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# NOVEL MATERIALS FOR MEMBRANE SEPARATION PROCESSES.

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DOCTOR OF PHILOSOPHY

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NOVEL MATERIALS FOR MEMBRANE SEPARATION PROCESSES.

Submitted For The Degree  
Of Doctor Of Philosophy

Michael Charles Lloyd  
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SUMMARY.

The aim of this work was to synthesise a series of hydrophilic derivatives of *cis*-1,2-dihydroxy-3,5-cyclohexadiene (*cis*-DHCD) and copolymerise them with 2-hydroxyethyl methacrylate (HEMA), to produce a completely new range of hydrogel materials. It is theorised that hydrogels incorporating such derivatives of *cis*-DHCD will exhibit good strength and elasticity in addition to good water binding ability.

The synthesis of derivatives was attempted by both enzymatic and chemical methods. Enzyme synthesis involved the transesterification of *cis*-DHCD with a number of trichloro and trifluoroethyl esters using the enzyme lipase porcine pancreas to catalyse the reaction in organic solvent. Cyclohexanol was used in initial studies to assess the viability of enzyme catalysed reactions.

Chemical synthesis involved the epoxidation of a number of unsaturated carboxylic acids and the subsequent reaction of these epoxy acids with *cis*-DHCD in DCC/DMAP catalysed esterifications. The silylation of *cis*-DHCD using TBDCS and BSA was also studied.

The rate of aromatisation of *cis*-DHCD at room temperature was studied in order to assess its stability and <sup>1</sup>H NMR studies were also undertaken to determine the conformations adopted by derivatives of *cis*-DHCD.

The copolymerisation of diepoxybutanoate, diepoxyundecanoate, dibutenoate and silyl protected derivatives of *cis*-DHCD with HEMA, to produce a new group of hydrogels was investigated. The EWC and mechanical properties of these hydrogels were measured and DSC was used to determine the amount of freezing and non-freezing water in the membranes. The effect on EWC of opening the epoxide rings of the comonomers was also investigated.

**Keywords:** *cis*-1,2-Dihydroxy-3,5-cyclohexadiene, Hydrogel, Epoxy Acid, Mechanical Properties, Esterification.

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**To Mum and Dad**

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## LIST OF ABBREVIATIONS.

AIBN	Azobisisobutyronitrile
br	Broad
BSA	Bis(trimethylsilyl)acetamide
CM	Cyclic Monomer
CWP	tris(cetylpyridinium)12-tungstophosphate
d (NMR)	Doublet
DCC	1,3-Dicyclohexylcarbodiimide
DCU	1,3-Dicyclohexylurea
dd (NMR)	Double Doublet
DEGDMA	Diethylene Glycol Dimethacrylate
DHCD	1,2-Dihydroxy-3,5-Cyclohexadiene
DHCD-EB	<i>cis</i> -1,2-Bis(2,3-epoxybutanoyloxy)-3,5-Cyclohexadiene
DHCD-EU	<i>cis</i> -1,2-Bis(10,11-epoxyundecanoyloxy)-3,5-Cyclohexadiene
DHCD-TBDMS	<i>cis</i> -1,2-Bis( <i>tert</i> -butyldimethylsiloxy)-3,5-Cyclohexadiene
DHCD-TMS	<i>cis</i> -1,2-Bis(trimethylsiloxy)-3,5-Cyclohexadiene
DHCD-UA	<i>cis</i> -1,2-Bis(10-undecenoyloxy)-3,5-Cyclohexadiene
DHCD-VA	<i>cis</i> -1,2-Bis(3-butenoyloxy)-3,5-Cyclohexadiene
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DSC	Differential Scanning Calorimetry
dt (NMR)	Double Triplet
$\delta$	Chemical Shift / ppm
E	Youngs Modulus
$\epsilon_b$	Elongation to Break

EDMA	Ethylene Glycol Dimethacrylate
EWC	Equilibrium Water Content
GC	Gas Chromatography
HEMA	2-Hydroxyethyl Methacrylate
J (NMR)	Coupling Constant
m (IR)	Medium
m (NMR)	Multiplet
<i>m</i> -CPBA	<i>m</i> -Chloroperoxybenzoic Acid
NAD	Nicotinamide Adenine Dinucleotide
PPL	Porcine Pancreas Lipase
PTC	Phase Transfer Catalysis
q (NMR)	Quartet
qt (NMR)	Quintet
s (IR)	Strong
s (NMR)	Singlet
$\sigma_b$	Tensile Strength
t (NMR)	Triplet
TBDCS	<i>tert</i> -Butyldimethylchlorosilane
TBDMS	<i>tert</i> -Butyldimethylsilyl
TEA	Triethylamine
TEGDMA	Tetraethylene Glycol Dimethacrylate
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
w (IR)	Weak
v/v	Volume / Volume
w/v	Weight / Volume
w/w	Weight / Weight
-ve (NMR)	Negative Peak
+ve (NMR)	Positive Peak

# **CHAPTER 1:**

## **Introduction.**

## 1. INTRODUCTION.

### 1.1 GENERAL INTRODUCTION.

Hydrogels have over the years become very important in the area of biomaterials, where their applications include such uses as catheters, hemodialysis membranes, degradable therapeutic systems, drug delivery systems and contact lenses.<sup>1-10</sup> It is of paramount importance that a biomaterial exhibits good biocompatibility, and this is accomplished with hydrogels by way of their high water content, which can be as high as 70%. Many of the current generation of hydrogel materials are based on poly(2-hydroxyethyl methacrylate) (polyHEMA). Such materials were first suggested by Wichterle<sup>11-13</sup> in 1961 and exhibit high water content and good oxygen permeability, but at the same time tend to suffer from poor mechanical properties. Much research has been directed at trying to modify existing hydrogels to overcome this problem.<sup>14-18</sup> The co-polymerisation of 2-hydroxyethyl methacrylate (HEMA) with conventional strengthening monomers such as styrene and methyl methacrylate has been shown to improve tear strength, but unfortunately it also causes a reduction in the hydrophilicity of the material which in turn causes a decrease in the water content of the swollen state.<sup>14-15</sup>

The compound *cis*-1,2-dihydroxy-3,5-cyclohexadiene (DHCD) has the potential to be the basis of a whole new range of hydrogel materials. Being a 1,3-diene it can be copolymerised with acrylic monomers to yield in-chain cyclohexene rings which would be expected to enhance both the toughness and the stiffness of the acrylic polymer. Furthermore, the diol moiety renders the monomer extremely hydrophilic. The reaction of these hydroxy groups by a number of well established processes allow them to be converted into a wide variety of derivatives.<sup>19-21</sup> The copolymerisation of hydrophilic derivatives of DHCD with HEMA would be expected to reduce the rotational mobility of the polymer chains, compared to polyHEMA itself, due to the larger steric interaction between pendant groups. It is theorised that these materials will exhibit

better mechanical strength and elasticity than polyHEMA whilst not losing any water binding ability. Hydrogels exhibiting such characteristics have been much sought after by researchers since the emergence of hydrogels as important biomaterials.

## **1.2 HYDROGELS.**

Hydrogels can most easily be described as water swollen polymer networks of either natural or synthetic origin. They are characterised by their hydrophilicity and insolubility in water. The hydrophilicity results from the presence of such groups as OH, CONH<sub>2</sub>, CONHR, CONR<sub>2</sub>, COOH and SO<sub>3</sub>H and allows water to be retained within the polymer network. The presence of a three dimensional network accounts for the insolubility of the polymer and the retention of shape when swollen in water to their equilibrium volume. The mechanical properties of hydrogels are dependent upon the amount of water in the polymer network.<sup>3</sup> For example, in the absence of water polyHEMA is hard, clear and glassy but on exposure to water the hydroxyl groups in polyHEMA take up water, converting it to a clear, flexible elastomeric gel.

### **1.2.1 Preparation of Hydrogels.**

The preparation of hydrogels is usually accomplished by the simultaneous polymerisation of monomer(s) and cross linking agent either in water, in another solvent or in the absence of solvent. An alternative method involves the introduction of cross links to a hydrophilic polymer in solution. The free radicals that are required to initiate these types of polymerisation may be generated by: a) chemical initiators such as azobisisobutyronitrile (AIBN) (or its ester derivatives), ammonium persulfate and benzoyl peroxide; b) UV radiation in the presence of a photosensitive chemical; or c) ionising radiation.<sup>22-24</sup>

The most commonly used cross linking agents in polyHEMA systems are dimethacrylate esters, ethylene glycol dimethacrylate (EDMA), diethylene glycol dimethacrylate (DEGDMA) and tetraethylene glycol dimethacrylate (TEGDMA).<sup>7-9</sup>

### 1.2.2 Network Structure.

The physical and other properties of a particular hydrogel will be dependent upon its network structure.<sup>23</sup> The majority of hydrogels that are used in biomedical applications are non-crystalline. An ideal network consists of a collection of randomly distributed chains between multifunctional cross-links. In a non-swollen network, the polymer chains may exist in one of three states:

1. unstrained
2. expanded (network swollen in a mixture of monomers)
3. supercoiled

However, real polymer networks deviate from this ideal situation and imperfections in the network occur from changes in cross-linking conditions, cross-linking of already cross-linked networks and end linking.

Imperfections generally fall into three categories:

1. Pre-existing order: This includes crystallites, non-randomly ordered chain segments, artificially orientated chains and the association of similar groups leading to supermolecular order. All of these structural features become fixed after cross-linking.
2. Network defects: These are closed loops, chain entanglements and unreacted functionalities, see Figure 1.1.
3. Phase separation structures: These occur when the quantity of solvent in the gel exceeds the maximum swelling capacity of the gel for the particular solvent and conditions in question. This leads to an exceeding of the critical value of the cross-linking density. Phase separation manifests itself as macro- and microsyneresis. Microsyneresis is more likely to occur in lightly cross-linked networks and results from

the slow relaxation of the network compared with the rate of establishing local phase separation within the gel. Macrosynersis on the other hand is more likely to occur in heavily cross linked networks, due to the reduction in the mobility of the polymer chains.

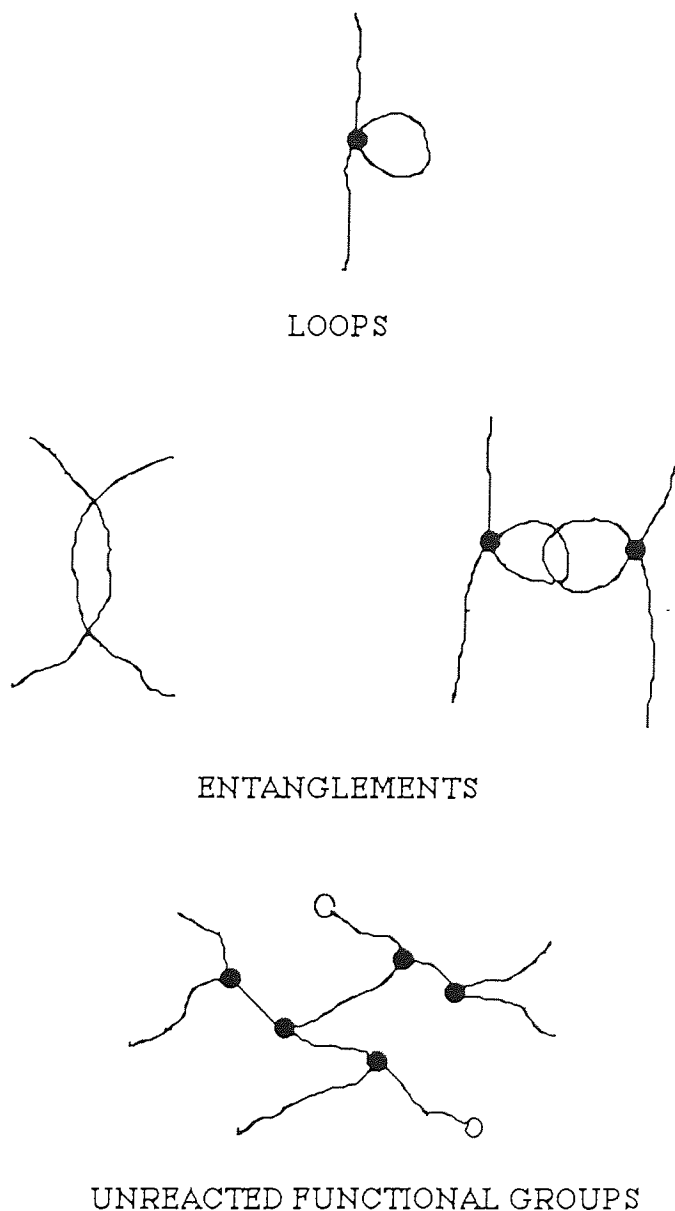


Figure 1.1: Network Defects.<sup>23</sup>

### 1.2.3 Hydrogel Materials as Contact Lenses.

In order for a material to be used in the eye, it must fulfil certain criteria relating to biocompatibility.<sup>4,9</sup> The material needs to be essentially an extension of the cornea, and as such it must allow the cornea to respire normally. Additionally, the material must resist the deforming force of the eyelid and permit a continuous tear film to be maintained on the lens. Any material in contact with biological fluid is prone to protein deposits and it is very important to minimise these accumulations. For a material to be used successfully in the eye, it must successfully mimic the properties of the cornea. The major properties of the cornea<sup>3</sup> are presented in Table 1.1.

**Table 1.1: Properties of the Cornea and its Environment.**

Water Content	81%
Critical surface tension	35mN m <sup>-1</sup>
Refractive index	1.37
Mechanical properties	Linear viscoelastic material at low stress; modulus of rigidity = $1.3 \times 10^7$ N m <sup>-2</sup>
Oxygen permeability (stroma)	$300 \times 10^{-10}$ cm <sup>3</sup> mm cm <sup>-2</sup> s <sup>-1</sup> cm <sup>-1</sup> Hg
Oxygen consumption	$3.5-7 \times 10^{-6}$ l cm <sup>-2</sup> h <sup>-1</sup>
Minimum oxygen tension for corneal clarity	12-18 mm Hg
Normal oxygen tension in tear fluid	155 mm Hg (open eye) 55 mm Hg (closed eye)
Surface tension of tears	46 mN m <sup>-1</sup>
Deforming force of eyelid	$2.6 \times 10^3$ N m <sup>-2</sup>

### 1.2.4 Water and Hydrogels.

In addition to being influenced by the structure of the polymer network, the properties of a hydrogel are also dependent upon the water imbibed within it, which acts just like a conventional plasticiser. The water also performs two other important functions. Firstly,



it acts as a bridge between the natural and the synthetic systems giving greater biocompatibility, and secondly it confers membrane properties on the hydrogel allowing the transport of oxygen and water soluble metabolites through the polymer matrix. It is the presence of water within hydrogels which makes them unique amongst biomaterials.

### **1.2.5 Equilibrium Water Content.**

The equilibrium water content (EWC) is defined as :

$$\text{EWC} = \frac{\text{Mass of Water in the Gel}}{\text{Mass of the Swollen Gel}} \times 100\%$$

It is argued that the EWC is the single most important property of a hydrogel, influencing the permeability, mechanical, surface and other properties of the gel. Unfortunately no simple relationship exists between the EWC and these properties.

### **1.2.6 Factors Affecting EWC.**

The nature of the monomer(s) is a major factor affecting the EWC of the gel, the more hydrophilic the monomer, the greater the EWC.<sup>16-18</sup> Inversely hydrophobic monomers such as styrene and methyl methacrylate will cause a drop in the EWC.<sup>14</sup> The cross link density also has a profound effect on the EWC.<sup>4</sup> An increase in the cross link density leads to a fall in the EWC because of a reduction in the chain length between cross links leading to a less elastic structure which can't imbibe as much water.

The temperature dependence of EWC for a particular hydrogel is thought to be determined by the competition of two processes.<sup>14</sup> In one process the hydrogen bonding between water and polar groups increases with increasing temperature, resulting in an increase in EWC. In the second process an increase in temperature causes an increase in the association of the hydrophobic portions of the polymer to form hydrophobic bonds, which effectively increases the cross link density and lowers the

EW. The nature of the particular hydrogel will dictate which process dominates and hence how the EW is effected by temperature.

### **1.2.7 Nature of Water in Hydrogels.**

There is much evidence to suggest that the water in hydrogels exists in more than one state.<sup>1,14,26-33</sup> The properties of the gel will be dependent upon the nature of the water imbibed by the gel. It is currently theorised that the water present in hydrogels exists in a continuum of states between two extremes.<sup>14,26-28</sup> At one extreme the water is strongly associated with the polymer by way of hydrogen bonding; water of this type is referred to as 'bound' or non-freezing water. At the other extreme there is water which has a much greater degree of mobility and is unaffected by the polymeric environment; water of this type is known as 'unbound' or freezing water. The water that exists between these extremes will show a wide variation in the nature of it's interactions with the polymer. Freezing water can be regarded as plasticising water because of it's greater relative effect on chain mobility.

### **1.2.8 Differential Scanning Calorimetry.**

The properties of hydrogels are strongly influenced by EW and the ratio of freezing:non-freezing water. This ratio is dependant upon the technique used to study water binding in the hydrogel. There are a large number of methods that can be used including: differential scanning calorimetry (DSC),<sup>14,26-29</sup> specific conductivity,<sup>29,34</sup> reverse osmosis,<sup>25,33</sup> dielectric refraction,<sup>34</sup> dilatometry,<sup>29,34</sup> diffusion techniques<sup>31</sup> and <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance spectroscopy.<sup>30</sup> Of these methods, DSC is the most convenient because of ease of sample preparation and speed with which measurements on samples can be made enabling rapid elucidation of water binding data.

DSC measures the gross phase changes of water within a polymer. When a hydrogel sample is cooled to -70°C, the free and interfacial water freezes but the bound water

remains in a non-frozen state as a consequence of its strong association with the polymer chain. DSC measures the freezing water content of the hydrogel, but if the EWC is known then the non-freezing water content can also be calculated.

### 1.2.9 Mechanical Properties.

Many of the hydrogel systems encountered exhibit low tensile strength and low Young's modulus, which limits their use in many applications. This is not surprising when you consider that hydrogels can often consist of over 50% water. Table 1.2 gives a comparison of the mechanical properties of a variety of materials.<sup>35</sup>

**Table 1.2: Mechanical Properties of a Range of Materials.**

	Tensile Strength /MPa	Young's Modulus /GPa	Elongation To Break /%
Titanium Alloy	690	120	0.15
Mild Steel	370	200	0.30
Nylon (polyamide)	90	2	1.0
Wrought Iron	310	190	-
Polystyrene	60	3.5	0.03
Polyethylene	12	0.2	5.0
Carbon Fibre Composite	1400	170	-
Poly HEMA	0.5	0.25x10 <sup>-3</sup>	198

The mechanical properties described are defined as follows:

$$\text{Tensile Strength } (\sigma_b) = \frac{\text{Load at Break}}{\text{Cross Sectional Area}}$$

$$\text{Young's Modulus (E)} = \frac{\text{Stress}}{\text{Strain}}$$

$$\text{Elongation To Break } (\epsilon_b) = \frac{\text{Extension of Gauge Length}}{\text{Original Gauge Length}} \times 100\%$$

$$\text{Stress} = \frac{\text{Load}}{\text{Cross Sectional Area}}$$

$$\text{Strain} = \frac{\text{Extension of Gauge Length}}{\text{Original Gauge Length}}$$

#### **1.2.10 Factors Affecting Mechanical Properties.**

The elastic behaviour and rigidity of hydrogels is strongly influenced by monomer structure<sup>3,5,6,9,15,18</sup> and effective cross link density,<sup>9,18</sup> which as well as covalent cross links also includes ionic, polar and steric interchain forces. An increase in the effective cross link density leads to an increase in both tensile strength and Young's modulus, but a decrease in the elongation to break of the material. These observations can be accounted for by a decrease in the chain length between cross links, which leads to a more rigid structure.

The incorporation of hydrophobic monomers such as methyl methacrylate and styrene into polyHEMA hydrogels leads to increased tensile strength and Young's Modulus but reduced elongation to break.<sup>15</sup> This is caused by a reduction in the amount of freezing or plasticising water in the hydrogel. Copolymerising hydrophobic derivatives with long side chains such as lauryl methacrylate tends to counteract this effect since the long flexible side chains act as an internal plasticiser.

#### **1.2.11 Poly HEMA.**

It was work by Wichterle and co-workers<sup>11-13</sup> in the 1960s that first indicated the usefulness of cross-linked polymers of 2-hydroxyethyl methacrylate in biomedical applications. Today, a large proportion of hydrogels are still based on polyHEMA.

There are a number of reasons to explain the dominance of polyHEMA in the hydrogel field:

1. Chemical Stability:- PolyHEMA is resistant to hydrolysis by acids and reactions with amines, and it only undergoes alkaline hydrolysis significantly at high pH and elevated temperatures.<sup>7</sup>
2. Mechanical Stability:- High compared to other hydrogel materials such as poly(N-vinylpyrrolidone).
3. Ease of Modification:- It is easy to incorporate a whole range of comonomers into the gels in order to alter chemical and mechanical properties.
4. Thermal Stability:- High stability allowing steam sterilisation.<sup>13</sup>
5. EWC:- The equilibrium water content of polyHEMA is little affected by degree and nature of cross-linking agent, temperature,<sup>14</sup> tonicity and pH of hydrating medium.<sup>1</sup>

The major problem encountered in the preparation of polyHEMA based hydrogels is that of obtaining the pure HEMA monomer. The impure monomer may contain methacrylic acid, ethylene glycol dimethacrylate and diethylene glycol dimethacrylate.<sup>7</sup> The presence of such impurities can have a profound effect on the properties of the hydrogel, leading to higher cross link densities and abnormal swelling.

The purification of the HEMA monomer is a highly complex procedure involving numerous extractions and reduced pressure distillation, in the presence of inhibitor, under a nitrogen atmosphere.

### 1.3 DHCD (1,2-DIHYDROXY-3,5-CYCLOHEXADIENE).

The compound 1,2-dihydroxy-3,5-cyclohexadiene (DHCD) exists in *cis*- and *trans*-isomeric forms. However, for the purpose of my work I have concentrated on the *cis*-form since its derivatives can be more easily polymerised than those of the *trans*- form.

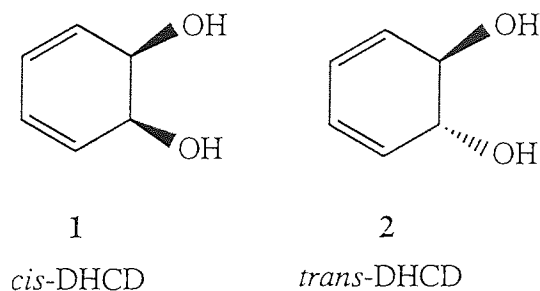
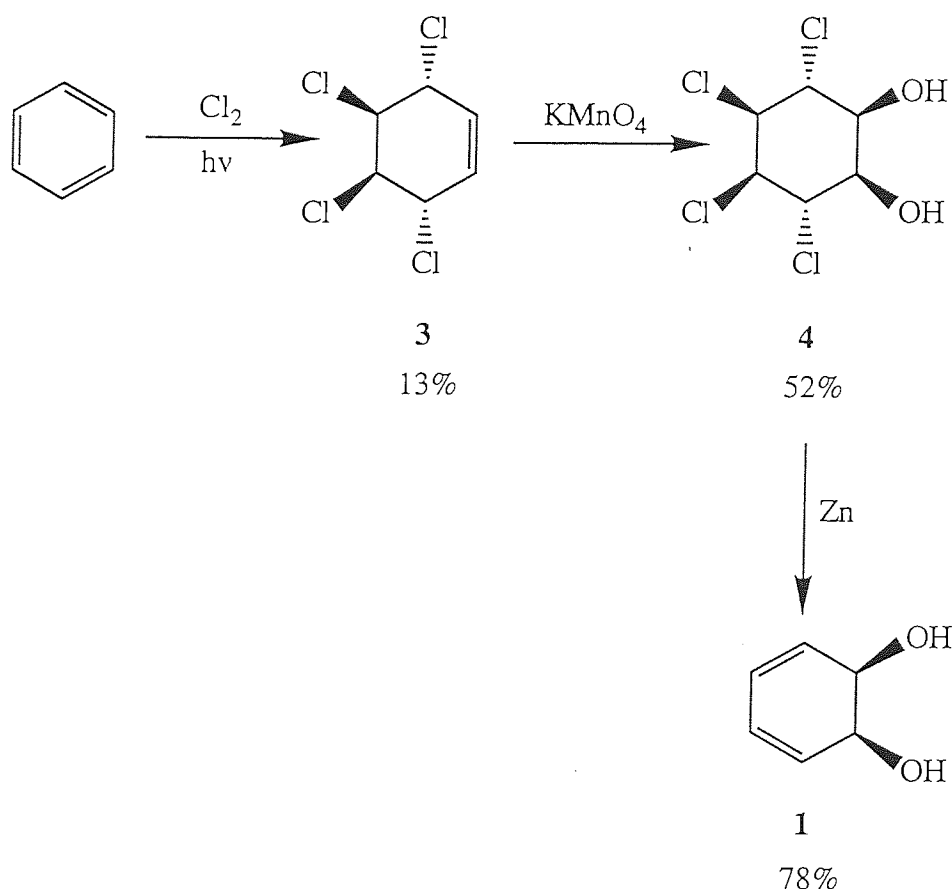


Figure 1.2: *cis*- and *trans*- Isomeric Forms of 1,2-Dihydroxy-3,5-Cyclohexadiene.

#### 1.3.1 *cis*-DHCD.

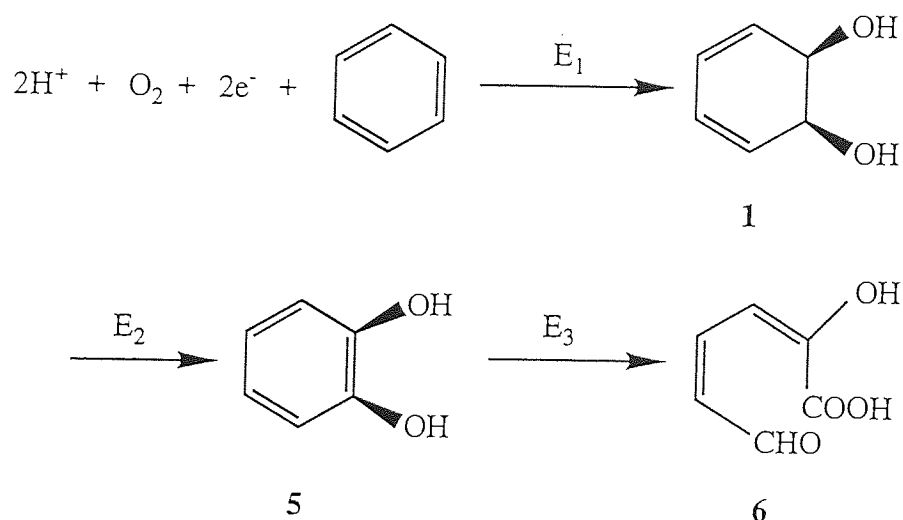
*cis*-DHCD exists as a white crystalline solid with a melting point of 60°C.<sup>36</sup> The most efficient method of production is via the microbial oxidation of benzene by a mutant of the *Pseudomonas putida* enzyme. This mutant strain was first isolated in 1970 by Gibson and coworkers,<sup>37</sup> and it has been shown to give almost 100% yield from benzene. A chemical synthesis route was reported by Nakajima and coworkers<sup>36</sup> in 1959 see scheme 1.1, and involves the polychlorination of benzene followed by treatment with potassium permanganate and elimination of chlorine. The overall yield of the process is 5%, making the synthesis prohibitively expensive for large scale production. Attempts at improving the yield have been unsuccessful.



**Scheme 1.1: Nakajima Synthesis of *cis*-DHCD.**

Micro-organisms that are capable of oxidising benzene have been known for some time, but because of their sensitivity and poor oxidation rates they have lacked the robustness needed for large scale production.<sup>37-42</sup> Enzyme studies have shown that benzene is metabolised via 1,2-dihydroxy-3,5-cyclohexadiene to catechol (see scheme 1.2).<sup>20</sup>

The ICI New Sciences Group were able to isolate a new organism, *Pseudomonas putida* 11767 from a manufacturing site that had over the decades been contaminated with hydrocarbons. This new organism was found to exhibit a higher rate of benzene oxidation and was substantially more tolerant of high benzene concentrations.

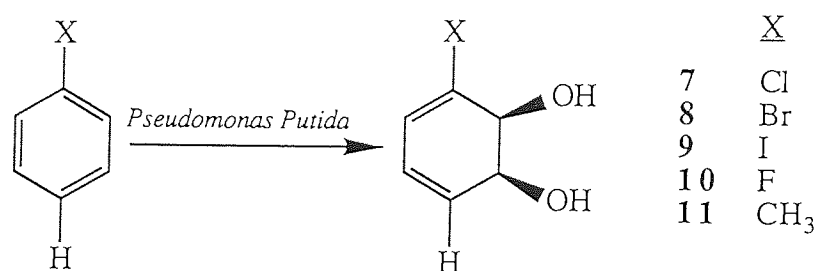


**Scheme 1.2: Microbial Oxidation of Benzene.**

The initial oxidation of benzene is brought about by the deoxygenase  $\text{E}_1$  with the assistance of the protonated form of nicotinamide adenine dinucleotide (NAD) as co-catalyst. A complete oxygen molecule reacts with benzene to form *cis*-DHCD. The *cis*-DHCD is then aromatised by the  $\text{E}_2$  enzyme to form catechol and the NAD is converted back to NADH. A third enzyme deoxygenase  $\text{E}_3$  then converts catechol into mucconic acid. By means of genetic manipulation it is possible to produce *Pseudomonas putida* 11767, which is a strain that lacks the  $\text{E}_2$  enzyme that is needed to oxidise *cis*-DHCD.

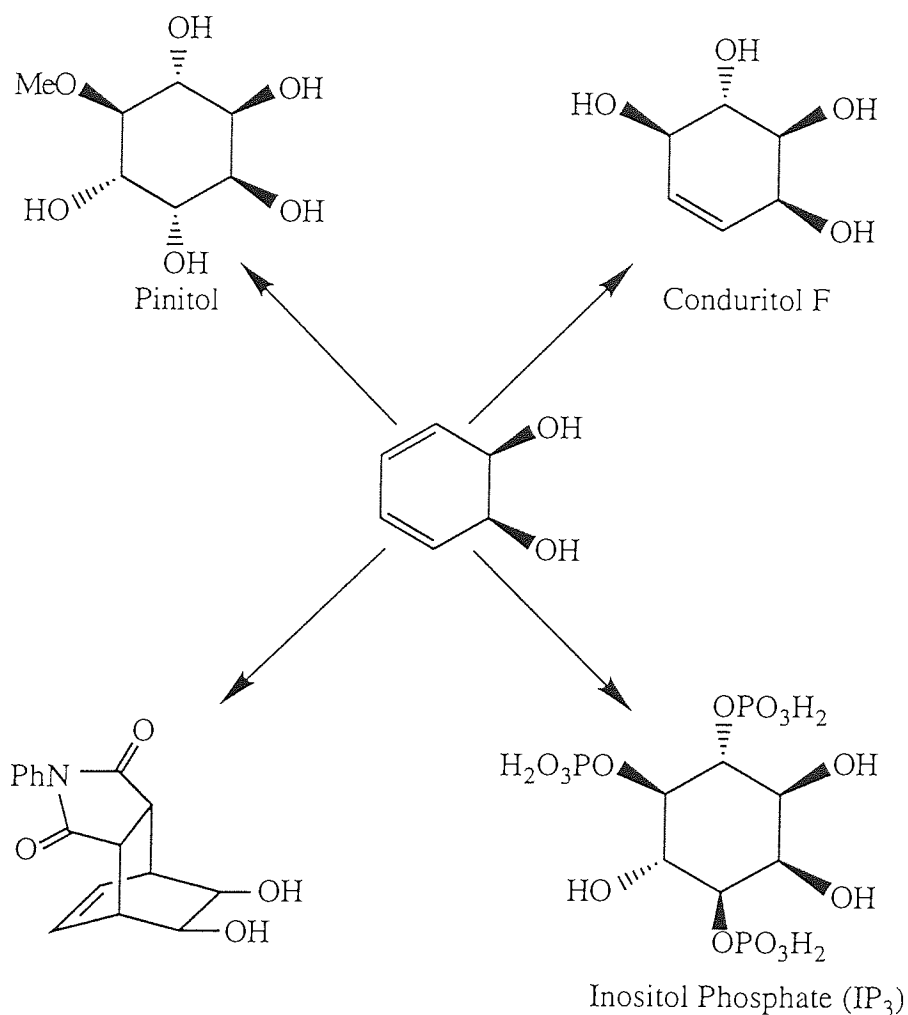
Gibson and coworkers<sup>37</sup> showed that this type of microbial oxidation is not limited to benzene. For aromatic rings containing substituents, the introduction of hydroxyl groups yields chiral molecules. A wide range of chiral nonracemic target molecules can be synthesised using aromatic rings as precursors to diols.





**Scheme 1.3: Enzymatic Preparation of Substituted Cyclohexadiene-1,2-diols.**

There has been extensive research into the use of DHCD and other diol derivatives chiral precursors in a wide number of reactions ranging from natural product synthesis<sup>43-52</sup> to Diels-Alder reactions<sup>53-54</sup> and other asymmetric synthesis.<sup>37,53,55</sup> The majority of the work has been performed by two research groups. Ley and coworkers at Imperial College have successfully converted *cis*-DHCD into pinitol<sup>49</sup> and conduritol F<sup>48</sup> by way of multi step processes. Similar research has been carried out by Hudlicky and coworkers<sup>50-52</sup> at Virginia Polytechnic and State University.



**Scheme 1.4: A Selection of Compounds Synthesised from *cis*-DHCD.**

### **1.3.2 Properties of *cis*-DHCD.**

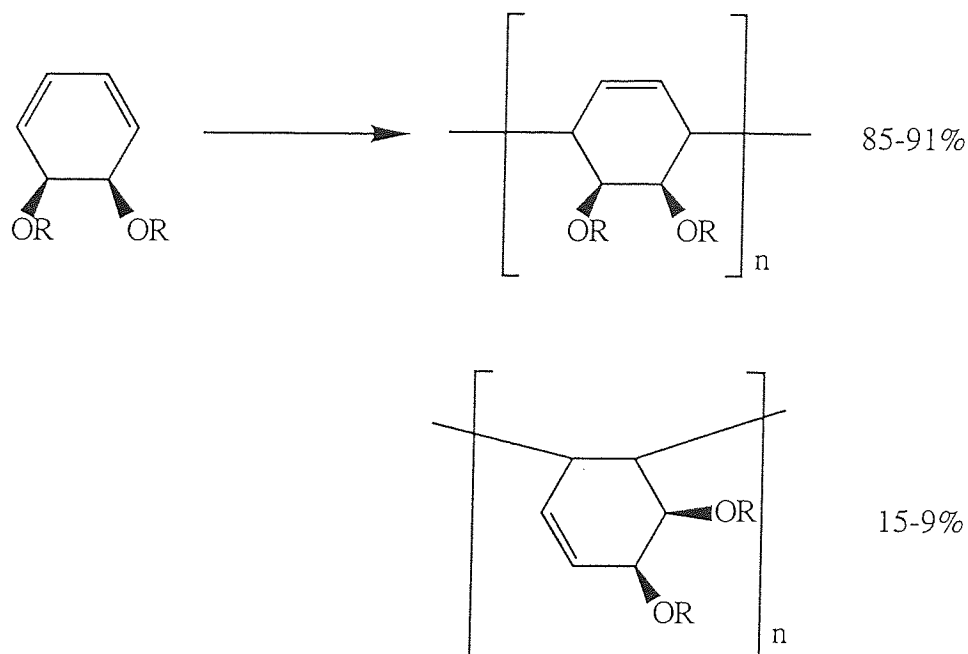
*cis*-DHCD is relatively unstable to heat, dehydrating to phenol and water at temperatures approaching 60°C. The presence of a mineral acid catalyses the aromatisation.<sup>20</sup> The stability of *cis*-DHCD has been investigated previously at Aston through the use of <sup>1</sup>H and <sup>13</sup>C N.M.R. spectroscopy.<sup>56</sup> It was found that *cis*-DHCD supplied by ICI contained only negligible amounts of phenol and after being stored at room temperature for three hours no decomposition had occurred. However, in the presence of water and traces of acid samples underwent 50% decomposition in the three hour time period. Samples of pure *cis*-DHCD have been stored at room temperature for several months before the formation of appreciable amounts of phenol. It was also

discovered that once a small quantity of phenol was produced, the aromatisation process was accelerated. Pure samples of *cis*-DHCD can be stored in a fridge for over 10 months, whereas impure samples decomposed in two to four weeks. Due to its instability, *cis*-DHCD is usually supplied in the form of a 20% w/w solution in ethyl acetate. A small amount of triethylamine is included as a stabiliser and the solution is stored below 4°C.

### 1.3.3 Derivatives of *cis*-DHCD.

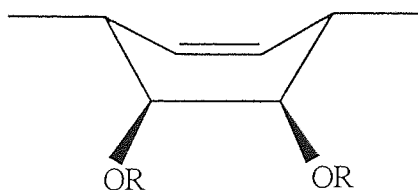
Due to the instability of *cis*-DHCD in acidic conditions, derivatives have to be produced in basic or neutral media. As a result the majority of reactions undertaken are base-catalysed such as acylations with acid chlorides and anhydrides using an organic tertiary base such as pyridine or triethylamine to neutralise the free acid yielded from such reactions.<sup>20,21,56</sup>

A number of derivatives of *cis*-DHCD have been well characterised: diacetate, dipivalate, dibenzoate, bis(*p*-nitrobenzoate), bis(*p*-bromobenzoate), dimethoxycarbonyl, diethoxycarbonyl and dimethyl ether.<sup>20,21,56</sup> Homopolymers of these derivatives have been produced<sup>19-21</sup> by using standard radical initiators such as azobisisobutyronitrile (AIBN) at lower than usual temperatures in order to minimise unwanted side reactions that the monomer may undergo at higher temperatures. The free radical polymerisation of derivatives of DHCD will yield a mixture of 1,4 and 1,2 repeat units (see scheme 1.5).



**Scheme 1.5: 1,4 and 1,2 Repeat Units of Polymers of DHCD Derivatives.**

Ballard<sup>20</sup> showed that polymerisation of the dimethyl carbonate derivative gave 85% 1,4-addition and 15% 1,2-addition. He further suggested that a boat conformation was adopted by the cyclohexene rings in the polymer chain. Work undertaken by McKean and Stille<sup>21</sup> used nmr techniques to estimate the amounts of the 1,4 and 1,2 repeat units in the polymers of DHCD derivatives. To date, there has been little use for these types of DHCD derivatives except as precursors for poly(p-phenylene).



**Figure 1.3: Boat Conformation Adopted by 1,4 Repeat Units.**

#### 1.3.4 Hydrogels Incorporating Derivatives of DHCD.

The direct incorporation of *cis*-DHCD into hydrogel membranes is not possible since it undergoes aromatisation at the temperatures required for polymerisation. However, DHCD derivatives exhibit greater stability and as a result can be incorporated into hydrogel membranes without the fear of aromatisation.

Research previously undertaken at Aston by St. Pourcain<sup>56</sup> has examined the effect on EWC and mechanical properties of incorporating diacetate, dimethylcarbonate and dipivalate derivatives of both *cis*- and *trans*- DHCD into HEMA based hydrogel membranes. Results indicate that increasing the amount of derivative in the hydrogel causes an increase in mechanical strength and Young's modulus but a reduction in equilibrium water content. This decrease in water content is expected as the amount of hydrophobic monomer in the polymer is increasing.

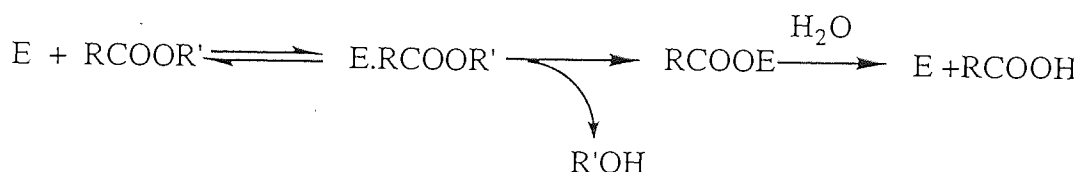
The synthesis of hydrophilic derivatives of DHCD and their subsequent copolymerisation with HEMA should yield hydrogels with the improved mechanical properties obtained with hydrophobic derivatives, but an equilibrium water content closer to that of polyHEMA.

#### 1.3.5 Hydrophilic Derivatives of *cis*-DHCD.

In synthesising hydrophilic derivatives of DHCD two factors have to be considered: the nature of the hydrophilic group and the length of the pendant chain. The most common type of hydrophilic group is hydroxyl but there are other groups including morpholine, amide and carboxylic acid. The use of carboxylic acid groups can cause problems due to their sensitivity to pH and the detrimental effect such groups have on biocompatibility. A long pendant chain can also be a problem due to the plasticising effect it has which counteracts the improved rigidity caused by the introduction of cyclic groups.

#### 1.4 USE OF ENZYMES IN ORGANIC SYNTHESIS.

Enzymes are complex natural products, mainly protein in nature that function as catalysts in biological reactions. Up until the mid 1980s it was generally thought that enzymes could only act as catalysts in aqueous media. However, pioneering work by Klibanov *et al.*<sup>57-69</sup> has disposed of this myth, suggesting that enzymes operate in a reverse mode when placed in organic solvents with low water content. For example, lipase enzyme will catalyse the hydrolysis of an ester in aqueous solutions but in organic solvents it catalyses the condensation of a free acid and alcohol. Such enzyme catalysed reactions take place under exceptionally mild conditions. The majority of enzymes used as catalysts in organic solvents to date have been hydrolases such as lipases and proteases.<sup>58,65,69-86</sup> These types of enzyme offer catalytic versatility, and are commercially available at relatively low cost. The general mechanism for the enzyme catalysed hydrolysis of an ester is shown in Scheme 1.6.<sup>61</sup>



**Scheme 1.6: Mechanism for the Enzyme Catalysed Hydrolysis of an Ester.**

where E is the hydrolytic enzyme, RCOOR' is the hydrolysable ester, E.RCOOR' is the noncovalent enzyme substrate complex (Michaelis complex) and RCOOE is the covalent acyl-enzyme intermediate. In water, the acyl-enzyme intermediate is rapidly hydrolysed thereby regenerating the free enzyme and producing the acid. In principle, other nucleophiles may compete with water for RCOOE but in aqueous solutions hydrolysis prevails. However, if organic solvents are used as the reaction media, then the acyl-enzyme intermediate can be exposed to any nucleophile without competition from water, and therefore hydrolysis can be replaced by a number of alternative reactions such as transesterification, aminolysis, oximolysis etc.<sup>58,65,71-73</sup> As a result of this a whole variety of compounds can be theoretically catalysed through the use of

enzymes. It has been established by Klibanov<sup>59-61,64</sup> and other workers<sup>71,72,81</sup> that a number of guidelines have to be observed if an enzyme is to act as an effective catalyst in organic media: i) hydrophobic solvents are generally superior to hydrophilic solvents, ii) some enzymes require exogeneous water addition to the dry solvent and iii) enzyme particles should be sufficiently small and suspensions should be continuously stirred in order to minimise diffusional limitations.

The initial step towards using enzymes in organic solvents involved the addition of water miscible organic solvents such as ethanol and acetone to an aqueous solution of the enzyme.<sup>65</sup> From here the next step involved the use of biphasic mixtures, consisting of an aqueous solution of the enzyme emulsified in a water immiscible solvent.<sup>57,85,86</sup> This was quickly followed by the use of nearly non-aqueous solvents as media for enzymatic reactions.<sup>59</sup> However, in all three cases the enzyme is essentially located in an aqueous milieu and its inherent properties are not too dissimilar to those exhibited in water. It is only by taking the ultimate step and moving to a system involving anhydrous organic solvents that we observe the enzyme's inverse behaviour.<sup>58,64</sup>

#### **1.4.1 The Role of Water.**

The simple act of replacing water with an organic solvent should distort the native protein structure of the enzyme and destroy its activity. An enzyme's chemical and physical properties are largely dependent upon the direct or indirect role of water in all non-covalent interactions such as hydrogen bonding and Van der Waals forces.<sup>59-61,72</sup> No truly anhydrous solvents have supported enzyme activity, and it appears that water is necessary for catalysis. What is unclear however is how much water is needed. It is theorised that an enzyme molecule requires a small hydration layer that acts as the primary component of the enzyme's micro environment.<sup>59,64</sup> The size of the hydration layer and hence the water requirement are enzyme dependent.

#### 1.4.2 Effect of Organic Solvent.

The solvent in which an enzyme is placed can effect it's activity in three ways.<sup>64,72,87,88</sup>

1. Direct interaction with the enzyme causing inactivation or inhibition. The solvent causes the native conformation of the enzyme to be altered, resulting in the disruption of hydrogen bonding and other interactions and leading to reduced activity and stability.
2. Interaction with diffusible substrates or reaction products.
3. Interaction directly with the enzyme's essential water. For example, highly polar solvents can strip the essential water from an enzyme, rendering it inactive.

#### 1.4.3 Applications.

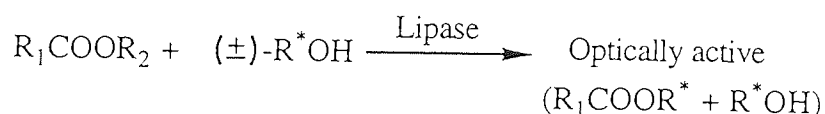
A major problem in the field of speciality chemicals is the production of optically active compounds. This area is expected to undergo rapid expansion, with the production of more commercially useful chiral compounds where only one enantiomer is biologically active.<sup>58,65,73,91</sup> Enzymes are ideal for the preparation of optically active compounds as they are built up of only L-amino acids. As a result their active centres constitute a dissymmetric environment which allows the enzyme to distinguish between enantiomers.<sup>63,89,90</sup> It is difficult to synthesise complex substances by enzyme catalysis since it would require the use of many different enzymes. At the same time it is also true that chemical synthesis usually lacks stereoselectivity which prevents it being generally applicable for optical compounds. A chemo-enzymatic approach<sup>66,92</sup> uses enzymes to prepare small optically active molecules ("synthons"), which are then put together by usual chemical methods.



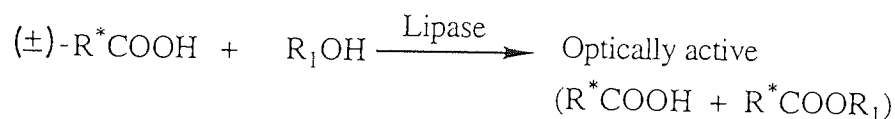
#### 1.4.4 Resolution of Racemates.

Over the past ten years, interest in the field of enzyme synthesis has expanded rapidly leading to the discovery of a whole host of new applications for enzymes in organic solvents. The most well documented being their use in the resolution of racemic mixtures by way of enzyme catalysed esterifications and transesterifications.

A whole host of racemic alcohols have been resolved via enzyme catalysed transesterifications:<sup>58,61,72,73</sup>



Racemic acids have also been resolved via enzyme catalysed esterifications:<sup>58,72</sup>



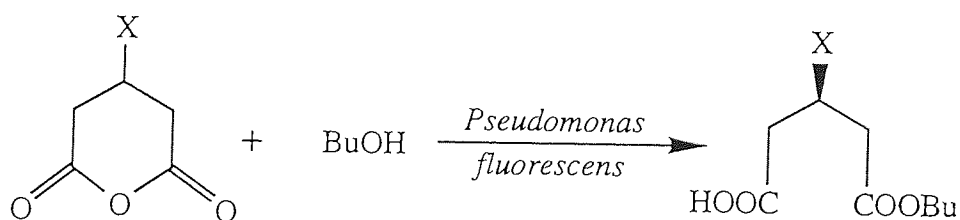
The resolution of enantiomers is possible due to the stereoselective nature of the enzyme, which effectively means that the enzyme catalyses the esterification of just one optical isomer. One enantiomer is converted into an ester while the second enantiomer remains in either the acid or alcohol form.

It appears from the literature that the use of enzymes in organic media is a fairly straightforward procedure. This is however not the case and I have observed that a particular enzyme and solvent combination that works for one reaction may not work in a second reaction. Our studies have indicated that in general, porcine pancreas lipase (PPL) will catalyse transesterifications whereas *candida cylindracea* lipase will tend to catalyse esterifications.<sup>58,71,72</sup>

In organic media, enzymes have been shown to catalyse a variety of synthetic reactions in high yields including esterifications,<sup>58,65,69,72,73,75</sup> transesterifications,<sup>57,58,65,71-73</sup> interesterifications (acyl exchange), lactonisation,<sup>61,71</sup> thiotransesterifications, aminolysis<sup>67,72</sup> and oximolysis. All of these reactions are possible in the presence of low water concentration solvents with low water activities.

Bianchi *et al.*<sup>76</sup> have investigated the use of anhydrides as acylating agents in lipase catalysed reactions. They have shown that enzyme catalysed acylations of both primary and secondary alcohols can be efficiently accomplished through the use of anhydrides. Furthermore, reaction rates were found to be higher than those obtained in the equivalent esterification and transesterification processes.

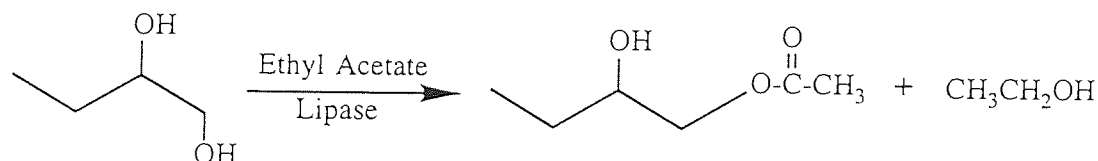
Oda and coworkers<sup>74</sup> found that *Pseudomonas fluorescens* catalyses the asymmetric ring opening of cyclic anhydrides. The reaction of derivatives of glutaric acid anhydride with primary alcohols yielded half ester product, and is a potential method for the synthesis of chiral synthons. Certain hydroxy acids undergo cyclisation to corresponding lactones in the presence of *Mucor michei* lipases. These same enzymes are used for the stereoselective ring closure of the appropriate hydroxy ester to the corresponding lactone.



Scheme 1.7: Enzyme Catalysed Acylation using Cyclic Anhydrides.

#### 1.4.5 Regioselective Synthesis.

Klibanov<sup>61</sup> has also used enzyme catalysed transesterifications to carry out a series of regioselective acylations of glycols.



Scheme 1.8: Regioselective Acylation of Butane-1,2-diol.

The presence of the enzyme causes acylation to occur at one specific hydroxy group. As with the previously described resolution of racemates, this is yet another example of the enzyme acting in a site specific manner.<sup>89,90</sup> Chemical synthesis of this type of product cannot be accomplished regiospecifically, mixtures of products are obtained and expensive protection and deprotection steps are required for regiospecific synthesis.

Regioselective synthesis can be applied to more than just simple diols, in fact regioselective acylations can be carried out with a whole host of natural materials such as sugars and steroids.<sup>61,72</sup> In these cases, there are large numbers of free hydroxyl groups in the molecules but the enzyme will only catalyse the acylation of one particular hydroxyl group.

#### 1.4.6 Enzyme Catalysed Polymerisations.

Dordick and co-workers<sup>92</sup> have used enzymes in the synthesis of sugar containing poly(acrylates) for hydrogel materials. The enzyme is used to modify the sugar with a polymerisable group, and the resulting monomer is polymerised via conventional free radical methods. The enzymatic step is stereoselective and the chemical polymerisation is facile and results in high molecular weights. Dordick has co-polymerised such sugar-containing monomers with other acrylic monomers including methacrylic acid and HEMA. Such materials were found to have exceptionally high water contents.

Wallace and Morrow<sup>93,94</sup> have been working on the synthesis of optically active epoxy-substituted polyesters by lipase catalysed polymerisation. The process involves the transesterification of an epoxy diester such as bis(2,2,2-trichloroethyl) (+)-3,4-epoxyadipate with 1,4-butanediol.

#### 1.4.7 Enzyme Catalysed Derivatisation of *cis*-DHCD.

The mild conditions required for enzyme catalysed reactions in organic solvents makes this type of synthesis a potential route for producing derivatives of DHCD. Enzyme catalysed esterifications and transesterifications make use of lipase which is both cheap and readily available. Such reactions are highly specific and will potentially lead to high product yields.

### 1.5 AIMS.

The main aim of this research is to synthesise a number of hydrophilic derivatives of *cis*-DHCD. Both enzyme and chemical methods will be examined as potential routes to these derivatives. Particular attention will be focused on enzyme catalysed transesterification reactions as a synthesis method.

Having produced a number of derivatives, it was my intention to co-polymerise them with HEMA to produce a new range of hydrogel contact lens materials that offer enhanced main chain stiffness, and hence improved mechanical properties without sacrificing hydrophilicity and water content.

The mechanical properties and hydrophilicity of the copolymers were determined by measuring the tensile strength, Young's modulus and equilibrium water content. differential scanning calorimetry was also used to calculate the ratio of freezing:non-freezing water in the gels

**CHAPTER 2:**  
**Enzyme Synthesis of Monomers.**

## 2. ENZYME SYNTHESIS OF MONOMERS.

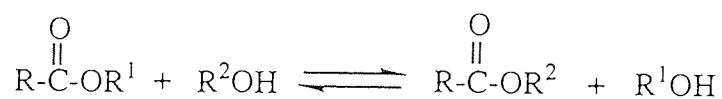
### 2.1 INTRODUCTION.

The use of enzymes as catalysts in organic solvents has been well documented,<sup>57-86</sup> and they offer a potential means by which hydrophilic groups can be attached to the DHCD molecule. Such groups could be attached by way of esterification and transesterification<sup>58,65,71,72</sup> reactions that avoid the usual harsh conditions that would bring about aromatisation of DHCD. In order to take advantage of these reactions I must use esters or carboxylic acids that contain hydrophilic groups.

Due to the high expense of *cis*-DHCD, it was decided to use a model compound in the initial stages of the research to assess the feasibility of using enzyme catalysts in my work.

### 2.2 TRANSESTERIFICATION.

Transesterification<sup>95</sup> involves the acid or base catalysed reaction of an alcohol and an ester leading to the production of a new alcohol and a new ester. The reaction process is an equilibria which generally results in low yields, since the products of the reaction can react together to reform the starting materials.

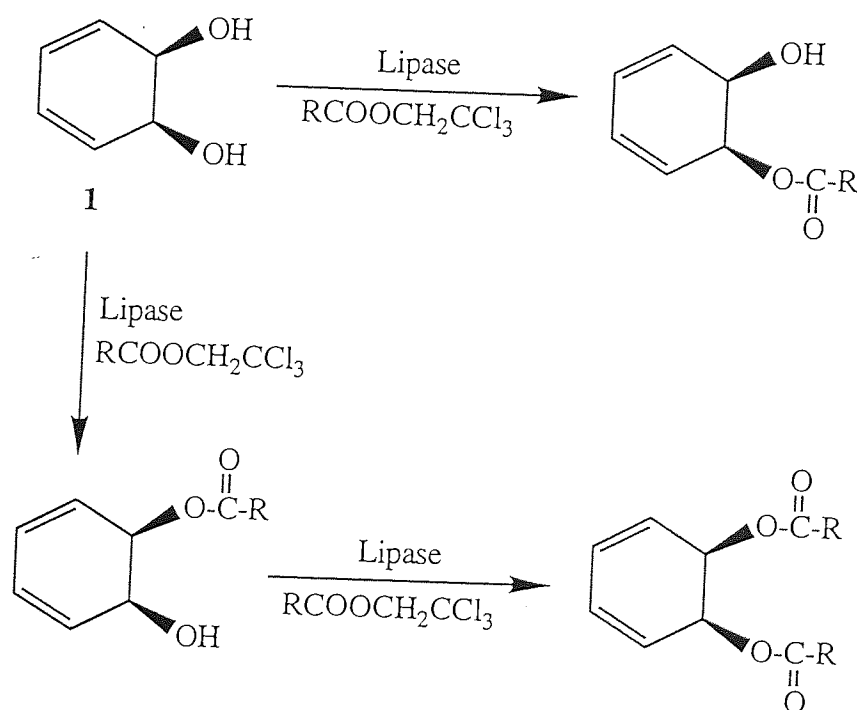


The elevated temperatures and acid catalysts used in this type of reaction make it an unsuitable method of esterifying *cis*-DHCD since under these conditions aromatisation would occur. However, the lipase enzyme porcine pancreas has been found to catalyse transesterification reactions at room temperature without the need for acid.<sup>58,72</sup> By using this enzyme as a catalyst it should be possible to esterify *cis*-DHCD under exceptionally mild conditions. However, in order to optimise yields we must ensure that the reaction equilibrium is pushed to the far right. This can be accomplished by using

activated esters that contain very good leaving groups and will hence push the equilibrium to the right.

### 2.3 ACTIVATED ESTERS.

In order to fully exploit the potential of enzymes it is essential to optimise the reaction conditions to afford the greatest possible yield. In the case of enzyme catalysed transesterifications, the yield from the reactions can be increased by using trichloroethoxy, trifluoroethoxy and enol esters.<sup>58,61,72,73</sup> Such esters contain very good leaving groups, and the alcohols produced are very poor nucleophiles, hence limiting the possible back reaction. Indeed in the case of the enol esters, the vinyl alcohol produced will tautomerise into an aldehyde, eliminating the possibility of a back reaction. The use of activated esters pushes the reaction towards completion and hence high yields are obtained. The regioselectivity of enzymes means that as a consequence of the diol moiety of DHCD, transesterifications may yield either mono- or disubstituted products, see scheme 2.1.



Scheme 2.1: Enzyme Synthesis of DHCD Derivatives.

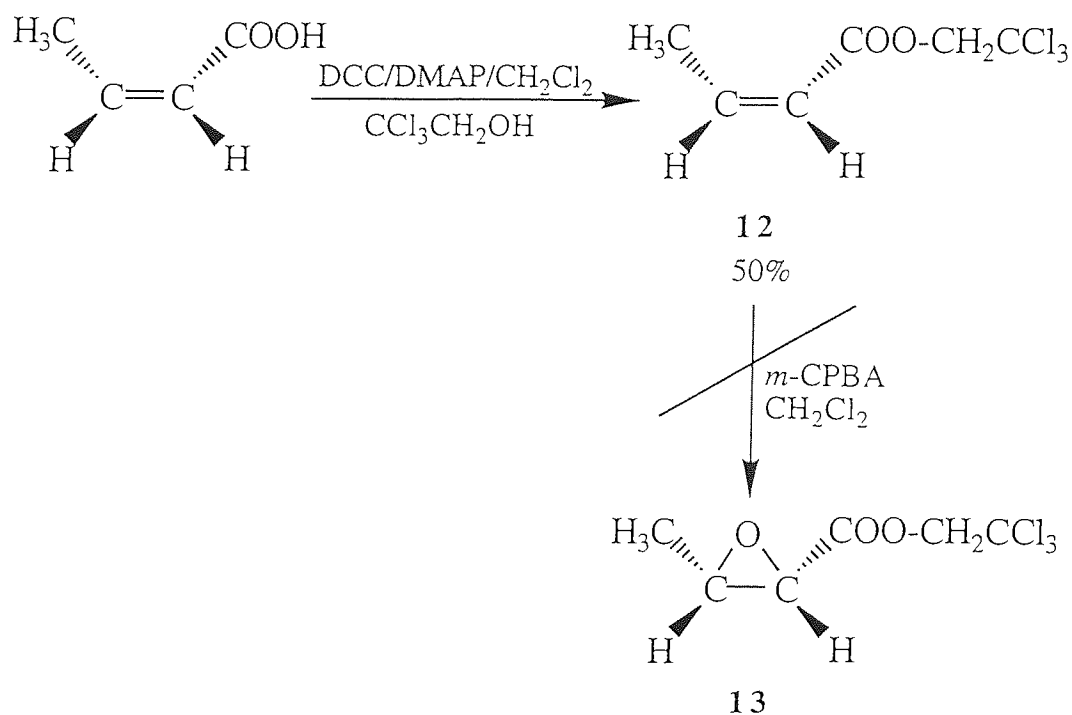
Since the objective of our work was to synthesise hydrophilic derivatives of DHCD, it was necessary to produce activated esters containing hydrophilic groupings. Unfortunately, the use of hydroxyl or amino esters may lead to reaction complications. However, the use of activated esters containing epoxide groups present no such problems and these can be easily synthesised by the esterification and subsequent epoxidation of unsaturated carboxylic acids.

The choice of carboxylic acid precursors for the synthesis of activated esters was dictated by a combination of cost and availability. Consequently, as a result of there low cost and ready availability the acids used were: crotonic acid, vinyl acetic acid and undecylenic acid.

### **2.3.1 Synthesis of 2,2,2-Trichloroethyl-2-butenate.**

The reaction of crotonic acid with 2,2,2-trichloroethanol in the presence of 4-(dimethylamino)pyridine (DMAP) and 1,3-dicyclohexylcarbodiimide (DCC) produced 2,2,2-trichloroethyl-2-butenate in a yield of 50%. The yield was lower than normally expected for this type of reaction because of the production of a solid white *N*-acylurea by-product. This by-product is produced by rearrangement of the DCC-carboxylic acid reaction intermediate.





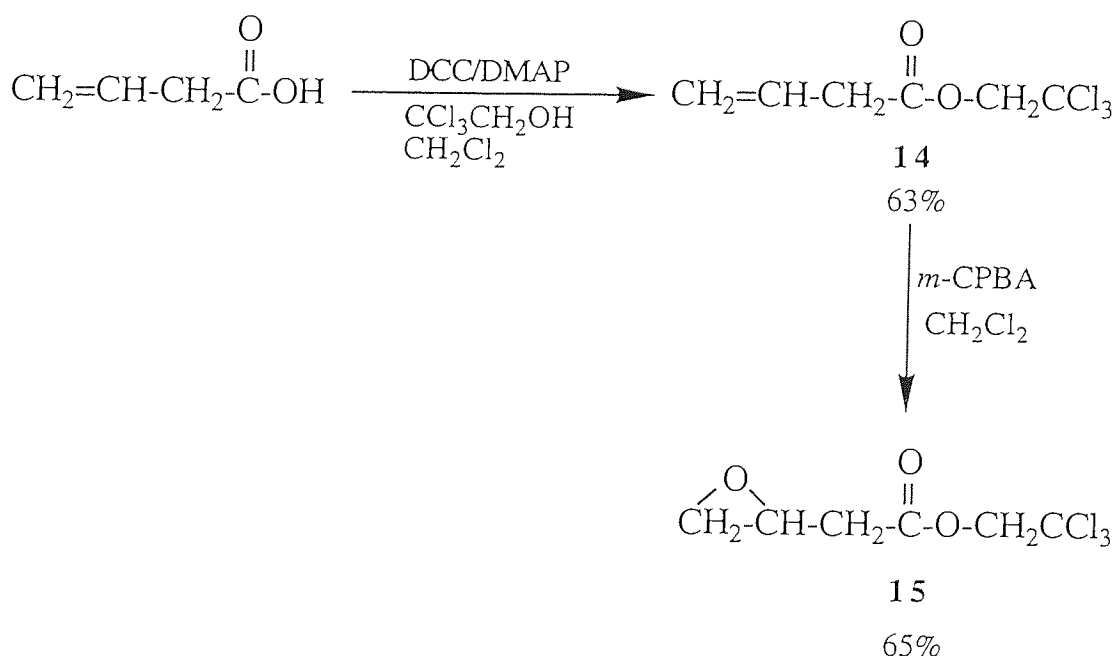
**Scheme 2.2: Synthesis of 2,2,2-Trichloroethyl-2-butenate.**

Several attempts were made at epoxidising 2,2,2-trichloroethyl-2-butenate using *meta*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane. Unfortunately no epoxide was isolated from any of the reactions. The lack of reactivity of the carbon-carbon double bond can be attributed to the conjugation that exists between the carbonyl and alkene groupings. Consequently, there is delocalisation which reduces the reactivity of the bond since epoxidation will disrupt the enhanced stability associated with delocalisation. The close proximity of the carbonyl group makes it more difficult for the carbon-carbon double bond to undergo attack by the peroxy acid nucleophile.

### 2.3.2 Synthesis of 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

The synthesis of 2,2,2-trichloroethyl-3,4-epoxybutanoate was accomplished via a two step process described by Morrow,<sup>96</sup> see scheme 2.3. The initial step leads to the production of the activated ester and involves the reaction of vinyl acetic acid with 2,2,2-trichloroethanol in the presence of 4-(dimethylamino)pyridine (DMAP) and 1,3-dicyclohexyldiimide (DCC) at room temperature. The product was initially recovered as a yellow oil which was purified by elution through a 4 inch silica column with dichloromethane to give a 63% yield of 2,2,2-trichloroethyl-3-butenate.

The second step involves the epoxidation of the activated ester and is accomplished by the reaction of 2,2,2-trichloroethyl-3-butenate with *m*-CPBA in dichloromethane. The reaction took place at room temperature in a nitrogen atmosphere and the product was purified by vacuum distillation to give a yield of 65%. The <sup>1</sup>H and <sup>13</sup>C N.M.R. spectra of this compound are displayed in figures 2.1a and 2.1b.



Scheme 2.3: Synthesis of 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

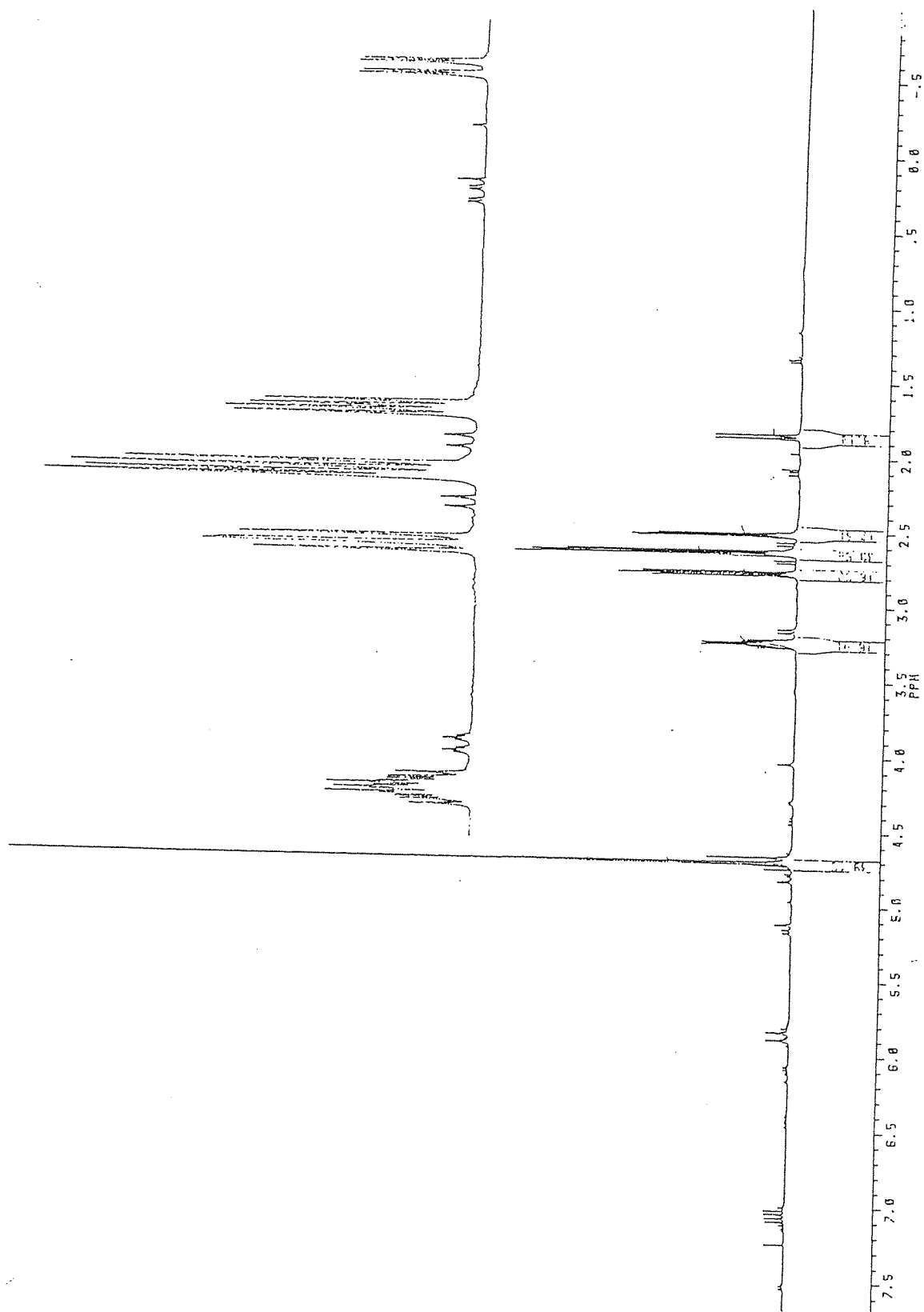


Figure 2.1a:  $^1\text{H}$  N.M.R. Spectra of 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

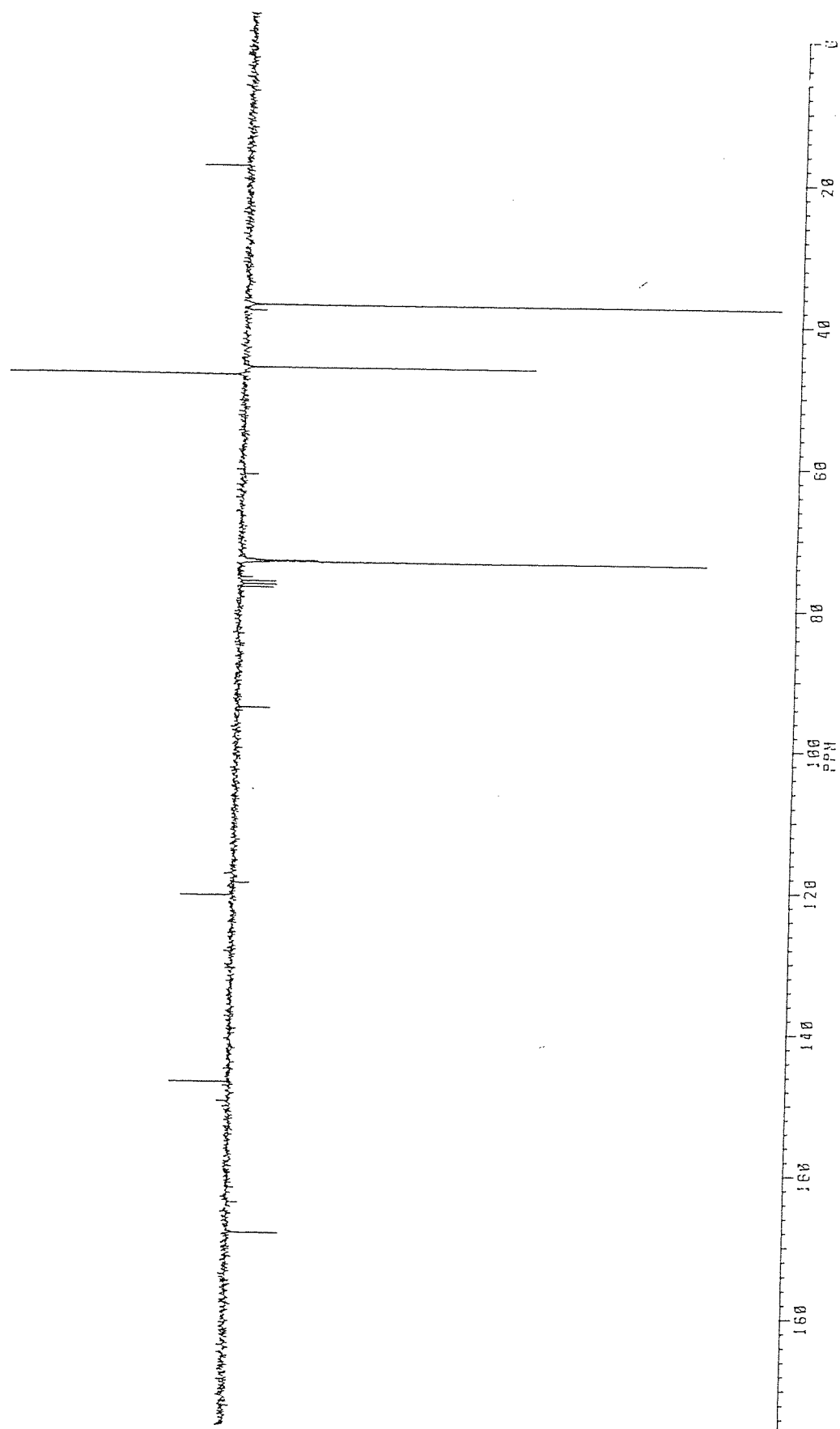
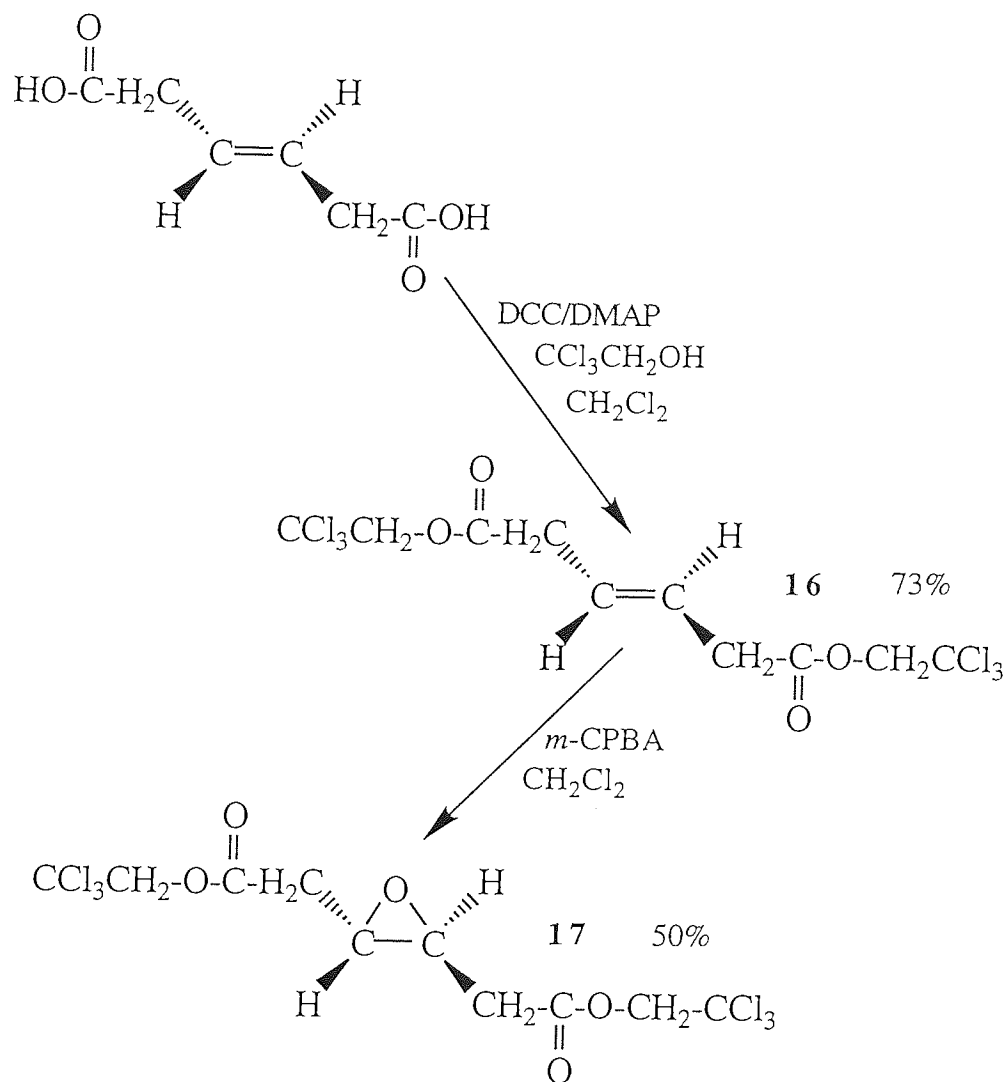


Figure 2.1b:  $^{13}\text{C}$  N.M.R. Spectra of 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

### 2.3.3 Synthesis of Bis(2,2,2-trichloroethyl)-(+)-epoxyadipate.

The diester bis(2,2,2-trichloroethyl)-(+)-epoxyadipate was produced via the two step procedure described by Morrow,<sup>93</sup> see scheme 2.4. The first step involves the reaction of *trans*-3-hexenedioic acid with 2,2,2-trichloroethanol in the presence of DMAP and DCC. The reaction took place at room temperature in a nitrogen atmosphere and gave a 73% yield of bis(2,2,2-trichloroethyl)-*trans*-3-hexenedioate.

The second step of the reaction involves the room temperature epoxidation of bis(2,2,2-trichloroethyl) *trans*-3-hexenedioate with recrystallised *m*-CPBA in dichloromethane. The reaction product was purified by flash chromatography, using a 1:1 hexane: dichloromethane elution system to give a yield of 50%.

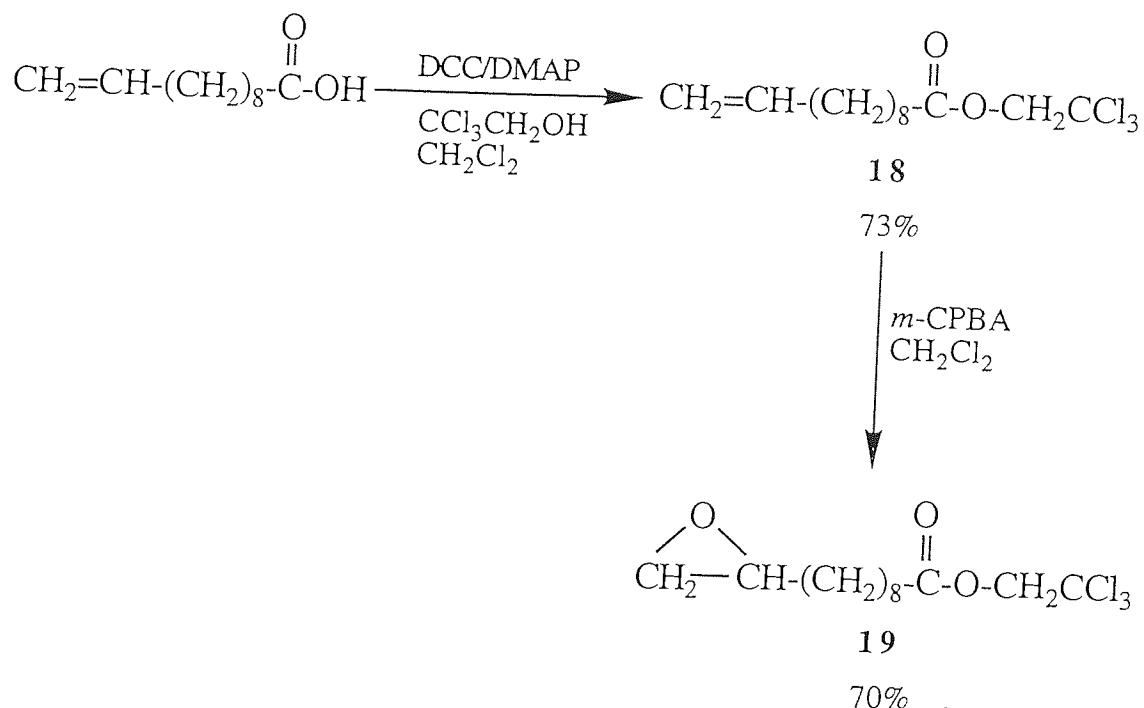


Scheme 2.4: Synthesis of Bis(2,2,2-trichloroethyl)-(+)-epoxyadipate.

#### 2.3.4. Synthesis of 2,2,2-Trichloroethyl-10,11-epoxyundecanoate.

The compound 2,2,2-trichloroethyl-10,11-epoxyundecanoate was produced via a two step procedure, see scheme 2.5. The initial step involved the esterification of undecylenic acid with 2,2,2-trichloroethanol in the presence of DCC and DMAP. The reaction took place at room temperature and gave a 73% yield of 2,2,2-trichloroethyl-10-undecenoate.

The second step of the reaction involved the epoxidation of 2,2,2-trichloroethyl-10-undecenoate with *m*-CPBA in dichloromethane. The reaction took place at room temperature and gave a yield of 70%.

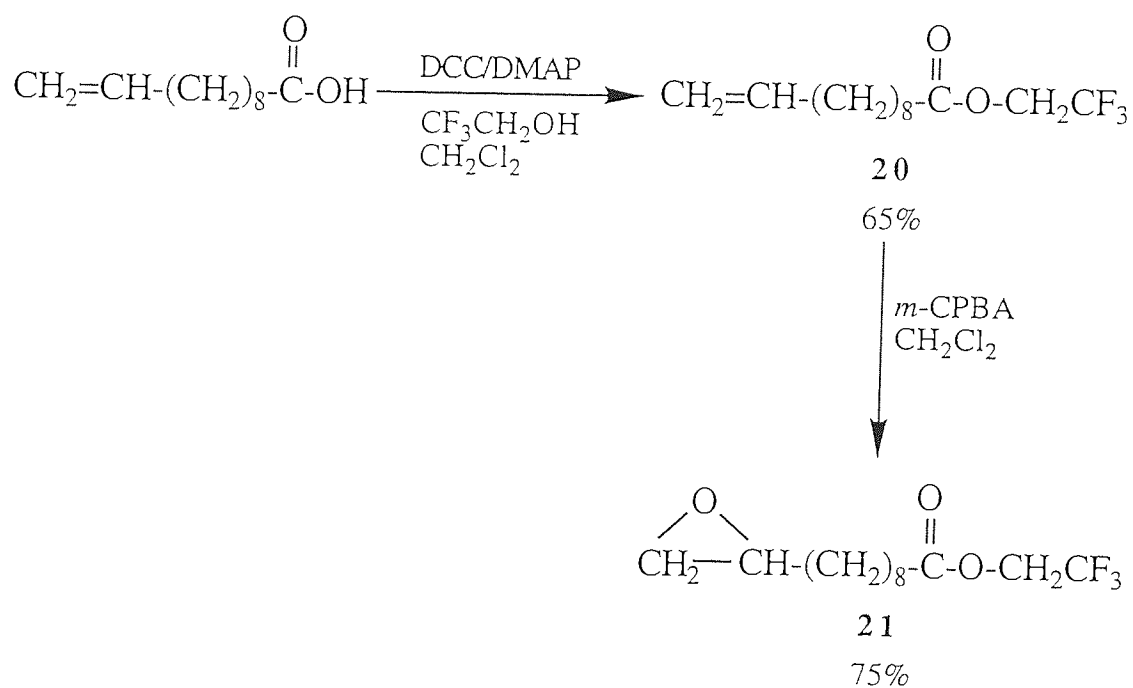


Scheme 2.5: Synthesis of 2,2,2-Trichloroethyl-10,11-epoxyundecanoate.

### 2.3.5 Synthesis of 2,2,2-Trifluoroethyl-10,11-epoxyundecanoate.

The synthesis of 2,2,2-trifluoroethyl-10,11-epoxyundecanoate was accomplished via a two step procedure, see scheme 2.6. The initial step involves the synthesis of 2,2,2-trifluoroethyl-10-undecenoate. This compound has previously been produced by Purgett *et al.*<sup>97</sup> by refluxing undecylenic acid with 2,2,2-trifluoroethanol and *p*-toluene sulphonic acid in benzene for two days. However, the harsh reaction conditions and poor yields make this an unfavourable synthesis. An alternative synthesis route makes use of the DCC/DMAP esterification procedure developed by Hassner,<sup>98</sup> and involves the reaction of undecylenic acid with 2,2,2-trifluoroethanol in the presence of DCC and DMAP. The reaction took place at room temperature, in an argon atmosphere and gave a yield of 65%.

The second step of the procedure involves the epoxidation of 2,2,2-trifluoroethyl-10-undecenoate with *m*-CPBA. The reaction took place at room temperature and a yield of 75% was obtained. The  $^1\text{H}$  and  $^{13}\text{C}$  N.M.R. spectra of this compound are displayed in figures 2.2a and 2.2b.



Scheme 2.6: Synthesis of 2,2,2-Trifluoroethyl-10,11-epoxyundecanoate.



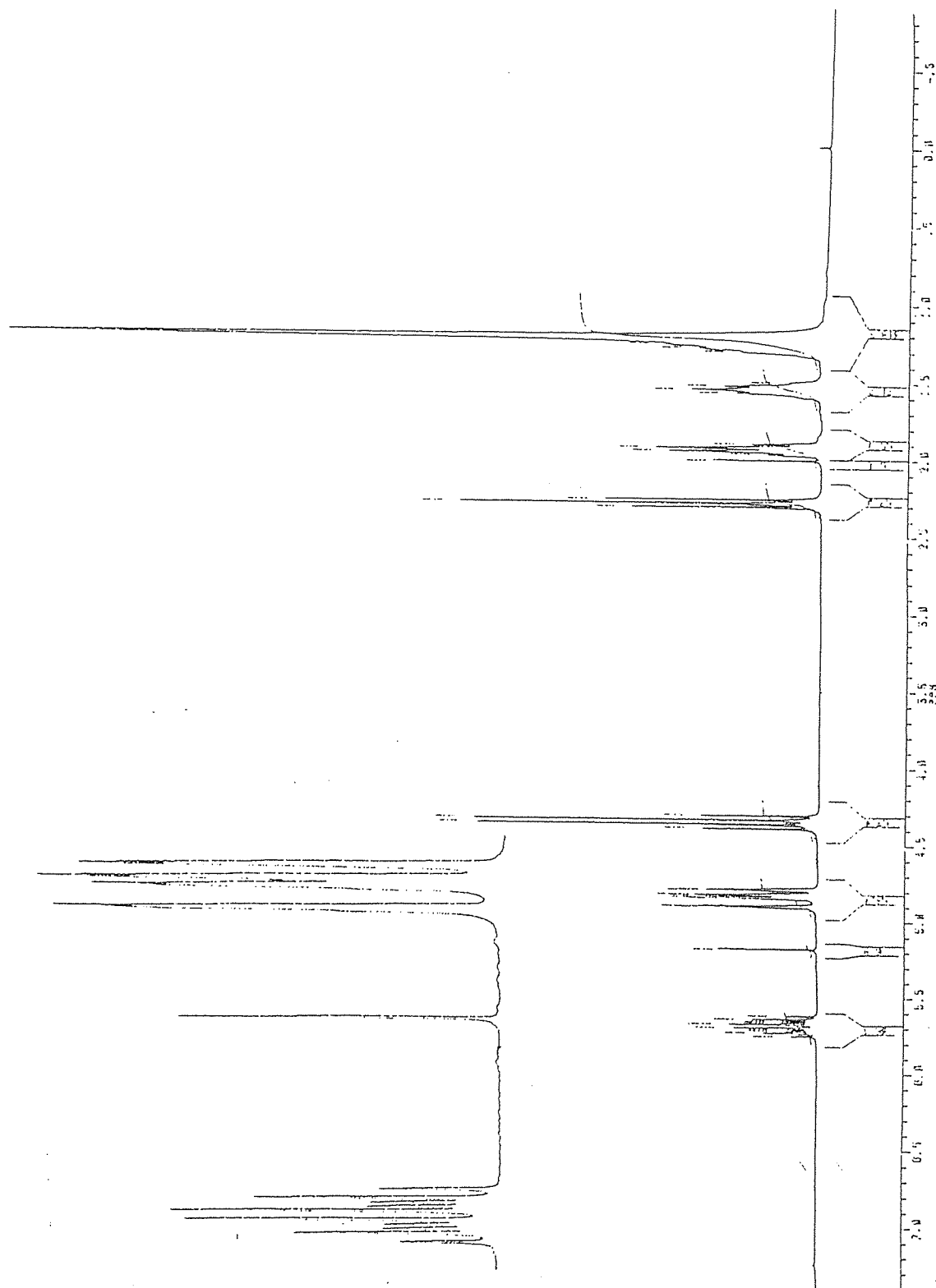


Figure 2.2a:  $^1\text{H}$  N.M.R. Spectra of 2,2,2-Trifluoroethyl-10,11-epoxyundecanoate.

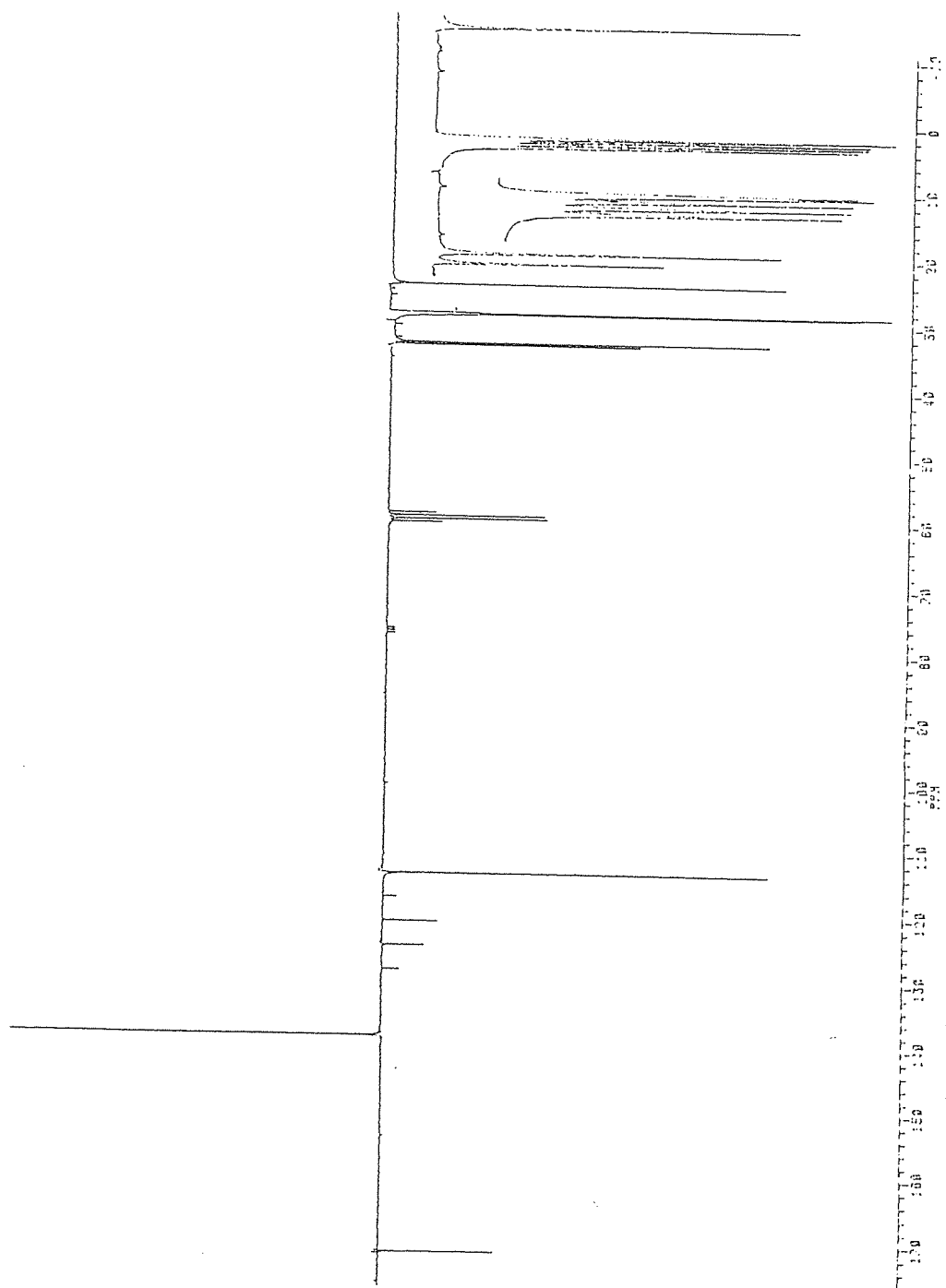


Figure 2.2b:  $^{13}\text{C}$  N.M.R. Spectra of 2,2,2-Trifluoroethyl-10,11-epoxyundecanoate.

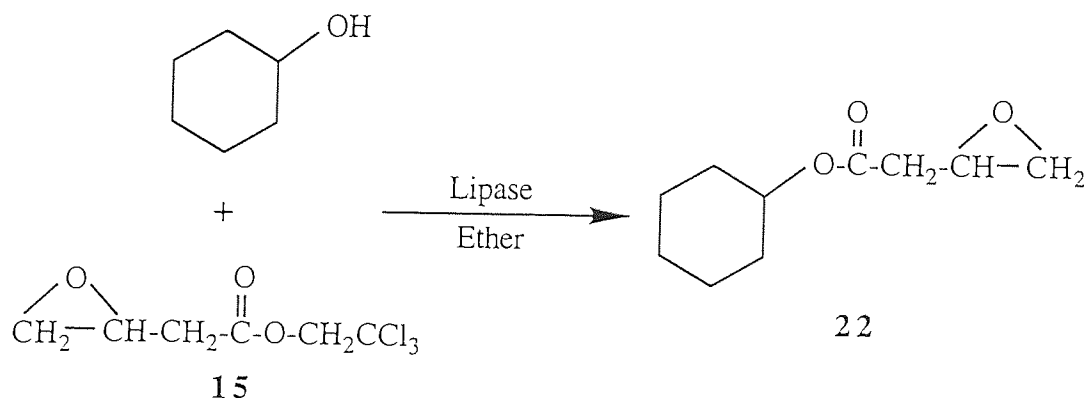
## 2.4 TRIAL EXPERIMENT.

Due to the expense of *cis*-DHCD and the uncertainty about the potential of enzyme catalysed reactions it was decided that a trial enzyme experiment should be conducted using cyclohexanol.

Cyclohexanol was chosen to assess the reactivity of cyclic secondary alcohols in enzyme catalysed reactions. It has been well documented that primary alcohols are catalysed in enzyme reactions more rapidly than secondary alcohols, but it was essential to determine whether enzymes will catalyse the transesterification of cyclic alcohols in high enough yields and in a reasonable time period.

The reaction of cyclohexanol with 2,2,2-trichloroethyl-3,4-epoxybutanoate in the presence of sodium dried ether and the enzyme porcine pancreas lipase (PPL) was studied to test the viability of using enzyme catalysed reactions and assess the kind of reaction conditions required. The reaction mixture was sealed into a flask and stirred continuously for seven days at room temperature. After this time the enzyme was filtered off and the solvent was removed. The resulting residue was studied using infra-red spectroscopy and gas chromatography (GC). The IR spectra of the residue indicated the presence of two ester carbonyl absorptions, one of which matched up with the starting ester. A mixture of the reagents was injected into the GC with a column temperature of 200°C and the resulting chromatogram was compared to that obtained for the reaction residue. The chromatogram of the residue showed the presence of an additional peak at higher retention time, which must be due to product.

From the GC trace it was determined that approximately 40% of the epoxide has reacted. Since the epoxide is racemic it is probable that the enzyme has catalysed the reaction of just one of the optical isomers. Therefore, assuming that there was initially a 50:50 mix of the two isomers, a maximum of 50% of the epoxide is going to react. As a result it can be claimed that 80% of the reactive isomer has reacted.



Scheme 2.7: Enzyme Catalysed Transesterification of Cyclohexanol.

### 2.5 REACTION CONDITIONS.

During the last few years there have been many conflicting reports concerning the ideal reaction conditions for enzyme catalysed reactions. Some researchers believe that wet solvents and long reaction times<sup>57,85,86</sup> give the best results whereas other researchers believe that very dry solvents and short reaction times are required.<sup>64,70,78</sup> Although this issue appears to be favouring the latter group of researchers, a range of reaction conditions were nevertheless investigated:

Quantity of enzyme used.

Solvent type and dryness.

Type of activated ester grouping.

Reaction time.

Reaction temperature.

## 2.6 ENZYME CATALYSED REACTIONS.

DHCD is a meso-diol, it contains two stereocentres and is prochiral. Work carried out by Theil<sup>99-100</sup> using *meso*-cyclopentenediols has shown that pancreatin catalysed transesterifications lead to a mixture of one mono substituted enantiomer and the disubstituted product. Since cyclopentenediol is a 5-membered analogue of DHCD it was thought that a similar mixture of products might be obtained in pancreatin catalysed transesterifications involving DHCD.

Enzyme catalysed transesterifications of DHCD were carried out using a series of activated esters and a variety of reaction conditions. Experiments involved the dissolution of DHCD and the appropriate activated ester in solvent and the subsequent addition of enzyme to the solution. Mixtures were then continuously stirred in sealed flasks for varying lengths of time. Table 2.1 describes the conditions used for each reaction and figures 2.3a and 2.2b are examples of the <sup>1</sup>H and <sup>13</sup>C N.M.R. spectra of reaction residues.

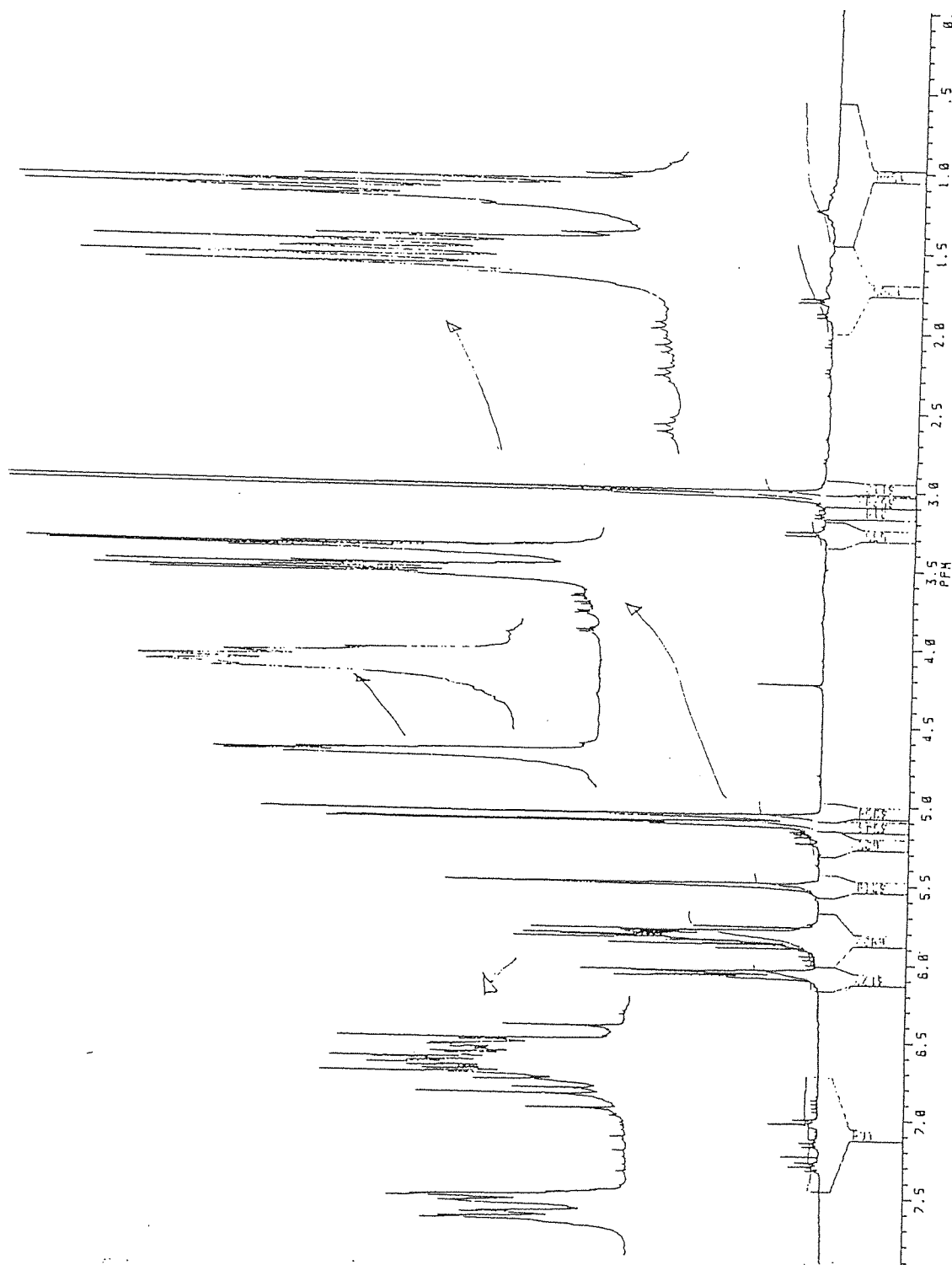


Figure 2.3a: <sup>1</sup>H N.M.R. Spectra of Residue from the Lipase Catalysed Transesterification of DHCD with 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

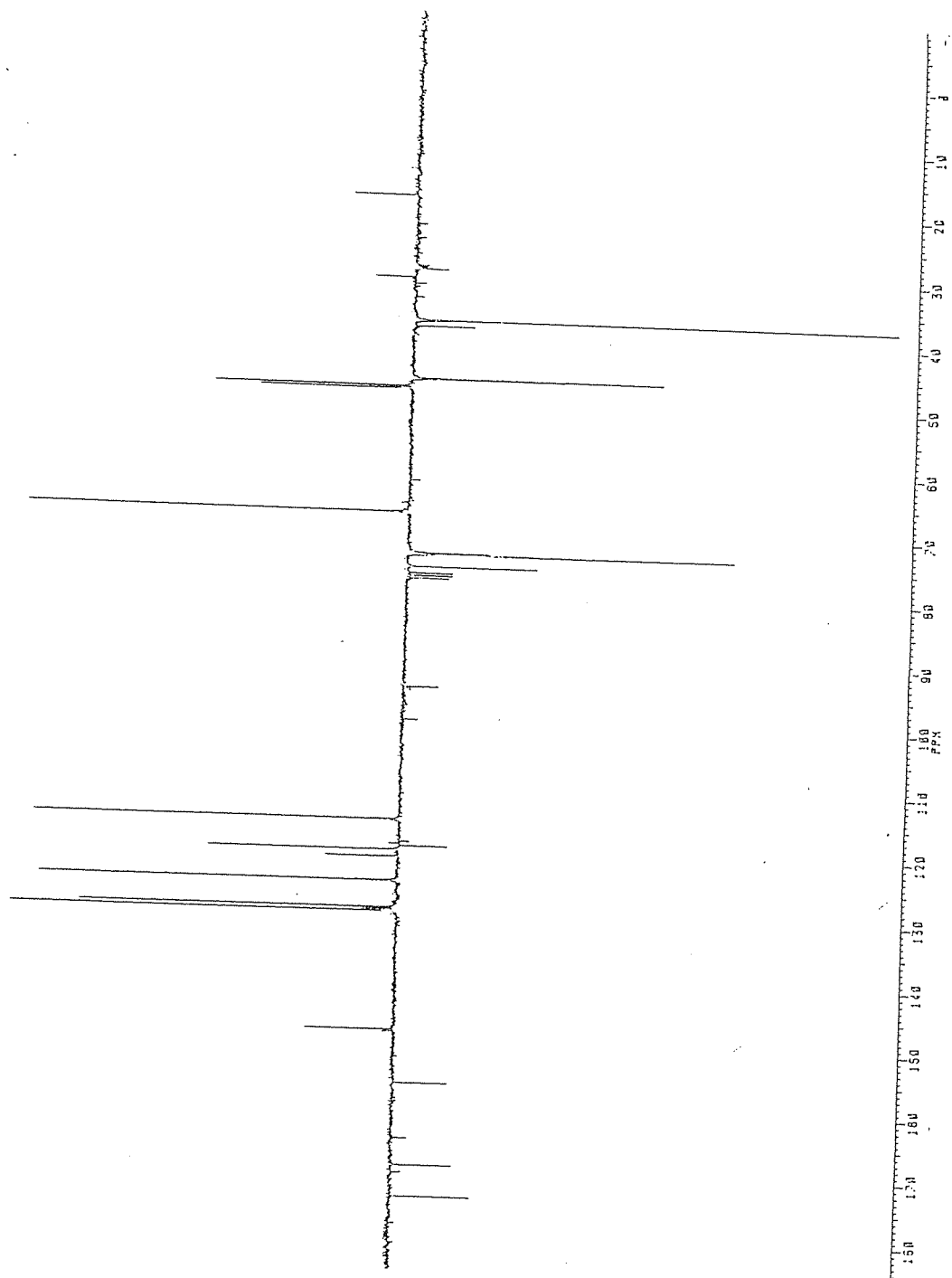


Figure 2.3b:  $^{13}\text{C}$  N.M.R. Spectra of Residue from Lipase Catalysed Transesterification of DHCD with 2,2,2-Trichloroethyl-3,4-epoxybutanoate.

Table 2.1: Enzyme Catalysed Transesterifications of DHCD.

Activated Ester Used	PPL	DHCD	Solvent	Reaction Time	Additional Information
2,2,2-trichloroethyl 3,4-epoxybutanoate	1.5g	0.37g	Na dried Ether	3 Weeks	Ambient Temperature
2,2,2-trichloroethyl 3,4-epoxybutanoate	2.5g	0.66g	Na dried Ether	3 Weeks	Ambient Temperature
2,2,2-trichloroethyl 3,4-epoxybutanoate	2.0g	0.6g	Ether	1 Week	Ambient Temperature
2,2,2-trichloroethyl 3,4-epoxybutanoate	2.5g	0.49g	Ether	1 Week	Ambient Temp. 10 drops TEA
2,2,2-trichloroethyl 3,4-epoxybutanoate	2.5g	1.22g	Na dried Ether	1 Week	Ambient Temp. 10 drops TEA
bis-2,2,2-trichloroethyl (±)-epoxyadipate	2.5g	0.49g	Na dried Ether	2 Weeks	Ambient Temperature
2,2,2-trichloroethyl 9,10-epoxyundecanoate	2.5g	1.26g	Na dried THF	1 Week	Ambient Temp. 1ml TEA
2,2,2-trichloroethyl 9,10-epoxyundecanoate	6.0g	1.26g	HPLC grade THF	3 Days	25°C; 1ml TEA
2,2,2-trichloroethyl 9,10-epoxyundecanoate	8.0g	1.2g	HPLC grade THF	24 Hours	25°C; 2ml TEA
2,2,2-trifluoroethyl 9,10-epoxyundecanoate	2.5g	1.29g	Na dried THF	3 Days	25°C; 1ml TEA
2,2,2-trifluoroethyl 9,10-epoxyundecanoate	3.0g	0.67g	HPLC grade THF	3 days	25°C; 1ml TEA
Control Experiment	1.2g	2.9g	Na dried Ether	10 days	Ambient Temperature

For each reaction the enzyme was removed by filtration and washed with the appropriate solvent. Solvents were then removed by rotary evaporator and the resulting residues were examined using a variety of techniques. Triethylamine was used in a



number of reactions since it has been shown to enhance the speed of transesterification for *cis*-2-cyclopenten-1,4-diol, the 5-member ring analogue of DHCD

### **2.6.1 Purification of Residues.**

The purity and composition of each residue was initially investigated using Thin Layer Chromatography (TLC). TLC plates were spotted with reaction residue, developed using a 2:1 mixture of ether:hexane and visualised by placing the plates into an iodine tank. The TLC plates of the residues indicated two or three distinct components which could potentially separated from one another. Each component was separated by Dry Flash Chromatography, using an elution mixture of hexane and ether that progressively became more polar. The eluent fractions were examined by TLC which indicated that a successful separation had been accomplished.

### **2.6.2 N.M.R. Analysis of Residues.**

Samples of each reaction residue and each separated component were examined by nmr. The spectra of each residue indicated that a mixture of compounds were present. However, each spectra indicated the presence of quantities of phenol far in excess of that expected from the room temperature decomposition of DHCD. For the majority of residues there was still evidence of DHCD, although in all cases none appeared to have reacted. The nmr spectra of residues involving trichloroethyl esters also indicated the presence of 2,2,2-trichloroethanol, which implied that some of the ester had undergone a reaction. Initial thoughts that the ester had reacted with either DHCD or phenol were quickly dispelled, since nmr data was consistent with unsubstituted phenol and unreacted DHCD. The presence of a new carbonyl peak at 174 ppm in the  $^{13}\text{C}$  nmr spectra of such residues also supports the hypothesis that some of the trichloroethyl ester has indeed reacted. The only feasible explanation for these observations is that the trichloroethyl ester has undergone partial hydrolysis.

Examination of the spectra of the components separated by Flash chromatography reveals no indication of product and the fractions consist of phenol and 2,2,2-trichloroethanol, unreacted activated ester and unreacted DHCD.

### **2.6.3 Control Experiment.**

A control experiment consisting of a mixture of porcine pancreatic lipase, DHCD and sodium dried ether was run for 10 days to ascertain whether the enzyme was responsible for the aromatisation observed in reactions of DHCD with activated esters. N.M.R. analysis of the residue from the control indicated that there was a phenol content of almost 5%. A sample of DHCD stored in a desicator at room temperature would take 100 days to develop a comparable phenol content. It therefore appears that the enzyme is catalysing the aromatisation of DHCD.

## **2.7 CONCLUSIONS.**

The enzyme catalysed transesterification of DHCD was theoretically a highly attractive means of synthesising hydrophilic derivatives. The mild conditions required for such reactions are ideally suited to DHCD.

Unfortunately, it appears from our experiments that the enzyme is catalysing the elimination of water from DHCD to yield phenol. A range of reaction conditions were investigated to discover whether the effect could be minimised, but alas in all cases phenol was produced in quantities far in excess of that expected from the simple decomposition of DHCD with time.

Once it became apparent that the production of phenol could not be minimised, it was decided to abandon enzyme methods of synthesis and concentrate on the chemical synthesis of derivatives.

Since the abandonment of enzyme experiments considerable research has been undertaken at Aston to optimise the ideal conditions for enzyme synthesis. However, if further attempts at synthesising DHCD derivatives were to be made, a greater understanding of the crude enzyme would be required.

**CHAPTER 3:**  
**Chemical Synthesis of DHCD  
Derivatives.**

### 3. CHEMICAL SYNTHESIS OF DHCD DERIVATIVES.

#### 3.1 INTRODUCTION.

The chemical synthesis of derivatives of *cis*-DHCD is severely limited by its inability to withstand reaction conditions involving strong acids or heat without undergoing aromatisation.<sup>20-21</sup> DHCD can be derivatised by converting the hydroxyl groups into either ether or ester functionality. The simplest method of producing ester derivatives is through the use of acid chlorides and anhydrides; such reactions have in the past used pyridine in the dual role of catalyst and acid trap. However, 4-dimethylaminopyridine (DMAP) has been shown to be a far more effective catalyst.<sup>101,102</sup> In DMAP catalysed reactions a tertiary amine such as triethylamine is employed to neutralise the acid produced. Unfortunately, most readily available acid chlorides and anhydrides will yield only hydrophobic derivatives.

The reaction of DHCD with cyclic anhydrides such as succinic acid will potentially yield derivatives containing hydrophilic carboxylic acid groups. However, carboxylic acid groups have a high protein affinity and as a consequence contact lenses containing such groups suffer from high protein absorption, resulting in a fall in the oxygen permeability of the lens.

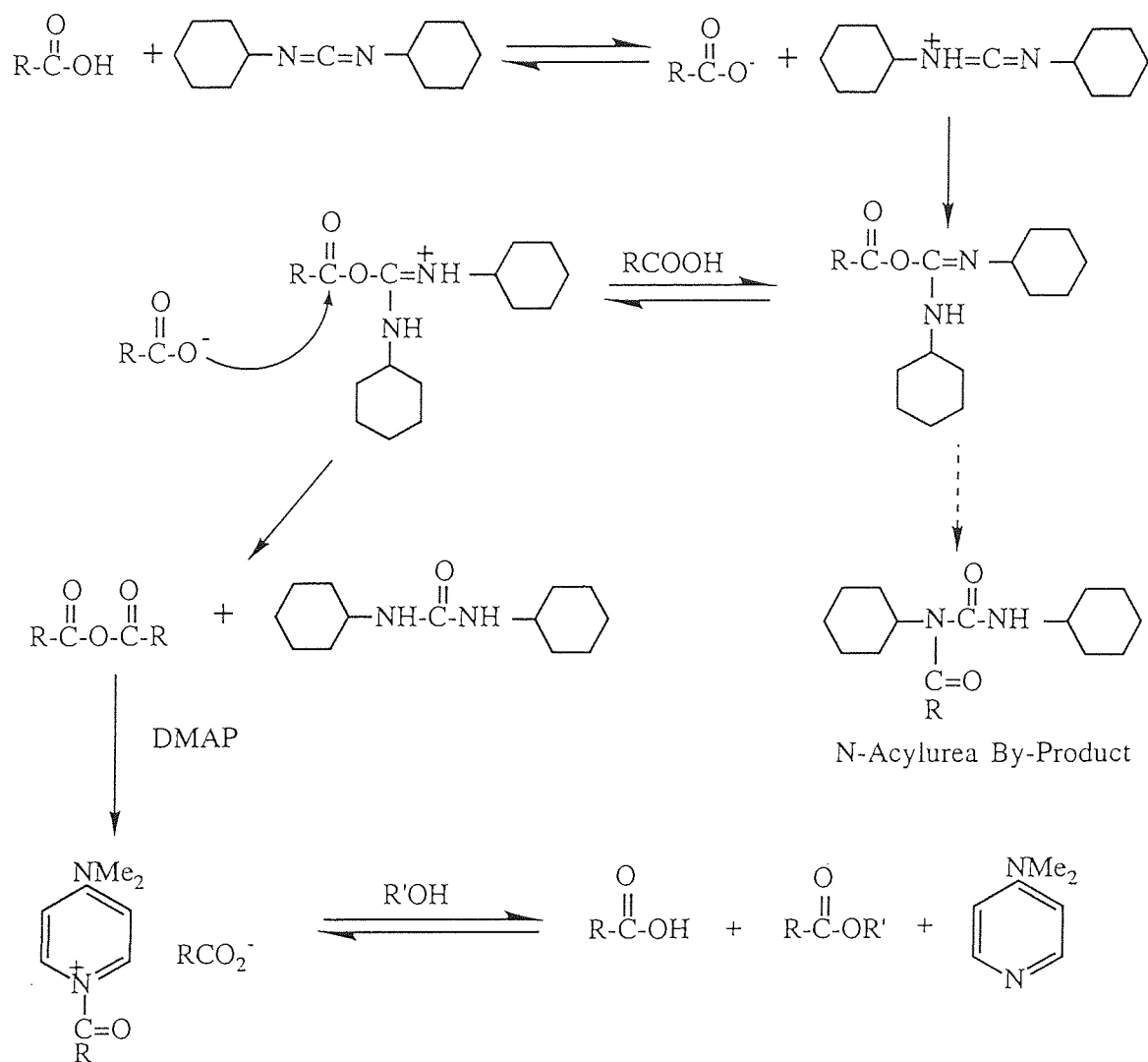
#### 3.2 ESTER SYNTHESIS.

The esterification of an alcohol and a carboxylic acid is catalysed by either acid or base and usually takes place at elevated temperatures. Such processes consist of equilibria involving alcohol and carboxylic acid reactants with ester and water products. In order to obtain the highest yield of product, the equilibrium must be shifted to the right.

There are three main methods of accomplishing this:

- i) the use of excess alcohol.
- ii) the removal of water or product by distillation.
- iii) the use of a dehydrating agent to remove the water.

The esterification of *cis*-DHCD with carboxylic acids is not possible under normal harsh esterification conditions. However, a procedure developed by Hassner<sup>98</sup> involving the use of 4-dimethylaminopyridine (DMAP) as a catalyst and 1,3-dicyclohexylcarbodiimide (DCC) as a dehydrating agent allows esterification reactions to be performed at ambient temperature without the need for an acid catalyst. DCC has long been recognised as an efficient dehydrating agent<sup>103</sup> but by itself it does not expedite the esterification reactions. Likewise DMAP has long been recognised as a very powerful acylation catalyst,<sup>101,102</sup> but only when these two compounds are combined is it possible to perform a wide range of esterifications at room temperature.<sup>98,103,104</sup>



**Figure 3.1: Mechanism for the DCC/DMAP Catalysed Esterification Process.**

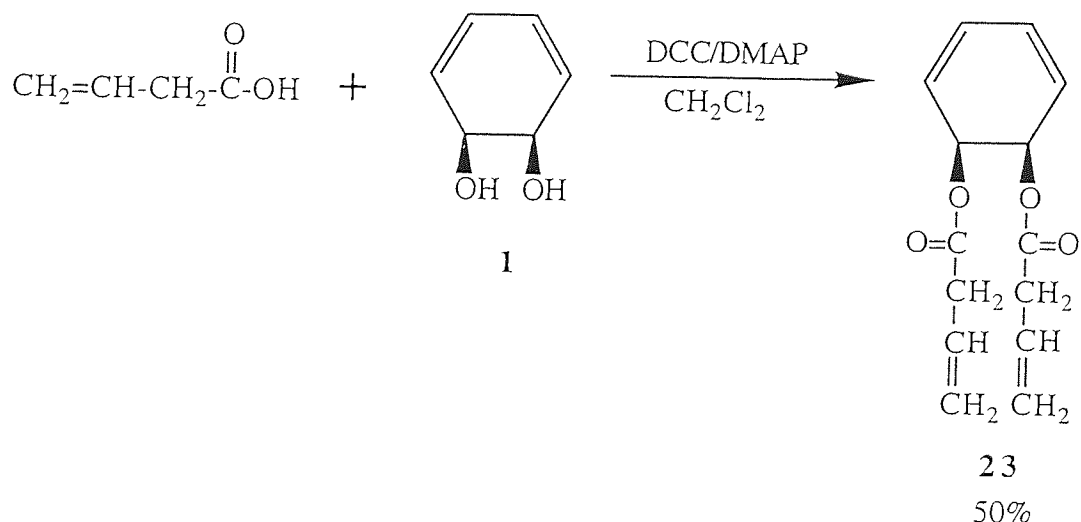
The mechanism<sup>103</sup> initially involves the protonation of DCC followed by an attack by the acid anion at the carbon atom of the cation formed. This leads to the formation of the highly active  $O$ -acylisourea, which can react further in one of two ways. Firstly, intramolecular migration of the acyl group from the oxygen atom to the nitrogen atom yields the  $N$ -acylurea byproduct. Secondly, after initial protonation of the  $O$ -acylisourea by a second acid molecule, the acid anion may attack the carbonyl carbon to yield the acid anhydride and 1,3-dicyclohexylurea (DCU). The acid anhydride forms a complex with DMAP which acts as the attacking species. Yields from such reactions are generally high, but they are retarded by the formation of the  $N$ -acylurea.

Although this type of process allows esterifications to be accomplished under mild conditions, the presence of functional groups such as OH and NH<sub>2</sub> on the acid will lead to unwanted side reactions.

To assess whether or not *cis*-DHCD could be used in these types of esterification reactions, a number of derivatives were produced by the reaction of *cis*-DHCD with several cheap and readily available unsaturated carboxylic acids.

### 3.2.1 Synthesis of *cis*-1,2-Bis(3-butenoyloxy)-3,5-cyclohexadiene.

The compound *cis*-1,2-bis(3-butenoyloxy)-3,5-cyclohexadiene was prepared by a slight modification of the procedure described by Hassner.<sup>98</sup> A yield of 50% was obtained by the reaction at room temperature of vinyl acetic acid with *cis*-DHCD under an argon atmosphere in the presence of DCC and DMAP. The <sup>1</sup>H and <sup>13</sup>C N.M.R. spectra of this compound are displayed in figures 3.2a and 3.2b. The yield was lower than usually obtained for this type of reaction, but this can be attributed to a combination of *N*-acylurea formation and the removal of any mono-substituted derivative during the washing procedure.



Scheme 3.1: Synthesis of *cis*-1,2-Bis(3-butenoyloxy)-3,5-cyclohexadiene.



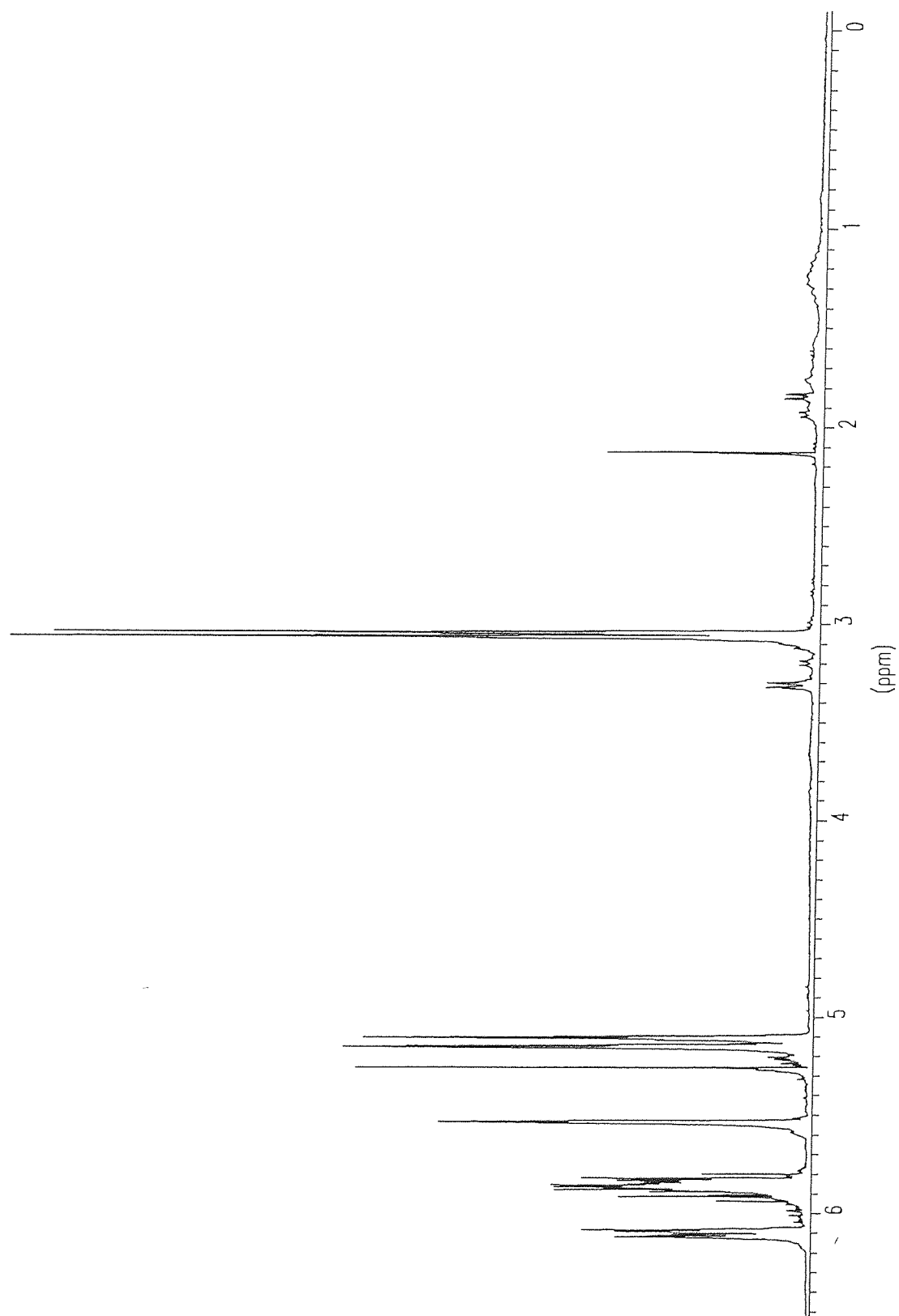


Figure 3.2a:  $^1\text{H}$  N.M.R. Spectra of *cis*-1,2-Bis(3-Butenyloxy)-3,5-Cyclohexadiene.

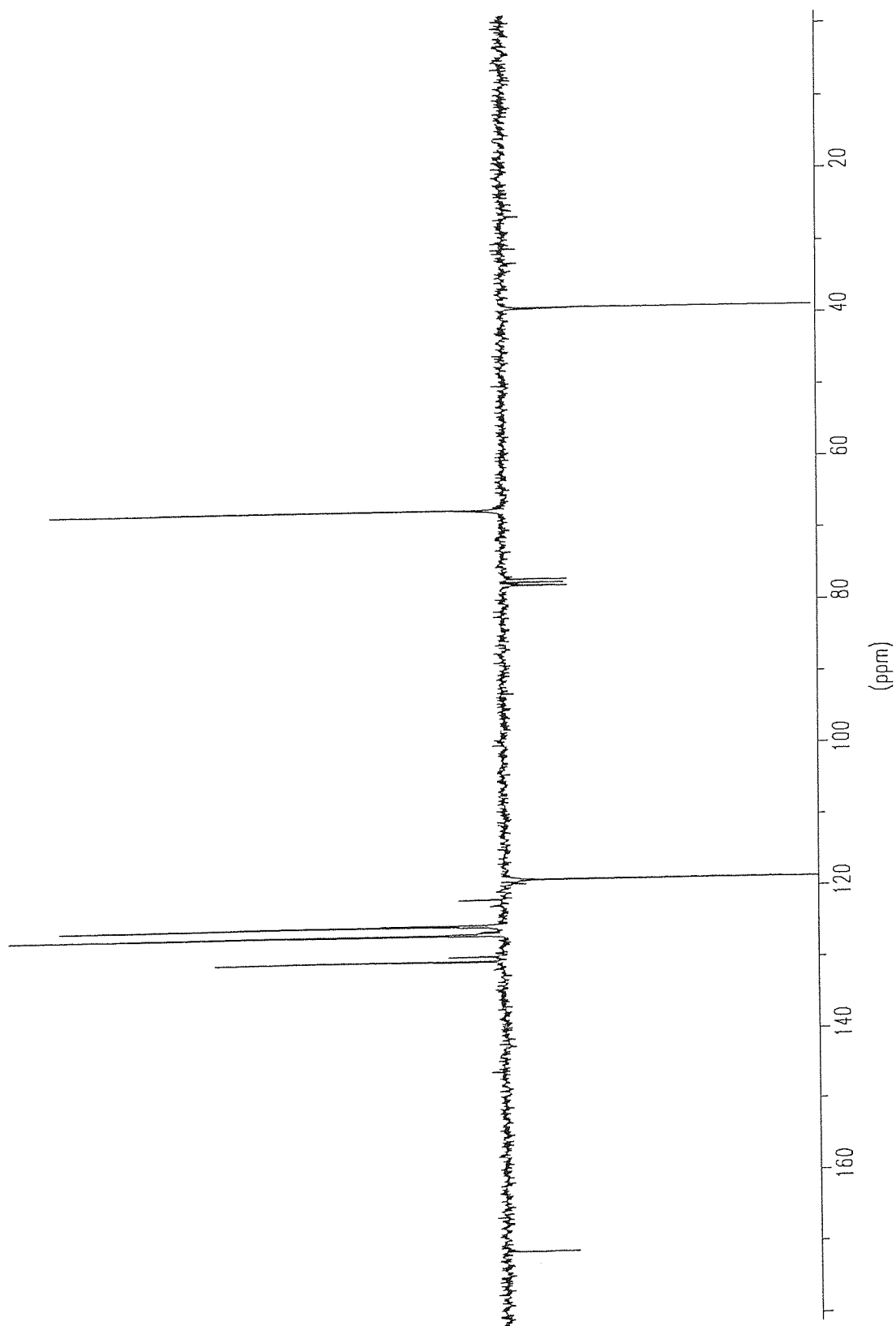
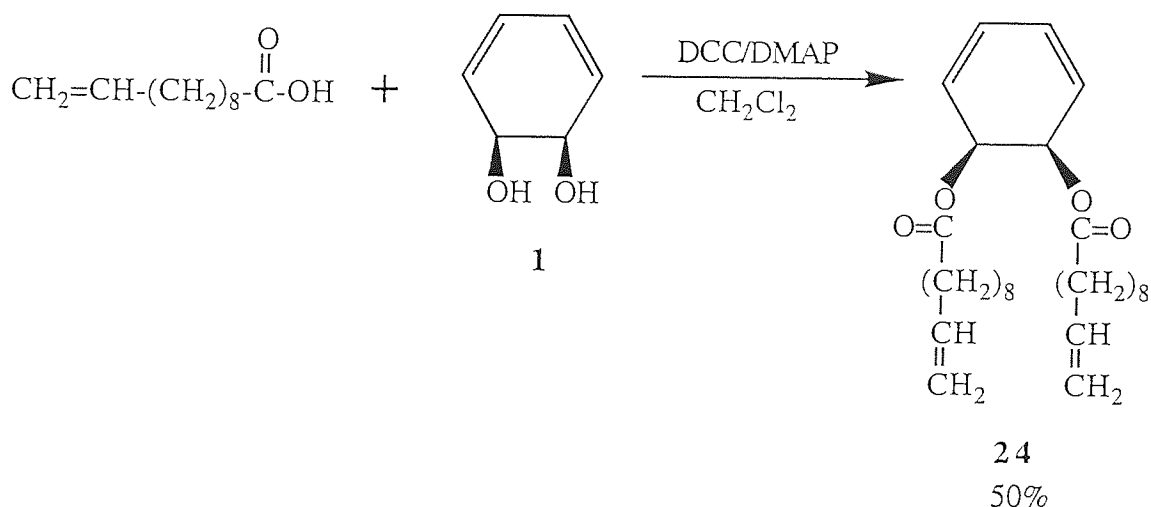


Figure 3.2b:  $^{13}\text{C}$  N.M.R. Spectra of *cis*-1,2-Bis(3-butenoyloxy)-3,5-Cyclohexadiene.

### 3.2.2 Synthesis of *cis*-1,2-Bis(10-undecenoyloxy)-3,5-cyclohexadiene.

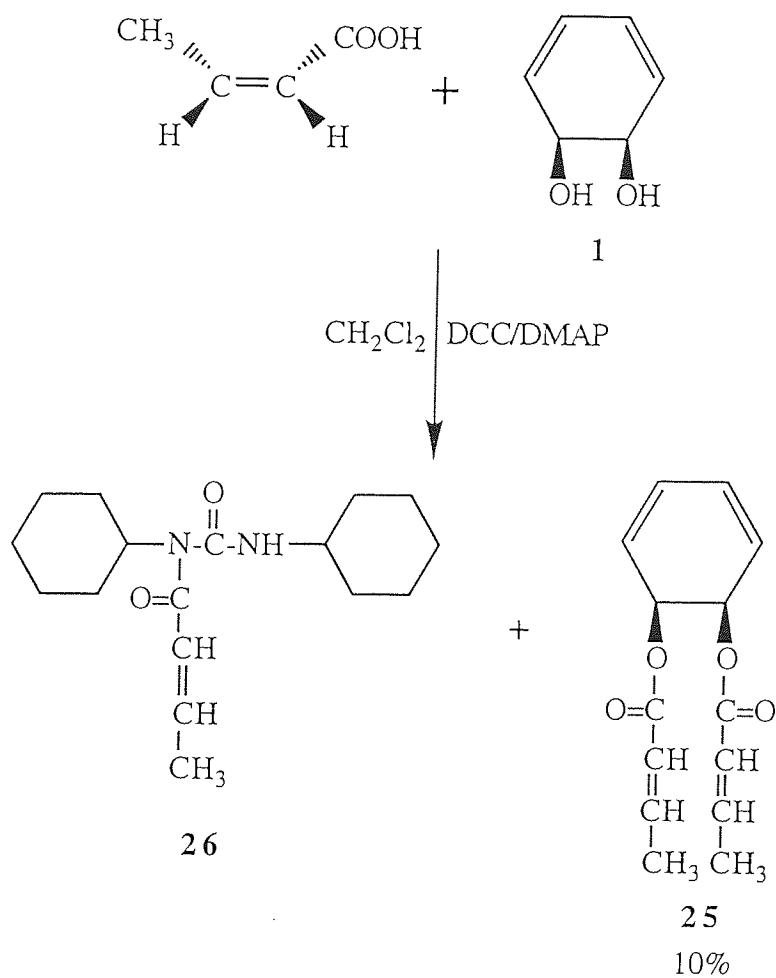
The compound *cis*-1,2-bis(10-undecenoyloxy)-3,5-cyclohexadiene was synthesised in a 63% yield by the reaction of *cis*-DHCD with undecylenic acid in an argon atmosphere in the presence of DCC and DMAP. The product was obtained as a low melting point solid and was recrystallised from chloroform.



Scheme 3.2: Synthesis of *cis*-1,2-Bis(10-undecenoyloxy)-3,5-cyclohexadiene.

### 3.2.3 Synthesis of *cis*-1,2-Bis(2-butenoyloxy)-3,5-cyclohexadiene.

The synthesis of *cis*-1,2-bis(2-butenoyloxy)-3,5-cyclohexadiene was attempted by the reaction of crotonic acid with DHCD in the presence of DCC and DMAP. Unfortunately the reaction yielded a substantial amount of *N*-acylurea byproduct which resulted in a product yield of 10%.



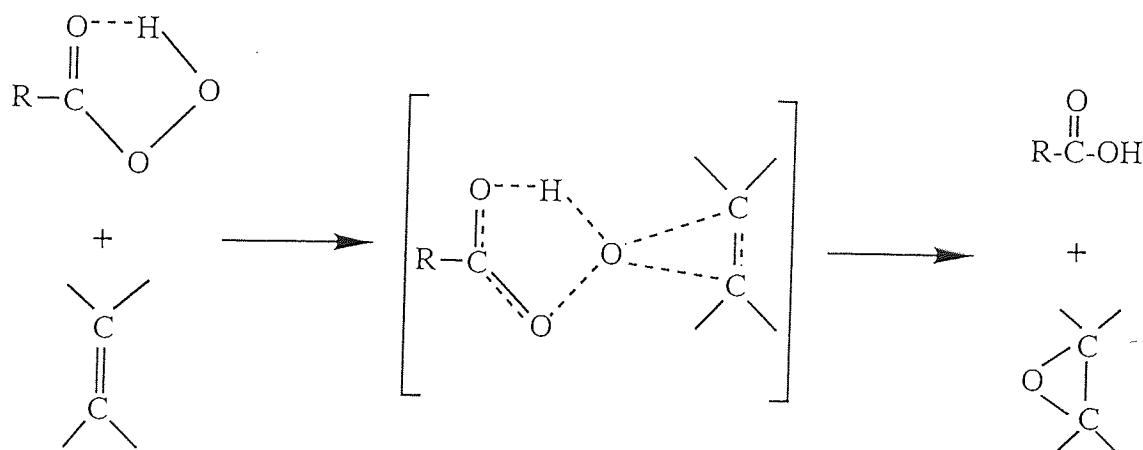
**Scheme 3.3: Synthesis of *cis*-1,2-Bis(2-butenoyloxy)-3,5-cyclohexadiene.**

### 3.3 EPOXIDATION.

Epoxides are three membered rings containing oxygen that are produced by the action of either peroxy acids<sup>105</sup> or hydrogen peroxide<sup>106-110</sup> on regions of unsaturation in a molecule. Epoxide rings are highly unstable and undergo both acid and base catalysed ring opening to yield glycols.<sup>114</sup> The epoxidation of unsaturated carboxylic acids offers a potential method of introducing hydrophilic derivatives into *cis*-DHCD, since epoxy acids can be used to esterify DHCD and the epoxide rings in the resulting derivatives can be subsequently opened to yield hydroxyl groupings.

#### 3.3.1 Epoxidation by Peroxy Acids.

There are a range of peroxy acids that can be used in epoxidation reactions, but the acid most frequently used is *meta*-chloroperoxybenzoic acid (*m*-CPBA).<sup>105</sup> This is due to a number of reasons including: ready availability, relative cheapness and stability for prolonged periods without decomposition.

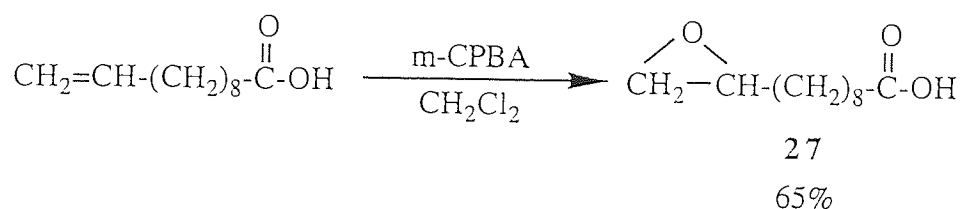


**Figure 3.3: Mechanism for Epoxidation by Peroxy Acid.**

The mechanism<sup>105</sup> for epoxidation involves the formation of a complex between the carbon-carbon double bond and the peroxy acid. A partial bond is formed between the oxygen and carbon atoms and this subsequently leads to the formation of the epoxide ring and the elimination of carboxylic acid.

### 3.3.1.1 Synthesis of 10,11-Epoxyundecanoic Acid.

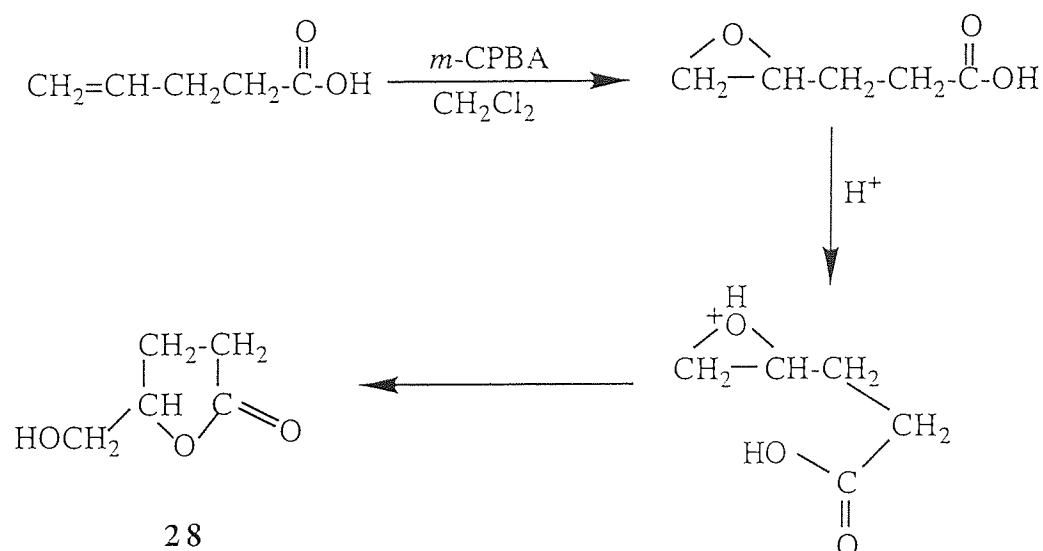
The compound 10,11-epoxyundecanoic acid was prepared by the reaction of undecylenic acid with *m*-CPBA in dichloromethane. The crude product was separated from the *m*-chlorobenzoic acid byproduct and purified by washing with cyclohexane to give a yield of 65%.



Scheme 3.4: Synthesis of 10,11-Epoxyundecanoic Acid.

### 3.3.1.2 Synthesis of 4,5-Epoxypentanoic Acid.

The synthesis of 4,5-epoxypentanoic acid was attempted by reacting 4-pentenoic acid with *m*-CPBA at room temperature. Unfortunately, examination of the I.R. and N.M.R. spectra of the product indicated that the epoxide ring of the racemic epoxy acid had been opened leading to the production of (*R,S*)-( $\pm$ )-dihydro-5-(hydroxymethyl)-2(3H)-furanone, see scheme 3.5. The lactonisation is believed to have been catalysed by protons from the *meta*-chlorobenzoic acid byproduct and attempts to prevent ring opening by the use of sodium benzoate buffer proved to be unsuccessful.



**Scheme 3.5: Epoxidation and Subsequent Lactonisation of 4-Pentenoic acid.**

The mechanism for the acid catalysed opening of an epoxide ring can be either S<sub>N</sub>1 or S<sub>N</sub>2. However, without undertaking far more detailed studies of the reaction it is not possible to decide which of the two mechanisms is occurring.

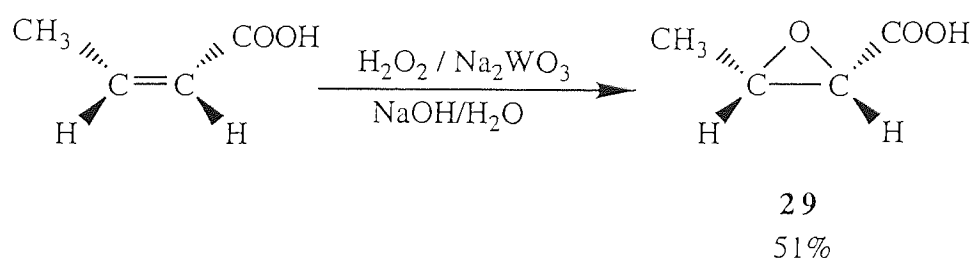
### **3.3.2 Epoxidation by Hydrogen Peroxide.**

Hydrogen peroxide is a poor oxidant, and requires a catalyst to successfully facilitate epoxidations. Typically such catalysts are transition metal complexes such as sodium tungstate<sup>106,107</sup> or tris(cetylpyridinium)-12-tungstophosphate<sup>108-110</sup> which behave as electron acceptors. It has been postulated that the mechanism for this type of epoxidation involves the formation of an inorganic peroxy acid. The coordination of the transition metal to a functional group adjacent to the double bond allows rapid transfer of oxygen and epoxide formation. Such epoxidations take place under aqueous or biphasic conditions and are hence suitable for the epoxidation of unsaturated carboxylic acids.

### 3.3.2.1. Synthesis of 2,3-Epoxybutanoic Acid.

The epoxidation of  $\alpha,\beta$ -unsaturated acids such as crotonic acid by the use of peroxy acids generally proceeds very slowly. This is due to the electron withdrawing effect of the carbonyl group which is directly attached to the ethylenic double bond and the resulting conjugation.

The compound 2,3-epoxybutanoic acid was produced by the reaction of crotonic acid with hydrogen peroxide solution in the presence of sodium tungstate catalyst. The reaction took place at 55°C in an aqueous solution with the pH kept above 4. The crude product had a purity of 75% but after recrystallisation from chloroform this was improved to almost 100%. The  $^1\text{H}$  and  $^{13}\text{C}$  N.M.R. spectra of the purified compound are displayed in figures 3.4a and 3.4b. Tungstates are polymeric in acid, base and neutral aqueous solutions, and as a consequence the pH of the solution is of paramount importance since under acidic conditions the ring opening of the epoxide will be catalysed.



Scheme 3.6: Synthesis of 2,3-Epoxybutanoic Acid.

The relatively high reactivity exhibited by  $\alpha,\beta$ -unsaturated acids in  $\text{H}_2\text{O}_2$ /sodium tungstate catalysed epoxidations is believed to be due to the formation of an intermediate cyclic complex involving the substrate and the inorganic peroxy acid. The oxidising species acts as an electrophile and coordination of the transition metal to the carbonyl group allows transfer of oxygen to rapidly occur.



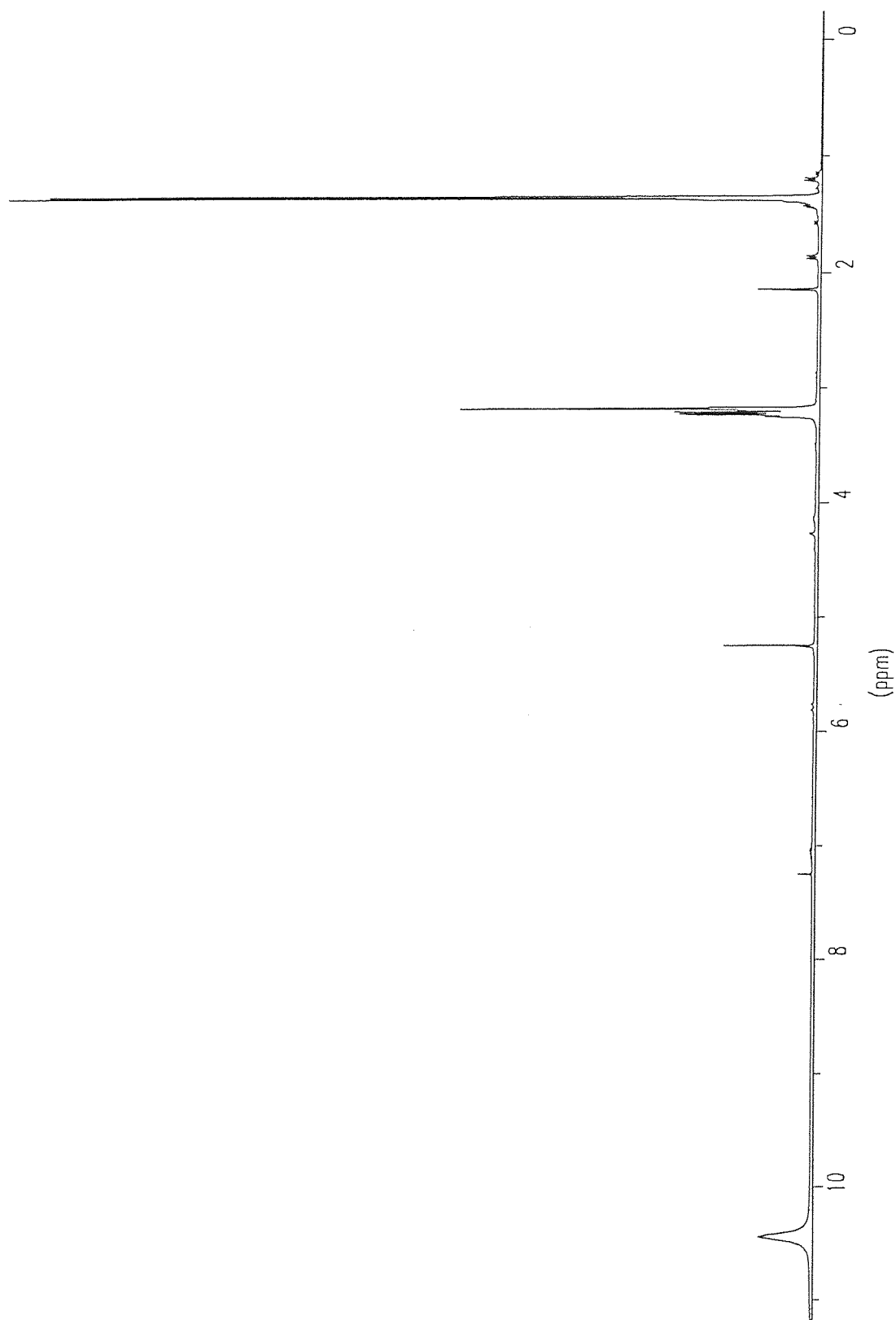


Figure 3.4a:  $^1\text{H}$  N.M.R. Spectra of 2,3-Epoxybutanoic Acid.

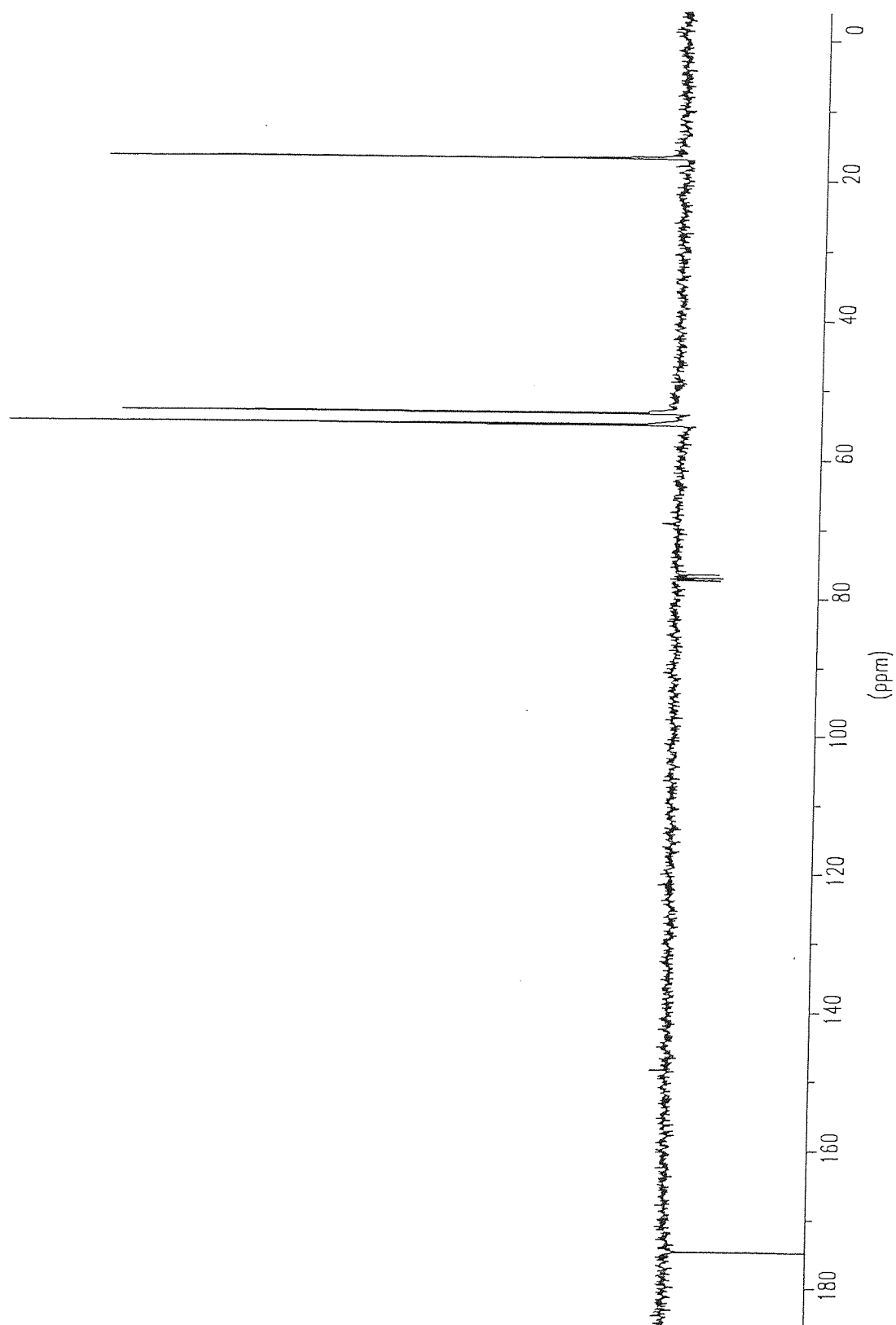


Figure 3.4b:  $^{13}\text{C}$  N.M.R. Spectra of 2,3-Epoxybutanoic Acid.

### 3.3.2.2 Synthesis of 3,4-Epoxybutanoic Acid.

The epoxidation of vinyl acetic acid was attempted by using three different methods. The first method involved the reaction of the acid with *m*-chloroperoxybenzoic acid in dichloromethane. However, the product obtained was heavily contaminated with *m*-chlorobenzoic acid and all attempts at purification proved unsuccessful.

The second method was similar to that used in the epoxidation of crotonic acid; the vinyl acetic acid was dissolved in sodium hydroxide solution and reacted with hydrogen peroxide in the presence of sodium tungstate catalyst. The oil recovered from the reaction was shown by nmr to consist of approximately 50% 3,4-epoxybutanoic acid and 50% vinyl acetic acid. Attempts to purify the epoxide by vacuum distillation led to lactonisation, and the two acids were not significantly different enough to be separated by flash chromatography. The slower reactivity of vinyl acetic acid compared with crotonic acid is explained by the lack of a functional group adjacent to the double bond for the transition metal to coordinate to. As a consequence the oxygen of the inorganic peroxy acid is not held in the vicinity of the double bond and as a result epoxidation is slower.

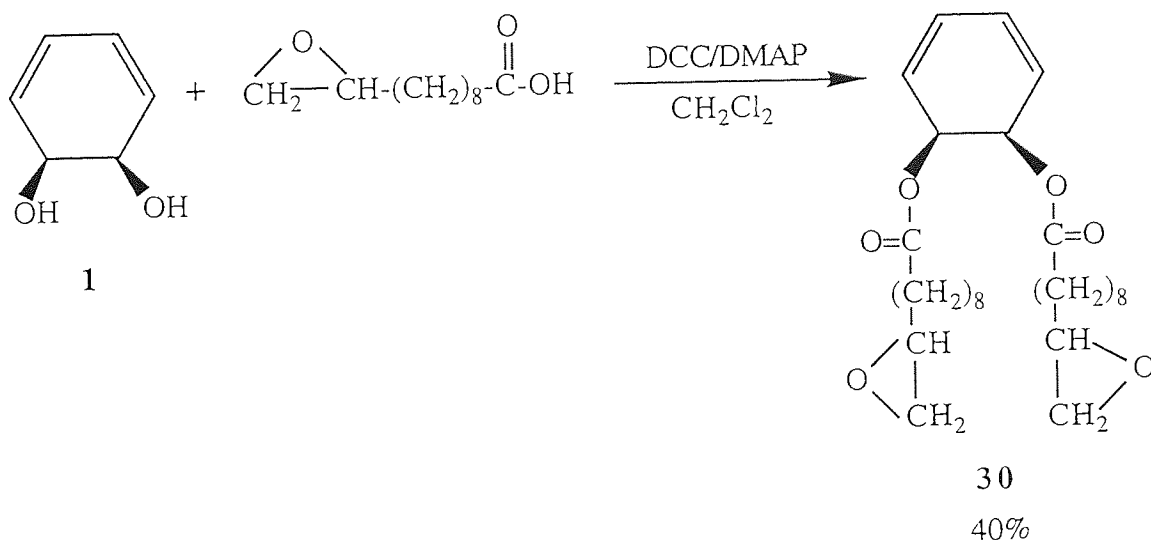
A third method of epoxidation involving the use of hydrogen peroxide in conjunction with tris(cetylpyridinium)-12-tungstophosphate (CWP); a heteropolyacid was also investigated. This method had been shown to be highly effective in the epoxidation of  $\alpha,\beta$ -unsaturated acids, and it was hoped that it would be equally effective for vinyl acetic acid. The method involved the dissolution of vinyl acetic acid in sodium hydroxide solution and reaction with hydrogen peroxide in the presence of CWP at 60°C. Upon addition of the hydrogen peroxide the solution turned a dark red colour, and over a period of 2 hours the colour faded to pale yellow. However, N.M.R. analysis of the oil recovered from the reaction showed very little evidence of any epoxide product, the majority of the oil being unreacted vinyl acetic acid.

### 3.4 ESTERIFICATION OF *cis*-DHCD WITH EPOXYACIDS.

It has already been demonstrated that the esterification process involving DCC and DMAP can be used to produce ester derivatives of *cis*-DHCD by reaction with unsaturated carboxylic acids. Unfortunately, this procedure is highly sensitive to hydrophilic groups such as OH and NH<sub>2</sub>. Consequently, carboxylic acids containing such functionality cannot be reacted with DHCD since a range of side reactions occur. The epoxide group may be considered as a protected diol moiety because of the ease with which the ring opens. Epoxy acids can be successfully reacted with DHCD under DCC/DMAP conditions without fear of side reactions. The epoxide rings can then be opened either before or after polymerisation to yield a multitude of hydroxy groups in the pendant chains of the DHCD derivative.

#### 3.4.1 Synthesis of *cis*-1,2-Bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene.

The reaction of 10,11-epoxyundecanoic acid with *cis*-DHCD in an argon atmosphere and in the presence of DCC and DMAP led to a 40% yield of *cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene. The product was obtained as an off-white solid which was recrystallised from chloroform. The <sup>1</sup>H and <sup>13</sup>C N.M.R. spectra of the purified compound are displayed in figures 3.5a and 3.5b.



**Scheme 3.7: Synthesis of *cis*-1,2-Bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene.**

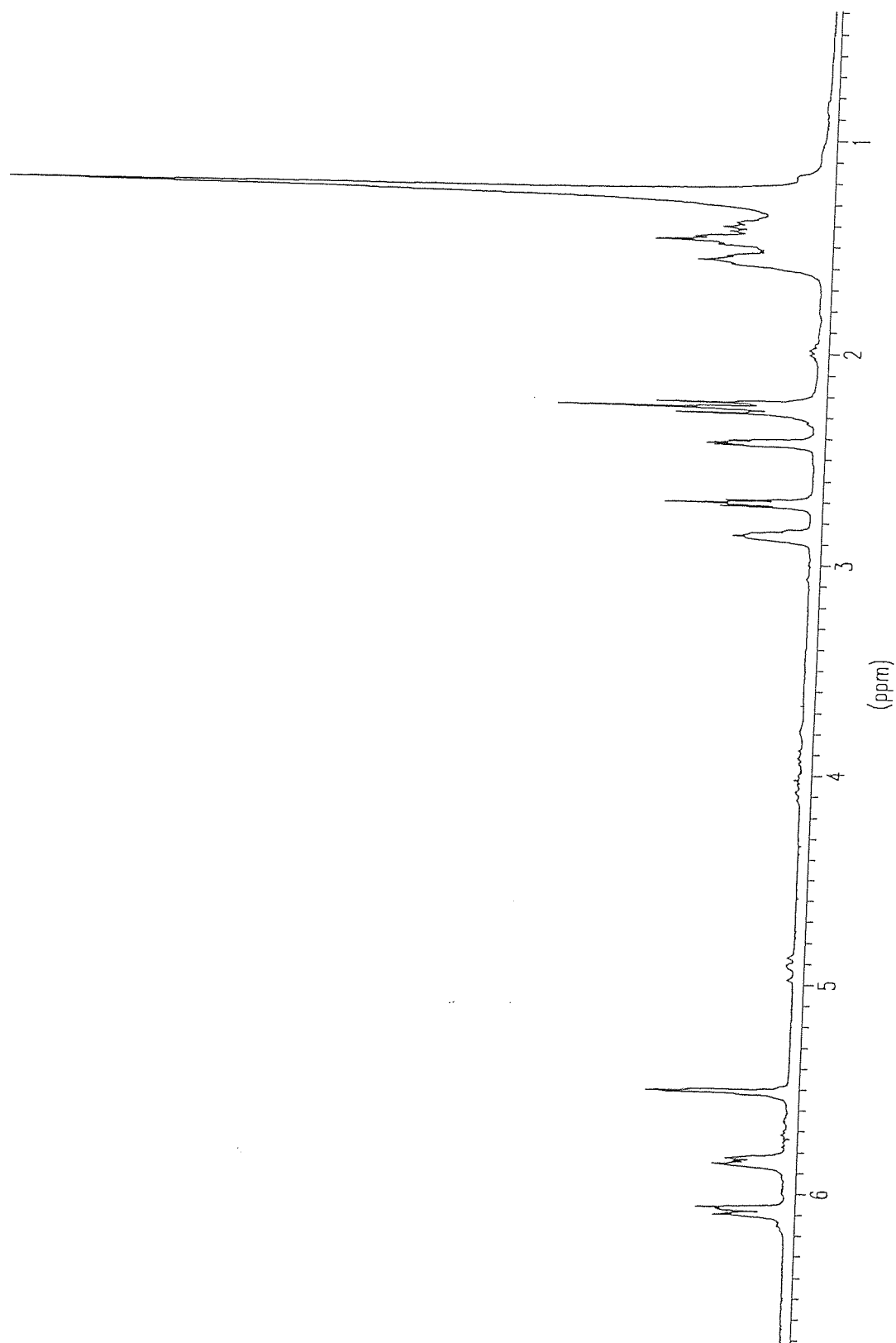


Figure 3.5a:  $^1\text{H}$  N.M.R. Spectra of *cis*-1,2-Bis(10,11-epoxyundecanoyloxy)-3,5-Cyclohexadiene.

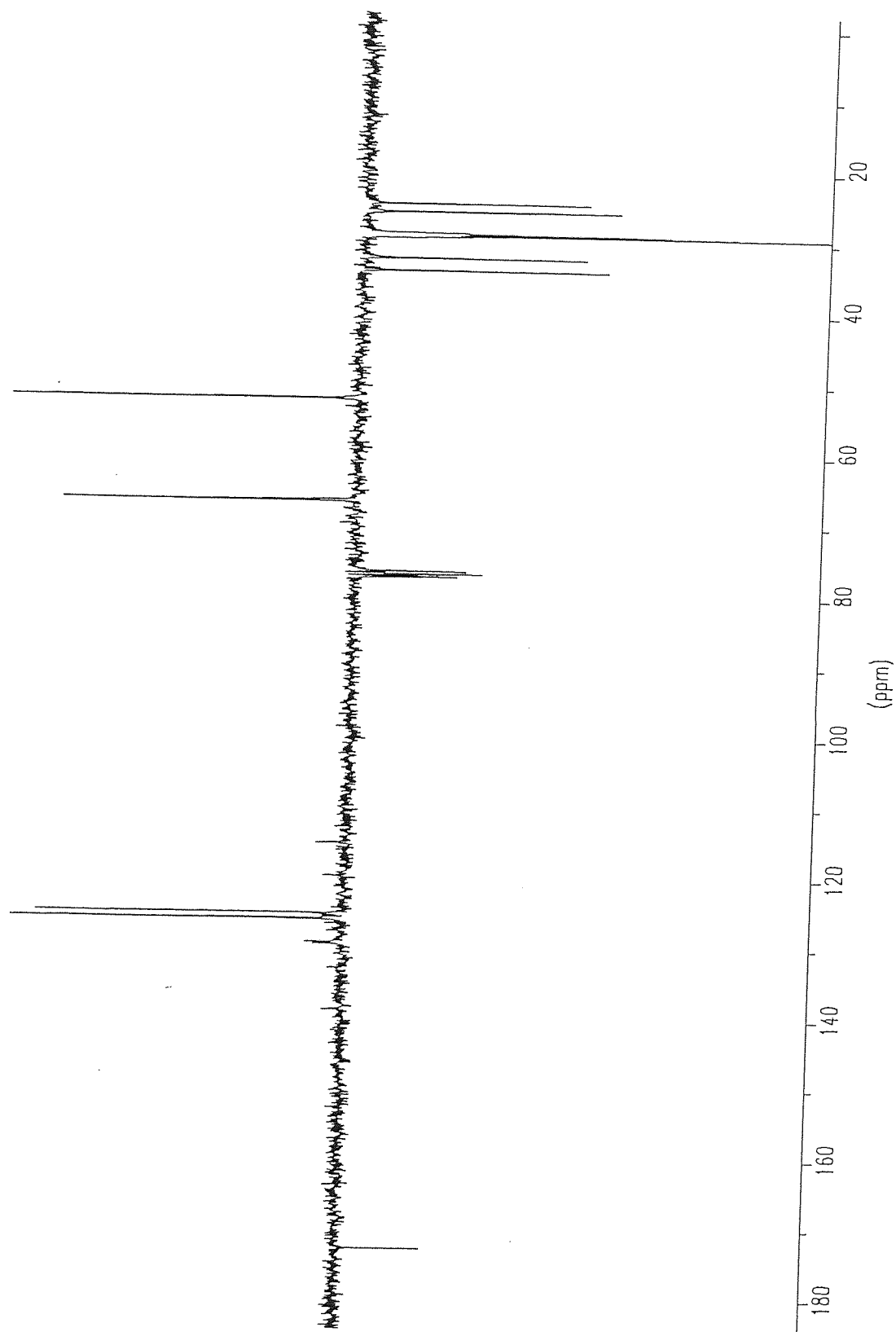
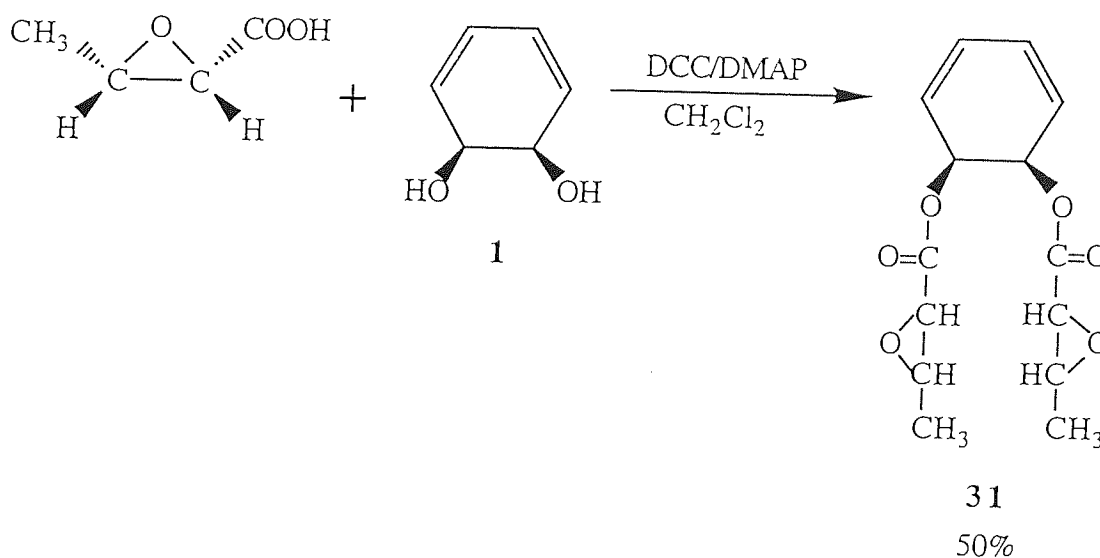


Figure 3.5b:  $^{13}\text{C}$  N.M.R. Spectra of *cis*-1,2-Bis(10,11-epoxyundecanoyloxy)-3,5-Cyclohexadiene.

### 3.4.2 Synthesis of *cis*-1,2-Bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene.

The synthesis of *cis*-1,2-bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene was accomplished by the reaction of 2,3-epoxybutanoic acid with *cis*-DHCD. The reaction took place at room temperature in the presence of DCC, DMAP and an argon atmosphere. The crude product was purified by flash chromatography using an ether/hexane elution system to give a yield of 50%. The low yield can be attributed to a combination of losses as the mono derivative and the *N*-acylurea.



Scheme 3.8: Synthesis of *cis*-1,2-Bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene.

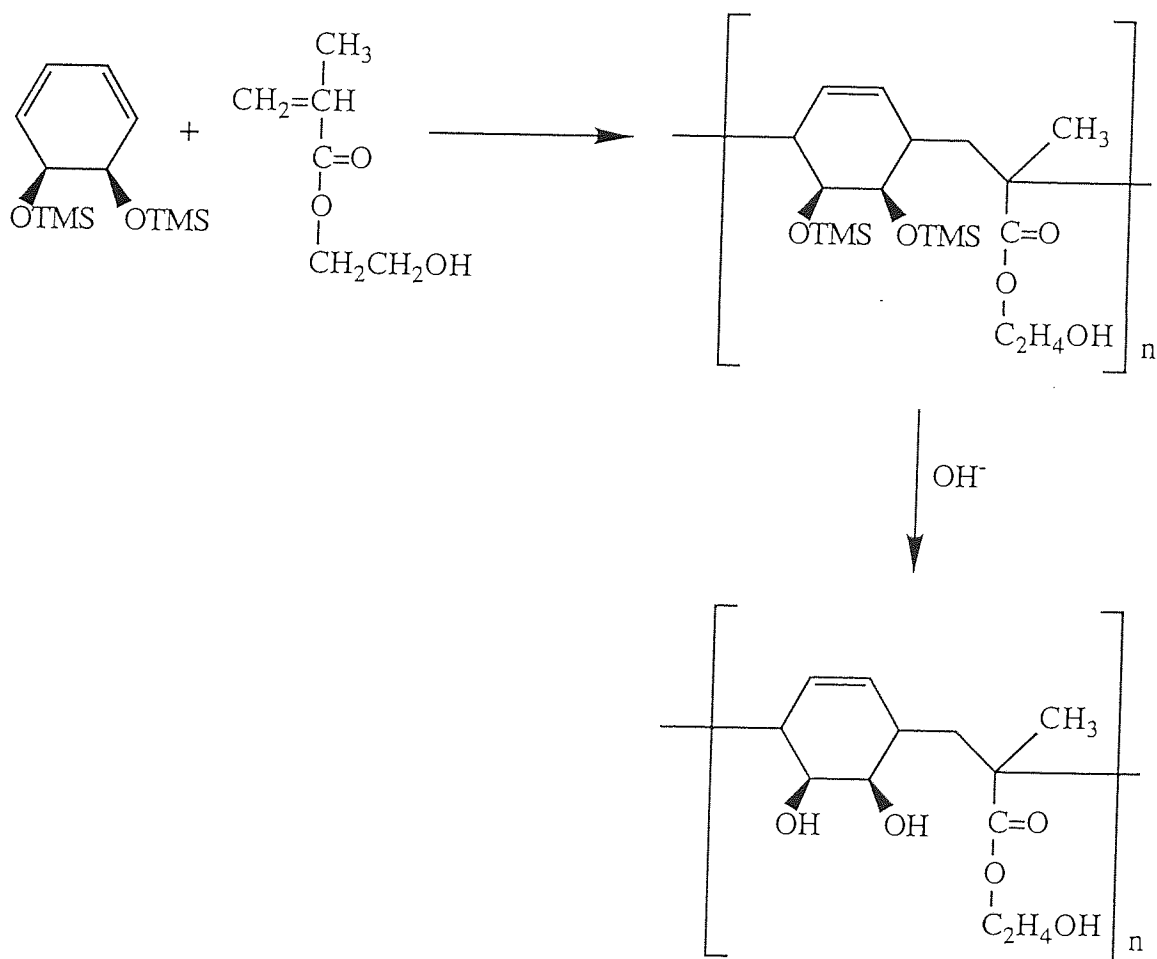
### 3.5 PROTECTING GROUPS.

The presence of more than one functional group in a molecule can often lead to unwanted side reactions during synthesis. Such side reactions can be minimised by the protection of functionality. In order to be effective, a protecting group must be introduced in high yield and must be impervious to all reagents in succeeding steps as well as easily removable. An effective method of protecting alcohols is to convert the hydroxyl group into a silyl ether (OSiR<sub>3</sub>).<sup>111-113</sup> The hydroxyl moiety can be easily regenerated once the synthesis is complete. The stability of silyl ethers is dependent upon R, with bulkier R groups producing more stable silyl ethers. It is also observed

that the rate of hydrolysis of secondary silyl ethers is significantly slower than that of primary silyl ethers.

### 3.5.1 Protection of *cis*-DHCD.

The instability of *cis*-DHCD can be accounted for by the ease with which the 1,2-diol moiety eliminates water to yield phenol. The use of silyl ether protecting groups should prevent the elimination of water and hence improve the stability of the molecule. The silyl protected DHCD can then be copolymerised with 2-hydroxyethyl methacrylate (HEMA) before having the protecting groups removed and the diol moiety regenerated.



**Scheme 3.9: Copolymerisation of Silyl Protected DHCD with HEMA and Subsequent Removal of Protecting Groups.**



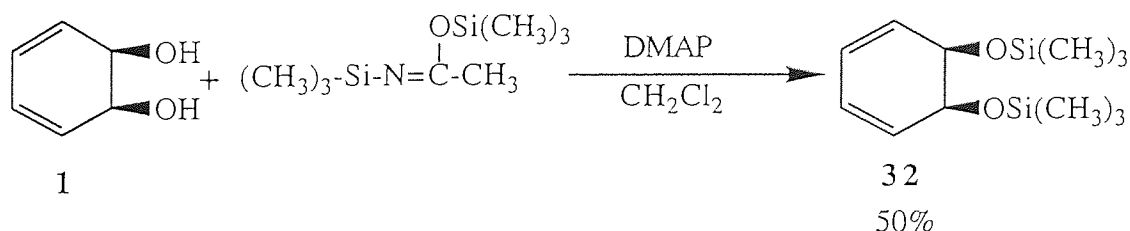
### 3.5.2 Trimethylsilyl Protecting Groups.

Trimethylsilyl ethers are highly susceptible to hydrolysis in either acidic or basic media, however the rate of hydrolysis of secondary silyl ethers is significantly slower than that of primary silyl ethers. As a consequence it was envisaged that trimethylsilyl protected derivatives of DHCD will exhibit sufficient stability to undergo polymerisation.

The compound bis(trimethylsilyl)acetamide (BSA) has been shown to be a very effective silylating agent.<sup>111</sup> Reactions involving BSA take place at low to moderate temperatures without the involvement of acid or base and no difficult to remove by-products are formed.

#### 3.5.2.1 Synthesis of *cis*-1,2-Bis(trimethylsiloxy)-3,5-cyclohexadiene.

The synthesis of *cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene was accomplished by the reaction of *cis*-DHCD with bis(trimethylsilyl)acetamide (BSA) in dichloromethane using DMAP as a catalyst. The reaction took place at room temperature and gave a yield of 50%. The by-product, *N*-(trimethylsilyl)acetamide was precipitated by the addition of hexane and removed by filtration. The purity of the product was determined initially by the absence of C=O and C=N bands in the IR spectra and secondly by N.M.R.



Scheme 3.10: Synthesis of *cis*-1,2-Bis(trimethylsiloxy)-3,5-cyclohexadiene.

### 3.5.3 *tert*-Butyldimethylsilyl Protecting Groups.

The stability of a silyl ether is dictated by the size of the silyl protecting group. Since bulkier groups give more stable silyl ethers, it is expected that *tert*-butyl dimethylsilyl (TBDMS) ethers will be more stable than trimethylsilyl ethers. The reason for this is that larger groups are more effective at shielding the silicon from attack by nucleophiles.

The established procedure for the preparation of TBDMS ethers involves the reaction of an alcohol with *tert*-butyldimethylchlorosilane (TBDCS) in the presence of imidazole in *N,N*-dimethylformamide (DMF) at room temperature<sup>112</sup>. However, the use of 4-dimethyl aminopyridine (DMAP) as a group transfer agent instead of imidazole allows a larger choice of reaction solvents<sup>113</sup>.

The favoured mechanism for DMAP catalysed silylations<sup>113</sup> involves the formation of a complex between the amine and the silyl chloride, which subsequently reacts with the alcohol to form the silyl ether. The presence of triethylamine allows the catalyst to be regenerated.

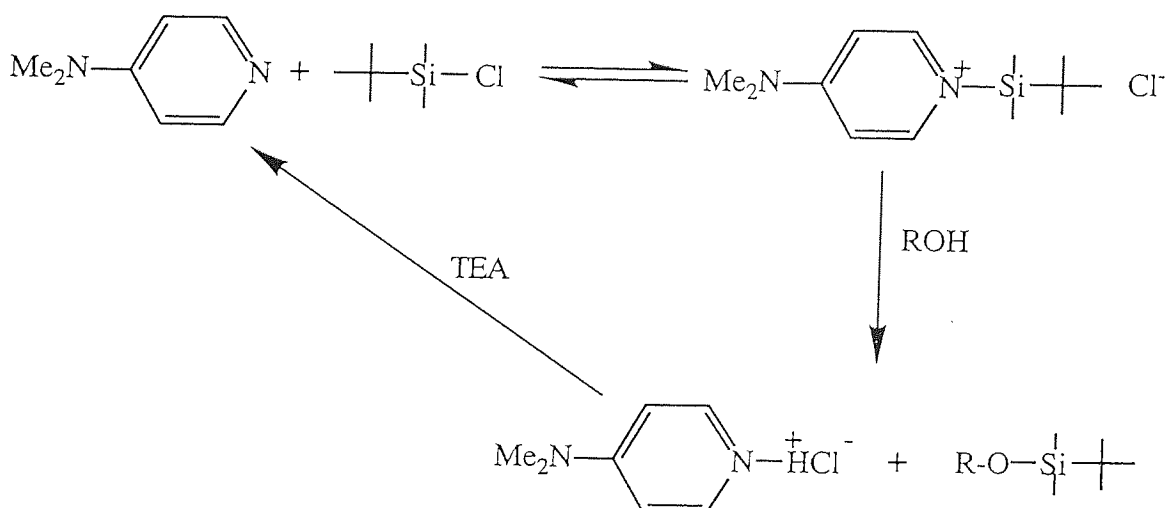
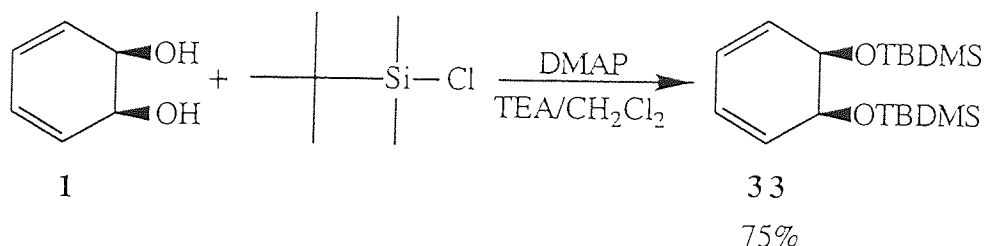


Figure 3.6: Mechanism for DMAP Catalysed Silylation of an Alcohol.

### 3.5.3.1 Synthesis of *cis*-1,2-Bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene.

The compound *cis*-1,2-bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene was synthesised from *cis*-DHCD and *tert*-butyldimethylsilyl chloride in a yield of 75%. The reaction took place at  $-78^{\circ}\text{C}$  in dichloromethane in the presence of DMAP and triethylamine. The  $^1\text{H}$  and  $^{13}\text{C}$  N.M.R. spectra of this compound are displayed in figures 3.7a and 3.7b.



Scheme 3.11: Synthesis of *cis*-1,2-Bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene.

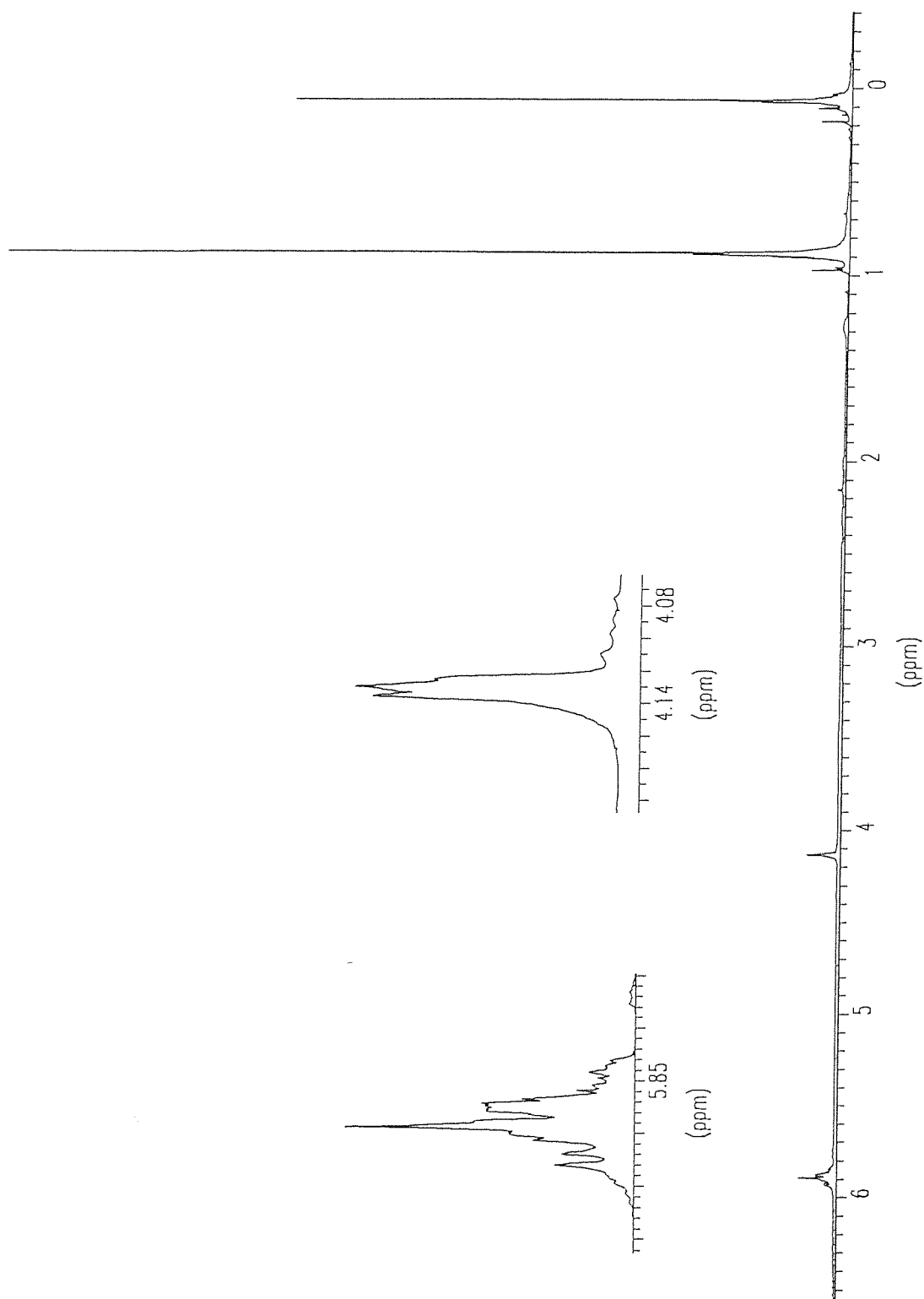


Figure 3.7a:  $^1\text{H}$  N.M.R. Spectra of *cis*-1,2-Bis(*tert*-butyldimethylsilyl)-3,5-Cyclohexadiene.

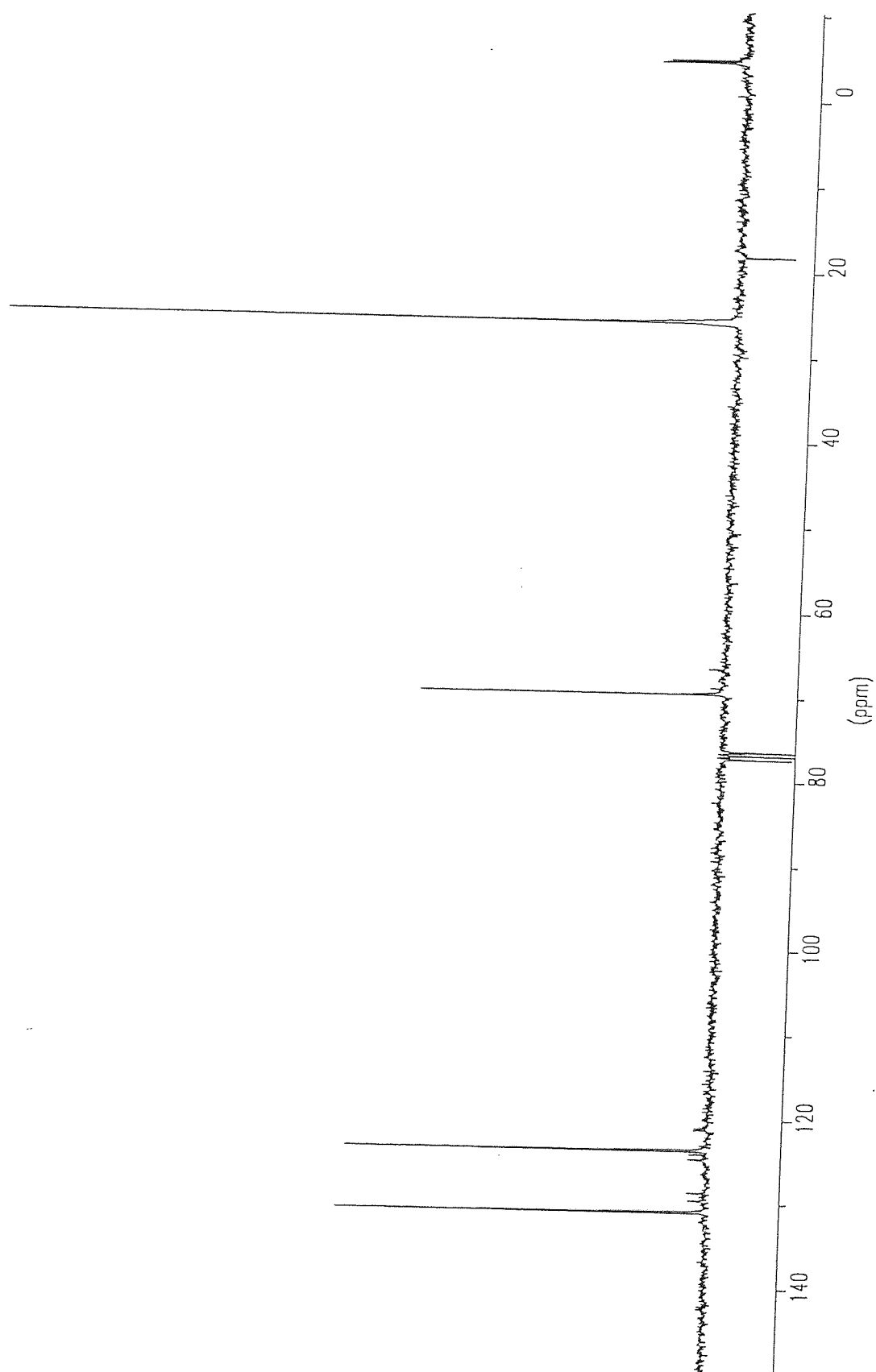
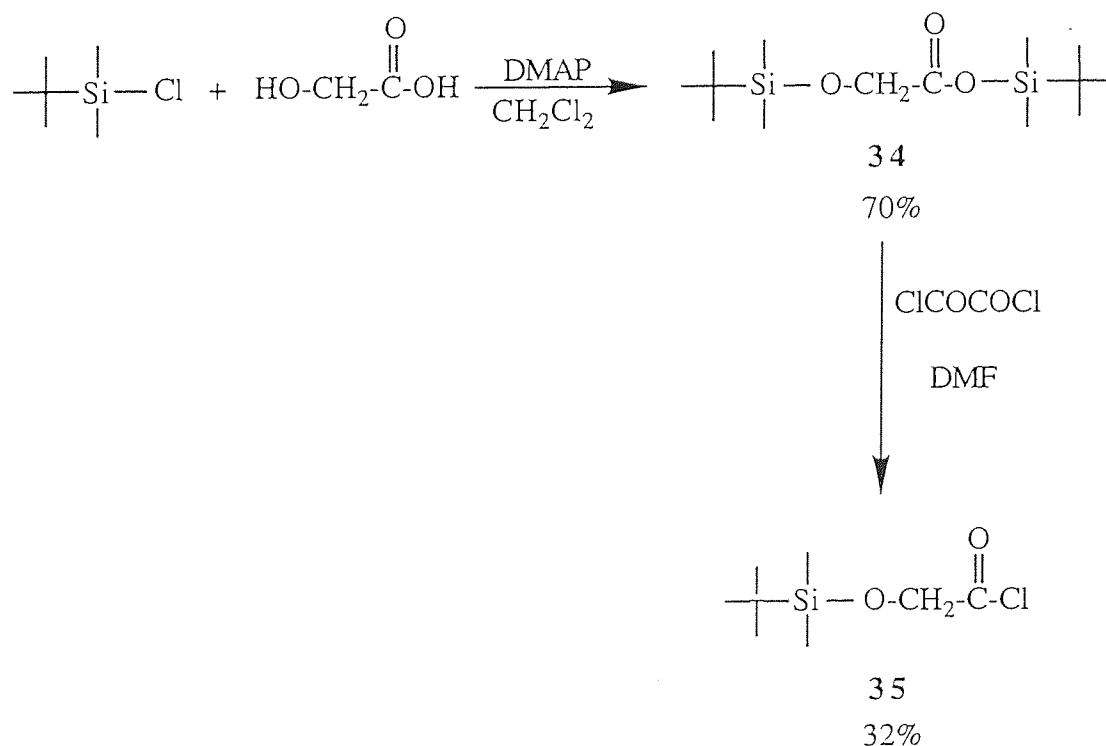


Figure 3.7b:  $^{13}\text{C}$  N.M.R. Spectra of *cis*-1,2-Bis(*tert*-butyldimethylsilyl)-3,5-Cyclohexadiene.

### 3.5.4 Protection of Hydroxy Acids.

The use of hydroxy acids is a potential route to the production of hydrophilic derivatives of *cis*-DHCD. Unfortunately such acids cannot be used in the DCC/DMAP type of esterification reaction because of the potential side reactions. However, by protecting the hydroxyl moiety in the form of a silyl ether it would be possible to eliminate unwanted side reactions. An additional complication is that the carboxylate moiety will be silylated in addition to the hydroxyl moiety leading to bisilylated compounds. Since silyl esters are less stable than silyl ethers, it is possible to react the silyl ester whilst leaving the silyl ether intact. This is illustrated by the production of acid chlorides with silyl ether groupings by the reaction of the bisilylated hydroxy acid with oxalyl chloride and DMF,<sup>115</sup> see scheme 3.12. Acid chlorides of this type should be easily reacted with *cis*-DHCD to afford derivatives that will be hydrophilic upon the removal of the protecting groups.



Scheme 3.12: Production of Silyl Protected Acid Chlorides from Hydroxy Acids.

#### 3.5.4.1 Synthesis of *tert*-Butyldimethylsilyl *tert*-Butyldimethylsiloxyacetate.

The compound *tert*-butyldimethylsilyl *tert*-butyldimethylsiloxyacetate was produced by the reaction of glycolic acid with *tert*-butyldimethylsilyl chloride in dichloromethane. The reaction took place at -78°C and produced a yield of 70%.

The silylation of glycolic acid was initially attempted by using trimethylsilyl chloride, but the trimethylsilyl ether protecting group proved to be far too unstable. Even when using TBDMS protecting groups it was found that washing procedures led to substantial losses of product. Consequently washing procedures were abandoned and the purity of samples was determined by nmr.

#### 3.5.4.2 Synthesis of *tert*-Butyldimethylsilyl Acetyl Chloride.

The compound *tert*-butyldimethylsilyl acetyl chloride was produced by the reaction of the bisilylated glycolic acid derivative with oxalyl chloride. The reaction took place at 0°C in the presence of a catalytic amount of DMF and gave a yield of 32%.

#### 3.5.4.3 Reaction of Silyl Protected Acid Chlorides with DHCD.

The reaction of *tert*-butyldimethylsilyl acetyl chloride with DHCD was attempted by two methods. The first method involved the isolation of *tert*-butyldimethylsilyl acetyl chloride and subsequent reaction with DHCD in the presence of DMAP and triethylamine. The reaction mixture was left stirring overnight, during which time a small amount of white crystals were produced. These were filtered off and IR analysis indicated that they were amine hydrochloride. The residue from the reaction was analysed by N.M.R. and this indicated an absence of any DHCD but the presence of aromatics.

The second method involved the production of *tert*-butyldimethylsilyl acetyl chloride in situ and addition of DHCD and triethylamine to the reaction mixture. On addition of DHCD the solution turned from pale yellow to dark orange. Unfortunately, examination

of the nmr of the residue of this reaction indicates the presence of a large quantity of *tert*-butyldimethyl silanol and a small amount of aromatic.

### 3.6 ETHER SYNTHESIS.

There are a number of methods of synthesising ethers, but the best method remains the Williamson synthesis<sup>116</sup> which involves the S<sub>N</sub>2 displacement of a primary alkyl halide by an alkoxide ion. The alkoxide may be derived from either primary and secondary alcohols but rigorous conditions are required.

An improved Williamson ether synthesis<sup>117</sup> makes use of phase transfer catalysis.<sup>118</sup> The optimum PTC conditions consists of a two phase system with at least a 5 times excess of 50% aqueous sodium hydroxide over alcohol, an excess of alkyl chloride and 3-5mol% of a tetrabutylammonium salt as catalyst. The reaction mixture is stirred at temperatures between 25 and 70°C.

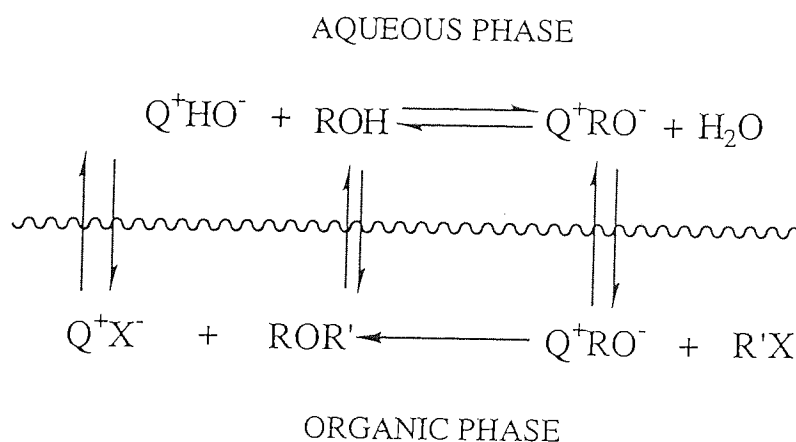


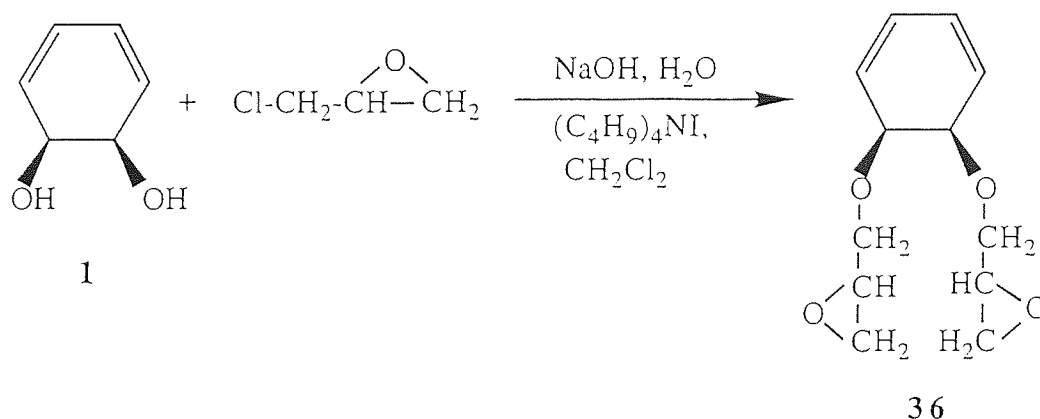
Figure 3.8: Phase Transfer Catalysis.

#### 3.6.1 Reaction of DHCD with Epichlorohydrin.

The technique of phase transfer catalysis (PTC) was investigated as a potential method of etherifying DHCD, and consequently the reaction of DHCD with epichlorohydrin under PTC conditions was studied. This involved an organic phase consisting of DHCD and epichlorohydrin dissolved in dichloromethane and an aqueous phase containing



sodium hydroxide and tetra-butylammonium iodide. The two phase system was continuously stirred at room temperature for 5 days before a reaction residue was recovered from the organic phase. The residue was analysed by N.M.R. but no evidence of DHCD was found, instead the spectra showed a large quantity of phenol.



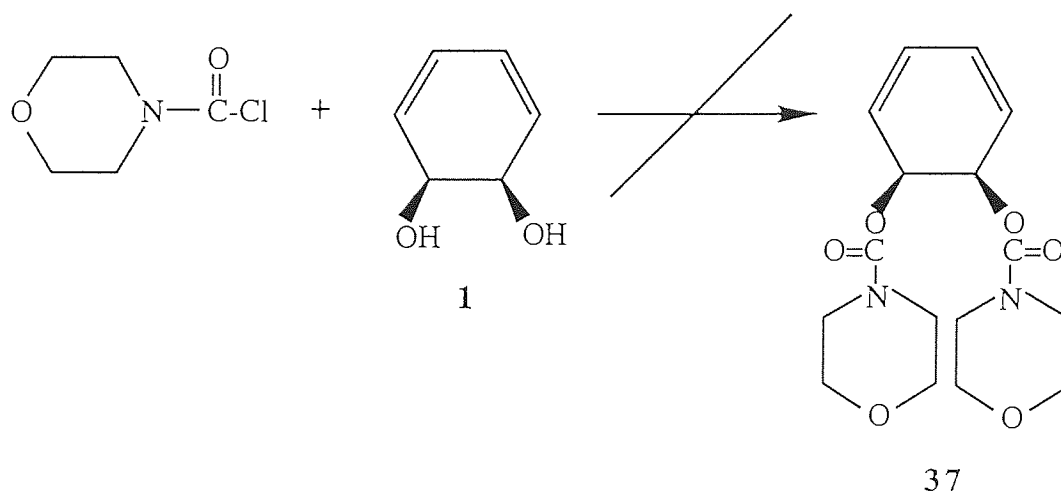
Scheme 3.13: Synthesis of *cis*-1,2-Bis(2,3-epoxypropoxy)-3,5-cyclohexadiene.

### 3.7. MORPHOLINE DERIVATISATION.

The morpholine group is a very interesting entity, since it is a heterocycle that contains both oxygen and nitrogen atoms. As a result it is hydrophilic in nature, and the possibility of producing derivatives of DHCD containing such a group is highly appealing.

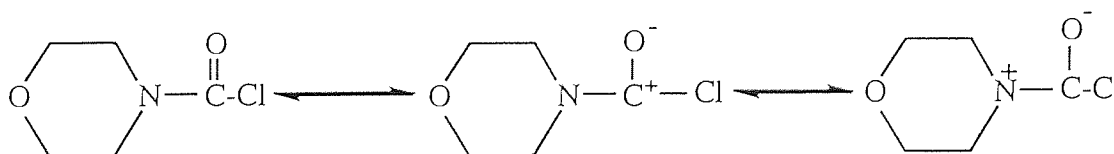
#### 3.7.1. Synthesis of *cis*-1,2-Bis(4-morpholinecarboxy)-3,5-cyclohexadiene.

An attempt to synthesise the compound *cis*-1,2-bis(4-morpholinecarboxy)-3,5-cyclohexadiene was made by reacting 4-morpholine carbonyl chloride with *cis*-DHCD in the presence DMAP and triethylamine at room temperature. Unfortunately, the only product obtained from the reaction was the anhydride of 4-morpholine carbonyl chloride.



**Scheme 3.14: Synthesis of *cis*-1,2-Bis(4-morpholinecarboxy)-3,5-cyclohexadiene.**

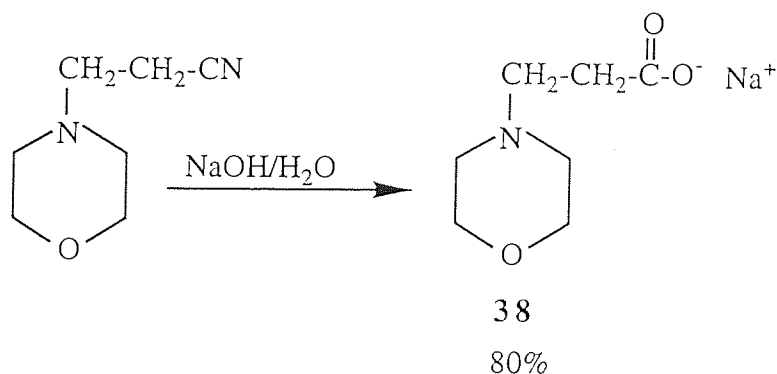
The failure of this reaction can be accounted for by the fact that 4-morpholine carbonyl chloride is far less reactive than many acid chlorides and this inertness can be attributed to the resonance structures that it forms, see figure 3.9. The delocalisation of a partial positive charge on the carbonyl carbon makes it far less electrophilic than other systems for which delocalisation is not possible. As a result it is far less susceptible to nucleophilic attack. An increase in the reaction temperature would make it more reactive but this would also have the effect of causing aromatisation of DHCD. Morpholine acid chlorides that contain a  $\text{CH}_2$  spacer group between the carbonyl and nitrogen would be expected to be more reactive since resonance structures will not be produced.



**Figure 3.9: Resonance Structures of 4-Morpholine Carbonyl Chloride.**

### 3.7.2 Synthesis of 4-Morpholine Propionic Acid.

The preparation of 4-morpholine propionic acid was attempted by hydrolysis of morpholine propionitrile with sodium hydroxide. It was envisaged that this carboxylic acid could be successfully reacted with DHCD using the DMAP/DCC esterification method. The reaction led to an 80% yield of the sodium salt, but unfortunately attempts to convert the salt into the carboxylic acid by acidification led to the production of the amine hydrochloride salt. Aqueous solutions of the sodium salt were neutralised to various pH values before being extracted with ether, but no carboxylic acid was obtained from the organic extracts.



**Scheme 3.15: Synthesis of Sodium Salt of 4-Morpholine Propionic Acid.**

It appears that a pH dependent equilibria analogous to that observed for amino acids has been set up. At high pH values the sodium salt dominates and at low pH values the amine hydrochloride dominates. For intermediate pH values there will either be a mixture of the two forms or a zwitterion form.

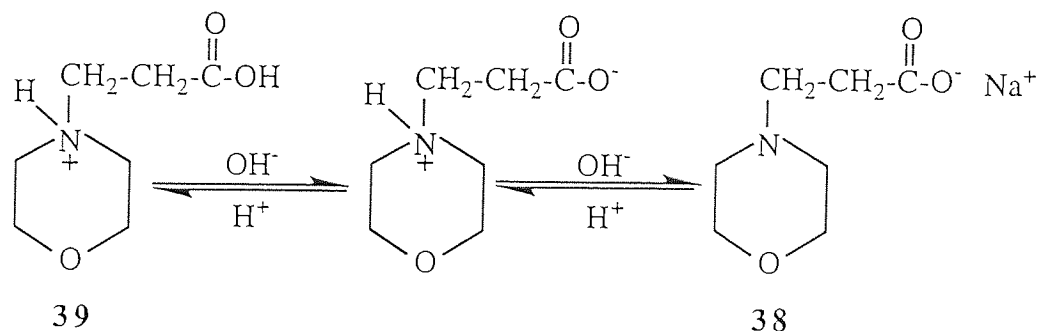


Figure 3.10: pH Dependence of Morpholine Derivatives.

### 3.7.3 Synthesis of 4-Morpholine Propionyl Chloride.

As a result of the problems encountered in trying to isolate 4-morpholine propionic acid, an attempt was made to produce 4-morpholine propionyl chloride from the sodium salt. This acid chloride would be expected to be more reactive than the previously used 4-morpholine carbonyl chloride since the resonance structures that stabilise this molecule are not possible with 4-morpholine propionyl chloride.

Two methods of preparation were examined using oxalyl chloride<sup>119</sup> and phosphorus oxychloride<sup>116</sup>:

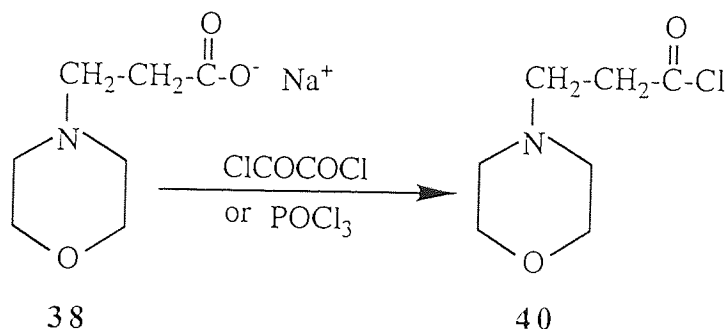
#### 1. Use of Phosphorus Oxychloride.

The slow addition of the sodium salt to a solution of phosphorus oxychloride in dichloromethane led to an exothermic reaction. It was thought that the reaction would yield a product that was soluble in the organic solvent. However, examination of the residue from the organic phase using infra-red indicated the presence of only phosphorus oxychloride.

#### 2. Use of Oxalyl Chloride.

The slow addition of the sodium salt to a solution of oxalyl chloride in dichloromethane led to an exothermic reaction. The reaction mixture was cooled in an ice bath but

examination of the IR spectra of the organic residue indicated that the amine hydrochloride had been produced.



Scheme 3.16: Synthesis of 4-Morpholine Propionyl Chloride.

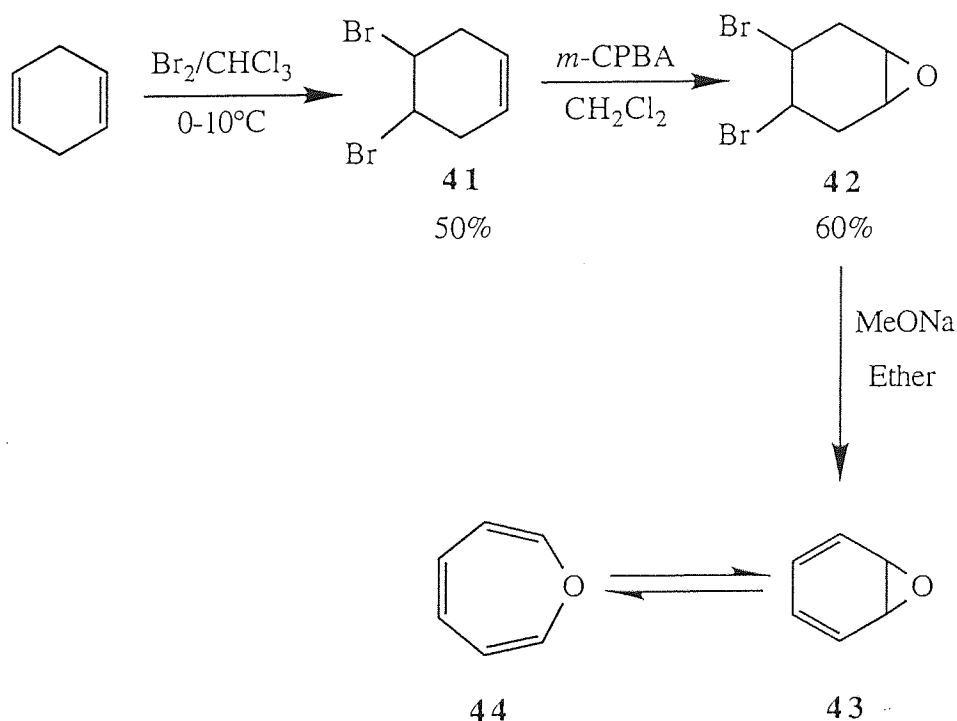
### 3.8 SYNTHESIS OF BENZENE OXIDE-OXEPIN.

The compound benzene oxide exists as a valence tautomer with oxepin and is an intermediate in the chemical synthesis of *trans*-DHCD from 1,4-cyclohexadiene.<sup>120-122</sup> However, the diene nature of benzene oxide marks it out as a possible alternative method of introducing cyclic hydrophilic molecules into hydrogel polymer backbones. The epoxide ring can be opened after polymerisation to yield hydroxyl groups.

The synthesis of benzene oxide-oxepin was attempted using a modification of the procedure described by Vogel,<sup>120</sup> see scheme 3.17. The three step synthesis initially involves the reaction of 1,4-cyclohexadiene with bromine in chloroform. The reaction takes place below 10°C and an excess of methanol is added at the end of the reaction to precipitate out any 1,2,4,5-tetrabromocyclohexane. The reaction produces 4,5-dibromocyclohex-1-ene in a yield of 50%.

The second step of the synthesis involves the epoxidation of 4,5-dibromocyclohex-1-ene with *m*-chloroperoxybenzoic acid to give a 60% yield of 4,5-dibromo-1,2-epoxycyclohexane.

The final step of the procedure involves dehydrohalogenation and is accomplished by reacting 4,5-dibromo-1,2-epoxycyclohexane with sodium methoxide in boiling ether. However, the residue recovered from the reaction did not show any evidence of either benzene oxide or oxepin. Spectral evidence indicates that the residue consists of 4,5-dibromo-1,2-epoxycyclohexane and phenol. Since oxepin readily isomerises to phenol in the presence of heat or acid it can be concluded that even if it had been possible to isolate the benzene oxide-oxepin tautomers, any attempt to copolymerise them with HEMA would have failed.



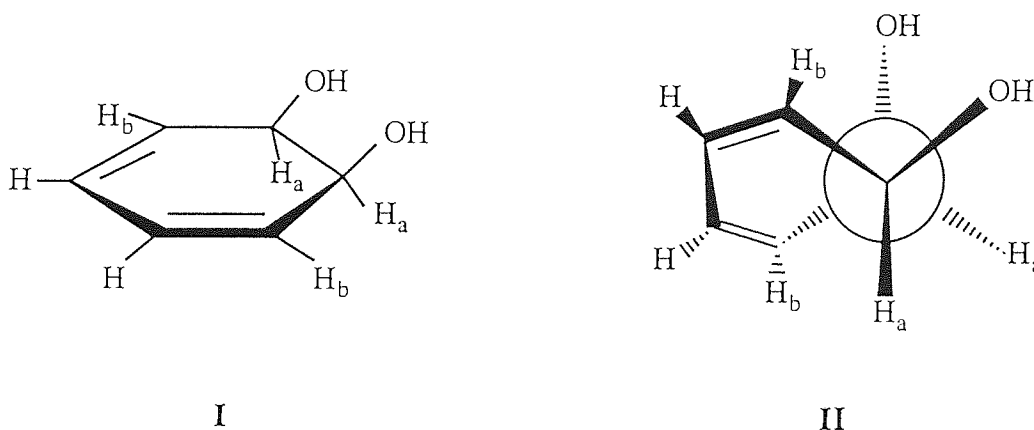
**Scheme 3.17: Synthesis of Benzene Oxide-Oxepin.**

### **3.9 CONFORMATION OF *cis*-DHCD AND IT'S DERIVATIVES.**

The two limiting conformations of *cis*-DHCD<sup>123</sup> are shown in Figure 3.11. In conformation I, there is ring angle strain since the bond angles of the  $\text{sp}^3$  carbons are distorted from their ideal value. The two hydroxyl groups in this conformation are eclipsed, resulting in steric strain and the partial negative charges on the oxygen atoms are closest together leading to a maximum Coulombic interaction (repulsion). However

this conformation is stabilised by the delocalisation of electrons around the 1,3-diene group. The dihedral angle between hydrogens **a** and **b** will be  $60^\circ$  in this conformation.

In conformation **II**, the ring is puckered to overcome both bond angle and steric strain. The bond angles of the  $sp^3$  carbons are closer to ideality and the two hydroxyl groups are gauche to one another. There is also a minimum Coulombic interaction since the partial negative charges on the oxygen atoms are furthest apart. However, this is all at the expense of the delocalisation of electrons within the 1,3-diene group which is disrupted by the puckering of the ring. The dihedral angle between hydrogens **a** and **b** will be  $90^\circ$  in this conformation.



**Figure 3.11: Limiting Conformations of *cis*-DHCD.**

The conformation of the six membered ring that is present in *cis*-DHCD and its derivatives can be determined from  $^1\text{H}$  N.M.R. spectra. By measurement of the vicinal coupling constant between hydrogens **a** and **b**, it is possible by using the Karplus equation to estimate the dihedral angle.<sup>124</sup> This in turn gives an indication of the conformation adopted by the ring.

The Karplus equation is defined as follows:

$$J_{ab} = J^0 \cos^2\Theta - 0.28$$

$J_{ab}$  = observed vicinal coupling constant between hydrogens **a** and **b**

$J^0 = 8.5$  the standard value for a dihedral angle ( $\Theta$ );  $0^\circ < \Theta < 90^\circ$

$\Theta$  = the dihedral angle between hydrogens **a** and **b**

Work previously performed at Aston by St.Pourcain<sup>123</sup> examined the dihedral angles of *cis*- and *trans*-DHCD and their diacetate and dimethylcarbonate derivatives. He found that the *cis* derivatives he examined all had dihedral angles of approximately  $60^\circ$  and so concluded that conformation **I** was being adopted by the ring.

The vicinal coupling constant  $J_{ab}$  and the dihedral angle ( $\Theta$ ) of a number of derivatives of *cis*-DHCD were calculated from the appropriate  $^1\text{H}$  N.M.R. spectra and the data is presented in table 3.1.

**Table 3.1: Conformational Data for Derivatives of *cis*-DHCD.**

	Multiplicity	$J_{ab}$ / Hz	Dihedral Angle
<i>cis</i> -DHCD-EU	Triplet	1.16	$66^\circ$
<i>cis</i> -DHCD-UA	Doublet	1.20	$65^\circ$
<i>cis</i> -DHCD-TMS	Triplet	0.92	$68^\circ$
<i>cis</i> -DHCD-TBDMS	Doublet	1.65	$62^\circ$
<i>cis</i> -DHCD-EB	Triplet	2.06	$58^\circ$
<i>cis</i> -DHCD-VA	Triplet	1.24	$65^\circ$

All nmr samples were run in  $\text{CDCl}_3$ .



From the above table it can be seen that all derivatives of *cis*-DHCD exhibit a dihedral angle approaching 60° which infers that they adopt conformation **I**. It appears that the preservation of the delocalisation of electrons in the conjugated diene is the overriding factor in determining conformation, outweighing the effects of steric and bond angle strain as well as the Coulombic force of repulsion. Even with bulky groups such as tert-butyldimethylsilyl attached, the ring still appears to prefer the planar conformation.

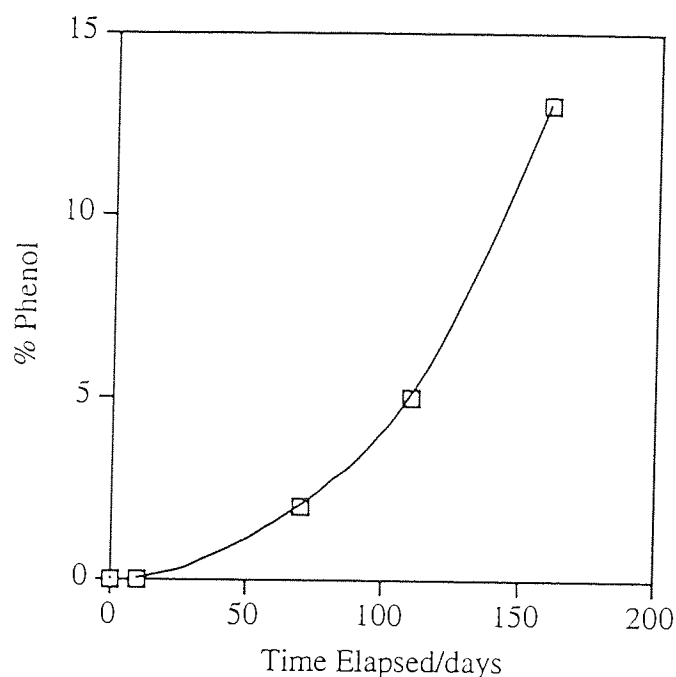
These results match up with those obtained by St. Pourcain,<sup>123</sup> who found that the diacetate and dimethylcarbonate derivatives of DHCD both exhibited dihedral angles close to 60°.

### **3.10 DECOMPOSITION OF *cis*-DHCD.**

It is well known that *cis*-DHCD is unstable to both heat and mineral acid,<sup>20,21</sup> resulting in its aromatisation to form phenol. Samples of *cis*-DHCD are usually obtained as 20% wt/vol solutions in ethyl acetate, and to minimise aromatisation samples are stored in a fridge.

An investigation was undertaken to examine the rate of decomposition of a sample of *cis*-DHCD stored at room temperature. The aromatisation was followed using <sup>1</sup>H N.M.R. spectroscopy by calculating the ratio of aromatic protons to DHCD protons.

The study showed that only after two months were detectable quantities of phenol produced. However, once a small amount of phenol was present in the sample, the rate of aromatisation increased dramatically. Figure 3.12 shows that after 50 days the sample of DHCD contains less than 2% phenol, yet after 150 days the phenol content has risen to nearly 15%.



**Figure 3.12: Changes in the Phenol Content of a Sample of DHCD with Time.**

### **3.11 CONCLUSIONS.**

The synthesis of DHCD derivatives remains a daunting prospect, it's instability in the presence of acids and heat dramatically limits the types of reactions that can be used.

The DCC/DMAP type of esterification method proved to be successful for the synthesis of a number of DHCD derivatives from unsaturated and epoxy acids. Those derivatives produced from epoxy acids will yield hydroxyl groups when the epoxide ring is opened and can thus be classed as hydrophilic. However, despite the success of this type of esterification reaction, the types of functional groups that can be incorporated into derivatives are still limited. Indeed the majority of DHCD derivatives that can be produced by this method will be hydrophobic in nature.

Attempts to protect the vulnerable hydroxyl groups of DHCD by silylation also proved successful and both trimethylsilyl and t-butyldimethylsilyl derivatives were isolated. These derivatives are of great interest since they are more stable than DHCD itself, and consequently can be copolymerised with HEMA without the problems of aromatisation

and phenol production. After polymerisation the silyl protection can be easily removed to regenerate the hydroxyl groups.

The possibility of attaching a morpholine group to DHCD was highly attractive due to the hydrophilic nature of the group. However, despite a variety of attempts attachment proved to be elusive. The inertness of 4-morpholine carbonyl chloride was attributed to resonance stabilisation and attempts to produce a more highly reactive morpholine acid chloride from 4-morpholine propionitrile led to the production of either the sodium or amine hydrochloride salt. The relative ease with which ionic morpholine species were produced meant that it was not possible to isolate a non-ionic morpholine carboxylic acid or acid chloride.

A study of the decomposition of DHCD at room temperature showed that it took two months for detectable amounts of phenol to form. However, once phenol was present in the sample the aromatisation process accelerated.

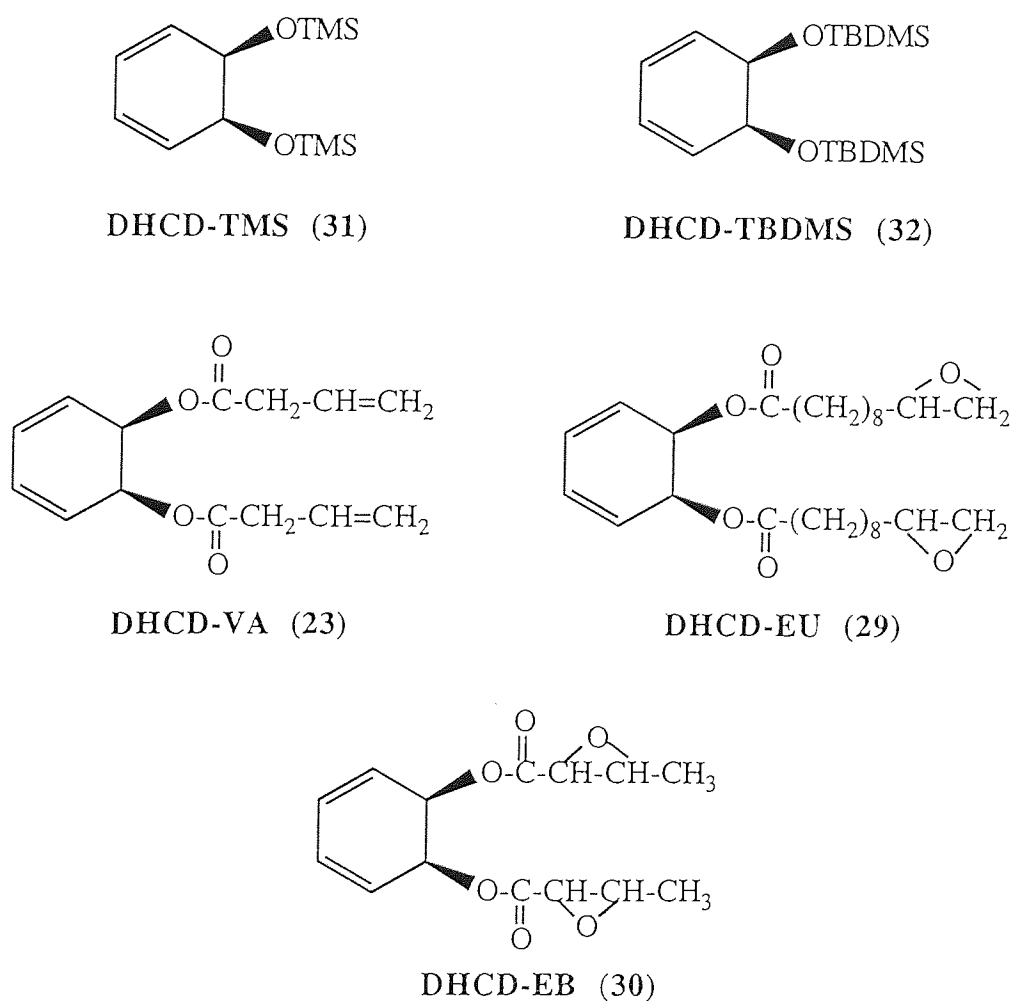
The conformational analysis performed on all of the synthesised derivatives of *cis*-DHCD indicated that the cyclohexadiene ring adopts a planar rather than puckered conformation. These results were in agreement with similar work performed by St.Pourcain on the diacetate and dimethylcarbonate derivatives of *cis*-DHCD. It can be concluded from the results that the retention of conjugation in the ring is the overriding factor that dictates conformation. The planar conformation exhibits both ring angle and steric strain in addition to a maximum Coulombic repulsion between the adjacent oxygen atoms but, these factors appear to be outweighed by the benefits of conjugation

**CHAPTER 4:**  
**Hydrogel Synthesis.**

## 4. HYDROGEL SYNTHESIS.

### 4.1 INTRODUCTION.

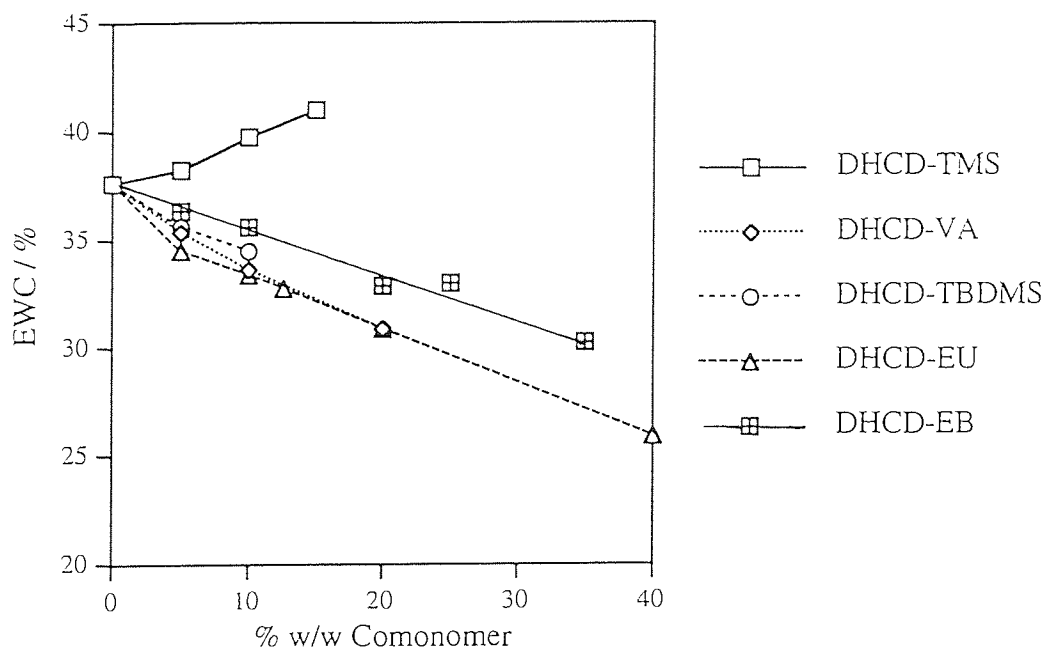
A number of new hydrogel polymers were synthesised by the free radical copolymerisation of HEMA with a variety of novel cyclic monomers (CM). The EWC, tensile strength, Youngs modulus and elongation to break of these gels were all measured and the effect of the cyclic monomer concentration on these properties was discussed. Differential Scanning Calorimetry (DSC) was also performed on a number of samples in order to calculate the quantity of freezing water in the gels. The data obtained is presented in graphical form in this chapter and tabular form in appendices 1-5.



**Figure 4.1: Cyclic Monomers.**

#### 4.2 EQUILIBRIUM WATER CONTENT.

The effect of CM concentration on the EWC was studied and the results are presented in figure 4.2. The results obtained were compared with the EWC of polyHEMA.



**Figure 4.2 : Effect of Comonomer Concentration on EWC.**

All of the CM exhibit similar decreases in EWC with increasing CM concentration except for DHCD-TMS which shows an increase in EWC with increasing comonomer concentration. The measured value of EWC for polyHEMA was 37.6%, and this is consistent with the literature values.<sup>14,18</sup> The decreases in EWC are to be expected since the CM are on the whole hydrophobic in nature. However, the decreases are not as dramatic as those observed with similar concentrations of methyl methacrylate or styrene comonomer.<sup>14,125</sup>

The optical clarity of each membrane after hydration was observed. It was found that membranes incorporating DHCD-EB were transparent at 5%, 10% and 20% w/w CM concentration, although at 25% and 35% concentrations the membranes became opaque. Membranes incorporating DHCD-EU and DHCD-TBDMS were found to be

opaque at all CM concentrations, and membranes incorporating DHCD-VA and DHCD-TMS were found to be transparent at concentrations of 5 and 10% CM but opaque at higher CM concentrations.

#### 4.3 TENSILE STRENGTH.

The effect of CM concentration on the tensile strength of polyHEMA based hydrogels was studied and the results are presented in figure 4.3. The tensile strength of polyHEMA is also recorded on the graph at 0% w/w comonomer.

The computing package used to produce the graphs of tensile strength, Young's modulus and elongation to break against % w/w comonomer uses straight lines to connect points. Such lines do not indicate trends and are used simply to make the data exhibited in the graphs easier to view.

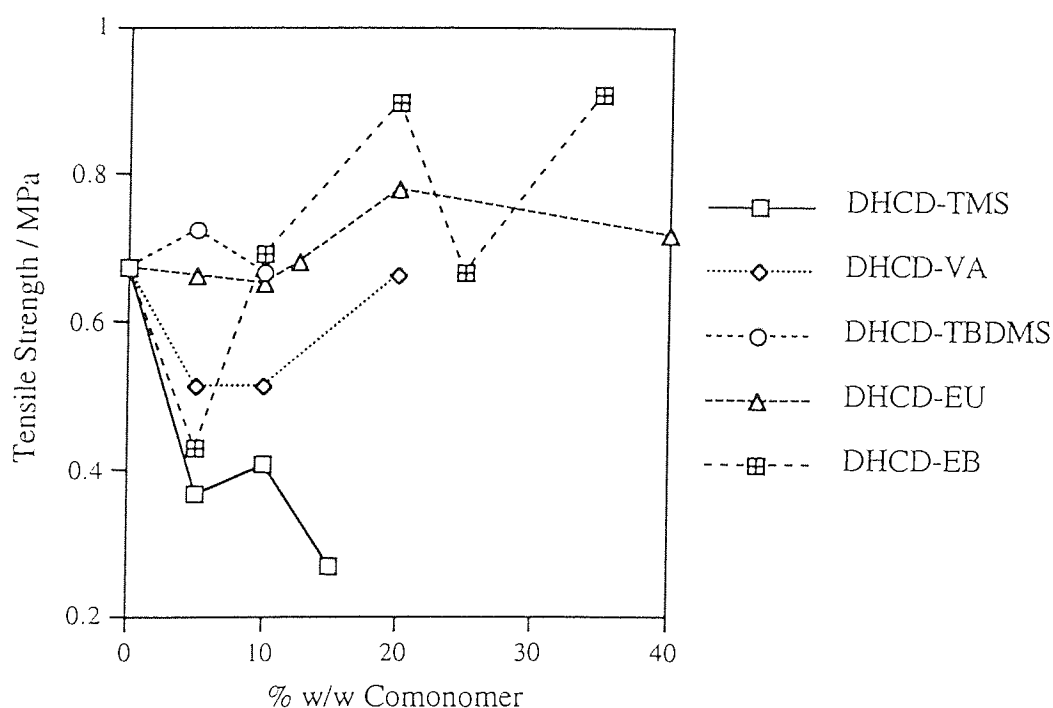
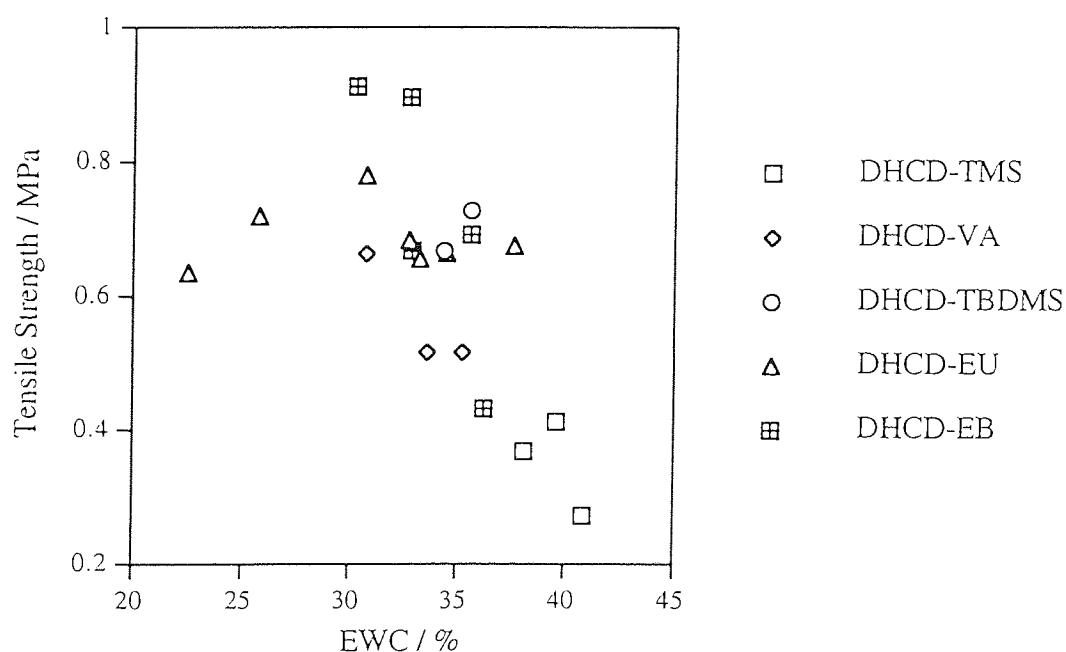


Figure 4.3 : Effect of Comonomer Concentration on Tensile Strength.

All derivatives with the exception of DHCD-EU show erratic changes in tensile strength with increasing CM concentration. The tensile strength of DHCD-EU remains fairly constant with increasing CM concentration but the tensile strength of DHCD-TMS gels falls off dramatically with increasing CM concentration. The tensile strength of polyHEMA was measured as 0.674 MPa and this is consistent with literature values.<sup>14,18</sup> Several derivatives exhibit reduced tensile strength compared to polyHEMA at low CM concentrations but improved tensile strength at higher CM concentrations.



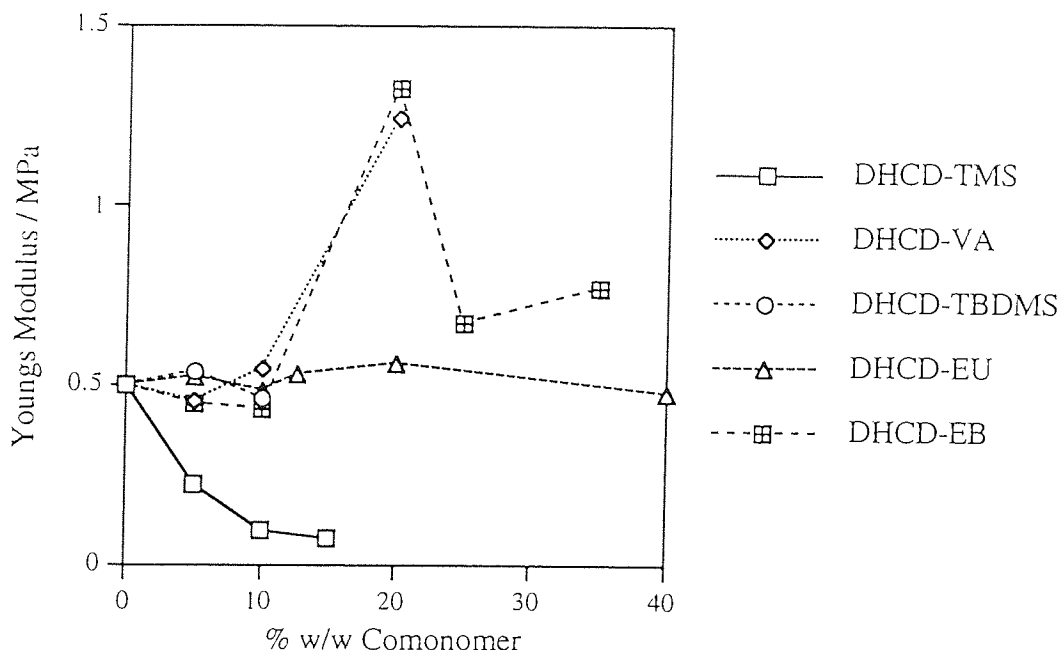
**Figure 4.4: Effect of EWC on Tensile Strength.**

The scatter plot in figure 4.4 shows the effect of EWC on tensile strength, and it follows the general trend that higher tensile strength corresponds to lower EWC.



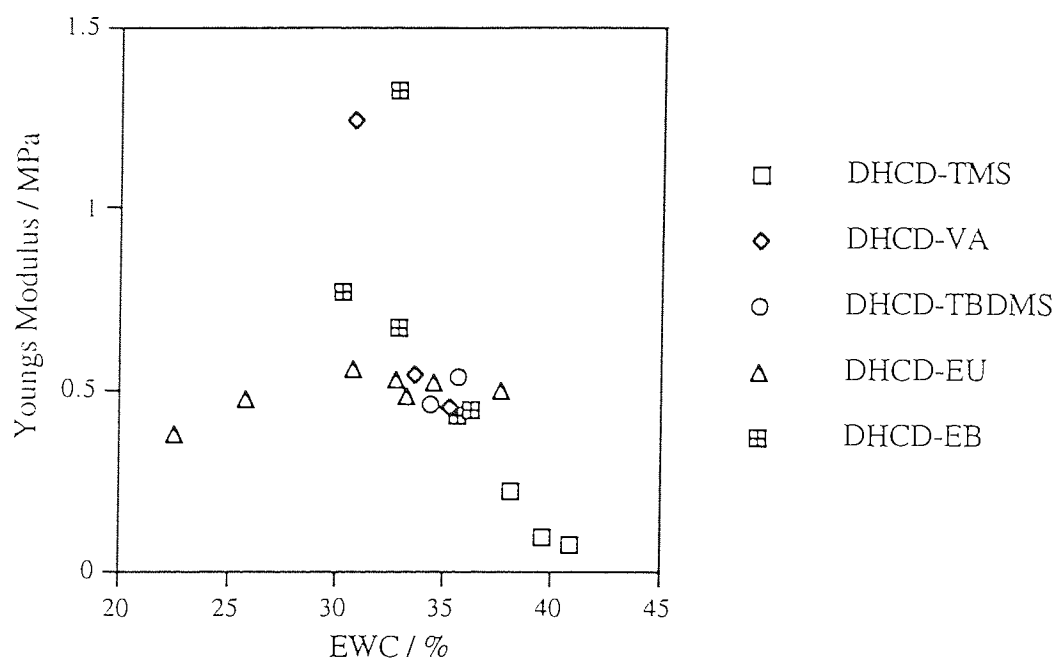
#### 4.4 YOUNG'S MODULUS.

The effect of CM concentration on the Young's modulus was studied and the results are presented in figure 4.5. The Young's modulus of a sample of polyHEMA is also included on the graph at 0% w/w comonomer.



**Figure 4.5 : Effect of Comonomer Concentration on Young's Modulus.**

Samples of DHCD-TBDMS and DHCD-EU show very little variation in modulus with changing comonomer concentration. The Young's modulus of DHCD-TMS decreases with CM concentration but both DHCD-VA and DHCD-EB show substantial increases at 20% w/w concentration. However, the modulus for DHCD-EB does drop off at higher concentrations. This may be the result of a degree of phase separation since gels with a DHCD-EB concentration of greater than 20% were found to be opaque. The value of Young's modulus measured for polyHEMA was 0.496MPa and this is consistent with quoted literature values<sup>14,18</sup>.

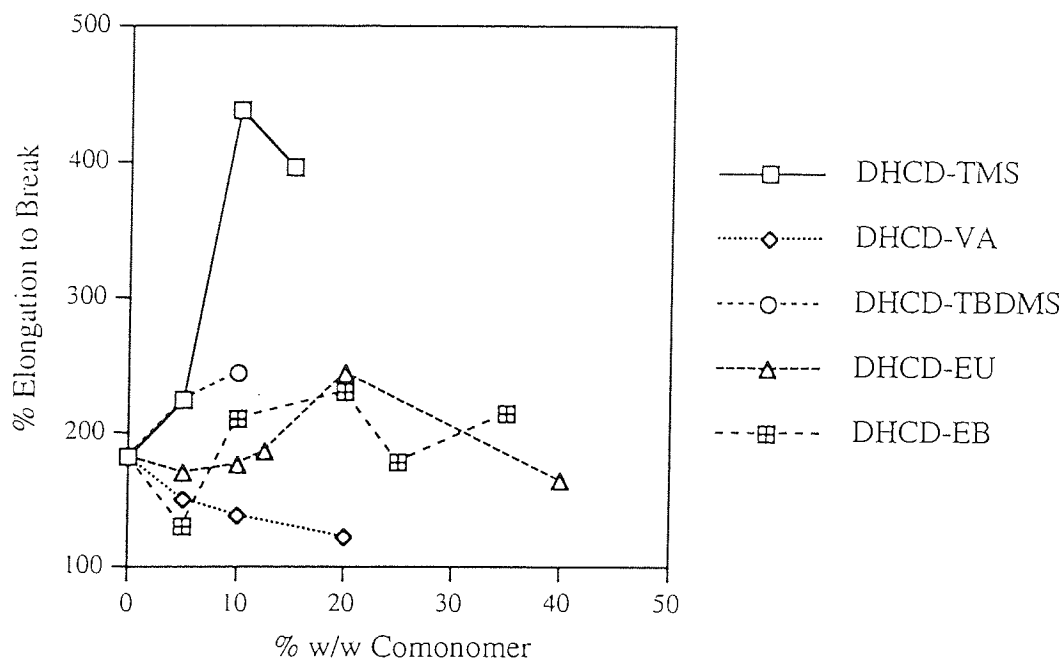


**Figure 4.6 : Effect of EWC on Young's Modulus.**

The scatter plot in figure 4.6 shows the effect of EWC on Young's Modulus. With the exception of DHCD-EU membranes, the plot generally follows the expected trend of higher modulus corresponding to lower EWC.

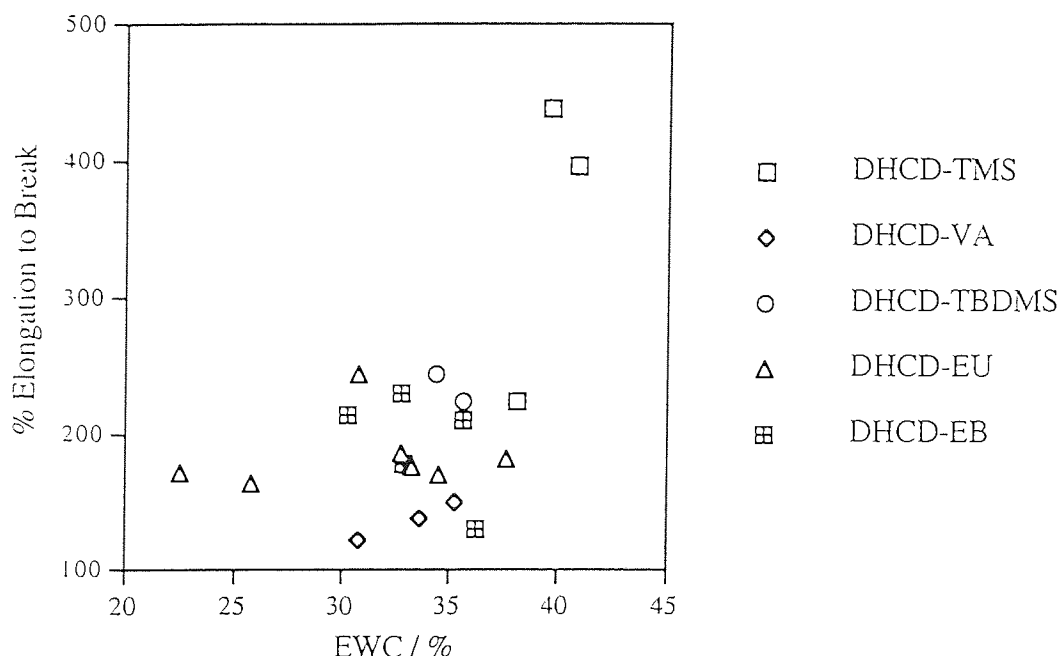
#### 4.5 ELONGATION TO BREAK.

The effect of CM concentration on the elongation to break was studied and the results are presented in figure 4.7. A value of elongation to break for polyHEMA is also recorded on the graph at 0% w/w comonomer.



**Figure 4.7: Effect of Comonomer Concentration on % Elongation to Break.**

The graph in figure 4.7 shows the effect of comonomer concentration on the % elongation to break. It can immediately be observed that the incorporation of DHCD-TMS leads to a dramatic increase in elongation, indeed at 10% w/w DHCD-TMS the elongation is over twice that of polyHEMA. This is indicative of a good network structure, implying that the CM has been successfully incorporated into the polymer matrix. The other comonomers show a somewhat erratic behaviour but all appear to exhibit elongation comparable to polyHEMA, although the elongation of DHCD-VA samples do exhibit a steady decline in moving to higher CM concentration.



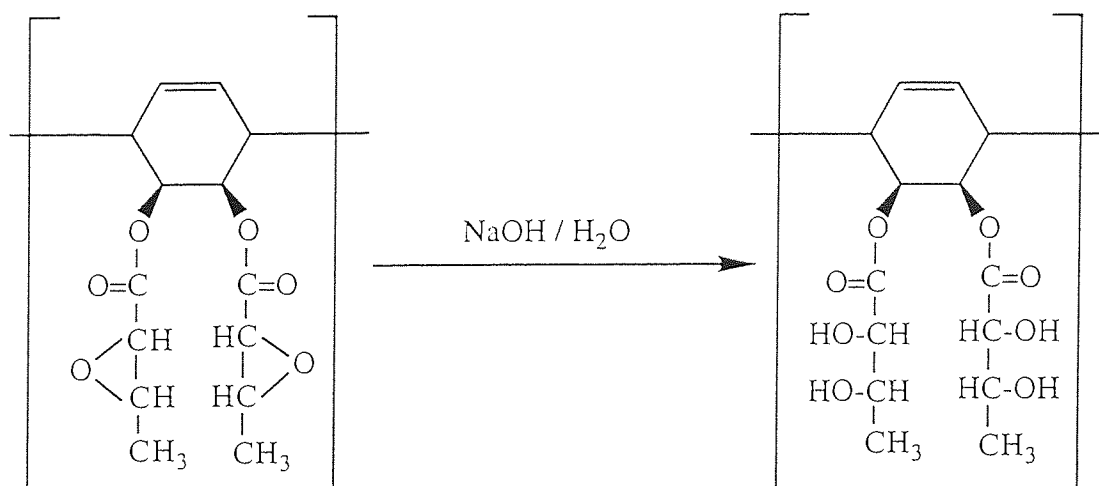
**Figure 4.8: Effect of EWC on % Elongation to Break.**

The scatter plot in figure 4.8 shows the effect of EWC on % elongation to break. There does not appear to be any direct relationship between EWC and elongation to break, and with the exception of DHCD-TMS containing hydrogels all elongations are within the range 100-250%. The high elongation exhibited by hydrogels containing DHCD-TMS indicates that these membranes are highly elastic.

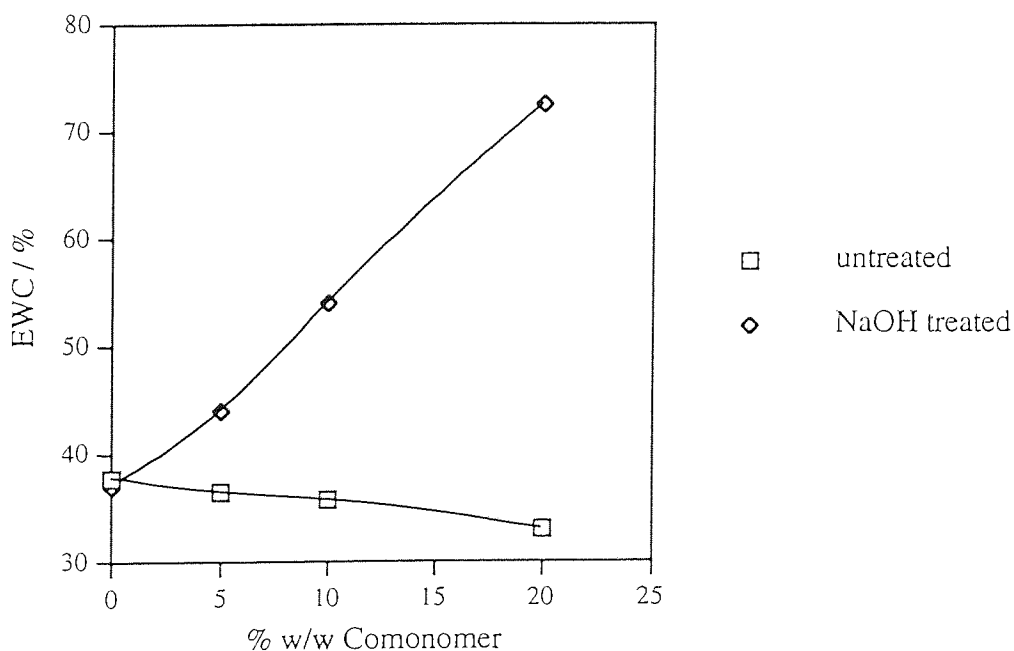
#### **4.6 NaOH TREATMENT.**

Several samples of DHCD-EB hydrogel membranes were soaked in 20% w/v aqueous sodium hydroxide overnight, in an attempt to open the epoxide rings contained within the comonomer units. The opening of the epoxide ring was expected to yield hydroxyl groups, and as a consequence the EWC of the sample would be expected to increase. After being treated with sodium hydroxide, samples were placed in distilled water to equilibrate for two weeks, with the water being changed daily. The effect on EWC of soaking polyHEMA membrane samples in sodium hydroxide solution was also

investigated. Equilibrium water contents of both treated and untreated membrane samples are shown in figure 4.10.



**Figure 4.9: Epoxide Ring Opening Facilitated by Sodium Hydroxide.**



**Figure 4.10: Effect of Epoxide Ring Opening on EWC.**

The treatment of hydrogel samples with sodium hydroxide solution leads to substantial increases in the EWC, and this is particularly evident in the case of 20% w/w of

DHCD-EB where the EWC of the treated gel is over 70%. If the epoxide rings of the comonomer are indeed being opened during soaking, then the largest EWC would be expected for the hydrogel with the largest proportion of comonomer. The results obtained fit this hypothesis.

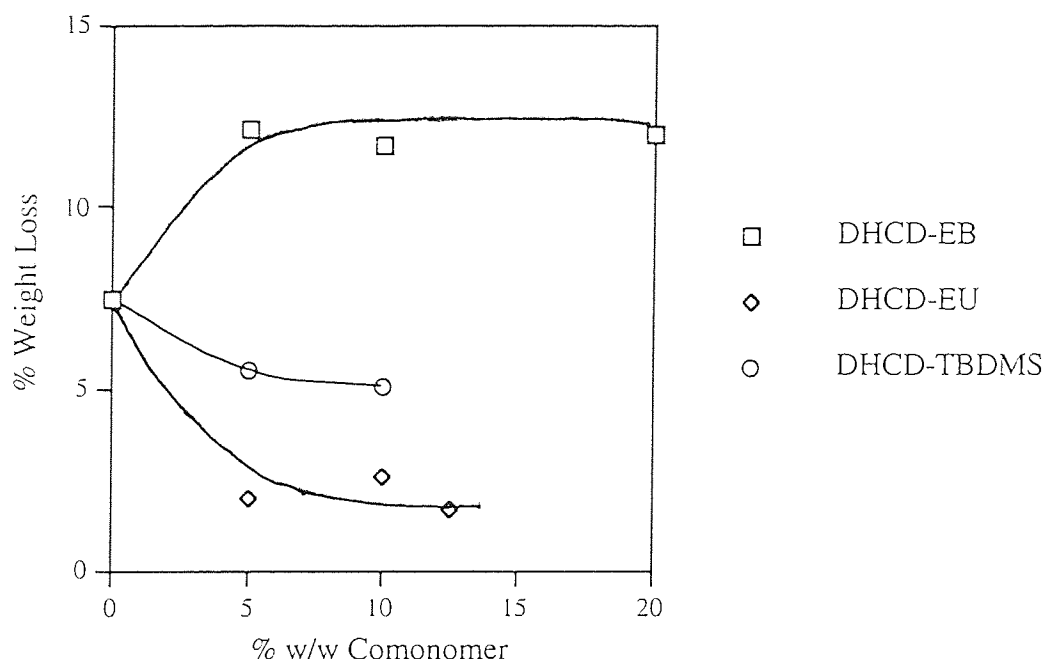
The increase in EWC observed as a consequence of epoxide ring opening would be expected to approximately double as the comonomer concentration doubles. In moving from 5-10% comonomer the increase in EWC doubles from 7.5% to 18.5% and again in moving from 10-20% comonomer the increase in EWC doubles from 18.5% to 39.5%. This observation is further evidence that the epoxide rings have been opened.

A sample of polyHEMA that was treated with sodium hydroxide showed no change in EWC, and this tends to suggest that the increases observed for other samples can be attributed to epoxide ring opening rather than the production of voids in the polymer network.

#### **4.7 WEIGHT LOSS BY EXTRACTION.**

During the soaking of hydrogels in water, low molecular weight material is extracted from the membrane. These extracts consist of unreacted monomer, short chain polymer and impurities. The amount of material extracted may be calculated by measuring the weight of a gel sample taken straight from the mould and the weight of the same sample after two weeks equilibrating in distilled water and subsequent dehydration. The percentage weight loss from the gel is given by the following expression:

$$\% \text{ Weight Loss} = \frac{\text{Weight of Sample from Mould} - \text{Weight of Dehydrated Sample}}{\text{Weight of Sample from Mould}} \times 100\%$$



**Figure 4.11: Effect of Comonomer Concentration on Weight Loss Through Extraction of Low Molecular Weight Material During Soaking.**

The graph in figure 4.11 shows the effect of CM concentration on the extraction of low molecular weight material during soaking. It is observed that for membranes containing DHCD-EB there is a greater weight loss than polyHEMA, although the weight loss does remain constant with increasing DHCD-EB concentration. For membranes containing either DHCD-EU or DHCD-TBDMS there is a lower weight loss than is observed for polyHEMA although, again the weight loss does not vary greatly with increasing CM concentration.

The weight loss from the gel gives an indication of the reactivity of the comonomers; the lower the weight loss the greater the reactivity of the comonomer and vice versa. These results indicate that DHCD-EU or DHCD-TBDMS are more highly reactive comonomers than DHCD-EB.

#### 4.8 DIFFERENTIAL SCANNING CALORIMETRY (DSC).

The water imbibed in hydrogel membranes exists in a continuum of states between two extremes. At one extreme there is the so-called bound or non-freezing water, which is water that is hydrogen bonded to hydrophilic sites on the polymer backbone and at the other we have bulk or freezing water which has greater mobility and is unaffected by the polymer environment. Between these two states there is interfacial water which exhibits properties associated with both states.

Although there are a number of methods of calculating freezing water contents,<sup>29-34</sup> DSC is the most convenient because of ease of sample preparation and speed with which measurements on samples can be made enabling rapid elucidation of water binding data.

DSC measures the gross phase changes of water within a polymer. When a hydrogel sample is cooled to  $-70^{\circ}\text{C}$ , the free and interfacial water freezes but the bound water remains in a non-frozen state as a consequence of its strong association with the polymer chain. DSC measures the freezing water content of the hydrogel, but if the EWC is known then the non-freezing water content is simply the difference.

The effect of comonomer concentration and sodium hydroxide treatment on freezing and non-freezing water contents was studied using DSC.

Sample DSC thermograms for polyHEMA and polyHEMA containing 10% w/w DHCD-EB are displayed in figures 4.12 and 4.13.



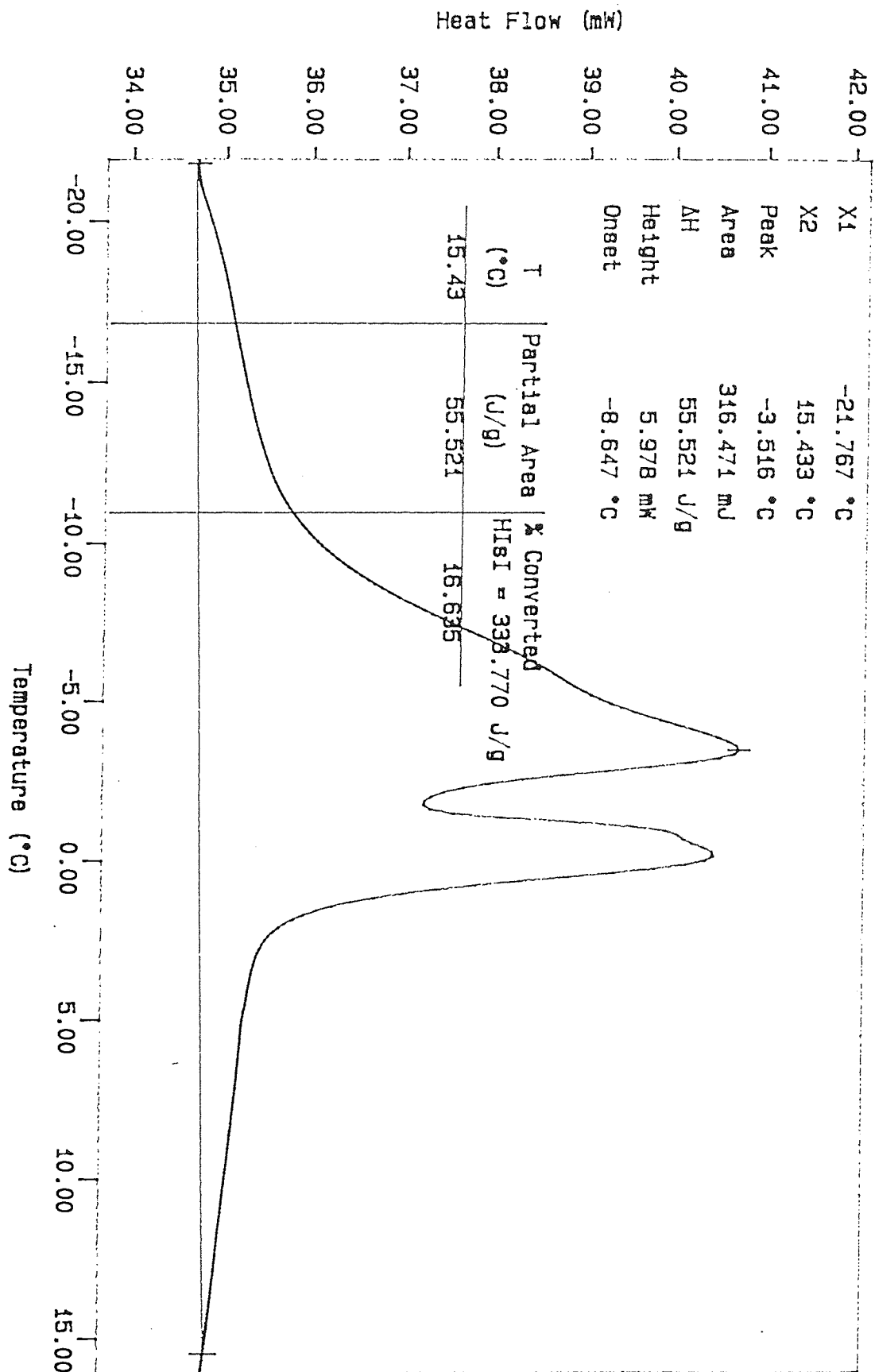


Figure 4.12: Differential Scanning Calorimetry Thermogram of PolyHEMA.

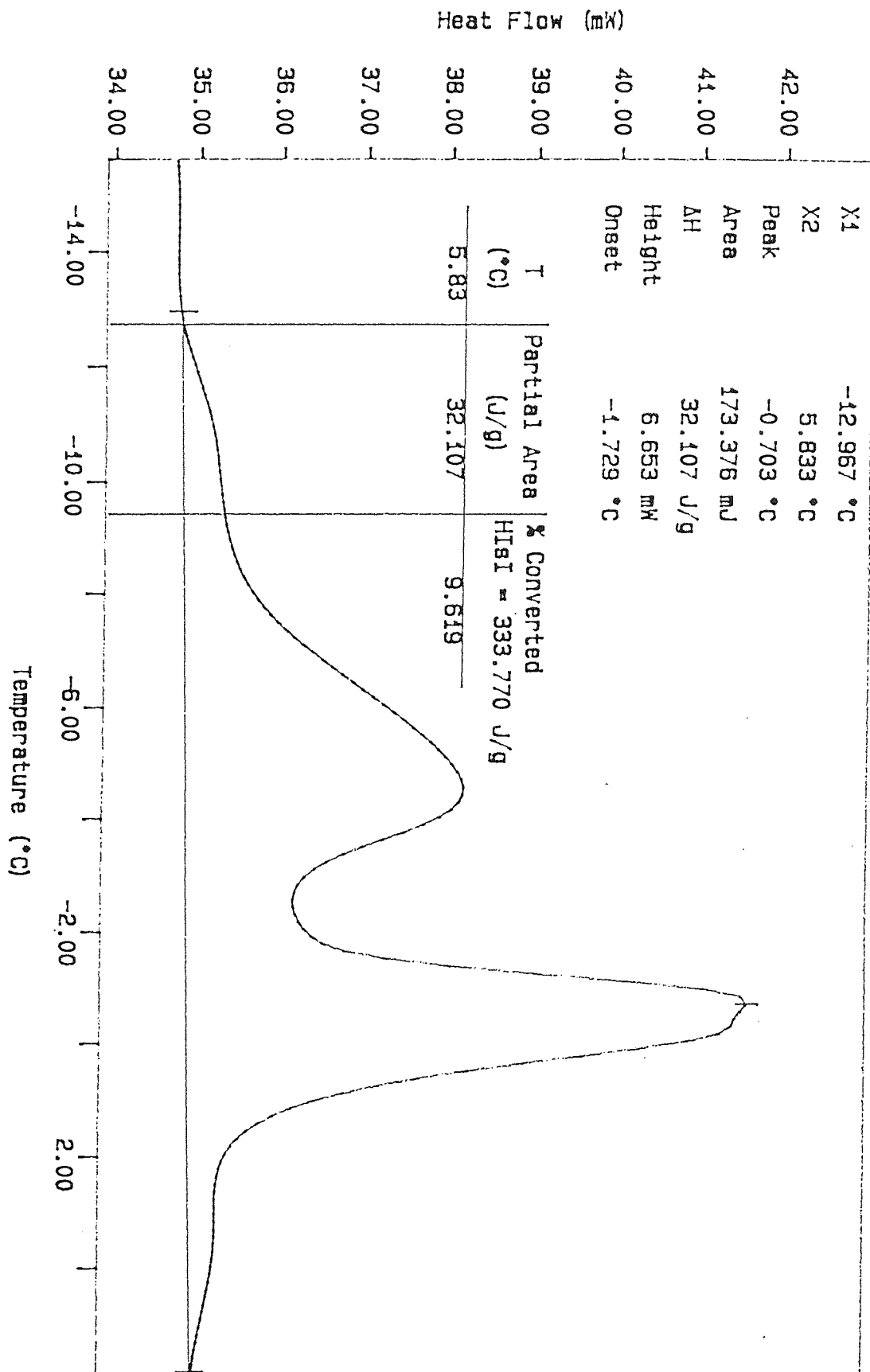
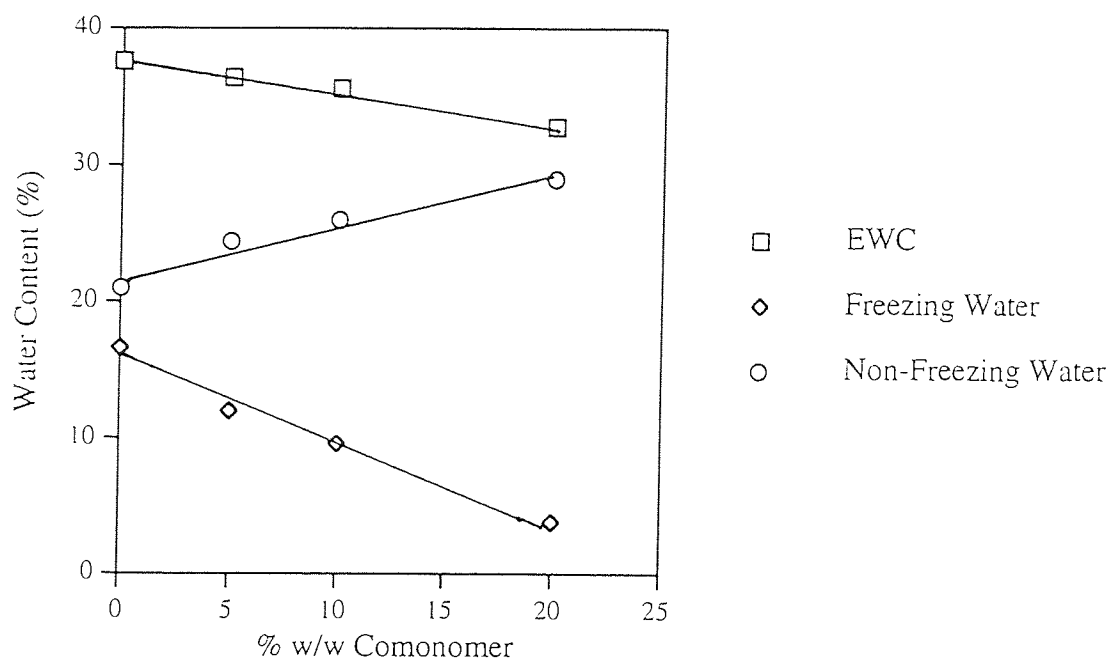
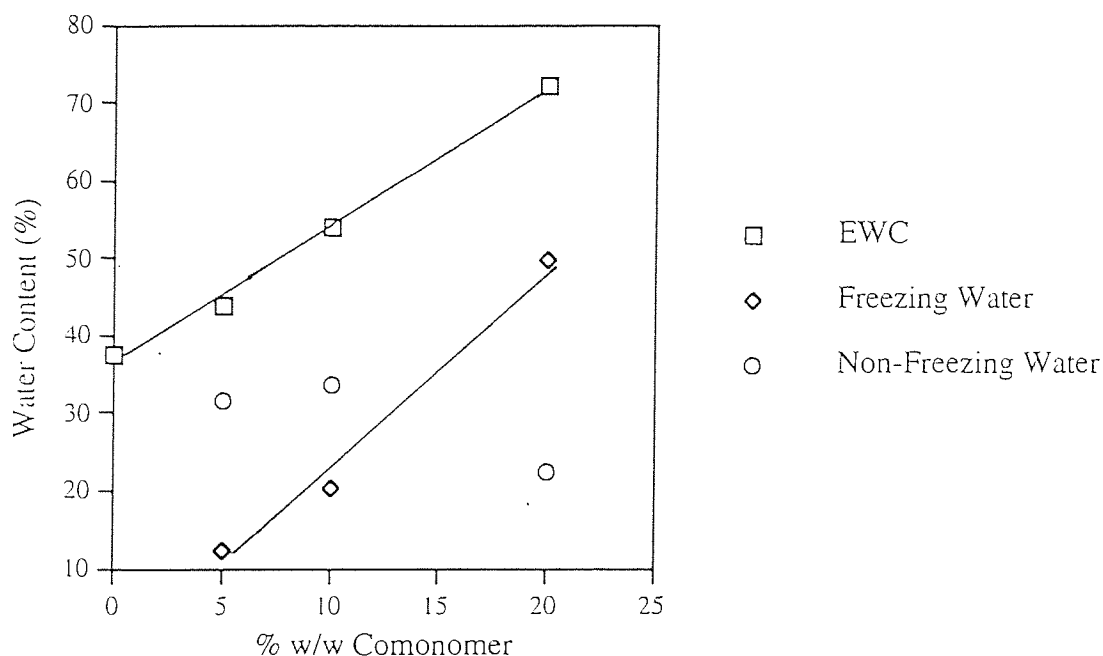


Figure 4.13: Differential Scanning Calorimetry Thermogram of PolyHEMA Hydrogel Containing 10% w/w DHCD-EB.



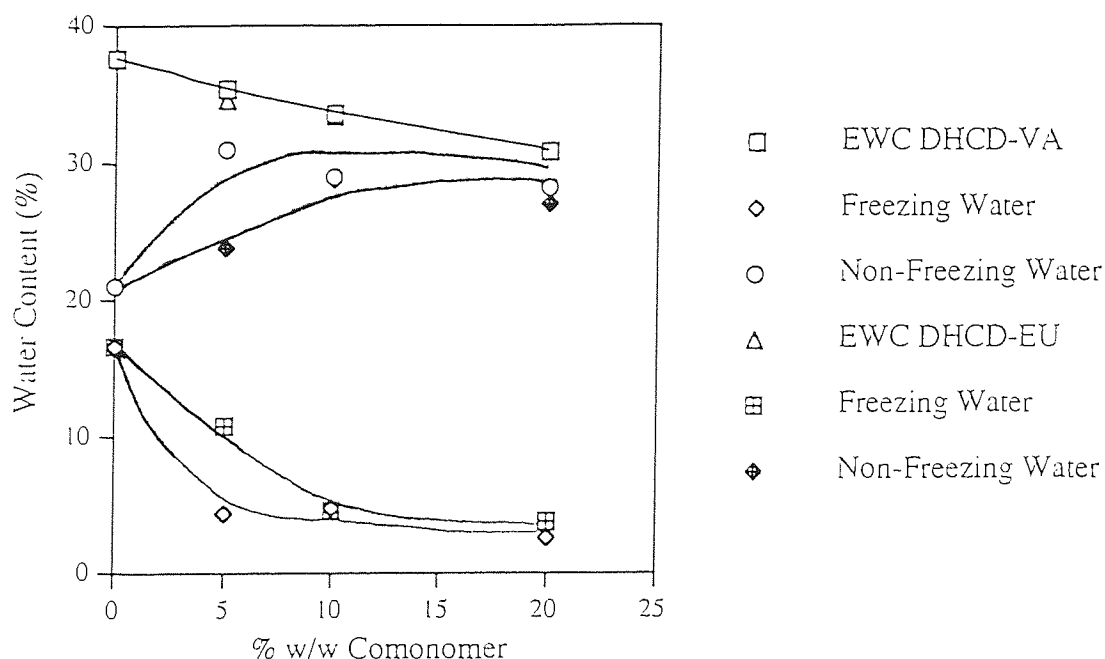
**Figure 4.14: Effect of DHCD-EB Concentration on Freezing and Non-Freezing Water Contents.**

The graph in figure 4.14 indicates that although the EWC reduces with increasing CM concentration, the non-freezing water content actually increases. At the same time the freezing water content also reduces. These observations imply that water binding to the epoxide groups of the comonomer. Consequently there will be more hydrogen bonded water molecules and an increase in the non-freezing water content.



**Figure 4.15: Effect of Comonomer Concentration on Freezing and Non-Freezing Water Contents of NaOH Treated PolyHEMA Hydrogels Containing DHCD-EB.**

The graph in figure 4.15 shows the effect of comonomer concentration on the water contents of sodium hydroxide treated hydrogels. It can be seen that EWC increases with increasing comonomer concentration and a similar trend is observed with the freezing water content. The non-freezing water content remains constant at low comonomer concentrations but at higher concentrations there is a visible drop off.



**Figure 4.16: Effect of Comonomer Concentration on the Water Content of PolyHEMA Hydrogels Containing DHCD-VA and DHCD-EU.**

The graph in figure 4.16 shows the effect of CM concentration on the EWC, freezing water content and non-freezing water content of polyHEMA hydrogels containing DHCD-VA and DHCD-EU. Both sets of hydrogels follow similar trends in water contents; that is a slight fall in EWC, a large increase in non-freezing water content and a large decrease in freezing water content. Since the non-freezing water contents of the copolymers are higher than the corresponding values for polyHEMA, it can be postulated that a larger quantity of water is being hydrogen bonded to the hydrophilic sites within the polymer.

The DSC melting endotherm of polyHEMA contains two peaks; one at approximately 0°C which represents the bulk water in the hydrogel and a second peak below 0°C which represents the interfacial water within the hydrogel. The incorporation of increasing amounts of DHCD-VA and DHCD-EU into the gel has the effect of substantially reducing the size of the lower temperature peak with respect to the higher

temperature peak. The inference from these results is that it is the interfacial water that is being primarily reduced by the introduction of CM.

The fall in EWC that is observed for both CMs is the result of introducing a largely hydrophobic comonomer into the polymer.

#### **4.9 CONCLUSIONS.**

Five derivatives of DHCD have been successfully incorporated into hydrogel membranes. The incorporation of these derivatives has generally led to a small decline in EWC. The tensile strengths and Young's moduli of these membranes are not too dissimilar to the values obtained for polyHEMA, although significant improvements are observed with 20% w/w DHCD-EB.

Weight loss experiments have indicated that the DHCD-EU and DHCD-TBDMS derivatives exhibit greater reactivity during copolymerisation with HEMA than the DHCD-EB derivative.

DSC analysis of the hydrogels indicates that the incorporation of derivatives causes an increase in the bound water content and a decrease in the freezing water content. The DSC thermogram indicates that the drop in freezing water content is primarily due to loss of interfacial water in the gels. These observations imply that the CM is acting as a spacer group in the polymer backbone, allowing more efficient packing around the hydrophilic groups in the polymer and an increase in bound water. As a result of more efficient packing there are likely to be less hydrophobic interactions and consequently the quantity of interfacial water in the membrane will fall.

An attempt to open the epoxide rings in hydrogels containing DHCD-EB by soaking the membranes in sodium hydroxide solution appears to have been successful. Substantial increases in EWC were observed, particularly at higher CM concentrations,

but this results in poor mechanical properties and samples were too fragile to test. The soaking of a sample of polyHEMA membrane in sodium hydroxide solution had no effect on the EWC, and this seems to confirm that the observed increases in EWC are as a result epoxide ring opening rather than voids being produced in the polymer network.

## **CHAPTER 5:**

### **Conclusions.**



## 5. CONCLUSIONS.

The original objective of this project was to synthesise hydrophilic derivatives of *cis*-1,2-dihydroxy-3,5-cyclohexadiene. Potential synthesis routes were severely limited by the inability of DHCD to withstand either heat or mineral acid without undergoing aromatisation. Enzyme catalysis was initially studied as a potential method of producing derivatives under mild conditions. Chemical methods of synthesis were also investigated.

The enzyme catalysed transesterification of cyclohexanol was successfully accomplished, and so the procedure was used with DHCD. A range of epoxy esters containing trichloroethoxy and trifluoroethoxy leaving groups were produced in high yields, and a series of attempts were made to react them with DHCD in the presence of lipase porcine pancreas catalyst. Unfortunately, despite varying reaction conditions no products were recovered but each residue contained substantial quantities of phenol, suggesting that the enzyme is catalysing the aromatisation process. A blank experiment consisting of *cis*-DHCD, ether and porcine pancreas lipase was performed and the results indicated that the enzyme does indeed expedite aromatisation. As a consequence enzyme work was abandoned and attention was focused on chemical synthesis routes.

A number of ester derivatives of *cis*-DHCD were successfully produced from unsaturated and epoxy acids by using the standard DCC/DMAP esterification procedure. This synthesis route has been shown to be an efficient method of producing esters under mild conditions. However, the range of derivatives that can be produced is limited by the sensitivity of the procedure to functional groups and the side reactions that can result from the presence of such groups.

The protection of the vulnerable hydroxyl groups of *cis*-DHCD as silyl ethers also proved fortuitous and both the trimethylsilyl and *tert*-butyldimethylsilyl derivatives have been isolated.

The hydrophilic nature of the morpholine group makes it highly attractive to produce derivatives containing such a group. Unfortunately, despite a variety of attempts, attachment proved to be elusive. The lack of reactivity observed with 4-morpholine carbonyl chloride was attributed to resonance stabilisation and attempts to produce a more highly reactive morpholine acid chloride only led to the production of either the sodium or amine hydrochloride salt. The pH sensitivity of these salts meant that neutral morpholine species could not be extracted.

A study of the rate of decomposition of *cis*-DHCD at room temperature revealed that detectable quantities of phenol did not appear in the sample for two months. However, once phenol is present in the sample the aromatisation process was accelerated.

Conformational analysis carried out on all of the synthesised derivatives of *cis*-DHCD indicated that the cyclohexadiene ring adopts a planar rather than a puckered conformation. The conformations were determined by calculating the dihedral angle from the Karplus equation, using the vicinal coupling constant, *J*, obtained from the <sup>1</sup>H NMR spectra of derivatives. This analysis is in agreement with similar work carried out by St.Pourcain on the diacetate and dimethylcarbonate derivatives of *cis*-DHCD. It would appear that retention of conjugation in the ring is the overriding factor that determines conformation. The stability associated with conjugation outweighs the forces of repulsion and strain that also accompany this type of conformation.

Five derivatives of DHCD were successfully copolymerised with HEMA to produce a new range of hydrogels. However, these hydrogels did not exhibit the anticipated combination of good mechanical properties and good water binding ability.

The EWC of these materials are seen to generally decline with increasing CM concentration. This is to be expected since the CM are mostly hydrophobic in nature and hydrophobic monomers tend to reduce EWC. The tensile strength and Young's moduli of these hydrogels are not too dissimilar to the values for polyHEMA. It was expected that the incorporation of such derivatives would cause an improvement in mechanical properties and the failure to observe this may be the result of phenolic impurities in the CM.

Weight loss experiments have shown that the DHCD-EU and DHCD-TBDMS derivatives are more reactive during the copolymerisation with HEMA than the DHCD-EB derivative.

DSC was used to determine the ratio of freezing:non-freezing water in each hydrogel membrane. It shows that for all derivatives the incorporation of CM into the hydrogel leads to an increase in bound water and a decrease in freezing water. These observations infer that water is being bound to the epoxide groups of the CM since I am decreasing the amount of HEMA present in the gel and still observing an increase in non-freezing water content. The DSC thermogram of polyHEMA shows two peaks: one below 0°C that represents interfacial water and one at 0°C that represents bulk water within the hydrogel. The thermograms of hydrogels containing CM show either substantial reduction or disappearance of the interfacial water peak. This indicates that reductions in freezing water are primarily due to loss of interfacial water.

The soaking of hydrogel membranes containing DHCD-EB in aqueous sodium hydroxide solution has effectively opened the epoxide rings contained in this monomer. Ring opening leads to an increase in the number of hydroxyl groups in the polymer and this results in large increases in EWC. The increase in EWC is most pronounced with 20% w/w CM, where I observed a doubling of the EWC from 33% to 73% as a consequence of the ring opening. Unfortunately, as a consequence of the high EWC, the membranes exhibit poor mechanical properties and indeed the membranes were too fragile to perform mechanical testing.

**CHAPTER 6:**  
**Materials and Methods.**

## 6. MATERIALS AND METHODS.

### 6.1 REAGENTS.

All reagents were used as supplied unless otherwise stated. Ether and tetrahydrofuran were both dried over sodium wire. Triethylamine was dried over potassium hydroxide and dichloromethane was distilled over calcium hydride prior to use. 2,2,2-trichloroethanol and 2,2,2-trifluoroethanol were both distilled prior to use.

Recrystallised *m*-chloroperoxybenzoic acid was obtained by dissolving the 50-60% pure compound supplied by Aldrich in dichloromethane to form a 10% w/v solution. The solution was then washed with 1.5 times the volume of phosphate buffer, pH 7.2, in three equal portions and dried over magnesium sulfate. Solvent removal yielded pure *m*-chloroperoxybenzoic acid

Table 6.1: Materials Used for Synthesis.

COMPOUND	R.M.M.	SUPPLIER
Acetic anhydride	102.10	Aldrich
Ammonium sulfate	132.14	Aldrich
11-Aminoundecanoic acid	201.30	Aldrich
Argon	39.95	BOC
Azobisisobutyronitrile	136.20	Fluka
Bis(trimethylsilyl)acetamide	203.43	Aldrich
Bromine	159.82	Aldrich
<i>tert</i> -Butyldimethylsilyl chloride	150.73	Fluorochem
Calcium hydride	42.10	Aldrich
Catechol	110.10	Aldrich

Cetylpyridinium chloride monohydrate	358.01	Aldrich
Chloroform	119.38	Fisons
<i>m</i> -Chloroperoxybenzoic acid	172.57	Sigma
Citric acid	192.12	Aldrich
Crotonic acid	86.10	Aldrich
1,4-Cyclohexadiene	80.00	Aldrich
Cyclohexane	84.16	Fisons
Cyclohexanol	100.16	Aldrich
Dichloromethane	84.93	Fisons
1,3-Dicyclohexylcarbodiimide	206.33	Aldrich
Diethyl ether (Ether)	74.12	Fisons
<i>cis</i> -1,2-Dihydroxycyclohexa-3,5-diene (20% w/v solution in ethyl acetate)	112	ICI
4-Dimethylaminopyridine	122.17	Aldrich
Dimethylformamide	73.10	Fisons
Epichlorohydrin	92.53	Aldrich
Ethyl acetate	88.11	Fisons
Ethylene glycol dimethacrylate	198.22	BDH
Glycolic acid	76.05	Aldrich
Hexane	86.18	Fisons
<i>trans</i> -3-Hexenedioic acid	144.13	Aldrich
Hydrochloric acid	36.46	Fisons
Hydrogen Peroxide 27.5% in water	34.02	Aldrich
2-Hydroxyethyl methacrylate	130.14	Vista Optics
Magnesium sulfate	120.37	Aldrich
Methanol	32.04	Fisons
4-Morpholinecarbonyl chloride	149.58	Aldrich
4-Morpholine propionitrile	140.19	Aldrich
Nitrogen	28.01	BOC

Oxalyl chloride	126.93	Aldrich
4-Pentenoic acid	100.12	Aldrich
Porcine pancreas lipase	-	Sigma
Potassium hydroxide	56.11	Fisons
Silica Gel (TLC Grade/no binder)	-	Aldrich
Sodium carbonate	105.99	Aldrich
Sodium chloride	58.44	Aldrich
Sodium hydrogen carbonate	84.01	Aldrich
Sodium hydroxide	40.00	Fisons
Sodium methoxide	54.02	Aldrich
Sodium sulfite	126.04	Hopkin+Williams
Sodium tungstate dihydrate	329.86	Aldrich
Succinic anhydride	100.07	Aldrich
Sulfuric acid	98.08	Fisons
Tetrabutylammonium iodide	369.38	Aldrich
Tetrahydrofuran	72.11	Fisons
2,2,2-Trichloroethanol	149.40	Aldrich
2,2,2-Trifluoroethanol	100.04	Vickers
Triethylamine	101.19	Aldrich
Trimethylchlorosilane	108.64	Vickers
12-Tungstophosphoric acid	2941	BDH
Undecylenic acid	184.28	Aldrich
Vinyl acetic acid	86.09	Aldrich



## 6.2 EXPERIMENTAL METHODS.

### 6.2.1 ISOLATION OF *cis*-1,2-DIHYDROXY-3,5-CYCLOHEXADIENE (1).

Into a 250ml conical flask was placed 50ml of 20% w/v solution of *cis*-1,2-dihydroxy-3,5-cyclohexadiene in ethyl acetate. The solution was cooled to below 10°C and 200ml of hexane was added. The resulting mixture was then sealed into the flask and placed into a fridge overnight. The white crystals of DHCD that had formed were decanted from the brown solid at the bottom of the flask, and dried in air. The brown solid was then redissolved in ethyl acetate and the recovery process repeated. The whole procedure was repeated approximately three times to give a total yield of between 30 and 60%. The recovered DHCD was stored in a fridge in sealed sample vials.

I.R. (cm<sup>-1</sup>; KBr):

3283(s;br); 3038(m); 2866(m); 1425(m); 1246(m); 1160(m);  
1083(s); 1024(m); 982(s); 925(m); 819(s); 726(s); 687(m);  
584(m); 504(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

3.66 (s; br; 2H; CH-OH); 4.18 (s; 2H; CH-OH); 5.84-5.95 (m;  
4H; CH=CH)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

67.44 (+ve; CH-OH); 124.54 (+ve; CH=CH-CH); 129.24 (+ve;  
=CH-CH-OH)

C<sub>6</sub>H<sub>8</sub>O<sub>2</sub> (112)

### 6.2.2 SYNTHESIS OF ACTIVATED ESTERS.

#### 2,2,2-trichloroethyl-2-butenolate (**12**).

To a 250ml 3-neck round bottom flask with a nitrogen inlet was added 5.94g (69mmol) of crotonic acid, 13ml (135mmol) of 2,2,2-trichloroethanol and 100ml of freshly distilled dichloromethane. To the stirred mixture was added 14.45g (70mmol) of DCC and 0.2g (1.64mmol) of DMAP dissolved in 75ml of dichloromethane. The reaction mixture was then stirred for 18 hours under a nitrogen atmosphere at ambient temperature, during which time a white precipitate formed. The precipitate was removed by filtration and the filtrate was washed successively with 2x100ml of 5% v/v of aqueous hydrochloric acid, 2x100ml of saturated sodium bicarbonate solution and 2x100ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and the solvents were removed by rotary evaporator to yield a white solid suspended in a pale yellow oil. The oil was distilled under vacuum to yield 6.5g (30mmol) 44% of (**12**). B.P. 45-48°@10mmHg.

I.R. (cm<sup>-1</sup>; neat):

2935(m); 2856(m); 1739(vs); 1659(vs); 1445(m); 1376(m);  
1296(s); 1244(s); 1164(vs); 1105(s); 1036(m); 969(s); 816(s);  
739(s); 721(s)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.77 (dd; 3H; CH<sub>3</sub>-CH=); 4.68 (s; 2H; CH<sub>2</sub>-CCl<sub>3</sub>); 5.81-5.89  
(m; 1H; CH<sub>3</sub>-CH=); 6.98-7.11 (m; 1H; =CH-C=O)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

18.0 (+ve; CH<sub>3</sub>-CH); 73.55 (-ve; CH<sub>2</sub>-CCl<sub>3</sub>); 94.91 (-ve; CCl<sub>3</sub>);  
120.95 (+ve; CH<sub>3</sub>-CH=); 147.39 (+ve; =CH-C=O); 164.35 (-ve;  
C=O)

C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>Cl<sub>3</sub> (217.5)

### 2,2,2-trichloroethyl-3-butenolate (14).

To a 250ml 3-neck round bottom flask was added 8.13g (94mmol) of vinyl acetic acid dissolved in 50ml of freshly distilled dichloromethane. The solution was then treated successively with 9.2ml (96mmol) of 2,2,2-trichloroethanol and 0.4g (3.3mmol) of DMAP. The mixture was then cooled to 0°C with an ice bath for 15 minutes, before the dropwise addition of 19.81g (96mmol) of DCC dissolved in 50ml of dichloromethane. The solution was continuously stirred and after the addition of the DCC was completed, the reaction mixture was allowed to warm up to room temperature and left stirring for 2 days. The white precipitate that had been produced was then removed by filtration and the filtrate was washed successively with 3x25ml of saturated citric acid solution, 2x25ml of saturated sodium bicarbonate solution and 1x25ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and solvents were removed by rotary evaporator to yield 12.8g (59mmol) 63% of a yellow oil (14).

I.R. (cm<sup>-1</sup>; neat):

3086(m); 2987(m); 2956(m); 1758(s); 1658(m); 1645(m);  
1295(m); 1240(m); 1157(s); 1105(m); 1060(m); 1031(m);  
926(m); 813(m); 740(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

3.03-3.23 (m; 2H; CH<sub>2</sub>-C=O); 4.68 (s; 2H; CH<sub>2</sub>-CCl<sub>3</sub>); 5.13-  
5.19 (m; 2H; CH<sub>2</sub>=); 5.81-5.89 (m; 1H; CH=)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

38.44 (-ve; CH<sub>2</sub>-C=O); 73.89 (-ve; CH<sub>2</sub>CCl<sub>3</sub>); 94.79 (-ve;  
CCl<sub>3</sub>); 119.27 (-ve; CH<sub>2</sub>-O); 129.08 (+ve; CH-O); 169.64 (-ve;  
C=O)

C<sub>6</sub>H<sub>7</sub>O<sub>2</sub>Cl<sub>3</sub> (217.5)

**2,2,2-trichloroethyl-3,4-epoxybutanoate (15).**

To a 250ml round bottom flask was added 15.0g (69mmol) of 2,2,2-trichloroethyl-3-butenate (**14**) and 50ml of dichloromethane. 23.6g (137mmol) of *m*-CPBA dissolved in 100ml of dichloromethane was then added dropwise to the stirred mixture. When the addition was completed, the reaction mixture was left stirring for 3 days at room temperature. After this time any excess *m*-CPBA was destroyed by the addition of an excess of saturated sodium sulfite solution. After 5 minutes a negative test was obtained with starch/iodide test paper, and the precipitate of *m*-chlorobenzoic acid was removed by filtration. The filtrate was then washed successively with 2x50ml of saturated sodium sulfite solution, 2x50ml of saturated sodium bicarbonate solution and 2x50ml saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and solvents were removed by rotary evaporator to yield 15g (64mmol) 93% of crude product (**15**). The product was then purified by vacuum distillation (B.P. 65-70°C @ 0.5mmHg; Lit. B.P. 65-70°C @ 0.1mmHg<sup>96</sup>) to give a yield of 65%.

I.R. (cm<sup>-1</sup>; neat):

3058(w); 3005(m); 2958(m); 2929(w); 1757(s); 1658(w);  
1423(m); 1408(m); 1372(m); 1324(m); 1266(m); 1242(m);  
1163(s); 1098(m); 1061(m); 1027(m); 882(m); 856(m); 844(m);  
799(m); 727(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

2.50-2.53 (m; 1H; O-HCH); 2.62-2.65 (m; 2H; -CH<sub>2</sub>-C=O);  
2.76-2.79 (t; 1H; O-HCH); 3.21-3.28 (m; 1H; O-CH); 4.70 (s;  
2H; CH<sub>2</sub>CCl<sub>3</sub>)

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

37.49 (-ve;  $\text{CH}_2\text{-C=O}$ ); 46.30 (-ve;  $\text{CH}_2\text{-O}$ ); 47.37 (+ve;  $\text{CH-O}$ );  
73.79 (-ve;  $\text{CH}_2\text{-CCl}_3$ ); 94.50 (-ve;  $\text{CCl}_3$ ); 168.54 (-ve;  $\text{C=O}$ )

$\text{C}_6\text{H}_7\text{O}_3\text{Cl}_3$  (233.5)

**bis(2,2,2-trichloroethyl)-*trans*-3-hexenedioate (16).**

To a 500ml 3-neck round bottom flask equipped with a magnetic stirrer and a nitrogen inlet was placed 10.0g (69mmol) of *trans*-3-hexenedioic acid, 13.5ml (140mmol) of 2,2,2-trichloroethanol and 100ml of anhydrous dichloromethane. To the resulting suspension was added 28.9g (140mmol) of DCC and 0.2g (1.64mmol) of DMAP dissolved in 75ml of dichloromethane. The mixture was stirred in a nitrogen atmosphere at ambient temperature for 12 hours, during which time a voluminous white precipitate of *N,N'*-dicyclohexylurea was produced. The precipitate was removed by filtration and the filtrate was washed successively with 2x100ml of 5% v/v of aqueous hydrochloric acid, 2x100ml of saturated sodium bicarbonate solution and 2x100ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and the solvent removed by rotary evaporator to give 20.3g (50mmol) 73% of a pale yellow oil (16).

IR ( $\text{cm}^{-1}$ ; neat):

3006(m); 2935(m); 2855(m); 1750(s); 1655(m); 1401(s);  
1313(s); 1246(m); 1210(m); 1171(s); 1055(m); 926(m); 809(s);  
725(s)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

3.24 (m; 4H;  $\text{CH}_2\text{-CH}$ ); 4.72 (s; 4H;  $\text{CH}_2\text{CCl}_3$ ); 5.74-5.79 (m;  
2H;  $\text{CH=CH}$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

37.22 (-ve;  $\text{CH}_2\text{-CH}$ ); 73.99 (-ve;  $\text{CH}_2\text{-CCl}_3$ ); 95.2 (-ve;  $\text{CCl}_3$ );  
169.66 (-ve;  $\text{C=O}$ )

$\text{C}_{10}\text{H}_{10}\text{O}_4\text{Cl}_6$  (407)

**bis(2,2,2-trichloroethyl) ( $\pm$ )-3,4-epoxyadipate (17).**

To a 500ml round bottom flask equipped with a magnetic stirrer and a nitrogen inlet was added 8.5g (20.9mmol) of bis(2,2,2-trichloroethyl) *trans*-3-hexenedioate (**16**) and 50ml of anhydrous dichloromethane. To the resulting solution was added 9.03g (52.3mmol) of recrystallised *m*-CPBA dissolved in 100ml of dichloromethane. The mixture was stirred in a nitrogen atmosphere at ambient temperature for 3 days. Any excess *m*-CPBA was then destroyed by the addition of an excess of saturated aqueous sodium sulfite solution. After 5 minutes a negative test was obtained with starch/iodide test paper. The precipitate of *m*-chlorobenzoic acid was removed by filtration and the filtrate was washed successively with 100ml of saturated sodium sulfite solution, 100ml of saturated sodium bicarbonate solution and 100ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and the dichloromethane was subsequently removed by rotary evaporator to give 4.2g (9.7mmol) 50% of a white solid (**17**). M.P. 50-51°C; Lit. M.P. 55-56°C<sup>93</sup>.

I.R. ( $\text{cm}^{-1}$ ; KBr):

3005(w); 2932(m); 2856(m); 1752(s); 1655(w); 1452(m);  
1401(m); 1312(m); 1246(m); 1231(m); 1170(s); 1055(m);  
925(m); 808(s); 725(s); 569(m)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

2.75 (t; 4H;  $J=5.2\text{Hz}$ ;  $\text{CH}_2\text{-CH}$ ); 3.22(t; 2H;  $J=5.1\text{Hz}$ ;  $\text{CH-O}$ );  
4.75 (s; 4H;  $\text{CH}_2\text{-CCl}_3$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

36.89 (-ve;  $\text{CH}_2\text{-CH}$ ); 53.09 (+ve;  $\text{CH-O}$ ); 74.03 (-ve;  $\text{CH}_2\text{CCl}_3$ ); 95.1 (-ve;  $\text{CCl}_3$ ); 168.33 (-ve;  $\text{C=O}$ )

$\text{C}_{10}\text{H}_{10}\text{O}_5\text{Cl}_6$  (433)

**2,2,2-trichloroethyl-10-undecenoate (18).**

To a 2-neck 500ml round bottom flask was added 17.5g (95mmol) of undecylenic acid, 14.34g (96mmol) of 2,2,2-trichloroethanol, 0.4g (3.3mmol) of DMAP and 100ml of freshly distilled dichloromethane. The resulting mixture was cooled in an ice bath for 15 minutes with constant stirring, after which time 19.81g (96mmol) of DCC dissolved in 75ml dichloromethane was added dropwise. During the addition a white precipitate formed. The mixture was then allowed to warm up to room temperature and left stirring for 3 days. The white precipitate was removed by filtration and the resulting filtrate was washed successively with 3x50ml saturated citric acid solution, 2x50ml saturated sodium bicarbonate solution and 1x50ml saturated sodium chloride solution. The organic layer was then dried over magnesium sulfate and solvents were removed by rotary evaporator leaving a 22.5g (71mmol) 75% yield of a yellow oil (18).

I.R. ( $\text{cm}^{-1}$ ; neat):

3077(m); 2976(m); 2926(vs); 2856(s); 1757(vs); 1641(m);  
1455(m); 1417(m); 1379(m); 1270(m); 1223(m); 1147(s);  
1118(s); 1061(m); 911(s); 805(s); 721(s)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

1.05-1.44 (s; br; 10H;  $(\text{CH}_2)_5$ ); 1.61 (qt; 2H;  $J=7.27\text{Hz}$ ;  $\text{CH}_2\text{CH}_2\text{CH}_2$ ); 1.95 (q; 2H;  $J=6.89\text{Hz}$ ;  $\text{CH}_2\text{CH}_2\text{CH=}$ ); 2.37 (t; 2H;  $J=7.46\text{Hz}$ ;  $\text{CH}_2\text{C=O}$ ); 4.66 (s; 2H;  $\text{CH}_2\text{CCl}_3$ ); 4.77-5.01 (m; 2H;  $\text{CH}_2=$ ), 5.57-5.83 (m; 1H;  $\text{CH=}$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

24.60 (-ve;  $\text{CH}_2$ ); 28.74 (-ve;  $\text{CH}_2$ ); 28.88 (-ve;  $\text{CH}_2$ ); 28.91 (-ve;  $\text{CH}_2$ ); 29.04 (-ve;  $\text{CH}_2$ ); 29.15 (-ve;  $\text{CH}_2$ ); 32.58 (-ve;  $\text{CH}_2$ ); 33.66 (-ve;  $\text{CH}_2$ ); 33.72 (-ve;  $\text{CH}_2$ ); 73.64 (-ve;  $\text{CH}_2\text{CCl}_3$ ); 95.03 (-ve;  $\text{CCl}_3$ ); 114.14 (-ve;  $\text{CH}_2=$ ); 138.79 (+ve;  $\text{CH}=$ ); 171.68 (-ve;  $\text{C=O}$ )

$\text{C}_{13}\text{H}_{21}\text{O}_2\text{Cl}_3$  (315.5)

**2,2,2-trichloroethyl-10,11-epoxyundecanoate (19).**

To a 2-neck round bottom flask was added 20g (63mmol) of 2,2,2-trichloroethyl-10-undecenoate (**18**) dissolved in 50ml of dichloromethane and 20g (116mmol) of *m*-chloroperoxybenzoic acid. The reaction mixture was then left stirring at ambient temperature for 3 days, during which time a white precipitate is produced. The precipitate is removed by filtration and the filtrate is treated with sodium sulfite solution to destroy any excess *m*-chloroperoxybenzoic acid. The filtrate was then washed successively with 3x50ml saturated sodium sulfite solution, 2x50ml saturated sodium bicarbonate solution and 1x50ml saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and solvents were removed by rotary evaporator giving 14.6g (44mmol) 70% yield of a pale yellow oil (**19**).

I.R. ( $\text{cm}^{-1}$ ; neat):

3049(m); 2931(s); 2857(s); 1757(s); 1714(m); 1465(m); 1456(m); 1413(m); 1379(m); 1268(s); 1220(m); 1142(s); 1114(s); 1060(m); 834(m); 805(s); 736(s); 721(s)



$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

1.06-1.25 (s; br; 8H;  $(\text{CH}_2)_4$ ); 1.25-1.44 (m; 4H  $(\text{CH}_2)_2$ ); 1.54 (qt; 2H;  $J=7.29\text{Hz}$ ;  $\text{CH}_2$ ); 2.2-2.42 (m; 2H;  $\text{CH}_2$  &  $\text{CH}$ ); 2.57 (t; 1H;  $J=4.02\text{Hz}$ ;  $\text{HCH}$ ); 2.63-2.82 (m; 1H;  $\text{HCH}$ ); 4.6 (s; 2H;  $\text{CH}_2\text{Cl}_3$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

24.34 (-ve;  $\text{CH}_2$ ); 25.59 (-ve;  $\text{CH}_2$ ); 28.59 (-ve;  $\text{CH}_2$ ); 28.72 (-ve;  $\text{CH}_2$ ); 28.97 (-ve;  $\text{CH}_2$ ); 32.1 (-ve;  $\text{CH}_2$ ); 33.46 (-ve;  $\text{CH}_2$ ); 51.86 (+ve;  $\text{CH-O}$ ); 73.38 (-ve;  $\text{CH}_2\text{CCl}_3$ ); 94.83 (-ve;  $\text{CCl}_3$ ); 171.51 (-ve;  $\text{C=O}$ )

$\text{C}_{13}\text{H}_{21}\text{O}_3\text{Cl}_3$  (331.5)

#### **2,2,2-trifluoroethyl 10-undecanoate (20).**

To a 2-neck round bottom flask was added 17.5g (95mmol) of undecylenic acid, 9.6g (96mmol) of 2,2,2-trifluoroethanol, 0.4g (3.3mmol) of DMAP and 100ml of freshly distilled dichloromethane. The resulting mixture was cooled in an ice bath for 15 minutes and a solution of 19.81g (96mmol) of DCC in 75ml of dichloromethane was added dropwise with stirring. The mixture was allowed to warm up to room temperature and left stirring for three days. The white precipitate that had formed was then filtered off and the filtrate was washed successively with 3x50ml of saturated citric acid solution, 2x50ml of saturated sodium bicarbonate solution and 1x50ml of saturated sodium chloride solution. The organic layer was then dried over magnesium sulfate and solvents were removed by rotary evaporator to yield 16.8g (63mmol) 67% of a pale yellow oil (20).

I.R. (cm<sup>-1</sup>; neat):

3079(w); 2929(s); 2857(s); 1762(s); 1642(m); 1414(m); 1283(s);  
1170(s); 979(m); 912(m); 842(w); 806(w)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.15-1.35 (m; 10H; (CH<sub>2</sub>)<sub>5</sub>); 1.55 (t; 2H; J=7.10Hz; CH<sub>2</sub>); 1.93  
(q; 2H; J=6.68Hz; CH<sub>2</sub>-CH=CH<sub>2</sub>); 2.28 (t; 2H; J=7.47Hz; CH<sub>2</sub>-  
C=O); 4.35 (q; 2H; J=8.55Hz; CH<sub>2</sub>-CF<sub>3</sub>); 4.77-4.95 (m; 2H;  
CH<sub>2</sub>=CH); 5.60-5.80 (m; 1H; CH=CH<sub>2</sub>)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

24.23 (-ve; CH<sub>2</sub>); 28.49 (-ve; CH<sub>2</sub>); 28.53 (-ve; CH<sub>2</sub>); 28.64 (-  
ve; CH<sub>2</sub>); 28.75 (-ve; CH<sub>2</sub>); 28.87 (-ve; CH<sub>2</sub>); 32.99 (-ve; CH<sub>2</sub>-  
CH=CH<sub>2</sub>); 33.37 (-ve; CH<sub>2</sub>-C=O); 59.49 (-ve; q; J=36.5Hz;  
CH<sub>2</sub>-CF<sub>3</sub>); 113.59 (-ve; CH<sub>2</sub>=); 122.75 (-ve; q; J=277Hz; CF<sub>3</sub>);  
138.46 (+ve; CH=); 171.31 (-ve; C=O)

C<sub>13</sub>H<sub>21</sub>O<sub>2</sub>F<sub>3</sub> (266)

#### **2,2,2-trifluoroethyl-10,11-epoxyundecanoate (21).**

To a 2-neck round bottom flask was added 15.0g (53mmol) of 2,2,2-trifluoroethyl 10-undecenoate (**20**) dissolved in 50ml of dichloromethane. A solution of 18.2g (106mmol) of *m*-CPBA dissolved in 125ml of dichloromethane was then slowly added to the stirred reaction flask. The mixture was left stirring for three days at room temperature, during which time a voluminous white precipitate formed. Any excess *m*-CPBA was then destroyed by the addition of an excess of saturated sodium sulfite solution. After five minutes a negative test was obtained with starch/iodide paper. The insoluble *m*-CBA was removed by filtration and the filtrate was washed successively with 100ml of saturated sodium sulfite solution, 100ml of saturated sodium bicarbonate

solution and 100ml of saturated sodium chloride solution. The organic layer was then dried over magnesium sulfate and solvents were removed by rotary evaporator to give 12g (40mmol) 75% yield of a pale orange oil (**21**).

I.R. ( $\text{cm}^{-1}$ ; neat):

3050(w); 2932(s); 2858(m); 1761(s); 1457(m); 1413(m); 1283(s);  
1169(s); 978(m); 917(w); 840(m); 739(m)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

1.02-1.32 (m; 10H;  $(\text{CH}_2)_5$ ); 1.43 (t; 2H;  $J=7.50$ ;  $\text{CH}_2$ ); 2.20 (m; 2H;  $\text{CH}_2$ ); 2.47 (t; 2H;  $J=3.98$  &  $1.27\text{Hz}$ ;  $\text{CH}_2\text{-O}$ ); 2.63-2.67 (m; 1H;  $\text{CH-O}$ ); 4.27 (q; 2H;  $J=8.61$ ;  $\text{CH}_2\text{CF}_3$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

24.18 (-ve;  $\text{CH}_2$ ); 25.50 (-ve;  $\text{CH}_2$ ); 28.44 (-ve;  $\text{CH}_2$ ); 28.64 (-ve;  $\text{CH}_2$ ); 28.89 (-ve;  $\text{CH}_2$ ); 32.04 (-ve;  $\text{CH}_2\text{-CH-O}$ ); 32.98 (-ve;  $\text{CH}_2\text{-C=O}$ ); 46.23 (-ve;  $\text{CH}_2\text{-O}$ ); 51.60 (+ve;  $\text{CH-O}$ ); 59.45 (-ve; q;  $J=36.4\text{Hz}$ ;  $\text{CH}_2\text{CF}_3$ ); 122.77 (-ve; q;  $J=277\text{Hz}$ ;  $\text{CF}_3$ ); 171.38 (-ve;  $\text{C=O}$ )

$\text{C}_{13}\text{H}_{21}\text{O}_3\text{F}_3$  (282)

### 6.2.3 SYNTHESIS OF EPOXY ACIDS.

#### 10,11-epoxyundecanoic acid (**27**).

To a round bottom flask with a condenser attached was added 50.0g (0.27mol) of undecylenic acid in 50ml of dichloromethane. To the solution 61.0g (0.35mol) of *m*-CPBA in 300ml of dichloromethane was added slowly with stirring. The solution was continuously stirred for two days at room temperature, during which time a white precipitate formed. The precipitate was removed by filtration and the filtrate was sealed

in a flask and placed in a freezer overnight in order to precipitate out any excess *m*-CPBA. Any further precipitate that has occurred is again removed by filtration and the solvent is removed by rotary evaporator, leaving a colourless oil that solidified on standing to yield 35.2g (176mmol) 65% of a white solid (**27**). M.P. 44-45°C.

I.R. (cm<sup>-1</sup>; KBr):

2929(s); 2850(s); 1707(s); 1466(m); 1434(m); 1322(m);  
1290(m); 1256(m); 1227(m); 1195(m); 911(m); 858(m); 844(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

0.95-1.55 (m; 14H; (CH<sub>2</sub>)<sub>6</sub>); 1.57 (t; 2H; J=7.08Hz; CH<sub>2</sub>); 2.30  
(t; 2H; J=7.48Hz; CH<sub>2</sub>); 2.44 (dd; 1H; J=2.80 & 4.95Hz; HCH);  
2.73 (t; 1H; J=4.49Hz; HCH); 2.89 (qt; 1H; J=5.34Hz & 3.38Hz;  
CH); 9.15-11.88 (S; br; 1H; COOH)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

24.53 (-ve; CH<sub>2</sub>); 25.80 (-ve; CH<sub>2</sub>); 28.88 (-ve; CH<sub>2</sub>); 29.01 (-  
ve; CH<sub>2</sub>); 29.21 (-ve; CH<sub>2</sub>); 32.37 (-ve; CH<sub>2</sub>); 33.96 (-ve; CH<sub>2</sub>);  
44.14 (-ve; CH<sub>2</sub>-O); 52.50 (+ve; CH-O); 179.99 (-ve; C=O)

C<sub>11</sub>H<sub>20</sub>O<sub>3</sub> (200.3)

### **2,3-epoxybutanoic acid (29).**

86g (1mole) of crotonic acid was added to a solution of 20g (50mmol) of sodium hydroxide in 250ml of water. The solution was filtered into a 3-neck round bottom flask equipped with stirrer, thermometer and dropping funnel. The solution was then heated to 55°C, and treated with 33g (0.10moles) of sodium tungstate dihydrate. To the stirred solution, 160ml (1.3moles) of 27.5% aqueous hydrogen peroxide was added dropwise over 5 minutes. The reaction temperature was held at 60-65°C by cooling and

the pH was kept above 4 by the dropwise addition of 30% w/v of aqueous sodium hydroxide solution as needed. After 1 hour at the same temperature, the mixture was cooled and acidified to a pH of 2.5 by the dropwise addition of 30% v/v of aqueous sulfuric acid. The solution was then saturated with ammonium sulfate and extracted with 5x200ml portions of ether. The ether extracts were combined and dried over magnesium sulfate. The ether was removed by rotary evaporator to yield a yellow oil which on standing yielded a white precipitate. The purity of the epoxy acid was estimated at 75% by nmr, with the impurity being made up of crotonic acid. The product was purified by recrystallisation from chloroform to give 52g (510mmol) 51% yield of white crystals (**29**). M.P. 84°C; Lit. M.P. 88.5°C and 84°C.<sup>106</sup>

I.R. (cm<sup>-1</sup>; KBr):

2984 (s); 2932 (s); 1708 (s); 1545 (w); 1447 (s); 1375 (m); 1338 (s); 1313 (s); 1265 (s); 1235 (s); 1162 (m); 1150 (m); 1058 (m); 1034 (s); 935 (s); 909 (s); 862 (s); 772 (m); 724 (m); 695 (s); 515 (m); 497 (m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.32-1.43 (m; 3H; CH<sub>3</sub>); 3.15-3.27; (m; 2H; CH-CH); 10.35-10.54 (s; 1H; COOH)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

16.94 (+ve; CH<sub>3</sub>); 53.30 (+ve; CH-O); 54.99 (+ve; O-CH); 174.66 (-ve; C=O)

C<sub>4</sub>H<sub>6</sub>O<sub>3</sub> (102)

**(R,S)-(+)-dihydro-5-(hydroxymethyl)-2(3H)-furanone (28).**

To a 3-neck 250ml round bottom flask was added 10g (100mmol) of 4-pentenoic acid dissolved in 30ml of freshly distilled dichloromethane. To the stirred solution was added 24.16g (140mmol) of *m*-CPBA dissolved in 120ml of dichloromethane. The resulting mixture was continuously stirred at room temperature for 2 days. The mixture was then filtered to remove the white precipitate formed, and the filtrate was sealed in a flask and placed in a freezer to precipitate out any remaining *m*-CPBA. The mixture was again filtered and the solvents were removed by rotary evaporator to yield a pale yellow oil that partially solidified on standing. The solid was again removed by filtration leaving 7.8g (67mmol) 67% yield of a yellow oil (28).

I.R. (cm<sup>-1</sup>; neat):

3430(s; br); 2942(m); 1767(vs); 1461(m); 1420(m); 1358(m);  
1192(s); 1158(m); 1101(m); 1065(m); 1028(m); 938(m); 917(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.84-1.95 (m; 1H; **HCH**-CH); 2.00-2.19 (m; 1H; **HCH**-CH);  
2.25-2.46 (m; 2H; **CH**<sub>2</sub>-COO); 3.42 (m; 1H; **HCH**-OH); 3.61 (dt;  
1H; J=2.92Hz & 12.50Hz; **HCH**-OH); 4.42-4.48 (m; 2H;  
**CH**&**OH**)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

22.59 (-ve; **CH**<sub>2</sub>-CH); 28.15 (-ve; **CH**<sub>2</sub>-OH); 63.15 (-ve; **CH**<sub>2</sub>-  
C=O); 80.87 (+ve; **CH**-O-C=O); 178.40 (-ve, C=O)

C<sub>5</sub>H<sub>8</sub>O<sub>3</sub> (116)

#### 6.2.4 SYNTHESIS OF DHCD ESTERS.

*cis*-1,2-bis(10-undecenoyloxy)-3,5-cyclohexadiene (24).

To a 3-neck 250ml round bottom flask with an argon inlet attached was added 1.7g (15mmol) of *cis*-DHCD (1) dissolved in 75ml of freshly distilled dichloromethane. To the resulting solution was added 5.9g (32mmol) of undecylenic acid and 0.1g (0.8mmol) of DMAP. The mixture was then cooled for 15 minutes in an ice bath before 6.6g (32mmol) of DCC dissolved in 75ml of dichloromethane was added dropwise to the solution. A white precipitate was observed to form immediately and upon completion of the addition of DCC, the mixture was allowed to warm up to room temperature and was continuously stirred in an argon atmosphere for two days. After this time, the white precipitate was filtered off and the filtrate was washed successively with 2x50ml of 5% v/v aqueous HCl, 2x50ml of saturated sodium bicarbonate solution and 1x50ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and solvents were removed by rotary evaporator to leave an orange oil that partially crystallised on standing giving 4.5g (9.5mmol) 63% yield of (24).

I.R. (cm<sup>-1</sup>; neat):

3075(w); 2927(s); 2855(s); 1741(s); 1641(m); 1237(m); 1163(s);  
1113(m); 990(w); 910(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.24 (s; 20H; (CH<sub>2</sub>)<sub>5</sub>); 1.57 (t; 4H; J=7.03Hz; CH<sub>2</sub>); 1.99 (q; 4H; J=6.95Hz; CH<sub>2</sub>); 2.26 (t; 4H; J=7.51Hz; CH<sub>2</sub>); 4.73-5.03 (m; 4H; CH=CH<sub>2</sub>); 5.51 (d; 2H; J=1.20Hz CH-O); 5.61-5.97 (m; 4H; CH=CH<sub>2</sub> & CH=CH); 6.06-6.22 (m; 2H; CH=CH)

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

24.80 (-ve;  $\text{CH}_2$ ); 28.79 (-ve;  $\text{CH}_2$ ); 28.98 (-ve;  $\text{CH}_2$ ); 29.14 (-ve;  $\text{CH}_2$ ); 29.21 (-ve;  $\text{CH}_2$ ); 33.69 (-ve;  $\text{CH}_2$ ); 34.13 (-ve;  $\text{CH}_2$ ); 66.67 (+ve;  $\text{CH-O}$ ); 114.08 (-ve;  $\text{CH=CH}_2$ ); 125.36 (+ve;  $\text{CH=CH}$ ); 126.09 (+ve;  $\text{CH=CH}$ ); 139.00 (+ve;  $\text{CH=CH}_2$ ); 172.96 (-ve;  $\text{C=O}$ )

$\text{C}_{28}\text{H}_{44}\text{O}_4$  (476.56)

*cis*-1,2-bis(3-butenyloxy)-3,5-cyclohexadiene (**23**).

To a 3-neck 250ml round bottom flask equipped with a magnetic stirrer and an argon inlet was placed 2.0g (18mmol) of *cis*-DHCD (**1**) and 50ml of anhydrous dichloromethane. To the resulting solution was added 3.28g (38mmol) of vinyl acetic acid and 0.15g (1.2mmol) of DMAP. The mixture was then cooled for 15 minutes in an ice bath before 7.84g (38mmol) of DCC dissolved in 50ml of anhydrous dichloromethane was added dropwise with stirring. A white precipitate was observed to form immediately and when the addition of the DCC solution was complete the mixture was allowed to warm up to room temperature. The reaction mixture was stirred continuously for two days under an argon atmosphere. After this time the white precipitate was filtered off and the filtrate was washed successively with 2x50ml 5% v/v aqueous HCl, 2x50ml saturated sodium bicarbonate solution and 1x50ml saturated sodium chloride solution. The organic phase was dried over magnesium sulfate and solvents were removed by rotary evaporator to leave 2.4g (9.5mmol) 53% yield of a yellow oil (**23**).

I.R. ( $\text{cm}^{-1}$ ; neat):

3082(w); 3055(w); 2983(w); 2925(m); 2857(w); 1741(s); 1643(m); 1410(m); 1326(m); 1251(m); 1164(s); 1112(m); 1051(m); 989(m); 925(m); 829(m)



$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

2.93-3.10 (dt; 4H;  $\text{CH}_2\text{-C=O}$ ); 4.98-5.10 (m; 2H;  $\text{HCH=CH}$ );  
5.10-5.16 (m; 2H;  $\text{HCH=CH}$ ); 5.44-5.58 (t; 2H;  $J=1.24\text{Hz}$ ;  $\text{CH-O}$ ); 5.77-5.91 (m; 4H;  $\text{CH=CH}$  &  $\text{CH=CH}_2$ ); 6.04-6.17 (m; 2H;  $\text{CH=CH}$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

38.72 (-ve;  $\text{CH}_2\text{-C=O}$ ); 66.86 (+ve;  $\text{CH-O}$ ); 118.96 (-ve;  $\text{CH}_2=\text{CH}$ ); 124.89 (+ve;  $\text{CH=CH}$ ); 126.15 (+ve;  $\text{CH=CH}$ );  
129.83 (+ve;  $\text{CH}_2=\text{CH}$ ); 170.57 (-ve;  $\text{C=O}$ )

$\text{C}_{14}\text{H}_{16}\text{O}_4$ (248)

***cis*-1,2-bis(2-butenoyloxy)-3,5-cyclohexadiene (25).**

To a 3-neck round bottom flask with an argon inlet attached was placed 2.7g (24mmol) of *cis*-DHCD (1), 4.48g (52mmol) of crotonic acid and 0.3g (2.5mmol) of DMAP dissolved in 50ml of freshly distilled dichloromethane. The resulting mixture was continuously stirred and cooled to  $0^\circ\text{C}$  for 15 minutes, after which time a solution of 10.73g (52mmol) of DCC dissolved in 75ml of dichloromethane was added dropwise. During the addition a white precipitate is formed. The mixture is then allowed to warm up to room temperature and left stirring for 3 days. The white precipitate was then removed by filtration and the filtrate was washed successively with 2x50ml of distilled water, 2x50ml of 5% v/v aqueous hydrochloric acid, 2x50ml of saturated sodium bicarbonate solution and 2x50ml of saturated sodium chloride solution. The organic layer was then dried over magnesium sulfate and solvents were removed by rotary evaporator to yield a solid suspended in an orange oil. The oil was dissolved in ether and the solid was removed by filtration. Removal of the ether yielded 0.6g (2.4mmol) 10% of a yellow oil (25).

I.R. (cm<sup>-1</sup>; neat):

2970(m); 2939(m); 2855(m); 1721(s); 1657(s); 1594(m);  
1440(m); 1261(m); 1167(s); 1102(s); 1058(m); 1003(m); 969(m);  
836(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.79-1.84 (m; 3H; CH<sub>3</sub>-CH=); 5.57 (s; 2H; CH-O); 5.75-5.85  
(dd; 1H; J=1.42Hz & 15.57Hz; CH<sub>3</sub>-CH=); 5.86-5.91 (m; 2H;  
CH=CH); 6.06-6.10 (m; 2H; CH=CH); 6.94 (st; 1H; J=7.3Hz;  
=CH-C=O)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

18.12 (+ve; CH<sub>3</sub>-CH); 66.88 (+ve; CH-O); 122.10 (+ve; CH<sub>3</sub>-  
CH=); 126.11 (+ve; CH=CH-CHO); 129.28 (+ve; CH=CH-  
CHO); 145.63 (+ve; =CH-C=O); 165.75 (-ve; C=O)

C<sub>14</sub>H<sub>16</sub>O<sub>4</sub> (248.18)

***cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene (30).**

A solution of 5.0g (45mmol) of *cis*-1,2-dihydroxy-3,5-cyclohexadiene (**1**) and 18.03g (90mmol) of 10,11-epoxyundecanoic acid (**27**) in 75ml of dichloromethane was added to a 3 neck round bottom flask with an argon inlet attached. 0.5g (4mmol) of DMAP were then added before cooling the mixture to 0°C in an ice bath for fifteen minutes. To the cooled mixture, a solution of 18.57g (90mmol) of DCC in 75ml of dichloromethane was added dropwise. A white precipitate was observed to form almost immediately after the addition of DCC. The reaction mixture was then allowed to slowly warm up to room temperature and was continuously stirred under argon for 2 days. The white precipitate was removed by filtration and the filtrate was washed successively with 2x50ml of distilled water, 2x50ml of 5% v/v aqueous hydrochloric acid, 2x50ml of saturated sodium bicarbonate solution and 2x50ml of saturated sodium chloride

solution. The organic phase was then dried over magnesium sulfate and solvents were removed by rotary evaporator to yield a yellow oil which solidified on standing to give 8.6g (18mmol), 40% of an off white solid (**30**), the purity of which was determined by nmr. M.P. 34-36°C

I.R. (cm<sup>-1</sup>; neat):

3052(m); 2930(s); 2856(s); 1738(vs); 1650(m); 1466(m);  
1412(m); 1371(m); 1257(m); 1160(s); 1105(m); 832(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

1.2-1.6 (m; 28H; (CH<sub>2</sub>)<sub>7</sub>); 2.26 (t; 4H; J=7.51Hz; CH<sub>2</sub>-C=O);  
2.42 (q; 2H; CH-O); 2.71 (t; 2H; J=4.05Hz; HCH-O); 2.85-2.87  
(qt; 2H; HCH-O); 5.51 (d; 2H; J=1.16Hz; CH-CH-O); 5.83-5.86  
(m; 2H; CH=CH); 6.06-6.10 (m; 2H; =CH-CH-O)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

24.77 (-ve; CH<sub>2</sub>); 25.85 (-ve; CH<sub>2</sub>); 28.95 (-ve; CH<sub>2</sub>); 29.07 (-  
ve; CH<sub>2</sub>); 29.27 (-ve; CH<sub>2</sub>); 32.36 (-ve; CH<sub>2</sub>); 34.11 (-ve; CH<sub>2</sub>);  
47.11 (-ve; CH<sub>2</sub>-O); 52.32 (+ve; CH<sub>2</sub>-CH-O); 66.7(+ve; =CH-  
CH-O); 125.33 (+ve; CH=); 126.12 (+ve; CH=); 172.98 (-ve;  
C=O)

C<sub>28</sub>H<sub>44</sub>O<sub>6</sub> (476)

*cis*-1,2-bis-(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene (**31**).

To a 3-neck round bottom flask with an argon inlet attached was added a mixture of 2.67g (24mmol) of *cis*-DHCD (**1**) and 5.1g (50mmol) of 2,3-epoxybutanoic acid (**29**) dissolved in 50ml of dichloromethane. 0.42g (4mmol) of DMAP were then added to the solution and the flask was cooled to 0°C for 15 minutes with an ice bath. After

cooling, a solution of 10.32g (50mmol) of DCC in 50ml of dichloromethane was slowly added with stirring. Upon addition of the DCC, a white precipitate was observed to form. The reaction mixture was allowed to warm up to room temperature and was continuously stirred for 24 hours. The white precipitate was removed by filtration and the resulting filtrate was washed successively with 3x50ml of saturated sodium bicarbonate solution and 2x50ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate, and solvents were removed by rotary evaporator to yield 3.1g (11mmol), 46% of an orange oil (**31**).

I.R. ( $\text{cm}^{-1}$ ; neat):

3058(m); 2975(m); 2932(m); 2858(m); 1752(vs); 1698(s);  
1648(m); 1593(m); 1558(m); 1489(m); 1427(s); 1379(m);  
1341(s); 1271(s); 1250(s); 1196(vs); 1146(s); 1055(s); 1034(s);  
996(m); 972(m); 927(m); 863(m); 833(m); 781(m); 735(s);  
703(m)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

1.25-1.32 (m; 6H;  $\text{CH}_3$ ); 3.07-3.17 (m; 4H;  $\text{CH-CH}$ ); 5.50-5.56  
(t; 2H;  $J=2.06\text{Hz}$ ;  $\text{CH-O}$ ); 5.78-5.86 (m; 2H;  $\text{CH=CH}$ ); 6.05-6.13  
(m; 2H;  $\text{CH=CH}$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

16.99 (+ve;  $\text{CH}_3$ ); 53.49 (+ve;  $\text{CH-CH}$ ); 54.67 (+ve;  $\text{CH-CH}$ );  
67.51 (+ve;  $\text{CH-O}$ ); 124.30 (+ve;  $\text{CH=CH}$ ); 126.69 (+ve;  
 $\text{CH=CH}$ ); 168.56 (-ve;  $\text{C=O}$ )

$\text{C}_{14}\text{H}_{16}\text{O}_6$  (280)

#### 6.2.5. SYNTHESIS OF SILYL ETHER DERIVATIVES OF DHCD.

*cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene (32).

To a 50ml round bottom flask with a condenser attached was added 2.27g (20mmol) of *cis*-DHCD (1), 10.4ml (42mmol) of bis(trimethylsilyl)acetamide and 15ml of anhydrous dichloromethane. The reaction mixture was left stirring at ambient temperature for 4 hours, during which time a white precipitate formed. The precipitate was removed by filtration and the filtrate was taken up in hexane, resulting in further precipitation of white crystals. After a second filtration, the solvents were removed leaving 2.65g (10mmol) 50% yield of a pale yellow oil (32).

I.R. (cm<sup>-1</sup>; neat):

3043(m); 2957(s); 2898(m); 2796(w); 1252(s); 1176(m);  
1119(s); 1052(m); 986(m); 971(m); 958(m); 926(s); 888(s);  
841(s); 754(m); 696(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

0.66 (s; 18H; Si(CH<sub>3</sub>)<sub>3</sub>); 4.05 (t; 2H; J=0.92Hz; =CH-CH-O);  
5.74-5.82 (m; 2H; CH=CH); 5.82-5.98 (m; 2H; =CH-CH-O)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

-0.096 (+ve; Si(CH<sub>3</sub>)<sub>3</sub>); 68.72 (+ve; CH-O); 123.72 (+ve;  
CH=CH); 130.15 (+ve; =CH-CH-O)

C<sub>12</sub>H<sub>24</sub>O<sub>2</sub>Si<sub>2</sub>(256)

*cis*-1,2-Bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene (33).

To a 250ml round bottom flask with a condenser attached was placed 2.27g (20mmol) of *cis*-DHCD (1), 0.3g (2.45mmol) of DMAP and 5.9ml (42mmol) of triethylamine dissolved in 50ml of dichloromethane. The mixture was cooled to 0°C and a solution of 6.33g (42mmol) of *tert*-butyldimethylsilyl chloride in 50ml of dichloromethane was added dropwise with stirring. The mixture was continuously stirred at 0°C for 1 hour before being allowed to warm up to room temperature and stirred for a further 18 hours. After this time the small amount of precipitate that had formed was filtered off and solvents were removed by rotary evaporator to yield a white solid suspended in an orange/red oil. The oil was dissolved in ether and the white solid was removed by filtration. The ether was then evaporated, leaving 5.1g (15mmol) 75% yield of an orange oil (33).

I.R. (cm<sup>-1</sup>; neat):

3042(w); 2955(s); 2929(s); 2893(s); 2857(s); 1472(m); 1254(s);  
1171(m); 1118(s); 1051(m); 940(m); 869(m); 836(s); 809(m);  
776(s); 665(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

0.06 (s; 12H; Si(CH<sub>3</sub>)<sub>2</sub>); 0.88 (s; 18H; Si-C(CH<sub>3</sub>)<sub>3</sub>); 4.12-4.14  
(d; 2H; J=1.65Hz; CH-O); 5.84-5.94 (m; 4H; CH=CH)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

-4.8 (+ve; Si(CH<sub>3</sub>)<sub>2</sub>); 18.27 (-ve; Si-C-(CH<sub>3</sub>)<sub>3</sub>); 25.89 (+ve; Si-  
C(CH<sub>3</sub>)<sub>3</sub>); 69.59 (+ve; CH-O); 123.71 (+ve; CH=CH); 131.00  
(+ve; CH=CH)

C<sub>18</sub>H<sub>36</sub>O<sub>2</sub>Si<sub>2</sub> (340.46)

### 6.2.6 MISCELLANEOUS SYNTHESSES.

#### *cis*-1,2-bis(4-morpholinecarboxy)-3,5-cyclohexadiene (37).

To a 100ml round bottom flask with a condenser attached was placed 2.4g (22mmol) of *cis*-DHCD (1), 0.75g (6.14 mmol) of DMAP and 6.4ml (46mmol) of triethylamine in 30ml of freshly distilled dichloromethane. The solution was cooled to 0°C and 5.4ml (46mmol) of 4-morpholinecarbonyl chloride was added dropwise with stirring. The reaction was stirred at 0°C for 30 minutes and a further 2 days at room temperature. The white precipitate was then removed by filtration and the solvents were removed from the filtrate to yield needle-like crystals. The crystals were then washed with hexane and dried in a desiccator and gave 2.2g (9mmol) 20% yield of anhydride.

I.R. (cm<sup>-1</sup>; neat):

3008(m); 2972(m); 2895(m); 2857(m); 1717(m); 1645(s);  
1436(m); 1417(m); 1263(m); 1111(s); 1071(m); 1037(m);  
882(m); 600(m); 549(m)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

3.22 (t; 8H; J=4.81Hz; CH<sub>2</sub>-N); 3.63 (t; 8H; J=4.78Hz; CH<sub>2</sub>-O)

<sup>13</sup>C N.M.R. (δ; CDCl<sub>3</sub>):

47.10 (-ve; CH<sub>2</sub>-N); 66.49 (-ve; CH<sub>2</sub>-O); 163.69 (-ve; C=O)

C<sub>10</sub>H<sub>16</sub>O<sub>5</sub>N<sub>2</sub> (244)

#### Sodium 4-Morpholine Propionate (38).

To a round bottom flask with a condenser attached was placed 50ml (370mmol) of 4-morpholine propionitrile and 100ml of 50% w/v aqueous solution of sodium hydroxide. The reaction mixture was vigorously stirred at reflux for 4 hours during which time a

white precipitate was formed. The precipitate was filtered off and dried in an oven at 60°C for 3 days, resulting in a yield of 42g (280mmol) 75% of white solid (38).

I.R. (cm<sup>-1</sup>; KBr):

3503(s); 2961(s); 2867(m); 2828(s); 1598(s); 1379(s); 1286(m);  
1115(m); 1076(m); 1010(m); 919(m); 868(m); 637(m)

<sup>1</sup>H N.M.R. (δ; D<sub>2</sub>O):

2.11-2.18 (m; 2H; CH<sub>2</sub>-COO<sup>-</sup>); 2.31 (s; 4H; CH<sub>2</sub>-N-CH<sub>2</sub>); 2.39-  
2.45 (m; 2H; N-CH<sub>2</sub>-CH<sub>2</sub>-COO<sup>-</sup>); 3.48-3.52 (m; CH<sub>2</sub>-O-CH<sub>2</sub>)

<sup>13</sup>C N.M.R. (δ; D<sub>2</sub>O):

36.58 (-ve; CH<sub>2</sub>-COO<sup>-</sup>); 54.53 (-ve; CH<sub>2</sub>-N-CH<sub>2</sub>); 56.93 (-ve;  
N-CH<sub>2</sub>-CH<sub>2</sub>-COO<sup>-</sup>); 68.42 (-ve; CH<sub>2</sub>-O-CH<sub>2</sub>); 183.42 (-ve;  
C=O)

C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>Na (151)

#### **Tricetylpyridinium 12-tungstophosphate (45).**

To a 250ml 3-neck round bottom flask was added 3.74g (10.4mmol) of cetylpyridinium chloride dissolved in 140ml of distilled water. A solution of 10g (3.4mmol) of 12-tungstophosphoric acid dissolved in 20ml of distilled water was added dropwise with stirring and a white precipitate formed immediately. The reaction mixture was continuously stirred for 3-4 hours at ambient temperature, after which time the mixture was filtered and washed several times with distilled water. The product was dried in vacuo to give 10.1g (2.7mmol) 80% yield of (45) as a white powder.



I.R. (cm<sup>-1</sup>; KBr):

2922(m); 2851(m); 1487(m); 1173(m); 1079(m); 977(m);  
895(m); 804(s); 679(m)

C<sub>63</sub>H<sub>114</sub>N<sub>3</sub>O<sub>40</sub>PW<sub>12</sub> (3791)

**1,2-dibromocyclohex-4-ene (41).**

To a 3-neck 1 litre round bottom flask was placed 25ml (260mmol) of 1,4-cyclohexadiene in 175ml of chloroform. The mixture was cooled to -5°C and a solution of 16.5ml (320mmol) of bromine in 40ml of chloroform was added dropwise with stirring. When the addition was complete, the mixture was allowed to warm up to room temperature and 600ml of methanol were added to precipitate any 1,2,4,5-tetrabromocyclohexane. The solution was then filtered and the solvents were removed by rotary evaporator leaving a white solid which upon washing with methanol gave 31.2g (130mmol) 50% yield of (41). M.P. 35°C; Lit. M.P. 35.1-35.2°C.

I.R. (cm<sup>-1</sup>; neat):

3003(m); 2953(m); 2905(m); 2855(m); 1433(m); 1416(s);  
1363(m); 1319(m); 1276(m); 1260(m); 1235(m); 1219(m);  
1182(m); 1166(s); 1140(m); 1009(s); 913(m); 889(s); 865(m);  
821(m); 786(s); 739(m); 565(s); 533(m); 509(s)

<sup>1</sup>H N.M.R. (δ; CDCl<sub>3</sub>):

2.41-2.69 (dt; 2H; **H**-C-H); 2.75-2.99 (dq; 2H; H-C-**H**); 4.49 (t;  
2H; **CH**-Br); 5.58-5.72 (m; 2H; **CH=CH**)

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

30.95 (-ve;  $\text{CH}_2$ ); 48.40 (+ve;  $\text{CH-Br}$ ); 121.99 (+ve;  $\text{CH=CH}$ )

$\text{C}_6\text{H}_8\text{Br}_2$  (239.95)

#### 1,2-dibromo-4,5-epoxycyclohexane (42)

To a 3 neck 250ml round bottom flask with a stirrer attached was added 15g (62.5mmol) of 1,2-dibromocyclohex-4-ene (**41**) in 50ml of freshly distilled dichloromethane. The solution was cooled to  $0^\circ\text{C}$  before the dropwise addition of 12.08g of *m*-CPBA dissolved in 100ml of dichloromethane. After the addition was completed, the mixture was kept at  $0^\circ\text{C}$  for 1 hour and at room temperature for a further 18 hours. During this time a white precipitate formed, which was removed by filtration. The filtrate was then washed successively with 2x50ml of saturated sodium bicarbonate solution and 2x50ml of saturated sodium chloride solution. The organic phase was then dried over magnesium sulfate and solvents were removed to yield 8.9g (34.4mmol) 55% of a white solid (**42**). M.P.  $52^\circ\text{C}$ .

I.R. ( $\text{cm}^{-1}$ ; KBr):

3003(m); 2953(m); 2905(m); 2855(m); 1433(m); 1416(s);  
1363(m); 1319(m); 1276(m); 1260(m); 1235(m); 1219(m);  
1182(m); 1166(m); 1140(m); 1009(s); 889(s); 786(s); 739(m)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

2.34-2.43 (m; 1H;  $\text{H-C-H}$ ); 2.53-2.61 (m; 1H;  $\text{H-C-H}$ ); 2.78-2.95  
(m; 2H;  $\text{H-C-H}$ ); 3.13-3.18 (m; 2H;  $\text{CH-Br}$ ); 4.09-4.16 (m; 1H;  
 $\text{CH-O}$ ); 4.20-4.26 (m; 1H;  $\text{CH-O}$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

32.41 (-ve;  $\text{CH}_2$ ); 33.36 (-ve;  $\text{CH}_2$ ); 47.44 (+ve;  $\text{CH-Br}$ ); 48.76  
(+ve;  $\text{CH-Br}$ ); 50.09 (+ve;  $\text{CH-O}$ ); 50.65 (+ve;  $\text{CH-O}$ )

$\text{C}_6\text{H}_8\text{Br}_2\text{O}$  (255.95)

*tert*-butyldimethylsilyl *tert*-butyldimethylsiloxyacetate (**34**).

To a 250ml round bottom flask was added 5.77g (76mmol) of glycolic acid, 25ml (180mmol) of triethylamine and 0.37g (3mmol) of DMAP dissolved in 50ml of dichloromethane. The solution was cooled to  $-78^\circ\text{C}$  with dry ice and a solution of 25g (170mmol) of *tert*-butyldimethylsilyl chloride in 40ml of dichloromethane was added dropwise with stirring. The mixture was continuously stirred at  $-78^\circ\text{C}$  for 30 minutes and at ambient temperature for a further 48 hours. The white precipitate produced was removed by filtration and solvents were removed from the filtrate by rotary evaporator leaving an orange oil containing a suspended white solid. The white solid was removed by filtration and the orange oil partially crystallised on standing to give 21.1g (53mmol) 70% yield of (**34**).

I.R. ( $\text{cm}^{-1}$ ; neat):

1746 (s); 1256 (s); 1220 (s); 1150 (s); 1007 (s); 939 (s); 841 (m);  
791(m)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

0.02 (s; 6H;  $\text{Si}(\text{CH}_3)_2$ ); 0.20 (s; 6H;  $\text{Si}(\text{CH}_3)_2$ ); 0.83 (s; 9H;  
 $\text{SiC}(\text{CH}_3)_3$ ); 0.85 (s; 9H;  $\text{SiC}(\text{CH}_3)_3$ ); 4.10 (s; 2H;  $\text{CH}_2$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

-4.61 (+ve;  $\text{Si}(\text{CH}_3)_2$ ); -3.67 (+ve;  $\text{Si}(\text{CH}_3)_2$ ); 17.47 (-ve;  $\text{SiC}(\text{CH}_3)_3$ ); 18.21 (-ve;  $\text{SiC}(\text{CH}_3)_3$ ); 25.34 (+ve;  $\text{C}(\text{CH}_3)_3$ ); 25.59; (+ve;  $\text{C}(\text{CH}_3)_3$ ); 62.12 (-ve;  $\text{OCH}_2$ ); 171.68 (-ve;  $\text{C}=\text{O}$ )

$\text{C}_{14}\text{H}_{32}\text{O}_3\text{Si}_2$  (304.18)

*tert*-butyldimethylsilyl acetylchloride (**35**).

To a 250ml round bottom flask was added 10g (33mmol) of *tert*-butyldimethylsilyl *tert*-butyldimethylsiloxoacetate (**34**) and 20 drops of dimethylformamide in 70ml of dichloromethane. The mixture was cooled to  $0^\circ\text{C}$  with an ice bath and 4.6ml (53mmol) of oxalyl chloride were added dropwise with stirring. The mixture was stirred continuously at  $0^\circ\text{C}$  for 30 minutes and then at ambient temperature for a further 3 hours. Solvents were removed by rotary evaporator to give 2.14g (10mmol) 32% yield of a dark brown/orange solid suspended in an orange oil (**35**).

I.R. ( $\text{cm}^{-1}$ ; neat):

1882(m); 1813(s); 1746(m); 1259(s); 1162(s); 1008(s); 939(s); 839(s); 782(s)

$^1\text{H}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

0.08 (s; 6H;  $\text{Si}(\text{CH}_3)_2$ ); 0.34 (s; 6H;  $\text{Si}(\text{CH}_3)_2$ ); 0.89 (s; 9H;  $\text{SiC}(\text{CH}_3)_3$ ); 0.96 (s; 9H;  $\text{SiC}(\text{CH}_3)_3$ ); 4.50 (s; 2H;  $\text{OCH}_2$ )

$^{13}\text{C}$  N.M.R. ( $\delta$ ;  $\text{CDCl}_3$ ):

-5.61 (+ve;  $\text{Si}(\text{CH}_3)_2$ ); -1.64 (+ve;  $\text{Si}(\text{CH}_3)_2$ ); 18.16 (-ve;  $\text{SiC}(\text{CH}_3)_3$ ); 18.97 (-ve;  $\text{SiC}(\text{CH}_3)_3$ ); 25.23 (+ve;  $\text{C}(\text{CH}_3)_3$ ); 25.49 (+ve;  $\text{C}(\text{CH}_3)_3$ ); 69.82 (-ve;  $\text{OCH}_2$ ); 173.00 (-ve;  $\text{C}=\text{O}$ )

$\text{C}_8\text{H}_{17}\text{O}_2\text{SiCl}$  (208.59)

### 6.3 HYDROGEL SYNTHESIS.

A mixture of HEMA, a % w/w comonomer, 1% w/w EDMA, and 0.5% w/w of AIBN were degassed by bubbling argon through the solution for 15 minutes. The solution was injected into the mould shown in figure 6.1, and the needle was then removed. The mould was placed in an oven at 60°C for three days followed by a three hour postcure at 90°C. The mould was then separated and the xerogel placed in 175ml of distilled water to equilibrate for at least two weeks, the water being changed daily. The mould consisted of two polyethylene gaskets (115mm x140mm external; 75mm x 100mm internal) sandwiched between two melinex (polyethylene terephthalate) sheets which had been attached to two glass plates by spray mount adhesive. The mould was held together by a series of bulldog clips.

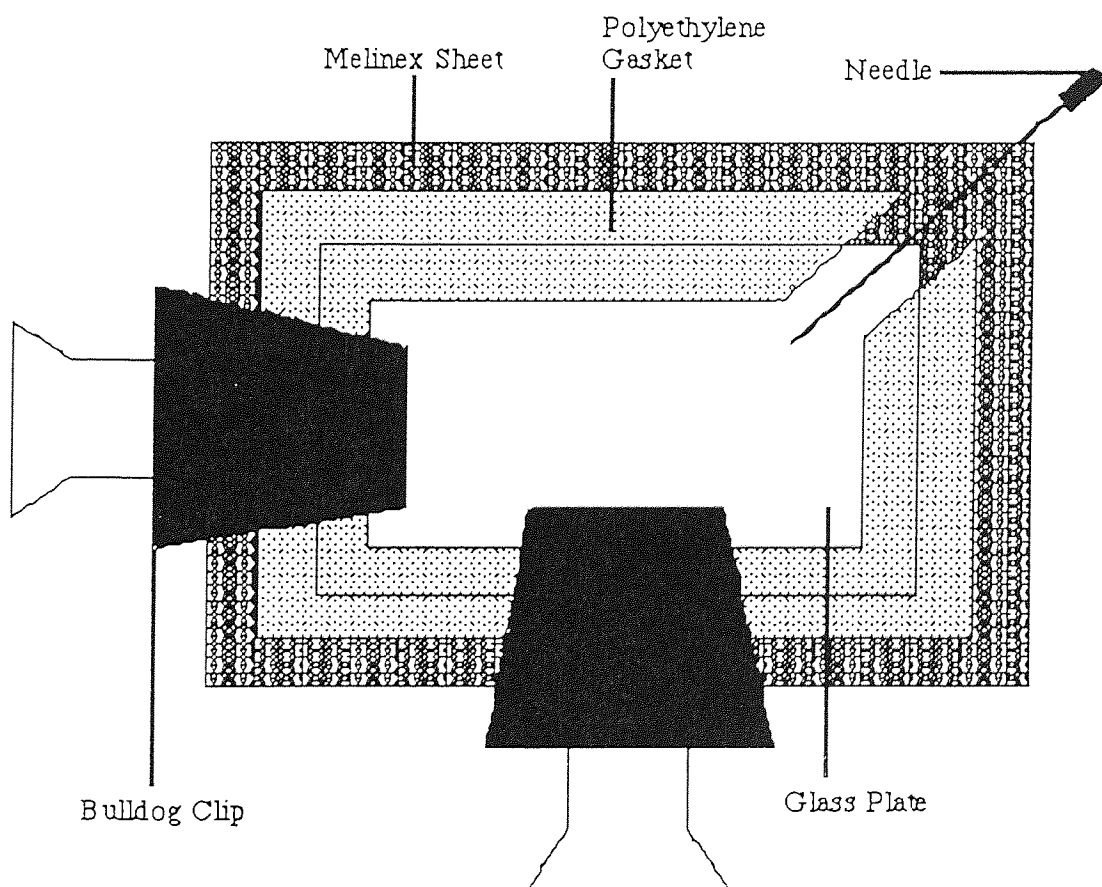


Figure 6.1: Membrane Mould.

## **6.4 METHODS OF ANALYSIS.**

### **6.4.1 Infra-Red Spectroscopy.**

All Infra-Red spectra were recorded on either a Perkin Elmer 1710 Fourier Transform Infrared Spectrometer or a Perkin Elmer Paragon 1000 Fourier Transform Infrared Spectrometer. Solid samples were prepared as KBr discs and liquids as thin films between sodium chloride plates.

### **6.4.2 Nuclear Magnetic Resonance Spectroscopy.**

All Nuclear Magnetic Resonance spectra were recorded on a Bruker AC300 spectrometer.  $^{13}\text{C}$  spectra were recorded as either APT (Attached Proton Test) or PENDANT (Polarization Enhancement Nurtured During Attached Nucleus Testing).

### **6.4.3 Melting Point Determination.**

Melting points were determined in capillary tubes with a Gallencamp Melting Point Apparatus, Model No. ME-370, and are uncorrected.

## **6.5 CHROMATOGRAPHIC TECHNIQUES.**

All chromatographic techniques depend upon the satisfactory separation of the various components of a mixture as it passes between two phases:- the mobile phase and the stationary phase. The mobile phase may be either a liquid or a gas and the stationary phase either a porous solid or liquid capable of retaining both solvents and solutes.

### **6.5.1 Thin Layer Chromatography (TLC).**

The analytical technique of thin layer chromatography is primarily used in the determination of material purity, but is also used for preliminary identification purposes. The results obtained using TLC allow us to determine the best solvent system for separations of mixtures by column chromatography. In TLC, the silica gel

absorbant is supported as a thin coating on a flat surface which may be a glass plate or more conveniently a sheet of aluminium or plastic.

The technique involves the spotting of samples onto the baseline of a TLC plate measuring approximately 8 x 2.5 cm. The TLC plate is then placed in a developing tank that contains approximately 20cm<sup>3</sup> of the developing solvent. It should be ensured that the level of the solvent in the developing tank does not cover the baseline on the plate as this will simply result in the sample dissolving rather than being carried up the plate. The solvent should be allowed to rise in a horizontal straight line up the plate by way of capillary action until it reaches about 1cm from the top. The plate is then removed from the developing tank, the solvent front is marked and excess solvent on the plate is allowed to evaporate off in a fume hood.

Unless the material being analysed is coloured it is necessary to treat the plate in some way in order to visualise the spots and measure the distance they have travelled. There are a number of methods of visualising TLC plates. these include: UV light , iodine vapour and spraying the plate with 50% H<sub>2</sub>SO<sub>4</sub> followed by heating. When using the iodine method, the plate is placed into a tank containing a few crystals of iodine and the various components in the sample appear as dark spots on the plate. Most compounds stain up within a few minutes but some may take several hours.

The retention factor is a useful measurement that can be obtained from a developed TLC plate. It is the relationship between the distance moved by the component spot and the distance moved by the eluting solvent.

$$R_f = \frac{\text{Distance moved by the product spot}}{\text{Distance moved by solvent front}}$$

### **6.5.2 'Dry Flash' Column Chromatography.**

This technique combines the speed and separation of 'flash' chromatography with the use of cheaper TLC grade silica gel. In 'dry flash' chromatography, the silica column is eluted by suction rather than using top pressure, removing the risk of bursting glassware. Another feature of this type of chromatography is that the column is eluted with predetermined volumes of solvent and is run dry before addition of the next fraction.

The column is made by producing a slurry of silica gel and the elution solvent which is poured into a sintered glass funnel. Suction is then applied and the column is compacted by pressing down firmly on the surface of the silica with a glass stopper. As the column continues to dry out, it is essential that any cracks are eliminated by continued packing. Once the column has been completely dried out, the sample is dissolved in the minimum amount of the pre-elution solvent and applied evenly to the surface of the silica with the column under suction. Once the sample has been absorbed onto the surface of the silica, the headspace of the funnel can be topped up with solvent and the column is ready for elution. The process is continued with increasingly more polar fractions and the progress of the separation is followed by TLC analysis of the fractions

## **6.6 MEASUREMENT OF THE PHYSICAL PROPERTIES OF HYDROGELS.**

### **6.6.1 Determination of Equilibrium Water Content.**

EWC determinations were carried out on four separate samples of gel and the average value calculated. A No.7 cork borer was used to cut out small discs of gel, which were then placed in a sample bottle of distilled water. For each determination the disc was blotted lightly with filter paper, to remove any surface water, and weighed. Dehydration of the gel was accomplished by placing it in a microwave oven for ten



minutes, after which the gel was reweighed. The EWC was then calculated using the equation in section 1.2.5.

#### **6.6.2 Mechanical Properties.**

All mechanical tests were performed on a Hounsfield Tensometer using dumbbell shaped samples and a test speed of 20 mm /minute. A minimum of three samples of each gel were tested and samples consisted of a gauge length of 8mm and a width of 3.3mm.

#### **6.6.3 Differential Scanning Calorimetry.**

The freezing water content of hydrogel samples was determined by using a Perkin-Elmer differential scanning calorimeter, DSC7, fitted with a liquid nitrogen sub-ambient accessory. The calorimeter was calibrated using the melting thermographs of indium and lead. 3-6mg samples of each gel were cut from hydrated sheets and the surface water was carefully removed with filter paper. The samples were then sealed into aluminium sample pans and the weight of each sample recorded. Each sample was then cooled to  $-70^{\circ}\text{C}$  at a rate of  $100^{\circ}\text{C}/\text{minute}$  in order to ensure that any supercooled water was frozen, and kept at that temperature for five minutes to attain equilibrium. Samples were then heated to  $-25^{\circ}\text{C}$  at a rate of  $20^{\circ}\text{C}/\text{minute}$  and subsequently to  $20^{\circ}\text{C}$  at a rate of  $10^{\circ}\text{C}/\text{minute}$ .

## Appendices.

## APPENDIX 1: EQUILIBRIUM WATER CONTENTS OF HYDROGELS.

*cis*-1,2-bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene (30).

% w/w comonomer	1/%	2/%	3/%	4/%	Av.EWC /%	$\sigma_{n-1}$
0	37.7	37.4	37.5	37.8	37.6	0.18
5	35.8	36.7	36.0	36.7	36.3	0.47
10	35.8	35.4	35.1	35.9	35.6	0.37
20	32.6	33.0	33.0	32.6	32.8	0.23
25	33.1	32.5	32.9	33.3	32.9	0.34
35	29.9	30.2	30.3	30.3	30.2	0.19

Sodium Hydroxide Treated Samples of (30).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.EWC /%	$\sigma_{n-1}$
0	36.7	36.9	36.9	36.6	36.8	0.15
5	44.0	44.2	43.5	43.4	43.8	0.39
10	53.3	54.1	53.9	53.7	53.8	0.34
20	72.0	72.3	72.0	72.7	72.3	0.23

*cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene (29).

Wt% Comonomer	1/%	2/%	3/%	4/%	Av.EWC /%	$\sigma_{n-1}$
5	34.6	34.2	34.6	34.7	34.5	0.22
10	33.6	33.3	33.7	32.7	33.3	0.45
12.5	32.6	32.9	32.6	32.7	32.7	0.14
20	30.5	30.8	30.4	31.4	30.8	0.45
40	25.0	27.0	26.1	25.1	25.8	0.94
60	22.9	22.5	22.0	22.4	22.5	0.37

*cis*-1,2-bis(3-butenoyloxy)-3,5-cyclohexadiene (23).

% w/w Comonomer	1 /%	2 /%	3 /%	4 /%	Av.EWC /%	$\sigma_{n-1}$
5	35.4	35.2	35.5	35.2	35.3	0.15
10	33.8	33.5	33.8	33.2	33.6	0.29
20	30.6	30.9	30.9	30.8	30.8	0.14

*cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene (31).

% w/w Comonomer	1 /%	2 /%	3 /%	4 /%	Av.EWC /%	$\sigma_{n-1}$
5	38.1	38.3	38.1	38.1	38.2	0.10
10	39.7	39.7	39.6	39.6	39.7	0.06
15	41.2	40.5	41.1	40.7	40.9	0.33

*cis*-1,2-bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene (32).

% w/w Comonomer	1 /%	2 /%	3 /%	4 /%	Av.EWC /%	$\sigma_{n-1}$
5	35.3	35.1	35.9	36.1	35.6	0.48
10	34.9	34.5	34.2	34.0	34.4	0.39

## APPENDIX 2: TENSILE STRENGTH OF HYDROGELS.

*cis*-1,2-bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene (30).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av. $\sigma_b$ /MPa	SD
0	0.702	0.693	0.627	-	0.674	0.041
5	0.286	0.555	0.652	0.231	0.431	0.204
9.3	0.467	0.688	0.647	0.556	0.590	0.098
10	0.618	0.752	0.836	0.561	0.692	0.125
20	0.887	0.988	0.906	0.802	0.896	0.076
25	0.672	0.767	0.466	0.758	0.666	0.140
35	0.843	0.882	0.961	0.950	0.909	0.056

*cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene (29).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av. $\sigma_b$ /MPa	SD
5	0.778	0.684	0.606	0.583	0.663	0.088
10	0.686	0.624	0.553	0.750	0.653	0.084
12.5	0.612	0.767	0.655	0.691	0.681	0.066
20	0.741	0.676	0.917	0.854	0.779	0.103
40	0.698	0.700	0.639	0.834	0.717	0.083
60	0.634					

*cis*-1,2-bis(3-butenoyloxy)-3,5-cyclohexadiene (23).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av. $\sigma_b$ /MPa	SD
5	0.466	0.558	0.512	0.523	0.515	0.038
10	0.352	0.644	0.667	0.389	0.513	0.166
20	0.724	0.653	0.705	0.570	0.663	0.069

*cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene (31).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av. $\sigma_b$ /MPa	SD
5	0.433	0.442	0.221	0.373	0.367	0.102
10	0.293	0.595	0.516	0.234	0.409	0.174
15	0.299	0.298	0.213	0.267	0.269	0.040

*cis*-1,2-bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene (32).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av. $\sigma_b$ /MPa	SD
5	0.946	0.675	0.619	0.658	0.725	0.15
10	0.740	0.757	0.649	0.512	0.665	0.11

### APPENDIX 3: YOUNG'S MODULUS FOR HYDROGELS.

*cis*-1,2-bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene (30).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av.E /MPa	SD
0	0.474	0.528	0.486	-	0.496	0.029
5	0.475	0.398	0.410	0.499	0.446	0.049
9.3	0.523	0.483	0.537	0.482	0.506	0.028
10	0.432	0.427	0.403	0.449	0.428	0.019
20	1.130	1.498	1.243	1.295	1.325	0.15
25	0.660	0.728	0.595	0.710	0.673	0.06
35	0.784	0.715	0.752	0.820	0.768	0.045

*cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene (29).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av.E /MPa	SD
5	0.484	0.519	0.542	0.530	0.519	0.025
10	0.498	0.500	0.444	0.494	0.493	0.031
12.5	0.519	0.572	0.505	0.514	0.528	0.030
20	0.594	0.488	0.589	0.551	0.557	0.038
40	0.467	0.476	0.486	0.488	0.479	0.010
60	0.379					



*cis*-1,2-bis(3-butenoyloxy)-3,5-cyclohexadiene (23).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av.E /MPa	SD
5	0.460	0.430	0.469	0.457	0.454	0.017
10	0.526	0.556	0.580	0.500	0.541	0.035
20	1.165	1.105	1.197	1.498	1.241	0.175

*cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene (31).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av.E /MPa	SD
5	0.214	0.194	0.241	0.232	0.220	0.021
10	0.102	0.092	0.083	0.104	0.095	0.010
15	0.069	0.060	0.091	0.074	0.073	0.013

*cis*-1,2-bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene (32).

% w/w Comonomer	1/MPa	2/MPa	3/MPa	4/MPa	Av.E /MPa	SD
5	0.516	0.504	0.566	0.554	0.535	0.030
10	0.451	0.459	0.450	0.499	0.465	0.023

#### APPENDIX 4: ELONGATION TO BREAK OF HYDROGELS.

*cis*-1,2-bis(2,3-epoxybutanoyloxy)-3,5-cyclohexadiene (30).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.E <sub>b</sub> /%	SD
0	192.5	176.3	176.3	-	181.7	9.4
5	82.5	166.9	210.6	61.3	130.3	70.3
9.3	121.3	183.8	185.0	149.4	159.8	30.6
10	188.8	227.5	256.3	164.4	209.2	40.7
20	221	224	246	225	229	11.5
25	190	205	116	203	178	42
35	204	211	221	218	213	8

*cis*-1,2-bis(10,11-epoxyundecanoyloxy)-3,5-cyclohexadiene (29).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.E <sub>b</sub> /%	SD
5	208	176	150	143	169	29
10	176	170	164	191	175	12
12.5	163	219	175	185	185	24
20	214	238	287	268	243	34
40	163	160	149	180	163	13
60	172					

*cis*-1,2-bis(3-butenoyloxy)-3,5-cyclohexadiene (23).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.E <sub>b</sub> /%	SD
5	134.4	166.9	144.4	146.9	148.1	13.6
10	88.8	175.0	181.3	100.6	136.5	48.5
20	140.0	121.3	127.5	95.6	121.1	18.7

*cis*-1,2-bis(trimethylsiloxy)-3,5-cyclohexadiene (31).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.E <sub>b</sub> /%	SD
5	263	285	128	218	223	70
10	365	538	539	305	437	120
15	430	464	303	382	395	70

*cis*-1,2-bis(*tert*-butyldimethylsiloxy)-3,5-cyclohexadiene (32).

% w/w Comonomer	1/%	2/%	3/%	4/%	Av.E <sub>b</sub> /%	SD
5	263	285	128	218	223	70
10	188	269	290	225	243	46

## APPENDIX 5: DIFFERENTIAL SCANNING CALORIMETRY RESULTS.

Comonomer	EWC / %	Freezing Water / %	Non-Freezing Water / %
pHEMA	37.6	16.6	21.0
5%w/w DHCD-EB	36.3	11.9	24.4
10%w/w DHCD-EB	35.6	9.6	26.0
20%w/w DHCD-EB	32.8	3.8	29.0
5%w/w DHCD-EB NaOH treated	43.8	12.4	31.4
10%w/w DHCD-EB NaOH treated	53.8	20.2	33.6
20%w/w DHCD-EB NaOH treated	72.3	49.8	22.5
5%w/w DHCD-VA	35.3	4.3	31.0
10%w/w DHCD-VA	33.6	4.7	28.9
20%w/w DHCD-VA	30.8	2.6	28.2
5%w/w DHCD-EU	34.5	10.7	23.8
10%w/w DHCD-EU	33.3	4.6	28.7
20%w/w DHCD-EU	30.8	3.8	27.0
5%w/w DHCD- TBDMS	35.6	7.3	28.3
10%w/w DHCD- TBDMS	34.4	1.4	33.0

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