = 3.5 Hz), 120.7 (q, J = 3.5 Hz),
123.9 (q, J = 33.3 Hz), 124.3 (q, J = 271.8 Hz), 127.6, 127.6, 128.5, 128.9, 139.0, 149.6, 170.8, 214.7 ppm; \( ^{19}F \text{ NMR} \) (282 MHz, CDCl₃): \( \delta \) −61.7 ppm; \( \text{LRMS} \) (+ESI) \( m/z \): 502.3 ([M+Na]⁺ 100%); \( \text{HRMS} \) (ESI)⁺ Calcd for C₂₆H₃₂F₃NO₄ [M+Na]⁺: 502.21811, found 502.21731.

6-((2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)amino)-2,2-dimethylhexan-3-ol 245

![Chemical Structure](image)

Prepared according to General Procedure D using 244 (1.0 g, 2.1 mmol). The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (830 mg, 85%) as a yellow oil, \( R_f \): 0.66 (3:7 ethyl acetate, hexane); \( \text{IR} \) (\( \nu_{\text{max}} \) (neat)): 2954, 2870, 1607, 1318, 1217, 1163, 1113, 735 cm⁻¹; \( ^1H \text{ NMR} \) (400 MHz, CDCl₃): \( \delta \) 0.86 (9H, s), 1.28-1.40 (1H, m), 1.43 (6H, s), 1.57-1.77 (2H, m), 1.79-1.92 (1H, m), 3.08-3.22 (3H, m), 3.97 (2H, s), 4.53 (2H, s), 6.75-6.80 (2H, m), 6.89 (1H, dd, 8.5, 2.1 Hz), 7.24-7.38 (5H, m) ppm; \( ^{13}C \text{ NMR} \) (100 MHz, CDCl₃): \( \delta \) 23.4, 23.5, 25.8, 27.1, 29.1, 35.1, 43.6, 64.6, 74.3, 75.0, 79.9, 105.9 (q, J = 3.1 Hz), 110.2, 113.2 (q, J = 3.6 Hz), 124.0 (q, J = 33.4 Hz), 124.9 (q, J = 271.1 Hz), 127.5, 127.7, 128.5, 139.2, 139.2, 148.3 ppm; \( ^{19}F \text{ NMR} \) (376 MHz, CDCl₃): \( \delta \) −61.6 ppm; \( \text{LRMS} \) (+ESI) \( m/z \): 486.3 ([M+H]⁺ 96%), 490.3 ([M+Na]⁺ 100%); \( \text{HRMS} \) (ESI)⁺ Calcd for C₂₆H₃₆F₃NO₃ [M+H]⁺: 468.27255, found 468.27207.
To a solution of 245 (150 mg, 320 μmol) and triethylamine (54 μL, 380 μmol) in anhydrous dichloromethane (5 mL) at 0 °C was added methyl chloroglyoxylate (44 μL, 480 μmol) and the mixture was warmed to room temperature and stirred for 2 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (15 mL) and extracted with dichloromethane (3 x 15 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the monoacylated product as a yellow oil.

The residue was taken up in dichloromethane (10 mL) and treated with Celite® followed by pyridium chlorochromate (72 mg, 340 μmol) and the mixture stirred at room temperature for 16 hours. The suspension was filtered through a pad of Celite® and the filtrate concentrated under reduced pressure to obtain the ketone intermediate.

The ketone intermediate was taken up in anhydrous tetrahydrofuran (3 mL) and added dropwise to a solution of potassium tert-butoxide in tetrahydrofuran (5 mL). The mixture was warmed to reflux and stirred for 2 hours. After cooling to room temperature the reaction was quenched with saturated aqueous ammonium chloride (15 mL), extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as a eluent to obtain the cyclised product 246 (125 mg, 75% over 3 steps) as an off-white waxy solid, Rf: 0.28 (3:2 chloroform, hexane); IR (νmax (neat)): 3598, 3522, 2989, 2971, 1665, 1610, 1333, 1318, 1267, 1114, 1041 cm⁻¹;¹H NMR (400 MHz, CDCl₃): δ 1.24 (9H, s), 1.41 (6H, s), 2.80 (2H, t, J = 6.6 Hz), 3.67 (2H, t, J = 6.6 Hz), 4.02 (2H, s), 4.50 (2H, s), 7.09 (1H, d, J = 8.4 Hz), 7.23-7.35 (5H, m), 7.55-7.62 (2H, m), 12.81 (1H, s) ppm;¹³C NMR (100 MHz, CDCl₃): δ 23.2, 25.0, 43.9, 48.6, 64.5, 74.6, 74.6, 112.5, 113.0, 123.5 (q, J = 33.9 Hz), 125.4 (q, J = 272.1 Hz), 126.3 (q, J = 3.9 Hz), 126.7 (q, J = 3.8 Hz), 127.5, 127.5, 128.4, 130.2, 139.0, 154.6, 156.3, 161.3, 210.8
ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –61.6 ppm; LRMS (+ESI) m/z: 542.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{28}$H$_{32}$F$_3$NO$_5$ [M+Na]$^+$: 542.21303, found 542.21211.

6-(2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-3-(tert-butyl)-5,6-dihydro-1H-pyrazolo[3,4-c]pyridin-7(4H)-one 247

To a solution of 246 (80 mg, 150 μmol) in ethanol (3 mL) was added acetic acid (1 drop) followed by hydrazine hydrate (9 μL, 190 μmol) and the mixture was heated at reflux for 4 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in ethyl acetate (30 mL), washed with water (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (80 mg, 100%) as a white solid, mp: 84.1-87.0 °C; $R_f$: 0.09 (3:7 ethyl acetate, hexane); IR ($\nu_{\text{max}}$ (neat)): 3191, 2969, 1658, 1614, 1563, 1470, 1333, 1115, 1079, 736 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.34 (15H, s), 2.95 (2H, t, $J = 6.4$ Hz), 3.77-3.92 (2H, m), 3.99 (2H, s), 4.45 (2H, s), 7.05 (1H, d, $J = 8.6$ Hz), 7.18-7.29 (5H, m), 7.53-7.60 (2H, m) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 22.3, 23.2, 29.7, 32.8, 51.5, 64.5, 74.7, 74.8, 112.9, 117.1, 123.4 (q, $J = 33.6$ Hz), 124.1 (q, $J = 271.8$ Hz), 126.3 (q, $J = 3.9$ Hz), 126.8 (q, $J = 3.8$ Hz), 127.4, 127.5, 128.4, 130.8, 139.2, 156.8, 159.5 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ –61.5 ppm; LRMS (+ESI) m/z: 538.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{28}$H$_{32}$F$_3$N$_3$O$_3$ [M+Na]$^+$: 538.22935, found 538.22852.
(R)-6-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-5,6-dihydro-1H-pyrazolo[3,4-c]pyridin-7(4H)-one 248

Prepared according to General Procedure L using 247 (83 mg, 160 μmol) and tosylate 213 (49 mg, 190 μmol) at 60 °C for 5 hours. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (70 mg, 71%) as a white solid, mp: 112.4-114.6 °C; \( R_f \): 0.57 (2:3 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 2961, 2867, 1658, 1410, 1387, 1365, 1163, 1136, 1092, 732 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.36 (9H, s), 1.37 (6H, s), 1.63-1.72 (1H, m), 1.77-1.97 (3H, m), 2.90-3.02 (2H, m), 3.71-3.92 (4H, m), 3.98-4.06 (2H, m), 4.36-4.46 (2H, m), 4.49 (2H, s), 4.63-4.76 (1H, m), 7.07 (1H, d, \( J = 9.2 \) Hz), 7.21-7.32 (5H, m), 7.53-7.59 (2H, m) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): 22.8, 23.2, 25.5, 29.0, 30.1, 33.1, 51.3, 54.3, 64.6, 68.2, 74.8, 75.0, 78.3, 112.9, 118.8, 123.4 (q, \( J = 33.1 \) Hz), 124.1 (q, \( J = 271.3 \) Hz), 126.2 (q, \( J = 3.1 \) Hz), 126.9 (q, \( J = 3.4 \) Hz), 127.4, 128.5, 128.4, 131.1, 131.8, 139.2, 155.0, 157.0, 169.2 ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \( \delta \) −61.5 ppm; LRMS (ESI) \( m/z \): 622.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{33}\)H\(_{40}\)F\(_3\)N\(_3\)O\(_4\) [M+Na]\(^+\): 622.28686, found 622.28559.

(R)-3-(tert-Butyl)-6-(2-hydroxy-2-methylpropoxy)-5-(trifluoromethyl)phenyl)-1-((tetrahydrofuran-2-yl)methyl)-5,6-dihydro-1H-pyrazolo[3,4-c]pyridin-7(4H)-one 183

Prepared according to General Procedure J using 248 (62 mg, 100 μmol) in the presence of acetic acid (5 drops). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (49 mg, 92%) as a colourless waxy solid, \( R_f \): 0.14 (2:3 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 3431,
2965, 1657, 1437, 1331, 1116, 1047 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.23 (6H, s), 1.36 (9H, s), 1.62-1.74 (1H, m), 1.76-1.89 (2H, m), 1.90-2.01 (1H, m), 3.05 (2H, t, $J = 6.7$ Hz), 3.68-3.77 (1H, m), 3.78-3.98 (5H, m), 4.31-4.53 (2H, m), 4.56-4.82 (1H, m), 7.04 (1H, d, $J = 8.6$ Hz), 7.50 (1H, d, $J = 2.2$ Hz), 7.54 (1H, dd, $J = 8.7, 2.2$ Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 22.3, 25.5, 26.1, 29.1, 30.1, 33.1, 51.9, 54.5, 68.2, 69.8, 77.3, 78.1, 113.1, 118.7, 123.7 (q, $J = 33.8$ Hz), 124.0 (q, $J = 271.5$ Hz), 125.7 (q, $J = 3.3$ Hz), 126.2 (q, $J = 3.7$ Hz), 131.5, 155.0, 156.9, 159.4 (one carbon signal was not observed due to peak broadening) ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -61.6 ppm; LRMS (+ESI) m/z: 532.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{26}$H$_{34}$F$_3$N$_3$O$_4$ [M+Na]$^+$: 532.23991, found 532.23906; [$\alpha$]$_D^{22}$: -25.9° (0.58, CHCl$_3$); HPLC: 99.8%, RT: 29.1 mins. (Method A, 254 nm).

**2-Fluoro-5-(trifluoromethyl)benzene-1-sulfonyl fluoride 249**

![Structure](image.png)

To a solution of 2-fluoro-5-trifluoromethylphenylsulfonyl chloride (180 mg, 690 μmol) in acetonitrile (5 mL) was added aqueous potassium hydrogen difluoride (2 M, 1.7 mL, 3.4 mmol) and the mixture was stirred at room temperature for 16 hours. The mixture was diluted with water (50 mL), extracted with ethyl acetate (3 x 40 mL), washed with saturated aqueous sodium chloride (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (150 mg, 88%) as a light yellow oil, $R_f$: 0.44 (1:9 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 2954, 2876, 1618, 1428, 1358, 1325, 1216, 1174, 1137, 1084, 962, 788 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.51 (1H, t, $J = 8.8$ Hz), 8.06 (1H, ddd, $J = 8.8, 4.4, 2.4$ Hz), 8.26 (1H, dd, $J = 6.0, 2.3$ Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 119.1 (d, $J = 21.5$ Hz), 122.5 (q, $J = 272.7$ Hz), 122.6-123.1 (m), 127.6-128.8 (m), 128.6-129.0 (m), 135.1-135.4 (m), 161.4 (d, $J = 268.3$ Hz) ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ 64.5, -62.5, -100.4 ppm.
(R)-3-(tert-Butyl)-6-((2-fluoro-5-(trifluoromethyl)phenyl)sulfonyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-indazole 250

To a solution of aryl bromide 229 (100 mg, 300 μmol) in anhydrous tetrahydrofuran (5 mL) at -78 °C was added n-butyllithium (2.5 M in hexanes, 180 μL, 450 μmol) dropwise and the mixture stirred for 15 minutes. A solution of sulfonyl fluoride 249 (72 mg, 300 μmol) in tetrahydrofuran (1 mL) was added and the mixture stirred for a further 3 hours. Saturated aqueous ammonium chloride (15 mL) was added and the mixture extracted with ethyl acetate (3 x 15 mL), washed with saturated aqueous sodium hydrogen carbonate (20 mL) and saturated aqueous sodium chloride (20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (72 mg, 50%) as a light yellow oil, \( R_f \): 0.20 (1:4 ethyl acetate, hexane); IR \( (v_{\text{max}} \text{ (neat)}) \): 2965, 1498, 1323, 1133, 1081 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.49 (9H, s), 1.69-1.91 (3H, m), 2.00-2.09 (1H, m), 3.65-3.81 (2H, m), 4.31-4.38 (1H, m), 4.42 (1H, dd, \( J = 14.3, 6.7 \) Hz), 4.51 (1H, dd, \( J = 14.4, 3.8 \) Hz), 7.23 (1H, t, \( J = 8.9 \) Hz), 7.57 (1H, dt, \( J = 8.8, 1.4 \) Hz), 7.80-7.76 (1H, m), 7.96 (1H, dd, \( J = 8.7, 0.7 \) Hz), 8.31-8.33 (1H, m), 8.43-8.47 (1H, m) ppm; \(^13\)C NMR (100 MHz, CDCl\(_3\)): 25.8, 29.0, 30.3, 33.9, 53.7, 68.4, 78.4, 112.6, 117.6, 118.4 (d, \( J = 22.4 \) Hz), 123.4, 124.2, 127.6 (m), 130.9 (m), 132.9 (m), 136.7, 140.8, 153.5, 161.2 (d, \( J = 258.3 \) Hz) (two carbon signals were not observed) ppm; \(^19\)F NMR (376 MHz, CDCl\(_3\)): \( \delta \) -62.2 (CF\(_3\)), -101.7 (CF) ppm; LRMS (+ESI) m/z: 507.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{23}\)H\(_{24}\)F\(_4\)N\(_2\)O\(_3\)S [M+Na]\(^+\): 507.13415, found 507.13323.
(R)-1-(2-((3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-indazol-6-yl)sulfonyl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 184

Prepared according to General Procedure E using 250 (55 mg, 110 μmol) and diol 93 at reflux for 6 hours. The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (40 mg, 63%) as a colourless foam, mp: 68.9-71.3 °C; Rf: 0.07 (3:7 ethyl acetate, hexane); IR (ν<sub>max</sub> (neat)): 3373, 2971, 1612, 1327, 1149, 1123, 1053 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.29 (3H, s), 1.33 (3H, s), 1.48 (9H, s), 1.61-2.87 (3H, m), 1.96-2.06 (1H, m), 3.17 (1H, s), 3.58-3.74 (2H, m), 3.93 (2H, s), 4.29-4.37 (1H, m), 4.41 (1H, dd, J = 14.4, 6.0 Hz), 4.47 (1H, dd, J = 14.2, 4.2 Hz), 7.03 (1H, d, J = 8.7 Hz), 7.52 (1H, dd, J = 8.7, 1.6 Hz), 7.76-7.82 (1H, m), 7.90-7.96 (1H, m), 8.82 (1H, dd, J = 1.7, 0.8 Hz), 8.45-8.48 (1H, m) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 25.7, 26.2, 29.0, 30.3, 33.9, 53.4, 68.4, 69.9, 78.4, 78.6, 111.6, 114.6, 117.2, 123.0, 123.6 (q, J = 272.6 Hz), 123.7 (q, J = 33.9 Hz), 123.9, 128.4 (q, J = 3.6 Hz), 129.8, 132.6 (q, J = 3.8 Hz), 137.9, 140.8, 153.5, 159.5 ppm; <sup>19</sup>F NMR (376 MHz, CDCl<sub>3</sub>): δ -61.8 ppm; LRMS (+ESI) m/z: 577.2 ([M+Na]<sup>+</sup> 100%); HRMS (ESI)<sup>+</sup> Calcd for C<sub>27</sub>H<sub>33</sub>F<sub>3</sub>N<sub>2</sub>O<sub>5</sub>S [M+Na]<sup>+</sup>: 577.19600, found 577.19498; [α]<sub>D</sub>: -14.7 ° (0.68, CHCl<sub>3</sub>); HPLC: 99.4%, RT: 30.6 mins. (Method A, 230 nm).

(R)-3-(tert-Butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole-4,5-dione 262

To a solution of 192 (600 mg, 2.7 mmol) in methanol (4.5 mL) was added potassium carbonate (74 mg, 530 μmol) followed by nitrosobenzene (290 mg, 2.7 mmol) and the mixture was heated at reflux for 3 hours. The solvent was removed under a stream of nitrogen and the residue taken up in dichloromethane (15 mL) and washed with saturated aqueous sodium chloride (2 x 10 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain a red solid that was used immediately without further purification or characterisation.
A solution of the imine intermediate in tetrahydrofuran (20 mL) was treated with aqueous hydrochloric acid (2 M, 6.8 mL, 13.5 mmol) and the mixture was stirred at room temperature for 1.5 hours. The mixture was diluted with water (30 mL), extracted with dichloromethane (5 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 1:1) as an eluent to obtain the title compound (230 mg, 35% over 2 steps) as a orange-red gummy mass, Rf: 0.62 (7:3 ethyl acetate, hexane); IR (vmax (neat)): 3261, 2961, 1703, 1559, 1364, 1288, 1063, 902 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 1.26 (9H, s), 1.59-1.72 (1H, m), 1.83-2.12 (3H, m), 3.54-3.65 (1H, m), 3.70-3.94 (3H, m), 4.20-4.31 (1H, m) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 25.6, 27.2, 29.2, 33.5, 49.5, 68.2, 75.6, 151.0, 151.7, 185.6 ppm; LRMS (+ESI) m/z: 239.2 ([M+H]+ 80%), 261.1 ([M+Na]+ 100%); HRMS (ESI)+ Cald for C₁₂H₁₈N₂O₃ [M+Na]+: 261.12151, found 261.12127.

(R)-7-(tert-Butyl)-3-(methylthio)-5-((tetrahydrofuran-2-yl)methyl)-5H-pyrazolo[3,4-e][1,2,4]triazine 263

To a solution of 262 (200 mg, 840 μmol) and potassium carbonate (140 mg, 1.0 mmol) in water (3 mL) was added thiosemicarbazide (84 mg, 920 μmol) and the mixture was stirred at room temperature for 1 hour then at reflux for 16 hours. The mixture was diluted with water (10 mL), acidified with acetic acid and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the thione intermediate, which was used immediately without further purification or characterisation.

A solution of the thione intermediate in anhydrous acetonitrile (3 mL) was treated with potassium carbonate (140 mg, 1.0 mmol) and methyl iodide (55 μL, 920 μmol) and the mixture was stirred at room temperature for 4 hours. The volatiles were removed under a stream of nitrogen and the residue purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:199) as an eluent to obtain the title compound (120 mg, 47% over 2 steps) as a yellow oil, Rf: 0.30 (1:4 ethyl acetate, hexane); IR (vmax (neat)): 2964, 2869, 1568, 1521, 1309, 1156, 1045, 1006 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ
1.58 (3H, s), 1.70-1.85 (1H, m), 1.86-1.96 (2H, m), 1.98-2.09 (1H, m), 2.69 (3H, s), 3.68-
3.78 (1H, m), 3.81-3.93 (2H, m), 4.17-4.29 (1H, m), 4.40-4.51 (1H, m) ppm; \(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\)): δ 14.1, 25.5, 29.4, 29.5, 30.5, 34.4, 50.4, 68.3, 139.1, 143.8, 155.3, 169.9 ppm; \(\text{LRMS}\) (+ESI) \(m/z\): 330.1 ([M+Na\(^+\) 100%); \(\text{HRMS}\) (ESI)\(^+\) Cald for C\(_{14}\)H\(_{21}\)N\(_5\)O\(_4\) [M+Na\(^+\)]: 330.13645, found 330.13613.

\(\text{2-(2-(Benzyloxy)-2-methylpropoxy)-5-(trifluoromethyl)phenyl} \)boronic acid 264

\[
\text{O}_\text{Bn} \\
\text{CF}_3 \\
\text{B(OH)}_2
\]

To a solution of 208 (500 mg, 1.2 mmol) in anhydrous diethyl ether (15 mL) at -78 °C was added n-butyllithium (2.4 M in hexanes, 630 μL, 1.5 mmol), dropwise and the mixture was stirred for 1 hour. Trisopropyl borate (910 μL, 3.6 mmol) was added, dropwise and the mixture was stirred for 1 hour at -78 °C and then a further 1 hour at room temperature. The reaction was quenched by the addition of saturated aqueous ammonium chloride (10 mL) and the mixture was stirred for 1 hour. The mixture was diluted with water (15 mL) and the aqueous phase extracted with diethyl ether (3 x 20 mL). The combined organic phases were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the resulting crude mass triturated with hexane to obtain the title compound (280 mg, 61%) as a white powder, mp: 125.7-128.7 °C; \(R_f\): 0.14 (1:9 ethyl acetate, hexane); \(\text{IR}\) (\(v_{\text{max}}\) (neat)): 3201, 1610, 1407, 1309, 1193, 1147, 1110, 1039, 742, 644 cm\(^{-1}\); \(^1\text{H NMR}\) (300 MHz, CDCl\(_3\)): δ 1.43 (6H, s), 4.02 (2H, s), 4.50 (2H, s), 6.15 (2H, s), 6.93 (1H, d, \(J = 8.7\) Hz), 7.20-7.44 (5H, m), 7.65 (1H, dd, \(J = 8.7, 2.4\) Hz), 8.11 (1H, d, \(J = 2.4\) Hz) ppm; \(^{19}\text{F NMR}\) (470 MHz, CDCl\(_3\)): δ -61.2 ppm; \(\text{LRMS}\) (-ESI) \(m/z\): 367.1 ([M-H\(^-\) 100%); \(\text{HRMS}\) (ESI)\(^-\) Cald for C\(_{18}\)H\(_{20}\)BF\(_3\)O\(_4\) [M-H\(^-\)]: 367.13285, found 367.13330.
A suspension of 263 (50 mg, 160 μmol), boronic acid 264 (120 mg, 330 μmol), copper(I)-thiophene-2-carboxylate (synthesised according to literature procedures using copper(I) oxide and 2-thiophencarboxylic acid,330 68 mg, 360 μmol), zinc acetate (36 mg, 190 μmol) and [1,1’-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (12 mg, 16 μmol) in anhydrous, degassed tetrahydrofuran (1.5 mL) was heated at 90 °C for 16 hours. The mixture was adsorbed onto Celite® and purified by flash column chromatography using a silica stationary phase with 3 cm of basic alumina loaded on the top of the column and ethyl acetate, dichloromethane (1:99 to 1:49) as an eluent to obtain the title compound (53 mg, 56%) as a yellow oil, 
\[ R_f = 0.73 \] (ethyl acetate, dichloromethane); IR \( v_{\text{max}} \) (neat): 2970, 2929, 1617, 1526, 1324, 1276, 1242, 1119, 1062, 823 cm\(^{-1}\); \( ^1H \) NMR (300 MHz, CDCl\(_3\)):
\[ \delta 1.36 \text{ (6H, s)}, 1.67 \text{ (9H, s)}, 1.80-2.08 \text{ (4H, m)}, 3.66-3.91 \text{ (2H, m)}, 4.07 \text{ (2H, s)}, 4.23-4.36 \text{ (1H, m)}, 4.44 \text{ (2H, s)}, 4.45-4.60 \text{ (2H, m)}, 7.07-7.32 \text{ (6H, m)}, 7.72 \text{ (1H, d, } J = 8.7 \text{ Hz)}, 8.20 \text{ (1H, s) ppm}; \]
\( ^{13}C \) NMR (75 MHz, CDCl\(_3\)):
\[ \delta 23.4, 25.6, 29.4, 29.7, 34.6, 50.5, 64.7, 68.3, 75.1, 75.6, 76.7, 113.0, 113.1, 123.2 \text{ (q, } J = 33.2 \text{ Hz)}, 125.0 \text{ (q, } J = 272.1 \text{ Hz)}, 127.3, 128.3, 128.7 \text{ (q, } J = 3.0 \text{ Hz)}, 139.4, 140.0, 143.6, 155.0, 159.7, 160.0 \text{ ppm (two carbon signals were not observed)}; \]
\( ^{19}F \) NMR (376 MHz, CDCl\(_3\)):
\[ \delta -61.6 \text{ ppm} \]
LRMS (+ESI) \( m/z \): 584.3 ([M+H]\(^+\) 100%), 606.3 ([M+Na]\(^+\) 10%); HRMS (ESI)\(^+\) Cald for C\(_{31}\)H\(_{38}\)F\(_3\)N\(_5\)O\(_3\) [M+H]\(^+\):
584.28485, found 584.28454.
(R)-1-(2-(7-(tert-Butyl)-5-((tetrahydrofuran-2-yl)methyl)-5H-pyrazolo[3,4-e][1,2,4]triazin-3-yl)-4-(trifluoromethyl)phenoxy)-2-methylpropan-2-ol 261

A solution of 265 (30 mg, 51 μmol) and durene (35 mg, 260 μmol) in toluene (1 mL) was treated with trifluoroacetic acid (500 μL) and the mixture heated at 90 °C for 16 hours. The reaction mixture was quenched with saturated methanolic ammonia (15 mL) and the volatiles were removed under a stream of nitrogen. The crude product was purified by flash column chromatography using ethyl acetate, dichloromethane (0:1 to 1:9) as an eluent to obtain the title compound (10 mg, 40%) as a yellow gummy mass, Rf: 0.10 (1:4 ethyl acetate, hexane); IR (νmax (neat)): 3412, 2971, 2872, 1527, 1323, 1275, 1117, 1064, 1006, 915, 823, 640 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 1.31 (6H, s), 1.66 (9H, s), 1.78-1.87 (1H, m), 1.88-1.98 (2H, m), 2.04-2.14 (1H, m), 3.74 (1H, ddd, J = 8.5, 7.3, 6.1 Hz), 3.87 (1H, dt, J = 8.5, 6.7 Hz), 4.11 (2H, s), 4.41 (1H, dd, J = 13.9, 4.3 Hz), 4.47-4.54 (1H, m), 4.62 (1H, dd, J = 13.9, 7.4 Hz), 7.16 (1H, d, J = 8.6 Hz), 7.74 (1H, dd, J = 8.7, 2.5 Hz), 8.33 (1H, d, J = 2.5 Hz) ppm; ¹³C NMR (125 MHz, CDCl₃): δ 25.6, 26.1, 29.4, 29.6, 34.6, 50.7, 68.4, 70.1, 77.0, 79.3, 115.1, 123.9 (q, J = 32.8 Hz), 124.3 (q, J = 273.5 Hz), 126.4, 129.2 (q, J = 3.4 Hz), 130.6 (q, J = 3.3 Hz), 140.0, 143.7, 155.3, 159.3, 161.0 ppm; ¹⁹F NMR (470 MHz, CDCl₃): δ -61.7 ppm; LRMS (+ESI) m/z: 494.3 ([M+H]+ 60%), 516.3 ([M+Na]+ 100%), 1009.4 ([2M+Na]+ 40%); HRMS (ESI)+ Calcd for C₂₄H₃₀F₃N₅O₃ [M+H]^+: 494.23790, found 494.23764; [α]D²²: +14.6 ° (1.2, CHCl₃); HPLC: 99.2%, RT: 31.3 mins. (Method A, 254 nm).

8.5.4 Synthetic Procedures from Chapter 5

5-(Benzyl oxy)pentan-1-ol 293

To a solution of 1,5-pentanediol (15.1 mL, 140 mmol) in anhydrous tetrahydrofuran (40 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil, 1.1 g, 28 mmol), portionwise and the mixture was stirred for 30 minutes before benzyl bromide (3.3 mL, 28
mmol) was added, dropwise. The mixture was warmed to room temperature and stirred for 16 hours. The reaction mixture was quenched with water (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with water (6 x 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (4.6 g, 85%) as a colourless oil, the characterisation data of which corresponded to that previously described.$^{331}$ *R*$_f$: 0.20 (1:4 ethyl acetate, hexane); LRMS (+ESI) *m/z*: 217.1 ([M+Na]$^+$ 100%).

(((5-Bromopentyl)oxy)methyl)benzene 294

To a solution of triphenylphosphine (6.8 g, 25.7 mmol) in dichloromethane (100 mL) at 0 °C was added bromine (1.3 mL, 25.7 mmol) and the mixture stirred for 30 minutes. A solution of the alcohol 293 (5.0 g, 25.7 mmol) in dichloromethane (20 mL) and pyridine (2.1 mL, 25.7 mmol) were added and the mixture was warmed to room temperature and stirred for 4 hours. The volatiles were removed and the residue suspended in water (200 mL), extracted with diethyl ether (3 x 100 mL) and dried over anhydrous magnesium sulfate. The ethereal extracts were concentrated (to ~100 mL) and hexane (100 mL) was added. The resulting precipitate was removed by filtration and the filtrate was aged at -30 °C for 24 hours. The large crystals of triphenylphosphine oxide were removed by filtration and the filtrate concentrated under reduced pressure to obtain the title compound (5.8 g, 88%) as a colourless oil, the characterisation data of which corresponded to that previously described.$^{332}$ *R*$_f$: 0.34 (1:19 ethyl acetate, hexane).

(S)-tert-Butyl (1-amino-3,3-dimethyl-1-oxobutan-2-yl)carbamate 295

Prepared according to General Procedure M using Boc-t-tert-leucine (2.0 g, 8.7 mmol) and ammonium chloride (690 mg, 13.0 mmol) to obtain the title compound (2.0 g, 100%) as a white foam, *R*$_f$: 0.55 (1:9 ethyl acetate, hexane); IR (v$_{max}$ (neat)): 3297, 2196, 2966, 1666, 1506, 1365, 1247, 1165, 1059, 1009 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$_d_6$): δ 0.89 (9H, s), 1.38 (9H, s), 3.79 (1H, d, J = 9.7 Hz), 6.29 (1H, d, J = 9.8 Hz), 7.02 (1H, bs), 7.31 (1H, bs) ppm; $^{13}$C NMR (75 MHz, DMSO-$_d_6$): 26.6, 28.2, 33.7, 61.8, 78.0, 155.2, 172.3 ppm; LRMS (+ESI) *m/z*: 253.3 ([M+Na]$^+$ 100%).
(S)-2-Amino-3,3-dimethylbutanamide hydrochloride 296

![Structural formula of (S)-2-Amino-3,3-dimethylbutanamide hydrochloride](image)

To a solution of the primary amide 295 (1.9 g, 8.3 mmol) in ethyl acetate (30 mL) was added hydrochloric acid (4 M in dioxane, 21 mL, 83 mmol) and the mixture stirred for 16 hours. The mixture was concentrated and the residue triturated with ethyl acetate and the resulting solid collected by filtration and dried in vacuo to afford the title compound (1.3 g, 95%) as a white solid, mp: 251.8-255.7 °C; IR (v_max (neat)): 3332, 3132, 2960, 1696, 1670, 1614, 1507, 1478 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 0.99 (9H, s), 3.49 (1H, s), 7.55 (1H, s), 7.95 (1H, s), 8.19 (3H, bs) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ 26.5, 32.5, 60.3, 169.0 ppm; LRMS (+ESI) m/z: 131.1 ([M+H]⁺ 100%).

(S)-Methyl 2-amino-3,3-dimethylbutanoate hydrochloride 297

![Structural formula of (S)-Methyl 2-amino-3,3-dimethylbutanoate hydrochloride](image)

To a solution of L-tert-leucine (1.0 g, 7.7 mmol) in methanol (60 mL) was added thionyl chloride (5.6 mL, 77 mmol) and the mixture was heated at reflux for 16 hours. The volatiles were removed under reduced pressure and the residue triturated with diethyl ether to obtain a white solid that was collected by filtration and dried in vacuo to obtain the title compound (1.1 g, 100%) as a gummy white hygroscopic solid, IR (v_max (neat)): 3383, 2961, 2365, 1735, 1613, 1517, 1248 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 1.00 (9H, s), 3.66 (1H, s), 8.31-9.00 (3H, m) ppm; ¹³C NMR (75 MHz, DMSO-d₆): 26.3, 33.1, 52.3, 60.7, 168.8 ppm; LRMS (+ESI) m/z: 146.1 ([M+H]⁺ 100%).

Ethyl 1-pentyl-1H-indole-2-carboxylate 298

![Structural formula of Ethyl 1-pentyl-1H-indole-2-carboxylate](image)

Prepared according to General Procedure L using ethyl indole-2-carboxylate (3.0 g, 15.9 mmol) and 1-bromopentane (2.4 mL, 19.1 mmol) to obtain the title compound (4.2 g, 100%) as a light yellow oil, Rf: 0.74 (1:4 ethyl acetate, hexane); IR (v_max (neat)): 2928, 1707, 1518, 1464, 1245, 1222, 1188, 1135, 1092, 745 cm⁻¹; ¹H NMR (300 MHz, CDCl₃):
δ 0.75-0.88 (3H, m), 1.19-1.42 (4H, m), 1.33 (3H, t, J = 7.1 Hz), 1.64-1.82 (2H, m), 4.30 (2H, q, J = 7.1 Hz), 4.42-4.56 (2H, m), 7.06 (1H, ddd, J = 8.0, 6.7, 1.2 Hz), 7.17-7.41 (3H, m), 7.56-7.65 (1H, m) ppm; 13C NMR (75 MHz, CDCl3): 14.2, 14.5, 22.6, 29.3, 30.5, 44.9, 60.6, 110.5, 110.6, 120.5, 122.7, 124.9, 126.1, 127.6, 139.2, 162.1 ppm; LRMS (+ESI) m/z: 282.2 ([M+Na]+ 100%).

*Ethyl 1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxylate 299*

Prepared according to General Procedure L using ethyl indole-2-carboxylate (3.0 g, 15.9 mmol) and alkyl bromide 294 (4.1 g, 15.9 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (4.6 g, 76%) as a light yellow oil, Rf: 0.55 (1:4 ethyl acetate, hexane); IR (ν max (neat)): 2928, 1707, 1454, 1246, 1178, 1092, 745 cm−1; 1H NMR (300 MHz, CDCl3): δ 1.31 (3H, t, J = 7.1 Hz), 1.30-1.43 (2H, m), 1.50-1.63 (2H, m), 1.67-1.80 (2H, m), 3.35 (2H, t, J = 6.4 Hz), 4.27 (2H, q, J = 7.1 Hz), 4.38 (2H, s), 4.43-4.52 (2H, m), 7.04 (1H, ddd, J = 8.0, 6.6, 1.3 Hz), 7.14-7.33 (8H, m), 7.53-7.62 (1H, m) ppm; 13C NMR (75 MHz, CDCl3): 14.5, 23.8, 29.6, 30.6, 44.8, 60.6, 70.3, 73.0, 110.5, 110.6, 120.5, 122.7, 124.9, 124.9, 126.1, 127.6, 127.6, 127.7, 128.4, 138.7, 139.1, 162.1 ppm; LRMS (+ESI) m/z: 388.2 ([M+Na]+ 100%); HRMS (ESI)+ Calcd for C23H27NO3 [M+Na]+: 388.18886, found 388.18814.

*I-Pentyl-1H-indole-2-carboxylic acid 300*

Prepared according to General Procedure B using ester 298 (4.0 g, 15.4 mmol) and aqueous sodium hydroxide (3 M, 26 mL, 77 mmol) in ethanol (50 mL) at reflux for 2 hours to obtain the title compound (3.4 g, 94%) as a white solid, mp: 108.2-111.4 °C; IR (ν max (neat)): 3032, 2952, 2859, 2601, 1677, 1519, 1426, 1265, 1225, 1133, 910, 740 cm−1; 1H NMR (300 MHz, CDCl3): δ 0.70-0.90 (3H, m), 1.16-1.37 (4H, m), 1.65-1.83 (2H, m), 4.50 (2H,
t, J = 7.4 Hz), 7.00-7.12 (1H, m), 7.22-7.45 (3H, m), 7.54-7.66 (1H, m) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): 14.1, 22.6, 29.2, 30.5, 45.0, 110.5, 112.9, 120.8, 123.1, 125.7, 126.1, 126.3, 139.8, 167.2 ppm; LRMS (-ESI) $m/z$: 230.0 ([M-H]$^-$ 100%).

**1-(5-(Benzyloxy)pentyl)-1H-indole-2-carboxylic acid 301**

![Structure of 1-(5-(Benzyloxy)pentyl)-1H-indole-2-carboxylic acid 301]

Prepared according to General Procedure B using ester 299 (4.0 g, 10.9 mmol) and aqueous sodium hydroxide (3 M, 18 mL, 54.7 mmol) in ethanol (50 mL) at reflux for 3 hours. The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (3.7 g, 100%) as a viscous yellow oil, $R_f$: 0.30 (1:4 ethyl acetate, hexane); IR ($\nu_{\text{max}}$ (neat)): 2921, 2859, 2588, 1677, 1519, 1237, 1093, 903, 728 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.39-1.61 (2H, m), 2.84 (2H, quin, J = 6.7 Hz), 1.89 (2H, quin, J = 7.7 Hz), 3.51 (2H, t, J = 6.3 Hz), 4.54 (2H, s), 4.58-4.68 (2H, m), 7.20 (1H, ddd, J = 7.9, 6.3, 1.5 Hz), 7.26-7.54 (8H, m), 7.75 (1H, d, J = 8.1 Hz) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$): 23.7, 29.6, 30.5, 44.8, 70.2, 73.0, 110.8, 112.9, 120.8, 123.1, 125.7, 126.0, 126.3, 127.6, 127.8, 128.5, 138.6, 139.8, 167.0 ppm; LRMS (+ESI) $m/z$: 360.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{21}$H$_{23}$NO$_3$ [M+Na]$^+$: 360.15756, found 360.15681.

**N-(Adamantan-1-yl)-1-pentyl-1H-indole-2-carboxamide 267**

![Structure of N-(Adamantan-1-yl)-1-pentyl-1H-indole-2-carboxamide 267]

Prepared according to General Procedure M using carboxylic acid 300 (120 mg, 520 μmol) and 1-adamantylamine (94 mg, 620 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (190 mg, 77%) as a white solid, the characterisation data of which corresponded to that previously described.$^{247}$ mp: 163.3-163.7 °C; $R_f$: 0.41 (1:19 ethyl acetate, hexane); IR ($\nu_{\text{max}}$ (neat)): 3290, 2906, 2853, 1632, 1535, 1454, 1343, 1280, 1220, 808, 747, 730 cm$^{-1}$; LRMS (+ESI) $m/z$: 387.3 ([M+Na]$^+$ 100%).

(S)-Methyl 3-methyl-2-(1-pentyl-1H-indole-2-carboxamido)butanoate 268

Prepared according to General Procedure M using carboxylic acid 300 (200 mg, 870 μmol) and L-valine methyl ester hydrochloride (170 mg, 1.0 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent and recrystallisation from hexane to obtain the title compound (190 mg, 64%) as a colourless solid, mp: 72.9-73.8 °C; Rf: 0.69 (2:3 ethyl acetate, hexane); IR (ν_max (neat)): 3365, 3282, 3195, 2957, 1650, 1630, 1460, 1303, 748 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.83-0.91 (3H, m), 1.00 (3H, d, J = 6.9 Hz), 1.04 (3H, d, J = 6.9 Hz), 1.25-1.37 (4H, m), 1.74-1.86 (2H, m), 2.24-2.36 (1H, m), 3.79 (3H, s), 4.48-4.56 (2H, m), 4.76 (1H, ddd, J = 8.9, 5.0 Hz), 6.66 (1H, d, J = 8.9 Hz), 6.95 (1H, s), 7.14 (1H, ddd, J = 7.9, 6.9, 1.0 Hz), 7.31 (1H, ddd, J = 8.4, 6.9, 1.2 Hz), 7.37-7.42 (1H, m), 7.65 (1H, dt, J = 8.0, 1.0 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 14.1, 18.1, 19.2, 21.6, 29.3, 30.5, 31.8, 44.7, 52.4, 57.2, 104.5, 110.6, 120.6, 122.1, 124.2, 126.2, 131.4, 138.6, 162.5, 172.6 ppm; LRMS (+ESI) m/z: 352.2 ([M+Na]+ 100%); HRMS (ESI)+ Calcd for C_{20}H_{28}N_{2}O_{3} [M+Na]+: 367.19976, found 367.19903; [α]_D^{27}: +35.2 ° (0.62, CHCl₃); HPLC: 95.8%, RT: 31.1 mins. (Method A, 230 nm).

(S)-Methyl 3,3-dimethyl-2-(1-pentyl-1H-indole-2-carboxamido)butanoate 270

Prepared according to General Procedure M using carboxylic acid 300 (250 mg, 1.1 mmol) and amine hydrochloride salt 297 (230 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound (210 mg, 54%) as a colourless oil, Rf: 0.42 (1:19 ethyl acetate, hexane); IR (ν_max (neat)): 3256, 2952, 1737, 1633, 1622, 1536, 1251, 1014, 741 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.83-0.89 (3H, m), 1.07 (9H, s), 1.26-1.37 (4H, m), 1.79 (2H,
quin, \( J = 7.7 \) Hz), 3.78 (3H, s), 4.49-4.55 (2H, m), 4.67 (1H, d, \( J = 9.6 \) Hz), 6.70 (1H, d, \( J = 9.5 \) Hz), 6.93 (1H, d, \( J = 0.8 \) Hz), 7.14 (1H, ddd, \( J = 7.9, 6.9, 1.0 \) Hz), 7.31 (1H, ddd, \( J = 8.4, 6.9, 1.2 \) Hz), 7.37-7.42 (1H, m), 7.65 (1H, dt, \( J = 8.0, 1.0 \) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta 14.1, 22.6, 26.8, 29.3, 30.5, 35.4, 44.8, 52.1, 60.0, 104.4, 110.6, 120.6, 122.0, 124.2, 126.2, 131.5, 138.6, 162.3, 172.2 \) ppm; LRMS (+ESI) \( m/z \): 381.2 ([M+Na]\(^+\) 100%), 739.3 ([2M+Na]\(^+\) 43%); HRMS (ESI)\(^+\) Cald for C\(_{21}\)H\(_{30}\)N\(_2\)O\(_3\) [M+Na]\(^+\): 381.21541, found 381.21466; \([\alpha]\)\(_D\)\(^{27}\): +21.1 ° (1.42, CHCl\(_3\)); HPLC: 99.3%, RT: 31.7 mins. (Method A, 230 nm).

\((S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-2-carboxamide 273\)

Prepared according to General Procedure M using carboxylic acid 300 (120 mg, 520 \( \mu \)mol) and L-valinamide hydrochloride (95 mg, 620 \( \mu \)mol). The crude product was purified by recrystallisation from ethyl acetate to obtain the title compound (170 mg, 100%) as a white solid, \( \text{mp}: 246.1-248.9 \) °C; \( R_f: 0.55 \) (9:1 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 3365, 3282, 3195, 2957, 1650, 1630, 1530, 1460, 1303, 748 cm\(^{-1}\); \(^1\)H NMR (400 MHz, DMSO-\( d_6\)): \( \delta 0.80 \) (3H, t, \( J = 7.1 \) Hz), 0.93 (3H, d, \( J = 6.8 \) Hz), 0.95 (3H, d, \( J = 6.8 \) Hz), 1.11-1.29 (4H, m), 1.65 (2H, quin, \( J = 7.4 \) Hz), 2.11 (1H, sept, \( J = 6.9 \) Hz), 4.26 (1H, dd, \( J = 8.9, 7.5 \) Hz), 4.51 (2H, t, \( J = 7.3 \) Hz), 7.05-7.13 (2H, m), 7.16 (1H, s), 7.25 (1H, ddd, \( J = 8.3, 6.9, 1.2 \) Hz), 7.44 (1H, bs), 7.53 (1H, dd, \( J = 8.4, 0.9 \) Hz), 7.64 (1H, d, \( J = 7.9 \) Hz), 8.14 (1H, d, \( J = 8.9 \) Hz) ppm; \(^{13}\)C NMR (100 MHz, DMSO-\( d_6\)): \( \delta 13.8, 18.5, 19.5, 21.9, 28.4, 29.9, 30.1, 43.5, 58.3, 105.0, 110.5, 120.0, 121.6, 123.4, 125.7, 131.7, 137.7, 161.8, 172.9 ppm; LRMS (+ESI) \( m/z \): 352.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{19}\)H\(_{27}\)N\(_3\)O\(_2\) [M+Na]\(^+\): 352.20010, found 352.19950; \([\alpha]\)\(_D\)\(^{27}\): +70.2 ° (0.57, DMSO); HPLC: 98.8%, RT: 26.9 mins. (Method A, 230 nm).
(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-indole-2-carboxamide 275

Prepared according to General Procedure M using carboxylic acid 300 (200 mg, 870 μmol) and amine hydrochloride salt 296 (170 mg, 1.0 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (190 mg, 64%) as a colourless solid, mp: 92.9-96.6 °C; \( R_f \): 0.31 (1:1 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 3370, 3196, 2955, 1638, 1523, 1399, 1228, 741 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)):\( \delta \) 0.86 (3H, t, \( J = 6.8 \) Hz), 1.12 (9H, s), 1.25-1.37 (4H, m), 1.72-1.82 (2H, m), 4.41-4.56 (2H, m), 4.61 (1H, d, \( J = 9.4 \) Hz), 5.99 (1H, bs), 6.42 (1H, bs), 6.96 (1H, s), 7.00 (1H, d, \( J = 9.5 \) Hz), 7.13 (1H, ddd, \( J = 7.9, 6.9, 1.1 \) Hz), 7.30 (1H, ddd, \( J = 8.4, 6.9, 1.2 \) Hz), 7.35-7.40 (1H, m), 7.61 (1H, dt, \( J = 8.0, 1.0 \) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)):\( \delta \) 14.1, 22.6, 26.8, 29.3, 30.5, 35.0, 44.7, 60.0, 104.9, 110.5, 120.7, 122.1, 124.2, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; LRMS (+ESI) \( m/z \): 366.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Calcd for C\(_{20}\)H\(_{29}\)N\(_3\)O\(_2\) [M+Na]\(^+\): 366.21575, found 366.21501; \([\alpha]_D^{27}\): +48.2 ° (0.52, DMSO); HPLC: 99.6%, RT: 28.1 mins. (Method A, 230 nm).

(S)-Methyl 2-(1-(5-(benzyloxy)pentyl)-1H-indole-2-carboxamido)-3-methylbutanoate 302

Prepared according to General Procedure M using carboxylic acid 301 (300 mg, 930 μmol) and methyl-L-valinate hydrochloride (190 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (260 mg, 62%) as a light yellow oil, \( R_f \): 0.15 (1:9 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 3278, 2932, 1738, 1656, 1523, 1455, 1204, 1090, 743 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)):\( \delta \) 1.02 (3H, d, \( J = 6.9 \) Hz), 1.06 (3H, d, \( J = 6.9 \) Hz), 1.38-1.53 (2H, m), 1.60-1.74 (2H, m), 1.85 (2H, quin, \( J = 7.7 \) Hz), 2.20-2.42 (1H, m), 3.46 (2H, t, \( J = 6.5 \) Hz), 3.81 (3H, s), 4.49 (2H, s), 4.52-4.62 (2H, m), 4.77 (1H, dd, \( J = 8.9, 4.9 \) Hz), 6.68 (1H, d, \( J = 8.9 \) Hz), 6.97 (1H, s), 7.17 (1H, ddd, \( J = 7.9, 6.8, 1.1 \) Hz), 7.25-7.48 (7H, m), 7.68 (1H, dt, \( J = 8.0, 1.0 \) Hz) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)):\( \delta \) 18.1, 19.2, 23.8, 29.6, 30.6, 31.8,
44.7, 52.4, 57.2, 70.3, 73.0, 104.6, 110.5, 120.6, 122.1, 123.2, 126.2, 127.6, 127.7, 128.5, 131.3, 138.6, 138.8, 162.4, 172.6 ppm; LRMS (+ESI) m/z: 473.3 ([M+Na]+ 100%); HRMS (ESI)+ Calcd for C_{27}H_{34}N_{2}O_{4} [M+Na]+: 473.24163, found 473.24102.

(S)-Methyl 2-(1-(5-(benzoyloxy)pentyl)-1H-indole-2-carboxamido)-3,3-dimethylbutanoate 303

Prepared according to General Procedure M using carboxylic acid 301 (300 mg, 930 μmol) and amine hydrochloride salt 297 (200 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound (170 mg, 40%) as a colourless oil, Rf: 0.25 (1:9 ethyl acetate, hexane); IR (νmax (neat)): 3260, 2939, 1736, 1639, 1534, 1453, 1085, 742 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 0.99 (9H, s), 1.27-1.43 (2H, m), 1.56 (2H, quin, J = 6.7 Hz), 1.75 (2H, quin, J = 7.7 Hz), 3.36 (2H, t, J = 6.5 Hz), 3.69 (3H, s), 4.39 (2H, s), 4.41-4.50 (2H, m), 4.58 (1H, d, J = 9.5 Hz), 6.62 (1H, d, J = 9.6 Hz), 6.85 (1H, s), 7.07 (1H, ddd, J = 8.0, 6.8, 1.1 Hz), 7.14-7.35 (7H, m), 7.57 (1H, d, J = 7.9 Hz) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 23.8, 26.8, 29.7, 30.6, 35.3, 44.7, 52.1, 60.0, 70.3, 73.0, 104.5, 110.6, 120.6, 122.1, 124.3, 126.2, 127.6, 127.7, 128.5, 131.4, 138.6, 138.8, 162.2, 172.1 ppm; LRMS (+ESI) m/z: 487.3 ([M+Na]+ 100%); HRMS (ESI)+ Calcd for C_{28}H_{36}N_{2}O_{4} [M+Na]+: 487.25728, found 487.25661.

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-(benzoyloxy)pentyl)-1H-indole-2-carboxamide 304

Prepared according to General Procedure M using carboxylic acid 301 (350 mg, 1.1 mmol) and l-valinamide hydrochloride (200 mg, 1.3 mmol). The crude product was purified by recrystallisation from ethyl acetate, hexane to obtain the title compound (320 mg, 68%) as
a white solid, mp: 179.1-182.5 °C; RF: 0.46 (4:1 ethyl acetate, hexane); IR (v<sub>max</sub> (neat)): 3404, 3278, 2856, 1673, 1532, 1455, 1255, 1088 cm<sup>-1</sup>; H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 0.94 (6H, d, J = 6.8 Hz), 1.19-1.38 (2H, m), 1.51 (2H, quin, J = 6.7 Hz), 1.67 (2H, quin, J = 7.1 Hz), 2.12 (1H, sept, J = 6.8 Hz), 3.35 (2H, t, J = 6.7 Hz), 4.28 (1H, t, J = 8.1 Hz), 4.49 (2H, s), 4.51 (2H, t, J = 7.2 Hz), 7.03-7.37 (9H, m), 7.45 (1H, bs, NH), 7.53 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 7.9 Hz), 8.13 (1H, d, J = 8.9 Hz) ppm; C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 18.5, 19.4, 23.0, 28.9, 30.1, 43.6, 58.3, 69.4, 71.7, 115.0, 110.6, 120.0, 121.6, 123.4, 125.7, 127.2, 127.3, 128.2, 131.6, 137.7, 138.7, 161.8, 172.9 ppm; LRMS (+ESI) m/z: 458.3 ([M+Na]+ 100%); HRMS (ESI) Calcd for C<sub>26</sub>H<sub>33</sub>N<sub>3</sub>O<sub>3</sub>[M+Na]+: 458.24196, found 458.24111.

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-((5-(benzyl)oxy)pentyl)-IH-indole-2-carboxamide 305

Prepared according to General Procedure M using carboxylic acid 301 (300 mg, 930 μmol) and amine hydrochloride salt 296 (190 mg, 1.1 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (2:3 to 1:1) as an eluent to obtain the title compound (290 mg, 70%) as a white foam, RF: 0.35 (1:1 ethyl acetate, hexane); IR (v<sub>max</sub> (neat)): 3251, 2939, 1638, 1536, 1453, 1084, 742 cm<sup>-1</sup>; H NMR (300 MHz, CDCl<sub>3</sub>): δ 1.13 (9H, s), 1.35-1.50 (2H, m), 1.65 (2H, quin, J = 6.7 Hz), 1.83 (2H, quin, J = 7.6 Hz), 3.45 (2H, t, J = 6.4 Hz), 4.46-4.57 (4H, m), 4.60 (1H, d, J = 9.4 Hz), 6.00 (1H, bs), 6.40 (1H, ds), 6.95-7.04 (2H, m), 7.16 (1H, ddd, J = 7.9, 6.7, 1.2 Hz), 7.25-7.45 (7H, m), 7.66 (1H, d, J = 8.0 Hz) ppm; C NMR (75 MHz, CDCl<sub>3</sub>): δ 23.8, 26.8, 29.6, 30.6, 35.0, 44.6, 60.0, 70.2, 73.0, 104.9, 110.5, 120.6, 122.1, 124.3, 126.2, 127.6, 127.7, 128.5, 131.3, 138.6, 138.7, 162.6, 172.8 ppm; LRMS (+ESI) m/z: 472.3 ([M+Na]+ 100%); HRMS (ESI) Calcd for C<sub>27</sub>H<sub>35</sub>N<sub>3</sub>O<sub>3</sub>[M+Na]+: 472.25761, found 472.25680.
(S)-Methyl 2-(1-(5-hydroxypentyl)-1H-indole-2-carboxamido)-3-methylbutanoate 269

Prepared according to General Procedure J using 302 (190 mg, 420 μmol) in tetrahydrofuran (10 mL). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (100 mg, 67%) as a white solid, mp: 87.1-90.8 °C; Rr: 0.10 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 3395, 3290, 2945, 1745, 1637, 1529, 1459, 1203, 1147, 1007, 748 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.01 (3H, d, J = 6.9 Hz), 1.04 (3H, d, J = 6.8 Hz), 1.33-1.43 (2H, m), 1.50-1.60 (2H, m), 1.70 (1H, bs), 1.83 (2H, quin, J = 7.4 Hz), 2.22-2.35 (1H, m), 3.59 (2H, t, J = 6.4 Hz), 3.79 (3H, s), 4.45-4.65 (2H, m), 4.72 (1H, dd, J = 8.9, 5.0 Hz), 6.66 (1H, d, J = 8.9 Hz), 6.95 (1H, d, J = 0.8 Hz), 7.14 (1H, ddd, J = 8.0, 6.9, 1.0 Hz), 7.31 (1H, ddd, J = 8.4, 6.9, 1.2 Hz), 7.36-7.41 (1H, m), 7.65 (1H, dt, J = 8.0, 1.0 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 18.1, 19.2, 23.3, 30.4, 31.6, 32.5, 44.5, 52.5, 57.3, 62.8, 104.7, 110.5, 120.7, 122.1, 124.3, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; LRMS (+ESI) m/z: 383.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Calcd for C₂₀H₂₈N₂O₄ [M+Na]⁺: 383.19467, found 383.19401; [α]D²⁷: +6.8 ° (0.75, CHCl₃); HPLC: 98.6%, RT: 25.1 mins. (Method A, 230 nm).

(S)-Methyl 2-(1-(5-hydroxypentyl)-1H-indole-2-carboxamido)-3,3-dimethylbutanoate 271

Prepared according to General Procedure J using 303 (120 mg, 260 μmol) in a mixture of methanol (5 mL) and tetrahydrofuran (5 mL). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:7) as an eluent to obtain the title compound (84 mg, 87%) as a colourless oil, Rr: 0.09 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 3357, 2935, 1732, 1647, 1523, 1458, 1320, 1215, 1163, 741 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.07 (9H, s), 1.33-1.43 (2H, m), 1.51-1.65 (3H, m), 1.83 (1H, quin, J = 7.4 Hz), 3.60 (2H, t, J = 6.4 Hz), 3.78 (3H, s), 4.47-4.62 (2H, m), 4.63 (1H, d, J = 9.5 Hz), 6.70 (1H, d, J = 9.5 Hz), 6.93 (1H, d, J = 0.8 Hz), 7.15 (1H, ddd, J = 7.9, 6.9, 1.0 Hz), 7.31 (1H, ddd, J = 8.3, 6.9, 1.2 Hz), 7.36-7.41 (1H, m), 7.65 (1H, dt, J = 8.0, 1.0 Hz) ppm;
**Chapter 8: Experimental**

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 23.3, 26.8, 30.4, 32.5, 32.5, 44.6 52.1, 60.1, 62.8, 104.6, 110.5, 120.7, 122.1, 124.3, 126.3, 131.5, 138.5, 162.4, 172.3 ppm; LRMS (+ESI) $m/z$: 397.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{21}$H$_{30}$N$_2$O$_4$ [M+Na]$^+$: 397.21033, found 397.20957; $[\alpha]_D^{27}$: +13.3 ° (0.58, CHCl$_3$); HPLC: 97.8%, RT: 26.4 mins. (Method A, 230 nm).

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-indole-2-carboxamide 274

![Chemical Structure](image)

Prepared according to General Procedure J using 304 (250 mg, 570 μmol) in a mixture of methanol (5 mL) and tetrahydrofuran (5 mL). The crude residue was purified by recrystallisation from isopropanol, diethyl ether to obtain the title compound (170 mg, 83%) as a white solid, mp: 220.8-221.2 °C; $R_f$: 0.10 (4:1 ethyl acetate, hexane); IR (νmax (neat)): 3382, 3263, 2934, 1622, 1536, 1265, 1253, 741 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 0.88-0.99 (6H, m), 1.17-1.30 (2H, m), 1.39 (2H, quin, $J$ = 6.9 Hz), 1.65 (2H, quin, $J$ = 7.4 Hz), 2.12 (1H, sept, $J$ = 6.8 Hz), 3.28-3.40 (2H, m), 4.23-4.35 (2H, m), 4.50 (2H, t, $J$ = 7.7 Hz), 7.05-7.14 (2H, m), 7.17 (1H, s), 7.25 (1H, t, $J$ = 7.7 Hz), 7.46 (1H, bs), 7.53 (1H, d, $J$ = 8.4 Hz), 7.64 (1H, d, $J$ = 7.9 Hz), 8.15 (1H, d, $J$ = 8.9 Hz) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 18.5, 19.5, 22.9, 22.9, 30.1, 30.2, 32.3, 43.7, 58.3, 60.6 105.0, 110.6, 120.0, 121.6, 123.5, 125.7, 131.6, 137.7, 161.8, 172.9 ppm; LRMS (+ESI) $m/z$: 368.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{19}$H$_{27}$N$_3$O$_3$ [M+Na]$^+$: 368.19501, found 368.19431; $[\alpha]_D^{27}$: +33.3 ° (0.75, DMSO); HPLC: 95.2%, RT: 21.5 mins. (Method A, 230 nm).

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-indole-2-carboxamide 276

![Chemical Structure](image)

Prepared according to General Procedure J using 305 (240 mg, 540 μmol) in a mixture of methanol (5 mL) and tetrahydrofuran (5 mL). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:2 to 7:3) as an eluent and
recrystallisation from dichloromethane, hexane to obtain the title compound (160 mg, 84%) as a white solid, \( \text{mp}: 140.8-144.7 \degree \text{C} \); \( R_t: 0.08 \) (3:2 ethyl acetate, hexane); \( \text{IR (v}_{\text{max}} \text{ (neat)}): 3415, 3331, 3189, 2933, 1632, 1527, 1457, 1403, 1354, 1314 \text{ cm}^{-1}; 1^1 \text{H NMR (400 MHz, DMSO-\text{d}_6)}: \delta 1.02 (9H, s), 1.18-1.29 (2H, m), 1.39 (2H, quin, \( J = 6.9 \) Hz), 1.65 (1H, quin, \( J = 7.5 \) Hz), 3.29-3.37 (2H, m), 4.31 (1H, t, \( J = 5.1 \) Hz), 4.41 (1H, d, \( J = 9.6 \) Hz), 4.46-4.53 (2H, m), 7.09 (1H, ddd, \( J = 7.9, 6.9, 0.9 \) Hz), 7.13-7.20 (2H, m), 7.26 (1H, ddd, \( J = 8.3, 7.0, 1.2 \) Hz), 7.50-7.56 (2H, m), 7.64 (1H, dt, \( J = 8.0, 0.9 \) Hz), 7.80 (1H, d, \( J = 9.6 \) Hz) ppm; \( 13^1 \text{C NMR (100 MHz, DMSO-\text{d}_6)}: \delta 23.3, 27.4, 30.7, 32.7, 34.6, 44.2, 60.6, 61.0, 105.4, 111.0, 120.5, 122.1, 124.0, 126.2, 132.1, 138.2, 162.1, 172.3 \text{ ppm}; \text{LRMS (ESI) m/z: 382.2 ([M+Na]^+ 100%); HRMS (ESI)}^+ \text{ Calcd for C}_{20}\text{H}_{29}\text{N}_3\text{O}_3 [M+Na]^+: 382.21066, found 382.20991; } [\alpha]D^{27}: +48.4 \degree \) (0.62, DMSO); \( \text{HPLC: 98.2%, RT: 22.7 mins. (Method A, 230 nm).} \)

\( (S)-N-(1\text{-amino-3,3-dimethyl-1-oxobutan-2-yl})-1-(5,5\text{-difluoropentyl})-1H\text{-indole-2-carboxamide 272} \)

\( \text{To a solution of alcohol 276 (70 mg, 196 \mu \text{mol}) in a mixture of dichloromethane (1 mL) and tetrahydrofuran (1 mL) at 0 \degree \text{C} \) was added Dess-Martin periodinane (100 mg, 236 \mu \text{mol}) and the mixture was warmed to room temperature and stirred for 16 hours. The solids were removed by filtration through a pad of silica and the filtrate concentrated under reduced pressure to obtain the crude aldehyde intermediate, which was used immediately without further purification or characterisation.

The crude aldehyde (50 mg, 140 \mu \text{mol}) was taken up in dichloromethane (2 mL) and cooled to 0 \degree \text{C}. Diethylaminosulfur trifluoride (43 \mu \text{L}, 322 \mu \text{mol}) was added slowly and the mixture was warmed to room temperature and stirred for 24 hours. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (10 mL) and the aqueous phase extracted with dichloromethane (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the crude product purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:1) as an eluent to obtain the title compound 272 (30 mg, 40%) as a colourless foam, \( R_t: 0.70 \) (7:3 ethyl acetate, hexane); \( \text{IR (v}_{\text{max}} \text{ (neat)): 3323, 3196, 2961, 2928, 1641, 1525,} \)
1455, 1403, 1026, 735 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.11 (9H, s), 1.42-1.57 (2H, m), 1.78-1.94 (4H, m), 4.45 (1H, d, $J = 9.3$ Hz), 4.51-4.60 (2H, m), 5.56 (1H, bs), 5.73 (1H, bs), 5.77 (1H, tt, $J = 56.8, 4.5$ Hz), 6.91 (1H, d, $J = 9.1$ Hz), 6.97 (1H, s), 7.15 (1H, ddd, $J = 8.0, 6.7, 1.0$ Hz), 7.29-7.40 (2H, m), 7.65 (1H, d, $J = 8.0$ Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 19.8 (t, $J = 5.7$ Hz), 26.8, 30.0, 33.9 (t, $J = 20.9$ Hz), 35.0, 44.3, 60.2, 105.0, 110.3, 117.3 (t, $J = 238.9$ Hz), 120.8, 122.3, 124.5, 126.3, 131.2, 138.5, 162.5, 172.4 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -115.9 ppm; LRMS (+ESI) m/z: 402.2 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{20}$H$_{27}$F$_2$N$_3$O$_2$ [M+Na]$^+$: 402.19690, found 402.19702; $[\alpha]_D^{27}$: +10.3 (0.66, CHCl$_3$); HPLC: 96.8%, RT: 22.4 mins.

$^{1}$-Pentyl-$N$-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide 277

Prepared according to General Procedure M using carboxylic acid 300 (150 mg, 650 μmol) and 1-methyl-1-phenylethylamine (110 μL, 780 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (130 mg, 58%) as a white solid, mp: 122.6-124.1 °C; $R_t$: 0.55 (1:4 ethyl acetate, hexane); IR ($v_{\text{max}}$ (neat)): 3412, 3253, 2928, 1635, 1538, 1457, 729 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.86 (3H, t, $J = 6.8$ Hz), 1.12 (9H, s), 1.20-1.36 (4H, m), 1.74 (2H, quin, $J = 7.5$ Hz), 1.83 (6H, s), 4.47-4.54 (2H, m), 6.50 (1H, bs), 6.87 (1H, d, $J = 0.8$ Hz), 7.14 (1H, ddd, $J = 8.0, 6.9, 1.0$ Hz), 7.23-7.33 (2H, m), 7.33-7.40 (3H, m), 7.46-7.51 (2H, m), 7.64 (1H, dt, $J = 8.1, 1.0$ Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.1, 22.6, 26.8, 29.3, 30.5, 35.0, 44.7, 60.0, 104.9, 110.5, 120.7, 122.1, 124.2, 126.3, 131.4, 138.5, 162.6, 172.8 ppm; LRMS (+ESI) m/z: 371.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{23}$H$_{28}$N$_2$O [M+Na]$^+$: 371.20993, found 371.20918; HPLC: 99.8%, RT: 31.8 mins. (Method A, 230 nm).
1-(5-(Benzyloxy)pentyl)-N-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide 306

Prepared according to General Procedure M using carboxylic acid 301 (220 mg, 695 μmol) and 1-methyl-1-phenylethylamine (120 μL, 830 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (260 mg, 62%) as a white solid, mp: 77.1-80.2 °C; Rf: 0.52 (1:4 ethyl acetate, hexane); IR (v_{max} (neat))): 3259, 2937, 1638, 1536, 1452, 1270, 747 cm\(^{-1}\); \textsuperscript{1}H NMR (300 MHz, CDCl\(_3\)): δ 1.32-1.48 (2H, m), 1.55-1.72 (2H, m), 1.74-1.86 (2H, m), 1.84 (6H, s), 3.44 (2H, t, J = 6.5 Hz), 4.48 (2H, s), 4.53 (2H, t, J = 7.4 Hz), 6.50 (1H, bs), 6.89 (1H, s), 7.13-7.21 (1H, m), 7.23-7.54 (12H, m), 7.66 (1H, d, J = 7.9 Hz) ppm; \textsuperscript{13}C NMR (75 MHz, CDCl\(_3\)): δ 23.7, 29.5, 29.6, 30.6, 44.3, 56.4, 70.3, 73.0, 103.7, 110.5, 120.5, 121.8, 123.9, 124.8, 126.2, 126.9, 127.6, 127.7, 128.5, 128.7, 132.7, 138.4, 138.8, 146.9, 161.9 ppm; LRMS (+ESI) m/z: 477.3 ([M+Na]+ 100%); HRMS (ESI)\(^+\) Cald for C\(_{30}\)H\(_{34}\)N\(_2\)O\(_2\) [M+Na]+: 477.25180, found 477.25092.

1-(5-Hydroxypentyl)-N-(2-phenylpropan-2-yl)-1H-indole-2-carboxamide 278

Prepared according to General Procedure J using 306 (140 mg, 310 μmol) in tetrahydrofuran (10 mL). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 2:3) as an eluent to obtain the title compound (90 mg, 80%) as a white solid, mp: 103.1-106.2 °C; Rf: 0.12 (3:7 ethyl acetate, hexane); IR (v_{max} (neat)): 3290, 2935, 1632, 1529, 1447, 1354, 1271, 981, 759 cm\(^{-1}\); \textsuperscript{1}H NMR (400 MHz, CDCl\(_3\)): δ 1.29-1.39 (2H, m), 1.49 (2H, m), 1.60 (1H, bs), 1.72-1.83 (2H, m), 1.82 (6H, s), 3.56 (2H, t, J = 6.5 Hz), 4.45-4.53 (2H, m), 6.53 (1H, bs), 6.88 (1H, d, J = 0.8 Hz), 7.14 (1H, ddd, J = 8.0, 6.9, 1.0 Hz), 7.23-7.32 (2H, m), 7.33-7.39 (3H, m), 7.45-7.50 (2H, m), 7.64 (1H, dt, J = 8.1, 1.0 Hz) ppm; \textsuperscript{13}C NMR (100 MHz, CDCl\(_3\)): δ 23.2, 29.5, 30.4, 32.4, 44.3, 56.4, 62.7, 103.8, 110.5, 120.5, 121.9, 124.0, 124.8, 126.2, 126.9, 128.7, 132.6, 138.3, 146.9, 161.9 ppm; LRMS (+ESI) m/z: 387.3 ([M+Na]+ 100%); HRMS (ESI)\(^+\) Cald for
C$_{23}$H$_{28}$N$_2$O$_2$ [M+Na]$^+$: 387.20485, found 387.20411; **HPLC**: 98.1%, RT: 26.8 mins. (Method A, 230 nm).

1-Pentyl-1H-pyrrolo[2,3-b]pyridine 307

![Structure of 1-Pentyl-1H-pyrrolo[2,3-b]pyridine]

Prepared according to General Procedure L using 7-azaidole (2.0 g, 16.9 mmol) and 1-bromopentane (2.5 mL, 20.5 mmol) to obtain the title compound (3.2 g, 100%) as a yellow oil, the characterisation data of which corresponded to that previously described.$^{333}$ $R_f$: 0.58 (2:3 ethyl acetate, hexane).

1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine 308

![Structure of 1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine]

Prepared according to General Procedure L using 7-azaindole (1.5 g, 12.7 mmol) and alkyl bromide 294 (3.9 g, 15.2 mmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (3.1 g, 85%) as a light yellow oil, $R_f$: 0.39 (1:4 ethyl acetate, hexane); **IR** ($v_{\text{max}}$ (neat)): 2935, 2859, 1509, 1425, 1306, 1094, 796, 773 cm$^{-1}$; **$^1$H NMR** (400 MHz, CDCl$_3$): $\delta$ 1.41-1.51 (2H, m), 1.63-1.73 (2H, m), 1.93 (2H, quin, $J$ = 7.4 Hz), 3.47 (2H, t, $J$ = 6.5 Hz), 4.32 (2H, t, $J$ = 7.2 Hz), 4.50 (2H, s), 6.47 (1H, d, $J$ = 3.5 Hz), 7.07 (1H, dd, $J$ = 7.8, 4.7 Hz), 7.23 (1H, d, $J$ = 3.5 Hz), 7.27-7.40 (5H, m), 7.93 (1H, dd, $J$ = 7.8, 1.6 Hz), 8.35 (1H, dd, $J$ = 4.7, 1.6 Hz) ppm; **$^{13}$C NMR** (100 MHz, CDCl$_3$): $\delta$ 23.7, 29.5, 30.3, 44.6, 70.2, 73.0, 99.4, 115.6, 120.7, 127.6, 127.7, 128.1, 128.8, 138.7, 142.7, 147.5 ppm; **LRMS (+ESI) m/z**: 295.2 ([M+H]$^+$ 62%), 317.2 ([M+Na]$^+$ 100%); **HRMS (ESI)$^+$** Cald for C$_{19}$H$_{22}$N$_2$O [M+Na]$^+$: 317.16298, found 317.16232.
To a solution of 307 (3.0 g, 15.9 mmol) in N,N-dimethylformamide (100 mL) at 0 °C was added aluminium chloride (10.6 g, 79.7 mmol) portionwise and the mixture stirred for 1 hour. Trifluoroacetic anhydride (3.4 mL, 23.9 mmol) was added, dropwise and the mixture stirred at room temperature for 3 hours. The reaction mixture was quenched with ice, diluted with water (150 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (3.5 g, 78%) as a yellow oil, the characterisation data of which corresponded to that previously described.\textsuperscript{334} Rf: 0.40 (1:4 ethyl acetate, hexane).

To a solution of 308 (2.4 g, 8.2 mmol) in anhydrous N,N-dimethylformamide (60 mL) at 0 °C was added aluminium chloride (5.4 g, 40.8 mmol), portionwise and the mixture stirred for 1 hour. Trifluoroacetic anhydride (1.7 mL, 12.2 mmol) was added, dropwise and the mixture was warmed to room temperature and stirred for 4 hours. The reaction mixture was poured in to a mixture of ice and water (100 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with water (3 x 100 mL) and lithium chloride (80 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (2.8 g, 88%) as a yellow oil, Rf: 0.28 (1:4 ethyl acetate, hexane); IR (v\textsubscript{max} (neat)): 2940, 2861, 1670, 1527, 1385, 1287, 1136, 882, 802, 714 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 1.44-1.55 (2H, m), 1.65-1.75 (2H, m), 1.94-2.04 (2H, m), 3.49 (2H, t, J = 6.3 Hz), 4.40 (2H, t, J = 7.4 Hz), 4.50 (2H, s), 7.25-7.38 (6H, m), 8.09 (1H, d, J = 1.7 Hz), 8.48 (1H, dd, J = 4.7, 1.7 Hz), 8.66 (1H, dd, J = 7.9, 1.6 Hz) ppm; \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ 23.6, 29.3, 29.9, 46.0, 69.9, 73.1, 108.0, 117.0 (q, J = 225.0 Hz), 108.8, 108.9, 128.1, 128.7, 135.7, 138.2, 138.7, 141.6, 142.9, 143.8, 144.2, 144.6 ppm.
290.6 Hz), 119.6, 119.8, 127.7, 127.7, 128.5, 131.2, 137.4 (q, \( J = 4.2 \) Hz), 138.5, 145.6, 148.1, 174.9 (q, \( J = 3.5 \) Hz) ppm; \(^{19}\text{F} \) NMR (376 MHz, CDCl\(_3\)): \( \delta \) -72.4 ppm; LRMS (+ESI) \( m/z \): 391.2 ([M+H]\(^+\) 52%), 413.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{21}\)H\(_{21}\)F\(_3\)N\(_2\)O\(_2\) [M+Na]\(^+\): 413.14528, found 413.14448.

\( \text{1-Pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid 311} \)

\[
\begin{align*}
\text{O} & \quad \text{OH} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{OH} & 
\end{align*}
\]

Prepared according to General Procedure B using 309 (3.5 g, 12.3 mmol) and aqueous lithium hydroxide (3 M, 10.3 mL, 30.8 mmol) in methanol (100 mL) at reflux for 48 hours to obtain the title compound (2.4 g, 84%) as an off-white solid, the characterisation data of which corresponded to that previously described.\(^{334} \) mp: 149.1-152.6 °C; \( R_f \): 0.07 (1:4 ethyl acetate, hexane); IR (\( \text{v}_{\text{max}} \) (neat)): 3102, 2931, 2502, 1689, 1532, 1257, 1138, 756 cm\(^{-1}\).

\( \text{1-(5-(Benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid 312} \)

\[
\begin{align*}
\text{O} & \quad \text{OH} \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{O} \\
\text{OH} & 
\end{align*}
\]

Prepared according to General Procedure B using 310 (2.5 g, 6.4 mmol) and aqueous lithium hydroxide (2 M, 16 mL, 32 mmol) in a mixture of methanol and tetrahydrofuran (1:1, 30 mL) at reflux for 72 hours to afford the title compound (1.6 g, 74%) as a light yellow solid, mp: 108.1-110.8 °C; \( R_f \): 0.28 (1:4 ethyl acetate, hexane); \( R_f \): 0.28 (1:4 ethyl acetate, hexane); IR (\( \text{v}_{\text{max}} \) (neat)): 3102, 2931, 2502, 1686, 1533, 1260, 1095, 800, 758, 730 cm\(^{-1}\); \(^{1}\text{H} \) NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.43-1.53 (2H, m), 1.69 (2H, quin, \( J = 6.6 \) Hz), 1.97 (2H, quin, \( J = 7.4 \) Hz), 3.47 (2H, t, \( J = 6.4 \) Hz), 4.38 (2H, t, \( J = 7.2 \) Hz), 4.50 (2H, s), 7.24-7.38 (6H, m), 8.06 (1H, s), 8.43 (1H, dd, \( J = 4.7, 1.7 \) Hz), 8.50 (1H, dd, \( J = 7.9, 1.6 \) Hz) ppm; \(^{13}\text{C} \) NMR (100 MHz, CDCl\(_3\)): \( \delta \) 23.7, 29.4, 30.0, 45.5, 70.0, 73.1, 73.1, 105.0, 118.4, 119.6, 127.7, 127.8, 128.5, 130.3, 135.4, 138.6, 144.2, 148.0, 169.3 ppm; LRMS (+ESI) \( m/z \): 361.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{20}\)H\(_{22}\)N\(_2\)O\(_3\) [M+Na]\(^+\): 361.15281, found 361.15205.
Prepared according to General Procedure M using carboxylic acid 311 (120 mg, 350 μmol) and adamantyl amine (64 mg, 420 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 2:3) as an eluent to obtain the title compound (110 mg, 65%) as a white gummy solid, \( R_f \) 0.29 (2:3 ethyl acetate, hexane); IR (\( v_{\text{max}} \) (neat)): 3365, 2907, 2848, 1620, 1536, 1516, 1422, 1278, 1251, 1095, 754 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.37-1.48 (2H, m), 1.59-1.80 (8H, m), 1.90 (2H, quin, \( J = 7.4 \) Hz), 2.10-2.21 (9H, m), 3.44 (2H, t, \( J = 6.4 \) Hz), 4.30 (2H, t, \( J = 7.2 \) Hz), 4.47 (2H, s), 5.55 (1H, bs), 7.17 (1H, dd, \( J = 7.9, 4.7 \) Hz), 7.23-7.36 (5H, m), 7.68 (1H, s), 8.27 (1H, dd, \( J = 8.0, 1.5 \) Hz), 8.35 (1H, dd, \( J = 4.7, 1.6 \) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 23.7, 29.4, 29.7, 30.2, 36.6, 42.3, 45.1, 52.3, 70.1, 73.0, 110.7, 117.3, 118.4, 127.7, 127.8, 128.5, 128.9, 130.1, 138.6, 143.7, 147.7, 163.9 ppm; LRMS (+ESI) m/z: 494.3 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{30}\)H\(_{37}\)N\(_3\)O\(_2\) [M+H]\(^+\): 494.27835, found 494.27749.

Prepared according to General Procedure J using 313 (70 mg, 148 μmol) and acetic acid (2 drops). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:1 to 3:2) as an eluent to obtain the title compound (49 mg, 86%) as a white solid, mp: 110.9-113.4 °C; \( R_f \): 0.16 (3:2 ethyl acetate, hexane); IR (\( v_{\text{max}} \) (neat)): 3320, 2904, 2849, 1618, 1534, 1517, 1425, 1290, 1160, 776 cm\(^{-1}\); \(^1\)H NMR (400 MHz,
CDCl$_3$): $\delta$ 1.34-1.45 (2H, m), 1.53-1.63 (2H, m), 1.66-1.79 (6H, m), 1.85-1.94 (3H, m), 2.09-2.20 (9H, m), 3.61 (2H, t, $J = 6.4$ Hz), 4.29 (2H, t, $J = 7.1$ Hz), 5.62 (1H, bs), 7.16 (1H, dd, $J = 7.9$, 4.7 Hz), 8.26 (1H, dd, $J = 7.9$, 1.5 Hz), 8.33 (1H, dd, $J = 4.7$, 1.5 Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 23.1, 29.7, 30.1, 32.4, 36.6, 42.3, 45.0, 52.4, 62.5, 110.6, 117.4, 118.4, 129.0, 130.3, 143.6, 147.6, 164.0 ppm; LRMS (+ESI) $m/z$: 404.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{23}$H$_{31}$N$_3$O$_2$ [M+Na]$^+$: 404.23140, found 404.23067; HPLC: 99.0%, RT: 24.5 mins. (Method A, 230 nm).

$\text{(S)-Methyl 3-methyl-2-(1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)butanoate}$

Prepared according to General Procedure M using carboxylic acid 311 (250 mg, 1.1 mmol) and L-methyl valinate hydrochloride (220 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 2:3) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (240 mg, 65%) as a white solid, mp: 86.9-89.8 °C; $R_f$: 0.20 (1:4 ethyl acetate, hexane); IR ($\nu_{\text{max}}$ (neat)): 3286, 2961, 2931, 1743, 1619, 1541, 1523, 1400, 1286, 1196, 1142, 776 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.87 (3H, t, $J = 6.9$ Hz), 1.01 (3H, d, $J = 6.9$ Hz), 1.04 (3H, d, $J = 6.9$ Hz), 1.26-1.41 (4H, m), 1.88 (2H, quin, $J = 7.4$ Hz), 2.22-2.37 (1H, m), 3.78 (3H, s), 4.26-4.34 (2H, m), 4.84 (1H, dd, $J = 8.6$, 4.9 Hz), 6.42 (1H, d, $J = 8.6$ Hz), 7.20 (1H, dd, $J = 8.0$, 4.7 Hz), 7.83 (1H, s), 8.32 (1H, dd, $J = 8.0$, 1.6 Hz), 8.37 (1H, dd, $J = 4.7$, 1.6 Hz), 8.48 (1H, s) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.0, 18.2, 19.2, 21.4, 29.0, 30.0, 31.8, 45.2, 52.4, 57.0, 108.9, 117.7, 118.3, 128.8, 131.0, 143.9, 147.8, 164.4, 173.3 ppm; LRMS (+ESI) $m/z$: 368.2 ([M+Na]$^+$ 100%), 713.3 ([2M+Na]$^+$ 43%); HRMS (ESI)$^+$ Calcd for C$_{19}$H$_{27}$N$_3$O$_3$ [M+Na]$^+$: 368.19501, found 368.19429; $[\alpha]_D^{27}$: +34.5 ° (0.58, CHCl$_3$); HPLC: 99.5%, RT: 25.9 mins. (Method A, 230 nm).
(S)-Methyl 3,3-dimethyl-2-(1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)butanoate 283

Prepared according to General Procedure M using carboxylic acid 311 (200 mg, 860 μmol) and amine hydrochloride salt 297 (190 mg, 1.0 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (140 mg, 45%) as a white solid, mp: 68.3-70.1 °C; Rf: 0.37 (2:3 ethyl acetate, hexane); IR (v max (neat)): 3334, 3101, 2956, 1738, 1614, 1537, 1515, 1426, 1267, 1217, 1140, 777 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 0.88 (3H, t, J = 6.9 Hz), 1.08 (9H, s), 1.28-1.41 (4H, m), 1.89 (2H, quin, J = 7.4 Hz), 3.76 (3H, s), 4.27-4.34 (2H, m), 4.76 (1H, d, J = 9.4 Hz), 6.42 (1H, d, J = 9.3 Hz), 7.21 (1H, dd, J = 7.9, 4.7 Hz), 7.83 (1H, s), 8.31 (1H, dd, J = 8.0, 1.5 Hz), 8.38 (1H, dd, J = 4.7, 1.6 Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 14.1, 22.4, 26.9, 29.0, 30.1, 35.3, 45.3, 52.1, 59.8, 108.9, 117.7, 118.2, 128.7, 131.1, 143.9, 147.8, 164.3, 172.9 ppm; LRMS (+ESI) m/z: 382.3 ([M+Na]$^+$ 100%), 741.4 ([2M+Na]$^+$ 32%); HRMS (ESI)$^+$ Cald for C$_{20}$H$_{29}$N$_3$O$_3$ [M+Na]$^+$: 382.21066, found 382.20991; [$\alpha$]$_D$$^{27}$: +30.5 ° (1.15, CHCl$_3$); HPLC: 99.8%, RT: 27.4 mins. (Method A, 230 nm).

(S)-N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 285

Prepared according to General Procedure M using carboxylic acid 311 (230 mg, 1.0 mmol) and L-valinamide hydrochloride (180 mg, 1.2 mmol). The crude product was purified by recrystallisation from ethyl acetate to obtain the title compound (240 mg, 73%) as a white solid, mp: 210.0-213.4 °C; Rf: 0.19 (ethyl acetate); IR (v max (neat)): 3372, 3293, 3193, 2934, 1649, 1621, 1530, 1516, 1426, 1297, 1144 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ
0.84 (3H, t, J = 7.1 Hz), 0.96 (3H, d, J = 6.7 Hz), 0.98 (3H, d, J = 6.7 Hz), 1.20-1.38 (4H, m), 1.84 (2H, quin, J = 7.2 Hz), 2.09 (1H, sept, J = 6.8 Hz), 4.19-4.38 (3H, m), 7.07 (1H, bs), 7.20 (1H, dd, J = 7.9, 4.7 Hz), 7.49 (1H, bs), 7.69 (1H, d, J = 8.8 Hz), 8.30 (1H, dd, J = 4.7, 1.7 Hz), 8.41 (1H, dd, J = 7.9, 1.7 Hz), 8.48 (1H, s) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 13.8, 18.5, 19.5, 21.7, 28.3, 29.2, 30.3, 44.1, 57.5, 107.9, 117.1, 118.9, 129.4, 131.2, 143.2, 147.1, 163.5, 173.4 ppm; LRMS (+ESI) m/z: 353.3 ([M+Na]$^+$ 100%); HRMS (ESI)$^+$ Cald for C$_{18}$H$_{26}$N$_4$O$_2$ [M+Na]$^+$: 353.19534, found 353.19461; $[\alpha]_D^{27}$: +22.8° (0.88, DMSO); HPLC: 96.3%, RT: 21.8 mins. (Method A, 230 nm).

$(S)$-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 286

Prepared according to General Procedure M using carboxylic acid 311 (250 mg, 1.1 mmol), amine hydrochloride salt 296 (220 mg, 1.3 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (9:1 to 1:0) as an eluent to obtain the title compound (310 mg, 84%) as a white solid, mp: 90.1-94.3 °C; R$_f$: 0.21 (ethyl acetate); IR (v$_{\text{max}}$ (neat)): 3334, 3187, 2954, 1674, 1620, 1535, 1513, 1398, 1367, 1261, 1140 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.84 (3H, t, J = 7.1 Hz), 1.01 (9H, s), 1.19-1.38 (4H, m), 1.85 (2H, quin, J = 7.3 Hz), 4.19-4.36 (2H, m), 4.48 (1H, d, J = 9.5 Hz), 7.11 (1H, bs), 7.21 (1H, dd, J = 7.9, 4.7 Hz), 7.40 (1H, d, J = 9.5 Hz), 7.55 (1H, bs), 8.31 (1H, dd, J = 4.7, 1.6 Hz), 8.39 (1H, dd, J = 7.9, 1.6 Hz), 8.55 (1H, s) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 13.8, 21.7, 26.9, 28.4, 29.2, 34.1, 44.1, 59.3, 107.8, 117.1, 118.8, 129.2, 131.4, 143.2, 147.2, 163.3, 172.4 ppm; LRMS (+ESI) m/z: 367.2 ([M+Na]$^+$ 100%), 711.3 ([2M+Na]$^+$ 20%); HRMS (ESI)$^+$ Cald for C$_{19}$H$_{28}$N$_4$O$_2$ [M+Na]$^+$: 367.21100, found 367.21025; $[\alpha]_D^{27}$: +25.5° (1.57, DMSO); HPLC: 98.4%, RT: 23.2 mins. (Method A, 230 nm).
Chapter 8: Experimental

(S)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-(benzyloxy)pentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 315

Prepared according to General Procedure M using carboxylic acid 312 (420 mg, 1.3 mmol) and amine hydrochloride salt 296 (250 mg, 1.5 mmol). The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (510 mg, 91%) as a colourless foam, \( R_f \): 0.29 (1:19 methanol, dichloromethane); \( \text{IR} (\nu_{\text{max}} \text{ (neat)})): 3103, 2938, 2856, 1685, 1535, 1260, 1096 \text{ cm}^{-1} \); \( ^1\text{H} \text{NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \): 1.16 (9H, s), 1.38-1.48 (2H, m), 1.66 (2H, quin, \( J = 6.7 \) Hz), 1.90 (2H, quin, \( J = 7.4 \) Hz), 3.45 (2H, t, \( J = 6.4 \) Hz), 4.29 (2H, td, \( J = 7.1, 1.9 \) Hz), 4.48 (2H, s), 4.79 (1H, d, \( J = 9.2 \) Hz), 5.98 (1H, bs), 6.81 (1H, d, \( J = 9.3 \) Hz), 6.94 (1H, bs), 7.19 (1H, dd, \( J = 8.0, 4.7 \) Hz), 7.24-7.37 (5H, m), 7.84 (1H, s, H7), 8.33 (1H, dd, \( J = 8.0, 1.6 \) Hz), 8.37 (1H, dd, \( J = 4.7, 1.6 \) Hz) ppm; \( ^{13}\text{C} \text{NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \): 23.6, 26.9, 29.4, 30.1, 34.9, 45.1, 59.9, 70.0, 73.0, 108.9, 117.7, 118.3, 127.6, 127.7, 128.5, 128.8, 130.9, 138.6, 143.9, 147.7, 164.5, 173.5 ppm; \( \text{LRMS (ESI)} m/z \): 473.3 ([M+Na]\(^+\) 100%); \( \text{HRMS (ESI)} \) \(^+\) Cald for C\(_{26}\)H\(_{34}\)N\(_4\)O\(_3\) [M+Na]\(^+\): 473.25286, found 473.25201.

(S)-Methyl 2-((1-(5-hydroxypentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamido)-3,3-dimethylbutanoate 284

The amide was formed according to General Procedure M using carboxylic acid 312 (36 mg, 106 μmol) and amine hydrochloride salt 297 (23 mg, 127 μmol). The crude product was taken on to the next step without further purification or characterisation. The benzyl ether group of 314 was removed according to General Procedure J with acetic acid (5 drops). The crude residue was purified by flash column chromatography using ethyl
acetate, hexane (1:1 to 1:4) as an eluent to obtain the title compound (16 mg, 40% over 2 steps) as a colourless foam, \( R_f \): 0.08 (1:1 ethyl acetate, hexane); IR (\( \nu_{\text{max}} \) (neat)): 3323, 2999, 2946, 2927, 1739, 1547, 1520, 1476, 1426, 1331, 1214, 1162, 1055 cm\(^{-1}\); \(^{1}\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.08 (9H, s), 1.40-1.51 (2H, m), 1.59-1.68 (2H, m), 1.92-2.02 (2H, m), 3.65 (2H, t, \( J = 6.2 \) Hz), 3.77 (3H, s), 4.34-4.50 (2H, m), 4.76 (1H, d, \( J = 9.3 \) Hz), 6.48 (1H, d, \( J = 9.3 \) Hz), 7.23-7.32 (1H, m), 7.87 (1H, s), 8.35-8.51 (2H, m) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 22.9, 26.9, 29.9, 31.9, 35.4, 52.1, 59.8, 62.5, 77.4, 109.9, 117.7, 117.8, 131.4, 143.3, 146.9, 163.8, 172.9 ppm; LRMS (+ESI) \( m/z \): 398.5 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{20}\)H\(_{29}\)N\(_3\)O\(_4\) [M+Na]\(^+\): 398.20591, found 398.20480; \([\alpha]_D^{27}\): +29.8 ° (1.1, CHCl\(_3\)); HPLC: 97.0%, RT: 26.6 mins. (Method A, 230 nm).

\( (S)\)-N-(1-Amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(5-hydroxypentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 287

Prepared according to General Procedure J using 315 (150 mg, 330 μmol) and both palladium on carbon (10% w/w, 35 mg, 33 μmol) and palladium hydroxide on carbon (20% w/w, 23 mg, 33 μmol) in methanol (10 mL). The crude residue was purified by flash column chromatography using methanol, dichloromethane (1:19) as an eluent to obtain the title compound (81 mg, 68%) as a white solid, mp: 90.1-92.2 °C; \( R_f \): (1:19 methanol, dichloromethane); IR (\( \nu_{\text{max}} \) (neat)): 3331, 3192, 2868, 1673, 1573, 1534, 1426, 1398, 1261, 1133 cm\(^{-1}\); \(^{1}\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.12 (9H, s), 1.31-1.41 (2H, m), 1.49-1.60 (2H, m), 1.80-1.90 (2H, m), 2.71 (1H, bs), 6.03 (1H, bs), 6.88-7.01 (2H, m), 7.14 (1H, dd, \( J = 7.7, 4.9 \) Hz), 7.89 (1H, s), 8.27-8.36 (2H, m) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 23.1, 27.0, 29.8, 32.0, 34.9, 45.0, 60.0, 62.4, 108.7, 117.7, 118.6, 129.1, 143.9, 147.7, 164.7, 173.8 ppm; LRMS (+ESI) \( m/z \): 383.2 ([M+Na]\(^+\) 100%); HRMS (ESI)\(^+\) Cald for C\(_{19}\)H\(_{28}\)N\(_3\)O\(_3\) [M+Na]\(^+\): 383.20591, found 383.20516; \([\alpha]_D^{27}\): +24.8 ° (0.91, DMSO); HPLC: 98.7%, RT: 23.1 mins. (Method A, 230 nm).
1-Pentyl-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 288

Prepared according to General Procedure M using carboxylic acid 311 (110 mg, 470 μmol) and 1-methyl-1-phenylethylamine (70 mg, 520 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (84 mg, 51%) as a white solid, mp: 171.8-174.3 °C; Rf: 0.30 (2:3 ethyl acetate, hexane); IR (v_{max} (neat)): 3328, 3093, 2923, 1620, 1538, 1516, 1426, 1270, 1198 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.91 (3H, t, J = 6.8 Hz), 1.29-1.46 (4H, m), 1.84-1.97 (8H, m), 4.33 (2H, t, J = 7.3 Hz), 6.13 (1H, bs), 7.20 (1H, dd, J = 7.9, 4.7 Hz), 7.26-7.31 (1H, m), 7.38 (2H, t, J = 7.7 Hz), 7.50-7.56 (2H, m), 7.77 (1H, s), 8.29 (1H, dd, J = 7.9, 1.6 Hz), 8.39 (1H, dd, J = 4.8, 1.5 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 14.1, 22.4, 29.1, 29.7, 30.1, 45.2, 56.4, 110.2, 117.4, 118.3, 124.9, 126.9, 128.7, 128.8, 130.6, 143.8, 147.4, 147.8, 163.8 ppm; LRMS (+ESI) m/z: 372.3 ([M+Na]⁺ 100%), 721.4 ([2M+Na]⁺ 46%); HRMS (ESI)⁺ Cald for C₂₂H₂₇N₃O [M+Na]⁺: 372.20518, found 372.20444; HPLC: 99.9%, RT: 27.4 mins. (Method A, 230 nm).

1-(5-(Benzylxy)pentyl)-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 316

Prepared according to General Procedure M using carboxylic acid 312 (250 mg, 740 μmol) and 1-methyl-1-phenylethylamine (250 mg, 890 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7 to 3:2) as an eluent to obtain the title compound 316 (290 mg, 85%) as a white solid, mp: 137.8-138.8 °C; Rf: (0.19 2:3 ethyl acetate, hexane); IR (v_{max} (neat)): 3337, 2926, 2854, 1621, 1537, 1275, 1094, 760 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.38-1.48 (2H, m), 1.60 (2H, m), 1.86 (6H, s), 1.86-
1.95 (2H, m), 3.44 (2H, t, \( J = 6.4 \) Hz), 4.30 (2H, t, \( J = 7.2 \) Hz), 4.46 (2H, s), 6.13 (1H, s),
7.16 (1H, dd, \( J = 8.0, 4.7 \) Hz), 7.22-7.55 (10H, m), 7.73 (1H, s, H7), 8.27 (1H, dd, \( J = 8.0, 1.6 \) Hz), 8.36 (1H, dd, \( J = 4.7, 1.6 \) Hz) ppm; \( ^{13} \text{C} \text{ NMR} \) (100 MHz, CDCl\(_3\)): \( \delta 23.7, 29.4, 29.7, 30.2, 45.1, 56.3, 70.1, 73.2, 110.2, 117.4, 118.3, 124.9, 126.8, 127.7, 127.7, 128.5, 128.6, 128.9, 130.4, 138.7, 143.8, 147.4, 147.8, 163.7 ppm;

\( ^{1} \text{H} \text{ NMR} \) (400 MHz, DMSO-\( d_6 \)): \( \delta 1.33 \) (2H, tt, \( J = 9.6, 6.0 \) Hz), 1.42-1.52 (2H, m), 1.69 (6H s), 1.86 (2H, quin, \( J = 7.4 \) Hz), 3.35-3.44 (2H, m), 4.29 (2H, t, \( J = 7.2 \) Hz), 4.37 (1H, bs), 7.11-7.19 (2H, m), 7.27 (2H, dd, \( J = 8.5, 7.0 \) Hz), 7.38-7.44 (2H, m), 7.98 (1H, s), 8.28 (1H, dd, \( J = 4.6, 1.6 \) Hz), 8.33 (1H, dd, \( J = 7.9, 1.7 \) Hz), 8.45 (1H, s) ppm; \( ^{13} \text{C} \text{ NMR} \) (100 MHz, DMSO-\( d_6 \)): \( \delta 22.8, 29.5, 29.9, 32.0, 44.1, 55.0, 60.4, 108.8, 116.9, 119.0, 124.7, 125.6, 127.8, 129.5, 130.9, 143.1, 147.1, 148.5, 163.1 ppm; LRMS (+ESI) \( m/z \): 366.3 ([M+H]+ 16%), 388.2 ([M+Na]+ 100%; HRMS (ESI)+ Cald for C\(_{22}\)H\(_{27}\)N\(_3\)O\(_2\) [M+Na]+: 388.20010, found 388.20103; HPLC: 99.7%, RT: 23.3 mins (Method A, 230 nm).

1-([5-Hydroxypentyl]-N-(2-phenylpropan-2-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide

Prepared according to General Procedure J using 316 (150 mg, 410 μmol) and both palladium on carbon (10% w/w, 43 mg, 41 μmol) and palladium hydroxide on carbon (20% w/w, 29 mg, 41 μmol) in a mixture of methanol (10 mL) and tetrahydrofuran (10 mL). The crude residue was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound 316 (106 mg, 71%) as a white solid, mp: 198.8-200.4 °C; \( R_f \): 0.25 (4:1 ethyl acetate, hexane); \( \text{IR} (\nu_{\text{max}} \text{ (neat)}) \): 3334, 2925, 2855, 1621, 1539, 1428, 1277, 760 cm\(^{-1}\); \( ^{1} \text{H} \text{ NMR} \) (400 MHz, DMSO-\( d_6 \)): \( \delta 1.33 \) (2H, tt, \( J = 9.6, 6.0 \) Hz), 1.42-1.52 (2H, m), 1.69 (6H s), 1.86 (2H, quin, \( J = 7.4 \) Hz), 3.35-3.44 (2H, m), 4.29 (2H, t, \( J = 7.2 \) Hz), 4.37 (1H, bs), 7.11-7.19 (2H, m), 7.27 (2H, dd, \( J = 8.5, 7.0 \) Hz), 7.38-7.44 (2H, m), 7.98 (1H, s), 8.28 (1H, dd, \( J = 4.6, 1.6 \) Hz), 8.33 (1H, dd, \( J = 7.9, 1.7 \) Hz), 8.45 (1H, s) ppm; \( ^{13} \text{C} \text{ NMR} \) (100 MHz, DMSO-\( d_6 \)): \( \delta 22.8, 29.5, 29.9, 32.0, 44.1, 55.0, 60.4, 108.8, 116.9, 119.0, 124.7, 125.6, 127.8, 129.5, 130.9, 143.1, 147.1, 148.5, 163.1 ppm; LRMS (+ESI) \( m/z \): 366.3 ([M+H]+ 16%), 388.2 ([M+Na]+ 100%; HRMS (ESI)+ Cald for C\(_{22}\)H\(_{27}\)N\(_3\)O\(_2\) [M+Na]+: 388.20010, found 388.20103; HPLC: 99.7%, RT: 23.3 mins (Method A, 230 nm).
Lithium diisopropylamide was prepared from \textit{n}-butyllithium (2.1 M, 16.6 mL, 34.8 mmol) and diisopropylamine (5.9 mL, 42.0 mmol) in tetrahydrofuran (10 mL) at -78 °C. To this solution was added 2-bromopyridine (5.0 g, 31.6 mmol) and the mixture was stirred for 4 hours before \textit{N},\textit{N}-dimethylformamide (10.6 mL, 137 mmol) was added. The mixture was stirred for 30 minutes before being warmed to room temperature and stirred for another 2 hours. Saturated aqueous ammonium chloride (100 mL) was added and the mixture was extracted with diethyl ether (3 x 100 mL), washed with water (3 x 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (2.2 g, 37%) as a colourless oil, which crystallised over time to form large colourless shards, the characterisation data of which corresponded to that previously described.\textsuperscript{335} \textbf{mp}: 74.6-76.6 °C; \textbf{Rf}: 0.29 (1:4 ethyl acetate, hexane); \textbf{IR} (\textit{v}_{\text{max}} \text{(neat)}): 3347, 3025, 2870, 1692, 1655, 1570, 1369, 1048 cm\textsuperscript{-1}; \textbf{LRMS} (+ESI) \textit{m/z}: 240.0/242.0 ([M+MeOH+Na]\textsuperscript{+} 100%) (observed as hemiacetal).

\textit{Ethyl 1H-pyrrolo[2,3-b]pyridine-2-carboxylate 319}

A mixture of glycine ethyl ester hydrochloride (10.0 g, 71.6 mmol), ethyl formate (33.7 mL, 420 mmol) and triethylamine (20 mL, 143 mmol) were heated at 60 °C for 16 hours. After cooling to room temperature the solid triethylamine hydrochloride was removed by filtration and the residue purified by vacuum distillation (10 mmHg, 140-145 °C). The intermediate formamide (confirmed by \textit{1H NMR} spectroscopy) was isolated as a colourless liquid and was taken on to the next step without further purification or characterisation. A solution of the formamide (780 mg, 5.9 mmol) and triethylamine (2.1 mL, 14.8 mmol) in dichloromethane (10 mL) at 0 °C was treated with phosphorus oxychloride (580 μL, 6.2 mmol) and the mixture warmed to room temperature and stirred for 1 hour. The solution was cooled again to 0 °C and aqueous sodium carbonate (10% w/v, 25 mL) was slowly added and the mixture was warmed to room temperature and stirred for 1.5 hours. The mixture was extracted with dichloromethane (3 x 30 mL), washed with water (2 x 50 mL),
dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the isocyanide intermediate (318) as an orange oil.

To a solution of aryl bromide 317 (1.0 g, 6.3 mmol), copper iodide (240 mg, 1.3 mmol) and cesium carbonate (4.1 g, 12.7 mmol) in dimethyl sulfoxide (6 mL) was added a solution of isocyanide 318 (790 mg, 7.0 mmol) in dimethyl sulfoxide (1 mL), slowly. The mixture was warmed to 80 °C and stirred for 16 hours. After cooling to room temperature, water (50 mL) was added and the mixture was extracted with ethyl acetate (3 x 50 mL), washed with water (3 x 100 mL) and aqueous lithium chloride (1 M, 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (3:7) as an eluent to obtain the title compound (660 mg, 55% over 2 steps) as a light green solid, the characterisation data of which corresponded to that previously described. mp: 160.8-164.2 °C; Rf: 0.36 (2:3 ethyl acetate, hexane); IR (vmax (neat)): 2976, 2969, 1707, 1522, 1424, 1271, 1208, 1023, 841, 769 cm⁻¹; LRMS (+ESI) m/z: 213.1 ([M+Na]+ 100%).

*Ethyl 1-pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxylate 320*

To a solution of 319 (100 mg, 530 μmol) in anhydrous N,N-dimethylformamide (5 mL) was added potassium carbonate (220 mg, 1.5 mmol) followed by 1-bromopentane (78 μL, 630 μmol) and the mixture stirred at 50 °C for 16 hours. After cooling to room temperature, water (20 mL) was added and the mixture was extracted with ethyl acetate (3 x 15 mL), washed with water (3 x 20 mL) and aqueous lithium chloride (1 M, 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19) as an eluent to obtain the title compound (86 mg, 63%) as a colourless oil, Rf: 0.30 (1:19 ethyl acetate, hexane); IR (vmax (neat)): 2956, 1711, 1458, 1223, 1189, 1093, 747 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.85-0.93 (3H, m), 1.31-1.41 (4H, m), 1.44 (3H, t, J = 7.1 Hz), 1.76-1.88 (2H, m), 4.42 (2H, q, J = 7.1 Hz), 4.71-4.78 (2H, m), 7.12 (1H, dd, J = 7.9, 4.6 Hz), 7.27 (1H, s), 8.00 (1H, dd, J = 7.9, 1.6 Hz), 8.50 (1H, dd, J = 4.6, 1.6 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 14.2, 14.5, 22.6, 29.2, 30.7, 43.5, 60.9, 108.3, 117.0, 118.6, 128.1, 130.9, 146.6, 149.2, 161.8 ppm; LRMS (+ESI) m/z: 261.2 ([M+H]+ 100%).
**Chapter 8: Experimental**

*1-Pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxylic acid 321*

Prepared according to General Procedure B using ethyl ester 320 (75 mg, 290 μmol) and aqueous sodium hydroxide (3 M, 480 μL, 1.4 mmol) in ethanol (3 mL) at reflux for 2 hours to obtain the title compound (61 mg, 91%) as an off-white solid, **mp**: 173 °C (decomposition); **IR** (ν<sub>max</sub> (neat)): 3193, 2946, 2485, 1696, 1514, 1220, 1063 cm<sup>-1</sup>; **<sup>1</sup>H** NMR (400 MHz, DMSO-<em>d</em><sub>6</sub>): δ 0.79 (3H, t, <em>J</em> = 7.0 Hz), 1.13-1.31 (4H, m), 1.70 (2H, quin, <em>J</em> = 7.5 Hz), 4.72 (2H, t, <em>J</em> = 7.3 Hz), 7.06 (1H, s), 7.11 (1H, dd, <em>J</em> = 7.9, 4.6 Hz), 8.02 (1H, dd, <em>J</em> = 7.9, 1.6 Hz), 8.35 (1H, dd, <em>J</em> = 4.6, 1.6 Hz) ppm; **<sup>13</sup>C** NMR (100 MHz, DMSO-<em>d</em><sub>6</sub>): δ 13.9, 21.9, 28.5, 30.1, 42.2, 105.5, 116.3, 118.3, 129.8, 129.9, 144.7, 148.3, 165.2 ppm; **LRMS** (-ESI) <em>m/z</em>: 231.2 ([M-H]<sup>-</sup> 100%).

*N-(Adamantan-1-yl)-1-pentyl-1H-pyrrolo[2,3-b]pyridine-2-carboxamide 290*

Prepared according to General Procedure M using carboxylic acid 321 (72 mg, 280 μmol) and 1-adamantylamine (51 mg, 340 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:9) as an eluent to obtain the title compound (60 mg, 59%) as a white solid, **mp**: 175.9-178.7 °C; **R<sub>r</sub>**: 0.52 (1:4 ethyl acetate, hexane); **IR** (ν<sub>max</sub> (neat)): 3318, 3098, 2924, 1740, 1613, 1540, 1517, 1450, 1305 cm<sup>-1</sup>; **<sup>1</sup>H** NMR (400 MHz, CDCl<sub>3</sub>): δ 0.86 (3H, t, <em>J</em> = 6.9 Hz), 1.23-1.38 (4H, m), 1.68-1.83 (8H, m), 2.10-2.19 (9H, m), 4.64-4.72 (2H, m), 5.88 (1H, bs), 6.66 (1H, s), 7.07 (1H, dd, <em>J</em> = 7.9, 4.6 Hz), 7.89 (1H, dd, <em>J</em> = 7.9, 1.6 Hz), 8.41 (1H, dd, <em>J</em> = 4.7, 1.6 Hz) ppm; **<sup>13</sup>C** NMR (100 MHz, CDCl<sub>3</sub>): δ 14.2, 22.6, 29.2, 29.7, 30.7, 36.5, 41.9, 43.0, 52.7, 101.0, 116.7, 118.8, 129.7, 134.0, 145.2, 148.8, 161.8 ppm; **LRMS** (+ESI) <em>m/z</em>: 366.3 ([M+H]<sup>+</sup> 46%), 388.3 ([M+Na]<sup>+</sup> 100%); **HRMS** (ESI)<sup>+</sup> Cald for C<sub>23</sub>H<sub>31</sub>N<sub>3</sub>O [M+Na]<sup>+</sup>: 388.23648, found 388.25383; **HPLC**: 99.6%, RT: 30.9 mins. (Method A, 230 nm).
3-Acetylpyridine 1-oxide 322

A suspension of 3-acetylpyridine (3.0 g, 24.8 mmol) and \(m\)-chloroperoxybenzoic acid (5.1 g, 29.7 mmol) in anhydrous dichloromethane (125 mL) was stirred for 16 hours. Triphenylphosphine (3.2 g, 12.4 mmol) was added and the mixture stirred for a further 4 hours. The volatiles were removed under a stream of nitrogen and the residue purified by flash column chromatography using methanol, ethyl acetate (1:9) as an eluent to obtain the title compound (2.0 g, 58%) as a white solid, the characterisation data of which corresponded to that previously described.\(^{337}\) \(R_f\): 0.23 (1:9 methanol, ethyl acetate); LRMS (+ESI) \(m/z\): 160.1 ([M+Na]\(^+\) 37%), 297.1 ([2M+Na]\(^+\) 100%).

1-(2-Chloropyridin-3-yl)ethanone 323

A solution of 322 (1.9 g, 13.9 mmol) in phosphorus oxychloride (23 mL, 249 mmol) was heated at 100 °C for 2 hours. Excess phosphorus oxychloride was removed under a stream of nitrogen and to the residue was added ice-water (200 mL) and the mixture extracted with diethyl ether (3 x 100 mL). The combined organic extracts were washed with water (2 x 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The product was isolated by flash column chromatography using ethyl acetate, hexane (1:4) as an eluent to obtain the title compound (1.1 g, 50%) as a yellow oil, \(R_f\) 0.55 (1:1 ethyl acetate, hexane); IR (\(\nu_{\text{max}}\) (neat)): 3020, 1683, 1574, 1559, 1276, 1116, 1063, 803 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 2.69 (3H, s), 7.34 (1H, dd, \(J = 7.6, 4.8\) Hz), 7.90 (1H, dd, \(J = 7.6, 2.0\) Hz), 8.49 (1H, dd, \(J = 4.8, 2.0\) Hz), 8.69 (1H, s) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 26.9, 124.7, 126.2, 135.8, 139.5, 142.4, 193.8 ppm.
3-Methyl-1H-pyrazolo[3,4-b]pyridine 324

A solution of pyridine 323 (1.0 g, 6.3 mmol) in ethylene glycol (5 mL) was heated at 165 °C for 16 hours. After cooling to room temperature, water was added (50 mL) and the mixture extracted with ethyl acetate (5 x 40 mL), washed with water (50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (680 mg, 81%) as a light yellow solid, mp: 144.9-150.7 °C; Rf: 0.20 (3:7 ethyl acetate, hexane); IR (v_max (neat)): 3134, 3097, 2893, 1590, 1394, 1285, 916, 764 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 2.62 (3H, s), 7.12 (1H, dd, J = 8.0, 4.6 Hz), 8.05 (1H, dd, 8.0, 1.5 Hz), 8.60 (1H, dd, J = 4.7, 1.5 Hz), 8.69 (1H, s), 12.89 (1H, bs) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 12.6, 115.1, 116.2, 129.9, 142.5, 148.7, 152.8 ppm; LRMS (+ESI) m/z: 156.1 ([M+Na]⁺ 100%).

3-Methyl-1-pentyl-1H-pyrazolo[3,4-b]pyridine 325

Prepared according to General Procedure L using 324 (300 mg, 2.3 mmol) and 1-bromopentane (340 μL, 2.7 mmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:4) as an eluent to obtain the title compound (210 mg, 46%) as a colourless oil, Rf: 0.38 (1:4 ethyl acetate, hexane); IR (v_max (neat)): 2929, 2859, 1599, 1574, 1500, 1459, 1388, 1267, 771 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.86 (3H, t, J = 7.0 Hz), 1.26-1.40 (4H, m), 1.87-1.98 (2H, m), 2.56 (3H, s), 4.41-4.47 (2H, m), 7.04 (1H, dd, J = 8.0, 4.5 Hz), 7.96 (1H, dd, J = 8.0, 1.6 Hz), 8.49 (1H, dd, J = 4.5, 1.6 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 12.5, 14.1, 22.5, 29.1, 29.8, 46.9, 115.1, 115.7, 129.4, 140.3, 148.6, 150.9 ppm; LRMS (+ESI) m/z: 204.2 ([M+H]⁺ 100%); HRMS (ESI)⁺ Calcd for C₁₂H₁₇N₃ [M+H]⁺: 204.15007, found 204.14946.
Chapter 8: Experimental

1-Pentyl-1H-pyrazolo[3,4-b]pyridine-3-carboxylic acid 326

To a suspension of 325 (300 mg, 1.5 mmol) in aqueous sodium hydroxide (1 M, 10.8 mL, 10.8 mmol) at 80 °C was added a solution of potassium permanganate (850 mg, 5.4 mmol) in water (10 mL) over 2 hours. The mixture was warmed to 90 °C and stirred for an additional 2 hours. The mixture was filtered, hot, through a pad of Celite®, washed with dichloromethane (20 mL), acidified with aqueous hydrochloric acid (6 M) and extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (120 mg, 36%) as an orange gummy mass, IR (νmax (neat)): 2955, 2929, 2553, 1702, 1581, 1501, 1252, 1177, 1146, 1124, 815 cm⁻¹; ¹H NMR (400 MHz, MeOD): δ 0.84-0.90 (3H, m), 1.21-1.41 (4H, m), 1.93-2.03 (2H, m), 4.59 (2H, t, J = 7.1 Hz), 7.37 (1H, dd, J = 8.1, 4.5 Hz), 8.52 (1H, dd, J = 8.2, 1.7 Hz), 8.60 (1H, dd, J = 4.5, 1.7 Hz) ppm; ¹³C NMR (100 MHz, MeOD): δ 14.2, 23.2, 29.9, 30.4, 48.8, 116.7, 120.3, 132.8, 135.4, 150.6, 161.7, 164.7 ppm; LRMS (+ESI) m/z: 278.1 ([M+2Na-H]+ 100%); HRMS (ESI)⁺ Calcd for C₁₂H₁₅N₃O₂ [M+2Na-H]⁺: 278.08814, found 278.08478.

N-(Adamantan-1-yl)-1-pentyl-1H-pyrazolo[3,4-b]pyridine-3-carboxamide 291

Prepared according to General Procedure M using carboxylic acid 326 (50 mg, 220 μmol) and 1-adamantylamine (41 mg, 270 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:19 to 1:4) as an eluent to obtain the title compound (42 mg, 52%) as a white solid, mp: 121.1-123.5 °C; Rf: 0.54 (3:7 ethyl acetate, hexane); IR (νmax (neat)): 3259, 2900, 2846, 1658, 1541, 1275, 1155 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.88 (3H, t, J = 7.0 Hz), 1.26-1.43 (4H, m), 1.66-1.80 (6H, m), 1.96 (2H, quin, J = 7.6 Hz), 2.11-2.22 (9H, m), 4.47-4.54 (2H, m), 6.80 (1H, bs), 7.20 (1H, dd, J = 8.1, 4.5 Hz), 8.53 (1H, dd, J = 4.5, 1.7 Hz), 8.65 (1H, dd, J = 8.1, 1.7 Hz) ppm; ¹³C NMR
(100 MHz, CDCl$_3$): $\delta$ 14.1, 22.4, 29.0, 29.6, 29.7, 36.6, 42.0, 47.7, 52.1, 114.8, 118.6, 132.3, 137.3, 149.1, 151.2, 161.5 ppm; LRMS (+ESI) $m/z$: 389.3 ([M+Na]$^+$ 100%), 755.4 ([2M+Na]$^+$ 53%); HRMS (ESI)$^+$ Calcd for C$_{22}$H$_{30}$N$_4$O [M+Na]$^+$: 389.23173, found 389.23101; HPLC: 99.2%, RT: 33.4 mins. (Method A, 230 nm).

2,2,2-Trichloro-1-(1H-pyrrolo[2,3-b]pyridin-3-yl)ethanone 327

To a solution of 7-azaindoles (500 mg, 4.2 mmol) in anhydrous dichloromethane (20 mL) at 0 °C was added aluminium chloride (2.8 g, 21.2 mmol), portionwise and the mixture was stirred for 15 minutes before trichloroacetyl chloride (710 μL, 6.4 mmol) was added, slowly. The mixture was warmed to room temperature and stirred for 4 hours then quenched with ice-cold water (80 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were washed with aqueous hydrochloric acid (1 M, 3 x 50 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (1.0 g, 91%) as a white solid, mp: 220 °C (decomposition); $R_f$: 0.24 (1:1 ethyl acetate, hexane); IR ($v_{\max}$ (neat)): 3139, 3011, 2833, 1661, 1411, 1292, 841, 742 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.37 (1H, dd, $J = 7.9$, 4.7 Hz), 8.42 (1H, dd, $J = 4.8$, 1.7 Hz), 8.51 (1H, dd, $J = 8.0$, 1.7 Hz), 8.67 (1H, s), 13.19 (1H, bs) ppm; $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 79.2, 103.8, 119.1, 119.5, 129.9, 137.0, 145.1, 148.4, 176.6 ppm; LRMS (+ESI) $m/z$: 263.0/265.0 ([M+H]$^+$ 100%).

$^1$H-Pyrrolo[2,3-b]pyridine-3-carboxylic acid 328

Prepared according to General Procedure B using 327 (3.0 g, 11.4 mmol) in aqueous sodium hydroxide (3 M, 65 mL, 200 mmol) at room temperature for 6 hours. The solution was acidified with aqueous hydrochloric acid (6 M) and the precipitate collected by filtration. The solid was dried in vacuo to obtain the title compound (1.8 g, 100%) as an off-white powder, the characterisation data of which corresponded to that previously described.$^{338}$
N-(Adamantan-1-yl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide 329

Prepared according to General Procedure M using carboxylic acid 328 (300 mg, 1.9 mmol) and 1-adamantylamine (340 mg, 2.2 mmol). The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (330 mg, 61%) as a white solid, mp: 274.1-280.2 °C; Rf: 0.35 (1:19 methanol, dichloromethane); IR (v<sub>max</sub> (neat)): 3322, 2905, 1626, 1531, 1416, 1302, 799, 773 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 1.57-1.76 (6H, m), 1.99-2.17 (9H, m), 7.10-7.17 (2H, m), 8.18-8.26 (2H, m), 8.41 (1H, dd, J = 7.9, 1.7 Hz), 11.97 (1H, bs) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>): δ 29.0, 36.2, 41.4, 51.0, 110.3, 116.6, 118.7, 127.9, 129.3, 143.2, 148.3, 163.6 ppm; LRMS (+ESI) m/z: 318.2 ([M+Na]<sup>+</sup> 100%).

N-(Adamantan-1-yl)-7-pentyl-7H-pyrrolo[2,3-b]pyridine-3-carboxamide 292

Prepared according to General Procedure O using 329 (50 mg, 170 μmol) and 1-bromopentane (110 μL, 850 μmol). The crude product was purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:49) as an eluent to obtain the title compound (37 mg, 60%) as a light yellow solid, mp: 158.1-160.0 °C; Rf: 0.31 (1:19 methanol, dichloromethane); IR (v<sub>max</sub> (neat)): 3269, 2904, 2849, 1618, 1558, 1456, 1358, 1307, 1178, 1145, 1096, 746 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.83-0.91 (3H, m), 1.30-1.40 (2H, m), 1.66-1.79 (6H, m), 1.98-2.08 (2H, m), 2.09-2.20 (9H, m), 4.67 (2H, t, J = 7.4 Hz), 5.64 (1H, bs), 7.04 (1H, dd, J = 7.6, 6.2 Hz), 7.67 (1H, dd, J = 6.2, 1.2 Hz), 8.06 (1H, s), 8.82 (1H, dd, J = 7.6, 1.2 Hz) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 14.0, 22.4, 28.9, 29.6, 29.8, 36.7, 42.4, 52.0, 53.9, 111.8, 112.2, 129.1, 130.2, 133.9, 145.0, 149.5, 165.0 ppm; LRMS (+ESI) m/z: 366.3 ([M+H]<sup>+</sup> 100%), 388.3 ([M+Na]<sup>+</sup> 43%), 731.4
([2M+H]^+ 35%); HRMS (ESI)^+ Calcd for C_{25}H_{31}N_{3}O [M+H]^+: 366.25454, found 366.25392; HPLC: 96.2%, RT: 22.6 mins. (Method A, 230 nm).

8.5.5 Synthetic Procedures from Chapter 6

4-Methyl-3-nitropyridin-2(1H)-one 340

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\]

To a suspension of 2-amino-4-methyl-3-nitropyridine (5.0 g, 32.6 mmol) in water (60 mL) at 0 °C was added concentrated sulfuric acid (5 mL), dropwise. A solution of sodium nitrite (3.4 g, 49.0 mmol) in water (10 mL) was added slowly and the mixture stirred at room temperature for 16 hours. The precipitate was collected by filtration and dried in vacuo to obtain the title compound (4.5 g, 90%) as a yellow solid, mp: 213 °C (decomposition); Rf: 0.56 (1:9 methanol, dichloromethane); IR (νmax (neat)): 3107, 2987, 2777, 1649, 1611, 1528, 1475, 1313, 929, 859, 797 cm\(^{-1}\); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 2.18 (3H, s), 6.26 (1H, d, \(J = 6.7\) Hz), 7.55 (1H, d, \(J = 6.7\) Hz), 12.49 (1H, bs) ppm; \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 17.0, 106.9, 137.2, 141.4, 145.0, 154.6 ppm; LRMS (-ESI) m/z: 153.4 ([M-H]^+ 100%).

3-Amino-4-methylpyridin-2(1H)-one 341

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\text{N} \\
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Prepared according to General Procedure H using 340 (1.5 g, 9.7 mmol) in methanol (100 mL) to obtain the title compound (1.1 g, 88%) as a brown solid, the characterisation data of which corresponded to that previously described\(^{177}\) mp: 141.6-144.1 °C; Rf: 0.52 (1:9 methanol, dichloromethane); IR (νmax (neat)): 3377, 3307, 2782, 1593, 1535, 1460, 1209, 949, 755 cm\(^{-1}\); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 16.7, 109.6, 119.7, 121.3, 135.3, 157.3 ppm; LRMS (+ESI) m/z: 147.1 ([M+Na]^+ 76%), 271.1 ([2M+Na]^+ 100%).
Chapter 8: Experimental

*N-(4-Methyl-2-oxo-1,2-dihydropyridin-3-yl)cycloheptanecarboxamide 342*

![Chemical structure of 342](image)

Prepared according to General Procedure C using cycloheptanecarboxylic acid (500 μL, 3.6 mmol) and 341 (300 mg, 2.4 mmol) to obtain the title compound (400 mg, 67%) as an off-white gummy mass, the characterisation data of which corresponded to that previously described.\(^{177}\) \(R_f\) 0.18 (1:49 acetic acid, ethyl acetate); \(\text{IR} (v_{\text{max}} \text{ (neat)}): 3254, 2918, 2402, 1635, 1604, 1582, 1519, 1423, 1209, 780 \text{ cm}^{-1}; \) \(^{13}\text{C NMR} (100 \text{ MHz, MeOD}): \delta 18.7, 27.9, 29.3, 32.8, 48.0, 111.0, 126.3, 132.5, 150.5, 162.4, 179.4 \text{ ppm}; \) \(\text{LRMS (+ESI) } m/z: 271.2 ([M+Na]^+) 100\%.

*N-(5-Bromo-4-methyl-2-oxo-1,2-dihydropyridin-3-yl)cycloheptanecarboxamide 343*

![Chemical structure of 343](image)

To a solution of 342 (80 mg, 320 μmol) in chloroform (500 μL) was added a solution of bromine (16 μL, 320 μmol) in chloroform (300 μL) and the mixture was stirred at room temperature for 16 hours. The mixture was diluted with chloroform (10 mL), washed with saturated aqueous sodium thiosulfate (4 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (56 mg, 53%) as an off-white solid, the characterisation data of which corresponded to that previously described.\(^{177}\) \(\text{mp: } 225.4-228.7 \degree \text{C; } R_f\) 0.68 (1:9 methanol, dichloromethane); \(\text{IR} (v_{\text{max}} \text{ (neat)}): 3270, 2920, 2850, 2678, 1663, 1634, 1505, 1475, 1406, 851 \text{ cm}^{-1}; \) \(^{13}\text{C NMR} (100 \text{ MHz, MeOD}): \delta 19.6, 27.8, 29.3, 32.8, 48.0, 103.9, 127.4, 133.8, 149.2, 161.1, 179.4 \text{ ppm}; \) \(\text{LRMS (+ESI) } m/z: 349.1/351.1 ([M+Na]^+) 100\%).
**Chapter 8: Experimental**

**N-(5-Bromo-1-(4-fluorobenzyl)-4-methyl-2-oxo-1,2-dihydropyridin-3-yl)cycloheptanecarboxamide 79**

![Chemical Structure](image)

To a solution of 343 (80 mg, 240 μmol) in N,N-dimethylformamide (3 mL) was added cesium fluoride (110 mg, 730 μmol). After stirring for 1 hour, 4-fluorobenzyl bromide was added and the mixture was stirred at 50 °C for 16 hours. The reaction mixture was poured into water (20 mL), extracted with ethyl acetate (3 x 15 mL), washed with water (3 x 40 mL) and aqueous lithium chloride (1 M, 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 3:7) as an eluent to obtain the title compound (63 mg, 60%) as a white solid, the characterisation data of which corresponded to that previously described.\(^{177}\) \(R_f\): 0.28 (3:7 ethyl acetate, hexane); \(\text{IR} (\nu_{\text{max}} \text{ (neat))}: 3330, 2920, 2852, 1680, 1651, 1507, 1460, 1222, 1155, 853 \text{ cm}^{-1}; \) \(^{19}\text{F NMR} (376 \text{ MHz, CDCl}_3): \delta -113.2 \text{ ppm}; \) \(\text{LRMS (+:ESI) } m/z: 457.1/459.1 ([M+Na]^+ 100%); \) \(\text{HPLC: 99.1%, RT: 28.2 mins. (Method A, 230 nm).}\)

**N-(5-Bromo-2-((4-fluorobenzyl)oxy)-4-methylpyridin-3-yl)cycloheptanecarboxamide 344**

![Chemical Structure](image)

To a solution of 343 (150 mg, 460 μmol) in a mixture anhydrous tetrahydrofuran (2 mL) and toluene (2 mL) was added silver carbonate (83 mg, 300 μmol) and 4-fluorobenzyl bromide (69 μL, 550 μmol) and the mixture was stirred in the absence of light at 66 °C for 16 hours. The mixture was filtered through a pad of Celite® and concentrated under reduced pressure to obtain a crude solid that was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent and recrystallisation from dichloromethane, diisopropyl ether to obtain the title compound (110 mg, 55%) as a white solid, \(\text{mp: 199.6-200.8 °C; } R_f: 0.59\) (3:7 ethyl acetate, hexane); \(\text{IR} (\nu_{\text{max}} \text{ (neat))}: 3270, 2921, 2854, 1655, 1505, 1433, 1358, 1220, 1082, 819 \text{ cm}^{-1}; \) \(\text{H NMR} (300 \text{ MHz, CDCl}_3): \delta 1.39-1.66 (6H, m), 1.66-1.84 (4H, m), 1.89-2.03 (2H, m), 2.26 (3H, s), 2.39-2.52 (1H, m), 5.29 (2H, s),
6.83 (1H, bs), 7.04 (2H, td, J = 8.6, 1.4 Hz), 7.33-7.43 (2H, m), 8.11 (1H, s) ppm; \(^{13}\text{C}\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 19.4, 26.7, 28.3, 31.8, 47.7, 67.8, 115.5 (d, \(J = 21.5\) Hz), 116.0, 120.4, 130.1, 132.7 (d, \(J = 3.1\) Hz), 144.9, 146.0, 157.1, 162.7 (d, \(J = 246.6\) Hz), 176.1 ppm; \(^{19}\text{F}\) NMR (282 MHz, CDCl\(_3\)): \(\delta\) -114.0 ppm; LRMS (+ESI) \(m/z\): 457.1/459.1 ([M+Na\(^+\)] 100%); HRMS (ESI)\(^+\) Calcd for C\(_{23}\)H\(_{23}\)BrFN\(_2\)O\(_2\) [M+Na\(^+\)]: 457.09029, found 457.09034; HPLC: 98.4%, RT: 31.3 mins. (Method A, 254 nm).

3-Nitro-\(1\text{H}\)-pyrrolo[2,3-\(b\)]pyridine 345

![3-Nitro-1H-pyrrolo[2,3-b]pyridine](image)

Prepared according to General Procedure N using 7-azaindole (1.0 g, 8.5 mmol). The crude product was recrystallised from ethanol, water to obtain the title compound (770 mg, 56%) as a light yellow solid, \(\text{mp}: 88.0-90.7 \, ^\circ\text{C}\); \(R_f\): 0.12 (2:3 ethyl acetate, hexane); IR (\(v_{\text{max}}\) (neat)): 3140, 3080, 2994, 2698, 1470, 1433, 1401, 1380, 1277, 1123, 749 cm\(^{-1}\); \(^{1}\text{H}\) NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 7.42 (1H, dd, \(J = 7.8, 4.9\) Hz), 8.38-8.50 (2H, m), 8.82 (1H, s), 12.4 (1H, bs) ppm; \(^{13}\text{C}\) NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 119.7, 122.6, 128.1, 130.8, 145.6, 146.7 ppm; LRMS (-ESI) \(m/z\): 162.1 ([M-H\(^-\)] 100%).

5-Bromo-3-nitro-\(1\text{H}\)-pyrrolo[2,3-\(b\)]pyridine 346

![5-Bromo-3-nitro-1H-pyrrolo[2,3-b]pyridine](image)

Prepared according to General Procedure N using 5-bromo-7-azaindole (3.0 g, 15.2 mmol). The crude product was purified by recrystallisation from ethanol, water to obtain the title compound (3.0 g, 82%) as an off-white solid, \(\text{mp}: 269.8-272.5 \, ^\circ\text{C}\); \(R_f\): 0.35 (2:3 ethyl acetate, hexane); IR (\(v_{\text{max}}\) (neat)): 3154, 2828, 2713, 1520, 1468, 1450, 1392, 1372, 1331, 1251, 1217, 1204, 901, 828 cm\(^{-1}\); \(^{1}\text{H}\) NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 8.41-8.60 (2H, m), 8.85 (1H, s), 13.45 (1H, bs) ppm; \(^{13}\text{C}\) NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 114.1, 115.0, 126.9, 128.1, 130.8, 145.6, 146.7 ppm; LRMS (+ESI) \(m/z\): 242.0/244.0 ([M+H\(^+\)] 100%).
**Chapter 8: Experimental**

1-(4-Fluorobenzyl)-3-nitro-1H-pyrrolo[2,3-b]pyridine 347

![Chemical Structure](image)

Prepared according to General Procedure L using 345 (1.0 g, 6.1 mmol) and 4-fluorobenzyl bromide (92 μL, 7.4 mmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (1.0 g, 61%) as a light yellow solid, mp: 154.1-157.7 °C; Rf: 0.54 (2:3 ethyl acetate, hexane); IR (v_max (neat)): 3132, 3055, 1523, 1509, 1474, 1431, 1377, 1322, 1218 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 5.51 (2H, s), 6.99-7.13 (2H, m), 7.30-7.44 (3H, m), 8.49 (1H, dd, J = 4.7, 1.6 Hz), 8.56 (1H, dd, J = 8.0, 1.6 Hz) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 48.3, 113.9, 116.3 (d, J = 21.8 Hz), 120.3, 127.7, 129.6, 129.6, 130.3 (d, J = 8.4 Hz), 131.1 (d, J = 3.2 Hz), 146.0, 146.0, 162.9 (d, J = 251.3 Hz) ppm; ¹⁹F NMR (282 MHz, CDCl₃): δ -112.7 ppm; LRMS (+ESI) m/z: 294.1 ([M+Na]⁺ 100%).

5-Bromo-1-(4-fluorobenzyl)-3-nitro-1H-pyrrolo[2,3-b]pyridine 348

![Chemical Structure](image)

To a solution of 346 (200 mg, 820 μmol) in anhydrous N,N-dimethylformamide (4 mL) was added potassium carbonate (340 mg, 2.4 mmol) followed by 4-fluorobenzyl bromide (124 μL, 980 μmol) and the mixture stirred at room temperature for 16 hours. The reaction mixture was diluted with water (50 mL), extracted with ethyl acetate (3 x 30 mL), washed with water (3 x 50 mL) and aqueous lithium chloride (1 M, 40 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography to obtain the title compound (200 mg, 69%) as a colourless oil, Rf: 0.24 (1:9 ethyl acetate, hexane); IR (v_max (neat)): 3057, 1523, 1501, 1474, 1397, 1342, 1222 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 5.47 (2H, s), 7.02-7.11 (2H, m), 7.29-7.36 (2H, m), 8.12 (1H, d, J = 2.2 Hz), 8.70 (1H, d, J = 2.2 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 48.6, 115.1, 116.5 (d, J = 21.8 Hz), 116.8, 126.9, 130.3, 130.3 (d, J = 8.4 Hz), 130.7 (d, J = 3.3 Hz), 131.7, 144.3, 147.0, 163.0 (d, J = 248.5 Hz) ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -112.3 ppm; LRMS (+ESI) m/z: 272.0/274.0 ([M+Na]⁺ 100%).
To a solution of 347 (100 mg, 370 μmol) in a mixture of ethanol and tetrahydrofuran (1:1, 6 mL) at 0 °C was added di-tert-butyl dicarbonate (160 mg, 740 μmol), 4-dimethylaminopyridine (4.5 mg, 37 μmol) and nickel chloride hexahydrate (8.8 mg, 37 μmol), followed by sodium borohydride (98 mg, 2.6 mmol), portionwise. The mixture was stirred for 15 minutes and then for a further 1 hour at room temperature. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (20 mL), extracted with ethyl acetate (3 x 15 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, dichloromethane (0:1 to 1:9) as an eluent to obtain the Boc-protected intermediate (60 mg, 39%) as a red oil. The unstable species (structure confirmed by ¹H NMR spectroscopy) was taken on to the next step without further purification or characterisation.

The intermediate was dissolved in anhydrous dichloromethane (2 mL) and to this was added trifluoroacetic acid (280 μL, 3.7 mmol), dropwise. The mixture was stirred for 2 hours then concentrated in vacuo to obtain the amine trifluoroacetate salt intermediate, which was taken on to the next step without further purification or characterisation.

To a solution of cycloheptyl carboxylic acid (30 μL, 219 μmol) in anhydrous dichloromethane (5 mL) was added N,N-dimethylformamide (2 drops) followed by oxalyl chloride (37 μL, 438 μmol) and the mixture was stirred at room temperature for 2 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in dichloromethane (2 mL) and added slowly to a solution of the amine trifluoroacetate salt intermediate in anhydrous dichloromethane (3 mL) at 0 °C. Diisopropylethylamine (127 μL, 730 μmol) and 4-dimethylaminopyridine (2 mg, 15 μmol) were added slowly and the mixture was warmed to room temperature and stirred for 16 hours. The mixture was diluted with saturated aqueous sodium hydrogen carbonate (15 mL), extracted with ethyl acetate (3 x 15 mL), washed with water (2 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column
chromatography using ethyl acetate, dichloromethane (0:1 to 1:9) as an eluent to obtain the title compound (40 mg, 30% over 3 steps) as a white solid, mp: 184.1-186.0 °C; Rf: 0.33 (1:9 ethyl acetate, dichloromethane); IR (νmax (neat)): 3262, 2924, 1636, 1602, 1555, 1509, 1455, 1340, 1223, 764 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.43-1.66 (6H, m), 1.72 (4H, m), 1.93-2.05 (2H, m), 2.40-2.52 (1H, m), 5.40 (2H, s), 6.95 (2H, t, J = 8.5 Hz), 7.07 (1H, dd, J = 7.9, 4.7 Hz), 7.21 (2H, dd, J = 8.3, 5.5 Hz), 7.33 (1H, bs), 7.75 (1H, s), 7.84 (1H, d, J = 7.9 Hz), 8.35 (1H, d, J = 4.7 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 26.7, 28.4, 32.0, 47.3, 47.7, 113.2, 113.8, 115.4, 115.7 (d, J = 21.6 Hz), 118.9, 125.7, 129.5 (d, J = 8.1 Hz), 133.5 (d, J = 3.3 Hz), 143.7, 144.9, 162.4 (d, J = 245.9 Hz), 175.2 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -114.8 ppm; LRMS (+ESI) m/z: 388.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Calcd for C₂₂H₂₄FN₃O [M+Na]⁺: 388.18011, found 388.18811; HPLC: 99.9%, RT: 26.6 mins. (Method A, 230 nm).

**N-(5-Bromo-1-(4-fluorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide**

![Structure](image)

To a solution of 348 (140 mg, 520 μmol) in a mixture of tetrahydrofuran (3 mL) and ethanol (1 mL) at 0 °C was added di-tert-butyl dicarbonate (230 mg, 1.0 mmol), 4-dimethylaminopyridine (6 mg, 52 μmol) and nickel chloride hexahydrate (12 mg, 52 μmol), followed by sodium borohydride (140 mg, 3.6 mmol), portionwise, over 15 minutes. The mixture was stirred for a further 5 minutes at 0 °C and then at room temperature for 1 hour. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (20 mL), extracted with ethyl acetate (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:4) as an eluent to obtain the Boc-protected intermediate, which was carried on to the next step without further purification or characterisation.

The intermediate (50 mg, 160 μmol) was dissolved in dichloromethane (2 mL) and to this was added trifluoroacetic acid (310 μL, 4.7 mmol), slowly. The mixture was stirred for 16
hours before the volatiles were removed in vacuo. The trifluoroacetate salt was obtained as a red oil and was used immediately without characterisation.

The trifluoroacetate salt was suspended in anhydrous dichloromethane (1 mL) at 0 °C and treated with cycloheptanecarboxylic anhydride (85 mg, 32 mmol), followed by 4-dimethylaminopyridine (2 mg, 16 μmol) and N,N-diisopropylethylamine (84 μL, 480 μmol) and the mixture stirred at room temperature for 2 hours. The volatiles were removed under a stream of nitrogen and the residue purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:4) as an eluent to obtain the title compound (30 mg, 13% over 3 steps) as an off-white solid, mp: 190.7-193.8 °C; Rf: 0.21 (1:4 ethyl acetate, hexane); IR (vmax (neat)): 3269, 2920, 2854, 1639, 1599, 1545, 1509, 1349, 1222, 1155 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.41-1.64 (6H, m), 1.72-1.85 (4H, m), 1.91-2.01 (2H, m), 2.40-2.50 (1H, m), 5.31 (2H, s), 6.94 (2H, t, J = 8.7 Hz), 7.18 (2H, dd, J = 8.5, 5.4 Hz), 7.45 (1H, bs), 7.70 (1H, s), 7.96 (1H, d, J = 2.1 Hz), 8.32 (1H, d, J = 2.1 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 26.7, 28.3, 31.9, 47.4, 47.6, 111.2, 112.6, 115.2, 115.8 (d, J = 21.6 Hz), 120.4, 128.0, 129.6 (d, J = 8.2 Hz), 133.0 (d, J = 3.2 Hz), 143.4, 144.3, 162.5 (d, J = 246.5 Hz), 175.5 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -114.4 ppm; LRMS (+ESI) m/z: 466.1/468.1 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Cald for C₂₂H₂₃BrFN₃O [M+Na]⁺: 466.09062, found 466.08980/468.08776; HPLC: 95.9%, RT: 31.4 mins. (Method A, 230 nm).

5-Bromo-3-iodo-4-methylpyridin-2-amine 349

To a solution of 5-bromo-4-methylpyridine-2-amine (5.0 g, 26.7 mmol) in acetic acid (32 mL) was added N-iodosuccinimide (6.1 g, 27.2 mmol) and trifluoroacetic acid (320 μL) and the mixture stirred at room temperature for 16 hours. Ice-water (150 mL) was added and the mixture neutralised with concentrated aqueous ammonia and the resulting precipitate collected by filtration to obtain the title compound (4.1 g, 49%) as an off-white solid, the characterisation data of which corresponded to that previously described. mp: 118.1-120.0 °C; Rf: 0.67 (1:19 methanol, dichloromethane); IR (vmax (neat)): 3462, 3282, 3145, 2918, 1622, 1557, 1441, 1407, 1371, 1034, 957, 740 cm⁻¹; ¹³C NMR (75 MHz, CDCl₃): δ 29.5, 84.8, 109.3, 148.6, 150.4, 157.6 ppm.
5-Bromo-4-methyl-3-((trimethylsilyl)ethynyl)pyridin-2-amine 350

Prepared according to General Procedure K using aryl iodide 349 (5.6 g, 17.7 mmol) and trimethylsilylacetylene (2.7 mL, 19.5 mmol) in a mixture of anhydrous, degassed tetrahydrofuran (25 mL) and triethylamine (110 mL) at room temperature for 16 hours. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (3.6 g, 71%) as an off-white solid, the characterisation data of which corresponded to that previously described.\(^{339}\) mp: 151.2-155.7 °C; \(R_f\): 0.52 (1:4 ethyl acetate, hexane); IR \((\nu_{\text{max}} \text{ (neat)})\): 3463, 3282, 3136, 2955, 2142, 1624, 1568, 1455, 1242, 837 cm\(^{-1}\); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ 0.1, 21.2, 98.9, 104.7, 106.0, 110.8, 148.6, 149.5, 158.5 ppm.

5-Bromo-4-methyl-1H-pyrro[2,3-b]pyridine 351

A solution of 350 (3.5 g, 12.4 mmol) in anhydrous \(N,N\)-dimethylformamide (50 mL) was slowly added to a stirred solution of potassium tert-butoxide (2.9 g, 25.9 mmol) in \(N,N\)-dimethylformamide (70 mL) at 80 °C. The mixture was stirred for 30 minutes, cooled to room temperature and quenched with saturated aqueous ammonium chloride (150 mL). The mixture was extracted with ethyl acetate (3 x 80 mL), washed with water (3 x 150 mL) and aqueous lithium chloride (1 M, 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (1.9 g, 73%) as a light orange solid, mp: 222.3-226.8 °C; \(R_f\): 0.42 (1:4 ethyl acetate, hexane); IR \((\nu_{\text{max}} \text{ (neat)})\): 3313, 2912, 2858, 1589, 1542, 1341, 949, 852 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 2.33 (3H, s), 6.20 (1H, s), 6.94-7.14 (1H, m), 8.04 (1H, s), 10.87 (1H, bs) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): δ 18.3, 98.6, 121.3, 125.4, 137.8, 143.4, 146.5 (one carbon signal was not observed) ppm; LRMS (+ESI) \(m/z\): 211.0/213.0 ([M+H]\(^+\) 100%).
5-Bromo-4-methyl-3-nitro-1H-pyrrolo[2,3-b]pyridine 352

Prepared according to General Procedure N using azaindole 351 (1.5 g, 7.1 mmol) to obtain the title compound (870 mg, 48%) as an orange solid, mp: 194.0-198.3 °C; Rf: 0.24 (3:7 ethyl acetate, hexane); IR (v_{\text{max}} (neat)): 3409, 2825, 2717, 1570, 1353, 1326, 1298, 1208, 855, 825 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.82 (3H, s), 8.47 (1H, s), 8.77 (1H, s), 13.33 (1H, bs) ppm; ^13C NMR (100 MHz, DMSO-d_6): δ 21.1, 112.7, 119.0, 128.5, 132.7, 139.3, 145.7, 146.7 ppm; LRMS (+ESI) m/z: 254.0/256.0 ([M-H]^{-} 100%); HRMS (ESI)^+ Cald for C_8H_5BrN_3O_2 [M-H]^{-}: 253.95651, found 253.95703/255.9550.

5-Bromo-1-(4-fluorobenzyl)-4-methyl-3-nitro-1H-pyrrolo[2,3-b]pyridine 353

To a solution of 352 (200 mg, 780 μmol) in anhydrous N,N-dimethylformamide (6 mL) was added potassium carbonate (320 mg, 2.3 mmol) followed by 4-fluorobenzyl bromide (120 μL, 940 μmol) and the mixture was stirred for 2 hours. Water (20 mL) was added and the mixture extracted with ethyl acetate (3 x 15 mL), washed with water (3 x 30 mL) and aqueous lithium chloride (1 M, 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:4) as an eluent to obtain the title compound (160 mg, 58%) as an off-white solid, mp: 162.4-165.9 °C; Rf: 0.67 (3:7 ethyl acetate, hexane); IR (v_{\text{max}} (neat)): 3140, 2922, 1526, 1511, 1354, 1219, 1159, 836 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 2.84 (3H, s), 5.54 (2H, s), 7.06-7.27 (2H, m), 7.35 (2H, m), 8.57 (1H, s), 9.10 (1H, s) ppm; ^13C NMR (100 MHz, DMSO-d_6): δ 21.2, 47.6, 113.2, 115.5 (d, J = 21.5 Hz), 119.6, 127.7, 130.0 (d, J = 8.3 Hz), 132.5 (d, J = 3.1 Hz), 134.7, 140.0, 144.3, 146.6, 162.7 (d, J = 248.2 Hz) ppm; ^19F NMR (376 MHz, DMSO-d_6): δ -114.3 ppm; LRMS (+ESI) m/z: 386.0/388.0 ([M+Na]^+) 100%; HRMS (ESI)^+ Cald for C_{15}H_{11}BrF_{3}N_{3}O_{2} [M+Na]^+: 385.99164, found 385.99092/387.98888.
N-(5-Bromo-1-(4-fluorobenzyl)-4-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide 332

Prepared according to General Procedure P using 353 (120 mg, 330 μmol). The crude residue was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 3:17) as an eluent to obtain the title compound (40 mg, 27%) as white solid, mp: 216.1-218.0 °C; Rf: 0.17 (1:4 ethyl acetate, hexane); IR (vmax (neat)): 3277, 2923, 1645, 1524, 1510, 1237, 1157, 852 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.46-1.70 (6H, m), 1.71-1.87 (4H, m), 1.96-2.07 (2H, m), 2.40-2.50 (1H, m), 2.70 (3H, s), 5.35 (2H, s), 6.96 (2H, t, J = 8.7 Hz), 7.14-7.23 (2H, m), 7.50 (1H, s), 8.34 (1H, s) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 18.5, 26.6, 28.4, 31.8, 47.4, 47.8, 112.7, 115.5, 115.7, 115.7 (d, J = 21.6 Hz), 122.4, 129.6 (d, J = 8.2 Hz), 133.1 (d, J = 3.2 Hz), 137.8, 144.1, 145.3, 162.5 (d, J = 246.2 Hz), 176.1 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -114.5 ppm; LRMS (+ESI) m/z: 480.2/482.2 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Cald for C₂₃H₂₅BrFN₃O [M+Na]⁺: 480.10627, found 480.10544/482.10338; HPLC: 95.3%, RT: 31.1 mins. (Method A, 230 nm).

7-(4-Fluorobenzyl)-3-nitro-7H-pyrrolo[2,3-b]pyridine 354

Prepared according to General Procedure O using 345 (500 mg, 3.1 mmol) and 4-fluorobenzyl bromide (3.8 mL, 30.2 mmol) to obtain the title compound (600 mg, 72%) as a brown solid, mp: 220.1-223.5 °C; Rf: 0.34 (3:2 ethyl acetate, hexane); IR (vmax (neat)): 3084, 1621, 1491, 1372, 1348, 1315, 1222, 1174, 1088 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 5.98 (2H, s), 7.18 (2H, t, J = 8.9 Hz), 7.47-7.65 (3H, m), 8.61-8.81 (3H, m) ppm; ¹³C NMR (75 MHz, DMSO-d₆): δ 54.8, 115.6 (d, J = 21.5 Hz), 116.2, 121.6, 127.9, 130.8 (d, J = 8.5 Hz), 131.5, 133.7, 134.9, 146.8, 147.9, 162.0 (d, J = 239.1 Hz) ppm; ¹⁹F NMR (282 MHz, DMSO-d₆): δ -113.6 ppm; LRMS (+ESI) m/z: 294.1 ([M+Na]⁺ 100%); HRMS (ESI)⁺ Cald for C₁₄H₁₀FN₃O₂ [M+Na]⁺: 294.06548, found 294.06483.
5-Bromo-7-(4-fluorobenzyl)-3-nitro-7H-pyrrolo[2,3-b]pyridine 355

Prepared according to General Procedure O using 346 (1.0 g, 4.1 mmol) and 4-fluorobenzyl bromide (5.2 mL, 41 mmol). The crude product was purified by flash column chromatography (neutral alumina) using ethyl acetate, hexane (1:9 to 3:2) as an eluent to obtain the title compound (720 mg, 50%) as an off-white solid, mp: 205.1-209.7 °C; Rf: 0.29 (2:3 ethyl acetate, hexane); IR (vmax (neat)): 3078, 1603, 1487, 1378, 1357, 1218, 1168, 1102 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 5.90 (2H, s), 7.10 (2H, t, J = 8.5 Hz), 7.43-7.50 (2H, m), 7.94 (1H, d, J = 1.8 Hz), 8.71 (1H, d, J = 1.8 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 55.9, 109.1, 116.8 (d, J = 21.9 Hz), 124.1, 128.1, 129.2 (d, J = 3.2 Hz), 131.1 (d, J = 8.5 Hz), 131.7, 136.4, 147.7, 149.1, 163.5 (d, J = 250.2 Hz) ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -110.7 ppm; LRMS (+ESI) m/z: 372.0/374.0 ([M+Na]+ 100%); HRMS (ESI)⁺ Cald for C₁₄H₉BrFN₃O₂ [M+Na]⁺: 371.97599, found 371.97525/373.97320.

5-Bromo-7-(4-fluorobenzyl)-4-methyl-3-nitro-7H-pyrrolo[2,3-b]pyridine 356

Prepared according to General Procedure O using 352 (300 mg, 1.2 mmol) and 4-fluorobenzyl bromide (1.5 mL, 11.7 mmol). The crude product was purified by flash column chromatography (neutral alumina) using ethyl acetate, hexane (0:1 to 3:7) as an eluent to obtain the title compound (270 mg, 63%) as a brown solid, mp: 214.3-216.1 °C; Rf: 0.56 (3:7 ethyl acetate, hexane, neutral alumina); IR (vmax (neat)): 3081, 1601, 1487, 1351, 1248, 1220, 1173, 1106, 793 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 3.09 (3H, s), 5.85 (2H, s), 7.05-7.13 (2H, m), 7.40-7.52 (2H, m), 7.98 (1H, s), 8.15 (1H, s) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 22.4, 55.3, 113.2, 115.4 (d, J = 21.9 Hz), 121.1, 129.3 (d, J = 3.2 Hz), 130.4, 130.8 (d, J = 8.4 Hz), 132.1, 147.6, 147.9, 150.0 163.3 (d, J = 249.8 Hz) ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -111.1 ppm; LRMS (+ESI) m/z: 386.0/388.0 ([M+Na]+
100%); **HRMS (ESI)**\(^+\) Cald for C\(_{15}\)H\(_{11}\)BrFN\(_3\)O\(_2\) [M+Na]\(^+\): 385.99164, found 385.99096/387.98898.

**N-(7-(4-Fluorobenzyl)-7H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide 333**

Prepared according to General Procedure P using 354 (50 mg, 180 \(\mu\)mol). The residue was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (24 mg, 36%) as yellow solid, mp: 175.3-177.1 \(^\circ\)C; \(R_f\): 0.46 (1:9 methanol, dichloromethane); IR (v\(_{\text{max}}\) (neat)): 3264, 2922, 2855, 1640, 1573, 1509, 1469, 1367, 1220, 1211, 1161, 1104, 849, 752 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.45-1.67 (6H, m), 1.76-1.89 (4H, m), 1.98-2.08 (2H, m), 2.44-2.54 (1H, m), 5.78 (2H, s), 6.79 (1H, dd, \(J = 7.5, 6.3\) Hz), 6.99 (2H, t, \(J = 8.6\) Hz), 7.34 (1H, dd, \(J = 8.5, 5.3\) Hz), 7.50 (1H, bs), 7.54 (1H, d, \(J = 6.2\) Hz), 7.84 (1H, s), 8.14 (1H, d, \(J = 7.5\) Hz) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 26.8, 28.3, 32.1, 47.7, 54.4, 109.2, 114.4, 116.1 (d, \(J = 21.7\) Hz), 124.5, 129.2, 130.5 (d, \(J = 8.3\) Hz), 130.9 (d, \(J = 3.2\) Hz), 131.0, 137.0, 145.7, 162.9 (d, \(J = 247.7\) Hz), 175.9 ppm; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)): \(\delta\) -112.9 ppm; LRMS (+ESI) \textit{m/z}: 366.3 ([M+H]\(^+\) 100%); **HRMS (ESI)**\(^+\) Cald for C\(_{22}\)H\(_{23}\)FN\(_3\)O [M+Na]\(^+\): 366.19817, found 366.19749; **HPLC**: 98.1%, RT: 20.9 mins. (Method A, 230 nm).

**N-(5-Bromo-7-(4-fluorobenzyl)-7H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide 334**

Prepared according to General Procedure P using 355 (200 mg, 740 \(\mu\)mol). The crude residue was purified by flash column chromatography using methanol, dichloromethane
(0:1 to 1:9) as an eluent to obtain the title compound (120 mg, 38%) as a light orange solid, **mp**: 99.6-102.7 °C; **Rf**: 0.55 (1:9 methanol, dichloromethane); **IR** (v<sub>max</sub> (neat)): 3262, 2921, 2854, 1643, 1561, 1509, 1474, 1226, 1102, 826 cm<sup>-1</sup>; **1H NMR** (400 MHz, CDCl<sub>3</sub>): δ 1.48-1.72 (6H, m), 1.77-1.90 (4H, m), 1.99-2.11 (2H, m), 2.42-2.52 (1H, m), 5.77 (2H, s), 7.04 (2H, t, J = 8.6 Hz), 7.22 (1H, bs), 7.36-7.42 (2H, m), 7.65 (1H, d, J = 1.8 Hz), 7.84 (1H, s), 8.23 (1H, d, J = 1.8 Hz) ppm; **13C NMR** (100 MHz, CDCl<sub>3</sub>): δ 26.8, 28.3, 32.1, 47.8, 54.1, 102.0, 114.1, 116.3 (d, J = 21.8 Hz), 125.4, 128.8, 130.4 (d, J = 3.3 Hz), 130.7 (d, J = 8.4 Hz), 133.4, 139.7, 145.1, 163.2 (d, J = 248.8 Hz), 175.8 ppm; **19F NMR** (376 MHz, CDCl<sub>3</sub>): δ -112.2 ppm; **LRMS** (+ESI) m/z: 466.1/468.1 ([M+Na]<sup>+</sup> 100%); **HRMS** (ESI)<sup>+</sup> Cald for C<sub>22</sub>H<sub>23</sub>BrFN<sub>3</sub>O [M+Na]<sup>+</sup>: 466.09062, found 466.08993/468.08788; **HPLC**: 99.1%, RT: 22.2 mins. (Method A, 230 nm).

N-(5-Bromo-7-(4-fluorobenzyl)-4-methyl-7H-pyrrolo[2,3-b]pyridin-3-y1)cycloheptanecarboxamide 335

Prepared according to General Procedure P using 356 (150 mg, 410 μmol). The crude residue was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:9) as an eluent to obtain the title compound (81 mg, 43%) as a yellow solid, **mp**: 108.1-110.8 °C; **Rf**: 0.52 (1:9 methanol, dichloromethane); **IR** (v<sub>max</sub> (neat)): 3244, 2920, 2854, 1654, 1561, 1509, 1448, 1261, 1224, 1098, 842, 798 cm<sup>-1</sup>; **1H NMR** (400 MHz, CDCl<sub>3</sub>): δ 1.48-1.70 (6H, m), 1.74-1.90 (4H, m), 2.01-2.12 (2H, m), 2.43-2.54 (1H, m), 2.67 (3H, s), 5.69 (2H, s), 7.01 (2H, t, J = 8.6 Hz), 7.09 (1H, bs), 7.35-7.41 (2H, m), 7.68 (1H, s), 7.71 (1H, s) ppm; **13C NMR** (100 MHz, CDCl<sub>3</sub>): δ 18.1, 26.8, 28.3, 31.9, 47.8, 54.1, 107.2, 113.1, 116.2 (d, J = 21.8 Hz), 125.2, 129.6, 130.6 (d, J = 3.4 Hz), 130.7 (d, J = 8.4 Hz), 142.7, 143.0, 145.1, 163.1 (d, J = 248.1 Hz), 177.8 ppm; **19F NMR** (376 MHz, CDCl<sub>3</sub>): δ -112.5 ppm; **LRMS** (+ESI) m/z: 458.2/460.2 ([M+H]<sup>+</sup> 100%); **HRMS** (ESI)<sup>+</sup> Cald for C<sub>23</sub>H<sub>25</sub>BrFN<sub>3</sub>O [M+H]<sup>+</sup>: 458.12433, found 458.12366/460.12158; **HPLC**: 95.2%, RT: 22.0 mins. (Method A, 230 nm).
**5-Bromo-4-methyl-3-nitropyridin-2-amine 361**

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To a solution of 5-bromo-4-methyl-2-aminopyridine (5.0 g, 26.7 mmol) in concentrated sulfuric acid (40 mL) at -10 °C was added concentrated nitric acid (28 mL), slowly and the mixture stirred for 30 minutes and then warmed to room temperature. Ice-water (150 mL) was added and the pH adjusted to 3 by the addition of concentrated aqueous ammonia. The precipitate was collected by filtration, washed with water, taken up in concentrated sulfuric acid (40 mL) and stirred for 2 hours. The solution was poured onto ice-water (200 mL), neutralised with concentrated aqueous ammonia and the precipitate collected by filtration. The filter cake was washed with hexane (2 x 50 mL) and dried in vacuo to obtain the title compound (3.9 g, 63%) as a yellow solid, the characterisation data of which corresponded to that previously described.{$^{340}$} **mp:** 157.9-162.8 °C; **Rf:** 0.83 (1:19 methanol, dichloromethane); **IR** ($v_{\text{max}}$ (neat)): 3475, 3285, 3124, 1630, 1580, 1532, 1509, 1454, 1317, 1222, 866, 776 cm$^{-1}$.

**5-Bromo-4-methylpyridine-2,3-diamine 359**

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\end{array}
\]

To a solution of 361 (1.0 g, 4.3 mmol) in a mixture of ethanol (5 mL), acetic acid (5 mL) and water (2.5 mL) was added iron powder (1.8 g, 32.3 mmol) and the mixture sonicated for 1 hour. The iron was removed by filtration and the filtrate diluted with saturated aqueous sodium hydrogen carbonate (50 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (3 x 100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the title compound (740 mg, 82%) as a orange-brown solid, the characterisation data of which corresponded to that previously described.{$^{340}$} **mp:** 154.3-157.1 °C; **Rf:** 0.17 (1:19 methanol, dichloromethane); **IR** ($v_{\text{max}}$ (neat)): 3392, 3357, 3177, 1658, 1593, 1438, 1283, 777, 741 cm$^{-1}$. 
**Ethyl 1-oxaspiro[2.6]nonane-2-carboxylate 362**

To a solution of ethyl chloroacetate (5.2 mL, 49.0 mmol) and cycloheptanone (5.3 mL, 44.6 mmol) in anhydrous acetonitrile (40 mL) at 60 °C was added sodium hydride (60% w/w dispersion in mineral oil, 2.0 g, 49.0 mmol), portionwise over 1.5 hours. The mixture was stirred for a further 1 hour, concentrated under a stream of nitrogen and the residue diluted with water (100 mL) and extracted with dichloromethane (3 x 50 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated under reduced pressure and the crude residue purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:19) as an eluent to obtain the title compound (5.2 g, 59%) as a colourless liquid, \( R_f: 0.48 \) (1:9 ethyl acetate, hexane); \( \text{IR} (v_{\text{max}} \text{ (neat))}: 2983, 2929, 2858, 1749, 1191, 1028 \text{ cm}^{-1}; \) \( ^1\text{H NMR} (400 MHz, CDCl}_3): \delta 1.28 (3H, t, J = 7.1 \text{ Hz}), 1.40-1.87 (12H, m), 3.30 (1H, s), 4.23 (2H, q, J = 7.1 \text{ Hz}) \text{ ppm}; \) \( ^{13}\text{C NMR} (100 MHz, CDCl}_3): \delta 14.4, 24.3, 24.7, 28.7, 28.8, 30.9, 37.1, 60.5, 61.3, 66.3, 168.7 \text{ ppm}; \) \( \text{LRMS (+ESI) m/z}: 221.1 ([M+Na]^+ 100%), 419.3 ([2M+Na]^+ 41%). \)

**Ethyl 2-cycloheptyl-2-hydroxyacetate 363**

To a solution of 362 (1.0 g, 5.0 mmol) in anhydrous dichloromethane (10 mL) at -40 °C was added trimethylsilyl trifluoromethanesulfonate (2 drops) and the mixture stirred for 1 hour. The reaction mixture was quenched with saturated aqueous sodium hydrogen carbonate (30 mL), extracted with ethyl acetate (3 x 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was taken on to the next step without further purification or characterisation.

The crude alkene (800 mg, 2.0 mmol) was dissolved in ethyl acetate and to this was added palladium on carbon (10% w/w, 400 mg, 400 μmol) under an atmosphere of nitrogen. The reaction vessel was evacuated and flushed with hydrogen three times and stirred for 16 hours. The palladium catalyst was removed by filtration through a pad of Celite® and the filtrate concentrated under reduced pressure. The crude residue was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound (800 mg, 80% over 2 steps) as a colourless oil, the characterisation data of
which corresponded to that previously described.\textsuperscript{341} \(R_f\): 0.57 (1:4 ethyl acetate, hexane); \textbf{LRMS} (+ESI) \(m/z\): 223.1 ([M+Na]\(^+\) 100%).

\textbf{2-Cycloheptyl-2-hydroxyacetic acid 360}

![Image of 2-Cycloheptyl-2-hydroxyacetic acid 360]

Prepared according to General Procedure B using ethyl ester 363 (700 mg, 3.5 mmol) and lithium hydroxide monohydrate (730 mg, 17.5 mmol) in a mixture of tetrahydrofuran and water (1:1, 30 mL) at room temperature for 16 hours to obtain the title compound (510 mg, 84\%) as a white solid, \(mp\): 81.8-85.5 °C; \textbf{IR} (\(v_{max}\) (neat)): 3377, 2920, 2855, 1725, 1444, 1230, 1091, 920 cm\(^{-1}\); \textbf{\(^1\text{H NMR}\)} (400 MHz, CDCl\(_3\)): \(\delta\) 1.38-1.83 (12H, m), 1.95-2.05 (1H, m), 4.02 (1H, \(d\), \(J = 3.3\) Hz), 5.56 (2H, bs) ppm; \textbf{\(^{13}\text{C NMR}\)} (100 MHz, CDCl\(_3\)): \(\delta\) 26.8, 27.1, 28.2, 28.2, 28.3, 31.4, 43.4, 75.8, 179.2 ppm; \textbf{LRMS} (+ESI) \(m/z\): 173.1 ([M+H]\(^+\) 100%).

\textbf{2-Acetoxy-2-cycloheptylacetic acid 364}

![Image of 2-Acetoxy-2-cycloheptylacetic acid 364]

To a solution of 360 (350 mg, 2.0 mmol) in anhydrous tetrahydrofuran (5 mL) at 0 °C was added acetic acid (580 μL, 10.2 mmol) followed by acetyl chloride (160 μL, 2.2 mmol) and the mixture was stirred at room temperature for 16 hours. The volatiles were removed \textit{in vacuo} and the residue purified by flash column chromatography using methanol, dichloromethane (1:19 to 1:9) as an eluent to obtain the title compound (440 mg, 100\%) as a light yellow oil, \(R_f\): 0.43 (1:9 methanol, dichloromethane); \textbf{IR} (\(v_{max}\) (neat)): 3498, 2921, 2855, 1712, 1372, 1226 cm\(^{-1}\); \textbf{\(^1\text{H NMR}\)} (400 MHz, CDCl\(_3\)): \(\delta\) 1.34-1.78 (12H, m), 2.06-2.15 (1H, m), 2.13 (3H, s), 4.92 (1H, \(d\), \(J = 3.9\) Hz), 5.63 (1H, bs) ppm; \textbf{\(^{13}\text{C NMR}\)} (100 MHz, CDCl\(_3\)): \(\delta\) 20.8, 26.6, 26.8, 28.3, 28.3, 29.0, 31.3, 77.5, 171.3 ppm; \textbf{LRMS} (-ESI) \(m/z\): 213.1 ([M-H] \(100\%\)); \textbf{HRMS} (ESI)\(^+\) Cald for C\(_{11}\)H\(_{18}\)O\(_4\) [M-H]\(^-\): 213.11269, found 213.11331.
(6-Bromo-7-methyl-3H-imidazo[4,5-b]pyridin-2-yl)(cycloheptyl)methanone 357

To a solution of the carboxylic acid 364 (320 mg, 1.5 mmol) in anhydrous dichloromethane (10 mL) at 0 °C was added \(N,N\)-dimethylformamide (2 drops) followed by oxalyl chloride (250 μL, 3.0 mmol) and the mixture was warmed to room temperature and stirred for 3 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in dichloromethane (1 mL) and added to a solution of 359 (300 mg, 1.5 mmol) and \(N,N\)-diisopropylethylamine (390 μL, 2.2 mmol) in dichloromethane (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 16 hours. Saturated aqueous sodium hydrogen carbonate (20 mL) was added and the mixture extracted with dichloromethane (3 x 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain a mixture of the two regioisomeric monoacylated products and some diacylated product. The monoacylated products were isolated by flash column chromatography using ethyl acetate, hexane (0:1 to 3:7) as an eluent to obtain the mixture of products as a yellow foam.

The monoacylated mixture (250 mg, 630 μmol) was heated at reflux in acetic acid (8 mL) for 16 hours. After cooling to room temperature the mixture was diluted with methanol (10 mL) and aqueous sodium hydroxide (3 M, 50 mL, 150 mmol) and the mixture stirred for 1 hour. The volatiles were removed under reduced pressure and the aqueous mixture extracted with dichloromethane (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to obtain the alcohol intermediate (358), which was taken on to the next step without further purification or characterisation.

The alcohol intermediate (160 mg, 470 μmol) was dissolved in chloroform (10 mL) and to this was added manganese dioxide (1.2 g, 14.2 mmol) and the mixture was stirred at reflux for 48 hours. The solids were removed by filtration through a pad of Celite® and the filtrate concentrated under reduced pressure to obtain a crude residue that was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent to obtain the title compound (50 mg, 10% over 4 steps) as a white solid, mp: 230 °C (decomposition); \(R_f\): 0.56 (1:4 ethyl acetate, hexane); IR (\(\nu_{\text{max}}\) (neat)): 3015, 2918, 2952, 1684, 1590, 1432, 1232, 1000, 913, 891 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.57-1.90 (10H, m), 2.04-2.16 (2H, m), 2.80 (3H, s), 3.91-4.01 (1H, m), 8.83 (1H, s), 14.3 (1H, bs) ppm; \(^13\)C NMR (100
MHZ, CDCl3): δ 17.2, 26.9, 28.6, 30.5, 46.6, 117.2, 137.2, 142.8, 146.1, 148.2, 148.6, 197.9 ppm; LRMS (+ESI) m/z: 358.1/360.1 ([M+Na]+ 100%).

(6-Bromo-4-(4-fluorobenzyl)-7-methyl-4H-imidazo[4,5-b]pyridin-2-yl)(cycloheptyl)methanone 336

Prepared according to General Procedure O using 357 (28 mg, 83 μmol) and 4-fluorobenzyl bromide (12 μL, 100 μmol). The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:99) as an eluent to obtain the title compound (32 mg, 86%) as an off-white solid, mp: 108.1-111.7 °C; Rf: 0.33 (1:19 methanol, dichloromethane); IR (νmax (neat)): 3035, 2921, 2851, 1677, 1603, 1510, 1284, 1226, 1110 cm⁻¹; ¹H NMR (400 MHz, CDCl3): δ 1.51-1.89 (10H, m), 1.96-2.15 (2H, m), 2.86 (3H, s), 3.96-4.09 (1H, m), 5.82 (2H, s), 7.02-7.14 (2H, m), 7.41-7.53 (2H, m), 7.95 (1H, s) ppm; ¹³C NMR (100 MHz, CDCl3): δ 18.4, 27.0, 28.6, 30.5, 48.0, 56.2, 110.7, 116.5 (d, J = 21.8 Hz), 129.6 (d, J = 3.4 Hz), 131.2 (d, J = 8.4 Hz), 132.5, 144.2, 146.9, 150.7, 163.3 (d, J = 249.4 Hz), 164.1, 201.8 ppm; ¹⁹F NMR (376 MHz, CDCl3): δ -111.3 ppm; LRMS (+ESI) m/z: 466.1/468.1 ([M+Na]+ 100%); HRMS (ESI)⁺ Calcd for C₂₂H₂₃BrFN₃O [M+Na]+: 466.09062, found 466.08984/468.08780; HPLC: 97.9%, RT: 23.7 mins. (Method A, 230 nm).

6-Bromo-7-methylimidazo[1,2-a]pyridin-8-amine 365

To a solution of 359 (350 mg, 1.7 mmol) in isopropanol (3 mL) was added chloroacetaldehyde (50% w/w in water, 240 μL, 1.9 mmol) and the mixture heated at reflux for 24 hours. After cooling to room temperature, saturated aqueous sodium hydrogen carbonate (30 mL) was added and the mixture was extracted with chloroform (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:19) as an eluent to obtain the title compound (320 mg, 82%) as
a green foam, $R_f$: 0.60 (1:9 methanol, dichloromethane); $\text{IR}(v_{\text{max}} \text{ (neat))}: 3328, 3206, 1620, 1522, 1478, 1322, 1134, 920, 710 \text{ cm}^{-1}$; $^1\text{H NMR}$ (300 MHz, CDCl$_3$): $\delta$ 2.26 (3H, s), 4.65 (2H, bs), 7.37-7.48 (2H, m), 7.84 (1H, s) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl$_3$): $\delta$ 15.6, 110.4, 112.7, 112.9, 115.8, 132.0, 133.1, (one carbon signal was not observed) ppm; $\text{LRMS (+ESI)} m/z$: 226.0/228.0 ([M+H]$^+$ 100%); $\text{HRMS (ESI)}^+$ Cald for C$_8$H$_8$BrN$_3$ [M+H]$^+$: 225.99798, found 225.99738/227.99532.

$N$-($6$-Bromo-$7$-methylimidazo[1,2-$a$]pyridin-$8$-yl)cycloheptanecarboxamide 366

![Chemical Structure](image)

To a solution of 365 (390 mg, 1.7 mmol) and cycloheptylcarboxylic acid (480 μL, 3.5 mmol) in anhydrous toluene (15 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (660 mg, 3.5 mmol) and the mixture was stirred at room temperature for 16 hours and then at reflux in the presence of 4-dimethylaminopyridine (21 mg, 170 μmol) for 16 hours. After cooling to room temperature the mixture was poured into water (50 mL) and extracted with dichloromethane (3 x 35 mL). The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (30 mL) and saturated aqueous ammonium chloride (30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using saturated methanolic ammonia, dichloromethane (0:1 to 1:99) as an eluent to obtain the title compound (250 mg, 41%) as a brown film, $R_f$: 0.50 (1:9 methanol, dichloromethane); $\text{IR}(v_{\text{max}} \text{ (neat))}: 3122, 2919, 2854, 1654, 1510, 1483, 1443, 1325, 1210, 1149, 993, 727 \text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl$_3$): $\delta$ 1.44-1.62 (6H, m), 1.68-1.84 (4H, m), 1.97-2.08 (2H, m), 2.30 (3H, s), 2.62-2.75 (1H, m), 7.43-7.53 (2H, m), 8.26 (1H, s), 9.48 (1H, bs) ppm; $^{13}\text{C NMR}$ (100 MHz, CDCl$_3$): $\delta$ 19.2, 26.7, 28.3, 31.8, 47.2, 112.9, 123.8, 123.9, 131.3, 132.3, 141.4, 176.6 (one carbon signal was not observed) ppm; $^{19}\text{F NMR}$ (376 MHz, CDCl$_3$): $\delta$ -111.1 ppm; $\text{LRMS (+ESI)} m/z$: 372.1/374.1 ([M+Na]$^+$ 100%); $\text{HRMS (ESI)}^+$ Cald for C$_{16}$H$_{20}$BrN$_3$O [M+Na]$^+$: 372.06874, found 372.06826/384.06621.
Chapter 8: Experimental

N-(6-Bromo-3-(4-fluorophenyl)-7-methylimidazo[1,2-a]pyridin-8-yl)cycloheptanecarboxamide 337

![Chemical Structure](image)

To a solution of 366 (70 mg, 200 μmol) in anhydrous N,N-dimethylformamide (5 mL) at 0 °C was added N-iodosuccinimide (49 mg, 220 μmol) and the mixture was stirred at room temperature for 16 hours. The mixture was poured into saturated aqueous sodium hydrogen carbonate (20 mL), extracted with ethyl acetate (3 x 15 mL), washed with saturated aqueous sodium hydrogen carbonate (30 mL), aqueous sodium thiosulfate (1 M, 30 mL) and aqueous lithium chloride (1 M, 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude, unstable product was taken on to the next step without further purification or characterisation.

The Suzuki cross coupling step was performed according to General Procedure G using the aryl iodide intermediate (40 mg, 84 μmol), 4-fluorophenylboronic acid (12 mg, 84 μmol) and potassium carbonate (23 mg, 168 μmol) in a mixture of degassed dioxane and water (9:1, 2 mL) at 100 °C for 5 hours. The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:9 to 1:4) as an eluent to obtain the title compound (26 mg, 69%) as a white solid, mp: 178.6-181.5 °C; Rf: 0.19 (1:4 ethyl acetate, hexane); IR (νmax (neat)): 3109, 2921, 2852, 1686, 1520, 1495, 1470, 1221, 1153, 832 cm⁻¹; 

\[ ^1H \text{ NMR} \ (400 MHz, CDCl}_3\] \( \delta \): 1.52-1.71 (6H, m), 1.76-1.89 (4H, m), 2.06-2.16 (2H, m), 2.38 (3H, s), 2.95-3.04 (1H, m), 7.28-7.43 (2H, m), 7.46-7.52 (2H, m), 7.61 (1H, s), 8.28 (1H, s) ppm; 

\[ ^{13}C \text{ NMR} \ (100 MHz, CDCl}_3\] \( \delta \): 19.7, 26.7, 28.3, 31.8, 47.4, 114.6, 117.1 (d, \( J = 21.9 \) Hz), 121.1, 123.2, 124.0, 125.7, 128.1 (d, \( J = 3.2 \) Hz), 130.8 (d, \( J = 8.5 \) Hz), 140.7, 163.4 (d, \( J = 250.9 \) Hz), 176.6 ppm; 

\[ ^{19}F \text{ NMR} \ (376 MHz, CDCl}_3\] \( \delta \): -110.4 ppm; 

LRMS (+ESI) \( m/z \): 466.1/468.1 \([\text{M+Na}]^+\) 100%; 

HRMS (ESI)+ Calcd for C\(_{22}\)H\(_{23}\)BrF\(_3\)N\(_3\)O \([\text{M+Na}]^+\): 466.09062, found 466.08992/468.08787; 

2-Chloro-3-(4-fluorophenyl)propanal 367

To a solution of 3-(4-fluorophenyl)propionaldehyde (350 mg, 2.3 mmol) in anhydrous dichloromethane (10 mL) at 0 °C was added proline (26 mg, 230 μmol) and N-chlorosuccinimide (290 mg, 2.2 mmol) and the mixture was stirred at room temperature for 16 hours. Hexane (30 mL) was added and the resulting precipitate removed by filtration. The filtrate was concentrated under reduced pressure and the residue purified by flash column chromatography using ethyl acetate, hexane (1:9) as an eluent to obtain the title compound (300 mg, 70%) as a colourless oil, which decomposed over time, $R_f$: 0.14 (1:9 ethyl acetate, hexane); $^1$H NMR (400 MHz, CDCl$_3$): δ 3.06 (1H, dd, $J$ = 14.6, 8.3 Hz), 3.34 (1H, dd, $J$ = 14.6, 5.5 Hz), 4.35 (1H, ddd, $J$ = 8.3, 5.6, 2.0 Hz), 6.98-7.06 (2H, m), 7.17-7.22 (2H, m), 9.54 (1H, d, $J$ = 2.0 Hz) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 37.6, 64.1, 115.7 (d, $J$ = 21.4 Hz), 131.2 (d, $J$ = 8.1 Hz), 131.2, 162.3 (d, $J$ = 246.0 Hz), 194.5 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): δ -115.2 ppm.

6-Bromo-3-(4-fluorobenzyl)-7-methylimidazo[1,2-a]pyridin-8-amine 368

To a solution of 359 (200 mg, 990 μmol) in isopropanol (1.5 mL) was added aldehyde 367 (200 mg, 1.1 mmol) and the mixture was heated at reflux for 3 hours. After cooling to room temperature the mixture was poured into saturated aqueous sodium hydrogen carbonate (20 mL), extracted with ethyl acetate (3 x 20 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using methanol, dichloromethane (0:1 to 1:49) as an eluent to obtain the title compound (160 mg, 48%) as a brown solid, mp: 129.1-132.7 °C; $R_f$: 0.60 (1:19 methanol, dichloromethane); IR (ν$_{max}$ (neat)): 3476, 3297, 3186, 1630, 1505, 1215, 813 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 2.21 (3H, s), 4.22 (2H, s), 5.77 (2H, bs), 7.06-7.18 (2H, m), 7.21-7.32 (3H, m), 7.38 (1H, s) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 15.7, 28.2, 107.9, 112.1, 112.8, 115.3 (d, $J$ = 21.3 Hz), 124.0, 129.2, 130.3 (d, $J$ = 8.0 Hz), 133.7 (d, $J$ = 3.0 Hz), 134.2, 137.5, 161.0 (d, $J$ = 242.8 Hz) ppm; $^{19}$F NMR (376 MHz,

$N$-(6-Bromo-3-(4-fluorobenzyl)-7-methylimidazo[1,2-a]pyridin-8-yl)cycloheptanecarboxamide 338

Prepared according to General Procedure C using cycloheptyl carboxylic acid (23 μL, 165 μmol) and 368 (50 mg, 150 μmol). The crude product was purified by flash column chromatography using ethyl acetate, hexane (1:4 to 2:3) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (50 mg, 72%) as a white solid, mp: 193.0-193.3 °C; $R_f$: 0.20 (3:7 ethyl acetate, hexane); IR ($v_{max}$ (neat)): 3107, 2919, 2848, 1675, 1505, 1469, 1431, 1225, 1154, 813 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): δ 1.39-1.60 (6H, m), 1.72-1.88 (4H, m), 2.01-2.12 (2H, m), 2.30 (3H, s), 2.68-2.79 (1H, m), 4.14 (2H, s), 6.95-7.05 (2H, m), 7.06-7.15 (2H, m), 7.27 (1H, s), 7.84 (1H, s), 9.55 (1H, bs) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): δ 19.3, 26.8, 28.3, 29.5, 31.9, 47.3, 112.7, 116.0 (d, $J$ = 21.5 Hz), 120.9, 123.2, 124.5, 129.8 (d, $J$ = 7.9 Hz), 130.1, 131.1, 131.7 (d, $J$ = 3.3 Hz), 142.2, 162.1 (d, $J$ = 245.9 Hz), 176.5 ppm; $^{19}$F NMR (376 MHz, CDCl$_3$): δ -116.1 ppm; LRMS (+ESI) m/z: 458.2/460.2 ([M+H]$^+$ 100%); HRMS (ESI)$^+$ Calcd for C$_{23}$H$_{25}$BrFN$_3$O [M+H]$^+$: 458.12433, found 458.12364/460.12156; HPLC: 99.7%, RT: 22.7 mins. (Method A, 230 nm).

2-(4-Fluorophenyl)acetimidamide hydrochloride 369

To a suspension of ammonium chloride (2.5 g, 47.4 mmol) in anhydrous toluene (20 mL) at 0 °C was added trimethylaluminium (2 M in heptane, 22 mL, 44.4 mmol) and the mixture was warmed to room temperature and stirred for 2 hours. 4-Fluorobenzylcyanide (3.6 mL, 29.6 mmol) was added and the mixture stirred at 80 °C for 16 hours. After cooling to room temperature, the mixture was poured into a slurry of silica (100 g) in dichloromethane (150 mL), filtered and washed with methanol (2 x 100 mL). The filtrate was concentrated under
reduced pressure and to the residue was added hydrochloric acid (4 M in dioxane, 8.9 mL, 35.5 mmol) and diethyl ether (100 mL). The resulting precipitate was collected by filtration and dried in vacuo to obtain the title compound (3.7 g, 66%) as a white hygroscopic solid, IR ($v_{\text{max}}$ (neat)): 3219, 3039, 1670, 1510, 1223, 1076, 823 cm$^{-1}$; $^1$H NMR (400 MHz, MeOD): $\delta$ 3.84 (2H, s), 7.07–7.16 (2H, m), 7.42–7.50 (2H, m), 8.63 (2H, bs), 9.05 (2H, bs) ppm; $^{13}$C NMR (100 MHz, MeOD): $\delta$ 38.6, 116.8 (d, $J = 21.8$ Hz), 130.5 (d, $J = 3.1$ Hz), 132.1 (d, $J = 8.3$ Hz), 163.9 (d, $J = 245.5$ Hz), 171.7 ppm; $^{19}$F NMR (376 MHz, MeOD): $\delta$ -116.2 ppm; LRMS (+ESI) m/z: 153.1 ([M+H]$^+$ 100%).

2-(4-Fluorobenzyl)-6-hydroxy-5-methyl-5,6-dihydropyrimidin-4(3H)-one 370

Sodium (910 mg, 39.8 mmol) was added to ethanol (50 mL) and the mixture stirred until hydrogen evolution had ceased. To this solution was added 369 (3.0 g, 15.9 mmol) and the mixture stirred for 5 minutes before diethyl methyl malonate (2.7 mL, 15.9 mmol) was added and the solution heated at reflux for 16 hours. The volatiles were removed under a stream of nitrogen and the residue diluted with water (50 mL) and acidified with aqueous hydrochloric acid (6 M). The precipitate was collected by filtration, washed with aqueous hydrochloric acid (1 M, 2 x 50 mL) and hexane (3 x 50 mL) and dried in vacuo to obtain the title compound (3.6 g, 97%) as a white solid, mp: >300 °C; IR ($v_{\text{max}}$ (neat)): 3445, 2576, 1624, 1605, 1429, 1288, 1124, 797 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.69 (3H, s), 3.78 (2H, s), 7.10-7.19 (2H, m), 7.30-7.38 (2H, m) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 7.6, 38.9, 95.3, 115.3 (d, $J = 21.2$ Hz), 130.7 (d, $J = 8.1$ Hz), 132.5 (d, $J = 3.1$ Hz), 157.2, 161.2 (d, $J = 242.8$ Hz), 164.8 ppm; $^{19}$F NMR (376 MHz, DMSO-$d_6$): $\delta$ -116.0 ppm.

4,6-Dibromo-2-(4-fluorobenzyl)-5-methylpyrimidine 371

A solution of 370 (1.0 g, 4.3 mmol) in phosphorus oxybromide (2.5 g, 8.5 mmol) was heated at 180 ºC in a sealed vessel for 1 hour. After cooling to room temperature, ice-water (50 mL) was added and the mixture extracted with dichloromethane (3 x 40 mL), dried
over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using dichloromethane, hexane (1:19 to 1:1) as an eluent to obtain the title compound (950 mg, 62%) as a white solid, mp: 119.7-121.5 °C; Rf: 0.45 (1:19 ethyl acetate, hexane); IR (v max (neat)): 2926, 1543, 1489, 1423, 1215, 1001, 894, 738 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 2.50 (3H, s), 4.14 (2H, s), 6.94-7.03 (2H, m), 7.30-7.37 (2H, m) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 21.2, 43.9, 115.5 (d, J = 21.3 Hz), 130.8 (d, J = 7.9 Hz), 131.8, 132.8 (d, J = 3.3 Hz), 154.4, 162.1 (d, J = 245.0 Hz), 167.5 ppm; ¹⁹F NMR (376 MHz, CDCl₃): δ -116.1 ppm.

6-Bromo-2-(4-fluorobenzyl)-5-methylpyrimidin-4-amine 372

Pyrimidine 371 (600 mg, 1.7 mmol) was suspended in saturated methanolic ammonia (5 mL) and the mixture heated at 100 °C in a sealed vessel for 72 hours. The volatiles were removed under a stream of nitrogen and the residue purified by flash column chromatography using methanol, dichloromethane (0:1 to 3:97) as an eluent to obtain the title compound (400 mg, 82%) as an off-white solid, mp: 190.2-193.5 °C; Rf: 0.57 (1:19 ethyl acetate, hexane); IR (v max (neat)): 3325, 3156, 2925, 1654, 1531, 1506, 1408, 1216, 1155, 1040, 791 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 2.05 (3H, s), 3.82 (2H, s), 6.88-7.17 (4H, m), 7.23-7.32 (2H, m) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ 15.1, 43.3, 109.9, 115.0 (d, J = 21.1 Hz), 130.6 (d, J = 8.0 Hz), 134.6 (d, J = 3.1 Hz), 151.4, 160.9 (d, J = 241.8 Hz), 163.1, 165.8 ppm; ¹⁹F NMR (376 MHz, DMSO-d₆): δ -117.0 ppm; LRMS (+ESI) m/z: 296.0/298.0 ([M+H]⁺ 74%), 318.1/320.1 ([M+Na]⁺ 100%), 614.9 ([2M+Na]⁺ 49%); HRMS (ESI)⁺ Cald for C₁₂H₁₁BrFN₃ [M+Na]⁺: 318.00181, found 318.00112/319.99906.
The requisite acid chloride was formed according to General Procedure C using cycloheptane carboxylic acid (96 mg, 680 μmol). The crude residue was taken up in dichloromethane (1 mL) and added to a solution of 372 (200 mg, 680 μmol) and N,N-diisopropylethylamine (180 μL, 1.0 mmol) in tetrahydrofuran (10 mL) and the mixture was heated at 60 °C for 2 hours. Thin layer chromatography indicated the formation of a single new product was found to have a mass corresponding to the imide, rather than the desired amide. Another equivalent of the acid chloride was therefore prepared as above and added to the reaction mixture and stirring was continued for another 2 hours. The volatiles were removed under a stream of nitrogen and the residue taken up in methanol (5 mL) and treated with aqueous lithium hydroxide (3 M, 450 μL, 1.4 mmol). After stirring for 30 minutes the volatiles were removed under a stream of nitrogen and the residue taken up in dichloromethane (30 mL) and washed with water (2 x 30 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate, hexane (0:1 to 1:9) as an eluent and recrystallisation from dichloromethane, hexane to obtain the title compound (120 mg, 42%) as a white solid, mp: 162.8-163.9 °C; \( R_f \): 0.11 (1:9 ethyl acetate, hexane); \( \text{IR} \) (\( \nu_{\text{max}} \) (neat)): 3268, 2924, 2858, 1672, 1564, 1537, 1507, 1487, 1398, 1284, 1219, 794 cm\(^{-1}\); \( ^{1}\text{H NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \) 1.40-1.85 (10H, m), 1.91-2.00 (2H, m), 2.20 (3H, s), 2.69-2.80 (1H, m), 4.09 (2H, s), 6.93-7.00 (2H, m), 7.22-7.30 (2H, m), 7.54 (1H, bs) ppm; \( ^{13}\text{C NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \) 17.6, 26.6, 28.3, 31.4, 44.1, 47.2, 115.4 (d, \( J = 21.4 \) Hz), 121.7, 130.7 (d, \( J = 7.9 \) Hz), 133.3 (d, \( J = 3.2 \) Hz), 156.5, 156.7, 161.9 (d, \( J = 244.8 \) Hz), 166.5, 176.5 ppm; \( ^{19}\text{F NMR} \) (376 MHz, CDCl\(_3\)): \( \delta \) -116.3 ppm; \( \text{LRMS} \) (+ESI) \( m/z \): 442.1/444.1 ([M+Na]\(^+\) 100%); \( \text{HRMS} \) (ESI\(^+\) Cald for C\(_{20}\)H\(_{23}\)BrFN\(_3\)O [M+Na]\(^+\): 442.09062, found 442.08979/444.08773; \( \text{HPLC} \): 95.1%, RT: 30.9 mins. (Method A, 230 nm).
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Appendix 1: 2D NMR Spectra
5-(5-bromofuran-2-yl)-3-(tert-butyl)-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazole \(214\), \(^1\)H HMBC, 500 MHz, CDCl\(_3\)

3-(tert-butyl)-6-chloro-1-((tetrahydrofuran-2-yl)methyl)-1H-pyrazolo[3,4-d]pyrimidine \(236\), \(^1\)H HMBC, 500 MHz, CDCl\(_3\)
N-(adamantan-1-yl)-7-pentyl-7H-pyrrolo[2,3-b]pyridine-3-carboxamide 292, $^1$H NOESY, 500 MHz, CDCl$_3$

N-(5-bromo-1-(4-fluorobenzyl)-4-methyl-2-oxo-1,2-dihydropyridin-3-yl)cycloheptanecarboxamide 79, $^1$H NOESY, 500 MHz CDCl$_3$
N-(5-bromo-2-((4-fluorobenzyl)oxy)-4-methylpyridin-3-yl)cycloheptanecarboxamide 344, $^1$H HMBC, 500 MHz, CDCl$_3$

N-(1-(4-fluorobenzyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide 330, $^1$H NOESY, 500 MHz, CDCl$_3$
N-(7-(4-fluorobenzyl)-7H-pyrrolo[2,3-b]pyridin-3-yl)cycloheptanecarboxamide 333, $^1$H HMBC, 500 MHz, CDCl$_3$

(6-bromo-4-(4-fluorobenzyl)-7-methyl-4H-imidazo[4,5-b]pyridin-2-yl)(cycloheptyl)methanone 336, $^1$H NOESY, 500 MHz, CDCl$_3$
(6-bromo-4-(4-fluorobenzyl)-7-methyl-4H-imidazo[4,5-b]pyridin-2-yl)(cycloheptyl)methanone 336, $^1$H HMBC, 500 MHz, CDCl$_3$