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APPLICATIONS OF BIOCATALYSTS IN THE  
RESOLUTION OF  
CHIRAL ORGANOMETALLIC COMPOUNDS.

AUTHOR

Christine M. Henderson

INSTITUTION  
and DATE

University of Warwick

1988

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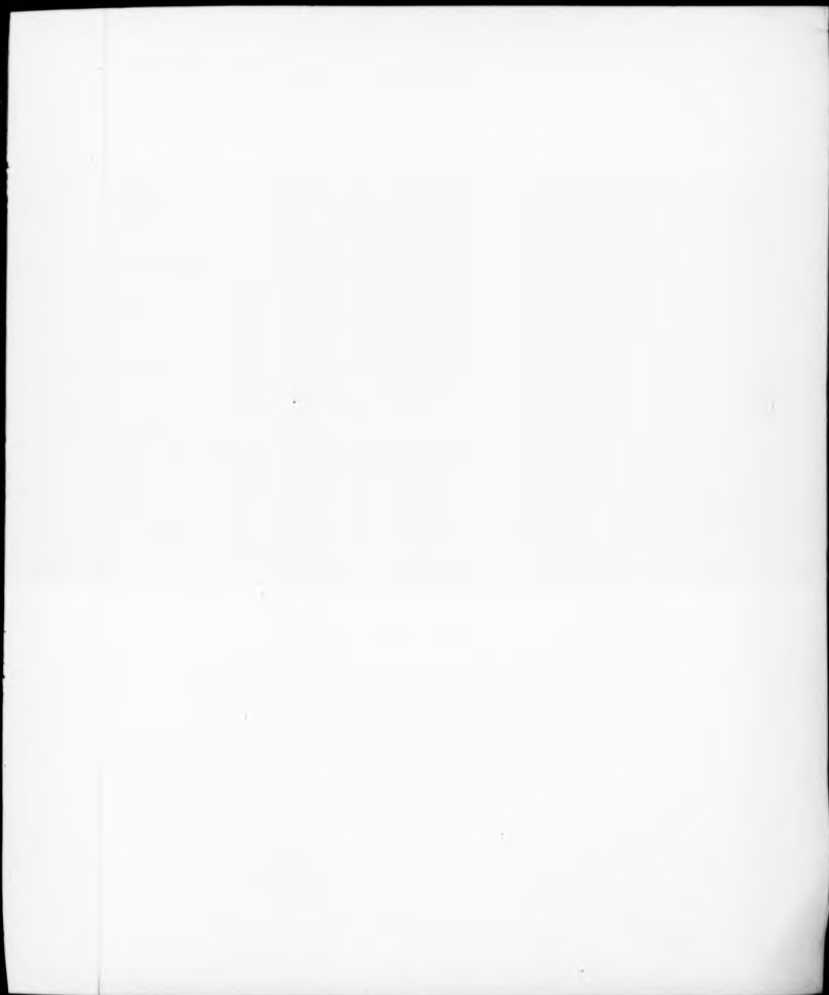
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**APPLICATIONS OF BIOCATALYSTS IN THE  
RESOLUTION OF  
CHIRAL ORGANOMETALLIC COMPOUNDS.**

**B y**

**Christine M. Henderson**

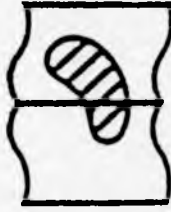
**Submitted for the degree of Doctor of Philosophy**

**University of Warwick**

**Department of Chemistry**

**September 1988.**

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## Contents

## Page

Abbreviations	iii
Acknowledgements	v
List of figures	vi
Declaration	vii
Publications	viii
Summary	ix

## Introduction

Part 1	Some uses of enzymes in organic synthesis	1
Part 2	The hydrolytic enzymes: Esterases and Lipases	8
Part 3	The application of optically active irontricarbonyl and ferrocene derivatives to organic synthesis	18

## Chapter 1    The enzymatic resolution of racemic irontricarbonyl complexes    44

1-1	Methyl sorbate irontricarbonyl	44
1-2	The enzyme-catalysed reactions of 1-1	48
1-3	1-1 as an enzyme substrate in organic solvent	49
1-4	Irontricarbonyl (2,4-hexadien-1-ol) acetate	55
1-5	The enzyme-catalysed reactions of 1-4	56
1-6	2-Carboethoxy-1,3-butadiene irontricarbonyl	62
1-7	The enzymatic hydrolysis of 2-acetoxy-1,3-butadiene irontricarbonyl	66
1-8	The enzymatic hydrolysis of 1-6	69
1-9	Determination of the absolute configuration of 2-carboxy-1,3-butadiene irontricarbonyl	72

## Chapter 2    The enzymatic resolution of racemic ferrocene derivatives    81

2-1	1,1'-Ferrocenedicarboxylic acid dimethyl ester	81
2-2	$\alpha$ -Methylferrocenecarboxylic acid	83

2-3	$\alpha$ -Methylferrocenecarboxylic acid methyl ester	86
2-4	$\alpha$ -Methylferrocenemethyl acetate/butyrate	87
2-5	The enzyme-catalysed reactions of 2-4	
(a)	In aqueous media	89
(b)	In organic media	90
2-6	The enzyme-catalysed transesterification of 2-4 (butyrate)	93
<u>Chapter 3</u>	Introduction to Experimental chapters	100
	Experimental to Chapter 1	103
<u>Chapter 4</u>	Experimental to Chapter 2	120
<u>Appendix</u>		131
<u>References</u>		132

**Abbreviations**

Me	Methyl
Et	Ethyl
Pr	Propyl
Ph	Phenyl
R	Alkyl or Aryl
NMR	Nuclear magnetic resonance
s	Singlet
d	Doublet
t	Triplet
q	quartet
m	multiplet
J	Coupling constant
ppm	parts per million
TMS	Tetramethylsilane
IR	Infra red
s	strong
m	medium
w	weak
UV	Ultra violet
MS	Mass spectrum
FAB	Fast atom bombardment
EI	Electron impact
cd	Circular dichroism
[ $\alpha$ ]	Specific rotation



c	Concentration (g/100ml)
ee	enantiomeric excess
epc	Enantiomerically pure compound
GLC	Gas-liquid chromatography
R <sub>T</sub>	Retention time
TLC	Thin-layer chromatography
mp	melting point
bp	boiling point
bz	<i>Benzene</i>
CDI	Carbonyldiimidazole
DMAP	Dimethylaminopyridine
DMSO	Dimethylsulfoxide
gly	Glycerol
MTPA	(-)-2-Methoxy-2-trifluoromethylphenylacetic acid
nba	<i>meta</i> -Nitrobenzylalcohol
PMSF	(Phenylmethyl)sulphonyl fluoride
THF	Tetrahydrofuran
Ac	Acetyl
PLE	Pig liver esterase
PPL	Pig pancreatic lipase
COL	<i>Candida cylindracea</i> lipase
<i>A. niger</i>	<i>Aspergillus niger</i>
<i>R. javanicus</i>	<i>Rhizopus javanicus</i>
EEAcE	Electric eel acetylcholinesterase
eu	Enzyme unit

### Acknowledgements

I would like to thank Professor D.H.G. Crout for his advice and constant encouragement which he gave throughout the course of this research. Also Dr. Susan Thomas for her invaluable guidance concerning the organometallic work.

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The financial assistance from the S.E.R.C. and Glaxo Group Research Plc. is gratefully acknowledged.

### List of figures

- Figure 7** Comparison of the  $^1\text{H}$  nmr spectra of methyl sorbate and its  $\text{Fe}(\text{CO})_3$  complexed derivative
- Figure 8** GLC trace following the PLE catalysed transesterification reaction between 3-methoxy-1-butanol and methyl propionate
- Figure 11** The cd spectrum of (-)-2-carboxy-1,3-butadiene irontricarbonyl
- Figure 12** The X-Ray structure of (+)-irontricarbonyl (1,3-butadien-2(S-(-)- $\alpha$ -methylbenzyl) amide
- Figure 12(a)** The unit cell of (+)-irontricarbonyl(1,3-butadien-2(S-(-)- $\alpha$ -methylbenzyl) amide
- Figure 14** The enzyme-catalysed transesterification of  $\alpha$ -methylferrocenemethyl butyrate with 1-heptanol
- Figure 16** 400MHz  $^1\text{H}$  nmr spectra of  $\alpha$ -methylferrocenemethyl aldehyde in the presence of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol

### Declaration

The work described in this thesis is the original of the author, except where acknowledgement has been made to results and ideas previously published. It was carried out in the Department of Chemistry, University of Warwick between October 1985 and September 1988 and has not been submitted previously for a degree at any institution.

Christine Henderson

## Publications

Parts of the research described in this thesis have appeared in the scientific literature as follows:

1. Enzymatic Resolution of a Chiral Organometallic Ester: Enantioselective Hydrolysis of 2-Ethoxycarbonylbuta-1,3-dienetricarbonyliron by Pig Liver Esterase.

Alcock, N.W., Crout, D.H.G., Henderson, C.M., Thomas, S.E., *J. Chem. Soc., Chem. Commun.* **1988**, 746-747.

## Summary

The aim of this thesis was to resolve fully a racemic organometallic complex, using a hydrolytic enzyme as the resolving agent. This was to demonstrate the vast substrates that biocatalysts will react with, and to provide a unique example of an enzyme recognising and differentiating between the two enantiomers of a molecule showing planar chirality. Irontricarbonyl-protected 2,4-dienes were chosen as the initial target molecules. Methyl sorbate irontricarbonyl was found not to be a substrate for PLE, neither in aqueous nor in organic media. Irontricarbonyl (2,4-hexadien-1-ol) acetate was shown to be a substrate for both esterases and lipases. The biocatalyst most effectively catalysing this hydrolysis in terms of rate and enantioselectivity was PPL, which gave a maximum of 43% e.e. when the reaction was conducted in an aqueous environment of pH 7 containing 10% MeOH. 2-Carboethoxy-1,3-butadiene irontricarbonyl and its corresponding acetate derivative were both found to be suitable enzyme substrates. 2-Acetoxy-1,3-butadiene irontricarbonyl was selectively hydrolysed to give the deprotected alcohol in a maximum of 40% e.e. This was achieved using the lipase *Aspergillus niger* as the biocatalyst in an aqueous media of pH 7. The e.e. was determined by  $^{31}\text{P}$  nmr spectroscopy of a phospholane derivative of the optically active alcohol. 2-Carboethoxy-1,3-butadiene irontricarbonyl was selectively hydrolysed by PLE in aqueous media of pH 7 containing 20% MeOH to give the corresponding acid in 85% e.e. Optically active 2-carboxy-1,3-butadiene irontricarbonyl was upon one recrystallisation gained in 100% e.e. The e.e. was verified by  $^1\text{H}$  nmr spectroscopy of an amide derivative of the optically pure acid, and its absolute configuration was determined by X-Ray structure analysis and by comparison of its cd.

The second type of organometallic complexes screened for enzyme-substrate feasibility were the ferrocenes. 1,1'-Ferrocenedicarboxylic acid dimethyl ester was shown to be a very non-ideal substrate, giving a mixture of mono- and di-acids as the reaction products.  $\alpha$ -Methylferrocenecarboxylic acid methyl ester was not an enzyme substrate in either aqueous nor organic media.  $\alpha$ -Methylferrocenemethyl acetate decomposed in aqueous conditions to give the corresponding  $\alpha$ -methylferrocenemethyl cation, yet in organic media was proved to be an adequate lipase substrate.

Studies of the comparative rates and selectivities of lipases either free in reaction media or immobilised on Biofix were carried out for the lipase-catalysed transesterification of  $\alpha$ -methylferrocenemethyl butyrate with 1-heptanol in iso-octane at 40°C. This showed that the rate of reaction and selectivity of the lipase varied dramatically as to which condition was used. In the case of both *A. niger* and *R. javanicus*, the lipase free in solution remained inactive whereas the lipase immobilised on Biofix catalysed the reaction rapidly and non-selectively. For PPL, the act of immobilising the lipase on Biofix reversed its enantioselectivity. The exception to this was CCL, which free in solution gave the highest e.e. of 50%. The e.e. was determined by  $^1\text{H}$  nmr spectroscopy of  $\alpha$ -methylferrocenemethyl aldehyde in the presence of chiral solvating agent 2,2,2-trifluoro-1-(9-anthryl) ethanol.

## Introduction

This thesis is divided into four main parts.

1. The first part, the Introduction, provides a general description of the use and versatility of enzymes in organic synthesis. It gives a detailed literature review of the published syntheses, resolutions and chiroptical properties of optically active irontricarbonyl and ferrocene derivatives.
2. The second part gives an account of the reactions of hydrolytic enzymes with irontricarbonyl complexes.
3. The third part gives an account of the reactions of hydrolytic enzymes with ferrocene derivatives.
4. The fourth part gives the experimental conditions in detail of parts 3 and 4.

## Part 1 Some uses of enzymes in organic synthesis

The application of enzymes in organic synthesis to replace conventional chemical reagents is beginning to be widely and successfully realised<sup>1,2</sup>. The word "Biotransformation" has been coined to encompass all such reactions.

The use and application of biotransformations in organic synthesis fall into four distinct categories.

1. The preparation of enantiomerically pure compounds. (e.p.c.s)
2. The carrying out of remote functionalisations.
3. For regiospecific reactions.

#### 4. The carrying out of reactions under very mild conditions.

There are six classes of enzymes: the oxidoreductases, the transferases, the hydrolases, the lyases, the isomerases and the ligases; which carry between them the potential to catalyse virtually all reactions of interest to the modern synthetic organic chemist. They can provide alternative and in many cases much simpler routes to synthetically useful molecules, and make a wide range of optically pure compounds readily available.

The four categories listed above will be exemplified below, as will a few selected examples of enzyme-catalysed reactions. This will highlight the importance of biotransformations in modern synthetic organic chemistry.

### 1. The preparation of e.p.c.s

The preparation of enantiomerically-pure compounds (e.p.c.s) is the most widely recognised and synthetically useful application of enzymes in organic synthesis. Due to their inherent and therefore unique substrate specificity, many organic and now organometallic compounds have been elegantly resolved. One widely used class of enzymes for the straightforward resolution of optical isomers has been the hydrolases, where the enantioselective hydrolysis of ester substrates in aqueous or organic media leads to one acidic antipode in optically pure form. The following separation of such compounds is then trivial. Extending this to the use of *meso* substrates<sup>3,4</sup> leads to optically pure compounds in (ideally) 100%



yield. (example 1, Fig. 1) This class of enzymes will be discussed in detail in Part 2 of this introduction.

The stereoselective synthesis of new chiral centres from achiral substrates by yeasts is now well established<sup>5</sup>, (example 2, Fig. 1) with the products in this example readily brought to >95% e.e. by recrystallisation.

The use of immobilised enzymes in organic solvents has more recently led to the resolution of compounds less stable or insoluble in aqueous media<sup>6</sup>. (example 3, Fig. 1.) By using alcohols in the place of water as nucleophiles, a variety of optically active products can be synthesised.

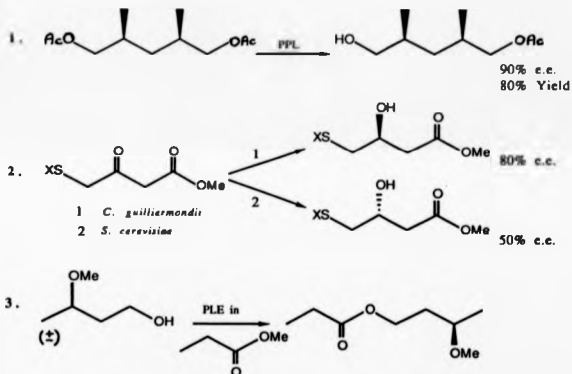


Figure 1.

## 2. The carrying out of remote functionalisations

Enzymes have been used to carry out remote functionalisations of specific target sites in many molecules. This would be difficult if not impossible to do chemically in some situations.

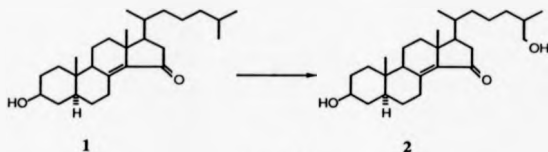


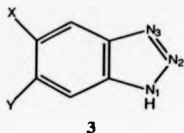
Figure 2

In the above example<sup>7</sup>, (Fig. 2) 3β-hydroxy-5α-cholest-8(14)-en-15-one **1** was specifically hydroxylated at the ~~26β~~ position with an enzyme from rat liver mitochondria to give 5α-cholest-8(14)-ene-3β, 26-diol-15-one **2** in a 4:1 mixture of 25R and 25S isomers. (60% e.e.)

## 3. For regioselective reactions

The acylation of sugars in aqueous media is thermodynamically unfavourable. Furthermore, regiospecific control involves the use of extensive protection and deprotection. α-D-glucose was regiospecifically butyrate solely at the 6-O position with PPL in pyridine<sup>8</sup>, and 1,6-anhydro-2,3,4-tri-O-acetyl-β-D-glucopyranose was regiospecifically deacylated<sup>9</sup> at C-3 with chymotrypsin and wheatgerm lipase; and at C-4 with liver esterase and pancreatic lipase.

Another example of the regiospecificity of enzymes is shown in the specific N-1-glycosidation of benzotriazoles **3** catalysed by NADase<sup>10</sup>. The glycosidation would have been expected to occur at the N-2 position due to electronic effects and effects of the substituents  $\lambda$  - as it does for indazoles, yet it glycosylates solely at the N-1 position under the above biocatalytic conditions.



#### 4. The carrying out of reactions under mild conditions

A further advantage of the use of biotransformations in organic synthesis is that they operate under very mild conditions. All reactions carried out in aqueous media are buffered, usually in the pH range 6-8, at temperatures generally not exceeding 45°C. The majority of synthetically used enzymes are non-toxic, and can be filtered off and re-used several times. Therefore waste is avoided, and in the present climate of chemical research, any move away from the use of toxic chemicals and harsh conditions (extremes of pH, high temperatures) is welcomed.

A few examples of the synthetic use of biotransformations

- (a) In peptide synthesis
- (b) In carbohydrate synthesis
- (c) In carbon-carbon bond formations

(a) In peptide synthesis

A recent and exciting new field of biotransformations is in the application of enzymes to peptide synthesis. This has several advantages over the well established chemical methods, in that it takes place under mild reaction conditions with no need for protection and activation, in the absence of racemisation and with all the regio- and stereoselectivities inherent in the nature of the biocatalyst. Extending this to enzymatic peptide synthesis in organic solvent opened up more interesting possibilities. It was found that the stereoselectivity of subtilisin relaxed sufficiently to allow the preparation of numerous peptides containing D-amino acids, which is impossible in water as proteases accept only L-amino acids<sup>11</sup>. Lipases were subsequently found to be wider ranging in their substrate specificity and utility in organic solvent, with the added advantage that lipases do not catalyse the secondary hydrolysis of peptides. The first example of this type<sup>12</sup> was concerned with the synthesis of N-acetyl-L-phenylalanyl-L-leucinamide from N-acetyl-L-phenylalanine-2-chloroethylester and L-leucinamide catalysed by PPL in dry toluene. This line of work has many exciting possibilities, with  $\alpha$ -chymotrypsin catalysed peptide synthesis in dichloromethane being

demonstrated competently and with great potential in our laboratories at the present time<sup>13</sup>.

#### (b) In carbohydrate synthesis

As mentioned briefly above, carbohydrate synthesis is another useful target for biotransformations. Due to the many complicated protection and deprotection stages during the chemical synthesis of oligo- and polysaccharides, very few of these compounds can be made easily, or have indeed been made.

$\beta$ -Galactosidase will selectively cleave the  $\beta$ -1,4 linkage between the galactose and glucose moieties of lactose, and will catalyse the transfer reaction of a nucleophile to this position. If another sugar molecule is present, a stereo- and regioselective transglycosylation reaction will be catalysed and a new disaccharide readily formed<sup>14,15</sup>. This process has great potential for the straightforward synthesis of oligo- and polysaccharides and is also being investigated in our laboratories at present.<sup>16</sup>

#### (c) In C-C bond formations

Another class of enzyme with great synthetic potential are the aldolases, which, as the name suggests, catalyse aldol-type C-C bond forming reactions<sup>17</sup>. (Fig. 3)

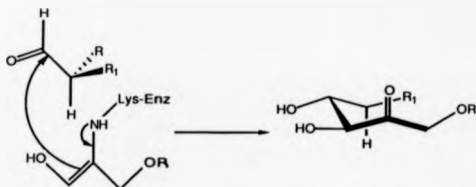


Figure 3

The most common enzyme used in synthetic applications is rabbit muscle aldolase, and given the current interest in synthetic organic chemistry of directed aldol reactions and the synthesis of carbohydrates, this enzyme is synthetically very important.

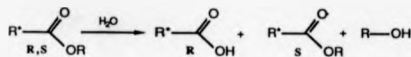
## **Part 2 The hydrolytic enzymes - Esterases and lipases**

Hydrolytic enzymes, or more specifically the hydrolases, are a large group of enzymes which act upon a vast range of substrates, catalysing the cleavage of a specific bond via hydrolysis. This group of enzymes contains esterases, thioesterases, phosphatases, glycosidases, peptidases and pyrophosphatases, with the bonds hydrolysed being ester links, thioester links, glycosidic bonds, peptides, acidic anhydrides and pyrophosphates<sup>18</sup>.

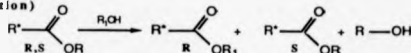
Of specific interest to the work presented in this thesis are the esterases. These are a group of enzymes (which include lipases) with a broad substrate specificity:- very stereoselective as to which particular bond is hydrolysed yet acting on a great range of

ester substrates. Their reactions catalysing the preparation of e.p.c.s are shown below.

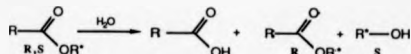
1. Ester hydrolysis



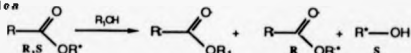
2. Ester interchange  
(transesterification)



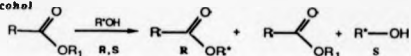
3. Acetate hydrolysis



4. Acetate  
transesterification



5. Transesterification  
with a chiral alcohol



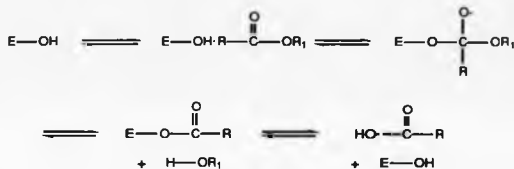
R\* = Asymmetric alkyl group

Figure 4

The enantiospecificity of the enzyme depends to a certain extent on its proximity to the asymmetric centre. The closer the hydrolytic bond to be cleaved is to this centre, the greater the chance of total enantiospecificity upon hydrolysis. If the bond to be cleaved is far away from the asymmetric centre, then often both enantiomers are attacked by the enzyme, albeit at different rates.

In order to use biocatalysts to their full potential in organic synthesis, *i.e.* to be able to predict substrate compatibility and the

enantiospecificity of a reaction, the electronic and physical configuration of the active site of an enzyme must be defined. Of the serine proteases,  $\alpha$ -chymotrypsin has been subjected to most attention insofar as the above ideals are concerned. The sequence of amino acids around the active site of  $\alpha$ -chymotrypsin has been elucidated, and *via* this chemical knowledge and subsequent X-Ray analysis, the physical shape and electronic structure of  $\alpha$ -chymotrypsin, and in particular of its active site, has been defined. From this information, and by an understanding of the kinetics of enzyme-catalysed hydrolysis reactions, a general mechanism has been proposed for the hydrolysis brought about by the serine proteases<sup>19</sup>. (Scheme 1)<sup>18</sup>



Scheme 1

The enzyme approaches the substrate, and binds to it, forming an *enzyme-substrate* complex. This is then attacked by the hydroxyl group of Ser-195, forming a high energy *tetrahedral intermediate*. This intermediate then collapses to form the *acylenzyme*, with release of the side product. Subsequent hydrolysis of the *acylenzyme* forms the *enzyme-product* complex, which dissociates to give the free enzyme and (generally) desired product of hydrolysis.



In the case of ester hydrolysis, the *acylenzyme* is rapidly formed. The rate determining step of the reaction is the subsequent hydrolysis and dissociation of the product acid.

Studies with inhibitors, in particular di-isopropyl fluorophosphate (DFP), a potent esterase inhibitor, have made specific recognition of the amino acids around the active site of the enzyme possible. DFP reacts with a single, unique serine residue, and through the elucidation of the sequence of amino acids around the enzyme active site, it has been shown that this is highly conserved in a number of mammalian esterases. (It is therefore thought that this class of enzymes come from a common ancestral esterase, and have diverged in their evolution to be specific for different substrates.)<sup>19</sup>

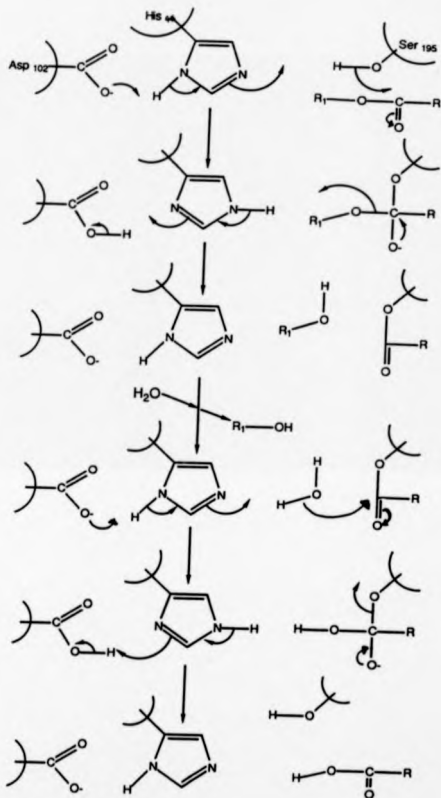
Because DFP reacts with only one specific Ser residue, it is thought that this amino acid has been activated in some way. Three-dimensional studies of  $\alpha$ -chymotrypsin show that there is a histidine residue (His-46) close to Ser-195, which is responsible for activating the serine group.

Serine is a poor nucleophile in its protonated form. Thus, it is thought that His-46 could function as a base, withdrawing a proton from the Ser, leaving it in its more reactive alkoxide form. X-Ray studies show an aspartate residue (Asp-102) situated close to the other imidazole nitrogen of His-46. This nitrogen atom is in a non-polar environment, and will tend to be protonated at normal pH. It was suggested<sup>20</sup> that this aspartate residue will act as a general base, removing the nearest proton from His-46, which in turn deprotonates and consequently polarises Ser-195.

This combination of three residues, Asp-102, His-46 and Ser-195, can be regarded as transferers of a negative charge from the non-

polar interior of the enzyme. This charge transfer resulting in the polar, alkoxide form of Ser-195 at the exterior of the protein, has been termed the *charge-relay* system.

A schematic representation of the above mechanism is shown below. (Scheme 2)"



Scheme 2

Simple esterases and lipases are mainly concerned with the hydrolysis of uncharged species. The main factors concerning their substrate tolerance are the lengths and shapes of the hydrophobic groups on either side of the ester link. Carboxyl esterases hydrolyse both aromatic and aliphatic esters, and speculation towards the shape of the active site has been made by looking at substrate specificities.<sup>21,23</sup>

Pig liver esterase is an important tool in biotransformations. It is broad ranging in its substrate specificity, highly enantiospecific and commercially available. The precise structure of the enzyme has never been elucidated, mainly owing to the difficulties encountered in obtaining a homogenous preparation. PLE is known to contain several iso-enzymes. However, models of its active site have been hypothesised<sup>23</sup>, and it appears to be generally agreed on that the active site (or sites) of the enzyme consist of one or two hydrophobic pockets with specific geometries, giving steric limitations to the configurations of the substrates available to it. The length of the alkyl chain of an ester plays an important part in the selectivity of PLE, as does the bulk of the acyl moiety. For cyclic diesters, PLE shows remarkable stereospecificity for the number of carbon atoms involved in the ring<sup>21</sup>. For cyclopropane and cyclobutane-1,2-diesters, PLE shows enantiospecificity for the S-centre groups with >97% e.e. For cyclohexane, the R-centre is enantiospecifically hydrolysed with >97% e.e. The switch-over point at cyclopentane becomes evident with hydrolysis occurring with only 17% e.e.

Lipases<sup>22,24</sup> catalyse the hydrolysis of long chain fatty acid esters. In nature they are concerned with the biological turnover of lipids. All lipolytic enzymes are ester hydrolases, and accept as substrates aliphatic esters with twelve or more carbon atoms, an "average" substrate being esters of oleic acid. The hypothetical distinction between a lipase and an esterase is the involvement of a lipid-water interface in the catalytic process of a lipase. The total sequence of amino acids in Pig Pancreatic Lipase is known. There is a single chain of 449 amino acids, with six disulphide bridges and two free thiol groups. The sequence of amino acids around the active site of PPL has been determined to be<sup>22,24</sup>

#### Leu-Ser(P)-Gly-His

and the same mechanism for hydrolysis as was discussed previously is thought to occur. The rate limiting step again being the hydrolysis of the *acylenzyme* intermediate.

The involvement of a lipid-water interface upon the activity of a lipase has deemed to be of importance. It has been postulated that, at the presence of an interface, a lipase acquires a different conformation to that which occurs in solution, which is more efficient. It is thought that the adsorption step of the lipase to the organic phase is controlled by a serine residue, essential for the recognition of the interface, and that there is a carboxyl group present of major importance for the stabilisation of the best active conformation of the lipase. Lipases are therefore ideal enzymes to act in organic solvents.

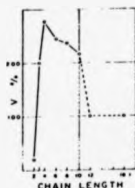


Figure 5

The influence of ester chain length upon the substrate specificity of a lipase is shown in the above figure. (Fig. 5) Triacetin is not a good substrate for PPL whilst tributyrin and tripropionin are rapidly hydrolysed. Tributyrin is taken as the ideal substrate for PPL. [The primary esters of a triglyceride are the only esters to be attacked by PPL, and the lipase is non-specific as to which (one or three) is hydrolysed. Glycerol is only very slowly produced, and only when both primary esters have been totally hydrolysed.]

Lipases in general will not tolerate much change in the fatty acid side of the ester link - usually this is pretty rigid in triglyceride substrates. Branching in the fatty acid portion of the molecule is found to be much more inhibitory than equidistant branching in the alcohol.

This is illustrated in Figure 6, as the ester on the left handside of the figure is a good lipase substrate, whereas the ester on the right hand side is not.

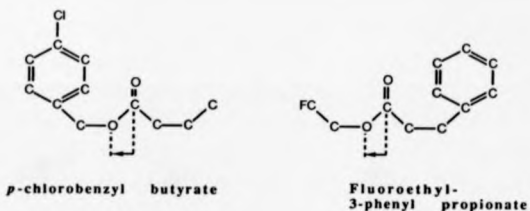


Figure 6. Directional fixation of substrates to pancreatic lipase

This two dimensional fixation of an ester in the active site of a lipase may result from the necessity to place the ether O of the ester within reach of the His proton. Therefore, fixation of the substrate in the direction of the arrow is obligatory for hydrolysis to occur.

Lipases can have widely diversified enzymatic properties and substrate specificities, depending upon their source of growth. (*i.e.*, fungal, mammalian, microbial).

The combination of esterases and lipases to catalyse reactions in aqueous and organic media in the preparation of e.p.c.s is an ideal one, and will be thoroughly explored and utilised during the course of this thesis.

Part 3 The application of optically active  
irontricarbonyl complexes and ferrocene  
derivatives to organic synthesis

"1,3-Diene irontricarbonyl complexes have many synthetic applications for the organic chemist. The irontricarbonyl group  $[\text{Fe}(\text{CO})_3]$  can act as a protecting group; it alters reactivities both to electrophilic and, indirectly through derived cations, to nucleophilic processes; it stabilises otherwise unstable structures, such as cyclohexadienones, and makes them available as the basis of reagents. Above all, it distinguishes between one side of a molecule and the other, often permitting totally stereospecific reactions, including the transfer of asymmetry to new chiral centres by the use of chiral complexes of nonchiral dienes"<sup>25</sup>

The above statement relies upon a wide range of 1,3-diene  $\text{Fe}(\text{CO})_3$  complexes being easily accessible in optically pure form. The few published methods of resolution are mainly tedious, involving several steps of synthesis followed by recrystallisations.

It was therefore decided to develop a system whereby such complexes could be fully resolved enzymatically, *i.e.* in one step, under mild conditions. This would be new and exciting for two reasons:

1. An enzyme has not yet resolved a compound with a plane of symmetry. (A "chiral plane", as distinct from a "chiral centre".)
2. An enzymatic resolution of an organometallic compound has not yet been achieved.



The high toxicity of irontricarbonyl complexes adds a further question to enzyme-substrate feasibility.

Ferrocenes were also chosen as target molecules for enzymatic resolution as they are thoroughly understood, and many resolutions of racemic derivatives have been published. Therefore, all attention could be placed upon the novelty of enzyme acceptance of an organometallic compound.

In this section, the properties, synthesis, optical resolutions and applications of irontricarbonyl-diene and ferrocene complexes in organic synthesis are discussed.

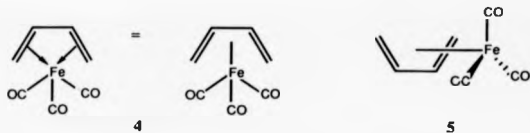
### Irontricarbonyl Complexes

Complexation by transition metals confers on an organic compound properties which differ markedly from those expected for the functional groups of the free ligand. Stabilisation of cationic species in such cases activates the ligand towards nucleophilic attack, and stereocontrol enforced by the  $\text{Fe}(\text{CO})_3$  fragment provides easy access to asymmetric synthesis. The position and nature of substituents on the ligand is also of importance to ensure regioselective control.

Consequently, optically active transition metal complexes are uniquely suitable to act as intermediates in asymmetric organic synthesis.

Irontricarbonyl complexes with dienes most commonly exist where all four carbon atoms are co-ordinated to the  $\text{Fe}(\text{CO})_3$  unit<sup>26</sup>. The first compound of this type, butadiene irontricarbonyl

4 was prepared in 1930<sup>27</sup>, but its structure was not elucidated until the work was taken up again 20 years later.



In 1942, compounds of this type (butadiene, isoprene, and 2,3-dimethylbutadiene irontricarbonyl ) were patented<sup>28</sup> as antiknock agents for motor fuels.

Most types of non-conjugated dienes will undergo rearrangement to the corresponding conjugated diene on reaction with ironcarbonyl reagents. The stability of the diene-irontricarbonyl unit is sufficiently great to let a variety of chemical reactions be performed on such complexes without rupture of the diene-irontricarbonyl bond. However, these complexes are generally air sensitive and will gradually decompose in contact with atmospheric oxygen. They are also particularly reactive towards electrophilic reagents.

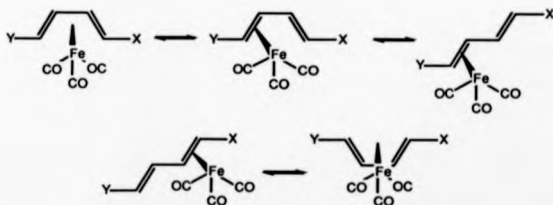
The original work was repeated,<sup>29</sup> and on the basis of these studies which included the relative thermal and chemical stability of butadiene irontricarbonyl 4 the now familiar *tetra-hapto* structure 4 was postulated. Soon after, <sup>1</sup>H nmr and uv spectroscopic studies revealed that the butadiene ligand remains intact when it is complexed to the irontricarbonyl unit. Later, the structure was solved by X-Ray crystallography<sup>30</sup> showing that the co-ordination of the iron atom in butadiene irontricarbonyl is

square pyramidal, with a carbonyl group in the apical position as is shown in structure 5.

Since this work, numerous 1,3-dienes have shown their ability to react with various ironcarbonyl compounds to form diene- $\text{Fe}(\text{CO})_n$  derivatives. The most widely used method to synthesise these compounds is to reflux the diene with  $\text{Fe}(\text{CO})_5$  or  $\text{Fe}_2(\text{CO})_9$  in high boiling solvents such as di-n-butylether, to give the complex in ~50% yield<sup>31</sup>. However, both reactants and products can be thermally unstable so other methods of synthesis have been determined. The photochemical induced reaction and the recent application of ultrasound<sup>32</sup> have been two milder and equally successful methods of complexing a 1,3-diene to an irontricarbonyl unit.

An asymmetrically substituted diene upon complexation with an  $\text{Fe}(\text{CO})_3$  group lacks a plane of symmetry and is therefore chiral. The resolution of such complexes is discussed in detail later.

Ease of racemisation varies from complex to complex, yet the majority of air stable diene- $\text{Fe}(\text{CO})_3$  compounds will not racemise at room temperature in neutral conditions. A mechanism for racemisation is shown below<sup>33</sup>. (Scheme 3)

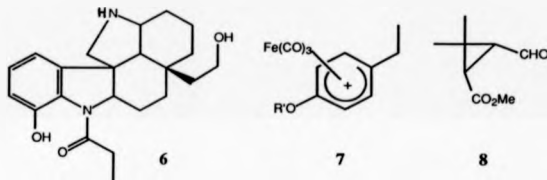


Scheme 3

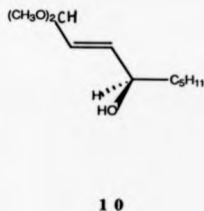
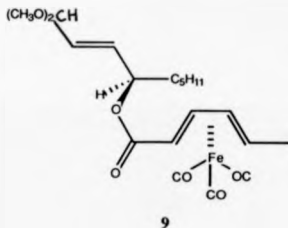
The requirement for this mechanism to occur is for the irontricarbonyl group to become detached at one of the double bonds. Racemisation of cyclic- $\eta$ -diene complexes is much slower, as the ligand must become completely decomplexed for racemisation to occur.

The use of chiral cyclic and acyclic  $(\eta^4\text{-diene})\text{Fe}(\text{CO})_3$  and  $[(\eta^5\text{-dienyl})\text{Fe}(\text{CO})_3]^+$  complexes in asymmetric synthesis has been well documented<sup>25,34</sup>.

Aspidosperma alkaloid 6, a natural product, has been synthesised using the optically active irontricarbonyl cation 7 to control the regio and stereospecificity of the preliminary reactions<sup>35</sup>.



Optically active sorbic aldehyde irontricarbonyl 16 was used in a stereo and enantioselective synthesis<sup>36</sup> of cis and trans hemicoronic aldehyde 8, and in a very recent publication<sup>37</sup>, optically active sorbic acid irontricarbonyl 13 was used to direct the asymmetric synthesis of 10 below.



The ironcarbonyl complex [in its (-) form] was used to form stereospecifically the diastereoisomers **9** which were separated by chromatography followed by reduction to synthesise **10**. Compound **10** is a key intermediate in the preparation of most of the lipoxygenation products at the n-6 position of polyunsaturated fatty acids.

The application of diene-Fe(CO)<sub>3</sub> compounds in enantioselective synthesis depends on the availability of a range of fully resolved complexes of known absolute configuration. Most reported methods of resolution rely on either the addition of chiral nucleophiles to cyclic dienyl systems, or on the classical resolution of complexes containing suitable functional groups. These methods are in general tedious, and are discussed in more detail below.

### Published resolutions of diene-Fe(CO)<sub>3</sub> complexes

Previous resolutions of racemic mixtures of irontricarbonyl complexes have been carried out using three major methods. These are:






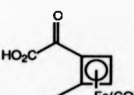
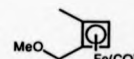
1. The synthesis and separation of diastereoisomers or diastereomeric salts. (classical resolutions.)
2. The direct separation of enantiomers via HPLC on a chiral support.
3. The asymmetric complexation of a diene from a less stable Fe(CO)<sub>3</sub> complex of an  $\alpha,\beta$ -unsaturated ketone.

These methods will be discussed in turn.

#### 1. The synthesis of diastereoisomers and their subsequent separation

Diastereomers can be formed in two ways. These are either (a) By the nucleophilic substitution of a substituent on the diene with a chiral nucleophile or (b) by the substitution of one CO ligand bonded to the iron atom with a chiral nucleophile, both methods forming separable diastereoisomers.

(a) The straightforward separations of diastereoisomers are listed in Table 1. The resolution of diene-Fe(CO)<sub>3</sub> complexes were most commonly brought about via the formation of diastereomeric salts using optically pure brucine or  $\alpha$ -methylbenzylamine. Of the cyclobutadiene complexes, optically active 19 was prepared from its camphor salts, and after two recrystallisations gave an e.e. of 30-40%. This was then modified to give optically active 20.

Compound	Resolving agent	Method of diastereomer separation	Absolute rotation	Lit. ref.
1 1 Fumaric acid $\text{Fe}(\text{CO})_4$	Brucine	Fractional crystallisation <i>EtOH/Acetone</i>	$[\alpha]_D = -593^\circ$ $+587^\circ$	3 8
1 2 Acrylic acid $\text{Fe}(\text{CO})_4$	Brucine	Fractional crystallisation <i>Benzene/hexane</i>	$[\alpha]_D = +372^\circ$	3 9
1 3 	(S)- $\alpha$ -methyl- benzylamine	Fractional crystallisation <i>Acetone</i>		4 0 4 1
1 4 	(S)- $\alpha$ -methyl- benzylamine	Chromatography	-	4 2
1 5 	(S)- $\alpha$ -methyl- benzylamine	-	-	4 2
1 6 	D camphor sulphonate	Fractional crystallisation <i>Acetone/CH2Cl2</i>	$[\alpha]_{278} = -66^\circ \pm n$	
1 7 	Ephedrine	Fractional crystallisation <i>Et2O</i>	$[\alpha]_D = +62^\circ$	4 3
1 8 	Quinine	-	-	4 4 4 5
1 9 	Camphor	Fractional crystallisation <i>EtOH/Et2O</i>	-	3 3

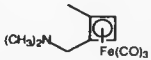
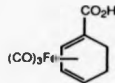
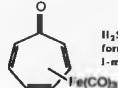
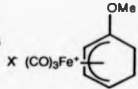
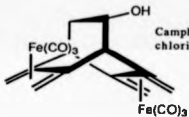
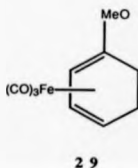
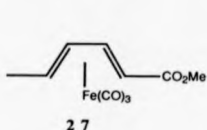
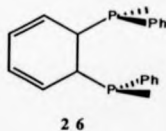
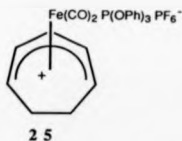
2 0		From 19			3 3
2 1		(S)- $\alpha$ -methylbenzylamine	Fractional crystallisation CHCl <sub>3</sub> /Acetone	$[\alpha]_D = +136^\circ$	4 6
2 2		H <sub>2</sub> SO <sub>4</sub> (cation formation) l-menthol	Medium pressure chromatography		4 7
2 3		l-menthol	Medium pressure chromatography	X = BF <sub>4</sub> $[\alpha]_D = -132^\circ, +136^\circ$ X = PF <sub>6</sub> $[\alpha]_D = +116^\circ$	48, 49 50, 51 52, 53
2 4		Camphanoyl chloride	HPLC	$[\alpha]_D = +25^\circ$	5 4, 5 5

Table 1







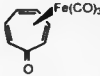

The cyclohexadiene 21 upon resolution<sup>46</sup> gave a total yield of 34%. This optically active acid was later used in the asymmetric synthesis of the natural product gabaculine<sup>56</sup>.

Synthetically useful are the cations 23, synthetic organic equivalents of cyclohex-2-enone cations. They have been resolved by several different methods, (a) classically<sup>48</sup> and (b) more recently with chiral nucleophiles<sup>50</sup>. phosphines, alkoxides and amines. The achiral salts were reacted with, for example,  $(+)\text{P}(\text{Ph})_2(\text{C}_{10}\text{H}_{19})$  (where  $\text{C}_{10}\text{H}_{19}$ =neomenthyl) and the diastereoisomers were separated by usual methods.

More recently, a system was described<sup>57</sup> where a chiral sulphoximine ester enolate reacted with the cation 25, forming diastereoisomers in unequal ratios. Following the manipulation of this chiral centre to form a non-chiral substituent, e.e.s as high as 50% were obtained. The e.e. was greatly dependent on the counter-cation used in generating the enolate anion, probably due to its strength of chelation, with strong chelation giving a low e.e. The cation 23, and its reaction with several chiral nucleophiles has been carefully studied. It was shown that diastereomer formation with R-(+)- $\alpha$ -methylbenzylamine in a 1:1 molar ratio gives rise to optical activity in the recovered salt<sup>51</sup>. No e.e.s were given as the diastereomeric protons were indistinguishable by  $^1\text{H}$  nmr spectroscopy. A second publication<sup>52</sup> using the same cation and this time using the chiral nucleophile 26 in a 1:0.5 molar ratio gave a 64:36 mixture of diastereomers, and the salt was recovered in 6% e.e. A third publication<sup>53</sup> with the same cation but this time using a chiral nucleophile with an alkyl rather than a P centre, again gave the unresolved (by  $^1\text{H}$ nmr spectroscopy) diastereoisomers, and with the recovered salt in 11% e.e. However, it is thought that the selectivity of the nucleophile on forming the diastereoisomers is significantly higher than this.

(b) Earlier this year, an elegant method for the resolution of methyl sorbate 27, sorbic aldehyde 16 and 1-methoxy cyclohexadiene 29 irontricarbonyl was published<sup>59</sup>. One CO ligand was exchanged for (+)-neomenthyl  $\text{P(Ph)}_2$  and the resulting diastereomers were separated by preparative TLC. In a previous publication<sup>60</sup>, the tropone complex 22 and 2-methylbutadiene  $\text{Fe(CO)}_3$  28 were resolved via the same method; separation of the resulting diastereomers was achieved by fractional crystallisation.

## 2. The direct separation of enantiomers by HPLC

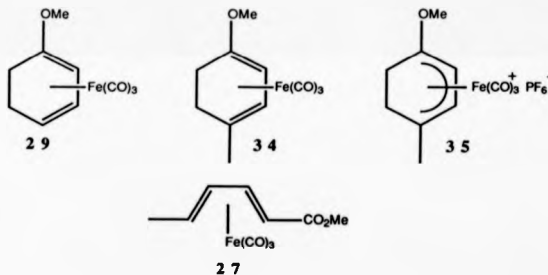
Compound	HPLC Column used	Absolute rotation	l.l. Ref.
3 1 	DIACEL CHIRALPAK OC <i>hexane/isopropanol</i> 9:1	$[\alpha]_D^{25} = +490^\circ$ $-486^\circ$	61
3 2 	DIACEL CHIRALPAK OB <i>hexane/isopropanol</i> 9:1	$[\alpha]_D^{25} = -526^\circ$	61
2 2 	CHIRALPAK OT <i>hexane/isopropanol</i> 0.97:0.3	—	62
3 3 	As 2 2	—	62

HPLC on chiral supports has more recently been used for the resolution of  $\text{Fe}(\text{CO})_3$  complexes. Diacel chiralpak OB, OC and OT were most widely used, with the eluant being various mixtures of hexane and isopropanol. No details of column packing nor solvent systems were given for the resolution of 30<sup>63</sup>. It was found that the pure fractions of 22 racemise slowly within a week when stored in the dark at room temperature.

### 3. Asymmetric complexation of dienes

This method of chiral induction exploits the phenomenon that  $\alpha,\beta$ -unsaturated ketones form less stable  $\text{Fe}(\text{CO})_3$  complexes than diene- $\text{Fe}(\text{CO})_3$  complexes. Therefore they preferentially transfer the  $\text{Fe}(\text{CO})_3$  group to a diene. Consequently if a suitably bulky and optically active  $\alpha,\beta$ -unsaturated ketone  $\text{Fe}(\text{CO})_3$  is treated with a diene, it will preferentially transfer the  $\text{Fe}(\text{CO})_3$  group to the diene, and, more specifically, to one face only of the diene, with the induction of optical activity.

The  $\alpha,\beta$ -unsaturated ketones first examined were (+)-pulegone and (-)-3 $\beta$ -acetyloxypregna-5,16-diene-20-one (16-DHA). The  $\text{Fe}(\text{CO})_3$  group was asymmetrically transferred to the complexes 29, 34 and 27 in less than 20% e.e.<sup>64</sup>.



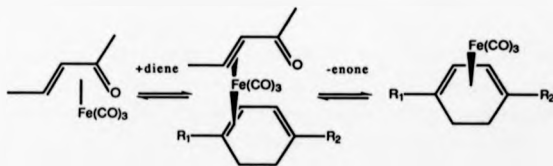
The absolute configurations of the asymmetrically synthesised complexes 29 and 34 were determined by the conversion to known terpenes via their respective cations<sup>65</sup>. Low e.e.s were gained using both (+)-pulegone and 16-DHA, though with 16-DHA

they were slightly higher, also giving the opposite absolute configuration to those synthesised with (+)-pulegone.

Modifying the system to work in benzene at 65°C and for 95 hours gave **34** in 40% e.e.<sup>66</sup>, and after hydride abstraction the salts **35** were recovered in the same optical yield.

This was further optimised,<sup>67</sup> and after obtaining **35** in 40% e.e. (as above) the salts were fractionally recrystallised three times from acetone and diethylether to give a maximum rotation of  $[\alpha]_D = -138^\circ$ . The optical purity of 100% was confirmed by  $^1\text{H}$  nmr spectroscopy in the presence of a chiral shift reagent  $[\text{Eu}(\text{fod})_3]$  and also by the transformation into a known natural product. This method of asymmetric induction was further investigated,<sup>68</sup> by varying the  $\alpha,\beta$ -unsaturated ketone  $\text{Fe}(\text{CO})_3$  donors with different diene substrates. 16-DHA was found to be the best  $\text{Fe}(\text{CO})_3$  donor for cyclic systems, (the best e.e. of 43% was found with **34**) and (-)-cholest-4-ene-3,6-dione most suitable for acyclic compounds. *i.e.* **27** was obtained in 19% e.e. The e.e.s varied with different substituents on the dienes, with the dienes themselves, and with the  $\text{Fe}(\text{CO})_3$  donor complexes.

A probable mechanism for the  $\text{Fe}(\text{CO})_3$  group transfer is shown below.

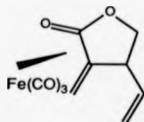
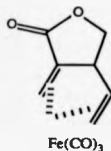
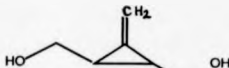


Scheme 4

The absolute configuration of the product is determined during the initial approach of the diene, and whilst the metal remains bound to this face of the diene, racemisation cannot occur.

### 3. Other methods of resolution

In a publication discussing two mechanisms for the transition-metal mediated olefin isomerism<sup>69</sup>, briefly mentioned was the preparation of complexes **36** and **37** in optically active form.

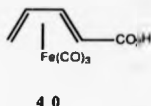
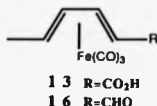
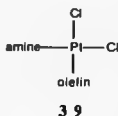
**36** $[\alpha]_D^{20} = +20$ **37** $[\alpha]_D^{20} = +150$ **38**

This was effected by the reaction of  $\text{Fe}_2(\text{CO})_9$  with partially resolved (-)-*trans*-2(R), 3(R)-bis(hydroxymethyl)methylene cyclopropane **38** in diethylether. No more details other than rotations were given.

Optical activity induced by the asymmetric destruction of one enantiomer of the tropone complex **22** by irradiation with circularly polarized light has been observed<sup>70</sup>. This led to an optical yield of 0.5% and an estimated absolute rotation of  $700^\circ$ .

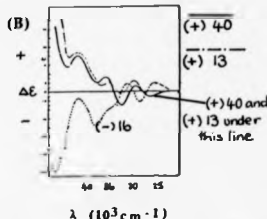
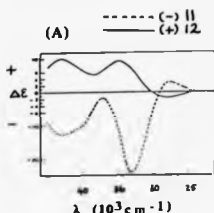
### Circular dichroism and its relationship to absolute configuration

A relationship between the cd and absolute configuration of an optically active transition metal  $\pi$ -complex was first noticed with respect to platinum-olefin complexes<sup>71</sup> 39.



It was observed that the olefin complex was symmetric. Therefore upon complexation with optically pure  $\alpha$ -methylbenzylamine diastereoisomers were not formed. The complex gave little or no cd in the region of the lowest energy  $d \rightarrow d$  electronic transition of Pt(II) at  $27,000\text{cm}^{-1}$ . However, the (+) and (-) diastereoisomers of asymmetric complexes gave respectively a positive and negative cd in the above mentioned region. The magnitude of this dichroism,  $(E_l - E_r)_{\text{max}}$ , was nearly the same for each diastereoisomer. It was subsequently found that the cd of the lowest energy  $d \rightarrow d$  electronic excitation of transition metal complexes is particularly sensitive to the absolute stereochemistry of the complex. Also, the magnitude of the cd could be related to the optical purity of the complex. A change in the configuration of the amine nucleophile used for diastereomer formation did not disturb the sign of the Cotton effect for the lowest energy cd<sup>72</sup>. This was later applied<sup>40</sup> to optically active ironcarbonyl complexes of prochiral olefins, and by comparing the cd's of (+)-sorbic acid

irontricarbonyl 13, (+)-*trans*-1,3-pentadienoic acid irontricarbonyl 40, (-)-sorbic aldehyde irontricarbonyl 16, (+)-acrylic acid  $\text{Fe}(\text{CO})_4$  12, and (-)-fumaric acid  $\text{Fe}(\text{CO})_4$  11, several interesting facts were observed.

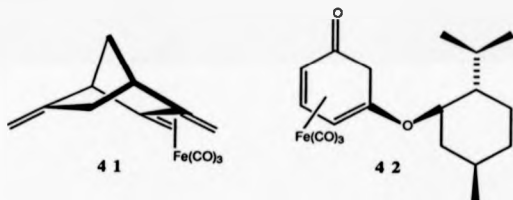


It can be seen by simple observation that the lowest energy  $d \rightarrow d$  transitions for acids 13 and 40 at 370-400nm lie on the negative side of the cd and are therefore of the same absolute configuration. The aldehyde 16 has the opposite absolute configuration, with its lowest energy  $d \rightarrow d$  transition lying in the positive region. Comparing the amplitudes of the lowest energy transitions of (-)-fumaric acid and (+)-acrylic acid (A) at ~360nm it can be seen that the amplitude of the former is about twice that of the latter. From this it can be concluded that each asymmetric carbon atom contributes by the same amount to the optical activity. Another interesting feature relating cd with absolute configuration<sup>73</sup> can be seen in the cd of (+)-sorbic acid. (B) Sorbic acid  $\text{Fe}(\text{CO})_3$  13 is of *trans, trans* geometry; its terminal carbon atoms have the opposite absolute configurations. They are both co-ordinated to the  $\text{Fe}(\text{CO})_3$  group. Consequently each half of the diene moiety generates optically active  $d \rightarrow d$  transitions showing as two absorbance maxima of opposite sign at 390 and 340nm on



the cd, reflecting the configurations of the  $\text{CH}=\text{CHOOH}$  and  $\text{CH}_3\text{CH}=\text{CH}$  groups. At the time of this publication, the assignment of the cd absorbance maxima had not been made. *Cis*, *trans* diene-ironcarbonyl complexes have the same absolute configuration at each terminal carbon atom. Thus the cd spectra shows only one maximum in this region<sup>42</sup>.

The cd of these complexes are solvent and temperature dependent, as the chiroptical properties of molecules are affected by solvation and aggregation effects. These effects are magnified if the complex contains alcoholic or similar H-bonding functionalities. The influence of temperature and solvent upon the *Cotton* effects of the ketone chromophore of **41** is much smaller than that found for the *Cotton* effects attributed to the tricarbonyl-diene chromophores.



The cd of complexes such as **41** were studied<sup>63,69</sup>, and they all showed temperature dependence. However, the corresponding uncomplexed compounds showed cd's which were temperature independent.

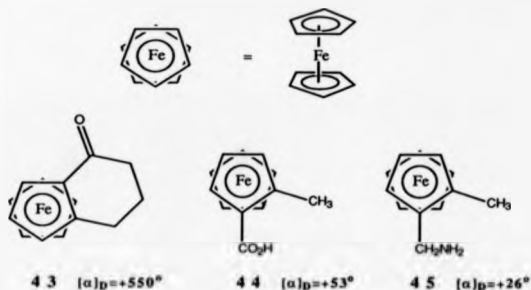
The *Cotton* effects observed above 270nm of similar complexes were significantly weaker than those observed for diene

irontricarbonyl complexes *i.e.* 13. The cd of diastereoisomers like 42 give curves that are almost identical except for the sign of the *Cotton* effect. This suggests that the absolute configuration of the metal-ligand  $\pi$ -bond is a dominant factor in the cd spectra of these compounds. Consequently the sign of the *Cotton* effect in specified regions can be used to <sup>predict</sup> confidently the absolute configuration of many transition metal  $\pi$ -complexes.

## Ferrocenes

Bis(cyclopentadienyl)iron was first discovered in 1951<sup>74</sup> and the name "ferrocene" was proposed one year later<sup>75</sup>. It was found to be electrically neutral, both on the rings and in the Fe atom itself, and of aromatic character, which reveals itself by ready electrophilic substitution. The rings of ferrocene lie staggered, with a C-C bond distance of 1.43 Å, an average Fe-C distance of 2.058 Å<sup>76</sup>, and an extremely small barrier to rotation *i.e.* 1.1 kcal/mole<sup>-1</sup>. However, in the vapour state at ~140° the rings are thought to be eclipsed. In substituted ferrocenes, the conformation of the rings is determined by the position and bulk of the various substituents. Additionally, ferrocene is relatively stable and non-toxic<sup>77</sup>.

Due to its unique sandwich-type structure, ferrocene is an extremely interesting model for stereochemical research, showing many examples of isomerism, including the classical geometric and positional isomers. Monosubstituted ferrocenes belong to the point group  $C_2$ <sup>78</sup>, but when disubstituted on one ring only <sup>with different substituents</sup> the symmetry becomes  $C_1$  and the molecule is chiral. (The term "chirality" was introduced into chemistry in 1964<sup>79</sup> and was applied to the metallocene field one year later)<sup>80</sup>. The first separation of a chiral ferrocene into its enantiomers was accomplished in 1959<sup>81</sup>, and the absolute configuration was determined *via Horeau's* method five years later<sup>82</sup>. This key compound, ferrocenocyclohexenone 43 was fractionally recrystallised from its menthydrazone salts to give the optically pure ketone after acid hydrolysis.



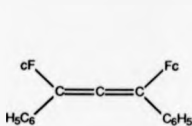
Since then, many hundreds of optically pure ferrocenes have been prepared by various methods, the most important of which are discussed below.

The most widely used method of resolution of a racemic ferrocene is classical, *i.e. via* the recrystallisation of diastereomeric derivatives, provided that suitable functional groups are available. For the carboxylic acids, *i.e.* 44, salts of  $\alpha$ -methylbenzylamine are separated; for amines 45 dibenzoyl tartrate salts are formed; and for racemic ketones and aldehydes 43, salts of the menthydrazones are most often used<sup>83</sup>. Using these methods, many ferrocenes have been totally resolved into their optical isomers.

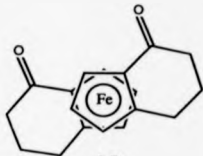
Countercurrent distribution, (with an eluant of cyclohexane/(+)-diethyltartrate)<sup>84</sup> and chromatography on partially acylated cellulose<sup>85</sup> have also been used to separate racemic mixtures of

43. Although optically active material was formed, the e.e.s were low. (maximum 10% e.e.)

A breakthrough was achieved with the use of medium pressure chromatography on microcrystalline triacetylcellulose (TAC) in ethanol at ambient temperatures<sup>86</sup>. Using this method, or extending it to a recycling mode several metallocenes (especially benzyl chromiumtricarbonyl complexes) have been fully resolved.



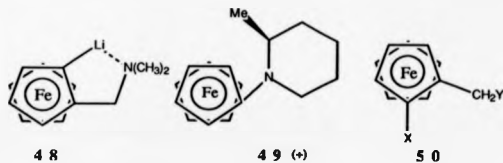
46



47

Diferrocenyl diphenylallene 46 was fully resolved after one passage through the column, whereas bis( $\alpha$ -ketotetramethylene)ferrocene 47 needed 16 cycles for full resolution.

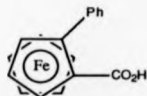
Asymmetric synthesis utilising directed lithiation has also been a successful method for the resolution of chiral ferrocenes. Lithiation<sup>87</sup> at the *ortho* position only of N,N-dimethylaminomethylferrocene 48 is attributed to the intramolecular co-ordination of the lone pair of electrons on the N, to the lithium atom forming a five membered chelate ring.



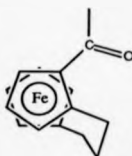
This was extended<sup>88</sup> by the synthesis of (+)-S-(N- $\alpha$ -pipecolylmethyl)ferrocene **49**. Directed lithiation, followed by three steps (electrophilic substitution with X<sup>+</sup>, methylation, and nucleophilic attack with Y<sup>-</sup>) gave the 1,2-disubstituted ferrocene **50** in 94% e.e.

Asymmetric (*ortho*) palladation has also been used, leading to optically active products with moderate e.e.s<sup>89</sup>. However, cyclo palladation of N,N-dimethylaminomethylferrocene in the presence of N-acetylvaline<sup>90</sup> or N-acetyl-L-leucine<sup>91</sup> gave rise to e.e.s as high as 79 and 84%.

The determination of the e.e. of optically active  $\alpha$ -methylferrocenecarboxylic acid **44** following resolution of its racemate has been determined via two independent methods. These methods were isotopic dilution, and <sup>1</sup>H nmr spectroscopy of the diastereomeric  $\alpha$ -methylbenzylamides<sup>92</sup>. Both methods gave identical results. As racemization of di-substituted ferrocenes is impossible without the breaking and formation of bonds, this key compound **44** has been directly or indirectly correlated to more than 150 optically active ferrocene derivatives, establishing their e.e.s.



51



52

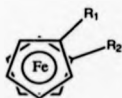
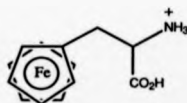
HPLC of the  $\alpha$ -naphthylethylamides has also been used to determine the e.e.s. of 44 and 51. Medium pressure chromatography on TAC provides a very simple and efficient method of determining the e.e. provided that full separation of enantiomers is achieved<sup>86</sup>.

The chiroptical properties of ferrocenes, mainly investigated for establishing configuration and conformation, have been extensively studied and are discussed in detail in several publications<sup>77,78</sup>. Interesting to note is that all metallocene cyclohexenones which have the absolute configuration of 1S exhibit +ve *Cotton* effects in the region of the metallocene chromophore, and give +ve rotations at 589nm. Their corresponding bridged-vinyl derivatives give the opposite signs. Obviously, temperature effects on the chiroptical properties of the "open" compounds are much greater than for the bridged derivatives. Similar behaviour was observed for chiral ferrocenophanes *i.e.* 52.

The absolute configuration of the dextrorotatory ketone 43 was determined *via* its reaction with racemic  $\alpha$ -phenyl butyric anhydride<sup>82</sup>. Subsequently, the absolute configurations of as many as 200 ferrocenes were deduced by comparison of their chiroptical properties with those of this key compound<sup>78</sup>. Another important

ferrocene derivative is the acid **44**, which, after kinetic resolution *via* the separation of its diastereomeric salts, gave results in accordance with those obtained *via* the ketone **43**. Since these earlier results, the introduction of the *Bijvoet* X-Ray technique confirmed unambiguously the absolute configurations previously determined, *via* comparisons with the above compounds. (**43,44**). The synthesis, complete resolution, and determination of the absolute configurations of many hundreds of ferrocenes have been well researched, understood and documented. Therefore, it was decided that a racemic ferrocene would be an ideal substrate for a demonstration of the versatility and selectivity of a hydrolytic enzyme. Full attention could be placed upon the enzymatic reaction.

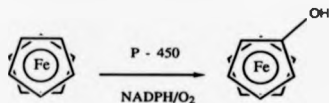
To explore the possibility of a ferrocene being an enzyme substrate, a thorough literature search revealed<sup>95</sup> a review outlining the use of metallocenes in biochemistry, microbiology and medicine. Concerning the cellular applications of metallocenes, various ferrocenes **53** with  $R_1$  and  $R_2$  as carbon acyl side chains, exhibited antimicrobial activity against a variety of bacteria, yeasts and fungi.

**53****54**



$\beta$ -Ferrocenylalanine 54 was found to be a non-competitive inhibitor with respect to L-phenylalanine, and a mixed inhibitor with respect to DMPH<sub>4</sub> in the phenylalanine hydroxylase system<sup>96</sup>. It was also found to be a competitive inhibitor of aromatic L-amino acid decarboxylase, again in comparison with phenylalanine.

Ferrocene has been oxidised to the ferrocenium cation  $(C_5H_5)_2Fe^+$  by horseradish peroxidase in the presence of hydrogen peroxide, and a second biotransformation involving the hydroxylation of ferrocene via cytochrome P-450 has been published<sup>97</sup>.



Ferrocene has thus been shown to be a suitable substrate for oxidase enzymes, yet no further enzyme work has been found. It could therefore be reasonable to expect and interesting to discover if ferrocene derivatives are suitable substrates for esterases or lipases and subsequently feasible for enzymatic resolution.

## Chapter 1.

### The enzymatic resolution of racemic irontricarbonyl complexes.

In the introduction was outlined the importance of optically active irontricarbonyl complexes and the present methods of their resolution. Most of the reported methods of resolution rely on either addition of chiral nucleophiles to cyclic dienyl systems<sup>50</sup>, or are classical resolutions of those compounds which contain suitable functional groups<sup>36</sup>.

None of these methods are simple, and they involve many synthetic steps.

The aim of this thesis is to demonstrate the versatility and broad substrate specificity of hydrolytic enzymes, in the simple and straightforward full resolution of an organometallic complex.

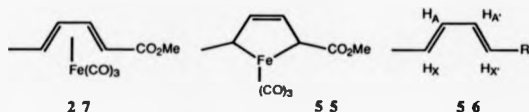
PLE was assayed according to the literature<sup>98</sup> against ethyl butyrate, and an activity of one enzyme unit to 71.35 $\mu$ l of PLE (lit. value 77 $\mu$ l) was recorded.

Lipase activity was checked using tributyrin as the substrate.

#### 1-1 Methyl sorbate irontricarbonyl.

An air-stable and easily accessible irontricarbonyl complex was desired to carry out initial reactions to test the feasibility of a biocatalyst to accept such a compound as a suitable substrate.

Thus the well known and fully characterised complex, methyl sorbate irontricarbonyl **27** was chosen.



Sorbic acid was esterified to its methyl ester by stirring with acetylchloride and methanol for several hours. The resulting ester was worked up via fractional distillation in a yield of 83%. Methyl sorbate was then complexed to Fe(CO)<sub>3</sub> as according to the literature<sup>99</sup>, by refluxing in di-n-butylether with Fe(CO)<sub>5</sub> followed by chromatographic work-up, to give methyl sorbate irontricarbonyl **27** in 33% yield.

It was of interest to compare the <sup>1</sup>H nmr spectra of methyl sorbate and its Fe(CO)<sub>3</sub> complexed derivative. (Fig. 7) The most prominent feature is the upfield shift of H-(C-2) and H-(C-5) from between 5 and 6ppm (usual for olefinic protons) to 0.9-1.5ppm. One explanation for the dramatic upfield shift of the *endo* protons could be that Fe(CO)<sub>3</sub> complexes with methyl sorbate in a 1,4-addition reaction thus saturating C-2 and C-5. Consequently, the chemical shifts of H-(C-2) and H-(C-5) would move to much higher regions of the spectrum. From this hypothesis, structure **55** would be the correct representation of the irontricarbonyl complex.

H-(C-3) and H-(C-4) also experience an upfield shift, but to a much lesser extent. In the light of the above argument, this could be due to the magnetic anisotropy of the neighbouring bonds.

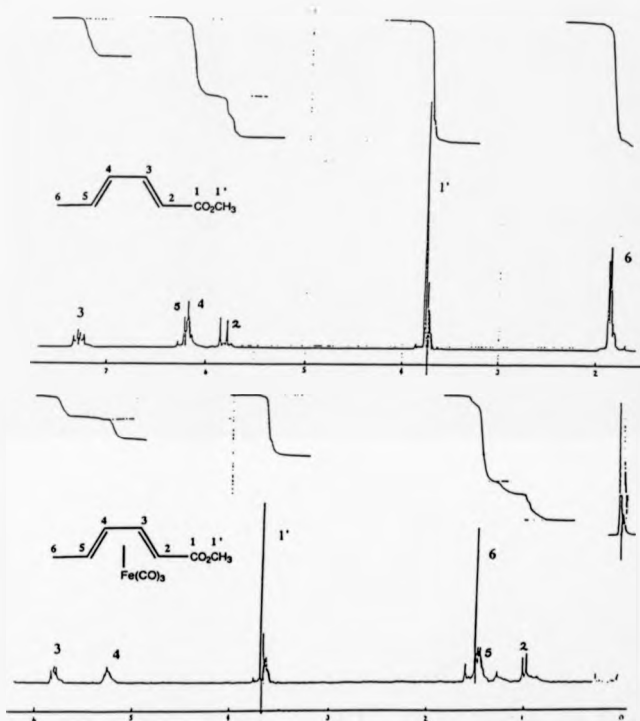


Figure 7

It was also interesting to note the H, H' coupling constants in the ironcarbonyl complex. For normal ethylene protons,  $J_{H,H'}(cis)=7-11\text{Hz}$  and  $J_{H,H'}(trans)=12-18\text{Hz}$ . In our case,  $J_{A,A'}=5\text{Hz}$  and  $J_{A,X}=J_{A',X'}=8.0\text{Hz}$ , which are values that are both too small for normal ethylenic bonds within complex **27**. This suggests a delocalisation of the double bonds between C-2 and C-5. However, it must be remembered that the vicinal coupling constants are influenced by adjacent functional groups, and the dihedral angle between  $H_A$  and  $H_X$  (or  $H_{A'}$  and  $H_{X'}$ ). This angle has been found to be  $-135^\circ$ <sup>100</sup>, thus the vicinal coupling constant can be expected not to be a maximum value.

The IR spectrum shows two very strong bands, one at  $2026\text{cm}^{-1}$  and the other at  $1998\text{cm}^{-1}$ . The latter has a shoulder at  $1980\text{cm}^{-1}$  which is also very strong, and it would be reasonable to assign these absorptions to the CO stretching frequencies of the  $\text{Fe}(\text{CO})_3$  moiety of the complex. Weak absorptions at  $1710\text{cm}^{-1}$  and  $1650\text{cm}^{-1}$  could be due to the ester carbonyl and olefinic C=C stretches respectively. The ester carbonyl of methyl sorbate also absorbs at  $1710\text{cm}^{-1}$ , and if structure **55** were correct, a change in stretching frequency due to loss of conjugation would have been expected.

X-Ray structure analysis shows no evidence of a bond between the  $\text{Fe}(\text{CO})_3$  group and the terminal carbon atoms of the ligand. From the above spectral evidence, it can be concluded that **55** is not the correct representation of the structure of an irontricarbonyl (olefin) complex. A far more likely suggestion is that of structure **57** below, with the large upfield shift of H-(C-2) and H-(C-5) explained by the electron-releasing effect of the  $\text{Fe}(\text{CO})_3$  group

shielding the protons in the vicinity of the  $\text{Fe}(\text{CO})_3$  fragment the most.



57

H-(C-2) and H-(C-5) actually point in towards the  $\text{Fe}(\text{CO})_3$ , and therefore experience the most dramatic upfield shift. Owing to the bulk of the  $\text{Fe}(\text{CO})_3$  group, each proton in the molecule is affected to some extent by its presence; thus the entire spectrum moves relatively upfield.

In this thesis, structure **27** will be the one used to represent all irontricarbonyl-diene complexes.

### 1-2 The enzyme-catalysed reactions of methyl sorbate and methyl sorbate irontricarbonyl.

On beginning the enzyme work, it was decided to test the substrate suitability of methyl sorbate before adding the further complication of the  $\text{Fe}(\text{CO})_3$  group.

100 $\mu$ l of PLE was added to a suspension of 0.1 mmoles of methyl sorbate in 10mls of buffer pH 8. This was stirred at room temperature and the reaction was followed by the continuous uptake of 0.01M NaOH from an autotitrator. The reaction was stopped after 26 minutes when the reaction had reached 100%, and was worked up via solvent extraction to give sorbic acid in 85% yield. Sorbic acid was characterised by  $^1\text{H}$  nmr spectroscopy.

showing an identical spectrum to that for methyl sorbate, with the 3H singlet missing at  $\delta=3.76$ .

Following the success of this reaction, similar conditions were employed for the attempted enzymatic hydrolysis of methyl sorbate irontricarbonyl 27. It was decided to use a co-solvent to help the highly hydrophobic complex dissolve in buffer, so two reactions, one pre-dissolving the complex in 50 $\mu$ l of methanol (1%), the other in 30% ethanol, were set up. Again the reactions were followed by NaOH uptake, but after 30 hours there was no visible reaction taking place so the reactions were stopped. Work up revealed 100% recovery of starting ester.

The failure of this reaction could be due to two possible reasons:

1. The insolubility of the irontricarbonyl complex, and therefore no access to the enzyme active site.
2. Non-substrate compatibility of the irontricarbonyl complex.

These two ideas were investigated.

### 1-3 Methyl sorbate irontricarbonyl as an enzyme substrate in organic solvent.

Work by Klibanov *et. al.* has pioneered the use of enzymes, particularly esterases and lipases, in organic media<sup>101</sup>. Thus a reaction of his was repeated to investigate the ease and reproducibility of such experimental techniques.<sup>6</sup>

Expt.	Ester	Alcohol	Time	Yield
1(a)	$\text{CH}_3\text{CH}_2\text{CO}_2\text{CH}_3$	$\text{CH}_3\text{CH}(\text{OCH}_3)(\text{CH}_2)_2\text{OH}$	5 hrs	42 %
2(b)	$\text{CH}_3\text{CH}_2\text{CO}_2\text{CH}_3$	$\text{CH}_3(\text{CH}_2)_6\text{OH}$	68 "	46 "
3(b)	$\text{CH}_3(\text{CH}=\text{CH})_2\text{CO}_2\text{CH}_3$	$\text{CH}_3(\text{CH}_2)_6\text{OH}$	85 "	6 "
4(b)	$\text{CH}_3(\text{CH}=\text{CH})_2\text{CO}_2\text{CH}_3$	$\text{CH}_3(\text{CH}_2)_6\text{OH}$	1 week	-

$\text{Fe}(\text{CO})_5$

(a) Ester as substrate/solvent

(b) Alcohol as substrate/solvent.

Table 2

PLE was immobilised on sepharose as according to the literature<sup>6</sup>, and the reactions were followed by GLC.

Methyl propionate and 3-methoxy-1-butanol (expt. 1. Table 2) were stirred together with PLE immobilised on sepharose. The reaction was followed by GLC, (Fig. 8) as the retention times between reactants and products were sufficiently large. After three hours, the ratios of the integrals of product to alcohol had reached 1:1. This ratio stayed constant over a further two hours. Optically active 3-methoxy-1-butyl propionate was extracted in 42% yield (out of a possible maximum of 50%) and had a rotation of  $[\alpha]_D = -19.2^\circ$ . This corresponded well with the literature result of  $[\alpha]_D = -16^\circ$ , although no e.e.s were given.

As the irontricarbonyl ester is the chiral substituent of the reaction to be investigated, the above work by Klivanov had to be modified insofar as an enzyme-acceptable achiral alcohol had to be found. Vigorously non-polar molecules are favoured by



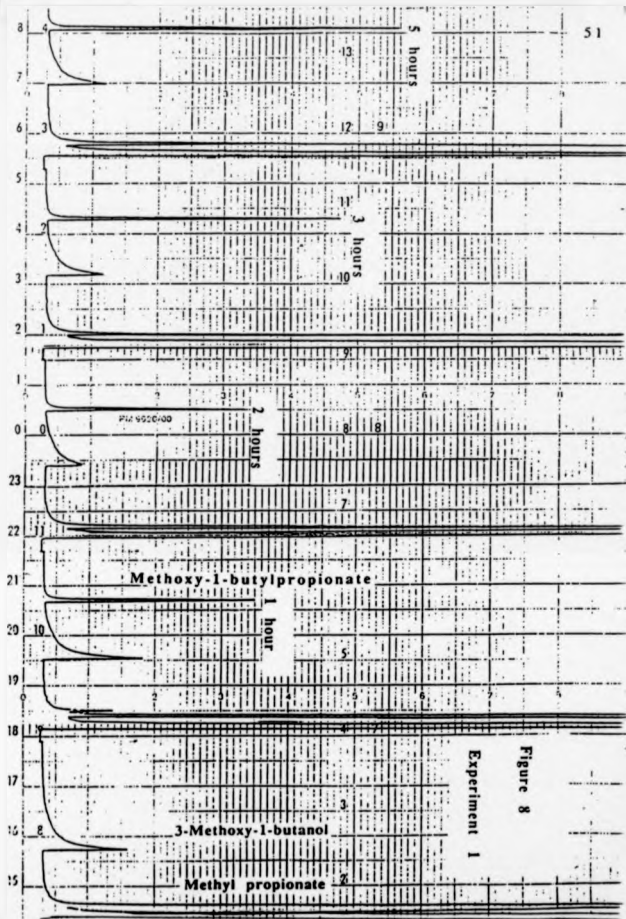


Figure 8

Experiment 1

enzymes as substrates, so that the essential aqueous monolayer surrounding the protein is not disturbed. Also, as esterases accept substrates with relatively short chain lengths, 1-heptanol was chosen as the substrate alcohol. 1-Heptanol had been used in a previous publication<sup>102</sup> as the alcohol moiety of a transesterification with tributyrin catalysed by PPL.

Thus experiment 2 (Table 2) was run, showing that although 1-heptanol is indeed an enzyme substrate, it is less ideal than the previously used alcohol. The reaction was again followed by GLC, and immediate product analysis by GC-MS confirmed the synthesis of heptyl propionate. (Table 3) Disappearance of the 3H singlet at  $\delta=3.70\text{ppm}$  ( $^1\text{H}$  nmr spectroscopy) and appearance of several multiplets at  $\delta=0.88$ , 1.31 and 1.59ppm also verified the correct product.

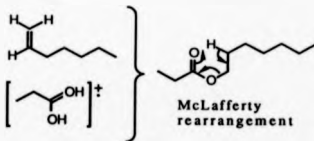
El $m/z$	Relative abundance (%)	Possible fragmentation
173	54.2	$[\text{MH}]^+$ Molecular ion
98	59	
75	70.7	
57	100	$[\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}]^+$ O-Acyl cleavage
115	16	
83	20.9	
41	39.3	
29	45.8	

Table 3

Methyl sorbate was the obvious choice of ester for the sequence of reactions to proceed, (expt 3, Table 2) thus the reaction was carried out in identical conditions to the two previous ones. This transesterification proceeded very slowly indeed, with only 6% product obtained *via* chromatographic work up after 85 hours of reaction. Heptyl sorbate was identified with  $^1\text{H}$  nmr spectroscopy, noting the disappearance of  $\text{OCH}_3$  at  $\delta=3.76\text{ppm}$  and the appearance of three multiplets at  $\delta=0.88$  (3H), 1.29 (8H) and 1.64 (2H)ppm; with the rest of the spectrum virtually identical to that of methyl sorbate.

The combination of non-ideal substrates (*i.e.* methyl sorbate and 1-heptanol) substantially slow down the rate of reaction under the above conditions.

To conclude the aim of this preliminary work, methyl sorbate irontricarbonyl **27** and 1-heptanol were stirred together with the immobilised enzyme. After one week, no reaction was observed by GLC.

It can therefore be concluded that option (1) - that the substrate is not getting to the active site of the enzyme due to its insolubility - cannot be true. Consequently, the remaining options remain open to investigation, which are that either the irontricarbonyl complex destroys the enzyme by irreversibly inhibiting it; or that the compound is simply not a substrate as it cannot fit into the active site of the enzyme.

This final ambiguity was resolved in the following experiment.

Experiment 1. (Table 2) was repeated in the presence of 0.13M equivalents of methyl sorbate irontricarboxyl. If the irontricarboxyl complex were any type of enzyme inhibitor, the reaction rate would change.

1. Time (hours)	Ratio SM : P	2. Time (hours)	RatioSM : P
-		0	
1	2.2 : 1	1	1.8 : 1
2	0.79 : 1	2	0.55 : 1
3	1.1 : 1	3	0.93 : 1
5	0.67 : 1	5	1 : 1

1. Expt. 1, Table 2.

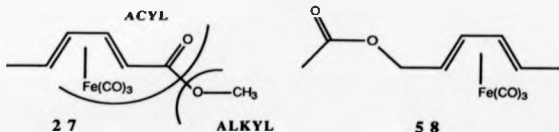
2. As 1. In the presence of methyl sorbate irontricarboxyl

Table 4

As can be seen from Table 4, the presence of the irontricarboxyl complex does not effect the rate of transesterification.

From this result it was concluded that methyl sorbate irontricarboxyl is not an inhibitor of PLE. It is simply not a substrate - the alkyl or acyl moiety of the ester could be of the wrong physical shape or is electronically incompatible with the active site of the enzyme.

Thus modification of the substrate structure must be next investigated.



The most straightforward method for structure modification was to reverse the alkyl and acyl functionalities of the ester. Consequently, if the acyl group at present was too large to fit into the active site of PLE, a simple acetylation would exchange the bulky diene- $\text{Fe}(\text{CO})_3$  moiety for a much smaller  $\text{CH}_3$  group and possibly allow the complex to bind to the enzyme.

#### 1-4 Irontricarbonyl (2,4-hexadien-1-ol) acetate.

Methyl sorbate irontricarbonyl was added slowly at  $-78^\circ\text{C}$  to a slurry of  $\text{LiAlH}_4$  in anhydrous diethyl ether, and stirred under  $\text{N}_2$  for two hours. The reaction was followed by TLC, and after this time it had reached completion. Quenching with ethyl acetate and water followed by diethyl ether extraction and column chromatography gave irontricarbonyl (2,4-hexadien-1-ol) **59** in 88% yield.



This product was characterised using  $^1\text{H}$  nmr, IR and MS; the MS showing the molecular ion at  $m/z=238$  followed by the consecutive losses of all three CO groups at  $m/z=210$ , 182 and 154. The ion of relative abundance of 100% falls at  $m/z=136$ , which is [M-

$((\text{CO})_3 + \text{H}_2\text{O})]^+$ . A sharp band at  $3621\text{cm}^{-1}$  and a broader one at  $3437\text{cm}^{-1}$  in the IR spectrum suggests H-bonding and intramolecular H-bonding of the hydroxyl group of the alcohol, and two very strong bands at  $2055$  and  $1977\text{cm}^{-1}$  due to the carbonyl groups of  $\text{Fe}(\text{CO})_3$  confirm the expected structure.

To a solution of freshly distilled pyridine and acetic anhydride in anhydrous diethyl ether was added an ethereal solution of irontricarbonyl (2,4-hexadien-1-ol) **59** at  $0^\circ\text{C}$ . The reaction was followed by TLC, and after eight hours the reaction had reached completion. Ether extraction of the basic followed by acidic aqueous fractions yielded the pure product irontricarbonyl (2,4-hexadien-1-ol) acetate **58** in 92% yield.

$^1\text{H}$  nmr spectroscopy showed a 3H singlet at  $\delta=2.05\text{ppm}$ , with the remainder of the spectrum virtually identical to that of irontricarbonyl (2,4-hexadien-1-ol) except for the slight downfield shift of H-(C-1) in the new complex. MS(FAB) had the molecular ion of  $m/z=280$ , followed by the successive losses of all three CO groups at 252, 224 and 196.  $^{13}\text{C}$  nmr, IR and UV spectroscopy with microanalytical results fully confirmed the new compound to be of the structure **58** as expected.

#### 1-5 The enzyme catalysed reactions of irontricarbonyl (2,4-hexadien-1-ol) acetate.

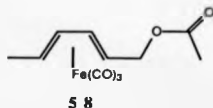
Preliminary experiments were carried out using the following biocatalysts to test for substrate suitability of the irontricarbonyl complex **58**.

- |                                       |             |             |
|---------------------------------------|-------------|-------------|
| (1) PPL                               | (2) CCL     | (3) PLE     |
| (4) LP 146(2)*                        | (5) LP 301* | (6) LP 150* |
| (7) Trypsin (immobilised on Eupergit) |             |             |

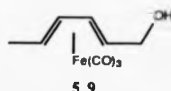
The complex was suspended in phosphate buffer, pH 7 and stirred rapidly at room temperature upon addition of the enzyme. In 20-40 hours, 50% of reaction had occurred in each case, with the exceptions being enzymes (5) and (7) where no hydrolysis was catalysed.

Thus the ironcarbonyl derivative 58 had been shown to be an enzyme substrate, and the enantiospecificities of each biocatalyst leading to optically active products were investigated. (Fig. 9).

Correlating the results of experiments (3) and (4) gave an absolute rotation of  $[\alpha]_D = -10^\circ$  for optically pure 59, thus the use of LP 150 (expt 1) looks favourable. However, LP 150 belongs to a private collection of lipases and was available only in very small amounts. So the best results for commercially available lipases were followed, in particular the hydrolysis using PPL.



Biocatalysis  
Room Temp.



Expt.	Buffer	Co-solvent	Lipase	Time <sup>f</sup>	Yield	[α] <sub>D</sub>	e.e. (%)
1	57mg, 5mls, pH7	-	LP 150*	41	54 %	+10.53	-
2	54mg, As 1	-	PLE	20	42	+5.3	-
3	105mg, 10mls, pH7	-	CCL	40	44	-2.5	-
4	149mg, 20mls, pH7	-	CCL	40	49	-	26
5	146mg, 20mls, pH8	-	PLE	66	62	-	6
6	70mg, 27mls, pH8	iso-octane 45°C	NOVO Liposyme	15	17	-	0
7	101mg, 10mls, pH7	-	PPL	36	42	+10.4 <sup>g</sup>	-
8	50mg, 4.5mls, pH7	10% MeOH	PPL	35	51	+0.24 <sup>h</sup>	-
						+5.3 <sup>e</sup>	-
9	99mg, 10mls, pH7	20% MeOH	PPL	41	54	-2.66 <sup>d</sup>	-
						+1.33 <sup>e</sup>	-
10	262mg, 63mls, pH7	10% MeOH	PPL	48	47	-	34
11	156mg, As 10	As 10	PPL (inhibited)	50	37	-	29
12	156mg, 30mls, pH7	-	As 11	32	11	-	16

(a) All reactions were followed by the continuous additions of 0.1M NaOH from an autotitrator. Work-up was done by ether extraction of the basified reaction mixtures, followed by passage through AlOx to separate acetate from alcohol.

(b) All rotations were measured in acetone, with  $c=1$ .

(c) E.e.s were determined by  $^{19}\text{F}$  nmr of the Mosher ester derivative of the product alcohol.

(d) Product alcohol.

(e) Recovered starting material.

(f) Time in hours.

\* Lipase from M. Schneider, Wuppertal.

Figure 9



PPL was suspended in a mixture of buffer and substrate, stirred at room temperature and the reaction was followed by NaOH from an autotitrator. After 36 hours, (31% hydrolysis by the autotitrator curve) no more reaction was taking place. A few drops of tributyrin were added to test for enzyme activity, which was confirmed by the immediate and rapid uptake of NaOH by the reaction mixture.

Product inhibition was the next factor to be considered therefore the above reaction plus another, identical one, with 0.2M equivalents of irontricarbonyl(2,4-hexadien-1-ol) added, were set up and reaction rates compared using autotitrators. After a set time interval of 6 hours and 40 minutes, the autotitrator with the alcohol present had added 0.228mls (12% hydrolysis) of NaOH and the other had added 0.268mls (14%). Thus negligible or only very slight product inhibition takes place.

Consequently, the question of substrate solubility <sup>3</sup>leading to the effect on rate and enzyme specificity due to the presence of a co-solvent was investigated.

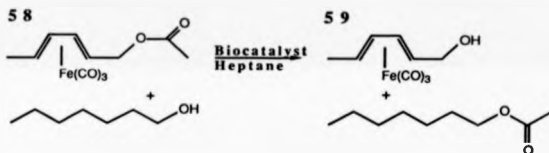
It became immediately apparent that using 10% methanol increased the rate of the reaction by a factor of at least two, and pushed the yield of hydrolysis to 50%. (expts 7,8) Increasing the concentration of methanol to 20% did not further effect the rate, but the sign of the rotation of the product alcohol was reversed. (expt 9.)

As the PPL used in this experiment was a very crude preparation containing <sup>4</sup>esterase and protease activity, an inhibited preparation of PPL<sup>103</sup> was used. Treating the lipase with PMSF irreversibly inhibited the serine protease activity of the lipase,

giving rise to a purer esterase. However, using the inhibited PPL in the above hydrolysis lowered both the rate and the selectivity of the reaction. (expt 11) Also, repeating the hydrolysis with inhibited PPL in the absence of a co-solvent (expt 12) lowered rate and selectivity further. Therefore it was shown that for maximum e.e. (34%) in the PPL catalysed hydrolysis of this particular substrate 58, the crude enzyme preparation and a co-solvent of methanol is of vital importance.

It was found that the irontricarbonyl acetate spontaneously hydrolyses to about 10% in either pure buffer or buffer with 10% methanol added in a period of 48 hours, thus an e.e. of 100% could never be attained using this particular system.

To avoid the problem of spontaneous hydrolysis, an enzyme catalysed transesterification in organic solvent was investigated.



A 1:1 molar ratio of ironcarbonyl complex 58 and 1-heptanol were stirred together with several enzymes. Heptane was chosen as solvent to try to prevent the known enzymatic alcohol inhibition by reducing the amount of alcohol present to a minimum. After reaction of one week, followed by GLC, only 2% transesterification was shown by PLE/Sephadex and 16% reaction with PPL. The other lipids (CCL, LP 150 and NOVO Liposyme) showed no reaction at all.

These two sections of work have shown that enzymes will indeed accept organometallic compounds as substrates, and that a biocatalyst can distinguish between the two faces of a compound with planar chirality. The conditions of the experiment need to be optimised to obtain a full enzymatic resolution of an organometallic complex.

It is of interest to note that the active sites of both PLE and several lipases will accept as a substrate the acetate of irontricarbonyl sorbic acid and not the ester. If the enzyme-substrate binding in the active site is directionalised as discussed in the introduction *i.e.*, along the acyl-O bond, then it is evident that the shape and bulk of the acyl portion of the ester greatly influences the outcome of a potential enzymatic hydrolysis. (Fig. 10)

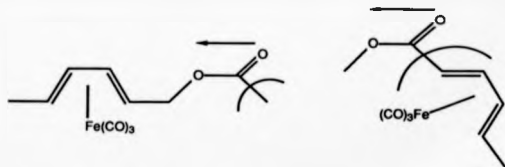
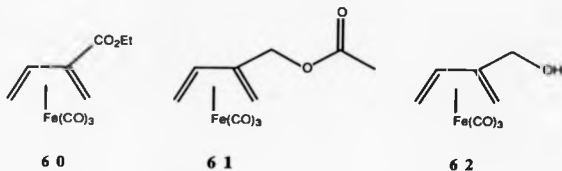


Figure 10

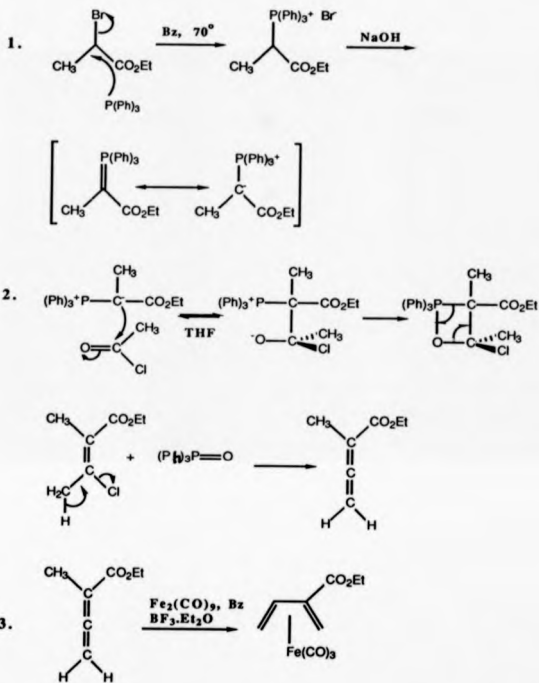
This is in total agreement with the results found with PPL and *p*-chlorobenzylbutyrate compared with fluoroethyl-3-phenylpropionate discussed in the introduction to this thesis.

1.6 2-Carboethoxy-1,3-butadiene irontricarbonyl.

The next substrate to be considered as an enzymatic target was 2-carboethoxy-1,3-butadiene irontricarbonyl **60**.



This compound was chosen as its synthesis is well explained in the literature<sup>104</sup>, (Scheme 5) and its slight difference in physical structure from the other complexes used will again evoke new information about the structure of the active site of PLE.



Scheme 5

The first stage of the synthesis<sup>104(a)</sup> was a simple *Wittig* reaction resulting in the formation of a phosphonium ylid (yellow solid) which was achieved in an overall yield of 60%. The ylid was then refluxed in THF with freshly distilled acetyl chloride for four hours, resulting in a precipitate, which was filtered off. The filtrate was condensed, and upon addition of ice cold light petroleum excess triphenylphosphonium oxide was precipitated out. After filtration, the residue was distilled to give the allene in 60% yield.

The third step of the synthesis<sup>104(b)</sup> involved the refluxing of the allene with  $\text{Fe}_2(\text{CO})_9$  in benzene, in the presence of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  for four hours. Column chromatography separated the ironcarbonyl complex in 32% yield. 2-Carboethoxy-1,3-butadiene irontricarbonyl was characterised via its MS(FAB) which gave the molecular ion at  $m/z=266$  and successive losses of all three CO groups at 238, 210 and 182 (100% ion).  $[\text{M}-\text{OC}_2\text{H}_5]^+$  was observed at  $m/z=137$ . IR confirmed the irontricarbonyl group by giving strong absorption bands at 2060 and  $1990\text{cm}^{-1}$ , and  $^1\text{H}$  nmr spectroscopy verified the remainder of the structure.

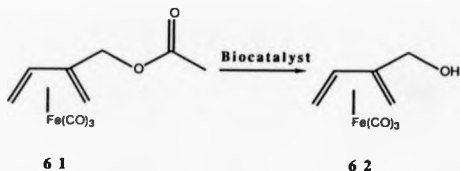
It was decided also to synthesise the acetate derivative 61 of this compound to compare enzyme activities towards each substrate.

An ethereal solution of irontricarbonyl complex 60 was stirred with  $\text{LiAlH}_4$  at  $-78^\circ\text{C}$  for two hours. After this time, the reaction had reached completion. It was worked up by extraction and purified by column chromatography to give 2-alkoxy<sup>metal</sup>-1,3-butadiene irontricarbonyl 62 in 76% yield. The complex was identified by its MS(FAB) which gave the molecular ion at  $m/z=224$  and other fragment ions at  $207[\text{M}-\text{OH}]^+$ ,  $196[\text{M}-\text{CO}]^+$ ,  $179[\text{M}-(\text{CO}+\text{OH})]^+$ ,  $168[\text{M}-(\text{CO})_2]^+$ ,  $151[\text{M}-((\text{CO})_2+\text{OH})]^+$  and  $140[\text{M}-$

$(\text{CO})_3]^+$   $^1\text{H}$  Nmr showed the loss of a three proton triplet at  $\delta=1.31\text{ppm}$  and a coupled multiplet at  $\delta=4.305\text{ppm}$  corresponding to the  $\text{OCH}_2\text{CH}_3$  of the ethyl ester complex 60; and the appearance of two doublets of doublets at  $\delta=4.26$  and  $4.53\text{ppm}$  due to the magnetic inequivalence of the two protons attached to C-1'. The remainder of the spectrum was virtually identical to that of complex 60. Thus the correct product was verified.

2-Alkoxy-<sup>methyl</sup>1,3-butadiene irontricarbonyl was acetylated by stirring with pyridine and acetyl chloride in diethyl ether at  $0^\circ\text{C}$  for 20 hours. Extraction followed by column chromatography isolated the pure product acetate 61 in 76% yield. The acetate was verified by the following spectral characteristics: MS showed a very weak molecular ion at  $m/z=266$  followed by the strong successive losses of all three CO groups at 238, 210 and 182. A large peak at  $m/z=149$  was due to  $[182-(\text{CH}_3+\text{H}_2\text{O})]^+$ .  $^1\text{H}$  Nmr spectroscopy showed an identical spectrum to that of iron tricarbonyl alcohol 62, excepting the addition of a 3H singlet at  $\delta=2.12\text{ppm}$  and the loss of alcoholic OH at  $\delta=1.9\text{ppm}$ . IR showed the usual strong tricarbonyl stretches at  $2050$  and  $1975\text{cm}^{-1}$ . Thus the desired product was confirmed.

1-7 The Enzymatic hydrolysis of 2-acetoxy-1,3-butadiene  
irontricarboxyl.



To assess for spontaneous hydrolysis of the irontricarboxyl acetate 61 in buffer at various values of pH, 30mg of the compound was suspended in 1ml of phosphate buffer at pH 6 and pH 8. Both reactions were gently stirred at room temperature for 67 hours and were followed by TLC. After this time, the reactions at pH 8 showed slight spontaneous hydrolysis whereas those at pH 6 showed none. Consequently, the preliminary reactions were carried out at this pH.



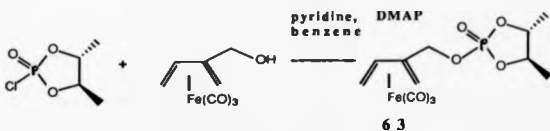
Expt.	Biocatalyst	Time(hrs)	Yield(%)	e.e.(%) <sup>a</sup>
1	PPL	17	13	-
2	<i>A. niger</i>	22.5	52	-
3	CCL	23	44	-
4	EEAcE	21	100	-
5	<i>R. japonicus</i>	20	7	-
6	<i>Pseudomonas</i>	25	28	-
7	<i>A. niger</i>	20	>50	40

(a) Typical conditions: 50-100mg **61** stirred at room temperature in 1ml buffer, pH6-7, with 12-20mg lipase. Work-up by basic extraction followed by chromatography. Reactions followed by additions of 0.1M NaOH from an autotitrator.

\* e.e.s determined via phospholane derivatisation and <sup>31</sup>P nmr spectroscopy.

Table 5

Six reactions were carried out, testing each lipase for its affinity to hydrolyse selectively the irontricarbonyl complex **61**. The reactions were followed by continuous additions of 0.1M NaOH from an autotitrator. From Table 5 it can be seen that the reaction stopping at ~50% (therefore the lipase is selectively hydrolysing one enantiomer of the complex only) is experiment 2, using *Aspergillus niger* as the biocatalyst. Therefore, the reaction was repeated on a larger scale and the e.e. was determined. This was carried out via the synthesis of the dioxaphospholane derivative **63** following a method previously described in the literature.<sup>105</sup>

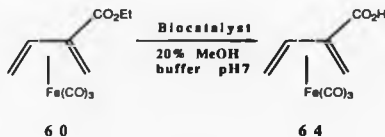


Complex **63** gave a  $^{31}\text{P}$  proton-decoupled nmr spectrum of two singlets, base-line separated at 14.688 and 14.678ppm, with equal ratios of integration for the racemic alcohol.

An e.e. of 40% was found when the alcohol used in synthesising **63** was recovered from the *Aspergillus niger* catalysed hydrolysis (expt 7). Unfortunately base-line resolution could not be obtained consistently, thus only one e.e. was recorded. From the autotitrator results, it can be seen that lipases appear to be more selective towards this substrate than the one esterase tried. (expt 4) EEAcE was totally non-selective and completely hydrolysed the acetate in 21 hours.

The reverse phenomenon was observed following the enzymatic resolution of 2-carboethoxy-1,3-butadiene irontricarbonyl **60**.

1-8 The Enzymatic hydrolysis of 2-carboethoxy-1,3-butadiene irontricarboxyl.



Expt. <sup>a</sup>	Biocatalyst	Time(hrs)	Yield(%)	e.e.(%) <sup>b</sup>
1	PPL	70	-	-
2	CCL	70	-	-
3	<i>A. niger</i>	70	-	-
4	<i>Pseudomonas</i>	70	-	-
5	<i>R. javanicus</i>	70	-	-
6	PLE	21	32	85
7	EEAcE	60	10	-

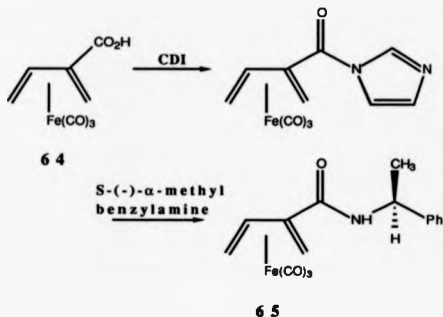
(a) Typical conditions: 100mg irontricarboxyl complex 60 in a total of 10mls 0.1M phosphate buffer pH 7 including 20% MeOH. All stirred at room temperature and monitored by TLC. Reactions worked up via solvent extraction.

(b) e.e.s determined by amide formation with S-(-)- $\alpha$ -methylbenzylamine followed by  $^1\text{H}$  nmr.

Table 6

Several lipases and esterases were tested for their suitability to catalyse enantioselectively the irontricarboxyl ester 60. As can be seen from Table 6, the ester was not a substrate for lipases, yet was selectively hydrolysed by PLE (expt 6) to give 2-carboxy-1,3-

butadiene irontricarboxyl **64** in 85% e.e. The conditions for the reaction are outlined in the above table. The e.e. was determined via formation of the amide derivative **65**.



Stirring the racemic irontricarboxyl acid **64** with CDI in  $\text{CDCl}_3$  for one hour, followed by the addition of 1.05M equivalents of *S*-(-)- $\alpha$ -methylbenzylamine and further stirring overnight, synthesised the irontricarboxyl amide **65** in quantitative yield.  $^1\text{H}$  nmr spectroscopy gave two doublets, one at  $\delta=1.53\text{ppm}$  and the other at  $1.575\text{ppm}$  which corresponded to the  $\text{CH}_3$  group of each diastereomer **65**. Baseline separation of each doublet allowed the e.e. of each enzymatic hydrolysis to be measured.

One recrystallisation of the acid obtained from the PLE catalysed hydrolysis of the irontricarboxyl ester (expt 6) gave the product in 100% e.e.

The absolute rotations of the fully resolved irontricarboxyl complex **64** are given in Table 7.

Compound	$[\alpha]_D$	m. pt.	R <sub>F</sub> <sup>e</sup>	$\delta$ ppm (CH <sub>3</sub> )
Acid <sup>a</sup>	-10.7° <sup>c</sup>	129-130°	-	-
Acid <sup>b</sup>	+11.0° <sup>d</sup>	133-134°	-	-
Amide <sup>f</sup>	-188° <sup>d</sup>	121°	0.25	1.575
Amide <sup>f</sup>	+42.4° <sup>d</sup>	115-116°	0.32	1.530

(a) PLE hydrolysis product

(b) Chemical hydrolysis product of residual ester 60

(c) c=0.5, CHCl<sub>3</sub> (d) c=1, CHCl<sub>3</sub>

(e) TLC on silica, eluant 95% toluene, 5%Et<sub>2</sub>O

(f) The amide diastereomers were separated by column chromatography and recrystallized to constant rotation and melting point.

Table 7

The acid formed in experiment 6 was of negative rotation,  $[\alpha]_D = -8.8^\circ$  and corresponded to 85% e.e. as determined by <sup>1</sup>H nmr spectroscopy of the respective amide derivative. This upon one recrystallisation gave a rotation of  $[\alpha]_D = -10.7^\circ$ , and showed only one set of doublets upon amide formation at  $\delta = 1.575$  ppm, verifying an e.e. of >98%. The residual ester from the enzymatic hydrolysis was chemically hydrolysed by stirring for four hours with LiOH/H<sub>2</sub>O in aqueous ethanol giving the acid with a rotation of  $[\alpha]_D = +11^\circ$ . This acid, upon amide formation followed by <sup>1</sup>H nmr spectrum analysis again showed only one set of doublets by <sup>1</sup>H nmr, centred at  $\delta = 1.530$  ppm.

Thus the first enzymatic total resolution of an organometallic complex had been carried out, making readily available each enantiomer in optically pure form. This also represents the first instance where an enzyme has distinguished fully between the enantiomers of a compound related by a plane of chirality.

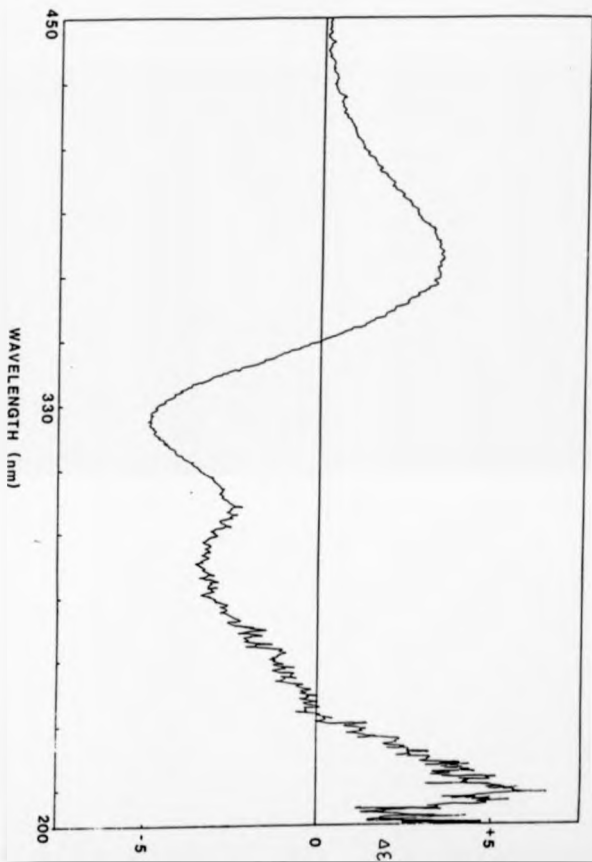
#### 1.9 Determination of the absolute configuration of 2-Carboxy-1,3-butadiene irontricarbonyl.

The absolute configuration of only a few irontricarbonyl complexes have been previously determined. Therefore it was of interest to correlate the rotation and absolute configuration by cd and X-ray structure analysis of 2-carboxy-1,3-butadiene irontricarbonyl.

As was discussed in detail in the introduction, the absolute configuration of an irontricarbonyl complex can be tentatively determined by the sign of the lowest energy  $d \rightarrow d$  transition in the cd spectrum. The lowest energy transition in the cd spectrum of (-)-2-carboxy-1,3-butadiene irontricarbonyl (Fig. 11) occurs at 380nm, and is strongly positive ( $\Delta E=+4$ ) in character. This is in full agreement with the cd spectra for (-)-fumaric acid  $\text{Fe}(\text{CO})_4$ , (-)-2, 4-hexadienoic acid  $\text{Fe}(\text{CO})_3$  and (-)-2, 4-hexadienal  $\text{Fe}(\text{CO})_3^{40}$ , which all exhibit strong, positive absorptions in this region. Thus it can be affirmed that the absolute configuration of each of these diene-ironcarbonyl complexes is the same.

The amplitude of this absorbance is directly related to the number of asymmetric carbon atoms co-ordinated to the  $\text{Fe}(\text{CO})_3$  group. There are two asymmetric olefins complexed in this case, giving  $\Delta E=+4$ . For acrylic acid  $\text{Fe}(\text{CO})_4$ ,  $\Delta E=2$ , and for fumaric acid

Figure 11

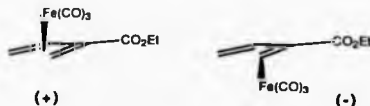


$\text{Fe}(\text{CO})_4 \Delta E=4$ . Thus the number of asymmetric carbon atoms is confirmed as two in this complex.

Also of relevance is the fact that the two asymmetric carbon atoms attached to the  $\text{Fe}(\text{CO})_3$  group are of different absolute configuration. This is reflected in the cd curve in the 310-390nm range, where there are two absorbances, each with a different sign. This reflects the configurations of the  $\text{CH}_2=\text{CH}_2$  and  $\text{CO}_2\text{Et}=\text{CH}_2$  groups, although which absorbance is due to which group is not known.

To confirm the absolute configurations of (+) and (-) 2-carboxy-1,3-butadiene irontricarbonyl, determination of the structure of (+)-irontricarbonyl(1,3-butadien-2(S-(-)- $\alpha$ -methylbenzyl)amide) (Fig. 12) by X-Ray analysis was carried out by Dr. N.W. Alcock of the Department of chemistry, University of Warwick.

It was immediately seen that the  $\text{Fe}(\text{CO})_3$  group is lying above the plane of the diene, which gives the absolute configuration of (+)-2-carboxy-1,3-butadiene irontricarbonyl as (2S,3R).



This is in total agreement with that which was predicted by cd determination.

From Figure 12(a), it can be seen that there are four independent molecules in the asymmetric unit, *i.e.* eight independent molecules per unit cell. The average Fe-C bond length is 2.085Å. The C-C



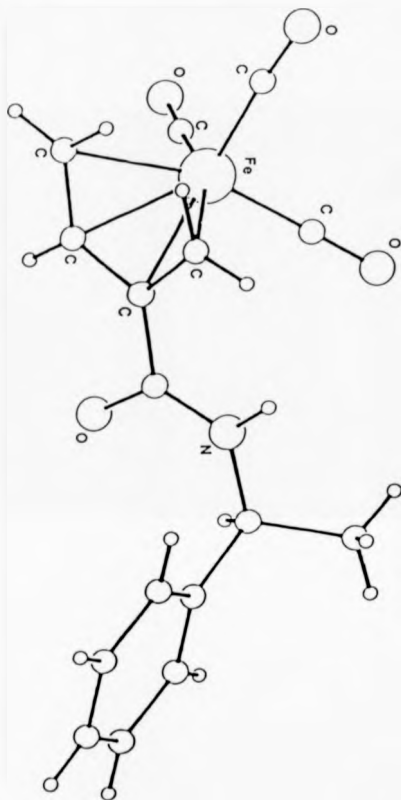


Figure 12

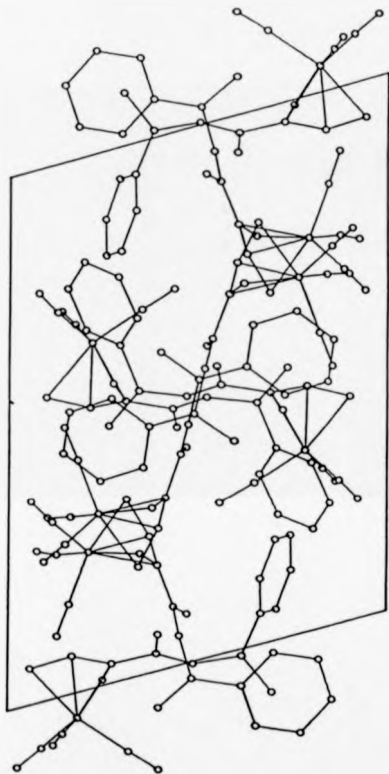


Figure 12(a)

# Bond Lengths (Å)

Fe(1)-C(1)	1.762(27)	Fe(1)-C(12)	1.797(19)
Fe(1)-C(17)	1.747(21)	Fe(1)-C(14)	1.76(22)
Fe(1)-C(13)	1.855(13)	Fe(1)-C(16)	1.875(13)
Fe(1)-C(17)	1.855(13)	Fe(2)-C(21)	1.853(17)
Fe(2)-C(22)	1.833(26)	Fe(2)-C(23)	1.882(27)
Fe(2)-C(24)	2.143(16)	Fe(2)-C(25)	2.056(16)
Fe(2)-C(26)	2.022(13)	Fe(2)-C(27)	2.057(16)
Fe(3)-C(31)	1.859(16)	Fe(3)-C(32)	1.745(24)
Fe(3)-C(33)	1.761(29)	Fe(3)-C(34)	2.120(18)
Fe(3)-C(35)	2.040(30)	Fe(3)-C(36)	2.027(17)
Fe(3)-C(37)	2.105(19)	Fe(4)-C(41)	1.820(29)
Fe(4)-C(42)	1.734(22)	Fe(4)-C(43)	1.774(20)
Fe(4)-C(44)	2.115(22)	Fe(4)-C(45)	2.066(19)
Fe(4)-C(46)	2.072(15)	Fe(4)-C(47)	2.086(17)
C(11)-C(11)	1.135(33)	C(12)-C(12)	1.121(26)
C(13)-C(13)	1.136(27)	C(14)-C(14)	1.380(25)
C(15)-C(16)	1.354(24)	C(16)-C(17)	1.417(13)
C(16)-C(18)	1.477(19)	C(18)-C(14)	1.217(19)
C(18)-N(1)	1.290(19)	N(1)-C(19)	1.475(19)
C(19)-C(110)	1.493(31)	C(19)-C(111)	1.559(19)
C(111)-C(112)	1.395	C(111)-C(116)	1.395
C(112)-C(113)	1.395	C(113)-C(114)	1.395
C(114)-C(115)	1.395	C(115)-C(116)	1.395
C(21)-C(21)	1.099(19)	C(22)-C(22)	1.109(35)
C(23)-C(23)	1.266(36)	C(24)-C(25)	1.408(26)
C(25)-C(26)	1.400(22)	C(26)-C(27)	1.335(32)
C(26)-C(28)	1.504(31)	C(28)-C(24)	1.225(19)
C(28)-N(2)	1.324(19)	N(2)-C(29)	1.452(19)
C(29)-C(210)	1.460(22)	C(29)-C(211)	1.487(20)
C(211)-C(212)	1.395	C(211)-C(216)	1.395
C(212)-C(213)	1.395	C(213)-C(214)	1.395
C(214)-C(215)	1.395	C(215)-C(216)	1.395
C(31)-C(31)	1.055(20)	C(32)-C(32)	1.166(30)
C(33)-C(33)	1.121(36)	C(34)-C(35)	1.398(31)
C(35)-C(36)	1.423(24)	C(36)-C(37)	1.401(24)
C(36)-C(38)	1.443(23)	C(38)-C(34)	1.255(21)
C(38)-N(3)	1.303(19)	N(3)-C(39)	1.498(21)
C(39)-C(310)	1.507(22)	C(39)-C(311)	1.530(22)
C(311)-C(312)	1.395	C(311)-C(316)	1.395
C(312)-C(313)	1.395	C(313)-C(314)	1.395
C(314)-C(315)	1.395	C(315)-C(316)	1.395
C(41)-C(41)	1.101(35)	C(42)-C(42)	1.164(29)
C(43)-C(43)	1.119(29)	C(44)-C(45)	1.382(24)
C(45)-C(46)	1.401(24)	C(46)-C(47)	1.430(24)
C(46)-C(48)	1.441(15)	C(48)-C(44)	1.263(18)
C(48)-N(4)	1.324(18)	N(4)-C(49)	1.442(18)
C(49)-C(410)	1.444(26)	C(49)-C(411)	1.507(18)
C(411)-C(412)	1.395	C(411)-C(416)	1.395
C(412)-C(413)	1.395	C(413)-C(414)	1.395
C(414)-C(415)	1.395	C(415)-C(416)	1.395

## Bond Angles (deg.)

C(1)-C(2)-C(3)	104.2(9)	C(11)-Fe(1)-C(13)	91.9(11)
C(1)-C(2)-C(4)	100.7(10)	C(11)-Fe(1)-C(14)	92.3(10)
C(1)-C(2)-C(5)	94.4(9)	C(13)-Fe(1)-C(14)	162.8(7)
C(1)-C(2)-C(6)	94.4(9)	C(12)-Fe(1)-C(15)	138.2(9)
C(1)-C(2)-C(7)	124.4(7)	C(14)-Fe(1)-C(15)	38.6(7)
C(1)-C(2)-C(8)	122.3(8)	C(12)-Fe(1)-C(16)	129.0(8)
C(1)-C(2)-C(9)	95.3(7)	C(14)-Fe(1)-C(16)	68.8(6)
C(1)-C(2)-C(10)	96.3(7)	C(11)-Fe(1)-C(17)	161.3(9)
C(1)-C(2)-C(11)	98.4(8)	C(13)-Fe(1)-C(17)	95.8(8)
C(1)-C(2)-C(12)	78.7(8)	C(15)-Fe(1)-C(17)	67.2(7)
C(1)-C(2)-C(13)	39.5(6)	C(21)-Fe(2)-C(22)	99.1(9)
C(1)-C(2)-C(14)	95.3(10)	C(22)-Fe(2)-C(23)	105.2(13)
C(1)-C(2)-C(15)	164.9(8)	C(22)-Fe(2)-C(24)	93.9(8)
C(1)-C(2)-C(16)	98.1(9)	C(21)-Fe(2)-C(25)	126.1(8)
C(1)-C(2)-C(17)	138.9(7)	C(23)-Fe(2)-C(25)	91.2(10)
C(1)-C(2)-C(18)	39.1(7)	C(21)-Fe(2)-C(26)	95.2(6)
C(1)-C(2)-C(19)	129.2(8)	C(23)-Fe(2)-C(26)	121.5(11)
C(1)-C(2)-C(20)	9.5(6)	C(25)-Fe(2)-C(26)	48.2(6)
C(1)-C(2)-C(21)	91.3(8)	C(22)-Fe(2)-C(27)	91.6(9)
C(1)-C(2)-C(22)	161.2(11)	C(24)-Fe(2)-C(27)	98.1(7)
C(1)-C(2)-C(23)	71.1(7)	C(26)-Fe(2)-C(27)	48.0(6)
C(1)-C(2)-C(24)	86.3(10)	C(31)-Fe(3)-C(33)	101.8(10)
C(1)-C(2)-C(25)	167.5(13)	C(31)-Fe(3)-C(34)	166.7(9)
C(1)-C(2)-C(26)	94.1(10)	C(33)-Fe(3)-C(34)	90.1(10)
C(1)-C(2)-C(27)	127.3(9)	C(32)-Fe(3)-C(35)	92.9(10)
C(1)-C(2)-C(28)	136.1(9)	C(34)-Fe(3)-C(35)	39.2(8)
C(1)-C(2)-C(29)	98.2(7)	C(32)-Fe(3)-C(36)	128.2(10)
C(1)-C(2)-C(30)	133.2(10)	C(34)-Fe(3)-C(36)	72.1(8)
C(1)-C(2)-C(31)	41.0(7)	C(31)-Fe(3)-C(37)	95.0(8)
C(1)-C(2)-C(32)	159.7(10)	C(33)-Fe(3)-C(37)	95.2(11)
C(1)-C(2)-C(33)	77.3(8)	C(35)-Fe(3)-C(37)	69.0(7)
C(1)-C(2)-C(34)	39.2(7)	C(41)-Fe(4)-C(42)	101.7(11)
C(1)-C(2)-C(35)	87.3(11)	C(42)-Fe(4)-C(43)	102.6(10)
C(1)-C(2)-C(36)	95.4(10)	C(42)-Fe(4)-C(44)	93.1(9)
C(1)-C(2)-C(37)	165.8(8)	C(41)-Fe(4)-C(45)	94.8(10)
C(1)-C(2)-C(38)	138.0(9)	C(43)-Fe(4)-C(45)	125.3(8)
C(1)-C(2)-C(39)	38.6(7)	C(41)-Fe(4)-C(46)	123.5(9)
C(1)-C(2)-C(40)	131.5(9)	C(43)-Fe(4)-C(46)	96.4(8)
C(1)-C(2)-C(41)	69.9(6)	C(45)-Fe(4)-C(46)	39.6(7)
C(1)-C(2)-C(42)	165.2(9)	C(42)-Fe(4)-C(47)	93.7(9)
C(1)-C(2)-C(43)	34.9(9)	C(44)-Fe(4)-C(47)	88.2(7)
C(1)-C(2)-C(44)	79.3(7)	C(46)-Fe(4)-C(47)	48.2(6)
Fe(1)-C(11)-C(12)	174.6(22)	Fe(1)-C(12)-C(13)	175.4(19)
Fe(1)-C(11)-C(13)	176.3(12)	Fe(1)-C(13)-C(14)	57.3(12)
Fe(1)-C(11)-C(14)	74.1(11)	Fe(1)-C(13)-C(15)	71.8(10)
C(14)-C(11)-C(12)	120.3(17)	Fe(1)-C(15)-C(16)	65.9(9)
Fe(1)-C(11)-C(15)	76.9(9)	C(15)-C(16)-C(17)	112.2(14)
Fe(1)-C(11)-C(16)	123.2(11)	C(15)-C(16)-C(18)	120.0(14)
C(17)-C(11)-C(12)	127.7(15)	Fe(1)-C(17)-C(18)	69.6(9)
C(17)-C(11)-C(15)	118.2(14)	C(16)-C(18)-N(1)	117.6(13)
C(17)-C(11)-N(1)	124.2(13)	C(18)-N(1)-C(19)	128.9(13)
N(1)-C(11)-C(12)	110.6(14)	N(1)-C(19)-C(11)	111.0(14)
C(12)-C(11)-C(13)	116.3(14)	C(19)-C(11)-C(12)	126.0(9)
C(12)-C(11)-C(15)	113.9(9)	C(112)-C(111)-C(116)	120.8
C(12)-C(11)-C(16)	120.0	C(112)-C(111)-C(117)	120.8
C(12)-C(11)-C(17)	120.0	C(114)-C(115)-C(116)	120.8
C(12)-C(11)-C(18)	120.0	Fe(2)-C(21)-C(22)	176.6(12)
C(12)-C(11)-C(19)	117.15	Fe(2)-C(21)-C(23)	177.4(12)

Fe(2)-C(24)-C(25) 67 1(9)  
 Fe(2)-C(25)-C(26) 68 6(8)  
 Fe(2)-C(26)-C(27) 71 2(9)  
 C(25)-C(26)-C(27) 117 6(14)  
 C(25)-C(26)-C(28) 117 5(13)  
 Fe(2)-C(27)-C(28) 68 7(9)  
 C(26)-C(28)-N(2) 118 5(13)  
 C(29)-N(2)-C(29) 123 7(13)  
 N(2)-C(29)-C(211) 111 1(11)  
 C(29)-C(211)-C(212) 119 1(7)  
 C(212)-C(211)-C(216) 120 0  
 C(212)-C(213)-C(214) 120 0  
 C(214)-C(215)-C(216) 120 0  
 Fe(3)-C(31)-C(31) 171 4(24)  
 Fe(3)-C(33)-C(33) 175 6(27)  
 Fe(3)-C(35)-C(34) 73 5(12)  
 C(34)-C(35)-C(36) 119 6(19)  
 Fe(3)-C(36)-C(37) 73 2(11)  
 Fe(3)-C(36)-C(38) 125 9(11)  
 C(37)-C(36)-C(38) 127 3(14)  
 C(36)-C(38)-C(34) 118 1(13)  
 O(34)-C(38)-N(3) 121 6(15)  
 N(3)-C(39)-C(310) 106 6(15)  
 C(310)-C(39)-C(311) 113 1(14)  
 C(39)-C(311)-C(316) 119 9(8)  
 C(311)-C(312)-C(313) 120 0  
 C(313)-C(314)-C(315) 120 0  
 C(311)-C(316)-C(315) 120 0  
 Fe(4)-C(42)-C(42) 175 5(20)  
 Fe(4)-C(44)-C(45) 68 8(12)  
 Fe(4)-C(45)-C(46) 70 4(10)  
 Fe(4)-C(46)-C(45) 70 8(9)  
 C(45)-C(46)-C(47) 116 4(14)  
 C(45)-C(46)-C(48) 119 3(15)  
 Fe(4)-C(47)-C(46) 69 4(9)  
 C(46)-C(48)-N(4) 122 2(13)  
 C(48)-N(4)-C(49) 125 0(12)  
 N(4)-C(49)-C(411) 109 9(13)  
 C(49)-C(411)-C(412) 119 5(8)  
 C(412)-C(411)-C(416) 120 0  
 C(412)-C(413)-C(414) 120 0  
 C(414)-C(415)-C(416) 120 0

Fe(2)-C(25)-C(24) 73 8(18)  
 C(24)-C(25)-C(26) 117 3(16)  
 Fe(2)-C(26)-C(27) 71 4(9)  
 Fe(2)-C(26)-C(28) 121 1(8)  
 C(27)-C(26)-C(28) 124 4(13)  
 C(26)-C(28)-O(24) 118 8(14)  
 O(24)-C(28)-N(2) 122 7(15)  
 N(2)-C(29)-C(210) 108 8(14)  
 C(210)-C(29)-C(211) 116 3(14)  
 C(29)-C(211)-C(216) 120 6(7)  
 C(211)-C(212)-C(213) 120 0  
 C(213)-C(214)-C(215) 120 0  
 C(211)-C(216)-C(215) 120 0  
 Fe(3)-C(32)-O(32) 178 5(23)  
 Fe(3)-C(34)-C(35) 67 3(11)  
 Fe(3)-C(35)-C(36) 69 0(10)  
 Fe(3)-C(36)-C(35) 70 0(11)  
 C(35)-C(36)-C(37) 112 4(15)  
 C(35)-C(36)-C(38) 120 3(15)  
 Fe(3)-C(37)-C(36) 67 2(10)  
 C(36)-C(38)-N(3) 120 3(15)  
 C(38)-N(3)-C(39) 122 8(13)  
 N(3)-C(39)-C(311) 109 4(12)  
 C(39)-C(311)-C(312) 120 1(8)  
 C(312)-C(311)-C(316) 120 0  
 C(312)-C(313)-C(314) 120 0  
 C(314)-C(315)-C(316) 120 0  
 Fe(4)-C(41)-O(41) 171 3(20)  
 Fe(4)-C(43)-O(43) 174 2(21)  
 Fe(4)-C(45)-C(44) 72 7(11)  
 C(44)-C(45)-C(46) 118 9(17)  
 Fe(4)-C(46)-C(47) 70 4(9)  
 Fe(4)-C(46)-C(48) 119 7(12)  
 C(47)-C(46)-C(48) 123 2(15)  
 C(46)-C(48)-O(44) 118 5(13)  
 O(44)-C(48)-N(4) 118 8(11)  
 N(4)-C(49)-C(410) 111 4(12)  
 C(410)-C(49)-C(411) 114 8(13)  
 C(49)-C(411)-C(416) 120 5(8)  
 C(411)-C(412)-C(413) 120 0  
 C(413)-C(414)-C(415) 120 0  
 C(411)-C(416)-C(415) 120 0

bond lengths of the diene are virtually identical (C(1)-C(2) 1.41 Å, C(2)-C(3) 1.40 Å, C(3)-C(4) 1.39 Å), indicating a delocalised system. The average length of these bonds is the same as the typical bond length in an aromatic system. (1.39 Å)

Therefore, structure 57 is an accurate representation of the Fe(CO)<sub>3</sub>-diene complex, confirming the spectral evidence given in the introduction to this chapter.

This work, to the best of our knowledge, describes the first example of an enzymatic resolution of an organometallic complex into its enantiomers in optically pure form. It also represents the first example of an enzyme recognising and differentiating between optical isomers related by a plane of symmetry.

## Chapter 2.

### The enzymatic resolution of racemic ferrocene derivatives.

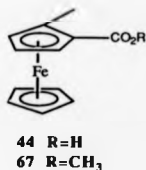
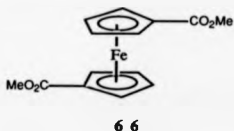
Following the successful resolution of an irontricarbonyl complex, it was decided to explore the ability of biocatalysts to accept as substrates a broad range of organometallic compounds.

Ferrocenes were chosen as the next target molecules, for the reasons detailed in the introduction to this thesis. In summary, it was because the stereochemistry of these compounds is complex and interesting, and that many hundreds of ferrocenes have been fully resolved into their enantiomers by chemical methods, and their chiroptical properties have been well documented.<sup>77,83</sup> Therefore, a ferrocene derivative enzymatically resolved would be a prime example of the versatility and use of biocatalysts in organic synthesis.

Also, as all the chiroptical properties are known, the emphasis of the experiment can be placed fully upon the enzyme-catalysed reaction.

#### 2.1 1,1'-Ferrocenedicarboxylic acid dimethyl ester.

The first substrate chosen as an enzyme target was 1,1'-ferrocenedicarboxylic acid dimethyl ester. <sup>66</sup> This compound, which is achiral, was chosen due to its easy synthesis, and to assess the possibility of a biocatalyst accepting a ferrocene ester as a substrate.



The diester **66** was synthesised from 1,1'-ferrocenedicarboxylic acid by stirring in a methanolic solution of acetyl chloride. The product diester **66** precipitated out of the reaction medium, and after filtration, was purified by column chromatography to give pure diester in 88% yield. This product was verified by its <sup>1</sup>H nmr spectrum which showed a six proton singlet at  $\delta=3.82\text{ppm}$  due to the two CO<sub>2</sub>CH<sub>3</sub> groups, and two multiplets of four protons each at  $\delta=4.42$  and  $4.83\text{ppm}$  corresponding to the aromatic protons of ferrocene. The IR spectrum showed strong bands at 1710, 1468 and  $1280\text{cm}^{-1}$ , due to the C-O stretching modes of the ester functionalities of the compound. Therefore the correct product **66** was confirmed.

Initial results of enzymatic hydrolysis of 1,1'-ferrocenedicarboxylic acid dimethyl ester **66** were discouraging. When 50mg of the diester was stirred in a 10% methanol solution of buffer pH 7, and 40 $\mu$ l PLE was added to catalyse the reaction, it took 1 month to hydrolyse the diester to give 32% yield. The products of the reaction were analysed by TLC, showing a mixture of mono and di-acids present. No reaction occurred in the absence of a biocatalyst, so it had been shown that the compound is, although very non-ideal, an enzyme substrate. However, due to the problems that arise from mono- and di-ester hydrolysis, it



was decided to focus attention on a chiral, mono ester ferrocene derivative.

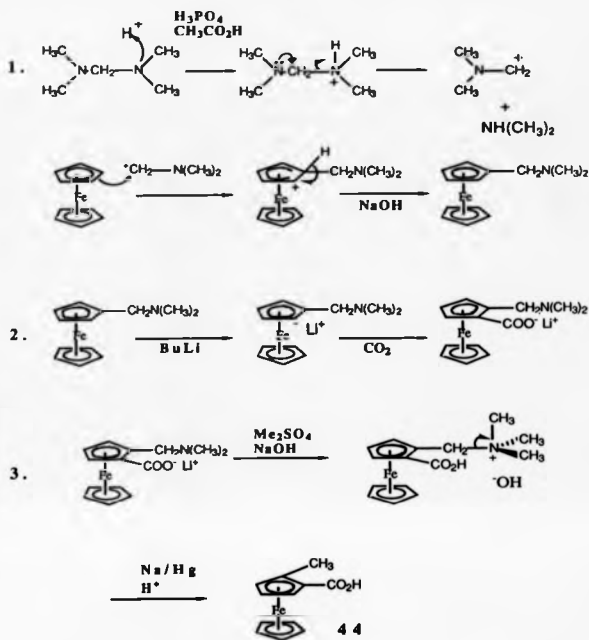
### 2-2 $\alpha$ -Methylferrocenecarboxylic acid.

The synthesis, although long, of  $\alpha$ -methylferrocene carboxylic acid **44** has been well documented.<sup>106,107</sup> Also reported is its chemical resolution into its optical isomers.<sup>77</sup>

The synthesis of  $\alpha$ -methylferrocene carboxylic acid can be divided into three stages (Scheme 6), the first of which was the preparation of N,N-dimethylaminomethylferrocene. This was synthesised by the stirring at 90°C of ferrocene and N,N,N',N'-tetramethylethylenediamine in a mixture of orthophosphoric acid and glacial acetic acid. The solution was cooled, extracted to remove residual ferrocene, and basified by the careful addition of solid NaOH. The solution was extracted to give N,N-dimethylaminomethylferrocene in 89% yield. This intermediate was characterised by its <sup>1</sup>H nmr spectrum, which showed the usual ferrocene protons as a multiplet centred at  $\delta=4.17$ ppm, and a six proton singlet arising from the two uncoupled N-CH<sub>3</sub> groups at  $\delta=2.21$ ppm. The CH<sub>2</sub>-N protons were observed as a singlet at  $\delta=3.33$ ppm.

The second step of the synthesis involved a directed lithiation using BuLi to <sup>(deprotonate)</sup>  $\Lambda$ regiospecifically the mono substituted ferrocene at the *ortho* position. Thus, N,N-dimethylaminomethylferrocene in an ethereal solution of BuLi were stirred together at room temperature for four hours. The reaction mixture was cooled to -100°C and was quenched with CO<sub>2</sub>. After gradual warming to room temperature, the resulting yellow solid was filtered off and washed, giving 52g of a mixture

of ferrocenolithium salts. In the final stage of the synthesis, 40g of the above lithium salts were dissolved in anhydrous methanol, which was made strongly basic by the addition of NaOH pellets. Dropwise addition of dimethyl sulphate gave an unstable trimethylaminoferrocene salt, which was used immediately. Crude  $\alpha$ -methylferrocene carboxylic acid **44** was liberated by the reduction of this salt with a Na/Hg amalgam. Extraction followed by chromatography gave the pure acid **44** in an overall yield of 26%.



Scheme 6

$\alpha$ -Methylferrocenecarboxylic acid **44** was confirmed as the purified product by MS(FAB) which showed the  $[M]^+$  at  $m/z=244$  and  $[M-OH]^+$  at 227. The  $^1H$  nmr spectrum showed a three proton singlet at  $\delta=2.34ppm$ , due to the ferrocene- $CH_3$  group, and the aromatic protons of the unsubstituted ring centred at  $\delta=4.23ppm$ . The substituted ring showed three multiplets of equal intensity (one proton) at  $\delta=4.35$ , 4.43 and 4.85ppm, corresponding to H-(C-3), H-(C-4) and H-(C-5) respectively. Consequently the desired product was concluded to have been formed.

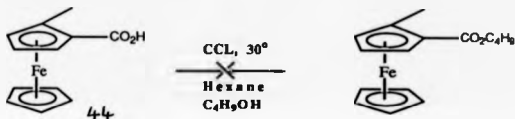
It was found that the addition of 4-5M equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol gave rise to a ~50% splitting of the  $CH_3$  singlet at  $\delta=2.34ppm$ , (shifted upfield to  $\delta=1.87ppm$  in the presence of the solvating agent) as seen by 220MHz  $^1H$  nmr spectroscopy. 400MHz  $^1H$  nmr spectroscopy would give baseline splitting of this signal. Thus, a very simple and straightforward method for the determination of the e.e. of an enzymatic resolution of this compound had been found.

### 2.3 $\alpha$ -Methylferrocenecarboxylic acid methyl ester.

$\alpha$ -Methylferrocenecarboxylic acid methyl ester **67** was synthesised by the straightforward methylation of the acid **44** with diazomethane in diethyl ether. The reaction was quenched by the addition of acetic acid, and upon chromatographic work up gave the pure ester in 78% yield. The desired product was justified by its  $^1H$  nmr spectrum, which was identical in all aspects to that of  $\alpha$ -methylferrocenecarboxylic acid, apart from the presence of a three proton singlet at  $\delta=3.86ppm$  due to the ester

methyl. Also, a slight upfield shift of  $-0.06\text{ppm}$  for each resonance of the spectrum indicated a loss of the acidic function.

The enzymatic hydrolysis of **67** was initially attempted on a small scale. This was dissolved in both 5mls and 10mls of a 20% MeOH solution of buffer, pH 7, with PLE and EEAcE as the biocatalysts. The reactions were followed by TLC, but after stirring for 5 days, no hydrolytic reaction was observed. Sonicating the reaction mixture before the addition of the enzyme was also unsuccessful. Following a method by Klibanov,<sup>108</sup> it was decided to try to esterify the acid **44** with butanol, in hexane, using CCL as the biocatalyst.



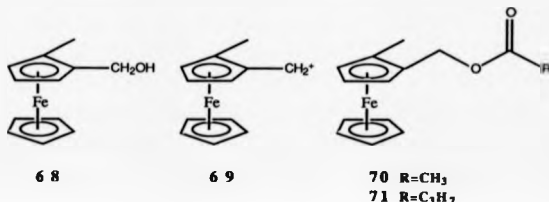
The reaction was again followed by TLC, yet after six days there was no observable reaction so the experiment was discontinued.

It was therefore concluded that this particular ester/acid is not an enzyme substrate under the above conditions. It was decided to synthesise the acetate, and again test for enzyme substrate acceptability.

#### 2-4 $\alpha$ -Methylferrocenemethyl acetate/butyrate.

4g of  $\alpha$ -methylferrocenecarboxylic acid methyl ester **67** was stirred in a slurry of  $\text{LiAlH}_4$  for thirty minutes, using TLC to follow

the reaction. After this time, the reaction had reached completion. It was quenched by the addition of wet ethyl acetate, and the product was purified by column chromatography to give a yield of 98%. The desired product,  $\alpha$ -methylferrocenemethyl alcohol **68** was confirmed by  $^1\text{H}$  nmr, IR, and MS(FAB, nba). The MS did not show the molecular ion, (presumably due to the simultaneous loss of OH forming the stabilised cation **69**) but at  $m/z=365$  a peak was present due to  $[(\text{M-OH})+\text{nba}]^+$ . The peak with relative abundance 100% was at 213, arising from  $[\text{M-OH}]^+$ .



The sea of delocalised electrons due to the aromatic character of the ferrocene rings, will readily stabilise the cation **69**. It was found that both the acetate and the butyrate derivatives of **68** (**70,71**) readily decomposed to give this cation, especially in aqueous or acidic conditions.

The  $^1\text{H}$  nmr spectrum of **68** showed the OH proton at  $\delta=1.38\text{ppm}$ , and a two proton multiplet centred at  $\delta=4.43\text{ppm}$  due to the  $\text{CH}_2$  group. The signal due to  $\text{CH}_3$  appeared at  $\delta=2.01\text{ppm}$ , and the signals due to the aromatic protons were observed at the usual ferrocenyl chemical shifts of  $\delta=4.16\text{ppm}$ . Hence the alcohol **68** was confirmed as the desired product.

$\alpha$ -Methylferrocenemethyl acetate **70** was synthesised by stirring the above alcohol **68** in a basic solution of acetic anhydride at 0°C. It was then worked up via solvent extraction. The desired product was purified by column chromatography (with some decomposition) to give the required acetate **70** in 66% yield.  $^1\text{H}$  nmr spectroscopy confirmed structure **70** as the purified product due to the following observations. The OH proton at  $\delta=1.38\text{ppm}$  of **68** was no longer present, and a three proton singlet at  $\delta=2.05\text{ppm}$  due to the acetyl  $\text{CH}_3$  group was observed. The  $\text{CH}_2$  resonance was shifted downfield with respect to the free alcohol **68** to  $\delta=5.02\text{ppm}$ , as expected. Thus the formation of the desired product was confirmed.

## 2.5 The enzyme-catalysed reactions of $\alpha$ -methylferrocenemethyl acetate.

### (a) In aqueous media.

50mg of  $\alpha$ -methylferrocenemethyl acetate **70** was stirred in a suspension of 20mls buffer pH 7, containing 20% MeOH. To this was added 40 $\mu\text{l}$  PLE. The reaction was followed by TLC, and was worked up after 66 hours by solvent extraction and column chromatography to give 18mg of  $\alpha$ -methylferrocenemethyl alcohol **68**, i.e., 40% hydrolysis had occurred. The rotation of the alcohol was low.  $([\alpha]_D^{25}=+1.5$ . This corresponds to 17% e.e. by comparison to the literature value of the absolute rotation of  $+9^\circ$ ). The recovered material other than the product alcohol was found not to be the starting acetate **70**, but the ferrocene derivative  $\alpha$ -methylferrocenemethoxymethylether **72** 26mg (57% yield, 3% e.e.) of the ether was recovered.

The reaction was repeated, identical in all aspects except that no biocatalyst was added. The same products, in the same yields ( $[\alpha]_D^{+0.25}$  for the alcohol) were recovered. It was concluded that no enzyme catalysed hydrolysis had taken place during the reaction. All the alcohol produced had come from the aqueous-catalysed decomposition of the acetate **70**, and the methyl ether from the quenching of the cation **68** with MeOH present in the solvent. (Fig. 13)

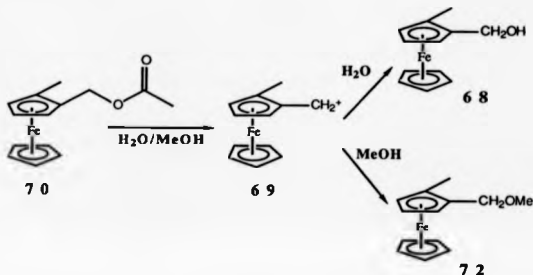


Figure 13

It was decided to carry out the enzyme-catalysed reaction in organic media, to get round the problem of substrate instability in aqueous media.

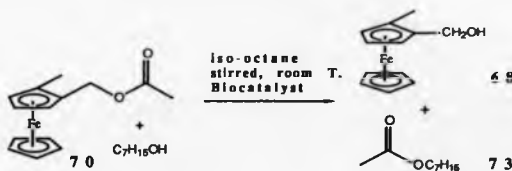
(h) In organic media.

3.6mg of  $\alpha$ -methylferrocenemethyl acetate **70** was dissolved in 1ml of freshly distilled, anhydrous iso-octane to which a 20% molar excess of 1-heptanol had been added. To each reaction flask, ~10mg of enzyme (20 $\mu$ l PLE) was added. The reactions were



stirred at room temperature, and were followed by GLC. Under the GLC conditions used, the ferrocene derivatives 70 and 68 were not observed, so the formation of heptyl acetate and the removal of heptanol were used to follow the reaction. As can be seen from Table 8, the best biocatalysts for this conversion were CCL and PLE. (expts 5,6) The other lipases, PPL, *Aspergillus niger* and *Rhizopus javanicus*, all catalysed the reaction, albeit very slowly.

Heptyl acetate 73 was chemically synthesised in 91% yield under the usual conditions from 1-heptanol. The  $R_T$  given by GLC for this compound was identical to the  $R_T$  for the reaction product in the transesterification reaction, confirming that the transesterification does indeed take place.



Expt.	Substrate	Biocatalyst	Results*
1	6mg	None	No reaction
2	6mg	<i>PPL</i>	Slight reaction
3	5mg	<i>A. niger</i>	Slight reaction
4	4mg	<i>R. javanicus</i>	Slight reaction
5	3mg	<i>CCL</i>	Over 50%
6	3mg	<i>PLE/Sephadex</i>	Good reaction

\* Extent of reaction was determined by GLC. by the appearance of heptylacetate. The reaction proceeded for three weeks.

Table 8

As the ferrocene acetate derivative 70 had been shown to be an enzyme substrate, it was decided to optimise the reaction conditions one step further by synthesising  $\alpha$ -methylferrocenemethyl butyrate 71. The increase in chain length should improve enzyme-acceptability.

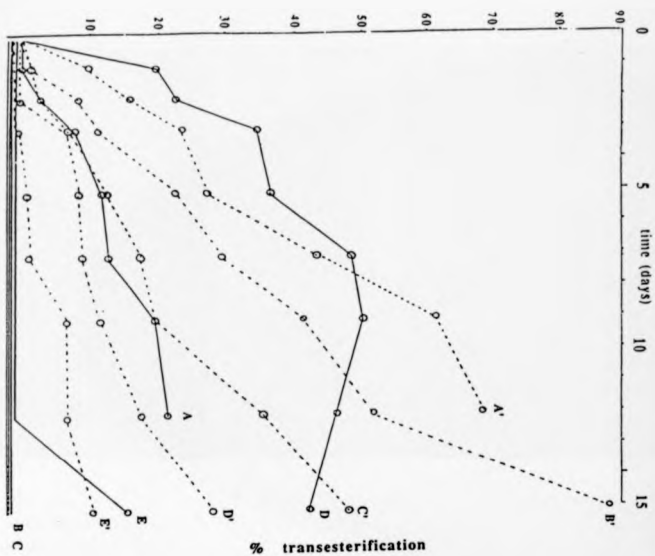
$\alpha$ -methylferrocenemethyl butyrate 71 was synthesised from 68 by stirring with a solution of pyridine, DMAP and butyric anhydride at 0°C for two hours. The product was isolated via

aqueous extraction, and was purified by Kugelrohr distillation to give the butyrate 71 in 70% yield. The MS gave the molecular ion at  $m/z=300$ , and the ion of 100% relative abundance was cation 69 at 213, due to loss of  $C_4H_7O_2$ .  $^1H$  nmr spectroscopy showed a three proton triplet at  $\delta=0.93ppm$  coupled to a two proton multiplet at  $\delta=1.65ppm$  due to  $CH_3$  and  $CH_2$  groups respectively, with the rest of the spectrum being identical to that of the acetate 70.

#### 2.6 The enzyme-catalysed transesterification of $\alpha$ -methylferrocenemethyl butyrate in organic media.

Preliminary reactions were carried out under the following conditions: -10mg of the above butyrate 71 was dissolved in 2mls of anhydrous iso-octane, and 20% molar excess of 1-heptanol was added. The reactions were stirred at room temperature, and ~20mg of the following lipases were added. PPL, *A. niger*, *R. javanicus*, CCL, *Pseudomonas* and the esterase, PLE immobilised on sepharose. The first five lipases were also immobilised on Biofix, (see Appendix) a porous ceramic support, and reactions containing these immobilised biocatalysts were also set up.

The reactions were followed by GLC, (which had been previously calibrated using heptyl butyrate) and the results are shown in Figure 14. It is immediately apparent and also of great interest to note the vast difference in enzyme activity between the lipase immobilised on Biofix and the lipase free in the reaction medium. The most extreme cases to note are those of *A. niger* and *R. javanicus*, where the lipase free in reaction media is totally non reactive, whereas the same lipases upon immobilisation on Biofix become extremely active and the reaction proceeded relatively rapidly to 100%. In general, immobilising the lipase upon Biofix



A PPL  
 B *Aspergillus niger*  
 C *Rhizopus javanicus*  
 D CCL  
 E *Pseudomonas*  
 A' PPL immobilised on Biofix  
 etc.

Figure 14

greatly increased the rate of reaction. The exception to this was with CCL, where the free lipase reacted steadily to ~50% conversion, yet the lipase on Biofix reacted at a much slower rate. PLE on sepharose did not accept 71 as a substrate.

The effect of immobilising a lipase upon Biofix must somehow drastically alter the shape or electronic conformation of the enzyme active site. There was no time to investigate this phenomenon during the course of this thesis, but it is a subject of great interest that is being investigated at present in our laboratories.

It was of interest to determine the enantioselectivities of the lipases towards the transesterification of 71, and to see how these were effected by the immobilisation of the lipase on Biofix.

$\alpha$ -Methylferrocenemethyl butyrate 71 was unstable to chromatography, as it decomposed to give the cation 69 and some alcohol 68. Therefore, chromatographic work-up to separate the reaction products of transesterification was not possible.

To get around this problem, it was decided to <sup>oxidise</sup> firstly the product alcohol *in situ* to the aldehyde 74, and then to deprotect the residual butyrate to the alcohol. Then the two relatively stable ferrocene derivatives could be separated chromatographically, and as racemisation is impossible in such a substituted derivatives, the e.e. of the reaction could be determined. (Fig. 15)

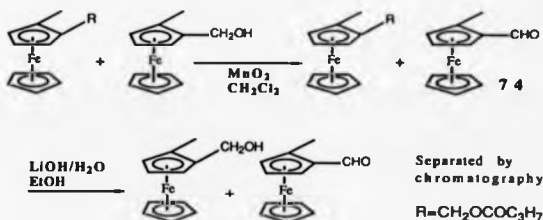
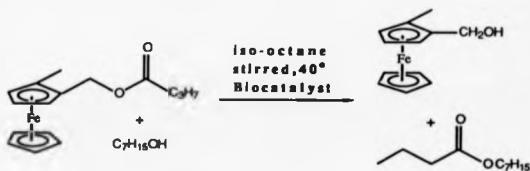


Figure 15

The entire reaction mixture was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> and was added to a stirred suspension of activated MnO<sub>2</sub>.<sup>109</sup> By TLC, the reaction had reached completion after one hour. After filtration, the reaction mixture was added to a solution of water and ethanol containing LiOH.H<sub>2</sub>O, and the reaction was followed by TLC for two hours. This was extracted, dried, and the solvent was removed by rotary evaporation. The alcohol and the aldehyde were separated by column chromatography, and the aldehyde **74** was Kugelrohr distilled prior to analysis.

It was found that the addition of an optimum of five molar equivalents of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol in d<sup>6</sup> benzene to the ferrocene aldehyde derivative **74** gave ~75% splitting of the ferrocene CH<sub>3</sub> singlet at  $\delta$ =2.30ppm by 220MHz <sup>1</sup>H nmr spectroscopy. At 400MHz, this splitting increased to baseline, and the enantiomeric excess of the various enzyme-catalysed transesterification reactions could be determined accurately. (to within 2%)

$\alpha$ -Methylferrocenemethyl butyrate **71** (~100mg) was dissolved in 10mls anhydrous iso-octane, and 60 $\mu$ l of heptanol was added. The reactions were stirred at 40°C upon addition of ~40mg of each lipase. (in the case of the lipases immobilised on Biofix, 40mg of lipase on 400mg of Biofix) The reactions were followed by GLC, which had previously been calibrated with heptyl butyrate, and the results are summarised in Table 9.



Expt	Lipase	Time	Yield (%)	$[\alpha]_D$	e.e. (%)
1	CCL	3 days	17	+115 <sup>c</sup>	50
2	CCL (Biofix)	6 days	50	+130 <sup>c</sup>	42
3	PPL	13 days	40	+7.1 <sup>d</sup>	26
4	PPL (Biofix)	5 days	50	-13 <sup>c</sup>	16
5	<i>A. niger</i> (Biofix)	6 days	50	+55 <sup>c</sup>	32
6	<i>R. javanicus</i> (Biofix)	6 days	50	+36 <sup>c</sup>	14

(a) Yield as determined by calibrated GLC.

(b) e.e. determined via oxidation to aldehyde followed by  $^1\text{H}$  nmr in the presence of chiral solvating agent.

(c)  $c=0.03$ , EtOH, 25°C

(d)  $c=0.014$ , EtOH, 25°C

Table 9

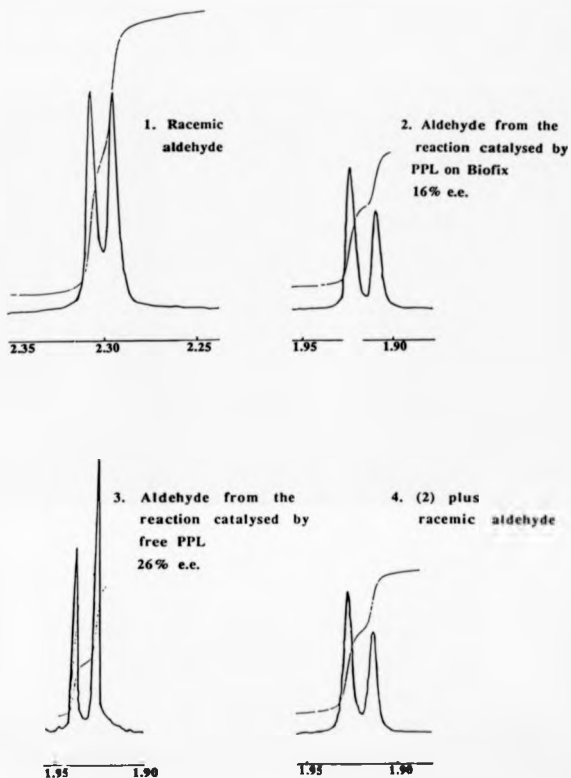
The lipase that gave the best enantioselectivity was CCL, as was expected from the preliminary results. The rotations and e.e. values correlated well (the literature value for the absolute rotation of  $\alpha$ -methylferrocenemethyl aldehyde  $[\alpha]_D=+220^\circ$ ), with the exception of experiment 2. The high rotation must have been due to inaccuracy in measurement, as the e.e. was definitely 42%



(by  $^1\text{H}$  nmr). Another very interesting phenomenon arising from immobilising PPL on Biofix as well as vastly increasing the rate of reaction, was that it reversed the lipase selectivity towards the ferrocene derivative. This was shown both in the rotation, and the  $^1\text{H}$  nmr spectroscopic measurements. (Fig. 16) This again opened up a vast field of speculation towards the role of Biofix in the immobilisation of a lipase.

This section of work has shown that a lipase will readily accept a ferrocene derivative as a substrate, and will react enantioselectively with it. The conditions have ideally still to be optimised to give 100% e.e. in the resolution, but the time was not available to do this for this thesis.

It is hoped that the work for this thesis has shown that the application of enzymes has a future in the resolution of organometallic complexes. Complexes which are difficult to resolve chemically can be straightforwardly resolved once the right conditions have been found. Also the diversity of an enzyme towards its substrate - that it can distinguish not only between centres of chirality, but also between a chiral plane.



100MHz  $^1\text{H}$  nmr spectra of  $\alpha$ -methylferrocenemethyl aldehyde in the presence of (S)-(+)-2,2,2-trifluoro-1-(9-anthryl) ethanol.

Figure 16

## Experimental

### Introduction

All solvents and starting materials were dried and purified prior to use following the procedures laid out in "Purification of Laboratory Chemicals" by D.D. Perrin, W.L.F. Armarego and D.R. Perrin (2nd ed., Pergamon Press, 1985) unless otherwise stated.

The enzymes used were obtained from the sources given below and had the listed activities:

Enzyme	Source	Activity
PLE (EC 3.1.1.1.)	Boehringer	10mg protein/ml solution 130 eu/mg protein
PPL (EC 3.1.1.3.)	Sigma, Type 2	35% protein 35-70 eu/mg protein
CCL (EC 3.1.1.3.)	Sigma, Type 7	35% protein $1.44 \times 10^6$ eu/mg protein
Acetylcholin- esterase (EC 3.1.1.7.)	Sigma, Type 6-S (Electric eel)	200-400 eu/mg protein
<i>Aspergillus niger</i>	Amano	Lipase A "Amano" 6 63.7 eu/mg protein
<i>Rhizopus javanicus</i>	Amano	Lipase N 155eu/mg protein
<i>Pseudomonas</i>	Amano	Lipase P "Amano" 34.5 eu/mg protein

Nmr spectra were recorded using the instruments listed below, operating at the frequencies given in the table:

Spectrometer	Frequency/MHz			
	<sup>1</sup> H	<sup>13</sup> C	<sup>19</sup> F	<sup>31</sup> P
Bruker WH400	400.13	100.62		
Bruker WH90			84.66	
Varian VXR400				161.9
Bruker AC250	200			
Perkin-Elmer R34	220			

The chemical shifts are quoted in ppm from TMS as the internal reference.

The spectral characteristics and physical data of the compounds involved were recorded using the instruments and methods listed below.

IR(CHCl <sub>3</sub> )	Perkin-Elmer 580-B Spectrophotometer
IR(CHBr <sub>3</sub> )	Perkin-Elmer 177
MS	Kratos MS80
[α]	Optical Activity Ltd. AA-1000 Polarimeter Rotations measured at 23°C, λ as specified using a 2dm path length cell
cd*	Jasco J40 CS Spectropolarimeter
mp	Gallenkamp apparatus, quoted uncorrected
X-Ray**	Nicolet P2 <sub>1</sub>

GLC	PYE-204 Gas Chromatograph. Columns 6ft length, N <sub>2</sub> as carrier gas at a flow rate of 30ml/min.
pH-Stat	Radiometer Copenhagen RTS882 recording titration system
TLC	Merck Kieselgel F254 on 0.2mm precoated plates. Spot detection by UV fluorescence quenching, potassium permanganate spray or phosphomolybdic acid/ethanol spray
Column Chromatography	Flash chromatography performed on Merck Kieselgel 60 silica gel (230-400 mesh). Approximately 100g silica to 1g compound. Flow of solvent through the column was approximately 4cm/min.

Light petroleum refers to the fraction boiling range 40-60°C unless otherwise stated.

\* Measured by Dr. A.F. Drake, Birkbeck College

\*\* Determined by Dr. N.W. Alcock, University of Warwick

### Chapter 3

#### Assay of PLE<sup>98</sup>

To 10ml of 0.0125M ethyl butyrate was added 100 $\mu$ l of PLE at 18°C. The reaction was stirred and the pH kept constant at 7.5 by the continuous additions of 0.01M NaOH from an autotitrator. After one and a half hours, the reaction was terminated. At optimum enzyme activity, *i.e.* the first 10% of reaction, PLE hydrolysed  $1.398 \times 10^{-6}$  moles of ethyl butyrate in one minute. Therefore, 1.398 $\mu$ moles of ethyl butyrate is hydrolysed by 100 $\mu$ l PLE per minute, *i.e.* 1 $\mu$ mole of ethyl butyrate is hydrolysed by 71.53 $\mu$ l PLE in one minute. Therefore, one enzyme unit (e.u.)=71.53 $\mu$ l PLE (literature value=77 $\mu$ l)

#### Methyl sorbate

50.021g (0.446 moles) of sorbic acid was added to a solution of 35ml acetyl chloride in 90mls dry MeOH at 0°C. The mixture was stirred at 40-50°C for six hours. The product was isolated *via* fractional distillation (77°C, 60mm Hg, lit. bp 180°C, 70<sup>20</sup>) to give 46.441g (83%) yield of methyl sorbate.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 1.87(d, J<sub>6,5</sub>=4.9, 3H, H-(C-6)); 3.76(s, 3H, H-(C-1')); 5.81(d, J<sub>2,3</sub>=16.7, 1H, H-(C-2)); 6.18(m, 2H, H-(C-5,4)); 7.29(dd, J<sub>3,2</sub>=16.2, J<sub>3,4</sub>=9.8, 1H, H-(C-3)).

#### Methyl sorbate irontricarbonyl<sup>31,27</sup>

3.5ml (0.027 moles) methyl sorbate in 25ml di-n-butylether with 2.5% by weight anhydrous AlCl<sub>3</sub> (0.082g) were heated under nitrogen to 100°C. To this was added 6mls Fe(CO)<sub>5</sub> (0.046 moles, 1.7 molar equivalents) and the mixture was refluxed (140°C)

under  $N_2$  for twelve hours. The solvent was removed and the resulting mixture eluted with 6% EtOAc in light petroleum (b.pt. 60-80) through 200g silica gel chromatography column to yield 2.409g (33.5%, lit. yield 33%) methyl sorbate irontricarboxyl.

$^1H$  nmr: (400MHz,  $CDCl_3$ ) 0.965(dd,  $J_{2,3}=8$ ,  $J_{4,5}=1.2$ , 1H, H-(C-2)); 1.450(s, 3H, H-(C-6)); 1.451(m, 1H, H-(C-5)); 3.635(s, 3H, H-(C-1)); 5.21(ddd,  $J_{4,5}=8$ ,  $J_{4,3}=5$ ,  $J_{4,2}=1.2$ , 1H, H-(C-4)); 5.76(ddd,  $J_{3,2}=8$ ,  $J_{3,4}=5$ ,  $J_{3,5}=1.2$ , 1H, H-(C-3)).

IR: ( $CHCl_3$ ) 2062 $cm^{-1}$ (s); 1998 $cm^{-1}$ (s); 1980 $cm^{-1}$ (s); 1710 $cm^{-1}$ (w); 1650 $cm^{-1}$ (w).

#### Hydrolysis of methyl sorbate with PLE

To 0.0162g ( $1.28 \times 10^{-4}$  moles) methyl sorbate dissolved in 10mls 0.1M phosphate buffer pH 8.02 was added 100 $\mu$ l (130 e.u.) PLE. The reaction mixture was stirred at 16°C for 26 minutes, the pH kept constant at 8.02 by the continuous addition of 0.01M NaOH from an autotitrator. 14.86mls 0.01M NaOH were added during the course of the reaction. The reaction mixture was acidified with 1M HCl to pH 1 followed by 3x20mls  $Et_2O$  extractions. The organic layer was dried over anhydrous  $MgSO_4$  and the ether removed by rotary evaporation. Preparative TLC with 80% light petroleum (bp 60-80) and 20%  $Et_2O$  separated out pure sorbic acid. (12mg, 84%)

$^1H$  nmr: (220MHz,  $CDCl_3$ ) 1.85(d,  $J_{5,6}=4.88$ , 3H, H-(C-6)); 5.8(d,  $J_{2,3}=15.64$ , 1H, H-(C-2)); 6.23(m, 2H, H-(C-5,4)); 7.34(dd,  $J_{3,4}=9.77$ ,  $J_{3,2}=16.62$ , 1H, H-(C-3)).

#### Immobilisation of PLE on sepharose beads<sup>6</sup>

1ml sepharose (purchased from Sigma, an aqueous suspension of 60-140 $\mu$ m beads, agarose concentration 4%) was washed with

water, then with 0.1M phosphate buffer pH 8.02. This was dried by suction on a glass filter, and the resulting resin cut into 2mm by 2mm squares. This was added to 1ml (1300 e.u.) of PLE and was left overnight at 4°C. The sepharose totally absorbed the enzyme.

GLC conditions and retention times for the following experiments

All temperature programmed runs on 3% SE 30

For A, B: Column temperature 55-200°C, 24°C per minute, 4 minutes initial temperature, 2 minutes final temperature.

For C: Column temperature 90-200°C, 16°C per minute, 3 minutes initial temperature, 2 minutes final temperature.

The retention times for the various compounds are as follows:

A: methyl propionate 89s; 3-methoxy 1-butanol 239s; 3-methoxy butylpropionate 364s

B: methyl propionate 8s; heptanol 320s; heptyl propionate 419s.

C: Heptanol 212s; methyl sorbate 247s; heptyl sorbate 626s.

A. Transesterification of methyl propionate and 3-methoxy 1-butanol with PLE<sup>6</sup>

To 3g (0.03 moles) 3-methoxy 1-butanol, 10g (0.1 moles) methyl propionate, 5 drops 0.1M phosphate buffer pH 8.1 was added 0.89ml (1157 e.u.) immobilised PLE. The reaction was stirred at room temperature for 19 hours, monitored by GLC. The enzyme was removed by filtration, washed with Et<sub>2</sub>O and the reaction mixture was extracted with 3x20mls H<sub>2</sub>O. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, then the solvent removed by rotary evaporation, to give the product in 42% (1.94g) yield.



<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 1.14(m, 6H, H-(C-3,4')); 1.77(m, 2H, H-(C-2)); 2.33(q, J<sub>2,3</sub>=7.35, 2H, H-(C-2)); 3.32(s, 3H, H-(C-5)); 3.41(m, 1H, H-(C-3')); 4.18(t, J<sub>1,2</sub>=6.86, 2H, H-(C-1')).  
[α]<sub>D</sub>=-19.2 (neat) (lit. [α]<sub>D</sub>=-16.3, neat).

B. Transesterification of methyl propionate and 1-heptanol

0.853g (0.01 moles) methyl propionate plus 4.452g (0.038 moles) 1-heptanol, 3 drops 0.1M phosphate buffer pH 8.1 were stirred with 297μl (373 e.u.) of immobilised PLE. The reaction was stirred at room temperature, and followed by GLC. After 68 hours the enzyme was removed by filtration, washed with ether and the reaction mixture was eluted down a chromatography column (350g silica gel, eluant 95% light petroleum bp 60-80, 5% Et<sub>2</sub>O). This gave 0.772g (45%) yield of heptyl propionate.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 0.88(m, 3H, H-(C-7)); 1.15(t, J<sub>3,2</sub>=6.9, 3H, H-(C-3)); 1.31(m, 8H, H-(C-3',4',5',6')); 1.59(m, 2H, H-(C-2)); 2.34(q, J<sub>2,3</sub>=6.9, 2H, H-(C-2)); 4.08(t, J<sub>1,2</sub>=5.4, 2H, H-(C-1')).

MS(EI): m/z= 173[MH]<sup>+</sup>; 115; 98[CH<sub>2</sub>CH(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>]; 75; 57[75-H<sub>2</sub>O]<sup>+</sup>; 41; 29.

C. Transesterification of 1-heptanol and methyl sorbate

To 0.5ml (0.004 moles) methyl sorbate, 1.14ml (0.01 moles) 1-heptanol, 50ml pH 8.02 0.2 M phosphate buffer, was added 133μl (173 e.u.) PLE immobilised on sepharose beads. The mixture was stirred at room temperature for 86 hours, then the enzyme was filtered off and the reaction mixture eluted down 100g chromatography column. (eluant: 95% light petroleum bp 60-80, 5% Et<sub>2</sub>O) 0.048g (6% yield) of heptyl sorbate was collected.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 0.88(m, 3H, H-(C-7)); 1.29(m, 8H, H-(C-3',4',5',6')); 1.64(m, 2H, H-(C-2'')); 1.85(d, J<sub>6,5</sub>=4.9, 3H, H-(C-6)); 4.14(t, J<sub>1',2</sub>=5.88, 2H, H-(C-1')); 5.79(d, J<sub>2,3</sub>=14.7, 1H, H-(C-2)); 6.17(m, 2H, H-(C-5,4)); 7.26(dd, J<sub>3,2</sub>=16.7, J<sub>3,4</sub>=9.8, 1H, H-(C-3)).

Irontricarboxyl (2,4-hexadien-1-ol)<sup>32</sup> 59

A solution of 1.188g (0.0045 moles) irontricarboxyl methyl sorbate in 2ml anhydrous Et<sub>2</sub>O was added with stirring to a slurry of 0.176g (0.0046 moles) LiAlH<sub>4</sub> in 10ml anhydrous Et<sub>2</sub>O at -78°C under a blanket of N<sub>2</sub>. The reaction mixture was stirred at -78°C for 2 hours. The reaction was followed by TLC (30% Et<sub>2</sub>O, 70% light petroleum), and after this time, all visible starting material had been used up. The reaction was quenched by the addition of 3ml EtOAc, was allowed to warm to room temperature and the reaction was filtered. Water added then it was extracted 4x50mls Et<sub>2</sub>O. The organic layer was dried by filtration through anhydrous MgSO<sub>4</sub>, and the solvent removed by rotary evaporation to give 0.935g (87% yield) of irontricarboxyl (2,4-hexadien-1-ol).

<sup>1</sup>H nmr: (200MHz, CHCl<sub>3</sub>) 1.1(q, J<sub>2,3</sub>=5.88, 1H, H-(C-2)); 1.27(m, 1H, H-(C-5)); 1.44(d, J<sub>6,5</sub>=7.35, 3H, H-(C-6)); 1.58(t, J<sub>1',1</sub>=4.9, 1H, H-(C-1')); 3.67(m, 2H, H-(C-1)); 5.13(m, 2H, H-(C-3,4)).

IR: (CHCl<sub>3</sub>) 3621cm<sup>-1</sup>(w); 3437cm<sup>-1</sup>(w); 2927cm<sup>-1</sup>(w); 2055cm<sup>-1</sup>(s); 1977cm<sup>-1</sup>(s).

MS(FAB): m/z= 238[M]<sup>+</sup>; 221[M-OH]<sup>+</sup>; 210[M-CO]<sup>+</sup>; 193[M-(CO+H<sub>2</sub>O)]<sup>+</sup>; 182[M-(CO)<sub>2</sub>]<sup>+</sup>; 165[M-((CO)<sub>2</sub>+OH)]<sup>+</sup>; 154[M-(CO)<sub>3</sub>]<sup>+</sup>; 136[M-((CO)<sub>3</sub>+H<sub>2</sub>O)]<sup>+</sup>.

Irontricarboxyl (2,4-hexadien-1-ol) acetate 58

To an ice cold solution of 0.474mg (1.99mmoles) irontricarboxyl (2,4-hexadien-1-ol) in 3ml anhydrous Et<sub>2</sub>O was added with stirring a solution of 2.344ml (14.5M equivalents) pyridine, 1ml (5M equivalents) Ac<sub>2</sub>O in 2ml anhydrous Et<sub>2</sub>O. This was stirred at 0°C for 8 hours, after which time there was no starting material left as shown by TLC. (eluant: 80% light petroleum, 20% Et<sub>2</sub>O) The reaction was allowed to warm to room temperature, then basified with saturated NaHCO<sub>3</sub> and extracted with Et<sub>2</sub>O. The pyridine was removed by washing the organic layer with 0.1M HCl, and the combined organic phase was dried over anhydrous MgSO<sub>4</sub>. The solvent was removed by rotary evaporation. This gave 0.512g (92%) yield of pure irontricarboxyl (2,4-hexadien-1-ol) acetate

<sup>1</sup>H nmr: (400MHz, CDCl<sub>3</sub>) 0.08(q, J<sub>4,5</sub>=7, 1H, H-(C-2)); 1.25(m, 1H, H-(C-5)); 1.4(d, J<sub>6,5</sub>=6.4, 3H, H-(C-6)); 2.05(s, 3H, H-(C-2')); 4.075(d, J<sub>1,2</sub>=7, 2H, H-(C-1)); 5.05(dd, J<sub>4,3</sub>=4.5, 1H, H-(C-4)); 5.2(dd, J<sub>3,4</sub>=4.2, 1H, H-(C-3)).

<sup>13</sup>C nmr: (100.62MHZ, CDCl<sub>3</sub>) 19.0(C-6); 20.8(C-2'); 54.2(C-2); 58.4(C-5); 66.0(C-1); 82.8(C-3); 86.4(C-4); 170.6(C-1'); 211.4(CO).

IR: (CHCl<sub>3</sub>) 2950cm<sup>-1</sup>(w); 2056cm<sup>-1</sup>(s); 1981cm<sup>-1</sup>(s); 1734cm<sup>-1</sup>(s); 1451cm<sup>-1</sup>(w); 1386cm<sup>-1</sup>(w); 1370cm<sup>-1</sup>(w); 1031cm<sup>-1</sup>(w).

MS (FAB): m/z= 280[M]<sup>+</sup>; 252[M-CO]<sup>+</sup>; 224[M-(CO)<sub>2</sub>]<sup>+</sup>; 196[M-(CO)<sub>3</sub>]<sup>+</sup>; 134; 91; 81; 56.

UV: λ<sub>max</sub> 307nm; 225nm; 210nm

Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>FeO<sub>5</sub>: C, 47.3; H, 4.5; Fe, 19.7. Found: C, 47.18; H, 4.32; Fe, 19.94.

Irontricarboxyl (2,4-hexadien-1-ol) Mosher ester

0.238g (1mmole) of irontricarboxyl(2,4-hexadien-1-ol) was stirred with a solution of 3ml pyridine, 235ml (1.5M equivalents) MTPA-Cl in 3ml anhydrous  $\text{CCl}_4$  under  $\text{N}_2$  at room temperature overnight. After this time, the reaction had reached completion by TLC. (eluant 80% light petroleum, 20%  $\text{Et}_2\text{O}$ ,  $R_F=0.06, 0.45$ ) The reaction mixture was poured into water and extracted  $3 \times \text{CHCl}_3$ . The combined organic layers were washed  $2 \times 0.5\text{M}$  HCl followed by  $1 \times$  saturated  $\text{Na}_2\text{CO}_3$ . It was dried over anhydrous  $\text{MgSO}_4$ , and solvent removed by rotary evaporation to give 0.549g of product. This was one spot pure by TLC thus no further purification was given. (It had previously been shown to decompose on preparative TLC).

$^1\text{H}$  nmr: (400MHz,  $\text{CDCl}_3$ ) 0.94(m, 1H, H-(C-2)); 1.25(m, 1H, H-(C-5)); 1.42(d,  $J_{6,5}=6$ , 3H, H-(C-6)); 3.545(s, 3H, H-(OCH<sub>3</sub>)); 4.225(m, 1H, H-(C-1)); 4.357(m, 1H, H-(C-1)); 5.075(m, 1H, H-(C-4)); 5.225(m, 1H, H-(C-3)); 7.40(m, 3H, H-(aromatic)); 7.50(m, 2H, H-(aromatic)).

$^{13}\text{C}$  nmr: (100.62MHz,  $\text{CDCl}_3$ ) 19.0(C-6); 51.6(C-2); 55.4(OCH<sub>3</sub>); 58.8(C-5); 68.2(C-1); 83.1(C-3); 87.2(C-4); 121.6(C-2'); 124.5(CF<sub>3</sub>); 127.2; 128.2; 129.4(aromatic); 166.2(C-1'); 211.0(CO).

$^{19}\text{F}$  nmr: (84.66MHz,  $\text{CDCl}_3$ ) 71.365(s, 3F, F-(F<sub>3</sub>-C)); 71.284(s, 3F, F-(F<sub>3</sub>C')).

IR: ( $\text{CHCl}_3$ ) 2960 $\text{cm}^{-1}$ (w); 2060 $\text{cm}^{-1}$ (s); 1990 $\text{cm}^{-1}$ (s); 1750 $\text{cm}^{-1}$ (m).

MS(FAB gly, EtOH):  $m/z = 426[\text{M}-\text{CO}]^+$ ; 395 $[\text{M}-(\text{CO}+\text{OMe})]^+$ ; 370 $[\text{M}-(\text{CO})_3]^+$ .

Anal. Calcd. for  $\text{C}_{19}\text{H}_{17}\text{FFeO}_6$ : C, 50.52; H, 3.77; F, 12.55; Fe, 12.30.

Found: C, 50.46; H, 4.03; F, 12.44; Fe, 12.65.

Enzymatic hydrolysis of irontricarbonyl (2,4-hexadien-1-ol acetate).

1. Preliminary experiment.

50mg of irontricarbonyl (2,4-hexadien-1-ol) acetate was suspended in 2mls 0.1M phosphate buffer pH 7. To this was added 10mg of lipase (or 40 $\mu$ l PLE) and the reaction was stirred at room temperature. It was followed by TLC (eluant: 80% light petroleum, 20% Et<sub>2</sub>O,  $R_F$ =0.04, 0.35). After 21 hours, considerable reaction had taken place.

Typical work-up: Basification of reaction mixture to pH 10 followed by 3xEt<sub>2</sub>O extraction. The organic layer was dried over anhydrous MgSO<sub>4</sub>, and the solvent removed by rotary evaporation. Preparative TLC (eluant as above) separated acetate from alcohol. The removed bands were extracted by stirring in Et<sub>2</sub>O for 10 minutes. This gave 42.5% irontricarbonyl(2,4-hexadien 1-ol) when the lipase used in the reaction was LP 150.

2. Conditions for experiment 10 (Fig. 9).

0.262mg irontricarbonyl complex 58 was suspended in a solution of 63ml 0.1M phosphate buffer pH 7 and 7ml MeOH. To this was added 45mg crude PPL and the reaction was stirred at room temperature, followed by the continuous addition of 0.1M NaOH from an autotitrator. The reaction had reached 47% hydrolysis after 48 hours, and was worked up as above (except column chromatography, AlOx, Grade V, 20g) to give 86mg irontricarbonyl alcohol and 113mg recovered acetate 58.

Inhibited PPL<sup>103</sup>

5g of crude PPL was stirred at 0-5°C for 30 minutes in 20ml 0.2M phosphate buffer pH 7.24. To this was added a solution of 10.5mg

(phenylmethyl)sulphonylfluoride (PMSF) in 0.25ml EtOH during a period of several minutes. This was stirred at the same temperature for a further two hours, then lyophilised overnight. The resultant powder was ground up and stored at 4°C.

Enzymatic transesterification of irontricarbonyl(2,4-hexadien-1-ol) acetate and 1-heptanol in heptane.

Typical run: 95mg ( $3.39 \times 10^{-4}$  moles) of irontricarbonyl acetate **58** and 77mg ( $6.62 \cdot 10^{-4}$  moles) 1-heptanol were stirred at room temperature in 2 ml heptane. To this was added 10 $\mu$ l 0.1M phosphate buffer pH 8, and 100 $\mu$ l PLE immobilised on sepharose. (or 50mg powdered lipase). The reactions were followed by GLC. (GLC conditions: Column 3% SE 30; temperature programmed run; 100-200°C, 12°C per minute, 1 minute initial temperature.  $R_T$  of acetate=499s, alcohol **59** 438s, 1-heptanol 110s.)

After 40 hours, the heptane was removed by high vacuum rotary evaporation at 30°C, and the products were separated from reactants by preparative TLC. (eluant 50% light petroleum, 50% Et<sub>2</sub>O) The bands were scraped off and extracted by stirring with Et<sub>2</sub>O for 2 hours, to give 58mg starting acetate **58** (61.1%) and 13mg irontricarbonyl alcohol. **59** (16.1%) Heptyl acetate gave the following spectra:

**<sup>1</sup>H nmr:** (220MHz, CDCl<sub>3</sub>) 0.9(m, 3H, H-(C-7)); 1.32(m, 8H, H-(C-3,4,5,6)); 1.64(m, 2H, H-(C-2)); 2.05(s, 3H, H-(C-2')); 4.08(t,  $J_{1,2}=5.9$ , 2H, H-(C-1)).

**IR:** (CHCl<sub>3</sub>) 3024cm<sup>-1</sup>(m); 2960cm<sup>-1</sup>(s); 2938cm<sup>-1</sup>(s); 2860cm<sup>-1</sup>(m); 1730cm<sup>-1</sup>(s); 1470cm<sup>-1</sup>(m); 1390cm<sup>-1</sup>(m); 1370cm<sup>-1</sup>(m); 1250cm<sup>-1</sup>(s); 1040cm<sup>-1</sup>(m).

MS(EI):  $m/z$  = 159[MH]<sup>+</sup>; 116[C<sub>7</sub>H<sub>15</sub>OH]<sup>+</sup>; 98[CH<sub>2</sub>=CHC<sub>5</sub>H<sub>11</sub>]<sup>+</sup>; 83[98-CH<sub>3</sub>]<sup>+</sup>; 70[98-C<sub>2</sub>H<sub>4</sub>]<sup>+</sup>; 61; 56[70-CH<sub>2</sub>]<sup>+</sup>; 43[98-C<sub>4</sub>H<sub>7</sub>]<sup>+</sup>(100%).

2-Carboethoxy-1,3-butadiene irontricarbonyl<sup>104,60</sup>

80g (0.305 moles) of recrystallised triphenylphosphine was dissolved in 150ml dried, distilled benzene and to this was added with stirring under N<sub>2</sub> 39.6ml (0.305 moles) ethyl-2-bromopropionate. This was stirred at 70°C for 16 hours, after which it was cooled, forming a white precipitate. This was filtered off, and dried for 48 hours at high vacuum. The precipitate was dissolved in one litre of water, and was again filtered. 2M NaOH was added to the filtrate, precipitating yellow crystals which were filtered off and dried in a desiccator over P<sub>2</sub>O<sub>5</sub>. The product was recrystallised from anhydrous ethyl acetate, to give 65.131g (60% yield) of ylid.

70g (0.193 moles) of the phosphonium ylid was dissolved with heating and stirring under N<sub>2</sub> in 225ml anhydrous THF to give a yellow solution. To this was added dropwise a solution of acetyl chloride (7ml, 0.1 moles) in 35ml anhydrous THF over a period of 30 minutes. The solution was refluxed under N<sub>2</sub> for four hours. After cooling to 0°C, the resulting brown precipitate was filtered off and was rinsed well with anhydrous Et<sub>2</sub>O. The solvent was removed via fractional distillation. (42-67°C) The brown residue was again shaken with 130ml anhydrous, ice-cold light petroleum and was filtered. The solvents were removed by fractional distillation. (40-60°C) The product allene was purified via Kugelrohr distillation (70°C, 40mm Hg) to give a clear and colourless liquid weighing 7.606g. (60.3% yield.)

7.606g (0.0603 moles) of allene was stirred under  $N_2$  in 500ml of anhydrous benzene, and was heated to boiling. To this was cautiously added 30.708g (1.4M equivalents)  $Fe_2(CO)_9$  and the mixture was kept boiling for 10 minutes. 49ml (6.57M equivalents)  $BF_3Et_2O$  was added dropwise, and the whole mixture was refluxed for four hours. After cooling, the solvent was removed via distillation (16°C, water pump pressure) to give a black oil. This oil was dissolved in a minimum of  $Et_2O$  and passed through ~3"  $AlOx$  to give a red-orange liquid. This was eluted down 500g flash silica column (eluant 5%  $Et_2O$ , 95% light petroleum) to give 5.1g (32% yield) of 2-carboethoxy-1,3-butadiene irontricarboxyl.

$^1H$  nmr: (400MHz,  $CDCl_3$ ) 0.225(m, 1H, H-(C-1endo)); 0.4(dm,  $J_{4,3(trans)}=9.8$ , 1H, H-(C-4endo)); 1.31(t,  $J_{2,1}=7$ , 3H, H-(C-2)); 1.96(dm,  $J_{4,3(cis)}=6.2$ , 1H, H-(C-4exo)); 2.425(m, 1H, H-(C-1exo)); 4.305(m, 2H, H-(C-1')); 6.25(t,  $J_{3,4}=8.4$ , 1H, H-(C-3)).

IR: ( $CHCl_3$ ) 2060 $cm^{-1}$ (s); 1990 $cm^{-1}$ (s); 1716 $cm^{-1}$ (m).

MS(FAB):  $m/z=266[M]^+$ ; 238[M-CO] $^+$ ; 210[M-(CO) $_2$ ] $^+$ ; 182[M-(CO) $_3$ ] $^+$ (100%); 221[238-OH] $^+$ ; 137[M-OC $_2$ H $_5$ ] $^+$ .

#### 2-Alkoxy-1,3-butadiene irontricarboxyl<sup>methyl</sup> 113 62

1.996g (7.503mmoles) 2-carboethoxy-1,3-butadiene irontricarboxyl was dissolved in 4ml anhydrous  $Et_2O$  and added dropwise with stirring to a solution of 0.29g (7.64mmoles)  $LiAlH_4$  in anhydrous  $Et_2O$  at -78°C. The reaction was stirred under  $N_2$  for two hours. The reaction was quenched by the addition of 6ml wet EtOAc, and brought to room temperature. It was filtered, and extracted 3x $Et_2O$ , dried over anhydrous  $MgSO_4$  and the solvent removed by rotary evaporation. The products were separated by



passage through 200g flash silica chromatography column (eluant: 20% Et<sub>2</sub>O, 80% light petroleum) to give 1.273g (76% yield) of the desired alcohol. (2% aldehyde formed as a by-product.)

<sup>1</sup>H nmr: (200MHz, CDCl<sub>3</sub>) 0.23(dd, J<sub>4,4</sub>=2.5, J<sub>4,3</sub>=10, 1H, H-(C-4<sub>endo</sub>)); 0.32(d, J<sub>1,1</sub>=2.5, 1H, H-(C-1<sub>endo</sub>)); 1.79(dd, J<sub>4,4</sub>=2.5, J<sub>4,3</sub>=10, 1H, H-(C-4<sub>exo</sub>)); 1.9(m, 2H, H-(C-1<sub>exo</sub>), H-(OH)); 4.26(dd, J<sub>2,2</sub>=15, J<sub>2,2'</sub>=7.5, 1H, H-(C-1'))); 4.53(dd, J<sub>2,2</sub>=15, J<sub>2,2'</sub>=10, 1H, H-(C-1'))); 5.55(t, J<sub>3,4</sub>=10, 1H, H-(C-3)).

IR: (CHCl<sub>3</sub>) 3570cm<sup>-1</sup>(w); 2050cm<sup>-1</sup>(s); 1975cm<sup>-1</sup>(s).

MS(FAB): m/z = 224[M]<sup>+</sup>; 207[M-OH]<sup>+</sup>; 196[M-CO]<sup>+</sup>; 179[M-(CO+OH)]<sup>+</sup>; 168[M-(CO)<sub>2</sub>]<sup>+</sup>; 151[M-((CO)<sub>2</sub>+OH)]<sup>+</sup>; 140[M-(CO)<sub>3</sub>]<sup>+</sup>.

#### 2-Acetoxy-<sup>metring</sup>1,3-butadiene irontricarboxyl 61

To 1g (4.46mmoles) 2-alkoxy-<sup>metring</sup>1,3-butadiene irontricarboxyl in 6ml anhydrous Et<sub>2</sub>O at 0°C was added with stirring a solution of 2.16ml (6M equivalents) pyridine, 0.84ml (2M equivalents) Ac<sub>2</sub>O in 4mls dry Et<sub>2</sub>O at 0°C. This was stirred under N<sub>2</sub> at 0°C for 20 hours. By TLC (eluant 20% Et<sub>2</sub>O, 80% light petroleum, R<sub>F</sub> of acetate=0.41) the reaction had reached completion after this time. The reaction mixture was basified to pH 8-9 with saturated NaHCO<sub>3</sub>, and extracted 3xEt<sub>2</sub>O. The combined ether layers were washed with 3x50mls 0.2M HCl, followed by 1x50ml brine. The organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and the solvent removed to give 0.943g. (79.5% yield) The product was further purified by column chromatography to give 0.905g yellow oil. (76%)

<sup>1</sup>H nmr: (200MHz, CDCl<sub>3</sub>) 0.20(dd, J<sub>4,4</sub>=2.5, J<sub>4,3</sub>=9, 1H, H-(C-4<sub>endo</sub>)); 0.29(d, J<sub>1,1</sub>=2.5, 1H, H-(C-1<sub>endo</sub>)); 1.76(dd, J<sub>4,4</sub>=2.5, J<sub>4,3</sub>=6.5, 1H, H-(C-4<sub>exo</sub>)); 1.9(d, J<sub>1,1</sub>=2.5, 1H, H-(C-1<sub>exo</sub>)); 2.12(s,

3H, H-(C-3')); 4.80(q,  $J_{1,1}=11$ , 2H, H-(C-1')); 5.47(t,  $J_{3,4}=7.5$ , 1H, H-(C-3)).

$^{13}\text{C}$  nmr: (100.62MHz,  $\text{CDCl}_3$ ) 20.8(C-3'); 38.8(C-1 or 4); 41.2(C-1 or 4); 66.7(C-1'); 55.7(C-3); 99.4(C-2); 170.5(C-2'); 210.7(CO).

IR: ( $\text{CHBr}_3$ )  $2900\text{cm}^{-1}(\text{w})$ ;  $2050\text{cm}^{-1}(\text{s})$ ;  $1975\text{cm}^{-1}(\text{s})$ ;  $1735\text{cm}^{-1}(\text{m})$ .

MS(FAB):  $m/z=266[\text{M}]^+$ ;  $238[\text{M}-\text{CO}]^+$ ;  $210[\text{M}-(\text{CO})_2]^+$ ;  $182[\text{M}-(\text{CO})_3]^+$ ;  $149[182-(\text{CH}_3+\text{H}_2\text{O})]^+$ .

UV: ( $\text{CH}_3\text{CN}$ )  $\lambda_{\text{max}}$  185nm; 285nm.

Enzymatic hydrolysis of 2-Acetoxy-<sup>methy</sup>l-3-butadiene irontricarboxyl.  
38mg ( $1.43 \times 10^{-4}$  moles) of irontricarboxyl complex 61 was stirred in 20ml 0.2M phosphate buffer pH 7. To this was added 20mg *Aspergillus niger*, and the reaction mixture was stirred at room temperature for 20 hours. The reaction was basified to pH 12 with 1M NaOH and extracted  $3 \times \text{Et}_2\text{O}$ . The combined organic layers were dried over anhydrous  $\text{MgSO}_4$ , and the solvent removed by rotary evaporation. A pipette column of ~2g  $\text{AlOx}$  (grade 1, neutral, activated) was used (eluant 80% light petroleum, 20 — 100%  $\text{Et}_2\text{O}$ ) to separate the product alcohol 62 from the starting acetate. 61. This gave 18.5mg (58% irontricarboxyl alcohol 62 and 16mg (42%) recovered acetate 61.

Dioxaphospholane derivative of 2-Alkoxy-<sup>methy</sup>l-3-butadiene irontricarboxyl<sup>105</sup>

To a solution of 7.5mg ( $3.3 \times 10^{-4}$  moles) irontricarboxyl alcohol 62 in freshly distilled, anhydrous benzene (0.33mls) was added 69 $\mu$ l triethylamine (1.5M equivalents) and 4mg (0.1M equivalents) DMAP dissolved in 200ml anhydrous benzene. 45 $\mu$ l (1.05M

equivalents) of 2-chloro-4(R),5(R)-dimethyl-2-oxo-1,3,2-dioxaphospholane was added, and the mixture was shaken for 30 seconds. It was left standing for several hours, then  $^{31}\text{P}$  nmr was run, showing two diastereomeric singlets.

$^{31}\text{P}$  nmr: (161.9MHz,  $\text{d}^6\text{Benzene}$ , proton decoupled)  $\delta=14.688$ , 14.678ppm. Ratio of peak intensities : 50.1:49.9

2-((S)-(-)- $\alpha$ -methylbenzylamido)-1,3-butadiene irontricarbonyl  
6.5

20mg ( $8.404 \times 10^{-5}$  moles) of 2-carboxy-1,3-butadiene irontricarbonyl was stirred in 0.75ml anhydrous  $\text{CDCl}_3$  under  $\text{N}_2$ . To this was added 14.3mg (1.05M equivalents) of carbonyldiimidazole, and the mixture was stirred under  $\text{N}_2$  for one hour at room temperature. By  $^1\text{H}$  nmr spectroscopy this stage of the synthesis had reached completion after this time. 11.4 $\mu\text{l}$  (1.05M equivalents) of S-(-)- $\alpha$ -methylbenzylamine were added, and the reaction was stirred under  $\text{N}_2$  at room temperature overnight.

Work-up: Excess amine was removed by extracting 3 times with 0.1M HCl pH 2. The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, and the solvent removed by rotary evaporation.  $^1\text{H}$  nmr showed four peaks with equal intensity due to the two diastereomeric  $\text{CH}_3$  doublets at  $\delta=1.6\text{ppm}$ .

$^1\text{H}$  nmr: (220MHz,  $\text{CDCl}_3$ ) 0.01(m, H, H-(C-1endo)); 0.37(m, 1H, H-(C-4endo)); 1.60(dd, 6H, H-( $\text{CH}_3$ )); 2.05(m, 2H, H-(C-1,4exo)); 5.32(m, 1H, H-(C-2')); 6.30(m, 2H, H-(C-3, NH)); 7.41(m, 5H, H-(aromatic)).

$^{13}\text{C}$  nmr: (100.62MHz,  $\text{CDCl}_3$ ) 21.1(C-3'); 36.64(C-1); 41.10(C-4); 49.39(C-2'); 87.76(C-3); 126-129(aromatic); 142.72(C-2); 167.45(C-1'); 210(CO).

IR: ( $\text{CHCl}_3$ )  $3450\text{cm}^{-1}(\text{m})$ ;  $1997\text{cm}^{-1}(\text{s})$ ;  $2070\text{cm}^{-1}(\text{s})$ ;  $1670\text{cm}^{-1}(\text{s})$ ;  $1515\text{cm}^{-1}(\text{s})$ .

UV: (MeOH)  $\lambda_{\text{max}} = 210\text{nm}$ ;  $300\text{nm}$ .

MS(EI):  $m/z = 342[\text{MH}]^+$ ;  $285[\text{M}-(\text{CO})_2]^+$ ;  $257[\text{M}-(\text{CO})_3]^+$ .

Anal. Calcd. for  $\text{C}_{16}\text{H}_{15}\text{FeNO}_4$ : C, 56.33; H, 4.43; N, 4.11; Fe, 16.37.

Found: C, 56.13; H, 4.66; N, 4.14; Fe, 16.58.

Enzymatic hydrolysis of 2-carboethoxy-1,3-butadiene irontricarboxyl.

50mg ( $1.88 \times 10^{-4}$  moles) irontricarboxyl ester **60** was stirred rapidly in a total of 20ml of a 20% MeOH solution in 0.2M phosphate buffer pH 7. To this was added 40 $\mu$ l PLE. The reaction was stirred at room temperature, and followed by TLC. (eluant: 20%  $\text{Et}_2\text{O}$ , 80% light petroleum) After 21 hours, the reaction was worked up *via* basifying the reaction mixture to pH 10-11 followed by  $\text{Et}_2\text{O}$  extraction, then acidification followed by  $\text{Et}_2\text{O}$  extraction. The separate organic layers were dried over anhydrous  $\text{MgSO}_4$  and the solvents removed after filtration by rotary evaporation. This gave 14mg (32%) of 2-carboxy-1,3-butadiene irontricarboxyl. (mp  $129-130^\circ\text{C}$ , 85% e.e. by amide **65** formation of the product alcohol followed by  $^1\text{H}$  nmr spectroscopy.) One recrystallisation from light petroleum and  $\text{Et}_2\text{O}$  gave the acid in 100% e.e. ( $[\alpha]_D = -10.7^\circ$ ,  $c=0.5$ ,  $\text{CHCl}_3$ ) The amide formed had a rotation of  $[\alpha]_D = -188^\circ$ ,  $c=1$ ,  $\text{CHCl}_3$  for optical purity. (mp  $121^\circ\text{C}$ )

2-Carboxy-1,3-butadiene irontricarboxyl<sup>113</sup> **64** (racemic)

2g of irontricarboxyl ester **60** (0.0075 moles) was dissolved in 30ml freshly distilled EtOH and was added with stirring at room temperature to a solution of 0.473g (0.011 moles, 1.5M

equivalents) LiOH.H<sub>2</sub>O in 60ml EtOH and 30ml H<sub>2</sub>O. It was stirred under N<sub>2</sub> in the dark for four hours. The reaction was followed by TLC, and had reached completion after this time. The reaction mixture was extracted with Et<sub>2</sub>O, the remaining aqueous phase was acidified, and extracted again with Et<sub>2</sub>O. The second organic phase was dried over anhydrous MgSO<sub>4</sub>, filtered, and the solvent removed by rotary evaporation. This gave 1.689g (95%) yield of pure 2-carboxy-1,3-butadiene irontricarbonyl.

<sup>1</sup>H nmr: (400MHz, CDCl<sub>3</sub>) 0.275(d, J<sub>1,1</sub>=2.6, 1H, H-(C-1*endo*)); 0.499(dd, J<sub>4,4</sub>=2.4, J<sub>4,3</sub>=10, 1H, H-(C-4*endo*)); 2.026(dd, J<sub>4,4</sub>=2.4, J<sub>4,3</sub>=7.2, 1H, H-(C-4*exo*)); 2.417(m, 1H, H-(C-1*exo*)); 6.307(t, J<sub>3,4</sub>=8.4, 1H, H-(C-3)).

IR: (CHCl<sub>3</sub>) 2060cm<sup>-1</sup>(s); 1995cm<sup>-1</sup>(s); 1700cm<sup>-1</sup>(w).

mp 144°C

UV: (CH<sub>3</sub>CN) λ<sub>max</sub>= 295nm; 210nm; 200nm.

MS(FAB, nba): m/z= 307[M+69]<sup>+</sup>; 239[MH]<sup>+</sup>; 210[M-CO]<sup>+</sup>; 182[M-(CO)<sub>2</sub>]<sup>+</sup>; 154[M-(CO)<sub>3</sub>]<sup>+</sup>; 136[M-((CO)<sub>3</sub>+H<sub>2</sub>O)]<sup>+</sup>.

Anal. Calcd. for C<sub>8</sub>H<sub>6</sub>FeO<sub>5</sub>: C, 40.38; H, 2.54; Fe, 23.47. Found: C, 40.41; H, 2.70; Fe, 23.47.

Separation of the diastereoisomers of 2-((S)-(-)-α-methylbenzylamido)-1,3-butadiene irontricarbonyl 65

5g of amide 65 were eluted through 200g flash silica column with 95% toluene and 5% Et<sub>2</sub>O. The faster running diastereomer (with TLC, eluant as above, the faster running spot had R<sub>F</sub>=0.32, the slower running spot R<sub>F</sub>=0.25) was recrystallised three times from light petroleum and Et<sub>2</sub>O to constant rotation [α]<sub>D</sub>=+42.5°, c=1, CHCl<sub>3</sub>; mp=115-116°C. (Yellow, needle-like crystals.)

The slower running diastereoisomer was recrystallised twice from  $\text{CHCl}_3$  and  $\text{Et}_2\text{O}$  to give a maximum rotation of  $[\alpha]_D = -188^\circ$ ,  $c=1$ ,  $\text{CHCl}_3$ .  $\text{Mp}=121^\circ\text{C}$ . (Yellow, round crystals.)

X-Ray structure analysis of (+)-6.5

Crystal data: monoclinic, space group  $P2_1$ ;  $a=11.479(4)$ ;  $b=18.413(7)$ ;  $c=16.645(4)$  Å,  $\beta=106.18(2)^\circ$ ,  $U=3379(2)$  Å<sup>3</sup>,  $z=8$  (four independent molecules in the asymmetric unit)

$R=0.073$  for 2606 unique observed  $[I/\sigma(I)] \geq 2.0$  reflections.

Principal dimensions (averages):

Fe-C(CO) 1.78(1); Fe-C(4) 2.13(1); Fe-C(3) 2.07(1); Fe-C(2) 2.05(1);  
Fe-C(1) 2.09(1); C(4)-C(3) 1.39(2); C(3)-C(2) 1.40(2); C(2)-C(1)  
1.41(2); C(2)-C(5) 1.46(2) Å.

## Chapter 4

### Ferrocene 1,1'-dicarboxylic acid dimethyl ester 66

4g (0.0146 moles) of 1,1'-ferrocenedicarboxylic acid was added at 0°C to a mixture of 50ml MeOH and 6.25ml (0.0876 moles) acetyl chloride. The reaction mixture was brought to 60-65°C with continuous stirring, and was stirred overnight. The reaction mixture was cooled, filtered, and the solvent removed by rotary evaporation. The compound was purified by column chromatography (eluant 30% EtOAc, 70% light petroleum) to give 3.87g (88% yield) of pure dimethyl ester. **66** It was recrystallised from light petroleum/EtOH.

mp 114-115°C (with sublimation at 109°C; lit.<sup>112</sup> mp 114-5°C)

<sup>1</sup>H nmr: (200MHz, CDCl<sub>3</sub>) 3.82(s, 6H, H-(CH<sub>3</sub>)); 4.42(m, 4H, H-(C-2,2',5,5')); 4.83(m, 4H, H-(C-3,3',4,4')).

IR: (CHBr<sub>3</sub>) 1723cm<sup>-1</sup>(s); 1710cm<sup>-1</sup>(s); 1468cm<sup>-1</sup>(s); 1430cm<sup>-1</sup>(m); 1370cm<sup>-1</sup>(m); 1280cm<sup>-1</sup>(s); 1027cm<sup>-1</sup>(m); 970cm<sup>-1</sup>(m); 898cm<sup>-1</sup>(m); 836cm<sup>-1</sup>(m); 825cm<sup>-1</sup>(m); 774cm<sup>-1</sup>(m).

### Enzymatic hydrolysis of 66

50mg of 1,1'-ferrocenedicarboxylic acid dimethyl ester was dissolved in a total of 20ml 0.2M phosphate buffer, pH7 containing 10% MeOH. To this was added 40μl PLE. The orange suspension was stirred at room temperature for one month. After this time, the reaction mixture was acidified, and extracted 4x Et<sub>2</sub>O. It was dried over anhydrous MgSO<sub>4</sub>, filtered, and solvent removed to give 47mg combined products. TLC (eluant: 50% EtOAc, 50% light petroleum) showed spots at R<sub>F</sub>=0.51, (starting diester) 0.15, 0.08, 0.02.

$\alpha$ -Methylferrocenecarboxylic acid 44<sup>106,107</sup>

1. 0.5g ( $2.69 \times 10^{-3}$  moles) of ferrocene was added to a vigorously stirred solution of 0.62ml ( $4.54 \times 10^{-3}$  moles)  $N,N,N',N'$ -tetramethylenediamine, 0.275ml phosphoric acid and 4.296ml acetic acid. The mixture was stirred under  $N_2$  and heated to  $90^\circ C$ . It was kept at this temperature for a further five hours then cooled to room temperature, diluted with  $H_2O$  and extracted  $4 \times Et_2O$ . The aqueous phase was cooled to  $0^\circ C$  followed by basification with 2.66g NaOH pellets. The resulting solution was extracted  $4 \times Et_2O$ , and the organic phase dried over anhydrous  $NaSO_4$ . This was filtered, and the solvent removed by rotary evaporation to give 0.585g (89.5%) yield of pure  $N,N$ -dimethylaminomethylferrocene.

$^1H$  nmr: (220MHz,  $CDCl_3$ ) 2.21(s, 6H, H-( $CH_3$ )); 3.33(s, 2H, H-( $CH_2$ )); 4.17(m, 9H, H-(aromatic)).

2. 36.77g (0.151 moles) of  $N,N$ -dimethylaminomethylferrocene was dissolved in 150ml freshly distilled anhydrous  $Et_2O$ . This was cooled to  $0^\circ C$ , and over a period of one hour, 217ml of 1.8M BuLi (0.39 moles) was added dropwise with stirring. The reaction was allowed to warm to room temperature, and was stirred under  $N_2$  for a further four hours. This was cooled to  $-100^\circ C$  and added with stirring to a mixture of 92g dry ice in 460ml anhydrous  $Et_2O$ . This was stirred at this temperature for 45 minutes, then was allowed to warm to room temperature overnight. The resulting yellow solid was filtered off, and washed twice with light petroleum. The yellow solid of lithium salts was dried under high vacuum for 24 hours. This gave 52.331g (lit. 50.56g) of a mixture of lithium salts.



3. 40g of the lithium salts were dissolved in one litre of freshly distilled anhydrous MeOH under  $N_2$ . To this was added 8g NaOH pellets, followed by the dropwise addition of 48ml (0.507 moles) dimethylsulphate. The yellow/brown solution was stirred at room temperature under  $N_2$  for three hours. The solvent was removed by rotary evaporation, and the residual brown gum was dissolved in 500ml distilled  $H_2O$  and cooled to  $0^\circ C$ . To this was added in portions with swirling and cooling, 1175g (lit. 2552g) of a 6.7% Na/Hg amalgam. This was decanted, the residual Hg washed well with  $H_2O$  and  $Et_2O$ , and the combined aqueous phase extracted  $4 \times Et_2O$ . The aqueous phase was acidified (with cooling) with  $H_3PO_4$ , followed by  $4 \times Et_2O$  extraction. The organic phase was dried over  $MgSO_4$ , filtered, and solvent removed by rotary evaporation. This gave 12.3g (36.8%) of  $\alpha$ -methylferrocenecarboxylic acid **44**, which was further purified by flash chromatography. (eluant: 95% light petroleum, 5% EtOH)

$^1H$  nmr: (220MHz,  $CDCl_3$ ) 2.34(s, 3H, H-(C-6)); 4.23(s, 5H, H-(C-1',2',3',4',5')); 4.35(m, 1H, H-(C-3)); 4.43(m, 1H, H-(C-4)); 4.85(m, 1H, H-(C-5)).

IR: ( $CHCl_3$ )  $3160-2840cm^{-1}(m)$ ;  $2640cm^{-1}(w)$ ;  $1680cm^{-1}(s)$ ;  $1475cm^{-1}(m)$ ;  $1458cm^{-1}(m)$ ;  $1445cm^{-1}(m)$ ;  $1300cm^{-1}(m)$ ;  $1228cm^{-1}(m)$ ;  $1100cm^{-1}(m)$ ;  $1010cm^{-1}(m)$ .

MS(FAB):  $m/z = 244[M]^+$ ;  $227[M-OH]^+$ ;  $185[C_{10}H_9Fe]^+$ .

mp  $149^\circ C$ .

Anal. Calcd. for  $C_{12}H_{12}FeO_2$ : C, 59.053; H, 4.955; Fe, 22.881. Found: C, 57.89; H, 4.97; Fe, 23.36.

$\alpha$ -Methylferrocenecarboxylic acid methyl ester<sup>107,67</sup>

1g (0.024 moles) of diazomethane dissolved in 80ml Et<sub>2</sub>O was added with swirling to a solution of 0.187g (0.766 mmoles)  $\alpha$ -methylferrocenecarboxylic acid in 20ml distilled Et<sub>2</sub>O at room temperature. After standing for 15 minutes, the solution was washed with acetic acid, dried over MgSO<sub>4</sub>, filtered and the solvent removed by rotary evaporation. This was purified by column chromatography (eluant: 80% light petroleum, 20% EtOAc) to give 0.155g (78.4%) yield of pure **67**.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 2.30(s, 3H, H-(C-6)); 3.86(s, 3H, H-(OCH<sub>3</sub>)); 4.18(s, 5H, H-(C-1',2',3',4',5')); 4.29(m, 1H, H-(C-3)); 4.37(m, 1H, H-(C-4)); 4.76(m, 1H, H-(C-5)).

IR: (CHCl<sub>3</sub>) 2960cm<sup>-1</sup>(w); 1710cm<sup>-1</sup>(s); 1446cm<sup>-1</sup>(m); 1420cm<sup>-1</sup>(w); 1380cm<sup>-1</sup>(w); 1235cm<sup>-1</sup>(m); 1165cm<sup>-1</sup>(w); 1100cm<sup>-1</sup>(m); 1040cm<sup>-1</sup>(w); 1006cm<sup>-1</sup>(w).

MS(FAB): m/z= 258[M]<sup>+</sup>; 227[M-OCH<sub>3</sub>]<sup>+</sup>; 213[227-CH<sub>2</sub>]<sup>+</sup>; 185[231-CO]<sup>+</sup>.

Anal. Calcd. for C<sub>13</sub>H<sub>14</sub>FeO<sub>2</sub>: C, 60.497; H, 5.467; Fe, 21.638. Found: C, 60.79; H, 5.73; nFe, 21.41.

#### $\alpha$ -Methylferrocenemethyl alcohol<sup>107,68</sup>

4g (0.0155 moles)  $\alpha$ -methylferrocenecarboxylic acid methyl ester dissolved in 35ml freshly distilled anhydrous Et<sub>2</sub>O was added dropwise over a period of 30 minutes to a slurry of 0.617g (0.017 moles) LiAlH<sub>4</sub> in 70ml anhydrous Et<sub>2</sub>O. The reaction was followed by TLC, and virtually immediately had gone to completion. (eluant: 10% Et<sub>2</sub>O, 90% CCl<sub>4</sub>, R<sub>F</sub> (ester)=0.41, (alcohol)=0.04) The reaction was quenched by the cautious addition of EtOAc, followed by H<sub>2</sub>O, then filtered. The solvent was removed by rotary evaporation. The

product was purified by column chromatography (150g, eluant as above) in quantitative yield.

$^1\text{H}$  nmr: (220MHz,  $\text{CDCl}_3$ ) 1.38(t,  $J=4.9$ , 1H, H-(OH)); 2.01(s, 3H, H-(C-6)); 4.16(m, 8H, H-(C-3,4,5,1',2',3',4',5')); 4.43(m, 2H, H-(CH<sub>2</sub>)).

IR: ( $\text{CHCl}_3$ ) 3620 $\text{cm}^{-1}$ (w); 3440 $\text{cm}^{-1}$ (w); 2930 $\text{cm}^{-1}$ (w); 1440 $\text{cm}^{-1}$ (w); 1385 $\text{cm}^{-1}$ (w); 1110 $\text{cm}^{-1}$ (w); 1040 $\text{cm}^{-1}$ (m); 1000 $\text{cm}^{-1}$ (m).

MS(FAB, nba):  $m/z$  = 365[M-(OH+nba)]<sup>+</sup>; 213[M-OH]<sup>+</sup>(100%); 121[C<sub>5</sub>H<sub>5</sub>Fe]<sup>+</sup>; 91[C<sub>7</sub>H<sub>7</sub>]<sup>+</sup>; 69[C<sub>5</sub>H<sub>9</sub>]<sup>+</sup>; 55[C<sub>4</sub>H<sub>7</sub>]<sup>+</sup>.

Anal. Calcd. for C<sub>12</sub>H<sub>14</sub>FeO: C, 62.64; H, 6.133; Fe, 24.272. Found: C, 63.11; H, 6.35; Fe, 25.4.

#### $\alpha$ -Methylferrocenemethyl acetate 7.9

0.600g (2.608 mmoles)  $\alpha$ -methylferrocenemethyl alcohol was dissolved in 20ml freshly distilled anhydrous Et<sub>2</sub>O and was cooled to 0°C. To this was added a solution of 2.53ml (12M equivalents) pyridine, 2mg DMAP, and 0.984ml (4M equivalents) Ac<sub>2</sub>O in 5ml anhydrous Et<sub>2</sub>O. This was stirred under N<sub>2</sub> at 0°C for 15 minutes, then the reaction was allowed to warm to room temperature and was stirred under N<sub>2</sub> overnight. The reaction was worked up by the addition of saturated NaHCO<sub>3</sub> followed by 3x Et<sub>2</sub>O extraction. The combined ether layers were washed 3x 0.1M HCl, dried over anhydrous MgSO<sub>4</sub>, filtered, and the solvent removed by rotary evaporation. The product was purified by column chromatography (eluant: 20% Et<sub>2</sub>O, 80% light petroleum) to give 0.449g (66.2%) yield of  $\alpha$ -methylferrocenemethyl acetate.

$^1\text{H}$  nmr: (220MHz,  $\text{CDCl}_3$ ) 2.039(s, 3H, H-(C-6)); 2.05(s, 3H, H-(CH<sub>3</sub>)); 4.13(m, 6H, H-(C-1',2',3',4',5',3)); 4.20(m, 1H, H-(C-4)); 4.25(m, 1H, H-(C-5)); 5.02(q,  $J=11.76$ , 2H, H-(CH<sub>2</sub>)).

Enzyme catalysed transesterification of  $\alpha$ -methylferrocenemethyl acetate with 1-heptanol

3-6mg  $\alpha$ -methylferrocenemethyl acetate was dissolved in 1ml Na distilled iso-octane and 3 $\mu$ l 1-heptanol was added. To each reaction was added ~10mg of lipase (or 20 $\mu$ l PLE immobilised on sepharose) and the reactions were stirred at room temperature. The reactions were followed by GLC. (GLC conditions: Column 3% SE 30, injector and detector temperatures 250°C, column temperature 100°C, temperature controlled run, 100-250°C, 16°C per minute, one minute initial temperature, 1-5 minutes final temperature.  $R_T$  of heptanol=132s, heptyl acetate=218s)

Heptyl acetate.

500mg (4.302mmoles) 1-heptanol were dissolved in 50ml freshly distilled, anhydrous Et<sub>2</sub>O, and was cooled to 0°C. To this was added 0.8ml (2M equivalents) Ac<sub>2</sub>O, 2.1ml (0.026 moles, 6M equivalents) pyridine and one small crystal of DMAP dissolved in 10ml anhydrous Et<sub>2</sub>O. The mixture was stirred under N<sub>2</sub>, and brought to room temperature. After 48 hours, the reaction had reached completion by TLC. (eluant: 50% Et<sub>2</sub>O, 50% light petroleum,  $R_F$  (heptanol)=0.28, (heptyl acetate)=0.59) The Et<sub>2</sub>O was removed by rotary evaporation, and the whole residue was separated by flash chromatography (eluant: 80% light petroleum, 20% Et<sub>2</sub>O) to give 0.619g (91% yield) of pure heptyl acetate.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 0.90(m, 3H, H-(C-7)); 1.32(m, 8H, H-(C-3,4,5,6)); 1.64(m, 2H, H-(C-2)); 2.05(s, 3H, H-(C-2')); 4.08(t,  $J_{1,2}$ =5.9, 2H, H-(C-1)).

IR: ( $\text{CHCl}_3$ )  $2960\text{cm}^{-1}(\text{s})$ ;  $2938\text{cm}^{-1}(\text{s})$ ;  $2860\text{cm}^{-1}(\text{m})$ ;  $1730\text{cm}^{-1}(\text{s})$ ;  $1470\text{cm}^{-1}(\text{m})$ ;  $1390\text{cm}^{-1}(\text{m})$ ;  $1370\text{cm}^{-1}(\text{m})$ ;  $1250\text{cm}^{-1}(\text{s})$ ;  $1040\text{cm}^{-1}(\text{m})$ .

MS(EI):  $m/z = 159[\text{MH}]^+$ ;  $116[\text{C}_7\text{H}_{15}\text{OH}]^+$ ;  $98[\text{C}_7\text{H}_{14}]^+$ ;  $83[98\text{-CH}_3]^+$ ;  $70[98\text{-C}_2\text{H}_4]^+$ ;  $56[70\text{-CH}_2]^+$ .

### $\alpha$ -Methylferrocenemethyl butyrate 71

A solution of 3.49ml (6M equivalents) pyridine, 10mg (0.01M equivalents) DMAP, 2.36ml (2M equivalents) butyric anhydride in 4ml freshly distilled anhydrous  $\text{Et}_2\text{O}$  was added to a stirred solution at  $0^\circ\text{C}$  under  $\text{N}_2$  of 1.656g (7.199mmoles)  $\alpha$ -methylferrocenemethyl alcohol in 10ml anhydrous  $\text{Et}_2\text{O}$ . The reaction was followed by TLC, (eluant: 98%  $\text{CH}_2\text{Cl}_2$ , 2%  $\text{Et}_2\text{O}$ ,  $R_F$  (alcohol)=0.19, (butyrate)=0.68) and after two hours, had reached completion. The reaction mixture was diluted with  $\text{Et}_2\text{O}$  and washed with 4x50ml 0.5M HCl followed by 3x50mls 1M  $\text{NaHCO}_3$ . The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, and solvent removed by rotary evaporation. The butyrate was distilled (0.2mm Hg,  $135\text{-}140^\circ\text{C}$ ) to give the product 71 in 70% yield.

$^1\text{H}$  nmr: (220MHz,  $\text{CDCl}_3$ ) 0.93(t,  $J_{11,10}=6.9$ , 3H, H-(C-11)); 1.65(m, 2H, H-(C-10)); 2.01(s, 3H, H-(C-6)); 2.28(t,  $J_{9,10}=7.38$ , 2H, H-(C-9)); 4.11(m, 6H, H-(C-1',2',3,3',4',5')); 4.16(m, 1H, H-(C-4)); 4.22(m, 1H, H-(C-5)); 5.01(q,  $J_{7,7}=11.8$ , 2H, H-(C-7)).

IR: ( $\text{CHCl}_3$ )  $3100\text{cm}^{-1}(\text{w})$ ;  $2970\text{cm}^{-1}(\text{s})$ ;  $2880\text{cm}^{-1}(\text{w})$ ;  $1728\text{cm}^{-1}(\text{s})$ ;  $1460\text{cm}^{-1}(\text{m})$ ;  $1375\text{cm}^{-1}(\text{m})$ ;  $1260\text{cm}^{-1}(\text{m})$ ;  $1180\text{cm}^{-1}(\text{s})$ ;  $1110\text{cm}^{-1}(\text{m})$ ;  $1040\text{cm}^{-1}(\text{m})$ ;  $1005\text{cm}^{-1}(\text{m})$ ;  $960\text{cm}^{-1}(\text{m})$ ;  $830\text{cm}^{-1}(\text{m})$ .

UV: ( $\text{CH}_3\text{CN}$ )  $\lambda_{\text{max}}$  436nm; 325nm; 252nm; 206nm.

MS(FAB gly, EtOH):  $m/z = 300[\text{M}]^+$ ;  $231[\text{M-C}_4\text{H}_7\text{O}_2]^+(100\%)$ ;  $235[\text{M-C}_5\text{H}_5]^+$ ;  $121[\text{C}_5\text{H}_5\text{Fe}]^+$ ;  $91[\text{C}_7\text{H}_7]^+$ ;  $77[\text{C}_6\text{H}_5]^+$ ;  $56[\text{Fe}]^+$ .

Anal. Calcd. for  $C_{16}H_{20}FeO_2$ : C, 64.02; H, 6.716; Fe, 18.605. Found: C, 64.72; H, 7.02; Fe, 21.4.

#### Heptyl butyrate.

500mg ( $4.3 \times 10^{-3}$  moles) 1-heptanol was dissolved in 10ml anhydrous  $Et_2O$ , stirred under  $N_2$  and cooled to  $0^\circ C$ . To this was added a solution of 2.1ml pyridine (6M equivalents), 1.4ml butyric anhydride (2M equivalents) and two small crystals of DMAP in 4ml anhydrous  $Et_2O$ . The reaction was allowed to warm to room temperature, and was stirred under  $N_2$  for two hours. After this time, the reaction mixture was diluted with  $Et_2O$ , extracted with a total of 100ml 0.5M HCl, followed by  $4 \times Na_2CO_3$  extraction, and was dried over anhydrous  $MgSO_4$ . The solution was filtered and the solvent removed by rotary evaporation. The product was purified by Kugelrohr distillation ( $50^\circ C$ , 0.1mm Hg) to give 0.624g (78% yield) of heptyl butyrate.

$^1H$  nmr: (220MHz,  $CDCl_3$ ) 0.95(m, 6H, H-(C-4',7')); 1.31(m, 8H, H-(C-3,4,5,6)); 1.64(m, 4H, H-(C-3',2)); 2.28(t,  $J_{2,3}=6.12$ , 2H, H-(C-2')); 4.18(t,  $J_{1,2}=5.9$ , 2H, H-(C-1)).

IR: ( $CHCl_3$ )  $3030cm^{-1}$ (m);  $2970cm^{-1}$ (s);  $2940cm^{-1}$ (s);  $2880cm^{-1}$ (m);  $2870cm^{-1}$ (m);  $1730cm^{-1}$ (s);  $1474cm^{-1}$ (m);  $1270cm^{-1}$ (m);  $1190cm^{-1}$ (s);  $1100cm^{-1}$ (m).

#### Preparation of activated $MnO_2$ <sup>109</sup>

A solution of 10.43g (0.066 moles)  $KMnO_4$  in 65.2ml  $H_2O$  was heated with stirring to  $60^\circ C$ . To this was added simultaneously and over a period of 40 minutes 12.7ml 10M NaOH and a solution of 12.065g  $MnSO_4$  in 16.3ml  $H_2O$ . This was stirred for four hours, then allowed to cool for one hour. The precipitated  $MnO_2$  was

recovered via centrifugation (10,000K, 10 minutes, room temperature) This was washed with H<sub>2</sub>O by heating to boiling with stirring for five minutes, then centrifuged again whilst still hot. The MnO<sub>2</sub> was washed six times in this fashion, after which time The washings had pH 10 and were brown and clear. It was dried overnight in an oven at 110°C, ground into a powder and stored above silica gel and P<sub>2</sub>O<sub>5</sub> in a desiccator. This gave 7.9g activated MnO<sub>2</sub>.

$\alpha$ -Methylferrocene aldehyde<sup>111 74</sup>

61.3mg (2.664x10<sup>-4</sup> moles)  $\alpha$ -methylferrocenemethyl alcohol was dissolved in 5ml freshly distilled anhydrous CH<sub>2</sub>Cl<sub>2</sub> and added to a stirred suspension of 0.301g (13M equivalents) activated MnO<sub>2</sub> under N<sub>2</sub>. By TLC (eluant: 90% CCl<sub>4</sub>, 10% Et<sub>2</sub>O R<sub>F</sub> (alcohol)=0.07, (aldehyde)=0.23) the reaction had reached 100% after one hour. The reaction mixture was filtered through hyflosupercel, and the solvent removed by rotary evaporation. The product was purified by column chromatography (eluant: 95% CCl<sub>4</sub>, 5% Et<sub>2</sub>O) followed by Kugelrohr distillation. (100°C, 0.2mm Hg) This gave 50.4mg (83% yield) of pure  $\alpha$ -methylferrocene aldehyde.

<sup>1</sup>H nmr: (220MHz, CDCl<sub>3</sub>) 2.30(s, 3H, H-(C-6)); 4.26(s, 5H, H-(C-1',2',3',4',5')); 4.53(m, 1H, H-(C-3)); 4.56(m, 1H, H-(C-4)); 4.76(m, 1H, H-(C-5)).

IR: (CHCl<sub>3</sub>) 2850cm<sup>-1</sup>(w); 1678cm<sup>-1</sup>(s); 1470cm<sup>-1</sup>(w); 1437cm<sup>-1</sup>(m); 1284cm<sup>-1</sup>(w); 1110cm<sup>-1</sup>(w); 1040cm<sup>-1</sup>(w); 1008cm<sup>-1</sup>(w).

MS(EI): m/z= 228[M]<sup>+</sup>; 200[M-CO]<sup>+</sup>; 121[C<sub>5</sub>H<sub>5</sub>Fe]<sup>+</sup>.

Anal. Calcd. for C<sub>12</sub>H<sub>12</sub>FeO: C, 63.197; H, 5.303; Fe, 24.487. Found: C, 63.64; H, 5.24; Fe, 23.2.

#### Immobilisation of lipases on Biofix.

20mg of lipase and 200mg Biofix (E1, RLO 3997) were placed in a 5ml round bottomed flask. To this was added 1.5ml H<sub>2</sub>O, and the slurry was gently shaken until all was thoroughly mixed. It was then put on a rotary evaporator at room temperature, rotated very slowly and the H<sub>2</sub>O removed. (20 minutes to half an hour) The white solids were then stored in a desiccator above silica gel at 4°C until used.

#### Enzymatic transesterification of $\alpha$ -methylferrocenemethyl butyrate.

~100mg of the above ferrocene butyrate was dissolved in 10ml iso-octane (distilled from Na), and 56 $\mu$ l 1-heptanol was added. To each reaction flask was placed 40mg of lipase (or 40mg of lipase immobilised on 400mg Biofix), the reactions were stirred at 40°C, and followed by GLC. (GLC conditions: Column 3% SE 30; column temperature 125°C; R<sub>T</sub>(heptanol)=66s, R<sub>T</sub>(heptyl butyrate)=261s.) The reactions were worked up by filtration of the enzyme, and solvent removed by rotary evaporation. The crude reaction mixture was Kugelrohr distilled (0.1mm Hg, 35°C, 2 hours) to remove the residual heptanol and heptyl butyrate; the residue was oxidised with activated MnO<sub>2</sub>, followed by deprotection with LiOH/H<sub>2</sub>O. The mixture of aldehyde and alcohol were separated by column chromatography (eluant: 98% CH<sub>2</sub>Cl<sub>2</sub>, 2% Et<sub>2</sub>O) The crude aldehyde was Kugelrohr distilled prior to analysis. (100°C, 0.2mm Hg)

#### Chemical hydrolysis of $\alpha$ -methylferrocenemethyl butyrate.



50mg ( $1.66 \times 10^{-4}$  moles) of the above butyrate dissolved in 1ml EtOH was added to a solution of 30mg (4.3M equivalents) LiOH/H<sub>2</sub>O in 1ml EtOH and 0.5ml H<sub>2</sub>O. This was stirred together for two hours at room temperature. After this time, the reaction had reached completion by TLC. (eluant 90% CCl<sub>4</sub>, 10% Et<sub>2</sub>O  $R_F(\text{alcohol})=0.13$ ,  $R_F(\text{butyrate})=0.58$ ,  $R_F(\text{by-product, } \alpha\text{-methylferrocenemethyl ether})=0.49$ ) The reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub>, dried over anhydrous MgSO<sub>4</sub>, filtered, and the solvent removed by rotary evaporation. The pure alcohol was purified by column chromatography (98% CH<sub>2</sub>Cl<sub>2</sub>, 2% Et<sub>2</sub>O) to give 35mg (92% yield) of pure alcohol. 68

Preparation of 6.7% Na/Hg amalgam<sup>110</sup>

80g of solid Na was cut up and placed in a two litre round bottomed flask equipped with a dropping funnel and an overhead stirrer. It was flushed with N<sub>2</sub>. 1196g Hg was placed in the dropping funnel. ~10ml of Hg was run into the flask, generating great heat and white fumes. The Na was melted by heating the round bottomed flask with two bunsen burners, then the rest of the Hg was quite rapidly added. This gave a molten, silvery amalgam which was immediately poured out onto a shallow board. As it cooled, it was hammered into small pieces. These were further crushed with a mortar and pestle, and sealed in a stoppered bottle. This gave 1175g of amalgam. (92% by weight.)

## **Appendix**

### **Biofix.**

Biofix, a kaolinite of chemical formula  $\text{Al}_2\text{O}_3\cdot 2\text{SiO}_2\cdot 2\text{H}_2\text{O}$  (from English China Clays) is a specially blended slurry of clay which has been formed into hollow microspheres. These microspheres have been calcinated and etched, giving rise to hollow microspheres with porous walls made up of an interconnecting network of little channels.

At present, Biofix is available in forms C1, C2 and E1, each a porous support as defined above yet with different cavity sizes and densities. Biofix C1 and C2 have been specially designed for whole cell immobilisation, whilst Biofix E1 has been designed for use in enzyme immobilisation. Thus Biofix E1 was used for the immobilisation of lipases used in this thesis.

Biofix E1 does not have an internal cavity as do C1 and C2, but consists of a macro-porous ceramic that is free of substrate diffusion limitations. It is stable in the pH range 1-14, is thermally stable up to  $1,000^\circ\text{C}$  and has a crush strength of  $>55\text{MPa}$ . The particles of Biofix E1 are of size  $20\text{-}50\mu\text{m}$ , and have a mean pore size of  $0.5\mu\text{m}$ . The surface area is  $5.0\text{m}^2\text{g}^{-1}$ , density of  $1.0\text{g}\text{cm}^{-3}$  and a percentage void volume of 50%.

The support can be activated with 2.5% by weight glutaraldehyde per g of support suspended in phosphate buffer, to form a covalent bond to the enzyme.

However, throughout the course of this work the lipases were simply adsorbed onto the support by slowly removing the water from a slurry of lipase and Biofix. The immobilised enzymes were stored over a desiccant at  $4^\circ\text{C}$  until used.

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