The Application of Lipase Mediated Resolutions to the Synthesis of the Optical Isomers of Bufuralol and Methadone

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Declaration

This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work is original, and has not been previously submitted, in whole or in part, for any degree at this, or any other university.
Acknowledgements

I would like to thank Dr Feodor Scheinmann and Professor Nicholas J. Turner for their support and encouragement throughout the course of my studies. I also thank Dr A. V. Stachulski and Dr S. Stokes for valuable discussions and for their continued support. Many thanks also to Jayne Law for discussions related to the bufuralol synthesis. I appreciate the help given by Dr G. N. Jenkins and for his tuition in the handling and use of whole cell microorganisms and Dr S. A. Brown for assistance with computerised searches. I am grateful to the analytical staff at the Universities of Exeter, Manchester, and Edinburgh for all their help. Thanks also to the members of the Turner - Flitsch group for making me feel very welcome whilst in Edinburgh and to the other staff at Ultrafine for words of encouragement. This PhD was partly funded by the BBSRC through the Teaching Company Scheme for which I am grateful. Finally, I thank my wife, Gail, for her patience and support for my studies.
Abstract

This thesis describes the application of lipase mediated kinetic resolutions in the synthesis of two biologically active and pharmaceutically important compounds:

Bufuralol has activity as a beta-blocker and recently it has become useful in metabolism studies as a substrate for a specific cytochrome P450 enzyme. Chapter three details how bufuralol, formerly marketed as a racemate, can be conveniently prepared in optically enriched form with a lipase resolution of a precursor as the key step.

Methadone is currently used worldwide as a treatment in the maintenance of patients with addictions to opiates. It is mainly administered as a racemic mixture although the activity of the drug is due to the levorotatory isomer only. Levo-methadone can be efficiently prepared by employing a lipase in the resolution of 1-dimethylamino-2-propanol which is an inexpensive starting material for the synthesis of methadone. This is described in chapter four. Since both isomers of 1-dimethylamino-2-propanol are isolated from the resolution dextro-methadone may also be prepared and although it shows little or no activity itself, dextro-methadone may be converted in two steps to levo-α-acetylmethadol which is used in the U.S. as a longer acting alternative to methadone.
The Teaching Company Scheme

This PhD was partly funded by the BBSRC as part of a Teaching Company Scheme (TCS) between the University of Edinburgh and UFC Ltd, Manchester (Ultrafine). The Teaching Company Scheme is a relatively unusual means by which to fund a higher degree in the sciences and it is appropriate to give a brief account explaining what a Teaching Company Scheme is and how it works.

Teaching Company was originally set up as a means of training engineering graduates in management and general commercial awareness within the workplace - the teaching company. The name "Teaching Company" comes from an analogy with the teaching hospital for students of medicine. The graduate or Teaching Company Associate (TCA) is given a placement in a company and a project to undertake over a period of two to three years. The project involves the transfer of new technology from an academic institution to the company and the TCA is supported in this task by an academic supervisor (expert in the field) and an industrial supervisor (manager - mentor). Also, a consultant from the Teaching Company Directorate (TCD) oversees the whole project. The TCA is based at the company for the most part and occasionally spends some time at the University when necessary. In terms of time spent in industry and time spent in academia a TCS may be considered to be opposite to an industrially sponsored CASE studentship. The TCA also attends various management training courses which are organised by the TCD. If the project is suitable then the TCA may register for a higher degree.

Over the last few years an increasing number of Teaching Company Schemes have been set up which are science based allowing science graduates to benefit from this new method of training and science companies to acquire new technologies being
developed at universities. The TCS between The University of Edinburgh and Ultrafine was set up to develop biotransformations as a new technology within Ultrafine. In fact this scheme was just the first of now three TCSs between Edinburgh and Ultrafine and currently Dr Stuart Brown is setting up a designated biotransformations laboratory at Ultrafine in which he will be able to handle microorganisms for specific biotransformations.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Å</td>
<td>angstroms</td>
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<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AGP</td>
<td>α-glycoprotein</td>
</tr>
<tr>
<td>AIDS</td>
<td>Auto Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>ANL</td>
<td>Aspergillus niger lipase</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>BASF</td>
<td>Badische Anilin und Soda Fabrik</td>
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<tr>
<td>Bp</td>
<td>boiling point</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>tBu</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>CALB</td>
<td>Candida antarctica lipase type B</td>
</tr>
<tr>
<td>cGMP</td>
<td>current good manufacturing practise</td>
</tr>
<tr>
<td>CLEC</td>
<td>cross linked enzyme crystals</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPA</td>
<td>chloropropanoic acid</td>
</tr>
<tr>
<td>d</td>
<td>dextrorotatory, doublet (NMR assignments)</td>
</tr>
<tr>
<td>DHP</td>
<td>Dihydropyran</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylaniline</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infra-red</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>hfc</td>
<td>3 (heptafluoropropylhydroxymethylene)- (+)-camphorate</td>
</tr>
<tr>
<td>His</td>
<td>histidine</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>l</td>
<td>levorotatory</td>
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</tbody>
</table>
LAAM  levo-α-acetylmethadol
LDA  lithium diisopropylamide
LSM  Life Science Molecules
M  molar
m  multiplet
Me  methyl
Mp  melting point
Ms  methanesulphonate
NCS  \textit{N}-chlorosuccinimide
NMR  nuclear magnetic resonance
Oct  octanoate
Pd-C  10\% palladium on carbon
PFL  \textit{Pseudomonas fluorescens} lipase
Ph  phenyl
ppm  parts per million
iPr  iso-propyl
q  quartet
s  singlet
Ser  serine
sp.  species
\textit{t}  triplet
\textit{t}/\textit{a}  tonnes per annum
TBDMS  \textit{tert}-butyldimethylsilyl
TCA  Teaching Company Associate
TCS  Teaching Company Scheme
Tf  triflate
TFE  trifluoroethyl
THF  tetrahydrofuran
THP  tetrahydropyran-2-yl
TMS  trimethylsilyl
Ts  toluenesulphonate
U.S.  United States
\textit{v}/\textit{v}  volume for volume
\textit{w}/\textit{w}  weight for weight
Contents

1.0 Introduction 1
  1.0.1 Introduction 1
1.1 Lipases - How they work 2
1.2 The lipase transformation of meso-substrates 6
1.3 Biocatalysis in Industry 16
  1.3.1 Avecia Life Science Molecules 17
  1.3.2 Eli Lilly & Co. 22
  1.3.3 Chiroscience 23
  1.3.4 BASF 27

2.0 Aims of the Project 29
  2.1 Preface to Chapter 3 29
  2.2 Preface to Chapter 4 30

3.0 Results & Discussion I (Bufuralol) 31
  3.1 Introduction 31
  3.2 Approaches to Bufuralol 32
  3.3 Resolution of Bufuralol 35
  3.4 Preparation of the chloroalcohol 80 36
  3.5 Lipase catalysed resolutions of the chloroalcohol 80 and conversion of the resolution products to bufuralol 39
  3.6 Conclusions and Suggestions for Future Work 43

4.0 Results & Discussion II (Methadone) 44
  4.1 Introduction 44
  4.2 Literature routes to homochiral methadones 45
    4.2.1 Classical resolution chemistry 45
    4.2.2 Synthesis from 'Chiral Pool' starting material 46
  4.3 A new lipase mediated approach to the synthesis of l-methadone 47
  4.4 Chiral HPLC analysis 55
  4.5 Chiral GC analysis of the resolution products 56
  4.6 Lipase screen 57
  4.7 Large scale resolution 59
  4.8 Levo-α-acetylmethadol 60
4.8.1 Introduction
4.8.2 The synthesis of LAAM from d-methadone
4.9 Conclusions and suggestions for future work

5.0 Experimental

5.0.1 General experimental

5.1 Bufuralol

5.1.1 The route to 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-hydroxyethane 80

5.1.1.1 Preparation of 1-Allyloxy-2-ethylbenzene 74
5.1.1.2 Preparation of 2-allyl-6-ethylphenol 75
5.1.1.3 Preparation of 3-Ethyl-2-hydroxy-5-methylstyrene 76
5.1.1.4 Ruthenium (III) chloride catalysed oxidative cleavage of styrene 76
5.1.1.5 Osmium (VIII) oxide catalysed oxidative cleavage of styrene 76
5.1.1.6 Preparation of 7-ethylbenzofuran-2-yl methyl ketone 78
5.1.1.7 Attempted preparation of 7-ethylbenzofuran-2-yl chloromethyl ketone 79 directly from ethylhydroxybenzaldehyde 77
5.1.1.8 Preparation of 7-ethylbenzofuran-2-yl chloromethyl ketone 79
5.1.1.9 Reaction of 7-ethylbenzofuran-2-yl methyl ketone 78 with sulphuryl chloride 73
5.1.1.10 Preparation of 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-hydroxyethane 80

5.1.2 Lipase catalysed resolutions using trifluoroethyl octanoate

5.1.2.1 Preparation of trifluoroethyl octanoate ester 74
5.1.2.2 Preparation of racemic 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl octanoate 81
5.1.2.3 Lipase catalysed esterification of the racemic chloroalcohol 80

5.1.3 Lipase catalysed resolutions using vinyl acetate as the acyl donor and subsequent conversion to bufuralol

5.1.3.1 Lipase AY catalysed acetylation of the chloroalcohol 80 with vinyl acetate 77
5.1.3.2 Lipase AY catalysed hydrolysis of (S)-2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl acetae (S)-82
5.1.3.3 Preparation of (R)-(+) Bufuralol (R)-60 from the (S)-alcohol (S)-80 isolated from the Lipase AY resolution 79
5.1.3.4 Lipase PS catalysed acetylation of the chloroalcohol 80 with vinyl acetate 80
5.1.3.5 Lipase PS catalysed hydrolysis of (S)-2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl acetae (S)-82
5.1.3.6 Preparation of (R)-(+) Bufuralol (R)-60 from the (S)-alcohol (S)-80 isolated from the Lipase PS resolution 82

5.2 Methadone
5.2.1 Resolutions with vinyl acetate

5.2.1.1 Resolution of 1-dimethylamino-2-propanol 89

5.2.1.2 Resolution of 1-dimethylamino-2-propanol 89

5.2.2 Resolution of 1-dimethylamino-2-propanol using vinyl propanoate and reaction of the resolution products to methadone 85

5.2.2.1 (R)-(−)-1-dimethylamino-2-propyl propanoate (R)-92

5.2.2.2 (R)-(−)-1-dimethylamino-2-propanol (R)-89

5.2.2.3 (S)-(+)1-dimethylamino-2-chloropropane hydrochloride (S)-90

5.2.2.4 (R)-(−)-2,2-diphenyl-4-dimethylaminopentanenitrile (R)-86

5.2.2.5 (R)-(−)-Methadone hydrochloride (R)-83

5.2.2.6 (S)-(+)1-dimethylamino-2-propanol (S)-89

5.2.2.7 (R)-(−)-1-dimethylamino-2-chloropropane hydrochloride (R)-90

5.2.2.8 (S)-(+)2,2-diphenyl-4-dimethylaminopentanenitrile (S)-86

5.2.2.9 (S)-(+)Methadone hydrochloride (S)-93

5.2.3 Lipase screen and chiral GC analysis of the propanoate ester 93

5.2.3.1 Preparation of racemic 1-dimethylamino-2-propyl propanoate 92

5.2.3.2 Lipase screen for the resolution of 1-dimethylamino-2-propanol 89

5.2.4 Large scale resolution and syntheses of chiral methadones 94

5.2.4.1 Resolution of 1-dimethylamino-2-propanol 89

5.2.4.2 (R)-(−)-1-Dimethylamino-2-propanol (R)-95

5.2.4.3 (S)-(+)1-Dimethylamino-2-chloropropane hydrochloride (S)-90

5.2.4.4 (R)-(−)-2,2-diphenyl-4-dimethylaminopentanenitrile (R)-86

5.2.4.5 (R)-(−)-Methadone hydrochloride (R)-83

5.2.4.6 (R)-(−)-1-dimethylamino-2-chloropropane hydrochloride (R)-90

5.2.4.7 (S)-(+)2,2-diphenyl-4-dimethylaminopentanenitrile (S)-86

5.2.4.8 (S)-(+)Methadone hydrochloride (S)-93

5.2.5 Levo-α-acetyl methadol (LAAM) 93

5.2.5.1 6-Dimethylamino-4,4-diphenyl-3-heptanol 94 by hydrogenation 102

5.2.5.2 6-Dimethylamino-4,4-diphenyl-3-heptanol 94

5.2.5.3 6-Dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride (levo-α-acetyl methadol hydrochloride) 93

5.2.5.4 6-Dimethylamino-4,4-diphenyl-3-heptanol 94 - large scale 104

5.2.5.5 6-Dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride (levo-α-acetyl methadol hydrochloride) 93 - large scale 105

6.0 References 107
1.0 Introduction

1.0.1 Introduction

There has been much interest over the past 10-15 years in the use of enzymes as catalysts for synthesis. In particular, due to the fact that enzymes provide their substrates with a unique chiral environment, they have been used extensively for the manufacture of optically active materials. Enzymes offer many advantages over more conventional techniques:

i) They can catalyse many different organic transformations

ii) They show high selectivity for -
   a) a particular functional group (chemoselective)
   b) the relative position of this group (regioselective)
   c) the absolute configuration of the group (enantioselective)

iii) They have a broad substrate tolerance

iv) They can be used under mild conditions

This thesis will concentrate on the application of lipases. Lipases are very versatile biocatalysts as they may be used in impure form without the need for expensive or unstable cofactors. These features allow them to be used economically and efficiently for large scale applications (as will be shown). Often lipases are used immobilised on a suitable support and this improves there ease of use as they can be easily filtered and recovered for re-use.

Chapter 1 will discuss firstly the mode of action of lipases and show how various investigators have revealed some detail of the mechanism of action and active site of these enzymes, secondly the many ways in which lipases have been used to prepare optically active materials will be reviewed, and finally some industrial applications of enzyme catalysis will be discussed.
1.1 Lipases - How they work

In nature, lipases are used to break down triacylglycerides into free fatty acids and glycerols - they catalyse the hydrolysis of ester bonds. In the laboratory, lipases can be used both for the hydrolysis of esters and the esterification of alcohols in the presence of a suitable acyl donor *i.e.* they can act reversibly.

The mechanism of action of all lipases is very similar and they are classed as serine esterases. Scheme 1 shows the mechanism for human pancreatic lipase as a representative example.\(^1\,2\)

![Scheme 1](image)
The active site of the enzyme consists of three amino acid residues known as the catalytic triad, namely aspartic acid-176, histidine-263 and serine-152. Hydrogen bonds are set up between these residues which increase the nucleophilicity of the oxygen of the serine and this can then attack the scissile ester bond forming a tetrahedral intermediate, 1. After displacement of the alcohol, R'OH by water (for hydrolysis) the catalytic triad act together again so that histidine deprotonates the water and at the same time releases a proton to Asp-176. The activated \( OH \) can attack the acylserine giving a second tetrahedral intermediate, 2. This intermediate can then breakdown to give the acid and regenerate the serine-OH so that the catalytic cycle can start again. In principle then, one molecule of enzyme can hydrolyse a large number of substrate ester molecules and this hydrolysis takes place in the specific chiral environment of the active site. Another important property of the active site is that there are no solvent molecules present which may otherwise solvate the activated species and lead to slower reactivity.

In order to understand more about the geometry around the active site, several workers began probing these enzymes with many different substrate molecules. This approach is known as substrate mapping. They developed a picture of the catalytic site and the area around it by changing the sizes and functionalities of the groups around the reactive centre of the substrate molecule and observing what effect this had on the rate of conversion and the enantiomeric purity of the products. By employing this empirical approach a reasonably accurate picture of the region around the catalytic site is obtained.\(^3\)\(^4\) It was found that the best substrates are those which have a substantial difference between the sizes of the two groups around the reactive centre (Figure 1). Also, the favoured enantiomer is that shown in Figure 1, and provided that the large group (L) has a higher priority over the small group (S) according to Prelog's rules then this is the (R)-isomer.
Over the past few years the high resolution X-ray crystal structures of several lipases have been reported including *Rhizomucor miehei* lipase,\(^5\) and human pancreatic lipase.\(^2\) These publications report the presence of a lid or flap which covers the catalytic site of these enzymes. A conformational change occurs at a lipid-water interface causing the lid to move and exposing the active site to the substrate molecule. It is thought that this is a common property of many lipolytic enzymes. It is also noted that once the lid is opened the area around the active site becomes more non-polar due to the hydrophobic residues on the underside of the lid being exposed, and this activated configuration is stabilised in an organic environment.

The group of Kaslauskas *et al.*,\(^6\) have examined the active site of *Candida rugosa* lipase and have established the mechanism by which this enzyme distinguishes between enantiomers. They used phosphonate analogs of \((R)\) and \((S)\)-menthyl esters to form covalently bound complexes with the lipase (Figure 2). Examination of each of these complexes in turn gave an understanding of why the \((R)\)-menthyl ester is preferred.
Figure 2 The X-ray crystal structure of *Candida rugosa* lipase with (R)-menthyl hexyl phosphonate bound to the active site.
The X-ray crystal structure analysis of the (R)-menthyl phosphonate bound to the lipase showed that the histidine residue in the catalytic triad is at a suitable distance to form hydrogen bonds with the serine group and the menthol oxygen (Figure 3). On the other hand, when the menthyl phosphonate has (S)-stereochemistry the isopropyl group points towards the imidazole ring of the histidine causing it to twist away and the distance to the menthol oxygen is too great (4.36Å) to form a hydrogen bond. The disruption of this hydrogen bond explains the much slower reactivity of the (S)-enantiomer of menthol. These workers go on to suggest that the enantiopreference of Candida rugosa lipase is due to the orientation of the catalytic triad and that this is very similar in other lipases. This is therefore a general mechanism of action for many lipases.

\[ \text{Introduction} \]

1.2 Lipase catalysed transformation of meso-substrates

Lipases are particularly effective catalysts for the transformation of prochiral or meso-substrates. The substrate is accommodated within the active site of the
enzyme so that only one of two enantiotopic groups can be transformed thereby creating an asymmetric centre in the molecule. As the substrate adopts an optimum geometry within the chiral environment of the active site it is, in theory, possible for the enzyme to convert an achiral compound into an optically pure product. The great advantage that this has over enzymic resolution of racemic compounds is that all of the substrate can be converted, theoretically, to a product with the same stereochemical configuration and therefore there are no difficulties in separating products with opposite stereochemistries as is the case in resolution procedures.

A wide range of substrates have been transformed into useful optically active building blocks using this type of technology. This work was originally developed by Sih et al.,\textsuperscript{7} in their preparation of (R) and (S)-mevalonic acids (Scheme 2).

![Scheme 2](image)

Here, the pro (R)-methyl ester group of 3 is selectively hydrolysed to give the asymmetric half ester 4.

Several other groups have used a similar approach in the preparation of chiral building blocks for the total synthesis of various natural products.\textsuperscript{8,9,10} Xie et al., have reported that 1,3-diacetoxy-2-methylpropane is a good substrate for \textit{Pseudomonas fluorescens} lipase.\textsuperscript{8,9} The reaction was allowed to proceed to 33% chemical conversion and the monoacetate 5 is obtained with ee >99%. The
unreacted diacetate is recovered. The conversion of 5 to a chiral C-5 isoprenoid, 10 is described which is itself a common unit in natural products such as tocopherol, phyloquinones phytol, and insect pheromones (Scheme 3).

\[
\begin{align*}
\text{HO} & \quad OAc \\
\text{THPO} & \quad OAc (\text{ii}) \\
\text{THPO} & \quad OH (\text{iii}) \\
\end{align*}
\]

\( (i) \text{ DHP, p-TsOH; (ii) K}_2\text{CO}_3, \text{MeOH; (iii) MsCl, pyridine; (iv) NaCN, DMSO; (v) aq. AcOH; (vi) N,N'-\text{carbonyldiimidazole, allyl bromide.} \)

Scheme 3

Firstly, the alcohol functionality was protected as the tetrahydropyran-2-yl ether 6. The acetate ester was then hydrolysed and the alcohol produced 7 was treated with mesyl chloride in pyridine to give the mesylate 8.

Conversion to the nitrile precursor 9 was effected by the reaction with sodium cyanide in DMSO. The THP group was removed with aqueous acetic acid and bromination was achieved by the reaction with allyl bromide and carbonyldiimidazole. (S)-4-Bromo-3-methylbutanenitrile 10 was thus obtained from the homochiral monoacetate 5 in 41% overall yield.

The preparation of (-)-muscone 16, from this C-5 unit is also shown using a novel three carbon ring expansion (Scheme 4).
Dodecanone, 11 was treated with LDA and the resulting enolate was reacted with ethyl cyanoformate to give the β-keto ester 12. This was easily deprotonated with potassium tert-butoxide to give a second enolate which condensed with the chiral isoprenoid 10 to produce 13. Further treatment with potassium tert-butoxide gave the three carbon ring expanded product 14. Acid hydrolysis of the nitrile group with subsequent decarboxylation together

Scheme 4

Dodecanone, 11 was treated with LDA and the resulting enolate was reacted with ethyl cyanoformate to give the β-keto ester 12. This was easily deprotonated with potassium tert-butoxide to give a second enolate which condensed with the chiral isoprenoid 10 to produce 13. Further treatment with potassium tert-butoxide gave the three carbon ring expanded product 14. Acid hydrolysis of the nitrile group with subsequent decarboxylation together
with hydrolysis of the ester functionality produced the 1,5-ketoacid. This was reduced to the alcohol with lithium aluminiumhydride and re-oxidised to give the aldehyde 15 by treatment with pyridinium chlorochromate. Finally, deformylation of 15 with Wilkinson’s complex afforded (-)-muscone 16. This example of a natural product synthesis employs a lipase catalysed desymmetrisation of a prochiral substrate as the method of choice for the generation of the asymmetric centre.

Mori and Chiba\textsuperscript{10} used a similar strategy for the synthesis of (S)-(-)-paraconic acid. They initially looked at the hydrolysis of two substrates, 17 and 18 (Figure 4) in the presence of several lipases in aqueous organic media.

The use of porcine pancreatic lipase gave the best results with the diacetate 18. The reaction was carried out in 30% aqueous acetone at pH 7 and the (R)-half ester, 19 was obtained in 56% yield with ee 86%. This was then oxidised with chromic acid to give the acid 20. Ozonolysis with oxidative work up afforded (S)-(+)\text{-}2\text{-}(acetoxymethyl)succinic acid 21 and treatment with dilute hydrochloric acid produced (S)-(--)\text{-}paraconic acid, 22 (Scheme 5). Absolute optical purity was achieved by crystallisation of its (R)-(+)\text{-}\text{a}-phenethylamine salt. Mori and Chiba stated that the classical resolution of racemic paraconic acid using either (S) or (R)-\text{a}-phenethylamine gave a very poor yield of the pure enantiomer and the use of this lipase catalysed asymmetric hydrolysis of the prochiral diacetate 18 was an improved approach to the chiral acid 22.
The regioselective acetylation of meso-diols, the reverse process, has been studied with the same substrate (Scheme 6).\textsuperscript{11,12}

Again the (R)-half ester, 19 is obtained with high ee (>94\%) but this time the yield is quoted as being quantitative. In the same series of compounds the 2-methylpropanediol has been acetylated in relatively poor yield and low enantiomeric excess.\textsuperscript{12} However, this substrate has been studied further by
Santaniello et al.,\textsuperscript{13} who discovered that the reaction can be pushed further to give a 40:60 mixture of monoacetate and diacetate with no remaining diol. The monoacetate isolated from this has an ee>98%. It is suggested that this is because the real substrate for the lipase is in fact the monoacetate which is resolved by the enzyme. The report confirms this by showing the results of an experiment in which the racemic monoacetate is treated with vinyl acetate in the presence of \textit{Pseudomonas fluorescens} lipase. Here, the unreacted monoacetate is isolated in 40% yield with ee>98%.

Cyclopentene-1,4-diol can be converted to (1S,4R)-4-hydroxycyclopent-2-enyl acetate in a similar fashion\textsuperscript{14} (Scheme 7) but this time pancreatin is the enzyme used. Again both monoacetates are formed but only one is converted to the diacetate.

\begin{equation}
\text{(i) trichloroethyl acetate, pancreatin, THF, NEt}_3, 23^\circ\text{C}
\end{equation}

\textit{Scheme 7}
The chiral monoacetate 24 can be converted to the enone 27 which is an important building block for the synthesis of prostaglandins (Scheme 8). Firstly, the alcohol is protected as the tert-butyldimethylsilyl ether 25. Then hydrolysis of the acetate ester followed by oxidation gives the enone 27. This versatile intermediate has been used for the syntheses of several prostaglandins \(^{15,16}\). A particularly attractive approach involves the conjugate addition to this enone and the subsequent trapping of the enolate produced with a suitable electrophile \(^{17}\). Reaction with the organozinc reagent 28 gives exclusively the 1,4 product 29 and the stereochemistry of the attack is directed by the bulky tert-butyldimethylsilyl group. The product of the conjugate addition is held as the metal enolate and reacted with the propargylic iodide 30. Again the stereochemistry is controlled by the neighbouring stereocentre and the attack by the electrophile is on the opposite face to the eight carbon side chain. The product 31 is then partially hydrogenated using a poisoned catalyst to give the precursor 32. Finally, sodium borohydride reduction of the ketone occurs on the less hindered face and after deprotection prostaglandin F\(_{2\alpha}\) 33 is obtained.

This represents a simple synthesis of a complex biologically active product with the stereochemistry for each step being controlled by the neighbouring chiral centre in the molecule. Essentially then, the asymmetric centre obtained from the initial lipase catalysed reaction controls the stereochemistry for the whole sequence.
Scheme 8 Prostaglandin F$_{2\alpha}$ 33
The asymmetry of meso compounds using lipases has been successful using larger more complex substrates. Xie et al.,\(^8\) took the meso-diacetate, 34 in the presence of Pseudomonas fluorescens lipase in a buffered solution and the monoacetate, 35 was produced in 79\% yield with ee 96\%. The product 35 was then used to synthesise the lactone 38 which has hunger modulating activities (Scheme 9).

Pyridinium dichromate oxidation of the alcohol produced the acid 36. The acetonide protection was removed in the presence of para-toluene sulphonate acid in dichloromethane and under these conditions ring closure to form the lactone 37 occurred. The acetate was then hydrolysed with potassium carbonate and the lactone reformed in the presence of dilute hydrochloric acid.

Heterocyclic systems can also make good substrates for regioselective lipase catalysed hydrolysates. Chênevert and Dickman\(^{18}\) report that meso-2.6-
substituted piperidines can be hydrolysed selectively in the presence of *Aspergillus niger* lipase (Scheme 10).

![Scheme 10](image)

It was noted that the addition of 5% acetonitrile improved the chemical yield and optical purity of the products. Also, it prevented the degradation of the enzyme.

### 1.3 Biocatalysis in Industry

Biocatalysis has become a core technology for many chemical firms, particularly pharmaceutical companies and others in the life sciences sector where enantiomerically pure chiral compounds are becoming more and more important. Indeed, biocatalysis is a key tool in the toolkit of any manufacturer interested in producing optically active materials.

There are many reasons why biocatalysis is becoming such an important technology:

i) It offers access to optically active materials. Often, alternative chemical routes may use patented technology such as Corey’s reduction of ketones using chiral oxazaborolidinones\(^{19}\) or Jacobsen’s asymmetric epoxidations.\(^{20}\)

ii) The number of available enzymes is increasing dramatically. Also natural
diversity is being exploited to access enzymes from different environments leading to the availability of biocatalysts which will tolerate a wide range of reaction conditions. For example, enzymes isolated from organisms which live deep in the ocean will be active at high pressure. Whereas others found in harsh environments such as in volcanic regions are stable at high temperatures and low pH.

iii) Advances in protein technology are allowing scientists to modify enzyme activities by mutagenesis of specific amino acids. This strategy is giving researchers the ability to 'fine-tune' enzymes for specific purposes.

iv) Enzymes are now commonly available in immobilised form or, from Altus Biologies Inc., as CLECs - cross-linked enzyme crystals. Immobilisation on solid support or formation of these cross linked enzyme crystals often gives increased activity. Also the biocatalyst can easily be filtered when the biotransformation is complete and may be recycled, thus giving large cost savings on an industrial scale.

In this section examples will be given of the use of biotransformations in industry for the production of chiral compounds.

1.3.1 Avecia Life Science Molecules
Avecia LSM (formerly Zeneca LSM) have developed a process for the manufacture of (S)-2-chloropropanoic acid ((S)-CPA) using a biotransformation. (S)-CPA is an important chiral building block and is used in the production of enantiopure phenoxypropanoic herbicides such as Mecoprop and Fluazifop.
Non-steroidal anti inflammatory drugs such as naproxen, ibuprofen, and ketoprofen all contain the same chiral propanoic acid group and so could be produced in optically active form using (S)-CPA. However, these compounds have either been marketed in the racemic form or prepared asymmetrically by an alternative route such as the chiral reduction of the acrylic acid using Noyori type asymmetric Ruthenium complexes.\textsuperscript{25} Avecia LSM have themselves developed a route to (R)-2'-benzyloxy-(2,4-difluoro)propiophenone 44 using (S)-CPA 41 as the chiral starting material.\textsuperscript{26}

This material is the key intermediate for the production of antifungal compounds for treating immunocompromised patients such as those with AIDS, or those undergoing chemotherapy or organ transplant.

Prior to the work carried out at Avecia LSM, (S)-CPA had been manufactured by a variety of methods. These included the fermentation of glucose using selected strains of \textit{lactobacillus} to give (R)-lactic acid which was converted to
(S)-CPA by esterification followed by chlorination. (R)-lactic acid is the unnatural isomer and although the bacterial strains which produce it are well characterised the process requires a much higher degree of fermentation control than that which produces the natural (S)-lactic acid. Another alternative synthesis is based on the lipase catalysed resolution of esters of CPA. However, long reaction times and a degree of conversion greater than 60% were required to obtain good optical purity.

Avecia LSM (then Zeneca LSM) developed an industrial scale process to produce (S)-CPA based on the enzymic resolution of the inexpensive racemic chloropropanoic acid using a dehalogenase (Scheme 12).

![Scheme 12]

Over a period of about 8 years, a multidisciplinary team developed this process from conception to production of greater than one thousand tonnes per annum of (S)-CPA. The first problem was to find a dehalogenase which was specific for (R)-CPA. After screening soil samples, several strains of bacteria were found which were capable of dehalogenating both isomers of CPA. One from a *Pseudomonas* species, *Pseudomonas putida*, was found to contain two dehalogenases. One enzyme was specific for (R)-CPA and the other for (S)-CPA. These proteins were separated by ion exchange chromatography and the (R)-CPA specific enzyme was studied to check its activity under various conditions. Avecia LSM found that both the enzyme and the host organism were good candidates for further development work.
The next decision to be made was whether to use the isolated enzyme or to use a whole cell system. The advantage of using whole cells would be the avoidance of having to extract and purify the enzyme but the (S)-CPA specific dehalogenase would need to be inactivated before this would be a viable approach. This was done by chemical mutagenesis which resulted in a strain with no (S)-CPA dehalogenase activity. After the selection of a suitable initiator - monochloroacetic acid - needed to induce the expression of the dehalogenase enzyme, Avecia had a biotransformation process capable of producing one thousand tonnes per annum of (S)-CPA.

Further development involved the use of molecular biology to identify the gene which encoded the (R)-CPA dehalogenase enzyme and this was expressed in a strain of *Escherichia coli*. This expressed much increased levels of active (R)-CPA dehalogenase and led to a 3-4 fold increase in productivity for the overall process.

This example illustrates how a biotransformation can be implemented on an industrial scale and how several hurdles can be overcome to give a robust and highly economical process.

Another example of the application of biotransformations on a commercial scale by Avecia LSM is in their process for producing an optically active intermediate used in the preparation of an anti-glaucoma drug. Here a whole cell microorganism is used to effect an asymmetric reduction of a keto group (Figure 5).
Baker's yeast or *Saccharomyces cerevisiae* is probably the most well known and most commonly used microorganisms to carry out asymmetric reductions of this type. Aveia screened this organism along with more than 40 other species including other yeasts, bacteria and fungi in order to identify both a good conversion rate and good selectivity to give the *trans* product 46.

A species of bacteria, *Lactobacillus plantarum*, gave a high percentage of the desired *trans* product but conversion rates were low. Further studies were carried out using this microorganism but it was found that in order to increase the conversion a near neutral pH was required and under these conditions the substrate was racemised. It was necessary to find an alternative microorganism which gave the required selectivity under acidic conditions. The fungus *Neurospora crassa* gave a *trans:cis* hydroxysulphone ratio of 71:29 in the initial screening experiments. *N. crassa* was known to be active at low pH and when tested at pH 4.3 the product was obtained in >80% yield and very high diastereomeric and optical purity (>98% of the trans product 46).

After optimisation this process was scaled up to plant scale and is proof in practise that biotransformations can be used commercially to produce complex optically pure materials.
1.3.2 Eli Lilly & Co.

Eli Lilly use a similar biotransformation to the one just discussed in their manufacture of an optically pure benzodiazepine 47.28

A key step in this process is the asymmetric reduction of the ketone 48.

After screening microbial libraries, Eli Lilly found that the yeast, Zygosaccharomyces rouxii, gave good conversion to the desired (S)-alcohol 49 with a high optical purity. However, it would only tolerate low concentrations of organic species. It is sometimes possible to overcome this problem by keeping the substrate and product separate from the active organism by using a two-phase system with an immiscible organic solvent. Unfortunately organic solvents were found to be too toxic for this particular yeast.

Eli Lilly devised an ingenious solution to this problem. They found that the reaction could be carried out with a 7-fold increase in substrate concentration by using a polymeric resin - XAD-7. At the start of the reaction the ketone was mostly adsorbed on the surface of the polymer with only a small amount in
the aqueous phase ~ 2 g/L. The yeast was added and as the product was formed it was adsorbed onto the resin. The XAD-7 effectively acted as a slow release mechanism for the substrate and a sponge for the product and in this way limited the exposure of either to the Z. rouxii. Furthermore, when the reaction was complete, as the product was adsorbed on the large (500μm) polymeric beads it was simply isolated using a suitably sized filter screen.

This example shows how a relatively sensitive microorganism can be used to carry out a very stereoselective reduction on a multi-kilogram scale by the use of some clever process engineering.

1.3.3 Chiroscience

Chiroscience (now Ascot Fine Chemicals via Chirotech Ltd) have always had an interest in the use of biocatalysis for the production of chiral materials. Two examples will be given where hydrolytic enzymes have been used to produce useful chiral intermediates. The first of these involves the enzymic resolution of a bicyclic lactam 50\(^24\) (Scheme 12). The single enantiomer lactam 51 can then be converted into optically active carbocyclic nucleosides commonly used as anti-viral agents e.g. carbovir (Scheme 13).

![Scheme 12](image)

Chiroscience developed two resolutions of this lactam. Firstly they used a whole cell system using a bacteria from *Pseudomonas* species. Here the cells were grown up by fermentation and the resulting biomass was frozen and stored. To carry out the resolution, all that was required was to add some of
the frozen cells to an aqueous solution of the racemic lactam. This gave the resolution products as shown in Scheme 12. An advantage of this process was that the fermentation was separate from the biotransformation so the organism could be grown, frozen, stored and used when required. Also the freeze-thaw process itself gave improved activity. This was because it caused some of the cells to break apart allowing some of the free enzyme to be liberated.

The second resolution had the opposite stereoselectivity. In this case, Chiroscience were able to isolate and immobilise the lactamase enzyme from an *Aureobacterium* species. The resolution could then be simply achieved by cycling an aqueous solution of the racemate through a column containing the immobilised enzyme until the (-)-enantiomer was completely hydrolysed. The use of an immobilised system like this allows for large cost savings as the catalyst can be re-used many times. The downstream processing is also greatly simplified as there is no need to extract the resolution products from an aqueous medium containing the enzyme. Once the transformation is complete the aqueous reaction stream is poured into acetone. The unhydrolysed lactam remains in solution and the amino acid crystallises out and can be collected by filtration.

The resolved lactam 51 may then be converted into the anti-viral agent carbovir 58 in ten steps. Firstly, the lactam is hydrolysed and then esterified to give the amino ester 53. To prevent the lactam from reforming, the amino group is acetylated by treatment with acetic anhydride. The ester is reduced with calcium borohydride to give the alcohol 55. After the removal of the acyl group by hydrolysis the free amine is coupled with 2-amino-4,6-dichloropyrimidine to produce 56. Formation of the diazocompound by treatment with *para*-chlorobenzenediazonium chloride followed by reduction
with zinc in acetic acid affords the triamine 57. The guanine fragment is then formed by treatment with ethyl ortho-formate and then sodium hydroxide (Scheme 13).

Scheme 13

Chiroscience have also developed other highly efficient enzyme catalysed resolutions. By combining their skills in enzyme isolation and immobilisation, with reactor design, they have come up with a cost effective and very stereoselective process for obtaining an enantiopure precursor to some important biologically active compounds.
Chiroscience make use of another hydrolytic enzyme for their preparation of an unnatural amino acid L-BOC-TAZ 59 which is used as a mimic for a histidine residue in renin inhibitors.

After initial attempts using an esterase to selectively hydrolyse an ester intermediate had been unsatisfactory, they performed a resolution on the N-acyl precursor (Scheme 14). Here, they use an aminoacylase from pig kidney.

The biotransformation forms part of a one-pot procedure starting from chloromethylthiazole and the acylase is able to carry out the resolution on a crude reaction mixture.

This example illustrates that provided that the enzyme is sufficiently stable a biotransformation can be an integral part of a multi-step chemical sequence and there is often no need to isolate and purify the substrate prior to the biotransformation.
1.3.4 BASF

BASF have been a major supplier of amines for many years. They consider amination reactions to be one of their core technologies. In 1993, they wished to develop the capability of producing optically pure amines. At the time lipases had been used extensively as acylation catalysts in the resolution of alcohols but there were very few examples in the literature of lipase catalysed resolutions of amines. BASF spent about one year carrying out screening experiments with 1-phenylethylamine as the substrate. They looked at using different lipases and different acylating agents. The choice of acylating agent was crucial to the success of the resolution and it was found that the ethyl ester of methoxyacetic acid was an excellent acyl group donor for this resolution (Scheme 15). The selectivity factor, or E-value, was >1000. Interestingly, using ethyl butanoate the rate of acylation is much slower and the ee's of the resolution products are greatly reduced. This suggests that the methoxyacetyl group is more tightly bound possibly due to hydrogen bonding between the oxygen and amino acid residues close to the active site of the lipase. This would make a slight change to the geometry around the active site which may lower the activation energy required to form the second quaternary intermediate and hence increase the rate of the reaction. Also, the selectivity for the (R)-isomer may well be increased by a small change in the shape of the active site.

Scheme 15
Using this enzymic resolution with a range of racemic amines, BASF were able to produce a wide range of optically active amines in quantities of greater than 100 t/a. They are currently developing this process in a dedicated plant with a view to producing chiral amines under strict cGMP guidelines by the end of 2001.
2.0 Aims of the Project

The general aim for the project was to develop some novel routes for the synthesis of target chiral molecules using enzyme catalysis. Ultrafine were interested in biotransformations as it would give unique access to chiral molecules. Ultrafine were also aware that competitors were using enzymes for chiral synthesis and were keen to see if this type of technology would be appropriate for the synthesis of some of their chiral targets.

Initially the project was planned to give a broad overview of biocatalysis and therefore to gain experience in the use of a range of different enzymes. The original plan was firstly, to use hydrolytic enzymes such as esterases and lipases for the kinetic resolution of appropriate substrates and then to move on to use other classes of enzymes such as reductases (both whole cell systems and isolated enzymes with co-factors). Although some time was spent looking at enantioselective reductions using Baker’s yeast and isolated enzymes such as horse liver dehydrogenase, the majority of time for the project was spent studying the use of lipases in the kinetic resolution of chiral secondary alcohols.

2.1 Preface to Chapter 3

The first target molecule was bufuralol which is a beta-blocker and is sold by Ultrafine as the hydrochloride salt in the racemic form. Bufuralol is a substrate for a specific P450 isozyme and is part of a range of substrates, inhibitors, and metabolites available from Ultrafine. Chapter 3 describes an enzymatic route to optically enriched bufuralol. A lipase catalysed kinetic resolution of the precursor is the key step.
2.2 Preface to Chapter 4

Methadone, the maintenance drug for heroin addicts, is generally prescribed as the racemic modification. The starting material in Bockmühl's original synthesis of methadone\(^{30}\) is 1-dimethylamino-2-propanol and an efficient lipase catalysed resolution of this aminoalcohol is described in chapter 4. The conversion of the resolution products to enantiomerically pure methadones is described and the subsequent conversion of the less active d-methadone to levo-\(\alpha\)-acetyl methadol, a longer acting alternative to methadone, is also discussed.
3.0 Results & Discussion I (Bufuralol)

3.1 Introduction
Bufuralol 60 was developed in the 1970's by Roche Products Limited as a β-adrenoreceptor blocking drug. There are many examples of this class of drug and they have similar structures. They all contain an ethanolamine side chain and an aromatic ring. These can either be directly bonded as is the case for sotalol 61, or there can be an oxymethylene spacer separating the ring from the side chain e.g. propranolol 62. The structure of bufuralol combines both of these elements. The benzofuran ring is bonded directly to the ethanolamine side chain but also the side chain is separated from the benzene ring by the oxygen and C-2 carbon of the furan ring.

 Bufuralol is a chiral molecule and the levorotatory (1'S) enantiomer is by far the more active isomer. The dextrorotatory (1'R) enantiomer shows little or no activity in adrenergic assays. Bufuralol was resolved into its optical isomers by the Roche workers and this is described in their original publications. They used a chiral acid - (+) and (-)-di-p-toluoyl tartaric acid - to form diastereomeric salts which were purified by crystallisation and then freed to give the optically pure enantiomers. The aim of our research was to
find an alternative route to (R) and (S)-bufuralol which used an enzyme catalysed transformation as the key step.

The metabolism of bufuralol has been widely studied and approximately fifteen oxidative metabolites have been identified. Some of these have been shown to be potent β-adrenoreceptor antagonists themselves and therefore contribute to the activity of the parent drug.

Bufuralol is a good substrate for the single isozyme cytochrome P-450 11D6 and this single enzyme has been the subject of much investigation. It is deficient or defective in 5-8% of the caucasian population. Ultrafine supplies bufuralol as the racemic hydrochloride salt as part of their range of substrates, inhibitors, and metabolites for specific isozymes in the cytochrome P-450 series. These compounds are sold worldwide as standards to medical research institutes. Ultrafine were interested in developing a route to (R) and (S)-bufuralol as their clients had requested these for use as standard substrates in their metabolism studies. Also Ultrafine were interested in using biotransformations as this would allow them to develop a new technology in house which may well prove useful for other synthetic targets.

3.2 Approaches to Bufuralol

The synthesis of bufuralol was first published as a communication in 1975 by workers at Roche Products Limited and this was later followed by a full paper in 1977. These reports give details of an eight step synthesis starting from 2-ethylphenol blocked in the para position with a bromine atom (Scheme 1). The bromine substituent was necessary in order to direct monoformylation to the ortho-position of the phenol in the first step and was readily removed in the final step by hydrogenation. Cyclisation of the salicylaldehyde was effected by ether formation with chloroacetone and ring closure under basic conditions. The methyl ketone produced was chlorinated by reaction with sulphuryl chloride and then reduced with
sodium borohydride to the chlorohydrin 67. Base treatment gave the epoxide 68 which was reacted with tert-butylamine to give the bromo-protected bufuralol 69. Some of the primary ethanolamine was also formed due to tert-butylamine attack at the more hindered end of the epoxide 68. It was reported that the presence of the bromine substituent on the benzofuran ring suppressed the formation of this unwanted isomer. The final step was the removal of this bromo group by hydrogenation at atmospheric pressure to give bufuralol 60.

\[
\begin{align*}
63 & \xrightarrow{(HCHO)_n} 64 \\
& \xrightarrow{\text{KOH, ClCH}_2\text{COCH}_3} \\
66 & \xrightarrow{\text{SO}_2\text{Cl}_2, \text{CHCl}_3 \text{ reflux}} 65 \\
& \xrightarrow{\text{NaBH}_4, \text{MeOH}} 67 \\
& \xrightarrow{\text{KOH, dry benzene}} 68 \\
& \xrightarrow{\text{H}_2 \text{ Pd-C, MeOH, EtOH, H}^+} 69
\end{align*}
\]

**Scheme 1**
This route to bufuralol, developed at Roche, has several low yielding steps and the overall yield for the process is 4%. An improved synthesis was published in 1991 by Nelson et al.\textsuperscript{34} These workers initially described a twelve step synthesis starting from 63 with an overall yield of 10%. They go on to give details of a more efficient shorter route (Scheme 2). The starting material and the first step are as described by the Roche workers, but Nelson et al. claim a higher yield of 61% (Roche 31 - 54%). The salicylaldehyde 64 can then react with bromoacetaldehyde diethyl acetal in DMF in the presence of potassium carbonate to give the O-alkylated product 70. Cyclisation to give the benzofuran 71 is achieved by acid catalysed intramolecular aldol condensation. Methylene transfer using dimethylsulphonium methyleide in tetrahydrofuran - dimethylsulphoxide affords the epoxide 68. The epoxide is then opened by reaction with tert-butylamine and the bromine removed by hydrogenolysis following the same procedure as the Roche workers.
3.3 Resolution of Bufuralol

As mentioned in the introduction to this chapter, bufuralol has been classically resolved into its optical isomers by the formation and crystallisation of diastereomeric salts using (+) and (-)-di-p-toluoyl tartaric acid. Our initial work centred on the use of a lipase mediated kinetic resolution in an attempt to resolve bufuralol itself. An initial experiment was carried out using Lipase PS (Pseudomonas cepacia) and vinyl acetate as acyl donor. The conditions were based on those used by Schneider et al., in their resolution of 2-phenylethanol. Unfortunately no ester formation was observed.
and only racemic bufuralol was recovered from the reaction. This was probably due to the bulky tert-butyl group preventing access to the active site of the lipase.

3.4 Preparation of the chloroalcohol 80

There was literature precedence for lipase resolution of the chloroalcohol precursor as other β-adrenoreceptor antagonists have been prepared as optical enantiomers using this methodology.\(^\text{36}\) *Candida antarctica* lipase type B was used for the resolution of the 1-aryloxy-3-chloro-propan-2-ol precursors to four beta-blockers. The resolutions were carried out both by hydrolysis/alkoholysis of the O-acetate derivatives and by acylation of the racemic alcohols using vinyl acetate as the acyl donor. The precursor to propranolol was efficiently resolved by *Candida antarctica* catalysed hydrolysis of the O-acetate derivative both the alcohol and unhydrolysed ester were obtained with 95% ee. Thus the chloroalcohol 80 became the first target molecule to try some lipase catalysed resolutions. The route chosen to generate the chloroalcohol 80 (Scheme 3) was similar to Ultrafine’s published synthesis of hydroxybufuralol - a metabolite.\(^\text{37}\)

Starting with 2-ethylphenol 73, the allyl ether 74 is formed in good yield by reaction with allyl bromide in the presence of potassium carbonate. Claisen rearrangement of this allyl ether has been reported as giving a 9:1 ratio of ortho:para products. The minor para regioisomer was observed in the NMR spectrum, the doublet signal from the methylene of the allyl group in the para product is shifted upfield by 0.11ppm compared to that of the major, 2-allyl-6-ethylphenol 75. The relative amounts of these two regioisomers were not quantified and they were not separated, simply reacted through the next step as a mixture. Palladium catalysed rearrangement of the double bond gave the styrene 76 as a mixture of cis and trans-isomers which could be seen in the
220MHz NMR spectrum. The key signal is the doublet of the methyl group which in the minor cis-isomer is shifted upfield by 0.19ppm. Oxidative cleavage of the double bond gave the benzaldehyde 77. It was found that using osmium tetroxide and sodium periodate gave the best yield but ruthenium trichloride and sodium periodate were also tried in a two-phase system with less success. Low temperature ozonolysis has been used before by Ultrafine and this gives similar yields but specialist equipment is required and this has always been carried out off site and so is less convenient. The benzaldehyde 77 was purified by chromatography.

Scheme 3

\[
\begin{align*}
73 & \underset{\text{Br}}{\longrightarrow} \underset{\text{K}_2\text{CO}_3}{\text{74}} \\
75 & \underset{\text{DMA}\text{, reflux}}{\longleftarrow} \text{Bis-(benzonitrile)-palladium (II) chloride} \\
76 & \underset{\text{Toluene, reflux}}{\longrightarrow} \text{77} \\
77 & \underset{\text{CHO}}{\longrightarrow} \underset{\text{K}_2\text{CO}_3, \text{Ethanol}}{\text{78}} \\
78 & \underset{\text{Et}_3\text{N}, \text{TMSTf}, \text{NCS}}{\longrightarrow} \underset{\text{NaBH}_4, \text{MeOH}}{\text{79}} \\
\end{align*}
\]
The next step is the condensation of the benzaldehyde 77 with chloroacetone to give the benzofuranyl methyl ketone 78. Similar conditions to those used by the Roche workers were employed but potassium carbonate was used as the base instead of potassium hydroxide because use of the stronger base was reported to give rise to a byproduct - the 2,2'-hydroxycinnamoylbenzofuran derivative 72 which we did not observe using potassium carbonate.

Attempts to form the chloromethyl ketone 79 directly by using dichloroacetone were unsuccessful. In Ultrafine's preparation of hydroxybufuralol the equivalent intermediate at this stage has a methyl ketone replacing the ethyl group at the 7-position and this compound reacts with dichloroacetone in the presence of a suitable base to give the chlorodiketone albeit in low yield and with the bis-benzofuranyl ketone as a significant side product. Why no reaction at all occurs in the bufuralol series is somewhat of a mystery.

The Roche workers report that the chlorination of the methyl benzofuranyl ketone proceeds smoothly by reaction with sulphuryl chloride in refluxing chloroform solution. We found that using these conditions for our substrate gave no sign of any product so a few drops of 2M hydrochloric acid were added. The reaction then proceeded and produced a mixture of mono and dichlorinated methyl ketones and it was difficult to control the reaction to stop at the monochlorinated stage. The monochlorinated product 79 was
prepared by firstly trapping the enol tautomer of the ketone as the silyl enol by reaction with triethylamine and trimethylsilyl triflate and then reacting with N-chlorosuccinimide. Under these conditions only the monochlorinated ketone 79 was produced but in only 23% yield.

Reduction of the chloroketone 79 with sodium borohydride in ethanol produced the desired chloroalcohol 80 in quantitative yield. It was now possible to investigate some lipase resolutions of this substrate.

3.5 Lipase catalysed resolutions of the chloroalcohol 80 and conversion of the resolution products to bufuralol

The chloroalcohol precursor to bufuralol 80 was subjected to transesterification using trifluoroethyl octanoate as the acyl donor and four different lipases obtained from Amano were used (Scheme 4). The trifluoroethyl octanoate was prepared by the reaction of trifluoroethanol with octanoyl chloride in pyridine with a catalytic amount of DMAP. In all four cases there was tlc evidence of ester formation. The Candida sp. lipase gave the greatest yield of octanoate ester 81 - 19%. A tiny amount of product was also isolated from the Pseudomonas sp. lipase reaction - 1%. The optical purities of these isolated esters was determined by shift reagent NMR spectroscopy using a chiral europium reagent - Eu(hfc)₃. A racemic sample of the octanoate ester 81 was firstly prepared by the reaction of octanoyl chloride with the chloroalcohol 80 in pyridine with DMAP as catalyst and the racemic ester was analysed by chiral shift NMR spectroscopy. The singlet from the proton at C-3 of the furan ring - δ 6.8 starts to split into two signals as chiral shift reagent is added. Unfortunately, as more shift reagent was added, baseline separation of these two signals was not observed before they started to coalesce with the other aromatic signals.
Chiral shift analysis of the octanoate ester 81 produced from the Lipase AY catalysed transesterification shows two unequal signals for the C-3 proton but they are not completely resolved. However, an approximate ee of 75% can be obtained from the relative integrals of these peaks. Analysis of the small amount of ester obtained from the Lipase PS (Pseudomonas) catalysed reaction showed only one peak after treatment with chiral shift reagent. So, although this lipase gives a very poor yield for the resolution, it is very selective and gives optically pure product. This early positive result was very encouraging and led us to further investigations using both Lipase AY and Lipase PS aiming to increase the selectivity and yield respectively.

Vinyl acetate is a widely used acylating agent for lipase catalysed transesterifications. This is due to the fact that the reaction is irreversible as the byproduct is acetaldehyde which is a gas and is lost to atmosphere. (This is convenient on a small scale but may cause problems on a large scale as
acetaldehyde is very toxic and would need to be contained.) The resolution of the chloroalcohol 80 was attempted using vinyl acetate and Lipase AY with vinyl acetate also acting as the solvent. Some of the literature examples have vinyl acetate as both solvent and acyl donor and others try a range of solvents and show that choice of solvent is important for both selectivity and yield. It may be useful for future work to optimise this resolution by investigating a range of different solvents. The mixture was stirred gently for 48h and the resolution products were separated by chromatography. The acetate ester (S)-82 obtained in 18% yield was hydrolysed back to the alcohol (S)-80 by Lipase AY catalysed hydrolysis. The lipase was used in both modes - for esterification and hydrolysis to optimise the optical purity of the product and the resolved (S)-alcohol (S)-80 was thus obtained in overall yield of 29% (Scheme 5). The enantiomeric excess of the alcohol was not measured at this stage - the material was further reacted to bufuralol itself using the same procedure used for the preparation of hydroxybufuralol.\textsuperscript{34} Potassium carbonate, tert-butylamine and a catalytic amount of potassium iodide were added to a solution of the (S)-alcohol (S)-52 in DMF and the mixture was heated at reflux for 7 hours. (R)-Bufuralol (R)-32 was isolated in 47% yield as a yellow oil with an enantiomeric excess of 50% as determined by chiral HPLC.
The same resolution strategy was employed using Lipase PS and vinyl acetate. Again the enzyme was used for both esterification and hydrolysis. (Scheme 6) The (S)-alcohol (S)-80 was obtained in 44% overall yield from the racemic alcohol 80 and was reacted through to (R)-bufuralol (R)-60. Lipase PS gave (R)-bufuralol (R)-60 with an ee of 90% as determined by chiral HPLC.
Results and Discussion

![Scheme 6](image)

3.6 Conclusions and Suggestions for Future Work

A novel route to optically enriched bufuralol has been described. The key step used to introduce the chirality is a lipase mediated kinetic resolution of the precursor, the chloroalcohol 80. (R)-Bufuralol (R)-60 was obtained with ee of 90% but in low yield. The resolution is the penultimate step in the synthetic sequence and it is expensive to lose at least half the weight at this stage. A better strategy would be to have the resolution as early in the synthesis as possible or alternatively to racemise the undesired isomer and repeat the resolution. This can sometimes be done in one pot and this is known as dynamic resolution and the theoretical maximum yield doubles to 100%.

This is certainly worth considering as an area for future work.

The route to the substrate for the resolution has several low yielding steps and there is certainly room for improvement. On going work at Ultrafine is looking at the direct orthoformylation of 2-ethylphenol. This would cut the first four steps to one step and would also avoid the low yielding oxidative cleavage of the styrene.
4.0 Results & Discussion II (Methadone)

4.1 Introduction

Methadone, or Amidone as it was originally named, was first prepared by I.G. Farbenindustrie workers at Hoechst-am-Main in Germany during the Second World War.\(^\text{30}\) It was developed as an analgesic with an activity similar to that of morphine. In fact methadone \(^83\) is an open chain analogue of morphine \(^84\) and obeys the 'morphine rule'.\(^\text{43}\) i.e. :-

i) It has a tertiary nitrogen with a small alkyl substituent

ii) It has a quaternary carbon

iii) It has a phenyl group bonded to the quaternary carbon

iv) There is a two carbon spacer between the quaternary carbon and the tertiary nitrogen (Figure 1)

![Figure 1](image)

Methadone is still a prescribed drug on the market today and is mostly used in the long term maintenance of heroin addicts. Heroin is the 3,6-diacetyl derivative of morphine and is 2-3 times more potent. Most of this increase in potency is due to its greater lipophilicity which leads to rapid CNS penetration. Methadone acts as a slow release heroin mimic. It accumulates in the lipid tissue outside of the CNS and has a slow onset and long duration of action.\(^\text{43}\) So although methadone does not give the same initial feeling of
euphoria as heroin it can be effective in reducing an addict's craving and therefore use of heroin.

Recently there has been some interest in the use of methadone in the treatment of cancer pain.\textsuperscript{44} Workers at the Pain Research Institute in Liverpool have shown that methadone can be particularly effective for the management of cancer pain for those patients who respond poorly to morphine based pain relief.

4.2 Literature routes to homochiral methadones

4.2.1 Classical resolution chemistry

Methadone, 6-dimethylamino-4,4-diphenyl-3-heptanone has one asymmetric carbon at C-6. The biologically active isomer is levomethadone. Several workers in the late 1940's reported resolutions of methadone into its optical isomers.\textsuperscript{45-48} Brode and Hill\textsuperscript{45} reported that methadone formed diastereomeric salts with D-tartaric acid and that these could be separated and freed to give pure L-methadone and D-rich methadone. The D-rich methadone was further purified by melting, the pure D-isomer has a higher melting point to the eutectic mixture of D and L-isomers. Larsen et al.\textsuperscript{46} also used D-tartaric acid as their resolving agent but they also studied the resolutions of isomethadone \textsuperscript{85} and the two nitrile precursors \textsuperscript{86} and \textsuperscript{87} (Figure 2). They stated that all four compounds were readily resolved into their optical isomers. However, a closer inspection of the experimental details reveals that the method for the resolution of the nitrile compounds is far simpler than that for isomethadone and methadone itself.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Figure 2}
\end{figure}
This is further corroborated in a report by Walton, Ofner, and Thorp \textsuperscript{47} in which they comment that "The resolution of methadone was attempted by means of a variety of optically active acids but without marked success" and also that "The penultimate cyanide, on the other hand was readily resolved by means of D-tartaric acid." Howe and Sletzinger \textsuperscript{48} used more exotic resolving agents in their resolutions of methadone and isomethadone. They found that d-methadone formed an insoluble d-\(\alpha\)-bromocamphor-\(\pi\)-sulphonate and that l-methadone could be obtained from the mother liquors. This was achieved by neutralising, forming the d-tartrate salt and allowing this to crystallise from an \(n\)-butanol solution. The l-methadone-d-tartrate was then directly converted to the hydrochloride by successive treatment with sodium hydroxide then hydrochloric acid. These workers also used \(p\)-nitrobenzoyl-L-glutamic acid for the resolution of isomethadone.

### 4.2.2 Synthesis from 'Chiral Pool' starting material

In a study of the stereochemistry of the synthesis of methadone,\textsuperscript{49} Barnett and Smirz obtained their chiral starting material (S)-(+)-1-dimethylamino-2-propanol (S)-\textsuperscript{89} from the ethyl ester of L-(−)-lactic acid \textsuperscript{88} (Scheme 1).\textsuperscript{50}

![Scheme 1](image)

They then essentially followed the procedure of Bockmuhl and Erhardt \textsuperscript{30} to give the optically pure nitrile precursor to methadone in two steps (Scheme 2).
Results and Discussion

\[
\begin{align*}
\text{(S)-89} & \quad \text{(i) SOCl}_2, \text{CHCl}_3, \text{reflux} \quad \text{(R)-90} \\
\text{(S)-86} & \quad \text{(ii) Ph}_2\text{CHCN, NaOH}
\end{align*}
\]

Scheme 2

The (S)-aminoalcohol (S)-89 was treated with thionyl chloride in chloroform and the mixture heated to reflux. The reaction proceeded with inversion of the stereocentre to give 1-dimethylamino-2-chloropropane (R)-90 as the hydrochloride salt. This was converted to the free base and then treated with sodium diphenylacetonitrile in toluene which gave a mixture of the two regioisomeric nitriles. The desired aminonitrile (S)-86 was isolated by chromatography. These workers do not describe the final step which would give chiral methadone but this could simply be achieved by Grignard reaction with ethylmagnesium bromide followed by acid hydrolysis as in the original procedure. 30

4.3 A new lipase mediated approach to the synthesis of 1-methadone

Although the activity of methadone is attributed to the levo-isomer it is generally prescribed as the racemate. The main reason for this is that the currently available methods (described in 4.2 above) are either impractical or too costly, or both. Resolution at the nitrile stage or on the final product itself (4.2.1) is particularly inefficient as this involves the loss of at least half of what is relatively expensive material. A much better approach would be to resolve the cheap starting material at the beginning of the synthesis. The racemic aminoalcohol 89 looked as though it would be a good substrate for a lipase catalysed resolution by transesterification as there is a reasonable difference in
the size of the two groups attached to the chiral centre. Provided that the selectivity is high this would give access to both optical isomers of the aminoalcohol \( \text{89} \) and hence a route to both \( \text{d} \) and \( \text{l} \)-methadones.

The initial resolution of 1-dimethylamino-2-propanol \( \text{89} \) was carried out on a small scale using Lipase PS (Amano) as the biocatalyst. The relative amount of enzyme preparation to substrate was 1:4 w/w and the acyl donor and solvent was vinyl acetate. The reaction mixture was allowed to stir at ambient temperature for two days and then the lipase was removed by filtration.

![Scheme 3](image)

The acetate ester \((\text{R})\)-91 was isolated by chromatography and analysed for optical purity by chiral shift reagent NMR spectroscopy using a chiral europium complex - Eu(hfc)\(_3\) \(39\). The shift reagent NMR spectrum for the racemic ester gave two equal peaks for the acetate signals. So the relative integrals of these peaks in the NMR spectrum of the resolved ester gave a value for the enantiomeric excess of the compound (Figure 3).
This first resolution gave a conversion of 60% and the ester \((R)-91\) had an ee of 46%. Thus Lipase PS shows only moderate selectivity in this resolution. The optical purity of the product ester could be improved by removing the lipase after only 16 hours reaction time. This gave a conversion of 33% but the acetate ester \((R)-91\) was obtained with an ee of 88%. (Figure 4)
Figure 4 Chiral shift NMR spectrum for the resolved acetate (R)-91 at 33% conversion

It is often the case, in lipase catalysed transesterifications, that the optical purity of the ester can be improved by stopping the reaction at less than 50% conversion. Conversely, the optical purity of the remaining alcohol can be optimised by pushing the resolution to beyond 50% conversion.

Unfortunately, for these initial resolutions, we were unable to isolate the unreacted alcohol (S)-89 from the chromatography column. Even eluting with 1:1 methanol : dichloromethane was not sufficient to recover the polar amino alcohol from the column.
Having achieved some selectivity using Lipase PS from Amano, it was now important to try other lipases to hopefully improve the selectivity. Novo Nordisk AS generously provided a sample of their Novozyme ® 435. This is an immobilised preparation of Candida antarctica lipase type B often abbreviated to CALB. There are many examples in the literature where this lipase has been used very effectively in resolution chemistry.51-53 It was also important to find an alternative to chromatography for isolating the resolution products. As we were unable to isolate the remaining amino alcohol and chromatography would make scale up very difficult.

It was found that using Novozyme ® 435 and vinyl propanoate as the acyl donor (Scheme 2) the resolution products could be conveniently separated by firstly filtering off the lipase and washing thoroughly with ethyl acetate and then by washing out the water soluble 1-dimethylamino-2-propanol \((S)-89\) with several portions of water. The ethyl acetate layer was then dried and evaporated to leave the propanoate ester \((R)-92\) as a yellow oil in 35% yield by weight (optimum yield 50%).

\[
\begin{align*}
\text{Me}_2\text{N} & \quad \text{Me}_2\text{N} \\
\text{OH} & \quad \text{OH} \\
\text{Novozyme} \, \circled{\text{435}} & \quad \text{(R)-92} \\
\text{(S)-89} & \quad \text{Me}_2\text{N} \\
\end{align*}
\]

Scheme 4

Several attempts were made to extract the resolved alcohol \((S)-89\) from the water layer using many organic solvents but without success so again we were unable to isolate the alcohol. The optical purity of the propanoate ester \((R)-92\) was not measured as chiral shift reagent NMR spectroscopy was unsuitable for the higher ester. The signals which were split by the europium reagent were multiplets and as more shift reagent was added it was impossible to obtain baseline separation before the signals broadened out and...
collapsed. Alternative methods for chiral analysis were developed later (4.5) but at this stage the material was reacted through to the 1-dimethylamino-2-chloropropane (S)-90 for which there was a literature value for its optical rotation (Scheme 5). The ester (R)-92 was firstly hydrolysed back to the alcohol (R)-89 by a Zemplen type methanolysis using a catalytic amount of freshly prepared sodium methoxide in methanol and then treated with thionyl chloride in chloroform and heated to reflux to give (S)-(+)1-dimethylamino-2-chloropropane (S)-90 as the hydrochloride salt.

\[
\begin{align*}
\text{Me}_2\text{N} & \quad \text{NaOMe} \quad \text{OH} \quad \text{SOCl}_2 \\
(R)-92 & \quad \text{MeOH} \quad \text{Me}_2\text{N} \quad \text{CHCl}_3 \text{ reflux} \quad \text{HCl} \\
(R)-89 & \quad (S)-90
\end{align*}
\]

Scheme 5

The optical rotation was measured after one recrystallisation from 2-propanol - [\(\alpha\)]\(_D\) = +59.1° (c 2.075, H\(_2\)O). This compares to the literature value of -65° (c 2.01, H\(_2\)O) for the (R)-(−)-isomer. A further two recrystallisations from 2-propanol gave material with [\(\alpha\)]\(_D\) = +65.9° (c 2.01, H\(_2\)O). At this stage we had obtained the opposite enantiomer to that which Barnett and Smirz used in their study examining the stereochemistry of the methadone synthesis. We assumed that these workers were working with precursors to the biologically active levomethadone. So we wished to isolate the unreacted (S)-alcohol (S)-89 from the resolution as we believed this to be the starting material for 1-methadone. It was also necessary to obtain some chiral analysis for the resolution products.

The resolution of 1-dimethylamino-2-propanol 89 was again carried out using Novozyme \(^\circledR\) 435 and vinyl propanoate as the acyl donor. The conditions were varied slightly so as to push the resolution to beyond 50% conversion in an attempt to give the required (S)-alcohol (S)-89 with higher optical purity.
The reaction was stirred slowly at 40°C for 3 days and a small excess of vinyl propanoate was used - 0.6 equivalents. The (S)-alcohol (S)-89 was isolated by distillation at reduced pressure and was obtained as a colourless oil in 32% yield based on a maximum desired yield of 50%. The second component to distil was the (R)-propanoate ester (R)-92 which distilled at 60°C at approximately 5mm Hg pressure and was obtained as a colourless oil in 36% yield. The optical rotation of the (S)-alcohol (S)-89 was measured in ethanol, \([\alpha]^D_0 = +23^\circ\) and this compared very well to the literature value of +24°.49 The (S)-alcohol was then converted through to homochiral methadone (Scheme 6).

\[
\text{Scheme 6}
\]

The (R)-1-dimethylamino-2-chloropropane (R)-90 was formed as the hydrochloride salt by the reaction of the alcohol (S)-89 with thionyl chloride in chloroform. This compound was then recrystallised from 2-propanol to give material with an optical rotation comparable to that given in the literature.49 The chloro compound (R)-90 was then added to a rapidly stirred mixture of diphenylacetonitrile in aqueous sodium hydroxide/dimethylsulphoxide with a catalytic amount of dibenzo-18-crown-6.55 The desired nitrile (S)-86 was obtained in low yield (33%). The mechanism for this step is thought to go via an aziridinium ion intermediate (Scheme 7).
As shown the diphenylacetonitrile anion can attack this reactive intermediate at two sites giving rise to two isomeric products. The desired product (S)-86 is favoured as this results from attack at the less hindered centre. The 18-crown-6 acts as a phase transfer catalyst which allows the reaction to be carried out at a lower temperature and favours formation of the desired aminonitrile (S)-86. This reaction was studied by Poupaert et al.\textsuperscript{55} and using these conditions they observed a 72:28 ratio in favour of the desired isomer (S)-86 using direct GC analysis of the reaction mixture, and they isolated the desired product in 44\% yield. In our hands, the aminonitrile derivative (S)-86 was isolated by crystallisation from hexane in 33\% yield and no attempt was made to isolate the unwanted isomer (S)-87. The optical rotation was measured as $[\alpha]_D = +52.9^\circ$ which compares to +49$^\circ$ in the report by Barnett and Smirz.\textsuperscript{49}

Conversion of the nitrile compound (S)-86 through to methadone was achieved by Grignard attack at the nitrile carbon followed by hydrolysis to give the ketone (S)-83 in 53\% yield. This was the same procedure as that used by Bockmuhl and Erhardt.\textsuperscript{30} The optical rotation was measured and found to be $[\alpha]_D = +136^\circ$. So by following the publication of Barnet and Smirz,\textsuperscript{49} the
inactive dextro rotatory isomer of methadone (S)-83 had been produced. It was wrong to assume that their report was based on the precursors to the active levomethadone.

The (S)-(+)1-dimethylamino-2-chloropropane (S)-90 had already been prepared from the ester (R)-92 isolated from the resolution. So to obtain the active l-methadone it was simply a matter of repeating the final two steps described above. l-Methadone was obtained as the hydrochloride salt ([α]D = -136°) in a comparable yield to its optical antipode.

4.4 Chiral HPLC analysis

Having obtained both d and l-methadones in high optical purity as demonstrated by their [α]D values we wished to explore a possible chiral HPLC method in order to obtain enantiomeric excess values for both optical isomers. Kristensen et al. 56 published a method for the determination of the enantiomers of methadone in blood serum using a chiral HPLC column from Chromtech. Using this column we were able to demonstrate that the enantiomers of methadone had different retention times. By testing a racemic sample obtained from Sigma using a mobile phase of 840:160 0.01M sodium phosphate buffer at pH 6.5: acetonitrile and a flow rate of 0.9ml/min two peaks were observed of equal intensity at just over 10 mins and 13.1 mins.

According to the Danish workers the first compound to elute was (R)-(−)-methadone followed by (S)-(+)methadone. On analysing the chiral methadones we had synthesised this was found to be the case and in both the HPLC traces no evidence for the presence of the opposite enantiomer was observed. So essentially using an enzymatic resolution of the starting amino alcohol optically pure (R)-(−)-methadone and (S)-(+)methadone have been prepared. The ee values are quoted as >99% because of the limit of detection of the HPLC instrument.
4.5 Chiral GC analysis of the resolution products

The optical purity of the methadones had been established yet there was still limited analytical data for the products of the resolution. So the resolution of 1-dimethylamino-2-propanol (S)-89 was carried out again and the reaction mixture was analysed periodically by chiral GC using a β-cyclodextrin column. The racemic 1-dimethylamino-2-propyl propanoate 92 was firstly prepared by the reaction of the racemic aminoalcohol 89 with propanoyl chloride. The ester isolated from this reaction was resolved into an even doublet by the GC column. Chiral GC analysis of the resolution reaction mixture after 2h showed that the conversion of aminoalcohol 89 to the propanoate ester (R)-92 was already 19% and the minor (S)-propanoate ester can only be seen as a tiny blip on the GC trace and was not integrated (Figure 5). Thus, an accurate ee value cannot be quoted but it can be assumed to be greater than 98%.

![Chiral GC trace for the racemic propanoate ester 92 and for the resolution reaction mixture after 2h](image)

**Figure 5** Chiral GC trace for the racemic propanoate ester 92 and for the resolution reaction mixture after 2h
After 4h the conversion was 32% and the ee of the propanoate ester (R)-92 was 95.9%. Further samples were taken at 16, 17, and 18 h and the results are represented in the graph below.

**Figure 6.** The resolution of 1-dimethylamino-2-propanol 89 using novozyme® 435 and vinyl propanoate

A final sample was analysed after a reaction time of 88h and this showed that the resolution was complete at 50% conversion and that the ee of the product ester was still high at 95.6%. Thus the Novozyme® 435 lipase preparation is extremely selective in this resolution. It is almost specific for the (R)-aminoalcohol (R)-89 and even after over 3.5 days reaction time only a very small amount of (S)-ester (S)-92 was detected.

### 4.6 Lipase screen

Having found a good method of analysis which gives both the degree of conversion to the ester and the ee of the ester and having obtained a chirazyme® lipase screening kit as a gift from Boehringer Mannheim it was now possible to carry out a screen using the substrate 1-dimethylamino-2-propanol 89 and the various enzyme preparations in the kit (Table 1).
Table 1. Origin of the lipases in the chirazyme® screening kit

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Burkholderia sp.</td>
</tr>
<tr>
<td>L2</td>
<td>Candida antarctica, fraction B</td>
</tr>
<tr>
<td>L3</td>
<td>Candida rugosa</td>
</tr>
<tr>
<td>L4</td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td>L5</td>
<td>Candida antarctica, fraction A</td>
</tr>
<tr>
<td>L6</td>
<td>Pseudomonas sp.</td>
</tr>
<tr>
<td>L7</td>
<td>Porcine pancreas</td>
</tr>
<tr>
<td>L8</td>
<td>Humicola sp.</td>
</tr>
</tbody>
</table>

Table 2. Results of the resolution of 1-dimethylamino-2-propanol 89 using the chirazyme® screening kit

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Time/h</th>
<th>Conversion/%</th>
<th>ee/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>2</td>
<td>6</td>
<td>81.9</td>
</tr>
<tr>
<td>2</td>
<td>L1</td>
<td>21</td>
<td>66</td>
<td>45.8</td>
</tr>
<tr>
<td>3</td>
<td>L2</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>L2</td>
<td>21</td>
<td>2</td>
<td>&gt;99</td>
</tr>
<tr>
<td>5</td>
<td>L3</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>L3</td>
<td>21</td>
<td>2</td>
<td>27.1</td>
</tr>
<tr>
<td>7</td>
<td>L4</td>
<td>2</td>
<td>37</td>
<td>88.5</td>
</tr>
<tr>
<td>8</td>
<td>L4</td>
<td>70</td>
<td>95</td>
<td>13.3</td>
</tr>
<tr>
<td>9</td>
<td>L5</td>
<td>2</td>
<td>0.5</td>
<td>&gt;99  *</td>
</tr>
<tr>
<td>10</td>
<td>L5</td>
<td>70</td>
<td>48</td>
<td>58.1 *</td>
</tr>
<tr>
<td>11</td>
<td>L6</td>
<td>2</td>
<td>2</td>
<td>&gt;99</td>
</tr>
<tr>
<td>12</td>
<td>L6</td>
<td>70</td>
<td>87</td>
<td>28.3</td>
</tr>
<tr>
<td>13</td>
<td>L7</td>
<td>2</td>
<td>3</td>
<td>&gt;99</td>
</tr>
<tr>
<td>14</td>
<td>L7</td>
<td>70</td>
<td>45</td>
<td>80.3</td>
</tr>
<tr>
<td>15</td>
<td>L8</td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>L8</td>
<td>70</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Results and Discussion II

It can be seen from the results given in Table 2 that most of the lipases showed some activity. In fact, only lipase L-8 gave no reaction at all and no product ester was observed in the GC trace. Lipases L-1, L-4, and L-6 showed good ee values for the product ester after short reaction times but after prolonged reaction the percentage conversion increased to beyond 50% and hence the selectivity fell. Lipases L-1 and L-2 gave slow rates for the resolution but L-2 showed a high selectivity. Out of all the lipases in the kit L-7 was the most promising (entries 13 and 14), initially showing very high selectivity and after 70 hours gave the ester with an ee of 80.3% and a degree of conversion of 45%. Curiously, lipase L-5 showed a reasonably strong preference for the (S)-aminoalcohol and after 3 days (entry 10) gave the (S)-propanoate ester (S)-92 with an ee of 58.1%.

4.7 Large scale resolution

The lipase screen has shown that none of the lipases in the Chirazyme ® kit are as effective as Novozyme ® 435 for the resolution of 1-dimethylamino-2-propanol 89 with vinyl propanoate as the acyl donor. Thus the resolution was scaled up using Novozyme ® 435 to establish whether or not the same methodology was appropriate for a kilogram scale transesterification.

1 Kg of the aminoalcohol 89 was treated with vinyl propanoate (0.5 equivalents) and 3% by weight Novozyme ® 435 was added. The mixture was stirred for three days and the resolution products were both isolated by distillation at reduced pressure. The unreacted (S)-aminoalcohol (S)-89 was the first compound to distill and was obtained as a colourless oil in 45% yield. This compares to a yield of 32% when the same resolution was carried out on a small scale. The (R)-propanoate ester (R)-92 distilled over as a colourless oil in 36% yield and again this is slightly higher than that obtained from the small scale resolution. An overall recovery of 81% was obtained for this large scale resolution. The resolution products were reacted through to the optical
isomers of methadone (R)-83 and (S)-83 using the same procedure as for the small scale syntheses. 94g of 1-methadone hydrochloride (R)-83 were obtained with an ee of 88.6%.

4.8 Levo-α-acetylmethadol

4.8.1 Introduction

Methadone was just one of a series of compounds which were studied as part of the research activities of I.G. Farbenindustrie at Hoechst-am-Main in Germany during the Second World War. Workers in America in the mid to late 1940's also produced a wide variety of related compounds. One of these, the acetoxy derivative had a similar potency to methadone. It was prepared by several groups in 1948 and 1949. Firstly, in 1948, May and Mosettig reported that amidone (methadone) could be reduced by hydrogenation with platinum oxide (Adam's catalyst) to give the carbinol and that only one of the two possible pairs of diastereoisomers were obtained - the α-methadols (Scheme 8).

A possible explanation for this is that methadone is held in a five-membered ring due to the attraction of the lone pair of electrons on the nitrogen to the carbon of the carbonyl group (Figure 7). Due to the free rotation of the bond between the quaternary carbon and the carbonyl carbon two conformations are possible. The 1,3-interaction between the methyl and ethyl groups is minimised when they are trans to each other and therefore this is the lowest energy conformation. Therefore (S)-methadone is reduced to (2S, 5S)-methadol and (R)-methadone gives (2R, 5R)-methadol.
May and Mosettig reported that acetylation of the methadol could be achieved by reaction with acetic anhydride in pyridine but this was low yielding. Better yields were obtained by firstly deprotonating with ethyl magnesium bromide and then reaction with acetic anhydride. This gave the O-acetyl derivative 93 in 80-90% yield. Pohland et al at the Lilly Research Laboratories, USA reported the synthesis of the O-acetyl derivative and also included some preliminary pharmacology tests carried out on the rat. These showed that although the α-methadols showed little activity compared to methadone, the α-acetyl methadols had similar activities. The d-isomer derived from the active l-methadone had double the activity of racemic methadone. The l-isomer 93 derived from d-methadone had a lower analgesic activity (5.4 times lower than the d-isomer) but had a delayed onset and a long duration of action. It is these properties which make the l-isomer a useful alternative to methadone in the maintenance of heroin addicts.
In the U.S., LAAM has been shown to be effective in maintenance programs when it is administered three times a week whereas methadone needs to be taken every day. As patients taking LAAM spend less time in the clinic they may have more 'normal' lifestyles and may be able to find employment. This would obviously help in the rehabilitation of the patient and offers a significant advantage over methadone.

4.8.2 The synthesis of LAAM from d-methadone

d-Methadone (S)-83 was obtained from (S)-(+-)1-dimethylamino-2-propanol (S)-89 in three steps, having isolated the homochiral aminoalcohol from a lipase mediated transesterification of the racemic material as described earlier (4.3). An initial attempt to reduce d-methadone was made using the conditions described by May and Mosettig in their original publication. Thus, d-methadone was hydrogenated in a Parr hydrogenation apparatus with platinum (IV) oxide as the catalyst. After overnight reaction, the product was isolated and found to be a mixture of methadone and the reduced methadol 94. The reduction of d-methadone was also tried using sodium borohydride as the reducing agent with a catalytic amount of the Lewis acceptor cerium (III) chloride and this proceeded very smoothly and the reduced methadol 94 was obtained in quantitative yield. As the borohydride reduction was so successful no further attempt was made to push the hydrogenation to completion. Conversion of the methadol 94 obtained to the acetate ester 93 was simply achieved by treatment with acetyl chloride and heating to reflux in ethyl acetate (Scheme 9).

\[
\text{Ph} \quad \text{Ph} \quad \text{NMe}_2 \quad \text{OH} \quad \xrightarrow{\text{AcCl, EtOAc, reflux}} \quad \text{Ph} \quad \text{Ph} \quad \text{NMe}_2 \quad \text{OAc} \quad .\text{HCl} \\
\text{94} \quad \xrightarrow{\text{AcCl, EtOAc, reflux}} \quad \text{93}
\]

Scheme 9
LAAM 93 was isolated as the hydrochloride salt in 84% yield. The \([\alpha]_D\) was measured as -60.6° which is slightly higher than the value quoted in the literature of -59°. Thus, using a slightly modified route (from that used by May and Mossettig) LAAM has been obtained in excellent yield and high optical purity based on its optical rotation.

4.9 Conclusions and suggestions for future work

A lipase catalysed resolution of 1-dimethylamino-2-propanol 89 has been described which is highly stereospecific. The resolution products have been reacted through to the enantiomerically pure isomers of methadone and the process has been scaled up. The initial resolution can be carried out using 1Kg of substrate aminoalcohol 89 which gives approximately 100g of the active 1-methadone (R)-83 and further scale up to pilot plant and even larger would present few difficulties. The d-isomer (S)-83 has been converted in two steps to levo-\(\alpha\)-acetylmethadol 93 which is prescribed as a longer acting alternative to methadone to heroin users in the U.S.

The possibilities for further work are quite considerable:-

i) The scope of the resolution itself could be studied. In particular, the effect of the presence (or absence) of the tertiary amine group in the substrate molecule on the selectivity and conversion rate could prove to be an interesting area of research. Several groups including our own have used nitrogen bases such as triethylamine or pyridine as additives in similar kinetic resolutions and in some cases they have a marked effect. The reason for this is a matter of some speculation but they are thought to prevent the inhibition of the enzyme by coordinating to any polar substances which may be present and which may otherwise coordinate to the amide bonds in the lipase and affect the activity of the catalyst. This topic has been recently reviewed by Theil.
Another area which could be suitable for further work is the use of alternative carbanions other than that of diphenylacetonitrile to attack the intermediate aziridinium ion which is the proposed intermediate formed in the penultimate step of the methadone synthesis (4.3 Scheme 7). This would generate a range of alternative compounds which may have biological activity as analgesics.

The lipase screen using the chirazyme® screening kit gave an interesting result in that lipase L-5 showed strong selectivity for the (S)-aminoalcohol (S)-89. This was opposite to all the other lipases screened and disaccords with Kaslauskas' empirical rules. As our objective was to improve the optical selectivity of the resolution, no further work was carried out using this lipase.
5.0 Experimental

5.0.1 General Experimental

$^1$H and $^{13}$C nmr were recorded on Perkin Elmer R34 220, Bruker AC 250, Bruker 300 or Bruker 400 instruments. Chemical shifts ($\delta_H$, $\delta_C$) are reported in ppm and coupling constants ($J$) are in Hertz (Hz). Chemical shifts were referenced to residual undeuterated solvent present in the deuteriated sample, *i.e.* CHCl$_3$ in CDCl$_3$ for all but those recorded on the continuous wave Perkin Elmer R34 220. Chemical shifts for those recorded on the Perkin Elmer R34 220 were referenced to tetramethylsilane as internal standard.

Fast atom bombardment (FAB) mass spectrometry was performed on a Kratos MS50TC spectrometer.

Infra-red spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer with a golden gate ATR. The frequencies (v) were measured in wavenumbers (cm$^{-1}$). Liquid samples were measured as thin films and solid samples were crushed in the diamond tipped probe and measured directly.

Melting points were measured on a Reichert-Jung micro hot stage apparatus, and are quoted in °C and are uncorrected.

Chiral HPLC analysis was carried out using Gilson hardware. A chiral-AGP column was used as the stationary phase for the methadone analyses, eluting with 0.01M sodium phosphate buffer pH 6.5: acetonitrile (840:160) with a flow rate of 0.9 ml min$^{-1}$. For the bufuralol analyses, a chiral Phenomenex 3022 column was used eluting with hexane: 1,2-dichloroethane: ethanol (88:10:2)
with 5% trifluoroacetic acid with a flow rate of 1.0 ml/min. Retention times \( (R_t) \) are quoted in minutes.

Optical rotations were measured on an Optical Activity poLAAr 2001 polarimeter having a readability of ± 0.001° (sodium 589 nm detection). Sample concentration was measured in g/100ml and \([\alpha]_D\) are quoted in \(10^{-1}\) deg cm\(^2\) g\(^{-1}\).

Thin layer chromatography (tlc) was carried out on aluminium plates coated with silica gel 60 F254, with detection by UV (254 nm) fluorescence, ammonium molybdate or potassium permanganate dips. Chromatography was carried out using silica gel 60 (Merck 7729).

All reagents were used as supplied by commercial sources unless stated. Novozyme ® 435 was received as a gift from Novo-Nordisk and the Chirazyme ® Lipase and Esterase Screening Kit was received as a gift from Boehringer Mannheim. Dry solvents were used when necessary and were obtained from commercial sources.

5.1 Bufuralol

5.1.1 The route to 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-hydroxyethane

5.1.1.1 Preparation of 1-Allyloxy-2-ethylbenzene
Potassium carbonate (425g, 3.07mol) was added to a stirred solution of 2-ethylphenol (250g, 2.05mol) in dimethylformamide (900ml) at 0°C. Allyl bromide (212.5ml, 2.46mol) and tetrabutylammonium bromide (10mg) were added and the mixture allowed to warm to room temperature and stirred for 16 hours. Tlc (EtOAc:hexane, 1:4) showed complete reaction so the mixture was poured into water (3L) and extracted with ethyl acetate (3 x 1L). The combined extracts were washed with water (1L) and brine (1L) and dried over magnesium sulphate. Filtration and removal of the solvent under reduced pressure afforded the title compound 74 as an orange oil (331.6g, 99.8%).

Rf (EtOAc:hexane, 1:4) 0.63

$\nu_{\text{max}}$ (neat)/cm$^{-1}$ 2965, 2930, 2872 (CH), 1237 (C-O)

$\delta_{\text{H}}$ (CDCl$_3$; 250 MHz) 7.20 (2 H, m, CH$_{ar}$), 6.90 (2 H, m, CH$_{ar}$), 6.12 (1 H, m, CH$_2$CH=CH$_2$H$_B$), 5.49 (1 H, dm, $J_{17.3}$, CH$_2$CH=CH$_2$H$_B$), 5.32 (1 H, dm, $J_{10.6}$, CH$_2$CH=CH$_2$H$_B$), 4.60 (2 H, dm, $J_{4.9}$, CH$_2$CH=CH$_2$H$_B$), 2.73 (2 H, q, $J_{7.5}$, CH$_3$CH$_2$), 1.27 (2 H, t, $J_{7.5}$, CH$_3$CH$_2$)

$\delta_{\text{C}}$ (CDCl$_3$; 63 MHz) 156.3 (ipso-Ar, CO), 132.9 (ipso-Ar), 133.7 (CH$_2$CH=CH$_2$), 129.0, 126.7, 120.6, 116.7 (CH$_{ar}$), 111.4 (CH$_2$CH=CH$_2$), 68.6 (CH$_2$CH=CH$_2$), 23.4 (CH$_2$CH$_3$), 14.2 (CH$_2$CH$_3$)

m/z (FAB) 91 (100%) 57 (18.3, OCH$_2$CH=CH$_2$)

5.1.1.2 Preparation of 2-Allyl-6-ethylphenol 75

A solution of 1-allyloxy-2-ethylbenzene 74 (331g, 2.04mol) was stirred at reflux in dimethylaniline (500ml) for 17 hours. Tlc (EtOAc:hexane, 1:4)
showed complete reaction so the solution was poured into concentrated hydrochloric acid (600ml) and ice (600ml) and this was shaken for 5 mins before ether (1L) was added. The layers were separated and the aqueous was extracted with more ether (2 x 1L) and the combined organics were washed with water (1L) and brine (1L) and dried (magnesium sulphate). The solvent was removed at reduced pressure to leave 75 as an orange/red oil (313.5g, 94.7%).

Rf (EtOAc:hexane, 1:4) 0.50

$\delta_H$ (CDCl$_3$; 250 MHz) 7.09 (1 H, dd, $J$ 7.4, 1.4, CH$_{ar}$), 7.01 (1 H, dd, $J$ 7.4, 1.6, CH$_{ar}$), 6.88 (1 H, t, $J$ 7.4, CH$_{ar}$), 6.03 (1 H, m, CH$_2$CH=CH$_2$), 5.25 (2 H, m, CH$_2$CH=CH$_2$), 5.06 (1 H, s, OH), 3.46 (2 H, d, $J$ 6.3, CH$_2$CH=CH$_2$), 2.67 (2 H, q, $J$ 7.5, CH$_3$CH$_2$), 1.27 (2 H, t, $J$ 7.5, CH$_3$CH$_2$).

[lit. 63] $\delta_H$ (CDCl$_3$; 100MHz) 6.86 (1H, t, p-H), 4.94 (1H, d, OH), 5.0 (2H, m, CH$_2$CH=CH$_2$), 5.2-5.8 (1H, m, CH$_2$CH=CH$_2$), 3.45 (2H, d, $J$ 6, CH$_2$CH=CH$_2$).

The NMR spectrum showed approximately 20% of the para-product.

$\delta_H$ (CDCl$_3$; 250 MHz) 6.73 (1 H, d, $J$ 8.1, CH$_{ar}$) 4.69 (1 H, s, OH) 3.34 (2 H, d, $J$ 6.7, CH$_2$CH=CH$_2$)

5.1.1.3 Preparation of 3-Ethyl-2-hydroxy-β-methylstyrene 76

Bis-(benzonitrile)-palladium (II) chloride (1g, cat.) was added to a solution of 2-allyl-6-ethylphenol 75 (163g, 1mol) in toluene (600ml) and the reaction mixture was stirred at reflux for 1 hour and then allowed to cool to room
temperature overnight. The toluene was removed at reduced pressure and the residue filtered through a plug of silica washing with diethyl ether (100ml). The ether was removed on the rotary evaporator and the styrene purified by distillation at reduced pressure (100-120°C @ 15 mm Hg). 3-Ethyl-2-hydroxy-β-methylstyrene 76 was obtained as a pale yellow oil (142g, 87%). The 250 MHz NMR spectrum showed this to be a mixture of the cis and trans products in a ratio of 1:2 respectively.

trans-isomer:-

Rf (EtOAc:hexane, 1:4) 0.48

δ_H (CDCl_3; 250 MHz) 7.16 (1 H, dd, J 7.5, 1.5, CH_ar), 7.05 (1 H, d, J 7.5, CH_ar), 6.87 (1 H, t, J 7.5, CH_ar), 6.60 (1 H, dd, J 15.8, 1.5, ArCH=CHCH_3), 6.20 (1 H, dq, J 15.8, 6.6, ArCH=CHCH_3), 5.08 (1 H, s, OH), 2.67 (2 H, q, J 7.5, CH_3CH_2), 1.95 (3 H, dd, J 6.6, 1.7, ArCH=CHCH_3), 1.27 (2 H, t, J 7.5, CH_3CH_2).

cis-isomer:-

δ_H (CDCl_3; 250 MHz) 7.11 (1 H, d, J 7.2, CH_ar), 6.98 (1 H, dd, J 7.5, 1.8, CH_ar), 6.88 (1 H, t, J 7.4, CH_ar), 6.43 (1 H, dd, J 11.2, 1.4, ArCH=CHCH_3), 6.07 (1 H, dq, J 11.2, 6.9, ArCH=CHCH_3), 5.11 (1 H, s, OH), 2.66 (2 H, q, J 7.5, CH_3CH_2), 1.75 (3 H, dd, J 6.9, 1.7, ArCH=CHCH_3), 1.27 (2 H, t, J 7.5, CH_3CH_2).

5.1.1.4 Ruthenium (III) chloride catalysed oxidative cleavage of styrene 76

To a 5L flange flask fitted with a mechanical stirrer was added styrene 76 (65g, 0.38mol), sodium periodate (347g, 1.62mol), carbon tetrachloride (1.5L), acetonitrile (1L), and water (1.5L). The resulting 2-phase mixture was stirred
and cooled to -4°C (ice/methanol bath) and anhydrous ruthenium (III) chloride (2g, cat.) was added in one portion. The temperature rose to 9°C over 5 minutes and then after removal of the cooling bath to 27°C over a further 15 minutes. The now darkened mixture was allowed to stir for 1.5 h to complete reaction. The solid was filtered off and the liquors extracted with ether (3 x 1L). The combined extracts were washed with water (1L), brine (1L), and dried (sodium sulphate). Evaporation to dryness afforded crude benzaldehyde 77 as a dark oil. This was chromatographed on silica eluting with 0-3% ethyl acetate in hexane. Product containing fractions were pooled and evaporated to leave 3-ethyl-2-hydroxybenzaldehyde 77 as a yellow oil (14.2g, 25%).

δ_H (CDCl_3; 220 MHz) 9.95 (1 H, s, H=O), 7.45 (1 H, d, J 8, CH_3), 7.00 (2 H, m, CH_2), 2.70 (2 H, q, J 7.5, CH_3CH_2), 1.25 (2 H, t, J 7.5, CH_3CH_2).

5.1.1.5 Osmium (VIII) oxide catalysed oxidative cleavage of styrene 76

The styrene 76 (49g, 0.302mol) was stirred in dioxane (200ml) at room temperature and a solution of osmium tetroxide ca. 0.8% (50ml) was added. Sodium periodate (132g, 0.617mol) was added in portions over 30 minutes. The mixture was stirred at ambient temperature for 70 h and then diluted with water (2L) and extracted with ethyl acetate (2 x 1.5L). The combined organics were washed with brine (2L) and dried (magnesium sulphate). Evaporation afforded a dark oil which was purified by chromatography on
silica eluting with 0-3% ethyl acetate in hexane. 3-Ethyl-2-hydroxybenzaldehyde 77 was obtained as a yellow oil (24.8g, 54.7%).

δ\text{H} (CDCl\textsubscript{3}; 220 MHz) 9.98 (1 H, s, HC=O), 7.41 (1 H, d, J\textsubscript{8, CH\textsubscript{ar}}), 6.95 (2 H, m, CH\textsubscript{ar}), 2.65 (2 H, q, J 7.5, CH\textsubscript{3}CH\textsubscript{2}), 1.25 (2 H, t, J 7.5, CH\textsubscript{3}CH\textsubscript{2}).

5.1.1.6 Preparation of 7-ethylbenzofuran-2-ylmethylketone 78

\begin{center}
\begin{tikzpicture}

% Diagram code here
\end{tikzpicture}
\end{center}

To a stirred solution of the 3-ethyl-2-hydroxybenzaldehyde 77 (1g, 6.6mmol) and chloroacetone (0.92g, 10.0mmol) in ethanol (10ml) was added potassium carbonate (1.84g, 13.3mmol) in portions over 30 minutes. The mixture was stirred at room temperature for 17 h. Water (100ml) was added and the mixture extracted with dichloromethane (3 x 100ml). The combined extracts were washed with 2M sodium hydroxide solution (2 x 100ml), water (100ml), brine (100ml), and dried (magnesium sulphate). The solvent was removed under reduced pressure to leave an orange oil which was chromatographed on silica eluting with 0-3% ethyl acetate in hexane. Fractions containing product were pooled and evaporated to dryness to leave a pale oil which crystallised on standing (520mg, 41.9%).

R\text{f} (EtOAc:hexane, 1:4) 0.39

Mp 56-58°C [lit. \textsuperscript{64} 54.5°C \textsuperscript{65} 58-60°C]

v\text{max} (neat)/cm\textsuperscript{-1} 1678 (C=O) [lit. \textsuperscript{65} v\text{max} 1682 cm\textsuperscript{-1}]
Experimental

\(\delta_H (\text{CDCl}_3; 250 \text{ MHz})\) 7.56 (1 H, dd, \(J = 7.5, 1.5\), \(\text{CH}_\text{ar}\)), 7.53 (1 H, s, \(\text{CH}_\text{furany}\)), 7.31 (2 H, m, \(\text{CH}_\text{ar}\)), 3.01 (2 H, q, \(J = 7.5\), \(\text{CH}_2\text{CH}_3\)), 2.64 (3 H, s, \(\text{CH}_3\text{CO}\)), 1.39 (3 H, t, \(J = 7.5\), \(\text{CHCH}_3\))

\(^1\text{H} \text{NMR comparable to literature}\).\(^{65}\)

\(\delta_C (\text{CDCl}_3; 63 \text{ MHz})\) 188.2 (\(\text{C} = \text{O}\)), 154.4, 152.4 (\(\text{C}-8, \text{C}-2\)), 129.0, 126.7, 127.2, 124.1, 120.6 (\(\text{CH}_\text{ar}\)) 113.3 (\(\text{C}-3\)), 26.5 (\(\text{CH}_3\text{CO}\)), 22.7 (\(\text{CH}_2\text{CH}_3\)), 14.0 (\(\text{CH}_2\text{CH}_3\))

\(m/z \) (FAB) 189 (100%, \(\text{MH}^+\)), 109 (28.8), 91 (99.1), 57 (24.7)

5.1.1.7 Attempted preparation of 7-ethylbenzofuran-2-ylchloromethylketone 79 directly from 3-ethyl-2-hydroxybenzaldehyde 77

The above procedure was followed but dichloroacetone was used instead of chloroacetone in an attempt to give the chloro ketone 79 directly. Unfortunately only baseline material was observed on tlc. The same reaction was tried using DBU as the base but again no product was observed by tlc. This route was abandoned.

5.1.1.8 Preparation of 7-ethylbenzofuran-2-ylchloromethylketone 79

\[\text{Methylketone } 78 (2g, 10.6\text{mmol}) \text{ was dissolved in dichloromethane (40ml)}
\]

and stirred under an argon atmosphere at 0°C. Trimethylsilyl triflate (2ml, 11mmol) was added followed by triethylamine (1.54ml, 11mmol). The mixture was stirred for 1.5 h. \(N\)-chlorosuccinimide (1.42g, 10.6mmol) was added and the mixture allowed to warm to room temperature over 1 h. The reaction mixture was diluted with ethyl acetate (90ml) and washed with 10% aqueous sodium thiosulphate (3 x 50ml), 10% aqueous sodium hydrog
carbonate (3 x 50ml), brine (50ml), and dried (magnesium sulphate). The solvent was evaporated off under reduced pressure to leave a pale yellow oil which crystallised from ethanol to afford 79 as white crystals in two crops (512mg, 22.8%).

Rf (EtOAc:hexane, 1:4) 0.43
\[ \delta_H \text{ (CDCl}_3; \text{ 220 MHz)} 7.70 (1 \text{ H, s, CH}_3\text{furain}), 7.60 (1 \text{ H, d, } J 7.6, \text{ CH}_2\text{ar}), 7.35 (2 \text{ H, m, CH}_3\text{ar}), 4.75 (2 \text{ H, s, ClCH}_2\text{CO}), 3.00 (2 \text{ H, q, } J 7.5, \text{ CH}_2\text{CH}_3), 1.38 (3 \text{ H, t, } J 7.5, \text{ CH}_2\text{CH}_3) \]

\(^1\text{H NMR comparable to literature.}^{65}

5.1.1.9 Reaction of 7-ethylbenzofuran-2-ylmethylketone 78 with sulphuryl chloride

To a stirred solution of the methylketone 78 (2g, 10.6mmol) in chloroform (30ml) was added sulphuryl chloride (1.2ml, 14.8mmol). The resulting solution was heated to reflux and a small amount of 2M hydrochloric acid (4 drops) was added. Heating at reflux was continued for a further 3 h and the solution was allowed to cool to room temperature overnight. Water (100ml) was added and the mixture extracted with dichloromethane (2 x 100ml). The combined organics were washed with brine (100ml), dried (magnesium sulphate), and evaporated to dryness. The residue crystallised from ethanol to give a mixture of 7-ethylbenzofuran-2-yl chloromethyl ketone 79, \( \delta_H \) (220MHz; CDCl\(_3\)) 7.2-7.7 (4 H, m, CH\(_{3}\)ar), 4.73 (2 H, s, ClCH\(_2\)CO), 3.0 (2 H, q, J 7.5 CH\(_2\)CH\(_3\)), 1.36 (3 H, t, J 7.5, CH\(_2\)CH\(_3\)) and 7-ethylbenzofuran-2-yl dichloromethyl ketone, \( \delta_H \) (220MHz; CDCl\(_3\)) 7.89 (1 H, s, CH\(_3\)furain), 7.2-7.7 (3 H, m, CH\(_{3}\)ar), 6.75 (1 H, s, Cl\(_2\)CHCO), 3.0 (2 H, q, J 7.5, CH\(_2\)CH\(_3\)), 1.36 (3 H, t, J 7.5, CH\(_2\)CH\(_3\)).
5.1.1.10 Preparation of 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-hydroxyethane

Sodium borohydride (85mg, 2.3mmol) was added to a stirred solution of the chloroketone 79 (510mg, 2.3mmol) in ethanol (15ml) at 10°C (cold water bath). The reaction was complete after 1h. The ethanol was evaporated at reduced pressure and the residue partitioned between water (50ml) and ethyl acetate (50ml). The aqueous was separated and extracted with more ethyl acetate (2 x 50ml). The combined organics were washed with brine (50ml), dried (magnesium sulphate), and evaporated to dryness to leave 80 as a yellow oil (512mg, 99.5%).

R_f (EtOAc:hexane, 1:4) 0.30

δ_H (CDCl_3; 250 MHz) 7.42 (1 H, dd, J 7.6, 1.5, CH_m), 7.15 (2 H, m, CH_m), 6.78 (1 H, s, CH_{2furan}), 5.12 (1 H, m, CH(OH)), 3.96 (2 H, m, CICH_2CH(OH)), 2.95 (2 H, q, J 7.5, CH_2CH_3), 2.70 (1 H, brs, OH), 1.36 (3 H, t, J 7.5, CH_2CH_3).

^1H NMR comparable with literature.65

5.1.2 Lipase catalysed resolutions using trifluoroethyl octanoate

5.1.2.1 Preparation of trifluoroethyl octanoate ester

To a solution of 2,2,2-trifluoroethanol (20g, 0.2mol) and DMAP (20mg, cat.) in dichloromethane (200ml) under argon was added pyridine (24ml, 0.3mol) and
octanoyl chloride (32.5g, 0.2mol) dropwise. Once the addition was complete the reaction was allowed to stir at room temperature for 1 h and then diluted with dichloromethane (200ml). The organic solution was washed with 0.1M hydrochloric acid (2 x 200ml), saturated sodium hydrogen carbonate solution (200ml), brine (100ml), and dried (magnesium sulphate). Evaporation to dryness followed by distillation at reduced pressure afforded the title compound as a clear oil (35.2g, 77.9%).

δ_H (CDCl_3; 220 MHz) 4.40 (2 H, m, OCH_2CF_3), 2.35 (2 H, t, J 7.5, CH_2CH_2CO), 1.60 (2 H, m, CH_2CH_2CO), 1.25 (8 H, br s, methylene Hs), 0.80 (3 H, br t, CH_3CH_2).

5.1.2.2 Preparation of racemic 2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl octanoate 81

Pyridine (1ml) and octanoyl chloride (90mg, 0.55mmol) were added to a solution of the chloroalcohol 80 (90mg, 0.4mmol) in dichloromethane (10ml) containing DMAP (2mg, cat.) under argon at room temperature. The reaction mixture was stirred for 1h and tlc (ethyl acetate:hexane, 1:4) showed complete conversion. The reaction mixture was diluted with dichloromethane (30ml) and washed with 0.1M hydrochloric acid (2 x 50ml), saturated sodium hydrogen carbonate (50ml), and brine (50ml). After drying over magnesium sulphate the solvent was removed at reduced pressure to leave the title compound 81 (55mg, 40%).
5.1.2.3 Lipase catalysed esterification of the racemic chloroalcohol 80

The resolution was carried out using four lipases from Amano. These were lipase AP, lipase AY, lipase M, and lipase PS. The following procedure is representative. The chloroalcohol 80 (90mg, 0.4mmol) and trifluoroethyl octanoate ester (230mg, 1mmol) were dissolved in benzene-hexane (1:9 v/v, 10ml). The lipase (300mg) was added and the mixture shaken at 45°C for 70 h. The progress of these reactions was monitored by tlc using ethyl acetate-hexane (1:4 v/v). The resolutions using lipase AP and lipase M showed only trace amount of ester and this was not isolated. Lipase AY showed approximately 20% conversion by tlc and lipase PS showed less than 10% conversion to the ester. The lipase from these reactions was removed by filtration and the octanoate ester and remaining alcohol were separated by chromatography on silica gel with ethyl acetate-hexane (3-5% v/v). Table 3 shows the amounts isolated for each lipase and the optical purity of the octanoate ester as determined by shift reagent NMR spectroscopy using a chiral europium reagent - Eu(hfc)₃.

The Rf value and the ¹H NMR spectrum at 300MHz for the octanoate esters obtained from these resolutions were identical to that of the racemic octanoate ester given above (5.1.2.2).
Table 3

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Amt of Ester 81</th>
<th>Amt of Alcohol 80</th>
<th>ee of Ester 81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase AY</td>
<td>26mg, 19%</td>
<td>60mg, 67%</td>
<td>75%</td>
</tr>
<tr>
<td>Lipase PS</td>
<td>2mg, 1%</td>
<td>82mg, 91%</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

5.1.3 Lipase catalysed resolutions using vinyl acetate as the acyl donor and subsequent conversion to bufuralol

5.1.3.1 Lipase AY catalysed acetylation of the chloroalcohol 80 with vinyl acetate

The chloroalcohol 80 (90mg, 0.401mmol) was dissolved in vinyl acetate (5ml). Lipase AY (500mg) was added and the mixture gently stirred at room temperature for 48h. After this time TLC (ethyl acetate:hexane, 1:4) showed approximately 50% conversion. The lipase was removed by filtration through celite and the residue was washed with ethyl acetate. The solution was evaporated to dryness at reduced pressure and the resolution products were separated by chromatography on silica gel eluting with ethyl acetate-hexane (3-5% v/v). The first compound to be eluted was the (S)-acetate (S)-82 and after removal of the solvent it was obtained as a clear oil (25mg, 18%).

\[
\text{R}_f (\text{EtOAc:hexane, 1:4}) = 0.50
\]

\[
\begin{align*}
\delta_{\text{H}} (\text{CDCl}_3; 250 \text{ MHz}) &\quad 7.42 (1 \text{ H, dd, } J 7.4, 1.8, \text{CH}_\text{ar}), \quad 7.18 (2 \text{ H, m, CH}_\text{ar}), \quad 6.81 (1 \text{ H, s, CH}_\text{furan}), \quad 6.20 (1 \text{ H, t, } J 6.4, \text{CH(OAc)}), \quad 4.01 (2 \text{ H, d, } J 6.4, \text{ClCH}_2\text{CH(OAc)}), \\
2.95 (2 \text{ H, q, } J 7.5, \text{CH}_2\text{CH}_3), &\quad 2.20 (3 \text{ H, s, CH}_3\text{C}=\text{O}), \quad 1.35 (3 \text{ H, t, } J 7.6, \\
\text{CH}_2\text{CH}_3). &\quad
\end{align*}
\]
Further elution afforded the more polar (R)-alcohol (R)-80 after evaporation as a clear oil (27mg, 27%).

Rf (EtOAc:hexane, 1:4) 0.30

\[ \delta_H (\text{CDCl}_3; 250 \text{ MHz}) 7.42 \ (1 \text{ H, dd, } J 7.6,1.5, \text{ CH}_2), \ 7.15 \ (2 \text{ H, m, CH}_3), \ 6.78 \ (1 \text{ H, s, CH}_2(\text{OH})), \ 5.12 \ (1 \text{ H, m, CH(OH)}), \ 3.96 \ (2 \text{ H, s, ClCH}_2\text{CH(OH)}), \ 2.95 \ (2 \text{ H, q, } J 7.5, \text{ CH}_2\text{CH}_3), \ 2.70 \ (1 \text{ H, brs, OH}), \ 1.36 \ (3 \text{ H, t, } J 7.5, \text{ CH}_2\text{CH}_3). \]

Optical purity was not measured at this stage and the assignment of absolute configuration was done by chiral HPLC analysis of the bufuralol produced from these compounds.

5.1.3.2 Lipase AY catalysed hydrolysis of (S')-2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl acetate (S)-82

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

The (S)-acetate (S)-82 (25mg, 0.094mmol) from the previous experiment (5.1.3.1) was dissolved in acetone (1ml) and 0.1M potassium phosphate buffer solution at pH 7.0 (5ml) was added. The mixture was stirred at room temperature and lipase AY (100mg) was added in one portion. The course of the reaction was monitored by tlc (ethyl acetate:hexane, 1:4) and the pH was checked periodically and maintained at pH 7 by the addition of small portions of 0.01M sodium hydroxide solution. After approximately 48h the reaction appeared to have reached 60% hydrolysis so diethyl ether (40ml) was added and the phases were allowed to separate. The aqueous layer was extracted with diethyl ether (3 x 40ml) and the combined extracts were washed with brine (30ml) and dried (magnesium sulphate). The solvent was removed.
under reduced pressure and the residue chromatographed over silica gel eluting with ethyl acetate-hexane (3-5% v/v). The (S)-alcohol (S)-80 was obtained as a colourless gum (13mg, 62%).

R_f (EtOAc:hexane, 1:4) 0.30
δ_H (CDCl_3; 250 MHz) 7.42 (1 H, dd, J 7.6, 1.5, CH_ar), 7.15 (2 H, m, CH_ar), 6.78 (1 H, s, CH_furan), 5.12 (1 H, m, CH(OH)), 3.96 (2 H, s, ClCH_2CH(OH)), 2.95 (2 H, q, J 7.5, CH_2CH_3), 2.70 (1 H, brs, OH), 1.36 (3 H, t, J 7.5, CH_2CH_3).

The unhydrolysed (S)-acetate (S)-82 was recovered and this was also isolated as a gum (10mg, 40%).

R_f (EtOAc:hexane, 1:4) 0.50
δ_H (CDCl_3; 250 MHz) 7.42 (1 H, dd, J 7.4, 1.8, CH_ar), 7.18 (2 H, m, CH_ar), 6.81 (1 H, s, CH_furan), 6.20 (1 H, t, J 6.4, CH(OAc)) 4.01 (2 H, d, J 6.4, ClCH_2CH(OAc)), 2.95 (2 H, q, J 7.5, CH_2CH_3), 2.20 (3 H, s, CH_3C=O), 1.35 (3 H, t, J 7.6, CH_2CH_3).

5.1.3.3 Preparation of (R)-(+) Bufuralol (R')-60 from the (S')-alcohol (S')-80 isolated from the Lipase AY resolution

Potassium carbonate (12mg, 0.087mmol), potassium iodide (5mg, 0.030mmol), and tert-butylamine (150μl, 1.45mmol) were added to a solution of the (S)-alcohol (S)-80 (13mg, 0.058mmol) in dimethylformamide (2ml) and the reaction mixture was heated at reflux for 7h. Tlc (methanol:dichloromethane,
Experimental

1:9) showed no remaining starting material so the reaction mixture was evaporated to dryness under reduced pressure (high vacuum). The residue was dissolved in ethyl acetate (20ml) and washed with water (2 x 30ml). The product was extracted with 5% citric acid (2 x 10ml) and the acid extracts were backwashed with ethyl acetate (10ml). The acid layer was then neutralised by the slow addition of 2M sodium hydroxide solution. The product was extracted with dichloromethane (3 x 20ml) and after drying over magnesium sulphate the solvent was removed at reduced pressure to afford (R)-(+-) Bufuralol (R)-60 (7mg, 47%) as a yellow oil. ee = 50%.

Rf (MeOH:CH2Cl2, 1:9) 0.41
[α]D +14° (c 0.25, EtOH)

δH (CDCl3; 250MHz) 7.37 (1 H, dd, J 1.5, 7.4, CHα), 7.14 (2 H, m, CHα), 6.63 (1 H, s, CHαHα), 4.13 (1 H, dd, J 9.0, 4.9, CHX(OH)CHαHα), 3.74 (1 H, dd, J 10.6, 4.9, CHX(OH)CHαHα), 3.60 (1 H, dd, J 10.6, 9.0, CHX(OH)CHαHα), 2.95 (2 H, q, J 7.6, CH2CH3), 2.83 (2 H, br s, NH and OH), 1.37 (3 H, t, J 7.6, CH2CH3), 1.16 (9 H, s, tBu)

5.1.3.4 Lipase PS catalysed acetylation of the chloroalcohol 80 with vinyl acetate

The chloroalcohol 80 (90mg, 0.401mmol) was dissolved in vinyl acetate (5ml). Lipase PS (500mg) was added and the mixture gently stirred at room temperature for 48h. After this time tlc (ethyl acetate:hexane, 1:4) showed approximately 50% conversion. The lipase was removed by filtration through
celite and the residue was washed with ethyl acetate. The solution was evaporated to dryness at reduced pressure and the resolution products were separated by chromatography on silica gel eluting with ethyl acetate-hexane (3-5% v/v). The first compound to be eluted was the (S)-acetate (S)-82 and after removal of the solvent it was obtained as a clear oil (45mg, 44%). Further elution afforded the more polar (R)-alcohol (R)-80 after evaporation as a clear oil (35mg, 39%). Optical purity was not measured at this stage and the assignment of absolute configuration was done by chiral HPLC analysis of the bufuralol produced from these compounds.

5.1.3.5 Lipase PS catalysed hydrolysis of (S)-2-chloro-1-(7-ethylbenzofuran-2-yl)-1-ethyl acetate (S)-82

The (S)-acetate (S)-82 (45mg, 0.169mmol) from the previous experiment was dissolved in acetone (1ml) and 0.1M potassium phosphate buffer solution at pH 7.0 (5ml) was added. The mixture was stirred at room temperature and lipase PS (100mg) was added in one portion. The course of the reaction was monitored by tlc (ethyl acetate:hexane, 1:4) and the pH was checked periodically and maintained at pH 7 by the addition of small portions of 0.01M sodium hydroxide solution. After approximately 48h the reaction appeared to have reached 50% hydrolysis so diethyl ether (40ml) was added and the phases were allowed to separate. The aqueous layer was extracted with diethyl ether (3 x 40ml) and the combined extracts were washed with brine (30ml) and dried (magnesium sulphate). The solvent was removed under reduced pressure and the residue chromatographed on silica gel
eluting with ethyl acetate-hexane (3-5% v/v). The (S)-alcohol (S)-80 was obtained as a colourless gum (20mg, 53%). The unhydrolysed (S)-acetate (S)-82 was recovered and this was also isolated as a gum (20mg, 44%).

5.1.3.6 Preparation of (R)-(−)-Bufuralol (R)-60 from the (S)-alcohol (S)-80 isolated from the Lipase PS resolution

Potassium carbonate (18mg, 0.134mmol), potassium iodide (7mg, 0.045mmol), and tert-butylamine (230µl, 2.23mmol) were added to a solution of the (S)-alcohol (S)-80 (20mg, 0.089mmol) in dimethylformamide (2ml) and the reaction mixture was heated at reflux for 7h. Tlc (methanol:dichloromethane, 1:9) showed no remaining starting material so the reaction mixture was evaporated to dryness under reduced pressure (high vacuum). The residue was dissolved in ethyl acetate (20ml) and washed with water (2 x 30ml). The product was extracted with 5% citric acid (2 x 10ml) and the acid extracts were backwashed with ethyl acetate (10ml). The acid layer was then neutralised by the slow addition of 2M sodium hydroxide solution. The product was extracted with dichloromethane (3 x 20ml) and after drying over magnesium sulphate the solvent was removed at reduced pressure to afford (R)-(−)-Bufuralol (R)-60 (13mg, 56%) as a yellow oil. ee = 90%.

R_t (MeOH:CH_2Cl_2, 1:9) 0.41
[α]_D +23° (c 0.25, EtOH)
δ_H (CDCl_3; 250MHz) 7.37 (1 H, dd, J 1.5, 7.4, CH_ar), 7.14 (2 H, m, CH_ar), 6.63 (1 H, s, CH_iurai), 4.13 (1 H, dd, J 9.0, 4.9, CH_x(OH)CH_yH_B), 3.74 (1 H, dd, J 10.6,
4.9, \text{CH}_X(\text{OH})\text{CH}_A\text{H}_B, 3.60 (1 \text{H, dd, } J 10.6, 9.0, \text{CH}_X(\text{OH})\text{CH}_A\text{H}_B), 2.95 (2 \text{H, q, } J 7.6, \text{CH}_B\text{CH}_3), 2.83 (2 \text{H, br s, NH and OH}), 1.37 (3 \text{H, t, } J 7.6, \text{CH}_2\text{CH}_3), 1.16 (9 \text{H, s, } t\text{Bu})

\(\delta_c (\text{CDCl}_3; 63 \text{MHz}) 157.5, 153.0, 128.0, 127.6, 123.3, 123.0, 118.4, 103.5, 64.4, 52.8, 52.2, 29.6, 23.0, 14.1\)

5.2 Methadone
5.2.1 Resolutions with vinyl acetate
5.2.1.1 Resolution of 1-dimethylamino-2-propanol 89

Racemic 1-dimethylamino-2-propanol 89 (2g, 19.4mmol) was stirred with vinyl acetate (10ml) at ambient temperature and Lipase PS (500mg) was added. The reaction mixture was stirred slowly for 42h and after this time tlc (10% methanol/ dichloromethane - visualise KMnO₄ solution) indicated that the reaction had gone to approximately 50% conversion. The enzyme was removed by filtration and the filter bed was washed with ethyl acetate (2 x 20ml). The solution was evaporated to leave a yellow oil which was chromatographed on silica (50g) eluting with 0-10 % methanol in dichloromethane to afford (-)-1-dimethylamino-2-propyl acetate 91 (1.7g, 60%) as a clear oil.

\(\delta_H (\text{CDCl}_3; 400 \text{ MHz}) 5.03 (1 \text{H, sextet, } J 7, \text{CH}_3\text{CH}(\text{OCOCH}_3)), 2.50 (1\text{H, dd, } J 10, 7, N-\text{CH}_A\text{H}_B\text{CH}(\text{OCOCH}_3)), 2.23 (6 \text{H, s, N(CH}_3)_2), 2.22 (1\text{H, dd, } J 10, 7, N-\text{CH}_A\text{H}_B\text{CH}(\text{OCOCH}_3)), 2.02 (3\text{H, s, CH}_3\text{CO}), 1.21 (3 \text{H, d, } J 7, \text{CH}_3\text{CH}(\text{OCOCH}_3))\)

ee = 46%. The ee of the product was determined by shift reagent NMR using Eu(hfc)₃.
The remaining alcohol could not be isolated from the column despite eluting with 50% methanol in dichloromethane.

5.2.1.2 Resolution of 1-dimethylamino-2-propanol 89
Racemic 1-dimethylamino-2-propanol 89 (1g, 9.7mmol) was stirred with vinyl acetate (3ml) at ambient temperature and Lipase PS (250mg) was added. The reaction mixture was stirred slowly for 16h and after this time tlc (10% methanol/ dichloromethane - visualise KMnO₄ solution) indicated that the reaction had gone to approximately 30% conversion. The enzyme was removed by filtration and the filter bed was washed with ethyl acetate (2 x 20ml). The solution was evaporated to leave a yellow oil which was chromatographed on silica (30g) eluting with 0-10 % methanol in dichloromethane to afford (−)-1-dimethylamino-2-propyl acetate 91 (471mg, 33%) as a clear oil.

δₜ (CDCl₃, 400 MHz) 5.03 (1 H, sextet, J 7, CH₃CH(OCOCH₃)), 2.50 (1 H, dd, J 10, 7, N-CH₃H₃CH(OCOCH₃)), 2.23 (6 H, s, N(CH₃)₂), 2.22 (1 H, dd, J 10, 7, N-CH₃H₃CH(OCOCH₃)), 2.02 (3 H, s, CH₃CO), 1.21 (3 H, d, J 7, CH₃CH(OCOCH₃))

ee =90%. The ee of the product was determined by shift reagent NMR using Eu(hfc)₃.

The remaining alcohol could not be isolated from the column despite eluting with 50% methanol in dichloromethane.
5.2.2 Resolution of 1-dimethylamino-2-propanol using vinyl propanoate and reaction of the resolution products to methadone

5.2.2.1 (R)-(-)-1-dimethylamino-2-propyl propanoate (R)-92

Racemic 1-dimethylamino-2-propanol 89 (100g, 0.97mol) was stirred with vinyl propanoate (106ml, 0.97mol) at ambient temperature and Novozyme ® 435 (6g) was added. The reaction mixture was stirred slowly for 70 hours and after this time tlc (10% methanol/dichloromethane - visualise KMnO₄ solution) indicated that the reaction had gone to 50% conversion. The enzyme was removed by filtration and the filter bed was washed with ethyl acetate (2 x 100ml). The organic layer was then washed with water (4 x 400ml) and the combined washes were back extracted with ethyl acetate (400ml). The organic layer was then washed with brine (200ml) and dried (MgSO₄). The ethyl acetate was removed in vacuo to leave 58.7g (0.37mol, 35% - maximum yield 50%) of (R)-(−)-1-dimethylamino-2-propyl propanoate (R)-92 as a yellow oil.

Rf (Methanol:dichloromethane, 1:9) 0.38

νₘₐₓ(neat)/cm⁻¹ 2978, 2941, 2821, 2768 (CH), 1732 (C=O)

δₚ (CDCl₃; 250 MHz) 5.05 (1 H, m, CH₃CH(OOC₂H₅)), 2.51(1 H, dd, J 12.9, 7.4, N-CH₃H₈CH(OOC₂H₅)), 2.30 (3 H, m, N-CH₃H₈CH(OOC₂H₅)), 2.25 (6 H, s, N(CH₃)₂), 1.21 (3 H, d, J 6.3, CH₃CH(OOC₂H₅)), 1.11 (3 H, t, J 7.5, OCOCH₂CH₃)

δc (CDCl₃; 63 MHz) 174.0 (C=O), 68.1 (CH-O), 64.0 (CH₂-N), 45.8 ((CH₃)₂N), 27.8 (COCH₂CH₃), 18.5 (CH₂CH₃), 9.0 (COCH₂CH₃)

The optical purity was not measured at this stage.
5.2.2.2 (R)-(−)-1-Dimethylamino-2-propanol (R)-89

\[ \text{Me}_2\text{N} \quad \text{OH} \]

A solution of sodium methoxide was made by dropping small pieces of sodium metal (200 mg) into methanol (5 ml) under an atmosphere of argon. This was then added to a stirred solution of (−)-1-dimethylamino-2-propyl propanoate (57.7 g, 0.36 mol) in methanol (200 ml). The mixture was stirred overnight and checked for completion by tlc (10% methanol/dichloromethane). The methanol was removed under reduced pressure to leave (R)-(−)-1-dimethylamino-2-propanol (R)-89 (32.8 g, 88%) as a dark oil.

R\text{f} (\text{Methanol:dichloromethane, 1:9}) \ 0.18 \\
\nu_{\text{max}} (\text{neat})/\text{cm}^{-1} \quad 3419 (\text{OH}) 2969, 2819, 2772 (\text{CH}) \\
\delta_H (\text{CDCl}_3; 250 \text{ MHz}) \quad 3.76 (1 \text{ H, m, CH}_3\text{CH(OH)}), 3.49 (1 \text{ H, brs, OH}), 2.24 (6 \text{ H, s, N(CH}_3)_2), 2.15 (2 \text{ H, m, NCH}_2) 1.10 (3 \text{ H, d, J 6.1, CH}_3\text{CH(OH)}) \\
\delta_C (\text{CDCl}_3; 63 \text{ MHz}) \quad 67.0 (\text{CH(OH)}), 63.0 (\text{CH}_2-\text{N}), 45.4 ((\text{CH}_3)_2\text{N}), 20.0 (\text{CH}_3\text{CH})

5.2.2.3 (S)-(+)/-1-Dimethylamino-2-chloropropane hydrochloride (S)-90 49, 66

\[ \text{Me}_2\text{HN}^+ \quad \text{Cl}^- \quad \text{Cl}^- \]

A solution of thionyl chloride (37 ml, 0.48 mol) in chloroform (20 ml) was added slowly, with stirring, to a cooled (ice/water) solution of (R)-(−)-1-dimethylamino-2-propanol (R)-89 (31.8 g, 0.32 mol) in chloroform (85 ml). When the addition was complete a precipitate formed. The mixture was allowed to warm to room temperature over 30 minutes and then heated to
reflux for a further 30 minutes. The precipitate redissolved on heating but then the product crystallised out from the boiling solvent as it formed. More chloroform (20ml) was needed to maintain the stirring. The cooled mixture was diluted with ether and filtered. The crude product (45.2g, 89%) was recrystallised from 2-propanol and decolourising charcoal was used. The product (33.1g, 65%) was obtained in 3 crops and the first crop (24.5g) was kept separate; [α]D +59.1° (c 2.075, H2O). This material was recrystallised twice more to give 15.7g, 31% of (S)-(−)-1-dimethylamino-2-chloropropane hydrochloride (S)-90.

Mp 192-193°C  
[α]D+65.9° (c 2.01, H2O)  
νmax(solid)/cm−1 2962 (CH)  
δH (D2O; 250 MHz) 4.42 (1 H, sextet, J 7.0, CH2CHClCH3), 3.40 (2 H, d, J 8.0, NCH2CHCl), 2.88 (6 H, d, J 8.4, N(CH3)2), 1.49 (3 H, d, J 6.5, CH3CHCl)  
δc (D2O, 63 MHz) 64.3 (CHCl), 52.0 (CH2N), 45.6 (CH3N), 41.8 (C4H9N), 22.3 (CH3CH)  
m/z (FAB) 124 (26.7%, MH+, 37Cl), 122 (100, MH+, 35Cl), 86 (8.5, M-Cl), 44 (11.0, N(CH3)2)

5.2.2.4 (R’)-(−)-2,2-Diphenyl-4-dimethylaminopentanenitrile (R)-86

A 50% w/v solution of sodium hydroxide in water (12.5ml, 0.32mol) was added to a mechanically stirred suspension of diphenylacetonitrile (15.0g, 0.08mol) and dibenzo-18-crown-6 (0.5g, cat.) in dimethylsulphoxide (12.5ml). The colour rapidly deepened to an orange/brown. (S)-(−)-1-dimethylamino-2-chloropropane hydrochloride (S)-90 (15g, 0.095mol) was added in portions
over 30 minutes, this caused the temperature to rise to 30°C. After the
addition was complete the mixture was warmed to 45-50°C (water bath) and
stirred for a further hour. The reaction mixture was then allowed to cool to
room temperature and was poured into ice/water (250ml) and extracted with
ethyl acetate (3 x 150ml). The combined extracts were dried (MgSO4), filtered,
and concentrated to ~100ml. The product was extracted into 1N HCl (100ml +
50ml) and this was back washed with ethyl acetate. The aqueous was basified
with 2M sodium hydroxide and extracted into ethyl acetate (3 x 100ml). The
extracts were washed with brine (70ml), dried (MgSO4), and evaporated to
yield a yellow oil. This was chilled and triturated with cold hexane (~50ml) to
give a white solid which was collected by filtration and washed thoroughly
with a further portion of cold hexane (100ml). The solid was recrystallised
from hexane to afford (R)-86 (7.0g, 32%).

Rf (Methanol:dichloromethane, 1:9) 0.45
Mp 100-101 °C
[α]D -50.2 (c 0.71, EtOH)
v_{max}(solid)/cm^{-1} 2972, 2938, 2819, 2774 (CH), 2229 (CN)
δH (CDCl3; 250 MHz) 7.38 (10 H, m, CHAr), 2.70 (1 H, dd, J 13.6, 6.4,
CHCHACHBCH2CPh2), 2.54 (1 H, sextet, J 6.3, CH3CHCHACHB), 2.25 (1 H, dd, J
13.6, 6.0, CHCHACHBCH2CPh2), 2.15 (6 H, s, N(CH3)2), 0.92 (3 H, d, J 6.5, CH3CH)
δC (CDCl3; 63 MHz) 141.2, 140.6 (ipso-Ar), 128.7, 128.6, 127.8, 127.6, 127.3,
127.1 (CHAr), 122.8 (CN), 55.4 (CH3CH), 49.6 (CPh2CN), 43.2 (N(CH3)2), 39.9
(CH2), 13.1 (CH3CH)
m/z (FAB) 289 (4.9%, MH+), 279 (100), 154 (23, Ph2), 137 (14), 72 (45,
CH3CHN(CH3)2)
5.2.2.5 (R)-(-)-Methadone hydrochloride (R)-83

All apparatus was dried and the reaction was carried out under an inert atmosphere of argon. A solution of (R)-(-)-2,2-diphenyl-4-dimethylamino-pentane-nitrile (R)-86 (5.0g, 0.018mol) in toluene (15ml) was added to a stirred solution of 3M ethylmagnesium bromide in ether (10.7ml, 0.03mol). The ether was removed under reduced pressure and the remaining solution heated at reflux (135-140°) for 3 hours. The solution went slightly cloudy but there was no significant precipitation. After cooling to room temperature 2N HCl (30ml) was added with care and then stirring was continued at 135-140° for a further 30 minutes. The two phases were allowed to separate and cool to room temperature. After scratching the sides of the flask a solid started to crystallise from the aqueous phase. The flask was cooled to complete crystallisation and the white solid was collected by filtration. This solid was recrystallised from water to yield 2.7g (43%) of (R)-(-)-methadone hydrochloride (R)-83 (6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride)

Rₙ 9.7 (99.8%) - Racemic - 10.0, 13.1
Mp 242-244°
[α]D -136° (c 2.04, EtOH)
ν max(solid)/cm⁻¹ 2935 (CH), 2462, 1702 (C=O)
δ H (d⁶DMSO; 250 MHz) 7.07-7.57 (10 H, m, CH₅r), 3.08 (1 H, brd, J 14.0, CHCH₅ACH₅CPh₂), 2.93 (1 H, m, CH₃CHCH₅CH₅CH₅B), 2.67 (6 H, brd, N(CH₃)₂), 2.40 (1 H, m, CHCH₅ACH₅CPh₂), 2.20 (2 H, q, J 7.3, CH₅CH₂CO), 0.73 (3 H, t, J 7.2, CH₅CH₂CO), 0.43 (3 H, d, J 6.6, CH₅CH)
δ (d<sup>6</sup>DMSO; 63 MHz) 140.7, 140.3 (ipso-Ar), 129.4, 129.2, 129.0, 128.7, 127.8, 127.0 (CH<sub>ar</sub>), 64.8 (CH<sub>2</sub>CH), 59.1 (CPh<sub>2</sub>CO), 38.5, 38.3 (CH<sub>2</sub>CH<sub>2</sub>CPh<sub>2</sub> and N(CH<sub>3</sub>)<sub>2</sub>), 32.6 (CH<sub>2</sub>CO), 14.9, 9.5 (CH<sub>3</sub>CH and CH<sub>3</sub>CH<sub>2</sub>)

m/z (FAB) 310 (100%, MH<sup>+</sup>), 265 (11, M-N(CH<sub>3</sub>)<sub>2</sub>), 154 (19, Ph<sub>2</sub>), 72 (15, CH<sub>3</sub>CHN(CH<sub>3</sub>)<sub>2</sub>)

Chiral HPLC shows no evidence of (S)-isomer (S)-83 - ee >99%

5.2.2.6 (S)-(+)1-Dimethylamino-2-propanol (S)-89

![Chemical Structure](image)

Racemic 1-dimethylamino-2-propanol 89 (100g, 0.97mol) was stirred with vinyl propionate (63.6ml, 0.58mol) at 40°C and Novozyme ® 435 (5g) was added. The reaction mixture was stirred slowly for 75 hours and after this time tlc (10% methanol/dichloromethane - visualise KMnO<sub>4</sub> solution) indicated that the reaction had gone to at least 50% conversion. The enzyme was removed by filtration and the filtrate was distilled at reduced pressure. (S)-(+)1-dimethylamino-2-propanol (S)-89 was obtained as a colourless oil (31.6g, 64%) bpt 35°C, 5mm Hg,

[α]<sub>D</sub> +23° (c 2.10, EtOH) [lit. 49° +24° (c 2.17, EtOH)]

R<sub>f</sub> (Methanol:dichloromethane, 1:9) 0.19

ν<sub>max</sub> (neat)/cm<sup>-1</sup> 2978, 2941, 2821, 2768 (CH), 1732 (C=O)

δ<sub>H</sub> (CDCl<sub>3</sub>; 250 MHz) 5.05 (1 H, m, CH<sub>3</sub>CH(OCOC<sub>2</sub>H<sub>5</sub>)), 2.51(1 H, dd, J 12.9, 7.4, N-CH<sub>2</sub>H<sub>2</sub>CH(OCOC<sub>2</sub>H<sub>5</sub>)), 2.30 (3 H, m, N-CH<sub>2</sub>H<sub>2</sub>CH(OCOC<sub>2</sub>H<sub>5</sub>)), 2.25 (6 H, s, N(CH<sub>3</sub>)<sub>2</sub>), 1.21 (3 H, d, J 6.3, CH<sub>3</sub>CH(OCOC<sub>2</sub>H<sub>5</sub>)), 1.11 (3 H, t, J 7.5, COCH<sub>2</sub>CH<sub>3</sub>)
\[ \delta_c (\text{CDCl}_3; \ 63 \text{ MHz}) \ 174.0 (\text{C}=\text{O}), \ 68.1 (\text{CH}=\text{O}), \ 64.0 (\text{CH}_2=\text{N}), \ 45.8 ((\text{CH}_3)_2\text{N}), \ 27.8 (\text{COCH}\text{CH}_3), \ 18.5 (\text{CH}_3\text{CH}), \ 9.0 (\text{COCH}_2\text{CH}_3) \]

5.2.2.7 (R)-(−)-1-Dimethylamino-2-chloropropane hydrochloride (R)-90 \(^{49,66}\)

\[
\text{Me}_2\text{HN}^+ \quad \begin{array}{c} \downarrow \text{Cl}^- \end{array} \quad \text{Cl}
\]

This was prepared following the same procedure as the (S)-isomer (5.2.2.3). 30.6g of (S)-89 were used and 45.0g (96%) of crude product was isolated. This was recrystallised from 2-propanol as in the other series to give 30.9g (65%) of (R)-90.

Mp 192-193°C [lit. \(^{49}\) 192-193°C]

\[ [\alpha]_D -65.8^\circ (c \ 2.0, \text{H}_2\text{O}) \] [lit. \(^{49}\) -65^\circ (c \ 2.01, \text{H}_2\text{O})]

\[ \nu_{\text{max}}(\text{solid})/\text{cm}^{-1} \ 2962 (\text{CH}) \]

\[ \delta_H (\text{D}_2\text{O}; \ 250 \text{ MHz}) \ 4.42 (1 \text{ H, sextet}, \ J 7.0, \text{CH}_2\text{CHClCH}_3), \ 3.40 (2 \text{ H, d, } J 8.0, \text{NCH}_2\text{CHCl}), \ 2.88 (6 \text{ H, d, } J 8.4, \text{N(CH}_3)_2), \ 1.49 (3 \text{ H, d, } J 6.5, \text{CH}_3\text{CHCl}) \]

\[ \delta_c (\text{D}_2\text{O, 63 MHz}) \ 64.3 (\text{CHCl}), \ 52.0 (\text{CH}_2\text{N}), \ 45.6 (\text{CH}_3\text{N}), \ 41.8 (\text{CH}_3\text{N}), \ 22.3 (\text{CH}_3\text{CH}) \]

\[ m/z (\text{FAB}) \ 124 (26.7%, \text{MH}^+, \text{Cl}^7), \ 122 (100, \text{MH}^+, \text{Cl}^5), \ 86 (8.5, \text{M-Cl}), \ 44 (11.0, \text{N(CH}_3)_2) \]

5.2.2.8 (S)-(−)-2,2-Diphenyl-4-dimethylaminopentanenitrile (S)-86 \(^{55}\)

\[
\text{Me}_2\text{N} \quad \text{CN} \quad \begin{array}{c} \backslash \backslash \text{Ph} \text{ Ph} \end{array}
\]

This was prepared following the same procedure as the (R)-isomer (5.2.2.4). \(^{55}\) 30g of (R)-90 were used and 14.65g (33%) of (S)-(−)-2,2-diphenyl-4-dimethylaminopentanenitrile (S)-86 were obtained.
Experimental

Mp 100-101°C [lit. \(^{49,46} 100-101°C\)]

\([\alpha]_D +52.9° (c 0.66, \text{EtOH})\) [lit. \(^{49} +49° (c 0.68, \text{EtOH})\)]

\(\nu_{\text{max}}(\text{solid})/\text{cm}^{-1}\) 2972, 2938, 2819, 2774 (CH), 2229 (CN)

\(\delta_H\) (CDCl\(_3\); 250 MHz) 7.38 (10 H, m, CH\(_{\text{ar}}\)), 2.70 (1 H, dd, \(J 13.6, 6.4,\), CH\(_{A}CH_{B}C\text{Ph}_2\)), 2.54 (1 H, sextet, \(J 6.3, \text{CH}_3\text{CHCH}_A\text{CH}_B\)), 2.25 (1 H, dd, \(J\) 13.6, 6.0, CH\(_A\text{CH}_B\text{CPh}_2\)), 2.15 (6 H, s, N(CH\(_3\))\(_2\)), 0.92 (3 H, d, \(J 6.5, \text{CH}_3\text{CH}\))

\(\delta_C\) (CDCl\(_3\); 63 MHz) 141.2, 140.6 (ipso-Ar), 128.7, 128.6, 127.8, 127.6, 127.3, 127.1 (CH\(_2\)), 122.8 (CN), 55.4 (CH\(_3\text{CH}\)), 49.6 (C\(_{\text{Ph}_2\text{CN}}\)), 43.2 (N(CH\(_3\))\(_2\)), 39.9 (CH\(_2\)), 13.1 (CH\(_3\text{CH}\))

\(m/z\) (FAB) 289 (4.9%, MH\(^+\)), 279 (100), 154 (23, Ph\(_2\)), 137 (14), 72 (45, CH\(_3\text{CHN(CH}_3\))\(_2\))

5.2.2.9 (S)-(+-)Methadone hydrochloride (S)-83 \(^{30}\)

![Structure of (S)-(+-)Methadone hydrochloride](image)

This was prepared following the same procedure as the (R)-isomer (5.2.2.5). 10g of (S)-86 were used and 6.6g (53%) of (S)-(+-)-methadone hydrochloride (S)-83 were obtained.

Rt 12.6 (99.9%) - Racemic - 10.0, 13.1

Mp 240-241°C [lit. \(^{58} 239-241°C\)]

\([\alpha]_D +136° (c 2.02, \text{EtOH})\)

\(\nu_{\text{max}}(\text{solid})/\text{cm}^{-1}\) 2935 (CH), 2462, 1702 (C=O)

\(\delta_H\) (d\(^6\)DMSO; 250 MHz) 7.07-7.57 (10 H, m, CH\(_{\text{ar}}\)), 3.08 (1 H, brd, \(J 14.0,\), CH\(_A\text{CH}_B\text{CPh}_2\)), 2.93 (1 H, m, CH\(_3\text{CHCH}_A\text{CH}_B\)), 2.67 (6 H, brd, N(CH\(_3\))\(_2\)),
2.40 (1 H, m, CHCH\textsubscript{2}CH\textsubscript{2}CPh\textsubscript{2}), 2.20 (2 H, q, J 7.3, CH\textsubscript{3}CH\textsubscript{2}CO), 0.73 (3 H, t, J 7.2, CH\textsubscript{3}CH\textsubscript{2}CO), 0.43 (3 H, d, J 6.6, CH\textsubscript{3}CH)

\[ \delta_c \text{ (d}^6\text{DMSO; 63 MHz)} \] 140.7, 140.3 (ipso-Ar), 129.4, 129.2, 129.0, 128.7, 127.8, 127.0 (CH\textsubscript{ar}), 64.8 (CH\textsubscript{2}CH), 59.1 (CPh\textsubscript{2}CO), 38.5, 38.3 (CH\textsubscript{2}CH\textsubscript{2}CPh\textsubscript{2} and N(CH\textsubscript{3})\textsubscript{2}), 32.6 (CH\textsubscript{2}CO), 14.9, 9.5 (CH\textsubscript{3}CH and CH\textsubscript{3}CH\textsubscript{2})

\[ \text{m/z (FAB) 310 (100\%, MH\textsuperscript{+}), 265 (11, M-N(CH}_3)_2, 154 (19, Ph}_2, 72 (15, CH}_3CHN(CH}_3)_2) \]

Chiral HPLC shows no evidence of (R)-isomer (R)-83, ee >99%

5.2.3 Lipase screen and chiral GC analysis of the propanoate ester

5.2.3.1 Preparation of racemic 1-dimethylamino-2-propyl propanoate 92

To a solution of 1-dimethylamino-2-propanol 89 (5g, 48.5 mmol) in dichloromethane (125ml) was added slowly with stirring a solution of propanoyl chloride (5g, 54.1 mmol) in dichloromethane (75ml). When the addition was complete stirring was continued for a further 30 mins and then the solvent was removed at reduced pressure to leave the hydrochloride salt as a white solid. This was dissolved in water (50ml) and the solution treated with saturated sodium hydrogen carbonate solution (50ml). The free base was extracted with ethyl acetate (3 x 70ml) and the combined organic layers were washed with brine (120ml) and dried (MgSO\textsubscript{4}). The ethyl acetate was removed by rotary evaporation at reduced pressure and the product purified by distillation at reduced pressure (bpt 132°C/∼10mmHg) to afford a colourless oil (5.7g, 74%).

R\textsubscript{t} 13.4, 14.0

\[ \delta_h \text{ (CDCl}_3; 220 MHz)} \] 5.09 (1 H, m, CH\textsubscript{3}CH(OCOC\textsubscript{2}H\textsubscript{5})), 2.10 - 2.60 (4H, m, N\textsubscript{-}CH\textsubscript{2}CH(OCOCH\textsubscript{2}CH\textsubscript{3})), 2.25 (6 H, s, N(CH\textsubscript{3})\textsubscript{2}), 1.21 (3 H, d, J 6, CH\textsubscript{3}CH(OCOC\textsubscript{2}H\textsubscript{5})), 1.13 (3 H, t, J 7.5, OCOCH\textsubscript{2}CH\textsubscript{3})
5.2.3.2 Lipase screen for the resolution of 1-dimethylamino-2-propanol 89

Racemic 1-dimethylamino-2-propanol 89 (103mg, 1 mmol) was stirred with vinyl propanoate (2ml) and a lipase from the Chirazyme ® kit was added. The reaction mixture was stirred slowly at room temperature. Periodically the reaction mixture was sampled, an aliquot (2 drops) was taken and diluted with diethyl ether (1ml). The samples were analysed by GC using a chiral β-cyclodextrin column. The results are discussed in chapter 4 (4.6).

5.2.4 Large scale resolution and syntheses of chiral methadones

5.2.4.1 Resolution of 1-dimethylamino-2-propanol 89

\[
\begin{align*}
\text{Me}_2\text{N} & \quad \text{OH} \\
\rightarrow & \\
\text{Me}_2\text{N} & \quad \text{OCOC}_2\text{H}_5 \\
\quad + & \\
\text{Me}_2\text{N} & \quad \text{OH}
\end{align*}
\]

Racemic 1-dimethylamino-2-propanol 89 (1003g, 9.74mol) was stirred with vinyl propionate (530ml, 4.87mol) and Novozyme ® 435 (30g) was added. The reaction mixture was stirred slowly for 70 hours and after this time tlc (10% methanol/dichloromethane - visualise KMnO₄ solution) indicated that the reaction had gone to 50% conversion. The enzyme was removed by filtration and the filtrate was distilled at reduced pressure. (S)-(+) 1-Dimethylamino-2-propanol (S)-89 was obtained as a colourless oil (450.6g, 45%) bpt 77°/~15mm,

\[R_f \ (\text{Methanol:dichloromethane, 1:9}) \ 0.16\]

\[\nu_{\text{max}}(\text{neat})/\text{cm}^{-1} \ 3419 \ (\text{OH}) \ 2969, \ 2819, \ 2772 \ (\text{CH})\]

\[\delta_H \ (\text{CDCl}_3; \ 250 \text{ MHz}) \ 3.78 \ (1 \text{ H, m, CH}_3\text{CH(OH)}), \ 3.50 \ (1 \text{ H, brs, OH}), \ 2.25 \ (6 \text{ H, s, N(CH}_3)_2), \ 2.15 \ (2 \text{ H, m, NCH}_2), \ 1.12 \ (3 \text{ H, d, J 6.1, CH}_3\text{CH(OH)})\]

\[\delta_C \ (\text{CDCl}_3; \ 63 \text{ MHz}) \ 67.0 \ (\text{CH(OH)}), \ 63.1 \ (\text{CH}_2-N), \ 45.4 \ ((\text{CH}_3)_2N), \ 20.1 \ (\text{CH}_3\text{CH})\]
(R)-(−)-1-Dimethylamino-2-propyl propanoate (R)-92 was the second component to distil (557.2g, 36%) bpt 105-108°C / ~15mm.

\(R_f\) (Methanol:dichloromethane, 1:9) 0.37

\(v_{\text{max}}(\text{neat})/\text{cm}^{-1}\) 2978, 2941, 2821, 2768 (CH), 1732 (C=O)

\(\delta\) (CDCl3; 250 MHz) 5.06 (1 H, m, CH$_3$CH(OCOC$_2$H$_5$)), 2.50 (1 H, dd, \(J\) 12.7, 7.2, N-CH$_3$H$_2$CH(OCOC$_2$H$_5$)), 2.30 (3 H, m, N-CH$_3$H$_2$CH(OCOCH$_2$CH$_3$)), 2.25 (6 H, s, N(CH$_3$)$_2$), 1.21 (3 H, d, \(J\) 6.2, CH$_3$CH(OCOC$_2$H$_5$)), 1.12 (3 H, t, \(J\) 7.5, OCOCH$_2$CH$_3$)

\(\delta\) (CDCl$_3$; 63 MHz) 174.0 (C=O), 68.1 (CH-O), 64.0 (CH$_2$-N), 45.8 ((CH$_3$)$_2$N), 27.8 (COCH$_2$CH$_3$), 18.5 (CH$_3$CH), 9.0 (COCH$_2$CH$_3$)

5.2.4.2 (R)-(−)-1-Dimethylamino-2-propanol (R)-89

![Structure of (R)-(−)-1-Dimethylamino-2-propanol](structure.png)

A solution of sodium methoxide was made by dropping small pieces of sodium metal (5g) into methanol (200 ml) under an atmosphere of argon. This was then added to a stirred solution of (R)-(−)-1-dimethylamino-2-propyl propanoate (R)-92 (557g, 3.5mol) in methanol (3.8L). The mixture was stirred overnight and checked for completion by tlc (10% methanol/dichloromethane). The methanol was removed under reduced pressure and the crude (R)-(−)-1-dimethylamino-2-propanol (342g) was purified by distillation at reduced pressure, bpt 75°C at ~15mmHg, to afford the title compound (R)-89 as a colourless oil (313.1g, 87%).

\(R_f\) (Methanol:dichloromethane, 1:9) 0.18

\(v_{\text{max}}(\text{neat})/\text{cm}^{-1}\) 3419 (OH) 2969, 2819, 2772 (CH)
δ

H (CDCl₃; 250 MHz) 3.76 (1 H, m, CH₃CH(OH)), 3.49 (1 H, brs, OH), 2.24 (6 H, s, N(CH₃)₂), 2.15 (2 H, m, NCH₂) 1.10 (3 H, d, J 6.1, CH₃CH(OH))
δ

C (CDCl₃, 63 MHz) 67.0 (CH(OH)), 63.0 (CH₂-N), 45.4 ((CH₃)₂N), 20.0 (CH₃CH)

5.2.4.3 (S)-(+-)1-Dimethylamino-2-chloropropane hydrochloride (S)-90

A solution of thionyl chloride (330ml, 4.55mol) in chloroform (400ml) was added slowly, with stirring, to a cooled (ice/water) solution of (R)-(−)-1-dimethylamino-2-propanol (R)-89 (312.4g, 3.03mol) in chloroform (800ml). When the addition was complete a precipitate formed. The mixture was allowed to warm to room temperature over 30 minutes and then heated to reflux for a further 30 minutes. The precipitate redissolved on heating but then the product crystallised out from the boiling solvent as it formed. The cooled mixture was diluted with ether (1.5L) and filtered. The crude product (440.4g, 92%) was recrystallised from 2-propanol and decolourising charcoal was used. The product (S)-90 was obtained in 2 crops (328.5g, 69%).

Mp 192-193°
[α]D +63.9° (c 2.01, H₂O)
ν

max (solid)/cm⁻¹ 2962 (CH)
δ

H (D₂O; 250 MHz) 4.42 (1 H, sextet, J 7.0, CH₂CHClCH₃), 3.40 (2 H, d, J 8.0, NCH₂CHCl), 2.88 (6 H, d, J 8.4, N(CH₃)₂), 1.49 (3 H, d, J 6.5, CH₃CHCl)
δ

C (D₂O, 63 MHz) 64.3 (CHCl), 52.0 (CH₂N), 45.6 (CH₃N), 41.8 (CH₃N), 22.3 (CH₃CH)

m/z (FAB) 124 (26.7%, MH+, ³⁷Cl), 122 (100, MH+, ³⁵Cl), 86 (8.5, M-Cl), 44 (11.0, N(CH₃)₂)
A 50% w/v solution of sodium hydroxide in water (300ml, 7.5mol) was added to a mechanically stirred suspension of diphenylacetonitrile (386.5g, 2.0mol) and dibenzo-18-crown-6 (12.7g, cat.) in dimethylsulphoxide (300ml). The colour rapidly deepened to an orange/brown. (S)-(−)-1-Dimethylamino-2-chloropropane hydrochloride (S)-90 (327g, 2.07mol) was added in portions over 30 minutes; this caused the temperature to rise and cooling was applied (cold water bath) to maintain the temperature below 50°C. After the addition was complete the mixture was stirred at 45-50°C (water bath) for a further hour. The reaction mixture was then allowed to cool to room temperature and was poured into ice/water (2.5L) and extracted with ethyl acetate (3 x 2L). The product was extracted into IN HCl (2.5L + 1.5L) and this was back washed with ethyl acetate. The aqueous was basified with 2M sodium hydroxide and extracted with ethyl acetate (3 x 2L). The organic extracts were washed with brine (1.5L), dried (MgSO4), and evaporated to give a clear yellow oil. This was dissolved in boiling hexane (~3L) and a white solid formed as this solution was allowed to cool. The suspension was cooled (ice/water) to complete crystallisation and the solid was collected by filtration and washed thoroughly with cold hexane (1L). The solid was dried under high vacuum and (R)-(−)-2,2-diphenyl-4-dimethylaminopentanenitrile (R)-86 was obtained as a white crystalline solid (197.4g, 34%).

Mp 100-101 °C
\([\alpha]_D -50.2^\circ \text{ (c 0.71, EtOH)}\)

\(\nu_{\text{max}}(\text{solid})/\text{cm}^{-1} \quad 2972, 2938, 2819, 2774 \text{ (CH)}, 2229 \text{ (CN)}\)
\[ \delta_H \text{ (CDCl}_3; 250 \text{ MHz)} 7.38 \text{ (10 H, m, CH}_2\text{)}, 2.70 \text{ (1 H, dd, } J 13.6, 6.4, \text{ CHCH}_2\text{CH}_3\text{)}, 2.54 \text{ (1 H, sextet, } J 6.3, \text{ CH}_2\text{CHCH}_2\text{CH}_2\text{)}, 2.25 \text{ (1 H, dd, } J 13.6, 6.0, \text{ CHCH}_2\text{CH}_3\text{)}, 2.15 \text{ (6 H, s, N(CH}_3\text{)}_2\text{)}, 0.92 \text{ (3 H, d, } J 6.5, \text{ CH}_3\text{)} \]

\[ \delta_C \text{ (CDCl}_3; 63 \text{ MHz)} 141.2, 140.6 \text{ (ipso-Ar)}, 128.7, 128.6, 127.8, 127.6, 127.3, 127.1 \text{ (CH}_2\text{)}, 122.8 \text{ (CN)}, 55.4 \text{ (CH}_3\text{CH)}, 49.6 \text{ (CPh}_2\text{CN)}, 43.2 \text{ (N(CH}_3\text{)}_2\text{)}, 39.9 \text{ (CH}_2\text{)}, 13.1 \text{ (CH}_3\text{CH)} \]

\[ m/z \text{ (FAB)} 289 \text{ (4.9\%, MH}^+)\text{, 279 (100)}, 154 \text{ (23, Ph}_2\text{)}, 137 \text{ (14)}, 72 \text{ (45, CH}_3\text{CHN(CH}_3\text{)}_2\text{)} \]

5.2.4.5 (R)-(−)-Methadone hydrochloride (R)-83. 30

\[
\text{Me}_2\text{N} \quad \text{Ph} \quad \text{Ph}
\]

All apparatus was dried and the reaction was carried out under an inert atmosphere of argon. A solution of (R)-(−)-2,2-diphenyl-4-dimethylaminopentanenitrile (R)-86 (197.0g, 0.71mol) in toluene (600ml) was added to a stirred solution of 3M ethylmagnesium bromide in ether (425ml, 1.275mol). The ether was removed under reduced pressure and the remaining solution heated at reflux (135-140°) for 3 hours. After cooling to room temperature 2N HCl (1.5L) was added with care and then stirring was continued at 135-140° for a further 30 minutes. The two phases were allowed to separate and cool to room temperature. After scratching the sides of the flask a solid started to crystallise from the aqueous phase. The flask was cooled to complete crystallisation and the white solid was collected by filtration. This solid was dried under high vacuum to yield 94g (38%) of (R)-(−)-methadone hydrochloride (R)-83 (6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride).
Experin:ielnal

Rt 6.3 (94.3%) 8.1 (5.7%)  ee = 88.6%  [racemic Rt 6.5, 8.0]

Mp 230-232°C

[α]D -111.3° (c 2.09, EtOH)

νmax(solid)/cm⁻¹  2935 (CH), 2462, 1702 (C=O)

δH (d⁹DMSO; 250 MHz) 7.07-7.57 (10 H, m, CH₆H), 3.08 (1 H, brd, J 14.0, CHCH₂CH₃CH₄CH₅), 2.93 (1 H, m, CH₃CH₂CH₄CH₅), 2.67 (6 H, brd, N(CH₃)₂), 2.40 (1 H, m, CHCH₂CH₃CH₄CH₅), 2.20 (2 H, q, J 7.3, CH₃CH₂CO), 0.73 (3 H, t, J 7.2, CH₃CH₂CO), 0.43 (3 H, d, J 6.6, CH₃CH)

δC (d⁹DMSO; 63 MHz) 140.7, 140.3 (ipso-Ar), 129.4, 129.2, 129.0, 128.7, 127.8, 127.0 (CH₆H), 64.8 (CH₃CH), 59.1 (CPh₂CO), 38.5, 38.3 (CHCH₂CPh₂ and N(CH₃)₂), 32.6 (CH₂CO), 14.9, 9.5 (CH₃CH and CH₃CH₂)

m/z (FAB) 310 (100%, MH⁺), 265 (11, M-N(CH₃)₂), 154 (19, Ph₂), 72 (15, CH₃CHN(CH₃)₂)

5.2.4.6 (R)-(−)-1-Dimethylamino-2-chloropropane hydrochloride (R)-90

This was prepared following the same procedure as the (S)-isomer (5.2.4.3). 450.6g of (S)-89 were used and 586.2g (84%) of crude product (R)-90 was isolated. This was not further purified.

νmax(solid)/cm⁻¹  2962 (CH)

δH (D₂O; 250 MHz)) 4.42 (1 H, sextet, J 7.0, CH₂CHClCH₃), 3.40 (2 H, d, J 8.0, NCH₂CHCl), 2.88 (6 H, d, J 8.4, N(CH₃)₂), 1.49 (3 H, d, J 6.5, CH₃CHCl)
5.2.4.7 (S)-(+)\textsuperscript{-}2,2\textsuperscript{-}Diphenyl-4\textsuperscript{-}dimethylaminopentanenitrile (S)-86.\textsuperscript{55}

![Structural diagram]

This was prepared following the same procedure as the (R)-isomer (5.2.4.4). 582.2g of (R)-90 were used and 297.0g (29\%) of (S)-(+)\textsuperscript{-}2,2\textsuperscript{-}diphenyl-4\textsuperscript{-}dimethylaminopentanenitrile (S)-86 were obtained.

Mp 100-101 °C [lit. \textsuperscript{47,49} 100-101 °C]

[α]D \textsuperscript{-50.2°} (c 0.71, EtOH) [lit. \textsuperscript{49} +49° (c 0.68, EtOH)]

ν\textsubscript{max}(solid)/cm\textsuperscript{-1} 2972, 2938, 2819, 2774 (CH), 2229 (CN)

δ\textsubscript{H} (CDCl\textsubscript{3}; 250 MHz) 7.38 (10 H, m, CH\textsubscript{ar}), 2.70 (1 H, dd, J 13.6, 6.4, CHCH\textsubscript{A}CH\textsubscript{B}CPh\textsubscript{2}), 2.54 (1 H, sextet, J 6.3, CH\textsubscript{3}CHCH\textsubscript{A}CH\textsubscript{B}), 2.25 (1 H, dd, J 13.6, 6.0, CHCH\textsubscript{A}CH\textsubscript{B}CPh\textsubscript{2}), 2.15 (6 H, s, N(CH\textsubscript{3})\textsubscript{2}), 0.92 (3 H, d, J 6.5, CH\textsubscript{3}CH)

δ\textsubscript{C} (CDCl\textsubscript{3}; 63 MHz) 141.2, 140.6 (ipso-Ar), 128.7, 128.6, 127.8, 127.6, 127.3, 127.1 (CH\textsubscript{ar}), 122.8 (CN), 55.4 (CH\textsubscript{3}CH), 49.6 (CPh\textsubscript{2}CN), 43.2 (N(CH\textsubscript{3})\textsubscript{2}), 39.9 (CH\textsubscript{2}), 13.1 (CH\textsubscript{3}CH)

m/z (FAB) 289 (4.9\%, MH\textsuperscript{+}), 279 (100), 154 (23, Ph\textsubscript{2}), 137 (14), 72 (45, CH\textsubscript{3}CHN(CH\textsubscript{3})\textsubscript{2})

5.2.4.8 (S)-(+)\textsuperscript{-}Methadone hydrochloride (S)-83.\textsuperscript{30}

![Structural diagram]

All apparatus was dried and the reaction was carried out under an inert atmosphere of argon. A solution of (S)-(+)\textsuperscript{-}2,2\textsuperscript{-}diphenyl-4\textsuperscript{-}dimethylaminopentanenitrile (S)-86 (297g, 1.07mol) in toluene (900ml) was added to a stirred solution of 3M ethylmagnesium bromide in ether (650ml,
Experimental

1.95 mol. The ether was removed under reduced pressure and the remaining solution heated at reflux (135-140°C) for 3 hours. After cooling to room temperature 2N HCl (1.8L) was added with care and then stirring was continued at 135-140°C for a further 30 minutes. The two phases were allowed to separate and cool to room temperature. After scratching the sides of the flask no solid precipitated from solution so the two layers were separated and after standing overnight crystals had formed in the toluene layer. The suspension was cooled in the fridge for two hours and the solid was collected by filtration, washed with cold toluene and dried. 220MHz NMR spectroscopy showed this to be (S)-(+) -methadone. It was dissolved in ether and treated with 1M ethereal HCl to form the salt. This was collected by filtration and dried under high vacuum to yield 51.6g (14%) of (S)-(+) -methadone hydrochloride (S)-83 (6-dimethylamino-4,4-diphenyl-3-heptanone hydrochloride).

R_t 7.8 (100%) ee >99% [racemic R_t 6.5, 8.0]

Mp 240-241°C

[α]_D +140.5° (c 2.03, EtOH)

ν max (solid)/cm⁻¹ 2935 (CH), 2462, 1702 (C=O)

δ_H (d⁶DMSO; 250 MHz) 7.07-7.57 (10 H, m, CH₉), 3.08 (1 H, brd, J 14.0, CHCH₂CHBCPh₂), 2.93 (1 H, m, CH₃CHCH₃CH₂CH₄), 2.67 (6 H, brd, N(CH₃)₂), 2.40 (1 H, m, CHCH₉CH₃CH₂CPh₂), 2.20 (2 H, q, J 7.3, CH₃CH₂CO), 0.73 (3 H, t, J 7.2, CH₃CH₂CO), 0.43 (3 H, d, J 6.6, CH₃CH)

δ_C (d⁶DMSO; 63 MHz) 140.7, 140.3 (ipso-Ar), 129.4, 129.2, 129.0, 128.7, 127.8, 127.0 (CH₉), 64.8 (CH₂CH), 59.1 (CPh₂CO), 38.5, 38.3 (CHCH₂CPh₂ and N(CH₃)₂), 32.6 (CH₂CO), 14.9, 9.5 (CH₃CH and CH₃CH₂)

m/z (FAB) 310 (100%, MH⁺), 265 (11, M-N(CH₃)₂), 154 (19, Ph₂), 72 (15, CH₃CHN(CH₃)₂)
Chiral HPLC shows no evidence of (R)-isomer, ee >99%.

5.2.5 Levo-α-acetyl methadol (LAAM) 93

5.2.5.1 6-Dimethylamino-4,4-diphenyl-3-heptanol 94 prepared by hydrogenation 57

(S)-(+) -Methadone hydrochloride (S)- 83 (200mg, 0.58mmol) was dissolved in distilled water (5ml) and platinum (IV) oxide (20mg) was added. This mixture was hydrogenated on the Parr apparatus overnight. Tlc (10% methanol/dichloromethane) seemed to show complete conversion so the catalyst was removed by filtration through celite. The filtrate was basified with 2M sodium hydroxide solution and then extracted with ethyl acetate (2 x 30ml). The combined organic extracts were washed with brine and dried (magnesium sulphate). The solvent was removed by evaporation at reduced pressure to leave a clear gum (160mg). The 220MHz NMR spectrum (CDCl3) showed this to be a mixture of methadone and the reduced methadol 94.

5.2.5.2 6-Dimethylamino-4,4-diphenyl-3-heptanol 94

(S)-(+) -Methadone hydrochloride (S)- 83 (600mg, 1.74mmol) was dissolved in ethanol (10ml) and the solution was stirred whilst sodium borohydride (140mg, 3.47mmol) was added portionwise over a period of 5 minutes. When
the addition was complete cerium (III) chloride heptahydrate (5mg, cat.) was added. The resultant solution was allowed to stir at room temperature for 30 minutes then the ethanol was removed under reduced pressure. The residue was partitioned between diethyl ether (40ml) and water (40ml). The aqueous layer was extracted with more diethyl ether (2 x 20ml) and then the combined organics were washed with brine (40ml) and dried (MgSO4). The ether was removed under reduced pressure to leave 6-dimethylamino-4,4-diphenyl-3-heptanol 94 (435mg, 80%).

δH (220MHz; CDCl3) 7.1-7.7 (10H, m, CHα), 3.85 (1H, dd, J 10, 3, CHOH), 2.7 (1H, dd, J 15, 7, CHCH2CPh2), 2.15 (6H, s, N(CH3)2), 1.9-2.3 (2H, m, CHCH2CPh2), 1.75 (1H, m, CH3CHAHB), 1.1 (1H, m, CH3CHAHB), 0.8 (6H, m, CH3CH3AHB and CH3CH).

5.2.5.3 6-Dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride (levorotatory methadone hydrochloride) 93

6-Dimethylamino-4,4-diphenyl-3-heptanol 94 (435mg, 1.40mmol) dissolved in ethyl acetate (10ml) was treated with acetyl chloride (183mg, 2.33mmol). The mixture was refluxed for 2 hours. After allowing the solution to cool to room temperature the solvent was removed under reduced pressure to leave a white foam, this crystallised from ethyl acetate to give 6-dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride 93 (420mg, 79%).
Experimental

Mp 206-207°C [lit. 215°C]  
[α]D -56° (c 0.2, H2O) [lit. -60° (c 0.2)]  
νmax(solid)/cm⁻¹ 2974 (CH), 2689, 1725 (C=O)  
δ (CDCl₃; 250 MHz) 12.03 (1 H, brs, (CH₃)₂NH⁺), 7.37 (10 H, m, CH₅), 5.73 (1 H, dd, J 10.3, 1.5, HCOOC=O), 3.01 (2 H, m, CH₂CH₂C₆H₄O and CH₂CH₂C₆H₄O), 2.63 (3 H, d, J 4.9, NCH₃), 2.53 (3 H, d, J 4.9, NCH₃), 2.10 (1 H, m, CH₂CH₂C₆H₄O), 1.00 (1 H, m, CH₂CH₂C₆H₄O), 0.79 (3 H, t, J 7.3, CH₃CH₃), 0.57 (3 H, d, J 6.6, CH₃CH₃)  
δ (CDCl₃; 63 MHz) 171.8 (C=O), 142.3, 140.2 (ipso-Ar), 130.1, 129.2, 128.2, 128.0, 127.4, 127.2 (CH₅), 77.8 (HCOAc), 59.3 (CH₂CH₃), 54.7 (C₆H₄), 41.3, 40.4, 37.6 (CH₂CH₂C₆H₄O and N(CH₃)₂), 24.4, 21.4 (CH₃CO and CH₃CH₂), 14.3, 10.9 (CH₃CH and CH₃CH₂)  
m/z (FAB) 354 (100%, MH⁺), 154 (26.3, Ph₂), 72 (41.1, CH₃CHN(CH₃)₂)  

5.2.5.4 6-Dimethylamino-4,4-diphenyl-3-heptanol 94 - large scale  
(S)-(++)-Methadone hydrochloride (S)-83 (20g, 57.9mmol) was dissolved in ethanol (120ml) and stirred at room temperature. Cerium (III) chloride heptahydrate (5mg, cat.) was added before sodium borohydride (140mg, 3.47mmol) was added portionwise over a period of 30 minutes. The reaction was quite exothermic so an ice bath was used to cool the mixture during the addition. The resultant solution was stirred at room temperature for 3h then the ethanol was removed under reduced pressure. The residue was partitioned between dichloromethane (250ml) and water (250ml). The aqueous layer was extracted with more dichloromethane (2 x 250ml) and then the combined organics were washed with brine (250ml) and dried (MgSO₄). The dichloromethane was removed under reduced pressure to leave 6-dimethylamino-4,4-diphenyl-3-heptanol 94 (18.6g, quant.).
δt (220MHz; CDCl3) 7.1-7.7 (10H, m, CH₃), 3.85 (1H, dd, J 10, 3, CHO), 2.7 (1H, dd, J 15, 7, CH₂CH₂C₂H₆), 2.15 (6H, s, N(CH₃)₂), 1.9-2.3 (2H, m, CH₂CH₂C₂H₆), 1.75 (1H, m, CH₃CH₂H₆), 1.1 (1H, m, CH₃CH₂H₆), 0.8 (6H, m, CH₃CH₂H₆ and CH₃CH)

5.2.5.5 6-Dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride (levo-α-acetyl methadol hydrochloride) 93 - large scale
6-Dimethylamino-4,4-diphenyl-3-heptanol 94 (15.6g, 50.2mmol) dissolved in ethyl acetate (150ml) was treated with acetyl chloride (6.57g, 83.7mmol). The mixture was refluxed for 2 hours and a white precipitate formed during this period. The resulting suspension was cooled in the fridge overnight. The white solid was collected by filtration and the mother liquors reduced to give a second crop. The two crops were combined and dried on the rotary evaporator (high vacuum) 6-dimethylamino-4,4-diphenyl-3-acetoxyheptane hydrochloride 93 (16.5g, 84%) was obtained as a white solid.

Mp 212-214°C [lit. 67 215°C]
[α]D -60.6° (c 0.216, H₂O) [lit. 67 -60° (c 0.2)]
νmax(solid)/cm⁻¹ 2974 (CH), 2689, 1725 (C=O)
δt (CDCl₃; 250 MHz) 12.03 (1H, brs, (CH₃)₂NH⁺), 7.37 (10H, m, CH₃), 5.73 (1H, dd, J 10.3, 1.5, HCOOCH₃), 3.01 (2H, m, CH(CH₃)CH₂C₂H₆ and CH₂CH₃CH₂C₂H₆), 2.63 (3H, d, J 4.9, NCH₃), 2.53 (3H, d, J 4.9, NCH₃), 2.10 (1H, m, CHCH₂CH₂C₂H₆), 2.14 (3H, s, CH₃CO), 1.82 (1H, m, CH₃CH₂H₆), 1.00 (1H, m, CH₃CH₂H₆), 0.79 (3H, t, J 7.3, CH₃CH₂H₆), 0.57 (3H, d, J 6.6, CH₃CH)
δC (CDCl₃; 63 MHz) 171.8 (C=O), 142.3, 140.2 (ipso-Ar), 130.1, 129.2, 128.2, 128.0, 127.4, 127.2 (CH₃), 77.8 (HCOAc), 59.3 (CH₃CH), 54.7 (CPh₂), 41.3, 40.4,
37.6 (CH\textsubscript{2}CH\textsubscript{2}CPh\textsubscript{2} and N(CH\textsubscript{3})\textsubscript{2}), 24.4, 21.4 (CH\textsubscript{3}CO and CH\textsubscript{2}CH\textsubscript{2}), 14.3, 10.9 (CH\textsubscript{3}CH and CH\textsubscript{3}CH\textsubscript{2})
m/z (FAB) 354 (100\%, MH\textsuperscript{+}), 154 (26.3, Ph\textsubscript{2}), 72 (41.1, CH\textsubscript{3}CHN(CH\textsubscript{3})\textsubscript{2})
6.0 References

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