

HOMOCHIRAL SYNTHESIS OF PARACONIC  
ACID AND THE BIOSYNTHESIS OF  
AMPHOTERICIN B

Thesis submitted for the degree of

Doctor of Philosophy

at

the University of Leicester

by

James Michael Crawforth, BSc (Leicester), MRSC, CChem

Department of Chemistry

University of Leicester

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## ABSTRACT

J. M. Crawforth

Homochiral (S)-(-)-paraconic acid was synthesised in 18 % overall yield from (4S)-4-isopropyl-2-oxazolidinone using titanium enolate methodology. Paraconic acid was used to synthesise A-factor. A-factor and analogues were fed to a culture of *Streptomyces nodosus* however no definitive enhancement, or a decrease in the lag time for the onset of metabolite production was observed.

Sodium acetate- $d_3$ , [1- $^{13}\text{C}$ ] and [3- $^{13}\text{C}$ ]-propionate were fed to a culture of *Streptomyces nodosus* and label incorporation into amphotericin B of 10-15 % was observed.

## Acknowledgements

I would like to thank my supervisor, Dr Bernard Rawlings for his assistance and support during the last three years. His encouragement and enthusiasm never waned even while facing repeated 'unexpected' results, and his Professor Baldwin impression will remain with me for a very long time.

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Finally I would like to thank the EPSRC for financial assistance, and SmithKline Beecham and Steve Box for their willingness to assist despite not being their CASE student.

Brian

*'When you part from your friend, grieve not;  
for that which you love most in him may be clearer in his  
absence, as the mountain to the climber is clearer from the plain'*

Kahil Gibran: The Prophet

## Abbreviations

Ac	acetyl
AIDS	acquired immune deficiency syndrome
AmA	amphotericin A
AmB	amphotericin B
Ar	aryl
atm	atmosphere
°C	degrees Celsius
CI	chemical ionisation
cm	centimetre
DEPT	distortionless enhancement by polarisation transfer
DMF	<i>N, N</i> -dimethylformamide
DMSO	dimethylsulfoxide
ee	enantiomeric excess
EI	electron impact
Et	ethyl
eV	electron volt
g	gram
HCl	hydrochloric acid
HPLC	high pressure liquid chromatography
Hz	Hertz
lit.	literature
L	litre
M	molar
M <sup>+</sup>	molecular ion

Me	methyl
MeOH	methyl alcohol
mg	milligram
MHz	megaHertz
mmol	millimole
m.p.	melting point
m/z	mass charge ratio
NAD(P)H	nicotinamide adenine dinucleotide
ng	nanogram
nm	nanometre
NMR	nuclear magnetic resonance
Ph	phenyl
PPL	porcine pancreatic lipase
ppm	parts per million
THF	tetrahydrofuran
$\delta$	chemical shift
$\mu$ l	microlitre
v	infrared vibration

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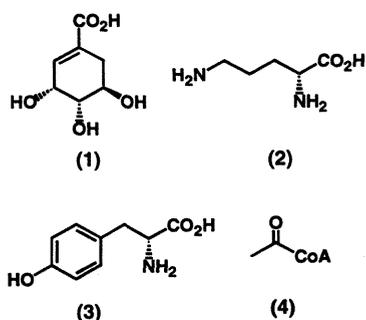
# CHAPTER 1

General Introduction

## 1.0 INTRODUCTION

### 1.1 General Introduction

All organisms synthesise and utilise a wide range of chemical compounds such as proteinogenic amino acids, fatty acids, sugars, nucleotides and the polymers derived from them: proteins, lipids, polysaccharides, RNA and DNA. These species are essential to the day-to-day survival and welfare of the organism and are commonly referred to as 'primary metabolites' with the processes involving their anabolism/catabolism known as 'primary metabolism'. Many organisms, occasionally and only at a specific period during their lifecycle also produce other compounds which appear to have no apparent utility in the routine life of the organism. These are referred to as 'secondary metabolites', and the pathways of their synthesis as 'secondary metabolism'. This classification of individual metabolites as either primary or secondary is a formalism and becoming increasingly ambiguous, for example some complex amino acids have been isolated<sup>1</sup> which are hard to place in either group. Polyamines such as putrescine, spermine and spermidine are ubiquitous in nature, have a high affinity for DNA and stimulate protein synthesis, and some microbial mutants exist that have a definite requirement for polyamines for growth. They appear to fill the criteria to be classified as primary metabolites yet there is no conclusive evidence to support their alleged cellular functions and therefore are defined as secondary metabolites.



(Fig 1) Secondary metabolism starting components:

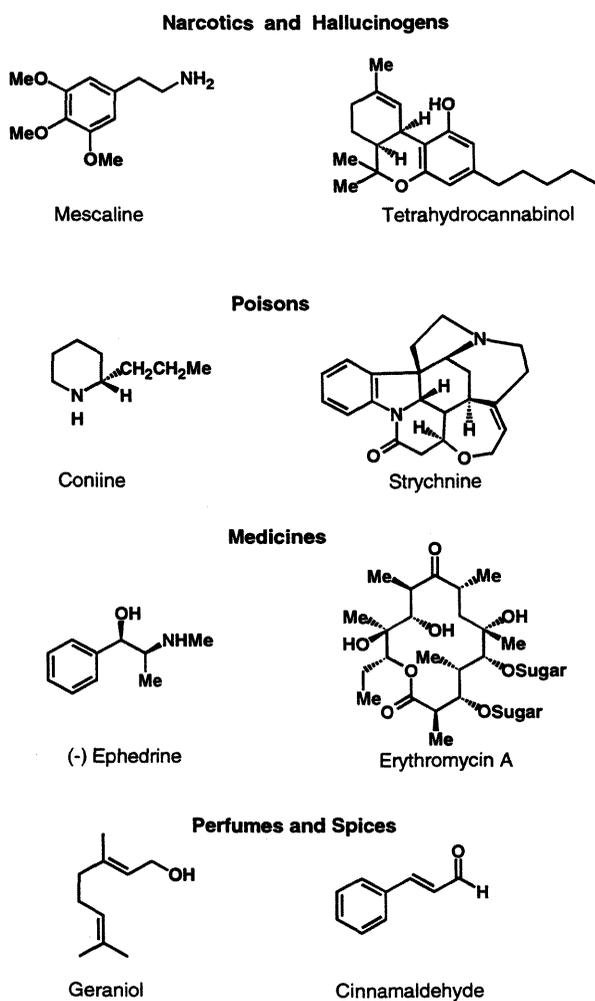
Shikimic acid (1), Ornithine (2), Tyrosine (3), Acetyl CoA (4).

Primary and secondary metabolism could be regarded as interconnected since the small molecules employed as starting materials for the important secondary metabolic pathways are in fact primary metabolites (Fig 1). For example the primary metabolite shikimic acid (1) is the precursor of both many secondary metabolites (lignins) as well as the proteinogenic aromatic amino acids. Amino acids such as ornithine (2) and tyrosine (3) lead to alkaloids and the penicillins, and acetyl CoA (4) is the precursor of polyketides, prostaglandins, macrocyclic antibiotics and the isoprenoids such as terpenes and steroids.

A multitude of these diverse secondary metabolites are extremely bioactive and may be produced by the organism to impart some ecological advantage over competitors in its surrounding environment. Few insects feed on plants containing nicotine and the repellent effects of alkaloids such as atropine and strychnine is well established. Rather than a defensive function a secondary metabolite, such as geraniol, (Fig 2) may have an attractive one and so aid in pollination by increasing the plant's appeal to insects. Soil bacteria from the genus *Streptomyces* produce a

large number of compounds with antibacterial properties presumably to engage in a form of 'chemical warfare' with surrounding bacteria in an attempt to increase their chances of survival. Many of these bioactive compounds have been found to have a beneficial effect in Man and find widespread use in industry as medicines or fragrances and in more illicit areas such as the hallucinogens (Fig 2).

The factors determining the onset and level of production of secondary metabolites are complex and as yet not fully understood. Generally for micro-organisms production is low during the growth phase (trophophase), and high in the exponential idiophase of arrested vegetative growth, eventually decreasing perhaps due to feedback regulation. Often there is a close relationship between the onset of secondary metabolism and morphological and cytological changes such as between antibiotic production and sporulation in fungi and filamentous bacteria. The simple bacterium *Escherichia coli* is found in the gut and therefore has an ample and sustained nutrient supply and as such it is under little ecological stress. It has a typical cell cycle involving co-ordinated division and separation,<sup>2</sup> and thus produces no antibiotics during its life cycle.



(Fig 2)

The genus soil bacteria *Streptomyces* generally exist within a more inimical environment and compete with other organisms for limited, and perhaps, inconsistent nutrient resources. Unlike simple bacteria they have a mycelial growth habit enabling relocation *via* sporulation and so an increased chance of species survival during times

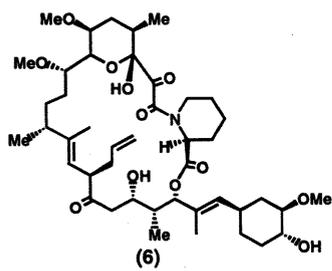
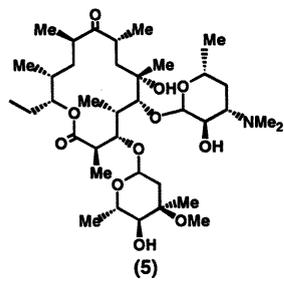
of ecological stress such as nutrient depletion. The substrate mycelium of the branching hyphae results from vegetative growth without cell separation and eventually generates dispersive units allowing propagation. *Streptomyces* achieve this differentiation by initially forming aerial mycelium which are both physically and nutritionally attached onto the substrate mycelium. Fragmentation of individual aerial hyphae into chains of hydrophobic spores containing a nucleoid<sup>3</sup> follows and the spores disperse. Aerial growth is accompanied by physiological changes in the colony such as a production of a range of secondary metabolites that include, among those produced by the compass of *Streptomyces* species, a multitude of antibiotics depending on the species. This aspect of streptomycetes developmental biology involves a range of regulatory influences. Essential nutrient depletion such as nitrogen starvation necessitates the need for sporulation and hence re-establishment in a different area with improved nutrient levels, thus ensuring the organisms' survival. In several *Streptomyces* species a fall in the guanine nucleotide pool, such as GTP levels (again caused by suitable nutrient starvation) has been suggested as a trigger for sporulation.<sup>3</sup> Cell lysis occurs during sporulation as the nutrients contained within the cells are required by the organism for the sporulation process. Antibiotic production coincides with this process presumably to impart some protection for the nutrients released during this cell lysis, and to gain a developmental advantage over other organisms present in the surrounding environment.

## 1.2 Autoregulators

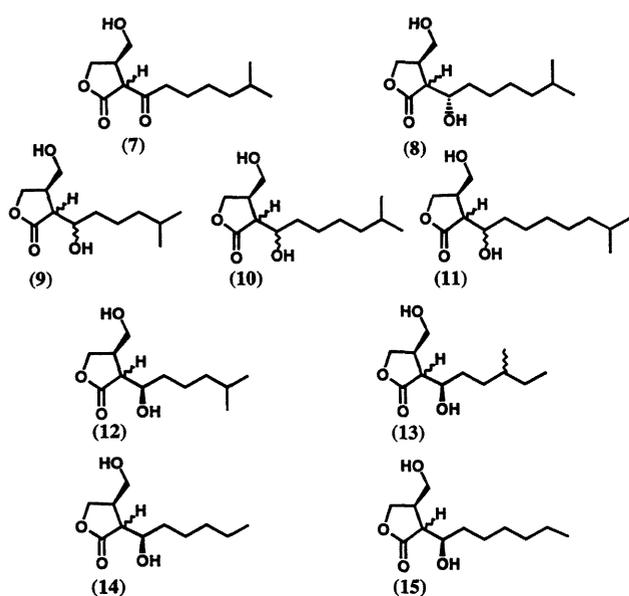
Streptomycetes and related bacteria are distributed widely throughout soils and are responsible for the production of a great many important biologically active secondary metabolites such as erythromycin B (5) a potent antibiotic used for

amongst others the treatment of Legionnaires' Disease, and FK506, an immunosuppressant (6) (Fig 3) which finds widespread clinical use.<sup>4</sup>

In 1973 A.S. Khokhlov<sup>5</sup> was working on *Streptomyces griseus*, producer of the antibiotic streptomycin (6). He noticed that colonies of blocked mutants that were normally unable to produce streptomycin, if incubated together on a petri dish with a wild type strain of *S. griseus*, had their ability to produce streptomycin restored when in close proximity to the wild strain. Later experiments involved the growth of the wild type *S. griseus* followed by complete extraction of its mycelia from the growth medium thus leaving within the whole broth *any compound* the wild type streptomycete had produced and released into the media. Subsequent inoculation of this media with the blocked mutant again led to the re-installation of streptomycin production and it was this observation that led Kokhlov to postulate that the wild strain produced an 'autoregulating factor' (A-factor), which was able to induce the biosynthesis of streptomycin in the inactive mutants. In 1976 A-factor was isolated<sup>6</sup> from the fermentation broth of *S. griseus* and its structure determined to be (3*R*)-2-(6-methylheptanoyl)-3-hydroxymethyl-4-butanolide (7) (Fig 4).<sup>6</sup>



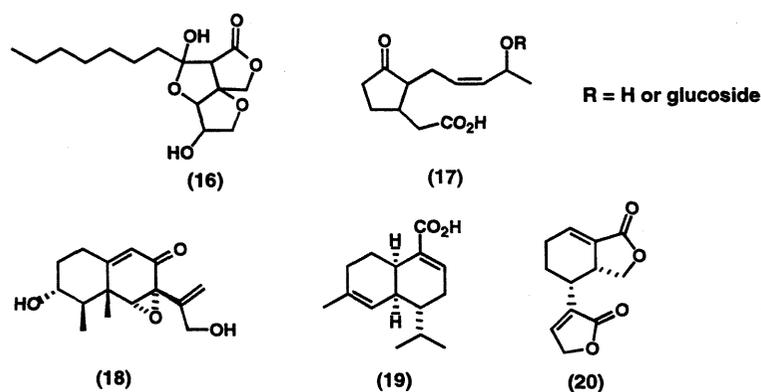
(Fig 3) Erythromycin B (*Saccharopolyspora erythraea*) (5) FK506 (*S. tsukubaensis*) (6)



(Fig 4) A-factor (7) Autoregulators from *S. viridochromogenes* (8) *S. bikiniensis* (9) and *S. cyaneofuscatus* (10), (11) and Virginiae Butanolides A (12), B (13), C (14), and D (15) from *S. virginiae*.

Since the discovery and structural elucidation of A-factor several other closely related 'autoregulators' of cytodifferentiation and antibiotic biosynthesis in streptomycetes have been isolated.<sup>7-12</sup> In 1979 Yanagimoto *et al* isolated inducers of staphylomycin production in *S. virginiae* (12 - 15) and in 1983 Grafe *et al* isolated a number of autoregulators from the *S. viridochromogenes*, *S. bikiniensis* and *S. cyaneofuscatus* (Fig 4). As can be seen above all share common structural features of a  $\gamma$ -lactone ring with a hydroxymethyl group with (*R*) stereochemistry in the 3 $\beta$  position, and a long hydrocarbon moiety in the  $\alpha$  position between six and eight carbon atoms in length.

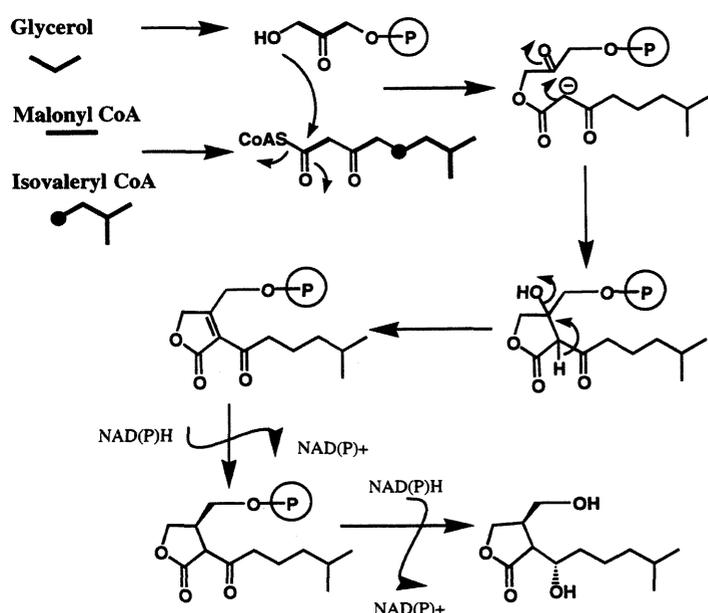
Other substances which appear to have a phenotropic function in nature have also been isolated from various species (Fig 5). *Syringolide Ps-1* (16) elicits the 'hypersensitivity response' of plant disease defences which include localised cell death, lignification of cell walls, the formation of antibiotics; some plants also accumulate jasmonic acids (17) as endogenous (secondary) signal molecules.<sup>13</sup> Probably the most important fungus in the Japanese fermentation industry is *Aspergillus oryzae* which is used extensively in the production of sake (rice wine), shoyu (soy sauce) and miso (fermented soy beans). The quality and yield of these fermentation processes are linked to the degree of sporulation of *A. oryzae* and research into the sporulation event led to the isolation of sporogen-AO 1 (18), a sporogenic sesquiterpene from the culture broth of *A. oryzae*<sup>14</sup>. Other bioregulators isolated include sclerosporin (19), a sporogenic substance from the fungi *Sclerotinia fructicola*, and differolide [4-(2',5'-dihydro-2'-oxo-3'-furanyl)-3a,4,5,6-tetrahydro-1(3*H*)-isobenzofuranone] (20),<sup>15</sup> a bioregulator for the formation of aerial mycelium and spores in *Streptomyces galucescens*.<sup>16</sup>



(Fig 5)

### 1.3 Autoregulator Biosynthesis

A-factor and related regulatory compounds are produced by an organism in extremely low concentrations. Due to the structural similarities between the autoregulators (7-15) it would not be unreasonable to assume that they also share a common biosynthesis. The extremely high potency of the autoregulators enables streptomycetes to require and therefore release only minute quantities to illicit the required response. This is indicated by the isolation of only 1.3 mg of Virginiae Butanolide D (15) from 1,450 litres of culture broth<sup>12</sup> of *S. virginiae*. This high potency and therefore low concentration of autoregulator within the broth made a biosynthetic study very difficult. In 1992 a strain of *S. antibioticus* was identified which produced several milligrams of Virginiae Butanolide A (12) *per* litre of culture, enabling Yasuhiro Yamada *et al*<sup>17-19</sup> to study its biosynthesis using normal stable isotope feeding experiments. Yamada showed that Virginiae Butanolide A was derived from two molecules of acetate and one molecule each of isovaleric acid and glycerol (Scheme 1).

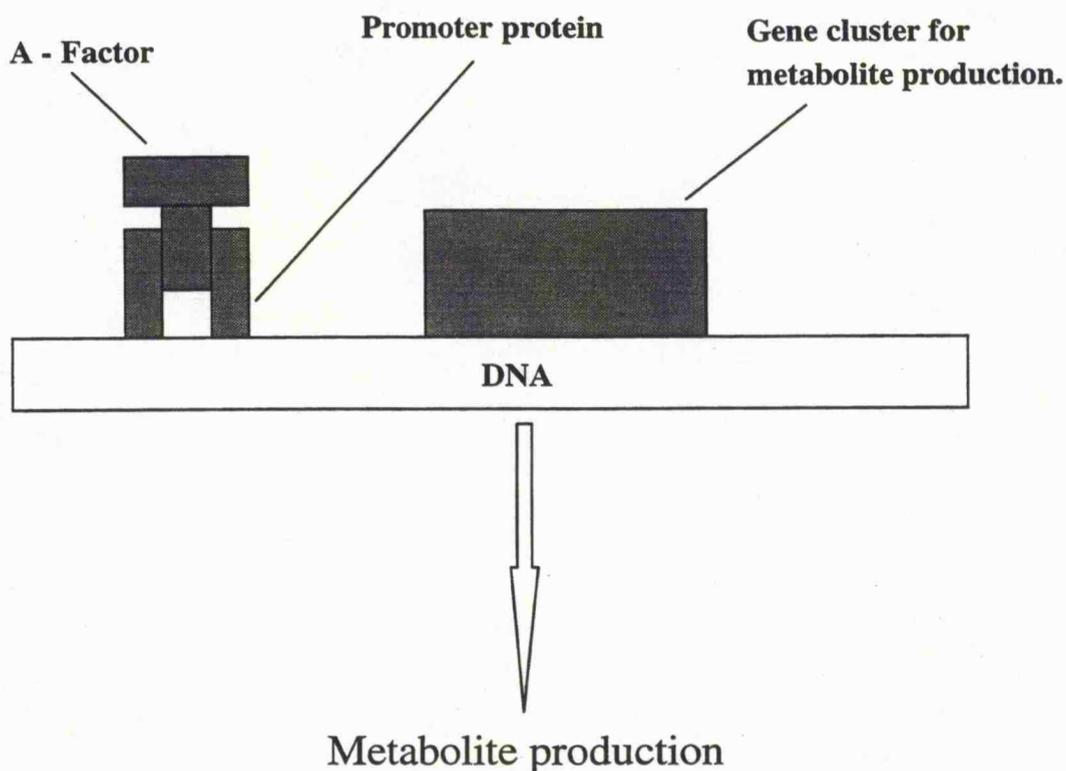


(Scheme 1) Biosynthesis of Virginiae Butanolide A.

#### 1.4 A-factor regulation

Streptomycetes produce between 60-70% of the important antibiotics in clinical use today.<sup>4</sup> The large number and pharmaceutical significance of these compounds has led to an interest in understanding the factors which effect the production of these metabolites. Research has centred on attempting to understand the fundamental mechanism by which A-factor exerts its pleiotropic effects. It is akin to hormones in eukaryotes as it activates several phenotypes such as metabolite production, resistance and sporulation in extremely low concentrations (as low as , for *S. griseus* ,  $10^{-9}$  M). It has been suggested<sup>2-3</sup> that autoregulators are released initially by parts of the colony that first sense an intracellular signal for differentiation. The lipid nature of the autoregulators allows them to diffuse through

the cell membrane into the media and then into neighbouring bacterial cells to form an intercellular signal for sporulation. Once the autoregulator has diffused through the cell membrane into the cytoplasm it binds to a promoter protein with high affinity, and this interaction promotes transcription of the gene cluster for metabolite production (Fig 6). Two research groups have now isolated proteins from *Streptomyces* species that will bind A-factor (7)<sup>20</sup> and Virginiae Butanolide C (14)<sup>21</sup>. This intercellular signal process starts a cascade of sporulation through the colony, allowing a relatively synchronous differentiation across the whole settlement. This synchronised sporulation of the *whole* colony increases the chances of survival for the species over lone spores released by localised areas of the mass. Removal of the autoregulator from the environment would then 'switch off' the metabolite production. In this way only a very small concentration of autoregulator is required (as one molecule of A-factor will result in thousands of transcriptions) for the regulatory process among the whole settlement.

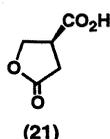


(Fig 6)

### 1.5 Synthesis of A-factor

Optically active A-factor was first synthesised in 1982 by Mori *et al*<sup>22</sup> followed in 1990 by other autoregulators,<sup>23</sup> both of these schemes employing enantiomerically pure (*S*)-(-)-paraconic acid (**21**) (Fig 7) as the key intermediate in the synthesis. Initially<sup>24</sup> (*S*)-(-)-paraconic acid was obtained from a racemic mixture by salt formation with a homochiral amine base. Repeated recrystallisation of the diastereomeric salt, followed by eventual liberation of the free acid gave chiral paraconic acid, however this process was extremely low yielding (3%). An improved synthesis of this important intermediate was reported in 1982<sup>25</sup> which employed an enantioselective hydrolysis of a prochiral diacetate with the enzyme porcine pancreatic lipase. Subsequent oxidation and hydrolysis steps yielded (*S*)-(-)-paraconic acid but in only 64 % enantiomeric excess. Again the low yielding

procedure of salt formation and recrystallisation as for the racemic paraconic acid was used to optically enrich the final product to near 100 % optical purity. Once optically pure (*S*)-(-)-paraconic acid was taken onto A-factor by reduction of the carboxylic acid functionality and protection of the resulting alcohol as the trimethylsilyl ether followed by LDA alkylation and final deprotection.



(Fig 7) (*S*)-(-)-Paraconic acid

### 1.6 Unregulated A-factor effects

A micro-organism normally produces autoregulators such as A-factor in response to an intracellular signal for sporulation (such as ecological stress due to nutrient starvation) to produce cytodifferentiation. However it has not been reported whether addition of A-factor at another period in the micro-organisms growth cycle would stimulate secondary metabolite production. The effects of an exogenous autoregulator on a *wild* type streptomycete rather than a blocked mutant has to our knowledge also not been investigated. It would be interesting to address the questions of whether the production of a secondary metabolite could be 'switched on' earlier than normal by addition of exogenous autoregulator, and whether a large excess, (relative to the natural wild type concentrations) would cause the regulation gene, and therefore production, to be active for longer thus giving an increased yield of the metabolite. Synthesis of A-factor and analogues followed by controlled feeding to a growing streptomycete prior to normal metabolite production and post feeding monitoring of the streptomycete's levels of antibiotic production may give an insight

into how much the autoregulators govern the organism's physical constitution. Conceivably this could add to the information necessary to fully understand the regulation processes apparent for secondary metabolism in streptomycetes. If an increase is apparent it may be of use in biotechnology to increase production, reduce the lag time for the onset of antibiotic production and so increase the turn over of the important antibiotics produced by streptomycetes.

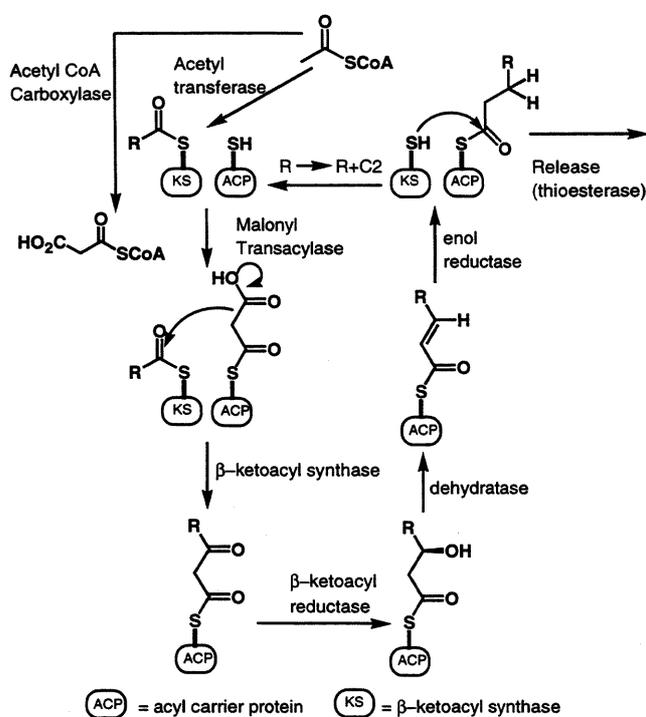
This proposal would require a source of optically active A-factor and hence, paraconic acid, with the ability to grow and monitor a *streptomycete*. As mentioned earlier, the present synthesis of A-factor depends upon a source of paraconic acid with complete optical purity and involves a low yielding optical enrichment process. Alternative methods produce A-factor with only partial<sup>26-28</sup> or no optical purity.<sup>29</sup> It was decided to address this problem by developing an asymmetric synthesis of this important intermediate thus avoiding the need for low yielding optical enrichment. The *streptomycete* chosen for this study was *S. nodosus*, the producer of the antibiotic amphotericin B (22) (Fig 8), as it is possible to monitor the production of its secondary metabolites amphotericin A (23) and B (22) using uv-vis spectroscopy. Both metabolites have characteristic absorptions between 260 and 410 nm from which it is possible to calculate their relative concentrations in the culture broth using their  $E_{1\text{cm}}^{1\%}$ <sup>30</sup>. Work was already under way within the group on the biosynthesis of amphotericin B, so conditions for the growth, monitoring and isolation of the metabolites were already developed. Therefore once the autoregulators were synthesised efforts could centre on monitoring their effects rather than developing a suitable protocol for the monitoring procedure.



relationship<sup>37</sup> between these metabolites. The polyketide synthase (PKS) for 6-methyl salicylic acid (6-MSA) has been isolated,<sup>38</sup> and using inhibition studies 6-MSA synthase was found to have two different thiol groupings analogous to the acyl carrier protein and  $\beta$ -ketosynthase thiols in fatty acid synthase. Cerulenin, an antibiotic isolated from *Cephalosporium caerulens*, is a potent inhibitor of fatty acid synthase systems and acts by inhibiting the  $\beta$ -ketoacyl synthase (KS), or condensing enzyme<sup>39</sup>. Cerulenin has also been found to inhibit the production of many polyketide synthases suggesting the condensing enzyme in polyketide biosynthesis resembles the analogous component in fatty acid biosynthesis<sup>40</sup>.

#### 1.7.2 Fatty Acid Biosynthesis

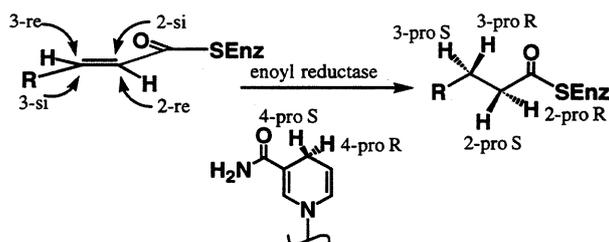
The synthesis of fatty acid occurs in most organisms and takes place by essentially a universal sequence of reactions (Scheme 2). There are two basic types of fatty acid synthase (FAS); Type I and Type II. Type I are generally found in eukaryotic organisms with each active site necessary for the whole fatty acid assembly process found in domains of large multifunctional proteins. In prokaryotic micro-organisms and plants Type II FAS operates. This comprises a dissociable group of discrete enzymes and an acyl carrier protein which can be separately isolated. In fatty acid biosynthesis it was initially thought that the first steps in the FAS cycle were the transfer of acetyl CoA onto an active thiol group of the  $\beta$ -ketosynthase; transfer of malonyl CoA (produced from the carboxylation of acetyl CoA) onto the acyl carrier protein; and a decarboxylative Claisen condensation to form an acetoacetyl group bound to the acyl carrier protein.



(Scheme 2) Fatty acid biosynthesis.

Recently however<sup>41</sup> a third condensing enzyme, 'condensing enzyme III' has been identified in *Escherichia coli* that catalyses the first condensation of acetyl CoA *directly* with malonyl bound to the acyl carrier protein. The direct condensation of acetyl CoA precludes the requirement for a transacylase activity, loading acetyl CoA onto a thiol residue of the condensing enzyme and this is proving a general phenomenon for Type II FASs of bacteria and plants. The  $\beta$ -ketosynthase is however utilised for subsequent condensations in the FAS cycle. After the condensation step a stereospecific NAD(P)H mediated reduction to give *only* the (3*R*)- $\beta$ -hydroxy intermediate is followed by dehydration to give the 2-(*E*)-enoyl species. The cycle is completed by a further NAD(P)H mediated reduction to produce the fully saturated

acyl group. The fully saturated acyl group is transferred onto the  $\beta$ -ketosynthase and a second condensation occurs. This cycle is repeated until the desired chain length is achieved, then a thioesterase releases the fatty acid. The stereochemical facet of each step has been studied and it has been shown that the carboxylation step occurs with retention of configuration while the condensation gives rise to an inversion of the configuration. The final reduction of the *trans*-2-enoyl species is variable in different organisms (Table 1) and enoyl reductases from different sources utilise different prochiral hydrogens of NAD(P)H and present hydrogens to varying faces of the double bond. This enables the hydrogens to add either suprafacially or antarafacially and with *re* or *si* face attack at C2 or C3 depending on the organism involved (Scheme 3).<sup>42</sup>



(Scheme 3) Stereospecific enoyl reduction with NAD(P)H.

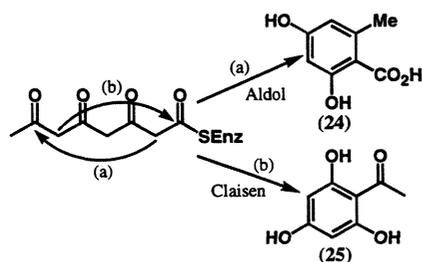
Source	NAD(P)H	C2	C3	Classification
<i>E. Coli</i>	4- <i>pro</i> S	<i>re</i>	<i>si</i>	Bacteria (Pro) suprafacial
<i>S. cerevisiae</i>	4- <i>pro</i> S	<i>si</i>	<i>si</i>	Yeast (Eu) antarafacial
Flour Beetle	4- <i>pro</i> R	<i>re</i>	<i>re</i>	Insect (Eu)
Rat liver	4- <i>pro</i> R	<i>si</i>	<i>re</i>	Mammal (Eu) suprafacial

(Pro = prokaryote, Eu = eukaryote)

(Table 1)

### 1.7.3 Aromatic Polyketides

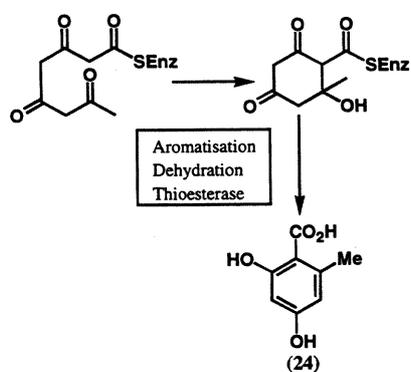
The majority of the polyketide secondary metabolites identified so far in Nature are the products of fungi or bacteria and of these a high percentage are aromatic, although some non-aromatic compounds exist. Aromatic polyketide formation occurs by repeated condensations of acetyl and malonyl CoA as for fatty acid biosynthesis but *without* reduction to the saturated methylene chain to produce a poly- $\beta$ -keto-thioester of the desired length. Folding of these poly- $\beta$ -keto-thioesters generated by the head-to-tail assembly of acetyl units followed by intramolecular Claisen or aldol-type condensations and dehydrative aromatisation leads to the aromatic polyketides (**Scheme 4a**). The aromatic polyketides can be formed from an unmodified poly- $\beta$ -keto-thioester chain such as in the formation of orsellinic acid (**24**) (**Scheme 4b**) or undergo modifications, either before aromatisation; such as reductions and C-methylation with S-adenosyl methionine (SAM) (as in the formation of flavipin (**26**) (**Scheme 5a**), or after aromatisation; such as O-methylation, oxidation and phenolic coupling as illustrated in the formation of usnic acid (**Scheme 5b**).



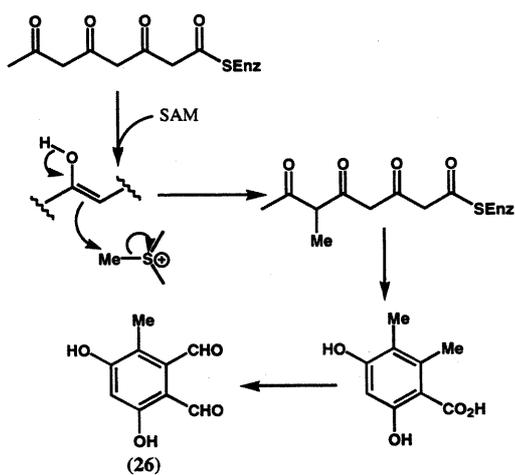
(**Scheme 4a**) Condensation of poly- $\beta$ -keto-thioester chains.

(a) Aldol type reaction to give orsellinic acid (**24**).

(b) Claisen type reaction to give phloroacetophenone (**25**)

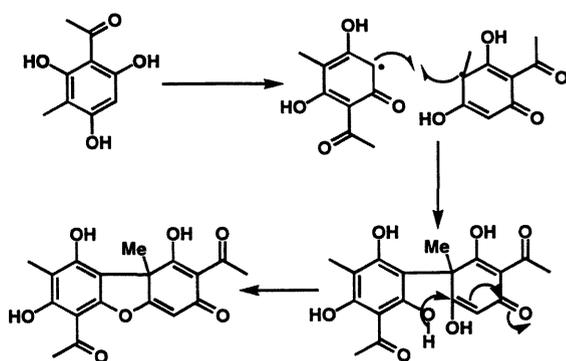


(Scheme 4b) Formation of orsellinic acid (24).



(Scheme 5a) SAM mediated C-methylation.

Formation of flavipin (26).

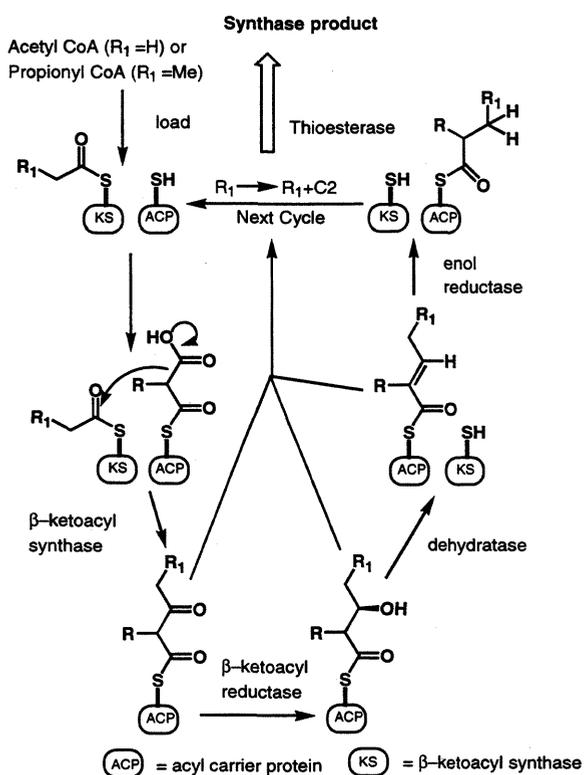


(Scheme 5b) Phenolic coupling to form usnic acid.

#### 1.7.4 Non-Aromatic Polyketides

The class of non-aromatic polyketides encompasses a vast degree of structural diversity and includes the polyethers, macrolides and reduced fungal metabolites. The structural complexity of these metabolites arises from the utilisation of starter units other than acetate such as propionate and butyrate during the chain extension, resulting in alkyl functionality along the polyketide backbone. The polyketide synthase can also either reduce or retain various levels of  $\beta$ -ketone functionality which arise during the assembly of the polyketide acyl chain. The synthase can utilise the whole complement of reducing enzymes involved in fatty acid biosynthesis. After each subunit condensation some of these reducing enzymes can be 'by-passed' so that intermediate reduction states are left intact. This enables functionality such as ketones, alcohols, double bonds and fully reduced methylene carbons all to be present in the final polyketide. The biosynthesis of polyketides is regarded as resembling that of fatty acid biosynthesis and two pathways, the '*processive*' (Scheme 6) and '*non-processive*' have been proposed. For the processive

pathway the decarboxylative Claisen condensation of a starter unit (acetyl or propionyl), with a chain extension unit (malonyl or methyl malonyl CoA), is followed by establishment of the required functionality stereospecifically from keto group to fully reduced  $-\text{CH}_2\text{CHR}-$  group *before* the next condensation. This cycle is repeated until the required length is achieved then releases the first enzyme-free intermediate. The whole assembly process is thought to take place on the polyketide synthase.



(Scheme 6) Polyketide synthase cycle.

The first enzyme-free intermediate is able to undergo specific tailoring modifications such as cyclisations, hydroxylation, oxidation and glycosidation with various sugars.

The non-processive pathway is thought to involve the repeated condensation of starter units and chain extension units until the polyketide chain, in the form of  $[\text{CH}_2\text{CO}]_n$ , reaches the desired length where the observed functionality, *via* an unspecified sequence of reductions and dehydrations occurs, doing so *after* the chain assembly is complete.

Strong evidence favouring the processive hypothesis now exists such as the intact incorporation of diketide chain intermediates<sup>43-48</sup> and the isolation of partially assembled chain intermediates from the mycinamicins.<sup>49</sup> Recent genetic studies have also provided a clearer understanding on a molecular basis of chain assembly and provide further evidence supporting the processive pathway.<sup>50</sup>

Studies of the genetics of the actinorhodin pathway<sup>51</sup> have led to the identification of genes coding for proteins which have a strong sequence homology with proteins of various fatty acid synthases and are probably the components of the polyketide synthase. Each of the individual genes are discrete, and therefore the corresponding enzyme activities are not linked as they are formed.

The actinorhodin polyketide synthase therefore resembles a Type II FAS of a dissociable set of co-operating enzymes. Only one gene codes for each catalytic activity in the FAS cycle which means that individual active sites in the polyketide synthase complex participate in successive cycles of chain assembly. Recently the complete sequence of the erythromycin polyketide synthase genes has been established.<sup>52</sup> It has been shown to consist of three large open reading frames, each coding for a large multifunctional protein with sufficient catalytic sites to carry out two chain extension cycles. There is an individual enzyme for *each* step carried out by the erythromycin polyketide synthase and these are organised in modules. Each module contains the active sites required for a condensation step and modification to

the desired functionality *before* passing onto the next module for a further condensation as in the processive pathway hypothesis.

#### 1.7.5 Study of Biosynthetic Pathways

The main reasons for studying the biosynthesis of secondary metabolites are:

- identification of the primary metabolite source used in the chain assembly
- determination of the mechanism by which these primary metabolites are incorporated into the secondary metabolite and the intermediates involved.
- to use the knowledge to develop new metabolites or increase production.

The major technique used for these studies is the use of isotopic tracers.<sup>53-58</sup> The isotopic label must have a low natural abundance, be easily detected even at low concentrations (preferably by non-destructive means), and to be relatively inexpensive. The experiments are usually performed by feeding the proposed precursor containing the label at a suitable site to the growing organism, and subsequent isolation and purification of the metabolite. If the metabolite contains the label at the expected site this would give some evidence for the intermediary of the labelled precursor in the biosynthetic pathway.

Tritium ( $^3\text{H}$ ) and carbon-14 ( $^{14}\text{C}$ ) are the most frequently used radioactive isotopes in biosynthetic studies. They are both  $\beta$ -emitters and have half lives of 12.3 and 5600 years respectively. Because of their low natural abundance and high sensitivity very low levels of label incorporation can be detected in the metabolite and this enables the use of very small quantities of precursor *per* feeding, resulting in minimal disturbance of the organisms normal metabolism. The exact position of the of the radioactive label, particularly carbon-14, is established by the chemical degradation and this is the main disadvantage when using radioactive labels. Doubly

labelled  $^3\text{H}$  and  $^{14}\text{C}$  precursors have been used. Comparison of the  $^3\text{H}:^{14}\text{C}$  ratio in both the precursor and enriched natural product (and if the ratios are the same), enables establishment of the intact incorporation of the labelled precursor but not the exact positioning of the label in the natural product without the need for chemical degradation.

The development of NMR and MS techniques has enabled the study of biosynthetic pathways using stable isotopes<sup>59</sup> such as  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{15}\text{N}$ , and  $^{18}\text{O}$  without the need for degradation studies of the isolated natural product. The only drawback is their relatively high natural abundance (**Table 2**) compared to radioactive isotopes, requiring a higher amount in each feeding which may lead to a disruption in the organism's metabolism and cause a decrease or complete cessation of metabolite production.

	$^2\text{H}$	$^{13}\text{C}$	$^{18}\text{O}$
Relative sensitivity	0.010	0.016	-
Nuclear spin	1	1/2	-
Natural abundance (%)	0.016	1.11	0.204

(Table 2)

Despite these problems, singly and doubly  $^{13}\text{C}$ -labelled precursors have become routine in biosynthetic studies since direct detection of  $^{13}\text{C}$  by NMR spectroscopy was made routine by the development of Fourier transform NMR and proton noise decoupling techniques. On feeding a  $^{13}\text{C}$  labelled precursor and accumulating the  $^{13}\text{C}$  spectrum, incorporation at any site will result in an enhancement of the corresponding  $^{13}\text{C}$  signal for the labelled carbon and this requires assignment of the

$^{13}\text{C}$  NMR spectrum of the metabolite. Also, because of the natural abundance of  $^{13}\text{C}$ , detection of small levels of incorporation (less than 1%) is difficult and a two-fold peak enhancement is required to conclude incorporation of label at a specific site with any certainty.

Deuterium ( $^2\text{H}$ ) NMR spectroscopy has enabled  $^2\text{H}$  incorporation to be detected directly<sup>60</sup> despite the problems of low sensitivity and the quadrupolar nucleus causing line broadening, and spectral crowding. The latter problem can be overcome by indirect observation using  $\alpha$  and  $\beta$ -shift techniques,<sup>60</sup> and measuring  $^2\text{H}$  indirectly by  $^{13}\text{C}$  NMR spectroscopy has become routine. For a proton decoupled  $^{13}\text{C}$  NMR spectrum of a carbon atom with one deuterium attached the signal appears as a 1:1:1 triplet with  $J_{\text{C-D}} \frac{1}{6}$  that of the corresponding  $J_{\text{C-H}}$ , with the signal shifted ( $\alpha$ -shift) 0.25-0.6 ppm upfield. Additional  $^2\text{H}$  atoms increase the multiplicity and the upfield shift induced is additive. However the often weak  $^{13}\text{C}$ - $^2\text{H}$  coupling is the limiting factor for this method. In the  $\beta$ -shift technique the telegraphing  $^{13}\text{C}$  nucleus is present  $\beta$  to the deuterium atom which can be detected by inducing an upfield shift of around 0.1 ppm in the reporter  $^{13}\text{C}$  nucleus for each  $^2\text{H}$  attached. The problems of poor signal intensity due to multiplicity and inefficient relaxation as found for the  $\alpha$ -shift method are removed, as two bond  $^{13}\text{C}$ - $^2\text{H}$  couplings are negligible and often hydrogen atoms attached to the reporter  $^{13}\text{C}$  nucleus aid relaxation. Oxygen-18 ( $^{18}\text{O}$ ) has no nuclear spin but can still be detected indirectly through  $^{13}\text{C}$  NMR spectroscopy as for deuterium and  $^{18}\text{O}$  induces an upfield shift of 0.01-0.035 ppm (relative to  $^{13}\text{C}$ - $^{16}\text{O}$ ) for singly bonded atoms, and shifts of 0.030-0.055 ppm in doubly bonded oxygens.

The incorporation of stable isotopes has also been studied using mass spectrometry. The low volatility of many metabolites for a number of years greatly restricted the use of this technique but the recent introduction of methods such as fast atom bombardment (FAB), and electrospray (ES) have generally overcome these

problems. These methods, although destructive, have the advantage of only requiring a very small sample size and the number of isotopic atoms incorporated *per* molecule can be determined. Low levels of incorporation are again hard to detect but with reasonable levels of label uptake this is possible. Incorporation is indicated by an enhancement of the [M+1] peak for a single incorporation of one  $^{13}\text{C}$  atom, an enhancement of [M+2] for incorporation of 2  $^{13}\text{C}$  atoms (at different sites), from feeding a singly labelled precursor or a doubly  $^{13}\text{C}$  labelled precursor being incorporated.

#### 1.7.6 Amphotericin B

Amphotericin B (22) is one of the polyene antibiotics. It is the drug of choice in the treatment of severe systemic fungal infections, particularly with immunocompromised patients such as AIDS sufferers and cancer patients undergoing chemotherapy. Amphotericin B is produced by the soil bacterium *Streptomyces nodosus* along with a co-metabolite amphotericin A (Fig 8), and was first isolated from the Orinoco river region of Venezuela in 1955.<sup>61-64</sup> The proposed mode of action<sup>65</sup> of amphotericin B is believed to be the complexation of eight steroid molecules, such as ergosterol, and eight amphotericin molecules to form a cyclic supramolecular complex which acts as a half pore within the fungal cell membrane. The dynamic nature of the cell membrane enables alignment of two such half pores forming an ionic channel causing the loss of potassium ions from within the cell. This loss of osmotic control leads to the cell's death. Mammalian cells also contain sterols (cholesterol) and amphotericin B acts on these cells as well as fungal cells which contain ergosterol. This lack of selectivity between host and fungal cell sterols leads to the toxicity of amphotericin B in humans, and side effects include

nephrotoxicity, neurological and cardiac disorders . Despite these adverse effects amphotericin B is still the drug of choice for serious deep seated fungal infections.

#### 1.7.7 The Biosynthesis of Amphotericin B

The proposed biosynthesis (Scheme 7) shows amphotericin B to be constructed from seventeen acetate and three propionate units assembled from processive head-to-tail condensation steps with the desired functionality inserted stereospecifically *before* the next condensation step in the processive manner. Once the desired chain length is reached cyclisation releases the first enzyme free intermediate as a 38 membered lactone ring. Subsequent tailoring enzymes hydroxylate the C8 position, glycosidation occurs with the amino sugar mycosamine on the C19 hydroxyl group, and the methyl group on C16 is oxidised to a carboxylic acid yielding the final metabolite. The order of the tailoring steps is unknown.

To date the only biosynthetic investigations into amphotericin B have involved radioactive labels<sup>66-68</sup> and no study using stable isotopes on this important natural product has been reported. Other studies have tended to centre on biological activity, structural confirmation by X-ray analysis,<sup>69,70</sup> and its total synthesis, which was completed in 1988 by Nicolaou.<sup>30,71-74</sup> We intend to initiate a biosynthetic study using stable isotopes initially looking at the propionate positions, by feeding [1-<sup>13</sup>C] and [3-<sup>13</sup>C]-propionate and measuring the incorporation using <sup>13</sup>C NMR spectroscopy and electrospray mass spectrometry. The <sup>13</sup>C and <sup>1</sup>H NMR spectrum for amphotericin B have been published<sup>75-78</sup> and most of the positions which may occur from propionate units in the biosynthesis have been assigned. On feeding 1-<sup>13</sup>C and 3-<sup>13</sup>C-propionate an enhancement of signals in the <sup>13</sup>C spectrum corresponding to carbons from a propionate unit will indicate label incorporation in these positions *and* also the role of propionate units in the biosynthesis.





Label incorporation can also be proven from the electrospray mass spectrum which would show an increase in intensity of the [M+1] peak for incorporation of a single  $^{13}\text{C}$  label, and an increase in the [M+2] peak for two labelled carbon atoms. The exact positioning of the  $^{13}\text{C}$  labels is not however possible using this technique.

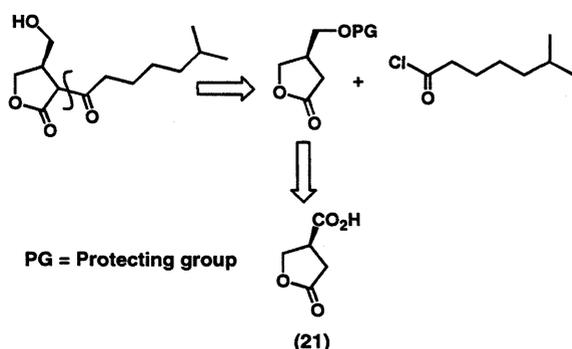
## CHAPTER 2

### Autoregulator Synthesis

## 2.0 Autoregulator synthesis

### 2.1 General Introduction

Autoregulators such as A-factor (**7**) are normally produced by the soil bacteria *streptomyces* at specific times in their life cycle in response to an intracellular signal to induce cytodifferentiation. The effects of an exogenous unregulated source of A-factor and analogues on a wild type *streptomyces* has not been reported and we intend to examine the effects of such a feeding experiment. As stated in the introduction for this proposal we required an optically active source of A-factor (**7**). The retrosynthetic analysis of A-factor (**Scheme 8**) highlights (*S*)-(-)-paraconic acid (**21**) as the key intermediate. Previous preparations of A-factor in the literature<sup>22-29</sup> have all employed paraconic acid and we too intend to follow this general strategy. We therefore required a convenient synthesis of homochiral (*S*)-(-)-paraconic acid. Early literature preparations of (**21**) involved the resolution of *racemic* paraconic acid by salt formation with (*R*)-(+)- $\alpha$ -phenethylamine.<sup>24</sup> Repeated recrystallisation of the salt and final release of the free acid using ion exchange resin gave (**21**), but in only 3% overall yield.



(Scheme 8) Retrosynthetic analysis of A-factor.

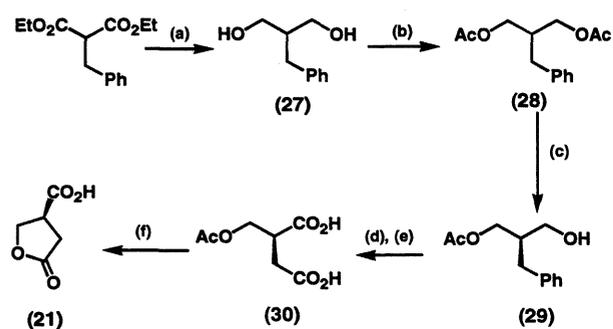
A more recent preparation of enantiopure paraconic acid<sup>25</sup> has been published that employs as the key step in the synthesis an enantioselective hydrolysis of a prochiral diacetate with porcine pancreatic lipase. The enantiomeric excess achieved however is reported as only 64% and again an extremely low yielding resolution was required to optically enrich the final paraconic acid to near 100% optical purity. Due to the limitations of the present literature preparations of enantiopure paraconic acid; the growing interest in autoregulators, and our requirement of optically active A-factor we decided to develop a convenient asymmetric synthesis of this intermediate, and therefore of A-factor itself.

## 2.2 Results and Discussion

### 2.2.1 Mori synthesis of (*S*)-(-)-Paraconic Acid

We began our program by initially repeating the synthesis of (*S*)-(-)-paraconic acid by Mori<sup>25</sup> in 1982 (Scheme 9). The synthesis involved porcine pancreatic lipase (PPL) in the key step to achieve an enantioselective hydrolysis of a prochiral diacetate. Repeating this synthesis would give an indication of the degree of enantiomeric excess possible from this enzymic method and its level of reproducibility. We reasoned that it may also be possible to increase the enantiomeric excess attainable from this system by altering the enzyme substrates. Mori *et al* did study the reaction conditions necessary to achieve the greatest enantiomeric excess varying the reaction temperature, solvent system and screening two other lipases (lipase MY and lipase Y) and it appears that these reaction variables have been optimised but a rational substrate study was not undertaken.

The initial steps in Mori's synthesis are both high yielding. The conversion of commercially available diethyl benzylmalonate to the corresponding diol (**27**) being achieved by a lithium aluminium hydride reduction in anhydrous THF followed by acetylation with acetic anhydride yielding the prochiral diacetate (**28**) in 85% overall yield for the two steps. The conditions requisite for the enzymic hydrolysis of the diacetate (**28**) involves stirring PPL and the substrate diacetate in a water/detergent/acetone mixture at 0 °C. The pH of the mixture is kept at 7.0 by neutralisation of the acetic acid liberated during the hydrolysis with dilute sodium hydroxide solution. The change in pH also gives an indication that the reaction is proceeding. Treatment of (**28**) with PPL under the conditions employed by Mori gave the desired alcohol (**29**) in 55-65% yield.



(Scheme 9) Mori synthesis of chiral paraconic acid. (a) LiAlH<sub>4</sub>, THF (b) Ac<sub>2</sub>O, Et<sub>3</sub>N (c) PPL, Triton-X100, Acetone, water pH = 7.0 (d) Jones reagent (e) Ozonolysis (f) HCl (2M)

The end point of the hydrolysis reaction is indicated by no further release of acetic acid. Comparison of the optical rotations of the sample of alcohol (**29**) and that prepared in the literature<sup>20</sup> indicated that the best enantiomeric excess obtained was

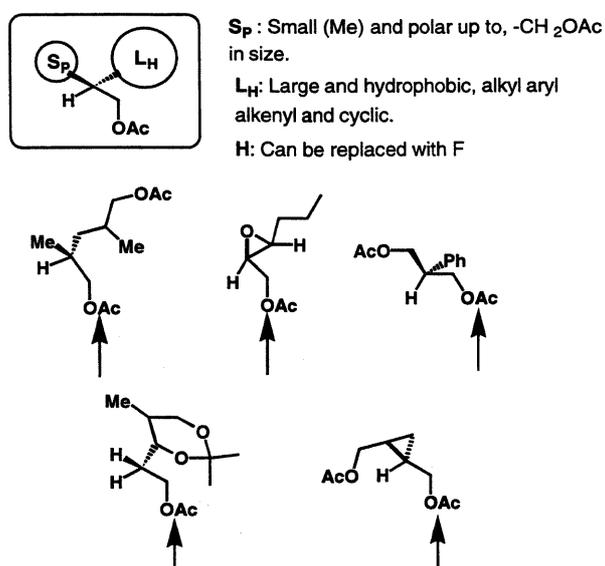
70%. This varied from 56-70% and was usually in the region of 60%. No *direct* measurement of the enantiomeric excess (ee) of (29), such as by fluorine-19 NMR on the Mosher's ester was carried out. However comparison of the literature optical rotation with that observed does give a reasonably accurate indication of the level of enantiomeric excess (ee). The enantiomeric excess can be further established, although tentatively, by examining the optical rotation of the final paraconic acid produced with that in the literature. If no racemisation occurs in the final stages then the (ee) from the alcohol will be carried through to the final paraconic acid. There is a chance however that during subsequent purification steps the enantiomeric excess may be enriched. Any comparisons therefore only serve as a guide and not proof of the initial (ee) of the starting alcohol (29). In Mori's synthesis the next steps involved a Jones oxidation of alcohol (29) followed by a three day ozonolysis of the phenyl ring to yield 2-acetoxy succinic acid (30). We found that during the Jones oxidation some loss of stereofidelity occurred. To overcome this problem and avoid a long ozonolysis the two oxidations steps were combined into a single reaction. Using ruthenium tetroxide as the oxidising agent generated catalytically *in situ* from ruthenium (III) chloride and periodic acid as the co-oxidant, both functional groups could be cleanly converted into the corresponding carboxylic acids without any loss of stereochemistry. This reaction system is well known in the literature<sup>79-81</sup> and provides an efficient and mild method of converting aromatic groups and double bonds into carboxylic acids enabling their use as carboxylate synthons. The final stage of the synthesis was the hydrolysis of (30) with dilute hydrochloric acid to yield (S)-(-)-paraconic acid (21) in 40% yield. Comparison of the observed optical rotation with that in the literature for optically pure paraconic acid enabled the enantiomeric excess to be determined as 59%. This is comparable to that observed for the alcohol (29) from the enzymic hydrolysis and indicates that no loss of stereofidelity had occurred in the subsequent oxidation and hydrolysis steps. It also shows that to

obtain (*S*)-(-)-paraconic acid in a completely homochiral form, the final paraconic acid, as Mori found, would have to undergo the extremely low yielding (3%) optical enrichment process. However the reactions were easy to perform, reasonably reproducible and gave moderate enantiomeric excesses.

## 2.3 Enzyme substrate studies

### 2.3.1 Strategy

The work carried out repeating Moris' synthesis of (*S*)-(-)-paraconic acid highlighted the advantages of an enzymic method for a chiral synthesis. The reactions were easy to perform; required only simple and cheap reagents; the conditions were mild, and the reactions reasonably reproducible. The only drawback being the degree of enantioselectivity observed. Porcine pancreatic lipase has been extensively studied<sup>32</sup> and in 1992 J. B. Jones *et al* proposed, from the results of a study on over one hundred esters, an active site model for this enzyme (Fig 9). This model contains a region which can accommodate a small polar group (Sp) and a region for a large hydrophobic group (LH). Using this model we decided to investigate the possibility of increasing the enantioselectivity of PPL in the synthesis by increasing the bulk of the large and hydrophobic group (LH) relative to the rest of substrate (28).

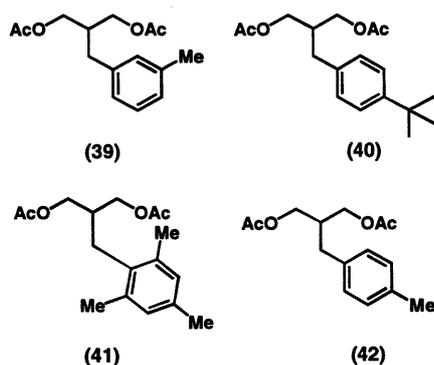


(Fig 9) Active site model for PPL and examples of enantioselective hydrolyses the model successfully explained.

If the enantioselectivity can be improved to an extent such that the requirement for optical enrichment of the final paraconic acid is removed ( $ee > 97\%$ ) then the strategy would become viable for the synthesis of optically active A-factor *via* homochiral paraconic acid.

The new enzyme substrates chosen for investigation (39-42) (Fig 10) have various levels of substitution around the phenyl ring, increasing its steric bulk. We reasoned that the increased bulk would enhance the selectivity of the enzyme by making the approach of the 'wrong' group of the prochiral diacetate into the active site of the lipase much less favourable due to steric interactions within the site. As the aromatic ring is later removed by oxidation to a carboxylic acid the type of

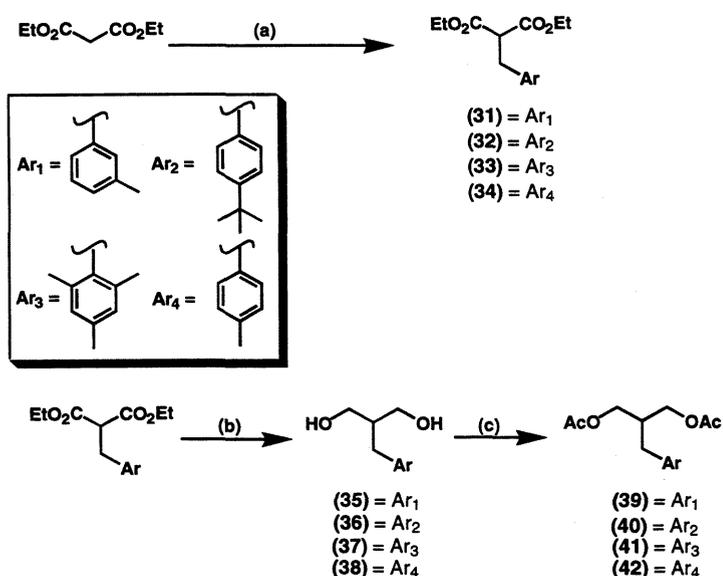
aromatic ring is unimportant. The ruthenium oxidation is also not adversely effected by the level of alkyl substitution around the ring.



(Fig 10) New enzyme substrates.

### 2.3.2 Enzyme substrates: synthesis and hydrolysis

The route chosen to the new enzyme substrates was *via* simple malonate chemistry (**Scheme 10**) as this class of coupling reactions is well preceded in the literature and usually achieves high yields. Coupling of diethyl malonate to various commercially available benzyl halides in anhydrous *N,N*-dimethylformamide and sodium hydride gave high yields (60-80%) of the corresponding aryl malonates (**31-34**). These coupling products were then reduced to the diols (**35-38**) with lithium aluminium hydride in dry tetrahydrofuran and acetylated with acetic anhydride and triethylamine yielding the desired prochiral diacetates (**34-37**) in good yields.



**(Scheme 10)** Synthesis of new prochiral enzyme substrates.

(a) NaH, DMF,  $\text{ArCH}_2\text{Cl}$  (b)  $\text{LiAlH}_4$ , THF (c)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ .

The new enzyme substrates were each screened for any uptake by PPL and hence enantioselective hydrolysis. The reactions were performed as for substrate (28) in Mori's synthesis of paraconic acid. The diacetates were dissolved in acetone/water/Triton X-100 (2:3:0.005) and the mixture homogenised in a sonic bath for 5 minutes. Porcine pancreatic lipase was added and the reaction mixture stirred at 0 °C, the pH being monitored and kept at 7.0. As mentioned previously an indication that the reaction was proceeding would be the release of acetic acid and hence a fall in the observed pH. If any of the new enzyme substrates were taken up by PPL and hydrolysis occurred, the reaction would be allowed to continue until the fall in pH (and therefore the release of acetic acid) was no longer detectable. At this

stage the reaction would be considered complete and the reaction mixture worked up.

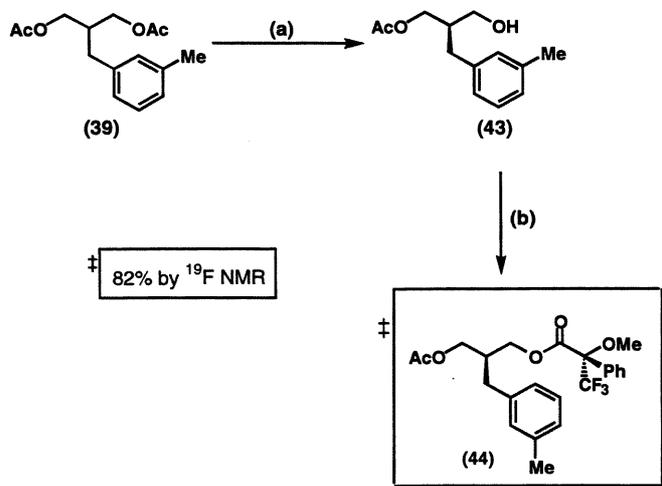
The results of the screen is summarised below (Table 3).

Substrate	Enzyme uptake/ Yield (%)	Reaction time (h)	ee obtained (%)*
(39)	Yes / 23	3	82
(40)	no reaction	no reaction	no reaction
(41)	no reaction	no reaction	no reaction
(42)	no reaction	no reaction	no reaction

\* By <sup>19</sup>F on the Mosher's ester

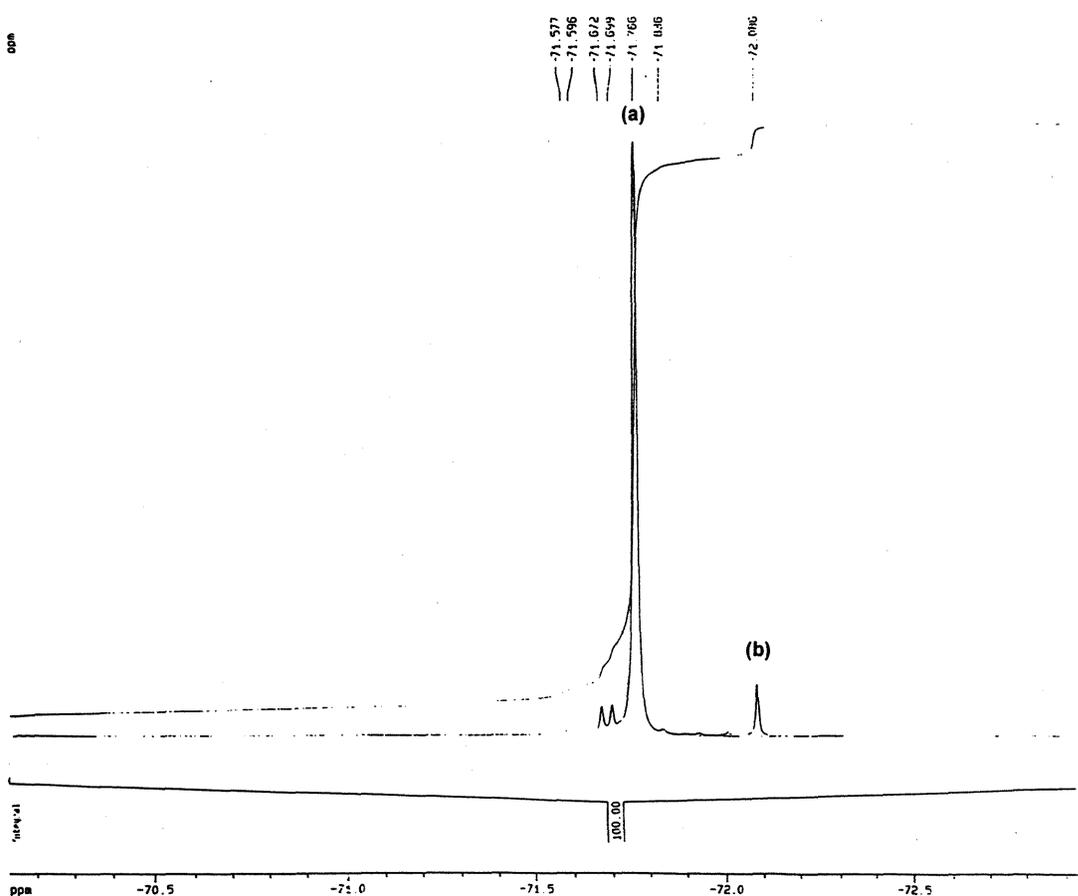
(Table 3)

As can be seen (Table 3) only one of the replacement diacetates (39) was a substrate for the enzyme. The acetates (40-42) gave none of the desired alcohol and in each case only starting material was recovered from the reaction mixture. The lack of uptake by PPL for the acetates (40-42) is probably a result of their increased steric bulk causing the substrate to no longer dock into the active site of the enzyme. However acetate (42) does have the same degree of substitution on the aromatic ring as for acetate (39) but, unlike (39) is *not* a substrate for the enzyme. This may indicate that the position of the alkyl substituents around the benzyl ring is important for substrate recognition by the enzyme and not just the steric bulk. The enantiomeric excess of the alcohol (43) was determined by the synthesis of the Mosher's ester (44) and examining its fluorine-19 NMR (Scheme 11).



**(Scheme 11)** Enantioselective hydrolysis of diacetate (39) with PPL and derivatisation to the Mosher's ester (44). (a) PPL, Acetone, water, Triton X-100 (b) Mosher's acid, DMAP, DCC.

From the <sup>19</sup>F NMR (Fig 11) the enantiomeric excess of (43) was determined as 82%. This was higher than we have previously been able to achieve with the simple phenyl ring *but* within the range of this simple system. The degree of enantioselectivity obtained was still low enough to demand that the final paraconic acid be optically enriched, and along with the reduced yield of only 23% made this route unviable even with this enhanced selectivity.



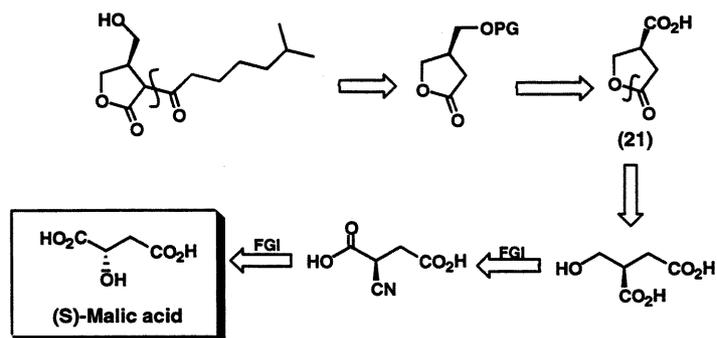
(Fig 11) Fluorine-19 NMR of the Mosher's ester (44).

((a) and (b) = diastereoisomers)

## 2.4 Malic acid as chiral precursor to (S)-(-)-Paraconic acid

### 2.4.1 Strategy

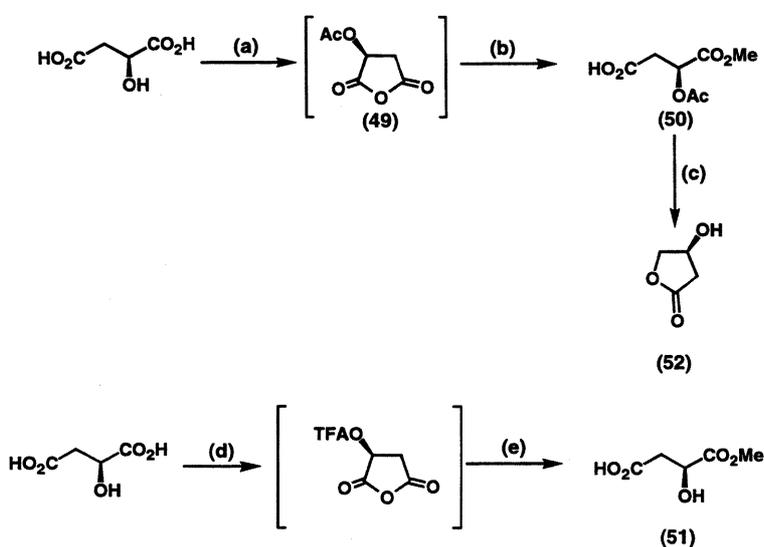
Due to the disappointing results obtained from an enzymic method for a homochiral synthesis of paraconic acid we decided to address the problem from a new direction. The retrosynthetic analysis of A-factor identifies the key intermediate as paraconic acid (21). If we carry the analysis on further and look at paraconic acid itself (Scheme 12) then one could reasonably suggest through suitable functional group interconversions that malic acid (45) would be an appropriate chiral synthon.



(Scheme 12) Retrosynthetic analysis  
of A-factor to (S)-malic acid (45).

Malic acid has been proven to be a valuable chiral starting material for enantiospecific synthesis and both enantiomers are commercially available in optically pure form. Examples of its use in the literature include the synthesis of



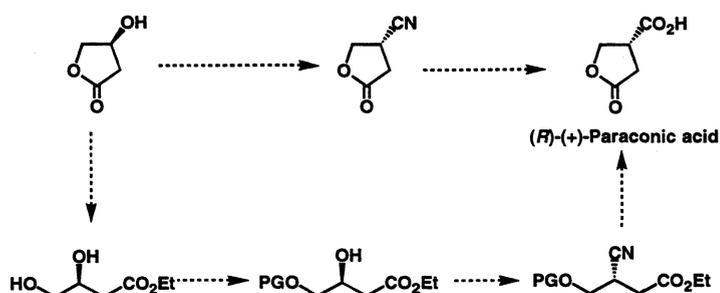


(Scheme 13) Selective protection and reduction of malic acid.

(a) AcCl (b) Dry MeOH (c) NaBH<sub>4</sub> (d) TFAA (e) Dry MeOH.

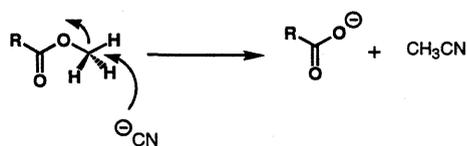
We reasoned that it may be possible to convert the hydroxyl group of the lactone alcohol (**52**) into a leaving group such as a sulfonate ester. Subsequent S<sub>N</sub>2 displacement and hydrolysis would then lead directly to paraconic acid. If this was not possible it seemed reasonable that the lactone (**52**) could be converted into a half ester, and the resulting primary alcohol protected (Scheme 14). This would then leave only the conversion of the secondary alcohol to a leaving group, displacement with cyanide and subsequent hydrolysis to convert the nitrile to a carboxylic acid. Unmasking of the primary alcohol would cause lactonisation and subsequent hydrolysis of the ester group yield paraconic acid. In the example shown the antipode of the desired paraconic acid acid enantiomer is formed however, the

correct enantiomer can be produced by starting with the other commercially available enantiomer of malic acid.



(Scheme 14) Hypothetical pathway to paraconic acid *via* lactone (52) produced from malic acid.

It is worth mentioning here that if the protecting group for the acid is a methyl ester, there is the possibility that during the cyanide displacement reaction the methyl ester may be cleaved to form the carboxylic acid. Methyl esters are known to be labile to cyanide; cleavage been thought to proceed by the attack of cyanide on the methyl carbon of the ester displacing the carboxylate anion. This provides an example of the rarely observed  $B_{AL2}$  mechanism<sup>88</sup> and a method of selectively cleaving a methyl ester in the presence of an ethyl ester (Fig 13).



(Fig 13) Cleavage of methyl esters with cyanide;  $B_{AL2}$  mechanism.

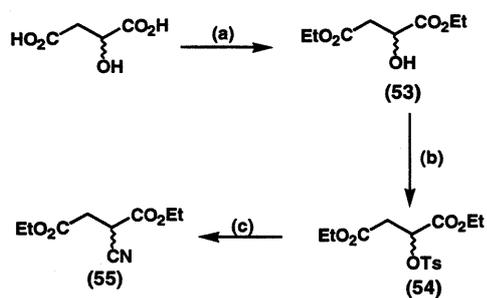
#### 2.4.2 Attempted Synthesis of Paraconic acid from Malic acid

We initially began the synthesis by showing that it was possible, using the literature precedent, to selectively protect the required acid functionality within malic acid and reduce the  $\gamma$ -carboxylic acid to the corresponding alcohol (Scheme 13). Treatment of (*S*)-(-)-malic acid with acetyl chloride at 45 °C gave the anhydride (49) in almost quantitative yield although it was not normally isolated. Reaction of (49) with anhydrous methanol gave the methyl ester (50) again in an almost quantitative yield. An analogous reaction was carried out using trifluoroacetic anhydride followed by dry methanol to yield the desired methyl ester (51). In this case the analogous anhydride was not isolated and also the methyl ester produced now contained the free  $\alpha$ -hydroxyl rather than the acetate as in (50). Both reactions indicate that it was possible to selectively protect the acid functionalities. The methyl ester (50) was reduced using a procedure developed by K. Soai *et al*<sup>89</sup> who was able to enhance the reactivity of sodium borohydride by the slow addition of methanol to a suspension of the reactants in *tert*-butanol at reflux. Reduction of the half ester (50) in this manner gave the  $\gamma$ -lactone alcohol (51) in high yield. At this stage we knew that the procedures for selective esterification and reduction were achievable. We decide to address the question of whether the cyanide displacement was possible, and whether the displacement, if successful, would proceed *via* an S<sub>N</sub>2 mechanism cleanly inverting the stereocentre.

We began by exploring the conditions requisite for the displacement reaction with *racemic* malic acid protecting both acid functionalities as the diethyl ester (53). There are numerous methods in the literature for the direct conversion of a hydroxyl group into a nitrile.<sup>90-93</sup> Of these methods the modified Mitsunobu reaction developed by Falck *et al*<sup>93</sup> looked promising as it is generally assumed that the Mitsunobu procedure involves an S<sub>N</sub>2-type displacement of an alkoxyphosphonium

species by the incoming nucleophile. If this mechanism holds for Falck's modified procedure then we could expect our nucleophile, in this case cyanide, to invert the stereocentre giving the desired stereochemistry at the  $\alpha$ -hydroxyl group. However we could not repeat this procedure and chose a two stage strategy of conversion of the hydroxyl group into a sulfonate ester followed by displacement with cyanide. The hydroxyl group was converted into the *p*-tolyl sulfonate ester (**54**) in high yield from the tosyl chloride and pyridine in chloroform. On heating a solution of the tosylate at 90 °C in dimethyl sulfoxide and potassium cyanide for five hours the corresponding nitrile (**55**) was produced in 85% yield (Scheme 15).

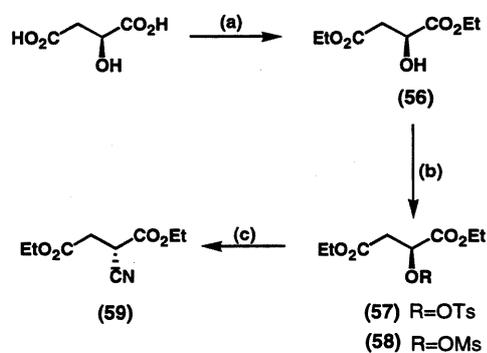
On determining ideal conditions for the displacement reaction we turned our attention to the optically pure malic acid in order to establish if the displacement was proceeding with the desired inversion of stereochemistry to furnish the absolute configuration required in the final paraconic acid.



(Scheme 15) (a) EtOH/H<sup>+</sup> (b) TsCl, Py (c) DMSO, KCN.

The same reaction sequence as for the racemic compound was followed employing optically pure malic acid as the starting material. Formation of the diethyl ester (**56**) from commercially available (*R*)-(+)-malic acid with ethanol and a catalytic amount of mineral acid proceeded in high yield. The hydroxyl group was converted into both

the methyl (**57**) and *p*-tolyl sulfonate esters (**58**) to determine which of the leaving groups would give the best results in the cyanide displacement reaction. The formation of the mesylate however gave only poor yields (43%) relative to the tosylate (89%) due to its greater susceptibility towards elimination to form diethyl fumarate and so work centred on the *p*-tolyl sulfonate derivative. Treatment of tosyl ester (**58**) under identical conditions as for the *racemic* analogue (**54**) (stirring in DMSO and potassium cyanide at 90 °C for five hours) gave the desired nitrile (**59**) in good yield (**Scheme 16**).

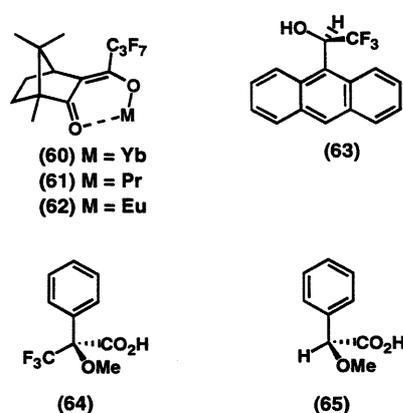


**(Scheme 16)** (a) EtOH/H<sup>+</sup> (b) TsCl  
(or MsCl), Pyridine (c) DMSO, KCN.

#### 2.4.3 Determination of enantiopurity utilising chiral shift reagents.

At this stage we had determined that it was possible to distinguish chemically between the carboxylic acid groups in our chiral precursor malic acid. This enabled the introduction of the desired functionality within the precursor ready for lactone formation which would occur spontaneously on 'unmasking' the acid and alcohol functionality by removal of the appropriate protecting groups. We had also

established a procedure for the formation of the nitrile by displacement of the *p*-tolyl sulfonate ester derivative. At this stage however the stereochemical outcome of the cyanide displacement reaction was unclear. There are numerous methods of determining the enantiomeric excess of a given compound. Techniques such as GC and HPLC with columns containing chiral packing material offer one method of direct measurement but many chemists use NMR methods and numerous examples can be found in the literature.<sup>94-97</sup> Enantiomers cannot be determined in an achiral medium due to the resonances of enantiotopic nuclei being isochronous. However it is possible to distinguish *diastereoisomers* if certain resonances of diastereotopic nuclei are anisochronous. This chemical shift non-equivalence of diastereotopic nuclei can therefore be measured using NMR by initially converting the enantiomers into a diastereomeric mixture using a suitable chiral auxiliary. If there is sufficient chemical shift non-equivalence to enable base line resolution then integration gives a direct measurement of diastereomeric composition and therefore the *enantiomeric* composition of the mixture. The main types of chiral auxiliary used are chiral lanthanide shift reagents (CLSR); chiral solvating agents (CSA); and chiral derivatising agents (CDA). Chiral lanthanide shift reagents such as ytterbium, praseodymium and europium heptafluorohydroxymethylene-d-camphorato (hfc) complexes (Fig 14) (60-62) act by forming a weak addition complex to the organic compound which is in fast exchange on the NMR time scale with the unbound organic substrate. The induced shift can be either to higher or lower frequency and is dependent on the distance and angle of the nucleus from the metal centre, and the metal itself.



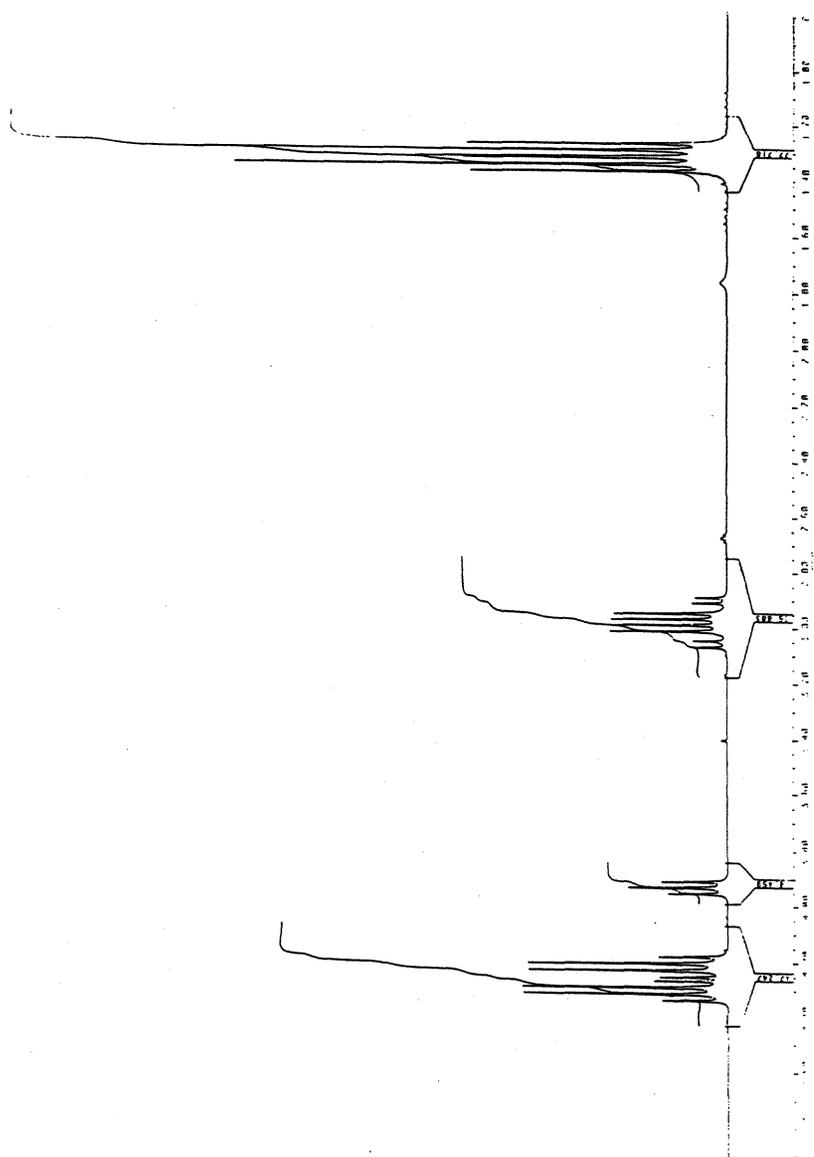
(Fig 14) Example chiral auxiliaries for enantiomeric excess determination

Chiral solvating agents such as 1-(9'-anthryl)-2,2,2-trifluoroethanol (**63**) act in a similar way to CLSR's by forming solvation complexes with solute enantiomers in a rapidly exchanging equilibrium with the bulk solvent. The chemical shift anisochronicity is caused by the relative position of the magnetically inequivalent groups (e.g. carbonyl groups) in the solution conformers to those in the diastereomeric complexes. The third class of chiral auxiliary, the chiral derivatising agent, is probably the most widely used of the three auxiliaries. Examples include  $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenyl acetic acid (Mosher's acid (**64**)) and (*S*)-O-methyl mandelic acid (**65**); although the list is extensive and enables observation of numerous nuclei other than proton, carbon and fluorine. Derivatisation of enantiomers with an enantiomerically pure auxiliary remains the most common assay of enantiomeric purity. Unlike CLSR and CSA such derivatisation covalently links the auxiliary to the organic substrate and yields discrete diastereoisomers. Chiral derivatisation agents have the advantage that the observed chemical shift non-equivalence is typically up to five times that observed with CSAs. They do require,

however, functionality within the organic substrate to provide a means of attachment for the auxiliary. The formation of the diastereoisomers must be carried out under conditions that exclude the chance of racemisation, kinetic resolution due to preferential reaction rates of the substrate enantiomers, and purification methods that avoid selective enrichment of one diastereoisomer.

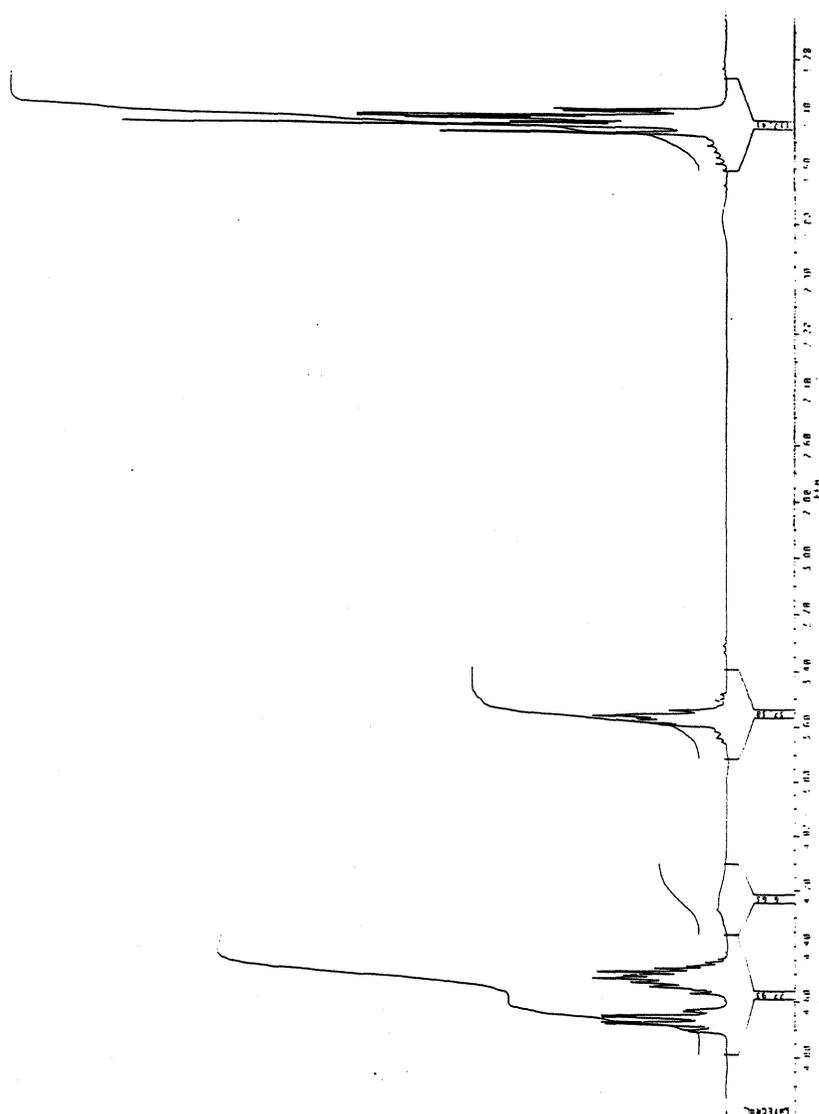
If we consider nitrile (**59**) then there is no easy functionality to which we could covalently attach an auxiliary such as Mosher's acid. The use of CDA strategy would therefore require some form of functional group interconversion to enable attachment of the auxiliary. Ester hydrolysis or reduction, to yield the carboxylic acid or alcohol respectively, or reduction of the nitrile group to give the corresponding amine would all yield groups suitable for derivatisation. A more direct method would be to utilise CLSR or CSA strategy requiring no functional group manipulation and avoiding the inherent risks of racemisation or optical enrichment. We decided to explore the possible use of chiral lanthanide shift reagents as their use in the literature<sup>94</sup> is widely documented. The shift reagents chosen were europium (III) heptafluorohydroxymethylene-d-camphorato ( $\text{Eu}(\text{hfc})_3$ ) (**62**) and praseodymium (III) heptafluorohydroxymethylene-d-camphorato ( $\text{Pr}(\text{hfc})_3$ ) (**61**) as both are commercially available from the Aldrich chemical company. The experiments were performed by initially obtaining a 'blank' proton NMR spectrum (Fig 15) of the nitrile (**59**) (60 mg in deuterated chloroform on a 300 MHz instrument) with no CLSR present. Increasing amounts of shift reagent were then added in portions (10 mg) and the new proton spectrum recorded between each addition until a suitable separation of a discernible signal became apparent, enabling the enantiomeric excess to be determined. Initially an europium lanthanide shift reagent was used but this gave poor results (Fig 16-17) and a clean separation of signals was not observable. However on changing to the praseodymium reagent a cleaner separation of signals was observed (Fig 18-19). On addition of 20 mg of shift reagent there was a

definitive change in the proton spectrum (Fig 18) compared to the 'blank' spectrum (Fig 15). The methyl triplets of the ester group at 1.20 ppm to 1.40 ppm were initially partially overlapping but began to separate out into discrete triplets. The methylene group originally at 3.00 ppm was shifted upfield to 2.75 ppm and each pair of doublets from the AB of an ABM system was further split each into doublets.

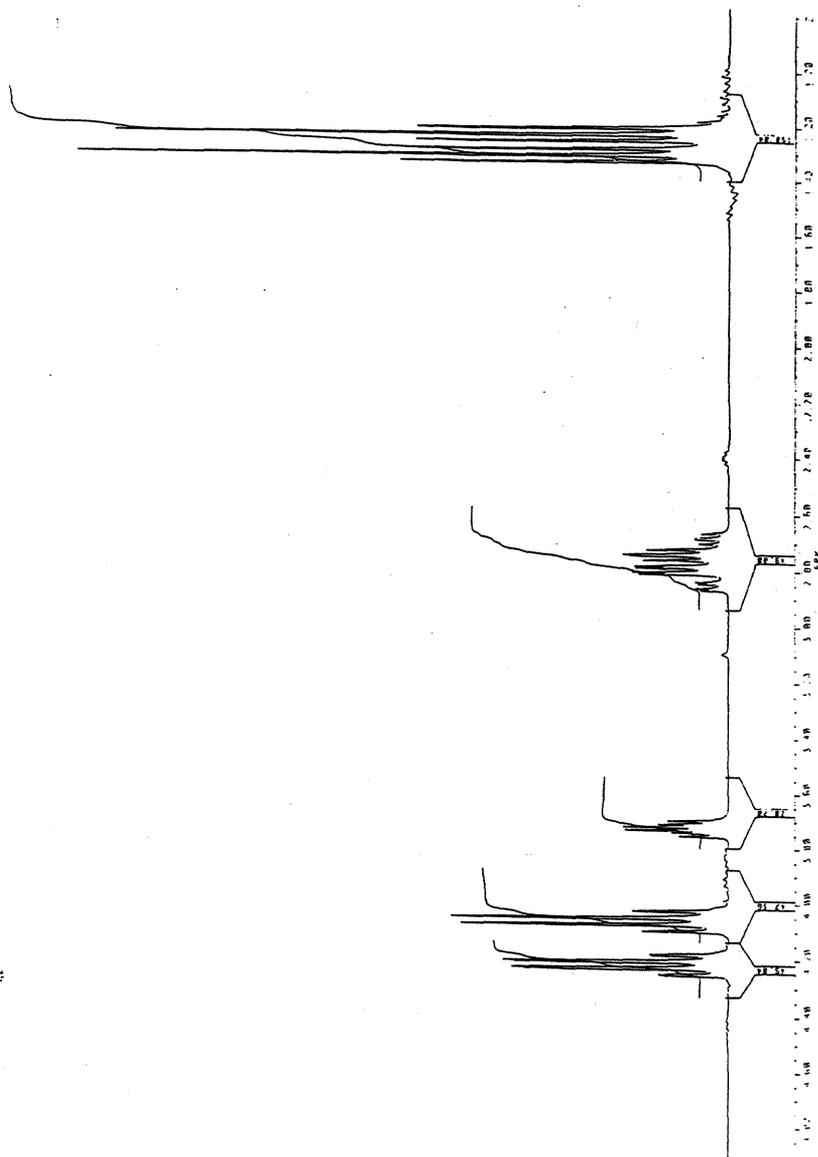


(Fig 15) Blank proton spectrum of nitrile (59)  
(no shift reagent present).

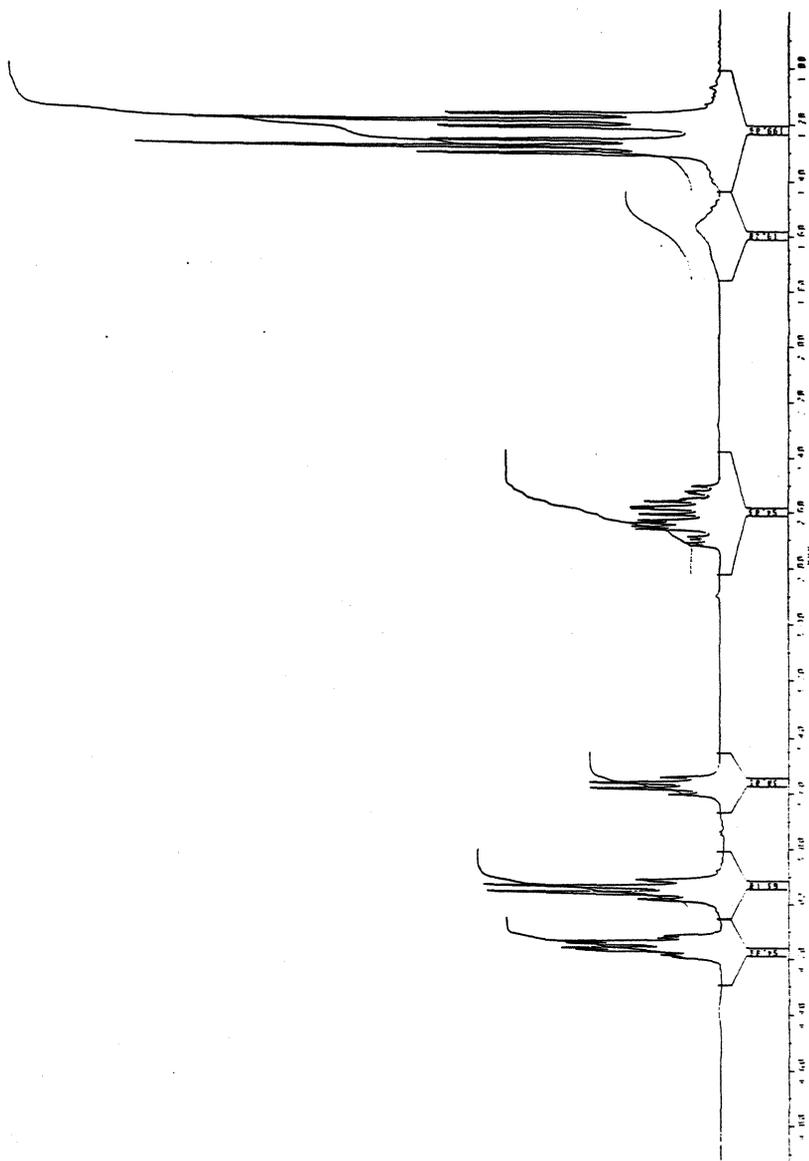




(Fig 17) Proton NMR spectrum of nitrile (59) with  
40 mg of Eu(hfc)<sub>3</sub> shift reagent present.



(Fig 18) Proton NMR spectrum of nitrile (59) with  
20 mg Pr(hfc)<sub>3</sub> shift reagent present.



(Fig 19) Proton NMR spectrum of nitrile (59) with 40 mg  $\text{Pr}(\text{hfc})_3$  shift reagent present.



The methyne proton signal was shifted upfield from 3.95 ppm to 3.75 ppm and became a complex multiplet while the close quartets of the methylene protons of the ethyl ester was also shifted upfield and separated further. Each peak of the quartet at 4.20 ppm also began to split into doublets and this along with the splitting pattern observed for the methylene group at 2.75 ppm indicated that the signals for the separate enantiomers of (59) were becoming observable. The optimum concentration of shift reagent was found to be 40 mg and higher levels gave only broad signals which yielded no serviceable information. When 40 mg of shift reagent was present (Fig 19) the methyl triplets from the ethyl ester were split such that each triplet was discrete, and that individual peaks within the triplet pattern were further split into doublets. The methylene protons were again shifted to 2.60 ppm and split such that the original AB part of the ABM pattern was further split so each signal was a doublet. The signal corresponding to the methyne proton was now at 3.55 ppm and appears as a quartet while the methylene protons of the ester group split such that each of the peaks within the quartet were split further into doublets.

The induced splitting throughout the chiral lanthanide shift reagent experiments always approximated to a 1:1 doublet. This indicated that the ratio of enantiomers was also (1:1) and that the nitrile (59) was essentially a racemic compound. Initial investigations on nitrile (59) showed it to possess an optical rotation, and this originally led to the assumption that the compound was optically active. In order to confirm the result obtained from the NMR studies we repeated the CLSR experiments on the racemate (55). Under identical experimental conditions the same splitting patterns were observed (Fig 20) confirming that nitrile (59) was essentially racemic; the observed optical rotation being due to either the nitrile having a small enantiomeric excess (< 2%), or a possible impurity in the sample at the time of measurement. This indicated that the displacement was either not proceeding *via* an S<sub>N</sub>2 mechanism as originally hoped, or that after displacement

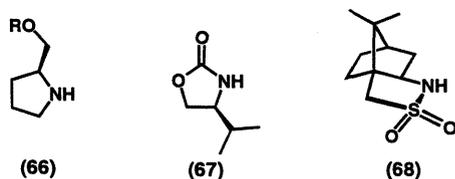
racemisation occurred, perhaps due to deprotonation of the labile methylene proton prior to work up.

## 2.5 Synthesis of homochiral paraconic acid from chiral auxiliaries

### 2.5.1 Strategy

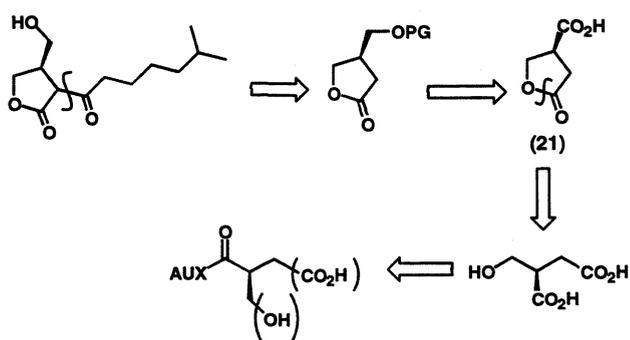
Recently methodology enabling asymmetric alkylation reactions using chiral auxiliaries has been developed. We decided to utilise this methodology in our synthesis of homochiral paraconic acid as these reagents are capable of achieving high yielding and highly diastereoselective reactions. The chiral auxiliaries (**66-68**) (**Fig 21**) all share the following features:

- Easily acylated with acyl halides in high yields.
- Undergo highly diastereoselective alkylation reactions from ordered transition states with fixed enolate geometry.
- Distinct auxiliaries are available which allow access to both enantiomers of the product.
- Easily removed under mild conditions and in high yields.



(Fig 21) Representative chiral auxiliaries.

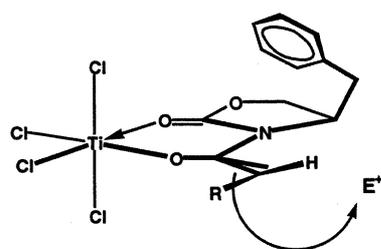
From the retrosynthetic analysis (**Scheme 17**) we required the ability, employing the chiral auxiliary to diastereoselectively attach a hydroxymethyl group; (either protected or as the free alcohol) to an acyl chain bound to the chiral auxiliary. The acyl chain could then be constructed to contain a carboxylic acid synthon that may be revealed as the free acid after the alkylation step. Subsequent removal of the auxiliary and hydroxyl protecting group (if used) would set up the lactonisation and so formation of paraconic acid. If the alkylation were to proceed with the desired diastereoselectivity then the paraconic acid produced would be of a very high enantiomeric purity, removing the requirement for optical enrichment.



(**Scheme 17**) Retrosynthetic analysis indicating possible auxiliary target. Brackets indicate final functionality required.

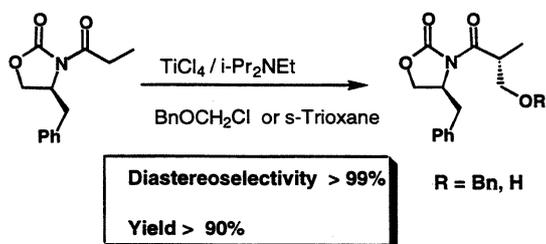
A precedent for this type of conversion was found in the literature from the work of D. Evans<sup>98</sup> who developed the 2-oxazolidinone class of auxiliaries (**67**). He described the use of a titanium enolate species generated from the precomplexation

of titanium (IV) chloride with the auxiliary followed by deprotonation with an amine base such as Hünig's base (diisopropylethylamine) or triethylamine (Fig 22).



(Fig 22) Titanium enolate complex.

The order of addition of the reagents was critical due to the irreversible formation of a titanium (IV) chloride-amine base complex, if the amine was added prior to the titanium chloride, and therefore none of the desired enolate formation. Of particular interest were the examples below (Fig 23) which showed the addition of both a protected and free hydroxymethyl group to the enolate with almost complete diastereoselectivity and in very high yield.

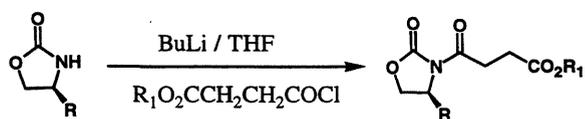


(Fig 23)

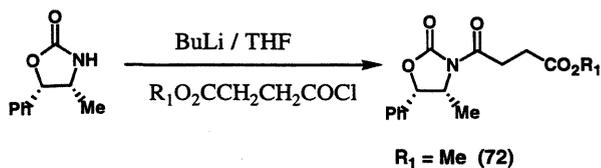
We intended to exploit this reaction in our synthesis of paraconic acid and hence A-factor by attaching to the acyl chain a veiled carboxylate functionality. Following the diastereoselective electrophilic addition to the titanium enolate, (employing either the benzyl chloromethyl ether or *s*-trioxane to give the protected or free hydroxymethyl group respectively), the acid functionality could be revealed. If, as with Evans' example, the alkylation proceeded with essentially full diastereoselectivity, the paraconic acid produced, following removal of the auxiliary and protecting groups, would be a single enantiomer.

#### 2.5.2 Simple esters as latent carboxylate functionalities.

We began by exploring the possibility that a simple ethyl or methyl ester would act as a suitable masking group for the acid functionality during the alkylation reaction and be easily removed by hydrolysis at the desired time during our synthetic scheme. In Evans' original paper a methyl ester was used as part of a *five* carbon acyl chain bound to an oxazolidinone chiral auxiliary and this was found to be stable to the reaction conditions, although the electrophile used was not either benzylchloromethyl ether or *s*-trioxane. This provided a suitable precedent for our strategy and therefore a reasonable starting point. Both methyl and ethyl succinyl chloride were commercially available and gave direct access to the desired acylated adduct from reaction with the *N*-lithium anion of the appropriate auxiliary. Originally the chiral auxiliaries were purchased from the Aldrich chemical company and three auxiliaries were tested including the non-chiral oxazolidinone (Scheme 18).



	(69)	(70)	(71)
R	H	Bn	Bn
R <sub>1</sub>	Et	Et	Me

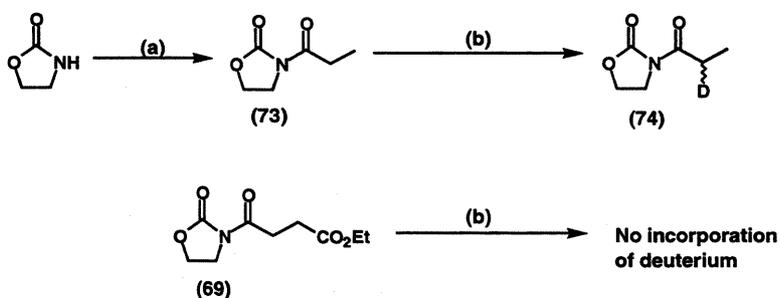


(Scheme 18)

Treatment of the appropriate auxiliary with *n*-butyl lithium in dry tetrahydrofuran at 0 °C to form the *N*-lithium anion followed by dropwise addition of the acid chloride at -78 °C gave the corresponding coupled products (69-72) as colourless crystalline solids in high yields (60-90%). Each adduct was tested in the titanium enolate reaction. The acylated auxiliaries (69-72) in dry dichloromethane under nitrogen were precomplexed with titanium (IV) chloride at 0 °C to give a deep orange solution of the complex. After a five minute delay Hünig's base was added furnishing a deep red/orange solution which was stirred at 0 °C for a further hour under nitrogen to ensure full enolate formation before addition of the electrophile. It is worth mentioning here that the benzylchloromethyl ether was originally purchased from the Fluka chemical company but due to a purity of only 60% (the NMR showed numerous impurities) the supplier was changed to TCI who were able to supply the benzylchloromethyl ether in greater than 98% purity. The titanium enolate solution

was treated with either *s*-trioxane or benzylchloromethyl ether and the reaction quenched after stirring at 0 °C for 20 hours. Examination of the reaction before and after quenching indicated numerous products and neither the desired alkylated product, nor starting material were recoverable from the reaction mixture. We reasoned that despite the literature precedent for using an ester functionality in the acyl chain the group was labile to the reaction conditions. Titanium (IV) chloride is a strong Lewis acid and was possibly attacking the ester group. No single product was actually isolated from the reaction mixture or any structure determined.

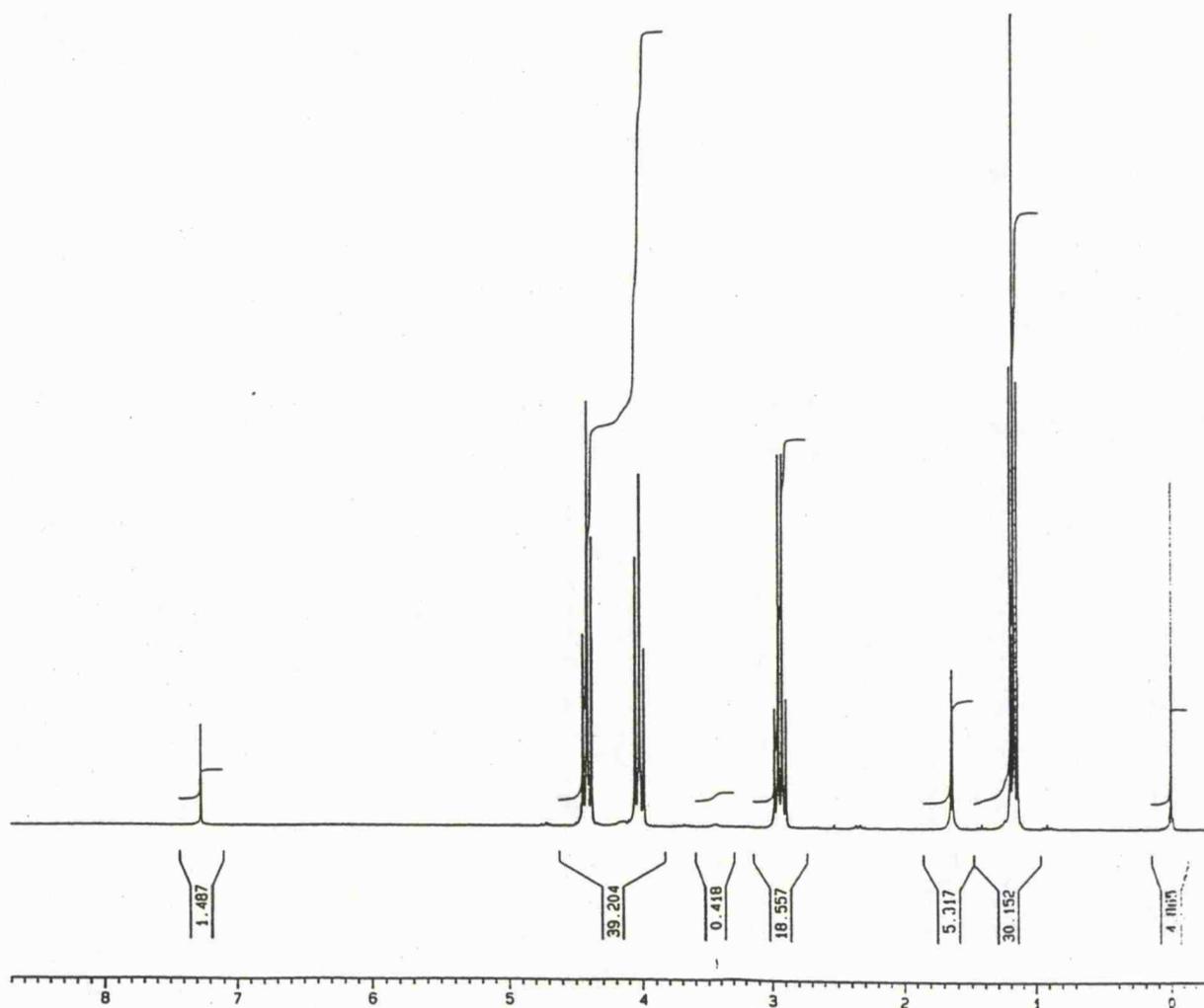
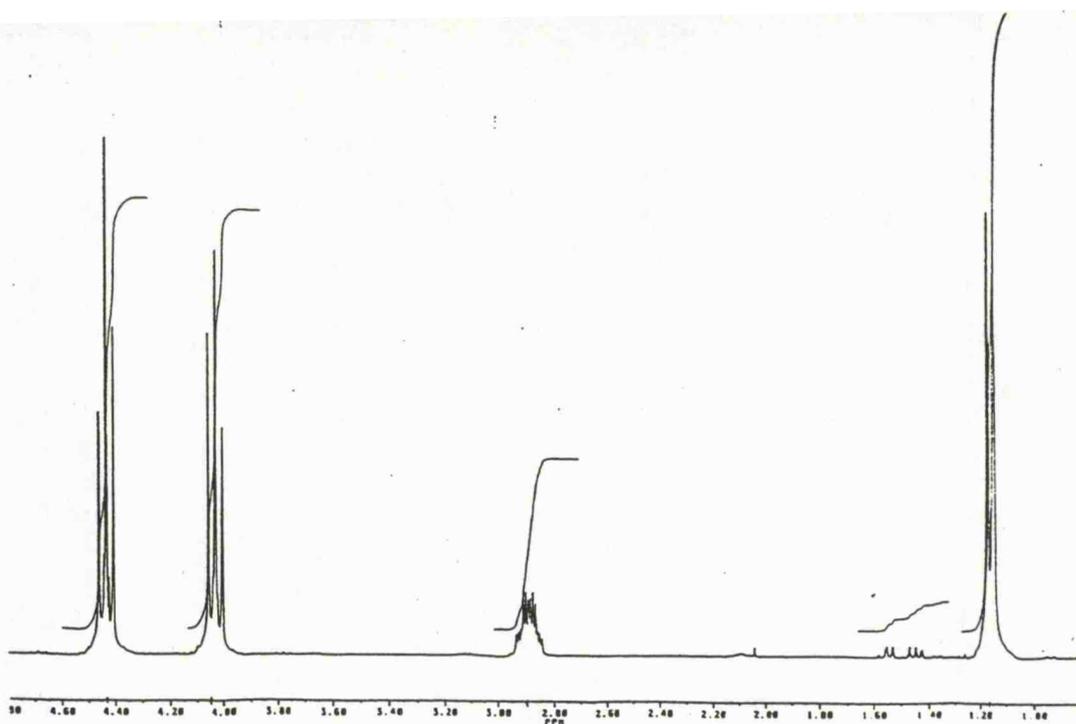
To determine if the starting material was labile to the conditions requisite for enolate formation reaction we synthesised the propyl acylated analogue (**73**) of auxiliary (**69**). This would enable a comparison between the reaction with a plain hydrocarbon acyl chain against the ester containing auxiliary. Treatment of 2-oxazolidinone with *n*-butyl lithium in anhydrous tetrahydrofuran at 0 °C followed by careful addition of propyl chloride gave (**73**) in 63% yield as a colourless solid. Both (**73**) and ester (**69**) were treated under identical conditions for the normal formation of the titanium enolate.



(Scheme 19) (a) BuLi, THF, Propionyl chloride,  
 (b) (i) TiCl<sub>4</sub>, Hünigs base, (ii) 37% DCl / D<sub>2</sub>O.

To the acylated auxiliaries (73) and (69) in anhydrous dichloromethane at 0 °C under nitrogen was added titanium (IV) chloride giving a yellow slurry and a orange solution respectively. After a five minute delay diisopropylethylamine was added and the resulting deep red solution stirred for a further hour at 0 °C before the enolate was quenched with deuterium chloride in deuterium oxide (37% v/v DCl/D<sub>2</sub>O) (Scheme 19) and the products isolated.

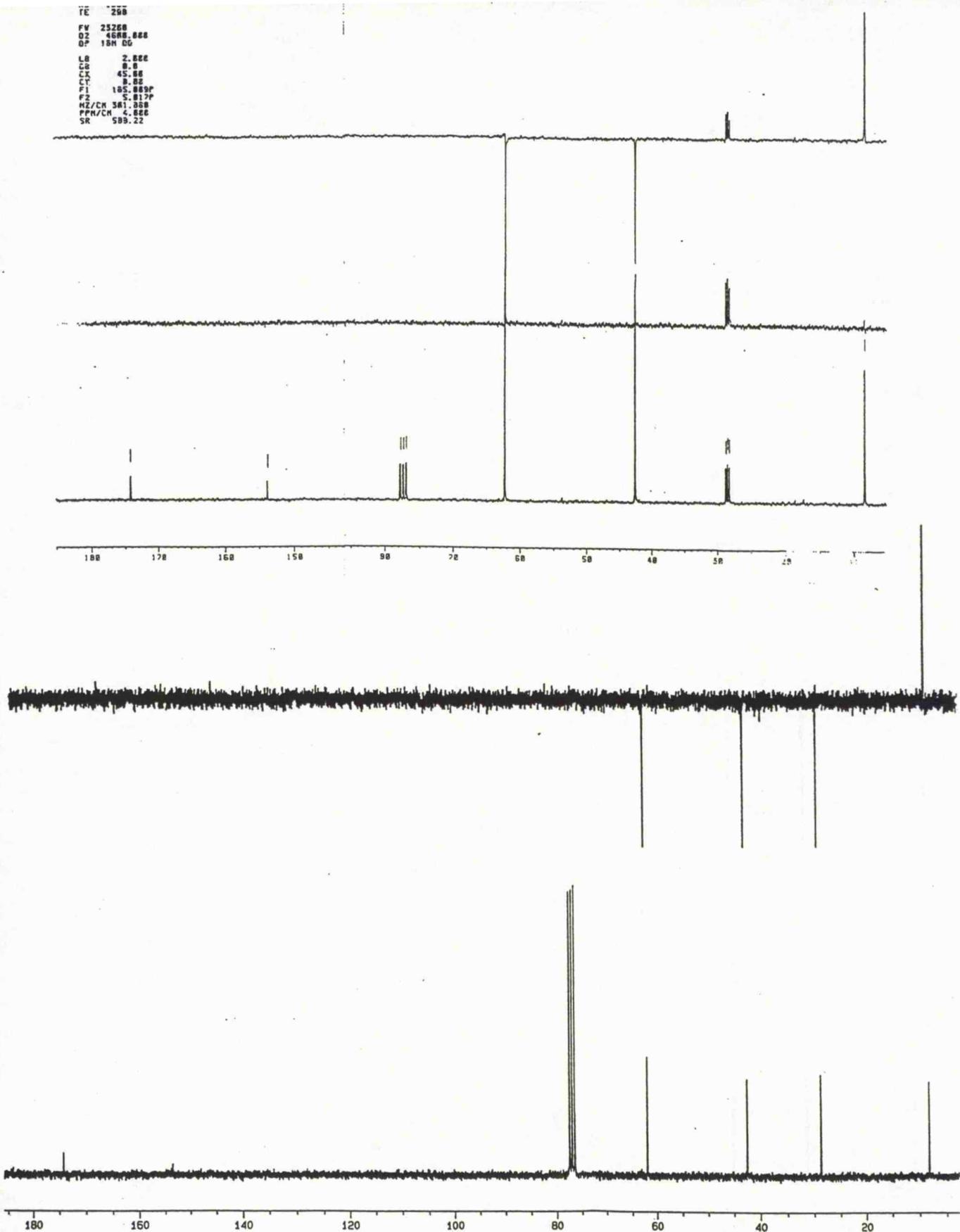
From the comparison of the proton (Fig 24a) and carbon spectra (Fig 24b) of (74) and (73) intact incorporation of a single deuterium atom can be clearly seen.



(Fig 24a) Top 300 MHz proton spectrum of (74).

Bottom 250 MHz proton spectrum of (73).

TE 298  
 FV 25268  
 QZ 4688.888  
 OP 18H 00  
 LB 2.888  
 CB 8.8  
 CX 45.88  
 CY 8.88  
 F1 185.888P  
 F2 5.817P  
 HZ/CH 381.388  
 PPM/CH 4.888  
 SR 589.22



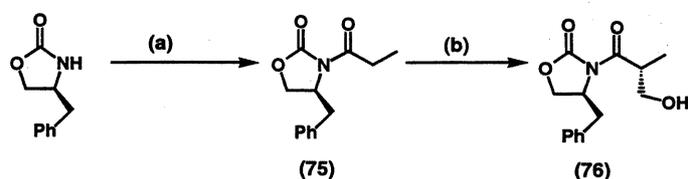
(Fig 24b) Top  $^{13}\text{C}$  DEPT (75.4 MHz) spectrum of (74).

Bottom  $^{13}\text{C}$  DEPT (62.9 MHz) spectrum of (73).

The signal from the chain methylene  $-CH_2-$  group was transposed from a simple quartet into a complex multiplet with small coupling (average  $J_{H-D} = 0-3.0$  Hz). The methyl triplet now appeared as a doublet of doublets ( $J_{H-H} = 7.4$  Hz,  $J_{H-D} = 0.9$  Hz) due to the induced asymmetry of the chain methylene protons. In the carbon DEPT spectra the signal corresponding to the  $-CHD-$  carbon was clearly visible at 28 ppm and appeared as a 1:1:1 triplet ( $J_{C-D} = 20$  Hz). The high resolution mass spectrum also indicated 100% deuterium incorporation ( $M^+ = 144$ ) and showed no sign of the starting material ( $M^+ = 143$ ) which could conceivably have remained during the recrystallisation. From the spectral data and the amount of deuterated product formed (90%) indicated complete enolate formation for the propyl analogue and that no disruption in the oxazolidinone moiety occurred. For the ethyl ester (**69**) however no single product (by TLC) was formed and we were not able to recover any of the starting material from the reaction mixture. This indicates that the ester moiety was unstable to the conditions necessary for formation of the enolate.

To ensure that we were able to diastereoselectively introduce the desired hydroxymethyl group into the acyl chain, and that the problems were associated with the choice of latent carboxyl group we tested one of the original substrates (**75**) from Evans' paper. Treatment of (4*R*)-4-benzyl-2-oxazolidinone with *n*-butyl lithium in anhydrous tetrahydrofuran followed by propionyl chloride gave (**75**) in 80% yield. Treatment with titanium (IV) chloride at 0 °C under nitrogen in dry dichloromethane produced a bright yellow slurry of the titanium complex. After a five minute delay diisopropylethylamine was added giving a deep blood red solution of the enolate. The enolate solution was stirred for a further hour at 0 °C to ensure full enolate formation; *s*-trioxane was added in dry dichloromethane, and the mixture stirred at 0 °C for 24 hours. Work-up and purification by flash chromatography on silica gel gave the desired product (**76**) in 62% yield (Scheme 20). From the NMR spectra the product appeared as a single diastereoisomer and no sign of the other diastereoisomer

was detectable. Whether the reaction was completely diastereoselective or that during the purification process some final resolution of the diastereoisomers occurred, is not clear as no NMR of the crude product was taken. This indicates that we were able to repeat the reaction and with full diastereoselectivity as reported. This also gave an indication that with the correct choice of masked carboxylate functionality, stable to the enolate formation conditions may have led to the desired alkylated acyl chain, and hence paraconic acid.



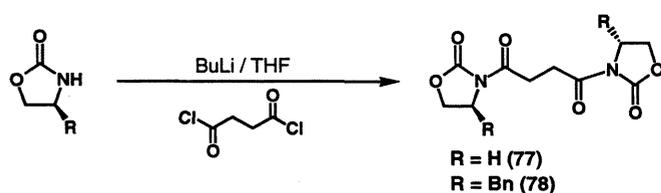
(Scheme 20) (a) *n*-BuLi, THF, Propionyl chloride.

(b) (i) TiCl<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, Hünig's base, (ii) *s*-Trioxane.

### 2.5.3 Bis-chiral auxiliaries.

From the deuterium quench experiments simple ester functional groups in the C4 position proved labile to the conditions requisite for the formation of the titanium enolate. Therefore a different synthon for the acid group must be determined. The deuterium experiment and the very nature of Evans' work indicated that the chiral auxiliaries themselves were stable to the enolate formation conditions *and* could be readily hydrolysed to yield the corresponding carboxylic acid. We therefore decided to synthesise the bis-chiral auxiliaries (77) and (78) to test whether these compounds would undergo the desired enolate formation, diastereoselective alkylation and

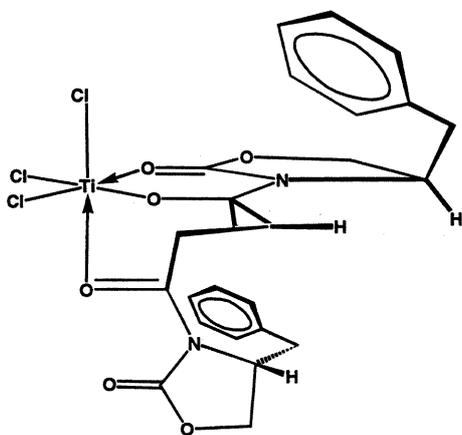
hydrolysis to yield paraconic acid (**21**). Treatment of these compounds with two equivalents of titanium (IV) chloride would presumably form the usual titanium-auxiliary complex across the oxygen atoms of the oxazolidinone and exocarbonyl groups. Addition of one equivalent of diisopropylethylamine would then produce the titanium enolate which could be trapped with the usual electrophiles. Hydrolysis of the auxiliaries with lithium hydroperoxide would give the diacid and subsequent deprotection (necessary only when benzylchloromethyl ether was the electrophile) would yield paraconic acid. The bis-2-oxazolidinones were prepared in good yield by the slow addition of succinyl chloride under nitrogen to the lithium anion of the auxiliary at -78 °C in anhydrous THF under nitrogen to give the bis-coupled adducts as white solids in 55-66% yield (**Scheme 21**).



(Scheme 21)

The bis-oxazolidinones (**77**) and (**78**) were treated in anhydrous dichloromethane under nitrogen with titanium (IV) chloride to give a yellow/orange slurry of the titanium-auxiliary complex. Addition of Hünig's base to the complex gave a deep blood red solution of the titanium enolate, to which (after stirring for one hour) was added the desired electrophile. In both cases after stirring the reaction for 24 hours *only* the starting materials (**77**) and (**78**) were recovered from the reaction mixture

and no product could be seen by TLC. This surprising lack of reactivity could be due to the steric bulk of the new titanium-auxiliary complex blocking *both* faces of the enolate to the approaching electrophile. The co-ordination of the titanium in the auxiliary complex has not been verified but was presumably five or six co-ordinate. It may therefore be possible on addition of titanium (IV) chloride for (77) and (78) to 'fold over' and form a complex where *three* of the carbonyl oxygens in the molecule are co-ordinated to *one* titanium atom. This would have the effect of rendering both faces of the enolate unapproachable to the large electrophiles used (Fig 25).



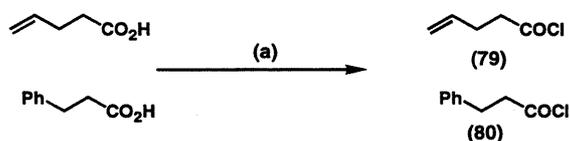
(Fig 25)

The lack of reactivity and the difficulty in predicting the geometry of the new titanium-auxiliary complex led to the abandonment of this strategy and work centred on utilising a hydrocarbon synthon for the carboxylic acid moiety with greater success.

#### 2.5.4 Phenyl and vinyl groups as carboxylate synthons.

From the deuterium quench experiments we have shown that compounds containing the simple ester functional groups, although suitable in terms of their formation and removal, were unstable to the conditions requisite to the enolate formation. These experiments also indicate that when the acyl chain moiety was a hydrocarbon, (such as propyl analogue (75)) the reaction was successful. Despite the stability of the bis-oxazolidinone auxiliaries (77) and (78) to the enolate formation conditions these compounds were found to be unreactive perhaps due to steric crowding in the titanium-enolate complex. These two observations led to the proposal of the use of a carboxylate synthon which contained only hydrocarbon functionality. From earlier work on the Mori synthesis (chapter 2.2.1) and the literature precedent<sup>79,80</sup> we knew that it was possible to oxidise an aryl ring to a carboxylic acid using ruthenium tetroxide. Under these conditions alkenes are also cleaved to carboxylic acids and we intended to use this strategy to mask the carboxylate functionality as either a phenyl ring or possibly an alkene. Alkylation of the acyl chain should occur as for the propyl analogue (76) with no attack on either the phenyl ring or the alkene double bond by the titanium (IV) chloride. The alcohol must be protected during the oxidation step and acetate has been shown to be stable under these oxidation conditions.<sup>79,80</sup> Subsequent oxidation and hydrolysis would 'unmask' the desired functionality and yield paraconic acid.

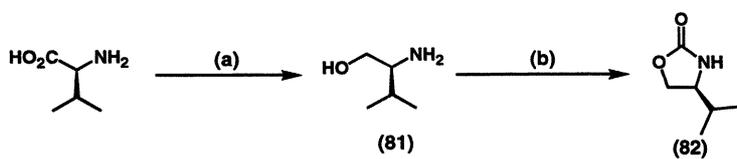
The two acyl chain moieties chosen were the pent-4-enoyl and hydrocinnamoyl which would provide aryl and alkene synthons for the carboxylic acid. Both the acyl chains were prepared as the acid chlorides from the parent commercially available carboxylic acids in high yield by reaction of thionyl chloride in the presence of a catalytic amount of *N,N*-dimethylformamide (Scheme 22).



(Scheme 22) (a)  $\text{SOCl}_2$ , *N,N*-dimethylformamide.

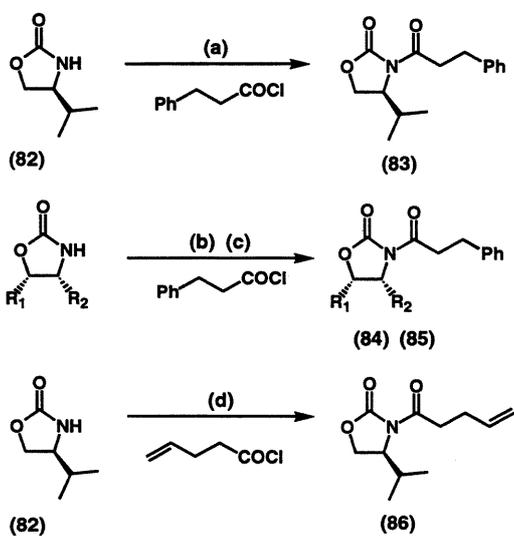
Initially we had been employing (4*S*)-4-benzyl-2-oxazolidinone as one of our auxiliaries and this compound, although commercially available was very expensive. We would require large quantities of the auxiliary, if the strategy was successful enabling the synthesis to be completed and decided to synthesise the corresponding (4*S*)-4-isopropyl-2-oxazolidinone auxiliary (**82**) from L-valine, which was less expensive. This would allow the test of this auxiliary in the initial stages of the synthesis along with our remaining sample of (4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinone and the non-chiral 2-oxazolidinone, finally concentrating on the less expensive auxiliary if this yielded a satisfactory level of diastereoselectivity. The paraconic acid produced initially would be the opposite (*R*)-(+)-enantiomer to that required, however this would still confirm the viability of the route, and the enantiopurity of the final paraconic acid. Repeating the synthesis from the other commercially available enantiomer of valine would yield the enantiomeric auxiliary and thus (*S*)-(-)-paraconic acid (**21**).

Reduction of (*S*)-valine to (*S*)-valinol (**81**) was achieved in 84% yield by following a procedure by A. Giannis and K. Sandhoff<sup>99</sup> who used lithium borohydride and trimethylsilylchloride to generate borane *in situ*. Reaction of valinol and diethylcarbonate in the presence of a trace amount of base gave the 2-oxazolidinone (**82**) in 61% yield after recrystallisation as a white solid (Scheme 23).



(Scheme 23) (a)  $\text{TMSCl}$ ,  $\text{LiBH}_4$

(b)  $(\text{EtO})_2\text{CO}$ ,  $\text{K}_2\text{CO}_3$



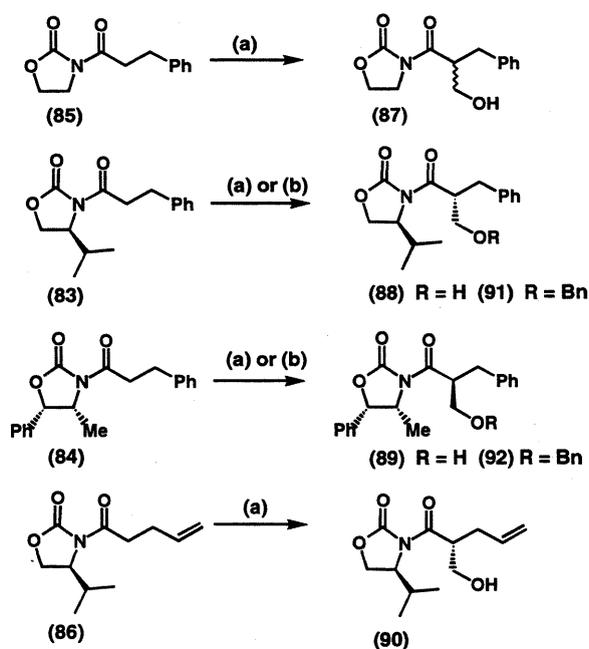
	(84)	(85)
$\text{R}_1$	Ph	H
$\text{R}_2$	Me	H

(Table 4)

(Scheme 24) (a)  $n\text{-BuLi}$ , THF

(b)  $n\text{-BuLi}$ , THF (c)  $n\text{-BuLi}$ , THF (d)  $n\text{-BuLi}$ , THF.

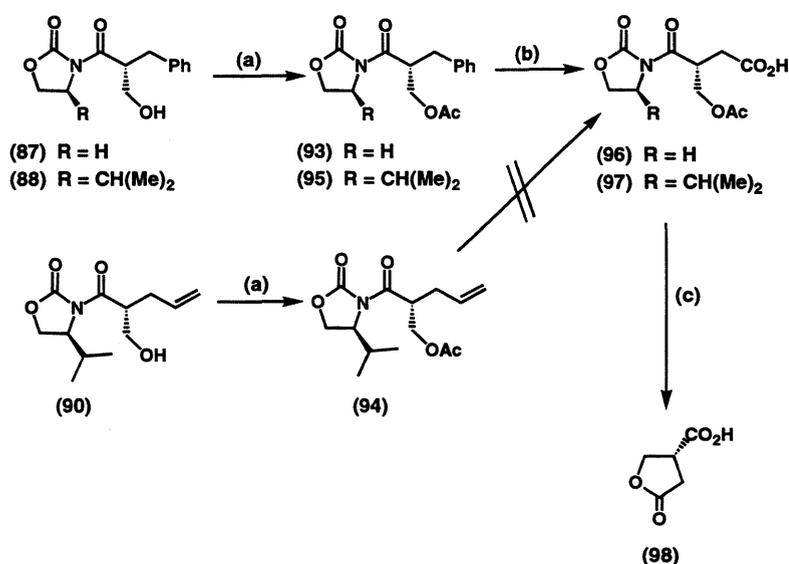
Initially hydrocinnamoyl chloride (**80**) was coupled to three oxazolidinones in the usual manner giving the corresponding adducts (**83-85**) in high yields (67-97%) (Scheme 24). Pent-4-enoyl chloride (**79**) was coupled to the (4S)-4-isopropyl-2-oxazolidinone (**82**) in order to draw comparisons between aryl and alkene carboxylate synthons (**83**) and (**86**) (Scheme 24). Once the auxiliary-acyl adducts had been synthesised each was tested in the diastereoselective alkylation reaction. Initially the non-chiral compound (**85**) was precomplexed with titanium (IV) chloride giving a bright yellow slurry which on treatment with diisopropylethylamine yielded a deep blood red solution of the titanium enolate. To the enolate solution was added *s*-trioxane and the reaction mixture stirred for 24 hours. Work-up and purification by flash chromatography over silica gel gave the desired hydroxymethyl alkylated acyl chain as a colourless oil (**87**) in 36% yield (Scheme 25).



(Scheme 25) (a)  $\text{TiCl}_4$ ,  $\text{CH}_2\text{Cl}_2$ , *s*-trioxane,  
 $i\text{Pr}_2\text{EtN}$ , (b)  $\text{TiCl}_4$ ,  $\text{CH}_2\text{Cl}_2$ ,  $\text{BnOCH}_2\text{Cl}$ .

Despite the disappointing yield in the hydroxymethylation of (85) we were encouraged by the ability to produce the desired alkylation product, and that the aryl functionality was unaffected by the enolate formation conditions. The reaction was repeated with the auxiliaries (83) (84) and (86) using *s*-trioxane as the incoming electrophile and all gave the analogous hydroxymethyl products (88) (89) (90) in 89%, 55% and 48% yield respectively (Scheme 25). Auxiliaries (83) and (84) were also tested using benzylchloromethyl ether as the electrophile yielding the corresponding protected alcohols (91) (92) and this enabled comparisons to drawn between the electrophiles used. We found that benzylchloromethyl ether gave the

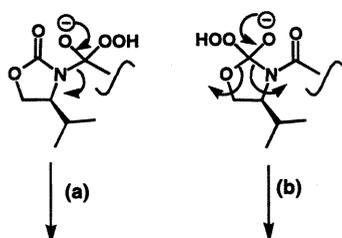
most reproducible results and generally higher yields. From the carbon-13 NMR of the alkylated products (88 - 92) both the protected and the free alcohol moieties were assembled as single diastereoisomers. This indicated that the alkylation reaction was proceeding with essentially full diastereoselectivity and more importantly, if no racemisation were to occur during the subsequent steps the paraconic acid produced would be a single enantiomer. This would negate the need for any optical enrichment process and enable the synthesis of optically pure (*S*)-(-)-paraconic acid and hence A-factor for our biological studies. At this stage from the <sup>13</sup>C spectra it was apparent that both the 4-isopropyl and 4-methyl-5-phenyl auxiliaries gave full diastereoselectivity in the alkylation reaction. Due to this observation work centred on the more accessible isopropyl auxiliary (82). The primary alcohol groups in the hydroxymethyl adducts (87) (88) and (90) were protected during the ruthenium tetroxide oxidation as the corresponding *O*-acetyl. Reaction with acetic anhydride in dichloromethane gave the acetates (93) (94) and (95) in 60%, 62% and 90% respectively. At this stage we were concerned that the conditions requisite to the aryl and alkene oxidation would attack the oxazolidinone moiety. Treatment of the oxazolidinones (93) and (95) with ruthenium tetroxide generated *in situ* from ruthenium (III) chloride and periodic acid as the co-oxidant, gave the desired carboxylic acids (96) (97) in reasonable yields (Scheme 26). It is worth mentioning however, that acetate (94) when treated under identical oxidation conditions gave no separable products and no starting material was recoverable from the reaction mixture. Due to the success of the oxidation reaction on acetate (95) this problem was not addressed and the project concentrated on the final hydrolysis step. The final cleavage of the auxiliary must be achieved without compromising the integrity of the new asymmetric centre.



**(Scheme 26)** (a) Ac<sub>2</sub>O, Et<sub>3</sub>N (b) RuCl<sub>3</sub> .x. H<sub>2</sub>O, H<sub>5</sub>IO<sub>6</sub>,  
 CCl<sub>4</sub>, CH<sub>3</sub>CN, H<sub>2</sub>O (c) (i) LiOH, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>O, THF (ii) HCl (6M).

Several methods for the cleavage of oxazolidinone systems have been developed and extend over oxidation, transesterification and reduction type reactions. We decided to remove the auxiliary using lithium hydroperoxide<sup>100</sup> to generate the free acid directly. This reagent is specific for the exocyclic carbonyl group and provides a mild and efficient cleavage of the auxiliary which may be recovered from the reaction mixture. The specific nature of this reagent can be explained in terms of the rate limiting breakdown of the tetrahedral intermediates formed (**Fig 26**). We decided not to isolate the diacid (or auxiliary) produced from the oxazolidinone hydrolysis but to cleave the acetate in the same reaction mixture. Acidification with hydrochloric acid would cause spontaneous cyclisation, directly yielding paraconic acid. Treatment of acid (**97**) with lithium hydroxide and hydrogen peroxide in anhydrous THF for 48 hours followed by acidification with hydrochloric acid gave

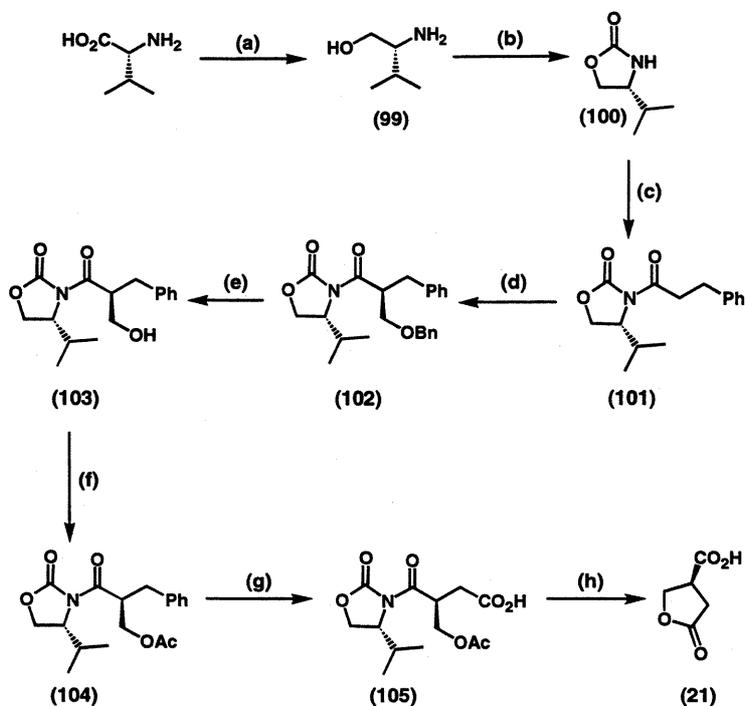
(*R*)-(+)-paraconic acid (**98**) as a white solid in 40% yield. Comparison of the observed and literature optical rotations showed the enantiomeric excess to be greater than ninety nine percent and the final paraconic acid to be a single enantiomer within ranges of detection. Further calculation of the enantiomeric excess of the paraconic acid (**98**) such as the use of chiral derivatisation agents or lanthanide shift reagents was not employed as the values of the optical rotations were such that the optical integrity of (**98**) was unambiguous.



(Fig 26) Lithium hydroperoxide hydrolysis: rate limiting tetrahedral intermediate breakdown (a) > (b).

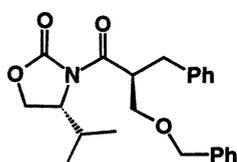
We had now developed a synthetic route using Evans' chiral auxiliary and titanium enolate chemistry to synthesise paraconic acid as a single enantiomer. The paraconic acid (**98**) was the opposite enantiomer to that which we would require in the synthesis of optically active A-factor. We therefore repeated the synthesis starting from the corresponding (*R*)-valine to produce the required (*S*)-(-)-paraconic acid and thus A-factor. The synthetic route is outlined below (Scheme 27). Reduction of (*R*)-valine to (*R*)-valinol (**99**) was carried out as for (**81**) to give the amino alcohol in 75% yield. Treatment of (**99**) with diethyl carbonate and potassium carbonate at reflux gave the (4*R*)-4-isopropyl -2-oxazolidinone (**100**) as a white solid in very good yield which on coupling with hydrocinnamoyl chloride in the usual manner gave the adduct (**101**) as a white solid. As mentioned previously we found

that quenching the titanium enolate with benzylchloromethyl ether rather than *s*-trioxane gave higher yields and more reproducible results. Despite this procedure requiring the extra step of deprotecting the alcohol prior to acetylation both of these reactions proceed almost quantitatively making the extra step trivial. Therefore treatment of **(101)** with titanium (IV) chloride in dry dichloromethane under nitrogen gave the characteristic complex as a yellow slurry which on addition of diisopropylethylamine yielded a deep blood red solution of the titanium enolate.



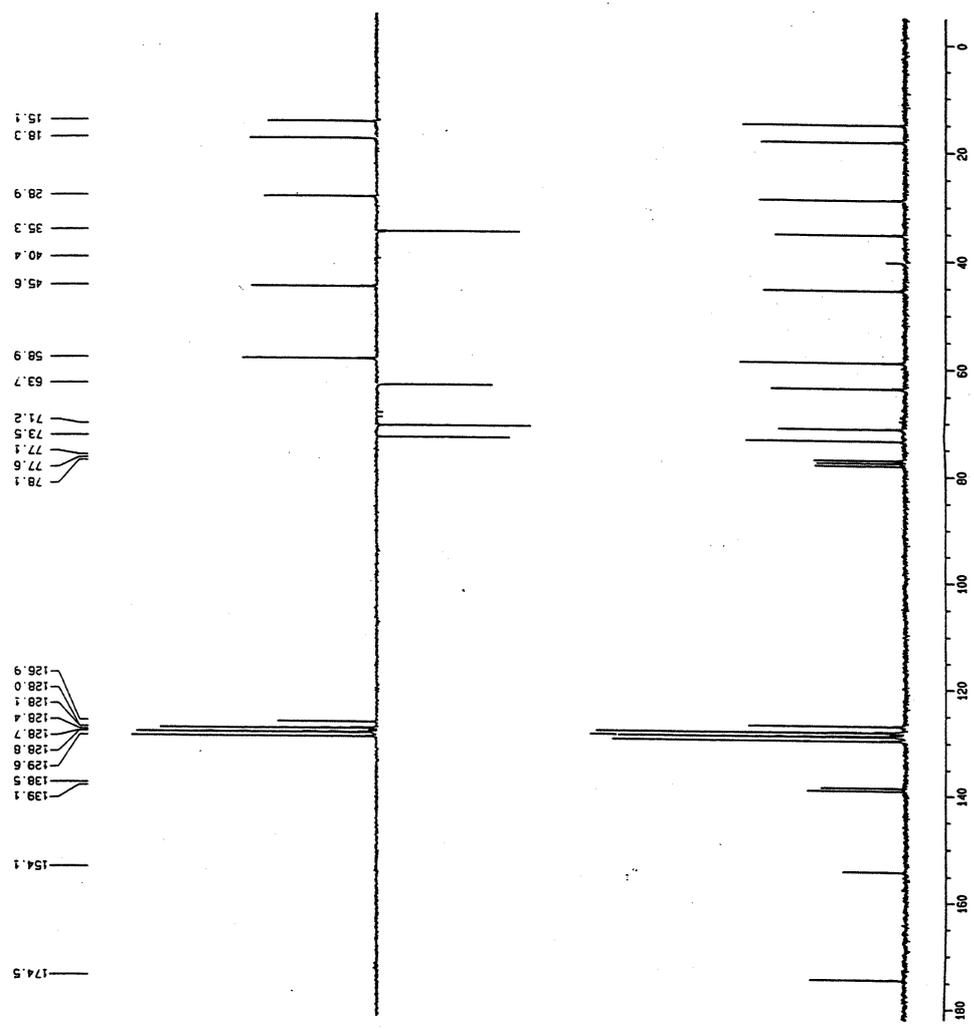
**(Scheme 27)** Synthesis of (*S*)-(-)-paraconic acid: (a)  $\text{LiBH}_4$ ,  $\text{TMSCl}$ , THF (b)  $(\text{EtO})_2\text{CO}$ ,  $\text{K}_2\text{CO}_3$  (c) *n*-BuLi, hydrocinnamoyl chloride (**80**) THF (d)  $\text{TiCl}_4$ ,  $\text{BnOCH}_2\text{Cl}$ ,  $\text{CH}_2\text{Cl}_2$  (e)  $\text{Pd/C}$ ,  $\text{H}_2$ ,  $\text{EtOAc}/\text{H}^+$  (f)  $\text{Ac}_2\text{O}$ , Py (g)  $\text{RuCl}_3 \cdot x\text{H}_2\text{O}$ ,  $\text{H}_5\text{IO}_6$ ,  $\text{CH}_3\text{CN}$ ,  $\text{CCl}_4$ ,  $\text{H}_2\text{O}$  (h) (i)  $\text{LiOH}$ ,  $\text{H}_2\text{O}_2$ , THF (ii)  $\text{HCl}$  (6M).

Addition of benzylchloromethyl ether to the enolate solution yielded the protected alcohol adduct (**102**) as a white crystalline solid in 94% yield after purification by chromatography over silica gel. At this stage we decided to confirm unambiguously the structure and stereochemistry of (**102**) by  $^1\text{H}$ - $^1\text{H}$  (COSY) and  $^1\text{H}$ - $^{13}\text{C}$  correlation NMR and by X-ray crystallography. This would indicate whether the alkylation was proceeding with the assumed full diastereoselectivity and so confirm the structure and stereochemical integrity the alkylated product. Carbon-13 NMR of the alkylated product *prior* to purification also showed no sign of the other diastereoisomer indicating that the reaction is itself fully diastereoselective and that the high levels of diastereomeric excess were not a result of resolution during purification. As the diastereomeric excess at this stage in the synthesis is crucial to the final enantiopurity of the paraconic acid we deemed it necessary to remove any ambiguity. Initially each carbon atom was assigned from the carbon-13 DEPT spectrum (Fig 27) which enabled assignment of individual methyl, methylene, methyne and carbonyl signals within the compound (**102**). The  $^1\text{H}$ - $^{13}\text{C}$  correlation spectrum (Fig 28) allowed each signal within the  $^1\text{H}$  NMR to be related to its corresponding signal in the  $^{13}\text{C}$  spectrum and this unambiguously assigned each signal in the  $^1\text{H}$  NMR spectrum. Finally the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Fig 29) gave information on the connectivity between neighbouring groups and determined which protons within the molecule were coupled. Combining all the spectroscopic data enabled the structure to be assigned unambiguously.

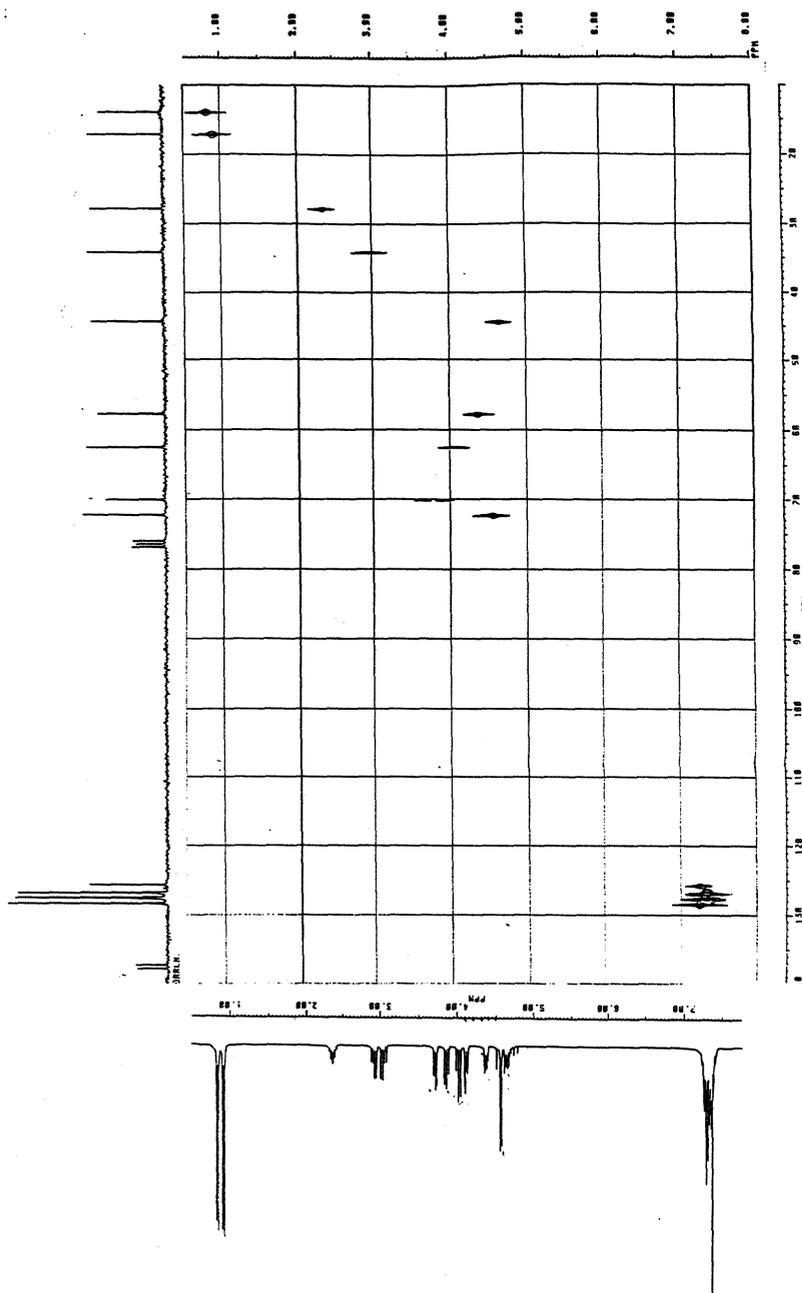


(**102**)

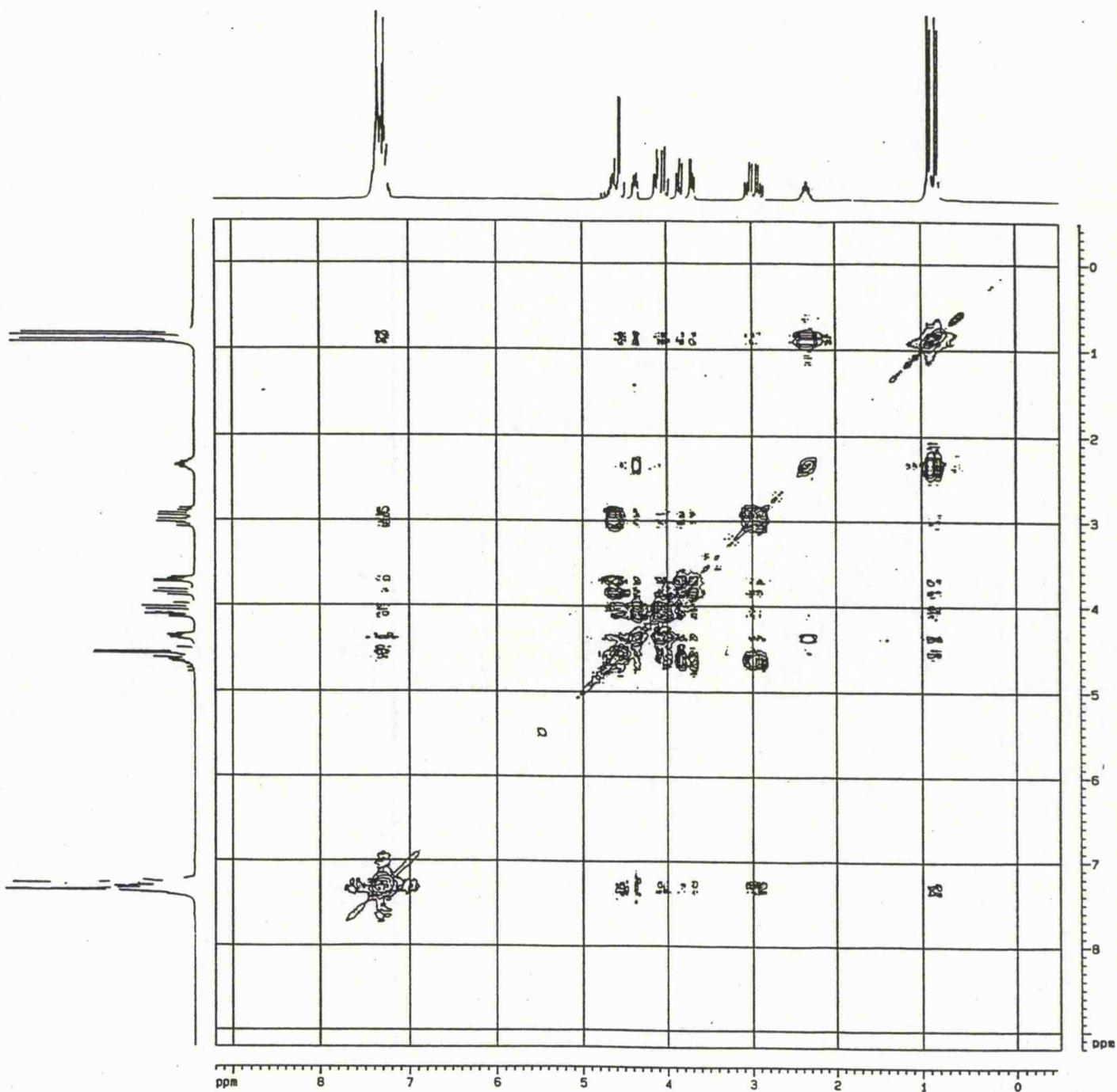
Although the spectroscopic data was able to unequivocally determine the gross overall structure it was not able to ascertain the absolute stereostructure of the asymmetric centres. As this assignment was crucial to the synthesis of paraconic acid an X-ray crystal structure was obtained (Fig 30). From the crystal structure the stereochemistry of the asymmetric centres was proven to be as drawn proving that the alkylation proceeded with complete diastereoselectivity and that (102) was a single diastereoisomer.



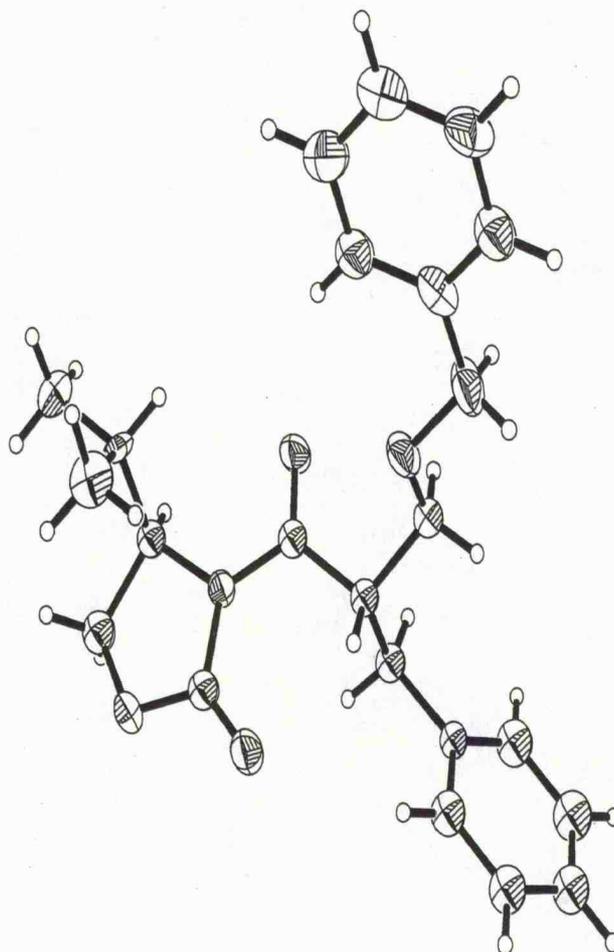
(Fig 27)  $^{13}\text{C}$  DEPT (62.9 MHz)  
spectrum of benzyl ether (102).



(Fig 28)  $^1\text{H}$ - $^{13}\text{C}$  correlation spectrum  
of benzyl ether (102).



(Fig 29)  $^1\text{H}$ - $^1\text{H}$  correlation spectrum  
of benzyl ether (102).

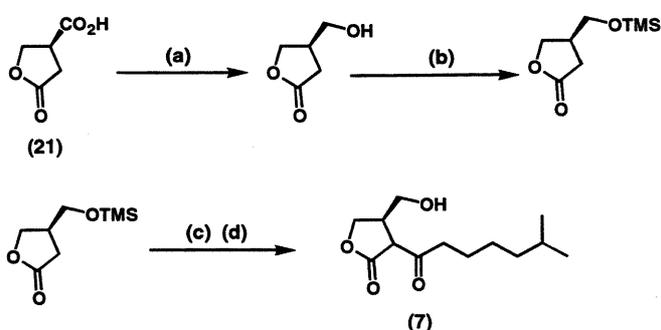


**(Fig 30)** Crystal structure of (4*R*,2'*S*)-3-[2'-benzyloxymethyl-1'-oxo-3'-phenylpropionyl]-4-isopropyl-2-oxazolidinone (**102**).

Once the structure and stereochemistry of the benzyl ether (102) had been determined the synthesis to optically pure (S)-(-)-paraconic acid was completed. Cleavage of the benzyl ether by catalytic hydrogenation with palladium on carbon (5%) followed by acetylation of the free hydroxyl (103) with acetic anhydride and pyridine gave the acetate (104) and both steps proceeded in almost quantitative yield. Oxidation of the aryl ring with ruthenium tetroxide generated *in situ* from ruthenium (III) chloride and periodic acid as the co-oxidant gave the carboxylic acid (105) cleanly in 65% yield. Again as for (97) a two step hydrolysis was employed with no attempt to recover the auxiliary to give (S)-(-)-paraconic (106) acid as a white solid in 40% yield. Comparison of the observed optical rotation with the literature value proved the paraconic acid to be the desired single (S)-(-)-enantiomer.

## 2.6 Synthesis of A-factor and analogues

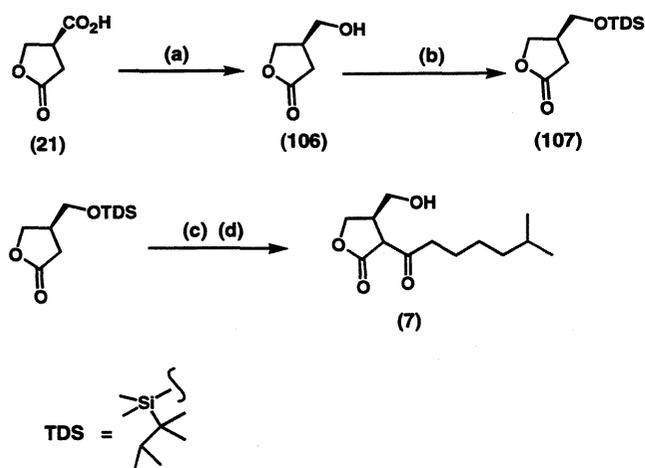
Once the synthesis of homochiral paraconic acid had been achieved, the next stage was the conversion to optically pure A-factor (7). Earlier literature preparations of A-factor<sup>22,24,25</sup> and other autoregulators<sup>25</sup> have all followed the same strategy indicated below (Scheme 28). We also intended to follow this general synthetic strategy but to employ a less labile silyl protecting group. This would enable purification of the protected lactone alcohol by flash chromatography and also ensure that no racemisation occurred at this stage due to ring opening and subsequent re-closing onto the now equivalent hydroxyl groups.



(Scheme 28) Mori synthesis of A-factor from (*S*)-(-)-paraconic acid. (a)  $\text{BH}_3\text{:SMe}_2$ , THF (b)  $\text{TMSCl}$ ,  $(\text{MeSi})_2\text{NH}$ , Pyridine (c)  $\text{LDA}$ ,  $\text{RCOCl}$  (d)  $\text{EtOH}$ ,  $\text{H}^+$ .

Reduction of (*S*)-(-)-paraconic acid (**21**) using borane dimethyl sulphide complex gave the desired alcohol (**106**) in good yield (60%) and it is important to note that at this stage the alcohol had to be kept at below 30 °C if racemisation was to be avoided. For this reason the alcohol was purified by flash chromatography and *not* distilled. The free hydroxyl group was protected as the hexyldimethylsilyl ether (**107**) in the usual manner from the corresponding silyl chloride and imidazole in high yield (87%) rather than the trimethylsilyl ether in Mori's case (52%). We decided that a less labile group would enable purification of the protected lactone by flash chromatography rather than distillation. The hexyldimethylsilyl group was chosen as the protecting group over the more common *tert*-butyldimethylsilyl group. Its choice here was due to its increased stability which was of the order of two to three times that of the *tert*-butyldimethylsilyl ether; its ease of handling as hexyldimethylsilyl chloride is a liquid while the *tert*-butyldimethylsilyl is a solid; and the cost, (twenty five grams of the hexyldimethyl silyl chloride is half that of the corresponding *tert*-butyldimethylsilyl chloride).<sup>101</sup> Treatment with lithium hexamethyldisilazide, alkylation with 6-methylheptanoyl chloride (**112**) and cleavage

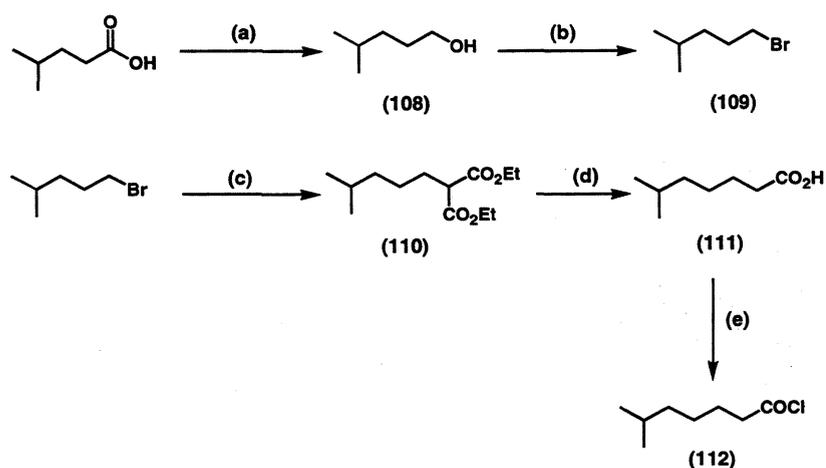
of the silyl ether with tetrabutylammounium fluoride gave A-factor in 43% yield from the silyl ether (**Scheme 29**). Comparison of the observed optical rotation with that in the literature<sup>24</sup> indicated that the A-factor produced was optically pure.



**(Scheme 29)** Synthesis of A-factor from (*S*)-(-)-paraconic acid. (a)  $\text{BH}_3\cdot\text{SMe}_2$ , THF (b)  $\text{TDSCl}$ , Imidazole (c) Lithium hexamethyldisilazide,  $(\text{CH}_3)_2\text{CH}(\text{CH}_2)_4\text{COCl}$  (d) TBAF.

6-Methylheptanoyl chloride (**112**) was synthesised from commercially available valeric acid (**Scheme 30**). Reduction of valeric acid with lithium aluminium hydride gave a good yield of 4-methylpropan-1-ol (**108**). Conversion of the primary alcohol to the bromide (**109**) was achieved in good yield using carbon tetrabromide and triphenylphosphine.<sup>102</sup> Displacement of the bromide by diethyl malonate to give the diester (**110**). Subsequent saponification and decarboxylation gave 6-

methylheptanoic acid (**111**) which was converted to the acid chloride (**112**) before coupling to the protected lactone (**107**).



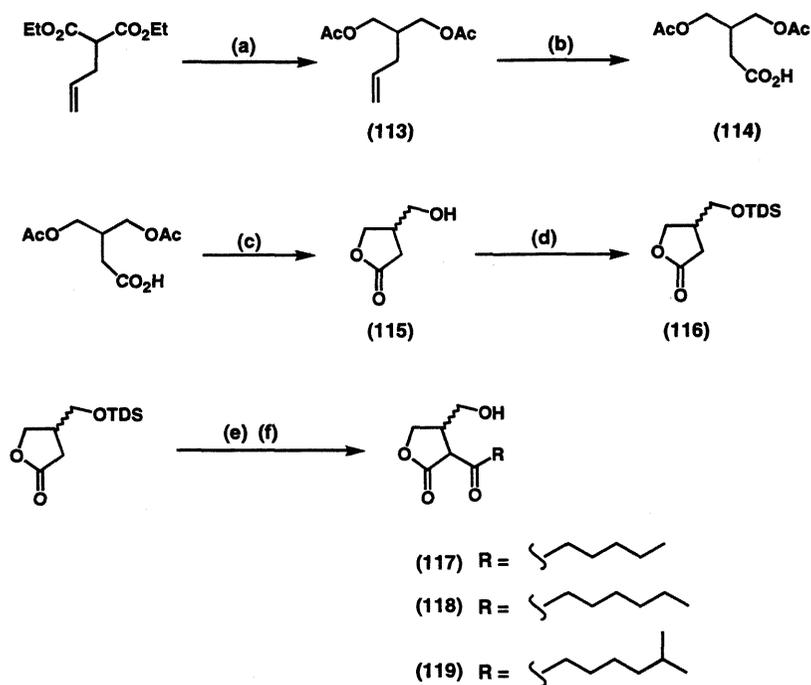
(Scheme 30). (a)  $\text{LiAlH}_4$ , THF (b)  $\text{CBr}_4$ ,  $\text{PPh}_3$ ,  $\text{CH}_2\text{Cl}_2$  (c)  $\text{NaH}$ , DMF, Diethyl malonate (d) (i) THF, Water,  $\text{NaOH}$  (ii)  $\text{HCl}$  (6M), (e)  $\text{SOCl}_2$ .

We now had a source of optically pure A-factor for the feeding experiments on *Streptomyces nodosus*. As mentioned in the introduction A-factor was originally isolated from *S. griesus* and not *S. nodosus*: to date an autoregulator from *S. nodosus* has not been isolated. Cross strain activity has been observed<sup>103</sup> among the autoregulators and is perhaps predictable due to the structural similarities between the autoregulators isolated to date. It is this observation that we feel gives credence to our argument that despite A-factor not being known as an endogenous autoregulator of *S. nodosus* a response to exogenous A-factor stimulation could still be observed. In order to further increase the possibility of a response to external

autoregulator stimulation we decided to synthesise two analogues of A-factor incorporating side chains from other autoregulators. The side chains chosen were the unbranched heptanoyl and hexanoyl moieties from the Virginiae Butanolides C (14) and D (15) as these combined with A-factor itself, span almost the entire range of chain length and type isolated. Due to the time already invested into the development and synthesis of homochiral paraconic acid and thus A-factor, repeating this synthesis to obtain sufficient material for the chiral synthesis of the analogues was not possible, and these analogues were synthesised in racemic form. It is *essential* to stress that initial experiments *must* be carried out with the optically active naturally occurring enantiomer of A-factor. A negative result from feeding a racemic mixture may not necessarily indicate that the autoregulator has no effect, as this relies on the *assumption* that the antipode of the naturally occurring A-factor is inactive: *i.e.* has zero efficacy. It is possible for a compound to have a *negative* efficacy value and therefore act as an antagonist at a receptor. In this case this would result in the antipode of A-factor competing with the naturally occurring active enantiomer for the active site. Once bound it could act as an antagonist by either suppressing the pleiotropic response or remain bound and inhibit the active enantiomer from binding. However if a response to the natural enantiomer of A-factor were observed it would be interesting to establish if the effect was reproducible with the racemic mixture which would be more accessible as an additive for the biotechnology industry. For this reason *racemic* A-factor was also synthesised.

The racemic analogues were synthesised as indicated below (Scheme 31). Reduction of the commercially available diethyl allylmalonate with lithium aluminium hydride in anhydrous tetrahydrofuran followed by acetylation of the diol formed with acetic anhydride gave the diacetate (113) in good yield. Cleavage of the double bond to the carboxylic acid (114) was achieved in high yield by catalytic ruthenium tetroxide oxidation generated *in situ* from ruthenium (III) chloride and

periodic acid as the co-oxidant. Cleavage of the acetate groups and acidification furnished the *racemic* 3-hydroxymethyl-4-butenolide (**115**) in reasonable yield (41%). The lactone alcohol was taken through to the racemic analogue of A-factor as described for the chiral compounds. The primary alcohol group was protected as the thexyldimethylsilyl ether (**116**) followed by alkylation at the C-2 position with the hydrocarbon moieties and subsequent desilylation to give the analogues (**117**) (**118**) and (**119**).



(Scheme 31). Synthesis of *racemic* A-factor and analogues.

(a) (i)  $\text{LiAlH}_4$ , THF (ii)  $\text{Ac}_2\text{O}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$  (b)  $\text{RuCl}_3 \cdot x \cdot \text{H}_2\text{O}$ ,  $\text{H}_5\text{IO}_6$ ,  $\text{CH}_3\text{CN}$ ,  $\text{CCl}_4$ ,  $\text{H}_2\text{O}$  (c)  $\text{K}_2\text{CO}_3$ , MeOH then HCl (6M)

(d) TDSCl, Imidazole, DMF (e) Lithium hexamethyldisilazide,  $\text{RCOCl}$  (f) TBAF.

We now had samples of optically active A-factor and its racemic analogue, and two other racemic analogues containing different side chain moieties. These compounds would enable a comparison, during our feeding experiments, of the response to exogenous autoregulator stimulation (if any) from the chiral and racemic A-factor and also its analogues. These analogues would also provide the means of increasing the probability of a stimulation response as the side chains incorporated (along with A-factor itself) span almost all of the side chain type and length isolated to date. With the synthesis of the autoregulators complete our attention turned to the feeding experiments on *S. nodosus*.

## CHAPTER 3

### Autoregulator Feeding

## 3.0 Autoregulator Feeding

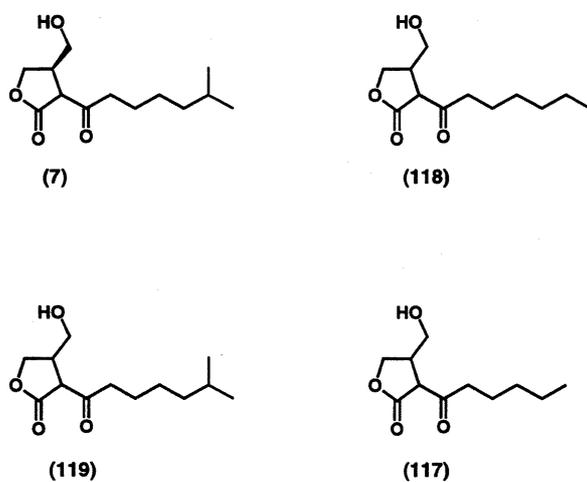
### 3.1 Introduction

As previously stated, autoregulators such as A-factor (7) are produced by the soil bacteria genus *Streptomyces* and impart a regulatory role over secondary metabolite production. These periods of autoregulator production occur at specific times in the life cycle of the developing bacterium, and as a response to an intracellular signal for cytodifferentiation, such as essential nutrient depletion, in the immediate environment. Autoregulators have been reported to re-introduce the biosynthesis of secondary metabolites in the blocked mutants of certain species of streptomyces and are thought to possess a fundamental role in the expression of proteins essential to secondary metabolite production. It has also been postulated<sup>2-3</sup> that the lipid nature of the autoregulators enables diffusion through the bacterial cell membranes into the surrounding medium, and therefore into neighbouring cells. This ensures an *intercellular* signal cascade, and an almost synchronous sporulation of the entire colony, increasing the chances of species survival.

To date, an investigation of the effect of an exogenous autoregulator on a wild type streptomycete has not been reported. It would be interesting to address the questions of whether an exogenous autoregulator would 'switch on' secondary metabolite production earlier than normal, and if the duration for metabolite production would be extended. Both effects if observed may be of significance to the biotechnology industry in decreasing lag time for the onset of metabolite production during the fermentation process, and increasing overall metabolite yields, many of which have important clinical applications.

As described previously (Chapter 2.6) homochiral A-factor (7) and analogues (117 - 119) (Fig 31) have been synthesised for our feeding studies. This enabled us

through our feeding experiments to screen both the chiral, and racemic A-factor for activity, and to determine the relative levels of efficacy. The analogues (117) (118) along with A-factor itself, also enabled us to encompass nearly the full range of chain lengths and type among the autoregulators isolated to date. This would be expected to increase the possibility of observing a response to the exogenous autoregulators during the feeding experiments.



(Fig 31)

### 3.2 Feeding Experiments and Assay

From our biosynthetic studies on amphotericin B (Chapter 4.0) we were able to establish the conditions requisite for metabolite production in *Streptomyces nodosus* in three different media. Each of these media was screened for exogenous autoregulator stimulation. As the different media give correspondingly different titres of metabolite, it would be interesting to establish if the levels of metabolite

production were sensitive to exogenous autoregulator in all three media, or if any one medium was more susceptible. The FCA media gave the highest titres of both amphotericin A (23) and B (22), typically in the order of 2000 and 3000 mg/Lt respectively. The complex and defined media yielded lower levels of both metabolites and these were typically in the order of 30% and 10% of that obtained from the FCA media, respectively. It is important to stress that the quoted concentrations of amphotericin A produced in these media were those obtained when a trace of ethanol (200  $\mu$ l) was present in the production medium (60 cm<sup>3</sup> broth). This level of ethanol in the production media has been reported<sup>68</sup> to suppress the concentrations of amphotericin A but *not* amphotericin B produced during fermentation.

From the biosynthetic studies on amphotericin B (Chapter 4.0) a determination of the standard profile for the fermentation of *S. nodosus* in terms of the pH profile of the medium, normal lag time for the onset of metabolite production, total overall titre during the production phase, and the duration of production was achieved. Under standard growth conditions metabolite production began between 10-14 hours after inoculation of the production broth, and this was found to be common to all three media employed. The levels of amphotericin A and B present in the broth generally increased, reaching their maximum concentrations around 110 hours for the complex and defined media, before decreasing. In the FCA media the concentration of metabolites in the culture broth generally reached the maximum levels around 160 hours. For the feeding experiments each autoregulator was added to the culture broth four hours into the growth. At this time the bacteria would be suitably established within the production media from the primary seed culture, and the reduction in lag time for metabolite production could still be established. Comparison of the time of onset for metabolite production in the autoregulator containing media, with that of a blank media containing no autoregulator, would

determine if the exogenous autoregulator was capable of 'switching on' metabolite production earlier than normally observed.

The amount of endogenous autoregulator normally present in the culture broth is in the order of 10 ng/Lt. In order to establish if the production of metabolites could be prolonged or increased during the fermentation process by an unregulated exogenous source of autoregulator in the broth, an aliquot (10 mg) of each autoregulator in ethanol (200  $\mu$ l) was added to the culture four hours into the fermentation (500 cm<sup>3</sup> Erlenmeyer flasks containing 60 cm<sup>3</sup> broth). At this concentration of autoregulator within the media, it is not unreasonable to assume that any regulatory process derived to govern the autoregulators anabolism/catabolism, and therefore levels within the media, would be unable to clear *all* the autoregulator from the broth. This would therefore provide a concentration of autoregulator within the broth, that for the full duration of the fermentation, exceeded that found for the endogenous autoregulator. This would enable us to determine from a single dosing if an undiminished supply of the autoregulator during fermentation is capable of enhancing, or prolonging, metabolite production. After feeding, the broth was assayed at regular intervals until the fermentation was considered complete. This would be determined by decrease in the levels of metabolites within the broth. The pH of the media was observed at each time interval and an aliquot of broth taken. The concentrations of amphotericin A and B within the broth was calculated as described in the experimental section (5.3), and the mean data for each feeding experiment tabulated (Appendix A).

### 3.3 Results and Discussion

Initial feeding experiments were carried out on the FCA media which produced high titres of both amphotericin A (23) and B (22) in the broth. These levels were typically in the order of 2000 mg/Lt for amphotericin A and 3000 mg/Lt for amphotericin B, in the presence of a trace of ethanol in the media (3.0 cm<sup>3</sup> per litre). It has previously been reported<sup>68</sup> that ethanol is capable of depressing the production of amphotericin A but *not* amphotericin B during fermentation. Other lower and higher alcohols depress the production of both metabolites, and the exact mechanism for this effect has not been reported. Each autoregulator was fed to the media in ethanol (200 µl) at four hours into the fermentation and a further aliquot of ethanol (200 µl) was added to the 'blank' media in order to form a baseline comparison. The concentrations of amphotericin A and B, and the pH of the broths was determined at regular intervals throughout the fermentation, as described in the experimental section, and the results expressed in graphical form. It can be clearly seen (Fig 32) (Fig 33) that addition of analogues (117) and (118) to the media does not decrease the lag time for metabolite production. There was a strong correlation between the production profile for both metabolites in the blank and exogenous autoregulator containing media. There was however a general overall decrease in the titre for both amphotericin A and B between the blank and autoregulator carrying media. Previous studies on this, and the other media, have shown that the titres of amphotericin produced vary up to ten percent between fermentations, the pH varied by five percent, and that the onset of metabolite production occurred between twelve and fifteen hours after inoculation. A resulting change in either lag time, or metabolite production, can therefore only be considered as statistically important if the lag time is reduced such that the onset occurs prior to ten hours, and that the change in the amphotericin titre within the broth is greater than fifteen percent. For

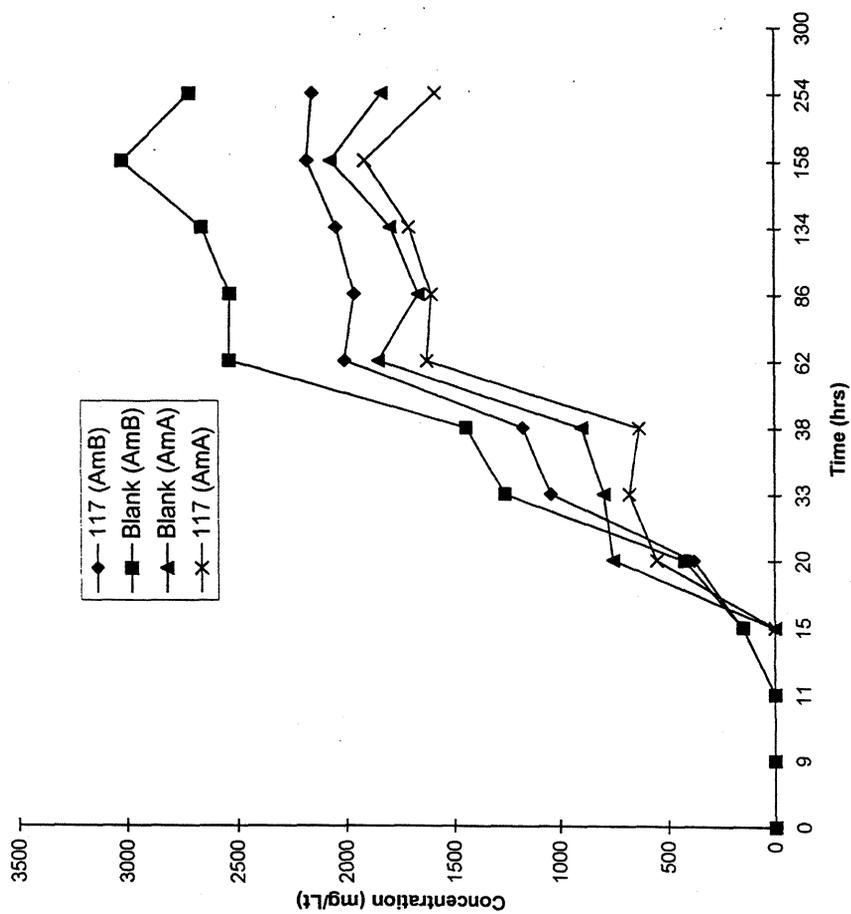
the autoregulators (117) (118) the titres of amphotericin A in the broth, although lower than in the blank media were within the fifteen percent accessible deviation. The levels of amphotericin B however for both (117) (118) were depressed by twenty eight and twenty three percent respectively, and this decrease is perhaps an indication that the exogenous autoregulator has a detrimental effect on metabolite production.

As explained earlier, A-factor (7) and the analogues (117) (118) (119) are not, to our knowledge, the endogenous autoregulators of *S. nodosus*, and this may account for their pernicious effect on amphotericin production. The pH profile (Fig 34) (Fig 35) for the blank media against the autoregulators (117) (118) containing media showed a good correlation, indicating that the pH of the media was unaffected by the presence of these exogenous autoregulators.

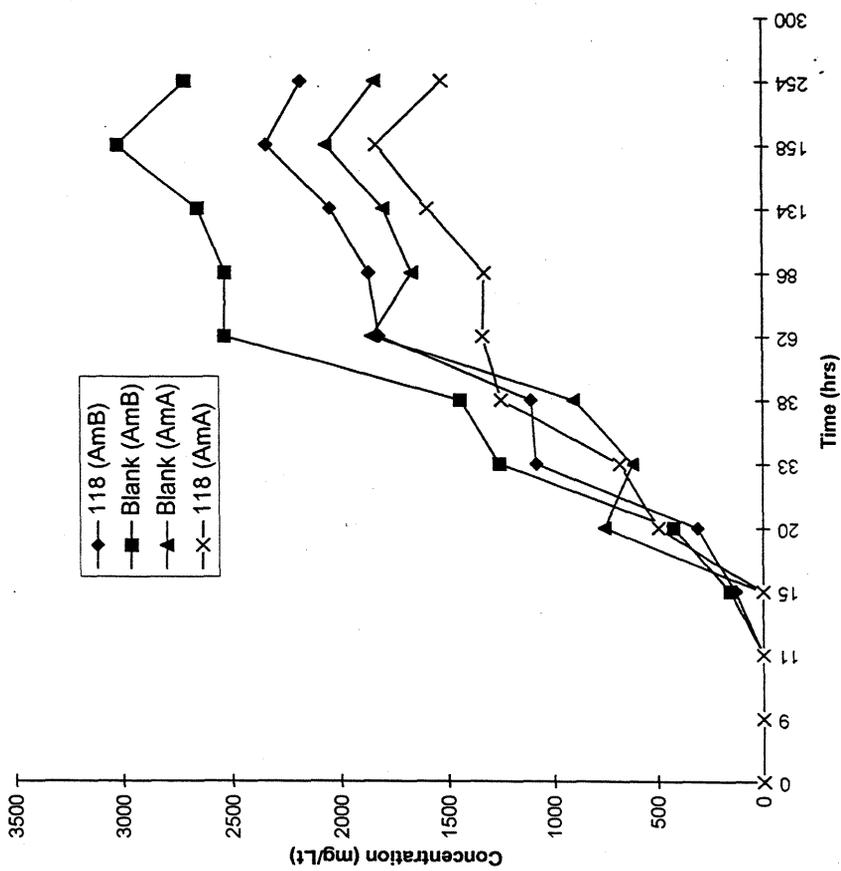
If we consider the results from the feeding experiment employing the homochiral naturally occurring A-factor (7) (Fig 36) and the racemic analogue (119) (Fig 37) neither appeared to enhance the absolute levels of amphotericin B in the broth. In the case of A-factor itself the titre of amphotericin B after twenty hours was consistently below that in the blank media containing no exogenous autoregulator. At fifteen hours the titre of amphotericin B within the A-factor receiving media exceeded that in the blank by a factor of two. This level of enhancement in the titre tended to suggest that exogenous autoregulator did exert some preliminary effect on the production of amphotericin B, and the absolute titre was twenty nine percent below that for the blank, again suggesting a final detrimental effect. The lag time for the onset of production for both metabolites was not however reduced. The concentrations of amphotericin A in the A-factor receiving media were within the expected range and the pH profile (Fig 38) also showed a good correlation with the blank media, suggesting no exogenous autoregulator stimulation. Considering the racemic analogue of the naturally occurring autoregulator (119) it would be expected

that the production profile, if a response was observed, would be comparable with A-factor (7) receiving media. The *racemic* A-factor (119) however gave no significant enhancement in the titre of amphotericin B during the fermentation and the absolute concentrations were within normal fermentation titres. The levels of amphotericin A produced after sixty two hours in the autoregulator containing broth exceeded those in the blank for the remainder of the fermentation. These levels, although higher, were again within the normal limits of the fermentation and therefore can not be thought of as statistically significant. The pH profile also showed a reasonable correlation between the autoregulator (119) receiving media and the blank which contained no exogenous autoregulator (Fig 39).

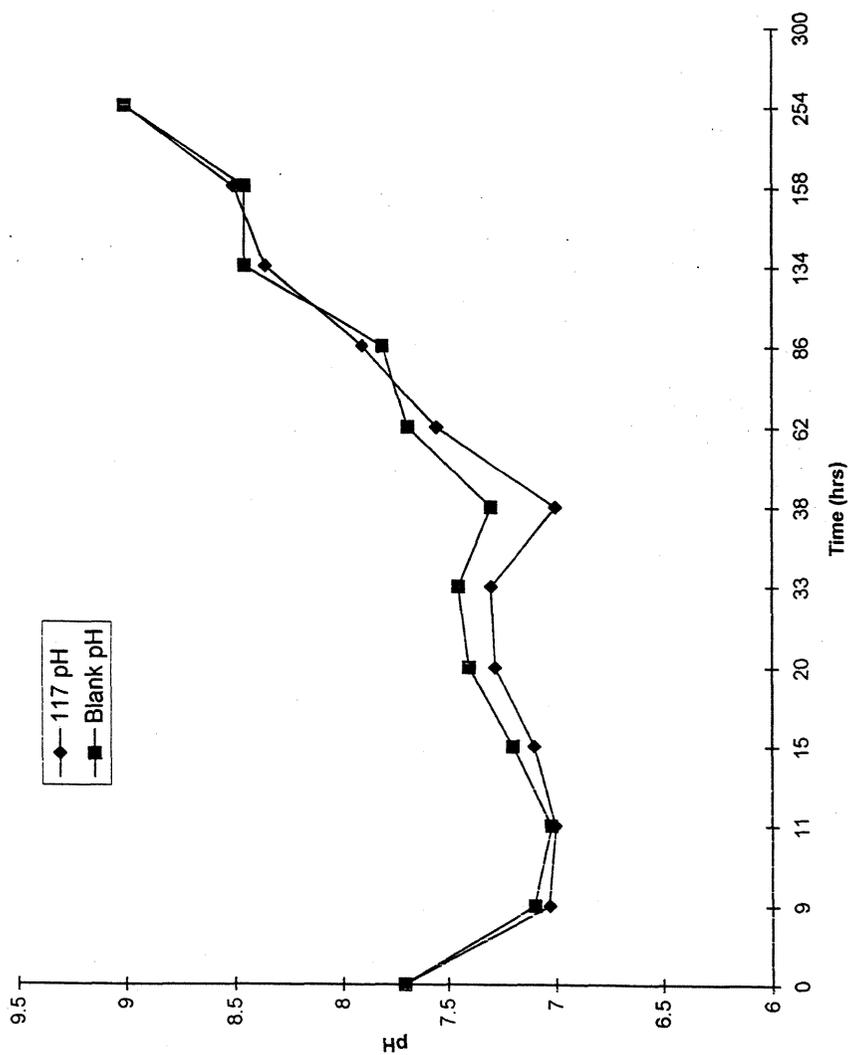
The feeding experiments on the FCA media indicated that no definitive effect on either the lag for onset of production, or the absolute concentration of metabolite produced was observed in this media. The only anomalies that were perceived during the feeding experiments were a general decrease in the titres for both amphotericin A and B in the broth. The only other aberration observed was a forty five percent higher titre of amphotericin B at fifteen hours in the media containing the natural A-factor (7) over the blank media. This was suggestive that increased secondary metabolite stimulation was occurring in the media containing the exogenous autoregulator (7) over the blank media. Although this was encouraging it is important to note that the value was an isolated point. The absolute concentration of amphotericin B concluded at a lower titre than in the blank, and the concentrations after fifteen hours in the autoregulator containing media were consistently below those in the blank media. Repeat experiments would therefore be required to verify the significance of the value.



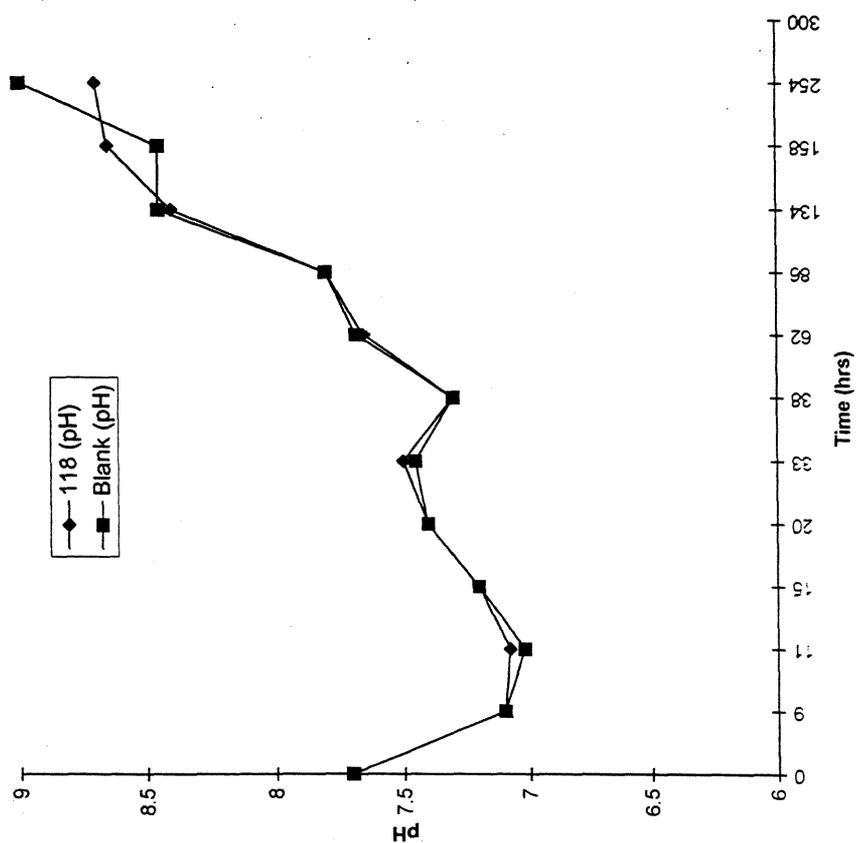
(Fig 32) Concentrations of amphotericin A and B in the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (117) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



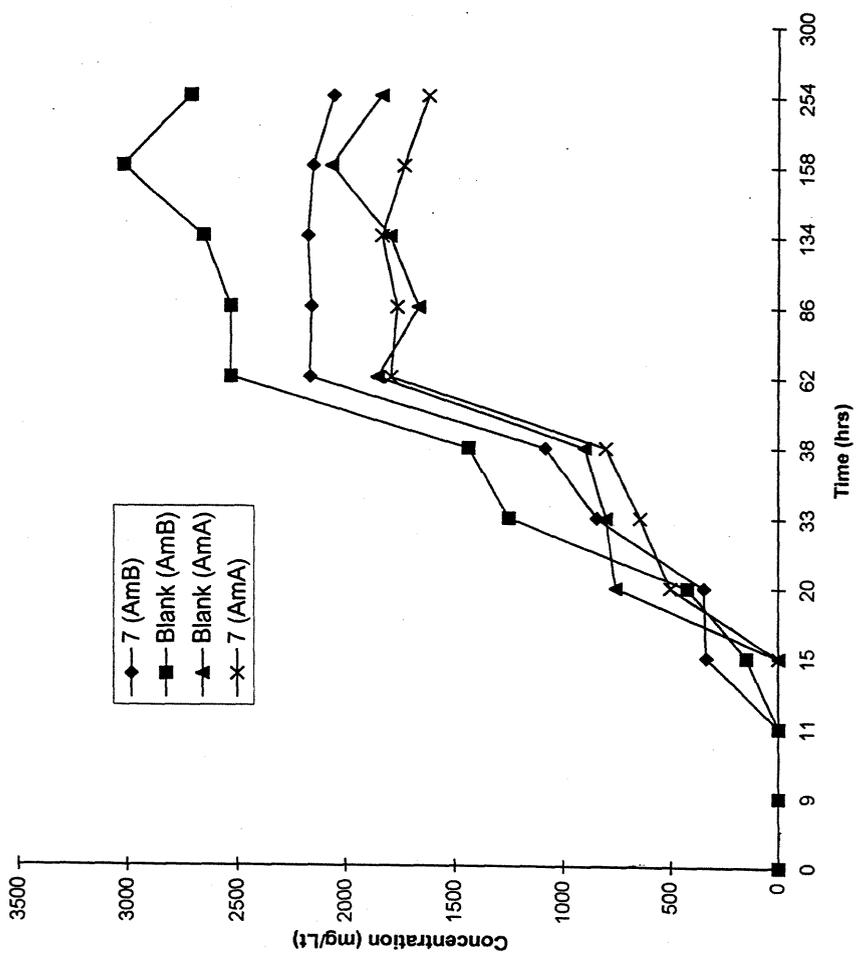
(Fig 33) Concentrations of amphotericin A and B in the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (118) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



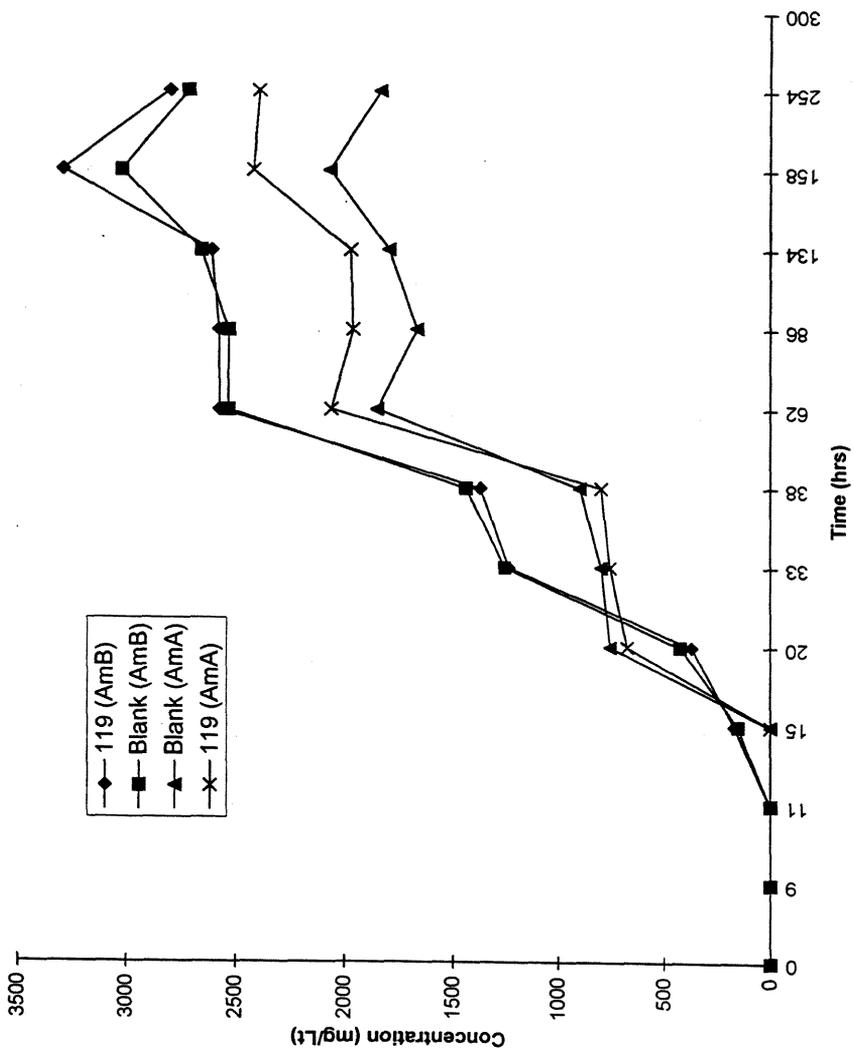
(Fig 34) pH of the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (117) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



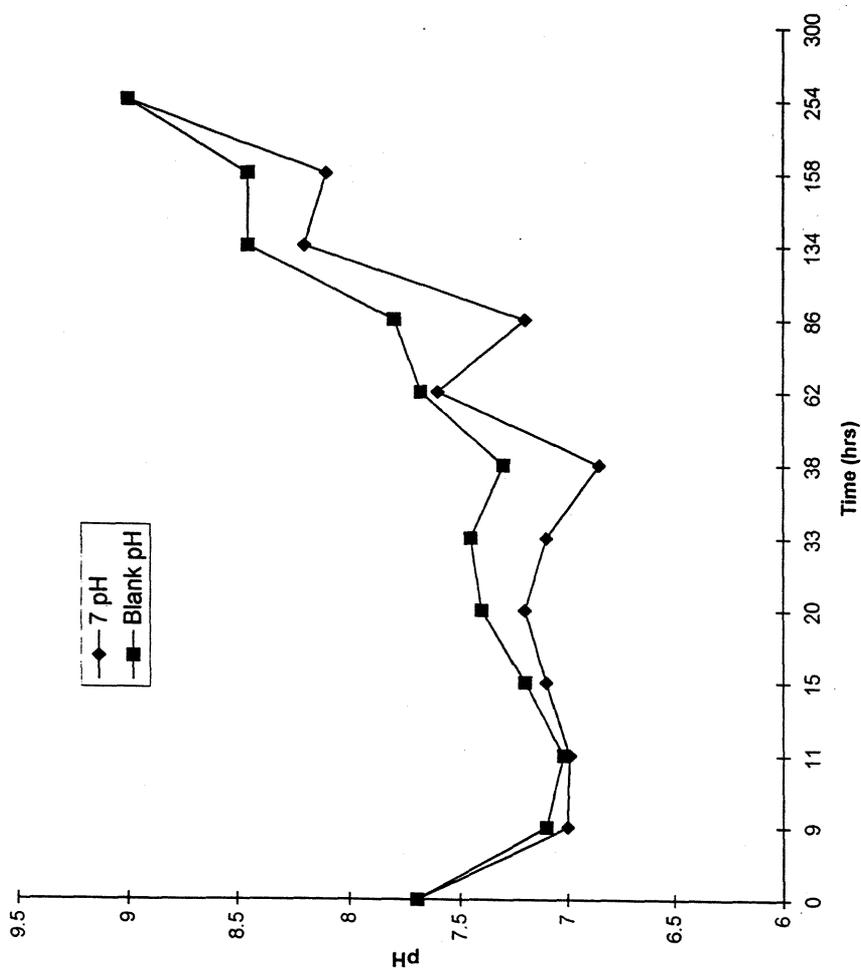
(Fig 35) pH of the FCA media containing 200 µl of ethanol (Blank) and autoregulator (118) (10 mg in 200 µl ethanol) from the time of inoculation.



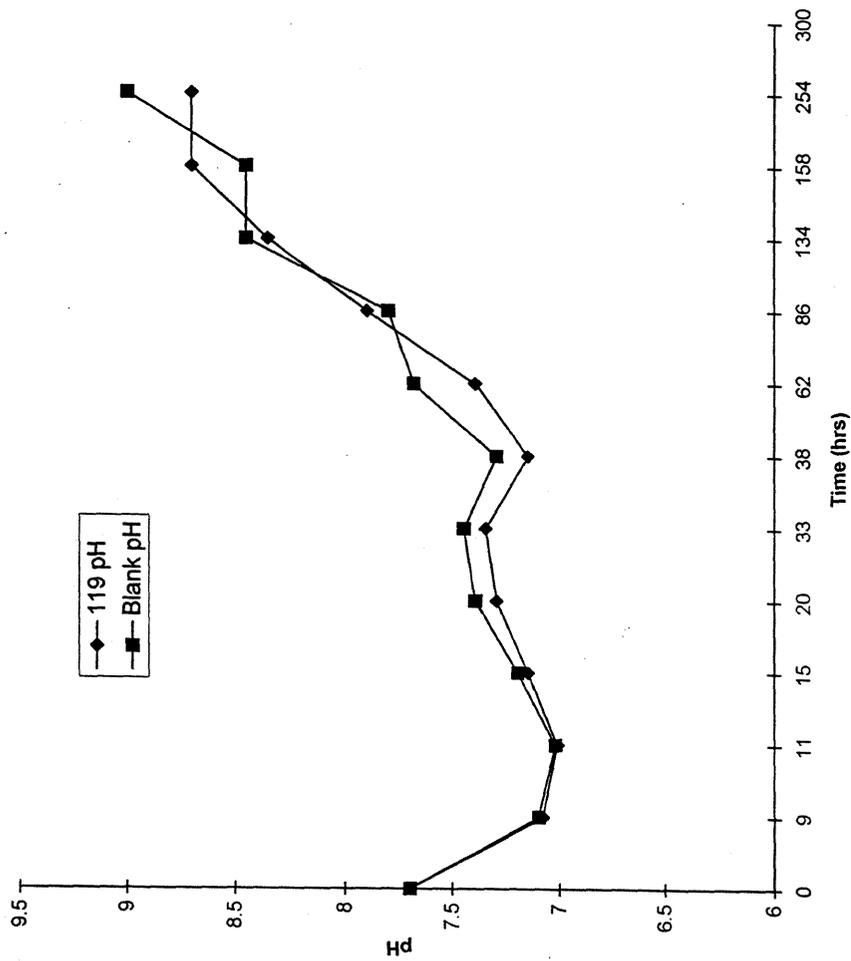
(Fig 36) Concentrations of amphotericin A and B in the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (7) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



(Fig 37) Concentrations of amphotericin A and B in the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.

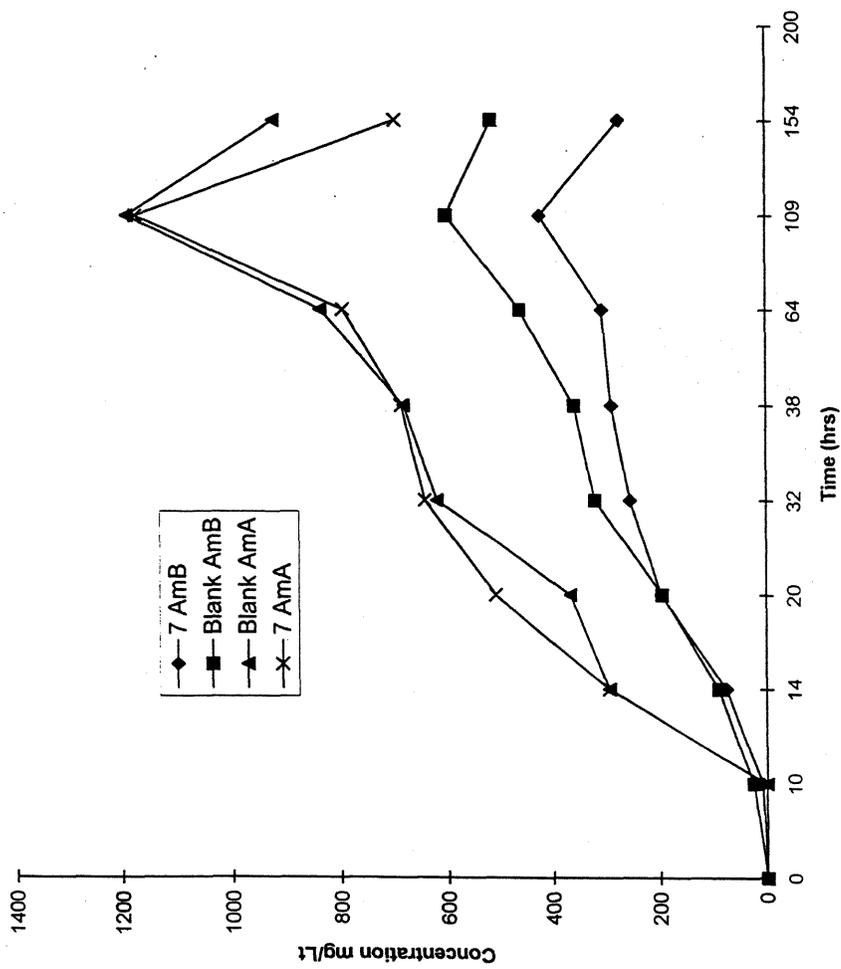


(Fig 38) pH of the FCA media containing 200 µl of ethanol (Blank) and autoregulator (7) (10 mg in 200 µl ethanol) from the time of inoculation.

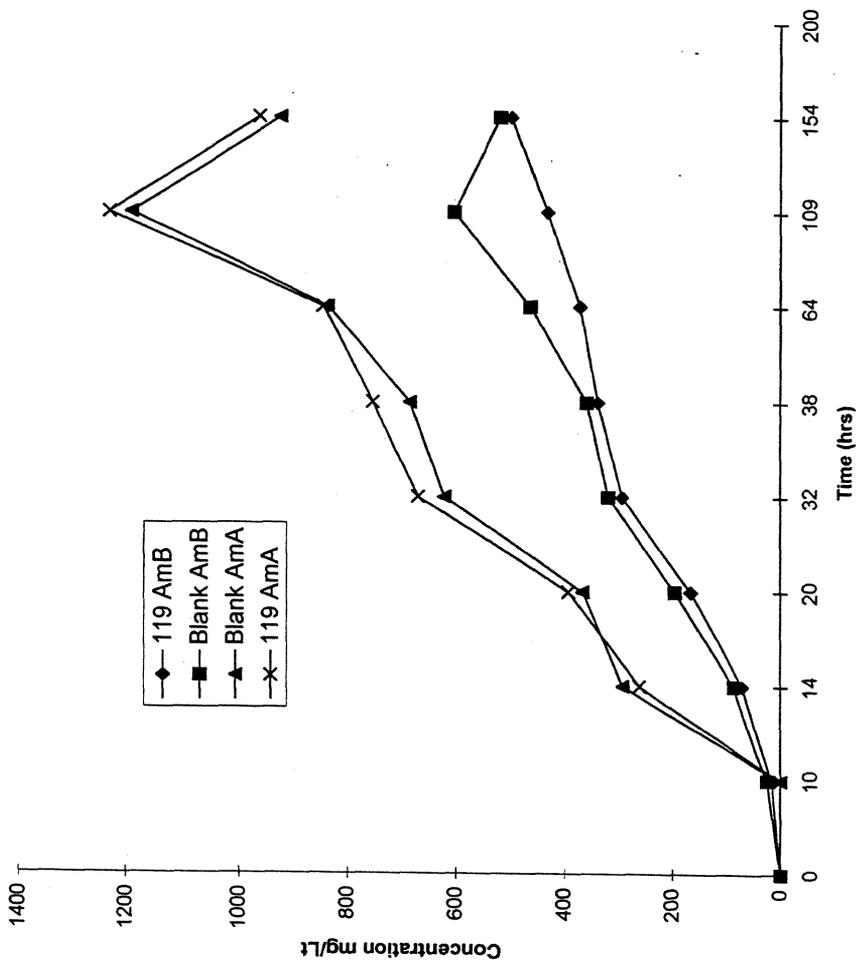


(Fig 39) pH of the FCA media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.

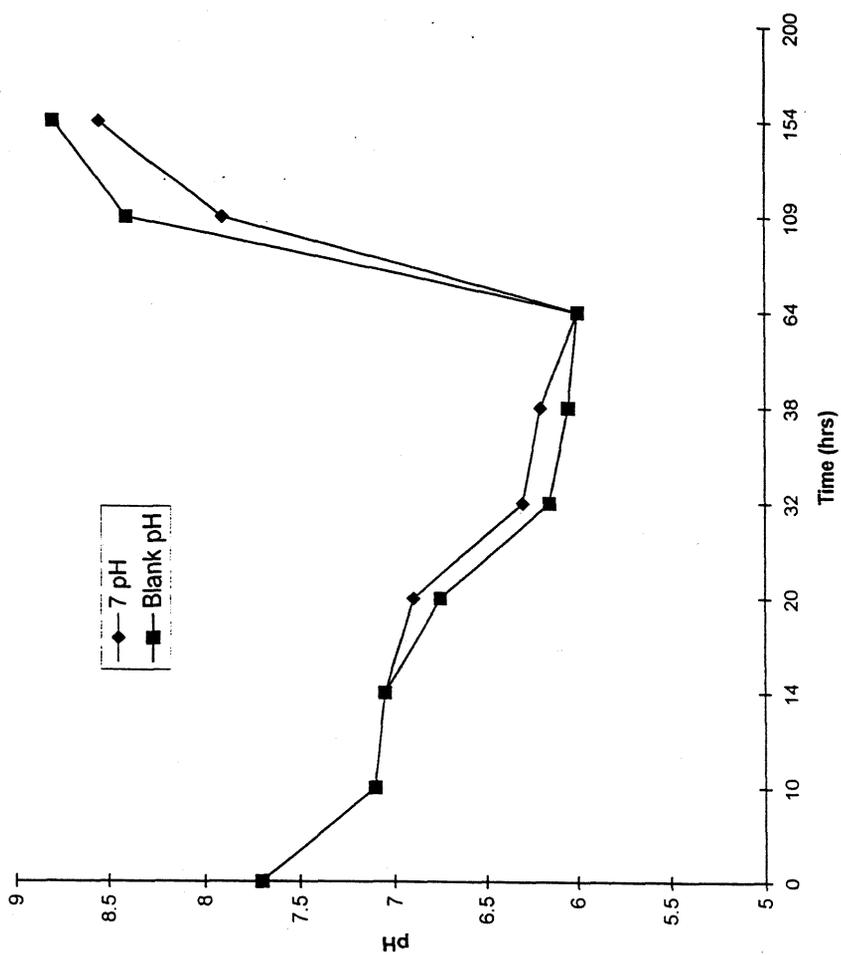
The complex media produces medium titres of amphotericin A and B, normally 1200 mg/Lt and 600 mg/Lt respectively. The feeding experiments were performed in an identical manner as described in the experimental section (5.3) for the FCA media. Addition of the natural A-factor (7) gave no significant increase in the titres of both amphotericin A or amphotericin B during the fermentation (Fig 40). A modest increase in the level of amphotericin A at twenty hours was observed but this was within the margins expected for the fermentation, and therefore no statistical importance could be placed on this value. The absolute concentrations of amphotericin A and B were both diminished in comparison to the blank media, however were within the range expected. Feeding the racemic analogue (119) of the naturally occurring A-factor gave comparable results (Fig 41) although in this case the absolute concentrations of amphotericin A and B in the autoregulator containing media were much closer to the blank media which contained ethanol alone. The pH profile of both the media containing A-factor (7) (Fig 42) and the racemic analogue (119) (Fig 43) showed a considerable correlation to the blank media, indicative that the pH of the media was unaffected by the exogenous autoregulators. Feeding analogue (118) to the media also gave no corroborative increase in the titres of amphotericin A and B during the fermentation (Fig 44). The pH profile again matched closely that of the blank media (Fig 45). The feeding experiments on the complex media with autoregulators (7) (119) (118) have generally indicated no significant aberration in either the titres of metabolite in the broth or the pH of the broth over the course of the fermentation, and generally expressed a diminished absolute concentration of the secondary metabolite. This may indicate that these exogenous autoregulators, if active, exerted a pernicious effect on the production of amphotericin A and B during the fermentation.



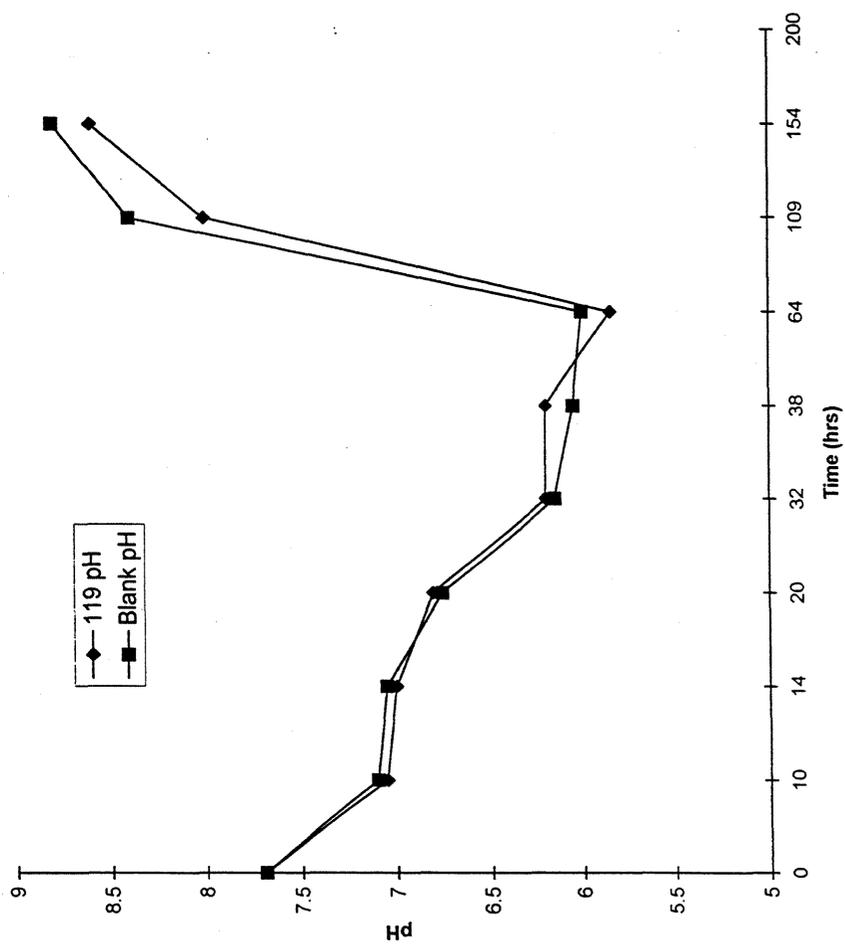
(Fig 40) Concentrations of amphotericin A and B in the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (7) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



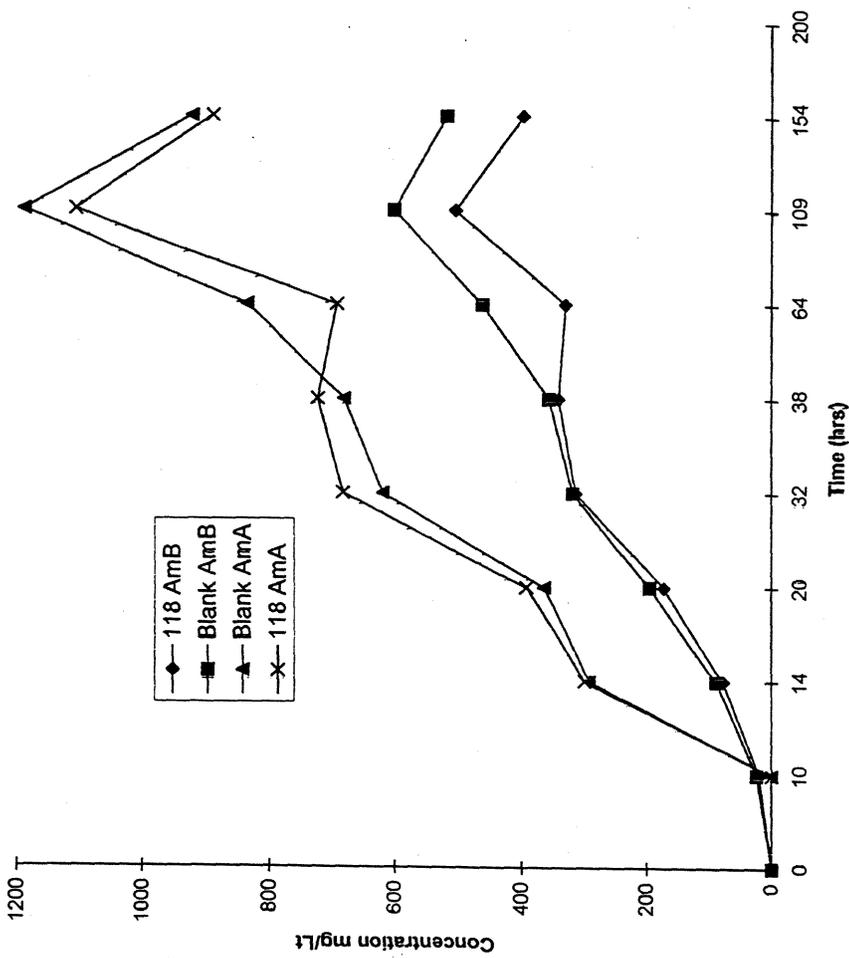
(Fig 41) Concentrations of amphotericin A and B in the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



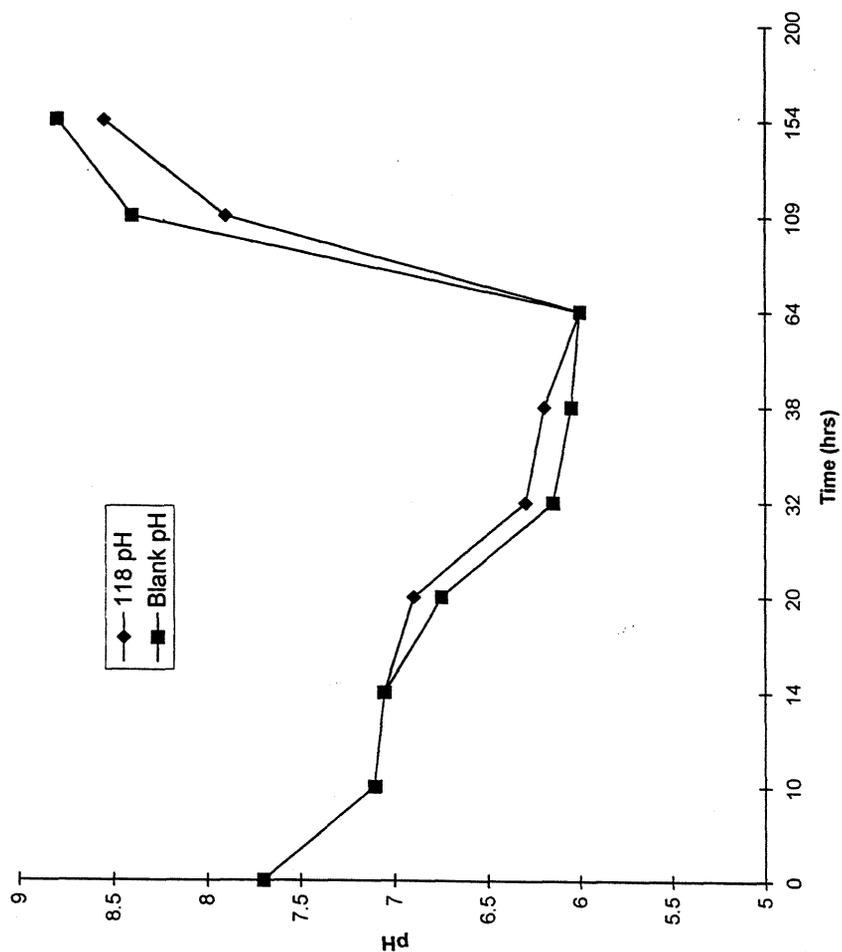
(Fig 42) pH of the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (7) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



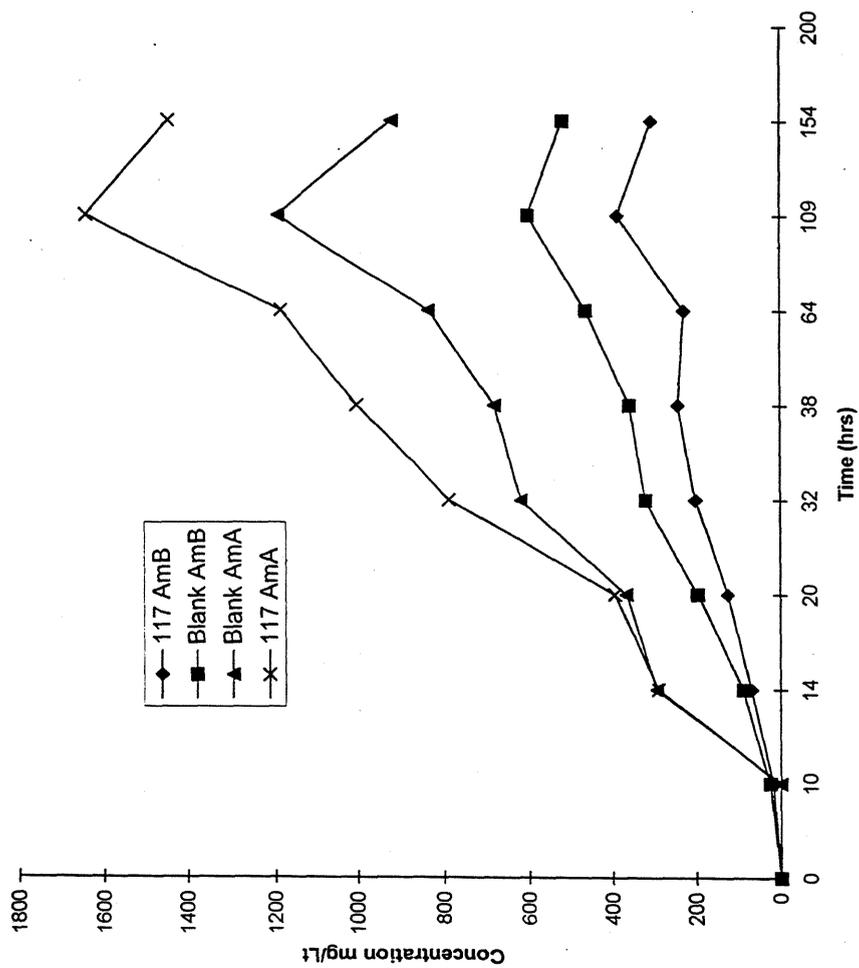
(Fig 43) pH of the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



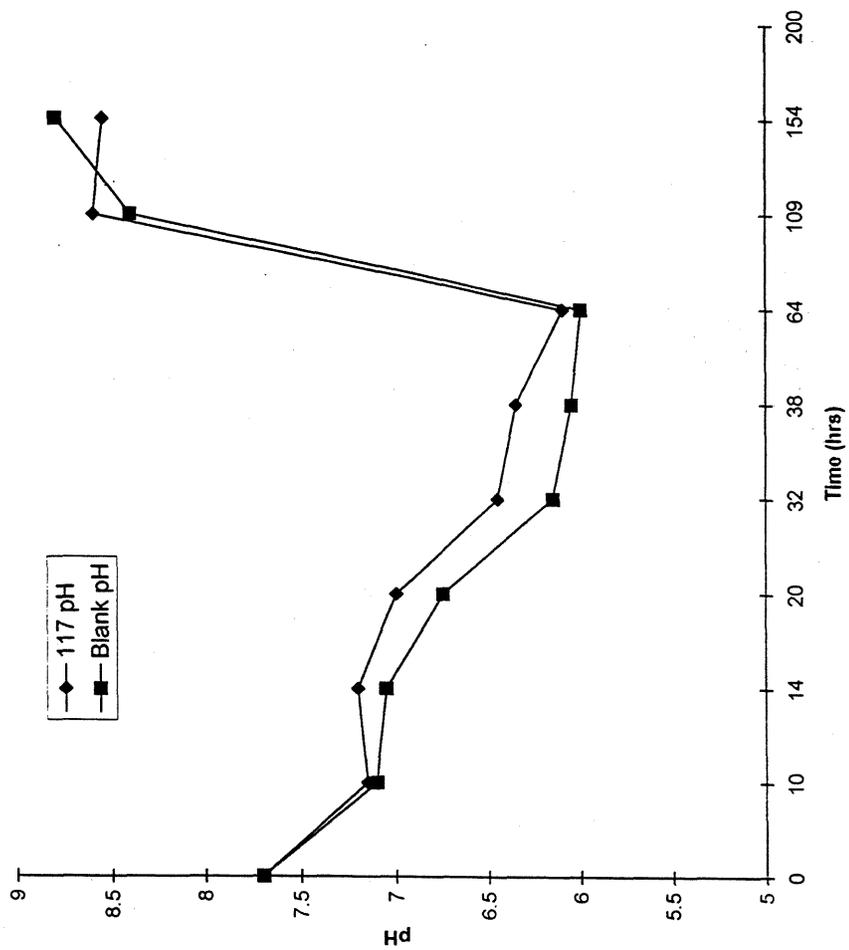
(Fig 44) Concentrations of amphotericin A and B in the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (118) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



(Fig 45) pH of the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (118) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



(Fig 46) Concentrations of amphotericin A and B in the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (117) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



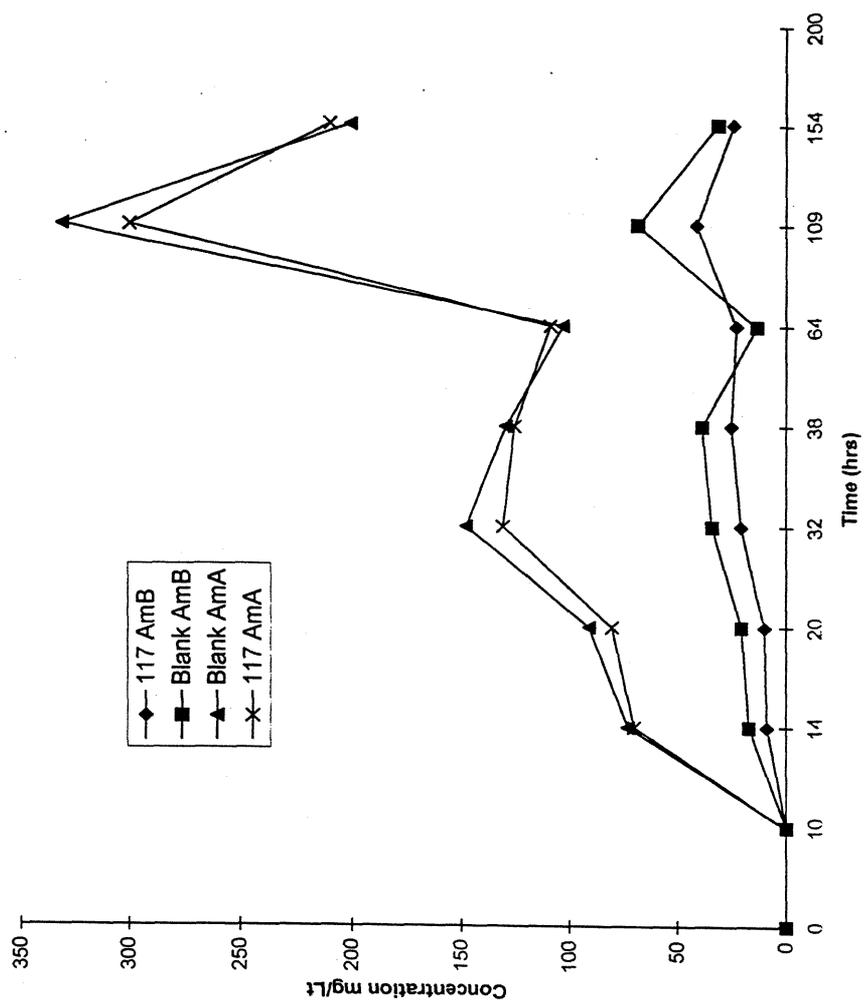
(Fig 47) pH of the complex media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (117) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.

An apparent anomaly appeared in the levels of amphotericin A in the media on feeding analogue (117), and the concentrations of amphotericin B were as previously experienced diminished relative to the blank media (Fig 46).

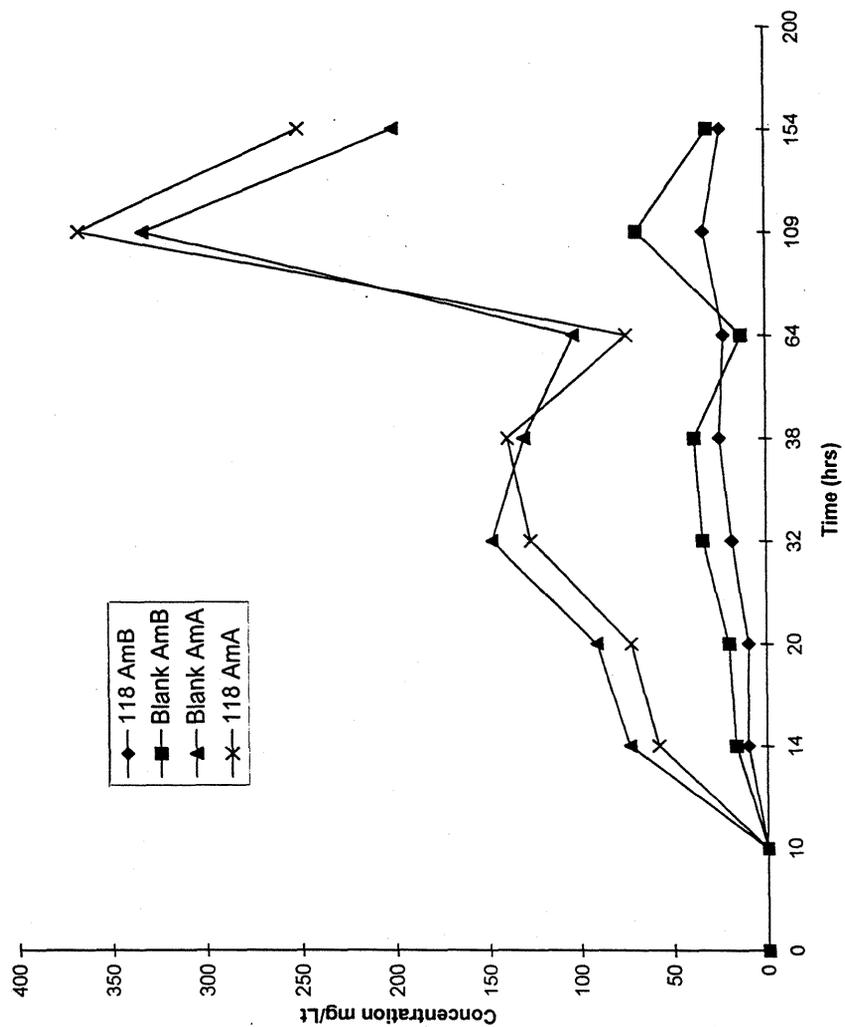
The titres of amphotericin A after twenty hours were however consistently between twenty one and thirty six percent greater than those obtained in the blank media containing no exogenous autoregulator (Fig 46). These levels would suggest that the exogenous autoregulator exerted a propitious and selective effect on the production of the secondary metabolite, increasing the concentration of amphotericin A within the broth. A repeat experiment would confirm this encouraging result. The pH profile of the media (Fig 47) showed a good correlation between the autoregulator containing media and the blank, registering that the pH of the media was unaffected during the fermentation by the exogenous autoregulator.

The defined media furnished the lowest titres of amphotericin A and B in the three media tested, typically in the order of 350 mg/Lt and 60 mg/Lt respectively. The feeding experiments were conducted in an identical manner as for the previous media and as described in the experimental section (5.3). Addition of analogues (117) and (118) to the media gave no significant increase in the titres of either amphotericin A or amphotericin B (Fig 48) (Fig 49) during the fermentation. The pH profile also closely correlated with that of the blank media containing no exogenous autoregulator (Fig 50) (Fig 51). This would suggest that these exogenous autoregulator analogues had no effect on the production of either amphotericin A or amphotericin B during the fermentation. Upon feeding the naturally occurring A-factor (7) to the culture broth the titres of amphotericin B showed a good correlation with the levels found in the blank media (Fig 52). This was also the situation in the media containing the racemic analogue (119) (Fig 53) where the concentration of amphotericin B was comparable with the titre in the blank media throughout the

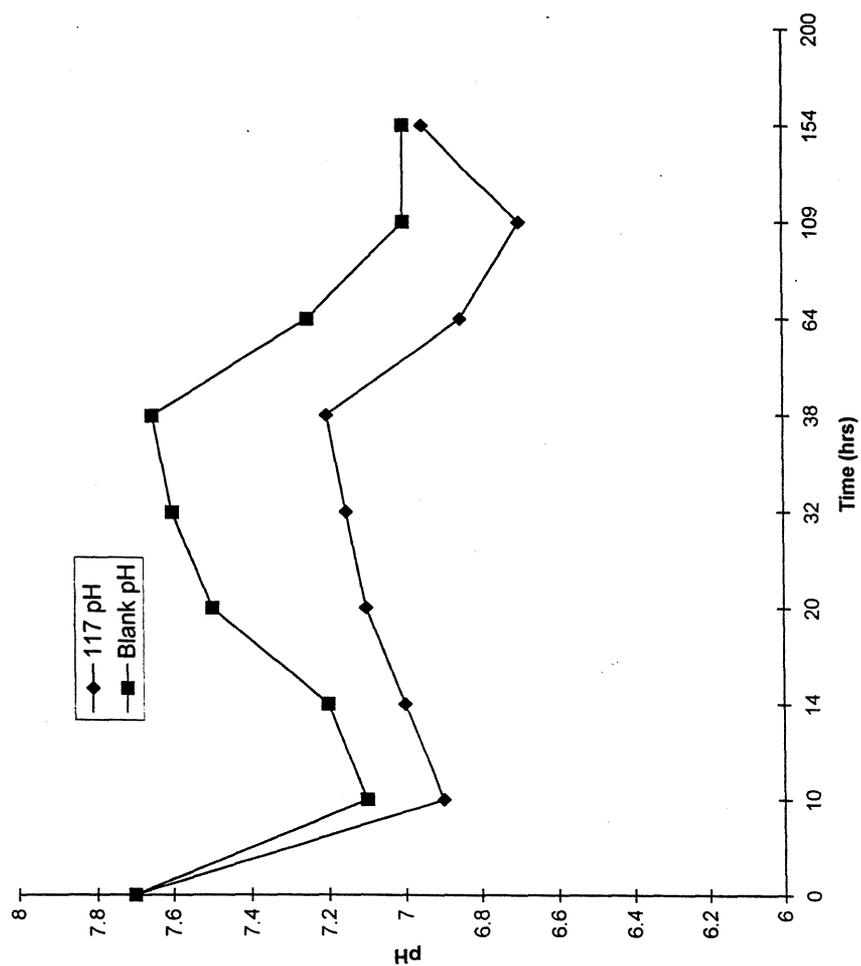
fermentation. Analysis of the concentration of amphotericin A in the media containing A-factor (7) and (119) indicated that the levels were in general comparable to those obtained in the blank media. The absolute concentrations in both these media however were greater than that observed within the blank media by twenty five and fifty percent respectively. This level of absolute concentration at this time in the fermentation suggests that the exogenous autoregulator altered the rate at which the concentration of metabolite decreases within the media, perhaps by exerting some effect on the feedback regulation mechanism apparent during the latter stages of the fermentation. Feedback regulation within streptomycete molecular biology is not well understood and therefore the exact mechanism by which the autoregulators could influence this process is not apparent. The pH profile for the media containing analogue (119) was comparable with that obtained from the blank media (Fig 54). This profile in the media containing the natural A-factor (7) did show a sharp drop in pH (Fig 55) relative to the blank media. Throughout the feeding experiments the pH profile of the media remained within the expected range and therefore the exact reason for this drop was not apparent, and a repeat feeding experiment would ascertain if this drop in pH was real.



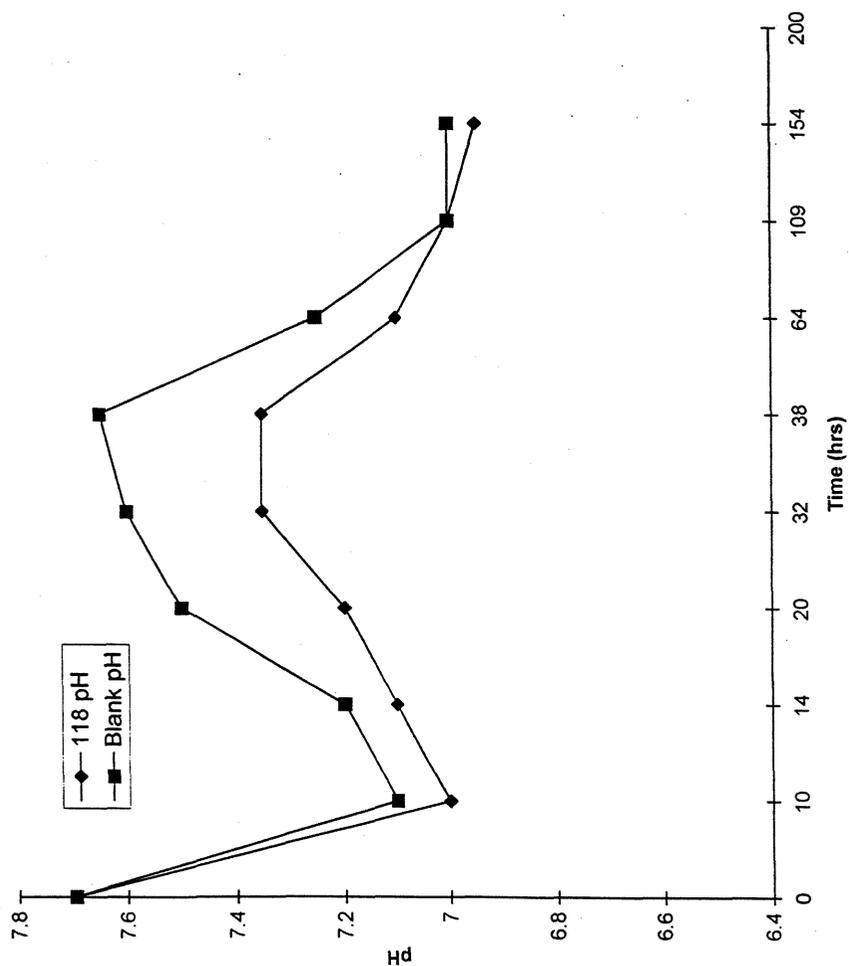
(Fig 48) Concentrations of amphotericin A and B in the defined media containing 200 µl of ethanol (Blank) and autoregulator (117) (10 mg in 200 µl ethanol) from the time of inoculation.



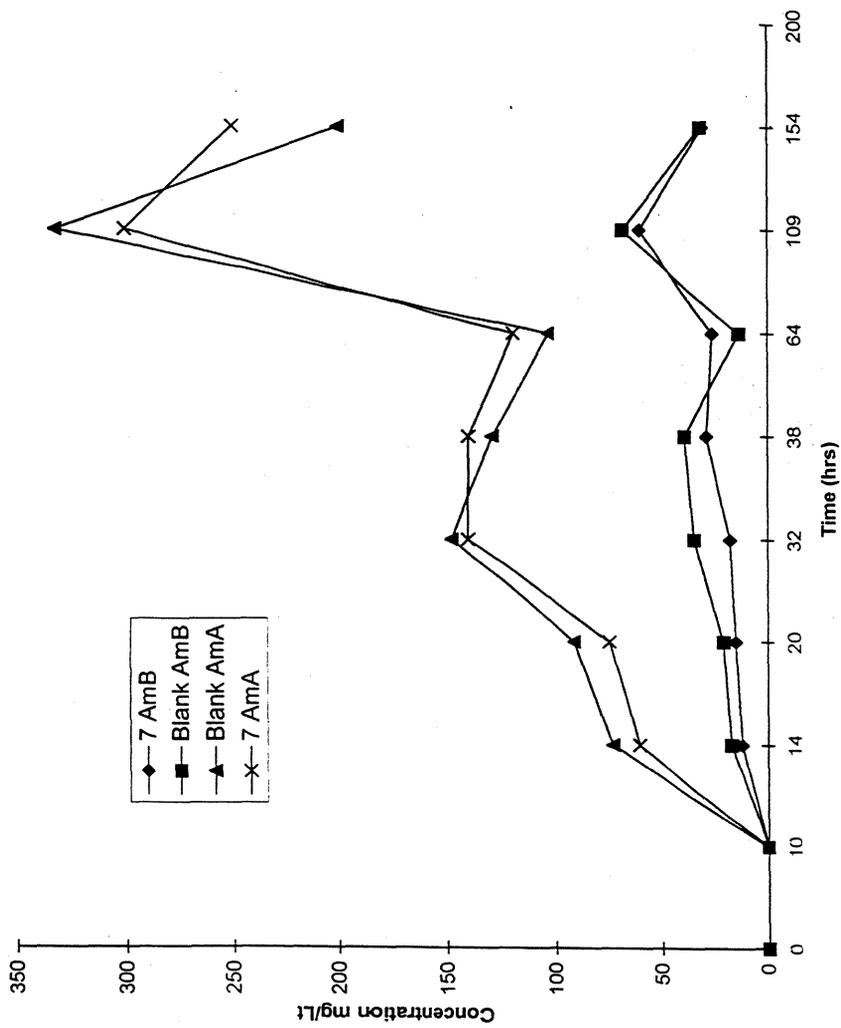
(Fig 49) Concentrations of amphotericin A and B in the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (118) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



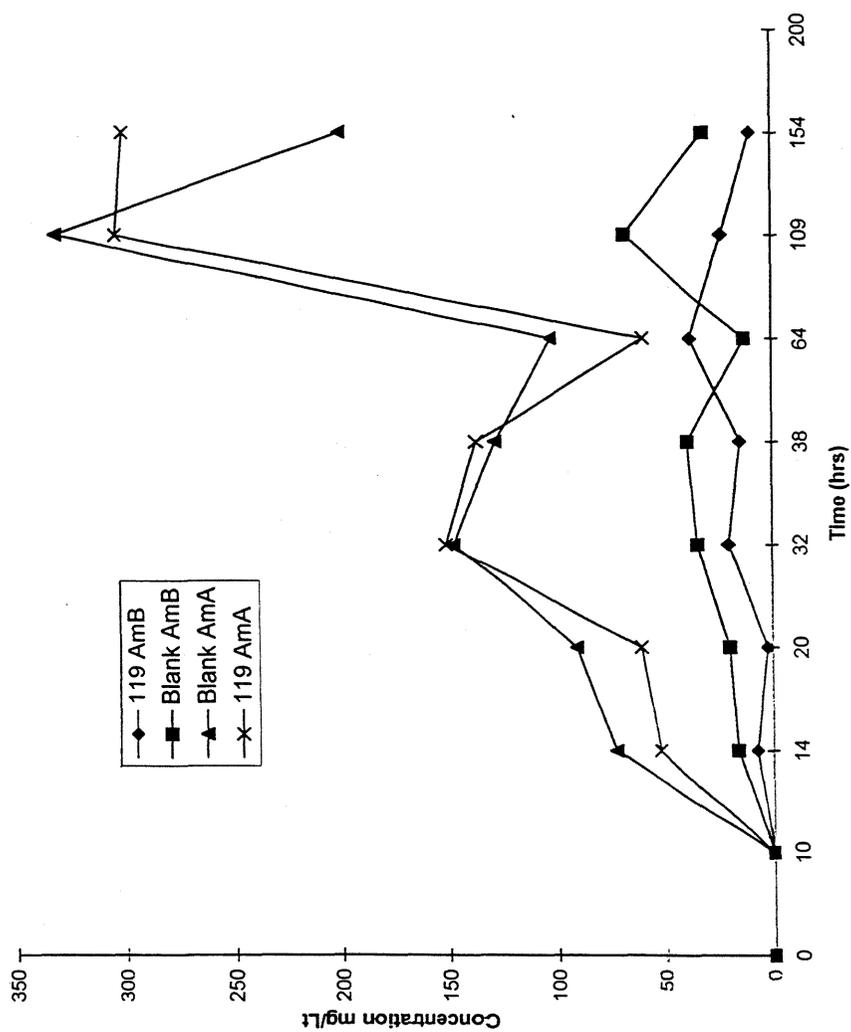
(Fig 50) pH of the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (117) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



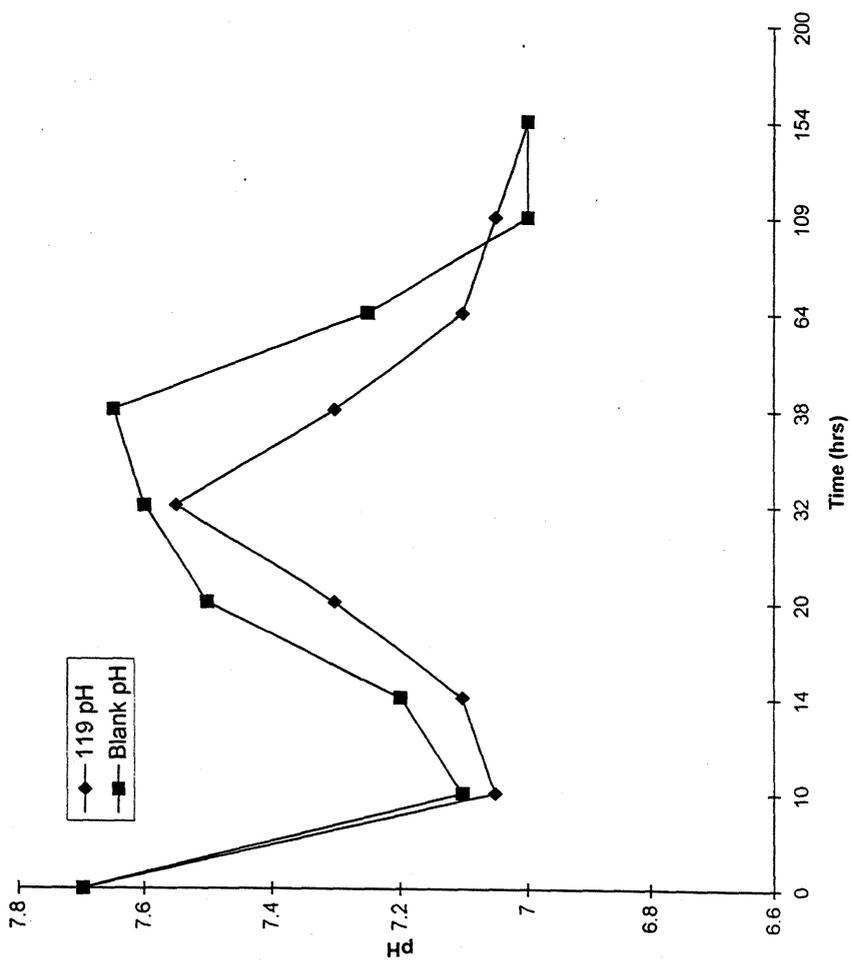
(Fig 51) pH of the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (**118**) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



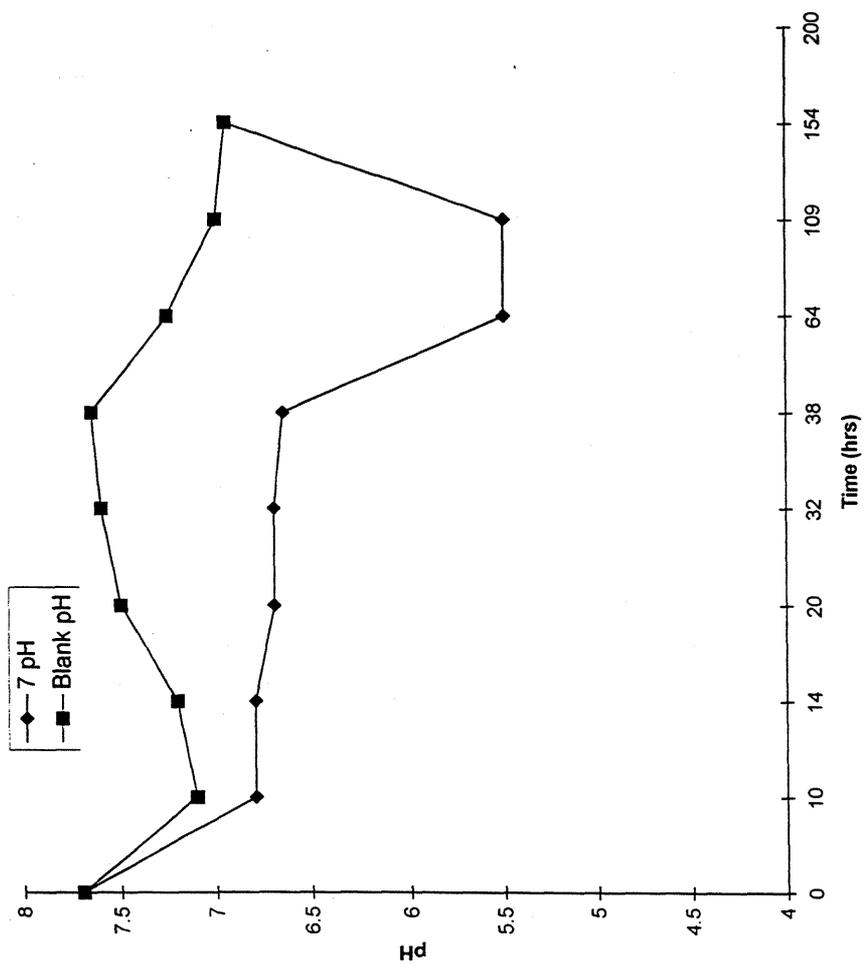
(Fig 52) Concentrations of amphotericin A and B in the defined media containing 200 µl of ethanol (Blank) and autoregulator (7) (10 mg in 200 µl ethanol) from the time of inoculation.



(Fig 53) Concentrations of amphotericin A and B in the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



(Fig 54) pH of the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (119) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.



(Fig 55) pH of the defined media containing 200  $\mu$ l of ethanol (Blank) and autoregulator (7) (10 mg in 200  $\mu$ l ethanol) from the time of inoculation.

### 3.3.1 Conclusion

The feeding experiments on the FCA, complex and defined media indicated that no decrease in the lag time for the onset of metabolite production was observed on addition of exogenous autoregulators. The addition in general also gave no comprehensive increase in the absolute titre of either amphotericin A or B during the fermentation, and in general furnished a diminished titre of both metabolites. Exceptions were expressed where local points indicated that the concentrations of amphotericin within the exogenous autoregulator containing media were higher than those within the blank media. Repeat experiments to confirm these results would indicate whether the effect was in fact a consequence of the autoregulator within the media. Encouraging results were also observed in the feeding of analogues (119) to the FCA media and analogue (117) to the complex media. A sustained higher concentration of amphotericin A in these media over that observed in the blank does suggest a beneficial effect imparted to this media by the presence of the exogenous autoregulator. Confirmation of the observations by repeating the feeding experiment would go some way to establishing the presupposition of exogenous stimulation. The autoregulator A-factor (7) and analogues tested (117) (118) and (119) are not known to be the endogenous autoregulator for *S. nodosus*. It may be necessary in repeat studies to use a streptomycete where the endogenous autoregulator is known,<sup>104</sup> and feed this autoregulator to test for an exogenous stimulation response.

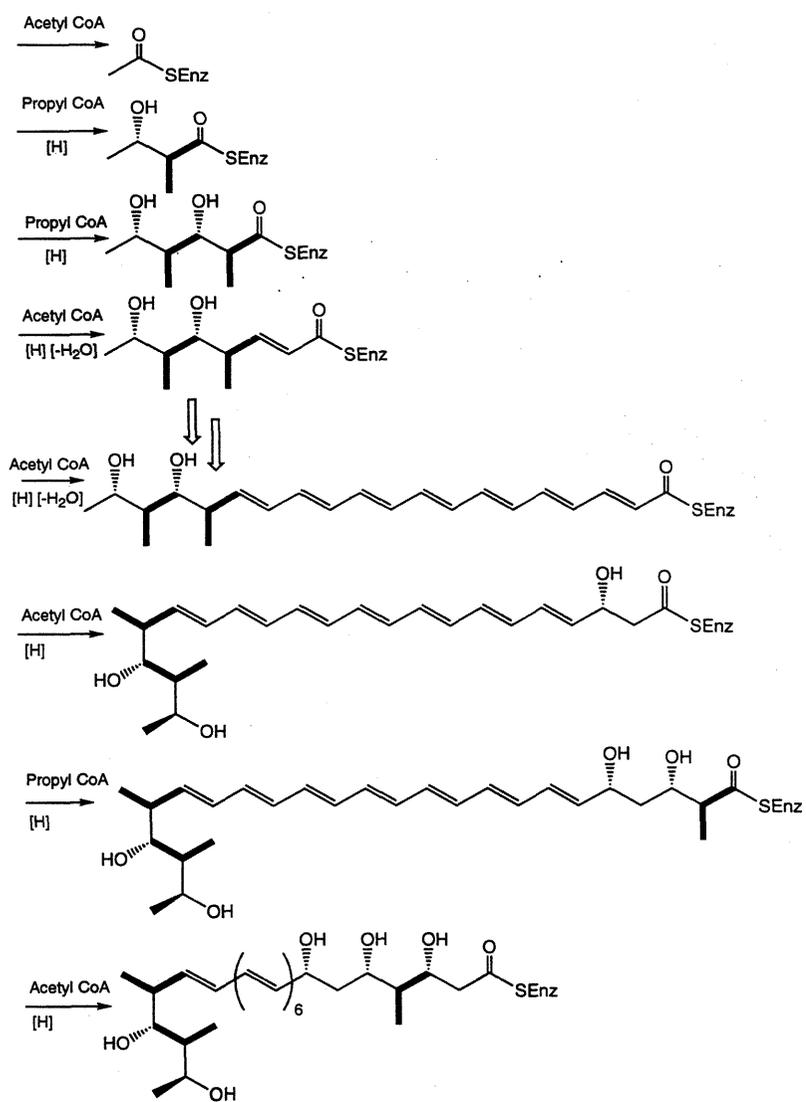
## CHAPTER 4

Biosynthetic Studies on Amphotericin B

## 4.0 Biosynthetic Studies on Amphotericin B

### 4.1 Introduction

Amphotericin B (22) is one of the class of polyene antibiotics and is used in the treatment of severe systemic fungal infections, particularly in patients that exhibit a compromised immune system, such as those suffering from AIDS, and those undergoing chemotherapy. It is produced by the soil bacterium *Streptomyces nodosus*, (along with a co-metabolite amphotericin A (23), that was first isolated from the soil of the Orinoco river region in Venezuela in 1955.<sup>61-64</sup> To date the only biosynthetic investigations on amphotericin B have been the incorporation of radiolabelled [<sup>14</sup>C]-acetate<sup>66-68</sup> and no study using stable isotopes has been reported. Other studies on this important natural product have tended to centre on its biological activity, structural confirmation by X-ray analysis,<sup>69,70</sup> and its total synthesis which was completed in 1988 by Nicolaou.<sup>30,71-74</sup> We intend to initiate a biosynthesis study on amphotericin B using stable isotope techniques, initially looking at the propionate positions by feeding [1-<sup>13</sup>C]; and [3-<sup>13</sup>C]-propionate. The proposed biosynthesis indicates amphotericin B to be constructed from seventeen acetate units and three propionate units. The positions derived from propionate units during the assembly are indicated below (Scheme 32). These units are assembled from head-to-tail condensation steps with the desired functionality inserted stereospecifically before the next condensation, in the processive manner (1.6.4). The carbon-13 and proton NMR spectrum for amphotericin B has been published<sup>75-78</sup> and most of the positions which may occur from the propionate units have been assigned. Feeding [1-<sup>13</sup>C] and [3-<sup>13</sup>C]-propionate to the bacterial broth during fermentation, harvesting the amphotericin B, and obtaining a carbon-13 spectrum



(Scheme 32a) Biosynthesis of amphotericin B.

Propionate positions are indicated in bold.

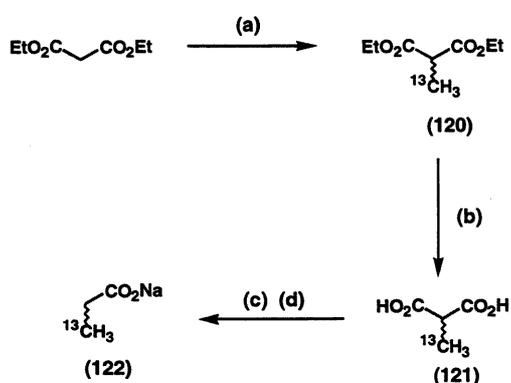


would, by an enhancement of the signals in the  $^{13}\text{C}$  NMR spectrum corresponding to carbons from a propionate unit, be indicative of label incorporation into these positions, and therefore the role of propionate units in the biosynthesis. This incorporation, although not the positioning, can also be proven by electrospray mass spectrometry. An increase in the intensity of the  $[M+1]$  peak would be indicative of incorporation of a single  $^{13}\text{C}$  label.

## 4.2 Results and Discussion

### 4.2.1 Synthesis of $[3-^{13}\text{C}]$ -propionate

Due to the restrictive price and commercial availability, sodium  $[3-^{13}\text{C}]$ -propionate was synthesised from carbon-13 labelled methyl iodide and diethyl malonate (Scheme 33). Addition of diethyl malonate to a suspension of sodium hydride in anhydrous *N,N*-dimethylformamide followed by  $[^{13}\text{C}]$ -methyl iodide gave the corresponding coupled adduct (120) in very good yield (82%). Saponification with sodium hydroxide gave the diacid (121) in high yield (88%). Decarboxylation by heating at reflux in hydrochloric acid (6 M) furnished the free acid which after distillation was converted into the corresponding sodium salt and freeze dried to give (122) in good yield (98%).



(Scheme 33) (a) NaH, DMF, [<sup>13</sup>C]-methyl iodide  
 (b) NaOH, THF, Water (c) HCl (6M) (d) NaOH.

#### 4.2.2 Biosynthesis of amphotericin B

Previous biosynthetic studies on amphotericin B using radiolabelled precursors<sup>66-68</sup> employed a glucose-yeast extract primary seed culture finally inoculating into the production medium. A complex media (5% glucose, 3% Pharmamedia, 1% calcium carbonate at pH 7.7), after sterilisation, was generally inoculated with 10% (v/v) of the primary seed and incubated at 28 °C 245 rpm for seven to eight days. The amphotericin B produced, in the region of 4500 to 3500 mg/Lt, was harvested by adjusting the broth pH to 6.5 with hydrochloric acid (2 M), and extracting with *n*-butanol. The *n*-butanol phase was reduced in volume and the amphotericin precipitated with diethyl ether. The crude precipitate, a mixture of amphotericin A and B, being further purified by countercurrent distribution to give a sample suitable for analysis.

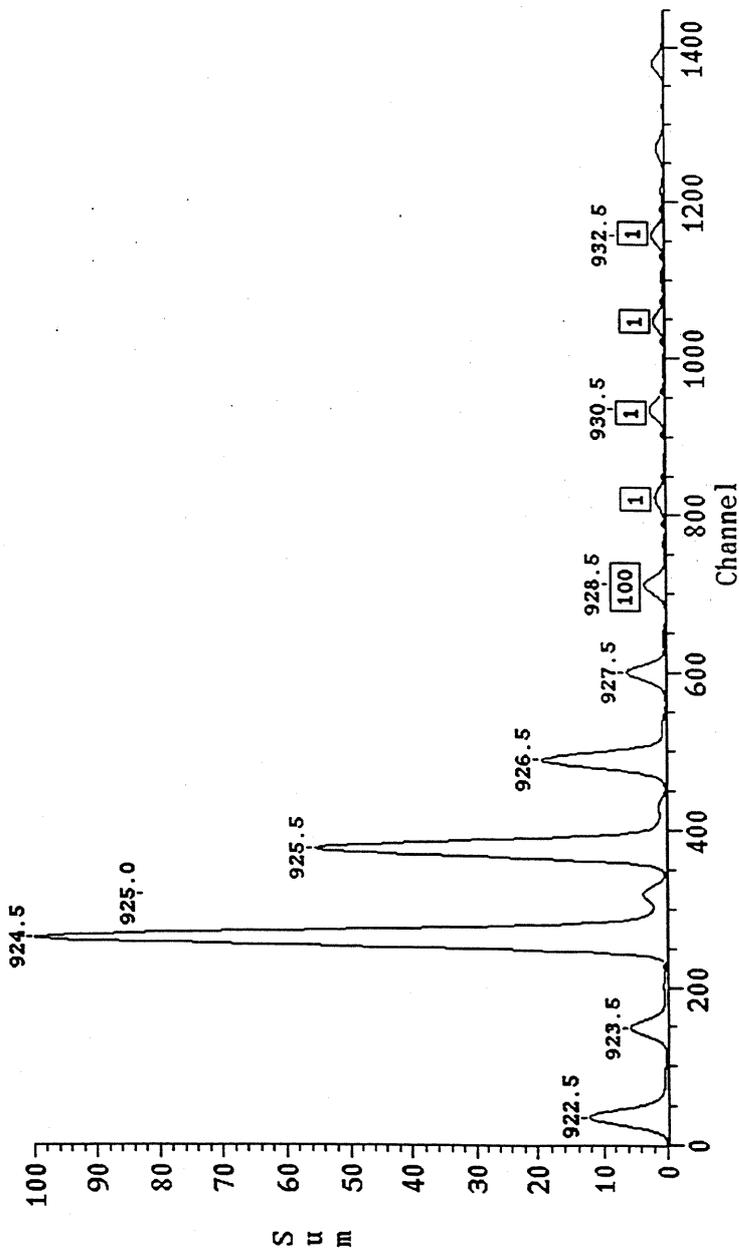
Initially an identical fermentation method was employed in our studies however the levels of amphotericin A and B observed during the fermentation were only 1000 and 500 mg/Lt respectively. Repeating the isolation procedure as described in the literature also gave poor yields, and the samples isolated were very impure, and difficult to purify further, even by HPLC. This level of amphotericin B production, although lower than that reported, would still be sufficient to enable stable isotope feeding experiments if a suitable isolation procedure could be developed. Repeated attempts at butanol extraction of the whole broth however gave mixed samples of amphotericin A and B which proved difficult to purify even after gel filtration and ion exchange resin chromatography on Dowex 50 resin. At this stage the *n*-butanol extraction procedure was repeated on a defined media (5% glucose, 1% calcium carbonate, 0.1% asparagine, 0.01% ferric sulfate and 0.01% manganese chloride). This media produced much lower titres of amphotericin A and B than the complex media (400 and 60 mg/Lt respectively). The absence of cottonseed flour (Pharmamedia) however as a constituent of the culture broth, a cleaner extraction from the whole broth was expected. Extraction of the defined culture broth with *n*-butanol as before, again gave samples which were difficult to purify and contained large amounts of impurity.

A collaboration with SmithKline Beecham gave access to a media and extraction method developed at their Brockham Park site.<sup>105</sup> This new media gave high titres of both amphotericin A and B, typically in the order of 3000 and 2000 mg/Lt respectively in shake flasks, and up to 5000 and 3500 mg/Lt during large scale (>20 Lt) fermentations. The media, (2% fructose, 6% Collofilm dextrin, 3% Arkasoy, and 1% calcium carbonate), was inoculated from a primary glucose-yeast culture and incubated as previously described in the experimental section (5.3). The concentrations of amphotericin A and B generally reached the highest titres 120-144 hours after inoculation, and this was found to be the optimum time for harvesting of

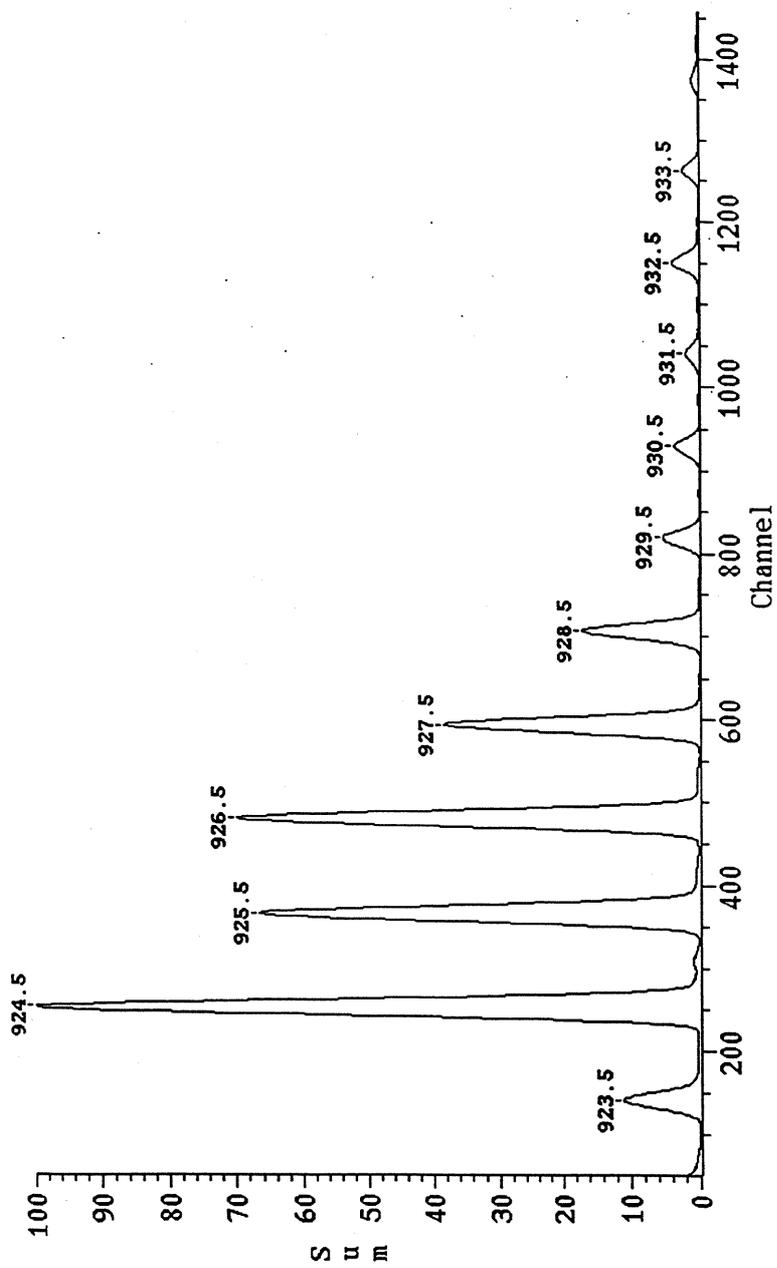
the metabolites. The isolation procedure involves adjusting the pH of the broth to 10.5 with sodium hydroxide solution (5 M) forming the carboxylate anion of amphotericin B. A solution of Aliquat 336<sup>®</sup> (7%, w/v) (tricaprylylmethylammonium chloride), a liquid ion exchange resin in a suitable water immiscible solvent was then added to the broth with vigorous stirring. Formation of an ion pair between the Aliquat and amphotericin A and B greatly increased their solubility in the organic solvent, enhancing the extraction. The mixture was spun on a centrifuge and the phases separated. Lowering of the pH of the organic phase containing the solution of Aliquat and amphotericin A and B would re-protonate the amphotericin, breaking the ion-pair, and causing the amphotericin to precipitate. In the original method by SmithKline Beecham *n*-butanol was employed as the organic solvent and in this case it was found that addition of a small amount of ethyl acetate (1% v/v) to the organic phase induced a precipitation of amphotericin B (22) as bright yellow spheres after 3-4 days. Resuspension of the precipitate in the mother liquor, filtration and washing with water, acetone, and methanol furnished amphotericin B with very high purity (>95%). On addition of ethyl acetate to the Aliquat-amphotericin solution the trace amounts of sodium hydroxide dissolved in the solvent causes saponification of the ethyl acetate. This releases acetic acid slowly lowering the pH of the organic phase and protonating the amphotericin which crystallises seeding on small particles of suspended dust within the solution, and falling to the base of the vessel as bright yellow spheres. It is important to stress that *no* detectable amounts of amphotericin A is precipitated during this time even though the two metabolites differ by only one double bond in the unsaturated back bone, absent in amphotericin A (23). The exact rationale for this highly selective precipitation is not obvious. If the pH is lowered manually by the addition of acetic acid then the amphotericin B precipitates as an amorphous fine solid and which is more difficult to harvest and has lower purity.

Repeating this fermentation and extraction procedure we found that lower titres of amphotericin were produced from our shake flask fermentations, typically 2000 mg/Lt amphotericin A and 1500 mg/Lt amphotericin B. Due to these lower titres of metabolite within the broth, ethyl acetate, rather than *n*-butanol, was found to be the most suitable organic solvent for the extraction process. This however negated the need for the addition of an external aliquot of ethyl acetate in order to initiate the precipitation, which occurred upon standing the organic phase for 3-4 days. The precipitation process using this solvent system still proved to be both effective *and* highly selective, furnishing pure samples of amphotericin B, which gave identical proton and carbon NMR spectral data, to that published.<sup>75-78</sup> We now had an effective procedure for the growth and isolation of pure samples of amphotericin B suitable for our biosynthetic studies.

To enable us to establish the conditions requisite for label incorporation, before committing samples of the more expensive [<sup>13</sup>C]-labelled propionates, initial feeding experiments were performed using commercially available deuterated sodium acetate (CD<sub>3</sub>CO<sub>2</sub>Na). The deuterated acetate was pulse fed to the FCA media as described in the experimental section (5.3). Amphotericin B was isolated, as described earlier, and analysed by electrospray mass spectrometry. An increase in the intensity of the [M+1] and [M+2] peaks, relative to a sample of amphotericin B isolated from a 'control' media, where no labelled acetate was added, would be indicative of label incorporation. Comparison of the mass spectra of the sample obtained from the 'control' media (Fig 56), with that obtained from the media pulse fed with sodium acetate-*d*<sub>3</sub> (Fig 57) clearly indicates successful label incorporation.

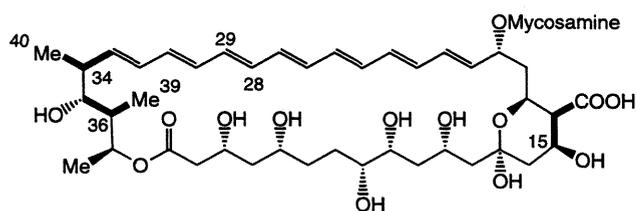


(Fig 56) Electrospray mass spectrum of amphotericin B obtained from 'control' media.



(Fig 57) Electrospray mass spectrum of amphotericin B obtained from FCA media pulse fed with sodium acetate- $d_3$ .

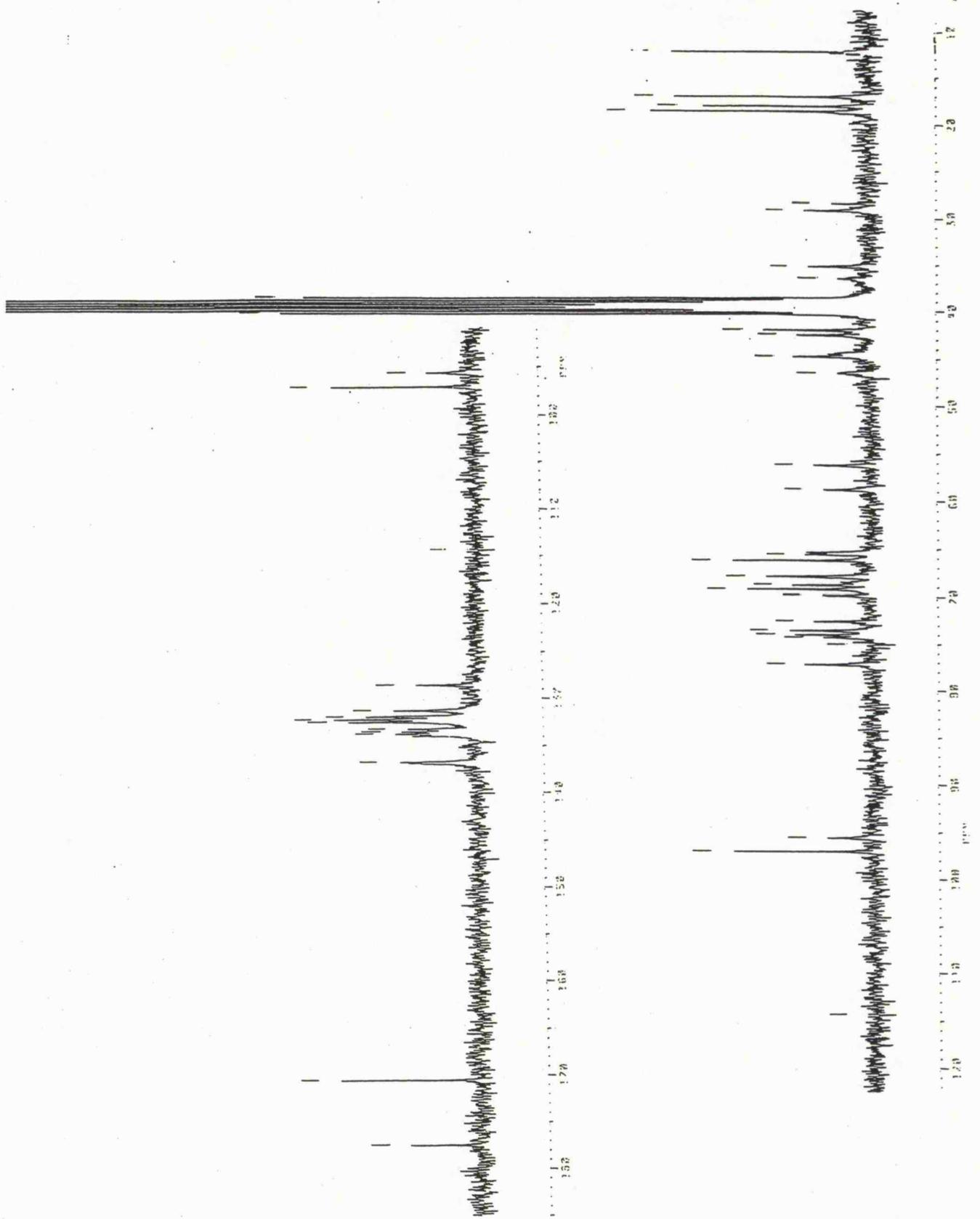
Once the conditions requisite for the feeding experiments had been determined our feeding studies turned to the labelled propionate units. Sodium [3-<sup>13</sup>C]-propionate (**122**) was pulse fed in aliquots (50 mg) at 22, 26, 30, 46, 50, 54 and 70 hours to the growing bacterium. After 120 hours the amphotericin B was isolated from the culture broth as described earlier. The proposed biosynthetic pathway (**Scheme 32**) indicates the carbon atoms within amphotericin B expected to originate from propionate units. The specific positions which will therefore be labelled on feeding [3-<sup>13</sup>C]-propionate (**122**), if intact incorporation of the precursor is achieved, are the methyl carbons (C-39), (C-40), and the carbon atom of the carboxylic acid moiety (**Fig 58**). The carboxylic acid group is derived by the oxidation of the methyl group of a propionate unit in the enzyme free intermediate by a tailoring enzyme, and intact incorporation at this position would be indicative that this oxidation process does not effect the label integrity at that centre.



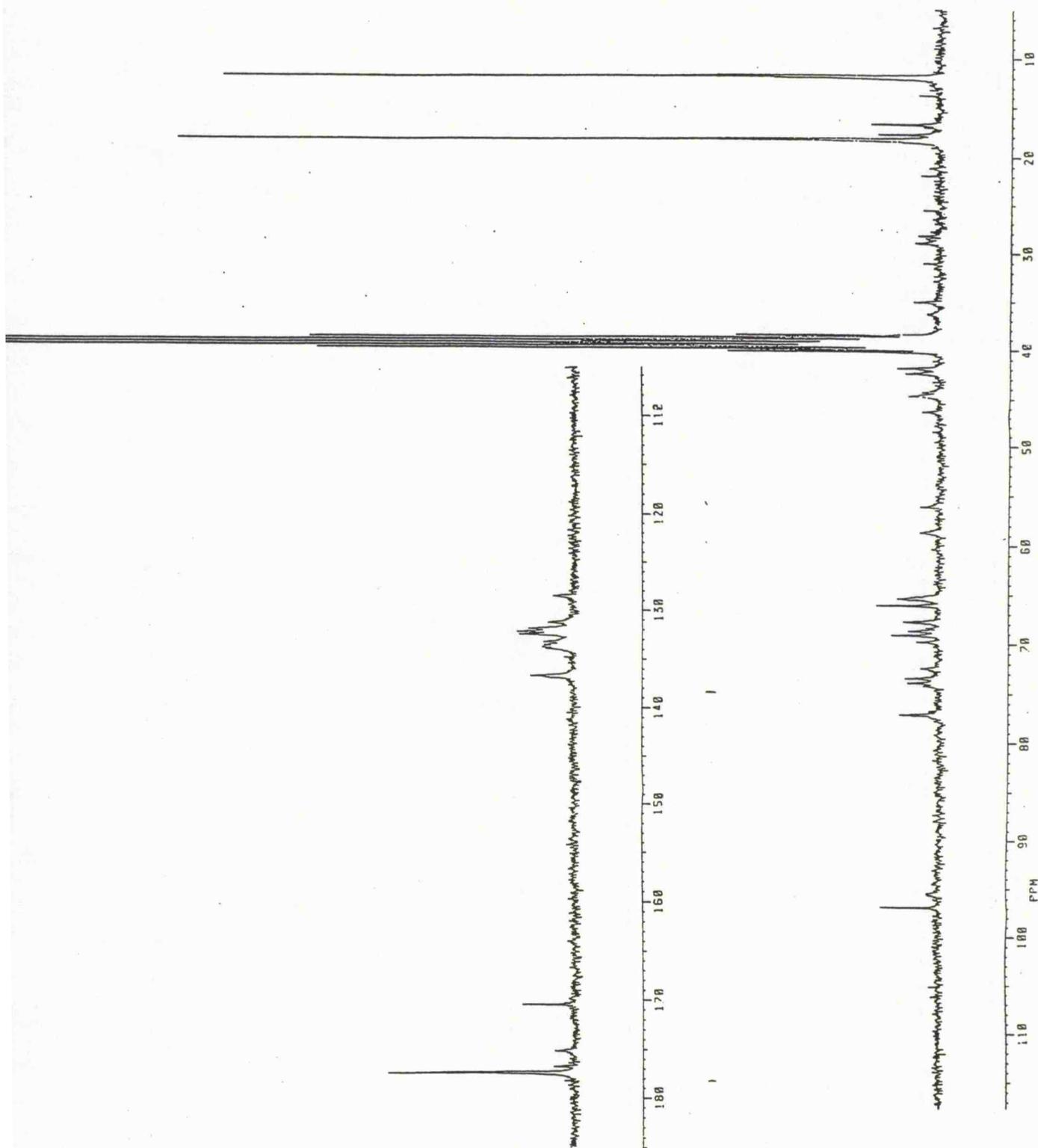
(**Fig 58**) Amphotericin B (**22**) with the propionate units indicated as bold lines.

The signals in the carbon-13 NMR of amphotericin B corresponding to these positions have been assigned.<sup>75</sup> Label incorporation would therefore be verified by the enhancement of these peaks in the carbon-13 NMR spectrum of amphotericin B isolated from the broth containing the labelled propionate, relative to the peak intensity apparent in a sample of 'unlabelled' amphotericin B, with a natural

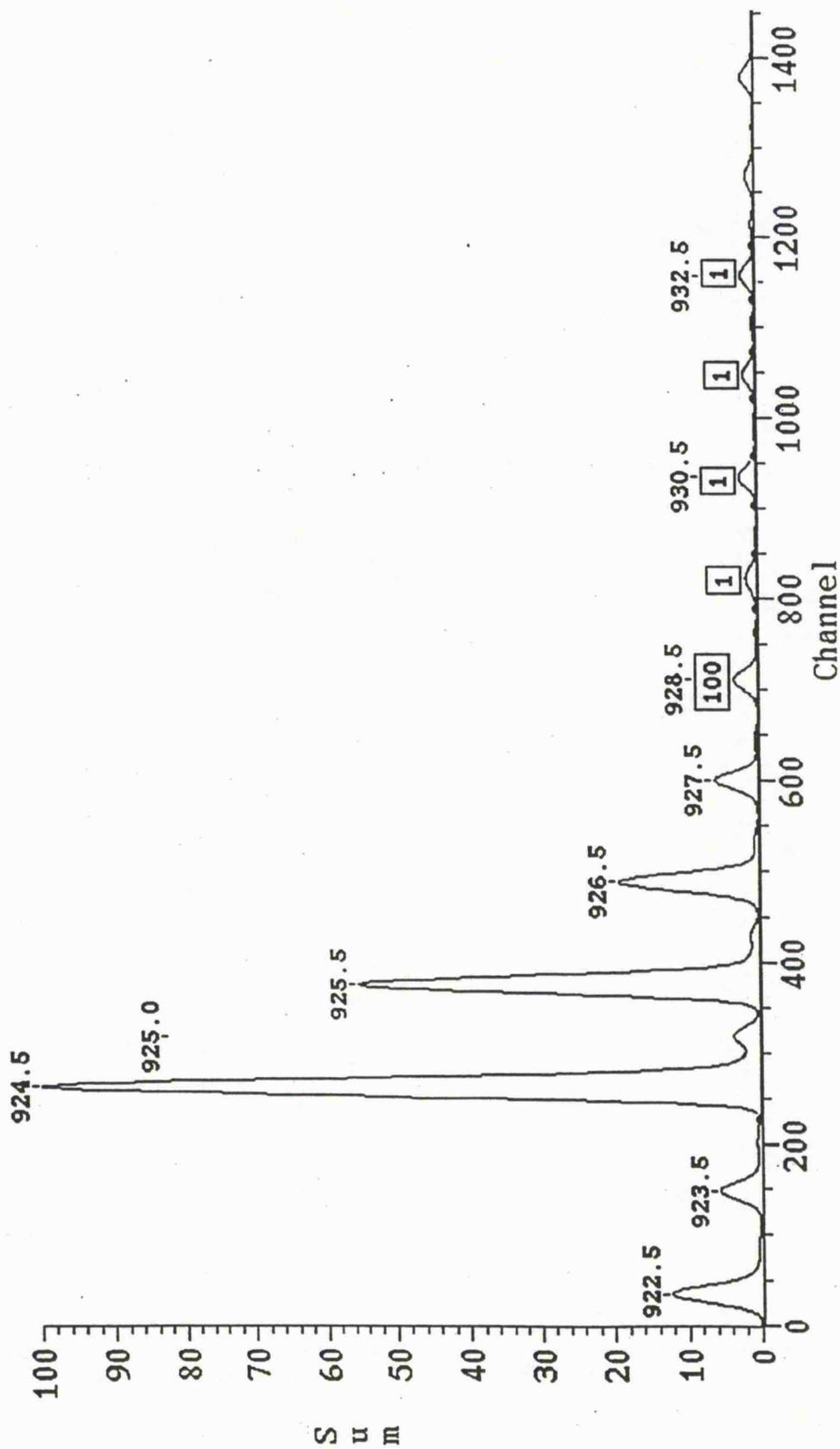
abundance of carbon-13 in these positions. Comparison of the carbon-13 spectra of the 'unlabelled' amphotericin B (Fig 59) with that obtained from the feeding experiments (Fig 60) shows a striking enhancement evident in both the methyl carbon signals, at 16.8 ppm (Me C-40) and 12.0 ppm (Me C-39), and in the carboxylate carbon at 176.6 ppm. This clearly illustrates incorporation of the carbon-13 label, and consequently the propionate units, into these positions. Further confirmation of label incorporation can be seen from the disparity in the electrospray mass spectra of the 'unlabelled' amphotericin B (Fig 61) and that obtained from the feeding experiment (Fig 62). The intensity of the [M+1] peak (925) in the amphotericin B from the feeding experiment presents a marked increase relative to that in the 'unlabelled' sample, and this is indicative of containing one carbon-13 label. The intensity of the [M+2] peak (926), and to a lesser extent the [M+3] peak (927), also register an enhancement, indicating the incorporation of two and three carbon-13 label atoms respectively. These levels of enhancement in the carbon-13 NMR and electrospray mass spectra indicate an incorporation of one [3-<sup>13</sup>C]-propionate (122) unit of approximately six to seven percent. An incorporation of two, and three labels was also evident, and these levels are around three and one percent respectively.



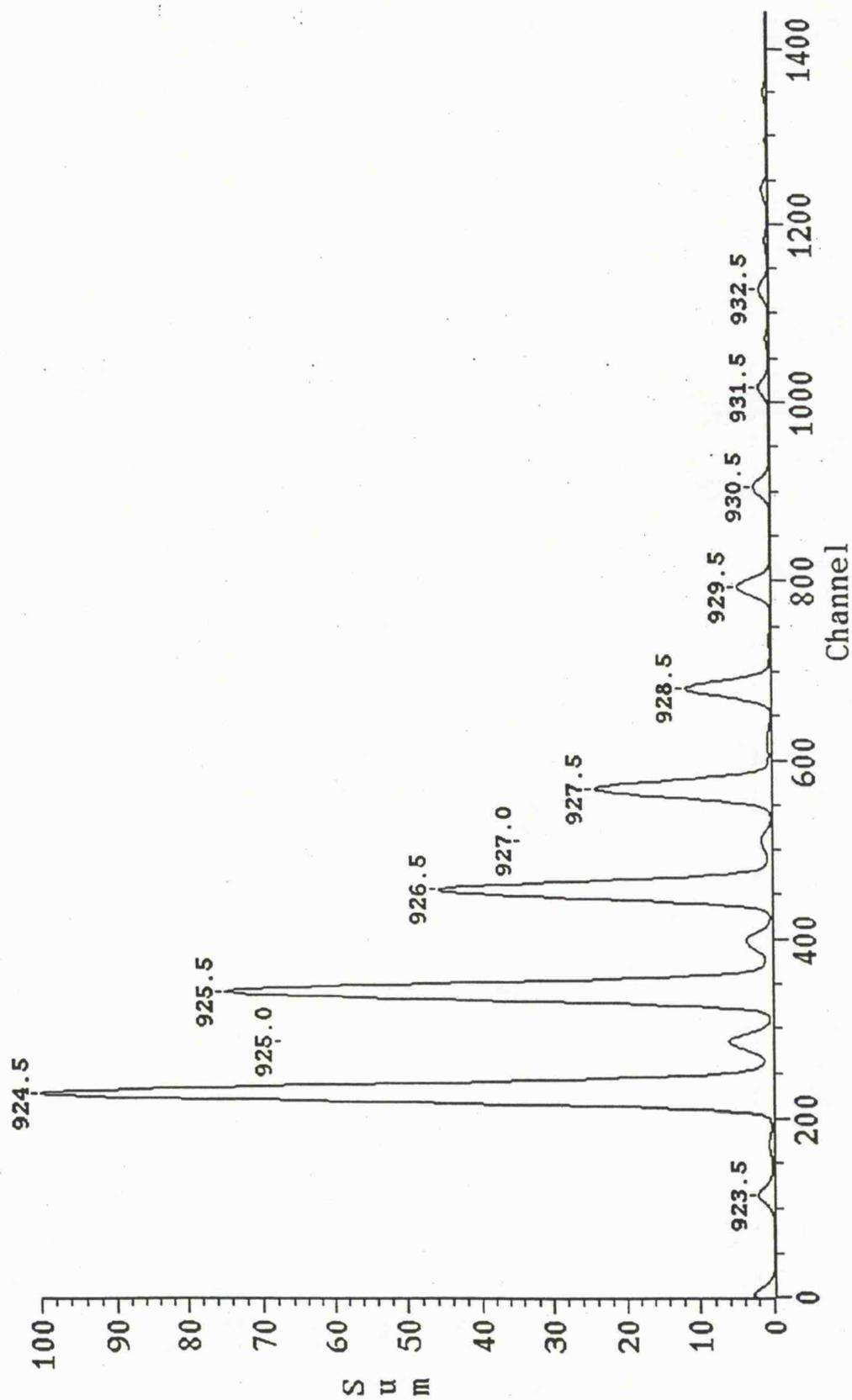
(Fig 59)  $^{13}\text{C}$  NMR spectrum (75.4 MHz,  $\text{DMSO-}d_6$ ) of 'unlabelled' amphotericin B.



(Fig 60)  $^{13}\text{C}$  NMR spectrum (75.4 MHz,  $\text{DMSO-}d_6$ ) of amphotericin B obtained from feeding  $[3-^{13}\text{C}]$ -propionate (122).



(Fig 61) Electrospray mass spectrum  
of 'unlabelled' amphotericin B.



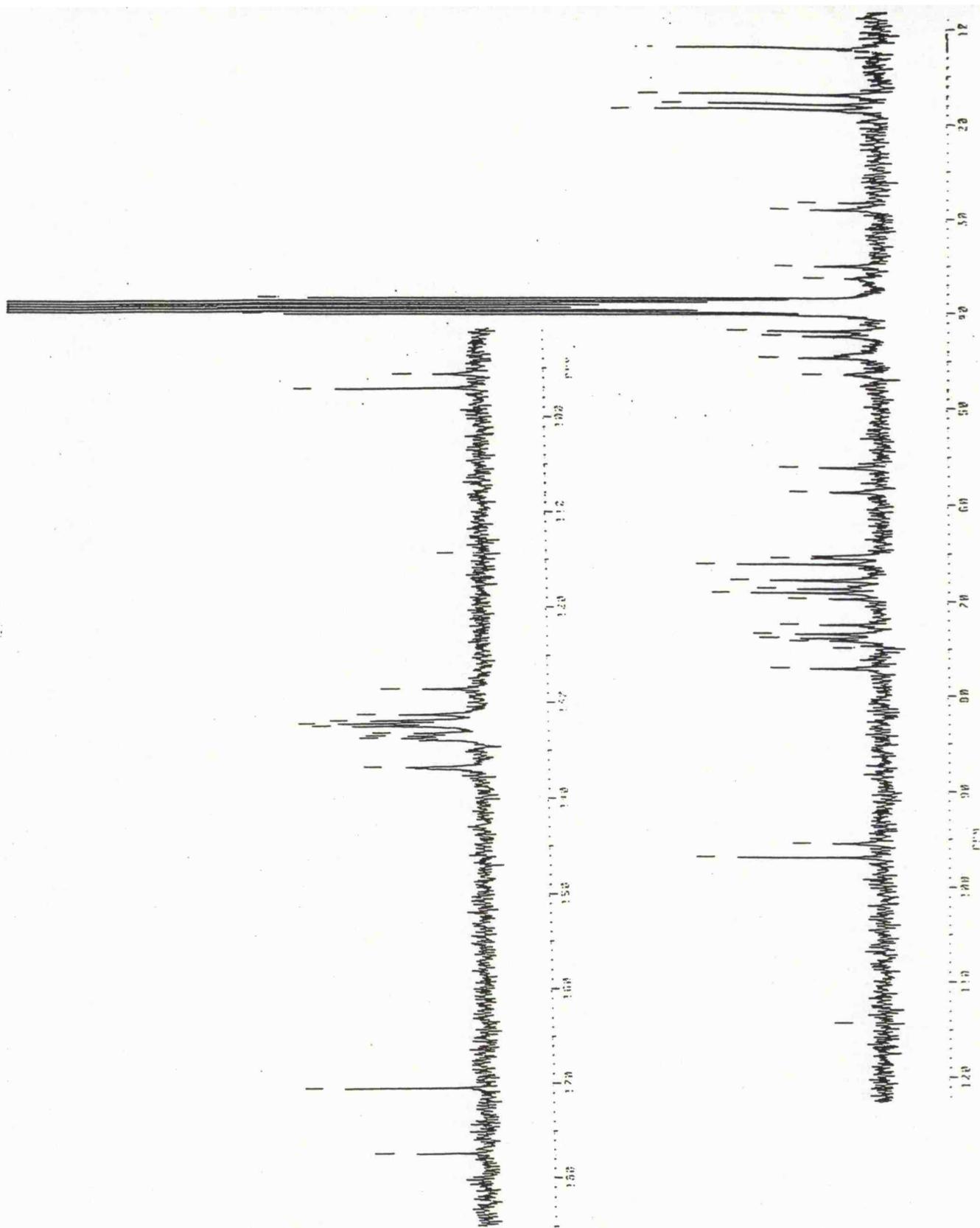
(Fig 62) Electrospray mass spectrum of amphotericin B  
 obtained from feeding [3-<sup>13</sup>C]-propionate (122).

The feeding experiments were repeated, pulse feeding commercially available sodium [1-<sup>13</sup>C]-propionate to the growing bacteria as described earlier for the [3-<sup>13</sup>C]-propionate (122) studies. The isolated amphotericin B was analysed for label incorporation by carbon-13 NMR, and electrospray mass spectroscopy, as before. For [1-<sup>13</sup>C]-propionate the positions in amphotericin B expected to show incorporation of the carbon-13 label were the last methyne carbon (C-33) in the conjugated double bond backbone, and the hydroxyl bearing methylene carbons (C-35) and (C-15) (Fig 58). The signals in the carbon-13 NMR spectrum for two of these positions, the two hydroxy methylene carbons, (C-15 65.4 ppm, C-35 77.0 ppm), have been assigned.<sup>75</sup> The signals in the methyne region of the carbon-13 NMR spectrum, (C-20 to C-33, 128-140 ppm), have however not been assigned, and an enhancement in this region would identify the carbon resonance of (C-33). Comparison of the carbon-13 NMR spectrum of the 'unlabelled' amphotericin B (Fig 63) with that obtained from the fermentation broth pulse fed with [1-<sup>13</sup>C]-propionate (Fig 64) again indicates high levels of label incorporation into the metabolite. A marked enhancement in the intensity of the peaks corresponding to the hydroxy methylene carbons, (C-15 65.5 ppm, C-35 77.1 ppm), was evident indicative of label incorporation into these positions. An enhancement was also evident at 135.0 ppm, within the methyne region of the carbon-13 NMR spectrum. This signal therefore concurred with label incorporation into C-33 and enabled unequivocal assignment of that carbon atom within the conjugated double bond back bone of amphotericin B. The levels of incorporation of [1-<sup>13</sup>C]-propionate (10-13%) were notably higher than those obtained in the [3-<sup>13</sup>C]-propionate feeding experiments, and were such that the signals from the naturally abundant carbon-13 atoms were no longer visible above the spectral noise. This high level of incorporation was also confirmed from the electrospray mass spectra. Comparison of the mass spectra obtained from the 'unlabelled' amphotericin B (Fig 65), with that isolated from the feeding experiments

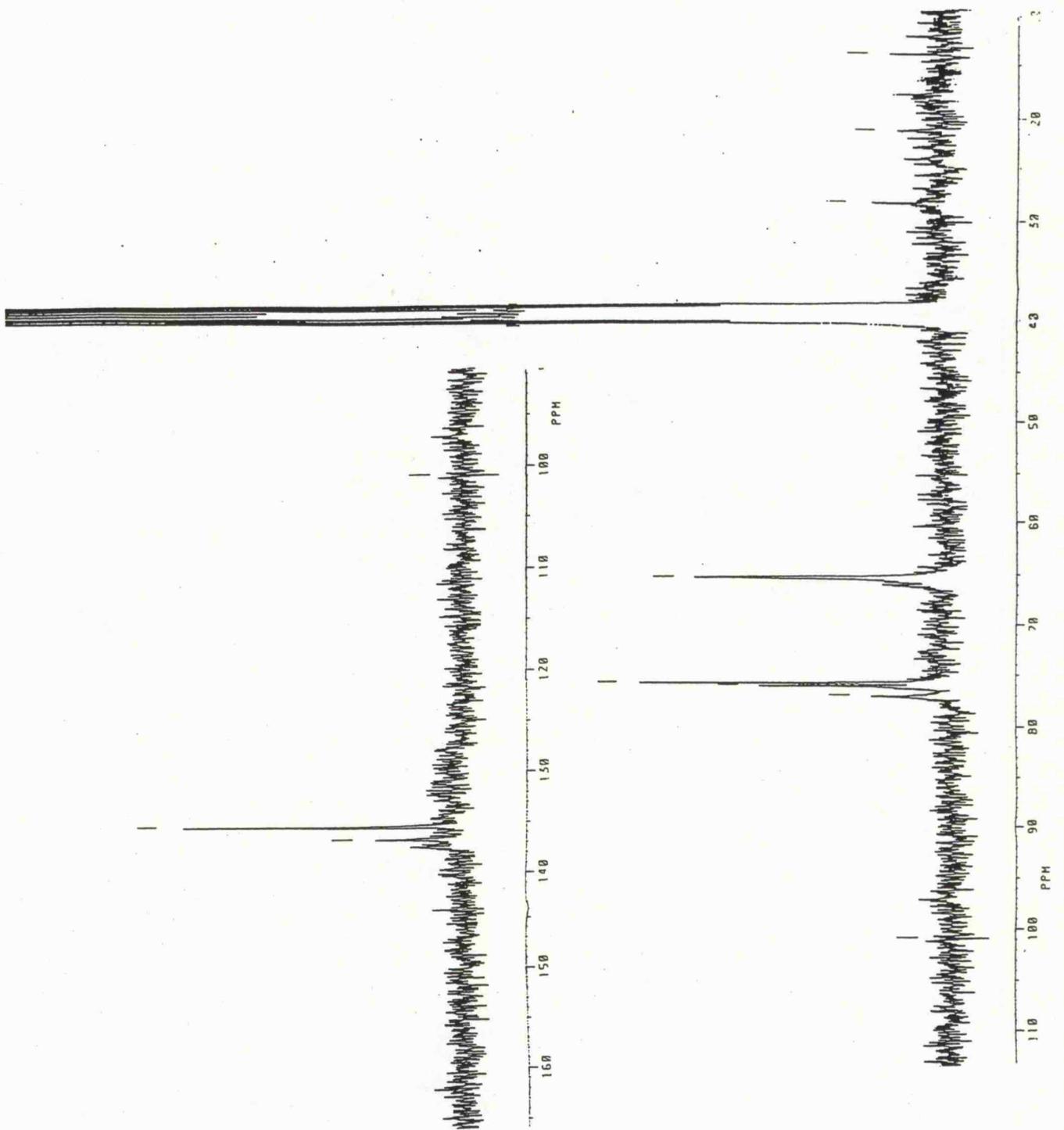
(Fig 66) clearly indicates enhancement of the [M+1], [M+2] and [M+3] peaks, and an incorporation of one [1-<sup>13</sup>C]-propionate unit of approximately ten to thirteen percent. From the electrospray mass spectrum it was also apparent that there was an incorporation of two and three labelled propionate units of four, and two percent respectively.

#### 4.2.3 Conclusion

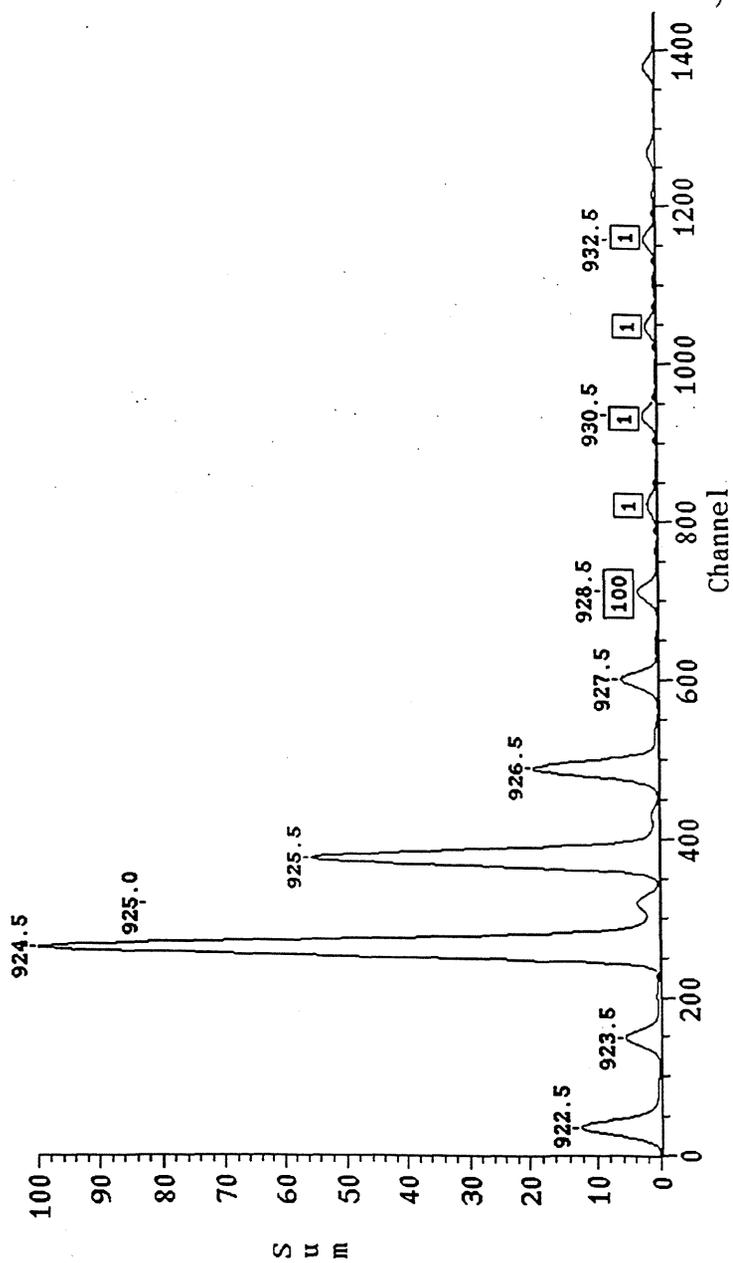
The feeding experiments using sodium [3-<sup>13</sup>C], and [1-<sup>13</sup>C]-propionate have clearly indicated the intermediacy of these units in the biosynthesis of amphotericin B, and have gone some way to proving our proposed biosynthetic scheme. The high levels of incorporation (seven and ten percent for [3-<sup>13</sup>C], and [1-<sup>13</sup>C]-propionate respectively), clearly indicates the potential of the developed stable isotope feeding protocol for further biosynthetic studies on amphotericin B. This 'pulse feeding' protocol has also established that no random incorporation of the stable isotope occurred due to degradation, and re-incorporation of the label. Also from the electrospray mass spectra a significant amount, (1-4%) of incorporation of two, and even three, labelled units was observed.



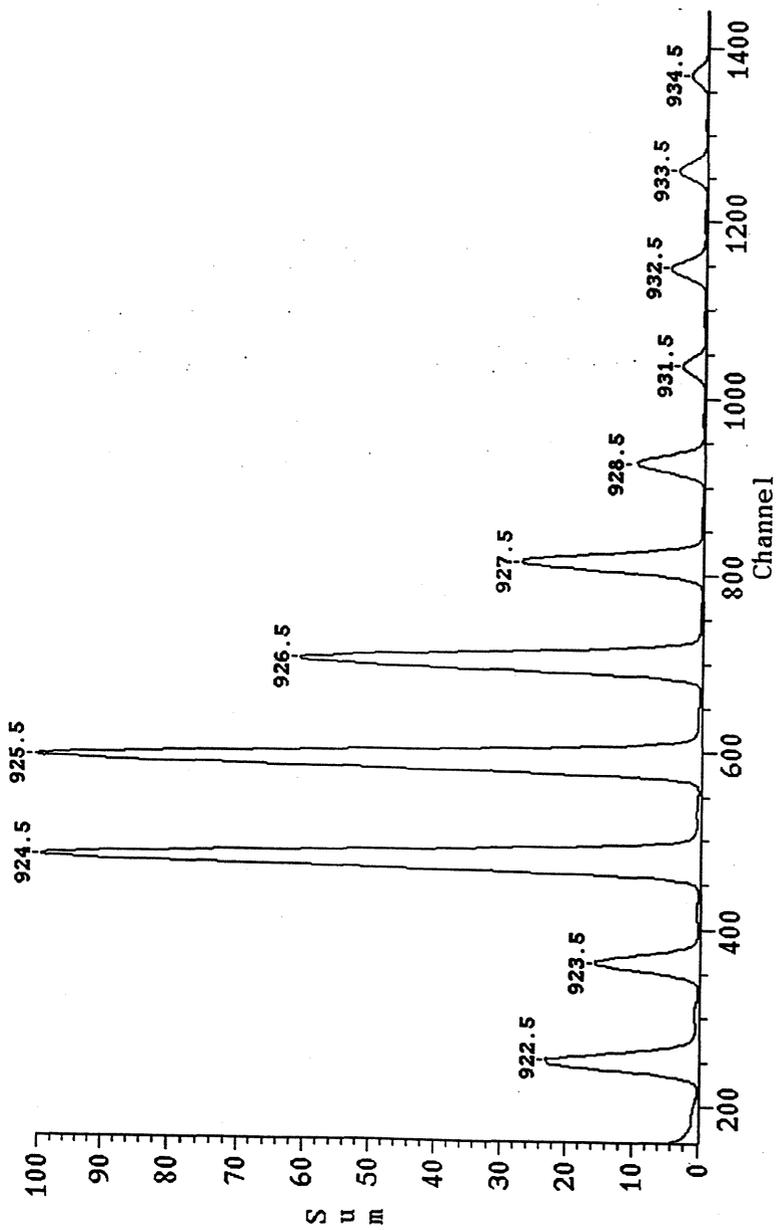
(Fig 63)  $^{13}\text{C}$  NMR spectrum (75.4 MHz, DMSO- $d_6$ ) of 'unlabelled' amphotericin B.



(Fig 64)  $^{13}\text{C}$  NMR spectrum (75.4 MHz,  $\text{DMSO-}d_6$ )  
of amphotericin B obtained from feeding  $[1-^{13}\text{C}]$ -propionate.



(Fig 65) Electrospray mass spectrum  
of 'unlabelled' amphotericin B.



(Fig 66) Electrospray mass spectrum of amphotericin B obtained from feeding [1-<sup>13</sup>C]-propionate.

## CHAPTER 5

### Experimental Details

## 5.0 EXPERIMENTAL DETAILS

### 5.1 General conditions

#### 5.1.1 Materials

Unless otherwise stated all chemicals were purchased from Aldrich Chemical Company Ltd, Lancaster Synthesis or Sigma Chemical Company Ltd. All components for the *streptomyces* broth media were purchased from the Sigma Chemical Company Ltd and were the cell tested variety unless otherwise stated. *Streptomyces nodosus* American type culture collection 14899 was obtained from SmithKline Beecham as a spore suspension.

#### 5.1.2 Solvents

Organic solvents where stated were dried as follows: Dichloromethane was distilled from calcium hydride. Tetrahydrofuran was distilled from sodium and benzophenone. Diisopropylamine and triethylamine were heated at reflux over potassium hydroxide, distilled and stored over molecular sieves. Diethyl ether was dried over sodium wire and used without distillation. Petroleum ether (petrol) unless otherwise stated was the 40-60 °C fraction. Dry *N,N*-dimethylformamide was purchased from Aldrich.

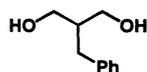
#### 5.1.3 Methods and instrumentation

Melting points were determined on a Koffler Hot Stage apparatus and are uncorrected. Infrared spectra were recorded on a perkin Elmer 298

spectrophotometer as solutions in the specified solvent; neat as films between salt disks or as KBr disks. The following abbreviations were used: s-strong, m-medium, w-weak and br-broad. Ultraviolet-visible spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer in the solvent specified using a 1 cm pathlength. For  $^1\text{H}$   $^{13}\text{C}$  and  $^{19}\text{F}$  NMR spectra the chemical shifts are quoted in ppm relative to tetramethylsilane and are reported as positive when downfield from the standard and negative when upfield. Coupling constants are quoted in Hertz. Proton NMR spectra were recorded using Varian EM 390 (90 MHz), Bruker ARX 250 (250 MHz) and a Bruker AM 300 (300 MHz) spectrometers in the specified solvents with trimethylsilane as internal standard. The following abbreviations were used: s-singlet, d-doublet, t-triplet, q-quartet, qu-quintet, sept-septet, m-multiplet and br-broad. Carbon-13 NMR spectra were recorded on Bruker ARX 250 (62.9 MHz) and Bruker AM 300 (75.4 MHz) spectrophotometers and were proton decoupled, multiplicities being determined by performing DEPT experiments at  $90^\circ$  and  $135^\circ$ . Fluorine-19 NMR spectra were recorded on a Bruker ARX 250 (235.3 MHz) and were proton decoupled. Optical rotation measurements were carried out at room temperature (*ca*  $22^\circ\text{C}$ ) using a Perkin Elmer 141 polarimeter in the solvent specified and the values are given in  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Mass spectra were recorded on a Kratos Concept H double focussing mass spectrometer using either electron impact (EI) at 70 eV and  $170^\circ\text{C}$ ; fast atom bombardment (FAB) at 6 kV using nitrobenzyl alcohol (NBA) as the matrix and xenon gas; or chemical ionisation (CI). The pH of aqueous solutions was measured with a single glass electrode. Flash chromatography was carried out as for Still's method using Merck Kieselgel 60, 230-400 mesh.

## 5.2 Chemical experimental details

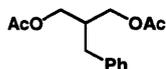
### 2-Benzyl-propan-1,3-diol (27)<sup>25</sup>



To a stirred ice-cooled suspension of lithium aluminium hydride (10.0 g, 260.0 mmol) in sodium dried ether (400 cm<sup>3</sup>) under nitrogen was added dropwise diethylbenzyl malonate (32.5 g, 130.0 mmol) in dry ether (100 cm<sup>3</sup>), and the reaction mixture stirred for 2 hours before quenching by careful addition of water (25 cm<sup>3</sup>), sodium hydroxide (10 cm<sup>3</sup>, 1 M), tetrahydrofuran (100 cm<sup>3</sup>), and the reaction mixture stirred for 1 hour before filtering through Celite. The filtrate was dried (magnesium sulfate), concentrated *in vacuo* and the residue re-crystallised, [petrol/diethylether, (1:2)], affording *the title compound* as a white solid (20.9 g, 96 %).

m.p. 66-67 °C (lit<sup>25</sup> 66-65 °C); (Found: MH<sup>+</sup> 167.10721, C<sub>10</sub>H<sub>13</sub>O<sub>2</sub> requires *M* 167.10815);  $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$  3200br m, 2950s, 1460m, 1375m, 1040m, 1020m, 750m, 700m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.25 (5 H, m, Ar-*H*), 3.70 (2 H, dd, *J* 4.0, 10.5, -CH<sub>2</sub>OH), 3.60 (2 H, dd, *J* 7.0, 10.5, -CH<sub>2</sub>OH), 2.70 (2 H, s, -OH), 2.60 (2 H, d, *J* 7.5, -CH<sub>2</sub>Ph), 2.05 (1 H, m, -CHCH<sub>2</sub>Ph);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 140.3 (-C=, Ph), 129.4, 128.7, 126.6 (3 x =C-H, Ph), 65.5 (2 x -CH<sub>2</sub>OH), 44.2 (-CHCH<sub>2</sub>OH), 34.7 (-CH<sub>2</sub>Ph); *m/z* (EI) 148 (M<sup>+</sup>, 4.3 %) 130 (21.0) 117 (78.0) 104 (20.8) 91 (100.0) 77 (10.6) 65 (14.8).

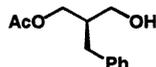
1,3-Acetoxy-2-benzylpropane (**28**)<sup>25</sup>



A solution of 2-benzylpropan-1,3-diol (**27**) (20.8 g, 125.0 mmol), triethylamine (53.0 cm<sup>3</sup>, 380.0 mmol), acetic anhydride (35.0 cm<sup>3</sup>, 371.0 mmol) and *N,N*-dimethylaminopyridine (1.0g, 8.2 mmol) in dichloromethane (150 cm<sup>3</sup>) was stirred at room temperature for 36 hours. Hydrochloric acid (200 cm<sup>3</sup>; 2 M) was added, and after stirring for 15 minutes the organic layer was separated, washed with water (100 cm<sup>3</sup>), a saturated solution of sodium hydrogen carbonate (100 cm<sup>3</sup>), and a further portion of water (100 cm<sup>3</sup>). The aqueous phase was dried (magnesium sulfate), concentrating *in vacuo*, and the residue distilled yielding *the title compound* (**28**) as a colourless oil (27.8 g, 89 %).

b.p. 126-127 °C (0.8 mbar) (Found:  $M^+$  250.12162, C<sub>14</sub>H<sub>18</sub>O<sub>4</sub> requires  $M$  250.12051);  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 2950m, 1740s, 1240m, 1040m, 745m, 700m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.20 (5 H, m, Ar-*H*), 4.08 (2 H, dd,  $J$  5.2, 11.1, -*CHHOAc*), 4.00 (2 H, dd,  $J$  6.2, 11.1, -*CHHOAc*), 2.70 (2 H, d,  $J$  7.0, -*CH*<sub>2</sub>Ph), 2.30 (1 H, m, -*CHCH*<sub>2</sub>Ph), 2.10 (6 H, s, -*COCH*<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.3 (2 x -*CH*<sub>2</sub>OCOCH<sub>3</sub>), 139.1 (-*C*=, Ph), 129.4, 128.9, 126.8 (4 x =*C*-H, Ph), 64.1 (2 x -*CH*<sub>2</sub>OAc), 39.5 (-*CHCH*<sub>2</sub>OAc), 35.0 (-*CH*<sub>2</sub>Ph), 21.1 (-*COCH*<sub>3</sub>);  $m/z$  (EI) 250 ( $M^+$ , 0.3 %) 190 (28.3) 149 (12.8) 130 (100.0) 117 (32.8) 104 (14.3) 91 (43.1) 65 (7.2).

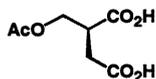
(2R)-3-Acetoxy-2-benzylpropan-1-ol<sup>25</sup>



To a solution of 1,3-acetoxy-2-benzylpropane (**28**) (10.0 g, 40.0 mmol) in acetone (150 cm<sup>3</sup>) and water (350 cm<sup>3</sup>) was added Triton X-100 (0.5 cm<sup>3</sup>) and the mixture homogenised in a sonic bath for 5 minutes. Porcine pancreatic lipase (Sigma Type II; 35.0 g) was added and the ice-cooled mixture vigorously stirred for 90 minutes with the pH being maintained at 7.0 by addition of sodium hydroxide (1 M). The reaction was stopped by the addition of ethyl acetate (400 cm<sup>3</sup>), filtered through Celite, the filtrate saturated with sodium chloride, and the organic layer separated. The aqueous phase was re-extracted with ethyl acetate (2 x 200 cm<sup>3</sup>), and the combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (1:1)] yielded *the title compound* (**29**) as a pale yellow oil (4.75 g, 57 %).

$[\alpha]_D = +19.7$  (*c* 1.1, CHCl<sub>3</sub> 70.4 % ee<sup>25</sup>), (lit<sup>25</sup> +17.5 *c* CHCl<sub>3</sub> 62.4 % ee);  $\nu_{\max}(\text{neat}) / \text{cm}^{-1}$  3440br m, 2900m, 1750s, 1240s, 1035m, 740, 700m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.25 (5 H, m, Ar-H), 4.07 (1 H, dd, *J* 4.9, 11.2, -CHHOAc), 4.17 (1 H, dd, *J* 6.3, 11.2, -CHHOAc), 3.60 (1 H, dd, *J* 4.7, 11.2, -CHHOH), 3.50 (1 H, dd, *J* 6.2, 11.2, -CHHOH), 2.70 (1 H, dd, *J* 7.6, 13.7, -CHHPh), 2.60 (1 H, dd, *J* 7.5, 13.7, -CHHPh), 2.47 (1 H, s, -OH), 2.12 (1 H, m, -CHCH<sub>2</sub>Ph), 2.06 (3 H, s, -COCH<sub>3</sub>);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 172.2 (CH<sub>3</sub>CO-), 139.8 (-C=, Ar), 129.5, 128.9, 126.9 (5 x =C-H, Ar), 65.8 and 62.4 (2 x -CH<sub>2</sub>O-), 42.8 (CH<sub>3</sub>CO-), 34.7 (-CH<sub>2</sub>Ph), 21.3 (-CHCH<sub>2</sub>OH).

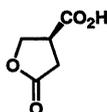
(2S)-(Acetoxymethyl) succinic acid (**30**)<sup>25</sup>



To a solution of (2R)-3-acetoxy-2-benzylpropan-1-ol (**29**) (5.6 g, 27.0 mmol), periodic acid (135 g, 394.0 mmol), water (165 cm<sup>3</sup>), acetonitrile (110 cm<sup>3</sup>), and carbon tetrachloride (110 cm<sup>3</sup>) was added ruthenium (III) chloride (0.21 g, 0.80 mmol), and the reaction mixture vigorously stirred at room temperature for 12 hours. The reaction was quenched by the careful addition of diethyl ether (100 cm<sup>3</sup>), filtered through Celite, and the organic layer separated. The aqueous phase was re-extracted with further portions of diethyl ether (2 x 80 cm<sup>3</sup>), the combined organic phases dried (sodium sulfate) and concentrated *in vacuo*. Flash chromatography on silica gel of the residue (ethyl acetate) gave the title compound (**30**) as a colourless oil (3.05 g, 59 %).

$[\alpha]_D = +11.6$  (*c* 1.0, MeOH) (lit +14.5 *c* 0.76 MeOH)<sup>25</sup>;  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3350br m, 2950m, 1790s, 1710s;  $\delta_{\text{H}}$  (300 MHz; Acetone-d<sub>6</sub>) 9.60 (2 H, br s, -CO<sub>2</sub>H), 4.36 (2 H, m, -CH<sub>2</sub>OAc), 3.50 (1 H, m, -CHCO<sub>2</sub>H), 2.70 (2 H, m, -CH<sub>2</sub>CO<sub>2</sub>H), 1.98 (3 H, s, CH<sub>3</sub>CO-);  $\delta_{\text{C}}$  (62.9 MHz; Acetone-d<sub>6</sub>) 173.42, 173.42 (-CO<sub>2</sub>H), 170.71 (CH<sub>3</sub>CO-), 64.38 (-CH<sub>2</sub>OAc), 41.23 (-CHCH<sub>2</sub>CO<sub>2</sub>H), 32.87 (-CH<sub>2</sub>CO<sub>2</sub>H), 20.49 (CH<sub>3</sub>CO-).

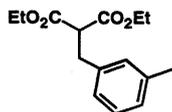
(S)-(-)-Paraconic acid (**21**)<sup>25</sup>



2-(Acetoxymethyl)succinic acid (**30**) (1.2 g, 6.3 mmol) was stirred in hydrochloric acid (40 cm<sup>3</sup>, 1 M) for 96 hours at room temperature. The solution was saturated with solid sodium chloride and extracted with ethyl acetate (2 x 75 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) and concentrated *in vacuo* to give the title compound as a pale yellow oil (0.34 g, 39 %).

[ $\alpha$ ]<sub>D</sub> = -32.87 (c 0.8 MeOH); e.e 59 % (lit<sup>25</sup> -59.6 c 0.61 MeOH);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000br m, 1785s, 1720s;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 9.55 (1 H, s, -CO<sub>2</sub>H), 4.55 (2 H, m, -CH<sub>2</sub>OCOCH<sub>2</sub>-), 3.75 (1 H, m, -CHCO<sub>2</sub>H), 2.85 (2 H, m, -CH(CO<sub>2</sub>H)CH<sub>2</sub>CO-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 176.7 and 174.0 (-CO<sub>2</sub>H, -OCOCH<sub>2</sub>-), 70.4 (-CH<sub>2</sub>OCO-), 40.8 (-CHCO<sub>2</sub>H), 31.7 (-CHCH<sub>2</sub>COO-).

Diethyl 3-methylbenzylmalonate (**31**)<sup>107</sup>

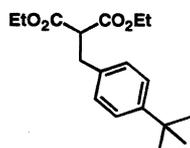


To a stirred suspension of sodium hydride (3.7 g, 91.6 mmol; 60 % dispersion in mineral oil) under nitrogen in dry *N,N*-dimethylformamide (30 cm<sup>3</sup>) at -40 °C was added diethyl malonate (11.05 g, 69.0 mmol), followed after a 5 minutes delay by the slow addition of 3-methylbenzyl chloride (10.6 g, 75.7 mmol). The reaction mixture was stirred for 1 hour and the internal temperature allowed to warm to room temperature before quenching by the addition of water (20 cm<sup>3</sup>), and extracting with

ethyl acetate (3 x 75 cm<sup>3</sup>). The combined organic phases were washed with water (3 x 60 cm<sup>3</sup>), dried (magnesium sulfate), and concentrated under reduced pressure to give *the title compound* as a colourless liquid (16.0 g, 80 %).

$\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.10 (4 H, m, Ar-H), 4.05 (4 H, q, *J* 4.5, -CH<sub>2</sub>CH<sub>3</sub>), 3.05 (2 H, s, -CH<sub>2</sub>Ar), 2.20 (3 H, s, CH<sub>3</sub>Ar), 3.20 (1 H, s, -CHCH<sub>2</sub>Ar), 1.15 (6 H, t, *J* 4.5, CH<sub>3</sub>CH<sub>2</sub>-).

*Diethyl (4'-tert-butylbenzyl)malonate (32)*

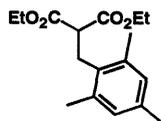


To a stirred suspension of sodium hydride (2.6 g, 65.6 mmol; 60 % dispersion in mineral oil) in dry *N,N*-dimethylformamide (40 cm<sup>3</sup>) at -40 °C under nitrogen was added diethyl malonate (5.0 cm<sup>3</sup>, 32.8 mmol), slowly followed after 5 minutes delay by the slow addition of 4-*tert*-butylbenzyl chloride (5.0 g, 27.4 mmol). The reaction mixture was stirred for 90 minutes while warming slowly to room temperature, before being quenched by the addition of water (40 cm<sup>3</sup>) and extracted with ethyl acetate (3 x 60 cm<sup>3</sup>). The combined organic phases were washed with water (3 x 50 cm<sup>3</sup>), dried (magnesium sulfate), and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (4:1)] gave *the title compound* as a colourless oil (5.1 g, 61 %).

(Found:  $M^+$  306.18311, C<sub>18</sub>H<sub>26</sub>O<sub>4</sub> requires *M* 306.18474);  $\nu_{\text{max}}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2950m, 1730s, 1370s, 1200m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.15 (4 H, m, Ar-H), 4.05 (4 H, q, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>), 3.10 (1 H, m, -CHCO<sub>2</sub>Et), 2.85 (2 H, d, *J* 6.0, -CH<sub>2</sub>Ar), 1.25 (9 H, s, -C(CH<sub>3</sub>)<sub>3</sub>), 1.05 (6 H, t, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.1 (-CO<sub>2</sub>Et), 149.6, 133.3 (2 x =C-, Ar), 129.8, 125.4 (4 x =C-H, Ar), 61.1 (2 x -

CH<sub>2</sub>CH<sub>3</sub>), 41.3 (-C(CH<sub>3</sub>)<sub>3</sub>), 38.5 (-CH<sub>2</sub>Ar), 31.9 (3 x -C(Me)<sub>3</sub>), 14.1 (-CH<sub>2</sub>CH<sub>3</sub>);  
*m/z* (EI) 306 (M<sup>+</sup>, 31.2 %) 291 (27.4) 259 (97.6) 232 (47.7) 217 (71.7) 147 (69.5) 97  
(35.2) 69 (44.9) 57 (100.0).

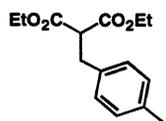
*Diethyl (2,4,6-trimethylbenzyl) malonate (33)*



To a stirred suspension of sodium hydride (2.2 g, 53.4 mmol; 60 % dispersion in mineral oil) in dry *N,N*-dimethylformamide (30 cm<sup>3</sup>) at -40 °C under a nitrogen atmosphere was added diethylmalonate (5.5 cm<sup>3</sup>, 35.6 mmol), slowly followed after 5 minutes by 2,4,6-trimethylbenzyl chloride (5.0 g, 29.6 mmol). The reaction mixture was stirred for 90 minutes while warming to room temperature, before being quenched by the addition of water (40 cm<sup>3</sup>) and extracted with ethyl acetate (3 x 60 cm<sup>3</sup>). The combined organic phases were washed with water (3 x 50 cm<sup>3</sup>), dried (magnesium sulfate), and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (5:1)] yielded *the title compound* as a pale yellow oil (4.8 g, 59 %).

(Found: M<sup>+</sup> 292.16749, C<sub>17</sub>H<sub>24</sub>O<sub>4</sub> requires *M* 292.16896);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  2900m, 1730s, 1430w, 1370w;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 6.71 (2 H, s, Ar-*H*), 4.15 (4 H, q, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>), 3.40 (1 H, t, *J* 6.0, -CHCH<sub>2</sub>Ar), 2.87 (2 H, d, *J* 6.0, -CH<sub>2</sub>Ar), 2.20, 2.15 (6 H, s, *o*-CH<sub>3</sub>Ar), 2.00 (3 H, s, *p*-CH<sub>3</sub>Ar), 1.25 (6 H, t, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.4 (2 x -CO<sub>2</sub>Et), 135.9, 136.7, 134.7 (4 x Me-C=, Ar), 130.8, 129.4 (2 x =C-H, Ar), 61.4 (2 x -CH<sub>2</sub>CH<sub>3</sub>), 51.4 (-CH(CO<sub>2</sub>Et)<sub>2</sub>), 29.7 (-CH<sub>2</sub>Ar), 20.8, 20.0 (3 x CH<sub>3</sub>-Ar), 14.1 (-CH<sub>2</sub>CH<sub>3</sub>); *m/z* (EI) 292 (M<sup>+</sup>, 6.6 %) 246 (3.5) 173 (14.6) 133 (100.0) 97 (8.4) 55 (15.3).

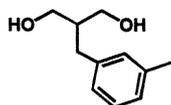
Diethyl 4-methylbenzyl malonate (**34**)<sup>108,109</sup>



To a stirred suspension of sodium hydride, (3.7 g, 91.6 mmol) in dry *N,N*-dimethylformamide (20 cm<sup>3</sup>) at -20 °C was added dropwise diethyl malonate (8.2 cm<sup>3</sup>, 54.0 mmol) followed after a 10 minute delay by 4-methylbenzyl bromide (10.0 g, 54.0 mmol). Stirring was continued for 2 hours the temperature being allowed to warm to ambient before the mixture poured into ice/water (50 cm<sup>3</sup>), and extracted with ethyl acetate (3 x 50 cm<sup>3</sup>). The combined organics were washed with water (3 x 50 cm<sup>3</sup>), dried (sodium sulfate), and concentrated *in vacuo* to give *the title compound* as a colourless oil (8.4 g, 59 %).

$\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  2950s, 1730s, 1510m, 1360m, 1200m, 1110w, 810w;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 6.95 (4 H, s, Ar-H), 4.10 (4 H, q, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>), 3.25 (1 H, s, -CH(CO<sub>2</sub>Et)<sub>2</sub>), 3.10 (2 H, s, -CH<sub>2</sub>Ar), 2.25 (3 H, s, CH<sub>3</sub>-Ar), 1.15 (6 H, t, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.1 (2 x -CO<sub>2</sub>Et), 136.4, 133.2 (2 x =C-, Ar), 130.0, 128.9 (4 x =C-H, Ar), 61.2 (-OCH<sub>2</sub>CH<sub>3</sub>), 60.2 (-CH(CO<sub>2</sub>Et)<sub>2</sub>), 38.3 (-CH<sub>2</sub>Ar), 21.0 (CH<sub>3</sub>-Ar), 13.9 (-CH<sub>2</sub>CH<sub>3</sub>).

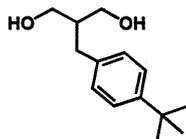
2-(3'-Methylbenzyl)propane-1,3-diol (35)



To an ice-cooled suspension of lithium aluminium hydride (5.0 g, 132.0 mmol) in sodium dried diethyl ether (150 cm<sup>3</sup>) was added dropwise diethyl 3-methylbenzyl malonate (31) (15.96 g, 60.4 mmol) in dry ether (75 cm<sup>3</sup>), and the reaction mixture stirred at 0 °C for 2 hours before being quenched by the careful addition of water (5 cm<sup>3</sup>), sodium hydroxide (10 cm<sup>3</sup>, 1 M), stirred for a further 30 minutes, then filtered through Celite. The filtrate was concentrated *in vacuo* to give the title diol as a colourless oil (8.8 g, 81 %).

$\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3400br m, 3000m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.10 (4 H, m, Ar-H), 3.00 (4 H, m, -CH<sub>2</sub>OH), 2.60 (2 H, m, -CH<sub>2</sub>Ar), 2.55 (1 H, m, -CHCH<sub>2</sub>Ar), 2.30 (3 H, s, CH<sub>3</sub>CO-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 138.4, 138.1 (2 x =C-, Ar), 131.7, 128.6, 128.5, 127.5 (4 x =C-H, Ar), 67.6, 65.9 (2 x -CH<sub>2</sub>OH), 43.8 (-CHCH<sub>2</sub>Ar), 39.8 (-CH<sub>2</sub>Ar), 21.9 (CH<sub>3</sub>-Ar).

2-(4-tert-Butylbenzyl)propan-1,3-diol (36)

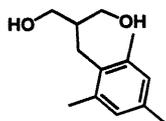


To a stirred ice-cooled suspension of lithium aluminium hydride (1.4 g, 36 mmol) in sodium dried ether (50 cm<sup>3</sup>) under nitrogen was added dropwise diethyl (4-tert-butylbenzyl) malonate (32) (5.0 g, 16.3 mmol) in dry ether (10 cm<sup>3</sup>), and the reaction

mixture stirred for 2 hours before quenching by careful addition of water (25 cm<sup>3</sup>), sodium hydroxide (10 cm<sup>3</sup>, 1 M) and tetrahydrofuran (100 cm<sup>3</sup>). Stirring was continued for 1 hour before filtering through Celite, and the filtrate dried (magnesium sulfate) and concentrated *in vacuo* to yield a sticky off-white residue which was recrystallised [petrol/diethyl ether (1:2)] affording *the title compound* as a white solid (3.46 g, 95 %).

m.p 161-163 °C;  $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$  3430br m, 1460m, 1040m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.15 (4 H, m, Ar-H), 3.45 (4 H, s, -CH<sub>2</sub>OH), 2.60 (2 H, s, -CH<sub>2</sub>Ar), 1.95 (1 H, s, -CHCH<sub>2</sub>Ar), 1.20 (9 H, s, -C(CH<sub>3</sub>)<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 149.5 (3 x =C-, Ar), 130.6, 125.8 (4 x =C-H, Ar), 67.5 (-CH<sub>2</sub>OH), 41.3 (-C(CH<sub>3</sub>)<sub>3</sub>), 39.3 (-CH<sub>2</sub>Ar), 31.9 (3 x -C(Me)<sub>3</sub>), 31.8 (-CHCH<sub>2</sub>Ar).

2-(2',4',6'-trimethylbenzyl)propan-1,3-diol (37)

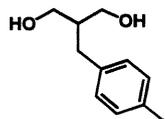


To a stirred ice-cooled suspension of lithium aluminium hydride (1.4 g, 36 mmol) in sodium-dried ether (50 cm<sup>3</sup>) under nitrogen was added dropwise diethyl (2,4,6-trimethylbenzyl)malonate (33) (4.80 g, 16.4 mmol) in dry ether (10 cm<sup>3</sup>), and the reaction mixture stirred for 2 hours before quenching by careful addition of water (25 cm<sup>3</sup>), sodium hydroxide (10 cm<sup>3</sup>, 1 M) and tetrahydrofuran (100 cm<sup>3</sup>). The reaction mixture was stirred for 1 hour before filtering through Celite, and the filtrate dried (magnesium sulfate), and concentrated *in vacuo* to yield *the title compound* as a colourless oil. (3.34 g, 98 %).

$\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3380br m, 1450m, 1020m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 6.75 (2 H, s, Ar-H), 3.95 (4 H, s, -CH<sub>2</sub>OH), 3.65 (2 H, m, -CH<sub>2</sub>Ar), 2.80 (1 H, m, -CHCH<sub>2</sub>Ar), 2.20

(6 H, s, *o*-CH<sub>3</sub>Ar), 2.00 (3 H, s, *p*-CH<sub>3</sub>Ar);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 138.2, 137.2, 135.2 (4 x =C-, Ar), 131.4, 130.0 (3 x =C-H, Ar), 66.0 (2 x -CH<sub>2</sub>OH), 43.2 (-CHCH<sub>2</sub>OH), 32.3 (-CH<sub>2</sub>Ar), 21.5, 17.6 (2 x CH<sub>3</sub>-Ar).

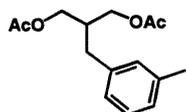
*2-(4-methyl benzyl)-propan-1,3-diol (38)*



To a stirred ice-cooled suspension of lithium aluminium hydride (2.5 g, 65.1 mmol) in sodium dried diethyl ether (75 cm<sup>3</sup>) was added dropwise a solution of (34) (7.8 g, 29.7 mmol) in ether (25 cm<sup>3</sup>) and the mixture stirred at room temperature for 2 hours before quenching by careful addition of water (10 cm<sup>3</sup>), tetrahydrofuran (80 cm<sup>3</sup>) and stirring for a further hour. The precipitates were removed by filtering through Celite, the filtrate dried (magnesium sulfate), and concentrated *in vacuo* to yield *the title diol* as a white solid. (4.7 g, 87 %)

$\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3400br m, 2980m, 1030m;  $\delta_H$  (90 MHz; CDCl<sub>3</sub>) 7.10 (4 H, s, Ar-H), 3.50 (4 H, s, -CH<sub>2</sub>OH), 2.65 (2 H, s, -CH<sub>2</sub>Ar), 2.30 (3 H, s, CH<sub>3</sub>-Ar), 2.10 (1 H, s, -CHCH<sub>2</sub>Ar);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 136.2, 134.9 (2 x =C-, Ar), 130.8, 129.3 (4 x =C-H, Ar), 67.4 (2 x -CH<sub>2</sub>OH), 43.9 (-CH<sub>2</sub>Ar), 39.4 (-CHCH<sub>2</sub>OH), 21.4 (CH<sub>3</sub>-Ar).

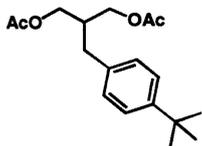
2-(Acetoxymethyl)-3-(3-methylphenyl)propyl acetate (39)



To a stirred solution of diol (35) (11.0 g 61.1 mmol) in dichloromethane (90 cm<sup>3</sup>) was added triethylamine (26.0 cm<sup>3</sup>, 183.4 mmol), acetic anhydride (17.0 cm<sup>3</sup>, 183.4 mmol), and 4-dimethylamino pyridine (catalytic amount), and the reaction mixture stirred for 36 hours at room temperature before being quenched with hydrochloric acid (50 cm<sup>3</sup>; 2 M). The organic phase was separated and washed with water (100 cm<sup>3</sup>), a saturated solution of sodium hydrogen carbonate (100 cm<sup>3</sup>), and a further portion of water (100 cm<sup>3</sup>), before being dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (20:1-15:1)] yielded *the title compound* as a colourless oil (11.3 g, 70 %).

$\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  2900m, 1740s, 1600m, 1370m, 1230m, 1040m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.20 (4 H, m, Ar-H), 4.00 (4 H, d, *J* 7.0, 2 x -CH<sub>2</sub>OAc), 2.65 (3 H, m, -CH<sub>2</sub>Ar, -CHCH<sub>2</sub>OAc), 2.30 (3 H, s, CH<sub>3</sub>Ar), 2.10 (6 H, s, CH<sub>3</sub>CO-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.1 (2 x CH<sub>3</sub>CO-), 138.2, 136.9 (2 x =C-, Ar), 131.7, 128.6, 127.8, 127.7 (4 x =C-H, Ar), 65.9 (2 x AcOCH<sub>2</sub>-), 41.7 (2 x CH<sub>3</sub>CO-), 39.8 (-CH<sub>2</sub>Ar), 21.9, 21.4 (CH<sub>3</sub>Ar, -CHCH<sub>2</sub>-).

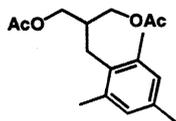
2-(Acetoxymethyl)-3-(4-tert-butyl phenyl) propyl acetate (**40**)



A solution of 2-(4-*tert*-butylbenzyl)propan-1,3-diol (**36**) (3.0 g, 13.5 mmol), triethylamine (5.6 cm<sup>3</sup>, 40.5 mmol), acetic anhydride (3.8 cm<sup>3</sup>, 40.5 mmol) and 4-dimethylaminopyridine (catalytic amount) in dichloromethane (50 cm<sup>3</sup>) was stirred at room temperature for 36 hours. Hydrochloric acid (200 cm<sup>3</sup>, 2 M) was added, and after stirring for 15 minutes the organic layer was separated and washed with water (100 cm<sup>3</sup>), a saturated solution of sodium hydrogen carbonate (100 cm<sup>3</sup>), and a further portion of water (100 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo*. Flash chromatography of the residue on silica gel [petrol/ethyl acetate (20:1)] gave *the title compound* as a colourless viscous oil (2.3 g, 55 %).

$\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000s, 1730s, 1235m, 1460m, 1030m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.10 (4 H, m, Ar-H), 3.78 (4 H, s, 2 x -CH<sub>2</sub>OAc), 2.65 (2 H, s, -CH<sub>2</sub>Ar), 2.2 (6 H, s, -COCH<sub>3</sub>), 2.1 (1 H, m, -CH(CH<sub>2</sub>OAc)), 1.25 (9 H, s, -C(CH<sub>3</sub>)<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.2 (2 x MeCO-), 149.8, 133.9 (2 x =C-, Ar), 130.5, 125.6 (4 x =C-H, Ar), 65.9 (2 x -CH<sub>2</sub>OAc), 41.8 (-C(Me)<sub>3</sub>), 39.5 (-CH<sub>2</sub>Ar), 31.8 (MeCO-), 21.4 (3 x -CH(CH<sub>3</sub>)<sub>3</sub>).

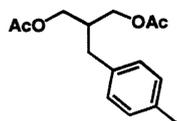
2-(Acetoxymethyl)-3-(2,4,6-trimethylphenyl)propyl acetate (**41**)



A solution of 2-(2',4',6'-trimethylbenzyl)propan-1,3-diol (**37**) (3.0 g, 14.4 mmol), triethylamine (6.0 cm<sup>3</sup>, 43.2 mmol), acetic anhydride (4.1 cm<sup>3</sup>, 43.2 mmol) and 4-dimethylaminopyridine (catalytic amount) in dichloromethane (50 cm<sup>3</sup>) was stirred at room temperature for 36 hours. Hydrochloric acid (200 cm<sup>3</sup>, 2 M) was added, and after stirring for 15 minutes the organic layer was separated and washed with water (100 cm<sup>3</sup>), a saturated solution of sodium hydrogen carbonate (100 cm<sup>3</sup>), and a further portion of water (100 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo*. Flash chromatography of the residue over silica gel [petrol/ethyl acetate (20:1)] gave *the title compound* as a colourless oil (1.6 g, 38%).

(Found  $M^+$  292.16745,  $C_{17}H_{24}O_4$  requires  $M$  292.16896);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  2900m, 1740s, 1370m, 1230m;  $\delta_{\text{H}}$  (90 MHz;  $\text{CDCl}_3$ ) 6.75 (2 H, s, Ar-H), 3.95 (4 H, d,  $J$  4.5, 2 x  $-\text{CH}_2\text{OAc}$ ), 2.85 (1 H, m,  $-\text{CHCH}_2\text{OAc}$ ), 2.65 (2 H, d,  $J$  7.0,  $\text{CH}_2\text{Ar}$ ), 2.15 (15 H, m,  $-\text{COCH}_3$ ,  $\text{CH}_3\text{-Ar}$ );  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 171.0 (2 x MeCO-), 137.6, 136.7, 135.4, 134.6 (4 x =C-, Ar), 132.7, 129.5 (2 x =C-H, Ar), 64.0 (2 x  $-\text{CH}_2\text{OAc}$ ), 38.1 ( $-\text{CHCH}_2\text{OAc}$ ), 29.7 ( $-\text{CH}_2\text{Ar}$ ), 21.0, 20.2 (3 x Me-Ar), 17.1 (MeCO-).

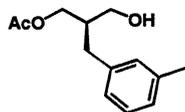
2-(Acetoxymethyl)-3-(4-methylphenyl)propyl acetate (**42**)



To a stirred solution of diol (**38**) (4.6 g, 25.8 mmol) in dichloromethane (70 cm<sup>3</sup>) was added triethylamine (11.0 cm<sup>3</sup>, 78.0 mmol) followed by acetic anhydride (7.5 cm<sup>3</sup>, 78.0 mmol) and the mixture stirred for 24 hours before being quenched by the addition of dilute hydrochloric acid (20 cm<sup>3</sup>, 2 M). The organic phases were separated and washed with water (3 x 50 cm<sup>3</sup>) dried (magnesium sulfate) and concentrated under vacuum. Chromatography of the residue over silica gel [petrol/ethyl acetate (18:1)] yielded *the title compound* as a colourless oil. (4.3 g, 63 %)

$\nu_{\text{max}}$ (neat)/cm<sup>-1</sup> 1740s, 1230m, 1040m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.00, 7.20 (4 H, d, *J* 9.0, Ar-*H*), 3.70 (4 H, s, -CH<sub>2</sub>OAc), 2.60 (2 H, s, -CH<sub>2</sub>Ar), 2.20 (3 H, s, *Me*-Ar), 2.15 (1 H, s, -CHCH<sub>2</sub>OAc), 2.00 (6 H, s, 2 x *Me*CO-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.1 (*Me*CO-), 136.5, 133.6 (=C- and =C-*Me*, Ar), 130.7, 129.4 (4 x =C-H, Ar), 65.8 (2 x -CH<sub>2</sub>OAc), 41.8 (-CHCH<sub>2</sub>OAc), 39.2 (-CH<sub>2</sub>Ar), 21.4, 21.3 (*Me*-Ar and *Me*CO-).

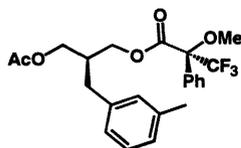
(2R)-2-(3-Methylbenzyl)-3-hydroxypropyl acetate (43)



To a solution of the diacetate (39) (6.5 g, 24.4 mmol) in acetone (90 cm<sup>3</sup>) and water (210 cm<sup>3</sup>) was added Triton X-100 (0.3 cm<sup>3</sup>), and the mixture homogenised in a sonic bath for 5 minutes. Porcine pancreatic lipase (Sigma Type II; 23.0 g) was added and the ice-cooled mixture vigorously stirred for 150 minutes with the pH being maintained at 7.0 by addition of sodium hydroxide (1 M), until the reaction was complete and ethyl acetate (300 cm<sup>3</sup>) was then added. The mixture was then filtered through Celite, the filtrate was saturated with sodium chloride, the organic layer separated, and the aqueous phase extracted with ethyl acetate (2 x 200 cm<sup>3</sup>). The combined organic fractions were dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (5:1)] yielded *the title alcohol* as a colourless oil (1.3 g, 23 %).

$[\alpha]_D = +9.61$  (*c* 1.2 CHCl<sub>3</sub>); (Found  $M^+$  222.12672, C<sub>13</sub>H<sub>18</sub>O<sub>3</sub> requires  $M$  222.12557);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3440br m, 1735s, 1240m, 740m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 7.00 (4 H, m, Ar-*H*), 4.10 (2 H, m, -CH<sub>2</sub>OH), 3.00 (2 H, m, -CH<sub>2</sub>OAc), 2.50 (3 H, m, -CH<sub>2</sub>Ar, -CHCH<sub>2</sub>OAc), 2.30 (3 H, s, -CH<sub>3</sub>CO), 2.05 (3 H, s, CH<sub>3</sub>Ar);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.2 (MeCO-), 139.8, 138.4 (=C-Me and =C-, Ar), 130.3, 128.8, 127.7, 126.5 (4 x =C-H, Ar), 64.6, 62.3 (2 x -CH<sub>2</sub>O-), 42.8 (MeCO-), 34.7 (-CH<sub>2</sub>Ar), 21.8, 21.3 (Me-Ar, -CHCH<sub>2</sub>OH);  $m/z$  (EI) 222 ( $M^+$ , 1.0 %), 204 (10.0), 162 (69.5), 144 (34.6), 131 (100.0), 118 (29.0), 105 (58.3), 91 (17.5).

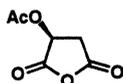
(2R)-2-(3-Methylbenzyl)-3-[(R)-(+)- $\alpha$ -methoxy- $\alpha$ -  
(trifluoromethyl)-phenyl acetyl]-propyl acetate (**44**)



To a stirred solution of (**43**) (165 mg, 0.74 mmol) in dry dichloromethane (10 cm<sup>3</sup>) was added (R)-(+)- $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)-phenylacetic acid (250 mg, 1.0 mmol) 1,3-dicyclohexylcarbodiimide (210 mg, 1.0 mmol), 4-dimethylaminopyridine (5 mg, 0.4 mmol) and the mixture stirred at room temperature for 24 hours. The organic phase was separated and washed with water (2 x 10 cm<sup>3</sup>), hydrochloric acid (10 cm<sup>3</sup>; 2 M), sodium hydrogen carbonate solution (10 cm<sup>3</sup> saturated solution), water (10 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo* to give the title ester as a colourless oil (215 mg, 68 %)

$\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2950m, 1740s, 1450m, 1370m, 1230m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.35 (5 H, m, Ar-H), 6.85 (4 H, m, Ar-H), 4.1 (4 H, m, -CH<sub>2</sub>OAc, -CH<sub>2</sub>OCO-), 3.45 (3 H, s, -OMe), 2.45 (2 H, m, -CH<sub>2</sub>Ar), 2.25 (4 H, m, -CHCH<sub>2</sub>Ar, CH<sub>3</sub>CO-), 1.90 (3 H, s, Me-Ar);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 171.2 (MeCO-), 166.9 (-OCOC(CF<sub>3</sub>)-), 138.6, 138.5, 132.5 (3 x =C-, Ar), 129.1, 128.9, 127.7, 126.4 (4 x =C-H, Ar), 65.6, 64.2 (2 x -CH<sub>2</sub>O-), 55.8 (MeCO-), 39.5 (-CH<sub>2</sub>Ar), 34.5 (-CHCH<sub>2</sub>Ar), 21.8 (Me-Ar), 21.1 (MeCO-);  $\delta_{\text{F}}$  (235.3 MHz; CDCl<sub>3</sub>) -71.76 (-CF<sub>3</sub>, 91.3 %), -72.08 (-CF<sub>3</sub>, 8.7 %) e.e = 82 %

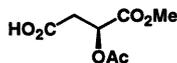
(3S)-3-Acetoxy succinic anhydride (**49**)<sup>86</sup>



(S)-Malic acid (10.0 g, 74.6 mmol) was dissolved in acetyl chloride (37.5 cm<sup>3</sup>, 525.0 mmol) and the mixture stirred in a 45 °C water bath for 2 hours before removing the excess acetyl chloride and acetic acid under high vacuum to give *the title anhydride* as a viscous oil (11.6 g, 98 %)

$[\alpha]_D = -26$  (c 5.11 CHCl<sub>3</sub>)<sup>86</sup>  $\delta_H$  (90 MHz; CDCl<sub>3</sub>) 5.45 (1 H, dd, *J* 9.0, 7.0, -CHOAc), 3.35 (1 H, dd, *J* 19.0, 9.0, -CH<sub>2</sub>CO-), 3.00 (1 H, dd, *J* 7.0, 19.0, -CH<sub>2</sub>CO-), 2.15 (3 H, s, CH<sub>3</sub>CO-);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 207.3 (CH<sub>3</sub>CO-), 171.4, 170.7 (-COOCO-), 69.6 (CH<sub>3</sub>CO<sub>2</sub>CHCO-), 36.7 (-CH<sub>2</sub>COO-), 20.9 (CH<sub>3</sub>CO-).

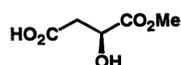
(3S)-3-Acetoxy-3-carboxymethyl propanoic acid (**50**)<sup>86</sup>



To anhydride (**49**) (11.6 g, 73.2 mmol) was added dry methanol (150 cm<sup>3</sup>) and the mixture stirred at room temperature for 24 hours. The methanol was removed *in vacuo* to give, after leaving under high vacuum overnight *the title ester* as a white solid. (13.7 g, 99 %).

$[\alpha]_D = -27.9$  (c 9.25 MeOH)<sup>86</sup>  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3400br, 3000m, 1735s, 1760s, 1380m;  $\delta_H$  (90 MHz; CDCl<sub>3</sub>) 9.51 (1 H, s, -CO<sub>2</sub>H), 5.45 (1 H, t, *J* 7.0, -CH(OAc)), 3.75 (3 H, s, CH<sub>3</sub>OCO-), 2.90 (2 H, d, *J* 7.0, -CH<sub>2</sub>CO<sub>2</sub>H), 2.15 (3 H, s, CH<sub>3</sub>CO-);  $\delta_C$  (62.9 MHz; Acetone-d<sub>6</sub>) 174.6, 174.5, 172.3 (CH<sub>3</sub>CO-, -CO<sub>2</sub>Me, -CO<sub>2</sub>H), 36.8 (-CH<sub>2</sub>OAc), 52.1 (-CO<sub>2</sub>CH<sub>3</sub>), 39.7 (-CH<sub>2</sub>CO<sub>2</sub>H), 21.2 (CH<sub>3</sub>CO-).

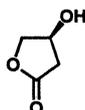
(3S)-3-hydroxy-3-carboxymethyl propanoic acid (**51**)<sup>87</sup>



(S)-Malic acid (10.0 g, 74.6 mmol) and trifluoroacetic anhydride (25.0 cm<sup>3</sup>, 177.0 mmol) were stirred with ice bath cooling for 2 hours before the excess trifluoroacetic acid and trifluoroacetic anhydride were removed under high vacuum the flask being kept at 0 °C. To the residue was added dry methanol (50 cm<sup>3</sup>) and the mixture stirred at room temperature for 3 hours before concentrating in vacuo to yield the title compound after recrystallisation [petrol/ethyl acetate (2:1)] as a white solid (9.1 g, 82 %)

m.p. 70-75 °C; (lit 79-80 °C)<sup>87</sup>  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3440m, 2950s, 1735s, 1460m, 1380m;  $\delta_{\text{H}}$  (90 MHz; Acetone-d<sub>6</sub>) 4.90 (1 H, t, *J* 6.0, -CH(OH)-), 3.70 (3 H, s, CH<sub>3</sub>OCO-), 2.70 (2, dd, *J* 6.0, 3.0, -CH<sub>2</sub>CO<sub>2</sub>H);  $\delta_{\text{C}}$  (62.9 MHz; Acetone-d<sub>6</sub>) 174.8, 173.2 (-CO<sub>2</sub>H, -CO<sub>2</sub>Me), 68.7 (-CH(OH)-), 53.0 (-CO<sub>2</sub>CH<sub>3</sub>), 39.8 (-CH<sub>2</sub>CO<sub>2</sub>H).

(3S)-3-Hydroxy-butano-4-lactone (**52**)<sup>86</sup>

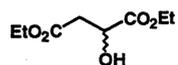


To a suspension of sodium borohydride (1.25 g, 33.0 mmol) at reflux in dry *tert*-butanol (20 cm<sup>3</sup>) was added a solution of the half ester (**51**) (1.55 g, 8.15 mmol) in dry *tert*-butanol (7 cm<sup>3</sup>) and dry methanol (2 cm<sup>3</sup>) and heated at reflux for 2 hours. To the cooled reaction mixture was added hydrochloric acid in methanol, [(40 cm<sup>3</sup>, made by the slow addition of acetyl chloride (6 cm<sup>3</sup>) to dry methanol (40 cm<sup>3</sup>)], until acidic and concentrated. To the residue was added ethyl acetate (50 cm<sup>3</sup>), the

mixture filtered and the filtrate neutralised by the slow addition of solid sodium hydrogen carbonate. The precipitates were filtered off and the filtrate concentrated in vacuo to give the title alcohol as an oil (0.6 g, 72 %).

$[\alpha]_{\text{D}} = -53.75$  ( $c$  1.8, EtOH) (lit<sup>86</sup>  $-81.0$   $c$  1.97 EtOH);  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 4.65 (1 H, m, -CH(OH)-), 4.35 (2 H, m, -CH<sub>2</sub>OCO-), 3.70 (1 H, d,  $J$  6.0, -CHOH-), 2.65 (2 H, m, -COCH<sub>2</sub>CH(OH)-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 177.9 (-OCOCH<sub>2</sub>-), 76.8 (-CH<sub>2</sub>OCO-), 67.7 (-CH(OH)-), 38.1 (-CH<sub>2</sub>COOCH<sub>2</sub>-).

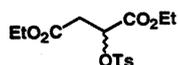
Diethyl (2R, S)-malate (53)<sup>106</sup>



(*R, S*)-Malic acid (5.0 g, 37.3 mmol) was treated with absolute ethanol (50 cm<sup>3</sup>) and sulfuric acid (1.0 cm<sup>3</sup>) as described for the preparation of (56) to give the title ester as a colourless liquid (5.9 g, 85 %).

$\nu_{\text{max}}$ (neat)/cm<sup>-1</sup>: 3480br, 2950m, 1740s, 1390m;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 4.30 (1 H, t,  $J$  4.5, -CH(OH)-), 4.10 (4 H, q,  $J$  7.0 -CH<sub>2</sub>CH<sub>3</sub>), 3.30 (1 H, s, -OH), 2.7 (2 H, d,  $J$  4.5, -CH<sub>2</sub>CO<sub>2</sub>Et), 1.2 (6 H, 2 x t,  $J$  7.0, -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.7, 170.4 (2 x -CO<sub>2</sub>Et), 67.6 (-CHOH-), 61.8, 60.9 (2 x -OCH<sub>2</sub>CH<sub>3</sub>), 39.1 (-CH<sub>2</sub>CO<sub>2</sub>Et), 13.8 (-CH<sub>2</sub>CH<sub>3</sub>).

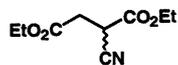
(R, S)-Diethyl O-*p*-toluenesulfonyl malate (**54**)<sup>110</sup>



Alcohol (**53**) (3.0 g, 16.0 mmol), was treated with *p*-toluenesulfonyl chloride (4.7 g, 24.4 mmol), pyridine (2.6 cm<sup>3</sup>, 32.6 mmol) in chloroform (30 cm<sup>3</sup>) as described in the preparation of (**57**) to give *the title compound* as a colourless oil (4.0 g, 82 %).

$\nu_{\max}$ (neat)/cm<sup>-1</sup> 3000m, 1740s, 1600s, 1370m, 1190m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.70 (2 H, d, *J* 8.3, Ar-*H*) 7.20 (2 H, d, *J* 8.0, Ar-*H*) 5.20 (1 H, t, *J* 6.0, -CH(OTs)) 4.10 (4 H, q, *J* 7.0, 2 x -CH<sub>2</sub>CH<sub>3</sub>) 2.70 (2 H, d, *J* 6.0, -CH<sub>2</sub>CO<sub>2</sub>Et) 2.3 (3 H, s, CH<sub>3</sub>-Ar) 1.2 (6 H, t, *J* 7.0, -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 168.0, 167.4 (2 x -CO<sub>2</sub>Et), 145.0 (-C=, Ar), 133.0 (Me-C=), 129.5, 127.9 (2 x =C-H, Ar), 73.5 (-CH(OTs)), 62.0, 61.3 (2 x -CH<sub>2</sub>CH<sub>3</sub>), 37.1 (-CH<sub>2</sub>CO<sub>2</sub>Et), 21.5 (CH<sub>3</sub>Ar), 13.7, 13.6 (2 x -CH<sub>2</sub>CH<sub>3</sub>).

Diethyl 2-cyanosuccinate (**55**)

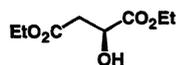


To a stirred solution of tosylate (**54**) (1.8 g, 5.2 mmol) in dimethylsulfoxide (30 cm<sup>3</sup>) was added potassium cyanide (0.5 g, 7.7 mmol) and the solution stirred at 100 °C for 5 hours under nitrogen before being allowed to cool to room temperature and poured into water (50 cm<sup>3</sup>). The aqueous solution was extracted with dichloromethane (2 x 50 cm<sup>3</sup>) the organic phase washed with water (3 x 75 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo* to give *the title compound* as a colourless oil (0.9 g, 85 %).

$\nu_{\max}$ (neat)/cm<sup>-1</sup> 3000m, 2250s, 1740s;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 4.30 (2 H, q, *J* 7.0, -CH<sub>2</sub>CH<sub>3</sub>), 4.20 (2 H, q, *J* 7.0, -CH<sub>2</sub>CH<sub>3</sub>), 3.90 (1 H, t, *J* 6.6, -CH(CN)-), 3.00 (1 H,

dd,  $J$  17.3, 6.6,  $-CHHCO_2Et$ ) 2.90 (1 H, dd,  $J$  17.3, 6.3,  $-CHHCO_2Et$ ), 1.30 (3 H, t,  $J$  7.0,  $-CH_2CH_3$ ), 1.2 (3 H, t,  $J$  7.0,  $-CH_2CH_3$ );  $\delta_C$  (62.9 MHz;  $CDCl_3$ ) 169.4, 165.4 (2 x  $-CO_2Et$ ), 116.2 ( $-CH(CN)-$ ), 63.6, 62.0 (2 x  $-CH_2CH_3$ ), 33.9 ( $-CH_2CO_2Et$ ), 33.3 ( $-CH(CN)-$ ), 14.4, 14.2 (2 x  $-CH_2CH_3$ ).

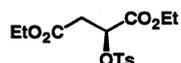
*Diethyl (R)-(+)-malate (56)*



A solution of (*S*)-(-)-malic acid (5.0 g, 37.3 mmol), absolute ethanol (50.0 cm<sup>3</sup>), and concentrated sulphuric acid (1.0 cm<sup>3</sup>) was heated at reflux for 3.5 hours, then stirred for 15 hours at room temperature before being concentrated under reduced pressure. The residue was poured into water (30 cm<sup>3</sup>), extracted with dichloromethane (2 x 40 cm<sup>3</sup>), and the combined organic phases washed with water (50 cm<sup>3</sup>), a saturated solution of sodium hydrogen carbonate (50 cm<sup>3</sup>), and a further portion of water (50 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo* to give *the title ester* as a colourless liquid (6.15 g, 86 %).

$[\alpha]_D = +10.64$  ( $c$  1.5 EtOH);  $\nu_{max}(neat)/cm^{-1}$  3480br, 2980m, 1740s, 1380m, 1100m;  $\delta_H$  (90 MHz;  $CDCl_3$ ) 4.40 (1 H, t,  $J$  4.5,  $-CH(OH)-$ ), 4.12 (4H, 2 x q,  $-CH_2CH_3$ ), 3.40 (1 H, s, OH), 2.75 (2 H, d,  $J$  4.5,  $-CH_2CO_2Et$ ), 1.22 (6H, 2 x t,  $J$  7.0,  $-CH_2CH_3$ );  $\delta_C$  (62.9 MHz;  $CDCl_3$ ) 173.7, 170.9 (2 x  $-CO_2Et$ ), 67.6 ( $-CHOH-$ ), 62.2, 61.2 (2 x  $-OCH_2CH_3$ ), 39.5 ( $-CH_2CO_2Et$ ), 14.4 ( $-CH_2CH_3$ ).

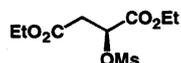
(R)-(-)-Diethyl-O-*p*-toluenesulfonyl malate (57)



To a solution of alcohol (56) (3.1 g 16.3 mmol) in chloroform (30 cm<sup>3</sup>) was added pyridine (2.6 cm<sup>3</sup>, 32.6 mmol) followed after a 15 minute delay by *p*-toluenesulfonyl chloride (4.7 g, 24.4 mmol) and the reaction mixture stirred at room temperature for 24 hours before being poured into water and the organic phase separated, washed with dilute hydrochloric acid (30 cm<sup>3</sup>; 2 M), water (2 x 30 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated in vacuo. Chromatography of the residue over silica gel [petrol/ethyl acetate (2:1)] yielded the title compound as a colourless oil (5.0 g, 89 %).

(Found: M<sup>+</sup> 344.09300, C<sub>15</sub>H<sub>20</sub>O<sub>7</sub>S requires M 344.09420);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3000m, 1740s, 1600s, 1370m, 1190m,  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.82 (2 H, d, *J* 8.3, Ar-H) 7.34 (2 H, d, *J* 8.0, Ar-H) 5.23 (1 H, t, *J* 6.2, -CH(OTs)) 4.15 (4 H, q, *J* 7.1, 2 x -CH<sub>2</sub>CH<sub>3</sub>) 2.89 (2 H, d, *J* 6.2, -CH<sub>2</sub>CO<sub>2</sub>Et) 2.45 (3 H, s, CH<sub>3</sub>-Ar) 1.21 (6 H, t, *J* 7.1, 2 x -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 168.0, 167.3 (2 x -CO<sub>2</sub>Et), 144.9 (-C=, Ar), 133.0 (Me-C=), 129.5, 127.9 (2 x =C-H, Ar), 73.6(-CH(OTs)), 62.0, 61.4 (2 x -CH<sub>2</sub>CH<sub>3</sub>), 37.0 (-CH<sub>2</sub>CO<sub>2</sub>Et), 21.5 (CH<sub>3</sub>-Ar), 13.8, 13.7 (2 x -CH<sub>2</sub>CH<sub>3</sub>); *m/z* (EI) 344 (M<sup>+</sup>, 2.0 %) 271 (14.8) 172 (12.8) 155 (100.0) 117 (30.8) 91 (92.8) 71 (24.0) 51 (14.8).

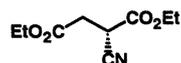
*Diethyl (R)-(-)-O-methane sulfonyl malate (58)*



To a solution of diethyl (*R*)-(+)-malate (**56**) (3.0 g, 15.8 mmol), in dichloromethane (20 cm<sup>3</sup>) at 0 °C was added triethylamine (3.3 cm<sup>3</sup>, 24.0 mmol), slowly, followed after 15 minutes by methanesulfonyl chloride (1.4 cm<sup>3</sup>, 17.6 mmol). The reaction mixture was stirred for 1 hour before being quenched with water (20 cm<sup>3</sup>) and the organic layer washed with hydrochloric acid (10 cm<sup>3</sup>; 2 M) water (2 x 10 cm<sup>3</sup>), dried (magnesium sulfate), and concentrated. Flash chromatography of the residue over silica gel [petrol/ethyl acetate (3:1-2:1)] gave *the title compound* as a colourless liquid (1.79 g, 43 %).

$[\alpha]_D = +46.04$  (c 1.6, EtOH); (Found:  $MH^+$  268.06167, C<sub>9</sub>H<sub>16</sub>O<sub>7</sub>S requires  $MH$  268.06264);  $\nu_{max}$ (neat)/cm<sup>-1</sup> 2950m, 1740s, 1360m, 1280s;  $\delta_H$  (90 MHz; CDCl<sub>3</sub>) 5.35 (1 H, t, *J* 7.0, -CH(OMs)-), 4.20 (2 H, m, -CH<sub>2</sub>CH<sub>3</sub>), 3.15 (3 H, s, CH<sub>3</sub>SO<sub>2</sub>-), 2.95 (2 H, d, *J* 7.0, -CH<sub>2</sub>CO<sub>2</sub>Et), 1.22 (6 H, m, -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 168.9, 168.0 (2 x -CO<sub>2</sub>Et), 73.9 (CH<sub>3</sub>SO<sub>2</sub>-), 62.5, 61.6 (2 x -OCH<sub>2</sub>CH<sub>3</sub>) 39.0 (-CH(OMs)-), 36.9 (-CH<sub>2</sub>CO<sub>2</sub>Et), 14.1 (-CH<sub>2</sub>CH<sub>3</sub>).

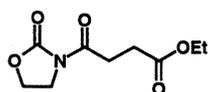
*Diethyl (2S)-2-cyano succinate (59)*



Tosylate (**57**) (3.5 g, 10.2 mmol) was treated with potassium cyanide (1.0 g, 15.5 mmol) and dimethylsulfoxide (50 cm<sup>3</sup>) as for the preparation of (**55**) to give *the title compound* as a colourless oil (0.9 g 89 %).

$[\alpha]_D = -2.06$  ( $c$  1.10, MeOH); (Found:  $M^+$  199.08443,  $C_9H_{13}NO_4$  requires  $M$  199.08527);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3000m, 2250s, 1740s;  $\delta_H$  (250 MHz;  $CDCl_3$ ) 4.30 (2 H, q,  $J$  7.0,  $-CH_2CH_3$ ), 4.20 (2 H, q,  $J$  7.0,  $-CH_2CH_3$ ), 3.90 (1 H, t,  $J$  6.6,  $-CH(CN)-$ ), 3.00 (1 H, dd,  $J$  17.3, 6.6,  $-CHHCO_2Et$ ) 2.90 (1 H, dd,  $J$  17.3, 6.3,  $-CHHCO_2Et$ ), 1.30 (3 H, t,  $J$  7.0,  $-CH_2CH_3$ ), 1.20 (3 H, t,  $J$  7.0,  $-CH_2CH_3$ );  $\delta_C$  (62.9 MHz;  $CDCl_3$ ) 169.4, 165.4 (2 x  $-CO_2Et$ ), 116.2 ( $-CH(CN)-$ ), 63.6, 62.0 (2 x  $-CH_2CH_3$ ), 33.9 ( $-CH_2CO_2Et$ ), 33.0 ( $-CH(CN)-$ ), 14.4, 14.2 ( $-CH_2CH_3$ ).

3-[3'-Ethoxycarbonyl-1'-oxo  
propanoyl]-2-oxazolidinone (69)

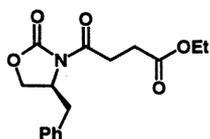


To a stirred solution of 2-oxazolidinone (4.0 g, 45.9 mmol) in dry tetrahydrofuran (60  $\text{cm}^3$ ) at 0  $^\circ\text{C}$  under nitrogen was added *n*-butyl lithium (1.6 M in hexanes; 30.0  $\text{cm}^3$ , 48.0 mmol) and the mixture stirred for 20 minutes before being cooled to  $-78$   $^\circ\text{C}$  and ethyl succinyl chloride (7.0  $\text{cm}^3$ , 49.0 mmol) added dropwise. Stirring was continued for a further 4 hours before the reaction was quenched with sodium hydrogen carbonate (60  $\text{cm}^3$ , saturated solution) and extracted with dichloromethane (4 x 50  $\text{cm}^3$ ). The combined organics were dried (magnesium sulfate) concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (3 to 2:1)] yielded *the title compound* as a white solid (8.0 g, 81 %).

m.p. 38-41  $^\circ\text{C}$ ; (Found: C 50.19, H 6.60, N 6.40,  $M^+$  215.07938,  $C_9H_{13}NO_5$  requires C 50.23, H 6.09, N 6.51 %,  $M$  215.08017);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1785s, 1735s, 1710s;  $\delta_H$  (250 MHz;  $CDCl_3$ ) 4.50 (2 H, t,  $J$  9.0,  $-CH_2OCON-$ ), 4.25 (2 H, q,  $J$  7.0,  $-CH_2CH_3$ ), 4.10 (2 H, t,  $J$  9.0,  $-CH_2NCO-$ ), 3.35, (2 H, t,  $J$  6.0, -

CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et), 2.78 (2 H, t, *J* 6.0, -CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et), 1.40 (3 H, t, *J* 7.0, -CH<sub>2</sub>CH<sub>3</sub>); δ<sub>C</sub> (62.9 MHz; CDCl<sub>3</sub>) 172.8, 172.3 (-NCOCH<sub>2</sub>- and -CO<sub>2</sub>Et), 154.0 (-OCON-), 62.7, 61.0 (-CH<sub>2</sub>OCON- and -CH<sub>2</sub>NCO-), 42.8, 30.7 (-CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Et), 28.6 (-CH<sub>2</sub>CH<sub>3</sub>), 14.5 (-CH<sub>2</sub>CH<sub>3</sub>); *m/z* (EI) 215 (M<sup>+</sup>, 2.3 %) 170 (35.0) 129 (100.0) 101 (91.7) 88 (20.5) 55 (41.1).

(4*S*)-3-[3'-Ethoxycarbonyl-1'-oxopropanoyl]-  
4-benzyl-2-oxazolidinone (70)

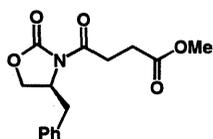


To a stirred ice-cooled solution of (4*S*)-4-benzyl-2-oxazolidinone (1.0 g, 5.6 mmol) in dry tetrahydrofuran (20 cm<sup>3</sup>) under nitrogen was added dropwise *n*-butyl lithium (1.6 M in hexanes; 3.5 cm<sup>3</sup>, 5.7 mmol) and the mixture stirred for 20 minutes before being cooled to -78 °C. Ethyl succinyl chloride (0.9 cm<sup>3</sup>, 6.3 mmol) was added slowly and the reaction mixture stirred for 4 hours, quenched with sodium hydrogen carbonate (20 cm<sup>3</sup>, saturated solution), and extracted with diethyl ether (3 x 70 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated. Chromatography of the residue over silica gel [petrol/ethyl acetate (3:1)] gave the title compound as a white solid (1.5 g, 86 %).

*m.p.* 55-58 °C; (Found: C 62.74, H 6.42, N 4.50, M<sup>+</sup> 305.12637, C<sub>16</sub>H<sub>19</sub>NO<sub>5</sub> requires C 62.94, H 6.27, N 4.59 %, *M* 305.12632); ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 2990s, 1775s, 1730s, 1690m; δ<sub>H</sub> (300 MHz; CDCl<sub>3</sub>) 7.28 (5 H, m, Ar-*H*), 4.67 (1H, m, -CHCH<sub>2</sub>Ph), 4.17 (4 H, m, -CH<sub>2</sub>CH<sub>3</sub> and -CH<sub>2</sub>OCON-), 3.24 (3 H, m, -CH<sub>2</sub>CO- and -CHHPh), 2.79 (1 H, dd, *J* 0.5, 13.4, -CHHPh), 2.69 (2 H, m, -CH<sub>2</sub>CO<sub>2</sub>Et), 1.27 (3

H, t,  $J$  7.1,  $-\text{CH}_2\text{CH}_3$ );  $\delta_{\text{C}}$  (75.4 MHz;  $\text{CDCl}_3$ ) 172.2, 171.7 ( $-\text{CO}_2\text{Et}$  and  $-\text{NCOCH}_2-$ ), 153.3 ( $-\text{OCON}-$ ), 135.0 ( $=\text{C}-$ , Ar), 129.3, 128.7, 127.1 (3 x  $=\text{C}-\text{H}$ , Ar), 66.1 ( $-\text{CH}_2\text{OCO}-$ ), 60.5 ( $-\text{CH}_2\text{CH}_3$ ), 54.9 ( $-\text{CHCH}_2\text{Ph}$ ), 37.6, 30.7, 28.2 ( $-\text{CH}_2\text{Ph}$  and  $-\text{CH}_2\text{CON}-$  and  $-\text{CH}_2\text{CO}_2\text{Et}$ );  $m/z$  (EI) 305 ( $\text{M}^+$ , 28.0 %) 260 (21.5) 129 (100.0) 117 (14.3) 101 (67.0) 91 (23.9).

(4*S*)-3-[3'-Methyloxycarbonyl-1'-oxopropanoyl]-  
4-benzyl-2-oxazolidinone (71)

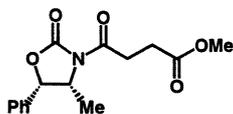


To a stirred solution of 4-benzyl-2-oxazolidinone (1.0 g, 5.64 mmol) in dry tetrahydrofuran (20  $\text{cm}^3$ ) under nitrogen at 0  $^\circ\text{C}$  was added *n*-butyl lithium (1.6 M in hexanes; 3.55  $\text{cm}^3$ , 5.68 mmol) dropwise and the mixture stirred for 20 minutes before cooling to  $-78$   $^\circ\text{C}$  and adding methyl succinyl chloride (0.8  $\text{cm}^3$ , 6.5 mmol) slowly. The reaction mixture was stirred for 3 hours, quenched with sodium hydrogen carbonate (30  $\text{cm}^3$ , saturated solution) and extracted with diethyl ether (4 x 40  $\text{cm}^3$ ) the combined organic phases dried (magnesium sulfate) and concentrated *in vacuo* to give the title compound after recrystallisation [petrol/ethyl acetate (4:1)] as a white solid (1.2 g, 71 %).

m.p. 90-92  $^\circ\text{C}$ ; (Found:  $\text{M}^+$  291.11067,  $\text{C}_{15}\text{H}_{17}\text{NO}_5$  requires  $M$  291.11071);  $\nu_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1785s, 1705s, 1390m;  $\delta_{\text{H}}$  (300 MHz;  $\text{CDCl}_3$ ) 7.31 (5 H, m, Ar-H), 4.69 (1 H, m,  $-\text{CHCH}_2\text{Ph}$ ), 4.21 (2 H, m,  $-\text{CH}_2\text{OCO}-$ ), 3.73 (3 H, s,  $\text{CH}_3\text{CO}-$ ), 3.27 (3 H, m,  $-\text{CH}_2\text{CON}-$  and  $-\text{CHHPH}$ ), 2.80 (1 H, dd,  $J$  13.4, 0.6,  $-\text{CHHPH}$ ), 2.73 (2 H, m,  $-\text{CH}_2\text{CO}_2\text{Me}$ );  $\delta_{\text{C}}$  (75.4 MHz;  $\text{CDCl}_3$ ) 172.7, 171.7 ( $-\text{CO}_2\text{Me}$  and  $-\text{NCOCH}_2-$ ), 153.3 ( $-\text{OCON}-$ ), 135.0 ( $=\text{C}-$ , Ar), 129.3, 128.7, 127.1 (3 x  $=\text{C}-\text{H}$ , Ar), 66.1 ( $-\text{CH}_2\text{OCO}-$ ), 60.5 ( $-\text{CH}_2\text{CH}_3$ ), 54.9 ( $-\text{CHCH}_2\text{Ph}$ ), 37.6, 30.7, 28.2 ( $-\text{CH}_2\text{Ph}$  and  $-\text{CH}_2\text{CON}-$  and  $-\text{CH}_2\text{CO}_2\text{Me}$ );  $m/z$  (EI) 305 ( $\text{M}^+$ , 28.0 %) 260 (21.5) 129 (100.0) 117 (14.3) 101 (67.0) 91 (23.9).

CO<sub>2</sub>Me and -NCOCH<sub>2</sub>-), 153.3 (-OCON-), 134.9 (=C-, Ar), 129.3, 128.8, 127.1 (3 x =C-H, Ar), 66.2 (-CH<sub>2</sub>Ph), 54.9 (-CHCH<sub>2</sub>Ph), 51.7 (CH<sub>3</sub>O-), 37.6 (-CH<sub>2</sub>OCON-), 30.7, 27.9 (-CH<sub>2</sub>CON- and -CH<sub>2</sub>CO<sub>2</sub>Me).

(4*R*, 5*S*)-3-[3'-Methoxycarbonyl-1'-oxopropanoyl]-  
4-methyl-5-phenyl-2-oxazolidinone (72)

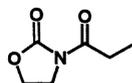


To an ice-cooled solution of (4*R*, 5*S*)-4-methyl-5-phenyl-2-oxazolidinone (2.8 g, 15.8 mmol) in dry tetrahydrofuran (40 cm<sup>3</sup>) was added dropwise *n*-butyl lithium (1.6 M in hexanes; 10.0 cm<sup>3</sup>, 16.0 mmol) and the mixture stirred for 20 minutes before cooling to -78 °C and adding methyl succinyl chloride (2.0 cm<sup>3</sup>, 16.2 mmol) dropwise. Stirring was continued for 3 hours the reaction quenched by sodium hydrogen carbonate (40 cm<sup>3</sup>, saturated solution) and extracted with diethyl ether (4 x 40 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate), concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (4 to 3:1)] yielded *the title compound* as a white solid (2.73 g, 59 %).

m.p. 71-72 °C; (Found: C 61.76, H 5.88, N 4.80, M<sup>+</sup> 291.11107, C<sub>15</sub>H<sub>17</sub>NO<sub>5</sub> requires C 61.85, H 5.88, N 4.81 % M 291.11068);  $\nu_{\max}$ (KBr)/cm<sup>-1</sup> 3000m, 1790s, 1740s, 1710s, 770m, 740m, 700m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.33 (5 H, m, Ar-*H*), 5.65 (1 H, d, *J* 7.3, -OCHPh), 4.75 (1 H, qu, *J* 6.7, -NCHMe), 3.70 (3 H, s, -COOMe), 3.25 (2 H, m, -CH<sub>2</sub>CON-), 2.70 (2 H, m, -CH<sub>2</sub>COOMe), 0.90 (3 H, d, *J* 6.7, -NCHMe);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 173.3, 172.0 (-NCOCH<sub>2</sub>- and -COOMe), 153.4 (-OCON-), 133.7 (=C-, Ar), 129.2, 129.1, 126.1 (3 x =C-H, Ar), 79.6 (-OCHPh), 55.2 (-COOMe), 52.3 (-NCHMe), 31.3, 28.5 (-NCOCH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me), 14.9 (-NCHMe),

*m/z* (EI) 291 (M<sup>+</sup>, 5.3 %) 260 (4.1) 134 (27.8) 115 (100.0) 87 (4.9) 70 (6.7) 55 (19.1).

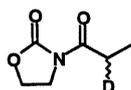
3-[1'-Oxopropanoyl]-2-oxazolidinone (73)<sup>111</sup>



To a stirred solution of 2-oxazolidinone (4.0 g, 45.9 mmol) in dry tetrahydrofuran (60 cm<sup>3</sup>) at 0 °C was added dropwise *n*-butyl lithium (1.6 M in hexanes; 30.0 cm<sup>3</sup>, 48.0 mmol) and the mixture stirred for 30 minutes. The reaction was cooled to -78 °C, propionyl chloride (4.0 cm<sup>3</sup>, 46.0 mmol) added slowly and stirring continued for 4 hours before quenching with sodium hydrogen carbonate (60 cm<sup>3</sup>, saturated solution) and extracting with dichloromethane (4 x 50 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo* to yield the title compound as a white solid which was recrystallised from petrol/ethyl acetate (3:1), (4.2 g, 63 %).

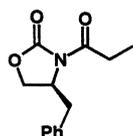
m.p. 74-77 °C; (Found: C 50.28, H 6.38, N 9.76, M<sup>+</sup> 143.05826, C<sub>6</sub>H<sub>9</sub>NO<sub>3</sub> requires C 50.35, H 6.34, N 9.79 %, M 143.05881);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1785s, 1710s;  $\delta_{\text{H}}$  (90 MHz; CDCl<sub>3</sub>) 4.30 (2 H, t, *J* 8.0, -CH<sub>2</sub>OCON-), 4.00 (2 H, t, *J* 8.0, -CH<sub>2</sub>NCO-), 2.85 (2 H, q, *J* 7.0, -CH<sub>2</sub>CH<sub>3</sub>), 1.15 (3 H, t, *J* 7.0 -CH<sub>2</sub>CH<sub>3</sub>); *m/z* (EI) 143 (M<sup>+</sup>, 19.4 %) 115 (22.0) 88 (54.6) 57 (100.0).

(2'-<sup>2</sup>H<sub>1</sub>)-3-[1'-Oxopropanoyl]-  
2-oxazolidinone (74)



To an ice-cooled solution of oxazolidinone (73) (0.5 g, 3.5 mmol) in dry dichloromethane (10 cm<sup>3</sup>) under nitrogen was added dropwise titanium (IV) chloride (0.4 cm<sup>3</sup>, 3.65 mmol) to give a yellow slurry followed after a five minute delay by dry diisopropylethylamine (0.8 cm<sup>3</sup>, 4.6 mmol) to give a deep blood red solution of titanium enolate. The reaction mixture was stirred for 1 hour and quenched with deuterium chloride in deuterium oxide (0.3 cm<sup>3</sup>; 37 % v/v DCI/D<sub>2</sub>O). Stirring was continued for 30 minutes, sodium hydrogen carbonate (20 cm<sup>3</sup> saturated solution) added the organic phase separated, and the aqueous phase re-extracted with a further portion of dichloromethane (30 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate), concentrated *in vacuo* and the residue recrystallised from petrol/ethyl acetate (3:1) to give the title compound as a white solid (0.45 g, 89 %). (Found: M<sup>+</sup> 144.06455, C<sub>6</sub>H<sub>7</sub>DNO<sub>3</sub> requires M 144.06502);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1785s, 1710s;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 4.43 (2 H, t, J 7.0, -CH<sub>2</sub>OCON-), 4.10 (2 H, t, J 7.0, -NCH<sub>2</sub>-), 2.90 (1 H, m, -CHDCH<sub>3</sub>), 1.16 (3 H, d, J 7.0, -CHDCH<sub>3</sub>);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 174.2 (-NCOCHD-), 153.7 (-OCON-), 62.2 (-CH<sub>2</sub>OCON-), 42.6 (-CH<sub>2</sub>NCO-), 28.7, 28.4, 28.2 (-CHDCH<sub>3</sub>), 8.26 (-CHDCH<sub>3</sub>); m/z (EI) 144 (M<sup>+</sup>, 11.1 %) 129 (24.1) 114 (100.0) 89 (19.0) 72 (99.1) 58 (55.1).

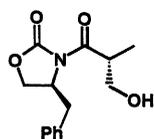
(4S)-3-[1'-Oxopropanoyl]-4-benzyl-2-oxazolidinone (75)<sup>112</sup>



To a solution of (4R)-4-benzyl-2-oxazolidinone (2.0 g, 11.3 mmol) in dry tetrahydrofuran (25 cm<sup>3</sup>) at 0 °C was added dropwise *n*-butyl lithium (1.6 M in hexanes; 7.1 cm<sup>3</sup>, 11.4 mmol) after stirring for 20 minutes the solution was cooled to -78 °C and propionyl chloride (1.0 cm<sup>3</sup>, 11.5 mmol) added slowly. Stirring was continued for 3 hours before the reaction was quenched with sodium hydrogen carbonate (30 cm<sup>3</sup>, saturated solution), extracted with diethyl ether (3 x 50 cm<sup>3</sup>), the combined organics were dried (magnesium sulfate), and concentrated. Chromatography of the residue over silica gel [petrol/ethyl acetate (5:1)] gave *the title compound* as a white solid (2.1 g, 79 %).

m.p. 44-46 °C; (Found: C 66.97, H 6.46, N 6.10, M<sup>+</sup> 233.10520, C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub> requires C 66.94, H 6.48, N 6.00 %, M 233.10615);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1770s, 1710s;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.27 (5 H, m, Ar-H), 4.65 (1 H, m, -NCHCH<sub>2</sub>-), 4.17 (2 H, m, -OCH<sub>2</sub>CH-), 3.30 (1 H, dd, *J* 3.2, 13.3, -CHHPh), 2.95 (2H, m, -CH<sub>2</sub>CO-), 2.75 (1 H, dd, *J* 4.8, 13.3, -CHHPh), 1.22 (3 H, t, *J* 7.4, -CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 174.0 (-NCOCH<sub>2</sub>-), 153.4 (-OCON-), 135.3 (=C-, Ar), 129.4, 128.9, 127.3 (3 x =C-H, Ar), 66.2 (-OCH<sub>2</sub>CH-), 55.1 (-NCHCH<sub>2</sub>Ph), 37.9 (-CH<sub>2</sub>CH<sub>3</sub>) 29.2 (-CH<sub>2</sub>CH<sub>3</sub>); *m/z* (EI) 233 (M<sup>+</sup>, 28.3 %) 142 (33.3) 91 (23.3) 57 (100.0).

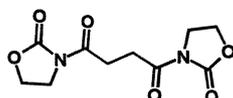
(4*S*, 2'*R*)-3-[2'-Hydroxymethyl-1'-oxopropanoyl]-  
4-benzyl-2-oxazolidinone (**76**)<sup>98</sup>



To a stirred ice-cooled solution of oxazolidinone (**75**) (1.0 g, 4.3 mmol) in dry dichloromethane (20 cm<sup>3</sup>) was added dropwise titanium (IV) chloride (0.5 cm<sup>3</sup>, 4.5 mmol) followed after a 5 minute delay by dry diisopropylethylamine (0.8 cm<sup>3</sup>, 4.6 mmol) to the resulting bright yellow slurry giving a deep blood red solution of the enolate. Stirring was continued for 1 hour at 0 °C and 1,3,5-trioxane (0.42 g, 4.7 mmol) added and a further portion of titanium (IV) chloride (0.5 cm<sup>3</sup>, 4.5 mmol) and the reaction mixture stirred for 2 hours before being quenched by the careful addition of sodium hydrogen carbonate (20 cm<sup>3</sup>). The organic phase was separated and the aqueous extracted with dichloromethane (2 x 20 cm<sup>3</sup>), the combined organics were dried (magnesium sulfate), concentrated *in vacuo*. Chromatography of the residue over silica gel [5 % methanol in chloroform] gave *the title alcohol* as a colourless oil (0.69 g, 62 %).

$\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 2900m, 1780s, 1700s, 1390m, 1350m;  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 7.30 (5 H, m, Ar-H), 4.67 (2 H, m, -CHBn and -OH), 4.18 (3 H, m, -CH<sub>2</sub>OCON- and -COCHMe), 3.85 (1 H, dd, *J* 7.6, 9.4, -CHHPh), 3.67 (1 H, dd, *J* 5.1, 9.4, -CHHPh), 3.25 (1 H, dd, *J* 3.2, 13.4, -CHHOH), 2.80 (1 H, dd, *J* 9.3, 13.4, -CHHOH), 1.2 (3 H, d, *J* 6.9, -CHMe);  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 174.9 (-NCOCHCH<sub>2</sub>OH), 153.0 (-OCON-), 135.1 (=C-, Ar), 129.3, 128.7, 127.0 (3 x =C-H, Ar), 69.4 (-CH<sub>2</sub>OCON-), 65.8 (-CH<sub>2</sub>OH), 55.0 (-NCHBn), 38.1 (-CHCH<sub>2</sub>OH), 37.6 (-CH<sub>2</sub>Ph), 13.8 (-CHCH<sub>3</sub>); *m/z* (EI) 263 (M<sup>+</sup>, 18.2 %) 245 (10.0) 228 (2.8) 178 (4.0) 154 (1.7) 117 (11.1) 86 (100.0) 69 (9.4) 59 (34.0) 51 (17.2).

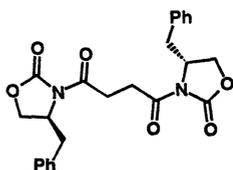
*Bis-(2-oxazolidin-3-yl)-succinate (77)*



To a solution of 2-oxazolidinone (3.8 g, 43.6 mmol) in dry tetrahydrofuran (75 cm<sup>3</sup>) at 0 °C was added slowly *n*-butyl lithium (1.6 M in hexanes; 30.0 cm<sup>3</sup>, 48.0 mmol) and after stirring for 20 minutes the reaction was cooled to -78 °C and succinyl chloride (2.3 cm<sup>3</sup>, 20.3 mmol) added dropwise and stirring continued for 4 hours. The reaction was quenched with ammonium chloride (70 cm<sup>3</sup>, saturated solution) extracted with dichloromethane (4 x 100 cm<sup>3</sup>) and the combined organic phases dried (magnesium sulfate) and concentrated *in vacuo* to give a brown residue which on trituration with cold dichloromethane/petrol (50:1) gave *the title compound* as a cream solid (3.4 g, 66 %).

m.p. 187-189 °C; (Found: M<sup>+</sup> 256.06952, C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>6</sub> requires *M* 256.07028);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  2990m, 1735s, 1640s, 1340m, 1310m, 1160m, 900m, 970m, 915m;  $\delta_{\text{H}}$  (300 MHz; DMSO-d<sub>6</sub>) 4.39 (4 H, m, -CH<sub>2</sub>OCON-), 3.88 (4 H, m, -CH<sub>2</sub>NCO-), 3.11 (4 H, s, -COCH<sub>2</sub>CH<sub>2</sub>CO-);  $\delta_{\text{C}}$  (75.4 MHz; DMSO-d<sub>6</sub>) 171.4 (-NCOCH<sub>2</sub>-), 153.6 (-OCON-), 62.3 (-CH<sub>2</sub>OCO-), 42.2 (-CH<sub>2</sub>NCO-), 29.0 (-CH<sub>2</sub>CON-); *m/z* (EI) 256 (M<sup>+</sup>, 0.3 %) 170 (100.0) 142 (20.4) 126 (18.3) 98 (11.9) 88 (24.9) 55 (55.4).

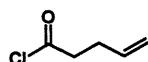
*Bis-[(4S)-4-benzyl-2-oxazolidin-3-yl]-succinate (78)*



To an ice-cooled solution of (4S)-benzyl-2-oxazolidinone (2.5 g, 14.1 mmol) in dry tetrahydrofuran (40 cm<sup>3</sup>) was added *n*-butyl lithium (1.6 M in hexanes; 11.0 cm<sup>3</sup>, 17.6 mmol) and the mixture stirred for 25 minutes before being cooled to -78 °C and succinyl chloride (0.8 cm<sup>3</sup>, 7.3 mmol) added dropwise. Stirring was continued for 4 hours and the reaction quenched with ammonium chloride (40 cm<sup>3</sup>, saturated solution) and extracted with dichloromethane (4 x 40 cm<sup>3</sup>), the combined organics were dried (magnesium sulfate) and concentrated *in vacuo* which after trituration of the residue with cold petrol/ethyl acetate (2:1) gave the title compound as a white solid (1.67 g, 54 %).

m.p. 129-131 °C; [ $\alpha$ ]<sub>D</sub> = +101.95 (*c* 0.06 CHCl<sub>3</sub>); (Found: C, 66.05, H, 5.47, N, 6.49 C<sub>24</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub> requires C, 66.05, H, 5.54, N, 6.42 %);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1790s, 1700s, 1380m, 1210m, 1190m;  $\delta_{\text{H}}$  (300 MHz; CDCl<sub>3</sub>) 7.26 (10 H, m, Ar-H), 4.67 (2 H, m, -CHBn), 4.17 (4 H, m, -CH<sub>2</sub>OCON-), 3.31 (4 H, s, -COCH<sub>2</sub>CH<sub>2</sub>CO-), 3.23 (2 H, dd, *J* 2.8, 13.4, -CHHPh), 2.80 (2 H, dd, *J* 9.3, 13.4, -CHHPh);  $\delta_{\text{C}}$  (75.4 MHz; CDCl<sub>3</sub>) 171.7 (-NCOCH<sub>2</sub>-), 153.4 (-OCON-), 135.0 (=C-, Ar), 129.3, 128.8, 127.1 (3 x =C-H, Ar), 66.2 (-CH<sub>2</sub>OCON-), 54.9 (-CHBn), 37.6, 29.9 (-NCOCH<sub>2</sub>CH<sub>2</sub>CON-); *m/z* (EI) 436 (M<sup>+</sup>, 10.3 %) 364 (19.2) 260 (27.2) 177 (7.9) 91 (87.4) 86 (100.0).

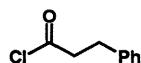
*Pent-4-enoyl chloride (79)*<sup>113</sup>



Pent-4-enoic acid (5.0 g, 49.9 mmol), thionyl chloride (5.5 cm<sup>3</sup>, 75.4 mmol) and a catalytical amount of *N,N*-dimethylformamide were stirred for 4 hours at room temperature and the excess thionyl chloride removed and the product distilled under reduced pressure to yield *the title acid chloride* as a colourless fuming liquid (4.1 g, 70 %).

b.p. 30 °C (20 mbar);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3000m, 1800s, 1400m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 5.80 (1 H, m, -CH=CH<sub>2</sub>), 5.10 (2 H, m, -CH=CH<sub>2</sub>), 3.00 (2 H, t, *J* 7.2, -CH<sub>2</sub>COCl), 2.45 (2 H, m, -CH<sub>2</sub>CH=CH<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 173.2 (-COCl), 134.7 (-CH=CH<sub>2</sub>), 116.9 (-CH=CH<sub>2</sub>), 46.2 (-CH<sub>2</sub>COCl), 28.9 (-CH<sub>2</sub>CH=CH<sub>2</sub>).

*Hydrocinnamoyl chloride (80)*<sup>113</sup>

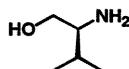


Hydrocinnamic acid (20.0 g, 133.1 mmol), thionyl chloride (20.0 cm<sup>3</sup>, 274.2 mmol) and a catalytical amount of *N,N*-dimethylformamide were combined and stirred at room temperature for 20 hours. The excess thionyl chloride was removed and the product distilled under reduced pressure to furnish *the title acid chloride* as a colourless liquid (21.2 g, 94 %).

b.p. 80-82 °C (5 mmHg); (Found: M<sup>+</sup> 168.03419, C<sub>9</sub>H<sub>9</sub><sup>35</sup>ClO, M<sup>+</sup> 170.03124 C<sub>9</sub>H<sub>9</sub><sup>37</sup>ClO, requires *M* 168.03482 (<sup>35</sup>Cl) *M* 170.03181(<sup>37</sup>Cl));  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3060s, 3025s, 1800s, 1610s, 1500s, 1460s, 1400s, 900s;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.24

(5 H, m, -Ph-H), 3.20 (2 H, t,  $J$  7.2, -CH<sub>2</sub>Ph), (2 H, t,  $J$  7.2, -CH<sub>2</sub>COCl);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 173.5 (-COCl), 139.1 (-C=, Ph), 129.2, 128.8, 127.3 (3 x =C-H, Ph), 49.0 (-CH<sub>2</sub>Ph), 31.4 (-CH<sub>2</sub>COCl);  $m/z$  (EI) 168 (M<sup>+</sup> (<sup>35</sup>Cl), 0.5 %) 150 (34.8) 133 (14.5) 104 (71.4) 91 (100.0) 77 (17.2) 65 (8.9) 51 (11.3).

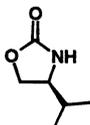
(S)-Valinol (**81**)<sup>99</sup>



To a solution of lithium borohydride (4.1 g, 189.2 mmol) in dry tetrahydrofuran (100 cm<sup>3</sup>) under nitrogen was added dropwise over 15 minutes trimethylsilyl chloride (50.0 cm<sup>3</sup>, 394.0 mmol), a precipitate of lithium chloride forms. (S)-Valine (11.12 g, 95.0 mmol) was added in portions and once the addition was complete the reaction mixture was stirred at room temperature for 24 hours. The reaction was cooled to 0 °C and quenched by the careful addition of methanol (125 cm<sup>3</sup>) the volatiles removed by distillation and the residue treated with potassium hydroxide solution (50 cm<sup>3</sup>; 20 % w/v), extracted with dichloromethane (3 x 80 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo* to yield the *title aminoalcohol* as a colourless oil (8.2 g, 84 %).

(Found: MH<sup>+</sup>(FAB) 104.10846, C<sub>5</sub>H<sub>13</sub>NO requires MH<sup>+</sup> 104.10754);  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 3400br m, 1410m, 1390m, 1370m, 1050m;  $\delta_H$  (250 MHz; CDCl<sub>3</sub>) 3.50 (1 H, dd,  $J$  3.7, 10.6, -CH<sub>2</sub>OH), 3.20 (1 H, dd,  $J$  8.4, 10.6, -CH<sub>2</sub>OH), 2.60 (3 H, br s, -OH and -NH<sub>2</sub>), 2.50 (1 H, m, -CHNH<sub>2</sub>), 1.50 (1 H, sept,  $J$  6.8, -CH(Me)<sub>2</sub>), 0.8 (6 H, d,  $J$  6.8, -CH(Me)<sub>2</sub>);  $\delta_C$  (62.9 MHz; CDCl<sub>3</sub>) 64.4 (-CH<sub>2</sub>OH), 58.9 (-CH<sub>2</sub>NH<sub>2</sub>), 31.2 (-CH(Me)<sub>2</sub>), 19.5, 18.6 (-CH(Me)<sub>2</sub>);  $m/z$  (CI) 104 (MH<sup>+</sup>, 100.0 %) 86 (11.0) 72 (96.0) 60 (43.0) 55 (13.0).

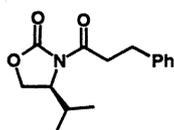
(4S)-4-Isopropyl-2-oxazolidinone (82)<sup>114</sup>



(S)-valinol (8.0 g, 77.6 mmol) diethylcarbonate (21.0 cm<sup>3</sup>, 173.3 mmol) and anhydrous potassium carbonate (1.2 g, 8.60 mmol) were combined and stirred at 140 °C for 2 hours before rearranging the apparatus and removing the ethanol produced by distillation over 2 hours. The residue was taken up in dichloromethane (100 cm<sup>3</sup>) and the organic phase washed with water (2 x 75 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated to yield *the title compound* as an off white solid which was recrystallised [petrol/ethyl acetate (4:1)] yielding a white crystalline solid (6.1 g, 61 %).

m.p. 69-71 °C; (lit<sup>114</sup> 71-71 °C); [ $\alpha$ ]<sub>D</sub> = +37.48 (c 1.24, CHCl<sub>3</sub>), (lit<sup>114</sup> +14.8 c 7.0 CHCl<sub>3</sub>); (Found: M<sup>+</sup> 129.07898, C<sub>6</sub>H<sub>11</sub>NO<sub>2</sub> requires M 129.07903);  $\nu_{\max}$ (KBr)/cm<sup>-1</sup> 3260br, 2990m, 1750s, 1410m, 1250m, 1090m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.40 (1 H, s, -NH) 4.35 (1 H, t, J 8.7, -CHNH), 4.00 (1 H, dd, J 6.4, 8.5, -CH<sub>2</sub>OCO-), 3.55 (1 H, dd, J 6.8, 14.8, -CH<sub>2</sub>OCO-) 1.65 (1 H, sept, J 6.7, -CH(Me)<sub>2</sub>), 0.85 (6H, d, J 6.7, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 161.4 (-CONH), 70.0 (-CH<sub>2</sub>OCO-), 58.6 (-CHNH), 33.0 (-CH(Me)<sub>2</sub>), 18.2, 17.9 (-CH(Me)<sub>2</sub>); m/z (EI) 129 (M<sup>+</sup>, 17.4 %) 105 (8.9) 97 (7.0) 86 (100.0) 77 (7.5) 57 (6.6).

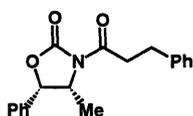
(4S)-3-[1'-Oxo-3'-phenylpropanoyl]-4-isopropyl-2-oxazolidinone (83)<sup>115</sup>



To an ice-cooled solution of (4S)-4-isopropyl-2-oxazolidinone (1.5 g, 11.6 mmol) in dry tetrahydrofuran (40 cm<sup>3</sup>) was added *n*-butyl lithium (1.6 M in hexanes; 8.0 cm<sup>3</sup>, 12.8 mmol) and the mixture stirred for 20 minutes before being cooled to -78 °C, hydrocinnamoyl chloride (1.8 cm<sup>3</sup>, 12.1 mmol) added dropwise and the reaction mixture stirred for 3 hours allowing to warm to room temperature. The reaction was quenched with sodium hydrogen carbonate (30 cm<sup>3</sup>, saturated solution), extracted with diethyl ether (3 x 40 cm<sup>3</sup>), and the combined organics dried (magnesium sulfate) concentrated *in vacuo* to yield a sticky off-white residue which on recrystallisation [petrol/ethyl acetate(40:1)] gave the title compound as a white solid (2.0 g, 67 %).

m.p. 60-62 °C (lit<sup>115</sup> 63-64 °C);  $[\alpha]_D = +70.94$  (*c* 0.89, CHCl<sub>3</sub>), (lit<sup>115</sup> +71.0 *c* 4.61 CH<sub>2</sub>Cl<sub>2</sub>); (Found: C 68.85, H 7.28, N 5.35, M<sup>+</sup> 261.13649, C<sub>15</sub>H<sub>19</sub>NO<sub>3</sub> requires C 68.94, H 7.33, N 5.36 %, *M* 261.13657);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  3100m, 1790s, 1600m, 1480m, 1380m, 1200s, 1000m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.44(5 H, m, Ar-*H*), 4.58 (1 H, m, -CH<sub>2</sub>CHNCO-), 4.37 (2 H, m, -CH<sub>2</sub>OCON-), 3.45 (2 H, m, -CH<sub>2</sub>CH<sub>2</sub>Ph), 3.16 (2 H, m, -CH<sub>2</sub>Ph), 2.51 (1 H, d x sept, *J* 6.97, 3.89, -CH(Me)<sub>2</sub>), 1.14 (6 H, dd, *J* 6.9, 15.8 -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.8 (-NCOCH<sub>2</sub>-), 154.5 (-OCON-), 140.9 (=C-, Ar), 129.0, 128.9, 126.6 (3 x =C-H, Ar), 63.8 (-CH<sub>2</sub>OCON-), 58.8 (-CH<sub>2</sub>CHNCO-) 37.5 (-COCH<sub>2</sub>-), 30.8 (-CH<sub>2</sub>Ph), 28.8 (-CH(Me)<sub>2</sub>) 18.3, 15.0 (-CH(CH<sub>3</sub>); *m/z* (EI) 261 (M<sup>+</sup>, 64.6 %) 193 (4.1) 150 (15.0) 142 (9.8) 130 (94.8) 104 (100.0) 91 (77.7) 77 (16.3) 65 (9.9) 51 (6.2).

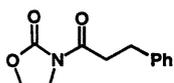
(4*S*, 5*R*)-3-[1'-Oxo-3'-phenylpropanoyl]-4-methyl-5-phenyl-2-oxazolidinone (**84**)<sup>115</sup>



A solution of (4*S*, 5*R*)-4-methyl-5-phenyl-2-oxazolidinone (2.0 g, 11.3 mmol) in dry tetrahydrofuran (40 cm<sup>3</sup>) was cooled to 0 °C, *n*-butyl lithium (1.6 M in hexanes; 7.2 cm<sup>3</sup>, 11.5 mmol) added and the solution stirred for 20 minutes before the temperature was reduced to -78 °C and hydrocinnamoyl chloride (1.8 cm<sup>3</sup>, 12.1 mmol) added dropwise. Stirring was continued for 2 hours and the reaction quenched with sodium hydrogen carbonate (30 cm<sup>3</sup>, saturated solution), extracted with diethyl ether (3 x 30 cm<sup>3</sup>) and the combined organics dried (magnesium sulfate) and concentrated. The crude product was recrystallised [petrol/ethyl acetate (14:3)] to give *the title compound* as a white solid (2.67 g, 76 %).

m.p. 97-98 °C (lit<sup>115</sup> 95-96 °C); (Found: C 73.74, H 6.18, N 4.52 M<sup>+</sup> 309.13649, C<sub>19</sub>H<sub>19</sub>NO<sub>3</sub> requires C 73.77, H 6.19, N 4.52 %, M 309.13657);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  3000m, 1780s, 1700s, 1380m, 1200m, 770m, 755m, 700m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.30 (10 H, m, Ar-H), 5.58 (1H, d, *J* 7.3, -OCH(Ph)-), 4.73 (1 H, qu, *J* 6.7, -NCH(Me)-), 3.27 (2 H, m, -COCH<sub>2</sub>CH<sub>2</sub>Ph), 3.00 (2 H, m, -CH<sub>2</sub>Ph), 0.87 (3 H, d, *J* 6.7, -NCH(Me)-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.1 (-NCOCH<sub>2</sub>-), 153.0 (-OCON-), 140.4, 133.3 (2 x =C-, Ar), 128.7, 128.5, 128.4, 126.2, 125.6 (5 x =C-H, Ar), 79.0 (-OCH(Ph)-), 54.7 (-NCH(Me)-), 37.2 (-NCOCH<sub>2</sub>-), 30.3 (-CH<sub>2</sub>Ph), 14.5 (-NCH(Me)-), (-NCOCH<sub>2</sub>CH<sub>2</sub>Ph), 28.8 (-CH(Me)<sub>2</sub>), 18.4, 15.0 (-CH(Me)<sub>2</sub>); *m/z* (EI) 309 (M<sup>+</sup>, 68.9 %) 219 (32.7) 180 (14.0) 159 (42.0) 133 (21.5) 118 (69.1) 104 (91.5) 91 (85.2) 61 (100.0) 51 (9.2).

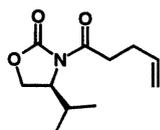
3-[1'-Oxo-3'-phenylpropanoyl]-  
2-oxazolidinone (85)<sup>116</sup>



To an ice-cooled solution of 2-oxazolidinone (2.0 g, 22.9 mmol) in dry tetrahydrofuran (50 cm<sup>3</sup>) was added *n*-butyllithium (1.6 M in hexanes; 15.0 cm<sup>3</sup>, 24.0 mmol), after stirring for 25 minutes the reaction mixture was cooled to -78 °C and hydrocinnamoyl chloride (3.6 cm<sup>3</sup>, 24.2 mmol) added slowly. Stirring was continued for 3 hours before the reaction was quenched with sodium hydrogen carbonate (50 cm<sup>3</sup>, saturated solution) and extracted with diethyl ether (3 x 60 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo* to give the title compound as a white solid which was recrystallised from petrol/ethyl acetate (8:3) (3.4 g, 67 %).

m.p. 101-103 °C (Found: C 65.63, H 5.94, N 6.37, M<sup>+</sup> 219.08954, C<sub>12</sub>H<sub>13</sub>NO<sub>3</sub> requires C 65.74, H 5.98 N 6.39 %, M 219.08959);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  3000m, 1790s, 1700s, 1390m, 1320m, 755m, 700m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.30 (5 H, m, Ar-H), 4.35 (2 H, t, *J* 8.0, -CH<sub>2</sub>OCON-), 3.98 (2 H, t, *J* 8.0, -CH<sub>2</sub>NCOO-), 3.25 (2 H, t, *J* 7.6, -COCH<sub>2</sub>-), 2.97 (2 H, t, *J* 7.6, -CH<sub>2</sub>Ph);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.5 (-NCOCH<sub>2</sub>-), 153.5 (-OCON-), 140.5 (=C-, Ar), 128.5, 128.3, 126.2 (3 x =C-H, Ar), 62.1 (-CH<sub>2</sub>OCON-) 42.5 (-CH<sub>2</sub>NCO-), 36.8 (-COCH<sub>2</sub>CH<sub>2</sub>Ph), 30.6 (-CH<sub>2</sub>Ph); *m/z* (EI) 219 (M<sup>+</sup>, 62.2 %) 132 (19.0) 104 (100.0) 88 (53.9) 77 (13.6) 65 (7.2) 51 (6.5).

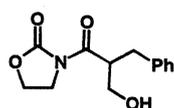
(4S)-3-[1'-Oxo-4'-pentenyl]-4-isopropyl-2-oxazolidinone (**86**)<sup>117</sup>



To a solution of (4S)-4-isopropyl-2-oxazolidinone (2.0 g, 15.5 mmol) in dry tetrahydrofuran (40 cm<sup>3</sup>) at 0 °C was added *n*-butyl lithium (1.6 M in hexanes; 10.0 cm<sup>3</sup>, 16.0 mmol) and the mixture stirred for 25 minutes, cooled to -78 °C and pent-4-enoyl chloride (1.9 g, 16.0 mmol) added slowly. Stirring was continued for 2 hours, the reaction quenched with sodium hydrogen carbonate (30 cm<sup>3</sup>, saturated solution) and extracted with diethyl ether (3 x 40 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo* to give the title compound as a colourless liquid which required no further purification (3.2 g, 97 %).

(Found: M<sup>+</sup>, 211.12085, C<sub>11</sub>H<sub>17</sub>NO<sub>3</sub> requires M 211.12091);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  2980m, 1790s, 1705s, 1380m, 1200m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 5.71 (1 H, m, -CH=CH<sub>2</sub>), 4.90 (2 H, m, -CH=CH<sub>2</sub>), 4.27 (1 H, m, -CHNCO-), 4.10 (2 H, m, -CH<sub>2</sub>OCON-), 2.90 (2 H, m, -NCOCH<sub>2</sub>-), 2.25 (3 H, m, -CH<sub>2</sub>CH=CH<sub>2</sub> and -CH(Me)<sub>2</sub>), 0.75 (6 H, dd, *J* 7.0, 10.8, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 172.9 (-NCOCH<sub>2</sub>-), 154.5 (-OCON-), 137.1 (-CH=CH<sub>2</sub>), 116.0 (-CH=CH<sub>2</sub>), 63.8 (-CH<sub>2</sub>OCON-), 58.8 (-CHNCO-), 35.1 (-COCH<sub>2</sub>CH<sub>2</sub>-), 28.8, 28.7 (-CH<sub>2</sub>CH=CH<sub>2</sub> and -CH(Me)<sub>2</sub>), 18.3, 15.0 (-CH(Me)<sub>2</sub>); *m/z* (EI) 211 (M<sup>+</sup>, 31.7 %) 183 (1.5) 168 (3.0) 143 (6.7) 130 (71.1) 100 (6.2) 85 (16.4) 69 (12.7) 55 (100.0).

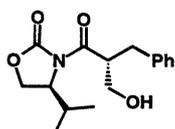
3-[2'-Hydroxymethyl-1'-oxo-3'-phenyl  
propanoyl]-2-oxazolidinone (87)



To an ice-cooled solution of oxazolidinone (85) (1.0 g, 4.6 mmol) in dry dichloromethane (10 cm<sup>3</sup>) under nitrogen was added titanium (IV) chloride (0.6 cm<sup>3</sup>, 5.5 mmol) giving a yellow slurry to which was added dry diisopropylethylamine (1.0 cm<sup>3</sup>, 5.7 mmol) and the resulting deep red enolate solution stirred for 1 hour at 0 °C before the addition of 1,3,5-trioxane (0.8 g, 8.9 mmol) in dry dichloromethane (10 cm<sup>3</sup>) and stirring continued for 24 hours at room temperature. The reaction was quenched with ammonium chloride (10 cm<sup>3</sup>, saturated solution) phases separated and the aqueous extracted with dichloromethane (2 x 10 cm<sup>3</sup>), the combined organics dried (magnesium sulfate) and concentrated. Chromatography of the residue over silica gel [5 % methanol in dichloromethane] furnished the title alcohol as a colourless oil (0.42 g, 36 %).

(Found: M<sup>+</sup> 249.10012, C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub> requires M 249.10015);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3600br, 3000m, 1780s, 1700s, 1400m, 760m, 700m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.27 (5 H, m, Ar-H) 4.30 (3 H, m, -CH<sub>2</sub>OCON- and -CHCH<sub>2</sub>OH), 3.95 (2 H, m, -CH<sub>2</sub>OH), 3.75 (2 H, m, -CH<sub>2</sub>NCOO-), 3.05 (1 H, dd, J 6.7, 13.3, -CHHPh), 2.85 (1 H, dd, J 8.4, 13.3, -CHHPh), 2.45 (1 H, s, -OH);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 174.4 (-NCOCH-), 153.8 (-OCON-), 138.7 (=C-, Ar), 129.5, 128.8, 126.9 (3 x =C-H, Ar), 67.9, 62.4 (-CH<sub>2</sub>OCON- and -CH<sub>2</sub>OH), 45.2 (-CHCH<sub>2</sub>OH), 43.1 (-CH<sub>2</sub>NCO-), 35.5 (-CH<sub>2</sub>Ph); m/z (EI) 249 (M<sup>+</sup>, 10.9 %) 231 (M<sup>+</sup>-H<sub>2</sub>O, 33.0 %) 218 (19.9) 176 (4.0) 144 (100.0) 131 (44.0) 116 (20.0) 103 (14.9) 91 (64.0) 77 (11.5) 65 (9.1) 51 (4.8).

(4*S*, 2'*R*)-3-[2'-Hydroxymethyl-1'-oxo-3'-phenyl  
propanoyl]-4-isopropyl-2-oxazolidinone (**88**)

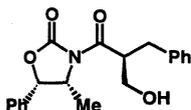


To an ice-cooled solution of oxazolidinone (**83**) (1.0 g, 3.83 mmol) in dry dichloromethane (10 cm<sup>3</sup>) was added titanium (IV) chloride (0.5 cm<sup>3</sup>, 4.55 mmol) giving a yellow slurry, followed after minute delay by dry diisopropylethylamine (0.8 cm<sup>3</sup>, 4.60 mmol) and the resulting deep blood red enolate solution stirred at 0 °C for 1 hour. 1,3,5-Trioxane (0.4 g, 4.44 mmol) was added in dry dichloromethane (10 cm<sup>3</sup>) and the reaction mixture stirred at room temperature for 24 hours before being quenched by the careful addition of ammonium chloride (10 cm<sup>3</sup>, saturated solution). The phases were separated and the aqueous extracted with dichloromethane (2 x 10 cm<sup>3</sup>), the combined organics dried (magnesium sulfate), and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (1:1)] furnished the title alcohol as a white solid (1.1 g, 89 %).

m.p. 83-85 °C; [ $\alpha$ ]<sub>D</sub> = +116.0 (*c* 0.5, CHCl<sub>3</sub>); (Found: M<sup>+</sup> 291.14706, C<sub>16</sub>H<sub>21</sub>NO<sub>4</sub> requires *M* 291.14713);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3500br, 3000m, 1780s, 1700s, 1380m, 1300m, 1200m, 905m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.25 (5 H, m, Ar-*H*), 4.34 (2 H, m, -CH<sub>2</sub>OH), 4.09 (2 H, m, -CHNCOO- and -CHCH<sub>2</sub>OH), 3.85 (2 H, m, -CH<sub>2</sub>OCON-) 3.00 (1 H, dd, *J* 5.8, 13.4, -CH<sub>2</sub>Ph) 2.94 (1 H, dd, *J* 5.6, 13.4, -CH<sub>2</sub>Ph), 2.36 (1 H, m, -CH(Me)<sub>2</sub>), 2.05 (1 H, d, *J* 5.9, -OH), 0.89 (6 H, t, *J* 7.4, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 175.5 (-NCOCH-), 154.5 (-OCON-), 138.9 (=C-, Ar), 129.5, 128.8, 126.9 (3 x =C-H, Ar), 63.9, 63.7 (2 x -CH<sub>2</sub>O-), 59.2 (-CH<sub>2</sub>CHNCO-), 47.4 (-CHCH<sub>2</sub>OH), 34.9 (-CH<sub>2</sub>Ph), 29.0 (-CH(Me)<sub>2</sub>), 15.1, 14.6 (-CH(Me)<sub>2</sub>); *m/z* (EI) 291

(M<sup>+</sup>, 8.0 %) 273 (32.8) 260 (31.5) 169 (7.2) 144 (100.0) 131 (54.4) 116 (17.7) 105 (14.9) 91 (74.2) 78 (8.2) 65 (7.4).

(4S, 5R, 2'S)-3-[2'-Hydroxymethyl-1'-oxo-3'-phenyl  
propanoyl]-4-methyl-5-phenyl-2-oxazolidinone (89)

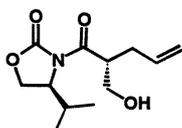


To an ice-cooled solution of oxazolidinone (84) (1.0 g, 3.2 mmol) in dry dichloromethane (10 cm<sup>3</sup>) was added titanium (IV) chloride (0.4 cm<sup>3</sup>, 3.6 mmol) followed after a 5 minute delay to the yellow slurry by dry diisopropylethylamine (0.6 cm<sup>3</sup>, 3.44 mmol) giving a deep blood red solution of the titanium enolate. Stirring was continued for 1 hour at 0 °C and 1,3,5-trioxane (0.35 g, 3.9 mmol) added in dry dichloromethane (10 cm<sup>3</sup>), the reaction mixture stirred for a further 24 hours at room temperature before being quenched by the careful addition of ammonium chloride (5 cm<sup>3</sup>, saturated solution) and the organic phase separated. The aqueous phase was extracted with dichloromethane (2 x 10 cm<sup>3</sup>) and the combined organic phases were dried (magnesium sulfate), concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/ethyl acetate (3:1)] yielded *the title alcohol* as a white foam (0.6 g, 55 %).

(Found: M<sup>+</sup> 339.14702, C<sub>20</sub>H<sub>21</sub>NO<sub>4</sub> requires M 339.14713);  $\nu_{\max}(\text{KBr})/\text{cm}^{-1}$  3500br, 3000m, 1780s, 1700s, 1450s, 1340m, 1200m, 700m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 7.30 (10 H, m, Ar-H), 5.33 (1 H, d, *J* 7.1, -OCH(Ph)-), 4.58 (1 H, m, -NCH(Me)-), 4.33 (1 H, m, -CHCH<sub>2</sub>OH), 3.83 (2 H, m, -CH<sub>2</sub>OH), 2.95 (2 H, m, -CH<sub>2</sub>Ph), 2.75 (1 H, br s, -OH), 0.87 (3 H, d, *J* 6.6, -CH(Me)-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 173.1 (-NCOCH-), 152.6 (-OCON-), 138.4, 133.2 (2 x =C-, Ar), 129.1, 128.7, 128.6, 128.4,

126.6, 125.6 (6 x =C-H, Ar), 78.7 (-OCH(Ph)-), 68.0 (-CH<sub>2</sub>OH), 54.9 (-NCH(Me)-), 45.1(-CHCH<sub>2</sub>OH), 35.5 (-CH<sub>2</sub>Ph), 14.5 (-NCH(Me)-); *m/z* (EI) 339 (M<sup>+</sup>, 16.7 %) 321 (M<sup>+</sup> - H<sub>2</sub>O, 59.3) 308 (54.0) 217 (24.8) 160 (20.7) 144 (38.8) 131 (68.2) 118 (100.0) 105 (21.7) 91 (90.5) 51 (20.8).

(4*S*, 2'*R*)-3-[2'-Hydroxymethyl-1'-oxo-pent-4-enoyl]-  
4-isopropyl-2-oxazolidinone (**90**)

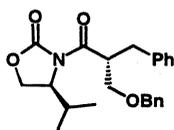


To a stirred solution of oxazolidinone (**86**) (1.0 g, 4.7 mmol) in dry dichloromethane (20 cm<sup>3</sup>) at 0 °C was added dropwise titanium (IV) chloride (0.6 cm<sup>3</sup>, 5.5 mmol) and to the resulting yellow slurry after a 5 minute delay dry diisopropylethylamine (1.0 cm<sup>3</sup>, 5.7 mmol) giving a deep blood red solution which was stirred for 1 hour and 1,3,5-trioxane (0.5 g, 5.5 mmol) added in dry dichloromethane (10 cm<sup>3</sup>). The reaction mixture was stirred for 24 hours at room temperature, quenched with ammonium chloride (10 cm<sup>3</sup>, saturated solution), the phases separated and the aqueous extracted with dichloromethane (2 x 10 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography the residue over silica gel [petrol/diethyl ether (1:1)] furnished *the title alcohol* as a colourless oil (0.55 g, 48 %).

(Found: M<sup>+</sup> 241.13143, C<sub>16</sub>H<sub>21</sub>NO<sub>4</sub> requires *M* 241.131147);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3600br, 3000m, 1780s, 1700s, 1640m, 1380m, 1200m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 5.77 (1 H, m, -CH=CH<sub>2</sub>), 5.05 (2 H, m, -CH=CH<sub>2</sub>), 4.45 (1 H, m, -CH<sub>2</sub>CHNCO-), 4.25 (3 H, m, -CH<sub>2</sub>OH and -CHCON-), 3.70 (2 H, m, -CH<sub>2</sub>OCON-), 2.35 (3 H, m, -CH<sub>2</sub>CH=CH<sub>2</sub> and -CH(Me)<sub>2</sub>), 0.90 (6 H, m, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>)

174.4 (-COCH-), 154.2 (-OCON-), 135.3 (-CH=CH<sub>2</sub>), 117.7 (-CH=CH<sub>2</sub>), 68.8, 63.6 (-CH<sub>2</sub>OH and -CH<sub>2</sub>OCON-), 58.9 (-CHNCO-), 43.3 (-CHCH<sub>2</sub>OH), 33.4 (-CH<sub>2</sub>CH=CH<sub>2</sub>), 28.9 (-CH(Me)<sub>2</sub>), 18.2, 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 241 (M<sup>+</sup>, 5.6 %) 223 (21.7) 210 (36.0) 196 (9.8) 169 (12.2) 130 (94.5) 113 (27.2) 95 (60.6) 86 (100.0) 67 (45.9) 55 (58.5).

(4*S*, 2'*R*)-3-[2'-Benzyloxymethyl-1'-oxo-3'-phenylpropanoyl]-  
4-isopropyl-2-oxazolidinone (**91**)

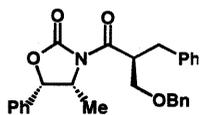


To an ice-cooled solution of oxazolidinone (**83**) (7.4 g, 28.3 mmol) in dry dichloromethane (40 cm<sup>3</sup>) was added titanium (IV) chloride (3.7 cm<sup>3</sup>, 33.7 mmol), and to the resulting yellow slurry after a 5 minute delay dry diisopropylethylamine (6.0 cm<sup>3</sup>, 34.4 mmol) and the deep blood red enolate solution stirred for 1 hour. Benzylchloromethyl ether (9.0 cm<sup>3</sup>, 64.7 mmol) was added and the reaction mixture stirred at room temperature for 24 hours before being quenched by ammonium chloride (60 cm<sup>3</sup>, saturated solution) and the phases separated. The aqueous phase was extracted with dichloromethane (2 x 40 cm<sup>3</sup>) and the combined organic phases dried (magnesium sulfate), and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (4:1)] yielded *the title compound* as a white solid (6.7 g, 62 %).

m.p. 73-75 °C [petrol/ether (3:1)]; [α]<sub>D</sub> = +59.4 (*c* 0.1, CHCl<sub>3</sub>); (Found: C 72.28, H 6.83, N 3.68, M<sup>+</sup> 381.19413, C<sub>23</sub>H<sub>27</sub>NO<sub>4</sub> requires C 72.42, H 7.13, N 3.67 *M* 381.19411); ν<sub>max</sub>(KBr)/cm<sup>-1</sup> 2950m, 1780s, 1710s, 1390m, 1360m, 1230m, 1200s, 1150m; δ<sub>H</sub> (250 MHz; CDCl<sub>3</sub>) 7.30 (10 H, m, Ar-*H*), 4.55 (3 H, m, -CH<sub>2</sub>OBn and -

CHCH<sub>2</sub>Ph), 4.35 (1 H, m, -NCHCH<sub>2</sub>O-), 4.11 (1 H, dd, *J* 5.9, 9.0, -CHHOCO-), 4.03 (1 H, dd, *J* 9.0, 17.0 -CHHOCO-), 3.82 (1 H, dd, *J* 1.7, 9.2, -CHHOBn), 3.67 (1 H, dd, *J* 4.4, 9.2, -CHHOBn), 2.95 (2 H, dd, *J* 7.3, 4.8, -CH<sub>2</sub>Ph), 2.33 (1 H, d sept, *J* 4.0, 6.9, -CH(Me)<sub>2</sub>), 0.89 (3 H, d, *J* 6.9, -CH(Me)<sub>2</sub>) 0.84 (3 H, d, *J* 6.9, -CH(Me)<sub>2</sub>); δ<sub>C</sub> (62.9 MHz; CDCl<sub>3</sub>) 174.5 (-NCOCH-), 154.2 (-OCON-), 139.1, 138.5 (2 x =C-, Ar), 129.6, 128.9, 128.0, 127.4, 126.9 (5 x =C-H, Ar), 73.5 (-CH<sub>2</sub>OBn), 71.3 (-OCH<sub>2</sub>Ph), 65.5 (-CH<sub>2</sub>OCON-) 59.0 (-CHNCO-), 45.6 (-CHCH<sub>2</sub>Ph), 35.3 (-CHCH<sub>2</sub>Ph), 29.0 (-CH(Me)<sub>2</sub>), 18.3, 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 381 (M<sup>+</sup>, 5.9 %) 290 (4.6) 273 (13.7) 184 (22.5) 171 (12.1) 143 (36.4) 131 (30.3) 117 (12.6) 91 (100.0) 55 (9.3).

(4R, 5S, 2'R)-3-[2'-Benzyloxymethyl-1'-oxo-3'-phenyl  
propanoyl]-4-methyl-5-phenyl-2-oxazolidinone (92)

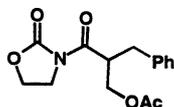


To an ice-cooled solution of oxazolidinone (84) (1.0 g, 3.2 mmol) in dry dichloromethane (15 cm<sup>3</sup>) was added titanium (IV) chloride (0.4 cm<sup>3</sup>, 3.6 mmol) followed after a 5 minute delay to the orange/yellow slurry by diisopropylethylamine (0.6 cm<sup>3</sup>, 3.4 mmol) and the reaction mixture stirred for 1 hour giving a blood red solution of the enolate to which was added benzylchloromethyl ether (1.0 cm<sup>3</sup>, 7.2 mmol) and the reaction mixture stirred for 24 hours at room temperature. The reaction was quenched by the careful addition of ammonium chloride (10 cm<sup>3</sup>, saturated solution) and the organic phase separated. The aqueous was extracted with dichloromethane (2 x 20 cm<sup>3</sup>), the organics phases were combined, dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue

over silica gel [petrol/diethyl ether (4:1)] yielded the title compound as a colourless oil (0.7 g, 51 %).

(Found:  $M^+$  429.19411,  $C_{27}H_{27}NO_4$  requires  $M$  429.19411);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3100br, 1780s, 1700s, 1490m, 1450m;  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 7.40 (15 H, m, Ar-H), 5.30 (1 H, d,  $J$  8.0, -OCH(Ph)-), 4.60 (4 H, m, -CH<sub>2</sub>OBn and -OCH<sub>2</sub>Ph), 3.90 (1 H, m, -COCHCH<sub>2</sub>Ph), 3.75 (1 H, m, -NCH(Me)-), 3.10 (2 H, m, -CH<sub>2</sub>Ph), 0.90 (3 H, d,  $J$  7.0, -NCH(Me)-);  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 174.3 (-NCOCH-), 153.1 (-OCON-), 139.1, 138.9, 138.7 (3 x =C-, Ar), 129.7, 129.1, 129.0, 128.8, 128.5, 128.3, 128.1, 127.1, 126.2 (9 x =C-H, Ar), 79.1 (-OCH(Ph)-), 73.5 (-CH<sub>2</sub>OBn), 71.1 (-OCH<sub>2</sub>Ph), 55.3 (-NCH(Me)-), 45.7 (-CHCH<sub>2</sub>Ph), 35.8 (-CH<sub>2</sub>Ph), 15.0 (-NCH(Me)-);  $m/z$  (EI) 429 ( $M^+$ , 3.4 %) 338 (4.9) 321 (12.1) 308 (10.7) 276 (3.6) 232 (20.8) 219 (8.7) 178 (7.5) 160 (33.6) 131 (20.5) 118 (100.0) 65 (6.7).

3-[2'-Acetoxymethyl-1'-oxo-3'-phenyl  
propanoyl]-2-oxazolidinone (93)

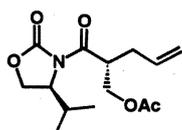


To a solution of alcohol (**87**) (0.25 g, 1.0 mmol) in dichloromethane (10 cm<sup>3</sup>) was added diisopropylethylamine (0.6 cm<sup>3</sup>, 3.4 mmol), acetic anhydride (0.3 cm<sup>3</sup>, 3.2 mmol) and a catalytical amount of 4-dimethylaminopyridine and the reaction mixture stirred at room temperature for 24 hours. The reaction was quenched with dilute hydrochloric acid (20 cm<sup>3</sup>, 2 M), the organic phase separated and washed with water (2 x 10 cm<sup>3</sup>), sodium hydrogen carbonate solution (2 x 10 cm<sup>3</sup>), water (2 x 10 cm<sup>3</sup>) dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the

residue over silica gel [petrol/diethyl ether (5:2)] yielded the *title compound* as a colourless oil (172 mg, 60 %).

$\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 7.32 (5 H, m, Ar-H), 4.40 (5 H, m,  $-\text{CH}_2\text{OCON}-$  and  $-\text{CHCH}_2\text{OAc}$ ), 4.00 (2 H, m,  $-\text{CH}_2\text{NCO}-$ ), 3.10 (1 H, dd,  $J$  7.0, 13.5,  $-\text{CHHPh}$ ), 2.87 (1 H, dd,  $J$  8.0, 13.5,  $-\text{CHHPh}$ ), 2.07 (3 H, s,  $-\text{COMe}$ );  $\delta_{\text{C}}$  (62.9 MHz;  $\text{CDCl}_3$ ) 174.1, 171.1 ( $-\text{NCOCH}-$  and  $-\text{COMe}$ ), 153.6 ( $-\text{OCON}-$ ), 138.3 ( $=\text{C}-$ , Ar), 129.5, 128.8, 127.1 (3 x  $=\text{C}-\text{H}$ , Ar), 64.1, 62.4 ( $-\text{CH}_2\text{OCON}-$  and  $-\text{CH}_2\text{OAc}$ ), 45.5 ( $-\text{CHCH}_2\text{OAc}$ ), 43.1 ( $-\text{CH}_2\text{NCO}-$ ), 35.4 ( $-\text{CH}_2\text{Ph}$ ), 21.4 ( $-\text{COMe}$ ).

(4*S*, 2'*R*)-3-[2'-Acetoxymethyl-1'-oxo-pent-4-enyl]-  
4-isopropyl-2-oxazolidinone (**94**)

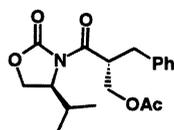


To a solution of alcohol (**90**) (460 mg, 1.9 mmol) in dichloromethane (20  $\text{cm}^3$ ) was added triethylamine (0.8  $\text{cm}^3$ , 5.7 mmol), acetic anhydride (0.5  $\text{cm}^3$ , 5.3 mmol) and a catalytic amount of 4,4-dimethylaminopyridine and the reaction mixture stirred at room temperature for 24 hours. Dilute hydrochloric acid (20  $\text{cm}^3$ , 2 M) was added and the organic phase separated and washed with water (3 x 20  $\text{cm}^3$ ), sodium hydrogen carbonate solution (2 x 20  $\text{cm}^3$ ), water (2 x 20  $\text{cm}^3$ ), dried and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (2:1)] gave the *title compound* as a colourless oil (335 mg, 62 %).

$\nu_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1780s, 1700s, 1380m, 1200m, 900m;  $\delta_{\text{H}}$  (250 MHz;  $\text{CDCl}_3$ ) 5.91 (1 H, m,  $-\text{CH}=\text{CH}_2$ ), 5.17 (2 H, m,  $-\text{CH}=\text{CH}_2$ ), 4.60 (1 H, m,  $-\text{CHNCO}-$ ), 4.37 (3 H, m,  $-\text{CH}_2\text{OAc}$  and  $-\text{CHCH}_2\text{CH}=\text{CH}_2$ ), 3.91 (1 H, dd,  $J$  1.6, 9.5,  $-\text{CH}_2\text{OCON}-$ ), 3.78 (1 H, dd,  $J$  4.6, 9.5,  $-\text{CH}_2\text{OCON}-$ ), 2.47 (3 H, m,  $-\text{CH}(\text{Me})_2$ )

and  $-CH_2CH=CH_2$ ), 2.36 (3 H, s,  $-COMe$ ), 1.00 (3 H, d,  $J$  7.0,  $-CH(Me)_2$ ), 0.9 (3 H, d,  $J$  7.0  $-CH(Me)_2$ );  $\delta_C$  (62.9 MHz;  $CDCl_3$ ) 174.0 ( $-NCOCH_2-$ ), 166.8 ( $-OCOMe$ ), 154.3 ( $-OCON-$ ), 135.3 ( $-CH=CH_2$ ), 117.7 ( $-CH=CH_2$ ), 68.9, 63.6 ( $-CH_2OAc$  and  $-CH_2OCO-$ ), 58.9 ( $-CH_2CHNCO-$ ), 43.3 ( $-CHCH_2OAc$ ), 33.4 ( $-CH_2CH=CH_2$ ), 28.8 ( $-CH(Me)_2$ ), 22.6 ( $-COMe$ ), 18.3, 15.0 ( $-CH(Me)_2$ ).

(4*S*, 2'*R*)-3-[2'-Acetoxymethyl-1'-oxo-3'-phenylpropanoyl]-  
4-isopropyl-2-oxazolidinone (**95**)

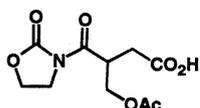


To a solution of oxazolidinone (**88**) (5.0 g, 17.3 mmol) in dichloromethane (15 cm<sup>3</sup>) was added pyridine (10 cm<sup>3</sup>, 123.6 mmol) and acetic anhydride (10 cm<sup>3</sup>, 106.0 mmol) and the reaction mixture stirred for 24 hours. Hydrochloric acid (80 cm<sup>3</sup>, 2 M) was added and after stirring for 30 minutes the organic layer was separated and washed with water (3 x 75 cm<sup>3</sup>), sodium hydrogen carbonate solution (2 x 75 cm<sup>3</sup>) and water (2 x 75 cm<sup>3</sup>), and the organic phase dried (magnesium sulfate). Chromatography of the residue was over silica gel [petrol/diethyl ether (2:1)] yielded the title compound as a colourless oil (5.2 g, 90 %).

$[\alpha]_D = +8.3$  (c 1.52,  $CHCl_3$ ); (Found:  $M^+$  333.15756,  $C_{18}H_{23}NO_5$  requires  $M$  333.15769);  $\nu_{max}$ (neat)/cm<sup>-1</sup> 3000m, 1780s, 1740s, 1700s, 1380m, 1300s, 1220m;  $\delta_H$  (250 MHz;  $CDCl_3$ ) 7.20 (5 H, m, Ar-*H*), 4.50 (1 H, m,  $-CHNCO-$ ), 4.26 (3 H, m,  $-CH_2OAc$  and  $-CHCH_2Ph$ ), 4.07 (1 H, dd,  $J$  6.3, 9.0,  $-CHHOCON-$ ), 3.95 (1 H, dd,  $J$  8.5, 9.0,  $-CHHOCON-$ ), 2.95 (1 H, dd,  $J$  5.7, 13.5,  $-CHHPH$ ), 2.76 (1 H, dd,  $J$  5.8, 13.5,  $-CHHPH$ ), 2.30 (1 H, m,  $-CH(Me)_2$ ), 1.95 (3 H, s,  $-COMe$ ), 0.85 (6 H, t,  $J$  6.2,  $-CH(Me)_2$ );  $\delta_C$  (62.9 MHz;  $CDCl_3$ ) 173.6 ( $-NCOCH-$ ), 170.8 ( $-OCOMe$ ), 154.0 (-

OCON-), 138.2 (=C-, Ar), 129.4, 128.9, 127.1 (3 x =C-H, Ar), 64.9, 63.8 (-CH<sub>2</sub>OAc  
 and -CH<sub>2</sub>OCON-), 59.0 (-CHNCO-) 44.4 (-CHCH<sub>2</sub>OAc), 35.4 (-CH<sub>2</sub>Ph), 28.0 (-  
 CH(Me)<sub>2</sub>), 21.0 (-OCOMe), 18.1, 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 333 (M<sup>+</sup>, 0.4 %) 273  
 (48.9) 260 (3.3) 169 (8.9) 144 (100.0) 131 (18.9) 117 (31.6) 103 (6.8) 91 (29.6) 77  
 (4.0) 55 (5.2).

3-[2'-Acetoxymethyl-3'-carboxy-1'-oxo-  
 propanoyl]-2-oxazolidinone (**96**)

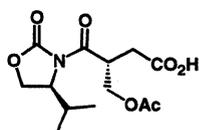


To a solution of oxazolidinone (**93**) (170 mg, 0.6 mmol) in water (3 cm<sup>3</sup>), acetonitrile  
 (2 cm<sup>3</sup>), and carbon tetrachloride (2 cm<sup>3</sup>) was added periodic acid (2.0 g, 8.8 mmol)  
 and ruthenium (III) chloride (6 mg, 0.024 mmol) and the reaction mixture stirred  
 vigorously for 3 hours at room temperature before the reaction was quenched with  
 diethyl ether (15 cm<sup>3</sup>) and the phases separated. The aqueous was re-extracted with  
 ether (2 x 10 cm<sup>3</sup>) and the combined organic phases dried (sodium sulfate), filtered  
 through Celite and the filtrate concentrated *in vacuo*. Chromatography of the residue  
 over silica gel [5 % methanol in dichloromethane] yielded *the title acid* as a  
 colourless oil (50 mg, 32 %).

$\nu_{\max}$ (neat)/cm<sup>-1</sup> 3500br, 3000m, 1780s, 1740s, 1710m, 1380m;  $\delta_{\text{H}}$  (250 MHz;  
 CDCl<sub>3</sub>) 9.30 (1 H, br s, -CO<sub>2</sub>H), 4.45 (4 H, m, -CH<sub>2</sub>OCON- and -CH<sub>2</sub>OAc), 4.05 (3  
 H, m, -CH<sub>2</sub>NCO- and -CHCH<sub>2</sub>OAc), 3.08 (1 H, dd, *J* 10.0, 17.5, -CHHCO<sub>2</sub>H), 2.60  
 (1 H, dd, *J* 4.5, 17.5, -CHHCO<sub>2</sub>H), 2.05 (3 H, s, -COMe);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>)  
 176.5, 172.2, 170.9 (-CO<sub>2</sub>H and -OCOMe and -NCOCH-), 153.5 (-OCON-), 63.5,  
 62.3 (-CH<sub>2</sub>OCON- and -CH<sub>2</sub>OAc), 42.8 (-CH<sub>2</sub>NCO-), 39.8 (-CHCH<sub>2</sub>OAc), 32.8

(-CH<sub>2</sub>CO<sub>2</sub>H), 20.7 (-COMe).

(4*S*, 2'*R*)-3-[2'-Acetoxymethyl-3'-carboxy-1'-oxo-  
propanoyl]-4-isopropyl-2-oxazolidinone (**97**)

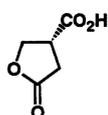


To a solution of (**95**) (3.8 g, 11.4 mmol) in water (36 cm<sup>3</sup>), acetonitrile (24 cm<sup>3</sup>), and carbon tetrachloride (24 cm<sup>3</sup>) was added periodic acid (39.0 g, 171.0 mmol) followed by ruthenium (III) chloride (125 mg, 0.5 mmol) and the reaction mixture stirred vigorously for 4 hours the temperature kept below 40 °C. The reaction was quenched by addition of diethyl ether (70 cm<sup>3</sup>), the phases separated and the aqueous re-extracted with a further portion of ether (2 x 50 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate), filtered through Celite and the filtrate concentrated under reduced pressure. Chromatography of the residue over silica gel [4 % methanol in dichloromethane] furnished the *title acid* as a colourless oil (2.1 g, 62 %).

[ $\alpha$ ]<sub>D</sub> = +49.2 (*c* 1.44, CHCl<sub>3</sub>); (Found: M<sup>+</sup> 301.11615, C<sub>13</sub>H<sub>19</sub>NO<sub>7</sub> requires *M* 301.11617);  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 3200br, 1780m, 1740m, 1710m, 1380m, 1220m;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 10.55 (1 H, br s, -CO<sub>2</sub>H), 4.25 (6 H, m, -OCH<sub>2</sub>CH- and -CH<sub>2</sub>OAc and -CH<sub>2</sub>CHN- and -CHCH<sub>2</sub>CO<sub>2</sub>H), 2.90 (1 H, dd, *J* 4.4, 17.5, -CHHCO<sub>2</sub>H), 2.50 (1 H, dd, *J* 4.1, 17.5, -CHHCO<sub>2</sub>H), 2.20 (1 H, m, -CH(Me)<sub>2</sub>), 1.93 (3 H, s, -COMe), 0.80 (6 H, t, *J* 7.0, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 176.9 (-CO<sub>2</sub>H), 172.6, 171.1 (-OCOMe and -NCOCH-), 154.3 (-OCON-), 64.4, 64.2 (-CH<sub>2</sub>OAc), 58.9 (-OCH<sub>2</sub>CH-), 39.5 (-CHCH<sub>2</sub>OAc), 33.0 (-CH<sub>2</sub>CO<sub>2</sub>H), 28.9 (-CH(Me)<sub>2</sub>), 20.9 (-COMe), 18.0, 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 302 (MH<sup>+</sup>, 0.5 %) 284

(M<sup>+</sup>-H<sub>2</sub>O, 0.5) 258 (3.8) 241 (22.6) 196 (9.3) 173 (20.6) 131 (27.8) 113 (22.9) 86 (100.0) 55 (10.4).

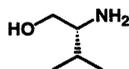
(R)-(+)-Paraconic acid (**98**)<sup>24</sup>



To a degassed solution of (**97**) (2.1 g, 7.0 mmol) in water (10 cm<sup>3</sup>) and tetrahydrofuran (30 cm<sup>3</sup>) under nitrogen was added hydrogen peroxide (100 vol; 3.0 cm<sup>3</sup>, 29.4 mmol) followed after a 5 minute delay by lithium hydroxide (270 mg, 11.2 mmol) and the reaction mixture stirred at room temperature for 48 hours hydrochloric acid added (20 cm<sup>3</sup>, 6 M) and stirring was continued for a further 36 hours. The tetrahydrofuran was removed *in vacuo* and the reaction mixture saturated with sodium chloride before being extracted with ethyl acetate (3 x 30 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (1:3)] yielded *the title acid* as a white solid (360 mg, 40 %).

m.p. 55-57 °C (lit<sup>19</sup> 57-58 °C); [ $\alpha$ ]<sub>D</sub> = +59.5 (c 1.05, MeOH) (lit<sup>24</sup> +59.6 c 0.614 MeOH); (Found: M<sup>+</sup> 130.02661, C<sub>5</sub>H<sub>6</sub>O<sub>4</sub> requires M 130.0268);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3000br m, 1785s, 1720s;  $\delta_{\text{H}}$  (250 MHz; CDCl<sub>3</sub>) 9.10 (1 H, br s, -CO<sub>2</sub>H), 4.40 (2 H, m, -CH<sub>2</sub>CO-), 3.60 (1 H, m, -CHCO<sub>2</sub>H), 2.80 (2 H, m, -CHCH<sub>2</sub>O-);  $\delta_{\text{C}}$  (62.9 MHz; CDCl<sub>3</sub>) 174.8, 174.1 (-CO<sub>2</sub>H and -OCOCH<sub>2</sub>-), 70.4 (-CH<sub>2</sub>OCO-), 40.8 (-CHCO<sub>2</sub>H), 31.8 (-CH<sub>2</sub>CO-); *m/z* (EI) 130 (M<sup>+</sup>, 6.5 %) 113 (3.7) 102 (26.4) 86 (18.9) 71 (46.0) 55 (100.0).

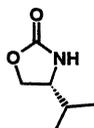
(R)-Valinol (99)



To a solution of lithium borohydride (2.6 g 119.5 mmol) in dry tetrahydrofuran (60 cm<sup>3</sup>) under nitrogen was added dropwise over 15 minutes trimethylsilyl chloride (30.0 cm<sup>3</sup> 236.4 mmol), a precipitate of lithium chloride results. (R)-valine (7.0 g 59.7 mmol) was added in portions and once the addition was complete the reaction was stirred at room temperature for 24 hours. The reaction was cooled to 0 °C and quenched by careful addition of methanol (90 cm<sup>3</sup>) the volatiles removed by distillation and the residue treated with potassium hydroxide solution (50 cm<sup>3</sup>, 20 % w/v), extracted with dichloromethane (3 x 80 cm<sup>3</sup>), dried (sodium sulfate) and concentrated *in vacuo* to yield the title compound as a colourless oil which required no further purification (4.6 g 75 %).

(Found: MH<sup>+</sup> 104.10754, C<sub>5</sub>H<sub>13</sub>NO requires MH 104.10846);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3400br, 1410m, 1390m, 1370m, 1050m;  $\delta_{\text{H}}$  (250 MHz CDCl<sub>3</sub>) 3.50 (1 H, dd, *J* 3.7, 10.6, -CH<sub>2</sub>OH), 3.20 (1 H, dd, *J* 8.4, 10.6, -CH<sub>2</sub>OH), 2.60 (3 H, br s, -OH and -NH<sub>2</sub>), 2.50 (1 H, m, -CHNH<sub>2</sub>), 1.50 (1 H, sept, *J* 6.8 -CH(Me)<sub>2</sub>), 0.80 (6 H, dd, *J* 2.2, 6.8, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz CDCl<sub>3</sub>) 64.4 (-CH<sub>2</sub>OH), 58.9 (-CH<sub>2</sub>NH<sub>2</sub>), 31.2 (-CH(Me)<sub>2</sub>), 19.5, 18.6 (-CH(Me)<sub>2</sub>); *m/z* (CI) 104 (MH<sup>+</sup> 100 %) 86 (11) 72 (96) 60 (43) 55 (13).

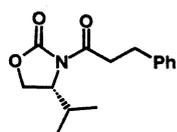
(4R)-4-isopropyl-2-oxazolidinone (**100**)<sup>115</sup>



(*R*)-valinol (8.6 g 83.8 mmol), diethyl carbonate (21.0 cm<sup>3</sup>, 173.3 mmol) and anhydrous potassium carbonate (1.2 g, 8.7 mmol) were combined and stirred at 140 °C for 2 hours before re-arranging the apparatus and removing the ethanol produced by distillation over 2 hours. The residue was taken up in dichloromethane (100 cm<sup>3</sup>) and the organic phases washed with water (2 x 75 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated. Recrystallisation [petrol/ethyl acetate (4:1)] gave *the title compound* as a white solid (9.1 g 84 %).

m.p 69-71 °C (lit<sup>115</sup> 69-70 °C); [ $\alpha$ ]<sub>D</sub> = -70.9 (c 1.86, CH<sub>2</sub>Cl<sub>2</sub>) (lit<sup>115</sup> -37.3 c 5.81 EtOH); (Found: M<sup>+</sup> 129.07898, C<sub>6</sub>H<sub>11</sub>NO<sub>2</sub> requires M 129.07903);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3460br, 3000m, 1710s, 1400w, 1230w, 1010w;  $\delta_{\text{H}}$  (250 MHz CDCl<sub>3</sub>) 7.40 (1 H, s, -NH), 4.35 (1 H, t, J 8.7, -CHNH), 4.00 (1 H, dd, J, 6.4, 8.5, -CH<sub>2</sub>OCO-), 3.55 (1 H, dd, J, 6.8, 14.8, -CH<sub>2</sub>OCO-), 1.65 (1 H, sept, J, 6.7, -CH(Me)<sub>2</sub>), 0.85 (6 H, dd, J 6.7, 16.1, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz CDCl<sub>3</sub>) 161.3 (-CONH), 69.0 (-CH<sub>2</sub>OCO-), 58.8 (-CHNH), 33.0 (-CH(Me)<sub>2</sub>), 18.2, 18.0 (-CH(Me)<sub>2</sub>); *m/z* (EI) 129 (M<sup>+</sup> 17.4 %) 105 (8.9) 97 (7.0) 86 (100) 77 (7.5) 57 (6.6).

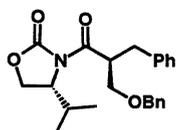
(4R)-3-[1'-Oxo-3'-phenylpropanoyl]-4-isopropyl-2-oxazolidinone (**101**)



To an ice-cooled solution of (**100**) (5.9 g 45.3 mmol) in dry tetrahydrofuran (90 cm<sup>3</sup>) under nitrogen was added *n*-butyl lithium (1.6 M in hexanes; 35.0 cm<sup>3</sup> 56.0 mmol) and the mixture stirred for 20 minutes before being cooled to -78 °C, hydrocinnamoyl chloride (8.0 cm<sup>3</sup> 53.8 mmol) added dropwise and the mixture stirred for 3 hours the temperature being allowed to warm to room temperature. The reaction was quenched with sodium hydrogen carbonate (100 cm<sup>3</sup> saturated solution), extracted with diethyl ether (3 x 150 cm<sup>3</sup>), and the combined organics dried (magnesium sulfate), and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (3:1)] yielded *the title compound* as a white solid (9.0 g 76 %).

m.p 59-61 °C; [ $\alpha$ ]<sub>D</sub> = -67.9 (*c* 0.86, CHCl<sub>3</sub>); (Found: C 68.94, H 7.33, N 5.36, M<sup>+</sup> 261.13651, C<sub>15</sub>H<sub>19</sub>NO<sub>3</sub> requires C 68.85, H 7.70, N 5.57 %, M 261.13771);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2950br m, 1780s, 1380m, 1250m, 1055w, 1030w;  $\delta_{\text{H}}$  (250 MHz CDCl<sub>3</sub>) 7.27 (5 H, m, Ar-H), 4.43 (1 H, m, -CHNCO-), 4.20 (2 H, m, -CH<sub>2</sub>OCO-), 3.30 (2 H, m, -CH<sub>2</sub>CH<sub>2</sub>Ph), 3.02 (2 H, m, CH<sub>2</sub>Ph), 2.36 (1 H, dxsept, *J* 6.9, 3.8, -CH(Me)<sub>2</sub>), 0.87 (6 H, dd, *J* 7.0 15.5, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$  (62.9 MHz CDCl<sub>3</sub>) 172.7 (-NCOCH<sub>2</sub>-), 154.5 (-OCON-), 140.9 (=C-, Ar), 129.4 129.0 128.9 126.6 (4 x =C-H, Ar), 63.8 (-CH<sub>2</sub>OCO-), 58.8 (-CHNCO-), 37.5 (-COCH<sub>2</sub>-), 30.8 (-CH<sub>2</sub>Ph), 28.8 (-CH(Me)<sub>2</sub>), 18.3, 15.0 (-C(Me)<sub>2</sub>); *m/z* (EI) 261 (M<sup>+</sup>, 37.0 %) 150 (10.8) 142 (8.3) 130 (76.1) 104 (100.0) 91 (82.4) 77 (19.2) 65 (11.1) 51 (6.6).

(4R, 2'S)-3-[2'-Benzyloxymethyl-1'-oxo-3'-phenylpropanoyl]-  
4-isoprpyl-2-oxazolidinone (**102**)

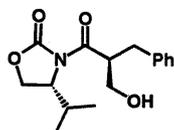


To an ice-cooled solution of (**101**) (4.0 g 15.3 mmol) in dry dichloromethane (50 cm<sup>3</sup>) under nitrogen was added titanium (IV) chloride (1.8 cm<sup>3</sup> 16.4 mmol). To the resulting yellow slurry after a 5 minute delay diisopropylethyl amine was added and the resulting blood red solution stirred for 1 hour at 0 °C. Benzylchloromethyl ether (4.8 cm<sup>3</sup>, 34.5 mmol) was added dropwise and the reaction mixture stirred over night the temperature being allowed to warm to ambient. The reaction was quenched by careful addition of ammonium chloride solution (40 cm<sup>3</sup> saturated solution), stirred for 10 minutes and the organic layer separated. The aqueous was re-extracted with a further portion of dichloromethane (2 x 30 cm<sup>3</sup>) and the combined organic phases dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue was over silica gel [petrol/diethyl ether (4:1)] furnished *the title compound* as a white solid (5.47 g, 94 %).

m.p. 72-74 °C; [ $\alpha$ ]<sub>D</sub> = -57.40 (c 1.22, CHCl<sub>3</sub>); (Found: C 72.25, H 7.19, N 3.75, M<sup>+</sup> 381.19406, C<sub>23</sub>H<sub>27</sub>NO<sub>4</sub> requires C 72.42, H 7.13, N, 3.67 % M 381.19411)  
 $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3000-2890br m, 1780s, 1700s, 1390m, 1200m, 1150m, 900w;  
 $\delta_{\text{H}}$ (250 MHz CDCl<sub>3</sub>) 7.30 (10 H, m, Ar-H), 4.55 (1 H, m, -CHCH<sub>2</sub>Ph), 4.71 (2 H, d, J 4.7, -OCH<sub>2</sub>Ph), 4.30 (1 H, m, -CHNCO-), 4.05 (1 H, dd, J 3.0, 9.0, -CH<sub>2</sub>OCO-), 3.95 (1 H, dd, J 8.5, 8.9, -CH<sub>2</sub>OCO-), 3.78 (1 H, dd, J 7.5, 9.1, -CH<sub>2</sub>OBn), 3.64 (1 H dd, J 4.7, 9.2, -CH<sub>2</sub>OBn), 2.96 (1 H, dd, J 8.1, 13.4, -CHCH<sub>2</sub>Ph), 2.83 (1 H, dd, J 7.3, 13.4, -CHCH<sub>2</sub>Ph), 2.30 (1 H, d x sept, J 4.1, 6.9, -CH(Me)<sub>2</sub>), 0.80 (6 H, dd, J 7.0, 20.0, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 174.5 (-NCOCHCH<sub>2</sub>Ph), 154.1 (-

OC(=O)N-, 139.1 138.5 (2 x =C-, Ar), 129.6 128.8 128.7 128.4 128.1 128.0 126.9 (7 x =C-H, Ar), 73.5 (-CH<sub>2</sub>OBn), 71.2 (-OCH<sub>2</sub>Ph), 63.7 (-CH<sub>2</sub>OCON-), 58.9 (-CHN-), 45.6 (-CHCH<sub>2</sub>Ph), 35.3 (-CH<sub>2</sub>Ph), 28.9 (-CH(Me)<sub>2</sub>), 18.3 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 381 (M<sup>+</sup>, 5.0 %) 273 (13.5) 184 (24.3) 171 (13.2) 143 (38.0) 117 (11.5) 91 (100.0) 55 (8.6).

(4*R*, 2'*S*)-3-[2'-Hydroxymethyl-1'-oxo-3'-phenylpropanoyl]-4-isopropyl-2-oxaolidinone (**103**)

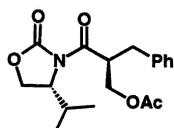


A mixture of (**102**) (9.4 g 2.5 mmol) and palladium on carbon (2.0 g, 5 % Pd/C) in ethyl acetate (100 cm<sup>3</sup>) containing a catalytical amount of hydrochloric acid was stirred under an atmosphere of hydrogen for 4 hours at room temperature before being filtered through Celite and concentrated *in vacuo* to furnish *the title alcohol* as a white solid (7.15 g, 99 %).

*m.p.* 85-87 °C; [ $\alpha$ ]<sub>D</sub> = -118.7 (*c* 0.55, CHCl<sub>3</sub>); (Found: C 65.76, H 7.38, N 5.01, M<sup>+</sup> 291.14706, C<sub>16</sub>H<sub>21</sub>NO<sub>4</sub> requires C 65.96, H 7.26, N 4.81 %, *M* 291.14839);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3485m, 2950m, 1780s, 1690s, 1370m 750 (m) 700 (m);  $\delta_{\text{H}}$  (250 MHz CDCl<sub>3</sub>) 7.25 (5 H, m, Ar-*H*), 4.39 (2 H, m, -CH<sub>2</sub>OH), 4.10 (2 H, m, -CHNCO- and -CHCH<sub>2</sub>OH), 3.85 (2 H, m, -CH<sub>2</sub>OCON-), 3.05 (1 H, dd, *J* 5.6, 13.2, -CH<sub>2</sub>Ph), 2.90 (1 H, dd, *J* 5.6, 13.4, -CH<sub>2</sub>Ph), 2.55 (1 H, br s, -OH), 2.39 (1 H, m, -CH(Me)<sub>2</sub>), 0.89 (1 H, t, *J* 7.2, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 175.5 (-NCOCH-), 154.5 (-CH<sub>2</sub>OCON-), 138.9 (=C-, Ar), 129.5 128.9 126.9 (3 x =C-H, Ar), 63.9, 63.8 (2 x -CH<sub>2</sub>O-), 59.2 (-CHNCO-), 47.4 (-CHCH<sub>2</sub>OH), 34.9 (-CH<sub>2</sub>Ph), 29.0 (-

CH(Me)<sub>2</sub>, 18.3 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 291 (M<sup>+</sup>, 3.2 %) 144 (96.4) 131 (61.1)  
116 (24.2) 91 (100.0) 78 (12.3) 65 (11.0).

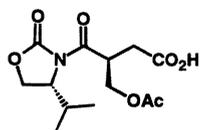
(4*R*, 2'*S*)-3-[2'-Acetoxymethyl-1'-oxo-3'-phenyl  
propanoyl]-4-isopropyl-2-oxazolidinone (**104**)



To a stirred solution of (**103**) (4.7 g 16.1 mmol) in dichloromethane (10 cm<sup>3</sup>) was added pyridine (5.0 cm<sup>3</sup> 61.8 mmol) and acetic anhydride (5.0 cm<sup>3</sup> 53.0 mmol) and the mixture stirred at room temperature for 24 hours before being quenched with hydrochloric acid (30 cm<sup>3</sup>, 2 M) and the aqueous phase extracted with dichloromethane (3 x 30 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate) concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (2:1)] yielded *the title compound* as a colourless oil (5.3 g, 99 %).

[ $\alpha$ ]<sub>D</sub> = -72.2 (c 1.09, CHCl<sub>3</sub>); (Found: M<sup>+</sup> 333.15765, C<sub>18</sub>H<sub>23</sub>NO<sub>5</sub> requires *M* 333.15907);  $\nu_{\max}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3000m, 1780s, 1740s, 1700s, 1380m, 1220m, 1100w, 1050w;  $\delta_{\text{H}}$ (250 MHz CDCl<sub>3</sub>) 7.22 (5 H, m, Ar-*H*), 4.55 (1 H, m, -CHNCO-), 4.30 (3 H, m, -CH<sub>2</sub>OAc -CHCH<sub>2</sub>Ph), 4.10 (1 H, dd, *J* 2.8, 9.1, -CH<sub>2</sub>OCO-), 4.00 (1 H, dd, *J* 2.8, 8.7, -CH<sub>2</sub>OCO-), 2.97 (1 H, dd, *J* 5.6, 13.4, -CH<sub>2</sub>Ph), 2.79 (1 H, dd, *J* 5.9, 13.4, -CH<sub>2</sub>Ph), 2.31 (1 H, m, -CH(Me)<sub>2</sub>), 1.96 (3 H, s, -COCH<sub>3</sub>), 0.86 (6 H, t, *J* 6.7, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 173.7 (-NCOCHCH<sub>2</sub>-), 170.8 (-OCOCH<sub>3</sub>), 154.0 (-OCON-), 138.1 (=C-, Ar) 129.5 128.9 127.1 (3 x =C-H, Ar), 64.9 63.8 (2 x -CH<sub>2</sub>O-), 59.0 (-CHNCO-), 44.4 (-CHCH<sub>2</sub>OAc), 35.4 (-CH<sub>2</sub>Ph), 29.4 (-CH(Me)<sub>2</sub>), 18.2 15.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 333 (M<sup>+</sup>, 0.3 %) 273 (36.3) 169 (7.0) 144 (100.0) 131 (13.6) 117 (20.5) 91 (20.1).

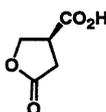
(4R, 2'S)-3-[2'-Acetoxymethyl-3'-carboxy-1'-oxo-  
propanoyl]-4-isopropyl-2-oxazolidinone (**105**)



To a solution of (**104**) (5.2 g 15.6 mmol) in water (50 cm<sup>3</sup>) acetonitrile (30 cm<sup>3</sup>) and carbon tetrachloride (30 cm<sup>3</sup>) was added periodic acid (51.0 g, 223.7 mmol) followed by ruthenium (III) chloride (0.125 g, 0.48 mmol) and the mixture stirred vigorously to ensure mixing of the phases for 6 hours keeping the temperature below 40 °C. Diethyl ether (100 cm<sup>3</sup>) was added carefully, the phases separated, and the aqueous phase re-extracted with a further portion of ether (2 x 75 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) and filtered through Celite before being concentrated under reduced pressure. Chromatography of the residue over silica gel [diethylether/petrol (2:1)] gave *the title acid* as a colourless oil (3.1 g, 65 %)

$[\alpha]_D = -41.3$  (c 1.21, CHCl<sub>3</sub>); (Found: M<sup>+</sup> 301.11615, C<sub>13</sub>H<sub>19</sub>O<sub>7</sub> requires M 301.11731);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3200-2950m, 1780s, 1740s, 1710s, 1380m, 1210m;  $\delta_{\text{H}}(250 \text{ MHz CDCl}_3)$  10.60 (1 H, s, -CO<sub>2</sub>H), 4.34 (4 H, m, -OCH<sub>2</sub>CHN- and -CH<sub>2</sub>OAc), 4.08 (2 H, m, -CHCH<sub>2</sub>CO<sub>2</sub>H and -CHNCO-), 2.85 (1 H, dd, J 7.2, 17.4, -CH<sub>2</sub>CO<sub>2</sub>H), 2.42 (1 H, dd, J 13.5, 17.5, -CH<sub>2</sub>CO<sub>2</sub>H), 2.15 (1 H, m, -CH(Me)<sub>2</sub>), 1.90 (3 H, s, -CO CH<sub>3</sub>), 0.75 (6 H, t, J 6.7, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}(62.9 \text{ MHz CDCl}_3)$  177.1 (-CO<sub>2</sub>H), 172.6 171.1 (-COCH<sub>3</sub> and -NCOCHCH<sub>2</sub>OAc), 154.3 (-OCON-), 64.4 64.2 (2 x -CH<sub>2</sub>O-), 58.9 (-CH<sub>2</sub>CHN-), 39.6 (-CHCH<sub>2</sub>OAc), 33.1 (-CH<sub>2</sub>CO<sub>2</sub>H), 28.9 (-CH(Me)<sub>2</sub>), 21.0 (-COCH<sub>3</sub>), 18.1 15.1 (-CH(Me)<sub>2</sub>); m/z (EI) 302 (MH<sup>+</sup>, 0.7 %) 241 (25.1) 196 (11.8) 173 (25.4) 131 (33.1) 113 (28.7) 86 (100.0) 69 (8.9) 55 (13.1).

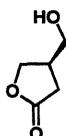
(S)-(-)-Paraconic acid (**21**)<sup>24,25</sup>



To a degassed solution of (**105**) (4.1 g, 13.6 mmol) in water (30 cm<sup>3</sup>) and tetrahydrofuran (90 cm<sup>3</sup>) under nitrogen was added hydrogen peroxide (100 vol; 10.0 cm<sup>3</sup>, 89.1 mmol) followed after 5 minutes by lithium hydroxide (1.3 g, 5.4 mmol) and the reaction mixture stirred at room temperature for 48 hours at which time hydrochloric acid was added (50 cm<sup>3</sup>, 6 M) and stirring continued for a further 36 hours. Tetrahydrofuran was removed and the reaction mixture saturated with sodium chloride before being extracted with ethyl acetate (3 x 75 cm<sup>3</sup>), the combined organic phases dried (sodium sulfate) and concentrated. Chromatography of the residue over silica gel [diethylether/petrol (3:1)] gave *the title acid* as a white solid (220 mg, 40 %).

m.p. 55-57 °C (lit<sup>19</sup> 56-57 °C);  $[\alpha]_D = -60.0$  (*c* 2.1, MeOH) (lit<sup>24</sup> -59.5, *c* 0.61 MeOH); (Found:  $M^+$  130.02661, C<sub>5</sub>H<sub>6</sub>O<sub>4</sub> requires  $M$  130.02694);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000br m, 1785m, 1710m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  9.40 (1 H, s, -CO<sub>2</sub>H), 4.30 (2 H, m, -CH<sub>2</sub>OCO-), 3.35 (1 H, m, -CHCO<sub>2</sub>H), 2.74 (2 H, m, -CHCH<sub>2</sub>CO-);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  176.7 (-CO<sub>2</sub>H), 174.0 (-OCOCH<sub>2</sub>-), 70.4 (-CH<sub>2</sub>OCO-), 40.8 (-CHCO<sub>2</sub>H), 31.7 (-CHCH<sub>2</sub>COO-);  $m/z$  (EI) 130 ( $M^+$  4.6 %) 113 (3.0) 102 (25.0) 100 (16.0) 86 (17.9) 71 (40.4) 55 (100.0).

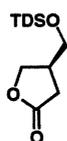
(3S)-(-)-3-hydroxymethyl-4-butenolide (**106**)<sup>24</sup>



To a stirred ice-cooled solution of (**21**) (750 mg, 5.7 mmol) in dry tetrahydrofuran (10 cm<sup>3</sup>) under nitrogen was added dropwise boranedimethylsulfide (0.9 cm<sup>3</sup>, 9.5 mmol) and the mixture stirred for 2 hours before the reaction was quenched with methanol (5 cm<sup>3</sup>) and concentrated *below* 30 °C to avoid racemisation. Chromatography of the residue over silica gel [petrol/ethyl acetate (1:2)] gave *the title alcohol* as a colourless oil (400 mg, 60 %).

$[\alpha]_D = -42.4$  (*c* 6.8, CHCl<sub>3</sub>) (lit<sup>24</sup>  $-46.3$  *c* 1.224 CHCl<sub>3</sub>); (Found: M<sup>+</sup> 116.04735, C<sub>5</sub>H<sub>8</sub>O<sub>3</sub> requires 116.04782);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3400br m, 2900m, 1760s, 1190m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  4.44 (1 H, dd, *J* 1.5, 9.4, -CHHOCO-), 4.24 (1 H, dd, *J* 4.0, 9.4, -CHHOCO-), 3.68 (2 H, m, -CH<sub>2</sub>OH), 2.78 (1 H, m, -CHCH<sub>2</sub>OH), 2.64 (1 H, dd, *J* 8.8, 17.6, -CHHCO-), 2.40 (1 H, dd, *J* 5.7, 17.6, -CHHCO-);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  178.6 (-OCOCH<sub>2</sub>-), 72.2 (-OCH<sub>2</sub>CO-), 63.3 (-CH<sub>2</sub>OH), 37.4 (-CHCH<sub>2</sub>OH), 31.3 (-CH<sub>2</sub>COO-); *m/z* (EI) 116 (M<sup>+</sup>, 4.2 %) 98 (7.8) 86 (9.9) 74 (53.5) 57 (100.0).

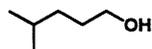
(3R)-(-)-3-(O-Dimethylhexylsilyl)-  
hydroxymethyl-4-butenolide (**107**)



To a solution of alcohol (**106**) (310 mg, 2.7 mmol) in *N,N*-dimethylformamide (5 cm<sup>3</sup>) was added imidazole (0.6 g, 8.8 mmol) followed after a 10 minute delay by hexyldimethylsilyl chloride (0.8 cm<sup>3</sup>, 4.0 mmol) and the reaction mixture stirred at room temperature for 24 hours before addition of dichloromethane (20 cm<sup>3</sup>). The organic phase was washed with water (3 x 10 cm<sup>3</sup>) dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (12:1)] gave *the title compound* as a colourless oil (0.6 g, 87 %).

$[\alpha]_D = -24.5$  (*c* 0.92, CHCl<sub>3</sub>); (Found:MH<sup>+</sup> 259.17295, C<sub>13</sub>H<sub>26</sub>O<sub>3</sub>Si requires *MH* 259.174.63);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2980s, 1780s, 1460m, 1250m, 830m;  $\delta_{\text{H}}(250 \text{ MHz CDCl}_3)$  4.28 (1 H, dd, *J* 7.5, 9.1, -CHHOCO-), 4.08 (1 H, dd, *J* 8.9, 9.1, -CHHOCO-), 3.52 (2 H, m, -CH<sub>2</sub>OTDS), 2.64 (1 H, m, -CHCH<sub>2</sub>OTDS), 2.47 (1 H, dd, *J* 8.8, 17.6, -CHHCOO-), 2.28 (1 H, dd, *J* 6.3, 17.6, -CHHCOO-), 1.51 (1 H, sept, *J* 6.9, -CH(Me)<sub>2</sub>), 0.75 (12 H, m, -C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 0.00 (6 H, s, -Si(Me)<sub>2</sub>-);  $\delta_{\text{C}}(62.9 \text{ MHz CDCl}_3)$  177.9 (-OCOCH<sub>2</sub>-), 71.2 (-CH<sub>2</sub>OCO-), 63.7 (-CH<sub>2</sub>OTDS), 37.8 (-CH(Me)<sub>2</sub>-), 35.3 (-CHCH<sub>2</sub>OTDS), 31.4 (-CH<sub>2</sub>CO-), 25.4 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-) 20.9, 19.2 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), -3.5 (-Si(Me)<sub>2</sub>-); *m/z* (FAB) 259 (MH<sup>+</sup>) 173, 159, 137.

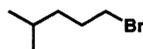
4-Methylpentan-1-ol (**108**)<sup>118</sup>



To an ice-cooled suspension of lithium aluminium hydride (3.1 g, 81.6 mmol) in sodium dried diethyl ether (100 cm<sup>3</sup>) was added dropwise valeric acid (10.0 cm<sup>3</sup>, 79.5 mmol) in dry diethyl ether (10 cm<sup>3</sup>) and the mixture stirred for 2 hours before being quenched by the careful addition of wet ether (50 cm<sup>3</sup>), water (4 cm<sup>3</sup>) and filtered through Celite. The filtrate was concentrated *in vacuo* and the residue distilled at ambient pressure to give *the title alcohol* as a colourless liquid (5.9 g, 72 %).

b.p. 160-165 °C;  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3340br s, 2930m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  3.60 (2 H, t, *J* 5.5, -CH<sub>2</sub>OH), 3.40 (1 H, br s, -OH), 1.56 (3 H, m, -CH<sub>2</sub>CH(Me)<sub>2</sub>), 1.22 (2 H, m, -CH<sub>2</sub>CH<sub>2</sub>OH), 0.89 (6 H, d, *J* 6.6, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  63.2 (-CH<sub>2</sub>OH), 35.5 (-CH<sub>2</sub>CH<sub>2</sub>OH), 30.9 (-CH<sub>2</sub>CH(Me)<sub>2</sub>), 28.8 (-CH(Me)<sub>2</sub>), 23.2 (-CH(Me)<sub>2</sub>).

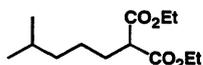
4-Methyl-1-bromopentane (**109**)<sup>118</sup>



To an ice-cooled solution of alcohol (**108**) (5.0 g, 48.9 mmol) and carbon tetrabromide (20.3 g, 61.2 mmol) in dichloromethane (50 cm<sup>3</sup>) was added carefully in portions triphenylphosphine (19.2 g, 73.4 mmol) and once the exothermic reaction was complete the mixture stirred for 10 minutes. Petrol (50 cm<sup>3</sup>) was added the precipitate removed by filtration, the filtrate concentrated *in vacuo*, and the product distilled to give *the title bromide* as a colourless liquid (6.75 g, 77 %).

b.p 60-62 °C (10 mmHg);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2950m, 1470m, 1340m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  33.9 (2 H, t,  $J$  6.9,  $-\text{CH}_2\text{Br}$ ), 1.86 (2 H, m,  $-\text{CH}_2\text{CH}_2\text{Br}$ ), 1.58 (1 H, sept,  $J$  6.6,  $-\text{CH}(\text{Me})_2$ ), 1.31 (2 H, m,  $-\text{CH}_2\text{CH}(\text{Me})_2$ ), 0.90 (6 H, d,  $J$  6.6,  $-\text{CH}(\text{Me})_2$ );  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  37.8 ( $-\text{CH}_2\text{Br}$ ), 34.7, 31.2 ( $-\text{CH}_2\text{CH}_2\text{CH}_2\text{Br}$ ), 27.9 ( $-\text{CH}(\text{Me})_2$ ), 23.0 ( $-\text{CH}(\text{Me})_2$ ).

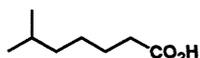
*Diethyl-(4-methylpentyl) malonate (110)*<sup>119</sup>



To an ice-cooled suspension of sodium hydride (60 % dispersion in mineral oil; 1.5 g, 37.5 mmol) in dry *N,N*-dimethylformamide (30 cm<sup>3</sup>) was added diethyl malonate (5.5 cm<sup>3</sup>, 32.5 mmol) followed after a 15 minute delay by bromide (**109**) (5.8 g, 32.4 mmol) and the reaction mixture stirred for 2 hours at room temperature before being quenched with water (100 cm<sup>3</sup>), extracted with dichloromethane (2 x 100 cm<sup>3</sup>) and the combined organic phases dried (magnesium sulfate) and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (12:1)] gave *the title compound* as a colourless oil (5.6 g, 70 %).

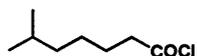
(Found:  $M^+$  244.16746,  $\text{C}_{13}\text{H}_{24}\text{O}_4$  requires  $M$  244.16896);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2950m, 1750s, 1470s, 1370m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  4.20 (4 H, q,  $J$  7.2,  $-\text{OCH}_2\text{Me}$ ), 3.32 (1 H, t,  $J$  7.5,  $-\text{CH}(\text{CO}_2\text{Et})_2$ ), 1.88 (2 H, q,  $J$  7.8,  $-\text{CH}_2\text{CH}(\text{CO}_2\text{Et})_2$ ), 1.54 (1 H, sept,  $J$  6.6,  $-\text{CH}(\text{Me})_2$ ), 1.25 (10 H, m,  $-\text{OCH}_2\text{Me}$  and  $-\text{CH}_2\text{CH}_2\text{CH}-$ ), 0.85 (6 H, d,  $J$  6.6,  $-\text{CH}(\text{Me})_2$ );  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  169.9 ( $-\text{CO}_2\text{Et}$ ), 61.6 ( $-\text{OCH}_2\text{Me}$ ), 52.4 ( $-\text{CH}(\text{CO}_2\text{Et})_2$ ), 38.8 ( $-\text{CH}_2\text{CH}(\text{CO}_2\text{Et})_2$ ), 29.3, 25.5 ( $-\text{CH}_2\text{CH}_2\text{CH}(\text{Me})_2$ ), 28.0 ( $-\text{CH}(\text{Me})_2$ ), 22.8 ( $-\text{CH}_2\text{Me}$ ), 14.4 ( $-\text{CH}(\text{Me})_2$ ).

6-Methylheptanoic acid (**111**)<sup>119</sup>



To a solution of (**110**) (5.8 g, 25.1 mmol) in water (60 cm<sup>3</sup>) and tetrahydrofuran (30 cm<sup>3</sup>) was added sodium hydroxide (10.0 g, 250.0 mmol) and the reaction mixture stirred for 48 hours where it was acidified with hydrochloric acid (6 M) and heated at reflux for a further 36 hours. The mixture was saturated with sodium chloride and extracted with ethyl acetate (3 x 75 cm<sup>3</sup>), the combined organics dried (sodium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (9:1)] furnished *the title acid* as a colourless liquid (2.3 g, 71 %).  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3000br s, 1700s;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  11.76 (1 H, br s, -CO<sub>2</sub>H), 2.35 (2 H, t, *J* 7.5, -CH<sub>2</sub>CO<sub>2</sub>H), 1.63 (3 H, m, -CH<sub>2</sub>CH(Me)<sub>2</sub> and -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), 1.22 (2 H, m, -CH<sub>2</sub>CH(Me)<sub>2</sub>), 0.90 (6 H, d, *J* 6.6, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  181.0 (-CO<sub>2</sub>H), 38.6 (-CH<sub>2</sub>CO<sub>2</sub>H), 34.8 (-CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>H), 28.1 (-CH(Me)<sub>2</sub>), 22.9 (-CH<sub>2</sub>CH(Me)<sub>2</sub>), 22.8 (-CH(Me)<sub>2</sub>).

6-Methylheptanoyl chloride (**112**)<sup>120</sup>

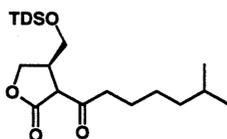


Thionyl chloride (1.0 cm<sup>3</sup>, 13.8 mmol) acid (**111**) (0.7 g, 4.8 mmol) and a catalytical amount of *N,N*-dimethylformamide were combined and stirred at room temperature for 24 hours and the excess thionyl chloride removed. Kugelrohr distillation furnished the *title acid chloride* as a colourless liquid (0.67 g, 85 %).

$\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2900s, 1800s, 1470w, 1370w;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  2.89 (2 H, t, *J* 7.2, -CH<sub>2</sub>COCl), 1.70 (2 H, quin, *J* 7.2, -CH<sub>2</sub>CH<sub>2</sub>COCl), 1.54 (1 H, sept, *J* 6.6, -

$CH(Me)_2$ , 1.35, 1.20 (4H, m,  $-CH_2CH_2CH(Me)_2$ ), 0.87 (6 H, d,  $J$  6.6,  $-CH(Me)_2$ );  
 $\delta_C$ (62.9 MHz  $CDCl_3$ ) 173.9 ( $-COCl$ ), 47.1 ( $-CH_2COCl$ ), 38.3 ( $-CH_2CH_2COCl$ ),  
 27.8 ( $-CH(Me)_2$ ), 26.2, 25.3 ( $-CH_2CH_2CH(Me)_2$ ), 22.5( $-CH(Me)_2$ ).

(3R)-(-)-3-O-(Dimethylhexylsilyl)hydroxymethyl-2-(  
 6'-methyl-1'-oxoheptanoyl)-4-butanolide (7a)

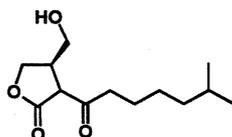


To a solution of (**107**) (460 mg, 1.8 mmol) in dry tetrahydrofuran (20  $cm^3$ ) under nitrogen at  $-78$  °C was added lithium hexamethyldisilazide (1.0 M in THF; 4.4  $cm^3$ , 4.4 mmol) followed after a 20 minute delay by acid chloride (**112**) (450 mg, 2.8 mmol) and the mixture stirred for 3 hours the temperature being allowed to warm to ambient. The reaction mixture was quenched with sodium hydrogen carbonate (20  $cm^3$ , saturated solution), extracted with diethyl ether (3 x 30  $cm^3$ ) and the organic phases dried (magnesium sulfate) and concentrated. Chromatography of the residue over silica gel [petrol/diethyl ether (13:1)] gave the title compound as a colourless oil (310 mg, 45 %).

$\delta_H$ (250 MHz;  $CDCl_3$ ) 4.30 (1 H, t,  $J$  8.8,  $-CH_2OCO-$ ), 4.03 (1 H, dd,  $J$  6.6, 8.8,  $-CH_2OCO-$ ), 3.53 (3 H, m,  $-CH_2OTDS$  and  $-COCHCO-$ ), 3.10 (1 H, m,  $-CHCH_2OTDS$ ), 2.87 (1 H, dt,  $J$  17.6, 7.5,  $-CHHCO-$ ), 2.52 (1 H, dt,  $J$  17.6, 7.2,  $-CHHCO-$ ), 1.51 (4 H, m,  $-COCH_2CH_2CH_2-$ ), 1.10 (4 H, m,  $-CH_2CH(Me)_2$  and  $-CH(Me)_2$ ), 0.77 (18 H, m,  $-Si(Me)_2C(Me)_2CH(Me)_2$  and  $-CH(Me)_2$ ), 0.00 (6 H, m,  $-OSi(Me)_2-$ );  $\delta_C$ (62.9 MHz  $CDCl_3$ ) 203.5 ( $-CHCOCH_2-$ ), 173.2 ( $-OCOCH-$ ), 69.9 ( $-CH_2OCO-$ ), 62.3 ( $-CH_2OTDS$ ), 55.3 ( $-COCHCO-$ ), 43.3 ( $-CH_2CO-$ ), 39.9 ( $-CHCH_2OTDS$ ), 39.3 ( $-COCH_2CH_2-$ ), 34.8 ( $-CH(Me)_2$ ), 28.5 ( $-CH(Me)_2$ ), 27.4,

25.8 (-CH<sub>2</sub>CH<sub>2</sub>CH(Me)<sub>2</sub>), 25.4 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-), 23.2, 20.9 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 19.1 (-CH(Me)<sub>2</sub>), -3.3 (-OSi(Me)<sub>2</sub>-).

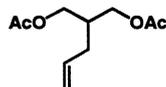
(3*R*)-(-)-3-Hydroxymethyl-2-(6'-methyl-1'-oxoheptanoyl)-4-butanolide [A-Factor] (7)<sup>24,25</sup>



Tetrabutylammonium fluoride (1.0 M in tetrahydrofuran; 5.0 cm<sup>3</sup>, 5.0 mmol) and (7a) (300 mg, 0.78 mmol) were combined and stirred at room temperature for 24 hours. Ammonium chloride (10 cm<sup>3</sup>, saturated solution) was added and the aqueous phase extracted with diethyl ether (3 x 10 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (1:2)] gave the title alcohol as a colourless oil (81 mg, 43 %).

[ $\alpha$ ]<sub>D</sub> = -16.33 (*c* 0.64; CHCl<sub>3</sub>) (lit<sup>24</sup> -13.1, *c* 1.2 CHCl<sub>3</sub>); (Found: M<sup>+</sup> 242.15183, C<sub>13</sub>H<sub>22</sub>O<sub>4</sub> requires *M* 242.15318);  $\nu_{\text{max}}$ (CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3400br m, 2950s, 1760s, 1380m, 1030w;  $\delta_{\text{H}}$ (250 MHz; CDCl<sub>3</sub>) 4.22 (1 H, m, -CHHOCO-), 3.94 (1 H, m, -CHHOCO-), 3.45 (3 H, m, -CH<sub>2</sub>OH and -COCHCO-), 2.98 (2 H, m, -CHCH<sub>2</sub>OH), 2.72 (1 H, dt, *J* 17.9, 7.2, -CHHCO-), 2.41 (1 H, dt, *J* 17.9, 7.5, -CHHCO-), 1.30 (3 H, m, -CH<sub>2</sub>CH<sub>2</sub>CO- and -CH(Me)<sub>2</sub>), 1.00 (4 H, m, -CH<sub>2</sub>CH<sub>2</sub>CH(Me)<sub>2</sub>), 0.63 (6 H, d, *J* 6.6, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 202.8 (-COCH<sub>2</sub>-), 174.6 (-OCOCH-), 68.8 (-CH<sub>2</sub>OCO-), 61.3 (-CH<sub>2</sub>OH), 54.6 (-COCHCO-), 42.2 (-CH<sub>2</sub>CO-), 39.3 (-CHCH<sub>2</sub>OH), 38.7 (-CH<sub>2</sub>CH<sub>2</sub>CO-), 27.5 (-CH(Me)<sub>2</sub>), 26.9, 23.5 (-CH<sub>2</sub>CH<sub>2</sub>CH(Me)<sub>2</sub>), 20.1 (-CH(Me)<sub>2</sub>); *m/z* (EI) 242 (M<sup>+</sup>, 3.2 %) 211 (40.1) 158 (21.3) 143 (41.4) 127 (59.0) 109 (91.6) 85 (100.0) 69 (26.4) 57 (85.3).

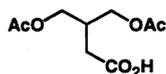
2-(Acetoxymethyl)-pent-4-enyl acetate (**113**)<sup>25</sup>



To an ice-cooled suspension of lithium aluminium hydride (5.5 g, 144.7 mmol) in sodium dried diethyl ether (300 cm<sup>3</sup>) was added diethylallyl malonate (20.0 cm<sup>3</sup>, 101.4 mmol) and the mixture stirred for 2 hours at room temperature. The reaction was quenched by careful addition of wet tetrahydrofuran (100 cm<sup>3</sup>) and water (20 cm<sup>3</sup>) and after stirring for 1 hour the precipitates were filtered and the filtrate dried (magnesium sulfate) and concentrated *in vacuo*. The residue was taken up in dichloromethane (50 cm<sup>3</sup>), triethylamine (26.0 cm<sup>3</sup>, 189.4 mmol), and acetic anhydride (16.0 cm<sup>3</sup>, 172.2 mmol) added and the reaction mixture stirred for 24 hours before hydrochloric acid (30 cm<sup>3</sup>, 2 M) was added and the phases separated. The organic phase was washed with water (2 x 30 cm<sup>3</sup>) sodium hydrogen carbonate solution (2 x 30 cm<sup>3</sup>) water (2 x 30 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated. The residue was distilled to give *the title compound* as a colourless oil (11.1 g, 55 %).

b.p. 62-65 °C (5 mbar); (Found:  $M^+$  200.10483, C<sub>10</sub>H<sub>16</sub>O<sub>4</sub> requires  $M$  200.10584);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2950m, 1740s, 1640m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  5.77 (1 H, m, -CH=CH<sub>2</sub>), 5.09 (2 H, m, -CH=CH<sub>2</sub>), 4.07 (4 H, m, -CH<sub>2</sub>OAc), 2.15 (3 H, m, -CHCH<sub>2</sub>CH=), 2.06 (3 H, s, -COMe);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  171.1 (-COMe), 135.2 (-CH=CH<sub>2</sub>), 117.7 (-CH=CH<sub>2</sub>), 64.1 (-CH<sub>2</sub>OAc), 37.3 (-COMe), 33.1 (-CH<sub>2</sub>CH=), 21.1 (-CHCH<sub>2</sub>OAc).

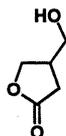
3,3-Diacetoxymethyl-propionic acid (**114**)



To a solution of (**113**) (5.0 g, 25.0 mmol) in water (75 cm<sup>3</sup>), carbon tetrachloride (50 cm<sup>3</sup>), acetonitrile (50 cm<sup>3</sup>) and sodium periodate (27.0 g, 126.2 mmol) was added ruthenium (III) chloride (200 mg, 0.76 mmol) and the reaction mixture vigorously stirred for 1 hour the temperature kept below 40 °C. Dichloromethane (100 cm<sup>3</sup>) was added the phases separated and the aqueous extracted with a further portion of dichloromethane (2 x 50 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate), filtered through Celite and the filtrate concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (1:1)] furnished the title acid as a colourless oil (3.8 g, 70 %).

$\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3200br m, 1730s, 1380m, 1040m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  10.80 (1 H, s, -CO<sub>2</sub>H), 4.10 (4 H, m, -CH<sub>2</sub>OAc), 2.55 (1 H, m, -CHCH<sub>2</sub>OAc), 2.45 (2 H, m, -CH<sub>2</sub>CO<sub>2</sub>H), 2.05 (6 H, s, -COMe);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  177.7, 171.4 (-CO<sub>2</sub>H, and -COMe), 64.1 (-CH<sub>2</sub>OAc), 34.5 (-CHCH<sub>2</sub>OAc), 33.4 (-CH<sub>2</sub>CO<sub>2</sub>H), 21.1 (-COMe).

3-Hydroxymethyl-4-butenolide (**115**)<sup>24</sup>

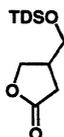


To a solution of acid (**114**) (3.7 g, 17.0 mmol) in methanol (30 cm<sup>3</sup>) and water (20 cm<sup>3</sup>) was added potassium carbonate (6.0 g, 43.4 mmol) and the reaction mixture stirred at room temperature for 2 hours before being acidified with hydrochloric acid

(2 M), saturated with sodium chloride and extracted with ethyl acetate (3 x 75 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) concentrated. Chromatography of the residue over silica gel [petrol/ethyl acetate (1:2)] yielded *the title alcohol* as a colourless oil (0.8 g, 41 %).

(Found: M<sup>+</sup> 116.04734, C<sub>5</sub>H<sub>8</sub>O<sub>3</sub> requires M 116.04782);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3400br m, 2950m, 1760s;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  4.44 (1 H, dd, *J* 1.4, 9.4, -CHHOCO-), 4.22 (1 H, dd, *J* 4.1, 9.4, -CHHOCO-), 3.67 (2 H, m, -CH<sub>2</sub>OH), 2.78 (1 H, m, -CHCH<sub>2</sub>OH), 2.64 (1 H, dd, *J* 8.8, 17.6, -CHCHHCOO-), 2.40 (1 H, dd, *J* 5.7, 17.6, -CHCHHCOO-);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  178.9 (-OCOCH<sub>2</sub>-), 71.4 (-CH<sub>2</sub>OH), 63.2 (-CH<sub>2</sub>OCO-), 37.3 (-CHCH<sub>2</sub>OH), 31.3 (-CHCH<sub>2</sub>COOCH<sub>2</sub>-).

3-(*O*-Dimethylhexylsilyl)-  
hydroxymethyl-4-butenolide (**116**)

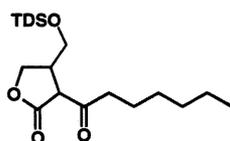


To a solution of (**115**) (180 mg, 1.5 mmol) in dry *N,N*-dimethylformamide (2.0 cm<sup>3</sup>) was added imidazole (0.2 g, 2.9 mmol) and hexyldimethylsilyl chloride (0.5 cm<sup>3</sup>, 2.3 mmol) and the mixture stirred for 24 hours at room temperature before the addition of dichloromethane (20 cm<sup>3</sup>). The organic phase was washed with water (2 x 20 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue over silica gel [petrol/diethyl ether (4:1)] gave *the title compound* as a colourless oil (240 mg, 40 %).

$\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2980s, 1780s, 1460m, 1250m, 830m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  4.28 (1 H, dd, *J* 1.0, 9.1, -CHHOCO-), 4.07 (1 H, dd, *J* 3.8, 9.1, -CHHOCO-), 3.52 (2 H, m, -CH<sub>2</sub>OTDS), 2.62 (1 H, m, -CHCH<sub>2</sub>OTDS), 2.47 (1 H, dd, *J* 8.8, 17.6, -

CHCHHCOOCH<sub>2</sub>-), 2.27 (1 H, dd, *J* 11.3, 17.6, -CHCHHCOOCH<sub>2</sub>-), 1.60 (1 H, sept, *J* 6.9, -CH(Me)<sub>2</sub>), 0.77 (6 H, d, *J* 6.9, -CH(Me)<sub>2</sub>), 0.74 (6 H, s, -C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 0.00 (6 H, s, -OSi(Me)<sub>2</sub>-); δ<sub>C</sub>(62.9 MHz CDCl<sub>3</sub>) 177.6 (-COCH<sub>2</sub>-), 70.9 (-CH<sub>2</sub>OCO-), 63.4 (-CH<sub>2</sub>OTDS), 37.5 (-COCH<sub>2</sub>CH-), 34.5 (-CH(Me)<sub>2</sub>), 31.1 (-CH<sub>2</sub>COO-), 25.5 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-) 20.6, 18.8 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), -3.8 (-OSi(Me)<sub>2</sub>-); *m/z* (CI) 276 (M + NH<sub>4</sub><sup>+</sup>) 259 (MH<sup>+</sup>) 173 155 75 55.

3-(*O*-Dimethylhexylsilyl)-hydroxymethyl-  
2-(1'-oxoheptanoyl)-4-butenolide (**117a**)

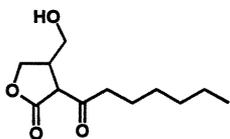


To a solution of (**116**) (575 mg, 2.2 mmol) in sodium dried diethyl ether (15 cm<sup>3</sup>) at -78 °C was added lithium hexamethyldisilazane (1.0 M in tetrahydrofuran; 3.0 cm<sup>3</sup>, 3.0 mmol) followed after a 20 minute delay by heptanoyl chloride (0.5 cm<sup>3</sup>, 3.2 mmol) and the reaction mixture stirred for 2 hours before being quenched with sodium hydrogen carbonate (20 cm<sup>3</sup>, saturated solution), extracted with diethyl ether (2 x 20 cm<sup>3</sup>) and the combined organic phases dried (magnesium sulfate) and concentrated. Chromatography of the residue over silica gel gave *the protected lactone alcohol* (540 mg, 66 %)

δ<sub>H</sub>(250 MHz CDCl<sub>3</sub>) 4.25 (2 H, m, -CH<sub>2</sub>OCO-), 3.55 (2 H, m, -CH<sub>2</sub>OTDS), 3.10 (1 H, m, -COCHCO-), 2.50 (2 H, m, -COCH<sub>2</sub>CH<sub>2</sub>-), 1.53 (2 H, m, -CH(Me)<sub>2</sub> and -CHCH<sub>2</sub>OTDS), 1.22 (8 H, m, 4 x -CH<sub>2</sub>- chain), 0.79 (15 H, m, -CH<sub>2</sub>Me, and -C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 0.00 (6 H, m, -OSi(Me)<sub>2</sub>-); δ<sub>C</sub>(62.9 MHz CDCl<sub>3</sub>) 203.2 (-CHCOCH<sub>2</sub>-), 172.9 (-OCOCH-), 69.6 (-CH<sub>2</sub>OCO-), 62.0 (-CH<sub>2</sub>OTDS), 55.1 (-

COCHCOCH<sub>2</sub>-), 43.0 (-COCH<sub>2</sub>CH<sub>2</sub>-), 39.6 (-CHCH<sub>2</sub>OTDS), 34.4 (-CH(Me)<sub>2</sub>), 31.9, 29.5, 23.7, 22.9 (4 x -CH<sub>2</sub>-, chain), 25.9 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-) 20.6, 18.9 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 14.4 (-CH<sub>2</sub>Me), -3.3 (-OSi(Me)<sub>2</sub>-).

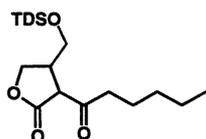
*3-Hydroxymethyl-2-(1'-oxo  
heptanoyl)-4-butenolide (117)*



To protected alcohol (**117a**) (400 mg, 1.1 mmol) was added tetrabutylammonium fluoride (1.0 M in tetrahydrofuran; 5.0 cm<sup>3</sup>, 5.0 mmol) and the mixture stirred at room temperature for 24 hours before ammonium chloride (10 cm<sup>3</sup>, saturated solution) added and the reaction mixture extracted with diethyl ether (3 x 10 cm<sup>3</sup>). The combined organic phases were dried (magnesium sulfate), concentrated. Chromatography of the residue gave *the title alcohol* as a colourless oil (98 mg, 40 %).

$\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3400br m, 2950m, 1760s, 1720s, 1380m, 1250w;  $\delta_{\text{H}}(250 \text{ MHz CDCl}_3)$  4.10 (2 H, m, -CH<sub>2</sub>OCO-), 3.45 (3 H, m, -CH<sub>2</sub>OH and -COCHCO-), 2.95 (1 H, m, -CHCH<sub>2</sub>OH), 2.60 (2 H, m, -CH<sub>2</sub>CO-), 1.35 (2 H, m, -CH<sub>2</sub>CH<sub>2</sub>CO-), 1.10 (6 H, m, 3 x -CH<sub>2</sub>-, chain), 0.70 (3 H, m, -CH<sub>2</sub>Me);  $\delta_{\text{C}}(62.9 \text{ MHz CDCl}_3)$  203.8 (-CHCOCH<sub>2</sub>-), 174.1 (-OCOCHCO-), 69.8 (-CH<sub>2</sub>OCO-), 61.9 (-CH<sub>2</sub>OH), 55.4 (-COCHCO-), 42.9 (-CH<sub>2</sub>CO-), 41.4 (-CHCH<sub>2</sub>OH), 32.0, 29.8, 24.0, 23.5 (4 x -CH<sub>2</sub>-, chain), 14.4 (-CH<sub>2</sub>Me).

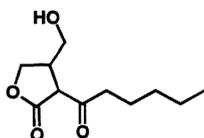
3-(*O*-Dimethylhexylsilyl)-hydroxymethyl-  
2-(1'-oxohexanoyl)-4-butenolide (**118a**)



Lithium hexamethyldisilazane (1.0 M in tetrahydrofuran; 1.2 cm<sup>3</sup>, 1.2 mmol), (**116**) (220 mg, 0.86 mmol), and hexanoyl chloride (0.2 cm<sup>3</sup>, 1.4 mmol) as described for the preparation of (**117a**) gave after chromatography over silica gel [petrol/diethyl ether (20:1)] (**118a**) (120 mg, 38 %).

$\delta_{\text{H}}$ (250 MHz CDCl<sub>3</sub>) 4.30 (1 H, dd,  $J$  0.6, 8.8, -CHHOCO-), 4.03 (1 H, dd,  $J$  2.2, 8.8, -CHHOCO-), 3.54 (3 H, m, -OCOCHCO- and -CH<sub>2</sub>OTDS), 3.10 (1 H, m, -CHCH<sub>2</sub>OTDS), 2.85 (1 H, d x t,  $J$  7.5, 17.6, -COCHHCH<sub>2</sub>-), 2.50 (1 H, d x t,  $J$  7.5, 17.9, -COCHHCH<sub>2</sub>-), 1.52 (3 H, m, -CH(Me)<sub>2</sub> and -COCH<sub>2</sub>CH<sub>2</sub>-), 1.22 (4 H, m, -CH<sub>2</sub>CH<sub>2</sub>Me), 0.80 (15 H, m, -C(Me)<sub>2</sub>CH(Me)<sub>2</sub> and -CH<sub>2</sub>Me), 0.00 (6 H, s, -OSi(Me)<sub>2</sub>-);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 203.2 (-COCH<sub>2</sub>CH-), 172.9 (-OCOCH-), 69.6 (-CH<sub>2</sub>OCO-), 62.0 (-CH<sub>2</sub>OTDS), 55.1 (-COCHCO-), 42.9 (-COCH<sub>2</sub>CH<sub>2</sub>-), 39.6 (-CHCH<sub>2</sub>OTDS), 34.5 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 31.5 (-COCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), 25.6 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-) 23.4, 22.8 (-COCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 20.6, 18.9 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 14.3 (-CH<sub>2</sub>Me), -3.3 (-OSi(Me)<sub>2</sub>-).

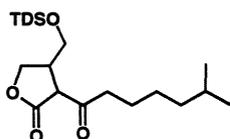
3-Hydroxymethyl-2-(1'-oxo-  
hexanoyl)-4-butanolide (**118**)<sup>121</sup>



Tetrabutylammonium fluoride (1.0 M in tetrahydrofuran; 6 cm<sup>3</sup>, 6.0 mmol) and (**118a**) (440 mg, 1.2 mmol) as described for the preparation of (**117**) gave after chromatography over silica gel [petrol/diethyl ether (1:1)] the title compound (**118**) as a colourless oil (216 mg, 49 %).

(Found:  $M^+$  214.12038, C<sub>11</sub>H<sub>18</sub>O<sub>4</sub> requires  $M$  214.12062);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3400br m, 1780s, 1720s, 1460m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  6.30 (1 H, br s, -OH), 3.25 (1 H, t,  $J$  7.3, -COCHCO-), 2.40 (2 H, m, -CH<sub>2</sub>OCO-), 1.65 (2 H, m, -CH<sub>2</sub>OH), 1.40 (2 H, m, -COCH<sub>2</sub>-), 1.10 (7 H, m, -CHCH<sub>2</sub>OH, 3 x -CH<sub>2</sub>-, chain), 0.75 (3 H, m, -CH<sub>2</sub>Me);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  210.0 (-CHCOCH<sub>2</sub>-), 172.2 (-OCOCH-), 61.0 (-COCHCO-), 42.9 (-CH<sub>2</sub>OCO-), 39.4 (-CHCH<sub>2</sub>OH), 31.5 (-CH<sub>2</sub>OH), 30.9 (-COCH<sub>2</sub>-), 29.9, 23.3, 22.8 (3 x -CH<sub>2</sub>-, chain), 14.1 (-CH<sub>2</sub>Me);  $m/z$  (EI) 214 ( $M^+$ , 0.7 %) 157 (5.8) 115 (86.4) 101 (17.6) 86 (15.3) 72 (100.0) 55 (19.5).

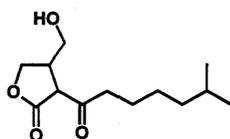
3-(*O*-Dimethylhexylsilyl)-hydroxymethyl-2-  
(6'-methyl-1'-oxoheptyl)-4-butenolide (**119a**)



Lithium hexamethyldisilazane (1.0 M in tetrahydrofuran; 3.5 cm<sup>3</sup>, 3.5 mmol), (**116**) (580 mg, 2.3 mmol) and 6-methylheptanoyl chloride (0.5 g, 3.4 mmol) as described for the preparation of (**7a**) gave after chromatography over silica gel [petrol/diethyl ether (20:1)] the title compound as a colourless oil (450 mg, 51 %).

$\delta_{\text{H}}$ (250 MHz CDCl<sub>3</sub>) 4.31 (1 H, t, *J* 8.8, -CH<sub>2</sub>OCO-), 4.00 (1 H, dd, *J* 6.6, 8.8, -CH<sub>2</sub>OCO-), 3.55 (3 H, m, -CH<sub>2</sub>OTDS and -COCHCO-), 3.10 (1 H, m, -CHCH<sub>2</sub>OTDS), 2.90 (1 H, dt, *J* 17.6, 7.5, -CHHCO-) 2.55 (1 H, dt, *J* 17.6, 7.2, -CHHCO-), 1.50 (4 H, m, -COCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), 1.10 (4 H, m, -CH<sub>2</sub>CH(Me)<sub>2</sub> and -CH(Me)<sub>2</sub>), 0.77 (18 H, m, -Si(Me)<sub>2</sub>C(Me)<sub>2</sub>CH(Me)<sub>2</sub> and -CH(Me)<sub>2</sub>), 0.00 (6 H, m, -OSi(Me)<sub>2</sub>-);  $\delta_{\text{C}}$ (62.9 MHz CDCl<sub>3</sub>) 203.7 (-CHCOCH<sub>2</sub>-), 173.1 (-OCOCH-), 69.9 (-CH<sub>2</sub>OCO-), 62.4 (-CH<sub>2</sub>OTDS), 55.2 (-COCHCO-), 43.3 (-CH<sub>2</sub>CO-), 39.9 (-CHCH<sub>2</sub>OTDS), 39.4 (-COCH<sub>2</sub>CH<sub>2</sub>-), 34.9 (-CH(Me)<sub>2</sub>), 28.5 (-CH(Me)<sub>2</sub>), 27.6, 25.8 (-CH<sub>2</sub>CH<sub>2</sub>CH(Me)<sub>2</sub>), 25.6 (-OSi(Me)<sub>2</sub>C(Me)<sub>2</sub>-), 23.1, 20.9 (-C(Me)<sub>2</sub>CH(Me)<sub>2</sub>), 19.1 (-CH(Me)<sub>2</sub>), -3.3 (-OSi(Me)<sub>2</sub>-).

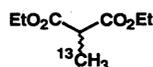
3-Hydroxymethyl-2-(6'-methyl-1'-  
oxoheptanoyl)-4-butenolide (119)



Tetrabutylammounium fluoride (1.0 M in tetrahydrofuran; 5.0 cm<sup>3</sup>, 5.0 mmol) and (119a) (450 mg, 1.1 mmol) as described for the preparation of (7) furnished after chromatography over silica gel [petrol/diethyl ether (1:1)] the title compound as a colourless oil (95 mg, 31 %).

(Found: M<sup>+</sup> 242.15181 C<sub>13</sub>H<sub>22</sub>O<sub>4</sub> requires M 242.15318);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3400br m, 2950s, 1760s, 1720s, 1380m;  $\delta_{\text{H}}(250 \text{ MHz } \text{CDCl}_3)$  4.34 (1 H, m, -COCHCO-), 4.05 (1 H, m, -CH<sub>2</sub>OCO-), 3.60 (1 H, m, -CHCH<sub>2</sub>OH), 3.20 (2 H, m, -CH<sub>2</sub>OH), 2.70 (2 H, m, -COCH<sub>2</sub>CH<sub>2</sub>-), 1.40 (1 H, m, -CH(Me)<sub>2</sub>), 1.10 (6 H, m, 3 x -CH<sub>2</sub>-, chain), 0.79 (6 H, d, J 6.6, -CH(Me)<sub>2</sub>);  $\delta_{\text{C}}(62.9 \text{ MHz } \text{CDCl}_3)$  203.4 (-COCH<sub>2</sub>-), 172.4 (-OCOCH-), 69.5 (-CH<sub>2</sub>OCO-), 62.1 (-CH<sub>2</sub>OH), 55.3 (-COCHCO-), 42.9 (-CH<sub>2</sub>CO-), 39.7 (-CHCH<sub>2</sub>OH), 39.0 (-CH<sub>2</sub>CH<sub>2</sub>CO-), 28.0 (-CH(Me)<sub>2</sub>), 27.1, 23.9 (-CH<sub>2</sub>CH<sub>2</sub>CH(Me)<sub>2</sub>), 20.0 (-CH(Me)<sub>2</sub>); m/z (EI) 242 (M<sup>+</sup>, 4.8 %) 211 (43.8) 158 (20.7) 143 (31.6) 127 (68.9) 109 (100.0) 85 (93.4) 57 (77.2).

[1-<sup>13</sup>CH<sub>3</sub>]-Diethyl methyl malonate (120)

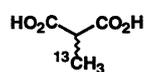


To a stirred ice-cooled suspension of sodium hydride (0.7 g, 17.5 mmol; 60 % dispersion in mineral oil) in dry *N,N*-dimethylformamide (20 cm<sup>3</sup>) was added slowly

diethyl malonate (2.2 cm<sup>3</sup>, 14.5 mmol) and the mixture stirred for 30 minutes. <sup>13</sup>C-Methyl iodide (1.0 cm<sup>3</sup>, 15.95 mmol) was added and the reaction mixture stirred at room temperature for 3 hours before being poured into ice/water (40 cm<sup>3</sup>) and extracted with dichloromethane (3 x 30cm<sup>3</sup>). The combined organic phases were washed with water (3 x 20 cm<sup>3</sup>), dried (magnesium sulfate) and concentrated *in vacuo*. Chromatography of the residue furnished *the title compound* as a colourless liquid (2.1 g, 82 %).

(Found: M<sup>+</sup> 175.09256, C<sub>7</sub><sup>13</sup>CH<sub>14</sub>O<sub>4</sub> requires M 175.09258);  $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3000m, 1750s, 1450m, 1370m;  $\delta_{\text{H}}(250 \text{ MHz CDCl}_3)$  4.17 (4 H, q, *J* 7.2, -CH<sub>2</sub>CH<sub>3</sub>), 3.40 (1 H, d x q, *J* 7.3, 7.2, -CH<sup>13</sup>CH<sub>3</sub>), 1.26 (6 H, t, *J* 7.2, -CH<sub>2</sub>CH<sub>3</sub>) 1.65, 1.15 (3 H, dd, *J* 7.3 *J*<sub>C-H</sub> 133.7, -<sup>13</sup>CH<sub>3</sub>CH-);  $\delta_{\text{C}}(75.4 \text{ MHz CDCl}_3)$  169.6 (CO<sub>2</sub>Et), 60.8 (-CH<sub>2</sub>CH<sub>3</sub>), 45.5 (d, *J* 34.3, -CH<sup>13</sup>CH<sub>3</sub>), 22.3 (-CH<sub>2</sub>CH<sub>3</sub>), 13.1 (-CH<sup>13</sup>CH<sub>3</sub>); *m/z* (EI) 175 (M<sup>+</sup>, 4.5 %) 170 (21.0) 101 (66.5) 91 (67.9) 83 (20.4) 73 (31.8) 69 (36.5) 55 (100.0).

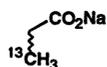
[1-<sup>13</sup>CH<sub>3</sub>]-methyl malonic acid (**121**)



To a stirred solution of malonate (**120**) (3.3 g, 18.8 mmol) in tetrahydrofuran (10 cm<sup>3</sup>) and water (10 cm<sup>3</sup>) was added sodium hydroxide (1.5 g, 37.5 mmol) and the reaction mixture stirred for 48 hours at room temperature. The solvents were removed and to the resulting white solid was added hydrochloric acid (20 cm<sup>3</sup> 2 M). The aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (3 x 20 cm<sup>3</sup>). The combined organic phases were dried (sodium sulfate) and concentrated *in vacuo* to give *the title acid* as a colourless liquid (1.9 g, 88 %).

(Found:  $M^+$  119.02997,  $^{13}\text{CC}_3\text{H}_6\text{O}_4$  requires  $M$  119.03030);  $\delta_{\text{H}}$ (250 MHz MeOD- $d_4$ ) 9.30 (2 H, br s,  $-\text{CO}_2\text{H}$ ), 3.40 (1 H, m,  $-\text{CH}(\text{CO}_2\text{H})_2$ ), 1.63, 1.10 (3 H, dd,  $J$  7.2  $J_{\text{C-H}}$  130.2,  $-\text{CH}^{13}\text{CH}_3$ );  $\delta_{\text{C}}$ (75.4 MHz MeOD- $d_4$ ) 172.4 ( $-\text{CO}_2\text{H}$ ), 46.8 (d,  $J_{\text{C-C}}$  81.6,  $-\text{CH}^{13}\text{CH}_3$ ), 14.0 ( $-\text{CH}^{13}\text{CH}_3$ );  $m/z$  (EI) 119 ( $M^+$ , 1.5 %) 102 (10.0) 75 (100.0) 57 (68.3).

3- $^{13}\text{CH}_3$ -sodium propionate (122)



A solution of diacid (**121**) (1.8 g, 15.1 mmol) in hydrochloric acid (30  $\text{cm}^3$ , 6 M) was heated at reflux for 30 hours. The cooled aqueous solution was saturated with sodium chloride and extracted with ethyl acetate (3 x 20  $\text{cm}^3$ ) the combined organic phases dried (sodium sulfate) and concentrated *in vacuo*. Distillation of the residue at atmospheric pressure gave *the free acid* as a colourless liquid (1.0 g, 88 %). To the free acid was added sodium hydroxide (0.52 g, 0.013 mmol) in water (10  $\text{cm}^3$ ) and the resulting solution freeze dried to give *the title sodium salt* as a white solid (1.2 g, 98 %).

$\delta_{\text{H}}$ (250 MHz  $\text{CDCl}_3$ ; Free acid) 10.3 (1 H, br s,  $-\text{CO}_2\text{H}$ ), 2.40 (2 H, m,  $-\text{CH}_2\text{CH}_3$ ), 1.4, 0.9 (3 H, d x t,  $J$  7.0,  $J_{\text{C-H}}$  130.0,  $-\text{CH}_2^{13}\text{CH}_3$ ).

### 5.3 Streptomycete experimental details

*General procedure for preparation  
of S. nodosus : vegetative suspension*

To a slant (ATCC 14899, SmithKline Beecham) was added a sterile solution of Tween 80 (5.0 cm<sup>3</sup>, 0.02 %, w/v Sigma) and the spores dislodged with a sterile loop. An aliquot of the spore suspension (2.0 cm<sup>3</sup>) was added to an Erlenmeyer flask (2 L) containing a glucose yeast-extract medium (500 cm<sup>3</sup>, [10 g glucose, 10 g yeast [SmithKline Beecham], 1000 cm<sup>3</sup> water] ) and incubated (28 °C, 300 rpm) for 48 hours. The culture broth was diluted 1:1 with sterilised CRY preservative (20 % glycerol, 10 % lactose, 70 % water, w/v) placed into aliquots (1.0 cm<sup>3</sup>) and stored at -40 °C.

*General procedure for the growth of  
S. nodosus : Primary seed*

A solution of glucose-yeast extract medium (1.0 g glucose, 1.0 g yeast, 100 cm<sup>3</sup> water) was adjusted to pH 7.1 with sodium hydroxide (5 M), sterilised in an autoclave for 20 minutes (2 atm 120 °C), and placed into an Erlenmeyer flask (500 cm<sup>3</sup>) with a cotton wool plug. An aliquot (1.0 cm<sup>3</sup>) of *S. nodosus* (ATCC 14899) vegetative suspension was added and the broth incubated (28 °C, 350 rpm) for 72 hours at which time the spores had developed into spheres of vegetative cells, and this suspension was used to inoculate the production media.

*General procedure for the growth  
of S. nodosus : Defined medium<sup>67</sup>*

A defined medium containing water (1000 cm<sup>3</sup>), calcium carbonate (10.0 g), glucose (50.0 g), asparagine (1.0 g), manganese (II) chloride (0.1 g), and ferric sulfate (0.1 g) was adjusted to pH 7.7 with sodium hydroxide (5 M) and sterilised in an autoclave for 20 minutes (2 atm, 120 °C). To aliquots (60 cm<sup>3</sup>) of the medium in Erlenmeyer flasks (500 cm<sup>3</sup>) with cotton wool plugs was added a suspension (3.0 cm<sup>3</sup>) of the primary seed and the broth incubated (28 °C; 400 rpm) for up to 7 days. Concentrations of amphotericin A and B generally peaked after 120 hours and this was found to be the optimum time for harvesting the metabolites.

*General procedure for the growth  
of S. nodosus : Complex medium<sup>67</sup>*

A complex medium containing cottonseed flour ( 30.0 g, SmithKline Beecham), glucose (50.0 g), calcium carbonate (10.0 g), and water (1000 cm<sup>3</sup>) was adjusted to pH 7.7 with sodium hydroxide (5 M) and sterilised in an autoclave for 20 minutes (2 atm, 120 °C). To aliquots (60 cm<sup>3</sup>) in Erlenmeyer flasks (500 cm<sup>3</sup>) with cotton wool plugs was added a suspension (3.0 cm<sup>3</sup>) of the primary seed and the broth incubated (28 °C; 400 rpm) for up to 7 days. Concentrations of amphotericin A and B generally peaked at 120 hours and this was found to be the optimum time for harvesting the metabolites.

*General procedure for the growth  
of S. nodosus : FCA medium<sup>105</sup>*

An FCA medium containing fructose (20.0 g, SmithKline Beecham), Collofilm dextrin (60.0 g, SmithKline Beecham), Arkasoy (30.0 g, SmithKline Beecham), calcium carbonate (10.0 g) and water (1000 cm<sup>3</sup>) was adjusted to pH 7.7 with sodium hydroxide (5 M) and sterilised in an autoclave for 20 minutes (2 atm, 120 °C). To aliquots (60 cm<sup>3</sup>) in Erlenmeyer flasks (500 cm<sup>3</sup>) with cotton wool plugs was added a suspension (3.0 cm<sup>3</sup>) of the primary seed and the broth incubated at 28 °C, 400 rpm for up to 8 days. Concentrations of amphotericin A and B generally peaked after 120 hours and this was found to be the optimum time for harvesting the metabolites. This media also gave the highest titres of both amphotericin A and B up to 3 g lt<sup>-1</sup> and 2 g lt<sup>-1</sup> respectively.

*Quantitative determination of amphotericin A and B  
directly from the culture broth<sup>67,74</sup>*

To an aliquot (1.0 cm<sup>3</sup>) of culture broth was added dimethylsulfoxide (9.0 cm<sup>3</sup>), the mixture sonicated for 30 minutes and stirred vigorously for one hour, then centrifuged (10,000 rpm). The clear yellow supernatant was diluted with methanol (0.2 cm<sup>3</sup> to 10 cm<sup>3</sup>) and the absorbance recorded in 1 cm quartz cuvettes with a wavelength range from 250 nm to 410 nm. Amphotericin A showed specific tetraene peaks at 280, 290, 304 and 320 nm; its concentration was calculated on the basis of the adsorbtion at 304 nm assuming an  $E_{1\text{cm}}^{1\%}$  of 0.850 (10µg/ml methanol). Amphotericin B showed typical heptaene peaks at 344, 364, 383, 406 nm; its concentration was calculated on the basis of the adsorbtion at 406 nm assuming an  $E_{1\text{cm}}^{1\%}$  of 1.740, (10µg/ml methanol).

*Isolation of amphotericin B  
from the culture broth<sup>105</sup>*

The harvested broth was adjusted to pH 10.5 with sodium hydroxide (5M) and mixed with one half its volume of ethyl acetate containing Aliquot 336<sup>®</sup> (7 % w/v) and the mixture stirred vigorously for one hour, the pH being maintained at 10.5 by further additions of sodium hydroxide (5 M). The mixture was centrifuged (10,000 rpm), the phases separated and the aqueous layer was discarded. The organic phases was left to stand in a fridge for 4-5 days whilst amphotericin B precipitated as bright yellow spherulites which sank to the bottom of the holding vessel. When the concentration of amphotericin B had fallen to 30 % of its original level the product was recovered. The precipitate was resuspended in the mother liquor by stirring and recovered by centrifugation. The solid was washed with water, aqueous acetone, anhydrous acetone and methanol before being dried under high vacuum to give amphotericin B as a bright yellow solid with a 95 % purity.

*Autoregulator feeding experiments.*

Production culture broths (defined, complex and FCA) were prepared as described above. At 4 hours into the incubation period the desired autoregulator (10 mg) was added in ethanol (200 µl). The production of amphotericin A and B and the pH of the culture broth was then monitored. A 'blank' culture broth was also prepared in the same manner except that only ethanol (200 µl) was added to provide a base line comparison.

*Procedure for fermentation  
with labelled acetate.*

Three Erlenmeyer flask (500 cm<sup>3</sup>) each containing FCA media (60 cm<sup>3</sup>) was prepared as previously outlined. The culture broth within each flask was pulse fed at 22, 26, 30, 46, 50, 54, and 70 hours into the incubation with sodium acetate-*d*<sub>3</sub> (50 mg each addition). After 120 hours the broth was harvested and the amphotericin B isolated as described above.

*Procedure for fermentation  
with labelled propionates.*

Three Erlenmeyer flask (500 cm<sup>3</sup>) each containing FCA media (60 cm<sup>3</sup>) were prepared as previously outlined. The culture broth within each flask was pulse fed at 22, 26, 30, 46, 50, 54, and 70 hours into the incubation with either 1-<sup>13</sup>C or 3-<sup>13</sup>C-sodium propionate (50 mg each addition). After 120 hours the broth was harvested and the amphotericin B isolated as described above.

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<sup>‡</sup> This paper describes the addition of A-factor to a *S. grievus* culture at the time of inoculation, inducing metabolite production 24 hours earlier than expected and became known at the time of binding of this thesis.

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## Appendix A

**FCA Feeding experiment  
Amphotericin B data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 406 nm		mg/Lt (7)		Abs 406 nm		mg/Lt (119)		Abs 406 nm		mg/Lt (118)		Abs 406 nm		mg/Lt (117)		Abs 406 nm		mg/Lt Blank	
		(7)	*0.0	*0.0	0.0	(119)	*0.0	*0.0	0.0	(119)	166.3	*0.057	*0.043	124.6	(118)	*0.052	*0.129	376.1	(117)	*0.052	*0.146
5	1	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0
9	5	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0
11	7	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0
15	11	*0.115	*0.115	334.2	334.2	*0.057	*0.057	166.3	166.3	*0.043	*0.043	124.6	124.6	*0.052	*0.052	151.3	151.3	*0.052	*0.052	150.0	150.0
20	16	*0.119	*0.119	345.1	345.1	*0.127	*0.127	368.9	368.9	*0.105	*0.105	304.4	304.4	*0.129	*0.129	376.1	376.1	*0.146	*0.146	422.7	422.7
33	29	*0.291	*0.291	844.3	844.3	*0.426	*0.426	1235.1	1235.1	*0.373	*0.373	1082.3	1082.3	*0.359	*0.359	1040.0	1040.0	*0.434	*0.434	1256.8	1256.8
38	34	*0.187	*0.187	1086.6	1086.6	*0.273	*0.273	1373.7	1373.7	*0.191	*0.191	1106.6	1106.6	*0.202	*0.202	1171.7	1171.7	*0.249	*0.249	1441.3	1441.3
62	58	*0.374	*0.374	2166.3	2166.3	*0.443	*0.443	2571.7	2571.7	*0.313	*0.313	1816.1	1816.1	*0.346	*0.346	2003.4	2003.4	*0.437	*0.437	2532.1	2532.1
86	82	*0.373	*0.373	2160.9	2160.9	*0.444	*0.444	2574.6	2574.6	*0.321	*0.321	1863.0	1863.0	*0.338	*0.338	1960.2	1960.2	*0.437	*0.437	2531.0	2531.0
134	130	*0.375	*0.375	2173.3	2173.3	*0.450	*0.450	2607.4	2607.4	*0.353	*0.353	2046.7	2046.7	*0.352	*0.352	2040.6	2040.6	*0.459	*0.459	2658.0	2658.0
158	154	*0.371	*0.371	2150.6	2150.6	*0.568	*0.568	3293.6	3293.6	*0.403	*0.403	2336.1	2336.1	*0.375	*0.375	2173.6	2173.6	*0.521	*0.521	3020.5	3020.5
254	250	*0.354	*0.354	2054.1	2054.1	*0.483	*0.483	2797.9	2797.9	*0.376	*0.376	2178.8	2178.8	*0.371	*0.371	2148.6	2148.6	*0.468	*0.468	2714.2	2714.2

\* 500 fold dilution. # 1000 fold dilution.

FCA Feeding experiment  
Amphotericin A data.

Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 304 nm (7)		Titre mg/Lt (7)		Abs 304 nm (119)		Titre mg/Lt (119)		Abs 304 nm (118)		Titre mg/Lt (118)		Abs 304 nm (117)		Titre mg/Lt (117)			
		*0.0	#0.109	0.0	503.3	*0.0	641.8	*0.0	758.0	*0.0	682.8	*0.0	494.2	*0.0	550.0	*0.0	681.4	*0.0	800.0
5	1	*0.0	#0.109	0.0	503.3	*0.0	641.8	*0.0	758.0	*0.0	682.8	*0.0	494.2	*0.094	*0.129	*0.136	800.0	*0.0	0.0
9	5	*0.0	#0.152	0.0	1792.4	*0.0	1792.4	*0.175	2059.5	*0.113	1333.8	*0.138	1624.5	#0.138	#0.157	#0.157	1851.4	*0.0	0.0
11	7	*0.0	#0.150	0.0	1763.5	*0.167	1958.3	*0.112	1325.9	*0.136	1603.7	#0.145	1708.6	#0.142	#0.153	#0.153	1795.4	*0.0	0.0
15	11	*0.0	#0.147	0.0	1731.3	*0.206	2417.9	*0.155	1827.9	*0.162	1910.5	#0.176	2065.5	#0.176	#0.176	#0.176	2065.5	*0.0	0.0
20	16	*0.085	#0.138	0.0	1620.9	*0.203	2389.3	*0.130	1523.6	*0.135	1584.9	#0.156	1833.1	#0.156	#0.156	#0.156	1833.1	*0.0	0.0
33	29	*0.109	#0.136	0.0	800.0	*0.068	800.0	*0.106	1247.8	#0.054	633.4	#0.077	900.0	#0.077	#0.077	#0.077	900.0	*0.0	0.0
38	34	#0.136	#0.152	0.0	1792.4	*0.175	2059.5	*0.113	1333.8	#0.138	1624.5	#0.157	1851.4	#0.157	#0.157	#0.157	1851.4	*0.0	0.0
62	58	#0.152	#0.150	0.0	1763.5	*0.167	1958.3	*0.112	1325.9	#0.136	1603.7	#0.142	1665.0	#0.142	#0.142	#0.142	1665.0	*0.0	0.0
86	82	#0.150	#0.156	0.0	1832.0	*0.168	1970.1	*0.136	1593.5	#0.145	1708.6	#0.153	1795.4	#0.153	#0.153	#0.153	1795.4	*0.0	0.0
134	130	#0.156	#0.147	0.0	1731.3	*0.206	2417.9	*0.155	1827.9	*0.162	1910.5	#0.176	2065.5	#0.176	#0.176	#0.176	2065.5	*0.0	0.0
158	154	#0.147	#0.138	0.0	1620.9	*0.203	2389.3	*0.130	1523.6	*0.135	1584.9	#0.156	1833.1	#0.156	#0.156	#0.156	1833.1	*0.0	0.0
254	250	#0.138	#0.138	0.0	1620.9	*0.203	2389.3	*0.130	1523.6	*0.135	1584.9	#0.156	1833.1	#0.156	#0.156	#0.156	1833.1	*0.0	0.0

\* 500 fold dilution # 1000 fold dilution.

**FCA Feeding experiment  
pH data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	pH (7)	pH (119)	pH (118)	pH (117)	pH Blank
0	0	7.70	7.70	7.70	7.70	7.70
9	5	7.00	7.08	7.10	7.03	7.10
11	7	6.99	7.01	7.08	7.00	7.02
15	11	7.10	7.15	7.20	7.10	7.20
20	16	7.20	7.30	7.40	7.28	7.40
33	29	7.10	7.35	7.50	7.30	7.45
38	34	6.85	7.15	7.30	7.00	7.30
62	58	7.60	7.40	7.65	7.55	7.68
86	82	7.20	7.90	7.80	7.90	7.80
134	130	8.20	8.35	8.40	8.35	8.45
158	154	8.10	8.70	8.65	8.50	8.45
254	250	9.0	8.70	8.70	9.0	9.0

**Complex media feeding experiment  
Amphotericin B data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 406 nm		mg/Lt (7)		Abs 406 nm (119)		mg/Lt (119)		Abs 406 nm (118)		mg/Lt (118)		Abs 406 nm (117)		mg/Lt (117)		Abs 406 nm		mg/Lt			
		(7)	(7)	*0.0	0.0	*0.027	15.5	*0.035	20.3	*0.030	17.5	*0.035	20.3	*0.030	17.5	*0.035	20.3	*0.030	17.5	*0.043	24.8	*0.043	24.8
5	1	*0.014	8.2	*0.027	15.5	*0.035	20.3	*0.030	17.5	*0.035	20.3	*0.030	17.5	*0.043	24.8	*0.043	24.8	*0.043	24.8	*0.043	24.8	*0.043	24.8
10	6	*0.127	73.8	*0.122	70.8	*0.135	78.1	*0.116	67.5	*0.135	78.1	*0.116	67.5	*0.152	88.4	*0.152	88.4	*0.152	88.4	*0.152	88.4	*0.152	88.4
14	10	*0.340	197.0	*0.288	166.8	*0.296	171.7	*0.215	124.7	*0.296	171.7	*0.215	124.7	*0.337	195.5	*0.337	195.5	*0.337	195.5	*0.337	195.5	*0.337	195.5
20	16	#0.219	254.1	#0.253	293.1	#0.271	314.5	#0.174	201.2	#0.271	314.5	#0.174	201.2	#0.275	319.2	#0.275	319.2	#0.275	319.2	#0.275	319.2	#0.275	319.2
32	28	#0.249	288.4	#0.291	337.9	#0.296	342.9	#0.208	241.0	#0.296	342.9	#0.208	241.0	#0.309	358.0	#0.309	358.0	#0.309	358.0	#0.309	358.0	#0.309	358.0
38	34	#0.106	305.8	#0.128	370.4	#0.114	331.0	#0.078	227.0	#0.114	331.0	#0.078	227.0	#0.160	462.5	#0.160	462.5	#0.160	462.5	#0.160	462.5	#0.160	462.5
64	60	#0.146	423.0	#0.172	497.4	#0.174	504.5	#0.134	387.2	#0.174	504.5	#0.134	387.2	#0.208	602.0	#0.208	602.0	#0.208	602.0	#0.208	602.0	#0.208	602.0
109	105	#0.095	274.0	#0.137	397.4	#0.138	398.8	#0.105	303.6	#0.138	398.8	#0.105	303.6	#0.179	517.6	#0.179	517.6	#0.179	517.6	#0.179	517.6	#0.179	517.6
154	150																						

\* 100 fold dilution. # 200 fold dilution ◆ 500 fold dilution.

**Complex media feeding experiment  
Amphotericin A data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 304 nm (7)		Titre mg/Lt (7)		Abs 304 nm (119)		Titre mg/Lt (119)		Abs 304 nm (118)		Titre mg/Lt (118)		Abs 304 nm (117)		Titre mg/Lt (117)		Abs 304 nm Blank		Titre mg/Lt Blank	
		*0.0	*0.0	0.0	0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	*0.0	0.0
5	1	*0.0	*0.0	0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	0.0	0.0
10	6	*0.0	*0.0	0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	0.0	0.0
14	10	*0.249	*0.222	292.7	260.8	*0.222	*0.222	260.8	260.8	*0.255	*0.255	300.1	300.1	*0.245	*0.245	288.4	288.4	*0.249	*0.249	292.3	292.3
20	16	*0.434	*0.333	510.0	392.1	*0.333	*0.333	392.1	392.1	*0.335	*0.335	393.5	393.5	*0.335	*0.335	393.4	393.4	*0.311	*0.311	366.1	366.1
32	28	#0.274	#0.284	643.6	667.1	#0.284	#0.284	667.1	667.1	#0.291	#0.291	684.0	684.0	#0.335	#0.335	788.7	788.7	#0.264	#0.264	619.8	619.8
38	34	#0.292	#0.320	686.9	752.3	#0.320	#0.320	752.3	752.3	#0.307	#0.307	723.2	723.2	#0.426	#0.426	1001.6	1001.6	#0.290	#0.290	683.0	683.0
64	60	#0.135	#0.144	795.2	844.1	#0.144	#0.144	844.1	844.1	#0.118	#0.118	693.5	693.5	#0.201	#0.201	1183.8	1183.8	#0.142	#0.142	836.3	836.3
109	105	#0.200	#0.209	1175.0	1231.3	#0.209	#0.209	1231.3	1231.3	#0.189	#0.189	1110.8	1110.8	#0.279	#0.279	1643.0	1643.0	#0.203	#0.203	1191.1	1191.1
154	150	#0.119	#0.164	697.5	962.4	#0.164	#0.164	962.4	962.4	#0.151	#0.151	889.2	889.2	#0.246	#0.246	1445.6	1445.6	#0.157	#0.157	922.3	922.3

\* 100 fold dilution. # 200 fold dilution. ◆ 500 fold dilution.

**Complex media feeding experiment  
pH data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	pH (7)	pH (119)	pH (118)	pH (117)	pH Blank
5	1	7.7	7.7	7.7	7.7	7.7
10	6	7.10	7.05	7.10	7.15	7.10
14	10	7.05	7.00	7.00	7.20	7.05
20	16	6.90	6.80	6.80	7.00	6.75
32	28	6.30	6.20	6.25	6.45	6.15
38	34	6.20	6.20	6.10	6.35	6.05
64	60	6.00	5.85	5.90	6.10	6.00
109	105	7.90	8.00	8.00	8.60	8.40
154	150	8.55	8.60	8.55	8.55	8.80

**Defined media feeding experiment  
Amphotericin B data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 406 nm		mg/Lt (7)		Abs 406 nm		mg/Lt (119)		Abs 406 nm		mg/Lt (118)		Abs 406 nm		mg/Lt (117)		Abs 406 nm		mg/Lt Blank	
		(7)	(7)	(7)	(7)	(119)	(119)	(118)	(118)	(119)	(119)	(118)	(118)	(117)	(117)	(117)	(117)	Blank	Blank		
5	1	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0
10	6	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0	*0.0	0.0
14	10	*0.021	12.0	*0.013	7.7	*0.018	10.4	*0.015	8.7	*0.017	9.8	*0.015	8.7	*0.029	16.7	*0.017	9.8	*0.015	8.7	*0.029	16.7
20	16	*0.025	14.3	*0.005	2.7	*0.017	9.8	*0.017	9.8	*0.017	9.8	*0.017	9.8	*0.035	20.3	*0.017	9.8	*0.017	9.8	*0.035	20.3
32	28	#0.015	17.2	#0.018	20.5	#0.016	18.4	#0.018	20.4	#0.016	18.4	#0.018	20.4	#0.029	33.9	#0.018	20.4	#0.018	20.4	#0.029	33.9
38	34	#0.024	28.0	#0.013	15.5	#0.022	25.0	#0.022	25.0	#0.022	25.0	#0.022	25.0	#0.033	38.3	#0.022	25.0	#0.022	25.0	#0.033	38.3
64	60	♦0.009	25.3	♦0.013	37.1	♦0.008	22.5	♦0.007	22.8	♦0.008	22.5	♦0.007	22.8	♦0.005	13.3	♦0.007	22.8	♦0.007	22.8	♦0.005	13.3
109	105	♦0.021	60.4	♦0.008	23.3	♦0.011	33.1	♦0.014	40.7	♦0.011	33.1	♦0.014	40.7	♦0.024	68.3	♦0.014	40.7	♦0.014	40.7	♦0.024	68.3
154	150	♦0.010	30.0	♦0.004	10.4	♦0.008	23.9	♦0.008	24.0	♦0.008	23.9	♦0.008	24.0	♦0.011	31.0	♦0.008	24.0	♦0.008	24.0	♦0.011	31.0

\* 100 fold dilution. # 200 fold dilution ♦ 500 fold dilution.

**Defined media feeding experiment  
Amphotericin A data.**

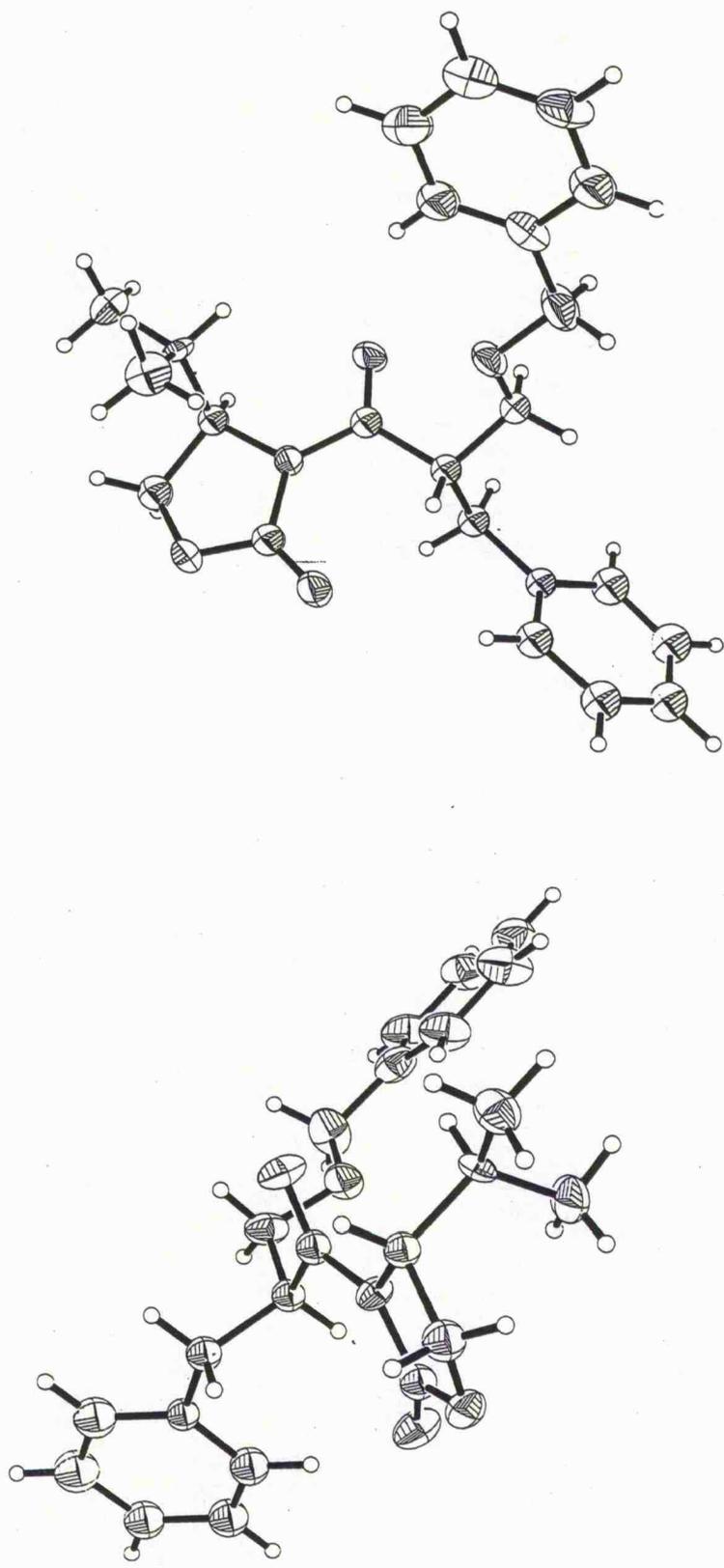
Total time from inoculation (hr)	Time from autoregulator addition (hr)	Abs 304 nm (7)		Titre mg/Lt (7)		Abs 304 nm (119)		Titre mg/Lt (119)		Abs 304 nm (118)		Titre mg/Lt (118)		Abs 304 nm (117)		Titre mg/Lt (117)	
		*0.0	*0.0	0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0	*0.0	*0.0	*0.0	0.0
5	1	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0
10	6	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0	*0.0	*0.0	0.0	0.0
14	10	*0.051	*0.051	60.5	60.5	*0.044	*0.044	52.0	52.0	*0.049	*0.049	57.5	57.5	*0.060	*0.060	70.0	72.3
20	16	*0.063	*0.063	74.3	74.3	*0.052	*0.052	61.3	61.3	*0.061	*0.061	71.9	71.9	*0.068	*0.068	80.1	90.7
32	28	#0.060	#0.060	140.0	140.0	#0.064	#0.064	151.0	151.0	#0.054	#0.054	126.0	126.0	#0.056	#0.056	130.9	147.6
38	34	#0.059	#0.059	139.9	139.9	#0.059	#0.059	137.6	137.6	#0.059	#0.059	138.5	138.5	#0.053	#0.053	125.5	129.6
64	60	◆0.020	◆0.020	120.0	120.0	◆0.010	◆0.010	60.0	60.0	◆0.013	◆0.013	74.0	74.0	◆0.019	◆0.019	108.8	103.0
109	105	◆0.051	◆0.051	300.4	300.4	◆0.052	◆0.052	304.0	304.0	◆0.062	◆0.062	366.9	366.9	◆0.005	◆0.005	30.0	331.7
154	150	◆0.043	◆0.043	250.1	250.1	◆0.051	◆0.051	300.3	300.3	◆0.043	◆0.043	250.2	250.2	◆0.036	◆0.036	210.1	200.3

\* 100 fold dilution. # 200 fold dilution ◆ 500 fold dilution.

**Defined media feeding experiment  
pH data.**

Total time from inoculation (hr)	Time from autoregulator addition (hr)	pH (7)	pH (119)	pH (118)	pH (117)	pH Blank
5	1	7.7	7.7	7.7	7.7	7.7
10	6	6.80	7.05	7.00	6.90	7.10
14	10	6.80	7.10	7.10	7.00	7.20
20	16	6.70	7.30	7.20	7.10	7.50
32	28	6.70	7.55	7.35	7.15	7.60
38	34	6.65	7.30	7.35	7.20	7.65
64	60	5.00	7.10	7.10	6.85	7.25
109	105	4.00	7.05	7.00	6.70	7.00
154	150	6.95	7.05	6.95	6.95	7.00

## Appendix B



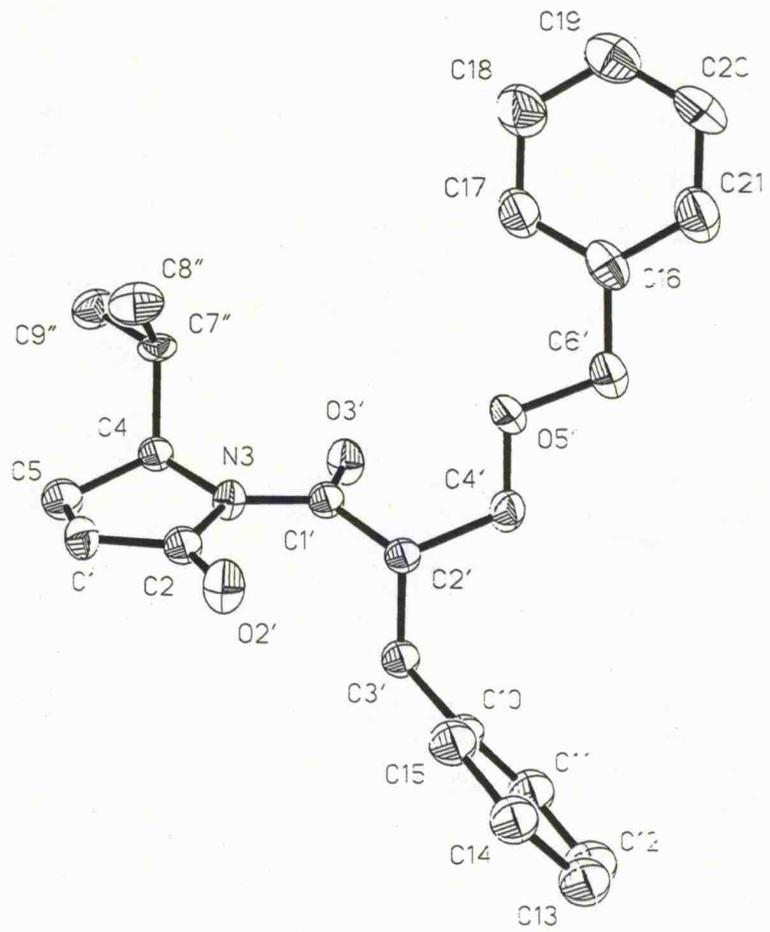


Table 1. Crystal data and structure refinement for  $C_{23}H_{27}NO_4$ .

Identification code	lab
Empirical formula	$C_{23}H_{27}NO_4$
Formula weight	381.46
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	$a = 6.272(5)$ Å $\alpha = 90^\circ$ $b = 9.88(2)$ Å $\beta = 90^\circ$ $c = 33.845(5)$ Å $\gamma = 90^\circ$
Volume	$2097(4)$ Å <sup>3</sup>
Z	4
Density (calculated)	$1.208$ Mg/m <sup>3</sup>
Absorption coefficient	$0.082$ mm <sup>-1</sup>
F(000)	816
Crystal size	0.48 x 0.18 x 0.07 mm
$\theta$ range for data collection	2.74 to $22.48^\circ$
Index ranges	$-1 \leq h \leq 6$ , $-1 \leq k \leq 10$ , $-1 \leq l \leq 36$
Reflections collected	2135
Independent reflections	1932 ( $R_{int} = 0.0214$ )
Refinement method	Full-matrix least-squares on $F^2$
Data / restraints / parameters	1932 / 0 / 169
Goodness-of-fit on $F^2$	1.041
Final R indices [ $I > 2\sigma(I)$ ]	$R1 = 0.0734$ , $wR2 = 0.1397$
R indices (all data)	$R1 = 0.1399$ , $wR2 = 0.1701$
Absolute structure parameter	4(5)
Largest diff. peak and hole	0.411 and $-0.204$ eÅ <sup>-3</sup>

Table 2. Atomic coordinates [ $\times 10^4$ ] and equivalent isotropic displacement parameters [ $\text{\AA}^2 \times 10^3$ ] for  $\text{C}_{23}\text{H}_{27}\text{NO}_4$ .  $U(\text{eq})$  is one third of the trace of the orthogonalized  $U_{ij}$  tensor.

	x	y	z	U(eq)
O(1)	6264 (9)	3145 (5)	-6 (2)	53 (2)
O(2')	6515 (11)	1459 (6)	437 (2)	62 (2)
O(3')	8470 (11)	4706 (5)	1156 (2)	58 (2)
O(5')	6079 (10)	2745 (6)	1726 (2)	58 (2)
N(3)	7231 (11)	3718 (6)	606 (2)	39 (2)
C(9'')	5051 (17)	7231 (8)	366 (3)	66 (3)
C(8'')	3007 (14)	5111 (9)	498 (3)	69 (3)
C(7'')	5067 (15)	5829 (7)	550 (2)	41 (2)
C(4)	6985 (15)	5030 (8)	404 (2)	42 (2)
C(5)	6833 (16)	4558 (8)	-25 (2)	55 (3)
C(2)	6658 (16)	2639 (9)	356 (3)	45 (2)
C(1')	8016 (15)	3656 (8)	991 (2)	43 (2)
C(2')	8343 (14)	2256 (8)	1177 (2)	40 (2)
C(3')	10555 (13)	1780 (8)	1054 (2)	46 (2)
C(10)	11007 (10)	304 (4)	1155 (2)	41 (2)
C(11)	12900 (9)	-33 (5)	1348 (2)	68 (3)
C(12)	13359 (8)	-1384 (6)	1433 (1)	78 (3)
C(13)	11923 (10)	-2397 (4)	1325 (2)	67 (3)
C(14)	10029 (9)	-2061 (5)	1133 (2)	65 (3)
C(15)	9571 (8)	-710 (6)	1048 (1)	60 (3)
C(4')	8176 (18)	2356 (9)	1624 (2)	56 (3)
C(6')	5768 (16)	2652 (12)	2141 (2)	81 (4)
C(16)	3848 (9)	3429 (7)	2266 (2)	62 (3)
C(17)	3028 (13)	4487 (8)	2039 (2)	74 (3)
C(18)	1264 (13)	5215 (6)	2173 (2)	94 (4)
C(19)	321 (10)	4885 (8)	2534 (3)	82 (4)
C(20)	1142 (13)	3826 (8)	2760 (2)	78 (4)
C(21)	2906 (13)	3099 (6)	2626 (2)	83 (4)

Table 3. Bond lengths [Å] and angles [°] for C<sub>23</sub>H<sub>27</sub>NO<sub>4</sub>.

O(1)-C(2)	1.348(9)	O(1)-C(5)	1.442(9)
O(2')-C(2)	1.200(9)	O(3')-C(1')	1.211(9)
O(5')-C(4')	1.413(10)	O(5')-C(6')	1.419(10)
N(3)-C(1')	1.395(9)	N(3)-C(2)	1.407(9)
N(3)-C(4)	1.473(9)	C(9 <sup>''</sup> )-C(7 <sup>''</sup> )	1.518(10)
C(8 <sup>''</sup> )-C(7 <sup>''</sup> )	1.485(11)	C(7 <sup>''</sup> )-C(4)	1.520(11)
C(4)-C(5)	1.531(10)	C(1')-C(2')	1.533(10)
C(2')-C(4')	1.518(10)	C(2')-C(3')	1.523(11)
C(3')-C(10)	1.525(9)	C(10)-C(15)	1.40
C(10)-C(11)	1.40	C(11)-C(12)	1.40
C(12)-C(13)	1.39	C(13)-C(14)	1.39
C(14)-C(15)	1.40	C(6')-C(16)	1.489(10)
C(16)-C(21)	1.39	C(16)-C(17)	1.39
C(17)-C(18)	1.40	C(18)-C(19)	1.40
C(19)-C(20)	1.39	C(20)-C(21)	1.40
C(2)-O(1)-C(5)	110.8(6)	C(4')-O(5')-C(6')	110.6(7)
C(1')-N(3)-C(2)	128.2(7)	C(1')-N(3)-C(4)	120.6(6)
C(2)-N(3)-C(4)	111.2(6)	C(8 <sup>''</sup> )-C(7 <sup>''</sup> )-C(9 <sup>''</sup> )	112.4(8)
C(8 <sup>''</sup> )-C(7 <sup>''</sup> )-C(4)	113.7(6)	C(9 <sup>''</sup> )-C(7 <sup>''</sup> )-C(4)	110.3(8)
N(3)-C(4)-C(7 <sup>''</sup> )	112.9(7)	N(3)-C(4)-C(5)	100.3(6)
C(7 <sup>''</sup> )-C(4)-C(5)	114.6(7)	O(1)-C(5)-C(4)	105.5(6)
O(2')-C(2)-O(1)	123.5(8)	O(2')-C(2)-N(3)	128.2(8)
O(1)-C(2)-N(3)	108.2(7)	O(3')-C(1')-N(3)	118.3(7)
O(3')-C(1')-C(2')	123.5(7)	N(3)-C(1')-C(2')	118.1(7)
C(4')-C(2')-C(3')	110.9(8)	C(4')-C(2')-C(1')	109.9(7)
C(3')-C(2')-C(1')	106.7(7)	C(2')-C(3')-C(10)	113.7(7)
C(15)-C(10)-C(11)	120.0	C(15)-C(10)-C(3')	120.5(5)
C(11)-C(10)-C(3')	119.4(5)	C(12)-C(11)-C(10)	120.0
C(13)-C(12)-C(11)	120.0	C(12)-C(13)-C(14)	120.0
C(13)-C(14)-C(15)	120.0	C(10)-C(15)-C(14)	120.0
O(5')-C(4')-C(2')	109.0(7)	O(5')-C(6')-C(16)	111.0(8)
C(21)-C(16)-C(17)	120.0	C(21)-C(16)-C(6')	118.1(7)
C(17)-C(16)-C(6')	121.9(7)	C(16)-C(17)-C(18)	120.0
C(17)-C(18)-C(19)	120.0	C(20)-C(19)-C(18)	120.0
C(19)-C(20)-C(21)	120.0	C(16)-C(21)-C(20)	120.0

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [ $\text{\AA}^2 \times 10^3$ ] for  $\text{C}_{23}\text{H}_{27}\text{NO}$ .  
 The anisotropic displacement factor exponent takes the  $-2\pi^2 [ (ha)^2 U_{11} + \dots + 2hka^* b^* U_{12} ]$

	U11	U22	U33	U23	U13	U12
O(1)	60(4)	61(4)	39(3)	-5(3)	-10(3)	3(4)
O(2')	89(5)	46(4)	51(3)	2(3)	-15(4)	-14(4)
O(3')	87(5)	39(3)	46(3)	-6(3)	-12(4)	-9(4)
O(5')	61(5)	77(5)	34(3)	-3(3)	7(3)	8(5)
N(3)	49(5)	31(3)	37(4)	3(3)	-7(4)	-7(4)
C(9'')	70(7)	51(6)	76(6)	8(5)	0(7)	11(7)
C(8'')	33(6)	78(7)	95(7)	-5(7)	0(6)	-10(7)
C(7'')	45(6)	26(5)	51(5)	-4(4)	4(6)	14(5)
C(4')	80(8)	44(6)	42(5)	-2(5)	-5(6)	10(7)
C(6')	78(8)	121(10)	43(6)	11(6)	7(6)	22(9)
C(16)	68(8)	72(8)	45(6)	-9(6)	7(6)	-22(8)
C(17)	103(10)	66(7)	53(6)	3(6)	12(8)	7(9)
C(18)	135(12)	74(8)	74(8)	-5(7)	15(8)	22(10)
C(19)	82(9)	88(10)	75(8)	-19(7)	10(8)	-6(9)
C(20)	85(9)	82(8)	66(7)	-14(7)	33(7)	-10(8)
C(21)	116(10)	78(8)	55(7)	1(6)	10(7)	4(9)

Table 5. Hydrogen coordinates (  $\times 10^4$  ) and isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for  $\text{C}_{23}\text{H}_{27}\text{NO}_4$ .

	x	y	z	U(eq)
H(9"A)	3834 (17)	7725 (8)	460 (3)	80
H(9"B)	6331 (17)	7702 (8)	439 (3)	80
H(9"C)	4978 (17)	7152 (8)	84 (3)	80
H(8"A)	1866 (14)	5668 (9)	595 (3)	80
H(8"B)	2784 (14)	4924 (9)	222 (3)	80
H(8"C)	3038 (14)	4276 (9)	642 (3)	80
H(7"A)	5258 (15)	5953 (7)	829 (2)	80
H(4A)	8255 (15)	5559 (8)	439 (2)	80
H(5A)	5763 (16)	5063 (8)	-165 (2)	80
H(5B)	8174 (16)	4674 (8)	-158 (2)	80
H(2'A)	7286 (14)	1639 (8)	1079 (2)	80
H(3'A)	11602 (13)	2340 (8)	1181 (2)	80
H(3'B)	10708 (13)	1900 (8)	774 (2)	80
H(11A)	13888 (12)	665 (7)	1422 (2)	80
H(12A)	14662 (9)	-1616 (9)	1565 (2)	80
H(13A)	12238 (14)	-3327 (5)	1384 (2)	80
H(14A)	9041 (12)	-2758 (6)	1059 (2)	80
H(15A)	8268 (9)	-478 (8)	915 (2)	80
H(4'A)	8505 (18)	1497 (9)	1741 (2)	80
H(4'B)	9176 (18)	3012 (9)	1721 (2)	80
H(6'A)	7002 (16)	2995 (12)	2275 (2)	80
H(6'B)	5593 (16)	1720 (12)	2213 (2)	80
H(17A)	3676 (18)	4715 (11)	1791 (2)	80
H(18A)	699 (20)	5943 (8)	2017 (3)	80
H(19A)	-892 (12)	5385 (11)	2626 (4)	80
H(20A)	494 (18)	3599 (12)	3009 (2)	80
H(21A)	3471 (19)	2370 (8)	2782 (3)	80

## Appendix C

## A Convenient Synthesis of Enantiopure Paraconic Acid- A Key intermediate in the Synthesis of A-Factor.

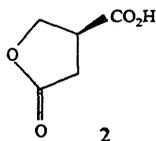
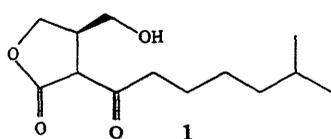
James M. Crawforth and Bernard J. Rawlings.\*

Department of Chemistry, University of Leicester, University Road, Leicester LE1 7RH, U.K. Email bjr2@le.ac.uk

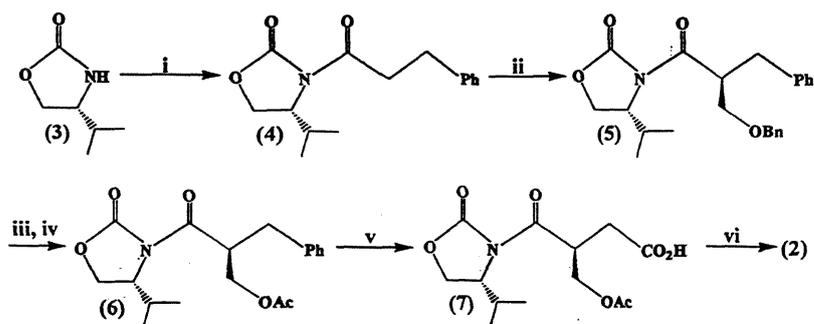
Key Words: A-Factor, paraconic acid.

**Abstract:** Enantiopure (S)-(-)-paraconic acid **2** has been synthesised by the stereospecific benzyloxymethylation of a titanium enolate derived from a homochiral oxazolidinone and dihydrocinnamoyl chloride.

(3*R*)-(-)-A-factor **1** is an inducer of cytodifferentiation in many streptomycetes. In *Streptomyces griseus*, it can induce the biosynthesis of streptomycin in inactive mutants and induce the formation of pores in asporophological modifications.<sup>1</sup> A-factor was first isolated from *S. griseus* in 1976 by Choklov<sup>2</sup>, its full stereostructure being determined by Mori in 1983 as (3*R*), with rapid epimerisation at C-2.<sup>3</sup> In 1982, Mori published a synthesis of enantiopure A-factor from enantiopure paraconic acid **2**,<sup>4</sup> obtained by resolution of the racemate with enantiopure phenethylamine in 3% yield.<sup>5</sup> In 1989, Mori developed an improved synthesis of enantiopure paraconic acid based upon the mono-hydrolysis of 2-acetoxymethyl)-3-phenylpropyl acetate using porcine pancreatic lipase in 62% ee,<sup>6</sup> followed by conversion of the resultant hydroxyacetate to optically active paraconic acid. This was then obtained in enantiopure form by repeated crystallisation with (R)-(+)- $\alpha$ -phenethylamine. (S)-(-)-Paraconic acid **2** can be converted into A-factor in four steps with complete stereofidelity in 15% yield.<sup>6,7</sup>



Parsons has recently published an ingenious synthesis of optically active A-factor based upon a Johnson-Claisen rearrangement of a ketene acetal derived from a chiral allylic alcohol.<sup>8</sup> However, the chiral allylic alcohol substrate was only available via an enantioselective reduction in 83% ee, and the resultant A-factor had less than 50% ee due to additional partial epimerisation of a later intermediate. This has prompted us to publish our synthesis of a key intermediate in the synthesis of enantiopure A-factor, enantiopure (S)-(-)-paraconic acid **2** (in 14% overall yield), Scheme 1.



Scheme 1

Reagents: i) BuLi, dihydrocinnamoyl chloride; ii)  $\text{TiCl}_4$ , di-isopropylethylamine,  $\text{BnOCH}_2\text{Cl}$ ; iii)  $\text{H}_2/\text{Pd/C}$ ; iv)  $\text{Ac}_2\text{O}/\text{pyridine}$ ; v)  $\text{H}_2\text{IO}_6/\text{RuCl}_3$ ; vi)  $\text{LiOH}/\text{H}_2\text{O}_2$ , then  $\text{HCl}$  (6M).

Oxazolidinone 3, derived from D-valine, is N-acylated with dihydrocinnamoyl chloride to give 4, m.p. 59–61<sup>o</sup> (from hexane/ethylacetate),  $[\alpha]_{\text{D}}^{25} = -67.9$  ( $c = 0.86$ ,  $\text{CHCl}_3$ ), in 90% yield.<sup>†</sup> Using procedures developed by Evans,<sup>9</sup> the addition of titanium tetrachloride and di-isopropylethylamine gave the Z-enolate, which was diastereospecifically alkylated by benzyloxymethyl chloride to give 5, m.p. 72–74<sup>o</sup> (from hexane/ether),  $[\alpha]_{\text{D}}^{25} = -57.4$  ( $c = 1.22$ ,  $\text{CHCl}_3$ ), in 65% yield, whose relative stereostructure was confirmed by X-ray analysis.<sup>10</sup> Hydrogenation and acetylation gave the corresponding acetate 6 as an oil,  $[\alpha]_{\text{D}}^{25} = -72.2$  ( $c = 1.09$ ,  $\text{CHCl}_3$ ), in 90% yield. Oxidation of the phenyl ring with ruthenium(VIII) oxide<sup>11</sup> gave the free acid 7 as an oil,  $[\alpha]_{\text{D}}^{25} = -41.3$  ( $c = 1.21$ ,  $\text{CHCl}_3$ ), in 65% yield. The chiral auxiliary was removed using lithium hydroperoxide<sup>12</sup> and the acetate was then hydrolysed (6M HCl) resulting in (S)-(-)-paraconic acid 2,  $[\alpha]_{\text{D}}^{25} = -60.0$  ( $c = 2.1$ , MeOH), (lit.,<sup>4</sup>  $[\alpha]_{\text{D}}^{23} = -59.6$ ,  $c = 0.61$ , MeOH), in 40% yield.

<sup>†</sup> All new compounds gave satisfactory spectral and combustion data.

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