A Medicinal Chemistry Investigation of 3,4-Methylenedioxyamphetamine (MDMA)

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Candidate Declaration

The work described in this thesis was carried out by the author in the School of Biomedical, supervision of Professor Robert Stick, Professor Allan McKinley and Associate Professor Matthew Piggott. Unless duly referenced, the work described is original.

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Katie D. Lewis

April 2011
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Summary

3,4-Methylenedioxymethamphetamine (MDMA) 1, the active chemical constituent of the illicit drug ecstasy, is a psychotropic agent whose effects are primarily modulated through the serotonergic system. In recent years numerous researchers have identified MDMA as possessing therapeutic activity towards a variety of disease states.

![MDMA 1](image)

Parkinson’s disease (PD) is a common and disabling neurodegenerative disorder. The primary symptomatic treatment of PD utilises the dopamine precursor levodopa. Long-term levodopa therapy typically elicits deleterious side-effects, the most significant being levodopa-induced dyskinesia (LID), the severity of which may negate the therapeutic benefit of levodopa. MDMA has been demonstrated in primate models to possess both anti-parkinsonian and anti-LID activity.

Burkitt’s lymphoma (BL) is a malignant disease of the lymphatic system, affecting B-cell lymphocytes in particular. Recent characterisation of a functioning immunoreactive serotonin reuptake transporter (SERT) in B-cell lines has been impetus for the investigation of SERT as a target for drug therapy in BL. MDMA, a known SERT substrate, exhibits an anti-proliferative and pro-apoptotic response in a BL cell line (L3055).

This thesis, prompted by recent reports of the therapeutic activity of MDMA in various disease states, details a medicinal chemistry investigation of MDMA, Chapters One, Two and Three document the analogues synthesised. These analogues were intended for evaluation as anti-neoplastics for Burkitt’s lymphoma and for evaluation as therapeutics for the treatment of PD. This work was conducted in collaboration with Prof. John Gordon and coworkers (MRC
Centre for Immune Regulation at The University of Birmingham), and Dr Jonathan Brotchie and coworkers (Toronto Western Research Institute).

Additionally a series of putative monoamine oxidase (MAO; EC 1.4.3.4) inhibitors was conceived, synthesised and assayed (Chapter Four). These compounds were structurally analogous to selegiline 10, a clinically useful MAO-B inhibitor.

![Selegiline 10](image_url)
Acknowledgements

I would like to thank my supervisor Professor Robert Stick, whose support and guidance have been invaluable. I would also like to thank my supervisor Professor Allan McKinley for his commitment to assisting my graduate studies.

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Thank you to Dr Lindsay Allet for his assistance during my candidature. Your professionalism is inspiring.

Finally I wish to thank my parents and my partner Jakub for their unwavering love, support and care through the triumphs and despair of this PhD candidature. Without whom my successes would not be possible. There is nothing I can write here to truly express my gratitude, excepting to say I love you.
Glossary

Abbreviations

5-HT  5-hydroxytryptamine (serotonin)
abs  absolute
anal.  analytical
aq  aqueous
ap.  apparent
br  broad
calcd  calculated
CI  confidence interval
d  doublet
DA  dopamine
DAT  dopamine transporter
dt  doublet of triplets
dd  doublet of doublets
ddd  doublet of doublet of doublets
ddq  doublet of doublet of quartets
DEPT  distortionless enhancement by polarisation transfer
DME  1,2-dimethoxyethane
DMF  dimethylformamide
EI  electron impact
ESI  electrospray ionisation
equiv  equivalent
eV  electron volt
FAB  fast atom bombardment
g  gram(s)
GC-MS  gas chromatography mass spectrometry
h  hour(s)
Hz  Hertz
HR-MS  high resolution mass spectrometry
IC50  half maximal inhibitory concentration
IR  infrared spectroscopy
Km  Michaelis-Menten constant
K\textsubscript{i}  inhibition constant
LID  levodopa-induced dyskinesia
lit.  literature
m  multiple
mg  milligram(s)
min  minute(s)
mL  milliliter(s)
mmol  millimole(s)
mol   mole(s)
mp    melting point
MPTP  1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NE    norepinephrine
NET   norepinephrine transporter
NIE   no inhibitory effect
NMR   nuclear magnetic resonance
PD    Parkinson’s disease
PEA   phenethylamine
q     quartet
quant. quantitative
rsf   rapid suction filtration
rt    room temperature
SAR   structure activity relationship
sat.  saturated
SEM   standard error of the mean
sept  septet
SERT  serotonin reuptake transporter
sext  sextet
st    strong
s     singlet
sh    sharp
sl    slightly
 t    triplet
tlc   thin layer chromatography
THF   tetrahydrofuran
\( V_{\text{max}} \) maximum rate
\( \delta \) chemical shift (parts per million)

**Functional Groups**

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<tr>
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</tr>
<tr>
<td>Ts</td>
<td>p-toluenesulfonyl (tosyl)</td>
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</tbody>
</table>
Introduction
Introduction

3,4-Methylenedioxymethamphetamine (MDMA) 1 is a psychotropic drug and the active chemical constituent of the illicit drug ecstasy. It is a ring-substituted derivative of methamphetamine 2, a similarly notorious substance of abuse. Both MDMA and methamphetamine belong to the entirely synthetic class of compounds, the amphetamines, all members of which possess the amphetamine scaffold 3. Amphetamines have been extensively studied and reported in the literature. They are most remarkable for their diverse and potent central nervous system (CNS) activity.

The social history of MDMA has been documented in many texts. In 1914 MDMA entered the chemical literature as an intermediate published in a Merck patent. There was little interest in MDMA until many decades later. By the late 1970s MDMA was being used in clinical settings in California, developing a reputation amongst psychotherapists as a useful adjunct to psychotherapy. Concurrently there were increasing numbers of recreational users consuming MDMA under the newly minted name ecstasy. The burgeoning appetite for ecstasy in the 1980s coincided with the growing popularity of the dance music counterculture, in particular with dance music events called raves, where ecstasy was the drug of choice.

In 1985, after a series of alarmist reports on ecstasy surfaced, the United States Drug Enforcement Agency listed MDMA on Schedule I of the Controlled Substances Act, for dangerous and addictive drugs without medical use. The scheduling of MDMA as a

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* More accurately the active chemical found in ecstasy is the hydrochloride of MDMA, MDMA.HCl.
† Methamphetamine is the active chemical constituent of the illicit drug commonly known as ice or speed.
‡ Australian regulatory bodies rapidly followed suit and restrictively scheduled MDMA in 1986.
controlled substance has not curtailed its popularity. The United Nations report on ecstasy production and consumption, *Ecstasy and Amphetamines Global Survey 2003*, declared ‘the huge increase in ecstasy consumption has continued globally over the past years’. The survey reported estimated worldwide ecstasy production at 125 tonnes a year, with eight million users.7

**Subjective Effects**

The phenomenal popularity of MDMA as a drug of abuse derives from the combination of effects that it elicits. As the name ecstasy implies, MDMA is both a euphoriant and an empathogen. In humans the effective dose range is between 100-160 mg (~2 mg/kg).8 A person who has consumed ecstasy is reported to experience feelings of intense pleasure, a lack of inhibition and heightened empathy and awareness.9 Indeed it is these subjective effects that some have argued lend MDMA so well to the role of an adjunct to psychotherapy.10 MDMA’s unique psychotropic properties prompted Nichols and coworkers to propose that it belonged to a novel drug class, designated the enactogens.11

**Pharmacology**

The pharmacology of MDMA has been studied extensively *in vitro* and *in vivo* and consequently has been the subject of periodic review. Recent noteworthy contributions include those by Green *et al.*12 (2003) and by Baumann and Rothman13 (2008). The principal pharmacological actions of MDMA are monoamine release, receptor binding and enzyme-linked effects.

MDMA is a substrate of the monoamine transporter proteins, the serotonin reuptake transporter (SERT), the dopamine transporter (DAT) and the noradrenaline transporter (NET). These proteins serve to transport the endogenous amines 5-hydroxytryptamine (5-HT, serotonin) 4, dopamine (DA) 5, and noradrenaline (NE) 6. MDMA causes release of endogenous amines

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7 A subjective MDMA experience has been documented, at length, by Shulgin.9
Introduction

from neurons in the central and peripheral nervous system.\textsuperscript{14-16} It is this pharmacology that
belies most of the observed activities.\textsuperscript{13}

\includegraphics{molecules}

\textbf{MDMA} is principally identified as a serotonergic agent acting on the serotonin pathways.
\textbf{MDMA} blocks reuptake of serotonin and reverses the flow at the SERT, resulting in enhanced
release of serotonin from nerve terminals.\textsuperscript{14,17} In addition to this \textbf{MDMA} also causes
transport-mediated release of dopamine, and to a lesser extent noradrenaline, in the CNS.
\textbf{MDMA} exhibits a plethora of less potent, direct receptor effects. It has highest affinity for
\(\alpha_2\)-adrenoreceptors, as well as affinity for various 5-HT\textsubscript{2}-serotonin, \(M_\text{1}\)-muscarinic and
\(H_1\)-histamine receptors.

Within the CNS \textbf{MDMA} has two principal enzyme effects. \textbf{MDMA} inhibits the monoamine
oxidases (MAO-A and MAO-B, EC 1.4.3.4), which catabolise monoamines after they are
released into the synapse. MAO inhibition therefore allows serotonin, dopamine and
noradrenaline released by \textbf{MDMA} to exert an effect over a longer period of time. \textbf{MDMA} also
acts to inactivate tryptophan hydroxylase (TPH, EC 1.14.16.4), which catalyses the rate-limiting
step in the biosynthesis of serotonin.\textsuperscript{12}
Introduction

**MDMA: A Burkitt’s Lymphoma Cytotoxin**

Burkitt’s lymphoma (BL) is a malignant disease of the lymphatic system; B-cell lymphocytes in particular are affected. BL exhibits an extraordinarily high mitotic index; a tumor can double in mass in one day.\(^{18}\)

Recent studies, characterising a functioning immunoreactive SERT in B-cell lines, are indicative of a regulatory role of serotonin in lymphocyte populations.\(^{19-21}\) Gordon and coworkers described one role of SERT in a model Burkitt’s lymphoma cell line (L3055), that of a conduit for serotonin-mediated apoptosis. In the presence of a selective serotonin reuptake inhibitor, fluoxetine, paroxetine and citalopram were investigated, apoptosis was blocked. This signaled an active transport mechanism.\(^{19}\)

These initial findings prompted the researchers to investigate further SERT as a target in the drug treatment of B-cell malignancies. Consequently, seventeen B-cell lines of diverse tumor origin were analysed for SERT expression; SERT was present at significantly higher levels in the malignant B-cell lines than in normal tonsilar B-cells. The seventeen cell lines were treated with known SERT substrates, including MDMA, to determine the type and magnitude of their anti-tumor effect. Twelve of the cell lines, including the BL cell line (L3055), revealed an anti-proliferative response to one or more of the SERT substrates. Uniquely a pro-apoptotic response to SERT substrates was observed only in the BL cell line (L3055). Importantly, viability of normal B-cells was not adversely affected in any of the experimental conditions.\(^{20}\)

In the BL cell line (L3055), MDMA was equipotent to the natural substrate serotonin in inducing apoptosis and arresting cell proliferation. The measured anti-proliferative activity of MDMA was 100-200 \(\mu\)M (expressed as an IC\(_{50}\) value). Gordon noted the findings augured for the further drug development of MDMA analogues for evaluation as novel anti-tumor chemotherapeutics.
Introduction

The Therapeutic Role of MDMA in Parkinson’s Disease

Parkinson’s disease (PD) is a common neurodegenerative disorder, characterised by disabling movement, including resting tremor, rigidity, postural instability and bradykinesia (slow movement). The major pathology of PD is the abnormal and progressive degeneration of neurons in the substantia nigra pars compacta, a site of dopamine production in the central nervous system. The cause of these pathogenic changes in the brain has not been identified; however, depletion of striatal dopamine is responsible for most of the observed motor deficits in PD sufferers.

The identification of the critical role of dopamine in normal locomotion enabled the successful development of dopamine replacement therapy (DRT) for the treatment of PD. DRT is administered as the dopamine precursor, L-3,4-dihydroxyphenylalanine (levodopa, L-DOPA). Levodopa is the most effective, and hence, the primary symptomatic treatment for PD.

Levodopa therapy acts to reduce the severity of parkinsonian symptoms and consequently has dramatically improved the quality of life of many PD sufferers. However, several major limitations associated with long-term levodopa therapy have been identified, including declining or fluctuating response to the therapy and the emergence of debilitating involuntary movement, called levodopa-induced dyskinesias (LID). LID has been reported to affect over 75% of patients within the first five years of dopamine replacement therapy and can be so...

** Dopamine itself is not an effective drug as it is unable to cross the blood-brain barrier. Levodopa is actively transported into the brain where it is decarboxylated to dopamine by the enzyme L-DOPA decarboxylase.
severe as to negate the benefits of therapy compliance.\textsuperscript{24} LID therefore represents the most significant problem in the symptomatic management of PD.\textsuperscript{22,††}

**MDMA** has been demonstrated to be efficacious in both the treatment of parkinsonism and in the management of LID, in an array of animal models of PD.\textsuperscript{26-32} In 2001, Kovar and coworkers first demonstrated the anti-parkinsonian activity of **MDMA**.\textsuperscript{26} In a subsequent report, they further explored the anti-parkinsonian activity of **MDMA** in haloperidol-induced cataleptic, 6-hydroxydopamine-lesioned rats.\textsuperscript{27,††} The study examined the activity of **MDMA** and its enantiomers (\textit{R})-**MDMA** and (\textit{S})-**MDMA**. The measured anti-parkinsonian activity was **MDMA** \( \gg \gg \) (\textit{S})-**MDMA \( \approx \) (\textit{R})-**MDMA**. Evidently the observed anti-parkinsonian effect was derived from pronounced synergism. The potent, dose-dependent effect was observed in the range 1-5 mg/kg.

\begin{center}
\begin{tabular}{c|c}
\textit{(R)}-**MDMA** & \textit{(S)}-**MDMA**
\end{tabular}
\end{center}

In another study, Lebsanft and coworkers\textsuperscript{29} assessed the anti-akinetic activity (another measure of anti-parkinsonian activity) of (\textit{R})-**MDMA** and (\textit{S})-**MDMA** in two animal models of PD, the catalepsy test and rotational behaviour in 6-hydroxydopamine-lesioned rats. Comparison of the enantiomers with the racemate provided mechanistic evidence that the anti-parkinsonian effect of **MDMA** was not mediated by either indirect dopamine agonism or 5-HT\textsubscript{2A}-receptor agonism. The researchers concluded that the enantiomers must mediate this activity via at least two different target sites.

The plausible hypothesis that the anti-parkinsonian effect of **MDMA** derives from either direct or indirect actions at nigrostriatal dopaminergic neurotransmission cannot be dismissed.

\textsuperscript{††} For a review of LID in PD see Brotchie \textit{et al.}\textsuperscript{25}

\textsuperscript{††} Haloperidol-induced catalepsy is a reliable model of parkinsonism. It is reversed by all clinically effective anti-parkinsonian drugs.
Introduction

However, Lebsanft and coworkers\(^2\) reasoned that: 1) the mechanism must necessarily exclude direct binding at D\(_1\) or D\(_2\) receptors in the striatum, as MDMA has a low binding affinity for these receptors; and 2) whilst MDMA releases dopamine, this too was excluded as a more potent dopamine releasing agent had no anti-parkinsonian activity.

Further mechanistic studies by Lebsanft \textit{et al.}\(^3\) came to the conclusion that opioid receptor-agonism may play a role in the MDMA-induced anti-parkinsonism.

MDMA has also been shown to possess potent anti-LID activity. The first report of this was in an isolated human case.\(^3\) In 2003 Iravani and coworkers corroborated this anecdotal evidence in a study of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets.\(^3\) In MPTP-treated marmosets MDMA dramatically reduced LID when given in conjunction with levodopa. Locomotor activity in the treated animals returned to the level observed in normal marmosets.

The mechanism of LID is complex, and several receptors and transporters have been implicated in the pathogenesis of LID. Iravani and coworkers demonstrated that treatment with the selective serotonin reuptake inhibitor, fluvoxamine, completely blocked the anti-LID effect of MDMA.\(^3\) Partial inhibition of the effect was observed with 5-HT\(_{1A/B}\) antagonists, leading the researchers to conclude that the anti-LID efficacy of MDMA derives from multiple actions within the serotonin system.\(^3\)

Walker and coworkers demonstrated partial, indirect 5-HT\(_{1A}\) mediation of the anti-LID activity of MDMA.\(^3\) Co-administration of MDMA with the selective 5-HT\(_{1A}\) antagonist, WAY100635, in 6-hydroxydopamine-lesioned rats reversed the anti-LID effect. Eskowa \textit{et al.}\(^3\) similarly focused their investigations on the role of the 5-HT\(_{1A}\) in alleviating LID.

Despite numerous studies, the precise mechanisms by which MDMA elicits an anti-parkinsonian and an anti-LID effect remain unresolved. This owes in part to the complex pharmacology of MDMA and to the limited understanding of the PD and LID.

\(^3\) The MPTP-treated primate is currently the best available animal model of PD. MPTP-induced destruction of primate dopamine systems produces symptomatology similar to that clinically observed.\(^3\)
Introduction

The Therapeutic Potential of MDMA

MDMA is unsuited to a clinical therapy application owing to its high abuse liability, subjective psychomimetic effects and neurotoxicity. However MDMA has been identified as a lead for drug development by Gordon and coworkers, as a BL cytotoxin, and by numerous researchers as an anti-parkinsonian and anti-LID agent in the treatment of PD. This represents a novel paradigm in the treatment of these disease states; a multiligand drug whose effect is primarily mediated through direct or indirect modulation of the serotonin system.

Purpose of the Project

Prompted by recent reports of the various therapeutic activities of MDMA and the identification of MDMA as a drug lead, the purpose of the project was to rationally design and synthesise analogues of MDMA for evaluation as therapeutics in a selection of in vivo and in vitro assays. The results of these assays were intended to assist elucidation of some fundamental structure-activity relationships (SARs) with respect to each therapeutic effect.

The therapeutic activities evaluated are listed below. Some of this work was conducted in conjunction with other researchers, who are indicated in parentheses.

- Anti-parkinsonian and anti-LID activity for the treatment of Parkinson’s disease (Dr Jonathon Brotchie and coworkers; Toronto Western Research Institute, Canada).
- Anti-proliferative and pro-apoptotic activity in L3055 BL cell lines (Prof. John Gordon and Dr Agata Wasik; University of Birmingham, UK).
- Monoamine oxidase inhibition activity.

Medicinal Chemistry Considerations and Structure-Activity Relationships of MDMA

The term privileged structure describes a chemical scaffold that produces diverse biological activity. The practice of manipulating privileged structures for the purpose of drug discovery
is both judicious and well proven. It has been calculated that half of all known drugs possess only thirty-two scaffold types.\textsuperscript{41}

Phenethylamine (PEA) 8 is a privileged structure.\textsuperscript{42} It is the foundation of a plethora of pharmacologically active compounds, including many pharmaceuticals (\textbf{Chart 1.1}). Consequently 8 is a strategic inclusion for rationally designed drug candidates. That MDMA possesses this privileged structure augured well for a medicinal chemistry investigation.

\begin{center}
\begin{tikzpicture}
\node (phenethylamine) at (0,0) {\includegraphics[width=0.5\textwidth]{phenethylamine.png}};\end{tikzpicture}
\end{center}

\textbf{Phenethylamine} 8

\begin{center}
\begin{tikzpicture}
\node (phentermine) at (0,0) {\includegraphics[width=0.5\textwidth]{phentermine.png}};\end{tikzpicture}
\end{center}

\textbf{Phentermine} 9

\begin{center}
\begin{tikzpicture}
\node (selegiline) at (0,0) {\includegraphics[width=0.5\textwidth]{selegiline.png}};\end{tikzpicture}
\end{center}

\textbf{Selegiline} 10

\begin{center}
\begin{tikzpicture}
\node (ritalin) at (0,0) {\includegraphics[width=0.5\textwidth]{ritalin.png}};\end{tikzpicture}
\end{center}

\textbf{Ritalin} 11

\textbf{Chart 1.1} Examples of pharmaceuticals that possess the phenethylamine 8 scaffold.

A medicinal chemistry investigation of MDMA is not without precedent. In 1986, Nichols and coworkers were the first researchers to identify MDMA as a potential drug lead, in that instance as a psychotherapeutic adjunct.\textsuperscript{43} The authors prepared two rationally designed analogues, 12 and 13, and studied their pharmacology, in order to explore if the reported psychotherapeutic activity of MDMA might be delineated from the ‘typical hallucinogen profile’, allowing a psychotherapeutic adjunct lacking abuse potential to be realised. Notably the hallucinogen profile was retained for both 12 and 13.
Introduction

Some SARs for the therapeutic locomotor effects of MDMA for PD have been reported. The analogues 13-15 have been shown to possess anti-parkinsonian effects. In a rotational behavioural model of PD, MDMA and 13-15 all induced ipsilateral rotations in 6-hydroxydopamine-lesioned rats. It is apparent that there is a degree of tolerance for modification of the amine or the α-position, for which therapeutic activity can be retained. Compound 14 was the most potent analogue studied, but it is a known hallucinogen and substance of abuse. Therefore, like MDMA, 14 is also a poor candidate for clinical application.

Amphetamine analogues substituted at the 4-position and the 3,4-position of the aromatic ring typically possess significant potency as serotonin-releasing agents. As the ability to act as a SERT substrate was hypothesised to play an important role in the locomotor effects on PD and the cytotoxicity of BL, it was prudent to retain the 3,4-methylenedioxy functionality. Only modifications of the side-chain were considered.

The SARs of MDMA with respect to psychoactivity have been studied extensively and reviewed on numerous occasions. The previously explored structural modifications of MDMA are illustrated in Figure 1.1. Some salient observations from the literature are that increasing the length of the N-substituent decreases psychomimetic activity in humans,
increasing the length of the α-substituent attenuates psychoactivity\textsuperscript{43,48} but not necessarily other central nervous system activity.\textsuperscript{49}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure1}
\caption{Previously examined structural modifications for MDMA psychoactivity SARs.}
\end{figure}

Whilst the intention of the project was not to produce a clinically useful drug but to investigate SARs of the various therapeutic activities of MDMA, it was rationalised that attenuation of the deleterious effects of MDMA would be desirable. Therefore in designing analogues for synthesis it was judicious to utilise existing MDMA psychoactivity SARs to obviate psychoactivity where possible. Accordingly analogues that were less likely to possess psychoactivity were given higher priority; initially a suite of MDMA analogues variously modified at the α-position and the amine was conceived.

\section*{Overview}

Prompted by recent reports of the therapeutic potential of MDMA for disease states modulated through serotonergic pathways, a series of MDMA analogues was designed, synthesised and evaluated for therapeutic activity using a selection of relevant \textit{in vivo} and \textit{in vitro} studies.

This thesis is divided into two parts. The first part describes the syntheses of MDMA and some MDMA analogues; \textit{Chapters One, Two and Three} detail α-substituted, N-modified and optically-active analogues, respectively. The second part reports selected \textit{in vitro} characterisation of the analogues prepared in the first part.
Introduction
Introduction
Chapter One

The Synthesis of Some α-Substituted Analogues of MDMA
Chapter One

Introduction

Previously in Piggott’s group α-substituted MDMA analogues 13, 17-21 were prepared (Figure 1.1).* These analogues were designed to examine the SARs of steric encumbrance, electronic properties and the length of the α-substituent with respect to therapeutic effect.

![Chemical structure of analogues](image)

**Figure 1.1** Piggott’s first generation α-substituted MDMA analogues.

Preliminary evaluation of analogues 13, 17-21 measuring levodopa-induced hyperactivity in monoamine depleted rats was conducted by Dr Jonathan Brotchie and coworkers (Toronto Western Research Institute).† The salient observation from this study was that 21 demonstrated a significant ability to minimise LID-equivalent behaviour without reduction of the normal movement restored by levodopa.⁵¹,⁵²

Prof. John Gordon and coworkers (University of Birmingham) evaluated analogues 13, 17-21 for cytotoxicity in an L3055 BL cell line using the procedure reported by Meredith *et al.*²⁰ (Table 1.1). Analogue 21 demonstrated the only notable improvement for cytotoxic efficacy, being seven-fold more potent than the parent compound. All other analogues (13, 17-20) possessed IC₅₀ values similar to that of MDMA.

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* Synthesis conducted by Keith Wagg (Australian National University) and Matthew McIlidowie (University of Western Australia).
† This animal model is not completely representative of human LID but the pharmalogical features are sufficiently analogous to be useful for identifying anti-LID activity in test compounds.⁴⁰
Table 1.1 IC\textsubscript{50} values for MDMA and α-substituted analogues for toxicity in L3055 BL cell line.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC\textsubscript{50} ± SEM (μM)\textsuperscript{a}</th>
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</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>507 ± 80</td>
</tr>
<tr>
<td>13</td>
<td>707 ± 23</td>
</tr>
<tr>
<td>17</td>
<td>379 ± 25</td>
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<td>801 ± 31</td>
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<td>447 ± 61</td>
</tr>
<tr>
<td>21</td>
<td>76 ± 5</td>
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</tbody>
</table>

\textsuperscript{a} Values reported by Gandy et al.\textsuperscript{55}

From the preliminary biological data 21 was designated the lead compound for design of further target analogues. Accordingly a suite of second generation α-aryl and α-heteroaryl analogues was conceived (Chart 1.1). In order to prepare the analogues a methodology that was succinct, general in scope (necessarily able to tolerate the inclusion of various heterocycles) and amenable to parallel synthesis, and therefore rapid analogue production, was sought.
Chart 1.1 Second generation MDMA analogues possessing an α-aryl or an α-heteroaryl moiety.

**Synthetic Routes to α-Substituted MDMA Analogues**

MDMA and other phenethylamines have stimulated significant interest as synthetic targets and accordingly have been prepared by a number of methods.\(^3\)\(^9\)\(^5\)\(^4\) The most common synthetic strategies toward α-substituted MDMA analogues are illustrated below (Schemes 1.1 and 1.2).\(^3\)\(^3\)\(^5\)\(^5\) The in Scheme 1.1 addition of an organometallic reagent 33 to piperonal 32 affords the alcohol 34, which dehydrates to give the styrene 35. The ketone 36 is furnished by oxidation of 35. Subsequent reductive amination of 36 affords the target amine 16.
Scheme 1.1 a) RCH\_2M 33 (where M = Mg or Li); b) dehydration; c) i) oxidation, ii) H\(^+\); d) reductive amination. A representative example is the synthesis of 17, the α-propyl MDMA analogue prepared by Shulgin:⁹ a) Mg, BuBr, Et\(_2\)O (quant.); b) KHSO\(_4\), Δ (62%); c) i) HCOOH, H\(_2\)O\(_2\), H\(_2\)O, (CH\(_3\))\(_2\)CO, ii) H\(_2\)SO\(_4\) (52%); d) Al, HgCl\(_2\), H\(_2\)O, ii) MeNH\(_2\)Cl, NaOH, t-PrOH, H\(_2\)O (50%; overall yield 16%).

An alternate route to 16 (Scheme 1.2) utilises a Henry reaction, whereby 32 condenses with the primary nitroalkane 37, followed by dehydration to yield an intermediate β-nitrostyrene 38. The β-nitrostyrene 38 is converted into the ketone 36 by means of a Nef reaction, and subsequent reductive amination affords the target amine 16. Alternatively 38 can be reduced to the primary amine 39 then reductively alkylated to give 16.⁵⁷
Scheme 1.2 a) RCH₂NO₂ 37; b) Nef reaction; c) reductive amination; d) reduction; e) reductive alkylation.

A representative example is the synthesis of MDMA by Gimeno and coworkers:⁵⁶ a) EtNO₂, CH₃COOH, c-HxNH₂ (40%); b) Fe, CH₃COOH (56%); c) Me₂NH₂Cl, NaCNBH₃, MeOH (45%; overall yield 10%).

Synthetic Strategy and Considerations

In designing a synthetic pathway to 16 it was judicious to retain the most advantageous elements of the known syntheses. Therefore the preparation of an intermediate ketone 36 followed by reductive amination to give 16 were to remain the key transformations. In addition the common substrate piperonal 32, which is both cheap and commercially available, was to be utilised.

The intermediacy of 36 is advantageous for several reasons. Firstly, it creates a late point of divergence in the synthesis, in that reductive amination with ammonium acetate, a primary or secondary amine could provide multiple analogues 39, 40 and 41, respectively (Figure 1.2).⁵⁸,†

† This idea was further explored and the results are reported in Chapter Two.
Secondly, inclusion of a prochiral intermediate would enable the enantiomers (R)-16 and (S)-16 to be expediently accessed via enantioselective synthesis or resolution.\textsuperscript{59} For synthetic ease target compounds were initially to be prepared as racemic mixtures.\textsuperscript{8} It was envisaged that where a target racemate exhibited favourable therapeutic activity, the enantiomers would subsequently be prepared for biological evaluation (as detailed in Chapter Three).

\textsuperscript{6} At the time the observed anti-LID effect of \textbf{MDMA} was observed with administration of the racemate,\textsuperscript{60} it was therefore hypothesised that synergism might have been necessary for optimal anti-LID activity. Work completed during the course of this PhD candidature has contributed to the enhanced understanding of roles of each enantiomer in the anti-LID mechanism of \textbf{MDMA}. Current knowledge is suggestive that the (R)-enantiomer is primarily responsible for the observed anti-LID activity.
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The routes described above (Schemes 1.1 and 1.2) were limited in their utility for this medicinal chemistry investigation. The former route (Scheme 1.1) possesses an early divergent step for the inclusion of the α-substituent and is thus unsuitable for the rapid production of analogues. In addition, accessing 36 via the alcohol 34 followed by the styrene 35 seemed unnecessarily protracted. The latter route (Scheme 1.2) lacks scope. For example, the reaction of piperonal and nitropropane was low yielding (< 20%) with significant by-product formation. It was also anticipated that the α-arylnitromethanes which would be required for the syntheses would be poor substrates for the Henry reaction owing to excessive resonance stabilisation and steric hindrance of the corresponding nitronate ions. Previous work within Piggott’s group has shown that α-nitrotoluene fails to react with piperonal under a variety of conditions.

The challenge for preparing analogues 16 therefore lay in developing an improved synthesis of the ketone 36. Having identified the primary considerations, succinctness, broad scope and amenability to parallel synthesis, alternative syntheses for preparing 36 were investigated.

Methods to Synthesise Benzyl Ketones

A simple and general method for the preparation of aryl ketones via aldehyde homologation was reported by Angle and Neitzel (Scheme 1.3). The in situ formation of piperonyltosylhydrazone 42 by condensation of 32 with tosylhydrazine, and subsequent addition of benzaldehyde and two equivalents of sodium ethoxide afforded 43 in 52% yield after two days at 55 °C. Previously, replication of this base-promoted modification of the Bamford-Stevens reaction within Piggott’s group gave poor yields (< 30%) and was therefore deemed unworthy of pursuing.
Palucki and Buchwald reported the first direct transition metal-catalysed arylation of ketones.\textsuperscript{64} Hamann and Hartwig independently discovered the same chemistry.\textsuperscript{65} This method promised to deliver ketone 36 in one step via direct coupling of the aryl halide 44 with various methyl ketones 45 in the presence of base and a transition metal catalyst (Scheme 1.4). The ketone 36 was required on a gram-scale, making the use of transition metal-catalysed chemistry potentially expensive. Nolan and coworkers showed that palladium catalysts bearing N-heterocyclic carbene ligands (Pd-NHC) could effect the desired transformation using low catalytic load (1 mol\%), which enhanced the potential applicability of ketone arylation.\textsuperscript{66,67} Regrettably previous efforts by this author found that Nolan’s method lacked scope; yields of heteroaryl ketones were poor. Coupling of 5-chloro-1,3-benzodioxole 44 (X = Cl) with 2-acetylfuran, 4-acetylpyridine and 2-acetylthiophene gave yields of 24\%, 15\% and 0\%, respectively.\textsuperscript{68}
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reagent 47 often results in facile generation of an undesired tertiary alcohol 49 (Scheme 1.5). One method to circumvent formation of 49 is to employ an organocuprate, which possesses softer nucleophilic character and therefore does not react with the desired ketone. 

Scheme 1.5

Dieter and coworkers reported treating Grignard reagents with stoichiometric amounts of CuCN.2LiCl to prepare organocuprate reagents, whereupon acylation with an acid chloride afforded ketones in excellent yields. Additionally, benzylic organocuprates have been prepared via transmetallation of organozinc reagents using the same THF-soluble CuCN.2LiCl complex. Transmetallation with CuCN.2LiCl complex considerably enhances the reactivity of organometallic species toward electrophiles. Exploiting this method, it was envisaged that a piperonyl organocuprate reagent, prepared from a Grignard reagent 50, could be treated with various acid chlorides 51 to furnish the desired ketones 36 (Scheme 1.6).

Scheme 1.6
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Overview

This chapter details the synthesis of a number of α-substituted analogues of MDMA via reductive amination of a piperonyl ketone intermediate 36. Various synthetic methods towards 36 were explored including acylation of a piperonyl organocuprate, derived from the Grignard reagent 50, with numerous acid chlorides 51.
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Results and Discussion

Piperonyl chloride 53 was prepared in good yield (82%) from piperonal 32 by standard methodology: reduction to afford the alcohol 52 followed by chlorination (Scheme 1.7).**

\[
\begin{align*}
32 & \xrightarrow{a} 52 & 52 & \xrightarrow{b} 53
\end{align*}
\]

Scheme 1.7 a) NaBH₄, EtOH (99%); b) SOCl₂, CH₂Cl₂ (83%; overall yield 82%).

Benzylic Grignard reagents have traditionally been difficult to prepare, owing to their propensity to form a coupled product via the reaction of the reactive benzylic halide and the Grignard reagent.⁷⁴ Piperonylmagnesium chloride 50 was efficiently furnished by using a ten-fold excess of mechanically activated magnesium, prepared by modification of the method of Baker et al.,⁷⁴ slow dropwise addition of piperonyl chloride over several hours, and ensuring the reaction remained ice-cold during the entire process (Scheme 1.8). Notably the reaction lacked a visible precipitate (MgCl₂), indicative that no coupled product had formed. The solution of 50 was used immediately without any attempt to determine the concentration and quantify the efficiency of the conversion.

\[
\begin{align*}
53 & \xrightarrow{a} 50
\end{align*}
\]

Scheme 1.8 a) Mg (stirred under Ar for 7 d), THF, 0 °C.

** Piperonyl chloride 53 has a limited shelf life and was kept stored under Ar at 4 °C and used within one month of preparation.
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Treatment of 50 with a stoichiometric amount of the copper(I) complex, CuCN.2LiCl, presumably afforded a piperonyl organocuprate species via transmetallation. Reaction of the piperonyl organocuprate with a variety of alkyl, aryl and heteroaryl acid chlorides 51 proceeded to afford the ketone 36 (Scheme 1.9, Table 1.2). A range of yields from modest (33%) to excellent (82%) was obtained.

Scheme 1.9 a) CuCN.2LiCl, THF, –20 ºC; b) RCOCl 51, THF, –20 ºC → rt.

Table 1.2 Yields of ketone 36 from reaction of a piperonyl organocuprate with various acid chlorides 51.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Yield (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>Ph</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>Me</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>c-Pr</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>1-naphthyl</td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>2-naphthyl</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>4-biphenyl</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>59</td>
<td>2-furyl</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>3-furyl</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>2-thienyl</td>
<td>62</td>
</tr>
</tbody>
</table>

** Yield is based on the substrate 53.

In the two instances where the yield of the ketones was particularly low (Table 1.2, entries 3 and 8), a significant amount of ester 62 was also isolated; this was indicative of oxygen contamination.
Despite considerable effort to maintain inert Schlenk conditions the formation of 62 was a sporadic frustration. Ultimately the source of inert gas used for the reactions was changed, after which no oxygen contamination products were observed.

Some difficulty was experienced in preparing the pyridyl acid chlorides. A method was therefore conceived whereby an organocuprate containing the pyridyl moiety was prepared and reacted with the piponeronyl acid chloride 68 (Scheme 1.10).

Scheme 1.10 a) BuLi, THF, $-100\, ^\circ\mathrm{C}$; b) CuCN.2LiCl, $-100\, ^\circ\mathrm{C} \rightarrow -20\, ^\circ\mathrm{C}$; c) 68, $-78\, ^\circ\mathrm{C} \rightarrow \mathrm{rt}$; d) $\mathrm{H}_3\mathrm{O}^+$.

The acid chloride 68 was accessed via the method shown in Scheme 1.11 (overall yield 77%). After several attempts the method (as shown in Scheme 1.10) did not yield the pyridyl ketone 65. It instead resulted in a complex mixture of products; by $^1\mathrm{H}$ NMR spectroscopy there was no evidence of the C2 methylene singlet in the complex mixture.
The method described in Scheme 1.9 allowed up to four ketones 36 to be made from a single batch of 50, however it was still cumbersome to perform. A more operationally simple method for the preparation of 36 was therefore sought.

**Addition of a Grignard Reagent to Nitriles**

Grignard reagents can be used to form ketones directly: nucleophilic addition to a Weinreb amide generates an unreactive tetrahedral intermediate or, alternatively, nucleophilic addition to a nitrile 69 forms a nucleophilic imine anion 70 that is unreactive towards the nucleophilic Grignard species 50. Upon the addition of acid the imine anion is protonated and readily hydrolysed to give the ketone 36 (Scheme 1.12).

**Scheme 1.11** a) NaCN, DMF (90%); b) NaOH, H2O, Δ (88%); c) CH2Cl2, SOCl2 (88%).

**Scheme 1.12** a) RCN 69, THF; b) H3O+.
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The method outlined in Scheme1.12 was operationally simple as it did not involve handling of hygroscopic LiCl and formation of the organocuprate species. In addition numerous nitriles 69 were readily available to allow various moieties to be installed at the α-position. This was unlike the acid chloride reagents 51 used in Scheme 1.9, which had to be prepared prior to use.

The reaction of benzonitrile with 50 afforded the ketone 43 in excellent yield (80%, Table 1.3, entry 3). Owing to the decreased electrophilic character of benzonitrile, as compared to benzoyl chloride, more vigorous reaction conditions (24 h reflux) were required.

Weberth and Hall demonstrated that the nucleophilic addition of Grignard reagents to nitriles is effectively catalysed by copper(I) salts.75 Using CuCN (15 mol%), piperonylmagnesium chloride was reacted with benzonitrile and the intermediate subsequently hydrolysed to afford 43 in slightly lower yield than in the absence of catalytic copper(I) (58% Table 1.3, entry 2).

Table 1.3 Yield of ketone 43 via various synthetic methods utilising Grignard reagent 50.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction Conditions†</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>80</td>
</tr>
</tbody>
</table>

†a) i) CuCN.2LiCl, THF, −20 °C; ii) PhCOCl, THF, −20 °C → rt.
b) PhCN, THF, CuCN, reflux.
c) PhCN, THF, reflux.
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Using heteroaryl nitriles the synthetic utility of the nucleophilic addition of 50 was not to be maintained; only benzonitrile gave an acceptable yield of the desired product (Table 1.4). The reaction of 2-thiophene nitrile with 50 gave ketone 61 in poor yield (17%, Table 1.4, entry 2). This was unacceptably low when compared to the coupling of piperonyl organocuprate and 2-thiophene carbonyl chloride, which gave ketone 61 in good yield (62%, Table 1.2, entry 8).

Table 1.4 Yields of ketone 36 from the addition of a Grignard reagent 50 to a heteroaryl nitrile.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ketone</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>Ph</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>2-thienyl</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>2-pyridyl</td>
<td>0*</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>3-pyridyl</td>
<td>0*</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>4-pyridyl</td>
<td>0*</td>
</tr>
</tbody>
</table>

* No ketone was detected in the crude product after workup. The characteristic CH$_2$ singlet resonance of the benzylic ketone (3-5 ppm) was not observed in the $^1$H NMR spectrum.

Attempted Preparation of Target Amines from Imine Ion Intermediates

Weiberth and Hall demonstrated it was possible to access primary amines from subsequent tandem alkylation-reduction reactions of Grignard reagents reacted with nitriles, by reducing the intermediate imine ion formed from the reaction. It seemed plausible then to obtain the desired methylamines 16 directly from the in situ methylation of the intermediate imine anion 74 and subsequent reduction of the imine 75 (Scheme 1.14). Some reactions exploring the viability of this proposed one-pot methodology were undertaken.
Addition of piperonylmagnesium chloride 50 to benzonitrile via the established procedure, followed by attempted methylation with methyl iodide and reduction with borane dimethyl sulfide, gave only the primary amine 76 (58%). Methylation was not effected. The procedure was repeated using the more electrophilic methylating reagent methyl tosylate; again no methylamine was obtained. The proposed methodology was not pursued any further, instead opting to pursue reductive amination of the existing piperonyl ketones to yield the desired methylamines 16.

Reductive Amination of Piperonyl Ketones

Target methylamines 16 were prepared by reductive amination of the piperonyl ketones 36 with methylamine. This transformation was affected using Borch reductive amination conditions (Scheme 1.15). The target amines 16 were furnished in good to excellent yield (69-91%).

++ The details of the reductive amination chemistry are discussed in further detail in Chapter Two.
Table 1.5 Table of yields of amines 16 via reductive amination of ketones 36.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph</td>
<td>43</td>
<td>21</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>Me</td>
<td>54</td>
<td>MDMA</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>c-Pr</td>
<td>55</td>
<td>18</td>
<td>69</td>
</tr>
<tr>
<td>4</td>
<td>1-naphthyl</td>
<td>56</td>
<td>31</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>2-naphthyl</td>
<td>57</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>6</td>
<td>4-biphenyl</td>
<td>58</td>
<td>29</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>2-furyl</td>
<td>59</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td>8</td>
<td>3-furyl</td>
<td>60</td>
<td>27</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>2-thienyl</td>
<td>61</td>
<td>26</td>
<td>81</td>
</tr>
</tbody>
</table>

The amines 16 were readily converted into the hydrochlorides 16.HCl by treatment with methanolic HCl and recrystallisation (Scheme 1.16). The hydrochlorides were more convenient to store, weigh and transport for biological testing purposes.

Scheme 1.16 a) MeOH, HCl.
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Overview

A series of ketones 36, variously substituted at the α-position with alkyl, aryl and heteroaryl moieties was prepared using methods coupling organometallic reagents with various electrophiles. From these ketones a number of target methylamines 16 was synthesised via reductive amination. Some of the compounds described were poised for further functionalisation.
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General Experimental

Solvents and Reagents

All solvents, except MeCN, were distilled prior to use and dried according to the methods of Burfield and coworkers.\textsuperscript{78-80} Lithium chloride was dried under reduced pressure (<0.5 mmHg) for 2 hr at 120 °C prior to use. Copper(I) iodide was used from a freshly opened bottle and within three months of opening. Potassium carbonate was dried overnight in an oven at 140 °C. Magnesium refers to reagent grade magnesium turnings (>98%) purchased from Sigma-Aldrich. Methanolic HCl (1 M) was prepared by the method detailed in Fieser and Fieser.\textsuperscript{81}

Procedures

'Standard workup' refers to dilution with water, repeated extraction with an organic solvent, sequential washing of the combined extracts with sat. aq NaHCO\textsubscript{3} (if necessary), aq HCl (1 M, if necessary), water and brine, drying the organic fraction over magnesium sulfate, followed by filtration and finally concentration of the filtrate by means of rotary evaporation at reduced pressure.

All reactions were performed using glassware that was dried in an oven at 140 °C prior to use. Procedures involving air or moisture sensitive species were performed under inert and anhydrous conditions, using standard Schlenk techniques.

Ultra-sonication conditions were created using a Soniclean 120T (50/60 Hz, 60 W).

Unless otherwise stated, the percentage yields of reactions are reported for only those compounds purified by chromatography or recrystallisation and the purity assessed by \textsuperscript{1}H NMR spectroscopy.
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Chromatography

Flash and rapid suction filtration (rsf) chromatography were conducted on Merck silica gel 60 (63-200 μm) using the specified eluents. Thin layer chromatography was conducted on Merck aluminium-supported silica sheets (silica gel 60 F254). Plates were routinely visualised using ultraviolet light (254 nm). Where appropriate, spot identification was also performed using stain preparations, including 2,4-dinitrophenylhydrazine (0.5%) in hydrochloric acid (2 M), ninhydrin (0.3%) and CH₃COOH (3%) in BuOH, and iodine (as solid crystals in a sealed chamber).

Instrumental Analyses

¹H and ¹³C NMR spectra were recorded using a Varian Gemini 200 (200 MHz for ¹H and 50.3 MHz for ¹³C), a Bruker AM300 (300.1 MHz for ¹H and 75.5 MHz for ¹³C), a Bruker ARX500 (500.1 MHz for ¹H and 125.8 MHz for ¹³C) or a Bruker AV600 spectrometer (600.1 MHz for ¹H and 150.9 MHz for ¹³C). Deuteriochloroform (CDCl₃) was used as solvent, unless otherwise stated. Chemical shifts are expressed in ppm relative to the residual solvent peak as stipulated by Gottelieb et al., for chloroform (¹H, δ 7.26; ¹³C, δ 77.16) and for acetone (¹H, δ 2.05; ¹³C, δ 206.26). Routine assignment of ¹³C spectra were made with the assistance of DEPT 135 and DEPT 90 experiments.

Infrared spectra were recorded on a Perkin-Elmer SpectrumOne FTIR spectrometer (4000-400 cm⁻¹). Microanalyses were conducted by Robertson Microlit Laboratories, New Jersey, USA. Melting points were determined using a Reichert hot stage melting point apparatus. Optical rotations were performed with a Perkin-Elmer 141 Polarimeter (1 mL, 10 cm pathlength). Mass spectra were recorded with a VG-Autospec spectrometer using either electrospray ionisation or fast atom bombardment ionisation technique. Single-crystal X-ray investigations were conducted on a Bruker AXS instrument. Mass spectra were recorded with a VG-Autospec spectrometer.
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Miscellaneous

Compounds were named using ACD Labs IUPAC Name Free 8.05 software.
Chapter One

Experimental

General Procedure A: Addition of a Piperonyl Organocopper Reagent to an Acyl Chloride or a Nitrile

A THF solution of piperonylmagnesium chloride 50 (1.0 mol equiv, 0.20 M) was added dropwise under Ar to a cold (−78 °C) stirred solution of anhydrous LiCl (2.0 mol equiv) and CuCN (1.0 mol equiv) in THF (5 mL/mmol of 50) whereupon a yellow solution that contained a suspended white precipitate formed. The mixture was allowed to warm gradually to −25 °C and stirred (10 min). The mixture was cooled (−78 °C) and the acyl chloride or nitrile (1.2 mol equiv) in THF (5 mL/mmol) was added dropwise. The reaction mixture was warmed gradually to 0 °C over 3 h then diluted with ice-cold aq HCl (0.3 M, 10 mL/mmol), whereupon a white precipitate formed. The mixture was filtered through a short column of Celite and the residue washed through with Et2O. The filtrate was subjected to a standard workup; flash chromatography gave the ketone 36.

General Procedure B: Addition of a Nitrile to a Grignard Reagent

The nitrile (1.0 mol equiv) in THF (5 mL/mmol) was added under Ar to a stirred THF solution of piperonylmagnesium chloride 50 (1.2 mol equiv, 0.20 M). The mixture was stirred (1 h at rt) and heated to reflux until tlc indicated that consumption of the nitrile was complete. The reaction mixture was diluted with ice-cold aq HCl (0.3 M, 10 mL/mmol) and subjected to a standard workup (Et2O); flash chromatography gave the ketone 36.

General Procedure C: Reductive Amination

Crushed, dried 3Å sieves (1 mg/mg of ketone 36) were added to a cold (0 °C) solution of the substrate ketone 36 (1.0 mol equiv) in THF (3 mL/mmol) and MeOH (3 mL/mmol). Under an Ar flow ethanolic methylamine (10 mol equiv, 33% w/v) was added dropwise with stirring, followed by dropwise addition of glacial CH3COOH (10 mol equiv); the mixture was stirred at 0 °C
(30 min). Sodium cyanoborohydride (1.0 mol equiv) was added and the mixture warmed (50 °C) and stirred until the reaction was observed to be complete by tlc (typically 24-36 h). The mixture was diluted with aq HCl (1 M) and stirred (10 min), then filtered through Celite, washing with MeOH, followed by concentration of the filtrate/washings. The concentrate was basified with aq NaOH (1 M) and subjected to a standard workup (Et₂O); chromatography gave the amine 16.

**General Procedure D: Amine Hydrochloride Preparation**

The substrate amine 16 was treated with a vast molar excess of methanolic HCl. The solvent and excess HCl were removed by evaporation and the resultant solid was recrystallised to afford the amine hydrochloride 16.HCl.

![MgCl₂](image)

**Piperonylmagnesium chloride 50**

Magnesium flakes (4.9 g, 0.20 mol) were vigorously stirred under a flow of Ar for 7 d during which time the Mg became finely divided, blackened and formed a mirror. The Mg was covered with THF (50 mL) and the suspension was cooled in an ice-water bath. Piperonyl chloride 84 53 (3.4 g, 0.020 mmol) in THF (50 mL) was added dropwise with vigorous stirring over 1 h and stirred for a further 2.5 h at 0 °C. The resultant suspension was filtered and transferred via cannula to yield the Grignard reagent 50, as a greenish-yellow coloured solution. The Grignard reagent was stored under Ar at 0 °C and used within 2 h. No attempt was made to determine the concentration of Grignard reagent. Subsequent calculations were based on an assumed quantitative yield (0.20 mol/L).
2-(1,3-Benzodioxol-5-yl)-1-phenylethanone 43

(a) Piperonylmagnesium chloride 50 (48 mL, 9.6 mmol) was added dropwise under a flow of Ar to an ice-cold solution of benzonitrile (0.83 mL, 8.0 mmol) in THF (10 mL) during which time the solution turned bright yellow; CuCN (14 mg, 2 mol%) was added. The reaction was stirred (0 °C, 3 h) and allowed to warm (rt) and stirred overnight. The following day TLC indicated that the benzonitrile was not consumed, so the reaction was heated to reflux (1 h). The reaction was quenched with water and subjected to a standard workup (EtOAc). Flash chromatography afforded the ketone 43 as a pale yellow solid (1.1 g, 58%). Recrystallisation of a small sample gave pale yellow crystals, mp 63.5-64.5 °C (MeOH). IR ν_max (KBr disc) 1687, 1503, 1255, 1041 cm⁻¹. 

¹H NMR (300 MHz): δ 8.04-7.98 (m, 2H, ArH), 7.60-7.43 (m, 3H, ArH), 6.79-6.68 (m, 3H, H₄', H₆', H₇'), 5.93 (s, 2H, OCH₂O), 4.20 (s, 2H, H₂). ¹³C NMR (125.6 MHz): δ 197.80 (C=O), 147.98, 146.70, 136.65 (Ar), 133.31, 128.78, 128.70 (ArH), 128.18 (Ar), 122.68, 110.03, 108.57 (ArH), 101.13 (OCH₂O), 45.20 (C2). HRMS (EI): m/z = 240.0785; [M]⁺ requires 240.0786. The ¹H and ¹³C NMR spectra were in agreement with those published.⁶²

(b) Piperonylmagnesium chloride 50 (180 mL, 36 mmol) and benzonitrile (3.1 mL, 30 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 43 as a yellow solid (5.8 g, 80%). The IR, ¹H and ¹³C NMR spectra agreed with those reported above.

(c) Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and benzoyl chloride (0.70 mL, 6. mmol) were treated according to General Procedure A
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[flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 43 as a yellow solid (1.1 g, 76%).

The IR, $^1$H and $^{13}$C NMR spectra agreed with those reported above.

1-(1,3-Benzodioxol-5-yl)acetone 54

Piperonylmagnesium chloride 50 (80 mL, 16 mmol), CuCN (1.4 g, 16 mmol), LiCl (1.4 g, 32 mmol) and acetyl chloride (1.3 mL, 19 mol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 54 as a colourless oil (1.9 g, 66%).

IR $\nu_{max}$ (film) 1712, 1503, 1248, 1039 cm$^{-1}$. $^1$H NMR (600 MHz): $\delta$ 6.77 (d, $J = 7.9$ Hz, 1H, H7$'$), 6.68 (dd, $J = 2.1$, 0.4 Hz, 1H, H4$'$), 6.66-6.62 (m, 1H, H6$'$), 5.95 (s, 2H, OCH$_2$O), 3.60 (s, 2H, H2), 2.15 (s, 3H, CH$_3$). $^{13}$C NMR (75.5 MHz): $\delta$ 206.76 (O), 148.04, 146.83, 127.93 (Ar), 122.66, 109.93, 108.63 (ArH), 101.20 (OCH$_2$O), 50.70 (C2), 29.34 (CH$_3$). HRMS (EI): m/z = 178.0624; [M]$^+$ requires 178.0630. The IR, $^1$H and $^{13}$C NMR spectra were in agreement with those previously reported.

2-(1,3-Benzodioxol-5-yl)-1-cyclopropylethanone 55

Piperonylmagnesium chloride 50 (190 mL, 38 mmol), CuCN (3.4 g, 38 mmol), LiCl (3.2 g, 76 mmol) and cyclopropylcarbonyl chloride$^{87}$ (4.2 mL, 46 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 55 as a colourless oil (3.0 g, 38%).

IR $\nu_{max}$ (film) 1693, 1489, 1247, 1038 cm$^{-1}$. $^1$H NMR (500 MHz): $\delta$ 6.77 (d, $J = 7.9$ Hz, 1H, H7$'$), 6.71 (dd, $J = 1.7$, 0.4 Hz, 1H, H4$'$), 6.69-6.66 (m, 1H, H6$'$), 5.95 (s, 2H, OCH$_2$O), 3.73 (s, 2H, H2), 1.99-1.93 (m, 1H, c-PrH), 1.05-1.01 (m, 2H, c-PrH), 0.88-0.83 (m, 2H, c-PrH). $^{13}$C NMR (125.8 MHz): $\delta$ 208.62 (C=O), 148.00, 146.73, 128.18 (Ar), 122.76, 110.03, 108.57 (ArH), 101.15 (OCH$_2$O), 50.40 (C2), 20.06, 11.48 (c-Pr). HRMS (EI): m/z = 204.0780; [M]$^+$ requires 204.0786.
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Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 1-naphthylcarbonyl chloride88 (1.1 g, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 56 as a pale yellow oil (1.0 g, 71%). IR νmax (film) 1680, 1488, 1243, 1036, 772 cm⁻¹. ¹H NMR (300 MHz): δ 8.62-8.53 (m, 1H, ArH), 8.01-7.82 (m, 3H, ArH), 7.63-7.45 (m, 3H, ArH), 6.83-6.72 (m, 3H, ArH), 5.92 (s, 2H, OCH₂O), 4.28 (s, 2H, H₂). ¹³C NMR (75.5 MHz): δ 201.55 (O=O), 147.8, 146.56, 135.44, 133.93, 132.76 (Ar), 130.38, 128.37, 128.02, 127.96, 127.78, 126.47, 125.76, 124.25, 122.61, 109.9, 108.4 (ArH), 100.96 (OCH₂O), 48.46 (C₂). HRMS (EI): m/z = 290.0938; [M]⁺ requires 290.0943.

Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 2-naphthylcarbonyl chloride89 (1.1 g, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 57 as a pale yellow oil (1.1 g, 79%). IR νmax (film) 1682, 1240, 836, 754 cm⁻¹. ¹H NMR (300 MHz): δ 8.54 (s, 1H, ArH), 8.06 (dd, J = 8.6, 1.7 Hz, 1H, ArH), 7.96 (d, J = 7.7 Hz, 1H, ArH), 7.90-7.84 (m, 2H, ArH), 7.64-7.52 (m, 2H, ArH), 6.84-6.76 (m, 3H, ArH), 5.92 (s, 2H, OCH₂O), 4.32 (s, 2H, H₂). ¹³C NMR
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(75.5 MHz): δ 197.52 (C=O), 147.73, 146.44, 135.44, 133.70 (Ar), 132.30, 130.19, 129.48, 128.43, 128.38, 128.06 (ArH), 127.63 (Ar), 126.68, 124.08, 122.47, 109.83, 108.32 (ArH), 100.87 (OCH₂O), 44.96 (C₂). HRMS (EI): m/z = 290.0942; [M]+ requires 290.0943.

2-(1,3-Benzodioxol-5-yl)-1-biphenyl-4-ylethanone 58

Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 4-biphenylcarbonyl chloride (1.3 g, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 58 as a pale yellow solid (1.3 g, 82%). A sample was recrystallised to give off-white crystals, mp 130-131 °C (MeOH).

IR v max (KBr disc) 1672, 1237, 1041 cm⁻¹.¹H NMR (300 MHz, (CD₃)₂CO): δ 8.19-8.11 (m, 2H, ArH), 7.85-7.76 (m, 2H, ArH), 7.75-7.68 (m, 2H, ArH), 7.55-7.38 (m, 3H, ArH), 6.88-6.76 (m, 3H, ArH), 5.96 (s, 2H, OCH₂O), 4.32 (s, 2H, H2). ¹³C NMR (75.5 MHz, (CD₃)₂CO): δ 197.55 (C=O), 148.65, 147.33, 146.16, 140.51, 136.41 (Ar), 130.04, 129.89 (ArH), 129.77 (Ar), 129.13, 127.98, 127.90, 123.54, 110.83, 108.91 (ArH), 101.90 (OCH₂O), 45.40 (C₂). Anal. calcd for C₂₁H₁₆O₃: C, 79.7; H, 5.1. Found: C, 79.5; H, 4.8. HRMS (EI): m/z = 316.1102; [M]+ requires 316.1099.

2-(1,3-Benzodioxol-5-yl)-1-(2-furyl)ethanone 59

Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 2-furancarbonyl chloride 59 (0.78 g, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 59 as a yellow solid.
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(0.95 g, 83%). A sample was recrystallised to give pale yellow crystals, mp 49.5-50.0 °C (MeOH).

IR \( \nu_{\text{max}} \) (KBr disc) 1673, 1247, 1038 cm\(^{-1}\). \(^1\)H NMR (300 MHz): \( \delta \) 7.58 (dd, \( J = 1.7, 0.8 \) Hz, 1H, H5\( '' \)), 7.21 (dd, \( J = 3.6, 0.8 \) Hz, 1H, H4\( '' \)), 6.80-6.73 (m, 3H, ArH), 6.51 (dd, \( J = 3.6, 1.7 \) Hz, 1H, H3\( '' \)), 5.90 (s, 2H, OCH2O), 4.01 (s, 2H, H2). \(^{13}\)C NMR (75.5 MHz): \( \delta \) 186.6 (C=O), 152.10, 147.70, 146.53, 146.52 (Ar), 127.40, 122.52, 117.81, 112.33, 109.79, 108.28 (ArH), 100.90 (OCH2O), 44.79 (C2). HRMS (EI): \( m/z = 230.0584; [M]^+ \) requires 230.0579.

2-(1,3-Benzodioxol-5-yl)-1-(3-furyl)ethanone 60

Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 3-furancarbonyl chloride\(^ {\dagger} \) (0.78 g, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 60 as a pale yellow solid (0.38 g, 33%). A sample was recrystallised to give large, pale yellow needles, mp 90.5-91.0 °C (MeOH). IR \( \nu_{\text{max}} \) (film) 1658, 1250 cm\(^{-1}\). \(^1\)H NMR (600 MHz): \( \delta \) 8.01 (dd, \( J = 1.5, 0.6 \) Hz, 1H, H2\( '' \)), 7.42 (dd, \( J = 1.9, 1.5 \) Hz, 1H, ArH), 6.78-6.74 (m, 3H, ArH), 6.71-6.69 (m, 1H, ArH), 5.93 (s, 2H, OCH2O), 3.94 (s, 2H, H2). \(^{13}\)C NMR (150.9 MHz): \( \delta \) 192.52 (C=O), 148.04 (Ar), 147.72 (ArH), 146.84 (Ar), 144.30 (ArH), 127.91, 127.37 (Ar), 122.61, 109.90, 109.11, 108.59 (ArH), 101.18 (OCH2O), 47.30 (C2). HRMS (EI): \( m/z = 230.0573; [M]^+ \) requires 230.0579.

2-(1,3-Benzodioxol-5-yl)-1-(2-thienyl)ethanone 61

(a) Piperonylmagnesium chloride 50 (25 mL, 5.0 mmol), CuCN (0.45 g, 5.0 mmol), LiCl (0.42 g, 10 mmol) and 2-thiophenecarbonyl chloride (0.64 mL, 6.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 61 a yellow solid (0.76 g, 62%). A sample was recrystallised to give pale yellow needles, mp 67.5-68.0 °C (EtOH). IR \( \nu_{\text{max}} \) (KBr disc) 1673, 1247, 1067 cm\(^{-1}\). \(^1\)H NMR (300 MHz): \( \delta \) 7.76 (dd, \( J = 3.8, 1.1 \) Hz,

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\(^ {\dagger} \) 3-Furoyl chloride was kindly provided by Katie Punch (University of Western Australia).
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1H, H3", 7.62 (dd, J = 5.0, 1.1 Hz, 1H, H5"), 7.11 (dd, J = 5.0, 3.9 Hz, 1H, H4"), 6.80-6.73 (m, 3H, ArH), 5.90 (s, 2H, OCH2O), 4.09 (s, 2H, H2). 13C NMR (75.5 MHz): δ 190.42 (C=O), 147.70, 146.5, 143.56 (Ar), 133.97, 132.53, 128.10 (ArH), 127.72 (Ar), 122.42, 109.68, 108.27 (ArH), 100.90 (OCH2O), 45.75 (C2). HRMS (EI): m/z = 246.0350; [M]+ requires 246.0351.

(b) Piperonylmagnesium chloride 50 (30 mL, 6.0 mmol) and 2-thiophene nitrile (0.47 mL, 5.0 mmol) were treated according to General Procedure A [flash chromatography (EtOAC/petrol, 1:9)] to afford the ketone 61 as a yellow solid (0.17 g, 17%). The IR, 1H and 13C NMR spectra agreed with those reported above.

![Image of 2-(1,3-Benzodioxol-5-yl)-1-phenylethanamine 76](image)

2-(1,3-Benzodioxol-5-yl)-1-phenylethanamine 76

Piperonylmagnesium chloride 50 (30 mL, 6.0 mmol) was added dropwise under a flow of Ar to an ice-cold solution of benzonitrile (0.52 mL, 5.0 mmol) in THF (10 mL) during which time the solution turned bright yellow. The reaction mixture was refluxed overnight. The following day TLC indicated that the benzonitrile was completely consumed. The reaction mixture was cooled (−78 °C) and MeI (0.37 mL, 6.0 mmol) was added dropwise with stirring. The reaction was warmed to room temperature and stirred (rt, 1 h). Borane dimethyl sulfide (0.60 mL, 6.0 mmol) was added and the reaction stirred overnight (rt) during which time a white precipitate formed. The reaction was quenched with aq HCl (0.3 M), followed by a standard workup (Et2O). The crude residue was subjected to rf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt3, 1:3:trace) to afford the amine 76 as a colourless oil (0.70 g, 58%). IR v_max (film) 3371, 1504, 1488, 1247, 1038 cm⁻¹.

1H NMR (600 MHz): δ 7.36-7.31 (m, 4H, ArH), 7.27-7.24 (m, 1H, ArH), 6.73 (d, J = 7.9 Hz, 1H,
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H7), 6.66 (d, J = 1.7 Hz, 1H, H4'), 6.62 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.93 (AB, J = 0.9 Hz, 2H, OCH2O), 4.13 (dd, J = 8.9, 5.0 Hz, 1H, H1), 2.92 (dd, J = 13.5, 5.0 Hz, 1H, H2), 2.74 (dd, J = 13.5, 8.9 Hz, 1H, H2), 1.58 (br s, 2H, NH2). 13C NMR (150.9 MHz): δ 147.76, 146.23, 145.68, 132.96 (Ar), 128.58, 127.25, 126.55, 122.45, 109.71, 108.32 (ArH), 100.98 (OCH2O), 57.79 (C1), 46.28 (C2). HRMS (EI): m/z = 241.1091; [M]+ requires 241.1103. The 1H NMR spectrum was consistent with that previously reported.91

2-(1,3-Benzodioxol-5-yl)-1-phenylethanamine hydrochloride 76.HCl

The amine 76 was treated according to General Procedure D to afford the hydrochloride 76.HCl as colourless needles, mp 229.5-231.0 °C (i-PrOH) [lit.92 mp 258-259 °C]. 1H NMR (600 MHz, d6-DMSO): δ 8.33 (br s, 3H, NH3), 7.44-7.41 (m, 2H, ArH), 7.39-7.31 (m, 3H, ArH), 6.75 (d, J = 7.9 Hz, 1H, H7'), 6.72 (d, J = 1.6 Hz, 1H, H4'), 6.55 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.94 (AB, J = 1.0 Hz, 2H, OCH2O), 4.44 (dd, J = 8.9, 6.2 Hz, 1H, H1), 3.19 (dd, J = 13.6, 6.0 Hz, 1H, H2), 3.00 (dd, J = 13.6, 9.0 Hz, 1H, H2). Anal. calcd for C15H16ClN02: C, 64.9; H, 5.8; N, 5.0. Found: C, 64.8; H, 5.8; N, 4.9.

1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine MDMA

The ketone 54 (0.71 g, 4.0 mmol) was treated with ethanolic methylamine (5.0 mL, 40 mmol), glacial CH3COOH (2.3 mL, 40 mmol) and NaCNBH3 (0.25 g, 4.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then
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EtOAc/petrol/NEt3, 1:3:trace) afforded the amine MDMA as a yellow oil (0.69 g, 90%). $^1$H NMR (600 MHz): δ 6.74 (d, $J = 7.9$ Hz, 1H, H7'), 6.68 (d, $J = 1.7$ Hz, 1H, H4'), 6.63 (dd, $J = 7.9$, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH2O), 2.76-2.68 (m, 1H, H1), 2.61 (dd, $J = 13.5$, 7.2 Hz, 1H, H2), 2.54 (dd, $J = 13.5$, 6.2 Hz, 1H, H2), 2.39 (s, 3H, NCH3), 1.52 (br s, 1H, NH), 1.04 (d, $J = 6.2$ Hz, 3H, CH3). $^{13}$C NMR (125.8 MHz): δ 147.76, 146.06, 133.35 (Ar), 122.31, 109.64, 108.30 (ArH), 100.95 (OCH2O), 56.57 (C1), 43.29 (C2), 34.14 (NCH3), 19.77 (CH3). HRMS (EI): $m/z = 193.1094$; [M]+ requires 193.1103. The $^1$H and $^{13}$C NMR spectra were consistent with those previously reported.

1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine hydrochloride MDMA.HCl

The amine MDMA was treated according to General Procedure D to afford the hydrochloride MDMA.HCl as colourless blocks, mp 153.0 °C (i-PrOH) [lit.47 mp 152-153°C (i-PrOH/Et2O)]. $^1$H NMR (600 MHz): δ 9.62 (v br s, 2H, NH2Cl), 6.74 (d, $J = 7.9$ Hz, 1H, H7'), 6.70 (d, $J = 1.7$ Hz, 1H, H4'), 6.67 (dd, $J = 7.9$, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH2O), 3.37 (dd, $J = 13.2, 4.1$ Hz, 1H, H2), 3.30-3.23 (m, 1H, H1), 2.76 (dd, $J = 13.2$, 10.5 Hz, 1H, H2), 2.69 (s, 3H, NCH3), 1.34 (d, $J = 6.5$ Hz, 3H, CH3). Anal. calcd for C11H16ClN02: C, 57.5; H, 7.0; N, 6.1. Found: C, 57.4; H, 7.1; N, 6.1. The $^1$H NMR spectrum was consistent with that previously reported.93

2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methylethanamine 18

The ketone 55 (0.41 g, 2.0 mmol) was treated with ethanolic methylamine (2.5 mL, 20 mmol), glacial CH3COOH (1.2 mL, 20 mmol) and NaCNBH3 (0.13 g, 2.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt3, 1:3:trace) afforded the amine 18 as a colourless oil (0.30 g, 69%). IR $\nu_{\text{max}}$ (neat) 3344 cm$^{-1}$. $^1$H NMR (300 MHz): δ 6.73 (d, $J = 7.8$ Hz, 1H, H7'), 6.67 (d, $J = 1.6$ Hz, 1H, H4'), 6.65 (dd, $J = 7.8$, 1.6 Hz, 1H, H6'), 5.92 (s, 2H, OCH2O), 2.83 (dd, $J = 13.6, 4.8$ Hz, 1H, H2), 2.66 (dd, $J = 13.6, 7.9$ Hz, 1H, H2), 2.44 (s, 3H, NCH3), 1.77 (ddd , $J = 8.3, 8.3, 4.9$ Hz, 1H, H1), 1.37 (br s,
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$^{1}H$, $^{13}C$ NMR (75.5 MHz): $\delta$ 147.52, 145.88, 133.10 (Ar), 122.16, 109.63, 108.11 (ArH), 100.82 (OCH$_2$O), 66.59 (C1), 41.40 (C2), 34.57 (NCH$_3$), 15.45, 1.64 (c-Pr). HRMS (EI): $m/z$ 219.1262; [M + H]$^+$ requires 219.1259.

2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methylethanamine hydrochloride 18HCl

The amine 18 was treated according to General Procedure D to afford the hydrochloride 18HCl as colourless needles, mp 155.5-157.5 °C ($i$-PrOH). $^1H$ NMR (300 MHz): $\delta$ 9.62 (br s, 2H, NH$_2$Cl), 6.76-6.71 (m, 3H, ArH), 5.93 (s, 2H, OCH$_2$O), 3.37 (dd, $J$ = 13.5, 4.5 Hz, 1H, H2), 3.05 (dd, $J$ = 13.5, 9.5 Hz, 1H, H2), 2.76 (apparent t, $J$ = 5.6 Hz, 3H, NCH$_3$), 2.44 (m, 1H, H1), 1.12-1.01 (m, 1H, c-PrH), 0.69 (m, 1H, c-PrH), 0.52-0.34 (m, 2H, c-PrH), -0.10 - -0.21 (m, 1H, c-PrH). Anal. calcd for C$_{13}$H$_{18}$ClN$_2$: C, 61.1; H, 7.1; N, 5.5: C, 61.1; H, 7.2; N, 5.6.

2-(1,3-Benzodioxol-5-yl)-N-methyl-1-phenylethanamine 21

The ketone 43 (1.7 g, 7.0 mmol) was treated with ethanolic methylamine (8.8 mL, 70 mmol), glacial CH$_3$COOH (4.0 mL, 70 mmol) and NaCNBH$_3$ (0.44 g, 7.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt$_3$, 1:3:trace) afforded the amine 21 as a pale yellow oil (1.6 g, 91%). IR $\nu_{\text{max}}$ (film) 3340 cm$^{-1}$. $^1H$ NMR (300 MHz): $\delta$ 7.38-7.21 (m, 5H, ArH), 6.71 (d, $J$ = 7.8 Hz, 1H, H7'), 6.64 (d, $J$ = 1.6 Hz, 1H, H4'), 6.59 (dd, $J$ = 7.9, 1.7 Hz, 1H, H6'), 5.92 (s, 2H, OCH$_2$O), 3.66 (dd, $J$ = 8.4, 5.6 Hz, 1H, H1), 2.83 (ddd, $J$ = 13.6, 8.4, 5.6 Hz, 2H, H2), 2.21 (s, 3H, NCH$_3$), 1.69 (br s, 1H, NH). $^{13}C$ NMR (75.5 MHz): $\delta$ 147.74, 146.09, 142.55, 132.30 (Ar), 128.42, 127.41, 127.22, 122.31, 109.50, 108.23(ArH), 100.82 (OCH$_2$O), 66.87 (C1), 44.51 (C2), 34.34 (NCH$_3$). HRMS (FAB): $m/z$ = 256.1341; [M + H]$^+$ requires 256.1338.

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2-(1,3-Benzodioxol-5-yl)-N-methyl-l-phenylethanamine hydrochloride 21.HCl

The amine 21 was treated according to General Procedure D to afford the hydrochloride 21.HCl as colourless needles, mp 212.0-213.0 °C (i-PrOH). $^1$H NMR (300 MHz): δ 10.32 (br s, 1H, NH), 10.02 (br s, 1H, NH), 7.46-7.32 (m, 5H, ArH), 6.56 (d, $J = 7.7$ Hz, 1H, H7'), 6.40 (m, 2H, H4',H6'), 5.84 (s, 2H, OCH20), 4.08 (m, 1H, H1), 3.47 (dd, $J = 13.0$, 6.9 Hz, 1H, H2), 3.32 (dd, $J = 13.0$, 11.1 Hz, 1H, H2), 2.49 (apparent t, $J = 5.3$ Hz, 3H, NCH3). Anal. calcd for C16H18ClNO2: C, 65.9; H, 6.2; N, 4.8. Found: C, 66.1; H, 6.2; N, 4.7.

2-(1,3-Benzodioxol-5-yl)-N-methyl-l-(2-thienyl)ethanamine 26

The ketone 61 (0.25 g, 1.0 mmol) was treated with ethanolic methylamine (1.2 mL, 10 mmol), glacial CH3COOH (0.6 mL, 10 mmol) and NaCNBH3 (0.06 g, 1.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt3, 1:3:trace) afforded the amine 26 as a pale yellow oil (0.21 g, 81%).

IR $\nu_{max}$ (film, CH2Cl2) 3335, 1490, 1248 cm⁻¹. $^1$H NMR (300 MHz): δ 7.23 (ddd, $J = 5.0$, 1.2, 0.6 Hz, 1H, H5''), 6.93 (dd, $J = 5.0$, 3.4 Hz 1H, H4''), 6.86 (ddd, $J = 3.5$, 1.2, 0.6 Hz, 1H, H3''), 6.72 (d, $J = 7.8$ Hz, 1H, H7'), 6.65 (d, $J = 1.6$ Hz, 1H, H4'), 6.61 (d, $J = 7.9$, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH2O), 4.01-3.96 (m, 1H, H1), 2.92 (m, 2H, H2), 2.30 (s, 3H, CH3). $^{13}$C NMR (75.5 MHz): δ 148.1, 147.6 (Ar) 146.22, 132.14 (Ar), 126.37, 124.52, 124.02, 122.38, 109.41, 108.22 (ArH), 100.95 (OCH2O), 62.31 (C1), 45.38 (C2), 34.45 (NCH3). HRMS (EI): $m/z = 261.0826$; [M]$^+$ requires 261.0823.
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2-(1,3-Benzodioxol-5-yl)-N-methyl-1-(2-thienyl)ethanamine hydrochloride 26.HCl

The amine 26 was treated according to General Procedure D to afford the hydrochloride 26.HCl as colourless crystals, mp 234.0 °C (i-PrOH). $^1$H NMR (600 MHz): $\delta$ 10.20 (v br s, 2H, NH$_2$), 7.36-7.29 (m, 2H, H1", H3"), 7.02 (dd, $J$ = 5.2, 3.6 Hz, 1H, H2"), 6.63-6.60 (m, 1H, ArH), 6.56-6.53 (m, 2H, ArH), 5.87 (s, 2H, OCH$_2$O), 4.38 (dd, $J$ = 11.3, 4.4 Hz, 1H, H1), 3.78 (dd, $J$ = 13.7, 4.7 Hz, 1H, H2), 3.37 (dd, $J$ = 13.8, 11.0 Hz, 1H, H2), 2.55 (s, 3H, NCH$_3$). Anal. calcd for C$_{14}$H$_{16}$ClNO$_2$S: C, 56.5; H, 5.4; N, 4.7. Found: C, 56.5; H, 5.3; N, 4.5.

2-(1,3-Benzodioxol-5-yl)-1-(3-furyl)N-methylethanamine 27

The ketone 60 (0.23 g, 1.0 mmol) was treated with ethanolic methylamine (1.2 mL, 10 mmol), glacial CH$_3$COOH (0.6 mL, 10 mmol) and NaCNBH$_3$ (0.06 g, 1.0 mmol) according to General Procedure C. Subsequent purification by rfs chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt$_3$, 1:3:trace) afforded the amine 27 as a colourless oil (0.17 g, 70%). IR $\nu$ max (film) 3420, 1505 cm$^{-1}$. $^1$H NMR (600 MHz, d$_6$-acetone): $\delta$ 7.47-7.45 (m, 1H, ArH), 7.32-7.30 (m, 1H, ArH), 6.71-6.67 (m, 1H, ArH), 6.62-6.60 (m, 1H, ArH), 6.44-6.42 (m, 1H, ArH), 5.93 (s, 1H, OCH$_2$O), 3.70-3.66 (m, 1H, H1), 2.85 (dd, $J$ = 13.5, 7.3 Hz, 1H, H2), 2.78 (dd, $J$ = 13.5, 6.7 Hz, 1H, H2), 2.22 (s, 3H, NCH$_3$). $^{13}$C NMR (600 MHz, d$_6$-acetone): $\delta$ 148.39, 146.84 (Ar), 143.84, 140.80 (ArH), 134.10, 128.73 (Ar), 123.13, 110.38, 110.04, 108.59 (ArH), 101.69 (OCH$_2$O), 58.75 (C1), 43.80 (C2), 34.51 (NCH$_3$). HRMS (EI): $m/z$ = 245.1060; [M]$^+$ requires 245.1052.

2-(1,3-Benzodioxol-5-yl)-1-(3-furyl)-N-methylethanamine hydrochloride 27.HCl

The amine 27 was treated according to General Procedure D to afford the hydrochloride 27.HCl as colourless needles, mp 219.5 °C (i-PrOH). $^1$H NMR (600 MHz): $\delta$ 9.98 (v br s, 2H, NH$_2$), 7.46-7.44 (m, 1H, ArH), 7.34-7.32 (m, 1H, ArH), 6.89 (dd, $J$ = 1.9, 0.8 Hz, 1H, ArH), 6.64 (dd, $J$ = 0.4, 7.8 Hz, 1H, H7'), 6.54-6.50 (m, 2H, H4', 6'), 5.89-5.88 (m, 2H, OCH$_2$O), 4.10 (dd, $J$ = 11.0, 4.1 Hz,
2-(1,3-Benzodioxol-5-yl)-1-(2-furyl)-N-methylethanamine 28

The ketone 59 (0.24 g, 1.0 mmol) was treated with ethanolic methylamine (1.2 mL, 10 mmol), glacial CH₃COOH (0.6 mL, 10 mmol) and NaCNBH₃ (0.06 g, 1.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt₃, 1:3:trace) afforded the amine 28 as a pale yellow oil (0.15 g, 62%).

IR \( \nu_{\text{max}} \) (film) 1488, 1250, 1037, 732 cm\(^{-1}\). \(^1\)H NMR (300 MHz): \( \delta \) 7.37 (dd, \( J = 1.8, 0.8 \) Hz, 1H, H5\(^\text{″} \)), 6.68 (dd, \( J = 7.6, 0.6 \) Hz 1H, ArH), 6.55 (m, 2H, ArH), 6.24 (dd, \( J = 3.2, 1.8 \) Hz, 1H, H4\(^\text{″} \)), 6.10 (ddd, \( J = 3.2, 0.8, 0.5 \) Hz, 1H, H3\(^\text{″} \)), 5.89 (s, 2H, OCH₂O), 3.76 (m, 1H, H1), 2.94 (m, 2H, H2), 2.28 (s, 3H, CH₃), 1.93 (br s, 1H, NH). \(^{13}\)C NMR (75.5 MHz): \( \delta \) 155.12, 147.47, 146.00 (Ar), 141.55 (ArH), 131.98 (Ar), 122.08, 109.84, 109.30, 108.06, 107.17 (ArH), 100.74 (OCH₂O), 59.68 (Cl), 40.72 (C2), 33.96 (NCH₃). HRMS (EI): \( m/z \) = 245.1058; \([\text{M}]^+ \) requires 245.1052.

2-(1,3-Benzodioxol-5-yl)-1-biphenyl-4-yl-N-methylethanamine 29

The ketone 58 (0.63 g, 2.0 mmol) was treated with ethanolic methylamine (2.5 mL, 20 mmol), glacial CH₃COOH (1.1 mL, 20 mmol) and NaCNBH₃ (0.13 g, 2.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then
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EtOAc/petrol/NEt₃, 1:3:trace) afforded the amine 29 as a pale yellow solid (0.48 g, 73%).

IR νmax (film) 1486, 1242, 1036 cm⁻¹. ¹H NMR (300 MHz): δ 7.65-7.54 (m, 4H, ArH), 7.47-7.31 (m, 5H, ArH), 6.73 (d, J = 7.9 Hz, 1H, H7'), 6.68 (d, J = 1.3 Hz, 1H, H4'), 6.62 (dd, J = 7.9, 1.4 Hz, 1H, H6'), 5.93 (s, 2H, OCH₂O), 3.74 (dd, J = 7.9, 6.0 Hz, 1H, H1), 2.91 (m, 2H, H2), 2.63-2.36 (br s, 1H, NH), 2.25 (s, 3H, CH₃). ¹³C NMR (75.5 MHz): δ 147.79, 146.27, 141.95, 141.00, 140.23, 132.45 (Ar), 128.87, 127.91, 127.31, 127.27, 127.16, 122.47, 109.62, 108.35 (ArH), 101.00 (OCH₂O), 66.70 (NCH₃), 44.62 (C2), 34.56 (C1). HRMS (ESI): m/z = 332.1639; [M + H]⁺ requires 332.1651.

2-(1,3-Benzodioxol-5-yl)-1-biphenyl-4-yl-N-methylethanamine hydrochloride 29.HCl

The amine 29 was treated according to General Procedure D to afford the hydrochloride 29.HCl as colourless plates, mp 218-220 °C (MeOH). ¹H NMR (600 MHz, (CD₃)₂SO): δ 9.85-9.35 (br s, 2H, NH₂), 7.72-7.67 (m, 4H, ArH), 7.59-7.55 (m, 2H, ArH), 7.47-7.44 (m, 2H, ArH), 7.39-7.35 (m, 1H, ArH), 6.75-6.72 (m, 2H, H4', H7'), 6.55 (dd, J = 8.0, 1.6 Hz, 1H, H6'), 5.93 (AB, J = 1.0 Hz, 2H, OCH₂O), 4.47 (dd, J = 10.3, 5.0 Hz, 1H, H1), 3.46 (dd, J = 13.5, 4.8 Hz, 1H, H2), 3.13 (dd, J = 13.5, 10.4 Hz, 1H, H2), 2.38 (s, 3H, NCH₃). Anal. calcd for C₂₂H₂₂ClN₂O₂: C, 71.8; H, 6.0; N, 3.8. Found: C, 71.5; H, 6.2; N, 3.7.

2-(1,3-Benzodioxol-5-yl)-N-methyl-l-(2-naphthyl)ethanamine 30

The ketone 57 (0.42 g, 1.4 mmol) was treated with ethanolic methylamine (1.8 mL, 14 mmol), glacial CH₃COOH (0.8 mL, 14 mmol) and NaCNBH₃ (0.09 g, 1.4 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3 then EtOAc/petrol/NEt₃, 1:3:trace) afforded the amine 30 as an off-white solid (0.37 g, 84%).

IR νmax (film) 3341, 1485 cm⁻¹. ¹H NMR (300 MHz): δ 7.86-7.78 (m, 3H, ArH), 7.74 (s, 1H, ArH), 7.52-7.42 (m, 3H, ArH), 6.73-6.68 (m, 2H, H4', H7'), 6.62 (dd, J = 7.8, 1.7 Hz, 1H, H6'), 5.93 (AB,
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$ J = 1.5 \text{ Hz}, 2H, \text{OCH}_2\text{O}$, 3.87 (dd, $ J = 8.1, 5.9 \text{ Hz}, 1H, H1), 2.95 (m, 2H, H2), 2.44 (br s, 1H, NH), 2.25 (s, 3H, CH3). 13C NMR (75.5 MHz): $\delta$ 147.79, 146.25, 140.38, 133.50 (Ar), 133.08 (ArH), 132.45 (Ar), 128.37, 127.90, 127.79, 126.48, 126.09 (ArH), 125.72 (Ar), 125.37, 122.45, 109.59, 108.35 (ArH), 100.98 (OCH2O), 67.15 (C1), 44.59 (C2), 34.62 (NCH3). HRMS (ESI): $m/z = 306.1486$; [M + H]$^+$ requires 306.1494.

2-(1,3-Benzodioxol-5-yl)-N-methyl-1-(2-naphthyl)ethanamine hydrochloride 30.HCl

The amine 30 was treated according to General Procedure D to afford the hydrochloride 30.HCl as a colourless powder, mp 242.0-244.0 °C (i-PrOH). $^1$HNMR (600 MHz, $d_6$-DMSO): $\delta$ 9.68-9.16 (br s, 2H, NH2), 7.96 (d, $ J = 8.6 \text{ Hz}, 1H, ArH), 7.94-7.91 (m, 2H, ArH), 7.88-7.85 (m, 1H, ArH), 7.67 (dd, $ J = 8.6, 1.7 \text{ Hz}, 1H, ArH), 7.57-7.53 (m, 2H, ArH), 6.71 (d, $ J = 1.7 \text{ Hz}, 1H, H^4$), 6.69 (d, $ J = 7.9 \text{ Hz}, 1H, H^7$), 6.53 (dd, $ J = 8.0, 1.7 \text{ Hz}, 1H, H^6$), 5.89 (AB, 1.0 Hz, 2H, OCH2O), 4.57-4.53 (m, 1H, H1), 3.46-3.44 (m, 1H, H2), 3.20 (dd, $ J = 13.6, 10.3 \text{ Hz} 1H, H2$), 2.39 (s, 3H, NCH3). Anal. calcd for C20H20ClNO2: C, 70.3; H, 5.9; N, 4.1. Found: C, 70.4; H, 5.8; N, 3.9.

2-(1,3-benzodioxol-5-yl)-N-methyl-1-(1-naphthyl)ethanamine 31

The ketone 56 (0.29 g, 1.0 mmol) was treated with ethanolic methylamine (1.2 mL, 10 mmol), glacial CH3COOH (0.6 mL, 10 mmol) and NaCNBH3 (0.06 g, 1.0 mmol) according to General Procedure C. Subsequent purification by rsf chromatography (EtOAc/petrol, 1:3) then afforded the amine 31 as a pale yellow oil (0.24 g, 79%).

IR $\nu_{max}$ (KBr disc) 3374 cm$^{-1}$. $^1$HNMR (300 MHz): $\delta$ 8.23 (d, $ J = 8.1 \text{ Hz }, 1H, ArH), 7.93-7.85 (m, 1H, ArH), 7.79 (d, $ J = 8.2 \text{ Hz}, 1H, ArH), 7.68 (d, $ J = 6.7 \text{ Hz}, 1H, ArH), 7.56-7.46 (m, 3H, ArH), 6.74-6.68 (m, 2H, H4', H7'), 6.65 (dd, $ J = 7.8, 1.7 \text{ Hz}, 1H, H6$), 5.92 (AB, $ J = 1.4 \text{ Hz}, 2H, OCH2O), 4.68 (dd, $ J = 8.4, 4.9 \text{ Hz}, 1H, H1$), 4.23-4.08 (br s, 1H, NH), 3.15 (dd, $ J = 13.8, 4.9 \text{ Hz}, 1H, H2$), 2.95 (dd, $ J = 13.8, 8.5 \text{ Hz}, 1H, H2$), 2.28 (s, 3H, NCH3). 13C NMR (75.5 MHz): $\delta$ 147.82, 146.35,
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137.46, 134.09, 132.31 (Ar), 129.22, 127.84, 126.12, 125.78, 125.52 (ArH), 122.68 (Ar), 122.48, 109.63, 108.39 (ArH), 101.00 (OCH₂O), 67.19 (Cl), 43.41 (C2), 34.19 (NCH₃). HRMS (ESI): m/z = 306.1486; [M + H]⁺ requires 306.1494.

2-(1,3-Benzodioxol-5-yl)-N-methyl-1-(1-naphthyl)ethanamine hydrochloride 31.HCl

The amine 31 was treated according to General Procedure D to afford the hydrochloride 31.HCl as colourless needles, mp 213.5-215 °C (i-PrOH). ¹H NMR (600 MHz, d₆-DMSO): δ 10.35-9.20 (br s, 2H, NH₂), 8.18-8.04 (br m, 2H, ArH), 7.96-7.91 (m, 2H, ArH), 7.65-7.59 (m, 1H, ArH), 7.52-7.48 (m, 2H, ArH), 6.72 (d, J = 1.1 Hz, 1H, H₄'), 6.60 (d, J = 7.9 Hz, 1H, H₇'), 6.51 (d, J = 7.8 Hz, 1H, H₆'), 5.83 (AB, J = 0.9 Hz, 2H, OCH₂O), 5.50-5.39 (m, 1H, H₁), 3.60 (dd, J = 13.5, 4.8 Hz, 1H, H₂), 3.31-3.23 (m, 1H, H₂), 2.43 (s, 3H, NCH₃). Anal. calcd for C₂₀H₂₀ClNO₂: C, 70.3; H, 5.9; N, 4.1. Found: C, 70.2; H, 5.9; N, 4.0.
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The Synthesis of Some $N$-Modified Analogues of MDMA
Chapter Two

Introduction

Gunn and coworkers\textsuperscript{94} first reported the biological activity of 14 in 1939. These researchers demonstrated that 14 was a stimulant in rabbit, mice and cats. Subsequently 14 has been reported to possess psychomimetic properties similar to the parent compound MDMA, as does the N-ethyl analogue 15.\textsuperscript{8,*} Accordingly both the analogues, 14 and 15, have received considerable interest as subjects of research and substances of abuse.\textsuperscript{96,97}

![Structures of 14 and 15](image)

In 1980 Braun \textit{et al.}\textsuperscript{11} published the most comprehensive investigation of the effect of modification of the N-moiety of MDMA. Twenty one N-modified analogues were synthesised and assessed for analgesia and motor activity in mice, and psychomimetic activity in humans. The salient observation from this study was that increasing the length of the N-substituent, to greater then N-ethyl, attenuates or abolishes the subjective psychoactivity reported by humans.\textsuperscript{46}

Of notable relevance, Lebsanft \textit{et al.}\textsuperscript{28} measured induced ipsilateral rotation in 6-hydroxydopamine unilaterally lesioned rats using MDMA and three of its analogues, including 14 and 15. This model is a powerful screening tool for anti-parkinsonian agents. Increasing strength of induced rotational behaviour was measured in the order, 15 < MDMA < 14. This result confirmed that modification of the N-moiety was a viable method for enhancing the potency of an observed therapeutic effect in MDMA analogues and validated an exploration of this nature for this study.

\textsuperscript{*} The World Health Organisation has proposed that the name ecstasy be inclusive of a small number of pharmacologically similar compounds, including MDMA, 14 and 15.\textsuperscript{95}
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Synthetic Routes to N-Modified MDMA Analogues

Various methods for functionalising the N-position of MDMA have been employed. Addition of hydrobromic or hydroiodic acid to safrole 77, an extract of the tree Sassafras albidum, affords the halopropane 78. The reaction of 78 with an amine nucleophile 79 produces the desired N-modified analogue 80 (Scheme 2.1). The reaction of an alkyl halide with a primary amine is not usually a feasible method for the preparation of a secondary amine, since the product is a stronger nucleophile than the starting material. The exception is where a vast excess of amine starting material is employed. This method however is useful for the preparation of tertiary amines.

\[
\text{Scheme 2.1 a) } HX (X = \text{Br, I); b) NHRR}_1 79. \]

MDMA and its differentially N-modified analogues 83 can be prepared by the Leuckart-Wallach reaction (Scheme 2.2). The first step involves treating the precursor ketone 54 with an N-alkylformamide 81 (N-methylformamide in the case of MDMA). The resultant formamide 82 can then be hydrolysed to afford the desired amine 83.

\[
\text{Scheme 2.2 a) } \text{RNHCHO 81;} \text{ b) HCl.} \]
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Reductive Amination of Ketones

Reductive amination is an important synthetic tool for the construction of carbon-nitrogen bonds. The reaction proceeds via condensation of a ketone 84 with an amine 85 under acidic conditions in the presence of a reducing agent to furnish the amine 86 (Scheme 2.3). Mechanistically, reductive amination occurs via a variety of pathways, dependant on the species involved.

![Chemical structure of Scheme 2.3](image)

**Scheme 2.3 a) Reduction [H], H_3O^+.

There is a broad range of ketone and amine reagents able to participate in reductive amination reactions. In general most primary and secondary, aliphatic and aromatic amines participate in reductive amination. Even poorly nucleophilic amines can be induced to react under select conditions. Equally there is a variety of reducing agents available to induce the transformation; these include metal hydrides, inorganic reducing agents, catalytic hydrogenation and borane reducing agents. The variety of reducing agents available is well illustrated by three popular routes of clandestine MDMA manufacture, utilising Al/Hg or sodium borohydride or sodium cyanoborohydride as the reducing agent.

By far the most common method for accessing MDMA analogues, variously substituted at the N-position, has been via reductive amination of the precursor ketone 54, using the conditions first reported in 1971 by Borch et al. Borch’s reductive amination is a simple, versatile, one-pot procedure that advantageously utilises the chemoselectivity of the reducing agent, sodium.
cyanoborohydride. The brevity of Borch’s method has made it amenable to rapid analogue production, as was necessitated in this study.

The mechanism of Borch’s reductive amination, as proposed by Borch and coworkers, is presented below using the representative example of MDMA synthesis (Scheme 2.4).

Condensation of ketone 54 with methylamine affords the hemiaminal 87; subsequent acid-catalysed dehydration gives the iminium ion 88, in equilibrium with its conjugate base, the imine 89. The cyanoborohydride ion chemoselectively reduces 88, in the presence of the ketone 54, to furnish the amine MDMA; selective reduction occurs at pH 6-8. This is the optimal pH range for the formation of 88 and one in which the reaction proceeds most efficiently.

Scheme 2.4 a) NaCNBH₃.

---

1 Reductive amination with borane reagents has been thoroughly reviewed.
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Target Analogues

A series of MDMA analogues was conceived (Chart 2.1). These analogues were intended to explore the effect of various alkyl and aryl N-modification of MDMA, as well as N-modification of the α-substituted analogues 18 and 21, on activity as PD therapeutics, MAO inhibition activity and BL cytotoxicity.†

![Chart 2.1 Target MDMA analogues.](image)

During the course of this study Brotchie and coworkers demonstrated that 18, a mixed serotonin and dopamine transporter, increases the quality of on-time provided by levodopa in reserpine-treated rat model of PD and parkinsonian (MPTP-lesioned) primates. In this respect 18 was more effective than MDMA. Indeed 18 was shown to be qualitatively superior to any currently available clinical drug to improve either quality or duration of on-time. Advantageously, unlike MDMA, 18 does not share MDMA’s propensity to reduce viability of serotonergic cells, elicit effects consistent with psychoactivity in a rat pre-pulse inhibition model or depress appetite; also, 18 did not substitute for MDMA in a drug discrimination assay. This work identified 18 as the prototype of a

† The analogues 80 whilst known in the literature have not been assessed with respect to the therapeutic activities of interest, anti-LID, MAO inhibition and BL cytotoxicity, which therefore warranted their preparation.
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new class of compound with therapeutic potential in the treatment of PD and prompted further study of this compound. Accordingly a small suite of analogues 90 were designed and synthesised.

Overview

A series of MDMA analogues, variously modified at the amine moiety, was conceived and synthesised. The target analogues were readily prepared by Borch reductive amination of the precursor ketones 43, 54 and 55 reported in Chapter One.

§ Further study of the therapeutic activity and mechanism of 18 in PD also required that the enantiomers of 18 be prepared. This work is documented in Chapter Three.
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Results and Discussion

Reductive Amination of Ketones

The ketone 54 was required on a multi-gram scale for a suite of reductive amination reactions. The acylation of a piperonyl organocuprate with acetyl chloride reported in Chapter One (Scheme 1.9) gave 54 in reasonable yield (66%, Table 1.2). However, the reaction’s lengthy Mg activation period (7 d) was somewhat unsatisfactory. A more expedient route to 54 was therefore undertaken.

Using a modification of a previously reported method, 54 was supplied but only in modest yield (22%; Scheme 2.5). This outcome served to confirm the superiority of the aforementioned acetylation of the piperonyl organocuprate to yield the requisite ketone 54.

\[ \text{CO}_2^+ \rightarrow \text{CCn} \rightarrow ^\circ \text{Oar} \]

Scheme 2.5 a) CH₃CH₂N₂, CH₃COONH₄ (64%); b) Fe, AcOH, H₂O (35%; overall yield 22%).

The nitrostyrene 92 was furnished by a Henry condensation of piperonal 32 and nitroethane in moderate yield (64%). Several variations of the reaction conditions did not bring about a higher yield. Notably, in all conditions the reaction suffered from formation of side-products. Previously both Shulgin and Nichols documented deleterious side-product formation for this type of reaction.

Additionally the hydrolysis of the nitrostyrene 92 did not proceed as expected. The desired ketone 54 was the minor product of the reaction (35%); the major product was the oxime 93 (45%) formed from the reduction of 92. The oxime 93 was isolated by standard flash chromatography as a
mixture of the $E$ and $Z$ isomers ($E$:$Z$, 1.0:3.3), distinguishable by their distinctly different methylene proton resonances, $\delta$ 3.42 and $\delta$ 3.66 ppm, respectively.$^{100,**}$

With gram quantities of the ketone 54 at hand (from the organocuprate approach), analogues 94-98 were prepared via the Borch reductive amination procedure using a series of amines (Table 2.1).$^{11}$ The analogues were encouragingly obtained in good to excellent yield (68-96%).

The optimised reaction conditions utilised: 1) a 10-fold excess of amine and 3Å sieves to encourage formation of the reducible imine intermediate. The excess of amine dually worked to prevent di-alkylation; 2) the amine and ketone reagents were stirred for a period (typically 0.5 h) prior to the addition of the sodium cyanoborohydride to allow preformation of the reducible intermediate imine species; 3) stoichiometric equivalent quantities of amine and glacial acetic acid were used to obtain the optimal pH (6-8).

** The relative quantity of $E$ and $Z$ isomers was determined by the integration ratio of the methylene proton in the $^1$H NMR spectrum.

$^{11}$ The amines described in Table 2.1 are a complement to MDMA analogues, also variously modified at the N-moietiy, prepared by fellow Piggott group member Blake Nguyen. These analogues are discussed in Chapter Four.
Table 2.1 Summary of yields for reductive amination reactions performed on the ketone 54.

<table>
<thead>
<tr>
<th>Product</th>
<th>R</th>
<th>R₁</th>
<th>Yield (%)</th>
<th>Lit. Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>Me</td>
<td>H</td>
<td>90</td>
<td>74</td>
</tr>
<tr>
<td>94</td>
<td>Me</td>
<td>Me</td>
<td>68</td>
<td>27</td>
</tr>
<tr>
<td>95</td>
<td>propargyl</td>
<td>H</td>
<td>71</td>
<td>39</td>
</tr>
<tr>
<td>96</td>
<td>allyl</td>
<td>Me</td>
<td>81</td>
<td>-</td>
</tr>
<tr>
<td>97</td>
<td>Ph</td>
<td>H</td>
<td>96</td>
<td>-</td>
</tr>
<tr>
<td>98</td>
<td>Bn</td>
<td>H</td>
<td>79</td>
<td>13</td>
</tr>
</tbody>
</table>

a) CH₃COOH, NaCNBH₃, MeOH, THF, 3Å sieves.
§ Reported isolated yields are for the hydrochlorides.

Using the same reaction conditions the ketones 43 and 55 were reductively aminated to afford a suite of analogues in similarly high yield (Table 2.2 and Table 2.3). The notable exception was the reaction of 43 with sterically hindered tert-butylamine. This reaction did not proceed appreciably (< 5%) even after the addition of excess amine and CH₃COOH (20 mole equivalents of each) in an effort to force the reaction to proceed. Prior to performing the reaction it was understood that sterically hindered amines make poor substrates for reductive aminations. However it was rationalised that if the product could be obtained even in poor yield (10-30%) then the effort to perform chromatographic separation on the reaction mixture would be worthwhile; it was not to be.
Table 2.2 Summary of yields for reductive aminations performed on the substrate ketone 43.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Product</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>H</td>
<td>82</td>
</tr>
<tr>
<td>21</td>
<td>Me</td>
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<td>Bu</td>
<td>86</td>
</tr>
<tr>
<td>102</td>
<td>i-Pr</td>
<td>77</td>
</tr>
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<td>103</td>
<td>t-Bu</td>
<td>&lt;5</td>
</tr>
<tr>
<td>104</td>
<td>allyl</td>
<td>83</td>
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<tr>
<td>105</td>
<td>propargyl</td>
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<tr>
<td>106</td>
<td>Bn</td>
<td>69</td>
</tr>
<tr>
<td>107</td>
<td>Ph</td>
<td>61</td>
</tr>
</tbody>
</table>

a) CH₃COOH, NaCNBH₃, MeOH, THF, 3Å sieves.
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Table 2.3 Summary of yields for reductive aminations performed on the substrate ketone 55.

<table>
<thead>
<tr>
<th>Product</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
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<td>18</td>
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<td>69</td>
</tr>
<tr>
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<tr>
<td>109</td>
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<td>90</td>
</tr>
<tr>
<td>110</td>
<td>i-Pr</td>
<td>57</td>
</tr>
</tbody>
</table>

a) CH₃COOH, NaCNBH₃, MeOH, THF, 3Å sieves.

Alkylation of Amines

The tertiary amines 111 and 112 were designed as part of a small suite of putative MAO inhibitors. These were not prepared by reductive amination of 54 owing to the expense of starting material amines, even though an analogous reaction, the reductive amination of 54 with N-allyl-N-methylamine, indicated that this would be a suitably high yielding route toward these analogues (81%, Table 2.1). An alternate method for preparing 111 and 112 via alkylation of the amines MDMA and 14 was investigated.

The analogue 111 was synthesized in good yield via propargylation of the free base of MDMA using one mole equivalent of propargyl bromide in the presence of K₂CO₃ in dry DMF (76%, Scheme 2.6).

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²⁴ For further discussion see Chapter Four.
²⁵ Fortuitously N-allyl-N-methylamine was at hand for use in reductive amination experiments.
To prepare **112**, **14** was first synthesised in gram quantities by the conventional method, LiAlH₄ reduction of the nitrostyrene **92** (Scheme 2.7).³,⁷,⁵,⁷ The analogue **112** was then furnished using similar propargylation conditions to those described above, using instead two mole equivalents of propargyl bromide (77%, Scheme 2.7). A higher yield of **112** was obtained when the reaction was performed with the substrate **14.HCl** in place of **14** (87%). Aside from the higher yield this procedural variation was preferable as the solid starting material was easier to handle and weigh than the oil of the free base.

In all of the propargylation reactions, efforts were made to minimise the formation of the quaternary salts*** by limiting propargyl bromide to stoichiometric quantities only and cooling of the reaction.

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*** The conversion of the tertiary amine into the quaternary ammonium salt is called the Menshutkin reaction.⁷⁰
mixture. Regardless, some quaternary salts did result in each case but were easily separable from the less polar tertiary amines by standard flash chromatography.

Overview

A series of MDMA analogues was prepared via Borch reductive amination of the precursor ketones 43, 54 and 55. Tertiary amines 111 and 112 were prepared by propargylation of MDMA and 14, respectively.
Chapter Two

Experimental

General Procedure A: Reductive Amination of a Ketone

Crushed, dried 3 Å sieves (1 mg/mg of ketone) were added to a cold (0 °C) solution of the substrate ketone (1.0 mol equiv) in THF (3 mL/mmol) and MeOH (3 mL/mmol). Under an Ar flow the amine (10 mol equiv) was added dropwise with stirring, followed by dropwise addition of glacial CH₃COOH (10 mol equiv); the mixture was stirred at 0 °C (30 min). Sodium cyanoborohydride (1.0 mol equiv) was added and the mixture warmed (50 °C) and stirred until the reaction was observed to be complete by tlc (typically 24-36 h). The mixture was diluted with aq HCl (1 M) and stirred (10 min), then filtered through Celite, washing with MeOH, followed by concentration of the filtrate/washings. The concentrate was basified with aq NaOH (1 M) and subjected to a standard workup (Et₂O); chromatography gave the amine.

General Procedure B: Amine Hydrochloride Preparation

The substrate amine was treated with a vast molar excess of methanolic HCl. The solvent and excess HCl were removed by evaporation and the resultant solid was recrystallised to afford the amine hydrochloride.

General Procedure C: Amine Propargylation

Potassium carbonate (1.5 mol equiv) was added under Ar to a stirred solution of the substrate amine (1.0 mol equiv) in anhydrous DMF (6 mL/mmol). The mixture was cooled (0 °C) and propargyl bromide (1.0 mol equiv, 80% w/w in PhMe) was added dropwise. The mixture was stirred (rt) and the progress of the reaction was monitored by tlc. Upon completion of the reaction the mixture was diluted (H₂O) and subjected to a standard workup (Et₂O); chromatography gave the propargylamine.
5-(2-Nitroprop-1-en-1-yl)-1,3-benzodioxole 92

Piperonal 32 (11.3 g, 75.0 mmol), CH₃COONH₄ (11.6 g, 150 mmol) and CH₃CH₂NO₂ (200 mL) were combined and the mixture refluxed, during which time an orange solution formed. After 4 h excess CH₃CH₂NO₂ was removed by distillation. The remaining reaction mixture was diluted (H₂O) and subjected to a standard workup (CH₂Cl₂, sat. aq NaHCO₃) to afford a residue; rsf chromatography (PhMe/petrol, 1:2) gave the nitrostyrene 92 as bright yellow crystals (9.93 g, 64%), mp 93.5-94.0 °C (CH₂Cl₂/petrol) [lit.¹⁰² mp 95 °C (EtOH)]. ¹H NMR (500 MHz): δ 8.02 (br s, 1H, CH), 6.98 (dd, J= 8.1, 1.7 Hz, 1H, H₆'), 6.95 (d, J= 1.7 Hz, 1H, H₄'), 6.89 (d, J= 8.1 Hz, 1H, H₇'), 6.05 (s, 2H, OCH₂O) 2.46 (d, J= 1.0 Hz, 3H, CH₃). The ¹H NMR spectrum was consistent with that previously reported.¹⁰³

1-(1,3-Benzodioxol-5-yl)acetone 54

Under an Ar flow a suspension of 92 (4.14 g, 20.0 mmol) in glacial CH₃COOH (100 mL) was added slowly to a stirred mixture of Fe powder (11.2 g, 200 mmol) and glacial CH₃COOH (50 mL). The reaction mixture was stirred for a further 1.5 h, after which tlc indicated complete consumption of the starting material; the mixture was filtered to remove excess Fe powder. The reaction was diluted (sat. aq NaHCO₃), subjected to a standard workup (CH₂Cl₂, sat. aq NaHCO₃) and followed by flash chromatography (EtOAc/petrol, 1:9); the first compound to elute was the ketone 54 as a colourless oil (1.25 g, 35%). The ¹H and ¹³C NMR spectrum were consistent with those reported in Chapter One. The second compound to elute was the oxime 93 as a solid (1.76 g, 45%) as a mixture
of the $E$ and $Z$ isomers, mp 89.5-92.0 °C (EtOAc/petrol) [lit.\textsuperscript{47} mp 84-87 °C (EtOH/H\textsubscript{2}O)]. \textsuperscript{1}H NMR (500 MHz): $\delta$ 6.77-6.66 (m, 6H, ArH), 5.94 (s, OCH\textsubscript{2}O, $E$ isomer), 5.93 (s, 2H, OCH\textsubscript{2}O, $Z$ isomer), 3.66 (s, 2H, CH\textsubscript{2}, $Z$ isomer), 3.42 (s, 2H, CH\textsubscript{2}, $E$ isomer), 1.83 (s, 3H, CH\textsubscript{3}, $Z$ isomer), 1.82 (s, 3H, CH\textsubscript{3}, $E$ isomer). The \textsuperscript{1}H NMR spectrum was consistent with that previously reported.\textsuperscript{104}

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$\text{1-(1,3-Benzodioxol-5-yl)-N,N-dimethylpropan-2-amine 94}$

The ketone 54 (0.36 g, 2.0 mmol) was treated with Me\textsubscript{2}NH (4.1 mL, 20 mmol, 33% v/v in EtOH), glacial CH\textsubscript{3}COOH (1.1 mL, 20 mmol) and NaCNBH\textsubscript{3} (0.13 g, 2.0 mmol) according to General Procedure A [30 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt\textsubscript{3}, 20:78:2)] to afford the amine 94 as a pale yellow oil (0.28 g, 68%). IR $\nu_{\text{max}}$ (film) 1514, 1489 cm\textsuperscript{-1}. \textsuperscript{1}H NMR (500 MHz): $\delta$ 6.72 (d, $J$ = 7.9 Hz, 1H, H7'), 6.67 (d, $J$ = 1.6 Hz, 1H, H4'), 6.61 (dd, $J$ = 7.9, 1.7 Hz, 1H, H6'), 5.92 (s, 2H, OCH\textsubscript{2}O), 2.88 (dd, $J$ = 13.3, 4.3 Hz, 1H, H2), 2.77-2.68 (m, 1H, H1), 2.33-2.28 (m, 7H, N(CH\textsubscript{3})\textsubscript{2}/H2), 0.91 (d, $J$ = 6.5 Hz, 3H, CH\textsubscript{3}). \textsuperscript{13}C NMR (125.6 MHz): $\delta$ 147.64, 145.79, 134.55 (Ar), 122.11, 109.63, 108.23 (ArH), 100.90 (OCH\textsubscript{2}O), 61.54 (C1), 40.82 (NCH\textsubscript{3}), 39.09 (C2), 13.78 (CH\textsubscript{3}). HRMS (ESI): $m/z = 208.1329$; [M + H]\textsuperscript{+} requires 208.1324.
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1-(1,3-Benzodioxol-5-yl)-N,N-dimethylpropan-2-amine hydrochloride 94.HCl

The amine 94 was treated according to General Procedure B to afford the amine hydrochloride 94.HCl as a colourless powder, mp 172.0 °C (EtOAc) [lit.47 mp 172-173 °C (i-PrOH/Et2O)].

$^1$H NMR (600 MHz); $\delta$ 6.75 (d, J = 7.9 Hz, 1H, H7'), 6.72 (d, J = 1.6 Hz, 1H, H4'), 6.67 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.95 (AB, J = 1.5 Hz, 2H, OCH2O), 3.52-3.47 (m, 1H, H1), 3.44-3.35 (m, 1H, H2), 2.76 (s, 6H, N(CH3)2), 2.51 (dd, J = 12.8, 11.0 Hz, 1H, H2), 1.26 (d, J = 6.7 Hz, 3H, CH3). Anal. calcd for C12H18ClN02: C, 62.8; H, 6.8; N, 5.2. Found: C, 63.0; H, 6.9; N, 5.2.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]prop-2-yn-l-amine 95

The ketone 54 (0.27 g, 1.5 mmol) was treated with propargylamine (1.0 mL, 15 mmol), glacial CH3COOH (0.86 mL, 15 mmol) and NaCNBH3 (0.09 g, 1.5 mmol) according to General Procedure A [24 h, rsf chromatography (EtOAc/petrol, 2:4 then EtOAc/petrol/NEt3, 30:68:2)] to afford the amine 95 as an almost colourless oil (0.23 g, 71%). IR $\nu_{max}$ (film) 3292, 1503 cm$^{-1}$. $^1$H NMR (600 MHz); $\delta$ 6.74 (d, J = 7.9 Hz, 1H, H7'), 6.70 (d, J = 1.6 Hz, 1H, H4'), 6.64 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH2O), 3.47 (dd, J = 17.2, 2.5 Hz, 1H, –CH2C≡C), 3.40 (dd, J = 17.2, 4.2 Hz, 1H, –CH2C≡C), 3.12-3.07 (m, 1H, H1), 2.61 (dd, J = 13.6, 7.3 Hz, 1H, H2), 2.57 (dd, J = 13.6, 6.3 Hz, 1H, H2), 2.18 (apparent t, J = 2.4 Hz, 1H, C≡CH), 1.53 (br s, 1H, NH), 1.05 (d, J = 6.2 Hz, 3H, CH3). $^{13}$C NMR (150.9 MHz); $\delta$ 147.81, 146.16, 132.90 (Ar), 122.36, 109.65, 108.32 (ArH), 100.97 (OCH2O), 82.14 (–C≡C), 71.40 (C≡CH), 52.72 (C1), 43.31, 35.72 (CH2), 16.24 (CH3). HRMS (EI): m/z = 217.1099; [M]$^+$ requires 217.1103.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]prop-2-yn-l-amine hydrochloride 95.HCl

The amine 95 was treated according to General Procedure B to afford the amine hydrochloride 95.HCl as colourless needles, mp 191.0-192.0 °C (i-PrOH) [lit.9 mp 189-190 °C (i-PrOH/Et2O)]. $^1$H NMR (600 MHz, $d_6$-DMSO); $\delta$ 9.43 (br s, 2H, NH2Cl), 6.97 (d, J = 7.9 Hz, 1H, H7'), 6.84 (d, J =...
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1.7 Hz, 1H, H4'), 6.69 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.99 (AB, J = 1.0 Hz, 2H, OCH2O), 3.99-3.90 (m, 2H, NCH2C=), 3.71 (apparent t, J = 2.4 Hz, 1H, C=CH), 3.44-3.36 (m, 1H, H1), 3.11 (dd, J = 13.3, 4.2 Hz, 1H, H2), 2.57 (dd, J = 13.3, 9.9 Hz, 1H, H2), 1.12 (d, J = 6.5 Hz, 3H, CH3). Anal. calcd for C13H16ClN02: C, 61.5; H, 6.4; N, 5.5. Found: C, 61.4; H, 6.2; N, 5.4.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N-methylprop-2-en-1-amine 96

The ketone 54 (0.36 g, 2.0 mmol) was treated with N-allyl-N-methylamine (1.9 mL, 20 mmol), glacial CH3COOH (1.1 mL, 20 mmol) and NaCNBH3 (0.13 g, 2.0 mmol) according to General Procedure A [36 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt3, 20:79:1)] to afford the amine 96 as a colourless oil (0.57 g, 81%). IR νmax (film) 1504, 1489 cm⁻¹. 1H NMR (500 MHz): δ 6.72 (d, J = 7.9 Hz, 1H, H7'), 6.66 (d, J = 1.7 Hz, 1H, H4'), 6.60 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.92-5.91 (m [condensed AB], 2H, OCH2O), 5.85 (ddt, J = 17.1, 10.1, 6.5 Hz, 1H, CH2-CH=CH2), 5.20 (apparent dq, J = 17.1, 1.9, 1.5 Hz, 1H, CH2–CH=CH2), 5.12 (ddt, J = 10.1, 1.9, 1.5 Hz, 1H, CH2–CH=CH2), 3.16-3.06 (m, 2H, CH2–CH=CH2), 2.94-2.84 (m, 2H, H2), 2.36-2.28 (m, 1H, H1), 2.25 (s, 3H, NCH3), 0.94-0.90 (d, J = 6.9 Hz, 3H, CH3). 13C NMR (125.8 MHz): δ 147.59, 145.75 (Ar), 136.94 (–CH=CH2), 134.62 (Ar), 122.12 (ArH), 117.04 (-C=CH2), 109.66, 108.19 (ArH), 100.88 (OCH2O), 59.66 (Cl), 57.16 (CH2–CH=CH2), 38.91 (C2), 36.75 (NCH3), 13.98 (CH3). HRMS (EI): m/z = 233.1410; [M]+ requires 233.1416.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N-methylprop-2-en-1-amine 96

![Diagram 97](image1.png)

![Diagram 98](image2.png)
Chapter Two

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]aniline 97

The ketone 54 (0.36 g, 2.0 mmol) was treated with aniline (1.8 mL, 20 mmol), glacial CH$_3$COOH (1.1 mL, 20 mmol) and NaCNBH$_3$ (0.13 g, 2.0 mmol) according to General Procedure A [48 h, flash chromatography (EtOAc/toluene/petrol, 1:25:25)] to afford the amine 97 as a yellow oil (0.49 g, 96%). IR $\nu_{\text{max}}$ (film) 3406, 1602, 1505, 1488, 1247, 1038 cm$^{-1}$. $^1$H NMR (600 MHz): $\delta$ 7.21-7.16 (m, 2H, ArH), 6.75 (d, $J = 7.9$ Hz, 1H, H$_7^\prime$), 6.71-6.67 (m, 2H, ArH), 6.65-6.59 (m, 3H, ArH), 5.94-5.93 (AB, $J = 1.0$ Hz, 2H, OCH$_2$O), 3.75-3.67 (m, 1H, H$_1$), 3.51 (br s, 1H, NH), 2.84 (dd, $J = 13.6$, 4.9 Hz, 1H, H$_2$), 2.63 (dd, $J = 13.6$, 7.2 Hz, 1H, H$_2$), 1.15 (d, $J = 6.4$ Hz, 3H, CH$_3$). $^{13}$C NMR (125.8 MHz): $\delta$ 147.71, 147.35, 146.16, 132.42 (Ar), 129.51, 122.52, 117.36, 113.51, 109.94, 108.27 (ArH), 100.97 (OCH$_2$O), 49.60 (C1), 42.15 (C2), 20.30 (CH$_3$). HRMS (ESI): 

$m/z = 256.1351$; [M + H]$^+$ requires 256.1338.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]aniline hydrochloride 97.HCl

The amine 97 was treated according to General Procedure B to afford the amine hydrochloride 97.HCl as colourless needles, mp 147.0-148.0 °C ($\beta$-PrOH). $^1$H NMR (600 MHz): $\delta$ 11.33 (br s, NH$_2$) 7.62-7.52 (m, 2H, ArH), 7.42-7.37 (m, 2H, ArH), 7.36-7.29 (m, 1H, ArH), 6.68 (d, $J = 7.9$ Hz, 1H, H$_7^\prime$), 6.58 (d, $J = 1.6$ Hz, 1H, H$_4^\prime$), 6.55(dd, $J = 7.9$, 1.7 Hz, 1H, H$_6^\prime$), 5.92-5.90 (AB, $J = 1.0$ Hz, 2H, OCH$_2$O), 3.62-3.55 (m, 1H, H$_1$), 3.41-3.33 (m, 1H, H$_2$), 2.71 (dd, $J = 13.1$, 11.1 Hz, 1H, H$_2$), 1.24 (d, $J = 6.5$ Hz, 3H, CH$_3$). Anal. calcd for C$_{18}$H$_{18}$ClNO$_2$: C, 65.9; H, 6.2; N, 4.8. Found: C, 65.6; H, 6.1; N, 4.8.

l-(1,3-Benzodioxol-5-yl)-N-benzylpropan-2-amine 98

The ketone 54 (0.23 g, 1.3 mmol) was treated with benzylamine (1.4 mL, 13 mmol), glacial CH$_3$COOH (0.74 mL, 13 mmol) and NaCNBH$_3$ (0.08 g, 1.3 mmol) according to General Procedure A [36 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt$_3$, 20:78:2)] to afford the...
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amine 98 as a pale yellow oil (0.28 g, 79%). IR ν\textsubscript{max} (film) 1504, 1488, 1247, 1040 cm\textsuperscript{-1}. \textsuperscript{1}H

NMR (600 MHz): δ 7.35-7.28 (m, 3H, ArH), 7.24-7.21 (m, 2H, ArH), 6.73 (d, J = 7.9 Hz, 1H, H\textsuperscript{7'}), 6.65 (d, J = 1.6 Hz, 1H, H\textsuperscript{4'}), 6.61 (dd, J = 7.9, 1.7 Hz, 1H, H\textsuperscript{6'}), 5.93 (AB, J = 1.5 Hz, 2H, OCH\textsubscript{2}O), 3.85 (d, J = 13.3 Hz, 1H, NCH\textsubscript{2}Ar), 3.72 (d, J = 13.3 Hz, 1H, NCH\textsubscript{2}Ar), 2.91-2.86 (m, 1H, H\textsubscript{l}), 2.67 (dd, J = 13.5, 7.2 Hz, 1H, H\textsubscript{2}), 2.57 (dd, J = 13.5, 6.3 Hz, 1H, H\textsubscript{2}), 1.60 (br s, 1H, NH), 1.09 (d, J = 6.2 Hz, 3H, CH\textsubscript{3}). \textsuperscript{13}C NMR (150.9 MHz): δ 147.73, 146.05, 140.63, 133.30 (Ar), 128.53, 128.13, 126.99, 122.31, 109.69, 108.27 (ArH), 100.94 (OCH\textsubscript{2}O), 53.91 (C1), 51.46, 43.42 (CH\textsubscript{2}), 20.27 (CH\textsubscript{3}). HRMS (ESI): m/z = 270.1492; [M + H]\textsuperscript{+} requires 270.1494.

1-((1,3-Benzodioxol-5-yl)-N-benzylpropan-2-amine hydrochloride 98.HCl

The amine 98 was treated according to General Procedure B to afford the amine hydrochloride 98.HCl as a colourless powder, mp 196.0-196.5 °C (i-PrOH) [lit.\textsuperscript{105} mp 195 °C]. \textsuperscript{1}H NMR (600 MHz): δ 9.62 (br s, 2H, NH\textsubscript{2}Cl), 7.64-7.61 (m, 2H, ArH), 7.41-7.36 (m, 2H, ArH), 7.34-7.27 (m, 1H, ArH), 6.72-6.68 (m, 1H, ArH), 6.58-6.54 (m, 2H, ArH), 5.91 (AB, J = 1.5 Hz, 2H, OCH\textsubscript{2}O), 4.08 (d, J = 13.5 Hz 1H, NCH\textsubscript{2}Ar), 4.00 (d, J = 13.5 Hz 1H, NCH\textsubscript{2}Ar), 3.37-3.30 (m, 1H, H\textsubscript{l}), 3.14-3.05 (m, 1H, H\textsubscript{2}), 2.72 (dd , J = 13.3, 10.5 Hz, 1H, H\textsubscript{2}), 1.31 (d, J = 6.5 Hz, 3H, CH\textsubscript{3}).

Anal. calcd for C\textsubscript{17}H\textsubscript{20}ClNO\textsubscript{2}: C, 66.8; H, 6.6; N, 4.6. Found: C, 66.7; H, 6.6; N, 4.5.
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2-(1,3-Benzodioxol-5-yl)-1-phenylethanamine 76

The ketone 43 (0.48 g, 2.0 mmol) was treated with CH₃COONH₄ (0.31 g, 40 mmol) and NaCNBH₃ (0.13 g, 2.0 mmol) using a modification of General Procedure B [48 h, no glacial CH₃COOH, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt₃, 20:77:3)] to give the primary amine 76 as a colourless oil (0.40 g, 82%). The IR, ¹H and ¹³C NMR spectra were consistent with those reported in Chapter One.

2-(1,3-Benzodioxol-5-yl)-N-ethyl-1-phenylethanamine 99

The ketone 43 (0.24 g, 1.0 mmol) was treated with methanolic ethylamine (5.0 mL, 10 mmol, 2.0 M), glacial CH₃COOH (0.57 mL, 10 mmol) and NaCNBH₃ (0.06 g, 1.0 mmol) according to General Procedure A [24 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt₃, 20:78:2)] to afford the amine 99 as a pale yellow solid (0.23 g, 84%), mp 52.0-53.5 °C. IR νmax (KBr disc) 3435, 1504 cm⁻¹. ¹H NMR (300 MHz): δ 7.35-7.20 (m, 5H, ArH), 6.70 (d, J = 7.9 Hz, 1H, H7'), 6.62 (d, J = 1.6 Hz, 1H, ArH), 6.57 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.92 (s, 2H, OCH₂O), 3.85-3.76 (m, 1H, H1'), 2.90-2.79 (m, 2H, CH₂), 2.53-2.35 (m, 2H, CH₂), 1.77 (br s, 1H, NH), 1.01 (t, J = 7.1 Hz, 3H, CH₃). ¹³C NMR (75.5 MHz): δ 147.69, 146.14, 143.63, 132.69 (Ar), 128.46, 127.41, 127.22, 122.41, 109.59, 108.24 (ArH), 100.90 (OCH₂O), 65.10 (C1), 44.90, 42.12 (CH₂), 15.29 (CH₃). HRMS (ESI): m/z = 270.1494; [M + H]⁺ requires 270.1494.

2-(1,3-Benzodioxol-5-yl)-N-ethyl-1-phenylethanamine hydrochloride 99·HCl

The amine 99 was treated according to General Procedure B to afford the amine hydrochloride 99·HCl as colourless blocks, mp 245.0 °C (i-PrOH). ¹H NMR (600 MHz, d₆-DMSO): δ 9.71 (br s, 1H, NH), 9.38 (br s, 1H, NH), 7.48-7.44 (m, 2H, ArH), 7.39-7.31 (m, 3H, ArH), 6.70 (d, J = 7.9 Hz, 1H, H7'), 6.62 (d, J = 1.6 Hz, 1H, H4'), 6.46 (dd, J = 8.0, 1.7 Hz, 1H, H6'), 5.92 (AB, J = 1.0 Hz, 2H, OCH₂O), 4.45-4.38 (m, 1H, H1), 3.46 (dd, J = 13.3, 4.2 Hz, 1H, H2), 2.70 (dd, J = 13.3, 10.9 Hz, 2H, OCH₂O).
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Hz, 1H, H2), 2.83-2.75 (m, 1H, NCH2), 2.65-2.58 (m, 1H, NCH2), 1.18 (t, J = 7.3 Hz, CH3). Anal. calcd for C17H20ClNO2: C, 66.8; H, 6.6; N, 4.6. Found: C, 66.8; H, 6.8; N, 4.3.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]propan-1-amine 100

The ketone 43 (0.24 g, 1.0 mmol) was treated with propylamine (0.82 mL, 10 mmol), glacial CH3COOH (0.57 mL, 10 mmol) and NaCNBH3 (0.06 g, 1.0 mmol) according to General Procedure A [24 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt3, 20:77:3)] to afford the amine 100 as a pale yellow oil (0.23 g, 83%). IR vmax (film) 3464 cm⁻¹. ¹H NMR (300 MHz): δ 7.35-7.21 (m, 5H, ArH), 6.71 (d, J = 7.9 Hz, 1H, H7'), 6.64 (d, J = 1.6 Hz, 1H, H4'), 6.58 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH20), 3.78 (dd, J = 8.1, 6.0 Hz, 1H, H1), 2.83 (ABM, J = 13.51, 8.1, 6.0 Hz, 2H, H2), 2.35 (m, 2H, NCH2), 1.61 (br s, 1H, NH), 1.37 (sext, 2H, CH2CH2CH3), 0.78 (t, J = 7.4 Hz, 3H, CH3). ¹³C NMR (75.5 MHz): δ 147.70, 146.12, 144.02, 132.83 (Ar), 128.43, 127.39, 127.13, 122.38, 109.58, 108.23 (ArH), 100.94 (OCH2O), 65.02 (Cl), 49.81, 45.10, 23.24 (CH3), 11.80 (CH3). HRMS (ESI): m/z = 284.1659; [M + H]⁺ requires 284.1651.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]propan-1-amine hydrochloride 100.HCl

The amine 100 was treated according to General Procedure B to afford the amine hydrochloride 100.HCl as colourless blocks, mp 238.0 °C (i-PrOH). ¹H NMR (600 MHz, d6-DMSO): δ 9.65 (br s, 1H, NH), 9.30 (br s, 1H, NH), 7.47-7.43 (m, 2H, ArH), 7.39-7.31 (m, 3H, ArH), 6.69 (d, J = 7.9 Hz, 79
The ketone 43 (0.48 g, 2.0 mmol) was treated with BuNH2 (2.0 mL, 20 mmol), glacial CH3COOH (1.1 mL, 20 mmol) and NaCNBH3 (0.13 g, 2.0 mmol) according to General Procedure A [24 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt3, 20:77:3)] to afford the amine 101 as a pale yellow oil (0.51 g, 86%). IR $\nu_{\text{max}}$ (film) 3413, 1489 cm$^{-1}$. $^{1}$H NMR (300 MHz): $\delta$ 7.38-7.21 (m, 5H, ArH), 6.71 (d, $J = 7.9$ Hz, 1H, H7'), 6.64 (d, $J = 16$ Hz, 1H, H4'), 6.58 (dd, $J = 7.9, 1.7$ Hz, 1H, H6'), 5.92 (s, 2H, OCH20), 3.78 (dd, $J = 8.0, 6.0$ Hz, 1H, HI), 2.83 (ABM, $J = 13.6, 8.0, 6.0$ Hz, 2H, H2), 2.45-2.31 (m, 2H, NCH2), 1.65 (br s, 1H, NH), 1.43-1.30 (m, 2H, CH2), 0.82 (t, $J = 7.2$ Hz, 3H, CH3). $^{13}$C NMR (75.5 MHz): $\delta$ 147.67, 146.10, 143.97, 132.80 (Ar), 128.41, 127.36, 127.11, 122.35, 109.56, 108.20 (ArH), 100.91 (OCH2O), 65.13 (C1), 47.63, 45.06, 32.28, 20.47 (CH2), 14.07 (CH3). HRMS (FAB): $m/z$ = 298.1807; [M + H]$^+$ requires 298.1807.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]butan-1-amine hydrochloride 101.HCl

The amine 101 was treated according to General Procedure B to afford the amine hydrochloride 101.HCl as a colourless powder, mp 229.0-230.0 °C (i-PrOH). $^{1}$H NMR (500 MHz): $\delta$ 10.60-9.81 (br s, 2H, NH$_2$Cl), 7.48-7.43 (m, 2H, ArH), 7.39-7.31 (m, 3H, ArH), 6.54 (d, $J = 8.3$ Hz, 1H, H7'), 6.39-6.35 (m, 2H, H4', H6'), 5.83 (AB, $J = 1.4$ Hz, 2H, OCH2O), 4.14-4.08 (m, 1H, H1), 3.98-3.87 (m, 1H, H2), 3.42-3.33 (m, 1H, H2), 2.72-2.64 (m, 2H, NCH2), 1.95-1.85 (m, 2H, NCH2CH3), 1.31-1.19 (m, 2H, CH2CH3), 0.78 (t, $J = 7.4$ Hz, 3H, CH3). Anal. calcd for C$_{19}$H$_{24}$ClNO$_2$: C, 68.4; H, 7.3; N, 4.2. Found: C, 68.4; H, 7.1; N, 4.2.
N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]propan-2-amine 102

The ketone 43 (0.48 g, 2.0 mmol) was treated with i-PrNH₂ (1.7 mL, 20 mmol), glacial CH₃COOH (1.1 mL, 20 mmol) and NaCNBH₃ (0.13 g, 2.0 mmol) according to General Procedure A [30 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt₃, 20:75:5)] to afford the amine 102 as an almost colourless oil (0.43 g, 77%). IR νmax (film) 3413, 1498 cm⁻¹. ¹H NMR (300 MHz): δ 7.36-7.20 (m, 5H, ArH), 6.66 (d, J = 7.9 Hz, 1H, H⁷'), 6.55 (d, J = 1.6 Hz, 1H, H⁴'), 6.49 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H, H⁶'), 5.90 (s, 2H, OCH₂O), 4.08 (br s, 1H, NH), 3.97 (apparent t, 7 = 7.1 Hz, 1H, H¹), 2.96 (dd, J = 13.4, 7.0 Hz, 1H, H²), 2.87 (dd, J = 13.5, 7.1 Hz, 1H, H²), 2.63 (sept, J = 6.3 Hz, 1H, CH(CH₃)₂), 1.01 (d, J = 6.3 Hz, 3H, CH(C₂H₅)), 0.98 (d, J = 6.3 Hz, 3H, CH(CH₃)₂). ¹³C NMR (75.5 MHz): δ 147.56, 146.07, 142.54, 132.29 (Ar), 128.50, 127.57, 127.37, 122.44, 109.64, 108.13 (ArH), 100.89 (OCH₂O), 62.09 (C₁), 46.10 (CH, CH(CH₃)₂), 43.51 (C₂), 23.69 (CH(CH₃)₂). HRMS (ESI): m/z = 284.1642; [M + H]⁺ requires 284.1651.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]propan-2-amine hydrochloride 102.HCl

The amine 102 was treated according to General Procedure B to afford the amine hydrochloride 102.HCl as colourless needles, mp 233.0-235.0 °C (i-PrOH).¹H NMR (600 MHz, d₆-DMSO): δ 9.51 (br s 1H, NH), 9.22 (br s, 1H, NH), 7.52-7.47 (m, 2H, ArH), 7.39-7.31 (m, 3H, ArH), 6.69 (d, J = 7.9 Hz, 1H, H⁷'), 6.64 (d, J = 1.6 Hz, 1H, H⁴'), 6.44 (dd, J = 7.9, 1.6 Hz, 1H, H⁶'), 5.92 (AB, J = 1.5 Hz, 2H, OCH₂O), 4.53-4.43 (m, 1H, H¹), 3.44-3.40 (m, 1H, H²), 3.12-3.03 (m, 1H, H²), 2.92-2.80 (m, 1H, CH(CH₃)₂), 1.29-1.18 (m, 6H, CH(CH₃)₂). Anal. calcd for C₁₈H₂₂ClNO₂: C, 67.6; H, 6.9; N, 4.4. Found: C, 67.5; H, 7.0; N, 4.2.
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N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]prop-2-en-1-amine 104

The ketone 43 (0.24 g, 1.0 mmol) was treated with allylamine (0.75 mL, 10 mmol), glacial CH₃COOH (0.57 mL, 10 mmol) and NaCNBH₃ (0.06 g, 1.0 mmol) according to General Procedure A [36 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt₃, 20:77:3)] to afford the amine 104 as a colourless oil (0.23 g, 83%). IR νmax (film) 3435, 1503 cm⁻¹. ¹H NMR (300 MHz): δ 7.38-7.21 (m, 5H, ArH), 6.71 (d, J = 7.9 Hz, 1H, H7'), 6.63 (d, J = 1.6 Hz, 1H, H4'), 6.58 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH₂O), 5.86-5.70 (m, 1H, C–CH=CH₂), 5.07-4.97 (m 2H, C–CH=CH₂), 3.84 (dd, J = 8.0, 6.0 Hz, 1H, H1), 3.14-3.04 (apparent tdd, J = 14.3, 5.3, 1.6 Hz, 1H, NCH₂–CH=CH₂), 2.95 (apparent tdd, J = 14.3, 6.6, 1.2 Hz, 1H, NCH₂–CH=CH₂), 2.84 (ABM, J = 13.5, 8.0, 6.0 Hz, 2H, H2), 1.57 (br s, 1H, NH).¹C NMR (75.5 MHz): δ 147.72, 146.17, 143.59 (Ar), 136.93 (C–CH=CH₂) 132.67 (Ar), 128.50, 127.47, 127.26, 122.41 (ArH), 115.80 (C–CH=CH₂), 109.61, 108.27 (ArH), 100.96 (OCH₂O), 64.08 (C1), 50.12, 45.03 (CH₂).HRMS (EI): m/z = 281.1422; [M]+ requires 281.1416.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]prop-2-en-1-amine hydrochloride 104.HCl

The amine 104 was treated according to General Procedure B to afford the amine hydrochloride 104.HCl as colourless prisms, mp 251.0-253.0 °C (i-PrOH). ¹H NMR (600 MHz, d₆-DMSO):

δ 9.96 (br s, 1H, NH), 9.62 (br s, 1H, NH), 7.46-7.42 (m, 2H, ArH), 7.40-7.32 (m, 3H, ArH), 6.70 (d, J = 7.9 Hz, 1H, H7'), 6.60 (d, J = 1.7 Hz, 1H, H4'), 6.45 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.96-5.88 (m, 3H, OCH₂O/C–CH=CH₂), 5.38-5.31 (m, 2H, C–CH=CH₂), 4.43-4.35 (m, 1H, H1), 3.53-3.46
(m, 3H, NH$_2$/H2), 3.07 (dd, $J = 13.4, 11.0$ Hz, 1H, H2). Anal. calcd for C$_{18}$H$_{20}$ClNO$_2$: C, 68.0; H, 6.3; N, 4.4. Found: C, 68.1; H, 6.3; N, 4.3.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]prop-2-yn-1-amine 105

The ketone 43 (0.24 g, 1.0 mmol) was treated with propargylamine (0.69 mL, 10 mmol), glacial CH$_3$COOH (0.57 mL, 10 mmol) and NaCNBH$_3$ (0.06 g, 1.0 mmol) according to General Procedure A [24 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt$_3$, 20:79:1)] to afford the amine 105 as a colourless oil (0.23 g, 84%). IR $\nu_{\text{max}}$ (film) 3292, 1502 cm$^{-1}$. $^1$H NMR (500 MHz): $\delta$ 7.36-7.24 (m, 5H, ArH), 6.72 (d, $J = 7.9$ Hz, 1H, H$^7$), 6.68 (d, $J = 1.6$ Hz, 1H, H$^4$), 6.62 (dd, $J = 7.9, 1.7$ Hz, 1H, H$^6$), 5.93 (s, 2H, OCH$_2$O), 4.07 (dd, $J = 8.9, 5.4$ Hz, 1H, H1), 3.32 (dd, $J = 17.2, 2.4$ Hz, 1H, CH$_2$C=CH), 3.05 (dd, $J = 17.2, 2.4$ Hz, 1H, CH$_2$C=CH), 2.90 (dd, $J = 13.7, 5.4$ Hz, 1H, H2), 2.80 (dd, $J = 13.7, 8.9$ Hz, 1H, H2), 2.17 (t, $J = 2.4$ Hz, 1H, CH$_2$C=CH), 1.64 (br s, 1H, NH). $^{13}$C NMR (125.8 MHz): $\delta$ 147.84, 146.31, 142.53, 132.25 (Ar), 128.60, 127.67, 122.42, 109.55, 108.34 (ArH), 100.99 (OCH$_3$O), 82.12 (CH$_2$C=CH), 71.48, 62.66 (C1, CH$_2$C=CH), 44.71, 35.90 (C2, CH$_2$C=CH). HRMS (EI): $m/z = 279.1265$; [M]$^+$ requires 279.1259.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]prop-2-yn-1-amine hydrochloride 105.HCl

The amine 105 was treated according to General Procedure B to afford the amine hydrochloride 105.HCl as colourless needles, mp 225.0-226.0 °C (i-PrOH). $^1$H NMR (500 MHz): $\delta$ 7.46-7.30 (m, 5H, ArH), 6.73-6.66 (m, 1H, ArH), 6.60-6.55 (m, 1H, ArH), 6.47-6.40 (m, 1H, ArH), 5.92 (s, 2H, OCH$_3$O), 4.43-4.35 (br m, 1H, H1), 3.66-3.60 (br m, 2H), 3.35-3.30 (m, 1H), 3.07-3.00 (m, 1H), 2.17 (br t, $J = 2.5$ Hz, 1H, CH$_2$C=CH). Anal. calced for C$_{18}$H$_{18}$ClNO$_2$: C, 68.5; H, 5.8; N, 4.4. Found: C, 68.4; H, 5.8; N, 4.4.
2-(1,3-Benzodioxol-5-yl)-N-benzyl-1-phenylethanamine 106

The ketone 43 (0.72 g, 3.0 mmol) was treated with benzylamine (3.3 mL, 30 mmol), glacial CH₃COOH (1.7 mL, 30 mmol) and NaCNBH₃ (0.19 g, 3.0 mmol) according to General Procedure A [36 h, flash chromatography (EtOAc/petrol, 1:9)] to afford the amine 106 as a pale yellow solid (0.69 g, 69%). IR vₚₓₜ (film, CH₂Cľ₂) 3415, 1489 cm⁻¹. ¹H NMR (300 MHz): δ 7.40-7.13 (m, 10H, ArH), 6.71 (d, J = 7.9 Hz, 1H, H⁷'), 6.61 (d, J = 1.6 Hz, 1H, H⁴'), 6.57 (dd, J = 7.9, 1.6 Hz, 1H, H⁶'), 5.93 (AB, J = 1.4 Hz, 2H, OCH₂O), 3.84 (dd, J = 8.4, 5.7 Hz 1H, H₁), 3.68 (d, J = 13.5 Hz 1H, NCH₂), 3.48 (d, J = 13.5 Hz 1H, NCH₂), 2.93-2.79 (m, 2H, H₂), 1.83 (br s, 1H, NH). ¹³C NMR (75.5 MHz): δ 147.68, 146.15, 143.64, 140.49, 132.60 (Ar), 128.53, 128.40, 128.09, 127.51, 127.28, 126.91, 122.36, 109.60, 108.23 (ArH), 100.93 (OCH₂O), 63.87 (C₁), 51.48, 45.07 (CH₂). HRMS (FAB): m/z = 332.1644; [M + H]⁺ requires 332.1651.

2-(1,3-Benzodioxol-5-yl)-N-benzyl-1-phenylethanamine hydrochloride 106.HCl

The amine 106 was treated according to General Procedure B to afford the amine hydrochloride 106.HCl as colourless, trapezoid blocks, mp 228.0 °C (i-PrOH/CHCl₃). ¹H NMR (600 MHz, d₆-DMSO): δ 10.24 (br s, 1H, NH), 9.79 (br s, 1H, NH), 7.51-7.34 (m, 10H, ArH), 6.69 (d, J = 7.9 Hz, 1H, H⁷'), 6.55 (d, J = 1.6 Hz, 1H, H⁴'), 6.42 (dd, J = 8.0, 1.5 Hz, 1H, H⁶'), 5.92 (AB, J = 1.1 Hz, 2H, OCH₂O), 4.48-4.36 (m, 1H), 4.13-4.00 (m, 1H), 3.80-3.70 (m, 1H), 3.66-3.55 (m, 1H, H₂) 3.15-3.05 (m, 1H, H₂). Anal. calcd for C₂₂H₂₂ClNO₂: C, 71.8; H, 6.0; N, 3.8. Found: C, 71.8; H, 6.0; N, 3.8.
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N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]aniline 107

The ketone 43 (0.24 g, 1.0 mmol) was treated with aniline (0.91 mL, 10 mmol), glacial CH₃COOH (0.57 mL, 10 mmol) and NaCNBH₃ (0.06 g, 1.0 mmol) according to General Procedure A [36 h, flash chromatography (EtOAc/petrol, 1:9)] to afford the amine 107 as a pale yellow oil (0.19 g, 61%). IR νmax (film) 3445, 1498 cm⁻¹. ¹H NMR (300 MHz): δ 7.37-7.22 (m, 5H, ArH), 7.11-7.03 (m, 2H, ArH), 6.73 (d, J = 7.7 Hz, 1H, H7'), 6.68-6.57 (m, 3H, ArH), 6.50-6.45 (m, 2H, ArH), 5.94 (AB, J = 1.4 Hz, 2H, OCH₂O), 4.56-4.48 (m, 1H, H1), 4.13 (br s, 1H, NH), 3.07 (dd, J = 14.0, 5.6 Hz, 1H, H2), 2.92 (dd, J = 14.0, 8.3 Hz, 1H, H2). ¹³C NMR (75.5 MHz): δ 147.90, 147.38, 146.50, 143.48, 131.52 (Ar), 129.15, 128.73, 127.22, 126.54, 122.36, 117.62, 113.76, 109.53, 108.34 (ArH), 101.06 (OCH₂O), 59.45 (C1), 45.03 (C2). HRMS (FAB): m/z = 318.1490; [M + H]+ requires 318.1494.

N-[2-(1,3-Benzodioxol-5-yl)-1-phenylethyl]aniline hydrochloride 107.HCl

The amine 107 was treated according to General Procedure B to afford the amine hydrochloride 107.HCl as fine, colourless needles, mp 164.5-165.0 °C (i-PrOH). ¹H NMR (600 MHz): δ 10.90 (br s, 2H, NH), 10.65 (br s, 1H, NH), 7.34-7.28 (m, 4H, ArH), 7.23-7.10 (m, 6H, ArH), 6.55 (br d, J = 8.1 Hz, 1H, H7'), 6.44-6.38 (m, 2H, H4'/H6'), 5.92 (AB, J = 1.2 Hz, 2H, OCH₂O), 4.46-4.40 (m, 1H), 3.90-3.54 (br m, 1H, H2), 3.44-3.30 (m, 1H, H2). Anal. calcd for C₂₁H₂₀ClNO₂: C, 71.3; H, 5.7; N, 4.0. Found: C, 71.2; H, 5.5; N, 4.0.

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2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-ethylethanamine 108

The ketone 55 (0.41 g, 2.0 mmol) was treated with methanolic EtNH\(_2\) (10 mL, 20 mmol, 2.0 M), glacial CH\(_3\)COOH (1.1 mL, 20 mmol) and NaCNBH\(_3\) (0.13 g, 2.0 mmol) according to General Procedure A [30 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NE\(_2\)t, 20:78:2)] to afford the amine 108 as a pale yellow oil (0.47 g, quant.). IR \(v_{\text{max}}\) (film) 2968, 1505 cm\(^{-1}\). \(^1\)H NMR (600 MHz): \(\delta\) 6.73 (d, \(J = 7.9\) Hz, 1H, H\(_7\)'), 6.70 (d, \(J = 1.6\) Hz, 1H, H\(_4\)'), 6.66 (dd, \(J = 7.9, 1.7\) Hz, 1H, H\(_6\)'), 5.93 (AB, \(J = 1.1\) Hz, 2H, OCH\(_2\)O), 2.88 (dq, \(J = 11.4, 7.2\) Hz, 1H, CH\(_2\)CH\(_3\)), 2.83 (dd, \(J = 13.7, 5.3\) Hz, 1H, H2), 2.73 (dd, \(J = 13.7, 7.7\) Hz, 1H, H2), 2.61 (dq, \(J = 11.4, 7.1\) Hz, 1H, CH\(_2\)CH\(_3\)), 2.24 (br s, 1H, NH), 1.94 (ddd, \(J = 9.2, 7.6, 5.3\) Hz, 1H, H1), 1.07 (t, \(J = 7.2\) Hz, 3H, CH\(_3\)) 0.76-0.69 (m, 1H, c-PrH), 0.58-0.54 (m, 1H, c-PrH), 0.46-0.40 (m, 1H, c-PrH), 0.27-0.21 (m, 1H, c-PrH), 0.01-0.04 (m, 1H, c-PrH). \(^{13}\)C NMR (150.9 MHz): \(\delta\) 147.72, 146.10, 133.00 (Ar), 122.46, 109.79, 108.28 (ArH), 100.95 (OCH\(_2\)O), 65.05 (C1), 41.88, 41.50 (CH\(_2\)), 15.72 (c-PrCH), 15.20 (CH\(_3\)), 5.11, 2.37 (c-PrCH\(_2\)). HRMS (ESI): \(m/z\) 234.1493; [M + H]\(^+\) requires 234.1494.

N-[2-(1,3-Benzodioxol-5-yl)-1-cyclopropylethyl]prop-2-yn-1-amine 109

The ketone 55 (0.41 g, 2.0 mmol) was treated with propargylamine (1.4 mL, 20 mmol), glacial CH\(_3\)COOH (1.1 mL, 20 mmol) and NaCNBH\(_3\) (0.13 g, 2.0 mmol) according to General Procedure A [40 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NE\(_2\)t, 20:78:2)] to afford the amine 109 as a colourless oil (0.44 g, 90%). IR \(v_{\text{max}}\) (film) 3296, 1505 cm\(^{-1}\). \(^1\)H NMR (600 MHz): \(\delta\) 6.73 (d, \(J = 7.9\) Hz, 1H, H\(_7\)'), 6.70 (d, \(J = 1.6\) Hz, 1H, H\(_4\)'), 6.66 (dd, \(J = 7.9, 1.7\) Hz, 1H, H\(_6\)'), 6.48 (t, \(J = 10.3\) Hz, 1H, H\(_2\)'), 5.92 (AB, \(J = 1.2\) Hz, 2H, OCH\(_2\)O), 2.86 (dq, \(J = 11.4, 7.3\) Hz, 1H, CH\(_2\)CH\(_3\)), 2.81 (dd, \(J = 13.7, 5.3\) Hz, 1H, H2), 2.72 (dd, \(J = 13.7, 7.7\) Hz, 1H, H2), 2.60 (dq, \(J = 11.4, 7.1\) Hz, 1H, CH\(_2\)CH\(_3\)), 2.24 (br s, 1H, NH), 1.93 (ddd, \(J = 9.2, 7.6, 5.3\) Hz, 1H, H1), 1.04 (t, \(J = 7.2\) Hz, 3H, CH\(_3\)) 0.76-0.69 (m, 1H, c-PrH), 0.57-0.56 (m, 1H, c-PrH), 0.44-0.41 (m, 1H, c-PrH), 0.28-0.21 (m, 1H, c-PrH), 0.01-0.03 (m, 1H, c-PrH). \(^{13}\)C NMR (150.9 MHz): \(\delta\) 147.72, 146.10, 133.00 (Ar), 121.59, 109.79, 108.28 (ArH), 100.95 (OCH\(_2\)O), 65.05 (C1), 41.88, 41.50 (CH\(_2\)), 15.72 (c-PrCH), 15.20 (CH\(_3\)), 5.11, 2.39 (c-PrCH\(_2\)). HRMS (ESI): \(m/z\) 234.1493; [M + H]\(^+\) requires 234.1494.
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5.93 (AB, J = 1.6 Hz, 2H, OCH₂O), 3.58 (dd, J = 17.2, 2.4 Hz, 1H, CH₂C≡CH), 3.50 (dd, J = 17.2, 2.4 Hz, 1H, CH₂C≡CH), 2.86 (dd, J = 13.8, 4.8 Hz, 1H, H2), 2.66 (dd, J = 13.8, 8.2 Hz, 1H, H2) 2.19-2.13 (m, 2H, H1/C≡CH), 1.58 (br s, 1H, NH), 0.68-0.60 (m, 1H, c-PrH), 0.59-0.54 (m, 1H, c-PrH), 0.46-0.42 (m, 1H, c-PrH), 0.38-0.33 (m, 1H, c-PrH), 0.05-0.00 (m, 1H, c-PrH). ¹³C NMR (150.9 MHz): δ 147.76, 146.14, 132.83 (Ar), 122.43, 109.73, 108.30 (ArH), 100.95 (OCH₂O), 82.46 (−C≡CH), 71.42 (−C≡CH), 62.30 (C1), 41.79, 35.93 (CH₂), 15.40 (c-Pr), 4.86 (c-Pr) 1.72 (c-Pr). HRMS (ESI): m/z = 244.1331; [M + H]⁺ requires 244.1338.

N-2-(1,3-Benzodioxol-5-yl)-1-cyclopropylethyl]prop-2-yn-1-amine hydrochloride 109.HCl

The amine 109 was treated according to General Procedure B to afford the amine hydrochloride 109.HCl as colourless plates, mp 155.5-157.0 °C (i-PrOH). ¹H NMR (600 MHz): δ 9.98 (br s, NH₂), 6.79-6.73 (m, 3H, ArH), 5.95 (AB, J = 1.5 Hz, 1H, OCH₂O), 5.94 (AB, J = 1.5 Hz, 1H, OCH₂O), 4.07 (dd, J = 16.8, 2.5 Hz, 1H, CH₂C≡CH), 3.98 (dd, J = 16.7, 2.5 Hz, 1H, CH₂C≡CH), 3.40 (dd, J = 13.6, 5.0 Hz, 1H, H2), 3.12 (dd, J = 13.6, 9.1 Hz, 1H, H2), 2.81-2.74 (m, 1H, H1), 2.55 (t, J = 2.5 Hz, 1H, CH₂C≡CH), 1.17-1.10 (m, 1H, c-PrH), 0.72-0.66 (m, 1H, c-PrH), 0.58-0.47 (m, 2H, c-PrH), −0.12−0.18 (m, 1H, c-PrH). Anal. calcd for C₁₅H₁₈ClN₂O₂: C, 64.4; H, 6.5; N, 5.0. Found: C, 64.4; H, 6.6; N, 5.1.

N-[2-(1,3-Benzodioxol-5-yl)-1-cyclopropylethyl]propan-2-amine 110

The ketone 55 (0.41 g, 2.0 mmol) was treated with methanolic i-PrNH₂ (1.7 mL, 20 mmol), glacial CH₃COOH (1.1 mL, 20 mmol) and NaCNBH₃ (0.13 g, 2.0 mmol) according to General Procedure A [30 h, rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt₃, 20:78:2)] to afford the amine 110 as a pale yellow oil (0.28 g, 57%). IR νmax (film) 2962, 1504 cm⁻¹. ¹H NMR (300 MHz): δ 6.73 (d, J = 7.9 Hz, 1H, H7'), 6.68 (d, J = 1.8 Hz, 1H, H4'), 6.64 (dd, J = 7.9 Hz, J = 1.8 Hz, 1H, H6'), 5.93 (s, 2H, OCH₂O), 3.01 (sept, J= 6.3 Hz, 1H, CH(CH₃)₂), 2.74 (ABM, J = 13.6 Hz, 6.9, 5.7
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Hz, 1H, 2H), 2.08-1.98 (m, 1H, H1), 1.64 (br s, 1H, NH), 1.03 (d, \(J = 6.3\) Hz, 6H, CH(CH₃)₂), 0.73-0.61 (m, 1H, c-PrH), 0.59-0.38 (m, 2H, c-PrH), 0.24-0.15 (m, 1H, c-PrH), 0.05-0.06 (m, 1H, c-PrH). \(^{13}\)C NMR (75.5 MHz): \(\delta 147.90, 145.80, 133.46\) (Ar), 123.02, 109.84, 108.77 (ArH), 101.23 (OCH₂O), 65.25 (C1), 45.20 (CH(CH₃)₂), 41.63 (C2), 15.80 (c-PrCH), 21.22 (CH(CH₃)₂), 4.98, 1.87 (c-PrCH₂). HRMS (ESI): \(m/z = 248.1655; [M + H]^+\) requires 248.1651

N-[2-(1,3-Benzodioxol-5-yl)-1-cyclopropylethyl]propan-2-amine hydrochloride 110.HCl

The amine 110 was treated according to General Procedure B to afford the amine hydrochloride 110.HCl as colourless blocks, mp 166.0-166.5 °C (i-PrOH). \(^1\)H NMR (600 MHz): \(\delta 9.43\) (br s, 2H, NH₂Cl), 6.76-6.70 (m, 3H, ArH), 5.93 (AB, \(J = 1.5\) Hz, 2H, OCH₂O), 3.67-3.60 (m, 1H), 3.57-3.51 (m, 1H), 3.19 (dd, \(J = 13.5\) Hz, \(J = 9.7\) Hz, 1H, H2), 2.53-2.47 (m, 1H), 1.55 (d, \(J = 6.5\) Hz, 3H, CH₃), 1.45 (d, \(J = 6.5\) Hz, 3H, CH₃), 1.26-1.18 (m, 1H, c-PrH), 0.70-0.63 (m, 1H, c-PrH), 0.48-0.42 (m, 1H, c-PrH), 0.37-0.31 (m, 1H, c-PrH), –0.20–0.27 (m, 1H, c-PrH). Anal. calcd for C₁₅H₂₂ClN₂O₂: C, 63.5; H, 7.8; N, 4.9. Found: C, 63.2; H, 8.0; N, 4.7.

![Diagram](image)

1-(1,3-Benzodioxol-5-yl)propan-2-amine 14

The nitrostyrene 92 (3.1 g, 15 mmol) in dry THF (50 mL) was added dropwise under Ar to a cooled (0 °C) suspension of LiAlH₄ (1.7 g, 45 mmol) in dry THF (50 mL); the reaction mixture was warmed to rt and refluxed overnight. The following morning the reaction mixture was cooled
(0 °C), quenched cautiously (H2O) and basified (aq NaOH 1M). The reaction mixture was passed through a Celite plug, washing the residue with Et2O (2 x); the filtrate/washings were subjected to a standard workup (Et2O) and purified by rsf chromatography (EtOAc/petrol, 1:4 then EtOAc/petrol/NEt3, 20:75:5) to afford the amine 14 as a pale yellow oil (2.4 g, 90%). ¹H NMR (600 MHz): δ 6.74 (d, J = 7.9 Hz, 1H, H7'), 6.67 (d, J = 1.7 Hz, 1H, H4'), 6.63 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H, H6'), 5.93 (s, 2H, OCH2O), 3.10 (ddq, J = 8.1, 6.3, 5.3 Hz, 1H, H1), 2.62 (dd, J = 13.4, 5.3 Hz, 1H, H2), 2.42 (dd , J = 13.4, 8.1 Hz, 1H, H2), 1.49 (br s, 2H, NH2), 1.10 (d, J = 6.3 Hz, 3H, CH3). ¹³C NMR (150.9 MHz): δ 147.74, 146.06, 133.58, 122.22, 109.63, 108.29 (ArH), 100.93 (OCH2O), 48.70 (C1), 46.41 (C2), 23.56 (CH3). HRMS (EI): m/z = 179.0950; [M⁺] requires 179.0946. The ¹H and ¹³C NMR spectra were consistent with those previously reported. 93

l-(1,3-Benzodioxol-5-yl)propan-2-amine hydrochloride 14.HCl

The amine 14 was treated according to General Procedure B to afford the amine hydrochloride 14.HCl as colourless plates, mp 191.0-192.0 °C (i-PrOH/Et2O) [lit. ⁹ mp 187-188 °C (i-PrOH/Et2O)]. ¹H NMR (600 MHz): δ 7.96 (br s, 3H, NH3Cl), 6.86 (d, J = 8.1Hz, 1H, H7'), 6.84 (d, J = 1.7 Hz, 1H, H4'), 6.69 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H, H6'), 5.99 (s, 2H, OCH2O), 3.34 (br m, 1H, H1), 2.89 (dd, J = 13.4, 5.7 Hz, 1H, H2), 2.60 (dd , J = 13.4, 8.5 Hz, 1H, H2),1.11 (d, J = 6.5 Hz, 3H, CH3). Anal. calcd for C10H14ClNO2: C, 55.7; H, 6.5; N, 6.5. Found: C, 55.9; H, 6.6; N, 6.3. The ¹H NMR spectrum was consistent with that previously reported. ⁹³

N-[2-(1,3-Benzodioxol-5-yl)-l-methylethyl]-N-methylprop-2-yn-1-amine 111

The amine MDMA (0.19 g, 1.0 mmol) was treated with propargyl bromide (0.11 mL, 1.0 mmol) and K₂CO₃ (0.21 g, 1.5 mmol) according to General Procedure C [2 h, flash chromatography (EtOAc/petrol 1:4)] to afford the propargylamine 111 as a colourless oil (0.18 g, 76%). IR vₘₐₓ (film) 3294, 1504 cm⁻¹. ¹H NMR (600 MHz): δ 6.72 (d, J = 7.9 Hz, 1H, H7'), 6.68 (d,
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\[ J = 1.6 \text{ Hz, 1H, H4'), 6.62 (dd, } J = 7.9, 1.6 \text{ Hz, 1H, H6'), 5.92 (AB, } J = 1.5 \text{ Hz, 2H, OCH}_2\text{O),} \]

3.44-3.37 (ABX, 2H, \( J = 17.0, 2.4 \text{ Hz, } CH_2C\equiv CH\)), 2.97-2.89 (m, 2H, H1/ H2), 2.40 (s, 3H, NCH3), 2.35-2.27 (m, 1H, H2), 2.24 (t, \( J = 2.4 \text{ Hz, 1H, C=CH}\)), 0.96 (d, \( J = 7.0 \text{ Hz, 3H, CH}_3\)). 13C NMR (150.9 MHz): 147.63, 145.86, 134.16 (Ar), 122.23, 109.72, 108.22 (ArH), 100.91 (OCH2O), 80.50 (C=CH), 72.69 (C=CH), 59.58 (C1), 43.31, 39.68 (CH2), 37.60 (NCH3), 15.16 (CH3). HRMS (EI): m/z = 231.1249; [M]+ requires 231.1259.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N-methylprop-2-yn-1-amine hydrochloride 111.HCl

The propargylamine 111 was treated according to General Procedure B to afford the propargylamine hydrochloride 111.HCl as colourless rods, mp 134.5-135.0 °C (i-PrOH). 1H NMR (600 MHz): 6 6.76 (d, \( J = 7.9 \text{ Hz, 1H, H7'}\)), 6.70 (d, \( J = 1.7 \text{ Hz, 1H, H4'}\)), 6.67 (dd, \( J = 7.9, 1.7 \text{ Hz, 1H, H6'}\)), 5.95 (AB, \( J = 1.2 \text{ Hz, 2H, OCH}_2\text{O}\)), 4.13-3.90 (br m, 2H, \( CH_2C\equiv CH\)), 3.57-3.54 (br m, 1H, H1), 3.47-3.39 (br m, 1H, H2), 2.87 (s, 3H, NCH3), 2.70 (dd, \( J = 13.0, 11.1, 1 \text{H, H2}\)), 2.68 (t, \( J = 2.7 \text{ Hz, 1H, C=CH}\)), 1.35 (d, \( J = 6.6 \text{ Hz, 3H, CH}_3\)). Anal. calcd for C14H18ClN02: C, 62.8; H, 6.8; N, 5.2. Found: C, 62.6; H, 6.9; N, 5.0.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N-prop-2-yn-1-ylprop-2-yn-1-amine 112

(a) The amine 14 (0.18 g, 1.0 mmol) was treated according to a modification of General Procedure C, using instead propargyl bromide (0.22 mL, 2.0 mmol) and K2CO3 (0.35 g, 2.5 mmol) [12 h, flash chromatography (EtOAc/petrol, 1:9)], to afford the dipropargylamine 112 as a colourless oil (0.20 g, 77%). IR v_max (film) 3292, 1503 cm\(^{-1}\). 1H NMR (600 MHz): 6 6.72 (d, \( J = 7.9 \text{ Hz, 1H, H7'}\)), 6.69 (d, \( J = 1.7 \text{ Hz, 1H, H4'}\)), 6.62 (dd, \( J = 7.9, 1.7 \text{ Hz, 1H, H6'}\)), 5.92 (AB, \( J = 1.5 \text{ Hz, 2H, OCH}_2\text{O}\)), 3.59 (d, \( J = 2.4 \text{ Hz, 4H, N(CH}_2\text{)}_2\)), 3.07 (ddq, \( J = 9.6, 6.5, 4.2 \text{ Hz, 1H, H1}\)), 3.00 (dd, \( J = 13.3, 4.2 \text{ Hz, 1H, H2}\)), 2.37 (dd, \( J = 13.3, 9.6 \text{ Hz, 1H, H2}\)), 2.24 (t, \( J = 2.4 \text{ Hz, 2H, C=CH}\)), 1.03 (d, \( J = 6.5 \text{ Hz, 3H, CH}_3\)). 13C NMR (150.9 MHz): 147.65, 145.93, 133.84 (Ar), 122.29, 109.73,
108.23 (ArH), 100.93 (OCH₂O), 80.38 (−C=CH), 72.89 (−C=CH), 58.59 (Cl), 40.20, 39.33 (CH₂), 16.24 (CH₃). HRMS (EI): m/z = 255.1255; [M]⁺ requires 255.1259.

(b) The amine hydrochloride 14.HCl (0.22 g, 1.0 mmol) was treated according to a modification of General Procedure C, using instead propargyl bromide (0.22 mL, 2.0 mmol) and K₂CO₃ (0.35 g, 2.5 mmol) [12 h, flash chromatography (EtOAc/petrol 1:4)], to afford the dipropargylamine 112 as a colourless oil (0.22 g, 87%). The ¹H and ¹³C NMR spectra were consistent with those reported above.

N-[2-(1,3-Benzodioxol-5-yl)-1-methylethyl]−prop-2-yn-1-ylprop-2-yn-1-amine hydrochloride 112.HCl

The dipropargylamine 112 was treated according to General Procedure B to afford the dipropargylamine hydrochloride 112.HCl as chunky, colourless crystals, mp 158.0-159.0 °C (i-PrOH). ¹H NMR (600 MHz): δ 6.75 (d, J = 7.9 Hz, 1H, H₇'), 6.71 (d, J = 1.7 Hz, 1H, H₄'), 6.68 (dd, J = 7.9, 1.7 Hz, 1H, H₆'), 5.95 (AB, J = 1.0 Hz, 2H, OCH₂O), 4.16-4.00 (br m, 4H, N(CH₂)₂), 3.75-3.63 (br m, 1H, H₁), 3.46-3.38 (br m, 1H, H₂), 2.83-2.74 (br m, 1H, H₂), 2.67 (br s, 2H, C=CH), 1.38 (d, J = 6.5 Hz, 3H, CH₃). Anal. calcd for C₁₆H₁₆ClNO₂: C, 65.9; H, 6.2; N, 4.8. Found: C, 65.8; H, 6.4; N, 4.7
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The Synthesis of Some Optically-Active Analogues of MDMA
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Introduction

MDMA is a chiral molecule. (R)-MDMA and (S)-MDMA exhibit direct receptor interactions and are known substrates for the monoamine transporters, SERT, DAT and NET (Table 3.1). The non-exocytotic release of the neurotransmitters serotonin 4, dopamine 5 and noradrenaline 6 is stereoselective. Of the two enantiomers the (S)-enantiomer is the more potent neurotransmitter releaser; it is the eutomer. 

![Chemical structures of MDMA, (R)-MDMA, and (S)-MDMA]

Table 3.1 Profile of MDMA and its enantiomers as monoamine transporter substrates.

<table>
<thead>
<tr>
<th>Neurotransmitter release</th>
<th>MDMA</th>
<th>(R)-MDMA</th>
<th>(S)-MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin (5-HT)</td>
<td>72 ± 5.6</td>
<td>340 ± 20</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>Noradrenaline (NE)</td>
<td>110 ± 10</td>
<td>560 ± 40</td>
<td>136 ± 9</td>
</tr>
<tr>
<td>Dopamine (DA)</td>
<td>278 ± 7</td>
<td>3700 ± 100</td>
<td>142 ± 4</td>
</tr>
</tbody>
</table>

*Values are extracted from the work of Setola et al.*

The first study of the psychopharmacology of MDMA and its enantiomers was reported by Anderson and coworkers. The potency of CNS activity was measured using evoked rectal hypothermia in rabbits and a subjective scaling measure in human subjects. The observed psychomimetic effect was distinctly stereoselective, with the (S)-enantiomer being the more potent. This outcome was unexpected; structurally analogous psychomimetics, such as amphetamine 3, typically possess the opposite absolute stereoselectivity.
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In accordance with the widely accepted role of serotonin in psychomimetic activity, Nichols and coworkers\textsuperscript{14} demonstrated that MDMA and its enantiomers released \([^{3}H]5-HT\) from rat brain synaptosomes. As predicted from earlier \textit{in vivo} psychopharmacology studies the (S)-enantiomer was the more potent releaser of \([^{3}H]5-HT\).

In light of the fact that the enantiomers of MDMA, and of other amphetamines, possess vastly different pharmacological and pharmacokinetic profiles\textsuperscript{2,12}, an investigation of therapeutic activities of these compounds was to necessarily include the preparation and evaluation of the enantiomers.\textsuperscript{*} The anti-parkinsonian activity of \((R)\)-MDMA and \((S)\)-MDMA has been examined in a haloperidol-induced Parkinsonism rat model.\textsuperscript{27} This study concluded that a strong synergism belies the mechanism of the anti-parkinsonian activity of racemic MDMA. Further investigations into the anti-parkinsonian actions of the enantiomers of MDMA, using rat rotational behaviour and the rat catalepsy test, demonstrated that the (S)-enantiomer was the eutomer and that \((R)\)-MDMA increased the actions of \((S)\)-MDMA.\textsuperscript{29} Unlike the anti-parkinsonian activity, the anti-LID activity of the enantiomers of MDMA has not been previously reported. An investigation of this nature was anticipated to provide further insight into the mechanism of anti-LID activity demonstrated by racemic MDMA.

The cytotoxicity of MDMA in a model L3055 Burkitt's lymphoma cell line (IC\textsubscript{50} 100-200 \(\mu\)M at 24 h) reported by Gordon \textit{et al}.\textsuperscript{20} has similarly not been studied for either enantiomer. The hypothesis that the major mechanism of cytotoxicity is via SERT interaction could be further corroborated by the study of \((R)\)-MDMA and \((S)\)-MDMA, given the distinctly different affinities they exhibit for SERT (Table 3.1).

The analogue 18, having been identified as a candidate for further development and investigation as a therapeutic for PD, was also earmarked for enantiomer studies. The enantiomers \((R)\)-18 and

\textsuperscript{*} Additionally, the decision to prepare enantiomers was guided by the knowledge that regulatory bodies dictate that individual enantiomers of drug candidates must be evaluated for the principal pharmacologic action.
(S)-18 were synthesised for both the purpose of *in vitro* pharmacology studies and *in vivo* characterisation.

This chapter details the preparation of the enantiomers of MDMA and the enantiomers of the analogue 18 for three distinct purposes: a) evaluation in MPTP-lesioned primate models for PD and *in vitro* pharmacological characterisation; b) assessment as cytotoxins in a model L3055 BL cell line assay; and c) *in vitro* quantification as inhibitors of monoamine oxidases. For each purpose, it was envisaged that investigation with enantiomers had the potential to deconvolute the pharmacological origins and hence mechanism of the observed therapeutic activity that belies both the enantiomers and the racemic counterpart.

Furthermore the enantiopure N-propargyl MDMA analogues *(R)*-111 and *(S)*-111 were also conceived. These compounds completed a series of putative MAO inhibitors possessing an N-propargyl moiety, as is present in the selective MAO-B inhibitor selegiline 10.†

† For further discussion of this topic see Chapter Four: MDMA Analogues as Monoamine Oxidase Inhibitors.
Previous Routes to the Enantiomers of MDMA and Enantiopure MDMA derivatives

A survey of the literature revealed a plethora of racemate resolution methods, both instrumental \(^{107,108}\) and classical \(^{109}\) to afford chiral amphetamines. The patented preparation of selegiline exemplifies a classical amphetamine resolution, whereby formation of the L-tartrate salt \(^{114}\) of methamphetamine 2 followed by fractional crystallisation and propargylation renders the desired enantiopure compound 10 (Scheme 3.1).\(^ {110}\)

Scheme 3.1 A commercial preparation of the chiral drug selegiline 10. a) i) \(\text{H}_3\text{O}^+, \text{H}_2\text{O}\); ii) resolution by fractional crystallisation; b) \(\text{NaOH}, \text{H}_2\text{O}\); c) \(\text{HC}≡\text{CCH}_2\text{Br}, \text{NaOH}\).

One synthetic pathway to chiral \(N\)-methylamphetamines utilises commercially available (R)- or (S)-\(\alpha\)-methylbenzylamine as a chiral auxiliary. This method was pioneered by Weinges and Graab\(^ {111}\) and later improved upon by Nichols and coworkers.\(^ {112}\) The method is high yielding and relatively simple, consequently it has featured repeatedly in the literature.\(^ {8,14,43,113}\) The only identified limitation has been the laborious nature of preparing some ketone substrates.\(^ {114,115}\)

An illustrative example of this methodology, reported by Nichols and coworkers,\(^ {43}\) showing the synthesis of (S)-MDMA from the ketone 54, is presented (Scheme 3. 2). Condensation of the chiral
auxiliary, \((S)-\alpha\)-methylbenzylamine \(115\) with the ketone \(54\), followed by Raney-nickel reduction and acidification, afforded a diastereomeric mixture of the secondary \(N\)-\(\alpha\)-methylbenzylamines as the hydrochlorides \(116\). Separation gave a single diastereomer \((S)-116\) in high purity and in a typical high yield (71%; for steps a and b). Subsequent catalytic cleavage of \((S)-116\) gave the chiral primary amine \((S)-14\). Treatment with methyl formate furnished the formamide \((S)-117\), which was reduced with lithium aluminium hydride to the desired enantiopure \(N\)-methylamine, \((S)-\text{MDMA}\) (55%; overall yield).

\[
\begin{align*}
\text{54} & \quad \xrightarrow{\text{a}} \quad \text{116} \\
\text{116} & \quad \xrightarrow{\text{b}} \quad (S)-116 \\
\text{c} \quad \xrightarrow{\text{d}} \quad (S)-14 & \quad \xrightarrow{\text{e}} \quad (S)-117 & \quad \xrightarrow{\text{f}} \quad (S)-\text{MDMA}
\end{align*}
\]

Scheme 3.2 Stereoselective synthesis of \((S)-\text{MDMA}\) from a chiral auxiliary.\(^{43}\) a) i) \((S)-\alpha\)-methylbenzylamine \(115\); ii) W-2 Raney Ni, \(\text{H}_2\); iii) \(\text{HCl}\); b) fractional crystallisation; c) \(\text{H}_2\), Pd/C, 3 atm; d) HCOOMe, \(\Delta\); e) LiAlH\(_4\) (overall yield 55%).

Multiple alternate methods for the preparation of chiral amphetamines are reported. However, many of these methods are limited in their utility because they furnish only one stereoisomer,\(^{115,116}\) require expensive chiral reagents,\(^{114}\) utilise toxic reagents\(^{117}\) or are indirect.\(^{114,115}\) The method published by Nenajdenko and coworkers was found to be a notable exception.\(^{118}\)
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These authors employed a chiral pool strategy to afford a variety of β-aryl(heteroaryl)alkylamines 121 from enantiopure amino acid precursors (Scheme 3.3). The critical transformation is a regioselective, nucleophilic ring-opening of an enantiopure N-tosylaziridine 118 by an organocuprate. The resultant sulfonamide 120 was then cleaved, without epimerisation, upon treatment with magnesium and methanol under ultrasonication conditions. The chiral β-aryl(heteroaryl)alkylamines 121 were obtained in yields ranging from good to excellent. The requisite enantiopure N-tosylaziridines 118 were prepared from commercially available, enantiopure amino acids. The impressive yields and simplicity of this method made it worthy of further exploitation.

\[ R_1 \text{N-Ts} + \text{Br} \rightarrow R_1 \text{N-Ts} \text{Br} \rightarrow R_1 \text{N-Ts} \rightarrow \text{R} \rightarrow \text{R} \rightarrow \text{R} \rightarrow \text{R} \rightarrow \text{NH}_2 \]

Scheme 3.3 a) i) Mg, THF; ii) Cul (15 mol%); iii) enantiopure aziridine, THF; b) Mg, MeOH,).

Synthetic Strategy and Considerations

The amines (R)-18 and (S)-18 would be accessible via a modification of the chiral auxiliary reductive amination method, utilising the previously optimised Borch conditions. Given ketone 55 was available in gram quantities, it seemed prudent to exploit this method. A truncation of this method was conceived whereby the N-methylamine was to be obtained from direct methylation of the secondary N-methylbenzylamine, followed then by hydrogenolysis of the chiral auxiliary

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* The use of naturally occurring amino acids for chiral strategy and asymmetric syntheses is extensively documented.

† Ring-opening reactions of aziridines have been recently reviewed by Hu.
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(Scheme 3.4). This would eliminate the two-step formylation/reduction otherwise required to introduce the \( N \)-methyl moiety.

\[ 
\text{Scheme 3.4} \quad a) \ i) \ CH_3COOH, \ MeOH, \ THF, \ 3\AA \ \text{sieves}; \ \text{ii)} \ NaCNBH}_3; \ b) \ \text{separation}; \ c) \ \text{methylation}; \ d) \ \text{reduction.} 
\]

\( \text{(R)-MDMA} \) and \( \text{(S)-MDMA} \) might well be considered pedestrian synthetic targets, given the frequency with which they appear in the literature. The challenge was to find novel syntheses of \( \text{(R)-MDMA} \) and \( \text{(S)-MDMA} \) that were succinct and high yielding, from affordable starting materials and amenable to gram-scale preparation. To this end, a modification of the procedure reported by Nenajdenko et al.\textsuperscript{118} was devised (Scheme 3.5). As above, the introduction of the \( N \)-methyl moiety was to be the penultimate step of the synthesis, this time by methylation of a sulfonamide. An added bonus of the proposed method was that the immediate precursor to either enantiomer is an \( N \)-methylsulfonamide 127. Such compounds are not subject to restrictive scheduling and could therefore be prepared in quantity and stored for future needs.
Finally, it was presumed that the analogues (R)-111 and (S)-111 were accessible from (R)-MDMA and (S)-MDMA by simple alkylation, a method having been previously useful in the synthesis of the racemate 111 (Scheme 2.6).

Overview

This chapter contains details of the preparation of the enantiomeric amines (R)-18 and (S)-18, novel syntheses of (R)-MDMA and (S)-MDMA, X-ray crystal structures of the aforementioned amines and the preparation of two enantiomeric N-propargyl MDMA analogues (R)-111 and (S)-111.
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Results and Discussion

Preparation of (R)-18 and (S)-18 via a diastereoselective reductive amination methodology

(R)-α-Methylbenzylamine 122 was reductively alkylated with the ketone 55 to afford the desired amines (R)-123 and (S)-123 as a diastereomeric mixture in excellent yield [99%, (R):(S); 1.0:2.0**] (Scheme 3.6).†† Initially the reductive amination was performed using a ten-fold excess of the chiral amine, which gave a yield of 83%. Subsequently a three-fold excess of amine was shown to provide both better economy and a better outcome (99%).

![Scheme 3.6](image)

The observed degree of stereocontrol was modest. From a search of the literature, other examples of Borch reductive aminations with enantiopure α-methylbenzylamine revealed comparable diastereoselectivity (dr; ≤ 2:1).58,121 The advantage of the modest stereoselectivity observed was that both enantiomers (R)-18 and (S)-18 were accessible from a single reductive amination reaction.

** The diastereomeric ratio was calculated using the integral values of the distinctive AB pattern arising from the diastereotopic protons of the acetal functionality. For (R)-123 these resonances were recorded at δ 5.96-5.95 ppm and for (S)-123 at δ 5.92-5.91 ppm.

†† The absolute stereochemistry of (R)-123 and (S)-123, and other intermediates in this synthetic pathway, were assigned retrospectively from the absolute stereochemistry of the final amine hydrochlorides, (R)-18.HCl and (S)-18.HCl, determined unambiguously by single crystal X-ray diffraction analyses.
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The unfavourable diastereomeric ratio made fractional crystallisation of the hydrochlorides (R)-123.HCl and (S)-123.HCl difficult potentially leading to a significant yield loss. The chromatographic resolution of the mixture of (R)-123 and (S)-123 was time-consuming although not impossible. A small portion of this mixture was resolved by flash chromatography to enable characterisation of these compounds. It was reasoned that methylation of the mixture of (R)-123 and (S)-123 would give a mixture of tertiary amines (R)-124 and (S)-124; this mixture would be less polar and presumably easier to resolve using standard chromatography. Additionally, prolonging the resolution of the diastereomers had the concomitant advantage of being a later point of divergence for the preparation of target compounds (R)-18 and (S)-18, thereby reducing the overall number of steps in the synthetic pathways. Methylation of the mixture of (R)-123 and (S)-123 gave (R)-124 and (S)-124 in satisfactory yield. As predicted, the chromatographic resolution of (R)-124 (30%) and (S)-124 (38%) was less challenging than the precursor amine mixture, although some mechanical loss was still experienced.

The amine (R)-124 was treated with hydrogen gas in the presence of palladium on carbon to effect hydrogenolysis of the N-α-methylbenzyl moiety. The transformation gave the desired chiral amine (R)-18 in good yield (80%). Amine (S)-18 was furnished in the same manner, this time in excellent yield (95%) (Scheme 3.7).
Scheme 3.7 a) MeI, K₂CO₃, DMF [(R)-123 (30%); (S)-123 (38%)]; b) H₂, Pd/C (5% w/w), EtOH, HCl [(R)-18 (80%); (S)-18 (95%)].

The amine hydrochlorides (R)-18.HCl and (S)-18.HCl were subjected to X-ray crystallographic analysis to elucidate the absolute stereochemistries (Figure 3.2).¹¹

¹¹ Crystallography was performed by Dr Brian Skelton at The University of Western Australia, Perth. Further details of the experimental and crystallographic data are provided in the Appendix.
Figure 3.2 Molecular structure of 1(R)-18.HCl. Cationic species only shown. Non-hydrogen ellipsoids have been shown at the 50% probability level. Hydrogen atoms are denoted as circles of arbitrary size. The molecular structure of (S)-18.HCl is shown in the Appendix (Figure A.1).

Chiral pool strategy for the synthesis of (R)-MDMA and (S)-MDMA

(R)-MDMA and (S)-MDMA were prepared using the proposed modification of Nenadjenko’s chiral pool strategy (Scheme 3.10). Provision of the requisite starting materials is outlined below. The aryl bromide 126 was afforded on a multi-gram scale and in excellent yield (89%) via bromination of benzodioxole 128 (Scheme 3.8).

\[
\begin{align*}
\text{CO} & \\
\text{CO}^{+}
\end{align*}
\]

Scheme 3.8 a) Br₂, DCM (89%).

Initial efforts to prepare the aziridine (S)-125 from L-alanine 129 followed the reported procedure of Berry and Craig (Scheme 3.9; reaction conditions a, b and c). The intermediates of this three-step procedure were obtained in yields comparable to the literature. However the final step could
not be emulated, even after several attempts. In each instance the O-tosylated species 131 was recovered. The recalcitrant nature of 131 toward cyclisation was noted by Berry and Craig. The sulfonamide 130 was amenable to cyclisation under Mitsunobu conditions. The overall yield of (S)-125 from L-alanine 129 was 48% (Scheme 3.9; reaction conditions a, b and d). The enantiomeric aziridine (R)-125 was prepared from D-alanine in higher yield (63%) using the same conditions.

\[
\begin{align*}
\text{COOH} & \quad \text{a, b} \quad \text{NH}_{2} \\
129 & \rightarrow \quad \text{OH} \\
131 & \quad \text{NHTs} \\
& \quad \text{TsN}
\end{align*}
\]

\(130\)

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\(338\)
The complete regioselectivity of the ring-opening step can be attributed to three factors: a) steric hindrance at C2 of the aziridine;\textsuperscript{125} b) the strong activating character of the tosyl group (SN$_2$ ring-opening is kinetically favoured by strong activation);\textsuperscript{126} and c) the ‘soft’ nucleophilic character of the organocuprate. The reaction of activated-aziridines with ‘harder’ nucleophiles, such as those generated by Grignard reagents, has been shown to give a mixture of regioisomers\textsuperscript{126,127} and lower yields.\textsuperscript{128} The probable mechanism of the SN$_2$ ring-opening is presented in Figure 3.3.
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\[ \text{S}_2 \text{ attack at the less hindered CI} \quad \text{Loose S}_2 \text{ transition state; negative charge resonance-stabilised by activating NTs group} \]

**Figure 3.3** The S$_2$ mechanism of nucleophilic ring opening of the N-tosyl-activated chiral aziridine. a) H$_3$O$^+$. 

The sulfonamides (R)-127 and (S)-127 were effortlessly converted into the corresponding N-methylsulfonamides, (R)-132 (quant.) and (S)-132 (99%), by treatment with methyl iodide and potassium carbonate in DMF (Scheme 3.10). The ease of N-alkylation of sulfonamides is well known. Introduction of the N-methyl moiety prior to detosylation allowed for more expedient synthesis of (R)-MDMA and (S)-MDMA.

The final step in the synthesis of the target enantiomers was detosylation of the respective N-methylsulfonamides (Scheme 3.10). The reaction conditions called for no racemisation of the purposefully installed stereogenic centre and was required to be complimentary with maintaining the methylenedioxy functionality, which is fortuitously recalcitrant. A summary of the detosylation reactions attempted is tabulated below (Table 3.2).
Table 3.2 A summary of detosylation reactions performed on compounds 157 and 158.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Experimental Conditions $^*$</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(R)-132</td>
<td>(R)-MDMA</td>
<td>a</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>(R)-132</td>
<td>(R)-MDMA</td>
<td>b</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>(S)-132</td>
<td>(S)-MDMA</td>
<td>c</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>(S)-132</td>
<td>(S)-MDMA</td>
<td>a</td>
<td>54</td>
</tr>
<tr>
<td>5</td>
<td>(S)-132</td>
<td>(S)-MDMA</td>
<td>d</td>
<td>50</td>
</tr>
</tbody>
</table>

$^*$ a) Mg, THF, MeOH)); b) 1,4-Dimethoxybenzene, NaBH$_4$, MeCN, EtOH, $\lambda = 254$ nm; c) Mg, MeOH, )))); d) Na, naphthalene, DME.

Initial efforts to detosylate (S)-132 focused on treatment with magnesium and MeOH under ultrasonication conditions. This method was identified by Anderson and Alonso$^{130}$ as sufficiently gentle as not to cause epimerisation of chiral aziridines during desulfonylation reactions and was later adapted by Nenajdenko and coworkers for their own purpose.$^{118}$ After several hours, the reaction stalled. The addition of further equivalents of magnesium and extended reaction time could not induce the reaction to proceed further. The overall yield of the reaction was 56% (Table 3.2; Entry 3). Several modifications were attempted to improve this method, each time the reaction stalled for no obvious reason (Table 3.2; Entries 1 and 4). In each instance the starting material was retrieved (>40%) during chromatography of the crude product.

Encouraged by the otherwise high yields obtained throughout the synthetic pathway, further detosylation methods were explored (Table 3.2; Entries 2 and 5). Cleavage of sulfonamides using treatment with the sodium naphthalenide radical-anion was first reported by Ji and coworkers.$^{131}$ The transformation is widely reported as a clean deprotection method.$^{132-134}$
with sodium naphthalenide gave the desired amine \((S)\)\text{-MDMA} in only modest yield (50\%, Table 3.2; Entry 5). One of the major issues encountered was that the ‘colourless endpoint’, frequently sighted in the literature for reactions of this type, was difficult to discern.

A photolytic cleavage of the tosyl group was attempted (Table 3.2; Entry 2). The sulfonamide \((R)-132\) was treated with 1,4-dimethoxybenzene and sodium borohydride in aqueous ethanol in a Rayonet reactor \((\lambda = 254 \text{ nm})\) using the conditions described by Lautens and coworkers.\textsuperscript{135} The reduction is reported to occur via an electron-transfer mechanism from the electron-donating aromatic to the sulfonamide.\textsuperscript{136} The isolated yield of the amine \((R)\text{-MDMA}\) was disappointingly low (33\%), given quantitative yields have been reported with similar substrates.\textsuperscript{137}

Detosylation of the precursor sulfonamides was the encumbrant step in the synthesis of \((R)\text{-MDMA}\) and \((S)\text{-MDMA}\). The arylsulfonamide is one of the most stable amine protecting groups and, consequently, can be difficult to remove.\textsuperscript{129} Further investigation of aziridine-activating groups that are more labile is warranted. This might judiciously include the nosyl group, which is widely accepted to be easier to remove than the tosyl group, and an excellent aziridine-activator,\textsuperscript{120} or a phosphonamidine as exploited by Hu \textit{et al.}\textsuperscript{138} An improvement of the final detosylation step promises to deliver high yielding, gram-scale synthesis for both \((R)\text{-MDMA}\) and \((S)\text{-MDMA}\) which, for the purpose of \textit{in vivo} testing, is highly desirable.

Crystallography of MDMA.HCl has been reported.\textsuperscript{139} The crystal structures of its enantiomers have not. This is remarkable given the interest in and comprehensive pharmacology of these compounds. An earlier report by Nichols \textit{et al.}\textsuperscript{43} alluded to having acquired this crystallographic data and preparing a relevant manuscript for publication. However, no subsequent publication from these authors on this subject was able to be located.
Herein, for the first time, the single crystal X-ray diffraction analysis of \((R)\)-MDMA.HCl and \((S)\)-MDMA.HCl is reported [Figure 3.4 and Figure A.2 (Appendix)]. The compounds showed the expected stereochemistry, confirming no loss of stereochemistry had occurred during the multi-step pathways (Scheme 3.10). Furthermore, the optical rotations for the synthesised amine hydrochlorides were consistent with the literature values.43

![Molecular projection of (R)-MDMA.HCl](image)

**Figure 3.4** Molecular projection of \((R)\)-MDMA.HCl. Cationic species only shown. The projection is approximately onto the plane of the rings. Non-hydrogen ellipsoids have been drawn at the 50% probability level. Hydrogen atoms are denoted as circles of arbitrary size. The molecular structure of \((S)\)-MDMA.HCl is shown in the Appendix (Figure A.2).

The crystal packing of \((R)\)-MDMA.HCl is depicted in Figure 3.5. There are hydrogen bonds between the NH2 and the chloride ion, forming a helical one-dimensional hydrogen-bonded polymer generated by the crystallographic 2_1 screw axis along the \(b\) direction. Bond distances between nitrogen atoms and chloride ions are in the order of 3Å (3.103±0.002 Å and 3.1154±0.018 Å). These values are consistent with those calculated for the racemate139 and other phenethylamines.140

§§ Refer to the Appendix for further detailing of the crystallography of \((R)\)-MDMA.HCl and \((S)\)-MDMA.HCl.
Figure 3.5 Hydrogen-bonded polymer of (R)-MDMA.HCl. The hydrogen-bonded polymer depicted is projected down the $\alpha$ axis with the $\beta$ axis horizontal. Nitrogen is depicted in blue, chloride in green and oxygen in red.

**Preparation of the chiral MDMA-selegiline hybrid analogues**

The chiral selegiline analogues (S)-111 and (R)-111 were prepared from (S)-MDMA and (R)-MDMA respectively. $N$-Propargylation reliably gave the analogues (S)-111 (70%) and (R)-111 (46%), albeit in lower than expected yield for the latter amine (Scheme 3.11).

\[ \text{MDMA} \xrightarrow{a} 111 \]

**Scheme 3.11** a) HC\(\equiv\)CCH\(_2\)Br, K\(_2\)CO\(_3\), DMF, PhMe.
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Experimental

General Procedure A: Nucleophilic Ring-Opening of an Aziridine by an Organocuprate

Magnesium flakes (10 mol equiv) were stirred under Ar (overnight). The following morning THF (2 mL/5 mmol of Mg) was added to the blackened Mg, followed by dropwise addition of 5-bromo-1,3-benzodioxole 126 (2.0 mol equiv) in THF (1 mL/mmol of 126). Initiation of the Grignard reagent formation was indicated by warming of the reaction vessel and darkening of the solution. The reaction mixture was stirred (4 h at rt then 1 h at 45 °C). The Grignard reagent was cannulated from the remaining Mg, to give a greenish-coloured solution, which was then cooled (−30 °C). Copper(I) iodide (0.30 mol equiv) was added and the pale yellow mixture stirred (45 min). The mixture was further cooled (−78 °C) and the aziridine substrate (1 mol equiv) in THF (4 mL/mmol of aziridine) was added dropwise with stirring. The mixture was allowed to warm gradually to rt and stirred (overnight). The mixture was diluted with sat. aq NH₄Cl, stirred (10 min) and subjected to a standard workup (Et₂O). Flash chromatography gave the sulfonamide.

General Procedure B: Sulfonamide Methylation

Potassium carbonate (3.0 mol equiv) and MeI (2.0 mol equiv) were loaded under Ar into a solution of the sulfonamide substrate (1.0 mol equiv) in anhydrous DMF (3 mL/mmol). The mixture was stirred at rt until tlc indicated the reaction was complete (typically > 12 h) and then subjected to a standard workup (Et₂O). Flash chromatography gave the N-methylsulfonamide.

General Procedure C: Desulfonylation

A solution of the N-methylsulfonamide (1.0 mol equiv) in dry solvent (MeOH or THF) was added under Ar to a suspension of Mg powder (10 mol equiv) in dry MeOH. The mixture was sonicated (1 h), during which time a colourless precipitate formed and the Mg appeared to have been...
consumed. Tlc analysis indicated the presence of a newly formed compound and unreacted starting material. Further Mg powder (5.0-10 mol equiv) was added and the mixture sonicated (1-2 h) until tlc indicated no further reaction had taken place. The reaction was diluted with sat. aq NaHCO₃, followed by a standard workup (Et₂O); chromatography gave the N-methylamine.

**General Procedure D: Reductive Amination**

Crushed, dried 3Å sieves (1 mg/mg of ketone) were added to a cold (0 °C) solution of the ketone (1.0 mol equiv) in THF (3 mL/mmol) and MeOH (3 mL/mmol). Under an Ar flow, the amine (3.0 mol equiv) was added dropwise with stirring, followed by dropwise addition of glacial CH₃COOH (3.0 mol equiv). The mixture was stirred at 0 °C (30 min). Sodium cyanoborohydride (1.0 mol equiv) was added and the mixture warmed (50 °C) and stirred until the reaction was observed to be complete by tlc (typically 24-36 h). The mixture was diluted with aq HCl (1 M) and stirred (10 min), then filtered through Celite, washing with MeOH, followed by concentration of the filtrate/washings. The concentrate was basified with aq NaOH (1 M) and subjected to a standard workup (Et₂O); chromatography gave the amine.

**General Procedure E: Propargylation**

Potassium carbonate (1.5 mol equiv) was added under Ar into a stirred solution of the substrate amine (1.0 mol equiv) in anhydrous DMF (6 mL/mmol of amine). The mixture was cooled (0 °C) and propargyl bromide (1.0 mol equiv, 80% w/w in PhMe) was added dropwise. The mixture was stirred (rt) and the progress of the reaction was monitored by tlc. Upon completion of the reaction the mixture was subjected to a standard workup (Et₂O); chromatography gave the propargylamine.
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General Procedure F: Amine Hydrochloride Preparation

The substrate amine was treated with a vast molar excess of methanolic HCl. The solvent and excess HCl were removed by evaporation and the resultant solid was recrystallised to afford the amine hydrochloride.

\[(R)-2-(1,3\text{-Benzodioxol-5-yl})-1\text{-cyclopropyl-N-}[1\text{-phenylethyl}]\text{ethanamine (R)-123}\]

\[(S)-2-(1,3\text{-Benzodioxol-5-yl})-1\text{-cyclopropyl-N-}[1\text{-phenylethyl}]\text{ethanamine (S)-123}\]

The ketone 55 (1.02 g, 5.00 mmol) was treated with (R)-1-phenylethylamine (1.94 mL, 15.0 mmol), glacial CH₃COOH (0.9 mL, 15 mmol) and NaCNBH₃ (0.31 g, 5.0 mmol) according to General Procedure D. Subsequent purification by rsf chromatography (EtOAc/petrol/NEt₃, 1:3:trace) afforded a pale yellow oil (1.53 g, 99%): the diastereomers (R)-123 and (S)-123 as a mixture [1.0:2.0 (as determined by ¹H NMR spectroscopy, comparing the integrals of the OCH₂AB resonances for each compound)]. The diastereomeric mixture was used in the proceeding step without further separation. An aliquot of the mixture was subjected to flash chromatography (EtOAc/petrol/NEt₃, 1:5:trace) to yield the pure amines. The first to elute was the minor diastereomer (R)-123 as a pale yellow oil. [α]D²⁰ = -59.2° (c 1.0, CHCl₃). IR νmax (film) 3323, 1503, 1488, 1246, 1040 cm⁻¹. ¹H NMR (600 MHz): δ 7.23-7.19 (m, 2H, ArH), 7.18-7.14 (m, 1H, ArH), 6.90-6.86 (m, 2H, ArH), 6.74 (d, J = 7.8 Hz, 1H, H7'), 6.58 (dd, J = 7.9, 1.5 Hz, 1H, H6'), 6.55 (d, J = 1.5 Hz, 1H, H4'), 5.96 (AB, J = 1.5 Hz, 1H, OCH₂O), 5.95 (AB, J = 1.5 Hz, 1H, OCH₂O), 4.14 (q, J = 6.8 Hz, 1H, CH), 2.78 (dd, J = 13.6, 4.2 Hz, 1H, H2), 2.55 (dd, J = 13.7, 9.2 Hz, 1H, H2), 1.74-
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1.69 (m, 1H, H1), 1.62 (br s, 1H, NH), 1.25 (d, J = 6.8 Hz, 3H, CH3), 0.73-0.66 (m, 1H, c-PrH), 0.60-0.54 (m, 1H, c-PrH), 0.37-0.32 (m, 1H, c-PrH), 0.16-0.10 (m, 1H, c-PrH), 0.09-0.09 (m, 1H, c-PrH). 13C NMR (150.9 MHz): δ 147.69, 146.08, 145.94, 133.19 (Ar), 128.36, 126.62, 126.34, 122.40, 109.83, 108.18 (ArH), 100.95 (OCH2O), 60.77, 55.00 (CH), 42.50 (C2), 25.71 (CH3), 16.19, 5.37, 1.18 (c-Pr). HRMS (FAB): m/z = 310.1814; [M + H]^+ requires 310.1807.

The next to elute was the major diastereomer \((S)-123\) as a pale yellow oil. \([\alpha]_D^{20} + 50.4^\circ\) (c 1.1, CHCl3). IR \(v_{\text{max}}\) (film) 3328, 1503, 1489, 1247, 1040 cm\(^{-1}\). 1H NMR (600 MHz): δ 7.33-7.29 (m, 2H, ArH), 7.25-7.21 (m, 3H, ArH), 6.71 (d, J = 7.9 Hz, 1H, H7'), 6.63 (d, J = 1.5 Hz, 1H, H4'), 6.60 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.92 (AB, J = 1.5 Hz, 1H, OCH2O), 5.91 (AB, J = 1.5 Hz, 1H, OCH2O), 3.89 (q, J = 6.6 Hz, 1H, CH), 2.77 (dd, J = 13.5, 5.3 Hz, 1H, H2), 2.71 (dd, J = 13.5, 6.4 Hz, 1H, H2), 1.72-1.67 (m, 1H, H1), 1.66 (br s, 1H, NH), 1.32 (d, J = 6.6 Hz, 3H, CH3), 0.67-0.61 (m, 1H, c-PrH), 0.36-0.27 (m, 2H, c-PrH), 0.11-0.16 (m, 1H, c-PrH), 0.18-0.23 (m, 1H, c-PrH). 13C NMR (150.9 MHz): δ 147.42, 146.12, 145.84, 133.31 (Ar), 128.56, 126.96, 126.58, 122.64, 110.16, 108.04 (ArH), 100.84 (OCH2O), 61.53, 55.21 (CH), 40.51 (C2), 24.72 (CH3), 16.72, 3.78, 3.71 (c-Pr). HRMS (FAB): m/z = 310.1799; [M + H]^+ requires 310.1807.

\[(R)-2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methyl-N-[(R)-1-phenylethyl]ethanamine (R)-124\]

\[(S)-2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methyl-N-[(R)-1-phenylethyl]ethanamine (S)-124\]

Methyl iodide (625 μL, 10.0 mmol) was added dropwise to a cold (0 °C) solution of the diastereomeric amines \((R)-123\) and \((S)-123\) (1.55 g, 5.00 mmol, \((R)-123:(S)-123 = 1.0:2.0\)) and
K$_2$CO$_3$ (1.73 g, 12.5 mmol) in DMF (50 mL). The mixture was stirred (6 h), gradually warming to rt, and then subjected to a standard workup (Et$_2$O) to give the diastereomers (R)-124 and (S)-124 as a pale yellow oil (1.40 g, 87%) [1.0:1.9 (as determined by $^1$H NMR spectroscopy, comparing the integrals of the OCH$_2$O AB resonances for each compound)]. The oil was subjected to flash chromatography (EtOAc/petrol/NEt$_3$, 1:9:trace) to afford the tertiary amines (R)-124 and (S)-124.

The first to elute was the minor diastereomer (R)-124 as a colourless oil (482 mg, 30%). \([\alpha]_D^{20} = 24.8^\circ (c 1.2, CHCl$_3$). IR \nu_{max} (film) 1503, 1488, 1248, 1040 cm$^{-1}$. $^1$H NMR (600 MHz): \(\delta 7.19-7.12 (m, 3H, ArH), 7.07-7.04 (m, 2H, ArH), 6.69 (d, J = 7.9 Hz, 1H, H7'), 6.54 (d, J = 1.6 Hz, 1H, H4'), 6.52 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.93 (AB, J = 1.5 Hz, 1H, OCH$_2$O), 5.92 (AB, J = 1.5 Hz, 1H, OCH$_2$O), 3.85 (q, J = 6.6 Hz, 1H, CH), 2.76 (dd, J = 13.6, 7.4 Hz, 1H, H2'), 2.59 (dd, J = 13.5, 6.7 Hz, 1H, H2), 2.37 (s, 3H, NCH$_3$), 2.05-2.00 (m, 1H, H1), 1.25 (d, J = 6.6 Hz, 3H, CH$_3$), 0.85-0.78 (m, 1H, c-PrH), 0.50-0.45 (m, 1H, c-PrH), 0.32-0.26 (m, 1H, c-PrH), 0.25-0.20 (m, 1H, c-PrH), −0.30−0.36 (m, 1H, c-PrH). $^{13}$C NMR (150.9 MHz): \(\delta 147.14, 146.79, 145.46, 135.08 (Ar), 128.18, 127.42, 126.45, 122.46, 110.22, 107.79 (ArH), 100.73 (OCH$_2$O), 66.11, 61.91 (CH), 38.67 (C2), 33.00 (NCH$_3$), 22.53 (CH$_3$), 11.17, 4.50, 3.06 (c-Pr). HRMS (ESI): $m/z = 324.1974; [M + H]^+$ requires 324.1964.

The next to elute was the major diastereomer (S)-124 as a colourless oil (623 mg, 38%). \([\alpha]_D^{20} + 17.5^\circ (c 1.1, CHCl$_3$). IR \nu_{max} (film) 1503, 1489, 1247, 1040 cm$^{-1}$. $^1$H NMR (600 MHz): \(\delta 7.30-7.24 (m, 4H, ArH), 7.23-7.19 (m, 1H, ArH), 6.67-6.64 (m, 1H, ArH), 6.52-6.49 (m, 2H, ArH), 5.90 (AB, J = 1.5 Hz, 1H, OCH$_2$O), 5.89 (AB, J = 1.5 Hz, 1H, OCH$_2$O), 3.77 (q, J = 6.6 Hz, 1H, CH), 2.85 (dd, J = 13.3, 5.5 Hz, 1H, H2), 2.55 (dd, J = 13.3, 8.1 Hz, 1H, H2), 2.37 (s, 3H, NCH$_3$), 2.03-1.97 (m, 1H, H1), 1.26 (d, J = 6.6 Hz, 3H, CH$_3$), 0.84-0.77 (m, 1H, c-PrH), 0.43-0.37 (m, 1H, c-PrH), 0.29-0.23 (m, 1H, c-PrH), 0.10-0.05 (m, 1H, c-PrH), −0.39−−0.44 (m, 1H, c-PrH). $^{13}$C NMR (150.9 MHz): \(\delta 147.22, 146.59, 145.41, 135.24 (Ar), 128.40, 127.43, 126.85, 122.32, 109.93, 107.86 (ArH), 100.73 (OCH$_2$O), 65.82, 61.52 (CH), 35.94 (C2), 33.70 (NCH$_3$), 22.03 (CH$_3$), 13.60, 4.81, 3.52 (c-Pr). HRMS (ESI): $m/z = 324.1970; [M + H]^+$ requires 324.1963.
Palladium on charcoal (390 mg, 5% w/w) and aq HCl (15 drops, 1 M) were added to an ethanolic solution (40 mL) of the amine (R)-124 (0.39 g, 1.2 mmol). The mixture was stirred under an atmosphere of H₂ (2.5 h, rt), after which it was filtered through Celite, washing with MeOH (3x), basified with NaOH (20 mL, 1 M) and partially concentrated. The residue was subjected to a standard workup (Et₂O) and rfs chromatography (EtOAc/petrol/NEt₃, 1:3:trace) to afford the amine (R)-IS (0.21 g, 80%). [α]D²⁰ = 1.1° (c 0.5, CHCl₃). IR νmax (film) 3335, 1503, 1488, 1248, 1040 cm⁻¹. 

H NMR (600 MHz): δ 6.73 (d, J = 7.9 Hz, 1H, H7'), 6.69 (d, J = 1.7 Hz, 1H, H4'), 6.65 (dd, J = 7.9, 1.7 Hz, 1H, H6'), 5.92 (s, 2H, OCH₂O), 2.82 (dd, J = 13.7, 4.8 Hz, 1H, H2), 2.66 (dd, J = 13.7, 7.9 Hz, 1H, H2), 2.44 (s, 3H, NCH₃), 1.80-1.75 (m, 1H, H1), 1.56 (br s, 1H, NH), 0.69-0.62 (m, 1H, c-PrH), 0.60-0.55 (m, 1H, c-PrH), 0.45-0.39 (m, 1H, c-PrH), 0.28-0.23 (m, 1H, c-PrH), 0.02—0.03 (m, 1H, c-PrH). ¹³C NMR (150.9 MHz): δ 147.69, 146.03, 132.28 (Ar), 122.39, 109.76, 108.24 (ArH), 100.92 (OCH₂O), 66.79 (C1), 41.61 (C2), 34.79 (NCH₃), 15.70, 5.18, 1.78 (c-Pr), HRMS (EI): m/z = 219.1263; [M]⁺ requires 219.1259.

The amine (R)-18 was treated according to General Procedure F to afford the amine hydrochloride (R)-18.HCl as colourless blocks, mp 161.5 °C (i-PrOH/petrol). [α]D²⁰ = +24.3° (c 1.0, CHCl₃). 

H NMR (600 MHz): δ 9.57 (br s, 2H, NH₂), 6.77-6.71 (m, 3H, ArH), 5.93 (AB, J = 1.5 Hz , 1H,
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OCH$_3$), 5.93 (AB, $J = 1.5$ Hz, 1H, OCH$_2$O), 3.36 (dd, $J = 13.5, 4.6$ Hz, 1H, H2), 3.05 (dd, $J = 13.6, 9.4$ Hz, 1H, H2), 2.75 (s, 3H, NCH$_3$), 2.47-2.42 (m, 1H, H1), 1.10-1.03 (m, 1H, c-PrH), 0.72-0.65 (m, 1H, c-PrH), 0.53-0.47 (m, 2H, c-PrH), −0.12—0.18 (m, 1H, c-PrH). Anal. calcd for C$_{13}$H$_{18}$ClN$_2$: C, 61.1; H, 7.1; N, 5.5. Found: C, 61.0; H, 6.9; N, 5.2.

(1S)-2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methylethanamine (S)-18

Amine (S)-124 (0.10 g, 0.31 mmol) was treated according to the same method described above for amine (R)-124 to afford the amine (S)-18 as a colourless oil (65 mg, 95%). $[\alpha]_D^{20} + 1.8^\circ$ (c 1.1, CHCl$_3$). HRMS (El): $m/z = 219.1262$; $[M]^+$ requires 219.1259. The IR, $^1$H and $^{13}$C NMR spectra agreed with those reported above for the enantiomer (R)-18.

(1S)-2-(1,3-Benzodioxol-5-yl)-1-cyclopropyl-N-methylethanamine hydrochloride (S)-18.HCl

The amine (S)-18 was treated according to General Procedure F to afford the amine hydrochloride (S)-18.HCl as colourless blocks, mp 161.5 °C (CHCl$_3$/petrol). $[\alpha]_D^{20} - 24.1^\circ$ (c 1.0, CHCl$_3$). Anal. calcd for C$_{13}$H$_{18}$ClN$_2$: C, 61.1; H, 7.1; N, 5.5; Found: C, 60.8; H, 7.3; N, 5.3. The $^1$H NMR spectrum was consistent with that reported above for the enantiomer (R)-18.HCl.

5-Bromo-1,3-benzodioxole 126

Bromine (5.1 mL, 0.10 mol) in CH$_2$Cl$_2$ (100 mL) was added dropwise over 6 h to a cold (0 °C), stirred solution of the benzodioxole 128 (11 mL, 0.10 mol) in CH$_2$Cl$_2$ (150 mL). The mixture was allowed to warm gradually and stirred overnight (rt). The following morning, GCMS analysis of an
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aliquot of the mixture showed the starting material to have been consumed. Sat. aq Na₂S₂O₇ (100 mL) was added and the mixture stirred (10 min). The reaction mixture was then subjected to a standard workup (CH₂Cl₂) and the crude residue was distilled at reduced pressure to afford the benzodioxole 126 as a pale yellow oil (18 g, 89%), bp 98-100 °C (3 mmHg) [lit.¹⁴¹ bp 85 °C (1 mmHg)]. The ¹H NMR spectrum was in agreement with that previously reported.¹⁴²

![Chemical structures](image)

N-[(R)-2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-4-methylbenzenesulfonamide (R)-127

The aziridine (R)-125 (2.11 g, 10.0 mmol) was treated according to General Procedure A [flash chromatography (EtOAc/petrol, 1:7)] to afford the sulfonamide (R)-127 as a colourless gum that, after a period of several months, solidified to give an amorphous, colourless solid (3.10 g, 93%), mp 59.0-62.5 °C. [α]D²⁰ + 6.1° (c 1.2, CHCl₃). IR νmax (film, CH₂Cl₂) 3286, 1490, 1248, 1159, 1039 cm⁻¹. ¹H NMR (600 MHz): δ 7.62-7.59 (m, 2H, ArH), 7.25-7.22 (m, 2H, ArH), 6.65 (d, J = 7.8 Hz, 1H, H7’), 6.46 (dd, J = 7.9, 1.7 Hz, 1H, H6’), 6.42 (d, J = 1.7 Hz, 1H, H4’), 5.92 (d, J = 1.4 Hz, 1H, OCH₂O), 5.91 (d, J = 1.4 Hz, 1H, OCH₂O), 4.24 (br d, J = 7.1 Hz, 1H, NH), 3.47-3.40 (m, 1H, H1), 2.59 (dd, J = 13.8, 6.3 Hz, 1H, H2), 2.55 (dd, J = 13.8, 6.9 Hz, 1H, H2), 2.42 (s, 3H, ArCH₃), 1.11 (d, J = 6.5 Hz, 3H, CH₃). ¹³C NMR (150.9 MHz): δ 147.82, 146.49, 143.30, 137.64, 130.89 (Ar), 129.65, 127.13, 122.49, 109.60, 108.36 (ArH), 101.05 (OCH₂O), 51.14 (C1), 43.21 (C2), 21.64, 21.63 (CH₃). HRMS (EI): m/z = 333.1028; [M]+ requires 333.1035.

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N-(1R)-2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N, 4-dimethylbenzenesulfonamide (R)-132

The sulfonamide (R)-127 (2.66 g, 8.00 mmol) was treated according to General Procedure B [20 h, flash chromatography (CH2Cl2)] to afford the N-methylsulfonamide (R)-132 as a colourless gum (2.77 g, 100%). \([\alpha]_D^{23} - 36.8^\circ\) (c 1.5, CHCl3). IR \(v_{\text{max}}\) (film, CH2Cl2) 3422, 1490, 1335, cm\(^{-1}\).

\(^1\)H NMR (600 MHz): \(\delta 7.60-7.56\) (m, 2H, ArH), \(7.25-7.21\) (m, 2H, ArH), 6.68 (d, \(J = 7.9\) Hz, 1H, H7\'), 6.58 (d, \(J = 1.7\) Hz, 1H, H4\'), 6.55 (dd, \(J = 7.9, 1.7\) Hz, 1H, H6\'), 5.93 (AB, \(J = 1.5\) Hz, 1H, OCH2O), 5.92 (AB, \(J = 1.5\) Hz, 1H, OCH2O), 4.24-4.17 (m, 1H, HI), 2.74 (s, 3H, NCH3), 2.61 (dd, \(J = 13.6, 6.5\) Hz, 1H, H2), 2.49 (dd, \(J = 13.6, 8.3\) Hz, 1H, H2), 2.40 (s, 3H, ArCH3), 0.95 (d, \(J = 6.7\) Hz, 3H, CH3). \(^{13}\)C NMR (150.9 MHz): \(\delta 147.72, 146.27, 143.04, 137.18, 132.14\) (Ar), 129.64, 127.18, 122.16, 109.53, 108.34 (ArH), 101.00 (OCH2O), 54.56 (C1), 40.81 (C2), 27.97, 21.62, 16.98 (CH3). HRMS (EI): \(m/z = 347.1186; [M]^+\) requires 347.1191.

(2R)-1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine (R)-MDMA

(a) The N-methylsulfonamide (R)-132 (2.72 g, 7.80 mmol) in THF (50 mL) was treated according to General Procedure C, using firstly Mg powder (1.90 g, 78.0 mmol) in dry MeOH (100 mL), then Mg powder (0.950 g, 39.0 mmol, 1 h), followed by rfs chromatography (Et2O/MeOH/NEt3, 96:2:2).

The first to elute was unreacted starting material, the N-methylsulfonamide (R)-132 as a colourless oil (1.42 g, 52%). The \(^{13}\)C and \(^1\)H NMR spectra were consistent with those reported above. The next to elute was the amine (R)-MDMA as a colourless oil (690 mg, 46%). \([\alpha]_D^{23} - 34.5^\circ\) (c 1.1, CHCl3).

\(^1\)H NMR (600 MHz): \(\delta 6.74\) (d, \(J = 7.9\) Hz, 1H, H7\'), 6.67 (d, \(J = 1.7\) Hz, 1H, H4\'), 6.63 (dd, \(J = 7.9, 1.7\) Hz, 1H, H6\'), 5.93 (s, 2H, OCH2O), 2.75-2.69 (m, 1H, H1), 2.61 (dd, \(J = 13.5, 7.2\) Hz, 1H, H2), 2.54 (dd, \(J = 13.5, 6.2\) Hz, 1H, H2), 2.39 (s, 3H, NCH3), 1.58 (br s, 1H, NH), 1.05 (d, \(J = 6.2\) Hz, 3H, CH3). \(^{13}\)C NMR (125.8 MHz): \(\delta 147.76, 146.06, 133.34\) (Ar), 122.31, 109.64, 108.31 (ArH), 100.96 (OCH2O), 56.57 (C1), 43.29 (C2), 34.14 (NCH3), 19.76 (CH3). HRMS (EI): \(m/z = 193.1102; [M]^+\) requires 193.1103. The \(^1\)H and \(^{13}\)C NMR spectra were in agreement with those previously reported for the racemate (Chapter One).
(b) The N-methylsulfonamide \((R)-132\) (0.17 g, 0.50 mmol), NaBH₄ (0.19 g, 5.0 mmol), 1,4-dimethoxybenzene (0.28 g, 2.0 mmol), MeCN (5 mL) and EtOH (10 mL) were loaded into a quartz tube. The reaction mixture was irradiated at 254 nm in a Rayonet reactor (2 h). After this time, tlc showed some starting material remained; the reaction was irradiated for a further 3 h. No further reaction was detected by tlc. The reaction mixture was concentrated azeotropically with EtOH and the crude product subjected to rsf chromatography (EtOAc/petrol/NEt₃, 25:73:2) to yield the amine \((R)-MDMA\) as a colourless oil (32 mg, 33%). \([\alpha]_D^{23} = -33.7^\circ\) (c 1.0, CHCl₃). The \(^1\)H and \(^{13}\)C NMR spectra were in agreement with those reported above.

\((2R)-1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine hydrochloride (R)-MDMA.HCl\)

\((R)-MDMA\) was treated according to General Procedure F to afford \((R)-MDMA.HCl\) as colourless shards, mp 185-186 °C (i-PrOH) [lit.\(^{43}\) mp 192-193 °C (EtOH/Et₂O)]. \([\alpha]_D^{23} = -19.3^\circ\) (c 1.1, H₂O) [lit.\(^{43}\) \([\alpha]_D = -17.5^\circ\) (c 1, H₂O)]. \(^1\)H NMR (600 MHz): \(\delta\) 9.64 (v br s, 2H, NH₂), 6.75 (d, \(J = 7.9\) Hz, 1H, H7'), 6.70 (d, \(J = 1.6\) Hz, 1H, H4'), 6.68 (dd, \(J = 7.9, 1.7\) Hz, 1H, H6'), 5.94 (AB, \(J = 1.5\) Hz, 1H, OCH₂O), 5.94 (AB, \(J = 1.5\) Hz, 1H, OCH₂O), 3.37 (dd, \(J = 13.2, 4.2\) Hz, 1H, H2), 3.30-3.22 (m, 1H, H1), 2.77 (dd, \(J = 13.2, 10.4\) Hz, 1H, H2), 2.69 (s, 3H, NCH₃), 1.34 (d, \(J = 6.5\) Hz, 3H, CH₃). The \(^1\)H NMR spectrum was consistent with that previously reported.\(^{43}\)
N-[(1S)-2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-4-methylbenzenesulfonamide (S)-127

The aziridine (S)-125 (1.06 g, 5.02 mmol) was treated according to General Procedure A [flash chromatography (EtOAc/petrol, 1:4)] to afford the sulfonamide (S)-127 as a colourless gum (1.62 g, 97%). [α]D20 = 6.3° (c 1.3, CHCl3). HRMS (EI): m/z = 333.1036; [M]+ requires 333.1035. The IR, 1H and 13C NMR spectra agreed with those reported above for the enantiomer (R)-127.

N-[(1S)-2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N,4-dimethylbenzenesulfonamide (S)-132

The sulfonamide (S)-127 (1.50 g, 4.50 mmol) was treated according to General Procedure B [23 h, flash chromatography (EtOAc/petrol, 1:8)] to afford the N-methylsulfonamide (S)-132 as a colourless gum (1.54 g, 99%). [α]D20 + 38.4° (c 1.2, CHCl3). HRMS (EI): m/z = 347.1189; [M]+ requires 347.1191. The IR, 1H and 13C NMR spectra agreed with those reported above for the enantiomer (R)-132.

(2S)-1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine (S)-MDMA

(a) A solution of the N-methylsulfonamide (S)-132 (695 mg, 2.00 mmol) in dry MeOH (15 mL) was treated according to General Procedure C, using firstly Mg powder (485 mg, 20.0 mmol) in dry MeOH (5 mL), then Mg powder (485 mg, 20.0 mmol, 1 h), followed by rsf chromatography (EtOAc/petrol/NEt3, 25:73:2). The first to elute was unreacted starting material, the N-methylsulfonamide (S)-132 as a colourless oil (217 mg, 56%). The 13C and 1H NMR spectra were consistent with those reported above. The next to elute was the amine (S)-MDMA as a colourless oil (169 mg, 44%). [α]D23 + 33.1° (c 1.2, CHCl3). HRMS (EI): m/z = 193.1096; [M]+ requires 193.1103. The 1H and 13C NMR spectra were consistent with those reported for the enantiomer (R)-MDMA.

(b) A solution of the N-methylsulfonamide (S)-132 (0.87 g, 2.5 mmol) in THF (10 mL) was treated according to General Procedure C, using firstly Mg powder (0.61 g, 25 mmol) in dry MeOH
(15 mL), then Mg powder (0.61 g, 25 mmol, 2 h), followed by rfs chromatography (Et₂O then Et₂O/NEt₃, 98:2). The first to elute was unreacted starting material, the N-methylsulfonamide (S)-132 as a colourless oil (0.27 g, 31%). The ¹³C and ¹H NMR spectra agreed with those reported above. The next to elute was the amine (S)-MDMA as a colourless oil (0.26 g, 54%). [α]D²³ + 32.7° (c 1.0, CHCl₃). The ¹H and ¹³C NMR spectra were consistent with those reported above for the enantiomer (R)-MDMA.

(c) Under an argon atmosphere, naphthalene (1.28 g, 10.0 mmol) and Na pieces (230 mg, 10.0 mmol) were covered with DME (50 mL); the surface of the Na immediately turned dark green. The sodium naphthalide solution was stirred (rt, 2 h), during which time the green colour darkened and persisted. The sodium naphthalide solution was added dropwise to a cold (-78 °C) solution of the N-methylsulfonamide (S)-132 (1.40 g, 4.03 mmol) in DME (30 mL). A persistent green endpoint was not discernable. The reaction was stirred at -78 °C (1 h) and then stirred at rt for a further 2 h. The reaction mixture was quenched with EtOH and concentrated to give a solid residue that was subjected to a standard workup (Et₂O) and rfs chromatography (petrol then EtOAc/petrol, 1:3 then EtOAc/petrol/NEt₃, 25:73:2). The first to elute was naphthalene. The second to elute was unreacted starting material, the N-methylsulfonamide (S)-132 as a colourless oil. The ¹³C and ¹H NMR were in agreement to those reported above. The next to elute was the amine (S)-MDMA as a colourless oil (392 mg, 50%). [α]D²³ + 33.0° (c 1.0, CHCl₃). The ¹H and ¹³C NMR spectra were consistent with those reported above for the enantiomer (R)-MDMA.

(2S)-1-(1,3-Benzodioxol-5-yl)-N-methylpropan-2-amine hydrochloride (S)-MDMA.HCl

(S)-MDMA was treated according to General Procedure F to afford (S)-MDMA.HCl as colourless crystals, mp 184.5-186.0 °C (MeOH/Et₂O) [lit. 43 mp 192-193 °C (EtOH/Et₂O)]. [α]D²³ + 18.3° (c 1.0, H₂O) [lit. 43 [α]D + 17.43° (c 1, H₂O)]. The ¹H NMR spectrum agreed with that reported above for the enantiomer (R)-MDMA.HCl.
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N-[(S)-2-(1,3-Benzodioxol-5-yl)-1-methylethyl]-N-methylprop-2-yn-1-amine (S)-111

(S)-MDMA (390 mg, 2.00 mmol) was treated according to General Procedure E [flash chromatography (EtOAc/petrol, 1:4)] to afford the propargylamine (S)-111 as a colourless oil (324 mg, 70%). \([\alpha]_D^{20} + 4.9^\circ \) \((c \ 1.0, \text{CHCl}_3)\). IR \(\nu_{max} \) (film) 3293, 1503, 1489, cm\(^{-1}\). \(^1\text{H} \text{NMR} \) (600 MHz): \(\delta \ 6.72 \ (d, \ J = 7.9 \ Hz, 1H, H^7'), \ 6.68 \ (d, \ J = 1.6 \ Hz, 1H, H^4'), \ 6.62 \ (dd, \ J = 7.9, 1.7 \ Hz, 1H, H^6'), \ 5.92 \ (AB, \ J = 1.5 \ Hz, 1H, OCH_2O), \ 5.92 \ (AB, \ J = 1.5 \ Hz, 1H, OCH_2O), \ 3.45-3.37 \ (m, 2H, -CH_2C=C), \ 2.97-2.88 \ (m, 2H, H_1, H_2), 2.40 \ (s, 3H, NCH_3), 2.25-2.23 \ (m, 1H, C=CH), \ 0.96 \ (d, = 7.0 \ Hz, 3H, CH_3). \ \(\text{13C NMR} \) (150.9 MHz): \(\delta \ 147.63, \ 145.86, \ 134.16 \) (Ar), 122.23, 109.72, 108.22 (ArH), 100.91 (OCH_2O), 80.50 (−C≡C), 72.68 (C≡CH), 59.57 (C1), 43.3, 39.67 (CH_2), 37.60 (NCH_3), 15.16 (CH_3). HRMS (EI): \(m/z = 231.1262; \ [M]^+ \) requires 231.1259.

The propargylamine (S)-111 was treated according to General Procedure F to afford the hydrochloride (S)-111.HCl as a colourless powder, mp 181.5-185.0 °C (i-PrOH). \([\alpha]_D^{20} + 5.4^\circ \) \((c \ 1.0, \text{MeOH})\). \(^1\text{H} \text{NMR} \) (600 MHz): \(\delta \ 6.76 \ (d, \ J = 7.9 \ Hz, 1H, H^7'), \ 6.70 \ (d, \ J = 1.7 \ Hz, 1H, H^4'), \ 6.67 \ (dd, \ J = 7.9, 1.6 \ Hz, 1H, H^6'), \ 5.95 \ (AB, \ J = 1.5 \ Hz, 2H, OCH_2O), \ 3.96 \ (br s, 2H, CH_2C≡C), \ 3.58-3.49 \ (m, 1H, H_1), \ 3.48-3.38 \ (m, 1H, H_2), \ 2.87 \ (s, 3H, NCH_3), 2.73-2.67 \ (m, 2H, C≡CH), 1.35 \ (d, = 6.5 \ Hz, 3H, CH_3). \ Anal. calcd for C_{14}H_{18}ClNO_2: \ C, 62.8; \ H, 6.8; \ ; \ N, 5.2. \ Found: \ C, 62.8; \ H, 6.7; \ N, 5.2.
N-[(lR)-2-(l,3-Benzodioxol-5-yl)-l-methylethyl]-N-methylprop-2-yn-1-amine (R)-111

(R)-MDMA (290 mg, 1.50 mmol) was treated according to General Procedure E [flash chromatography (EtOAc/petrol, 1:4)] to afford the propargylamine (R)-111 as a colourless oil (161 mg, 46%). \([\alpha]_D^{20} - 4.6^\circ\) (c 1.2, CHCl3). HRMS (EI): \(m/z = 231.1251; \text{[M]}^+\) requires 231.1259. The IR, \(^1\)H and \(^{13}\)C NMR spectra agreed with those reported above for the enantiomer (S)-111.

N-[(lR)-2-(l,3-Benzodioxol-5-yl)-l-methylethyl]-N-methylprop-2-yn-1-amine hydrochloride (R)-111.HCl

The propargylamine (R)-111 was treated according to General Procedure F to afford the hydrochloride (R)-111.HCl as a colourless powder, mp 183.0-185.0 °C (i-PrOH). \([\alpha]_D^{20} - 5.4^\circ\) (c 1.0, MeOH). Anal. caled for C14H18ClNO2: C, 62.8; H, 6.8; N, 5.2. Found: C, 62.9; H, 6.6; N, 5.0. The \(^1\)H NMR spectrum agreed with that reported above for the enantiomer (S)-111.HCl.
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Introduction

Monoamine oxidases [MAOs; amine: oxygen oxidoreductase (deaminating) (flavin-containing); EC 1.4.3.4] are flavoenzymes, integral to the outer-mitochondrial membrane, that catalyse the oxidative deamination of a myriad of exogenous and endogenous amines, including the neurotransmitters serotonin 4, dopamine 5 and noradrenaline 6.143 This is a major inactivation pathway for these neurotransmitters and consequently MAOs serve a critical role in neurophysiological development and regulation.144

![Chemical structures of serotonin, dopamine, and noradrenaline](image)

The oxidative deamination catalysed by MAO is shown below (Scheme 4.1).145 A primary, secondary or tertiary amine 133 furnishes an aldehyde 134, an amine 135 (ammonia in the case of a primary amine) and hydrogen peroxide.

![Scheme 4.1](image)

In mammals there are two known isozymes, MAO-A and MAO-B; both are present in a variety of human tissues, being distributed in varying proportions.145 The two isozymes were originally
distinguished by their distinct substrate specificities and sensitivities to the acetylenic inhibitors, chlorgyline 136 and selegiline 10. For example, serotonin 4 is a substrate of MAO-A, which is selectively inhibited by chlorgyline 136. Conversely, phenethylamine 8 is a substrate of MAO-B, which is selectively inhibited by selegiline 10. Dopamine 5 and noradrenaline 6 are substrates for both isozymes (Table 4.1).

![Phenethylamine 8](image1) ![Selegiline 10](image2) ![Chlorgyline 136](image3)

**Table 4.1** MAO-A and MAO-B substrate specificities in human cerebral cortex and inhibitor sensitivities in rat frontal cortex.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MAO-A</th>
<th>MAO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ ($\mu$M)</td>
<td>$V_{max}$</td>
</tr>
<tr>
<td>5-HT§</td>
<td>137 ± 24</td>
<td>228 ± 31</td>
</tr>
<tr>
<td>PEA§</td>
<td>140 ± 22</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>DA§</td>
<td>212 ± 33</td>
<td>680 ± 123</td>
</tr>
<tr>
<td>NE§</td>
<td>284 ± 17</td>
<td>561 ± 42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50}$ (nM)</th>
<th>$IC_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorgyline*</td>
<td>1.0 (0.7-1.3)</td>
<td>700 (600-800)</td>
</tr>
<tr>
<td>selegiline*</td>
<td>1400 (700-310)</td>
<td>5.8 (3.4-9.9)</td>
</tr>
</tbody>
</table>

§ Values extracted from Youdim et al.; $V_{max}$ is measured in pmol/min/mg protein.
* Values extracted from Saura et al.; 95% confidence interval is shown in parentheses.
In 1988, cloning experiments conducted by Bach and coworkers\textsuperscript{148} unequivocally proved that MAO-A and MAO-B were distinct polypeptides coded for by separate genes on the X chromosome. The genes shared a 71% common sequence identity.

The first X-ray crystal structure of MAO was reported in 2002,\textsuperscript{149} being that of pargyline-inhibited human MAO-B. A report of the X-ray structure of rat MAO-A soon followed.\textsuperscript{151} Both enzymes were revealed to be dimeric and possessed similar chain folds, as expected from their high sequence homology. Subsequently human MAO-B\textsuperscript{152,153} and human MAO-A\textsuperscript{154} have been co-crystallised with a variety of other inhibitors and the resultant complexes have also been structurally resolved.

X-ray crystallographic data have provided insights into the mechanism of MAO catalysis, extending the knowledge acquired from earlier kinetics experiments.\textsuperscript{150,155} Previously there had been two preferred postulates, a single electron transfer (SET) mechanism\textsuperscript{156} and a polar nucleophilic mechanism.\textsuperscript{157,4} Current structural evidence is consistent with the latter.\textsuperscript{150,155}

The critical transformation of MAO catalysis via a polar nucleophilic mechanism, proposed by Edmondson and coworkers\textsuperscript{158} and supported by X-ray crystal data, is detailed in Figure 4.1. Nucleophilic attack of the substrate amine 138 at C(4a) of the enzyme-bound flavin coenzyme 137 yields the adduct 139. Proton abstraction by the flavin N(5) is likely to be a concerted transfer step, yielding an intermediate iminium ion 140 and the deprotonated flavin-bound enzyme 141.

\textsuperscript{*} X-ray crystallography studies were enabled by developments in MAO production. The methylotrophic yeast \textit{Pichia pastoris} expression system produces high levels of human recombinant MAO-B. This was needed for successful crystallisation of the enzyme.\textsuperscript{150}

\textsuperscript{†} The postulated mechanisms of MAO catalysis are from work conducted on MAO-B. The homology of the two isozymes and the presence of an identical flavin cofactor have led to the assumption that both enzymes function by similar mechanisms.\textsuperscript{158}
**Monoamine Oxidase Inhibitors as Pharmacotherapeutics**

Monoamine oxidase inhibitors (MAOIs) are a class of compounds that have historically demonstrated significant therapeutic utility. In the 1950s, iproniazid 142 was the first MAOI to be successfully clinically employed, being used for the treatment of depression. This was the dawn of modern psychiatric pharmacotherapy.\(^{159}\) Since then MAOIs have been primarily used in the treatment of psychiatric disorders (depressive and anxiety disorders) and neurological diseases (Parkinson’s and Alzheimer’s disease), as illustrated in Table 4.2.\(^{160}\) The variety of clinical applications of MAOIs reflects the important role MAOs serve in CNS development and function.\(^{160}\)
Table 4.2 Selected MAO inhibitors and their therapeutic utility.

<table>
<thead>
<tr>
<th>Name</th>
<th>Isozyme Selectivity</th>
<th>Inhibition Type</th>
<th>Target Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>selegiline†</td>
<td>MAO-B</td>
<td>irreversible</td>
<td>Parkinson’s disease/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anxiety disorders</td>
</tr>
<tr>
<td>rasagiline</td>
<td>MAO-B</td>
<td>irreversible</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>ladostigil</td>
<td>MAO-A and MAO-B</td>
<td>irreversible</td>
<td>Alzheimer’s disease,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depression</td>
</tr>
<tr>
<td>tranylcypromine†</td>
<td>MAO-A and -B</td>
<td>irreversible</td>
<td>Depression</td>
</tr>
<tr>
<td>amiflamine†</td>
<td>MAO-A</td>
<td>reversible</td>
<td>Depression</td>
</tr>
<tr>
<td>chlorgyline</td>
<td>MAO-A</td>
<td>irreversible</td>
<td>Depression</td>
</tr>
</tbody>
</table>

† These drugs belong to the amphetamine class.

Most simplistically, the therapeutic activity of MAOIs derives from potentiation of a requisite amine by inhibiting its degradation.144 For example, selegiline acts to potentiate dopamine by inhibiting its degradation.† Because of dopamine’s central role in normal movement, dopamine potentiation allows normal movement to be temporarily restored in PD patients.162

Recent discoveries and insights into the structure, mechanism and inhibition of MAOs have served to reinvigorate research in the field of MAOs and their inhibitors.146,163-166 The therapeutic potential of MAOIs has been the subject of a recent review by Youdim et al.146 They observed that as the intricacies of neurotransmitter and MAO function continue to be unfurled, the invaluable nature of MAOs as a drug target and the clinical scope of MAOIs are certain to increase.

† Dopamine potentiated by selegiline can be endogenous or derived from the decarboxylation of levodopa, when selegiline is prescribed as an adjunct to levodopa therapy.161
Amphetamines as Monoamine Oxidase Inhibitors

Many MAOIs possess the amphetamine scaffold; examples include the clinical pharmacotherapies amiflamine 143, tranylcypromine 144 and selegiline 10. Indeed, amphetamines exhibit potent and diverse modes of MAO inhibition. Amphetamine 3 itself is a competitive, reversible and selective MAO-A inhibitor. In this respect amphetamine exhibits stereoselectivity, (S)-amphetamine being the eutomer. MDMA is also a competitive, reversible and selective MAO-A inhibitor. However unlike amphetamine, MDMA does not exhibit stereoselectivity (Table 4.3).

Selegiline 10 is the N-propargyl derivative of (R)-methamphetamine (R)-2. Selegiline possesses potent and selective MAO-B inhibitory activity, being a one thousand-fold more potent inhibitor than (R)-methamphetamine in this respect (Table 4.3). The N-propargyl moiety is well known for imparting MAO inhibition activity.
Chapter Four

Table 4.3 MAO inhibition values for the enantiomers of MDMA and amphetamine.

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>( K_i ) (( \mu \text{M} \pm \text{SD} ))</th>
<th>IC\textsubscript{50} (( \mu \text{M} \pm \text{SEM} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-Amphetamine</td>
<td>203 ± 7</td>
<td>44 ± 6.06</td>
</tr>
<tr>
<td>(S)-Amphetamine</td>
<td>33.8 ± 4</td>
<td>378 ± 6.29</td>
</tr>
<tr>
<td>MAO-A</td>
<td>180 ± 3</td>
<td>370 ± 4.86</td>
</tr>
<tr>
<td>MAO-B</td>
<td>161 ± 32</td>
<td>56 ± 8.24</td>
</tr>
</tbody>
</table>

*Values reported by Leonardi and Azmitia.\textsuperscript{168}
†Values reported by Robinson.

Purpose of the Study

The MDMA analogues documented in Chapters One, Two and Three were assayed to determine their MAO-A and MAO-B inhibitory activity. The purpose of these experiments was three-fold:

1) To establish if the observed therapeutic activity of MDMA and its analogues might, in part, be due to MAO inhibition and therefore potentiation of the endogenous monoamines 4, 5 and 6.

2) To determine some fundamental SARs, namely if structural modification of MDMA caused an increase in either potency or selectivity for MAO inhibition of either isozyme when compared to the parent compound.

3) Additionally MAOs are valuable drug targets in their own right.\textsuperscript{171} Accordingly a suite of putative MAOIs, incorporating the \( N \)-propargyl moiety, was conceived (Chart 4.1). Studies of a similar nature, examining the effects of structural modification of propargylamines on MAOI-B, have been previously reported.\textsuperscript{169,170}

Braun and coworkers\textsuperscript{47} demonstrated that 95 lacks a psychoactivity profile in human subjects. It was reasoned therefore that if the anti-LID and anti-parkinsonian profile of the parent structure MDMA could be retained, whilst MAO inhibitory activity was enhanced and psychoactivity and abuse liability attenuated, a potentially useful dual PD therapeutic may be realised.
Experimental Considerations

MAO assays have been the subject of extensive review in Current Protocols in Pharmacology\textsuperscript{172} and elsewhere.\textsuperscript{173,174} There are numerous methods available for screening MAOIs. All of the methods rely on direct or indirect monitoring of either reactant consumption or product formation in a MAO-catalysed oxidation (Scheme 4.1).

In planning the assay the major considerations were: the source of the enzyme to be used; the number of compounds being tested (~ thirty five); the availability of equipment and the overall operational simplicity of the selected assay. Existing methods were evaluated with respect to the identified considerations; the most appropriate method was required to be sensitive, operationally simple and amenable to testing and screening a small library of analogues.
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Polarographic methods, used to detect oxygen consumption, lacked the necessary sensitivity.\textsuperscript{172} Radiochemical methods whilst sensitive and robust were not considered owing to a lack of operational simplicity. Working with radioactive substances would necessitate specialist training and equipment and incur unnecessary safety issues.

The number of compounds to be assessed favoured an assay that was amenable to high-throughput screening. Spectrophotometric and fluorimetric assays are amenable to high-throughput screening because both can be performed using multi-well plates on plate-reading instruments.\textsuperscript{172}

A survey of the literature for studies of a similar nature to the one proposed, MAOI screening of an analogue library, found a number of occurrences. These utilised a variety of methods including LC/MS/MS\textsuperscript{175} and HPLC detection of metabolites,\textsuperscript{165,176,177} radiochemical assay\textsuperscript{178} and fluorimetric assays.\textsuperscript{164,179} The most promising report was of a one-step fluorescence assay for high throughput screening of MAOIs by Guang and Du.\textsuperscript{179} This was further investigated, the details of which are presented below.

In 1997, Zhou and coworkers\textsuperscript{180} reported a stable H$_2$O$_2$ probe, Amplex Red 145 (10-acetyl-10H phenoxazine-3,7-diol); they simultaneously published a one-step fluorometric MAO assay utilising 145.\textsuperscript{181} The assay works via indirect measurement of H$_2$O$_2$ using a horseradish peroxidase (HRP)-coupled reaction system. Hydrogen peroxide generated from MAO-catalysed oxidation (Scheme 4.1) oxidises 145 to Resofurin 146 (7-hydroxy-3H-phenoxazin-3-one) in the presence of catalytic HRP (Scheme 4.2). Resofurin is readily detected by fluorimetry, allowing MAO activity to be indirectly quantified.
Zhou's method possesses excellent sensitivity, being shown to detect commercial MAO-B activity as low as $1.2 \times 10^{-5}$ U/ml, using reaction volumes of only 100 µL. This method has been subsequently validated as a simple and sensitive assay for high-throughput screening of putative MAOIs.\textsuperscript{179}

The Amplex Red fluorimetric assay satisfied all the strategic requirements: it was sensitive, operationally simple and amenable to testing of a library of analogues. Using this assay the MAO-A and MAO-B inhibitory activities of the MDMA analogues prepared in \textit{Chapters One, Two and Three} were determined.
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Results and Discussion

Source of Enzyme

The enzyme assays were initially attempted using a crude suspension of bovine heart mitochondria as the source of MAO-A and MAO-B. The mitochondrial suspension was prepared with fresh material obtained from a slaughterhouse using the procedure reported by Blair. Amplex Red assays were performed using the known MAO inhibitors selegiline 10 and chlorgyline 136 but reasonable inhibition values (IC₅₀) could not be obtained. Considerable substrate deamination was consistently observed in the presence of the inhibitors (10⁻⁶ M) at all the assayed enzyme dilutions. This was postulated to be owing to the presence of semicarbazide-sensitive amine oxidase (SSAO) in the enzyme preparation. SSAO deaminates both the substrates, benzylamine and p-tyramine, yielding hydrogen peroxide and is known to be present in high proportion in bovine smooth cardiac muscle. Semicarbazide (10⁻⁴ M) was added to the assay to inhibit SSAO present; despite this the observed interference was not corrected. Owing to this difficulty, the crude mitochondrial preparation was abandoned as the enzyme source. The issue was rectified using purer, commercially obtained MAO-A and MAO-B.

Optimisation of the Assay Methodology

Initially the assay methodology was optimised for a number of parameters: the concentration of MAO, the concentration of horse radish peroxidase (HRP) and the concentration of substrate. The validity of the optimised conditions was then evaluated by determining the IC₅₀ values of the known MAO inhibitors selegiline 10 and chlorgyline 136.

The optimal concentration of MAO-A for each assay was determined to be 5.0 x 10⁻¹ U/mL (final concentration in assay). At this concentration resofurin fluorescence was detected and demonstrated a linear relationship over the 60 minutes of continuous fluorescence intensity measurements.

Additionally, complete inhibition could be effected with the selective MAO-A inhibitor chlorgyline
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136 (10⁻⁶ M). At higher MAO-A concentrations resofurin fluorescence intensity was not linear over the measured time period nor could complete inhibition be effected with chlorglyline 136 (10⁻⁶ M). At lower MAO-A concentration detection of resofurin fluorescence intensity was problematic.

MAO-B assays were performed using the same concentration of enzyme (5.0 x 10⁻¹ U/mL). Complete inhibition of MAO-B with the selective MAO-B inhibitor selegiline 10 (10⁻⁶ M) could be effected at this concentration.

The ability to detect the resofurin fluorescence intensity and a demonstrated linearity of fluorescence intensity over the 60 minutes of continuous measurements were used to determine optimal concentration of HRP (1.0 U/mL) and the substrate (1.0 mM) for each assay.

The IC₅₀ values of selegiline 10 and chlorglyline 136 were determined using the optimised assay conditions and compared to values cited in the literature (Table 4.4). The IC₅₀ values corresponded with those reported by Guang and Du, using an Amplex Red fluorescence method. These researchers noted that their values matched those reported using traditional assays.

Table 4.4 Experimentally determined IC₅₀ values for MAO inhibitors chlorglyline and selegiline.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MAO-A IC₅₀ (nM ± 95% CI)</th>
<th>Lit.¹⁷⁹ (nM)</th>
<th>MAO-B IC₅₀ (nM ± 95% CI)</th>
<th>Lit.¹⁷⁹ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selegiline 10</td>
<td>-</td>
<td>-</td>
<td>5.75 ± 4.02-6.96</td>
<td>7.04</td>
</tr>
<tr>
<td>Chlorglyline 136</td>
<td>3.58 ± 2.53-5.06</td>
<td>2.99</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹⁷⁹ Guang and Du.
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**Determination of the Kinetic Parameters $K_m$ and $V_{\text{max}}$ of MAO-A and MAO-B**

Using the optimised experimental conditions the kinetic parameters $K_m$ and $V_{\text{max}}$ for MAO-A and MAO-B were determined. The Michaelis-Menten plots for both enzymes are shown below (Figure 4.2). For the substrate $p$-tyramine, MAO-A displayed a Michaelis constant ($K_m$) of $117 \pm 20 \, \mu\text{M}$ (lit. $107 \pm 15 \, \mu\text{M}$) and a maximum reaction velocity ($V_{\text{max}}$) of $154 \pm 6 \, \text{nmol/min/mg protein}$. For the substrate benzylamine, MAO-B displayed a Michaelis constant of $170 \pm 12 \, \mu\text{M}$ (lit. $130 \, \mu\text{M}$) and a maximum reaction velocity of $28 \pm 1 \, \text{nmol/min/mg protein}$.

![Michaelis-Menten Curves for MAO-A and MAO-B](image)

**Figure 4.2** Michaelis-Menten Curves for MAO-A (left) and MAO-B (right).

**Monoamine Oxidase Inhibition Studies**

MDMA and its analogues were initially screened for the presence of MAO-A and MAO-B inhibitory activity at a concentration of $100 \, \mu\text{M}$ of the test compound. For analogues that exhibited inhibition at this concentration further characterisation was undertaken: inhibition assays were performed at seven or eight concentrations, a dose-response curve prepared and the $IC_{50}$ value
calculated. Compounds that exhibited no inhibition at 100 \mu M of the test compound are reported as having no inhibitory effect (NIE). The MAO-A and MAO-B IC\textsubscript{50} values for MDMA and its analogues are reported below (Tables 4.5-4.9).

**Table 4.5 MAO IC\textsubscript{50} values for N-substituted MDMA analogues.**

![Diagram of MDMA analogues]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R\textsubscript{1}</th>
<th>MAO-A IC\textsubscript{50} (\mu M ± 95% CI)</th>
<th>MAO-B IC\textsubscript{50} (\mu M ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>Me</td>
<td>H</td>
<td>293 (200-429)</td>
<td>638 (573-709)</td>
</tr>
<tr>
<td>(R)-MDMA</td>
<td>Me</td>
<td>H</td>
<td>162 (191-204)</td>
<td>569 (484-670)</td>
</tr>
<tr>
<td>(S)-MDMA</td>
<td>Me</td>
<td>H</td>
<td>116 (75-178)</td>
<td>703 (665-784)</td>
</tr>
<tr>
<td>94</td>
<td>Me</td>
<td>Me</td>
<td>277 (226-341)</td>
<td>NIE*</td>
</tr>
<tr>
<td>147*</td>
<td>Et</td>
<td>H</td>
<td>399 (311-512)</td>
<td>NIE</td>
</tr>
<tr>
<td>148*</td>
<td>Pr</td>
<td>H</td>
<td>951 (582-1560)</td>
<td>NIE</td>
</tr>
<tr>
<td>149*</td>
<td>i-Pr</td>
<td>H</td>
<td>965 (456-2040)</td>
<td>NIE</td>
</tr>
<tr>
<td>150*</td>
<td>Bu</td>
<td>H</td>
<td>650 (386-1100)</td>
<td>NIE</td>
</tr>
<tr>
<td>96</td>
<td>allyl</td>
<td>H</td>
<td>228 (172-302)</td>
<td>NIE</td>
</tr>
<tr>
<td>98</td>
<td>Bn</td>
<td>H</td>
<td>154 (121-197)</td>
<td>NIE</td>
</tr>
<tr>
<td>97</td>
<td>Ph</td>
<td>H</td>
<td>687 (462-1020)</td>
<td>47.4 (35.8-62.7)</td>
</tr>
</tbody>
</table>

*No inhibitory effect (NIE) at 10^{-5} M.

* Compounds prepared by Hung Nguyen at The University of Western Australia.
### Table 4.6 MAO IC\textsubscript{50} values for α-cyclopropyl MDMA analogues.

![Chemical structure](attachment:image.png)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>MAO-A IC\textsubscript{50} ((\mu)M ± 95% CI)</th>
<th>MAO-B IC\textsubscript{50} ((\mu)M ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Me</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>(R)-18</td>
<td>Me</td>
<td>430 (304-609)</td>
<td>NIE</td>
</tr>
<tr>
<td>(S)-18</td>
<td>Me</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>110</td>
<td>i-Pr</td>
<td>488 (285-837)</td>
<td>NIE</td>
</tr>
</tbody>
</table>

### Table 4.7 MAO IC\textsubscript{50} values for α-phenyl MDMA analogues.

![Chemical structure](attachment:image.png)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>MAO-A IC\textsubscript{50} ((\mu)M ± 95% CI)</th>
<th>MAO-B IC\textsubscript{50} ((\mu)M ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>76</td>
<td>H</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>21</td>
<td>Me</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>99</td>
<td>Et</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>100</td>
<td>Pr</td>
<td>842 (623-1140)</td>
<td>NIE</td>
</tr>
<tr>
<td>102</td>
<td>i-Pr</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>101</td>
<td>Bu</td>
<td>760 (383-1510)</td>
<td>NIE</td>
</tr>
<tr>
<td>104</td>
<td>allyl</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>106</td>
<td>Bn</td>
<td>65.5 (51.0-84.3)</td>
<td>292 (204-417)</td>
</tr>
<tr>
<td>107</td>
<td>Ph</td>
<td>61.5 (39.2-96.4)</td>
<td>NIE</td>
</tr>
</tbody>
</table>
Table 4.8 MAO IC\textsubscript{50} values for α-aryl MDMA analogues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>MAO-A IC\textsubscript{50} (\mu M ± 95% CI)</th>
<th>MAO-B IC\textsubscript{50} (\mu M ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>4-biphenyl</td>
<td>65.2 (58.1-73.3)</td>
<td>5.00 (4.02-6.21)</td>
</tr>
<tr>
<td>31</td>
<td>1-naphthyl</td>
<td>173 (122-245)</td>
<td>NIE</td>
</tr>
<tr>
<td>30</td>
<td>2-naphthyl</td>
<td>362 (261-502)</td>
<td>NIE</td>
</tr>
<tr>
<td>27</td>
<td>3-furyl</td>
<td>NIE</td>
<td>NIE</td>
</tr>
<tr>
<td>26</td>
<td>2-thienyl</td>
<td>NIE</td>
<td>NIE</td>
</tr>
</tbody>
</table>
### Table 4.9 MAO IC$_{50}$ values for N-propargyl MDMA analogues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MAO-A IC$_{50}$ (µM ± 95% CI)</th>
<th>MAO-B IC$_{50}$ (µM ± 95% CI)</th>
<th>MAO-B selectivity$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td><img src="image1" alt="Structure" /></td>
<td>0.388 (0.366-0.446)</td>
<td>0.112 (0.086-0.145)</td>
<td>3.5</td>
</tr>
<tr>
<td>(S)-111</td>
<td><img src="image2" alt="Structure" /></td>
<td>15.7 (12.4-20.0)</td>
<td>2.47 (2.08-2.94)</td>
<td>6.4</td>
</tr>
<tr>
<td>(R)-111</td>
<td><img src="image3" alt="Structure" /></td>
<td>0.209 (0.168-0.259)</td>
<td>0.078 (58-104)</td>
<td>2.7</td>
</tr>
<tr>
<td>112</td>
<td><img src="image4" alt="Structure" /></td>
<td>111 (88-140)</td>
<td>206 (149-285)</td>
<td>0.54</td>
</tr>
<tr>
<td>95</td>
<td><img src="image5" alt="Structure" /></td>
<td>544 (412-719)</td>
<td>55.3 (20.8-147)</td>
<td>9.8</td>
</tr>
<tr>
<td>109</td>
<td><img src="image6" alt="Structure" /></td>
<td>393 (295-524)</td>
<td>82.2 (62.8-108)</td>
<td>4.8</td>
</tr>
<tr>
<td>105</td>
<td><img src="image7" alt="Structure" /></td>
<td>NIE</td>
<td>102 (78-135)</td>
<td>-</td>
</tr>
</tbody>
</table>

$^*$MAO-B selectivity calculated as IC$_{50}$(MAO-A) / IC$_{50}$(MAO-B).
Chapter Four

As demonstrated previously by Leonardi et al., MDMA and its enantiomers are selectively potent for MAO-A inhibition (Table 4.5). This trend for selective MAO-A inhibition was observed in the majority of the evaluated analogues, with the following exceptions: 97 (Table 4.5), 29 (Table 4.8) and most notably, albeit unsurprisingly, analogues possessing an N-propargyl moiety (Table 4.9). The analogue most selective for MAO-B inhibition is 97, with a calculated MAO-B selectivity of 14.5; the selective analogue 97 exhibits only modest potency for MAO-B inhibition (IC₅₀ 47.4 μM).

Somewhat predictably most of the evaluated MDMA analogues exhibit MAO inhibitory activity approximately equipotent, if not less potent, than the parent compound MDMA (Tables 4.5-4.8). Indeed the majority of analogues exhibited no inhibitory effect (NIE) at 100 μM. For the remaining analogues the IC₅₀ values were, for the most part, modest, being in the micromolar range only. This result is not without significance; the analogue 18 and its enantiomers (R)-18 and (S)-18 lack appreciable MAO-B inhibitory activity. It is not possible therefore that their observed efficacy as PD therapeutics stems from dopamine potentiation in this manner.

A comparison of the various N-propargyl MDMA analogues (Table 4.9) illustrates that several other structural elements influence MAO inhibition: 1) the nature of the α-substituent; 2) the substitution pattern of the amine; 3) the chirality of the α-stereocentre.

Possessing a larger α-substituent fractionally negates the MAO-B inhibitory activity imparted by an N-propargyl moiety. The analogues 109 (82.2 μM) and 105 (102 μM), possessing an α-cyclopropyl and α-phenyl moieties, respectively, both exhibit less potent MAO-B inhibition than 95 (55.3 μM), which has an α-methyl moiety.

N-Methylation of the propargylamine results in a dramatic increase in both MAO-A and MAO-B inhibitory activity, as seen in a comparison of IC₅₀ values obtained for analogues 95 (MAO-A IC₅₀ 544 μM and MAO-B IC₅₀ 55.3 μM) and 111 (MAO-A IC₅₀ 388 nM and MAO-B IC₅₀ 112 nM).

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§ MAO-B selectivity calculated as IC₅₀ (MAO-A) / IC₅₀ (MAO-B).
Disubstitution of the amine is itself not critical for enhancing potency of MAO inhibition. The $N,N$-dipropargyl analogue 112 has a lower MAO-B inhibitory activity than the $N$-propargyl analogue 95, having MAO-B IC$_{50}$ values of 206 μM and 55.3 μM, respectively.

The stereoselectivity of MAO-B inhibition by propargylamines is highlighted by the IC$_{50}$ values obtained for the enantiomers (S)-111 (2.47 μM) and (R)-111 (78 nM). The observed eudismic ratio is 32, which is approximately equal to the eudismic ratio for the enantiomers of selegiline reported by Robinson.$^{167}$

The $N$-propargyl analogues 111 and (R)-111 were the most potent inhibitors of MAO-B having IC$_{50}$ values of 112 nM and 78 nM, respectively (Table 4.9). The appreciable activity of the analogues was however greater than ten-fold less potent than selegiline 10 assayed under the same conditions (IC$_{50}$ 5.75 nM, Table 4.4). This observation contradicts the conclusions of Knoll and coworkers,$^{185}$ who found that exchanging the benzene ring of selegiline 10 with an alternate aromatic moiety had no significant influence on the observed MAO-B inhibitory effect.

Future studies could include co-crystallisation of MAO-B with (R)-111 and structural resolution, to provide insight into the mechanism of inhibition; which, this author postulates, is likely to be analogous to the mechanism of MAO-B inhibition by selegiline 10.

The observation that analogues 111 and (R)-111 exhibit appreciable MAOI activity warrants further investigation of these compounds as potential therapeutics for PD. Ideally further study would demonstrate that 111 and (R)-111 retain the remarkable anti-dyskinetic activity of the parent compound MDMA but lack a psychoactive profile, making them suitable for a clinical application. Dually the analogues would act to potentiate dopamine in the substantia nigra of the PD patient via their MAO-B inhibitory activity and thereby act as a symptomatic treatment and lower the dose of levodopa required to restore normal movement.
Chapter Four

Overview

A fluorescence assay for MAO inhibition studies was optimised. Under the optimised conditions the kinetic parameters of human MAO-A (\(K_m\) 117 ± 20 \(\mu\)M, \(V_{\text{max}}\) 154 ± 6 nmol/min/mg protein) and human MAO-B (\(K_m\) 170 ± 12 \(\mu\)M, \(V_{\text{max}}\) 28 ± 1 nmol/min/mg protein) were determined. MDMA and its analogues were assayed for MAO inhibition activity. Compounds that demonstrated a degree of inhibition at a concentration 100 \(\mu\)M were subjected to further evaluation and IC\(_{50}\) values calculated. The novel analogues 111 (MAO-A IC\(_{50}\) 388 nM and MAO-B IC\(_{50}\) 112 nM) and (\(R\))-111 (MAO-A IC\(_{50}\) 209 nM and MAO-B IC\(_{50}\) 78 nM) were the most potent with respect to MAO-A and MAO-B inhibition activity.
Chapter Four

Experimental

Reagents

Amplex Red Monoamine Oxidase Assay Kits were purchased from Invitrogen. Selegiline hydrochloride was purchased from Sigma-Aldrich.

MOA-B (human, recombinant, expressed in baculovirus infected BTI insect cells: 5.0 mg/mL protein by Lowry, kynuramine deamination enzymatic activity 23 U/mg protein) and MAO-A (human, recombinant, expressed in baculovirus infected BTI insect cells: 5.0 mg/mL protein by Lowry, kynuramine deamination enzymatic activity 92 U/mg protein) were purchased from Sigma-Aldrich. One unit (1 U) of kynuramine deamination activity is defined as deamination of 1 nanomole of kynuramine per minute at pH 7.4 at 37 °C.

Horse radish peroxidase was purchased from Invitrogen as part of the Amplex Red Monoamine Oxidase Assay Kit. One unit (1 U) is defined as the amount of enzyme that will form 1 milligram of purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20 °C.

Prior to assay, the purity of all amine hydrochlorides synthesised in Part One was determined by elemental analysis (C H N) performed by Robertson Microlit Laboratories (New Jersey, USA).

Instrumentation

Fluorimetry was performed using a BMG Labtech FLUOstar Optima multi-well plate reader and recorded with BMG Labtech Optima Software version 2.10 R2. Resofurin fluorescence was measured at excitation (544 nm) and emission (590 nm).
Chapter Four

Experimental Analysis and Statistics

Determination of the enzyme kinetic parameters, $K_{\text{m}}$, and $V_{\text{max}}$, was performed with Grafit 7 using non-linear regression analysis; all measurements were performed in triplicate.

MAO IC$_{50}$ calculations were performed with GraphPad Prism 5 using non-linear regression analysis of the dose response curve (fractional activity vs log concentration of the inhibitor). A minimum of seven drug concentrations per curve were used; all measurements were performed in triplicate.

MAO-B selectivity ratios for the test compounds were calculated as IC$_{50}$ (MAO-A)/IC$_{50}$ (MAO-B).

General Procedure for Amplex Red Fluorescence Assay

Assays were conducted in a 96-well microplate; total reaction volume for each well was 200 µL.

Each assay contained the enzyme (MAO-A or MAO-B) in pH 7.4 sodium phosphate buffer (90 µL, 1.1 x 10$^{-1}$ U/mL). The final concentration of enzyme in each assay was 5.0 x 10$^{-2}$ U/mL. The enzyme was pre-incubated with milliQ H$_2$O (10 µL) or with the test compound in milliQ H$_2$O (10 µL) for 30 minutes at 37 °C. At the end of the pre-incubation period Amplex Red mixture (100 µL) was added to each assay. Amplex Red mixture contained Amplex Red (400 µM), HRP (2 U/mL) and the substrate, benzylamine for MAO-B or p-tyramine for MAO-A in pH 7.4 sodium phosphate buffer (2.0 mM). The contents of each well were protected from light and mixed by agitation on an oscillating table for 1 minute, followed by incubation at 37 °C. Resofurin fluorescence was measured as either an endpoint measurement, 45 minutes after the addition of the Amplex Red mixture, or as continuous measurements conducted for a 1 hour period at 5 minute intervals from the addition of the Amplex Red mixture.

Additionally for each 96-well microplate the following wells were prepared and measured according to the above described procedure:
A negative enzyme control contained pH 7.4 sodium phosphate buffer (90 µL), milliQ H₂O (10 µL) and the Amplex Red mixture (100 µL).

A negative inhibition control contained the enzyme (MAO-A or MAO-B) in pH 7.4 sodium phosphate buffer (90 µL, 1.1 x 10⁻¹ U/mL), milliQ H₂O (10 µL) and the Amplex Red mixture (100 µL).

A positive MAO-A inhibition control contained MAO-A in pH 7.4 sodium phosphate buffer (90 µL, 1.1 x 10⁻¹ U/mL), chloroglyline in milliQ H₂O (10 µL, 20 µM) and the Amplex Red mixture (100 µL).

A positive MAO-B inhibition control contained MAO-B in pH 7.4 sodium phosphate buffer (90 µL, 1.1 x 10⁻¹ U/mL), selegiline in milliQ H₂O (10 µL, 20 µM) and the Amplex Red mixture (100 µL).

A background fluorescence correction was made by subtracting the value for the negative enzyme control (MAO-A or MAO-B, as appropriate) from each measurement for each test compound.

The enzyme activity was expressed by either of two methods:

1) As a percentage of the activity relative to the control experiment conducted simultaneously without addition of the test compound (negative inhibition control);

2) By quantification of the product formed. A seven point resofurin standard curve (0.00 µM, 1.00 µM, 2.50 µM, 5.00 µM, 7.50 µM, 10.0 µM, 12.5 µM) was prepared using pH 7.4 sodium phosphate buffer, which allowed the measured fluorescence intensity to be converted into the amount of resofurin produced.
**Chapter Four**

**Optimisation of MAO Inhibition Assays**

**Enzyme Optimisation**

The enzyme optimisation experiment was performed using a modification of the *General Procedure for Amplex Red Fluorescence Assay*. Six concentrations of MAO-A were assayed. The final concentrations of enzyme in the assays were 1.0 U/mL, 5.0 x 10^-1 U/mL, 1.0 x 10^-1 U/mL, 5.0 x 10^-2 U/mL, 1.0 x 10^-2 U/mL, 5.0 x 10^-3 U/mL, 1.0 x 10^-3 U/mL and 5.0 x 10^-4 U/mL. For each MAO-A concentration, the enzyme in pH 7.4 sodium phosphate buffer (90 µL) was pre-incubated with chlorglyline (10 µL, 10^-6 M) and alternatively milliQ H2O (10 µL) for 30 minutes at 37 °C, after which time Amplex Red mixture (100 µL) was added. Continuous resofurin fluorescence measurements were conducted for a 1 hour period at 5 minute intervals from the addition of the Amplex Red mixture. For each enzyme concentration inhibition, was expressed as the percentage of the activity relative to the control experiment conducted simultaneously without addition of chlorglyline (negative inhibition control).

**Substrate Optimisation**

Substrate optimisation experiments were performed using a modification of the *General Procedure for Amplex Red Fluorescence Assay*. The Amplex Red mixture was prepared containing varying amounts of the substrate, benzylamine for MAO-B or p-tyramine for MAO-A (2.0 mM, 1.5 mM, 1.0 mM, 0.50 mM, 0.20 mM, 0.10 mM, 0.05 mM). Continuous resofurin fluorescence measurements were conducted for a 1 hour period at 5 minute intervals from the addition of the Amplex Red mixture. Enzyme activity was quantified by equating measured fluorescence with resofurin formation using a seven-point resofurin standard curve.
Horse Radish Peroxidase Optimisation

Horse radish peroxidase (HRP) optimisation experiments were performed using a modification of the General Procedure for Amplex Red Fluorescence Assay; five concentrations of HRP were assayed. The final concentrations of HRP in the assays were 1.0 U/mL, 5.0 x 10^{-1} U/mL, 2.0 x 10^{-1} U/mL, 1.0 x 10^{-1} U/mL and 0.0 x 10^{-1} U/mL. Continuous resofurin fluorescence measurements were conducted for a 1 hour period at 5 minute intervals from the addition of the Amplex Red mixture. Enzyme activity was quantified by equating measured fluorescence with resofurin formation using a seven-point resofurin standard curve.

Determination of the Kinetic Parameters $K_m$ and $V_{max}$ for MAO-A and MAO-B

For each enzyme (MAO-A and MAO-B) the kinetic parameters $K_m$ and $V_{max}$ were determined using a modification of the General Procedure for Amplex Red Fluorescence Assay. The enzyme was pre-incubated with milliQ H$_2$O (10 μL) for 30 minutes at 37 °C. The Amplex Red mixture was prepared containing varying amounts of the substrate, benzylamine for MAO-B or p-tyramine for MAO-A (2.0 mM, 1.5 mM, 1.0 mM, 0.50 mM, 0.20 mM, 0.10 mM, 0.05 mM). Continuous resofurin fluorescence measurements were conducted for a 1 hour period at 5 minute intervals from the addition of the Amplex Red mixture. Enzyme activity was quantified by equating measured fluorescence with resofurin formation using a seven-point resofurin standard curve.

Monoamine Oxidase Inhibition Assays

Determination of Enzyme Inhibition Activity of a Test Compound at 0.10 mM

The enzyme inhibition activities (MAO-A and MAO-B) for each test compound were determined using the General Procedure for Amplex Red Fluorescence Assay. For each test compound a final concentration of 0.10 mM was used. For the test compounds 29, 30, 31, 99, 101, 102, 104, 105, 106 and 107, the stock solutions (10^{-2} M) were prepared in DMSO owing to limited aqueous solubility.
of the compounds. For test compounds whose stock solutions were made up in DMSO, positive and negative controls were made to contain an equal quantity of DMSO. Endpoint resofurin fluorescence was measured 45 minutes after the addition of the Amplex Red mixture. The enzyme activity was expressed as the percentage of the activity relative to the control experiment conducted simultaneously without addition of the test compound (negative inhibition control). Test compounds that possessed no measurable enzyme inhibition activity at 0.10 mM were not subjected to further assays.

**Determination of IC$_{50}$ Values of a Test Compound**

The IC$_{50}$ value for each test compound for each enzyme (MAO-A and MAO-B) was determined using the General Procedure for Amplex Red Fluorescence Assay. For each test compound seven or eight serial dilutions were used. The final concentrations ranged from $10^{-3}$ M to $10^{-10}$ M. For the known inhibitors chlorgyline and selegiline, ten serial dilutions were used. Final concentrations ranged from $10^{-5}$ M to $10^{-13}$ M. Endpoint resofurin fluorescence was measured, 45 minutes after the addition of the Amplex Red mixture. The enzyme activity was expressed as the percentage of the activity relative to the control experiment conducted simultaneously without addition of the test compound (negative inhibition control).
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Epilogue

**MDMA analogues as cytotoxins and pro-apoptotic agents in B-cell lymphomas**

The amine hydrochloride analogues detailed in *Chapters One and Two* were sent to Prof. John Gordon and coworkers at the MRC Centre for Immune Regulation at The University of Birmingham, UK. Evaluation of the compounds as cytotoxins and pro-apoptotic agents in B-cell malignancies was performed in a number of *in vitro* cell assays. Attention was also given to determining the mechanisms and pathways to cell death.

The analogues 29 and 30 possessed the greatest L3055 BL cell killing potency, having IC\textsubscript{50} values of 6.0 ± 1 μM and 6.6 ± 0.4 μM, respectively. This is an appreciable (100-fold) increase in potency compared to the parent compound MDMA (IC\textsubscript{50} 0.2-1.0 mM).

These compounds represent the most promising new leads toward the effort to produce MDMA analogues with enhanced BL cytotoxic efficacy.

Some analogue syntheses and preliminary biological results have been communicated in ‘Redesigning the designer drug ecstasy: non-psychoactive MDMA analogues exhibiting Burkitt’s lymphoma cytotoxicity’ published in *MedChemComm* (Appendix). A second article further detailing these findings was published in *Investigational New Drugs*, under the title ‘Enhancing the anti-lymphoma potential of 3,4-methylenedioxymethamphetamine (‘ecstasy’) through iterative chemical redesign: mechanisms and pathways to cell death’ (Appendix).

**MDMA analogues in the treatment of Parkinson’s disease**

The amine hydrochloride analogues prepared in *Chapters One, Two and Three* were sent to Dr Jonathon Brotchie and coworkers at the Toronto Western Research Institute at Toronto Western Hospital, Canada. The anti-parkinsonian and anti-dyskinesia benefit of some analogues was assessed in a reserpine-treated rat model of PD and MPTP-lesioned primates with levodopa-induced dyskinesia. The pharmacological binding profile of analogues was assessed *in vitro* across a select...
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panel of relevant transporter and receptor targets, including SERT, DAT, NET and various 5-HT receptors. Serotonergic neurotoxicity of analogues was characterised by in vitro assay in a serotonergic cell line, human colorectal adenocarcinoma CCL-220 cells. Further characterisation and evaluation of select analogues as chemotherapeutics for PD is ongoing.

The syntheses and biological studies of the enantiomers of MDMA has been published in ‘Characterisation of 3,4-methylenedioxymethamphetamine (MDMA) enantiomers in vitro and in the MPTP-lesioned primate: R-MDMA reduces severity of dyskinesia whereas S-MDMA extends duration of ON-time’ in The Journal of Neuroscience (Appendix). The key findings in animal model studies were that when motor disability (parkinsonism and dyskinesia) and duration of anti-parkinsonian benefit was evaluated following the administration of (R)-MDMA and (S)-MDMA, the data was suggestive that the primary mechanism of the anti-dyskinetic action of racemic MDMA is likely to derive from the partial 5-HT₂A agonism by (R)-MDMA. In addition, the SERT/DAT inhibition activity of (S)-MDMA is likely responsible for the action of MDMA to extend anti-parkinsonian action of levodopa.

Additionally during the course of this PhD it has been determined by Brotchie and coworkers that the analogue 109 has demonstrated exceptional in vitro activity as an inhibitor of the dopamine transporter (DAT). The modification of 18 by inclusion of an N-propargyl moiety has served to impart both DAT and MAO-I inhibitory activity. This exciting finding warrants further development and investigation of 109 as a potential therapeutic for PD and disease states whose pathology is characterised by dopamine deficiency.
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References
References


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References


References


(68) Lewis, K. D., Synthesis of α-Substituted Analogues of MDMA ('Ecstasy'), Honours Thesis: University of Western Australia, 2005.

(69) Aldabbagh, F. Ketones Bearing an α,β-Aryl or -Heteroaryl substituent; Elsevier: Amsterdam, 2005; Vol. 3.


References

References

(122) Berry, M. B.; Craig, D. Synlett 1992, 41-44.
References

References


References


Appendix
Appendix

Crystallographic Data for (R)-18.HCl and (S)-18.HCl

The crystal data for (R)-18.HCl and (S)-18.HCl are summarized in Table A.2. Crystallographic data for the structures were collected at 100(2) K on an Oxford Diffraction Xcalibur diffractometer fitted with Mo Kα radiation. The structures were refined against $F^2$ with full-matrix least-squares using the program SHELXL-97. All H-atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atom. Anisotropic displacement parameters were employed for the non-hydrogen atoms. The value of the Flack parameter for (R)-18.HCl refined to -0.01(5), thus defining the absolute stereochemistry. The value of the Flack parameter for (S)-18.HCl refined to 0.00(5), thus defining the absolute stereochemistry. Relevant geometric parameters are given in Table A.3.

Figure A.1 Molecular structure of (S)-18.HCl. Cationic species only shown. Non-hydrogen ellipsoids have been shown at the 50% probability level. Hydrogen atoms are denoted as circles of arbitrary size.
Appendix

Crystallographic Data for (R)-MDMA.HCl and (S)-MDMA.HCl

The crystal data for (R)-MDMA.HCl and (S)-MDMA.HCl are summarized in Table A.1. Crystallographic data for the structures were collected at 110(2) K on an Oxford Diffraction Gemini diffractometer fitted with Cu Kα radiation. The structures were refined against $F^2$ with full-matrix least-squares using the program SHELXL-97. The amine H atoms were allowed to refine freely. All remaining H atoms were added at calculated positions and refined by use of a riding model with isotropic displacement parameters based on those of the parent atom. Anisotropic displacement parameters were employed for the non-hydrogen atoms. The value of the Flack parameter for (R)-MDMA.HCl refined to -0.009(16), thus defining the absolute stereochemistry. The value of the Flack parameter for (S)-MDMA.HCl refined to -0.003(14), thus defining the absolute stereochemistry. Relevant geometrical parameters are given in Table A.3.

Figure A.2 Molecular projection of (S)-MDMA.HCl. Cationic species only shown. The projection is approximately onto the plane of the rings. Non-hydrogen ellipsoids have been drawn at the 50% probability level. Hydrogen atoms are denoted as circles of arbitrary size.
Table A.1 Crystal data and structural refinements for (R)-MDMA.HCl and (S)-MDMA.HCl.

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<th>(S)-MDMA.HCl</th>
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<td>C\textsubscript{11}H\textsubscript{16}ClNO\textsubscript{2}</td>
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<td>\textit{P}2\textsubscript{1}</td>
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<td>Unit cell dimensions</td>
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<td>2</td>
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<td>1.280 Mg/m\textsuperscript{3}</td>
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<td>Crystal size</td>
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Appendix

Table A.2 Crystal data and structural refinements for (R)-18.HCl and (S)-18.HCl.

<table>
<thead>
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<th>Parameter</th>
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<td>( P_2_1 )</td>
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<tr>
<td></td>
<td>( c = 10.7362(4) \text{ Å} )</td>
<td>( c = 10.7293(3) \text{ Å} )</td>
</tr>
<tr>
<td></td>
<td>( \beta = 101.733(3)^\circ )</td>
<td>( \beta = 101.639(3)^\circ )</td>
</tr>
<tr>
<td>Volume</td>
<td>662.65(4) Å(^3)</td>
<td>661.20(3) Å(^3)</td>
</tr>
<tr>
<td>( Z )</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Density</td>
<td>1.282 Mg/m(^3)</td>
<td>1.284 Mg/m(^3)</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.279 mm(^{-1})</td>
<td>0.279 mm(^{-1})</td>
</tr>
<tr>
<td>( F(000) )</td>
<td>272</td>
<td>272</td>
</tr>
<tr>
<td>Crystal size</td>
<td>( 0.21 \times 0.08 \times 0.03 \text{ mm}^3 )</td>
<td>( 0.31 \times 0.08 \times 0.04 \text{ mm}^3 )</td>
</tr>
<tr>
<td>( \theta ) range for data collection</td>
<td>3.35 to 36.13(^\circ)</td>
<td>3.35 to 37.44(^\circ)</td>
</tr>
<tr>
<td>Index ranges</td>
<td>(-14 \leq h \leq 14, -11 \leq k \leq 11, -16 \leq l \leq 17)</td>
<td>(-14 \leq h \leq 14, -12 \leq k \leq 12, -16 \leq l \leq 18)</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>13185</td>
<td>15969</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>5608 [R(int) = 0.0499]</td>
<td>6486 [R(int) = 0.0527]</td>
</tr>
<tr>
<td>Completeness to ( \theta )</td>
<td>( \theta = 35.75^\circ, 98.9% )</td>
<td>( \theta = 37.00^\circ, 98.4% )</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
<td>multi-scan</td>
</tr>
<tr>
<td>Max. / min. transmission</td>
<td>1.00/0.94</td>
<td>1.00/0.92</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on ( F^2 )</td>
<td>Full-matrix least-squares on ( F^2 )</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>5608 / 1 / 156</td>
<td>6486 / 1 / 155</td>
</tr>
<tr>
<td>Goodness-of-fit on ( F_2 )</td>
<td>0.850</td>
<td>0.881</td>
</tr>
<tr>
<td>Final R indices ([I&gt;2\sigma(I)])</td>
<td>( R_1 = 0.0463, wR_2 = 0.0691 )</td>
<td>( R_1 = 0.0501, wR_2 = 0.0746 )</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>( R_1 = 0.0994, wR_2 = 0.0816 )</td>
<td>( R_1 = 0.1092, wR_2 = 0.0898 )</td>
</tr>
<tr>
<td>Absolute structural parameter</td>
<td>0.01(5)</td>
<td>(-0.15(5))</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.818 and (-0.308 \text{ e.Å}^{-3})</td>
<td>0.816 and (-0.408 \text{ e.Å}^{-3})</td>
</tr>
</tbody>
</table>
### Appendix

Table A.3 Crystallographic data for relevant hydrogen bond distances and angles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>N-H…Cl</th>
<th>Distance (N-H)(Å)</th>
<th>Distance (H-Cl)(Å)</th>
<th>Distance (N…Cl)(Å)</th>
<th>Angle (NHCl)(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-MDMA.HCl</td>
<td>N(1)-H(1A)...Cl(1)</td>
<td>0.93(3)</td>
<td>2.18(3)</td>
<td>3.103(2)</td>
<td>171(2)</td>
</tr>
<tr>
<td></td>
<td>N(1)-H(1B)...Cl(1)</td>
<td>0.95(2)</td>
<td>2.17(3)</td>
<td>3.1154(18)</td>
<td>173(2)</td>
</tr>
<tr>
<td>(S)-MDMA.HCl</td>
<td>N(1)-H(1A)...Cl(1)</td>
<td>0.92(3)</td>
<td>2.18(3)</td>
<td>3.0992(17)</td>
<td>174.4(19)</td>
</tr>
<tr>
<td></td>
<td>N(1)-H(1B)...Cl(1)†</td>
<td>0.92(2)</td>
<td>2.20(2)</td>
<td>3.1154(15)</td>
<td>175.1(18)</td>
</tr>
<tr>
<td>(R)-18.HCl</td>
<td>N(1)-H(1A)...Cl(1)</td>
<td>0.92</td>
<td>2.19</td>
<td>3.1099(13)</td>
<td>176.1</td>
</tr>
<tr>
<td></td>
<td>N(1)-H(1B)...Cl(1)†</td>
<td>0.92</td>
<td>2.24</td>
<td>3.1363(15)</td>
<td>165.7</td>
</tr>
<tr>
<td>(S)-18.HCl</td>
<td>N(1)-H(1A)...Cl(1)</td>
<td>0.92</td>
<td>2.23</td>
<td>3.1328(12)</td>
<td>165.7</td>
</tr>
<tr>
<td></td>
<td>N(1)-H(1B)...Cl(1)</td>
<td>0.92</td>
<td>2.19</td>
<td>3.1099(10)</td>
<td>176.9</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms: † -x+1,y+1/2,-z; ‡ -x+1,y-1/2,-z+2; *2-x,y-1/2,1-z; † -x, y/2+y,1-z

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Appendix
Burkitt's lymphoma (BL) is a particularly aggressive cancer that primarily affects African children. Unfortunately, effective and affordable treatment is out of reach of most of the afflicted. The illicit psychoactive drug methylenedioxymethamphetamine (MDMA, 'ecstasy') is cytotoxic to BL cell lines, but its low potency, psychoactivity and neurotoxicity preclude consideration as a therapeutic drug candidate. This paper describes the discovery of novel α-aryl analogues of MDMA that lack psychoactivity and reduce BL cell line viability with significantly more potency than the lead compound. Preliminary in vitro studies also indicate that the compounds are non-toxic to a relevant neuronal cell line.

Introduction

Burkitt’s lymphoma (BL) is an extremely aggressive cancer that primarily affects children and young adults. The sporadic form of the disease is rare, with an incidence of 0.002-0.003% of population per year, although its prevalence has increased rapidly in recent times due to the susceptibility of HIV-infected individuals, who have a 1000-fold greater incidence relative to the general population. In equatorial Africa, where it is associated with Epstein-Barr virus and malarial infection, BL is endemic and the most common childhood cancer, with a frequency of 0.005-0.020%. As the population of the ‘lymphoma belt’ exceeds 500 million, this equates to 25-100,000 cases per year. Endemic BL commonly manifests as horrific facial tumours, which can double in size in one day (Fig. 1).

Current therapies for BL involve aggressive combination chemotherapy and frequent hospitalisation. While offering cure rates of 80%, these therapeutic regimes are not readily accessible to poor Africans, are not nearly as effective in AIDS sufferers and patients with disseminated tumours, and are associated with significant toxicity. Accordingly, there is an urgent demand for BL treatments that are cheaper, more efficacious and more amenable to patient compliance. Despite this need, BL is a very low priority disease for pharmaceutical companies due to its low profitability.

Thus, it seems that the burden of BL drug discovery falls on academia. Indeed, some reasonably simple compounds with very potent activity against BL cell lines, notably 2-benzoxazolylhydrazones and α-styrylbenzylsulfones, have recently been discovered by academic research groups. However, given the many barriers to successful drug development, a multipronged attack is probably required.

In 2005, the illicit drug MDMA ('ecstasy', 1) (Fig. 2) was shown to induce apoptosis in BL cell lines. Although MDMA is cheap to make and has excellent pharmacokinetic properties, it is not suitable for development as a treatment for BL. Firstly, its potency with respect to killing BL cells (IC50 0.2-1 mM) is...
Methylenedioxymethamphetamine (MDMA, ‘ecstasy’). The hydrochloride is the most commonly ‘marketed’ form. Inadequate. Secondly, MDMA is both psychoactive and neurotoxic. Accordingly, we set out to discover MDMA analogues with enhanced anti-BL potency and selectivity but without psychoactivity and neurotoxicity. Herein we detail our initial progress towards this goal. Although the structure-psychoactivity relationships of MDMA analogues have been explored, to the best of our knowledge, the use of MDMA as a lead compound in a medicinal chemistry program has not been reported previously.

Target choice

Our rationale for the choice of MDMA analogue targets has been guided by the brave and extensive work of Shulgin and coexperimenters. Two salient observations were apparent from their studies: extension of the α- or β-substituent of MDMA to anything larger than an ethyl group abolished psychoactivity (at least anecdotally). As part of our goal was to dissociate psychoactivity from anti-BL activity, modification of these positions provided a good starting point. Herein we focus on the α-substituent; our investigation of variously N-substituted MDMA analogues is ongoing.

Synthesis

Because of the wide interest in amphetamine derivatives, abundant syntheses of the simpler members of the family exist, most of which proceed via reductive amination of benzyl ketones. Two of the most common routes to such ketones involve Knoevenagel-Walter condensation (an Henry reaction followed by dehydration) to give a β-nitrostyrene, followed by reductive hydrolysis; or epoxidation of a styrene followed by acid catalysed rearrangement. While the latter strategy lacks efficiency because of an early divergent step, and is probably not applicable to regioselective synthesis of benzyl aryl ketones, we have found that the Knoevenagel-Walter reaction lacks scope (see also ref. 16). For example, the reaction of α-nitrotoluene (2) with piperonal (4) failed to give the required β-nitrostyrene 5 under a variety of conditions (Fig. 3). This is presumably due to the combined effects of the stereoelectronically-stabilised intermediate nitronate 3 and relatively unreactive, electron-rich aldehyde 4. Accordingly, a more general and efficient synthetic strategy was investigated.

Our initial plan involved the reaction of the known piperonylmagnesium chloride (6) with various nitriles, which, after hydrolytic workup, would give a series of piperonyl ketones (7) (Fig. 4). In practice, the reaction of 6 with benzonitrile gave the desired acetophenone 7g in good yield, but with butyronitrile the yield of the ketone 7c was only 29%, presumably due to competing α-deprotonation, as supported by the isolation of the proto-demetalated product, 3,4-methylenedioxytoluene. An attempt to attenuate the basicity of the organometallic reagent by transmetallation with ceric chloride failed to improve the yield of 7e. Furthermore, heteroaromatic nitriles lacking an α-proton also gave very low yields of the corresponding ketones. Knochel and co-workers have shown that benzylic organozinc reagents can be transmetallated with the THF-soluble complex CuCN.2LiCl, and that the resulting organocuprates react cleanly with acid chlorides to give benzyl ketones in excellent yields. However, they were unable to prepare piperonylzinc halides, as homocoupling predominates. This problem was solved by using a piperonylzinc phosphate.


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We have found that the organocuprate 8 derived from the Grignard reagent 6 also adds smoothly to acid chlorides (Fig. 5). Thus, various acid chlorides were added, in parallel, to solutions of the organocuprate 8, giving the piperonyl ketones 7 in fair to excellent yields (Tables 1 and 3). The reaction of 8 with phenylacetyl chloride gave an intractable mixture, thus benzyl piperonyl ketone (7p) was prepared using Baldwin’s acyl anion-equivalent incorporation as per ref. 9; mean value ± SEM, n = 3 except for 1 and 7g, where n = 14 and 8, respectively.

Results and discussion

Series 1 analogues: Anti-BL activity

MDMA (1) and the analogues llb-t were assessed for toxicity to the L3055 Burkitt’s lymphoma cell line.\(^7\) Initial studies began with llb-g (Table 1), which were chosen to explore the effects of an incremental increase in the size of the α-substituent. The n-Ph analogue 11g was the standout performer, being almost seven times more potent than MDMA. Before making analogues of 11g, it was important to establish whether the negative attributes of MDMA had been retained in this compound, and thus it was assessed for potential to be psychoactive and neurotoxic.

Psychoactivity

MDMA was originally described as inducing “an easily controlled altered state of consciousness with emotional and sensual overtones”.\(^24\) However, it is most likely the associated intense euphoria that has made MDMA such a popular recreational drug.\(^13\) Whilst mild euphoria might be a side-effect with some benefit in a cancer treatment, the powerful psychoactivity of MDMA is probably excessive for this purpose and has abuse liability. Thus, as mentioned above, one of our goals was to dissociate this psychoactivity from the BL cytotoxicity.

We assessed potential psychoactivity with prepuke inhibition of the acoustic startle reflex (PPI), which is a reduction in the magnitude of the obligatory startle reflex induced by loud abrupt sounds produced by preceding the startling sound with a quiet, non-startling sound (the prepulse), by 100 ms in the present case. In this study, a wide range of intensities of startling stimuli were used, from below threshold to the asymptotic range, and we measured the prepulse-induced reduction in the asymptotic magnitude of response.\(^27,28\)

A reduction in PPI is a widely replicated endophenotype of schizophrenia (for reviews see ref. 27–29), which may correlate with psychotic symptoms.\(^30–32\) In particular, we have observed prepuke-induced inhibition of the asymptotic startle magnitude in patients with schizophrenia,\(^14\) and decreased PPI of this measure in people is associated with poor attention and inhibitory control of irrelevant stimuli in the Stroop test.\(^33\) The most common animal models of psychosis produce a similar PPI deficit\(^34\) with psychotomimetic or hallucinogenic drugs,\(^35–37\) including dopaminergic drugs like amphetamines,\(^38\) MDMA\(^39\) or other psychotomimetics such as N-methyl-D-aspartate (NMDA) receptor antagonists like phencyclidine, ketamine and MK801.\(^40–44\) On the other hand, antipsychotic drugs have been shown to reverse the effects of some of these psychotomimetic compounds on PPI,\(^45\) and some antipsychotics increase PPI on their own.\(^46\) Thus, drug-induced reductions in PPI in rats correlate well with their psychotomimetic and hallucinogenic effects, and PPI provides an objectively quantifiable and reliable measure of psychoactivity of MDMA and related compounds. para-Methoxymetamphetamine (PMA) was included as a control as it is a related amphetamine analogue that has extremely potent hallucinogenic effects, and PPI provides an objectively quantifiable and reliable measure of psychoactivity of MDMA and related compounds.

As indicated in Fig. 6, the human-psychoactive drugs MDMA and PMA reduce prepulse inhibition in a dose-dependent manner. Conversely, compound 11g clearly shows the opposite trend, marginally increasing prepulse inhibition, much like some antipsychotic drugs (see above). These data strongly suggest that 11g does not exhibit psychoactivity. By extrapolation, other MDMA analogues with large α-substituents (see below) are also unlikely to be psychoactive (and therefore were not tested for this purpose).

Receptor binding studies

In a preliminary investigation of the mode-of-action against the L3055 cell line, and to explain its lack of psychoactivity, the binding of 11g to a panel of receptors and transporters was assessed. MDMA is known to interact directly with several serotonin (5-hydroxytryptamine, SHT) receptors,\(^45–46\) as well as

Table 1 Series 1 MDMA analogues: yields of piperonyl ketones and reductive aminations (see Fig. 5); IC\(_{50}\) data = BL cells for the hydrochlorides

<table>
<thead>
<tr>
<th>R</th>
<th>Ketone % Yield</th>
<th>Amine % Yield</th>
<th>IC(_{50}) nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me</td>
<td>7a</td>
<td>85</td>
<td>1</td>
</tr>
<tr>
<td>Et</td>
<td>7b</td>
<td>87</td>
<td>1</td>
</tr>
<tr>
<td>Pr</td>
<td>7c</td>
<td>85</td>
<td>1</td>
</tr>
<tr>
<td>c-Pr</td>
<td>7d</td>
<td>65</td>
<td>1</td>
</tr>
<tr>
<td>i-Pr</td>
<td>7e</td>
<td>84</td>
<td>1</td>
</tr>
<tr>
<td>Hg</td>
<td>7f</td>
<td>64</td>
<td>1</td>
</tr>
<tr>
<td>Ph</td>
<td>7g</td>
<td>97</td>
<td>11g</td>
</tr>
</tbody>
</table>

* Yields are based upon on Grignard concentration (determined by titration).\(^9\) The optimized conditions were not used for all reactions, hence the variable yields. \(^*\) Based on reduced [%H]thymidine incorporation as per ref. 9; mean value ± SEM, n = 3 except for 1 and 7g, where n = 14 and 8, respectively.
Fig. 6 Effect of MDMA, p-methoxyamphetamine (PMA) and 11g on prepulse inhibition of R\textsubscript{MAX} (maximum response achievable from a subject under a given drug and prepulse condition). Dose-response curves were fitted using the equation \( y = y_0 + (R_{\text{MAX}} - y_0)(1 + 10^{(x - x_0)/\text{ES50}}) \), where \( y_0 \) was defined by the percent prepulse inhibition under the control condition, and \( R_{\text{MAX}} \) by the percent prepulse inhibition at the maximum dose. Dose-response curves for MDMA and PMA showed good agreement with observed values (\( r^2 = 0.862 \) and 0.966, respectively), while that for 11g did not (\( r^2 = 0.261 \)). *Significantly different from MDMA and PMA, \( p < 0.05 \); + significantly different from saline (y-intercept), \( p < 0.05 \).

Fig. 7 Effect of MDMA and 11g on SH-SY5Y cell viability. SH-SY5Y cells were exposed to various concentrations of MDMA or 11g (1, 30, 300, 600 \( \mu \text{M} \)) for 24 h. Cell viability was assessed using Alamar Blue. Data are presented as mean ± SEM (\( n = 6 \)). Two way analysis of variance using concentration and compound as factors showed significant effects of concentration (\( p < 0.001, F_4 = 8.10 \)) and compound (\( p < 0.001, F_3 = 92.37 \)) and a significant interaction between the two (\( p < 0.001, F_{4,12} = 9.44 \)). Bonferroni post hoc showed significant effects of MDMA at 1-600 \( \mu \text{M} \), and 11g at 300 and 600 \( \mu \text{M} \), on cell viability compared to vehicle (media). ##, ### indicates a \( p < 0.01 \) and \( p < 0.001 \), respectively for 11g compared to vehicle. **, *** indicates a \( p < 0.01 \) and \( p < 0.001 \), respectively for MDMA compared to vehicle.

**Table 2** Neuroreceptor/transporter binding affinity (\( K_i \) in \( \mu \text{M} \)) of MDMA and 11g.*

<table>
<thead>
<tr>
<th>Receptor/transporter</th>
<th>MDMA</th>
<th>11g</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHT\textsubscript{1A}</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>SHT\textsubscript{1D}</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>SHT\textsubscript{2C}</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>SHT\textsubscript{1B}</td>
<td>1.23 ± 0.71*</td>
<td></td>
</tr>
<tr>
<td>NET</td>
<td>&gt;10</td>
<td>0.70 ± 0.2</td>
</tr>
<tr>
<td>SERT</td>
<td>0.21 ± 0.2</td>
<td>1.32 ± 0.4</td>
</tr>
<tr>
<td>DAT</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

* The affinity is provided as the half-maximal inhibitory constant (\( K_i \) in \( \mu \text{M} \)). The error is the SEM of the \( K_i \) provided by triplicate experiments. * \( P = 0.04 \) (Student \( t \) test).
Table 3  Series 2 MDMA analogues: yields of piperonyl ketones and reductive aminations (see Fig. 5); IC<sub>50</sub> data for the hydrochlorides

<table>
<thead>
<tr>
<th>R</th>
<th>Ketone</th>
<th>% Yield&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Amine</th>
<th>% Yield</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;/μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>7h</td>
<td>57</td>
<td>11h</td>
<td>76</td>
<td>65 ± 8</td>
<td></td>
</tr>
<tr>
<td>7i</td>
<td>88</td>
<td>11i</td>
<td>83</td>
<td>51 ± 6</td>
<td></td>
</tr>
<tr>
<td>7j</td>
<td>67</td>
<td>11j</td>
<td>83</td>
<td>42 ± 3</td>
<td></td>
</tr>
<tr>
<td>7k</td>
<td>58</td>
<td>11k</td>
<td>86</td>
<td>92 ± 8</td>
<td></td>
</tr>
<tr>
<td>7l</td>
<td>68</td>
<td>11l</td>
<td>74</td>
<td>80 ± 3</td>
<td></td>
</tr>
<tr>
<td>7m</td>
<td>49</td>
<td>11m</td>
<td>64</td>
<td>69 ± 3</td>
<td></td>
</tr>
<tr>
<td>7n</td>
<td>71</td>
<td>11n</td>
<td>70</td>
<td>63 ± 2</td>
<td></td>
</tr>
<tr>
<td>7o</td>
<td>64</td>
<td>11o</td>
<td>85</td>
<td>81 ± 8</td>
<td></td>
</tr>
<tr>
<td>7p</td>
<td>36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11p</td>
<td>88</td>
<td>69 ± 4</td>
<td></td>
</tr>
<tr>
<td>7q</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11q</td>
<td>67</td>
<td>36.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>7r</td>
<td>63</td>
<td>11r</td>
<td>70</td>
<td>12.6 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>7s</td>
<td>75</td>
<td>11s</td>
<td>84</td>
<td>6.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>7t</td>
<td>71</td>
<td>11t</td>
<td>83</td>
<td>6 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Based upon 100% conversion of piperonyl chloride into organocuprate B; as the actual conversion is somewhat lower, the yields shown here are lower estimates.<br><sup>b</sup> Prepared using Baldwin’s acyl anion equivalent methodology from 9.<br><sup>c</sup> Prepared by self-condensation of homopiperonylic acid (10). Mean value ± SEM, n = 3 except for 11q where n = 2.

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is not always sufficient to predict in vivo toxicity, especially where metabolism and hyperthermia are implicated,66 the reduced toxicity of 11g relative to MDMA was encouraging, and led us to adopt it as the new lead compound.

Series 2 analogues
The enhanced potency and reduced toxicity of 11g to the neuronal cell line, coupled with the likelihood that it is not psychoactive, made it the basis for further structural modifications. Series 2 analogues that explored tolerance to steric (11b-j) and (stereo)electronic (11k-o) modifications to, and reduction (11o) or extension (11p) of, the phenyl substituent did not have a dramatic effect on activity (Table 3). However, a modest improvement in potency as like substituents were 'shifted' from the ortho-meta-para position (11b-m) was noted.

Symmetrical analogue 11q was roughly twice as potent as the series 2 lead 11g, which could reflect an effective doubling of concentration due to the absence of chirality, or simply an enhancement due to a larger a-substituent. The latter reasoning is certainly supported by the results for 11r-s, which are approximately ten fold more potent than 11g, and nearly two orders of magnitude more potent than MDMA.

Neurotoxicity – series 2 analogues
Two of the most promising leads, 11r and 11s, were assessed for toxicity to the SH-SYSY cell line alongside MDMA, as described above for 11g. As indicated in Fig. 8, there was no significant effect of 11r or 11s on cell viability compared to vehicle. This suggests that modifications that enhance toxicity to the BL cell line, namely incorporation of large aromatic a-substituents, also eliminate inherent neurotoxicity. Of course, further studies are required to see if the lack of toxicity in this cell-based assay is replicated in animal models.

Conclusion
Readily synthesised analogues of the illicit, psychoactive drug MDMA bearing alternative substituents (to methyl) at the a-position, reduced viability of L3055 Burkitt’s lymphoma (BL) cells at micromolar concentrations. Pre-pulse inhibition studies indicate that the most potent series 1 analogue 11g, with an a-phenyl substituent, is very unlikely to be psychoactive. However, 11g is toxic to the dopaminergic neuroblastoma cell line SH-SYSY, albeit less so than MDMA. Series 2 analogues, based on 11g (11h-11l), also killed BL cells, in some instances (11r-11t) with low micromolar potency. Furthermore, 11r and 11s exhibited no toxicity in the dopaminergic cell line and are unlikely to be psychoactive. Accordingly, 11r and 11s are promising new drug leads for Burkitt’s and related lymphomas.

Our efforts to further improve selective BL-killing potency and determine the mode of action of this class of compounds are ongoing. Future studies will include an examination of the importance of configuration (i.e. evaluation of individual enantiomers as opposed to the racemates used in this study), receptor/transporter functionality studies for the more potent analogues, further optimisation of the a-substituent, and modification of other parts of the amphetamine skeleton. The toxicity of these compounds to a broader range of cancerous cells lines is also of interest.

Moreover, in this paper we have shown, for the first time, that it is possible to dissociate the psychoactivity exhibited by MDMA from one of its useful biological properties. This opens the door to the use of MDMA as a lead compound in other indications for which it has shown promising activity; for example, in Parkinson’s disease,66 and post-traumatic stress disorder.4,67

Acknowledgements
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References
Characterization of 3,4-Methylenedioxymethamphetamine (MDMA) Enantiomers In Vitro and in the MPTP-Lesioned Primate: R-MDMA Reduces Severity of Dyskinesia, Whereas S-MDMA Extends Duration of ON-Time

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3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective treatment for Parkinson’s disease, but long-term L-DOPA administration is marred by the emergence of motor complications, namely, dyskinesia and a shortening of antiparkinsonian benefit (wearing-OFF). 3,4-Methylenedioxymethamphetamine (MDMA) is unique in that it exerts antidyskinetic effects and may enhance antiparkinsonian actions of L-DOPA. MDMA is composed of two enantiomers with different pharmacological profiles; here, we describe a novel enantiomer-specific synthesis of the two enantiomers and expand on the previous characterization of their pharmacology. R-MDMA (rectus-MDMA) is relatively selective for 5-HT2A receptors, whereas S-MDMA (sinister-MDMA) inhibits both serotonin (SERT) and dopamine transporters (DAT; SERT/DAT ratio of 10 to 1). R- or S-MDMA (1, 3, and 10 mg/kg, s.c.) was administered in combination with L-DOPA (15 mg/kg, s.c.) to female common marmosets (Callithrix jaccus) rendered parkinsonian by MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) administration. Motor disability, including parkinsonism and dyskinesia, and duration of antiparkinsonian benefit (ON-time) were evaluated. After the administration of R-MDMA (3 and 10 mg/kg), the severity of peak-dose dyskinesia was decreased (by 33 and 46%, respectively; p < 0.05); although total ON-time was unchanged (~220 min), the duration of ON-time with disabling dyskinesia was decreased by 90 min when compared to L-DOPA alone (69% reduction; p < 0.05). S-MDMA (1 mg/kg) increased the total ON-time by 88 min compared to L-DOPA alone (34% increase; p < 0.05), though dyskinesia were exacerbated. These data suggest that racemic MDMA exerts simultaneous effects, reducing dyskinesia and extending ON-time, by 5-HT2A antagonism and SERT-selective mixed monoamine uptake inhibition, which arise from its R and S enantiomers, respectively.

Introduction

The motor manifestations of Parkinson’s disease (PD) are caused by striatal dopamine depletion (Hornykiewicz and Kish, 1987). Dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) is the most effective treatment against the motor symptoms of PD. However, with increased duration of therapy, motor complications develop and afflict 95% of patients after 15 years (Hely et al., 2005). In an anecdotal report (BBC, 2001), 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”), a widely used drug of abuse, reduced severity of L-DOPA-induced dyskinesia and increased duration of antiparkinsonian benefits (ON-time) in a patient with early-onset PD. Of these two actions, the antidyskinetic activities of MDMA were confirmed in subsequent animal studies. Thus, when added to L-DOPA therapy, MDMA reduced dyskinesia-like behaviors in 6-hydroxydopamine (6-OHDA)-lesioned rats (Bishop et al., 2006) and dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset (Irwani et al., 2003). The precise mechanism by which MDMA alleviates dyskinesia is unknown. MDMA is composed of two enantiomers with distinct pharmacological profiles. R-MDMA (rectus-MDMA) has affinity for the serotonergic type 2A (5-HT2A) receptors (Lyon et al., 1986), at which it acts as a low efficacy partial agonist (Nash et al., 1994). S-MDMA (sinister-MDMA) binds to the serotonin (5-HT; SERT), dopamine (DAT), and noradrenaline transporters (NET), inhibits monoamine uptake, and reverses the action of the transporters.
Huot et al. • target agonists clozapine and quetiapine reduced dyskinesia- associated behaviors in the 6-OHDA-lesioned rodent and dyskinesia in the MPTP-lesioned primate (Oh et al., 2002; Visani et al., 2006). In human studies, low-dose clozapine alleviated dyskinesia (Durif et al., 2004). The S-HT_{1A} receptor inverse agonist pimavanserin (ACP-103) also reduced dyskinesia in MPTP-lesioned macaques (Vanover et al., 2008) and human PD subjects (Roberts, 2006). On the other hand, an indirect action on S-HT_{1A} (serotoninergic type 1A) receptors, mediated by MDMAs-induced 5-HT release, was suggested to play a role in MDMAs antidyskinetic actions (Bishop et al., 2006); such a mechanism could be mediated by S-MDMAs.

This study was undertaken to determine which, if either, of the two enantiomers of MDMAs was responsible for the antidyskinetic properties of the compound. We hypothesized that the antidepressant action of racemic MDMAs was primarily mediated by its R enantiomer. In the current study, we executed a novel enantiospecific synthesis of R- and S-MDMAs; established their pharmacological profile in vitro at S-HT_{1A} receptors and monoamine transporters using selective, well-characterized ligands; and administered them, in combination with L-DOPA, to MPTP-lesioned common marmosets.

Materials and Methods

R- and S-MDMAs synthesis

Methodology developed by Nenajdenko et al. (2001) was adapted to the synthesis of R- and S-MDMAs. The key step involved the enantiospecific and high-yielding ring opening of the enantiopure aziridines 2 and 3, conveniently derived from L- and D-alanine, respectively, by the Grignard reagent 1 (Fig. 1). Subsequent methylation and detosylation provided R- and S-MDMAs, which were converted to their hydrochlorides for pharmacological characterization.

In vitro pharmacology

Tissue preparation. Female Sprague Dawley rats (250–275 g, Charles River) were housed, with access to food and water ad libitum, in a temperature (19–21°C), humidity (55%), and light-controlled (12 h light/dark cycle; lights on 7:00 A.M.) environment. All procedures were performed in accordance with an Institutional Animal Care and Use Committee (IACUC) approved by University Health Network Animal Care Committee and in accordance with the regulations defined by the Canadian Council on Animal Care. All reasonable efforts were made to reduce animal numbers used and minimize their suffering.

Rats were killed by decapitation after CO₂ narcosis. Brains were immediately removed and placed into ice-cold Krebs’ buffer containing the following (in mM): 134 NaCl, 5 KCl, 1.3 CaCl₂, 1 MgSO₄, 25 NaHCO₃, 1.25 KH₂PO₄, and 10 glucose. Various brain regions (cerebral cortex, striatum, and substantia nigra) were dissected on ice and placed separately into ice-cold Tris buffer, pH 7.4, and centrefuged for 20 min at 4°C (20,000 × g 

The receptor and transporter binding assays performed in the current study targeted only the receptor transporters to which racemic MDMAs was shown to display affinity in a previous experiment performed by our group (Gandy et al., 2010). Even though MDMAs did not display high affinity at S-HT_{1A} receptors in that experiment, we nevertheless performed S-HT_{1A} receptor binding for both enantiomers, as it has been suggested that MDMAs’ antidyskinetic effect involved a 5-HT_{1A}-mediated mechanism (Bishop et al., 2006). Binding parameters relating to brain region used, ligand and ion concentration, as well as incubation conditions employed for each of the assays are summarized in Table 1. Incubations were conducted in 380 μl 96-well plates. Brain membranes were incubated with R- and S-MDMAs (1 nM to 1 μM) in the presence of the radioligand.

After incubation, the membranes were rapidly washed in 50 μl Tris buffer, pH 7.4 (20 s wash at 10,000 X g) and filtered under vacuum through Whatman glass fiber (GF/F) filters (GE Healthcare Canada) presoaked in 50 μl Tris buffer, pH 7.4, using a cell harvester (Brandel). NET and DAT assays, GF/F filters were presoaked in 50 μl Tris buffer solution containing 0.1% polyethyleneimine (Sigma-Aldrich) and were washed with a 50 μl Tris solution containing 0.1% bovine serum albumin (Sigma-Aldrich). Filters were then immersed in scintillation fluid (40 μl Insta-Gel, Bevco) as counts per minute.

Determination of R- and S-MDMAs affinity at selected receptor/transporter values of displacement of R- and S-MDMAs were determined in three independent experiments, each in triplicate, and displacement was expressed as a percentage of specific binding. Dose–response curves were constructed and the half maximal inhibitory concentration (IC50) was determined via nonlinear regression analysis using the software GraphPad Prism 5.03. The inhibition constant (Ki) was calculated using the Cheng and Prusoff (1973) equation.

Behavioral assessment of R- and S-MDMAs in the MPTP-lesioned nonhuman primate

Induction of parkinsonism and dyskinesia in the common marmoset. Six female common marmosets (Callithrix jaccus Harlan) weighing 350–500 g were housed in groups of two or three under conditions of controlled temperature (25 ± 2°C) and a 12 h light/dark cycle (lights on at 8:00 A.M.) Animals were cared for in accordance with an IACUC approved by University Health Network Animal Care Committee and with the regulations defined by the Canadian Council on Animal Care. Animals had unlimited access to food, fresh fruit supplements, and water, and their home cage was enriched with primate toys, perches, and auditory stimuli. Before the start of studies, animals were acclimatized to handling, administered a standardized baseline treatment, as well as transfer to observation cages for assessment of behavior.

Animals were rendered parkinsonian by administration of MPTP hydrochloride (Sigma-Aldrich) according to a standard protocol (Silverdale et al., 2005) (2 mg/kg, s.c., daily, for 5 consecutive days). After MPTP treatment, marmosets entered a 12 week recovery period to allow parkinsonian symptoms to develop and stabilize. The animals included in the study exhibited a severe and homogeneous degree of parkinsonism.
Table 1. Experimental parameters for receptors/transporters binding assays

<table>
<thead>
<tr>
<th>Receptor/transporter</th>
<th>Brain tissue</th>
<th>Radioligand (concentration)</th>
<th>Non-specific displacer</th>
<th>Ionic conditions</th>
<th>Incubation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>cerebral cortex</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]-WAY-100,635 (3.0 nM)</td>
<td>B-OHPAT</td>
<td>60 min, ambient T°, in the presence of 10 nM L-745,870</td>
<td></td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>cerebral cortex</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]-ketanserin (2.5 nM)</td>
<td>(SKF 38393)</td>
<td>60 min, ambient T°, in the presence of 10 nM L-745,870</td>
<td></td>
</tr>
<tr>
<td>NET</td>
<td>cerebral cortex</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]-nisoxetine (2.0 nM)</td>
<td>(paroxetine)</td>
<td>60 min, ambient T°, in the presence of 10 nM L-745,870</td>
<td></td>
</tr>
<tr>
<td>SERT</td>
<td>ROB</td>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]-citalopram (2.5 nM)</td>
<td>(paroxetine)</td>
<td>60 min, ambient T°, in the presence of 10 nM L-745,870</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>striatum</td>
<td>(&lt;sup&gt;3&lt;/sup&gt;H)-GBR 12909 (3.0 nM)</td>
<td>(paroxetine)</td>
<td>60 min, ambient T°, in the presence of 10 nM L-745,870</td>
<td></td>
</tr>
</tbody>
</table>

The following compounds were used to determine the binding affinity: 5-HT<sub>1A</sub> (WAY-100,635, 5-HT<sub>2A</sub> (ketanserin), NET (nisoxetine), SERT (citalopram) and DAT (GBR 12909) at the concentrations indicated. Incubations were performed for 60 min at ambient temperature, in the presence of 10 nM L-745,870, washed and harvested for binding analysis. Results are expressed as the mean of triplicates ± standard deviation.

The dyskinesia score given reflected the most disabling dyskinesia observed, whether chorea or dystonia, in any 5 min period of assessment. Psychosis-like behaviors were rated in a similar manner to dyskinesia. The following behaviors were scored: hypokinesia, response to nonpostural stimuli (halucinatory behavior), repetitive grooming, and stereotypies. Each of these was rated from zero to four (0, absent; 1, mild; present <30% of time, and animal is still able to eat, drink, and perform normal activities; 2, moderate, present >30% of time, and animal is still able to eat, drink, and perform normal activities; 3, marked, present >30% of time, and animal is unable to eat, drink, and perform normal activities; 4, severe, present >90% of time, and animal is unable to eat, drink, and perform normal activities). For psychosis, the score attributed to each observation period was the most disabling of any of the four subscores observed in that 5 min period.

Parkinsonian disability, dyskinesia, and psychosis-like behavior scores were converted for each hour across the entire 6 h of observations and during the peak effect period (60–150 min after L-DOPA administration). The duration of antiparkinsonian benefit, i.e., ON-time, was defined as the number of minutes for which bradykinesia was absent. ON-time was further divided as "good" or "bad" quality, depending on the severity of dyskinesia present. Thus, good-quality ON-time was defined as the number of minutes when bradykinesia was zero, and dyskinesia were either absent, mild, or considered of low intensity (scores of 0, 1, and 2), whereas bad-quality ON-time was defined as the number of minutes during which bradykinesia was zero, and dyskinesia were either marked or severe (scores of 3 and 4). Similarly, ON-time with psychosis-like behavior was defined as the number of minutes for which bradykinesia was zero and psychosis-like behavior was either absent, mild, or moderate in intensity (scores of 0, 1, and 2), whereas ON-time with disabling psychosis-like behavior was defined as the number of minutes during which bradykinesia was zero and psychosis-like behavior was severe or marked (scores of 3 and 4).

Statistical analysis. Categorical, discontinuous scores for parkinsonian disability, dyskinesia, and psychosis-like behavior severity were analyzed using nonparametric Friedman's followed by Dunn's multiple comparison post hoc tests. ON-time data were analyzed by one-way repeated-measures (RM) ANOVA followed by Tukey's multiple comparison post hoc tests. Time-course data for parkinsonian disability and dyskinesia scores were ranked by marmoset across each of the four treatments and analyzed by a two-way RM ANOVA followed by Bonferroni's multiple comparison tests. Statistical significance was assigned when p < 0.05. Analyses were performed using GraphPad Prism 5.02.

Results.

Pharmacological profile of R- and S-MDMA

R-MDMA exhibited moderate affinity for 5-HT<sub>1A</sub> receptors (K<sub>i</sub> = 4.7 ± 1.1 µM) and lower affinity for SERT (K<sub>i</sub> = 24.5 ± 0.8 µM). R-MDMA exhibited no appreciable affinity for either 5-HT<sub>2A</sub> receptors, NET, or DAT (K<sub>i</sub> > 50 µM) (Table 2). In contrast, S-MDMA exhibited no affinity at 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (K<sub>i</sub> > 50 µM), but high affinity for SERT (K<sub>i</sub> = 222 ± 62 nm), and moderate affinity at both DAT and NET (K<sub>i</sub> = 2.3 ± 0.4 µM and 7.8 ± 2.1 µM, respectively) (Table 2).
Table 2. Enantiomers binding profiles

|            | 
|------------|------------|
| R-MDMA     | S-MDMA     |
| 5-HT1A     | 3.5±1.1    | 3.6±1.2 |
| 5-HT1B     | 310±70     | 78±21   |
| NET        | 2.2±0.6    | 2.1±0.4 |
| SERT       | 24±5.8     | 24±5.8  |

The affinity is provided as the mean Kd (μM) ± SEM of three independent experiments, each performed in triplicate.

Figure 2. A. Time course of dyskinesia in marmosets treated with L-DOPA and R-MDMA (1, 3, and 10 mg/kg) or vehicle. R-MDMA (3 and 16 mg/kg) significantly reduced the severity of L-DOPA-induced dyskinesia during the first 2 h of treatment, compared to L-DOPA vehicle treatment (p < 0.001 from 0–40 min; p < 0.01 from 40–60 min; and L-DOPA 1 mg/kg R-MDMA treatment; p < 0.05 for 10 mg/kg R-MDMA from 60–90 min, and p < 0.01 for 10 mg/kg R-MDMA from 90–120 min). Each point represents the cumulated dyskinesia score for every 5 min observation period during the preceding 60 min. The maximal possible score (most severe disability) was 216. On the y-axis, 54 is mild, 108 is moderate, 162 is marked, and 216 is severe. The cross on the graph indicates a time point for which there is significance.

B. Time course of parkinsonism in marmosets treated with L-DOPA and S-MDMA (1, 3, 10 mg/kg) or vehicle. S-MDMA had no effect on the antiparkinsonian action of L-DOPA (p > 0.05). Each time point represents the cumulated parkinsonism score for every 5 min observation period during the preceding 60 min. The maximal possible score (most severe disability) was 216. On the y-axis, 54 is mild, 108 is moderate, 162 is marked, and 216 is severe. C. Time course of parkinsonism in marmosets treated with L-DOPA and R-MDMA (1, 3, 10 mg/kg) or vehicle. R-MDMA had no effect on the antiparkinsonian action of L-DOPA (p > 0.05). Each time point represents the cumulated parkinsonism score for every 5 min observation period during the preceding 60 min. The maximal possible score (most severe disability) was 216. On the y-axis, 54 is mild, 108 is moderate, 162 is marked, and 216 is severe. D. Time course of parkinsonism in marmosets treated with L-DOPA and S-MDMA (1, 3, 10 mg/kg) or vehicle. S-MDMA had no effect on the antiparkinsonian action of L-DOPA (p > 0.05). Each time point represents the cumulated parkinsonism score for every 5 min observation period during the preceding 60 min. The maximal possible score (most severe disability) was 216. On the y-axis, 54 is mild, 108 is moderate, 162 is marked, and 216 is severe.

R-MDMA decreases the severity of L-DOPA-induced dyskinesia without reducing the antiparkinsonian action of L-DOPA. S-MDMA (3 and 10 mg/kg) significantly reduced the severity of dyskinesia in the first 2 h of the observation period when administered with l-DOPA (Fig. 2A). Thus, the severity of dyskinesia was reduced by 33 and 46%, respectively, during the first hour, when 3 and 10 mg/kg R-MDMA was compared to L-DOPA alone (F_{(2,9)} = 10.14, p < 0.0001; and F_{(2,9)} = 2.685, p < 0.01; two-way RM ANOVA, both p < 0.001, Bonferroni’s post hoc test), and by 33% when 10 mg/kg R-MDMA was compared to 1 mg/kg R-MDMA (p < 0.01, Bonferroni’s post hoc test). R-MDMA (3 and 10 mg/kg) also reduced the severity of dyskinesia (by 29%) during the second hour of observation, when compared to animals treated with L-DOPA alone (both p < 0.01, Bonferroni’s post hoc test.). When L-DOPA was combined with 3 and 10 mg/kg R-MDMA, the severity of peak-dose dyskinesia was reduced by 27 and 34%, respectively, compared to L-DOPA alone (Freidman’s statistic (FS) = 11.63, p < 0.01; both p < 0.05; Dunn’s post hoc test (Fig. 2A)). At no time during the observation period did R-MDMA impair the antiparkinsonian efficacy of l-DOPA (Fig. 2C). R-MDMA did not decrease the peak antiparkinsonian efficacy of l-DOPA (Fig. 4A). Additionally, R-MDMA did not alter duration of the ON-time (Fig. 5A). However, the duration of the ON-time with disabling dyskinesia was significantly reduced when R-MDMA (3 and 10 mg/kg) was compared to l-DOPA alone (by 69 and 65%, respectively; F_{(2,15)} = 8.061, p < 0.01, one-way RM ANOVA; both p < 0.01, Tukey’s post hoc test). R-MDMA at 3 and 10 mg/kg also significantly increased the duration of the ON-time with nondisabling dyskinesia, by 152 and 147%, respectively (F_{(2,15)} = 6.773, p < 0.01, one-way RM ANOVA; both p < 0.01, Tukey’s post hoc test), when compared to l-DOPA alone (Fig. 5C). Duration of the ON-time without disabling dyskinesia was also significantly enhanced by R-MDMA at 1, 3 and 10 mg/kg (F_{(2,15)} = 10.67, p < 0.01, one-way RM ANOVA; p < 0.05 for 1 mg/kg R-MDMA, p < 0.01 for 3 mg/kg R-MDMA, p < 0.01 for 10 mg/kg R-MDMA, Tukey’s post hoc test, data not shown).

S-MDMA extends duration of antiparkinsonian action of l-DOPA, but worsens the severity of dyskinesia. S-MDMA significantly extended the duration of antiparkinsonian action of l-DOPA. Thus, S-MDMA (1 mg/kg) increased duration of ON-time, by 35%, compared to vehicle (F_{(2,15)} = 11.86, p < 0.001, one-way RM ANOVA; p < 0.01, Tukey’s post hoc test) (Fig. 5D). This extension of ON-time was not observed with higher doses of S-MDMA (3 and 10 mg/kg) with which duration of ON-time was significantly lower than in the 1 mg/kg treatment (by 21 and 30%, respectively; F_{(2,15)} = 11.86, p < 0.001, one-way RM ANOVA;
**Discussion**

A novel synthesis of MDMA enantiomers allowed us to confirm that they exhibit different pharmacological profiles and demonstrate that they produce qualitatively different behavioral effects when administered in combination with L-DOPA to the MPTP-lesioned marmoset. R-MDMA, a relatively selective compound for 5-HT\(_{2A}\) receptors, decreased severity of peak-dose dyskinesia and increased duration of good ON-time; S-MDMA, a compound exhibiting high affinity for SERT and moderate affinity for DAT, extended total duration of ON-time but exacerbated dyskinesia. These results demonstrate that reducing 5-HT\(_{2A}\) receptor-mediated transmission is likely the primary mechanism by which racemic MDMA reduces dyskinesia, and identify dual SERT > DAT inhibitors as promising agents to increase ON-time duration.

**Technical considerations: R- and S-MDMA binding profile**

The affinity of R-MDMA for 5-HT\(_{2A}\) receptors, 4.7 \(\mu\)M, is in accordance with previous studies (Lyon et al., 1986; Battaglia et al., 1988). These two studies also demonstrated that MDMA and its enantiomers bind to 5-HT\(_{2A}\) receptors; however, they used \(^{3}H\)-5-HT as the radioligand and unlabeled 5-HT as the displacer, making the assays nonspecific. Very few studies addressed the affinity of MDMA enantiomers for the monoamine transporters and, to our knowledge, our study is the first to assess the affinity of R- and S-MDMA at a range of monoamine transporters using the modern, well-characterized, and highly selective radioligands \(^{3}H\)-citalopram, \(^{3}H\)-GRB 12935, and \(^{3}H\)-nisoxetine. Although there are some minor discrepancies in absolute affinities reported, the relative affinities of the compounds for any given receptor and transporter are in agreement with the literature (Lyon et al., 1986; Battaglia et al., 1988; Verrico et al., 2007).

**5-HT\(_{2A}\) receptors and dyskinesia**

The effects and pharmacology of R-MDMA support a role for 5-HT\(_{2A}\) antagonists as antidyskinetic agents (Maerten de Noordhout and De Waide, 1986; Mezo et al., 1988; Roberts, 2006; Visanji et
MDMA would prevent this increase in corticostriatal glutamatergic mediated depolarization (Rahman and Neuman, 1993), so en- glial, resulting in dyskinesia. By antagonizing 5-HT\textsuperscript{A} receptors, R-MDMA would prevent this increase in corticostriatal glutamatergic neurotransmission. Such a modulation of corticostriatal glutamatergic transmission by the selective 5-HT\textsubscript{A} antagonist voltinserin (M100,907) was demonstrated in the MPTP-lesioned mouse (Ferguson et al., 2010).

Another mechanism by which 5-HT\textsubscript{A} antagonists alleviate L-DOPA-induced dyskinesia severity might be via an interaction with dopaminergic D_{3} receptors, which are thought to play a central role in the pathophysiology of dyskinesia (Jenner, 2008). Indeed, in the 6-OHDA-lesioned rat, M100,907 effectively reduced hyperlocomotion (Bishop et al., 2005) and controlateral rotations (Taylor et al., 2006) induced by the D_{3} agonist N-allyl-b-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzapine-7,8-diol (SKF-82958).

However, R-MDMA is not a 5-HT\textsubscript{A} antagonist, but a 5-HT\textsubscript{A} partial agonist that elicits 10% of 5-HT-elicited response (Nash et al., 1994). Dopamine is also a partial agonist at 5-HT\textsubscript{A} receptors, at which it elicits 60% of 5-HT-elicited response (Bhattacharya et al., 2006). Thus, although R-MDMA elicits a weak response at 5-HT\textsubscript{A} receptors, it acts as an antagonist when compared to dopamine, as it dampens dopamine-induced activation of 5-HT\textsubscript{A} receptors.

Our results do not support a 5-HT\textsubscript{A}-mediated mechanism of action for MDMA antidyskinetic efficacy (Bishop et al., 2006). Direct actions are unlikely, since neither of the two enantiomers exhibit affinity for 5-HT\textsubscript{A} receptors. Second, an indirect 5-HT\textsubscript{A}-mediated mechanism would be mediated by S-MDMA, which inhibits S-HT\textsubscript{A} receptors in the current study, S-MDMA exacerbated dyskinesia severity.

Serotonergic and dopaminergic transporters inhibition and ON-time extension
Monamine reuptake inhibitors represent promising compounds in PD treatment. Thus, the selective DAT inhibitor GBR 12909 improved parkinsonism as monotherapy in the MPTP-lesioned marmoset (Hansard et al., 2002). In small clinical studies, the dual DAT/NET inhibitor nomifensine improved parkinsonism as monotherapy (Park et al., 1981), whereas the triple monoamine reuptake blocker tesofensine was not effective (Hausser et al., 2007). In another clinical study, nomifensine, in combination with L-DOPA, produced mild antiparkinsonian benefit but worsened dyskinesia (Park et al., 1977), whereas it failed to show any benefit in another trial (Bedard et al., 1977). These studies suggest that although DAT inhibition might provide some antiparkinsonian benefit, the relative activity of compounds across the three transporters is probably critical for modulating L-DOPA actions. Unlike GBR 12909, tesofensine, and nomifensine, S-MDMA preferentially inhibits SERT and DAT (SERT/DAT ratio of 10/1). Such a monoamine reuptake inhibition profile might be more suitable to extend ON-time duration. However, although
S-MDMA did not increase the severity of peak-dose dyskinesia; it increased the duration of ON-time with dyskinesia, somewhat offsetting the benefits of ON-time extension. Nonetheless, benefits of reduction of OFF-time have been appreciated in the clinic, even if the supplementary ON-time is associated with dyskinesia (Rinne et al., 1998; Parkinson Study Group, 2005).

The relative contribution of SERT and DAT inhibition to ON-time extension achieved with S-MDMA is unclear. Although DAT inhibition is an appealing mechanism, MPTP administration to marmosets leads to >90% reduction in striatal dopamine uptake (Jenner et al., 1994), making a striatal site of action unlikely. More likely, though hypothetically, would be an action where dopamine terminals are likely to participate in the reuptake of dopamine synthesized from L-DOPA (Berger, 1978a; Berger and Glowinski, 1978). Thus, SERT inhibitors could prolong dopamine actions, accounting for the extension of ON-time observed with S-MDMA. However, this in itself is insufficient as selective SERT inhibitors do not produce similar effects (Hansard et al., 2002).

S-MDMA action on SERT likely accounts for the fact that ON-time extension was compromised by dyskinesia. Conversion of L-DOPA into dopamine in raphespinal terminals is thought to be an important determinant in dyskinesia pathophysiology (Carter et al., 2007). Thus, inhibiting SERT would exacerbate nonphysiologic dopaminergic transmission, as striatal serotonergic terminals participate in the reuptake of dopamine. Furthermore, although speculative, inhibiting SERT would also result in less 5-HT being reuptaken into the presynaptic cleft, allowing for more dopamine to be packed into vesicles, which would exacerbate nonphysiologic dopamine release. However, a study performed in the 6-OHDA-lesioned rat demonstrated that chronic treatment with the SERT inhibitor cloropram reduces dyskinesia severity after 2 months of daily administration (Kuo et al., 2008). It remains to be established whether chronic daily treatment with a dual SERT > DAT inhibitor would also reduce dyskinesia severity.

The extension of ON-time obtained with S-MDMA 1 mg/kg was not maintained at higher doses. This phenomenon might be
explained by neurotransmitter depletion at higher doses of S-MDMA. Indeed, after its binding with monoamine transporters, MDMA inhibits monoamine uptake and reverses transport direction, promoting neurotransmitter release (Fleckenstein et al., 2007) and depleting presynaptic vesicles (Milnar and Corrado, 2003). It is thus possible that higher doses of S-MDMA led to an early exhaustion of presynaptic neurotransmitters, explaining their failure to extend ON-time.

MDMA enantiomers and dopaminergic psychosis-like behaviors

R-MDMA exerted a favorable effect on l-DOPA-induced psychosis-like behavior. These results support the potential efficacy of 5-HT2A antagonists against PD dopaminergic psychosis demonstrated previously with ACP-103 (Meltzer et al., 2010). The data are also in accordance with two recent anatomical studies which found increased 5-HT2A receptor levels in the temporal cortex of PD patients with visual hallucinations (Ballanger et al., 2010; Huot et al., 2010b).

The findings of the present study demonstrate that dual SERT > DAT inhibitors do not worsen the severity of dopaminergic psychosis-like behavior in the parkinsonian marmoset. This is important to know from a drug-development perspective, as enhancing dopaminergic transmission carries the risk of triggering psychiatric features (Merims and Giladi, 2008).

Concluding remarks

Racemic MDMA possesses unique activity since it alloviates l-DOPA-induced dyskinesia and, in an anecdotal case report, extended l-DOPA antiparkinsonian benefits. The data presented here expand our understanding of the mechanisms of MDMA actions in parkinsonism. Thus, MDMA antidyskinetic action likely comes from R-MDMA partial agonist activity at 5-HT2A receptors, whereas S-MDMA SERT > DAT inhibitory activity probably accounts for the ability of MDMA to extend l-DOPA antiparkinsonian action. Unfortunately, MDMA and its enantiomers are good candidates for clinical development for PD because of psychoactivity (Shulgin and Nichols, 1978) and potential for neurotoxicity (O’Hearn et al., 1988). However, studies such as ours help to refine the understanding of interactions between the serotonin and dopaminergic systems in PD, and might define pharmacological profiles of novel molecules that could represent leads for drug discovery programs.

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Enhancing the anti-lymphoma potential of 3,4-methylenedioxymethamphetamine (‘ecstasy’) through iterative chemical redesign: mechanisms and pathways to cell death

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Summary While 3,4-methylenedioxymethamphetamine (MDMA/‘ecstasy’) is cytostatic towards lymphoma cells in vitro, the concentrations required militate against its translation directly to a therapeutic in vivo. The possibility of ‘redesigning the designer drug’, separating desired anti-lymphoma activity from unwanted psychoactivity and neurotoxicity, was therefore mooted. From an initial analysis of MDMA analogues synthesized with a modified α-substituent, it was found that incorporating a phenyl group increased potency against sensitive, Bcl-2-deplete, Burkitt’s lymphoma (BL) cells 10-fold relative to MDMA. From this lead, related analogs were synthesized with the ‘best’ compounds (containing 1- and 2-naphthyl and para-biphenyl substituents) some 100-fold more potent than MDMA versus the BL target. When assessed against derived lines from a diversity of B-cell tumors MDMA analogues were seen to impact the broad spectrum of malignancy. Expressing a BCL2 transgene in BL cells afforded only scant protection against the analogues and across the malignancies no significant correlation between constitutive Bcl-2 levels and sensitivity to compounds was observed. Bcl-2-deplete cells displayed hallmarks of apoptotic death in response to the analogues while BCL2 overexpressing equivalents died in a caspase-3-independent manner. Despite lymphoma cells expressing monoamine transporters, their pharmacological blockade failed to reverse the anti-lymphoma actions of the analogues studied. Neither did reactive oxygen species account for ensuing cell death. Enhanced cytotoxic performance did however track with predicted lipophilicity amongst the designed compounds. In conclusion, MDMA analogues have been discovered with enhanced cytotoxic efficacy against lymphoma subtypes amongst which high-level Bcl-2—often a barrier to drug performance for this indication—fails to protect.

Keywords Apoptosis • Bcl-2 • Cytotoxicity - Lymphoma • MDMA

Abbreviations
ABC Activated B-Cell-like
BL Burkitt’s lymphoma
DAT Dopamine transporter
DLBCL Diffuse large B-cell lymphoma
Introduction

The incidence of B-cell lymphomas, constituting around 95% of all the non-Hodgkin lymphomas (NHL), is increasing steadily year-on-year. NHL is a heterogeneous group of neoplasia ranging from indolent examples like slow growing follicular lymphoma (FL) to highly aggressive, rapidly proliferating entities exemplified by diffuse large B-cell lymphoma (DLBCL) - the most common of the NHL in Europe, Australasia and the US—and Burkitt’s lymphoma (BL) rare in the West but endemic in the World’s malarial belt. The diversity of tumors reflects a composite of factors including the differentiation stage of the target B-cell and the mutations/translocations arising therein. Multiple profiling platforms such as gene array are disclosing additional heterogeneity within previously considered single clinical entities which can be manifested molecularly, cellurally and prognostically. DLBCL for example is now considered a composite of disease subtypes comprising primarily ‘Activated B-Cell-like’ (ABC) cases and those that are ‘Germinal B-Cell-like’ (GCB): survival rates among the former being substantially worse than the latter. Moreover, within ABC DLBCL constitutive expression of the pro-survival gene BCL2 further discriminates a substantially inferior subgroup with regards overall survival even in the face of intense therapy.

Anti-apoptotic BCL2, originally identified as the gene translocating to the IGH locus on chromosome 14 in the hallmark t(14;18) of FL, offers a considerable barrier to drug efficacy in lymphoma treatment. BL, while extremely aggressive, lacks genetic alterations in BCL2, is deplete in Bcl-2 protein and has a high cure rate using combination chemotherapy. Over the past decade we have adopted BL as a template on which to explore novel therapeutic opportunities for lymphoma: BL offering a sensitive monitor of pro-apoptotic/anti-proliferative activities and at the same time being a tumor that is readily adaptable to tissue culture with derived lines remaining ‘biopsy-like’ when maintained in early passage. Transfection of BCL2 on a constitutive promoter into these cells allows the opportunity to model directly on an otherwise isogenic background the impact of its dysregulated, high level expression on the efficacy of promising new drug candidates. Within this context we have been investigating compounds which target components of neurotransmitter pathways that can be found in immune cells and their cancers: most notably the transporters for serotonin and dopamine (SERT and DAT, respectively), each expressed in a broad range of the NHL subtypes and other B-cell malignancies [1–5].

Amongst such compounds, the amphetamine derivatives fenfluramine and 3,4-methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) were found to be anti-proliferative against B-cell lines of diverse malignant B-cell origin. It was shown (at least with fenfluramine) that in Bcl-2-deplete BL cells, growth arrest was accompanied by apoptotic cell death following activation of caspase-3: these latter features were reversing on introducing BCL2 as a transgene [4]. Unfortunately the concentrations of the amphetamine derivatives required to elicit anti-proliferative/pro-apoptotic activity in vitro were too high for safe translation to a cancer therapeutic in vivo. Therefore we mooted for MDMA the potential of "redesigning the designer drug" to enhance lymphoma killing while reducing neurotoxicity and psychoactivity.

Research by Shulgin and co-workers [6–9] suggests that extending the α-or N-substituent of MDMA to anything larger than an ethyl group abolishes the drug’s psychoactivity. Nash and Nichols, studying acute effects in rats, showed that a simple substitution of the methyl group at the α-C of MDMA with an ethyl substituent, creating MBDB, significantly diminishes the amount of dopamine released in the striatum [10]. The α-substituent was therefore deemed a rational plinth for redesign. We now describe improved cytotoxic performance of MDMA analogues with modified α-substituents against a spectrum of B-cell malignancies giving attention to the mechanisms and pathways to cell death, including the impact of anti-apoptotic Bcl-2. A companion study details in foil the chemistry and synthesis of the analogues while providing evidence for diminished neurotoxicity and psychoactivity of selected compounds, together with a brief description of their rank potency in targeting a BL cell line [11].

Materials and methods

Compounds

MDMA and analogues with modified α-substituents were synthesized by reductive amination of the corresponding piperonyl ketones as described recently [11]. All target amines were converted to their hydrochlorides and were tested as such.
Cell culture

Cell lines deriving from different B-cell malignancies and variants of the L3055 BL cell line were as described previously [4]. EBV-transformed lymphoblastoid cell lines were from the School of Cancer Sciences, University of Birmingham U.K. All cell lines were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 10% v/v FCS, 100 U/ml penicillin, 100 U/ml streptomycin under 5% CO₂ at 37°C and passaged three times weekly.

Cellular cytotoxicity

Cellular cytotoxicity/viability was assessed by staining treated cells with propidium iodide (PI, a DNA binding dye incapable of penetrating intact cell membranes, (Sigma Aldrich, Dorset, UK)) at a final concentration of 0.85 μg/ml or 1.15 μg/ml prior to flow cytometric analysis (FACS Calibur BD) of PI^* versus PI^V cells. Results were analysed using FlowJo 8 software for Macintosh.

Apoptosis

Apoptosis was assessed by dual staining of cells with PI and PhiPhiLux (Oncoimmunin, Gaithersburg, MD, USA) an indicator of active caspase-3 followed by analysis on FACS exactly as described previously [4]. Activation of caspase-3 was additionally assessed by staining cells with a rabbit antibody specific for the active form of caspase-3 (BD Pharmigen, Oxford, UK), followed by FACS analysis; non-immune rabbit IgG (control) was from Sigma Aldrich. Cells were pre-treated using the FIX and PERM kit for intracellular staining (Caltag, Invitrogen, Paisley, UK) according to the manufacturer’s instructions. Cleavage of poly(ADP-ribose) polymerase-1 (PARP-1) as determined by Western blot and mitochondrial membrane permeability as assessed by JC-1 staining were performed as detailed elsewhere [3]. Bcl-2 protein content of cells was determined by Western blot as described previously [3].

Treatment with antioxidants

Cells were pre-treated with catalase (Sigma Aldrich, Dorset, UK) for 1 h or PEG-catalase (Sigma Aldrich, Dorset, UK) for 1.5 h before seeding cells onto 96-well plates containing MDMA/MDMA analogue. Cells at a final density of 10^5/ml were incubated with drug for 24 h and cell viability was assessed by PI uptake analysed by flow cytometry.

Lipophilicity calculations

Estimates of lipophilicity were obtained from the “average log P” value output by the applet ALOGPs 2.1 available online [12]. A plot of average log P versus pIC₅₀ showing the SEM in each variable was constructed and a curve was fitted by weighted linear regression using Grafit 4, where the weighting of each point was inversely proportional to the respective error in pIC₅₀. Due to the uniformity in the magnitude of errors of average log P across the dataset, these were ignored when the weighted curve was fitted.

Pharmacological interpretation and statistics

Pharmacological interpretation of cytotoxicity assays to generate the pIC₅₀ and Hill coefficient of a compound’s activity against L3055 cells was performed using a four parameter logistic equation with iterative fitting using Kaleida Graph [13]. Regression analysis for cytotoxic response vs Bcl-2 expression was calculated as a ratio between remaining cell viability (assessed as in 2.3 above) following treatment with MDMA and analogues and the optical density (computed using ImageJ for Macintosh) of Bcl-2 vs calnexin protein bands as determined by Western blot. Graphs were created in OriginPro 8 (OriginLab, Northampton, MA).

Results

Substitutions at the α-carbon in MDMA can augment cytotoxic performance against L3055 Burkitt’s lymphoma cells

The first generation of α-substituted MDMA analogues synthesized contain either novel alkyl/cycloalkyl groups (compounds 1-5) or, in the case of compound 6, a phenyl substituent. When assessed for anti-lymphoma potential against L3055, a prototype early-passage BL cell line, compound 6 was the most potent (approximately 10-fold > MDMA) both in inhibiting 3H-thymidine incorporation (data not shown) and in its cytotoxic efficacy (Fig. 1b): pIC₅₀=4.12±0.03 versus pIC₅₀=3.39±0.09 for MDMA. Compound 6 therefore formed the template on which to design the next generation of compounds in the quest for a lymphoma therapeutic based on MDMA.

Larger aromatic α-substituents enhance cytotoxic potential towards L3055 cells

The second generation of α-modified MDMA analogues all contain aromatic rings (two in the case of compounds 16, 17, 18), apart from compound 7, which possesses a cyclohexyl group (Fig. 2). The substitutions in this series of MDMA analogues differ from each other with respect to three-dimensional structure, rigidity, and electron density:
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Fig. 1 Cytotoxic efficacy of MDMA and Series 1 (first generation) MDMA analogues versus L3055 Burkitt’s lymphoma cells.

a Chemical structure of MDMA with α-carbon (α-C) indicated (and in tabulated right hand panel) MDMA analogues with the first iteration of α-C substituents constituting Series 1 compounds 1-6 as shown, together with calculated pIC50 ± SEM and Hill coefficients ± SEM from response curves as generated in (b) with number of separate experiments performed with each compound given as n.

The benzene ring possessing all six carbons within one plane (sp²-hybridized) by contrast to the cyclohexyl group, where the carbon atoms are sp²-hybridized and therefore non-planar and conformationally flexible. Compound 8 has an α-benzyl group and thus an additional sp²-hybridized carbon between the main carbon chain and the aromatic α-substituent. This provides additional flexibility compared to phenyl substituents, and extends the aromatic ring from the main chain, exploring the depth of a putative hydrophobic pocket in the target receptor(s).

Compounds 9, 10 and 11 are more polar than their parent (6) due to the addition of a methoxy group. The lone pairs of electrons make the methoxy oxygen a hydrogen bond acceptor and therefore adds to the hydrophobicity of the molecule [14, 15]. Metabolic stability is also increased by the inclusion of fluorine.

Compounds 16, 17 and 18 have much larger hydrophobic substituents at the α-position of MDMA, and therefore increased lipophilicity. The naphthyl group (compounds 16 and 17) is highly rigid as all the carbon atoms are positioned in one plane, whereas the biphenyl group differs from compound 6 by the addition of a para-phenyl group and therefore both of the benzene rings are able to rotate around the axis of the bond between them.

From results presented in Fig. 2 it can be noted that from the second generation of MDMA analogues modified at the α-carbon, compounds 16-18 were by far the most potent regards cytotoxicity towards L3055 cells; compounds 17 and 18 being the most efficacious and equipotent with a pIC50 = 5.18 ± 0.03 and 5.22 ± 0.08; representing a ~10-fold and ~100-fold improvement over compound 6 and MDMA respectively. Similar rank potency of these analogues was observed when assessed for their capacity to inhibit H-thymidine incorporation into L3055 cells (data not shown). It should be noted that all compounds tested for concentration-dependent cytotoxicity generated steep response curves yielding relatively high Hill coefficients (Figs. 1 and 2) suggesting deviation from simple mass interaction [16].

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Fig. 2 Cytotoxic efficacy of Series 2 (second generation) analogues versus L3055 Burkitt's lymphoma cells. As in Fig. 1 but here with Series 2 compounds 7-18; MDMA again included for comparison.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ör Substituent</th>
<th>%IC_{50} ± SEM</th>
<th>Hill coefficient ± SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td><img src="image" alt="Compound 7" /></td>
<td>4.10 ± 0.04</td>
<td>6.56 ± 0.45</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Compound 8" /></td>
<td>4.16 ± 0.03</td>
<td>5.92 ± 0.58</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Compound 9" /></td>
<td>4.04 ± 0.04</td>
<td>4.62 ± 0.38</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Compound 10" /></td>
<td>4.10 ± 0.01</td>
<td>6.05 ± 0.16</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td><img src="image" alt="Compound 11" /></td>
<td>4.16 ± 0.02</td>
<td>5.60 ± 0.73</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td><img src="image" alt="Compound 12" /></td>
<td>4.20 ± 0.02</td>
<td>5.89 ± 1.12</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td><img src="image" alt="Compound 13" /></td>
<td>4.20 ± 0.05</td>
<td>6.68 ± 0.73</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td><img src="image" alt="Compound 14" /></td>
<td>4.29 ± 0.05</td>
<td>5.05 ± 0.17</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td><img src="image" alt="Compound 15" /></td>
<td>4.37 ± 0.03</td>
<td>5.69 ± 0.27</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td><img src="image" alt="Compound 16" /></td>
<td>4.50 ± 0.02</td>
<td>4.04 ± 0.74</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td><img src="image" alt="Compound 17" /></td>
<td>5.18 ± 0.03</td>
<td>4.38 ± 0.73</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td><img src="image" alt="Compound 18" /></td>
<td>5.22 ± 0.08</td>
<td>5.05 ± 0.97</td>
<td>3</td>
</tr>
</tbody>
</table>

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Cytotoxic efficacy of selected α-substituted MDMA analogues towards B-cell lines of different malignant derivation

The constituent cells of B-cell lines from a diverse range of malignancies were treated with MDMA and six of the α-substituted MDMA analogues (selected according to their activity versus sensitive L3055 cells and applied at a concentration at or close to their maximal cytotoxic performance against this cell line) then analysed for remaining viability (Fig. 3a). Given the resistance often afforded to therapeutic regimens by dysregulated/overexpressed BCL2 in B-cell lymphoma, cells were simultaneously assessed for Bcl-2 protein content (v calnexin standard) by Western blotting. The origin of cells spanned patients additional to those diagnosed with BL (L3055 series; KHM2B): precursor acute lymphoblastic leukemia (LILA), pro-lymphocytic leukemia (JVM2), mantle cell lymphoma (Rec-1; NCEB-1), primary mediastinal B-cell lymphoma (K1106), diffuse large B-cell lymphoma (K422; DoHH2), multiple myeloma (KMS11; H929). Immortalized B-cell lines generated from the peripheral blood of three donors by transformation with Epstein-Barr virus (EBV) were also included (HCD1; SF-N; YW6)—EBV being invariably linked to endemic BL, PTLD (post- transplant lymphoproliferative disease) and a high proportion of HIV-associated lymphoma.

MDMA and its analogues were set at concentrations displaying maximal/near-maximal impact on L3055 cell viability to serve as a reference. At these concentrations each of the analogues tested displayed (albeit a varying degree of) cytotoxicity against the spectrum of malignancies included. The best compound (18) showed a consistently substantive impact against each of the subtypes. As reported previously [4], Bcl-2 content showed some degree of correlation with a cell’s ability to resist killing from MDMA. With each of the analogues, however, there was scant correlation between Bcl-2 protein level and extent of cytotoxicity observed (Fig. 3a). To assess the influence of Bcl-2 directly, a detailed concentration-dependent response was established for the cytotoxic efficacy of the analogues against L3055 cells transfected with empty vector versus cells expressing the BCL2 transgene. The latter were only marginally more resistant (approximately a single log2 difference) to each of the analogues than cells negative for Bcl-2 expression (Fig. 3b). This was consistent for cells plated at relatively low or high starting density.

Mode of cell death induced by selected first and second generation α-substituted MDMA analogues

When assessing cell integrity in response to compound 6 at 300 μM and compound 18 at 31.25 μM by dual PhiPhiLux (primarily detecting active caspase-3) and propidium iodide (plasma membrane permeability) staining, L3055 BL cells transfected with empty vector showed classic progression from early to late apoptosis over the course of the 6 h monitored (Fig. 4a). While at the fixed concentration of the analogues used L3055-Bcl-2 cells again showed a degree of resistance to their cytotoxic actions, nevertheless the death that occurred failed to register an ‘early apoptosis’ stage at any time point as indicated by cells staining as PhiPhiLux+/PI-. While no PhiPhiLux positivity was developed with compound 6, compound 18 progressively moved a portion of cells to what is conventionally considered a ‘late apoptotic’ stage: PhiPhiLux+/PI+. However, assessing engagement of the apoptotic machinery by alternative more direct methods gave no evidence for compound 18 provoking this pathway in L3055-Bcl-2 cells. Thus the specific detection of active caspase-3 by antibody revealed its appearance in response to compounds 6 and 18 in L3055-VC but not in L3055-Bcl-2 cells (Fig. 4b). Likewise, the cleavage of poly (ADP-ribose) polymerase (PARP) [17], as shown occurring in L3055-VC cells with the well characterized apoptosis-inducing agent anti-IgM, was also seen on application of MDMA and here more potently with compounds 16, 17, and 18 whereas L3055-Bcl-2 cells revealed little if any PARP cleavage in response to any of the agents applied (Fig. 4c). JC-1 staining to indicate collapse of mitochondrial potential [1] similarly supported the different routes to cell death by analogs depending on the expression of Bcl-2 in L3055 BL cells (data not shown).

Mechanisms and pathways to lymphoma cell killing by selected α-substituted MDMA analogues

Depending upon cell type and system studied, MDMA has been purported to provoke toxicity via a diverse array of...
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**Figure a**

- L3055-WT
- L3055-VC
- L3055-Bcl2
- KHM28
- LILA
- JVM2
- Rec-1
- NCEB1
- K1106
- K422
- DSHH2
- KMS11
- H520
- H201
- AT-4V
- VVWo

**Figure b**

- MDMA
- compound 6
- compound 12
- compound 15
- compound 16
- compound 17
- compound 18

**Graphs**

- Bcl-2/calnexin
- Bcl-2/calnexin

**Cells**

- L3055-VC
- L3055-BC12

**Legend**

- R=0.48
- R=0.12
- R=0.13
- R=0.06
- R=0.17
- R=0.20
- R=0.17

**Notes**

- Viability (% of control)
- 10^5/ml
- 5x10^5/ml

**References**

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Fig. 4 Mode of cell death in L3055-VC and L3055/Bcl2 cells in response to MDMA analogues 6 and 18. a Cells from L3055 variant lines indicated were cultured with compound 6 (500 µM) or compound 18 (125 µM) for 6 h before dual staining with PPL (light grey) and PI (apoptotic; bottom left quadrants), PI/PPL (late apoptotic; top right), and PI/PPL (necrotic; top left). The lower set of graphs illustrate similar analyses arising from exposing cells to the compounds over 1-6 h with the data represented as the % of cells arising in each quadrant at the different times of harvest: viable marked in white, early apoptotic marked light grey, late apoptotic marked dark grey, necrotic marked black. Data are the mean of three independent experiments (n=3) ± SEM with the values shown obtained after subtracting vehicle control; b L3055-VC and L3055-Bcl2 cells at 5 x 10^5/ml treated for 2 h with compound 6 at 500 µM and compound 18 at 31.25 µM (black line) or vehicle control (shaded) then stained with antibody to active caspase-3 and intensity of staining analysed by FACS. A representative example shown. c Western blot analysis of PARP cleavage in L3055-VC and L3055-Bcl2 cells plated at 10^6/ml and treated for 6 h with MDMA at 2000 µM or compounds 6, 17 or 18 at 31.25 µM; upper 117 kDa band = intact PARP; lower 97 kDa band = cleaved PARP; anti-IgM (25 ng/ml) >s a positive control and treated for 6 h with MDMA at 2000 µM or compounds 16, 17 or 18 at 31.25 µM; upper 117 kDa band = intact PARP; lower 97 kDa band = cleaved PARP; anti-IgM (25 ng/ml) is a positive control treatment known to signal IARP cleavage in L3055-VC cells via cell surface BCR. This experiment was performed twice with a representative example shown.

Discussion

Analogues of MDMA with modified α-substituents were iteratively designed and synthesised, and found to be up to 10-fold (first generation) and 100-fold (second generation) more potent than the parent amphetamine derivative at promoting lymphoma cell death: the goal and driver to this study. Impressively, forced over-expression or high constitutive levels of anti-apoptotic Bcl-2 failed to protect, to any significant degree, the anti-lymphoma actions of the analogues; this despite their ability to promote apoptotic cell death in Bcl-2-deplete cells. Thus, in the face of high-level Bcl-2, death still occurred but in a caspase-3-, PARP-independent fashion that was similarly independent from a collapse in mitochondrial membrane potential. It should be noted, however, that while analogues of MDMA efficiently generated active caspase-3 within 4-6 h of exposure in the bulk of native L3055 BL cells, a majority of their Bcl-2-overexpressing counterparts were still alive at 6 h. Thus, at least for BL, if translated to an in vivo therapeutic, these compounds show potential to reduce tumor burden through efficient apoptotic clearance without the attendant inflammatory side effects of necrotic death.

Importantly, improved cytotoxic performance against lymphoma cells does not simply reflect a generally enhanced, non-specific toxicity profile of the compounds. A companion study shows that the most active compound versus lymphoma cells from Series 1 (compound 6) and two of the even more active ones from Series 2 (compounds 16 and 17) are in fact less toxic than MDMA to SH-SY5Y.
Fig. 5 Investigation of potential pathways through which MDMA analogues elicit cytotoxicity in L3055 cells. a Impact of monoamine transporter (MAT) inhibitors. L3055 cells at 10^5/ml were pre-incubated with MAT inhibitors for 1 h before adding MDMA or compounds 15 and 16 at 125 µM and 31.25 µM respectively then culturing for 20 h prior to assessing cell viability as in Fig. 1c; b Influence of scavenging extracellular ROS with catalase. L3055 cells at 10^5/ml were pre-treated with catalase at concentrations shown for 1 h before adding H_2O_2 or compounds indicated (compound 6, 500 µM; compound 12, 250 µM; compound 15, 125 µM; compounds 16, 17 and 18, 31.25 µM) and then culturing for 20 h prior to assessing viability as above; c Influence of scavenging intracellular ROS with PEG-catalase. L3055-VC cells at 10^5/ml were pre-treated with PEG-catalase for 1.5 h before adding H_2O_2, MDMA, or compound 6 at concentrations indicated and then culturing for 24 h prior to assessing viability.

The literature around MDMA and the mechanisms underlying its toxicity is large, varied and occasionally contradictory [23–31]: the cell system, cellular origin, animal species, drug concentration and other elements all contributing confounding factors. Here we scrutinized several of the major candidate pathways proposed for MDMA for their potential contribution to the toxic action of the analogues versus B-lymphoma cells. The current study was predicated on the discovery that B lymphoma cells express both SERT and DAT, the transporters for serotonin and dopamine, respectively, and to which MDMA binds in the human with high affinity as it also does to NET,

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Fig. 6 Calculated lipophilicities of MDMA and analogues versus cytotoxic performance. Relationship between average log P ± SEM and pIC50 ± SEM for MDMA (○), alkyl α-substituted analogues 1-5 (●), monocyclic aromatic α-substituted analogues 6-15 (△) and polycyclic aromatic α-substituted analogues 16-18 (□). The curve was fitted to all data points shown using weighted linear regression that gave an r² value of 0.88.

against the norepinephrine transporter [30, 32]. Against serotonergic JAR cells for example, MDMA's cytotoxicity is delivered via SERT: being inhibited by imipramine, a monoamine transporter blocker with highest affinity for SERT [33]. The capacity of serotonin to drive apoptosis in BL cells is reversed by SERT blockade with e.g. the selective serotonin reuptake inhibitor, fluoxetine [1]. However, adopting the approach of pharmacological transporter blockade in this work, neither MDMA nor two of its more potent redesigned analogues were seen to be delivering their toxic hit to lymphoma cells via any of the three monoamine transporters probed. Moreover, Montgomery and colleagues [34] examining the action of MDMA and several MDMA analogues on 5-HT and NA uptake in cells transfected with SERT or NET reported a Hill coefficient for inhibition by MBDB (our compound 3) of −1 for both HEK-SERT and PC12-NET compared to that generated from its anti-lymphoma action in this study of >3. Others have shown that MDMA is capable of promoting cell death independently of SERT expression or activity [35]. A second major mechanism for MDMA's cellular toxicity in other systems was similarly ruled out here for both the lead compound and the more (anti-lymphoma) potent synthe­sized analogues: namely the direct or indirect, production of reactive oxygen species. Inhibitors of extracellular ROS which have previously been shown to reverse the anti-lymphoma actions of dopamine [36] did not protect against MDMA and its analogues in this respect. Similar failure of PEGylated SOD and catalase to inhibit death delivered from the compounds under study equally argued against intracellular ROS formation contributing to the lymphoma cell killing observed.

If not through ROS generation or from entering via monoamine transporters, how are MDMA and its redesigned analogues attacking the lymphoma cells? Screening against the sensitive L3055 cell line revealed no significant difference in the cells' response to compounds containing α-substituents with either different steric (13-15, 16-17) or stereoelectronic (9-12) properties. Instead, the addition of further aromatic rings, thereby increasing the size of substituents at the α-carbon of MDMA, appeared a unifying factor to increasing potency: i.e. compound 6 in Series 1 with a single aromatic ring and compounds 18, 17 and 16 in Series 2 with two aromatic rings being the most potent from each iteration. That said, the non-aromatic cyclohexyl substituent confers equipotency to phenyl. Size of the α-substituent and overall lipophilicity of the compound may therefore be primary determinants of potency. In an earlier study we noted from a seemingly otherwise disparate set of compounds capable of killing lymphoma cells the shared feature of being cationic amphiphiles [4]. This class of compounds has the capacity to disrupt cellular membranes, as do amphiphilic molecules generally. Greater lipophilicity also enhances entry into cells, thereby increasing the effective intracellular concentration, and entropically favours complex formation (the hydrophobic effect) and thus, potentially, affinity of drug for intracellular receptors/targets. Numerous studies indicate a selectivity of lipophilic compounds for impacting rapidly proliferating cancer cells over normal cells [37, 38] and others show, amongst related series of compounds, a clear correlation between anti-proliferative activity/cytotoxicity and degree of lipophilicity [39-41]. When this relationship was examined for the newly synthesized analogues of MDMA, a strong correlation was indeed observed with anti-lymphoma potency closely track­ing calculated lipophilicity, at least for those compounds with aromatic α-substituents. We are currently exploring precisely how this physiochemical property of the compounds translates mechanistically to improved lymphoma killing in order to assist further rational design of MDMA analogues as anti-neoplastics.

Irrespective of relative anti-lymphoma potency all compounds including MDMA generated steep inhibition curves with Hill coefficients >3 indicating a high degree of cooperativity in their action. Similar behaviour has been observed from SSRIs and tricyclic antidepressants (Sera­feim Blood 2003; Meredith FASEB J 2005) and at least with the former class of compound we know that cell death is preceded by the stimulation of Ca2+ entry. Preliminary data (unpublished) indicate similarly altered Ca2+ flux in...
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