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The Asymmetric Synthesis of Decalin Synthons for Use in Flavour and Fragrance Compounds

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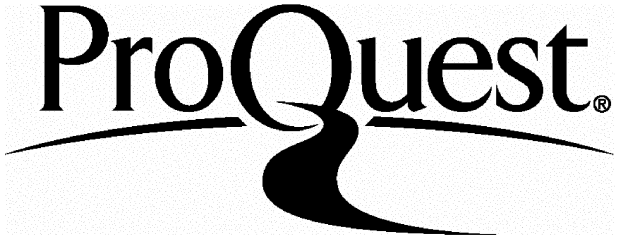
PhD 1998



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LIST OF ABBREVIATIONS

aq	**aq**ueous
BBA	**B**ush **B**oake **A**llen
CCL	**C**andida **c**ylindracea **l**ipase
CMC	**C**ritical **M**icellar **C**oncentration
CTAB	**C**etyl**t**rimethyl**a**mmonium **b**romide
DCM	**D**ichloromethane
DMSO	**D**imethyl sulfoxide
DMF	**D**imethyl **f**ormamide
e.e.	**E**nantiomeric **e**xcess
EI	**E**lectron **I**mpact
FAB	**F**ast **a**tom **b**ombardment
g	**G**ram
GLC	**G**as-**L**iquid **C**hromatography
HPLC	**H**igh **P**eformance **L**iquid **C**hromatography
hr	**H**our
IPA	**I**sopropyl **a**lcohol
IR	**I**nfra **R**ed
l	**L**itre
LDA	**L**ithium **d**iisopropyl**a**mine
M	**M**olar
m-CPBA	***m*-C**hloro**p**eroxy**b**enzoic **a**cid
MDEB	(-)-**d**odecyl-N-methyl**e**phedrinium **b**romide
Me	**M**ethyl
MeOH	**M**ethanol
mg	**M**illi**g**ram
min	**M**inute
MHz	**M**ega**H**ertz

ml	**Millilitre**
mM	**Millimolar**
M	**Molar**
MS	**Mass Spectroscopy**
Ms	**Mesyl = Methane Sulfonyl**
nm	**Nanometer**
NMR	**Nuclear Magnetic Resonance**
Pg	**Picogram**
Ph	**Phenyl**
PPL	**Porcine pancreatic lipase**
psi	**Pounds per square inch**
rpm	**Revolutions per minute**
rt	**Room temperature**
THF	**Tetrahydrofuran**
TLC	**Thin Layer Chromatography**
Ts	**Tosyl = toluene sulphonyl**
UCL	**University College London**
UV	**Ultra Violet**
w.r.t.	**With respect to**

ABSTRACT

In the pursuit of synthons for the preparation of the secondary metabolites *geosmin* and *dehydrogeosmin*, four synthetic strategies were evaluated. To support this study, the preparation of a chiral auxiliary and new surfactant media were also investigated:

Techniques for the production of laboratory scale quantities of the enantiomeric forms of butan-2,3-diol were identified: The bacterial fermentation of sugars by *Bacillus subtilis* strains to access optically pure D-(-)-(2*R*,3*R*)-butandiol and the kinetic enzymatic resolution of a commercially available isomeric mixture of the diol to recover the L-(+)-(2*S*,3*S*)-isomer in optically pure form.

Two optically pure amino acid derived surfactants were synthesised, characterised and a measurement of their critical micellar concentration undertaken.

A novel strategy to construct the dehydrogeosmin skeleton was designed, employing a 'biomimetic' intramolecular polyene cyclisation as the key step. The preparation of the *trans* olefinic cyclisation precursor was investigated and metal facilitated carbonylation was used to generate a requisite aldehydic intermediate. The biomimetic cyclisation was tested and generated the predicted bicyclic octalin ether in a chemical yield and an enantiomeric excess of 22% and >99% respectively.

The novel Diels-Alder disconnection of geosmin was investigated, through the preparation of several diene systems and testing their reactivity in the [4π+2π] cycloaddition reaction. A similar disconnection was applied to the dehydrogeosmin system and optically pure ketals of butan-2,3-diol used in lithium perchlorate solution to generate a precursor in 70% enantiomeric excess.

A study of the Hajos-Parrish reaction - an amino acid catalysed intramolecular cyclisation - was undertaken to evaluate the effects of solvent and amino acid choice on enantioselectivity. Conditions were identified to form the target bicyclic ketone intermediate in a chemical yield of 72% and an enantiomeric excess of 74%. The formation of the cyclisation precursor, from the Michael addition of 2-methylcyclohexane-1,3-dione to ethyl vinyl ketone, was found to be greatly enhanced by performing the reaction in micellar media with a yield of >99% in surfactant solution compared to 55% in water. The use of surfactant solutions in studies of Robinson annelations was also undertaken.

The enantioselective dehydration of a decalol, using an optically pure amino acid as a catalytic dehydrator, was carried out to prepare a key geosmin precursor in a chemical yield and an enantiomeric excess of >99% and 54% respectively. As part of the study into the effect of solvent and amino acid choice on selectivity, new insights into the mechanism of action of the Hajos-Parrish reaction were gained.

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To my friends, both near and far.

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INTRODUCTION

The thesis describes an investigation into the asymmetric synthesis of decalin synthons, for use in the total synthesis of two flavour and fragrance compounds possessing earthy type odour characteristics, geosmin and dehydrogeosmin. The decalin skeleton of these naturally occurring metabolites, nominally the synthetic targets in the project, provided a focus for evaluating new synthetic routes to this bicyclic backbone. Four generic synthetic strategies were derived from the different disconnections possible. The project was thus divided into several key areas, reflected in the presentation of this thesis:

Chapter One - Geosmin and Dehydrogeosmin

This chapter introduces geosmin and dehydrogeosmin, the focus of this project from both a synthetic and commercial point of view. These molecules have significant value as flavour and fragrance ingredients of earthy type character, and the reported work into their synthesis is described, with a discussion of the successful synthetic pathways to date.

Four generic strategies were selected to establish the bicyclic decalin skeleton in the desired synthons, based on different retrosynthetic analyses of geosmin and dehydrogeosmin. Each disconnection is explained in the relevant chapter describing the chosen methodology, the selected four being:

Chapter Four - 'Biomimetic' polyene cyclisation

A strategy was devised to synthesise, with absolute stereocontrol, a key precursor to both geosmin and dehydrogeosmin, through an intramolecular cyclisation of an optically pure polyene derivative. Three experimental protocols were investigated to

establish the required *trans* stereochemistry in the target olefin. The most successful employed a metal facilitated carbonylation to produce an aldehydic precursor that was derivatised to an optically pure acetal, in order to test the stereoselectivity of the key 'biomimetic' cyclisation step.

Chapter Five - Diels-Alder Cycloaddition

The decalin skeleton of both geosmin and dehydrogeosmin can be disconnected back to a [4π+2π] Diels-Alder cycloaddition. This chapter describes the study that was made into this disconnection, the preparation of the necessary parent dienes and the performance of the key cycloaddition reactions. The use of lithium perchlorate solution as a reaction medium, with optically pure dienophiles derived from ketals of enantiomerically pure butan-2,3-diol, was also investigated, in order to evaluate whether asymmetric induction is possible with systems of this type.

Chapter Six - Asymmetric Cyclisation

An investigation was made into the factors affecting amino acid catalysed cyclisations of triketone intermediates, precursors in the establishment of the bicyclic skeleton. The study provided insights into the design of amino acid based surfactants and their use in Michael Addition reactions.

Chapter Seven - Enantioselective Dehydration

The unusual application of naturally occurring amino acids as enantioselective catalytic dehydrators was studied with a view to preparing, in optically enriched form, a synthon for onward manipulation to geosmin. The application of chiral surfactants to aqueous Michael addition reactions was also investigated, and their potential use in asymmetric dehydration reactions discussed.

To support these synthetic strategies, and as projects in their own right, two subsidiary investigations were undertaken in the course of this work:

Chapter Two - Butan-2,3-diol

Butan-2,3-diol is a valuable chemical, particularly in optically pure form when this diol can act as an effective chiral handle for directing the stereochemical course of many reactions. This type of application was central to two of the synthetic strategies outlined above - the 'biomimetic' cyclisation and the Diels-Alder cycloaddition. Access to the enantiomeric forms of butan-2,3-diol was therefore critical to the project and this chapter details the extensive study that was made, through the application of fermentation and enzymatic resolution techniques, into the production of laboratory scale quantities of this chiral diol in optically pure form.

Chapter Three - Aqueous and Micellar Phase Reactions

This chapter introduces the concept of aqueous and micellar media, and describes the synthesis and characterisation of two optically pure amino acid derived surfactants. The basis on which these aqueous media are now seen as potential substitutes for organic solvent systems is also discussed, an application that was a key theme of this project. Where micellar media were used, the effects on reaction stereoselectivity and efficacy are discussed in the appropriate section of the synthetic strategy studied.

CHAPTER ONE: INTRODUCTION TO GEOSMIN AND DEHYDROGEOSMIN

1.0 Introduction to geosmin

A contraction of two Greek words, *geo*-earth and *osme*-odour, geosmin is a naturally occurring compound of exceptionally low odour threshold that has variously been identified as the chemical responsible for the smell of freshly ploughed soil[1], and the 'muddy' taste in water[2] and trout[3].

Geosmin was initially isolated as a metabolite of the actinomycetes strain *Streptomycetes griseus*[1], with confirmation of its structure obtained through the racemic synthesis of Marshall and Hochstetler in 1968[4].

Given its extremely low odour threshold (quantified by Kasier and Nussbaumer at $2x10^{-11}$ g/l air[5]), geosmin has since found a commercial use as a perfumery chemical. This was the innovation of *Firmenich*, the flavour and fragrance company which, on the basis of the Marshall[4] and Ayer[6] syntheses, patented in 1979 the use of geosmin as an enhancer of ambery notes in perfumes and synthetic essential oils[7].

The work of subsequent years into this chemical has been to obtain stereochemically pure geosmin (the Gosselin synthesis of 1989[8]) and, most recently (with the Revial[9] and Swarts syntheses[10]), the naturally occurring enantiomer, (-)-geosmin. Reported to have an odour threshold value 11 times lower than (+)-geosmin[11], the pursuit of enantiomerically pure natural geosmin is therefore of greater potential commercial value than simple access to the racemate.

1.1 Syntheses of geosmin

Gerber's analysis of geosmin isolated from the actinomycetes microorganism[1] suggested, by comparison with the spectroscopic data for a series of dimethyl decalols synthesised independently by Marshall[12], that the structure of this metabolite was a 1,10-dimethyl-9-decalol. The relative stereochemistry was confirmed Marshall in 1968[4], with the absolute configuration being determined by

Ayer in 1976[6]. Thus naturally occurring geosmin is now known to be (-)-(1*S*,4a*R*,8a*S*)-*trans*-4,8a-dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-4a-ol (**1**).

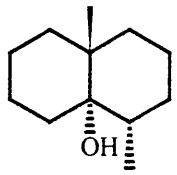
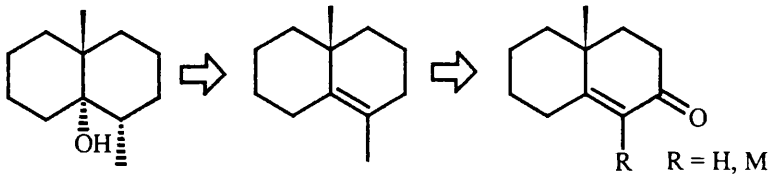


Figure 1: Natural geosmin

A simple examination of geosmin identifies a number of structural characteristics that have been suggested to play an important role in the structure-odour relationship for earthy-type odourants. These being:

- a rigid bicyclic structure of 10-15 carbons[13]
- an axial or semi-axial tertiary hydroxyl group[14]
- a methyl group adjacent to the carbinol group[15]

A retrosynthetic analysis suggests an unsaturated octalone type precursor, derived from *argosmin* (**2**), the dehydration product of geosmin. This has been the key intermediate in the published syntheses, typically accessed *via* an intermolecular Michael addition (between an α,β-unsaturated ketone and an appropriately functionalised cyclohexanone) and intramolecular Aldol reaction sequence (scheme 1).



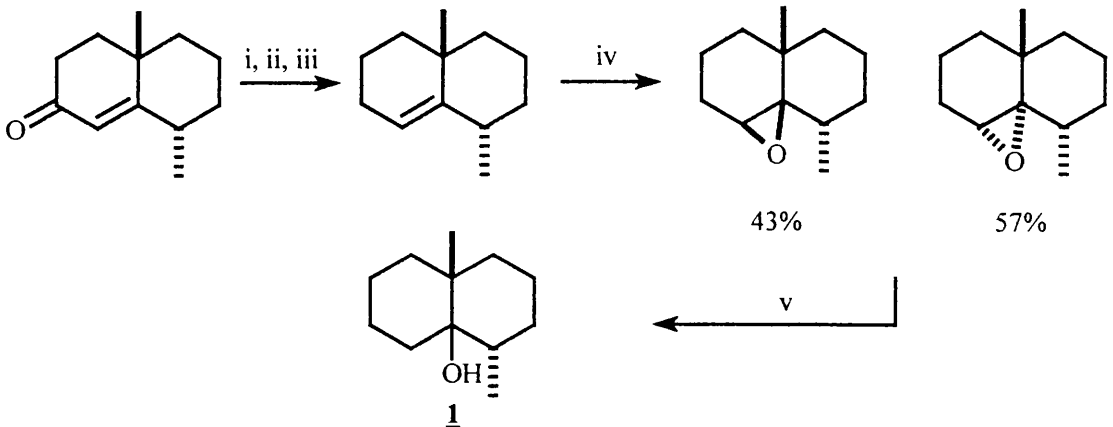
1 **2** an octalone

Scheme 1: Octalone disconnection of geosmin

The key structural features that must be obtained in any stereoselective synthesis of geosmin are a *bis*-angularly substituted *trans* ring junction, with a *syn* relationship between the hydroxyl and vicinal methyl group. In achieving an enantioselective

synthesis, there is the further requirement that the stereochemistry of the methyl group at the bicyclic ring junction be set absolutely.

Marshall's racemic synthesis[4] used a commercially available dimethyl octalone as its starting material and racemic geosmin was isolated from a diastereomeric mixture by column chromatography (scheme 2).



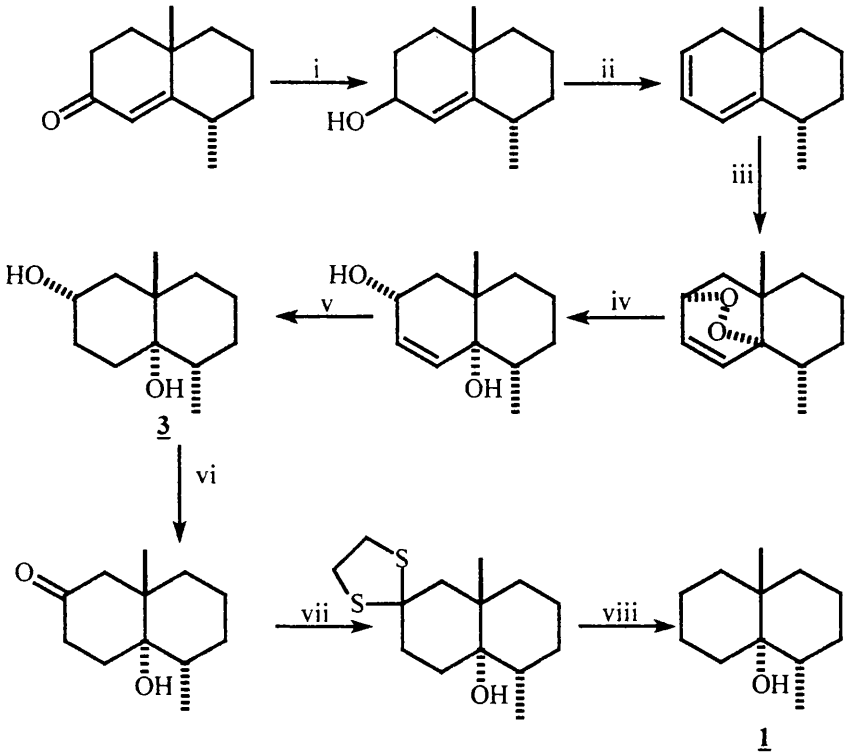
1

(i):$LiAlH_4$ (ii):Ac_2O (iii):$Li/EtNH_2$ (iv):*m*-$ClC_6H_4CO_3H$ (v):$LiAlH_4$

Scheme 2: Marshall's synthesis of geosmin

This synthesis, the first to geosmin, also provided a synthetic route to the other isomeric decalols which were, themselves, found to exhibit interesting olfactory properties.[4,7] However, the key epoxidation was not stereospecific (step (iv), scheme 2) and separation methodology was necessary in order to isolate the desired isomer.

Ayer's synthesis of geosmin[6] arose from research into another bacterial metabolite, *trans*-5,8a-dimethyl-octahydro-naphthalene-2,4a-diol 3, a terpenoid isolated from the bird's nest fungi *Cyathus bulleri*[16]. The racemic synthesis is shown in scheme 3:



(i):$LiAlH_4$, Et_2O (ii):Alumina, heat (iii):O_2, hν, CH_2Cl_2 (iv):Al amalgam

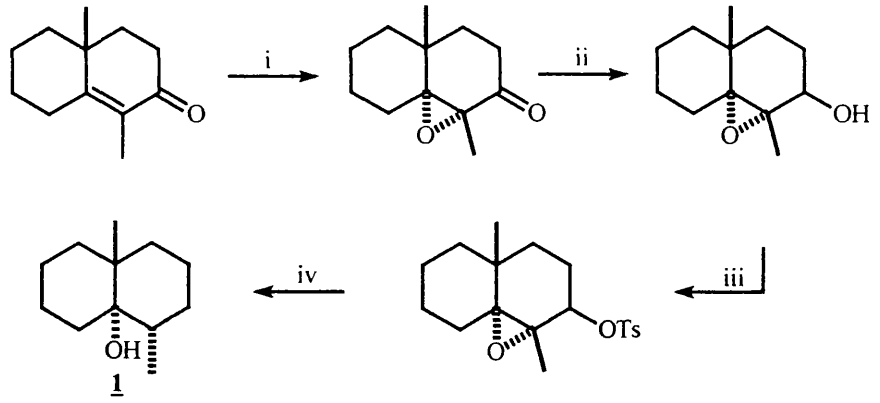
(v):H_2, Pt (vi):CrO_3, acetone (vii):CH_2SHCH_2SH, AcOH, BF_3-etherate

(viii):H_2, Raney-Ni

Scheme 3: Ayer's synthesis of geosmin

The importance of this synthesis resulted from the confirmation it gave of the relative stereochemistry of geosmin. Synthetically it was clearly derived from a procedure to another target compound (**3**) and was thus a relatively long route to geosmin.

Gosselin's approach[8] was, again, to take a commercially available octalone[17] and to *direct* the epoxidation of its double bond to give, upon opening, a *trans* ring junction and the required *syn* relationship between the hydroxyl and methyl group explained earlier. The synthesis (scheme 4) was stereoselective, though not enantioselective.



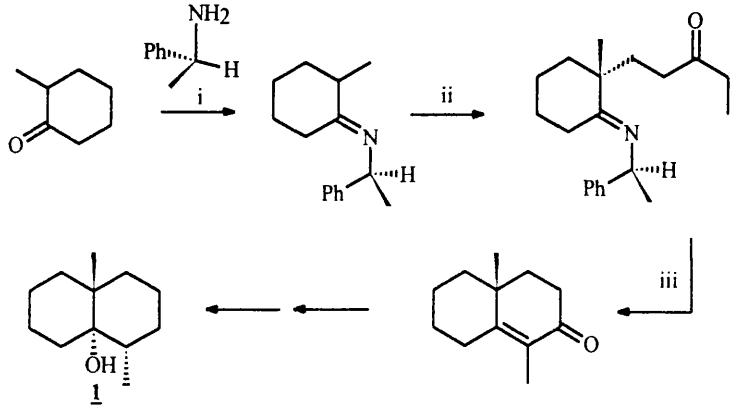
(i):*m*-CPBA, CH_2Cl_2, 20 °C, 10 hrs (ii):$NaBH_4$, MeOH, 0 °C, 7hrs

(iii):TsCl, pyridine, $CHCl_3$, 5 °C, 12 hrs (iv):$LiAlH_4$, THF, reflux, 3 hrs

Scheme 4: Gosselin's route to geosmin

In the course of developing this procedure, Gosselin investigated the use of different epoxidising agents to direct the key epoxidation step. He identified that *m*-chloroperoxybenzoic acid gave a diastereomeric excess of 92% (compared to 35% with hydrogen peroxide based systems) in the desired bicyclic epoxyketone.

In order or achieve an asymmetric synthesis, Revial took a very similar approach utilising many of the advances of Gosselin in effecting a stereospecific deoxygenation. By preparing the octalone precursor in optically pure form, Gosselin was able to set the stereochemistry at the ring junction absolutely (scheme 5).[9]



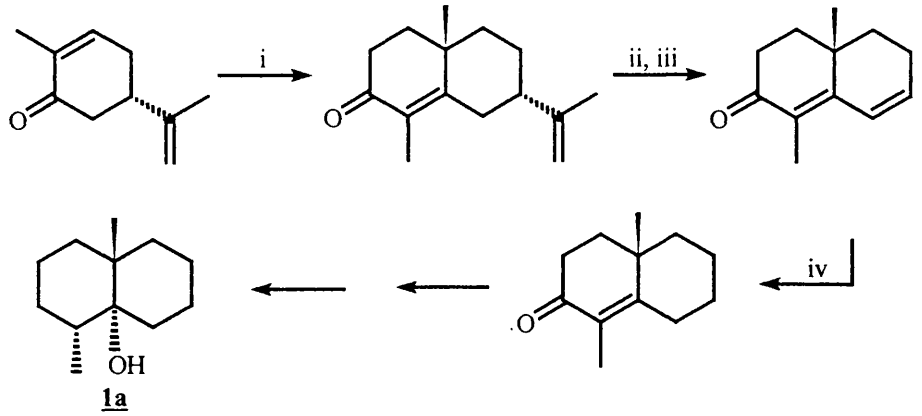
(i):(*S*)-(-)-α-methylbenzylamine, toluene (ii):Ethyl vinyl ketone, r.t.

(iii):20% $AcOH_{(aq)}$

Scheme 5: Revial's asymmetric synthesis of geosmin

This methodology - the use of chiral imines - is now patented[18] and has become standard procedure for invoking chiral Robinson annelations. Its utility arises from the availability of both forms of the chiral amine at reasonable cost and the efficiency of the procedure that allows near total recovery of these amines for reuse. The approach was successfully used by Saito[9a], who diastereoselectively reduced a Revial octalone and manipulated it to enantiomerically pure natural geosmin.

The Swarts synthesis[10] is particularly noteworthy as it, too, involves the preparation of the same optically pure octalone as Revial's, but uses a natural terpene, (*S*)-(+)-carvone, as the starting material in an approach to unnatural (+)-geosmin (**1a**).



1a

(i):Ethyl vinyl ketone (ii):O_3, MeOH, Ac_2O, Et_3N, DMAP (iii):$NaOCH_3$

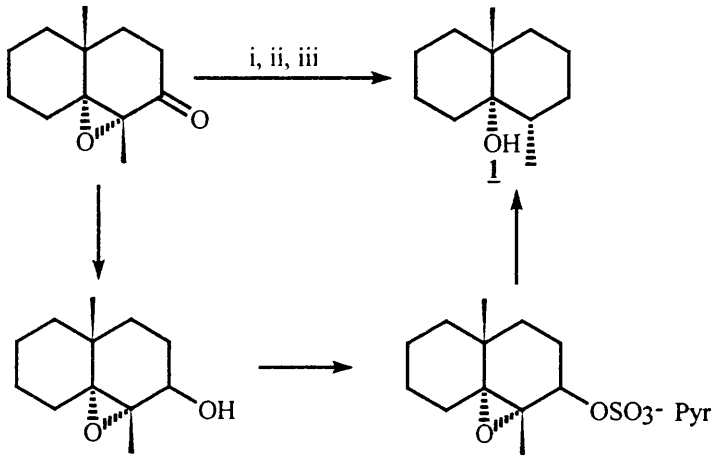
(iv):L-selectride, DMPU

Scheme 6: The Swarts synthesis of unnatural geosmin

As an approach to natural geosmin, this route was limited by the availability of the less common (*R*)-(-)-carvone but Swarts' work was of importance for demonstrating the use of the chirality present in some terpenes, as handles with which to direct reactions. Furthermore, the work established procedures for the removal of such chiral handles and demonstrated the different ways in which the products could be manipulated.

The only other reported work into geosmin synthesis has been an investigation by Hansson *et al* into improving the efficiency of the conversion of octalone precursors

into geosmin.[10a] Hannson demonstrated a four-step, one-pot experimental protocol for the conversion of an octalone to geosmin in 35% overall yield (compared to yields of 10-27% when carried out as discrete steps):

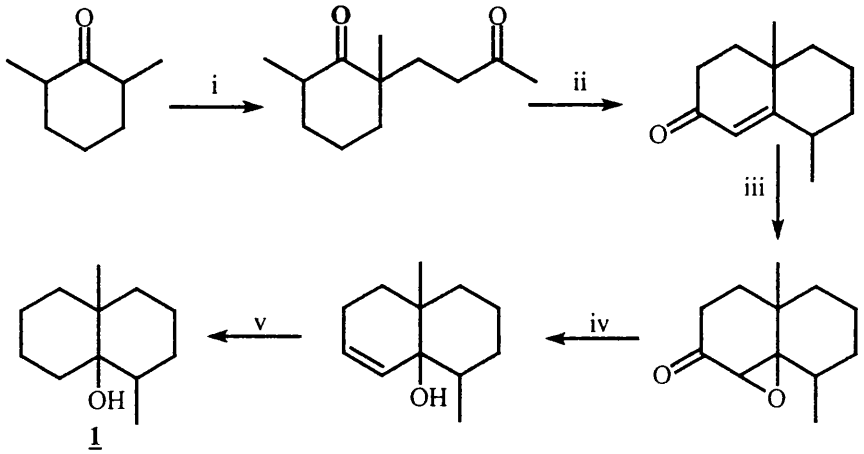


(i): $LiAlH_4$, THF (ii): SO_3.Pyridine (iii):$LiAlH_4$, THF

Scheme 7: Hannson's one-pot protocol

1.2 Bush Boake Allen and geosmin

Since 1990, with the expiration of the Firmenich patent[7], Bush Boake Allen (BBA) has been the only commercial supplier of geosmin for use as a perfumery chemical, with the racemic material currently priced at ~£2000/kilo. In common with many of the reported syntheses, the BBA route (scheme 8) involves an octalone precursor.

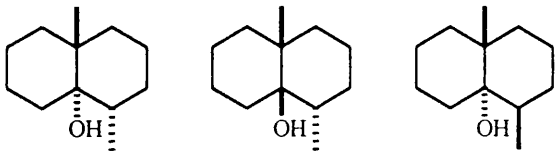


(i):Methyl vinyl ketone, acid (ii):MeOH, NaOMe (iii):H_2O_2, NaOH

(iv):NH_2NH_2, H_2O (v):H_2-Pt

Scheme 8: BBA route to geosmin

The BBA route is not stereoselective and an analysis of the BBA product shows it to be the isomeric mixture given in figure 2.[19]



12% 80% 8%

Racemic Geosmin 1

Figure 2: BBA geosmin - Isomer Mix

1.3 Introduction to dehydrogeosmin

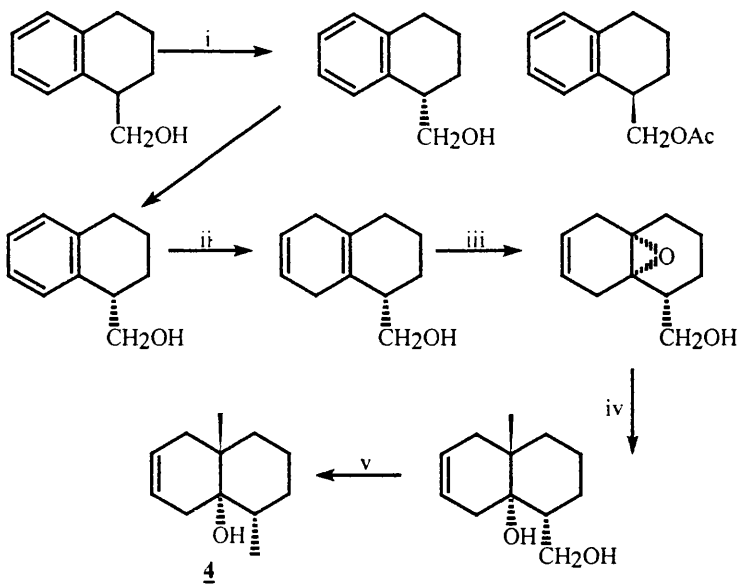
Structurally identical to geosmin but with the addition of an unsaturation in one of the carbocyclic rings, *dehydrogeosmin* is another strongly odoriferous metabolite possessing the characteristic musty-earthy odour of geosmin.

The discovery of this natural product was particularly interesting, having been identified by Kaiser and Nussbaumer[20] in the flower scents of species belonging to the genera of several Cataceae, for example *Rebutia*, *Sulcorebutia*, *Dolichothele* and *Mammillaria.*

The odour threshold level of dehydrogeosmin is comparable to that of geosmin, at $2x10^{-11}$ g/l, and being another naturally occurring molecule, there is similar potential for its use as a flavour or fragrance ingredient.

1.4 Syntheses of dehydrogeosmin

The relative stereochemistry of dehydrogeosmin was elucidated by Kaiser[20] who analysed the headspace isolate of *Rebutia marsoneri* and compared the spectral data with that of synthetic by-products of geosmin[21]. The only reported synthesis of dehydrogeosmin is that of Huber *et al*[22], which was novel in its approach to generating the *bis*-angularly substituted *trans* octalin skeleton by employing a regio- and stereo- selective opening of an epoxide across the ring junction of the bicyclic system. The application of enzymatic resolution techniques to the alcohol starting material enabled an enantioselective synthesis of the target compound to be achieved (scheme 9).



(i):*Candida Cylindracea Lipase*, vinyl acetate (ii):$NH_{3(liq)}$, Li

(iii):$[VO(acac)_2]$, ^tBuOOH (iv) MeMgBr, CuI (v): CH_3SO_2Cl, NaI/Zn

Scheme 9: Huber *et al* synthesis of natural dehydrogeosmin

The Huber synthesis of dehydrogeosmin was important because, being asymmetric, it permitted the definitive establishment of the absolute stereochemistry of this molecule. By GLC analysis of the synthetic enantiomers on a chiral stationary phase and comparison of the spectra with that obtained with a sample of dehydrogeosmin collected from the headspace of *Rebutia marsoneri*, the naturally occurring isomer was identified to be (+)-(4*S*,4a*S*,8a*S*)-1,2,3,4,4a,5,8,8a-octahydro-4,8a-dimethylnaphthalen-4a-ol (**4**).

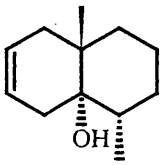


Figure 3: Natural dehydrogeosmin

With both enantiomers characterised, Huber[22] was able to perceive a much fresher and camphoraceous tone in the natural (+)-isomer though interestingly, unlike

geosmin, the unnatural (-)-isomer was found to possess a lower odour threshold value (10 pg/l air compared to 140 pg/l).

1.5 This project

Geosmin has been in use as an ingredient in fragrance preparations for many years and the olfactory similarity of dehydrogeosmin, and its relatively recent discovery, would suggest a similar application for this compound that has yet to be commercially exploited. Since both molecules are natural in origin and possess extremely low threshold values, it was believed that both geosmin and dehydrogeosmin could possess highly desirable organoleptic properties, and would therefore find a valuable commercial use as fine chemicals in flavour preparations. Whilst legislation allows the use of any synthetic chemical in fragrance preparations (provided it passes statutory standards for safety and toxicity in use), chemicals for flavour use are, however, more stringently regulated.

Current US and EU legislation, controlling the world's largest markets, permits only the use of *natural* or *nature identical* chemicals in all new flavours. Within this context, natural is defined as a chemical extracted in accordance with a limited number of stipulated procedures. For example, solvent extraction of vanilla pods to obtain natural vanillin. In addition, any chemical prepared by a bio-transformation, such as bacterial fermentation, is considered natural and is therefore permitted.

Chemicals prepared through standard chemical means in the laboratory are also allowed provided they can be shown to exist in nature. An interesting consequence for these so-called nature identical chemicals is that they may be used at concentrations far in excess of that which occurs naturally.

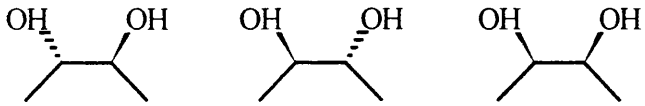
Whilst examples are known that demonstrate the difference in organoleptic properties between enantiomers of flavour chemicals (for example, the terpene carvone), there is no legislative requirement for optically pure flavour chemicals. This may change, following the precedent of pharmaceutical legislation, though the apparently low toxicological risk makes this appear unlikely.

The challenge for this project was therefore to identify and investigate key synthetic strategies to preparing both geosmin and dehydrogeosmin in stereochemically pure form. Such work is of importance to permit an evaluation of both chemicals as potential flavour ingredients, in view of the strict legislative requirements governing such use. At the same time, through the investigation of alternative synthetic methodologies towards cleaner and more selective syntheses of both geosmin and dehydrogeosmin, the immediate value of these chemicals as perfumery ingredients will be enhanced.

CHAPTER TWO: BUTAN-2,3-DIOL

2.0 Objective

The pursuit of a 'biomimetic' route to geosmin, expounded in chapter four, utilises the optically pure forms of butan-2,3-diol as effective chiral auxiliaries in a diene cyclisation reaction. The three isomeric forms (L-(+)-(2*S*,3*S*)-**5a**, D-(-)-(2*R*,3*R*)-**5b** and *meso*-**5c**) are available commercially at considerable cost.



5a **5b** **5c**

Figure 4: Butan-2,3-diol structures

Experience at BBA suggests prices of between £17 and £40 per gram (depending on the isomer) yet contaminant odours have been detected in these commercial samples, preventing their use in fragrance synthesis and evaluation. Access to laboratory scale quantities of the pure isomers was therefore essential if the proposed biomimetic route was to be evaluated.

Beyond this particular research, there is also substantial interest in the vast array of potentially interesting flavour and fragrance chemicals that could be generated in optically pure form from simple acetal formation with this diol. These targets are widespread in nature, such as the acetal of vanillin found in vanilla extract (of obvious importance to Bush Boake Allen as one of the world's leading producers of this flavour ingredient).

Butan-2,3-diol is increasingly finding industrial applications, as a solvent, chemical intermediate[23] and, in optically active form, for directed asymmetric syntheses (for example, in boronic esters[24], Diels-Alder reactions[25] and chiral acetal auxiliaries[26]).

Thus the establishment of laboratory scale preparative techniques to the three isomeric forms of butan-2,3-diol had potential application beyond the scope of both this chapter and this research.

2.1 Approaches

Being a natural product, a vast body of work exists in the literature into the biosynthesis of this target chemical[27] and its biosynthetic pathways have been elucidated. Such an understanding is useful in providing the starting points for strategies to meet the stated objected. To this end, a review of the literature identified three approaches for investigation:

Chemical synthesis

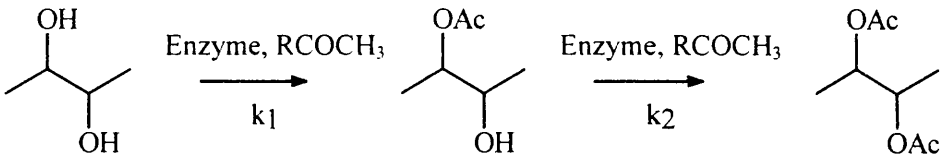
This was the approach which resulted in the establishment of the absolute stereochemical configurations of the butan-2,3-diol isomers. Rubin *et al*[28] transformed D- and L-mannitols through a series of unequivocal reactions to optically active butan-2,3-diols, comparing the optical rotations of their di-*p*-nitrobenzoates derivatives with those of natural laevorotatory butandiol to establish the isomeric forms as L-(+)-(2*S*,3*S*)-butandiol and D-(-)-(2*R*,3*R*)-butandiol.

Subsequent synthetic routes to the various stereoisomers of butan-2,3-diol have included a synthesis from diethyl tartarate[29], asymmetric opening of 2,3-epoxybutane using camphorsulfonic and tartaric acids[29a], and the hydrogenation of 2,3-butandione with BINAP as a chiral auxilliary[30] to access L-(+)-(2*S*,3*S*)-butandiol.

Enzymatic enantioselective resolution

The basis of such techniques is the derivatisation of the racemate, usually to an ester, followed by hydrolysis in the presence of an enzyme that effects a kinetic resolution by de-esterifying one enantiomer faster than the other. After separation of the alcohol, now enantiomerically enriched, chemical hydrolysis of the remaining ester yields the other enantiomeric form.

Examples of this approach are provided by Caron[31] and Bisht[32], who produced the two chiral forms of butan-2,3-diol with enantiomeric excesses of 40% through such a sequence (figure 5).



$k_1(2S,3S) \neq k_1(2R,3R) \neq k_1(meso)$

and $k_2(2S,3S) \neq k_2(2R,3R) \neq k_2(meso)$

Figure 5: Kinetic enzymatic resolution of butan-2,3-diol

Physical enantioselective resolution

Various degrees of success have been obtained when using chromatographic techniques to separate the three isomeric forms of butan-2,3-diol. Gas chromatography has been successfully employed to separate L-(+)-(2*S*,3*S*)-butandiol from mixtures contaminated with the *meso*-form[33] (though the utility of this technique is limited, by restrictions on scale, to analytical applications only).

Liquid chromatography, as a technique, can operate at preparative scales and this has been used successfully to isolate *meso*-butan-2,3-diol from its racemic mixture.[34] Separation of the enantiomers using non-bound chiral stationary phases of cellulose derivatives on silica is possible, though prohibitively expensive in an academic environment.

Enzymatic enantioselective oxidation/reduction

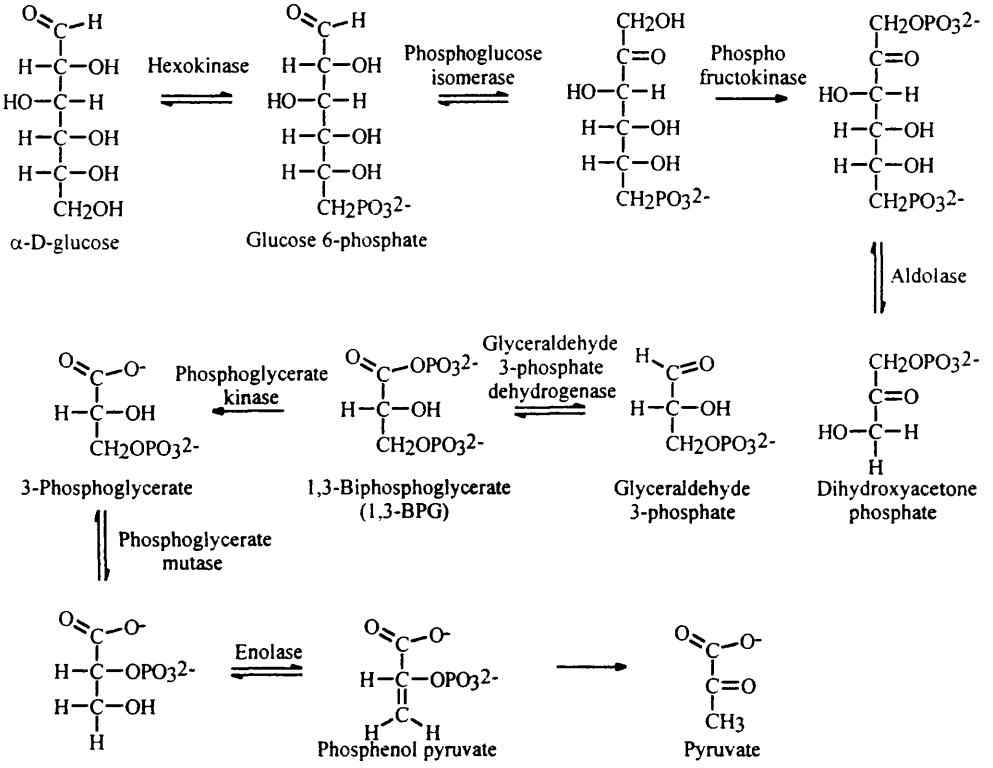
Enzymatic transformations of this type depend on the establishment of a coupled substrate to regenerate the cofactor NADPH. The optically pure alcohol may be obtained by direct reduction or, in the context of butan-2,3-diol production, exploiting the absence of such a dehydrogenase. This was the approach of Ui[35], who resolved a racemate of this diol by oxidising a mixture in the presence of *Brevibacterium saccharolyticum* C-1012, known to possess a strictly stereospecific L-(+)-(2S,3*S*)-butandiol dehydrogenase.

L-(+)-(2*S*,3*S*)-Butan-2,3-diol + NAD^+ $\rightleftharpoons$ L-(2*S*,3*S*)-(+)-Acetoin + NADH + H^+

Figure 6: Enzymatic reduction to butan-2,3-diol

Bacterial fermentation

This approach has been extensively researched and an informative overview of this area is provided in a review by Maddox.[27] Research has concentrated on the metabolic pathway from glucose to pyruvate, with the glycolytic pathway summarised below. [36]



Scheme 10: Glycolytic pathway from glucose to pyruvate

Additionally, the biosynthetic pathway from pentose sugars to pyruvate is known through work carried out by Jansen and Tsao, who showed that the pathways converge at glyceraldehyde-3-phosphate, after which point glycolysis occurs in an identical fashion to that for the hexose sugars.[37]

Selected approach

Of the five methodologies described above, the two selected for investigation in the context of this project were bacterial fermentation and enzymatic resolution.

2.2 Bacterial fermentation approach

The commercial potential for bacterial fermentation is well known in the flavour industry which has been using such technology, and exploiting the legislative advantages of labelling such products obtained as natural, for years in the production of flavour compounds such as the γ-lactones. The technology has proven scalabilty and great potential since the raw starting materials for fermentative processes are typically readily available and quite often by-products of other industries.

For the production of butan-2,3-diol by such a means, three bacterial families are known to produce the diol in reasonable quantities[27] - *Aeromonas* (*Pseudomonas*), *Klebsiella* and *Bacillus*.

The stereochemical distribution of the products varies greatly with a number of factors and much effort has been expended into establishing the effect each has on the quantity and isomer of butan-2,3-diol produced. Such investigations have included:

The carbon source

A range of different carbon food sources has been investigated with the families of bacteria described - not only simple sugars (for example, glucose, sucrose, lactose) but also polymeric materials such as whey[38] and molasses[39]. However, the exact specificity exhibited by each organism appears to be case specific, dependent on the combination of all experimental parameters used.[27] The selection of a particular sugar has therefore been driven more by the desire to exploit a potentially valuable by-product (for example, whey from dairy waste[40]) than to optimise production of a particular butan-2,3-diol isomer.

The temperature

Bacterial growth is optimal within a defined temperature range, typically 30 °C to 37 °C depending on the individual strain. Little attention has been given to studying the effects of temperature optimisation on butan-2,3-diol production though Barrett *et al* observed that raising the fermentation temperature of a *Klebsiella* strain from 33 °C to 37 °C lowered butan-2,3-diol production by 66%.[41] Such an observation has

profound consequences on maximising the yield of any fermentation reaction though there appears to be no effect on the actual stereochemistry of the isomer obtained.

The rate of aeration

In effect, this relates to the availability of oxygen to the culture and has been extensively investigated.[42,43] Generally, when oxygen availability is high, biomass formation is favoured over butan-2,3-diol production (typically during the initial growth phase of the bacteria). When the oxygen supply is subsequently limited, incomplete oxidation of the available sugars takes place and butan-2,3-diol production commences.

In optimising the production of butan-2,3-diol, the optimal oxygen concentrations and supply rates are specific to each combination of strain, carbon source and other experimental parameters.[27] The effect of aeration does not, however, extend to the stereochemistry of the diol produced.

2.2.1 Origin of stereoselectivity in fermentations

It has been observed that in the fermentation products of certain families of bacteria there is a predominance of one or more of the stereoisomers of butan-2,3-diol. The product distribution dependence by bacterial strain can be broadly represented by the following table:

Strain	L-(+)-(2*S*,3*S*)-	D-(-)-(2*R*,3*R*)-	*Meso*
Klebsiella pneumoniae	■		■
Aeromonas hydrophila		■	
Bacillus subtilis		■	■
Bacillus polymyxa		■	
Serratia marescens			■

Table 1: Selectivity of bacterial strains

From the glycolytic pathway (scheme 10), it can be seen that chirality is conferred to the desired product (butan-2,3-diol) in the final stages. The path to a particular stereoisomer of butan-2,3-diol is dependent on the transformation of acetoin which,

in the glycolysis of glucose, is invariably D-(-)-acetoin. Stereocontrol is dependent on the presence (or absence) of a dehydrogenase that is specific through its action on a substrate with the D- stereochemistry only.

Such biological processes are reversible and a specific dehydrogenase can equally oxidise a particular form of butan-2,3-diol to the corresponding acetoin (for example, D-(-)-butan-2,3-diol to D-(-)-acetoin). However, specific dehydrogenases are not capable of interconverting different chiral forms through a reduction-oxidation-reduction cycle, acting only on and forming only stereoisomers of like configuration.

Various groups have investigated the presence of specific dehydrogenases in different bacterial strains, and their models for the mechanism of formation of different stereoisomers of butan-2,3-diol from D-(-)-acetoin are shown below:

Existence of an acetoin racemase

Taylor and Juni demonstrated the presence of a D-(-)-dehydrogenase in *Bacillus polymyxa* to explain its specificity for D-(-)-butan-2,3-diol production during fermentation.[44] The presence of D-(-)-, L-(+)- and *meso*-butan-2,3-diol isomers in the product mix from fermentations of *Aerobacter aerogens* was therefore attributed to the presence of both D-(-)- and L-(+)- specific dehydrogenases and, additionally, the presence of an acetoin racemase. This racemase was postulated to allow for L-(+)-acetoin, produced from D-(-)-acetoin.

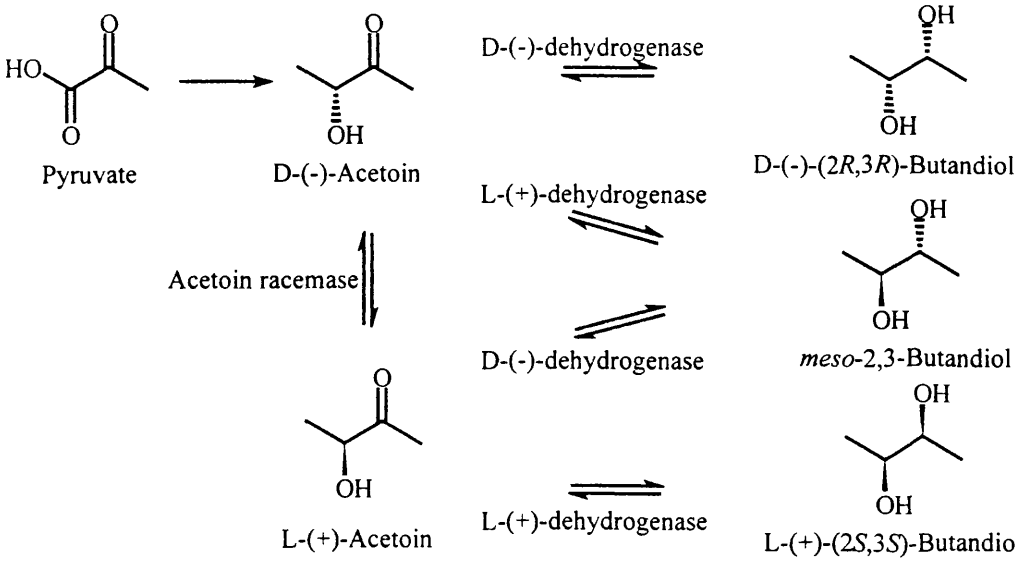


Figure 7: Existence of an acetoin racemase

To explain the presence of the non-chiral *meso*-diol in the product distribution, it was proposed that this isomer possesses both D- and L- arranged hydroxyl groups, each capable of being recognised and acted upon by the respective specific reductases present in the bacteria.

Presence of an acetoin racemase was supported by Voloch *et al*[45], who identified L-(+)-butan-2,3-diol when investigating fermentations involving the bacteria *Klebsiella pneumoniae*.

Absence of an acetoin racemase

Production of L-(+)-butan-2,3-diol from D-(-)-acetoin does not automatically require the presence of an acetoin racemase. Under appropriate physical conditions, chemical oxidation of D-(-)-acetoin to diacetyl is possible and an L-(+)-specific reductase present could be responsible for reducing this to L-(+)-acetoin and subsequently to the L-(+)-butan-2,3-diol.[46] Hohn-Bentz *et al* questioned the specificity of the acetolactate decarboxylases present in different bacterial strains and suggested that in, for example, *Enterobacter aerogens*, there could be a decarboxylase capable of producing L-(+)-acetoin for reduction by an L-(+)-dehydrogenase present.

Ui[47] proposed a model for the specificity of the strain *Klebsiella pneumoniae* that suggested D-(-)- and L-(+)- were interconvertible, through the action of two separate specific dehydrogenases, with *meso*-butan-2,3-diol (possessing both D- and L-hydroxyl configurations) as the common intermediate.

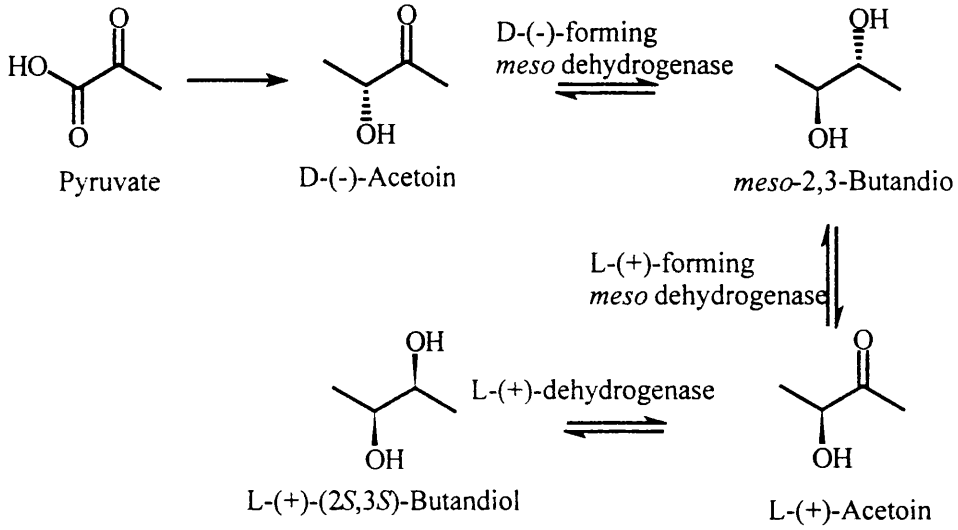


Figure 13: Absence of an acetoin racemase

In conclusion, the origin of the specificity exhibited by a particular bacterial family cannot be stated unequivocally and is highly dependent on the strain under investigation. It was against this background that a study was undertaken to evaluate a *Bacillus subtilis*, with the objective of exploiting any specificity observed, rather than to deduce its origin.

2.3 Results and discussion

The fermentation study was carried out with a sample of the bacterium *Bacillus subtilis (JK)* (kindly supplied by Dr J. Kinderlerer of the University of Sheffield). The strain, isolated from coconut waste[49a], is understood to be thermophilic in nature, potentially promoting rapid metabolism. While the strain has been shown to produce butan-2,3-diol, exact isomer determination has not been carried out. The study therefore also provided the opportunity to identify any isomer specificity exhibited by the bacterium.

The protocol adopted was to resuscitate the bacterium on standard nutrient agar slopes (enriched with yeast extract and glucose) at 50 °C for three days. After this time, the bacteria were grown further by incubation in nutrient broths of the same composition as the agar for seven days at 30 °C. Controlled volumes of the suspended bacteria were then used to inoculate nutrient broths containing the carbon source. In this work, a limited range of simple sugars was investigated, shown in figure 8.

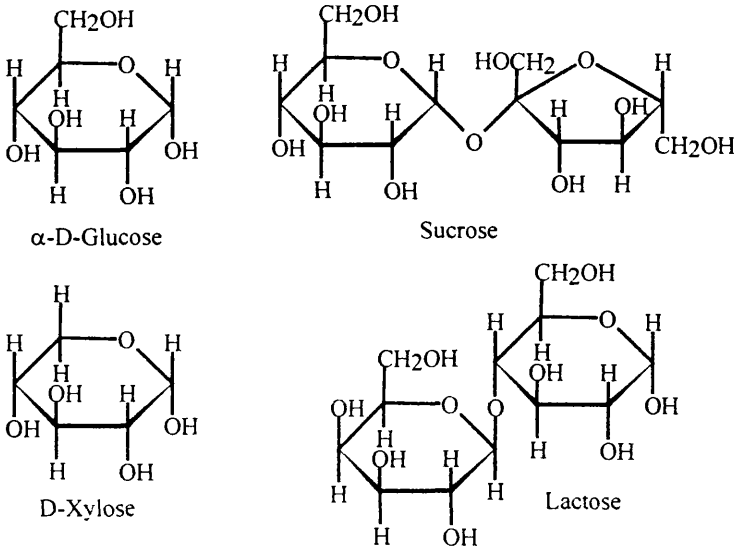


Figure 8: Carbon sources investigated

2.3.1 Glucose Fermentations

The primary carbon source investigated in the study was α-D-glucose. Nutrient broths of different glucose concentration were inoculated with the suspended bacteria then incubated under the following experimental conditions:

Reaction time

Incubation times of 5 and 8 days were tried.

Initial inoculum concentration

It was considered possible that diol production could vary with concentration of bacteria within the fermentation broth. The butan-2,3-diol pathway may also be activated at an earlier or later stage in the bacteria's growth.

Initial glucose concentration

Variation of the initial sugar concentration was made to monitor its effect on diol production.

Agitation

Both static and shaken incubation regimes were investigated, as a simple study into the effect of oxygen supply on diol production.

Aeration

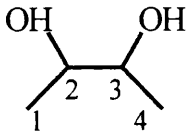
A limited study was carried out whereby the fermentation reactors - one litre conical flasks - were 'anaerobically' sealed (by stoppering with a bubbler) to allow the escape of fermentation gases but to prevent the entry of atmospheric oxygen.

Analysis

All fermentation broths were worked up by extraction with identical quantities of dichloromethane. After removal of solvent, the concentrated extracts were analysed by a combination of techniques.

NMR was used as the primary means for identification. Under all regimes, the sole products of each experiment were found to be acetoin and butan-2,3-diol alone, with no other metabolites isolated. The three isomeric forms of the diol were found, surprisingly, to differ in their ^{1}H NMR spectra, with the position of the hydroxyl and

carbinol protons available as markers to define the isomer produced in each fermentation experiment (table 2). The phenomena were observable both for isomers in isolation and when part of isomeric mixtures.



Signal	C2-H, C3-H			C2-O*H*, C3-O*H*		
Isomer	5a	5b	5c	5a	5b	5c
Appearance	multiplet	Multiplet	multiplet	Broad singlet	broad singlet	broad singlet
Position	3.48	3.47	3.74	2.29	2.92	2.97

Table 2: ^{1}H NMR signals for butan-2,3-diol isomers

The origin of this effect was unclear: Distinction between the *meso* and the racemate of butan-2,3-diol by ^{13}C NMR was observed by Voloch *et al* and attributed to the possibility of intramolecular hydrogen bonding in the non-chiral form.[34] A possible explanation for the effect seen in this study was the formation, in solution, of aggregates of the two enantiomeric forms. This implies that commercially available samples of the individual enantiomers are not optically pure. This possibility, and the variable appearance of the hydroxyl protons in ^{1}H NMR spectra, made it unsound to use the technique as the sole measure of optical purity of the butan-2,3-diol obtained in the fermentation study. The use of NMR spectroscopy was therefore limited to identification of the metabolites alone.

Gas-Liquid Chromatography (SP2100 packed silica column) was employed as a rapid means of assessing the quantity of butan-2,3-diol in the fermentation extract. This was achieved through the use of commercially available samples as standards against which to compare the fermentation extracts.

The determination of the optical purity of the diols extracted was carried out using a chiral HPLC technique. Commercial samples of the three isomeric forms of butan-2,3-diol were used as standards and derivatisation of these diols to diacetates (by direct reaction with acetyl chloride) allowed their detection by UV during HPLC analysis on a chiral column. Retention times of derivatives of diols isolated from the

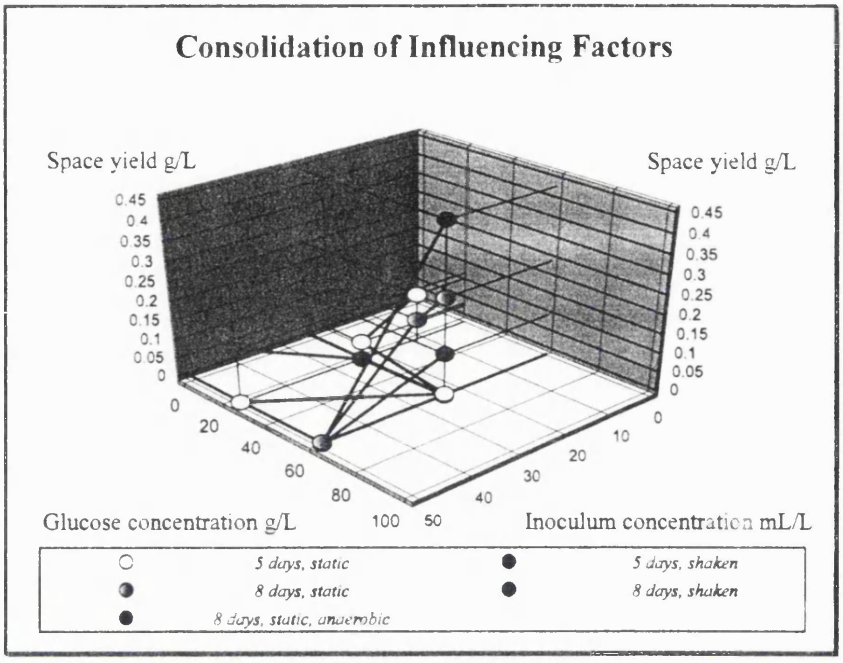
fermentation broths were compared with those obtained from commercially available isomeric samples to allow a measure of optical purity to be made. This technique was selected for its rapidity and cost effectiveness (particularly against NMR based determinations of optical purity, such as ester formation with Mosher's acid).

Results

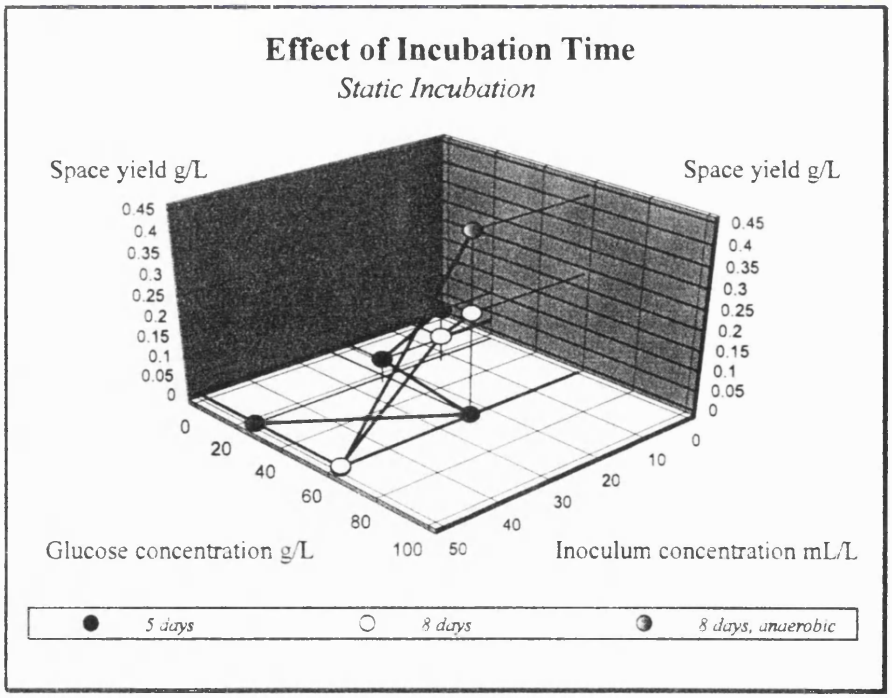
The consolidated results are tabulated below and shown graphically by experimental parameter. (***Space yield g/l*** in these studies refers to the unit mass of butan-2,3-diol isolated per volume of fermentation broth (grams of butan-2,3-diol per litre of broth).

Glucose g/l	Inoculum ml	Incubation	Diol mass (by GLC) mg	Space yield mg/l
12.5	25	Static	49.6	124
25	25	Static	18.9	47.3
50	25	Static	0	0
25	62.5	Static	22.1	5.5
25	25	Shaken	0	0
50	25	Shaken	56.3	140.8
250	62.5	Shaken	0	0
12.5	25	Static	21.4	53.5
25	62.5	Static	102.1	255.3
50	62.5	Static	1.9	4.8
25	62.5	Shaken	4.5	112.3
50	62.5	Shaken	0	0
25	62.5	Static, anaerobic	172.1	430.2

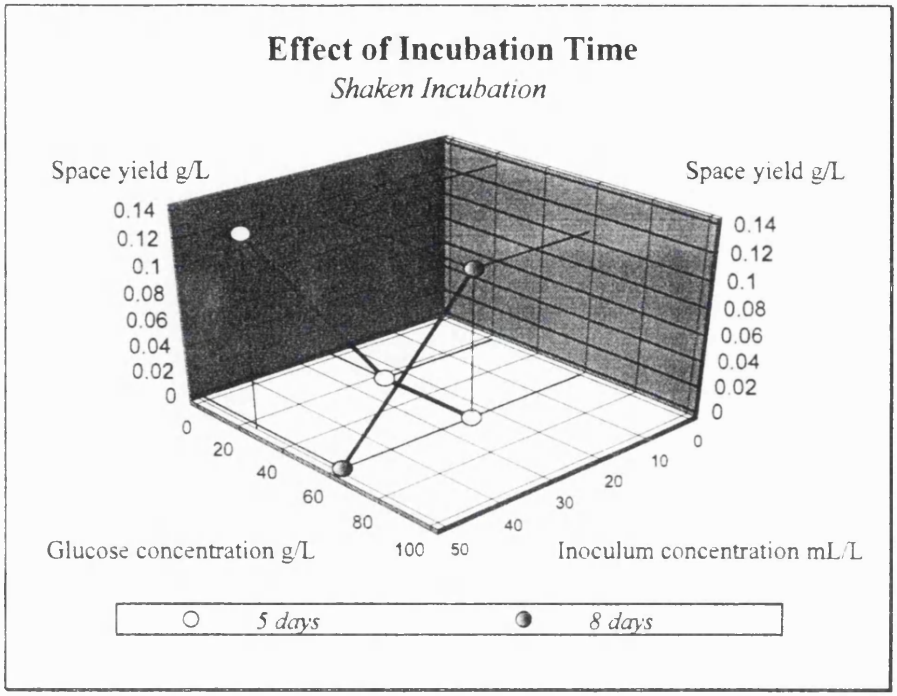
Table 3: Fermentation study results



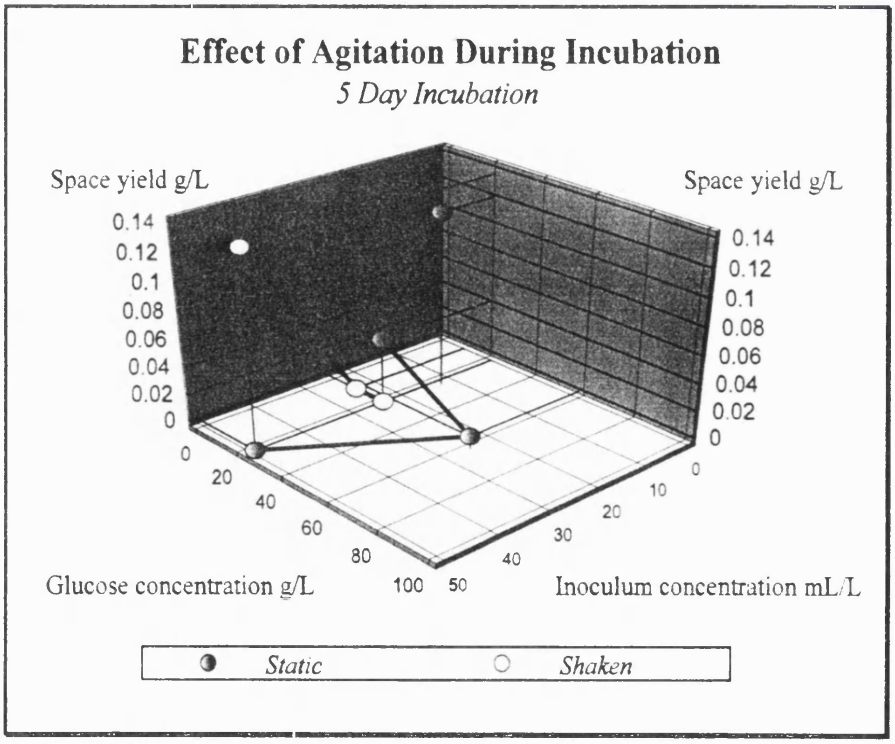
Graph 1: Consolidated results



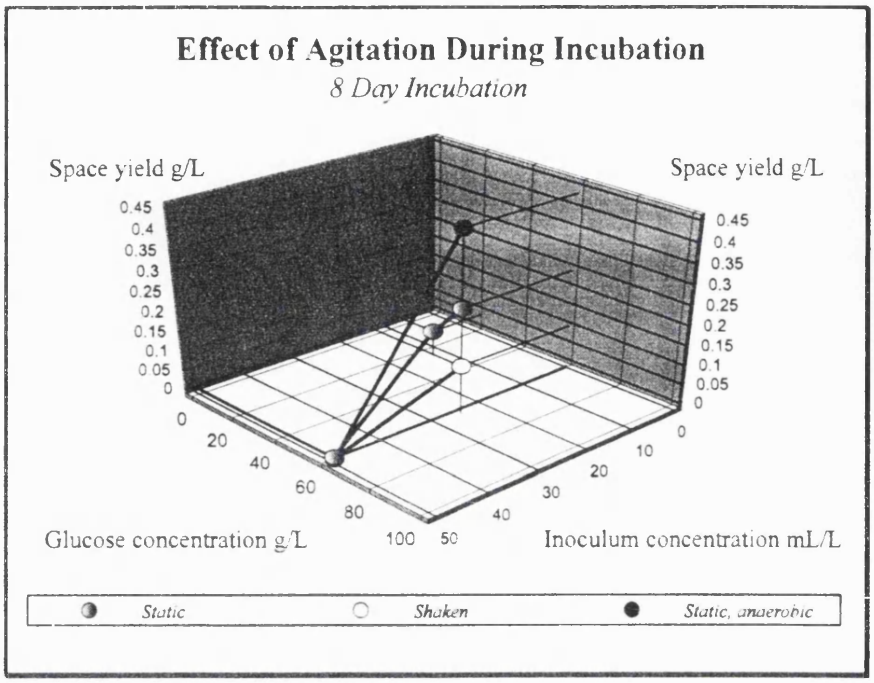
Graph 2: Effect of incubation time - static incubation



Graph 3: Effect of incubation time - shaken incubation



Graph 4: Effect of agitation - 5 day incubation



Graph 5: Effect of agitation - 8 day incubation

2.3.2 Inferences

Effect of time (graph 2 and graph 3)

In general, the quantity of diol produced increased with the incubation time, though the difference was most marked with the static, rather than shaken, incubation.

Effect of agitation (graph 4 and graph 5)

Where shaken incubation was employed, greater mass yields were obtained but this was through acetoin, and not butan-2,3-diol, production. This was rationalised as the 'oxygen rich' conditions under agitation favouring the more oxidised product. In all cases, where otherwise identical conditions were employed, greater yields of the diol were obtained when incubation occurred without agitation.

Effect of inoculum Concentration

Over 5 days, initial inoculum concentration showed a positive correlation with diol production in the case of shaken incubation, yet the opposite relationship over 8 days. This suggested that over the shorter time period, once a certain cellular

concentration had been reached in the fermentation medium, aerobic conditions (brought about by the agitation) favoured the metabolism of glucose to the diol during the bacteria's growth stage. After this time, the increase in diol production was limited.

This inference was supported by the case in which the incubation was static, where the relationship between initial inoculum concentration and quantity of diol produced was seen to be negative over both time scales. This again suggested that anaerobic conditions encouraged the metabolism of glucose to the diol at an early stage in the bacteria's growth cycle, but even though this growth was slowed (compared to the aerobic case), the overall metabolism to the diol was made more efficient.

Effect of initial glucose concentration

Over the 5 day period, diol production was favoured under an agitated regime when initial glucose concentration was low, yet over 8 days the opposite relationship was observed. Where incubation was static, no discernible relationship existed.

Effect of aeration

Under the limited range of conditions tried, the highest yield of diol was obtained when using an anaerobic regime. This was consistent with the metabolic pathway from glucose to butan-2,3-diol, where the diol is the final anaerobic product.

Isomer specificity

In all cases, butan-2,3-diol was confirmed to be an isolate of the fermentation experiments and determined, by chiral HPLC analysis against standard samples, to be *exclusively* the D-(-)-(2*R*,3*R*)-isomer. No contamination by the L-(+)-(2*S*,3*S*)- and *meso*-isomers was observed in any of the fermentation runs.

2.3.3 Further investigations

Extraction procedure

It is common in bacterial fermentations for one of the metabolites produced to be toxic to the producing organism to the extent that further production of that chemical

is inhibited when a certain concentration is reached in the fermentation medium. Jansen *et al* observed for butan-2,3-diol production by the organism *Klebsiella oxytoca* that complete inhibition of diol production occurs when a concentration of 60 g/l is reached.[48] To overcome this problem, *in situ* extraction has been investigated as a means of removing the inhibitory product as it is formed by the bacteria.

The extracting solvent may, itself, be toxic to the bacteria, preventing further growth and metabolite production. Eiteman and Gainer investigated the continuous extraction of butan-2,3-diol from *Klebsiella oxytoca* with a number of organic solvents, balancing toxicity against affinity for the diol.[49] Their study also compared *in situ* extraction to that carried out in an external extraction column (before recycling the aqueous residues to the fermentation reactor) and selected dodecanol as the optimal solvent in an external extraction environment. This technique was found to be less harmful to the bacteria, minimising contact between the extracting organic solvent and the biologically active fermentation broth.

In situ or external column extraction, in the context of this research, was not seen as a useful way to proceed since the observed concentrations of D-(-)-(2*R*,3*R*)-butan-2,3-diol were much lower than in literature studies and did not warrant the investment in specialist apparatus. Instead, simple procedural variations to the extraction procedure were looked at, altering the physical conditions under which the diol was extracted from the aqueous fermentation medium.

A study was performed to compare the efficiency of different solvents in extracting a known mass of butan-2,3-diol from a controlled volume of water:

Solvent	Volume	Diol Mass	Extracted
	Ml	Mg	%
Dichloromethane	50	7	0.7
Diethyl ether	50	8	0.8
Ethyl acetate	50	38	3.8
Ethyl acetate *	50	105	10.5

* NaCl saturation prior to extraction

Table 4: Results of Extraction Study

Saturation of the solution with a salt (such as sodium chloride) prior to extraction substantially increased the mass of diol that could be removed in this way. Ethyl acetate was also seen to be a more effective extraction solvent than dichloromethane. These findings were applied to a number of fermentation runs with the result that isolated space yield was raised to 0.88 g butan-2,3-diol per litre (from 0.43 g/l extracting with dichloromethane previously).

Carbon source

The premise of this investigation was that variation of the carbon source may influence the selectivity of the conversion of pyruvate to a particular isomer of butan-2,3-diol, by invoking the expression of different reductases. It was also the aim to extend the characterisation of the isomer specificity of *Bacillus subtilis (JK)* to sugars other than glucose.

The experimental protocol employed was identical to that used in the glucose study and fermentation broths of lactose, D-xylose and sucrose were prepared and incubated under identical conditions to those used in section 2.3.1.

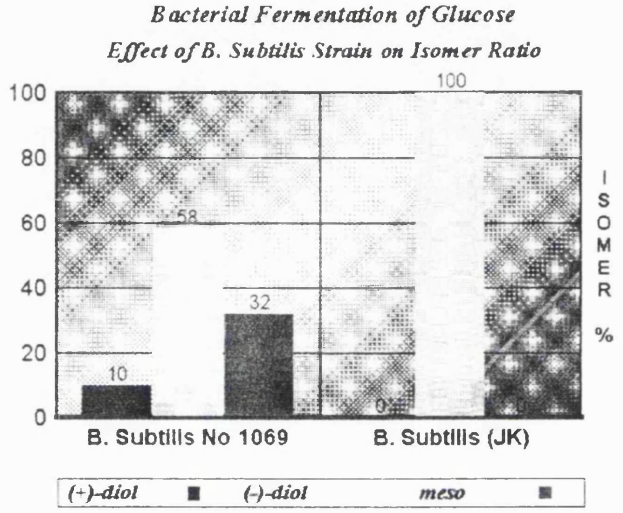
In the case of the sucrose and lactose fermentations, mass yields of the diol were respectively 10% and 20% lower than that obtained in an identical glucose fermentation. For both sugars, the exclusively D-(-)-(2*R*,3*R*)-butan-2,3-diol was identified. From the xylose fermentation, negligible material was recovered.

Whilst this study could have been extended to a greater variety of sugar skeletons, it was felt that greater success may be achieved using different bacterial strains. Future work could involve the use of a different carbon source at the resuscitation stage.

Variation of strain

It was proposed that within the *Bacillus* family, production of a different butan-2,3-diol isomer could be invoked by a variation in the *Bacillus* strain. This would be achieved through a specific reductase capable of interconverting the two enantiomeric forms of acetoin, following any of the models outlined in section 2.2.1. To this end, a commercial sample of another *Bacillus subtilis (1069)* was purchased

and a fermentation was carried out under identical conditions to those used for the *Bacillus subtilis (JK)*, with glucose as the carbon source under the conditions identified in section 2.3.1. and the improved extraction regime of section 2.3.3.



Graph 6: Dependency of isomer ratio on bacterial strain

- *Bacillus subtilis (1069)* produced a mixture of stereoisomers of butan-2,3-diol, with the D-(-)-(2*R*,3*R*)-form dominating
- *Bacillus subtilis (JK)* produced the D-(-)-(2*R*,3*R*)-form of butan-2,3-diol specifically and exclusively

There was a clear variation in the specificity exhibited by each strain of bacteria, emphasising the uniqueness of each combination of parameters that determine the course of any fermentation reaction. The reductases of strain *1069* exhibited different specificity to those of strain *JK*, explicable by any of the models proposed in section 2.2.1. For the purposes of this project, however, the origin of these other stereoisomers was unimportant since *Bacillus subtilis (1069)* had been shown to be non-specific and could not be exploited to access the L-(+)-(2*S*,3*S*)-butan-2,3-diol *via* a simple fermentation strategy.

2.3.4 Conclusions of the fermentation study

Fermentation conditions (time, temperature, concentrations, extraction) were established for a system based on *Bacillus subtilis (JK)* metabolising α-D-glucose

that produced a space yield of 0.88 g butan-2,3-diol per litre of fermentation broth. The diol isolated was identified as being exclusively the D-(-)-(2*R*,3*R*)-butandiol. demonstrating that this bacterial strain shows remarkable selectivity in the conversion of pyruvate to the target isomer.

Despite the variation of carbon sources and *Bacillus* strain, the opposite enantiomeric form - L-(+)-(2*S*,3*S*)-butandiol - could not be produced in the necessary quantities or optical purity. The effect of other parameters could have been explored but it was decided to investigate an alternative approach to the fermentation strategy to access this target isomer.

2.4 Enzymatic resolutions

The second approach selected in this project to access the enantiomeric forms of butan-2,3-diol was the use of enzymatic enantioselective resolution technology (section 2.1), which appeared to be a facile and effective means of separating the three stereoisomers of the target diol and thus provide access to the L-(+)-(2*S*,3*S*)-form. The approach is not unprecedented and Bisht[32], Mattson *et al*[50] and Caron *et al*[31] have achieved considerable success in resolving racemic butan-2,3-diol through this method. The approach adopted was a sequential kinetic resolution of a commercially available isomeric mixture of the diol, using an acyl donor under enzymatic control. Discrimination was achieved by exploiting the different rate at which each butan-2,3-diol enantiomer accepts an acyl group from an "acyl-enzyme combine" to form an ester (figure 5).

2.4.1 Mode of action

Lipases are a class of enzymes that are used by living matter to digest triglyceride fats[51]. For the synthetic chemist, their utility comes from their ability to accept a broad range of artificial substrates and to catalyse the hydrolysis of these esters with degrees of speed and selectivity remarkably similar to those shown with their natural substrates. For this purpose, there are now over 20 different commercially available lipases that have their origins in plant, animal and microbial sources.

Reactions involving enzymes have typically been carried out in aqueous media because, being protein structures, it was assumed that the position and shape of the active site at which a reaction took place - the enzyme pocket - is affected by the conformation adopted by the enzyme. Naturally hydrophobic regions of the protein, such as amino acid side chains, prefer to be buried in the interior whilst charged hydrophilic regions orientate towards the surface of the enzyme, in contact with the polar aqueous medium.[52] The conformational dependence of the enzyme pocket is the origin of specificity in enzyme catalysis and has been exploited to alter the selectivity observed in enzyme catalysed reactions.

Changes to the enzyme structure can be made directly, through the use of protein engineering techniques, or by induced changes to the enzyme structure, through alteration of the reaction medium and its interaction with protein structure. This latter approach has the advantage of simplicity (which is significant when modifying the selectivity of an enzyme to accept a new substrate) and has been extensively researched such that three solvent systems are routinely used in enzymatic conversions.[52]

Water-miscible organic co-solvent system

Addition of a water miscible co-solvent, such as dimethylsulfoxide or an aliphatic alcohol, increases substrate solubility[53] and decreases the dielectric constant of the medium (altering the hydration of the ionic parts of the enzyme's protein structure[54]). Addition of excess organic co-solvent, however, has the effect of denaturing the enzyme, causing a loss of stereospecificity in the catalysed reaction.

Anhydrous organic solvents

A quantity of water is required to maintain various types of non-covalent bonding interactions that confer conformational rigidity to the enzyme structure, enabling it to perform a catalytic role with stereochemical specificity.[55,56] However, use of a near anhydrous organic solvent as the reaction medium has been found to enhance the structural rigidity of the active enzyme pocket and promote higher enantioselectivity in the catalysed reaction.[57] In the case of reversible lipase

catalysed hydrolysis reactions, an organic medium also drives the reaction towards product formation by limiting the extent of the reverse reaction, which relies on the availability of water in the system to act as a competing nucleophile.

Biphasic systems of water and a water immiscible organic solvent

In such systems, the enzyme is solubilised in the aqueous phase and surrounded by an organic solvent containing the substrate. The catalytic conformation of the enzyme is maintained and catalysis is dependent on physical agitation of the reaction medium to bring the substrate and enzyme into contact.

2.4.2 Specificity in organic solvents

Lipases are interfacial catalysts that utilise a lipid-water interface in the catalytic process.[58] This gives the lipase a unique affinity for hydrophobic environments and is a reason they remain active and selective in non-aqueous environments where they might otherwise be considered to become easily denatured.

Interfacial enzyme catalysis is understood through the work of Verger *et al*[59] who modelled the process as two successive equilibria. The first of these is the penetration of the substrate interface by the enzyme (Enz) to adopt a new conformation (Enz^*). The penetrated enzyme then binds with the substrate (Su) in a reversible way (Enz^*Su) before catalysis occurs to regenerate the penetrated form of the enzyme (Enz^*), liberating products (Pr) irreversibly. In enantioselective reactions, selectivity is therefore dependent on the relative rates of irreversible breakdown of each enzyme-enantiomer complex. This model has shown good agreement with the observed kinetic data from lipase catalysed reactions in near anhydrous/biphasic reaction media where the substrate concentration is effectively infinitely low.[60]

2.4.3 Enzyme selection

In selecting a suitable lipase for the enantioselective esterification of racemic butan-2,3-diol, the issue is one of experimentation since the degree of selectivity an enzyme will exhibit is dependent on its acceptance of the substrate (in this case, the

diol) into the enzyme pocket, whose shape, as has been explained, is itself highly dependent on the choice of reaction media and acylating agent.

Empirical rules to predict the selectivity exhibited with a particular substrate have been determined for a number of lipases.[61,62,63] In general, active site models predict the greatest degree of enantioselectivity in substrates that possess substantial steric and/or electrochemical differences between groups at the stereocentre where the catalysed reaction occurs. This is not unlike the Prelog rule[64], the earliest and simplest enzyme model that still provides the underlying logic for newer, more refined models. Their utility is questionable, however, due to contradiction and the variable purity in which commercially available lipase are supplied.

The Enzymatic Resolution Study

2.5 Results and discussion

Enzyme and acyl donor selection

Three lipases were selected: *Porcine Pancreatic Lipase (PPL)* and *Candida Cylindracea Lipase (CCL)* are both commercially available and have a demonstrated tolerance of a wide range of substrates.[65] *Candida antarctica*, in immobilised form on a polymer support, was obtained from Professor M. Turner of University College London and screened with the substrate under investigation. Being immobilised, it was proposed that *Candida antarctica* would be more stable and therefore exhibit greater enantioselectivity and show better retention of activity for reuse.

The use of alternative acyl donors was studied, employing both isopropenyl and vinyl acetates: The achievement of high enantioselectivity requires the minimisation of the reverse reaction, hydrolysis, since this negates the kinetically favoured esterification of one enantiomer over the other. The chosen acyl donors were selected on the basis that alcohols, poorer competing nucleophiles than water, are formed during the esterification. This allows the equilibrium of the reaction to be pushed towards product formation. Use of these acylating agents as both substrate and solvent enhances this effect, by creating a near anhydrous environment and

preventing the alcohol produced, potentially a competing nucleophile, at an effectively negligible concentration in the reaction system.

Experimental protocol

The experimental protocol adopted was to stir the substrate, a commericially available isomeric mixture of butan-2,3-diol, in excess acyl donor (acting as the reaction solvent) with a quantity of lipase, at a defined temperature for a period of time. The reaction mixtures were filtered to remove the lipase then, after isolation, the reaction products purified by flash column chromatography.

Control experiments, reacting the substrate and acyl donor in the absence of an enzyme catalyst, were not carried out as it was considered unlikely that the esterification would proceed very far, and with any stereoselectivtiy, in the absence of a chiral catalyst.

Analysis

NMR was used as the primary means for identification. Under all regimes, the products of each resolution experiment were found to be mixtures of butan-2,3-diol, 2-acetyl-3-butanol (the monoacetate form) and 2,3-diacetylbutane (the diacetate form) alone.

As in the fermentation experiments, chiral HPLC analysis was employed to directly measure the degree of enantioselectivity that had been achieved in each step of each reaction. Products isolated from the resolution were converted, where necessary, to the diacetate by chemical means and retention times compared with those of derivatives of the standard samples to allow a measure of optical purity to be made. Converging the isomeric forms of the diol at the diacetate (rather than any other derivative) enabled a single analytical technique to be used for each reaction isolate (which included the diacetate).

Performing the analysis on the commerically available isomeric mixture of the butan-2,3-diol, the three stereoisomers were found to be in the approximate ratio:

meso-form : D-(-)-(2*R*,3*R*)-form : L-(+)-(2*S*,3*S*)-form

3 : 1 : 1

This indicated that only 25% of the isomeric diol used in the resolution experiments would be available, limiting the theoretical yield of the target L-(+)-(2*S*,3*S*)-isomer to this figure.

2.5.1 Results: *Candida cylindracea and Porcine pancreatic lipases*

Candida cylindracea lipase (CCL)		**Reaction isolates**					
		Diol		**Monoacetate**		**Diacetate**	
Acyl donor	**Reaction conditions**	**Mole %**	**e.e. %**	**Mole %**	**e.e. %**	**Mole %**	**e.e. %**
Isopropenyl acetate	Room temp, 7 days	95	0	5	8	0	-
Isopropenyl acetate	37 °C, 8 days	92	1	8	9	0	-
Vinyl acetate	Room temp, 7 days	93	1	7	9	0	-
Vinyl acetate	37 °C , 8 days	90	2	10	13	0	-

Table 5: Resolution of isomeric butan-2,3-diol with *CCL*

Porcine pancreatic lipase (PPL)		**Reaction isolates**					
		Diol		**Monoacetate**		**Diacetate**	
Acyl donor	**Reaction conditions**	**Mole %**	**e.e. %**	**Mole %**	**e.e. %**	**Mole %**	**e.e. %**
Isopropenyl acetate	Room temp, 7 days	95	0	5	2	0	-
Isopropenyl acetate	37°C, 8 days	93	1	7	4	0	-
Vinyl acetate	Room temp, 7 days	90	2	10	13	0	-
Vinyl acetate	37°C , 8 days	88	2	12	16	0	-

Table 6: Resolution of isomeric butan-2,3-diol with *PPL*

- In all cases, L-(+)-(2*S*,3*S*)-butandiol was found to be in excess of the D-(-)-(2*R*,3*R*)-isomer.
- Mole % refers to the percentage of a particular reaction isolate out of the total mixture.

2.5.2 Inferences

With both *CCL* and *PPL*, L-(+)-(2*S*,3*S*)-butandiol was found to be in excess of the D-(-)-(2*R*,3*R*)-butandiol in the underivatised diol, though contaminated with the *meso* form (30-40%). The rate of the acylation reaction was slow with both enzymes and, over the time scales of the experiment, not enough of the kinetically more active *meso*- and D-(-)- forms of the diol were reacting to form acetate derivatives to leave the L-(+)- isomer unreacted.

The modest selectivity and low kinetic activity exhibited by both *CCL* and *PPL* was attributed to poor discrimination between the three stereoisomers of butan-2,3-diol within the active pocket of these enzymes. If this were the case, manipulation of the solvent medium (or other physical changes to the reaction system) may have invoked a change in the conformation of the enzyme active site (see section 2.4.1) thereby altering its stereospecificity. Instability of enzyme protein structures in polar organic reaction media has also been cited as a cause of poor enzyme specificity and immobilisation has been demonstrated as a means of overcoming such biocatalytic instability.[66]

In preference to 'solvent engineering', it was hoped that *Candida antarctica*, being in immobilised form on a polymer support, would be more stable in the organic medium and therefore exhibit greater enantioselectivity. The resolution experiments were therefore repeated under the most successful conditions arising from the *CCL/PPL* studies: Vinyl acetate as the acylating agent, incubating the reactions at 37 °C over a range of timescales.

2.5.3 Results: *Candida antarctica* lipase

Product distribution over time

Time	Reaction isolates		
	Diol	Monoacetate	Diacetate
	Mole %	Mole %	Mole %
2 hours	56	44	0
4 hours	17	80	3
5 hours	11	84	5
3 days	0	52	48

Table 7: Product distribution with *Candida antarctica*

Candida antarctica was seen to be more effective in catalysing the acetylation of butan-2,3-diol, converting the substrate to both the mono- and di- acetates in a much shorter time than with either *CCL* or *PPL*.

Lipase selectivity

Time	Monoaetate			Diol			Maximum e.e.	Preferred Form
	(2*S*,3*S*) %	(2*R*,3*R*) %	*meso* %	(2*S*,3S) %	(2*R*,3*R*) %	*meso* %	%	
2 hours	13	14	73	40	10	50	60	(2*S*,3*S*)-
4 hours	0	57	43	13	0	72	100	(2*S*,3*S*)-
5 hours	19	9	72	99	0	1	100	(2*S*,3*S*)-
3 days	7	58	35	20	1	79	91	(2*S*,3*S*)-

Table 8: Resolution of butan-2,3-diol with *Candida antarctica*

In the limited time study carried out, an incubation time of 5 hours was found to be optimal for the *Candida antarctica* catalysed resolution of commercially available isomeric butan-2,3-diol. The target L-(+)-(2*S*,3*S*)-isomer was recoverable uncontaminated as the unreacted diol, in 11% isolated yield (based on the quantity of

isomeric mixture used), or 35% theoretical yield (based on availability of the target isomer).

2.5.4 Activity of catalyst

An issue in evaluating a strategy involving enzymatic catalysis is the retention of catalytic activity after repeated use. To test the stability of *Candida antarctica* and its potential for reuse, a resolution was repeated under optimal conditions with a quantity of the enzyme that had been used previously, isolated from the reaction, and dried in air before storage at low temperature (3 °C to 5 °C).

After the resolution, the recovered unreacted diol was found to have an e.e. of 91% in favour of the L-(+)-(2*S*,3*S*)-form though this was contaminated with the *meso* isomer (which made up 32 mole % of the mixture).

This demonstrated that *Candida antarctica* was robust and able to withstand the potentially denaturing conditions of the organic medium though a separate time study would be necessary to optimise any resolution using the recycled enzyme.

2.5.5 Conclusions of the enzymatic resolution study

A facile, scalable and repeatable procedure for the enzymatic kinetic resolution of commercially available isomeric butan-2,3-diol was established using a system of immobilised *Candida antarctica* in a non-aqueous environment of vinyl acetate as both substrate and solvent. This generated the L-(+)-(2*S*,3*S*)-isomer in an enantiomeric excess and an isolated yield of >99% and 11% (25% based on available isomer) respectively.

2.6 Conclusions of the chapter

The combination of the fermentation and enzymatic resolution procedures established in this chapter provided access to laboratory scale quantities of isolated L-(+)-(2*S*,3*S*)- and D-(-)-(2R,3*R*)- butan-2,3-diol isomers. These enantiomerically pure diols were thus available as chiral auxiliaries for use in other aspects of this project.

CHAPTER THREE: AQUEOUS AND MICELLAR PHASE REACTIONS

3.0 Introduction

As an organic reaction medium, water offers the possibility of a clean, safe and cheap medium and such a use is very much in tune with rising environmental concerns in synthetic chemistry. The use of water as a solvent is not unprecedented. Indeed, Diels and Alder, when investigating the cycloaddition that now bears their names, performed this reaction in aqueous media back in the 1930s. [67]

The 'rediscovery' of water as a solvent in 1980 is credited to Breslow[68] who discovered a remarkable rate enhancement of the Diels-Alder reaction between cyclopentadiene and methyl acrylate; performing the reaction in water, instead of isoctane, enhanced the rate of cylcoaddition by over 700 times. This was later followed by the observation that aqueous media could also have a striking effect on the stereoselectivity of such Diels-Alder reactions.[69,70]

3.1 Aims

It was an objective of this project to apply the use of aqueous based reaction media wherever possible, in the hope of extending its applicability in synthetic methodology. Such practice represents a BATNEEC (*B*est *A*vailable *T*echnique *N*ot *E*ntailing *E*xcess *C*ost) under the International Pollution Control regulations, which have been incorporated into the Environmental Protection Act of this country.

This chapter therefore introduces the concept of water as a reaction medium, and the concept of micellar based media derived from the aqueous solubilisation of surfactants. The synthesis and characterisation of two chiral surfactants was also undertaken in this part of the project, providing new micellar media with their own unique substrate solubilisation properties.

The concepts introduced in this chapter have therefore been applied, wherever possible, in the other areas of the project, described in subsequent chapters. The

scope of this work was, however, limited to the use of ionic surfactants operating in aqueous media and the fields of non-ionic surfactants and 'reverse' micelle behaviour (through use of polar and non-polar solvent systems) were not explored.

3.2 Water as a medium - the hydrophobic effect

Water is a 'structured' liquid, in which strong, directional hydrogen bonds link each molecule to four neighbouring molecules. This highly structured network is of low entropy and low density, in which many cavities exist. This model may be contrasted with that of the 'unstructured' liquids where weak, non-directional Van der Waals forces are responsible for linking molecules together in a regular manner with greater overall space efficiency.[71]

Water possesses the highest cohesive energy (550 Cal/ml or 22,000 atm) of any liquid, a large surface tension and a high heat capacity.[72] These physical characteristics manifest themselves in the unique structure of water and, from a synthetic organic perspective, an insolubility of organic compounds in this highly polar medium. As a consequence, organic reactions carried out in water may be expected to proceed slowly, if at all, and in poor yield.

These physical characteristics also give rise to an associated effect, termed the *hydrophobic effect*. First applied by Kauzmann in 1959 to rationalise the folding of proteins in aqueous systems, this describes the driving force behind the aggregation of charged molecules to minimise unfavourable interactions between species of disparate polarity.[73]

Where apolar organic molecules are even only slightly soluble, as may occur if a cavity in the water structure is infiltrated, they may aggregate together as a consequence of the hydrophobic effect and, in doing so, cause an increase in the local concentration of organic molecules. From a synthetic organic perspective, these reactants may now be in sufficiently close proximity to permit a reaction to occur.

Thus the hydrophobic effect can promote the formation of transition states between apolar hydrophobic reactants, the polarity of water stabilising the transition

states with a minimal penalty due to the dilution of these activated complexes by the solvent.[74] A second rate enhancing effect arises when the activation volume of the reaction is negative whereby the high cohesive energy of water acts in the same way as an externally applied pressure, forcing the reactants together.[72] This 'internal pressure' of water has, however, a limit, beyond which the solutes are literally squeezed out of solution.

Apart from eliciting rate enhancement, the hydrophobic effect has also been shown to have a profound influence on the stereochemical course of organic reactions that proceed in water *via* such an effect, whereby the dominant products are those of the smallest electrostatic volume (thus minimising unfavourable interactions in, and beyond, the transition state). An example of this has been observed for certain Diels-Alder cycloadditions which, when carried out in water, show a marked preference for the spatially more compact *endo* product.[75]

3.3 Micelles

The utility of the hydrophobic effect is limited by the extent to which aggregation of the reacting molecules occurs. This is dependent on the affinity molecules have for aggregates of their own type, as compared to their affinity with aggregates of other reactants.

These problems may be overcome by the addition of surfactants (*surf*ace *act*ive re*agents*) to the reaction medium that allow the solubilisation of organic molecules in bulk water. Literature sources variously describe these additives as 'detergents', 'surface-active molecules' or 'amphiphiles'; all are synonymous with 'surfactants', which remains the preferred (though ultimately arbitrary) choice of terminology for this project.

Surfactant molecules are characterised by a long aliphatic chain (eight to twenty carbons), typically with a smaller polar head group at one end (though surfactants of other types have also been synthesised. For example; 'double headed'[76], 'double tailed' or bolaform[77], with functional groups at both ends of the hydrocarbon chain).

The nature of the head group may be cationic (*e.g.* ammoniums), anionic (*e.g.* sulphates), amphoteric (*e.g.* betaines) or uncharged (*e.g.* polyoxyethanes).

The exact configuration the surfactant adopts in solution, in the presence of other surfactant molecules, is therefore dependent on the balancing of attractive interactions between the non-polar portions of the molecules and the repulsive forces between the head groups, within the polar environment of the bulk aqueous solution.

It is generally accepted that, at low concentrations, the preferred conformation of surfactant molecules in an aqueous environment is as single monomers.[78] At higher surfactant concentrations, as a consequence of the hydrophobic effect[79] - the tendency to minimise entropically unfavourable interactions and contact between molecules of disparate polarity[80] - aggregation of these monomer units occurs with the formation of surfactant aggregates (termed *micelles*). The surfactant concentration at which this aggregation occurs is termed the *critical micellar (or micellisation) concentration* (*CMC*).

It is accepted that this initial aggregation involves between 30-150 individual surfactant molecules but beyond this, there is no consensus as to the structural features of the micelles formed[81] or even the concept of a sharply defined CMC below which surfactant molecules remain monomeric[82].

It is beyond the CMC, the point at which micellisation occurs, that the useful properties of surfactants are realised since it is through the dissolution of hydrophobic material within the micellar interior that enables surfactants to solubilise organic material within a bulk aqueous environment.

This concept of micelle formation has enabled surfactants to find application, not only as detergents (in the purely domestic sense)[83], but also as carriers for the delivery of aqueous insoluble drugs[84] and in organic synthesis[85].

3.4 Micellar structure and the CMC

The concept of micellisation, and the description of micellar structure in aqueous solution at different concentrations, are highly contested subjects in the chemical literature. There is near universal disagreement on every aspect of micellar study

and the following section attempts to report the most frequently proposed models for describing the structure of surfactant aggregates at the CMC.

When ionic surfactant molecules are dissolved in aqueous solution, three broad behavioural regimes can be described with increasing concentration: monomeric at low concentration, roughly spherical micelles at the CMC, and larger, longer rod shaped aggregations at higher surfactant concentrations:[86]

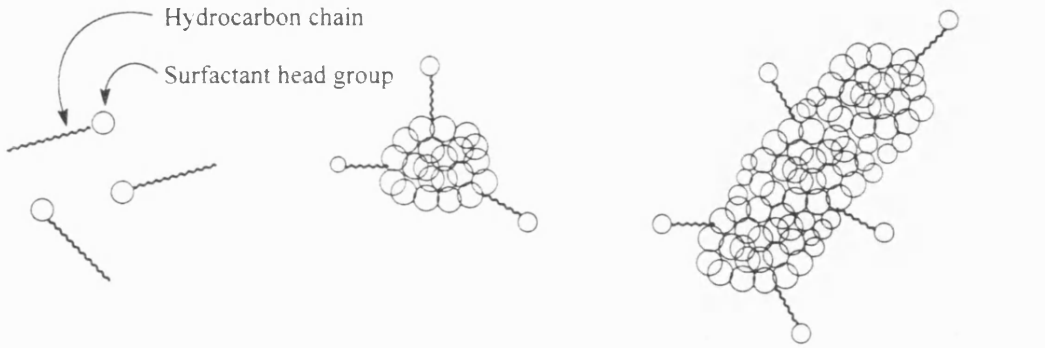


Figure 9: Surfactant behaviour at different concentrations

The acceptance of the existence of monomeric surfactant molecules at low concentration is not universal and has been contested by arguments in favour of dimeric, trimeric and pre-micellar aggregations.[87]

3.4.1 Behaviour at the CMC

The Hartley model

The first universally accepted hypothetical structure for micelles formed at the CMC was that proposed by Hartley and the model describes an approximately spherical hydrocarbon core encased by a shell of hydrated ions.[78] In the context of the Hartley model, the CMC is therefore the surfactant concentration at which spherical surfactant aggregates form:

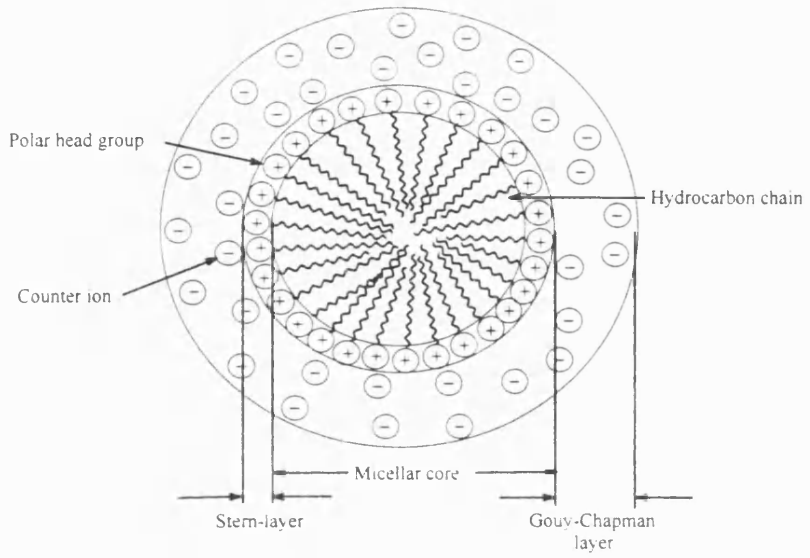


Figure 10: The Hartley model of a micelle - cationic surfactant example

It was proposed that the micellar interior, formed by the aggregation of the hydrophobic carbon chains of the surfactant molecules, gives rise to a spherical cell diameter of around 10 to 30 Å. This minimises water-hydrocarbon interactions between the carbon chains and the bulk aqueous solution and provides an apolar environment within the core of the micelle.

Surrounding this is a very thin layer (a few Å)- *the Stern layer* - made up of the more polar head groups of the surfactant molecules. Since these are hydrophilic in nature, they point towards the aqueous solution, and are hydrated by solvent molecules, forming a charged shell surrounding the micellar core.

Beyond this is a thicker (several hundred Å), diffuse region - *the Gouy-Chapman layer* - composed of unbound counter ions to the head groups that eventually gives way to the bulk aqueous exterior.

Alternative models

The Hartley model, useful as a conceptual model and a description of the features of the micellar structure, has been criticised for being unrealistic. At the very least, the near all-*trans* geometry of the hydrocarbon tails of the surfactant molecules is not spatially possible within the confines of the micellar core.

Research into the study of micellar structure has been reviewed by a number of authors.[80,85,88] In proposing alternatives to the classical Hartley structure, attempts have been made to reconcile the steric demands of individual surfactant molecules within the micelle and the question of water penetration within the micellar core. The basic tenets for micellar structure are not new and the work of recent years has therefore sought only to lend support to one model over another, rather than offer any new perspectives.

At one extreme is the so-called 'fjord' model, advocated by Sevens and Rosenholm, which proposed complete percolation of water molecules throughout the micellar core.[89] At the other extreme is that put forward by Stigter, who suggested the 'reef' model.[90] In this scenario, there is no penetration of water whatsoever into the micellar core, a view supported by Wennerström and Mahieu *et al.*[90a,90b]

To overcome the steric objections to the Hartley model, Dill and Flory applied a statistical model for molecular organisation within of a micelle and derived a lattice packed structure, approximately crystalline at the core and with a smooth spherical shell containing the ionic head groups.[91] Internal folding and looping of surfactant chains was proposed, overcoming the objection to the Hartley model that the micellar core contains only *trans*- configured hydrocarbon chains. This results in a significant probability that mid-chain and terminal methylene groups may reside on the micellar surface in contact with the bulk aqueous medium.

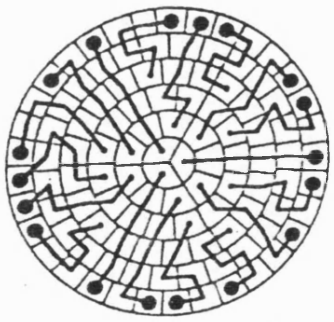


Figure 11: The Dill and Flory model

Incorporating many of the competing ideas, Menger proposed a 'porous cluster' model of the micelle, capable of trapping large volumes of water in its interior, with

coiling and disorder amongst the hydrocarbon chains placing chain termini in the Stern layer [81,92]

For the purposes of discussion, where micellar media have been used, the Hartley model has been assumed from a descriptive point of view to distinguish between a predominantly polar micellar region (containing charged hetero functionality) and a natively hydrocarbon region (that may reside somewhere within the micellar interior). Whilst a far from accurate model, in light of the conflicting experimental evidence into micellar behaviour, the Hartley model more than adequately describes the phenomenon of micellisation.

3.5 Surfactants in organic synthesis

The solubilisation brought about by surfactants can be understood as a dynamic process whereby organic molecules move in and out of the micelles formed, finding temporary solubility in the hydrocarbon micellar core. This engenders a number of properties to micelles:[93]

- **local concentration effects** - hydrophobic molecules aggregate in the hydrocarbon interior of the micelle increasing the concentration of reactant molecules in this region
- **cage effects** - two reactive intermediates may be held temporarily, but for sufficiently long, by the micelle to allow reaction to occur
- **preorientational effects** - stereochemistry may be induced into reactions by orientation of organic molecules such that their polar groups interact with the Stern-layer whilst their non polar parts reside in the micellar core
- **electrostatic effects** - reactions between charged species may be propagated by their aggregation within the Stern-layer
- **polarity effects** - solvent polarity dependent reactions may be influenced by the polarity of the micellar interior which, whilst less polar than water, is more polar than hydrocarbon solvents
- **microviscosity effects** - viscosity sensitive reactions may be affected by micelles since the viscosity within the micelles is higher than that in the bulk solution

With aggregation and dissolution of monomers occurring at the same time, the concept of a uniquely defined micellar boundary is probably arbitrary in itself.[94a,94b]

3.6 Surfactant syntheses

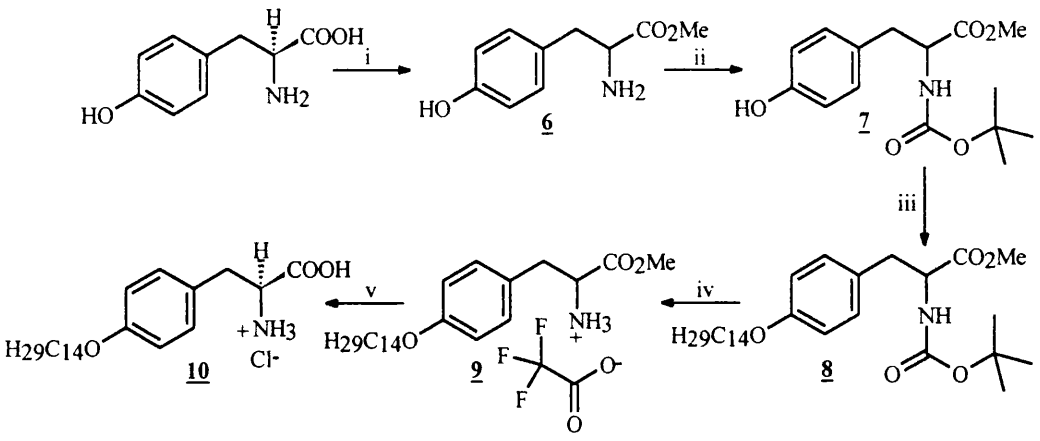
Amino acids are readily available synthons from the chiral pool for use in asymmetric synthesis. For the purposes of this project, two optically active surfactants were synthesised from these materials, with the objective of providing alternative reaction media in which to study the chemical effects (for example, yield and stereoselectivity) of performing a number of different organic reactions in micellar media. The simple premise to be tested was that an optically active surfactant might generate a chiral micelle that could invoke a transfer of asymmetry to a reaction taken place within it.

3.6.1 *O-tetradecanyl-(S)*-tyrosine (10)

The etherification of the phenol group in tyrosine is not unprecedented in the literature and an attempt was made to form the C-14 ether by adapting a procedure used in the synthesis of *O*-benzyl tyrosine [95]. However when tetradecanyl bromide was substituted for benzyl bromide as the alkylating agent, the reaction did not proceed and starting materials alone were recovered. This was attributed to stabilisation of the S_N2 transition state by secondary orbital overlap of the benzylic π orbitals in the case of benzyl bromide, and the enhanced leaving group ability of bromine in benzyl bromide compared to alkyl bromides.

This view was supported by several additional failures to form the tetradecanyl ether **10** directly with tyrosine and the tetradecanyl bromide in basic media (deprotonating with carbonate/hydroxide).

Following the failure of a direct etherification method, it was decided to investigate the formation of the tyrosine ether through initial protection of the amino acid functionalities; the corresponding methyl ester for the acid group and BOC (*t*-butoxyl carbonyl) group for the amine, which can be readily cleaved under acidic conditions (scheme 11).



(i):MeOH, $SOCl_2$, 0 °C (ii):$NaOH_{(aq)}$, di-*t*-butyldicarbonate (iii):NaH, THF, 1-bromohexadecane (iv):trifluoroacetic acid, DCM (v):$NaOH_{(aq)}$

Scheme 11: Synthesis of *O*-tetradecanyl-tyrosine

The methyl ester **6** was prepared, in near quantitative yield (95% after recrystallisation from hexane), *via* the *in situ* formation of the acid chloride of tyrosine and the BOC (*t*-butoxyl carbonyl) protecting group introduced in good yield (72%)[96].

With the protected tyrosine molecule **7** obtained, a standard Williamson type procedure[97], using sodium hydride as a base, was applied to introduce the tetradecanyl chain at the phenolic position. This reaction was only low yielding (2%). Milder conditions and deprotonating with potassium carbonate were ineffective, as was deprotonating with sodium hydride in THF. As an alternative, THF was replaced with DMF (thereby increasing the basicity of sodium hydride) and as a result, the phenolic ether **8** was obtained in an acceptable 64% yield after isolation and purification.

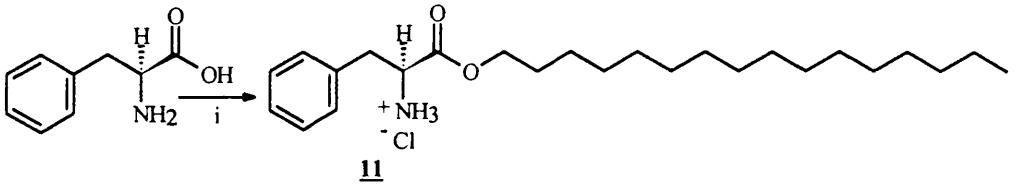
The two deprotection steps were carried out sequentially, without purification of the intermediate **9**, by treatment with trifluoroacetic acid and sodium hydroxide to remove the BOC and methyl ester groups respectively. By washing the resulting product with saturated sodium chloride, the target surfactant was liberated (in 64%

yield over the two steps from the corresponding protected derivative) as the ammonium chloride salt of the tyrosine ether **10**.

The use of both the acid and basic conditions described in scheme 10 may have compromised the optical purity of surfactant **10**. Whilst not undertaken in this work, the measurement of the optical purity of this surfactant would have been necessary if the effects of the chiral environment formed on micellisation, in a synthetic context, were to be understood.

3.6.2 (*S*)-phenylalanine hexadecanyl ester hydrochloride (11)

A second chiral surfactant was synthesised, based on a simple ester of phenylalanine. The C-16 analogue was selected and prepared by the direct esterification of tyrosine with hexadecanyl (cetyl) alcohol under acid catalysis. The surfactant **11** was obtained in 42% yield as the ammonium chloride salt (scheme 12).



11

(i):Hexadecanyl alcohol, *p*-toluenesulfonic acid, toluene

Scheme 12: Synthesis of (*S*)-phenylalanine hexadecanyl ester hydrochloride

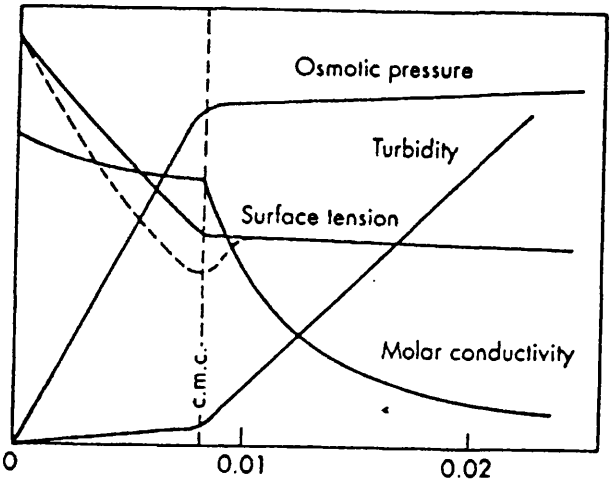
As with surfactant **10**, the acid conditions used in scheme 11 may have compromised the optical purity of surfactant **11**. Measurement of its optical purity would be essential if the effects of a chiral micellar were to be studied and if chiral inductions were observed through its use.

3.7 CMC determination of micelles

If a reaction is to be operated in the micellar phase, it is necessary that the concentration of surfactant molecules present in solution be at, or above, that required for the aggregation to occur and micelles to be formed - the critical micellar concentration (CMC).

Many diverse methods have been developed to measure this concentration and all depend upon the observance of a marked change in some physical property of the

surfactant solution when the CMC is reached.[86] Physical properties which alter as a function of surfactant concentration can be grouped into three types; those that show an increase (turbidity, solubilisation, magnetic resonance), decrease (equivalent conductivity, self-diffusion) or uniformity (osmotic pressure, surface tension) above the CMC (graph 7).



Graph 7: Variation of physical properties with surfactant concentration

The determination of the CMC is never unambiguous[88] and the physical change on which the CMC is determined has an enormous influence on the value obtained. Indeed, Mukerjee and Mysels identified 71 different measures.[98] The measurement of the CMC is therefore conceptual and arbitrary.

3.7.1 Dye solubilisation

A particularly facile method for CMC determination of cationic surfactants was developed within the research group by M. Diego-Castro[99] who utilised the discovery of Dutta that the UV-absorption of methyl orange in distilled water (λ_{max}=460 nm) is shifted in the presence of a cationic surfactant (λ_{max}=360 nm)[100]. In water, the maximum UV absorption arises from conjugation of the double bond system across the whole molecule, from the amine group at one end to the sulfate at the other, and results in an absorption peak at 460nm.

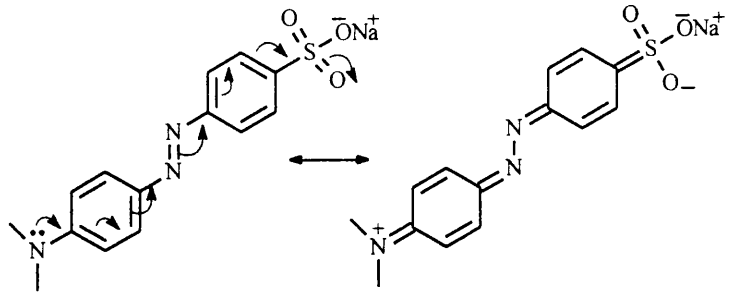


Figure 12: Delocalisation of methyl orange in 'free solution'

Methyl orange has been shown to undergo a blue shift when exposed to polyelectrolytes.[101] In solutions of CTAB, Dutta found that this phenonenon occurs at the CMC of the surfactant and postulated that alignment of methyl orange and surfactant molecules was occurring. The change in the microenvironment of the methyl orange molecule causes an observed decrease in absorption maximum *i.e.* a blue shift in the UV spectrum (to shorter wavelengths).[100] Whilst indicative of a decrease in the extent of delocalisation of charge across the methyl orange molecule, other mechanisms (such as the adoption of the *cis* conformation) have been proposed.[102,103]

As a means of CMC determination, the monitoring of such spectral changes accompanying solubilisation of dyes by surfactant micelles is not new and the shortcomings of the technique have been reviewed by Mukerjee.[98] The principle objection to the technique is its accuracy since the dye may itself alter the micellar structure of the surfactant or even induce micellisation. However, as Reeves pointed out, where a CMC lower than that measured in the absence of the dye is obtained, the magnitude of the error is not usually significant.[104] Furthermore, since it was the intention of the project to use surfactants with reaction substrates, the interaction of surfactant and dye molecules may well be a good model for the surfactant's actual behaviour when in use.

Despite the drawbacks of the technique, dye solubilisation was selected as the standard procedure for CMC determination within the research group, owing to the rapidity of the technique and the arbitrariness inherent in any CMC determination.

3.7.2 CMC determination *via* dye solubilisation

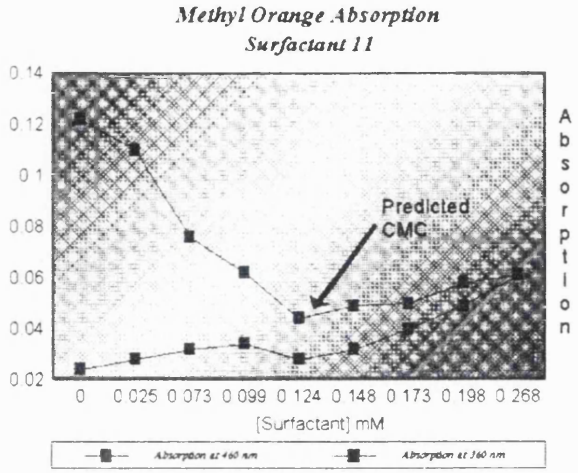
The dye solubilisation tecnhique was applied to the determination of the CMC of the chiral surfactants synthesised in this project (section 3.6) and the procedure employed was as follows:

A solution was formed by dissolving a known quantity of the surfactant **11** to obtain a solution of known concentration. This was divided into portions which were further diluted by the introduction of methyl orange (5 mg/100 ml) and distilled water, in accordance with the table 9.

Surfactant solution ml	Methyl orange Ml	Water ml	[Surfactant] mM
0	1	10	0
1	1	9	0.026
2	1	8	0.049
3	1	7	0.073
4	1	6	0.099
5	1	5	0.124
6	1	4	0.148
7	1	3	0.173
8	1	2	0.198
9	1	1	0.251

Table 9: CMC determination of surfactant **11**

The UV absorption spectrum for each solution was recorded and the absorptions for the bands λ=460 nm and λ=360 nm plotted against the corresponding surfactant concentration for that solution.



Graph 8: CMC determination of surfactant **11**

Examining the relationship between the concentration of **11** and UV absorption, from the graph it can be observed that the gradual shift in absorption for the 460 nm band is complete at a surfactant concentration of 0.125 mM. This was assigned to be the CMC of surfactant **11**.

Other studies carried out in the research group, measuring changes in conductance to detect the CMC, were found to give results consistent with the dye solubilisation method. This suggested accuracy of the CMC measured for surfactant **11**, despite the possibility that mixed micelle formation was occurring between the surfactant and methyl orange molecules.

The use of methyl orange dye was found to be incompatible with surfactant **10** in determining its CMC. This may have been due to its zwitterionic nature, resulting in low solubility in water at pH 7, where the CMC is measured. Alternatively, the acidic proton in the amino acid moiety may bind to any amine group within the methyl orange molecule, altering its UV absorption. This manifests itself as a pink colour in the surfactant test solutions and renders methyl orange useless for CMC determination where the surfactant contains acid protons. The use of acid insensitive dyes is a potentially simple way of overcoming this problem[105], though in this project the CMC determination of **10** was not taken any further.

CHAPTER FOUR: BIOMIMETIC CYCLISATIONS

4.0 Introduction

In total synthesis, polyene cyclisations are a valuable alternative to the step-by-step, ring-by-ring type annelation strategies for the construction of polycyclic natural products. The strategy involves the formation of a number of rings in a stereospecific fashion *via* a single intramolecular telomerization step of an acyclic chain of olefinic bonds of defined stereochemistry.[106] This is a process that has been shown to occur naturally as part of the biosynthesis of stereochemcially pure polycyclic compounds.[106,107] Whilst such cyclisations in nature are under enzyme control, there is a substantial body of work in the literature that has investigated whether the origin of this stereochemical control is implicit in the cyclisation itself, and whether the use of chiral auxiliaries can act to mimic the enantiomeric control exhibited by enzymes. Hence synthetic strategies of this type are frequently described as 'biomimetic cyclisations' after the biological processes they seek to mimic.

4.1 Approach

The discovery of enzymatically controlled polyene cyclisations in nature arose out of extensive research into the elucidation of the terpene biosynthetic pathway from acetate to cholesterol.[107] The intramolecular cyclisation of squalene oxide was considered a key transformation since the product was shown to be a precursor to cholesterol.[108,109]

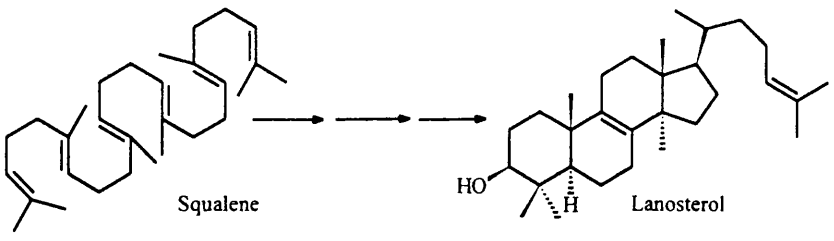


Figure 13: Schematic transformation of squalene to lanosterol

Stork[110] and Eschenmoser[111], working independently in the 1950s, rationalised the stereochemical control seen in polyene cyclisations on stereoelectronic grounds, whereby all *trans* polyolefins (such as squalene) have an intrinsic susceptibility to cyclise stereospecifically to give a product with a *trans* fused configuration. In such a case, the cyclisation proceeds *via* chair-like conformations of the emerging rings with the addition to each double bond occurring in an antiparallel fashion. The corollary of this hypothesis (which bears the names of both its proponents, Stork and Eschenmoser) is that a *cis* olefinic bond will give rise to a *cis*-fused ring system.

4.2 Cyclisation initiation groups

Polyene cyclisation reactions of this type, involving 1,5-dienes, are biased towards the formation of six-membered rings (6-*endo*-trig) over five-membered variants (5-*exo*-trig). [112] The initiation of polyene cyclisations can occur by a number of means, such as double bond protonation, interaction with Lewis acids[113] or the addition of electrophiles[112]. However, in terms of preparatively useful polyene systems, the use of stabilised carbonium ion initiating groups by Johnson[114] marked the introduction of the technique to general synthetic methodology. Subsequent innovations have been the use of allylic carbonium ions[115], allylic ions[116] and sulphur stabilised species[117] but it was the use of an oxonium ion initiator[118,119,120] that was of interest to this research.

Oxonium ions, being derived from acetals, have the potential to be functionalised in an optically pure form by the simple reaction of an aldehyde with an optically pure diol. A high degree of asymmetric induction has been demonstrated in the cyclisation of 1,5-diene derived chiral acetals[121] and it was with this choice of initiating group that the polyene cyclisation approach held the possibility of a regio-, stereo- and enantio- selective strategy to an octalin synthesis.

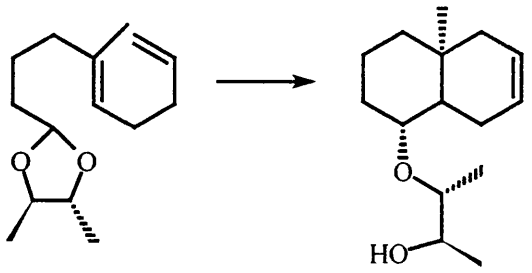
Given the availability of both enantiomeric forms of the diol butan-2,3-diol from the study described in chapter 2, and in order to achieve an enantioselective route to dehydrogeosmin and its analogues, the use of optically active acetals derived from this diol therefore appeared an excellent approach to investigate.

4.3 Objective of the chapter

Polyene cyclisations have been successfully employed in steroid syntheses[122,123,124] for the construction of bi, tri and higher order ring systems with defined and predictable stereochemistry. For the purpose of this project, and the synthesis of geosmin, dehydrogeosmin and their isomers, it was proposed to investigate the construction of the key bicyclic octalin skeleton, with absolute stereochemistry defined through such a polyene cyclisation sequence.

Part of the investigation necessarily entailed the synthesis of a suitable polyene precursor and its functionalisation as an acetal cyclisation precursor using the optically pure butan-2,3-diol isomers prepared in chapter two.

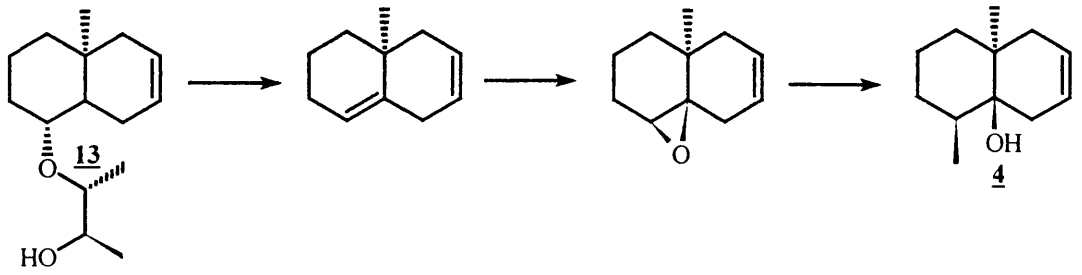
The key cyclisation step to be tested in this chapter, in the context of a manipulable target to dehydrogeosmin, was therefore:



12 13

Figure 14: Biomimetic cyclisation step under test

The precedent of Williamson[121] suggested that the octalin formed would possess a (4a*R*)-methyl group at the ring junction. Such an intermediate **13** provides an effective synthon for the onward transformation to natural dehydrogeosmin **4** (and its isomers) by established procedures (scheme 13).[4,8,9,10]



Scheme 13: Proposed manipulation to dehydrogeosmin

4.5 Routes to the acyclic precursor

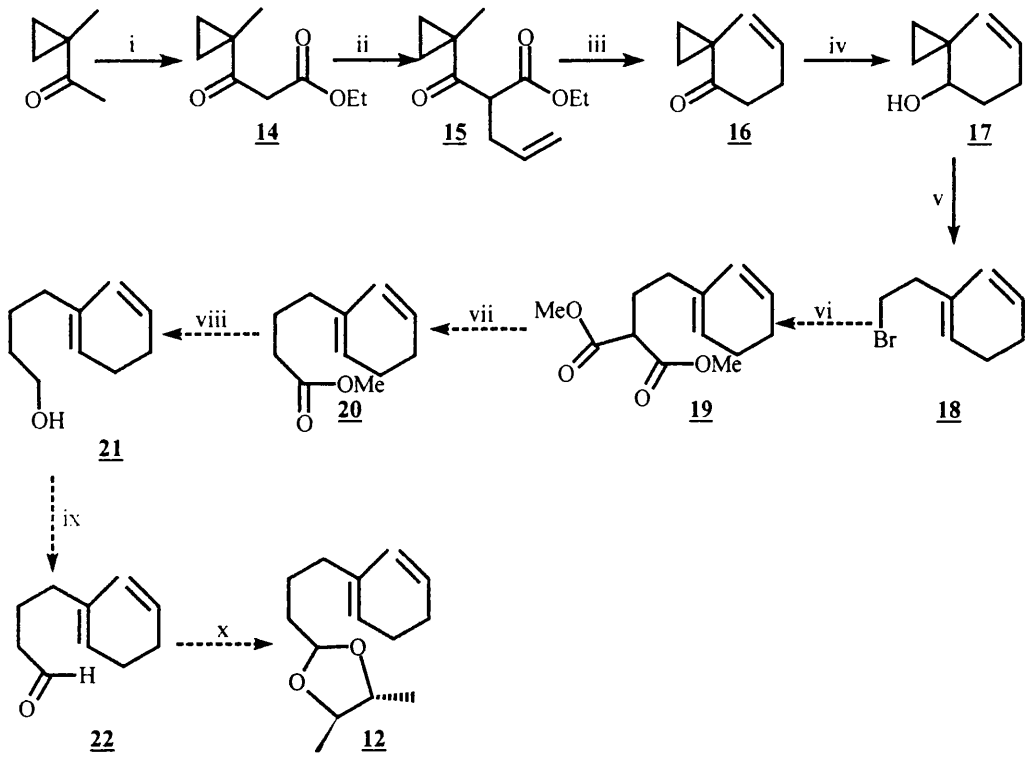
In the course of this investigation, three separate routes emerged that were followed, in parallel, to access the model acyclic diene precursor **12** - the acetal derived from butan-2,3-diol and (5*E*)-5-methyl-5,9-decandiene-1-al:

- Cyclopropyl carbinol rearrangement
- Wittig olefination
- Claisen rearrangement

In all three cases, the key synthetic challenge of each strategy was the establishment of a trisubstituted double bond in the 5-position of the acyclic precursor **12** with the highest degree of stereoselectivity (that is, *E*:*Z* selectivity) possible. This latter condition was important since, whilst both *cis* and *trans* olefins have been shown to undergo cyclisation, a meaningful measure of asymmetric induction, attributable to the use of an optically active acetal handle, could only be made with a stereochemically pure cyclisation precursor.

4.5.1 Cyclopropyl carbinol rearrangement

Initially, it was proposed to synthesise the acetal cyclisation precursor **12** *via* the trisubstituted olefinic homoallylic bromide **18**, modifying a procedure by Johnson (scheme 14) [121,125]. The key step in the construction of purely *E*- geometry in this olefinic precursor was the stereoselective rearrangement of the parent cyclopropyl carbinol **17**, a procedure developed by Julia.[125a]



(i):NaH, THF, diethyl carbonate (ii):NaH, THF, allyl bromide (iii):EtOH, H_2O, $Ba(OH)_2.8H_2O$ (iv):$LiAlH_4$, Et_2O (v):LiBr, collidine, PBr_3, Et_2O (vi):NaH, DMF, dimethyl malonate

Scheme 14: Cyclopropyl carbinol rearrangement route to the acyclic precursor

Commercially available 1-acetyl-1-methylcyclopropane was used as the starting material in the sequence and was successfully added to diethyl carbonate, under base catalysed conditions with displacement of ethoxide, in 81% isolated yield (of intermediate **14**). Enolate chemistry was used again for the addition of this keto ester to allyl bromide with displacement of the halide (intermediate **15** in 96% isolated yield). Decarbethoxylation was then carried out by treatment with barium hydroxide to give the cyclopropyl ketone **16** in 93% yield.

This olefinic ketone was reduced to the corresponding carbinol (**17** in 83% yield) before treatment with a lithium bromide/zinc bromide/phosphorus tribromide combination to open the carbinol. The resulting bromodiene **18** was obtained in 74% isolated yield, and NMR analysis of this material indicated the presence of one

singlet at 1.61 ppm corresponding to the C-6 methyl group, consistent with literature references to exclusively *E*- stereochemistry in this product [125].

The subsequent, and apparently simple, step of addition of a malonate group to this diene with displacement of the bromine, resulted in the isolation of a single product; a material which, whilst possessing a strong, sharp odour and appearing as a single compound by both 1-D and 2-D TLC analyses, gave few signals when analysed by NMR. In addition, no functional groups were discernible by IR spectroscopy. MS analysis gave no further clues as to the structure of this molecule, which was clearly not the malonate precursor **19** to the desired *trans*-decadiene **20**.

Without an explanation as to the unexpected path this reaction sequence had taken, it was felt prudent to pursue alternative strategies in parallel.

4.5.2 Wittig approach

The Wittig olefination reaction is perhaps *the* established methodology for olefin synthesis where regio- and stereo-selectivity are of paramount importance. In essence, the reaction is that between aldehydes or ketones with phosphonium ylides to form olefins with complete predictability in the positioning of the double bond.[126,127] The proliferation of research into this area following the initial disclosures of Wittig and his co-workers has resulted in considerable sophistication being applied to reactions of this type, with the result that high stereoselectivity in the resulting olefins, *i.e.* *Z*- and *E*- selectivity, is routinely achievable.[128]

There is a substantial precedent to demonstrate that Wittig type reactions can be successfully carried out in aqueous and biphasic media - water/benzene mixtures[129], water/dichloromethane[130] and water/alcohol systems[131,132,133] have all been shown to be compatible. By extension, the use of micellar aqueous media has been shown to be possible, though with inconclusive effects on the yield and stereoselectivity of these reactions.[134]

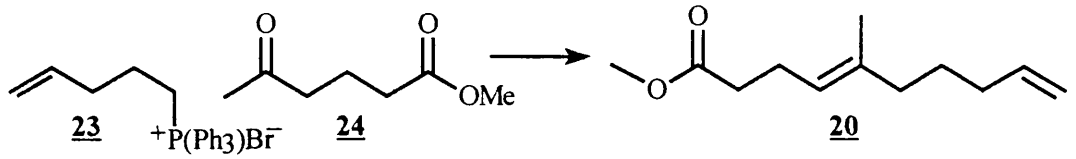
In keeping with the objectives of this project and the aims set out in chapter three, the use of aqueous and micellar mediums was therefore included, where possible, in the following study to prepare the target decadiene **22** for the biomimetic reaction

sequence. This provided insights into the relative merits of organic *versus* aqueous systems in relation to the system under study, and solvent dependency effects on yield and stereoselectivity.

4.5.2.1 Substrates

The chosen strategy to prepare the target decadiene precursor was to react an ester-protected oxohexanoic acid with a phosphonium salt of 5-bromo-pent-1-ene (scheme 15). The Wittig acceptor, methyl 5-oxohexanoate **24**, was prepared in 67% yield (from the corresponding oxoacid). Triphenyl phosphonium bromide **23**, prepared in 27% yield, was selected as the unstabilised Wittig reagent.

Whilst unstabilised Wittig reagents derived from tri-*n*-butylphosphine are reportedly more reactive[135], these were not prepared due to the possibility of forming competing ylids.



Scheme 15: Proposed wittig route to decadiene precursor

From the outset, this approach presented several challenges:

Cis/trans selectivity

Unstabilised ylids of the type prepared give predominantly *cis* olefins. This was accepted in view of the directness of the Wittig approach and the fact that *cis* to *trans* isomerisation of olefins is precedented in the literature (section 4.5.2.3).

Trisubstituted double bond formation

The formation of trisubstituted olefins typically requires modification of the reacting ylid to increase its nucleophilicity. It was hoped that the influence of aqueous and micellar media may alter the reactivity of the unstabilised systems tested here.

Ylid formation

The formation of an ylid of an unstabilised phosphonium salt in aqeuous media was envisaged to be difficult - the pK_a of such a Wittig reagent is typically far too high for proton abstraction by an aqueous compatible base to take place. However, aqueous reaction conditions have been used successfully with unstabilised Wittig reagents[134a,134b] and experience in the research group suggested that micellar media may act promote the formation of unstabilised ylids under aqueous conditions[134c].

Despite the identified deficiencies, it was felt that the directness of the Wittig strategy made investigation worthwhile, as any success would minimise the number of discrete synthetic required to access the target olefinic ester.

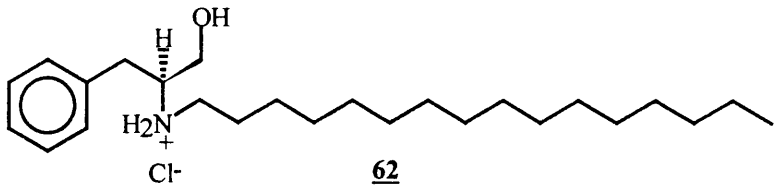
4.5.2.2 Results with unstabilised Wittigs

Organic solvent

The reaction of triphenylphosphonium bromide **23** with the ketoester **24**, however, produced the target decadienic ester **20** in 15% isolated yield, though the reaction was not scalable. ^{1}H NMR analysis indicated that ester **20** was a mixture of *cis* and *trans* isomers in the approximate ratio 1:1. There was insufficient material at this stage to proceed with the biomimetic sequence.

Aqueous and micellar media

'In situ' (preparing the ylid in the presence of the ketone group by basification of the reaction medium) and 'preformed' (external preparation of the ylid prior to reaction with carbonyl compound) methods were attempted to generate the reacting ylid species of **23**. Both 'in situ' and 'preformed' methods were applied to reactions in a surfactant medium (a solution of the surfactant **62** at the CMC)[134d] and an aqueous medium of distilled water.



62

Despite several modifications to the physical conditions (elevated temperature, use/absence of solvent, concentration) of the reaction, in no case was the target ester **20** isolated. In the case of the 'preformed' experiments carried out in aqueous and micellar media, the absence of the deep red colour associated with ylids was strongly indicative that the ylid formation had not occurred under the relatively mild basic conditions.

4.5.2.3 Daylight lamp Irradiation

One reported way of generating exclusively *E*-olefins is through the use of daylight lamp irradiation during the preparation of the olefin, work carried out by Matikanien *et al.*[136] The same group has also reported that the conversion of *Z*-olefins to an *E*- geometry can readily be achieved by a similar photoirradiation, vindicating the use of unstabilised ylids as a direct strategy to the target olefinic ester.

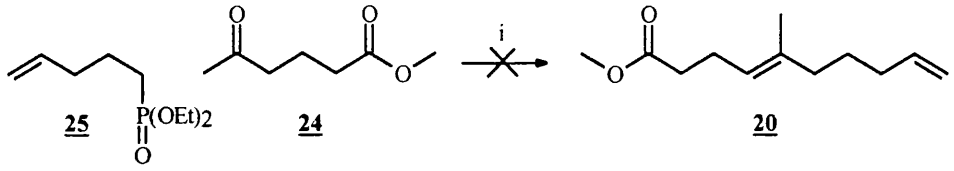
The Matikanien procedure was applied to the system under test, reacting phosphonium salt **23** and methyl 5-oxohexanoate **24** in moist dioxane with sodium carbonate as the base. A control experiment (where all light was excluded from the reaction vessel) was also carried out in tandem.

Both reactions failed to show any of the desired decadienic ester **20** by TLC analysis and subsequent work-up and purification returned the unreacted ketoester **24**.

4.5.2.4 Alternative Wittig reagents - dialkyl phosphonates

The Horner modification[137] was selected to overcome the difficulties encountered with the unstabilised Wittig system. 'Horner-Wittig' methodology utilises the diphenyl phosphoryl group, which directs the course of the separable Horner-Wittig addition and elimination reactions with stereochemcial control.[138] Horner-Wittig olefinations have been successfully applied to the preparation of trisubstituted alkenes[139], though even this pathway can be low yielding[138].

The Horner-Wittig addition reaction between diethyl phosphonate **25** (prepared in 44% by the Arbusov reaction[140]) and **24** was therefore attempted. This returned the unreacted ketoester **24** as the only isolable product from the reaction.



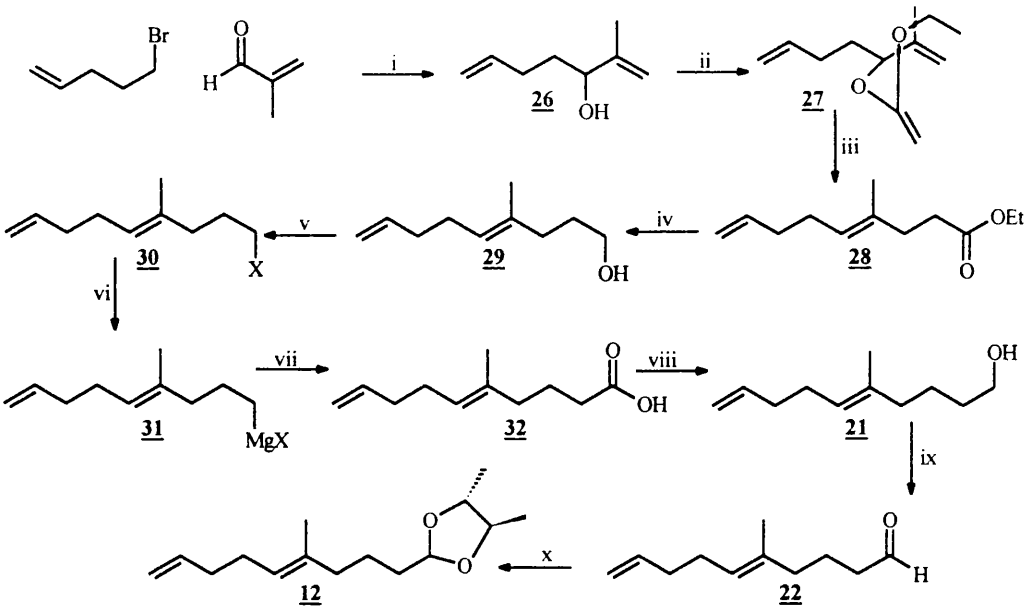
(i):NaH, THF

Scheme 16: Attempted Horner-Wittig Reaction

Despite the failure of the organic system, there has been substantial work into the compatibility of the Horner-Wittig reaction with aqueous systems, with or without phase transfer catalysts, and in mixed solvent and pure water systems.[141,142,143,144] In previous work, carbonate has emerged as a base of choice, reducing the propensity for side reactions to occur.[144] As with organic systems, however, aqueous based Horner-Wittig reactions with ketones are also noted as being particularly low yielding. This is attributed to the reduced electrophilicity of the ketone group *vis-a-vis* aldehydes and the associated steric hinderance. The use of a weak base (such as potassium carbonate) is dependent on the acidity of the methylenic protons in diethyl phosphonate **25** and in the system under investigation, these protons, being otherwise unfunctionalised, were not considered labile enough to be reactive.

4.5.3 Claisen rearrangement route

A third approach to forming the dienic ester precursor was investigated, utilising a version of the Claisen rearrangement to achieve the critical *trans*-trisubstituted olefinic bond in the cyclisation precursor **12** (scheme 17).



Where X = halide

(i):Mg, Et_2O (ii):Ethyl orthoacetate, propionic acid (iii):$LiAlH_4$, Et_2O (iv):$P(Ph)_3$, *N*-bromosuccinimide, THF (v):CO_2, Mg, Et_2O (vi):$LiAlH_4$, Et_2O (vii):D-(-)-(2*R*,3*R*)-butandiol, $CaSO_4.2H_2O$, $BF_3.Et_2O$, THF (viii):$SnCl_4$, benzene

Scheme 17: Claisen rearrangement route to the acyclic precursor

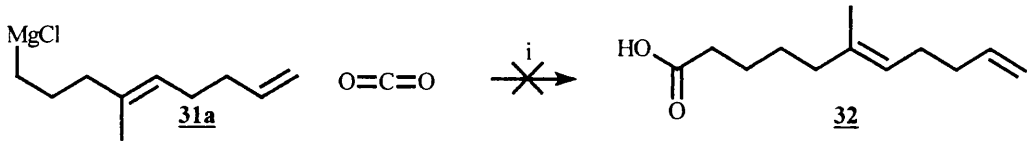
4.5.3.1 Initial steps

A procedure by Trust and Ireland[145] was followed to prepare the *trans* dienic ester **28** in two steps: The allylic alcohol **26** was prepared in 87% isolated yield through a Grignard type coupling of 4-chloro-1-butene and methacrolein. This was heated in triethylorthoacetate to form the allyl vinyl ether **27** *in situ* which underwent rearrangement to the dienic ester **28** in 64% isolated yield. ^1H NMR analysis of this material indicated the presence of exclusively ethyl (4*E*)-4-methyl-4,8-nonadieneoate by the presence of a single singlet at 1.59 ppm (which is reported to correspond to the C-4 methyl group in a *Z*- geometry).[145]

The lithium aluminium hydride reduction of this ester gave the corresponding nonadienic alcohol **29** in 97% isolated yield. This alcohol provided a stock of material for onward conversion to a series of halides for subsequent transformation along the reaction sequence.

4.5.3.2 Chloro routes

Initially the chloro analogue, (*4E*)-1-chloro-4-methyl-4,8-nonadiene **30a**, was prepared by reaction with triphenylphosphine in dry carbon tetrachloride in 60% yield from the corresponding alcohol **29**. Following the proposed reaction scheme, a Grignard type coupling of this halide with dry carbon dioxide was attempted (scheme 18) but found to be unsuccessful, with a recovery of the alkyl halide.



(i):$CO_{2(s)}$, THF

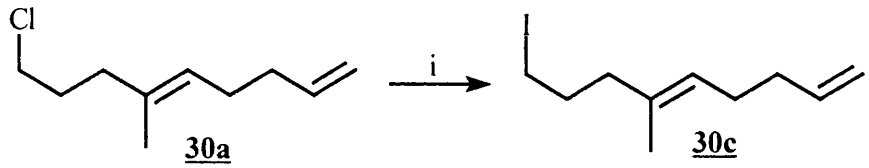
Scheme 18: Attempted Grignard coupling

This was attributed to the relative inactivity of the chlorodiene, failing to form the chloromagnesium Grignard species. This view was supported by the failure of the catalytic iodine to decolourise in the reaction mixture.

Since the stability of organometallic Grignard species can be solvent dependent, a solvent modification - replacement of diethyl ether with tetrahydrofuran - was attempted. Whilst the Grignard reagent was likely to have been formed (as indicated by iodine decolourisation), the excessive heat required to initiate this reaction was sufficient to promote side reactions with no recovery of the desired addition product **32**.

4.5.3.3 Iodo routes

With the objective of preparing a more reactive halide that would form the desired Grignard species under mild conditions, the potentially more reactive iodo analogue of the halodiene **30c** was prepared instead, from the chloride **30a**. This was achieved in 65% isolated yield following the Finkelstein procedure, by displacing chloride with sodium iodide in acetone (scheme 19).[146]



(i):NaI, acetone

Scheme 19: Finkelstein preparation of the iodide

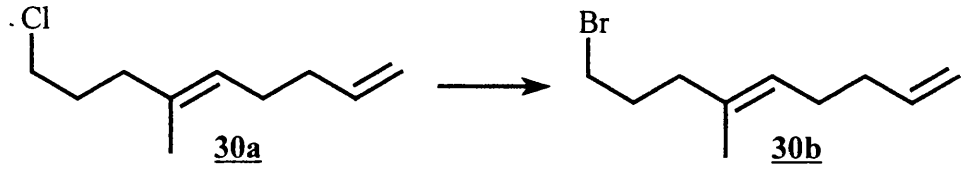
However, this halide also failed to react with magnesium, a possible explanation being the instability of the iodomagnesium species **31c**.

4.5.3.4 Bromo routes

It was logical to assume that the bromodiene would lie between the analogous chloro and iodo derivatives in reactivity such that is would be reactive enough to form a Grignard species with magnesium and yet stable enough to go on to couple with carbon dioxide. Therefore a synthetic strategy to (*4E*)-1-bromo-4-methyl-4,8-nonadiene **30b** was required.

Via the chloride

In attempting to access the olefinic bromide **30b** from the chloride **30a**, a procedure by Ishchenko *et al* was followed which reported that halide exchange between chloride and bromide can be promoted by using sodium bromide in excess under phase transfer conditions (scheme 20).[147]



Scheme 20: Bromide formation from chloride

Triethylbenzylammonium chloride was used as the phase transfer catalyst in a biphasic water-dichloromethane mixture. However the chloride **30a** was recovered unreacted from this procedure.

An alternative approach was provided by Babler and Spina[148], who reported that primary alkyl chlorides can be converted to the corresponding bromide by treatment

with sodium bromide in a mixture of *N*,*N*-dimethylformamide and dibromomethane (with the DMF acting as a chloride scavenger). Application of this procedure to the chlorononadiene system **30a** returned a mixture of the desired bromodiene **30b** and the unexchanged chloro analogue **30a** in the approximate ratio of 1:7. Since the halide exchange was incomplete, it was not possible to utilise the material obtained in any subsequent steps along the proposed reaction pathway and a more efficient route to the bromodiene was still required.

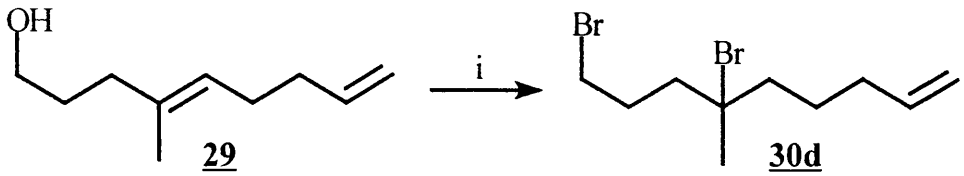
Via the alcohol

The difficulties experienced with halide exchange reactions necessitated the alternative approach of accessing the bromide directly from the parent alcohol, (*4E*)-4-methyl-4,8-nonadiene-1-ol **29**.

Direct conversion of alcohols to bromides is commonly effected by the use of HBr[149], PBr_3[150] or $(Ph)_3PBr_2$[151]. In the first instance, preparation of the bromide **30b** in an analogous way to that of the chloride - *via* a triphenylphosphine/carbon tetrabromide protocol - was attempted, but resulted in an unidentifiable product and an unreconcilable mass balance for the reaction. It had been necessary to employ the use of a solvent to solubilise the substrates and it was highly likely that the selection of tetrahydrofuran had resulted in side reactions taking place.

A procedure of Hwa and Sims[152], who looked at the bromination of hexadiene, was followed employing a combination of phosphorus tribromide and hydrobromic acid as the brominating agent. However, NMR analysis of the isolated product suggested that dibromination had occurred, adding hydrogen bromide across the trisubstituted double bond (scheme 21).

Olah *et al* developed the use of halotrimethylsilanes, generated *in situ*, for the conversion of alcohols to the corresponding halides. This approach was applied to the current system using chlorotrimethylsilane and lithium bromide to generate the brominating agent, bromotrimethylsilane.[153] However, the product of this reaction was, again, identified to be the dibrominated compound, 1,4-dibromo-4-methyl-8-nonene **30d**.



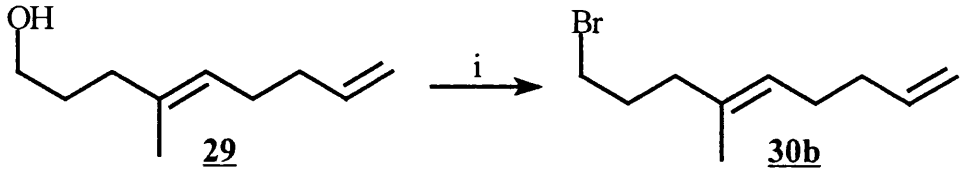
29 30d

(i):PBr_3, HBr or $SiMe_3Cl/LiBr$

Scheme 21: Dibromide formation

Sodium carbonate and triethylamine, as mild bases, were employed in an attempt to dehydrobrominate the tertiary bromide without displacing the primary halide. This approach was unsuccessful and probably ill-founded, since there would have been no stereocontrol in the resulting diene (negating the efforts to ensure a *trans* diene relationship).

As an alternative, a different brominating reagent - *N*-bromosuccinimide - was tried in combination with triphenylphosphine following a procedure by Schweizer *et al* (scheme 22).[154]



29 30b

(i):*N*-bromosuccinimide, THF, $P(Ph)_3$

Scheme 22: Bromide formation from the alcohol

This returned the desired monobromide, (*4E*)-1-bromo-4-methyl-4,8-nonadiene **30b**, albeit in low (30%) isolated yield. Despite making procedural variations, such as altering the reaction time, order and rate of addition, this yield could not be improved upon and it was necessary to accept this inefficiency to be able to drive the project towards the next step in the proposed reaction sequence.

4.5.3.5 Grignard routes

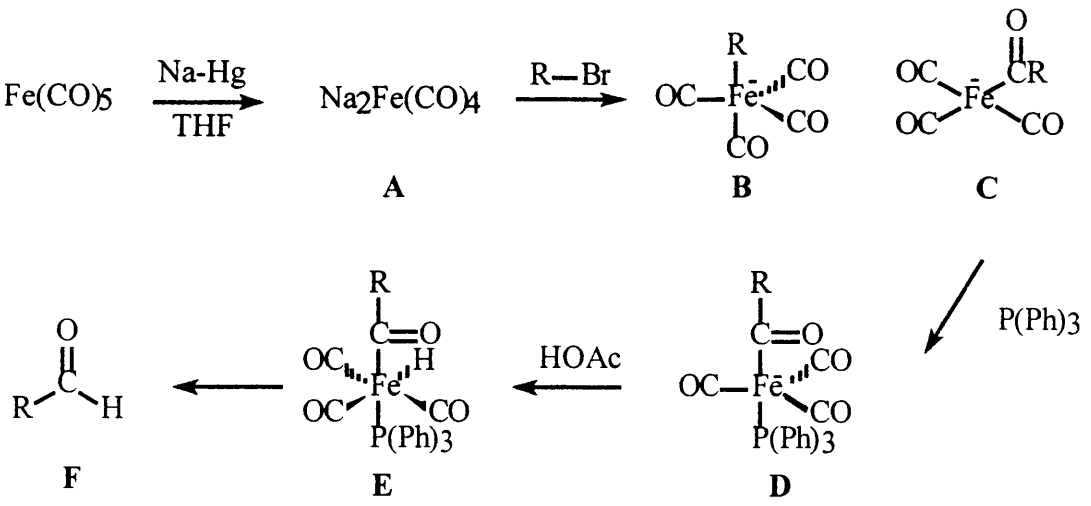
It was possible that the failure of the chloro- and iodo- dienic Grignard reagents (**30a** and **30c**) to react may have been due, in part, to the acceptor selected. A slurry

of solid carbon dioxide and tetrahydrofuran was used at low temperatures which could easily have acted as a moisture trap, quenching any Grignard species instantly.

In attempting a similar Grignard reaction with the olefinic bromide **30b** obtained, sensible modifications would be to bubble gaseous carbon dioxide into the reactor (having first dried the gas over molecular sieves), or to use of more reactive acceptors (such as ethyl chloroformate). However, the single addition of the alkyl magnesium halide species **31** to ethyl chloroformate would be difficult to control and successive nucleophilic substitutions could take place. Furthermore, even with the successful execution of the Grignard step, three further synthetic steps (reduction, oxidation, acetalisation) would still be required to access the cyclisation precursor **12**.

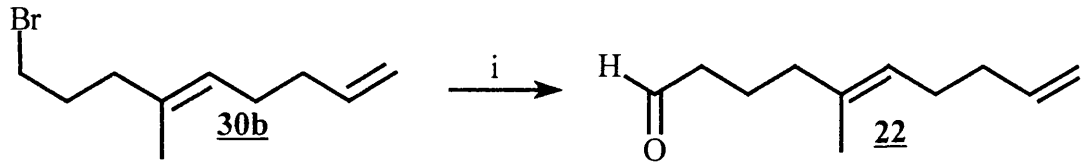
4.5.3.6 Metal carbonylation

A more direct route to the homologated aldehyde **22** was selected - the direct formylation of the alkyl bromide, (*4E*)-1-bromo-4-methyl-4,8-nonadiene **30b**[155]. This approach involved the use of the d^{10} anion from iron pentacarbonyl as the reagent for insertion of a formyl group into the alkyl halide, with displacement of the halogen. The mechanism by which the reaction is believed to proceed is stated in scheme 23:



Scheme 23: Proposed mechanism of carbonylation

For the nonadiene system under investigation, the proposed transformation to be effected by this methodology was therefore the synthesis of the aldehyde, (5*E*)-5-methyl-5,9-decadienal **22**, with the advantage that its success would shorten the overall sequence to the target acetal by combining the homologation-reduction-oxidation steps into a one-pot conversion of the bromononadiene **30b** into the decadienal **22** (scheme 24).



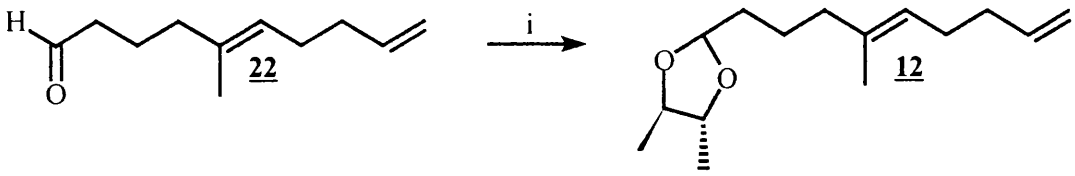
(i):$Na_2Fe(CO)_4$, THF, $P(Ph)_3$, N_2

Scheme 24: Direct formylation of **30b**

In practice, this transformation was achieved smoothly in a 74% isolated yield of the aldehyde **22**, by the reaction of commercially available disodium tetracarbonyl ferrate with the prepared bromononadiene **30b** under an inert atmosphere of nitrogen. This was achieved without disruption to the double bond systems, showing that such methodology may be applied to formylation of olefinic systems of the type used.

4.5.3.7 Acetal formation

The derivatisation of (5*E*)-5-methyl-5,9-decadiene-1-al **22** to the optically active acetal **12** was carried out using a sample of optically pure D-(-)-(2*R*,3*R*)-butandiol **5b** obtained through the fermentation of glucose by the bacteria *Bacillus subtillis* (the details of the study being given in Chapter 2). The protection was carried out under Lewis acid conditions, with physical removal of the water generated using anhydrous calcium sulphate (*Drierite*®). The target cyclisation precursor **12** was thus isolated in 77% isolated yield (scheme 25).

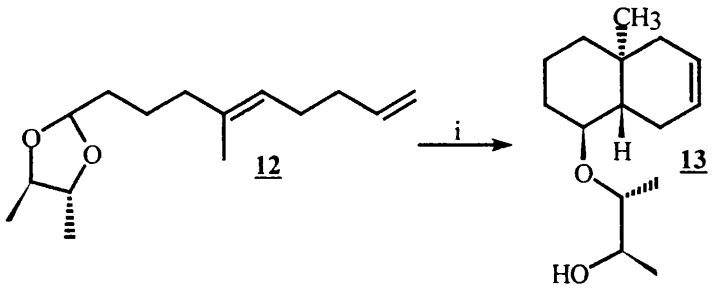


(i):*Drierite*®, THF, $Et_2O.BF_3$, 0 °C

Scheme 25: Acetal formation

4.6 Biomimetic cyclisation

Having obtained a quantity of the olefinic cyclisation precursor **12**, the aim of the final step of the sequence was to study the degree of asymmetric induction exerted by the terminal optically active butyl acetal group on the stereochemistry at the ring junction of the resulting bicyclic octalin system. This objective was carried out according to experimental conditions followed in the initial inspiring paper, performing the cyclisation in anhydrous benzene, with the aid of stannic chloride as a Lewis acid catalyst (scheme 26).[121]



(i):Benzene, $SnCl_4$, rt

Scheme 26: Biomimetic cyclisation step

After work-up and isolation, the major product of the reaction was identified to be exclusively *trans* fused (1*S*,4a*R*)-1,2,3,4,4a,5,6,8,8a-octahydro-4a-naphthalene-1-yl-((1*R*',2*R*')-2'-hydroxy-1'-methylpropyl) ether **13**. This assignment was made on the basis of the position in the NMR spectrum of the singlet corresponding to the methyl group at the ring junction (1.12 ppm) and by correlation with analogues reported in the literature.[121,177] The hydroxy ether moiety was similarly assigned to be in the equatorial position.

^{1}H and ^{13}C NMR analyses were indicative of a single diastereoisomer present. Chiral HPLC was used to confirm that selectivity had taken place during the cyclisation and the Chiralcel OB column, which has been shown to separate decalone enantiomers bearing structural similarity to the octalin **13**[155a], was selected for the analysis. The observance of a single spectral peak when the hydroxyether **13** was subjected to chiral HPLC analysis therefore supported the view that a very high optical purity had been achieved.

This result indicated a high degree of steric control exerted by the chiral acetal handle. Consistent with the principles expounded by Stork[110] and Eschenmoser[111], the following transition state was envisaged to account for the assigned stereochemistry in this cyclisation step:

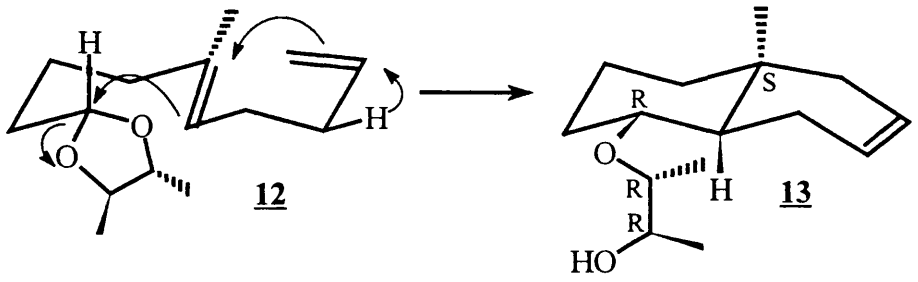


Figure 15: Preferred cyclisation intermediate

4.7 Conclusions of the chapter

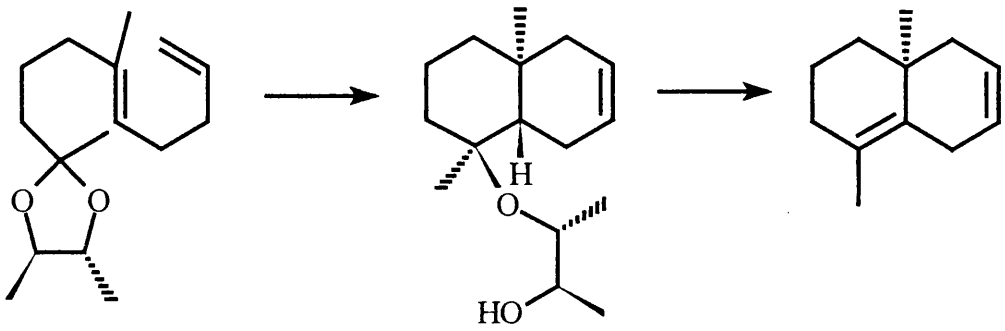
This study has demonstrated that optically active acetals derived from butan-2,3-diol are effective chiral handles with which to effect directed self-cyclisation reactions with considerable asymmetric induction. The use of a suitable decadienic system provides a useful and novel way to access the octalin backbone of the target molecule in this chapter, dehydrogeosmin.

4.8 Future work

Implicit in this work is the equivalence of both enantiomeric forms of butan-2,3-diol in their effectiveness as chiral handles during the cyclisation process. Since both isomers are available (through the work described in chapter two), the methodology tested could be repeated to access the opposite enantiomeric form of

the octalin skeleton. This would assist in confirming the direction of the cyclisation of **12** carried out in this study.

In the context of a dehydrogeosmin synthesis, the cyclisation of a precursor bearing greater similarity to the target molecule could be tested. This would logically involve a ketal in the key cyclisation step (scheme 27):



Scheme 27: Proposed cyclisation of a dehydrogeosmin precursor

CHAPTER FIVE: DIELS-ALDER STRATEGIES

5.0 Introduction

The Diels-Alder reaction has, since its discovery in 1928[156], become one of the most important reactions in synthetic organic chemistry. The reaction is now understood to be an orbital controlled [4π+2π] cycloaddition between an alkene (dienophile) and a conjugated diene, for which orbital symmetry rules require that the stereochemistry of the addition be *cis* with respect to the diene and dienophile (figure 16).[157]

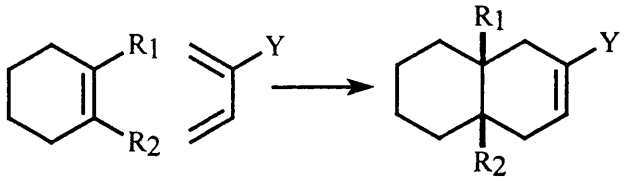


Figure 16: Stereochemistry of Diels-Alder Reactions

The utility of the reaction in organic synthesis comes from the remarkable selectivity that may be achieved, with the simultaneous and regioselective formation of two C-C bonds and the creation of up to four chiral centres at the addition site with predictable stereochemistry. The reaction has therefore been extensively reviewed [158,159] and it is not the intention here to summarise the enormous effort that has gone into exploring every aspect and application of this methodology.[160]

Molecular orbital theory explains the essence of this cycloaddition as being a donation of electrons from the **H**ighest **O**ccupied **M**olecular **O**rbital (HOMO) in the diene to the **L**owest **U**noccupied **M**olecular **O**rbital (LUMO) in the dienophile in a thermally allowed process. Whether the reaction proceeds, or not, is therefore determined by the energy separation between these interacting orbitals.

This requirement for comparable orbital energies explains why the Diels-Alder reaction typically involves a diene with electron releasing substituents and a dienophile with electron withdrawing substituents. In the former, both the HOMO

and LUMO are raised in energy, in the latter, both HOMO and LUMO are lowered. These effects work in cooperation with each other, the net effect being to significantly reduce the energy separation between the diene HOMO and the dienophile LUMO and therefore the propensity to interact to form the cycloadduct.

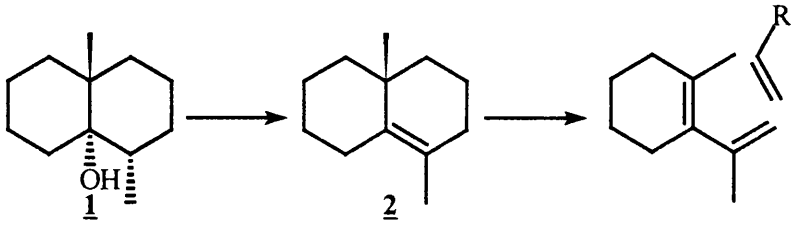
Cases exist where the electron donating group appears in the dienophile and the electron withdrawing group in the diene. In these cases the energy differences are reversed and it is the LUMO of the diene and the HOMO of the dienophile which are brought sufficiently close together in energy to allow a reaction to occur. Such systems are termed as reverse electron demand.

5.1 Aims

The six membered carbon rings that make up the backbone of the bicyclic skeletons of both geosmin and dehydrogeosmin would appear to be ideal candidates for construction *via* a Diels-Alder strategy. Yet, to our knowledge, there is no reference in the literature to such an approach to form these target compounds. The work in this chapter therefore describes an investigation into using the rapidity and stereoselectivity of the [4π+2π] cycloaddition as a route to the formation of key octalin intermediates for onward manipulation to geosmin and dehydrogeosmin.

5.2 Strategies to geosmin

Retrosynthetically, argsomin may be viewed as a Diels-Alder adduct, formed from the cycloaddition of 1-isopropenyl-2-methyl-cyclohex-1-ene and an ethene derivative (scheme 28).



Scheme 28: Diels-Alder disconnection of geosmin

There are no reported strategies to geosmin that utilise this disconnection yet such a system may be expected to react readily, since it is reported that alkenes may be

more reactive when the diene moiety is exocyclic, rather than endocyclic.[161] Furthermore, ethene derivatives are numerous and through the introduction of electron withdrawing groups (such as ketones ($R=CHOR_1$) or esters ($R=CO_2R_1$)), this dienophile may be manipulated to react more favourably with different dienes.

In evaluating the Diels-Alder reaction in the context of a synthetic route to geosmin, the strategy employed in this project was to design a model system for argosmin with which to test the general feasibility of the cycloaddition approach, before applying it to an argosmin precursor.

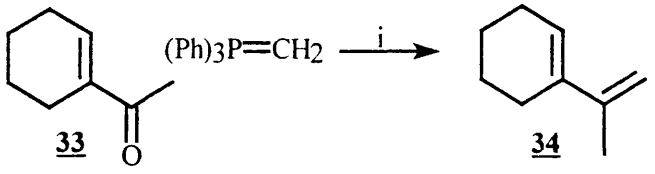
Methyl acrylate was selected as the dienophile in every diene-dienophile system investigated, owing to its reactivity in cycloaddition reactions. This arises from the electron withdrawing characteristics of the ester functionality, activating the olefinic system towards cycloaddition reactions (in line with molecular orbital rationalisation set out in section 5.0). Furthermore this dienophile is not sterically crowded at the alkene moiety. As part of the study, modifications were also made to both the model and argosmin diene systems, with a view to increasing their reactivity.

5.3 Argosmin model system

The model diene-dienophile system was based on the commercial availability of 1-acetyl-1-cyclohexene as the precursor to the exocyclic diene **34**. This was selected for its similarity to the diene required for the true argosmin type system, 1-isopropenyl-2-methyl-1-cyclohexene **37**, lacking only a ring methyl group at the terminus of the olefinic system.

5.3.1 Diene synthesis

Diene **34** was prepared in a 30% isolated yield by the Wittig olefination of 1-acetyl-1-cyclohexene with triphenylphosphonium bromide (scheme 29)[162].



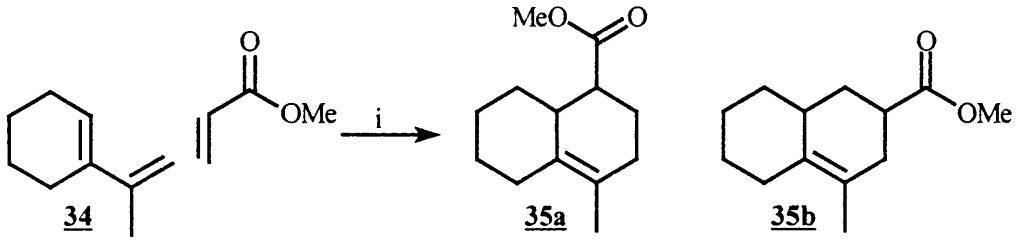
(i):*n*-BuLi, Et_2O

Scheme 29: Diene synthesis – model system

The yield obtained was consistent with literature preparations of cyclohexanyl olefins, though it is reported that use of the methanesulfonyl carbanion as a base, formed from NaH and DMSO, can increase this to ~70%.[163]

5.3.2 Diels-Alder reaction

The Diels-Alder cycloaddition step was attempted, initially without the aid of catalysis, in dichloromethane as the solvent. However, in common with a study by Mayelvaganan *et al* (who investigated the cycloaddition of vinylcyclohexenes with conjugated ketones), the reaction only proceeded in the presence of a Lewis acid catalyst (aluminium trichloride) (scheme 30).[164]



(i):$AlCl_3$, DCM

Scheme 30: Diels-Alder reaction – model system

The cycloadduct **35** was formed in 70% isolated yield and identified as a mixture of the 1- and 2-methylcarboxylate isomers **35a** and **35b** in the approximate ratio of 5:1 (determined by the relative intensities of the corresponding methoxy signals at 3.66ppm and 3.60ppm in the ^1H NMR spectrum), with a single stereoisomer (presumed to be the *endo* adduct) dominating.

The presumed regio- and stereochemical preference for the 1-methylcarboxylate isomer of **35a** was rationalised by an examination of the electronic characteristics of the system[158] and the *endo* preference exhibited by Diels-Alder reactions (due to secondary orbital overlap between the diene and dienophile) (figure 16):

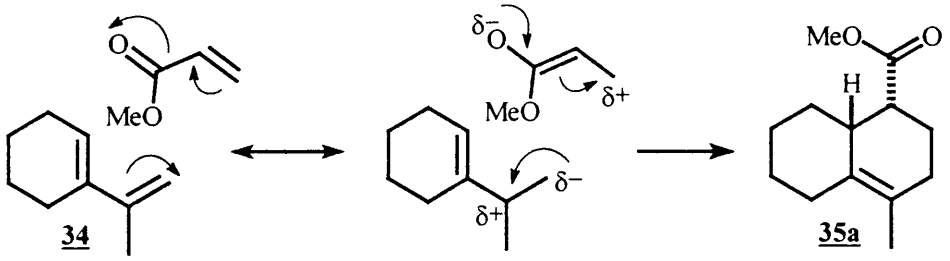


Figure 16: Rationalisation of stereochemistry - model system

It is noteworthy that the cycloadduct **35**, as a mixture of isomers, possessed a *sweet, fruity* odour. This suggested that through simple modification of the dienophile (for example, reacting with the ethyl acrylate), a whole range of potentially interesting fragrance chemicals could be generated. The existence of any such chemicals in nature would also allow their evaluation as potential flavour ingredients.

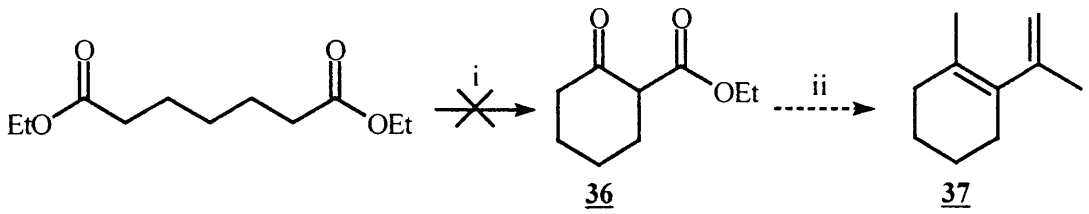
5.4 Argosmin system

The success of the argosmin model system (scheme 30) suggested that, by modifying the exocyclic diene appropriately, the Diels-Alder cycloaddition could be applied to the synthesis of an argosmin precursor. The target diene required was the methylated analogue **37** and three synthetic strategies were identified for its preparation:

1. *Via* a Dieckmann condensation of an appropriate acyclic diester,
2. *Via* a Grignard coupling onto an appropriate cyclohexenone,
3. *Via* an appropriate exocyclic acetylcyclohexene.

5.4.1 Dieckmann strategy

The first route to the diene to be investigated was the Dieckmann condensation of diethyl pimelate[165], followed by the addition of a methyl organometallic reagent with subsequent dehydration to the diene (scheme 31).



36 37

(i): *see* table 10 (ii): Methylmagnesium bromide, THF

Scheme 31: Attempted Dieckmann sequence

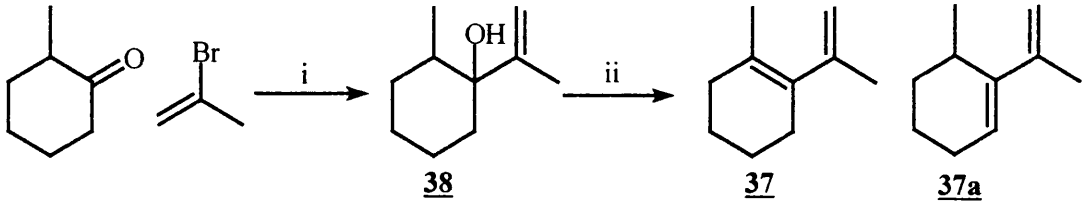
Base	Solvent	Conditions
Na	toluene	reflux
NaOH	MeOH	reflux
KOH	toluene	water removal

Table 10: Attempted Dieckmann conditions

Several attempts were made to generate **36**, using different base-solvent combinations, as shown above. NMR and MS analyses did show the presence of the desired exocyclic ester but the efficiency of the reaction, under the regimes attempted, was insufficient to warrant any further work into this route. This was particularly valid, given that the subsequent Grignard addition steps could not be assumed facile.

5.4.2 Grignard coupling

A second route to the target diene **37** was investigated whereby a metal facilitated addition of 2-bromo-1-propene to 2-methylcyclohexanone was applied to generate a tertiary alcohol that could be dehydrated to the thermodynamically most favoured, and the desired, diene (scheme 32).



38 37 37a

(i):Mg, I_2, Et_2O (ii):Toluene, orthophosphoric acid

Scheme 32: Attempted Grignard sequence

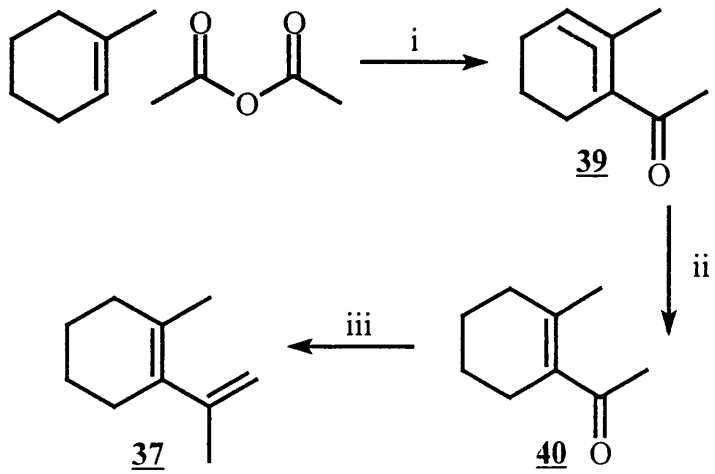
The Grignard reaction produced a mixture of both the hydroxyl alkene **38** and the diene **37** and **37a** in a 63% combined yield. However, aqueous acid dehydration was insufficiently selective and a mixture of both the thermodynamic (**37**) and kinetic (**37a**) dienes was generated.

The preparation of the hydroxyl alkene **38** was also investigated under aqueous conditions, applying the methodology of Einhorn and Luche to the allylation of carbonyl compounds.[166] For the purposes of this investigation, tin and zinc were used as the metal surfaces and sonication conditions employed in the case of tin.

In both cases, the reaction did not proceed and starting material was isolated after the experiment. Einhorn and Luche's study found a broad tolerance to a range of carbonyl substrates but the reactivity of different allyl halides was more restricted.[166] The failure of this system was therefore attributed to the parent halide, 2-bromopropene, which, being a vinyl halide, would generate a radical inherently less stable than the allyl radicals investigated in the Einhorn and Luche study.

5.4.3 Exocylic acetylcyclohexenone approach

The third strategy to prepare the diene **37** was *via* the acetylation of 1-methyl-1-cyclohexene with subsequent double bond migration and olefination of the corresponding exocyclic ketone **40** (scheme 33).



(i):$ZnCl_2$, ZnI_2, Ac_2O (ii):Al_2O_3 (iii):$MeP(Ph)_3^+Br^-$, *see* table 11

Scheme 33: Synthesis of argosmin precursor

The procedure of Hudlicky and Srnak, who looked into the aluminium oxide catalysed isomerisation of acylated cycloalkenes[167], was used to prepare 1-acetyl-2-methyl-1-cyclohexene **40**. Acetic anhydride/zinc chloride is an effective acylating system for cyclic alkenes but gives rise to a mixture of conjugated and deconjugated enones, even after base catalysed isomerisation.[168] By performing the isomerisation on a surface of neutral aluminium oxide, Hudlicky and Srnak isolated solely the conjugated enone quantitatively from the isomeric mixture. Using this approach, intermediate **40** was prepared in 60% isolated yield from the parent alkene.

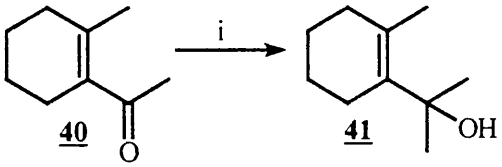
Olefination of the acetyl group was initially investigated through application of Wittig methodology, using the commercially available phosphonium salt, methyltriphenylphosphonium bromide. The base/solvent combinations tried, without success, are listed in table 11:

Organic Media	n-BuLi/THF
	NaH/Et_2O
	$n\text{-BuLi}/Et_2O$
	NaH/DMSO
Aqueous Media	$NaOH/H_2O$

Table 11: Attempted base/solvent conditions

In no case was the desired diene positively identified and the starting material (2-methyl-1-acetyl-1-cyclohexene **40**) was recovered unreacted. Since the olefination of the unmethylated analogue, 1-acetyl-1-cyclohexene **34**, had proceeded smoothly, this indicated that the inactivity with **40** did not arise from difficulties in the formation of the methyltriphenylphosphonium ylid. The failure of this system to react was therefore attributed to an unexpectedly strong steric effect, caused by the ring methyl group, blocking the approach of the ylid to the carbonyl group.

A second approach to the target diene **37** from the exocyclic ketone **40** was *via* the synthesis of the corresponding tertiary alcohol through a Grignard type addition of methylmagnesium bromide. The resulting alcohol would then be available for dehydration to the desired conjugated diene (scheme 34).

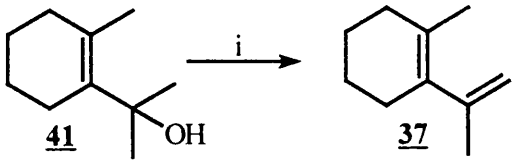


(i):MeMgBr, Et_2O

Scheme 34: Tertiary alcohol formation

The Grignard addition step was carried out successfully in an 82% crude yield. Further purification was not necessary and the alcohol **41** was dehydrated immediately. It was noted that **41** possessed a strong *minty, earthy* odour, very similar to that of geosmin.

The dehydration was attempted under a range of conditions, again investigating organic and aqueous mono- and biphasic media (scheme 35):



(i):*see* table 12

Scheme 35: Tertiary alcohol dehydration

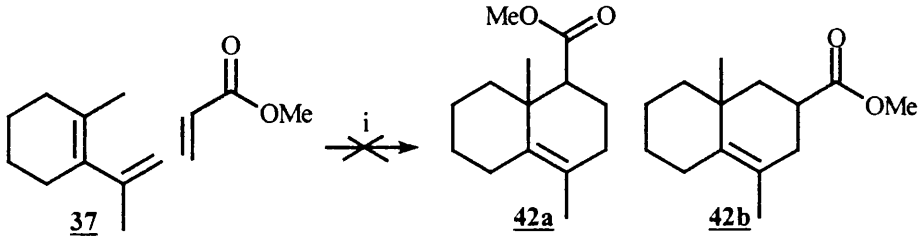
Aqueous	H_2SO_4/H_2O
Biphasic	Catalytic H_2SO_4/THF/H_2O
Organic	H_2SO_4/DCM
	Catalytic H_2SO_4/DCM

Table 12: Attempted dehydration conditions

The biphasic THF/H_2O system was found to be most efficacious with an isolated yield of the diene **37** of 50%. Efforts to increase the isolated yield through extension of the reaction time were unsuccessful since, after 3 hours, TLC analysis of the reaction mixture indicated the formation of by-products. These were most likely to arise from acid catalysed isomerisations, or as a result of self-condensation.

5.4.4 Diels-Alder reaction

The Diels-Alder reaction with methyl acrylate was attempted in dichloromethane, at room and reflux temperatures, and in the presence and absence of aluminium trichloride as a Lewis acid catalyst (which had been successful in the model system, section 5.3.2) (scheme 36).



(i):$AlCl_3$, DCM or DCM, heat

Scheme 36: Attempted Diels-Alder reaction – argosmin system

In no case were the desired cycloadducts **42a** and **42b** detected; mixtures of starting materials and self-condensation products of methyl acrylate were isolated.

This was disappointing, in view of the success of the model system, and attributed to the presence of a methyl group at the end of the diene system in intermediate **37** (absent from the corresponding diene **34** in the model system), blocking the approach of the dienophile during the cycloaddition.

The presence of this methyl group alone, at the emergent ring junction of the cycloadduct, was not thought sufficient to explain its steric influence since the Mayelvaganan study[164] successfully employed dienophiles of this type. However diene **37** possesses a second methyl group as part of the exocyclic isopropene group, exerting its own steric influence in that region of the molecule. When the role of the Lewis acid catalyst is taken into consideration, the opportunity for sufficient orbital overlap between the approaching methyl acrylate dienophile and diene **37** to permit a reaction to occur is extremely limited.

5.5 Diene modification

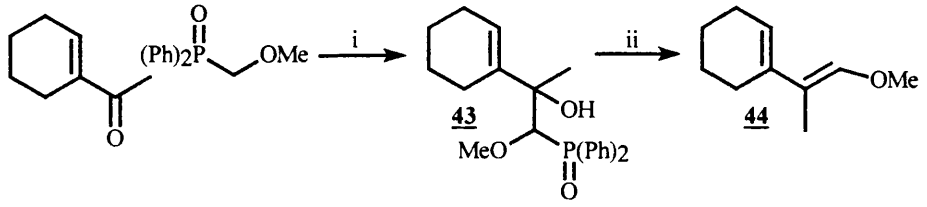
Cycloadditions involving acrylate esters, as electron withdrawing dienophiles, can be expected to react more readily with electron rich dienes since, as explained in

section 5.0, the combined effect is to bring the levels of the reacting HOMO of the diene and LUMO of the dienophile closer together in energy.

With the systems under test in this study, an example of such an activated diene is the synthetic equivalent of a conjugated vinyl ether. It was therefore decided to investigate the preparation and reactivity of this type of diene, and to consider whether their reactivity would be sufficient to form cycloadducts without the need of Lewis acid catalysts. This was of particular value in the argosmin system, where the unactivated diene had failed to undergo cycloaddition.

5.5.1 Model system - activated diene

The use of activated dienes was again investigated with the model system first, through formation of the methyl vinyl ether of 1-acetyl-1-cyclohexene **44**. This was achieved in 32% yield over two steps through a Horner-Wittig type coupling with methoxymethyldiphenyl phosphine oxide, applying standard procedures (scheme 37).[169]

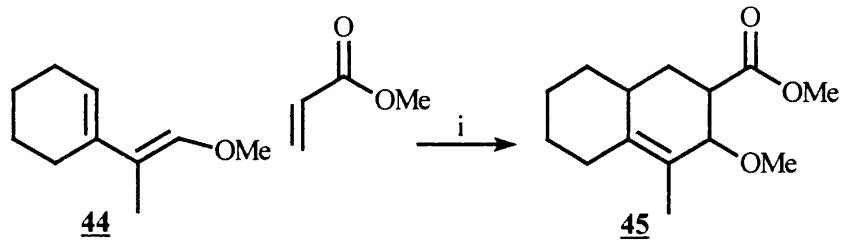


(i):LDA, THF (ii):NaH, THF, rt

Scheme 37: Horner-Wittig preparation of activated model diene

Accordingly, the tertiary alcohol adduct **43** was formed in 63% yield from the addition of methoxymethyldiphenylphosphine oxide to 1-acetyl-1-cyclohexene, preforming the ylid through deprotonation with lithium diisopropylamine as the base. The adduct was then transformed to the vinyl ether **44**, in 50% isolated yield, by treatment with a single equivalent of sodium hydride base. This reaction is understood to proceed stereospecifically, with a single diastereoisomer of the ether being formed.[169]

The diene **44** was considered electron donating in character, owing to the mesomeric effect of the methoxy group at the terminal position of the diene group and therefore compatible with methyl acrylate, an electron deficient dienophile (scheme 38).

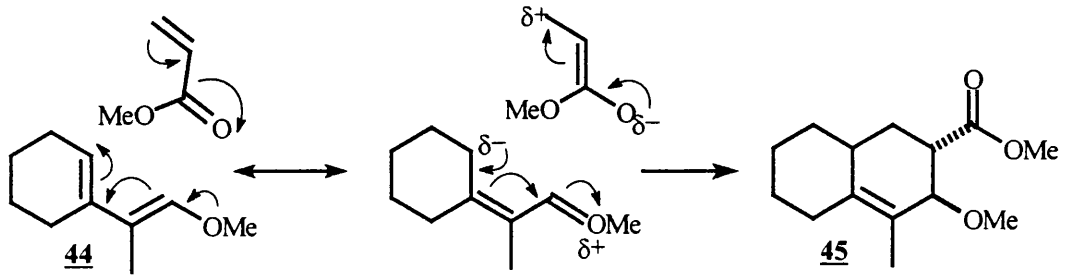


44 **45**

(i):DCM

Scheme 38: Diels-Alder reaction - activated model system

This premise was shown to be valid, borne out by the rapidity with which the cycloadduction occurred, without the need for external heating. The reaction showed good selectivity and the cycloadduct **45** was obtained in 40% isolated yield. A single methyl ester signal at 3.67 ppm in the ^{1}H NMR spectrum suggested the product was regio- and stereochemically pure. By a similar rationalisation to that made for the unactivated model diene system[158], the predominant isomer was assigned to be the 2-methylcarboxylate cycloadduct (figure 13):

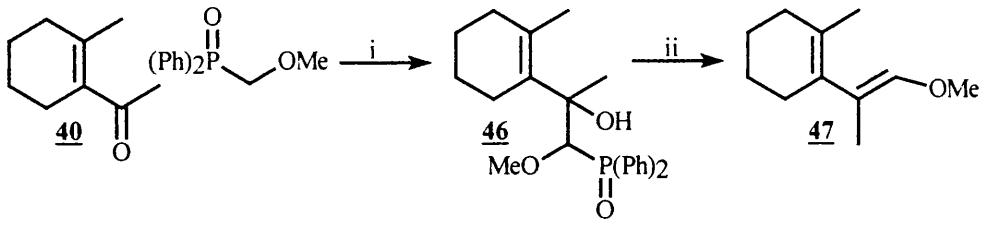


44 **45**

Figure 13: Rationalisation of stereochemistry - activated model system

5.5.2 Argosmin system - activated diene

The corresponding methyl vinyl ether **47** was selected as the modified diene for the argomsin system (scheme 39).



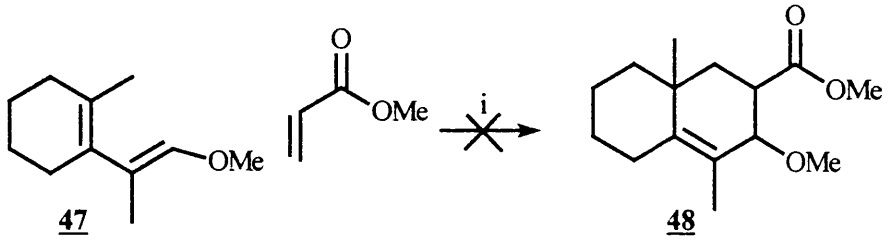
(i) and (ii):LDA, THF

Scheme 39: Synthesis of activated diene – argosmin system

In the first instance, the diene **47** was prepared applying identical procedures to those of section 5.5.1, through a standard Horner-Wittig type coupling[169] between methoxymethyldiphenyl phosphine oxide and 1-acetyl-2-methyl-1-cyclohexene **40**.

The methoxy diene **47** was obtained in a single step in 26% yield, without the isolation of the intermediate **46** (contrary to the experience with the corresponding unmethylated analogue **43** in section 5.5.1). Whilst not high yielding, this procedure provided sufficient material with which to test the cycloaddition step.

In attempting the cycloaddition between the isolated diene **47** and the selected dienophile, methyl acrylate, both the presence and absence of aluminium chloride as a Lewis acid catalyst were tried (scheme 40).



(i):$AlCl_3$, DCM or DCM, heat

Scheme 40: Attempted Diels-Alder reaction – activated argosmin system

Despite prolonged reaction times and the application of external heating, TLC analysis failed to indicate that any reaction had occurred.

5.6 Steric influence of the methyl group

The failure of the cycloadditions involving both **37** and **47**, *vis-a-vis* the unmethylated analogues **34** and **44**, was rationalised in terms of the methyl group at the terminus of the olefinic system blocking the approach of the methyl acrylate dienophile and preventing orbital overlap from occurring.

5.7 Future work

The precedent of Mayelvaganan *et al*[164] is strongly indicative that Diels-Alder cycloadditions involving cyclohexanyl dienes can proceed to generate bicyclic systems with methyl groups at the ring junction. It is therefore likely that conditions and catalysts exist that are compatible with the systems studied in this chapter and that the desired adducts **42** and **48**, manipulable to geosmin, may be prepared *via* such a Diels-Alder strategy. Future work would therefore focus on identifying appropriate conditions to effect these reactions.

Transformation to geosmin

Use of methyl acrylate as the dienophile leaves an exocyclic ester group that must be removed if the adduct is to be transformed into argosmin (for which onward procedures to geosmin in two discrete steps exist[4,8,10,10a]).

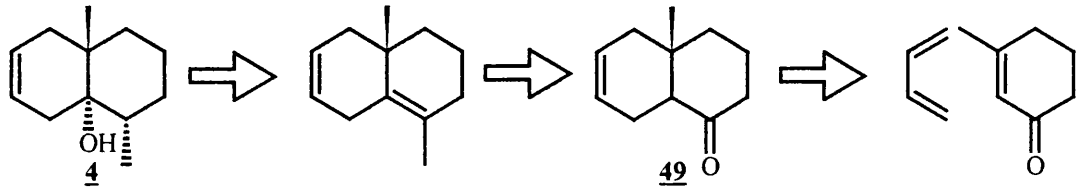
One suitable way of effecting this transformation is by decarboxylation of the corresponding carboxylic acid. This could be achieved *via* a radical initiated reduction of the acid chloride[170], or the ester of *N*-hydroxypyridine-2-thione[171].

Asymmetric induction

The Diels-Alder investigations in this chapter have not addressed the question of a chiral synthesis of geosmin, for which it is crucial to set the stereochemistry of the methyl group at the ring junction with absolute stereospecificity. The approach identified – a cycloaddition using an acrylic ester system as a dienophile - lends itself very well to asymmetric functionalisation and this strategy has been reported to effect asymmetric Diels-Alder reactions with good enantioselectivity. Examples of successful acrylic ester functionalisations[172] used in this way include Evans chiral oxazolidinones[173] and camphor based esters[174].

5.8 Routes to dehydrogeosmin

The Diels-Alder strategies investigated thus far have looked to synthesise a octalin skeleton that may be manipulated to argosmin, and onward to geosmin. In chapter four, where biomimetic strategies towards dehydrogeosmin were studied, the key precursor to this target molecule was the octalone **49** - 4a-methyl-3,4,4a,5,8,8a-hexahydro-2*H*-naphthalen-1-one - which, when disconnected, could also be accessible *via* a [4π+2π] cycloaddition (scheme 41).



Scheme 41: Diels-Alder disconnection of dehydrogeosmin

The simplest disconnection of target octalone **49** shows it to be the adduct of 1,3-butadiene and 3-methyl-1-cyclohexanone. This cyclic ketone, however, is reported to be completely non-reactive with acyclic ketones under both thermal and Lewis acid cycloaddition conditions.[175,176] This inactivity may be attibuted to the presence of the methyl group at the end of the end of the olefinic system, exerting both steric and electronic effects: As was seen with the geosmin type systems in section 5.6, the presence of any group other than hydrogen at the emerging ring junction position acts to block the approach of the diene to the dienophile. Furthermore the electron donating character of this alkyl group acts against the electronic withdrawing effect of the ketone functionality, deactivating this dienophile.

Work carried out by Aben *et al*, however, suggested that a combination of heat, catalysis and externally applied pressure, can achieve some success in promoting Diels-Alder cycloadditions with this unreactive dienophile.[177]

A second experimental modification to improve the reactivity of methylcyclohexenone systems towards Diels-Alder reactions comes from the use of highly polar reaction media. Lithium perchlorate in diethyl ether was shown by Sauer and Braun to accelerate Diels-Alder reactions with a polar transition state and

to elicit *endo* selectivity in the cycloaddition.[177a] This acceleration was attributed to an effect similar to that seen with water, whereby 'internal pressure' of the solvent causes a compression of the reactants together.[72]

Grieco utilised lithium perchlorate etherate solutions to accelerate and promote cycloadditions that would otherwise not proceed under normal conditions.[178,179] By derivatisation to the ketal, it was found that even the unreactive 2-methyl-2-cyclohexenone could be made to undergo Diels-Alder cycloaddition reactions in good yield. Dailey *et al* speculated that the acceleration due to lithium perchlorate was not a solvent effect but 'lithium ion induced', whereby the lithium cation acted as a Lewis acid catalyst.[179a] On this basis, Grieco postulated that the ketal cycloaddition was occurring *via* an ionic dienophile, the lithium perchlorate solution catalysing the formation of an oxygen stabilised cation (figure 14).[180]

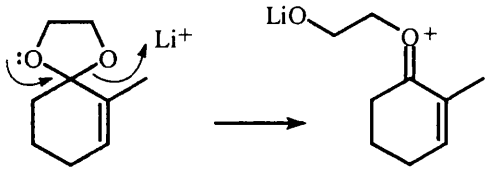


Figure 14: Postulated oxygen stabilised cationic dienophile

5.9 Strategy

The objective of this part of the project was therefore to investigate the use of the Diels-Alder cycloaddition in the synthesis of octalone **49**, or a derivative of it, to provide access to this key dehydrogeosmin precursor. To achieve this, the strategy was to extend the Grieco study[180] to the 3-methyl-2-cyclohexenone system, which has not been investigated in the context of lithium perchlorate catalysed cycloaddition reactions.

Given the availability of an optically pure butan-2,3-diol (through the fermentation and enzymatic studies described in chapter two), it was also the objective of this work to examine whether an asymmetric modification to this reaction would be possible. It was envisaged that the use of an optically pure ketal would influence the stereoselectivity of the cycloaddition, the extent to which this would occur depending on the lifetime of the proposed allyl cation intermediate. Were the cation

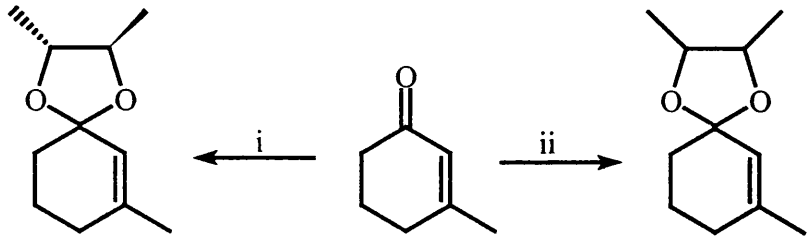
to exist for a short period, the asymmetric centres would be relatively close to the site of addition. A longer lifetime for the cation would allow rotation of the chiral groups away from the addition site, diminishing their influence.

5.10 Model systems

For the purposes of evaluating the use of lithium perchlorate solution in Diels-Alder reactions of this type, it was decided to investigate model systems using 2,3-dimethyl-1,3-butadiene and isoprene, since 1,3-butadiene is gaseous under standard laboratory conditions and therefore more difficult and costly to handle.

5.10.1 Ketal formation

The ketals **50a** and **50a-c** were prepared by reacting 3-methyl-2-cyclohexene-1-one with, respectively, optically pure D-(-)-(2*R*,3*R*)-butandiol (obtained from fermentation studies of chapter two) and a commercially available isomeric mixture of the diol (scheme 42). Standard acid catalysed conditions were applied, with the mechanical removal of water formed using a Dean-Stark apparatus.



50a **50a-c**

(i):D-(-)-(2*R*,3*R*)-butandiol, *p*-toluenesulfonic acid, toluene

(ii):Isomeric butan-2,3-diol, *p*-toluenesulfonic acid, toluene

Scheme 42: Ketal formation

Both racemic isomeric (**50a-c**) and optically pure (**50a**) ketals were obtained in between 50-60% isolated yield.

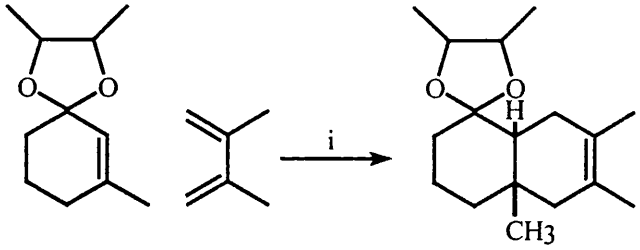
5.10.2 Lithium perchlorate reactions

For each ketal, a Diels-Alder reaction was carried out with both 2,3-dimethyl-1,2-butadiene and isoprene, following the experimental protocol of Grieco, using a 4M

$LiClO_4.Et_2O$ solution with catalytic (*S*)-camphorsulfonic acid.[180] The reactants were stirred at room temperature for a number of hours under an inert atmosphere before quenching with water, work-up and purification by flash column chromatography. Each isolated product was analysed by NMR to determine which of the cycloadducts had formed. This was relatively straightforward in the case of the optically pure ketal systems **51a-b** and **52a-b**/**53a-b**, but considerably harder in the case of the racemic adducts (owing to the presence of all the different isomers possible).

5.10.3 Results

2,3-Dimethyl -1,3-butadiene systems

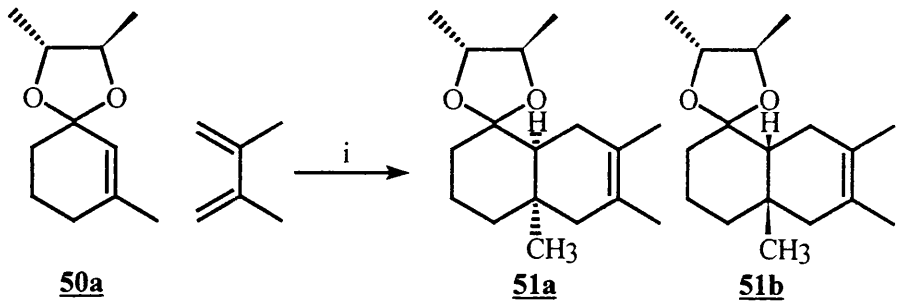


50a-c **51a-f**

Combined isolated yield 20%

Relative isomer ratio indeterminable by NMR

In the cycloadditions involving the ketals **50a-c**, the ^1H and ^{13}C NMR spectra of the ketal cycloadducts were too complicated to enable discernment of the isomer ratio in the products, **51a-f**.



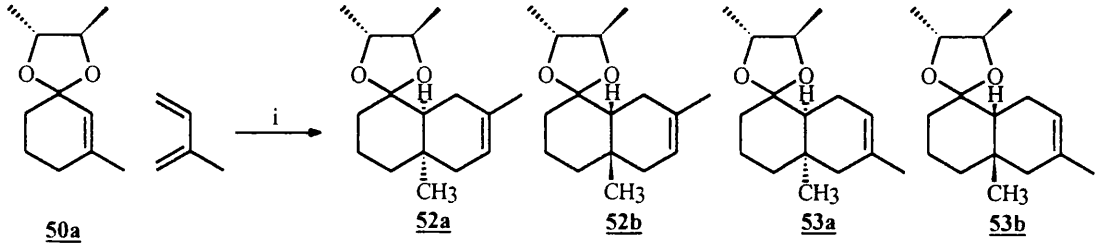
50a **51a** **51b**

Combined isolated yield 39%

Relative isomer ratio 6:1

^{1}H and ^{13}C NMR analysis of the products of the reaction involving the optically pure ketal **50a**, however, showed the clear presence of the two diastereomers **51a** and **51b**. Whilst the stereochemistry of each isomer could not be assigned absolutely, the two were present in the mixture in the ratio of 6:1, suggesting that a diastereomeric excess of ~70% had been obtained in the course of the cycloaddition.

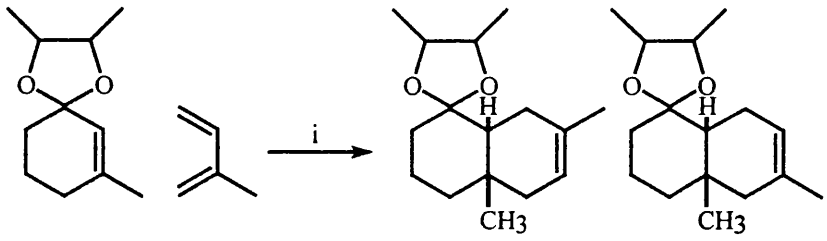
Isoprene systems



50a **52a** **52b** **53a** **53b**

Combined isolated yield 33%

Relative isomer ratio indeterminable by NMR



50a **52a-f** **53a-f**

Combined isolated yield 37%

Relative isomer ratio indeterminable by NMR

In interpreting the NMR spectra of the isolated cycloadducts from the isoprene systems, it was not possible to discern the exact isomer ratio of the products formed due to their complexity. This was due to the possibilty of regioisomers, in addition to diastereoisomers, and was particularly difficult for the isomeric ketal system.

For both model systems, the yields obtained were considerably lower than those of the Grieco study, where even the relatively hindered 2-methyl-2-cyclohexen-1-one

ketal formed an adduct with isoprene in 86% yield.[180] The was attributed to negative steric effects in the dienophile system (which possesses a methyl group at the emergent ring junction (section 5.6) and a more hindered ketal moiety than that used by Grieco). Furthermore, the reactions carried out in this study were not optimised for yield and in the course of the investigation, reactions involving lithium perchlorate were found to be greatly affected by the quality, age and concentration of the solutions (in line with reported observations).[180,180a]

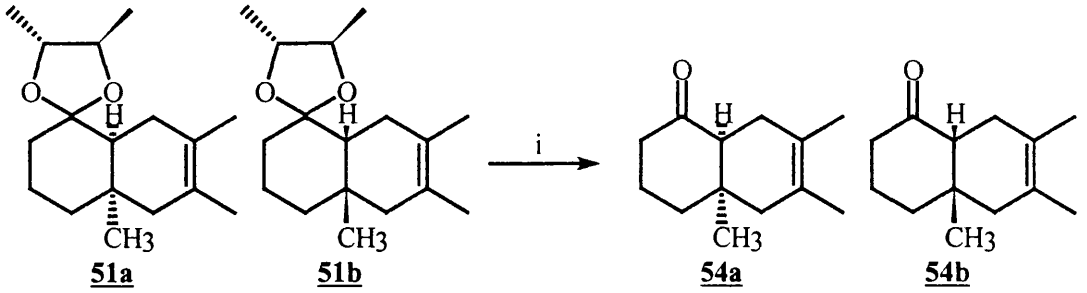
5.10.4 Measuring asymmetric induction

In order to quantify and corroborate the NMR assessment of the degree of selectivity that had been obtained with the chiral ketal system **50a**, all the isolated ketal cycloadducts **51-53** were subjected to HPLC analysis. However none of these compounds were found to be compatible with this method of analysis.

In an effort to improve their stability on the silica chromatography columns, these ketals were deprotected by acid catalysed hydrolysis. Correlation of their NMR spectra with those in the literature[177] showed the presence of exclusively the *cis* fused octalones, indicating that no epimerisation of the ring junction had occurred as a result of the acidic conditions. This result also confirmed the stereochemistry assigned to the parent ketals, **51-53**.

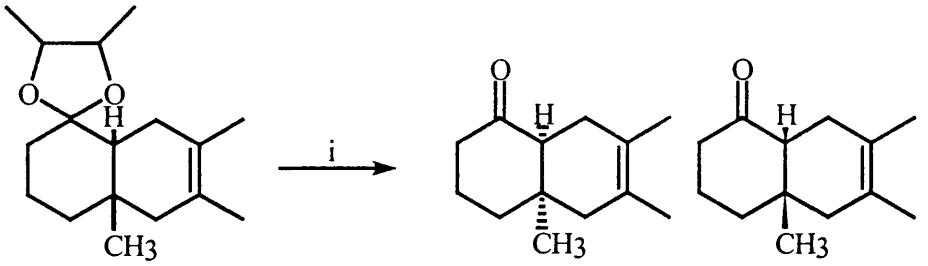
Being enantiomers, the deprotected ketones **54a-b** were no longer separately discernible by NMR. Chiral HPLC was therefore employed to measure the enantiomeric excess in these octalones, the technique having been shown to separate structurally related decalone enantiomers.[155a]

2,3-Dimethyl-1,3-butadiene systems



51a **51b** **54a** **54b**

Combined isolated yield 9%

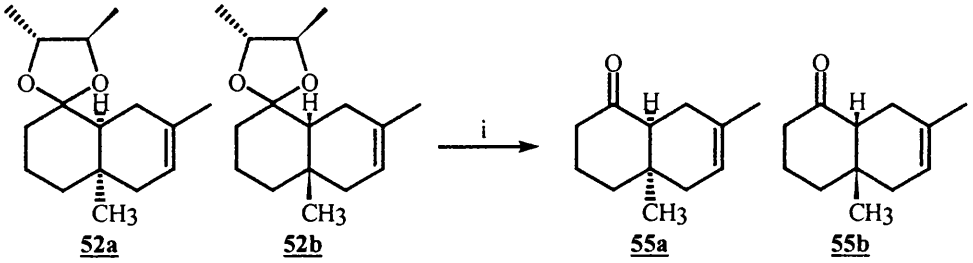


51a-f **54a** **54b**

Combined isolated yield 24%

The isolated octalones **54a** and **54b** were found to be incompatible with any of the chiral HPLC columns available in the laboratory and broke down on analysis. Being mirror images of each other, the enantiomers formed were not separately discernible by NMR analysis and the ratio of **54a** : **54b** could not be determined.

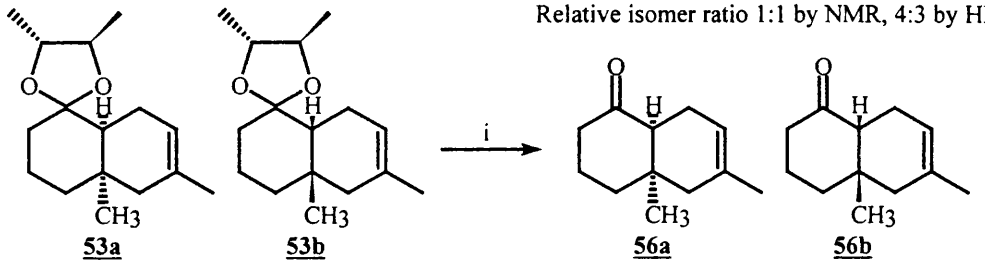
Isoprene systems



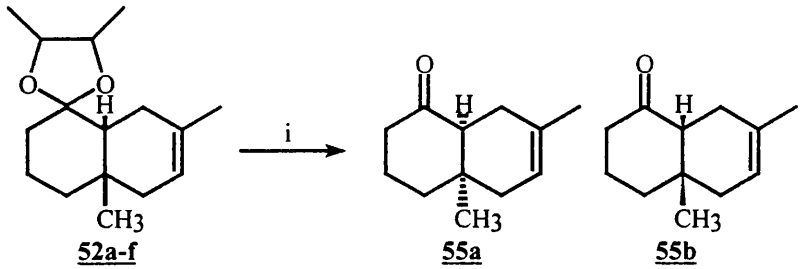
52a 52b 55a 55b

Combined isolated yield (55 and 56) 9%

Relative isomer ratio 1:1 by NMR, 4:3 by HPLC



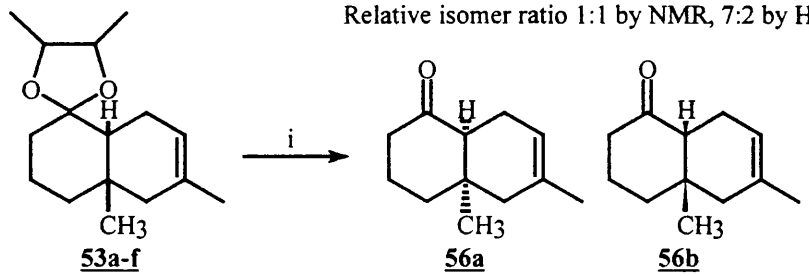
53a 53b 56a 56b



52a-f 55a 55b

Combined isolated yield (55 and 56) 21%

Relative isomer ratio 1:1 by NMR, 7:2 by HPLC



53a-f 56a 56b

It was possible to differentiate between the regioisomers 55 and 56 using an HPLC technique. This allowed a comparison to be made with measures obtained through ^{1}H NMR analysis of the octalones, where the olefinic methyl signal was used as a marker with which to measure the relative abundance of each regioisomer.

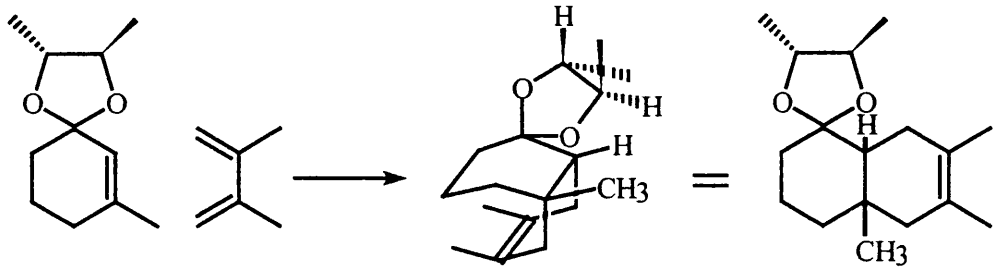
In the case of the chiral system, both HPLC and ^{1}H NMR measurements were in agreement that no regioselectivity had occurred in the initial cycloaddition. HPLC

and ^{1}H NMR measurements made on the isomeric system, however, were not in agreement; accepting HPLC to be more accurate in this respect, the result suggested that the stereochemistry of the *meso*-butan-2,3-diol ketal may have had an effect in influencing the regioselectivity of the Diels-Alder reaction. This was not investigated any further.

5.10.5 Proposed cycloaddition transition state

In order to rationalise the stereoselectivity of the cycloaddition between the chiral ketal **50a** and 2,3-dimethyl-1,3-butadiene to form **51**, models were constructed on the premise that the allyl oxonium cation proposed by Grieco (figure 14) could form, however transiently. The steric hindrance caused by the rotation of the opened ketal was judged to be greater for the top face of the dieneophile, necessitating the approach of the diene from below. Were this formation of the allyl oxonium cation not to occur, the methyl groups of the chiral ketal moiety appeared to be too remote from the site of addition to have an influence on the approaching diene.

Consistent with the principle that *endo* isomers are favoured by the polar medium of the lithium perchlorate solution used[177a], the following relative stereochemistry was assigned to the cycloadduct **51** (figure 15):



50a **51**

Figure 15: Assigned stereochemistry in the cycloadduct

On the basis of models alone, it was not possible to unequivocally assign the absolute stereochemistry of the octalin ketal **51**. Correlation to known molecules would therefore reveal further insights into the stereocontrol exerted during the addition and also aid elucidation of the mechanism of the reaction.

5.11 Conclusions

This investigation demonstrated that the use of highly polar lithium perchlorate solution allows 3-methyl-2-cyclohexen-1-one, generally considered to be an inert dieneophile, to undergo Diels-Alder cycloadditions in moderate yields when protected as the ketal. Through the use of an optically active diol, it was demonstrated that an asymmetric cycloaddition could occur; with the D-(-)-(2*R*,3*R*)-butandiol system tested in this study, an enantiomeric excess of 70% was achieved in the cycloadduct **51**. Further work is required to confirm the direction of the selectivity.

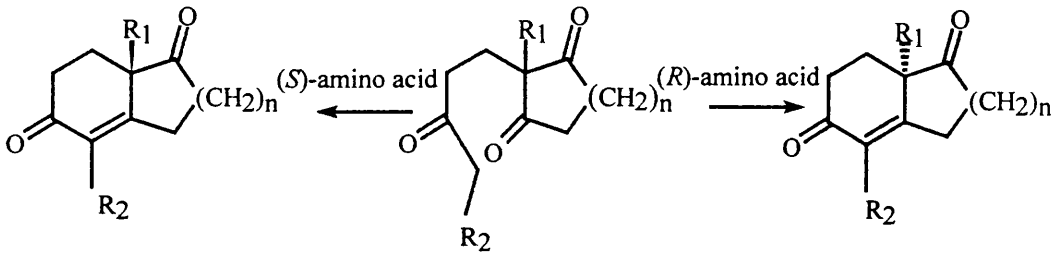
The model systems tested in this study demonstrate the feasibility of this approach in the preparation of the target octalone **49**, a key precursor to dehydrogeosmin. The cycloadducts obtained in this study are themselves precursors to dehydrogeosmin analogues and could be converted to worthwhile target molecules to gain an understanding of the structure-odour relationship in dehydrogeosmin type systems.

In developing a strategy to dehydrogeosmin itself, the next logical step would be the extension of the methodology to systems capable of being manipulated to this target compound. This would require the use of 1,3-butadiene equivalents, such as the gas itself, or possibly 1-acetoxy-1,3-butadiene. With the availability of both enantiomeric forms of butan-2,3-diol from the work of chapter two, the Diels-Alder study carried out in this chapter offers the prospect of an asymmetric route to both natural and unnatural dehydrogeosmin.

CHAPTER SIX: ASYMMETRIC CYCLISATION

6.0 Introduction

The use of the Robinson annelation in the construction of fused ring ketone systems is well accepted and has provided a synthetically useful approach to the preparation of such compounds.[181] A variant of this reaction, which has proved useful in the preparation of optically active steroid units, has been the introduction of chirality into the fused ring system by performing the intramolecular aldol condensation of prochiral triketones asymmetrically through the presence of an amino acid catalyst (scheme 43).[182]



Scheme 43: The Hajos-Parrish-Eder amino acid catalysed asymmetric cyclisation

This innovation was discovered independently by Eder [183] and Hajos and Parrish[184] and together, their work established the basic parameters (substrate, amine component, acid and solvent choice) controlling the degree of chiral induction. The generic reaction in figure 41 has been shown to be tolerant to a range of substrates, and asymmetric induction has been observed for a range of substituents R_1, R_2 and n.[182]

6.1 Use of surfactants

The use of surfactant based aqueous systems as reaction media in organic synthesis was introduced in chapter three and in this part of the project, these themes were explored in relation to two organic methodologies; the Michael reaction and the Robinson annelation.

Michael reaction

Such reactions may benefit from the polarity effects, both within the micelle and the bulk solution, whereby enolate anions formed are stabilised by the polar environment. Concentration and cage effects may also bring the reactants into sufficient proximity to favour reaction.

Studies into the use of aqueous media, in the context of the Michael reaction, are scarce[71] but significant enhancements in rate and yield have been observed through the replacement of organic media with water[185].

Of all the variants of the Michael reaction, the addition of an enolate system to an α,β–unsaturated ketone in a conjugate fashion is perhaps the most studied and utilised form in synthetic organic chemistry. Through an investigation carried out by Bassetti *et al*, the application of surfactant media to this fundamental area is not unknown.[186] The focus of their study was the alkylation of β-dicarbonyl species in a micellar phase, the reaction exploiting an apparent decrease in the pK_a of the reacting species as a result of the micellar environment and thereby negating the need for any external base. This was an exciting proposition since the uncatalysed Michael reaction had been considered impossible for many years.[187]

Whilst the Bassetti investigation[186] was limited to acyclic dicarbonyl species, in the context of this project it was thought that the methodology could equally be applied to a cyclic dicarbonyl system of a type that could form the basis of a synthetic route to geosmin *via* an asymmetric intramolecular cyclisation; that is, the addition of 2-methylcyclohexane-1,3-dione to ethyl vinyl ketone.

Robinson annelation

Micelles may induce stereochemical control due to preorientational effects within the Stern-layer. Where the Stern-layer is chiral in character (through the use of a chiral surfactant), this may invoke a transfer of asymmetry in reactions taking place within this region of the micelle.

The Robinson annelation is an example of an intramolecular aldolisation and is a reaction routinely performed under acidic or basic conditions in organic media.[188a] However, by applying the use of aqueous media to aldolisation reactions, in a

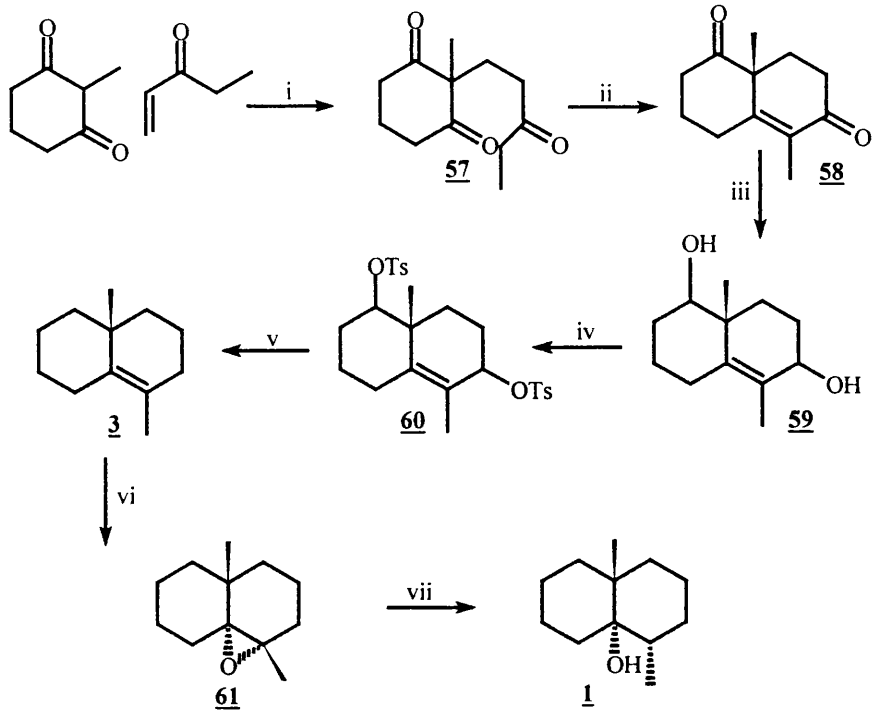
number of instances it has enabled the formation and isolation, under neutral conditions, of addition products which would ordinarily be extremely sensitive to conventional acidic or basic media. For example, the aldol addition product of acrolein.[188b]

Another example of the way in which water may promote aldolisation reactions is the Mukaiyama reaction, in which a silyl enol ether derviative adds to a carbonyl group.[189] This reaction typically requires forcing conditions (high external pressure)[190] or Lewis acid catalysis[189] in order to proceed in organic solvents. The application of aqueous media enables this reaction to take place under mild and neutral conditions, with increased selectivity compared to that elicited by organic reaction conditions.[191]

These examples illustrate the yield and selectivity gains that may be realised through the substitution of organic solvents with aqueous systems and it is a logical extension to expect these benefits to be applicable to Robinson annelation type aldolisations. In utilising such a strategy to form the bicyclic backbone of a geosmin precursor, there appears to be no literature example of a case where the use of a chiral micellar environment as a reaction medium has been successful in exerting any kind of asymmetric control in this addition.

6.2 A route to geosmin

Geosmin can be viewed as being derived from the diketone derivative **58**, accessible *via* an amino acid induced asymmetric cyclisation. The proposed synthetic route utlilising this transformation is shown in scheme 44.



(i):H_2O, hydroquinone, AcOH (ii):(*R*)-amino acid (iii):$NaBH_4$, MeOH, rt (iv):TsCl, pyridine, $CHCl_3$ (v):$LiAlH_4$, THF, reflux (vi):*m*-CPBA, CH_2Cl_2 (vii):$LiAlH_4$, THF, reflux

Scheme 44: Proposed asymmetric cyclisation route to natural geosmin

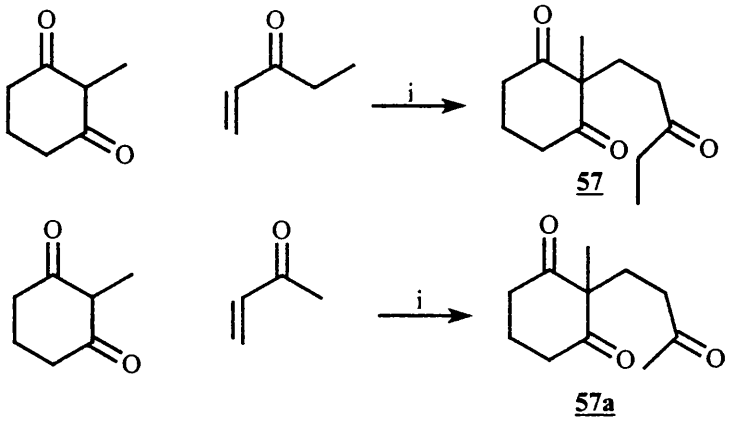
The objective in this part of the project was therefore to evaluate this synthetic scheme, being concise and of low cost. This necessitated revisiting the work of Eder and Hajos-Parrish to optimise the cyclisation of intermediate **57** to **58,** forming an intermediate that has not been exploited as an optically active precursor to geosmin. The second objective was to investigate the effect of amino acid based surfactants as reaction media in this key cyclisation step, extending the application of aqueous media described in section 6.1. This was based on the assumption that amino acids, when incorporated into surfactant head-groups, would retain their catalytic activity as part of the micellar Stern-layer. It was further proposed that, as a result of the hydrophobic effect, a transfer of asymmetry may occur.

6.3 Triketone preparation

The intermediate **57** possesses a quaternary prochiral centre, which forms one of the positions at the bicyclic ring junction once chirality has been induced through the intramolecular Robinson type annelation step.

A limited study was carried out comparing the formation of this intermediate **57** by the standard literature procedure (which, interestingly, is an example of a Michael type addition performed in water[192]) with the use of surfactant media. The study was also extended to a closely related analogue, which forms the precursor to the synthetically useful Wieland-Miescher ketone, this being the Michael addition product of 2-methyl-1,3-cyclohexanedione and methyl vinyl ketone.[193,194,195]

6.3.1 Aqueous medium



(i):H_2O, hydroquinone, AcOH,

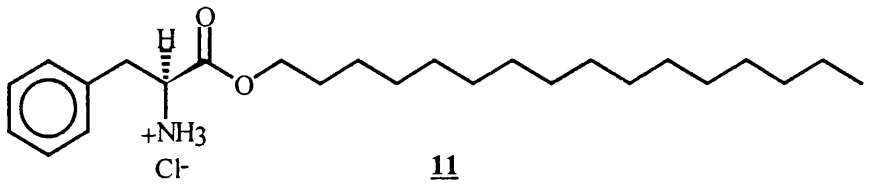
Scheme 45: Michael addition study

The procedure of Gutzwiller[192] was followed for both systems, performing the reaction in deionised water with a trace of acidity. Triketones **57** and **57a** were prepared in 55% and 70% isolated yield respectively, providing a benchmark against which to measure the efficacy of surfactant based media.

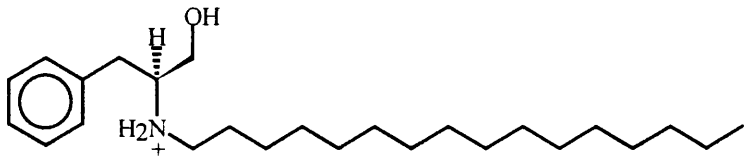
6.3.2 Surfactant medium

The Bassetti study was limited to Michael additions performed in solutions of the commercially available surfactant CTAB.[186] Test reactions, using CTAB under the same experimental conditions as Bassetti, *i.e. quantitatively* w.r.t. reactants, found

that the large quantity of surfactant complicated the work-up, making measurements of yield unreliable. For the purpose of this study, it was therefore decided to investigate the use of two amino acid derived surfactants – **11** and **62** - that had been synthesised in the research group.



11

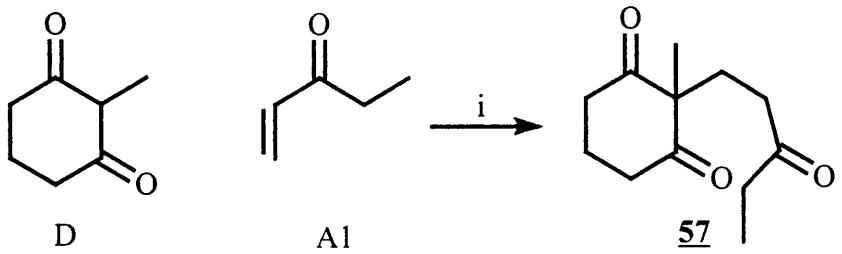


62

In common with Bassetti's work[186], no external base was added to the media and the reactions were performed by simply stirring the Michael donor (D) with the Michael acceptor (A) in surfactant solutions at elevated temperatures (80 °C). However, in a departure from Bassetti's protocol, surfactants **11** and **62** were arbitrarily used at their CMC (which is temperature dependent).

6.3.2.1 Results

Surfactant media versus water



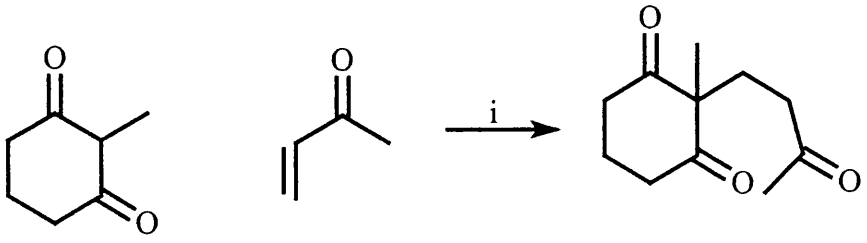
D A1 **57**

(i):*see* table 16

Scheme 45: Michael addition – ethyl vinyl ketone system

Medium	D:A1	Yield %	D:A1	Yield %
11	1:2	86	1:1	90
62	1:2	95	1:1	96
Water	1:2	51		

Table 16: Michael addition yields – ethyl vinyl ketone system



D A2 **57a**

(i):*see* table 17

Scheme 46: Michael addition – methyl vinyl ketone system

Medium	D:A2	Yield %	D:A1	Yield %
11	1:2	99	1:1	95
62	1:2	99	1:1	92
Water	1:2	70		

Table 17: Yields of Michael addition reaction – methyl vinyl ketone system

The yield of triketone was significantly higher in the surfactant systems, with no real difference between surfactants **11** or **62**. In addition, when using micellar media, there appeared to be no improvement in yield when using an excess of the Michael acceptor with respect to Michael donor. It also appeared adequate to operate with surfactant solutions at their CMC and no higher.

6.3.2.2 Conclusions of surfactant study

This investigation provided a means of preparing the first intermediate to geosmin in high yield whilst minimising the quantities of starting materials required (literature procedures requiring a one equivalent excess of the Michael acceptor[192]). Furthermore, it demonstrated that the methodology of Bassetti could be successfully extended beyond simple acyclic diketones to include cyclic systems.

In comparing surfactant systems to the reference aqueous reaction system, the propensity for the Michael acceptor to self-condense was significantly reduced in the micellar media (presumably due to the neutral conditions possible). This explained why reactions performed in surfactant media did not require an excess of Michael acceptor.

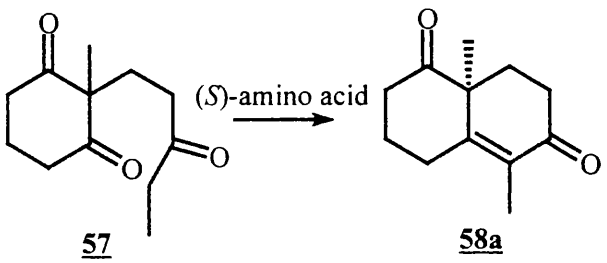
6.4 Asymmetric Robinson annelation

It was with the objective of obtaining diketone **58** for the evaluation of synthetic scheme 44 that the cyclisation of triketone **57** was investigated. The reaction is not unprecedented and Yamada *et al* reported the (*S*)-proline pyrrolidide cataysed preparation of **58** in 50% chemical and 60% optical yield.[196] This was contested by Uma *et al*[197], who reported their own improved synthesis employing (*S*)-phenylalanine as a chiral catalyst in acetic acid to generate the diketone in 80% chemical and 83% optical yield.[198]

In addition to simply preparing a quantity of **58**, the objective in this part of the study was therefore to re-examine the requirements of this reaction, in terms of amino acid choice and solvent selection, with a view to extending the use of aqueous media to the Robinson annelation.

6.4.1 Amino acid cyclisations

It was decided to experiment with a range of natural amino acids - proline, phenylalanine, valine, phenylglycine and glutamic acids - that have all been successfully used with other triketones.[183,184,192] With the exception of phenylglycine, the naturally occurring amino acids tested were of the (*S*)-configuration and were expected to confer (*S*)-configuration to the resulting bicyclic diketones (scheme 47).[182] Any optimised reaction that resulted from this study would therefore require the use of the more expensive (*R*)-forms of the amino acids in order to obtain the required stereochemistry for natural (-)-geosmin **1**).



57 58a

Scheme 47: Stereochemistry with (*S*)-amino acid

Inital reactions were attempted using the procedures of Gutzwiller[192], whereby the triketone was heated in DMSO with the amino acid under an inert atmosphere, and Eder[183], where the solvent system was DMF with a trace of perchloric acid. Neither were successful and the triketone was recovered unreacted. A second system used by Eder[183] - employing glacial acetic acid as the solvent - was found to be more successful and three amino acids exhibited catalytic activity; phenylglycine, valine and phenylalanine.

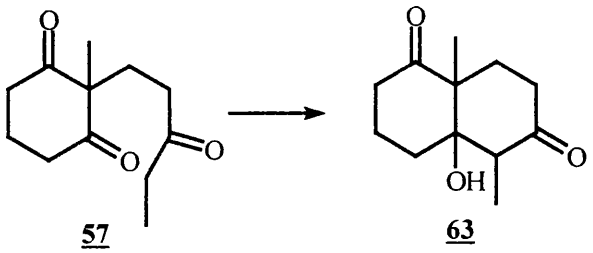
The cyclisation reactions were repeated with different catalyst concentrations to study the effect on yield and optical purity of the product. After standard aqueous work-up, all the isolated products were analysed by NMR to confirm their structural identity, and by chiral HPLC (Chiralcel OD column) to assess the degree of enantioselectivity that had occurred during the cyclisation.

Glutamic acid

(*S*)-Glutamic acid was tried, with acetic acid as solvent, but failed to induce any kind of cyclisation and the triketone was recovered unreacted.

Proline

Proline was ineffective as a catalyst for the triketone system in DMSO, DMF and acetic acid solvent systems. In the case of the DMF system, however, a white solid, was produced in 5% isolated yield. This was identified as the tertiary alcohol, **63** (scheme 48).



57 63

Scheme 48: Triketone cyclisation in the presence of proline

The dehydration of this alcohol to the parent diketone **58** allowed its analysis by chiral HPLC, though this showed that the cyclisation had proceeded racemically.

Phenylalanine

Amino Acid mole equiv.	Enantiomeric Excess %	Isolated Yield %
0.25	64	72
0.5	65	75
1	72	70
2	70	70

Table 18: Triketone cyclisation in the presence of phenylalanine

The isolated yield obtained with this catalyst was consistently >70% and a weak positive correlation between enantiomeric excess and amino acid concentration was exhibited. These data suggested that phenylalanine was an effective catalyst in acetic acid and one mole equivalent gave an optimal balance between yield and optical purity of the product.

Valine

Amino Acid mole equiv.	Enantiomeric Excess %	Isolated Yield %
0.25	58	60
0.5	63	66
1	62	51
2	61	83

Table 19: Triketone cyclisation in the presence of valine

No relationship was apparent between amino acid concentration and enantiomeric excess, and chemical yields were generally lower than those for phenylalanine. On balance, valine was therefore judged to be slightly inferior to phenylalanine.

Phenylglycine

Amino Acid mole equiv.	Enantiomeric Excess %	Isolated Yield %
0.25	14	76
0.5	21	62
1	31	61
2	30	90

Table 20: Triketone cyclisation in the presence of phenylglycine

Whilst the chemical yields obtained with phenylglycine were acceptable, the catalyst was markedly poorer than either phenylalanine or valine. This was disappointing since the (*R*)-configuration of the naturally occuring (*R*)-phenylglycine induces the required (*R*)-stereochemistry for natural (-)-geosmin, giving this catalyst a potential cost advantage in any commercial process based on this cyclisation.

6.4.1.1 Conclusions

From these experiments, it appeared that the simplest amino acids, alanine derivatives, were most successful in inducing a chiral cyclisation. Any additional heterofunctionality, such as in proline or glutamic acid, prevented any reaction occurring. This may be understood in terms of the complex formed between the amino acid and the triketone, explained in section 6.5.

6.4.2 Solvent optimisation

6.4.2.1 Effect of pH

It was apparent that solvent selection influenced the course of the cyclisation reaction. The role of acidity was judged to be key and an investigation of the effect of pH on yield and optical purity of the cyclisation product was undertaken. Two

solvent systems selected for study, in both cases using dilute hydrochloric acid to control the pH of the medium;

- deionised water
- surfactant solution, CTAB

Having been identified as the most effect catalyst, phenylalanine was heated at with triketone **57** in both solvent systems, with one molecular equivalent of amino acid present. After standard aqueous work-up, the isolated product was analysed by chiral HPLC (Chiralcel OD column) to determine the enantiomeric excess.

Water

Solution pH	Enantiomeric Excess %	Isolated Yield %
1.1	0	60
3.02	23	25
5.15	48	11
7.23	0	0

Table 21: Effect of pH – water

Unacidified water, at pH 7.23, induced no cyclisation at all and the triketone was recovered unreacted. There was a general increase in yield with decreasing pH, however enantiomeric excess was adversely affected by increasing acidity.

The trend of decreasing enantiomeric excess with decreasing pH, to the point of a racemic product at pH 1, suggested a change to a simple protonation mechanism that did not involve the amino acid.

CTAB solution

Solution pH	Enantiomeric Excess %	Isolated Yield %
1.01	0	70
3.11	41	10
5.10	39	8
6.85	0	0

Table 22: Effect of pH – CTAB

In the case of the surfactant solutions, very similar trends were observed to those seen in the simple aqueous system, with yield positively correlated to acidity and enantiomeric excess inversely so. These trends again highlighted the importance of acidity with a presumed shift to a "simple" protonation mechanism, not involving the amino acid in the cyclisation at the lower pHs.

6.4.2.2 Micellar phase cyclisation

It was suggested in section 6.2 that the use of chiral surfactants, generating chiral micellar media, may invoke a transfer of asymmetry for reactions occurring in this chiral environment. Based on the intramolecular cyclisation studies detailed in section 6.3, it was hoped that the amino acid moiety, when incorporated in a surfactant molecule, would induce a preorientational effect within the charged Stern-layer of the micelles formed in solution. Such an induction was envisaged to occur through the favourable interaction between the amine functionality on the surfactant head groups and enolate anions embedded within the Stern-layer.

(*S*)-phenylalanine was identified both in this study and by Uma[197] as exhibiting the highest inductive effect with triketone substrate **57**. Surfactants based on this naturally occurring amino acid, **11** and **62** (previously utilised in the Michael addition study of section 6.3), were therefore used to investigate whether the micellar environment could invoke any asymmetric control in the cyclisation of intermediate **57** to **58**.

Experimentally, all the test cyclisation reactions were carried out in an identical way to the amino acid/acetic acid studies - heating the triketone **57** in the surfactant solution at 80 °C with agitation of the reaction mixture. After aqueous work-up, TLC and NMR analyses were used to determine the extent of reaction.

Results

Surfactant	Solvent	Medium pH	Isolated yield
11	H_2O	7	0
62	H_2O	7	0
11	H_2O, AcOH	5	0
62	H_2O, AcOH	5	0
11	H_2O, HCl	1	0

Table 23: Cyclisation in surfactant media

The absence of any reaction strongly implied that the presence of micelles did not act to lower the pK_a of the reacting monoketone system sufficiently to promote the formation of a reacting enolate species of the **57**. However, the recovery of the starting material in all cases, including the pH 1 system (which may have been assumed to promote enolate formation better than a neutral system), suggested the importance of having an amino acid catalyst present in the reaction media.

An alternative explanation for the failure of these reactions was suggested by examination of the structure of the head-group of the surfactant molecules: **11** and **62** possess two heteroatoms at the assumed active site, oxygen and nitrogen derived from the parent amino acid moiety. If the co-ordination of both these heteroatoms to the substrate is required for catalysis during cyclisation to occur, both surfactants effectively have one of these co-ordination sites blocked by a long alkyl chain - **62** at the nitrogen and **11** at the oxygen.

6.4.3 Other cyclisations

To demonstrate unequivocally that the amino acid functionality was required for cyclisation to occur, the cyclisation of triketone **57** was tested in the absence of this catalyst under basic and acidic, and under racemic catalysed conditions.

Basic Cyclisation, with an absence of amino acid, was attempted following a procedure used by Revial with methoxide as the deprotonating agent.[9] Despite prolonged heating at reflux, and a range of base concentrations, substantial amounts of residue formed and yet uncyclised **57** remained.

Performing the cyclisation in acetic acid, with an absence of amino acid, recovered the triketone **57** unreacted. When repeating the experiment with acetic acid and a mixture of racemic (*RS*)-phenylalanine, the racemic diketone **58** was obtained in 79% isolated yield.

Inferences

The racemic cyclisation suggested the need to have both amino acid and acidic conditions for the cyclisation to proceed. This was supported by the failure to cyclise under both basic conditions (emphasising the role of acidity and protonation) and in absence of an amino acid (emphasising the necessity of this catalyst).

6.5 Mechanism of annelation

The mechanistic aspects of this type of intramolecular aldol condensation are not fully understood.[199] The critical dilemma in this reaction, recognised by Hajos and Parrish[184] in their pioneering work, is whether the amino acid catalyst activates a ring carbonyl by forming a carbinolamine intermediate prior to cyclisation, or interacts with the side chain carbonyl to form an enammonium group.

Enamine intermediate

In this model, the enamine type intermediate formed between the catalyst and triketone could be envisaged as the following (illustrated with proline as the amino acid):

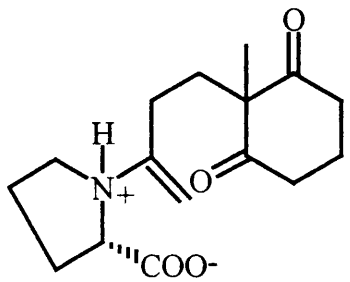


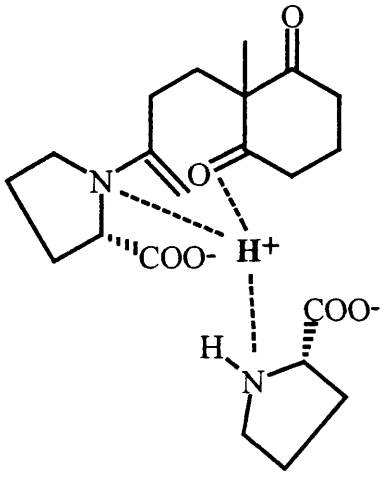
Figure 17: Enamine Intermediate

Efforts made to invoke asymmetric cyclisation with other catalysts, such as chiral amines and esters and amides of natural amino acids have been successful and in

such cases, intermediates that are enamine in nature have even been isolated [199]. This would suggest that a similar pathway is operating with amino acids but, to date, no such intermediates of the type postulated have been isolated.

If the reaction does proceed *via* such an intermediate, the use of acetic acid in this study would explain why catalytic quantities of amino acid were sufficient to achieve complete reaction, the solvent system presumably containing sufficient free water to allow the release of the catalyst from the enamine cyclisation product. The longer reaction times observed when using anhydrous aprotic solvents would therefore be indicative of the reduced presence of free water to regenerate the amino acid for reuse in the reaction system.

The existence of an enamine pathway was supported by Agami *et al* [200], who studied X-ray structures of ketols produced from proline-catalysed triketones. Subsequent work by this group proposed that the amino acid may have a dual role, not only in a side chain enamine species, but forming a three centre hydrogen bonded transition state involving a second amino acid molecule.[201]



------ H-Bonding interaction

Figure 18: Three Centre Transition State

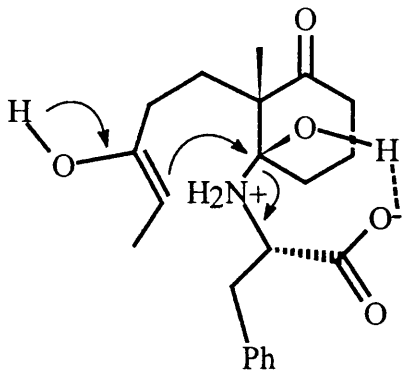
Whilst a reasonable assertion in an aprotic solvent environment, such a model is incompatible with the high selectivity observed in the protic environment of acetic

acid, where random hydrogen bonding is prevalent and unlikely to be so stereospecifically defined.

Via a carbinolamine

In this study, since triketone **57** is prochiral, both cyclic keto groups are identical and the catalyst could co-ordinate to either through the amine group. Asymmetric control would therefore result from the chiral catalyst holding the exocyclic enol group in one preferential orientation to the ring whilst the co-ordinated ring carbonyl is held, *via* hydrogen bonding (and hence the strong solvent/protic dependence of the reaction), by the free carboxylate group on the amino acid.

For example, with phenylalanine:



------ H-bonding interaction

Figure 19: Carbinolammonium Transition State

This type of bimolecular co-ordinate is supported by the study of Jung, who studied the related cyclisation to the Wieland-Miescher ketone with proline.[202] Such a model can help to explain the decrease in enantiomeric excess with decreasing pH in the case of the water and surfactant based cyclisations, despite the presence of an amino acid catalyst. Clearly the pH of acetic acid, when used as a solvent, is sufficient to allow keto-enol tautomerism of the exocyclic ketone system yet not so strongly acid as to suppress complexation of the amino acid to one of the cyclic ketone groups. Even a simple mechanism, whereby the low pHs of mineral acid systems induces protonation of the amine moiety in the catalyst, is sufficient to

explain its inability to complex and confer some rigidity to the transition state that directs the cyclisation.

Significantly, cyclisations catalysed by amino acids tend to proceed with greater asymmetric control than those involving the corresponding amines or amides.[199] This emphasises the crucial role played by the carboxylic acid moiety, arguably more important in the carbinolamine model than the proposed enamine transition state. This is supported by the pH study carried out in section 6.4.2.1, in which enantiomeric excess in the cyclised product was seen to peak for reactions carried out in media of between pH 3 and pH 5. It is precisely within this range that the carboxylic moiety of phenylalanine becomes completely dissociated, enabling it to hydrogen bond effectively (as shown in figure 19).

Concluding comments

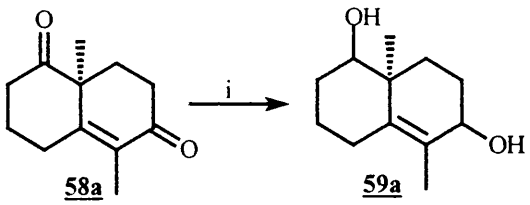
The preceding discussion has sought to align the results of this experimental study against the two most likely mechanisms by which the asymmetric cyclisation proceeds. On the basis of the study carried out in this chapter, it was not possible to categorically favour one mechanism over the other and both an enamine or carbinolamine type intermediate are consistent with the results obtained. In the following chapter, however, a study was carried out into the cyclisation of monoketone analogues, the results of which were strongly indicative of solvent dependency of the actual mechanism.

6.6 Towards geosmin

In continuing the evaluation of the proposed route to geosmin (scheme 44), the deoxygenation of the optically enriched diketone **58a** was required to generate the octalin skeleton of argosmin, *via* a three step sequence involving reduction, esterification to a suitable leaving group and displacement by hydride.

6.6.1 Bicyclic diol

The generation of intermediate **59a**, the bicyclic diol, was achieved in 38% isolated yield from **58a** (scheme 49).



58a 59a

(i):$NaBH_4$, IPA

Scheme 49: Formation of bicyclic diol

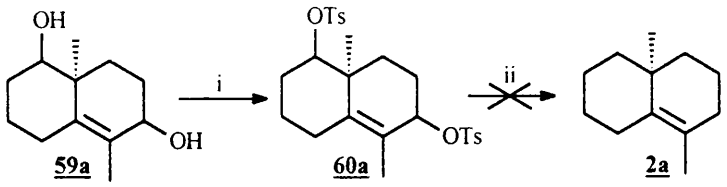
The relatively low yield prompted an investigation into the use of alternative solvent systems (methanol, propanol, water and mixtures of these) for this reduction but no noticeable improvement was achieved. The low yields were therefore attributed to difficulties in isolating and extracting the diol from the polar reaction media and were tolerated whilst the synthetic route was being evaluated.

Diol **59a** was recrystallised from dichloromethane, a technique which has been reported to raise the optical purity of the Wieland-Miescher ketone.[192] **59a** was found to be incompatible with any of the chiral HPLC columns available and a direct assessment of whether enrichment had occurred was not possible. Chiral HPLC of the diacetate derivative was inconclusive.

6.6.2 Towards argosmin

The precedent of Gosselin suggested that *p*-toluene sulfonyl group would be a suitable candidate for a leaving group easily displaced by hydride.[8] Gosselin removed a single secondary tosyl group in an allyl position in high yield though in the system under investigation in this study, a second tosyl group in a neopentyl position would also have to be displaced. S_N2 substitution at neopentyl positions can be 10^{-5} times slower than at unhindered primary positions. Gustafson, however, reported that lithium aluminium hydride may satisfactorily displace tosyl groups from such positions[203a], supporting the strategy.

The diol **59a** was therefore treated with *p*-toluenesulfonyl chloride in the presence of pyridine to generate the ditosyl diester **60** in 60% isolated yield. With this material several attempts were made to prepare argosmin, displacing the tosyl groups with lithium aluminium hydride (scheme 50).

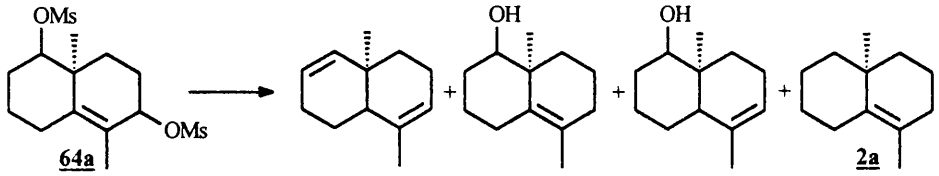


(i):*p*-toluenesulfonyl chloride, pyridine, DCM (ii):$LiAlH_4$, THF

Scheme 50: Attempted displacement of tosyl groups

This failure prompted the investigation of an alternative leaving group (the dimesyl diester **64a**) and a range of hydride sources for which there was a literature precedent (lithium triethylborohydride ('Superhydride')[203,204], lithium aluminium hydride and chloroaluminium hydride ('Alane'), the latter of which has been shown to reduce allyl alcohols such as **59a** directly to the alkene[205]).

In all cases, very little of the desired product **2a** was detected by TLC. NMR analysis of the products of the mesyl removal reactions suggested mixtures of mono-deoxygenated and rearranged analogues of the starting material, as shown in scheme 51:



Scheme 51: Attempted mesyl removal

Of the systems tested, direct 'alane' reduction of the underivatised diol appeared to be the most successful but a maximum yield of only 10% of Argomsin **2a** was achieved, contaminated with other dehydration and rearrangement products.

The difficulties experienced in displacing the leaving groups were attributed to the axial methyl group at the ring junction exerting a strong steric effect, typical in neopentyl systems, blocking the backside approach of hydride ions.

6.7 Conclusions of the chapter

A synthetic scheme to optically enriched geosmin was evaluated. Robinson type annelation was optimised for the system under study such that an enantiomeric excess of 74% was obtained in the key diketone intermediate **58a**. Furthermore, the application of surfactant media to the preparation of the triketone cyclisation precursor **57** achieved near quantitative yields without the need for excess reactants. This study therefore demonstrated that micellar media may promote Michael type reactions in cyclic diketone systems.

Whilst micellar media were unsuccessful in inducing the asymmetric cyclisation step itself, insights into the mechanism of this reaction were gained through the study into the role of acidity, amino acid and surfactant structure on this key step.

In evaluating the proposed synthetic route to geosmin, significant difficulties were encountered in the deoxygenation step of this sequence and an alternative strategy was sought.

6.8 Future work

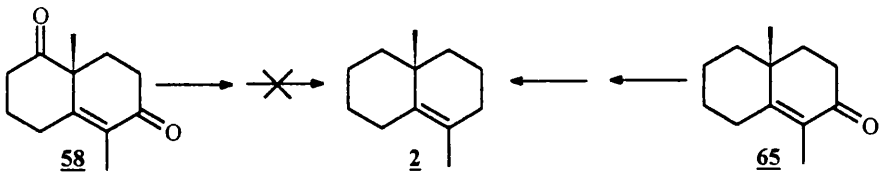
Surfactant **10**, synthesised in chapter three, is structurally identical to phenylalanine at the amino acid moiety and would be the next logical surfactant to test in the asymmetric intramolecular cyclisation of triketone **57**.

In transforming diketone **58** to argosmin, the alternatives to a hydride displacement sequence are limited, given the precedent in the literature. Standard protocols, such as the Clemmensen or Wolff-Kishner reductions, when applied to substrates similar to those studied in this research, have produced similar rearrangements and dehydrations. The viability of the proposed strategy to geosmin (scheme 44) would therefore require the investigation of alternative reduction procedures, such as electrochemical[206] or samarium iodide[207] reductions, which have shown some success with the types of system being investigated.

CHAPTER SEVEN: ENANTIOSELECTIVE DEHYDRATION

7.0 Background

In evaluating scheme 19, the preparation of geosmin through the manipulation of a bicyclic diketone obtained from the asymmetric cyclisation of a prochiral triketone, considerable difficulty was encountered in the deoxygenation of diketone **58** to Argsomin **2**. An alternative synthetic strategy was therefore sought and the precedent in the literature of syntheses that rely upon the manipulation of a monoketone precursor **65**[4,6,8,9,10,10a] suggested this to be an appropriate route to investigate (scheme 52).

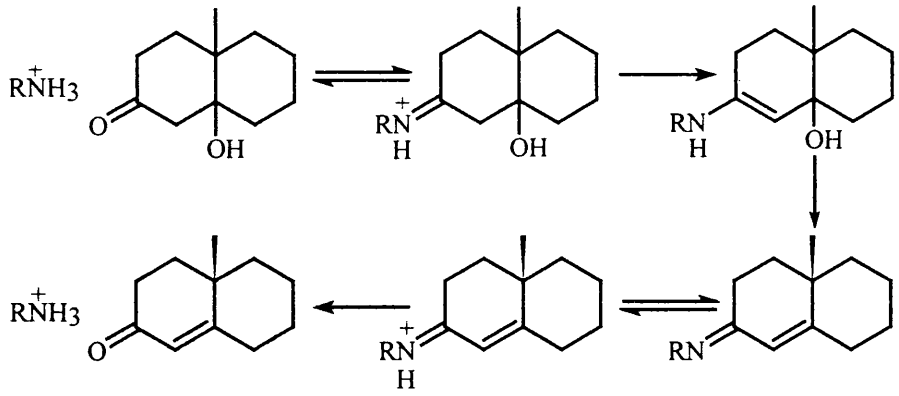


Scheme 52: Monoketone argosmin precursor

7.1 Enantioselective dehydration

A monoketone precursor to geosmin, (4a*R*)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3*H*-naphthalen-2-one **65**, was prepared in high yield and optically pure form by Revial and Pfau, who investigated the application of chiral imines to Michael-type alkylations.[9,18] The approach was extremely efficient in its use of optically pure amines as chiral auxiliaries that were recyclable after use.

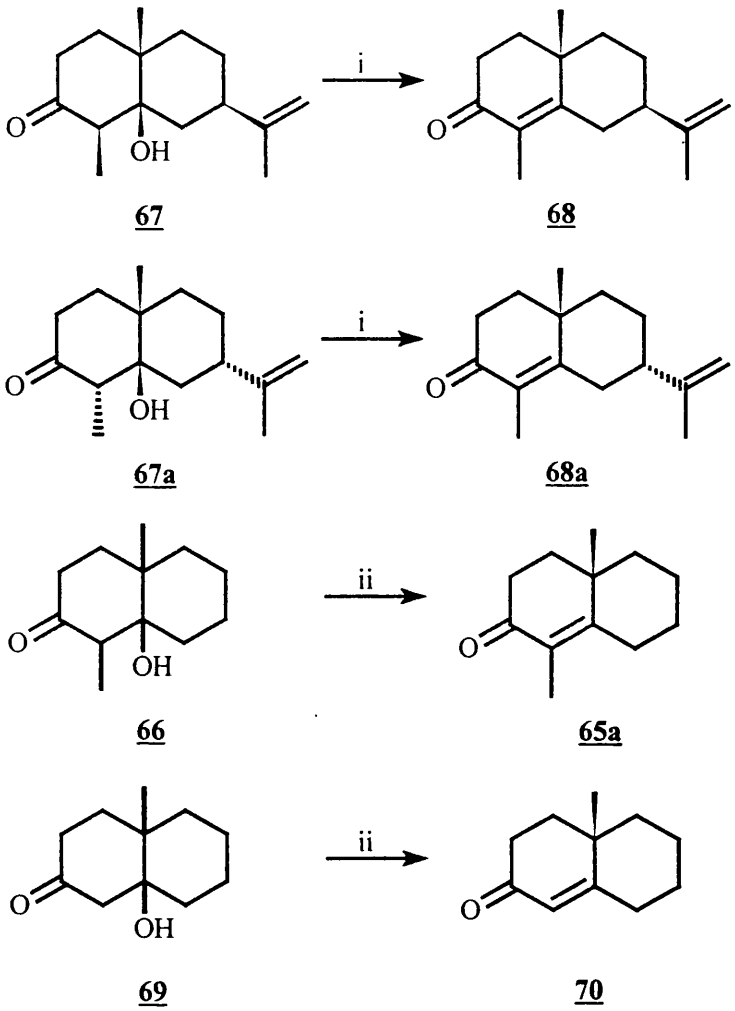
An alternative approach to access this octalone is *via* the dehydration of the corresponding β-ketol. This amine catalysed reaction was extensively studied by Spencer *et al* who postulated that the mechanism involves the deprotonation of an immonium ion formed (scheme 53).[208,209]



Scheme 53: Dehydration of β–Ketol

The scope of Spencer's work was extended by Agami *et al* who substituted amines with amino acids, to act as enantioselective dehydrating agents.[200] The reaction was presumed to proceed *via* the immonium type intermediates postulated by Spencer[208,209] and arose out of a study into the mechanism of the asymmetric Robinson cyclisation studied in chapter six.

Agami discovered that by employing (*S*)-proline as a catalyst in the aprotic solvent DMSO and limiting the extent of the conversion, enantioselectivity could be achieved in the dehydration of bicyclic β-ketols. The study (scheme 54) showed that stereoselectivity was only observed in ketols bearing a methyl group *geminal* to the hydrogen being abstracted, and that the reaction behaved as a kinetic resolution does, with the highest enantioselectivity being observed in the early stages of the reaction.



67 68

67a 68a

66 65a

69 70

(i):(*S*)-phenylalanine, perchloric acid, acetonitrile (ii): (*S*)-proline, DMSO

Scheme 54: The Agami Study[200]

Ketol	Enone	Yield %	Enantiomeric excess %
67	68	31	25
67a	68a	0	0
66	65a	37	87
69	70	44	0

Table 24: Results of the Agami Study[200]

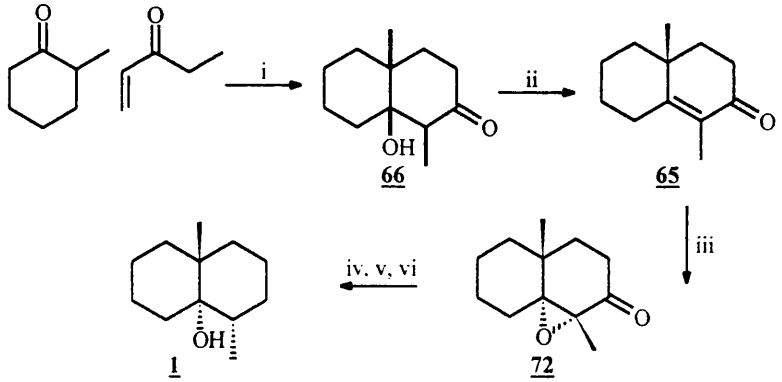
7.3 Aims

The focus of the study described in this chapter was the preparation of monoketone **65**, utilising amino acids as enantioselective dehydrating agents. It was envisaged that this octalone intermediate would be easier to transform to geosmin, overcoming the problems encountered in chapter six with the deoxygenation of the analogous diketone analogue **58**.

The Agami study was extremely limited in scope, investigating only the use of a single catalyst, proline, in a single solvent, DMSO.[200] The approach in this study was therefore to test a range of amino acids and solvent systems, with the objective of obtaining a better understanding of the relationship between solvent, amino acid structure and enantioselectivity during the dehydration.

In keeping with the themes of chapter three, and extending the investigation of micellar promoted Michael additions begun in chapter six, the use of surfactant media was also applied to the formation of the parent hydroxymonoketol **66**, and to the target octalone **65** itself, through the application of surfactant media to the dehydration step.

In order to qualify the strategy as a viable route to geosmin, established literature procedures were applied to the manipulation of octalone **65** to this target molecule, as presented in scheme 55:



(i):Na, EtOH (ii):(*R*)-amino acid, DMSO (iii):*m*-CPBA, CH_2Cl_2 (iv):$LiAlH_4$, THF (v):SO_3.Pyridine (vi):$LiAlH_4$, THF, reflux

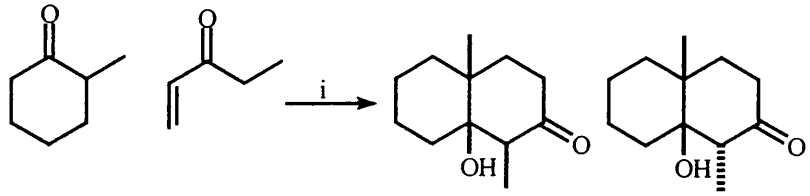
Scheme 55: Proposed asymmetric dehydration route to geosmin

7.4 Ketol preparation

The target ketol, 1,4a-dimethyl-8a-hydroxy-1,4,4a,5,6,7,8,8a-octahydro-3*H*-naphthalen-2-one **66**, can be prepared by the conjugate addition of 2-methylcyclohexanone to ethyl vinyl ketone. This Michael type addition was investigated in order to examine the effect of organic and aqueous solvent systems on yield, extending the study of section 6.3.2 to cyclic monoketone systems.

7.4.1 Organic solvent

Ketol **66** was prepared in 26% isolated yield from 1-methylcyclohexanone and ethyl vinyl ketone, following a procedure by Marshall who reported formation of exclusively *cis* fused ketol, by reacting the thermodynamic enolate with sodium ethoxide in ethanol at reduced temperatures (scheme 56).[210]



66

(i):NaOEt, EtOH

Scheme 56: Ketol preparation

The *cis* fused ketol was obtained in 36% isolated yield as a mixture of diastereoisomers (corresponding to axial and equatorial methyl groups at the α–position to the carbonyl group).

The use of aprotic media for this reaction has been investigated by Ziegler and Hwang as a means of improving the efficiency of this reaction.[211] They studied the use of an LDA/THF system under thermodynamic conditions but were able to isolate only a mixture of both *cis* and *trans* fused ketols, together with the aldolised and dehydrated bicyclic enone.

7.4.2 Aqueous media

Bassetti *et al* investigated the Michael reaction of 2-methylcyclohexanone and methyl vinyl ketone in a solution of the surfactant CTAB but, in that study, failed to

identify either the ketol **66** or the aldol product **65**.[186] Work carried out in the research group by Monteleone, however, showed that the addition of a mild base (potassium hydroxide or potassium carbonate) could yield either the Michael adduct or the annelation product. Despite the use of chiral surfactants, the study found that no asymmetric induction had taken place.[212]

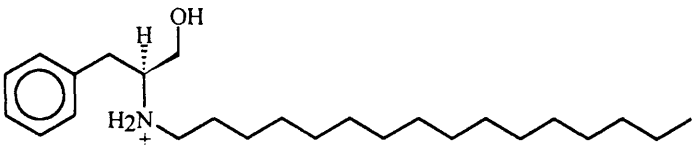
7.4.3 Use of chiral amine bases

It is well reported in the literature that chiral bases, such as lithium amides, may effect transfers of asymmetry.[213] The Monteleone study[212] had demonstrated that under micellar conditions, far milder bases could successfully deprotonate cyclic ketones of a type pertinent to this project.

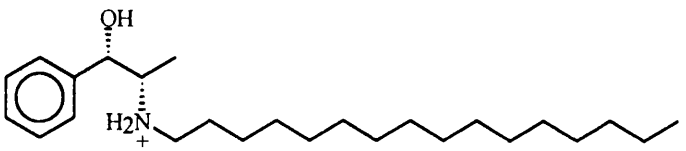
Combining these two themes, it was postulated the Michael addition sequence of scheme 56 could be repeated using chiral amines as mild bases to deprotonate cyclic ketones under micellar conditions. It was envisaged that within the micelle, the amine would complex, in a stereoselective manner, to the prochiral enolate carbanion such that the subsequent conjugate addition step would proceed with stereocontrol.

7.4.4 Initial studies

The Michael addition of 2-methylcyclohexanone to ethyl vinyl ketone (scheme 56) was attempted in aqueous and surfactant media, in the presence of a chiral amine base. Solutions of the chiral surfactants **62** and commercially available MDEB were used, operating at concentrations slightly above the critical micellar concentration. A control reaction was carried out in deionised water.



62



MDEB

Of the commercially available chiral amines, phenylethylamine is available at moderate cost in both its enantiomeric forms. Therefore (*S*)-α-phenylethylamine was used in 0.6 mole equivalence (so as to be above the catalytic but below the quantitative levels) and the reactions were carried by simple stirring of the reactants at room temperature.

7.4.4.1 Results

NMR analysis of the isolated reaction products identified them to be the aldolised and dehydrated Robinson annelation product, **65**. This was a divergence from the Monteleone study (which isolated mainly the corresponding ketol), and was attributed to the presence of the amine base in the reaction system. Chiral HPLC (Chiralcel OB column) was employed to determine any degree of enantioselectivity obtained in the cyclisation.

Amine	Reaction Medium	Yield / %
(*S*)-α-phenylethylamine	**62**	28
(*S*)-α-phenylethylamine	MDEB	5
(*S*)-α-phenylethylamine	Water	2

Table 25: Results with chiral amine/aqueous systems

Yields for all three reactions were low, though **62** performed significantly better than the other systems. In all three cases, chiral HPLC analysis of the isolated enones indicated that the Michael addition had proceeded without asymmetric induction

The comparative deficiency of MDEB was rationalised by an examination of the head group of this surfactant. Its two resolved chiral centres, when orientated in the Stern-layer of a micelle, can be expected to exert more steric demand on an embedded chiral amine-enolate complex than in the case of the singularly resolved centres of the head groups present in the Stern-layer of the micelles formed by **62**. This steric demand may result in the Michael donor, a complexed amine-enolate species, being unable to penetrate the Stern-layer of the micelle and react with a

Michael acceptor within. The situation therefore approaches that of the free water case, which was shown to give a very low yield of product.

7.4.5 Further studies

To test the assertion that the addition is promoted when this reaction takes place in the Stern-layer, the investigation was extended to alternative reaction conditions. MDEB was disgarded and investigations centred on **62** and water, to compare the efficacy of a micellar versus simple aqueous system. Elevated temperatures and lithium chloride were tried. This salt is understood to increase the hydrophobic effect and 'salt' reactants into the micelle and away from the bulk solution.[214]

7.4.5.1 Results

Effect of elevated temperature

Amine	Reaction Medium	Reaction Temperature	Yield
(*S*)-α-phenylethylamine	**62**	room temp	32
(*S*)-α-phenylethylamine	**62**	80°C	22
(*S*)-α-phenylethylamine	Water	room temp	2
(*S*)-α-phenylethylamine	Water	80°C	48

Table 26: Effects of elevated temperature

A raised temperature was found to increase the yield of Robinson adduct in the case of water, but to decrease it when applied to the surfactant system. This was rationalised in terms of the temperature dependence of the CMC, altering the solubility of the reactants in the micelles. In all cases, chiral HPLC analysis indicated that no asymmetric induction had occurred during the Michael addition step.

Effect of 'salting in' agent

Amine	Reaction Medium	Yield %	
		With LiCl	*Without LiCl*
(*S*)-α-phenylethylamine	**62**	45	32
(*S*)-α-phenylethylamine	Water	28	19

Table 26: Effect of lithium chloride

Chiral HPLC analysis of the adducts formed indicated that no asymmetric induction had occurred during the Michael addition step. Lithium chloride, as a 'sating in' agent, acted to significantly increase the yield of the Robinson adduct in both the surfactant and, interestingly, the plain water system.

Whilst the 'salting in' effect may be invoked to drive the Michael donor and acceptor together within the micellar environment, promoting reaction, the conducive effect seen in the water system was attributed to the increase in ionic strength of this solution, stabilising any amine-enolate complex formed.

Accepting the action of the lithium chloride in promoting reaction within the micellar environment, this added weight to the suggestion that the Michael addition was taking place further into the micellar interior and away from the chiral environment of the Stern-layer.

7.4.6 Comparison of aqueous and organic systems

In a micellar medium and the presence of a mild inorganic base, such as carbonate or hydroxide, the Michael addition progressed with a subsequent aldol condensation to form the corresponding octalone **65**. However, this reaction was generally low yielding and proceeded without asymmetric induction (though these factors may be improved upon through the use of salting-in reagents and, possibly, more sterically demanding micelles).

The use of chiral amine bases in conjunction with micellar media was shown to be conducive to promoting the Michael addition, but the effect appeared to be

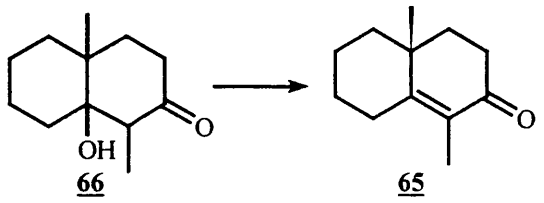
dependent on specific surfactant-base combinations and no asymmetric induction was detected.

In conclusion, from the point of view of ketol formation *via* a Michael type addition, the use of micellar media appeared to drive the reaction to the corresponding octalone dehydration product in a not wholly predictable way.

Thus, with 1,4a-dimethyl-8a-hydroxy-1,4,4a,5,6,7,8,8a-octahydro-3*H*-naphthalen-2-one **66** the appropriate target for this particular investigation, an organic alkoxide/alcohol reaction system, whilst only moderately yielding, was judged preferable to the aqueous media tested.

7.5 Enantioselective dehydration

An investigation was made into the enantiocontrol exerted by amino acids in the dehydration of ketol **66** to the corresponding octalone, (4a*R*)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3*H*-naphthalen-2-one **65** (scheme 57).



66 **65**

Scheme 57: Enantioselective dehydration

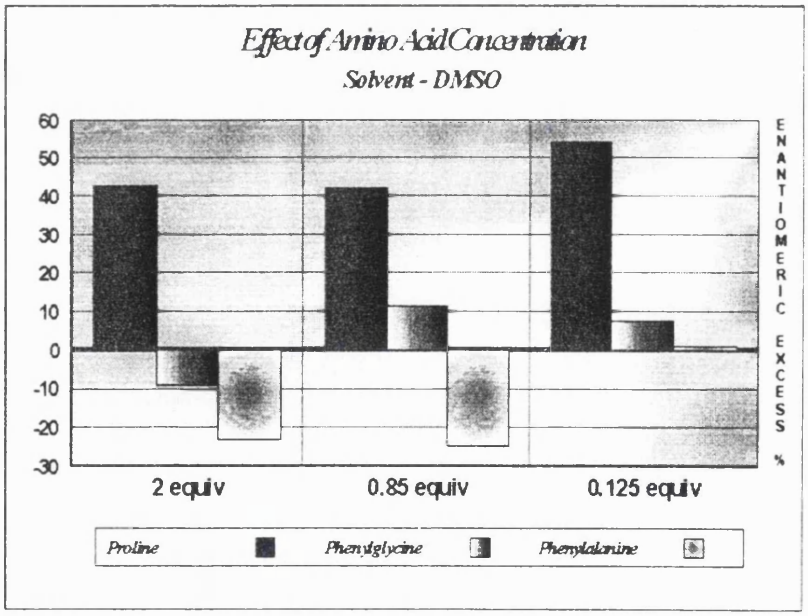
The experimental protocol employed was that of Agami, whereby the ketol was heated in a solvent of choice with the amino acid present.[200] The investigation involved the variation of amino acid, its concentration, and the solvent employed, while keeping the reaction time and temperature constant (48 hours at 65 °C). Chiral HPLC analysis was selected as a rapid means of measuring the degree of any enantioselectivity obtained in the resulting octalones. From the Agami study, it is known that racemic **66** is dehydrated to (+)-(4a*S*)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3*H*-naphthalen-2-one **65a** with (*S*)-proline.[200]

7.5.1 Results

Notes on the graphs:

1. A high percentage of recovered alcohol was indicative that the dehydration had not proceeded very far during the course of the reaction. A figure of 50 would be required for the ideal recovery, whereby 50% of the racemate was recovered unreacted.
2. The yield of octalone **65**, the dehydration product, was calculated on the basis of the amount of **66** consumed *i.e.* after recovery of any unreacted decalol. It was therefore a measure of the efficiency of the dehydration step.
3. A negative figure for enantiomeric excess indicated that (-)-(4a*R*)-**65** was the enantiomer in excess, a positive figure (+)-(4a*S*)-**65a**.

Effect of amino acid choice on enantiomeric excess



Graph 9: Effect of amino acid choice on enantioselectivity

- The greatest optical enrichment was seen with proline, with a maximum e.e. of 54%.
- Phenylglycine exerted essentially no enantiocontrol.

- Phenylalanine gave a product enriched in the opposite enantiomer to that with proline. This was rationalised by studying models that showed the aromatic ring of the phenylalanine was able to fold round onto the decalol **66** in a way the rigid proline molecule was unable to do (see section 7.5.2, figure 20).
- Enantiomeric excess showed no relationship with amino acid concentration for proline and phenylglycine. However,
- At low amino acid concentration, the dehydration product was racemic with phenylalanine.

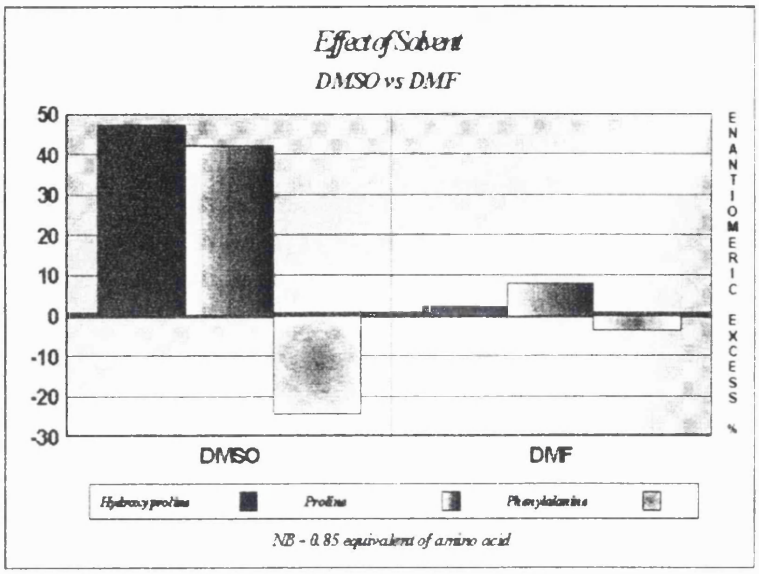
Effect of amino acid choice on yield of octalone

Amino acid	Catalyst concentration Equiv	Solvent	Octalone yield %	Recovered ketol %
(*S*)-Phenylalanine	2.0	DMSO	99	58
(*S*)-phenylalanine	0.85	DMSO	93	93
(*S*)-Phenylalanine	0.125	DMSO	45	45
(*R*)-Phenylglycine	2.0	DMSO	32	47
(*R*)-Phenylglycine	0.85	DMSO	65	82
(*R*)-Phenylglycine	0.125	DMSO	33	64
(*S*)-Proline	2.0	DMSO	83	68
(*S*)-Proline	0.85	DMSO	99	87
(*S*)-Proline	0.125	DMSO	63	90

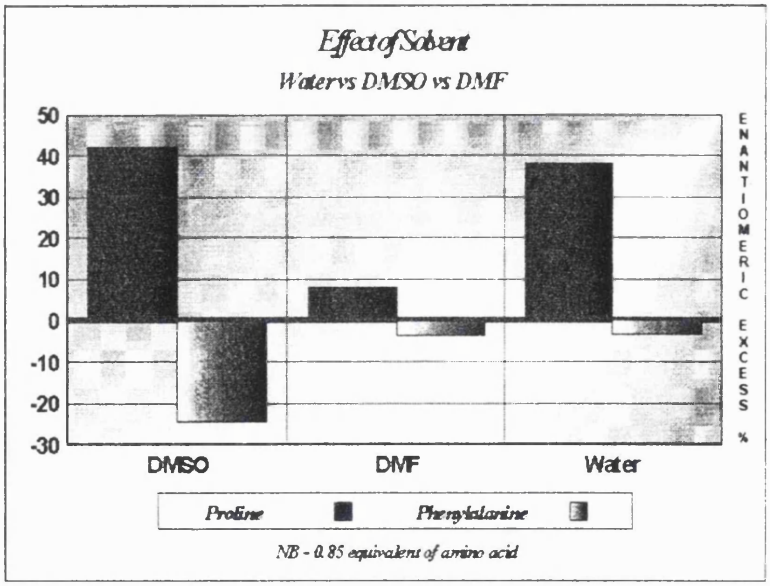
Table 27: Effect of amino acid choice on yield

- Conversion of the decalol to the dehydration product showed a weakly positive correlation with amino acid concentration.
- Recovered unreacted alcohol exhibited a weakly negative relationship with amino acid concentration, indicating greater conversion with increasing amino acid present.

Effect of solvent on enantiomeric excess



Graph 10: Effect of solvent on enantiomeric excess



Graph 11: Effect of solvent on enantiomeric excess

- Enantiomeric excess exhibited a strong solvent dependency, where enantiomeric excess in DMF << in DMSO.
- Enantiomeric excess in water was slightly reduced compared to DMSO, though greater than in DMF.

Effect of solvent on yield

Amino acid	Catalyst concentration equiv	Solvent	Octalone yield %	Recovered ketol %
(*S*)-Phenylalanine	0.85	DMSO	93	48
(*S*)-Phenylalanine	0.85	DMF	99	58
(*S*)-Phenylalanine	0.85	Water	21	87
(*S*)-Proline	0.85	DMSO	99	87
(*S*)-Proline	0.85	DMF	49	49
(*S*)-Proline	0.85	Water	8	79
4-Hydroxyproline	0.85	DMSO	21	85
4-Hydroxyproline	0.85	DMF	20	83

Table 28: Effect of solvent on yield

(Note: The 4-hydroxyproline isomer used was the (2*S*,4*R*)-(-)-4-hydroxypyrrolidine-2-carboxylic acid)

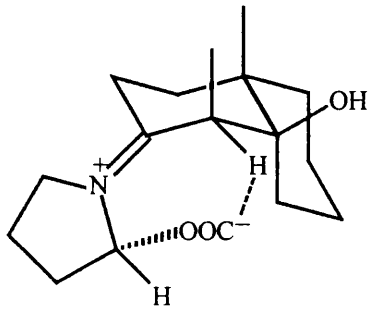
- Yield was reduced in DMF (*versus* DMSO) for proline and phenylglycine, though comparable for phenylalanine and hydroxyproline.
- Yield in water was significantly lower, with the reaction not progressing very far in the timescale of the experiment.

These results indicated a positive correlation between enantiomeric excess and the polarity of the solvent used. Yield, on the other hand, seemed to be significantly increased by the use of aprotic, as opposed to protic solvents. This second observation was unsurprising in view of mechanism for the dehydration proposed by Spencer *et al*, whereby aprotic conditions increase the basicity of the amino acid carboxylate group when deprotonating at the position α- to the immonium group formed with the amine part of the catalyst.[208,209]

7.5.2 Initial conclusions

Optimal conditions - proline at one equivalent concentration in DMSO - resulted in a maximum enantiomeric excess of 54% in the octalone **65** but a high recovery of

the ketol starting material **66** (87% of that used). The low conversion was observed in many of the experiments and attributed to the need for this ketol to possess an equatorial hydrogen for the amino acid to co-ordinate to and deprotonate, *via* the imine (figure 19).

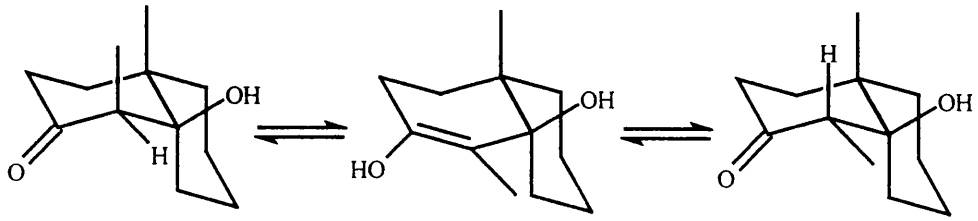


------ H-Bonding interaction

Figure 19: Co-ordination in transition state

Since the decalol **66** is a mixture of isomers, with both axial and equatorial hydrogens adjacent to the carbonyl group, the enantioselective reaction is limited to that diastereoisomer which possesses this axial methyl, equatorial hydrogen arrangement. It is thus a classical kinetic resolution process.

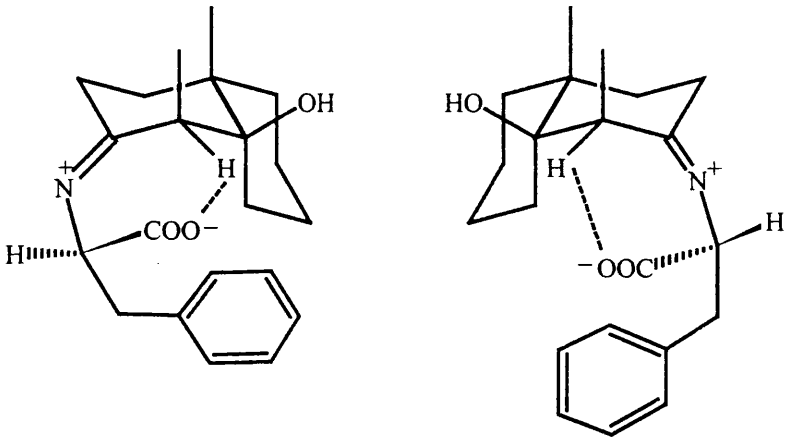
It was therefore thought that the reaction could be driven further, with no loss of enantiocontrol (which, ultimately, depends on the stereochemistry at the ring junction) if this α-position could be epimerised such that all the hydrogens present could be in an equatorial position, however transiently, to react (scheme 58).



Scheme 58: Epimerisation of α-position

In rationalising the opposite stereoselectivity observed with (*S*)-phenylalanine to that of (*S*)-proline, models suggested that the larger and more flexible backbone of phenylalanine destabilised the transition state in the 'favoured' isomer, through

interaction between the phenyl and carbocyclic rings. This effect was thought to allow the less favourable hydrogen bonding interaction in the 'less-favoured' isomer to dominate (figure 29).



'Favoured' stereoisomer' 'Unfavoured' stereoisomer'

------ H-Bonding interaction

Figure 20: Proposed transition state with phenylalanine

This was an extremely important observation. Agami *et al* used the role of the amino acid as a catalytic dehydrator as direct support for an enamine type intermediate in the cyclisation of triketone in the Hajos-Parrish reaction (figure 41).[200] This premise would require the phenylalanine to elicit the same stereoselectivity with the monoketone analogue **66** to that with the triketone **57**. This had clearly been shown not to be the case and intriguingly, gave a new insight into the mechanism of the original Hajos-Parrish cyclisation.

In chapter six, two mechanisms were described to rationalise the stereoselectivty of the Hajos-Parrish reaction. In order to rationalise the differing stereoselectivity exhibited by phenylalanine, the choice of solvent regime may now be understood to influence the transition states through which both the dehydration (**66** to **65**) and cyclisation (**57** to **58**) reactions proceed. For example, an enamine type intermediate when the reaction proceeds in an aprotic environment and a carbinol type complex when the cyclisation is carried out in a polar solvent. At the very least, this divergence indicated that without isolating other possible effects (such as secondary

complexation through other carbonyl groups present in 57), the monoketone system 66 may not be a suitable model for probing the Hajos-Parrish reaction

7.5.3 Epimerising conditions - acetic acid as solvent

In order to achieve the epimerisation of the position adjacent to the carbonyl group in the starting material 66, acetic acid was chosen as a protic solvent, employing the proline (as the most successful catalyst). This reaction was carried out against a control experiment, in the absence of a catalytic dehydrator, in order to isolate the epimerisation effect due to the solvent.

Catalyst	Solvent	Octalone yield %	Enantiomeric excess %	Recovered decalol %
(S)-Proline	Acetic acid	82	2	10
None	Acetic acid	90	0	70

Table 29: Effect of epimerising conditions

- Both products were found to be racemic by chiral HPLC analysis.
- In the presence of a catalyst, a high yield of octalone and high consumption of the starting material was consistent with epimerisation at the α- position.
- In the absence of proline, the corresponding conversion of ketol 66 to octalone 65 was significantly lower.

This final point demonstrated that amino acids must act as catalytic dehydrators in the rate enhancement of the dehydration step, even when the stereochemistry at the α-position is favourable under acidic, epimerising conditions.

In the case of the proline system, since this reaction proceeded beyond the theoretical maximum of 50% for maximum enantiomeric excess, higher optical purity may have been obtained had the reaction been halted sooner. Other factors of influence could include reduction of the acetic acid concentration to limit this type of uncontrolled dehydration, or the use of catalytic amounts of acid, sufficient to

promote interconversion of the axial and equatorial α-hydrogens, in other solvents systems.

7.6 Conclusions of the catalytic dehydration study

The catalytic dehydration study provided access to optically enriched (+)-(4a*S*)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3*H*-naphthalen-2-one **65a** through an enantioselective dehydration of the corresponding β-ketol. Factors affecting both the yield and degree of optical enrichment of the resulting octalone were studied and the combination of proline as the amino acid catalytic dehydrator, in a solvent of DMSO, was identified as being optimal. This generated the octalone **65a** in an enantiomeric excess of 54% and a yield of >99% (based on consumption of **66**).

In extending the study, most obviously the catalytic properties of other amino acids could be investigated and, as alluded to in section 7.5.3, the catalytic use of acid, in other solvent systems (to effect interconversion of the α-hydrogens in the parent ketol and therefore promote conversion to the octalone).

The similarity of the results of proline with those of hydroxyproline (yield of octalone excepted) suggested that the catalytic properties of these two amino acids may be considered the same, by virtue of their shared structural characteristics at the amino acid head of these molecules.

In view of the mechanism by which the dehydration of the β-ketol is believed to proceed[200,208,209] and the lessons learnt in chapter six regarding the need to maintain this amino functionality intact to preserve catalytic ability, hydroxyproline would appear an ideal candidate from which to prepare a surfactant derivative. The presence of a hydroxy group on the heterocyclic ring provides a site through which to attach a long aliphatic chain, without obstructing the key amino acid functionalities that catalyse this dehydration.

Such a surfactant could be envisaged to be as effective as proline, the most successful of the amino acids investigated in this study, as a result of their structural similarity. The use of an aqueous system was demonstrated in this investigation to be effective in inducing enantiocontrol with proline as a catalyst. The presence of

rigid micelles formed by such an *O*-alkyl hydroxyproline surfactant may enhance the catalytic ability of the proline headgroup and address the comparatively low yield of the aqueous reactions to date.

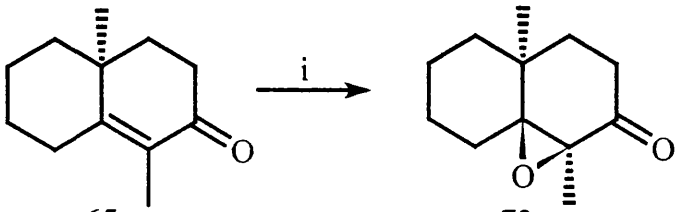
7.7 Manipulation to geosmin

To demonstrate the integration of the catalytic dehydration study into a viable synthetic strategy to geosmin, a quantity of the octalone **65a** resulting from that work was transformed to the unnatural analogue **1a** of this target molecule, through a sequence of reported experimental steps that have become the standard procedure for such syntheses.

7.7.1 Epoxide formation

In order to introduce the tertiary hydroxy group at the ring junction, the established procedure, since the first geosmin synthesis by Marshall, has been through expoxidation and subsequent opening.[4] In order to achieve this stereospecifically from the intermediate **65a**, the 4a-methyl group at the ring junction is used as a stereochemical handle to direct transformations occurring at the enone moiety.

Epoxidation may be achieved with a variety of oxidising agents, which have each been shown to exert their own effect on the stereochemistry of the epoxide obtained.[215] Where the stereochemistry of the 4a-methyl group at the ring junction is defined absolutely, however, this group is able to direct the formation of a single disastereoisomer when epoxidising under standard experimental conditions with *m*-CPBA in dichloromethane (scheme 59).



65a **72a**

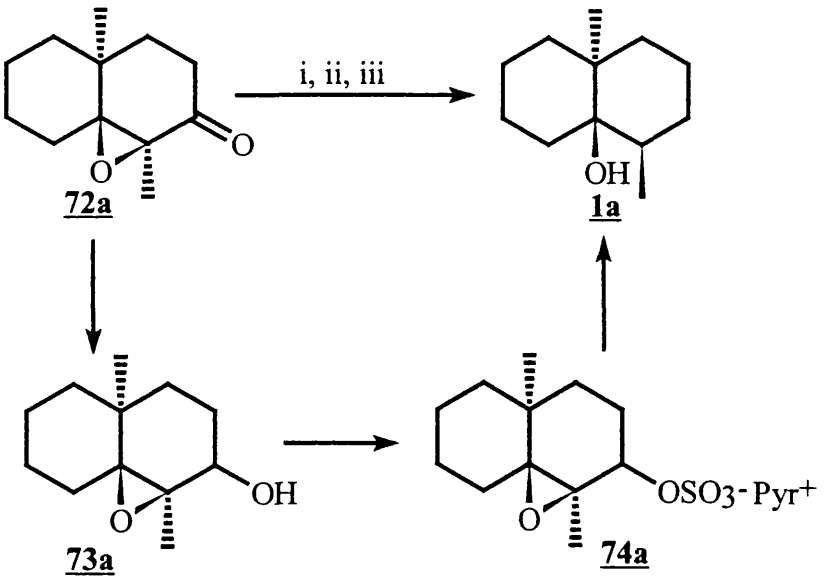
(i):*m*-chloroperbenzoic acid, DCM

Scheme 59: Epoxidation of octalone

Thus (1*S*,4a*S*,8a*S*)-1,4a-dimethyl-1,8a-epoxyperhydronaphthalen-2-one **72a** was prepared in 92% isolated yield from the corresponding octalone (obtained from the dehydration study).

7.7.2 Geosmin

The most experimentally efficient transformation of the bicyclic epoxide **72a** to geosmin is by the procedure of Hansson and Carlson, who carried out the four step conversion following a single-pot reaction protocol.[10a] The sequence involves the deoxygenation of the ketone functionality through conversion, firstly to the alcohol and then derivatisation to the sulfonyl ester leaving group, followed by simultaneous oxirane cleavage and reductive elimination of this leaving group. This can be achieved in one pot, owing to the high yields for each discrete step, shown in scheme 60::



(i):$LiAlH_4$, THF (ii):SO_3.Pyridine (iii):$LiAlH_4$, THF

Scheme 60: Transformation to geosmin

Thermodynamic control is used to limit the initial reduction to the ketone only, and to control the sulfonyl ester synthesis. In this respect, the use of the bulky (and non-nucleophilic) pyridine as a base ensures that the epoxide functionality remains intact under these basic conditions.

With the stereochemistry of the epoxide group in **72a** set by its opposition to the axial methyl group at the bicyclic ring junction, oxirane opening by hydride occurs at the most accessible site (C-8a), with inversion at this centre, to generate the *syn* methyl-hydroxyl group relationship in geosmin.

Thus, following the Hansson experimental procedure[10a], optically enriched, unnatural geosmin **1a** was prepared in 40% yield from the expoxy intermediate, (1*S*,4a*S*,8a*S*)-1,4a-dimethyl-1,8a-epoxyperhydronaphthalen-2-one **72a**. The optical rotation of the unnatural geosmin obtained was measured and found to be $[\alpha]_{25}$ = +7.5°. This compared with a literature value of $[\alpha]_{25}$ = -15.5° for the natural isomer[1] and a theoretical maximum of $[\alpha]_{25}$ = +8.4° (given that the parent octalone **65a** had an enantiomeric excess of 54%). This data therefore demonstrated that optical purity had not been seriously compromised during the deoxygenation process.

7.8 Conclusions of the chapter

A synthetic route to optically enriched geosmin was demonstrated, with the key transformation in setting the stereochemical course of the sequence being the amino acid catalysed dehydration of the β-ketol, 1,4a-dimethyl-8a-hydroxy-1,4,4a,5,6,7,8,8a-octahydro-3*H*-naphthalen-2-one **66**. The preparation of this intermediate was studied, with the view to extending the success of micellar media in promoting Michael addition reactions involving cyclic ketones begun in chapter six.

The effects of amino acid and solvent choice on the optical and chemical yields of the dehydration reaction were also investigated, with the identification of ways in which surfactant technology could be applied to this dehydration. Arising from this study, a postulate for solvent dependency of the mechanism by which the Hajos-Parrish proceeds was stated.

APPENDIX A: EXPERIMENTAL

Mass spectrometry was carried out at the London School of Pharmacy where spectra were recorded on a VG micromass 305 electron impact (EI) mass spectrometer. Proton and carbon nuclear resonance spectra (^{1}H- and ^{13}C-NMR) were recorded on a Varian VXR-400 (400 MHz) spectrometer, the chemical shifts (δ) being recorded in ppm (parts per million). Infra red (IR) spectra were obtained on a Nicolet 205 FT-IR spectrometer. Chiral HPLC analysis was carried out using a Chiralcel OD, OB and AD columns with hexane/isopropyl alcohol mixtures as the mobile phase and UV detection. GLC analysis was performed with the help of Bush Boake Allen, using a temperature Chromosorb (SP2100) packed column fitted to a flame ionisation detector under isothermal conditions (120 °C, carrier gas N_2 at 60 psi back pressure). Commercially available Merck TLC glass sheets (silica gel 60 F_{254}) were used for thin layer chromatography. Visualisation was carried out by a mixture of techniques – UV, I_2, and staining (aqueous potassium permanganate solution, 5% $(NH_4)_6Mo_7O_{24}$ + 0.2% $Ce(SO_4)_2$ in 5% aqueous H_2SO_4, 0.5 g vanallin + 0.5 ml H_2SO_4 + 9 ml ethanol). Flash chromatography was performed using BDH flash silica (particle size 40-63 μm) as the stationary phase. Unless otherwise stated, where reactions were carried out at reduced temperature, acetone-dry ice cooling baths mixtures were employed. The use of anhydrous conditions refers to reactions carried out under an inert atmosphere of nitrogen.

Butan-2,3-diol

FERMENTATION STUDIES

The following is representative of the procedure employed in carrying out the resuscitation and initial growth of the *Bacillus subtilis* strains:

Growth of Bacillus subtilis on agar slopes

Standard nutrient agar slopes of the following composition were prepared and autoclaved at 120 °C for 20 mins before allowing to set: peptone (Difco) 20 g/l; yeast extract (Difco) 3 g/l; sodium chloride 5 g/l; α-D-glucose (Sigma) 5 g/l; agar (Sigma) 20 g/l. These slopes were then inoculated with the freeze dried *Bacillus subtilis* strain, suspended in a small volume of growth medium (excluding the agar), then incubated at 50 °C for 3 days to resuscitate the bacteria.

Growth of Bacillus subtilis in liquid medium

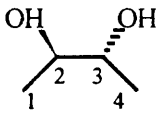
The aqueous medium used to further cultivate the bacteria had the following composition: peptone (Difco) 20 g/l; yeast extract (Difco) 3 g/l; sodium chloride 5 g/l; α-D-glucose (Sigma) 5 g/l; This medium was autoclaved at 120 °C for 20 mins before use. Colonies from the agar slant tube cultures were used to inoculate 250 ml Erlenmayer flasks containing 50 ml of the above medium and the resulting broths were rotated in an orbtial incubator (250 rpm) at 30 °C for 4 days.

Growth of Bacillus subtilis in high sugar liquid Media and osolation of metabolites

The following is representative of the general procedure employed in the investigation of the physical parameters affecting the fermentation of different sugars by strains of *Bacillus subtilis*:

High glucose concentration broths of the following composition were prepared, autoclaving at 120 °C for 20 mins before use: peptone (Difco) 20 g/l; yeast extract (Difco) 3 g/l; sodium chloride 5 g/l; α-D-glucose (Sigma), variable (or other sugar - see section 2.4). Known volumes of *Bacillus subtilis* cultures in liquid media (above) were used to inoculate the high glucose broths before incubating at 30 °C under the conditions given in sections 4.5 and 4.8. After this incubation time the culture broth was diluted with dichloromethane (300 ml/l broth) in a separating funnel. After separation, the aqueous layer was extracted twice more with similar volumes of solvent before combining the organic extracts and drying over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude product analysed by GLC (9' 10% SP2100 packed silica, 120 °C isothermal) against standard samples to determine the concentration of the butan-2,3-diol present. The crude isolates were then were purified by flash chromatography (ethyl acetate-petroleum ether 40-60 °C, 1:1) to isolate the butan-2,3-diol and acetoin, the only other product of the fermentation.

Laboratory scale production of (D)-(-)-(2R,3R)-butandiol (5b) by Bacillus subtilis (JK):



High glucose concentration broths of the following composition were prepared, autoclaving at 120 °C for 20 mins before use: peptone (Difco) 20 g/l; yeast extract (Difco) 3 g/l; sodium chloride 5 g/l; α-D-glucose (Sigma) 25 g/l. 62.6 ml/l volumes of *Bacillus subtilis (JK)* cultures in liquid media were used to inoculate the high glucose broths before incubating at 37 °C (150 rpm) for 11 to 12 days. After this incubation time the culture broth was saturated with sodium chloride then diluted with ethyl acetate (300 ml/l broth) in a separating funnel. After separation, the aqueous layer was extracted twice more with similar volumes of solvent before combining the organic extracts and drying over anhydrous magnesium sulfate. The

solvent was removed *in vacuo* and the crude product purified by flash chromatography (ethyl acetate-petroleum ether 40-60 °C 1:1) to isolate the desired butan-2,3-diol.

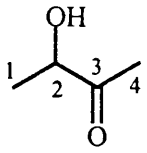
(Found: M^+, 90.0680. $C_4H_{10}O_2$ requires: *M*, 90.0681);

ν_{max}(film) 3400s, 2972s;

δ_H (400MHz; $CDCl_3$ 3.48 (2H, m, C2 and C3-H), 2.30 (2H, br s, C2 and C3-O*H*), 1.15 (6H, d, *J* 6.0 Hz, C1 and C4-H_3),

δ_C (100MHz; $CDCl_3$) 76.01 (C2 and C3), 15.83 (C1 and C4);

Acetoin - isolated from fermentation broths



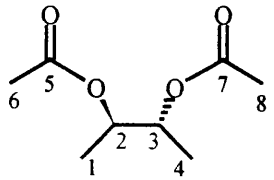
(Found: M^+, 88.0520. $C_4H_8O_2$ requires: *M*, 88.0524);

ν_{max}(film) 3384s, 2972s, 1730s;

δ_H (400MHz; $CDCl_3$) 4.23 (1H, q, *J* 7.0 Hz, C2-H), 2.18 (3H, s, C4-H_3), 1.36 (3H, d, *J* 7.0 Hz, C1-H_3);

δ_C (100MHz; $CDCl_3$) 198.02 (C3), 81.23 (C2), 28.12 (C4), 16.48 (C1);

(2R,3R)-Butanediacetate



For the purpose of measuring the optical purity of the butan-2,3-diol isolated from the fermentation broths by chiral HPLC analysis, derivatisation to the diacetate was carried out in accordance with the following procedure:

To a sample of the isolated butan-2,3-diol (0.300 g, 3.33 mmol) was added 1 ml of acetyl chloride and the mixture shaken. When effervescing had ceased water was added and the mixture shaken again. The diacetate was isolated by extraction with ethyl acetate (10 ml) which, after drying and removal of solvent *in vacuo*, was analysed by chiral HPLC, against standard samples of the three enantiomeric forms of the diacetate, to determine enantiomeric excess.

(Found: M^+, 174.0896. $C_8H_{14}O_4$ requires: *M*, 174.0892);
ν_{max}(film) 3002s, 1675s;
δ_H (400MHz; $CDCl_3$) 4.95 (2H, m, C2 and C3-H), 2.16 (6H, 2 x s, C6 and C8-H_3), 0.98 (6H, m, C1 and C4-H_3);
δ_C (100MHz; $CDCl_3$) 172.69 (C5 and C7), 77.15 (C2 and C3), 17.87 (C6 and C8), 16.30 (C1 and C4);
Chiral HPLC (Chiralcel OD column, hexane:propan-2-ol, 99:1, 1 ml/min, UV: 226 nm) 5.92 min (*meso*-2,3-butanediacetate), 6.43 min ((2*R*,3*R*)-butanediacetate), 6.68 min ((2*S*,3*S*)-butanediacetate).

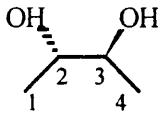
ENZYMATIC RESOLUTION STUDIES

The following is representative of the procedure employed for the investigations into the enzymatic kinetic resolutions of the racemic diol:

Commercially available isomeric butan-2,3-diol (2.70 g, 30.0 mmol) was charged to a round bottomed flask with the selected acylating agent (35 ml) and lipase (0.5 g) under investigation. The reagents were stirred at the specified temperature for the specified time before stopping the reaction by filtration to physically remove the enzyme catalyst. The mixture was concentrated *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 2:1) to isolate the diacetylated, monoacetylated and unreacted butan-2,3-diol isomers. The derivatised

forms were later analysed by chiral HPLC (Chiralcel OD) to identify the degree of enantioseparation that had occurred in the resolution.

Optimised procedure for the preparation of (L)-(+)-(2S,3S)-butan-2,3-diol (5a)



Commercially available isomeric butan-2,3-diol (27.0 g, 300.0 mmol) was charged to a Erlenmayer flask with vinyl acetate (350 ml) and Novozyme 435 (5 g), stoppering the vessel lightly with a cotton wool bung secured with aluminium foil. The flask was placed in an orbital incubator and shaken (100 rpm) at 37 °C for 5 hrs. After this time, the mixture was filtered to remove the supported enzyme and the vinyl acetate removed *in vacuo* to leave the crude mixture of unreacted and monoacetylated butan-2,3-diol. This was purified by flash chromatography (hexane-ethyl acetate, 2:1) to yield L-(+)-(2*S*,3*S*)-butandiol (**5a**) in its unreacted form (2.50 g, 9% recovery, ~35% theoretical yield (based on 25% available enantiomer in the isomeric mixture)).

(Found: M^+, 90.0680. $C_4H_{10}O_2$ requires: *M*, 90.0681);

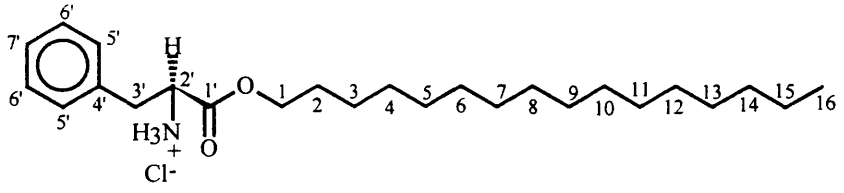
ν_{max}(film) 3408s, 2969s;

δ_H (400MHz; $CDCl_3$) 3.47 (2H, m, C2 and C3-H), 2.90 (2H, br s, C2 and C3-O*H*), 1.13 (6H, d, *J* 5.0 Hz, C1 and C4-H_3);

δ_C (100MHz; $CDCl_3$) 76.01 (C2 and C3), 15.83 (C1 and C4);

Aqueous and micellar phase reactions

(S)-phenylalanine hexadecanylester hydrochloride (11)



(*S*)-phenylalanine (3.48 g, 21.2 mmol), hexadecyl alcohol (5.59 g, 23.3 mmol) and a few crystals of *p*-toluenesulfonic acid were charged, in list order, to a flask containing toluene (150 ml) and the mixture heated at reflux with a Dean-Stark apparatus for 48 hrs. After cooling to room temperature, white crystals appeared. These were isolated by reduced pressure filtration and washed with hexane (100 ml). The solid was recrystallised twice from ethyl acetate to yield the *title compound* as colourless crystals (4.82 g, 42%).

$[\alpha]_{25}$ = +12.13° (c 0.4, EtOH)

(Found: M^+, 425.3058. $C_{25}H_{44}NO_2Cl$ requires: *M*, 425.3060)

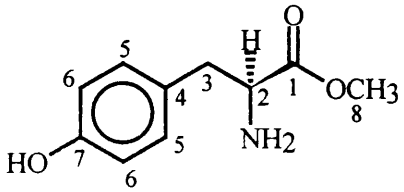
ν_{max}(KBr disc) 3165s, 2910s, 1738s, 1489m, 1465m cm^{-1};

δ_H(400MHz, CD_3OD) 7.40 (1H, m, C7'-H), 7.28 (2H, d, *J* 8.0 Hz, 2 x C5'-H_2), 7.20 (2H, d, *J* 7.2 Hz, 2 x C6'-H_2), 4.33 (1H, t, *J* 7.2 Hz, C2'-H), 4.18 (2H, t, *J* 7.0 Hz, C1-H_2), 3.17 (2H, br m, C3'-H_2), 1.60 (2H, t, *J* 7.0 Hz, C2-H_2), 1.25 (28H, m, C3 to C15-H_2), 0.92 (3H, t, *J* 7.2 Hz, C16-H_3);

δ_C(100MHz, CD_3OD) 168.90 (C1'), 140.45 (C4'), 128.98 (C5'), 126.41 (C6'), 125.90 (C7'), 69.21 (C2'), 64.45 (C1), 39.74 (C3'), 31.19, 30.90, 30.68, 30.66, 30.21, 29.57, 28.99, 28.57, 27.49, 27.11, 26.39, 26.00, 24.54, 23.47, 17.89 (C16);

m/z (EI) 149 (M^+-Cetyl-$NH_3^+Cl^-$, 85%), 97 (53), 83 (63), 57 (100).

(S)-tyrosine methylester hydrochloride (6)

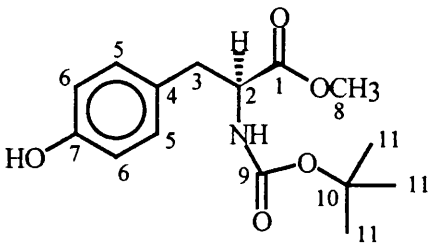


(*S*)-tyrosine (3.00 g, 17.0 mmol) was dissolved in dry methanol (150 ml) and the solution chilled to –5 °C by means of an external ice-salt bath. Thionyl chloride (3.10 ml, 42.5 mmol) was added slowing over 30 mins, maintaining a flask temperature of 0 °C. The temperature of the solution was increased to room temperature and stirred for 18 hrs. After this time, the solvent was removed *in vacuo*

to give the crude product, which was purified by recrystallisation from hexane. This gave the *title compound* as a creamy white solid (3.74 g, 95% yield).

ν_{max}(KBr disk) 3339s, 3200br s, 2919s, 2849s, 1741s, 1613m, 1514m, 1450s cm^{-1};
δ_H (400MHz; CD_3OD) 11.25 (1H, s, C7-O*H*), 7.10 (2H, d, *J* 9.2 Hz, 2 x C5-H), 6.70 (2H, d, *J* 9.2 Hz, 2 x C6-H), 4.10 (1H, dd, *J* 6.4 and 7.2 Hz, C2-H), 3.65 (3H, s, C8-H_3), 3.00 (2H, m, C3-H_2);
m/z (FAB) 196 (M^++1, 70%), 182 ((Tyrosine)$^+$, 78), 154 ((C_6H_5)-CH_2-CH-$NH_2)^+$, 23), 136 (HO-(C_6H_5)-CH_2-CH-$NH_2)^+$, 14), 107 (HO-(C_6H_5)-$CH_2)^+$, 40).

N-t-butoxycarbonyl (S)-tyrosine methylester (7)



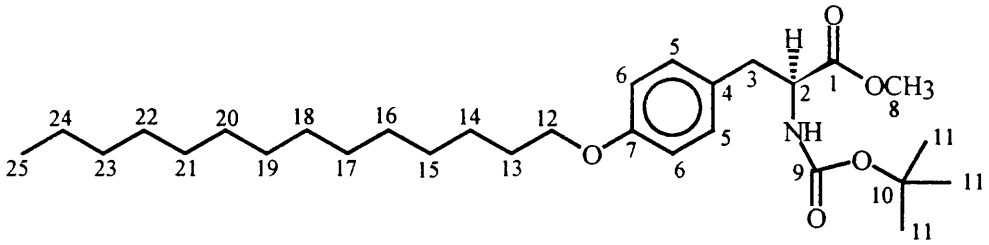
(*S*)-tyrosine methylester hydrochloride (**6**) (2.00 g, 78.0 mmol) was dissolved in a mixture of dioxane (20 ml) and water (10 ml). To this was added an aqueous solution of sodium hydroxide (0.70 g in 20 ml water) and the resulting mixture cooled to 0 °C. Di-*t*-butyldicarbonate (3.00 g, 156 mmol) was added in one portion, the external cooling bath removed, and the reaction mixture stirred at room temperature for 18 hrs. After this time, the temperature was again decreased to 0 °C and the reaction mixture acidified to pH 3 by the addition of 1N hydrochloric acid. The product was extracted into ethyl acetate (3 x 40 ml), washed with brine (1 x 10 ml) and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 1:1) to leave the *title compound* as a pale yellow oil (1.66 g, 72%).

ν_{max}(paraffin oil) 3406br s, 2925s, 2854s, 1746m, 1686m, 1616m, 1518m 1458s, 1675m 1236m cm^{-1};

δ_H (400MHz; [d_6]DMSO) 12.5 (1H, s, C7-O*H*), 9.15 (1H, s, C2-N*H*), 7.05 (2H, d, *J* 9.0 Hz, 2 x C5-H), 6.65 (2H, d, *J* 9.0 Hz, 2 x C6-H), 3.81 (1H, dd, *J* 7.0 and 5.3 Hz, C2-H), 3.35 (3H, s, C8-H_3), 2.85 (1H, d, *J* 7.0 Hz, C3-H), 2.70 (1H, d, *J* 5.3 Hz C3-H), 1.35 (9H, s, 3 x C11-H_3);

m/z (FAB) 295 (M^+, 67%), 107 ((HO-(C_6H_5)-CH_2)$^+$, 50), 77 ((C_6H_5)$^+$, 24), 61 (COO-NH_3)$^+$, 20), 57 ((CH_3)$_3$C)$^+$, 30).

N-t-butoxycarbonyl (S)-tyrosine methylester O-tetradecanylether (8)



Performing the reaction under anhydrous conditions, sodium hydride (60% dispersion in mineral oil) (0.37 g, 9.1 mmol) was washed several times with dry hexane then covered with dry THF (30 ml). *N-t*-butoxycarbonyl (*S*)-tyrosine methyl ester (7) (2.50 g, 8.3 mmol) in dry THF (60 ml) was added to the reaction vessel over 15 mins and the resulting mixture stirred at room temperature for 1hr. After this time, 1-bromohexadecane (3.06 g, 100 mmol) in dry THF (20 ml) was added over 10 mins. The reaction mixture was stirred at room temperature for 18 hrs and then quenched by the addition of water (30 ml). After further dilution, the product was extracted into ethyl acetate (3 x 80 ml) then dried over anhydrous magnesium sulfate. After removal of the solvent *in vacuo* the crude material was purified by flash chromatography (hexane-ethyl acetate 6:1) to leave the *title compound* as an oily liquid (2.68 g, 64% yield).

ν_{max}(paraffin oil) 3357br s, 2928s, 2854s, 1748m, 1684m, 1616m 1517m 1458s, 1259m cm^{-1};

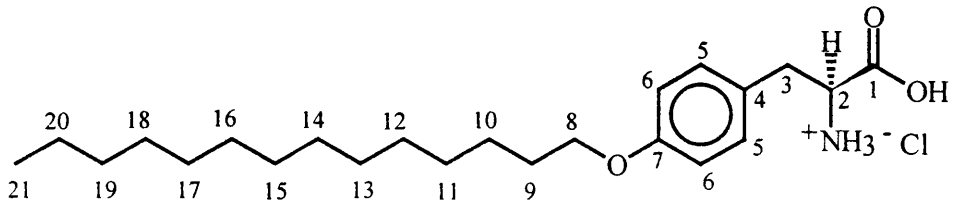
δ_H (400MHz; [d_6]DMSO) 7.27 (2H, d, *J* 9.0 Hz, 2 x C5-H), 6.82 (2H, d, *J* 9.0 Hz, 2 x C6-H), 4.40 (1H, br s, C2-H), 3.93 (2H, d, *J* 2.8 Hz, C3-H_2), 3.72 (3H, s, C8-H_3),

3.41 (2H, t, *J* 6.7 Hz, C12-H_2), 3.05 (1H, br s, C2-N*H*), 2.85 (2H, m, C13-H_2), 1.43 (9H, s, 3 x C11-H_3), 1.21 (22H, m, C14 to C24-H_3), 0.89 (3H, t, *J* 5.6 Hz C25-H_3); *m/z* (FAB) 493 (M^++1, 40%), 178 (M^+-tetradecanyl ether-CH_3-CH_3, 50), 107 ((HO-C_6H_5-CH_2)$^+$, 25), 77 ((C_6H_5)$^+$, 24), 57 (($(CH_3)_3C$)$^+$, 32).

N-Triflate (S)-tyrosine methylester O-hexadecanylether (9)

N-t-butoxycarbonyl (*S*)-tyrosine methylester *O*-tetradecanylether (**8**) (2.67 g, 5.40 mmol) was dissolved in dichloromethane (100 ml). Trifluoroacetic acid (~30 ml) was added over 3 hrs and the reaction mixture was stirred overnight. After this time, TLC analysis indicated that the reaction had reached completion and the solvent was removed *in vacuo*. The residue was washed with diethyl ether, which was itself removed *in vacuo*. By repeating this procedure several times, excess trifluoroacetic acid was removed from the crude product by co-distillation with the ether. The residue (12.3 g) was dried in air and under high vacuum before being used without further purification.

O-hexadecanyl-(S)-tyrosine (10)



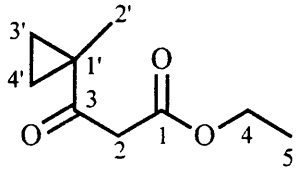
To the crude *N*-triflate tyrosine methylester *O*-tetradecanylether (**9**) (12 g) in THF (250 ml) was added aqueous sodium hydroxide solution (~5 g in 25 ml H_2O) over a 3 hrs period. TLC analysis after this time indicated that the reaction had gone to completion and the brine (80 ml) was added to quench the reaction. The organic phase was washed with brine (2 x 80 ml) and upon addition of a small amount of

ethyl acetate, a product precipitated out. This was isolated by filtration under reduced pressure to give the first crop. The filtrate was diluted with ethyl acetate and dried over anhydrous magnesium sulfate. As the solvent was removed *in vacuo*, a second crop of product precipitated out and was isolated by filtration. Both crops were dried in air and under high vacuum to leave the *title compound* as a flaky white solid (1.42 g in total, 64% yield).

(Found: M^+, 378.3020. $C_{23}H_{40}NO_3Cl^-$ requires: *M*, 378.3020);
ν_{max}(paraffin oil) 3349br s, 2920s, 2851s, 1742s, 1510m, 1480m cm^{-1};
δ_H (400MHz; [d_6]DMSO) 8.48 (1H, br s, C1-O*H*), 7.19 (2H, d, *J* 9.0 Hz, 2 x C6-H), 6.89 (2H, d, *J* 9.0 Hz, 2 x C5-H), 4.09 (1H, br s, C2-H), 3.93 (2H, t, *J* 7.2 Hz, C8-H_2), 3.38 (3H, br s, C2-NH_3), 3.04 (2H, d, *J* 0.9 Hz, C3-H_2), 1.70 (2H, m, C9-H_2), 1.25 (22H, m, C10-H_2 to C20-H_2), 0.83 (3H, t, *J* 6.8 Hz, C21-H_3);
δ_C (100MHz; [d_6]DMSO) 177.35 (C1), 156.30 (C7), 130.68 (C6), 128.68 (C5), 114.56 (C4), 74.36 (C2), 67.47 (C8), 40.48 (C3), 36.21 (C9), 31.36, 29.09, 28.78, 27.90, 27.56, 27.01, 26.39, 25.86, 25.69, 24.51, 22.16, 14.02 (C21);
m/z (EI) 378 (M^+-Cl, 100%), 365 (36), 332 (42), 289 (43), 239 (61).

Biomimetic cyclisations

3-(1-Methyl-cyclopropyl)-3-oxo-propionic acid ethyl ester (14)

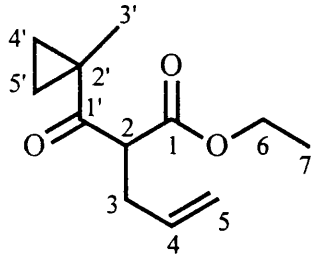


Performing the reaction under anhydrous conditions, sodium hydride (60% dispersion in mineral oil, 1.01 g, 42.3 mmol) was charged to a round bottomed flask under nitrogen and 5 ml of dry tetrahydrofuran added. The suspension was stirred and dry diethyl carbonate (20 ml) was added to the vessel followed by a mixture of 1-acetyl-1-methylcyclhexanone (1.00 g, 10.2 mmol) in a further 3 ml of diethyl

carbonate and 2 drops of absolute ethanol. The resulting solution was stirred for 15 minutes then heated with a water bath for 1hr, by which time the evolution of gas from the reaction mixture had ceased. The vessel was cooled to 0 °C with an external ice bath and the reaction quenched by addition of a solution of diethyl ether (10 ml) and acetic acid (2 ml), followed by water (50 ml). The layers were separated and the aqueous layer extracted twice into diethyl ether (2 x 50 ml). The combined organic extracts were then washed with saturated sodium hydrogencarbonate solution (60 ml) and saturated brine (60 ml). The extracts were dried over anhydrous magnesium sulfate and the solvent removed *in vacuo*. The crude product was purified by distillation under reduced pressure to remove unreacted diethyl carbonate (18 ml collected, b.p. 30-38 °C @ 10 mmHg) then by flash chromatography (hexane-ethyl acetate 5:1) to leave the *title compound* as a pale orange oil (1.14 g, 65 % yield).

ν_{max}(film) 2978m, 1742s, 1692s, 1619m cm^{-1};
δ_H (400MHz; $CDCl_3$) 4.19 (2H, q, *J* 6.1 Hz, C4-H_2), 3.40 (2H, s, C2-H_2), 1.37 (3H, s, C2'-H_3), 1.31 (2H, m, C3'-H and C4'-H), 1.28 (3H, t, *J* 6.1 Hz, C5-H_2), 0.80 (2H, m, C3'-H and C4'-H);
δ_C (100MHz; $CDCl_3$) 204.19 (C9), 167.57 (C1), 61.28 (C4), 45.28, 27.00, 19.50, 18.67, 14.10 (C2');
m/z (EI) 133 (13), 71 (65), 55 (55), 43 (100), 29 (100).

1-Methylcyclopropyl-1-carbethoxy-3-butenyl ketone (15)

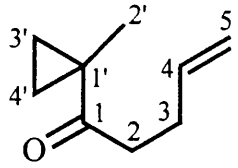


Performing the reaction under anhydrous conditions, sodium hydride (60% dispersion in mineral oil, 0.27 g, 6.80 mmol) was charged to a round bottomed flask

under nitrogen and 5 ml of dry tetrahydrofuran added. The suspension was cooled to 0 °C with an external ice bath and a solution of the keto ester (**14**) (1.10 g, 6.47 mmol) in dry tetrahydrofuran (5 ml) added over 35 mins. The temperature of the mixture was increased to room temperature before allyl bromide (0.86 g, 7.12 mmol) in tetrahydrofuran (5 ml) was added over 15 mins. The resulting solution was stirred for 18 hrs at room temperature then heated to reflux for 1 hr. After cooling the vessel with an external ice bath, the reaction was quenched by the addition of water (15 ml). The aqueous layer was extracted with diethyl ether (2 x 20 ml) and the combined organic extracts were washed once with saturated brine (50 ml) then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* to leave the *title compound* as an oil (1.30 g, 96% yield) which was used without further purification.

ν_{max}(film) 3081m, 2975s, 1741s, 1683s, 1643m cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.72 (1H, m, C11-H), 5.10 (2H, m, C15-H_2), 4.16 (2H, q, *J* 6.2 Hz, C6-H_2), 3.7 (1H, t, *J* 7.3 Hz, C2-H), 2.56 (2H, m, C3-H_2), 1.38 (3H, s, C3'-H_3), 1.35 (2H, m, C4'-H and C5'-H), 1.25 (3H, t, *J* 6.2 Hz, C7-H_3), 0.77 (2H, m, C4'-H and C5'-H);
δ_C (100MHz; $CDCl_3$) 205.69 (C1'), 169.12 (C1), 134.68 (C4), 117.26 (C5), 61.29 (C6), 52.47 (C2), 33.17 (C3), 27.05 (C3'), 19.65 and 18.19 (C4' and C5'), 14.01 (C7);
m/z (EI) 212 (M^++2, 18%), 83 ($(CH_3C(CH_2\text{-}CH_2)CO)^+$, 82), 55 (75), 43 (100).

1-(1-Methyl-cyclopropyl)-pent-4-en-1-one (16)



The above crude allyl keto ester (**15**) (1.25 g, 5.95 mmol) was dissolved in ethanol (7.5 ml) and water (20 ml) and to the solution was added barium hydroxide

octahydrate (3.75 g, 11.9 mmol). The mixture was heated at reflux under nitrogen for 18 hrs, cooled to room temperature, then poured into a solution of water (50 ml), diethyl ether (50 ml) and concentrated hydrochloric acid (12.5 ml). The aqueous layer was extracted with diethyl ether (2 x 40 ml) and the combined organics were washed with saturated sodium hydrogen carbonate solution (40 ml) then saturated brine (40 ml) before drying over anhydrous magnesium sulfate. The solvent was removed *in vacuo* to leave the crude *title compound* as an oil (0.57 g, 70% yield) that was used without further purification.

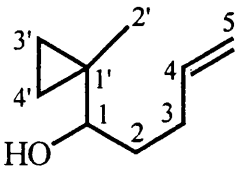
ν_{max}(film) 3080m, 2925s, 1689s, 1643m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.78 (1H, m, C4-H), 4.99 (1H, dd, *J* 14.1 and 1.7 Hz, C5-H_{trans}), 4.93 (1H, dd, *J* 10.2 and 1.7 Hz, C5-H_{cis}), 2.45 (2H, t, *J* 7.0 Hz, C2-H_3), 2.28 (2H, m, C3-H_2), 1.32 (3H, s, C2'-H_3), 1.20 (2H, m, C3'-H and C4'-H), 0.67 (2H, m, C3'-H and C4'-H);

δ_C (100MHz; $CDCl_3$) 210.89 (C1), 137.51 (C4), 114.99 (C5), 37.09 (C2), 29.68 (C3), 28.00, 26.47, 19.75 and 18.02 (C3' and C4');

m/z (EI) 137 (M^+-1, 15%), 125 (24), 111 (50), 97 (66), 83 ($(CH_3\text{-}C(CH_2\text{-}CH_2)\text{-}CO)^+$, 82), 57 (95), 43 (100).

1-(1-Methyl-cyclopropyl)-pent-4-en-1-ol (16)

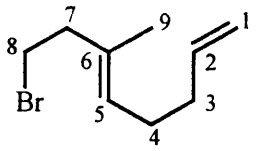


Performing the reaction under anhydrous conditions, 1-methylcyclopropyl-3-butenyl ketone (**15**) (0.25 g, 1.81 mmol) was charged to a round bottomed flask containing dry diethyl ether (15 ml) and lithium aluminium hydride (0.07 g, 1.81 mmol) was added gradually over 5 mins. The mixture was stirred at room temperature for 2 hrs and then 0.25 ml saturated sodium sulfate solution was added to quench the reaction. Solid anhydrous sodium sulfate was added and the mixture left to stand for 30 mins.

After filtration, the solvent was removed *in vacuo* to leave the *title compound* as a colourless liquid (0.21 g, 83% yield) which was used without further purification.

ν_{max}(film) 3355br s, 2932s, 1640s cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C4-H), 5.02 (1H, dd, *J* 15.8 and 1.8 Hz, C5-H_{trans}), 4.94 (1H, dd, *J* 8.2 and 2.0 Hz, C5-H_{cis}), 2.82 (2H, t, *J* 6.0 Hz, C1-H), 2.22 (2H, m, C2-H_2), 2.10 (2H, m, C3-H_2), 1.64 (2H, m, C3'-H and C4'-H), 1.23 (1H, br s, C1-O*H*), 1.01 (3H, s, C2'-H_3), 0.35 (2H, m, C3'-H and C4'-H);
δ_C (100MHz; $CDCl_3$) 138.62 (C4), 114.65 (C5), 78.42 (C1), 33.25 (C2), 30.52 (C3), 29.69 (C2'), 17.09 (C1'), 11.92 and 11.18 (C3' and C4');
m/z (EI) 140 (M^+, 6%), 122 (M^+-H_2O, 80), 57 (90), 43 (100).

(5E)-8-bromo-6-methyl-1,5-octadiene (18)



Hydroxycyclopropylalkene (17) (0.200 g, 1.43 mmol) was added to a mixture of 2,4-collidine (0.140 g), lithium bromide (0.250 g) and dry diethyl ether (5 ml). This was then cooled to –60 °C by means of an external cardice-acetone bath whilst phosphorus tribromide (0.09 ml) was added. The temperature of the reaction mixture was increased to 0 °C and stirred at this temperature for 1½ hrs. Collidine (2 ml) and water (2 ml) were added to quench the reaction and the mixture was poured into a water/diethyl ether mixture (30 ml/30 ml). The product was extracted twice with ether (2 x 20 ml) and the combined extracts washed with water (20 ml), saturated sodium hydrogencarbonate solution (20 ml) and brine (20 ml) before drying over anhydrous sodium sulfate. After removal of the solvent *in vacuo*, the crude material was added slowly to zinc bromide (0.28 g) in dry diethyl ether (5 ml), maintaining a temperature of –60 °C. After warming to 0 °C and stirring at this temperature for a further 1½ hrs, the reaction was quenched by the addition of ether (15 ml) and 50% (w/v) aqueous sodium chloride solution (15 ml). The resulting

aqueous phase was extracted with ether (15 ml) and after washing the combined extracts with brine and drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* to leave the *title compound* as a colourless liquid (0.22 g, 74% yield). This was used without further purification.

(Found: M^+, 202.0388. $C_9H_{15}Br^{\cdot}$ requires: *M*, 202.0357);

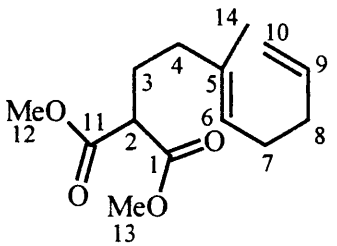
ν_{max}(film) 2925s, 2854s, 1640m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C2-H), 5.21 (1H, m, C5-H), 5.00 (1H, dd, *J* 17.0 and 2.0 Hz, C1-H_{trans}), 4.94 (1H, dd, *J* 11.0 and 2.1 Hz, C1-H_{cis}), 3.41 (2H, t, *J* 7.4 Hz, C8-H_2), 2.51 (2H, t, *J* 7.4 Hz, C7-H_2), 2.08 (4H, m, C3-H_2 and C4-H_2), 1.61 (3H, s, C9-H_3);

δ_C (100MHz; $CDCl_3$) 138.38 (C2), 132.23 (C6), 127.17 (C5), 114.64 (C1), 42.87 (C8), 33.67, 31.70, 27.39, 15.65 (C9);

m/z (EI) 203 ($M^{\cdot}$, 80%), 188 (M^+-CH_3, 42), 123 ($M^{\cdot}$-Br, 95), 108 (M^+-Br-CH_3, 28), 80 (Br, 20), 55 (90).

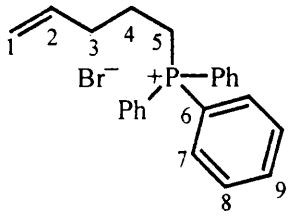
Attempted formation of (5E)-2-methylcarboxylate-5-methyldeca-5,9-diene carboxylic acid methyl ester (19)



Performing the reaction under anhydrous conditions, sodium hydride (2.07 g, 46.0 mmol) was charged to a 100 ml round bottomed flask and washed with several portions of dry hexane. Dry DMF (35 ml) was then added followed by dimethyl malonate (7.78 g, 48.9 mmol) over a 45 min period. The mixture was then stirred for 30 mins and then (*5E*)-*8*-bromo-6-methyl-1,5-octadiene (**18**) (1.00 g, 4.9 mmol) in dry tetrahydrofuran (4 ml) was added over a further 30 mins. The resulting mixture was heated at 45-50 °C for 3 hrs before external cooling with an ice bath.

Ice water (20 ml) was then added to quench the reaction and the product extracted into pentane (3 x 100 ml). After drying over anhydrous magnesium sulfate, and removal of solvent *in vacuo*, the crude product was purified by filtration through a pad of silica (hexane as eluent) to leave 0.85 g of an unidentifiable colourless liquid.

1-Pentene-5-triphenylphosphonium bromide (23)



1-Bromo-5-pentene (4.00 g, 26.8 mmol) was dissolved in 40 ml of toluene and triphenylphosphine was (7.12 g, 27.2 mmol) added. The solution was then heated at reflux for 18 hrs and then cooled to room temperature. The precipitated solid was isolated by reduced pressure filtration, washed with hexane and dried in air. This *title compound* (2.81 g, 24% yield) was used without further purification.

(Found: M^+, 331.1610. $C_{23}H_{24}P^+$ requires: *M*, 331.1616);

m.p. 189 °C (*Lit.*[216] 191 °C)

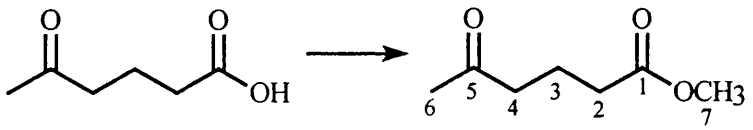
ν_{max}(KBr disc) 2910m, 1645w, 1582w, 1490s, 1430s, 1110s cm^{-1};

δ_H (400MHz; $CDCl_3$) 7.72 (15H, m, 3 x (C7-H and C8-H and C9-H), 5.69 (1H, m, C2-H), 5.03 (2H, m, C1-H_2), 3.73 (2H, m, C5-H_2), 2.38 (2H, m, C3-H_2), 1.70 (2H, m, C4-H_2);

δ_C (100MHz; $CDCl_3$) 136.21 (C6, *J* 12.2 Hz), 134.97 (C7, *J* 19.8 Hz), 133.48 (C9), 130.37 (C8, *J* 7.1 Hz), 118.52 (C1), 116.87 (C2), 33.78 (C5), 22.13, 21.73;

m/z (FAB) 743 ($2M^+$+2, 55%), 331(M^+, 100).

Methyl 5-oxohexanoate (24)



1-Acetylbutyric acid (3.00 g, 23.1 mmol) was dissolved in diethyl ether (25 ml) to which methanol (0.810 g, 25.5 mmol), *N,N*-dicyclohexylcarboimide (5.22 g, 25.5 mmol) and 4-dimethylaminopyridine (0.31 g, 2.55 mmol) was added. The mixture was stirred at room temperature overnight then filtered under reduced pressure. The filtrate was washed with water (10 ml), 5% aqueous acetic acid (10 ml) and again with water (10 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and the solvent removed *in vacuo*. The crude material was purified by flash chromatography (hexane-ethyl acetate 5:1) to leave the *title compound* as a pale yellow liquid (2.13 g, 64% yield).

(Found: M^+, 144.0788. $C_7H_{12}O_3$ requires: *M*, 144.0786);

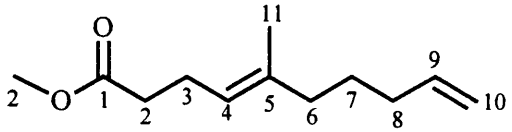
ν_{max}(film) 2953m, 1736br s cm^{-1};

δ_H (400MHz; $CDCl_3$) 3.67 (3H, s, C7-H_3), 2.51 (2H, t, *J* 7.3 Hz, C2-H_2), 2.34 (2H, t, *J* 7.3 Hz, C4-H_2), 2.14 (3H, s, C6-H_3), 1.90 (2H, m, C3-H_2);

δ_C (100MHz; $CDCl_3$) 210.10 (C5), 173.63 (C1), 53.29 (C7), 42.47 (C4), 33.01 (C2), 29.97 (C6), 18.90 (C3);

m/z (EI) 144 (M^+, 25%), 129 (M^+-CH_3, 42), 113 (M^+-OCH_3, 100).

Methyl 5-methyldeca-4,9-dieneoate (20)

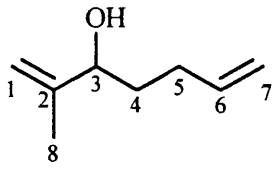


Performing the reaction under anhydrous conditions, 1-pentenyl-5-triphenylphosphonium bromide **23** (0.43 g, 1.04 mmol) was charged to a round bottomed flask under nitrogen and dissolved in dry tetrahydrofuran (15 ml). The solution was cooled to –78 °C and a solution of 2M *n*-butyl lithium in hexane (0.16ml, 1.14mmol) added. After stirring for 15 mins at -78°C, a solution of the prepared keto ester **24** (0.15g, 1.04 mmol) in dry tetrahydrofuran (5 ml) was added. The reaction mixture was warmed to room temperature and stirred for 18 hrs. The reaction was quenched by the addition of water (10 ml) and the product extracted

into ethyl acetate. After drying over anhydrous magnesium sulphate, the solvent was removed *in vacuo* and the material purified by flash chromatography (hexane-ethyl acetate 9:1) to leave the *title compound* as a pale yellow oil (0.03 g, 15% yield).

ν_{max}(film) 2985s, 2799s, 1745m, 1665w, 1644w cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.83 (1H, m, C9-H), 5.13 (1H, m, C4-H), 4.98 (2H, m, C10-H_2), 3.68 (3H, s, C12-H_3), 2.51 (2H, t, *J* 7.3 Hz, C2-H_2), 2.35 (2H, t, *J* 6.8 Hz, C6-H_2) 2.28 (2H, m, C3-H_2), 2.08 (3H, m, C11-H_3), 2.00 (2H, m), 1.90 (2H, m);
m/z (EI) 196 (M^+, 3%), 181 (M^+-CH_3, 8), 163 (26), 109 (51), 55 ($(CH_2CH_2CHCH)^+$, 100).

2-Methyl-3-hydroxy-1,6-heptadiene (26)

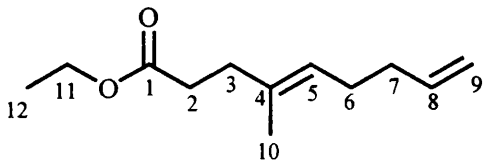


Performing the reaction under anhydrous conditions, magnesium turnings (0.840 g, 35.0 mmol) were charged to a 100 ml round bottomed flask containing dry diethyl ether (26 ml) and a crystal of iodine. 4-Bromo-1-butene (4.00 g, 29.6 mmol) in 2 ml of dry diethyl ether was added to initiate the reaction (as identified by the decolouration of the reaction mixture) and then at such a rate as to maintain the mixture at a gentle reflux. External heating was then applied to maintain reflux for a further 30 mins then the heating was stopped and methacrolein (2.18 g, 31.5 mmol) in 2 ml dry diethyl ether was added dropwise at a rate to maintain reflux under the heat of the reaction. After the addition, reflux was maintained for 1½ hrs with an external water bath before the reaction mixture was cooled in ice and quenched by the slow addition of 5% aqueous hydrochloric acid (25 ml). The resulting solution was diluted with water (50 ml) and after separation, the aqueous phase was extracted with diethyl ether (2 x 80 ml). The organic extracts were washed with saturated

sodium hydrogen carbonate solution (1 x 100 ml) and saturated brine (1 x 100 ml). After drying over anhydrous magnesium sulphate, the solvent was removed *in vacuo* and the crude product purified by flash chromatography (hexane-ethyl acetate 8:1) to leave the *title compound* as a colourless liquid (0.589 g, 18% isolated yield).

ν_{max}(film) 3375br s, 1676s, 1641s cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C6-H), 4.95 (4H, m, C7-H_2 and C1-H_2), 4.06 (1H, t, *J* 6.5 Hz, C3-H), 1.71 (3H, s, C8-H_3), 1.64 (4H, m, C4-H_2 and C5-H_2);
δ_C (100MHz; $CDCl_3$) 147.40 (C2), 138.38 (C6), 114.87 (C7), 111.15 (C1), 75.47 (C3), 34.01 (C5), 29.97 (C4), 17.58 (C8);
m/z (EI) 111 (M^+-CH_3, 100%), 54 (30).

(4E)-ethyl 4-methyl-4,8-nonadieneoate (28)



To a 25 ml round bottomed flask fitted with a Claisen head and condenser was charged 2-methyl-3-hydroxy-1,6-heptadiene (**26**) (0.500 g, 3.97 mmol), ethyl orthoacetate (3.69 g, 24.6 mmol) and propionic acid (0.014 g, 0.189 mmol). The mixture was heated to maintain a vapour temperature of 135-140 °C for 2 hrs, after which time the ethanol had ceased to distill over. The reaction mixture was cooled to room temperature and the distillation residue purified by flash chromatography (hexane-ethyl acetate 8:1) to leave the *title compound* as a colourless oil (0.462 g, 64% isolated yield).

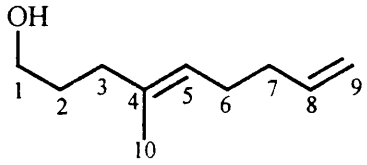
ν_{max}(film) 2976s, 2922s, 1736s, 1639s cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.80 (1H, m, C8-H), 5.15 (1H, m, C5-H), 4.97 (1H, dd, *J* 16.5 Hz and 3.8 Hz, C9-H_{trans}), 4.91 (1H, dd, *J* 10.6 Hz and 3.8 Hz, C9-H_{cis}), 4.09 (2H, q,

J 7.0 Hz, C11-H_2), 2.35 (4H, m), 2.01 (4H, m), 1.59 (3H, s, C10-H_3), 1.22 (3H, t, *J* 7.0 Hz, C12-H_3);

δ_C (100MHz; $CDCl_3$) 173.58 (C1), 138.51, 133.76, 124.50, 114.55, 60.26 (C11), 34.69, 33.88, 33.23, 27.31, 16.00 (C10), 14.23 (C12);

m/z (EI) 196 (M^+, 13%), 151 (M^+-OCH_2CH_3, 24), 109 (M^+-$CH_2COOCH_2CH_3$, 100), 81 (95).

(4E)-4-methyl-4,8-nonadiene-1-ol (29)



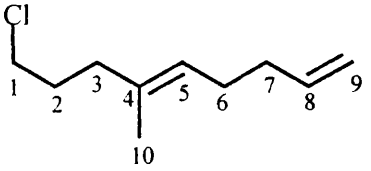
Performing the reaction under anhydrous conditions, ethyl (4*E*)-4-methyl-4,8-nonadieneoate (**28**) (0.400 g, 2.05 mmol) was charged to a 50 ml round bottomed flask containing dry diethyl ether (10 ml). Lithium aluminium hydride (0.008 g, 2.1 mmol) was added gradually over 30 mins and the resulting mixture was stirred at room temperature for 18 hrs. After this time, the reation was quenched with a few drops of saturated sodium sulphate solution. Solid anhydrous sodium sulphate was then added to the flask and after standing for 2 hrs, the mixture was filtered, washing the collected aluminate salts thoroughly with diethyl ether. The filtrate was concentrated *in vacuo* to leave the *title compound* as a colourless oil (0.306 g, 97% yield) that was used without further purification.

ν_{max}(film) 3333br s, 2935s, 2865s, 1639s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.82 (1H, m, C8-H), 5.15 (1H, m, C5-H), 4.98 (1H, dd, *J* 15.2 Hz and 1.2 Hz, C9-H_{trans}), 4.92 (1H, dd, *J* 10.2 Hz and 1.2 Hz, C9-H_{cis}), 3.61 (2H, t, *J* 6.4 Hz, C1-H_2), 2.08 (4H, m), 1.59 (3H, s, C10-H_3), 1.58 (4H, m);

δ_C (100MHz; $CDCl_3$) 138.65 (C4), 135.08 (C8), 124.43 (C5), 114.25 (C9), 62.97 (C1), 35.99, 33.95, 30.62, 27.31, 15.97 (C10);

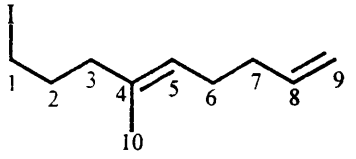
(4E)-1-chloro-4-methyl-4,8-nonadiene (30a)



Performing the reaction under anhydrous conditions, (4*E*)-4-methyl-4,8-nonadiene-1-ol (**29**) (1.60 g, 10.4 mmol) was charged to a round bottomed flask containing triphenylphosphine (2.98 g, 11.4 mmol) and dry carbon tetrachloride (10 ml). The mixture was heated at reflux for 72 hrs and after this time, diluted with petroleum spirit (30-40°) and filtered to remove precipitated triphenylphosphine oxide. The solvent was removed from the filtrate by ordinary distillation at atmospheric pressure and the *title compound*, isolated as a pale yellow liquid (1.80 g, >99% yield) that was used without further purification.

ν_{max}(film) 2958s, 2929s, 1666w, 1640m, 912m cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C8-H), 5.18 (1H, m, C5-H), 5.02 (1H, dd, *J* 17.5 Hz and 1.3 Hz, C9-H_{trans}), 4.98 (1H, dd, *J* 11.9 Hz and 1.3 Hz, C9-H_{cis}), 3.51 (2H, t, *J* 6.7 Hz, C1-H_2), 2.10 (4H, m), 1.92 (4H, m), 1.61 (3H, s, C10-H_3);
δ_C (100MHz; $CDCl_3$) 138.44 (C4), 131.95 (C8), 128.35 (C5), 114.35 (C9), 44.77 (C1), 36.15, 33.08, 30.68, 27.81, 15.48 (C10);
m/z (EI) 136 (M^+-HCl, 12%), 95 (M^+-$CH_2CH_2CH_2Cl$, 100), 82 (53), 54 (72).

(4E)-1-iodo-4-methyl-4,8-nonadiene (30c)

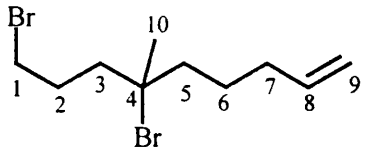


(*4E*)-1-chloro-4-methyl-4,8-nonadiene (**30a**) (1.50 g, 8.7 mmol) was heated with sodium iodide (3.91 g, 26.1 mmol) in acetone (25 ml) at reflux for 20 hrs, during which time sodium chloride precipitated out of the solution. This was subsequently removed by filtration, and the solvent removed from the filtrate *in vacuo* to leave the

title compound as a bright yellow liquid (1.50 g, 65% yield) that was used without further purification.

ν_{max}(film) 2927s, 2850s, 1666w, 1639m, 911s cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C8-H), 5.20 (1H, m, C5-H), 5.04 (1H, dd, *J* 16.6 Hz and 1.9 Hz, C9-H_{trans}), 4.98 (1H, dd, *J* 12.2 Hz and 1.8 Hz, C9-H_{cis}), 3.15 (2H, t, *J* 7.0 Hz, C1-H_2), 2.10 (4H, m), 1.92 (4H, m), 1.60 (3H, s, C10-H_3);
δ_C (100MHz; $CDCl_3$) 138.55 (C4), 132.21 (C8), 125.93 (C5), 114.85 (C9), 39.09 (C1), 33.92, 31.44, 27.14, 15.81 (C2), 6.77 (C10);
m/z (EI) 123 (50%), 109 (21), 95 (M^+-$CH_2CH_2CH_2I$, 100).

1,4-Dibromo-4-methyl-8-nonene (30d)



Phosphorus tribromide (0.22 g, 0.8 mmol) and 30% hydrobromic acid in acetic acid (2 drops) were charged to a round bottomed flask and cooled to ice temperature by means of an external cooling bath. (*4E*)-4-methyl-4,8-nonadiene-1-ol (**29**) (0.300 g, 1.90 mmol) was added over 5 mins, maintaining external cooling, and the temperature of the mixture was increased to room temperature and stirred for 18 hrs. The reaction was quenched by the addition of water (20 ml) and the product extracted into diethyl ether (3 x 10 ml). The extracts were washed until neutral with saturated sodium carbonate solution (2 x 20 ml) and water (2 x 20 ml) before drying over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude product purified by flash chromatography (hexane-ethyl acetate 8:1) to leave the *title compound*, *i.e.* the dibrominated product (0.17 g, 30% yield) and not the monobromide as desired.

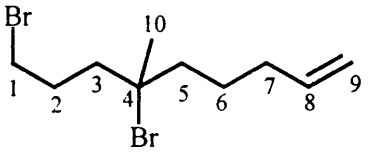
ν_{max}(film) 2942s, 1641m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.80 (1H, m, C8-H), 5.03 (1H, dd, *J* 16.4 Hz and *J* 1.6 Hz, C9-H_{trans}), 4.98 (1H, dd, *J* 10.8 Hz and *J* 1.6 Hz, C9-H_{cis}), 3.45 (2H, t, *J* 6.4 Hz, C1-H_2), 2.10 (4H, m), 1.72 (3H, s, C10-H_3), 1.60 (6H, m);

δ_C (100MHz; $CDCl_3$) 138.11 (C8), 115.61 (C9), 44.98 (C1), 43.48 (C4), 33.35, 33.34, 31.54, 29.04, 24.99, 22.71 (C10);

m/z (EI) 217 (M^+-Br, 13%), 137 (M^+-2Br, 88), 95 (M^+-$CH_2CH_2CH_2Br$-Br, 70), 55 (95), 27 (98).

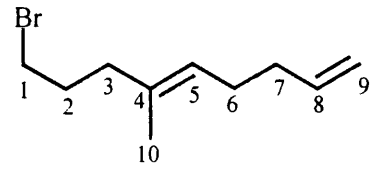
1.4-Dibromo-4-methyl-4,8-nonadiene (30d)



Performing the reaction under anhydrous conditions, lithium bromide (0.340 g, 3.90 mmol) was dissolved in dry acetonitrile (20 ml) in a round bottomed flask. To this was added 1N chlorotrimethysilane in tetrahydrofuran (0.530 g, 4.8 ml, 4.90 mmol) followed by (*4E*)-4-methyl-4,8-nonadiene-1-ol (**29**) (0.300 g, 1.90 mmol) in dry acetonitrile (5 ml). After the addition, the reaction mixture was heated at reflux for 18 hrs and then quenched by the addition addition of water (20 ml). The product was extracted into diethyl ether (3 x 20 ml) then washed until neutral with 5% aqueous sodium hydrogen carbonate solution (2 x 20 ml) and water (2 x 20 ml) before drying over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude product purified by flash chromatography (hexane-ethyl acetate 8:1) to leave the *title compound*, *i.e.* the undesired dibrominated product (0.26 g, 45% yield).

Spectral data obtained was identical to that given above.

(4E)-1-bromo-4-methyl-4,8-nonadiene (30b)



(*4E*)-4-methyl-4,8-nonadiene-1-ol (**29**) (0.30 g, 2.0 mmol) was charged to a round bottomed flask and diluted with toluene (20 ml). Triphenylphosphine (0.70 g, 2.7 mmol) was added over 10 mins and the resulting mixture stirred for 15 mins. After this time, *N*-bromosuccinimide was added over 30 mins. The reaction was then allowed to stir at room temperature for 6 hrs before being filtered to remove any precipitated triphenylphosphine oxide. The filtrate was concentrated *in vacuo* before purification by flash chromatography (pure hexane) to leave the *title compound* as a colourless liquid (0.13 g, 30% yield).

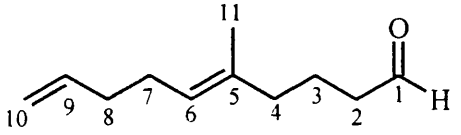
ν_{max}(film) 3076m, 2961s, 2917s, 2847m, 1640m, 1407s, 911s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.81 (1H, m, C8-H), 5.20 (1H, m, C5-H), 5.05 (1H, dd, *J* 16.8 Hz and *J* 1.5 Hz, C9-H_{trans}), 4.98 (1H, dd, *J* 10.1 Hz and *J* 1.5 Hz, C9-H_{cis}), 3.39 (2H, t, *J* 6.7 Hz, C1-H_2), 2.10 (4H, m), 1.95 (4H, m), 1.57 (3H, s, C10-H_3);

δ_C (100MHz; $CDCl_3$) 138.54 (C4), 133.43 (C8), 125.58 (C5), 114.53 (C9), 37.18 (C1), 33.28, 33.42, 30.80, 27.95, 15.58 (C10);

m/z (EI) 176 (25%), 154 (100), 136 (M^+-HBr, 90), 107 (43).

(5E)-5-methyl-5,9-decadienal (22)

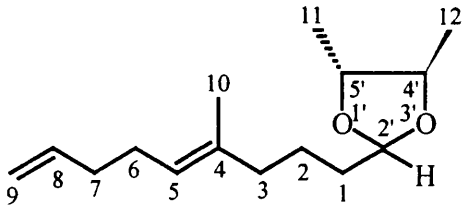


Performing the reaction under anhydrous conditions, the initial charging of materials was carried out in a glove box: Disodiumirontetracarbonyldioxan complex (4.80 g, 13.9 mmol) was charged to a Schlenk tube followed by dry tetrahydrofuran (100 ml). Triphenylphosphine (3.22 g, 12.3 mmol) in tetrahydrofuran (10 ml) was then added followed by (*4E*)-1-bromo-4-methyl-4,8-nonadiene (**30c**) 4.80 g, 13.9 mmol)

in dry tetrahydrofuran (10 ml). The tube was sealed and transferred to a Schlenk line where it was allowed to stir at room temperature overnight. After this time, the reaction was quenched by the addition of acetic acid (1200 μλ) to decompose the complex formed. The reaction mixture was poured quickly into water (250 ml) and the product extracted into pentane (3 x 100 ml). After washing with water (200 ml) and drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 10:1) to leave the *title compound* as an oil (1.22 g, 74% yield).

δ_H (400MHz; $CDCl_3$) 9.78 (1H, s, C1-H), 5.41 (2H, m, C6-H and C9-H), 5.13 (2H, m, C10-H_2), 2.68 (2H, t, *J* 5.8 Hz, C2-H_2), 2.41 (2H, dt, *J* 6.2 and 5.2 Hz, C7-H_2), 2.00 (2H, m, C8-H_2), 1.60 (3H, s, C11-H_3), 1.31 (2H, m, C3-H_2), 0.89 (2H, t, *J* 5.7 Hz, C4-H_2);
δ_C (100MHz; $CDCl_3$) 210.64 (C1), 132.13 (C5), 132.08 (C9), 124.15 (C6), 123.16 (C10), 48.78, 44.99, 40.76, 20.26, 17.89, 14.23 (C11);
m/z (EI) 149 (20%), 114 (44), 41 (100).

2'-((4E)-4-methyl-4,8-nonadienyl)-(4'R,5'R)-dimethyl-1',3'-dioxolane (12)



(*5E*)-5-Methyl-5,9-decadienal (1.22 g, 7.4 mmol) was added to a round bottomed flask containing D-(-)-(2*R*,3*R*)-butan-2,3-diol (6.91 g, 76.7 mmol) and dry tetrahydrofuran (80 ml) and Drierite (anhydrous calcium sulphate) (22.7 g). The reaction mixture was cooled to 0 °C and boron trifluoride etherate (316 μl) added. Stirring was continued for a further 18 hrs, maintaining this temperature by means of a Cryocool apparatus. The reaction mixture was then filtered to remove the Drierite and the filtrate diluted with water (100 ml) before extraction of the product into diethyl ether (3 x 100 ml). After drying over anhydrous magnesium sulfate and

removal of the solvent *in vacuo*, the crude material was purified by flash chromatography (hexane-ethyl acetate 10:1) to leave the *title compound* as a pale liquid (1.35 g, 77% yield).

(Found: M^+, 238.1946. $C_{15}H_{26}O_2$ requires: *M*, 238.1932);

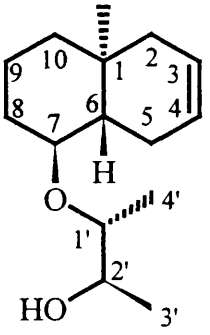
ν_{max}(film) 2899s, 1641m, 1608m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.40 (1H, m, C8-H), 5.14 (1H, br s, C5-H), 5.10 (1H, t, *J* 5.8 Hz, C-2'), 5.08 (1H, d, *J* 15.1 Hz, $C9_{trans}$-H), 5.03 (1H, d, *J* 9.7 Hz, $C9_{cis}$-H), 3.58 (2H, dq, *J* 8.4 Hz and *J* 5.1 Hz, C5'-H and C4'-H), 2.08 (2H, m), 2.02 (2H, m), 1.62 (4H, m), 1.59 (3H, s, C10-H_3), 1.52 (2H, m), 1.29 (3H, d, *J* 5.1 Hz), 1.22 (3H, d, *J* 5.1 Hz);

δ_C (100MHz; $CDCl_3$) 135.31, 124.59, 124.05, 114.31, 101.10 (C2'), 79.68 and 78.03 (C4' and C5'), 39.44, 34.26, 33.92, 22.14, 17.81, 17.25, 16.91, 15.21;

m/z (EI) 149 (70%),71 (50), 56 (100), 41 (75).

(1S,4aR)-1,2,3,4,4a,5,8,8a-octahydro-4a-naphthalene-1-yl-((1R',2R')-2'-hydroxy-1'-methylpropyl) ether (13)



2'-((*4E*)-4-Methyl-4,8-nonadienyl)-(4'*R*,5'*R*)-dimethyl-1',3'-dioxolane (**12**) (1.30 g, 5.50 mmol) was diluted with anhydrous benzene (130 ml) in a round bottomed flask. To this was added stannic chloride (2.84 g, 1.3 ml, 10.9 mmol) and the resulting solution stirred at room temperature for 4hrs. After this time the reaction was quenched by the addition of water (200 ml) and the product was extracted into pentane (3 x 100 ml). After drying over anhydrous magnesium sulfate, the solvent

was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 10:1) to yield the *title compound* as a coloured oil (0.28 g, 22% yield).

$[\alpha]_{25}$ = -5.15° (c 0.7, EtOH)

(Found: M^+, 238.1950. $C_{15}H_{26}O_2$ requires: *M*, 238.1932);

ν_{max}(film) 3472br s, 2929s, 1601m, 1084s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.48 (2H, m, C6-H and C7-H), 3.67 (1H, m, C2'-H), 3.49 (1H, m, C1'-H), 3.28 (1H, dt, *J* 6.2 Hz and *J* 1.4 Hz, C1-H), 3.13 (1H, dt, *J* 6.3 Hz and 1.6 Hz, C8a-H), 1.85 (4H, m, C5-H_2 and C8-H_2), 1.16 (3H, d, *J* 3.6 Hz), 1.12 (3H, s, C9-H_3), 1.10 (6H, m), 1.08 (3H, d, *J* 5.0 Hz);

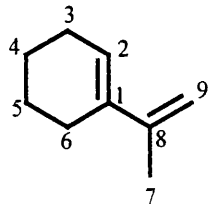
δ_C (100MHz; $CDCl_3$) 128.23, 126.84, 73.98, 71.31, 71.18, 51.36, 44.76, 44.48, 34.91, 32.24, 29.46, 26.46, 24.47, 22.71, 19.91;

m/z (EI) 239 (M^++1, 5%), 227 (100), 149 (81), 93 (72), 81 (56), 54 (57).

Chiral HPLC (Chiralcel OB hexane-isopropyl alcohol mixture 98:2; UV detection 254nm) 4.155 mins - **13**.

Diels-Alder strategies

1-Isopropenyl-1-cyclohexene (34)



The reaction was carried out under anhydrous conditions whereby methyl triphenylphosphonium bromide (1.44 g, 4.03 mmol) was added to a flask containing 10 ml of dry diethyl ether and *n*-butyl lithium (2 ml of 2.0 M in hexanes, 4.00 mmol), cooled to –78 °C. The temperature of the mixture was warmed to room temperature and then stirred for 4 hrs. A solution of 1-acetyl-1-cyclohexene (0.5 g,

4.03 mmol) in 2 ml dry diethyl ether was then added and the resulting yellow solution heated at reflux overnight. This was filtered, diluted with water (50 ml) and extracted twice (2 x 50 ml) with diethyl ether. The extracts were washed once with saturated brine (50 ml) then dried over anhydrous magnesium sulphate. After removal of the solvent *in vacuo*, the crude material was purified by flash chromatography (pure hexane) to leave the *title compound* as a colourless liquid (0.165 g, 23% yield).

(Found: M^+, 122.1081. C_9H_{14} requires: *M*, 122.1096);

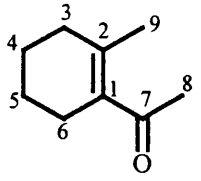
ν_{max}(film) 2926s, 2834s, 1636s, 1607s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.82 (1H, d, *J* 3.9 Hz, C2-H), 4.86 (2H, br s, C9-H_2), 1.5-2.2 (4H, m), 1.82 (3H, s, C7-H_3), 1.38 (2H, m), 0.90 (2H, d, *J* 7.3 Hz);

δ_C (100MHz; $CDCl_3$) 143.81 (C8), 137.56 (C1), 124.85 (C2), 109.34 (C9), 30.60, 26.20, 25.95, 25.86, 22.41 (C7);

m/z (EI) 164 ($2M^+$-$C(CH_2)CH_3$, 57%), 122 (M^+, 13), 121 (M^+-1, 45), 82 (M^+-$C(CH_2)(CH_3)$, 28), 71 (55), 57 (100).

1-Acetyl-2-methyl-1-cyclohexene (40)



1-Methyl-1-cyclohexene (5.00 g, 52.0 mmol) was dissolved in acetic anhydride (32 ml) in a 100 ml round bottomed flask and stirred at 0 °C by means of an external ice bath. A few crystals of zinc iodide (~10 mg) were added and the mixture left for 15 mins before zinc chloride (7.26 g, 52.0 mmol) was added to the vessel. Stirring was maintained for 2 hrs at 0 °C before the reaction was quenched by the careful addition of ice. The mixture was extracted with diethyl ether (2 x 100 ml) and the extracts washed with 10% aqueous sodium hydroxide solution until the washings were basic. The combined organic extracts were dried over anydrous magnesium sulphate and

after removal of the solvent *in vacuo*, the crude material was loaded (with a small quantity of dichloromethane to aid the transfer) onto a pad of neutral aluminium oxide. (Volume of aluminium oxide to crude organic phase was 3:1.) After leaving for 18 hrs, the pad was washed thoroughly with dichloromethane and after removal of this solvent *in vacuo*, the crude product was purified by flash chromatography (hexane-ethyl acetate 8:1) to yield the *title compound* as a colourless liquid (4.30 g, 60% isolated yield).

(Found: M^+, 138.1040. $C_9H_{14}O$ requires: *M*, 138.1045);

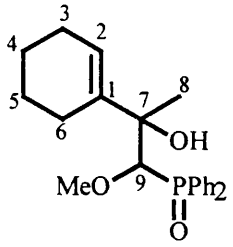
ν_{max}(film) 2931s, 2861s, 1711s, 1692s cm^{-1};

δ_H (400MHz; $CDCl_3$) 2.32 (8H, m, C3 to C6-H_2), 2.01 (3H, s, C8-H_3), 1.83 (3H, br s, C9-H_3);

δ_C (100MHz; $CDCl_3$) 204.31 (C7), 141.11 (C2), 133.71 (C1), 33.40, 26.27, 22.73, 22.32, 21.06, 19.65 (C9);

m/z (EI) 138 (M^+, 65%), 137 (M^+-1, 46), 123 (M^+-CH_3, 100)

Methoxymethyl(1-cyclohexenyl-1-hydroxyethyl)diphenylphosphine oxide (43)

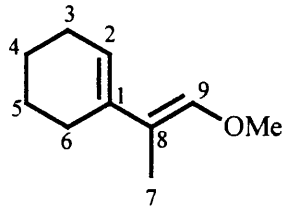


Performing the reaction under anhydrous conditions, methoxymethyldiphenylphosphine oxide (0.500 g, 2.03 mmol) was dissolved in 15 ml of dry tetrahydrofuran in a 50 ml round bottomed flask and cooled to 0 °C. Lithium diisopropylamine solution (1.2 ml of 2.0 M in heptane/THF, 2.44 mmol) was added and after stirring for 10 mins, the mixture was cooled to –78 °C. A solution of 1-acetyl-1-cyclohexene (0.252 g, 2.03 mmol) in dry THF (2.5 ml) was added over 5 mins and then the resulting mixture was warmed to room temperature

over 2 hrs. The reaction was quenched by the addition of saturated ammonium chloride solution (1 ml) followed by water (20 ml). The mixture was extracted with diethyl ether (2 x 30 ml) and the extracts washed once with saturated sodium chloride solution (30 ml). After drying over anhydrous magnesium sulphate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 1:1) to leave the *title compound* as a white solid (0.471 g, 63% isolated yield).

δ_H (400MHz; $CDCl_3$) 7.74 (10H, m, 2 x phenyl), 5.60 (1H, br s, C2-H), 3.92 (1H, d, *J* 7.1 Hz, C9-H), 3.18 (3H, s, OC-H_3), 1.31 (3H, s, C8-H_3), 1.24 (8H, m);
δ_C (100MHz; $CDCl_3$) 142.12 (C1), 139.81, 132.33, 131.97, 130.99, 128.36 (C2), 85.29 (C9), 77.84, 62.01, 25.41, 25.22, 24.85, 22.45, 21.43 (C8);
m/z (FAB) 371 (M^++1, 26%), 353 (M^+-OH, 31), 201 (100), 151 (80).

1-(2-Methoxy-1-methylvinyl)-1-cyclohexene (44)



Performing the reaction under anhydrous conditions, methoxymethyl(1-cyclohexenyl-1-hydroxyethyl)diphenylphosphine oxide (**43**) (0.400 g, 1.08 mmol) was stirred in 20 ml of dry tetrahydrofuran with sodium hydride (60% dispersion in mineral oil, 0.089 g, 2.16 mmol) at room temperature overnight. The reaction was quenched by the addition of a few drops of water followed by anhydrous sodium sulphate. After filtration, the solvent was removed *in vacuo* from the filtrate and the crude material purified by flash chromatography (hexane-ethyl acetate 20:1) to leave the *title compound* as a colourless liquid (0.080 g, 49% isolated yield).

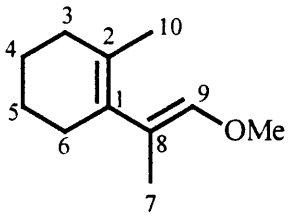
(Found: M^+, 151.1281. $C_{10}H_{16}O$ requires: *M*, 151.1201);
ν_{max}(film) 2926s, 1649s, 1219s, 1110s cm^{-1};

δ_H (400MHz; $CDCl_3$) 6.94 (1H, s, C9-H), 5.65 (1H, m, C2-H), 3.57 (3H, s, O*C*-H_3), 2.29 (2H, m), 1.71 (3H, d, *J* 1.0 Hz, C7-H_3), 1.48 (6H, m);

δ_C (100MHz; $CDCl_3$) 143.42, 143.35, 123.27, 120.78, 57.17 (O*C*H_3), 25.58, 25.63, 22.49, 22.75, 10.02 (C7);

m/z (EI) 153 (M^-, 13%), 147 (31), 97 (48), 81 (54), 54 (100).

2-Methyl-1-(2-methoxy-1-methylvinyl)-1-cyclohexene (47)



Methoxymethyldiphenylphosphine oxide (3.57 g, 14.5 mmol) was dissolved in 90ml dry tetrahydrofuran in a 150 ml round bottomed flask and cooled to 0 °C by means of an external ice bath. Lithium diisopropylamine solution (8.7 ml of 2.0 M in heptane/THF, 17.4 mmol) was added and after stirring for 10 mins, the mixture was cooled to –78 °C. A solution of 2-methyl-1-acetyl-1-cyclohexene (1.83 g, 13.3 mmol) in dry THF (10 ml) was added over 5 mins, then the temperature of the resulting mixture was increased to room temperature over 2 hrs. After this time, the reaction was quenched by the addition of saturated ammonium chloride solution (5 ml) followed by water (100 ml). The mixture was extracted with diethyl ether (2 x 60 ml) and the extracts washed once with brine (50 ml). After drying over anhydrous magnesium sulphate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 8:1) to recover the starting material (2-methyl-1-acetyl-1-cyclohexene, 0.33 g, 18%) and the *title compound* as a colourless liquid (0.781 g, 26% isolated yield based on ketone consumed).

(Found: M^+, 166.1350. $C_{11}H_{18}O$ requires: *M*, 166.1358);

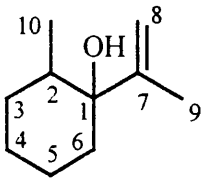
ν_{max}(film) 2927s, 1681s, 1661s, 1218s, 1127s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.69 (1H, s, C9-H), 3.53 (3H, s, OCH_3), 1.94 (3H, br s, C10-H_3), 1.58 (3H, d, *J* 1.4 Hz, C7-H_3), 1.50 (6H, m);

δ_C (100MHz; $CDCl_3$) 143.67, 140.08, 130.88, 115.38, 59.73 (OCH_3), 31.85, 29.84, 23.42, 23.12, 17.03 (C10), 12.80 (C7);

m/z (EI) 166 (M^+, 100%), 151 (M^+-CH_3, 55).

1-Isopropenyl-2-methylcyclohexan-1-ol (38)



Performing the reaction under anhydrous conditions, magnesium turnings (0.240 g, 9.80 mmol) were charged to a 25 ml round bottomed flask with 10 ml of dry diethyl ether and a crystal of iodine and stirred for 5 mins. 2-Bromopropene (1.10 ml, 10.7 mmol) in 2 ml of dry ether was added gradually at a rate to maintain gentle reflux of the reaction mixture. After the addition was complete, reflux was maintained for 45 mins by use of a warm water bath. After this time, methylcyclohexanone (1.00 g, 8.90 mmol), in 5 ml of dry ether, was added dropwise to the reactor over a period of 10 mins. External heating was again applied to keep the reaction mixture at reflux for a further 35 mins. The mixture was then cooled to room temperature and quenched by the careful addition of water (10 ml) followed by dilute hydrochloric acid (5 ml). The resulting layers were separated and the aqueous phase extracted once with 30 ml of ether before the organics were combined and dried over anhydrous magnesium sulfate. After solvent removal, *in vacuo*, the crude product was purified by flash chromatography (hexane-ethyl acetate 99:1) to give the *title compound* as a colourless oil (0.35 g, 56% based on methylcyclohexanone consumed).

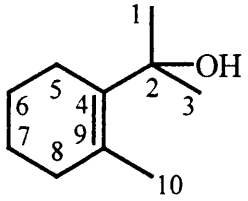
ν_{max}(film) 3490s, 2933s, 2853s, 1640s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.03 (1H, s, C8-H), 4.81 (1H, s, C8-H), 1.71 (3H, s, C9-H_3), 1.51 (9H, m), 0.70 (3H, d, *J* 6.8 Hz, C10-H_3);

δ_C (100MHz; $CDCl_3$) 138.50 (C7), 107.09 (C8), 64.89 (C1), 37.50, 35.41, 28.08, 27.30, 21.74, 15.12, 14.38 (C10);

m/z (EI) 135 (M^+-H_2O, 23%), 123 (60), 113 (M^+-$C(CH_2)CH_3$, 100), 95 (M^+-$C(CH_2)CH_3$-H_2O, 74), 73 (65), 56 (46).

2-(2-Methylcyclohex-1-enyl)-propan-2-ol (41)



Performing the reaction under anhydrous conditions, 1-acetyl-2-methyl-1-cyclohexene (4.00 g, 29.0 mmol) was dissolved in dry diethyl ether (35 ml) in a round bottomed flask. This was cooled to 0 °C and preformed 3N methyl magnesium bromide in diethyl ether (10.6 ml, 31.9 mmol) was added over 5 mins. The temperature of the reaction mixture was allowed to increase to room temperature over 15 mins then stirred at ambient temperature for a further three hours. After this time the reaction was quenched by the addition of ice water (20 ml). The mixture was acidified with 10% (v/v) aqueous hydrochloric acid and the product extracted into diethyl ether (3 x 70 ml). The extracts were washed with brine (2 x 60 ml) then dried over anhydrous magnesium sulfate. The solvent was then removed *in vacuo* to leave the *title compound* as a colourless oil (3.65 g, 82% yield) that was used without further purification.

Found: M^+, 154.1370. $C_{10}H_{18}O$ requires: *M*, 154.1358);

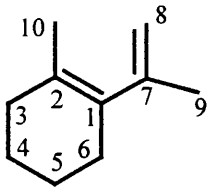
ν_{max}(film) 3392br s, 2926s, 2836s, 1616w cm^{-1};

δ_H (400MHz; $CDCl_3$) 1.85 (3H, br s, C10-H_3), 1.75 (8H, m), 1.34 (1H, br s, C2-O*H*), 1.26 (3H, s), 1.18 (3H, s);

δ_C (100MHz; $CDCl_3$) 134.63 (C9), 126.85 (C4), 49.45 (C2), 34.16, 29.55, 26.09, 25.34, 23.54, 22.87, 20.53 (C10);

m/z (EI) 154 (M^+, 3%), 137 (M^+-OH, 12), 59 ($(CH_2)COH(CH_2)^+$, 35), 43 (100).

2-Isopropenyl-2-methyl-1-cyclohex-1-ene (37)



2-(2-methylcyclohex-1-enyl)-propan-2-ol (0.500 g, 3.25 mmol) was stirred in dichloromethane (10 ml) containing one drop of concentrated sulfuric acid for 18 hrs. After this time, the reaction was quenched by addition of water (40 ml) and the product was extracted into dichloromethane (3 x 20 ml), washed with saturated aqueous sodium hydrogen carbonate solution (1 x 40 ml) and finally water (2 x 40 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and the solvent removed *in vacuo*. The crude product was then purified by flash chromatography (pure hexane) to give the *title compound* as a colourless oil (0.22 g, 50% yield).

(Found: M^+, 137.1334. $C_{10}H_{16}$ requires: *M*, 137.1330

Found: $2M^+$, dimer, 272.2500. $C_{20}H_{32}$ requires: 2*M*, 272.2504);

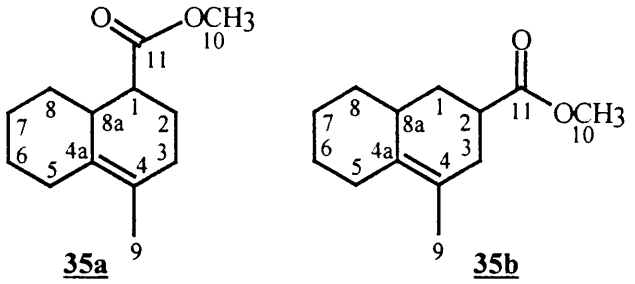
ν_{max}(film) 2940s, 2809s, 1638s, 1621s cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.18 (1H, s, C8-H), 5.01 (1H, s, C8-H), 1.73 (3H, s, C9-H_3), 1.60 (3H, s, C10-H_3), 1.18 (8H, m);

δ_C (100MHz; $CDCl_3$) 134.55, 133.90, 131.44, 126.09, 31.10, 28.91, 25.32, 23.38, 22.57, 20.46;

m/z (EI) 272 ($2M^+$, 85%), 207 (100), 137 (M^+, 83), 95 (M^+-$C(CH_2)CH_3$, 78).

4-Methyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-1-methylcarboxylate (35a) and 4-Methyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-2-methylcarboxylate (35b)



35a **35b**

1-(Isopropenyl)-1-cyclohexene (**34**) (0.100 g, 8.20 mmol), excess methyl acrylate (1.00 ml) and catalytic aluminium trichloride (0.10 g) were charged in list order to a flask containing in dichloromethane (10 ml) and the resulting mixture was heated at reflux for 12 hrs. After this time, the reaction mixture was quenched with water (15 ml) and extracted twice with dichloromethane (2 x 20 ml). The combined organic extracts were washed twice with water (2 x 20 ml) and dried over anhydrous magnesium sulfate. After solvent removal *in vacuo*, the crude product was purified by flash chromatography (hexane-diethyl ether 20:1) to leave the *title compounds* **35a** and **35b** as a mixture, in the approximate ratio 5:1 respectively (0.127 g, 77% yield).

(Found: M^+, 208.1469. $C_{13}H_{20}O_2$ requires: *M*, 208.1463);

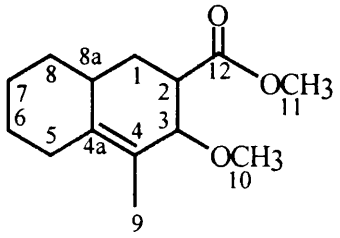
ν_{max}(film) 2997s, 2855s, 1736s, 1436m, 1165m, 1197m cm^{-1};

δ_H (400MHz; $CDCl_3$) 3.66 and 3.60 (3H, 2 x s, C10-H), 2.73 (1H, m, C1-H), 1.60 (3H, s, C9- H_3), 1.23 (12H, m);

δ_C (100MHz; $CDCl_3$) 210.31 and 209.41 (C11), 125.43 (C4), 122.28 (C4a), 51.52 and 51.04 (C10), 41.60, 31.87, 31.00, 30.60, 28.29, 28.15, 26.29, 23.17, 20.49, 20.57;

m/z (EI) 208 (M^+, 16%), 190 (M^+-CH_3), 148 (M^+-$CO(OCH_3)$-2H, 50), 147 (100), 105 (63).

3-Methoxy-4-methyl-1,2,3,5,6,7,8,8a-octahydro-naphthalene-1-methylcarboxylate (45)



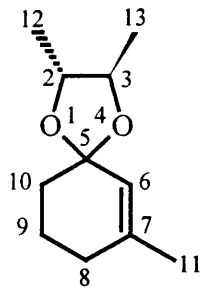
1-(2-Methoxy-1-methylvinyl)-1-cyclohexene (**44**) (0.08 g, 0.53 mmol), excess methyl acrylate (1.00 ml) and catalytic aluminium trichloride (0.07 g, 0.05 mmol) were added in list order to a flask containing dichloromethane (15 ml) and the resulting mixture was heated at reflux for 12 hrs. After this time, the reaction mixture was quenched with water (15 ml) and extracted twice with dichloromethane (2 x 20 ml). The combined organic extracts were washed twice with water (2 x 20 ml) and dried over anhydrous magnesium sulfate. After solvent removal *in vacuo*, the product was analysed by NMR to identify the *title compound* as a colourless liquid (0.05 g, 40% yield).

ν_{max}(film) 3000s, 2882s, 1738s, 1618m cm^{-1};

δ_H (400MHz; $CDCl_3$) 3.79 (1H, d, *J* 5.3 Hz, C3-H), 3.67 (3H, s, C11-H_3), 3.65 (3H, s, C10-H_3), 2.45 (1H, dt, *J* 5.3 and 3.9 Hz, C2-H), 1.58 (3H, s, C9-H_3), 1.46 (11H, m);

m/z (EI) 194 (22%), 178 (41), 159 (78), 145 (100), 136 (98).

(2R,3R)-dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (50a)

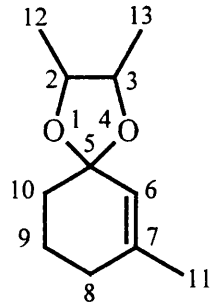


3-Methyl-2-cyclohexene-1-one (2.30 g, 20.9 mmol) was charged to a round bottomed flask containing D-(-)-(2*R*,3*R*)-butandiol (2.83 g, 31.3 mmol), *p*-

toluenesulfonic acid (0.200 g, 1.06 mmol) and toluene (100 ml). The mixture was heated at reflux for 24 hrs, removing the water formed by means of a Dean-Stark apparatus. The reaction was quenched with a few drops of triethylamine and the solvent removed *in vacuo*. The crude product obtained was purified by flash chromatography (hexane-ethyl acetate 8:1) to yield the *title compound* as a pale liquid (2.24 g, 59% yield).

(Found: M^+, 182.1304. $C_{11}H_{18}O_2$ requires: *M*, 182.1307);
ν_{max}(film) 3001s, 2986s, 2851s, 1658m, 1199s, 1082m cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.36 (1H, s, C6-H), 3.65 (1H, dq, *J* 7.2 and 3.9 Hz, C2-H), 3.61 (1H, dq, *J* 7.2 and 3.9 Hz, C3-H), 1.93 (2H, br s, C8-H_2), 1.69 (3H, s, C11-H_3), 1.74 (4H, m), 1.26 (3H, d, *J* 7.2 Hz), 1.24 (3H, d, *J* 7.2 Hz);
δ_C (100MHz; $CDCl_3$) 141.13 (C7), 123.67 (C6), 105.56 (C5), 34.67, 29.90, 23.95, 20.59, 16.57, 16.28;
m/z (EI) 181 (M^+-H, 23%), 146 (36), 121 (58), 107 (100).

2,3-Dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (50a-c)



3-Methyl-2-cyclohexene-1-one (1.91 g, 17.3mmol) was charged to a round bottomed flask containing commercially available isomeric butan-2,3-diol (2.35 g, 26.0mmol), *p*-toluenesulfonic acid (0.16 g, 0.9 mmol) and toluene (60 ml). The mixture was heated at reflux for 24 hrs, removing the water formed by means of a Dean-Stark apparatus. The reaction was quenched with a few drops of triethylamine and the solvent removed *in vacuo*. The crude product obtained was purified by flash

chromatography (hexane-ethyl acetate 8:1) to yield the *title compound*, a pale liquid (1.74 g, 55% yield), as a mixture of three diastereoisomers.

(Found: M^+, 182.1304. $C_{11}H_{18}O_2$ requires: *M*, 182.1307);

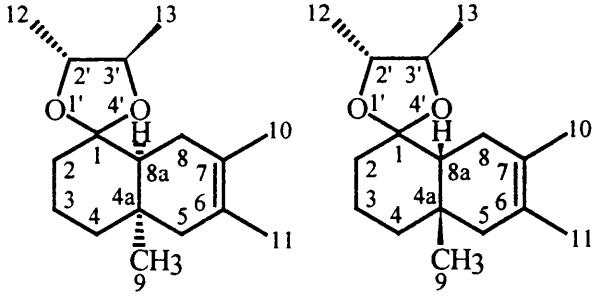
ν_{max}(film) 2973s, 2932s, 2869s, 1670m, 1654m, 1190m, 1102m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.87, 5.38 and 5.42 (1H, 3 x s, C6-H), 3.80, 3.65 and 3.51 (2H, 3 x m, C2-H and C3-H), 1.68 (3H, s, C11-H_3), 1.23 and 1.25 (3H, 2 x d, *J* 5.9 Hz), 1.14 and 1.13 (3H, 2 x d, *J* 5.9 Hz);

δ_C (100MHz; $CDCl_3$) 131.75 (C7), 126.65 (C6), 105.64 (C5), 77.43, 77.32, 75.08, 74.56, 74.90, 73.21, 40.68, 24.04, 20.91, 17.99, 17.62, 17.03, 16.92, 16.83, 15.97, 15.61;

m/z (EI) 182 (M^+, 23%), 154 (M^+-CH_2CH_3, 80), 114 (45), 82 (100).

4a,6,7-Trimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (2R,3R)-butandiol ketal (51a) and (51b)



51a **51b**

(2*R*,3*R*)-dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (**50a**) was added to 4M lithium perchlorate solution (4.40 ml) followed by 2,3-dimethyl-1,3-butadiene (0.360 g, 4.40 mmol) and 0.5 M solution of (*S*)-camphorsulfonic acid in THF (0.02 ml). The reaction mixture was thus 0.25 M w.r.t.the ketal, 1.00 M w.r.t. the diene and 1.0 mol % camphorsulfonic acid catalyst w.r.t. the ketal. The reaction mixture was stirred at room temperature for 3 hrs before quenching by the addition of water (10 ml). The product was extracted into diethyl ether (2 x 20 ml), washed with brine (1 x 20 ml) then dried over anhydrous magnesium sulfate. The

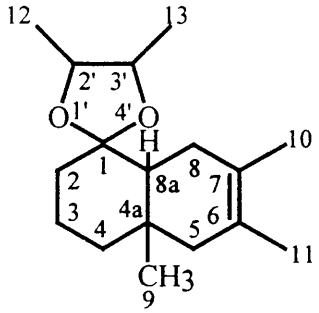
solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 20:1) to leave the *title compound*, a pale liquid (0.02 g, 7% yield neglecting recovered unreacted ketal), as a mixture of isomers **51a** and **51b**.

Whilst **51a** and **51b** were found to be in the ratio 6:1, it was not possible to unequivocally assign which is isomer was in excess.

Spectral data for the major isomer:
(Found: M^+, 264.2080. $C_{17}H_{28}O_2$ requires: *M*, 264.2089);
ν_{max}(film) 2968s, 2980s, 2879s, 1650w, 1008m cm^{-1};
δ_H (400MHz; $CDCl_3$) 3.35 (2H, m, C2' and C3'-H), 2.03 (1H, t, *J* 5.9 Hz, C8a-H), 1.56 and 1.54 (6H, 2 x s, C10-H_3 and C11-H_3), 1.37 (3H, d, *J* 8.1 Hz), 1.20 (3H, d, *J* 6.2 Hz), 0.95 (3H, s, C9-H_3), 1.0-1.7 (10H, m);
δ_C (100MHz; $CDCl_3$) 128.31, 124.23, 111.83 (C1), 78.51, 78.01, 60.13, 46.83, 37.66, 30.30, 30.05, 29.61, 29.54, 24.84, 19.34, 18.71, 18.01, 17.59 (C9);
m/z (EI) 264 (M^+, 100%).

Spectral data for the minor isomer:
(Found: M^+, 264.2080. $C_{17}H_{28}O_2$ requires: *M*, 264.2089);
ν_{max}(film) 2968s, 2980s, 2879s, 1650w, 1008m cm^{-1};
δ_H (400MHz; $CDCl_3$) 3.54 (2H, m, C2' and C3'-H), 2.28 (1H, t, C2-H, *J* 5.8 Hz), 1.58 and 1.53 (6H, 2 x s, C10-H_3 and C11-H_3), 1.39 (3H, d, *J* 8.0 Hz), 1.24 (3H, d, *J* 6.9 Hz), 0.96 (3H, s, C9-H_3), 1.0-1.7 (10H, m);
δ_C (100MHz; $CDCl_3$) 128.31, 124.23, 110.69 (C1), 77.98, 77.34, 58.27, 46.83, 37.66, 30.30, 30.05, 29.61, 29.54, 24.84, 19.34, 18.71, 17.76, 17.04 (C11);
m/z (EI) 264 (M^+, 100%).

4a,6,7-Trimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one 2,3-butandiol ketal (51a-f)

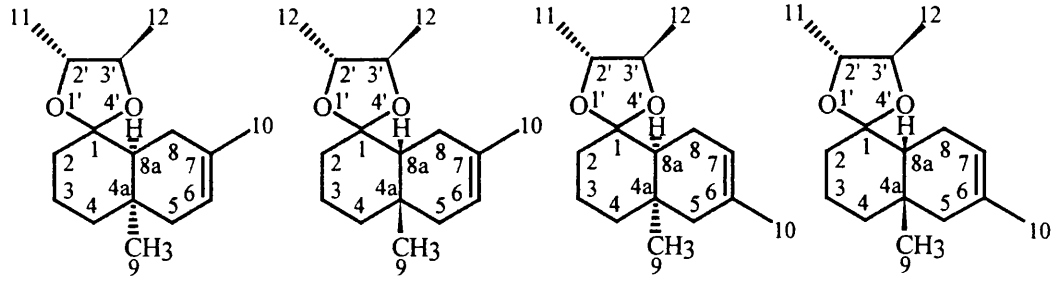


51a-f

2,3-Dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (**50a-c**) (0.200 g, 1.10 mmol) was added to 4 M lithium perchlorate solution (4.4 ml) followed by 2,3-dimethyl-1,3-butadiene (0.360 g, 4.40 mmol) and 0.5 M solution of (*S*)-camphorsulfonic acid in THF (0.02 ml). The reaction mixture was thus 0.25 M w.r.t. the ketal, 1.00 M w.r.t. the diene and 1.0 mol % camphorsulfonic acid catalyst w.r.t. the ketal. The reaction mixture was stirred at room temperature for 3 hrs before quenching by the addition of water (10 ml). The product was extracted into diethyl ether (2 x 20 ml), washed with brine (1 x 20 ml) then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 20:1) to leave the *title compound* as a pale liquid (0.03 g, 11% yield neglecting recovered unreacted ketal).

Spectroscopic data (based on **51a** and **51b** above) was consistent with the proposed structure, but indicated a mixture of isomers, **51a**, **51b**, **51c**, **51d**, **51e** and **51f**.

4a,6-Dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (2R,3R)-butandiol ketal (52a-b) and 4a,7-dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (2R,3R)-butandiol ketal (53a-b)



52a **52b** **53a** **53b**

(2*R*,3*R*)-dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (**50a**) (0.50 g, 2.75 mmol) was added to 4 M lithium perchlorate solution (11 ml) followed by isoprene (0.75 g, 11.0 mmol) and 0.5 M solution of (*S*)-camphorsulfonic acid in THF (0.05 ml). The reaction mixture was thus 0.25 M w.r.t. the ketal, 1.00 M w.r.t. the diene and 1.0 mol % camphorsulfonic acid catalyst w.r.t. the ketal. The reaction mixture was stirred at room temperature for 3 hrs before quenching by the addition of water (20 ml). The product was extracted into diethyl ether (2 x 30 ml), washed with brine (1 x 20 ml) then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to yield the *title compounds*, a pale liquid (0.22 g, 32% yield neglecting recovered unreacted ketal), as a mixture of isomers **52a-b** and **53a-b** in the ratio 1:1.

It was not possible to unequivocally assign spectral data to each enantiomeric pair **52a-b** and **53a-b**.

Spectral data for first isomer pair:

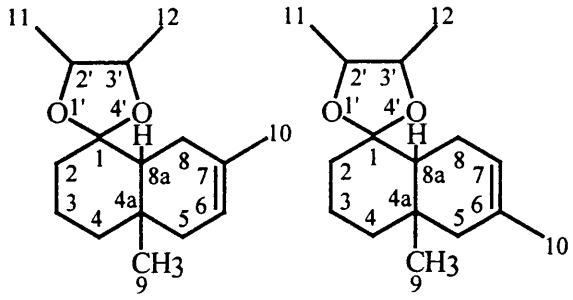
ν_{max}(film) 2968s, 2926s, 2867s, 1652w, 1105m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.12 (1H, m, C6-H), 3.54 (2H, m, C2' and C3'-H), 2.28 (1H, t, *J* 5.9 Hz, C8a-H), 1.67 and 1.65 (6H, 2 x s, C10-H_3), 1.39 (3H, d, *J* 8.1 Hz), 1.35 (10H, m), 1.24 (3H, d, *J* 6.9 Hz), 0.96 (3H, s, C9);

δ_C (100MHz; $CDCl_3$) 129.15, 115.84, 110.97 (C1), 79.81, 78.59, 60.98, 47.36, 39.08, 32.78, 38.12, 32.86, 31.81, 27.07, 20.41, 19.18, 17.98, 17.23, 17.06;
m/z (EI) 249 (M^+-1, 18%), 195(18), 137 (52), 114 (70), 81 (83), 69 (100).

Spectral data for second isomer pair:
ν_{max}(film) 2968s, 2926s, 2867s, 1652w, 1105m cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.08 (1H, m, C7-H), 3.35 (2H, m, C2' and C3'-H), 2.03 (1H, t, *J* 5.8 Hz, C9a-H), 1.63 and 1.60 (6H, 2 x s, C10-H_3), 1.37 (3H, d, *J* 8.0 Hz), 1.35 (10H, m), 1.20 (3H, d, *J* 6.2 Hz), 0.95 (3H, s, C9);
δ_C (100MHz; $CDCl_3$) 127.81, 114.13, 108.86 (C1), 80.11, 79.64, 61.10, 44.68, 37.18, 37.01, 36.87, 32.16, 31.80, 26.48, 20.89, 20.01, 18.01, 17.45, 17.27;
m/z (EI) 249 (M^+-1, 18%), 195(18), 137 (52), 114 (70), 81 (83), 69 (100).

4a,6-Dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one 2,3-butandiol ketal (52a-f) and 4a,7-Dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one 2,3-butandiol ketal (53a-f)



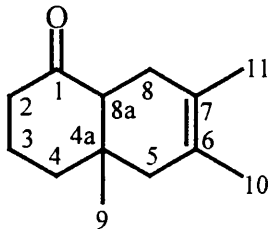
52a-f **53a-f**

2,3-Dimethyl-7-methyl-1,4-dioxaspiro[4:5]dec-6-ene (**50a-c**) (0.50 g, 2.75mmol) was added to 4 M lithium perchlorate solution (11 ml) followed by isoprene (0.75 g, 11.0 mmol) and 0.5 M solution of (*S*)-camphorsulfonic acid in THF (0.05 ml). The reaction mixture was thus 0.25 M w.r.t. the ketal, 1.00 M w.r.t. the diene and 1.0 mol % camphorsulfonic acid catalyst w.r.t. the ketal. The reaction mixture was stirred at room temperature for 3 hrs before quenching by the addition of water (20 ml). The product was extracted into diethyl ether (2 x 30 ml), washed with brine (1

x 20 ml) then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to leave the *title compounds*, a pale liquid (0.25 g, 36% yield neglecting recovered unreacted ketal), as a mixture of isomers.

Spectroscopic data obtained (based on **52a-b** and **53a-b** given above) was consistent with a mixture of isomers **52a-f** and **53a-f**.

4a,6,7-Trimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (54)



4a,6,7-Trimethyl-2,3,4,4a,5,8,8a-hexahydro-2*H*-naphthalen-1-one (2*R*,3*R*)-butandiol ketal (**51a-b**) (0.270 g, 1.00 mmol) was heated for 6 hrs at 70 °C in 10% aqueous hydrochloric acid (30 ml). After this time, the product was extracted into diethyl ether (3 x 20 ml) and washed with brine (3 x 20 ml). After drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to leave the *title compound* as a pale liquid (0.02 g, 10% yield).

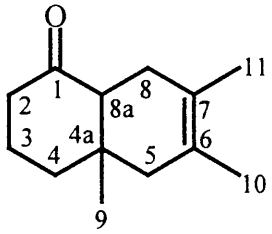
ν_{max}(film) 2968s, 2920s, 1718s, 1660m cm^{-1};

δ_H (400MHz; $CDCl_3$) 2.35 (3H, m), 2.18 (2H, s, C5-H_2), 1.74 (6H, m), 1.61 (6H, s, C10-H_3 and C11-H_3), 0.98 (3H, s, C9-H_3);

δ_C (100MHz; $CDCl_3$) 211.52 (C1), 128.86, 127.93, 60.55 (C8a), 40.51, 38.99, 37.85, 36.57, 31.42, 23.51, 20.14, 17.64, 17.53.

m/z (EI) 141 (23%), 114 (100), 81 (31), 69 (40), 41 (60).

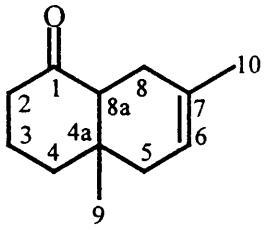
4a,6,7-Trimethyl-3,4,4a,5,8,8a-hexahydro-2H-napthalen-1-one (54)



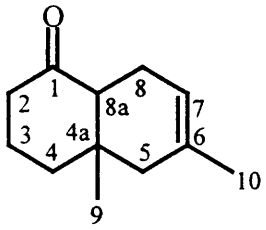
4a,6,7-Trimethyl-2,3,4,4a,5,8,8a-hexahydro-2*H*-naphthalen-1-one (2*R*,3*R*)-butandiol ketal **51a-f** (0.140 g, 0.50 mmol) was heated for 6 hrs at 70 °C in 10% aqueous hydrochloric acid (20 ml). After this time, the product was extracted into diethyl ether (3 x 15 ml) and washed with brine (3 x 15 ml). After drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to leave the *title compound* as a pale liquid (0.02 g, 18% yield).

Spectroscopic data was identical to that given above.

4a,7-Dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (55) and 4a,6-dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (56)



55



56

A isomeric mixture of ketals **52a-f** and **53a-f** (0.240 g, 1.00 mmol) was heated for 6 hrs at 70 °C in 10% aqueous hydrochloric acid (30 ml). After this time, the product was extracted into diethyl ether (3 x 20 ml) and washed with brine (3 x 20 ml). After drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to leave the *title compounds*, a pale liquid (0.04 g, 23% yield), as a mixture of regioisomers in the ratio 1:1.

It was not possible to unequivocally assign regioisomers **56** and **55** individually.

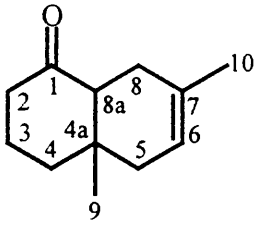
ν_{max}(film) 2957s, 2928s, 1715s, 1676m cm^{-1};

δ_H (400MHz; $CDCl_3$) 5.20 (1H, m), 2.33 (1H, m), 2.17 (2H, m), 1.68 (8H, m), 1.60 and 1.56 (3H, 2 x s, C10-H_3), 0.96 (3H, s, C9-H_3);

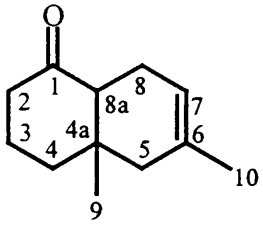
δ_C (100MHz; $CDCl_3$) 215.51 (C1), 122.42, 122.28, 53.23 (C8a), 40.46, 39.94, 38.71, 36.70, 34.84, 28.67, 27.47, 19.98, 18.71 (C9).

m/z (EI) 178 (M^+, 2%), 152 (43), 133 (98), 117 (81), 105 (38).

4a,7-Dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (55) and 4a,6-dimethyl-3,4,4a,5,8,8a-hexahydro-2H-naphthalen-1-one (56)



55



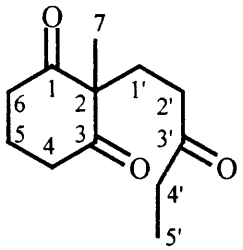
56

An isomeric mixture of ketals **52a-b** and **53a-b** was heated for 6 hrs at 70 °C in 10% aqueous hydrochloric acid (30 ml). After this time, the product was extracted into diethyl ether (3 x 20 ml) and washed with brine (3 x 20 ml). After drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 25:1) to leave the *title compounds*, a pale liquid (0.02 g, 14% yield), as a mixture of regioisomers in the ratio 1:1.

Spectral data obtained was identical to that given above.

Asymmetric cyclisations

2-Methyl-2-(3-oxopentyl)-cyclohexan-1,3-dione (*51*)



2-Methylcyclohexan-1,3-dione (10.0 g, 83.3 mmol) was stirred with hydroquinone (0.242 g), acetic acid (0.274 g) and deionised water (70 ml) at room temperature under a nitrogen atmosphere. To this, ethyl vinyl ketone (13.7 g, 16.3 ml, 163 mmol) was added over 5 mins and the resulting mixture heated at reflux (80-84 °C) for 6½ hrs. The reaction was cooled to room temperature and extracted into dichloromethane (3 x 80 ml). The combined organic extracts were washed with water (2 x 70 ml) before drying over anhydrous magnesium sulfate. Solvent was removed *in vacuo* and the material purified by filtration through a pad of silica (hexane-ethyl acetate 5:1) to give the *title compound* as a yellow oil (9.39 g, 54%).

(Found: M^+, 210.1253. $C_{12}H_{18}O_3$ requires: *M*, 210.1256);

ν_{max}(film) 2974m, 2940m, 1717s, 1686s cm^{-1};

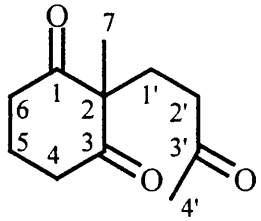
δ_H (400MHz; $CDCl_3$) 2.62 (4H, m, C4-H_2 and C6-H_2), 2.33 (2H, q, *J* 2.3 Hz, C4'-H_2), 2.26 (2H, t, *J* 7.6 Hz, C2'-H_2), 2.01 (2H, t, *J* 7.3 Hz, C1'-H_2), 1.86 (2H, m, C5-H_2), 1.18 (3H, s, C7-H_3), 0.96 (3H, t, *J* 2.0 Hz, C5'-H_3);

δ_C (100MHz; $CDCl_3$) 213.85, 213.90, 208.21 (C3'), 37.66, 36.89, 35.86, 32.87, 30.94, 29.71, 19.75, 17.52, 7.62 (C5');

m/z (EI) 210 (M^+, 15%), 139 (27), 111 (43), 57 (100).

The following is representative of the procedures used to investigate the micellar phase Michael addition reaction of section 6.3.2.

2-Methyl-2-(3-oxobutyl)-cyclohexan-1,3-dione (51a)



2-Methylcyclohexan-1,3-dione (1.00 g, 8.30 mmol) was stirred in 50 ml of surfactant solution at room temperature for 15 mins. After this time, methyl vinyl ketone (0.72 g, 8.3 mmol) was added and the resulting mixture heated at reflux (80-84 °C) for 12 hrs. The reaction was cooled to room temperature and extracted into dichloromethane (3 x 30 ml). The combined organic extracts were washed with water (2 x 30 ml) before drying over anhydrous magnesium sulfate. Solvent was removed *in vacuo* and the material purified by filtration through a pad of silica (hexane-ethyl acetate 5:1) to give the *title compound* as a yellow oil (1.57 g, 96%).

(Found: M^+, 196.1094. $C_{11}H_{16}O_3$ requires: *M*, 196.1099);

ν_{max}(film) 2970m, 2950m, 1730s, 1719s cm^{-1};

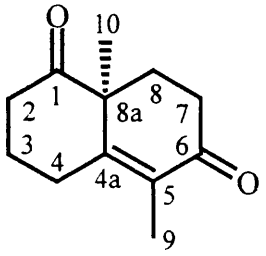
δ_H (400MHz; $CDCl_3$) 2.62 (4H, m, C4-H_2 and C6-H_2), 2.31 (2H, t, *J* 7.2 Hz, C2'-H_2), 2.10 (3H, s, C4'-H_3), 2.01 (2H, t, *J* 7.2, C1'-H_2), 1.89 (2H, m, C5-H_2), 1.20 (3H, s, C7-H_3);

δ_C (100MHz; $CDCl_3$) 211.32 (C3'), 207.93, 207.81, 62.48 (C2), 40.61, 37.35, 38.04, 24.59, 20.37, 18.79, 17.69 (C7);

m/z (EI) 196 (M^+, 83%), 181 (M^+-CH_3, 27), 178 (M^+-H_2O, 95), 168 (M^+-CO, 35), 154 (52), 126 (95).

The following is representative of the general procedure employed in section 6.4.1 to investigate the Hajos-Parrish reaction:

(8aS)-5,8a-dimethyl-3,4,8,8a-tetrahydro-2H,7H-naphthalene-1,6-dione (58a)



Triketone (**57**) (0.250 g, 1.19 mmol) was added to a flask containing (*S*)-phenylalanine (0.197 g, 1.19 mmol) and acetic acid (10 ml) and the resulting mixture was stirred at 75 °C to 80 °C for 5 hrs. After cooling to room temperature, the reaction mixture was diluted with water (20 ml) and extracted with ethyl acetate (3 x 20 ml). The combined extracts were washed with water (3 x 20 ml) then saturated sodium hydrogencarbonate solution (2 x 20 ml) before drying over anhydrous magnesium sulfate. Removal of the solvent *in vacuo* left the crude product, purified by filtration through a silica pad (hexane-ethyl acetate 5:1) to give the *title compound* as an orange coloured oil (0.159 g, 70%).

(Found: M^+, 192.1152. $C_{12}H_{16}O_2$ requires: *M*, 192.1150);

ν_{max}(film) 2871s, 2953s, 1709s, 1667s, 1610s cm^{-1};

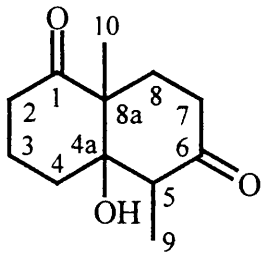
δ_H (400MHz; $CDCl_3$) 2.83 (2H, m), 2.65 (2H, m), 2.44 (2H, m), 2.08 (2H, m, C8-H_2), 2.01 (2H, m, C3-H_2), 1.76 (3H, s, C9-H_3), 1.37 (3H, s, C10-H_3);

δ_C (100MHz; $CDCl_3$) 212.01 (C1), 197.63 (C6), 155.96 (C4a), 127.93 (C5), 37.35, 33.31, 29.78, 29.61, 23.66, 21.51, 19.82 (C9), 11.28 (C10):

m/z (EI) 192 (M^+, 100%), 177 (M^+-CH_3, 35), 149 (51), 136 (85).

Chiral HPLC (Chiralcel OD column, hexane:propan-2-ol, 99:1, 1 ml/min, UV: 226 nm) 21.02 min ((8a*S*)-**58**), 21.97 min ((8a*R*)-**58**)

4a-Hydroxy-5,8a-dimethyl-hexahydro-naphthalene-1,6-dione (63)



Triketone (**57**) (2.00 g, 9.52 mmol) was stirred at 70 °C to 80 °C with (*S*)-proline (0.520 g, 4.52 mmol) in acetonitrile (26 ml) with a trace of 1 N perchloric acid (2.6 ml) for 25 hrs under a nitrogen atmosphere. After cooling, the reaction mixture was washed once with saturated brine (10 ml) and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (petroleum ether 60-80°-ethyl acetate 1:1) to give the *title compound* as a white solid (0.09 g, 5%, 11% based on recovered starting material).

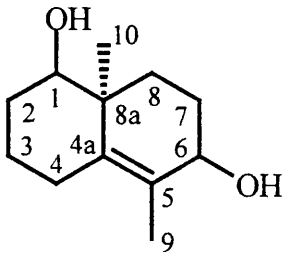
(Found: M^+, 210.1255. $C_{12}H_{18}O_3$ requires: *M*, 210.1256)

ν_{max}(KBr disc) 3448br s, 2870s, 2878s, 1711s, 1701s cm^{-1};

δ_H (400MHz; $CDCl_3$) 2.65 (2H, m), 2.41 (2H, m), 2.37 (1H, m, C5-H), 2.28 (2H, m), 2.01 (2H, m), 1.80 (2H, m, C3-H_2), 1.28 (3H, s, C10-H_3), 1.03 (3H, d, *J* 6.7 Hz, C19-H_3);

m/z (EI) 210 (M^+, 38%), 139 (100), 127 (196), 111 (62).

(8aS)-5,8a-dimethyl-1,2,3,4,6,7,8,8a-octahydro-naphthalene-1,6-diol (59)



(8a*S*)-5,8a-dimethyl-3,4,8,8a-tetrahydro-2*H*,7*H*-naphthalene-1,6-dione (**58**) (9.60 g, 50.0 mmol) was dissolved in 500 ml of isopropyl alcohol to which sodium borohydride (4.00 g, 100 mmol) was added. The mixture was stirred at room

temperature for 24 hrs then quenched by the careful addition of dilute hydrochloric acid (20 ml). After a further 10 mins of stirring, the product was extracted into ethyl acetate (3 x 80 ml) and dried over anhydrous magnesium sulfate. After solvent removal *in vacuo* the crude product was purified by flash chromatography (hexane-ethyl acetate 5:1) to give the *title compound* as a white solid (3.70 g, 38% yield).

(Found: M^+, 210.1460. $C_{12}H_{18}O_3$ requires: *M*, 210.1463)

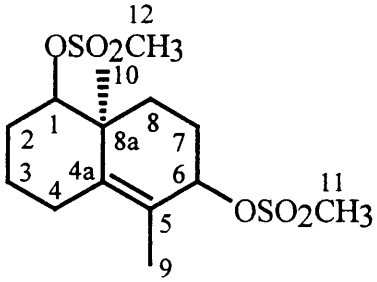
ν_{max}(film) 3351s, 2968m, 2406s, 2835m cm^{-1};

δ_H (400MHz; $CDCl_3$) 3.5-3.6 (2H, m), 1.71 (3H, s, C9-H_3), 1.42 (10H, m), 0.97 (3H, s, C10-H_3);

δ_C (100MHz; $CDCl_3$) 145.12 (C4a), 138.24 (C5), 79.27, 78.03, 49.80, 33.89, 31.10, 30.60, 29.38, 27.89, 20.01 (C9), 16.87 (C10);

m/z (EI) 196 (M^+, 35%), 178 (M^+-H_2O, 25), 163 (M^+-CH_3-H_2O, 8), 145 (M^+-H_2O-H_2O-CH_3, 47), 107 (78), 83 (100).

(8aS)-5,8a-dimethyl-1,2,3,4,6,7,8,8a-octahydro-naphthalene-1,6-dimethanesulfonyl ester (64a)



(8a*S*)-5,8a-dimethyl-1,2,3,4,6,7,8,8a-octahydro-naphthalene-1,6-diol (**59**) (1.00 g, 5.10 mmol) was dissolved in 100 ml of dichloromethane and cooled to 0 °C. To this was added triethylamine (1.60 ml, 20.4 mmol) and the mixture stirred for 10 mins. Methanesulfonyl chloride (1.20 ml, 15.3 mmol) was then added and stirring continued for 4 hrs at 0 °C . After this time, the reaction temperature was allowed to increase to room temperature and stirred for 18 hrs before quenching by the addition of water (10 ml). The product was extracted into dichloromethane (3 x 20 ml) and washed once with water (10 ml). The combined organic extracts were dried over

anhydrous magnesium sulfate and the solvent removal, *in vacuo*. The material was purified by flash chromatography (hexane-ethyl acetate 19:1) to give the *title compound* as a coloured oil (0.240 g, 16% yield).

ν_{max}(film) 2936m, 1369s, 1173s cm^{-1};
δ_H (400MHz; $CDCl_3$) 5.49 (1H, t, *J* 6.9 Hz, C6-H), 4.59 (1H, t, *J* 7.3 Hz, C1-H), 3.65 (3H, s, C11-H_3), 3.01 (3H, s, C12-H_3), 1.75 (3H, s, C9-H_3), 1.61 (10H, m), 1.02 (3H, s, C10-H_3);
m/z (EI) 264 (48%), 199 (100), 145 (36), 121 (57).

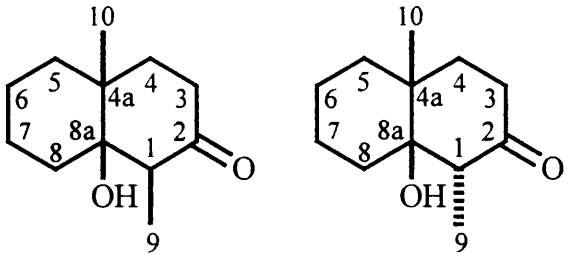
Attempted hydride displacement reactions

The following is representative of the hydride displacement procedure employed.

Performing the reaction under anhydrous conditions, dimesyl ester (**64a**) (0.70 g, 2.00 mmol) was dissolved in 50 ml of dry tetrahydrofuran under an atmosphere of nitrogen. The hydride source, for example lithium aluminium chloride (0.26 g, 6.00 mmol), was added gradually over 5 mins. The reaction mixture was then heated at reflux for 4hrs before cooling to room temperature. After quenching with water (2 ml) and dilute sodium hydroxide solution (2 ml), the reaction mixture was filtered through a pad of celite and extracted into diethyl ether (3 x 10 ml) and then dried over anhydrous magnesium sulfate. After solvent removal *in vacuo*, TLC analysis of the crude product against standard samples indicated an absence of the desired product. Purification by flash chromatography (pure hexane) gave three products (total mass 0.120 g) the NMR spectra of which indicated a mixture of dehydration and monohydroxy products.

Enantioselective dehydration

1,4a-Dimethyl-8a-hydroxy-1,4,4a,5,6,7,8,8a-octahydro-3H-naphthalen-2-one (66)



2-Methylcyclohexanone (8.00 g, 71.4 mmol) was added to 1 M solution of sodium ethoxide (1.31 ml, 1.31 mmol) and stirred at –5 °C for 5 mins. Ethyl vinyl ketone (8.00 g, 71.4 mmol) was then introduced to the mixture over a period of 30 mins and the resulting solution stirred at -20 °C to –5 °C for 6 hrs. The reaction was then quenched by the addition of water (20 ml) and the product was extracted into diethyl ether (3 x 60 ml). The combined organic extracts were washed with saturated brine (50 ml) then dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 5:1) to recover unreacted methylcyclohexanone (2.72 g, 34%) and the *title compound*, an oil, as a mixture of isomers in the ratio 1:1 (2.39 g, 26% yield based on consumption of ketone).

(Found: M^+, 196.1470. $C_{12}H_{20}O_2^+$ requires: *M*, 196.1463);

ν_{max}(film) 3511br s, 2936s, 2867s, 2364w, 1708s cm^{-1};

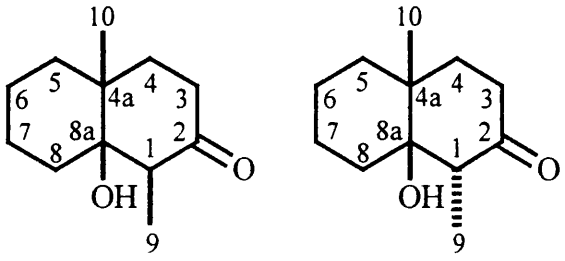
δ_H (400MHz; $CDCl_3$) 2.97 and 2.84 (1H, 2 x q, *J* 3.4 Hz, C1-H_{ax} and C1-H_{eq}), 2.51 and 2.29 (2H, 2 x m, C3-H_{2ax} and C3-H_{2eq}), 2.07 (2H, m), 1.48 (6H, m), 1.19 and 1.04 (3H, 2 x s, C10-H_3), 0.99 and 0.97 (3H, 2 x d, *J* 3.4 Hz, C9-H_{3ax} and C9-H_{3eq});

δ_C (100MHz; $CDCl_3$) 211.33 (C2), 90.08 (C8a), 55.34, 47.71, 36.41, 35.06, 33.03, 28.97, 24.27, 21.00, 16.01 (C10), 5.62 (C9);

m/z (EI) 196 (M^+, 12%), 112 (100).

The following is representative of the general procedure employed for investigating the effect of using a surfactant medium with a chiral base ((*S*)-α-phenylethylamine).

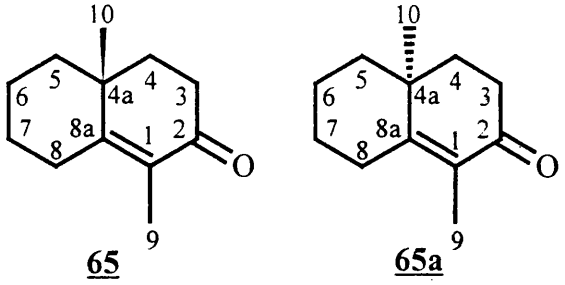
1,4a-Dimethyl-8a-hydroxy-1,4,4a,5,6,7,8,8a-octahydro-3H-naphthalen-2-one (66)



2-Methylcyclohexanone (0.80 g, 7.10 mmol) was stirred with (*S*)-α-phenylethylamine (0.52 g, 4.30 mmol) in an aqueous solution of the surfactant **62** (25 ml) at its CMC. To this was added ethyl vinyl ketone (0.73 g, 8.60 mmol) over 5 mins. The solution was stirred overnight at room temperature before quenching by the addition of water (20 ml). The product was extracted into diethyl ether (3 x 60 ml) and the combined extracts were washed with saturated brine (50 ml). After drying over anhydrous magnesium sulfate, the solvent was removed *in vacuo* and the crude material purified by flash chromatography (hexane-ethyl acetate 5:1) to recover unreacted methylcyclohexanone (0.13 g, 16%) and the *title compound* as an oil (0.33 g, 28% yield based on consumption of ketone).

Spectral data obtained was identical to that recorded above.

(4aR)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one (65) and (4aS)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one (65a)



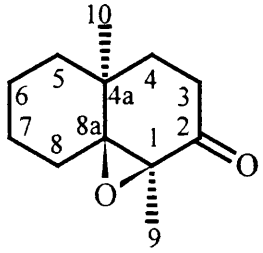
65 **65a**

The following is representative of the general procedure for investigating the enantioselective dehydration of section 7.6.

Hydroxydecalone (**66**) (0.60 g, 3.10 mmol) was added to (*S*)-proline (0.30 g, 2.61 mmol) dissolved in 25 ml of DMSO. The mixture was heated at 60 °C for 48 hrs then cooled to room temperature. The product was poured into water (100 ml) and extracted twice with diethyl ether (2 x 40 ml). The combined organic extracts were washed with saturated brine (2 x 50 ml) then dried over anhydrous magnesium sulfate. After solvent removal *in vacuo*, the crude product was purified by flash chromatography (hexane-ethyl acetate 5:1) to give the *title compounds* as a pale yellow oil (0.07 g, 97 % yield based on recovered **66** (0.52 g, 87% of original)).

(Found: M^+, 178.1350. $C_{12}H_{18}O^+$ requires: *M*, 178.1358);
ν_{max}(film) 2928s, 2858s, 1664s, 1611s cm^{-1};
δ_H (400MHz; $CDCl_3$) 1.73 (3H, s, C9-H_3), 1.49 (12H, m), 1.19 (3H, s, C10-H_3);
δ_C (100MHz; $CDCl_3$) 199.24 (C2), 163.03 (C8a), 128.36 (C1), 42.14 (C9), 37.70, 36.23, 33.86, 27.75, 26.88, 22.49, 15.98 (C9), 10.87 (C10);
m/z (EI) 178 (M^+, 12%), 163 (70), 136 (76), 121 (46), 91 (52), 83 (55);
Chiral HPLC (Chiracel OB column, hexane-isopropyl alcohol, 98:2; UV detection, 226 nm) 6.852 mins (4a*S*)-(+)-**65a**, 6.103 mins (4a*R*)-(-)-**65**.

(1S,4aS,8aS)-1,4a-dimethyl-1,8a-epoxyperhydronaphthalen-2-one (72a)



Optically enriched (4a*S*)-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3*H*-naphthalen-2-one (**65a**) (0.300 g, 1.69 mmol) was dissolved in dichloromethane (30 ml) and *m*-chloroperbenzoic acid (57-84%) (0.623 g, 2.52 mmol) added over a 10 mins. The

mixture was stirred at room temperature for 18 hrs then diluted with 5N aqueous sodium hydroxide solution (3 ml) to quench the reaction. After 10 mins, the organic layer was separated and washed with water (10 ml) before the addition of further 5N sodium hydroxide solution (3 ml). After another 10 mins, the organic layer was separated again and washed with water (3 x 10 ml) before drying over anhydrous magnesium sulfate. After solvent removal *in vacuo*, the *title compound* was left was an oil (0.320 g, 98% yield) that was used without further purification.

(Found: M^+, 194.1310. $C_{12}H_{18}O_2^+$ requires: *M*, 194.1307);

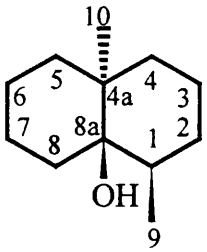
ν_{max}(film) 2940s, 2870m, 1705s, 1450m cm^{-1};

δ_H (400MHz; $CDCl_3$) 1.31 (3H, s, C9-H_3), 1.20-2.40 (12H, m), 1.00 (3H, s, C10-H_3);

δ_C (100MHz; $CDCl_3$) 207.74 (C2), 71.79 (C8a), 65.27 (C1), 38.02 (C4a), 34.43, 33.21, 31.99, 26.27, 23.91, 20.90, 20.40 (C10), 11.11 (C9);

m/z (EI) 194 (M^+, 5%), 176 (M^+-H_2O, 14), 133 (16), 109 (100), 81 (25), 67 (49), 55 (24).

(1R,4aS,8aR)-1,4a-dimethyl-1,2,3,4,5,6,7,8-octahydro-naphthalen-8a-ol (1a)



Performing the reaction under anhydrous conditions, optically enriched (1*S*,4a*S*,8a*S*)-1,4a-dimethyl-1,8a-epoxyperhydronaphthalen-2-one (**72a**) (0.300 g, 1.55 mmol) was charged to a round bottomed flask containing tetrahydrofuran (5 ml) and the mixture cooled to –30 °C. 1M lithium aluminium hydride in THF (0.45 ml, 0.450 mmol) was added and the temperature of the reaction increased to room temperature over 1 hr. After a further hour of stirring, sulfur trioxide pyridine complex (0.286 g, 1.78 mmol) was added to the reaction mixture and stirring continued at ambient temperature for 2 hrs. Further 1M lithium aluminium hydride

solution (4 ml) was introduced and the resulting mixture heated at reflux temperature for 18 hrs. After cooling to room temperature, the reaction was quenched by the careful addition of saturated aqueous sodium sulfate solution followed by solid anhydrous sodium sulfate. After 20 mins of stirring, the mixture was filtered, washing the precipitated lithium salts with diethyl ether (3 x 20 ml), before drying the combined organics over anhydrous magnesium sulfate. After solvent removal *in vacuo*, the material was purified by flash chromatography (hexane-ethyl acetate 10:1) to yield the *title compound* as a colourless liquid (0.113 g, 40% yield).

$[\alpha]_{25}$ = +7.5° (c 0.5, $CHCl_3$) (*Lit.*[1] $[\alpha]_{25}$ = -15.5° (-)-geosmin **1**)
(Found: M^+, 182.1675. $C_{12}H_{22}O^+$ requires: *M*, 182.1671);
ν_{max}(film) 3630w, 3520br, 2940s, 2865s, 1465m, 1380m, 945m cm^{-1};
δ_H (400MHz; $CDCl_3$) 1.40 (15H, m, C2 to C4-H_2, C1-H and C5 to C8-H_2), 1.24 (1H, s, C8a-O*H*), 1.03 (3H, s, C10-H_3), 0.77 (3H, d, *J* 6.9 Hz, C9-H_3);
δ_C (100MHz; $CDCl_3$) 74.41 (C8a), 37.28 (C4a), 35.69 (C1), 35.07, 34.28, 30.41, 29.08, 21.07, 20.74, 20.60, 20.23 (C10), 14.89 (C9);
m/z (EI) 182 (M^+, 4%), 125 (13), 112 (100), 111 (24), 97 (16), 55 (35), 43 (44), 41 (44).

APPENDIX B: REFERENCES

1. Gerber N. N.; Lechevalier H. A., *Applied Microbiology*, **1965**, *13*, 935.
2. Medsker L.; Jenkins D.; Thomas J., *Environ. Sci. Technol.*, *2*, **1968**, 461.
3. Yurkowski M.; Tabachek J-A., *J. Fish Res. Board Can.*, *31*, **1974**, 1815.
4. Marshall J. A.; Hochstetler A. R., *J. Org. Chem.*, **1968**, *33*, 2593.
5. Kaiser R.; Nussbaumer C., *Helvetica Chimica Acta*, **1990**, *73*, 133.
6. Ayer W. A.; Browne L. M.; Fung S., *Canadian J. Chem.*, **1976**, *54*, 3276.
7. Esher S. D.; Morris A. F., on behalf of Firmenich SA, United States Patent, No 4,248,742.
8. Gosselin P.; Joulain D.; Laurin P.; Rouessac F.; *Tetrahedron Letters.*, **1989**, *48*, 2775.

8a. Saito A.; Tanaka A.; Oritani T., *Tetrahedron Asymmetry*, **1996**, *7*, 2923.

9. Revial G., *Tetrahedron Letters*, **1989**, *30*, 4121.
10. Swarts H. J. K.; Haaksma A. A.; Jansen B. J. M.; de Groot A., *Tetrahedron*, **1992**, *48*, 5497.

10a. Hannson L.; Carlson R., *Acta Chemica Scandinavica*, **1990**, *44*, 1042.

11. Polak E. H.; Provasi J., *Chemical Senses*, **1992**, 17, *1*, 23.
12. Marshall J. A.; Hochstetler A. R., *J. Org. Chem.*, **1966**, *31*, 1020.
13. Polak E.; Trotier D.; Baliguer E., *Chemical Senses and Flavour*, **1978**, 3, *4*, 369.
14. Moorkerjee B.; Light K.; Hill I., *Essential Oils*, 247, Allured Publishing Corp., **1981**.
15. Brunke E.; Kappey C.; Klein E., *Proceedings of the IX International Congress of Essential Oils*, **1983**.
16. Ayer W. A.; Paice M. G., *Can. J. Chem.*, **1976**, *54*, 910.
17. Zoretic P.; Branchaud B.; Maestrone T., *Tetrahedron Letters*, **1975**, 527.
18. Pfau M.; Guingant A.; Revial G.; Organisation Mondiale de la Propriete Intellectuelle, Patent No WO 85/04873, **1985**.

19. Source: BBA analytical services.
20. Kaiser R.; Nussbaumer C., *Helv. Chim. Acta*, **1990**, *73*, 133.
21. Nussbaumer C., unpublished work
22. Huber U.; Boland W.; König W. A.; Gerhcke B., *Helv. Chim. Acta*, **1993**, *76*, 1993.
23. Long S. K.; Patrick R., in Umbreit W. W., *Advances in Applied Microbiology*, **1963**, *3*, 135.
24. Matteson D. S., *et al*, *J. Org. Chem.*, **1985**, *15*, 281.
25. Boehler M.; Konopelski J., *Tetrahedron*, **1991**, *47*, 4519.
26. Alexakis A.; Mangeney P., *Tetrahedron Asymmetry*, **1990**, *1*, 477.
27. Maddox I. S., *Biotechnology 6b.*, **1988**, 31.
28. Rubin L. J.; Lardy H. A.; Fischer H. O. L., *J. Am. Chem. Soc.*, **1952**, 425.
29. Plattner J.; Rapoport H., *J. Am. Chem. Soc.*, **1971**, *93*, 1758.
29a. Wilson L., *J. Am. Chem. Soc.*, **1936**, *58*, 2401.
30. Kitamura M.; Ohkuma T.; Inoue S.; Sayo N.; Kumobayashi H.; Akutagawa S.; Ohta T.; Takaya H.; Noyori R., *J. Am. Chem. Soc.*, **1988**, *110*, 629.
31. Caron G.; Kazlauskas R. J., *Tetrahedron Asymmetry*, **1993**, *4*, 1995.
32. Bisht K.; Crout D., *Tetrahedron Asymmetry*, **1993**, *4*, 957.
33. Ui S.; Masuda H.; Muraki H., *Agric. Biol. Chem.*, **1984**, *48*, 2835.
34. Voloch M.; Ladisch M. R.; Rodwell V. W., Tsao G., *Biotechnology and Bioengineering*, **1981**, Vol XXIII, 1289.
35. Ui S.; Masuda H.; Murak H., *Agric. Biol. Chem.*, **1984**, *48*, 2837.
36. Stryer L., in *Biochemistry*, 490, 4th Edition, W.H. Freeman.
37. Jansen N. B.; Tsao G. T., *Adv. Biochem. Eng. Biotechnology*, **1983**, *27*, 85.
38. Krishnamurthy S.; Brown H., *J. Org. Chem.*, **1983**, *48*, 3085.
39. Holder R.; Matturro M., *J. Org. Chem.*, **1977**., *42*, 2166.
40. Lee H. K.; Maddox I. S., *Biotechnology Letters*, **1984**, *6*, 815.
41. Barrett E. L.; Collins E. B.; Hall B. J.; Matoi S. H., *J. Dairy Sci.*, **1983**, 66, 2407.

42. Magee R. J.; Kosaric N., 'The Microbial Production of 2,3-Butandiol' in *Advances in Applied Microbiology*, **1987**, *32*, ed Laskin A. I., 1077, 89.

43. Voloch M.; Jansen N. B.; Ladisch M. R.; Tsao G. T.; Narayan R.; Rodwell V. W., '2,3-Butandiol' in *Comprehensive Biotechnology*, ed M. Moo-Young, **1985**, 933.

44. Taylor M. B.; Juni E., *Biochim. Biophys. Acta.*, **1960**, *39*, 448.

45. Voloch M.; Ladisch M. R.; Rodwell V. W.; Tsao G. T., *Biotechnol. Bioeng.*, **1981**, *25*, 173.

46. Hohn-Bentz H.; Radler F., *Arch. Microbiol.*, **1978**, *116*, 197.

47. Ui S.; Matusyama N.; Masuda H.; Muraki H., *J. Ferment. Technol.*, **1984**, *62*, 551.

48. Jansen N. B.; Flickinger M. C.; Tsao G., T., *Biotechnol. Bioeng.*, **1984**, *26*, 362

49. Eiteman M. A.; Gainer J. L., *Appl. Microbiol. Biotechnol.*, **1989**, *30*, 614.

49a. Kinderlerer J. K.; Kellard B., *Chemistry and Industry*, August **1987**, 567.

50. Mattson A.; Öhrner N.; Hult K.; Norin T., *Tetrahedron Asymmetry*, **1993**, *4*, 925.

51. Eigtved P.; Hansen T. T.; Miller C. A., *Paper presented to the World Conference on Biotechnology for the Fats and Oils Industry*, **29th September 1987**.

52. Chen C-S.; Sih C., *Angew. Chem. Int. Ed. Engl.*, **1989**, *28*, 695.

53. Empie M.; Gross A., *Annu. Rep. Med. Chem.*, **1988**, *23*, 305.

54. Homandberg G.; Mattis J.; Laskovski M., *Biochemistry*, **1978**, *17*, 5220.

55. Zaks A.; Kilbanov A., *J. Biol. Chem.*, **1988**, *263*, 3194.

56. Kilbanov A., *CHEMTECH*, **1986**, 354.

57. Parida S.; Dordick J., *J. Am. Chem. Soc.*, **1991**, *113*, 2253.

58. Bruckman H.; Borgström H., *Lipases*, Elsevier, Amsterdam, **1984**, 3.

59. Verger R.; Mieras M.; DeHaas G., *J. Biol. Chem.*, **1973**, *248*, 4023.

60. Lavayre J.; Verrier J.; Baratti J., *Biotechnol. Bioeng.*, **1982**, *24*, 2175.

61. Kazlauskas R.; Weissfloch A.; Rappaport A.; Cuccia L., *J. Org. Chem.*, **1991**, *56*, 2656.
62. Hutlin P.; Jones B., *Tetrahedron Letters*, **1992**, *33*, 1399.
63. Wimmer Z., *Tetrahedron*, **1992**, *48*, 8431.
64. Prelog V., *Pure Appl. Chem.*, **1964**, *9*, 119.
65. Klibanov A., *Acc. Chem. Res.*, **1990**, *23*, 114.
66. Laane C.; Boeren S.; Hilhorst R.; Veeger C., in *Stud. Org. Chem. (Amsterdam)*, **1987**, *29*.
67. Diels O.; Alder K., *Liebigs Ann. Chem.*, **1931**, *490*, 243.
68. Breslow R.; Rideout D. C., *J. Am. Chem. Soc.*, **1980**, *102*, 7816.
69. Breslow R.; Maitra U.; Rideout D., *Tetrahedron Letters*, **1983**, *24*, 1901.
70. Breslow R.; Maitra U., *Tetrahedron Letters*, **1984**, *25*, 1239.
71. Lubineau A.; Augé J.; Queneau Y., *Synthesis*, **1994**, 743.
72. Lubineau A., *Chemistry & Industry*, **1996**, 123.
73. Kauzman M., *Adv. Protein Chem.*, **1959**, 141.
74. Breslow R., *Acc. Chem. Res.*, **1991**, 159.
75. Grieco P. A.; Garner P.; He Z., *Tetrahedron Letters*, **1983**, *24*, 1900.
76. Brisset F.; Garelli-Clavert R.; Azema C.; Rico-Lattes I.; Lattes A.; Moisand A., *New J. Chem.*, **1996**, *20*, 595.
77. Comeau E. K.; Beck E. J.; Caplan J. F.; Howley C. V.; Marangoni G. D., *Can. J. Chem.*, **1995**, *73*, 174.
78. Hartley G., *Aqueous Solutions of Paraffin Chain Salts*, **1936**, Hermann et Cie, Paris.
79. Tanford C., *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, **1975**, Whiley Interscience Publishers, New York.
80. Ramnath N.; Ramesh V.; Ramamurthy V., *Journal of Scientific and Industrial Research*, 44, **1985**, 199.
81. Menger F. M., *Acc. Chem. Res.*, 12, *4*, **1974**, 111.
82. Clunie J.; Goodman J.; Symons P., *Trans. Faraday. Soc.*, **1967**, 63, 754.
83. McBain J., *Adv. Colloid. Sci.*, **1942**, *1*, 99.

84. Elworthy P.; Florence A.; Macfarlane C., *'Solubilisation by Surface Active Agents'*, **1968**, Chapman and Hall, London.

85. Tascioglu S., *Tetrahedron*, **1996**, 52, *34*, 11113.

86. Lindemann B.; Wennerstroem H.; *Topics in Current Chemistry*, **1980**, *87*, 3.

87. Mukerjee P., *Adv. Colloid Interface Sci.*, **1967**, *1*, 241.

88. Fisher L. R.; Oakenfull D. G., *Chem. Soc. Rev.*, **1977**, *6*, 25.

89. Sevens B.; Rosenholm B., *J. Colloid Interf. Sci.*, **1973**, *44*, 495.

90. Stigter D., *J. Phys. Chem.*, **1974**, *78*, 1480.

90a. Wennerström H.; Lindman B., *J. Phys. Chem.*, **1979**, *83*, 2931.

90b. Mahieu N.; Tekely P.; Canet D., *J. Phys. Chem.*, **1993**, *97*, 2764.

91. Dill K.; Flory P., *Proc. Natl. Acad. Sci. USA*, **1981**, *78*, 676.

92. Menger F. M.; Doll D. W., *J. Am. Chem. Soc.*, **1984**, *106*, 1109.

93. *Topics in Current Chemistry – Photochemistry and Organic Synthesis*, **1991**, *129*, 60.

94a. Thomason M.; Bloor D.; Wyn-Jones E., **1991**, *J. Phys. Chem.*, *95*, 6017.

94b. Frindi M.; Michels B.; Zana R., **1994**, *J. Phys. Chem.*, *98*, 6607.

95. Greene T., in *Protective Groups in Organic Synthesis*, 232, **1981**, J. Wiley & Sons.

96. Morley J. *J. Chem. Soc. Chem. (C)*, **1967**, 2410.

97. see Hassner A.; Stumer C., *Organic Syntheses based on Name Reactions and Unnamed Reactions*, **1994**, Pergamon Press.

98. Mukerjee P.; Mysels J., *Critical Micelle Concentrations in Aqueous Surfactant Systems, Nat. Stand. Ref. Data Ser., Nat. Bur. Stand. (U.S.)*, **1971**, 36.

99. Diego-Castro M. J., unpublished results

100. Klotz I. M., *Biochemistry*, **1969**, *8*, 47.

101. Dutta K. R.; Bhat S. N., *Bull. Chem. Soc. Jpn.*, **1993**, *66*, 2457.

102. Quadrifoglio F.; Crescenzi V., *J. Colloid Interface Sci.*, **1971**, *35*, 447.

103. Lovrien R.; Peshenck P.; Tisel W., *J. Am. Chem. Soc.*, **1974**, *96*, 245.

104. Reeves R. L., *J. Am. Chem. Soc.*, **1975**, *97*, 6019.

105. Hailes H. C.; Foley L., unpublished work
106. Johnson W. S., *Bioinorganic Chemistry*, **1976**, *5*, 51.
107. see Clayton R., *Quart.Rev (London)*, **1965**, *19*, 168
108. Van Tamelen E.; Willett J.; Clayton R.; Lord K., *J. Am. Chem. Soc.*, **1966**, *88*, 4752.
109. Corey E. J.; Russey W.; Ortiz de Montellano P., *J. Am. Chem. Soc.*, **1966**, *88*, 4750.
110. Stork G.; Burgstahler W., *J. Am. Chem. Soc.*, **1955**, *77*, 5068.
111. Eschenmoser A.; Ruzicka L.; Jeger O.; Arigoni D., *Helv. Chim. Acta.*, **1955**, *38*, 1890.
112. Sutherland J. K., *The Tilden Lecture*, London, **1979**.
113. Van Tamelen E.; Hessler E., *J. C. S. Chem. Comm.*, **1966**, 411.
114. Johnson W. S., *Bio-Org. Chem.*, **1976**, *5*, 51.
115. Johnson W. S.; Lunn H.; Fitzi K., *J. Am. Chem.Soc.*, **1964**, *86*, 1972.
116. Harding K.; Cooper J., *Tetrahedron Letters.*, **1977**, 3321.
117. Corey E. J.; Walinsky S., *J. Am. Chem. Soc.*, **1972**, *94*, 8932.
118. Van der Gen A.; Weidhaup K.; Swoboda J.; Dunathan H.; Johnson W., *J. Am. Chem. Soc.*, **1973**, *95*, 2656.
119. Ireland R. E.; Libinski C.; Kowalski C.; Tilley J.; Walba D., *J. Am. Chem. Soc.*, **1974**, *96*, 3333.
120. Marshall J.; Wuts P., *J. Org. Chem.*, **1977**, *42*, 1794.
121. Johnson W. S.; Harbert C. H.; Stipanovic R. D., *J. Am. Chem. Soc.*, **1976**, *98*, 6188.
122. Johnson W. S.; Semmelhack M.; Sultanbawa U.; Dolak L. *J. Am. Chem. Soc.*, **1968**, *90*, 2944.
123. Johnson W. S.; McBarry B., *J. Am. Chem. Soc.*, **1971**, *93*, 4332.
124. Ireland R. E.; McKenzie T. C.; Trust R. L., *J. Org. Chem.*, **1975**, 40, *8*, 1007.
125. Brady S. F.; Ilton M. A.; Johnson W. S., *J. Am. Chem. Soc.*, **1968**, *90*, 11, 2882.
125a. Julia M.; Julia S.; Tchen S-Y., *Bull. Soc. Chim. France*, **1961**, 1849.

126. Wittig G.; Geissler G., *Liebigs. Ann. Chem.*, **1953**, *44*, 580.

127. Wittig G.; Schöllkopf U., *Chem. Ber.*, **1954**, *87*, 1318.

128. Gosney I.; Rowley A., *Organophosphorus Reagents in Organic Synthesis*, Cadogen J. ed., Academic Press, New York, **1979**, 17-153.

129. Tagaki W.; Inoue I.; Yano Y.; Okonogi T., *Tetrahedron Letters.*, **1974**, *30*, 2587.

130. Märkl G.; Merz A., *Synthesis*, 1973, 295, Hünig S., Stemmler I, *Tetrahedron Letters*, **1974**, *36*, 3151.

131. Schmitt M.; Bourguignon J.; Wermout C., *Tetrahedron Letters*, **1990**, *31*, 2145.

132. Smith D.; Trippett S., *J. Chem. Soc. Commun.*, **1972**, 191.

133. Schweizer E.; Crause D.; Minami T.; Wehman A., *Chem. Commun.*, **1979**, 1000.

134a. Markl G.; Merz A., *Synthesis*, **1973**, 295.

134b. Tagaki W.; Inoue I.; Yano Y.; Okonogi T., *Tetrahedron Letters*, **1974**, 2587.

134c. Hailes H. C.; Eames A., unpublished work.

134d. Synthesised by M. Diego-Castro.

135. Johnson A.; LaCount R., *Tetrahedron*, **1960**, *9*, 130.

136. Matikanien J. K.; Kaltia S.; Hase T., *Synlett.*, **October 1994**, 817.

137. Horner H.; Hoffman H.; Wippel G.; Klahre G., *Chem. Ber.*, **1959**, *92*, 2499.

138. Clayden J.; Warren S., *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 241.

139. Buss A. D.; Greeves N.; Mason R.; Warren S., *J. Chem. Soc. Perkins Trans I*, **1987**, 2569.

140. Arbusov A., *J. Russ. Phys. Chem. Soc.*, **1906**, *38*, 687.

141. Schmitt M.; Bourguignon J.; Wermuth C., *Tetrahedron Lett.*, **1990**, *31*, 2145.

142. Mouloungui Z.; Delmas M.; Gaset A., *J. Org. Chem.*, **1989**, *54*, 3936.

143. Rambaud M.; Del Vecchio A.; Villieras J., *Synth. Commun.*, **1984**, *14*, 833.

144. Villieras J.; Rambaud M., *Synth. Commun.*, **April 1983**, 301.

145. Trust R. L.; Ireland R. E., *Org. Synth. Coll.*, **1973**, *Vol 53*, 116.

146. Finkelstein H., *Ber. Dtsch. Chem. Ges.*, **1910**, *43*, 1528.

147. Ishchenko R.; Kovalev B.; Khusid A., *Zhurnad Organicheskoi Khimii*, **1987**, *28*, 6, 1190.

148. Babler J. H.; Spina K. F., *Synthetic Communications*, **1984**, *14*, 1313.

149. Reid E.; Ruhoff J.; Burnett R., *Org. Synth. Coll.*, **1943**, *2*, 246.

150. Bradsher C.; Brown F.; Leake P., *J. Am. Chem. Soc.*, **1957**, *79*, 1468.

151. Wiley G.; Hershkowitz R.; Rein B.; Chung B., *J. Am. Chem. Soc.*, **1964**, *86*, 964.

152. Hwa J. C. H.; Sims H., *Org. Synth. Coll*, **1973**, *5*, 608.

153. Olah G. A.; Gupta B. B. G.; Malhotra R.; Narang S. C., *J. Org. Chem.*, **1980**, *45*, 1638.

154. Schweizer E. E.; Creasey W. S.; Light K. A.; Shaffer E. T., *J. Org. Chem.*, **1969**, *34*, 213.

155. Cooke M. P., *J. Am. Chem. Soc.*, **1970**, *92*, 6080.

155a. see 'Application Guide for Chiral Column Selection', *2nd Edition*, Daicel Chemical Industries Ltd.

156. Diels O.; Alder K., *Justus Liebigs Ann. Chem.*, **1928**, *460*, 98.

157. Woodward R. B., *Angew. Chem.*, **1969**, *81*, 797.

158. Martin J. G.; Hill R. K., *Chem. Rev.*, **1961**, *61*, 537.

159. Carruthers W., *Cycloaddition Reactions in Organic Synthesis*, Pergamon Press, Oxford, **1988**.

160. see March J., *Advanced Organic Chemistry*, 745, 3rd Edition, J. Wiley & Sons.

161. Oppolzer W., *Synthesis*, **1978**, 793.

162. Wittig G.; Schoellkopf U., **1954**, *Chem. Ber.*, *87*, 1318.

163. Greenwald R.; Chaykovsky M.; Corey E., *J. Org. Chem.*,**1963**, *28*, 1128.

164. Mayelvaganan T.; Shreeshailkumar S. B.; Bhat S. V., *Tetrahedron*, **1997**, *53*, 6, 2185.

165. Schaefer J.; Bloomfield J., *Org. React.*, **1967**, *15*, 1.

166. Einhorn C.; Luche J-L, *Journal of Organometallic Chemistry*, **1987**, 322, 177.

167. Hudlicky T.; Srnak T., *Tetrahedron Letters*, **1981**, 22, *35*, 3351.

168. Groves J.; Jones N., *Tetrahedron Letters*, **1970**, 1161.

169. Earnshaw C.; Wallis C. J.; Warren S., *J. Chem. Soc. Perkins Trans I.*, **1979**, 3099.

170. Billing N.; Jackson R.; Malek F., *J. Chem. Soc. Perkins Trans I.*, **1979**, 1137.

171. Barton D. H. R.; Crich D.; Motherwell W. B., *J. Chem. Soc. Chem. Comm.*, **1983**, 939.

172. see Oppolzer W., *Angew. Chem. Int. Ed. Eng.*, **1984**, *23*, 876.

173. Evans D. A.; Chapman K. T.; Bisaha J., *J. Am. Chem. Soc.*, **1988**, 110, *4*, 1238.

174. Oppolzer W., *Tetrahedron*, **1987**, 43, *9*, 1969.

175. Fringuelli F.; Pizzo F.; Taticchi A.; Wenkert E., *J. Org. Chem.*, **1983**, *48*, 2802.

176. Angell E.; Fringuelli F.; Pizzo F.; Minuti L.; Taticchi A.; Wenkert E., *J. Org. Chem.*, **1989**, *54*, 1217.

177. Aben R. W. M.; Minuti L.; Scheeren H. W.; Taticchi A., *Tetrahedron Letters*, **1991**, 32, *44*, 6445.

177a. Braun J.; Sauer J.; *Chem. Ber.*, **1986**, *119*, 1269.

178. Grieco P. A.; Nunes J.; Gaul M., *J. Am. Chem. Soc.*, **1990**, 112, 4595.

179. Grieco P. A.; Handy S.; Beck J., *Tetrahedron Letters*, **1994**, *24*, 59.

179a. Forman M.; Dailey W., *J. Am. Chem. Soc.*, **1991**, *113*, 2761.

180. Grieco P. A.; Collins J. L.; Handy S. T., *Synlett.*, **1995**, 1155.

180a. Grieco P. A.; Nunes J.; Gaul M., *J. Am. Chem. Soc.*, **1990**, *112*, 4595.

181. Bergmann E.; Ginsburg D.; Pappo R., *Org. React.*, **1959**, *10*, 179.

182. Drauz K.; Kleeman A.; Martens J., *Angew. Chem. Int. Ed.*, **1982**, *21*, 548.

183. Eder U.; Sauer G.; Wiechert R., *Angew. Chem. Int. Ed.*, **1971**, *10*, 496.

184. Hajos Z. G.; Parrish D. R., *J. Org. Chem.*, **1974**, *39*, 1615.

185. Lubineau A.; Augé J., *Tetrahedron Letters*, **1992**, *33*, 8073.

186. Bassetti M.; Cerichelli G.; Floris B., *Gazzetta Chimica Italiana.*, **1991**, *121*, 527.

187. Kohler E., *J. Am. Chem. Soc.*, **1916**, *38*, 889.

188a. Denmark S.; Lee W., *Tetrahedron Letters*, **1992**, *33*, 7729.

188b. Lubineau A; Meyer E., *Tetrahedron*, **1988**, *44*, 6065.

189. Mukaiyama T.; Banno K.; Naraska K., *J. Am. Chem. Soc.*, **1974**, *96*, 7503.

190. Yamamoto Y.; Maruyama K.; Matsumoto K., *J. Am. Chem. Soc.*, **1974**, *105*, 6963.

191. Lubineau A., *J. Org. Chem.*, **1986**, *51*, 2142.

192. Gutzwiller P.; Buschacher P.; Fürst A., *Synthesis: Communications*, **1977**, 167.

193. Li Y.; Nassim B.; Crabbé P., *J. Chem. Soc. Perkins Trans. 1*, **1983**, 2349.

194. Ihara M.; Toyota M.; Fukumoto K.; Kametani T., *J. Chem. Soc. Perkins Trans. 1*, **1986**, 2151.

195. Kim M.; Gross R.; Sevestre H.; Dumlap N.; Watt D., *J. Org. Chem.*, **1988**, *53*, 93.

196. Hiroi K.; Yamada S., *Chem. Pharm. Bull. (Japan)*, **1975**, *23*, 1103.

197. Uma R.; Rajagopalan K.; Swaminathan S., *Tetrahedron*, **1986**, *42*, 2757.

198. Uma R.; Rajagopalan K.; Swaminathan S., *Tetrahedron Letters*, **1984**, *25*, 5825.

199. Cohen N., *Acc. Chem. Res.*, **1976**, *9*, 417.

200. Agami C.; Meyner F.; Puchot C.; Guilhem J.; Pascard C., *Tetrahedron*, **1984**, *40*, 1031.

201. Agami C.; Levisalles J.; Puchot C., *J. Chem. Soc. Chem. Commun.*, **1985**, 441.

202. Jung M., *Tetrahedron*, **1976**, *32*, 3.

203. Krishnamurthy S.; Brown H. C., *J. Org. Chem.*, **1983**, *48*, 3085.

203a. Gustafson E., *Tetrahedron Letters*, **1961**, 401.

204. Holder R. W.; Matturro M. G., *J. Org. Chem.*, **1977**., *42*, 2166.

205. Brewster J. H.; Bayer H. O., *J. Org. Chem.*, **1964**, *29*, 116.

206. Throop L., *J. Am. Chem. Soc. Comm.*, **1967**, 4789.

207. Inanaga J.; Ishikawa M.; Yamaguchi M., *Chemistry Letters*, **1987**, 1485.

208. Hupe D. J.; Kendall M. C. R.; Spencer T. A., *J. Am. Chem. Soc.*, **1972**, 94, 1254.

209. Ferran H. E.; Drake D. A.; Spencer T. A., *J. Org. Chem.*, **1975**, 40, 2017.

210. Marshall J.; Fanta W., *J. Org. Chem.*, **1964**, *29*, 2501.

211. Ziegler F. E.; Hwang K., *J. Org. Chem.*, **1983**, *48*, 3349.

212. Hailes H. C.; Monteleone A., unpublished results.

213. Cox P. J.; Simpkins N. S., *Tetrahedron Asymmetry*, **1993**, *2*, 1.

214. Von Hippel P. H.; Schleich T., *Acc. Chem. Res.*, **1969**, *2*, 257.

215. Hannson L.; Carlson R.; Sjöberg A-L., *Acta Chemica Scandinavica*, **1990**, *44*, 1036.

216. Taber D. F.; Rahimizadeh M.; You K. K.; *J. Org. Chem.*, **1995**, *60*, 529.