Novel asymmetric microenvironments for the separation of enantiomers of chiral drugs and natural products

Thesis

NOVEL ASYMMETRIC MICROENVIRONMENTS FOR THE SEPARATION OF ENANTIOMERS OF CHIRAL DRUGS AND NATURAL PRODUCTS

A THESIS SUBMITTED BY

DUNCAN ADAM RIMMER (BSC)

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Date of submission: 9th July 1990

Date of award: 13th September 1990

OPEN UNIVERSITY

CHEMISTRY DEPARTMENT

JULY 1990

ABSTRACT

The determination of enantiomeric composition is of great importance in the pharmaceutical and natural product industries where two enantiomeric forms of a chiral molecule can have very different biological activities.

Homochiral derivatization of enantiomeric compounds followed by chromatographic separation of the resultant diastereoisomers on conventional achiral columns has proved to be a cheap and useful method for enantioquantification.

Reactions of simple alkyl halides with alcohols, acids, thiols and amines are known. Novel homochiral derivatization procedures based on two such reactions have been investigated. In these reactions, homochiral alkyl halide reagents, some of which are prepared here for the first time, have been based on proline and lactone ring systems and chiral esters.

Another derivatization approach utilising highly reactive and novel homochiral chloromethyl ether reagents has proved successful. For example, racemic secondary alcohols when derivatized with (S)-(+)-tetrahydro-5-oxo-2-furanmethyl chloromethyl ether, have been separated to baseline. Quantification of 1% of one enantiomer in the presence of 99% of its antipode has been achieved. The presence of 5 bonds between the chiral centres of such diastereisomeric derivatives makes their separation an interesting result. Indeed, further work on the effects of spacing between chiral centres indicated that the separation efficiency of these systems were in the order 3>4<5 (bonds). An order of 3>4>5 is expected.

The chloromethyl ether reagents, unlike the alkyl halides, do not have a proton on the atom β to the halide and thus cannot undergo dehydro-

halogenation as a side reaction. The mild reaction conditions, employing diisopropylethylamine as a hindered non nucleophilic base make such derivatizations amenable to compounds containing acid and base sensitive functions.

Assessment of the performances of both the alkyl halide and chloromethyl ether reagents are made by comparison with enantioseparations achieved when using N-acetyl-(S)-(-)-prolyl chloride and (+)-(trans)-chrysanthemoyl chloride reagents. Synthetic procedures for the preparation of all the homochiral reagents are reported.

NMR techniques were investigated by employing modified cyclodextrins in an attempt to form diastereoisomeric inclusion complexes with chiral analytes. It was hoped that differences in their spectra would enable enantioquantification.

Initial attempts at preparing novel homochiral polyesters by the condensation polymerisation of polyethylene glycol (400) with homochiral diacids were made. Such polymers may have use as homochiral chromatographic stationary phases.

ACKNOWLEDGEMENTS

Of the many people whom I have to acknowledge in this section, Dr Malcolm Rose has to be put first. I thank him for his enthusiastic and excellent supervision. His taste in music (deemed obscure by many) to which I have partially succumbed, will live on in my record collection for many a year!!!

I am very grateful to the S.E.R.C. for supplying me with a grant for my studies.

Thirdly, my thanks goes out to the research department of I.C.I. Pharmaceuticals, for their help, interest and collaboration in my project. Several people in particular deserve a mention, these being Fred Bailey and Phil Britton for establishing contact, Lee Edwards and Kevin McKiernan for maintaining the collaboration and John Mulley and David Clayton for their assistance with the chromatographic work I performed in their labs. I should also thank them for giving me the opportunity to give a lecture to their department.

I would like thank all the staff in the Open University Chemistry Department, especially those who chipped in with their thoughts and ideas on some of the problems that were encountered. Gordon Oats, for his invaluable contribution in the recording of NMR spectra, Graham Jeffs for his patience with chromatographic hiccups and Brandon Cook for being a suffering lab technician deserve particular mention.

I am particularly grateful to Sue Hegarty for her excellent work in typing this thesis.

My best wishes for the future go out to the many students who have undergone the PhD process at the O.U., and in particular to Steve Lowes for being a tolerant

flatmate and temporary ambulanceman!!!

As well as receiving further education at the Open University, I hope that I have given something back in return - a Ski Club! The setting up of the club could not have happened without the dedication of Charles Gowing and many other people. The many new friends I have made and the running of the club gave me an outside interest that kept me sane. I would like to thank them all for their help, and I hope the club has a long future.

I would especially like to thank my parents and Claire (+ family) for all their patience and support throughout the writing of this thesis.

CONTENTS

Definitions of abbreviations and terms

α	Separation factor
CD	Cyclodextrin
CI(MS)	Chemical ionisation (mass spectrometry)
CME	Chloromethylether
CZE	Capillary zone electrophoresis
DCM	Dichloromethane
DME	Dimethylester
DMOE	Dimethoxyethane
EI(MS)	Electron ionisation (mass spectrometry)
GC	Gas chromatography
GLC	Gas liquid chromatography
GPC	Gel permeation chromatography
H	Height equivalent to one theoretical plates
HMPA	Hexamethylphosphoramide
HPLC	High-performance liquid chromatography
IR	Infrared (spectroscopy)
LSR	Lanthanide shift reagent
MTM	Methyl thiomethyl
N	Number of theoretical plates

NMR	Nuclear magnetic resonance (spectroscopy)
PEG	Polyethylene glycol
Py	Pyridine
Rs	Resolution
r.t.	Room temperature
SFC	Supercritical fluid chromatography
TFA	Trifluoroacetyl
THF	Tetrahydrofuran
TLC	Thin-Layer chromatography
TPC	Tifluoracetyl-(S)-prolylchloride
t_R	Retention time
UV	Ultraviolet (spectrophotometry)
VPO	Vapour phase osmometry
W	Width of chromatographic peak at its base

A homochiral compound, reagent, stationary phase, etc. is one enantiomerically pure form of the chiral compound, reagent, stationary phase, etc.

Enantioseparation is a process in which the enantiomers of a chiral compound are physically resolved.

Enantioquantification is a process by which the relative amounts of the two enantiomers of a chiral compound are determined

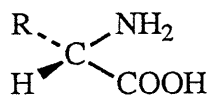
1 INTRODUCTION

1.1 Enantiomers and chirality

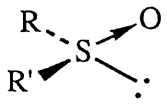
The existence of mirror-image (enantiomorphous) crystals of compounds such as quartz provoked speculation, during the early 19th century, as to the different natures of their composition. The resolution by recrystallisation of a racemic mixture (containing equal quantities of each enantiomer) of sodium ammonium tartrate into two enantiomorphic sets of crystals was achieved by Pasteur. This led him to propose that the composite molecules of the crystals exist themselves as mirror-images.[1]

An appreciation of 3-dimensional molecular structure led Vant Hoff and Le Bell to independently propose that the four valencies of carbon are directed towards the vertices of a tetrahedron,[2,3] and that if four different atoms or groups of atoms are bound to the central atom then the existence of two non-superimposable mirror-images forms is feasible. The two mirror-image forms of such a molecule are called enantiomers, and are referred to as being chiral (a term introduced by Lord Kelvin in 1884 to describe the property of handedness of these molecules).[4] Enantiomers are classified as stereoisomers that are identical in chemical constitution but that have different spacial arrangements of their atoms or groups of atoms. The two enantiomeric forms of a compound having identical internal energies thus have identical physical and chemical properties. They do, however, differ in their ability to rotate plane-polarized light. Each enantiomerically pure form is described as being optically active, one producing a positive rotation, the other a negative rotation. An equal mixture of both (a racemate) will produce no net rotation. This ability of enantiomers to rotate plane-polarized light forms the basis of polarimetry, and can be used to quantify enantiomeric composition.

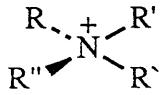
Figure 1



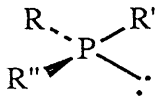
An amino acid



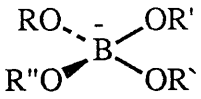
A sulphoxide



An ammonium ion



A phosphine



A borate ion

(R, R', R"and R` are dissimilar groups which will produce a molecule with four different groups attatched to the central atom)

1.2 Chirality and molecular structure

Chirality at a molecular level results from the presence of an asymmetric atom (centre), axis or plane within the molecular structure. Energy barriers prevent interconversion between the mirror-image molecular structures.

1.2.1 Atom-centred chirality

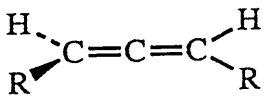
Non-planar covalently bonded molecular structures may contain an atom which forms a chiral centre. Typically formed from atoms of elements such as carbon, silicon and nitrogen, the chirality occurs due to their being tetrahederally bound to four different atoms or groups of atoms. Some common examples of molecular structures exhibiting atom-centered chirality are shown in Figure 1.

Group V and VI elements notably phosphorus and sulphur when complexed with three different ligands can also form non-planar chiral structures, the lone pair of electrons completing the tetrahedral arrangement. However, the ability for planar conformations to exist in such structures causes interconversions between the enantiomeric states (ie. racemisation).

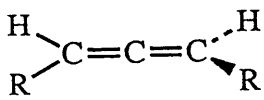
1.2.2 Axial chirality

Axial chirality can occur when the rotation to a bond (or bonds) within the molecule is hindered. This is most common in allenes, biaryls and spiranes.

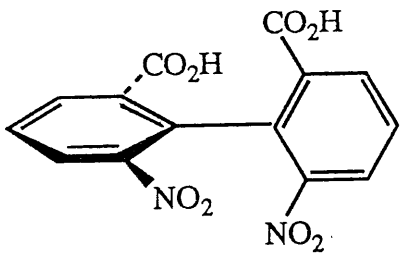
Here the two mirror images are non-superimposable and the fixed double bonds of compound (1) prevent rotation, and hence interconversion between the two enantiomeric forms.



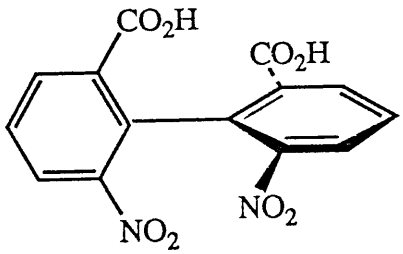
(1a)



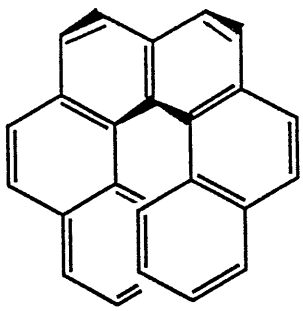
(1b)

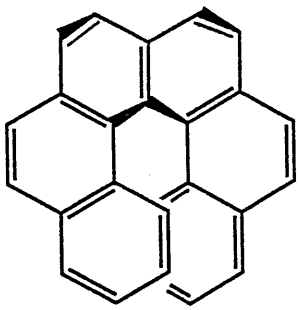


(2a)



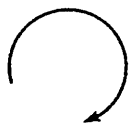
(2b)





-ve rotation

(3)



+ve rotation

The steric influence of the substituents in o,o'-dinitrodiphenic acid (Compound (2), a substituted biaryl) prevents rotation around the central bond, again inducing chirality.

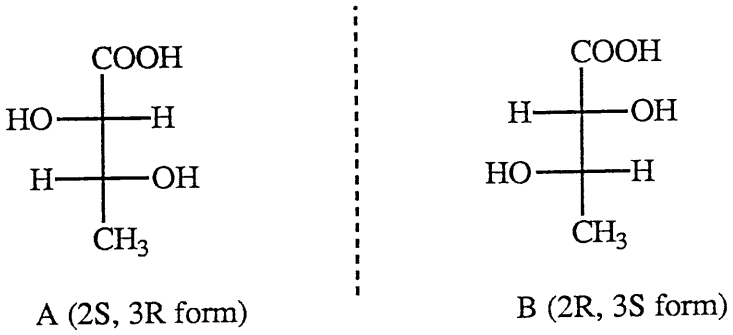
1.2.3 Planar chirality

This occurs in compounds such as ansa-compounds or trans cycloolefins,[5] which possess a chiral plane. Helocene (3) is an example of a cycloolefin which contains a plane of chirality (in the plane of the paper).

1.2.4 Diastereoisomers

If a molecule has more than one chiral centre, the number of possible stereoisomers is correspondingly increased. For example 2,3-dihydroxy-butanoic acid can exist in 4 stereoisomeric forms (see Figure 2). Isomers A and B are enantiomeric (i.e. they are mirror-image forms) and thus have identical physical and chemical properties. This is also the case for isomers C and D. Isomers A and C or A and D are however not mirror-image (enantiomeric) forms and are not superimposable. They are called diastereoisomers and are found to have different physical and chemical properties (i.e. different internal energies). If these properties are sufficiently different then they can be separated by conventional means (e.g. GC, HPLC, TLC, distillation etc.). The two diastereoisomeric forms may also have differences in their NMR spectra which can enable their quantification. The full possibilities of the enantiomeric and diastereoisomeric relationships for the 2,3-dihydroxybutanoic acid isomers is shown in Figure 3.

Figure 2



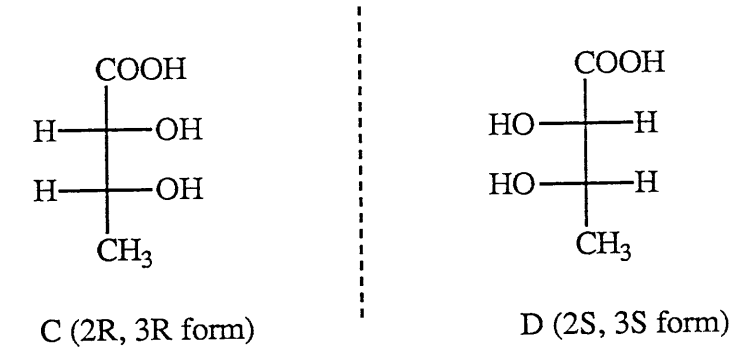
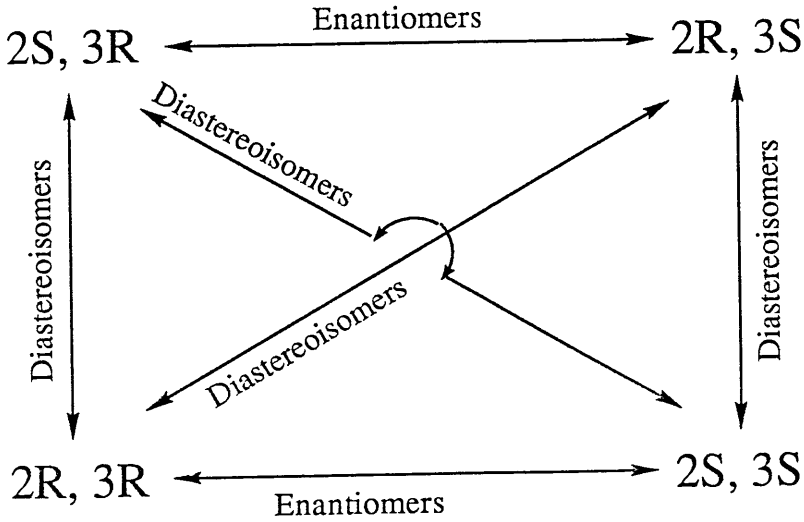
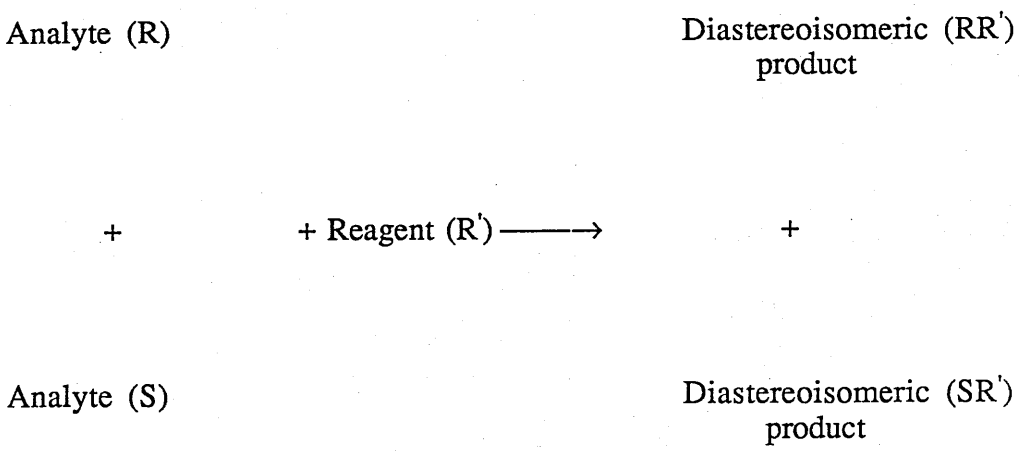


Figure 3



The ability to quantify the diastereoisomeric composition of a mixture through conventional separative or non-separative methods has been exploited for the determination of enantiomeric composition. This has been achieved through the use of homochiral derivatizing agents which when reacted with a mixture of enantiomers will form diastereoisomers. The use of a homochiral reagent means that only two diastereoisomeric products will be formed, the proportions of which will represent the composition of the original enantiomeric mixture (see Figure 4).

Figure 4



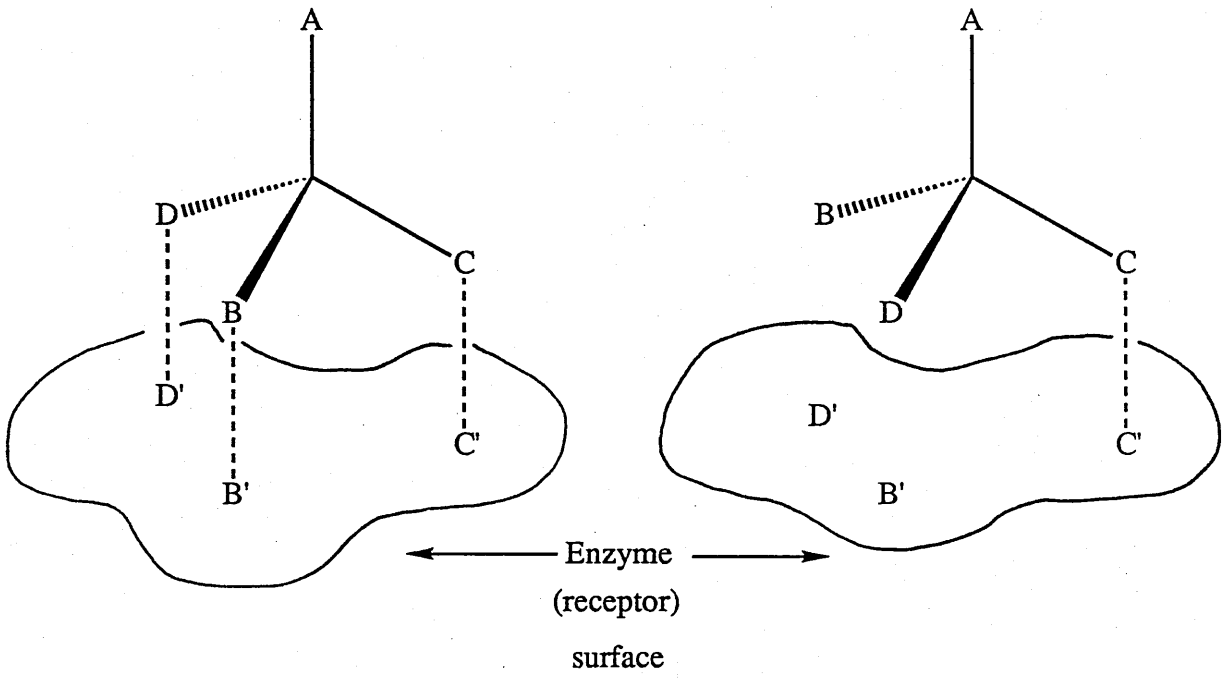
The formation of diastereoisomeric molecules is not always required for the quantification of enantiomeric composition. If the enantiomers of an analyte can undergo suitable (diastereoisomeric) interactions with a homochiral compound which is incorporated into a chromatographic stationary phase, separations of the enantiomers can be achieved. Diastereoisomeric interactions of enantiomers of an analyte with a homochiral compound added to the mobile phase of a liquid chromatographic system or to a NMR solvent can also enable the quantification of the enantiomeric composition (by LC or NMR analyses).

1.3 Asymmetry in nature

From a molecular to a universal scale, the presence of asymmetry is apparent. Universally, it is recognised by significant enantiomorphic excesses of S type (anticlockwise rotating) over Z type (clockwise rotating) spiral galaxies.[6-9] On a smaller scale the importance of asymmetry is recognised by the predominance of one enantiomeric form over its opposite (mirror-image) form in naturally occurring organic molecules. In fact almost all important biomolecules, including biopolymers such as polysaccharides, polynucleotides and proteins are found to be optically active.[5] This predominance of one enantiomeric form over the other stems from nature's ability to convert chiral compounds with remarkable stereo-specificity. This is generally due to the action of an enzyme (an asymmetric biological catalyst) in one or more biosynthetic steps. As enzymes are themselves produced by other enzymes, the origin and evolution of nature's asymmetry is thus unclear and complex.[6]

Enzymes, being protein-based, inherently have asymmetric active sites. For a chiral substrate to undergo an enzyme-catalysed reaction it must have the appropriate stereochemistry to enable the stereoselective interactions with

Figure 5 Three Point Interaction Model for Enzymes or Chiral Receptors

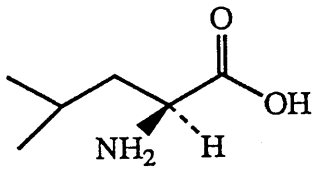


Three points of interaction.

Maximum of two points of interaction

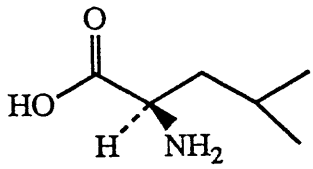
these active sites. Thus while one enantiomer interacts strongly with the enzyme and undergoes the biosynthetic conversion, the other does not.

Kinetic and X-ray crystallographic studies of enzymes have led to a greater understanding of the importance of spatial arrangements in these multipoint active site-substrate interactions. The simplest model illustrating these lock-and-key type mechanisms or chiral substrate-receptor interactions requires at least three points of interaction.[10] Figure 5 illustrates schematically that only

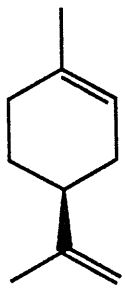


(L)-Leucine (Bitter)
Naturally occurring

(4)

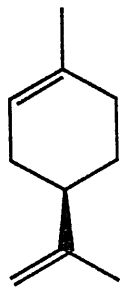


(D)-Leucine (Sweet)
Unnatural



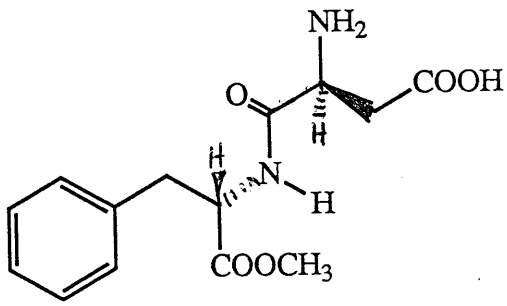
(S)-(-)-Limonine
(Lemon smell)

(5)



(R)-(+)-Limonine
(Orange smell)

Both enantiomers are naturally occurring



Aspartame (6)

(N-L- α-aspartyl-L-phenylalanine 1-methyl ester)

one enantiomeric form of the substrate has the correct spatial arrangement to interact with the asymmetric receptor.

Similar interactions between small chiral molecules and protein receptors often results in different biological activities. This is illustated by differences in the taste and smell of different antipodes of some natural products (e.g. compounds (4) and (5)).

1.4 Asymmetry in industry

The different effects on (or responses felt by) a biological system in contact with the different antipodes of a chiral compound have been exploited, or enhanced in the natural product and pharmaceutical industries. The use of artificial sweeteners is one of many thousands of examples in the natural product industry. The synthetic dipeptide Aspartame (6) in its L,L-form induces a sweet taste, whereas its D,L- form, its L,D- form and its D,D forms do not.[11] This is due to L,L-Aspartame undergoing stereoselective interactions with the protein receptors responsible for tasting sweetness.[12] The sweetness experienced from some of the artificial sweetners can be up to 3000 times that of sucrose, but aspartame is only 100-200 times sweeter.[11-14] The lower quantities of these compounds required to produce the same sweetness, and the fact that they do not undergo the same metabolic pathway (some are not metabolised), ensure that these sweetners are non-nutritive, and are thus used by diabetics and dieters.

The pharmaceutical industry is a multibillion pound business. The fact that about 50% of all pharmaceuticals are chiral illustrates the scale of the importance of enantiomers within this industry.[5] Their biological activity is again dependent on their stereochemistry. For instance, only the

(+)-enantiomer of the hormone estrone produces a physiological response. The (-)-enantiomer is inactive.

It has been estimated that about 80% of all the chiral pharmaceuticals are marketed as racemates. This may be due to a lack of knowledge of the different activities of each enantiomer of a chiral drug at the time of introduction, or because efficient stereoselective syntheses or separations yielding the enantiomerically pure products are not yet available. Whatever the reason, use of racemates can cause real problems, due to the enantiomers possibly having:

1. different biological activities. They can be active and beneficial, inactive, or active and harmful;

2. different protein binding and transport. This may cause the different enantiomers to be transported to different parts of the body, where again they may have undesirable effects;

3. different rates of metabolism. This may produce a harmful accumulation of the undesired enantiomer if the dosage is over a long period;

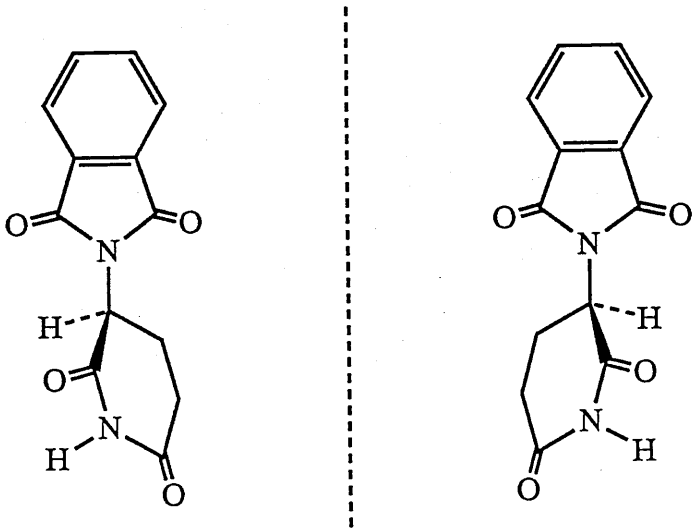
4. harmful metabolites;

5. different clearance rates. This again may cause a harmful accumulation of the undesired enantiomer.

The use of racemic pharmaceuticals is acceptable if the contaminating enantiomer and its metabolites are biologically inactive, and are passed out of the body at a similar rate to those of the useful enantiomer. They may also be utilised if the beneficial properties of the desired enantiomer outweigh any side-

effects caused by the other enantiomer. A tragic example of failing to appreciate the problems involved when using a racemic mixture was shown when the sedative and sleeping drug thalidomide (7) was administered in the early 1960s. It was found to cause serious malformations in the foetuses of mothers who used the drug in the early stages of pregnancy. The teratogenic action was found to be possessed only by the (S)-(-)-enantiomer.[15,16] Thus if the enantiomerically pure (R)-(+)-enantiomer had been administered, the drug would possibly still be in safe use today. While other racemic pharmaceuticals may produce less drastic side-effects, there are still pressures for the separation and pharmacological testing of the individual enantiomers.[17]

Thalidomide (7)



(S)-enantiomer (R)-enantiomer

By using efficient preparative separation techniques or asymmetric syntheses, the number of enantiomerically pure pharmaceuticals is on the increase. With this comes the need to guarantee the enantiomeric purity of the products. Methodology and techniques for the determination of enantiomeric composition are thus receiving considerable attention.

The reasons why companies within the natural product and pharmaceutical industries are showing particular interest in production of enantiomerically pure products are summarised below.

(i) Humanitarian - Both industries strive to provide and ensure a better quality of life without causing suffering.

(ii) Legislation - The number of new laws governing the testing of new products, and the guarantee of their quality are increasing.

(iii) Legal - Any errors in these industries often result in big law suits which can seriously damage the reputation of a company, and thus affect sales of their other products.

(iv) Financial - The possible legal costs and damage to sales incurred if an enantiomerically impure product is found to have undesired side-effects can run into hundreds of millions of pounds. On a more economic point, a company marketing a racemate is, in effect, selling a product that is only 50% pure (ie. 50% of the feedstuffs are wasted).

1.5 Determination of enantiomeric purity

Enantiomeric purity is a measure of the excess of one enantiomer over the other, i.e

$$\text{Enantiomeric purity} = (F+ - F-)/(F+ + F-)$$

$$= 2F+ - 1$$

where F+ and F- are the mole fractions of the enantiomers. F+ is taken as that enantiomer in excess. The value of enantiomeric purity is equal to the value of optical purity which will be defined later (Section 1.6.1).

Methods for the determination of enantiomeric purity can be placed into two categories: (i) those not involving separation; (ii) those involving separation. The methods within these categories are discussed separately.

1.6 Methods not involving separation

These use absolute values for properties of enantiomers, and rely on the analytes being chemically pure. If a mixture of different chiral analytes is present such methods can be very unreliable. The main three methods using polarimetry, NMR and enzyme techniques are discussed here. Less common methods incorporating isotope dilution analysis or calorimetry have also been used.[18,19]

1.6.1 Polarimetry

Biot was the first to discover (in 1815) that the plane of oscillation of a beam of plane-polarised light is rotated by solutions of certain compounds (e.g. sugars).[20] This ability to rotate plane-polarised light was later found to be a property possessed by enantiomers.

The measured rotation of a solution is dependent on factors such as solute concentration, optical pathlength, solvent used, temperature, wavelength used, etc. For the method to be used for quantitative analysis of an optically active

mixture of enantiomers the conditions must be specified, and should be constant between samples. This will be apparent later. Introducing these factors allows the specific rotation to be calculated thus:

$$[\alpha]_D^T = \frac{100\alpha}{lc}$$

where α = measured optical rotation, T = temperature (^oC), D = wavelength of the polarised light used (D inferes that a sodium lamp was used), l = cell length (dm), c = concentration of solute (g/dm^3). As the specific rotation can be very solvent and concentration dependent, they are usually quoted along with the value. The equation in this form has been used to calculate all the specific rotations quoted in this thesis.

The optical purity (P %) of a compound can be determined using the following equation

$$P = \frac{[\alpha]}{[\alpha]_{max}} \times 100\ \%$$

where $[\alpha]_{max}$ is the specific rotation of the optically pure compound and $[\alpha]$ is the specific rotation of the unknown enantiomeric composition.

The drawbacks of using polarimetry are several fold:

1. An enantiomerically pure sample of the compound of interest must be obtained in order to determine $[\alpha]_{max}$. Many $[\alpha]_{max}$ values are quoted in catalogues, but use of these values may introduce errors, as the exact optical rotation may differ slightly from polarimeter to polarimeter. $[\alpha]_{max}$ can be determined by indirect means (i.e. the isotope dilution method[21]) if required.

2. Optical purity has been found to be linearly related to enantiomeric purity only when there is no interaction between the enantiomeric forms in solution.[22]

3. The technique is not amenable to mixtures of different compounds.

4. The accuracy of the technique is not as high as those involving separation of the enantiomers.

5. The technique is not suitable for determining trace levels of enantiomeric purities due to the inherent inaccuracies involved.

1.6.2 Nuclear magnetic resonance spectroscopy

The use of nuclear magnetic resonance spectroscopy for the determination of enantiomeric composition was first utilized during the late 1960s. The technique requires the enantiomers to be transformed into a diastereoisomeric states. This is done in any of three ways:

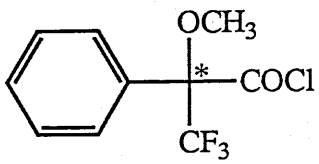
1. The formation of diastereoisomers by chemical reaction with homochiral reagent.

2. The use of homochiral solvents which can interact with the enantiomeric analytes (producing short-lived diastereoisomeric associates).

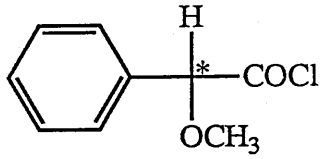
3. Use of homochiral lanthanide shift reagents to form diastereoisomeric complexation.

In these diastereoisomeric states, differences between their corresponding chemical shifts are observed. This is due to the different environments

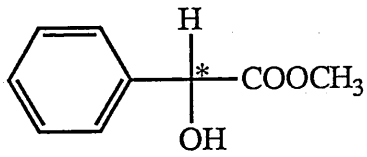
experienced by the atoms in the different states.[23] These different signals and their corresponding integration results allow the determination of the enantiomeric ratio present. The choice of the enantiomerically pure reagent for the formation of diastereoisomers by reaction is dependent on the functional groups present in the analyte. Reagents such as α-methoxy-α-trifluoro-methylphenylacetyl chloride (8)[24] or R- and S- methyl mandelyl chloride (9)[23] have been employed to derivatize alcohols and amines.



(8)



(9)



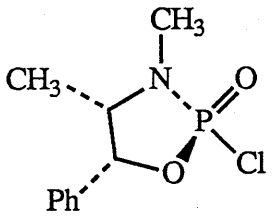
(10)

(*= chiral centres, either R or S)

Other reagents such as (R)-(-)- or (S)-(+)-methyl mandelate (10) have been used as derivatizing agents for the determination of the enantiomeric purity of α-deuteriated carboxylic acids, alcohols and amines.[25]

The use of phosphorus containing homochiral derivatizing reagent such as (11) has enabled ^{31}P NMR techniques to be used for the determination of enantiomeric purities of alcohols and amines.[26] Use of ^{31}P NMR techniques

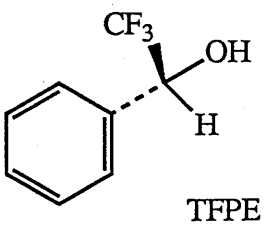
have proved to be beneficial for analyses where ^{1}H and ^{13}C nmr spectra are complex.[27]



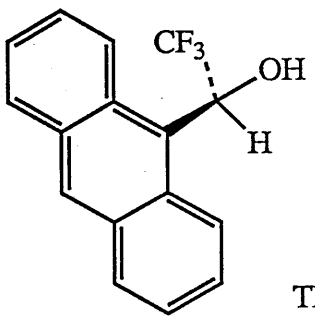
(11)

Homochiral solvents/solvating agents, have successfully produced differences between corresponding NMR signals of two enantiomers. This is considered to be due to the ability of one enantiomer to undergo preferential interactions with the solvent[28] which puts the atoms into different environments distinguishable by NMR techniques. It was studies into these preferential interactions which led Pirkle and co-workers to the design of suitable homochiral stationary phases for HPLC,[29] as discussed later.

Enantiomerically pure NMR solvents include (R)-(-)-2,2,2-trifluoro-1-phenyl-ethanol (12) and (S)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (13).



TFPE (12)



TFAE (13)

Figure 6 shows portions of the ^{1}H spectrum of a (S)- enriched mixture of (S)- and (R)-methylalanate in (R)-(-)-TFPE (Compound 12) (2 equivalents).[30]

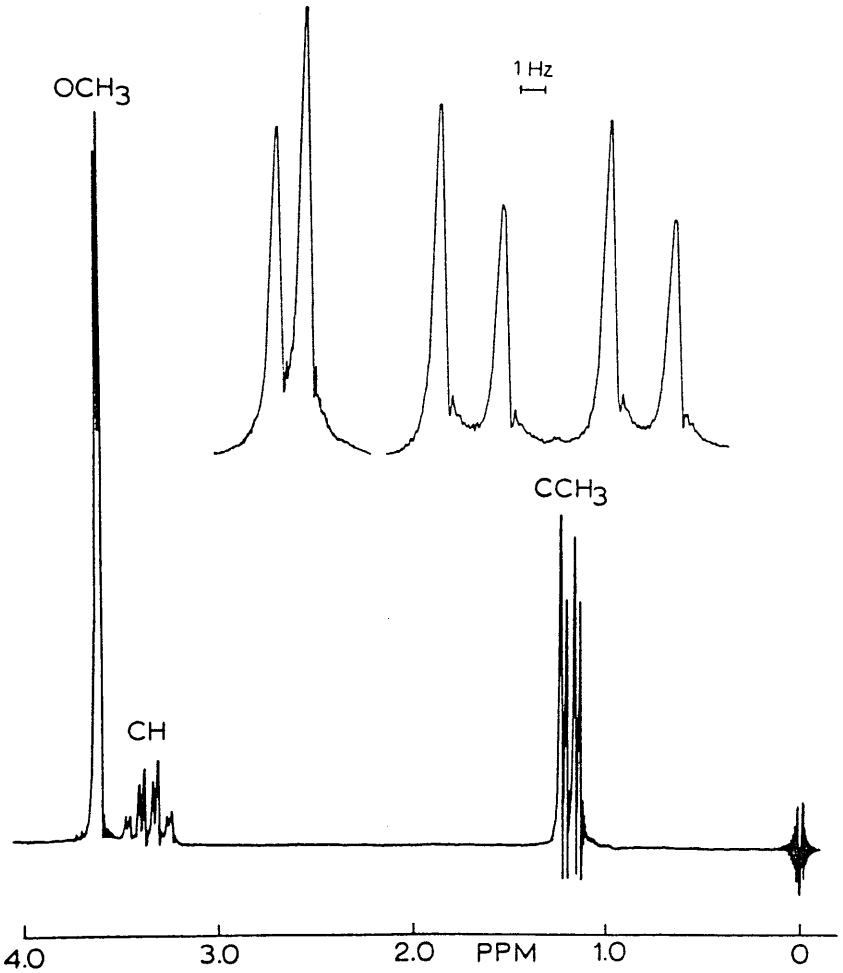


Figure 6. Portions of the 100 MHz NMR spectrum of the partially resolved mixture of (S)- and (R)-methyl alanate ((S)- enriched) in (R)-(-)-TFPE (12). The upper traces are scale expansions of the O-methyl (left) and C-methyl (right) resonances. Reprinted with permission from Pirkle, W.H., Beare, S.D., J. Am. Chem. Soc., 1969, **91**, 5150-5155.[30] Copyright 1969 American Chemical Society.

The 1.43:1 mixture of enantiomers (17.8% enantiomeric excess) was quantified from the relative intensities of the two sets of signals.

One other feature of homochiral solvating agents is that while ideally they should be enantiomerically pure, in practice they do not have to be. The use of an enantiomerically impure reagent only reduces the differences between the shifts of the two sets of signals, and not their intensities.

A similar effect to that observed when using homochiral NMR solvents occurs when a racemic analyte interacts with itself. Under appropriate conditions, "self discrimination" has been used for the determination of enantiomeric composition of 1,2-diols.[31]

The use of homochiral lanthanide shift reagents (LSRs) for the determination of enantiomeric composition has been well documented.[32-35] The magnitudes of the shift differences between enantiomers in the presence of the optically active LSRs have been reported to be generally greater than those for the other NMR methods.[32] The shifts induced, which increase the resolution, arise from both contact and dipolar interactions.[36,37] The differences in shifts may be caused by the different diastereoisomeric complexes having different equilibrium constants (K_S and K_R, see Figure 7). The complex having larger binding constant will show a larger shift.[38] The two diastereosimeric complexes may also experience differences in their shifts resulting from the different environments experienced by their atoms in the different geometries they may assume.

Tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium (III) [$Eu(tfc)_3$] (14) is a typical lanthanide shift reagent in current use.[34] Compound (15) is another lanthanide shift reagent which has been succesfully utilized for the quantification of enantiomeric mixtures of compounds such as α-phenyl-ethylamine (see Figure 8).[33] Again, the quantification of enantiomeric

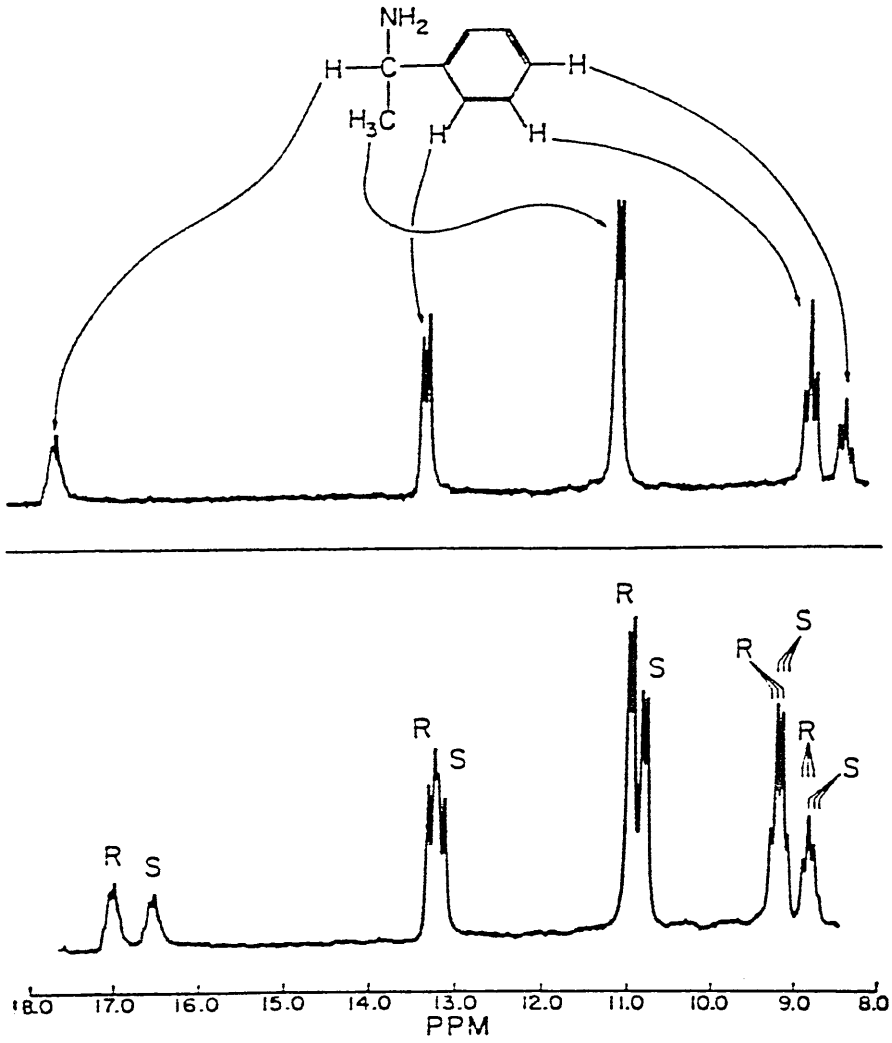
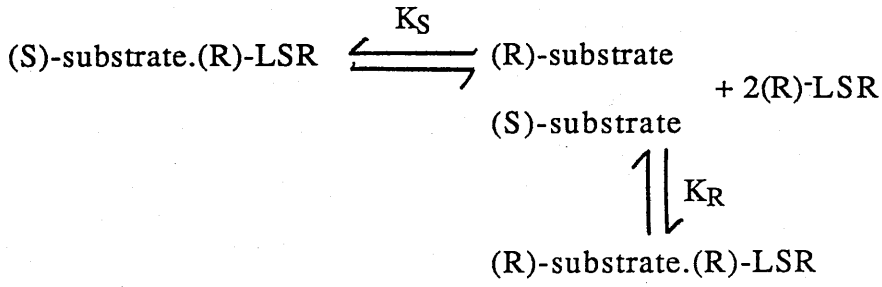
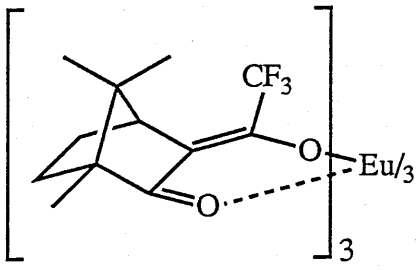


Figure 8. Spectra of solutions prepared from (S)-α-phenylethylamine (10μl) (upper), and a mixture (R)- and (S)-α-phenylethylamine (7μl and 5μl, respectively) (lower), in 0.3 ml of a carbon tetrachloride solution of (15) (~0.15M). The chemical shift scale applies only to the spectrum of the mixture; that of the pure S enantiomer was displaced slightly to lower field due to differences in concentrations of the samples. Reprinted with permission from Whitesides, G.M., Lewis, D.W., J. Am. Chem. Soc., 1970, **92**, 6979-6980.[33] Copyright 1970 American Chemical Society.

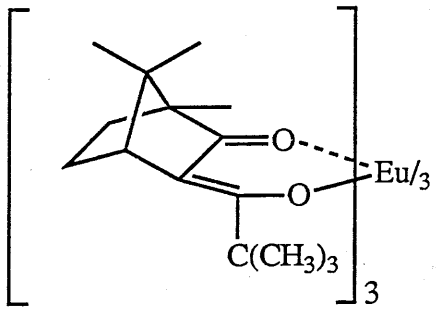
Figure 7



composition can be performed using the relative intensities of the two sets of signals (i.e. the integration results).



(14)



(15)

There are several drawbacks in all of the NMR techniques available.

(a) They lack sensitivity (typically >0.01 mmol of sample is required using a 90 MHz instrument, although with modern higher field instruments, the quantity required can be considerably less).

(b) As the techniques rely on a separation of signals along the δ scale, and not on physical separation, they are generally inappropriate for all but the most simple mixtures of different chiral compounds. This is purely due to possible overlapping of signals and the complexity of the resultant spectra for mixtures.

(c) The differences between the signals may only be small, which could affect the integration results especially when low levels of one enantiomer are being determined in the presence of its antipode.

1.6.3 Enzyme techniques

The action of enzymes, as already discussed, tends to be highly stereoselective. This is particularly so with amino acids where many enzymes act selectively on one amino acid enantiomer in the presence of the other.[39,40] Knowledge of this has led to the preparation of peptides in which one or more of the normal L-amino acids have been replaced by their D-analogues. This has in some cases produced retention of their pharmacological activity *(in vitro)*, and a greater activity than the natural peptide *(in vivo)* due to their resistance proteolytic breakdown.[41,42]

Enantiospecific enzyme-catalysed transformations have also been used for the accurate determination of high enantiomeric purities. Based on the study of the reaction of the contaminating enantiomer, it is possible to detect 0.1% in the presence of 99.9% of its optical antipode. Examples of such enantiospecific reactions involve an enzyme such as an acetylase. Hog renal acetylase immobilised on a cellulose support has been used for the catalysed hydrolysis of *N*-acetyl-L-amino acids in the presence of their D-enantiomers.[43] (see Figure 9)

Figure 9

$$\text{(D,L)-R'CONHCHRCO}_2\text{H} \longrightarrow \text{(D)-R'CONHCHRCO}_2\text{H} + \text{(L)-H}_3\overset{+}{\text{N}}\text{CHRCO}_2^-$$

Streptomyces olivacens can be used for the catalysed hydrolysis of an N-acetyl-D-amino acid in the presence of its L-enantiomer.[44]

However, for some transformations such as decarboxylation, only one enantiomer (the L-form in this case) can be determined. This is due to the lack of an enzyme to catalyse the transformation of the other enantiomer.

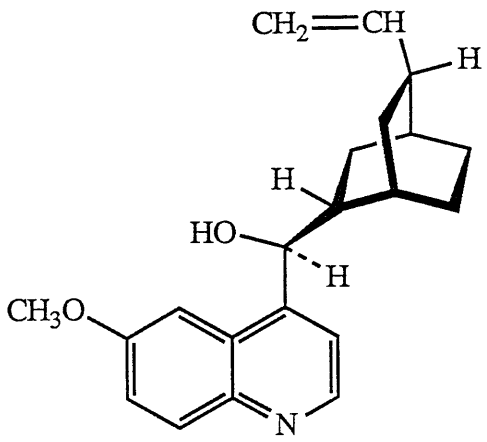
The drawbacks in the enzyme technique are that it is only applicable to compounds which can undergo enzymic transformations, and that enzymes for some transformations of specific enantiomers cannot be obtained.

1.7 Methods involving separation

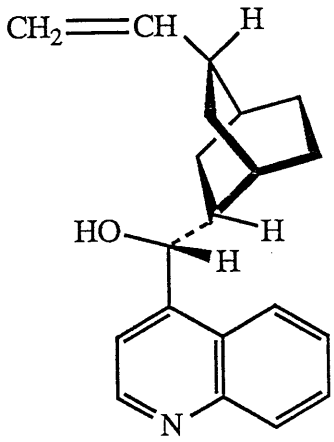
1.7.1 Crystallisations

Crystallisation has been used to separate enantiomers and diastereoisomers. As neither separation procedure is quantitative, errors can occur if used for enantiomeric composition determinations. Crystallisations, especially of diastereoisomers, have thus mainly been used as a preparative technique, to separate enantiomers and to ensure their enantiomeric purity. The technique is particularly important for the preparation of compounds requiring a high enantiomeric purity (e.g. for optically active derivatizing agents[45]).

The discovery by Pasteur of the spontaneous resolution of racemic sodium ammonium tartate[1] is only applicable to specialized examples where the formation of separable asymmetric, homochiral crystals occurs. The problems associated with spontaneous resolution such as recognition and separation of the enantiomorphic crystals may be overcome by controlling crystal growth using chiral growth inhibitors.[46] Pasteur also worked on the formation of diastereoisomers from enantiomers using optically active reagents such as paragine, quinine, strychnine etc, noting that the two diastereoisomeric forms



(-)-Quinine (16)



(+)-Cinchonine (17)

had different properties (e.g. solubility, crystal form, specific gravity etc.).[47] Diastereoisomeric quinine (16) or cinchonine (17) salts of racemic acids have been successfully separated by careful fractional crystallisation. Acidification of the isolated salts yields pure enantiomers of the original racemic acid.[48,49]

The drawbacks associated with crystallisation for the determination of enantiomeric purity, as pointed out, are numerous. Much more effective qualitative techniques are covered in the next subsection.

1.7.2 Chromatographic techniques - General

Chromatography as a means for the determination of enantiomeric composition is currently the most applicable and popular technique in this field. The extraordinarily rapid development of GC and LC instrumentation and column technology has almost made chromatography a science of its own.

Chromatographic theory will not be discussed in depth here, as most modern standard analytical texts cover the subject. Indeed there are many texts dedicated to chromatography,[50,51] and a few to the chromatographic separation of stereoisomers.[5,6,18,52] The chromatographic terminology used for enantio-separations is the same as that for any other chromatographic separation.

Recent improvements in packed analytical LC columns and the introduction and subsequent improvements in capillary GC columns have opened up the field of chromatography for enantiomeric separations. This is mainly due to the improved efficiencies of modern columns which has in turn improved their resolving capabilities. The efficiency of a column is thus a measure of its ability to transport a compound with little peak broadening. It is expressed as height equivalent to one theoretical plate (H) and can be calculated from a chromatogram thus:

$$N = 16\,(t_R/W)^2, \qquad H = {}^{L}/_{N}$$

Where N is the number of theoretical plates, t_R and W are the retention time and baseline width of the peak respectively and L is the length of the column. Typical H values of 0.025mm are achieved in modern columns, and N values of 2×10^5 theoretical plates are not uncommon in capillary GC columns that are 20-200 metres in length.

When considering the separation of any two components (see Figure 10) two terms are of particular importance, these being the separation and resolution factors (α and Rs respectively).

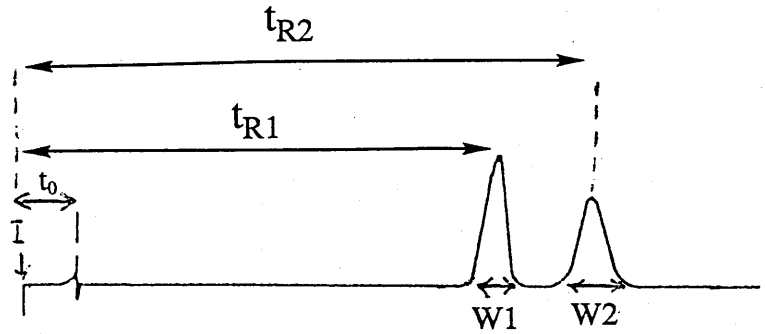


Figure 10 showing the retention and peak width parameters used for evaluation of column efficiency (H), separation factor (α) and resolution (Rs). The retention time of an unretained compound is t_o.

The separation factor can be calculated from the retention times of the two components:

$$\alpha = \frac{t_{R2}-t_o}{t_{R1}-t_o}$$

As the separation factor is a measure of the relative peak separation, it is thus related to the difference in the interaction of the two compounds with the stationary phase, and remains constant under a given set of analytical conditions (e.g. stationary phase, mobile phase, temperature, etc). As α is a comparison of the extent of interaction of the two components with a stationary phase, it can also be written as

$$\alpha = \frac{K_2}{K_1}$$

where K_1 and K_2 are the distribution constants for the two components.

Since $\Delta G = -RT \ln K$, an expression $\Delta\Delta G = -RT \ln \alpha$ can be obtained. This indicates that the separation factor is equivalent to an energy quantity which may be expressed in kJ/mole. This quantity represents the difference between the energies of the two components as they interact with the stationary phase. In terms of diastereoisomeric separations on an achiral stationary phase, it represents the difference between the internal energies of the two diastereoisomers. In terms of enantiomeric separations using homochiral stationary phases (or homochiral mobile phase additives) it represents the difference in binding (interaction) energies of the two enantiomers with a given homochiral site. This may be due to different steric effects producing different conformational changes in the two enantiomeric forms on interaction with the stationary phase (or mobile phase additive).

Table 1 lists the free energy differences required to produce the corresponding separation factors.[53]

α	ΔΔG (J/mol)
1.01	25
1.05	121
1.10	236
1.50	1005
2.00	1717

The resolution value for the two components, unlike their separation factor, is dependent on factors such as flow rate, column dimensions, etc. It is thus dependent on the column efficiency as well as the separation factor, and can be evaluated from a chromatogram (Figure 10) thus

$$Rs = \frac{2(t_{R2}-t_{R1})}{W_1+W_2}$$

Sometimes it is more convenient to measure the peak width at $^1/_2$ peak height ($W_{(0.5)}$). As $W = 1.7\ W_{(0.5)}$ the equation becomes

$$Rs = \frac{1.18\ (t_{R2}-t_{R1})}{W_{(0.5)1} + W_{(0.5)2}}$$

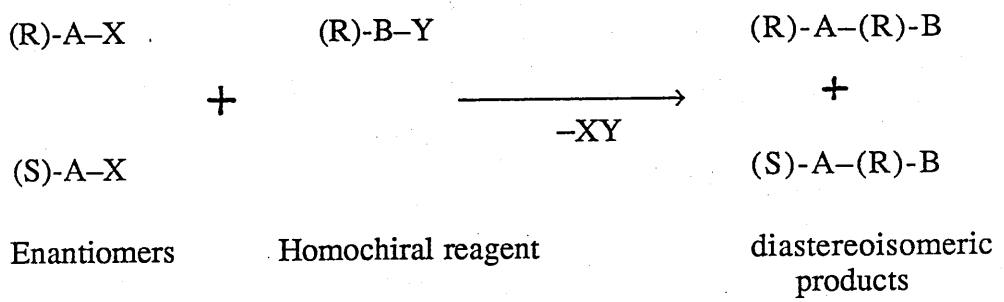
For chromatographic enantioseparations α values tend to be low (due to the small energy differences involved). Thus, in order to resolve the components, highly efficient columns must be used. Resolution values are found to be more useful in appreciating the extent of a separation as the width of the peaks as well as their retention times are considered. A resolution of 1.5 infers baseline

separation, but values down to 0.8 may be usable for the determination of enantiomeric composition (e.g. for measuring ~10% of one enantiomer in the presence of 90% of the other).

1.7.3 Chromatographic techniques: separation of diastereoisomeric derivatives

Until quite recently most LC and GC enantioseparations were carried out using conventional achiral columns. This is achieved by reacting the analytes with homochiral reagents to form diastereoisomeric derivatives (Figure 11).

Figure 11



The diastereoisomers having different internal energies and conformations have different physical and chemical properties. They thus experience different interactions with the stationary phase enabling separation.

Many non-chiral derivatization reactions and techniques have been developed since the introduction of gas chromatography, mainly to enhance volatility of the analytes. The use of such reactions, but using homochiral reagents for the formation of diastereoisomers, has been exploited. Examples of the common derivatization reactions employed, the functional groups involved and suitable homochiral reagents for GC and LC analyses are considered below.

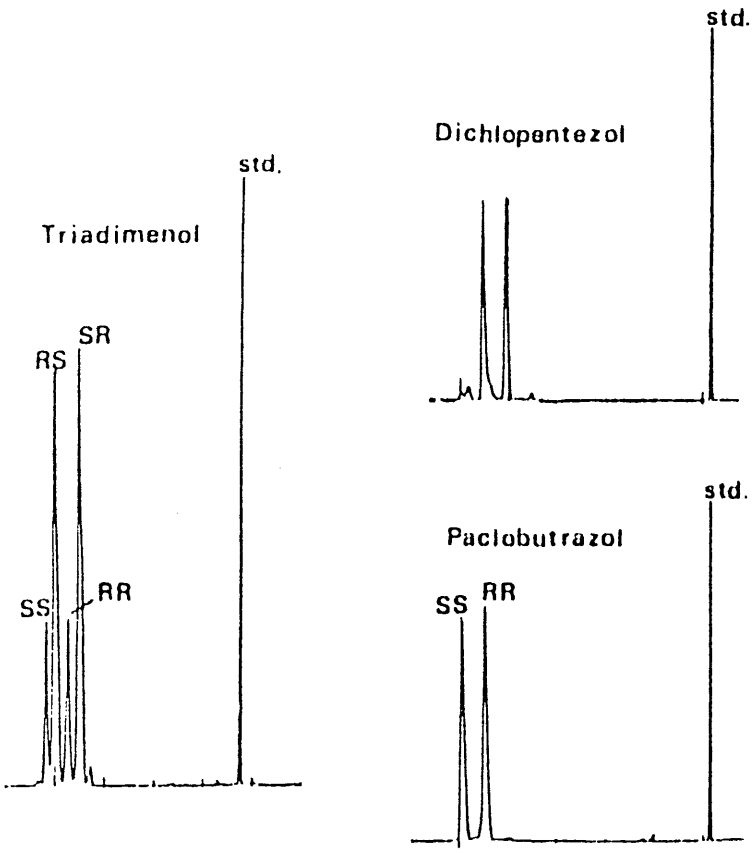
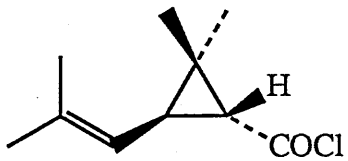


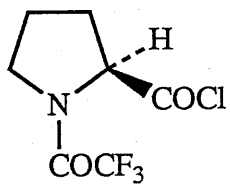
Figure 12. Gas chromatograms on an SE-52 capillary column of racemic triazole alcohols after esterification with (1S, 3S)-(trans)-chrysanthemoyl chloride. The internal standard was a C_{24} n-alkane. Enantiomeric assignments were made through separate experiments with single enantiomers. std.=Standard. Reproduced with permission from Burden, R.S., Deas, A.H.B., Clark, T., J. Chromatogr., 1987, **391**, 273-279.[56] Copyright 1987 by Elsevier Scince Publishers.

The derivatization reaction most commonly employed in diastereoisomer formation involves the use of acid chlorides. The reaction can involve a homochiral acid chloride to derivatize enantiomeric alcohols and amines (forming esters and amides respectively). Alternatively a homochiral alcohol or amine can be utilized to form derivatives with enantiomeric acids (via their acid chlorides) forming esters or amides, respectively.

An example of homochiral acid chlorides includes (+)-(trans)-chrysanthemoyl chloride (18) which has enabled the quantification of enantiomers of pheromones,[54] terpenes (e.g. menthol)[55] and triazole alcohols[56] (see Figure 12) and various amines (e.g. amphetamine)[55] by gas chromatography. Another, prepared from the cheap and readily available amino acid (S)-proline is *N*-trifluoroacetyl-(S)-prolyl chloride (19). This reagent has been used widely for the separation of enantiomers of numerous alcohols and amines by GC[46,54,57-59] and HPLC.[60,61] Use of *N*-heptafluoroacetyl-(S)-prolyl chloride has also been reported.[62]

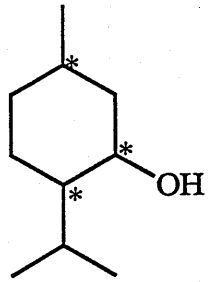


(18)



(19)

Examples of homochiral alcohols employed for the derivatization of enantiomers of acids (via their acid chlorides) for GC analysis include (-)-menthol (20),[63-65] (+)-3-methylbutanol[66,67] and enantiomerically pure 2-alcohols.[68-70] Figure 13 illustrates the results that can be obtained when using (-)-menthol to enable the quantification of chiral amino acids.[63]



(20) (* = Chiral centre, 1S, 2R, 5S)

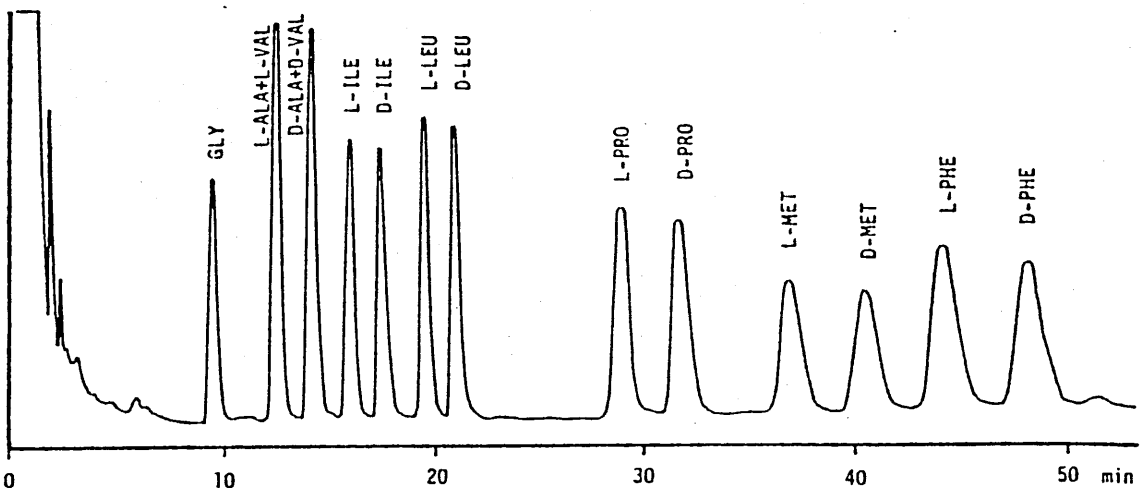


Figure 13. Simultaneous analysis of aliphatic and aromatic amino acid (-)-menthyl esters. *Conditions;* PEG-adipate column (4 m x 3 mm i.d.), 140-200^{O}C at 2^{O}C min^{-1}, flow = 10 ml min^{-1} (He), sample size = 1 µl. Reproduced with permission; Hasegawa, M., Matsubara, I., Anal. Biochem., 1975, **63**, 308-320 (Fig 2).[63] Copyright by Academic Press, Inc.

HPLC analysis of enantiomers of chiral acids (via their acid chlorides) have utilized homochiral reagents such as (R)- or (S)-1-phenylethylamine.[71]

Another derivatization reaction involves the use of chloroformates. The most notable example of a homochiral reagent with this functional group is

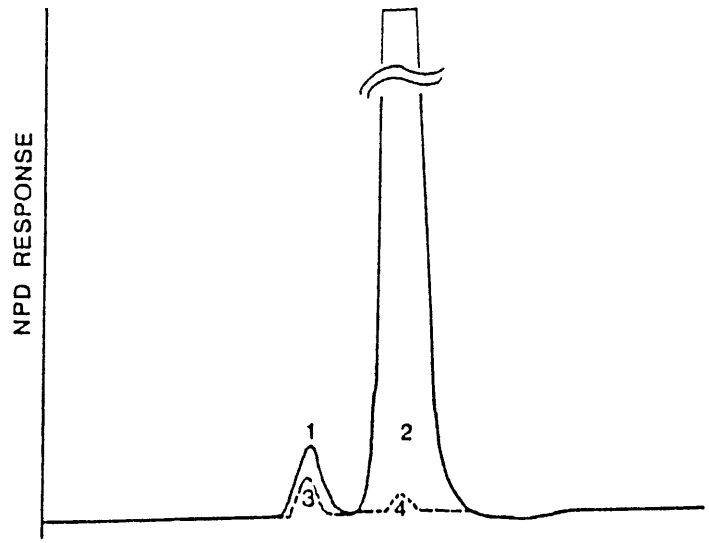
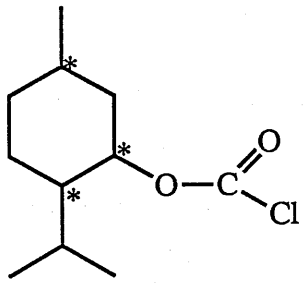


Figure 14. Representation of a typical chromatogram of aspartic acid TPC derivative of a brain sample (D / L-Asp < 0.050) analysed on an achiral DB-5 column. Peaks 1 and 2 are the two expected diastereoisomers. Peaks 3 and 4 (dashed lines) are the two dipeptides formed when TPC is not enantiomerically pure (i.e. after partial racemisation by excess triethylamine). Peaks: 1 = N-TFA-L-Pro-D-Asp$(OMe)_2$; 2 = N-TFA-L-Pro-L-Asp$(OMe)_2$; 3 = N-TFA-D-Pro-L-Asp$(OMe)_2$; 4 = N-TFA-D-Pro-D-Asp$(OMe)_2$; Reproduced with permission; Payan, I.L., Cadilla-Perezrios, R., Fisher, G.H., Man, E.H., Anal. Biochem., 1985, **149**, 484-491 (Fig 2).[45] Copyright by Academic Press, Inc.

(-)-menthylchloroformate (21) which has been used to derivatize enantiomeric amino or hydroxy-containing compounds for GC and HPLC analysis.[72,73]



(21) (* = Chiral centre, 1S, 2R, 5S)

Other less common derivatization reactions applicable to the formation of diastereoisomers are known. Examples of homochiral reagents for these reactions along with further examples of reagents used in the aforementioned reactions are listed in several texts.[74-76]

While the chromatographic analyses of diastereoisomers has produced some very good separations, the technique has several drawbacks:

1. Unless the reagent used is 100% optically pure any contaminating diastereoisomer peak may be due to an impurity in the reagent, and not in the analyte.[45] (see Figure 14 and discussion in Chapter 4).

2. Racemisation of the reagents can occur under certain reaction conditions which again may produce misleading results.[45]

3. Kinetic resolution can occur, and in some cases when the reaction has not run to completion and one enantiomer has reacted more rapidly than the other an incorrect result will be obtained.[77]

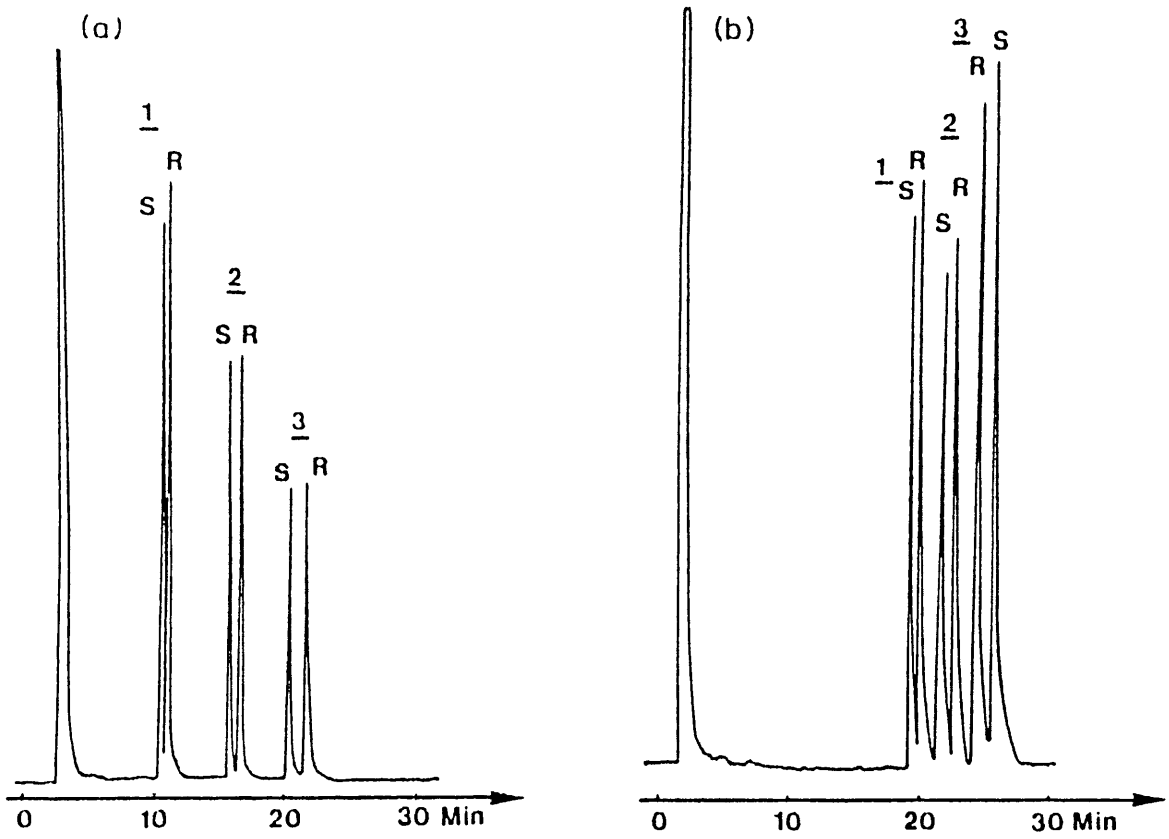


Figure 15. Separation of secondary alcohols as their urethane derivatives. Reagents (R)-(+)-1-phenylethyl isocyanate (a) and (R)-(-)-1-naphthylethyl isocyanate (b) on a DB-210 fused silica column (25 m), carrier gas N_2, 0.8 bar. Conditions: (a): 170°C isothermal; 1 = hexan-2-ol (α = 1.036); 2 = heptan-2-ol (α = 1.051); 3 = octan-2-ol (α = 1.0671); (b): 180°C at 2°C min^{-1} ; 1 = hexan-2-ol (α = 1.0305); 2 = heptan-2-ol (α = 1.0363); 3 = octan-2-ol (α = 1.0412). Reproduced with permission from Deger, W., Gessner, G., Hensinger, G., Singer, G., Mosandl, A., J. Chromatogr., 1986, **366**, 385-390.[78] Copyright 1986 by Elsevier Scince Publishers.

4. Fewer bonds between the two chiral centres of diastereoisomers produce better separations. Three bonds is the lowest number possible when using homochiral derivatization. Any more than four bonds between the chiral centres are reported as often producing unsatisfactory separations.[5]

5. The different diastereoisomers may have different detector responses (although unlikely) which may produce false results.

6. Different classes of analytes require different reagents (i.e. many methods are specific to just one class of compound).

7. An enantiomerically enriched sample of the analyte must be obtainable in order to determine the order of elution of the diastereoisomers (by derivatizing it separately). This should be carried out for each component of a mixture, even in a homologous series. This is because it does not always follow that the elution order will be the same for different homologues (see Figure 15).[78]

These potential drawbacks can be minimised by the choice of a suitable reagent and suitable reaction conditions.

The advantages of homochiral derivatization are also several fold:

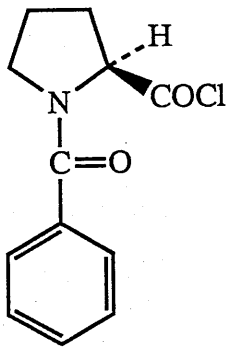
a) The reagents are usually obtained from cheap and readily available starting materials.

b) The separations can be achieved on conventional, relatively cheap achiral columns.

c) The order of elution of the diastereoisomeric products can be changed readily by using the other optical antipode of the homochiral reagent.

d) Different analogues of the homochiral reagents can be synthesised in order to enhance factors such as volatility of the reagent or to improve the sensitivity or selectivity of the analysis (e.g. the use of a reagent such as compound (22) may improve the UV detection for HPLC analyses).

While investigations into the synthesis and utilisation of novel homochiral reagents and reactions still receives attention, the use of homochiral chromatographic stationary phases, or mobile phase additives has become the more popular approach due to their inherent benefits (as discussed in Sections 1.7.4 and 1.7.5)



(22)

(22) Contains a chromophoric group which should enhance the sensitivity for HPLC analyses of chiral compounds.

1.7.4 Chromatographic techniques: Use of homochiral stationary phases

The use of homochiral stationary phases for GC and LC enantioseparations has received considerable attention over the past two decades. The different types of stationary phases available, the proposed chiral recognition mechanisms involved and many representative examples (with references) receive in-depth coverage in several modern texts and reviews.[5,6,18,79,80] For this reason a fairly brief discussion of the techniques available and representative examples are given here.

Chromatographic separations are based on differences in the retentions of the individual components. The retentions are dependent on the molecular interactions between the analytes and the stationary phase. Thus the types of interaction involved in chiral separations should be considered if an understanding of the recognition mechanisms is to be gained.

Models accounting for optical resolutions by GC and LC have often been based on the 3-point interaction theory (see the discussion on enzymes and Figure 1 in Section 1.3).[81] The model requires a minimum of 3 spatially significant contacts or interactions which can include steric interactions as well as non-covalent attachments. In gas chromatography this can include hydrogen bonding, hydrophobic and steric interactions. Liquid chromatography can involve the same interactions, but has the advantage in that the polarity of the mobile phase can be altered to enhance some of the interactions.

The resolution of enantiomers is achieved through their reversible diastereoisomeric association with the chiral environment of the stationary phase. The many different types of molecular interactions able to produce differences in the association of enantiomers with a homochiral stationary phase are reflected by the number and variety of chiral stationary phases available.

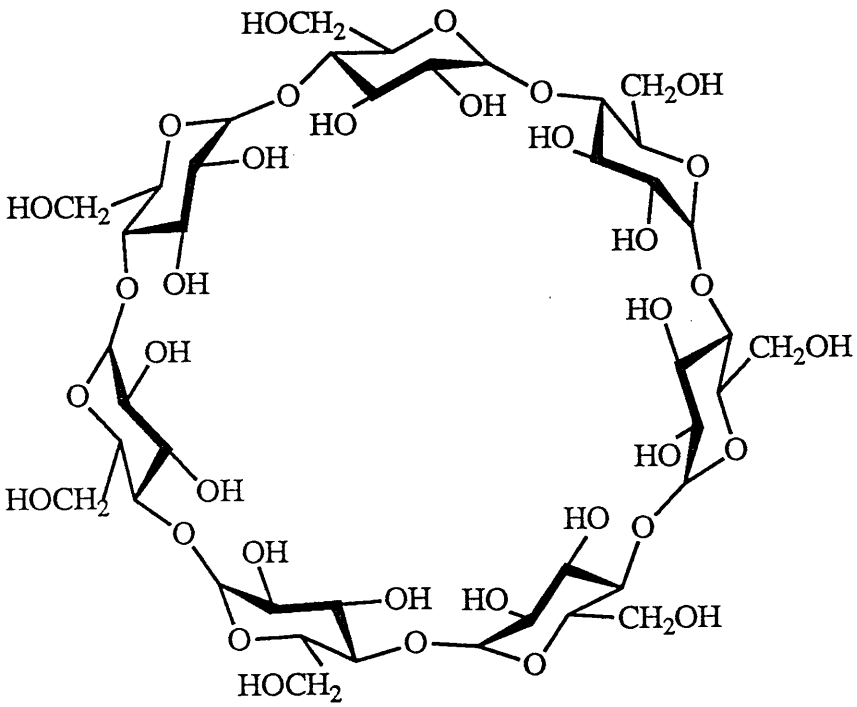


Figure 16. The Structure of β-Cyclodextrin

Some important types of binding are now briefly introduced before assessing their role in enantioseparations using various GC and LC homochiral stationary phases.

a) Coordination to transition metals

Transition metal coordination complexes can be formed using an immobilised homochiral ligand to produce a homochiral metal complex phase. Diastereo-isomeric mixed ligand sorption complexes will form by displacement or exchange mechanisms when a suitable racemate mixture passes through the column.

b) Charge transfer interactions

Aromatic compounds have the ability to form stable charge transfer complexes with their ring systems. They require π electron system interactions, acting as donor and receptor components. These aromatic π-π interactions alone, have been sufficient to enable separation of condensed aromatic hydrocarbons such as helocenes (showing planar chirality). The enantioselectivity (and applicability) of such interactions is greatly increased by further bonding interactions.[82] Homochiral stationary phases utilising these interactions have thus proved to be of great importance in LC enantioseparations.

c) Inclusion phenomena

The inclusion of guest molecules within the structure of certain host compounds is known. Cyclodextrins are chiral crystalline degradation products of starch (see Figure 16), the α, β and γ forms being composed of 6, 7 and 8 glucose units. The different forms are able to include compounds of differing sizes.[83-85] Thus for example the α-form can include iodine or benzene, but not

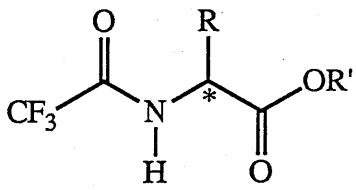
bromobenzene. Bromobenzene, is however included within the β-form (inducing precipitation).

Cyclodextrins, having a hydrophobic cavity and hydrophilic exterior, allow inclusion of hydrocarbon-rich parts of molecules. As structural demands enabling inclusion are not high, enantioselection is thought to occur through hydrogen-bonding effects and steric interactions with the chiral substituents present in the chiral structure at the entrance to the cavity.[86]

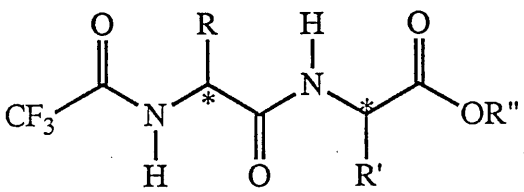
Homochiral GC phases

Examples of homochiral stationary phases employed in gas chromatographic enantioseparations are now considered.

The first homochiral stationary phases used for gas chromatographic enantio-separations were based on N-TFA-amino acid esters (23)[87] and N-TFA-dipeptide esters (24).

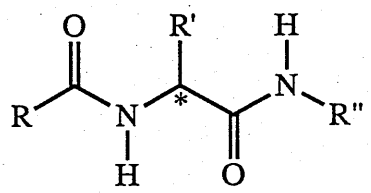


(23)



(24)

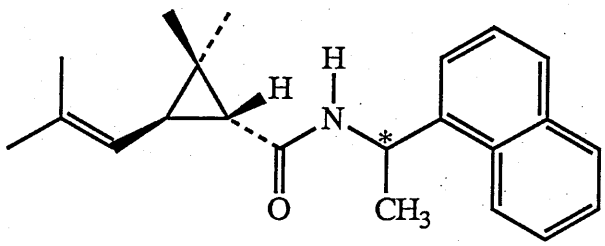
While such stationary phases resolved a number of N-TFA-D, L-amino acid esters by virtue of hydrogen-bonding interactions, their practical applicability was limited due to their volatility and low thermal stability. This was somewhat overcome by use of diamide phases (25) which were thermally stable up to 130°C and displayed high enantioselectivity.



(25)

The approach of adapting the structures of the stationary phase to the structures of the substrates produced some successes.[5]

Other useful low molecular weight homochiral stationary phases (e.g. 26) have been developed by Oi *et al.*[88]

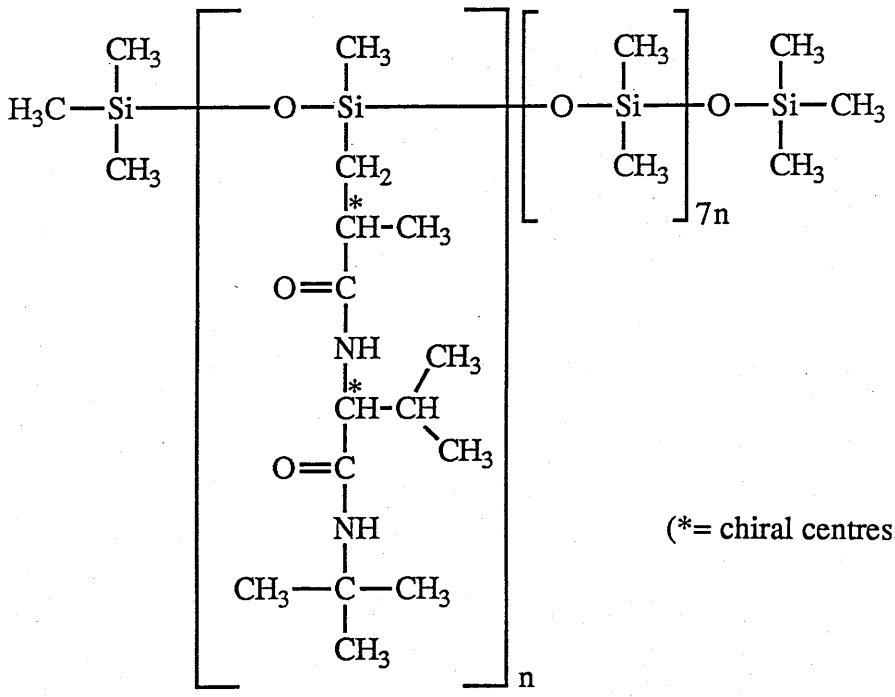


(26)

(*= chiral centres, R or S)

The preparation of 'Chirasil Val' (see Figure 17) by Frank, Nicholson and Bayer in 1977[89] was a significant development in homochiral GC stationary phases. Prepared by the covalent attachment the low molecular weight diamide, (S)-valine tertiary butyl amide, to a silicone polymer backbone it displayed unsurpassed thermal stability (up to 220°C) while showing excellent enantioselectivity in many cases.[90-93]

Figure 17. The Structure of "Chirasil-Val"



A typical chromatogram showing the separation of the enantiomers of nine N-TFA amino acids isopropyl esters is shown in Fig. 18.[93a] Another example illustrating the separation of derivatives of dipeptides on a Chirasil Val column

Figure 18. Courtesy of Macherey-Nagel, D-5160 Dueren, Germany.

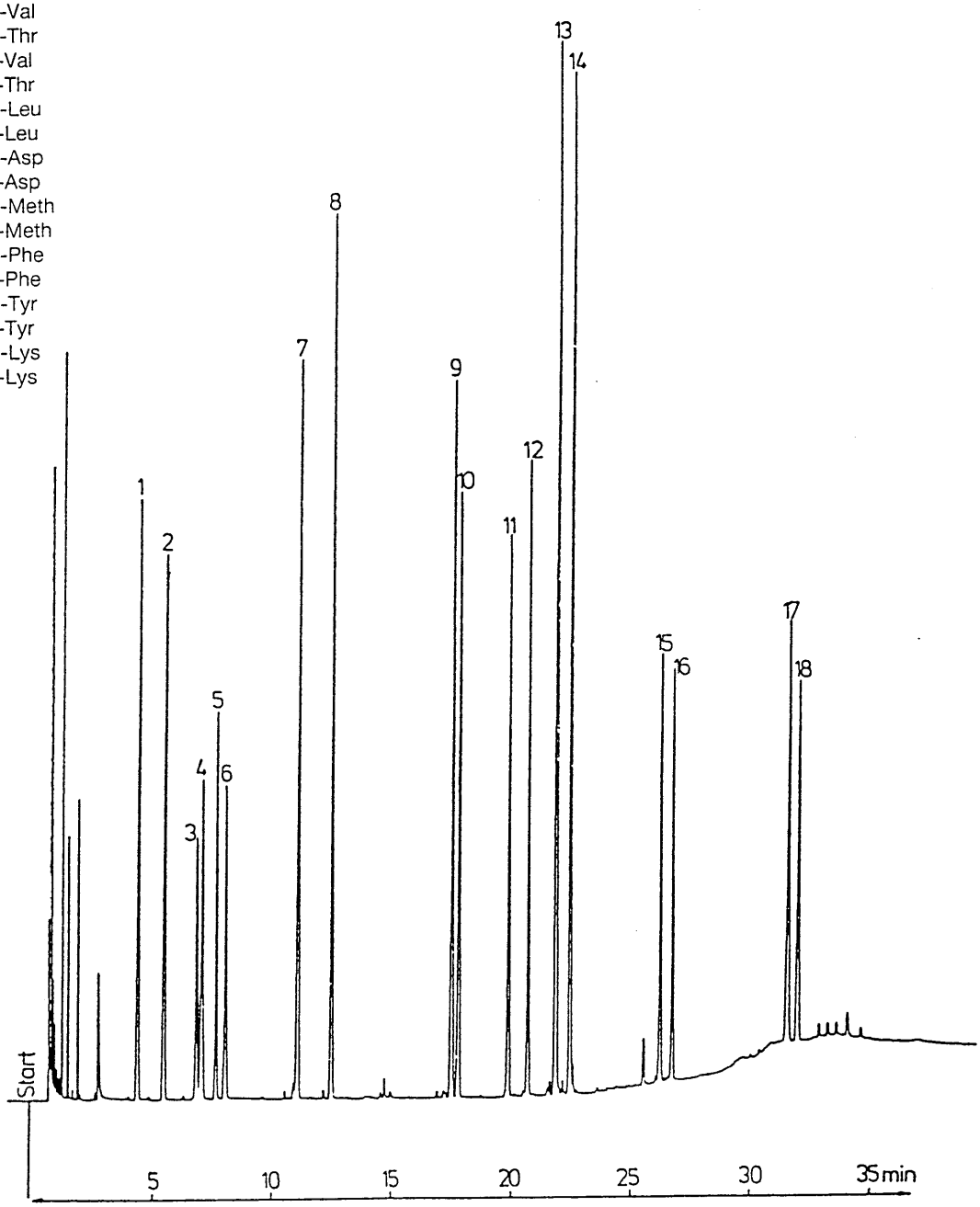
Enantiomeric separation of TFA amino acid isopropyl esters

Chromatographic conditions:

Capillary column:	PERMABOND® L-CHIRASIL-VAL, 25 m x 0.25 mm ID, max. temperature 220/240 °C;
Injection volume:	0.5 µl
Carrier gas:	0.4 bar H_2
Split:	about 1 : 40
Temperature:	80 °C (5 min) to 190 °C (10 min) with 4 °C/min
Detector:	FID 260 °C, 10 x 4

Peaks:
TFA amino acid isopropyl esters of

1. D-Ala
2. L-Ala
3. D-Val
4. D-Thr
5. L-Val
6. L-Thr
7. D-Leu
8. L-Leu
9. D-Asp
10. L-Asp
11. D-Meth
12. L-Meth
13. D-Phe
14. L-Phe
15. D-Tyr
16. L-Tyr
17. D-Lys
18. L-Lys



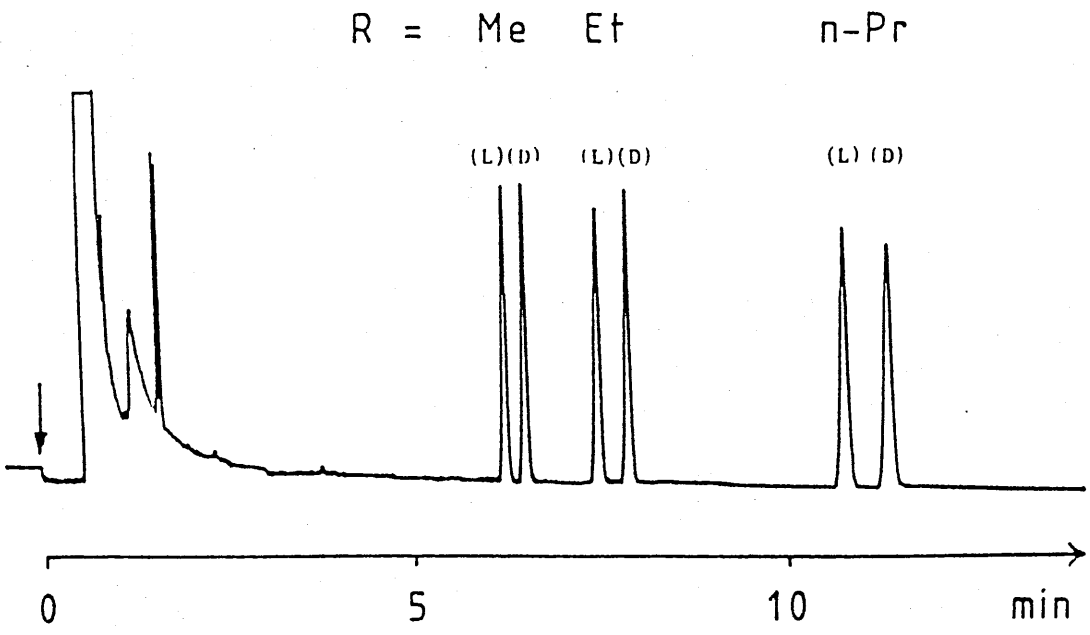
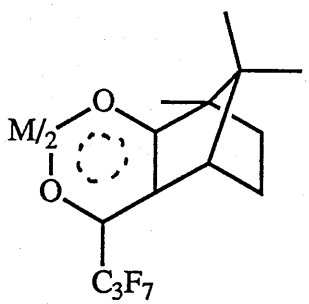


Figure 19. Enantiomer resolution of N-TFA-Gly-Val-OR by GC on D-Chirasil-Val (H40); isothermal 160^{O}C; 0.5 bar hydrogen; dead time 0.69 min. Me = methyl; Et = ethyl; n-Pr = n-propyl. Reproduced with permission from Koppenhoefer, B., Allmendinger, B., Chang, H., Cheng, L.B., J. Chromatogr., 1988, **441**, 89-98.[93]

is shown in Figure 19.[93] Many versatile homochiral phases have more recently been prepared by modification of the polysiloxane XE-60.[94-96]

Complexation GC (based on homochiral metal complexes) has proved useful for many compound classes that cannot be resolved on chiral amide phases (due to the lack of suitable functional groups for diastereoisomeric interaction), or by homochiral derivatization.[97,98] Separations are achieved by enantioselective interactions with chiral organometallic chelate complexes such as heptafluoro-butyryl-(1R)-camphor (27) which result in the formation diastereoisomeric association complexes.



(27) (M= e.g. Ni(II), Co(II) or Mn(II))

Cyclodextrins and their derivatives are increasing in popularity as enantioselective GC stationary phases.[99-101] Separation is thought to be achieved by the different ability of the enantiomers to be included within the cavity of the macrocycle.[102] Examples of some separations achieved on perpentylated α-cyclodextrin columns are shown in Figure 20.[100]

Homochiral LC phases

Enantiodifferentiation by chiral recognition of enantiomers by a homochiral stationary phase in LC is perhaps the most versatile of all the techniques

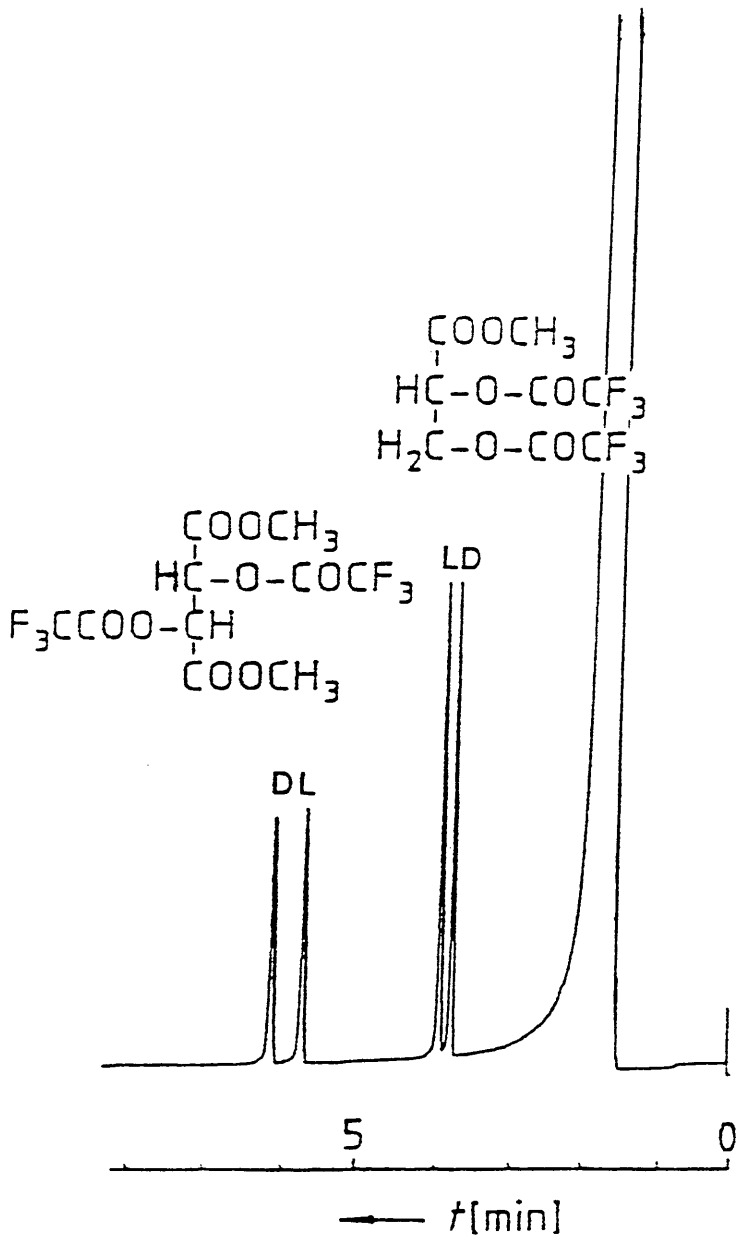


Figure 20. Enantiomer separation of glyceric acid and of tartaric acid after esterification with methanol and trifluoroacetylation. 40-m glass capillary with perpentylated α-cyclodextrin. Column temperature 90°C; Carrier gas: 1 bar H_2.[100] Permission requested from Konig, W.A., Lutz, S., Wenz, G., Angew. Chem., 1988, **27**, 7, 979-980. Copyright 1988 by VCH Verlagsgesellschaft mbH.

discussed so far.[103] It offers the ability for preparative as well as analytical separations using phases based on natural polymers (and their derivatives) as well as synthetic phases. Low molecular weight chiral selectors as well as the polymers have been bound to silica to produce improved column performances.

Early realisation of the asymmetric nature of some natural polymers, especially carbohydrates and polysaccharides led to the use of lactose to produce the first (nearly complete) chromatographic resolution reported in literature.[104] The occasional observation of the resolution of racemic amino acids in paper chromatography indicated the potential resolving capacity of cellulose (and polysaccharides in general).[105,106] Starch, has also been used for column chromatography of polar aromatic compounds (e.g. 2,2'-dinitrodiphenic acid (2)[107]) with separations showing a pronounced dependence on the nature and ionic strength of the mobile phase. Derivatives of carbohydrates and polysaccharides have also been used.

Synthetic polymers that either have a homochiral backbone or have homochiral substituents have been prepared. Polymers containing homochiral substituents are produced by firstly derivatizing a suitable monomer with the substituent followed by polymerisation of these monomer derivatives.[108,109] Use of homochiral catalysts has enabled the formation of homochiral 'helical' polymer backbones which have proved to be excellent for the optical resolution of racemic aromatic hydrocarbons showing linear or planar chirality.[110,111] Synthetic polymers containing asymmetric cavities imitating enzyme binding sites have also been produced.[112] Other natural polymers that have shown promise in enantioseparations are proteins. Current interest is centred on immobilized α_1-acid glyco-protein.[113,114]

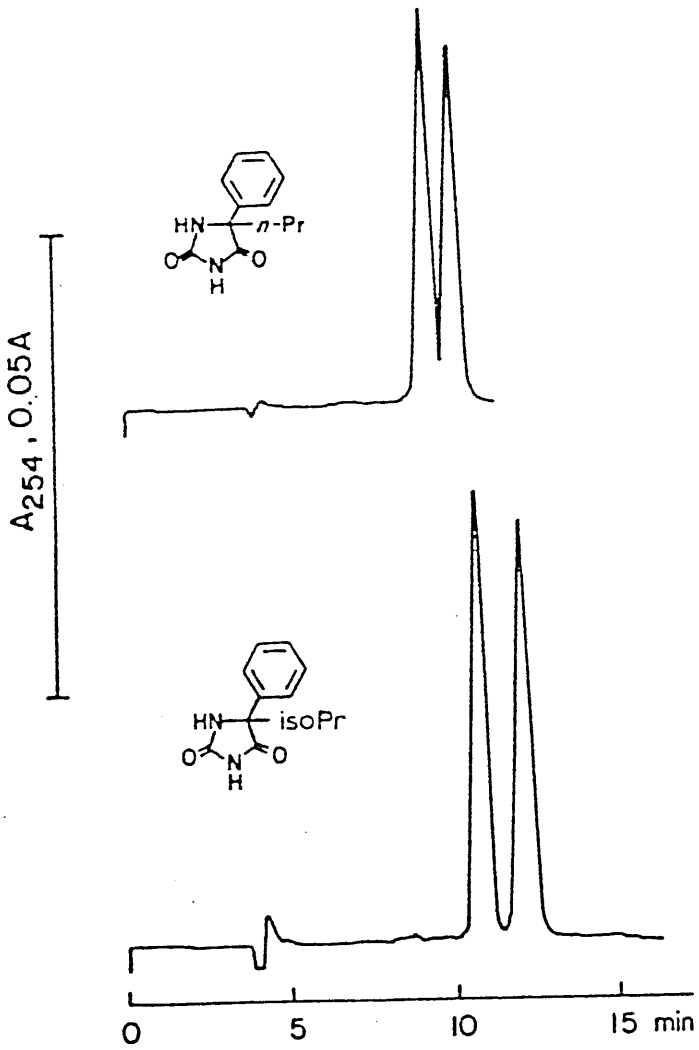


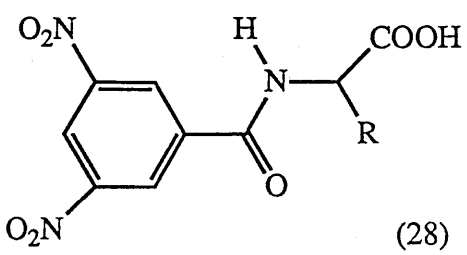
Figure 21. Chromatograms of the enantiomeric resolution of 5-(isopropyl)- and 5-(n-propyl)-5-phenylhydantoins on a β-cyclodextrin HPLC column with 20% methanol as eluent. Pr = Propyl. Reprinted with permission from Maguire, J.H., J. Chromatogr., 387, 453-485, 1987. Copyright 1987 by Elsevier Science Publications.

The choice of selector can be rationalised by NMR studies on their enantioselective properties (see Section 6.2).

The separation of enantiomers by virtue of inclusion (host-guest complexation) phenomena has been effected with cyclodextrins and asymmetric crown ethers covalently bound to a silica support. Stationary phases of these types have received considerable attention recently.[115-125] Figure 21 shows examples of the separations that can be achieved on cyclodextrin LC stationary phases.[119]

Chiral ligand-exchange chromatography was developed in the late 1960s and early 1970s. The phases are composed of (S)-proline immobilized on chloromethylated styrene-divinylbenzene copolymer. Separation is brought about by diastereoisomeric complexation of amino acid ligands with a transition metal complexed to the immobilized (S)-proline. This method has more recently been adapted for TLC analyses.[126]

Homochiral selectors using charge-transfer complexation have proved to be very versatile. Pirkle and coworkers have led the field, introducing many new phases, producing many different separations, and rationalizing the chiral recognition mechanisms at work.[108,127,128] Examples of immobilized charge transfer acceptor ligands include homochiral *N*-(3,5-dinitrobenzoyl)amino acids (28).[82]



(28)

The very succesful selector principle for these types of stationary phases was proposed by Pirkle et al.[82] is shown below in Figure 22.

Figure 22. Pirkles selector principle for homochiral stationary phases which utilise charge-transfer complexation.

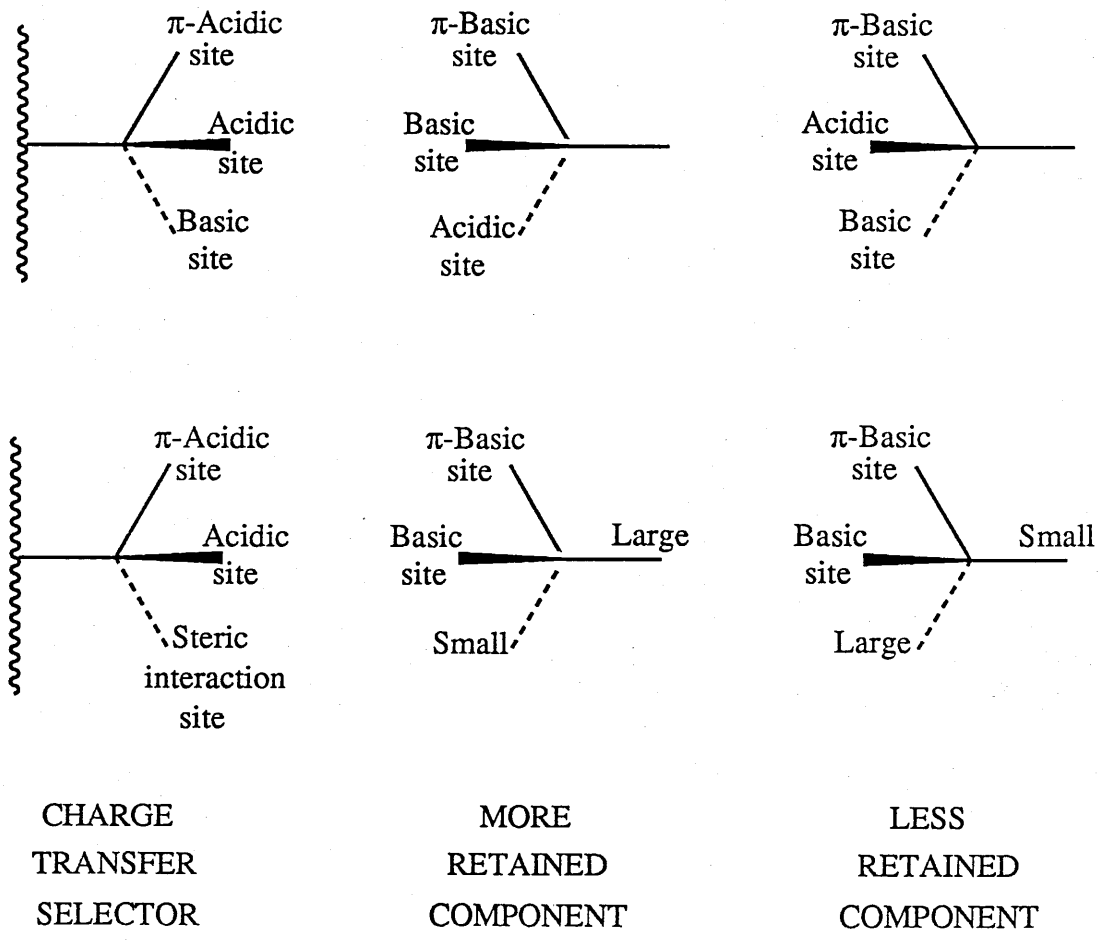
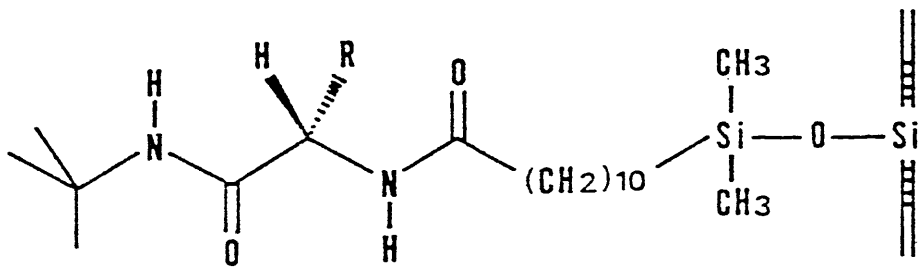


Figure 23. Optical resolution of racemic N-(4-nitrobenzoyl)leucine isopropyl ester on CSP1 (see below). Reprinted with permission from Dobashi, A., Dobashi, Y., Kinoshita, K., Hara, S., Anal. Chem., 1988, **60**, 1985-1987.[130] Copyright 1988 American Chemical Society.



CSP1, R=i-Pr;

The final main catagory of homochiral stationary phases developed for LC are ones relying on hydrogen bonding. The mechanism is similar in principle to that for the homochiral amide phases used in GC. The stationary phases have included *N*-acyl derivatives of (S)-valine covalently bound to 3-aminopropyl silica.[129] Separations reported on homochiral diamide phases have been good[130] (see example in Figure 23).

The use of homochiral stationary phases has several advantages:

1. Derivatization with homochiral reagents is not required.

2. For reason 1, errors in the calculation of the enantiomeric composition resulting from the formation of undesired diastereoisomers (due to the use of impure homochiral reagents) cannot occur.

3. For LC systems, preparative scale techniques are amenable.

4. The smaller underivatized analytes or their simple derivatives are more conveniently analysed by GC due to their higher volatility (than those requiring the larger (homochiral) derivatives).

There are however disadvantages for homochiral stationary phases:

a) The columns tend to be expensive (eg. Chirasil Val columns typically cost £700 or more).

b) The GC columns tend to have low upper temperature limits. Some are usable only below 130°C, but Chirasil Val can be utilized at temperatures up to 230°C.

c) Derivatization to improve the volatility of analytes is still often required for GC analyses. It may also be required to improve the sensitivity or selectivity for HPLC analyses.

1.7.5 Chromatographic techniques: Use of homochiral mobile phase additives

The formation of ion pairs in organic media is a well known phenomenon, where the partition of one ion may be greatly influenced by the nature of its counter-ion. The use of enantiomerically pure counter-ions enable the formation of diastereoisomeric ion pairs with certain enantiomers, enabling their separation on a conventional reversed phase LC column.[131] Their use as homochiral amphiphilic additives (ones that interact with the stationary phase) which effectively convert the achiral sorbent into a homochiral one is common.

Diastereoisomeric interactions can occur through metal complexation (i.e. in homochiral ligand exchange chromatography),[132] hydrogen bonding (using diamide type compounds)[133] or using ion pairing techniques (e.g. using homochiral counter-ions such as (+)-10-camphorosulphonic acid).[134-136]

1.7.6 Thin layer chromatography

The use of TLC for the separation of enantiomers has been performed. Methods have incorporated reversed phase plates pretreated with a poly-alkyl α-amino acid derivatives/copper (II) complexes.[126] Others have used cyclodextrin mobile phase additives.[137] The use of TLC for the determination of enantiomeric composition is however limited in that mixtures containing low quantities of one enantiomer (i.e. <5%) in the presence of its antipode may not be easily detected. Quantification is also difficult in comparison with other methods.

1.7.7 Supercritical fluid chromatography, capillary zone electrophoresis and other recent developments

Supercritical fluid chromatography (SFC)

While supercritical fluid chromatography (SFC) was first reported in 1962,[138] its use as an analytical technique was very limited. This was mainly due to the poor efficiences (and stabilities) of the columns and the unavailability of suitable high pressure instrumentation (required for SFC) at this time.

The advent of capillary GC columns in the early 1980s and improvements in chromatographic instrumentation led to an increase in interest in the technique. This was due to the inherent advantages of SFC over GC and HPLC systems, notably

a) the solutes having higher diffision coefficients in supercritical fluids than in liquids,

b) the supercritical fluids having lower viscosities than liquids,

c) the supercritical fluids having good solvating properties.[139]

These properties, being intermediate to those of GC or LC systems, can produce higher chromatographic efficiencies (as capillary GC columns can be used) and faster analyses than LC systems while having the ability to separate high molecular weight (involatile) molecules which are not amenable to GC analysis. Both HPLC and GC detection systems have been employed.[140]

Within the last five years, some work on the separation of enantiomers using supercritical and subcritical fluid chromatography has been reported.[141-145] Some excellent separations have been achieved on Pirkle-type homochiral LC

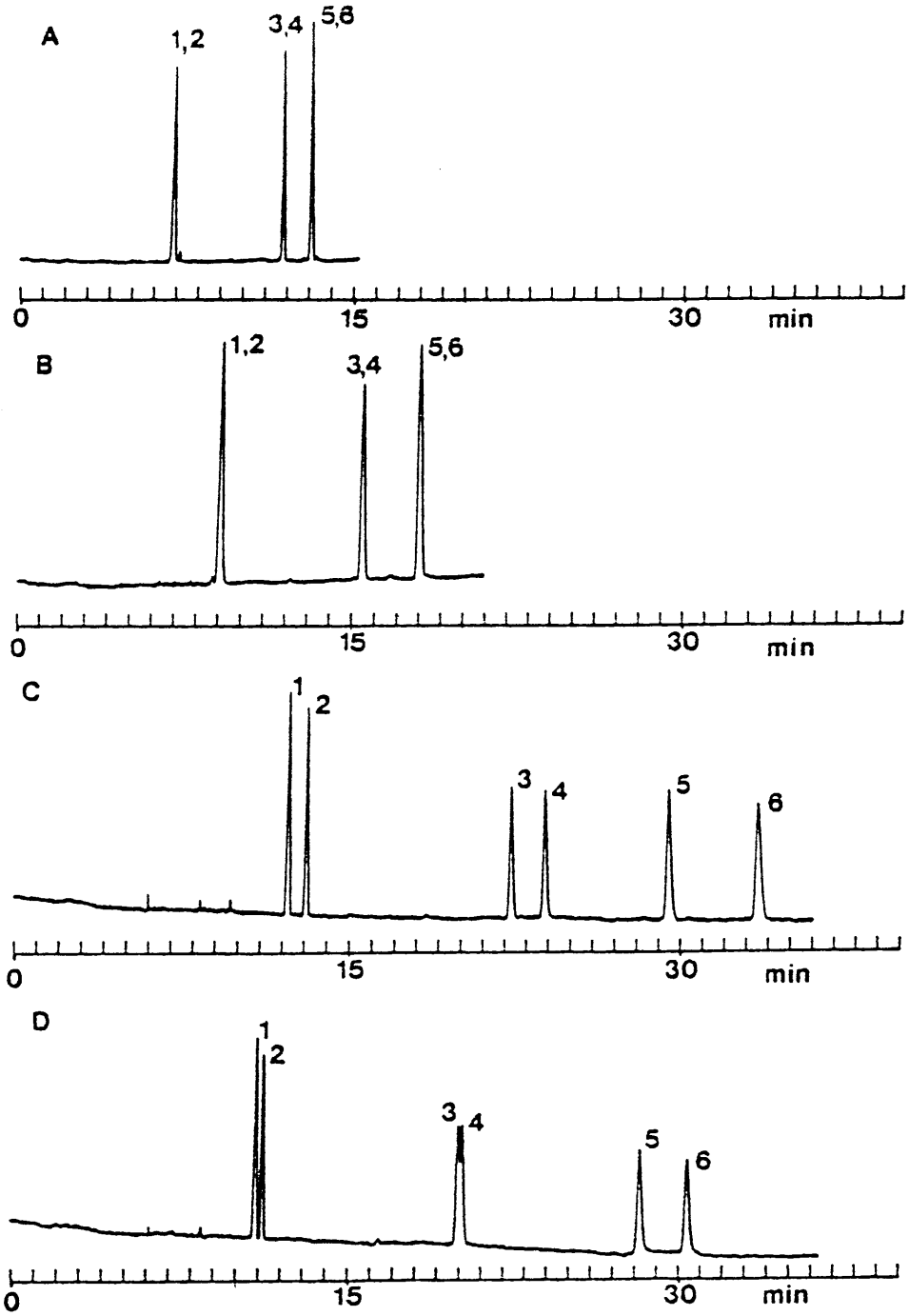


Figure 24. Separation of dansylated-DL-amino acids. 1 = Dns-L-Glu; 2 = Dns-D-Glu; 3 = Dns-L-Ser; 4 = Dns-D-Ser; 5 = Dns-L-Leu; 6 = Dns-D-Leu. (A) Buffer: 0.1M Tris-0.25M boric acid (pH 8.3), 7M urea, Gel: T = 5%, C = 3.3%, 0.1M Tris-0.2M boric acid (pH 8.3), 7M urea. Capillary: 150 mm x 0.075 mm I.D., 400 V/cm, 8μA. Electroinjection: 250 V/cm, 8μA,30s, detection wavelength, 254 nm. (B) Addition of 75 mM α-CD to the buffer and the gel mixture. (C) Addition of 75 mM β-CD to the buffer and the gel mixture. (D) Addition of 75 mM γ-CD to the buffer and the gel mixture.

columns,[141,142,144] or using homochiral ion-pairing modifiers (with achiral columns).[145] As SFC is in its infancy with regard to its analytical use, it is envisaged that many more applications to enantiomeric separations will be published within the next 5-10 years.

Capillary zone electrophoresis (CZE)

Another technique that is likely to have a profound influence on the quantification of enantiomeric compositions over the next decade is capillary zone electrophoresis. The technique has been developed over the last 15 years and exploits the migration properties shown by ionic substances in capillary columns (20-200 μm i.d.) under the influence of an applied field (10-40 kV).[146] This produces a separation system showing extremely high sensitivity and efficiency, with theoretical plate values of 2.7×10^6 and 3.3×10^6 reported for separations of dansylated glycine and lysine respectively.[147] CZE instrumentation has also successfully been coupled for mass spectrometric detection using an electrospray interface.[147]

Enantioseparations have recently been reported using CZE with cyclodextrins incorporated into polyacrylamide columns[148] (effectively a homochiral stationary phase) and homochiral metal chelate micelles[149] (effectively a chiral solvent). Figure 24 illustrates the excellent enantioseparations of dansylated amino acids that can be achieved using the β-cyclodextrin columns.[148] The use of α and γ-cyclodextrins does not produce equivalent separations due to these CD cavities not being the optimum size to allow the inclusion of the dansyl groups. CZE, offering such high efficiencies, will undoubtably receive considerable attention in the future for enantioseparations.

Chirooptical detectors

Small volume (<0.5ml) polarimeter cells that can be utilized for HPLC detection of optically active compounds have been developed.[150] Such detectors have enabled the determination of enantiomeric purity from overlapped peaks and mixtures not amenable to polarimetry measurements. They are likely to become more popular in the future as they will enable easier characterisation of the enantiomers eluting from homochiral HPLC analyses.

Theoretical and modelling studies

Dalton's three-point interaction model for explaining in simple terms how chromatographic enantiomeric separations occur, is in fact an oversimplification of the actual processes occurring. Recently there has been considerable research effort, often employing computer modelling in an attempt to obtain a better understanding of the complex mechanisms involved.[151-156] Further research into such theoretical aspects will undoubtedly continue in the future and computer modelling will become more amenable as more powerful computer systems become available.

1.8 Current status

The work reported in this thesis has concentrated on improving existing procedures (that have been used for the determination of enantiomeric composition) by attempting novel approaches to overcome some of their inherent problems.

Firstly, while homochiral derivatizing agents are produced from cheap and readily available starting materials, and separation of their diastereoisomeric derivatives can be performed on conventional achiral GC and HPLC columns,

their use has several drawbacks (see Section 1.7.3). One of the main drawbacks results from the fact that the reactive functional groups of the homochiral reagents will only react with certain functionalities. The reagents are thus often limited in their application and are chosen depending on the functional groups present in the analyte. The synthesis of novel homochiral derivatizing agents that are reactive with a wider range of functional groups under the same set of reaction conditions (and hence are more generally applicable) is desirable. Investigations were thus undertaken to utilise several lesser known synthetic reactions as derivatization reactions employing some novel homochiral reagents.

Homochiral chromatographic stationary phases incorporating cyclodextrins are becoming increasingly popular. The enantioseparations achieved are considered to result from the fact that different molecules can form energetically different inclusion complexes. As NMR techniques for the quantification of enantiomeric composition rely on the formation of energetically different diastereoisomers or diastereoisomeric complexes (with a homochiral solvent), the potential use of cyclodextrins and their derivatives as homochiral complexation reagents for NMR analyses consequently deserved investigation.

The rapid improvements in chromatographic systems and in column technology over the past 20 years have produced major advances in enantioseparations. This is particularly evident with the development of Chirasil Val, and other homochiral GC phases using capillary columns. While the different homochiral stationary phases developed for LC systems have been well characterised and their applicability has been widely investigated, GC systems have not received as much attention. This is mainly due to the limitations of GC analyses such as the requirement for volatile analytes and the inability to use mobile phase additives. Early homochiral GC stationary phases also had low upper

temperature limits (i.e. <130°C). The introduction of Chirasil Val however, enabled enantiomeric separations to be carried out at upto 230°C (200°C, isothermal) and considerably extended the range of chiral compounds amenable to GC analysis.

The use of both HPLC and GC homochiral columns has advantages over the use of other methods, especially homochiral derivatization, in that the results directly reflect the enantiomeric composition, and errors arising from the use of enantiomerically impure derivatizing agents[47] cannot occur. Considerable research into the development of novel homochiral stationary phases and the subsequent characterisation of the chiral recognition mechanisms is still receiving attention.

Most homochiral stationary phases have incorporated amino acids, usually into side chains attached to a polymer backbone. Another approach, incorporating the amino acid residues into the polymer chain itself has received little attention. Some novel approaches for the formation of such amino acid based polymers was thus investigated with the intention of using them as stationary phases. The theoretical costs of producing such polymers (and thus the columns) should be substantially lower than the prices charged for commercially available homochiral columns (e.g. £700 for a Chirasil Val column in 1989).

2 HOMOCHIRAL ACID CHLORIDE DERIVATIZING AGENTS

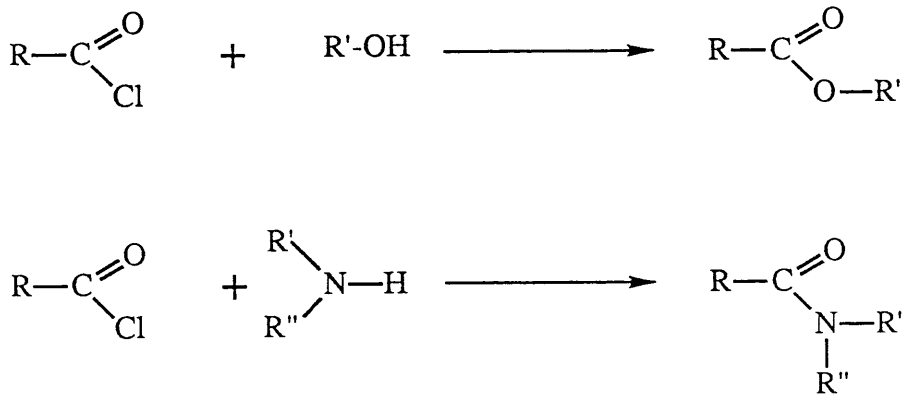
Hydroxy and amino functional groups are common in pharmaceuticals and natural products. Their reactivity towards acid chlorides is well known[157] and has been utilized in their protection as esters and amides (Scheme 1).[158] The use of homochiral acid chlorides for the preparation of diastereoisomers from enantiomeric compounds containing hydroxy or amine functions has thus proved to be popular.

(+)-(trans)-Chrysanthemoyl chloride (18) and N-trifluoroacetyl-(S)-(-)-prolyl chloride (19) were among the first homochiral acid chlorides to be used.[55,57] Their diastereoisomeric ester and amide products have been well separated by GC[54,55,56,59] and HPLC,[60,61] indicating their utility in the determination of enantiomeric compositions.[75]

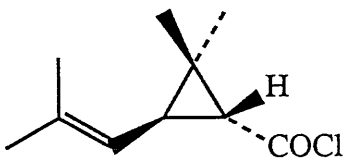
Both (18) and (19) contain only one reactive functional group and contain features which allow interaction with a suitable non-chiral stationary phase. In the case of (+)-(trans)-chrysanthemoyl derivatives, the rigid, non-polar three-membered ring system will probably interact with a non-polar stationary phase. However in the case of the N-trifluoroacetyl-(S)-(-)-prolyl derivatives, the amide function may experience hydrogen bonding interactions with a medium to high polarity stationary phase.

If the hydroxy or the amine function of the analyte is directly bonded to its chiral centre, then three bonds will be present between the two chiral centres of their (+)-(trans)-chrysanthemoyl or N-trifluoroacetyl-(S)-(-)-prolyl derivatives. This is the minimum number of bonds that can be present between the chiral centres of diastereoisomers, without disturbing the stereochemistry of either chiral centre during derivatization (which may produce erroneous results). It has been suggested in a study on the

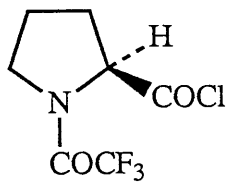
SCHEME 1



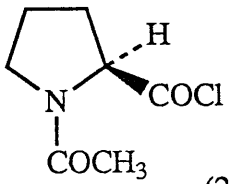
(where R=alkyl or aryl, R' and R"= H, alkyl or aryl)



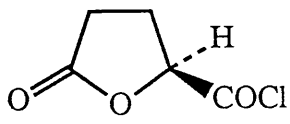
(18)



(19)



(29)



(30)

separation of the diastereoisomers of N-TFA-amino acid menthyl esters that steric factors can enable two different low energy conformations to exist for the two diastereoisomers[63,152] (see Figure 25). This assumes that the ester function remains in a fixed orientation to the menthyl ring of the derivatizing group. This may also apply to the 3-membered ring in (18) or to the prolyl ring in (19). Further interactions via H-bonding of the ester function and other steric non-polar, polar, and charge transfer interactions of the analyte group with the stationary phase may enable differentiation between the different conformations of the diastereoisomers (i.e. 3 or more points of interaction will exist - see Section 1.3 and Figure 5).

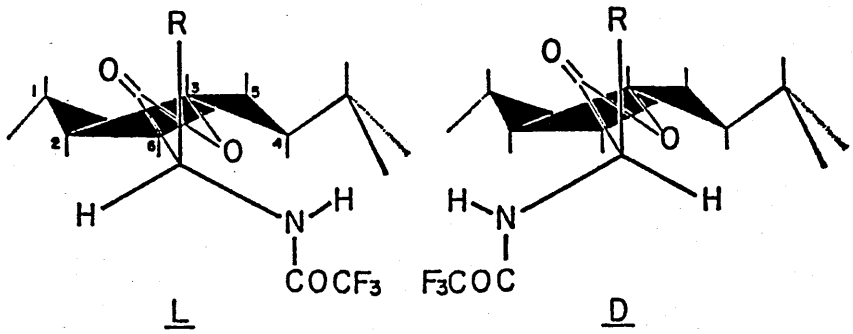
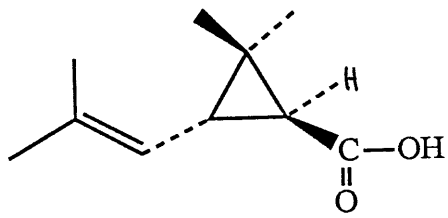


Figure 25. Proposed preferable conformations of N-TFA menthyl esters of L- and D- amino acid. The menthyl group (painted out in black) is seen over the α-carbon of amino acid moiety. Reproduced with permission; Hasegawa, M., Matsubara, I., Anal. Biochem., 1975, **63**, 308-320 (Fig 8).[63] Copyright by Academic Press, Inc.

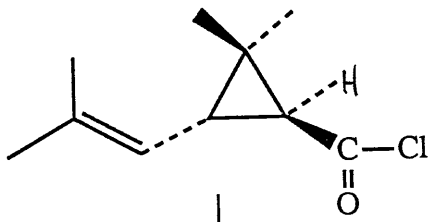
The third homochiral acid chloride reagent chosen for investigation was (S)-(+)-tetrahydro-5-oxo-2-furan carbonyl chloride (30). Derivatizations of secondary alcohols with this reagent have produced diastereoisomers which

SCHEME 2



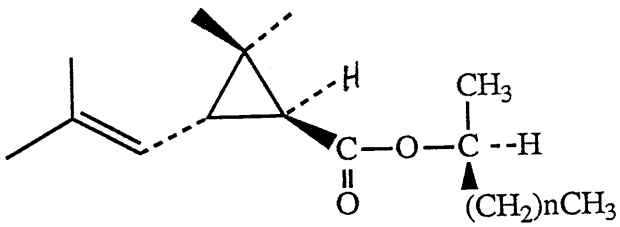
(+)-(trans)-Chrysanthemic acid

$SOCl_2$ / DCM



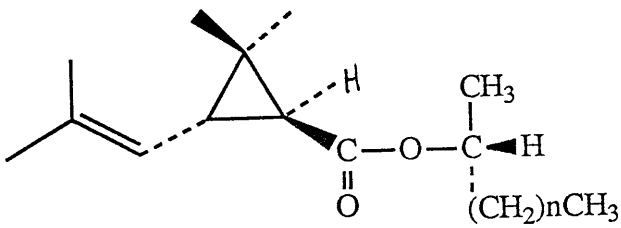
(18)

Racemic Secondry Alcohols / DCM



(31a)

+



(31b)

(n = 2 -> 5 for 2-pentanol -> 2-octanol)

are reported to be difficult to separate on common non-polar achiral columns.[159] Very polar (SP2340 and C_pCC) GC columns or use of HPLC were required to enable their separation. The possible reasons for the difficulties encountered in (and the chiral recognition mechanisms responsible for) the separation of the derivatives are discussed in Chapter 3.

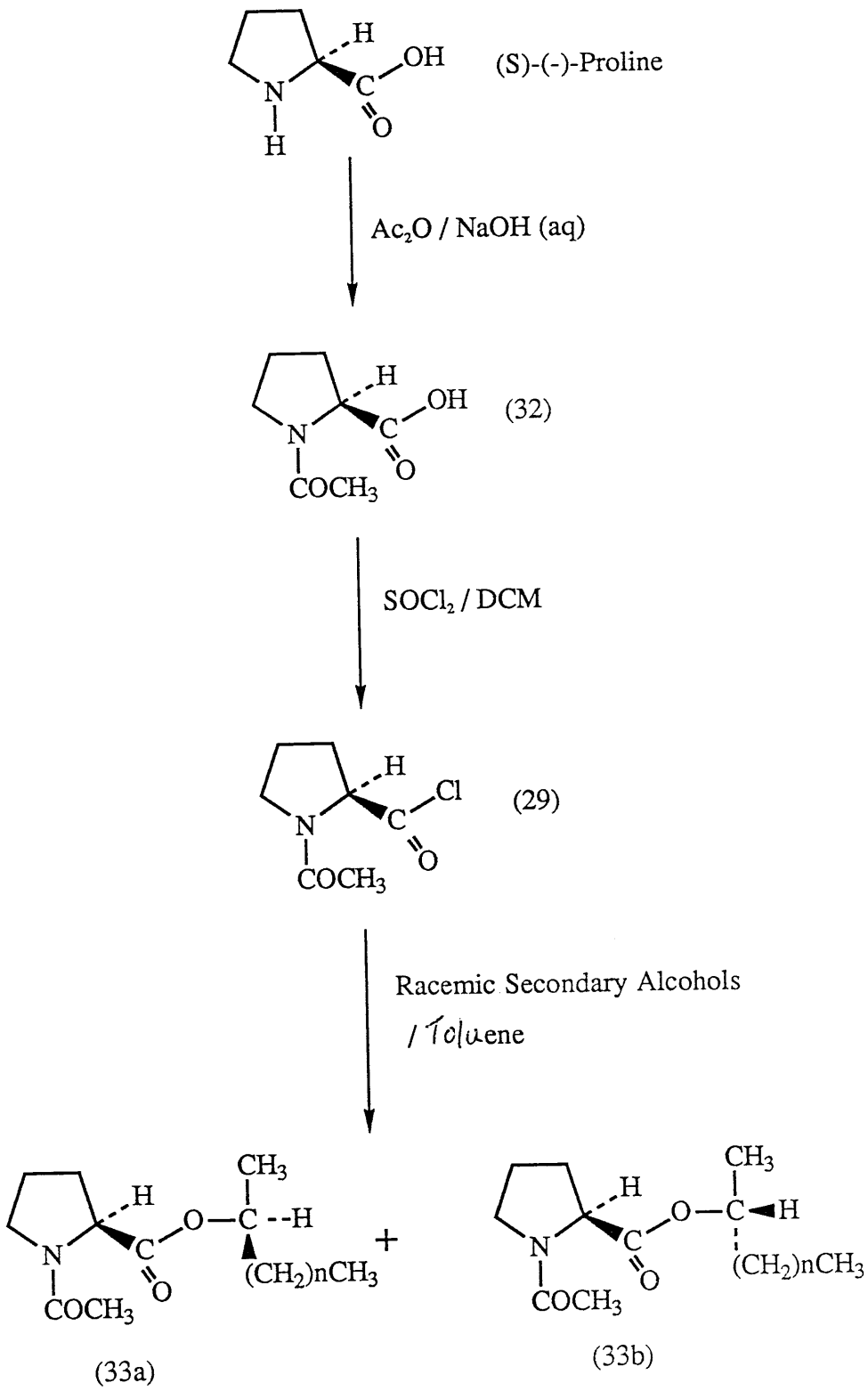
Work was undertaken to synthesise acid chlorides (18), (29) (30) and to use them to derivatize a range of racemic secondary alcohols. The GC results obtained in separating the resultant diastereoisomers would serve as useful comparisons with those obtained using novel derivatizing agents and reactions (Chapters 3 and 4). Secondary alcohols were chosen as models for several reasons.

1. Their availability (both as racemic mixtures and as pure enantiomers).

2. Their simplicity. This enables investigations into how the resolution varies with varying carbon chain length of the alcohol unit (i.e. the bulk dissimilarity).

3. Recent work on the preparation of methyl ketones by the action (enzyme catalysis) of the microorganism *Aspergillus ruber* on short-chain fatty acids revealed that under anaerobic conditions, the methyl ketones were further metabolized to secondary alcohols.[160] As enzyme-catalysed reactions of this nature can often be highly stereoselective, a knowledge of the enantiomeric composition of the secondary alcohol products would be of interest.

SCHEME 3


(S)-(-)-Proline
Ac2O / NaOH (aq)
(32)
SOCl2 / DCM
(29)
Racemic Secondary Alcohols
/ Toluene
(33a)
(33b)
(n = 2 -> 5 for 2-pentanol -> 2-octanol)

An equimolar mixture of racemic 2-pentanol, 2-hexanol, 2-heptanol and 2-octanol was prepared for use in these derivatization reactions and ones reported in subsequent chapters. (+)-(trans)-Chrysanthemoyl chloride (18) was synthesised from (+)-(trans)-chrysanthemic acid, and reacted directly with the alcohol mixture (Scheme 2). Chromatogram C1, shown in the Appendix, is typical of the GC results obtained (in this case an alcohol mixture up to 2-undecane was used). Table 2 lists the retention times (t_R), the separation factors (α) and the resolution values (Rs) for each of the 2-pentanol to 2-octanol derivatives.

N-Acetyl-(S)-(-)-prolyl chloride (29) was synthesised from (S)-(-)-proline via N-acetyl-(S)-(-)-proline (32) (Scheme 3).[161] The analytical data for acid (32) and acid chloride (29) were consistent with the correct products. However, it was noticed that in their ^{1}H NMR spectra, some of the signals appeared more complex than would be expected. The ^{13}C NMR spectra also appeared to have more signals than the expected products should have. Acid (32) for instance has two signals (at δ = 46.6 and 48.3 ppm) in its ^{13}C NMR spectrum which corresponds to the -$\underline{C}H_2$-N carbon. This is a known phenomenon[162] and is due to the different environments experienced by the atoms in the different rotameric forms of the chiral molecule. It has also been observed numerous times in the NMR spectra of other homochiral compounds (see experimental, Chapter 8), and where it has occurred, the two signals have been noted (e.g. 46.6/48.3). The N-acetyl-(S)-(-)-prolyl chloride (29), upon synthesis was utilised directly (as it was found to decompose rapidly) for the derivatization of the alcohol mixture (Scheme 3). Table 3 lists the t_R, α and Rs values for each alcohol derivative.

Table 2 **Showing t_R, α and Rs values for the GC separations of the diastereoisomeric (+)-(trans)-chrysanthemoyl esters (31) of 2-pentanol, 2-hexanol, 2-heptanol and 2-octanol.**

Alcohol	n	t_R	α	Rs
2-Pentanol	2	4.83	-	-
2-Hexanol	3	5.93/5.99	1.013	0.62
2-Heptanol	4	7.27/7.40	1.022	0.93
2-Octanol	5	8.94/9.15	1.028	1.32

- = Diastereosiomers not resolved

Separations carried out on an SE-30 glass capillary column (33m x 0.25 mm) using the Varian 6000 chromatograph.

Temperature program = 150 $\longrightarrow$ 185 (10 min) at $5^oC\ min^{-1}$.

Table 3 **Showing t_R, α and Rs values for the GC separations of the diastereoisomeric N-acetyl-(S)-(-)-prolyl esters (33) of 2-pentanol, 2-hexanol, 2-heptanol and 2-octanol. The figures in brackets are for the separations on the BP-1 column.**

Alcohol	n	t_R	α	Rs
2-Pentanol	2	4.06/4.17	1.031	0.75
		(3.81/3.89)	(1.025)	(-)
2-Hexanol	3	5.72/5.95	1.045	1.25
		(5.27/5.48)	(1.045)	(0.71)
2-Heptanol	4	8.31/8.73	1.054	1.64
		(7.52/7.88)	(1.052)	(1.06)
2-Octanol	5	12.20/12.90	1.06	1.73
		(10.88/11.49)	(1.059)	(1.44)

- = Diastereoisomers not sufficiently resolved.

Separations carried out on a BP-5 fused-silica capillary column (12m x 0.33 mm; 0.5 μm film) at an isothermal temperature of 170°C or on a BP-1 fused-silica capillary column (12.5m x 0.32 mm, 1.0μm film) at an isothermal temperature of 175°C. Both analyses were carried out using the Carlo Erba gas chromatgraph.

The results show that the α values are similar for separations on both the BP-1 and the BP-5 columns, but that the resolutions are considerably better on the BP-5 column. This indicates that the efficiency of the BP-5 column is considerably greater than that of the BP-1 for these compounds. As both the BP-1 and the BP-5 columns are non-polar, the amide function of the proline group will not be capable of hydrogen bonding with the stationary phase. Other non-polar interactions must be occurring, with the amide and ester functions possibly involved in repulsive interactions to enable stereo differentiation.

Mass spectra obtained for the two separated 2-octanol derivatives (MS1 and MS2) confirm the identity of the two diastereoisomeric products. It is interesting to note that their mass spectra are indistinguishable.

It is apparent from chromatogram C1 and Tables 2 and 3 that the α and Rs values increase with either the retention time, the carbon chain length of the alcohol group or both. With there being two possible variables, no clear conclusions can be drawn from the trends in the α and Rs values. If however one of the variables was fixed then useful information could be obtained. It is known that an increase in retention time can produce a better resolution. It was thus decided to investigate the effect of chain length on α and Rs by eluting the various derivatives at a fixed retention time. These

Table 4 **Showing t_R, α and Rs values for the 2-pentanol ⟶ 2-octanol derivatives (31)[a] and (33)[b]**

Alcohol	n	isothermal oven temp (°C)	t_R	α	Rs
2-Pentanol	2	- (148)	- (8.72/9.03)	- (1.038)	- (0.62)
2-Hexanol	3	150 (160)	8.44/8.60 (8.66/9.05)	1.02 (1.048)	0.86 (0.94)
2-Heptanol	4	160 (170)	8.71/8.98 (9.03/9.47)	1.036 (1.052)	1.23 (1.21)
2-Octanol	5	170 (180)	8.91/9.22 (9.04/9.54)	1.04 (1.059)	1.49 (1.40)

a) Separations were performed on an SE-30 glass capillary column (33m x 0.25mm) using the Varian 6000 gas chromatograph.

b) Separations were performed on a BP-1 fused-silica capillary column (12.5m x 0.32mm, 1.0 μm film) using the Carlo Erba gas chromatgraph. The corresponding results are given in brackets.

analyses were performed on both the (+)-(trans)-chrysanthemoyl and the N-acetyl-(S)-(-)-prolyl ester derivatives ((31) and (33) respectively). The results are given in Table 4 (with the values for the (33) derivatives in brackets). These results are also illustrated on Graphs 1 and 2.

The results from Table 4 and Graphs 1 and 2 indicate that at a fixed retention time:

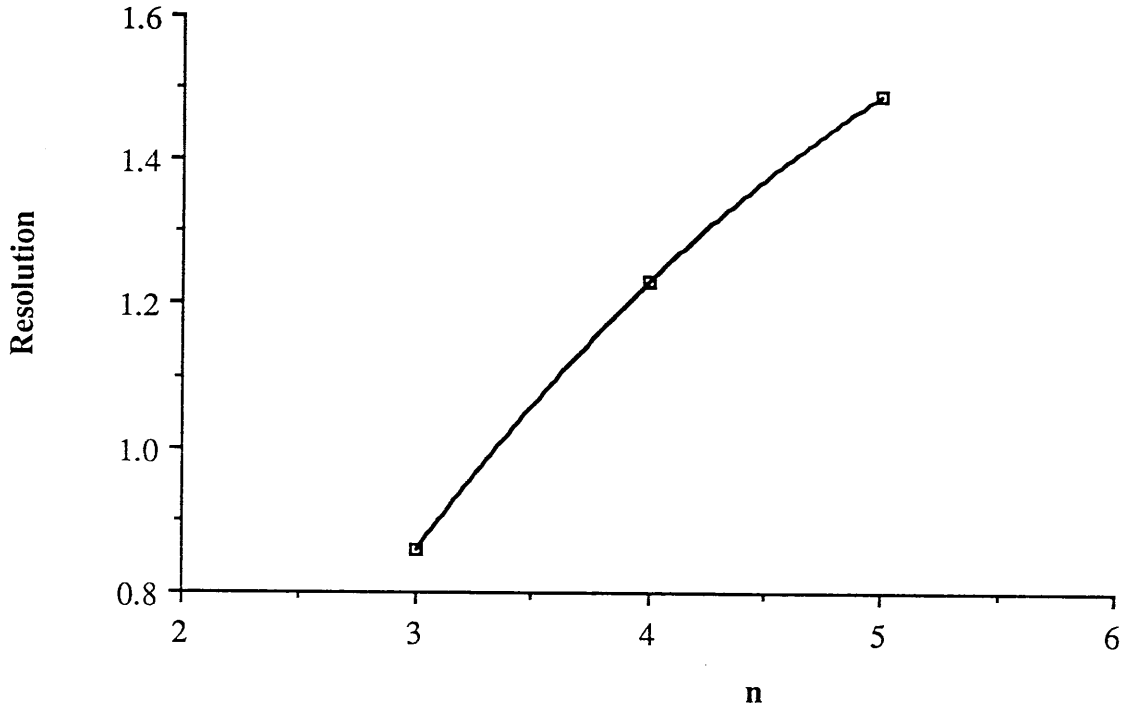
a) The separation factor (α values) appear to vary little over the range of carbon chain lengths used.

b) The resolution (Rs) of the diastereoisomers increases with increasing carbon chain length.

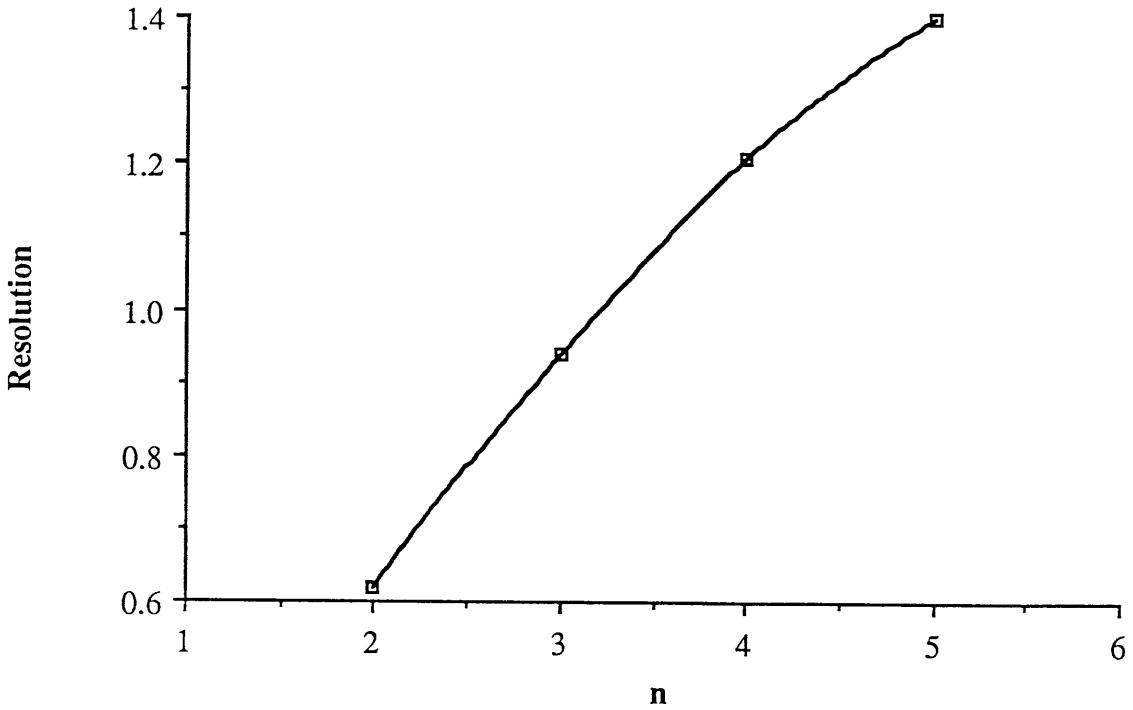
c) Derivatives (31a) and (31b) have comparable resolutions to those of (33a) and (33b) but the α values for (33a) and (33b) are greater on the columns used.

A hypothesis explaining why the resolution increases with increasing carbon chain lengths can be formed by considering the structures of compounds (31a) and (31b). Assuming that the two units of the carbon chain (i.e. the $-CH_3$ and the $-(CH_2)_nCH_3$ groups) interact with the non polar stationary phase, and are involved in the chiral recognition mechanism, then the greater is the dissimilarity of the two groups (i.e. as n increases) the greater the differentiation between the diastereoisomers. Thus, when n=0, (31a) and (31b) are identical molecules and are inseparable, but as n increases, the binding sites are increasingly able to differentiate a methyl group and a larger chain. This is reflected by the increase in the resolution values, as observed in GRAPH 1 and GRAPH 2. However, as the carbon chain length increases, the differences in the dissimilarity will become less

Graph 1. Showing how GC resolution varies with carbon chain length of the secondary alcohol derivatives (31) at a fixed retention time.



Graph 2. Showing how GC resolution varies with carbon chain length of the secondary alcohol derivatives (33) at a fixed retention time.



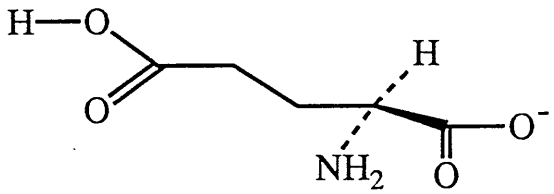
apparent and increasing n by one among the higher homologues gives little improvement in molecular recognition by the binding site. Hence the resolution values would be expected to become constant with larger values of n. For example, the differences in the resolution values between n=2 and n=3 (from GRAPH 2) is 0.32, but the difference between n=4 and n=5 is 0.19. Thus a diastereoisomeric derivative of a secondary alcohol containing 20 carbons will have virtually the same resolution value as its equivalent secondary alcohol derivative containing 21 carbons. Both GRAPHS 1 and 2 show a levelling off of the resolution values consistent with this hypothesis.

From Tables 2 and 4 it is noticed that the α and Rs values for 2-octanol are markedly different. While the same column was employed in both analyses, and similar retention times are observed, the only difference between them is that one was carried out using a ramped temperature program, the other was isothermal. This clearly shows that use of an isothermal temperature program produces better results.

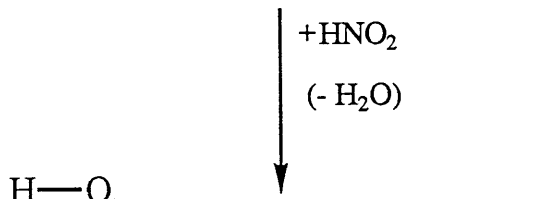
The lactone acid chloride (30) was synthesised from (S)-(+)-glutamic acid. Deamination of (S)-(+)-glutamic acid induces a cyclisation reaction to occur with the formation of lactone acid (34) (Scheme 4).[163] Treatment of the acid with oxalyl chloride produces the acid chloride (30) in good yield (Scheme 5).[164] This reagent was found to be stable for up to six months in a sealed container.

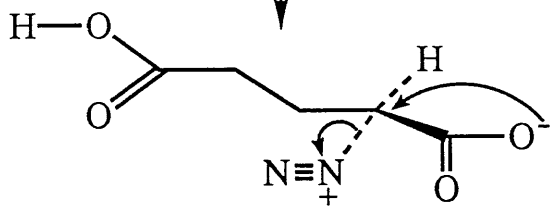
Derivatization of 2-heptanol with the lactone acid chloride (30) gave diastereoisomers (35a) and (35b) (Scheme 5).[159] GC/MS analysis failed to separate the diastereoisomers. The mass spectra obtained clearly identified them as being the desired products. A hypothesis as to why the diastereoisomeric products were not separated is given in Chapter 3.

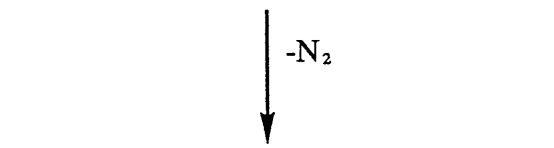
SCHEME 4.

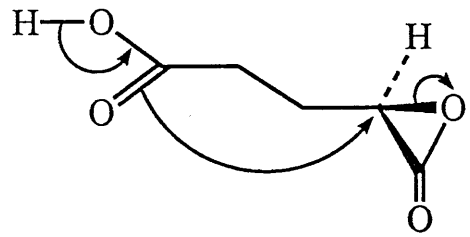


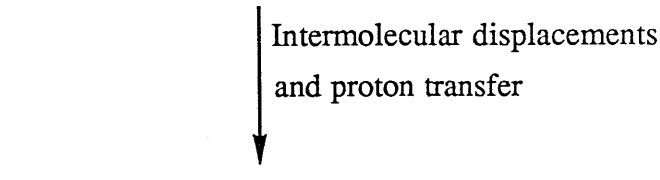
(S)-(+)-Glutamic acid

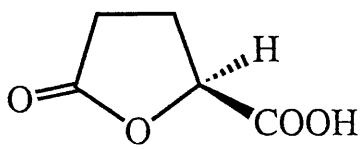






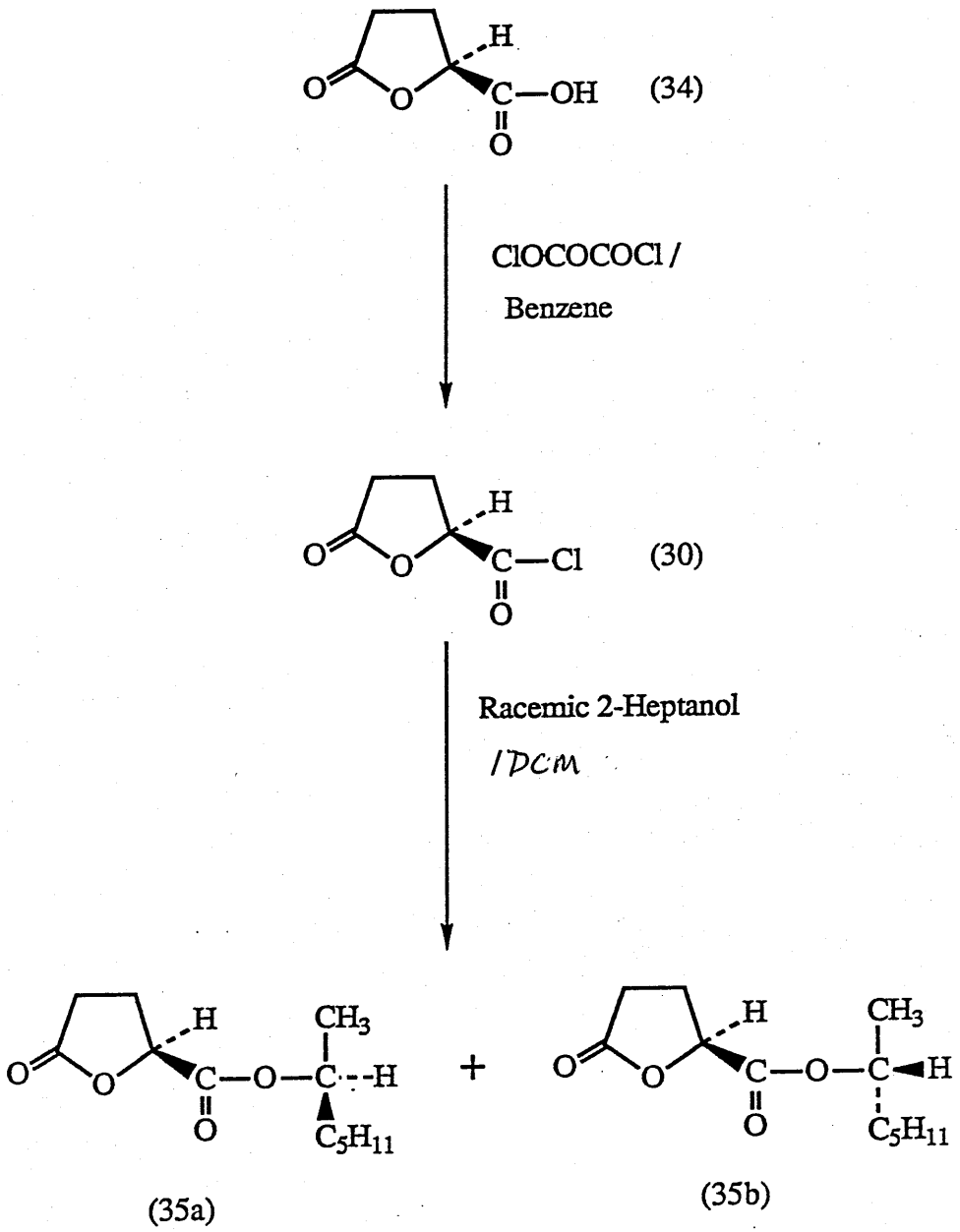






(34)

SCHEME 5



The work discussed in this chapter illustrates the usefulness of the homochiral acid chlorides (18) and (29) in enantioseparations. Baseline separations of their diastereoisomeric products have been achieved at relatively low GC (oven) temperatures and short retention times. The separation of diastereoisomers formed from acid chloride (30) was not however achieved. This illustrates that structural (and functional group) differences between homochiral derivatizing agents can have a very large effect on the enantioseparations.

The use of homochiral acid chloride reagents is limited in that only analytes containing hydroxy, thiol or amino groups are suitable for derivatization. This has not been such a large problem as many drugs and natural products do contain these functions.

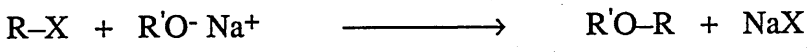
While acid chlorides (29) and (30) are produced from cheap and readily obtainable starting materials, acid chloride (18) is not.* The synthesis of (+)-(trans)-chrysanthemic acid (precursor to (18)) has been reported but is time consuming and complex.[165] This is one of the reasons for the increasing popularity of the proline-based reagents.

* The (+)-(trans)-chrysanthemic acid used here was a kind gift from Professor G Pattenden (University of Nottingham)

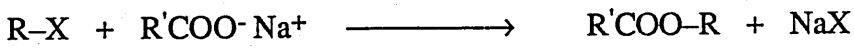
3 HOMOCHIRAL ALKYL HALIDE DERIVATIZING AGENTS

While alkyl halides generally have a low reactivity towards uncharged nucleophiles,[167] they are found to be reactive with nucleophiles bearing a negative charge at the heteroatom site. Their reaction with alkoxide or aroxide ions (prepared from their corresponding alcohols or phenols) will, for example, produce ethers (Williamson reaction,[168,169] see Scheme 6). Likewise carboxylates (prepared from their corresponding acids) have been used to prepare esters (see Scheme 7). Both reactions have been shown to follow an S_N2 mechanism when using primary alkyl halides. If secondary or tertiary alkyl halides are used, the reaction may follow either S_N1 or S_N2 mechanisms (depending mainly on the steric influences of the halide). Such a reaction may cause racemisation of the alkyl halide unit if the carbon attached to the halide (the α carbon) is chiral and if the sample is homochiral to start with.[170]

SCHEME 6



SCHEME 7



The counter-ions for the nucleophiles in these reactions are usually sodium or potassium for alkoxides and sodium, potassium, silver(I), mercury(II), copper(I), etc. for carboxylates.

Reactions of this type have been used widely for preparative purposes, generally using simple nucleophiles,[167,169] (e.g. silver or mercury acetates, and sodium methoxide or ethoxide). This has generally been due to their ease of preparation or commercial availability. The preparation of both carboxylates or alkoxides is however limited to molecules which do not contain base-sensitive functions (such as esters or lactones) as these may react with the nucleophile, forming further charged nucleophiles which will yield undesired by-products on reaction with the alkyl halide (see Section 3.2).

The effects of the structure of the halide on the relative rates and yields of the alkylation reaction are also very important.[171] With both α and β branching in the structure, dehydrohalogenation (elimination) reactions are reported to occur, and can predominate, giving low yields of the desired ester or ether products.[169,171] For this reason, simple primary alkyl halides have generally been used.

As these reactions follow an S_N2 mechanism, the leaving group involved must also be considered. Iodide is known to be a good leaving group and fluoride is not. The order is thus $I^- > Br^- > Cl^- > F^-$.

The problems associated with the preparation of more complex alkoxides or carboxylates and the fact that side-reactions can occur with more complex alkyl halides have meant that little or no work on the use of alkyl halides as homochiral derivatizing agents has been reported. Potentially, the use of homochiral alkyl halides as derivatizing agents does have advantages over other homochiral reagents (and reactions), especially with the range of functional groups (nucleophiles) amenable to reaction. To this extent, an

investigation into the synthesis and use of homochiral alkyl halides was undertaken.

Several structural aspects were considered important for an alkyl halide to be a successful derivatizing agent, these being:

(a) It should be primary because secondary or tertiary alkyl halides are more likely to follow an S_N1 mechanism which can cause racemisation. Elimination reactions are also more likely with secondary and tertiary alkyl halides.

(b) Ideally, its β-atom should be the chiral centre. This would enable a minimum number of three bonds to exist between the chiral centres of the diastereoisomeric products. The β-branching may however cause problems as it can promote elimination reactions. If an alkyl halide with a chiral α-carbon was used, its stereochemistry would be affected by the nucleophilic attack (inverted if pure S_N2 and racemised if pure S_N1).

(c) It should not contain other functional groups that are reactive under the reaction conditions.

Two reactions were chosen for study (Schemes 8 and 9). Both were one-pot reactions, not requiring the formation of the alkoxides or carboxylates prior to reaction with the alkyl halides.

One used solid potassium hydroxide in DMSO for the alkylation of phenols, alcohols, amides and acids,[172,173] the other used a mercury(II) tetrafluoroborate complex in DCM for the alkylation of alcohols and acids.[174,175] The first reaction which incorporates basic conditions was

SCHEME 8

$$R\text{–}X + R'OH \xrightarrow{KOH/DMSO} R'\text{–}O\text{–}R + H_2O + KX$$

(X = Cl, Br, I)

SCHEME 9

$$R\text{–}X + R'OH \xrightarrow[DCM]{Hg(BF_4)_2} R'\text{–}O\text{–}R$$

(X = Cl, Br, I)

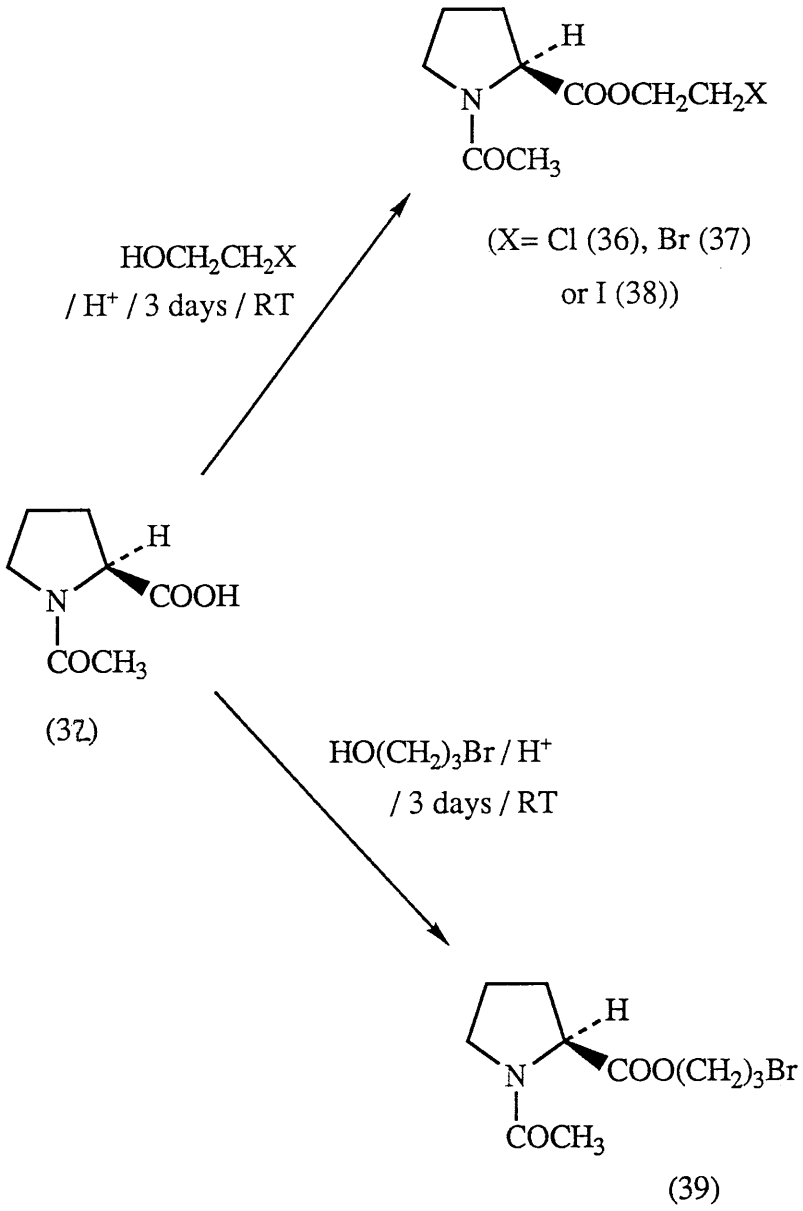
found to be suitable only for reagents (and analytes) not containing base-sensitive functions (e.g. esters). The second which is carried out under neutral conditions is, in theory, more widely applicable.

3.1 Synthesis of homochiral alkyl halides

The synthesis of a range of homochiral alkyl halides from cheap and readily available starting materials was undertaken.

The alkyl halides chosen for synthesis contain sub-structures which are incorporated into known homochiral acid chloride derivatizing agents, some of which have been discussed in Chapter 2. Thus for example, several alkyl halides containing the N-acetyl-(S)-(-)-prolyl unit have been prepared. The synthesis of these and other homochiral alkyl halides is now discussed.

SCHEME 10

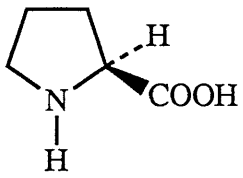

H
N
COOCH2CH2X
COCH3
HOCH2CH2X
/ H+ / 3 days / RT
(X= Cl (36), Br (37)
or I (38))
H
N
COOH
COCH3
(37)
HO(CH2)3Br / H+
/ 3 days / RT
H
N
COO(CH2)3Br
COCH3
(39)

N-Acetyl-(S)-(-)-proline (32) was synthesised from (S)-(-)-proline (Chapter 2). Acid-catalysed esterification with 2-haloethanols gave alkyl halides (36)-(38) in acceptable yields (33-44%) (Scheme 10). Likewise alkyl halide (39) was synthesised using 3-bromopropanol. The analytical data for the alkyl halides (36)-(39) were consistent with the correct products. Elemental analyses were not obtained for these compounds because they eventually proved to be of little use as homochiral derivatizing agents (Section 3.2).

Alkyl halides (42) and (43) should be the most useful proline-type derivatives for direct comparison with the known N-acetyl-(S)-(-)-prolyl chloride reagent (Chapter 2). These were prepared from (S)-(-)-proline by firstly reducing the acid to give (S)-(+)-2-pyrrolidine methanol (40) in acceptable yield (67%).[176] Selected acetylation of the amine function produced N-acetyl-(S)-(-)-2-pyrrolidine methanol (41) in good yield (73%). Alkyl halides (42) and (43) were then prepared by the reaction of alcohol (41) with the corresponding thionyl halide (Scheme 11).[177-179] Difficulties were experienced in the purification of the halide products due to their hygroscopic or water soluble nature. This was particularly so with the bromide (43) (where problems also occurred in the removal of the excess thionyl bromide (some remained trapped in the thick oily product ,even under high vacuum)). Many other methods for the preparation of alkyl halides from alcohols (e.g. using reagents such as PBr_3, or methods such as the Finkelstein reaction) may prove more convenient.[180-184]

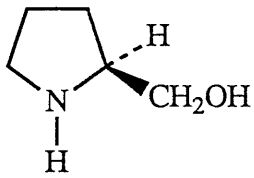
Analytical data for (42) and (43) were consistent with the correct products, and again due to their poor performance as derivatizing agents, elemental analyses were not obtained. It is interesting to note that the NMR signals for the acetyl protons ($\underline{CH}_3$-CO) shifted considerably with respect to the corresponding signal in the alcohol (41). The $C{=}O_{STR}$ absorbance in the IR

SCHEME 11



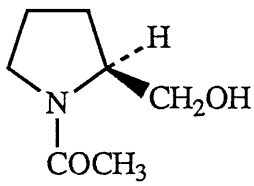
(S)-(-)-Proline

$LiAlH_4$ / THF



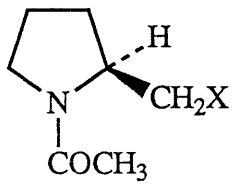
(40)

Ac_2O / MeOH



(41)

SOX_2 / DCM

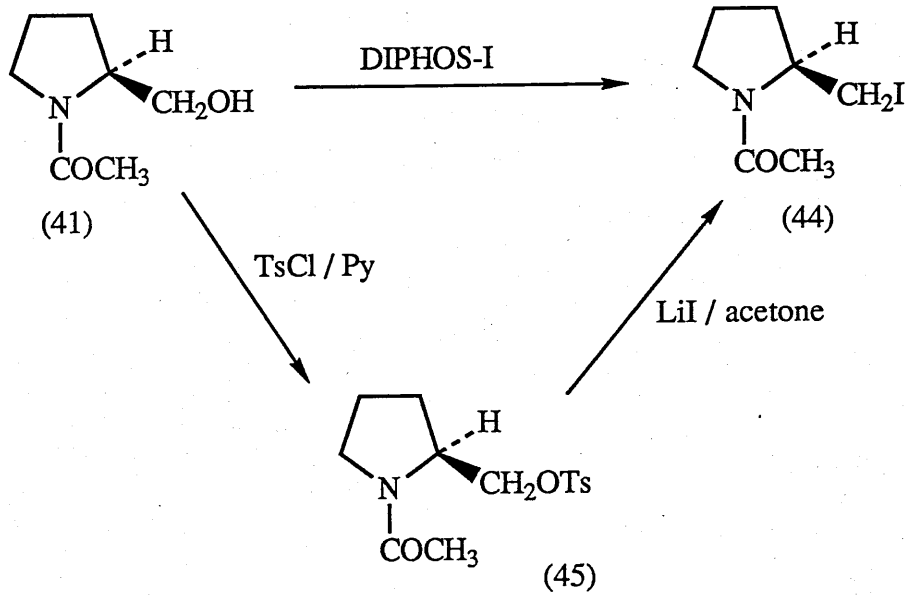


(X= Cl (42), Br (43))

spectra of the halides also shifted. This suggests that any intra- or inter-molecular interactions which involve the acetyl function (probably intramolecular H-bonding in the alcohol) have been removed. This may explain why these halides are solids whereas the alcohol (41) (which has intramolecular H-bonding interactions) is not.

The synthesis of the corresponding iodide (44) was attempted, following two different routes (see Scheme 12). The first used the reagent 1,2-bis(diphenylphosphino)ethane tetraiodide (DIPHOSI) which was prepared

SCHEME 12



$(C_6H_5)_2PCH_2CH_2P(C_6H_5)_2$ = DIPHOS

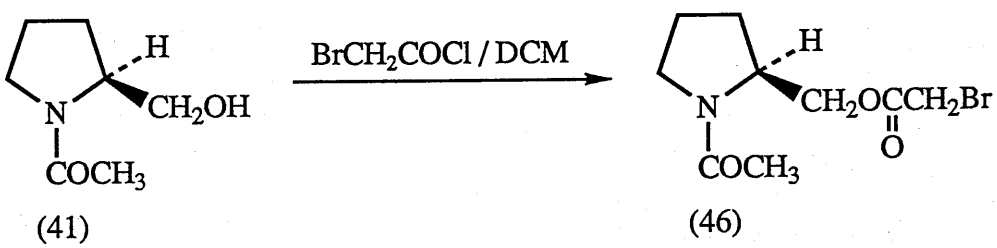
(DIPHOS-I = DIPHOS tetraiodide)

in turn by the reaction of 1,2-bis(diphenylphosphino)ethane (DIPHOS) with iodine.[184] This route failed, as it relied on the product being soluble in the pentane/ether/DCM solvent system employed in the work-up. This was not the case with the iodide product (44) and it was thus precipitated along with the phosphorous by-products. Other work-up procedures may not succeed due to the possible hygroscopic nature of the iodide and its expected high boiling point. TLC results indicated that a complex mixture of products were present.

The second route involved the attempted synthesis of tosylate (45).[163,185,186,187] Nucleophilic attack on the tosylate by iodide ions should then yield iodide (44).[186] However, the synthesis of tosylate (45) proved unsuccessful and thus the second step was not tried.

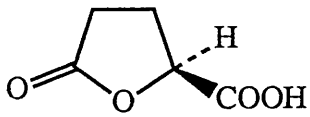
N-Acetyl-(S)-(-)-prolylmethylbromoacetate (46) was synthesised from alcohol (41) in good yield (72%) (Scheme 13). Again, due to this compound showing poor performance as a homochiral derivatizing agent (Section 3.2), a C, H and N analysis was not performed.

SCHEME 13



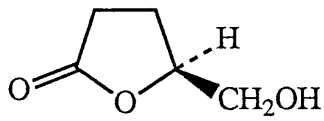
(41) (46)

SCHEME 14



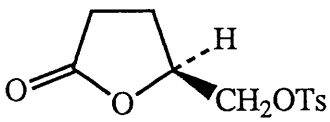
(34)

BH_3SMe_2 / THF



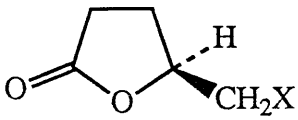
(47)

TsCl / Py / 0^0 C



(48)

LiX / Acetone



X = I (49)

X = Br (50)

Another structural unit which has been incorporated into a homochiral acid chloride derivatizing agent is the γ-lactone ring (see Compound (3), Chapter 2). The synthesis of alkyl halides (49) and (50) was thus undertaken (Scheme 14). Lactone acid (34), synthesised from (S)-(+)-glutamic acid was the starting material for the halides. Selective reduction of the acid group (in the presence of the lactone function) using a borane methyl sulphide complex gave lactone alcohol (47).[163] Tosylate (48) was synthesised[163,186] then converted to halides (49) and (50) by reaction with lithium iodide or bromide.[186]

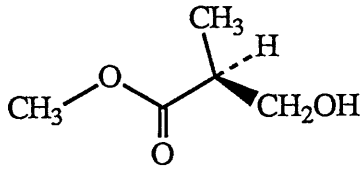
Analytical data for iodide (49) was in agreement with those reported,[186,188] except for the optical rotation. A reading of $[\alpha]_D$ -15.4° (c = 1.75, $CHCl_3$) was obtained in this work, but others[188] reported a value of $[\alpha]_D$ +2.3° (c = 2.0, CH_2Cl_2). It is not known why these figures should be of opposite sign, but use of different solvents can have considerable effects on optical rotation values.[18]

The structure of bromide (50) was confirmed from the analytical data obtained. No reference to this compound has been found in the literature, and hence the data cannot be checked.

Both the iodide (49) and the bromide (50) were produced in low yields (36% and 20%) from lactone acid (34). However, due to the low price and availability of the starting material, (S)-(+)-glutamic acid, the syntheses were carried out on a large scale. The low yields were thus of little concern.

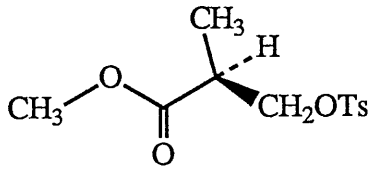
Iodide (53) was synthesised from alcohol (51) in acceptable yields (43%) (Scheme 15). The choice of this compound for assessment as a potential homochiral derivatizing agent was made due to it having a similar structure to (S)-α-acetoxypropanoyl chloride (54) (formed from (S)-(+)-lactic acid)

SCHEME 15



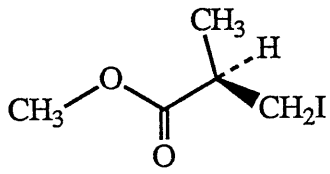
(51)

TsCl / Py / 0°C

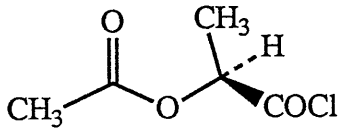


(52)

LiI / acetone / reflux



(53)



(54)

which is a known homochiral derivatizing agent.[152,159,190,191] The analytical data for iodide (53) confirmed its identity.

3.2 Derivatizations using homochiral alkyl halides

3.2.1 Model reactions using KOH/DMSO reaction conditions

The reaction of simple primary alkyl halides with alcohols, acids, amides and phenols in the presence of solid KOH in DMSO has been studied.[172] The low solubility of the KOH in DMSO coupled with the fact that esters have been formed under these conditions suggests that the reaction is occurring on the surface of the KOH (as an ester would normally by hydrolysed under basic conditions).

Other papers describe the use of a (similar) NaOH/DMSO system for the peralkylation of pentaerythritol.[173,192] Again the reaction appears to progress well when using simple primary alkyl halides in various polar aprotic solvents. The principle reactions involved using this system are shown in Scheme 16, (e.q. 1 - e.q. 5).

It has been suggested that in these solvents, large and polarizable anions are solvated better than small anions (i.e. $OH^- < RO^- < X^-$, where X is a halide).[193] Thus the reactivity of the OH^- ions would be very high in polar aprotic solvents and their concentration very low. The OH^- ions produced in reaction (eq. 1) will be consumed in reactions (eq. 2) and (eq. 5). If reaction (eq. 5) proceeds to any extent, reactions (eq. 6) and (eq. 7) may also occur.

If this is the case then both the alkyl halide and the KOH may be depleted at a greater rate than desired. This coupled with the fact that the dehydrohalogenation reaction (eq. 8) can also occur (when more complex

SCHEME 16

$NaOH + solv \rightleftharpoons Na^+(solv) + OH^-(solv)$ (eq.1)

$ROH + OH^- \rightleftharpoons RO^- + H_2O$ (eq.2)

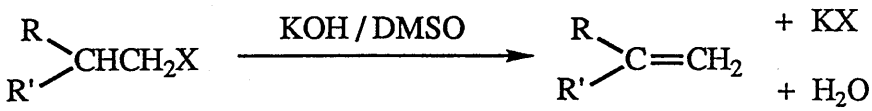
$R'X + solv \rightleftharpoons R'X\ (solv) \rightleftharpoons R'^+(solv) + X^-(solv)$ (eq.3)

$RO^- + R'X \longrightarrow ROR' + X^-$ (eq.4)

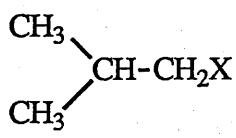
$OH^- + R'X \longrightarrow R'OH + X^-$ (eq.5)

$R'OH + OH^- \rightleftharpoons R'O^- + H_2O$ (eq.6)

$R'O^- + R'X \longrightarrow R'OR' + X^-$ (eq.7)

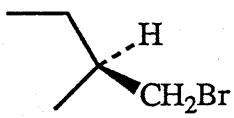


(eq 8)



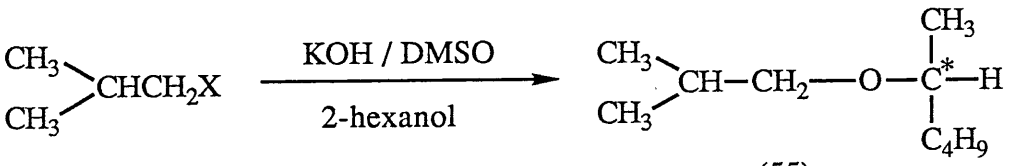
(X= Cl, isobutyl chloride)

(X= Br, isobutyl bromide)

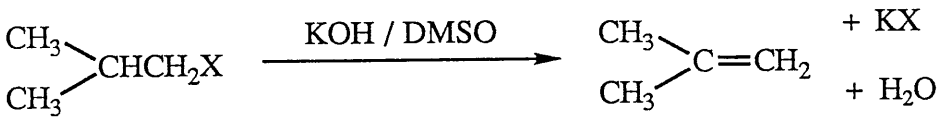


(S)-(+)-1-Bromo-2-methylbutane

SCHEME 17



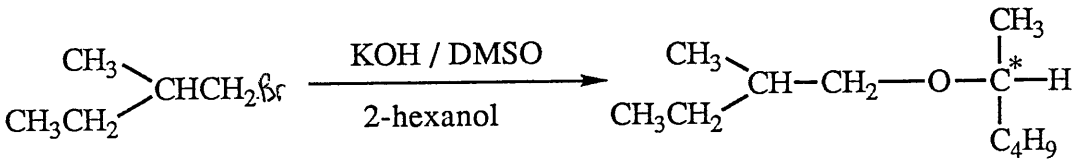
(55)



(56)

(X = Cl or I)

SCHEME 18



(57)

(*= chiral centre, R and S)

alkyl halides are used),[172,173] means that both the reagents should be added in excess with respect to the alcohol being derivatized.

To obtain a better understanding of how the reactions would proceed when using the homochiral derivatizing agents, model alkyl halide reagents were used. Isobutyl chloride and iodide, and (S)-(+)-1-bromo-2-methylbutane were chosen due to their having sterically similar reaction sites to the homochiral reagents.

Isobutyl chloride and iodide were reacted with 2-hexanol (Scheme 17) and sampled after various periods of time. The samples were worked-up and analysed by GC. In both cases by-product peaks were not observed in the chromatograms. The desired ether product (55) was found to increase, and the halide peak to decrease with reaction time in both cases. As no internal standard was used, an estimate of the % yield of the ether product was calculated from the following equation.

$$\% \text{ yield} = \frac{\text{area of product peak}}{\text{area of (2-hexanol + product) peaks}} \times 100 \quad \text{(eq. 9)}$$

Isobutyl chloride, produced at best a 12.7% yield (after 19 hours at 60°C) and isbutyl iodide a 10% yield (after 19 hours at room temperature). It was noticed that the quantity of the alkyl halides decreased more rapidly than the ether product (55) was being formed (even though they were added in a 2 molar excess to the 2-hexanol). This was paticularly noticeable with the isobutyl iodide reaction. It was concluded that this was probably due to the halides undergoing a dehydrohalogenation (elimination) reaction (Scheme 17) to yield alkene (56). The alkene was not observed in the chromatograms due to its being highly volatile and either lost from the reaction mixture, or coeluting with the solvent peak. More rapid depletion of the isobutyl iodide compared to the isobutyl chloride was observed. This is

consistent with alkyl iodides being more reactive (both in substitution and elimination reactions) than alkyl chlorides. Low yields of ether (55) were experienced in both cases.

(S)-(+)-1-Bromo-2-methylbutane, being very similar to isobutyl chloride and iodide, was reacted with 2-hexanol (Scheme 18). This was done to ascertain whether the bromide would have reactivity intermediate to that of the chloride and iodide (i.e. to see if the ether product (57) would be formed at a faster rate than when using isobutyl chloride while the competing elimination reaction is slower than when using isobutyl iodide). Again the reaction was monitored at different times by sampling, extracting the products, then analysing by GC. A rough % yield of the ether product (57) was estimated using (eq. 9) and the % alkyl bromide in the mixture was calculated from (eq. 10) below.

$$\% \text{ bromide} = \frac{\text{area of bromide peak}}{\text{area of (bromide + 2-hexanol + ether product) peaks}} \times 100 \quad \text{(eq. 10)}$$

The results obtained are summarised in Table 5.

The results indicate that the bromide has reacted more favourably under the KOH/DMSO conditions than the similar chloride or iodide.

Further model reactions using 1-halobutanes were carried out to confirm that alkyl bromides are the most suitable alkylating reagents under KOH/DMSO reaction conditions.

The 1-halobutanes were reacted with 2-hexanol (Scheme 19) and the reactions were monitored by GC over a 19 hour period.

TABLE 5 Showing how the % yield of ether (57) and % (S)-(+)-1-bromo-2-methylbutane vary with reaction time

	TIME		
	30 min	90 min	19 hrs
% Yield	6.5	11.5	30
% Bromide	37	44*	0.2

* The 2-hexanol and (S)-(+)-1-bromo-2-methylbutane peaks were very close together in the chromatograms (0.08 minute difference) which may have led to an inaccurate integration results.

The chromatograms obtained show that four components are present, these being:

(i) The 1-halobutane (eluting at 2.12 minutes for the chloride, 2.53 minutes for the bromide and 3.25 minutes for the iodide)

(ii) 2-Hexanol (3.05 minutes)

(iii) A by-product (4.02 minutes)

(iv) The desired product (5.94 minutes)

SCHEME 19

$$CH_3CH_2CH_2CH_2X + HOCH(CH_3)C_4H_9 \xrightarrow{KOH/DMSO} CH_3CH_2CH_2CH_2OCH(CH_3)C_4H_9 \quad (58)$$

The by-product was identified (by GC/MS) as being dibutyl ether. The formation of this by-product is consistent with the reactions (5), (6) and (7) in Scheme 16.

Integration of the GC peaks allowed an estimation of the % quantities of each component (at the time of sampling) using the following equations (eq. 11) - (eq. 13)

$$\% \text{ product} = \frac{\text{area of product peak}}{\text{area of (product + by-product + 2-hexanol) peaks}} \times 100 \quad \text{(eq. 11)}$$

$$\% \text{ halide} = \frac{\text{area of halide peak}}{\text{area of (product + by-product + 2-hexanol + halide) peaks}} \times 100 \quad \text{(eq. 12)}$$

$$\% \text{ by-product} = \frac{\text{area of by-product peak}}{\text{area of (product + by-product + 2-hexanol) peaks}} \times 100 \quad \text{(eq. 13)}$$

The results for the % product and % by-product (dibutyl ether) are given in Table 6. Results for the % halide remaining are given in Table 7.

As expected the results indicate that the order of reactivity is RI>RBr>RCl as the yield of the ether product (58) at a given time is found to increase in going from the chloride to the iodide. This is also confirmed by the more

rapid decrease in the quantity of the halide in going from the chloride to the iodide. This rapid depletion of the iodide, again suggests that dehydrohalogenation is also occurring, and at a greater rate than the bromide or chloride (see Scheme 16, (eq. 8)).

The more rapid increase in the quantity of the dibutyl ether by-product when using the bromide reagent suggests that, unfortunately it is more susceptible to the hydrolysis reaction (Scheme 16, eq. 5)) than the chloride or iodide.

TABLE 6 **Showing the % yields of the ether product (58) and the dibutyl ether by-product (figures in brackets) vary with reaction time.**

TIME	HALIDE		
	R-Cl	R-Br	R-I
2 MIN	- -	- -	24% (2.5%)
15 MIN	20.1% (2.4%)	37.4% (7.8%)	35% (4.3%)
$1^1/_2$ HOURS	24.6% (4.1%)	40.5% (11.6%)	44.2% (9.2%)
3 HOURS	28.5% (4.5%)	42% (15%)	49% (9.9%)
19 HOURS	48.5% (12%)	44% (40%)	- -

TABLE 7 Showing how the % halide in the reaction mixture varies with the reaction time.

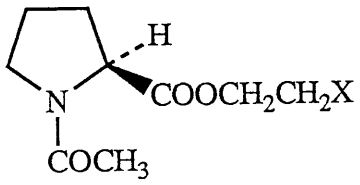
TIME	HALIDE		
	R-Cl	R-Br	R-I
2 MIN	-	-	41%
15 MIN	52%*	52%	34.6%
1½ HOURS	57%*	32%	10.5%
3 HOURS	41%	11.8%	NIL
19 HOURS	NIL	NIL	-

* Further repetitions of this experiment would probably show one of these results to be an anomaly

The reaction using 1-chlorobutane was slow in comparison to that when using the corresponding bromide or iodide, requiring 19 hours to produce similar (ie. 40-50%) yields (cf. 1½ hours for the bromide or iodide). The use of chlorides for a rapid derivatization procedure would not appear to be viable. The 1-iodobutane, on the other hand, produced the best yields, and after only 3 hours reaction. However when using more complex alkyl halides (e.g. isobutyl iodide), the dehydrohalogenation (elimination) reaction proceeded rapidly and predominated over the desired reaction.

Taking all the factors into account, it was concluded that the alkyl bromides exhibit the most desirable reactive properties under the KOH/DMSO conditions. However, the other halides were also incorporated in the homochiral reagents in a further attempt to find the most suitable reagent.

SCHEME 20

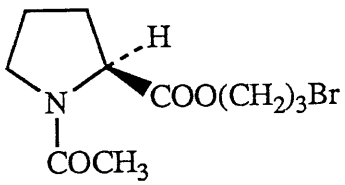


2-hexanol

KOH / DMSO

PRODUCTS

(X= Cl (36), Br (37) or I (38))

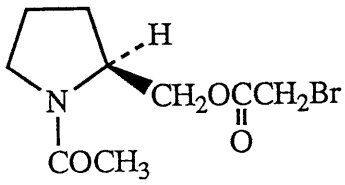


2-hexanol

KOH / DMSO

PRODUCTS

(39)



2-hexanol

KOH / DMSO

PRODUCTS

(46)

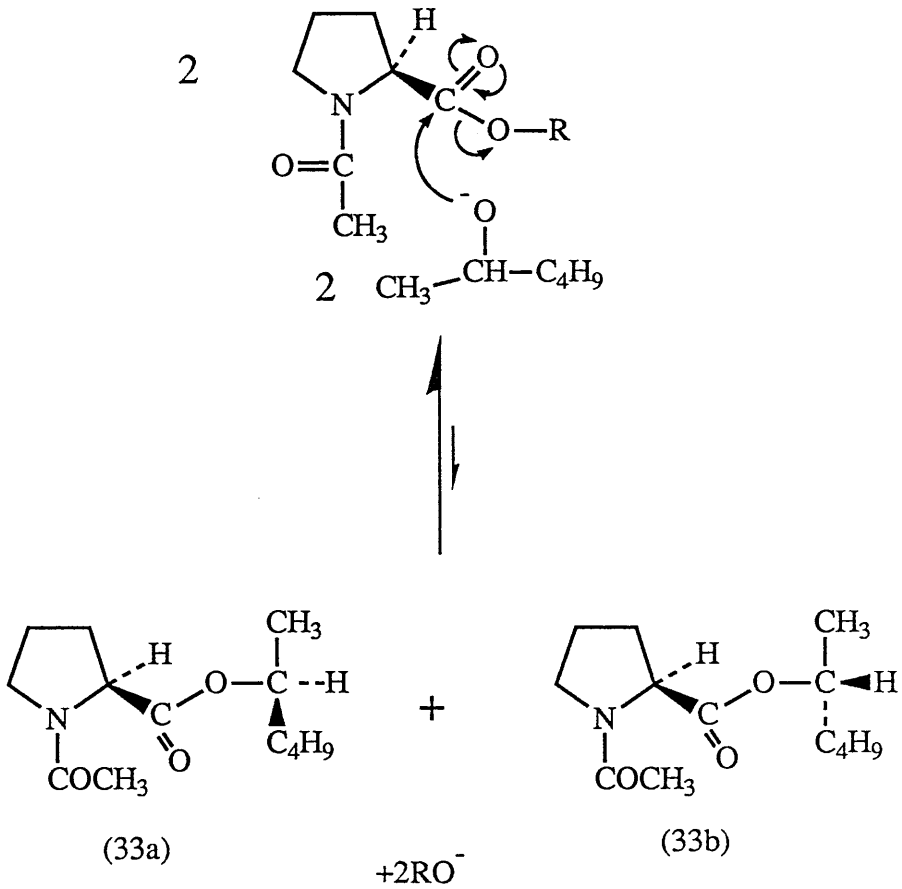
3.2.2 Use of homochiral alkyl halides under the KOH/DMSO reaction conditions

Derivatizations of racemic 2-hexanol with alkyl halides (36)-(38), (39) and (46) (Scheme 20) failed under the KOH/DMSO reaction conditions, with none of the desired ether products being formed. This was probably due to the hydrolysis of the ester function under the basic conditions. The chromatograms of the reaction mixtures using halides (36)-(38) and (39) do however show peaks that are due to diastereoisomeric products (and which are separated). The retention times of the diastereoisomers are found to be the same in each case irrespective of the halide used. This would be expected when using halides (36)-(38) as they have a common product, but not with bromide (39). This indicates that the reactions occurring involve the loss of the halide-containing side chain (i.e. that the ester function is reacting). It is proposed that a transesterification reaction is taking place (Scheme 21) to a small extent. The fact that the diastereoisomeric peaks are small in comparison to the 2-hexanol peak in the chromatograms is consistent with the proposed equilibria.

The identity of the diastereoisomeric products (33) was confirmed by injecting a sample containing the diastereoisomers (33), as prepared using the N-acetyl-(S)-(-)-prolyl chloride reagent (Chapter 2). The retention times were observed to be the same in each case.

Reaction of the lactone iodide (49) and bromide (50) with racemic 2-hexanol under the KOH/DMSO conditions produced a complex mixture of products. As well as the possible dehydrohalogenation and hydrolysis side-reactions (with the possible formation of the dialkyl ether product (see Scheme 16 (eq. 7)), the lactone ring itself could also undergo hydrolysis or

SCHEME 21

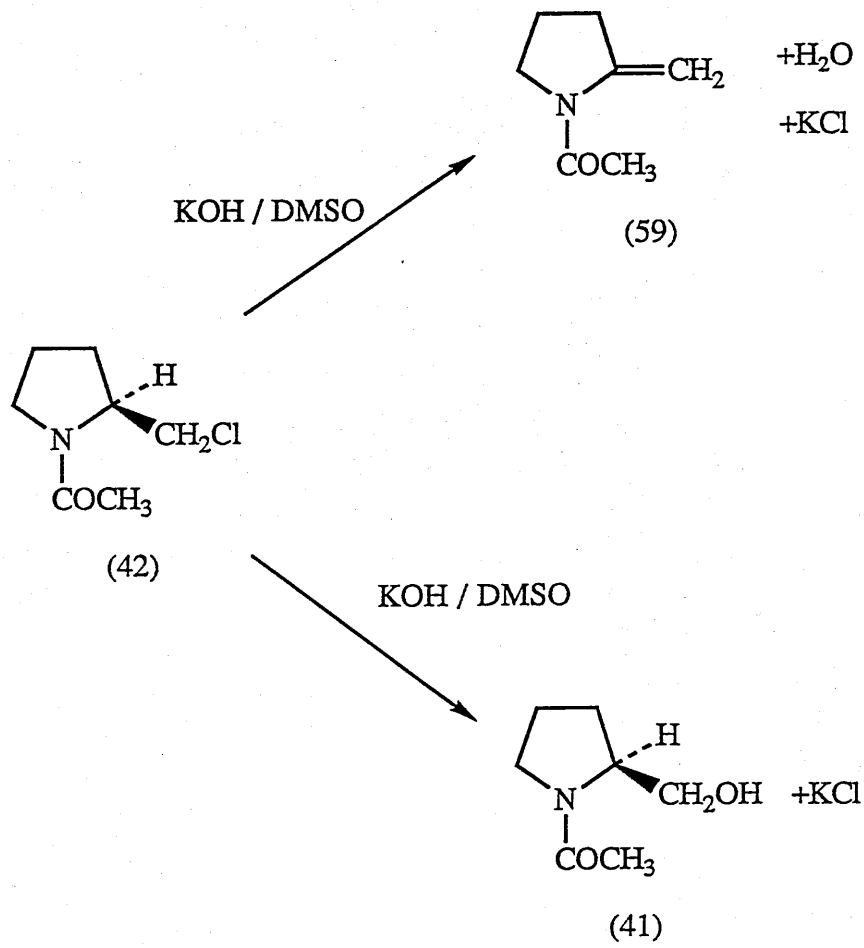


(33a) (33b)

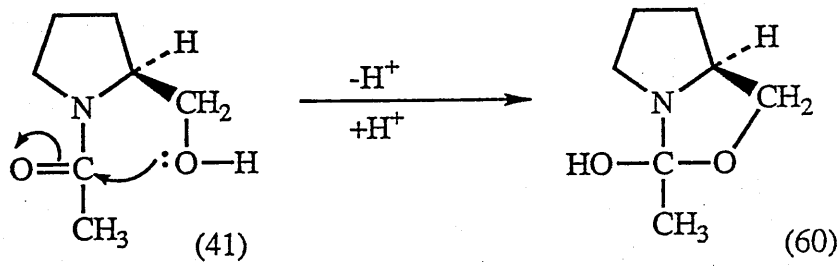
$+2RO^-$

(R= CH_2CH_2X (36)-(38), $(CH_2)_3Br$ (39))

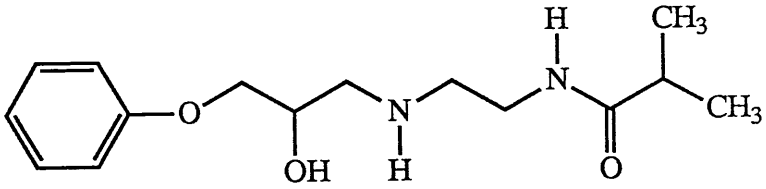
SCHEME 22



SCHEME 23



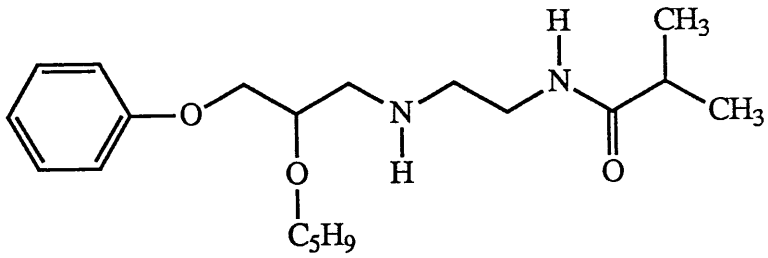
SCHEME 24



(61)

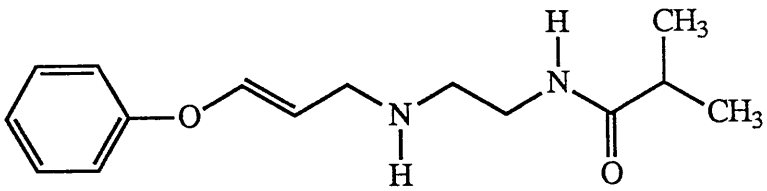
$BrCH_2CH(CH_3)C_2H_5$

KOH / DMSO



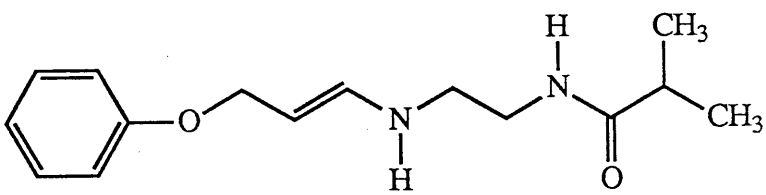
(62)

+



(63a)

and / or



(63b)

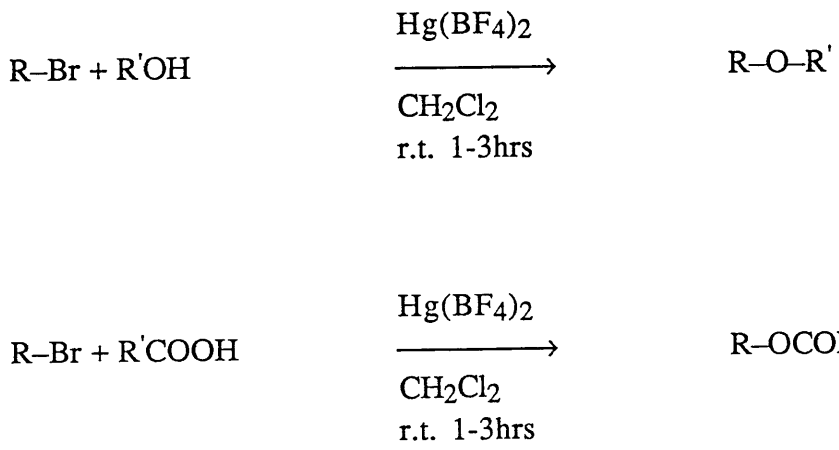
transesterification reactions. The use of these reagents for alcohol derivatizations under the KOH/DMSO conditions was thus deemed unsuitable. Iodide (53) was not reacted as similar results were anticipated.

Chloride (42) and bromide (43) were reacted with 2-hexanol under the KOH/DMSO conditions. GC/MS analysis indicated that both dehydrohalogenation and hydrolysis reactions were occurring (Scheme 22). Alcohol (41) produced from the hydrolysis reaction then appears to form the bicyclic compound (60) (see Scheme 23). The resultant chromatogram (C2) and mass spectra (MS3 and MS4) were consistent with the by-products (59) and (60). The mass spectrum of the cyclised by-product (MS4) is seen to be quite different from that of the isomeric alcohol (41) from which it was formed (see MS5).

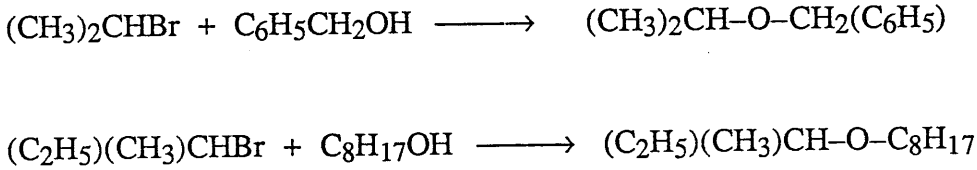
Finally, to ascertain whether a homochiral alkyl halide could be used to derivatize a molecule more complex than 2-hexanol, (S)-(+)-1-bromo-2-methylbutane was reacted with racemic compound (61) under the KOH/DMSO conditions (Scheme 24). The GC/MS results (see chromatogram C3) indicated that while a desired product (62) had been formed (MS6), the analyte itself had undergone a dehydration reaction to form the alkene products (63a) and/or (63b) (MS7). This type of reaction is known to occur in the presence of either KOH or DMSO.[194] Separation of the diastereoisomeric products was not observed on the three GC columns tried.

The mass spectrum MS6 indicated that the derivatization reaction was occurring only on the alcohol group, and not on the secondary amine or amide groups. This conclusion is based on fragment ions such as *m/z* 114, 143 and

SCHEME 25



SCHEME 26



171 which are rationalised in terms of ions with a secondary amine group. The reason for this selectivity may be steric.

It was concluded that the use of alkyl halides as homochiral derivatizing agents under the KOH/DMSO reaction conditions was unsuitable due to the occurrence of many side reactions in the reagents or more complex analytes.

3.2.3 Use of homochiral alkyl halides under the $Hg(BF_4)_2$ reaction conditions

The use of alkyl halides for the alkylation of carboxylates and alkoxides in dipolar aprotic solvents is well known. While the reactions have been reported to work well using simple primary halides and/or non-sterically hindered carboxylates or alkoxides, it has been found that when using more complex reagents, yields are reduced.[172,195] Difficulties can also occur in the preparation of the more complex alkoxides or carboxylates, especially if base-sensitive functions are present.

The reactivity of alkyl halides has been shown to be enhanced by the presence of heavy metal salts. Mercury(II) perchlorate has been reported to be particularly useful, but its use can prove to be dangerous due to its tendency to undergo explosive decomposition.[167]

Barluenga et. al. have reported the use of a mercury tetrafluoroborate reagent ($Hg(BF_4)_2$)[196] which is highly electrophilic in nature and has anions of low nucleophicity. This has been found to enhance the alkylating ability of alkyl bromides, and has been used for the preparation of ethers and esters (Scheme 25).[174,175]

The published yields for two reactions (Scheme 26) are given in Table 8.[174]

TABLE 8 **Percentage yields of the ether product of the reaction of an alcohol with a bromide (after 1hr at room temperature) under the $Hg(BF_4)_2$ reaction conditions (as reported)[174]**

Alcohol	Bromide	
	Isopropyl bromide	2-Bromobutane
Benzyl alcohol	80%	-
1-Octanol	-	55%

TABLE 9 **Percentage yields (estimated from chromatographic results using (eq. 9)) of the ether product of the reaction of an alcohol with a bromide (at room temperature) under the $Hg(BF_4)_2$ reaction conditions**

Alcohol	Bromide	
	Isopropyl bromide	2-Bromobutane
Benzyl alcohol	60% (3 days)	-
1-Octanol	-	4% (12 hrs)

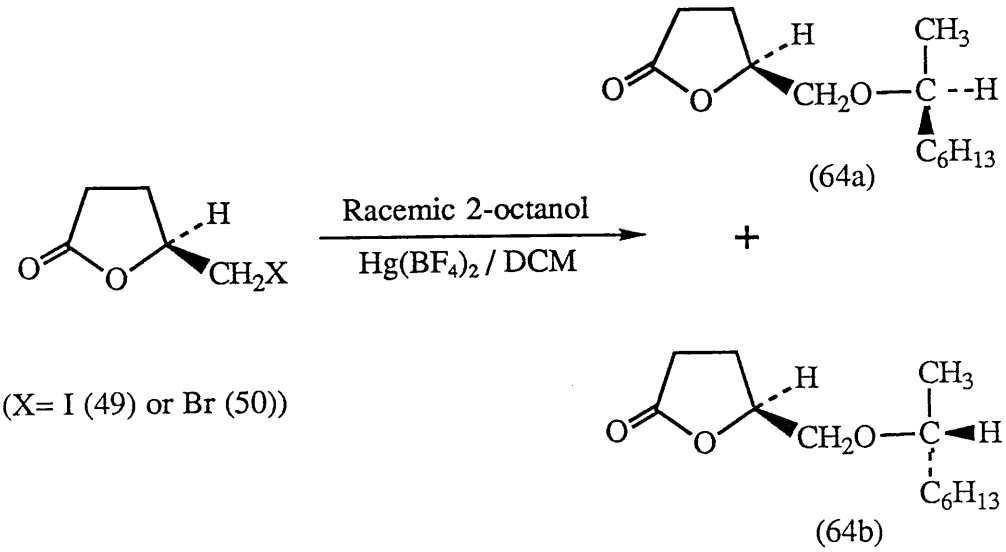
As a comparison with the published results, the reactions were repeated under identical conditions. The estimated yields (from chromatographic results using (eq. 9)) and reaction times required to achieve them are given in Table 9. As can be seen, lower yields were experienced even with the considerably longer reaction times used. It is not known why there should be such a discrepancy between the reported results and those obtained upon checking.

The reaction of iodides (49) and (53) and bromide (50) with 2-octanol under the $Hg(BF_4)_2$ reaction conditions were performed (Scheme 27). Again, low yields were experienced (typically 10%). GC/MS analyses indicated that no significant side reactions were taking place (i.e. even after an overnight reaction the main components remaining were the starting materials and the diastereoisomeric products). The diastereoisomeric products (64a) and (64b) were separated by GC and found to have virtually identical mass spectra (see MS8 and MS9). This is not an unexpected result, as any energy differences which may produce differences in the spectra will be obviated at the high inlet and ion source temperatures. They also lack strong neighbouring group effects (which would influence spectra markedly) during fragmentation of these molecules.

Chromatograms C4 and C5 show that the diastereoisomers (64a) and (64b) are well separated (i.e. to the baseline). The Rs and α values for the chromatographic separation of these diastereoisomers and the diastereoisomers (65a) and (65b) are given in Table 10. The corresponding chromatographic conditions are also given.

Commercially available, enantiomerically pure (R)- and (S)-2-octanols (from Aldrich) were derivatized separately using the lactone iodide (49) to establish the order of elution of the diastereoisomers. The chromatographic

SCHEME 27


H
CH3
O
O
CH2O—C --H
C6H13
(64a)
H
O
O
CH2X
Racemic 2-octanol
Hg(BF4)2 / DCM
+
(X= I (49) or Br (50))
H
CH3
O
O
CH2O—C—H
C6H13
(64b)

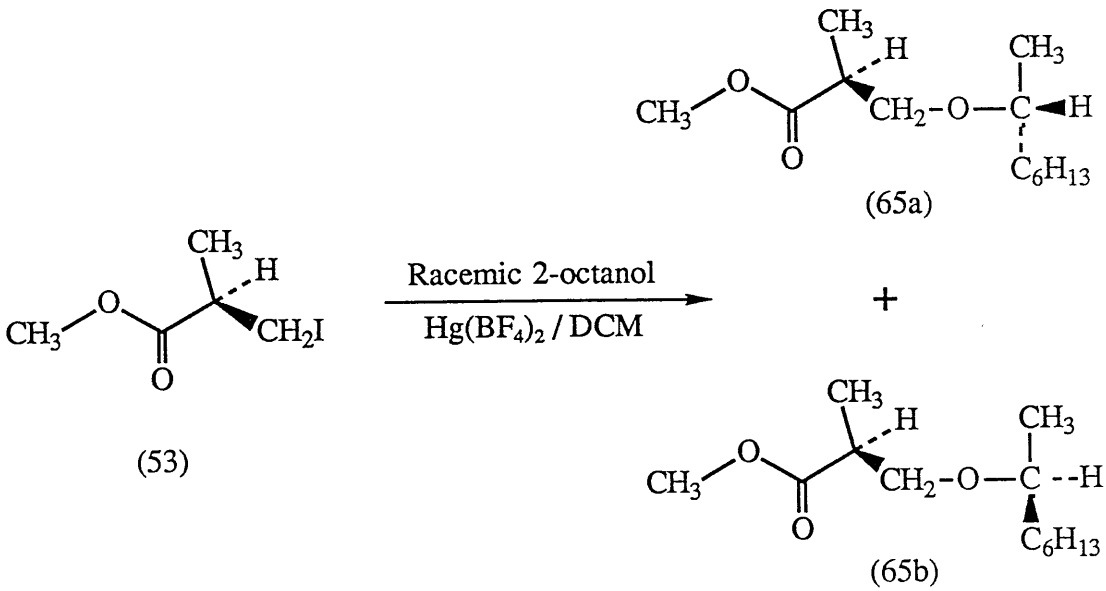

CH3
H
CH3
O
CH3
CH2—O—C—H
O
C6H13
(65a)
CH3
H
O
CH3
CH2I
O
Racemic 2-octanol
Hg(BF4)2 / DCM
+
(53)
CH3
H
CH3
O
CH3
CH2—O—C --H
O
C6H13
(65b)

TABLE 10 Separation factor (α), resolution values (Rs) and other GC data for the separation of diastereoisomers (64a) and (64b) and diastereoisomers (65a) and (65b)

Diastereoisomers	Column and column temp.	t_R	α	Rs
(64)	BP-1[(a)] 80 (2min)→ 220°C(at 10°C min^{-1})	10.02/10.22	1.021	1.57
(65)	BP-5[(b)] iso 103°C	16.28/16.73	1.028	1.1

(a) 12m x 0.22mm, 0.25μm film thickness performed on the Carlo Erba 226204.

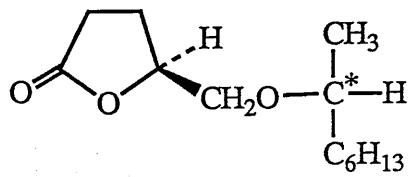
(b) 12m x 0.33mm, 0.5μm film thickness performed on the Carlo Erba 226204.

results showed that the diastereoisomer formed from (S)-2-octanol (i.e. the S,S product (64a)) was the first to elute and that the diastereoisomer formed from the (R)-2-octanol (i.e. the S,R product (64b)) eluted second.

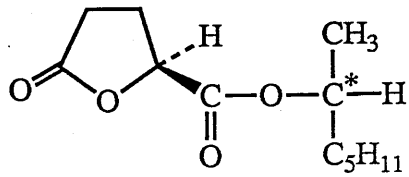
The results obtained from these derivatizations showed that under the $Hg(BF_4)_2$ reaction conditions, diastereoisomeric ether products can be formed from alkyl halides (49), (50) and (53) and secondary alcohols such as 2-octanol. Good gas chromatographic separations of the diastereo-isomeric ether products (64a) and (64b) as well as (65a) and (65b) were

achieved on conventional achiral columns. The yields of the ether products were however, found to be low (<10%) even after lengthy reaction times. This may cause problems in real-life analyses where sensitivity may be lost or kinetic resolution may occur and produce a false result. Even so chromatograms C4 and C5 do show equal peak areas, as expected for the racemic mixture used, despite the low yield. This suggests that little or no kinetic resolution is occurring.

The fact that the diastereoisomeric products (64a) and (64b) containing an ether linkage were separable, but the diastereoisomeric products (35a) and (35b) containing an ester linkage (see Chapter 2) were not indicates that different chiral mechanisms are occurring (with which the ether and ester linkages must be directly associated).

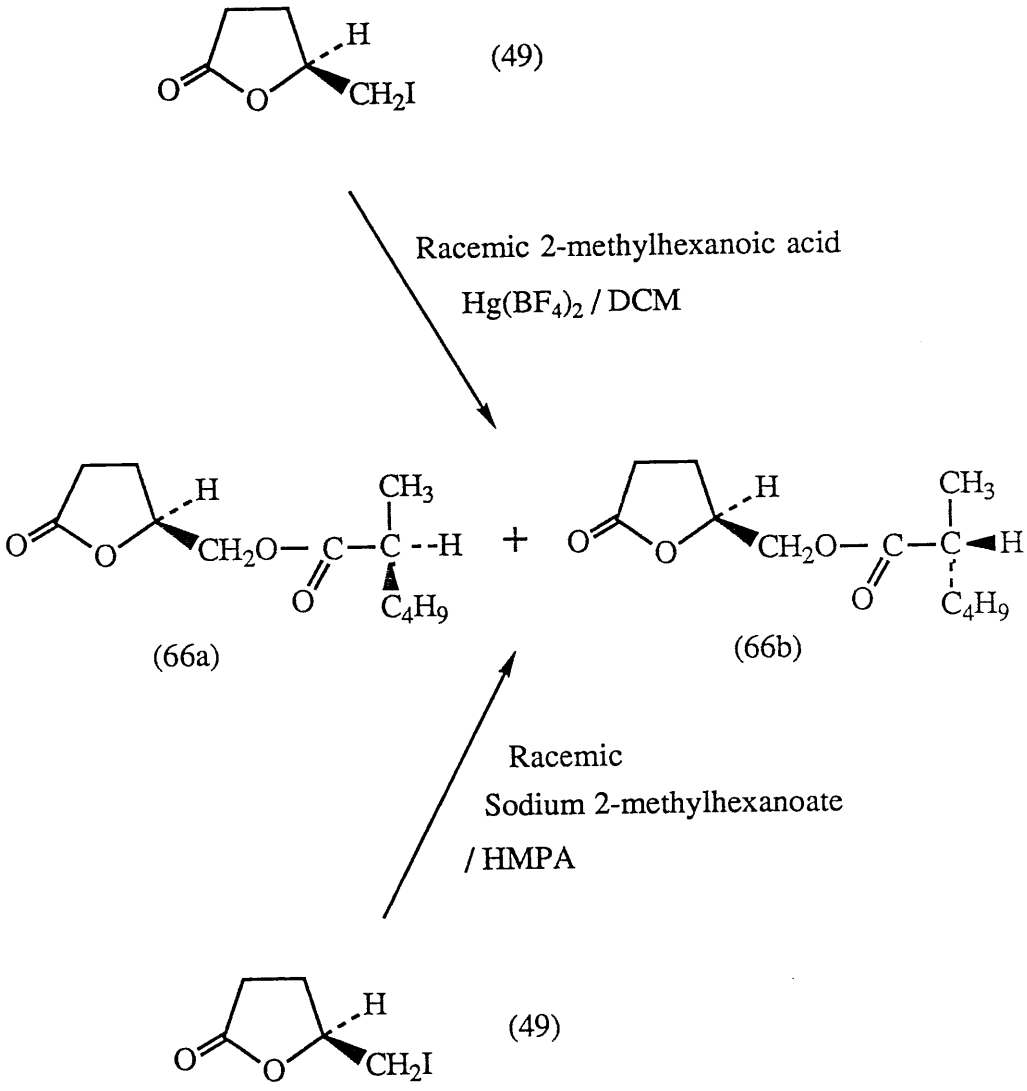


(*= chiral centre, S (64a) or R (64b))

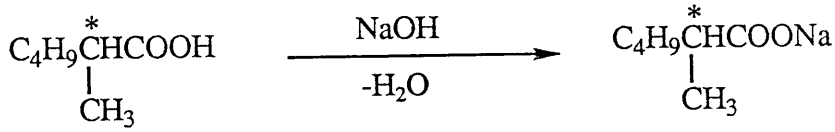


(*= chiral centre, S (35a) or R (35b))

SCHEME 28



SCHEME 29



(*= chiral centre, R or S (racemic mixture))

A hypothesis as to the recognition mechanism assumes that one strong polar interaction (via the C=O in the lactone structure) and two weak (hydrophobic) interactions (near the ether linkage) complete a very selective 3-point interaction of the ethers with the stationary phase. If another strong polar interaction (via the C=O in the ester linkage) is introduced, it may predominate over, and may change the orientation of the other two differentiating (weaker) interactions. The two-point interaction thus set up will be far less selective. It would require considerable experimental and molecular modelling work to verify this hypothesis.

The other homochiral alkyl halide reagents were not reacted under the $Hg(BF_4)_2$ conditions for two reasons. Firstly, halides such as (42) and (43) have been found to be difficult to prepare, and sufficient quantities for assessment were not available. Secondly, derivatizations using alkyl halides (36)-(39) and (46) would yield diastereoisomeric products containing six or more bonds between the chiral centres. As it is accepted that an increased number of bonds between the chiral centres of diastereoisomers causes a reduction (or loss) in their chromatographic resolution,[18,152] these reagents are unlikely to produce the desired results.

Reaction of lactone iodide (49) with racemic 2-methylhexanoic acid (Scheme 28) under the $Hg(BF_4)_2$ reaction conditions gave several products. The retention time of one of the product peaks matched that of the diastereoisomeric products (66a) and (66b) from the reaction of lactone iodide (49) with racemic sodium 2-methylhexanoate in HMPA[171] (Schemes 28 and 29). In neither case were the diastereoisomeric products separated. Again, this could be due to the fact that when an ester linkage is in close proximity to a lactone function, 2-point polar interactions take precedence over diagnostic 3-point interactions with the GC stationary phase, thus

preventing chiral recognition (as previously discussed). In addition, there are now four bonds between the chiral centres, rather than three in diastereoisomers (64) and (65). It should be stressed, though, that even with three bonds between chiral centres the diastereoisomeric esters (35) could not be resolved. It appears that type of linkage, ether or ester, is a more critical factor than the number of bonds between chiral centres in determining the ability to resolve the isomers chromatographically.

4 HOMOCHIRAL CHLOROMETHYL ETHER (CME) DERIVATIZING AGENTS

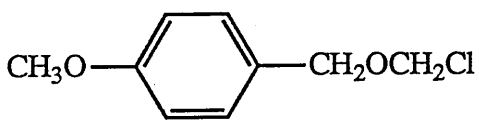
The two approaches investigated for utilizing homochiral alkyl halides as derivatizing agents were found to be unsuitable (see Chapter 3). Under the KOH/DMSO conditions, side-reactions were found to predominate with little or none of the desired products being formed in all but the simplest cases (ie. when simple haloalkanes were used). Under the milder $Hg(BF_4)_2$ conditions, while the correct products were formed, and some were separable on achiral stationary phases, yields were found to be low (<10%). Its use for quantitative/qualitative analyses would thus be inappropriate. The need for a homochiral derivatizing agent that is reactive with a range of functional groups under mild conditions was apparent.

4.1 Chloromethyl ether (CME) derivatizing agents

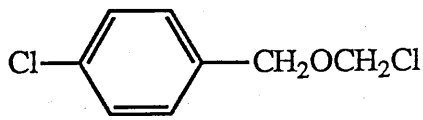
Methoxyethoxy methyl chloride (MEM-Cl) (A) and derivatives of benzyl chloromethyl ether such as p-methoxybenzyl chloromethyl ether (B) or p-chlorobenzyl chloromethyl ether (C) have been used as protecting groups for alcohols.[197-201]

$CH_3OCH_2CH_2OCH_2Cl$ = MEM-Cl

(A)



(B)



(C)

The use of such reagents has several advantages over other methods of protection, these being as follows.

(a) Due to the reactivity of the reagents (see later) the protection step is carried out under mild conditions.[202]

(b) The absence of a proton on the atom β to the functional group prevents the possibility of dehydrohalogenation (elimination).

(c) Primary, secondary and tertiary alcohols react to give acetals in high yield (typically >90%).

(d) The acetals produced are stable to a variety of conditions including those attending the use of strong bases, reducing agents, organometallic agents, many oxidising agents and mild acids.[199]

(e) The acetals can be selectively cleaved in the presence of a range of other protecting groups and functional groups.

(f) As with other protecting agents, the lack of chirality of compounds (A)-(C) prevents any stereochemical complications.

The reactivity of CMEs under conditions favouring S_N1 and S_N2 mechanisms is considered to be due to the ability of the alkoxyl group to stabilize the transition state (S_N2) or intermediate cations (S_N1) by resonance stabilization as shown below in Fig. 26.[202]

Figure 26

$$R-O-\overset{+}{C}H_2 \quad <\text{---------}> \quad R-\overset{+}{O}=CH_2$$

The hydrolysis of chloromethyl methyl ether for example is estimated to occur 10^{13} times faster than that of 1-chloropropane.[203]

CMEs can react directly with an alcohol using diisopropylethylamine as a hindered non-nucleophilic base (eq. 14), or via its triethylammonium salt (eq. 15).[198,199] They are also reactive to sodium salts of the alcohols (eq. 16).[199] Little reference has been made to the reactivity of chloromethyl ethers with other functional groups (such as thiols or acids).

SCHEME 30

$$ROCH_2Cl + R'OH \xrightarrow[DCM]{EtNPr_2^i} ROCH_2OR' + HCl \qquad \text{(eq.14)}$$

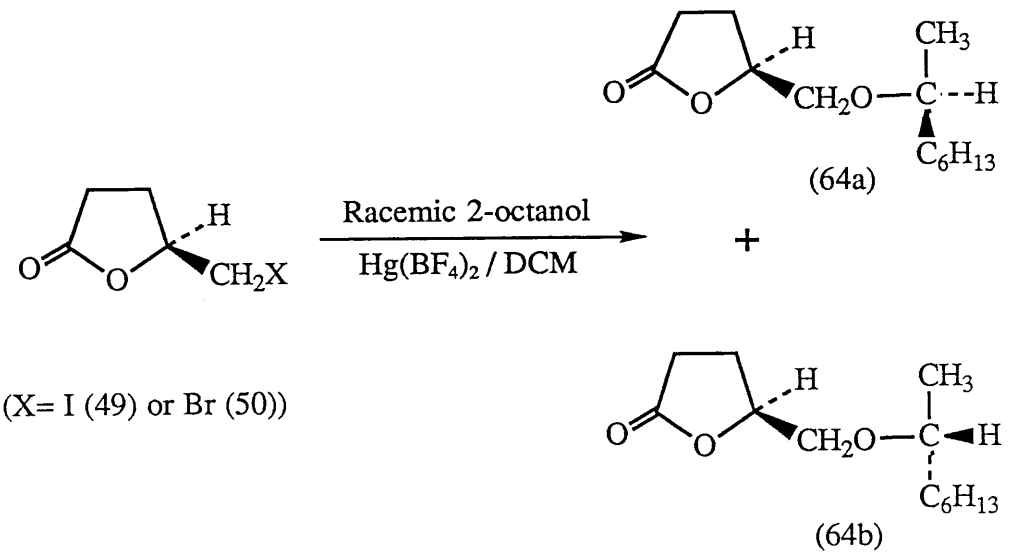
$$ROCH_2Cl \xrightarrow[Et_2O]{Et_3N} ROCH_2NEt_3.Cl \xrightarrow[CH_3CN]{R'OH} ROCH_2OR' + HNEt_3.Cl \qquad \text{(eq.15)}$$

$$ROCH_2Cl + R'ONa \xrightarrow{THF \text{ or } DMOE} ROCH_2OR' + NaCl \qquad \text{(eq.16)}$$

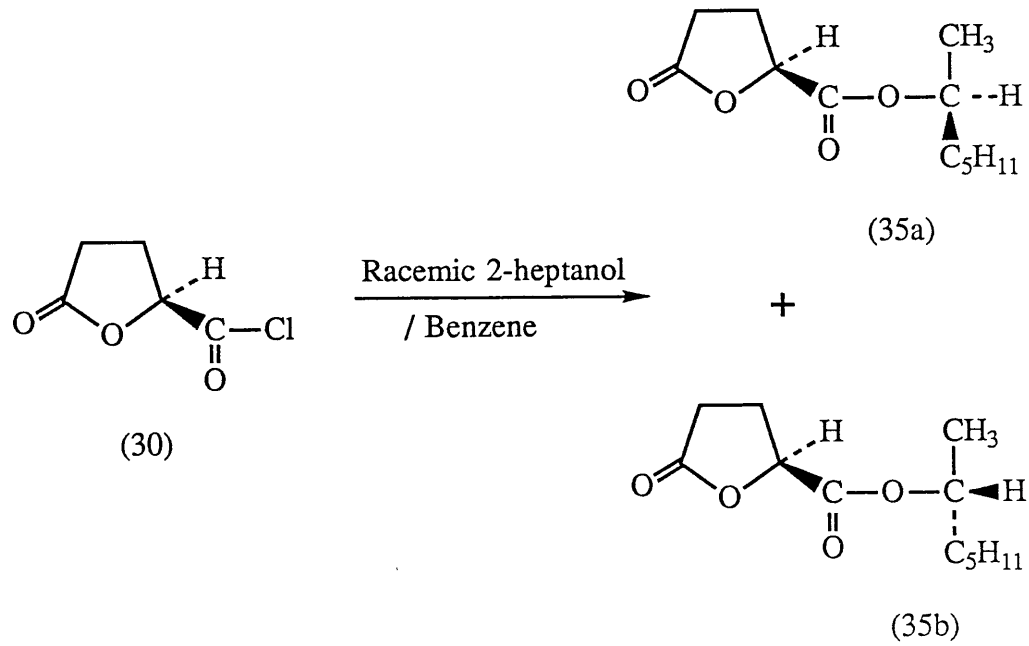
4.2 Chiral chloromethyl ether derivatizing agents

The use of homochiral CMEs as chiral derivatizing agents for GC analyses has had no attention in the literature. This is probably due to the assumption that because the resulting diastereoisomers will have 4 or more bonds between the chiral centres, they will be indistinguishable by gas chromatography.[18,152]

SCHEME 31

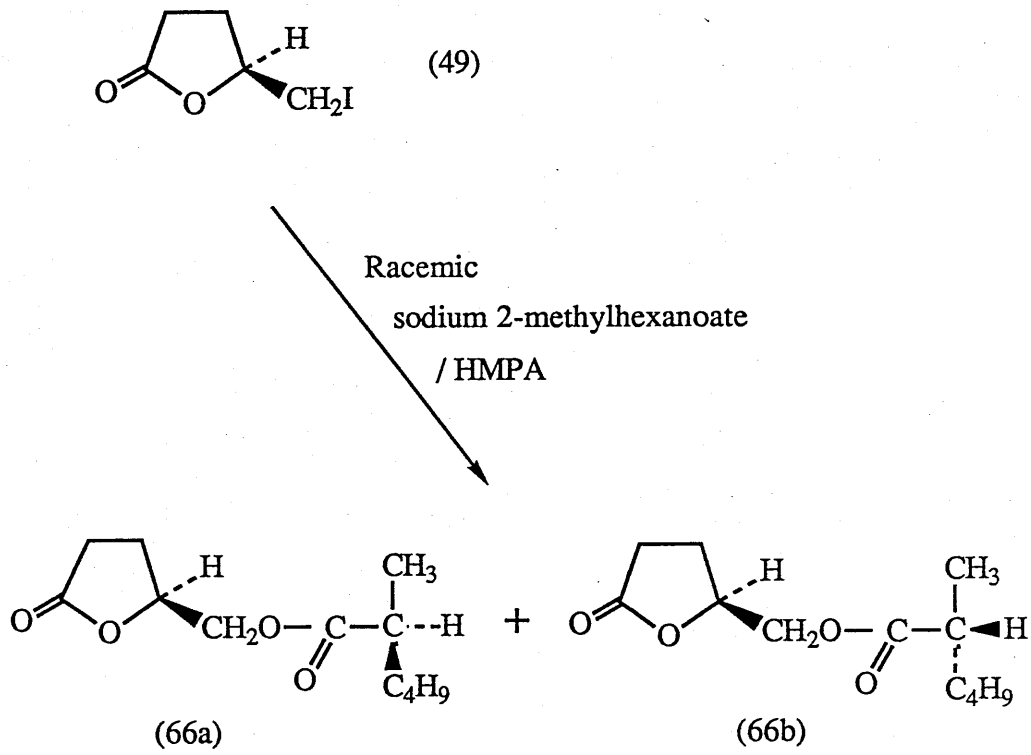


Diastereoisomers (64a) and (64b) <u>were</u> separable.



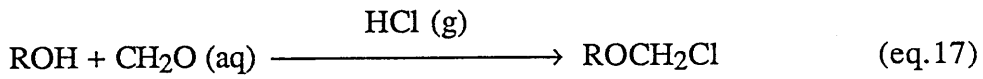
Diastereoisomers (35a) and (35b) <u>were not</u> separable.

SCHEME 31b

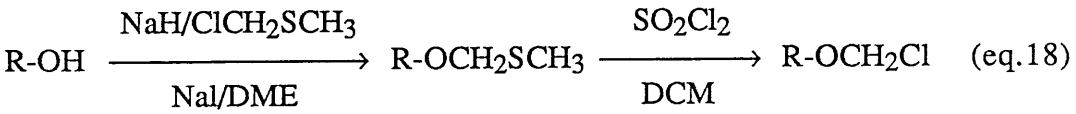


Diastereoisomers (66a) and (66b) were not separable.

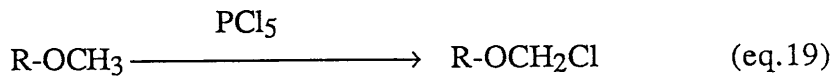
SCHEME 32



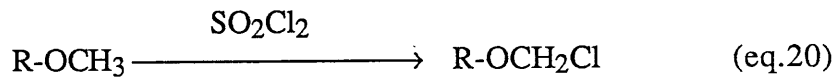
(eq.17)



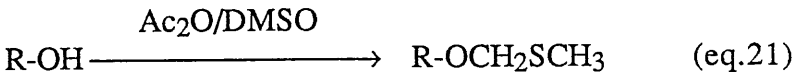
(eq.18)



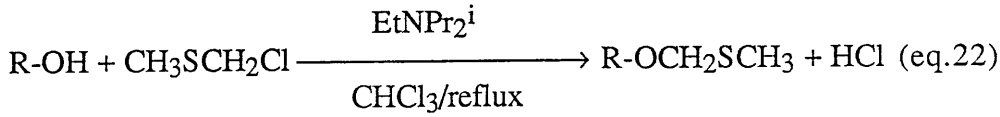
(eq.19)



(eq.20)



(eq.21)



(eq.22)

As already shown in Chapter 3, diastereoisomeric ether derivatives of chiral alcohols were found to be separable, whereas in some cases their corresponding ester derivatives were not (see Scheme 31 and 31b and the discussion on the chiral recognition mechanism involved (Chapter 3)). Homochiral CMEs employed for such derivatizations would yield diastereosimers which contain an acetal linkage. The good separation results shown in Chapter 3 for ethers were thus encouraging for the possible separation of such diastereoisomeric acetals. The use of homochiral CMEs as derivatizing agents for enantiomeric alcohols, acids, thiols and amines thus deserved investigation.

4.3 Synthesis of chiral chloromethyl ethers

Several routes for the preparation of CMEs from alcohols have been reported (Scheme 32).[177,198-200,204,]

Reaction of hydrogen chloride (gas) with an alcohol in aqueous formaldehyde solution is reported to yield the CME (eq. 17).[199,204,205] Another route developed by Undheim et al. proceeds via their methyl thiomethyl (MTM-) ether derivatives (eq. 18).[200,201] Finally, CMEs have been prepared by the reaction of phosphorus pentachloride or sulphuryl chloride with the methyl ether derivative of an alcohol ((eq. 19) and (eq. 20)).

The synthesis of homochiral CMEs following the first two routes ((eq. 17) and (eq. 18)) was undertaken. The five alcohols selected as the starting materials were:

(i) (1S,2R,5S)-(+)-menthol

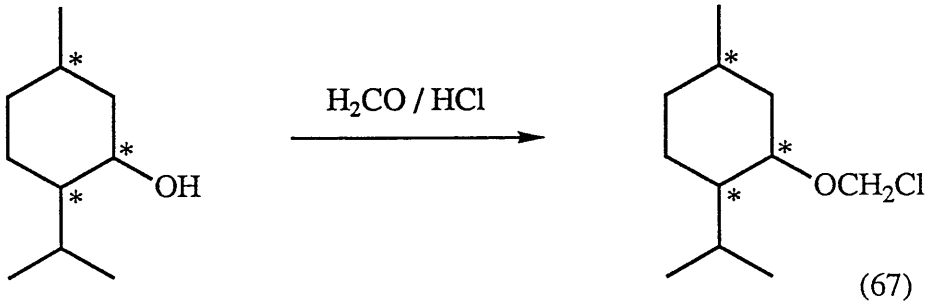
(ii) N-acetyl-(S)-(-)-pyrrolidinemethanol

(iii) (S)-(+)-tetrahydro-5-oxo-2-furanmethyl alcohol

(iv) (S)-(+)-methyl-3-hydroxy-2-methyl propionate

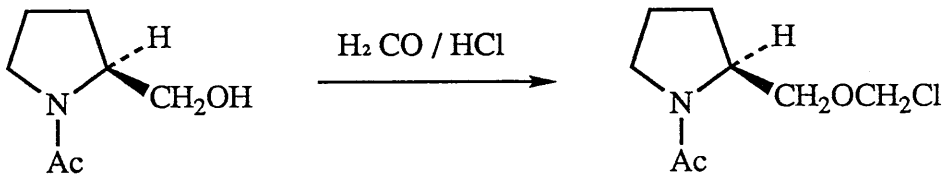
(v) (S)-(+)-methyl lactate

SCHEME 33.

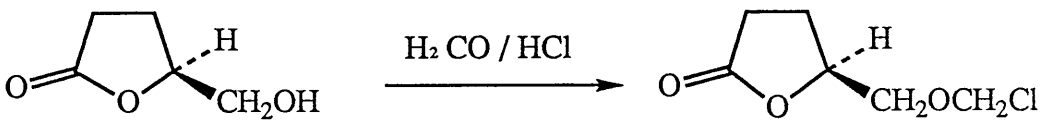


(67)

(*= chiral centres, 1S, 2R, 5S)



(41) (68)



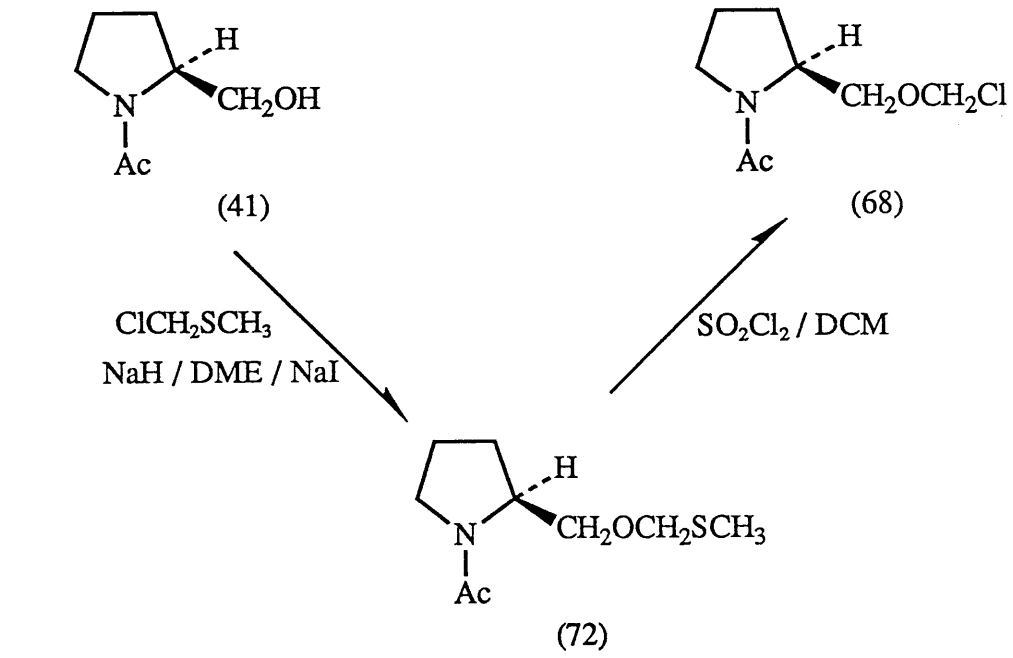
(47) (69)

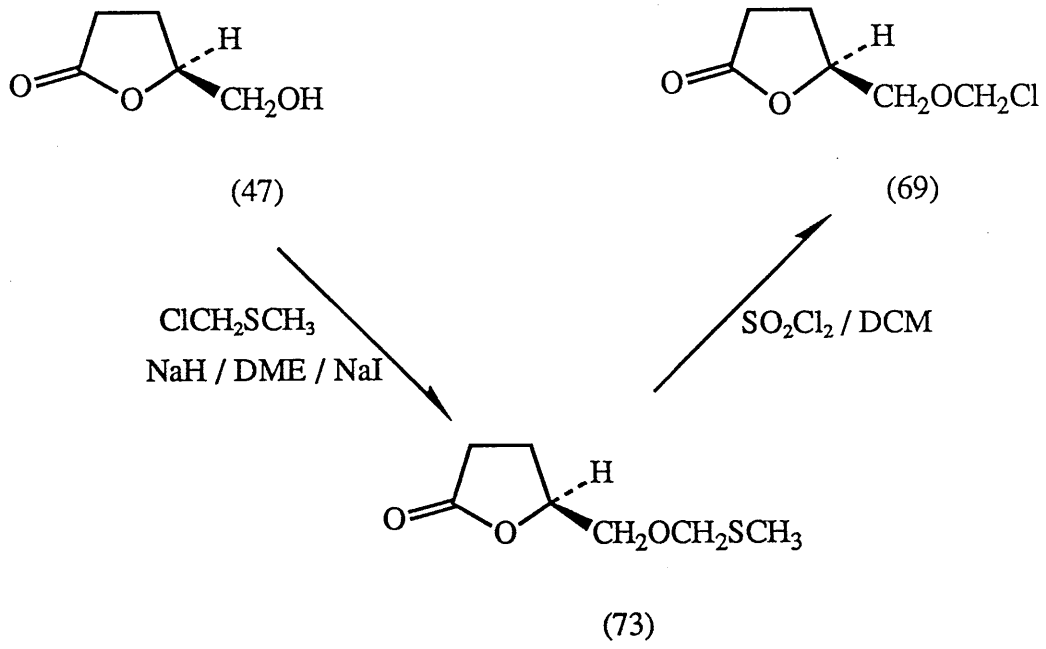
Each contain functions or structures which are present in existing homochiral acid chloride reagents.

In order to improve on the CME synthesis reported by Undheim et al.,[200,201] two other methods for the sythesis of MTM ethers were investigated. The first was developed by Yamada et al.[207] (eq. 21) and the second was a novel method (eq. 22) which will be discussed in more detail later. Other methods for the synthesis of MTM-ethers have been reported[208,209] but were not attempted.

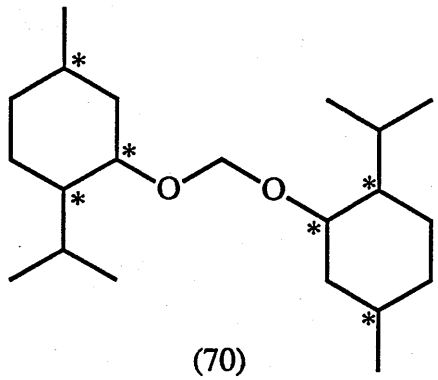
Problems arose during the direct synthesis of compounds (67)-(69) by the reaction of formaldehyde and hydrogen chloride with the homochiral alcohols in aqueous media (Scheme 33). The raw yield of compound (67) appeared to be good (7.198g; 81%), but inspection of the proton NMR led to the conclusion that a mixture of products was present (signal at δ= 4.7 ppm indicated that a O-C$\underline{H_2}$-O function may exist as well as the O-C$\underline{H_2}$-Cl function which has a singlet at δ=5.4 ppm). TLC analysis (silica plates, various solvents) did not indicate two products, but this could be due to the very non-polar nature of the two products preventing separation. Vacuum distillation gave two products, one a solid (with a ^{1}H NMR signal at δ= 4.7 ppm) and the other a liquid (with a proton NMR signal at δ= 5.4 ppm). The yield of the liquid product, which appeared pure by proton NMR analysis was low (12%). GC/MS performed on the liquid product indicated that several compounds were present. Two of the compounds were identified, one as being menthol (m/z 155 $[M-H]^+$) and the other the desired product (m/z 189 $[M-CH_3^+]$; this peak was very small and the expected chlorine isotope peak at m/z 191 appeared to be below the detection limit). The product structure was confirmed by the resonance stabilised chlorine-containing fragment ions with m/z values of 119/121 ($CH_3CH=C\overset{+}{H}CHOCH_2Cl$ <--> $CH_3CH=CHCH=O^+CH_2Cl$). The other peaks appeared to be dialkyl diether type compounds. The mass spectra

SCHEME 34


H
N
CH2OH
Ac
(41)
H
N
CH2OCH2Cl
Ac
(68)
ClCH2SCH3
NaH / DME / NaI
SO2Cl2 / DCM
H
N
CH2OCH2SCH3
Ac
(72)

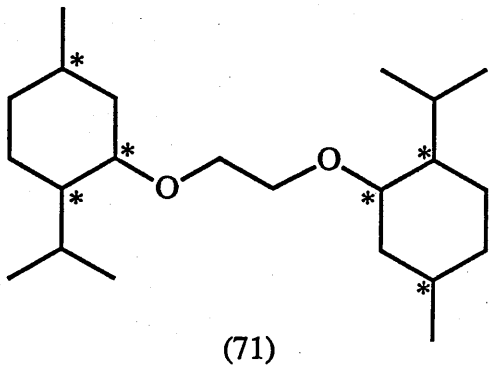

H
O
O
CH2OH
(47)
H
O
O
CH2OCH2Cl
(69)
ClCH2SCH3
NaH / DME / NaI
SO2Cl2 / DCM
H
O
O
CH2OCH2SCH3
(73)

obtained for these were not conclusive (due to the absence of molecular ions), but suggested that products (70) and (71) had formed.



(70)

(*= chiral centres)

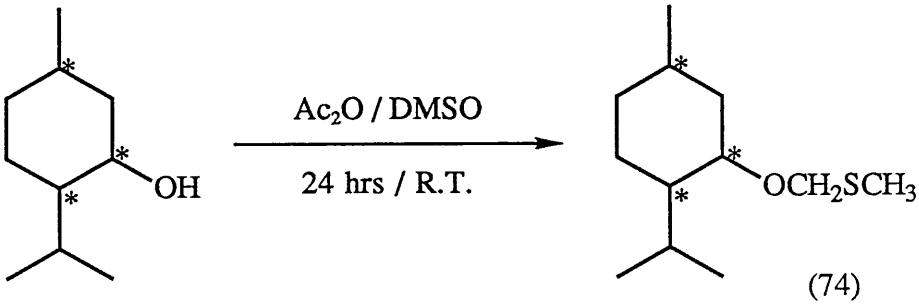


(71)

Compounds (70) and (71) may be formed by the reaction of CME (67) with any residual menthol (which had distilled over with it) in the hot injector zone of the gas chromatograph. GC analysis of the same product sample using on-column injection produced a much cleaner chromatogram showing only the desired CME product (67) and a small quantity of menthol (<5%).

The synthesis of CMEs (68) and (69) failed under the formaldehyde/HCl conditions. This was probably due to the amide and lactone functions being sensitive to the highly acidic conditions. Their synthesis via their MTM ether derivatives[200,201] was thus attempted (Scheme 34).

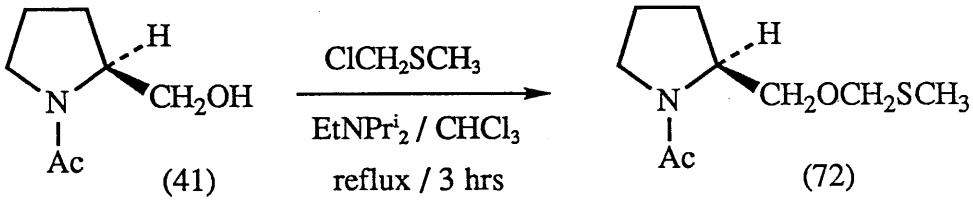
SCHEME 35



(74)

(* = Chiral centre, 1S, 2R, 5S)

SCHEME 36a



(41) (72)

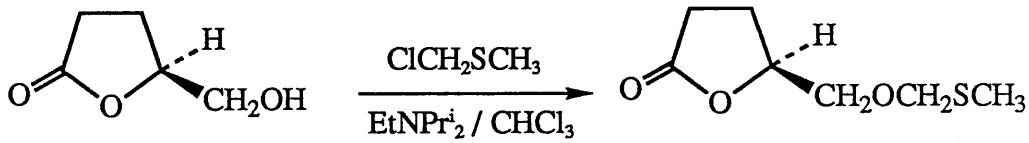
MTM ether (72) was obtained in low yields (~5%) under the sodium hydride conditions. The subsequent conversion to CME (68) appeared successful by NMR (in ~100% yield). The synthesis of MTM ether (73) was not attempted as ester functions (thus lactones) are known to be reactive to sodium hydride.

The low yields and/or incorrect products obtained in the preparation of MTM ether (72) suggest that this route is not viable for alcohols containing base-sensitive functions. Alternative methods for the synthesis of MTM ethers were thus considered.

Menthyl thiomethyl ether (74) was prepared in good yield (78%) following the procedure reported by Yamada et. al.[207] (Scheme 35). While the NMR results confirmed the identity of the product, the elemental analysis indicated the presence of impurity (i.e. C -0.68 and H + 0.36%). Gas chromatography (BP-5, 120-220°C at 20°C min^{-1}) confirmed the presence of an impurity (~5%), at a retention time very close to that of the desired product (3.09 and 3.24 minutes respectively). This could lead to difficulties if distillation was chosen as a purification method as the two products will have similar boiling points. If the by-product contained a menthyl unit, separation by column chromatography would be difficult as the polarities of the two products would probably be similar (low). As the assessment of the analytical performance of the menthyl chloromethyl ether (67) derived from the thiomethyl ether (see later) was the prime objective, it was decided that initial use of the menthyl thiomethyl ether in its 95% pure state would be acceptable.

Chiral MTM ethers (72), (73), (75) and (76) were prepared using a novel approach (Schemes 36a and 36b). This strategy was developed by analogy with the derivatization step (for alcohols) using chloromethyl ethers reported by Undheim et al.[200] If chloromethyl ethers are reactive to nucleophilic attack by alcohols

SCHEME 36b



(47) (73)

$CH_3OCO\overset{*}{C}H(CH_3)CH_2OH \xrightarrow[EtNPr^i_2 / CHCl_3,\ reflux / 3\ hrs]{ClCH_2SCH_3} CH_3OCO\overset{*}{C}H(CH_3)CH_2OCH_2SCH_3$

(75)

(* = chiral centre, S)

$CH_3OCO\overset{*}{C}H(CH_3)OH \xrightarrow[EtNPr^i_2 / CHCl_3,\ reflux / 3\ hrs]{ClCH_2SCH_3} CH_3OCO\overset{*}{C}H(CH_3)OCH_2SCH_3$

(76)

(* = chiral centre, S)

under the diisopropylethylamine conditions, then it is likely that chloromethyl methyl sulphide will also be reactive under the same conditions. This is due to the similar ability of the sulphide group to stabilize the transition state (S_N2) or intermediate cations (S_N1), as shown (for S_N1) below.

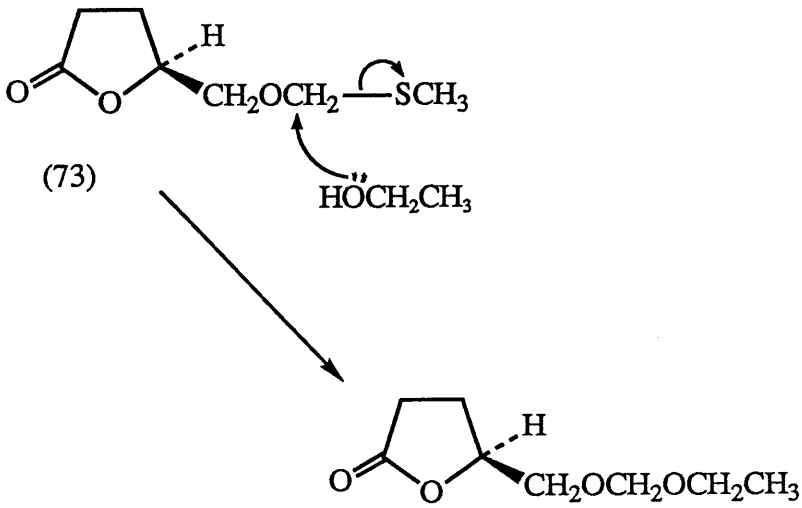
$$R - S - \overset{+}{C}H_2 \quad <--------> \quad R - \overset{+}{S} = CH_2$$

The reactions were monitored by GC until the product had maximized (to typically 60%) with respect to the starting alcohol and to two by-products. One by-product had a shorter retention time than the corresponding MTM-ether, and the other a longer retention time.

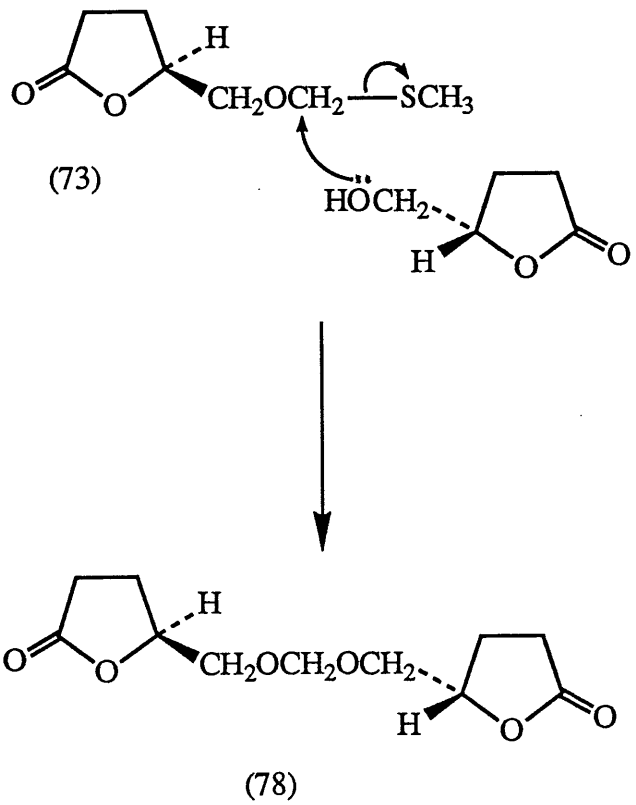
The mass spectral and ^1H NMR data obtained for the by-product with the shorter retention time (isolated by vacuum distillation) from the preparation of the lactone MTM ether (73) was consistent with compound (77). Nucleophilic attack of ethanol on the required product, MTM ether (73) would produce compound (77) (Scheme 37) and methyl thiol which would explain the results. The presence of ethanol in the chloroform solvent as a stabilizer (at ~1%, i.e. ~0.6 ml (~0.45g, 10 mmol) in 60 ml) explains its origin. In reality, the processes occurring would be more complex because the ethanol would also react with the chloromethyl methyl sulphide producing ethyl MTM ether. The homochiral alcohol (starting material) could also act as the nucleophile to produce the dialkyl diether by-product (78) (Scheme 37). This would account for the by-product with the longer retention time. Such dialkyl diether by-products have been isolated and identified (see Chapter 5).

The use of distillation to purify the MTM ethers produced 25-46% yields of (72), (73), (75) and (76). The analytical data obtained were consistent with the correct products. The low yields were due to difficulties in removing the by-product with the lower boiling point (the differences between them being small).

SCHEME 37



(73) (77)



(73) (78)

Decomposition may also have occurred where higher distillation temperatures were required (especially noticeable with MTM ether (72)). The use of a more efficient vacuum system would reduce this.

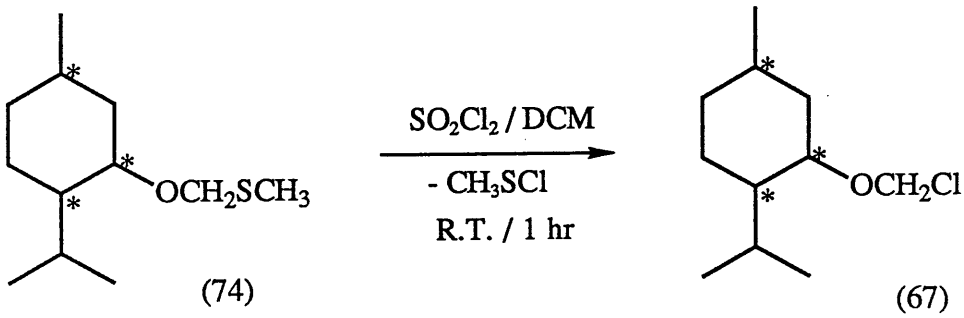
As will be shown in Chapter 5, the use of ethanol-free chloroform prevents the formation of the ethoxymethyl ether by-products.

Synthesis of chloromethyl ethers (67)-(69), (79) and (80) (Schemes 38 and 38b)

MTM ethers (72)-(76), when treated with sulphuryl chloride in DCM at room temperature, underwent selective cleavage of the carbon-sulphur bond to yield the corresponding CMEs (67)-(69), (79) and (80) in good yields (>90%). The methane sulphenyl chloride by-product was removed along with the solvent using rotary evaporation then applying a high vacuum.

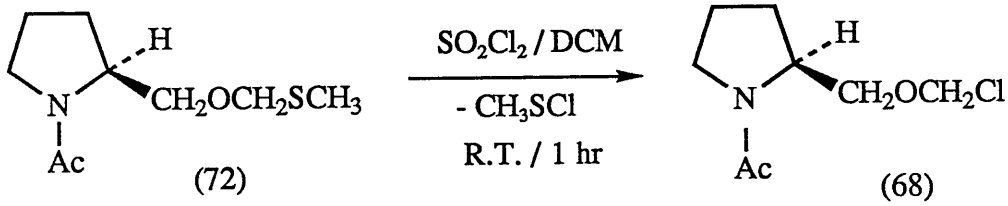
The products appeared pure by GC analysis, and the ^{1}H and ^{13}C NMR results indicated that the correct products were present. Loss of the singlet due to the $OC\underline{H_2}S$ protons (δ = 4.6 ppm) and appearance of a singlet due to the $OC\underline{H_2}Cl$ protons (δ = 5.4 ppm) in the ^{1}H NMR spectra confirms the identity of compounds (67)-(69), (79) and (80). Difficulties were encountered in obtaining consistent mass spectral and elemental composition data. It would appear that Undheim et al. encountered similar problems[200], describing the CMEs as possibly 'being unstable, decomposing on heating or storage'. As samples of CMEs have been seen to decompose in sealed containers when left at room temperature overnight, it can be assumed that the CMEs sent away for elemental composition determination to were decomposing before analysis. An exception was CMEs (80) on which a satisfactory C and H composition was found (±0.3%).

SCHEME 38



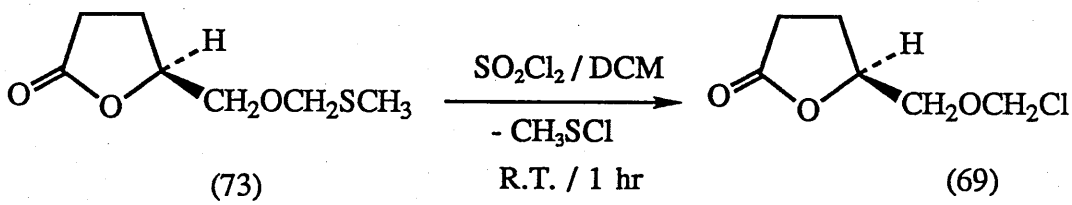
(74) (67)

(* = Chiral centre, 1S, 2R, 5S)

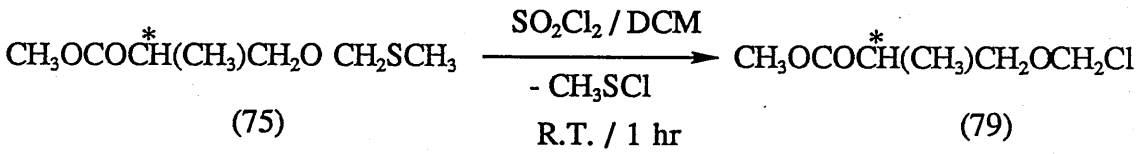


(72) (68)

SCHEME 38b

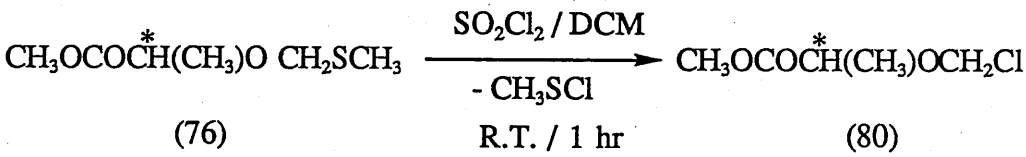


(73) (69)



(75) (79)

(* = chiral centre, S)

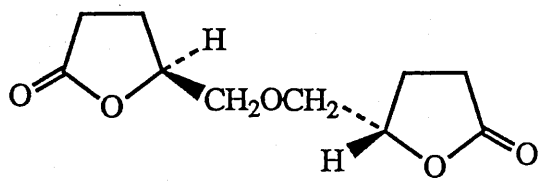


(76) (80)

(* = chiral centre, S)

Use of high resolution mass spectrometry to obtain the relative molecular mass (and thus the molecular formulae) of the CMEs may prove to be difficult, due to their thermal instability and low molecular ion abundance (due to loss of HCl in EI mode). Use of CI may produce better molecular ion information, but samples would again have to be sent off for analysis, which would in turn allow decomposition to occur.

The mass spectra obtained for compounds (68) and (69) were consistent with the correct structures. However, after some time on the probe tip the mass spectrum of compound (69) showed an ion at m/z 214. This could possibly indicate the presence of compound (81) as a thermal reaction product. Ions with even higher m/z values than 214 have also been observed indicating that even larger products may have formed in the ion source.



(81)

The problems encountered due to the instability of the chloromethyl ethers have meant that GC and NMR are the most reliable methods to hand for determining the composition and identity of the products. As already discussed, these would seem to indicate that the correct products are present and in a pure form.

Because only small quantities of chlormethyl ethers were available and because of their probable thermal instability, the use of distillation to ensure the purity of

compounds (67)-(69), (79) and (80) was not attempted. They were thus prepared when required and used immediately for the derivatization reactions.

4.4 Derivatization of enantiomeric mixtures of chiral alcohols, amines, acids and thiols

Chiral CMEs ethers (67)-(69), (79) and (80) were used to derivatize the following compounds, which contained a range of functional groups:

(i) An equimolar mixture of racemic 2-pentanol, 2-hexanol, 2-heptanol and 2-octanol

(ii) Racemic 2-methylhexanoic acid

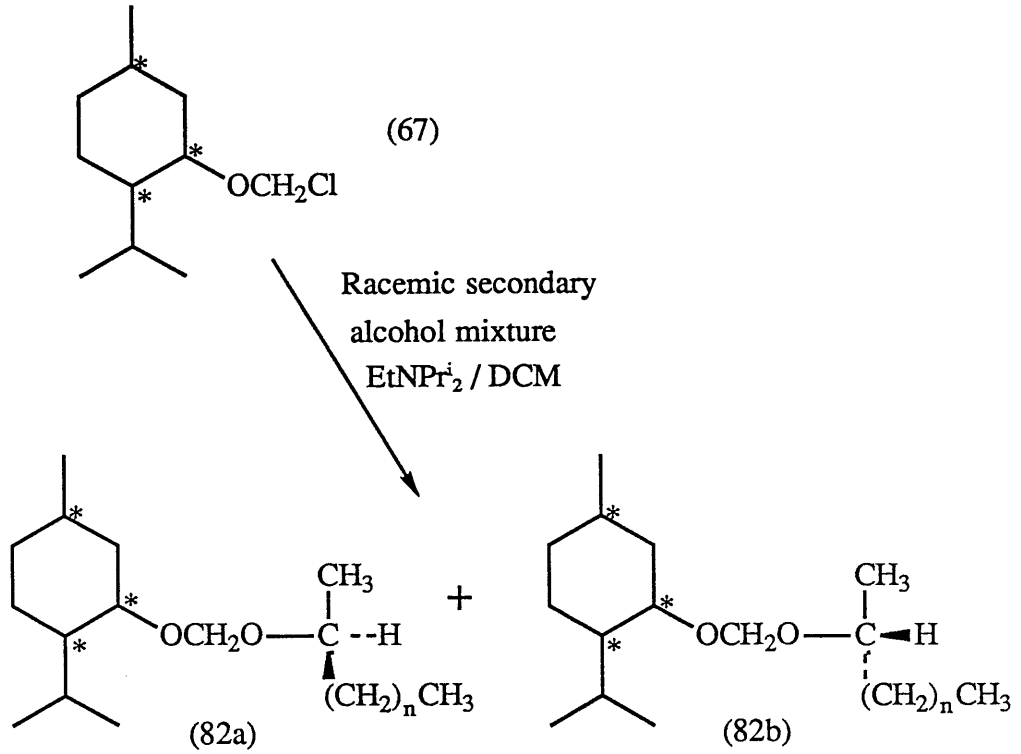
(iii) Aniline (non chiral)

(iv) Racemic 2-methyl-1-butanethiol

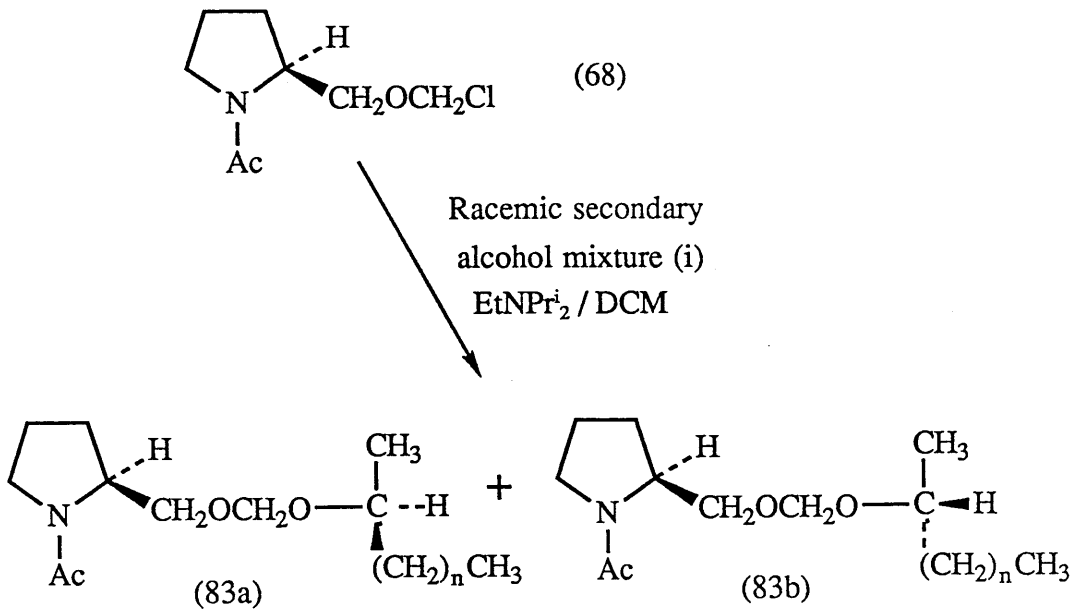
(v) Non-racemic mixtures of R- and S-2-octanol (derivatization using chiral CME (69) only)

(vi) Commercial 'enantiomerically pure' (99% from Aldrich) R- and S-2-octanol (derivatization using chiral CME (69) only)

SCHEME 39a

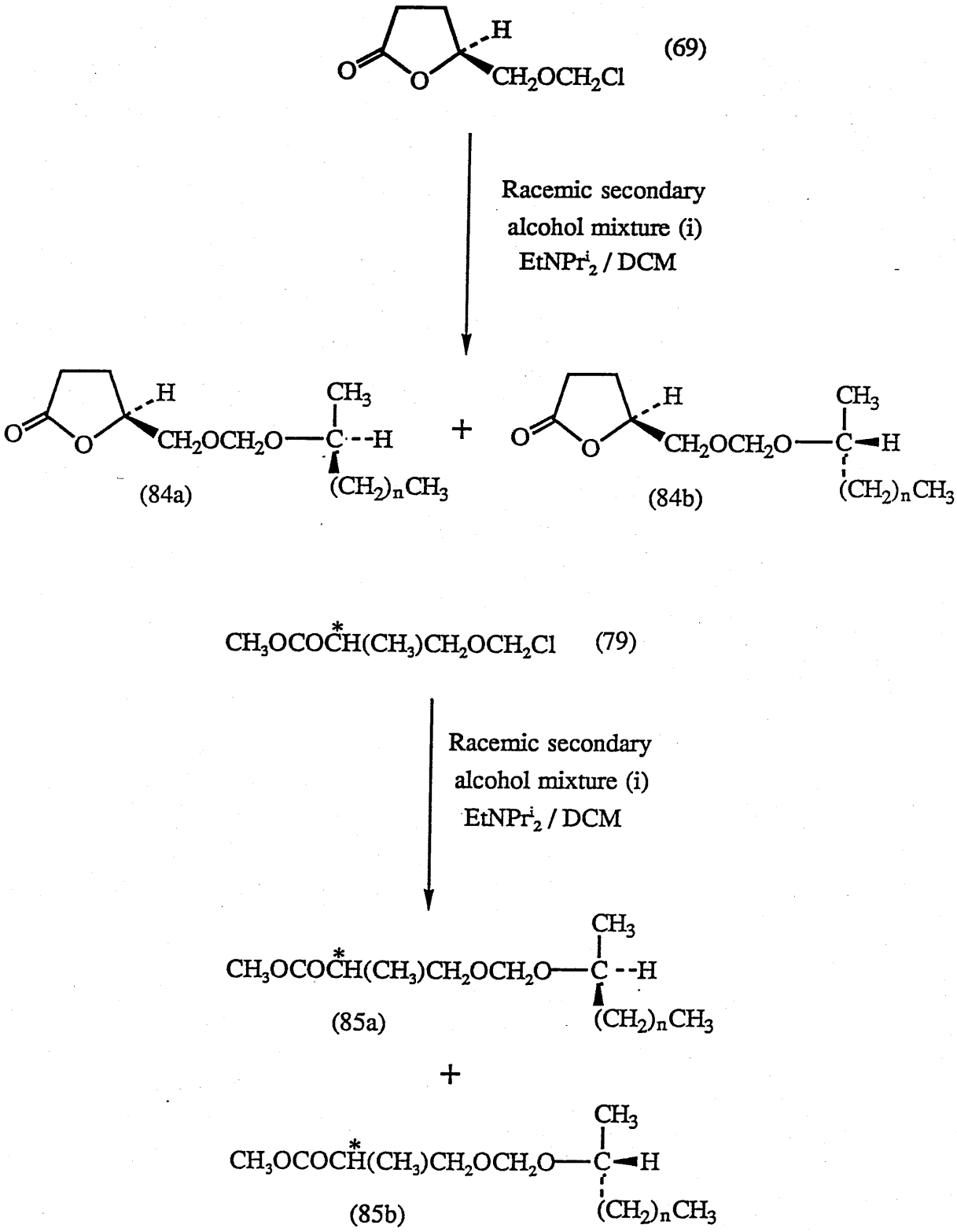


(* = Chiral centres, 1S, 2R, 5S)



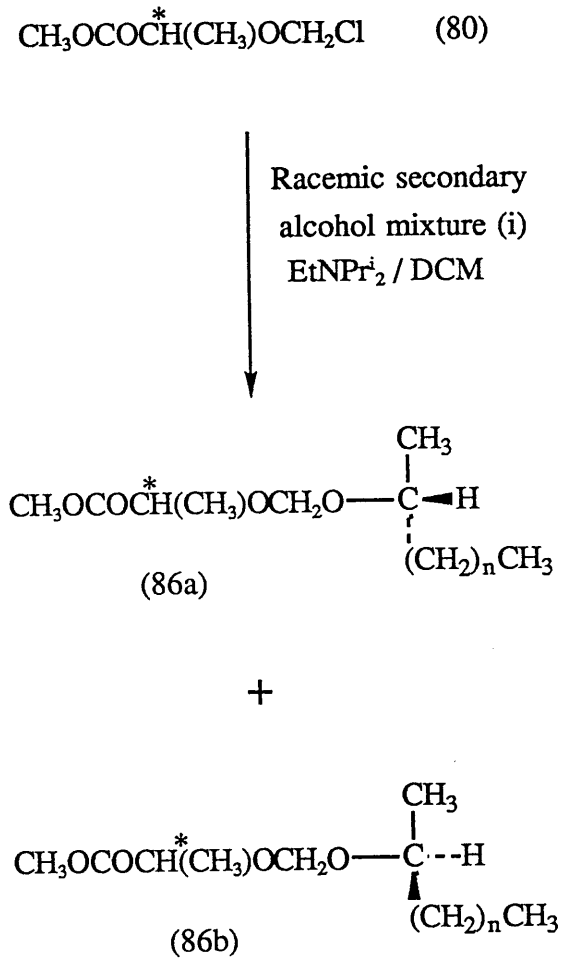
(n = 2 to 5 for 2-pentanol to 2-octanol derivatives)

SCHEME 39b



(n = 2 to 5 for 2-pentanol to 2-octanol derivatives ; * = chiral centre, S)

SCHEME 39c



(n = 2 to 5 for 2-pentanol to 2-octanol derivatives ; * = chiral centre, S)

(i) Derivatives of the mixture of racemic secondary alcohols (2-pentanol ⟶ 2-octanol)

The mixture of racemic secondary alcohols was derivatized using chiral CMEs (67)-(69), (79) and (80) to produce diastereoisomeric products (82)-(86) (Scheme 39a-39c). GC analysis indicated that in all cases the reaction yield was 90-100% after 5 hours. GC/MS confirmed the identity of the products. In the cases where diasteroisomeric separation occurred (e.g. with (84a) and (84b), also (85a) and (85b), see chromatograms C6-C9, the mass spectral data for the individual diastereoisomers were indistinguishable (see mass spectra, MS10 and MS11). Assignment of the SR and SS configurations for the separated diastereoisomers can be made (as shown later for the 2-octanol derivatives) by preparing the derivatives of the enantiomerically pure forms of the secondary alcohols.

Data obtained from the chromatograms enabled the calculation of the separation factor (α) and resolution (Rs) values for the diastereoisomers. Table 11 lists the retention times and α values (in brackets) for the individual diastereoisomers. The corresponding Rs values are listed in Table 11a. The results indicate the following:

(a) The diastereoisomers formed using CMEs (69) and (79) were separated to a similar extent (see diastereoisomeric products (84) and (85)).

(b) There is little or no separation of the diastereoisomers formed from CMEs (67), (68) and (80) (see diastereoisomeric products (82), (83) and (86)).

(c) Rs values increase with increasing carbon chain length of the secondary alcohol units and/or retention time.

TABLE 11 Showing the retention times for diastereoisomers (82)-(86) along with the calculated α-values (in brackets)

		Diastereoisomeric Products				
Alcohol	n	82[a]	83[b]	84[c]	85[d]	86[e]
2-Pentanol	2	7.52 (N)	- -	7.15/7.22 (1.011)	6.32/6.37 (1.009)	5.14 (N)
2-Hexanol	3	9.38 (N)	-	9.77/9.90 (1.015)	9.55/9.66 (1.013)	7.15 (N)
2-Heptanol	4	11.34 (N)	- -	13.65/13.87 (1.018)	14.87/15.10 (1.017)	9.23 (N)
2-Octanol	5	13.37 (N)	14.41/14.55 (1.01)	19.41/19.76 (1.019)	23.77/24.20 (1.019)	11.30/11.36 (1.005)

TABLE 11a Showing the corresponding resolution values for diastereoisomers (82)-(86)

		Diastereoisomeric Products				
Alcohol	n	82[a]	83[b]	84[c]	85[d]	86[e]
2-Pentanol	2	N	-	0.767	N	N
2-Hexanol	3	N	-	0.93	0.7	N
2-Heptanol	4	N	-	1.11	1.06	N
2-Octanol	5	N	X	1.36	1.25	X

N = Diastereoisomers not resolved

- = Analysis not performed

X = Not sufficiently separated to calculate Rs

(a) BP-5 (12m x 0.33mm; 0.5μm film) 120→220 at 5°C min^{-1}
(b) FFAP-CB (25m x 0.32mm; 0.3μm film) iso 200°C
(c) and (d) DB-1701 (30m x 0.25mm; 0.2μm film) (c) iso 180°C (d) iso 130°C
(e) BP-5 (12m x 0.33mm; 0.5μm film) 80 (2min)→220 at 5°C min^{-1}

TABLE 12 Showing the t_R, α and Rs values for the 2-pentanol→2-octanol diastereoisomeric derivatives (84)

Alcohol	n	Oven Temp. (°C)	t_r (minutes)	α	R_S
2-Pentanol	2	iso 170	9.77/9.88	1.013	0.77
2-Hexanol	3	iso 180	9.77/9.00	1.015	0.93
2-Heptanol	4	iso 190	9.84/9.97	1.015	1.00
2-Octanol	5	iso 200	9.77/9.89	1.014	1.036

These analyses were performed on a DB-1701 fused silica column (30m x 0.25mm; 0.2μm film) using a Perkin Elmer 5840 gas chromatograph.

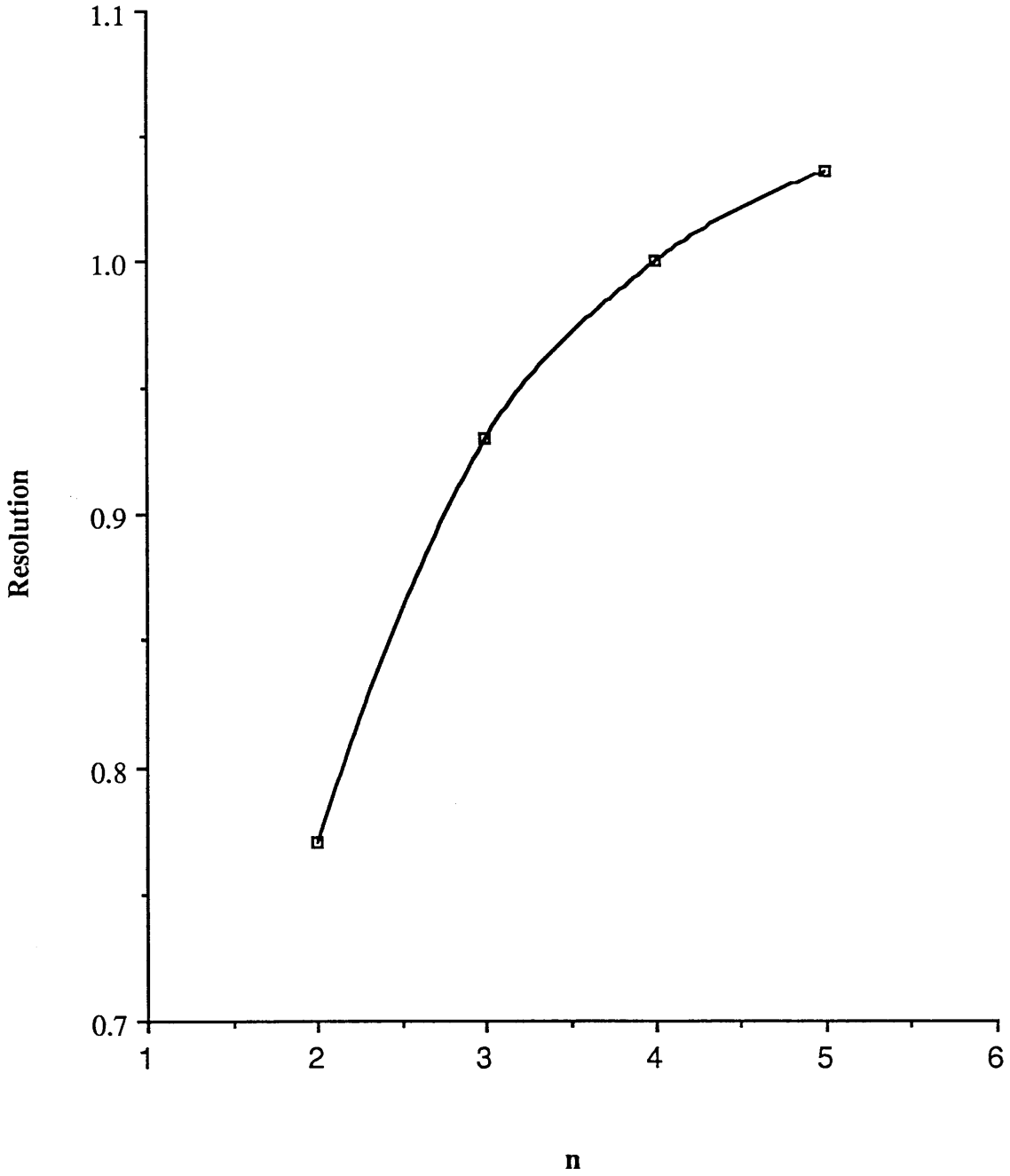
(d) α values increase slightly with increasing chain length of the secondary alcohol units and/or retention time.

(e) It would seem that the derivatives using CMEs (69) and (79) (i.e. derivatives (84) and (85)), containing 5 bonds between the chiral centres show greater separations than those using CMEs (67) and (80) (i.e. derivatives (82) and (86)), which contain 4 bonds between the chiral centres. This interesting result is discussed in more detail later in this chapter.

No clear conclusions can be drawn from the trends in the α and Rs values due to there being two variables (retention time and carbon chain length). It was decided, therefore that if the retention time for the diastereoisomers could be kept constant, then the variation of α and Rs values with the carbon chain length of the secondary alcohol units could be assessed. Thus the diastereoisomeric products (84a) and (84b) were subjected to further GC analyses. The diastereoisomers containing the 2-pentanol unit (n=2) were made to elute at ~9.8 minutes (isothermal 170°C). Three more GC runs were then performed at different isothermal temperatures to elute the other derivatives containing the 2-hexanol (n=3) to 2-octanol (n=5) units at a similar retention time to that of the 2-pentanol derivative. Table 12 summarises the results obtained for the lactone CME derivatives (84).

The results in Table 12 can be compared with those in Table 4 for the (+)-(trans)-chrysanthemoyl and N-acetyl-(S)-(-)-prolyl derivatives ((31) and (33) respectively, as discussed in Chapter 2). Likewise Graph 3 (a plot of how the resolution varies with the carbon chain length of the alcohol unit) can be compared with Graphs 1 and 2.

Graph 3. Showing how GC resolution varies with carbon chain length of the secondary alcohol derivatives (84) at a fixed retention time.



While the resolution values were lower than those for the other derivatives (see Table 4), the same trends in the separation factor (α) and resolution (Rs) values are observed, these being:

(a) The separation factor (α values) appear to be constant, or show a small increase over the range of carbon chain lengths used.

(b) The resolution (Rs) of the diastereoisomers increases with increasing carbon chain length.

The hypothesis given in Chapter 2 explaining the trends in the resolution values also applies to those observed for the lactone CME derivatives (84).

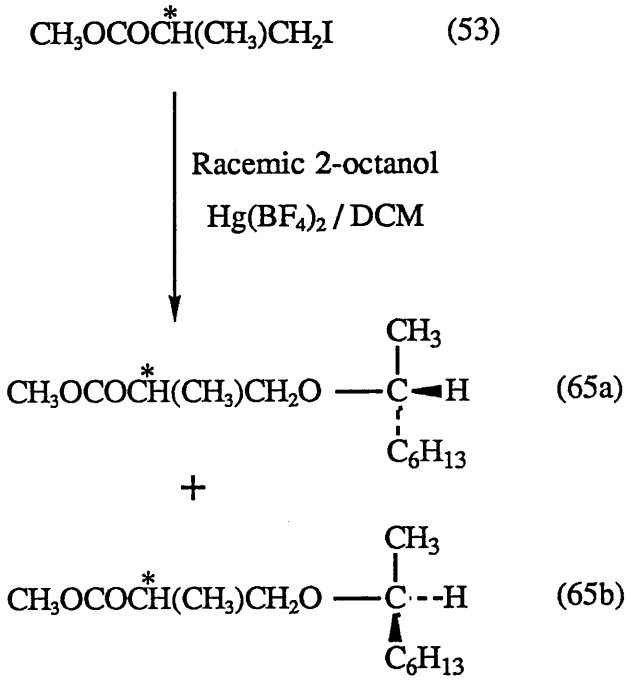
The considerably greater resolution values shown by the acid chloride derivatives (31) and (33) compared to those for the lactone CME derivatives (84) may be due to:

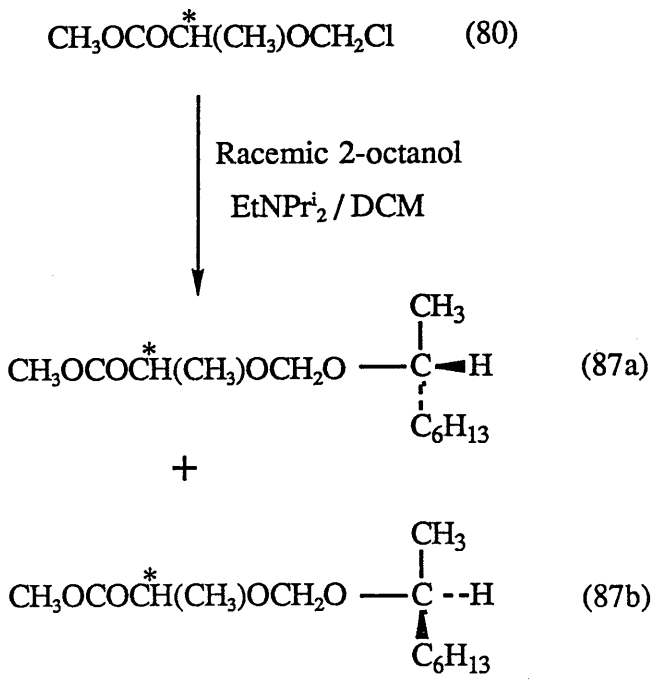
(i) The different diastereoisomers containing a different number of bonds between the chiral centres (i.e. 3 for (31a) and (31b) and 5 for (84a) and (84b)

(ii) Diastereoisomers (31) and (33) having more rigid structures (due to the 3 bond ester function) enable them to exist in well defined conformations which (along with their other functional groups and structures) are differentiated to a greater extent by the GC stationary phase.

(iii) The stationary phase and column conditions for the separation of the diastereoisomers of compound (84a) and (84b) having not yet been optimised.

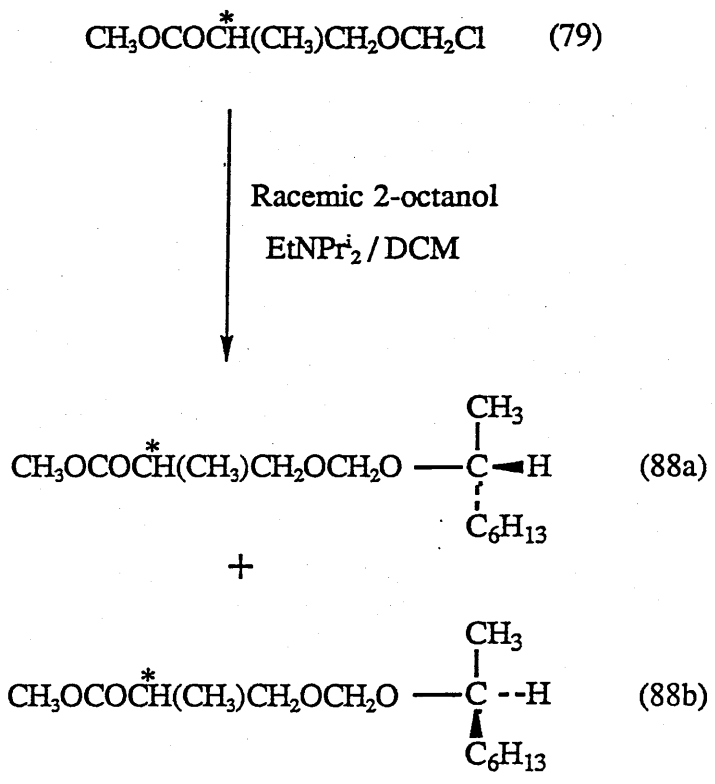
SCHEME 40





(* = chiral centre = S)

SCHEME 40b



(* = chiral centre = S)

TABLE 13 Showing the α values for diastereoisomers (65), (87) and (88) with their retention times (min, in brackets) and the isothermal column temperatures on a range of different columns

Diastereoisomers	GC columns		
	BP-1[a]	BR-5[b]	BP-20[c]
65	1.026 (18.15/18.62) 105°C	1.028 (16.28/16.73) 103°C	1.036 (18.72/19.4) 93°C
87	- (19.25) 115°C	1.012 (17.5/17.71) 113°C	- (17.38) 115°C
88	1.012 (15.86/16.06) 130°C	1.018 (16.79/17.09) 125°C	- (19.45) 123°C

- = not resolved

(a) 12.5 m x 0.32mm, 1.0μm film

(b) 12 m x 0.33mm, 0.5μm film

(c) 12 m x 0.32mm, 0.5μm film

Explanations (i) and (ii) appear to be the more likely. Optimising the column and conditions is unlikely to improve the separation of (84a) and (84b) to the extent achieved with (33a) and (33b) (as will be shown later in this chapter).

Chiral separations - Dependence on the number of bonds between the chiral centres

The differences in the separations of diastereoisomers containing different numbers of bonds between the chiral centres was investigated by preparing the derivatives (65), (87) and (88) (Scheme 40a and 40b). While derivatives (87) and (88) were prepared in the usual way, reacting CMEs (80) and (79) (respectively) with 2-octanol, derivative (65) was prepared by reacting iodide (53) with 2-octanol (see Chapter 3).

On looking at the structures of the diastereoisomers (65), (87) and (88) it is apparent that the products are very similar except for the differing number of bonds between the chiral centres. Any differences in the separation of the diastereoisomers therefore, should mainly be a result of the different number of bonds present. GC analyses of the resulting diastereoisomeric derivatives using a range of columns (BP-1 (non-polar), BP-5 and BP-20 (polar)) gave the results shown in Table 13. α Values and the retention times of the diastereoisomers (in brackets) are listed being with the isothermal temperature (in ^{o}C) used in the GC analysis.

The results indicate that:

(a) Diastereoisomers (65a) and (65b) with 3 bonds between the chiral centres show the best separations on all the columns used.

(b) The separations of diastereoisomers (65a) and (65b) appear to increase as the polarity of the columns increases.

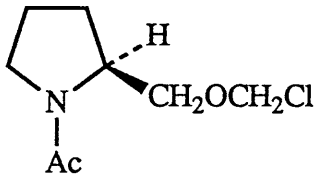
(c) The BP-5 column appears to be the most appropriate column for separations of diastereoisomers (87a) and (87b) as well as (88a) and (88b).

(d) Diastereoisomes (88a) and (88b) (containing 5 bonds between the chiral centres) are separated to a greater extent than diastereoisomers (87a) and (87b) (which contain 4 bonds between the chiral centres).

The separations of diastereoisomers (65a) and (65b) being greater than those for compounds (87) and (88) is consistent with the known phenomenon of fewer bonds between chiral centres producing greater separations.[152] This concept does not appear to explain the differences in the separations of the diastereoisomers of (87) and (88), where the diastereoisomers with 5 bonds between the chiral centres (88a) and (88b) are separated to a greater extent than those containing 4 bonds between the chiral centres. This result may be explained if it is assumed that the separations are due to interactions between the chiral centres. This fits with the theory that the fewer bonds between the chiral centres, the closer their proximity, the greater their interactions and thus the greater is their separation. If however, more distant chiral centres can be brought into close proximity by intramolecular interactions then separations will also be improved. In other words, chiral centres that are distant through bonds could acquire conformations that bring them close through space. It is likely that the chiral centres of diastereoisomers (88a) and (88b), with 5 bonds between them can approach closer in space than the chiral centres of diastereoisomers (87a) and (87b) with only 4 bonds between them. This would lead to stronger interactions in products (88) than in products (87), and hence the observed efficiencies of separation.

Molecular modelling could ascertain whether low energy conformations with intramolecular bonding, bringing the chiral centres into closer proximity actually

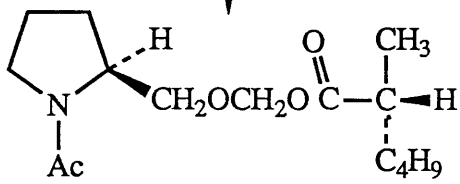
SCHEME 41



(68)

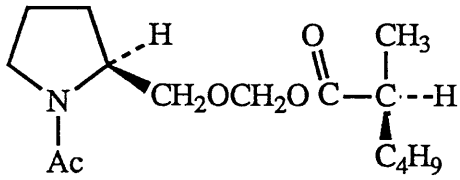
Racemic 2-methylhexanoic acid

$EtNPr^i_2$ / DCM

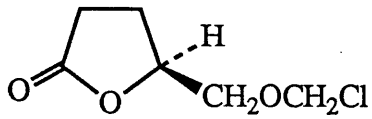


(68a)

+



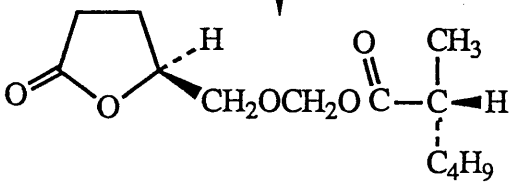
(68b)



(69)

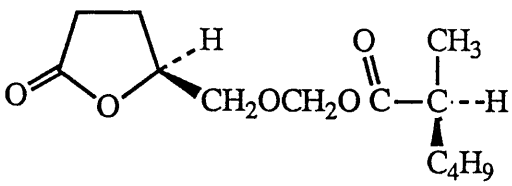
Racemic 2-methylhexanoic acid

$EtNPr^i_2$ / DCM



(89a)

+



(89b)

exist. However the relevance of any such (low energy) conformations at the high temperatures experienced in GC and in the presence of an interactive stationary phase would be unclear.

(ii) Derivatives of racemic 2-methylhexanoic acid

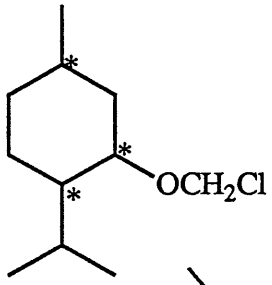
GC analyses indicated that the reaction of CMEs (68) and (69) with 2-methylhexanoic acid (e.g. Scheme 41) had in each case produced 90 to 100% yield after 5 hours. GC/MS was used to confirm the identity of the products, but separations of the resulting diastereoisomers were not observed on the columns used (BP-1 (non-polar), BP-5, CP-SIL 19 CB, and FFAP (most polar)).

The results indicate that while diastereoisomers containing an ester group with 3 bonds between the chiral centres can be separated (e.g. (+)-(trans)-chrysanthemyl esters[55,56] or N-trifluoroacetyl (or N-heptafluorobutyryl) -S-prolyl esters,[54,59,62] ones containing 5 or more bonds cannot (cf the separations of the corresponding diastereoisomeric ether products which contain 5 bonds). Again, considerable molecular modelling would be required to ascertain why chiral recognition is not taking place.

(iii) Derivatives of aniline (non-chiral)

The reaction of CMEs with tertiary amines is known to produce (involatile) quaternary ammonium salts.[199] This would obviously be undesirable for analytical techniques such as GC which requires the analyte to be volatile. In model reactions with aniline, the CME reagents (67) and (69) were thus added in quantities lower than those required to form the ammonium salts. GC and GC/MS analyses were unclear, as little or no peaks were present in the chromatograms. This may be a result of solubility problems in the solvent extraction used, with

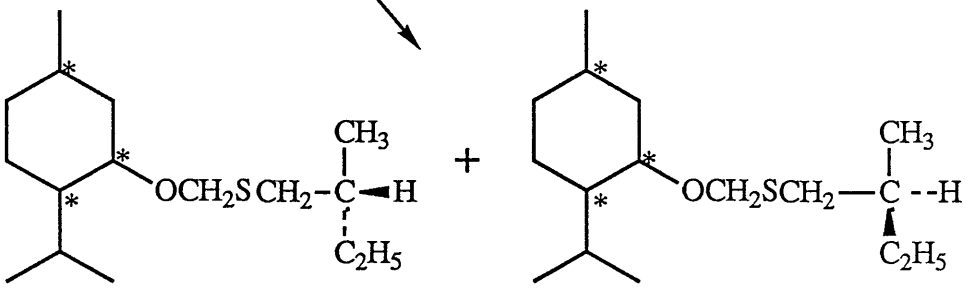
SCHEME 42



(67)

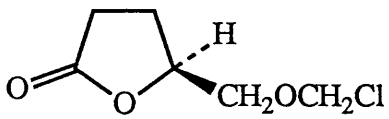
Racemic 2-methyl-1-butane thiol

$EtNPr^{i}_2$ / DCM



(90a) (90b)

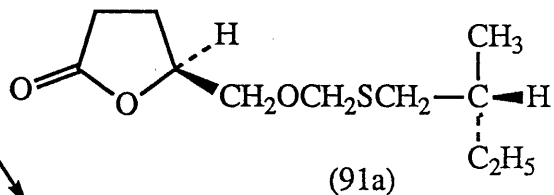
(* = Chiral centres, 1S, 2R, 5S)



(69)

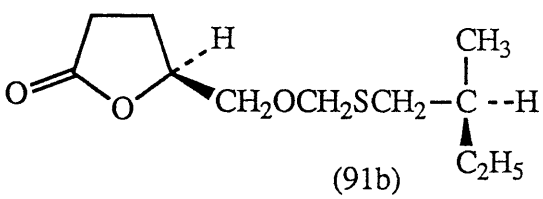
Racemic 2-methyl-1-butane thiol

$EtNPr^{i}_2$ / DCM



(91a)

+



(91b)

the amine starting material and products possibly being soluble in the water phase. This derivatization procedure does not therefore appear to be applicable for amine analytes.

(iv) Derivatives of racemic 2-methyl-1-butanethiol

GC analyses indicated that the reaction of the thiol with CMEs (67) and (69) (Scheme 42) had produced a 90-100% yield after 5 hours. The GC/MS data obtained confirmed the identity of the products (e.g. diastereoisomers (90a) and (90b)). Separation of the products on the columns used was not achieved. This could be due to:

(a) There being 6 bonds between the chiral centres of the products (91) when using lactone chloromethyl ether (69) as the chiral derivatizing agent.

(b) The menthyl chloromethyl ether reagents being inadequate for these separations (see Table 11 and 11a)

(c) There being insufficient dissimilarity of the alkyl groups in the thiol unit to enable chiral recognition of the different diastereoisomers (see discussion in Chapter 2)

(d) The optimum stationary phase having not been found

Selection of a thiol with a structure comparable to the secondary alcohols used earlier may produce separations.

(v) and (vi) Derivatives of non-racemic mixtures of R- and S-2-octanol, and of the commercially available enantiomerically pure R- and S- forms

CME (69), having produced the most promising separation results was chosen as the chiral reagent for these analyses.

GC results showing near baseline separations were obtained when reagent (69) was reacted with non-racemic mixtures of 2-octanol or the commercial (entiomerically pure) 2-octanol. By matching the main GC peak present to the main component of the non-racemic mixtures, the identities of the peaks were established (as labelled on the chromatograms C10-C13). It was found that the SR derivative (where the first symbol, here S, refers to the configuration of the reagent and the second, here R, refers to the configuration of the analyte) eluted before the SS isomer. It is interesting to note that this elution order is opposite to that obtained for the analogous derivatives containing a three-bond ether linkage between the chiral centres (i.e. diastereoisomers (64a) and (64b)). An explanation for this observation cannot be made. It may be noted that there are no hard and fast rules about elution orders, as different orders have been observed even within a homologous series.[78]

Commercial S-2-octanol, before and after spiking with small amounts of commercial R-2-octanol, was subjected to derivatization with reagent (69) and analysed by GC using a DB-1701 column. Samples containing commercial R-2-octanol, were likewise treated with the S-isomer and analysed.

The GC integration results obtained for the derivatives of the non-racemic mixtures (and the commercially 'pure' enantiomers) enabled calculation of the enantiomeric impurities present. These results are summarised in Table 14.

TABLE 14 Listing the % levels of contaminating enantiomers found in standard non-racemic mixtures of 2-octanols

R-Mixture			S-Mixture		
% S-added	% S-found	% extra	% R-added	% R-found	% extra
5.0	6.4	1.4	5.0	6.54	1.54
1.0	2.15	1.15	1.0	2.74	1.7
0.2	1.5	1.3	0.2	1.4	1.2
0.0	1.1	1.1	0.0	1.3	1.3

Separations performed using a DB-1701 CB (30m x 0.25mm, 0.2μm film) iso 180°C

Assuming that the diastereoisomers have identical responses at the FID detector, the results all indicate that there is more of the contaminating enantiomer present than expected. On average 1.24% more of the S enantiomer was present in the R mixtures, and 1.44% more of the R enantiomer was present in the S mixtures. These apparent impurities were also observed in the commercially available enantiomers. Initially, one may conclude from these results that the commercial R- and S-2-octanols are not enantiomerically pure, containing the other enantiomer at ~1% level (which is consistent with the manufacturers claim of their product being ~99% pure). However, if the chiral (S) derivatizing agent contained a small quantity (~1%) of its (R) form, this will lead to the formation of R'S and R'R diastereoisomers (R' = contaminating enantiomer of the reagent). If it is assumed that the R- and S-2-octanols are enantiomerically pure, then the diastereo-isomeric products present will be SR (~99%) and R'R (~1%) for the R-2-octanol and SS (~99%) and R'S (~1%) for the S-2-octanol (see ref. 45 and Figure 14, Section 1.7.3).

As the R'S and the SR products are enantiomers of each other they will have the same retention time on the achiral columns used. Likewise the R'R and the SS products will not be separated. The origin of the 1% impurity peak could thus be due to an impurity in the reagent, the analyte or both.[45] Analysis of the mixtures on a homochiral stationary phase should distinguish these possibilities because all four isomers would have slightly different retention times.

As the absolute enantiomeric purity of the chiral derivatizing reagent is not known and purification procedures to ensure the enantiomeric purity of the reagents were not used, then the identity of the ~1% contaminating peaks cannot be established with an achiral stationary phase. This is a known drawback to using chiral derivatizing agents as opposed to other methods (eg. use of homochiral stationary phases in GC or HPLC).[5,45,110] Derivatization of a chiral alcohol known to be

100% enantiomerically pure would verify the enantiomeric composition of the reagent, and thus confirm the source of the contaminating peak, using an achiral stationary phase.

The work described in this Chapter has shown that homochiral CMEs can:

a) be prepared conveniently and in acceptable yields from their corresponding alcohols.

b) be employed as derivatizing agents for GC analyses of chiral compounds containing alcohol, acid and thiol functions.

c) enable the quantification of enantiomeric mixtures of chiral alcohols down to 1% levels of one enantiomer in the presence of 99% of the other.

d) possibly be employed as homochiral derivating agents for NMR analyses (see Chapter 5 and 6.2)

e) produce diastereoisomers that are separable even with 5 bonds between chiral centres (the ease of resolution decreasing in the order 3 bonds>5 bonds>4 bonds between chiral centres)

The homochiral CMEs do however have several drawbacks:

(i) They are unstable and have to be synthesised from their MTM ether derivatives as required.

(ii) Other non-chiral CMEs are reported to be toxic. It may thus follow that the homochiral CMEs are also toxic and should be handled with due care.

(iii) Amines did not react satisfactorily with the CMEs and thus could not undergo GC analyses.

(iv) Diastereoisomeric derivatives of acids and thiols (as studied) were not separated to the same extent as the alcohol derivatives. This may be due to an increased number of bonds between the chiral centres of the derivatives in the case of the acids, and use of an inappropriate example of a thiol.

(v) Different homochiral CMEs produced diastereoisomers which were separated to different extents. Some produced little or no separation (e.g. when the proline or menthyl CMEs were used) in comparison to others (e.g. when the lactone CME (69) was used).

(vi) As with other homochiral derivatizing agents, their enantiomeric purity must be ensured. Results obtained from their diastereoisomeric peak areas in their chromatograms may otherwise be incorrect.

Other work described in this Chapter has shown how the distance between chiral centres (i.e. the number of bonds) and the type of linkage (ether or ester) are of great importance in the separation of diastereoisomeric derivatives. Some unusual results have been observed where the magnitude of separation (i.e. their Rs and α values) has differed from expected results i.e. the order of increased separation which was expected to be 3>4>5 (numbers = number of bonds between the chiral centres) was found to be 3>4<5. Generally, an ester group between the chiral centres prevented resolution while analogous ether linkages permitted separation.

The preparation of MTM ethers formed a crucial part of the synthetic work of the CMEs. The novel method developed to synthesise MTM ethers was thus considered in more detail (see Chapter 5).

5 SYNTHESIS OF NON-CHIRAL METHYL THIOMETHYL ETHERS

The novel method for the preparation of MTM ethers had proved useful in the synthesis of the homochiral CME reagents, where acid- or base-sensitive functions were present. The promising results obtained (albeit with low yields) prompted further investigation into the usefulness of this reaction.

The novel method was used to prepare a range of non-chiral MTM ethers (92)-(96) (Scheme 43) in order to compare results with those obtained by Corey[199] and Undheim.[200] The synthetic usefulness of the reaction could then be assessed by comparison of yields, ease of reaction and applications.

The novel reaction was originally carried out using bench chloroform as a reagent. As the reactions proceeded, two by-products were formed, one having a lower boiling point than the MTM ether and the other a higher. The reactions were thus allowed to proceed for approximately 3 hours until the optimum yield of desired product with respect to the by-products and the starting alcohol was obtained (typically 60% by GC). Eventual yields were found to be low due to:

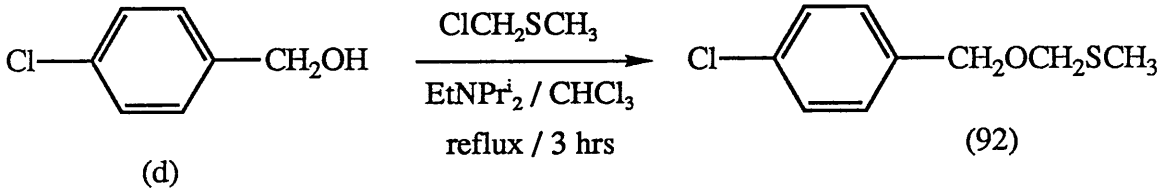
(i) Difficulties in removal of the lower boiling point by-product.

(ii) Decomposition during distillation in some cases.

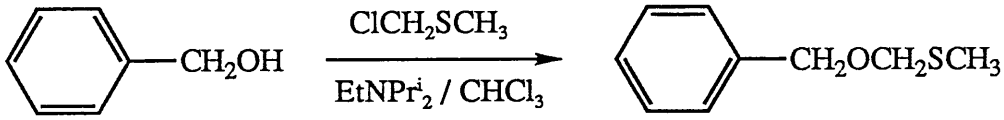
(iii) Substantial formation of the higher boiling point by-product.

The lower boiling point by-products were found to be the ethoxymethyl ethers (e.g. (97) produced from (93), as identified by its mass spectrum and proton NMR). These were formed by nucleophilic attack on their MTM-ethers by ethanol, with loss of methane thiol (see Section 4.3).

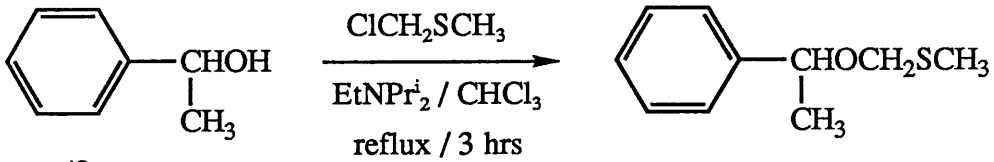
SCHEME 43



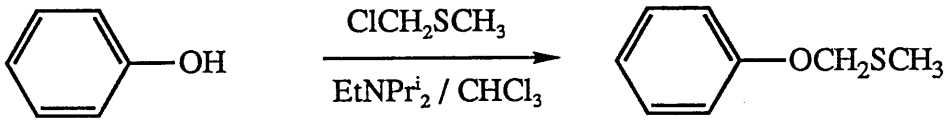
(d) (92)



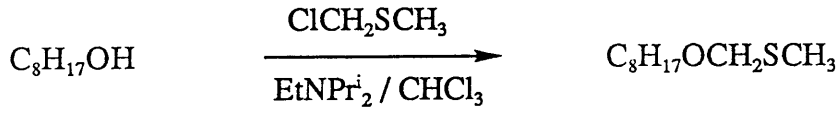
(e) (93)



(f) (94)



(g) (95)



(h) (96)

TABLE 15

Compound	Yield Method A[a]	Yield Method B[b]	Undheim[200]
92	33	47	55
93	51	42	56
94	32	40	61
95	28.5[c]	16[c]	-
96	39	-	-

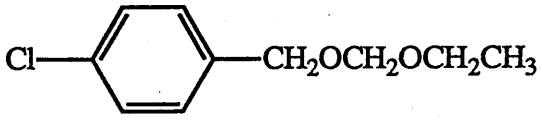
- = Synthesis not performed

(a) Using bench chloroform (containing 1-2% ethanol)

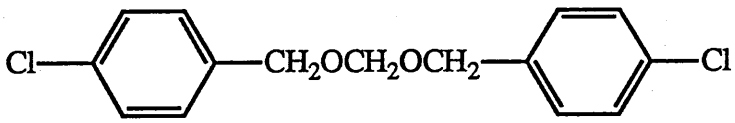
(b) Using ethanol-free chloroform

(c) Decomposition of product on distillation

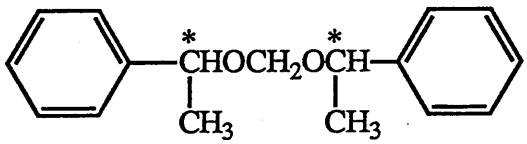
The higher boiling point by-products were found to be dialkyl diether compounds (e.g.(98) and (99) formed from (92) and (94) respectively). These were formed by nucleophilic attack on their MTM-ethers by the chiral alcohols (starting materials), with loss of methane thiol (see discussion 3.2). The dialkyl diether by-product (99) has two chiral centres allowing diastereoisomeric forms to exist. This is observed in the NMR spectra (see NMR 1 and NMR 2, Chapter 6), where the diastereoisomers have different shifts. This is discussed in more detail in Chapter 6.



(97)



(98)



(99)

(*= chiral centre, R and S)

The reactions were repeated using ethanol-free chloroform,[211] thus preventing the formation of the ethoxy methyl ether by-products. After 7-8 hours reaction, little or no alcohol remained in the reaction mixtures. The products were thus easier to purify by distillation than previously, as there were no lower boiling point components to remove.

Table 15 gives the percentage yields obtained for the novel reaction when using bench chloroform (reacting for 3 hours), ethanol free-chloroform (reacting for 7 hours), and those reported by Undheim.[200]

The results show that the use of ethanol free-chloroform does improve yields generally, but not by a large amount. This was due to higher yields of the dialkyl diether by-products achieved in the 7 hour reaction. Yields of the MTM-ethers may be increased by reducing the reaction times to optimise their yields. This may then produce yields greater than those achieved by Undheim.

While the Undheim method, using sodium hydride as the base for the reaction has been found to produce acceptable yields of MTM-ethers from simple aromatic alcohols, it has been found to be unsuitable for alcohols which also contain base-sensitive functions such as esters[209] (see Section 4.3). The novel method, although not achieving the yields as reported by Undheim, is performed under mild conditions (suitable for base-sensitive reagents) and has thus proved to be more generally applicable.

6 HOMOCHIRAL NMR SHIFT REAGENTS

The different approaches for the determination of enantiomeric composition using NMR techniques were discussed in Chapter 1. Procedures based on two of the approaches have been investigated.

6.1 NMR-cyclodextrin inclusion complexes

As already discussed (Chapter 1), cyclodextrins (CDs) and their derivatives have been employed as homochiral stationary phases for the HPLC analyses of a wide range of enantiomeric compounds.[86,118-122,124,125] They have also been used as homochiral mobile phase additives for LC systems.[117,137]

Due to the high melting point of CDs they are only suitable as stationary phases for gas-solid chromatography.[100] The use however, of formamide as a matrix medium for the CDs has enabled their use as GLC stationary phases. Good enantioseparations have been reported with such phases.[99,102]

Derivatives of CDs have also been utilised as homochiral GLC stationary phases (having relatively low melting points but high boiling points). Peralkylated or acetylated derivatives in particular have proved popular for enantioseparations of a wide range of chiral compounds.[100,101,212,213]

The geometry of the CD molecules can be represented as a hollow, truncated cone. The primary and secondary alcohol groups of the constituent glucose units are located on the edges of the wider and narrower openings of the cone respectively, the interior of the cavity being hydrophobic. A separation is achieved when the enantiomers or any other analyte molecules

differ in their ability to be accommodated in the cavity of the CD molecules. It has also been reported that in addition to the hydrophobic binding site, successful resolution requires polar interactions of the secondary alcohol groups of the β-CD with substituents on the analyte.[86,120] This is not always necessary, as shown by the successful separation of the enantiomer of α-pinene on unmodified CD phases.[99,102] The successful use of peralkylated CD phases also indicates that the polar interactions are not mandatory. Steric factors may be important in these cases.

The size of the CD cavity is also considered important as a snug fit of the non-polar part of the analyte in the cavity is required to enable diastereoisomeric complexation to occur. A study into the structural basis for the enantiomeric resolutions when using CD mobile phases recently incorporated the use of ^{1}H NMR techniques. Differences in the shifts of the two enantiomeric forms of pseudoephedrine were observed.[214] Another paper also describes the use of various asymmetric compounds for the formation of host guest complexes enabling the ^{1}H NMR quantification of enantiomeric composition.[215] Investigations into whether CDs could be employed to form diastereoisomeric inclusion complexes quantitatively with compounds containing non-polar ring moieties were undertaken. NMR analyses were used in an attempt to observe any differences between the spectra of enantiomeric molecules.

Free CDs are only sparingly soluble in water and DMSO. They are insoluble in most of the other commonly used NMR solvents. Modified cyclodextrins which would be more soluble in organic solvents were thus investigated.

SCHEME 44

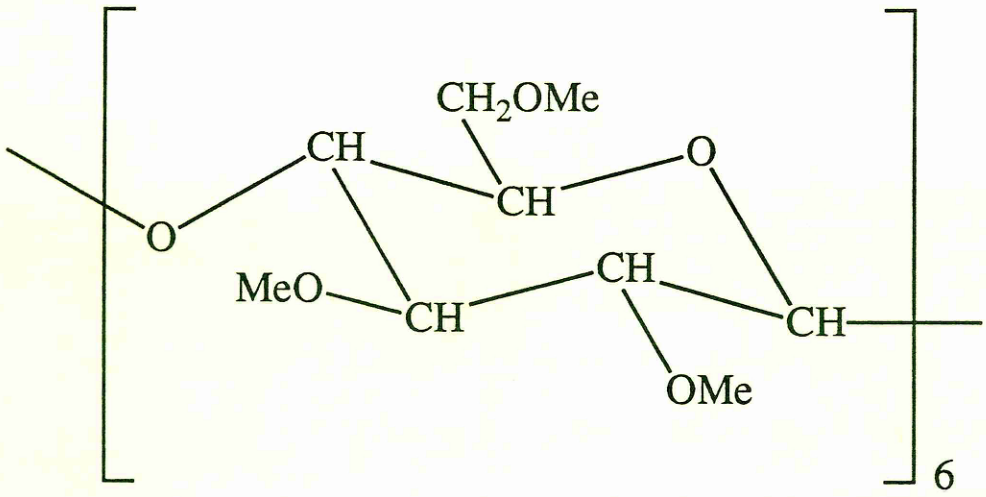
α-Cyclodextrin	KOH/DMSO	Permethylated

(18 -OH groups)	MeI	α-cyclodextrin (100)

β-Cyclodextrin	KOH/DMSO	Permethylated

(21 -OH groups)	MeI	β-cyclodextrin (101)

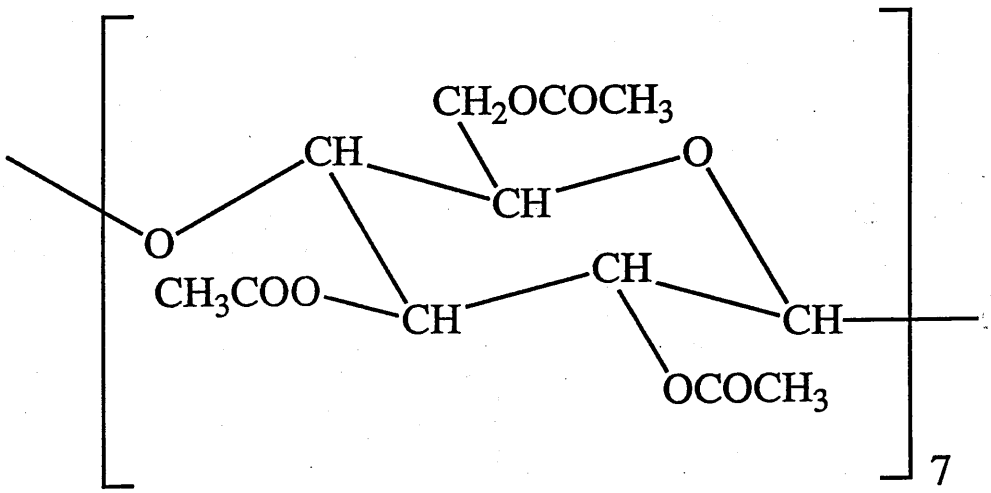
Permethylated α-Cyclodextrin (100).



SCHEME 45

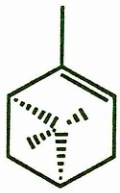
β-Cyclodextrin $\xrightarrow[CH_3COCl]{}$ Acetylated β-cyclodextrin (102)

Acetylated β-Cyclodextrin (102).



SCHEME 46

(R)-(+)-α-Pinene.

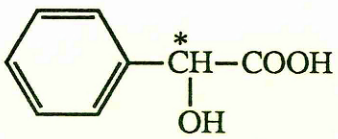


(S)-(-)-α-Pinene.

α-pinene + Permethylated β-cyclodextrin $\underset{CD_3OD}{\rightleftharpoons}$ α-Pinene/permethylated β-cyclodextrin inclusion complex

α-pinene + Permethylated β-cyclodextrin $\underset{CD_3OD}{\rightleftharpoons}$ α-Pinene/permethylated β-cyclodextrin inclusion complex

SCHEME 47



Mandelic acid (*= chiral centre)

Mandelic acid + permethylated β-cyclodextrin $\underset{CDCl_3}{\rightleftharpoons}$ Mandelic acid/ permethylated β-cyclodextrin inclusion complex

Permethylated α- and β- CDs as well as acetylated β-CD were prepared (Schemes 44 and 45). The permethylated CDs, lacking hydroxyl functions, are readily soluble in deuteriated methanol and chloroform. The reduction in their polarity around the opening of the cavity prevents any possible interactions with any polar groups an analyte may contain, and may in fact hinder the formation of diastereoisomeric complexes. The initial analyte chosen for analysis was thus the hydrocarbon α-pinene (Scheme 46). It was hoped that non-polar (hydrophobic) interactions would produce the desired diastereoisomeric complexation.

The ^{1}H NMR spectrum of permethylated α- and β- CDs have signals in the 2.8-4.2 δ and 4.85-5.1 δ regions. The rest of the spectrum is clear and hence useful for observing any signals resulting from the complexed analyte. When pinene is mixed with permethylated CDs in a ratio of 1:2 (pinene: CD), the spectra of both enantiomerically pure (S)-(-)-α-pinene and of the racemic mixture of pinene contain two unsplit methyl signals at 0.85 δ and 1.25 δ. Had the desired diastereoisomeric complexation occurred, then each of these signals in the racemate should have resulted in two peaks (the integration of which would have been used to determine the enantiomeric composition). The fact that this was not observed indicated that an inclusion complex has not been formed, or if it had, that sufficient diastereoisomeric interactions were not occurring for resolution by the 90 MHz instrument available.

In another experiment, an enantiomerically pure sample and the racemate of mandelic acid were employed as the analyte (Scheme 47). Again, the number of analyte signals remained the same inferring that sufficient diastereoisomeric complexation had not occured.

The ^{13}C NMR spectra for the α-pinene and the mandelic acid complexation experiments also showed no increase in the number signals for the analytes in the presence of methylated CDs.

The acetylated β-CD is readily soluble in deuteriated methanol and chloroform. It still has polar functions around the opening of the hydrophobic cavity and may thus be more suitable for the formation of diastereoisomeric inclusion complexes with mandelic acid. In both the ^{13}C NMR and ^{1}H NMR spectra of racemic mandelic acid, mixed with the β-CD derivative, signals from the acetylated β-CD and the mandelic acid were observed. The signals for the mandelic acid were not changed. This suggests that if complexation is in fact occurring then diastereoisomeric interactions are not. The use of acetylated α-CD may prove more successful as the aromatic ring may then be included in the cyclodextrin cavity in a more fixed position (i.e. the α-CD cavity may be a more suitable size).

The use of underivatized CDs in deuteriated water or DMSO for the complexation of various analytes should be investigated. This would reduce the size of the signals corresponding to the CD molecule (compared with those for the modified CDs) enabling easier observation of the analyte signals. The more polar solvent environment may also promote complexation as the aromatic or hydrocarbon rings will more preferably be included within the hydrophobic CD cavity.

Further investigations into the use of acetylated CDs are required. If trifluoroacetyl derivatives were employed the ^{1}H NMR spectra would be greatly simplified. The use of higher field nmr instrumentation would also improve (or bring about) resolutions.

It is clear from the results obtained that there are many difficulties associated with the employment of CDs as homochiral complexation reagents for NMR analyses, these being:

a) Very large quantities of CDs are required for complexation. This is due to their having high relative molecular masses and because the CDs are added in excess to try to encourage complexation. The quantity used is dependent on the solvent system (i.e. on how soluble the CD is in a particular solvent) and the type of cyclodextrin (i.e. whether it is free or modified) being used. This may limit the applications of the technique as the quantity of analyte undergoing analysis will be limited, which will thus reduce the sensitivity.

b) The NMR signals resulting from the CD reagent may obscure some of the analyte signals that may be of interest.

c) Analyses are dependent on at least part of the analyte being included in the hydrophobic cavity of the CD, and that the fit within the cavity should hold the included part (usually an aromatic or hydrocarbon ring) in a fixed orientation. Thus the correct CD (α, β or γ) should be chosen for the ring system to be included.

d) The sensitivity of the technique is very limited, and quantification of the enantiomeric composition using the integration results would be subject to considerable errors.

6.2 NMR - homochiral derivatizing agents

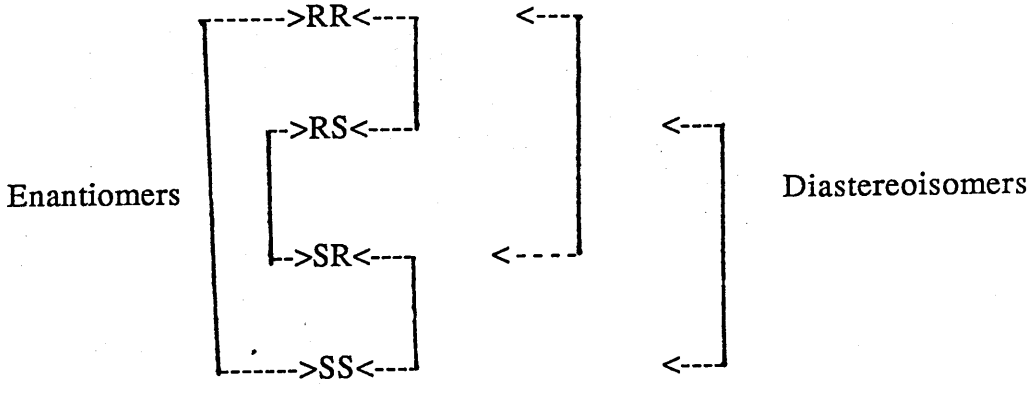
As already discussed (Chapter 1), homochiral derivatization can produce diastereoisomers which have differences in their NMR spectra.

Enantiomeric composition of the original mixture can be determined by using the integration results for the diastereoisomers. Again this initially requires the use of diastereoisomers of known configuration in order to assign sets of signals to individual diastereoisomers.

While a homochiral derivatizing agent was not prepared, diastereoisomeric resolution was observed in the spectrum of the by-product (99) from the reaction of sec-phenylethyl alcohol with chloromethyl methyl sulphide (see Chapter 5).

Observation of chiral resolution in an NMR spectrum

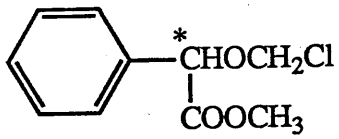
On looking at the proton NMR spectrum for the dialkyl diether product (99) (see NMR 1) twice the number of signals than expected are observed. This is also apparent in the ^{13}C NMR spectrum (see NMR 2). Noting that the sec-phenylethyl alcohol used was a racemic mixture and hence that the dialkyl diether by-products will be diastereoisomeric, the different signals were then assigned to the different diastereoisomeric combinations present. The following combination of products will exist in equal quantities:



While both the RR and SS and the RS and SR forms are enantiomeric and are not differentiated, two sets of diastereoisomers exist, and these can be differentiated. Thus, one set of signals seen in the ^{1}H NMR spectrum (see NMR 1) is due to the RR and SS enantiomers and the other is due to the RS and SR enantiomers.

This result is encouraging for the use of homochiral chloromethyl ethers as chiral NMR reagents. If for example, sec-phenylethyl alcohol was obtained in an enantiomerically pure form (e.g. the R enantiomer) and its chloromethyl ether was prepared, it could then be reacted with other chiral alcohols (to form dialkyl diethers) to quantify their enantiomeric composition by nmr spectroscopy. This would be demonstrated by reacting the homochiral chloromethyl ether (derived from enantiomerically pure (R)-sec-phenylethyl alcohol) with racemic sec-phenylethyl alcohol. The resulting product would be the dialkyl diether (99), which would have an identical NMR spectrum to that already obtained, but in this case one set of signals would be due to protons from the RR diastereoisomer, the other from the RS diastereoisomer. This could also be used for the quantification of non-racemic mixtures (as the signals are well separated in the NMR spectra). As already stated, differences also occurred in the ^{13}C NMR spectrum of compound (99) (NMR 2) which could also be used in the determination of the enantiomeric composition of the alcohol.

Another possibility would be to synthesise the optically active chloromethyl ether (103) from the readily available (+)-mandelic acid (via its methyl ester). Use of this reagent to derivatize racemic sec-phenylethyl alcohol is likely produce similar differences between the signals of its



(103)

(*= chiral centre, R or S)

diastereoisomeric products to those observed with compound (99), enabling the determination of enantiomeric composition of the alcohol.

The use of homochiral chloromethyl ethers for this kind of NMR work opens up a whole new area of research and requires further investigation.

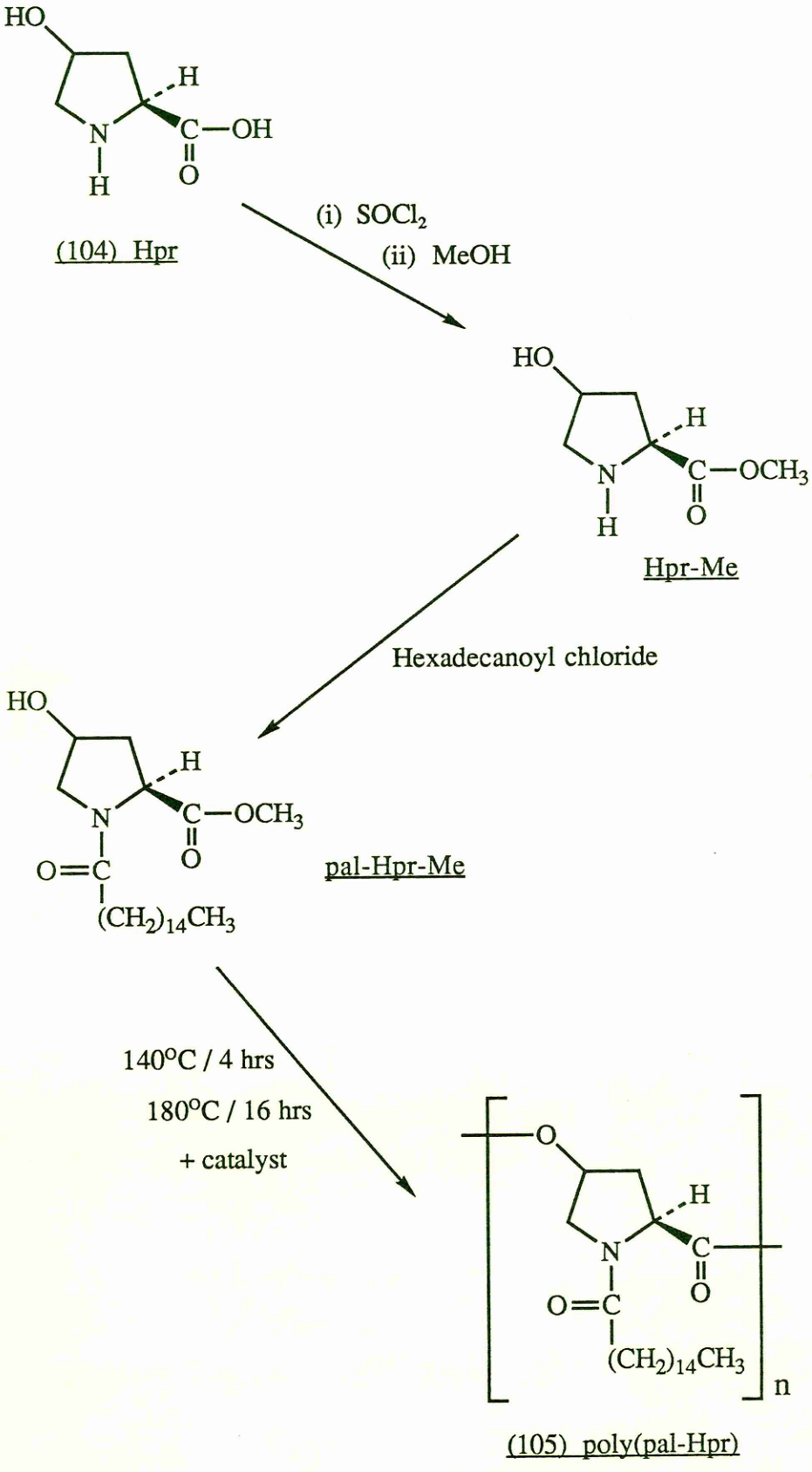
7 HOMOCHIRAL POLYMERIC MATERIALS FOR GAS CHROMATOGRAPHIC STATIONARY PHASES

Many different homochiral materials have been prepared for use as homochiral GC stationary phases (see Section 1.7.4). The lower molecular weight N-TFA-amino acid (23), N-TFA-dipeptide (24) and diamide phases (25) tend to have low thermal stability or are volatile at typical GC operating temperatures. The upper temperature limit for columns prepared using such phases is usually less than 130°C (drastically limiting their application). The introduction of Chirasil Val columns in the late 1970s enabled separations to be performed at temperatures up to 220°C. While Chirasil Val columns show excellent enantioselectivity to a wide range of compounds, the upper temperature limit is still restrictive.

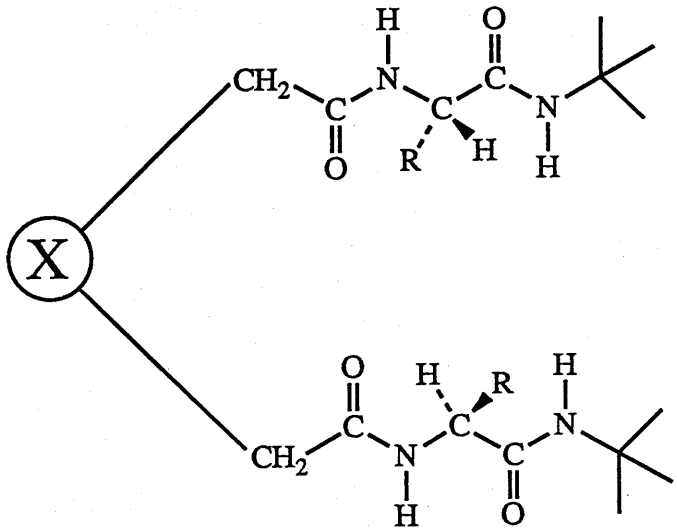
Most of the homochiral polymeric materials employed as stationary phases to date have utilised achiral polymer backbones (such as alkyl polysiloxanes) which have been modified by homochiral substitution (usually with amino acid residues). The use of synthetic polymeric materials containing amino acid residues within the polymer backbone have received little attention for use as homochiral stationary phases.

Poly[(1-palmitoyl-4,(S)-2-pyrrolidinediyl)carbonyloxy] (105) has been prepared from trans-4-hydroxy-(S)-proline (104) (Scheme 48).[216] This polyester (Mn = 8450; Mw = 15500) had a melting range of 95-110°C and was found to be thermally stable up to 320°C. As such it may be suitable as a homochiral stationary phase for GC. This has not been investigated.

SCHEME 48


HO
H
N
H
C—OH
O
(104) Hpr
(i) $SOCl_2$
(ii) MeOH
HO
H
N
H
C—OCH_3
O
Hpr-Me
Hexadecanoyl chloride
HO
H
N
C—OCH_3
O
O=C
$(CH_2)_{14}CH_3$
pal-Hpr-Me
140°C / 4 hrs
180°C / 16 hrs
+ catalyst
O
H
N
C
O
O=C
$(CH_2)_{14}CH_3$
n
(105) poly(pal-Hpr)

Oligo-oxaalkanoyl tetraamides (106) derived from L-phenylalanine have been prepared.[217] The resolution of amino acid n-butyl esters was found to be greatest when there are 14 bonds between the chiral centres, (i.e. when n=2).



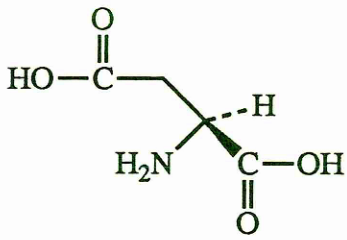
(106)

R = $-CH_2C_6H_5$

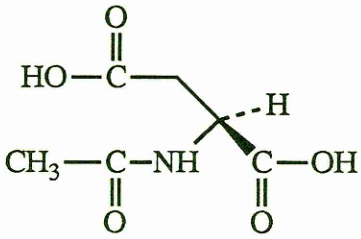
X = -O-, $-OCH_2CH_2O-$ or $-O(CH_2CH_2O)_n-$ (where n = 2 or 3)

The ability to vary the distance between the chiral centres (by using ethylene glycols of different molecular weights) is appealing as the different materials may then be applicable to different enantiomeric separations. It is interesting to note that 26 bonds exist between the chiral centres of Chirasil Val.

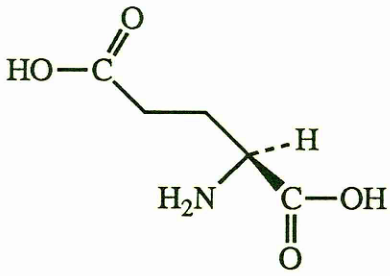
Amino acid containing polyester materials such as poly[(1-palmitoyl-4,L-2-pyrrolidinediyl)carbonyloxy] (105) have not been investigated extensively for use as homochiral GC stationary phases. This may be due to the chiral centres being separated by only 5 bonds.



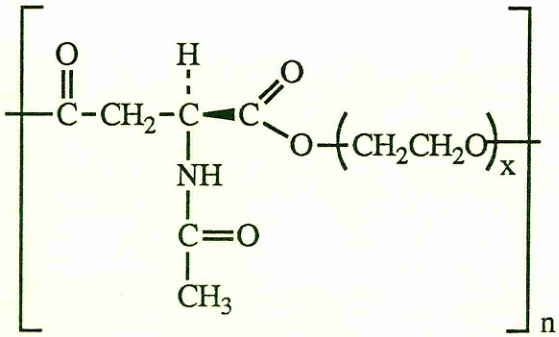
(107) (S)-(+)-Aspartic acid



(107a) N-acetyl-(S)-(-)-Aspartic acid



(108) (S)-(+)-Glutamic acid



(109) Polyester

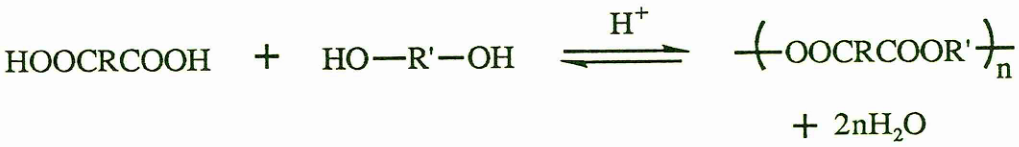
One route to the preparation of homochiral (amino acid containing) polyester materials with the chiral centres sufficiently separated would be to copolymerise a homochiral diacid with an achiral diol. Suitable readily available amino acids which contain two acid functions include (S)-(+)-aspartic acid (107) and (S)-(+)-glutamic acid (108). Use of their N-acetylated derivatives (eg. (107a)) would prevent polyamide formation.

Diols such as n-ethylene (or polyethylene) glycols are also readily available and the fact that their higher analogues (eg. PEG-20000) have already been employed as achiral GC stationary phases makes them all the more attractive as monomers for the copolymerisation.

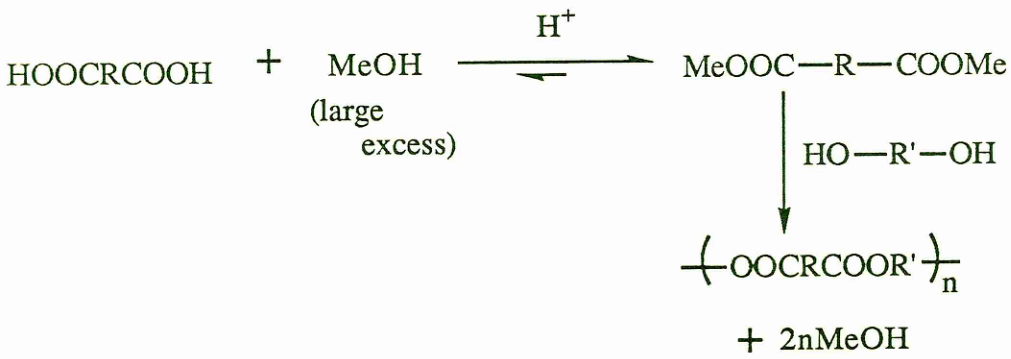
The polyester (109) formed by the reaction of N-Ac-(S)-Asp, and diol (as discussed above) would have several features that would assist in its ability to distinguish between (and thus separate) two enantiomers. In each repeating unit there are 4 sites that can undergo hydrogen bonding interactions with an analyte (ie. the two ester carbonyls and the amide carbonyl are hydrogen bond acceptors and the amide proton is a hydrogen bond donor). The possibility of substituting the acetyl group for other acyl units like a benzoyl group would introduce the possibility for steric interactions to play an influence in separations. The value of x could be varied easily by selecting an appropriate polyethylene glycol starting material.

SCHEME 49

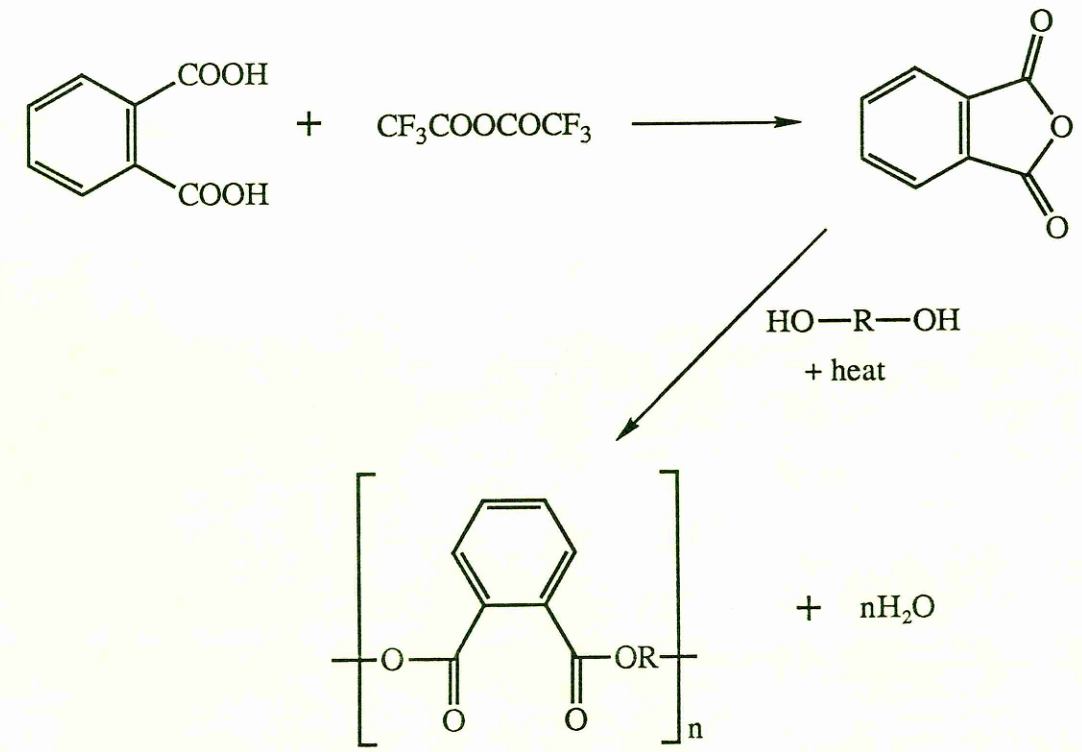
ROUTE A



ROUTE B



ROUTE C



7.1 Synthesis of polyesters

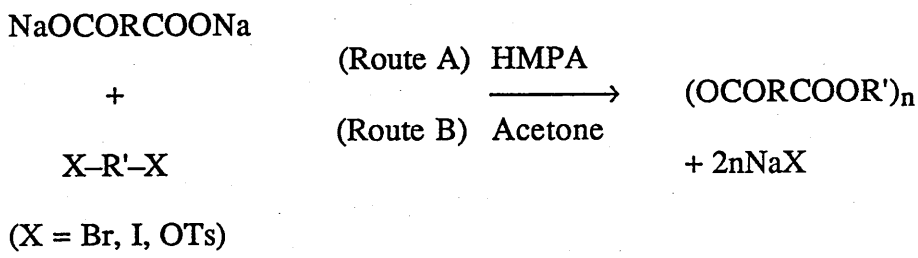
Polyesters have generally been synthesised from diacids and diols following three main routes (Scheme 49, Routes A-C). The routes all involve condensation reactions, where the equibrium is pushed in favour of the products by heating the reactions to up to 200°C. At this temperature the by-products are distilled off. Acid catalysis is also commonly employed (e.g. using H_2SO_4 or HCl). Zinc acetate, stannous 2-ethylhexanoate, tetraisopropyl orthotitanate and aluminium isopropoxide are also reported to catalyse the condensation reaction.[216]

The reaction utilising dimethyl diesters (route B) proceedes more favourably than that when the corresponding acid is used (route A) due to the methanol by-product being more volatile than water and thus easier to remove. Polymerisations employing succinic anhydride derivatives (e.g. phthalic anhydride) also proceed (route C) more favourably because the first ester bond is formed without yielding a by-product.[218] The formation of the other ester bond again gives yield to water as a by-product. Other possible routes to polyesters exist, but are not commonly used or have not been investigated.

Alkyl halides are known to react with sodium carboxylates in solvents such as HMPA.[167,170] Thus, a disodium dicarboxylate if reacted with an alkyl dibromide or iodide (synthesised from a diol) in HMPA should produce a polyester (Scheme 50 (Route A)). Ditosylates may also be of use for this reaction. Polymerisations employing this type of reaction have not been reported in literature.

The reaction of alkyl halides with sodium alkoxides in acetone is known.[181] A similar reaction employing disodium dicarboxylates to react with dihalocompounds should yield the desired polyester (Scheme 50 (Route B)).

SCHEME 50

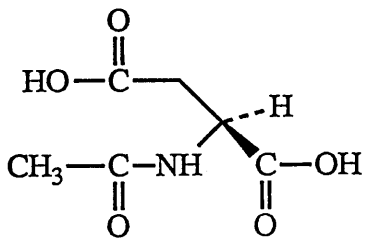


Investigations into the use of such reactions for the formation of homochiral polyesters were undertaken.

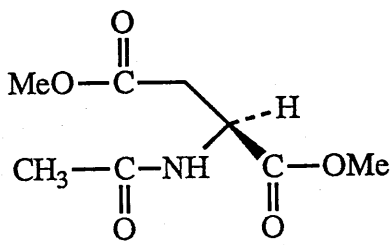
7.2 Synthesis of monomeric materials

(S)-(+)-Aspartic acid derivatives were synthesised and employed as the diacid monomers.

N-Acetyl-(S)-(-)-aspartic acid (107a) was obtained commercially and used for the direct polymerisations with diols. Also, its dimethyl ester N-Ac-(S)-(+)-Asp-DME (110), was synthesised in good yields (84%) by the acid-catalysed esterification reaction of diacid (107a) with methanol (in a large excess). The analytical data for (110) was consistent with the desired product.



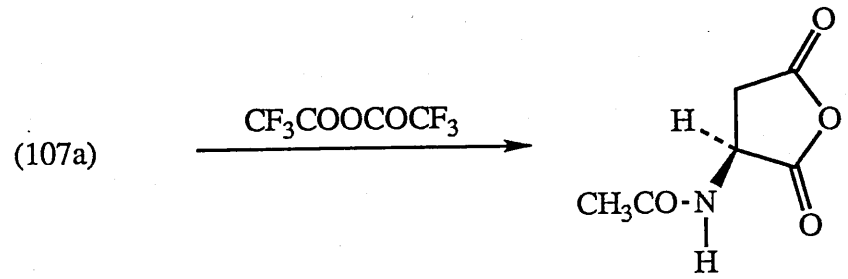
(107a)



(110)

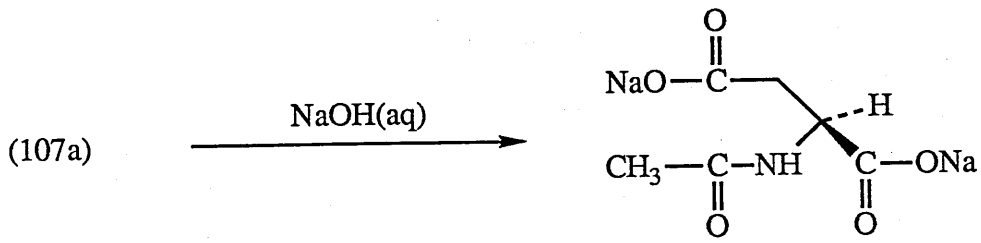
The (S)-2-acetamido succinic anhydride (111) monomer was conveniently prepared in good yield (97%) by the reaction of N-Ac-Asp (107a) with trifluoroacetic anhydride (Scheme 51). The analytical data for the product indicated that (111) had been formed.

SCHEME 51



(111)

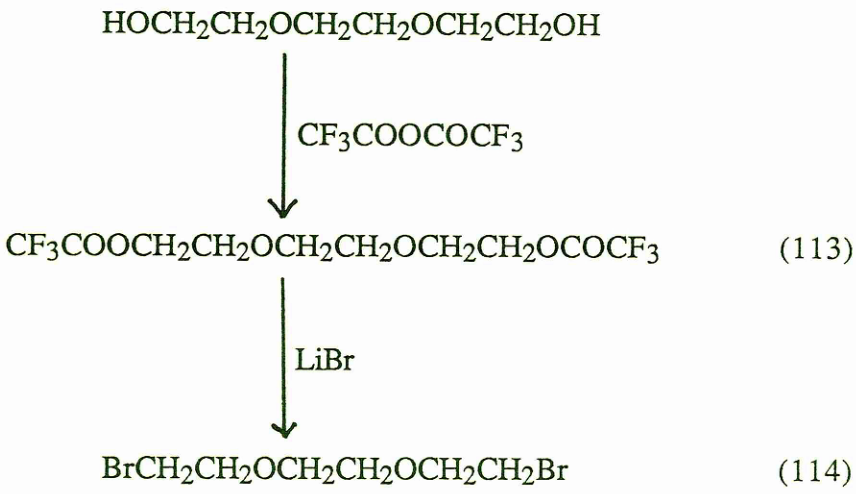
SCHEME 52



(112)

Disodium N-acetyl-(S)-aspartamate (112) was prepared by the reaction of diacid (107a) with sodium hydroxide solution (Scheme 52). On removal of the water, a white solid remained in 109% yield. Infrared analysis of the product showed a loss of the OH_{STR} absorbance resulting from the acid function. However a OH_{STR} signal was present, and indicated that water of crystallisation may be responsible both for the IR signal and the high yield.

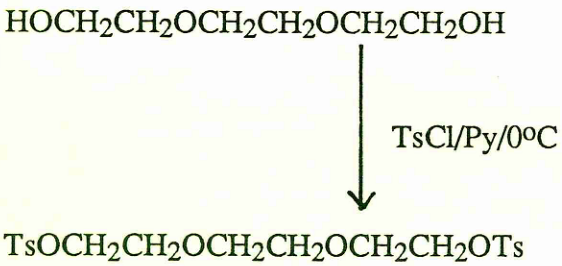
SCHEME 53



(113)

(114)

SCHEME 54



(115)

The various n-ethylene glycol monomers were readily available commercial samples. Triethylene glycol (TRIGOL) and polyethylene glycol-400 (PEG-400) were chosen for investigation. Polymerisations with PEG-400 would on average result in 30-33 bonds between the chiral centres of the product.

The 1,2-bis(2-bromoethoxy)ethane monomer (114) was prepared in acceptable yield (46%) from triethylene glycol (Scheme 53) The first step involved the synthesis of its di-trifluoroacetyl derivative (113). Substitution of the trifluoroacetyl groups with bromide ions gave the desired bromide.[183] The I.R. and NMR results were consistent with the correct product.

1,2 Bis(2-tosylethoxy)ethane (115) was synthesised from triethylene glycol[163] in acceptable yields (27%) (Scheme 54). The low cost of the starting materials enable this reaction to be carried out on a large scale if desired. The analytical data for (115) were consistent with the desired product.

7.3 Polymerisation reactions

All the polymerisation reactions discussed in Section 7.1 were attempted except for that involving the use of the triphenyl phosphine reagent. In all cases, the ratio of the two monomers was precisely 1:1 in order to achieve as higher relative molecular mass as possible.

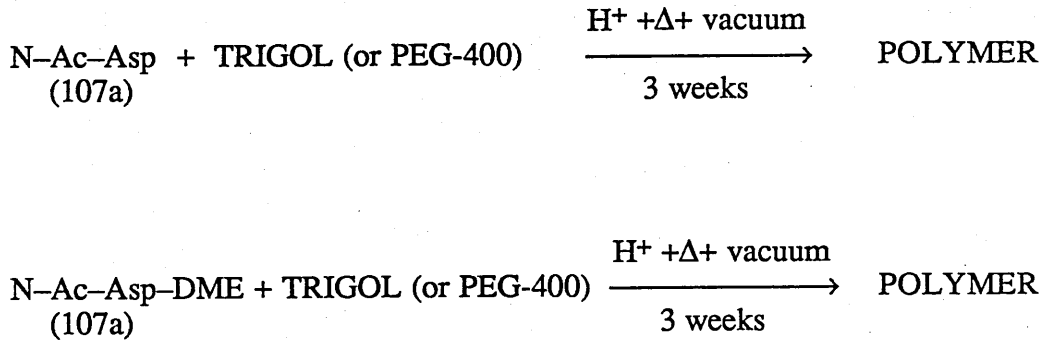
The acid catalysed polyesterifications of N-acetyl-(S)-(-)-aspartic acid (107a) or its dimethyl ester, N-ac-(S)-(+)-Asp-DME (110) with TRIGOL or PEG-400 were carried out by mixing the components (plus 2 drops of conc. H_2SO_4) then heating the reactions while applying a vacuum (<1 mmHg) to remove the water or methanol by-products (Scheme 55). The temperature to which the reactions were heated was limited by the volatility of the TRIGOL and to a

TABLE 16 Acid-catalysed polymerisation results

Compound	Molecular Weight (AV)		Optical Rotation
	^{13}C NMR	VPO	
N-Ac-Asp	na	na	-9^0
N-Ac-Asp-DME	na	na	$+67^0$
Polymer (TRIGOL +N-Ac-Asp-DME)	658	-	-5.4
Polymer (TRIGOL +N-Ac-Asp-DME)	965	-	-5.0
Polymer (TRIGOL +N-Ac-Asp-DME)	1800	-	-9.3
Polymer (TRIGOL +N-Ac-Asp-DME)	2135	1865	-
Polymer (PEG-400 +N-Ac-Asp-DME	-	2089	-
Polymer (TRIGOL +N-Ac-Asp	754	-	-
Polymer (PEG.400 +N-Ac-Asp	-	1458	-
PEG 1025	na	1477	na
PEG 2000	na	1117	na

na = not applicable
- = analysis not performed
= measurements were not made due to the high colouration of the products, or due to the quantities of the sample being too small to obtain usable readings.

SCHEME 55

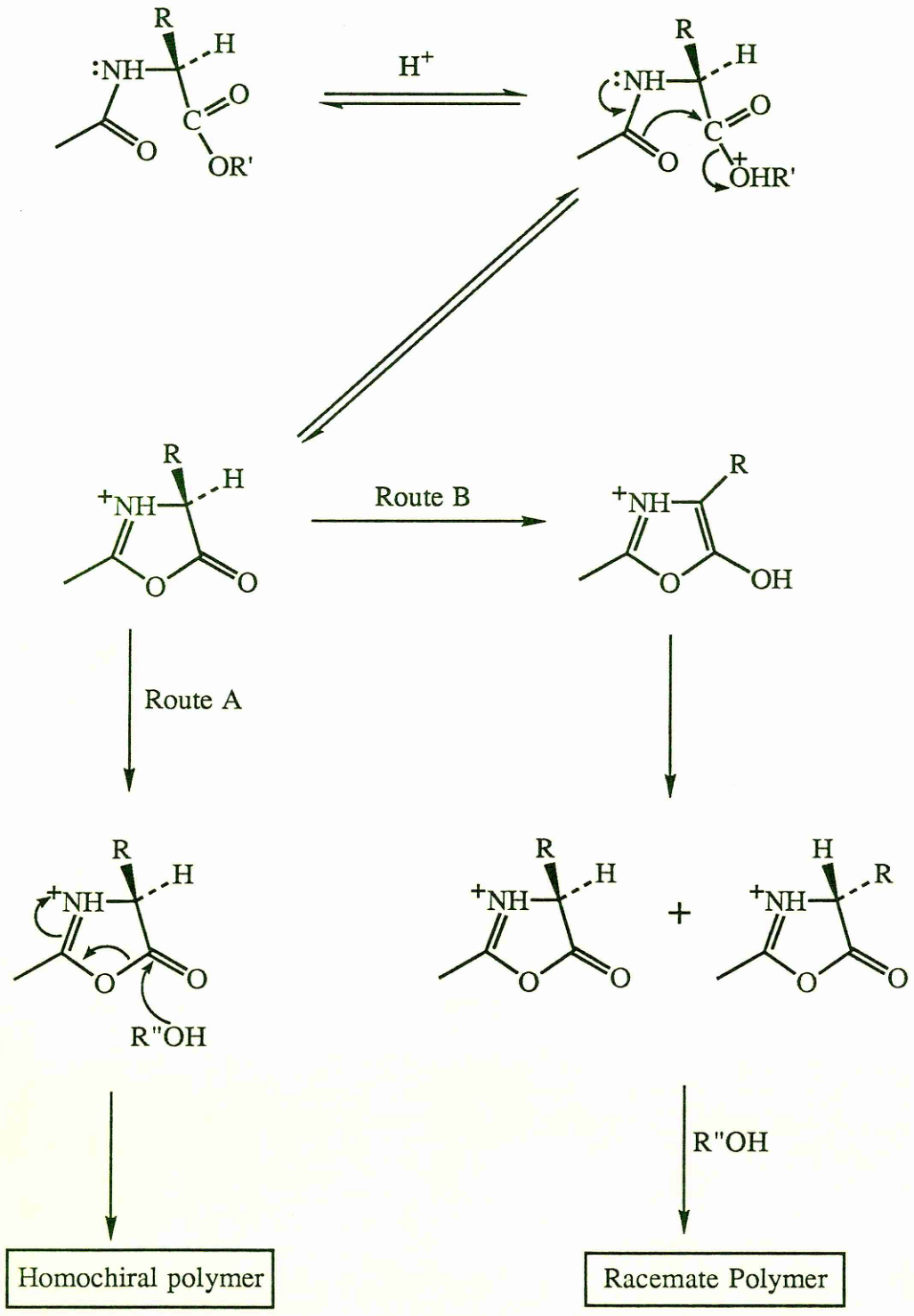


lesser extent of the PEG-400. The reactions were thus usually performed between 60°C and 80°C.

Initially the reaction mixtures were clear and could be readily stirred, but after 2-3 weeks some of the mixtures had become resinous solids, while others (the reactions with PEG-400) became viscous. In all cases, coloration was observed. After 3 weeks reaction the products were analysed by ^{13}C NMR and Vapour-phase osmometry (V.P.O.) in order to determine their relative molecular masses (see Section 7.4 for ^{13}C NMR method). Standard reference materials (e.g. PEG-2000 and PEG-1025) also had their molecular weights determined by V.P.O. to establish accuracy. Some of the products also had their optical rotation measured. The results from these experiments are summarised in Table 16.

The optical rotation measurements were performed in order to establish whether a racemisation process was occurring (i.e. via Route B, Scheme 56) or not (i.e. reaction via Route A). The results in Table 16 appear to show that racemisation was not occurring. The results however, may be prone to errors because the rotations were low in value and the coloration in the polymeric

SCHEME 56



(R' = H or polymer chain, R" = polymer chain or n-ethylene glycol)

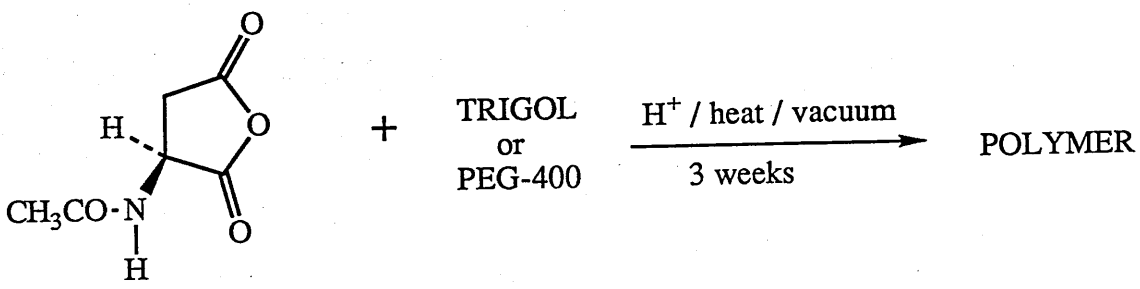
products made measurements difficult. The colourisation also prevented the use of cells with longer path lengths (to improve the rotation readings).

Two different methods were employed for determining the average relative molecular mass. The ^{13}C NMR method (described in Section 7.4) and the VPO method produced similar results for the one analysis where they were both used (i.e. 2135 and 1865 respectively for one of the TRIGOL/N-ac-Asp-DME products - see Table 16). While the ^{13}C NMR method will have inherent problems which may produce an inaccurate result, the V.P.O. method should not. However, the V.P.O. measurements of the molecular weights of two standards (PEG-1025 and PEG2000) were found to be considerably different from the expected values. Others have also reported that the reliability of V.P.O. as a method for determining the average relative molecular mass of polyethylene glycols and thus possibly their product polymers may be in question.[219] If higher molecular weights were achieved then gel permeation chromatography would have been used to ensure that the results were correct. As molecular weights of 10000 daltons or more are desirable in order to use the polymeric materials as GC stationary phases, the polymers produced (with Mw = 2000) were not considered suitable for this application. Other approaches for the synthesis of polyesters were thus considered in an attempt to yield materials with higher relative molecular masses.

None of the compounds reported to catalyse polyesterification[216] were of value for the reaction required here. For example, the use of zinc acetate gave a product after 5 weeks at 60°C under vacuum that had an average molecular weight of 370 (see Section 7.4).

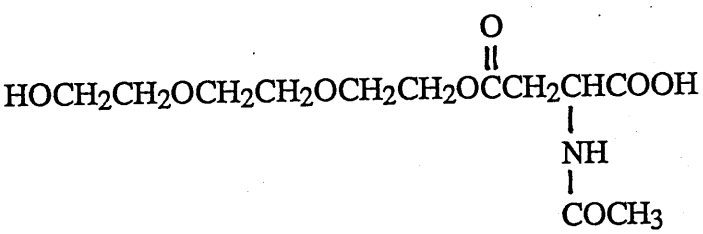
Reaction of the succinic anhydride derivative (111) with triethylene glycol (Scheme 57) proceeded fairly rapidly at first. After 24 hours, on average, one

SCHEME 57



(111)

ester bond had been formed between each of the two monomers resulting in product (116). However, heating for a further 3 weeks under vacuum (in order to promote the further condensation reactions) did not increase the molecular weight significantly. A product with an average relative molecular mass of 754 was achieved for one of the polymerisations using this method. The difficulties encountered in increasing the molecular weights of the products may result from the secondary acid groups of (116) being considerably less reactive than either the anhydride (111) or the primary acid groups of (107a).

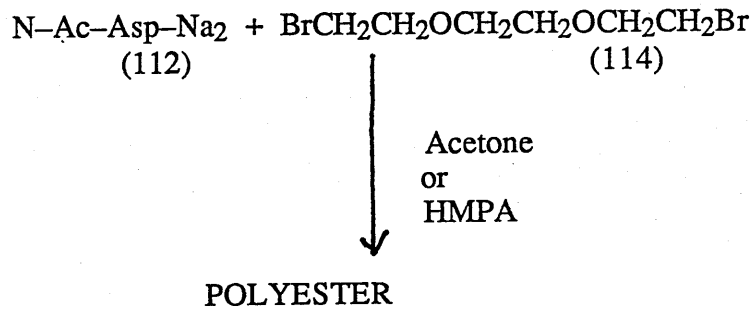


(116)

The highest average molecular weights achieved were in the region of 2000-2100 daltons and resulted from the polymerisation of N-ac-(S)-(+)-Asp-DME (110) with either TRIGOL or PEG400.

The reaction of disodium dicarboxylate (112) with dibromide (114) was attempted in two different solvents (Scheme 58).

SCHEME 58



HMPA has been reported as a useful solvent for the formation of esters from alkyl halides and sodium carboxylates.[167,170] When employed as solvent for the reaction between disodium dicarboxylate (112) and dibromide (114), the desired esterification occurred. However after 3 weeks the average relative molecular mass of the product was estimated at only 400 daltons (by the ^{13}C NMR method). The length of time that would be required to form polyester (109) thus makes this approach unsuitable.

Acetone was employed in an attempt to drive the forward reaction by precipitating the sodium bromide by-product (similar to the Finkelstein reaction). However the disodium dicarboxylate (112) was found to have low solubility in the acetone, thus preventing the reaction from taking place. Reactions employing ditosylate (115) were not performed due to the solubility problems experienced with the disodium salt (112).

All the approaches investigated in order to produce polyester (109) failed to yield the desired average molecular weight of ~20000 daltons. Some of the reactions produced materials with average molecular weights of up to 2000 daltons, and with longer reaction times and/or higher temperatures (and vacuum) may have produced higher molecular weights. One reason for the slow rates of reaction lies in the fact that the secondary acid function, or its derivatives are considerably less reactive than the primary acid function. This is observed in the ^{13}C NMR spectra for the products where a considerable imbalance in two forms of ester-bound carbon atoms exists after a short reaction time. The reaction rate will also decrease as the chain length of the polymer and the viscosity of the reaction mixture both increase.

Other methods for the synthesis of polyesters do exist and may produce more encouraging results. One method in particular, employing triphenylphosphine (as a catalyst) in hexachloroethane has been reported to yield polyesters in good yield directly from diacids and diols and deserves investigation.[220] Another route employing the di(nitrophenyl)ester of N-acetyl-(S)-(-)-aspartic acid may yield a polyester more favourably as considerably better leaving groups will be present.

7.4 Molecular weight determinations employing ^{13}C NMR techniques

^{13}C NMR data were used to estimate the extent of esterification that has occurred during the reaction between the monomeric units. This was achieved by firstly identifying signals within the product spectra which correspond to carbon atoms originating from the TRIGOL unit close to the ester function of the product. Signals corresponding to the original carbon atoms in monomers (or oligomer end groups) in an unbound state can also be identified. By comparison of these relative peak intensities, models incorporating various combinations of

molecules (oligomers/polymers/monomers) can be proposed which satisfy the observed result while also satisfying the 1:1 molar ratio of monomer units used. Use of these models can enable calculation of the average relative molecular mass from the following equation (eq. 21)

$$\text{Av Mr} = \frac{\Sigma \text{Mr of individual molecules}}{\text{number of molecules}} \qquad \text{(eq. 21)}$$

It is important to note that these calculations will only yield approximate values. This is due to the inaccuracies involved comparing the integrals of different ^{13}C NMR signals (as they may have different relaxation times). The inaccuracies are likely to be small in this case, where the signals being compared result from carbon atoms in similar environments. This will become evident in the following examples of average relative molecular mass determination:

Example 1. Product from the zinc acetate catalysed reaction

^{13}C NMR spectra were obtained for TRIGOL, N-Ac-Asp-DME (110) and the product. The signals were identified and are labelled on the structures shown with the spectra, NMR 3-5.

Upon bonding, signals (D) and (E) (in the spectrum of N-Ac-Asp-DME, see: NMR 3) would be expected to decrease as the methyl groups are lost on reaction. Signals (A) and (B) (in the spectrum of TRIGOL, see NMR 4) would also be expected to decrease, but at the same time other signals should apear (and increase accordingly) resulting from the different environments experienced by the corresponding polyester-bound carbon atoms. Signals (L) and (M) (in the spectrum of the product, see spectrum NMR 5) thus correspond to signals (A) (of the TRIGOL) and likewise (N) corresponds to (B). Signals (L) and (M) have different shifts due to the slightly different environments

experienced by these carbon atoms (which is also seen with signals (D) and (E) of the N-Ac-Asp-DME).

Having identified the carbon atoms which undergo changes in their shift values upon reaction (i.e. (A) and (B) becoming (L), (M) and (N)), the extent of polyester bond formation can be estimated.

^{13}C NMR integrals are generally inaccurate for quantitative purposes. Different carbon atoms in different environments will have different relaxation times. If a carbon atom has a long relaxation time it will produce a small signal and vice versa. So if a mixture of two compounds in the same concentration have their ^{13}C NMR spectra recorded, but two carbon atoms with different relaxation times (in different environments) are chosen to quantify the mixture ratio, then the integral data will be different. The integral data will indicate an unequal mixture which is a false result. For this procedure to work the two carbon atoms being observed must be in a very similar environment. Fortunately in this reaction the following changes occur.

$$\underset{\text{(B)}\quad\text{(A)}}{\text{–O–CH}_2\text{CH}_2\text{–O–H}} \longrightarrow \underset{\text{(N)(LorM)}}{\text{–O–CH}_2\text{CH}_2\text{–O–COR}}$$

(R = Remainder of the aspartic acid unit)

As can be seen, the environments of the carbon atoms (A) and especially (B) do not change very much in the reaction. Thus it is probably valid to compare the integrals of atoms (A) to (L) and (M), and of (B) to (N) to give an **approximate** ratio of the bound:unbound sites. Calculations using this ratio only give an estimate of the molecular weights involved, but are still useful. V.P.O. and Gel Permeation Chromatography (G.P.C.) are the more commonly used techniques, but as already seen (Table 16) V.P.O. may produce false results.

If, using the ^{13}C NMR spectrum of the product (spectrum 3), the integral of peak (A) represents the unbound reaction sites and the sum of the integrals of peaks (L) and (M) represents the bound reaction sites, then the ratio of the bound to unbound reaction sites can be estimated.

The integration results give:

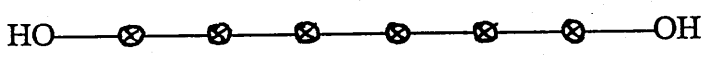
	15.2	:	11.53
approximating to	8	:	6

By treating peaks (B) and (N) in the same way to cross check the result the bound to unbound ratio is:

	17.6	:	11.4
or	9	:	6

The two calculated ratios are in quite close agreement.

Using the bound to unbound ratio of 8 : 6 as well as the 1 : 1 molar ratio (of monomers employed) the following molecules can be drawn which satisfy these conditions.



6 BOUND : 2 UNBOUND

TRIGOL ASP TRIGOL ASP TRIGOL ASP TRIGOL Mr = 1017

Plus

2 BOUND : 2 UNBOUND

TRIGOL ASP TRIGOL Mr = 439

Plus

TRIGOL 2 UNBOUND

Mr = 150

Plus

3x N–Ac–Asp–DME Mr 203

⊗ = Polyester Bond

(ASP and TRIGOL as labelled are used to represent the bound N–Ac–Asp–DME and TRIGOL units within the polymer chain).

The final reaction mixture in this case has a total of 8 bound and 6 unbound sites (thus satisfying the 8 : 6 bound to unbound ratio), while containing a 1 : 1 molar ration of TRIGOL and N–Ac–Asp–DME (7 units of each, as represented). Six molecules exist in this model.

As both ratios have been satisfied the average relative molecular mass is calculated thus:

$$\text{AvMr} = \frac{1017 + 439 + 150 + (3 \times 203)}{6}$$

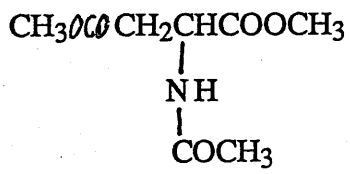
$$= 369$$

This is only one of many models existing which would satisfy the ratios. For instance a chain containing 80 monomers could exist, but the large molecular weight of this chain would be offset by the many monomer units required to satisfy the bound to unbound site ratio (40 unbound TRIGOL units are required), and the
1 : 1 molar ratio (40 unbound N-Ac-asp units would also be required). A molecule as large as an 80 unit chain is unlikely to exist in this case because statistically the many monomer units present would have reacted before the 80 unit chain had formed. Whatever the model, the average relative molecular mass would still be 369.

Clearly this result falls well below the desired molecular weight of > 10000. Although no V.P.O. measurements were made to verify this result, the example is a useful demonstration of how ^{13}C NMR can be used to calculate molecular weights. All other calculations from ^{13}C NMR data follow the procedures outlined in this example (see example 2 later).

The ^{13}C NMR spectra, as well as providing average molecular weights also provide information about how the constituents are reacting. Considering the product spectrum (spectrum NMR 5), it is noticed that an imbalance in peaks (D) and (E) as well as peaks (L) and (M) exists. This indicated that the reaction is proceeding more favourably at one of the N–Ac–Asp–DME reactive sites. By looking at the N–Ac–Asp–DME molecule it is seen that it contains a primary and a secondary ester as its reaction sites.

(D) (P) (S) (E)



(P = primary ; S = secondary)

It is known that the rates of reactions for secondary esters are slower than those for primary esters.[221] This is simply due to steric effects. Thus any polymer chain containing an aspartic acid unit as an end group will tend to have ester (S) as the unreacted site. This would lead to the carbon (E) peak being larger than that of carbon (D) in the ^{13}C NMR spectrum, as observed. Likewise peak (L) is observed to be larger than peak (M), as expected due to the primary ester reacting more favourably to form the polyester bond.

For molecules of higher molecular weight where the polyester bonds predominate, the difference in the peak heights becomes less, as more of the primary and secondary ester sites become incorporated within the chain.

Example 2. Product from the acid-catalysed reaction of N-Ac-Asp-DME with TRIGOL.

The ratio of bound to unbound reactive groups (of the TRIGOL unit) obtained from the ^{13}C NMR spectrum of the product (using the method shown in example 1) was

13.92 : 1.4

or 10 : 1 (using signals (A), (L) and (M))

This indicates an average relative molecular mass of 1,781 (by the calculations outlined in the previous example)

The ratio also worked out as

	17.4	:	1.4	
or	16	:	1	(using signals (B) and (N))

This indicates an average molecular weight of 2488 (by the calculations outlined in the previous example 1)

The mean of the two give an average relative molecular mass of 2135 (see Table 16 in Section 7.3).

8 EXPERIMENTAL

8.1 Instrumentation

The following instruments have been used throughout the research. Any operating conditions not listed below are given on the spectra or chromatograms.

(a) *Gas Chromatographs*

Gas chromatography work was performed on the following gas chromatographs:

Varian 6000 using a split injector (250°C) and FID detector (260°C). The data was processed using a Spectra-Physics 4100 integrator.

Carlo-Erba, GC6000 Vega Series 2 using an on-column injector and FID detector (250°C). The data were processed using a Milton Roy Cl-10CB integrator.

Hewlett Packard 5890 using a split injector (275°C) and FID detector (270°C). The data were processed using a Hewlett Packard HP5895A work station.

The various columns and temperature programs used are given on chromatograms.

(b) *Mass Spectrometers*

Probe work and GC/MS work were both performed using a VG305 magnetic sector mass spectrometer and a VG 20-250 quadrupole mass spectrometer

both in the electron ionisation mode. Scans were made every 2.5 seconds (VG 305) and just over one per second (VG 20-250). GC/MS work was performed using Carlo Erba Fractovap 2150 and Hewlett Packard 5890 gas chromatographs coupled to the VG 305 and VG 20-250 mass spectrometers (respectively) by a direct column insertion. The columns and temperature programs used are given on chromatograms.

(c) *Infrared Spectrophotometers*

Perkin Elmer 1420 ratio recording and Pye Unicam SP3-100 Infrared Spectrophotometers were used with 3 minute scan times.

(d) *Melting Point Apparatus*

An Electrothermal digital melting point instrument was used.

(e) *Nuclear Magnetic Resonance Spectrometers*

The instruments used were:

(i) A Jeol PMX60si (60 MHz) for ^{1}H NMR spectroscopy

(ii) A Jeol FX90Q FT NMR (90 MHz) for ^{13}C NMR and higher resolution ^{1}H NMR work.

(iii) A Bruker 90 MHz NMR instrument for other ^{13}C NMR and higher resolution ^{1}H NMR work.

(f) *Polarimeter*

A half shadow polarimeter (Lippich type) was used for optical rotation measurements.

(g) *Chemicals*

All the chemicals used were of standard lab reagent quality mainly obtained from Aldrich Chemical Company, and were usually used without further purification.

(h) *Elemental Analyses*

Elemental analyses were performed by Medac Ltd, Brunel University, Uxbridge, Suffolk.

8.2. Synthesis and use of homochiral derivatizing agents containing an acid chloride function

8.2.1 Synthesis of homochiral acid chloride derivatising agents

Synthesis of (+)-(trans)-chrysanthemoyl chloride (18)

To (+)-(trans)-chrysanthemic acid (56mg; 0.34 mmol) in sodium-dried toluene (3 ml) in a dry round-bottomed flask was added thionyl chloride (2 ml). The flask was flushed with nitrogen and the mixture was refluxed for 1 hour. The solvent and excess thionyl chloride were then removed using a stream of dry nitrogen. The deep orange oily product was used directly for derivatizations (see later).

Synthesis of N-acetyl-(S)-(-)-proline (32)

(S)-(-)-Proline (4g; 0.035 mol) was dissolved in 2M NaOH (15 ml). The stirred solution was cooled in ice while acetic anhydride (20 ml) was added dropwise.

The reaction was left to stand for 18 hours before acidifying with 2M H_2SO_4. The product was extracted into chloroform (2 x 150 ml) which was then dried ($MgSO_4$). After filtering, the solvent was removed by rotary evaporation. Ethyl acetate (40 ml) was added to the residue and the white crystalline product was filtered then washed with cold ethyl acetate.

The product was dried in an oven at 40°C yielding 3.611g (66%) as a white solid. **Mpt** = 116-118°C (Lit.[166] = 118°C). $[\alpha]_D^{20}$ - 101° (c=2.0, H_2O) (commercial N-acetyl-L-proline $[\alpha]_D^{20}$ -100° (c=2.0, H_2O)). Lit $[\alpha]_D^{20}$ = -115° (C=2.0, H_2O)[166]; **IR**: ν_{max} (KBr disc) 3600→2300 (O-H_{STR}, acid), 1720 (C=O_{STR}, acid) and 1600 cm^{-1} (C=O_{STR}, amide); **NMR;** δ_H ($CDCl_3$) 2.1 ($C\underline{H}_3CO$, s, 3H), 1.8-2.3 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 3.5 (N-$C\underline{H}_2$, m, 2H), 4.4 ($C\underline{H}$, d.t., 1H) and 8.5 ($COO\underline{H}$, s, 1H); **NMR**; δ_c ($CDCl_3$) 22.0/22.8 and 24.6 ($\underline{C}H_2$-$\underline{C}H_2$), 28.7 ($\underline{C}H_3CO$), 46.6/48.3 ($\underline{C}H_2$-N), 59.3/60.4 ($\underline{C}H$), 171.7 and 173.6 ($\underline{C}$=O, acid and $\underline{C}$=O, amide); **EIMS**: m/z (probe) 157 (2%, $M^{+\bullet}$), 113 (28%), 112 (25%, $[M-{}^{\bullet}COOH]^+$), 85 (16%), 70 (100%, $[M-({}^{\bullet}COOH + CH_2CO)]^+$) 43 (63%, CH_3CO^+).

Synthesis of N-acetyl-(S)-(-)-prolyl chloride (29)

To a stirred solution of N-acetyl-(S)-(-)-proline (32) (1.56g, 10 mmol) in dry (ethanol-free) dichloromethane (40 ml) was added dropwise thionyl chloride (2.3 g, 20 mmol). The reaction was stirred for 1 hour at room temperature before removing the solvent excess thionyl chloride by rotary

evaporation. After adding dry diethyl ether and again removing the solvent, the product was put under high vacuum and used without further purification.* The product was a light orange thick oil with a yield of 1.83g (104%) indicating that some thionyl chloride may still be present (trapped in the oil). $[\alpha]_D^{20}$ was not recorded due to the product not being pure; **IR**:ν_{max} thin film loss of absorbance at 3600→2300 (OH_{STR}, acid), 1798 ($C{=}O_{STR}$, acid chloride) and 1650 cm^{-1} ($C{=}O_{STR}$, amide); **NMR**: δ_H ($CDCl_3$) 1.8-2.4 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.15 ($C\underline{H}_3CO$, s, 3H), 3.65 (-$C\underline{H}_2$-N, d.t., 2H) and 4.75 ($C\underline{H}$, t, 1H); **NMR**: δ_C 21.8/22.3 and 24.5 ($\underline{C}H_2$-$\underline{C}H_2$), 28.8/31.2 ($\underline{C}H_3CO$), 47.0/48.0 (-$\underline{C}H_2$-N), 67.3/69.1 ($\underline{C}H$), 170.3 and 173.5 (C=O, amide and C=O, acid chloride); **EIMS**: m/z 139 (18%, $[M\text{-}HCl]^{+\bullet}$), 112 (48%, $[M\text{-}{}^{\bullet}COCl]^{+}$) 70 (100%, $[M\text{-}(CH_2CO + {}^{\bullet}COCl)]$), 43 (22%, CH_3CO^{+}) and 36/38 (3:1, 5%, HCl^{+}).

* N-acetyl-(S)-(-)-prolyl chloride decomposed on distillation, and colorised soon after synthesis if not stored under vacuum thus indicating its sensitivity to atmospheric moisture.

Preparation of (S)-(+)-tetrahydro-5-oxo-2-furancarboxylic acid (34) from (S-)-(+)-glutamic acid

A solution of sodium nitrite (126 g; 1.83 mol) in water (270 ml) was added dropwise to a mixture of (S)-(+)-glutamic acid (180 g; 1.22 mol) in water (480 ml) and concentrated (37%) hydrochloric acid (180 ml) at 0-5°C in a 2 litre flask fitted with a mechanical stirrer. On addition, the stirred solution cleared and effervesced (loss of N_2 and NO_2). After three hours stirring, the mixture was allowed to stand overnight at O°C, then at room temperature for 5 hours.

The water was removed by freeze drying to yield a pale yellow oil and colourless fine crystals. Hot acetone was added (600 ml) until all the oil was dissolved, leaving a fine suspension of sodium chloride. This was filtered off and the filtrate was concentrated to 300 ml. Anhydrous magnesium sulphate was added and the mixture was allowed to stand overnight at 3°C. The solids were removed by filtration and the solvent was removed. The oily residue was taken up in ethyl acetate (500 ml), and any further insoluble material was removed by filtration. The solvent was removed and the residue was taken up in a minimum of warm ethyl acetate (200 ml). An equal volume of benzene was added and the mixture was allowed to crystallise in a freezer (after seeding). The crystalline product was filtered and washed with anhydrous diethyl ether. The combined filtrates were again seeded and cooled to give a further crop of crystals.

The total yield was 80g (50%); **Mp** 55°C (lit. 70-72°C)[163]; $[\alpha]_D^{20}$ + 14.6° (c=2.0, EtOH) (lit.[7] + 15.6°); **IR**: ν_{max} (nujol) 3600→2350 (OH_{STR}, acid) ($C{=}O_{STR}$, lactone), 1720 ($C{=}O_{STR}$ acid), 1180 cm^{-1} ($C\text{-}O_{STR}$); **NMR**: δ_H (CD_3OD) 2.2-2.7 ($C\underline{H}_2\text{-}C\underline{H}_2$, m, 4H), 5.0 ($C\underline{H}$, m, 1H) 5.4 ($O\underline{H}$, s, 1H); **NMR**: δ_c (CD_3OD) 28.5 and 29.5 ($\underline{C}H_2\text{-}\underline{C}H_2$), 79.0 ($\underline{C}H\text{-}O$), 175.1 and 180.8 ($\underline{C}{=}O$ lactone and acid).

(S)-(+)-Tetrahydro-5-oxo-2-furancarbonyl chloride (30) from (S)-(+)-tetrahydro-5-oxo-2-furancarboxylic acid (34)

Acid (34) (5 g; 0.0385 mol) was heated with oxalyl chloride (6.71 ml; 0.077 mol) in dry benzene (10 ml) at 60-70°C for 5 hours (using reflux apparatus).

Benzene and excess oxalyl chloride were removed under vacuum (methanol being used to decompose the excess oxalyl chloride before disposal) and the residual oil was distilled under vacuum (110-120°C/0.2 mm Hg (lit.[164] 76-

82°C/0.02 mm Hg)) to yield 4.971 g (87%) of (30) as a clear oil; $[\alpha]_D^{20}$ + 4.0°C (c=2, $CHCl_3$); **IR:** ν_{max} (thin film) 1800 ($C{=}O_{STR}$, lactone and acid chloride) 1170 and 1140 (C-O), 970 and 910 (C-Cl); **NMR:** δ_H ($CDCl_3$) 2.3-2.8 ($\underline{CH_2}$-$\underline{CH_2}$, m, 4H) 5.1 ($\underline{CH}$, m, 1H), **EIMS:** m/z 149 (1.2%, $M^{+\bullet}$), 85 (100%, $[M\text{-}^{\bullet}COCl]^+$), 36/38 (13%, HCl^+).

Preparation of the equimolar mixture of secondary alcohols

2-Pentanol (0.88g, 10 mmol), 2-hexanol (1.02g, 10 mmol), 2-heptanol (1.16g, 10 mmol) and 2-octanol (1.30g, 10 mmol) were mixed together to produce an equimolar mixture.

8.2.2 Derivatizations using homochiral acid chloride reagents

Derivatization of the racemic secondary alcohol mixture using (+)-(trans)-chrysanthemoyl chloride (18)

A 0.5M solution of the secondary alcohol mixture was prepared by diluting the alcohol mixture (0.109 g, 1.0 mmol) with toluene to 2 ml. An aliquot of 120µl of this solution (0.06 mmol of alcohols) was added to the freshly prepared acid chloride (18) (0.34 mmol) in toluene (2 ml). The reaction was refluxed for 2 hours before removing the solvent using dry nitrogen. The brown oily residue was diluted with ethyl acetate (1 ml) and used directly in the GC analyses. Results are summarised and discussed in Chapter 2.

A similar procedure was also used for the 2-pentanol→2-undecanol mixture (see GC1).

Derivatization of the racemic secondary alcohol mixture using N-acetyl-(S)-(-)-prolyl chloride (29)

To acid chloride (29) (0.351 g, 2 mmol) in toluene (15 ml) was added to the alcohol mixture (27 mg, 0.25 mmol). The reaction was stirred at room temperature for 1 hour. A 1ml sample was taken and the solvent was removed. Ethyl acetate was added and then washed with water. This was then dried ($MgSO_4$) and filtered (cotton wool) then analysed by GC and GC/MS. The GC results are discussed in Chapter 2 and representative mass spectra for the two diastereoisomers are given (MS1 and MS2).

Derivatization of 2-heptanol using (S)-(+)-tetrahydro-5-oxo-2-furan carbonyl chloride (30).

Work was performed on the derivatization of 2-heptanol following a method described by R E Doolittle et al.[159] 2-Heptanol (20µl; 0.14 mmol) was added to pyridine (80µl) and the mixture was stirred and cooled to 0°C. A 2M solution of acid chloride (30) in dichloromethane (100µl; 0.2 mmol) was added and the reaction mixture was allowed to warm to room temperature. Two drops of 1M hydrochloric acid solution was added followed by hexane (2 ml). The layers were separated and the organic layer was dried by passing through anhydrous magnesium sulphate. This solution was then analysed directly by GC and GC/MS. Chromatographic resolution of the diastereoisomeric products (35a) and (35b) was not observed on the columns used (BP-5) (12 m x 0.33 mm, 0.5µm film) and BP-1 (12.5m x 0.32mm, 0.5µm film). The mass spectrum for the product peak contained the following diagnostic ions, **EIMS**: m/z 229 $[M+H]^+$, 213 $[M-{}^{\bullet}CH_3]^+$, 185 $[M-C_3H_7]^+$, 157 $[M-C_5H_{11}]^+$, 143 $[M-C_6H_{13}]^+$ and 85 $[M-{}^{\bullet}COOC_7H_{15}]^+$.

8.3 Homochiral alkyl halide derivatizing agents

8.3.1 Syntheses of homochiral alkyl halide reagents

Preparation of 2-haloethyl N-acetyl-(S)-(-)-prolinates (36-38) and 3-bromopropyl N-acetyl-(S)-(-)-prolinate (39) from N-acetyl-(S)-(-)-proline (30): General procedure

To N-acetyl-(S)-(-)-proline (30) (500 mg, 3.2 mmol), 2-chloroethanol (1 ml, 15 mmol), 2-bromoethanol (0.5 ml; 7 mmol), 2-iodoethanol (1 ml, 12.8 mmol) or 3-bromopopan-1-ol (2.5 ml, 28 mmol) was added. One drop of concentrated sulphuric acid was added to catalyse the reaction. After 4 days stirring at room temperature, water (20ml) was added. The product was extracted into chloroform (50 ml) then back-washed with water (5 x 10 ml) to remove any 2-haloethanol or 3-bromopropan-1-ol remaining. The product solution was dried by filtering through cotton wool and rotary evaporation gave compounds (36)-(39).

2-Chloroethyl N-acetyl-(S)-(-)-prolinate (36).

This compound was isolated in 0.23g (33%) yield as a clear oil. **IR:** ν_{max} (thin film) 1740 (C=O_{STR}, ester) 1620 (C=O_{STR}, amide), 1170 cm^{-1} (C(=O)-O-C_{STR}); **NMR:** δ_H ($CDCl_3$) 1.8-2.5 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.05 ($C\underline{H}_3$-CO, s, 3H), 3.3-3.8 ($C\underline{H}_2$-Cl and $C\underline{H}_2$-N, 2xt, 4H), 4.15-4.6 (O-$C\underline{H}_2$ and $C\underline{H}$, t + m, 3H); **NMR:** δ_C ($CDCl_3$) 22.2/22.8 and 24.8 ($\underline{C}H_2$-$\underline{C}H_2$), 29.4/31.5 ($\underline{C}H_3$-CO), 41.6 ($\underline{C}H_2$-Cl), 46.3/47.7 ($\underline{C}H_2$-N), 58.6/60.1 ($\underline{C}H$), 64.3/64.8 (O-$\underline{C}H_2$), 169.4 and 171.9 ($\underline{C}$=O, ester and amide); **EIMS:** m/z 219/221 (8%, $M^{+\bullet}$, 3:1 ratio indicating that the compound is chlorine containing), 176/178 (1%, $[M\text{-}CH_3CO^{\bullet}]^+$, 3:1 ratio is present, indicating that the fragment ion contains chlorine), 140 (7%, $[M\text{-}^{\bullet}OCH_2CH_2Cl]^+$), 112 (60%, [M-

$^{\bullet}COOCH_2$-$CH_2Cl]^{+)}$, 70 (100%, $[M-(^{\bullet}COOCH_2CH_2Cl + CH_2CO)]^{+}$) and 43 (12%, CH_3CO^{+}).

2-Bromoethyl N-acetyl-(S)-(-)-prolinate (37)

Isolated in 0.37g (44%) yield as a clear oil. **IR:** ν_{max} (thin film) 1735 ($C=O_{STR}$, ester), 1635 (C=O, amide), 1180 cm^{-1} ($C(C=O)-O-C_{STR}$); **NMR:** δ_H ($CDCl_3$) 1.7-2.25 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.0 ($C\underline{H}_3$-CO, s, 3H), 3.2-3.7 ($C\underline{H}_2$-N and $C\underline{H}_2$-Br, m, 4H), 4.15-4.45 (O-$C\underline{H}_2$ and $C\underline{H}$, m, 3H); **NMR:** δ_C ($CDCl_3$) 20.3/21.5 and 23.2 ($\underline{C}H_2$-$\underline{C}H_2$), 27.3 and 28.0 ($\underline{C}H_3$-CO and $\underline{C}H_2$-Br), 45.2/46.5 ($\underline{C}H_2$-N), 57.3/58.9 ($\underline{C}H$) 61.1/62.7 (O$\underline{C}H_2$), 168.5 and 170.1 ($\underline{C}$=O, ester and amide).

2-Iodoethyl N-acetyl-(S)-(-)-prolinate (38)

Isolated in 0.35g (35%) yield as an orange oil. **IR:** ν_{max} (thin film), 1735 ($C=O_{STR}$, ester), 1625 ($C=O_{STR}$, amide), 1180 cm^{-1} ($C(=O)-O-C_{STR}$); **NMR:** δ_H ($CDCl_3$) 1.8-2.3 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.0 ($C\underline{H}_3$-CO, s, 3H), 3.2 ($C\underline{H}_2$-I, t, 2H), 3.2-3.7 ($C\underline{H}_2$-N, m, 2H), 4.3 (O-$C\underline{H}_2$, t, 2H), 4.35 ($C\underline{H}$, t, 1H); **NMR:** δ_C ($CDCl_3$) 0.4 ($\underline{C}H_2$-I), 22.2/22.8 and 24.8 ($\underline{C}H_2$-$\underline{C}H_2$), 29.5/31.6 ($\underline{C}H_3CO$), 46.3/47.8 ($\underline{C}H_2$-N), 58.7/60.2 ($\underline{C}H$), 64.9 (O-$\underline{C}H_2$), 169.5 and 171.7 (C=O, ester and amide); **EIMS:** m/z 311 (4%, $M^{+\bullet}$), 155 (4%, $ICH_2CH_2^{+}$), 128 (5.5%, $HI^{+\bullet}$), 127 (6.5%, I^{+}), 112 (85%), $[M-{}^{\bullet}COOCH_2CH_2I]^{+}$) and 70 (100%, $[M-(^{\bullet}COOCH_2CH_2I + CH_2CO)]^{+}$).

3-Bromo-1-propyl N-acetyl-(S)-(-)-prolinate (39)

Isolated in 0.405g (46%) yield as a clear oil. $[\alpha]_D^{20}$ -36% (c=2.0, EtOH). **IR:** ν_{max} (thin film) 1750 ($C=O_{STR}$, ester), 1650 ($C=O_{STR}$, amide), 1180 (C(=O)-O-C); **NMR:** δ_H ($CDCl_3$) 1.8-2.35 ($C\underline{H}_2$-$C\underline{H}_2$ and CH_2-$C\underline{H}_2$-CH_2Br, m, 6H), 2.0 ($C\underline{H}_3$-CO, s, 3H), 3.2-3.7 ($C\underline{H}_2$-Br and $C\underline{H}_2$-N, m, 4H), 3.9-4.5 (O-$C\underline{H}_2$ and $C\underline{H}$, m, 3H); **NMR:** δ_C ($CDCl_3$), 22.2/22.9 and 24.9 ($\underline{C}H_2$-$\underline{C}H_2$), 29.5 ($\underline{C}H_3$-CO and $CH_2\underline{C}H_2CH_2Br$), 31.7 ($\underline{C}H_2$-Br), 46.3/47.8 ($\underline{C}H_2N$), 58.7/60.2 ($\underline{C}H$), 62.7/63.3 (O-$\underline{C}H_2$), 169.4 and 172.3 ($\underline{C}$=O, ester and amide); **EIMS;** m/z 277/9 (8%, $M^{+\bullet}$, 1:1 ratio is present, indicating the ion is bromine containing), 234/6 (1%, $[M-{}^{\bullet}COCH_3]^+$, 1:1 ratio is again present), 140 (3%, $[M-BrCH_2CH_2CH_2O^{\bullet}]^+$), 112 (80%, $[M-{}^{\bullet}COOCH_2CH_2CH_2Br]^+$) and 70 (100%, $[M-({}^{\bullet}COOCH_2CH_2CH_2Br + CH_2CO)]^+$).

Preparation of (S)-(+)-pyrrolidine methanol (40)

In a 500 ml, two-necked round-bottomed flask equipped with a stopper, reflux condensor (+ drying tube) and heating mantle/magnetic stirrer was placed dry THF* (250 ml) and lithium aluminium hydride (6.0 g, 0.156 mol). The suspension was heated under reflux for 15 minutes before switching the heat off. Powdered (S)-(-)-proline (11.51 g, 0.1 mol) was added in small portions (stoppering after each addition) at such a rate as to maintain reflux. This was then refluxed for a further hour.

* *Dry, peroxide free THF was prepared by refluxing over KOH pellets for 2 hours, distilling then storing it over lithium aluminium hydride until used).*

Excess hydride was decomposed by the addition of a solution of potassium hydroxide (2.8 g, 0.05 mol) in water (11.2 ml) through a pressure equilising dropping funnel to the boiling mixture (without external heating). Precipitation of white aluminium salts made stirring difficult. After complete addition, the mixture was refluxed for 15 minutes then filtered, and the filtrate retained. The precipitate was refluxed in fresh THF (50 ml) and again filtered. The filtrates were combined and the solvent removed by rotary evaporation (<30°C) to yield 12.88 g of crude product as a pale yellow oil. Vacuum distillation (80°C/1.0 mmHg) gave 6.78 g (67%) of pure product as a clear oil. $[\alpha]_D^{20}$ -31° (C=1, $C_6H_5CH_3$) (lit.[222] $[\alpha]_D^{20}$ -31° (c=1, $C_6H_5CH_3$)). **IR:** ν_{max} (thin film) 3250 (OH_{STR}, alcohol and N-H_{STR}, amine), loss of absorbance at ~1720 cm^{-1} ($C{=}O_{STR}$, acid); **NMR:** δ_H ($CDCl_3$) 1.1-2.0 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.7-3.05 ($C\underline{H}_2$-N, t, 2H), 3.1-3.7 ($C\underline{H}_2$-O, N-$\underline{H}$ and $C\underline{H}$, m, 4H), 3.65 ($O\underline{H}$, s, 1H); **NMR:** δ_C($CDCl_3$) 25.8 and 27.6 ($\underline{C}H_2$-$\underline{C}H_2$), 46.3 ($\underline{C}H_2$-N), 59.9 ($\underline{C}H_2$-O), 64.6 ($\underline{C}H$); **EIMS:** m/z 101 (1.5%, $M^{+\bullet}$), 100 (2%, $[M\text{-}H^{\bullet}]^+$) 83 (27%, $[M\text{-}H_2O]^{+\bullet}$) and 70 (100%, $[M\text{-}{}^{\bullet}CH_2OH]^+$).

Preparation of N-acetyl-(S)-(-)-pyrrolidinemethanol (41)

To (S)-(+)-pyrrolidinemethanol (10.1g, 0.1 mol) in methanol (100 ml) was added acetic anhydride (9.53 ml, 0.101 mol). The reaction progressed exothermically. After 30 minutes, the reaction mixture had cooled back to room temperature. Infrared analysis showed an absorbance at ν_{max} = 1610 cm^{-1} ($C{=}O_{STR}$, amide) indicating that the reaction had run to completion (confirmed by TLC using silica plates and methanol as the solvent). The methanol was removed by rotary evaporation and 1M sodium hydroxide solution (50 ml) was added to the residue. The product was extracted into chloroform (2 x 50 ml then 2 x 20 ml) and dried ($MgSO_4$). The solvent was

removed to yield 10.3g (73%) of (41) as a clear oil $[\alpha]_D^{20}$ -52.5° (c=2.0, EtOH). **IR:** ν_{max} (thin film) 3360 (OH_{STR}, alcohol), 1610 cm^{-1} ($C{=}O_{STR}$, amide); **NMR:** δ_H ($CDCl_3$) 1.6-2.2 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.1 ($C\underline{H}_3$-CO, s, 3H), 3.35-3.7 ($C\underline{H}_2$-O and $C\underline{H}_2$-N, m, 4H), 4.15 ($C\underline{H}$, m, 1H), 7.75 ($O\underline{H}$, s, 1H); **NMR:** δ_C ($CDCl_3$) 22.9 and 24.3 ($\underline{C}H_2$-$\underline{C}H_2$), 28.3 ($\underline{C}H_3CO$), 48.9 ($\underline{C}H_2$-N), 60.7 ($\underline{C}H_2$-O), 66.2 ($\underline{C}H$), 171.8 ($\underline{C}$=O, amide); **EIMS:** m/z 143 (1%, $[M^{+\bullet}]$), 125 (12%, $[M\text{-}H_2O]^{+\bullet}$), 112 (30%, $[M\text{-}{}^{\bullet}CH_2OH]^{+}$) and 70 (100%, $[M\text{-}({}^{\bullet}CH_2OH + CH_2CO)]^{+}$).

Preparation of N-acetyl-(S)-(-)-pyrrolidinemethyl chloride (42)

To N-acetyl-(S)-(-)-pyrrolidinemethanol (0.715 g, 5 mmol) in dry DCM (10 ml) was added thionyl chloride (0.615 ml, 8.5 mmol). On addition, effervescence was observed and the reaction mixture warmed up.

After 1 hour stirring at room temperature, the solvent and excess thionyl chloride were removed by rotary evaporation. Further DCM was added and again removed before applying a high vacuum to the product. The white hygroscopic powder product (0.61g, 79.4%) was recrystallised from ethyl acetate, and the resultant needle-like crystals were filtered off under an atmosphere of nitrogen to yield 178 mg (22%) of (42) (95% pure by GC). Mpt = 121-125°C. $[\alpha]_D^{20}$ -48° (c = 2, $CHCl_3$). **IR:** ν_{max} (nujol) loss of absorbance at 3360 (OH_{STR}, alcohol), 1665 ($C = O_{STR}$, amide; moved from 1610), 730 cm^{-1} ($C\text{-}Cl_{STR}$); **NMR:** δ_H ($CDCl_3$) 1.9-2.3 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.55 ($C\underline{H}_3$-CO, s, 3H), 3.5-3.9 ($C\underline{H}_2$-N and $C\underline{H}_2$-Cl, m, 4H), 4.4 ($C\underline{H}$, m, 1H); **NMR:** δ_C ($CDCl_3$) 20.7 and 23.5 ($\underline{C}H_2$-$\underline{C}H_2$), 28.2 ($\underline{C}H_3$-CO), 43.9 ($\underline{C}H_2$-Cl), 50.0 ($\underline{C}H_2$-N), 60.0 ($\underline{C}H$), 173.4 ($\underline{C} = O$); **EIMS:** m/z 161/3 (3%, $M^{+\bullet}$, 3:1 isotope ratio indicates that it is a chlorine containing ion), 126 (1.7%, $[M\text{-}Cl^{\bullet}]^{+}$), 118/20 (1.5%, $[M\text{-}{}^{\bullet}COCH_3]^{+}$, 3:1 chlorine isotope ratio

is present), 112 (33%, $[M-{}^{\bullet}CH_2Cl]^+$), 70 (100%, $[M-({}^{\bullet}CH_2Cl + CH_2CO)]^+$) and 43 (7%, CH_3CO^+).

Preparation of N-acetyl-(S)-(-)-pyrrolidinemethyl bromide (43)

The procedure was the same as that used to prepare its chloride analogue (42) except that it was performed on a smaller scale (i.e. using 50 mg (0.32 mmol) of alcohol (41) and thionyl bromide (0.15 ml, 2 mmol) with the other solvents, etc. scaled down accordingly). Isolation of the solid product proved to be a problem as the thionyl bromide was difficult to remove (even under high vacuum). Washing the orange oily (contaminated) product several times with diethyl ether removing the supernatant each time eventually gave 43.5 g (66%) of the bromide (43) as a pale brown hygroscopic solid. Mpt = 150-155°C (with decomposition). **IR:** ν_{max} (nujol) loss of OH_{STR} (3360), 1670 ($C{=}O_{STR}$, amide); **NMR:** δ_H ($CDCl_3$) 1.8-2.4 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.8 ($C\underline{H}_3$-CO, s, 3H), 3.5-4.0 ($C\underline{H}_2$-Br and $C\underline{H}_2$-N, m, 4H), 4.6 ($C\underline{H}$, m, 1H); **EIMS:** m/z 205/7 (1.5%, $M^{+\bullet}$, 1:1 isotope ratio indicates that the ion contains bromine), 162/4 (0.7%, $[M-{}^{\bullet}COCH_3]^+$, 1:1 bromine ratio present), 126 (7%), $[M-Br^{\bullet}]^+$), 112 (70%, $[M-{}^{\bullet}CH_2Br]^+$), 80/82 (25%, HBr^+, 1:1 bromine isotope ratio confirms the identity of the ion), 70 (100%, $[M-({}^{\bullet}CH_2Br + CH_2CO)]^+$) and 43 (17%, CH_3CO^+).

Attempted synthesis of N-acetyl-(S)-(-)-pyrrolidinemethyl iodide (44) from alcohol (41) using the DIPHOSI reagent

The DIPHOSI reagent was synthesised by the dropwise addition of a solution of iodine (3.0456g, 24 mmol) in DCM (10 ml) to a solution of 1,2-bis(diphenylphosphino)-ethane (DIPHOS) (4.78g, 12 mmol) in DCM (50 ml) at 0°C under an argon atmosphere. This was carried out at a rate which

maintained the reaction temperature below 10°C. A solution of alcohol (41) (2.86g, 20 mmol) in DCM (10 ml) was added to the reaction mixture containing the DIPHOSI reagent and the temperature was allowed to reach 25°C. After stirring for 2 hours, ether (140 ml) was added followed by pentane (280 ml) to precipitate the phosphorus by-products. The mixture was filtered and the solids washed with ether/pentane. The combined extract when evaporated gave little or no product, possibly due to its co-precipitation with the by-products. TLC results on the dissolved solids were also unclear. The possible hygroscopic nature of the product and the solubility problems involved prevented work from being undertaken to isolate iodide (44).

Attempted synthesis of N-acetyl-(S)-(-)-pyrrolidinemethyl tosylate (45) from alcohol (41)

p-Toluenesulphonyl chloride (2.812g; 14.7 mmol) was added to a stirred solution of alcohol (41) (1.24g; 8.7 mmol) in dry pyridine (8 ml) at 0°C. After 30 minutes stirring the mixture was left to stand at 0°C for 22 hours. The mixture was then filtered and the filtrate was poured into cold 5% sodium bicarbonate solution (15 ml). The product was extracted into ethyl acetate (2 x 10 ml), and the combined extracts were washed with 5% sodium bicarbonate solution (10 ml), water (10 ml) and brine (10 ml). After drying with anhydrous magnesium sulphate and filtering, the solvent was removed to yield 0.23g (8.9%) as a thick orange oil. The product was found to be impure, by proton NMR showing many more signals than would be expected. The main signals do possibly indicate that the correct product was present.

Preparation of N-acetyl-(S)-2-prolylmethyl bromoacetate (46) from alcohol (41)

To alcohol (41) (0.5 g; 3.5 mmol) in dichloromethane (1 ml) was added bromoacetyl chloride (0.57 g, 3.6 mmol). An initial warming was observed after which the solution was stirred for 2 hours. Water (5 ml) was added and the product was extracted with dichloromethane. After drying ($MgSO_4$) and filtering, the solvent was removed to yield 0.6618 g (72%) of product (46) as a light brown oil (giving one spot by TLC on silica plates, using chloroform or methanol as a solvent). **IR:** ν_{max} (thin film) loss of absorbance at 3400 (OH_{STR}, alcohol), 1740 ($C = O_{STR}$, ester), 1635 (C = O, amide), 1170 ($C(=O)\text{-}O\text{-}C_{STR}$), 730 cm^{-1} (C-Br); **NMR:** δ_H ($CDCl_3$) 1.6-2.2 ($C\underline{H}_2\text{-}C\underline{H}_2$, m, 4H), 2.0 ($C\underline{H}_3\text{-}CO$, s, 3H), 3.25-3.55 ($C\underline{H}_2\text{-}N$, m, 2H), 3.85 ($C\underline{H}_2\text{-}O$, d, 2H), 4.25 ($C\underline{H}_2\text{-}Br$, s, 2H), 4.25 ($C\underline{H}$, m, 1H); **EIMS:** m/z 263/5 (0.15%, $M^{+\bullet}$, 1:1 isotope ratio indicates that the ion is bromine containing), 220/2 (0.4%, $[M\text{-}^{\bullet}COCH_3]^+$, contains 1:1 bromine isotope ratio), 192/4 (0.15%, $[M\text{-}CH_3CON{=}CH_2]^{+\bullet}$, contains the 1:1 bromine isotope ratio), 142 (2%, $[M\text{-}^{\bullet}COCH_2Br]^+$), 125 (20%, $[M\text{–}BrCH_2COOH]^+$), 112 (80%, $[M\text{-}^{\bullet}CH_2OCOCH_2Br]^+$) and 70 (100%, $[M\text{-}(^{\bullet}CH_2OCOCH_2Br + CH_2CO]^+$).

Preparation of (S)-(+)-tetrahydro-5-oxo-2-furanmethyl alcohol (47) from acid (34).

To a three necked 500 ml round bottomed flask, set up with a magnetic stirrer, septum, stopper and reflux condenser connected to an argon bubbler, was added acid (34) (21.6 g, 166 mmol), followed by dry THF (140 ml). After flushing the system with argon, a 2M (THF) solution of borane methyl sulphide complex (95 ml, 190 mmol) was injected slowly (over 1 hour).

After 3 hours stirring, the mixture was quenched by cautious addition of anhydrous methanol (60 ml). Most of the solvent was removed by rotary evaporation and a further portion of methanol (200 ml) was added then removed. Vacuum distillation (125-135°C/0.6 mm Hg) yielded 16.71g (87%) of alcohol (47) as a clear oil. $[\alpha]_D^{20}$ + 25.5° (c = 2.0, EtOH) (lit. [163] $[\alpha]_D^{20}$ + 29.6° (c = 0.4, EtOH)). **IR:** ν_{max} (thin film) 3400 (O-H_{STR}), 1765 cm^{-1} (C = O_{STR} lactone); **NMR:** δ_H ($CDCl_3$) 2.25 and 2.6 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 3.75 ($C\underline{H}_2$-O, m, 2H), 4.1 (O-$\underline{H}$, s, 1H), 4.6 ($C\underline{H}$, m, 1H); **NMR:** δ_C ($CDCl_3$) 23.0 (C-$\underline{C}H_2$-C), 28.4 (O_2C-$\underline{C}H_2$-C), 63.6 (O-$\underline{C}$H-C), 80.9 ($\underline{C}H_2$-OH), 178 ($\underline{C}$=O).

Preparation of (S)-(+)-tetrahydro-5-oxo-2-furanmethyl tosylate (48) from alcohol (47)

The procedure was the same as that used in the attempted synthesis of tosylate (45), but using alcohol (47) (15 g, 0.13 mmol), p-toluenesulphonyl-chloride (42 g, 0.22 mmol) and pyridine (100 ml). The quantities of the solutions etc. used in the work-up were scaled-up accordingly.

The orange oil produced was crystallised twice from benzene/hexane to yield 16.2g (47%) of tosylate (48) as fine white needles. **Mpt:** = 83°C (lit.[163] = 85-87°C). $[\alpha]_D^{20}$ + 43 (c = 1, $CHCl_3$) (lit.[163] $[\alpha]_D^{20}$ + 47° (c = 1.6, $CHCl_3$)). **IR:** ν_{max} (KBr disc) 1760 (c = O_{STR} lactone), 1355 (S = O), 1170 (S = O), 975 cm^{-1} (S-O-C); **NMR:** δ_H ($CDCl_3$) 2-2.6 ($C\underline{H}_2C\underline{H}_2$, m, 4H), 2.45 ($C\underline{H}_3$, s, 3H), 4.15 ($C\underline{H}_2$-O, dd, 2H), 4.65 ($C\underline{H}$-O, m, 1H), 7.25 and 7.8 (aromatic $C\underline{H}$, 2xd, 4H); **NMR:** δ_c ($CDCl_3$) 21.6 ($\underline{C}H_3$), 23.4 and 27.8 ($\underline{C}H_2$-$\underline{C}H_2$), 69.9 ($\underline{C}$H-O), 76.3 ($\underline{C}H_2$-O), 127 and 130 (aromatic $\underline{C}$H), 132.2 ($\underline{C}$-CH_3), 145.3 ($\underline{C}$-S), 175.9 ($\underline{C}$=O).

(S)-(-)-Tetrahydro-5-oxo-2-furanmethyl iodide (49) from tosylate (48)

Lithium iodide (24 g, 0.18 mmol) was added to a solution of tosylate (48) (12 g, 44 mmol) in acetone (150 ml). The mixture was stirred and heated under reflux for 5 hours, when the reaction was seen to be complete by TLC (on silica plates using methanol or chloroform as solvent). The acetone was removed by rotary evaporation and the orange/brown residue was dissolved in water (20 ml). The product was extracted twice with ethyl acetate (15 ml), and the combined extracts were washed with sodium thiosulphate and brine solutions. After drying with anhydrous magnesium sulphate and filtering, the ethyl acetate was removed by rotary evaporation to yield 9.33g (94%) as a pale orange oil; $[\alpha]_D^{20}$ -15.4° (C = 1.75, $CHCl_3$) (lit.[188] $[\alpha]_D^{20}$ + 2.3 (C = 2.4, CH_2Cl_2); **IR:** ν_{max} (thin film) 1760 cm^{-1} (C = O_{STR}, lactone); **NMR:** δ_H ($CDCl_3$) 2-3 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 3.5 (CH-$C\underline{H}_2$-I, d, 2H), 4.65 ($C\underline{H}$-O, m, 1H); **NMR:** δ_c (CD_3OD) 7.9 ($\underline{C}H_2$-I), 28.0 and 28.8 ($\underline{C}H_2$-$\underline{C}H_2$), 78.4 ($\underline{C}$H-O), 176.2 ($\underline{C}$=O).

(S)-(+)-Tetrahydro-5-oxo-2-furanmethyl bromide (50) from tosylate (48)

The procedure was exactly the same as that used for the preparation of iodide (49), except that it was carried out on a 4 mmol scale. Thus tosylate (48) (1.066g, 4 mmol), acetone (12 ml) and lithium bromide (1.303 g, 15 mmol) were used giving 0.335 g (47%) of bromide (50) as a clear oil. $[\alpha]_D^{20}$ + 3.6° (c = 1.4, $CHCl_3$); **IR:** ν_{max} (thin film), 1780 cm^{-1} (C = O_{STR}, lactone); **NMR:** δ_H ($CDCl_3$) 2.0-2.8 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 3.6 ($C\underline{H}_2$-Br, d, 2H), 4.75 ($C\underline{H}$O, m, 1H); **NMR:** δ_C ($CDCl_3$) 26.2 and 28.4 ($\underline{C}H_2$-$\underline{C}H_2$), 34.1 ($\underline{C}H_2$-Br), 77.1 ($\underline{C}$H), 176.1 ($\underline{C}$=O).

Preparation of (S)-(+)-methyl 3-tosyl-2-methyl propionate (52) from alcohol (51)

The procedure was exactly the same as that used for the preparation of tosylate (48) except that it was performed on a 13 mmol scale. Thus (S)-(+)-methyl 3-hydroxy-2-methyl propionate (51) (1.534g, 13 mmol, commercially available from Aldrich), pyridine (10 ml) and tosyl chloride (4.2g, 22 mmol) were used. The work-up, also scaled accordingly gave 2.413g, (68%) as a pale orange oil (pure by GC) (Found: C, 52.88; H, 5.92. $C_{12}H_{16}O_5S$ requires C, 52.93; H, 5.92). $[\alpha]_D^{20}$ + 9.0° (C = 2.0, MeOH); **IR:** ν_{max} (thin film) loss of OH_{STR} (~3400), 1745 (C = O_{STR}, ester), 1600/1495 (aromatic $C\underline{---}C_{STR}$), 1365 (S = O), 1210 (C(=O)-O-C_{STR}), 1180 (S = O), 975 (S-O-C), 750 (C-H_b), 670 cm^{-1} ($C\underline{---}C_b$); **NMR:** δ_H ($CDCl_3$) 1.15 ($C\underline{H}_3CH$, d, 3H), 2.4 (Ar-$C\underline{H}_3$, s, 3H), 2.85 ($C\underline{H}$, q, 1H), 3.6 ($C\underline{H}_3$-O, s, 3H), 4.1 ($CHC\underline{H}_2O$, 2H), 7.35 and 7.8 (aromatic $C\underline{H}$s, 2xd, 4H); **NMR:**δ_C ($CDCl_3$) 13.6 ($\underline{C}H_3CH$), 21.6 (Ar-$\underline{C}H_3$), 39.2 ($\underline{C}H$), 52.0 ($\underline{C}H_3O$), 70.8 ($\underline{C}H_2O$), 127.9 and 129.9 (aromatic $\underline{C}H$), 132.8 (aromatic $\underline{C}$-CH_3), 145.0 (aromatic $\underline{C}$-S), 173.0 ($\underline{C}$=O); **EIMS:** m/z 272 (8%, $M^{+\bullet}$), 241 (2%, [M-$CH_3O^{\bullet}]^+$), 187 (10%), 172 (12%, $T_sOH^{+\bullet}$), 155 (80%, $CH_3(C_6H_4)\overset{+}{S}O_2$), 117 (57%, [M-$CH_3(C_6H_4)^{\bullet}SO_2]^+$), 91 (100%, $C_7H_7^+$), 85 (30%, $CH_3OC\overset{+}{O}C{=}CH_2$, and 69 (20%, O=C=C ($CH_3$) $\overset{+}{C}H_2$).

Preparation of (R)-(+)-methyl 3-iodo-2-methylpropionate (53) from tosylate (52).

The procedure was exactly the same as that used for the preparation of iodide (49) except that it was performed on a 5 mmol scale. Thus, lithium iodide (2.737 g, 20.45 mmol), tosylate (52) (1.351g, 5 mmol) and acetone (20 ml) were used. The work-up, also scaled accordingly, gave 0.764 g

(63%) of iodide (53) as a pale yellow liquid (pure by GC). (Found: C, 26.68; H, 4.05. $C_5H_9IO_2$ requires C, 26.34; H, 3.98). $[\alpha]_D^{20}$ + 21° (c=2.0, MeOH); **IR:** ν_{max} (thin film) 1740 ($C{=}O_{STR}$, ester), 1210 and 1160 cm^{-1} ($C(=O)\text{-}O\text{-}C_{STR}$); **NMR:** δ_H ($CDCl_3$) 1.25 ($C\underline{H}_3$-CH, d, 3H), 2.80 (C<u>H</u>, m, 1H), 3.3 ($C\underline{H}_2$-I, m, 2H), 3.7 ($C\underline{H}_3$-O, s, 3H); **NMR:** δ_C ($CDCl_3$) 6.8 ($\underline{C}H_2$-I), 18.1 ($\underline{C}H_3$-CH), 42.2 (<u>C</u>H), 52.0 ($\underline{C}H_3$-O), 173.7 (<u>C</u>=O); **EIMS:** m/z 228 (28%, $M^{+\bullet}$), 197 (8%, $[M\text{-}CH_3O^{\bullet}]^+$), 169 (26%, $[M\text{-}{}^{\bullet}COOCH_3]^+$), 127 (9%, I^+), 101 (100%, $[M\text{-}I^{\bullet}]^+$), 73 (20%), 59 (75%, $CH_3OC{\equiv}O^+$) and 41 (30%).

8.3.2 Derivatizations using achiral and homochiral alkyl halides under KOH/DMSO reaction conditions

Derivatization reaction; general procedure

To finely ground potassium hydroxide (0.2244g, 4 mmol) in a dry 5ml round bottomed flask was added DMSO (2ml). After 5 minutes stirring, racemic 2-hexanol (0.126 ml, 1 mmol) was added followed by the alkyl halide (2 mmol). The reaction was stirred and samples (0.5 ml) were taken at specified times (see later). Each sample was added to water (1 ml) and the product was extracted into chloroform. After drying (cotton wool), the product was analysed by GC and in some cases by GC/MS.

Reactions using the model (achiral) reagents.

The following quantities of alkyl halides were used:

(a) Isobutyl chloride (0.12 ml, 2 mmol)

(b) Isobutyl iodide (0.238 ml, 2 mmol)

(c) (S)-(+)-1-bromo-2-methylbutane (0.25 ml, 2 mmol)

The reaction mixtures were sampled after 30 minutes, 90 minutes and 19 hours. The GC results obtained for reaction (c) are summarised in Table 5 (Chapter 3).

Reactions using 1-halobutanes

The following quantities of each halobutane were used:

(a) 1-chlorobutane (0.21 ml, 2 mmol)
(b) 1-bromobutane (0.215 ml, 2 mmol)
(c) 1-iodobutane (0.23 ml, 2 mmol)

The reaction mixtures were sampled after 2 minutes, 15 minutes, 90 minutes, $3^1/_2$ hours and 19 hours. The GC results are summarised in Tables 6 and 7 (Chapter 3).

Reactions using the homochiral reagents

The following quantities of each halide were used:

(a) chloride (36) (70 mg, 0.31 mmol)
(b) bromide (37) (82 mg. 0.31 mmol)
(c) iodide (38) (96 mg, 0.31 mmol)
(d) bromide (39) (85 mg, 0.31 mmol)
(e) bromide (46) (81 mg, 0.31 mmol)
(f) lactone iodide (49) (0.226g, 1 mmol)
(g) lactone bromide (50) (0.179g, 1 mmol)
(h) chloride (42) (50 mg, 0.31 mmol)
(i) bromide (43) (64 mg, 0.31 mmol)

The quantities of the other reagents were: 2-hexanol (18.5µl, 0.15 mmol), KOH (35 mg, 0.62 mmol) and DMSO (1 ml) for reactions (a)-(e) and (h)-(i). For reactions (f) and (g), 2-hexanol (30 mg, 0.3 mmol), KOH (0.1122g, 2 mmol) and DMSO (1 ml). The reaction mixtures were sampled after 30 minutes and 19 hours. The samples were worked-up (as described earlier) and the products from reactions (a)-(e) were analysed by GC on a SE-30 glass capillary (33 m x 0.25 mm) using the Varian 6000 gas chromatograph (180$\longrightarrow$240°C at 5°C min^{-1}). Incorrect products were observed.

Reactions (f) and (g) were analysed by GC/MS (VG 20-250), with the results indicating that incorrect products had been formed. Reactions (h) and (i) were analysed by GC/MS (see C2, MS3 and MS4). Incorrect products were identified (see Chapter 3).

Reaction of (S)-(+)-1-bromo-2-methylbutane with racemic compound (61)

Racemic compound (61) (8.4 mg, 0.03 mmol) was derivatized with (S)-(+)-1-bromo-2-methylbutane (15 mg, 0.12 mmol) under the KOH/DMSO reaction conditions (10 mg, 0.18 mmol in 0.5 ml respectively). After 3 hours the reaction mixture was worked-up then analysed by GC/MS. The results indicated that the reaction had been partially successful (see C3, MS6 and MS7).

8.3.3 Derivatizations using achiral and homochiral alkyl halides under the $Hg(BF_4)_2$ reaction conditions

Preparation of the $Hg(BF_4)_2$ reagent[196]

$$Hg^{II}O + 2HBF_4 \longrightarrow Hg(BF_4)_2 + H_2O$$

Yellow mercury(II) oxide (4.3 g, 20 mmol) was added, with stirring, to 48% tetrafluoroboric acid (7.3 g, 40 mmol). The resultant yellow solution was put under high vacuum to remove the water. The white very hygroscopic solid was kept under vacuum until required.

General derivatization procedure

A solution of alkan-2-ol (0.5 mmol) and halide (1 mmol) in dichloromethane (2 ml) was added to dry mercury(II) tetrafluoroborate (0.19 g; 0.5 mmol) in a 5 ml round bottomed flask. The mixture was stirred for 2 hours at room temperature before being treated with 3M potassium hydroxide until basic. The regenerated mercury (II) oxide was filtered off and the organic layer was separated and dried before undergoing GC and GC/MS analyses.

The following reagents were used:

(a) Benzyl alcohol (0.54 g, 5 mmol) and isopropyl bromide (1.23 g, 10 mmol).

(b) n-Octanol (0.65 g, 5 mmol) and 2-bromobutane (1.37 g, 10 mmol).

(c) Racemic 2-octanol (65 mg, 0.5 mmol) and lactone iodide (49) (0.226g, 1 mmol).

(d) (R)-(-)-2-octanol (65 mg, 0.5 mmol) and lactone iodide (49) (0.226 g, 1 mmol).

(e) (S)-(+)-2-octanol (65 mg, 0.5 mmol) and lactone iodide (49) (0.226 g 1 mmol).

(f) Racemic 2-octanol (65 mg, 0.5 mmol) and iodide (53) (0.244 g, 1 mmol).

The estimated % yields and chromatographic results are summarised and discussed in Section 3.2.3 (see also chromatograms C4 and C5 and mass spectra MS8 and MS9).

Reaction of racemic 2-methylhexanoic acid with lactone iodide (49)

The procedure was the same as that used for the alcohol derivatizations except that racemic 2-methylhexanoic acid (65 mg, 0.5 mmol) was used. GC analyses (BP-5, 12m x 0.33mm, 0.5μm film, at 100 (2 min)$\longrightarrow$230 ^{o}C at 10^{o}C min^{-1}) showed 4 product peaks, one with a retention time of 11.36 min (see the results from the reaction of lactone iodide (49) with sodium 2-methylhexanoate).

Synthesis of racemic sodium 2-methylhexanoate

To 2-methylhexanoic acid (2.604 g, 20 mmol) was added 4M sodium hydroxide solution (5 ml, 20 mmol). The reaction mixture warmed initially. After 2 hours stirring, the water was removed under high vacuum (0.1 mmHg for several hours) to yield 2.826 g (93%) of racemic sodium 2-methyl hexanoate as a white solid Mpt=48-52^{o}C. **IR:** ν_{max} (KBr disc) 3360 (OH_{STR}, water of crystallisation), loss of absorbance at 3500-2300 (OH_{STR}, acid), loss of absorbance at 1710 ($C{=}O_{STR}$, acid) and gain of band at 1550 cm^{-1} ($C{=}O_{STR}$, carboxylate).

Reaction of lactone iodide (49) with racemic sodium 2-methylhexanoate

To racemic sodium 2-methylhexanoate (70 mg, 0.5 mmol) in dry HMPA (1 ml) in a flask under a nitrogen atmosphere and cooled on ice, was added lactone iodide (49) (190mg, 0.75 mmol). The reaction mixture was slowly allowed to warm to room temperature then stirred for 3 hours. Diethyl ether

(5 ml) was added and the mixture was washed with water (2x5 ml) and sodium thiosulphate solution (5 ml). After drying ($MgSO_4$), the solution was reduced to 1 ml by rotary evaporation and analysed directly by GC (BP-5 column, 12m x 0.33mm, 0.5μm film, at 100 (2 min)⟶230°C at 10°C (min^{-1}). Three peaks were observed at retention times corresponding to those of 2-methylhexanoic acid, lactone iodide (49) and diastereoisomeric products (66a) and (66b) (at a retention time of 11.47 minutes) which were not separated.

8.4 Synthesis and use of homochiral chloromethyl ether derivatizing agents

8.4.1 Synthesis of homochiral chloromethyl ethers

(1S,2R,5S)-(+)-Menthyl chloromethyl ether (67) from (1S,2R,5S)-(+)-menthol

This method follows work performed to prepare benzyl CME.[204] Menthol (6.876 g, 0.044 mol) was added to aqueous formaldehyde solution (37 wt. %) (4.8 g, 0.04 mol of usable formaldehyde*) with extra water (10 ml). The mixture was stirred and cooled to between -5° and 0°C using an ice salt bath. HCl gas was slowly bubbled through the mixture while keeping the temperature below 10°C until saturation. At this stage it was noted that the solid menthol phase that had been floating on top of the aqueous phase had liquefied (i.e. the products were in a liquid form).

(* Formaldehyde solution contains 10-15% methanol. This will also react with the formaldehyde to form methyl CME which is fairly volatile and is thus lost on work-up. Quantities of formaldehyde solution take into account the methanol present).

After a further hour of HCl gas treatment the mixture was allowed to stand. The organic layer was separated, and the aqueous layer was extracted once with diethyl ether. The extract was combined with the organic product and this was dried with anhydrous magnesium sulphate, filtered and rotary evaporated to yield 7.198 g (81%) as a clear oily product. **NMR:** δ_H analysis of the raw product indicated that a mixture of products had been formed but that the mixture contained the required product as shown by peaks at 5.4 (O–C$\underline{H_2}$–Cl) and a singlet at 4.7 (possibly O–C$\underline{H_2}$–O).

Vacuum distillation (68°C/0.2 mmHg) gave 1.044 g (12%) of a product that appeared pure by NMR. **NMR:** δ_H ($CDCl_3$) peak at 5.4 observed but peak at 4.7 not seen). **EIMS:** analysis of this product indicated that a mixture of compounds was present (or had been formed in the hot injection zone of the GC or in the hot ion source of the mass spectrometer (see discussion 3.2)); **GC:** with an on-column injection system indicated that the product was 95% pure and that menthol was the only volatile contaminant.

Attempted synthesis of N-acetyl-(S)-(-)-prolyl chloromethyl ether (68) from N-acetyl-(S)-(-)-pyrrolidine methanol (41)

The procedure was the same as for the preparation of (1R, 2S, 5R)-(+)-menthyl CME except that it was carried out on an 11 mmol scale (using 1.57 g of alcohol). On completion of the reaction it was found that the products were water soluble, and that isolation of the products by solvent extraction was ineffective. Water was removed under vacuum to leave a thick brown oil. **IR:** ν_{max} (thin film) 1600 ($C{=}O_{STR}$, amide), 735 cm^{-1} (C–Cl); **EIMS:** m/z 125 $(M–HOCH_2Cl)^+$; 112 $(M-{}^{\bullet}CH_2OCH_2Cl)^+$, 70 $(M–{}^{\bullet}CH_2OCH_2Cl–CH_2CO)^+$, Cl isotope splitting not observed; **NMR:** δ_H (CD_3OD) singlet at 5.4 (OC$\underline{H_2}$Cl) is not observed.

Attempted synthesis of (S)-(+)-tetrahydro-5-oxo-2-furanmethyl chloromethyl ether (69) from (S)–(+)–tetrahydro-5-oxo-2-furanmethyl alcohol (47).

The procedure was the same as that used for the preparation of (1R, 2S, 5R)–(+)–menthyl CME except that it was carried out on a 5.6 mmol scale (using 0.65 g of alcohol). **NMR:** δ_H ($CDCl_3$) no peak observed at 5.4 (O–CH_2–Cl); **NMR:** δ_C ($CDCl_3$) 11 peaks observed (only 6 expected).

Synthesis of N-acetyl-(S)-(-)-prolyl chloromethyl ether (68) from N-acetyl-(S)-(-)-pyrrolidine methanol (41) via its MTM-ether derivative (72)

Work was performed following procedures described in literature,[200,201] the first step being the formation of an O,S-acetal (MTM-ether).

N-Acetyl-(S)-(-)-pyrrolidine methanol (41) (1.43 g, 10 mmol) in dimethoxy ethane (DMOE; 6 ml) was added to a magnetically stirred suspension of sodium hydride (0.48 g, 20 mmol) in DME (9 ml) at 0°C under argon.

Sodium iodide (1.48 g, 9.87 mmol) and chloromethyl methyl sulphide (0.95 g, 9.87 mmol) were added (which form iodomethyl methyl sulphide *in situ*) and the reaction was left stirring for 1 hour at 0°C. The reaction mixture was then allowed to warm up to room temperature over 4 hours, before being poured into water (20 ml) and extracted into diethyl ether (2x10 ml). The combined extracts were washed with brine, dried with anhydrous potassium sulphate and the solvent was removed by rotary evaporation to yield a mixture of products by TLC (silica plates, chloroform or methanol as solvents). The product was purified on a silica column, eluting with chloroform to yield 95 mg (4.7%) as an oil. **NMR:** δ_H ($CDCl_3$) showed a peak at 4.68 (may indicate the presence of O–C<u>H</u>$_2$–S).

The O,S-acetal (MTM-ether (72)) was converted into its CME derivative by reaction with sulphuryl chloride (55 mg; 41 mmol) in DME (1 ml) at room temperature. This was complete after 20 minutes, and removal of the solvent gave 88 mg (~100%) as a dark liquid. **NMR:** δ_H ($CDCl_3$) indicates a loss of the peak at 4.6 (O–C<u>H</u>$_2$–S), and appearance of a peak at 5.4 (O–C<u>H</u>$_2$–Cl).

Synthesis of (1S, 2R, 5S)–(+)–menthyl thiomethyl ether (74)

To (1S, 2R, 5S)–(+)–menthol (1.872 g, 12 mmol) in a round-bottomed flask, was added DMSO (60 ml) and acetic anhydride (60 ml). The reaction was stirred at room temperature for 30 hours (monitoring by GC) before pouring into water (200 ml). The product plus excess acetic anhydride were extracted into chloroform (100 ml), which was then washed 5 times with water (100 ml). After drying ($MgSO_4$), the solvent and acetic anhydride were removed by rotary evaporation (at 80°C). Applying a high vacuum and warming the flask removed the last traces of any remaining menthol (starting material) to leave 2.146 g (78%) as a clear liquid (Found: C, 65.93; H, 11.54. $C_{12}H_{24}OS$ requires C, 66.61; H, 11.18%). $[\alpha]_D^{20}$ + 199^0 (c = 2.0, EtOH); **IR:** ν_{max} (thin film) loss of O–HSTR, (3400, alcohol), 1050 cm^{-1} (C–O–CSTR, ether); **NMR:** δ_H ($CDCl_3$) 0.7–2.7 (C<u>H</u>$_3$, C<u>H</u>$_2$ and C<u>H</u>, various complex signals from menthyl unit, 18H), 2.15 (S–C<u>H</u>$_3$, s, 3H), 3.5 (C<u>H</u>–O, dt, 1H), 4.6 (O–C<u>H</u>$_2$–S, s, 2H); **NMR:** δ_C ($CDCl_3$) 12 signals observed as expected. Diagnostic signals at 14.1 (S<u>C</u>H$_3$), 72.3 (<u>C</u>HO), 75.9 (O<u>C</u>H$_2$S); **EIMS:** m/z 216 (1.5%, $M^{+\bullet}$), 169 (18%, $[M–CH_3S^{\bullet}]^+$), 139 (24%, $[M–{}^{\bullet}CH_2OCH_2SCH_3]^+$), 111 (5%, $C_8H_{15}^+$), 97 (18%, $C_7H_{13}^+$), 83 (100%, $CH_2{=}CH\text{-}\overset{+}{C}H–CH(CH_3)_2$), 69 (27%, $C_5H_9^+$), 61 (22%, $CH_2\text{-}\overset{+}{S}CH_3$), 55 (25%, $C_4H_7^+$) and 41 (10%, $C_3H_5^+$).

Synthesis of homochiral MTM-ethers (72), (73), (75) and (76): general procedure

To the homochiral alcohol (20 mmol) in chloroform (60 ml) was added diisopropylethylamine (12.9 g (17.5 ml), 100 mmol) and chloromethyl methyl sulphide (5.8 g (5.1 ml), 60 mmol) under an argon atmosphere. The mixture was refluxed for 3-4 hours until the reaction mixture contained a maximum quantity of product (typically 60-70%) with respect to the starting material and by-products (as monitored by GC). The cooled reaction mixture was washed with 1M hydrochloric acid (100 ml) and water (200 ml) then dried ($MgSO_4$). The solvent was removed and the product purified by distillation. The yields and analytical data for compounds (72), (73), (75) and (76) are as follows.

N-Acetyl-(S)-(-)-2-pyrrolidinemethyl methylthiomethyl ether (72)

Prepared from N-acetyl-(S)-(-)-2-pyrrolidinemethanol (2.86 g, 20 mmol). Distillation (140°C/0.1 mm Hg) afforded 1.04 g (26%) MTM-ether (72) as a pale yellow oil (Found: C, 52.23; H, 8.40; N, 6.73; S, 13.56. $C_9H_{17}O_2S$ requires C, 53.17; H, 8.43; N, 6.89; S, 15.8%). $[\alpha]_D^{20}$ -80° (c = 0.35, EtOH); **IR:** ν_{max} (thin film) loss of O-HSTR, (3400, alcohol), 1,650 (C=OSTR, amide), 1420 (C-NSTR, amide), 1070 cm^{-1} (C-O-CSTR, ether); **NMR:** δ_H ($CDCl_3$) 1.6-2.1 ($C\underline{H}_2$–$C\underline{H}_2$, m, 4H), 2.05 ($C\underline{H}_3C$=O, s, 3H), 2.15 (S–$C\underline{H}_3$, s, 3H), 3.2-3.75 ($C\underline{H}_2$-O + $C\underline{H}_2$-N, m, 4H), 4.25 ($C\underline{H}$, m, 1H), 4.6 (O–$C\underline{H}_2$–S, s, 2H); **NMR:** δ_C ($CDCl_3$) 14.0/14.39 (S–$\underline{C}H_3$), 22.4 and 22.8/22.9 ($\underline{C}H_2$-$\underline{C}H_2$), 27.86/28.95 ($\underline{C}H_3C$=O), 45.6/48.5 ($\underline{C}H_2$–N), 56.1/57.6 and 68.1/69.1 ($\underline{C}H_2$–O + $\underline{C}$H–N), 75.6 (O–$\underline{C}H_2$–S), 169.4 ($\underline{C}$=O); **EIMS:** m/z 188 (1%, $[M-{}^{\bullet}CH_3]^+$), 156 (3%, $[M-CH_3S^{\bullet}]^+$), 142 (24%, $[M-{}^{\bullet}CH_2SCH_3]^+$), 127 (34%, $[M-CH_3SCH=O]^{+\bullet}$), 112 (40%, $[M-{}^{\bullet}CH_2OCH_2SCH_3]^+$), 100

(13%), 84 (16%), 70 (100%, [M – ($CH_2C{=}O$ + $^{\bullet}CH_2OCH_2SCH_3$)]$^+$) and 61 (13%, $CH_2{=}\overset{+}{S}CH_3$).

(S)-(+)-Tetrahydro-5-oxo-2-furan methyl methyl thiomethyl ether (73)

Prepared from (S)-(+)-tetrahydro-5-oxo-2-furan methylalcohol (2.32 g, 20 mmol). Distillation (130°C/0.15 mm Hg) afforded 1.625 g (46%) MTM-ether (73) as a pale yellow liquid (Found: C, 47.65; H, 7.01; S, 18.08. $C_7H_{12}O_3S$ requires C, 47.71; H, 6.86; S, 18.18%). $[\alpha]_D^{20}$ + 20° (c = 1.0, EtOH); **IR:** ν_{max} (thin film) loss of O-HSTR, (3400, alcohol), 1,780 (C=OSTR, lactone), 1180 (C(=O)–O–CSTR, ester), 1080 cm^{-1} (C–O–CSTR, ether); **NMR:** δ_H ($CDCl_3$) 2.0–2.7 ($C\underline{H}2–C\underline{H}_2$, m, 4H), 2.15 ($S–C\underline{H}_3$, s, 3H), 3.6 ($C\underline{H}_2–O$, m, 2H), 4.6 ($O–C\underline{H}_2–S$, s, 2H + $C\underline{H}$, m, 1H); **NMR:** δ_C ($CDCl_3$) 14.1 ($S–\underline{C}H_3$), 24.3 ($CH_2–\underline{C}H_2–CH$), 28.6 ($\underline{C}H_2C{=}O$), 69.5 (O–$\underline{C}H$), 75.9 ($O–\underline{C}H_2–S$), 79.0 ($\underline{C}H_2–O$), 177.6 ($\underline{C}{=}O$); **EIMS:** m/z 176 (28%, $M^{+\bullet}$), 146 (43%, [M – CH_2O]$^{+\bullet}$), 129 (57%, [M – $CH_3S^{\bullet}$]$^+$), 114 (16%, [M – CH_3SCH_3]$^{+\bullet}$), 99 (100%, [M – $CH_3SCH_2O^{\bullet}$]$^+$), 85 (83%, [M – $^{\bullet}CH_2OCH_2SCH_3$]$^+$), and 61 (1%, $CH_2{=}\overset{+}{S}CH_3$).

Mass spectral data used to identify compound (77) as by-product of methyl thiomethyl ether (73)

EIMS: m/z 173 (1%, [M – $H^{\bullet}$]$^+$), 144 (3%, [M – CH_3CH_3]$^{+\bullet}$), 129 (7%, [M – $CH_3CH_2O^{\bullet}$]$^+$), 100 (39%, [M – $CH_3CH_2OCH{=}O$]$^+$), 85 (47%, [M – $^{\bullet}CH_2OCH_2OCH_2CH_3$]$^+$), and 59 (100%, $CH_3CH_2\overset{+}{O}{=}CH_2$).

(S)-(+) 2-Acetoxy propyl methyl thiomethyl ether (75)

Prepared from (S)-(+) methyl 3-hydroxy-2-methyl propionate (2.36 g, 20 mmol). Distillation (50-55°C/0.25 mm Hg) afforded 0.9 g (25%) MTM-

ether (75) as a clear liquid (Found: C, 47.14; H, 8.11; S, 17.89. $C_7H_{14}O_3S$ requires C, 47.17; H, 7.92; S, 17.9%). $[\alpha]_D^{20}$ + 27° (c = 0.6, MeOH); **IR:** ν_{max} (thin film) loss of O-H$_{STR}$ (3400, alcohol), 1720 (C=O$_{STR}$, ester), 1190 (C(=O)-O-C$_{STR}$, ester), 1060 (C-O-C$_{STR}$, ether); **NMR:** δ_H ($CDCl_3$) 1.1 ($C\underline{H}_3CH$, d, 3H), 2.1 (S-$C\underline{H}_3$, s, 3H), 2.7 ($C\underline{H}$, m, 1H), 3.7 ($C\underline{H}_3$-OCO + $C\underline{H}_2$-O, s + m, 5H), 4.6 (O-$C\underline{H}_2$-S, s, 2H); **NMR:** δ_C ($CDCl_3$) 13.73 ($\underline{C}H_3$-CH), 14.02 (S-$\underline{C}H_3$), 39.92 ($\underline{C}H$), 51.76 ($\underline{C}H_3OCO$), 69.74 ($\underline{C}H_2$-O), 75.37 (O-$\underline{C}H_2$-S), 175.1 ($\underline{C}$=O).

(S)-(-)-1-Acetoxy ethyl methyl thiomethyl ether (76)

Prepared from (S)-(-)-methyl lactate (2.08 g, 20 mmol). Distillation (68°C/5.5 mm Hg) afforded 1.292 g (40%) MTM-ether (76) as a clear liquid (Found: C, 43.84; H, 7.37; S, 19.28. $C_6H_{12}O_3S$ requires C, 43.88; H, 7.37; S, 19.51%). $[\alpha]_D^{20}$ - 158° (c = 2.0, $CHCl_3$); **IR:** ν_{max} (thin film) loss of O-H$_{STR}$, (3400, alcohol), 1730 (C=O$_{STR}$, ester), 1200 (C(=O)-O-C $_{STR}$, ester), 1100 cm^{-1} (C-O-C$_{STR}$, ether); **NMR:** δ_H ($CDCl_3$) 1.4 ($C\underline{H}_3$-CH, d, 3H), 2.15 (S-$C\underline{H}_3$, s, 3H), 3.75 ($C\underline{H}_3$-OCO, s, 3H), 4.35 ($C\underline{H}$, q, 1H), 4.7 (O-$C\underline{H}_2$-S, s, 2H); **NMR:** δ_C ($CDCl_3$) 13.9 (S-$\underline{C}H_3$), 18.4 ($\underline{C}H_3$-CH), 51.9 ($\underline{C}H_3OCO$), 71.1 ($\underline{C}$H-O), 74.3 (O-$\underline{C}H_2$-S), 173.3 ($\underline{C}$=O); **EIMS:** m/z 117 (100%, [M - $CH_3S^{\bullet}$]$^+$), 89 (95%, $CH_3OCOCH{=}\overset{+}{O}H$), 70 (30%), 61 (13%, $CH_2{=}\overset{+}{S}CH_3$), 59 ($^+COOCH_3$) and 45 (55%).

Synthesis of homochiral chloromethyl ethers (67)-(69), (79) and (80): general procedure

The CMEs were prepared in small quantities from the MTM-ethers when required. Sulphuryl chloride (0.34 g 2.5 mmol) in DCM (5 ml) was added dropwise to a stirred solution of the homochiral MTM-ether (72)-(76) (2.5 mmol) in DCM (8 ml). A small amount of effervescence (SO_2) was

observed on addition. After stirring for 30 minutes at room temperature, the solvent and methanesulphenyl chloride (by-product) were removed by rotary evaporation, and high vacuum (see discussion). The CME products were 90-95% pure by GC analysis, and were used in the derivatization reactions without further purification. Yields and analytical data are presented as follows.

(1S, 2R, 5S)-(+)-Menthyl chloromethyl ether (67)

Prepared from (1S, 2R, 5S)-(+)-menthyl MTM ether (74) (0.54g, 2.5 mmol). Yield: 0.468 g (91%) as a clear liquid. $[\alpha]_D^{20}$ +31° (c = 1.0, $CHCl_3$); **IR:** ν_{max} (thin film) 1,115 (C-O-CSTR, ether), 640 cm^{-1} (C-Cl); **NMR:** δ_H ($CDCl_3$) 0.7-2.3 ($C\underline{H}_3$, $C\underline{H}_2$ and $C\underline{H}$, various complex signals from the menthyl unit, 18H), 3.55 ($C\underline{H}$-O, dt, 1H), 5.55 (O-$C\underline{H}_2$-Cl, s, 2H); **NMR:** δ_C ($CDCl_3$) 15-48 (9 signals from the menthyl unit), 78.97 ($\underline{C}$H-O), 81.2 (O-$\underline{C}H_2$-Cl).

N-Acetyl (S)-(-)-2-pyrrolidine methyl chloromethyl ether (68)

Prepared from N-acetyl-(S)-(-)-2-pyrrolidinemethyl MTM ether (72) (0.51 g, 2.5 mmol). Yield: 0.464 g (97%) as a clear oil (Found: C, 40.81; H, 7.11; N, 5.38; Cl, 18.52. $C_8H_{14}NO_2Cl$ requires C, 50.14; H, 7.36; N, 7.31; Cl, 18.50%). **IR:** ν_{max} (thin film) 1640 (C=OSTR, amide), 1420 (C-NSTR, amide), 1040 (C-O-CSTR, ether), 640 cm^{-1} (C-ClSTR); **NMR:** δ_H ($CDCl_3$) 1.8-2.2 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 2.2 ($C\underline{H}_3CO$, s, 3H), 3.5 ($C\underline{H}_2$-N, t, 2H), 3.75 ($C\underline{H}_2$-O, d, 2H), 4.25 ($C\underline{H}$, m, 1H), 5.4 (O-$C\underline{H}_2$-Cl, s, 2H); **EIMS:** m/z 191/3 (1%, $M^{+\bullet}$), 156 (13%, $[M - Cl]^+$), 126 (28%, $[M - ClCH_2O^\bullet]^+$), 112 (95%, $[M - {}^\bullet CH_2OCH_2Cl]^+$), 70 (100%, $[M - (CH_2C{=}O + {}^\bullet CH_2OCH_2Cl)]^+$) and 36/38 (20%, HCl^+).

(S)-(+)-Tetrahydro-5-oxo-2-furan methyl chloromethyl ether (69)

Prepared from (S)-(+)-tetrahydro-5-oxo-2-furan methyl MTM ether (73) (0.44 g, 2.5 mmol). Yield: 0.3908 g (95%) as a clear liquid (Found: C, 43.89; H, 6.04; Cl, 18.63. $C_6H_9O_3Cl$ requires C, 43.79; H, 5.51; Cl, 21.54%). $[\alpha]_D^{20}$ + 23° (c = 1.0, $CHCl_3$); **IR:** ν_{max} (thin film) 1780 (C=OSTR, lactone), 1,180 (C(=O)-O-CSTR, ester), 1070 (C-O-CSTR, ether), 650 cm^{-1} (C-ClSTR); **NMR:** δ_H ($CDCl_3$) 2.05-2.8 ($C\underline{H}_2$-$C\underline{H}_2$, m, 4H), 3.8 ($C\underline{H}_2$-O, m, 2H), 4.65 ($C\underline{H}$, m, 1H), 5.45 (O-$C\underline{H}_2$-S, s, 2H); **NMR:** δ_C ($CDCl_3$) 23.7 (CH_2-$\underline{C}H_2$-CH), 28.2 ($\underline{C}H_2C$=O), 69.2 (O-$\underline{C}$H), 78.6 ($\underline{C}H_2$-O), 95.4 (O-$\underline{C}H_2$-Cl), 177.0 ($\underline{C}$=O); **EIMS:** m/z 129 (7%, $[M - Cl^{\bullet}]^+$), 114 (7%, $[M - CH_3Cl]^{+\bullet}$), 99 (7%, $[M - ClCH_2O^{\bullet}]^+$), 85 (100%, $[M - {}^{\bullet}CH_2OCH_2Cl]^+$), 49/51 (6%, CH_2Cl^+) and 36/38 (4%, HCl^+).

(S)-(+)-2-Acetoxy propyl chloromethyl ether (79)

Prepared from (S)-(+)-2-acetoxy propyl MTM ether (75) (0.445 g, 2.5 mmol). Yield: 0.387 g (93%) as a pale yellow liquid. **IR:** ν_{max} (thin film) 1745 (C=OSTR, ester), 1200 (C(=O)-O-C STR, ester), 1125 (C-O-CSTR, ether), 640 cm^{-1} (C-ClSTR); **NMR:** δ_H ($CDCl_3$) 1.2 ($C\underline{H}_3CH$, d, 3H), 2.7 ($C\underline{H}$, m, 1H), 3.7 ($C\underline{H}_3$-OCO + $C\underline{H}_2$-O, s + d, 5H), 5.4 (O-$C\underline{H}_2$-Cl, s, 2H); **NMR:** δ_C ($CDCl_3$) 13.84/14.0 ($\underline{C}H_3$-CH), 39.5/40.1 ($\underline{C}$H), 51.78/51.9 ($\underline{C}H_3OCO$), 69.7/71.9 ($\underline{C}H_2$-O), 82.8 (O-$\underline{C}H_2$-Cl), 175.1 ($\underline{C}$=O).

(S)-(-)-1-Acetoxy ethyl chloromethyl ether (80)

Prepared from (S)-(-)-1-Acetoxy ethyl MTM ether (76) (0.41 g, 2.5 mmol). Yield: 0.365 g (95%) as a pale yellow liquid (Found: C,39.49; H, 6.10. $C_5H_9O_3Cl$ requires C, 39.36; H, 5.95%). $[\alpha]_D^{20}$ -129° (c = 1.0, $CHCl_3$); **IR:** ν_{max} (thin film) 1730 (C=OSTR, ester), 1200 (C(=O)-O-CSTR, ester),

1100 cm^{-1} (C-O-CSTR, ether); **NMR:** δ_H ($CDCl_3$) 1.45 ($C\underline{H}_3CH$, d, 3H) 3.8 ($C\underline{H}_3$-OCO, s, 3H), 4.45 (C$\underline{H}$, q, 1H), 5.55 (O-$C\underline{H}_2$-Cl, s, 2H); **NMR:** δ_C ($CDCl_3$) 18.0 ($\underline{C}H_3$-CH), 52.0 ($\underline{C}H_3OCO$), 72.7 ($\underline{C}$H-O), 80.8 (O-$\underline{C}H_2$-Cl), 172 ($\underline{C}$=O).

8.4.2 Derivatization of enantiomeric mixtures of chiral alcohols, amines, acids and thiols using homochiral chloromethyl ethers

Preparation of the equimolar mixture of racemic secondary alcohols

The mixture was prepared according to the procedure given in Section 8.1.3.

Preparation of the non-racemic mixtures of R- and S-2-octanol

99.8, 99.0 and 95.0% mixtures of R- and S-2-octanol were prepared using commercially available enantiomerically pure R- and S-2-octanol (99%, Aldrich) as follows.

Separate stock solutions containing R- and S-2-octanol in dichloromethane (320 mg in 10 ml) were prepared (i.e. 0.5 ml contains 16 mg (0.125 mmol) alcohol). The required mixtures were prepared using the two stock solutions thus:

998 µl of one solution + 2 µl of the other produces a 99.8% mixture
990 µl of one solution + 10µl of the other produces a 99.0% mixture
950 µl of one solution + 50µl of the other produces a 95.0% mixture

Derivatization procedure (general)

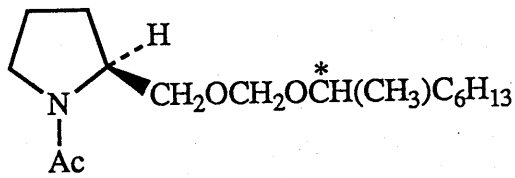
To a solution of the analyte (i)-(vi) (0.125 mmol) in DCM (0.5 ml) under an argon atmosphere was added diisopropylethylamine (80 mg, 0.62 mmol) and

a chloromethyl ether (67)-(69), (79) or (80) (0.377 mmol) in DCM (0.5 ml). The mixture was allowed to react for 5 hours before taking a sample (0.2 ml). The solvent was removed from the sample (Ar gas) and the product was extracted from hydrochloric acid (2 ml of a 0.5M solution) into ethyl acetate (1 ml). After washing with water and drying (through cotton wool) the sample underwent GC/MS and/or GC analysis.

Equimolar mixture of racemic secondary alcohols (i) derivatized with CMEs (67)-(69), (79) and (80).

The reaction was performed using the mixture of secondary alcohols (14 mg, 0.125 mmol) producing a yield ~90-100% (by GC) after 5 hours.
See C6-C9 for examples of chromatograms obtained.
Also see MS10 and MS11 for examples of mass spectra.
See Tables 11 and 11a (Section 4.4) for an assessment of the chromatographic results obtained. Mass spectra confirming the identity of the individual diastereoisomers were obtained using GC/MS.

EXAMPLE 1 Diastereoisomers (83) (Mr = 285).

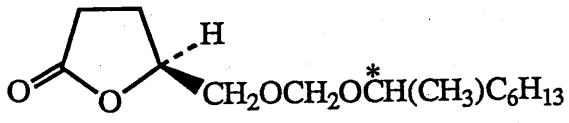


(*= chiral centre, R or S)

EIMS: m/z 285 (0.2%, $M^{+\bullet}$), 254 (0.43%), 242 (0.2%, $[M - {}^{\bullet}COCH_3]^+$), 172 (2%, $[M - C_8H_{17}]^+$), 156 (15%, $[M - C_8H_{17}O^{\bullet}]^+$), 142 (16%, $[M - C_8H_{17}O\dot{C}H_2]^+$), 126 (18%, $[M - C_8H_{17}OCH_2O^{\bullet}]^+$), 112 (78%, $[M - C_8H_{17}OCH_2O\dot{C}H_2]^+$) and 70 (100%, [M - ($C_8H_{17}OCH_2O\dot{C}H_2$ +

$CH_2CO)]^+$). Both the SS and the SR diastereoisomers had virtually identical mass spectra.

EXAMPLE 2 Diastereoisomers (84) (Mr = 258)



(*= chiral centre, R or S)

EIMS: m/z 214 (0.03%, $[M - CO_2]^{+\bullet}$), 199 (0.2%, $[M - (CO_2 + {}^{\bullet}CH_3)]^+$), 173 (6%, $[M - C_6H_{13}{}^{\bullet}]^+$), 143 (5%, $C_6H_{13}CH(CH_3)\overset{+}{O}{=}CH_2$), 129 (100%, $[M - C_6H_{13}CH(CH_3)O^{\bullet}]^+$), 99 (30%, $[M - C_6H_{13}CH(CH_3)\text{-}OCH_2O^{\bullet}]^+$), 71 (32%, $C_5H_{11}^+$), 57 (30%, $C_4H_9^+$) and 43 (30%, $C_3H_7^+$). Both the SS and the SR diastereoisomers had virtually identical mass spectra.

EXAMPLE 3 Diastereoisomers (87) (Mr = 246)

$CH_3OCOCH(CH_3)OCH_2\text{-}OCH(CH_3)C_6H_{13}$

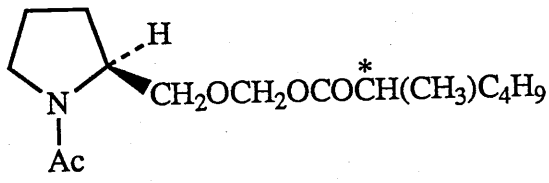
(* = chiral centres, R and S)

EIMS: m/z 187 (1.6%, $[M - {}^{\bullet}COOCH_3]^+$), 143 (10%, $C_6H_{13}CH(CH_3)\overset{+}{O}{=}CH_2$), 117 (100%, $[M - C_6H_{13}CH(CH_3)O^{\bullet}]^+$), 89 (70%, $CH_3OCOCH{=}\overset{+}{O}H$), 71 (58%, $C_5H_{11}^+$) and 57 (40%, $C_4H_9^+$).

Racemic 2-methylhexanoic acid (ii) derivatized with chloromethyl ethers (67)-(69)

The reaction was performed on 2-methylhexanoic acid (16 mg, 0.125 mmol) producing a yield of 90-100% (by GC) after 5 hours. Separation of the diastereoisomers was not achieved (see Section 4.4). Mass spectra confirming the identity of the diastereoisomers were obtained using GC/MS.

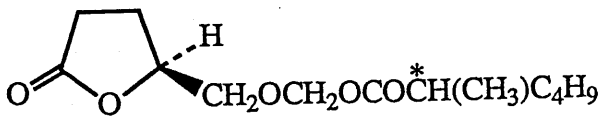
EXAMPLE 1 Diastereoisomers (68a) and (68b) (Mr = 285)



(*= chiral centre, R or S)

EIMS: m/z 285 (0.25%, $M^{+\bullet}$), 242 (0.1%, $[M - {}^{\bullet}COCH_3]^+$), 172 (0.75%, $[M - {}^{\bullet}COC_6H_{13}]^+$), 156 (14%, $[M - C_6H_{13}COO^{\bullet}]^+$), 142 (3%, $[M - C_6H_{13}COO\dot{C}H_2]^+$), 126 (29%, $[M - C_6H_{13}COOCH_2O^{\bullet}]^+$), 112 (62%, $[M - C_6H_{13}COOCH_2O\dot{C}H_2]^+$), 84 (22%, $[M - (C_6H_{13}COOCH_2O^{\bullet} + CH_2CO)]^+$), 70 (100%, $[M - (C_6H_{13}COOCH_2O\dot{C}H_2 + CH_2CO)]^+$).

EXAMPLE 2 Diastereoisomers (89) (Mr = 258)



(*= chiral centre, R or S)

EIMS: m/z 202 (2%, $[M - C_4H_8]^{+\bullet}$), 172 (1.5%, $C_6H_{13}COOCH_2\overset{+}{O}=CH_2$), 145 (3.7%, $[M - {}^{\bullet}COC_6H_{13}]^{+}$), 129 (75%, $[M - C_6H_{13}COO^{\bullet}]^{+}$), 113 (28%, $C_6H_{13}CO^{+}$), 99 (65%, $[M - C_6H_{13}COOCH_2O^{\bullet}]^{+}$) and 85 (100%, $[M - C_6H_{13}COOCH_2\overset{\bullet}{O}CH_2]^{+}$).

Aniline (iii) derivatized with chloromethyl ethers (67) and (69)

The reacton was performed on aniline (12 mg, 0.125 mmol) and (a) CME (69) (61 mg, 0.37 mmol), (b) CME (69) (21 mg, 0.127 mmol), and (c) CCME (67) (25 mg, 0.125 mmol)

GC analyses indicate that the correct products have possibly been formed, but a large peak due to aniline is also seen, indicating low reaction yields (see Section 4.4).

Racemic 2-methyl-1-butanethiol (iv) derivatized with chloromethyl ethers (67) and (69)

The reaction was performed 2-methyl-1-butanethiol (13 mg, 0.125 mmol) and (a) CME (67) (77 mg, 0.375 mmol), and (b) CME (69) (62 mg, 0.375 mmol). GC analyses show a single peak at a retention time that would be expected for the diastereoisomeric products but no separations were observed (see Section 4.4). GC/MS was used to confirm the identity of the product.

EXAMPLE Diastereoisomers (90) (Mr = 272)

EIMS: m/z 272 (0.4%, $M^{+\bullet}$), 199 (1.7%, $[M - (C_5H_{11}^{\bullet} + H_2)]^+$), 169 (17%, $[M - {}^{\bullet}SC_5H_{11}]^+$), 138 (25%, $[M - ({}^{\bullet}OCH_2SC_5H_{11} + H_2)]^+$), 83 (100%, $CH_2{=}CH\overset{+}{C}HCH(CH_3)_2$), 69 (40%, $C_5H_9^+$), 57 (35%, $C_4H_9^+$), 55 (38%, $C_4H_7^+$), 43 (36%, $C_3H_7^+$), 41 (37%, $C_3H_5^+$).

Non-racemic mixtures of R- and S-2-octanol (v) derivatized with chloromethyl ether (69)

The reactions were performed using the solutions of the non-racemic mixtures (0.5 ml containing 16 mg, 0.125 mmol) and CME (69) (62 mg, 0.375 mmol). See C10-C12 for examples of the chromatograms obtained. See Section 4.4, Table 14 for an assessment of the results.

Commercial R- and S-2-octanol (vi) derivatized with chloromethyl ether (69)

The reactions were performed using commercially available R- and S-2-octanols (16 mg, 0.125 mmol) and CME (69) (62 mg, 0.125 mmol). See C13 for an example of the chromatographic results. See Section 4.4, Table 14 for an assessment of the results.

8.5 Synthesis of non-chiral methyl thiomethyl ethers

General procedure

To alcohols (d)-(h) in Scheme 43 (20 mmol) in chloroform (60 ml) were added diisopropylethylamine (17.5 ml, 0.1 mol) and chloromethyl methyl sulphide (5.1 ml, 60 mmol). The mixture was refluxed for 3.5 - 4 hours, while monitoring the reaction by GC to optimise the yield. The cooled reaction mixture was washed with 1M hydrochloric acid (100 ml) and water (200 ml) then dried ($MgSO_4$). The solvent was removed and the product

purified by distillation. The yields and analytical data for compounds (92)-(96) were as follows.

The above method was also repeated using ethanol-free chloroform which was prepared thus. Chloroform (200 ml) was washed with concentrated sulphuric acid (2 x 10 ml), water (60 ml), saturated $NaHCO_3$ solution (40 ml) and water again (60 ml). The chloroform was dried using anhydrous magnesium sulphate then distilled over calcium hydride. The dry and ethanol-free chloroform was stored in a brown bottle in a dark place and used within 3 days.

p-Chlorobenzyl methyl thiomethyl ether (92)

The reaction using p-chlorobenzyl alcohol (2.85 g, 20 mmol) gave 1.321 g (33%) of MTM ether (92) as a clear oil (distilled at 120-125°C/0.4 mm Hg). **IR:** ν_{max} (thin film) loss of O-H$_{STR}$ (3400, alcohol), 1600 and 1495 (C=C$_{STR}$, aromatic ring), 1070 (C-O-C$_{STR}$, ether), 810 (C-Cl$_{STR}$), 730 (C-H$_b$, aromatic) and 685 cm^{-1} (C-C$_b$, aromatic); **NMR:** δ_H ($CDCl_3$) 2.15 (S-C$\underline{H}_3$, s, 3H), 4.55 and 4.65 (Ar-C$\underline{H}_2$-O and O-C$\underline{H}_2$-S, 2 x s, 4H), 7.3 (aromatic C$\underline{H}$, s, 4H); **NMR:** δ_C ($CDCl_3$) 13.9 (S-$\underline{C}H_3$), 68.6 ($\underline{C}H_2$-O), 74.5 (O-$\underline{C}H_2$-S), 128.6, 129.31 (aromatic $\underline{C}$'s), 133.5, 136.1 (aromatic $\underline{C}$-Cl and $\underline{C}$-CH_2); **EIMS:** m/z 202/4 (2.2%, $M^{+\bullet}$), 171/3 (2.7%), 154/6 (14%, [M - $CH_3SH]^+$), 141/3 (20%, [M - $CH_3S\dot{C}H_2]^+$), 125/7 (100%, [M - $CH_3SCH_2O^\bullet]^+$), 119 (20%, [M - (HCl + $CH_3S^\bullet$)]$^+$), 91 (28%, $C_7H_7^+$), 89 (17%, $C_7H_5^+$), 77 (9%, $C_6H_5^+$).

Analytical data on by-product (97)

The remaining oily residue (97) from the distillation of MTM was analysed by NMR and MS. **NMR:** δ_H ($CDCl_3$) 1.25 ($CH_2C\underline{H}_3$, t, 3H), 3.65

($C\underline{H}_2CH_3$, q, 2H), 4.55 (Ar-$C\underline{H}_2$-O, s, 2H), 4.7 (O-$C\underline{H}_2$-O, m, 2H), 7.3 (aromatic C$\underline{H}$'s, m, 4H); **EIMS:** m/z 154/6 (40%, $[M - CH_3CH_2OH]^{+\bullet}$), 125/7 (100%, $[M - CH_3CH_2OCH_2O^{\bullet}]^+$), 119 (51%, $[M - (CH_3CH_2OH + Cl^{\bullet})]^+$), 91 (40%, $C_7H_7^+$), 89 (27%, $CH_3CH_2OCH_2\overset{+}{O}=CH_2$), 77 (16%, $C_6H_5^+$), 59 (46%, $CH_3CH_2\overset{+}{O}=CH_2$).

Benzyl methyl thiomethyl ether (93)

The reaction using benzyl alcohol (2.16 g, 20 mmol) gave 1.6 g (51%) of MTM ether (93) as a pale yellow oil (distilled at 50-55°C/0.1 mm Hg). **IR:** ν_{max} (thin film) loss of HSTR (3400, alcohol), 1060 (C-O-CSTR, ether), 730 (C-H_b, aromatic) and 690 cm^{-1} (C-C_b, aromatic); **NMR:** δ_H ($CDCl_3$) 2.15 (S-$C\underline{H}_3$, s, 3H), 4.5 and 4.6 (Ar-$C\underline{H}_2$-O and O-$C\underline{H}_2$-S, 2 x s, 4H), 7.2 (aromatic C$\underline{H}$, s, 5H); **NMR:** δ_C ($CDCl_3$) 13.84 (S-$\underline{C}H_3$), 69.4 ($\underline{C}H_2$-O), 74.4 (O-$\underline{C}H_2$-S), 127.7, 127.9, 128.0 and 128.4 (aromatic $\underline{C}$'s); **EIMS:** m/z 168 ($M^{+\bullet}$), 138, 120 $(M - CH_3SH)^+$, 91 ($C_6H_5CH_2^+$), 77 ($C_6H_5^+$), 61 ($CH_3\overset{+}{S}=CH_2$) (% not given as the sample was overloaded).

Analytical data on by-product (98)

The remaining oily residue (98) from the distillation of MTM ether (93) was analysed further by NMR. **NMR:** δ_H ($CDCl_3$) 4.55 (2 x Ar-$C\underline{H}_2$-O, s, 4H), 4.75 (O-$C\underline{H}_2$-O, s, 2H), 7.25 (2 x aromatic rings C$\underline{H}$'s, s, 10H).

sec-Phenylethyl methyl thiomethyl ether (94)

The reaction using sec-phenylethyl alcohol (2.44 g, 20 mmol) gave 1.244 g (32%) of MTM ether (94) as a clear oil (distilled at 78-82°C/0.1 mm Hg). **IR:** ν_{max} (thin film) loss of O-HSTR (3400, alcohol), 1495 (C---CSTR, aromatic ring), 1080 (C-O-CSTR, ether), 760, 730 (C-H_b, aromatic), 700 and

685 cm^{-1} (C-C_b, aromatic); **NMR:** δ_H ($CDCl_3$) 1.45 ($C\underline{H}_3CH$, d, 3H), 2.15 (S-$C\underline{H}_3$, s, 3H), 4.45 (O-$C\underline{H}_2$-S, q (long range splitting), 2H), 4.85 (Ar-$C\underline{H}$-O, q, 1H), 7.2 (aromatic $C\underline{H}$, s, 5H); **NMR:** δ_C ($CDCl_3$) 13.8 (S-$\underline{C}H_3$), 23.5 ($\underline{C}H_3CH$), 72.3 ($\underline{C}H$-O), 74.1 (O-$\underline{C}H_2$-S), 126.6, 127.6, 128.5 (aromatic $\underline{C}$'s), and 142.7 (aromatic $\underline{C}$-CHO); **EIMS:** m/z 182 (8%, $M^{+\bullet}$), 152 (15%), 134 (15%, $[M - CH_3SH]^{+\bullet}$), 121 (22%, $[M - CH_3S\dot{C}H_2]^+$), 105 (100%, $C_8H_9^+$), 91 (7%, $C_6H_5CH_2^+$), 77 (14%, $C_6H_5^+$).

Analytical data on by-product (99)

The remaining oily residue (99) from the distillation of MTM ether (94) was analysed further by NMR. **NMR:** δ_H ($CDCl_3$) 1.25 and 1.45 ($C\underline{H}_3CH$, 2 x d (indicating diastereoisomeric forms), 6H), 4.42 and 4.65 (O-$C\underline{H}_2$-O, 2 x s (indicating diastereoisomeric forms), 2H), 4.55 and 4.75 (Ar-$C\underline{H}_2$-O, 2 x m (indicating diastereoisomeric forms), 4H), 7.25 (2 x aromatic ring $C\underline{H}$'s, s, (possibly starting to show 2 signals), 10H). **NMR:** δ_C ($CDCl_3$) 23.1 and 24.0 (diastereoisomeric forms of $\underline{C}H_3$), 73.8 and 74.6 (diastereoisomeric forms of $\underline{C}H$-O), 126.3, 126.5, 127.4, 127.5 and 128.4 (diastereoisomeric forms of aromatic $\underline{C}$-H's), 143.21 and 143.85 (diastereoisomeric forms of atomatic $\underline{C}$-CH).

Phenyl methyl thiomethyl ether (95)

The reaction using phenol (1.88 g, 20 mmol) gave 0.78 g (28%) of MTM ether (95) as a clear oil (distilled at 85°C/0.3 mm Hg) plus a decomposition product (NMR does not show the dialkyl diether by-product). **IR:** ν_{max} (thin film) loss of O-HSTR (3400, alcohol), 1595 and 1495 (C---CSTR, aromatic ring), 1205 (C-O-CSTR, ether) 780 and 755 (C-H_b, aromatic) and 690 cm^{-1} (C-C_b, aromatic); **NMR:** δ_H ($CDCl_3$) 2.2 (S-$C\underline{H}_3$, s, 3H), 5.25 (O-$C\underline{H}_2$-S, s, 2H), 6.7-7.4 (aromatic $C\underline{H}$, s, 5H); **NMR:** δ_C ($CDCl_3$) 14.5

(S-$\underline{C}H_3$), 72.4 (O-$\underline{C}H_2$-S), 116.0, 121.8, 129.4 (aromatic $\underline{C}$'s) and 157.1 (aromatic $\underline{C}$-O); **EIMS:** m/z 154 (25%, $M^{+\bullet}$), 107 (100%, $[M - CH_3S^{\bullet}]^+$), 77 (40%, $C_6H_5^+$), 61 (50%, $CH_3\overset{+}{S}=CH_2$).

Octanyl thiomethyl ether (96)

The reaction using 1-octanol (2.6 g, 20 mmol) gave 1.485 g (39%) of MTM ether (96) as a clear oil (distilled at 90°C/0.3 mm Hg). **IR:** ν_{max} (thin film) loss of O-HSTR (3400, alcohol), 1090 (C-O-CSTR, ether); **NMR:** δ_H ($CDCl_3$) 1.9 ($C\underline{H}_3CH_2$, t, 3H), 1.1-1.7 (($C\underline{H}_2)_6$, m, 12H), 2.15 (S-$C\underline{H}_3$, s, 3H), 3.5 ($C\underline{H}_2O$, t, 2H), 4.65 (O-$C\underline{H}_2$-S, s, 2H); **NMR:** δ_C ($CDCl_3$) 13.9 (S-$\underline{C}H_3$), 22-32 various signals ($\underline{C}H_3(\underline{C}H_2)_6$, some overlapping), 68.2 ($\underline{C}H_2$-O), 75.2 (O-$\underline{C}H_2$-S).

8.6 Homochiral NMR shift reagents

8.6.1 Synthesis of modified cyclodextrins

Preparation of permethylated α-cyclodextrin and β-cyclodextrins (100) and (101) from α–cyclodextrin and β-cyclodextrin respectively.[172]

α-CD has 18-OH groups and β-CD has 21-OH groups. The procedure used follows the method adopted as the derivatization reaction with the alkyl halides (see the KOH/DMSO reactions in Chapter 3). A molar ratio of 1:4:2, cyclodextrin -OH groups: KOH: MeI was used as illustrated in the following detailed procedure for permethylated α-CD.

To a stirred mixture of finely ground dry potassium hydroxide (8.06 g, 144 mmol) in DMSO (15 ml), was added α-CD (1.946 g, 2 mmol (36 mmol -OH

groups)) followed by iodomethane (4.48 ml, 72 mmol). The initial warming was reduced by immersion of the reaction flask in an ice bath. When cooled to room temperature the reaction was left stirring for 2 hours. Water (100 ml) was then added and the product was extracted into chloroform (20 + 15 + 10 ml). The combined extracts were washed with water (5 x 20 ml), dried using anhydrous magnesium sulphate, filtered and the solvent was removed to yield 2.45 g (91%) of (100) as a fine white crystalline solid. **Mpt** = 200-202°C (lit.[223] = 205°C) . **IR:** ν_{max} (KBr disc) loss of peak at 3450 cm^{-1} (OH_{STR}, alcohol groups). **NMR:** δ_H ($CDCl_3$) 3.4, 3.5, 3.6 (3 x $C\underline{H}_3$-O, 3 x s, 9H), 3-4.2 (2 x C-C$\underline{H}$-O and C-C$\underline{H}_2$-O, broad signal, 6H) and 4.95 (O-C$\underline{H}$-O, s, 1H).

The **IR** and **NMR** spectra (δ_H) were exactly the same for the permethylated β-CD (101). **Mpt** = 85-87°C (lit.[224] = 86-87.5°C and lit.[225] = 88°C). Another paper[223] reports the melting point of (101) to be 156°C. The melting point of the permethylated β-CD appeared low in comparison to that of the permethylated α-CD, and that some widely differing results had been reported.[223-225] It had been noted that the melting points of the permethylated CDs were dependent on the solvents used for crystallisation.[225]

It was suggested that solvent molecules were being included within the CD cavity and that this may be responsible for the observed differences. The melting points reported in this thesis may thus result from chloroform being included within the permethylated β-CD cavity but not within the permethylated α-CD cavity.

Preparation of acetylated β-cyclodextrin (102)

To β-CD (4.54 g, 4 mmol containing 84 mmol hydroxyl groups) in a 50 ml round bottomed flask was added acetyl chloride (12 ml, 168 mmol). The mixture was warmed to 50°C with the evolution of gas observed (HCl). On reaction the β-CD becomes soluble in the acetyl chloride. When the evolution of gas ceased and the reaction mixture became clear the excess acetyl chloride was removed under high vacuum to yield 6.82g, (97%) of (102) as a white solid. **Mpt** = 135-138°C. **IR:** ν_{max} 1740 ($C{=}O_{STR}$, ester), 1230 ($C(=O)\text{-}O\text{-}C_{STR}$, acetate), 1040 cm^{-1} (C-O-C, ether); **NMR:** δ_H ($CDCl_3$) 2.0 (3 x $C\underline{H}_3$-COO, 3 x s, 9H), 3.6-5.4 (various C<u>H</u>-O and $C\underline{H}_2O$ signals, d's and dd's, 7H); **NMR:** δ_C ($CDCl_3$) 20.7 (large signal, 3 x $\underline{C}H_3$-COO overlapped), 62.56, 69.6, 69.9, 70.89, 76.8 (<u>C</u>H-O and $\underline{C}H_2$-O signals), 96.7 (O-<u>C</u>H-O), 169.4, 170.4 and 170.7 (3 x <u>C</u>=O).

8.6.2 Formation of diastereoisomeric inclusion complexes using permethylated or acetylated cyclodextrins

Complexation of α-pinenes using permethylated β-cyclodextrin

The same procedure was performed on: (a) a racemic mixture of R, S-α-pinene, and (b) a sample of enantiomerically pure (S)-(-)-α-pinene.

To α-pinene ((a) or (b)) (34 mg; 0.25 mmol) in a small sample vial was added permethylated β-CD (0.72g; 0.5 mmol) and deuteriated methanol (0.5 ml). The vial was capped and shaken, immediately dissolving the permethylated cyclodextrin. The α-pinene remained as a separate phase (it is insoluble in methanol), and only dissolved (possibly due to inclusion) after considerable shaking of the sample. Tetramethylsilane (TMS) was

added to the mixture before transferring it to a NMR tube for ^{1}H and ^{13}C NMR analyses using the 90 MHz NMR instrument.

Complexation of α-pinenes using permethylated α-cyclodextrins

The procedure was exactly the same as that used for the permethylated β-CD complexation except that permethylated α-CD (0.62g; 0.5 mmol) was used.

Complexation of mandelic acid using permethylated β-cyclodextrin

The procedure was the same as that used for the complexation of α-pinene using permethylated CD's except that (a) a racemic mixture of S, R mandelic acid (38 g, 0.25 mmol) and, (b) enantiomerically pure (S)-(+)-mandelic acid (38 g, 0.25 mmol) were used in place of the α-pinene. Deuteriated chloroform (0.5 ml) was also used in place of the methanol.

Complexation of mandelic acid using acetylated β-cyclodextrin

The procedure was the same as that used for the complexation of mandelic acid using permethylated β-CD except that acetylated β-CD (0.882 g, 0.5 mmol) was used. The complexations were performed on enantiomerically pure (S)-(+)-mandelic acid and a racemic mixture of S,R-mandelic acid in both deuteriated methanol and deuteriated chloroform.

8.7 Synthesis of homochiral polymeric materials for use as GC stationary phases

8.7.1 Synthesis of monomers

Preparation of N-acetyl-(S)-(+)-aspartic acid dimethyl ester (110)

To *N*-acetyl-(S)-(-)-aspartic acid (107a) (5.0 g; 0.0286 mol) in a 25 ml round bottomed flask, methanol (15 ml; 0.371 mol) and 4 drops of concentrated sulphuric acid were added. After stirring for 5 days at room temperature, the methanol was removed and the product was extracted twice with chloroform (20 ml) from water (50 ml). The chloroform extract was washed with water (2 x 20 ml), then rotary evaporated to leave a thick gum. On further evaporation under high vacuum, 4.88 g (84%) of the dimethyl ester was isolated as a white waxy solid. **Mpt** 56-58°C. $[\alpha]^{20}$ +65° (c = 2.0, $CHCl_3$). **IR:** υ_{max} (thin film) 3350 (NH_{STR}, amide), 1735 ($C{=}O_{STR}$, ester), 1650 cm^{-1} ($C{=}O_{STR}$, amide); **NMR:** δ_H ($CDCl_3$) 2.0 ($C\underline{H}_3CO$, s, 3H), 2.85 ($C\underline{H}_2CH$, dd, 2H), 3.65 (2 x $C\underline{H}_3OCO$, 2 x s, 6H), 4.8 ($C\underline{H}$, m, 1H), 6.5 ($N\underline{H}$, d, 1H); **NMR:** δ_C ($CDCl_3$) 23.0 ($\underline{C}H_3CO$), 36.2 ($\underline{C}H_2CH$), 48.6 ($\underline{C}H$) 52 and 52.8 (2 x $\underline{C}H_3OCO$), 170.1, 171.3 and 171.6 ($\underline{C}{=}O$, signals from 2 x ester and 1 x amide function).

Preparation of (S)-(-)-2-acetamido succinic anhydride (111)

N-acetyl-(S)-(-)-aspartic acid (107a) (5.2542 g; 0.3 mol) was placed in a 25 ml round bottomed flask set up with reflux apparatus. Trifluoroacetic anhydride (4.245 ml; 0.03 mol) was added down the condenser while stirring, and cooling the flask in an ice bath. The reaction was allowed to reach room temperature over 1 hour with stirring. The trifluoroacetic acid (by-product) was removed under vacuum (+ heat) to yield 4.56 g (97%) of (111) as a white solid. **Mpt** 131-134°C. $[\alpha]^{20}$ -25° (c = 2.6M HCl). **IR:** υ_{max} (KBr disc) loss of absorbance in the range 3900-2500 (OH_{STR}, acid), 3400 ($N\text{-}H_{STR}$), 1800 and 1870 (smaller) ($C{=}O_{STR}$, 5 membered cyclic anhydride) and

1650 cm^{-1} ($C{=}O_{STR}$, amide); **NMR:** δ_H (d6-acetone) 2.00 ($\underline{CH}_3$, s, 3H), 2.05 ($\underline{NH}$, m, 1H), 3.2 ($\underline{CH}_2$, m, 2H), 4.75 ($\underline{CH}$, dd, 1H); **NMR:** δ_C (d_6-acetone) 21.9 ($\underline{C}H_3CO$), 35.5 ($\underline{C}H_2CH$), 50.8 ($\underline{C}H$), 170.4, 171.5 and 172 (3 x $\underline{C}$=O, amide and anhydride carbonyls); **EIMS:** m/z 157 (10%, $M^{+\bullet}$), 85 (17%, $[M-(CO + CO_2)]^{+\bullet}$), 43 (100%, CH_3CO^+).

Preparation of N-acetyl-(S)-aspartic acid di-sodium salt (112)

4M Sodium hydroxide solution (5 ml; 0.02 mol) was added to N-acetyl-(S)-(-)-aspartic acid (1.75 g, 0.01 mol (0.02 mol acid groups)) with stirring. After 1 hour, when the reaction had reached room temperature it was placed under high vacuum to remove the water. The resulting white solid weighed 2.393 g (109%). The high yield may be due to water of crystallisation as shown by the O-H peak in the IR. **IR:** ν_{max} (KBr disc) lack of OH_{STR} (acid), 3450 (OH_{STR}, water) and 1600 cm^{-1} (carboxylate C=O); **NMR:** δ_H (CD_3OD) 2.0 ($\underline{CH}_3CO$, s, 3H) 2.7 ($\underline{CH}_3$, d, 2H), 4.5 ($\underline{C}HCH_2$, t, 1H); $\underline{NH}$ signal out of range; **NMR:** δ_C (CD_3OD) 23 ($\underline{CH}_3CO$), 42.1 ($\underline{C}H_2$), 53.9 ($\underline{CH}$) 172.6 ($\underline{C}OCH_3$) and 180.4 (2 x $\underline{C}OONa$).

Preparation of 1,2-bis(2-bromoethoxy)ethane (114)

To triethylene glycol (4.505 g; 30 mmol) in THF (25 ml) was added trifluoroacetic anhydride (9.95 ml; 70 mmol). After 15 minutes stirring at room temperature the solvent, excess trifluoroacetic anhydride and trifluoroacetic acid (by-product) were removed by rotary evaporation to yield di-trifluoroacetate (113). Dry THF (25 ml) and HMPA were directly added followed by dry lithium bromide (25 g; 288 mmol). The reaction was refluxed for 3 hours then left overnight.

The THF was removed by rotary evaporation and water (20 ml) was added to the residue. The product was extracted into hexane (2 x 20 ml) then washed with water (3 x 10 ml) and dried. Rotary evaporation gave 5.36 g. The product was passed through a silica column eluting with hexane then chloroform to yield 3.833 g (46.3%) of the pure product (115) as a clear oil. **IR:** (thin film) loss of 3500 ($O\text{-}H_{STR}$, alcohol); **NMR:** δ_H ($CDCl_3$) 3.7 (2 x $C\underline{H}_2Br$ and 4 x $C\underline{H}_2O$, m, 12H); **NMR:** δ_C ($CDCl_3$) 30.4 ($\underline{C}H_2Br$) (cf triethylene glycol $\underline{C}H_2OH$ signal at 61.9), 70.5 and 71.2 ($\underline{C}H_2O$).

Preparation of 1,2 bis(2-tosylethoxy)ethane (115) from triethylene glycol

To triethylene glycol (3 g; 0.02 mol) in pyridine (20 ml) at 0°C was added para-toluenesulphonyl chloride.[53] After stirring for 2 hours the reaction was left to stand overnight at 2°C. The solution was then filtered and the filtrate poured into a cold 5% sodium bicarbonate solution (40 ml). Ethyl acetate was used to extract the product (20 ml then 10 ml). The extract was washed with 5% sodium bicarbonate solution (20 ml), water (20 ml) and brine (20 ml). After drying over anhydrous magnesium sulphate and filtering, the solvent was removed to yield a crude product (with triethylene glycol contaminating). The product was shaken with diethyl ether (20 ml) and filtered. This was repeated yielding a purer product by nmr. Final yield of (115) was 2.5 g (27.3%) as colourless solid. **Mpt** = 79-80°C, **IR:** (KBr disc) loss of alcohol O-H (3500 cm^{-1}); **NMR:** δ_H ($CDCl_3$) 2.4 ($C\underline{H}_3$-Ar, s, 6H), 3.6 ($C\underline{H}_2OC\underline{H}_2$, m, 8H), 4.1 ($C\underline{H}_2OTs$, t, 4H), 7.5 (disubstituted aromatic C<u>H</u>'s. 2 x d, 8H); **NMR:** δ_C ($CDCl_3$) 21.6 ($\underline{C}H_3$-Ar), 70.3, 70.7 and 72.4 ($\underline{C}H_2O$), 127.9, 130, 132 and 144 (aromatic <u>C</u>H's).

8.7.2 Polymerisation reactions

Acid catalysed melt polymerisations

The following reactants were combined in round bottomed flasks and 2 drops of concentrated sulphuric acid (catalyst) was added to each.

(a) N-Ac-asp (107) (172.5 mg; 0.985 mmol) + TRIGOL (148 mg; 0.985 mmol)

(b) N-Ac-asp (107) (172.5 mg; 0.985 mmol) + PEG-400 (394 mg; 0.985 mmol)

(c) N-Ac-asp-DME (110) (200 mg; 0.985 mmol) + TRIGOL (148 mg; 0.985 mmol)

(d) N-Ac-asp-DME (110) (200 mg; 0.985 mmol) + PEG-400 (394 mg; 0.985 mmol)

The reactions were left for 3 weeks under vacuum at 60°C. Stirring with magnetic fleas became difficult as the reactions progressed. After 3 weeks ^{1}H NMR, ^{13}C NMR and Mr (by V.P.O.) data were obtained (see Section 7.3 for the results and discussion).

Melt polymerisations -various catalysts

Various catalysts have been reported as useful for this type of polymerisation.[216] In all four reactions N-Ac-Asp-DME (110) (200 mg; 0.985 mmol) was added to TRIGOL (148 mg; 0.985 mmol) in a 5 ml round bottomed flask. To each of the four reactions was added a different catalyst (~1% w/w), these being (i) Zinc acetate (3.5 mg), (ii) Stannous 2-ethyl-hexanoate (1.5 mg), (iii) Tetraisopropyl orthotitanate (3.0 mg), and (iv) Aluminium isoproxoxide (3.5 mg).

The four reactions were left for 5 weeks under vacuum at 60°C. Samples were taken and from the ^{13}C NMR spectra obtained the relative molecular masses of the products were estimated (see Section 7.3 and 7.4).

Melt polymerisations using S-2-acetamidosuccininc anhydride (111)

To S-2-acetamidosuccinic anhydride (111) (1.57 g, 0.01 mol) was added TRIGOL (1.5 g, 0.01 mol) and one drop of conc. sulphuric acid. The reaction mixture was heated to 50°C and put under high vacuum. Samples were taken after 1 day, 1 week and 4 weeks. The average relative molecular mass of the product was estimated from the ^{13}C NMR spectra obtained (see Section 7.4 for results).

Solution polymerisation of disodium dicarboxylate (112) with dibromide (114) using HMPA as solvent.

Disodium dicarboxylate (112) (1.1 g, 5 mmol) was added to HMPA (3 ml) in a round bottomed flask with only a small quantity dissolving. The mixture was cooled to 0°C before the dibromide (114) (1.38 g, 5 mmol) in HMPA (1 ml) was added. The reaction was stirred at room temperature and samples were taken after 1 week, 4 weeks and 2 months, and ^{13}C NMR spectra were obtained. The results indicated that due to the low solubility of the disodium salt in the HMPA, the reaction was progressing slowly. The soluble product (with solids removed) was mainly the dibromide with some ester products.

Attempted solution polymerisation of disodium dicarboxylate (112) with dibromide (114) using acetone as solvent.

The attempted polymerisation of disodium dicarboxylate (112) (1.1 g, 5 mmol) with dibromide (114) (1.38 g, 5 mmol) failed due to (112) being insoluble in acetone. After 3 days reaction, ^{13}C NMR analysis indicated that only dibromide (114) was present in solution.

REFERENCES

1. Pasteur, L., *Ann. Chim. Physique.*, 1848, **24**, 442.

2. Le Bel, J.A., *Bull. Soc. Chim., France*, 1874, **2**, 22, 337.

3. Van't Hoff, J.H., *Bull. Soc. Chim., France*, 1875, **2**, 23, 295.

4. Lord Kelvin, *Baltimore Lectures*, 1884, C J Clay and Sons, London, 1904, 436 and 619.

5. Konig, W.A., *The Practice of Enantiomer Separation by Capillary Gas Chromatography*, Heidelberg: Huthig, 1987.

6. Krustulovic, A.M., *Chiral Separations by HPLC-applications to pharmaceutical compounds*, Ellis Horwood, 1989, p.27-28.

7. Yamagat, T.M., Hambe, M., and Iye, M., *Angular Momentum Orientations of Spiral Galaxies, Tokyo Observatory Annals.*, 1981, **18**, 164-174.

8. Borchkhadze, T.M., Kogoshvili, N.G., *The Anisotropy of Spiral Galaxy Orientation, Astronomy and Astrophysics*, 1976, **53**, 431-433.

9. MacGillivray, H.T., Dodd, R.J., *The Anisotropy of the Spatial Orientations of Spiral Galaxies in the Local Supercluster, Astronomy and Astrophysics*, 1985, **145**, 269-274.

10. Dalgliesh, J., *Chem. Soc.*, 1952, 137.

11. M, R.H., Schlatter, J.M., Goldkamp, A.H., *J. Am .Chem. Soc.*, 1969, **91**, 10, 2684-2691.

12. Cloninger, M.R., Baldwin, R.E., *Science*, 1970, **170**, 81-82.

13. Chedd, G., *New Scientist*, 1974, 299-302.

14. Selinger, B., *Chemistry in the Market Place,* Harcourt Brace Jovanovich, 1986, 379-382.

15. Blashke, G., Kraft, H.P., Fickenscher, K., Kohler, F., *Arzneim-Forsch,* 1979, **29**, 1690.

16. Blashke, G., Kraft, H.P., Markgraf, H., *Chem. Ber.,* 1980, **113**, 2318-2322.

17. Drayer, D.E., *Therapeutic Drug Monitoring,* 1988, **10**, 1-7.

18. Allenmark, S.G., *Chromatographic Enantioseparations,* Ellis Horwood, 1988, 31-33.

19. Raban, M., Mislow, K., *Top. Stereochem.,* 1967, **2**, 202-207 and 226-228.

20. Biot, J.B., *Bull. Soc. Philomath.*, Paris, 1815, 190.

21. Ref 19, p. 202-207.

22 Consiglio, G., Pino, P., Flowers, L.I., Pittman Jr., C.U., *J. Chem. Soc., Chem. Commun.,* 1983, 612.

23 Raban, M., Mislow, K., *Top. Stereochem.,* 1967, **2**, 216-226.

24 Dale, J.A., Dull, D.L., Mosher, H.S., Mosher, J., *J. Org. Chem.,* 1969, **34**, 2543-2549.

25 Parker, D., *J. Chem. Soc., Perkin Trans.,* 1983, **2**, 83-88.

26 Johnson, C.R., Elliot, R.C., Penning, T.D., *J. Am. Chem. Soc.,* 1984, **106,** 5019-5020.

27 Feringa, B.L., Smaardijk, A.B., Wynberg, H., *J. Amer. Chem. Soc.,* 1985, **107**, 4798-4799.

28 Pirkle, W.H., Hoover, D.J., *Top. Stereochem.,* 1982, **13**, 263-331.

29 Allenmark, S.G., *Chromatographic Enantioseparations,* Ellis Horwood, 1988, p.29.

30 Pirkle, W.H., Beare, S.D., *J. Am. Chem. Soc.,* 1969, **91**, 5150-5155.

31 Luchinat, C., Roelens, S., *J. Am. Chem. Soc.,* 1986, **108**, 4873-4878.

32 Sullivan, G.R., *Top. Stereochem.,* 1978, **10**, 287-331.

33 Whitesides, G.M., Lewis, D.W., *J. Am. Chem. Soc.,* 1970, **92**, 6979-6980.

34 Yeh, H.J.C., Balani, S.K., Yagi, H., Green, R.M., Sharma, N.D., Boyd, D.R., Jerina, D.M., *J. Org. Chem.,* 1986, **51**, 5439-5443.

35 Bounoure, J., Souppe, J., *Analyst,* 1988, **113**, 1143-1144.

36 Flockhart, B.D., *C.R.C. Crit. Reviews in Analytical Chemistry*, 1976, **5**, 69.

37 Cockerill, A.F., Davis, G.L.O., Harden, R.C., Rockham, D.M., *Chemical Reviews,* 1973, **73**, 553.

38 McCreary, M.D., Lewis, D.W., Wernic, D.L., Whitesides, G.M., *J. Am. Chem. Soc.,* 1974, **96**, 1038.

39 Greenstein, J.P., Winitz, M., *Chemistry of Amino Acids,* Vol.2, Wiley, New York, 1961, p.1738.

40 Barrett, G.C., *Chemistry and Biochemistry of the Amino Acids,* Chapman and Hall, 1985, p.345-348.

41 Ref. 40, p.239.

42 Morley, J.S., *Trends in Pharmacol. Sci.,* 1980, 463.

43 Barth, T., Maskova, H., *Coll. Czech. Chem. Commun.,* 1971, **36**, 2398.

44 Sugie, M., Suzuki, H., *Agricultural and Biological Chemistry,* 1980, **4**, 1089.

45 Payan, I.L., Cadilla-Perezrios, R., Fisher, G.H., Man, E.H., *Anal. Biochem.,* 1985, **149**, 484-491.

46 Addadi, L., Weinstein, S., Gati, E., Weissbush, I., Lahav, M., *J. Am. Chem. Soc.,* 1982, **104**, 4610.

47 Translation by Richardson, G.M., *Scientific Memoirs (Pasteur),* The Foundation of Stereochemistry, American Book Co., 1901, 28.

48 Adams, R., Hauserman, F.B., *J. Am. Chem. Soc.,* 1952, **74**, 694-699.

49 Mori, K., *Tetrahedron,* 1975, **31**, 1381-1384.

50 Poole, C.F., Schuette, S.A., *Contemporary Practice of Chromatography,* Elsevier, Amsterdam, 1984.

51 Smith, R.M., *Gas and Liquid Chromatography in Analytical Chemistry,* Wiley, Chichester, 1988.

52 Souter, R.W., *Chromatographic Separations of Stereoisomers,* C.R.S. Press, Boca Raton, 1985.

53 Allenmark, S.G., *Chromatographic Enantioseparations, Methods and Applications*, Ellis Horwood, 1988, p.71.

54 Attygalle, A.B., Morgan, E.D., Evershed, R.P., Rowland, S.J., *J. Chromatogr.,* 1983, **260**, 411-417.

55 Brooks, C.J.W., Gilbert, M.T., Gilbert, J.D., *Anal. Chem.,* 1973, **45**, 896-902.

56 Burden, R.S., Deas, A.H.B., Clark, T., *J.Chromatogr.,* 1987, **391**, 273-279.

57 Halpern, B., Westley, J.W., *J. Chem. Soc., Chem. Commun.*, 1966, 34-35.

58 Souter, R.W., *J. Chromatogr.*, 1975, **108**, 265.

59 Liu, R.H., Ku, W.W., Fitzgerald, M.P., *J. Assoc. Off. Anal. Chem.*, 1983, **66**, 6, 1443-1446.

60 Hermansson, J., von Bahr, E., *J. Chromatogr.*, 1980, **221**, 109.

61 Silber, B., Riegelman, J., *J. Pharm. Exp. Ther.*, 1980, **215**, 643.

62 Roy, S.D., Lim, H.K., *J. Chromatogr.*, 1988, **431**, 210-215.

63 Hasegawa, M., Matsubara, I., *Anal. Biochem.*, 1975, **63**, 308-320.

64 Klein, R., *J. Chromatogr.*, 1979, **170**, 468.

65 Horiba, M., Kitaharo, H., Takahashi, H., Yamamota, S., Muramo, A., Oi, N., *Agric. Biol. Chem.*, 1979, **43**, 2311.

66 Konig, W.A., Rahn, W., Eyem, J., *J. Chromatogr.*, 1977, **133**, 141.

67 Ref. 5, p.71.

68 Raulin, F., Khave, B.N., *J. Chromatogr.*, 1973, **75**, 13.

69 Kamerling, J.P., Duran, M., Gerwig, G.J., Ketling, D., Bruinvis, L., Hiegenhart, J.F.G., Wadman, S.K., *J. Chromatogr.*, 1981, **222**, 276.

70 Schweer, H., *J. Chromatogr.*, 1982, **243**, 149.

71 Tamura, S., Kuzann, S., Kawai, K., Kishimato, S., *J. Pharm. Pharmacol.*, 1981, **33**, 701.

72 Westley, J.W., Halpern, B., *J. Org. Chem.*, 1968, **33**, 3978-3980.

73 Jeyaraj, G.L., Porter, W.R., *J. Chromatogr.*, 1984, **315**, 378-383.

74 Ref 18, p.51-61.

75 Blau, K., King, G.S., *Handbook of derivatives for chromatography,* Heyden, London, 1978.

76 Ref. 5, p.71-83.

77 Kagan, H.B., Fiaud, J.C., *Top. Stereochem.,* 1988, **18**, 249-331.

78 Deger, W., Gessner, G., Heusinger, G., Singer, G., Mosandl, A., *J. Chromatogr.,* 1986, **366**, 385-390.

79 Schurig, V., *Kontakte (Darmstadt)*, 1986, **1**, 3-22.

80 Lochmüller, C.H., Souter, R.W., *J. Chromatogr.,* 1975, **113**, 283-302.

81 Dalgliesh, C., *J. Chem. Soc.,* 1952, 137.

82 Pirkle, W.H., Hugn, M.H., Bank, B., *J. Chromatogr.,* 1984, **316**, 585.

83 Sybilska, D., Smolková-Keulemansová, *Inclusion Compounds,* 1984, **3**, 229-243.

84 Arad-Yellin, R., Green, B.S., Knossour, M., Tsoucaris, G., *Inclusion Compounds,* 1984, **3**, 265-272.

85 Szejtli, J., *Inclusion Compounds,* 1984, **3,** 373-389.

86 Armstrong, D.W., Ward, T.J., Armstrong, R.D., Beesley, T.E., *Science,* 1986, **232**, 1132-1135.

87 Gil-Av, E., Feibush, B., Charles-Siegler, R., *Tet. Lett.,* 1966, 1009.

88 Oi, N., Kitahar, H., Doi, T., *J. Chromatogr.,* 1983, **254**, 282.

89 Frank, H., G.J., Bayer, E., *J. Chromatogr., Sci.,* 1977, 174.

90 Koppenhoefer, B., Bayer, E., *J. Chromatogr. Lib.,* 1985, **32**, 1.

91 Konig, W.A., Schmidt, E., Krebber, R., *Chromatographia,* 1984, **18**, 12, 698-700.

92 Jemal, M., Cohen, A.I., *J. Chromatogr.,* 1987, **392**, 442-446.

93 Koppenhoefer, B., Allmendinger, H., Chang, L.P., Cheng, L.B., *J. Chromatogr.,* 1988, **441**, 89-98.

93a Separation of chiral molecules and control of optical purity by TLC, GLC and HPLC, Macherey-Nagel GMbH and Co catalogue, 1988-89, p.12.

94 Konig, W.A., *J. High Resolut. Chromatogr. Chromatogr. Commun.,* 1982, **5**, 588.

95 Konig, W.A., Benneke, I., Sievers, S., *J. Chromatogr.,* 1982, **217**, 71.

96 Frank, H., *J. High Resolut. Chromatogr. Chromatogr. Commun.,* 1988, **11**, 787-792.

97 Halterman, R.K., Roush, W.R., Hoong, L.K., *J. Org. Chem.,* 1987, **52**, 1152-1155.

98 Schurig, V., Weber, R., *J. Chromatogr.,* 1984, **289**, 321.

99 Koscielski, T., Sybilska, D., Jurkzak, J., *J. Chromatogr.,* 1983, **283**, 131-134.

100 Konig, W.A., Latz, S., Wenz, G., *Angew. Chem.,* 1988, **27**, 979-980.

101 Schurig, V., Nowotny, H.-P., *J. Chromatogr.,* 1988, 441, 155-163.

102 Koscielski, T., Sybilska, D., Jurkzak, J., *J. Incl. Phenom.,* 1987, **5**, 69-72.

103 Armstrong, D.W., *Anal. Chem.,* 1987, **59**, 84A-91A.

104 Prelog, V., Wieland, P., *Helv. Chim. Acta,* 1944, **27**, 1127.

105 Dent, C.E., *Biochem. J.,* 1948, **43**, 169.

106 Fukuhara, T., Isoyama, M., Shimada, A., Itoh, M., Yuasa, S., *J. Chromatogr.,* 1987, 387, 562-565.

107 Hess, H., Burger, G., Musso, H., *Angew. Chem.,* 1978, **90**, 645.

108 Blashke, G., Broker, W., Fraenkel, W., *Angew. Chem. Int. Ed., Engl.,* 1986, **25**, 830-831.

109 Blashke, G., *J. Liq. Chromatogr.,* 1986, **9**, 341.

110 Yuki, H., Okamoto, Y., Okamoto, I., *J. Am. Chem. Soc.,* 1980, **102**, 6556.

111 Okamoto, Y., Hatada, K., *J. Liq. Chromatogr.,* 1986, **9**, 369.

112 Walff, G., Poll. H.G., Minarik, M., *J. Liq. Chromatogr.,* 1986, **9**, 385.

113 Hermansson, J., *Eur. Pat. Appl.,* No. 84850169.8, Publ. No. EP0128886A2.

114 Schill, G., Wainer, I.W., Barkan, S.A., *J. Liq. Chromatogr.,* 1986, **9**, 641.

115 Curtis, W.D., Laidler, D.A., Stoddart, J.F., Jones, G.H., *Chem. Commun.,* 1975, **883**, 835.

116 Sonsa, L.R., Sogah, G.D.Y., Hoffman, D.H., Cram, D.J., *J. Am. Chem. Soc.,* 1978, **100**, 4569.

117 Debowski, J., Sybilska, D., Jurczac, J., *Chromatographia,* 1982, **16**, 198-200.

118 Tanaka, M., Okazaki, J., Ikeda, H., Shono, T., *J. Chromatogr.,* 1986, **370**, 293-301.

134 Petterson, C., Schill, G., *J. Chromatogr.,* 1981, **204**, 179.

135 Petterson, C., Schill, G., *Chromatographia,* 1982, **16**, 192.

136 Petterson, C., Josefsson, M., *Chromatographia,* 1986, **21**, 321-326.

137 Armstrong, D.W., He, F.-Y., Han, S.M., *J. Chromatogr.,* 1988, **448**, 345-354.

138 Klesper, E., Corwin, A.H., Turner, D.A., *J. Org. Chem.,* 1962, **27**, 700.

139 Randall, L.G., *Ultra High Resoln. Chromatogr.,* 1984, 135-169.

140 Jackson, W.P., Richter, B.E., Fjeldsted, J.C., Kong, R.C., Lee, M., *Ultra High Resoln. Chromatogr.,* 1984, 121-134.

141 Mouriere, P.A., Eliot, E., Caude, M.H., Rosset, R.H., *Anal. Chem.,* 1985, **57**, 14, 2819-2823.

142 Macaudiere, P., Caude, M.H., Rosset, R., Tambute, A., Alembik, M.A., Wainer, I.W., *J. Chromatogr.,* 1986, **371**, 177-193.

143 Hara, S., Dobashi, A., Kinoshita, K., Hondo, T., Saito, M., Senda, M., *J. Chromatogr.,* 1986, **371**, 153.

144 Macaudiere, P., Caude, M.H., Rosset, R., Tambute, A., Chiral Sep., Chromatographic Society Symposium, Stevenson, D., Wilson, D., (Editors), Plenum, 1988, p.115-120.

145 Steuer, W., Schindler, M., Schill, G., Emi, F., *J. Chromatogr.,* 1988, **447**, 287-296.

146 Ewing, A.G., Wallingford, R.A., Olefiroicz, T.M., *Anal. Chem.,* 1989, **61**, 4, 292A-303A.

147 Smith, R.D., Olivares, J.A., Nguyen, N.T., Udseth, H.R., *Anal. Chem.,* 1988, **60**, 436-441.

148 Guttman, A., Paulus, A., Cohen, A.S., Grinber, N., Karger, B.L., *J. Chromatogr.*, 1988, **448**, 41-43.

149 Cohen, A.S., Paulus, A., Karger, B.L., *Chromatographia,* 1987, **24**, 15-24.

150 Mannschreck, A., Andert, D., Eiglsperger, A., Gmahl, E., Buchner, H., *Chromatographia,* 1988, **25**,182-188.

151 Koppenhoefer, B., Bayer, E., *Chromatographia,* 1984, **19**, 123-130.

152 Rose, H.C., Stern, R.L., Karger, B.L., *Anal. Chem.,* 1966, **38**, 469-472.

153 Lipkowitz, K.B., Demeter, D.A., Zegarra, R., Larter, R., Darten, T., *J. Am. Chem. Soc.,* 1988, **110**, 3446-3452.

154 Boehm, R.E., Martire, D.E., Armstrong, D.W., *Anal. Chem.,* 1988, **60**, 522-528.

155 Lipkowitz, K.B., Demeter, D.A., Parish, C.A., *Anal. Chem.,* 1987, **59**, 1731-1733.

156 Wainer, I.W., Stiffin, R.M., Shibata, T., *J. Chromatogr.,* 1987, **411**, 139-151.

157 Streitwieser, A.J., Heathcook, C.H., *Introduction to Organic Chemistry (2nd Ed)*, Collier MacMillan, 1981, p.546-550.

158 Green, T.W., *Protective Groups in Organic Synthesis,* Wiley Interscience, 1981.

159 Doolittle, R.E., Heath, R.R., *J. Org. Chem.,* 1984, **49**, 5041-5050.

160 Kinderlerer, J.L., *Phytochemistry,* 1987, **26**, 1417.

161 duVigneaud, V., Meyer, C.E., *J. Biol. Chem.,* 1932, **98**, 295.

162 Barret, G.C., Davis, J.S., *Chemistry and Biochemistry of Amino Acids,* Chapman and Hall, 1985, p.525-544.

163 Ravid, U., Silverstein, R.M., Smith, L.R., *Tetrahedron,* 1978, **34**, 1449-1452.

164 Doolittle, R.E., Tumlinson, J.M., Proveaux, A.T., Heath, R.R., *J. Chem. Ecol.,* 1980, **6**, 473-485.

165 Gopichand, Y., Khanra, A.S., Mitra, R.B., Chakravarti, K.K., *Indian J. Chem.,* 1975, **13**, 433-436.

166 Dictionary of Organic Compounds, Chapman and Hall, 5th edn. 1982, p.4773.

167. McKillop, A., Ford, M.E., *Tetrahedron,* 1974, **30**, 2467-2475.

168. Patai, *Chemistry of the Ether Linkage*, Interscience, New York, 1967, p.446-450 and 460-468.

169. March, J., *Advanced Organic Chemistry*, Wiley, Interscience, 1985, p.342-343 and 353-354.

170. Kemp, D.S., Vellaccio, F., *Organic Chemistry,* Worth Publishers Inc., 1980, p.185-194.

171. Lavcock, R.C., *J. Org. Chem.,* 1974, **39**, 3721-3727.

172. Johnstone, R.A.W., Rose, M.E., *Tetrahedron,* 1979, **35**, 2169-2173.

173. Ciucanu, I., Gabris, P., *Chromatographia,* 1987, **23**, 574-578.

174. Barluenga, J., Alonso-Cires, L., Campos, P.J., Asensio, G., *Synthesis*, 1983, 53-55.

175. Barluenga, J., Alonso-Cires, L., Campos, P.J., Asensio, G., *Synthesis*, 1983, 649-651.

176. Enders, D., Fey, P., Kipphardt, *Organic Synthesis*, **65**, 173-174.

177. Fieser and Fieser, *Reagents for Organic Synthesis* II, p.1160-1161.

178. Ref. 169, p.382-383.

179. Elderfield, R.C., Kremer, C.B., Kupcham, S.M., Birstein, O., Cortes, G., *J. Am. Chem. Soc.*, 1947, **69**, 1258-1260.

180. Hodge, P., Khoshdel, E., *J. Chem. Soc., Perkin Trans.*, 1984, 195-198.

181. Example of the Finkelstein reaction - Schurink, H.B., *Organic Synthesis, II*, p.476-478. (see also ref. 169, p.381-382).

182. Noller, C.R., Dinsmore, R., *Organic Synthesis, II*, p.358-360.

183. Camps, F., Gasol, V., Guerrero, A., *Synth. Commun.*, 1987, 511-512.

184. Schmidt, S.P., Brooks, D.W., *Tet. Lett.*, 1987, **28**, 767-768.

185. Hawdegger, E., Ott, H., *Helv. Chim. Acta*, 1955, **36**, 312.

186. Mori, K., *Tetrahedron*, 1975, **31**, 3011-3012.

187. Baldwin, J., *Tetrahedron*, 1984, **40**, 4513.

188. Vigneran, J.P., Meric, R., *Tet. Lett.*, 1982, **23**, 5051-5054.

189. Ref. 18, p.28.

190. Frank, H., Nicholson, G.J., Bayer, E., *J. Chromatogr.*, 1978, **146**, 197.

191. Julia, S., Sans, J.M., *J. Chromatogr. Sci.*, 1979, **17**, 651.

192. Ciucanu, I., Kerek, F., *Carbohydrate Research*, 1984, **131**, 209.

193. Martin, D., Hauthal, H.G., *Dimethyl Sulfoxid, Akademie Verlag,* Berlin, 1971, p.67-78.

194. Ref. 169, p.902.

195. Pfeffer, P.E., Silbert, L.S., *J. Org. Chem.,* 1976, **41**, 1373-1379.

196. Barluenga, J., Alonso-Cires, L., Arsensio, G., *Synth. Commun.,* 1979, 962-964.

197. Green, T.W., Protective Groups in Organic Synthesis. Wiley and Sons 1981.

198. Kozikowski, A.P., Wu, J.P., *Tetrahedron Lett.,* 1987, **28**, 5125-5128.

199. Corey, E.J., Gras, J.L., Ulrich, P., *Tetrahedron Lett.,* 1976, **11**, 809-812.

200. Undheim, K., Strande, P., Benneche, T., *Synthesis*, 1983, 762.

201. Corey, E.J., Bock, M.G., *Tetrahedron Lett.,* 1975, **38**, 3269-3270.

202. Barton, D., Ollis, W.D., Comprehensive Organic Chemistry, 1, Permagon Press, 1979, 841-843.

203. Ballinger, P.B.D., de la Mare, G., Kohnstram, G., Prestt, B.M., *Journal of the Chemical Society,* 1955, 3641-3647.

204. Connor, D.S., Klein, G.W., Taylor, G.N., *Organic Synthesis,* 1972, **52**, 16-19.

205. Marvel, C.S., Porter, P.K., *Organic Syntheses,* **I**, 2nd Ed, 1967 377-379.

206. Gross, H., Burger, W., *Organic Syntheses,* V, 1973, 221-223.

207. Yamada, K., Kato, K., Nagase, H., Hirato, Y., *Tetrahedron Letters,* 1976, 65-66.

208. Suzuki, K., Inamaga, J., Yamaguchi, M., *Chemical Letters,* 1979, 1277.

209. Pojer, D.P.M., Angyal, S.J., *Australian Journal of Chemistry,* 1978, 1031-1040.

210. Lindner, W., *Chromatographic Science (Chromatographic Chiral Separations),* 1988, **40,** 91-130 (p.96).

211. Perrin, D.D., Armarego, W.L.F., Perrin, D.R., *Purification of Laboratory Chemicals,* Permagon Press (2nd ed.)

212. Konig, W.A., Lutz, S., Wenz, G., von der Bey, E., *J. High Resolution Chromatogr. and Chromatogr. Commun.,* 1988, **11**, 506-509.

213. Konig, W.A., Lutz, S., Mischnic-Lubbecke, P., Brassat, B., Wenz, G., *J. Chromatogr.,* 1988, **447**, 193-197.

214. Mularz, E.A., Cline-Love, L.J., Petersheim, M., *Anal. Chem.,* 1988, **60**, 2751-2355.

215. Doda, F., Movi, K., Okada, J., *Chem. Lett.,* 1988, 131-134.

216. Kohn, J., Langer, R., *J. Am. Chem. Soc.,* 1987, **109**, 817-820.

217. Palla, G., Dossena, A., Marchell, R., *J. Chromatogr.,* 1985, **349**,

218. Morisson, R.T., Boyd, R.N., *Organic Chemistry,* Fifth Edition, Allyn and Bacon, p.1249-1250.

219. Taleb-Bendiab, S.A., Vergnaud, J.M., *J. Applied Polymer Science,* 1980, **25**, 499-510.

220. Yasuda, S., Wu, G-C., Tanaka, H., Sanui, K., Ogata, N., *J. Polymer Science, Polymer Chem., Edn.,* 1983, **21**, 2609-2616.

221. Ref. 169, p199.

222. Aldrich 1988-89 Catalogue, p.1300

223. Casu, B., Reggiani, M., Gallo, G.G., Viegevani, A., *Tetrahedron,* 1968, **24**, p.803-821.

224. Johnson, J.R., Shankland, N., Sadler, I.H., *Tetrahedron,* 1985, **41**, p.3147-3152.

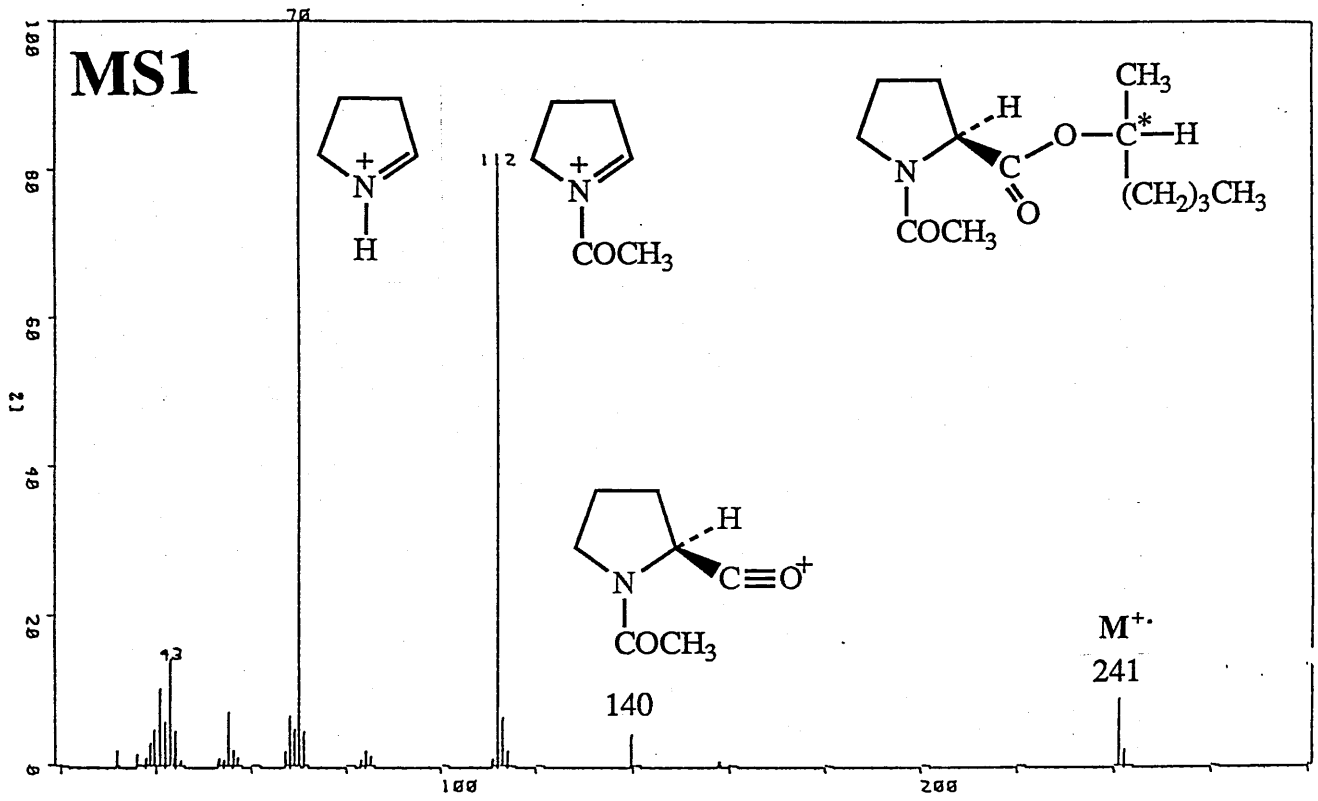
225. Casu, B., Reggiani, M., Sanderson, G.R., *Carbohydr. Res.,* 1979, **76**, p.59.

APPENDIX

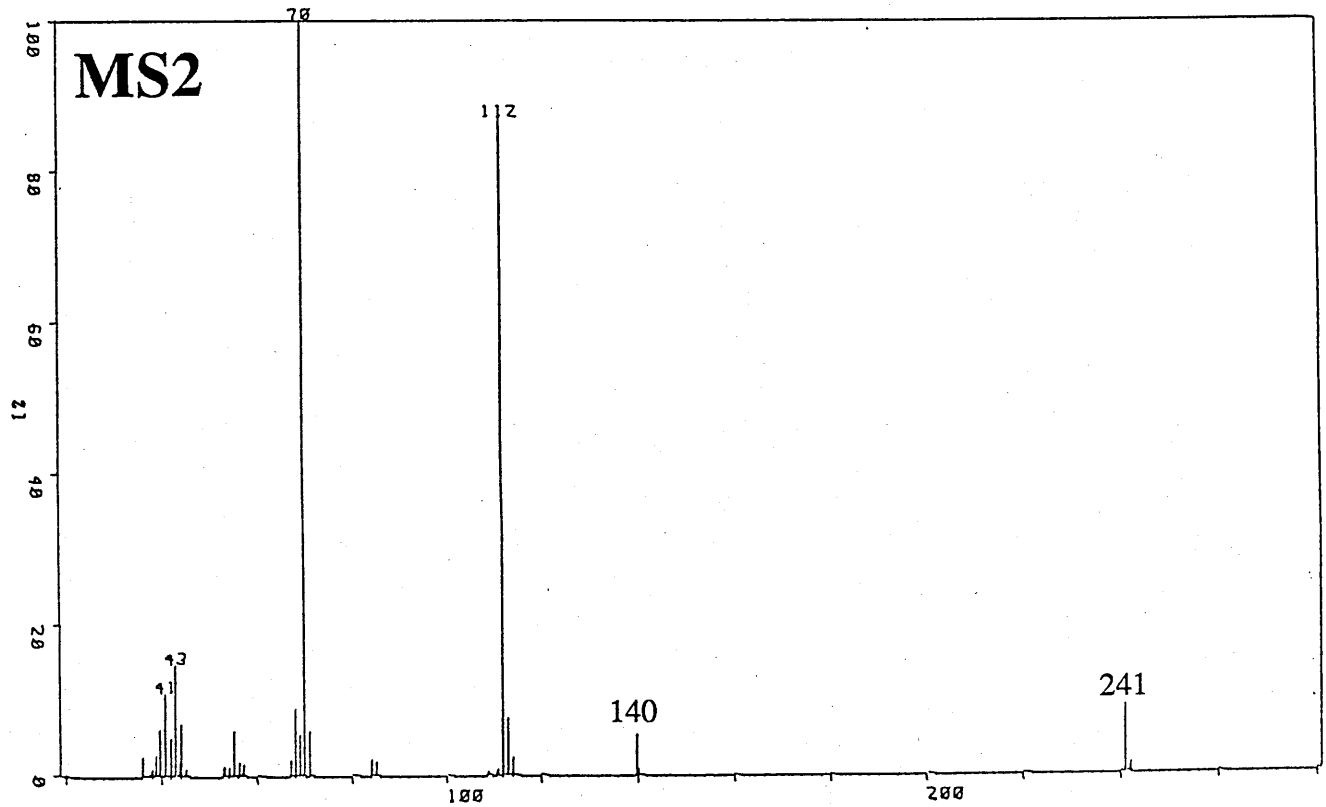
SPECTRA AND CHROMATOGRAMS

Mass spectra of resolved N-acetyl-(S)-(-)-prolyl esters (33) of 2-hexanol.

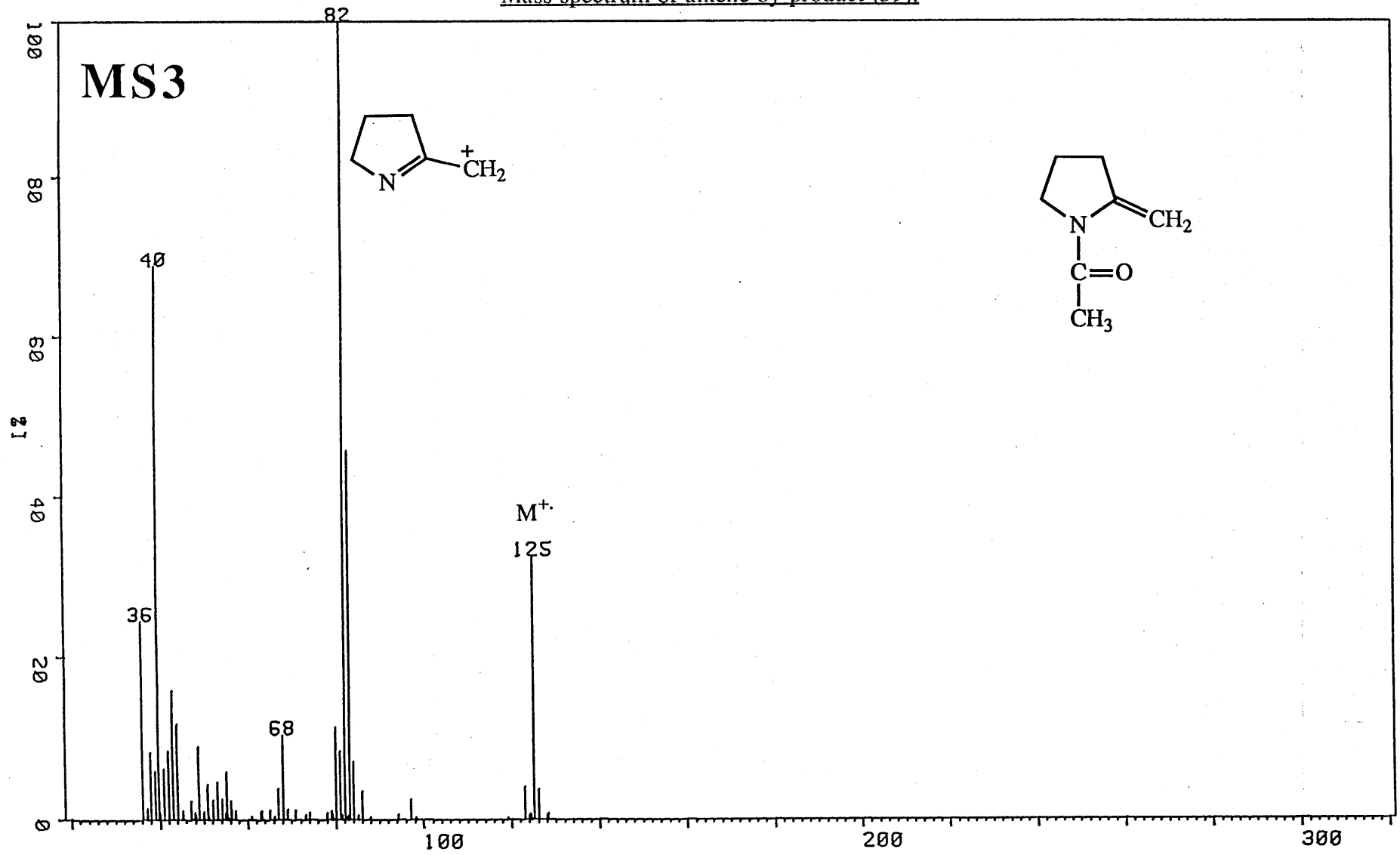
SCAN 413



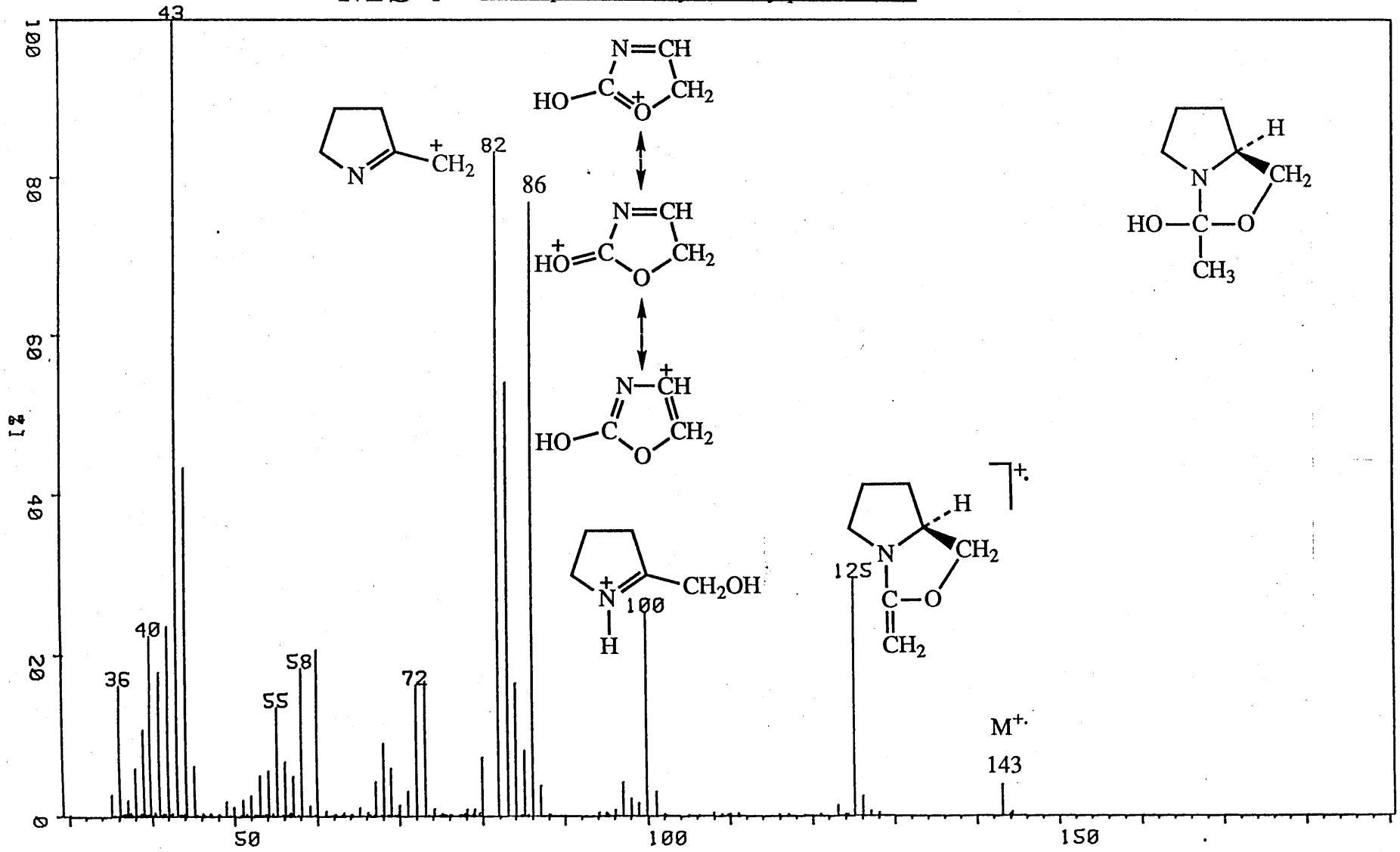
SCAN 424

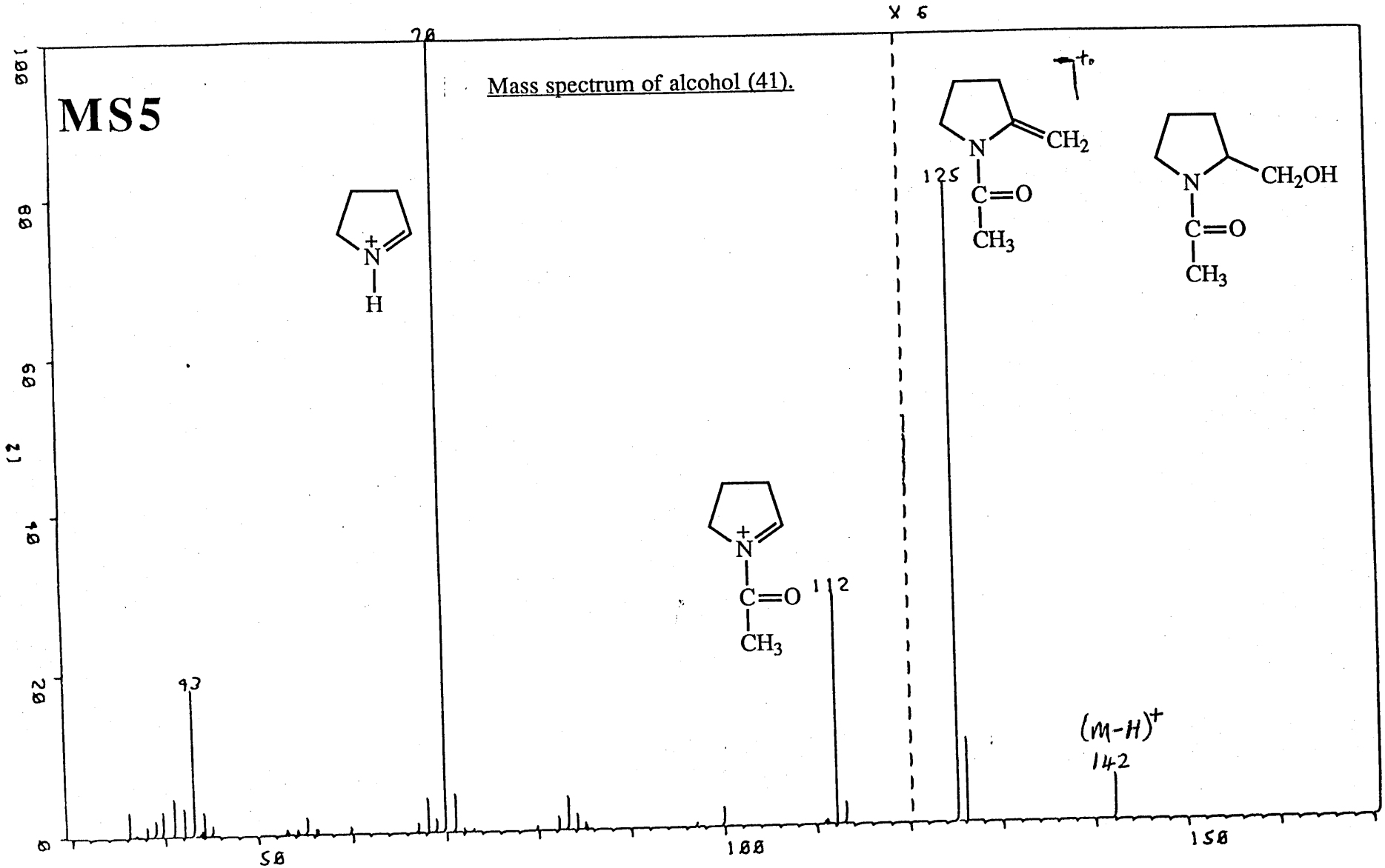


Mass spectrum of alkene by-product (59).

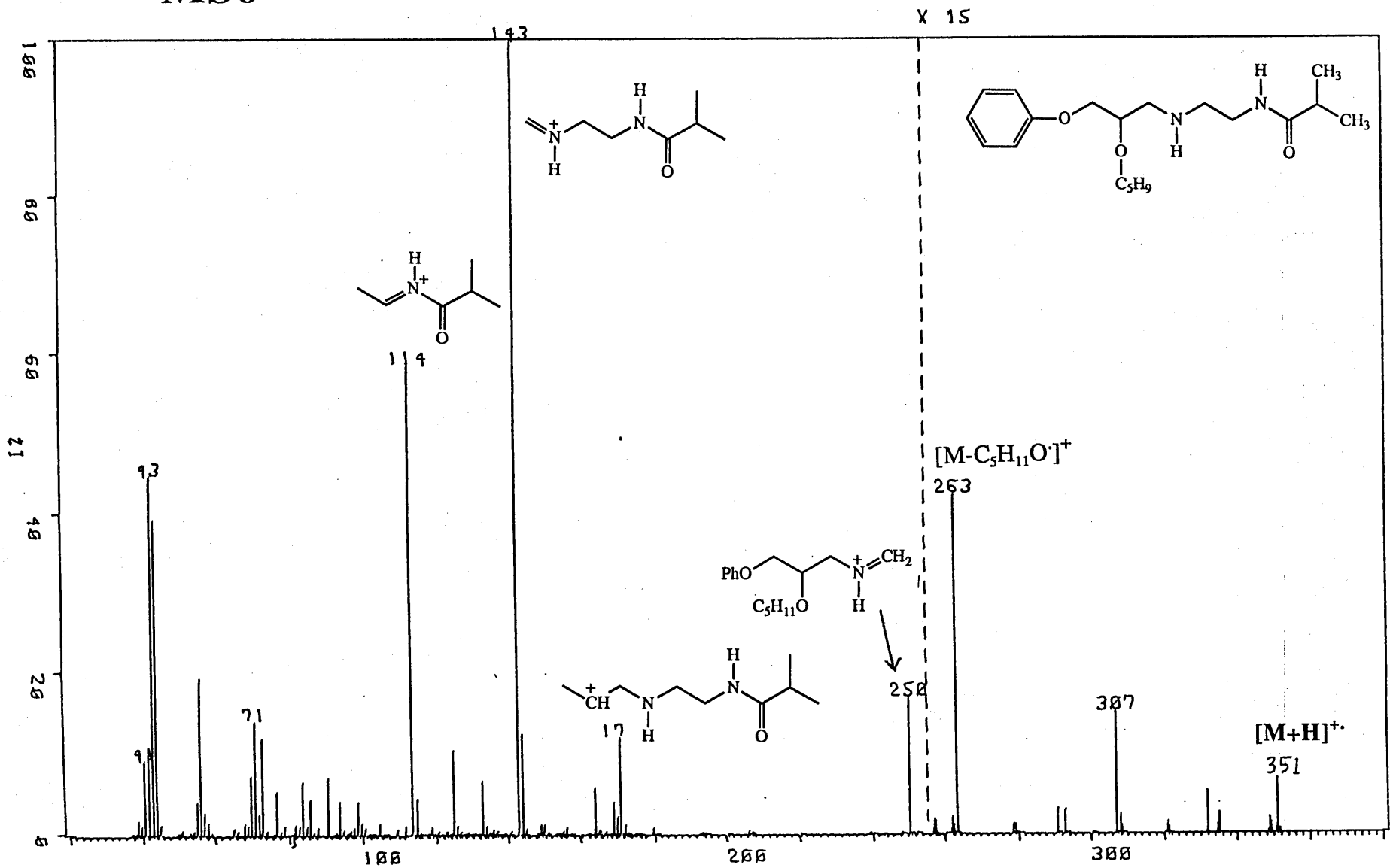


MS4 Mass spectrum of cyclised by-product (60).

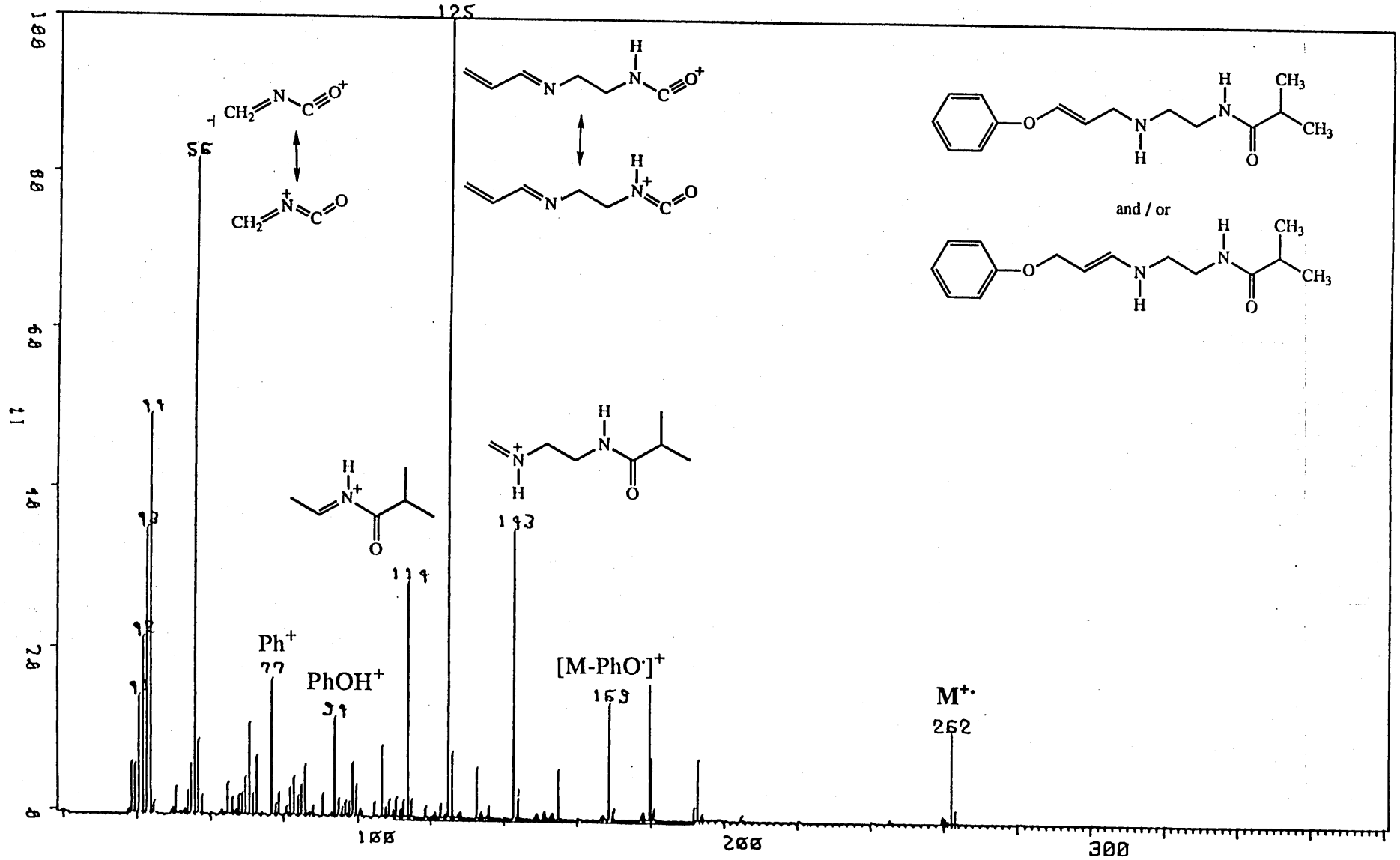



MS5
Mass spectrum of alcohol (41).
X 5
100
80
60
40
20
0
%
70
125
112
93
142
$(M-H)^+$
50
100
150
N
H
C=O
CH_3
CH_2
CH_2OH

MS6 Mass spectrum of the unresolved diastereoisomeric compound (62)

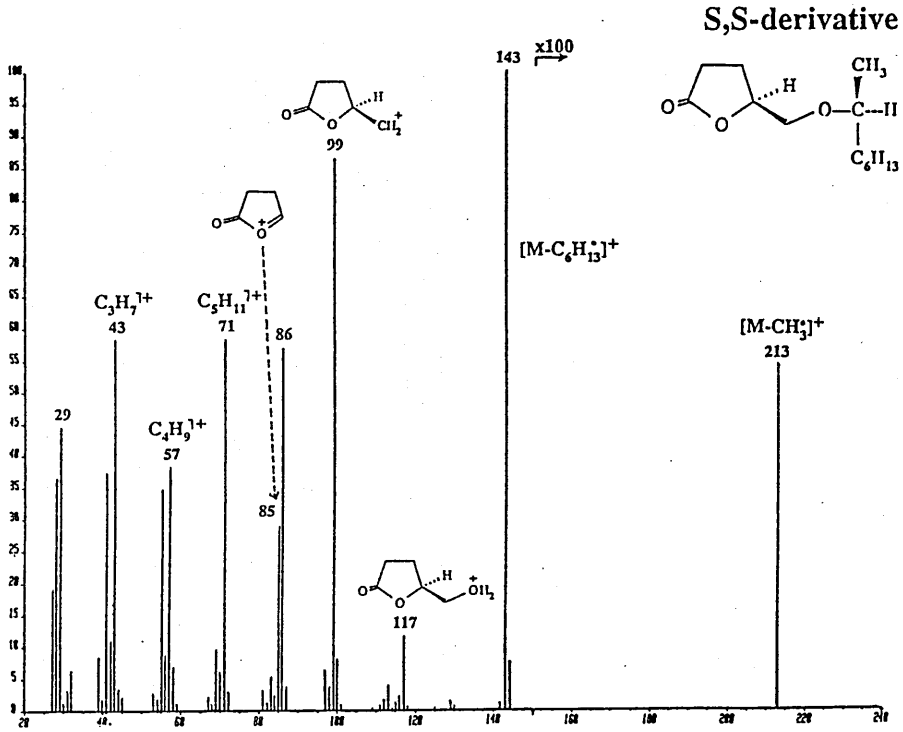


MS7 Mass spectrum of the alkene (dehydration) by-product (63a) or (63b)



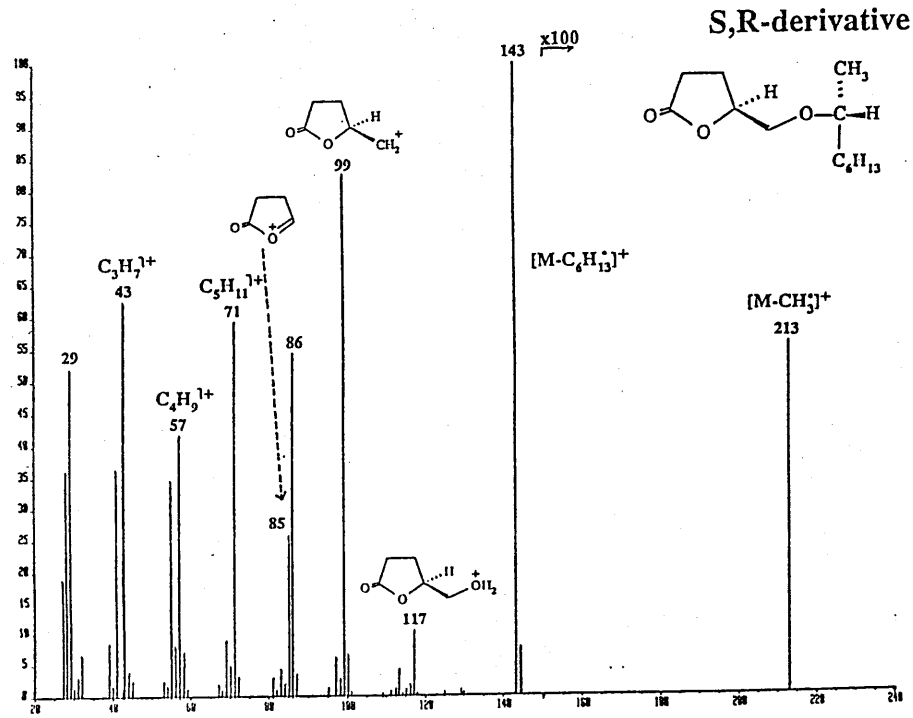
Mass spectrum of the resolved S,S-diastereoisomer (64) (2-octanol derivative).

MS8



Mass spectrum of the resolved S,R-diastereoisomer (64) (2-octanol derivative).

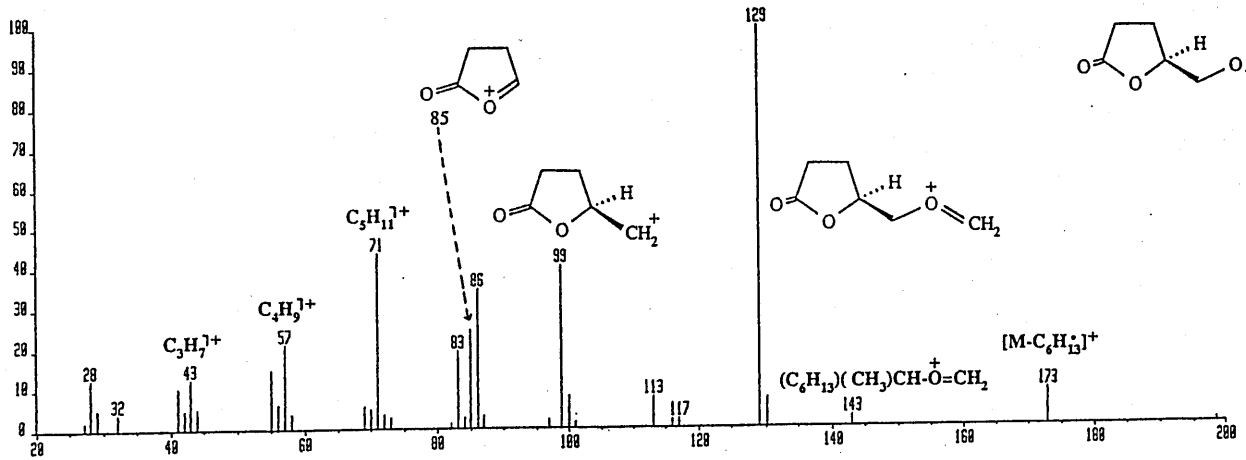
MS9



Mass spectrum of the resolved S,R-diastereoisomer (84) (2-octanol derivative).

MS10

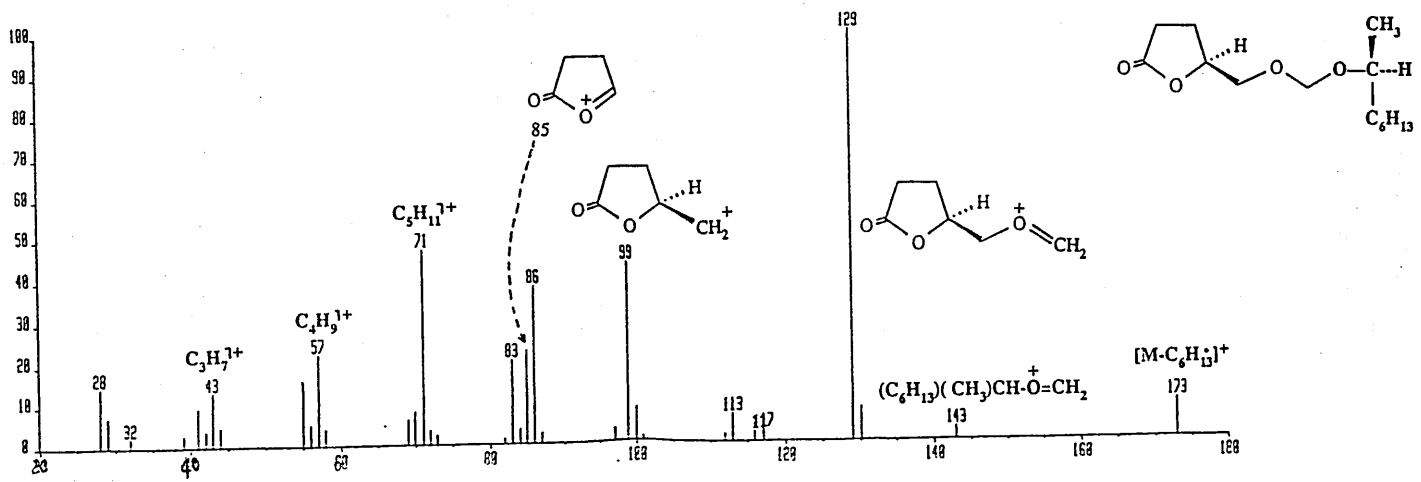
S,R-derivative

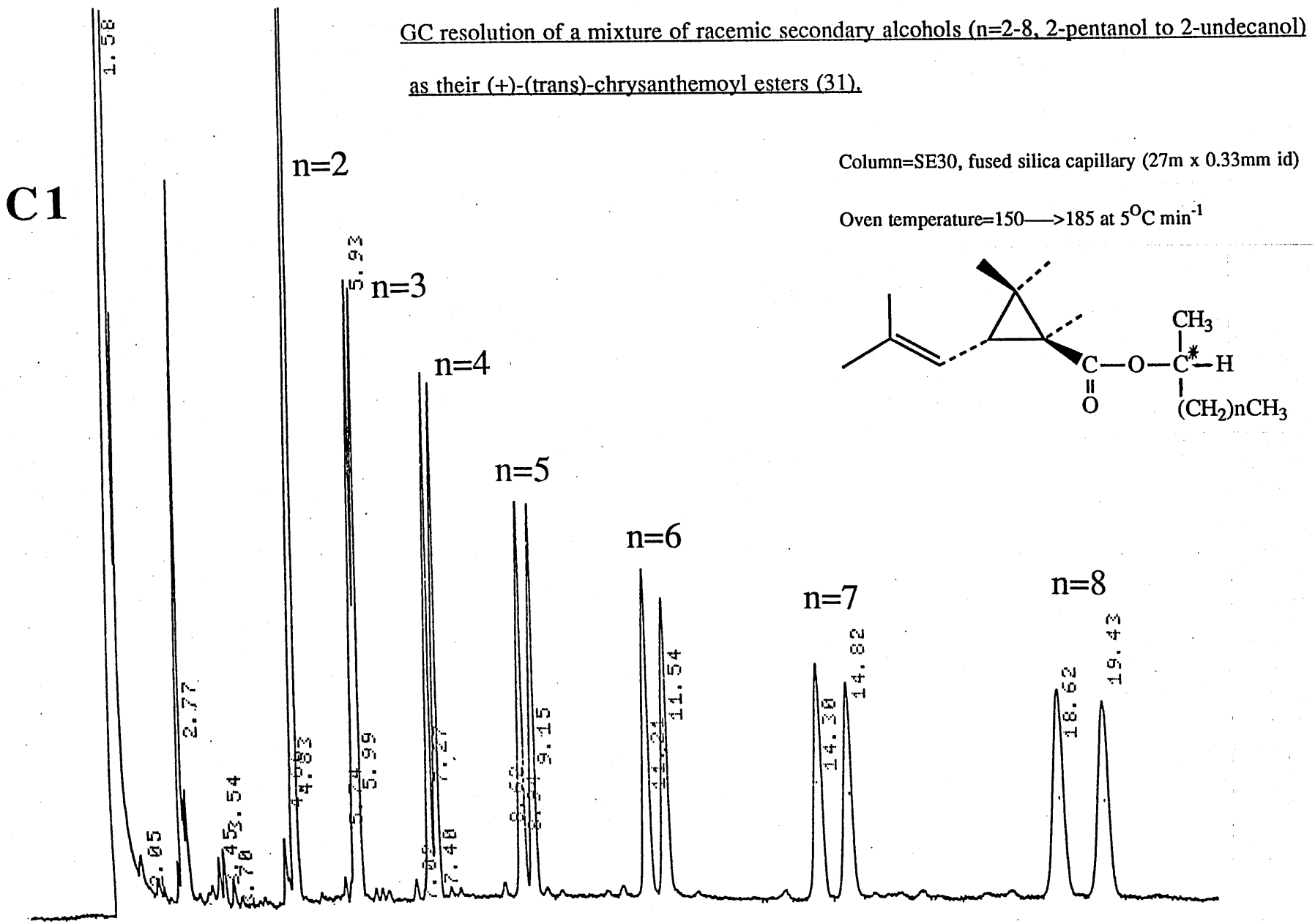


Mass spectrum of the resolved S,S-diastereoisomer (84) (2-octanol derivative).

MS11

S,S-derivative





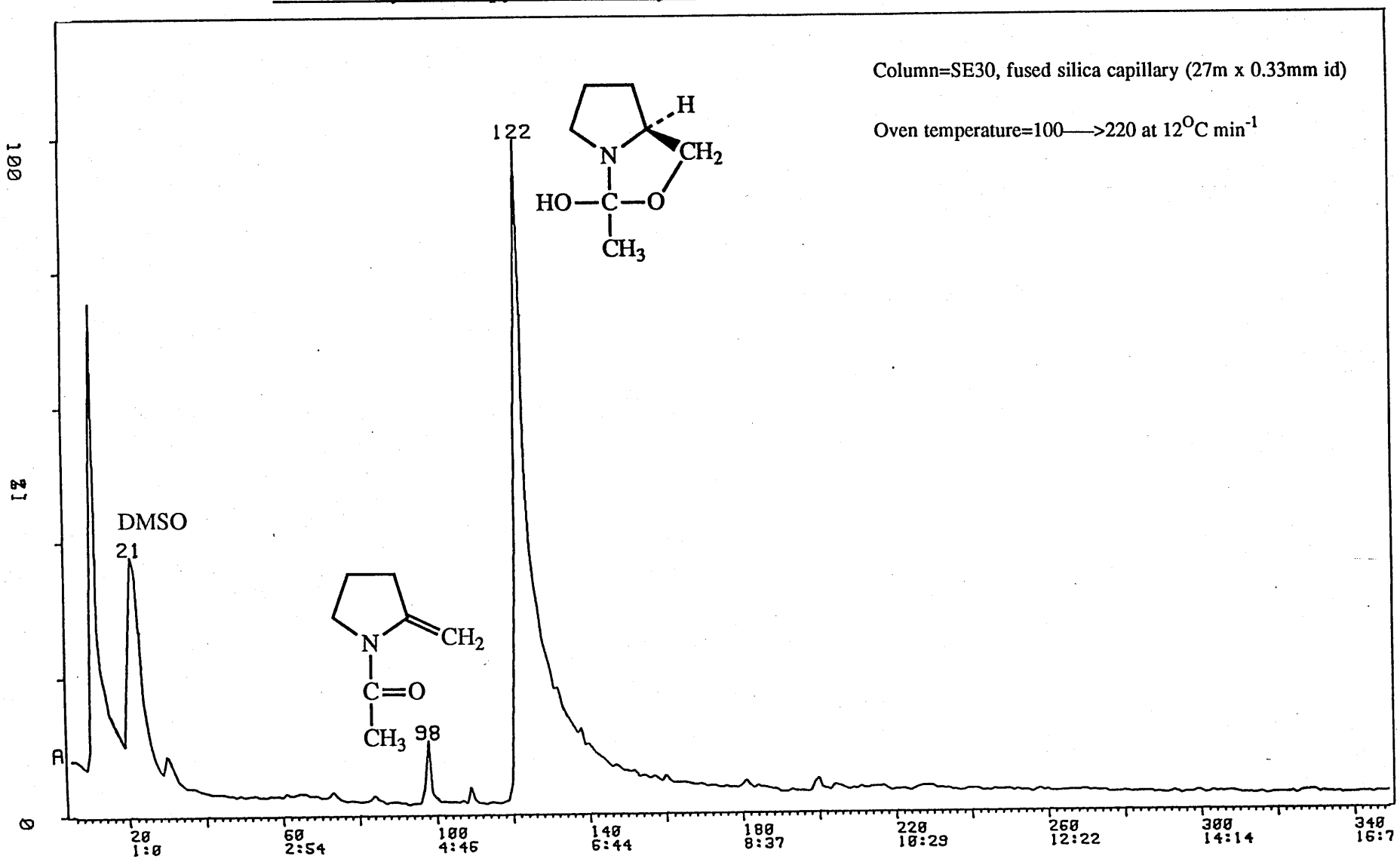
GC resolution of a mixture of racemic secondary alcohols (n=2-8, 2-pentanol to 2-undecanol) as their (+)-(trans)-chrysanthemoyl esters (31).

Column=SE30, fused silica capillary (27m x 0.33mm id)

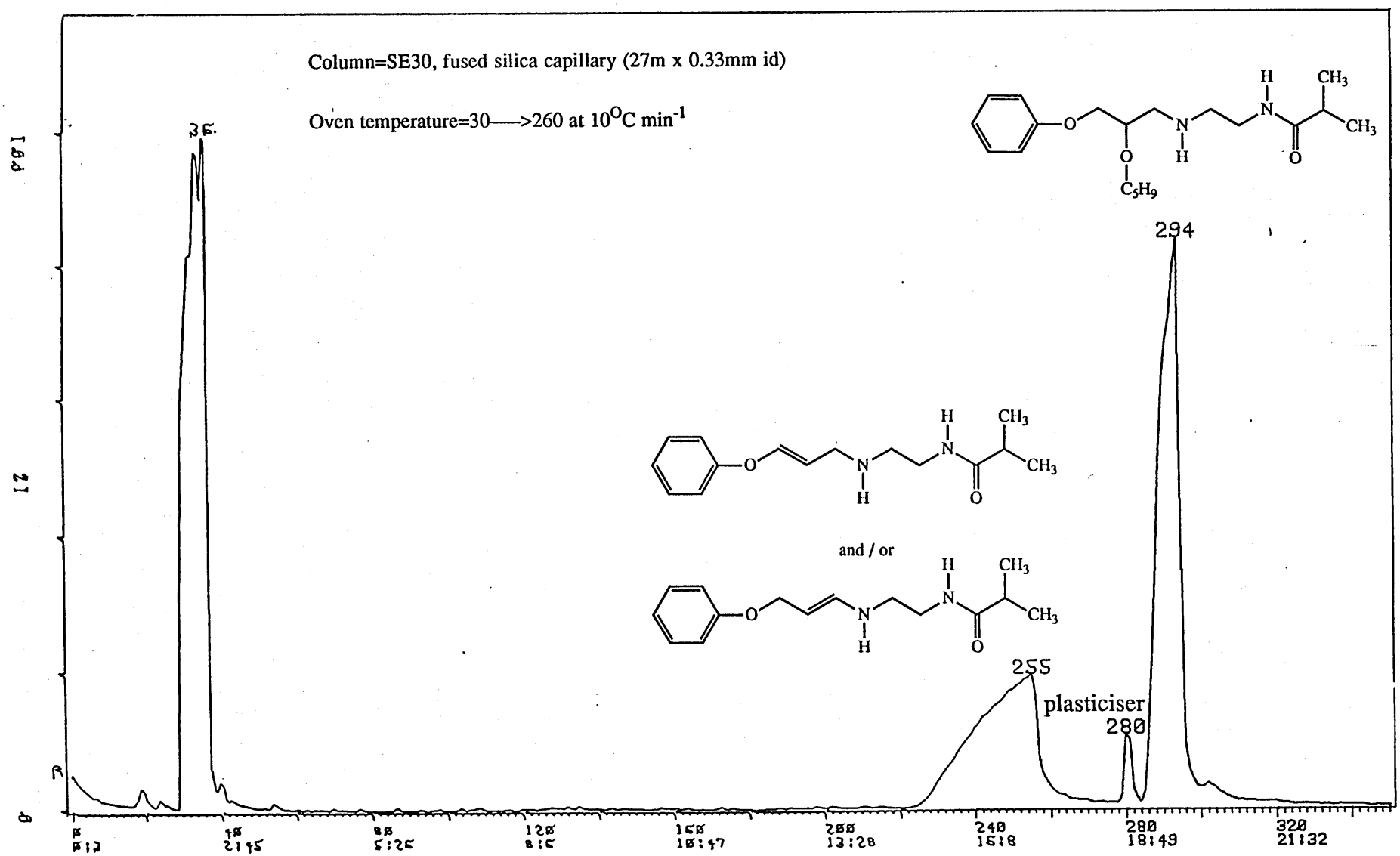
Oven temperature=150——>185 at 5^{O}C min^{-1}

C2

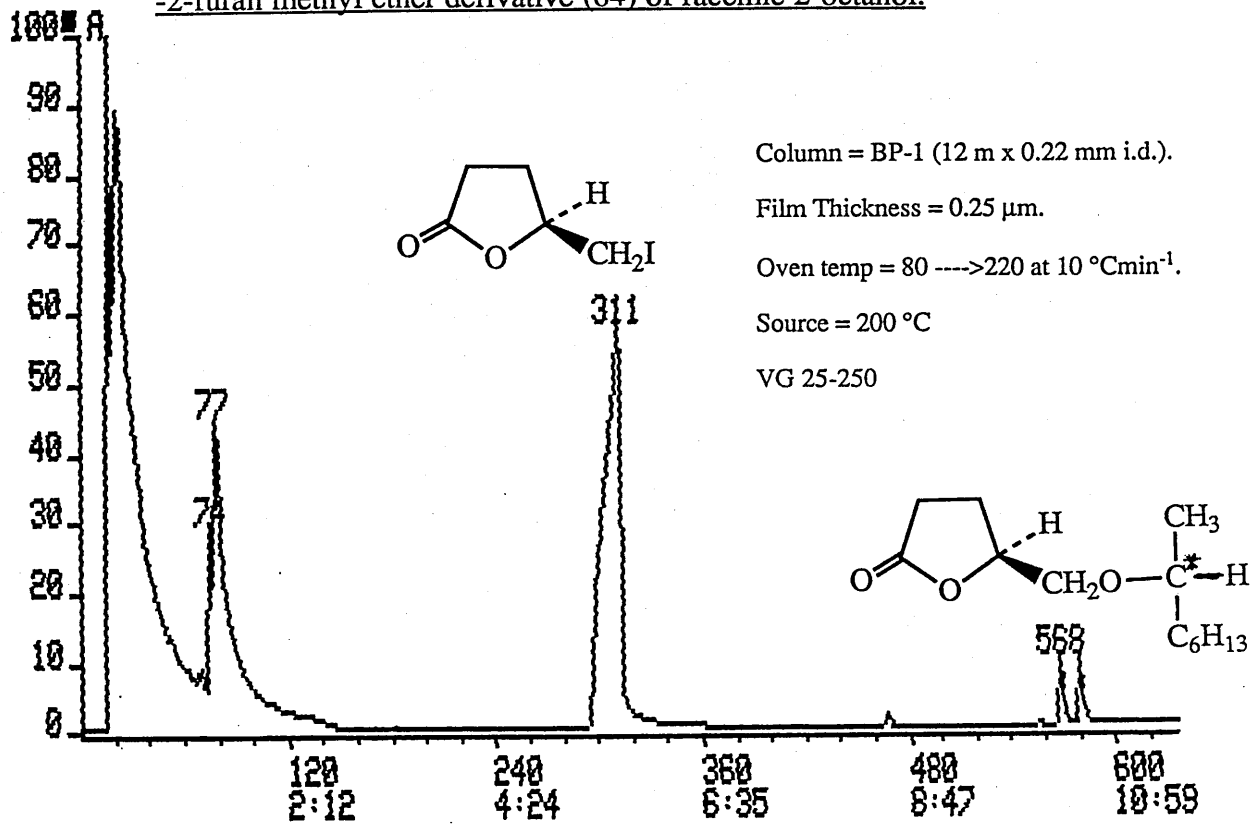
Chromatogram of the product mixture from the attempted derivatization of racemic 2-hexanol with N-acetyl-(S)-(-)-pyrrolidine methylchloride (42).



C3 Chromatogram of the product mixture from the derivatization reaction of racemic compound (61) with (S)-(+)-1 bromo-2-methyl butane.



C4 Chromatograms showing the GC resolution of the (S)-(+)-tetrahydro-5-oxo--2-furan methyl ether derivative (64) of racemic 2-octanol.



Column = BP-1 (12 m x 0.22 mm i.d.).

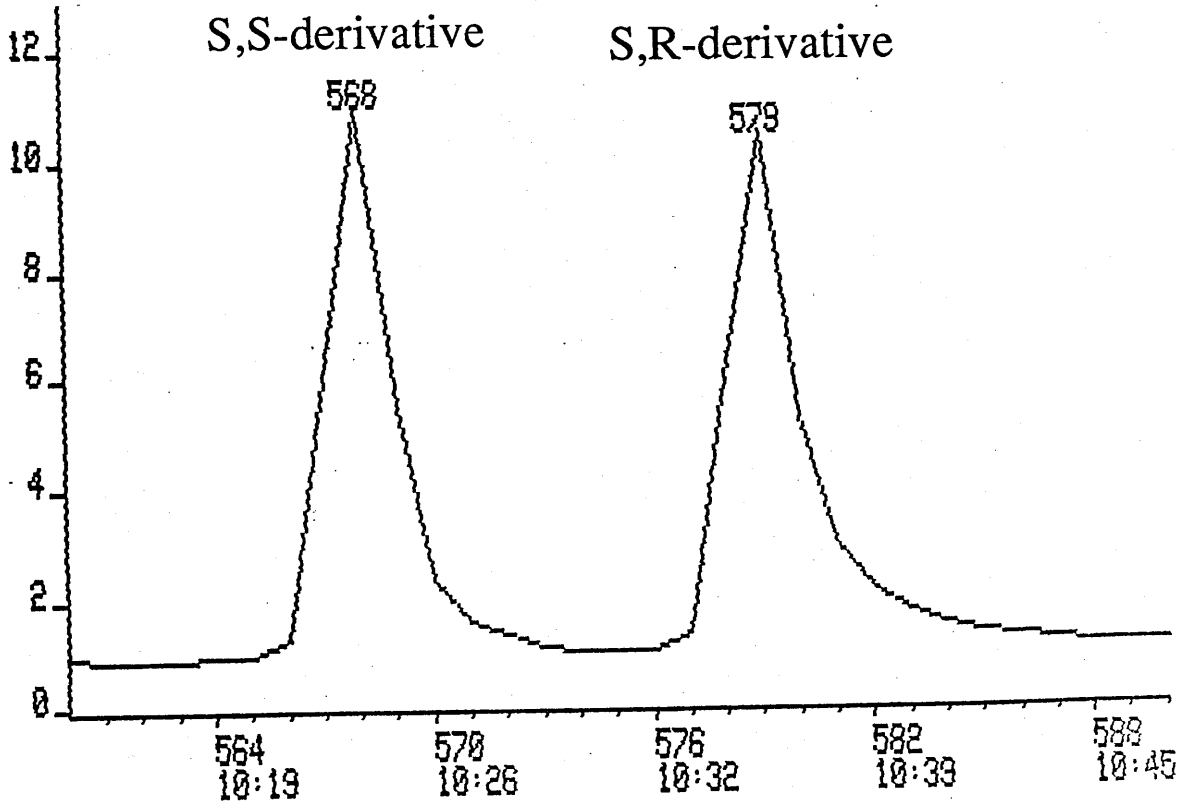
Film Thickness = 0.25 µm.

Oven temp = 80 ---->220 at 10 °Cmin^{-1}.

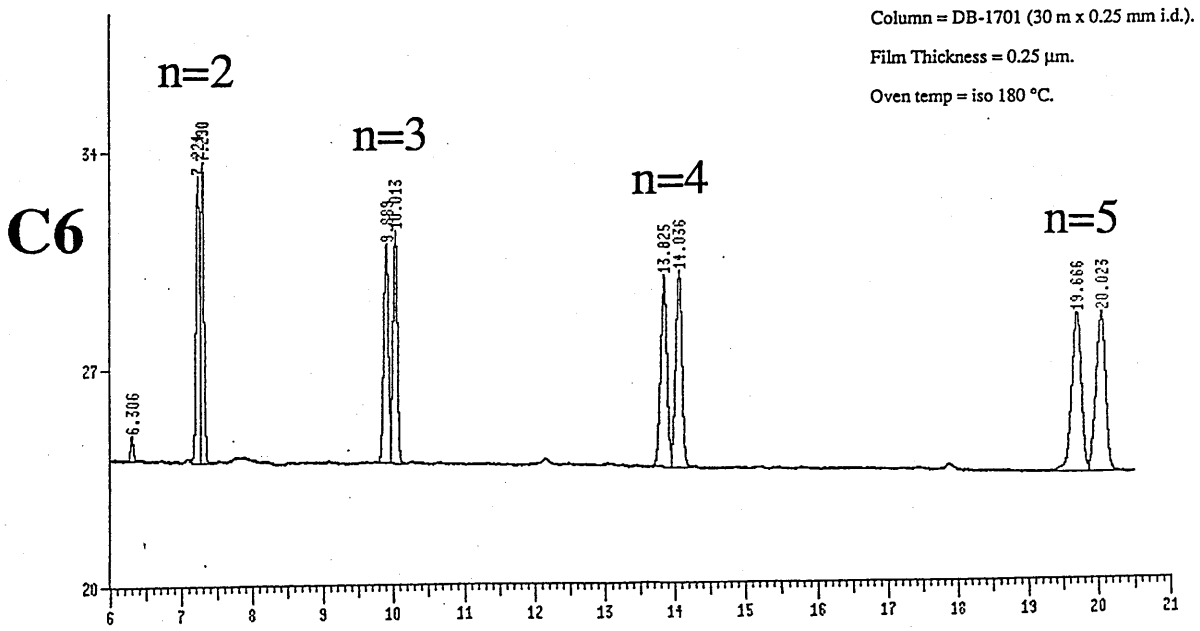
Source = 200 °C

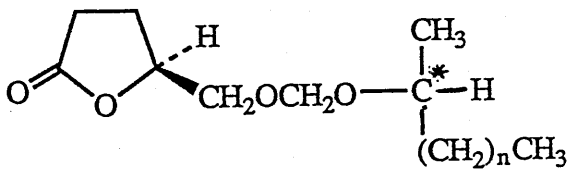
VG 25-250

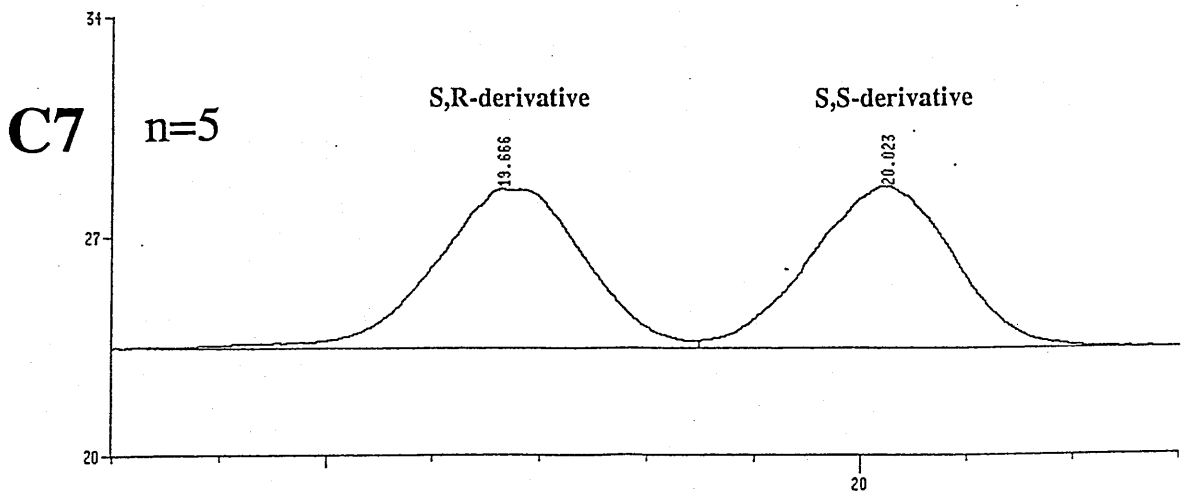
C5



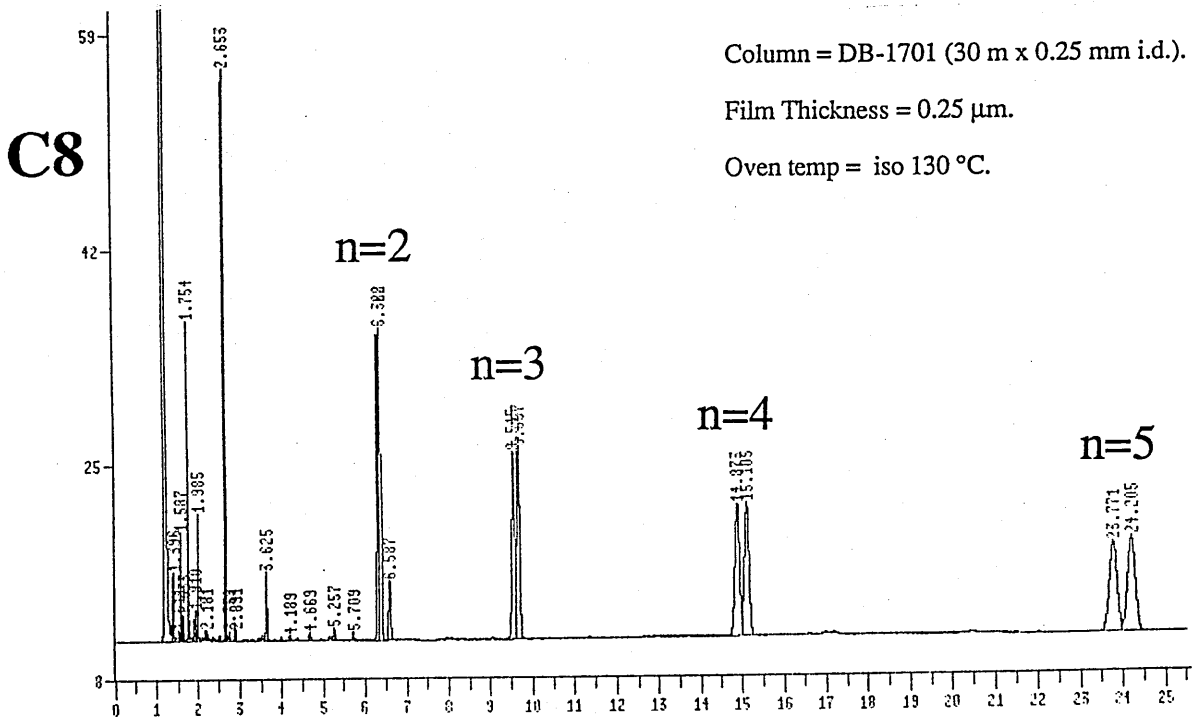
Chromatograms showing the GC resolution of a mixture of racemic secondary alcohols (n=2-5, 2-pentanol to 2-octanol) derivatized with CME (69) (ie diastereoisomers (84)).

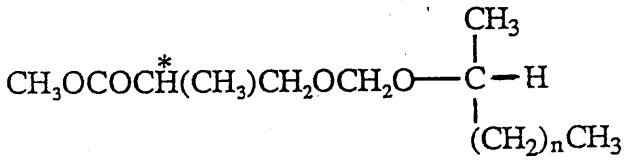


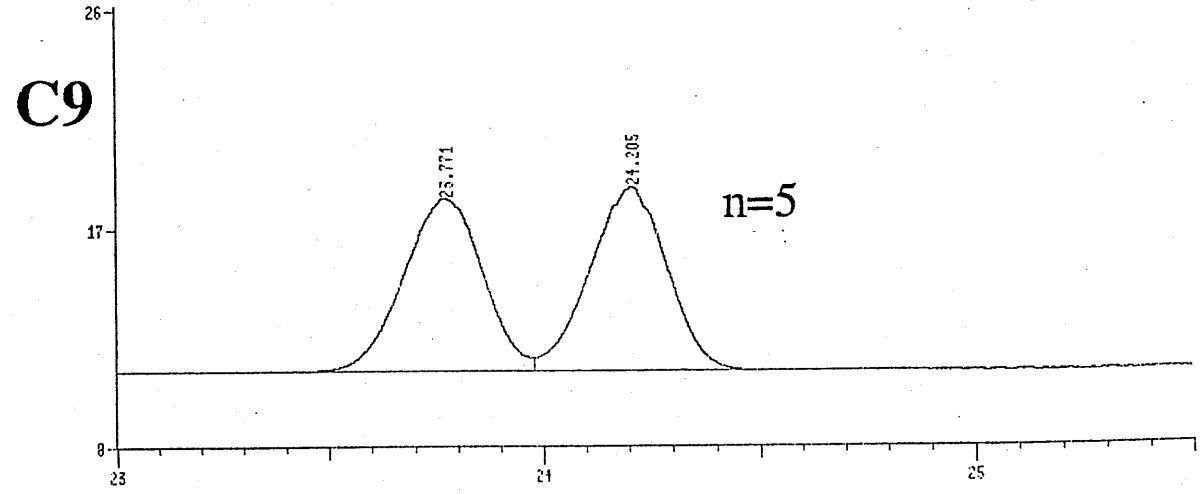




Chromatograms showing the GC resolution of a mixture of racemic secondary alcohols (n=2-5, 2-pentanol to 2-octanol) derivatized with CME (79) (ie diastereoisomers (85)).



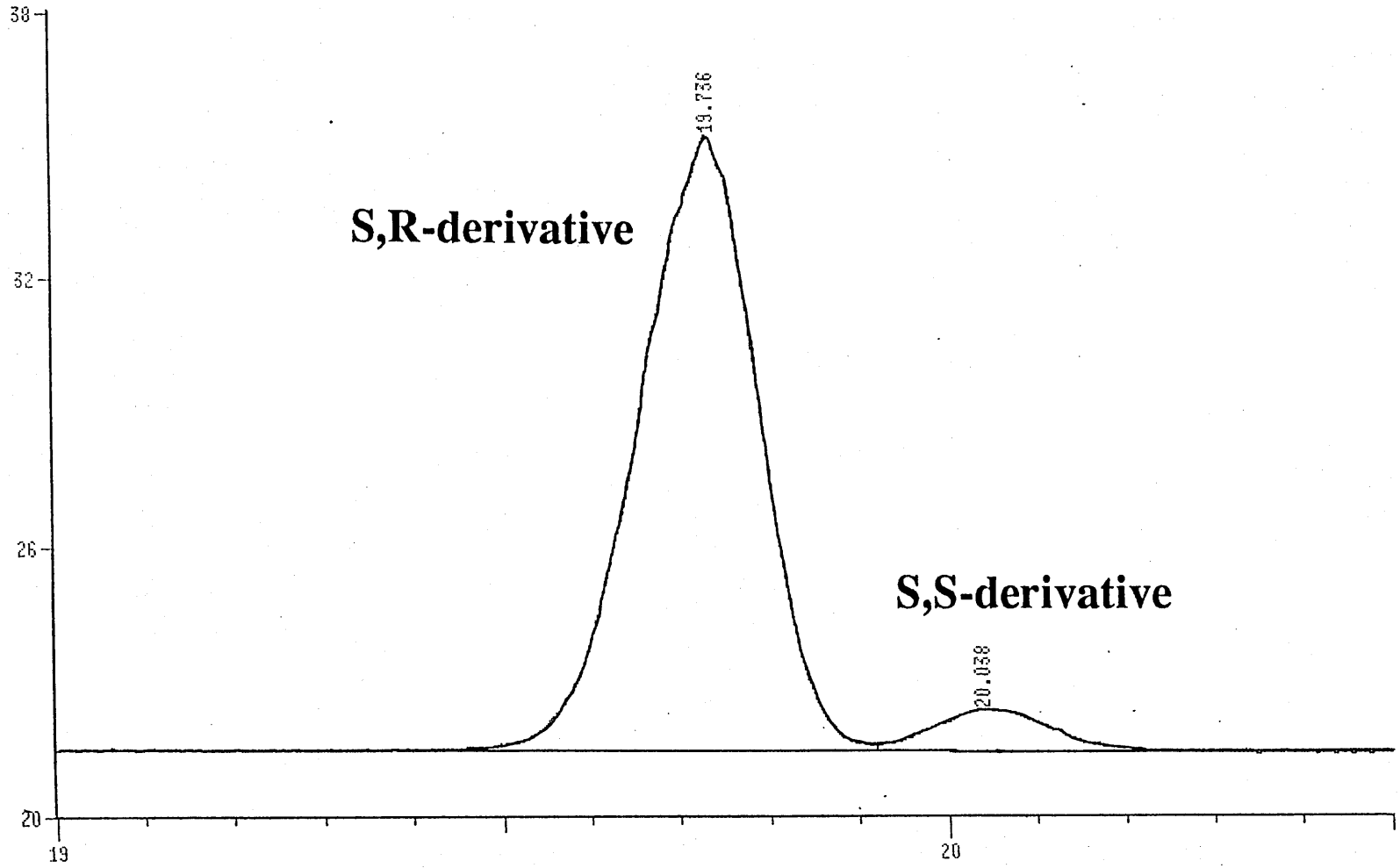




C10

GC resolution of R-2-octanol (95%) and S-2-octanol (5%) derivatized using CME (69).

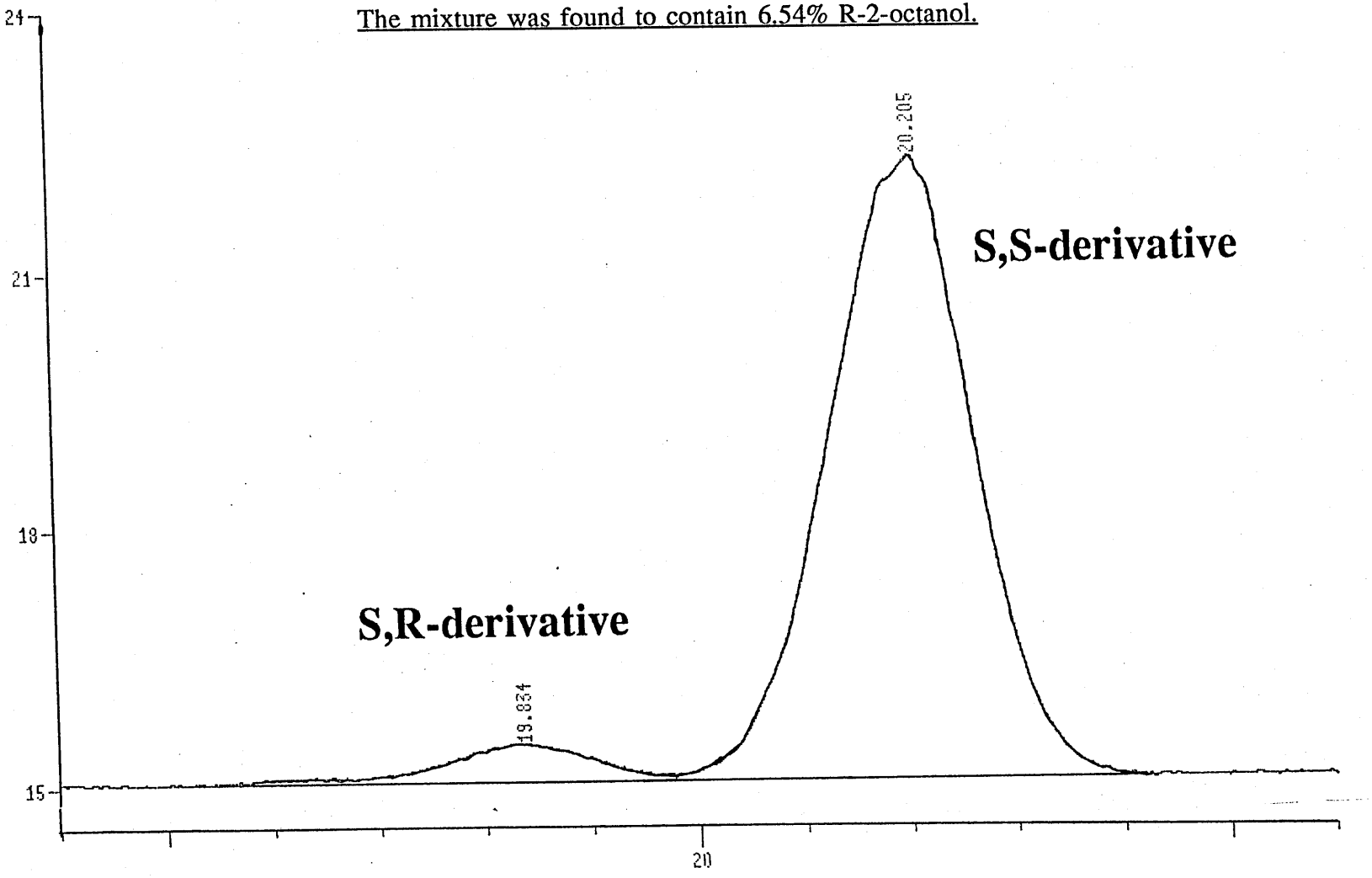
The mixture was found to contain 6.4% S-2-octanol.



C11

GC resolution of S-2-octanol (95%) and R-2-octanol (5%) derivatized using CME (69).

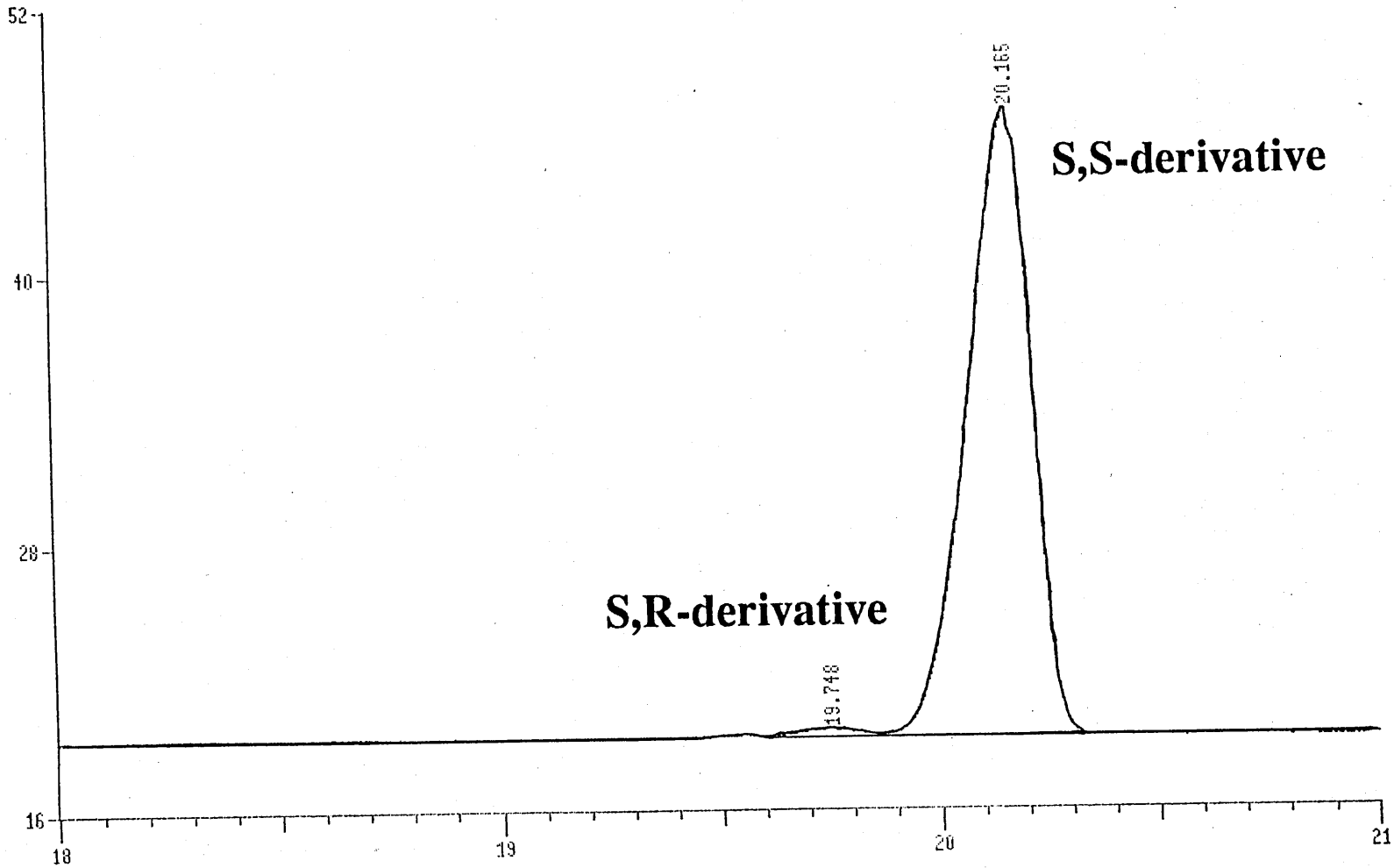
The mixture was found to contain 6.54% R-2-octanol.



C12

GC resolution of S-2-octanol (99.8%) and R-2-octanol (0.2%) derivatized using CME (69).

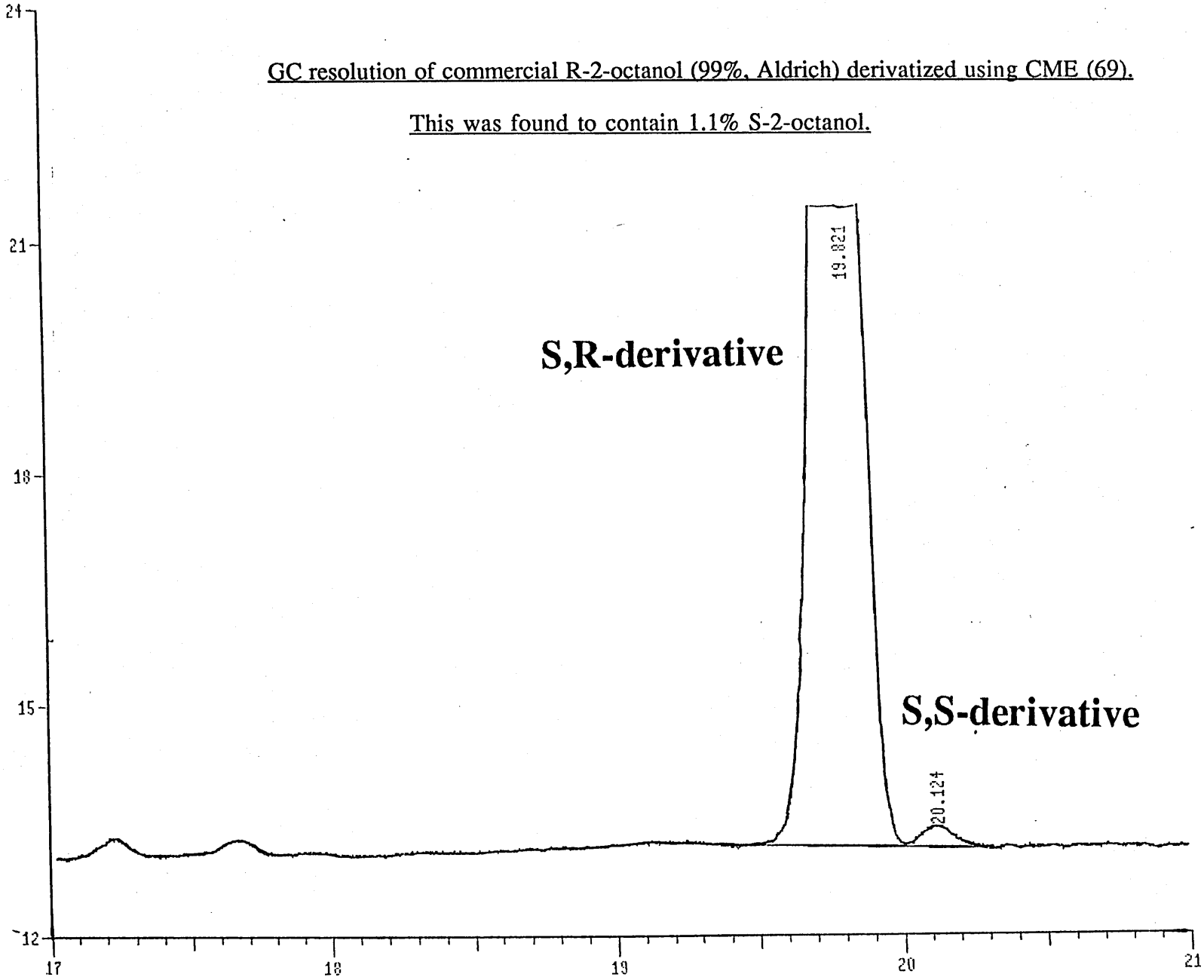
The mixture was found to contain 1.4% R-2-octanol.



C13

GC resolution of commercial R-2-octanol (99%, Aldrich) derivatized using CME (69).

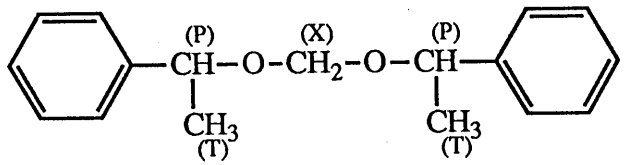
This was found to contain 1.1% S-2-octanol.



NMR1

^{1}H nmr spectrum Of dialkyl diether by-product (99).

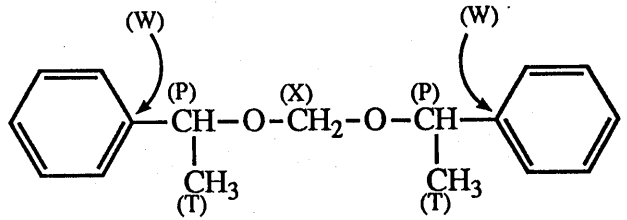
NO	FREQ(HZ)	PPM	INT%
1	655.82	7.310	6949
2	652.58	7.283	6495
3	437.81	4.877	606
4	430.41	4.803	1013
5	423.09	4.721	993
6	421.63	4.705	848
7	415.83	4.631	772
8	406.73	4.539	900
9	400.87	4.473	2125
10	192.62	2.149	487
11	137.69	1.536	3062
12	134.27	1.498	413
13	131.10	1.463	2957
14	118.65	1.324	3690
15	112.06	1.250	3543
16	3.41	0.038	416
17	0.00	0.000	8375



NMR2

^{13}C nmr spectrum Of dialkyl diether by-product (99).

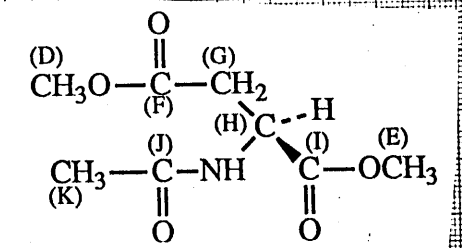
NO	FREQ(HZ)	PPM	INT%
1	3241.26	143.846	628
2	3227.82	143.214	668
3	2893.05	128.393	8397
4	2872.34	127.474	2708
5	2869.75	127.359	3250
6	2856.81	126.784	371
7	2850.34	126.497	4260
8	2846.45	126.325	5482
9	2059.44	91.397	1520
10	2034.84	90.306	1175
11	1768.19	78.472	953
12	1735.83	77.035	1145
13	1703.47	75.599	1196
14	1681.46	74.623	2214
15	1669.81	74.106	424
16	1663.34	73.818	2164
17	539.77	23.955	2405
18	530.71	23.553	444
19	521.65	23.150	2847
20	0.00	0.000	1567



NMR3

^{13}C nmr spectrum of N-acetyl-(S)-(+)-aspartic acid dimethyl ester (110).

E	CURSOR	FREQ	PPM	INTEGRAL	INTENSITY
1	1034	3457.052	171.5746	1.815	4.098
2	1039	3451.240	171.2861	1.720	3.578
3	1059	3426.628	170.0646	1.613	3.616
4	2566	1587.067	78.7666	3.075	5.132
5	2592	1555.118	77.1810	3.630	5.412
6	2618	1523.145	75.5941	3.806	5.138
7	2995	1063.017	52.7578	5.202	10.015
8	3008	1047.428	51.9842	4.901	10.397
9	3064	979.211	48.5985	7.627	15.244
10	3269	728.688	36.1650	7.868	18.162
11	3486	463.645	23.0109	4.510	9.145
12	3866	-.029	-.0014	6.018	14.742



(H)

(G)

TMS

(D&E)

(K)

Solvent

(F, I&J)

2ϕϕ

1ϕϕ

ϕ

NMR4

^{13}C nmr spectrum of triethylene glycol.

E	CURSOR	FREQ	PPM	INTEGRAL	INTENSITY
1	2605	1478.759	73.3910	10.746	14.591
2	2620	1460.021	72.4611	.1259	.308
3	2641	1433.835	71.1615	12.043	18.000
4	2794	1247.485	61.9129	11.022	14.482
5	2957	1049.033	52.0637	.322	.275
6	3974	1027.710	51.0054	.712	.749
7	2992	1006.149	49.9353	1.231	1.377
8	3009	984.902	48.8808	1.475	1.639
9	3027	963.323	47.8124	1.289	1.434
10	3044	942.100	46.7566	.478	.511
11	3062	920.533	45.6900	.319	.290
12	3916	-5.105	.0052	1.993	2.827

(C)

(A)

(B)

(A) (B) (C) (C) (B) (A)

$HOCH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2OH$

TMS

Solvent

200 100 0

NMR5

^{13}C nmr spectrum of the product mixture from the polymerisation reaction of N-ac-(S)-(+)-Asp-DME and TRIGOL using zinc acetate as a catalyst.

L	CURSOR	FREQ	PPM	INTEGRAL	INTENSITY
1	1039	3452.046	171.3261	4.940	3.956
2	1049	3440.142	170.7353	5.571	6.485
3	1055	3433.147	170.3881	5.545	4.222
4	2564	1590.683	78.9461	2.685	3.023
5	2590	1558.584	77.3530	2.813	2.504
6	2617	1526.232	75.7474	3.363	2.936
7	2669	1462.298	72.5743	11.374	12.512
8	2703	1420.373	70.4936	18.535	16.000
9	2707	1416.210	70.2869	9.691	13.320
10	2731	1386.250	68.8000	17.596	13.255
11	2800	1302.656	64.6512	9.304	9.358
12	2812	1287.416	63.8948	5.894	4.747
13	2819	1279.609	63.5074	1.896	2.025
14	2852	1238.820	61.4830	11.531	11.216
15	2998	1061.140	52.6647	3.967	4.682
16	3010	1046.587	51.9424	8.077	7.818
17	3062	982.491	48.7613	16.655	10.469
18	3271	728.121	36.1369	16.017	9.958
19	3491	459.319	22.7961	9.832	10.819
20	3867	-.157	-.0078	3.974	4.100