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Design and synthesis of novel alpha1 adrenoceptor modulators

Susan J. McGinty

University of Wollongong

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Design and Synthesis of Novel Alpha$_1$-Adrenoceptor Modulators

Susan J. McGinty B.Med.Chem (Hons)

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

Department of Chemistry

University of Wollongong
Wollongong, Australia

February 2003
Declaration

The work described in this Thesis does not contain any material that has been submitted for the award of any higher degree in this or any other University, and to the best of my knowledge and belief contains no material previously published by any other person, except where due reference has been acknowledged.

Susan J. McGinty

February, 2003
Acknowledgements

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My most heartfelt thanks goes to my wonderful husband, Michael, whose patience, encouragement and support over the last three years has been unwavering, and without whom my days would be dreary.
List of Abbreviations

(1,4,5)-IP$_3$  inositol (1,4,5)-triphosphate
Å  angstrom
AgNO$_3$  silver nitrate
AIBN  2,2'-azobisisobutyronitrile
AlMe$_2$CH$_2$Ph  diethylbenzyl
AR  adrenoceptor
ArH  aromatic protons
ArMgBr  aryl magnesium bromide
BBr$_3$  boron tribromide
BCl$_3$  boron trichloride
BF$_3$.Et$_2$O  boron trifluoride etherate
BI$_3$  boron triiodide
Bi(NO$_3$)$_3$.5H$_2$O  bismuth nitrate heptahydrate
Boc  tert-butoxycarbonyl
bs  broad singlet (spectroscopy)
Bu  butyl
BuLi  butyl lithium
' BuOH  tert-butanol
' BuOOH  tert-butanoic acid
Bu$_3$SnH  tributyltin hydride
°C  degrees celcius
C  carbon
$^{13}$C  carbon NMR spectroscopy
CAM  constitutively active mutant
cAMP  cyclic adenosine monophosphate
CDCl$_3$  deuterio chloroform
CEC  chloroethylclonidine
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<tr>
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<tr>
<td>δ</td>
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<td>d</td>
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<td>DAG</td>
<td>diacyl glycerol</td>
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<td>DCC</td>
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<td>dichloromethane</td>
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<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
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<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
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<td>gHMBC</td>
<td>gradient heteronuclear multiple bond correlation</td>
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<td>GPCR</td>
<td>guanidine nucleotide binding protein</td>
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<tr>
<td>GTP</td>
<td>guanidine triphosphate</td>
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H  hydrogen/proton
'H  proton NMR spectroscopy
H₂  hydrogen
HBA  hydrogen bond acceptor
HBD  hydrogen bond donor
HBr  hydrobromic acid
HCl  hydrochloric acid
HEM buffer  biological buffer
HEPES  biological buffer
HOBt  1-hydroxybenzotriazole
H₃PO₄  phosphoric acid
HRMS  high resolution mass spectroscopy
Hypo  hypothesis
Hz  Hertz
J  coupling constant (nmr spectroscopy)
K₂CO₃  potassium carbonate
KH₂PO₄  potassium dihydrogen phosphate
KOH  potassium hydroxide
LDA  lithium diisopropylamine
LiAlH₄  lithium aluminium hydride
LiAlH₄-BF₃  lithium aluminium hydride – boron trifluoride
LiN(J-Pr)₂  lithium diisopropylamine
m  multiplet (spectral)
M  molar (moles per litre)
Me  methyl
MeOH  methanol
MgCl₂  magnesium chloride
min  minute/s
mL  milliliters
mmol  millimoles
mol  moles
Mp, mp  melting point
MS  mass spectroscopy
Mu  mutant (with respect to the Cys^{128}Phe α_{1B}-adrenoceptor)
m/z  mass to charge ratio (mass spectroscopy)
NaBH_{4}  sodium borohydride
NaCNBH_{3}  sodium cyanoborohydride
Na_{2}CO_{3}  sodium carbonate
Na[Et_{2}Bn_{2}(NH_{2})Al]  sodium diethyl dibenzylamido aluminate
NaH  sodium hydride
Na_{2}HPO_{4}  sodium hydrogen phosphate
NaN(SiMe_{3})_{2}  sodium bistrimethylsilyl amine
nBu_{4}NI  tetrabutyl ammonium iodide
NEt_{3}  triethylamine
nM  nanomolar
NMR  nuclear magnetic resonance spectroscopy
NOE  nuclear Overhauser effect
NOESY  nuclear Overhauser effect spectroscopy
OH  hydroxyl
OMe  methoxy
O/N  overnight
PA  phosphotidic acid
Pd (II)  palladium (II)
Pd/C  palladium on carbon
PdCl_{2}  palladium chloride
PdCl_{2}(CH_{3}CN)_{2}  palladium (II) chloride bisacetonitrile
PdH  palladium hydride
Pd(PPh_{3})_{4}  tetrakis(palladium(0))triphenylphosphine
PhCH_{2}OH  benzyl alcohol
Ph_{3}Cl  lithium triphenylmethane
Ph_{3}P  triphenyl phosphine
PKC  protein kinase C
PLA₂     phospholipase A₂
PLC      phospholipase C
PLD      phospholipase D
ppm      parts per million
Ptd Ins (4,5)-P₂  phosphotidyl inositol (4,5)-diphosphate
p-TsOH   para-toluene sulphonic acid
Rᶠ       retention factor (chromatography)
RT       room temperature
s        singlet (spectroscopy)
sat.     saturated
t        triplet (spectroscopy)
TBDMS    tert-butyldimethylsilyl
TFA      trifluouro acetic acid
THF      tetrahydrofuran
TLC      thin layer chromatography
TM       transmembrane
+ve      positive
WT       wild-type (with respect to the α₁B-adrenoceptor)
# Amino Acids

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Abstract

A constitutively active Cys\textsuperscript{128}Phe mutant of the \(\alpha\text{}_{\text{IB}}\)-adrenoceptor has been shown to not only activate G-protein coupling in the absence of an agonist, but also activate only a single effector pathway (phospholipase C but not phospholipase A\textsubscript{2}). This suggests that in the case of the \(\alpha\text{}_{\text{IB}}\)-adrenoceptor, a single receptor subtype forms multiple conformations for G-protein interactions that are specific for a particular G-protein/effector pathway. The existence of this receptor opens up possibilities for the specific modulation of the receptor pathway. Specifically, the development of small molecule ligands which activate a single active conformer would thus provide new signalling-specific therapeutics with pharmacological diversity.

In order to achieve this, ligands which bound selectively to the Cys\textsuperscript{128}Phe mutant \(\alpha\text{}_{\text{IB}}\)-adrenoceptor over the wild-type \(\alpha\text{}_{\text{IB}}\)-adrenoceptor were required. Through molecular modelling studies and pharmacophore development a range of conformationally restrained target ligands have been developed which mimic varying conformations of the endogenous ligand adrenaline. This range of target ligands included a simple cyclic series, a bicyclic series, a spirocyclic series and a spiro-fused series. Members of the bicyclic and spirocyclic series were predicted to have particular selectivity potential for the Cys\textsuperscript{128}Phe mutant \(\alpha\text{}_{\text{IB}}\)-adrenoceptor.

The synthesis of the simple cyclic derivatives was via pyrrolidinone and piperidone frameworks. A series of nine new bicyclic tropane-based target derivatives were prepared from 6-hydroxytropinone. The major step in the approach to the synthesis of the
spirocyclic target ligands was a pro spirocycle-forming intramolecular palladium-assisted cyclisation. One such spirocyclic compound was made. The synthetic procedure for the spiro-fused target derivatives also began from pyrrolidinone and piperidone frameworks, involving the formation of a hydroxymethyl aryliodide derivative, and a crucial palladium-catalysed intramolecular cyclisation. While this last approach was not completed, a novel indeno-pyrrole derivative was prepared.

Pharmacological testing of the synthesised simple cyclic and bicyclic derivatives indicated that whilst some of the simple cyclic ligands exhibited selectivity for the Cys$^{128}$Phe mutant $\alpha_{1B}$-adrenoceptor, the bicyclic ligands displayed no selectivity for this mutant receptor, and had only weak affinity for both $\alpha_{1B}$-adrenoceptors. The increased rigidity and steric bulk of the bicyclic ligands appeared not to be favourable. However, the set of bicyclic ligands have provided a promising new lead for selective $\alpha_{1D}$-adrenoceptor ligands.
# Table of Contents

Declaration i  
Acknowledgements ii  
List of Abbreviations iv  
Amino Acids ix  
Abstract x  

Chapter One: Introduction 1  
1.1 The Adrenergic Receptor System 2  
1.1.1 Introduction and Pharmacology 2  
1.1.2 G-protein coupled receptors 4  
1.1.3 Development of sub-type-selective ligands for the $\alpha_1$ adrenoceptors 6  
1.2 Adrenergic Receptor Activation 7  
1.2.1 Interaction of catecholamines: Mechanisms in adrenoceptor activation 7  
1.2.2 The process of GPCR-G protein coupling 13  
1.2.3 G-proteins as cellular signallers 15  
1.3 Theory of Receptor Activation 17  
1.3.1 The Ternary Complex Model 17  
1.3.2 Multiple effector responses and agonist-directed trafficking: extension of the two-state model to a multi-state model 18  
1.4 Implications of a constitutively active mutant $\alpha_{1B}$ adrenoceptor 20  
1.4.1 Constitutively active mutants of GPCRs 20  
1.4.2 Constitutively active mutant receptors in pathology 23  
1.4.3 Constitutively active adrenoceptors and the $\text{Cys}^{128}\text{Phe} \alpha_{1B}$ adrenoceptor 23  
1.5 Project Aims 28
## Chapter Two: Molecular Modelling and Pharmacophore Generation

2.1 Introduction 31

2.2 Generation of the Training Set 32

2.2.1 Conformer generation 36

2.3 Pharmacophore Development 37

2.4 Design of Target Ligands 42

2.5 Pharmacophore Re-modelling 45

## Chapter Three: Synthesis of Simple Cyclic Derivatives

3.1 Introduction and Design Rationale 55

3.2 Synthesis of Simple Cyclic Derivatives 61

3.2.1 Retrosynthesis of simple cyclic derivatives 61

3.2.2 Synthesis of 3-phenyl-pyrrolidin-3-ol and 3-phenyl-piperidin-3-ol 62

3.2.3 Expansion of the set of simple cyclic derivatives:
- Synthesis of 3-(3,4-dihydroxyphenyl)-piperidin-3-ol 64
  - 3.2.3.1 Methods for deprotection of methylenedioxy groups 66

## Chapter Four: Synthesis of Bicyclic Tropane Derivatives

4.1 Introduction 72

4.2 Design of Bicyclic Tropane derivatives as α1B-AR ligands 78

4.2.1 Retrosynthesis and Proposed Syntheses 82

4.3 Synthesis of 3α-phenyltropan-3-ol 84

4.4 Preparation of 6β-arylhydroxynortropane derivatives 87

4.4.1 Synthesis of 6β-hydroxytropan-3-one 87

4.4.2 Synthesis of 6-Arylhydroxytropane derivatives from 6β-hydroxytropan-3-one 89

4.4.2.1 Protection of 6β-hydroxytropan-3-one 91

4.4.2.2 Protection of the 3-ketone 94

4.4.2.3 Oxidation of protected 6-hydroxytropane derivative 73 98
4.4.2.4 Arylation of 75 to provide 6-arylhydroxytropane derivatives 99
4.4.2.6 Removal of Ketal protecting group 103

4.4.3 Revision of Synthesis of 6β-arylhydroxynortropane derivatives 110
4.4.3.1 Incorporation of dimethyl ketal protecting group 110
4.4.3.2 Synthesis of Aryl Derivatives 112

4.4.4 Selective ketone reduction to yield 3β-hydroxytropane derivatives 117

Chapter Five: Synthesis of Spirocyclic Derivatives 129

5.1 Introduction 130

5.2 Known Syntheses of Biologically Useful Spirocyclic Derivatives 132

5.3 Retrosynthetic Analysis of the Spirocyclic Target Derivatives 136

5.4 Initial steps in the Synthesis of Target Spirocyclic Derivatives 138

5.5 Methods of Cyclisation for Spiro Formation 146

5.6 Revised Synthetic Strategy 167

5.6.1 Reduction of benzylamide 139 168
5.6.2 Palladium-catalysed intramolecular cyclisation of 161b 174
Chapter Six: Synthesis of Benzo-Fused Spirocyclic Derivatives

6.1 Introduction and Design Rationale

6.2 General Therapeutic Uses for Spiro[isobenzofurans]

6.3 Synthetic Methodologies for the Preparation of Spiro[isobenzofurans]

6.3.1 Previous Syntheses of Spiro[isobenzofuran-piperidines]

6.3.2 Synthetic Design for the Spiro[isobenzofuran-pyrrolidines] and Spiro[isobenzofuran-piperidines]

6.4 Synthetic approaches to the Spiro[isobenzofurans]

6.4.1 Preparation of 3-O-substituted pyrroli dine spiro precursors

6.4.2 Preparation of 2-O-substituted pyrroli dine spiro precursors

6.4.3 Preparation of piperidine spiro precursors

Chapter Seven: Pharmacological Assessment of Target Ligands

7.1 Introduction

7.2 General Methods

7.3 Results of binding affinity assays upon wild-type (WT2) and Cys^{128}Phe mutant (Mu2) $\alpha_{1B}$-AR.

7.4 Discussion

7.4.1 Binding of the simple cyclic derivatives

7.4.2 Receptor binding of the bicyclic derivatives

7.4.3 Discussion of other $\alpha_{1}$-adrenoceptor selective ligands

7.5 Examination of design motif

7.6 Examination of pharmacophore

Chapter Eight: Conclusions and Future Directions

Chapter Nine: Experimental Methods and Data

9.1 Methods for Pharmacophore Development

9.2 Methods for Pharmacological Assessment of Ligands

9.3 General Experimental for Synthesis
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4 Experimental for Chapter Three: Synthesis of Monocyclic Derivatives</td>
<td>226</td>
</tr>
<tr>
<td>9.5 Experimental for Chapter Four: Synthesis of Bicyclic Derivatives</td>
<td>239</td>
</tr>
<tr>
<td>9.6 Experimental for Chapter Five: Synthesis of Spirocyclic Derivatives</td>
<td>260</td>
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<tr>
<td>9.7 Experimental for Chapter Six: Synthesis of Spiro{isobenzofuran} Derivatives</td>
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References 285

Appendix 312
Chapter One

Introduction
1.1 The Adrenergic Receptor System

1.1.1 Introduction and pharmacology

The adrenergic receptor system consists of adrenoceptors, their endogenous ligands, and the effects they mediate. The adrenergic receptor system is vital for control of the heart and plays a major role in the control of most smooth muscle tissues including blood vessels and bronchi. The last few decades has seen much research aimed at the design of selective ligands for the adrenergic system which may provide targeted chemotherapeutic assistance in controlling the adrenergic system in disease states.

Adrenoceptors (ARs) are membrane-bound, guanine nucleotide binding protein (G protein)-coupled receptors located throughout the body, on both neuronal and non-neuronal tissues (for an example, see Figure 1.1, Schematic of the hamster \(\alpha_{1b}\)-AR). Adrenoceptors mediate a diverse range of functional responses to the endogenous catecholamines adrenaline (also known as epinephrine), a circulating hormone released from the adrenal medulla, and noradrenaline (also known as norepinephrine), a neurotransmitter released from the pre-synaptic junction of sympathetic nerve terminals upon stimulation. These responses include increased rate and force of cardiac activity, constriction and dilation of smooth muscle, and relaxation of the gastrointestinal tract and other organ tissues.

Initial investigations subdivided the adrenoceptor family into two subtypes, \(\alpha\) and \(\beta\), based upon pharmacological studies in isolated tissues\(^1\). The \(\alpha\)-adrenoceptors were later further subdivided based upon their anatomical classification, with those \(\alpha\)-ARs located on post-synaptic nerve terminals designated as \(\alpha_1\)-adrenoceptors, and those existing on peripheral sympathetic nerve terminals designated as \(\alpha_2\)-adrenoceptors\(^2\). The \(\alpha_2\)-adrenoceptors were also later found to occur on liver cells, platelets and smooth muscle
cells of blood vessels. α₁-adrenoceptors are coupled mainly to phospholipase C, and produce their effects mainly by the release of intracellular calcium; their physiological effects centre mainly on smooth muscle contraction. The α₂-adrenoceptors are mainly coupled negatively to adenylate cyclase, and reduce cyclic AMP (cAMP) formation, leading to a reduction in intracellular calcium; physiological effects include the inhibition of transmitter release, hypotension, anaesthesia and vasoconstriction.

Pharmacological differences between the two α-adrenoceptor classes were soon identified, notably the ability of yohimbine and rauwolscine to act as antagonists upon the α₂-adrenoceptor. Further subdivision of the α₁-adrenoceptors has been facilitated by both molecular biological and pharmacological techniques. The initial classification of the α₁-adrenoceptors as α₁A- and α₁B-subtypes was determined from differences in the binding characteristics of both the competitive antagonist WB4101 and the site-directed alkylating agent chloroethylclonidine (CEC). Radioligand binding studies revealed that the α₁A subtype has a high affinity for WB4101 and is CEC sensitive, whilst the α₁B subtype is insensitive to CEC and exhibits a low affinity to WB4101. Additionally, three different cDNA's were isolated, characterised and shown to code for three functional α₁-adrenoceptors: α₁A, α₁B, and a third subtype α₁D, which exhibits a low affinity for oxymetazoline in contrast to the α₁A subtype, and is only partially sensitive to CEC. In the heart, the α₁A-adrenoceptors appear to promote automaticity and arrhythmias during myocardial ischaemia. The α₁A-adrenoceptors are involved in activation of the sodium/potassium pump which leads to hyperpolarisation of the cell and a decreased tendency for heart rhythm abnormality.

The first α₂-AR cDNA was isolated from human platelet and designated as α₂C10. Related genes, designated as α₂C2 and α₂C4 were also shown to encode for α₂-
adrenoceptors. These genes were subsequently classified as corresponding to the $\alpha_{2a}$-AR ($\alpha_{2C10}$)$^{16}$, the $\alpha_{2b}$-AR ($\alpha_{2C12}$)$^{15,17}$ and the $\alpha_{2c}$-AR ($\alpha_{2C4}$)$^{18}$.

The $\beta$-adrenoceptors have been classified as $\beta_1$$^{19}$, $\beta_2$$^{20}$ and $\beta_3$$^{21}$ using cDNA cloning techniques. $\beta_1$-receptors are found mainly in the heart, where they control the positive inotropic and chronotropic effects of catecholamines via stimulation of adenylate cyclase. $\beta_2$-receptors are found in the smooth muscle of many organs, where they cause relaxation, also via stimulation of adenylate cyclase. For an extensive review of $\alpha$- and $\beta$-ARs, including biology, classification, structure-activity relationships and therapeutic applications, see Hieble et al.$^{16}$ and Ruffolo et al.$^{22}$.

### 1.1.2 G-protein coupled receptors

The functional responses produced by activation of adrenoceptors are effected by the interaction of the receptor proteins with G-proteins. Adrenoceptors are thus known as G protein-coupled receptors (GPCRs). GPCRs comprise a superfamily of more than 1000$^{23}$ cell-surface receptors which mediate diverse effects of extracellular signalling molecules. They share a common structure, characterized by seven transmembrane (TM) helices with three intracellular and extracellular hydrophilic loops, an extracellular N-terminus and an intracellular C-terminus (See Figure 1.1 for an example). GPCRs are involved in almost all of the physiological systems of the body, including the endocrine system, cardiovascular and brain functions, taste, smell and light sensing, and are major targets for drugs currently used or in development$^{24}$. Each
Figure 1.1: Secondary structure of the hamster $\alpha_{1B}$-AR$^{27}$. Note the highly conserved DRY motif at the intracellular end of the transmembrane III helix.

GPCR is specifically activated by a different ligand, including cations, monoamines, neurotransmitters, lipids, odorant molecules, peptides of various lengths and proteins.
Accordingly, GPCRs have been classified into one of 5 families (Class A-E)\textsuperscript{24}. The adrenoceptors fall into Class A, biogenic amines. The majority of receptors in this class contain a highly conserved Glu/Asp-Arg-Tyr (E/DRY) motif located at the cytosolic end of transmembrane helix III (see in Figure 1.1). To date there exists only one crystal structure for any GPCR, that of bovine rhodopsin\textsuperscript{25,26}.

1.1.3 Development of sub-type-selective ligands for the $\alpha_1$-adrenoceptors

The clinical uses of adrenergic compounds are vast. $\alpha_1$-antagonists such as indoramin and prazosin, and the $\alpha_2$-agonist clonidine have antihypertensive activity; clonidine is often used as an adjunct to general anaesthetics. $\alpha_1$-Adrenoceptor antagonists are also used in the treatment of benign prostatic hypertrophy.

In order to discover more about the role of $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$ adrenoceptors in disease and to improve therapeutic applications, research has been aimed at developing ligands selective for each of these subtypes. Although there are some selective $\alpha_{1A}$ antagonists known, such as SNAP5089 and (+)niguldipine, there are no antagonists showing substantial selectivity for either the $\alpha_{1B}$ or $\alpha_{1D}$ adrenoceptors (See Table 1.1). There is thus a need for selective agonists and antagonists for both $\alpha_{1B}$ and $\alpha_{1D}$ adrenoceptors.
Table 1.1: Information about $\alpha_1$-adrenoceptors$^{28}$; NA = Noradrenaline

<table>
<thead>
<tr>
<th>subtype</th>
<th>$\alpha_{1A}$</th>
<th>$\alpha_{1B}$</th>
<th>$\alpha_{1D}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency Order</td>
<td>Adrenaline = NA</td>
<td>Adrenaline = NA</td>
<td>Adrenaline = NA</td>
</tr>
<tr>
<td>Agonists</td>
<td>A61603</td>
<td>None available</td>
<td>None available</td>
</tr>
<tr>
<td>Antagonists</td>
<td>KMD3213 (+)niguldipine SNAP5089 SNAP 5272 RS17053</td>
<td>None available</td>
<td>BMY7378 SKF105854</td>
</tr>
<tr>
<td>Coupling</td>
<td>Gq/11</td>
<td>Gq/11</td>
<td>Gq/11</td>
</tr>
</tbody>
</table>

1.2 Adrenergic Receptor Activation

1.2.1 Interaction of catecholamines: mechanisms in adrenoceptor activation

Gether and Kobilka$^{29}$ have suggested a general model for the activation of rhodopsin-like GPCRs, which includes adrenoceptors. Most studies directed towards elucidating the mode of interaction of agonists and antagonists with adrenoceptors have been based upon the $\beta$-adrenoceptor, specifically the $\beta_2$-AR subtype, as it is perhaps the best characterised. The $\alpha$- and $\beta$-adrenoceptors have similar molecular structure (see Figure 1.2). The $\beta$-ARs range from 388-477 amino acids in length. The $\alpha$-ARs (see Figure 1.1), particularly $\alpha_{1B}$- and $\alpha_{1D}$-subtypes, are larger than the $\beta$-ARs, ranging from 501-560 amino acids in length, with longer extracellular amino and cytoplasmic carboxyl termini. The $\alpha_{2}$-ARs are comparable in size to the $\beta$-ARs (450-461 amino acids) but with shorter amino and carboxyl termini and very long third intracellular loops.
The transmembrane (TM) domains of the β₂-AR create the ligand binding pocket. This has been shown by the co-expression of two β₂-AR fragments, TM helices I-V and TM helices VI-VII, which resulted in a functional membrane receptor capable of binding β₂-AR radioligand \([^{125}\text{I}]\text{iiodocyanopindolol}\) (\([^{125}\text{I}]\text{ICP}\)) and mediating adenylate cyclase. Neither fragment alone recognised a β₂-AR ligand. Site-directed mutagenesis has demonstrated that Asp\(^{113}\) in TMIII is required for both agonist and antagonist binding. It is believed that the free carboxyl group of Asp\(^{113}\) interacts with the protonated amine of adrenergic ligands. The \textit{meta-} and \textit{para-}hydroxyl groups of catecholamines are postulated to form H-bonds to Ser\(^{204}\) and Ser\(^{207}\) respectively, located in the TMV helix. Binding to both Ser\(^{204}\) and Ser\(^{207}\) appears necessary for full agonist activity at the β-AR. More recently Ser\(^{204}\) and Ser\(^{207}\) have been implicated not only in the docking site for the agonist, but also in control of the equilibrium of the receptor from inactive to active forms. A hydrogen bond interaction between the chiral benzyllic \(\beta\)-hydroxyl group of catecholamines and Asn\(^{293}\) in TMVI of the β₂-AR may be responsible for the stereoselective binding of agonists. In the TMVI helix of β₂-ARs the phenyl ring of Phe\(^{290}\) (equivalent to Phe\(^{311}\) in the \(\alpha\)₁β-AR) is postulated to participate in binding the aromatic ring of agonist ligands.
The $\beta_1$-AR is predicted to have the same structural orientation as the $\beta_2$-AR and contains Asp$^{138}$, Ser$^{229}$, Ser$^{232}$ in corresponding positions to those shown to be important for the binding of agonists in the $\beta_2$-AR$^{16}$. It has not been possible to localize any specific changes in the receptor protein responsible for the relatively subtle differences in pharmacology that exist between the $\beta_1$- and $\beta_2$-AR subtypes$^{16}$. It is likely that the pharmacological differences between $\beta_1$- and $\beta_2$-AR are not due to specific amino acid substitutions, but rather to subtle changes in orientation of the primary binding sites, resulting in a slightly different binding pocket$^{16}$.

Intramolecular interactions contributing to the stability of the inactive receptor conformation of the $\alpha_{1B}$-AR have been identified between Asp$^{125}$ in TMIII and Lys$^{331}$ in TMVII$^{36b-38}$ (shown in Figure 1.3). Interactions shown to be important in the binding of catecholamines and activation of the $\alpha_{1B}$-AR include an ionic interaction between the
protonated amine of adrenaline and Asp\textsuperscript{125} in TMIII (equivalent to Asp\textsuperscript{113} in the β\textsubscript{2}-AR), and a hydrogen bond between the catechol meta-hydroxyl of adrenaline and Ser\textsuperscript{207} in TMV\textsuperscript{27} (Figure 1.3). In contrast, hydrogen bond interactions involved in the binding of agonists to the α\textsubscript{1A}-AR occur between the meta-hydroxyl and Ser\textsuperscript{188} (equivalent to Ser\textsuperscript{203}, not Ser\textsuperscript{204} in the β\textsubscript{2}-AR), accounting for 70-90% of the wild-type response, and the para-hydroxyl and Ser\textsuperscript{192} (equivalent to Ser\textsuperscript{207} in the β\textsubscript{2}-AR), which contributes only minimally to receptor activation\textsuperscript{39}. Although the α\textsubscript{1}-ARs preserve the stereoselectivity of catecholamine binding and activation displayed by the β-ARs, the determinants of stereoselectivity (i.e. interaction with the chiral benzylic β-hydroxyl substituent) have not been defined\textsuperscript{27} and the Asn\textsuperscript{293} equivalent is replaced by a residue which lacks hydrogen-bonding potential (either leucine or methionine)\textsuperscript{27}.

Further modelling and experimental studies by Chen et al.\textsuperscript{27} have indicated that Phe\textsuperscript{310} in TMVI, which points into the agonist-binding pocket, interacts directly with the catechol ring of the catecholamine agonists (Figure 1.3). Although this interaction was shown not to be necessary for spontaneous receptor isomerization from the basal state, R, to a partially activated conformation, R’, it was essential for isomerization from R’ to the fully activated R* state. Phe\textsuperscript{163} (TMIV) and Phe\textsuperscript{187} (TMV) (residues novel to the α\textsubscript{1B}-AR) have been reported as binding contacts in the agonist binding pocket, but not as being directly involved in receptor activation\textsuperscript{40}. 


Several different experimental and modelling studies suggest that both negatively and positively charged residues of the highly conserved E/DRY motif may be involved in the activation process of GPCRs\textsuperscript{41,42}. An alternate theoretical model of α\textsubscript{1B}-AR activation\textsuperscript{43,44}, which deviates from the one just presented, has indicated a network of hydrogen-bonding interactions among polar residues forming a “polar pocket” near the cytosol (Asn\textsuperscript{63} in TMI, Asp\textsuperscript{91} in TMII, Asn\textsuperscript{344} and Tyr\textsuperscript{348} in TMVII) and Arg\textsuperscript{143} of the E/DRY motif in TMIII. This set of interactions is suggested\textsuperscript{45} to constrain the receptor in its ground state by controlling the degree of cytosolic exposure by Arg\textsuperscript{143}. The main role of Arg\textsuperscript{143} is thus indicated to be mediation of receptor activation, allowing amino acids of the intracellular loops to attain the correct configuration for G-protein
interaction. Mutations of Arg\textsuperscript{143} were indicated to drive the $\alpha_{1B}$-AR into different activation states\textsuperscript{45}, further suggesting a role of this residue in the activation process of the receptor. The mechanism of this activation is hypothesised to consist of protonation of the aspartate (Asp\textsuperscript{142}) and a shift of the arginine (Arg\textsuperscript{143}) out of the "polar pocket". Recent results\textsuperscript{46} support the hypothesis that a salt bridge between the highly conserved arginine Arg\textsuperscript{143} of the E/Dry motif of TMIII and a conserved glutamate (Glu\textsuperscript{289}) in TMVI constrains the $\alpha_{1B}$-AR in the inactive state. Mutations of Glu\textsuperscript{289} that weakened the Arg\textsuperscript{143}-Glu\textsuperscript{289} interaction constitutively activated the receptor. Residues located in the environment of the interface between the cytosolic extension of helices III and VI of $\alpha_{1B}$-AR are indicated to be largely involved in the activation of the receptor\textsuperscript{46}. This indicates that a rearrangement of helices III and VI represents an important step in receptor activation.

In the case of the $\alpha_{2}$-AR, Asp\textsuperscript{113} in TMIII is also required for specific binding of ligands\textsuperscript{47}, by an ionic interaction with the protonated amine of the ligand. The interaction of Ser\textsuperscript{204} (corresponding to Ser\textsuperscript{207} in $\beta_{2}$-AR) with the $p$-hydroxyl of catecholamine agonists is important, but the interaction of Ser\textsuperscript{200} (corresponding to Ser\textsuperscript{204} in $\beta_{2}$-AR) with the $m$-hydroxyl is not. An interaction of the $m$-hydroxyl with Cys\textsuperscript{201} in the $\alpha_{2}$-AR has also been postulated\textsuperscript{47}.

A detailed model for the binding of catecholamines to the $\alpha_{2A}$-AR receptor, based upon structural, pharmacological, and theoretical evidence, has been proposed\textsuperscript{48}. In this model, the most important interaction in the binding process is that formed between the positively charged amine of the catecholamine and the negatively charged carboxyl chain of Asp\textsuperscript{113} in TMIII (as for other adrenoceptors). The $\beta$-hydroxyl group is positioned such that it can interact with one side chain oxygen of Asp\textsuperscript{113}. The $N$-methyl
group of adrenaline was shown to pack against Phe\textsuperscript{411} and Phe\textsuperscript{412} in TMVII in docking simulations. These parts of the TMVII are exposed to the binding cavity, favourable for hydrophobic, aromatic and methyl contacts. The ring plane of the aromatic ring is proposed to pack against TMVI (as is the case with the $\alpha_{1B}$-AR\textsuperscript{27}), with additional interactions with TMIII and TMV. GRID calculations predict interactions between the aromatic ring and Phe\textsuperscript{391} and Tyr\textsuperscript{395} in TMVI, and partially with Phe\textsuperscript{205} and Cys\textsuperscript{201} in TMV. The catecholic hydroxyl groups can co-ordinate with Thr\textsuperscript{118} in TMIII, and Ser\textsuperscript{200}, Ser\textsuperscript{204} and Cys\textsuperscript{201} in TMV. The inactive receptor conformation model suggests the length of the phenethylamine ligands is too short to easily reach both Asp\textsuperscript{113} in TMIII and Ser\textsuperscript{200}, Ser\textsuperscript{204} and Cys\textsuperscript{201} in TMV. A proposed rotation of TMV occurring in the active receptor form therefore exposes both Ser\textsuperscript{200} and Ser\textsuperscript{204} to the ligand-binding cavity.

1.2.2 The process of GPCR-G protein coupling

G-proteins physically and functionally connect the receptor and its effector system. In its resting state, a G-protein exists as a heterotrimer, containing $\alpha$, $\beta$ and $\gamma$ subunits, with guanidine diphosphate (GDP) occupying the guanine nucleotide binding site on the $\alpha$ subunit. When a receptor is occupied by an agonist molecule (endogenous or synthetic) a conformational change occurs, causing it to acquire high affinity for $\alpha\beta\gamma$. Association of $\alpha\beta\gamma$ with the receptor causes a GDP/GTP (guanidine triphosphate) exchange followed by dissociation of $\alpha$-GTP from the G-protein. The $\beta\gamma$ subunit is very hydrophobic and remains complexed with the cytoplasmic surface of the membrane. $\alpha$-GTP (the active form of the protein) diffuses in the membrane and associates with various enzymes and ion channels, causing activation or inactivation (this will be
discussed more shortly). The process is terminated when GTP is hydrolysed to GDP: the resulting α-GDP dissociates from the effector and reassociates with βγ, completing the cycle. Each subunit of the G-protein contains structural diversity, and consequently the G-protein family is large and both structurally and functionally diverse.

Adrenoceptors interact preferentially with three classes of G-proteins: Gs (β-adrenoceptor) mediating activation of adenylyl cyclase, Gi (α2-adrenoceptors) mediating inhibition of adenylyl cyclase, and Gq (α1-adrenoceptors) mediating activation of phospholipase C16. Different receptor subtypes may, however, interact preferentially with different G-proteins, for example the α2C-adrenoceptor may interact with Go rather than with Gi as observed with the other α2-adrenoceptor subtypes49. Adrenoceptors may also be coupled to other effector systems, such as ion channels perhaps through Go, and single adrenoceptor subtypes may be coupled to multiple effector systems through different G-proteins.

It has been indicated that adrenoceptors and G-proteins interact through the second (i2) and third (i3) intracellular loops44,50, which is the most structurally diverse portion of the adrenoceptors. The character of this loop determines which G-protein the receptor will preferentially bind to9,30,51-53. For example, replacing a 27 amino acid segment of the N-terminus of the i3 loop of the β2 AR with the α1B sequence resulted in a mutant capable of activating both phospholipase C and adenylate cyclase54. Similarly, small peptides derived from the intracellular loops of adrenoceptors have been shown to bind and activate G-proteins55,56, suggesting that in the native receptor these regions may be shielded from the G-protein until agonist binding induces a change in the conformation of the receptor57. Point mutations in the i3 carboxy terminus of the α1B AR can result in constitutively active receptors58, receptors which are activated in the absence of agonist
This small change in receptor conformation is obviously enough to induce receptor-G-protein interaction. Other studies have identified hydrophobic amino acids as contributing to the receptor-G protein interface. Recently, Arg and Lys in the third intracellular loop were hypothesised to be contact sites on the $\alpha_{1B}$ receptor for the $\alpha$-subunit of Gq, playing a direct role in receptor-G-protein coupling.

Evidence has also been given that members of the $\alpha_1$-AR may bind antagonists on the extracellular surface of the receptor. Further replacement of the third extracellular loop of the $\beta$-AR with the corresponding region of the $\alpha_{1A}$ subtype gave the chimeric receptor (see Figure 1.2) a higher binding affinity for agonists, an increased potency, and an agonist-independent higher basal activity of adenylate cyclase. This activity (referred to as ‘constitutive activity’; see section 1.4.1) suggested a possible role of the third extracellular loop of the $\beta_2$-AR in controlling receptor/G protein activity, most likely by indirectly influencing the arrangement of TMVI and TMVII in the membrane bilayer.

1.2.3 G-proteins as cellular signallers

Stimulation of $\alpha_1$-ARs and their G-proteins results in the activation of various effector enzymes, including Phospholipase C (PLC), Phospholipase A$_2$ (PLA$_2$) and Phospholipase D (PLD), as well as activation of Ca$^{2+}$ channels, Na$^+$-H$^+$ and Na$^+$-Ca$^{2+}$ exchange, and activation or inhibition of K$^+$ channels. In most cells, the primary functional response of all $\alpha_1$-ARs to activation is an increase in intracellular Ca$^{2+}$. The complexity of $\alpha_1$-AR signalling is due, in part, to the ability of the individual subtypes
to couple to different effectors via distinct G proteins. The $\alpha_1$-ARs preferentially bind to GPL$_{\alpha_q}$, PLC mediating G-proteins, but also couple to PLA$_{2}$ mediating G-protein G$_i$.

PLC is a membrane-bound enzyme which when activated by G$_{q}$ acts on phosphatidylinositol (4,5)-diphosphate (Ptd Ins (4,5) P$_2$) in the membrane to produce two second messengers: inositol (1,4,5)-triphosphate (1,4,5-IP$_3$), which causes the release of intracellular Ca$^{2+}$, and diacyl glycerol (DAG) which activates protein kinase C (PKC)$^3$. (1,4,5-IP$_3$) is then dephosphorylated producing inositol, and DAG is phosphorylated to produce phosphatidic acid (PA). Inositol and PA couple, and through a series of phosphorylation reactions, complete the cycle to produce Ptd Ins (4,5) P$_2$. The increased Ca$^{2+}$ levels effected by the 1,4,5-IP$_3$ can result in physiological changes such as smooth muscle contraction, increased force of cardiac muscle contraction, secretion from exocrine glands and neurotransmitter release from neurones and hormone release$^3$. PKC controls phosphorylation of serine and threonine residues of a variety of intracellular proteins$^{63-66}$. The physiological effects of PKC activation include (but are not limited to): release of hormones; increase or decrease in neurotransmitter release and in neuronal excitability; contraction or relaxation of smooth muscle; inflammatory responses; tumour promotion; and receptor desensitisation.

Receptor mediated activation of PLA$_{2}$ leads to the production of arachidonic acid metabolites$^{67}$, which function as intracellular messengers, controlling potassium channel function in certain neurons$^{68}$ and local hormones communicating between cells. It can be seen therefore, that the stimulation of the $\alpha_1$-B-AR can result in the activation of a number of second messengers, and a myriad of functional responses. In the search for more selective ligands and improved therapeutics for the adrenergic system (and in fact any GPCR system) it will be necessary to target the selective activation of G-proteins.
1.3 Theory of Receptor Activation

1.3.1 The ternary complex model

For many years the classical receptor theory described the process of ligand-receptor interaction and resulting effector responses. The classical receptor theory\textsuperscript{69-71} is based upon the existence of a single population of receptors that are activated only when a ligand possessing both affinity and efficacy interacts with the receptor. The interacting ligand induces a conformational change in the receptor which results in G-protein coupling and effector stimulus. From this theory developed the ternary complex model, now the most widely accepted model describing the activation of GPCRs, proposing that receptor activation requires the agonist-promoted formation of an active "ternary" complex of agonist (A), receptor (R) & G-protein (G)\textsuperscript{72}:

\[ A + R \rightarrow AR + G \rightarrow ARG \]

A further extension of this model\textsuperscript{73} referred to as the "two-state model"\textsuperscript{74}, proposes receptors exist in equilibrium between two conformations, an inactive (R) form, which displays low agonist affinity and does not couple to G-protein, and an active (R*) form which displays high agonist affinity and couples to G-protein\textsuperscript{73}:

\[ A + R \rightleftharpoons R^* + A \rightleftharpoons AR^* + G \rightleftharpoons AR^*G \]

In this model the different efficiencies of agonists to promote second messenger activation are explained by supposing that stronger agonists favour binding to R* to a greater extent than weaker agonists, and hence more readily form the AR*G ternary complex. Inverse agonists bind to and stabilise the inactive R conformation, leading to a reduction in basal effector activity\textsuperscript{75,76}. Neutral antagonists bind equally well to both R and R* and do not alter effector activity\textsuperscript{77}. In a strict two-state model, the affinity of G
Chapter One Introduction

for R is identical to that for AR and its affinity for \( R^* \) is identical to that for \( AR^* \) because \( R \) and \( R^* \) represent unique defined conformational states. The \( AR^*G \) conformation in a strict two-state model is the same for all agonists, which differ only in the ratio of \( AR^*G \) to \( ARG_{total} \) (where \( ARG_{total} = ARG + AR^*G \))\(^{74}\).

1.3.2 Multiple effector responses and agonist-directed trafficking: extension of the two-state model to a multi-state model

Agonists are capable of providing multiple stimuli upon receptor activation. There are several examples of a single receptor coupling to more than one cellular signal transduction pathway, including: the 5-HT\(_{1A} \) receptor, which both inhibits adenylyl cyclase activity\(^ {78} \) and opens K\(^+ \) channels\(^ {79} \); members of the serotonin 5-HT\(_2 \) receptor family couple to both PLC and PLA\(_2 \)^{80,81}; the thyroid-stimulating hormone (TSH) receptor stimulates both adenylyl cyclase and PLC\(^ {82} \). \( \alpha_2 \)-AR agonists have been observed to both stimulate and inhibit adenylyl cyclase\(^ {83-86} \) and the receptor has been shown to couple both physically and functionally to \( G_5 \) as well as \( G_i \)^{87}. The magnitude of each of the multiple stimuli elicited can be different and agonist-dependent. It has been suggested that each agonist stabilises a unique set of receptor conformations with a range of G-protein-activating abilities\(^ {88,89} \). Indeed, functionally different agonists have been shown to induce distinct conformations in the G-protein coupling domain of the \( \beta_2 \) AR\(^ {90} \).

This concept has been termed “agonist-directed trafficking of receptor stimulus” and has been discussed in detail by Kenakin\(^ {91,92} \). Agonist-directed trafficking predicts that “the efficiency of receptor coupling to each of multiple effector pathways in a cell is a
function of the agonist\textsuperscript{93} and as a consequence agonist relative efficacy could be
effectector pathway dependent.

Although the two-state model of agonist action allows for a receptor to couple to
multiple cellular effectors, the single active receptor conformation does not allow for
activation of effectors differentially in an agonist-dependent manner\textsuperscript{77}. To allow for this,
the two-state model has been extended to a three-state model (or multi-state model) in
which receptors are proposed to exist in equilibrium between at least two active
conformations (R* and R**) and an inactive (R) conformation\textsuperscript{94}. In a model with
multiple active conformation states, each of the active conformations is proposed to
interact with a different effector pathway; in this way agonists can promote the
formation of different receptor conformations by having differential affinity for these
conformations. The fluorescent labelling of the β₂ AR indicates the receptor can exist in
at least two conformational states\textsuperscript{88}: unliganded and agonist-bound. This data could
support the two-state model, or it could support a multi-state model, in which the
biological efficacy of an agonist is a consequence of the magnitude of conformational
change it induces in the receptor.

Experimental evidence provides support for agonist-directed trafficking\textsuperscript{95,96} and the
concept of agonist-selective receptor states\textsuperscript{74,88,97} is becoming more accepted. Data for
5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors treated with a series of agonists strongly supports the
hypothesis that agonists may traffic the receptor stimulus differentially to multiple
effectector pathways coupled to the same receptor\textsuperscript{93}. The most likely mechanism by which
this may occur is through differential G-protein coupling. Although the agonist-directed
trafficking hypothesis allows for a different receptor conformational state unique to
each agonist, the analysis of the 5-HT\textsubscript{2} receptors\textsuperscript{93} suggests that two active receptor
conformations are sufficient to explain pathway-dependent agonist efficacy. The basis for differential agonist efficacies is therefore different efficacies among agonists at the two active forms $R^*$ and $R^{**}$ (Scheme 1.1).

$$
\begin{align*}
  A_1 + R^* & \quad A_2 + R^{**} \\
  \downarrow & \quad \downarrow \\
  A_1R^* & \quad A_2R^{**} \\
  \downarrow \downarrow & \quad \downarrow \downarrow \\
  E_1 & \quad E_2 \quad E_1 \quad E_2
\end{align*}
$$

**Scheme 1.1** Diagrammatic explanation of the multi-state model of receptor activation. The red and blue represent two different receptor conformation states and their G-protein coupled effector pathways; each activated receptor may interact with either effector pathway $E_1$ or $E_2$.

Agonist directed trafficking has important implications for both normal and pathological physiological function and for therapeutic drug design. If drugs can be designed which upon coupling to a receptor preferentially activate or inactivate a specific G-protein and effector pathway, they should provide more selective results with minimised side effects.

### 1.4 Implications of a constitutively active mutant $\alpha_{1B}$-adrenoceptor

#### 1.4.1 Constitutively active mutants of GPCRs

Constitutive activity is defined as ligand-independent activity, resulting in the production of a second messenger, even in the absence of an agonist$^{24}$. It is also referred to as a ‘gain-of-function’ phenotype. In a constitutively active mutant receptor (CAM),
there is an increase in basal activity relative to the wild-type receptor. The structure adopted by CAMs may therefore often be an approximation of the real active conformation of the receptor.

Activating mutations can affect any part of the receptor, and there has been an enormous number of constitutively active mutant GPCRs either found by accident or created. The positioning of mutations within a GPCR can highlight areas of conformational constraint in inactive receptor conformations and identify intramolecular interactions. CAMs have been used to identify constraining intramolecular interactions that keep ligand-free receptors inactive. For example, CAMs of rhodopsin have indicated that TMVI and TMVII provide important conformational constraints. Details of these interactions have been reviewed recently. Similarly, CAMs of the angiotensin AT₁ receptor indicate conformational constraints between TMIII and TMVII. Random mutagenesis of the whole angiotensin II AT₁A receptor has shown that most activating mutations are present in transmembranes, largely on one side of TMIII. Most CAMs of M5 muscarinic receptors affect the 2nd intracellular loop of TMVI in a pattern suggesting the existence of interacting surfaces in the inactive conformation. Many Class A GPCRs contain a highly conserved D/ERY motif at the cytoplasmic end of the TMIII. Through characterisation of several CAMs, in which the first residue of this motif was mutated, including rhodopsin, α₁B-AR, β₂-AR, gonadotropin-releasing hormone and M1 muscarinic receptors, this motif has been indicated as a major interaction constraining the inactive conformation of the receptors.

CAMs may become active by one of two methods. The current hypothesis supports the view that CAMs release the conformational constraints of the GPCR inactive state.
Whilst in the wild-type receptor the intramolecular interactions that constrain the receptor in an inactive state are postulated to be overcome by new interactions created by the presence of an agonist, many CAMs may be activated by simply disrupting the interaction of the inactive conformation. The conformations of those CAMs may therefore only be approximations of the real active conformation, giving no indication of the interactions involved in the ligand-induced active conformation(s). Conversely, some CAMs may create new interactions which stabilize the active state. CAMs resulting from an additional intramolecular interaction may be closer models of ligand-activated conformations, and may be more stable than those CAMs which result from the loss of an intramolecular interaction²⁴.

Mutations at different positions of the parathyroid receptor present an example of the two ways to produce CAMs. Almost all the substitutions of threonine⁴¹⁰ result in CAMs, in agreement with the disruption of inactivating interactions involving TM6. Alternatively, substitutions of His²²³ with only Arg or Lys result in CAMs¹¹³. Computer modelling studies suggest these mutations create a new interaction between TM2 and TM⁵¹¹⁴.

Constitutive phosphorylation has been demonstrated for many CAMs, including those of the luteinising hormone receptor¹¹⁵,¹¹⁶ and adrenergic receptors¹¹⁷,¹¹⁸. This suggests that the conformation they adopt is a good enough representation of the ligand-receptor active state to be recognized by the specific and non-specific kinases, as well as the desensitisation and internalisation machineries. Some CAMs however, for example of the AT₁ receptor, do not impart either constitutive or agonist-induced phosphorylation, indicating that the adopted conformation of the CAM is not that of the agonist-induced wild-type receptor¹¹⁹. The further possibilities of constitutive downregulation²⁴ and constitutive internalisation and recycling¹²⁰-¹²² have recently been addressed.
1.4.2 Constitutively active mutant receptors in pathology

The unregulated and ligand-independent activity of CAMs has been linked with autonomous hormonal secretion in several endocrine diseases: hyperfunctioning thyroid adenoma (thyroid-stimulating hormone)\(^1\); diabetes insipidus (V2 vasopressin receptor)\(^2\); retinitis pigmentosa and night blindness due to the degradation of retina cells (rhodopsin)\(^3\); and defects in chondrocyte differentiation (parathyroid hormone receptor)\(^4\). Disease has also been associated with CAMs of the luteinising hormone receptor\(^5\), follicle-stimulating hormone receptor\(^6\) and Ca\(^{2+}\) sensing receptor\(^7\). The phenotype resulting from these mutations may be either gain-of-function or loss-of-function. In many of these cases, the mutation results in a misfolded protein, which can block other cell functions\(^8\).

1.4.3 Constitutively active adrenoceptors and the Cys\(^{12}\) Phe \(\alpha_{1B}\)-adrenoceptor

Some constitutively active mutants of the \(\beta_2\)-AR\(^{36,54}\) and the \(\alpha_{1B}\)-AR\(^{58}\) have already been presented in Section 1.2.2. Their mutation regions (i3 loop and e3 loop) were implicated in being important for the structural rigidity of the inactive receptor conformation. Further replacement of the C-terminal portion of the third transmembrane helix of the \(\beta_2\)-adrenoceptor (residues 266-272) with the homologous region of the \(\alpha_{1B}\) adrenoceptor \([\text{L}^{266}\text{S}, \text{K}^{267}\text{R}, \text{H}^{269}\text{K}, \text{L}^{272}\text{A}]\) led to the formation of a CAM which indicated activation of adenylyl cyclase\(^{57}\). This CAM exhibited an increased affinity for agonists (even in the absence of G-protein), but not antagonists, an increased potency of agonists for the stimulation of adenylyl cyclase, and an increased intrinsic activity of
partial agonists. Further constitutive activation of adrenoceptors is also conferred by other mutations in the third intracellular loop\textsuperscript{45,58,130}.

A Cys\textsuperscript{128}Phe mutation in the third transmembrane domain of the $\alpha_{1B}$-adrenoceptor also imparts constitutive activity\textsuperscript{89}. This residue is located approximately one helical turn below Asp\textsuperscript{125} in the third transmembrane domain (Figure 1.4). Because Asp\textsuperscript{125} is the putative counter ion that binds the protonated amine of adrenergic ligands, it is postulated that an agonist induced conformational change of the third TM segment is involved in receptor signalling. Disruption of this critical interaction, as may be the case with the Cys\textsuperscript{128}Phe mutation, might induce changes in the conformation of this helix which may mimic the activated ($R^*$ or $R^{**}$) (also referred to as $R_1^*$ and $R_2^*$) form of the receptor. The constitutive activity of the Cys\textsuperscript{128}Phe mutant indicates that it may very well be representative of an active form of the receptor.

The constitutive activity of this receptor was initially suggested by the finding that it binds the (agonist) catecholamines adrenaline and noradrenaline and other phenethylamines with higher affinity than the wild-type receptor, whilst being unaltered in its binding of a range of diverse antagonists (Table 1.2)\textsuperscript{89}. As with other CAMs, the increase in agonist affinity was demonstrated to not be due to enhanced G protein coupling. Interestingly, the Cys\textsuperscript{128}Phe $\alpha_{1B}$-AR activates only a single effector pathway, the PLC pathway (See Scheme 1.2). This is in contrast to another constitutively active $\alpha_{1B}$-AR mutant Ala\textsuperscript{293}Glu, which activates both the PLC and PLA\textsubscript{2} effector pathways\textsuperscript{89}. This data was strong evidence for the formation of the multi-state model of receptor activation.
Figure 1.4: Model of the constitutively active Cys$^{128}$Phe $\alpha_{1B}$-AR mutant (Perez et al.), showing the cysteine residue in TMIII.

$$G_1AR^* \rightarrow G_1 + AR^* \rightarrow A + R^* \rightarrow R \rightarrow R_2^* + A \rightarrow AR_2^* + G_2 \rightarrow G_2AR_2^*$$

Surprisingly, the affinity of the Cys$^{128}$Phe $\alpha_{1B}$-AR mutant for the imidazoline class of adrenoceptor agonists was largely unaltered from that observed with the wild-type receptor (Table 1.2). With the imidazolines containing a constrained basic nitrogen (see Figure 1.5), the freely rotating ethylamine side chain of the phenethylamines seems to be the important factor, suggesting that they are able to adopt a conformation preferred
by the mutation induced activated Cys_{128}^{Phe} receptor. The Ala_{293}^{Glu} mutant displayed a similar ligand binding phenotype to that of the Cys_{128}^{Phe} mutant: the affinity for phenethylamine agonists was increased with the mutants to an extent related to their degree of intrinsic activity at the wild-type receptor, whilst the affinity for imidazoline ligands was largely unaltered from that of the wild-type receptor (Table 1.2). The increased affinity observed with the phenylethylamine ligands is thus "not due merely to changes in the ligand binding pocket but rather indicates that the mutations induce conformations that partially mimic the R* state". Because PLC and PLA_{2} are activated by two distinct G proteins, these findings demonstrate preferential activation of G proteins (and coupled effectors) in the mutant Cys_{128}^{Phe} \alpha_{1B}-AR by specific agonists (phenethylamines, but not imidazolines). This information suggests that it should be possible to develop agonists that selectively activate only a single effector pathway by trapping either R*_{1} or R*_{2} or inverse agonists that selectively inhibit signalling by either the R*_{1} or R*_{2} conformations (Scheme 1.2).

![General structures of the phenylethylamine and imidazoline ligands.](image)

**Figure 1.5** General structures of the phenylethylamine and imidazoline ligands.
Table 1.2: Binding affinities ($K_i$) for a range of known $\alpha_1$-AR agonists and antagonists upon the wild-type $\alpha_{1B}$-AR and the Cys$^{128}$Phe $\alpha_{1B}$-AR. Data shown in bold indicates a significantly different binding affinity for the Cys$^{128}$Phe $\alpha_{1B}$-AR (indicative of selectivity for the receptor).

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_i$ (nM) Wild-type AR</th>
<th>$K_i$ (nM) Cys$^{128}$Phe AR</th>
<th>$K_i$ (nM) Ala$^{393}$Glu AR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agonist</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R$-Adrenaline (1)</td>
<td>2223</td>
<td>133</td>
<td>2223</td>
</tr>
<tr>
<td>$S$-Adrenaline (2)</td>
<td>3700</td>
<td>373</td>
<td>3700</td>
</tr>
<tr>
<td>Methoxamine (4)</td>
<td>247000</td>
<td>16000</td>
<td>247000</td>
</tr>
<tr>
<td>Phenylephrine (5)</td>
<td>450000</td>
<td>47000</td>
<td>450000</td>
</tr>
<tr>
<td>(-)-Dobutamine (6)</td>
<td>10360</td>
<td>1913</td>
<td>10360</td>
</tr>
<tr>
<td>SKF(-)-89748 (7)</td>
<td>1230</td>
<td>550</td>
<td>1230</td>
</tr>
<tr>
<td>Oxymetazoline (8)</td>
<td>2399</td>
<td>2042</td>
<td>2399</td>
</tr>
<tr>
<td><strong>Imidazoline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymetazoline (8)</td>
<td>596</td>
<td>560</td>
<td>596</td>
</tr>
<tr>
<td>Cirazoline (9)</td>
<td>1337</td>
<td>1066</td>
<td>1337</td>
</tr>
<tr>
<td>Clonidine (10)</td>
<td>1506</td>
<td>1113</td>
<td>1506</td>
</tr>
<tr>
<td>SKF-35886 (11)</td>
<td>1995</td>
<td>1122</td>
<td>1995</td>
</tr>
<tr>
<td>SKF-43315</td>
<td>15850</td>
<td>7943</td>
<td>15850</td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.35</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>5-Methylurapidil</td>
<td>99</td>
<td>60</td>
<td>99</td>
</tr>
<tr>
<td>WB 4101</td>
<td>15</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>2993</td>
<td>1660</td>
<td>2993</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>1230</td>
<td>1323</td>
<td>1230</td>
</tr>
</tbody>
</table>
1.5 Project Aims

The overall aim of this project was therefore to design, synthesise and evaluate small molecule ligands which would selectively bind to the Cys<sup>128</sup>Phe mutant α<sub>1B</sub>-AR over the wild-type α<sub>1B</sub>-AR. Such ligands would selectively interact with only a single
effector pathway, as agonists that would selectively trap $R^*_1$ or $R^*_2$. These selective ligands may therefore be able to induce activation of only a single effector pathway in the wild-type $\alpha_{1B}$AR. In disease states where constitutively active receptors are present, neutral antagonists would not provide any selective control, as they bind to both the basal and activated states with the same affinity. However, inverse agonists, which bind to the basal receptor state with higher affinity than the activated state, could trap the receptor in the basal state, and thus prevent signalling of the active state. This process might be slow as it requires the constitutively active receptor to first isomerise to the basal conformation, which is an infrequent occurrence.

A good initial lead for the design of such ligands was the experimental evidence that phenylethylamines but not imidazolines bound with greater affinity to the Cys$^{128}$Phe mutant $\alpha_{1B}$-AR$^{89}$. This suggested that selective ligands need to mimic a specific conformation of the adrenaline motif. It was therefore proposed to prepare a range of conformationally restricted ligands which mimic extended or folded conformations of the phenylethylamine-based endogenous ligand adrenaline (Figure 1.6). To ensure a particular conformation, rigidity is needed in the molecules, which can be provided by the use of heterocyclic frameworks.

![Design Motif](image)

**Figure 1.6**: Design Motif incorporating the adrenaline structure for the design of small molecule heterocyclic ligands

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Hydrophobic bonding region (Aromatic)</td>
<td>B = Hydrogen Bond acceptor (HBA)/Hydrogen Bond Donor (HBD) region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C = Carbon spacer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D = Basic Amine (forms H-bond reinforced salt bridge when protonated)</td>
<td></td>
</tr>
</tbody>
</table>
The use of molecular modelling was likely to be helpful in determining the important spatial and conformational differences between the phenethylamine agonists and the imidazoline agonists. Moreover, the generation of ligand-based pharmacophores for the Cys$^{128}$Phe mutant $\alpha_{1B}$AR and the wild-type $\alpha_{1B}$AR (based upon data from Table 1.2) may provide valuable leads as to conformational and three-dimensional requirements for selectivity for the Cys$^{128}$Phe mutant $\alpha_{1B}$AR. Catalyst® was therefore to be used to assist in the design of small molecule heterocyclic ligands based on the adrenaline motif, which may provide selective binding to the Cys$^{128}$Phe mutant $\alpha_{1B}$AR and specificsignalling of the PLC pathway.

Finally, the synthesised ligands were to be assessed for their binding affinities and selectivities on both the Cys$^{128}$Phe mutant $\alpha_{1B}$AR and wild-type $\alpha_{1B}$AR, as well as for signalling-specificity. It was hoped these results could then be used in the further development of more selective ligands, and in the generation of more developed pharmacophore models. The long term goal of this work was the development of signalling-specific cardiovascular therapeutics, containing greater selectivity and imparting fewer side effects than those currently available.
Chapter Two

Molecular Modelling

and Pharmacophore Generation
2.1 Introduction

Over the past decade ligand-based drug design has become a popular tool for the medicinal chemist, especially where the three-dimensional structure of the target receptor is not known\textsuperscript{132,133}. With little known about the tertiary structure of the $\alpha_{1B}$ adrenoceptor (AR), or the bioactive conformation of adrenaline, it may be advantageous to use computer modelling techniques to provide a template for ligands which might prefer the Cys$^{128}$Phe $\alpha_{1B}$-AR over the wild-type $\alpha_{1B}$-AR. This may be initiated by developing pharmacophores for both receptor types. In preparing pharmacophores for the differing receptors, it may be possible to distinguish differences in binding patterns, which may lead to the design of ligands selective for the Cys$^{128}$Phe $\alpha_{1B}$-AR over the wild-type $\alpha_{1B}$-AR, and prediction of the bioactive conformations of adrenaline may be possible.

These aims may be achieved by utilising ligand-based drug design methods using CATALYST\textsuperscript{\textregistered} by Accelrys to rationalise the differences between binding patterns of the two receptors. Experimentally determined affinities of known $\alpha_{1B}$ AR agonists upon the two receptors were used to derive a pharmacophore model that describes the three-dimensional structure properties required to obtain optimal interactions within the Cys$^{128}$Phe $\alpha_{1B}$ AR.

2.2 Generation of the Training Set

The principal lead in this project was the selectivity displayed by phenylethylamine-type agonists over imidazoline-type agonists for the constitutively active and signalling-specific Cys$^{128}$Phe $\alpha_{1B}$-AR\textsuperscript{89}. Experimentally determined binding affinities of the two
classes of ligands, as well as TIQ (12), for both the Cys\(^{128}\)Phe \(\alpha_{1B}\)-AR and the wild-type \(\alpha_{1B}\)-AR are given in Table 2.1, and the structures of the agonists are given in Figure 2.1. This work, undertaken by collaborators, was the first indication of an \(\alpha_{1B}\)-AR displaying signalling-specificity, and the first suggestion of a chemical structural type exhibiting selectivity for a mutant \(\alpha_{1B}\)-AR over the wild-type. It was therefore apparent that this set of derivatives would provide the best training set for ligand-based drug design.

Table 2.1 Binding Affinities for Training Set Ligands: data shown in bold indicates phenylethylamine ligands displaying selectivity for the Cys\(^{128}\)Phe \(\alpha_{1B}\)-AR.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Affinity (\text{WT} \alpha_{1B})-AR (K_i (nM))</th>
<th>Affinity (\text{Cys}^{128})Phe (\alpha_{1B})-AR (K_i (nM))</th>
<th>Selectivity for mutant over WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-adrenaline (1)</td>
<td>2223</td>
<td>133</td>
<td>20</td>
</tr>
<tr>
<td>(R)-noradrenaline (2)</td>
<td>3700</td>
<td>373</td>
<td>10</td>
</tr>
<tr>
<td>(S)-noradrenaline (3)</td>
<td>247000</td>
<td>16000</td>
<td>20</td>
</tr>
<tr>
<td>Methoxamine (4)</td>
<td>450000</td>
<td>47000</td>
<td>10</td>
</tr>
<tr>
<td>Phenylephrine (5)</td>
<td>10360</td>
<td>1913</td>
<td>5</td>
</tr>
<tr>
<td>(S)-dobutamine (6)</td>
<td>1230</td>
<td>550</td>
<td>2</td>
</tr>
<tr>
<td>SKF89748 (7)</td>
<td>2399</td>
<td>2042</td>
<td>1</td>
</tr>
<tr>
<td>Oxymetazoline (8)</td>
<td>596</td>
<td>560</td>
<td>1</td>
</tr>
<tr>
<td>Cirazoline (9)</td>
<td>1337</td>
<td>1066</td>
<td>1</td>
</tr>
<tr>
<td>Clonidine (10)</td>
<td>1506</td>
<td>1113</td>
<td>1</td>
</tr>
<tr>
<td>SKF35886 (11)</td>
<td>1995</td>
<td>1122</td>
<td>2</td>
</tr>
<tr>
<td>TIQ (12)</td>
<td>73600</td>
<td>20400</td>
<td>5</td>
</tr>
</tbody>
</table>

![Phenylethylamines](image1)

![Imidazolines](image2)
Selection of the training set molecules is one of the most important exercises performed in hypothesis generation. The ideal training set must contain at least 16 compounds to assure statistical power. Additionally, activities should span 4 orders or
magnitude, with each order of magnitude being represented by at least 3 compounds. There should be no redundant information included, and no excluded volume problems. If two compounds have similar structures (collections of features), they must differ in activity by an order of magnitude to be included in the training set. Similarly, if two compounds have similar activities (within one order of magnitude), they must be structurally distinct (from a chemical feature point of view) in order to be included.

CATALYST® favours models where estimated and measured activities correlate well. By providing a training set that represents each order of magnitude equally, imbalances in data correlation may be prevented. CATALYST® also favours the most active compounds in the training set when it generates the chemical feature space relevant to a particular experiment. This space is defined as the subset of possible chemical feature-based models likely to contain the model which provides the best explanation of the training set data. CATALYST® assumes this subset can be constructed from the most active molecules of the training set, which are those compounds meeting the condition:

\[
\frac{\text{Activity}}{\text{Uncertainty}} \leq \text{Activity of most active compound} \times \text{Uncertainty}^{136}
\]

CATALYST® tries to map all functions in a hypothesis to one of the most active training set molecules\(^{136}\). Any other molecule in the training set should map to at least one function in the hypothesis. Therefore, in order to have an unbiased active set of molecules, the top order of magnitude of activity must be well represented in the training set.

The statistical validity of the training set used here is questionable. There are, by necessity, only twelve compounds included, rather than the required minimum sixteen, indicating that the odds of computing a chance correlation are quite high. The wild-type \(\alpha_{1B}\)-AR data spans four orders of magnitude, however the Cys\(^{128}\)Phe \(\alpha_{1B}\)-AR data spans
only three orders of magnitude. These orders of magnitude are not equally represented, with 58% (7/12) of the molecules for the wild-type set in the same order of magnitude, and 42% (5/12) of the Cys\textsuperscript{128}Phe set in the same order of magnitude. Perhaps more significant is the fact that the most active molecule in the wild-type set (oxymetazoline) is the only molecule representing that order of magnitude, leading to a very biased hypothesis. For the Cys\textsuperscript{128}Phe set, of the four most active molecules (all within the same order of magnitude: \(\alpha\)-adrenaline, \(\alpha\)-noradrenaline, \(S\)-dobutamine, oxymetazoline) three have similar structures. The hypothesis will therefore be biased toward the chemical features of \(\alpha\)-adrenaline.

Given the fact that the compounds in the presented training set are the only compounds to have experimental affinity data for both the wild-type \(\alpha\textsubscript{1B}\) AR and Cys\textsuperscript{128}Phe \(\alpha\textsubscript{1B}\)-AR, it is not possible to expand or alter the training set. It must therefore be acknowledged and kept in mind that this is a minimal, statistically unfavourable training set, and it will provide only very preliminary hypotheses.

### 2.2.1 Conformer generation

In this Thesis, CATALYST\textsuperscript{®} 4.0, CATALYST\textsuperscript{®} 4.5 and CATALYST\textsuperscript{®} 4.7 versions have been used. CATALYST\textsuperscript{®} 4.0 and CATALYST\textsuperscript{®} 4.5 were used on a Silicon Graphics Workstation, CATALYST\textsuperscript{®} 4.7 was used on a Silicon Graphics SGI Fuel Workstation.

Because the biologically active conformation of our training set molecules are not known, CATALYST\textsuperscript{®} may be used to generate a conformational model for each molecule that represents the flexibility of the molecule. The conformational model
consists of a representative set of conformers from the range of energetically reasonable conformations of the molecule, using a standard 20 kcal/mol limit (relative to the lowest energy conformer found). CATALYST® provides two methods of conformational analysis: fast quality and best quality. Best conformational analysis is recommended if the conformational models are to be used for hypothesis generation, and was the method used in this work.

The conformational model for each of the training set molecules was generated using CATALYST® 4.0. These conformational models are outlined in Table 2.1. All the conformers of each compound were employed in the generation of a set of pharmacophore hypotheses.

### 2.3 Pharmacophore Development

CATALYST® is useful as a drug design tool in its ability to generate a hypothesis. A CATALYST® hypothesis is a model which represents the distinguishing chemical features of a class of compounds. These features are displayed as coloured spheres (location constraints) in a three-dimensional spatial orientation, and are representative of characteristics of the set of training molecules that participate in important binding interactions. Estimated activities are then computed by comparing how well the chemical features of a subject molecule overlap with the chemical features in the hypothesis. In terms of hypothesis significance, the difference between the magnitude of the cost of any returned hypothesis and the null hypothesis represents the validity of the data correlation. If this difference is >60 bits, there is a 90% probability of representing a true correlation in the data; if this difference is reduced to 40-60 bits, the
probability drops to 75-90%; if the difference falls below 40 bits, the probability of representing a true correlation is less than 50%.

Using experimental affinity values \((K_i, \text{nM})\) and the conformational models for each of the training set compounds (Table 2.1, 1 – 12), two separate pharmacophores were generated for the WT \(\alpha_{1B}\)-AR and the Cys\(^{128}\)Phe \(\alpha_{1B}\)-AR using CATALYST® 4.0\(^{137}\). An uncertainty value for the training set of three was used. A minimum of four features was nominated for the WT \(\alpha_{1B}\)-AR pharmacophore, and a minimum of three features for the Cys\(^{128}\)Phe \(\alpha_{1B}\)-AR pharmacophore. The selected features were hydrophobic, ring aromatic, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD) and positive ionisable groups. A ring aromatic feature is one which maps 5- and 6-membered aromatic rings, and a positive ionisable feature represents any group that is either positively charged or can become positively charged (through protonation at physiological pH)\(^{136}\). Catalyst assumes that all chemical features contribute equally in providing binding energy, therefore all features were weighted equally. All remaining hypothesis options used were CATALYST® default parameters\(^{136}\).

Each pharmacophore returned ten hypotheses, which were each reduced to two different hypotheses, based on their lower cost values. The lowest cost hypothesis is considered to be the best, however hypotheses with costs within 10-15 bits of the lowest cost hypothesis should be considered good candidates for visual analysis\(^{136}\) (hypothesis cost differences could not be provided here due to loss of data from a computer malfunction, but they were very low, representative of the biased training set, and suggestive of high chance correlation). From these two hypotheses, the best was determined by comparing the estimated affinities of selected training set molecules with the experimental values. Therefore, as shown in Table 2.2, the estimated affinities of \(R\)-adrenaline,
oxymetazoline and SKF89748 provided by the wild-type and Cys\textsuperscript{128}Phe \(\alpha_{1B}\)-AR were compared to their experimental binding affinities. This provided WT-hypo1 and Mutant-hypo4 as the chosen pharmacophores. The pharmacophores differed largely in the nature of their features: the wild-type pharmacophore (to be referred to as WT1) contains two hydrophobic regions, a hydrogen bond acceptor (HBA) region, and a hydrogen bond donor (HBD) region; the Cys\textsuperscript{128}Phe pharmacophore (to be referred to as Mu1) contains a hydrophobic aromatic ring region, and two HBD regions.

**Table 2.2** Comparison of pharmacophores via estimated binding affinities (\(K_i\)) of \(\beta\)-adrenaline, oxymetazoline and SKF89748\textsuperscript{137}.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Molecule</th>
<th>(K_i) Estimated (nM)</th>
<th>(K_i) Experimental (nM)</th>
<th>Ratio Exp./Est.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type-hypo1</td>
<td>R-adrenaline (1)</td>
<td>2000</td>
<td>2200</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Oxymetazoline (8)</td>
<td>1100</td>
<td>600</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>SKF89748 (7)</td>
<td>3000</td>
<td>2300</td>
<td>0.8</td>
</tr>
<tr>
<td>Wild-type-hypo4</td>
<td>R-adrenaline</td>
<td>3300</td>
<td>2200</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Oxymetazoline</td>
<td>4300</td>
<td>600</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>SKF89748</td>
<td>3500</td>
<td>2300</td>
<td>0.7</td>
</tr>
<tr>
<td>Mutant-hypo2</td>
<td>R-adrenaline</td>
<td>38</td>
<td>130</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Oxymetazoline</td>
<td>1200</td>
<td>560</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>SKF89748</td>
<td>200</td>
<td>2000</td>
<td>10</td>
</tr>
<tr>
<td>Mutant-hypo4</td>
<td>R-adrenaline</td>
<td>67</td>
<td>130</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Oxymetazoline</td>
<td>1600</td>
<td>560</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>SKF89748</td>
<td>1600</td>
<td>2000</td>
<td>1.3</td>
</tr>
</tbody>
</table>

These pharmacophores are shown with \(\beta\)-adrenaline overlaid in Figure 2.2 below. The predicted binding affinities for each training set derivative upon both pharmacophores are given in Table 2.4. What is noticeably different about the two pharmacophores is the positioning of these features relative to each other. As detailed in Table 2.3, the distance...
between the HBD and the HBA/HBD region is 2.9-4.9 Å in the WT pharmacophore and 3.1-5.1 Å Cys\textsuperscript{128}Phe α\textsubscript{1B}-AR; between the HBA/HBD and hydrophobic/ring aromatic regions 2.0-4.0 Å (WT) and 3.9-5.9 Å (Cys\textsuperscript{128}Phe); and between the HBD and hydrophobic/ring aromatic features 4.7-6.7 Å (WT) and 2.8-4.8 Å (Cys\textsuperscript{128}Phe). The positioning of this last feature is perhaps the most important difference in the two pharmacophores, because, as can be seen in Figure 2.2, it places the adrenaline molecule in an extended conformation on the WT pharmacophore, but in a much more folded conformation on the Cys\textsuperscript{128}Phe pharmacophore. This folded conformation, which reflects the selectivity of the more flexible phenylethylamine derivatives over the more rigid imidazoline derivatives, provided an initial lead three-dimensional structure upon which to base the development of novel α\textsubscript{1B} AR ligands selective for the Cys\textsuperscript{128}Phe α\textsubscript{1B}-AR over the WT α\textsubscript{1B}-AR. These crude pharmacophores suggest that the bioactive conformer of adrenaline, bound to the constitutively active Cys\textsuperscript{128}Phe α\textsubscript{1B}-AR, might be similar to that represented by the Cys\textsuperscript{128}Phe pharmacophore.
Figure 2.2a: R-adrenaline (adopting an extended conformation) overlayed on the wild-type $\alpha_{1b}$-AR pharmacophore, displaying interactions with three of the four proposed binding regions; mesh spheres represent location constraints (where the feature should be in space); Hydrophobic refers to a hydrophobic bonding region, HBD refers to a hydrogen bond donor region, HBA refers to a hydrogen bond acceptor region. In the HBA and HBD regions, the smaller sphere represents the interacting feature of the ligand, and the larger one represents the interacting feature of the protein\textsuperscript{137}.

Figure 2.2b: R-adrenaline (adopting a folded conformation) overlayed on the Cys$^{128}$Phe $\alpha_{1b}$-AR pharmacophore, displaying interactions with all three of the proposed binding regions. For explanation of binding features see Figure 2.2a. Additionally, in this diagram the Hydrophobic feature refers to a hydrophobic ring aromatic feature: the second hydrophobic sphere indicates the interacting ring aromatic pi system of the protein\textsuperscript{137}.
Table 2.3 Distances (Å) between important binding regions of the two α₁β-AR pharmacophores.

<table>
<thead>
<tr>
<th>Binding regions</th>
<th>α₁β-AR WT Pharmacophore</th>
<th>α₁β-AR Cys¹²⁸Phe Pharmacophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBA/HBD - HBD</td>
<td>2.90 - 4.9</td>
<td>3.1 - 5.1</td>
</tr>
<tr>
<td>HBA/HBD - Hydrophobic</td>
<td>2.0 - 4.0</td>
<td>3.9 - 5.9</td>
</tr>
<tr>
<td>Hydrophobic/Ring Aromatic - HBD</td>
<td>4.7 - 6.7</td>
<td>2.8 - 4.8</td>
</tr>
</tbody>
</table>

2.4 Design of Target Ligands

Rather than constructing structural frameworks to fit the exact dimensions of the three-dimensional outline provided by the Cys¹²⁸Phe pharmacophore, a number of known heterocyclic frameworks were chosen, derivatives constructed, and these derivatives assessed against both the WT and Cys¹²⁸Phe mutant pharmacophores. The purpose of this method was to discover compounds which not only predicted acceptable affinities for the receptors, but also selectivity for the Cys¹²⁸Phe pharmacophore over the WT pharmacophore. These derivatives could then be synthesised, assessed for experimental affinity, and utilised in the development of a larger training set and a more accurate pharmacophore. Hopefully, this set of derivatives might also provide a lead compound on which to base further SAR development.

The only lead in the selective and signalling-specific activation of the Cys¹²⁸Phe α₁β-AR receptor was the preference of the receptor for the phenylethylamines over imidazolines. It therefore seemed logical, and necessary, given the lack of previous work in this area, to also take cognisance of this observation in designing target ligands, rather than just trying to construct a molecule to fit the Cys¹²⁸Phe α₁β-AR pharmacophore. Target ligands would therefore need to mimic the structure of adrenaline, in varying rigid conformations. Target ligands incorporated the three
features of the pharmacophores, and were based on the structural motif of adrenaline as shown below:

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{HO} & \quad \text{C}^{(R)} \\
\text{A} & \quad \text{B} \quad \text{C} \quad \text{D}
\end{align*}
\]

A = Hydrophobic bonding region (aromatic)  
B = Hydrogen bond acceptor (HBA)/Hydrogen bond donor (HBD) region  
C = Carbon spacer  
D = Basic amine (H-bond reinforced salt bridge formed when protonated)

Heterocyclic molecules could provide the desired rigidity. We also wished to have a range of new ligands with the ionisable nitrogen in a ring system, the benzo-ring either fused or non-fused, and the HBA/HBD feature (oxygen or nitrogen based) either in a ring or as a substituent. Other conformationally constrained analogues of adrenaline and noradrenaline (including the imidazoline agonists and IQC used in the training set) have been made and studied in the past\textsuperscript{12,13}, however we were interested in greater structural variety.

![Figure 2.3 Target frameworks based on the adrenaline design motif](Image)

**Figure 2.3** Target frameworks based on the adrenaline design motif
The heterocyclic frameworks chosen for the target ligands incorporated different extended and folded conformations of the adrenaline motif (Figure 2.3). They consisted of 4 major frameworks: a simple cyclic framework, consisting of pyrrolidine \((n = 1)\) and piperidine \((n = 2)\) derivatives; a bicyclic framework, consisting of tropane derivatives; a rigid spirocyclic framework; and a [benzo-spirofused] framework, consisting of spiro[isobenzofuran-pyrrolidine] and spiro[isobenzofuran-piperidine] derivatives. Each derivative was subjected to a conformational model, best fit, generation within a 20kcal/mol energy range (performed in CATALYST® 4.0). These molecules were then all assessed for predicted affinities on both the WT and Cys\(^{128}\)Phe \(\alpha_{1B}\) -AR pharmacophores, and selectivities calculated. Some derivatives with interesting predicted binding profiles, those selected for synthetic development, are given in Table 2.4, together with their structures shown below.
Table 2.4 Derivatives chosen for synthetic development and their predicted pharmacophore binding profiles\textsuperscript{137}; values in bold represent significant predicted selectivity.

<table>
<thead>
<tr>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epinephrine</strong></td>
</tr>
<tr>
<td>Mono cyclic 13</td>
</tr>
<tr>
<td>Mono cyclic 14</td>
</tr>
<tr>
<td>Bicyclic 15</td>
</tr>
<tr>
<td>Bicyclic 16</td>
</tr>
<tr>
<td>Spirocyclic 17</td>
</tr>
<tr>
<td>Spirocyclic 18</td>
</tr>
<tr>
<td>Spiro fused 19</td>
</tr>
<tr>
<td>Spiro fused 20</td>
</tr>
<tr>
<td><strong>K\textsubscript{i} (nM) Wild-type Pharmacophore</strong></td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>S</td>
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<tr>
<td>R</td>
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<td>S</td>
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<tr>
<td>R</td>
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<tr>
<td>R</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td><strong>K\textsubscript{i} (nM) Cys\textsuperscript{128}Phe Pharmacophore</strong></td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>R</td>
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<td>R</td>
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<tr>
<td>R</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>R</td>
</tr>
<tr>
<td><strong>Selectivity for Cys\textsuperscript{128}Phe mutant AR</strong></td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>150</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>70</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>310</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>560</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>250</td>
</tr>
<tr>
<td>530</td>
</tr>
<tr>
<td>300</td>
</tr>
<tr>
<td>2860</td>
</tr>
<tr>
<td>260</td>
</tr>
<tr>
<td>1460</td>
</tr>
</tbody>
</table>

2.5 Pharmacophore Re-modelling

In order to gain some estimation of the validity of the pharmacophores, we investigated their reproducibility. This exercise was not only a validation of the legitimacy of our pharmacophores, but also of the reproducibility of different versions of the CATALYST\textsuperscript{®} program. Additionally, we wished to investigate the effect of using charged compounds (basic N protonated), as would be the case in a biological system, as well as be able to test new ligand designs. Therefore, protonated training set ligands were re-built in CATALYST\textsuperscript{®} 4.5 (1999) and their conformational models generated again using the best fit method and a 20 kcal/mol energy range. This set of molecules was then submitted again to both WT and Cys\textsuperscript{128}Phe pharmacophore generation.
(CATALYST® 4.5), using the same parameters as previously. A total of nine hypotheses were returned for both the Cys\textsuperscript{128}Phe and WT pharmacophores. For all hypotheses (both Cys\textsuperscript{128}Phe and WT) the cost differences were very low, less than three bits. In terms of hypothesis significance, this indicated the hypotheses are quite likely chance correlated rather than a true correlation. The nine hypotheses were reduced to one acceptable hypothesis for both the WT pharmacophore (WT2) and the Cys\textsuperscript{128}Phe pharmacophore (Mu2), based upon the comparison of estimated binding affinities with experimental affinities and previously estimated affinities (on WT1 and Mu1 pharmacophores) of \(\Sigma\)-noradrenaline and \(\Sigma\)-noradrenaline. These two training set ligands were chosen for the fact that both previously estimated affinities and experimental binding affinities displayed selectivity for the \(R\) enantiomer over the \(S\) enantiomer. Data for the comparison of estimated binding affinities of training set ligands on the two sets of WT and Cys\textsuperscript{128}Phe pharmacophores is given in Table 2.6.

Surprisingly, these pharmacophores (referred to from here on in as WT 2 and Mu 2) were different to the original pharmacophores (WT 1 and Mu 1) (Figure 2.4). Mu 1 and Mu 2 contain only one (of three) common features. Both had a hydrophobic ring aromatic region (mapped by the aromatic ring). Where Mu1 had a HBD region (mapped by the sidechain \(\beta\)-hydroxyl), Mu2 had a HBA region (also mapping the sidechain \(\beta\)-hydroxyl). Whilst the basic nitrogen of \(R\)-adrenaline was mapped onto a HBD region in the Mu1 pharmacophore, in the Mu2 pharmacophore the protonated nitrogen of \(R\)-adrenaline mapped onto a positive ionisable feature. Despite these differences in chemical features, the spatial orientation of the two pharmacophores was not too dissimilar, although the distances between the features do differ somewhat (Table 2.5). The WT2 and Mu2 pharmacophores are shown comparatively in Figure 2.4. The two wild-type pharmacophores differ less dramatically. Both pharmacophores contain two
hydrophobic features, one of which maps the aromatic ring, the other is not mapped for adrenaline. The sidechain β-hydroxyl substituent is mapped onto a HBA feature in both pharmacophores. Whereas the WT1 pharmacophore has the nitrogen mapped onto a HBD region, in WT2 this is a positive ionisable feature. The spatial orientations of these features in the two pharmacophores are very similar (Figure 2.4 and Table 2.5).

**Table 2.5** Comparison of distances (in Å) between important binding regions in the two sets of pharmacophores.

<table>
<thead>
<tr>
<th>Binding regions</th>
<th>WT 1 Pharmacophore</th>
<th>WT 2 Pharmacophore</th>
<th>Cys\textsuperscript{128} Phe 1 Pharmacophore</th>
<th>Cys\textsuperscript{128} Phe 2 Pharmacophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBA/HBD - HBD/+ve ionisable</td>
<td>2.9 - 4.9</td>
<td>2.9 - 4.9</td>
<td>3.1 - 5.1</td>
<td>1.8 - 3.8</td>
</tr>
<tr>
<td>HBA/HBD - Hydrophobic/Ring Aromatic</td>
<td>2.0 - 4.0</td>
<td>2.4 - 4.4</td>
<td>3.9 - 5.9</td>
<td>2.6 - 4.6</td>
</tr>
<tr>
<td>Hydrophobic/Ring Aromatic - HBD/+ve ionisable</td>
<td>4.7 - 6.7</td>
<td>3.4 - 5.4</td>
<td>2.8 - 4.8</td>
<td>2.6 - 4.6</td>
</tr>
</tbody>
</table>
Figure 2.4a: R-adrenaline overlayed on the WT 2 pharmacophore: Red sphere (+ve ionisable) represents a positive ionisable feature; blue sphere (hydrophobic) represents a hydrophobic feature; green sphere (HBA) represents a hydrogen bond acceptor feature, the smaller sphere indicates the hydrogen bond acceptor on the ligand (in this case a OH group), the larger sphere indicates the interacting hydrogen bond donor on the protein.

Figure 2.4b: R-adrenaline overlayed on the Cys$^{128}$Phe mutant 2 pharmacophore: Red sphere (+ve ionisable) represents a positive ionisable feature; orange sphere (hydrophobic) represents a hydrophobic ring aromatic feature, the second unmapped sphere represents the interacting pi system in the protein; green sphere (HBA) represents a hydrogen bond acceptor feature, the smaller sphere indicates the hydrogen bond acceptor on the ligand (in this case a OH group), the larger sphere indicates the interacting hydrogen bond donor on the protein.
The predicted binding affinities of the training set compounds on WT2 and Mu2 are given in Table 2.6, along with a comparison of WT1 and Mu1. Whilst the predicted affinities of Mu1 and Mu2 are comparable, those of WT2 are approximately one order of magnitude lower than WT1, leading to obvious differences between selectivity 1 and selectivity 2.

Table 2.6 Comparison of predicted affinities ($K_i$) of training set ligands for the two sets of pharmacophores.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Predicted Affinity $K_i$ (nM)</th>
<th>Predicted Affinity $K_i$ (nM)</th>
<th>Selectivity for Cys$^{128}$Phe AR 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT1</td>
<td>WT2</td>
<td>Cys$^{128}$Phe Mu1</td>
</tr>
<tr>
<td>R-adrenaline (1)</td>
<td>8300</td>
<td>7000</td>
<td>310</td>
</tr>
<tr>
<td>R-noradrenaline (2)</td>
<td>3500</td>
<td>6200</td>
<td>260</td>
</tr>
<tr>
<td>S-noradrenaline (3)</td>
<td>170000</td>
<td>32000</td>
<td>2100</td>
</tr>
<tr>
<td>Methoxamine (4)</td>
<td>16000</td>
<td>9000</td>
<td>2000</td>
</tr>
<tr>
<td>Phenylephrine (5)</td>
<td>15000</td>
<td>13000</td>
<td>2000</td>
</tr>
<tr>
<td>S-dobutamine (6)</td>
<td>1600</td>
<td>5300</td>
<td>1700</td>
</tr>
<tr>
<td>SKF89748 (7)</td>
<td>4100</td>
<td>8600</td>
<td>1700</td>
</tr>
<tr>
<td>Oxymetazoline(8)</td>
<td>2200</td>
<td>3900</td>
<td>1700</td>
</tr>
<tr>
<td>Cizazoline (9)</td>
<td>4100</td>
<td>260</td>
<td>2000</td>
</tr>
<tr>
<td>Clonidine (10)</td>
<td>1700</td>
<td>4800</td>
<td>1800</td>
</tr>
<tr>
<td>SKF35886 (11)</td>
<td>1900</td>
<td>4900</td>
<td>1700</td>
</tr>
<tr>
<td>TIQ (12)</td>
<td>17000</td>
<td>29000</td>
<td>76000</td>
</tr>
</tbody>
</table>

It was certainly curious that the same set of training set compounds had produced different pharmacophores, and it was thus of interest to investigate the reason for this occurrence. Considering that the parameters of the pharmacophore generation process used had been identical (apart from the charge on the nitrogen), and the variables had
been the different versions of CATALYST®, it appears that different versions of CATALYST® may generate both different conformational models for each compound as well as different hypotheses.

To begin investigating this hypothesis, a new (3rd) set of conformers of the training set molecules was generated on CATALYST® 4.5, and compared to the 2nd set. More than half the molecules showed differences in the number of conformers generated within the 20 kcal/mol energy range (Table 2.7). This fact provides support that different conformational models of the same set of compounds may possibly provide different pharmacophores.

**Table 2.7 Comparison of conformers and energy ranges for the two new conformer sets.**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>2nd Conformer set</th>
<th>3rd Conformer set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Conformers</td>
<td>Energy Range (kcal/mol)</td>
</tr>
<tr>
<td>R-adrenaline (1)</td>
<td>25</td>
<td>19.695</td>
</tr>
<tr>
<td>R-noradrenaline (2)</td>
<td>16</td>
<td>19.348</td>
</tr>
<tr>
<td>S-noradrenaline (3)</td>
<td>15</td>
<td>16.700</td>
</tr>
<tr>
<td>Methoxamine (4)</td>
<td>69</td>
<td>19.830</td>
</tr>
<tr>
<td>R-phenylephrine (5)</td>
<td>32</td>
<td>19.053</td>
</tr>
<tr>
<td>S-dobutamine (6)</td>
<td>171</td>
<td>19.546</td>
</tr>
<tr>
<td>SKF89748 (7)</td>
<td>21</td>
<td>19.851</td>
</tr>
<tr>
<td>Oxymetazoline (8)</td>
<td>26</td>
<td>19.784</td>
</tr>
<tr>
<td>Cirazoline (9)</td>
<td>45</td>
<td>19.674</td>
</tr>
<tr>
<td>Clonidine (10)</td>
<td>23</td>
<td>19.611</td>
</tr>
<tr>
<td>SKF35886 (11)</td>
<td>31</td>
<td>19.765</td>
</tr>
<tr>
<td>TIQ (12)</td>
<td>27</td>
<td>19.473</td>
</tr>
</tbody>
</table>
To further investigate the reproducibility of pharmacophores, separate pharmacophores were generated (CATALYST® 4.5) from different conformational models (both CATALYST® 4.5) of the training compounds. This experiment produced wild-type hypotheses which were essentially identical (same features, same distances between features, similar cost values) but gave slightly different estimated activities for the training set molecules. The mutant hypotheses produced were very different from each other: whereas the Mu2 pharmacophore consisted of three features, HBA, positive ionisable and ring aromatic (as shown in Fig. 2.4b), the pharmacophore for the 3rd set of training set conformers (referred to as Mu3) consisted of 4 features, 2 hydrophobic regions, a HBA and a positive ionisable feature (Figure 2.5). The orientation of the adrenaline molecule was also quite different, with the para-hydroxyl of the aromatic ring, rather than the benzylic β-hydroxyl, mapping the HBA region.

Figure 2.5  R-adrenaline overlayed on the Cys128Phe Mu3 pharmacophore: Red sphere (+ve ionisable) represents a positive ionisable feature; blue spheres (hydrophobic) represent hydrophobic features, the second feature is unmapped; green sphere (HBA) represents a hydrogen bond acceptor feature, the smaller sphere indicates the hydrogen bond acceptor on the ligand (in this case a OH group), the larger sphere indicates the interacting hydrogen bond donor on the protein.
To establish the level of reproducibility between the same set of conformers, and different versions of CATALYST® hypothesis generation (CATALYST® 4.5 and CATALYST® 4.7), the 3rd set of training set conformers was re-subjected to hypothesis generation in CATALYST® 4.7. The WT hypotheses (WT4) proved to be identical, having the same features, with the same 3-dimensional arrangement of features and distances between features as the WT3 pharmacophore (Table 2.8). The two pharmacophores map the same conformers of R-adrenaline, and provide only slightly different estimated activities for the training set molecules. The mutant agonists also provided the same hypotheses in both versions of CATALYST®, mapping the same conformer of R-adrenaline, although the estimated binding activities were again not exactly the same.

<table>
<thead>
<tr>
<th>Pharmacophore</th>
<th>HBA - Hydrophobic</th>
<th>HBA - +ve ionisable</th>
<th>Hydrophobic - +ve ionisable</th>
<th>Adrenaline conformer mapped</th>
<th>Est. affinity Adrenaline ( K_\text{d} ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT 3</td>
<td>2.4 - 4.4</td>
<td>2.8 - 4.8</td>
<td>3.4 - 5.4</td>
<td>9</td>
<td>3300</td>
</tr>
<tr>
<td>WT 4</td>
<td>2.4 - 4.4</td>
<td>2.8 - 4.8</td>
<td>3.4 - 5.4</td>
<td>9</td>
<td>3800</td>
</tr>
</tbody>
</table>

From this piece of work, there has emerged a trend which indicates that the reproducibility of pharmacophores is questionable. The same set of molecules can provide different conformational models. Whilst the same conformer model provided very similar hypotheses on different CATALYST® versions (versions 4.5 and 4.7) for the mutant pharmacophore, slightly different hypotheses were provided for the WT pharmacophore. Different conformer models provided very similar WT hypotheses in
the same version of CATALYST®, but very different hypotheses for the mutant pharmacophore. It is therefore not surprising that the WT2 and Mu2 pharmacophores differ from the WT1 and Mu1 pharmacophores, which were not only generated on a different version of CATALYST®, but also from different conformational models, as well as from basic (neutral) training set molecules.

After the synthesis and biological testing of the designed target ligands, a new and more significant training set of molecules was expected to be available, from which new pharmacophore models could be derived.
Chapter Three

Synthesis of Simple Cyclic Derivatives
3.1 Introduction and Design Rationale

To begin investigations of the selective-signalling pathway hypothesis, it was desired to examine a small set of simple cyclic derivatives (based on 13 and 14) for their binding selectivities and affinities upon the $\alpha_{1B}$ Cys$^{128}$Phe mutant adrenoceptor. These derivatives are based upon simple cyclic analogues of the adrenaline motif presented earlier. The use of the cyclic system provides a more rigid environment for the important nitrogen atom than adrenaline does, yet still allows some flexibility in the rest of the molecule without adding too much extra steric bulk.

Conformational modelling of the monocyclic derivatives was undertaken using fast conformational analysis in Catalyst. This exercise provided insight into the preferred conformations of the compounds. For each ligand a conformational model, containing a set of conformers representative of the range of energetically reasonable conformations of the molecule, within a 20kcal/mol range, was generated. Of particular interest were the conformations of the adrenaline mimics provided by these molecules. As Figure 3.1 shows, the global energy minimum of 13 (Figure 3.1b) and 14 (Figure 3.1c) contain the adrenaline motif in a more constrained conformation than that of $R$-adrenaline itself (Figure 3.1a). The pyrrolidine derivative 13 consistently displayed a more folded
conformation than that of the piperidine derivative 14, whose extra ring carbon can be seen to have a significant effect on the nitrogen positioning. The most folded conformation of 13 (Fig. 3.1b, right) is not significantly different from that of the global minimum (Fig. 3.1b, left), and in fact all three conformers shown are similar, especially with respect to the spatial disposition of the pyrrolidine rings. Despite the fact that the Cys¹²⁸Phe mutant pharmacophore prefers a folded rather than an extended conformation of the adrenaline motif, it is not the most folded conformer of 13 which interacts most favourably with the pharmacophore (Fig. 3.1b, right). Similarly, the most folded conformer of 14 does not interact most favourably with the pharmacophore either (Fig. 3.1c, right). There is considerable difference between the conformers of 14 shown, and the preferred piperidine derivative adopts a more extended conformation (Figure 3.1), with the chair conformation of the piperidine ring lying in the same plane as the phenyl ring.

The overlaying of the conformational models of these simple cyclic ligands upon the mutant pharmacophore, shown in Figure 3.2, allows these features to be visualised. The most striking feature is the fact that the ligands map only two of the binding features: the positively ionisable nitrogen, and the alpha-hydroxy functionalities. The hydrophobic feature remains unmapped, and the phenyl rings do not interact at all with the pharmacophore. So although the conformations of the adrenaline mimics are rigid, they are not the correct conformation required for active binding to the Cys¹²⁸Phe mutant receptor.
Chapter 3 Synthesis of Simple Cyclic Derivatives

Figure 3.1a: Left: R-Adrenaline shown in the lowest energy (global energy minimum) conformation (extended); Right: R-Adrenaline shown in the folded conformation preferred by the mutant pharmacophore (18.426 kcal/mol);

Figure 3.1b: Left: 13 shown in the lowest energy (global energy minimum) conformation; Middle: 13 shown in the conformation preferred for the mutant pharmacophore (12.388 kcal/mol); Right: 13 shown in the most folded conformation adopted by the molecule (9.547 kcal/mol).

Figure 3.1c: Left: 14 shown in the lowest energy (global energy minimum) conformation; Middle: 14 shown in the conformation preferred for the mutant pharmacophore (2.020 kcal/mol); Right: the most folded conformation of 14 (10.687 kcal/mol).
Figure 3.2a: *R*-Adrenaline (preferred folded conformation) overlayed on the Cys$^{128}$Phe mutant pharmacophore. Red sphere represents a positive ionisable feature; orange sphere represents a hydrophobic ring aromatic feature, the second unmapped sphere represents the interacting pi system in the protein; green sphere represents a hydrogen bond acceptor feature, the smaller sphere indicates the hydrogen bond acceptor on the ligand (hydroxyl substituent), the larger sphere indicates the interacting hydrogen bond donor on the protein.

Figure 3.2b: 13 (preferred conformation) overlayed on the Cys$^{128}$Phe mutant pharmacophore. The hydrophobic regions of the pharmacophore are not mapped; the phenyl ring does not interact. For explanation of spheres, see Figure 3.2a.

Figure 3.2c: 14 (preferred extended conformation) overlayed on the Cys$^{128}$Phe mutant pharmacophore. The hydrophobic regions of the pharmacophore are not mapped; the phenyl ring does not interact with the pharmacophore. For explanation of spheres, see Figure 3.2a.
Data calculations revealed that the simple cyclic derivatives display only moderate affinity for both the wild-type and mutant adrenoceptors when compared to adrenaline, and poor selectivity for the mutant adrenoceptor over the wild-type (Table 3.1). However, because these derivatives do mimic varied extended and folded conformations of adrenaline, and are straightforward to synthesise, they would allow a quick initial testing of our hypothesis and our computer experimental methods, via the comparison between experimental and computer calculated affinity and selectivity data. Analysis of these derivatives will thus indicate if the chosen testing methods are correct.

Table 3.1: Affinities ($K_i$, nM) and selectivities for simple cyclic derivatives on wild-type (WT1) and Cys$^{128}$Phe mutant (Mu1) $\alpha_{1B}$-AR pharmacophores.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Affinity on $\alpha_{1B}$-AR WT Pharmacophore (WT1)</th>
<th>Affinity on $\alpha_{1B}$-AR Cys$^{128}$Phe Pharmacophore (Mu1)</th>
<th>Selectivity for Cys$^{128}$Phe $\alpha_{1B}$-AR over WT $\alpha_{1B}$-AR</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>$S$ 74000 $R$ 230000</td>
<td>$S$ 2900 $R$ 3600</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>$S$ 350000 $R$ 11000</td>
<td>$S$ 2400 $R$ 2300</td>
<td>150</td>
</tr>
<tr>
<td>25</td>
<td>$S$ 1700 $R$ 1500</td>
<td>$S$ 4500 $R$ 3000</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>$S$ 150000 $R$ 21000</td>
<td>$S$ 2100 $R$ 760</td>
<td>70</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>$R$ 3500</td>
<td>$S$ 2600</td>
<td>10</td>
</tr>
</tbody>
</table>
The set of simple cyclic derivatives to be investigated initially included 3-phenylpyrrolidin-3-ol (13), 3-phenylpiperidin-3-ol (14) and their N-benzyl derivatives 23 and 25. The 3-aryl-piperidin-3-ol derivative 27 had previously been investigated for potential \( \alpha \)-adrenoceptor binding properties\(^{138} \). It was developed as an analogue of 1-aryl-2-aminoethanol 28 and the corresponding morpholine analogue 29, with the aim of comparing pharmacological properties, and to propose a steric model for the stimulation of the \( \alpha \)-adrenergic receptor. Compound 27 was shown to contain activity comparable with those of 28 and 29, although specific \( \alpha \)-adrenoceptor sub-types were not specified.

For other potential therapeutic uses, the hydrochloride salt of 1-benzyl-3-phenylpiperidin-3-ol (26) and the quaternary salt 1-benzyl-1-isobutyl-3-phenylpiperidin-3-ol bromide (30) have been assessed as inhibitors of cholinergic transmission\(^{139} \), proving to have a post-junctional blocking activity which masked any pre-junctional effect at the cholinergic nerve terminals. The salt 3-(4-fluorophenyl)-piperidin-3-ol hydrochloride
(31) has been investigated as a sedative<sup>140</sup>, and <i>N</i>-alkyl derivatives of 3-phenylpiperidin-3-ol such as 32 have been prepared as antidepressants<sup>141</sup>.

Neither 3-phenyl-3-hydroxy-pyrrolidine nor other simple aryl-pyrrolidine derivatives are known as potential pharmacological ligands at the α-adrenergic receptor or indeed in any other biological system.

### 3.2 Synthesis of Simple Cyclic Derivatives

#### 3.2.1 Retrosynthesis of simple cyclic derivatives

Retrosynthetically, the preparation of the monocyclic piperidine and pyrrolidine derivatives is quite straightforward (Scheme 3.1). Incorporation of the phenylhydroxy moiety into the ring may be achieved readily via a simple Grignard reaction of the corresponding <i>N</i>-protected ketones. These ketones may be provided by commercially available <i>N</i>-benzyl-3-pyrrolidinone (33) and <i>N</i>-benzyl-3-piperidone (34).

![Scheme 3.1: Retrosynthetic analysis of Monocyclic derivatives 13 and 14.](attachment:image.png)
3.2.2 Synthesis of 3-phenyl-pyrrolidin-3-ol and 3-phenyl-piperidin-3-ol

Beginning then from \( N \)-benzyl-3-pyrrolidinone and \( N \)-benzyl-3-piperidone, the preparation of 3-phenyl-3-hydroxy-pyrrolidine (13) and 3-phenyl-3-hydroxy-piperidine (14) involved incorporation of the phenylhydroxy functionality and deprotection of the benzyl protecting group. When Hemsworth et al. prepared 3-hydroxy-3-phenyl-piperidine from its \( N \)-benzyl derivative, they reported using phenyl lithium in preference to the lower yielding arylmagnesium halide method previously established by Iselin and Hoffman in the original synthesis of the compound (74% compared with ~ 54% respectively). More recently, Sui et al. reported using the arylmagnesium halide method for the preparation of \( N \)-benzyl-3-hydroxy-3-phenyl-piperidine as an intermediate in the synthesis of \( N \)-aryloyl-3-phenyltetrahydropyridines as potential non-steroidal bone formation promoting agents. They reported only low yields of \( N \)-benzyl-3-phenyl-piperidin-3-ol formation from \( N \)-benzyl-3-piperidone (45%). Despite these reported low yields, it was decided to initially investigate levels of success with the Grignard reaction to incorporate the desired phenylhydroxy moiety into the simple cyclic target derivatives.

\( N \)-benzyl-3-pyrrolidinone (33) was therefore treated with phenylmagnesium bromide, affording the desired alcohol 23 (C\(_{17}\)H\(_{19}\)NO) as a dark orange oil in 69% yield (Scheme 3.2). \(^1\)H NMR spectroscopy revealed the introduction of the phenylhydroxy functionality to the pyrrolidine ring, via an additional five protons seen in the aromatic region and a broad singlet at \( \delta 2.85 \). The \(^{13}\)C NMR spectrum indicated the absence of the ketone peak at \( \delta 206 \), and the presence of the 6 new phenyl carbons at \( \delta 125.5 \) - 129.0, and \( \delta 144.4 \). Mass spectral analysis (Cl\(^+\)) confirmed the identity of the newly formed pyrrolidine derivative as C\(_{17}\)H\(_{19}\)NO \textit{via} a molecular ion at m/z 254 (M+1).
Chapter 3 Synthesis of Simple Cyclic Derivatives

Reagents and Conditions: a) phenylmagnesium bromide (3.0M in ether, 1.2 eq.), ether, RT, 3hrs, 69-86% yield; b) Pd/C, H₂, ethanol, RT, 44 hrs, 68-100% yield.

Scheme 3.2: Preparation of 3-phenyl-3-pyrrolidinol and 3-phenyl-3-piperidinol

The same method was used for the preparation of the desired phenylpiperidinol derivative 25 (Scheme 3.2). Thus, treatment of N-benzyl-3-piperidone (34) with phenylmagnesium bromide afforded 25 (C₁₈H₂₁NO) as off-white crystals in 86% yield after chromatographic purification, much improved upon previously reported literature yields.¹⁴² ¹H NMR spectroscopy again revealed the addition of the phenylhydroxy functionality to the piperidine ring via an additional five protons at δ7.26 – 7.52 and a broad singlet at δ3.98. The ¹³C NMR spectrum also indicated the absence of the ketone peak at δ207, and the presence of the 6 new phenyl carbons at δ127.2 - 128.9, and δ145.6. Mass spectral analysis (CI⁺) confirmed the molecular formula of 25 to be C₁₈H₂₁NO via a molecular ion at m/z 268 (M+1).

Although both 23 and 25 intermediates will provide additional interesting ligands in themselves for pharmacological evaluation, N-benzyl protecting groups need to be removed so as to provide the desired targets 13 and 14. This step was achieved via the use of palladium on carbon in a hydrogen atmosphere in ethanol solution at room temperature.¹⁴³ Both 3-phenyl-pyrrolidin-3-ol (13) (C₁₀H₁₃NO) and 3-phenyl-piperidin-
3-ol (14) \((\text{C}_{11}\text{H}_{15}\text{NO})\) were obtained as colourless oils in yields of 100% and 68% respectively. \(^1\text{H}\) NMR spectroscopy clearly revealed the loss of the benzyl methylene at \(\delta 3.73\) (13) and \(\delta 3.57\) (14) and the loss of five aromatic protons in the spectra of both 13 and 14. Mass spectral analysis \((\text{CI}^+)\) confirmed the identity of both the pyrroldine 13 and piperidine 14 target derivatives \textit{via} \(\text{MH}^+\) ions at \(m/z\) 164 \((\text{C}_{10}\text{H}_{13}\text{NO})\) and \(m/z\) 178 \((\text{C}_{11}\text{H}_{15}\text{NO})\) respectively.

The hydrochloride salts of the target derivatives 13, 23, 14 and 25 were prepared for pharmacological testing purposes. These salts were readily made by the addition of hydrogen chloride in diethyl ether to the respective bases.

3.2.3 \textit{Expansion of the set of simple cyclic derivatives: synthesis of 3-(3,4-dihydroxyphenyl)-piperidin-3-ol}

After receiving some encouraging pharmacological results for the piperidinol derivatives 14 and 25 (which shall be presented and discussed in Chapter 6), it was decided to expand the set of simple cyclic derivatives by preparing the 3-(3,4-methylenedioxyphenyl)- and 3-(3,4-dihydroxyphenyl)-piperidin-3-ol derivatives 35 and 36. The longer term goal was then to unmask the catechol moieties at a later stage to give catechols closer in structure to adrenaline. Therefore, following the method used previously, but altering the Grignard reagent, \(N\)-benzyl-3-piperidone (34) was treated with 3,4-methylenedioxyphenylmagnesium bromide (Scheme 3.3). After chromatographic purification the mixture afforded 36 \((\text{C}_{19}\text{H}_{21}\text{NO}_3)\) as a yellow oil in 52% yield. The new 3,4-methylenedioxyphenyl substituent in 36 was displayed in the \(^1\text{H}\) NMR spectrum as a 2 proton singlet at \(\delta 5.91\) and three aromatic peaks at \(\delta 6.75\),
δ6.93 and δ7.02, and with spin-spin couplings characteristic of a 1,2,3-substituent disposition in the aromatic ring. Mass spectral analysis (Cl+) confirmed the identity of 36 via a molecular ion at m/z 312, consistent with the molecular formula C_{19}H_{21}NO_{3}.

![Chemical structure and reactions](image)

Reagents and Conditions: a) 3,4-methylenedioxyphenylmagnesium bromide (1M in THF, 1.2 eq.), THF, RT, 3hrs, 52% yield; b) Pd/C, H₂, ethanol, RT, 44 hrs, % yield; c) BCl₃/n-Bu₄NI or BBr₃ in DCM.

**Scheme 3.3**: Preparation of substituted 3-phenyl-3-hydroxy-piperidine derivatives

Subsequent removal of the benzyl protecting group (Pd/C, H₂) provided the target intermediate 35 (C_{12}H_{15}NO_{3}) as a colourless oil in 45% yield. Confirmation of the benzyl group having been removed was evident in the ¹H NMR, where the absence of signals for the benzyl methylene singlet at δ3.56 and the five aromatic protons at δ7.29 was apparent. Mass spectral (Cl+) analysis further confirmed the identity of 35 via a molecular ion peak at m/z 222, consistent with the molecular formula C_{12}H_{15}NO_{3}. 35 was also assessed pharmacologically as the methylenedioxy group could provide some interesting SAR data in comparison to the dihydroxy derivative 26.
The dihydroxy derivative 26 may be accessed via either methylenedioxy deprotection of benzyl protected 36 to produce the catechol derivative 27, or deprotection of the methylenedioxy derivative 35 to produce the catechol derivative 26, as outlined in Scheme 3.3. It was thus necessary to investigate methods for the removal of the methylenedioxy group from the aryl functionality to provide the desired catechol derivative.

3.2.3.1 Methods for deprotection of methylenedioxy groups

A number of known methods are available for effecting the dealkylation of aryl ethers, a process regarded as one of the most popular deprotecting methods in organic synthesis, including boron tribromide, sodium ethylsulfide/DMF, trimethylsilyl iodide, pyridine-HCl and hydrobromic acid/acetic acid. Routine methods for methylenedioxy cleavage include the use of boron tribromide, aluminium tribromide with ethylsulfide and boron trichloride in the presence of dimethylsulfide. Boron trichloride alone has been used in refluxing dichloromethane, however, as it is not effective at low temperatures, Brooks et al. have developed a mild reagent combining BCl₃ with nBu₄NI, which exhibits a greatly enhanced reactivity between that of BBr₃ and BI₃ at low temperatures. The vigorous reagents BBr₃ and BI₃ presumably dissociate more readily than BCl₃ "to provide oxonium ion activation (ArOR'BX₂)+ and free nucleophilic halide ion (X⁻). With the BCl₃/nBu₄NI reagent combination, iodide may act as both a stabilising ligand on boron, favouring formation of a reactive oxonium ion species (ArOR'BIX)+, and a source of potent nucleophilic iodide. Hwu et al. have developed alkali organoamides NaN(SiMe₃)₂ and LiN(I-Pr)₂ as efficient deprotecting agents for benzodioxole derivatives, obtaining 93-99% yields of catechols.
in a THF:DMEU (1,3-dimethyl-2-imidazolidinone) mixture in a sealed tube at 185°C for 12 hours.

Of these known cleavage methods, the boron trichloride/tetrabutylammonium iodide method\textsuperscript{163}, being both mild and reactive, seemed to be the most appropriate for our tertiary alcohol derivatives. Whilst sulfides are unpleasant to work with, high temperature reactions are also unsuitable due to the presence of other protecting groups on our substrates and the possibility of side-reactions and decomposition. Therefore, following the method of Brooks \textit{et al.}\textsuperscript{63}, \textbf{36} was treated with \textit{n}Bu\textsubscript{4}NI and BCl\textsubscript{3} at low temperature (Scheme 3.4) to afford a crude yellow oil. Both \textsuperscript{1}H NMR spectroscopy and MS(CI\textsuperscript{+}) (m/z 213, 294) revealed \textbf{36} only, with no loss of the methylenedioxy protecting group.

[Diagram showing chemical structures]

\textbf{36} -> \textbf{37}

\textbf{No Reaction}

Reagents and Conditions: \textit{n}Bu\textsubscript{4}NI (1.1 eq.), BCl\textsubscript{3} (2.5 eq.), -78°C, 1 hr; OR \textit{n}Bu\textsubscript{4}NI (3.1 eq.), BCl\textsubscript{3} (5 eq.), -78°C, 1 hr;

\textbf{Scheme 3.4}: Attempted methylenedioxy cleavage of \textbf{36} using BCl\textsubscript{3}/\textit{n}Bu\textsubscript{4}NI.

Considering that derivative \textbf{36} contains two additional basic sites at which the boron may complex (Figure 3.2), 4.5 – 5 molar equivalents may be needed in the reaction to ensure effective cleavage of the methylenedioxy moiety. Thus the reaction was repeated with 5.0 equivalents BCl\textsubscript{3} and 3.1 equivalents \textit{n}Bu\textsubscript{4}NI. The isolated crystalline product
again proved to be 36 via $^1$H NMR spectroscopy (methylene peak at $\delta 5.91$) and mass spectral analysis ($\text{Cl}^+$) ($m/z$ 312, 294, C$_{19}$H$_{21}$N0$_3$).

* basic sites at which boron may complex

**Figure 3.2:** The basic sites upon 36 where boron may complex are indicated by an asterisk (*).

Given the difficulties encountered with this reaction, it was decided to investigate the reaction conditions with a model compound, the simple methylenedioxy derivative piperonal (38). Thus, as outlined in the generic Scheme 3.5, piperonal was treated with tetrabutylammonium iodide and boron trichloride. Unfortunately TLC analysis indicated no reaction had occurred, and mass spectral analysis ($\text{Cl}^+$) confirmed the presence of only recovered 38 (C$_8$H$_6$O$_3$) via a molecular ion base peak at $m/z$ 151.

**Scheme 3.5:** Attempted deprotection of piperonal (38) using $n$Bu$_4$NI and BCl$_3$.

Given that cerium chloride heptahydrate is reported to smoothly cleave 1,3-dioxolane protecting groups$^{165}$ and bismuth nitrate pentahydrate readily cleaves dimethyl ketal
protecting groups\textsuperscript{166}, it was reasoned that these reagents might also effect cleavage of aryI ethers, in particular methylenedioxy ethers. To investigate this, 36 was initially treated with bismuth nitrate pentahydrate in ethanol, stirring at 70°C for a total of 70 hours. Mass spectral analysis (Cl\textsuperscript{+}) displayed an ion at \(m/z\) 312 (M+1, 36). The reaction mixture was subsequently refluxed in acetonitrile for 2 days, with the addition of further bismuth nitrate pentahydrate. Mass spectral analysis (Cl\textsuperscript{+}) displayed only a high molecular mass peak at \(m/z\) 357. To investigate if cerium chloride heptahydrate may effect the desired deprotection, 36 was treated with cerium chloride heptahydrate and sodium iodide in acetonitrile at room temperature for 24 hours. Mass spectral (Cl\textsuperscript{+}) analysis indicated that still only starting material 36 was recovered: \(m/z\) 312, 294 (C\textsubscript{10}H\textsubscript{21}N\textsubscript{3}O\textsubscript{3}).

Further investigations of both bismuth nitrate pentahydrate and cerium chloride heptahydrate were undertaken upon the model compound piperonal (38). Piperonal and bismuth nitrate pentahydrate (2 equivalents) were refluxed in acetonitrile for 19 hours. Mass spectral analysis (Cl\textsuperscript{+}) displayed a small amount of product 39 (C\textsubscript{7}H\textsubscript{6}O\textsubscript{3}) at \(m/z\) 139 (M+1, 17\%) with a major peak at \(m/z\) 151 as expected for piperonal, suggesting only a small conversion rate of product. Although reflux continued, no further cleavage was observed. Microwave irradiation of the reaction was therefore investigated. The reaction mixture was exposed to microwave radiation at 100°C for 30 minutes. No ether cleavage was observed, with mass spectral analysis (Cl\textsuperscript{+}) displaying the piperonal molecular ion at \(m/z\) 151 (100\%). The cerium chloride heptahydrate was also investigated with piperonal under microwave conditions. A mixture of piperonal, cerium chloride heptahydrate and sodium iodide in acetonitrile was exposed to microwave radiation at 100°C for 30 minutes. Again, mass spectral (Cl\textsuperscript{+}) analysis revealed only piperonal (38) in the reaction mixture, via a base peak at \(m/z\) 151. 36 was
also treated under analogous conditions, but again failed to furnish any of desired catechol 37.

Boron tribromide was thus investigated as a more reactive methylenedioxy-cleavage reagent. Following the method of Neumeyer\textsuperscript{154d} 36 was treated with BBr\textsubscript{3} under anhydrous reflux conditions. Unfortunately, the crude product proved by \textit{H} NMR to be unreacted 36 (C\textsubscript{19}H\textsubscript{21}NO\textsubscript{3}). The reaction was repeated on model compound piperonal (38) at \(-78^\circ\text{C}\) for 1 hour. The resultant crude residue turned black, and proved to be insoluble in methylene chloride. However, stirring of the suspension in methylene chloride overnight, followed by filtration and concentration \textit{in vacuo}, provided a colourless oil which rapidly turned brown. The residue proved by mass spectral analysis (CI\textsuperscript{+}) to contain desired dihydroxy derivative 39 \textit{via} a molecular ion base peak at \textit{m/z} 139. A small amount of unreacted 38 was also noted (\textit{m/z} 151, 10%). \textit{H} NMR analysis revealed the crude product to be a 1:1 mixture of 38 and 39: this was evidenced by two peaks for the aldehyde proton, of equivalent integration (1H each) at \(\delta 9.77\) and \(\delta 9.82\), and a peak of 1H integration, ascribed to the methylenedioxy protons, at \(\delta 6.08\). Additionally, the \textit{H} NMR spectrum also contained a large amount of hydrocarbon contamination, in the regions \(\delta 1.25 - 2.09\) and \(\delta 1.71 - 2.09\). The source of this contamination could not be determined. Although the crude mixture was not purified, the evidence of some methylenedioxy deprotection having occurred indicates that this method is of potential use in preparing future catechol derivatives. Unfortunately, due to time constraints, the initial target catechol derivatives 35 and 37 could not be accessed.
Chapter Four

Synthesis of Bicyclic Tropane Derivatives

_Duboisia myoporoides_ (source of scopolamine)
4.1 Introduction

The tropane system is a well-known member of the alkaloid class of natural products. Alkaloids are chemically defined as secondary metabolites of plant, microbial or animal origin, and typically contain one or more nitrogen atoms, usually in a heterocyclic ring, have a pronounced basic reaction and generally possess strong and varied pharmacological effects when administered to humans and animals\textsuperscript{167}. There are over 12,000 known alkaloids, with many more being discovered each year. Tropane alkaloids are structurally related natural products which have in common the azabicyclo[3.2.1]octane structure; the systematic name for tropane is therefore 8-methyl-8-azabicyclo[3.2.1]octane.

The majority of tropane alkaloids are esters of organic acids and hydroxytropanes, with 3α-hydroxytropane (known as tropine) being the amino alcohol most frequently encountered. In addition, its 3β-isomer (pseudotropine), the di-(3,6- 3,7- or 6,7-) and trihydroxylated 3,6,7-tropanes, the 6,7-epoxide and the corresponding \textit{N-nor} derivatives occur in numerous plant species, in either free base form, or more often esterified with a wide variety of aliphatic, aromatic and heterocyclic acids\textsuperscript{167}. Dimeric and trimeric tropane alkaloids have also been found\textsuperscript{168}. More than 200 tropane alkaloids are known; their distribution in the plant kingdom has been investigated\textsuperscript{167} and their biosynthesis extensively studied over the last few decades\textsuperscript{169}.
A new group of tropane alkaloids, called calystegines, was isolated and identified in 1988, from the roots of the morning glory *Calystegia sepium*\textsuperscript{170}. These alkaloids are characterized as polyhydroxylated nortropmes with a bridgehead hydroxyl substituent\textsuperscript{171-175}. The hydroxyl groups vary in both position and stereochemistry. To date, at least 16 calystegines have been isolated and structurally characterised\textsuperscript{167}. The natural occurrence and therapeutic applications of other known polyhydroxylated alkaloids has been reviewed\textsuperscript{176}.

![Calystegine skeleton](image)

The most widely recognised tropane alkaloids from a pharmacological point of view are (-)-scopolamine (hyoscine), (-)-hyoscyamine, atropine and cocaine. Pharmacologically important tropane alkaloids such as these, in which the nitrogenous base is esterified with tropic acid (or a derivative), are apparently unique to the solanaceae family\textsuperscript{177}.

![(-)-hyoscyamine](image) ![atropine](image) ![(-)-scopolamine](image) ![(-)-cocaine](image)
Numerous synthetic approaches to the tropane skeleton have been developed, from the classical synthesis of tropine by Willstatter\textsuperscript{178} at the beginning of the century and comprehensively reviewed by Holmes\textsuperscript{179}, to the effective synthesis of tropinone by Robinson\textsuperscript{180}. Modification of this latter synthesis by Lehmann\textsuperscript{181} and then Seebach\textsuperscript{182}, further led to more recent developments dealing with asymmetric deprotonation of tropinone, and utilisation of chiral lithium amide bases for the enantioselective synthesis of a range of tropanes\textsuperscript{183}.

The optical activity of hyoscyamine and scopolamine arises from the chiral centre in the acid portion of the molecule, ($S$)-tropic acid. Although tropine itself contains stereogenic centres, it is a symmetrical and optically inactive molecule and may be regarded as a 	extit{meso} isomer. Under basic catalysis, racemisation can take place at the asymmetric centre of the acyl component, and in the case of (-)-hyoscyamine this leads to the racemic alkaloid atropine. The plant sources generally contain only the enantiomerically pure alkaloids.

All the main tropic acid-esterified tropane alkaloids including hyoscyamine, atropine, scopolamine, $N$-butylscopolamine and $N$-ethylscopolamine, show antimuscarinic activity. Their mode of action is competitive antagonism of acetylcholine and other muscarinic agonists such as pilocarpine, physostigmine or arecoline, which are also natural alkaloids. Generally, the most active forms are the (-)-isomers, with the peripheral effects of (-)-hyoscyamine being 10 to 20 times stronger than those of (+)-hyoscyamine, and with CNS effects 8 to 50 times stronger. The main therapeutic applications of tropane alkaloids include treatment of motion sickness (scopolamine), prevention of vagal reflexes (atropine), preanesthetic medication to aid respiration (atropine), and the treatment of intestinal hypermotility and spasms of the urinary tract (atropine). Cocaine is a powerful CNS stimulating agent, with both local anaesthetic
and local vasoconstriction effects. Medicinally, it is used as its hydrochloride salt in 0.1-4% aqueous solution as a local anaesthetic for topical application. Calystegines, as polyhydroxylated alkaloids, are currently of interest as glycosidase inhibitors and particularly as anti-viral agents (Asano, 1996; Asano, 1997). Their role in this context appears to derive largely from their ability to alter glycoprotein structure by interfering with the processing of the oligosaccharide moiety (Nash, 1996).

Figure 4.1 Semisynthetic & synthetic tropane alkaloids

Some semisynthetic and synthetic derivatives of scopolamine and atropine have been prepared with the aim of improving peripheral and reducing CNS effects (Figure 4.1). For instance, homatropine is 10 times weaker than atropine with correspondingly decreased toxicity. Likewise the quaternary ammonium tropanes, ipratropium bromide (a derivative of atropine), oxitropium bromide and tiotropium bromide (derivatives of...
scopolamine) and flutropium bromide, a fluoroethyl derivative, used mainly in the
treatment of chronic obstructive pulmonary disease, have decreased central effects as they do not readily cross the blood-brain barrier. N-alkylated scopolamine derivatives such as N-butylscopolamine bromide, N-methylscopolamine bromide and N-methylhomotropine bromide have been used to relieve gastrointestinal spasms.

![Chemical structures](image)

There has been a considerable amount of work done on the synthesis of new tropane derivatives in the search for novel compounds with pharmacological activity. The earlier work investigated tropane analogues as narcotic antagonists (e.g. 40), analgesics \(^{184-187}\), and potential opiate agonists and antagonists (e.g. 41) \(^{188}\). There has also been interest shown in the use of tropanes as anticholinergic, anticonvulsant and anti-inflammatory agents (e.g. 42) \(^{189-191}\), as well as being considered for new membrane-active substances at the acetylcholine receptor \(^{192}\). More recently, there has been much investigation into the synthesis of cocaine analogues as cocaine antagonists and potential dopamine transporter (DAT) antagonists (e.g. 43 \(^{193}\), 44 \(^{194}\)) \(^{195-199}\). Such analogues may be effective as therapeutics in the treatment of cocaine abuse and neurological disorder, including depression and Parkinson’s disease.
A new class of amphibian alkaloid, epibatidine, isolated in 1992 from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*, has been reported to be a highly potent non-opioid analgesic, *via* its potent agonistic activity at the nicotinic acetylcholine receptor. The azabicyclic[2.2.1]heptane (7-azanorbornane)-based ring system of epibatidine, however, displays high toxicity levels, generating much interest in the preparation of analogues which may be selective nicotinic receptor analgesics with reduced toxicity. This has led to numerous syntheses of epibatidine and related 7-azabicyclo[2.2.1]heptane analogues.

Interestingly, the related compound homoepibatidine, containing the 6-pyridinyl-8-azabicyclo[3.2.1]octane (tropane) system, displays comparable analgesic activity to epibatidine. Therefore, in recent years, there has been interest in the development of tropane-based analogues of epibatidine and homo-epibatidine, including 2-pyridyl derivatives, 3-pyridyl derivatives such as, and 5-pyridyl derivatives such as. The therapeutic potential of these nicotinic acetylcholine receptor agonists extends beyond analgesia, and includes the possibility of preventing the neurodegeneration associated with Alzheimer’s disease.
4.2 Design of Bicyclic Tropane Derivatives as $\alpha_{1B}$-AR Ligands

An important structural feature of tropanes is that of rigidity. Whilst the 6-membered ring portion of the molecule may equilibrate between chair and boat forms, the fixed conformation of the 5-membered pyrrolidine ring provides a rigid framework. This feature renders bicyclic bridged derivatives such as tropanes useful frameworks when a fixed chemical geometry is desired. This was certainly appealing in the present work with respect to mimicking a fixed conformation of the adrenaline motif.

Generation of the Cys$^{128}$Phe $\alpha_{1B}$-AR mutant pharmacophore (Chapter 2) has revealed that the predicted conformation of adrenaline upon the Cys$^{128}$Phe $\alpha_{1B}$-AR has a folded conformation, as discussed in Chapter 2. In the development of ligands which encompass a folded conformation that contains the necessary ionisable nitrogen atom, derivatives with a tropane framework present an appropriate choice. The tropane framework may provide a similar geometry to that of a folded conformation of adrenaline (Figure 4.2), with the additional feature of being held in a relatively rigid heterocycle.

Figure 4.2 Folded conformation of R-Adrenaline (structure from CATALYST®).
The folded conformation of adrenaline appears to be an essential feature for receptor selectivity, therefore the incorporation of this adrenaline motif is necessary into the design of our target molecules. In transferring this motif to the tropane skeleton (Figure 4.3), substitution at the 6 and 2 positions would fulfil this requirement. Additionally, substitution at the 3-position would allow investigation of the effect on the mutant model of extending the motif by one carbon. Both 2- and 6-substituted tropane derivatives would provide the adrenaline motif in a folded conformation, whereas the 3-substituted tropane derivative mimics a more extended conformation of the motif. Studying the binding profiles of these related derivatives would allow further investigation into the hypothesis that adrenaline adopts a folded conformation upon the mutant receptor.

![Adrenaline motif, 6-substituted tropane, 2-substituted tropane, 3-substituted tropane](image)

**Figure 4.3** Presence of the adrenaline motif in bicyclic tropane derivatives

The tropane-derived ligands to be investigated computationally were therefore based upon the 3- and 6-substituted tropane derivatives, together with substituted derivatives of some of these bicyclic ligands. Whilst 2-arylhydroxytropane derivatives fit the desired adrenaline motif, their synthetic production would be via a lengthy route from cocaine derivatives, and was deemed unfeasible. Table 4.1 displays the range of ligands
investigated and their predicted activity data upon both the wild-type (WT1) and Cys\textsuperscript{128}Phe (Mul) \( \alpha_{1B} \)-AR pharmacophores.

**Table 4.1** Predicted binding affinities and selectivities of tropane derivatives on wild-type (WT1) and Cys\textsuperscript{128}Phe (Mul) pharmacophores.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Predicted WT Affinity</th>
<th>Predicted Cys\textsuperscript{128}Phe Affinity</th>
<th>Selectivity (for Cys\textsuperscript{128}Phe over WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_i ) ( (nM) )</td>
<td>( K_i ) ( (nM) )</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>340000</td>
<td>1100</td>
<td>310</td>
</tr>
<tr>
<td>S</td>
<td>12000</td>
<td>570</td>
<td>20</td>
</tr>
<tr>
<td>R</td>
<td>340000</td>
<td>610</td>
<td>560</td>
</tr>
<tr>
<td>50</td>
<td>10000</td>
<td>380</td>
<td>30</td>
</tr>
<tr>
<td>S</td>
<td>18000</td>
<td>2100</td>
<td>10</td>
</tr>
<tr>
<td>R</td>
<td>21000</td>
<td>700</td>
<td>30</td>
</tr>
<tr>
<td>51</td>
<td>6400</td>
<td>3900</td>
<td>2</td>
</tr>
<tr>
<td>S</td>
<td>4400</td>
<td>4500</td>
<td>1</td>
</tr>
</tbody>
</table>

The most appropriate ligands to be synthesised were chosen to be 50 and 52. In making this choice synthetic viability, as well as the aim of testing the validity of our pharmacophores, was taken into account. Target ligands for the bicyclic series was therefore to include 6-arylhydroxytropane derivatives 50 and 53, with incorporation of
both phenyl and catechol substituents, and their intermediates 54-60, as well as the 3-phenylhydroxytropane ligand 52, containing an extra carbon spacer between C and D (Figure 4.3), as shown in Figure 4.4.

Examination of the literature revealed that compounds similar to 50 had been investigated previously; 6β-aryltropan-6-ols based on 61 were explored some twenty years ago as low potency morphine-like analgesics\textsuperscript{215} and later as low potency opiate
agonists/antagonists\textsuperscript{188} 62. Further 6-phenyl-8-azabicyclo[3.2.1]octanes and octenes based on 61 were developed, displaying potential antidepressant, analgesic and unspecified cardiovascular activity\textsuperscript{216}. Whilst there was no evidence for 2-aryltropan-2-ol derivatives such as 63 having previously been developed, 3-phenyltropanes such as 64 were investigated as antispasmodic agents some time ago\textsuperscript{217}.

4.2.1 Retrosynthesis and proposed syntheses

The initial retrosynthetic analysis for the tropane derivative 52 focussed on a crucial C-C disconnection to give a suitable protected ketone as represented in Scheme 4.1. Thus synthesis of 52 was to be achieved \textit{via} a relatively short synthetic pathway from commercially available tropinone. N-Demethylation of tropinone and subsequent protection of the nitrogen were necessary, as not only may the N-methyl provide steric hindrance to direct Grignard reagent attack on tropinone, but additionally the nitrogen protecting group must be removable under mild conditions. A suitable protecting group for the basic nitrogen was therefore 2,2,2-trichloroethyl carbamate, as its introduction would N-demethylate and protect the nitrogen in the one step, and the conditions of removal (zinc/acetic acid) would be sufficiently mild to leave the aryl hydroxyl moiety intact. Alternatively, the ethyl carbamate protecting group could also provide N-demethylation, however the aryl hydroxyl moiety would not withstand the removal conditions (trimethylsilyl iodide)\textsuperscript{218}.
Scheme 4.1 Retrosynthetic analysis and proposed synthesis of 3-phenylnortropan-3-ol (52)

Initial analysis of the retrosynthesis of the target 6-aryl substituted tropane diol derivative 50, indicated that it could be synthesised via commercially available 6β-hydroxytropan-3-one, which could be synthesised via the Mannich type condensation reaction of 2,5-dimethoxy-2,5-dihydrofuran with acetone dicarboxylic acid and methylamine hydrochloride (Scheme 4.2). Alternatively, 6β-hydroxytropan-3-one could also be accessed from the natural product 6β-hydroxyhyoscyamine. All three of these sources will be discussed (section 4.4.1).

Scheme 4.2 Retrosynthetic analysis of 6-arylhydroxytropan-3-ol (50).
4.3 Synthesis of 3α-Phenyltropan-3-ol

As outlined in the retrosynthetic scheme (Scheme 4.1), N-demethylation was a crucial step in the synthesis of the 3-aryltropan-3-ol derivative 52. N-demethylation may be effected by a number of reagents, and it would be most advantageous to not only N-demethylate before any Grignard addition, but also to carry out the demethylation and the protection of the nitrogen in the same step. For many years the synthetic route to nortropanes lay primarily in N-demethylation of tropanes by KMnO₄, K₃Fe(CN)₆ or cyanogen bromide. The use of chloroformate esters, such as ethyl chloroformate or phenyl chloroformate, has often proved to be superior to earlier methods of tertiary amine dealkylation because of their increased selectivity and cleaner reactions, and are thus the method of choice. Whilst poor yields were encountered with the use of benzyl chloroformate in the N-demethylation of tropan-3-one, the use of ethyl chloroformate also posed a problem because the hydrolytic step failed to furnish any of the desired nortropinone. 2,2,2-Trichloroethyl chloroformate was reported to be the reagent of choice for tropinone N-demethylation, producing the trichloroethyl carbamate from tropinone in a high yield (95%). The carbamate protecting group may then be easily cleaved by zinc in methanol or acetic acid to give the nortropane in a moderate (62%) yield. This carbamate was thus ideal for both the desired N-demethylation of tropan-3-one and successive protection of the nitrogen moiety during subsequent chemistry, particularly as the deprotection conditions are sufficiently mild that they would not be expected to dehydrate the arylhydroxyl moiety of target ligand 52.

Accordingly, following the method of Montzka as shown in Scheme 4.3, tropinone was heated under reflux in a suspension of 2,2,2-trichloroethylchloroformate and
Chapter Four Synthesis of Bicyclic Tropane Derivatives

anhydrous potassium carbonate in dry benzene under argon for 68 hours. The product 65 was isolated as crystalline material in 83% yield. $^1$H NMR analysis revealed the loss of the N-methyl singlet peak at $\delta 2.63$, and the presence of the methylene peak of the trichloroethyl carbamate was seen at $\delta 4.67$ as a doublet. Mass spectral analysis (Cl$^+$) indicated the molecular formula of 65 to be $C_{10}H_{12}NO_3Cl_3$, with distinct chlorine isotope ions at m/z 300/302/304.

![Diagram of reaction](image)

**Scheme 4.3 Synthesis of 3α-phenyltropan-3-ol 52**

Having removed the N-methyl substituent, it was then possible to arylate the tropane ring of 65 via Grignard addition to the carbonyl. Although we wished to investigate both phenyl and catechol ring derivatives, it was initially decided to prepare the 3-phenyl-tropan-3-ol derivative 52 as a model study for both synthetic procedures and as a potential ligand for the $\alpha_{1B}$-AR receptors. As noted in Scheme 4.3, 65 was treated with phenylmagnesium bromide to furnish the arylated 66 in 82% yield. $^1$H NMR spectroscopy revealed the expected aromatic 5H multiplet of the newly formed derivative at $\delta 7.20-7.53$. $^{13}$C NMR spectroscopy supported this with the disappearance of the ketone cabonyl peak at $\delta 207.2$, and the additional aromatic peaks at $\delta 124.6-128.6$. The reaction proved to be selective for the 3α-phenyltropan-3-ol derivative, as
shown in Scheme 4.3, and as determined by NOESY 2D NMR (spectrum given in Appendix). This is not surprising considering the presence of the bulky carbamate group would compete sterically with the phenylmagnesium bromide reagents. High resolution mass spectral analysis (CI⁺) confirmed the molecular formula of 66 as C₁₆H₁₈NO₃Cl₃.

With removal of the carbamate functionality from the nitrogen, the target ligand 67 would be achieved. A common reagent for the effective removal of trichloroethyl carbamates has been 48% hydrogen bromide/acetic acid. However, as mentioned a milder reagent such as zinc in acetic acid is more suitable for derivative 66 so as to avoid dehydration of the tertiary hydroxyl group. Zinc in 90% acetic acid is reported to effect smooth deprotection of trichloroethyl carbamates (90% yield). An improved method for cleavage of the trichloroethoxycarbonyl group uses cadmium dust in acetic acid:DMF (1:1 mixture) instead of zinc dust in acetic acid. Further, these authors also reported the trichloroethoxycarbonyl group to be unstable to hydrogenation catalysed by Pd-C. With the consideration that a number of compounds may be unstable in, or be difficult to isolate from, acetic acid, formic acid or DMF, an alternate method developed by Just & Grozinger involves cleavage of 2,2,2-trichloroethylcarbamates to amines with zinc in a THF/1M KH₂PO₄/Na₂HPO₄ buffer in 30 mins at pH 4.2, or for 18h at pH 5.5. Investigation into the application of this method for the preparation of the derivative 52 found that use of Zn/THF/ KH₂PO₄ at pH 4.2 with stirring at RT for 5 hours, failed to produce any of the desired nortropane derivative.

Therefore, the cleavage of the 2,2,2-trichloroethylcarbamate in 66 was achieved via the method of Montzka et al. As shown in Scheme 4.3, 66 was treated with zinc dust in 90% acetic acid to afford 52 in 60% yield. NMR analysis clearly indicated the loss of the carbamate moiety peaks at δ4.64-4.72 (CH₂) in the ¹H NMR spectrum, and at
\( \delta 151.7 \) (NC=O), \( \delta 95.2 \) (CCl\(_3\)) and \( \delta 74.8 \) (CH\(_2\)) in the \(^{13}\)C NMR spectrum. The product 52 was further supported by mass spectrometry (Cl\(^+\)) via an MH\(^+\) ion at \( m/z \) 204, consistent with the formula C\(_{13}\)H\(_{17}\)NO. Compound 52 rapidly dehydrated under heating during the mass spectral analysis process, and high resolution mass spectral analysis (EI\(^+\)) of the dehydration product (C\(_{13}\)H\(_{16}\)N\(^+\)) confirmed the molecular formula of 52.

### 4.4 Preparation of 6\( \beta \)-Arylhydroxynortropane Derivatives

#### 4.4.1 Synthesis of 6\( \beta \)-hydroxytropan-3-one

Initial investigations into the synthesis of 6\( \beta \)-hydroxytropan-3-one (67) followed literature procedures\(^{196,227}\), and details are shown in Scheme 4.4. 2,5-dimethoxy-2,5-dihydrofuran was treated with 3N hydrochloric acid then 6N sodium hydroxide, followed by a mixture of acetone dicarboxylic acid, methylamine hydrochloride and anhydrous sodium acetate at pH 4.0 – 5.0. Intense extraction of the reaction mixture with diethyl ether resulted in the isolation of crude 6\( \beta \)-hydroxytropan-3-one (67) as a brown oil. Recrystallisation from 2-propanol was unsuccessful as the resulting solid still contained some impurities. Chromatography partially isolated some product, however clean separation of 6\( \beta \)-hydroxytropan-3-one from other impurities proved difficult. The resulting yield was very low (1.2%), with 6\( \beta \)-hydroxytropan-3-one being obtained as cream crystals. The \(^1\)H NMR data of 67 agreed with literature data\(^{227}\), and mass spectral analysis (Cl\(^+\)) revealed the molecular ion at \( m/z \) 156 (MH\(^+\), C\(_8\)H\(_{13}\)NO\(_2\)).
Chapter Four Synthesis of Bicyclic Tropane Derivatives

This synthesis posed 2 major problems regarding efficiency and success. The first was the high water solubility of $6\beta$-hydroxytropan-3-one, which rendered difficult the extraction of the crude product from the reaction mixture, even with strict control of the pH at 9. Consequently the crude yield obtained never exceeded 11%. Zhao et al.\textsuperscript{228} also experienced this solubility problem, but still managed purified yields of 42%; others reported yields of 20\%\textsuperscript{227} and 49\%\textsuperscript{189}. Impurity of the crude extract posed another problem, as it made recrystallisation difficult. Chromatography also proved to be inefficient with the presence of a number of components of similar polarity in the crude mixture. The extremely low isolated yields of $6\beta$-hydroxytropan-3-one from this synthesis, which was repeated four times, compromised the feasibility of the route, so an alternate source of $6\beta$-hydroxytropan-3-one was needed.

It may have been possible to synthesise $6\beta$-hydroxy-tropan-3-one via a multi-step synthesis from the natural alkaloid $6\beta$-hydroxyhyoscyamine (68), a derivative of scopolamine, as shown in Scheme 4.5. However, this method was not utilised because of the difficulty in obtaining 68.
4.4.2 Synthesis of 6-arylhydroxytropane derivatives from 6β-hydroxytropan-3-one

Due to the difficulty experienced in the synthesis of 6β-hydroxytropan-3-one, it was decided to source the derivative commercially (despite its high cost).

As previously noted in Scheme 4.2, retrosynthetic analysis of the target 6-aryl-6-hydroxytropan-3-ol derivatives indicated that N-demethylation and subsequent N-protection was required. This analysis is shown in more detail in Scheme 4.6. The 3-ketone would give rise to the 3-hydroxyl derivatives, however, this carbonyl group would need protection before the oxidation of the 6-hydroxyl substituent to a carbonyl, and the subsequent Grignard attack to incorporate the aryl moiety.
The forward synthesis planned is outlined in Scheme 4.6. The 6β-hydroxy functionality was first protected with a robust tert-butyldimethylsilyl functionality, followed by the \( N \)-demethylation and re-protection of the 6β-hydroxytropan-3-one amine functionality as a 2,2,2-trichlorethylchloroformate. Protection of the carbonyl group as a ketal was to be followed by deprotection of the silyl group to afford the free 6-hydroxy. Oxidation of this secondary alcohol would provide the ketone anchor needed for the Grignard attack and insertion of the arylhydroxy moiety. Deprotection of the 3-carbonyl and reduction to an alcohol, followed by the removal of the carbamate, would then provide the desired target ligands.

Scheme 4.6 Retrosynthetic analysis of 6-arylhydroxytropan-3-ol derivatives.
4.4.2.1 Protection of 6β-hydroxytropin-3-one

Due to the secondary nature of the hydroxyl group of 67, and the close proximity of the N-methyl substituent, the 6β-hydroxyl may be considered relatively hindered. The tert-butyldimethylsilyl functionality is a common protecting group for hydroxyl substituents, largely due to its stability to many reaction conditions\textsuperscript{218}, and the ease of removal using tetrabutylammonium fluoride (TBAF). The hindered 6β-hydroxyl group may be protected with a tert-butyldimethylsilyl functionality, utilising the highly reactive tert-butyldimethylsilyl trifluoromethansulfonate (TBDMSOTf) and 2,6-lutidine in dichloromethane, as shown in Scheme 4.7. The reaction afforded 69 as a pale yellow oil in 86% yield. The newly silylated product was identified via \textsuperscript{1}H NMR spectroscopy, with the tert-butyl group of the tert-butyldimethylsilyl functionality appearing as a typical singlet at $\delta$0.87 and the dimethyl silyl methyl protons as a singlet at $\delta$0.04. High resolution mass spectral analysis (CI\textsuperscript{+}) confirmed the molecular formula of 69 as C\textsubscript{14}H\textsubscript{27}N\textsubscript{O\textsubscript{2}}Si.

\begin{align*}
6\beta\text{-hydroxytropin-3-one} \rightarrow a \rightarrow 69 \rightarrow b \rightarrow 72
\end{align*}

Reagents & Conditions: (a) 2,6-Lutidine, tert-butyldimethylsilyl trifluoromethanesulfonate, DCM, O\textdegree C-RT, N\textsubscript{2}, 3hrs, 86% yield; (b) 2,2,2-trichloroethyl chloroformate, K\textsubscript{2}CO\textsubscript{3} (anhydrous), benzene (anhydrous), reflux, N\textsubscript{2}, 4hrs, 85% yield.

\textbf{Scheme 4.7} Synthesis of protected 6β-hydroxytropinone derivative 72.
On one occasion, bis-silylation occurred when excess tert-butyldimethylsilyl-trifluoromethansulfonate was used (2.0 equivalents, instead of 1.5 equivalents) and resulted in formation of the bis-silylated enol ether 70 and its isomer 71 (Scheme 4.8) in 62% yield. With the excessive presence of the active base lutidine, and the excess of very reactive silylating agent, it is not surprising that the ketone enol ether functionality was silylated. The bis-silylated products were evidenced by both $^1$H and $^{13}$C NMR spectroscopy: the $^1$H NMR spectrum clearly displayed the additional 3 methyl peaks at $\delta$0.84 (9H), 2 additional dimethylsilyl peaks at $\delta$0.09 (6H), and the two vinylic protons at $\delta$4.73 (d, $J$ = 5.7 Hz) (H2 in 70) and $\delta$4.83 (d, $J$ = 5.7 Hz) (H4 in 71) in a ratio of 2:1 respectively. The H1 proton is seen in the $^1$H NMR spectrum as two peaks, at $\delta$3.05 (d, $J$ = 5.4) and $\delta$3.09 (d, $J$ = 5.4), in a ratio of 1:2 respectively. The downfield peak is representative of the H1 proton in isomer 70 (C2=C3), indicating 70 as the major isomer, and identifying the =CH peak at $\delta$4.73 as belonging to H2. The $^{13}$C NMR spectrum evidenced the shift of the C3 peak from $\delta$208.4 (C-O) to $\delta$146.3/148.4, and clearly indicated the presence of the silyl enol ether at $\delta$4.5 ((CH$_3$)$_2$), $\delta$18.0 (C(CH$_3$)$_3$), $\delta$26.1 ((CH$_3$)$_3$) and $\delta$102.5/$\delta$107.0 (=CH). Mass spectral analysis (Cl$^+$) of 70 and its isomer indicated an M+1 peak at $m/z$ 364. High resolution mass spectral analysis (Cl$^+$) confirmed the molecular formula of 70 and its isomer as C$_{22}$H$_{41}$NO$_2$Si$_2$. 


Chapter Four Synthesis of Bicyclic Tropane Derivatives

With the hydroxyl group protected, N-demethylation and carbamate formation was carried out in the same way as previously, according to Scheme 4.7. The carbamate 72 was afforded as a white solid in 85% yield. NMR spectroscopy revealed the loss of the N-methyl singlet at δ2.63 in the $^1H$ NMR spectrum and δ38.2 in the $^{13}C$ NMR spectrum. The methylene protons of the newly formed carbamate derivative 72 appeared as a multiplet at δ4.67-4.77 in the $^1H$ NMR, and in the $^{13}C$ NMR this methylene carbon appeared at δ74.7, whilst the carbonyl of the carbamate was seen at δ152, and the trichlorinated quaternary carbon at δ95.8. Mass spectral (Cl+) analysis of 72 indicated the presence of 3 chlorine atoms in the molecular ion of C$_{16}$H$_{26}$NO$_{4}$SiCl$_{3}$ at m/z 430/432/434, with relative abundances expected for the chlorine isotopes.
In an attempt to reduce the reaction time, the $N$-demethylation/carbamate formation reaction was investigated under microwave reaction conditions. A mixture of 69, potassium carbonate and 2,2,2-trichloroethylchloroformate in acetonitrile under argon in a sealed atmosphere was exposed to microwave radiation at 100°C for 20 mins. The desired product 72 was furnished, and although the yield was lower, 60%, this method could produce 72 in minutes rather than days.

4.4.2.2 Protection of the 3-ketone

The next synthetic step was to first protect the 3-ketone group before removing the silyl protecting group ready for hydroxyl oxidation (Scheme 4.10). In protecting the carbonyl, a ketal functionality may be appropriate, or alternatively the use of reductive etherification would provide direct access later to the desired 3-hydroxy derivative. Recent work reported by Miura et al.\textsuperscript{229} indicated that alkoxydimethylsilanes (ROSiHMe\textsubscript{2}) can be used for the Lewis acid-catalysed reductive etherification of various aldehydes and ketones. Of the four alkoxydimethylsilanes investigated (\(\text{ROSiHMe}_2\): \(R = \text{Bu; Ph(CH}_2)_2; \text{cyclohexane; benzyl}\)), the benzyloxydimethylsilane would be most appropriate in this circumstance, as it may be cleaved under mild conditions using palladium and hydrogen. However, the yields of ethers reported as being formed using benzyloxydimethylsilane were disappointingly low (35-41%).

We thus returned to ketal groups for the protection of the 3-ketone. As important carbonyl protecting groups in organic synthesis, many methods have been introduced for both their formation and removal\textsuperscript{218}. The choice of which ketal group to use will largely depend on available mild deprotection methods, which would retain the tertiary hydroxyl group in the target molecules in this study. Thus aqueous acidic hydrolysis of
the ketal was not considered suitable. Although the use of reagents such as molybdenum (IV) oxide acetylacetonate (MoO$_2$(acac)$_2$)$_{230}$, montmorillonite K10$^{231}$, FeCl$_3$.6H$_2$O$^{232}$, CuCl$_2$.2H$_2$$_{233}$ and cerium ammonium nitrate$^{234}$ have been reported, methods using neutral conditions were required for the deprotection of the potentially acid-sensitive substrates.

Cyclic 1,3-dioxolanes have been used previously for the protection of 3-tropanone derivatives$^{235}$. Whilst they may be integrated into the tropane skeleton via a number of methods, a mild method for removal is needed once the aryl group is incorporated, without causing the dehydration of the accompanying alcohol. Ko and Park$^{236}$ report the use of Magtrieve$^\text{TM}$ for the deprotection of ketals under neutral conditions; whilst the cyclic 1,3-dioxolane protecting group was inert to the reagent, acyclic dimethyl ketalts were cleaved in good yields (>90%). Bartoli et al.$^{165}$, report cerium (III) chloride to function as an efficient Lewis acid for the cleavage of 1,3-dioxolanes to carbonyl compounds under neutral conditions, and furthermore, bismuth nitrate pentahydrate$^{166}$ was shown to be an efficient reagent under neutral conditions for the selective deprotection of acyclic ketals such as dimethyl ketals, although not with cyclic 1,3-dioxolanes. Therefore both 1,3-dioxolane and dimethyl ketal protecting groups were considered potentially suitable for use in the protection of the ketone of 72, due to their reported ease of removal under neutral conditions. It was decided to investigate firstly the 1,3-dioxolane functionality.

There are a number of methods available for the 1,3-dioxolane protection of a carbonyl functionality, including ethanediol and $p$-toluenesulfonic acid$^{235}$, ethanediol with BF$_3$.Et$_2$O in acetic acid$^{237}$, and ethanediol and trichloromethylsilane$^{238}$ to name a few. It was important however to employ a method which would not attack the TBDMS and carbamate protecting groups.
Initially, the method of Fieser & Stevenson$^{237}$, using 1,2-ethanediol and boron trifluoride etherate in acetic acid was employed to form the 1,3-dioxolane derivative (Scheme 4.9). However, the presence of the BF$_3$.Et$_2$O also led to the cleavage of the silyl protecting group to afford 73 in 42% yield. This was evidenced in the $^1$H NMR spectrum by the loss of the typical tert-butyldimethylsilyl peaks at $\delta$0.04 ((CH$_3$)$_2$Si) and $\delta$0.87 ((CH$_3$)$_3$). The 1,3-dioxolane ketal was represented in the $^1$H NMR spectrum by a one proton triplet at $\delta$3.83 (OCH), a one proton multiplet at $\delta$3.91-3.99 (OCH) and a two proton multiplet at $\delta$4.09-4.21 (OCH$_2$). The identity of 73 was further confirmed as C$_{12}$H$_{16}$NO$_5$Cl$_3$ via mass spectral analysis (CI$^+$), with the molecular ion seen at m/z 360/362/364. Although this synthetic method shortened the procedure by one step, the low yield did not render it useful.

![Scheme 4.9 1,3-dioxolane protection of ketone 72](image)

An alternate, non-acidic synthetic procedure for the protection of ketones as 1,3-dioxolanes uses ethanediol and chlorotrimethylsilane$^{238}$ and will not attack the tert-butyldimethylsilyl functionality. As shown in Scheme 4.10, 72 was treated with ethanediol and chlorotrimethylsilane with heating under reflux to furnish the desired product 74 in 86% yield. $^1$H NMR spectroscopy revealed the presence of the newly formed cyclic ketal via a methylene multiplet at $\delta$4.09-4.21 and a one proton triplet and
a one proton multiplet at δ3.83 and δ3.91-3.99 respectively. The presence of the tert-butylidemethylsilyl group was confirmed via the typical singlets in the $^1$H NMR at δ0.06 and δ0.86. In the $^{13}$C NMR, the cyclic ketal methylene peaks appeared at δ63.5 and δ64.5, whilst the C3 carbon had shifted from the typical ketone chemical shift at δ206 upfield to δ106.7. The molecular formula of 74 was confirmed as C$_{18}$H$_{30}$NO$_5$Cl$_3$ via mass spectral analysis (CI$^+$), revealing the molecular ion at $m/z$ 474/476/478.

![Scheme 4.10 Preparation of 6β-hydroxy ketal derivative 73 via 74.](image)

Reagents and Conditions: (a) ethanediol, tert-butyldimethylsilyl chloride, DCM, reflux, 48hrs, 86% yield; (b) TBAF, THF, 69% yield.

The next synthetic step was to remove the tert-butyldimethylsilyl protecting group. The most common method for removal of this protecting group is via reaction with tetrabutylammonium fluoride. Therefore, as shown in Scheme 4.10, 74 was treated with tetrabutylammonium fluoride to yield the desired product 73 in 69% yield.

In order to reduce the reaction time when synthesising 74, it was decided to attempt the reaction under microwave conditions. Accordingly, 72, ethanediol and tert-butyldimethylsilyl chloride in dry acetonitrile under argon in a sealed reaction vessel, was exposed to microwave radiation at 80°C for 20 mins. Surprisingly, under the novel reaction profile achievable with microwave radiation, not only did the reaction product contain the desired 1,3-dioxolane ring, but cleavage of the tert-butyldimethylsilyl group...
had also occurred, resulting in derivative 73. Whilst cleavage of the tert-butyldimethylsilyl group suggested there may have been some hydrogen chloride formed in situ, this reaction might also be due to superheating effects of the microwave radiation\(^{239,240}\). That this cleavage had in fact occurred was established by \(^1\)H NMR spectroscopy on the product, which clearly revealed the loss of the typical tert-butyldimethylsilyl methyl singlets at \(\delta 0.06\) and \(\delta 0.86\), and the gain of the two methylene doublet of doublets of the cyclic ketal at \(\delta 3.82\) and \(\delta 3.92\). Mass spectral analysis (CI\(^+\)) confirmed the molecular ion of 73 at \(m/z\) 360/362/364, consistent with the molecular formula \(\text{C}_{12}\text{H}_{16}\text{N}_{05}\text{Cl}_{3}\).

With an efficient route to cyclic ketal derivative 73 under microwave conditions in place, it remained to oxidise the secondary 6-hydroxyl to a ketone, to allow for incorporation of the aryl substituents at the 6 position of the tropane ring.

### 4.4.2.3 Oxidation of protected 6-hydroxytropane derivative 73

The literature contains a plethora of methods for the oxidation of secondary alcohols, from standard oxidising agents such as chromium (VI) reagents\(^{241}\), manganese dioxide\(^{242}\) and Raney Nickel\(^{243}\), to sodium hypochlorite with acetic acid\(^{244}\) or ruthenium tetroxide\(^{245}\), or catalytic systems including for example \(\text{RuCl}_2(\text{PPh}_3)_3\text{BuOOH}\)^\(^{246,247}\), pyridinium dichromate-\(\text{Me}_3\text{SiOSiMe}_3\)\(^{248}\) and \(\text{R}_4\text{N}^+\text{Br}_4\text{MoO}_5^-\text{BuOOH}\)^\(^{249,250}\). The oxidation of the secondary 6-hydroxyl in tropane systems has previously been undertaken via a number of methods, including chromic acid\(^{188}\), Pfitzner-Moffat oxidation, using DCC, phosphoric acid & DMSO\(^{184,251,252}\), and the popular Swern oxidation\(^{196}\), utilising oxalyl chloride in DMSO. Initially the method of Pfitzner-
Moffat as used by Montzka was chosen to explore oxidation of the 6-hydroxy group in 73 to the 6-ketone derivative.

The treatment of 73 with H₃PO₄ in DMSO resulted in formation of the desired product 75 as a white solid in 78% yield. ¹H NMR spectroscopy confirmed the loss of the 6-hydroxy multiplet peak previously at δ4.88-5.02, and the C-6 carbon in the ¹³C NMR shifted from δ73.5 downfield to δ202.9, a position typical of a ketone carbonyl. Mass spectral analysis (Cl⁺) confirmed the formula C₁₂H₁₅NO₅Cl₃ via an MH⁺ ion at m/z 358/360/362.

\[
\begin{align*}
\text{CO}_2\text{CH}_2\text{CCl}_3
\end{align*}
\]

Reagents and Conditions: H₃PO₄, DMSO, RT, 3hrs, 78% yield.

**Scheme 4.11 Oxidation of 6β-hydroxy tropane 73 to 6-tropinone derivative 75.**

**4.4.2.4 Arylation of 75 to provide 6-arylhydroxytropane derivatives**

With the derivative 75 in hand, 6-arylhydroxytropane derivatives were then accessed via Grignard addition to the 6-ketone functionality. As depicted in Scheme 4.12, attack at the C6 carbonyl with either phenylmagnesium bromide or 3,4-methylenedioxyphenylmagnesium bromide gave rise to 6-arylhydroxy derivatives 76 and 77. Reaction conditions were initially investigated using phenylmagnesium bromide, affording the desired product 76 in 77% yield. NMR spectroscopy evidenced the formation of 76, with the ¹H NMR spectrum clearly displaying the phenyl protons at...
87.20-7.52, and the tertiary hydroxyl proton was assigned to the broad singlet at δ4.29. The $^{13}$C NMR spectrum showed a loss of the C6 carbonyl peak at δ202.9 and the additional phenyl carbons at δ123.9-128.1. High resolution mass spectral analysis (CI$^+$) confirmed the molecular formula C$_{18}$H$_{20}$NO$_5$Cl$_3$.

Molecular modelling conformational data was obtained for 75 using global mechanics in the Spartan program. The lowest energy conformers of 75 were found to be that shown in Figure 4.3c, with the cyclic ketal ring positioned in part below the bicyclic tropane ring. This conformation blocks the concave face of the tropane ring, suggesting that the large phenyl Grignard reagent would attack solely from the top face of the tropane skeleton, leading to formation of only the one diastereomer 76. This expectation was proven to be the case, as NOESY 1D $^1$H NMR experiments revealed the OH proton to have a NOE interaction with the H5, H4 and H2 protons (Figure 4.3a), whereas the aromatic ortho protons displayed NOE interactions with only the H5 and H7 protons (Figure 4.3b). This clearly indicated that the phenyl ring functionality is exo to the tropane skeleton and the hydroxyl group is endo.
Figure 4.3a NOESY 1D NMR spectrum of 76: the irradiated OH proton displays a NOE interaction with H5, H4 and H2, indicating the exo stereochemistry of the aryl functionality.

Figure 4.3b NOESY 1D NMR spectrum of 76: the irradiated aromatic ortho protons display a NOE interaction with H5 and H7, indicating exo stereochemistry of the aryl functionality.
Synthesis of 77, with the protected catechol moiety, followed the same synthetic route (Scheme 4.12): treatment of 75 with 3,4-methylenedioxyphenylmagnesium bromide in THF yielded 77 in 75% yield. High resolution mass spectral (Cl+) analysis indicated the molecular formula to be C_{19}H_{20}NO_{7}Cl_{3}. ^1H NMR analysis of 77 confirmed the additional aromatic hydrogens at 6.7-7.05, and the presence of the methylenedioxy function through a downfield singlet at 85.92.

With derivatives 76 and 77 synthesised, access to six target derivatives for testing was feasible (Figure 4.4). Direct hydrolysis of the carbamate protecting group from derivatives 76 and 77 would yield the ligands 58 and 60. Removal of the cyclic ketal from 76 and 77 would provide ligands 78 and 79, which may be either hydrolysed to afford ligands 55 and 57, or reduced to afford the β-hydroxylic derivatives 80 and 81, which in turn could be converted to target derivatives 50 and 54.
4.4.2.6 Removal of ketal protecting group

With the target ligands now in sight, the next step was the removal of the 1,3-dioxolane protecting group. As previously discussed neutral reaction conditions were required for this cleavage step, and, in this context, cerium trichloride heptahydrate and sodium iodide was considered, based on the report of Bartoli et al.\textsuperscript{165}. Lanthanide salt-mediated
Lewis acid reactions have gained increased interest over recent years\textsuperscript{253} due to their low toxicity and ease of handling, making them attractive alternatives to classical reagents such as titanium tetrachloride. Bartoli and co-workers studied the use of cerium trichloride heptahydrate in organic reactions and have developed new methods for the deprotection of a number of functional groups, including trialkylsilyl ethers\textsuperscript{254}, \textit{p}-methoxybenzyl ethers\textsuperscript{255} and 1,3-dioxolanes\textsuperscript{165}.

For 1,3-dioxolane derivatives, it has been shown\textsuperscript{165} that cerium trichloride heptahydrate and a catalytic amount of sodium iodide transforms 1,3-dioxolanes to their parent ketones in high yields under refluxing conditions (Scheme 4.13). Mechanistically, it is proposed\textsuperscript{165} that while the Lewis acid-like species cerium trichloride polarises the dioxolane oxygen, the presence of water effects the deprotection (Scheme 4.13). The deprotection did not proceed with anhydrous cerium trichloride, however the catalytic role of the sodium iodide is not clear. It was expected that upon treatment of 76 with cerium trichloride heptahydrate and sodium iodide in acetonitrile, as shown in Scheme 4.14, the ketone 78 would be furnished. However, after 20 hours at room temperature in the presence of 1.5 equivalents of cerium trichloride heptahydrate and 0.15 equivalents of sodium iodide, followed by 22 hours of reflux, no cleavage had occurred, with the \textsuperscript{1}H NMR spectrum clearly displaying a quantitative amount of the two cyclic methylene proton signals at 83.97 (dd) & 84.02-4.18 (m).

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\textbf{Scheme 4.13} Mechanism of 1,3-dioxolane removal by CeCl$_3$.7H$_2$O};
\end{tikzpicture}
\end{center}
To take account of the possible chelation sites in 76, the amount of cerium trichloride heptahydrate (7.5 molar equivalents) and sodium iodide (0.75 molar equivalents) was increased. Additionally, although Bartoli et al.\textsuperscript{65} used refluxing acetonitrile conditions to obtain quantitative 1,3-dioxolane cleavage, this temperature may not provide enough energy for the reaction of the more sterically demanding bicyclic derivatives. In order to achieve a higher reaction profile, it was desired to investigate the cleavage of the 1,3-dioxolane derivatives 76 and 77 under microwave conditions. As has been previously discussed, microwave radiation can provide novel temperature and reaction profiles for given reaction mixtures, often, in fact, by superheating effects\textsuperscript{239,240}. It was hoped that such superheating effects might aid in the cerium trichloride-mediated cleavage of the 1,3-dioxolane ring. Accordingly, as shown in Scheme 4.15, derivatives 75 and 76 were separately treated with cerium trichloride heptahydrate (7.5 equivalents) and sodium iodide (0.75 equivalents) in acetonitrile under microwave radiation at a range of temperatures (100°C-120°C) for time periods ranging from 20 to 100 minutes.

Scheme 4.14 Deprotection of the 1,3-dioxolane derivative 76
Chapter Four Synthesis of Bicyclic Tropane Derivatives

![Synthesis Scheme 4.15](image)

Reagents and Conditions: CeCl₃·7H₂O (7.5 eq.), NaI (0.75 eq.), microwave radiation, 100-120°C, 20-100 mins.

Scheme 4.15 Microwave reaction attempts to remove the 1,3-dioxolane substituent

In the first microwave reaction attempt, 76 was exposed to microwave radiation initially for 20 minutes at 100°C. Mass spectral analysis (Cl⁺) of the reaction mixture indicated a small peak at m/z 392/394/306 for the ketone 76 (C₁₆H₁₆NO₄Cl₃), with a major peak for 78 (m/z 436/438/440, C₁₈H₂₀NO₅Cl₃). Microwave radiation continued for 60 minutes at 100°C, followed by another 60 minutes at 120°C. Mass spectral analysis (Cl⁺) of the reaction mixture indicated only 76 (m/z 392/394/306) to be present, and ¹H NMR analysis of the purified product revealed it to be 76 only, with the two dioxolane methylene peaks indicated at δ 3.97 (dd) & δ 4.02-4.18 (m). Repeating the reaction, at 120°C for 60 mins failed to furnish any of the desired ketone 78, with ketal peaks clearly remaining in the ¹³C NMR, at δ 65.4 and δ 65.7, and no C=O at δ 200.

In microwave reactions it is optimal to have a homogeneous rather than a heterogeneous mixture to provide a uniform heating pattern. Due to the dielectric heating method of microwave chemistry, polar solvents and/or ions are required for microwave heating. The use of highly polar solvents and the addition of ionic salts to the reaction mixture allows the attainment of increased heating rates and reaction profiles. In analysing this
reaction system then, it may be seen that whilst the ionic salts cerium trichloride heptahydrate and sodium iodide are both present, they are not soluble in acetonitrile, which is not a highly polar solvent in comparison to aqueous solvents. Therefore, to increase the reaction profile of the system it would theoretically be advantageous to use a more polar solvent, one which would solubilize both the substrate and the reagents. An acetonitrile:methanol:water (6:3:1) system was chosen, and the resulting reaction mixture, using 75 as the substrate, was exposed to microwave radiation at 100°C for 20 minutes (Scheme 4.15). Mass spectral (Cl+) analysis revealed some of the desired product 82 to have formed, via an M+1 peak at m/z 314/316/318 (C_{10}H_{10}NO_{4}Cl_3), though a significant amount of 75 still remained (m/z 358/360/362). Microwave irradiation was continued for a further 30 minutes at 100°C, and then for 50 minutes at 110°C, however, the reaction did not go to completion (as monitored by mass spectral analysis) and only starting material could be recovered from the reaction mixture.

An alternate method for 1,3-dioxolane deprotection was thus needed, however with no other suitably mild and neutral method apparent, it was decided to substitute the cyclic dioxolane ketal protecting group with the acyclic dimethyl ketal, which is reported to be easily cleaved by bismuth nitrate under neutral conditions. An alternate method for 1,3-dioxolane deprotection was thus needed, however with no other suitably mild and neutral method apparent, it was decided to substitute the cyclic dioxolane ketal protecting group with the acyclic dimethyl ketal, which is reported to be easily cleaved by bismuth nitrate under neutral conditions. To investigate the ease with which the dimethyl ketal may be introduced to and removed from the 3-carbonyl group in the tropane skeleton, the carbamate protected tropinone derivative 65 was firstly investigated as a model compound (Scheme 4.16). Following the method used by Paquette et al., dimethyl ketal formation was achieved by means of trimethylorthoformate and p-toluenesulfonic acid, providing 83 as a colourless oil in a good yield. \(^1\)H NMR spectroscopy of 83 revealed the two methyl ketal peaks at δ3.12 and δ3.19 and \(^{13}\)C NMR spectroscopy displayed the loss of the ketone peak at δ207.2, and the new methoxy groups were assigned to peaks at δ47.3 and δ48.4. Mass spectral
(Cl⁺) analysis of the newly formed derivative 83 evidenced an isotopic cluster for the MH⁺ ion at m/z 347/349/351, consistent with the molecular formula C₁₂H₁₈NO₄Cl₃.

\[
\begin{align*}
\text{CO}_2\text{CH}_2\text{CCl}_3 & \quad \rightarrow \quad \text{CO}_2\text{CH}_2\text{CCl}_3 \\
65 & \quad \rightarrow \quad 83 & \quad \text{OCH}_3 & \quad \rightarrow \quad 65 \\
\end{align*}
\]

Reagents and Conditions: (a) Trimethylorthoformate, p-TsOH (cat.), MeOH, RT, O/N, 80% yield; (b) Bi(NO₃)₃·5H₂O, DCM, RT, O/N, 100% yield.

**Scheme 4.16** Dimethyl ketal protection (and deprotection) of ketone 65

With no problems obtaining the dimethyl ketal derivative 83, investigation of the cleavage of the protecting ketal was then explored. The method of Eash,\(^{166}\) reported to cleave dimethyl ketals with ease under neutral conditions employing bismuth nitrate pentahydrate, furnished ketone 65 in quantitative yield (Scheme 4.16). \(^1\)H NMR and \(^13\)C NMR spectroscopy indicated a loss of the two methyl ketal peaks at δ3.12, δ3.19 and δ47.3, δ48.4 respectively, and the ketone carbonyl was indicated by a peak in the \(^13\)C NMR spectrum at δ207.

Having proven that the use of the dimethyl ketal protecting group was suitable for these tropane derivatives, the synthetic scheme was revised to incorporate this protecting functionality, as shown in Figure 4.5.
Figure 4.5 Revised Synthetic Scheme
4.4.3 Revision of Synthesis of 6β-arylhydroxynortropane derivatives

4.4.3.1 Incorporation of dimethyl ketal protecting group

Previously, when reacting the ketone under microwave conditions to give the 1,3-dioxolane 72, the tert-butyldimethylsilyl protecting group was cleaved, leaving the 6-hydroxy derivative 73. This was advantageous as deprotecting the 6-hydroxy functionality was the next step in the synthesis. It would also prove advantageous if cleavage of the tert-butyldimethylsilyl group could be achieved during the formation of the dimethyl ketal derivative. With this reaction requiring the presence of catalytic p-toluenesulfonic acid, acid hydrolysis of the hydroxyl protecting group was certainly a possibility, the mechanism of which is outlined in Scheme 4.18.

![Scheme 4.17 Preparation of dimethyl ketal protected derivative 85](image)

Reagents and Conditions: (a) Trimethylorthoformate, p-TsOH (cat.), MeOH, RT, O/N, 80% yield; (b) H₃PO₄, DMSO, RT, 3hrs, 78% yield.

Scheme 4.17 Preparation of dimethyl ketal protected derivative 85

![Scheme 4.18 Mechanism for silyl ether cleavage of 72 by p-toluenesulfonic acid](image)
Ketone 72 was therefore treated with trimethylorthoformate and p-toluenesulfonic acid monohydrate at room temperature (Scheme 4.17). During the course of the reaction silyl ether cleavage did in fact occur, and the desired product 84 was achieved in 85% yield. The presence of the newly formed dimethyl ketal derivative was clearly indicated in the \(^1\)H NMR spectrum of the product by new signals at \(\delta 3.11\) and \(\delta 3.15\) for the two methoxy groups, and in the \(^{13}\)C NMR spectrum by the shift of the C3 carbon from a signal at \(\delta 206.0\) to one upfield at \(\delta 98.3\) The high resolution mass spectrum also was consistent with the molecular formula. Also noticeable was the loss of the tert-butyldimethylsilyl protecting group from the 6 position via the absence of methyl peak signals at \(\delta -4.7\) and \(\delta 25.8\) in the \(^1\)H NMR spectrum. The high resolution mass spectrum (CI\(^+\)) of 84 was consistent with the molecular formula \(\text{C}_{12}\text{H}_{18}\text{NO}_5\text{Cl}_3\).

The oxidation of the secondary 6-hydroxyl group in 84 to the desired ketone had been established previously with derivative 73 using the Pfitzner-Moffatt oxidation method\(^{251}\), providing ketone 75 in good yield. Using analogous reaction conditions, the dimethyl ketal derivative 84 was oxidized to afford 85 in 61% yield. Confirmation that the oxidation had taken place was forthcoming from the \(^{13}\)C NMR spectrum, in which the signal for C6 at \(\delta 73.2\) was absent and a new signal, typical of a ketone, appeared at \(\delta 203.0\). Mass spectral analysis (CI\(^+\)) of 85 also indicated oxidation had occurred with an MH\(^+\) ion at \(m/z\) 360/362/364, consistent with the molecular formula \(\text{C}_{12}\text{H}_{16}\text{NO}_5\text{Cl}_3\), confirmed by high resolution mass spectral analysis.
4.4.3.2 Synthesis of aryl derivatives

With the 6-ketone derivative 85 in hand, the next step in the synthesis led into the set of aryl target derivatives and intermediates. The plan was to synthesise target ligands 50 and 54 via intermediates 86 and 87, 78 and 79, and 80 and 81 (Figure 4.6). In order to obtain some structure activity relationship (SAR) data, intermediate derivatives 88 and 89, and 55 and 57 were also to be investigated for their binding activities upon both WT and mutant α₁B-adrenergic receptors.

Figure 4.6 Attainment of target ligands 50 and 54
Introduction of the aryl moiety into the tropane skeleton of derivatives like 85 (dimethyl ketone) had previously been established via Grignard reactions of the dioxolane analogue 75. Therefore, using similar conditions, 85 was treated with the Grignard reagents phenylmagnesium bromide and 3,4-methylenedioxyphenylmagnesium bromide, resulting in the formation of 86 and 87 in good yields (Scheme 4.19). $^1$H NMR spectroscopy clearly identified the additional 5 phenyl protons of 86 at $\delta$7.19-7.54, and in the case of 87 the 3 aromatic protons at $\delta$6.72-7.05 and the methylene protons at $\delta$5.92. The high resolution mass spectra of 86 (CI$^+$) and 87 (EI$^+$) confirmed molecular formulae of C$_{18}$H$_{22}$NO$_5$Cl$_3$ and C$_{19}$H$_{22}$NO$_5$Cl$_3$ respectively, via ion peaks representative of the rapid loss of a methoxy substituent from the derivative, a trend also seen in the low resolution mass spectral data.

As with the cyclic dioxolane derivative 75, assessment of the lowest energy conformer of dimethyl ketone derivative 85 (as determined using the Spartan program) suggested that Grignard reagent attack by phenylmagnesium bromide on the ketone would occur preferentially from the exo face (Figure 4.7).
Having established the chemistry of dimethyl ketal deprotection using bismuth nitrate (section 4.4.2.6, Scheme 4.16) on a similar tropinone derivative (83), it was now possible to deprotect both the dimethyl ketal derivatives 86 & 87. To ensure the same reaction conditions were appropriate, to a small-scale solution of 86 stirring in dichloromethane was added 0.25 equivalents bismuth nitrate pentahydrate with stirring continuing for 20 hours at room temperature. Analysis of the reaction mixture (by both TLC and mass spectrum) revealed starting material. Despite a further 1.5 molar equivalents of bismuth nitrate pentahydrate being added and the mixture heated under reflux for 2 days, no product 78 was observed.

\[ CO_2CH_2CCl_3 \]

Figure 4.7 Lowest energy conformer of 85 (left) & preferred exo-aryl trope derivative 86.
The reaction was repeated, using 25 mol% of bismuth nitrate pentahydrate in dichloromethane at room temperature, and after 30 minutes yielded a new spot which corresponded (TLC analysis) with neither 86 or 78. Chromatographic separation of this compound and characterization using NMR spectroscopy and mass spectrometry revealed it to be the cyclic acetal 90. The $^1$H NMR spectrum of 90 displayed only one methoxy singlet at δ3.53, suggesting the cyclic acetal 90 had formed. Additionally, the signal assigned to the C3 in the $^{13}$C NMR spectrum was a quaternary carbon (from DEPT analysis), and this signal had moved downfield from δ99.3 to δ106.4. Finally, mass spectral (Cl+) analysis of 90 confirmed it to be C$_{17}$H$_{18}$NO$_4$Cl$_3$ via M+1 chlorine isotope peaks at m/z 406/408/410. The formation of the cyclic acetal derivative 90 suggests that the bismuth nitrate may have complexed with the tertiary hydroxyl oxygen of 86, rather than with the methoxy oxygen, thus enabling attack of the complexed oxygen at the C3 carbon, resulting in loss of one methoxy substituent and formation of the cyclic acetal 90.

An alternative method for dimethyl ketal deprotection was thus needed. Ellison et. al.$^{257}$ used 50% aqueous trifluoroacetic acid (TFA) in chloroform to remove dimethyl ketal protecting groups. Thus, as shown in Scheme 4.20, 86 was treated with 50% aqueous TFA in chloroform (1:2) to produce a single product (TLC) in very high yield, indicated by high resolution mass spectral analysis (Cl+) to be C$_{16}$H$_{16}$NO$_4$Cl$_3$. NMR analysis revealed that this product was actually a mixture of ketone 78 (as the minor isomer) and cyclic hemi-ketal 91 (as the major isomer), both compounds having the same R$_f$ value (TLC). The predominance of 91 was evident in the $^{13}$C NMR spectra, in particular the signal at δ203.1 in the $^{13}$C NMR ascribed to the C3 carbonyl was weak, with a new signal ascribed to the C3 carbon in the cyclic hemi-ketal at δ103.6 (See Figure 4.8).
Surprisingly, when the methylenedioxyphenyl derivative 87 was treated with the TFA:chloroform reagent, two products of quite different Rf value (TLC) were isolated. The first was the crystalline ketone derivative 79 (Rf 0.20), obtained in 24% yield. The signal representative of the C3 carbonyl was seen in the $^{13}$C NMR at $\delta$204.2. Mass

**Figure 4.8** $^{13}$C NMR spectrum of 91
spectral analysis (Cl+) of 79 indicated a molecular ion at m/z 436/438/440, indicative of the molecular formula C_{17}H_{16}NO_{6}Cl_{3}. The second, more polar product (Rf 0.08), obtained in 62% yield, was the crystalline cyclic hemiketal derivative 92, on the basis of the signal for the C3 carbon at δ103.6. Mass spectral analysis (Cl+) of 92 also indicated the molecular formula C_{17}H_{16}NO_{6}Cl_{3}, via a molecular ion at m/z 436/438/440.

The ring system embodied in the new cyclic hemi-ketal is that of the tropane alkaloid deoxynorscopoline. The related 7-hydroxylated compound, oscine (scopoline), is a direct product of the hydrolysis of scopolamine^{258a}. The synthesis of both deoxynorscopoline and oscine have been investigated from non-alkaloid sources^{258}.

\[
\begin{align*}
\text{deoxynorscopoline} & \quad \text{oscine}
\end{align*}
\]

**4.4.4 Selective ketone reduction to yield 3β-hydroxytropane derivatives**

The initial target tropane derivative was that exemplified by structure 50, containing a β-hydroxy substituent at the 3-position of the tropane skeleton. Initial pharmacophore work predicted that this 3β-hydroxy derivative of the S-enantiomer would be 65 times more selective for the mutant pharmacophore over the WT pharmacophore than the 3-α-hydroxy derivative 51, (Table 4.1) and 1.8 times more selective than the 3-dihydro derivative 49. Compound 50 was predicted to display an affinity for the mutant receptor in the same region as that of 49 and 51. The β-hydroxy derivative was therefore
preferred, and so it was necessary that a method be found for selective reduction of ketone derivatives 78 and 79 (and their hemi-ketal derivatives) to the β-hydroxy derivatives.

![Chemical structures](image)

Many options are available for the selective and stereospecific control of ketone reduction\textsuperscript{259,260}. Since the first report of reduction by diborane\textsuperscript{261}, metal hydrides have dominated the reduction of aldehydes and ketones to the corresponding alcohol. Opportunities for variation in the metal, ligands, counterion and reaction conditions have enabled most problems of stereo-, regio- or chemo-selectivity in synthesis to be overcome\textsuperscript{259a}. Detailed investigations of carbonyl reductions utilising borane and derivatives, metal borohydrides, metal aluminohydrides and transition metal hydrides\textsuperscript{262-266} have been undertaken. The stereoselective reduction of cyclic ketones is profoundly influenced by conformational effects, and diastereoselectivity is possible by the correct choice of reagent\textsuperscript{258}. Both conformational homogeneity and temperature control are also recognized as important factors in achieving high diastereoselectivity. The search for increased selectivity in synthesis has led to the development of reducing agents that will discriminate between ketones and other carbonyl groups and between various classes of ketone\textsuperscript{259a}, including cyclic ketones.

Unhindered cyclohexanones exhibit an intrinsic preference for axial attack by small hydride reagents (sodium borohydride (NaBH\textsubscript{4}), lithium aluminium hydride (LiAlH\textsubscript{4}))
leading to the equatorial alcohol. Monoalkylborohydrides proved to be superior to these traditional reagents, but selectivity declined as the ketone became more hindered.\textsuperscript{267,268} Comparatively, axial alcohols, via equatorial attack, have been produced from both hindered and unhindered ketones with bulky hydride reducing agents, the most successful being highly branched alkylborohydrides generally with a lithium counterion\textsuperscript{269} (for example lithium trisiamlyborohydride, \textit{tris}(trans-methylcyclopentyl)borohydride, L-selectride and lithium dimesitylborylhydride\textsuperscript{271}). In the reduction of bridged bicyclic ketones, hydride attack from the less hindered side of the carbonyl group with reagents such as LiAlH\textsubscript{4}, LiAlH(OC\textsubscript{4}H\textsubscript{9}-t)\textsubscript{3}, or NaAlH\textsubscript{2}(OCH\textsubscript{2}CH\textsubscript{2}OCH\textsubscript{3})\textsubscript{2}, may be enhanced with the use of LiAlH(OCH\textsubscript{3})\textsubscript{3}\textsuperscript{260}. In the reduction of 9-isocedran-2-one (93) (which is of similar conformation to those in the tropanones 78, 79), use of LiAlH(OCH\textsubscript{3})\textsubscript{3} directs hydride attack almost exclusively from the \textit{exo} side (Fig. 4.9), giving the axial hydroxyl derivative (-)-neoisocendran-2-ol (94) in 99% isomeric purity, whereas LiAlH\textsubscript{4} yields only 93.6% of this product\textsuperscript{272}.

![](image)

**Figure 4.9** Stereoselectivity of lithium methoxyborohydride reduction of 9-isocedran-2-one (93)\textsuperscript{272}.

Lanthanoid chlorides, particularly cerium chloride, have been shown to have dramatic effects on the regioselective and stereoselective outcomes of sodium borohydride ketone reduction\textsuperscript{273-275}. In the case of \textit{\alpha}-enones sodium borohydride/cerium chloride provides highly regioselective 1,2 reduction\textsuperscript{273}, and stereoselectively shows a greater preference
of equatorial alcohols\textsuperscript{273,276}. Saturated ketones were also demonstrated to be attacked on the axial side, with selectivity greatly enhanced by Ce\textsuperscript{3+}.

In the synthesis of tropan-3-ols with alkyl, alkenyl and alkynyl groups at the bridgehead, reduction of the ketone (±)-1-methoxymethyltropan-3-one (95) to the 3β-alcohol 96 was achieved in a 90% yield employing sodium and ethanol in boiling toluene\textsuperscript{192}, with the high degree of stereocontrol being thermodynamically based. Less harsh conditions were desired for arylhydroxy derivatives 78 and 79, and the use of sodium borohydride in the presence of cerium trichloride appeared appropriate.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{reaction.png}
\caption{Reduction of ketone 95 to alcohol 96.}
\end{figure}

The basis for the selective reduction with the sodium borohydride/cerium trichloride complex appeared to lie in the formation of alkoxyborohydride species\textsuperscript{273}. Alkoxyborohydrides, and particularly monosubstituted BH\textsubscript{3}OR\textsuperscript{−} species, are known to be more reactive than BH\textsubscript{4}\textsuperscript{−}\textsuperscript{277}. In methanol, the reaction rate for ketone reduction was very high\textsuperscript{273}, and the formation of NaBH\textsubscript{4−}(OCH\textsubscript{3})\textsubscript{n} is favoured, with BH\textsubscript{4−} being decomposed before reduction of the ketone occurs. This decomposition of BH\textsubscript{4−} by the solvent is catalysed by Ce\textsuperscript{3+} and is the rate-determining step of the reaction. The substitution of hydrides in BH\textsubscript{4−} by alkoxy groups increases the "hardness" of the reagents. Stereoselectivity of the reduction is related to the hardness of the hydride with axial attack in cyclohexanones being favoured with harder reagents\textsuperscript{278}. Gemal \textit{et al.}
observed that most enone reductions performed in the presence of Ce$^{3+}$ favoured the formation of equatorial alcohols$^{273}$.

To investigate the selectivity achievable with the sodium borohydride/cerium trichloride reagent on the tropane derivatives, 65 (tropinone) was treated with sodium borohydride and cerium trichloride heptahydrate in methanol at room temperature at varying concentrations of these two reagents (Scheme 4.21). The most favourable conditions (see Table 4.2) were those employing a cerium trichloride heptahydrate concentration of 0.066M, which yielded a $\beta$-hydroxyl group with 95% selectivity. This was established by $^1$H NMR spectroscopy. The $\alpha$-hydrogen of 97 resonates at $\delta4.14$ as a dddd with an axial-axial coupling with H2/H4 of 10.5Hz and an axial-equatorial coupling with H2/H4 of 5.7Hz. The $\beta$-hydrogen of 98, on the other hand, is characterized by a $\delta3.74$ chemical shift, with an equatorial-axial coupling constant of 6.6Hz, and an equatorial-equatorial coupling constant of 2.4Hz.

Scheme 4.21 Reduction of ketone 65 to the $\beta$-hydroxy derivative 97.
Table 4.2 Reduction of 65 with sodium borohydride/cerium chloride heptahydrate.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>NaBH₄ (mol. Eq.)</th>
<th>[CeCl₃·7H₂O]a</th>
<th>65 recovered %b</th>
<th>Yield β-hydroxy %b</th>
<th>Yield α-hydroxy %b</th>
<th>Total Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10c</td>
<td>2</td>
<td>0.22 M</td>
<td>-</td>
<td>75</td>
<td>25</td>
<td>98</td>
</tr>
<tr>
<td>10d</td>
<td>1.2</td>
<td>0.13M</td>
<td>50</td>
<td>33.33</td>
<td>16.67</td>
<td>96</td>
</tr>
<tr>
<td>10c</td>
<td>1.0</td>
<td>0.066M</td>
<td>-</td>
<td>95</td>
<td>5</td>
<td>97</td>
</tr>
</tbody>
</table>

a in methanol; b as determined by ¹H NMR spectroscopy; c reaction was undertaken at RT; d reaction began at 0°C for 5 mins, then was warmed to RT for 5 mins.

The 3,4-methylenedioxyphenyl ketone derivative 79 was treated with 0.066M cerium chloride heptahydrate in methanol, followed by sodium borohydride to afford the β-hydroxy derivative 81 (Scheme 4.22). ¹H NMR spectroscopy indicated only the β-hydroxyl substituent had formed, via the α-hydrogen indicated by the dddd signal at δ3.94 in the spectrum, with an axial-axial coupling of 9.6Hz and an axial-equatorial coupling of 6.3Hz. The axial H3 proton was in fact represented by two very close but separate dddd peaks: one at δ3.94 (0.75H integration) and the other at δ4.07 (0.25H integration). This downfield peak initially suggested the presence of either numerous rotamers, or more than one conformer. NOESY 1D NMR of the separate peaks further indicated that whilst the peak at δ3.94 displayed interactions with H2, H4, H7ax and the tertiary hydroxyl proton, the H3 peak at δ4.07 displayed interactions with only H2ax/eq and H4ax/eq. This indicated that the tropane was now possibly partially favouring the
boat conformation, as depicted in Figure 4.10, as a result of H-bonding between the 3-hydroxyl and the carbamate carbonyl group. H3 in this conformation now sits equatorial, and is too far from the 6-hydroxy and H7_\text{ax} to have a NOE interaction. The equatorial conformation of H3 is reflected in the H2/H4 equatorial-axial coupling constant values of 5.4Hz, whilst the equatorial-equatorial coupling constant was too small to be seen. Confirmation of the product 81 as C_{17}H_{18}NO_{6}Cl_{3} was obtained via high resolution mass spectrometry (Cl\(^+\)).

Scheme 4.22 Selective $\beta$-reduction of 79, 92 and 91, furnishing diol derivatives 81 and 80.
Chapter Four Synthesis of Bicyclic Tropane Derivatives

In the case of the reduction of hemi-ketal derivative 92, the hemi-ketal functionality must first isomerise to the ketone prior to any reduction. This isomerisation may in fact be assisted by the presence of cerium chloride in the reaction mixture: cerium chloride is proposed to complex to the tertiary C6 oxygen, thus promoting formation of the carbonyl bond as depicted in Scheme 4.23. Following isomerisation, axial attack of the methoxyborohydride species may then proceed. Hemi-ketal 92 was therefore treated with a solution of cerium trichloride heptahydrate in methanol followed by sodium borohydride to afford only the $\beta$-hydroxy derivative 81 in 84% yield (based on 43% recovered starting material). $^1$H NMR spectroscopy indicated that only one conformer of the $\beta$-hydroxy had formed, via the $\alpha$-hydrogen indicated by the broad doublet at $\delta 4.19$. High resolution mass spectral analysis (CI+) confirmed the molecular formula of 81 to be C$_{17}$H$_{18}$NO$_6$Cl$_3$.

There were two major differences in the reduction of the ketone versus the hemiketal derivative. The first was the reaction time, with the hemi-ketal derivative needing a longer reaction time than the ketone (4 hours for partial reduction of 92, compared to 30
minutes for full reduction of 79), due presumably to the isomerisation factor. The second was the concentration of the cerium chloride solution. Whilst the ketone derivative 79 used a 0.066M cerium chloride solution, the hemi-ketal derivative 92 required a 0.4M cerium chloride solution, with less complete reaction.

Scheme 4.23 Assistance of cerium chloride in the isomerisation of 92 to 79.

It was fortunate that in these reduction reactions only the β-hydroxyl derivatives were being produced. It is likely that the presence of the 6-hydroxy substituent was further directing axial hydride delivery, and perhaps even preventing equatorial hydride delivery, by interacting with the borohydride species as depicted in Fig. 4.11. Additionally, the bulky N-carbamate may hinder somewhat equatorial attack of the methoxyborohydride species.

Figure 4.11 Borohydride complexing to OH provides axial hydride delivery.
Reduction of the ketone/hemi-ketal mixture 78/91 with 0.4M cerium chloride heptahydrate and sodium borohydride in methanol afforded, after an extended reaction time (16 hours), the β-hydroxy derivative 80. Again, 1H NMR spectroscopy indicated that only the β-hydroxy derivative had formed, with the axial α-hydrogen ascribed to the broad multiplet at δ4.24-4.27. High resolution mass spectral analysis (Cl+) confirmed the molecular formula of the product as C_{16}H_{18}NO_{4}Cl_{3}.

With the desired dioxolane ketal (76, 77), dimethyl ketal (90, 91), cyclic hemi-ketal (91, 92) and dihydroxy (80, 81) derivatives in hand, it now remained to remove the carbamate protecting groups to afford the desired target derivatives for the receptor binding studies. This was achieved utilising zinc in acetic acid, to afford target ligands and intermediates 50, 54, 58, 60, 99, 100, 101 and 102, in yields of 60-100%. Both 1H and 13C NMR spectroscopic analysis of the products confirmed the loss of the carbamate group. Surprisingly, treatment of the dimethyl ketal derivatives 90 and 91 with zinc in acetic acid led not only to removal of the carbamate moiety, but also to the loss of one of the methoxy substituents, resulting in the hemi-ketal derivatives 101 and 102 shown in Scheme 4.24. This was clearly seen in the 1H NMR spectra, where both derivatives displayed only one methoxy peak at δ3.48. Additionally, DEPT analysis of the derivatives clearly indicated that the C3 carbon was quaternary. High resolution mass spectral analysis (Cl+) confirmed the molecular formulae of 101 (C_{14}H_{17}NO_{2}) and 102 (C_{13}H_{17}NO_{4}).
Scheme 4.24 Removal of the carbamate group of target precursors to afford the target ligands.
Chapter Four Synthesis of Bicyclic Tropane Derivatives

High resolution mass spectral data for the above derivatives were in accordance with expected values, and are shown in Table 4.3. Each derivative was converted into its hydrochloride salt for biological testing upon the $\alpha_{1B}$-adrenoceptors.

Table 4.3 High resolution mass spectral data for target ligands.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Molecular Formula</th>
<th>Molecular Mass</th>
<th>High Resolution Molecular Mass Observed (CI'), M+H$^+$</th>
<th>Calculated Values M+H$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>C$<em>{13}$H$</em>{19}$NO$_3$</td>
<td>261.3208</td>
<td>-$^a$</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>C$<em>{16}$H$</em>{19}$NO$_5$</td>
<td>305.3335</td>
<td>305.1254$^b$</td>
<td>305.1263</td>
</tr>
<tr>
<td>101</td>
<td>C$<em>{14}$H$</em>{17}$NO$_2$</td>
<td>231.2945</td>
<td>231.1266$^b$</td>
<td>231.1259</td>
</tr>
<tr>
<td>102</td>
<td>C$<em>{13}$H$</em>{17}$NO$_4$</td>
<td>275.3043</td>
<td>276.1240</td>
<td>276.1236</td>
</tr>
<tr>
<td>99</td>
<td>C$<em>{13}$H$</em>{15}$NO$_2$</td>
<td>217.2676</td>
<td>218.1175</td>
<td>218.1181</td>
</tr>
<tr>
<td>100</td>
<td>C$<em>{14}$H$</em>{15}$NO$_4$</td>
<td>261.7744</td>
<td>261.1002 (EI')</td>
<td>261.1001</td>
</tr>
<tr>
<td>50</td>
<td>C$<em>{13}$H$</em>{17}$NO$_2$</td>
<td>219.2835</td>
<td>220.1335</td>
<td>220.1338</td>
</tr>
<tr>
<td>54</td>
<td>C$<em>{14}$H$</em>{17}$NO$_4$</td>
<td>263.2933</td>
<td>-$^a$</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ The high resolution mass spectrum could not be determined;

$^b$ An M$^+$ ion was observed in the mass spectral data.
Chapter Five

Synthesis of Spirocyclic Derivatives
5.1 Introduction

Molecular modelling work indicated that spirocyclic derivatives displayed some remarkable selectivities for the $\alpha_{1B}$ Cys$^{128}$Phe mutant adrenoceptor pharmacophore (Mu2, Chapter Two). In particular, derivative 18b was shown to be the most selective of all first generation target ligands, displaying a selectivity for the mutant receptor over the WT receptor of 2860, and an affinity for the mutant receptor of 350nM ($R$-enantiomer) (Table 5.1). The spirocyclic target ligands of interest for evaluation of their binding affinities and selectivities upon the $\alpha_{1B}$ Cys$^{128}$Phe mutant adrenoceptor are shown below, and their calculated data is given in Table 5.1.

![Spirocyclic Derivatives](image)

**Table 5.1 Calculated data for spirocyclic ligands in comparison to monocyclic & bicyclic ligands. $S$ and $R$ refers to the stereochemistry at the spirocentre for 18 and 17, and at the aryl-substituted carbon for 13, 14 and 50. Predictions for 103 were not made.**

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Predicted WT Affinity</th>
<th>Predicted Cys$^{128}$Phe Affinity</th>
<th>Selectivity (for Cys$^{128}$Phe over WT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_i$ (nM)</td>
<td>$K_i$ (nM)</td>
<td></td>
</tr>
<tr>
<td>18a S</td>
<td>370000</td>
<td>3900</td>
<td>100</td>
</tr>
<tr>
<td>18a R</td>
<td>1400000</td>
<td>4700</td>
<td>300</td>
</tr>
<tr>
<td>18b S</td>
<td>330000</td>
<td>2900</td>
<td>110</td>
</tr>
<tr>
<td>18b R</td>
<td>1000000</td>
<td>350</td>
<td>2860</td>
</tr>
<tr>
<td>17 S</td>
<td>350000</td>
<td>8100</td>
<td>40</td>
</tr>
<tr>
<td>17 R</td>
<td>340000</td>
<td>2200</td>
<td>160</td>
</tr>
<tr>
<td>13 S</td>
<td>350000</td>
<td>2400</td>
<td>150</td>
</tr>
<tr>
<td>13 R</td>
<td>11000</td>
<td>2300</td>
<td>5</td>
</tr>
<tr>
<td>14 S</td>
<td>150000</td>
<td>4500</td>
<td>1</td>
</tr>
<tr>
<td>14 R</td>
<td>21000</td>
<td>3000</td>
<td>1</td>
</tr>
<tr>
<td>50 S</td>
<td>340000</td>
<td>1100</td>
<td>310</td>
</tr>
<tr>
<td>50 R</td>
<td>10000</td>
<td>570</td>
<td>20</td>
</tr>
</tbody>
</table>
The spirocyclic derivatives provide a good model for selective ligands due to the constrained folded conformation provided by the quaternary spirocyclic carbon, resulting in the two rings lying in planes at 90° to each other, as shown in Figure 5.1. Catalyst pharmacophore generation indicated that the adrenaline molecule probably adopted a folded conformation on the mutant adrenoceptor, as was discussed in Chapter Two. The spirocyclic framework enables a good approach to mimicking such a folded conformation in a synthetic ligand. The orthogonal spatial orientation of the rings places the phenyl ring directly opposite one ionisable nitrogen, as depicted in Figure 5.1; the other nitrogen could serve as a mimic of the alpha-oxygen functionality in adrenaline. That this folded conformation is indeed likely to be favoured by the mutant adrenoceptor is reflected in the calculated selectivity values of these spirocyclic derivatives over some other target ligands containing a more extended conformation of the adrenaline mimic (for example the monocyclic derivatives, Table 5.1).

![Figure 5.1](image)

**Figure 5.1** Lowest energy conformer of target ligand 18b, indicating the two nitrogen-containing rings orthogonal to each other, and the folded conformation of the design motif contained within the rigid heterocycle.
Figure 5.2 depicts 18b overlayed on the mutant pharmacophore. All three features of the pharmacophore (HBD, ring aromatic and positive ionisable groups) are mapped, and the ligand is a good fit for the pharmacophore. The incorporation of the methyl substituent in the target ligand 18 improves the selectivity for the Cys\textsuperscript{128}Phe mutant pharmacophore over the wild-type pharmacophore dramatically compared to ligand 17 which lacks the methyl substituent (see data in Table 5.1).

![Figure 5.2 Target ligand 18b overlayed on the Cys\textsuperscript{128}Phe mutant α1B-AR pharmacophore. The ligand interacts with all three binding functions.](image)

### 5.2 Known Syntheses of Biologically Useful Spirocyclic Derivatives

Due to their highly constrained ring structures, spirocyclic derivatives are likely to play diverse functions in medicinal chemistry where selectivity is desired. Because of the conformational rigidity of their structures, where small rings are involved, such spirocyclic derivatives may have a role as not only small molecule ligands, but also peptide-receptor antagonists, peptide-mimetics and even DNA-interacting molecules. For example spirocycles can act as type-II β-turn mimics\textsuperscript{279-284} and thus may provide a
popular option for use in peptide- and protein-interacting molecules. Ward et al.\textsuperscript{282} have reported the synthesis of a proline-based derivative as a potent and highly selective neurokinin antagonist: the (S)-spirolactam (104a) was shown to be an effective replacement for the (I+1)th residue of a classical type-II $\beta$-turn\textsuperscript{279,280} and as such was incorporated into peptide analogues. The spiro-forming cyclisation of precursor derivative 105 was a simple amide bond formation mediated by the use of $N$-[(dimethylamino)propyl]-$N'$-ethyloxycarbodimide (EDCI) (Scheme 5.1) and producing the diastereomeric spirocycles 104a and 104b.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme_5_1}
\caption{Cyclisation step in the synthesis of neurokinin antagonist 104a}
\end{figure}

Another peptidomimetic containing a spirolactam type-II $\beta$-turn mimic was reported by Genin et al.\textsuperscript{284}. The synthetic route to their peptidomimetic 106 was analogous to that previously reported by Hinds et al.\textsuperscript{281}. The cyclisation step was achieved under Mitsunobu reaction conditions, as shown in Scheme 5.2. Again based upon the amino acid proline, this synthesis provides a simple, somewhat generic spirocyclic lactam, and indicated a possible strategy for the synthetic targets in our work.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme_5_2}
\caption{Cyclisation step in the synthesis of neurokinin antagonist 104a}
\end{figure}
Urban and colleagues have repeated work on the development of spirocyclic amino acids and esters as GABA-analogues. These selective GABA uptake inhibitors consisted of spirocyclic amino acids (107-110) which incorporated the GABA skeleton into a heterocyclic ring (Figure 5.3).

The spirocyclic lactam 111 was prepared from β-keto ester 112 via the use of benzylamine in toluene at reflux, as shown in Scheme 5.3. Molecular sieves were used to trap the expelled ethanol, resulting in a high yield (98%) of the spirocyclic ketone (111) and minimising formation of the pyrrolidinone 113. The spirocyclic ketone 114 was then made by enolisation of the ketone functionality of 111 with

---

**Scheme 5.2** Cyclisation towards a spirolactam type-II β-turn mimic

**Figure 5.3** Spirocyclic GABA uptake inhibitors
triphenylmethyllithium, and subsequent reduction of the non-enolisable lactam group with lithium aluminium hydride.

Scheme 5.3 Preparation of the spirocyclic ketone 111 via lactam 112.

More recent development upon this synthesis\textsuperscript{285} utilised the methods depicted in Scheme 5.4. The $\beta$-keto amide 114 already contained the nitrogen functionality in the desired position for the preparation of the spirocyclic lactam (115). Thus conjugate addition of the $\beta$-keto amide to acrolein afforded the aldehyde 116 in excellent yields, which slowly underwent intramolecular addition of the amide to the formyl functionality. Reduction of the aldehyde 116 (or the already cyclised 115) with LiAlH\textsubscript{4} resulted in a mixture of the spirocyclic alcohols 117\textsubscript{a} and 117\textsubscript{b} (3:1, 85\%). It was apparent then that established chemistry for the preparation of aza spirocyclic molecules existed: it just remained to find the best synthetic pathway for our related target ligands.
5.3 Retrosynthetic Analysis of the Spirocyclic Target Derivatives

In order to establish a successful synthetic strategy for the spirocyclic class of derivatives, restrosynthetic analysis must take into consideration the differences between the target spirocyclic derivatives. Specifically, it was the substitution at the C8* position which needed to be taken into account when planning the synthesis.

Retrosynthetic analysis, as depicted in Scheme 5.5 indicated that the precursor derivative to the intramolecular spiro-cyclisation is a crucial step. As may be seen, in the case of the 8-methyl derivative, cyclisation may be affected via an allyl derivative, providing the desired methyl functionality. Similarly, the 8-unsubstituted spiro derivative may be synthesised from a saturated primary halo- or hydroxy-side chain.
derivative, whilst the 8-hydroxy derivative may be achieved via cyclisation of the amide nitrogen with an aldehyde.

Scheme 5.5 Retrosynthetic Analysis of Spiroyclic Target Ligands
Although there was some established chemistry for the synthesis of derivatives similar to the 8-hydroxy and 8-unsubstituted spiro derivatives 103 and 17, there was no previous known work outlining the synthesis of 8-methyl- or such substituted spiro derivatives. At first glance, however, it appeared that cyclisation between the benzylamide secondary nitrogen and the allyl methine carbon should facilitate spirocyclic formation with the desired methyl substitution.

Retrosynthetically then, it was planned that the cyclisation precursors may be derived from functionalised proline-benzylamide derivatives 118, 119 and 120, and that the Boc-protected amino acid proline would provide these derivatives. Because the $S$ stereochemistry at the carbon of the spiro centre was desired ultimately, stereosynthesis may therefore begin with readily commercially available L-proline. However, initially it was intended to establish a route to the targets with separation of stereoisomers to be undertaken at appropriate stages.

5.4 Initial steps in the Synthesis of Target Spirocyclic Derivatives

As seen from molecular modelling studies, the stereochemistry of the spiro derivatives is important, with the $S$-stereochemistry at the spirocyclic chiral carbon providing much greater selectivity than the $R$-stereochemistry (see Table 5.1). This feature has also been noted by others reflected by their preparation of $S$-stereospecific spirocyclic analogues.

Beginning with (S)L-proline, then, it was desired to keep the stereochemistry anchored throughout the synthesis without risk of racemisation. Enantiomerically pure amino acids have been reported to pose a problem undergoing $\alpha$-alkylation without
racemisation\textsuperscript{289,290} and without the use of a chiral auxiliary\textsuperscript{291}. However, Seebach \textit{et al.}\textsuperscript{292} have reported the preparation of enantiomerically pure \(\alpha\)-substituted proline derivatives, \textit{via} \(\alpha\)-alkylation without the loss of optical activity. The synthesis was based upon the acid-catalysed condensation of \(L\)-proline with pivaldehyde to yield a single stereoisomer of 2-\textit{tert}-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (121) in a 92\% yield (Scheme 5.6). The bicyclic compound 122 was extremely sensitive to hydrolysis, leading to the regeneration of proline; thus the azeotropic removal of water during the reaction was of the utmost importance. Compound 122 was thus reported to be very air sensitive, with insoluble proline forming spontaneously \textit{via} hydrolysis; it is also thermally labile, losing \(\text{CO}_2\) above 100\(^\circ\text{C}\). Deprotonation of 122 with LDA led to the formation of the chiral lithium enolate and subsequent alkylation with various electrophiles, including iodomethane, allylbromide, benzyl bromide, \(\text{N, N}\)-dimethylammonium chloride, and \(\alpha\)-halo acetic ester and amide, yielded products represented by 123 as a single diastereomer. With the (more reactive) electrophiles just mentioned, yields were reported as moderate to excellent, with 123 (allyl derivative) obtained in an 87\% yield. These enolate derivatives were reported to be much more stable toward both hydrolysis and thermal decarboxylation, with hydrolysis of the alkylated derivatives to their corresponding acids (like 124) requiring acidic reaction conditions of 15\%-48\% hydrogen bromide at temperatures varying from 25\(^\circ\text{C}\) to heating at reflux.
Chapter Five Synthesis of Spirocyclic Derivatives

Reagents & conditions: (i) TFA, pivaldehyde, pentane; (ii) LDA, THF, -78°C; (iii) allylbromide; (iv) 15%-48% aq. HBr, 8hr, RT.

Scheme 5.6 Chiral synthesis of (R)-2-allylproline from (S)-proline via 122

This procedure therefore provides the allylated chiral proline derivative 124 required for the generation of the target spirocyclic system, as was depicted in Scheme 5.6. It has also been used successfully by others in the synthesis of novel spiro-bicyclic systems. With this support it was decided to follow a similar path and use this method to begin the synthesis. The first intended intermediate was therefore (R)-2-allylproline (124), via the intermediate (2R,5R)-5-allyl-2-tert-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (123).

5.4.1 Attempted synthesis of (2R,5R)-5-allyl-2-tert-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (123) and (R)-2-allylproline (124)

The synthesis of (2R,5R)-5-allyl-2-tert-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (123) was therefore attempted following the method of Seebach et al. (1983) as outlined in Scheme 5.6. Crude 122 was obtained as a colourless oil in a low (47%) yield, which at room temperature very quickly began to hydrolyse on being even only slightly exposed to air, back to white solid proline. The mixture was identified via mass spectral
(Cl⁺) analysis, which displayed molecular ion peaks for 121 (proline) and 122 at m/z 184 (M+1) and 116 (M+1) respectively.

This material was nonetheless taken on to be allylated, following the method outlined in Scheme 5.6. Separation of the crude product gave a low yield of an impure mixture of allylated 123 and unreacted 121, identified by mass spectral (Cl⁺) analysis (m/z 184 (M+1, 121), 224 (M+1, 123), 288, 367).

With low yields and stability problems encountered in the attempted preparation of the initial (2R,5R)-5-allyl-2-tert-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one, it was necessary to find an alternate route to the preparation of the desired chiral allylproline intermediate 124.

Dhaon et al.²⁹³ have investigated the esterification of N-protected α-amino acids with respect to the effect on racemisation. Previous reports²⁹⁴ had observed "some racemisation" in the esterification of N-(benzyloxy carbonyl) α-amino acids with tert-butyl alcohol, but noted that racemisation may be "largely avoided at lower temperatures and with shorter reaction times". Dhaon et al.²⁹³ however, reported, amongst other things, that N-(benzyloxy carbonyl)L-proline readily formed its tert-butyl ester with no racemisation, using catalytic dimethylamino pyridine (DMAP) (0.1 or 0.5 equivalents), EDCI and tert-butyl alcohol with stirring overnight. They also reported other Boc-protected amino acids to withstand racemisation during esterification. We therefore chose to prepare the N-Boc proline tert-butyl ester 125 via this method. Accordingly, as shown in Scheme 5.7, L-proline was firstly N-Boc protected using di-tert-butyl dicarbonate to afford 126, then a solution of 126 in dichloromethane was treated with DMAP, tert-butanol and EDCI at room temperature overnight to afford the desired tert-butyl ester 125 as a colourless oil. The ¹H NMR spectrum displayed the
typical tert-butyl signal as a singlet at an upfield chemical shift of δ1.47, typical of the ester moiety. Mass spectral (Cl⁺) analysis confirmed the identity of 125 with a MH⁺ peak at m/z 273 indicative of the product C₁₄H₂₆N₂O₄.

\[
\begin{align*}
\text{N} & \text{COOH} \\
\text{(S)} & \text{H} \\
121 & \\
\end{align*}
\]

\[
\begin{align*}
\text{N} & \text{COOH} \\
\text{(S)} & \text{H} \\
\text{Boc} & \\
126 & \\
\end{align*}
\]

Reagents and Conditions: a): 10% NaOH, (Boc)₂O, dioxane/H₂O, 0°C 5hr, RT O/N, 91%; b) ROH, DMAP, EDCI, DCM, 0°C 2hr, RT O/N.

**Scheme 5.7 Preparation of N-protected ester derivatives**

It was also desired to determine if this method could prepare other esters (namely ethyl and methyl esters) of the N-Boc-proline without racemisation. The degree of racemisation is dependent on the alcohol used with the order of increased racemisation being methanol < ethanol < benzyl alcohol < tert-butyl alcohol. Additionally, racemisation levels may be controlled by employing only 0.1 equivalents of DMAP and shorter reaction times. It is apparent then that the method of Dhaon et al.²⁹³ may also be applied to the successful preparation of methyl and ethyl ester derivatives of L-proline 127 and 128. Reaction conditions analogous to those previously used (Scheme 5.7), substituting tert-butyl alcohol with ethanol and methanol, yielded the desired esters 127 (55% yield) and 128 (93% yield) respectively. ¹H NMR analysis verified the esterification, displaying the ethyl ester peaks at δ4.12-4.32 (2H, m) and δ1.28 (3H, t) and the methyl ester peak at δ3.70 (3H, s). Mass spectral analysis (Cl⁺) confirmed the identities of the new proline ester products, with an MH⁺ peak at m/z 244 for 127.
(C_{12}H_{21}NO_4) and an MH^+ peak at m/z 230 for 128 (C_{11}H_{19}NO_4). The optical rotation for 128 was measured as -58.11 (at 22°C in ethanol) (compared with the literature value of -54.54^{295} at 25°C in chloroform), reflecting the retention of the S-stereochemistry at the α-carbon.

While methyl ester production was the higher yielding of the esterification reactions, for subsequent synthetic steps it was not certain which of the proline ester derivatives would prove most suitable for further derivatisations to allow cyclisation. Urban and co-workers^{285,286} utilised the bromoethyl substituted β-keto ester 129 to provide the spirocyclic lactam 130 (Scheme 5.8), a method which may be adopted to the proline derivatives in this work. Similarly, they also used the β-keto ester 131 (containing an ethyl ester) in an aminolysis reaction to produce the β-keto amide 132, which further yielded aldehyde 133 upon conjugate addition to acrolein, and resulting in isomerization to the spirocyclic lactam 134 (Scheme 5.9).

Reagents & Conditions: benzylamine, toluene, 4A molecular sieves, reflux, 96%.

Scheme 5.8 Preparation of spirolactam 130 from β-keto ester 129^{285}
In their preparation of spirocyclic derivatives, Ward et al. employed the proline allyl butyl ester derivative 135 as the precursor to the spirolactam derivatives 136a and 136b as shown in Scheme 5.10, with cyclisation occurring between the ester carbonyl and the leucine amine nitrogen.

In order to facilitate 8-methyl spiro formation via either the bromoester proline derivative 138 or the allylated proline benzylamide derivative 139, an allyl side chain must be incorporated into the α-position of the proline skeleton (derivative 140) (see retrosynthetic Scheme 5.5). The allyl derivatives of all three proline ester derivatives
125, 127 and 128, were therefore prepared and their suitability for the desired synthesis assessed.

5.4.2 Allylation of proline ester derivatives

Having established that the synthesis of the chiral allylated derivative 124 by the method of Seebach et al. was unsuitable due to instability and poor yields, it may be seen that with the protected proline ester derivatives 125, 127 and 128 in hand, a simple α-alkylation employing a strong base and an allyl halide would provide the desired allylated derivative, albeit in a racemic form. At this stage, it was aimed to undertake at least partial synthesis of the racemic target ligands (in particular the intramolecular cyclisation), before proceeding further with chiral syntheses, which could be completed once racemic syntheses had been established. Ward et al. used lithium diisopropylamine (LDA) to lithiate the α-H of proline before allylating with allyl iodide. Therefore, a solution of the proline ester derivative 125 was treated with LDA followed by allyl bromide, as shown in Scheme 5.11, to afford 141 as a yellow oil. 'H NMR analysis revealed the loss of the α-H at δ4.11, and the presence of the distinctive allyl H’s at δ5.66-5.76 (=CH, m, 1H), δ5.10 (=CH2, 2H, dd) and δ2.52 and δ2.95 (CH2, 2 x dd). 'C NMR displayed the three new carbon atoms downfield at δ133.4 (=CH) & δ118.3 (=CH2) and upfield at δ38.2 (CH2). Mass spectral (Cl⁺) analysis revealed an MH⁺ peak at m/z 312, consistent with the newly formed derivative 141, C₁₇H₂₉NO₄.
The other two allyl derivatives 142 and 143 (methyl) were prepared in a similar manner, in yields of 71% and 75% respectively. The additional allyl protons of 142 and 143 were observed in the $^1$H NMR and $^{13}$C NMR at identical chemical shifts to that of 141. Mass spectral analysis (CI$^+$) of 142 revealed an MH$^+$ ion at m/z 284, confirming its identity as C$_{15}$H$_{25}$NO$_4$; whilst the identity of 143 was confirmed by an MH$^+$ ion seen at m/z 270 (for that of C$_{14}$H$_{23}$NO$_5$).

5.5 Methods of Cyclisation for Spiro Formation

5.5.1 Introduction

Various methods for the production of some spirocyclic derivatives have been introduced (section 5.2). However, whilst aiming to prepare the spirocycles, it was also of interest to investigate novel methods of spirocycle formation. The key step in the spirocyclic derivative synthesis is the cyclisation step, producing a spiro centre and a new 5-membered nitrogen-containing heterocycle. For the 8-dihydro (17) and 8-hydroxy (103) target ligands the chemistry for such cyclisations is known, and may be
adapted for the synthesis of the 8-methyl spiro cyclisation. It was therefore proposed that cyclisation of bromo derivative 138 may be possible, as might cyclisation of the benzylamide derivative 139.

As was revealed in the retrosynthetic analysis of the target ligands (Scheme 5.5), derivative 119 may be achieved from 144 via the method of Bendl et al. and 120 may be achieved from 145 via the methods of Hollauf et al. For the 8-methyl substituted derivative 118, for which no established preparation methods were known, cyclisation between the benzylamide secondary nitrogen and a tertiary carbon in the alkyl side chain could be facilitated via a number of different reagents and intermediates. For the bromo derivative 139, analogous reaction conditions to that of Bendl et al. could provide the desired bond formation as shown in Scheme 5.12.

![Scheme 5.12 Methods for Spiro cyclisation](image-url)
Alternatively, cyclisation between the benzylamide secondary nitrogen and an activated double bond, in the case of the allyl side chain in 139, should facilitate the desired 8-methyl substituted spiro ring. A variety of procedures, including organometallic-mediated cyclisation (eg palladium and mercury), or incorporating radical cyclisation, are available for cyclisation of the allyl benzylamide derivative 139.

5.5.2 Spiro cyclisation via 2-bromopropyl-proline derivative 138

Utilising the method of Bendl et al.\textsuperscript{285} and Hollauf et al.\textsuperscript{286}, it was expected that the secondary bromoalkyl sidechain would cyclise with benzylamine in the presence of toluene and molecular sieves to yield the desired spirolactam 118 containing the 8-methyl substitution. Addition of hydrogen bromide to the double bond would therefore need to preferentially provide the desired tertiary bromo-substituted Markovnikov product rather than the undesired secondary bromo-substituted anti-Markovnikov product.

Carbon-carbon double bonds readily react with hydrogen bromide, however competing radical and ionic reactions generally result in a mixture of products, unless the system is symmetrically substituted. Ionic addition may be controlled to provide the Markovnikov product by use of phase-transfer conditions at high temperature\textsuperscript{296}. Alternatively, competing radical reactions may be avoided by conducting the reaction on the surface of silica or alumina gels\textsuperscript{297-299}. Surface-mediated additions afford stereoselectivities often unattainable in solution, and the use of hydrogen bromide precursors with silica or alumina gel allows for convenient generation of hydrogen bromide \emph{in situ}\textsuperscript{297}. The use of this latter method should then easily provide the desired 2-bromopropyl-proline derivative 138 from the allyl derivative 143. Therefore, following the method of Kropp
et al., a model reaction employing the treatment of cyclohexane (146) with trimethylsilyl bromide in the presence of anhydrous alumina was undertaken, to afford bromocyclohexane (147) in high yield (Scheme 5.13). Similarly, 143 was treated with trimethylsilyl bromide in the presence of dried alumina. The reaction afforded recovered starting material and a small amount of the Boc-deprotected derivative 148. Unfortunately repeating the reaction a number of times did not furnish any of the brominated 138. Iodination of the double bond of 143 was also attempted utilising trimethylsilyl iodide instead of trimethylsilyl bromide, however again unreacted 143 was recovered. This result was unexpected considering the double bond is not sterically encumbered.

\[
\begin{align*}
\text{cyclohexene} & \rightarrow \text{Br} \\
146 & \rightarrow 147
\end{align*}
\]

Reagents and Conditions: anhydrous alumina, 1M trimethylsilyl bromide, dichloromethane, RT, 2 hrs, 90% yield

\[\text{Scheme 5.13 Model reaction: surface-mediated bromination of cyclohexene}\]

Instead of further pursuing methods for Markovnikov bromination of the allyl chain, it was decided to investigate an alternate route for the formation of the spirocycle.
functionality, namely *via* the intramolecular cyclisation between an amide moiety and the allyl sidechain.

5.5.3 Preparation of allylbenzylamide derivative 139

The next section of work focused upon preparing benzylamide derivative 139 for cyclisation. Classically, amides have been prepared from either an ester or an amine, or from an acid. It would be advantageous to prepare the benzylamide derivative directly, in one step, and as outlined in Scheme 5.14, 139 may be achieved *via* a number of methods, from either the ester derivative 143 or the acid 149.

![Scheme 5.14 Routes to benzylamide 139 via acid or ester derivatives](image)

5.3.3.1 Preparation of benzylamide 139 from ester 143

Because the ester derivative 143 was available, it was decided to initially investigate the synthesis of 139 from the ester 143. Analysis of the literature provided a number of possible reagents suitable for this transformation. Cossy & Thelland\(^\text{300}\) reported a one-pot procedure for the high-yield conversion of β-ketoesters into β-ketoamides, using the catalyst DMAP with a variety of primary and secondary amines in refluxing toluene. This method may be applied to the proline esters 141, 142 and 143. Accordingly, a
mixture of 143, benzylamine and DMAP were heated in refluxing toluene, as shown in Scheme 5.15. The resultant crude oil was shown to contain 143 and benzylamine only (unchanged ¹H NMR). Repeated attempts at this reaction increasing quantities of benzylamine and reaction times failed to furnish any of the desired amide product.

Among many of the methods reported for direct transformation of carboxylic acid esters to the corresponding amides the preparation involving dimethylaluminium amides was regarded to give good yields of amides under mild conditions. Sim and Yoon found sodium diethyldiamidoaluminate, readily prepared from sodium diethyl dihydroaluminate and diethylamine, to be an excellent reagent for amide synthesis from esters. All esters examined, both aliphatic and aromatic, gave very good yields of the corresponding amides in 1-6 hours by refluxing in toluene or THF-toluene (1:1) using a 10% excess of sodium diethyldiamidoaluminate (10% excess). Primary, secondary and tertiary amines used gave equally good results, and sterically hindered esters were also readily converted to the corresponding amide by increasing the amount of reagent. This method should therefore successfully provide the desired benzylamide derivative 139. To investigate this, the t-butyl ester derivative 141 was treated with sodium diethyldibenzylamidoaluminate, as shown in Scheme 5.16.
To freshly prepared sodium diethyldibenzylamidolinate was added a solution of the t-butyl ester 141 in toluene and the mixture refluxed for 1-6 hours (multiple reactions). $^1$H NMR analysis of the crude oily product indicated that the reaction had failed to furnish any of the desired amide product 139, as the t-butyl ester peak at $\delta 1.28$ was still present, and no peaks attributable to the benzylamide were seen. This disappointing indication was further supported by the mass spectral (Cl$^+$) data, which clearly showed an $m/z$ peak at 312, corresponding to that of the ester 141 ($C_{17}H_{29}NO_4$). Investigations therefore moved to the ethyl ester derivative 142. Employing analogous reaction conditions, freshly prepared sodium diethyldibenzylamidolinate and 142 in toluene were refluxed for 16-20 hours. The reaction yielded a crude yellow oil which proved to be solely unreacted starting material as evidenced by the presence of the ethyl peaks in the $^1$H NMR at $\delta 1.27$ and $\delta 4.05-4.25$, and the lack of any benzylamide peaks. Mass spectral analysis (Cl$^+$) also clearly displayed the M+1 peak of 142 at $m/z$ 284 ($C_{15}H_{25}NO_4$).

To determine if the reaction conditions and reagents being used were effective, a model reaction using freshly prepared sodium diethyldibenzylamidolinate and methyl
phenylacetate 150 was undertaken (Scheme 5.17). The reaction provided an off-white solid in 93% yield, and $^1$H NMR analysis confirmed the benzylamide derivative to have formed, with the loss of the methyl ester singlet peak at $\delta$3.70, and the presence of the benzylamide NH singlet at $\delta$5.75, as well as an aromatic multiplet at $\delta$7.17-7.38, and a methylene peak at $\delta$4.41/4.43. Mass spectral analysis (Cl⁺) revealed an MH⁺ peak at $m/z$ 226, consistent with the molecular formula of the newly formed derivative 151 (C₁₅H₁₅NO).

![Chemical structure](image)

Reagents and Conditions: sodium diethylbenzylaminoaluminate, toluene:THF, reflux, 1hr, 93% yield

Scheme 5.17 Synthesis of benzylamide 151 using diethylbenzylamidoaluminate

Without having yet achieved the desired derivative 139, it was decided to try a slightly different reagent, dimethylaluminium benzylamide, as used by Basha et al.³⁰⁵, to attempt smooth amidation. Accordingly, to a solution of freshly prepared dimethylaluminium benzylamide was added a solution of methyl ester 143 in dichloromethane and the mixture heated at reflux (Scheme 5.18). $^1$H NMR analysis of the resultant product unfortunately revealed only starting material present, with no amide formation having occurred.
A return to the sodium diethyldibenzylamidoaluminate reagent was therefore made, and its reaction with the methyl ester \(143\) was investigated. Having first-hand confirmation that the amidation reaction works, using analogous reaction conditions to those previously used (Scheme 5.16) \(143\) was refluxed with sodium diethyldibenzylamidoaluminate (in a 20% excess) in toluene for a total of 60 hrs. The reaction provided a 30% yield of \(152\) (\(C_{16}H_{18}N_2O_2\)) as a pale yellow oil, indicated by mass spectral analysis (CI\(^+\)) \((m/z \ 271 \ (M+1))\), and NMR spectroscopy. \(^1\)H NMR indicated loss of the \(t\)-butyl group at 1.27 and the methyl ester at 83.70, and the addition of the benzyl group by aromatic protons at 87.24-7.35, and the methylene protons at 84.59-4.71. The absence of the NH proton from the spectrum indicated that it had become a tertiary nitrogen. The \(^{13}\)C NMR spectrum further indicated the presence of two keto groups (8159.8 and 8175.3), and the loss of the Boc C-O at 879.5. It thus appears that once the benzylamide had formed the nitrogen immediately attacked the Boc group, leading to an intramolecular cyclisation and production of \(152\) (Scheme 5.19). It was apparent then that it would be necessary to investigate the synthesis of benzylamide derivative \(139\) from the acid derivative \(149\).
**Chapter Five Synthesis of Spirocyclic Derivatives**

**Scheme 5.19** Intramolecular cyclisation to produce bicyclic spirolactam 152

5.5.3.2 *Preparation of benzylamide 139 from acid 149*

Acid derivative 149 was prepared readily from the methyl ester 143 via base hydrolysis in high yield and purity (Scheme 5.20). Both the $^1$H NMR and $^{13}$C NMR spectra for 149 displayed the loss of the methyl ester peak at 81.43 and 8175.1 respectively. Mass spectral (CI) analysis revealed the MH$^+$ ion of the acid at m/z 256, consistent with the molecular formula (C$_{13}$H$_{21}$NO$_4$).

**Scheme 5.20** Preparation of Acid derivative 149.
Chapter Five Synthesis of Spirocyclic Derivatives

The synthesis of the benzylamide derivative 139 from 149 was initially undertaken using the common method of dicyclohexylcarbodiimide (DCC) coupling, employing catalytic dimethylaminopyridine, DCC and benzylamine (Scheme 5.21). DCC is a common reagent in peptide bond formation, assisting in the linkage of carbonyl and amino functionalities. The reaction yielded a crude pale yellow oil, which from mass spectral analysis (CI\(^+\)) \(m/z \ 256, \text{MH}^+ \ C_{13}H_{21}NO_4\) was shown to be the acid 149. Repeating the reaction with an increase in temperature failed to furnish any of the desired product (mass spectrum (CI\(^+\)): expected \(m/z \ 345 \ C_{20}H_{28}N_2O_3\)). Further temperature increases upon the reaction, to refluxing in toluene overnight, again failed to produce any of the desired amide 139.

![Scheme 5.21 Attempted preparation of 139 via DCC coupling](image)

Reagents and Conditions: benzylamine, DMAP, DCC, toluene 0°C-reflux.

1-Hydroxybenzotriazole (HOBt) may aid in the formation of amide bonds. It was decided to first investigate if a procedure utilising this reagent would effect amide coupling with the un-substituted derivative 126 (Scheme 5.22). Accordingly, 126 was treated with benzylamine, HOBt, triethylamine and DCC in chloroform at room temperature. The desired product 153 was afforded as a UV-fluorescing white solid in 67% yield. \(^1\text{H} \text{NMR analysis revealed the benzylamide protons as a multiplet at } \delta 7.25-7.32 \text{ (aromatic protons), and a multiplet at } \delta 4.22-4.57 \text{ (methylene protons). The NH}
proton was not observed. The $^{13}$C NMR displayed the aromatic carbons at $\delta 127.4$-$128.6$, and the slight upfield shift of the acid carbonyl of 126 from $\delta 180.5$ to the amide carbonyl of 153 at $\delta 174.0$. Mass spectral analysis of 153 (CI$^+$) indicated an MH$^+$ ion at $m/z$ 305, supporting the molecular formula C$_{17}$H$_{24}$N$_2$O$_3$.

![Chemical Structures](image)

Reagents and Conditions: a) benzylamine, HOBt, NEt$_3$, DCC, CHCl$_3$, 67% yield; b) LDA, THF, -78°C; c) allylbromide, THF, -78°C -- -30°C, 10% yield.

**Scheme 5.22** Successful preparation of the proline benzylamide 139

With this method proving successful, and with the benzylamide derivative in hand, it was decided to attempt allylation of 153 in the $\alpha$-position with allyl bromide (Scheme 5.22). If successful, this would provide the desired derivative 139 in only 3 steps from the L-proline starting material, in comparison to the 5 steps needed in the synthesis going via the ester derivative 143. Therefore, a solution of 153 (amide) was treated with freshly prepared lithium diisopropylamine (1.5 equivalents) followed by allyl bromide. The crude product was extracted as a cream solid, and $^1$H NMR analysis indicated a mixture of mainly starting material with a small amount (10%) of desired allylated product 139, represented by a multiplet at $\delta 5.65$ (=CH), $\delta 5.14$ (=CH$_2$) and $\delta 2.61$ (CH$_2$). Mass spectral (CI$^+$) analysis displayed an MH$^+$ peak at $m/z$ 305 (153, C$_{17}$H$_{24}$N$_2$O$_3$) and 345 (C$_{20}$H$_{28}$N$_2$O$_3$, 10%) representative of 139.
Given the low yield of this reaction, it was decided to return to amidation procedures with the already allylated acid derivative 149.

Using the successful HOBt/DCC coupling method, allyl derivative 149 was treated with equivalent amounts of benzylamine, HOBt, triethylamine and DCC at room temperature, yielding the desired product 139 as a white solid in 95% yield. Mass spectral (CI\(^+\)) analysis confirmed the identity of 139 via the presence of an MH\(^+\) ion at \(m/z\ 345\) (C\(_{20}\)H\(_{28}\)N\(_2\)O\(_3\)). The \(^1\)H NMR spectrum clearly displayed the methylene protons of the benzyl substituent at \(\delta\ 4.34\)–4.52, the aromatic protons at \(\delta\ 7.28\) and the amide NH at \(\delta\ 2.98\)–3.10. The \(^{13}\)C NMR displayed the shift of the carbonyl peak from \(\delta\ 180.5\) for the acid to \(\delta\ 173.8\) for the amide derivative.

![reaction scheme](image)

Reagents and Conditions: benzylamine, HOBt, NEt\(_3\), DCC, CHCl\(_3\), RT, 16 hrs, 95% yield.

**Scheme 5.23** High yield preparation of benzylamide derivative 139

### 5.5.4 Palladium-assisted cyclisation of benzylamide derivative 139 to form spirocycles

It was aimed to facilitate intramolecular cyclisation of 139 by utilising the double bond of the allyl side chain. By rendering the double bond electron deficient, the lone pair of electrons on the tertiary nitrogen atom may interact with and form a bond with the methine carbon atom, producing the desired 5-membered spirocycle. Alternatively,
intramolecular 5-exo-trig radical cyclisation from a nitrogen-centred radical may also effect the desired cyclisation.

5.5.4.1 Introduction

Transition metal organometallic reagents have been used extensively in the synthesis of heterocyclic compounds (for reviews of work in this area see Hegedus\textsuperscript{308-310}). Palladium (II) has frequently been used to activate simple carbon-carbon double bonds to undergo attack by a variety of nucleophiles\textsuperscript{311}. Many nucleophiles, including acetate, chloride and alkoxide, as well as alcohols and amines\textsuperscript{312} have been reacted with Pd (II) complexed olefins. Intramolecular adaptations of this reaction have provided effective methods for synthesising heterocycles. For example, 2-allylphenols have been converted to benzofurans\textsuperscript{313}, 2-allylamines to indoles\textsuperscript{314}, \(\alpha,\beta\)-unsaturated ketoximes to isoxazoles\textsuperscript{315}, \(o\)-hydroxychalcones to flavones\textsuperscript{316}, and \(\alpha,\beta,\gamma,\delta\)-unsaturated carboxylic acids to pyrones\textsuperscript{317} by treatment with a form of Pd(II) and base to generate a nucleophilic heteroatom.

Hegedus and coworkers\textsuperscript{310,311,314,318-320} extensively investigated the role of Pd(II) in the synthesis of heterocycles, particularly via olefin complexation. They have used palladium-assisted cyclisation in the synthesis of functionalised heterocycles\textsuperscript{310,318,319}, including indoles\textsuperscript{314,320} and isocoumarins\textsuperscript{321}, dihydroisocoumarins and isoquinolines\textsuperscript{311}. Most of this work employed the use of olefins as both intermolecular and intramolecular reaction points. Treatment of a number of 2-allylbenzoic acids (such as 154) with PdCl\(_2\)(CH\(_3\)CN)\(_2\) and Na\(_2\)CO\(_3\) in THF led to production of 2-alkylisocoumarins such as 155 in high yield via nucleophilic attack of a carboxylate on the Pd-complexed olefin\textsuperscript{311}.
The reaction was thought to proceed via "co-ordination of the olefin to Pd(II), generation of the free carboxylate by sodium carbonate, attack of carboxylate on Pd(II) complexed olefin to produce ring closure and a σ-alkyl palladium complex, followed by elimination of PdH and rearrangement to the observed isocoumarin"\(^{311}\).

![Scheme 5.24 Synthesis of 2-alkylisocoumarins](image)

Similar mechanisms have been proposed for the formation of indoles from 2-allylanilines\(^{314}\) and benzofurans from 2-allylphenols\(^{313}\). The cyclisation was also made catalytic with respect to palladium by the addition of cupric acetate and \(\text{O}_2\) to provide a means for oxidation of the Pd(0) produced in the cyclisation step. This catalytic method was further developed in subsequent work\(^{310,320}\).

This method was also used for the intramolecular cyclisation of some amide derivatives: in particular 2-(2-propenyl)benzamide to 3-methylisocarbostyril (Scheme 5.25) and 2-(2-propenyl)-\(\text{N}\)-methylbenzamide to 3-methyl-\(\text{N}\)-methylisoquinoline, by treatment with \(\text{PdCl}_2(\text{CH}_3\text{CN})_2\) and sodium hydride in THF\(^{311}\).
The functional group similarity between the allyl and amide reacting groups of 2-(2-propenyl)benzamide and 2-(2-propenyl)-N-methylbenzamide, and the allylprolinebenzylamide derivative 139 suggested that intramolecular cyclisation of 139 may be achievable using this olefin-Pd complex methodology. Investigations thus began into the cyclisation of 139 using PdCl$_2$(CH$_3$CN)$_2$ and sodium hydride in THF.

**5.5.4.2 Attempts at palladium assisted cyclisation of 139**

Initial investigations into the Pd-assisted cyclisation of 139 utilised the method of Korte et al.$^{311}$ Thus, 139, PdCl$_2$(CH$_3$CN)$_2$ and sodium hydride were stirred in THF at room temperature (Scheme 5.26). The reaction yielded a crude mixture which $^1$H NMR spectroscopic analysis revealed to be mainly 139, with the allylic protons being ascribed to the peaks at $\delta$5.64 (1H, dddd, =CH) and $\delta$5.14 (2H, dd, =CH$_2$). The reaction was attempted a second time utilising the same reaction conditions. TLC analysis indicated no 139 remaining and the formation of two new products, one on the
baseline, the other just above the baseline. $^1$H NMR analysis suggested a 1:1 mixture of cyclic product 139 (peak for the methyl substituent at $\delta 1.26$ (1.5H, s, CH$_3$) and the vinyl proton at $\delta 5.54$ (0.5H, t, $J = 2.4$, =CH)) and allylated 139 (allyl protons present at $\delta 5.58-5.72$ (0.5H, m, =CH), $\delta 5.15$ (1H, dd, $J = 7.5$, 9.3, =CH$_2$). The altered TLC $R_f$ suggested perhaps a palladium complex of 139. Mass spectral analysis (CI$^+$) of the reaction mixture revealed ions at $m/z$ 345 (MH$^+$, 139), 346, 343 (MH$^+$, 156), 289 (fragment of 139), 287 (fragment of 156).

Reagents and Conditions: PdCl$_2$(CH$_3$CN)$_2$, NaH, THF, RT-reflux, 1-10 days

Scheme 5.26 Attempted cyclisation of 139 using PdCl$_2$(CH$_3$CN)$_2$ and sodium hydride.

Repeating the reaction at 40°C for 4 days again failed to furnish any of the desired product, mass spectral (CI$^+$) analysis revealing only a loss of the Boc protecting group as indicated by a peak at $m/z$ 273 (C$_{17}$H$_{19}$N$_2$O$_2$). Increasing the reaction temperature to 55°C (5 days stirring) still failed to furnish the desired product 156, and a further increase to reflux for 5 days produced a crude mixture which mass spectral (CI$^+$) analysis revealed to contain only fragments of 149 at $m/z$ 345 (MH$^+$), 289, 245.

Subsequent modifications by Hegedus et al. to their initial methodology have been made in the case of different substrates. In their synthesis of indoles utilising olefin-Pd complex cyclisation of amines$^{310}$ they found that addition of triethylamine to the reaction was necessary for the formation of any indole. They also noted that with the
simple allyl side chain, cyclisation produced only indoles from attack at the secondary
carbon chain of the olefins. This fits in with our requirement for cyclisation via attack at
the secondary olefin carbon, and the subsequent production of a 5-membered ring. It
was thus decided to try the triethylamine reaction system, and see if this would furnish
any of the desired spirocycle 156.

Accordingly, as outlined in Scheme 5.27, to a slurry of PdCl2(CH3CN)2 in THF was
added 139 and sodium hydride. Triethylamine was added at hourly intervals, and
immediately produced a deep cherry red, homogenous solution which began to deposit
metallic palladium, and yielded a crude yellow oil. TLC analysis revealed baseline
product which suggested the compound was still palladium-complexed. Mass spectral
analysis of the reaction mixture revealed ions at \textit{m/z} 343 ((MH\textsuperscript{+}, 156 C\textsubscript{20}H\textsubscript{26}N\textsubscript{2}O\textsubscript{3}),
345 (MH\textsuperscript{+}, 139) suggesting the presence of some cyclized product 156. A clean sample
of 156 could not be isolated.

![Scheme 5.27 Cyclisation of 139 using PdCl\textsubscript{2}(CH\textsubscript{3}CN)\textsubscript{2}, sodium hydride and triethylamine.](image)

The reaction was repeated at a higher temperature (50°C, 2 days) to try to force the
cyclisation, but without the use of sodium hydride (Scheme 5.28). Mass spectral (Cl\textsuperscript{+})
analysis showed the presence of peaks at \textit{m/z} 343 (C\textsubscript{20}H\textsubscript{26}N\textsubscript{2}O\textsubscript{3}, MH\textsuperscript{+}), corresponding to
the desired product 156, as well as m/z 305, 287, 343, and 156 was isolated in a 30% yield. The newly formed methyl substituent of the spirocyclic derivative was seen in the 

$^1$H NMR as a singlet at $\delta 1.24$ ($^{13}$C NMR $\delta 23.8$), and the single vinyl peak also as a singlet at $\delta 5.29$ ($^{13}$C NMR $\delta 130.9$). The NH proton of the benzylamide precursor was absent from the $^1$H NMR ($\delta 2.98-3.10$). High resolution mass spectral analysis (EI$^+$) confirmed the molecular formula of 156 as C$_{20}$H$_{26}$N$_2$O$_3$. Attempts to improve this reaction yield increased reaction temperatures (to refluxing) and reaction times, but was not achieved.

![Image of chemical structures](image)

Scheme 5.28 Cyclisation of 139 using PdCl$_2$(CH$_3$CN) and triethylamine

Given the efficiency which Hegedus et al.$^{310}$ had achieved in these reactions (yields up to 85%) it appeared that conformational restriction of the amide bond might be a problem. Given the structural nature of our amide derivative 139, containing a fixed quaternary carbon and a rotationally hindered carbonyl, it may be that the conformational restriction of the molecule is such that cyclisation is too unfavourable to achieve more than a 30% yield. The steric bulk of the phenyl ring may add to this effect. In comparison, the planar nature of the benzamide derivative (157) used$^{310}$ lends itself to cyclisation. Additionally, the hindered rotation of the amide carbonyl may in fact
improve the structural conformation of the molecule for attack of the olefin by the nitrogen.

The conformational factors involved in hindering the cyclisation of 139 were investigated using molecular modelling with the Spartan program. The lowest energy conformers of 139 are such that cyclisation between the amide and the double bond is highly unfavoured, as can be clearly seen in Figure 5.4a, where the two functionalities are pointing away from each other. The rotational hindrance about the amide C-N bond in 139 means the nitrogen atom has less potential for interacting with the allyl double bond. Indeed, to achieve a conformation which is favourable for cyclisation (conformer 69), where the nitrogen and allyl methine are pointing toward one another, an energy increase of 7.04 kcal/mol is required (see Figure 5.4b, Table 5.2). Additionally, the low energy conformers of 139 favour hydrogen-bonding occurring between the Boc C=O and N-H, holding the nitrogen down and away from the allyl double bond. This makes the N to C=C distance of 4.41 Å too far for intramolecular interaction and hinders cyclisation (Table 5.2). It should be noted however that with sodium hydride present in the reaction, amide ion formation would be possible thus negating this H-bond stabilisation. It was thus surprising that the cyclisation reactions employing sodium hydride were not successful. The most favourable conformer of 139 for cyclisation is conformer 87, which has the smallest distance from N to C=C of 3.17 Å (Table 5.2).
This is also one of the highest energy conformers, with a relative energy of 8.61 kcal/mol.

Figure 5.4a Spartan Molecular Model of lowest energy conformer of 139; note H-bond interaction (NH to C=O distance = 1.80 Å) and hindered rotation; distance N to C=C = 4.41 Å.

Figure 5.4b Spartan Molecular Model of conformer 69 of 139, the lowest energy conformer favourable for cyclisation: there is no H-bond interaction between the Boc C=O and the N-H; the distance from N to C= is 3.24 Å allowing for intramolecular interaction; a higher energy of 7.42 kcal/mol is required for this conformation.
Chapter Five Synthesis of Spirocyclic Derivatives

5.6 Revised Synthetic Strategy

With the cyclisation of benzylamide derivative 139 proving to be low yielding, the next alternative appeared to be reduction of the amide functionality and cyclisation of the more freely rotating resultant benzylamine derivative. Reduction of the benzylamide carbonyl functionality would remove the amide rotamer problem and allow the nitrogen atom to interact with the allyl double bond. Although there is still potential for hydrogen bonding between the carbamate carbonyl oxygen and the N-H in 158, the free rotation in the amide sidechain of 158 allows for interaction between the N-H and the allyl double bond, and resulting cyclisation (Figure 5.5). Surprisingly, the energy of the lowest energy conformer was higher than that of the amide derivative 139 (see Table 5.2).

Figure 5.5 Conformer 61 of amine 158 (relative energy = 5.05kcal/mol); the amine is free of hindered rotation and the N is in a good position to interact with the C=C (distance = 3.17Å).
A revised synthetic outline for entry to the desired spirocyclic derivative 18 thus incorporated reduction of amide 158 before effecting palladium-assisted cyclisation, Grignard reaction, reduction and deprotection as shown in Scheme 5.29.

\[ \text{Scheme 5.29 Revised synthetic procedure for cyclisation preparation of 18} \]

5.6.1 Reduction of benzylamide 139

In considering methods for the reduction of the amide functionality to the desired amine, it must be remembered that both the Boc group and the allyl group may be sensitive to many reduction agents. For this reason, a selective amide reducing agent was required. Such reagents include diborane\textsuperscript{321-324} and lithium aluminium hydride\textsuperscript{325-328}. Diborane proved to be highly reactive towards the Boc carbamate in refluxing THF, reducing it to the unprotected derivative 159 (Scheme 5.30). Evidence for the formation

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### Table 5.2 Important intramolecular distances and energies for cyclisation precursors

<table>
<thead>
<tr>
<th>Derivative</th>
<th>N to C=C distance (Å)</th>
<th>N-H to C=O distance (Å)</th>
<th>Energy (kcal/mol)</th>
<th>Relative Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>139 (conf. 1)</td>
<td>4.41</td>
<td>1.80</td>
<td>0.38</td>
<td>0.00</td>
</tr>
<tr>
<td>139 (conf. 69)</td>
<td>3.24</td>
<td>4.75</td>
<td>7.41</td>
<td>7.04</td>
</tr>
<tr>
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<td>3.17</td>
<td>6.17</td>
<td>8.99</td>
<td>8.61</td>
</tr>
<tr>
<td>158 (conf. 61)</td>
<td>3.22</td>
<td>-</td>
<td>25.99</td>
<td>5.05</td>
</tr>
</tbody>
</table>

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"Chapter Five Synthesis of Spirocyclic Derivatives"
of 159 included the loss of the entire Boc group from both the $^1$H NMR spectrum ($\delta$1.34 (3 x CH$_3$)) and the $^{13}$C NMR spectrum ($\delta$155.0 (C=O), $\delta$80.5 ((CH$_3$)$_3$CO), $\delta$28.3 (3 x CH$_3$)), and the presence in the spectra of the remaining amide carbonyl at $\delta$176.5 ($^{13}$C NMR) and the new NH proton at $\delta$2.31 ($^1$H NMR). Mass spectral (CI$^+$) analysis of the product supported the formation of 159 (C$_{15}$H$_{20}$N$_2$O) via a molecular ion peak at $m/z$ 245.

![Scheme 5.30 Carbamate Reduction of 139 using diborane](image)

Lithium aluminium hydride has been used to reduce amides to amines, and is reported to be unreactive towards carbamates. It was therefore desired to attempt reduction of benzylamide 139 using lithium aluminium hydride. Accordingly, a solution of 139 (amide) was treated with a solution of lithium aluminium hydride (3 molar equivalents) in THF (Scheme 5.31). After refluxing for 2 hours, the resultant yellow oil was shown to be the unprotected amine 160, with the complete loss of the Boc group accompanying reduction of the amide functionality. This was evidenced by the loss of all the Boc peaks in the $^1$H NMR (9H, s, $\delta$1.34, (CH$_3$)$_3$), and the $^{13}$C NMR ($\delta$28.3 (3 x CH$_3$), $\delta$155.0 (C=O), $\delta$80.5 ((CH$_3$)$_3$CO)), as well as the amide carbonyl peak at $\delta$173.8). The peak for the additional NH proton was evident in the $^1$H NMR at $\delta$2.46. The newly formed methylene from the amide reduction was evidenced at $\delta$2.24 in the $^1$H NMR and $\delta$64.3
in the $^{13}$C NMR. Mass spectral analysis (Cl$^+$) of 160 confirmed the identity of the newly formed derivative as C$_{15}$H$_{20}$N$_2$O via a peak at m/z 229 (M, 100%).

\[
\begin{align*}
\text{139} & \quad \text{160 84% yield} \\
\end{align*}
\]

Reagents and Conditions: 1M LiAlH$_4$/THF (3eq.), 0°C, reflux 2hrs.

**Scheme 5.31** Attempted reduction of 139 using LiAlH$_4$

A second attempt at lithium aluminium hydride reduction of the amide carbonyl reduced the amount of lithium aluminium hydride in THF (1 molar equivalent) to try to contain reduction to the carbonyl group only (Scheme 5.32). However, upon $^1$H NMR analysis of the product it was found that it was in fact the bicyclic derivative 161 which had been formed, and not amine 160. This was evidenced by the loss in the $^1$H NMR spectra of the tert-butyl peak at δ1.34, yet the presence in the $^{13}$C NMR of a urea carbonyl peak downfield at δ163.3. Instead of the expected newly formed methylene peak seen in the $^1$H NMR, it was a methine proton singlet at δ4.63. Additionally, the newly formed tertiary carbon (established by DEPT NMR analysis) was seen at δ82.6 in the $^{13}$C NMR. This unexpected derivative 161 was confirmed by mass spectral (Cl$^+$) analysis, which displayed an MH$^+$ peak at m/z 273 (100%), consistent with the molecular formula C$_{16}$H$_{20}$NO$_2$, which was confirmed by high resolution mass spectral (Cl$^+$) analysis.
The newly formed tertiary carbon in 161 is also a stereogenic centre, and thus with the other stereogenic centre at the ring fusion point, can give rise to cis and trans diastereomers. The two diastereomers were easily separated via chromatography on silica gel. The less polar crystalline product was identified by X-ray crystallography (Professor A.H. White; University of Western Australia) to be the trans diastereomer 161a, as shown in Figure 5.6a, with the allyl side chain and the hydroxyl functionality cis to one another and the benzylamide functionality in the ring. The other oily product appeared to be the cis diastereomer 161b, with the allyl side chain and the hydroxyl functionality trans to one another, on the basis of a very similar series of chemical shifts and multiplicities in the $^1$H NMR spectra. The main difference was that whilst the H3 proton on the proline ring of 161b resonated at $\delta$1.57, in 161a this proton resonated upfield at $\delta$1.04-1.12. Additionally, whilst the peak of the NH proton of 161b was seen at $\delta$3.03-3.15, in 161a it was downfield at $\delta$4.46. High resolution mass spectral analysis (CI$^+$) of both diastereomers 161a and 161b confirmed their molecular formula as C$_{16}$H$_{20}$NO$_2$. 

Scheme 5.32 Preparation of bicyclic diastereomers 161a and 161b via reduction of 139; relative stereochemistry shown.
The diastereomers were characterised with the assistance of gHMBC (Appendix) and NOESY 1D (Figure 5.6b, 5.6c) NMR analysis. Upon irradiation of the methine CH proton at δ4.63, NOESY 1D analysis of 161a indicated NOE interactions between this proton and one of the benzyl protons at δ4.13, the =CH methine proton at δ5.82-5.94 and H3 at δ1.04-1.12 (Figure 5.6b). This supports the methine proton being in a \textit{trans} arrangement to the allyl side chain, as is the case in 161a. Irradiation of the methine proton at δ4.70 in 161b indicated a NOE interaction between this proton and one of the benzyl protons at δ4.13, and the CH$_2$ protons of the allyl side chain at δ2.20 (Figure 5.6c). Further irradiation of the =CH proton of 161b displayed NOE interactions between it and the methine proton at δ4.70, as well as the other allyl protons: =CH$_2$ at δ4.98 and CH$_2$ at δ2.20 (Figure 5.6d). This data supports the methine proton and the allyl proton residing \textit{cis} to each other, as is the case in 161b.

\textbf{Figure 5.6a} X-ray structure of the \textit{trans} diastereomer 161a.
Figure 5.6b NOESY 1D NMR spectrum of 161a: irradiation of the CH proton at δ4.63 indicates NOE interactions with the allyl methine =CH, the H3 protons, and one benzyl CH.

Figure 5.6c NOESY 1D NMR spectrum of 161b: irradiation of the CH proton at δ4.70 indicates NOE interactions with one benzyl CH proton at δ4.13 and the allyl CH2 protons.

Figure 5.6d NOESY 1D NMR spectrum of 161b: irradiation of the allyl =CH proton at δ5.65 indicates NOE interactions with the allyl CH2 protons, the =CH2 protons and the CH at δ4.70.
The *trans* diastereomer 161a shown in Figure 5.6a should easily be cyclized to produce the spiro derivative 162, whereas the *cis* diastereomer 161b is conformationally restricted and would not be expected to undergo cyclisation due to high levels of ring strain.

### 5.6.2 Palladium-catalysed intramolecular cyclisation of 161b

Before the true structural identity of 161b (*cis*) was known, it had been cyclised using PdCl₂(CH₃CN)₂ and triethylamine at room temperature by the method previously used (as shown in Scheme 5.33), to yield the 6-membered ring derivative 163. For 161b to have formed it was first necessary for it to isomerise to the other diastereomer, before undergoing palladium-catalysed cyclisation. That 163 was the 6-membered ring derivative was evidenced by the presence of an additional methylene peak (2H, s, δ2.48) in the ¹H NMR spectrum (δ36.2 in the ¹³C NMR spectrum, confirmed via DEPT analysis) rather than the expected methyl peak of the five-membered ring 162 at δ2.00.

That this methylene singlet represented a cyclic CH₂ of 163 rather than an exocyclic =CH₂ of the intermediate 164 is indicated by its upfield position. This upfield chemical shift of the methylene singlet also indicates that the double bond resides in the enol ether position. Additionally, whereas the exocyclic =CH₂ would reside downfield at approximately δ107, and the accompanying quaternary C= at approximately δ150, the

![Chemical structures](image-url)
additional methylene carbon in question is seen at δ36.2 in the $^{13}$C NMR spectrum, and there are no new quaternary carbons present.

\[ \text{Scheme 5.33 Cyclisation to yield 163 using PdCl}_2(\text{CH}_3\text{CN})\text{ and triethylamine} \]

It appeared then that at room temperature cyclisation conditions furnished the 6-endo-trig cyclisation product 163. With this indication, the reaction was repeated under reflux conditions, in an attempt to yield the 5-exo-trig spirocycle 162. The product of the reaction displayed a molecular ion at \( m/z \) 271 in the mass spectrum (CI$^+$), which was confirmed by high resolution mass spectrometry (CI$^+$) to be representative of the molecular formula C$_{16}$H$_{18}$N$_2$O$_2$, suggestive of desired derivative 162 (C$_{16}$H$_{18}$N$_2$O$_2$). Indeed the $^1$H NMR displayed a methyl singlet at δ2.00, suggestive of 5-exo-trig cyclisation. However, the $^{13}$C NMR contained a carbonyl peak downfield at δ195 which
could not be assigned to the 5-exo-trig spirocycle 162, and the identity of the carbon adjacent to the nitrogen in the pyrrolidine ring (C2) as a methine carbon (DEPT analysis) indicated that the spirocycle had not formed.

Further extensive NMR analysis, including HMBC and $^{15}$N HMBC experiments (kindly undertaken by Dr. Brent Copp at the University of Auckland), revealed that the most likely structure for the product of the cyclisation reaction was that of 165.

![Scheme 5.34 Cyclisation of 161b yields 165.](image)

The HMBC experiments undertaken indicated that the carbonyl signal at δ195 in the carbon NMR spectrum displayed interactions with the singlet representative of the methyl substituent at δ2.00, and some of the pyrrolidine methylene protons (δ1.93-2.08 and δ2.63). The singlet at δ5.57 in the $^1$H NMR spectrum, ascribed to a –CH proton, displayed HMBC interactions with the peaks representative of the carbonyl at δ195.5, the quaternary carbon at δ156.8 (NC=) and the methine proton at δ64.3. The HMBC spectrum did not indicate that the bicyclic parent structure of 161b had been altered.

The $^{15}$N HMBC experimental data of the product 165 indicated interactions of the $^{15}$N peak at δ103, ascribed to that of the pyrrolidine nitrogen, with the methine doublet (δ4.82), and the methylene protons of the pyrrolidine ring (δ3.19, δ3.62, δ2.63, δ2.08). The other $^{15}$N peak at δ132, ascribed to that of the benzyl nitrogen, indicated
interactions with only the benzyl protons (84.60) and the methine proton represented by the peak at 85.37. This data confirms that the bicyclic parent structure of 161b was indeed present in 165, and supports the structure of 165 as shown. The gCOSY, HMBC, $^{15}$N NMBC spectra is given in the appendix.

Figure 5.7a indicates the $^1$H and $^{13}$C assignments for the derivative 165, as assigned by the Chemdraw program; these chemical shifts do not agree entirely with that obtained from the product 165. A three-dimensional representation of the structure of 165 is given in Figure 5.7b (courtesy of Dr. Brent Copp).

Due to time restrictions, the further investigation into the mechanism of this reaction, and the completion of the synthesis of the spirocyclic target ligand 18 could not be completed.

![Figure 5.7a: $^1$H NMR and $^{13}$C NMR chemical shifts assigned by Chemdraw for 165]
Figure 5.7b: Three-dimensional representation of the structure of 165
Chapter Six

Synthesis of Benzo-Fused Spirocyclic Derivatives
Chapter Six Synthesis of Benzofused Spirocyclic Derivatives

6.1 Introduction and Design Rationale

To further extend the range of target ligands, a new set of derivatives which incorporated varied conformations of the adrenaline motif were designed. With a view to increasing the rigidity of the motif, particularly in the positioning of the nitrogen and the aromatic ring, these proposed new derivatives contained spiro[isobenzofuran] structures. Specifically, this new set of target ligands were based upon spiro[isobenzofuran-1(3H), 3'-pyrrolidine] (166) and spiro[isobenzofuran-1(3H),3'-piperidine] (167).

![Adrenaline motif](image)

**Figure 6.1** Spiro[isobenzofuran] target ligands based upon a constrained adrenaline motif

These molecules mimic the adrenaline motif (Figure 6.1), retaining the oxygenated-amine sidechain function (B, C and D) within the two spirocycles. The use of both piperidine and pyrrolidine rings as the nitrogen source would provide slightly different geometrical environments for the ionisable nitrogen and thus for the motif as a whole.

Additional target ligands to be incorporated in the spiro[isobenzofuran] set included the N-methyl 3'-pyrrolidine (168) and 3'-piperidine (169) derivatives, as well as the 4'-piperidine (170), N-methyl-4'-piperidine (171), 2'-pyrrolidine (172) and N-methyl-2'-pyrrolidine (173) derivatives, as well as catechol derivatives 19 and 20. The 2'-piperidine and N-
methyl-2’-piperidine derivatives will not be assessed because the 2-piperidinone precursor required was not commercially available. The addition of N-methyl substituents mimics the adrenaline N-methyl, and will provide information on the effect of alkyl substitution on the nitrogen. Positioning the nitrogen one carbon closer to or one carbon further from the aryl ring will indicate structure-activity relationships specifically between the ionisable nitrogen and the aryl ring. We could thus evaluate the tolerance of the length of the carbon chain, and whether this tolerance is related to or independent of the conformation of the molecule.
To investigate structural aspects of the proposed ligands more clearly, conformational molecular modelling was undertaken using CATALYST®. The rigidity of these ligands is reflected in the lower number of conformers each one was able to generate within the 20kcal/mol energy range, as shown in Table 6.1.

### Table 6.1 Conformational data for the spiro[isobenzofuran] target ligands

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Conformers</th>
<th>Energy Range (kcal/mol)</th>
<th>Derivative</th>
<th>Conformers</th>
<th>Energy Range (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>166</td>
<td>11</td>
<td>17.366</td>
<td>167</td>
<td>11</td>
<td>16.432</td>
</tr>
<tr>
<td>168</td>
<td>8</td>
<td>18.241</td>
<td>169</td>
<td>11</td>
<td>18.204</td>
</tr>
<tr>
<td>19</td>
<td>9</td>
<td>18.715</td>
<td>20</td>
<td>10</td>
<td>18.488</td>
</tr>
<tr>
<td>172</td>
<td>7</td>
<td>14.559</td>
<td>170</td>
<td>9</td>
<td>17.754</td>
</tr>
<tr>
<td>173</td>
<td>6</td>
<td>16.296</td>
<td>171</td>
<td>14</td>
<td>17.400</td>
</tr>
</tbody>
</table>

Representative conformers of 19 and 20 in their most folded arrangements are shown in Figure 6.2. There is only a small difference in the number of conformations accessible with the pyrrolidine and piperidine ring derivatives. The presence of either an N-methyl substituent or diphenolic substituents appears to have little effect on conformation.
How then do these derivatives fit onto the pharmacophore model? Initial pharmacophores (WT1 and Mu1, see Chapter Two) predicted some of the spiro[isobenzofuran] derivatives to have remarkable selectivity for the Cys$^{128}\text{Phe } \alpha_{1B}$ AR (Table 6.2). This trend was also seen in the WT2 and Mu2 pharmacophores. As can be seen in Table 6.2, the majority of these ligands only map two of the Cys$^{128}\text{Phe } \alpha_{1B}$ AR pharmacophore (Mu2) features: the positive ionisable nitrogen and the HBA (represented by the oxygen of the furan ring). In contrast to this, they generally map three of the WT $\alpha_{1B}$ AR pharmacophore (WT2) features. The mappings on to the WT2 and Mu2 pharmacophores of selected target ligands are shown in Figures 6.3a-d. This indicated that this class of derivatives would not provide a suitable paradigm for our model of folded adrenaline mimics. Despite this fact, these ligands are still of interest for obtaining biological data (experimental affinities) and thus
testing our pharmacophore predictions, and building a larger training set for next generation pharmacophore development.

Table 6.2 Predicted binding affinities and selectivities of the spiro[isobenzofuran] target ligands 19, 20, 166-173 upon the wild-type (WT) and Cys\textsuperscript{128}Phe mutant (Mu) pharmacophores.

| Derivative | WT Pharmacophore | | Cys\textsuperscript{128}Phe Pharmacophore | | Selectivity (for mutant over WT) |
|------------|------------------|------------------|------------------|------------------|
|            | WT Pharmacophore | Cys\textsuperscript{128}Phe Pharmacophore | | |
|            | WT1 | WT2 | Mu1 | Mu2 | 1 | 2 |
| 166        | 26000 | 97000 | 3 | 2200 | 4200 | 2 | 10 | 20 |
| 168        | - | 96000 | 3 | - | 4100 | 2 | 20 |
| 172        | - | 490000 | 2 | - | 10000 | 2 | 50 |
| 173        | - | 500000 | 2 | - | 13000 | 2 | 40 |
| 19         | 560000 | 630000 | 2 | 2200 | 4100 | 2 | 260 | 150 |
| 167        | 21000 | 71000 | 3 | 2600 | 3500 | 2 | 10 | 20 |
| 169        | - | 44000 | 3 | - | 3500 | 2 | 10 |
| 170        | - | 25000 | 3 | - | 1900 | 3 | 10 |
| 171        | - | 34000 | 3 | - | 5600 | 3 | 10 |
| 20         | 2900000 | 520000 | 2 | 3300 | 3100 | 2 | 880 | 170 |

*\(K_i\) (nM) * Of a total of 4 features * Of a total of 3 features
Figure 6.3a 166 mapped on WT α1B AR Pharmacophore (WT2), displaying interactions with three of the four proposed binding regions; mesh spheres represent location constraints (where the feature should be in space); Hydrophobic refers to a hydrophobic bonding region; positive ionisable refers to a positive charge feature which interacts with a negative charge on the receptor protein; HBA refers to a hydrogen bond acceptor region. In the HBA region, the smaller sphere represents the interacting feature of the ligand, and the larger one represents the interacting feature of the protein.

Figure 6.3b 166 mapped on Cys128Phe α1B AR Pharmacophore (Mu2) displaying interactions with two of the three four proposed binding regions; mesh spheres represent location constraints (where the feature should be in space); Ring aromatic refers to an aromatic bonding region, the second unmapped sphere represents the interacting pi system in the protein; positive ionisable refers to a positive charge feature which interacts with a negative charge on the receptor protein; HBA refers to a hydrogen bond acceptor region. In the HBA region, the smaller sphere represents the interacting feature of the ligand, and the larger one represents the interacting feature of the protein.
Chapter Six Synthesis of Benzofused Spirocyclic Derivatives

Figure 6.3c 171 mapped on WT $\alpha_{1B}$ AR Pharmacophore (WT2), interacting with three of the four bonding regions. For identification of the coloured spheres, see Figure 6.3a.

Figure 6.3d 171 mapped on Cys$^{128}$Phe $\alpha_{1B}$ AR Pharmacophore (Mu2) interacting with two of the three bonding regions. For identification of the coloured spheres, see Figure 6.3b.
6.2 General Therapeutic Uses for Spiro[isobenzofurans]

There are no accounts in the literature on bioactivity studies of spiro[isobenzofuran-1(3H),3'-pyrrolidines] or spiro[isobenzofuran-1(3H),2'-pyrrolidines] such as target ligands 166 and 172. However, there is an abundance of spiro[isobenzofuran-1(3H),3'-piperidine]- and spiro[isobenzofuran-1(3H),4'-piperidine]- based ligands which have been prepared for potential therapeutic use.

Compounds such as 174 have been prepared as modulators of NPY5 receptor activity, useful in the treatment of a variety of disorders including obesity, bulimia, psychiatric disorders, diabetes and hypertension\(^{329}\). Spiro-fused piperidines such as 175 have been developed as antagonists of the orphanin FQ receptor, useful for the treatment of a number of diverse disorders, including memory and attention deficits, anxiety, stress, depression,
dementias, epilepsy, convulsions, pain, drug withdrawal, control of water balance, Na⁺
excretion and arterial blood pressure disorders, and metabolic disorders such as obesity³³⁰.
The compound YM-35375 (176)³³¹ displays antagonistic binding to both the NK2 receptor
(IC₅₀ 84nM) and NK1 receptor (IC₅₀ 710nM). It was used as a lead compound in the
development of the more potent NK2 receptor antagonist³³² YM-38336 (177), which shows
10 times more potent NK2 receptor binding affinity. Spiro piperidines such as 178 have
been reported to promote the release of growth hormone in humans, and are of potential use
for the treatment of physiological or medical conditions characterized by a deficiency in
growth hormone secretion³³³.

Substituted 1,3-dihydropyrido[isobenzofurans] such as 179 and 180 have been reported as
effective tranquilisers and analgesics³³⁴. The compound 181 was developed as a potential
nervous system agent, inhibiting tetrabenazine-induced depression in mice with an ED₅₀
comparable to that of omipramine and desmethyldimipramine³³⁴b.
Potent affinity of spiro[isobenzofuran-1(3H),4'-piperidine] derivatives at the σ1 and σ2 receptor binding sites has been achieved with 182, which displays IC$_{50}$($σ1$) of 17nM and IC$_{50}$($σ2$) of 0.12nM$^{335}$. This ligand also displays affinity for serotonin 5-HT$_{1A}$ (IC$_{50}$ = 21000nM) and 5-HT$_{2A}$ (IC$_{50}$ = 2000nM), dopamine D$_2$ (IC$_{50}$ = 800nM) and, interestingly, at adrenergic $α_{1A}$ (IC$_{50}$ = 330nM) receptors.

### 6.3 Synthetic Methodologies for the Preparation of Spiro[isobenzofurans]

#### 6.3.1 Previous syntheses of spiro[isobenzofuran-piperidines]

Preparations of spiro[isobenzofuran-1(3H),3'-piperidines]$^{336}$ similar to the target ligands we wished to prepare have been described in the literature. Both 3-substituted and 4-substituted spiro[isobenzofuran-piperidines] have been prepared by Marxer et al.$^{336}$, as outlined in Schemes 6.1 and 6.2. The 4-substitued piperidone derivatives were accessed via both Method A and Method B. The method of Meyers$^{337}$ (Method A, Scheme 6.1), employed the magnesium derivatives of 2-(2-bromophenyl)-4,4-dimethyloxazoline (183) and N-alkylpiperidones (184a, 184b) to yield the piperidinols 185a, 185b, with subsequent hydrolysis to give the phthalides 186a, 186b. The yields of piperidinols 185 were low (~35%), but were improved by using a modification of the method of Hauser$^{338}$ (Method B), whereby the aryllithium compound 187 was reacted with N-alkylpiperidones to provide, upon hydrolysis, the desired phthalide intermediates 186a, 186b. Phthalides 186a, 186b were then reduced to provide the corresponding phthalans which underwent reductive cleavage of the benzyl group to afford the parent secondary amine derivative.
spiro[isobenzofuran-1(3H),4'-piperidine] (170), which may be further alkylated on nitrogen.

\[
\begin{align*}
\text{Method A:} \\
&\text{183} + \text{184a, b} \rightarrow \text{185a, b}.
\end{align*}
\]

\[
\begin{align*}
&\text{Method B:} \\
&\text{187} + \text{184a, b} \rightarrow \text{186a, b}.
\end{align*}
\]

**Scheme 6.1** Methods for the preparation of spiro[isobenzofuran-1(3H),4'-piperidines].

190
In investigating the synthesis of spiro[isobenzofuran-1(3H),3'-piperidines] it was also reported that Method B furnished practically none of the isomeric phthalide 188 from N-benzyl-3-piperidone (189), whilst method A (Scheme 6.2) provided piperidinol 190 in 47% yield. Subsequent acid hydrolysis furnished phthalide 188, which may similarly undergo reduction, followed by reductive removal of the N-benzyl group, to produce the spiro[isobenzofuran-1(3H),3'-piperidine] (167). This then provides a known and successful method whereby target ligands 167 (3-NH), 169 (3-NCH₃), 170 (4-NH), 171 (4-NCH₃) may be achieved directly.

\[
\begin{align*}
\text{Scheme 6.2 Methods for the preparation of spiro[isobenzofuran-1(3H),3'-piperidines].}
\end{align*}
\]

Alternatively, Parhem et al.³³⁹ have prepared spiro[isobenzofuran-1(3H),4'-piperidine] (171) via a method involving coupling of o-bromobenzoic acid with N-methyl-4-piperidone to afford phthalan 186a, followed by reduction with lithium aluminium hydride (Figure 6.4).
Compound 171 was also prepared by cyclisation of 4-[o-(hydroxymethyl)phenyl]-1-methyl-4-piperidinol (191).

\[
\begin{align*}
\text{o-bromobenzoic acid} & \quad \xrightarrow{a, b} \quad \text{186a} & \quad \xrightarrow{c} \quad \text{171} \\
& \quad \text{Reagents and Conditions: a) BuLi; b) N-methyl-4-piperidone; c) LiAlH}_4-BF_3
\end{align*}
\]

**Figure 6.4** Alternate methods for the preparation of spiro[isobenzofuran-1(3H),4'-piperidine] 171.

6.3.2 Synthetic design for the spiro[isobenzofuran-pyrrolidines] and spiro[isobenzofuran-piperidines]

Whilst neither Marxer et al.\textsuperscript{336} nor Parham et al.\textsuperscript{339} extended their methodologies to investigate the synthesis of spiro[isobenzofuran-pyrrolidine] derivatives, it was possible these methods could be useful for the preparation of the set of pyrrolidine target derivatives including 166. Spiro[isobenzofuran-pyrrolidine] derivatives such as 166 and 172 have not been described previously in the literature. Looking at a retrosynthetic analysis (Figure
6.5), it can be seen that the desired spirocycle may be effected via an intramolecular cyclisation between an aryl halide substituent and the pyrrolidine (or piperidine) portion of a bicyclic compound. This is a similar tactic to that employed by Parham et al.\textsuperscript{339} In investigating novel methods, we further looked to the method of Jin et al.\textsuperscript{340}, who prepared annulated vinyl sulfone derivatives such as 192 via a key intramolecular Heck reaction of aryl iodides to vinyl sulfones. As shown in Scheme 6.3, the O-alkylated precursor 193 was prepared from the corresponding arylbenzyl bromide derivative 194 and the desired hydroxy vinyl sulfone 195.

Figure 6.5 Retrosynthetic analysis of spiro[isobenzofuran-1(3H),3'-pyrrolidines].
Chapter Six Synthesis of Benzofused Spirocyclic Derivatives

Scheme 6.3 Preparation of annulated vinyl sulfone 192 via intramolecular Heck cyclisation.²⁴⁰

This method may be modified and applied to the synthesis of target spiro pyrrolidine-derivatives such as 166 and 172, as outlined in Scheme 6.4. Reaction of o-iodobenzyl bromide (196) with the enolate anion of benzyl-3-pyrrolidinone (197) (generated in situ) should afford the spiro precursor 198. Enolate anion generation and selective O-alkylation of N-benzyl-3-pyrrolidinone has successfully been undertaken with the use of both lithium diisopropylamine and lithium hexamethyldisilazide,²⁴¹,²⁴² to selectively form in the presence of triethylsilyl chloride, the triethylsilyl enol ether. Butyl lithium may also generate the desired enolate anion. An intramolecular Heck reaction at the enamine carbon of 198 should afford the desired spiro derivative 199. Removal of the benzyl protecting group would then give access to the target spiro[isobenzofuran-1(3H),3'-pyrrolidine] (166), which may be further methylated if desired to the spiro[isobenzofuran-1(3H),3'-(N-methyl)-pyrrolidine] (168). The commercial availability of precursors o-iodobenzyl alcohol and N-benzyl-3-pyrrolidinone allows for a direct 4-5 step synthesis.
**Scheme 6.4** Proposed synthesis for the preparation of spiro pyrrolidine derivatives 166 and 168.

Furthermore, the incorporation of a methylenedioxy group into the iodobenzyl bromide derivative would potentially furnish, after coupling, Heck reaction and deprotection, the desired catechol derivatives 19 and 200, as shown in Scheme 6.5. Substitution of the N-benzyl-3-pyrrolidinone skeleton with N-benzyl-2-pyrrolidinone would in turn generate catechols of derivatives spiro[isobenzofuran-1(3H),2’-pyrrolidine] (172) and spiro[isobenzofuran-1(3H),2’-(N-methyl)-pyrrolidine] (173).
This approach could also provide a route for the preparation of the spiro-piperidine target ligands. Following the same methodology as just discussed, and as outlined in Scheme 6.6, the synthesis may begin with o-iodobenzyl bromide (with or without phenolic substituents) and commercially available N-benzyl-3-piperidone, to furnish the desired target ligands spiro[isobenzofuran-1(3H),3’-piperidine] (127) and spiro[isobenzofuran-1(3H),3’-(N-methyl)-piperidine] (169), and their catechol derivatives 20 and 205.
Scheme 6.6 Proposed synthesis for the preparation of spiro piperidine derivatives 167, 20, 169 and 208.

Again, substitution of the benzyl-3-piperidone skeleton with \(N\)-benzyl-4-piperidone would in turn generate spiro[isobenzofuran-1(3\(H\)),4'-piperidine] (170) and spiro[isobenzofuran-1(3\(H\)),4'-(\(N\)-methyl)-piperidine] (171), with or without the catechol moiety.
6.4 Synthetic approaches to the Spiro[isobenzofurans]

The synthesis of spiro[isobenzofuran-1(3H),3’-pyrrolidine] (166) and spiro[isobenzofuran-1(3H),3’-piperidine] (167) and derivatives were investigated initially using the modified method of Jin et al., employing the Heck reaction for effecting intramolecular cyclisation, as detailed in Schemes 6.4 and 6.5. For synthetic ease, the methodology was to be established firstly on the phenyl derivatives, before incorporating the catechol derivatives. Unfortunately, time restraints meant that the synthesis of this set of target ligands remained largely unfinished.

6.4.1 Preparation of 3-O-substituted pyrrolidine spiro precursors

The synthesis of 166 therefore began with the high yield preparation of o-iodobenzyl bromide (196) from o-iodobenzyl alcohol (209) as detailed in Scheme 6.7. $^1$H NMR analysis of 196 reflected the loss of the alcohol at $\delta$2.04 from the o-iodobenzyl alcohol spectrum, and a slight upfield shift of the benzyl methylene protons (0.68 ppm) consistent with substitution by the less deshielding bromine. This shift was also clearly displayed in the $^{13}$C NMR spectrum, where the methylene resonance appeared at $\delta$69.3 in 209 and $\delta$38.8 in 196. Mass spectral analysis (Cl+) supported the formula C$_7$H$_6$Ibr for 196 via a molecular ion at m/z 296 ($^{79}$Br) and 298 ($^{81}$Br).
Coupling of o-iodobenzyl bromide to the desired pyrrolidinone (either N-benzyl-3-pyrrolidinone 197 or N-benzyl-2-pyrrolidinone 210) should then lead to formation of the spiro precursors 198 and 211. In the event, reaction of N-benzyl-3-pyrrolidinone with butyl lithium, followed by o-iodobenzyl bromide (as outlined in Scheme 6.8) afforded not the desired 198 (C\textsubscript{18}H\textsubscript{18}NOI), but rather the o-iodobenzyl dimer 212 (C\textsubscript{14}H\textsubscript{12}I\textsubscript{2}). Dimer formation was indicated by the (Cl\textsuperscript{+}) mass spectrum (MH\textsuperscript{+} m/z 434) and in the \textsuperscript{1}H NMR spectrum by the presence of only 2 benzyl methylene peaks at δ3.00 (2H, d, J = 6.3Hz) and δ3.03 (2H, d, J = 6.3Hz), and 8 aromatic methine peaks. There was no evidence of any pyrrolidine derivative whatsoever. This was also reflected in the \textsuperscript{13}C NMR, which displayed only 2 methylene peaks (δ36.5 and δ41.0) and the 12 aromatic carbons for the two aryl rings. The fact that the two benzyl methylenes were represented as separate peaks in both the \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra indicates that the dimer is not able to freely rotate. Given the steric bulk of the two iodo-substituents, it is not surprising that rotation is hindered.

The reaction was repeated twice more, each time the dimer 212 was the product. It appeared that the butyl lithium had not reacted with N-benzyl-3-pyrrolidinone to form the
required enolate anion, but instead an exchange reaction took place with o-iodobenzyl bromide followed by coupling (either via nucleophilic displacement or free radical intermediates)\textsuperscript{143} to afford the substituted dibenzyl derivative (Scheme 6.8). The excess of butyl lithium (1.5 equivalents) allows for this side reaction to have occurred. However, given the excess of o-iodobenzyl bromide also used (1.5 equivalents), and the fact that the dimer was formed in only a 39% yield, it was expected that 98 should still be formed in up to 90% yield.

\[
\begin{align*}
\text{benzyl-3-pyrrolidinone} & \xrightarrow{\text{a, b}} 198 \\
\begin{bmatrix}
\text{Li} & \text{Br} \\
\text{I} & \text{I}
\end{bmatrix} & \xrightarrow{-\text{LiBr}} 212
\end{align*}
\]

Reagents and Conditions: a) BuLi, THF, -78°C, 30 mins; b) 196, THF, -78°C to -30°C, 3hrs, 39% yield

Scheme 6.8 Formation of 212 in the attempted synthesis of spiro pyrrolidine precursor 198.
6.4.2 Preparation of 2-O-substituted pyrrolidine spiro precursors

In view of the difficulties with the preparation of 198 it was decided to move on to the preparation of the 2-pyrrolidine derivative 172. Using the same conditions, N-benzyl-2-pyrrolidinone (210) was treated with butyl lithium, followed by o-iodobenzylbromide. After chromatography, the crude reaction mixture was shown to consist not of the desired O-alkylated benzyl derivative 211, but rather the C-alkylated derivative 213 (C_{18}H_{18}NOI)(Scheme 6.9). This was made evident in the $^{13}$C NMR spectrum, where the presence of the carbonyl functionality remained unchanged at $\delta$175.2. Additionally, the expected $=\text{CH}$ methine proton of the O-alkylated product was absent from both the $^1$H NMR and $^{13}$C NMR spectra, whilst the methine proton of the C-alkylated carbon was observed as a doublet of doublets ($J = 3.0, 7.2$ Hz) at $\delta$2.86 in the $^1$H NMR and the carbon itself at $\delta$42.7 in the $^{13}$C NMR. That C-alkylation had occurred $\alpha$ to the carbonyl functionality, and not on the benzyl carbon (as in 214) was indicated by the N-benzyl protons resonating in the $^1$H NMR as doublets ($J = 9.6$ Hz) at $\delta$2.86 and $\delta$3.42, as would be expected for 213. Additionally, the two o-iodobenzyl protons resonated as a singlet at $\delta$4.47, only slightly upfield from their position in the $^1$H NMR spectrum of o-iodobenzyl bromide. If C-alkylation had produced 214, a single N-benzyl proton would be expected in the $^1$H NMR as a triplet or a doublet of doublets, and the two o-iodobenzyl protons would probably also be exhibited as triplets or doublets of doublets. High resolution mass spectral analysis (CI$^+$) confirmed that the molecular formula of 213 was indeed C_{18}H_{18}NOI. The methine proton at the alkylated carbon of 213 is represented by a doublet of doublets at $\delta$2.86 in the $^1$H NMR, with coupling constants to the adjacent carbon of 3.0 Hz and 7.2 Hz, indicative of equatorial-equatorial and axial-equatorial coupling respectively.
Chapter Six Synthesis of Benzofused Spirocyclic Derivatives

\[ \text{N-benzyl-2-pyrrolidinone} \rightarrow \text{a, b} \rightarrow \text{213} + \text{215} \]

84% yield

15% yield

Reagents and Conditions: a) BuLi, THF, \(-78^\circ\text{C}\), 30 mins; b) 196, THF, \(-78^\circ\text{C}\) to \(-30^\circ\text{C}\), 3hrs.

Scheme 6.9 Synthesis of C-alkylated 213 and the indeno-pyrrole derivative 215.
While the lithium counter ion is known\textsuperscript{343} to favour $C$-alkylation of enolate anions in non-polar solvents through strong $O\cdots Li^+$ complexing, it was thought some $O$-alkylation might still be observed\textsuperscript{344,345}. However, repeating the reaction a number of times led to a mixture of $C$-alkylated product 213 and another derivative being produced in yields of 84\% and 15\% respectively. The products were easily separated via chromatography. NMR spectroscopy established the structure of the new product as 215 ($C_{25}H_{24}NOI$). $^1H$ NMR confirmed the presence of thirteen aromatic protons, four benzylic protons, two next to $N$ as doublets of doublets at $\delta3.57$ ($J = 13.5, 14.7 \text{ Hz}$), the other two next to $O$ as doublets of doublets at $\delta3.70$ ($J = 13.8 \text{ Hz}$), and seven other additional methylene and methine protons, mostly in complex multiplets. There were no double bonds indicated. The $^{13}C$ NMR of 215 and DEPT analysis indicated the presence of two benzylic carbons at $\delta51.3$ and $\delta62.8$, 3 methylene carbons at $\delta28.9$, $\delta32.7$ and $\delta58.3$, and only one methine carbon at $\delta45.6$. The $^{13}C$ NMR and DEPT spectra of 215 also indicated thirteen aromatic CH carbons and six quaternary carbons: suggesting the presence of three aromatic rings, two of which were disubstituted, and only one of which was iodinated ($\delta100.3$). The lack of a ketone carbonyl peak in the $^{13}C$ NMR indicated that formation of an enolate anion followed by $O$-alkylation had occurred. Mass spectrometry ($Cl^+$) analysis gave a molecular ion peak at $m/z$ 482, which correspond to a molecular formula of $C_{25}H_{24}NOI$, confirmed by high resolution mass spectrometry analysis ($Cl^+$). This data supports the structure of the indeno-pyrrole derivative 215. A proposed mechanism for the formation of 215 is given in Scheme 6.9: reaction of the iodo-substituent with butyl lithium would lead to intramolecular cyclisation to provide an alkoxy intermediate, which may react with iodobenzyl bromide by nucleophilic displacement to provide indeno-pyrrole 215.
The relative stereochemistry of 215 is cis ring fusion (as shown), resulting from the steric constraint factors present in the intramolecular aryl anion attack on the lactam carbonyl in 213. Having established the structure of the major C-alkylation product of the reaction as 213 rather than 214, it can be concluded that 215, and not 216 (arising from intramolecular aryl anion attack of 214 and coupling with o-iodobenzyl bromide by the same mechanism as shown above), which may also fit the NMR spectral data, is the correct structure.

O-Alkylated indenopyrroles like 215 have not been reported previously in the literature. N-Alkylated indenopyrrole derivatives 217 and 218 have been reported. The amine 217 was prepared by the reductive amination of 1-oxo-2-indenylacetic acid, followed by reduction and alkylation. Derivatives like 218 were prepared via the condensation of o-allylbenzaldehyde with N-methylamino acids and esters. Amines 218c and 218d exhibit weak oral hypoglycaemic activity, and interestingly 218a inhibits epinephrine biosynthesis. Another known indenopyrrole derivative is the tricyclic lactam 219, stereoselectively prepared via condensation of (E)-3-pentenamide and benzaldehyde in phosphoric acid.
6.4.3 Preparation of piperidine spiro precursors

Although known methodology for the preparation of both spiro[isobenzofuran-3-piperidines] and spiro[isobenzofuran-4-piperidines] has been presented, we wished to investigate the preparation of these derivatives utilising the same Heck coupling as was planned for the pyrrolidinone derivatives (as was shown in Scheme 6.6).

The synthesis began with the coupling of o-iodobenzylbromide (196) and N-benzyl-3-piperidone (189). Accordingly, N-benzyl-3-pyrrolidinone was treated with butyl lithium followed by o-iodobenzylbromide (Scheme 6.10). Purification of the crude crystalline product again furnished the dimer 212 (C_{14}H_{12}I_{2}) as a white solid in 36% yield. High resolution mass spectral and NMR analysis supported the formation of the dimer. This once again suggested that enolate anion formation had not been successful.

Reagents and Conditions: a) BuLi, THF, -78°C, 30 mins; b) 196, THF, -78°C to -30°C, 3hrs, 36% yield

Scheme 6.10 Formation of 212 in the attempted preparation of spiro piperidine precursor 220.
Unfortunately time constraints did not allow for further investigation into these \(O\)-alkylation reactions of iodobenzyl bromide and piperidone and pyrrolidinone derivatives, or any further synthesis of this set of target derivatives. For the preparation of 2-pyrrolidine spiro derivatives, sodium hexamethyldisilazide should be considered as a base to attempt to induce more \(O\)-alkylation of the enolate anion intermediate from 210 and \(\sigma\)-iodobenzyl bromide. For the preparation of 3-pyrrolidine and piperidine spiro derivatives, it may be necessary to investigate an alternate synthesis, or to use one of those reported in the literature for the preparation of spiro-piperidine derivatives.
Chapter Seven

Pharmacological Assessment of Target Ligands
7.1 Introduction

With the successful synthesis of the simple cyclic derivatives and the bicyclic tropane derivatives it was possible to obtain experimental in vitro affinities for these target ligands upon both the wild-type (WT) and Cys^{128}Phe mutant (Mu) \( \alpha_{1B} \)-AR. In this way it would be possible to begin to assess the accuracy of the pharmacophores developed and the success of the design process used. To further examine the potential of these ligands as alternate \( \alpha \)-adrenoceptor ligands, they were also tested upon the \( \alpha_{1A} \) and \( \alpha_{1D} \)-adrenoceptors.

7.2 General Methods

All binding affinity experiments were undertaken by Dr. Songhai Chen, Dr. Angela Finch, or Dr. Edwin Willems at the Victor Chang Cardiac Research Institute, Sydney.

The ligand binding characteristics of membrane expressed receptors (\( \alpha_{1A} \), \( \alpha_{1B} \), Cys^{128}Phe \( \alpha_{1B} \) and \( \alpha_{1D} \)) (Chinese Hamster ovary cells) were determined in a series of radioligand binding studies using [125I]-HEAT, an alpha-1-specific antagonist as the radioligand, as previously described\(^{89,350}\). For competition studies, 200pM of [125I]-HEAT was used with increasing amounts of non-radioligand competing ligand. The membrane concentration used in these studies was selected to allow binding of less than 10% of the total radioligand added. After incubation for 1 hour, the reactions were stopped by addition of HEM-buffer and the membranes collected on Whatman GF/C glass filters using a Brandel Cell Harvester. The filters were washed 3 times with HEM to remove unbound radiolabel and then counted for bound activity using a Packard Auto-gamma
5000 counter. Binding data were analysed using the iterative, non-linear, curve-fitting program, Prism. The HEM buffer was made up as follows: 20mM HEPES pH7.5, 1.4mM EGTA and 12.5mM MgCl₂.

Although predicted binding affinities for the target ligands were measured in Kᵢ values (a feature of CATALYST®), in vitro binding affinities were not measured in Kᵢ, for two reasons: the Kᵢ determination experiments are time consuming, and because many of the ligands bound weakly, the experiments were not warranted. Instead the affinity was measured in terms of IC₅₀, which represents the concentration of unlabelled ligand which inhibits 50% of the radioligand binding. IC₅₀ and Kᵢ are related by the equation:

\[ Kᵢ = \frac{IC₅₀}{1+C/K_d} \]

where K_d is the dissociation constant of the radioligand of concentration C. It is expected that the IC₅₀ value was of the same order of magnitude as the Kᵢ value, and therefore provided a comparative measurement against our preliminary pharmacophore predicted values.

### 7.3 Results of binding affinity assays upon wild-type (WT2) and Cys₁₂⁸ Phe mutant (Mu2) \( \alpha_{1B}-AR \).

The results from the experimental binding assays of the simple cyclic and bicyclic target ligands are given in Table 7.1 and Table 7.2 respectively. They have been compared with the predicted binding affinities of the WT2 and Mu2 pharmacophores. All experimental
values are for the racemic compounds. Additionally, experimental data for the bicyclic ligands and some of the monocyclic ligands is given in the Appendix.

Figure 7.1 shows an example of the dose-concentration curve of the assay for adrenaline (epinephrine), and 14.

**Figure 7.1** Dose-concentration curves for adrenaline (Epi), 100 (4.93, left graphs) and 99 (4.82, right graphs) upon the $\alpha_{1B}$-AR wild-type (top graphs) and Cys$^{128}$Phe (bottom graphs) receptors.
Table 7.1 Predicted and Experimental binding affinities for the simple cyclic target ligands.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>WT α₁B-AR Affinity (nM)</th>
<th>Cys^{128}Phe α₁B-AR</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est. ($K_i$)</td>
<td>Exp. (IC₅₀)</td>
<td>Est. ($K_i$)</td>
</tr>
<tr>
<td>13</td>
<td>$R$</td>
<td>23000</td>
<td>&gt;100000</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>30000</td>
<td>3700</td>
</tr>
<tr>
<td>23</td>
<td>$R$</td>
<td>22000</td>
<td>25000</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>24000</td>
<td>3000</td>
</tr>
<tr>
<td>14</td>
<td>$R$</td>
<td>15000</td>
<td>174000</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>22000</td>
<td>3700</td>
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<tr>
<td>24</td>
<td>$R$</td>
<td>12000</td>
<td>&gt;100000</td>
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<td></td>
<td>$S$</td>
<td>2700</td>
<td>3100</td>
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<tr>
<td>35</td>
<td>$R$</td>
<td>17000</td>
<td>100000</td>
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<td></td>
<td>$S$</td>
<td>14000</td>
<td>18000</td>
</tr>
<tr>
<td>36</td>
<td>$R$</td>
<td>6400</td>
<td>W.B.#</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>27000</td>
<td>3100</td>
</tr>
</tbody>
</table>

* The selectivity could not be determined due to the poor binding affinity data.
# W.B. The binding affinity was too weak to determine an effective IC₅₀.
### Table 7.2 Predicted and Experimental binding affinities for the bicyclic target ligands.

<table>
<thead>
<tr>
<th>Ligand*</th>
<th>WT $\alpha_{1B}$-AR Affinity (nM)</th>
<th>Cys$^{128}$Phe $\alpha_{1B}$-AR</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est. ($K_i$)</td>
<td>Exp. (IC$_{50}$)</td>
<td>Est. ($K_i$)</td>
</tr>
<tr>
<td>52</td>
<td>$R$</td>
<td>20000</td>
<td>3700</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>2700</td>
<td>3000</td>
</tr>
<tr>
<td>58</td>
<td>$R$</td>
<td>22000</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>18000</td>
<td>5200</td>
</tr>
<tr>
<td>60</td>
<td>$R$</td>
<td>18000</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>14000</td>
<td>3200</td>
</tr>
<tr>
<td>101</td>
<td>$R,S$</td>
<td>110000</td>
<td>&gt;30000</td>
</tr>
<tr>
<td>102</td>
<td>$R,R$</td>
<td>81000</td>
<td>&gt;30000</td>
</tr>
<tr>
<td>99</td>
<td>$R,R$</td>
<td>180000</td>
<td>&gt;30000</td>
</tr>
<tr>
<td>100</td>
<td>$R,R$</td>
<td>79000</td>
<td>&gt;30000</td>
</tr>
<tr>
<td>50</td>
<td>$R$</td>
<td>11000</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>$S$</td>
<td>&gt;10000</td>
<td>3100</td>
</tr>
<tr>
<td>54</td>
<td>$R,S$</td>
<td>12000</td>
<td>&gt;30000</td>
</tr>
</tbody>
</table>

* The stereochemistry defined is for the aryl ring; where there are two stereogenic centres present, the first stereocentre denoted is for the aryl ring.

# W.B. The binding affinity was too weak to determine an effective IC$_{50}$.

* The selectivity could not be determined due to the poor binding affinity data.
Chapter Seven Pharmacological Assessment of Target Ligands

13

23

14

24

35

36

52

58

60

101

102

99

100

50

54
Table 7.3 Experimental binding affinities for the bicyclic target ligands on $\alpha_{1A}$- and $\alpha_{1D}$-adrenoceptors.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$\alpha_{1A}$-AR Affinity</th>
<th>$\alpha_{1D}$-AR Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td>IC$_{50}$ (nM)</td>
</tr>
<tr>
<td>58</td>
<td>W.B.*</td>
<td>360</td>
</tr>
<tr>
<td>60</td>
<td>W.B.*</td>
<td>W.B.*</td>
</tr>
<tr>
<td>101</td>
<td>&gt;30000</td>
<td>2840</td>
</tr>
<tr>
<td>102</td>
<td>&gt;30000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>99</td>
<td>43900</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>100</td>
<td>&gt;30000</td>
<td>2210</td>
</tr>
<tr>
<td>50</td>
<td>13100</td>
<td>2910</td>
</tr>
<tr>
<td>54</td>
<td>&gt;30000</td>
<td>&gt;10000</td>
</tr>
</tbody>
</table>

# W.B. The binding affinity was too weak to determine an effective IC$_{50}$. 


7.4 Discussion

7.4.1 Binding of the simple cyclic derivatives

The simple cyclic target ligands bound only weakly to the wild-type $\alpha_{1B}$-AR, and more weakly than was predicted by the pharmacophore. Only one ligand, 23, showed an experimental affinity the same as that predicted. Of significance was that generally the simple cyclic ligands bound better to the Cys$^{128}$Phe $\alpha_{1B}$ mutant receptor than to the wild-type receptor, which was the trend predicted by the pharmacophores. However, the experimental affinities for the Cys$^{128}$Phe $\alpha_{1B}$-AR were generally not as good as had been predicted. The exceptions were the phenyl-piperidinols 14, 36, and to a lesser extent 35, for which the predicted affinity on the Cys$^{128}$Phe $\alpha_{1B}$-AR was very close to the experimental affinity. Additionally, 14 also demonstrated significant experimental selectivity for the Cys$^{128}$Phe $\alpha_{1B}$-AR mutant (a value of 50). Encouragingly, the methylenedioxy (catechol protected) piperidinol derivative 35 also demonstrated selectivity for the Cys$^{128}$Phe $\alpha_{1B}$-AR mutant (a value of 17). The lower selectivity and binding values indicate two major facts: the additional steric bulk of the methylenedioxy substituent is somewhat encumbering the binding process, and the presence of only hydrogen bond acceptor rather than hydrogen bond donor or acceptor functions in the protected methylenedioxy group does not improve binding. Coupled together, these facts present a derivative that has poorer binding ability than the non-functionalised 3-phenyl-piperidin-3-ol 14.

Interestingly, the binding affinities of the N-benzyl derivatives 23 and 36 did not differ markedly from their parent derivatives in either predicted or experimental affinity. Only 24 displayed a markedly decreased affinity on the mutant receptor. This trend suggests that
while the extra aromatic ring is not enhancing affinity of the simple cyclic ligands, it may not be hindering it either.

The 3-phenyl-piperidin-3-ol 14 therefore presents a suitable lead for further structure activity relationship investigations for selective and signalling-specific \( \alpha_{1B} \)-AR ligands. The 3-phenyl-3-piperidinol derivatives have previously been demonstrated to contain potential \( \alpha \)-adrenergic receptor stimulation properties\(^{138} \) (as discussed in chapter 3). In particular, the catechol derivatives of 14 and 35 should be synthesised and evaluated for Cys\(^{128}\)Phe \( \alpha_{1B} \)-AR binding affinity.

7.4.2 Receptor binding of the bicyclic derivatives

The 3-phenyl-3-tropanol derivative 52 displayed poor experimental binding affinity for both the wild-type and the Cys\(^{128}\)Phe \( \alpha_{1B} \)-adrenoceptors. The experimental affinity was one to two orders of magnitude worse than that predicted, and there was no notable selectivity by 52 for the Cys\(^{128}\)Phe over the wild-type \( \alpha_{1B} \)-AR. This indicated that the extra carbon spacer between the aryl moiety and the protonated nitrogen in the 3-phenyl-3-tropanol model (in comparison to the design motif) may be detrimental to binding.

The pharmacophores predicted similar affinities and selectivities for the ketal protected 6-phenyl-6-tropanol derivatives 58 and 60. Unfortunately, these derivatives exhibited very weak binding on both the wild-type and the Cys\(^{128}\)Phe \( \alpha_{1B} \)-adrenoceptors, so effective IC\(_{50}\) values could not be determined. It appears that the extra steric bulk of the ketals present may hinder binding at the adrenaline binding site in these \( \alpha_{1B} \)-adrenoceptors.
The remaining bicyclic ligands all exhibited weak affinity (>30000nM) on both the wild-type and Cys\textsuperscript{128}Phe mutant \(\alpha_{1B}\)-adrenoceptors, indicating no selectivity for the mutant receptor as had been predicted. This lack of difference in binding results restricts any structure-activity relationship trends from being apparent, except for the general trend that the tropane ligands appear to be too bulky to effectively bind to the \(\alpha_{1B}\)-adrenoceptors. It also appears that the lack of selectivity for the Cys\textsuperscript{128}Phe mutant \(\alpha_{1B}\)-adrenoceptor exhibited by these ligands is due either to the steric bulk of the tropane ring, or it may be a reflection of the molecules being too rigid, presenting the potentially interacting features in an incorrect orientation in space.

7.4.3 Discussion of other \(\alpha_{1}\)-adrenoceptor selective ligands

Given the rigidity of the adrenaline motif within the framework of the bicyclic tropane ligands, it was also of interest to assess their pharmacological activities on the \(\alpha_{1A}\)- and \(\alpha_{1D}\)-adrenoceptors. The bicyclic ligands did not exhibit any selectivity for the \(\alpha_{1A}\)-adrenoceptor. However, a number of tropane-based derivatives, 100, 101, 50 and 58, exhibited selectivity for the \(\alpha_{1D}\)-adrenoceptor over the \(\alpha_{1A}\)-, \(\alpha_{1B}\)- and Cys\textsuperscript{128}Phe \(\alpha_{1B}\)-adrenoceptors. Whilst 100, 101 and 51 displayed selectivities for the \(\alpha_{1D}\)-AR of 10- to 15-fold, 58 exhibited 100-fold selectivity for the \(\alpha_{1D}\)-AR, with an IC\textsubscript{50} of 360nM. Given the lack of selective \(\alpha_{1D}\)-AR ligands available, these derivatives therefore provide a promising new lead for the development of selective \(\alpha_{1D}\)-AR ligands.
7.5 Examination of the design motif

From the structure-activity relationship data obtained, it appeared that whilst some of the simple cyclic ligands provided a satisfactory model for the design motif, the conformation of adrenaline in these derivatives was quite extended. In comparison, the increased rigidity of the bicyclic tropane derivatives appeared to cause too much steric bulk to provide effective receptor binding, and in fact no selectivity for those derivatives was seen at all experimentally, despite being predicted by the pharmacophores. To assess the design motif properly, further experimental structure-activity relationship data on the rigid spirocyclic and spiro-fused sets of derivatives is required.

7.6 Examination of pharmacophore

The preliminary pharmacophores developed were only crude, and this is reflected in the poor correlation between predicted affinities and experimental affinities for both the simple cyclic and bicyclic derivatives. With a larger set of experimental affinity data for the Cys^{128}\text{Phe } \alpha_{1B}-adrenoceptors having been obtained, new pharmacophores may be developed which may provide more accurate information with respect to the design motif.
Chapter Eight

Conclusions and Future Directions
A constitutively active Cys\textsuperscript{128}Phe mutant of the α\textsubscript{1B}-AR had previously been identified as a signalling specific receptor, activating only one biochemical signalling pathway, the PLC pathway, and in the absence of ligand\textsuperscript{89}. It had also been shown that this mutant receptor favoured the binding of phenethylamine-type ligands over imidazoline ligands\textsuperscript{89}.

From this lead a set of preliminary pharmacophores was developed to assist in the generation of novel compounds which were predicted to display selective binding to the Cys\textsuperscript{128}Phe α\textsubscript{1B}-AR over the wild-type α\textsubscript{1B}-AR. Although crude, the pharmacophores suggested a folded conformation of adrenaline may be important for stronger binding to the mutant receptor. This was then used as a basis for new ligand design. Ligands were therefore designed as conformationally restricted heterocycles, incorporating the adrenaline design motif. To allow the investigation of a wider range of adrenaline mimic conformations (including folding ones), the ligands were based upon four heterocyclic frameworks: simple cyclic derivatives, bicyclic (tropane) derivatives, spirocyclic derivatives, and benzo-fused spirocyclic derivatives, the syntheses of which were undertaken. Some of these derivatives displayed encouraging predicted selectivities for the Cys\textsuperscript{128}Phe mutant receptor.

In the first category, a total of two pyrrolidinols and four piperidinols were prepared, based on Grignard reagent additions to introduce the aryl and hydroxyl groups related to those in adrenaline. These simple cyclic derivatives displayed some interesting experimental binding affinities, in particular the 3-phenylpiperidin-3-ol derivative 14. Future work should ensure the preparation of the catechol derivative to allow for analysis of the importance of the catechol moiety. Being a direct mimic of adrenaline, the catechol derivative should provide improved binding affinity and interesting selectivity data.
In the second category, stereocontrolled syntheses were developed to a range of 3- and 3,6-disubstituted tropane derivatives, including $6\beta$-phenyltropane-$3\beta,6\alpha$-diol (50). Some new related cyclic hemi-ketal and cyclic ketal compounds were also prepared. These compounds displayed only weak binding to the $\alpha_1$-adrenoceptors, including the wild-type and Cys$^{128}$Phe mutant, however a few compounds, in particular the ketal protected $6\beta$-phenyltropan-6-ol derivative 58, displayed interesting selectivity for the $\alpha_{1D}$ receptor. This compound may provide an initial lead for the design of selective $\alpha_{1D}$ ligands, of which there are very few. Future work on this set of ligands should again focus on the preparation of the catechol target derivative 53, and an investigation of its binding affinity and selectivity properties on the wild-type and Cys$^{128}$Phe $\alpha_{1B}$-ARs. It would also be of interest to assess the binding properties of this ligand on the $\alpha_{1A}$- and $\alpha_{1D}$-ARs.

Synthesis of the spirocyclic class of target derivatives was, unfortunately, not completed. The synthetic pathway to the spirocyclic precursor 2-(2-propenyl)-l-pyrrolidinecarboxylic acid,l-(l,l-dimethylethyl)-2-benzamide (139) has been established in high yield, and subsequent palladium-assisted cyclisation to yield 1-benzyl-5-methyl-2,3-dihydropyrrol-2-one-3-spiro-2'-pyrrolidine-1'-carboxylic acid,l-(1,1-dimethylethyl) ester (156) has been achieved, albeit in low yield. This cyclisation should be repeated, and the spirocyclic target ligand 18 accessed via Grignard reaction and deprotection. In this way the binding affinity of 18 may be determined. To improve the quality of the preparation of 18, the reduction of the benzamide 139 to the corresponding amine, 2-(2-propenyl)-1,2-pyrrolidinedicarboxylic acid,l-(1,1-dimethylethyl) ester (159) could be further investigated. If experimental binding affinities support those predicted, the stereoselective synthesis of 18a (and its catechol derivative) should be pursued, as 18a provides a good lead for selective binding to the
Cys$^{128}$Phe $\alpha_{1B}$-AR. The 8-dihydro (17) and 8-hydroxy (103) ligands (and their catechol derivatives) should also be prepared and assessed.

Syntheses of the benzo-fused spirocyclic ligands were also unfinished. The desired $O$-alkylation of the N-benzyl pyrrolidinones and N-benzyl piperidone was not achieved and methods for $O$-alkylation need to be reassessed with alternate reagents. Alternatively, the synthesis of these derivatives may be pursued via known literature methods, to allow for the assessment of binding affinities. The $C$-alkylation of N-benzyl pyrrolidine-2-one with iodobenzylbromide, followed by intramolecular cyclisation and further $O$-alkylation, led to the formation of a new heterocyclic compound, 
\[\text{cis-1-benzyl-8b-(2-iodobenzyloxy)-}1,2,3,3\text{a,4,8b-hexahydroinden}[1,2-b]\text{pyrrole (215)}.\]

The experimental binding affinities for 15 new ligands upon the wild-type and Cys$^{128}$Phe $\alpha_{1B}$-ARs have been obtained. Although most of the ligands displayed weak affinity, and some very weak binding, the data will allow for a more realistic, less biased, training set, especially with the larger range of structural variation now available. From the new training set, second generation pharmacophores may be developed. Unfortunately time constraints did not allow for the new pharmacophore to be generated here. These pharmacophores may be compared with the pharmacophores used in this Thesis, and should be used to further design ligands selective for the Cys$^{128}$Phe $\alpha_{1B}$-adrenoceptor.

Such selective ligands should conform with the activated state of the Cys$^{128}$Phe receptor, and it is thought that these ligands might display signalling-specificity in the wild-type $\alpha_{1B}$-AR.
Chapter Nine

Experimental Methods and Data
9.1 Methods for Pharmacophore Development

All pharmacophore development was undertaken using the CATALYST® program, versions 4.0, 4.5, and 4.7, from Accelrys. CATALYST® 4.0 and 4.3 were used on a Silicon Graphics Workstation, CATALYST® 4.7 was used on a Silicon Graphics SGI Fuel Workstation. See Chapter Two for methods used for generation of the training set (section 2.2), conformer generation (section 2.2.1) and pharmacophore development and evaluation (sections 2.3 and 2.5).

9.2 Methods for Pharmacological Assessment of Ligands

Methods for the pharmacological assessment of synthesised target ligands upon α₁-adrenoceptors (including α₁A, α₁B, α₁B-Cys¹²⁸Phe and α₁D) are given in Chapter Seven (7.2).

9.3 General Experimental for Synthesis

Melting point determinations were carried out on a Griffin melting point apparatus. Optical rotation values were determined on a Jasco DIP-300 polarimeter. Low resolution chemical ionisation (isobutane, CI⁺) mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer by a direct insertion technique with an electron beam of 70eV. High-resolution mass spectra (HRMS) (methane, CI⁺ and EI⁺) were determined on a VG
Autospec spectrometer using PFK (perfluorokerosene) as the internal standard. The $m/z$ values are stated with their peak intensities as a percentage in parentheses.

Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity 300 MHz or a Varian Mercury 300 MHz spectrometer. Two dimensional NOESY spectra were obtained on a Varian Innova 500 MHz spectrometer. HMBC $^{15}$N NMR experiments were undertaken by Dr. Brent Copp at The University of Auckland, on a 400 MHz NMR, according to published methods\textsuperscript{351}. Spectra were recorded in deuterated chloroform, unless otherwise specified, and referenced to the residual non-deuterated solvent signal. Chemical shifts ($\delta$) in ppm were measured relative to the internal standard. Where samples exhibited rotamers, the minor form is recorded in parentheses and is indicated by an asterisk.

X-Ray crystallography was undertaken by Prof. A.H. White at The University of Western Australia.

Analytical thin layer chromatography (TLC) was carried out on Merck Silica gel 60 F\textsubscript{254} pre-coated aluminium plates with a thickness of 0.2mm. Preparative TLC was done on Merck Silica gel 60 PF\textsubscript{254} coated onto glass plates with a thickness of 0.1mm. Chromatography refers to column chromatography, performed under ‘flash’ conditions on Merck Silica gel 60 (230-400 mesh). Chromatography solvent mixtures were measured by volume, and are reported in parentheses. The $R_f$ values given refer to the determination of a sample on TLC in the same solvent as noted for the chromatography.

Organic extracts were dried with anhydrous sodium sulphate, and the solvent was removed under reduced pressure (\textit{in vacuo}) with a Büchi rotary evaporator.
Anhydrous solvents were purified and dried according to Perrin and Armarego\textsuperscript{352} and distilled immediately before use. Other solvents used were AR grade, and were used as received, with the exception of dichloromethane (DCM) and hexane, which were LR grade and were distilled before use. Starting materials and reagents were purchased from Sigma Aldrich Pty Ltd and were used as received.

Microwave reactions were performed in a Milestone Ethos Sel Microwave Solvent Extractor, employing Easywave software, and using internal reaction temperature control. Sealed Teflon reaction vessels were used, and were washed with concentrated nitric acid between uses.

Molecular modelling was undertaken using Spartan version 5.0 (Wavefunction Inc.) on a Silicon Graphics workstation.

For referencing of the proton and carbon NMR data for all the derivatives in the spirocyclic section, the numbering system used is based upon that of pyrrolidine.

\textbf{9.4 Experimental for Chapter Three: Synthesis of Monocyclic Derivatives}

\textit{Synthesis of 1-benzyl-3-phenyl-pyrrolidin-3-ol (23).}

To 1-benzyl-3-pyrrolidinone (0.092g, 0.525mmol) in anhydrous diethyl ether (5mL) under nitrogen was added phenylmagnesium bromide (3M in ether, 0.2lmL, 0.63mmol) dropwise via syringe. Stirring continued at room temperature for 3 hours, after which time the reaction mixture was taken up in ethyl acetate (20mL) and water (20mL) and separated. The aqueous layer was extracted with ethyl acetate (3x50mL). The combined organic
extracts were washed with sodium chloride (sat.) (50mL), water (2x50mL), then dried (sodium sulphate) and concentrated in vacuo to yield a brown oil. Chromatography (1:8 ethyl acetate:hexane) provided 23 (C_{17}H_{19}NO) as a brown oil (0.092g, 69% yield). $^1$H NMR (δ): 2.16-2.25 (1H, m, H4); 2.37 (1H, ddd, J_4',4 = 9.6, J_{4',5} = 9.3, J_{4',5'} = 4.8, H4'); 2.58 (2H, ddddd, J = 9.6, 9.3, 6.0, 6.3, 3.3, H5, H5'); 2.85 (1H, bs, OH); 2.98 (1H, d, J = 9.6, H2); 3.14 (1H, ddd, J = 9.3, 4.8, 4.8, H2'); 3.73 (2H, s, CH_{2}Ph); 7.24-7.36 (8H, m, ArH x 8); 7.48 (1H, d, J = 1.2, ArH); 7.50 (1H, d, J = 0.9, ArH). $^{13}$C NMR (δ): 42.1 (CH_{2}, C4); 52.9 (CH_{2}, C5); 60.1 (CH_{2}, C2); 68.7 (CH_{2}Ph); 80.9 (C3); 125.5, 127.3, 127.4, 128.4, 128.6, 129.0 (ArCH x 10); 138.9 (ipso C (benzyl)); 144.4 (ipso C). MS (Cl^+): m/z 254 (M+1, 100%); 236 (-H_{2}O, 55%). HRMS (Cl^+): Found: 254.1533; Required: 254.1545 for C_{17}H_{20}NO.

**Synthesis of 3-phenyl-pyrrolidin-3-ol (13).**

A suspension of 23 (0.400g, 1.58mmol) and Pd/C (10%) (0.165g) in anhydrous ethanol (15mL) was flushed with nitrogen, followed by hydrogen, and stirred under a hydrogen atmosphere at room temperature for 44 hours. The reaction mixture was filtered through celite and concentrated to yield 13 (C_{10}H_{13}NO) as a yellow oil, (0.277g, 100% yield). No purification was needed. $^1$H NMR (δ): 2.09-2.13 (1H, m, H4); 2.16-2.19 (1H, m, H4'); 2.94 (1H, d, J = 11.4, H2); 3.06-3.14 (4H, m, NH, OH, H5, H5'); 3.28 (1H, m, J = 11.4, H2'); 7.26-7.37 (3H, m, ArH x 3); 7.45 (1H, d, J = 1.5, ArH); 7.47 (1H, d, J = 1.5, ArH). $^{13}$C NMR (δ): 42.0 (CH_{2}, C4); 46.2 (CH_{2}, C5); 58.3 (CH_{2}, C2); 82.3 (C3); 125.5, 127.4, 128.6, (ArCH x 5); 144.5 (ipso C). MS (Cl^+): m/z 164 (M+1, 100%); 146 (-H_{2}O, 100%). HRMS
Found: 164.1070; Required: 164.1075 for C\textsubscript{10}H\textsubscript{14}NO. The hydrochloride salt was made.

**Synthesis of 1-benzyl-3-phenyl-piperidin-3-ol (25).**

To 1-benzyl-3-piperidone (0.862g, 4.55mmol) in anhydrous diethyl ether (15mL) under nitrogen was added phenylmagnesium bromide (3M in ether, 1.82mL, 5.46mmol) dropwise via syringe. Stirring continued at room temperature for 3 hours, after which time the reaction mixture was taken up in ethyl acetate (20mL) and water (20mL) and separated. The aqueous layer was extracted with ethyl acetate (3x50mL). The combined organic extracts were washed with sodium chloride (sat.) (50mL), water (2x50mL), then dried (sodium sulphate) and concentrated \textit{in vacuo} to yield a brown oil. Chromatography (1:4 ethyl acetate:hexane) provided 25 (C\textsubscript{18}H\textsubscript{21}NO) as a white solid, which was recrystallised from ethanol (1.04g, 86% yield). Mp: 116-119°C. \textit{\textsuperscript{1}H NMR (δ)}: 1.65-1.71 (1H, m, H4); 1.76-1.81 (2H, m, H5, H5'); 1.95-2.12 (2H, m, H4', H6); 2.33 (1H, d, J = 11.1, H2); 2.76 (1H, d, J = 11.1, H2'); 2.94 (1H, dd, J = 1.8, 7.5, H6'); 3.59 (2H, 2 x s, CH\textsubscript{2}Ph); 3.98 (1H, s, OH); 7.26-7.38 (8H, m, ArH x 8); 7.50 (1H, d, J = 1.2, ArH); 7.52 (1H, d, J = 1.2, ArH). \textit{\textsuperscript{13}C NMR (δ)}: 21.9 (CH\textsubscript{2}, C5); 36.2 (CH\textsubscript{2}, C4); 53.0 (CH\textsubscript{2}, C6); 62.6 (CH\textsubscript{2}, C2); 65.1 (CH\textsubscript{2}Ph); 71.1 (C3); 124.7 (Benzyl ArCH); 127.2 (ArCH); 128.1 (Benzyl ArCH x 2); 128.3 (ArCH x 2); 128.9 (ArCH x 2); 137.8 (ipso C (benzyl)); 145.6 (ipso C). MS(CI\textsuperscript{+}): m/z 268 (M+1, 100%); 250 (-H\textsubscript{2}O, 50%). HRMS (CI\textsuperscript{+}): Found: 268.1700; Required: 268.1701 for C\textsubscript{18}H\textsubscript{22}NO.
Synthesis of 3-phenyl-3-piperidin-3-ol (14).

A suspension of 25 (0.550g, 2.06mmol) and Pd/C (10%) (0.55g) in anhydrous ethanol (15mL) was flushed with nitrogen, followed by hydrogen, and stirred under a hydrogen atmosphere at room temperature for 44 hours. The reaction mixture was filtered through celite and concentrated to yield 14 (C_{11}H_{15}NO) as a colourless oil (0.249g, 68% yield). No purification was needed. $^1$H NMR (δ): 1.61 (1H, bd, J = 9.0, H4); 1.85-1.97 (3H, m, H5, H5', H4'); 2.60 (1H, dd, J = 9.9, 11.1, H6); 2.87 (1H, s, OH); 3.05-3.10 (3H, bm, H2, H2', H6'); 3.67 (1H, d, J = 6.3, NH); 7.24-7.36 (3H, m, ArH x 3); 7.49 (2H, s, ArH x 2). $^{13}$C NMR (δ): 22.4 (CH_{2}, C5); 36.9 (CH_{2}, C4); 46.1 (CH_{2}, C6); 57.8 (CH_{2}, C2); 70.8 (C3); 124.9, 127.2, 128.4 (ArCH x 5); 146.5 (ipso C). MS(Cl$^+$): m/z 178 (M+1, 100%); 160 (-H$_2$O, 85%). HRMS (Cl$^+$): Found: 178.1228; Required: 178.1232 for C_{11}H_{16}NO. The hydrochloride salt was made.

Synthesis of 1-benzyl-3-(3,4-methylenedioxyphenyl)-piperidin-3-ol (36).

To 1-benzyl-3-piperidone (0.39g, 2.06mmol) in anhydrous THF (15mL) under nitrogen was added 3,4-methylenedioxyphenylmagnesium bromide (1M in THF, 2.47mL, 2.47mmol) dropwise via syringe. Stirring continued at room temperature for 3 hours, after which time the reaction mixture was taken up in ethyl acetate (20mL) and water (20mL) and separated. The aqueous layer was extracted with ethyl acetate (3x50mL). The combined organic extracts were washed with sodium chloride (sat.) (50mL), water (2x50mL), then dried (sodium sulphate) and concentrated in vacuo to yield a brown oil. Chromatography (1:8 ethyl acetate:hexane) provided 36 (C_{19}H_{21}NO_{3}) as a yellow oil, which crystallised to a white solid upon standing, and was recrystallised from ethanol.
(0.33 g, 52% yield). Mp: 82-84°C. $^1$H NMR (δ): 1.61-1.81 (3H, m, H4, H5, H5'); 1.90-2.07 (2H, m, H6, H4'); 2.24 (1H, d, J = 11.1, H2); 2.70 (1H, d, J = 11.1, H2'); 2.89 (1H, dd, J = 8.7, H6'); 3.56 (2H, 2 x s, CH2Ph); 4.93 (1H, s, OH); 5.91 (2H, s, OCH2O); 6.75 (1H, d, J = 7.8, aryl CH); 6.93 (1H, d, J = 7.8, aryl CH); 7.02 (1H, s, aryl CH); 7.29 (5H, s, ArH x 5).

$^{13}$C NMR (δ): 22.0 (CH2, C5); 36.4 (CH2, C4); 53.0 (CH2, C6); 62.6 (CH2, C2); 65.3 (CH2Ph); 71.1 (C3); 100.8 (OCH2O); 105.8, 107.7, 117.7 (aryl ArCH x 3); 127.1, 128.2, 128.8 (Benzyl ArCH x 5); 137.7 (ipso C aryl); 139.8 (ipso C benzyl); 146.1 (ipso C aryl); 147.3 (ipso C aryl). MS (Cl$^+$): m/z 312 (M+1, 100%); 294 (-H2O, 70%). HRMS (Cl$^+$): Found: 312.1604; Required: 312.1600 for C19H22N03.

**Synthesis of 3-(3,4-methylenedioxyphenyl)-piperidin-3-ol (35).**

A suspension of 36 (0.200 g, 0.642 mmol) and Pd/C (10%) (0.200 g) in anhydrous ethanol (6 mL) was flushed with nitrogen, followed by hydrogen, and stirred under a hydrogen atmosphere at room temperature for 44 hours. The reaction mixture was filtered through celite and concentrated to yield 35 (C12H15N03) as a colourless oil (0.100 g, 65% yield). $^1$H NMR (δ): 1.56-2.03 (5H, m, H4, H4', H5, H5', NH); 2.27-2.73 (2H, m, H6, H6'); 2.78 (1H, s, OH); 3.02 (2H, d, J = 13.2, H2, H2'); 5.91 (2H, s, OCH2O); 6.75 (1H, dd, J = 8.1, 2.7, aryl CH); 6.94 (1H, ddd, J = 8.1, 9.3, 1.5, aryl CH); 7.03 (1H, dd, J = 1.8, 11.1, aryl CH). $^{13}$C NMR (δ): 22.5 (CH2, C5); 36.8 (CH2, C4); 46.0 (CH2, C6); 58.0 (CH2, C2); 70.3 (C3); 100.7 (OCH2O); 105.7, 107.7, 117.6 (aryl ArCH x 3); 140.5 (ipso C aryl); 146.0 (ipso C aryl); 147.3 (ipso C aryl). MS (Cl$^+$): m/z 222 (M+1, 100%); 204 (-H2O, 95%). HRMS (Cl$^+$): Found: 222.1116; Required: 222.1130 for C12H16N03. The hydrochloride salt was made.

230
Attempted synthesis of 1-benzyl-3-(3,4-dihydroxyphenyl)-piperidin-3-ol (37) utilising boron trichloride/tetrabutylammonium iodide.

To a stirred mixture of 36 (0.020g, 0.064mmol) and tetrabutylammonium iodide (0.073g, 1.984mmol) in anhydrous DCM (1mL) at -78°C under nitrogen was added boron trichloride (1M in DCM, 0.32mmol) dropwise over 2 mins. After 5 mins the solution was slowly warmed to room temperature and stirred for 1.5 hours. The reaction mixture was quenched with ice and stirred for 30 mins, diluted with aqueous sodium hydrogen carbonate (sat.) (at which point the solution turned blue in colour) and extracted with DCM. The combined organic extracts were washed with water, dried (sodium sulphate) and the solution concentrated in vacuo to yield a mixture of colourless crystalline material and some blue residue (0.070g). $^1$H NMR indicated the presence of neither 37, nor unreacted 36. The residue was not identified. $^1$H NMR (δ): 1.01 (4H, t, $J$ = 7.2); 1.46 (3H, q, $J$ = 7.2, 15.0, 7.8); 1.63-1.71 (3H, m); 3.35 (3H, dd, $J$ = 8.4, 9.0); $^{13}$C NMR (δ): 13.6; 19.7; 24.2; 59.0; 138.0.

Alternatively, to a stirred mixture of 0.2M 36 (0.22g, 0.707mmol) in anhydrous DCM (2.36mL) and tetrabutylammonium iodide (0.81g, 2.192mmol) at -78°C under nitrogen was added boron trichloride (1M in DCM, 3.54mL) dropwise over 2 minutes. Stirring was continued at -78°C for 1 hour. The reaction mixture was quenched with ice and stirred for 30 mins, then diluted with aqueous sodium hydrogen carbonate (sat.) (10mL) and extracted with DCM (2x20mL). The combined organic extracts were washed with water, dried (sodium sulphate) and the solution concentrated in vacuo to yield a yellow oil which crystallised upon standing. Chromatography (ethyl acetate, followed by 1:10
methanokethyl acetate, \( R_f 0.06 \) yielded colourless crystals (0.190g), which proved to be unreacted 36. \(^1\)H NMR (\( \delta \)): 1.61-1.81 (3H, m, H4, H5, H5'); 1.90-2.07 (2H, m, H6, H4'); 2.24 (1H, d, \( J = 11.1 \), H2); 2.70 (1H, d, \( J = 11.1 \), H2'); 2.89 (1H, dd, \( J = 8.7 \), H6'); 3.56 (2H, 2 x s, \( CH_2Ph \)); 4.93 (1H, s, OH); 5.91 (2H, s, OCH2O); 6.75 (1H, d, \( J = 7.8 \), aryl CH); 6.93 (1H, d, \( J = 7.8 \), aryl CH); 7.02 (1H, s, aryl CH); 7.29 (5H, s, ArH x 5).

**Attempted synthesis of 1-benzyl-3-(3,4-dihydroxyphenyl)-piperidin-3-ol (37) utilising boron tribromide.**

A 1M solution of boron tribromide in DCM (1.64mL) was added to a stirred solution of 36 (0.100g, 0.529mmol) in anhydrous DCM (10mL) under nitrogen. Stirring was continued for 22 hours with heating at reflux. To the cooled solution was added methanol (10mL), and the solvent was removed \textit{in vacuo} to yield a crude red oil, which proved to be unreacted 36 (C\(_{19}\)H\(_{21}\)N\(_{2}\)O\(_3\)). \(^1\)H NMR (\( \delta \)): 1.61-1.81 (3H, m, H4, H5, H5'); 1.90-2.07 (2H, m, H6, H4'); 2.24 (1H, d, \( J = 11.1 \), H2); 2.70 (1H, d, \( J = 11.1 \), H2'); 2.89 (1H, dd, \( J = 8.7 \), H6'); 3.56 (2H, 2 x s, \( CH_2Ph \)); 4.93 (1H, s, OH); 5.91 (2H, s, OCH2O); 6.75 (1H, d, \( J = 7.8 \), aryl CH); 6.93 (1H, d, \( J = 7.8 \), aryl CH); 7.02 (1H, s, aryl CH); 7.29 (5H, s, ArH x 5).

**Attempted synthesis of 1-benzyl-3-(3,4-dihydroxyphenyl)-piperidin-3-ol (37) utilising bismuth nitrate pentahydrate.**

To a stirred solution of 36 (0.100g, 0.321mmol) in ethanol (10mL) was added bismuth nitrate pentahydrate (0.117g, 0.241mmol). Stirring was continued at 70°C for 70 hours. MS (Cl\(^+\)) analysis of a sample of the reaction mixture showed no reaction had taken place (\( m/z 312 \)). The reaction mixture was then concentrated, taken up in acetonitrile (10mL), further bismuth nitrate pentahydrate (0.100g, 0.206mmol) was added and the mixture was heated at
reflux for 2 days. MS (Cl⁻) analysis of the reaction mixture still showed no reaction had taken place.

**Attempted synthesis of 1-benzyl-3-(3,4-dihydroxyphenyl)-piperidin-3-ol (37) utilising cerium chloride heptahydrate.**

To a stirred suspension of 36 (0.020g, 0.064mmol) and sodium iodide (0.0014g, 0.010mmol) in acetonitrile (2mL) was added cerium chloride heptahydrate (0.036g, 0.096mmol) and the mixture stirred at room temperature for 24 hours, followed by 16 hours heating at reflux. MS (Cl⁻) analysis of the crude reaction mixture showed unreacted 36 only: m/z 312 (M+1 (C₁₉H₂₁N₃O₃)), 294 (-H₂O).

**Attempted synthesis of 3,4-dihydroxybenzaldehyde (39) utilising boron trichloride/tetrabutylammonium iodide.**

To a stirred solution of piperonal (38) (0.100g, 0.666mmol) and tetrabutylammonium iodide (0.271g, 0.733mmol) in anhydrous DCM (1.33mL) at -78°C was added boron trichloride (1M in DCM, 1.665mL) dropwise. Stirring was continued at -78°C for 1 hour, and then the mixture was warmed to room temperature with stirring for 40 hours. TLC of the reaction mixture showed unreacted 38 only (C₈H₆O₃). MS (Cl⁺): m/z 151 (M+1, 100%).

**Attempted synthesis of 3,4-dihydroxybenzaldehyde (39) using boron tribromide.**

To a stirred solution of piperonal (0.100g, 0.666mmol) in anhydrous DCM (2mL) at -78°C was added boron tribromide (1M in DCM, 2.33mL) dropwise. Stirring was continued at -78°C for 1 hour, after which time the mixture was poured into DCM (20mL) and water (10mL). The organic extract was separated, dried (sodium sulphate) and concentrated in
to yield a black residue. The black residue was stirred in DCM overnight, filtered and concentrated in vacuo to yield a colourless oil, which rapidly turned to a brown residue (0.048g). The crude mixture proved to be a 1:1 mixture of 39 and 38 by $^1$H NMR; MS (Cl$^+$): m/z 139 (M+1, 39, 100%); 151 (M+1, 38, 5%).

**Attempted synthesis of 3,4-dihydroxybenzaldehyde (39) using bismuth nitrate pentahydrate.**

a) A solution of piperonal (38) (0.050g, 0.333mmol) and bismuth nitrate pentahydrate (0.323g, 0.666mmol) in acetonitrile (5mL) was heated at reflux for 17 hours. MS (Cl$^+$) analysis of the reaction mixture: m/z 139 (M+1, 39, 10%), 151 (M+1, 38, 100%).

b) A solution of piperonal (38) (0.025g, 0.167mmol) and bismuth nitrate pentahydrate (0.041g, 0.084mmol) in acetonitrile (8mL) was irradiated with microwave radiation at 100°C for 30 mins. MS (Cl$^+$) analysis of the reaction mixture: m/z 151 (100%).

9.5 Experimental for Chapter Four: Synthesis of Bicyclic Derivatives

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyclo[3.2.1]octan-3-one (65).**

Tropinone (2.0g, 14.38mmol), 2,2,2-trichlorehylchloroformate (2.18ml, 15.82mmol) and dry potassium carbonate (2.0g, 14.38mmol) were heated at reflux in anhydrous benzene (30mL) under argon for 22 hours. Benzene was removed in vacuo, azeotroping with methanol. The crude reaction mixture was taken up in ethyl acetate (100mL) and water (100mL) and separated. The aqueous fraction was extracted with ethyl acetate (3x100mL)
and the combined organic fractions were washed with sodium chloride (sat.) (100mL), water (2x100mL), dried (sodium sulphate) and concentrated in vacuo to yield 65 \((C_{10}H_{12}NO_3Cl_3)\) as a pale yellow oil, which crystallised under high vacuum to an off-white solid (3.59g, 83% yield). Further purification was not required. Mp: 144-146°C. \(^1\)H NMR (\(\delta\)): 1.76 (2H, q, H7a, H6a, \(J_{H7a,7e} = 8.1\), \(J_{H6a,6e} = 8.1\), \(J_{H7a,6e} = J_{H6a,7e} = 3.6\)); 2.18 (2H, d, H6e, H7e, \(J = 4.8\)); 2.42 (2H, d, H2e, H4e, \(J_{H2a,2e} = J_{H4a,4e} = 16.2\)); 2.75 (2H, d, H2a, H4a, \(J_{H2a,2e} = J_{H4a,4e} = 16.2\)); 4.67 (2H, dd, \(J_{CH,CH'} = 4.8\)); 4.71 (1H, H1, \(J_{H1,7} = 12.0\)); 4.94 (1H, H5, \(J_{H5,6} = 12.0\)). \(^{13}\)C NMR (\(\delta\)): 28.4 (CH2, C7(C6)); 29.3 (CH2, C6(C7)); 48.6, 49.1 (2x CH2, C2, C4); 53.4 (2xCH, C1, C5); 74.7 (OCH2), 151.6 (NC=O); 207.2 (C=O, C3). MS (CI\(^+\)): \(m/z\) 300/302/304 (MH\(^+\), \(^{34}\)Cl, 24%); 302 (MH\(^+\), \(^{35}\)Cl); 304(MH\(^+\), \(^{37}\)Cl); 264/266 (\(^{34}\)Cl,10%); 126 (100%). HRMS (CI\(^+\)): Found: 299.9945; Required: 299.9961 for \(C_{10}H_{13}NO_3^{35}\)Cl3.

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-3α-phenyl-8-azabicyclo[3.2.1]octan-3β-ol (66).**

To a stirred solution of 65 (1.00g, 3.33mmol) in anhydrous diethyl ether (15mL) under a nitrogen atmosphere was added phenylmagnesium bromide (3M in diethyl ether) (1.33mL, 4.00mmol) dropwise. Stirring was continued at room temperature for 4 hours. The reaction mixture was filtered of precipitated magnesium hydroxide, taken up in ethyl acetate (50mL) and water (50mL) and separated. The aqueous fraction was extracted with ethyl acetate (3x100mL), and combined organic extracts were washed with sodium chloride (sat.) (100mL), water (2x100mL), dried (sodium sulphate) and concentrated in vacuo to yield a crude yellow oil. Chromatography (1:4 ethyl acetate:hexane; \(R_f\) 0.57) yielded 66.
(C₁₆H₁₈NO₃Cl₃) as a pale yellow oil (0.900g, 71% yield). \(^1\)H NMR (δ): 1.74 (1H, d, H₆a, \(J = 4.2\)); 1.91 (2H, dd, H₇e, H₇a, \(J = 12.0, 13.5\)); 2.16 (1H, d, H₆e, \(J = 4.5\)); 2.40 (3H, dd, H₂e, H₄a, H₂a, \(J_{H₂a,2e} = J_{H₄a,4e} = 16.5\)); 2.73 (1H, dd, H₄e, \(J_{H₄e,4a} = 16.5\)); 4.49 (1H, bd, H₁, \(J = 4.2\)); 4.64-4.72 (2H, m, CH₂); 4.95 (1H, dd, H₅, \(J_{H₅,5a} = 15.6, J_{H₅,6c} = 4.2\)), 7.20-7.53 (m, 5H, ArHₙ); \(^1\)C NMR (δ): 27.8 (28.5*) (CH₂, C₄); 28.7 (29.6*) (CH₂, C₂); 44.4 (44.5*) (CH₂, C₇); 48.9 (49.4*) (CH₂, C₆); 53.7 (CH, C₁); 54.0 (CH, C₅); 73.8 (C₃); 74.8 (74.0*) (CH₂CCl₃), 95.2 (CCl₃); 124.5, 127.3, 128.6 (ArCH x 5); 149.4 (ipso C); 151.7 (151.9*) (NC=O). MS (Cl⁺): \(m/z\) 378 (MH⁺, 35Cl, 30%); 380 (MH⁺, 35.5Cl); 382 (MH⁺, 37Cl); 360/362/364 (-H₂O, 7%); 300/302/304 (-Ph, 100%). HRMS (Cl⁺): Found: 380.0403; Required: 380.0401 for C₁₆H₁₉NO₃₃₅Cl₂₃₇Cl.

**Synthesis of 6β-hydroxytropan-3-one (67).**

A solution of 2,5-dimethoxy-2,5-dihydrofuran (26.00g, 0.2mol) in 3M hydrochloric acid (360mL) was stirred at room temperature for 18 hours, the neutralised with 6M sodium hydroxide (200mL). This mixture was added to a stirred solution of sodium acetate (140.00g), acetone dicarboxylic acid (58.00g, 0.4mol), and methylamine hydrochloride (27.00g, 0.4mol) in water (1250mL). The pH was adjusted to 3, and the mixture was left stirring at room temperature for 4 days. The solution was neutralised with potassium carbonate and sodium chloride (150.00g) was added. The solution was extracted extensively in 250mL portions with diethyl ether (2x200mL) followed by chloroform (2x200mL). The combined organic extracts were dried (sodium sulphate) and concentrated in vacuo to yield a brown oil. Chromatography (10:1 DCM:methanol) isolated 67 (C₈H₁₃NO₂) as an off-white crystalline material (0.37g, 1% yield). Mp: 120-122°C (lit. mp:
121-121°C\textsuperscript{353).} \textsuperscript{1}H NMR (δ): 2.01-2.25 (6H, m, H2e, H2a, H4e, H4a, H7e, H7a); 2.63 (3H, s, N-CH\textsubscript{3}); 2.71-2.74 (1H, bs, OH); 3.38 (1H, d, H1, J = 4.8); 3.60 (1H, bs, H5); 4.08 (1H, dd, H6, J = 3.6). MS (Cl\textsuperscript{+}): m/z 156 (MH\textsuperscript{+}, 60%).

**Synthesis of 6β-O-tert-butyldimethylsilyl-8-methyl-8-azabicyclo[3.2.1]octan-3-one (69).**

6β-Hydroxytropan-3-one (0.554g, 3.57mmol) was dissolved in anhydrous DCM (20mL) and cooled to 0°C under nitrogen with stirring. 2,6-lutidine (0.833mL, 7.14mmol) and tert-butyldimethylsilyl-trifluoromethanesulfonate (1.23mL, 5.35mmol) were added dropwise consecutively via syringe, and stirring was continued for 3 hours as the solution was allowed to warm to room temperature. The reaction was quenched by the addition of water (40mL) and saturated aqueous sodium hydrogen carbonate (10mL). Extraction with DCM (100mL), drying (sodium sulphate) and concentration in vacuo yielded a yellow liquid. Chromatography (ethyl acetate; R\textsubscript{f} 0.31) provided the ketone 69 (C\textsubscript{14}H\textsubscript{27}N\textsubscript{0}2Si) as a pale yellow oil (0.83g, 86% yield). \textsuperscript{1}H NMR (δ): 0.04 (6H, s, (CH\textsubscript{3})\textsubscript{3}Si); 0.87 (9H, s, (CH\textsubscript{3})\textsubscript{3}); 2.01-2.06 (2H, m, H2e, H4e); 2.13 (2H, dd, H2a, H4a, J\textsubscript{H2a,2e} = J\textsubscript{H4a,4e} = 15.9); 2.63 (3H, s, N-CH\textsubscript{3}); 2.57-2.65 (2H, m, H7, H7'); 3.28 (1H, d, H1, J = 4.8); 3.55 (1H, ddd, H5, J\textsubscript{H5,5a} = 1.5, J\textsubscript{H5,6} = 4.2, J\textsubscript{H5,4a} = 6.0); 4.08 (1H, dd, H6, J\textsubscript{H6,7e} = 3.6). \textsuperscript{13}C NMR (δ): -4.7 ((CH\textsubscript{3})\textsubscript{3}Si); 18.1 ((CH\textsubscript{3})\textsubscript{3}CSi); 25.9 ((CH\textsubscript{3})\textsubscript{3}); 38.2 (N-CH\textsubscript{3}); 41.3 (CH\textsubscript{2}, C4); 44.4 (CH\textsubscript{2}, C2); 46.3 (CH\textsubscript{2}, C7); 60.8 (CH, C1); 69.4 (CH, C5); 79.6 (CH, C6); 208.4 (C=O, C3). MS (Cl\textsuperscript{+}): m/z 270 (M+1, 100%); 256 (39%); 224 (26%). HRMS (Cl\textsuperscript{+}): Found: 270.1903; Required: 270.1889 for C\textsubscript{14}H\textsubscript{28}N\textsubscript{0}2Si.
Synthesis of 3-O-tert-butyldimethylsilyl-6β-O-tert-butyldimethylsilyl-8-methyl-8-azabicyclo[3.2.1]octane (70).

Following the above procedure, and using an excess of tert-butyldimethylsilyl-trifluoromethanesulfonate (1.5 equivalents), the bis-silylated tropinone derivative 70 (C_{20}H_{41}NO_2Si_2) and its isomer 71 were produced in 62% yield as a yellow oil; ^1H NMR (δ): 0.01 (3H, s, (CH_3)Si); 0.02 (3H, s, (CH_3)Si); 0.08 (3H, s, (CH_3)Si); 0.10 (3H, s, (CH_3)Si); 0.84 (9H, s, (CH_3)3); 0.87 (9H, s, (CH_3)3); 1.96-1.97 (2H, m, H2(H4)); 2.28 (2H, ddd, H7a, H7e, J_{H7a,l} = 7.5, J_{H7e,l} = 4.2, J_{H7e,6} = 7.2); 2.37 (3H, s, N-CH3); 3.05 (0.33H, d, H1, J = 5.4); 3.09 (0.66H, d, H1, J = 5.4); 3.30 (1H, dd, H5, J = 4.2, 3.6); 4.11-4.14 (1H, m, H6); 4.73 (0.66H, d, =CH, H2, J = 5.4); 4.83 (0.33H, d, =CH, H4, J = 5.4). ^13C NMR (δ): -4.6, -4.5, -4.2, -4.2 ((CH_3)2Si); 18.0, 18.3, 18.6 ((CH_3)3CSi); 25.7, 26.0, 26.1 ((CH_3)3); 31.7, 33.1 (CH2, C4); 35.0, 35.3 (CH2, C2); 43.3 (N-CH3); 48.0 (CH2, C7); 57.1, 58.5 (CH, C1); 66.0, 66.6 (CH, C5); 78.9, 79.2 (CH, C6); 102.5, 107.0 (C3); 146.3 (=CH, C2(C4)); 148.4 (=CH, C4(C2)). MS (Cl⁺): m/z 384 (M+1, 24%); 252 (42%, -TBDMSOH); 173 (53%); 133 (100%); HRMS (Cl⁺): Found: 384.2739; Required: 384.2754 for C_{20}H_{42}NO_2Si_2.

Synthesis of 6-exo-O-tert-butyldimethylsilyl-8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyclo[3.2.1]octan-3-one (72).

The base 69 (1.33g, 4.94mmol), 2,2,2-trichloroethyl chloroformate (0.748ml, 5.43mmol) and anhydrous potassium carbonate (0.683g, 4.94mmol) were heated at reflux in anhydrous benzene under nitrogen for 4 days. The benzene was then azeotroped with methanol, and the crude reaction mixture taken up in ethyl acetate (100mL) and water (100mL) and separated. The aqueous fraction was extracted with ethyl acetate (3x100mL) and combined organic extracts were washed with sodium chloride (sat.) (100mL), water (100mL) dried
(sodium sulphate) and concentrated in vacuo to yield a brown oil which crystallised upon standing. Chromatography (1:4 ethyl acetate:hexane; Rf 0.46) provided 72 (C\textsubscript{16}H\textsubscript{26}NO\textsubscript{4}SiCl\textsubscript{3}) as a white solid (1.80g, 85% yield). Mp: 68-70°C. \textsuperscript{1}H NMR (δ): 0.05 (6H, s, (CH\textsubscript{3})\textsubscript{2}Si); 0.86 (9H, s, (CH\textsubscript{3})\textsubscript{3}); 2.04-2.13 (2H, m, H2e, H4e); 2.40 (2H, dd, H2a, H4a, J\textsubscript{H2a,2e} = J\textsubscript{H4a,4e} = 16.2); 2.65-2.74 (2H, m, H7a, H7e); 4.18 (1H, dd, H1, J\textsubscript{H1,2a} = 12.6, J\textsubscript{H1,2e} = 5.4); 4.39 (1H, dd, H5, J\textsubscript{H5,4a} = 5.7, J\textsubscript{H5,4a} = 6.9); 4.67-4.77 (2H, m, CH\textsubscript{2}); 4.92 (1H, dd, H6, J = 11.7). \textsuperscript{13}C NMR (δ): -4.7 ((CH\textsubscript{3})\textsubscript{2}Si); 18.1 ((CH\textsubscript{3})\textsubscript{3}CSi); 25.8 ((CH\textsubscript{3})\textsubscript{3}); 41.4 (42.0*) (CH\textsubscript{2}, C4); 45.5 (46.0*) (CH\textsubscript{2}, C2); 48.5 (48.9*) (CH\textsubscript{2}, C7); 53.0 (53.2*) (CH, C1); 62.5 (62.5*) (CH, C5); 74.7 (75.4*) (CH\textsubscript{2}CCl\textsubscript{3}); 74.8 (75.4*) (CH, C6); 95.8 (CCl\textsubscript{3}); 152.0 (NC=O); 206.0 (C=O, C3). MS (Cl\textsuperscript{+}): m/z 430 (MH\textsuperscript{+}, \textsuperscript{35}Cl); 432 (MH\textsuperscript{+}, \textsuperscript{35}Cl); 434 (MH\textsuperscript{+}, \textsuperscript{37}Cl); 396/398 (-34Cl); 372/374/376; 298/300/302; 282. HRMS (Cl\textsuperscript{+}): Found: 430.0747; Required: 430.0775 for C\textsubscript{16}H\textsubscript{26}NO\textsubscript{4}Si\textsuperscript{35}Cl\textsubscript{3}.

Microwave Method: To 69 (0.83g, 3.08mmol) in anhydrous acetonitrile (15mL) under argon was added 2,2,2-trichloroethyl chloroformate (0.467mL, 3.39mmol) and anhydrous potassium carbonate (0.426g, 3.08mmol). The reaction mixture was exposed to microwave radiation for 2 minutes warming to 100°C, followed by 18 mins at 100°C. The reaction mixture was allowed to cool, then taken up in ethyl acetate (50mL) and water (50mL) and separated. The aqueous fraction was extracted with ethyl acetate (3x50mL) and combined organic extracts were washed with sodium chloride (sat.) (50mL), water (50mL) dried (sodium sulphate) and concentrated in vacuo to yield a brown oil. Chromatography (1:4 ethyl acetate:hexane; Rf 0.46) provided 72 (C\textsubscript{16}H\textsubscript{26}NO\textsubscript{4}SiCl\textsubscript{3}) as a white solid (0.796g, 60% yield). All spectral and melting point data agreed with that previously determined for 72.
Synthesis of $6\beta$-O-tert-butyldimethylsilyl-8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyc[3.2.1]3-spiro-2'-1',3'-dioxolane)-octane (74).

To a stirred solution of ethanediol (0.27mL, 4.91mmol) and anhydrous DCM (15mL) under nitrogen was added 72 (0.96g, 2.23mmol). To this mixture was added chlorotrimethylsilane (1.25mL, 9.82mmol) and the mixture was heated at reflux for 72 hours. A 5% aqueous solution of sodium hydrogen carbonate (20mL) was added and the mixture extracted with diethyl ether (3x100mL). The combined organic extracts were washed with sodium chloride (sat.) (100mL), water (100mL), dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:6 ethyl acetate:hexane; $R_f$ 0.69) provided 74 ($C_{18}H_{30}NO_5SiCl_3$) as a pale yellow oil (0.90g, 86% yield). $^1$H NMR (δ): 0.06 (6H, s, (CH$_3$)$_3$Si); 0.86 (9H, s, (CH$_3$)$_3$); 1.67-1.90 (2H, m, H2e, H4e); 1.97-2.30 (1H, m, H2a(H4a)); 2.26-2.42 (1H, m, H4a(H2a)); 2.46-2.75 (2H, m, H7a, H7e); 3.83 (1H, t, OCH, $J = 6.3$); 3.91-3.99 (1H, m, OCH); 4.09-4.21 (2H, m, OCH$_2$); 4.39 (1H, t, H1, $J = 5.7, 7.2$); 4.47-4.59 (1H, m, H5); 4.62-4.74 (2H, m, CH$_2$); 4.82-4.98 (1H, m, H6). $^{13}$C NMR (δ): 4.7 ((CH$_3$)$_2$Si); 10.1 ((CH$_3$)$_3$CSi); 25.8 ((CH$_3$)$_3$); 39.5 (40.1*) (CH$_2$, C4); 45.5 (46.0*) (CH$_2$, C2); 48.5 (48.9*) (CH$_2$, C7); 53.0 (53.5*) (CH, C1); 62.5 (62.6*) (CH, C5); 63.5 (OCH$_2$); 64.5 (OCH$_2$); 74.0 (74.7*) (CH, C6); 74.8 (75.4*) (CH$_2$CCl$_3$); 95.8 (CCl$_3$); 106.7 (C3); 151.7 (152.0*) (NC=O). MS (Cl$^+$): $m/z$ 474 (MH$^+$, $^{35}$Cl); 476 (MH$^+$, $^{35.5}$Cl); 478 (MH$^+$, $^{37}$Cl); 416($^{35}$Cl); 418 ($^{35.5}$Cl); 420 ($^{37}$Cl); 342 ($^{35}$Cl); 344 ($^{35.5}$Cl); 346 ($^{37}$Cl); 327; 300; 177. HRMS (Cl$^+$): Found: 474.1037; Required: 474.0952 for $C_{18}H_{31}NO_5Si^{35}Cl_3$. 
Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyclo[3.2.1]octan-3-spiro-2'-(1',3'-dioxolane)-6β-ol (73).

To a stirred solution of 74 (0.13g, 0.274mmol) in anhydrous THF (5mL) under nitrogen at 0°C was added tetrabutylammonium fluoride (1M in THF). The reaction was stirred at room temperature for 3 hours, excess ammonium chloride was added and the mixture stirred for 10 minutes. The mixture was filtered and the THF was removed in vacuo. The residue was extracted with ethyl acetate (50mL), washed with water (3x50mL), dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:1 ethyl acetate:hexane; Rf 0.30) provided 73 (C₁₂H₁₆N₀₅Cl₃) as a colourless oil (0.068g, 69% yield). ¹H NMR (δ): 1.73-2.15 (2H, m, H₂e, H₄e); 2.32-2.56 (2H, m, H₂a, H₄a); 2.67-2.76 (2H, m, H₇a, H₇e); 3.82 (2H, dd, OCH₂, J=6.0); 3.96 (2H, dd, OCH₂, J=6.0); 4.17-4.26 (1H, m, H₁); 4.48 (1H, d, H₅, J₅,₆ = 6.3); 4.55-4.70 (2H, m, CH₂); 4.88-5.02 (1H, m, H₆). ¹³C NMR (δ): 38.4 (40.9*) (CH₂, C₄); 45.0 (45.5*) (CH₂, C₂); 48.3 (48.9*) (CH₂, C₇); 52.9 (53.6*) (CH, C₁); 62.2 (62.5*) (CH, C₅); 63.5 (OCH₂); 64.5 (OCH₂); 73.5 (74.3*) (CH, C₆); 74.6 (74.7*) (CH₂CCl₃); 95.8 (CCl₃); 106.5 (C₃); 152.2 (NC=O). MS (Cl⁺): m/z 360 (MH⁺, 35Cl); 362 (MH⁺, 35,35Cl); 364 (MH⁺, 37Cl); 316/318/320 (M+1 – CH₂CH₂O); 298/300/302 (-H₂O); 282/284 (-34Cl). HRMS (Cl⁺): Found: 360.0153; Required: 360.0173 for C₁₂H₁₇N₀₅Cl₃. 

Microwave method: To a solution of 72 (0.680g, 1.578mmol) in anhydrous acetonitrile (10mL) under an argon stream was added ethanediol (0.194mL, 3.472mmol) followed by chlorotrimethylsilane (0.878mL, 6.944mmol). The reaction mixture was exposed to microwave radiation: warming to 80°C for 2 minutes, followed by heating at 80°C for 23
minutes. The reaction mixture was allowed to cool, then diluted with 5% aqueous sodium hydrogen carbonate (20mL) and extracted with diethyl ether (3x50mL). The combined organic fractions were washed with water (2x50mL), dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:1 ethyl acetate:hexane; Rf 0.43) yielded 73 \( \text{C}_{12}\text{H}_{16}\text{N}_{5}\text{O}_{5}\text{Cl}_{3} \) as a colourless oil (0.470g, 83% yield). All spectral data agreed with that previously determined for 73.

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyclo[3.2.1]octan-3-spiro-2’-(1’,3’-dioxolane)-6-one (75).**

The alcohol 73 (0.46g, 1.28mmol) was dissolved in a stirred solution of anhydrous dimethylsulfoxide (5mL) and dicyclohexylcarbodiimide (0.79g, 3.83mmol). Phosphoric acid (anhydrous) (0.063g, 0.64mmol) in anhydrous dimethylsulfoxide (1mL) was added dropwise and the mixture stirred at room temperature for 3 hours. Ethyl acetate (30mL) was added, followed by a solution of oxalic acid (0.25g) in methanol (10mL) and stirring continued for 30 minutes. The precipitated dicyclohexylurea was removed by filtration, washed with ethyl acetate (100mL) and the solution was extracted with aqueous sodium hydrogen carbonate (sat.) (50mL), water (50mL), dried (sodium sulphate) and concentrated in vacuo to yield an off-white semi-solid. Chromatography (1:3 ethyl acetate:hexane; Rf 0.40) provided 75 \( \text{C}_{12}\text{H}_{14}\text{N}_{5}\text{O}_{5}\text{Cl}_{3} \) as a white solid (0.324g, 71% yield). Mp: 87-90°C. \(^1\)H NMR (δ): 1.90 (1H, d, H7e, J7e,7a = 13.5); 2.22-2.42 (2H, m, H2e, H4e); 2.51-2.69 (2H, m, H2a, H4a); 2.89 (1H, m, H7a); 3.88-3.91 (2H, m, OCH2); 3.92-3.97 (2H, m, OCH2); 4.60 (1H, d, H1, J1,2e = 2.1); 4.66-4.91 (2H, m, CH2); 5.03 (1H, bd, H5, J5,6 = 6.0). \(^13\)C NMR (δ): 38.9 (39.4*) (CH2, C7); 40.1 (CH2, C4); 42.3 (42.9*) (CH2, C2); 51.4 (CH, C1); 59.7 (60.0*) (CH, C5); 64.1 (OCH2); 64.7 (OCH2); 74.7 (74.9*) (CH2CCl3); 95.8 (CCl3); 105.9
(C3); 151.8 (NC=O); 202.9 (C=O, C6). MS (Cl\(^+\)): \(m/z\) 360 (M+1, \(^{35}\)Cl); 362 (M+1, \(^{35}\)Cl); 364 (M+1, \(^{37}\)Cl); 342/344/346 (-H\(_2\)O); 316/318/320; 298/300/302. HRMS (Cl\(^+\)): Found: 358.0018; Required: 358.0016 for C\(_{12}\)H\(_{15}\)NO\(_5\)Cl\(_3\).

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6\(\beta\)-phenyl-8-azabicyclo[3.2.1]octan-3-spiro-2\'-(1\',3\'-dioxolane)-6\(\alpha\)-ol (76).**

The ketone 75 (0.300g, 0.837mmol) was dissolved in anhydrous diethyl ether (10mL) under nitrogen with stirring. Phenylmagnesium bromide (3M in diethyl ether) (0.558mL, 1.637mmol) was added dropwise and stirring continued for 3 hours. The reaction mixture was taken up in water (20mL) and extracted with diethyl ether (3x50mL). The combined organic extracts were washed with sodium chloride (sat.) (50mL), water (50mL), dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:2 ethyl acetate:hexane; R\(_f\) 0.65) provided 76 (C\(_{18}\)H\(_{20}\)NO\(_5\)Cl\(_3\)) as a white solid (0.280g, 77% yield). 76 was recrystallised from ethyl acetate/hexane. Mp: 110-112°C. \(^1\)H NMR (\(\delta\)): 2.00 (1H, dd, H\(_7\)a, \(\delta_{7\text{a},7\text{e}} = 8.4, J_{7\text{a},5} = 7.5\)); 2.14-2.23 (2H, m, H\(_2\)e, H\(_4\)e); 2.36-2.46 (2H, m, H\(_2\)a, H\(_4\)a); 2.74 (1H, dt, H7, \(J_{7\text{e},1} = 8.7, J_{7\text{a},1} = 4.8\)); 3.97 (2H, dd, OCH\(_2\), \(J = 6.3, 1.5\)); 4.02-4.18 (2H, m, OCH\(_2\)); 4.29 (1H, bs, OH); 4.55 (1H, dd, H1, \(J_{1,2} = 10.8, J_{1,7\text{e}} = 8.4\)); 4.70-4.92 (2H, m, CH\(_2\)); 5.02 (1H, d, H5, \(J = 2.7\)); 7.20-7.35 (3H, m, ArHx3); 7.52 (2H, t, ArHx2, \(J = 7.2\)). \(^{13}\)C NMR (\(\delta\)): 36.9 (37.7\(*)(CH\(_2\), C7); 40.5 (41.4\(*)(CH\(_2\), C4); 44.9 (45.8\(*)(CH\(_2\), C2); 52.7 (52.8\(*)(CH, C1); 62.8 (63.1\(*)(CH, C5); 63.8 (OCH\(_2\)); 64.7 (OCH\(_2\)); 74.4 (74.5\(*)(CH\(_2\)CCl\(_3\)); 79.8 (80.3\(*)(C6); 95.5 (95.6\(*)(CCL\(_3\)); 107.0 (C3); 123.9, 124.0, 125.2, 126.7, 128.1 (ArCHx5); 150.8 (151.1\(*)(NC=O). MS (Cl\(^+\)): \(m/z\) 436/438/440 (M+1, 29%); 418/420/422 (-H\(_2\)O, 100%).
Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6β-(3,4-methylenedioxyphenyl)-8-azabicyclo[3.2.1]octan-3-spiro-2'- (1',3'-dioxolane)-6α-ol (77).

To a stirred solution of 75 (0.090g, 0.251mmol) in anhydrous diethyl ether (4mL) under nitrogen was added 3,4-methylenedioxyphenylmagnesium bromide (1M in THF:toluene) (0.301mL, 0.301mmol) dropwise, and stirring continued for 3 hours. The reaction mixture was taken up in water (10mL) and extracted with diethyl ether (3x25mL). The combined organic extracts were washed with sodium chloride (sat.) (25mL), water (25mL), dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:2 ethyl acetate:hexane; Rf 0.45) provided 77 (C19H20NO7Cl3) as a colourless oil (0.90g, 75% yield). $^1$H NMR ($\delta$): 1.94-2.44 (5H, m, H2a, H2e, H4a, H4e, H7a); 2.63-2.76 (1H, m, H7e); 3.96-4.00 (2H, m, OCH2); 4.05-4.18 (2H, m, OCH2); 4.23 (1H, dd, OH, $J = 3.6$); 4.53 (1H, dd, H1, $J = 11.4, 9.9$); 4.70-4.90 (2H, m, CH2); 4.99 (1H, s, H5); 5.92 (2H, s, OCH2O); 6.70-6.80 (1H, m, ArH); 6.85-6.98 (1H, m, ArH); 7.05 (1H, dd, ArH, $J = 1.8$). $^{13}$C NMR ($\delta$): 36.9 (37.7*) (CH2, C7); 40.4 (41.4*) (CH2, C4); 45.0 (45.8*) (CH2, C2); 52.6 (52.8*) (CH, C1); 53.1 (53.3*) (CH, C5); 63.9 (OCH2); 64.7 (OCH2); 74.5 (74.6*) (CH2CCl3); 79.8 (80.4*) (C6); 95.5 (95.6*) (CCl3); 100.8 (OCH2O); 105.4 (ArCH); 107.0 (C3); 107.6 (ArCH); 117.1 (ArCH); 142.3, 146.1, 147.5 (ipso C x3); 150.8 (151.2*) (NC=O). HRMS (Cl$^+$): Found: 483.0247; Required: 483.0246 for C19H21NO735Cl37Cl2.

Attempted Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6α-hydroxy-6β-phenyl-8-azabicyclo[3.2.1]octan-3-one (78).

To a stirred suspension of 76 (0.08g, 0.183mmol) and sodium iodide (0.004g, 0.0275mmol) in acetonitrile (2mL) was added cerium chloride heptahydrate (0.102g, 0.275mmol).
mixture was stirred at room temperature for 20 hours and then heated at reflux for 22 hours. The reaction mixture was diluted with diethyl ether (20mL) and treated with 0.5M HCl (10mL). The organic layer was separated and the aqueous layer extracted with diethyl ether (3x20mL). The combined organic fractions were dried (sodium sulphate) and concentrated in vacuo to yield an off-white semi-solid (0.04g). The crude sample was shown by $^1$H NMR to consist of 76 only. MS (CI+) of the crude sample indicated the presence of some 78: $m/z$ 436/438/440 (76, M+1, 29%); 418/420/422 (-H$_2$O, 100%); 392/394/396 (78, M+1, 20%); 384/386/388 (-H$_2$O, 30%).

Microwave Method: To a suspension of 76 (0.05g, 0.114mmol) and sodium iodide (0.006g, 0.0043mmol) in acetonitrile (8mL) was added cerium chloride heptahydrate (0.064g, 0.172mmol) and the reaction mixture was exposed to microwave radiation in the following sequence: 2 minutes to 100°C, followed by 18 minutes at 100°C. The reaction mixture was allowed to cool, then it was diluted with diethyl ether (20mL) and treated with 0.5M HCl (10mL). The organic layer was separated and the aqueous layer extracted with diethyl ether (3x20mL). The combined organic fractions were dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil (0.05g). MS (CI+) of the crude product indicated the presence of some 78 (C$_{16}$H$_{16}$NO$_4$Cl$_3$): $m/z$ 436/438/440 (76, M+1, 29%); 418/420/422 (-H$_2$O, 100%); 392/394/396 (78, M+1, 20%); 384/386/388 (-H$_2$O, 30%). Chromatography (1:2 ethyl acetate:hexane, R$_f$ 0.65) provided 76 (C$_{18}$H$_{20}$NO$_5$Cl$_3$) as the only major product (0.04g, 80% recovery). Spectral data agrees with that of 76. No 78 (C$_{16}$H$_{16}$NO$_4$Cl$_3$) was isolated.
Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-3,3-dimethoxy-8-azabicyclo[3.2.1]-octane (83).

A stirred solution of 65 (0.100g, 0.333mmol) in anhydrous methanol (3mL) was treated with trimethylorthoformate (0.23mL, 2.083mmol) and p-toluenesulfonic acid monohydrate (0.004g, 0.023mmol) and the resulting solution was stirred at room temperature for 16 hours. The reaction mixture was treated with diethyl ether (10mL) and potassium carbonate (0.116g), filtered through a plug of flurosil and concentrated to yield 83 (C_{12}H_{18}N_{2}O_{4}Cl_{3}) as a colourless oil (0.098g, 85% yield). \(^1\)H NMR (δ): 1.83-1.96 (6H, m, H2a, H2e, H4a, H4e, H6a, H7a); 2.06 (2H, dd, H6e, H7e, J = 7.5); 3.12 (3H, s, OCH3); 3.19 (3H, s, OCH3); 4.36 (2H, d, CH2, J_{CH,CH'} = 13.5); 4.75 (2H, H1, H5, J_{H1,7} = J_{H5,6} = 12.0). \(^1\)C NMR (δ): 27.0 (CH2, C6(C7)); 27.9 (CH2, C7(C6)); 37.8 (CH2, C2(C4)); 38.5 (CH2, C4(C2)); 47.3 (OCH3); 48.4 (OCH3); 53.0 (2 x CH, C1, C5); 74.6 (OCH2CCl3); 95.8 (CCl3); 98.9 (C3); 151.1 (NC=O).

Preparation of 8-(2,2,2-trichloroethylformate)-8-azabicyclo[3.2.1]-octan-3-one (65) from 83.

To a stirred solution of 83 (0.040g, 0.115mmol) in DCM (2mL) was added bismuth nitrate pentahydrate (0.014g, 0.029mmol) and stirring continued at room temperature for 21 hours. The reaction mixture was filtered, washed with 10% aqueous sodium hydrogen carbonate (10mL), sodium chloride (sat.) (10mL), water (10mL), then dried (sodium sulphate), and concentrated \textit{in vacuo} to yield 65 (C_{10}H_{12}NO_{3}Cl_{3}) as an off-white solid (0.033g, 95% yield). No further purification was required. NMR and mass spectral data agreed with that of previously prepared 65.
**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6β-hydroxy-3,3-dimethoxy-8-azabicyclo[3.2.1]-octane (84).**

Following the method used for 83, 84 (C_{12}H_{19}NO_5Cl_3) (0.300g, 85% yield) was prepared from 72 (0.420g, 0.975mol) as a pale yellow oil after chromatography (ethyl acetate; R_f 0.79). $^1$H NMR (δ): 1.73-1.77 (1H, m, H7e); 1.84 (2H, dd, H2e, H4e, J = 4.2); 1.96-2.00 (1H, m, H2a); 2.12-2.20 (1H, dd, H7a, J = 7.2); 3.11 (3H, s, OCH_3); 3.15 (3H, s, OCH_3); 4.13 (1H, ddd, H1, J = 7.2, 14.4); 4.46 (2H, dd, CH_2, J = 6.3, 7.8); 4.47 (1H, H5, J = 7.2); 4.90 (1H, t, H6, J = 11.7). $^{13}$C NMR (δ): 35.8 (36.4*) (CH_2, C2(C4)), 36.5 (37.2*) (CH_2, C4(C2)); 39.5 (40.0*) (CH_2, C7); 47.3 (OCH_3); 48.1 (OCH_3); 53.1 (53.2*) (CH, C1); 62.0 (62.2*) (CH,C5); 73.2 (74.0*) (C6); 74.5 (74.6*) (OCH_2CCl_3); 95.5 (CCl_3); 98.3 (C3); 151.8 (NC=O). MS (CI⁺): m/z 362 (M+1, ^35Cl), 364 (M+1, ^35^5Cl), 366(M+1, ^37Cl); 330/332/334 (-CH_3OH); 312/314/316 (-H_2O); 296/298; 278/280. HRMS (CI⁺): Found: 363.0221; Required: 363.0207 for (C_{12}H_{19}NO_5^35Cl_2^37Cl).

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-3,3-dimethoxy-8-azabicyclo[3.2.1]-octan-6-one (85).**

Following the method used for 75, 85 (C_{12}H_{17}NO_5Cl_3) (0.490g, 71% yield) was prepared from 84 (0.700g, 1.93moles) as a colourless oil which crystallised to a white solid after chromatography (1:2 ethyl acetate:hexane; R_f 0.74). Mp: 62-63°C. $^1$H NMR (δ): 1.75 (1H, dd, H2e, $J_{2e1} = 13.8$); 2.03-2.19 (2H, m, H4e, H7e); 2.42-2.65 (3H, m, H2a, H4a, H7a); 3.04 (3H, s, OCH_3); 3.16 (3H, s, OCH_3); 4.21 (1H, m, H1); 4.72-4.88 (3H, m, H5, CH_2). $^{13}$C NMR (δ): 36.2 (36.8*) (CH_2, C4), 36.9 (37.6*) (CH_2, C2); 42.7 (43.1*) (CH_2, C7); 47.4 (OCH_3); 47.7 (OCH_3); 51.4 (51.8*) (CH, C1); 59.3 (60.3*) (CH,C5); 74.7 (OCH_2CCl_3);
95.2 (CCl₃); 98.3 (C3); 151.4 (NC=O); 203.0 (C=O, C6). MS (Cl⁺): m/z 360 (M+1,³⁵Cl),
362 (M+1,³⁵Cl, ³⁷Cl), 364 (M+1, ³⁷Cl); 342/344/346 (-H₂O); 328/330/332 (-CH₃OH); 294/296.
HRMS (Cl⁺): Found: 359.0103; Required: 359.0094 for (C₁₂H₁₈N₀₅³⁵Cl₃).

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-3,3-dimethoxy-6β-phenyl-8-azabicyclo[3.2.1]-octan-6α-ol (86).**

Following the method used for 76, 86 (C₁₈H₂₂N₀₅Cl₃) (0.293g, 88% yield) was prepared
from 85 (0.273g, 0.757moles) as a colourless oil after chromatography (1:4 ethyl
acetate:hexane; Rf 0.42), which crystallised to a white solid upon standing. Mp:100-101°C.

¹H NMR (δ): 1.97 (1H, dd, H7a, J = 3.9); 2.02-2.15 (2H, m, H2a, H4e); 2.22-2.31 (1H, m,
H2a(H4a)); 2.51-2.61 (1H, m, H4a(H2a)); 2.75 (1H, dddd, H7e, J₇e,₇a = 8.4, J₇e,₁ = 5.1); 3.23
(3H, s, OCH₃); 3.33 (3H, s, OCH₃); 4.27 (1H, dd, H₁ J = 4.2, 5.4); 4.46-4.52 (1H, m, H5);
4.79 (2H, dddd, CH₂, J = 10.8, 12.0); 7.19-7.36 (3H, m, ArHx3); 7.49-7.54 (2H, m,
ArHx2). ¹³C NMR (δ): 35.3 (35.7*) (CH₂, C4), 36.1 (36.5*) (CH₂, C2); 45.1 (46.0*) (CH₂,
C7); 47.8 (OCH₃); 48.1 (OCH₃); 52.3 (52.5*) (CH, C1); 62.5 (62.8*) (CH, C5); 74.4
(74.6*) (OCH₂CCl₃); 79.5 (80.1*) (CH, C6); 95.5 (CCl₃); 99.3 (C3); 124.0, 124.1, 126.7,
128.1, 128.3 (ArCxC); 147.8 (ipso C); 150.8 (151.1*) (NC=O). MS (Cl⁺): m/z 406 (M+1-
CH₃OH,³⁵Cl); 408 (M+1-CH₃OH, ³⁵Cl); 410 (M+1-CH₃OH, ³⁷Cl); 372/374/376; 354.
HRMS (Cl⁺): Found: 406.0363; Required: 406.0380 for C₁₇H₁₉N₀₄³⁵Cl₃ (loss of CH₃OH).
Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-3,3-dimethoxy-6β-(3,4-methylenedioxyphenyl)-8-azabicyclo[3.2.1]octan-6α-ol (87).

Following the method used for 77, 87 (C_{19}H_{22}NO_{7}Cl_{3}) (0.199g, 84% yield) was prepared from 85 (0.177g, 0.491moles) as a colourless oil after chromatography (1:4 ethyl acetate:hexane; Rf 0.28). \[^1^H\text{NMR (δ): 1.92-1.99 (1H, m, H7a); 2.02-2.13 (1H, m, H2e); 2.19-2.30 (m, 1H, H4e); 2.49-2.59 (2H, m, H2a, H4a); 2.68 (1H, ddd, H7e, J_{7e,7a} = 8.4, J_{7e,7e} = 5.7); 3.22 (3H, s, OCH₃); 3.32 (3H, s, OCH₃); 4.21 (1H, dd, H1, J = 3.3, 12.3); 4.43-4.50 (1H, m, H5); 4.79 (2H, dddd, CH₂, J₁ = 10.2, 12.0); 5.28 (1H, s, OH) (exchangeable with D₂O) 5.92 (2H, s, OCH₂O); 6.72 (1H, dd, ArH, J = 1.8); 6.93-6.98 (1H, m, ArH); 7.05 (1H, dd, ArH, J = 1.8, 8.4). \[^{13}C\text{NMR (δ): 35.2 (35.7*) (CH₂, C4), 36.1 (36.5*) (CH₂, C2); 45.1 (46.0*) (CH₂, C7); 47.5 (47.8*) (OCH₃); 48.1 (OCH₃); 52.2 (52.4*) (CH, C1); 62.8 (63.0*) (CH, C5); 74.5 (74.6*) (OCH₂CCl₃); 79.5 (80.2*) (CH, C6); 95.5 (CCl₃); 99.3 (C3); 100.8 (OCH₂O); 105.4, 107.6 (ArCH x 2); 117.0 (ArCH); 141.2, 146.1, 147.5 (ipso C x 3); 151.1 (NC=O). MS (Cl⁺): m/z 390/392/394; 360/362/364; 342/344/346; 328/330/332; 294/296. HRMS (EI⁺): Found: 449.0157; Required: 449.0200 for C_{18}H_{18}NO_{6}^{35}Cl_{3} (loss of CH₃OH).}


Following the method used for the deprotection of 83, 79 (0.02g, 0.0455mmol) was treated with 0.25 equivalents of bismuth nitrate pentahydrate in DCM (2mL) and stirred at room temperature for 2 hours, after which time all the substrate had reacted (via TLC). The usual work-up procedure afforded 90 (C_{17}H_{18}NO_{6}Cl_{3}) as a colourless oil (Rf 0.56, 1:4 ethyl acetate:hexane) (0.018g, 100 % yield). \[^1^H\text{NMR (δ): 1.89 (1H, dd, H7a, J_{7a,7e} = 6.0, J_{7e,1} =}
2.4; 2.00 (2H, s, H2e, H4e); 2.18 (2H, dd, H2a, H4a, J = 2.7, 3.6); 2.31 (1H, dd, H7e, J7c,1 = 4.8); 3.53 (3H, s, OCH3); 4.56 (1H, d, H1 J = 4.8); 4.72-4.89 (3H, m, H5, CH2); 7.25-7.45 (5H, m, ArHx5). 13C NMR (δ): 37.1 (37.6*) (CH2, C4), 39.8 (40.4*) (CH2, C2); 45.8 (46.1*) (CH2, C7); 50.0 (50.0*) (OCH3); 55.6 (56.1*) (CH, C1); 65.5 (65.5*) (CH, C5); 74.6 (OCH2CCl3); 87.8 (88.5*) (C6); 95.7 (CCl3); 106.4 (106.5*) (C3); 124.6, 124.7, 127.5, 128.3 (ArC5); 140.5 (140.6*) (ipso C); 150.6 (NC=O). MS (CI+): m/z 406 (M+1,35Cl, 90%); 408 (M+l, 35Cl); 410 (M+l, 37Cl); 372/374/376 (- MeOH, 40%); 232 (55%). HRMS (CI+): Found: 406.0376; Required: 406.0380 for C17H19N04Cl3.

Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6β-phenyl-8-azabicyclo[3.2.1]-3-oxo-octan-6-ol (78) and 4-(2,2,2-trichloroethoxycarbonyl)-6α-phenyl-2,5-methano-hexahydro-2H-furo[3,2-b]pyrrol-6α,2β-diol (91).

A stirred solution of 86 (0.103g, 0.235mmol) in a 2:1 mixture of chloroform:trifluoroacetic acid (50% aq.) (4mL) was heated at reflux for 20 hours. The cooled solution was poured onto chloroform, separated, and the organic layer washed with water (5mL), dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:4 EtOAc:Hexane; Rf 0.19) yielded a colourless oil (0.090g, 98% yield), which proved to be an inseparable mixture of 78 (C16H16N04Cl3) and 91 (C16H16N04Cl3). 1H NMR (δ): 1.93 (1H, d, H7a, J=11.4); 2.02-2.19 (5H, m, H2e, H4e, H7e, H2a, H4a); 3.97 (1H, bs, OH); 4.56 (1H, s, H1); 4.72-4.88 (3H, m, CH2, H5); 7.26-7.43 (5H, m, ArH x 5); 13C NMR (δ): 38.4 (38.5*) (CH2, C4), 44.6 (44.9*) (CH2, C2); 45.3 (45.6*) (CH2, C7); 55.4 (55.8*) (CH, C1); 65.7 (65.7*) (CH, C5); 74.6 (74.9*) (OCH2CCl3); 88.4 (89.1*) (C6); 95.5 (95.6*) (CCl3); 103.6 (103.7*) (C3); 124.7, 124.8, 127.6, 128.3, 128.5 (ArCH x 5); 140.1 (140.2*)
(ipso C); 150.6 (151.0*) (NC=O); 203.1 (C=O, C3). MS (Cl⁺): 392/394/396 (M+1); 374/376/378 (-H2O); 358/360/362; 340/342/344; 314/316/318; 218; 200.

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6α-hydroxy-6-β-(3,4-methylenedioxyphenyl)-8-azabicyclo[3.2.1]octan-3-one (79) and 4-(2,2,2-trichloroethoxycarbonyl)-6αβ-(3,4-methylenedioxyphenyl)-2,5-methano-hexahydro-2H-furo[3,2-b]pyrrol-6αβ,2β-diol (92).**

A solution of 87 (0.179g, 0.371mmol) in a 2:1 mixture of chloroform:trifluoroacetic acid (50% aq.) (4mL) was heated at reflux for 20 hours. The cooled solution was poured into chloroform, separated, and the organic layer washed with water (2x5mL), dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:4 EtOAc:Hexane) isolated 2 products: the ketone derivative 79 and the hemi-ketal derivative 92 (4.87fl8-32). 79 (C₁₇H₁₆NO₆Cl₃) was isolated as an off-white solid, recrystallised from DCM/diethyl ether (0.039g, 24% yield); Rf 0.20 (1:4 EtOAc:Hexane). Mp: 106-108°C. ^1H NMR (δ): 2.19-2.28 (1H, m, H7a); 2.45-2.60 (3H, m, H2e, H4e, H7e); 2.83-2.92 (2H, m, H2a, H4a); 4.72 (1H, dd, H1, J = 4.8); 4.94-5.09 (2H, dddd, CH₂, J = 10.2, 12.0); 5.27 (1H, s, H5); 5.98 (2H, s, OCH₂O); 6.31 (1H, dd, OH, J = 2.4, 5.7) (exchangeable with D₂O); 6.77-6.89 (3H, m, ArH x 3). ^13C NMR (δ): 44.4, 45.0, 45.2, 45.8 (CH₂, C4, C2, C7); 57.1 (CH, C1, C5); 74.7 (OCH₂CCl₃); 84.5 (CH, C6); 95.4 (CCl₃); 101.3 (OCH₂O); 105.8, 108.4 (ArCH x 2); 119.8 (123.3*) (ArCH); 125.0 (ipso C); 145.4, 148.2 (ipso C x 2); 150.3 (NC=O); 204.2 (C=O). MS (Cl⁺): m/z 436/438/440 (M+1, 10%); 418/420/422 (-H₂O, 15%); 384/386/388 (20%); 314/316/318 (20%); 262 (70%); 244 (100%). HRMS (El⁺): Found: 435.0026; Required: 435.0043 for C₁₇H₁₆NO₆Cl₃.
92 (C_{17}H_{16}NO_{6}Cl_{3}) was isolated as a colourless oil (0.100g, 62% yield); R_f 0.08 (1:4 EtOAc:Hexane); ^1H NMR (δ): 1.89 (1H, bm, H7a); 2.06-2.13 (4H, m, H2e, H4e, H2a, H4a); 2.19-2.31 (1H, m, H7e); 3.75 (1H, d, J = 3.0, OH); 4.50 (1H, s, H1); 4.70-4.76 (2H, m, CH_{2}CCl_{3}); 4.87 (1H, dd, J = 6.6, H5); 5.95 (2H, s, OCH_{2}O); 6.78 (1H, 2xd, J = 4.5, ArH); 6.85 (1H, dd, J = 4.5, 1.5, ArH); 6.89 (1H, d, J = 3.0, ArH). 13C NMR (δ): 38.5 (38.8*) (CH_{2}, C4), 44.6 (45.0*) (CH_{2}, C2), 45.1 (45.3*) (CH_{2}, C7); 55.3 (55.7*) (CH, C1); 65.6 (65.7*) (CH, C5); 74.6 (OCH_{2}CCl_{3}); 88.4 (89.1*) (CH, C6); 95.5 (CCl_{3}); 101.1 (OCH_{2}O); 103.6 (103.7*) (C3); 105.7 (105.8*) (ArCH); 108.0 (ArCH); 118.0 (118.1*) (ArCH); 134.2 (ipso C); 146.5, 148.0 (ipso C x 2); 150.8 (NC=O). MS (Cl^+): m/z 436/438/440 (M+1, 60%); 418/420/422 (-H_{2}O, 100%); 384/386/388 (60%). HRMS (El^+): Found: 435.0049; Required: 435.0043 for C_{17}H_{16}NO_{6}Cl_{3}.

Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-8-azabicyclo[3.2.1]octan-3β-ol (97).

To a stirred solution of 65 (0.050g, 0.167mmol) and 0.4M cerium chloride heptahydrate (0.167mmol) in methanol (2.5mL) at room temperature was added sodium borohydride (0.007g, 0.167mmol). Stirring continued for 10 minutes, at which time 5% aqueous hydrochloric acid (1mL) was added and the mixture extracted with DCM (3x5mL). The combined organic extracts were washed with water (2x5mL), dried (sodium sulphate) and concentrated in vacuo to yield 97 (C_{10}H_{14}NO_{3}Cl_{3}) as an off-white solid (0.049g, 97% yield) in 95% stereoselectivity. Mp: 84-86°C. ^1H NMR (δ): 1.60-1.71 (3H, m, H2a, H4a, OH); 1.73-1.84 (2H, m, H6a, H7a); 1.94-2.06 (3H, bm, H6e, H7e, H2e); 2.11-2.25 (1H, m, H4e); 4.14 (1H, dddd, H3a, J_{H2a,3} = J_{H4a,3} = 10.5, J_{H2e,3} = J_{H4e,3} = 5.7); 4.31-4.43 (2H, m, CH_{2}); 4.67 (1H, dd, H1, J = 10.2); 4.84(1H, dd, H1, J = 10.2). 13C NMR (δ): 27.8 (27.8*) (CH_{2},
C7(C6)); 28.5 (28.6*) (CH₂, C6(C7)); 38.4 (39.0*) (CH₂, C2(C4)); 40.3 (40.9*) (CH₂, C2(C4)); 53.3 (53.4*) (CH, C3); 63.7 (CH, C1); 64.9 (CH, C5); 74.5 (74.6*) (CH₂CCl₃), 95.7 (CCl₃); 151.2 (NCO). MS (CI⁺): m/z 302/304/306 (M+1, 100%); 284/286/288 (-H₂O, 15%); 268; 270 (loss of 34Cl, 40%). HRMS (CI⁺): Found: 304.0072; Required: 304.0088 for C₁₂H₁₅N₀₃Cl₂Cl₂.

**Synthesis of 8-(2,2,2-trichloroethylformate)-6β-phenyl-8-azabicyclo[3.2.1]octane-3β,6α-diol (80).**

To a stirred solution of 0.4M cerium chloride heptahydrate in methanol (5mL) under nitrogen was added 78 (0.120g, 0.306mmol), followed a few minutes later by sodium borohydride (0.021g, 0.547mmol). Stirring continued for 4 hours, after which time 5% aqueous hydrochloric acid was added to the mixture, and then the mixture poured onto DCM (10mL) and extracted. The organic layer was washed with water (10mL), dried (sodium sulphate) and concentrated *in vacuo* to yield a colourless oil which crystallised upon standing to a white solid. Mp: 131-132°C. Chromatography (1:2 ethyl acetate:hexane) yielded unreacted 78 (0.072g, 60% yield) and 80 (C₁₆H₁₈N₀₄Cl₃) as a colourless oil (Rf 0.43, 1:2 ethyl acetate:hexane, 0.049g, 40% yield, 100% yield based on recovered 78). ¹H NMR (δ): 1.87 (1H, m, H₇a); 2.16-2.38 (3H, m, H₂e, H₄e, H₇e); 2.48 (1H, dd, J₂a₂e = 8.7, H₂a); 2.73 (1H, dddd, J₄a₄e = 9.0, H₄a); 3.70 (1H, 2 x bs, OH); 4.24-4.27 (2H, m, H₁, H₃a); 4.39-4.47 (1H, m, H₅); 4.79 (2H, dddd, J = 12.0, CH₂CCl₃); 5.55 (1H, bd, OH, J = 12.3); 7.22-7.32 (3H, m, ArH x 3); 7.48-7.54 (2H, m, ArH x 2). ¹³C NMR (δ): 33.4 (34.1*) (CH₂, C₄), 38.0 (38.9*) (CH₂, C₂); 45.8 (46.6*) (CH₂, C₇); 52.6 (52.9*) (CH, C₁); 62.9 (63.1*) (CH, C₃); 63.8 (63.9*) (CH, C₅); 74.4 (74.5*) (OCH₂CCl₃); 79.7 (80.3*) (C₆);
95.5 (CCl₃); 124.0, 126.8, 126.8, 128.2, 128.2 (ArCH x 5); 140.1 (ipso C); 151.0 (NC=O).

MS (Cl⁻): m/z 394/396/398 (M⁺, 60%). HRMS (Cl⁻): Found: 395.0274; Required: 395.0272 for C₁₆H₁₈NO₄⁵Cl₂³⁷Cl.

**Synthesis of 8-(2,2,2-trichloroethoxycarbonyl)-6β-(3,4-methylenedioxyphenyl)-8-azabicyclo[3.2.1]octane-3β,6α-diol (81).**

To a stirred solution of 0.066M cerium chloride heptahydrate in methanol (3mL) at room temperature was added 79 (0.049g, 0.112mmol), followed a few minutes later by sodium borohydride (0.009g, 0.224mmol). Stirring continued for 30 minutes, after which time 5% aqueous hydrochloric acid (2mL) was added to the mixture, and the mixture was poured onto DCM (10mL) and extracted. The organic layer was washed with water (10mL), dried (sodium sulphate) and concentrated *in vacuo* to yield the crude product as a colourless oil. Chromatography (1:1 ethyl acetate:hexane; Rᵢ = 0.48) yielded 81 (C₁₇H₁₈NO₆Cl₃) as a white solid, recrystallised from ethyl acetate (0.048g, 98% yield). Mp: 131-132°C. 

1H NMR (δ): 1.65-1.85 (3H, m, H7a, H2e, H4e); 1.93-2.39 (3H, m, H2a, H4a, H7e); 3.94 (0.75H, dddd, J₃a,₂e = 6.3, J₃a,₂a = 9.6, H₃a); 4.07 (0.25H, dd, J₃e,₂a = 5.4, H₃e); 4.69 (1H, dd, J = 6.0, H1); 4.80-4.96 (2H, m, CH₂CCl₃); 5.05 (1H, dd, J = 3.3, H5); 5.97 (2H, s, OCH₂O); 6.76-6.96 (3H, m, ArH x 3). 13C NMR (δ): 33.9, 34.1, 34.6, 34.9 (CH₂ x 3, C4, C2, C7); 58.2 (58.3*) (CH, C1); 64.8 (CH, C3); 65.4 (CH, C5); 74.6 (OCH₂CCl₃); 95.6 (CCl₃); 101.3 (101.3*) (OCH₂O); 105.7 (ArCH); 108.4 (108.6*) (ArCH); 119.7 (119.8*), 121.3 (121.5*) (ArCH); 124.6 (C6), 126.0 (126.1*) (ipso C); 142.0 (142.1*) (ipso C); 147.8 (148.1*) (ipso C); 149.9 (NC=O). MS (Cl⁻): m/z 438/440/442 (M⁺, 5%); 420/422/424 (-
H₂O, 100%); 402/404/406 (-H₂O, 13%); 386/388/390 (25%). HRMS (Cl⁺): Found: 421.0040; Required: 421.0065 for C₁₇H₁₆NO₅³⁵Cl₂³⁷Cl (loss of H₂O).

In the same way, using a 0.4M solution of cerium chloride heptahydrate in methanol (5mL), 92 (0.100g, 0.229mmol) yielded, after chromatography (1:2 ethyl acetate:hexane), unreacted 92 (0.043g, 43% yield) and 81 as a colourless oil (0.048g, 48% yield, 84% yield based on recovered 92). All spectral data agreed with that reported above.

**General procedure for the removal of the 2,2,2-trichloroethylformate protecting group to yield secondary amine derivatives:**

The 2,2,2-trichloroethoxycarbonyl derivative (0.10-1.50mmol) and zinc dust (10 equivalents) were stirred in 90% acetic acid (2-20mL) at 40°C for 2 days. The reaction mixture was basified (ammonia, pH 9) and extracted with diethyl ether (3x100mL). The combined organic extracts were washed with sodium chloride (sat.) (100mL), water (2x100mL), dried (sodium sulphate) and concentrated in vacuo to give the desired secondary amine derivatives. In this way derivatives 52, 72, 60, 101, 102, 99, 100, 50 and 54 were prepared.

**General procedure for hydrochloride salt formation:**

The aryl tropanol derivatives (0.10-1.00mmol) were dissolved in diethyl ether (1-2mL), and treated dropwise with 1M hydrochloric acid in diethyl ether (0.50-1.00mL). The precipitate was filtered and dried under high vacuum to yield the desired hydrochloride salt.
3α-phenyl-8-azabicyclo[3.2.1]octan-3-ol (52).

Reaction of 66 (0.575g, 1.52mmol) yielded 52 (C₁₃H₁₇NO) as a pale yellow oil (0.184g, 60% yield). ¹H NMR (δ): 1.74-1.78 (2H, m, H₆e, H₇e); 1.85 (2H, d, H₆a, H₇a, J = 15.9); 2.22 (1H, d, OH, J = 3.9); 2.27 (1H, d, NH, J = 3.9); 2.30-2.40 (4H, m, H₂a, H₂e, H₄a, H₄e); 3.61 (2H, s, H₁, H₅); 7.18-7.22 (m, 2H, ArH); 7.27-7.35 (2H, m, ArHx₂); 7.45-7.51 (2H, m, ArHx₂). ¹³C NMR (δ): 28.9 (CH₂, C₄); 30.1 (CH₂, C₂); 40.0 (CH₂, C₇); 50.9 (CH₂, C₆); 54.3 (CH, H₁); 54.9 (CH, H₅); 73.3 (C₃); 124.4, 126.7, 127.2, 128.2, 128.7 (ArCH x 5); 129.5 (ipso C). MS (CI⁺): m/z 204 (M+1, 29%); 186 (-H₂O, 50%); 126 (100%). HRMS (EI⁺): Found: 186.1268; Required: 186.1283 for C₁₃H₁₆N (loss of H₂O). The hydrochloride salt was made.

6β-phenyl-8-azabicyclo[3.2.1]octan-3-spiro-2'-(1',3'-dioxolane)-6-ol (58).

Reaction of 76 (0.075g, 0.17mmol) yielded 58 (C₁₅H₁₉NO₃) after chromatography (ethyl acetate) as a colourless oil (0.025g, 51% yield). ¹H NMR (δ): 1.78 (2H, bs, OH, NH); 1.93 (2H, dd, H₂e, H₄e, J = 4.2); 2.05 (1H, d, H₇a, J = 7.2); 2.27 (1H, d, H₂a(H₄a), J = 14.4); 2.45 (1H, d, H₄a(H₂a), J = 14.4); 2.62 (1H, dd, H₇e, J = 7.8, 8.1); 3.38 (1H, s, H₁); 3.71 (1H, t, H₅ J = 3.9, 7.8); 3.92-3.99 (2H, m, OCH₂); 4.03-4.14 (2H, m, OCH₂); 7.23 (1H, dd, ArH, J = 7.2); 7.33 (2H, dd, ArHx₂, J = 7.2, 8.1); 7.67 (2H, d, ArHx₂, J = 8.4). ¹³C NMR (δ): 39.5 (CH₂, C₇); 43.4 (CH₂, C₄); 44.9 (CH₂, C₂); 53.5 (CHx₂, C₁, C₅); 63.6 (OCH₂); 64.4 (OCH₂); 82.2 (C₆); 107.5 (C₃); 124.7, 126.4, 127.9 (ArCHx₅); 148.9 (ipso-C). MS (CI⁺): m/z 262 (M+1); 244 (-H₂O); 184. The hydrochloride salt was made.
6β-(3,4-methylenedioxy)-8-azabicyclo[3.2.1]octan-3-spiro-2'-(1',3'-dioxolane)-6-ol (60).

Reaction of 77 (0.09g, 0.19mmol) yielded 60 (C_{16}H_{19}NO_{5}) after chromatography (ethyl acetate) as a white solid (0.021g, 37% yield). Mp: 218-220°C. ^1H NMR (δ): 1.74 (2H, bs, OH, NH); 1.91 (2H, dd, H2e, H4e, J = 4.2); 2.03 (1H, d, H7a, J = 6.6); 2.24 (1H, d, H2a(H4a), J = 14.4); 2.41 (1H, d, H4a(H2a), J = 14.4); 2.55 (1H, dd, H7e, J = 8.1); 3.38 (1H, d, H1, J = 4.2); 3.69 (1H, dd, H5 J = 4.2); 3.94-3.99 (2H, m, OCH_{2}); 4.02-4.16 (2H, m, OCH_{2}); 5.92 (2H, s, OCH_{2}O) 6.75 (1H, d, ArH_{5}, J = 8.1); 7.10 (1H, dd, ArH_{6}, J = 1.8, 8.1); 7.27 (1H, d, ArH_{2}, J = 2.1). ^13C NMR (δ): 37.4 (CH_{2}, C_{7}); 40.8 (CH_{2}, C_{4}); 44.2 (CH_{2}, C_{2}); 56.2 (CHx_{2}, C_{1}, C_{5}); 61.3 (OCH_{2}); 67.3 (OCH_{2}); 66.5 (C_{6}); 101.2 (OCH_{2}O); 105.7, 106.2 (ArCHx_{2}); 107.6 (C_{3}); 118.1 (ArCH); 135.8, 147.0, 147.9 (ipso-C x3). MS (C_{1}^+): m/z 306 (M+1); 288 (-H_{2}O); 244, 184. HRMS (C_{1}^+) Found: 305.1254; Required: 305.1263 for C_{16}H_{18}NO_{5}. The hydrochloride salt was made.


Reaction of 90 (0.05g, 0.114mmol) yielded 101 (C_{14}H_{17}NO_{2}) after chromatography (ethyl acetate) as a pale yellow oil (0.022g, 85% yield). ^1H NMR (δ): 1.78 (1H, d, H7a, J = 10.5); 1.92-2.04 (3H, m, H2e, H4e, H7e); 2.23-2.29 (2H, m, H2a, H4a); 2.50 (1H, bs, NH); 3.49 (3H, s, OCH_{3}); 3.79 (1H, d, H1, J = 5.1); 3.88 (1H, d, H5, J = 2.7); 7.24-7.46 (5H, m, ArHx5). ^13C NMR (δ): 38.1 (CH_{2}, C_{4}); 41.4 (CH_{2}, C_{2}); 48.5 (CH_{2}, C_{7}); 49.7 (OCH_{3}); 56.4 (CH, C_{1}); 67.3 (CH, C_{5}); 88.7 (C_{6}); 106.2 (C_{3}); 124.6, 126.9, 128.1, 128.3 (ArC_{x5}); 142.0 (ipso C). MS (C_{1}^+): m/z 232 (M+1, 100%); 200 (M+1 – MeOH, 40%). HRMS (C_{1}^+): Found: 231.1266; Required: 231.1263 for C_{14}H_{17}NO_{2}. The hydrochloride salt was made.
2-methoxy-6αβ-(3,4-methylenedioxyphenyl)-2,5-methano-hexahydro-2H-furo[3,2-b]pyrrol-6α-ol (102).

Reaction of 91 (0.058g, 0.120mmol) yielded 102 (C_{15}H_{17}NO_{4}) as a pale yellow oil (0.025g, 78 % yield). ^1H NMR (δ): 1.91-2.01 (3H, m, H2e, H4e, H7a); 2.11-2.28 (3H, m, H2a, H4a, H7e); 2.62 (1H, bs, NH); 3.48 (3H, s, OCH_{3}); 3.74 (1H, bd, H1, J = 3.6); 3.87 (1H, bs, H5); 5.95 (2H, s, OCH_{2}O); 6.77-6.80 (1H, m, ArH); 6.88-6.95 (2H, m, ArH x 2). ^13C NMR (δ): 37.9 (CH_{2}, C4), 41.4 (CH_{2}, C2); 48.0 (CH_{2}, C7); 49.7 (OCH_{3}); 56.2 (CH, C1); 67.1 (CH, C5); 88.6 (C6); 100.9 (OCH_{2}O); 105.8, 106.2 (ArCH x 2); 107.9 (C3); 117.8 (ArCH); 136.0, 146.4, 147.5 (ipso C x 3). MS (Cl^+): m/z 276 (M+1, 70%); 244 (M+1 – MeOH, 65%); 154 (55%). HRMS (Cl^+): Found: 276.1240; Required: 276.136 for C_{15}H_{18}NO_{4}. The hydrochloride salt was made.


Reaction of 93 (0.090g, 0.229mmol) yielded hemi-ketal 99 (C_{13}H_{15}NO_{2}) after chromatography (10:2 ethyl acetate:methanol; Rf 0.12) as a colourless oil (0.015g, 30% yield). ^1H NMR (δ): 1.88 (1H, d, H7a, J = 12.3); 1.89-2.20 (4H, m, H2e, H4e, H2a, H4a); 2.24 (1H, d, J =11.7, H7e); 2.99 (2H, bs, NH, OH); 3.81 (1H, s, H1); 3.89 (1H, s, H5); 7.23-7.44 (5H, m, ArHx5). ^13C NMR (δ): 39.5 (CH_{2}, C4), 46.1 (CH_{2}, C2); 47.7 (CH_{2}, C7); 56.1 (CH, C1); 67.3 (CH, C5); 89.3 (C6); 103.5 (C3); 124.8, 126.8, 127.1, 127.9, 128.2 (ArCH x 5); 141.6 (ipso C). MS (Cl^+): m/z 218 (M+1, %); 200 (M+1 – H_{2}O, %). HRMS (Cl^+): Found: 218.1175; Required: 218.1181 for C_{13}H_{16}NO_{2}. The hydrochloride salt was made.
6αβ-(3,4-methylenedioxyphenyl)-2,5-methano-hexahydro-2H-furo[3,2-b]pyrrol-6αα,2β-diol (100).

Reaction of 94 (0.040g, 0.092mmol) yielded 100 (C14H15NO4) after chromatography (10:2 ethyl acetate:methanol; Rf 0.13) as a colourless oil (0.014g, 58% yield). 1H NMR (δ): 1.84 (1H, dd, J7a,7e = 12.0, J7a,1 = 3.3); 1.95-2.03 (4H, m, H2e, H4e, H2a, H4a); 2.18 (1H, d, J7a,7e = 11.7, H7e); 3.07 (2H, bs, NH, OH); 3.75 (1H, s, H1); 3.86 (1H, s, H5); 5.93 (2H, s, OCH2O); 6.76 (1H, d, J = 8.1, ArH); 6.66 (1H, d, J = 1.5, ArH); 6.90 (1H, dd, J = 8.1, 1.5 ArH). 13C NMR (δ): 39.3 (CH2, C4), 46.1 (CH2, C2); 47.3 (CH2, C7); 55.9 (CH, CI); 67.1 (CH, C5); 89.3 (C6); 101.0 (OCH2O); 103.5 (C3); 106.0 (ArCH); 107.9 (ArCH); 118.1 (ArCH); 135.7, 146.7, 147.8 (ipso Cx3). MS (CI): m/z 262 (M+1, 40%); 244 (M+1 - H2O, 25%). HRMS (CI): Found: 261.1002; Required: 261.1001 for C14H15NO4. The hydrochloride salt was made.

6β-phenyl-8-azabicyclo[3.2.1]octane-3α,6β-diol (50).

Reaction of 80 (0.059g, 0.149mmol) yielded 50 (C13H17NO2) after chromatography (10:2 ethyl acetate:methanol, Rf 0.18) as a colourless oil, which was recrystallised from DCM (0.012g, 36% yield); mp: 1H NMR (δ): 1.89 (1H, dd, J = 11.1, 14.7, H7a); 2.03 (1H, 2 x dd, J = 4.5, 4.8, H4e); 2.13 (1H, 2 x dd, J = 4.5, 4.8, H2e); 2.36 (2H, dd, J = 14.4, 15.6, H2a, H4a); 2.64 (1H, dd, J = 7.5, H7e); 3.29 (1H, s, OH); 3.53-3.56 (4H, m, H1, H5, NH, OH); 4.17 (1H, dd, J = 5.1, 10.2, H3a); 7.25 (1H, d, J = 7.2, ArH); 7.34 (2H, dd, J = 7.2, 7.8, ArH x 2); 7.60 (2H, dd, J = 7.2, 1.2, ArH x 2). 13C NMR (δ): 33.5 (CH2, C4), 40.6 (CH2, C2); 45.5 (CH2, C7); 53.0 (CH, C1); 63.7 (CH, C3); 64.1 (CH, C5); 82.8 (C6); 124.7, 126.7, 128.3 (ArCH x 5); 148.7 (ipso C). MS (CI): m/z 220 (M+1, 40%); 202 (-H2O,
100%). HRMS (Cl\textsuperscript{+}): Found: 220.1335; Required: 220.1338 for C\textsubscript{13}H\textsubscript{18}N\textsubscript{2}O\textsubscript{2}. The hydrochloride salt was made.

6\beta-(3,4-methylenedioxyphenyl)-8-azabicyclo[3.2.1]octane-3\alpha,6\beta-diol (54).

Reaction of 81 (0.048g, 0.109mmol) yielded 54 (C\textsubscript{14}H\textsubscript{17}N\textsubscript{2}O\textsubscript{2}) after chromatography (10:2 ethyl acetate:methanol; R\textsubscript{f} 0.12) as a colourless oil which was recrystallised from diethyl ether, (0.015g, 52% yield); mp: \textsuperscript{1}H NMR (\delta): 1.90 (1H, dd, J = 15.0, 16.5, H7a); 2.05 (1H, 2 x dd, J = 3.9, H4e); 2.16 (1H, 2 x dd, J = 4.2, H2e); 2.35 (2H, dd, J = 14.4, H2a, H4a); 2.60 (1H, dd, J = 7.8, H7e); 3.23 (1H, s, OH); 3.56 (1H, dd, J = 3.9, H1); 3.77 (3H, bs, H5, NH, OH); 4.18 (1H, dd, J = 4.5, 9.9, H3a); 5.94 (2H, s, OCH\textsubscript{2}O); 6.75 (1H, d, J = 8.4, ArH); 7.00 (1H, dd, J = 1.8, 8.1, ArH); 7.19 (1H, dd, J = 1.8, ArH). \textsuperscript{13}C NMR (\delta): 35.2 (CH\textsubscript{2}, C4), 40.4 (CH\textsubscript{2}, C2); 45.4 (CH\textsubscript{2}, C7); 52.8 (CH, C1); 63.7 (CH, C3); 64.1 (CH, C5); 82.5 (C6); 100.9 (OCH\textsubscript{2}O); 106.3 (ArCH); 107.5 (ArCH); 117.5 (ArCH); 142.8 (ipso C); 146.3 (ipso C); 147.8 (ipso C). MS (Cl\textsuperscript{+}): m/z 246 (M+1-H\textsubscript{2}O, 10%). The hydrochloride salt was made.

9.6 Experimental for Chapter Five: Synthesis of Spiroyclic Derivatives

Synthesis of (2R,5R)-5-allyl-2-tert-butyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one (122).

L-Proline (anhydrous) (5.0g, 0.043mol) was suspended in anhydrous n-pentane (160mL) under argon. Pivaldehyde (23ml, 0.215mol) was added, followed by trifluoroacetic acid (0.077mL, 1.892mmol). The mixture was heated at reflux for 66 hours with the azeotropic removal of water using a dean-stark apparatus. The clear solution was filtered from the
remaining white solid and concentrated \textit{in vacuo} to yield \textbf{122} \((\text{C}_{10}\text{H}_{17}\text{NO}_2)\) as a colourless oil \((3.66\text{g, 47\% yield})\). The colourless oil immediately began to hydrolyse, producing a white solid \(\text{(proline)}\), and was used immediately without purification. Freshly prepared lithium diisopropylamine \((21\text{mL, 0.021mol})\) was added to a 0.17M solution of \textbf{122} \((3.66\text{g, 0.02mol})\) in anhydrous THF \((120\text{ml})\) at \(-78^\circ\text{C}\). After 30 mins, allyl bromide \((1.9\text{mL, 0.022mol})\) was added dropwise and the temperature was allowed to warm to \(-30^\circ\text{C}\) over 3 hours. The mixture was partitioned between DCM \((100\text{mL})\) and water \((100\text{mL})\). The organic layer was washed with water \((100\text{mL})\), dried \(\text{(sodium sulphate)}\) and concentrated \textit{in vacuo} to yield a crude yellow oil which was dried under high vacuum. Chromatography \((1:1\ \text{DCM:hexane})\) yielded a yellow powder \((0.11\text{g})\), which \(^1\text{H NMR}\) indicated consisted of a mixture of \textbf{123} \((\text{C}_{13}\text{H}_{21}\text{NO}_2)\) and some \textbf{121} \((\text{C}_{10}\text{H}_{17}\text{NO}_2)\). MS \((\text{Cl}^+): m/z\) 184 \((\text{M+1, 121, 60\%}); 216 \text{ (40\%); 224 (M+1, 123, 20\%)}\).

\textit{Synthesis of S-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)ester (126).}

Proline \((2.0\text{g, 17.37mmol})\) was dissolved in distilled water \((45\text{mL})\) and cooled in an ice-bath. To this solution was added 10\% sodium hydroxide \((9\text{mL})\), followed by a solution of di-\textit{tert}-butyldicarbonate \((4.32\text{g, 19.80mmol})\) in dioxane \((45\text{mL})\). The reaction was stirred at 0\(^\circ\text{C}\) for 5 hours and room temperature overnight. The mixture was washed with diethyl ether \((100\text{mL})\) and the aqueous phase acidified with 2M hydrochloric acid to pH3, and extracted with ethyl acetate \((4\times150\text{mL})\). Combined organic extracts were washed with sodium chloride \((\text{sat.})\) \((150\text{mL})\), water \((150\text{mL})\), the dried \(\text{(sodium sulphate)}\) and concentrated \textit{in vacuo} to yield \textbf{126} \((\text{C}_{10}\text{H}_{17}\text{NO}_4)\) as a white solid, which was recrystallised form diethyl ether \((3.42\text{g, 91\% yield}); \text{m.p. 133\^\circ C (lit. m.p. 133-135\^\circ C)}\). \(^1\text{H NMR}\ (\delta):\)
Chapter Nine Experimental Methods and Data

1.43 (3H, s, CH₃); 1.49 (6H, s, 2xCH₃); 1.92-2.00 (2H, m, H₃, H₃’); 2.03-2.14 (1H, m, H₄,); 2.26-2.32 (1H, m, H₄’); 3.35-3.57 (2H, m, H₅, H₅’); 4.25 (4.35*) (1H, dd, H₂, J₂,₃ = 3.9 (2.7*), J₂,₃’ = 8.1); 9.27 (1H, bs, COOH). ¹³C NMR (δ): 23.6 (24.3*) (CH₂, C₄); 28.3 (3 x CH₃); 30.8 (CH₂, C₃); 46.3 (46.9*) (CH₂, C₅); 59.0 (C₂); 80.3 (81.2*) ((CH₃)₃CO); 153.9 (165.2*) (OC=O); 175.2 (178.7*) (C=O, COOH). MS (Ctf): m/z 216 (M+1, 29%); 198 (-H₂O, 10%); 160 (100%); 116 (60%).

Procedure for the esterification of 126

Synthesis of S-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-methyl ester (128).

A solution of 126 (3.42g, 15.89mmol), dimethylaminopyridine (0.97g, 7.94mmol) and methanol (1.93mL, 47.67mmol) was cooled in an ice-bath with stirring. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.35g, 17.48mmol) was added and the mixture was stirred at 0°C for 2 hours and room temperature overnight. The reaction mixture was evaporated to dryness, taken up in ethyl acetate (100mL) and water (100mL), separated, and the water fraction further extracted with ethyl acetate (4x100mL). Combined organic fractions were washed with sodium chloride (sat.) (2x100mL), water (2x100mL), then dried (sodium sulphate) and concentrated in vacuo to yield a colourless oil which partially crystallised upon standing. Chromatography (1:4 ethyl acetate:hexane; Rf 0.27) yielded 128 (C₁₁H₁₉NO₄) as a colourless oil (3.39g, 93%); α_D = -58.110 (ethanol, 22°C, 5cm² path length); ¹H NMR (δ): 1.39 (6H, s, 2 x CH₃); 1.44 (3H, s, CH₃); 1.81-1.98 (3H, m, H₃, H₃’, H₄); 2.15-2.23 (1H, m, H₄’); 3.38-3.55 (2H, m, H₅, H₅’); 3.70 (3.75*)
(3H, s, OCH₃); 4.20 (4.31*) (1H, dd, H2, J₂,₃ = 3.9 (3.3*)). ¹³C NMR (δ): 23.6 (24.3*) (CH₂, C4); 28.2 (3 x CH₃); 29.9 (30.8*) (CH₂, C3); 46.3 (46.5*) (CH₂, C5); 51.9 (52.1*) (OCH₃); 58.7 (59.1*) (CH, C2); 79.8 ((CH₃)₂CO); 153.8 (NC=O); 173.4 (173.7*) (C=O, COOMe). MS (Cl⁺): m/z 230 (M+1, 100%).

**Synthesis of S-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-ethyl ester (127).**

In the same manner, 126 (2.04g, 9mmol) was reacted to form 127 (C₁₂H₂₁NO₄), which was isolated after chromatography (1:4 ethyl acetate:hexane; Rf 0.48) as a colourless oil (1.00g, 55% yield). ¹H NMR (δ): 1.28 (3H, t, CH₃, J = 7.2); 1.42 (6H, s, 2 x CH₃); 1.47 (3H, s, CH₃); 1.83-2.08 (3H, m, H₃, H₃', H₄); 2.15-2.27 (1H, m, H₄'); 3.36-3.59 (2H, m, H₅, H₅'); 4.12-4.32 (3H, m, H₂, OCH₂). ¹³C NMR (δ): 14.1 (14.2*) (CH₂CH₃); 23.6 (24.2*) (CH₂, C4); 28.2, 28.4 (3 x CH₃); 29.9 (30.8*) (CH₂, C3); 46.3 (46.5*) (CH₂, C5); 58.8 (59.1*) (CH, C2); 60.8 (OCH₂); 79.8 ((CH₃)₂CO); 153.8 (154.3*) (NC=O); 172.9 (173.2*) (C=O). MS (Cl⁺): m/z 244 (M+1, 100%).

**Synthesis of S-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-tert-butyl ester (125).**

In the same manner, 126 (3.542g, 16.5mmol) was reacted to form 125 (C₁₄H₂₆NO₄), which was isolated after chromatography (1:4 ethyl acetate:hexane; Rf 0.59) as a colourless oil (2.478g, 55% yield). ¹H NMR (δ): 1.44 (6H, s, 2 x CH₃); 1.46 (3H, s, CH₃); 1.47 (9H, s, 3 x CH₃); 1.85-1.97 (3H, m, H₃, H₃', H₄); 2.13-2.25 (1H, m, H₄'); 3.38-3.56 (2H, m, H₅, H₅'); 4.11 (4.19*) (1H, dd, H₂, J = 3.6 (3.0*), 8.4). ¹³C NMR (δ): 23.4 (24.2*) (CH₂, C4); 27.0 (3 x CH₃); 28.3 (3 x CH₃); 29.8 (30.8*) (CH₂, C3); 46.2 (46.4*) (CH₂, C5); 59.6 (CH,
C2); 79.3 (79.5*) ((CH₃)₃CO); 80.7 ((CH₃)₃CO); 153.9 (NC=O); 172.3 (C=O). MS (Cl⁺): m/z 272 (M+1, 100%).

**Procedure for the allylation of 128, 127 and 125.**

THF (4.82mL) was syringed into a flask under argon. Diisopropylamine (1.48mL, 10.5mmol) was added and the solution cooled to 0°C with stirring. n-Butyl lithium (2.5M in Hexane, 4.2mL, 10.5mmol) was added and the solution was stirred for 30 mins at 0°C. A 1M solution of lithium diisopropylamine was thus prepared.

Freshly prepared lithium diisopropylamine (1M, 1.5 molar equivalents) was added to a 0.17M stirred solution of the Boc-protected proline ester substrate in anhydrous THF under an argon atmosphere at -78°C. After 30 minutes, allyl bromide (1.5 molar equivalents) was added, and the solution stirred at -78°C, with the temperature warming to -30°C over 3hrs. The mixture was partitioned between DCM (150mL) and water (150mL), and the organic layer was washed with water (2x100mL), then dried (sodium sulphate) and concentrated in vacuo, leaving the crude product as a yellow oil. Chromatography provided the desired product as a pale yellow oil.

**Synthesis of (±) 2-(2-propenyl)-1,2-pyrrolidinedicarboxylic acid, 1-(1,1-dimethylethyl)-2-methyl ester (143).**

The methyl ester 128 (1.45g, 6.32mmol) was reacted to afford 143 (C₁₄H₂₃NO₄) after chromatography (1:4 ethyl acetate:hexane; Rf 0.55) as a yellow oil (1.28g, 75% yield). α₀ = -0.033 (ethanol, 22°C, 5cm² path length); ¹H NMR (δ): 1.40 (6H, s, 2 x CH₃); 1.43 (3H, s, CH₃); 1.78-1.87 (3H, m, H3, H3', H4); 2.01-2.19 (2H, m, H4', CH); 2.58 (1H, dd, J = 8.4,
CH'); 2.90 (3.08*) (1H, dd, J = 6.6, H5); 3.30-3.40 (1H, m, H5); 3.70 (3H, s, OCH3); 5.07-5.13 (2H, m, =CH2); 5.67-5.79 (1H, m, =CH). 13C NMR (δ): 22.6 (23.0*) (CH2, C4); 28.3, 28.4 (3 x CH3); 35.6 (36.9*) (CH2, C3); 38.3 (39.5*) (CH2); 48.4 (48.5*) (CH2, C5); 52.1 (52.1*) (OCH3); 66.9 (67.4*) (C2); 79.5 (80.0*) ((CH3)3CO); 118.7 (119.0*) (=CH2); 133.2 (133.6*) (=CH); 153.5 (NC=O); 175.1 (C=O). MS (Cl\(^{+}\)): m/z 270 (M+1, 60%); 242 (-iBu, 10%); 214 (100%).

**Synthesis of (+) 2-(2-propenyl)-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-ethyl ester (142).**

The ethyl ester 127 (3.471g, 14.27mmol) was reacted to afford 142 (C15H25N04) after chromatography (1:7 ethyl acetate:hexane; Rf 0.43) as a yellow oil (2.87g, 71% yield). 1H NMR (δ): 1.27 (3H, t, CH3, J = 7.2); 1.41 (6H, s, 2 x CH3); 1.44 (3H, s, CH3); 1.77-1.87 (2H, m, H3, H3'); 2.01-2.19 (2H, m, H4, H4'); 2.58 (1H, dd, J = 8.1, CH); 2.92 (3.08*) (1H, dd, J = 6.6, CH'); 3.23-3.41 (1H, m, H5); 3.54-3.61 (3.64-3.72*) (1H, m, H5'); 4.05-4.25 (2H, m, OCH2); 5.11 (2H, dd, J = 6.0, 12.3, =CH2); 5.67-5.81 (1H, m, =CH). 13C NMR (δ): 14.2 (CH2CH3); 22.5 (23.1*) (CH2, C4); 28.3, 28.4 (3 x CH3); 35.7 (37.1*) (CH2, C3); 38.2 (39.4*) (CH2); 48.5 (CH2, C5); 60.9 (61.0*) (OCH2); 66.9 (67.4*) (C2); 79.4 (80.0*) ((CH3)3CO); 118.6 (118.9*) (=CH2); 133.4 (133.8*) (=CH); 153.8 (OC=O); 174.2 (C=O). MS (Cl\(^{+}\)): m/z 232 (100%), 228 (-iBu, 80%).

**Synthesis of (±) 2-(2-propenyl)-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-tert-butyl ester (141).**

The tert-butyl ester 125 (1.681g, 6.17mmol) was reacted to afford 141 (C17H29N04), after chromatography (1:4 diethyl ether:hexane; Rf 0.36) as a yellow oil (0.782g, 85% yield,
based upon 48% recovered). $^1$H NMR (δ): 1.44, 1.46, 1.47 (18H, s, 6 x CH$_3$); 1.73-1.88 (2H, m, H3, H3'); 1.96-2.06 (1H, m, H4); 2.09-2.18 (m, 1H, H4'); 2.52 (2.55*) (1H, dd, J = 9.0, 14.1, CH); 2.95 (3.05*) (1H, dd, J = 6.6, 14.1, CH'); 3.29-3.41 (1H, m, H5); 3.49-3.57 (3.63-3.71*) (1H, m H5'); 5.10 (2H, dd, =CH$_2$, J = 8.4, 12.3); 5.66-5.76 (1H, m, =CH). $^{13}$C NMR (δ): 22.3 (23.1*) (CH$_2$, C4); 27.9 (3 x CH$_3$); 28.3 (3 x CH$_3$); 35.6 (37.2*) (CH$_2$, C3); 38.2 (39.0*) (CH$_2$); 48.5 (48.6*) (CH$_2$, C5); 67.1 (67.7*) (C2); 79.1 (79.9*) ((CH$_3$)$_3$CO); 80.5 (80.8*) ((CH$_3$)$_3$CO); 118.3 (118.8*) (=CH$_2$); 133.4 (134.0*) (=CH); 153.7 (NC=O); 173.4 (173.6*) (C=O). MS (Cl$^+$): m/z 312 (M+1, 5%); 256 (-tBu, 10%); 200 (20%); 100 (100%).

**Synthesis of bromocyclohexane (146).**

Alumina (2.00g) was equilibrated with the atmosphere at 120°C for 68 hours. The flask was stoppered and the contents cooled to room temperature. A solution containing cyclohexane (0.101mL, 1.00mmol) in dichloromethane (1mL) was added and the resulting suspension was stirred under nitrogen. A solution of 1M trimethylsilyl bromide (0.528ml, 4mmol, in 4mL anhydrous DCM) was added dropwise over 20 minutes, then stirring continued for 2 hours. The suspension was filtered, and the filtrate was washed with DCM (3x25mL). The combined organic fractions were washed with sodium hydrogen carbonate (sat.) (2x50mL), followed by water (50mL), then dried (sodium sulphate) and concentrated in vacuo to yield 146 (C$_6$H$_n$Br) as a colourless oil (0.147g, 90% yield). $^1$H NMR (δ): 1.25 (1H, s, CH); 1.34-1.43 (2H, m, CH$_2$); 1.55-1.60 (1H, m, CH); 1.76-1.88 (4H, m, 2 x CH$_2$); 2.13-2.18 (2H, m, CH$_2$); 4.16-4.23 (1H, m, HC-Br).
**Synthesis of (+)-2-(2-bromopropyl)-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl)-2-methyl ester (138).**

Following the above procedure, a solution of 143 (0.500g, 1.855mmol) in anhydrous DCM (15mL) was added to dried alumina and the resulting suspension was stirred at room temperature. A 1M solution of trimethylsilyl bromide (7.42mmol) in anhydrous DCM was added dropwise over 20 minutes and the solution was stirred at room temperature for 5 hours. After the usual work-up, chromatography (1:4 ethyl acetate:hexane) afforded recovered 143 (0.300g, 60% yield) and 148 (C₉H₁₅NO₂) as a pale yellow oil (Rᶠ 0.06, 1:4 ethyl acetate:hexane) (0.100g, 32% yield). Spectral data for 148: 

\[ ^1H \text{NMR (δ): 1.63-1.78} \]
\[ (3H, m, H₂, H₃', H₄'); 2.09-2.14 (1H, m, H₄'); 2.29 (1H, dd, J = 8.4, CH); 2.49 (1H, s, NH); 2.52 (1H, dd, J = 8.4, CH); 2.93 (2H, dd, J = 6.9, H₂, H₂'); 3.66 (3H, s, OCH₃); 5.00-5.07 (2H, m, =CH₂); 5.62-5.73 (1H, m, =CH); \]
\[ ^13C \text{NMR (δ): 27.8 (CH₂, C₄); 35.1 (CH₂, C₃); 43.9 (CH₂); 46.3 (CH₂, C₅); 52.1 (OCH₃); 69.1 (C₂); 118.1 (=CH₂); 133.7 (=CH); 177.0 (C=O). MS(Cl): m/z 170 (M⁺, 100%); 128 (30%).} \]

**Synthesis of N-(phenylmethyl)benzamide (151).**

Sodium diethyl dibenzylamidoaluminate (1.83mmol) was prepared by heating at reflux under argon for 1 hour the mixture of 2M toluene sodium diethylhydroaluminate (0.915mL, 1.83mmol) and benzylamine (0.398mL, 3.66mmol) in THF:toluene (1:1) (4mL). 150 (0.500g, 3.33mmol) in THF:toluene (1:1) (1mL) was added, and the mixture was heated at reflux for 1 hour. The reaction mixture was quenched with dilute hydrochloric acid (5mL) and extracted with ethyl acetate (3x50mL). Combined extracts were washed with water (2x50mL), then dried (sodium sulphate) and concentrated in vacuo to yield 151.
(C_{15}H_{15}NO) as an off-white solid (0.70g, 93% yield). Mp: 104-105°C (lit. Mp: 104-106°C\textsuperscript{353}) \textsuperscript{1}H NMR (δ): 3.64 (2H, s, PhCH\textsubscript{2}O); 4.41 (1H, s, NCHPh); 4.43 (1H, s, NCHPh); 5.75 (1H, bs, NH); 7.17-7.38 (10H, m, ArH x 10). \textsuperscript{13}C NMR (δ): 43.6, 43.8 (PhCH\textsubscript{2} x 2); 127.4, 127.5, 128.6, 129.0, 129.4 (ArCH x 10); 134.8 (ipso C); 138.1 (ipso C); 170.8 (C=O). MS (Cl\textsuperscript{+}): \textit{m/z} 226 (M+1, 100%).

\textit{Attempted synthesis of (±) 2-(2-propenyl)-1-pyrrolidinecarboxylic acid,1-(1,1-dimethylethyl)-2-benzamide (139) via sodium diethyldibenzylamidoaluminate.}

Sodium diethyldibenzylamidoaluminate (1.49mmol) was prepared by heating at reflux under argon for 1 hour the mixture of 2M toluene sodium diethyldihydroaluminate (0.740mL, 1.49mmol) and benzylamine (0.324mL, 2.97mmol) in toluene (4mL). 141 (0.827g, 2.70mmol) in toluene (2mL) was added, and the mixture refluxed for 21 hours. The reaction mixture was quenched with dilute hydrochloric acid (5mL) and extracted with ethyl acetate (3x50mL). The combined extracts were washed with water (2x50mL), then dried (sodium sulphate) and concentrated \textit{in vacuo} to yield a crude yellow oil. Chromatography (1:4 ethyl acetate) recovered 141 as a yellow oil (0.65g, 79% recovery), identified by \textsuperscript{1}H NMR.

In an analogous preparation, 143 (0.100g, 0.41mmol) was added to a solution of freshly prepared sodium diethyldibenzylamidoaluminate (0.615mmol) in toluene and heated at reflux for 21 hours. The usual work up, followed by chromatography (preparative TLC, 1:8 ethyl acetate:hexane; \textit{Rf} 0.17) afforded 152 (C\textsubscript{16}H\textsubscript{18}N\textsubscript{2}O\textsubscript{2}) as a pale yellow oil (0.033g, 30% yield). \textsuperscript{1}H NMR (δ): 1.26-2.10 (3H, m, H3, H3', H4); 2.19-2.30 (1H, m, H4'); 2.38 (1H, dd, \textit{J} = 6.6, 13.8, CH); 2.56 (1H, dd, \textit{J} = 7.5, 13.8, CH'); 3.15-3.24 (1H, m, H5); 3.73-3.82 (1H,
m, H5'); 4.59 (2H, dd, J = 7.2, 14.7, PhCH2); 4.71 (1H, dd, J = 15.0, 7.5, PhCH); 5.08 (2H, 
dd, J = 10.2, 15.9, =CH2); 5.54-5.66 (1H, m, =CH); 7.24-7.35 (5H, m, ArH x 5). 13C NMR 
(δ): 26.1 (CH2, C4); 32.1 (CH2, C3); 39.5 (CH2); 42.5 (CH2Ph); 45.0 (CH2, C5); 72.2 (C2); 
121.1 (=CH2); 127.6, 128.1, 128.4 (ArCH x 5); 130.9 (=CH); 135.8 (ipsoC); 159.8 
(NC(N)=O); 175.3 (C=O). MS (CI'): m/z 271 (M+1, 100%), 229 (30%).

**Attempted synthesis of 139 via dimethylaluminium benzylamide.**

Dimethylaluminium benzylamide was prepared by slowly adding trimethylaluminium (2M in 
hexane, 0.288mL, 0.576mmol) to a stirred solution of benzylamine (0.078mL, 0.72mmol) in anhydrous DCM (3mL) at room temperature under nitrogen. The mixture 
was stirred at room temperature for 15 minutes, then 143 (0.100g, 0.36mmol) in DCM 
(1mL) was added dropwise. The mixture was heated at reflux for 16 hours. The reaction 
was quenched with dilute hydrochloric acid (2mL) and extracted with DCM (3x20mL). The 
combined extracts were washed with water (5mL), then dried (sodium sulphate) and 
concentrated *in vacuo* to recover 143 as a yellow oil (0.100g), as identified by 1H NMR.

**Synthesis of (±)-2-(2-propenyl)-1,2-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl) 
ester (149).**

Lithium hydroxide method: A solution of 143 (1.09g, 4.05mmol) in dry THF (5mL) was 
stirred in a 0.2M solution of lithium hydroxide (100mL) at 55°C for 24 hours. The reaction 
mixture was evaporated to dryness, taken up in diethyl ether (50mL) and water (50mL) and 
separated. The aqueous mixture was acidified with dilute hydrochloric acid and extracted 
with diethyl ether (4x100ml). The combined organic extracts were washed with sodium 
chloride (sat.), water, then dried (sodium sulphate) and concentrated *in vacuo* to afford 149
(C13H22NO4) as an off-white solid (0.60g, 58% yield). Unreacted 143 was recovered from the initial diethyl ether separation (0.46g, 42% recovery). 143 was recrystallised from ethanol-water to afford white crystals. Mp: 94-97°C. 1H NMR (δ): 1.41, 1.45 (9H, s, 3 x CH3); 1.75-1.98 (3H, m, H3, H3’, H4); 2.13 (1H, dd, J = 6.9, H4’); 2.40-2.46 (1H, m, CH); 2.51-2.69 (1H, m, CH’); 2.88-2.98 (1H, m, H5); 3.23-3.40 (1H, m H5’); 5.14 (2H, ddd, =CH2, J = 6.3, 9.0, 15.3); 5.58-5.78 (1H, m, =CH); 7.77 (1H, bs, OH). 13C NMR (δ): 22.8 (22.9*) (CH2, C4); 28.5 (28.6*) (3 x CH3); 35.0 (37.3*) (CH2, C3); 38.3 (39.4*) (CH2); 48.9 (49.4*) (CH2, C5); 66.1 (67.1*) (C2); 80.8 (81.7*) ((CH3)3CO); 119.4 (120.1*) (=CH2); 132.2 (133.3*) (=CH); 153.9 (NC=O); 176.5 (180.5*) (C=O, COOH). MS (Cl+): m/z 256 (M+1, 10%); 214 (100%); 200 (40%); 170 (80%).

Sodium hydroxide method: To a solution of 143 (3.65g, 13.55mmol) in THF (40mL) was added 1M sodium hydroxide (40mL) and the mixture was stirred at 80°C for 72 hours. The reaction mixture was concentrated in vacuo, then taken up in diethyl ether (100mL) and water (100mL). The diethyl ether layer was separated and the aqueous fraction was acidified to pH3 with 2M hydrochloric acid, and extracted with diethyl ether (4x100mL). The combined organic fractions were washed with sodium chloride (sat.) (2x100mL), water (2x100mL), then dried (sodium sulphate) and concentrated in vacuo to yield 149 (C13H22NO4) as a pale yellow oil which crystallised off-white upon standing, and was recrystallised from ethanol-water (3.07g, 89% yield); All spectroscopic and melting point data agreed with that given above.
**Synthesis of 139 from 149.**

The acid **149** (1.50g, 5.875mmol), benzyamine (0.642mL, 5.875mmol), 1-hydroxybenzotriazole (0.794g, 5.875mmol) and triethylamine (0.819mL, 5.875mmol) were dissolved in anhydrous chloroform (30mL). A solution of dicyclohexylcarbodiimide (1.21g, 5.875mmol) in anhydrous chloroform (10mL) was added and the solution was stirred at room temperature under nitrogen for 16 hours. Precipitated dicyclohexylurea was filtered off, and the filtrate was diluted with chloroform (100mL) before being washed with 1M aqueous sodium hydrogen carbonate (50mL), 10% aqueous citric acid (50mL), sodium chloride (sat.) (50mL) and water (50mL), then dried (sodium sulphate) and concentrated *in vacuo* to yield an orange oil which crystallised upon standing. Chromatography (1:4 ethyl acetate:hexane; Rf 0.41) provided **139** (C_{20}H_{28}N_{2}O_{3}) as a white solid (2.00g, 99% yield), which was recrystallised from diethyl ether/hexane to yield white crystals. Mp: 184-186°C.

$^1$H NMR (δ): 1.34 (1.43*) (9H, s, 3 x CH$_3$); 1.73-1.96 (3H, m, H3, H3', H4); 2.19-2.31 (1H, m, H4'); 2.61 (2.75*) (2H, dd, J = 7.5, 8.1, CH, CH'); 2.98-3.10 (1H, m, NH); 3.21-3.43 (1H, m, H5); 3.45-3.52 (3.61-3.64*) (1H, m H5'); 4.34-4.52 (2H, bm, CH$_2$Ph); 5.14 (2H, dd, J = 16.8, 9.0, =CH$_2$); 5.65 (1H, dddd, J = 8.1, 9.3, 8.7, 7.2, =CH); 7.28 (5H, s, ArH x 5). $^{13}$C NMR (δ): 22.2 (22.5*) (CH$_2$, C4); 28.3 (3 x CH$_3$); 34.5 (37.6*) (CH$_2$, C3); 38.6 (38.7*) (CH$_2$); 43.5 (43.7*) (CH$_2$Ph); 49.0 (49.5*) (CH$_2$, C5); 68.3 (69.9*) (C2); 80.1 (80.8*) ((CH$_3$)$_3$CO); 119.3 (119.5*) (=CH$_2$); 126.9, 127.1, 127.4, 127.8, 128.3 (ArCH x 5); 132.5 (132.7*) (=CH); 138.4 (ipsoC); 155.0 (NC(O)=O); 173.8 (NC=O amide). MS (Cl$^+$): m/z 345 (M+1, 64%), 289 (=Bu, 100%), 245 (80%). HRMS (Cl$^+$): Found: 345.2180; Required: 345.2180 for C$_{20}$H$_{29}$N$_2$O$_3$. 

271
Alternatively, to a stirred solution of 149 (0.08g, 0.313mmol) in anhydrous DCM (5mL) was added dimethylaminopyridine (0.004g, 0.031mmol) and benzylamine (0.041mL, 0.376mmol) and the mixture was cooled to 0°C. Dicyclohexylcarbodiimide (0.071g, 0.345mmol) was added and stirring continued at 0°C for 5 minutes and 40°C for 24 hours. Precipitated dicyclohexylurea was filtered off and the filtrate was evaporated in vacuo. The residue was taken up in DCM (30mL) and further filtered of precipitated urea. The residue was washed with dilute hydrochloric acid (30mL), aqueous sodium hydrogen carbonate (sat.) (30mL), water (2x30mL), then dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil. Chromatography (1:4 ethyl acetate:hexane; Rf 0.41) provided 139 (C20H28N2O3) as a yellow oil (0.035g, 32% yield). All spectroscopic data agreed with that above for 139.

**Synthesis of S-2-[(phenylmethyl)amino]carbonyl]-1-pyrrolidinedicarboxylic acid,1-(1,1-dimethylethyl) ester (153).**

The acid 126 (1.00g, 4.64mmol), benzylamine (0.507mL, 4.64mmol), 1-hydroxybenzotriazole (0.627g, 4.64mmol) and triethylamine (0.647mL, 4.64mmol) were dissolved in anhydrous chloroform (30mL) under nitrogen. A solution of dicyclohexylcarbodiimide (0.957g, 4.64mmol) in anhydrous chloroform (15mL) was added and the reaction was stirred at room temperature for a total of 48 hours. Precipitated dicyclohexylurea was filtered off, the filtrate was diluted with chloroform (50mL), washed with 1M aqueous sodium hydrogen carbonate (100mL), 10% aqueous citric acid (100mL), water (100mL), then dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow solid. Chromatography (1:4 ethyl acetate:hexane; Rf 0.07) provided the amide 153 (C17H24N2O3) as a white solid (0.95g, 67% yield). Mp: 130-131°C (lit. mp: 130-131°C354).
\(^1\)H NMR (\(\delta\)): 1.40 (1.43\(^*\)) (9H, 2 x s, 3 x CH\(_3\)); 1.90 (3H, bs, H3, H3', H4); 2.18 (2.37\(^*\)) (1H, 2 x s, H4'); 2.98-3.10 (1H, m. NH); 3.42 (2H, bs, H5, H5'); 4.22-4.57 (3H, m, CH\(_2\)Ph, H2); 7.25-7.32 (5H, s, ArH x 5). \(^{13}\)C NMR (\(\delta\)): 24.6 (25.6\(^*\)) (CH\(_2\), C4); 28.2 (3 x CH\(_3\)); 31.0 (33.9\(^*\)) (CH\(_2\), C3); 43.2 (CH\(_2\)Ph); 47.1 (48.9\(^*\)) (CH\(_2\), C5); 59.8 (61.3\(^*\)) (C2); 80.5 ((CH\(_3\))\(_3\)CO); 127.4, 128.6 (ArCH x 5); 138.2 (ipsoC); 153.8 (NC(O)=O); 174.0 (NC=O amide). MS (Cl\(^+\)): \(m/z\) 305 (MH\(^+\), 80%), 249 (MH\(^+\)-'Bu 100%), 205 (85%).

**Synthesis of 139 from 153.**

Freshly prepared lithium diisopropylamine (1M in THF:hexane, 3.28mL, 3.28mmol) was added to a 0.17M solution of 153 (0.50g, 1.64mmol) in anhydrous THF (9.65mL) under nitrogen at -78°C with stirring. After 30 minutes allylbromide (0.14mL, 1.64mmol) was added, and the solution was stirred at -78°C, with the temperature rising to -30°C over 3 hours. The mixture was partitioned between DCM (100mL) and water (100mL), and the organic layer was washed with water (2x100mL), then dried (sodium sulphate) and concentrated in vacuo to yield the crude product as an off-white solid. TLC of the crude product (1:4 ethyl acetate:hexane) revealed the presence of 153 (R\(_f\) 0.07) and a new product (R\(_f\) 0.41). \(^1\)H NMR and MS (Cl\(^+\)) analysis of the crude product mixture revealed 153 and some 139 (approx. 10%). MS (Cl\(^+\)): \(m/z\) 345 (MH\(^+\), 10%), 305 (MH\(^+\), SM), 249 (100%), 205.
Attempted synthesis of \(1\)-(2,2-dimethylethyl)-7-benzyl-8-methyl-6-oxo-1,7-diazaspiro[4.4]non-8-en-1-carboxylate (156) utilising palladium (II) chloride bisacetonitrile and sodium hydride.

A solution of \(139\) (0.100g, 0.290mmol), sodium hydride (0.0115g, 0.479mmol) and palladium (II) chloride bisacetonitrile (0.113g, 0.435mmol) were stirred in THF at room temperature under nitrogen for 16 hours. The mixture was filtered of palladium(O) and concentrated to yield a crude brown oil (0.150g) which \(^1\)H NMR indicated consisted of a 1:1 mixture of 156 and 139. TLC analysis (1:4 ethyl acetate:hexane) revealed three components: R\(_f\) 0.64, R\(_f\) 0.33, R\(_f\) 0.03. \(^1\)H NMR (\(\delta\))\(\delta\): 1.26 (1.5H, s, CH\(_3\)); 1.34 (3H, s, CH\(_3\)); 1.44 (6H, s, 2 x CH\(_3\)); 1.80-1.94 (3H, m, H\(_3\), H\(_3\), H\(_{4}\)); 2.28 (1H, dd, \(J=7.2, 7.8, H_{4}'\)); 2.56-2.60 (0.5H, m, CH); 2.72-2.81 (0.5H, m, CH); 3.03 (0.5H, bs, NH); 3.28-3.30 (1H, m, H\(_5\)); 3.48-3.53 (0.5H, m, H\(_5\)); 3.71-3.75 (0.5H, m, H\(_5\)); 4.35 (1H, dd, \(J=6.6, 7.2, CH_{2}Ph\)); 4.45-4.52 (1H, m, CH\(_2\)Ph); 5.15 (1H, dd, \(J=7.5, 9.3, =CH_{2}\)); 5.54 (0.5H, t, \(J=2.4, =CH\)); 5.58-5.72 (0.5H, m, =CH); 7.28 (5H, s, ArH x 5); MS(CI\(^+\)): \(m/z\) 345 (M+1, 139, 50%), 343 (M+1, 156, 15%), 287 (30%), 245 (90%).

Attempted synthesis of 156 utilising palladium (II) chloride bisacetonitrile, sodium hydride and triethylamine.

Palladium (II) chloride bisacetonitrile (0.113g, 0.435mmol) was transferred to a flask, stoppered and blown with nitrogen for 10 mins. Anhydrous THF (5mL) was added and the mixture was stirred under nitrogen for 10 minutes. \(139\) (0.100g, 0.290mmol) in anhydrous THF (4mL) was added via syringe and the mixture was stirred at room temperature for 45 minutes. Sodium hydride was added and the mixture was stirred at 50°C for 45 minutes.
Triethylamine (0.061ml, 0.435mmol) was added via syringe and the mixture was heated to reflux. Further triethylamine (0.061ml, 0.435mmol) was added at 1 hour intervals for 2 hours. The mixture continued to be heated at reflux for 20 hours. The reaction mixture was filtered of precipitated palladium (0) through celite and rinsed with ethyl acetate (3x25mL). The combined filtrate and ethyl acetate was washed with water (2x50mL), then dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:4 ethyl acetate) yielded 156 (C_{20}H_{26}N_{2}O_{3}) (0.008g), however a clean NMR sample could not be obtained. The sample was identified by MS (CI⁺): m/z 343 (M+1, 20%); 305 (55%); 287 (100%).

*Synthesis of 156 utilising palladium (II) chloride bisacetonitrile and triethylamine.*

Palladium (II) chloride bisacetonitrile (0.150g, 0.578mmol) was transferred to a flask, stoppered and blown with nitrogen for 10 minutes. Anhydrous THF (8.09mL) was added and the mixture was stirred under nitrogen for 10 minutes. A solution of 139 (0.199g, 0.578mmol) in THF (2.02mL) was added via syringe and the mixture was stirred at 40°C for 90 minutes, after which time triethylamine (0.080mL, 1.734mmol) was added via syringe. After 90 mins triethylamine (0.080mL) was again added, and after another 60 mins further triethylamine (0.200mL) was added. The mixture was heated at 50°C for 48 hours. The reaction mixture was filtered of precipitated palladium (0) through celite and rinsed with ethyl acetate (3x20mL). The combined filtrate and ethyl acetate was washed with water (2x50mL), then dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:2 ethyl acetate:hexane; Rf 0.05) afforded 156 as a yellow oil (0.06g, 30% yield). 

\[ \text{^{1}H NMR} (\delta): 1.24 (3H, s, CH3); 1.43 (6H, s, 2 x CH3); 1.47 (3H, s, CH3); 1.79-1.88 (3H, m, H3, H3', H4); 1.94-2.00 (1H, m, H4'); 3.54 (2H, ddd, J = 4.5, 4.5, \]
5.7, H5, H5'); 4.32 (1H, d, J = 15.6, CHPh); 4.82 (1H, d, J = 15.6, CHPh); 5.29 (1H, s, =CH); 7.21-7.30 (5H, m, ArH x 5); \(^{13}\)C NMR (δ): 23.0 (23.7*) (CH\(_2\), C4); 23.8 (CH\(_3\)); 28.8 (28.9*) (3 x CH\(_3\)); 36.5 (37.0*) (CH\(_2\), C3); 44.0 (44.4*) (CH\(_2\)Ph); 48.0 (48.3*) (CH\(_2\), C5); 64.7 (65.3*) (C2); 85.5 (85.8*) (=CCH\(_3\)); 79.7 (80.5*) ((CH\(_3\))\(_3\)CO); 127.5, 127.7, 128.1, (128.3*), 128.8, 128.9 (ArCH x 5); 130.9 (=CH); 135.8 (ipsoC); 153.5 (153.6*) (NC(O)=O); 175.0 (175.2*) (NC=O amide). MS (CI\(^+\)): \(m/z\) 343 (M+1, 20%); 305 (55%); 287 (100%). HRMS (EI\(^+\)): Found: 342.1934; Required: 342.1940 for (C\(_{20}\)H\(_{26}\)N\(_2\)O\(_3\)).

**Synthesis of 2-(2-propenyl)-2-benzyl-pyrrolidine-2-carboxamide (159): reduction of 139 using diborane.**

To a solution of diborane in THF (1M, 0.484mL, 0.484mmol) under nitrogen was added a solution of 139 (0.100g, 0.29mmol) in anhydrous THF (2mL) over 15 minutes, with the temperature being maintained at 0°C. The solution was then heated at reflux for 2 hours. The reaction mixture was cooled to room temperature and hydrochloric acid (4.5M, 10mL) was added. The THF was removed with rotary evaporation at atmospheric pressure. Sodium hydroxide pellets were added to the saturated aqueous phase, and the aqueous phase was extracted with ethyl acetate (3x30mL). The combined extracts were washed with water (2x50mL), then dried (sodium sulphate), concentrated *in vacuo* and dried under high vacuum to yield crude 159 (C\(_{15}\)H\(_{20}\)N\(_2\)O) as a yellow oil (0.080g). \(^1\)H NMR (δ): 1.80-2.04 (3H, m, H3, H3', H4); 2.14-2.31 (3H, m, H4', CH\(_2\)); 2.77-2.87 (2H, m, H5, H5'); 3.00-3.03 (1H, m, NH); 4.41 (2H, d, J = 6.0, CH\(_2\)Ph); 5.10 (2H, dd, J = 12.3, 2.1, =CH\(_2\)); 5.63-5.77 (1H, m, =CH); 7.23-7.35 (5H, m, ArH x 5); \(^{13}\)C NMR (δ): 26.2 (CH\(_2\), C4); 36.3 (CH\(_2\), C3); 42.8 (43.1*) (CH\(_2\)); 47.0 (CH\(_2\)Ph); 61.7 (62.5*) (CH\(_2\), C5); 68.6 (C2); 118.9 (=CH\(_2\)); 127.0,
127.2, 128.4 (ArCH x 5); 133.3 (=CH); 138.4 (ipsoC); 176.5 (C=O). MS (Cl⁻): m/z 245 (M+1, 15%); 149 (60%).

**Synthesis of 2-(benzylaminomethyl)-2-(2-propenyl)-pyrrolidine (160): Reduction of 139 using excess lithium aluminium hydride.**

In an argon atmosphere a stirred solution of 139 (0.100g, 0.29mmol) in anhydrous THF (2mL) was cooled to 0°C, and lithium aluminium hydride (1M, 0.033g) in anhydrous THF (0.87ml) was added dropwise. Stirring continued for 16 hours at room temperature. The precipitate was filtered off and washed with ethyl acetate (50mL). The combined organic filtrates were dried (sodium sulphate) and concentrated in vacuo to yield crude 160 (C₁₅H₂₂N₂) as a yellow oil (0.054g, 84% yield). ¹H NMR (δ): 1.67-1.88 (3H, m, H₃, H₃', H₄); 2.17 (1H, d, J = 8.7, H₄'); 2.24 (2H, d, J = 7.5, CH₂); 2.46 (1H, s, NH); 2.70-2.81 (2H, m, H₅, H₅'); 2.96 (1H, bm, NH); 3.12-3.18 (1H, m, CH); 3.24 (1H, d, J = 7.2, CH'); 3.53 (2H, dd, J = 11.7, 12.0, CH₂Ph); 5.02-5.10 (2H, m, =CH₂); 5.70-5.82 (1H, m, =CH); 7.24-7.38 (5H, m, ArH x 5); ¹³C NMR (δ): 25.7 (CH₂, C₄); 37.1 (CH₂, C₃; CH₂); 45.9 (CH₂Ph); 56.8 (CH₂, C₅); 64.3 (N-CH₂); 72.4 (C₂); 117.3 (=CH₂); 126.7, 127.9, 128.1, 128.2 (ArCH x 5); 135.1 (=CH); 139.0 (ipsoC). MS (Cl⁻): m/z 229 (M+1, 100%), 245, 259.

**Synthesis of 2-benzyl-2,3,3a,4,5,6-hexahydro-3-(R)-hydroxy-3a-(S)-(2-propenyl)-1H-pyrrolo[1,2-c]imidazol-1-one (161a) and 2-benzyl-2,3,3a,4,5,6-hexahydro-3-(S)-hydroxy-3a-(S)-(2-propenyl)-1H-pyrrolo[1,2-c]imidazol-1-one (161b).**

A solution of 139 (1.11g, 3.22mmol) in anhydrous THF (15mL) was cooled to 0°C under a nitrogen atmosphere, and lithium aluminium hydride (0.158g, 4.186mmol, 1M) in THF (4.19ml) was added dropwise. After heating at reflux for 4 hours, water (2mL) was added...
and the mixture was stirred at room temperature for 1 hour. The precipitate was filtered off and washed with ethyl acetate (100mL). The ethyl acetate solution was washed with water (50mL), then dried (sodium sulphate) and concentrated in vacuo to yield a pale yellow oil.

Chromatography (1:1 ethyl acetate:hexane) isolated two compounds (diastereomers): 161a (trans diastereomers) and 161b (cis diastereomers). 161a (C₁₆H₂₀N₂O₂) (Rₜ 0.63, 1:1 ethyl acetate:hexane) was isolated as a white solid, and was recrystallised from ethanol (0.148g, 17% yield). Mp: 118-120°C. ¹H NMR (δ): 1.04-1.12 (1H, m, H3); 1.81-1.94 (3H, m, H3’, H4, H4’); 2.19 (1H, dd, J = 13.5, 9.3, CH); 2.67 (1H, bd, J = 13.2, CH’); 3.00-3.09 (1H, m, H5); 3.77-3.85 (1H, m, H5’); 4.13 (1H, d, J = 15.0, PhCH); 4.46 (1H, bs, NH); 4.63 (1H, s, CH); 4.77 (1H, d, J = 15.0, PhCH); 5.11 (2H, dd, J = 15.9, 9.3, =CH₂); 5.82-5.94 (1H, m, =CH); 7.23-7.34 (5H, m, ArH x 5). ¹³C NMR (δ): 23.7 (CH₂, C4); 31.0 (CH₂, C3); 36.2 (CH₂); 43.9 (CH₂Ph); 44.7 (CH₂, C5); 70.3 (C2); 82.6 (HC(O)N); 118.5 (=CH₂); 127.3, 128.1, 128.5 (ArCH x 5), 134.2 (=CH); 136.8 (ipsoC); 163.3 (NC(O)=O). MS (Cl⁺): m/z 273 (M+1, 100%); 255 (-H₂O, 25%); 231 (20%). HRMS (Cl⁺): Found: 273.1600; required: 273.1600 for C₁₆H₂₁N₂O₂.

161b (C₁₆H₂₀N₂O₂) (Rₜ 0.33, 1:1 ethyl acetate:hexane) was isolated as a yellow oil (0.550g, 63% yield). ¹H NMR (δ): 1.57 (1H, ddd, J = 6.3, 6.6, 12.6, H3); 1.93 (2H, m, H4, H4’); 2.04-2.12 (1H, m, H3’); 2.20 (2H, J = 7.5, CH₂); 3.03-3.15 (2H, m, NH, H5a); 3.66 (1H, ddd, J₅e₅a = 9.0, J₅e₄a = 6.0, J₅e₄e = 2.4, H5e); 4.11 (1H, d, J = 15.3, PhCH); 4.70 (1H, s, CH); 4.75 (1H, d, J = 15.3, PhCH); 4.98 (2H, ddd, J = 15.6, J = 11.4, J = 4.2, =CH₂); 5.58-5.72 (1H, m, =CH); 7.25-7.31 (5H, m, ArH x 5). ¹³C NMR (δ): 26.1 (CH₂, C4); 29.1 (CH₂, C3); 42.9 (CH₂); 43.4 (CH₂Ph); 45.9 (CH₂, C5); 69.8 (C2); 82.9 (HC(O)N); 118.9 (=CH₂); 127.2, 127.9, 128.3 (ArCH x 5); 132.6 (=CH); 137.1 (ipsoC); 161.6 (NC(O)=O). MS (Cl⁺):
m/z 273 (M+1, 100%); 255 (-H2O, 25%); 231 (20%). HRMS (CI⁺): Found: 273.1603; required: 273.1603 for C₁₆H₂₁N₂O₂.

**Synthesis of 6-benzyl-1,2-dihydro-5H,10H-pyrano[2,3-c]pyrrolo[1,2-c]imidazol-5-one (163).**

Using similar conditions to those used for the synthesis of 156, 161b (0.090g, 0.330mmol) in THF (5mL) was added via syringe to a stirred slurry of palladium (II) chloride bisacetonitrile (0.094g, 0.363mmol) in THF (5mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 45 minutes, after which time triethylamine (0.046mL, 0.330mmol) was added via syringe. Stirring at room temperature continued, and after 1 hour triethylamine (0.046mL) was again added, and after another 1 hour further triethylamine (0.046mL) was added. Stirring continued at room temperature for 16 hours. The reaction mixture was filtered of precipitated palladium (0) through celite and rinsed with ethyl acetate (2x30mL). The combined filtrate and ethyl acetate was washed with water (2x30mL), then dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:1 ethyl acetate:hexane; Rf 0.25) afforded 163 (C₁₆H₁₈N₂O₂) as a yellow oil (0.078g, 88% yield). ¹H NMR (δ): 1.50-1.58 (1H, m, H₃); 1.65-1.95 (3H, m, H₃', H₄, H₄'); 2.48 (2H, s, CH₂); 3.05 (1H, ddd, J₅a,₅e = 9.0, J₅a,₄a = 8.7, J₅a,₄e = 5.4, H₅a); 3.81 (1H, ddd, J₅e,₅a = 9.0, J₅e,₄a = 6.3, J₅e,₄e = 2.4, H₅e); 4.38 (1H, d, J = 14.7, PhCH); 4.62 (1H, s, CH); 4.82 (1H, d, J = 14.7, PhCH); 5.50 (1H, d, J = 15.0, =CH); 5.87-5.96 (1H, m, =CH); 7.21-7.26 (5H, m, ArH x 5). ¹³C NMR (δ): 24.9 (CH₂, C4); 35.2 (CH₂, C3); 44.3 (CH₂Ph); 46.2 (CH₂, C5); 73.1 (=CH); 75.9 (C2); 107.4 (HC(O)N); 127.3 (=CH); 128.3, 128.4 (ArCH x 5); 137.2 (ipsOC); 163.2 (NC(O)=O). MS(CI⁺): m/z 271 (M+1, 30%); 255 (100%).
Synthesis of 2-benzyl-3-(2-oxopropylidene)-2,3,3a,4,5,6-hexahydro-1H-pyrrolo[1,2-c]imidazole-1-one (165).

Palladium (II) chloride bisacetonitrile (0.158g, 0.610mmol) was transferred to a flask, stoppered and blown with nitrogen for 10 minutes. Anhydrous THF (5mL) was added and the mixture was stirred under nitrogen for 10 minutes. 161b (0.151g, 0.554mmol) in THF (4mL) was added via syringe and the mixture was stirred at room temperature for 45 minutes, after which time triethylamine (0.116mL, 0.831mmol) was added via syringe. The mixture was heated at reflux, and after 1 hour triethylamine (0.116mL) was again added, and after another 1 hour further triethylamine (0.116mL) was added. Heating at reflux continued for 2 hours. The reaction mixture was filtered of precipitated palladium (0) through celite and rinsed with ethyl acetate (2x30mL). The combined filtrate and ethyl acetate was washed with water (2x30mL), then dried (sodium sulphate) and concentrated in vacuo to yield a yellow oil. Chromatography (1:1 ethyl acetate:hexane; Rf 0.51) gave 165 (C_{16}H_{18}N_{2}O_{2}) as the major product, as a yellow oil (0.045g, 30% yield). $^1$H NMR (δ): 1.17-1.30 (1H, m, H3); 1.93-2.08 (2H, m, H3', H4); 2.00 (3H, s, CH$_3$); 2.63 (1H, ddd, J = 6.3, 6.3,1.5, H4'); 3.19 (1H, ddd, J = 1.2, 6.9, H5); 3.62 (1H, ddd, J = 1.1.4, 10.8, 3.3, H5'); 4.60 (2H, s, PhCH$_2$); 4.82 (1H, dd, J = 1.5, 6.6, CH); 5.37 (1H, s, =CH); 7.12-7.28 (5H, m, ArH x 5); $^{13}$C NMR (δ): 26.9 (CH$_2$, C4); 30.3 (CH$_2$, C3); 31.5 (CH$_3$); 45.4 (CH$_2$, C5); 45.8 (CH$_2$Ph); 64.3 (CH, C2); 97.6 (HC=C); 126.8, 127.1, 127.9, 129.0, (ArCH x 5); 135.3 (ipsoC); 156.8 (C=CH); 160.5 (NC(N)=O); 195.5 (C=O). MS (CI$^+$): m/z 271 (M+1, 100%); 287 (30%). HRMS (CI$^+$): Found: 271.1447; Required: 271.1447 for (C$_{16}$H$_{19}$N$_2$O$_2$).
9.7 Experimental for Chapter Six: Synthesis of Spiro[isobenzofuran] Derivatives

**Synthesis of o-iodobenzyl bromide (196)**

A stirred solution of o-iodobenzyl alcohol (2.00g, 8.56mmol) in anhydrous DCM (15mL) was cooled to -30°C under nitrogen, and boron tribromide (1M in DCM, 9.00mmol) was added dropwise via syringe. The mixture was allowed to warm to room temperature, and stirring continued for 3 hours. The mixture was carefully poured onto ice-water (100mL) and extracted with DCM (50mL), then dried (sodium sulphate) and concentrated *in vacuo* to yield a white solid. Chromatography (1:4 ethyl acetate:hexane; Rf 0.91) afforded 196 (C₇H₆BrI) as a white solid (2.34g, 92% yield), recrystallised from diethyl ether. Mp: 55-56°C (lit. mp: 55-57°C355). ¹H NMR (δ): 4.60 (2H, s, CH₂PhI); 6.98 (1H, ddd, J = 1.5, 1.8, 7.5, ArH); 7.33 (1H, ddd, J = 1.5, 1.8, 7.5, ArH); 7.47 (1H, dd, J = 1.5, 8.1, ArH); 7.85 (1H, dd, J = 0.9, 8.1, ArH); ¹³C NMR (δ): 38.8 (CH₂Br), 99.8 (ArC-I); 128.8 (ArCH, C₅, para to I); 130.0 (ArCH, C₆); 130.4 (ArCH, C₄); 139.1 (ArCH, C₃); 140.0 (ipso C, ArCH₂Br). MS (Cl⁺): m/z 296/298 (M⁺, 79Br, 81Br, 17%); 217 (100%, loss of Br).

**Synthesis of 1,1′-(1,2-ethandiyl)bis-[2-iodo]benzene (212).**

To a stirred solution of benzyl-3-pyrrolidinone (0.17M, 0.157g, 0.898 mmol) in anhydrous THF (5.30mL) at -78°C under nitrogen was added butyl lithium (2.5M in hexane, 1.347mmol). After 30 minutes, o-iodobenzyl bromide (0.400g, 1.347mmol) in anhydrous THF (1.0mL) was added and the solution was stirred at -78°C, with the temperature rising to -30°C over 3 hours. The mixture was partitioned between DCM (50ml) and water (50ml), and the organic layer was washed with sodium chloride (sat.) (50mL), followed by
water (50mL), and was then dried (Na₂SO₄) and concentrated in vacuo, providing the crude product as a brown oil which crystallised upon standing. Chromatography (1:4 ethyl acetate:hexane; Rᵣ 0.92) afforded 212 (C₁₄H₁₂I₂) as an off-white crystalline solid (0.252g, 39% yield). Mp: 102-103°C (lit. m.p. 102°C). ¹H NMR (δ): 3.00 (2H, d, J = 6.3, CH₂Ph); 3.03 (2H, d, J = 6.3, CH₂Ph); 6.87-6.93 (1H, m ArH); 7.04-7.10 (1H, m, ArH); 7.18-7.25 (4H, m, ArH x 4); 7.55 (1H, d, J = 8.1, ArH); 7.83 (1H, d, J = 8.1). ¹³C NMR (δ): 36.5 (CH₂, CH₂Ph); 41.0 (CH₂Ph); 100.4 (ArC-I); 127.3, 127.7, 127.9, 128.3, 129.6, 130.5, 132.7, 139.4 (ArCH x 8); 140.3 (ipso C), 140.4 (ipso C). MS (Cl⁺): m/z 434 (M⁺, 21%); 386, 340; 217 (10%); 185 (50%). HRMS (Cl⁺): Found: 433.9018; Required: 433.9029 for (C₁₄H₁₂I₂).

Additionally, to a stirred solution of benzyl-3-piperidone (0.17M, 0.170g, 0.898 mmol) in anhydrous THF (5.30mL) at -78°C under nitrogen was added butyl lithium (2.5M in hexane, 1.347mmol). After 30 minutes, o-iodobenzyl bromide (0.400g, 1.347mmol) in anhydrous THF (10.0mL) was added and the solution was stirred at -78°C, with the temperature rising to -30°C over 3 hours. The usual work-up provided, after chromatography (1:4 ethyl acetate:hexane; Rᵣ 0.87), 212 (C₁₄H₁₂I₂) as a white crystalline solid (0.211g, 36% yield). All spectral data agreed with that previously reported for 212.

**Synthesis of N-benzyl-3-(2-iodobenzyl)-pyrrolidin-2-one (213) and cis-1-benzyl-8b-(2-iodobenzyloxy)-1,2,3,3a,4,8b-hexahydroindeno[1,2-b]pyrrole (215).**

To a stirred solution of benzyl-2-pyrrolidinone (0.17M, 0.314g, 1.796 mmol) in anhydrous THF (10.60mL) at -78°C under nitrogen with stirring was added butyl lithium (2.5M in hexane, 2.694mmol). After 30 minutes, o-iodobenzyl bromide (0.700g, 2.358mmol) in
anhydrous THF (1.0mL) was added and the solution was stirred at -78°C, with the temperature rising to -30°C over 3 hours. The mixture was partitioned between DCM (50mL) and water (50mL), and the organic layer was washed with sodium chloride (sat.) (50mL), followed by water (50mL), and was then dried (sodium sulphate) and concentrated in vacuo, providing the crude product as a yellow oil. Chromatography (1:4 ethyl acetate:hexane) isolated \textbf{215} ($\text{C}_{25}\text{H}_{24}\text{NOI}$, R$_f$ 0.70) as a pale yellow oil (0.127g, 15% yield) and \textbf{213} ($\text{C}_{18}\text{H}_{18}\text{NOI}$, R$_f$ 0.24) also as a pale yellow oil (0.590g, 84% yield).

\textbf{213}: $^1$H NMR ($\delta$): 1.71-1.81 (1H, m, H4); 1.96-2.03 (1H, m, H4'); 2.86 (1H, dd, $J$ = 3.0, 7.2, CH); 2.86 (1H, d, $J$ = 9.9, CHPh); 2.86 (1H, dd, $J$ = 8.7, 5.4, H5, H5'); 3.42 (1H, d, $J$ = 9.6, CHPh); 4.47 (2H, s, N-CH$_2$Ph); 6.87-6.92 (1H, m, ArH); 7.21-7.34 (7H, m, ArH x 7); 7.82 (1H, d, $J$ = 7.8, ArH). $^{13}$C NMR ($\delta$): 24.3 (CH$_2$, C4); 41.2 (CH$_2$, C5), 42.7 (CH, C3); 44.8 (CH$_2$Ph), 46.8 (CH$_2$Ph), 101.4 (ArC-I); 127.4, 127.9, 128.0, 128.2, 128.5, 130.5 (ArCH x 8); 136.4 ($ipso$ C); 139.5 (ArCH, C3, ortho to C-I); 142.1 ($ipso$ C), 175.2 (C=O).

MS (Cl$^+$): m/z 392 (M+1, 100%); 264/266 (10%); 174 (40%). HRMS (El$^+$): Found: 391.0395; Required: 391.0433 for ($\text{C}_{18}\text{H}_{18}\text{NOI}$).

\textbf{215}: $^1$H NMR ($\delta$):2.17-2.37 (2H, m, CH$_2$ $\beta$ to N); 2.50 (1H, dd, $J$ = 3.9, 8.1, H $\alpha$ to N); 2.61-2.66 (2H, m, CH$_2$ $\alpha$ to phenyl); 2.72-2.78 (1H, m, CH); 2.95 (1H, dd, $J$ = 8.1, H' $\alpha$ to N); 3.57 (2H, dd, $J$ = 13.5, 14.7, CHPh, CHPh); 3.70 (2H, dd, $J$ = 13.8, CHPh, CHPh); 6.93 (2H, t, $J$ = 7.5, ArH x 2); 7.26-7.37 (7H, m, ArH x 7); 7.49-7.58 (2H, m, ArH x 2); 7.77 (2H, dd, $J$ = 2.1, 8.1, ArH x 2). $^{13}$C NMR ($\delta$): 28.9 (CH$_2$, $\beta$ to N); 32.7 (CH$_2$, $\alpha$ to N), 45.6 (CH, $\gamma$ to N); 51.3 (CH$_2$Ph); 58.3 (CH$_2$, $\alpha$ to phenyl); 62.8 (O-CH$_2$Ph), 100.3 (ArC-I); 123.7 (ArCH, para to I); 126.4, 126.8, 127.2, 128.0, 128.1, 128.5, 128.9, 130.3 (ArCH x 9);
131.3 (ipso C); 134.4 (ArCH); 136.7 (ipso C); 139.1, 139.2 (ArCH x 2); 141.5 (ipso C),
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18. Bylund, D.B.; Blaxall, H.S.; Iversen, L.J.; Caron, M.G.; Lefkowitz, R.J.; Lomasney,

19. Frielle, T.; Collins, S.; Daniel, K.W.; Caron, M.G.; Lefkowitz, R.J.; Kobilka, B.K.

20. Dixon, R.A.F.; Kobilka, B.K.; Strader, D.J.; Benovic, J.L.; Dohlman, H.G.; Frielle,
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21. Emorine, L.J.; Marullo, S.; Briend-Sutron, M.-M.; Patey, G.; Tate, K.; Delavier-


        Le Trong, I.; Teller, D.C.; Okada, T.; Stenkamp, R.E.; Yamamoto, M.; Miyano, M.


98. Han, M.; Smith, S.O.; Sakmar, T.P. *Biochemistry* (1998), **37** (22), 8253.


References


134. Rules for the generation of training sets were available at www.msi.com/support/catalyst/hypogen.html. This web site however no longer exists.


References


References


Appendix 1: NOESY 2-Dimensional Spectrum of 66
Appendix 2a: gHMBC spectrum of 161a
Appendix 2b. gHMBC spectrum of 161b
Appendix 3a: gCOSY spectrum of 165
Appendix 3b: HMBC spectrum of 165
Appendix 3c: $^{15}$N HMBC spectrum of 165
Appendix 4: Data for Pharmacological Testing of Ligands

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 (SJB 4.92)</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$, concentration of compound resulting in 50% of maximum inhibition

$^b$ mean log concentration ratio ± SE (-logIC$_{50}$ Epinephrine minus -logIC$_{50}$ compound)

$^c$ relative affinity of compound as % Epinephrine
Appendix

% Maximum Specific Binding (I\textsuperscript{125}I HEAT)

log Conc. (M)

% Maximum Specific Binding (I\textsuperscript{125}I HEAT)

log Conc. (M)

% Maximum Specific Binding (I\textsuperscript{125}I HEAT)

log Conc. (M)

% Maximum Specific Binding (I\textsuperscript{125}I HEAT)

log Conc. (M)
<table>
<thead>
<tr>
<th>α1B C128F</th>
<th>-log IC$_{50}$</th>
<th>IC$_{50}$ (µM)$^a$</th>
<th>Δ$^b$</th>
<th>Relative affinity$^c$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>7.48±0.07</td>
<td>0.03</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>100 (SJB 4.93)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>99 (SJB 4.82)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>54 (SJB 4.05)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>101 (SJB 4.26)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>102 (SJB 4.25)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>50 (SJB 4.92)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ concentration of compound resulting in 50% of maximum inhibition
$^b$ mean log concentration ratio ± SE (−logIC$_{50}$ Epinephrine minus −logIC$_{50}$ compound)
$^c$ relative affinity of compound as % Epinephrine
<table>
<thead>
<tr>
<th>α1A</th>
<th>-log IC₅₀</th>
<th>IC₅₀ (µM)ᵃ</th>
<th>Δᵇ</th>
<th>Relative affinityᶜ</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>5.10±0.04</td>
<td>8.00</td>
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<td>3</td>
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<tr>
<td>100 (SJB 4.93)</td>
<td>&gt;300</td>
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<td>3</td>
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<tr>
<td>99 (SJB 4.82)</td>
<td>3.36±0.15</td>
<td>439</td>
<td>2.86±1.08</td>
<td>0.001</td>
<td>3</td>
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<tr>
<td>54 (SJB 4.05)</td>
<td>&gt;300</td>
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<td></td>
<td>3</td>
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<tr>
<td>101 (SJB 4.26)</td>
<td>&gt;300</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>102 (SJB 4.25)</td>
<td>&gt;300</td>
<td></td>
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<td>3</td>
</tr>
<tr>
<td>50 (SJB 4.92)</td>
<td>3.88±0.03</td>
<td>131</td>
<td>2.51±1.25</td>
<td>0.003</td>
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</tr>
</tbody>
</table>

ᵃ IC₅₀, concentration of compound resulting in 50% of maximum inhibition  
b mean log concentration ratio ± SE (-logIC₅₀ Epinephrine minus -logIC₅₀ compound)  
c relative affinity of compound as % Epinephrine
<table>
<thead>
<tr>
<th>α1D</th>
<th>-log IC$_{50}$</th>
<th>IC$_{50}$ (μM)$^a$</th>
<th>Δ$^b$</th>
<th>Relative affinity$^c$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine</td>
<td>6.10±0.57</td>
<td>0.79</td>
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</tr>
<tr>
<td>100 (SJB 4.93)</td>
<td>4.66±0.20</td>
<td>22.1</td>
<td>1.45±0.77</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>99 (SJB 4.82)</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>54 (SJB 4.05)</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>101 (SJB 4.26)</td>
<td>4.55±0.34</td>
<td>28.4</td>
<td>1.56±0.92</td>
<td>0.03</td>
<td>2</td>
</tr>
<tr>
<td>(SJB 4.25)</td>
<td>&gt;100</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>50 (SJB 4.92)</td>
<td>4.54±0.27</td>
<td>29.1</td>
<td>1.57±0.84</td>
<td>0.03</td>
<td>2</td>
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</tbody>
</table>

$^a$ IC$_{50}$, concentration of compound resulting in 50% of maximum inhibition

$^b$ mean log concentration ratio ± SE (-logIC$_{50}$ Epinephrine minus -logIC$_{50}$ compound)

$^c$ relative affinity of compound as % Epinephrine