## *Enantiomer discrimination and ion receptors*

Bates, Paul Stephen

# ENANTIOMER DISCRIMINATION AND ION RECEPTORS

by

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A Thesis submitted for the degree of Doctor of Philosophy at the University of Durham

April 1993

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Dedicated to my wife Maureen

# ABSTRACT

## ENANTIOMER DISCRIMINATION AND ION RECEPTORS

Highly lipophilic α-, β- and γ- cyclodextrin derivatives were prepared in order to obtain enantiomer selective ionophores for β-aryl ammonium ions and selective ionophores for tetrahedral ammonium ions. The extent of cyclodextrin functionalisation and the homogeneity of the products was investigated by chemical depolymerisation, $^{1}H$ and $^{13}C$ NMR and (+)-FAB-, FD- and ES- mass spectral analysis. For each cyclodextrin, the products of alkylation were found to consist of several constitutional isomers and homologues.

These highly lipophilic molecules were incorporated into solvent polymeric membranes and investigated as electrochemical sensors for chiral molecules incorporating an aryl ring. Electrodes using BBPA as the plasticizer were stable and well defined with a limit of detection for ephedrine of -log[c] = 6.5. Interference from serum levels of $Na^{+}$, $K^{+}$, $Ca^{2+}$ and $Mg^{2+}$ is minimal ($-\log K^{POT} = 3.9$). The electrodes were highly enantioselective in binding ephedrine and closely related homologues. The mechanism of enantiomer discrimination was investigated by several multi-nuclear NMR techniques and the complexation process was investigated by ES-MS.

Lipophilic per-O-octylated α-, β- and γ- cyclodextrins exhibit size selectivity and cation discrimination in the binding of $^{+}NH_4$, $^{+}NMe_4$ and $^{+}NEt_4$. Sensors based on per-O-octyl-β-cyclodextrin show excellent sensitivity and good selectivity for $^{+}NMe_4$ over metal cations and may be used for the detection of cationic surfactants. Complexation was studied *in situ* and competitively by ES-MS as well as by $^{1}H$ and $^{14}N$ relaxation time acquisition.

The possibility of developing several chiral crown ether and cyclam based ionophores was also investigated.

Paul Stephen Bates
April 1993

# ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof. David Parker, for his support, endless enthusiasm and encouragement throughout the course of my research.

My special thanks go to Dr. Ritu Kataky who performed the potentiometric studies. Without her knowledge, patience and expertise the more informative sections of this thesis would be incomplete.

I am indebted to Dr. Ray Matthews for his help in obtaining useful NMR information from both the AC 250 and AMX 500 instruments, to Mrs Julia Say for running numerous NMR spectra and to Dr. A Kenwright for his insight into the pit-falls of using novel NMR pulse sequences.

I would also like to thank Dr. Brian Green (Fisons Instruments) for running the many electrospray mass spectra detailed in this thesis, Dr. Mike Jones for several informative discussions about the finer points of mass spectral analysis and Miss L. Turner for carrying out the chemical ionisation mass spectral analyses. My thanks also go to Messrs. R. Hart and G. Haswell for supplying excellently crafted glassware and Mrs J. Dostal for carrying out the elemental analyses.

Finally I feel obliged to mention the continual 'support' of all my fellow researchers in lab 27, especially Luke, Mark and Russell.

I would like to thank S. E. R. C. for financial support of this project.

CONTENTS

# CHAPTER ONE
# INTRODUCTION

## 1.1 ENANTIOMER DISCRIMINATION AND ION RECEPTORS

At the outset of this project several receptor frameworks were known which had been shown to interact with achiral and chiral substrates in a size-, structural and enantiomer selective manner[1]. Some of the more successful approaches are summarized in the following sections of this chapter, although natural ionophores[2] will only be referred to if necessary in the context of the discussion. The majority of the discussion concentrates on the cyclodextrins and in particular their derivatives which have formed the basis of the experimental investigation and complexation studies detailed in chapters two, three and four.

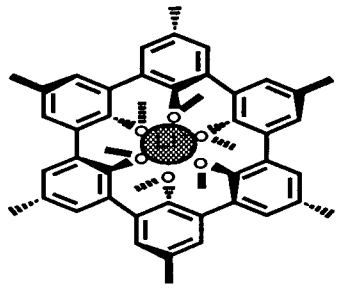
## 1.2 HOST DESIGN

The design of artificial receptor molecules[3] requires manipulation of the energetic and stereochemical features of noncovalent intermolecular forces (such as electrostatic interactions, hydrogen bonding, van der Waals forces and London forces) within a defined molecular architecture. In order to achieve selective recognition it is desirable that receptor and substrate be in contact through interaction at several binding sites. This occurs when the receptor is able to wrap around or encapsulate its guest so as to establish numerous noncovalent binding interactions and to sense its molecular size, shape and architecture.

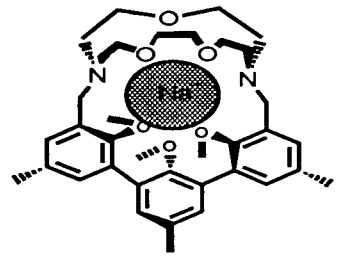
### 1.2.1 Preorganisation of Binding Sites

The degree of host binding site preorganisation has a profound influence upon the binding strength and selectivity of the host for a range of guest species[4]. Whilst this term suggests that it is entropic effects which are important the enthalpic effects of binding site preorganisation cannot be

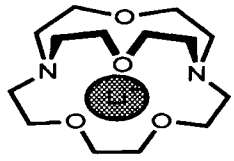
ignored. That is, enthalpic destabilisation of the ground state as a result of preorganisation may be a significant driving force in complex formation[5]. Figure 1.1 shows several synthetic host molecules which differ in the degree of binding site preorganisation. Comparison of the binding strengths for these, and many other hosts[6], illustrates that binding site preorganisation is a central determinant of the host binding power.



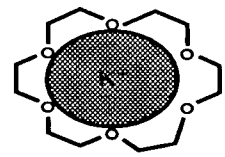
Spherand
$-\Delta G° > 90$ kJ mol$^{-1}$



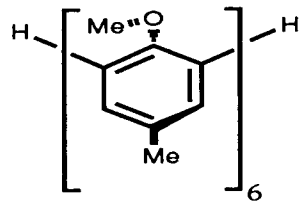
Cryptaspherand
$-\Delta G° \approx 80$ kJ mol$^{-1}$



Cryptand
$-\Delta G° \approx 65$ kJ mol$^{-1}$



Crown Ether
$-\Delta G° \approx 45$ kJ mol$^{-1}$




Podand
$-\Delta G° < 25$ kJ mol$^{-1}$ for $Na^+$, $K^+$, $Li^+$ as guests

Figure 1.1. Host structures arranged in order of deceasing $-\Delta G°$ values for binding of their most complementary guest.

The most preorganised example, the spherand[7], possesses a single conformation ideally arranged for binding $Li^+$. Its oxygen binding sites are buried deep within a hydrocarbon shell which prevents solvation and hence the need for desolvation upon complex formation. The free energy cost of binding site organisation has already been 'paid' during synthesis and therefore will not contribute to a less favourable $\Delta G°$ for complex formation. This spherand is preorganised for binding with its ground state conformation closely resembling that of its complex. Moving down the series to the less preorganised crown ether and acyclic podand the less favourable $\Delta G°_{complex}$ can be attributed to a greater need for binding site reorganisation and ether oxygen desolvation for complex formation to occur.

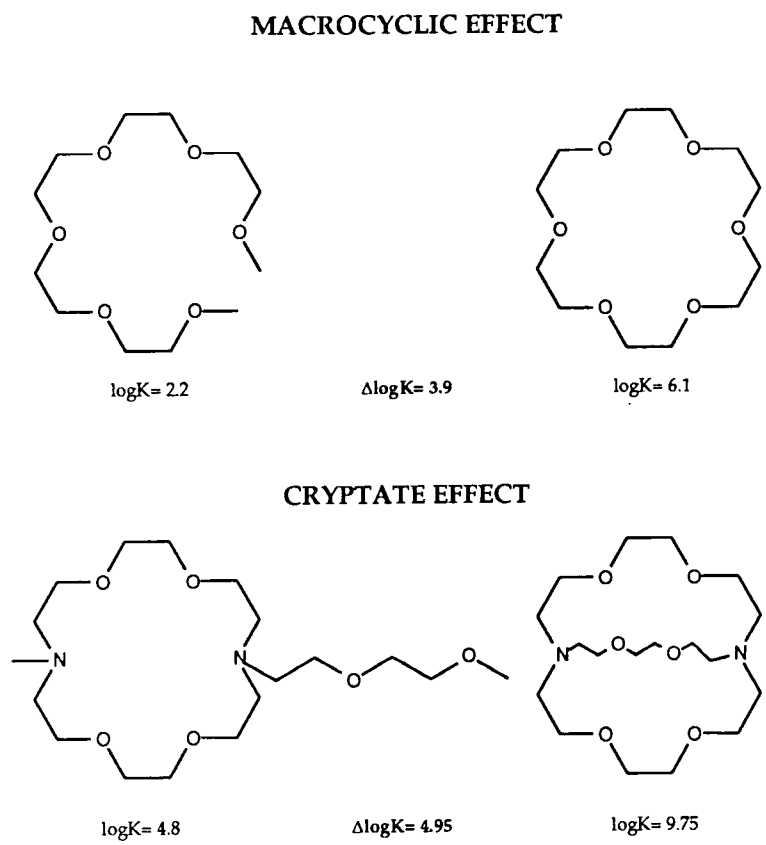



Figure 1.2. The macrocyclic and cryptate effect expressed as $\Delta$logK for $K^+$ as guest in $H_2O$ at 25°C.

What is evident however is a large increase in complex stability in moving from a non macrocyclic to a macrocyclic host and then from a macrocyclic to a macrobicyclic host. These two effects of host preorganisation have become known as the macrocyclic and cryptate effect respectively, figure 1.2. Although macrocyclisation does contribute significantly to a favourable free energy change for complexation, a large amount of reorganisation is still required for guest complexation. Figure 1.3 shows the crystal structure of 18-crown-6 and its potassium complex.

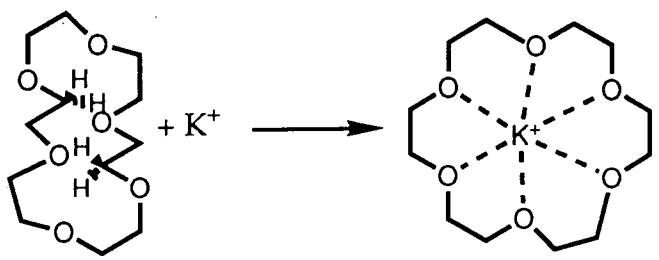
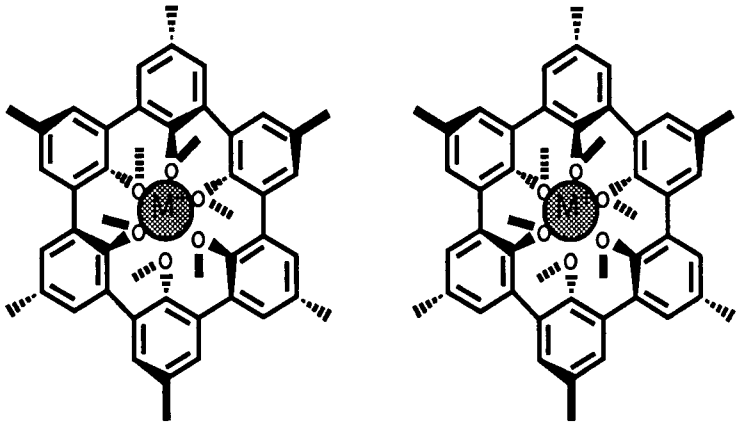



Figure 1.3. Crystal Structure of 18-Crown-6 and its Potassium Complex.

In the crystal structure of 18-Crown-6 the uncomplexed crown possesses inward turning methylene groups that fill the 'hole'[8] with no convergent arrangement of binding sites evident. In the complex the oxygens all turn inward and the ethylene glycol units possess a gauche conformation[9]. It is this need for reorganisation that leads to a less stable complex through reduction in conformational freedom. The conformational mobility of the uncomplexed crown also allows the binding sites to be highly solvated in solution. This may lead to a reduction in complex stability through the need for desolvation prior to complex formation[10].

### 1.2.2 Structuring of Complexes: Host - Guest Complementarity

Although binding site preorganisation determines the binding power of the host it is the complementarity of the host and guest binding sites which determines the extent of structural recognition. The binding energy at a single contact site is at most a few kilojoules per mole, much less than that of a covalent bond. Thus contact at several sites between the host and guest is required for structuring of complexes. The extent of such contacts depends on the complementary placement of binding sites in the complexing partners[11]. Figure 1.4 shows structural recognition of alkali metal ions by spherands, as determined by Cram.



$Li^+/Na^+$ >600 $Na^+/K^+$ $>10^{10}$

Figure 1.4. Structural recognition by Spherands[12] measured by $K_a^A/K_a^{A'}$ values for alkali metal picrates at 25 °C in $CDCl_3$ saturated with $D_2O$.

Structural recognition is a fundamental aspect of chiral recognition in complexation. In such a case the host is chiral and binding site complementarity has a chiral as well as structural aspect. In order for selective chiral discrimination to occur the chiral binding sites of the host

must be complementary to one enantiomer of the guest but involve some disfavoured, often steric, interaction with the other enantiomer. Chiral recognition is discussed further in section 1.4 in relation to the recognition of primary ammonium ions.

### 1.2.3 Chemical Sensors

Although selective recognition of a guest species is the primary aim of any potential host it is also important that the host should contain a chemical moiety capable of translating the binding event into a detectable physical change. This physical change may, for example, be electrochemical[13], UV-VIS spectrophotometric[14] or fluorescent in nature.

## 1.3 RECOGNITION OF INORGANIC IONS

The simplest recognition process involves either a positively charged metal cation or a negatively charged halide anions with a synthetic or natural receptor. This implies spherical recognition. The complexation chemistry of alkali cations has developed rapidly over the past two decades with the discovery of several classes of more or less powerful synthetic macrocycles such as crown ethers and spherands as well as macropolycycles such as cryptands, prototypical examples of these frameworks have already been given in figure 1.1.

The problem of spherical recognition is that of selecting a given spherical ion from amongst a collection of different spheres of the same charge. Some of the more successful synthetic approaches for the discrimination of, in particular, alkali and alkaline earth metal cations are discussed below. The

thermodynamics and kinetics of the cation - macrocycle interaction have been reviewed by Izatt[15].

### 1.3.1 Crown Ether Derivatives

In 1967 Pedersen[16] reported the synthesis and properties of a wide range of macrocyclic polyethers. Dibenzo-18-Crown-6, figure 1.5, was the first crown ether synthesized and the first neutral synthetic compound shown to be capable of complexing metal ions. Since that time, crown ethers have become a very important member of the class of molecules known as organic hosts[17].

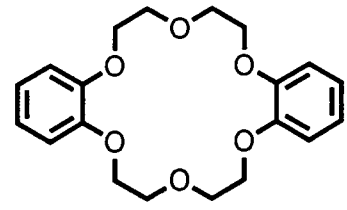


Figure 1.5 Dibenzo 18-Crown-6

Numerous crown ether derivatives have subsequently been synthesized over the past two decades. The aim has been to modify the basic framework in order to facilitate the selective complexation of inorganic cations, such as lithium, sodium and potassium (or primary organic ammonium ions). Peripheral substituents on a crown framework add a 'third dimension' to the receptor. This influences the binding site preorganisation within the macrocyclic ring and changes the overall lipophilicity of the host, often reducing the extent of binding site solvation. Such modification is often manifested in an increased selectivity towards guest molecules. In 1975 Lehn synthesized the chiral crown ether N, N, N′, N′, N″, N″, N‴, N‴-octamethyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxamide.

This crown derivative formed a complex with calcium[18] (K = 100, 26°C, $H_2O$) which was considerably more stable than that formed by dicyclohexyl 18-crown-6 (K ≈ 3). The inclusion selectivity is also affected by the basicity of the heteroatoms.

Several ingenious sensing mechanisms have been used in attempts to develop crown ether based chemical sensors, these include fibre optics, fluorescence and UV-Shifts. However perhaps the most successful approach has been to develop lipophlic crown ether derivatives for incorporation into an ion selective electrode. Investigations along such lines have been studied in detail by several workers such as Simon[19]. This work concentrated upon the development of neutral, and therefore pH insensitive lipophilic derivatives which were suitable for incorporation into a PVC polymeric membrane. Despite the fact that the electrochemical behaviour of such derivatives was good, the potentiometric selectivity for one cation in the presence of several others was often relatively poor. However, such work did show that the potentiometric selectivity of these sensors for a given inorganic cation was primarily governed by the complexation specificity of the ionophore involved and not merely the lipophilicity of the system.

In 1990 Parker and coworkers further developed these observations by introducing carboxamide side chains into a 14-crown-4 framework which were able to interact with a complexed lithium ion as well as confer lipophilicity. This modified both the physical properties and the inclusion selectivity of the crown ether derivative and resulted in a lithium sensor with a $10^{3.25}$:1 selectivity over sodium[20], figure 1.6.

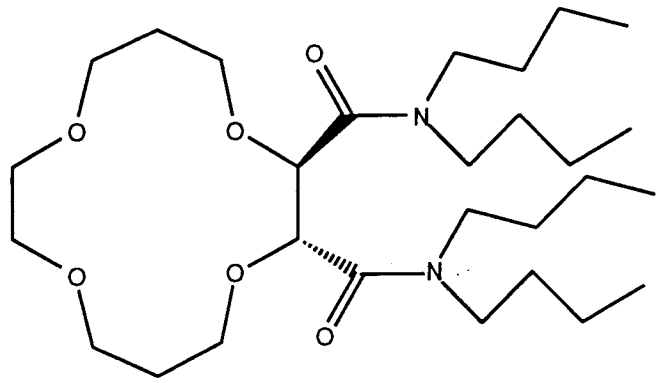


Figure 1.6. Parker's Lithium Ion Sensor.

### 1.3.2 Cryptands

Macrobicyclic cryptands[21] form highly stable and selective cryptates with alkali and alkaline earth metal cations whose size is complementary to that of the cavity. This results in cation encapsulation as illustrated by figure 1.7. Quaternary ammonium derivatives of such macrobicycles, free of oxygen donors, also bind spherical anions[22].

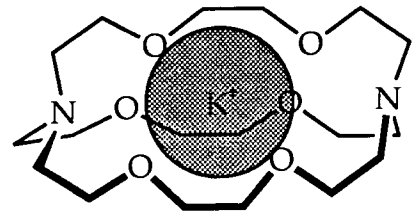


Figure 1.7. Potassium Ion Encapsulation.

## 1.4 RECOGNITION OF AMMONIUM IONS AND RELATED SUBSTRATES

In view of the important role played by substituted ammonium ions in chemistry and biology, the development of receptor molecules capable of recognising such substrates is of special interest. Several receptor frameworks have been employed and many derivatives investigated.

### 1.4.1 Crown Ethers

Macrocyclic polyethers bind primary ammonium ions by anchoring the $^{+}NH_3$ group via three $^{+}N\text{-}H\cdots O$ hydrogen bonds[23]. This interaction is essentially of an ion-dipole nature. With 18-crown-6 it involves the $C_3$ related oxygens[24]; Figure 1.8

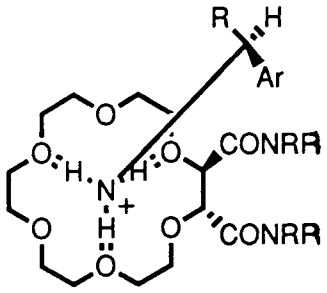



Figure 1.8. Binding of a Primary Ammonium ion by 18-crown-6

An aliphatic 18-membered macrocyclic polyether is particularly suitable for complexing organic primary ammonium cations[3]. Crowns containing aromatic ethers generally form less stable complexes than those containing only aliphatic ethers owing, at least in part, to the weaker basicity of the aromatic ether oxygens and to ring deformations introduced by the aromatic moiety.

The introduction of side chains, as discussed in relation to spherical recognition, confers a 'third dimension' to the macrocycle allowing analogies to be drawn with the macropolycyclic cryptands[25] which display much stronger complexation towards alkali, alkaline earth and ammonium cations than unsubstituted crown ethers. For example, the tetracarboxylate

shown in figure 1.9 conserves the desirable [18]-$O_6$ ring and adds electrostatic interactions. This macrocycle is said to form the most stable metal ion and ammonium ion complexes of any polyether macrocycle[26].

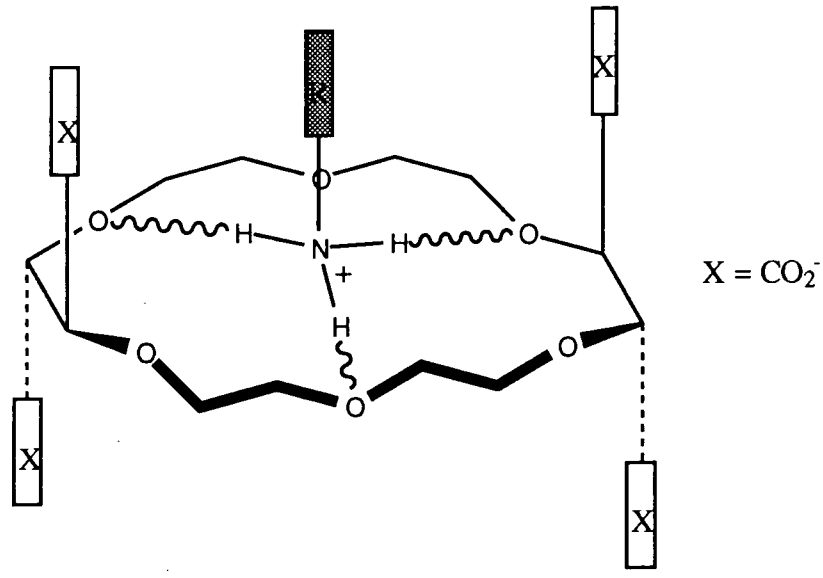
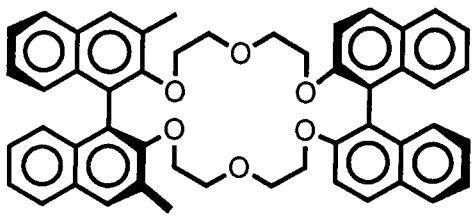



Figure 1.9 Lehn's '3-dimentional' Tetracarboxylate

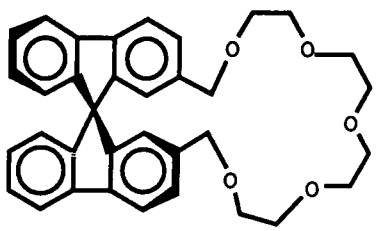
Indeed, the so-called 'lariat ethers'[27] were designed originally to incorporate the binding dynamics of a crown ether with the three - dimensionality of cryptands. It is desirable that the substituted macrocycle be chiral with known absolute configuration in order to allow the development of enantioselective as well as structurally selective ionophores. The synthesis of chiral crown ether derivatives from natural and synthetic sources of chirality has been reviewed[28].

Chiral macrocyclic polyethers which bind chiral ammonium ions with high enantiomer selectivity and behave has as ionophores have been developed by several workers[29, 3]. The most successful approaches have been those taken by Stoddart[30], Prelog[31], Cram[3] and Lehn[32] and this has been recognised

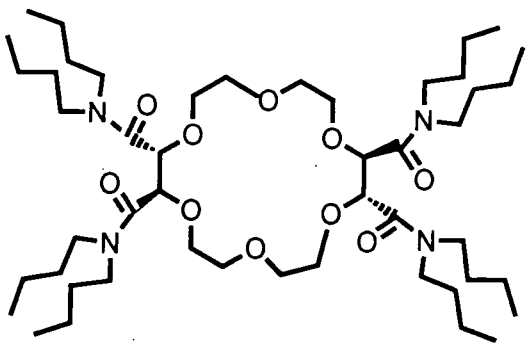
by the award of the 1987 Nobel prize for chemistry to the latter two and Pedersen[33] for his discovery of the crown framework. The molecules which have been developed most successfully as ionophore receptors are depicted in figure 1.10.



Cram[34]



Prelog[35]



Lehn[36]

Figure 1.10 Chiral Crown Ether Derivatives.

All three of these polyether based ionophores show good structural and enantiomeric discrimination for primary ammonium ions but unfortunately suffer from severe alkali cation interference. For example the tetracarboxamide 18-crown-6 derivative developed by Lehn[37] shows good enantioselectivity for the α-phenylethylammonium ion, $PEA^+$ and good selectivity over competing primary ammonium ions such as the ephedrinium ion, $EPH^+$, table 1.4.1A, but only shows a selectivity of 3:1 over potassium cations, table 1.4.1B.

Table 1.4.1A Enantiomer Selectivity of Lehn's tetracarboxamide expressed as a potential difference, $\Delta\Delta E$

| CATION | $\Delta\Delta E=\Delta E_+-\Delta E_-$ |
|---|---|
| $PEA^+$ | 25.1 |
| $EPH^+$ | 2.3 |
| $\psi EPH^+$ | 4.2 |

Table 1.4.1B Selectivity Factors, $_{log}K^{pot}$ for membranes with Lehn's tetracarboxamide (0.1M solution)

| Ion J | $^{log}K^{pot}_{PEAJ}$ |
|---|---|
| $K^+$ | -0.53 |
| $NH_4^+$ | -1.80 |
| $Mg^{2+}$ | -3.28 |

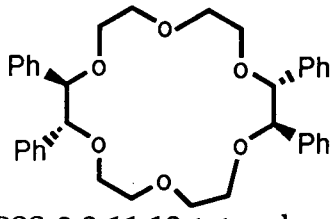
Hence such receptors generally complex alkali metal cations more strongly and therefore selective binding by such a receptor in the presence of serum levels of alkali and alkaline earth cations is difficult to envisage. The interaction of chiral crown ether derivatives with organic ammonium salts has been reviewed[38].

## 1.5 APPLICATIONS OF CHIRAL CROWN ETHER DERIVATIVES

### 1.5.1 Enantioselective Catalysis

Chiral crown ether derivatives have been the subject of several investigations as potential chiral auxiliaries in enantioselective catalysis[39]. The attraction of such compounds is that noncovalent supramolecular structures are numerous and well investigated. Consequently enantioselective catalysis is, at least in principle, feasible.

An example of such an investigation is that of Stoddart and coworkers[40]. Here ammonia-borane complexes of chiral tetraphenyl 18-Crown-6 derivatives were used for the enantioselective reduction of prochiral ketones leading to secondary alcohols. The resulting enantiomeric excesses obtained were an improvement on most of those which had been reported previously with nonmacrocyclic chiral auxiliaries[41]. However, the major advantage of using a chiral crown ether derivative as the chiral auxiliary is that it is not covalently bound to the reactants and hence can easily be recycled.




SSSS-2,3,11,12-tetraphenyl 18-crown-6

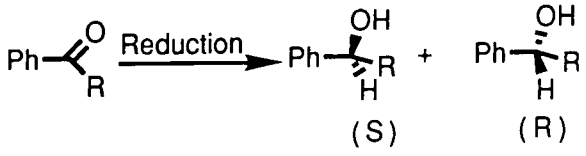



Figure 1.11 Enantioselective Reduction of Aromatic Ketones.

**Table 1.5.1A Reduction of aromatic ketones with $NH_3BH_3$ adducts of 2,3,11,12-Tetraphenyl-18-Crown-6.**

| Crown Ether configuration | Ratio $NH_3BH_3$ : Crown Ether | Ketone | % yield | ee | absolute configuration |
|---|---|---|---|---|---|
| RRRR | 1:1 | PhCOMe | 70 | 28 | S |
| SSSS | 1:1 | PhCOMe | 70 | 20 | R |
| SSSS | 1:1 | $PhCOPr^i$ | 71 | 67 | R |
| SSSS | 1:1 | $PhCOBu^t$ | 73 | 64 | R |

Such derivatives of crown ethers are often employed because of their $C_2$ symmetry which confers facial equivalence to the macrocycle. This, and many similar investigations, has led to enantiomeric excesses in the region of 65%.

### 1.5.2 Application of Crown Ethers in Chromatography

One of the first successful applications of crown ethers to chromatography was described by Cram[42] in 1979. It involved the immobilisation of a bisbinaphthyl host, figure 1.10, in a polystyrene based resin and resulted in enantiomeric resolution of several amino acid salts. Separation factors, $\alpha$, ranging from 26 to 1.4 were reported.

Crown ethers have also been employed successfully in liquid chromatography[43] for selective separations as components of either the stationary or mobile phase. However, their use in gas chromatography has been quite limited[44]. Simolkova-Keulemansova[45] has investigated the use of benzo and benzopyridine crown ether derivatives in gas chromatography stationary phases for the separation of alcohols, amines, and alkanes. This work resulted in elution times dependent upon the ability of the sorbate to hydrogen bond to, and hence form an inclusion type complex with, the crown ether stationary phase.

## 1.6 CYCLODEXTRINS

### 1.6.1 Physical Properties

Cyclodextrins are α-1,4-linked oligosaccharides[46] formed from starch by the action of the glucosyltransferase enzyme[47]. They are toroidal in shape[48] with each chiral glucose residue possessing a rigid $^4C_1$ chair conformation[49]. The three major cyclodextrins are crystalline, homogeneous, non-hygroscopic substances, built up from six, seven and eight glucopyranose units as indicated in figure 1.12. Several names have been given to these dextrins, the most popular being α-, β- and γ- cyclodextrin or cyclomaltohexaose, -heptahose and octaose. Table 1.6.1A summarises some of the more important physical properties of these compounds.

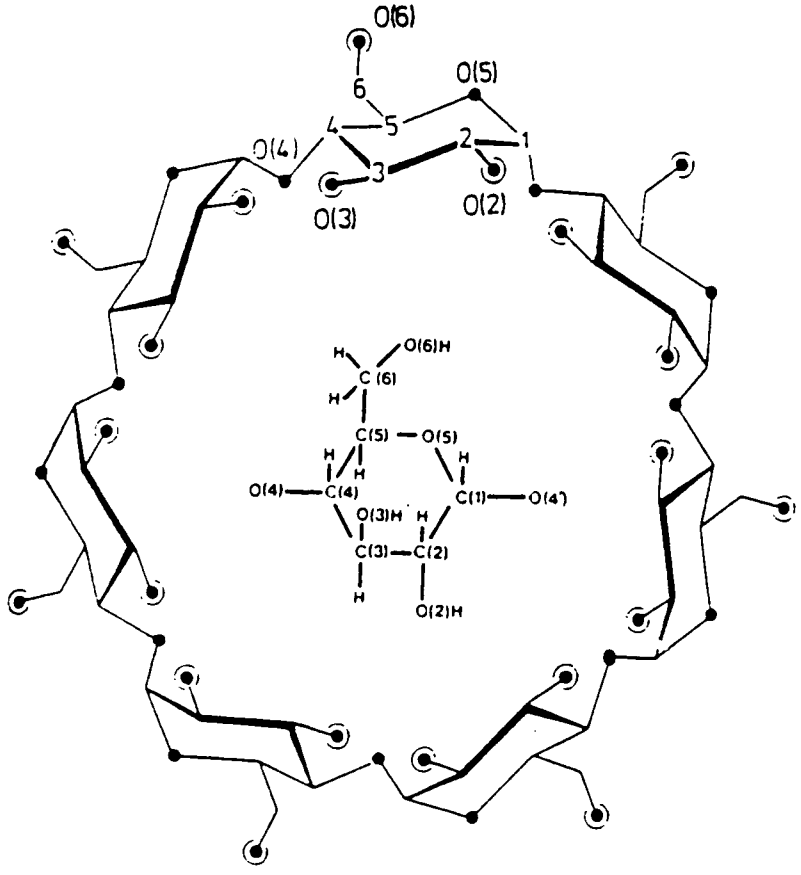



Figure 1.12 Chemical Structure and Numbering Scheme for β-cyclodextrin.

**Table 1.6.1A Physical properties of cyclodextrins**

| | CYCLODEXTRIN | | |
|---|---|---|---|
| | α | β | γ |
| Number of glucose units | 6 | 7 | 8 |
| Number of chiral centres | 30 | 35 | 40 |
| Molecular mass | 972.86 | 1135.01 | 1297.15 |
| External diameter (pm) | 1370-1460 | 1530-1540 | 1690-1750 |
| Internal diameter (pm) | 470-520 | 600-650 | 750-850 |
| Volume of cavity($nm^3$) | 0.176 | 0.346 | 0.510 |
| pKa of hydroxyl groups | | 12.1-12.6 (all) | |
| Solubility in water (grams per $100cm^3$, 25°C) | 14.50 | 1.85 | 23.20 |
| Molarity of saturated solution [M] | 0.114 | 0.016 | 0.179 |
| Melting and decomposition point (K) | 551 | 572 | 540 |

It has been demonstrated by Sundararajan and Rao[50], using conformational energy maps, that cyclodextrins with less than six glucose residues cannot be formed as a consequence of steric ring strain and the six fold nature of the starch helix[51]. Higher homologues, containing up to twelve glucose units have been reported by French[52]. The macrocyclic rings of these higher homologues will have high flexibility making selective complexation, and hence purification, unlikely.

As figure 1.13 shows, a consequence of the $C_1$ conformation of the glucopyranose units is that all secondary hydroxyl groups are situated on one edge of the macrocyclic cavity and all primary hydroxyls are on the other. The cavity interior is lined by the hydrogen atoms of C(3) and C(5) and the glycosidic oxygen bridges. The lone pairs on the glycosidic oxygens point into the cavity. The overall consequences of these structural feature are a hydrophobic, slightly Lewis basic cavity flanked by two hydrophilic rims.

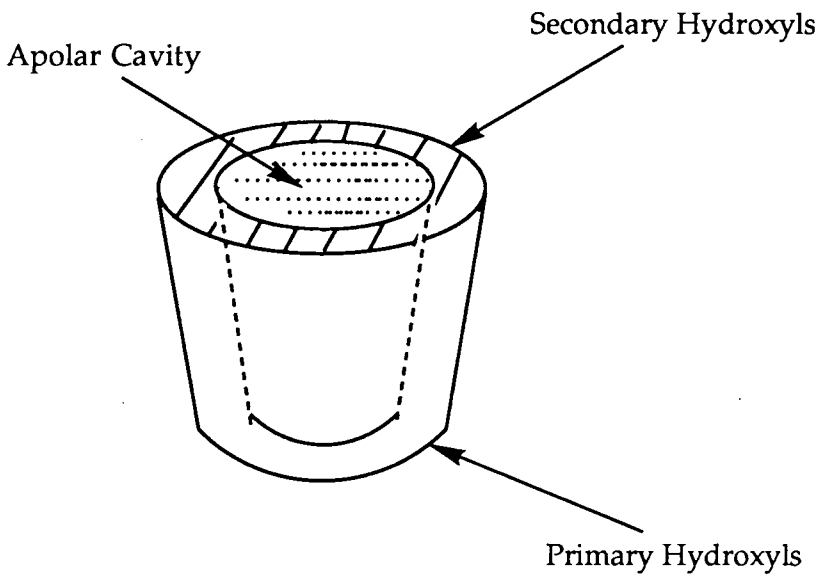



Figure 1.13 Functional structural scheme of cyclodextrins

### 1.6.2 Intramolecular hydrogen bonding

As figure 1.14 depicts, the (2)-O group of one glucose subunit can hydrogen bond with the (3)OH' of an adjacent glucose unit[53]. Within the cyclodextrin molecule a complete secondary belt is formed by these hydrogen bonds, making the whole macrocycle a rigid structure[50]. Conformational energy maps have demonstrated that such hydrogen bonding results in a lowering of energy in α-cyclodextrin (84 kJ $mol^{-1}$) and in β-cyclodextrin (125 kJ $mol^{-1}$).

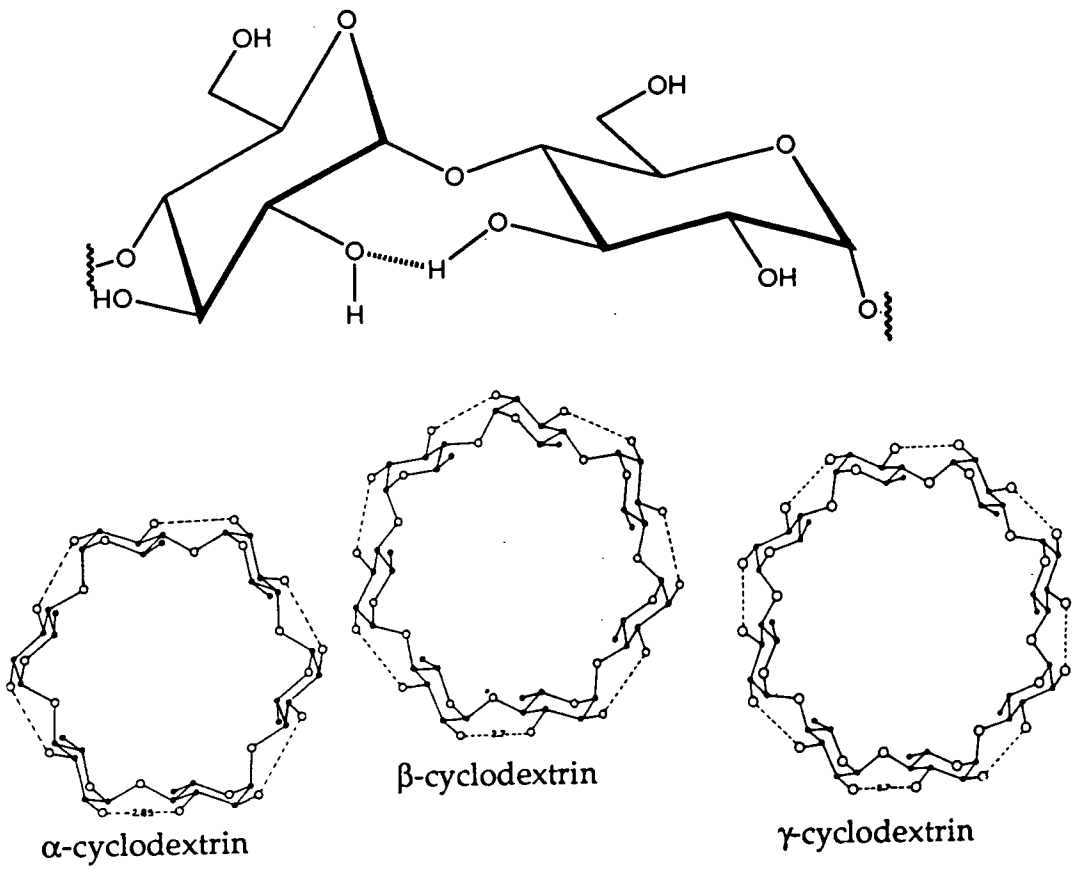



Figure 1.14. O(2)···HO(3) intramolecular hydrogen bonding in cyclodextrins.

### 1.6.3 Solution State Properties

Comparing the water solubilities of the three native cyclodextrins, shown in table 1.6.1A, it is apparent that they behave in a rather anomalous way. The low solubility of β-cyclodextrin, by comparison to α- and γ-, is explained by the ability of β-cyclodextrin to form all seven possible O(2)···OH(3)' hydrogen bonds. This confers a more rigid, less accommodating, structure than for α- and γ- cyclodextrin which are unable to fulfil all their potential hydrogen bonds[54]. In the presence of organic molecules the solubility of all three cyclodextrins generally decreases as a result of complex formation.

### 1.6.4 Industrial Applications

Cyclodextrins are used in the pharmaceutical and food additive industries. In both cases the cyclodextrin can be present free, or as is usually the case, as an inclusion complex to improve the solubility and/or taste of a drug, flavour or other such guest species. Such complexation is also termed 'microencapsulation'[55].

## 1.7 CYCLODEXTRIN INCLUSION COMPLEXES

Cyclodextrins are able to interact with a variety of ionic and molecular species[56]. As discussed earlier in a general sense, the cyclodextrin host and potential guest must possess complementary molecular architecture. That is, the convergent functionality of the cyclodextrin must be complementary to and able to interact with the divergent binding sites of the guest. Figure 1.15 shows a representation of cyclodextrin inclusion complex formation with *p*-xylene.

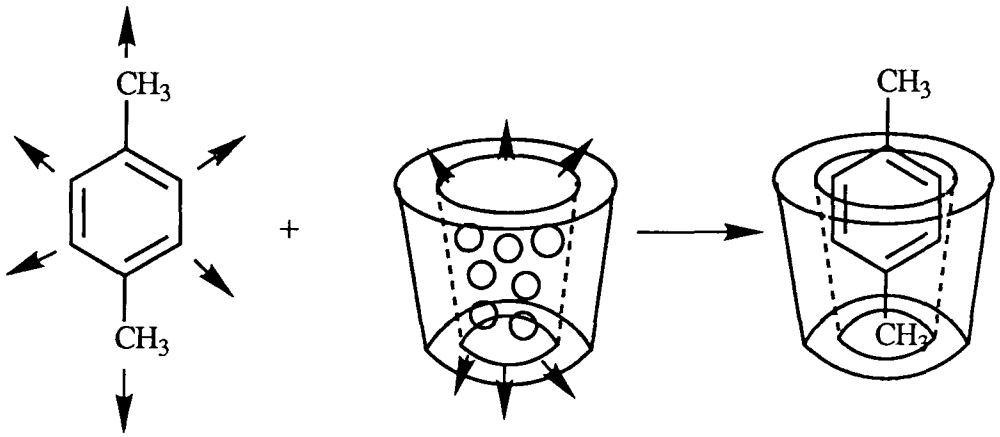



Figure 1.15 Schematic representation of cyclodextrin inclusion complex formation. *p*-Xylene is the guest molecule, O indicates included water.

The inclusion complexes of cyclodextrins with aromatic molecules have been studied by techniques such as U.V. absorption, induced C.D.[57], fluorescence[58] and NMR spectroscopy[59].

### 1.7.1 Geometric Compatibility of Guest

Cyclodextrins are capable of forming inclusion complexes with molecules having a size compatible with the dimensions of the cavity. Hence it is geometric rather than chemical factors that are decisive in determining the types of guest which can penetrate into the cyclodextrin cavity. Thus, as was demonstrated to be the case with other receptors such as spherands, crown ethers and cryptands, the cyclodextrin cavity operates on the basis of size selectivity. The α- β- and γ- cyclodextrins with their different size cavities are able to accommodate molecules of different sizes. This is illustrated by table 1.7.1A.

**Table 1.7.1A Complex Forming Ability of Cyclodextrins with Various Guest Molecules**

| guest | α-cyclodextrin | β-cyclodextrin | γ-cyclodextrin |
|---|---|---|---|
| $CH_3CH_2COOH$ | ✓ | – | – |
| $CH_3CH_2CH_2COOH$ | ✓ | ✓ | – |
| Biphenyl | ✓ | ✓ | ✓ |
| cyclohexane | ✓ | ✓ | ✓ |
| naphthalene | – | ✓ | ✓ |
| anthracene | – | – | ✓ |
| $Cl_2$ | ✓ | – | – |
| $Br_2$ | ✓ | ✓ | – |
| $I_2$ | ✓ | ✓ | ✓ |

✓ indicates ability to form a 1:1 complex

The structures of cyclodextrin complexes may differ significantly in the crystalline state to those in the solution state[60]. In solution a guest molecule may sit inside the cavity and the whole complex is surrounded by a solvate shell. In the crystalline state, the guest molecules can be accommodated not only in the cavity but also in the intermolecular spaces formed by the crystal lattice, or sandwiched between two complex molecules. The included guest is normally oriented in the host in such a way as to achieve the maximum contact between the hydrophobic part of the guest and the apolar cyclodextrin cavity[61]. The hydrophilic part of the guest remains, as far as possible, at the outer edge of the cavity to insure maximum contact with the solvent and the polar hydroxyl groups of the cyclodextrin.

Complex formation with molecules significantly larger than the cavity is also possible[62]. Here only certain groups or even side chains penetrate into the cyclodextrin cavity. This is the case with β-cyclodextrin - steroid derivative and β-cyclodextrin - pharmaceutical drug complexes such as that formed with indomethacin[63]. Although indomethacin is far too large to fit into the cyclodextrin cavity there is inclusion complexation with part of the framework, as figure 1.16 shows.

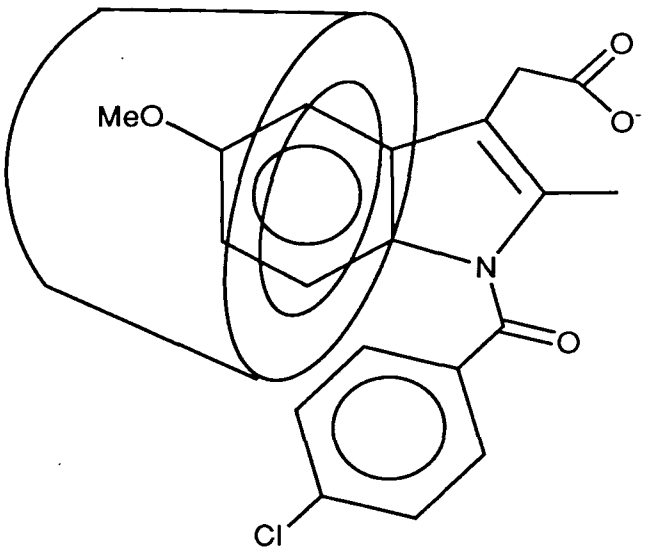



Figure 1.16 Indomethacin inclusion by β-cyclodextrin (in $D_2O$ at pD=7.8)

### 1.7.2 The Driving Force of Complexation

The process of guest inclusion in a cyclodextrin cavity involves the substitution of the included water molecules, or some other such solvent, by the less polar guest. This process is an energetically favoured interaction and both entropy and enthalpy changes play an important role[64]. The exact driving force of complexation is, as yet, not fully understood. It is however understood that that the driving force is a combination of various effects which play a lesser or more important role depending on the guest functionality, solvent polarity, and the extent of derivatisation of the cyclodextrin. All these factors determine the extent to which non-specific van der Waals interactions[65], hydrogen bonding[66] and London forces[67] contribute to a favourable enthalpy change. The dependence of the binding constant upon substrate polarisability[68] indicates that, in general, van der Waals interactions predominate. However, the role of the substitution of water molecules by a guest of the appropriate size, shape and polarity appears quite universal[69]. Water molecules within the cyclodextrin cavity cannot

satisfy their tetrahedral hydrogen bonding capacity as can those in bulk water. Consequently these included water molecules may be regarded as being of relatively high energy[70]. Upon expulsion, complex formation is favoured by a gain in entropy as well as by a gain in potential energy[71].

The release of ring strain upon complexation is only significant in the case of α-cyclodextrin as a contribution to the driving force[72], since the structure of α-cyclodextrin hydrate is distorted whereas those of β- and γ- cyclodextrin are not[70, 73].

### 1.7.3 The Mechanism of Complex Formation

Complex formation by cyclodextrins in aqueous solution has been investigated extensively[74] and has been shown to comprise the following fundamental steps:

1) Water molecules escape from the cyclodextrin cavity and move to an energy level, corresponding to that in the gaseous state. As a consequence van der Waals interactions and the number of hydrogen bonds are decreasing, whilst the translational and rotational degrees of freedom of the water molecules are increasing.

2)The conformation energy of the cyclodextrin ring decreases on relaxing.

3)The guest molecule sheds its hydrate shell and also assumes the state of an ideal gas. This empty hydrate shell collapses and rearranges.

4)The guest molecule, regarded as being in the ideal gaseous state, enters the empty cyclodextrin cavity and the complex is stabilised by van der Waals interactions, and perhaps hydrogen bonding. The guest molecule retains a one-dimensional freedom.

5)The displaced water molecules condense from the gaseous state to the liquid state.

6)The structure of water is restored around the exposed part of the guest molecule, and integrated with the cyclodextrin ring hydrate shell.

### 1.7.4 Thermodynamics of Inclusion

For native cyclodextrin complexes in aqueous solution the thermodynamic parameters $\Delta S$ and $\Delta H$ can be obtained from the temperature dependence of the dissociation constant[75]. The $\Delta H$ value for complex formation is always negative, as a consequence of the release of high energy water[64] and the $\Delta S$ value can be either positive or negative depending on the guest. This indicates that,as suggested earlier, several forces are contributing to complex formation.

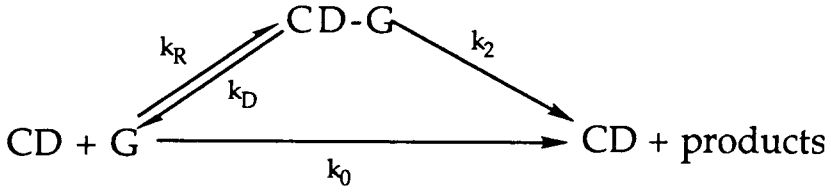
Although thermodynamic data relating to cyclodextrin complexes is often obtained in this way, more accurate results may be obtained via calorimetric investigation. Liveri[76] and co-workers have investigated complex formation between surfactants and cyclodextrins by a calorimetric method. This investigation allowed direct determination of complex stoichiometries and molar enthalpies of formation for several cyclodextrin - surfactant complexes in aqueous solution. It was shown that a single complex of high stability constant is formed.

### 1.7.5 Cyclodextrins as Enzyme Models

### -Catalytic Phenomena

Cyclodextrins and, more often, their derivatives have been proposed as models for enzyme - substrate binding[77]. That is, the apolar cavity of the cyclodextrin is a specific discriminating and orientating site; the cyclodextrin -OH group(s), or other substituent then represents a potential reactive site. Inclusion catalysis by cyclodextrins shows several characteristics of enzyme catalysed reactions such as saturation limit, competitive inhibition and Michaelis-Menton type kinetics[78]. For example, studies of cyclodextrin catalysis of pyrophosphate cleavage[79] and phenyl ester cleavage[80] have led to the conclusion that these reactions are good models for the action of chymotrypsin.

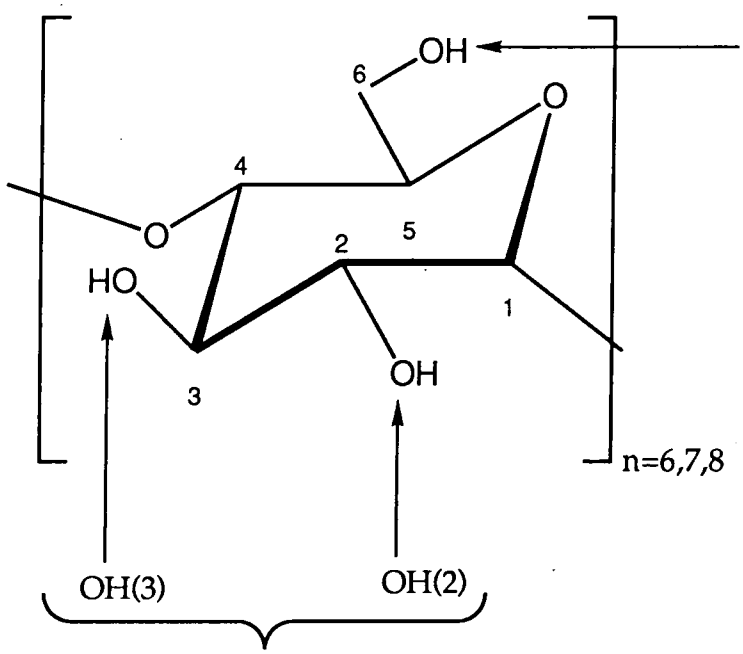
The kinetics of a cyclodextrin catalysed reaction may be represented as:




## 1.8 CYCLODEXTRIN DERIVATIVES

Although the usual chemistry of polysaccharides such as starch and cellulose can be related to that of cyclodextrins, the unique structure and complexation properties of these compounds have directed the synthetic efforts towards selective modification of each type of alcoholic function, figure 1.17. Modification of cyclodextrins has been intensely investigated by several workers with the, often successful, aim of improving their complex forming

and perhaps catalytic abilities. Properties of modified cyclodextrins are hence usually only discussed in terms of their complex forming and/or catalytic activity in relation to introduced groups. However, such substitution affects the conformation and geometry of the host-guest interaction. Consequently, the cyclodextrin host cannot, however tempting, be considered as an immobilised receptor onto which other useful groups can be added if required.




OH(6) Primary hydroxyl and hence most reactive towards substitution.

Although OH(2) and OH(3) are both secondary hydroxyl groups, OH(3) is generally less reactive towards substitution as a result of intramolecular hydrogen bonding.

Figure 1.17. Reactivity of Cyclodextrin Hydroxyl Groups

### 1.8.1 Synthesis of Cyclodextrin Derivatives

As a result of the ability of cyclodextrins to form inclusion complexes with innumerable guests they have been seized upon as 'ideal' substances for the design of synthetic molecular receptors. They can be modified in a variety of ways such as:

-substitution of the hydrogen atoms of the primary and/or secondary hydroxyls (eg. ethers and esters)

-substitution of one or more primary and/or secondary hydroxyl (eg. amino, deoxyhalogeno-)

-oxidation: C(5)-$CH_2OH \Rightarrow$ C(5)-COOH

In chapter two application of the Williamson ether synthesis to the formation of highly lipophilic cyclodextrin derivatives will be discussed in detail. However, in this chapter a very brief overview of the many types of possible derivatives synthesized to date is presented. A full discussion of the synthesis and properties has been reported previously, with such derivatives been treated in detail elsewhere[81].

**The Variety of Cyclodextrin Derivatives**

Figure 1.18 schematically illustrates the structural variety of cyclodextrin derivatives.

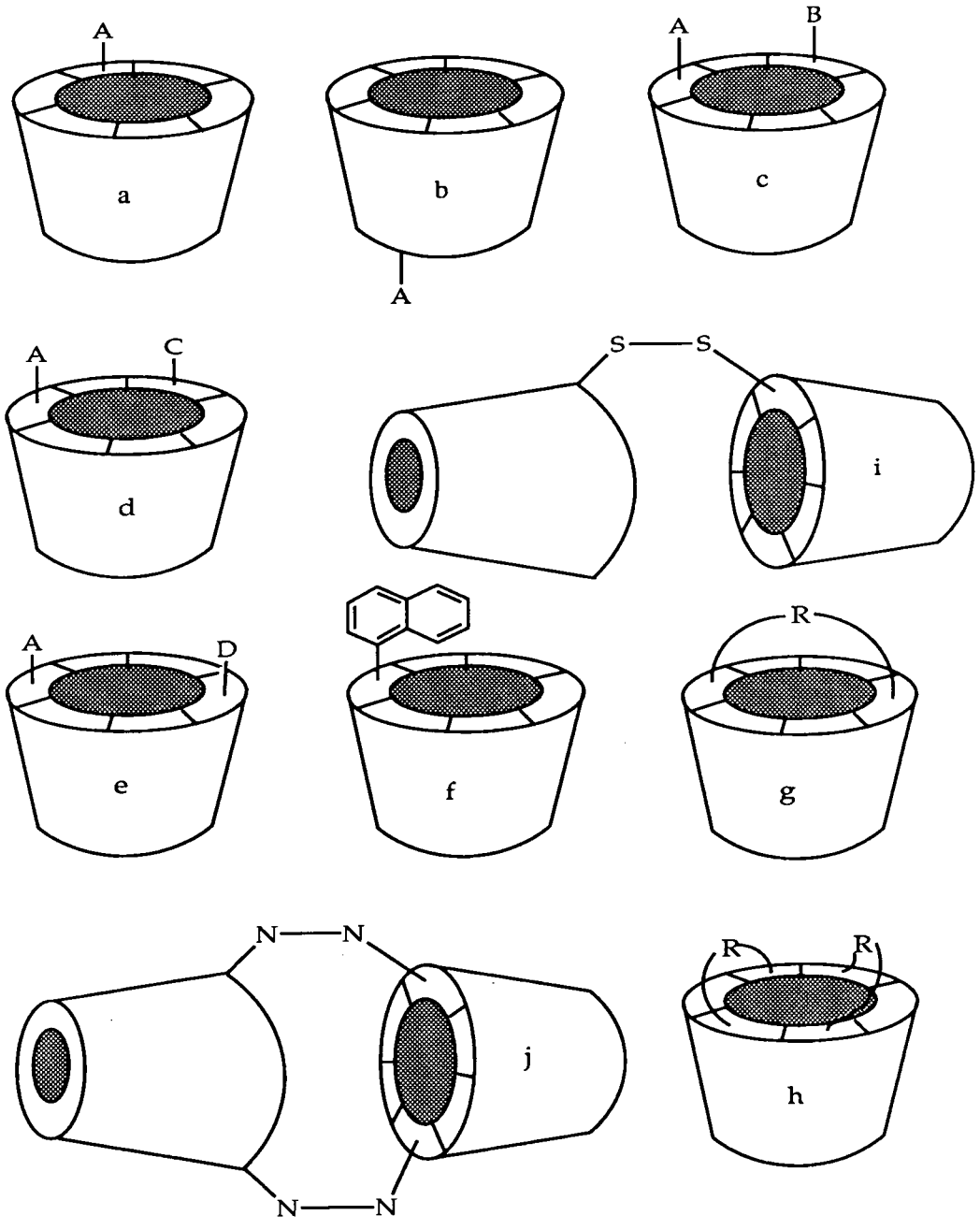



Figure 1.18 Cyclodextrin Derivative Structures; a=monosubstituted on primary hydroxyl side. b=monosubstituted on secondary hydroxyl side. c=A-B, d=A-C and e=A-D disubstituted. f=appended, g=capped. h=double capped. i=duplex with single bridge. j=duplex with double bridge.

## 1.9 INCLUSION CHEMISTRY OF ALKYLATED CYCLODEXTRINS AND RELATED DERIVATIVES

Native cyclodextrins form diastereoisomeric complexes by the inclusion of chiral guests within their cavity. Consequently several attempts have been made to use them as reagents for chiral resolution. Though cyclodextrins consist of six or more optically active D-glucose subunits, the cavity which accommodates guest molecules is a round symmetrical feature. This may cause difficulties in recognising the chirality of included guests, which is suggested by the fact that, with few exceptions[82], only low optical resolution is achieved by the precipitation of racemic compounds with cyclodextrins[83] leading to enantiomeric excesses in the region of 60%. However, functionalisation of the large number of available hydroxyl groups which line the perimeter of the cyclodextrin cavity has two very beneficial results. Firstly the cyclodextrin can be made highly lipophilic , rendering it suitable for chromatography applications, immobilisation in a non aqueous solvent[84] or solvent polymeric membrane. Secondly it has been shown by Harata[85] that methylation, and hence by extrapolation other derivatives, leads to distortion of the regular polygonal symmetry of the parent cyclodextrin in the crystalline state through the removal of the aforementioned intramolecular hydrogen bonds. This distortion of the cavity geometry has been postulated to confer greater enantioselective properties. These effects have been investigated by comparing the crystal structures of native and per-O-methylated cyclodextrins with chiral guests such as 1-phenyl ethanol. It was found that slight structural differences were not sufficient for α-cyclodextrin to discriminate the R and S isomers and include one of them exclusively[86].

Harata found that upon methylation the conformation of both α- and β-cyclodextrins is markedly effected[87]. The removal of the 2(O)···3(OH)' hydrogen bonding causes the glycosidic oxygens to become less planar resulting in increased tilting of the individual pyranose residues with respect to the mean z-axis of the cavity. This tilting causes narrowing of the cavity on the O(6) side and leads to considerable reduction in the symmetry of the system in the crystalline state. The distortion results in a more flexible macrocyclic structure and it is thought that this may be related to greater chiral discrimination. That is, the reduction in the pre-organisation of the cavity upon functionalisation allows closer host-guest interaction which, through induced fit conformational changes of the host, is likely to allow a closer host-guest contact for one enantiomer of a pair.

With achiral guests the complexation geometry may also be influenced by functionalisation of the cyclodextrin host[88]. This is illustrated by the examples in figure 1.19 which shows schematically the inclusion features of α-cyclodextrin and per-O-methylated α-cyclodextrin with *p*-nitrophenol determined by crystallographic analysis[89]. It is apparent that the whole sense of inclusion is reversed upon methylation of α-cyclodextrin. Such changes in inclusion geometry have been ascribed to changes in the shape and size of the host cavity upon alkylation.

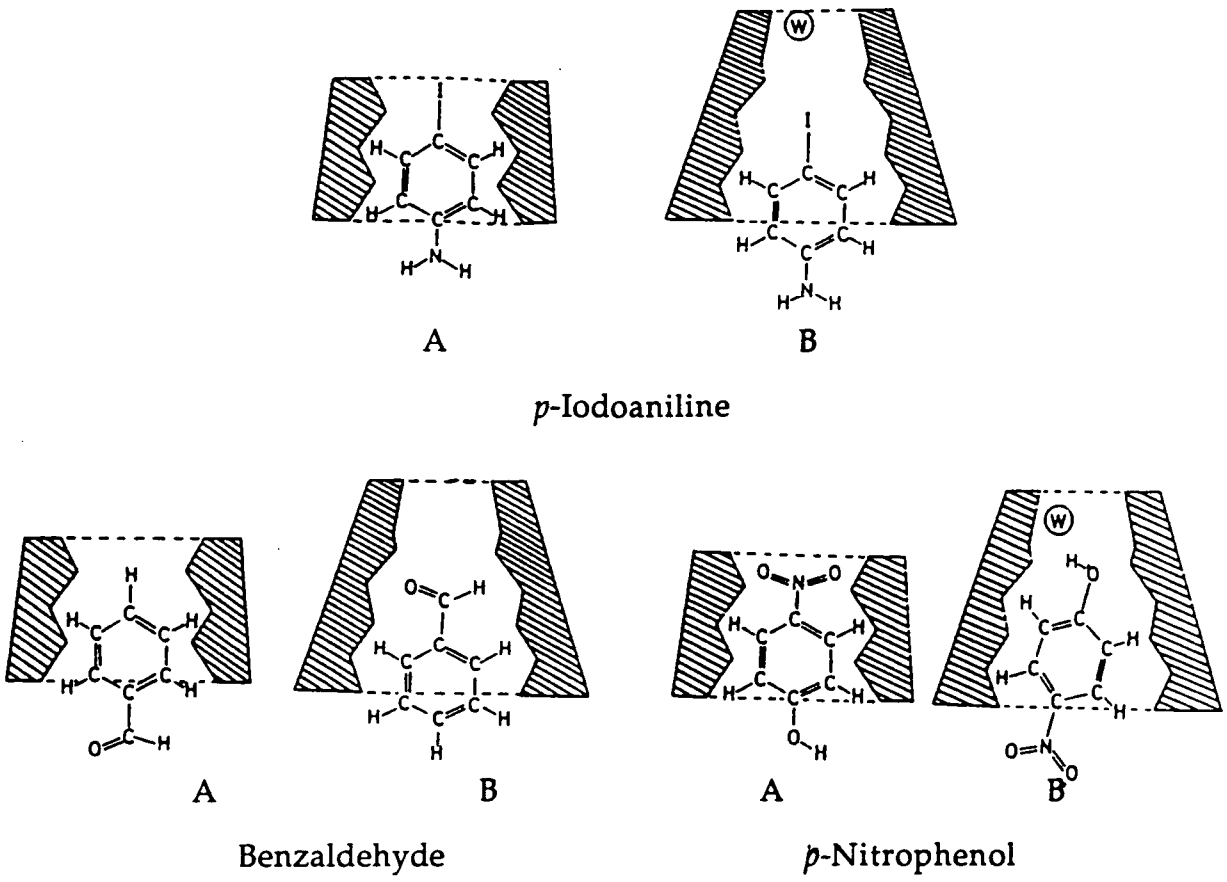
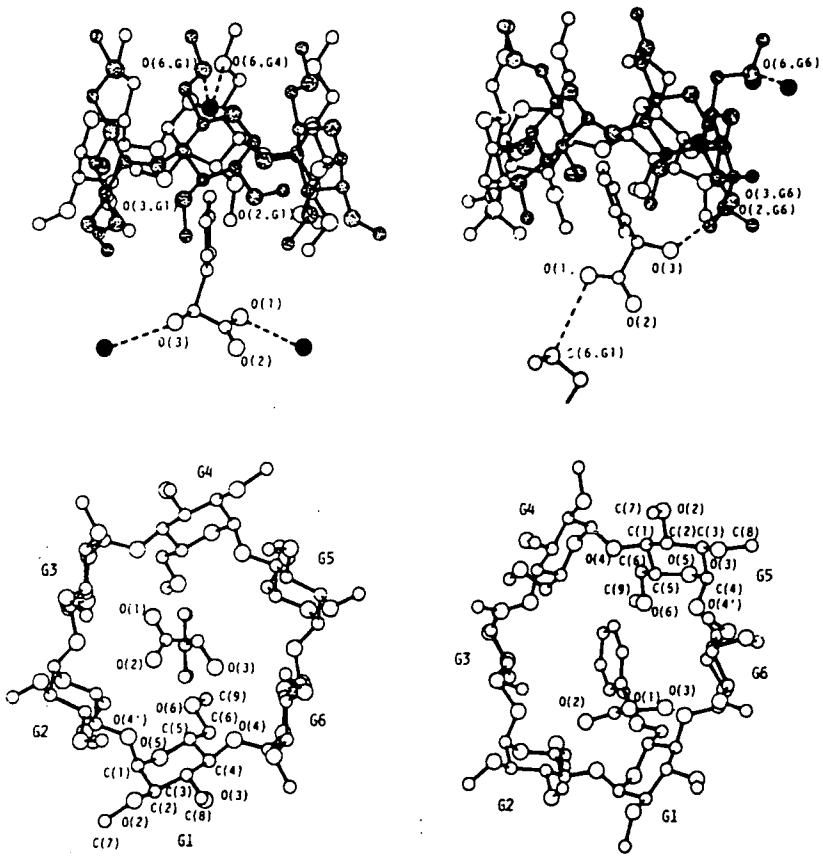



Figure 1.19 Schematic Drawings of the Inclusion Features of α-cyclodextrin (A) and per-O-methyl-α-Cyclodextrin (B) Complexes. Water Molecules are Denoted W

### 1.9.1 Chiral Recognition Through Induced Fit

Per-O-methylated cyclodextrins form inclusion complexes with a variety of guests, but the geometry of the hosts - guest interaction differs from that of the parent cyclodextrin as discussed above. A very good example is the complex between per-O-methyl α-cyclodextrin and mandelic acid[90]. It was found that with the R-enantiomer the cavity is less symmetrical than with the S-enantiomer where there is a pseudo $C_2$ axis of symmetry, figure 1.20.




S-Mandelic Acid R-Mandelic Acid

Figure 1.20 Structures of Per-O-Methylated α-Cyclodextrin Complexes with S- and R- Mandelic Acid

There are many other differences detailed by Harata concerning the geometry of inclusion. The most striking, and enantiodiscriminating, difference is the orientation of the mandelic acid phenyl ring within the cavity. With the S-enantiomer the phenyl group is included in the classical sense, parallel to the pseudo $C_2$ axis of the cavity whereas in the case of R-mandelic acid the phenyl group is inclined at an angle of ≈20° to this axis. The cyclodextrin derivative is therefore capable of recognising the chirality of mandelic acid. Comparison of such crystal structures with those of parent dextrins and chiral guests[87] shows that the methyl groups play an important role in the chiral recognition process, largely through the removal of intramolecular hydrogen bonding leading to a reduction of cavity symmetry.

Chiral recognition by methylated cyclodextrins has also been observed by X-ray crystallography for the per-O-methylated β-cyclodextrin complex with the enantiomers of flurbiprofen[91].

## 1.10 APPLICATIONS OF LIPOPHILIC CYCLODEXTRIN DERIVATIVES IN CHIRAL CHROMATOGRAPHY

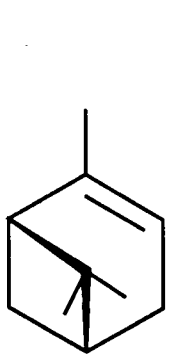
The problem of determining enantiomeric compositions (enantiomeric excess, ee) is central to all contemporary research concerned with the synthesis, characterisation and use of chiral compounds. Cyclodextrins are used in many chromatographic procedures[83] for the separation of compounds, including enantiomers. The current chromatographic applications of cyclodextrins and their derivatives have recently been reviewed by Purdy[92].

### 1.10.1 Liquid Chromatography

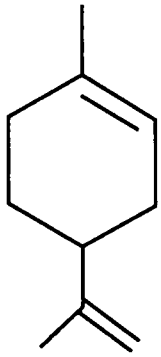
The use of cyclodextrins in HPLC has proven to be especially successful[93]. Much of the pioneering work in this area, particularly concerned with the use of modified cyclodextrins, has been carried out by Armstrong[94]. Unlike many of the well known (often polyamide) stationary phases, the cyclodextrin stationary phases described so far are compatible with aqueous and strongly polar mobile phases in the reverse phase mode of operation. Cyclodextrin bonded stationary phases have been demonstrated to be particularly adept at separating constitutional isomers and homologues[95]. For instance, α-cyclodextrin has proved useful as a chiral bonded stationary phase in liquid chromatography for the separation of aromatic amino acids[96].

### 1.10.2 Gas Chromatography

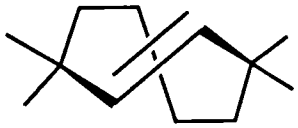
In gas chromatography, both immobilised cyclodextrins[97] and their derivatives[98] and cyclodextrin polymers[99] have been used as stationary phases[100]. Modified cyclodextrins have been employed by König and co-workers for the separation of structurally related and even enantiomeric compounds by gas chromatography. It was recognised that highly hydrophobic derivatives had the potential to act as enantioselective hosts for enantiomeric alkenes[101] such as:



α-Pinene



Limonene



3,3,8,8-Tetramethyl-trans-Cyclooctene

Highly lipophilic per-O-pentyl-cyclodextrin derivatives were prepared[102]. Such derivatives proved to be ideal for applications as chiral stationary phases in gas chromatography since they are highly viscous oils which allows them to be coated onto the glass capillary columns, and are of high thermal stability(>200°C). Figure 1.21 shows the separation of 3,3,8,8-tetramethyl-trans-cyclooctene using a per-O-pentyl γ-cyclodextrin stationary phase.

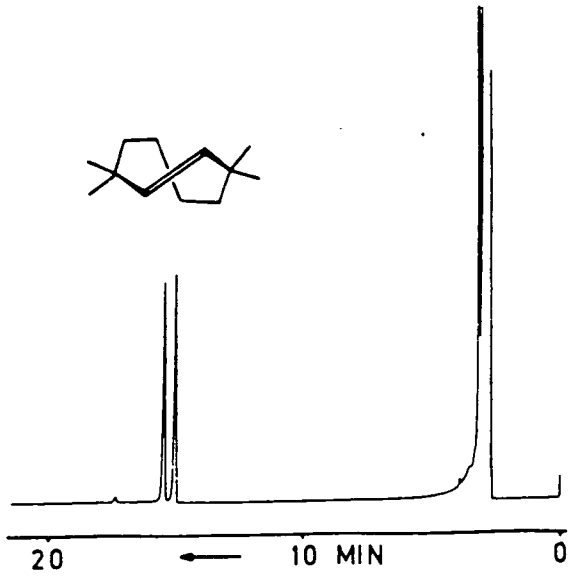



Figure 1.21 Separation of 3,3,8,8-tetramethyl-trans-cyclooctene enantiomers using a per-O-pentyl γ-cyclodextrin stationary phase.

Per-O-pentyl-β-cyclodextrin was found to separate the enantiomers of acyclic, monocyclic and bicyclic alkenes, provided that the chiral centre is α to the double bond. Upon changing the functionality of the β-cyclodextrin to 3-O-acetyl-2,6-di-O-pentyl it was found that, as might be expected, the acetyl group was able to act as a hydrogen bond acceptor[103]. This allowed the separation of the enantiomers of more polar compounds such as α- and β- chiral amines, amino alcohols and β-amino acids. In fact König was able to demonstrate that the nature of the O(3) substituent plays an important role in chiral recognition under the conditions of gas chromatography[104].

From an extensive investigation of these, and many other systems, König has concluded that molecular inclusion is essential for enantioselective separation by the cyclodextrin stationary phase in gas chromatography[104]. This was confirmed by the observation that per-O-pentylated acyclic oligosaccharides such as maltotriose exhibited only weak enantioselectivity, if any, towards chiral substrates[104].

König has also shown that there is a definite size selectivity dependence on the ability of the cyclodextrin to give enantiomer separation. He investigated an homologous series of 2-hydroxyacid methyl esters using both α- and β-cyclodextrin hydrophobic derivatives. It was found that enantioselective separation was dependent upon the size and shape of the guest species. Other investigations using homologous 2-hydroxyacid esters and alkyl halides showed a chain length dependence on the observed enantioselectivity[102b].

As a result of this work König concluded that the functionality of the guest, its size and shape were all factors which may affect enantioselective inclusion. However, he has been unable to rationalise these findings in terms of a unifying mechanism(s) of host-guest interaction.

Armstrong has also investigated the use of lipophilic cyclodextrin derivatives as chiral stationary phases for gas chromatography[105]. The approach is similar to that of König, concentrating on the study of homologous series. An example of the type of results which can be obtained in this way is given in table 1.10.2A for the alkyl esters of 2-bromobutanoic acid.

**Table 1.10.2A Separation of 2-Bromobutanoic Acid Esters by Gas Chromatography using Cyclodextrin Based Stationary Phases.**

| Ester of 2-Bromo butanoic acid | Column temp.°C | k′ | α‡ | Stationary Phase |
|---|---|---|---|---|
| Methyl | 80 | 5.07 | 1.56 | β |
| | 80 | 6.71 | 1.57 | γ |
| Ethyl | 80 | 5.25 | 1.29 | β |
| | 80 | 9.93 | 1.16 | γ |
| n-Hexyl | 80 | 66.4 | 1.16 | β |
| | 80 | 80.0 | 1.09 | γ |

As would be expected the retention times, k′, increase exponentially within the series with increase in molecular weight. However, the enantioselectivity factor, α, is largely independent of the retention time. Armstrong was able to draw two important conclusions from these results[106]:

i) longer carbon chains effect the retention time but not the enantioselectivity.

ii) the size of the cyclodextrin cavity can effect both retention and enantioselectivity.

‡ $-\Delta(\Delta G°) = RT\ln\alpha$

## 1.11 SURFACTANT INCLUSION BY MODIFIED β-CYCLODEXTRIN

Especially strong binding is found between β-cyclodextrin and long chain ionic surfactants where the hydrophobic carbon chain is either coiled up within the host cavity, linearly inserted within it[107] or, as suggested by Park, encapsulated by as many cyclodextrin molecules as are required to accommodate the extended chain. Reinsborough and co-workers have investigated the inclusion of surfactants by modified cyclodextrins using conductivity determinations[108]. Representative binding constants for such complexes are shown in figure 1.22.

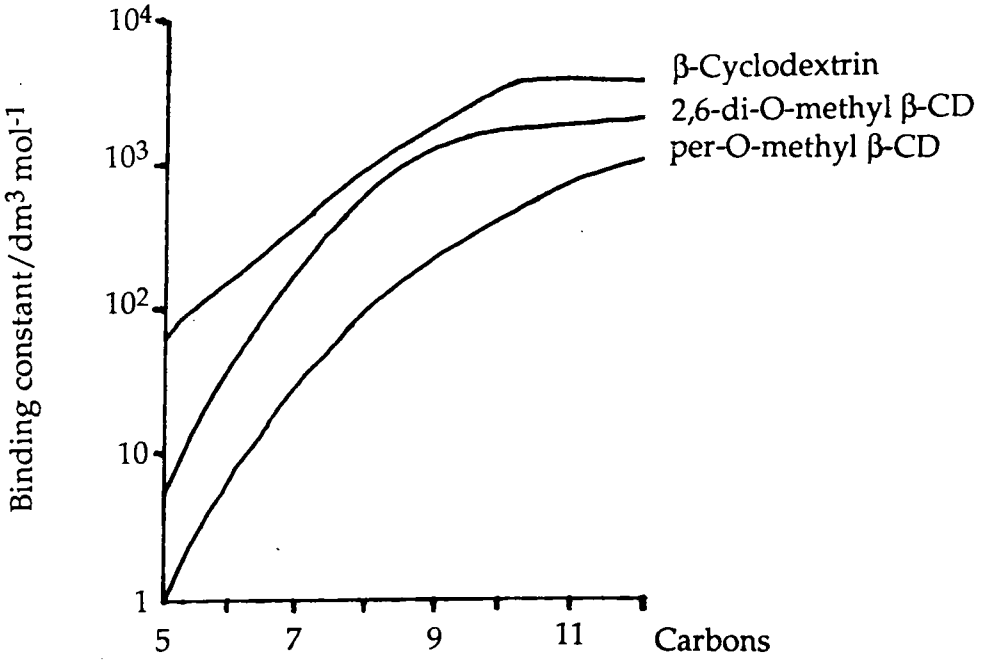



Figure 1.22 Binding constants for β-Cyclodextrin/alkane-1-sulphonate complexes[109] at 25°C

It can be concluded from these results that substitution of the hydroxyl functions dramatically reduces the binding strengths for the alkanesulphonate series. Per-O-methylation of the cyclodextrin, for example, reduces the binding constant for the entire series by an order of magnitude.

Satake and co-workers[110] have shown that with alkanesulphonates longer than $C_{10}$, the binding constant is independent of chain length, for both α- and β- cyclodextrin. This has been attributed to the filling of the cyclodextrin cavity with a ten carbon hydrophobic surfactant tail in aqueous solution. This levelling off of the binding constant also indicates that complexation is primarily hydrophobically driven[111]. Such levelling off at $C_{10}$ is not observed with methylated cyclodextrins as a result of an increase in cavity depth upon methylation from 8Å to 11Å. It is worth noting here that the α-cyclodextrin cavity can accommodate, at most, five methylene groups of a fully extended alkyl chain in its hydrophobic cavity[112]. The levelling off of the binding constant at $C_{10}$ for surfactant complexation suggests that some of the methylene groups interact with other parts of the cyclodextrin molecule, most probably the lateral hydrophilic surfaces.

## 1.12 INVESTIGATIONS OF MOLECULAR RECOGNITION BY CYCLODEXTRINS

The work of Armstrong and König in particular has sparked great interest in the use of highly functionalised, usually lipophilic cyclodextrins for the separation of structurally related and enantiomeric species. Although these workers have developed empirical guide-lines to govern the ability of cyclodextrins to differentiate between homologues and enantiomers, they have not been able to suggest any form of mechanism by which the recognition may take place.

### 1.12.1 Application of Mass Spectrometry

The development of several new ionisation techniques and inlet systems over the past ten years or so has seen a huge expansion in applications of mass spectrometry. Recently several 'soft' ionisation techniques such as electrospray (ES), fast atom bombardment (FAB), plasma desorption (PD), field desorption (FD) and thermospray (TS) have allowed applications to high molecular weight, involatile macromolecules such as proteins, enzymes and, of particular interest, highly functionalised cyclodextrin derivatives. These soft ionisation techniques are so called because they allow ionisation without the use of high temperatures or very high field gradients[113]. This allows ionisation of involatile molecules without immediate fragmentation and hence allows molecular ion detection.

Mass spectrometry has previously suggested that β-cyclodextrin forms an adduct with an aromatic phosphate ester based insecticide[114]. In 1987 Yaozu and co-workers reported, what appears to be, the first successful direct application of FAB-MS to the native α-, β- and γ- cyclodextrins[115] showing clear and unambiguous molecular ion peaks, $[M+H]^+$, for all three. Also evident, particularly in the case of α-cyclodextrin, were $[CD+glycerol]^+$ peaks perhaps indicating cyclodextrin-matrix complexation. This important observation was followed by demonstration of adduct formation between methylated cyclodextrins and organic ammonium salts by Stoddart[116]. This work clearly showed the existence of $[DM\alpha CD+RNH_3]^+$ adducts (R=$Bu^t$, $PhCH_2$, $PhCH_2CH(CO_2CH_3)$), indicating that complexation was occurring under the conditions of FAB-MS.

### 1.12.2 Application of NMR

The application of nuclear magnetic resonance (NMR) techniques to the investigation of complexation phenomena with cyclodextrins[117] and their derivatives is central to an understanding of the solution state structure[118] and dynamics of complexation[119]. In 1970 Demarco and Thakkar first demonstrated that the aromatic moiety of a guest molecule is included within the cyclodextrin cavity[120]. It was observed that the protons located inside the cavity (H(3) and H(5)) are susceptible to anisotropic shielding by an aromatic ring leading to a shift in their resonances to higher frequency. Protons located on the exterior of the cavity (H(2), H(4) and H(6)) are relatively unaffected.

With the advent of Fourier transform NMR and, in particular, the increase in attainable magnetic field strengths more detailed investigations of cyclodextrins have appeared since the mid 1980's[118]. In 1985 Sadler[117a] reported a full assignment of the $^{1}H$ and $^{13}C$ spectra for per-O-methyl-β-cyclodextrin through the use of two dimensional homo- and hetero- nuclear correlation spectroscopy (COSY and HETCOR). Since that time other workers have applied such highly informative NMR techniques to several other cyclodextrin derivatives.

In recent years NMR has been applied to the study of cyclodextrin complexes[121] in attempts to track down the important intermolecular host-guest interactions which lead to recognition and hence allow postulation of a mechanism for such recognition in the solution state. Several researchers such as Perly[122] have applied high resolution $^{1}H$, $^{13}C$, COSY and HETCOR to a variety of cyclodextrin and methylated cyclodextrin complexes in $D_2O$. As

an example, Perly has shown that β-cyclodextrin forms true inclusion complexes with steroids such as prednisolone. It was also demonstrated that the inclusion stoichiometries can be fully rationalized by considering the nature and position of the substituents on the steroid skeleton. Figure 1.23 shows the effect of change in the CD:steroid ratio on the partial 500MHz proton spectrum of the cyclodextrin as obtained by Perly.

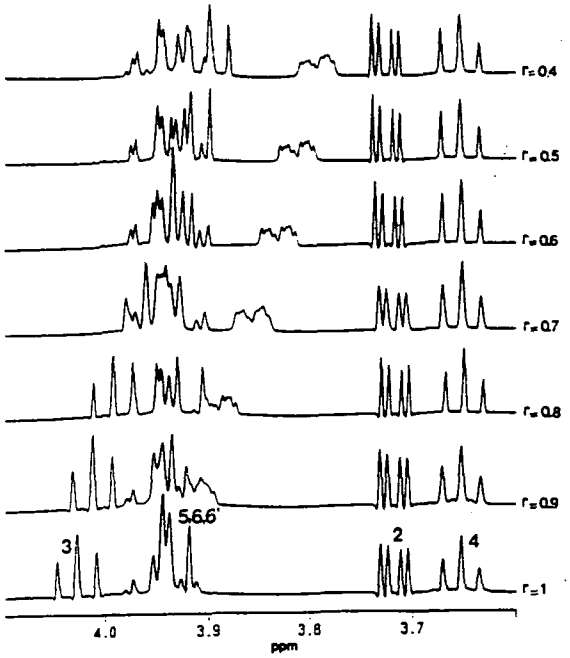



Figure 1.23 Influence of Prenisolone:CD ratio on the Partial 500MHz Proton Spectrum (302K) of β-CD. The Total Concentration of species is 5mM and the molar ratios are given as CD:CD+steroid.

From such spectra Δδ for a particular cyclodextrin resonance can be correlated to the host-guest ratio and an approximate equilibrium constant for complexation can be obtained from Benesi-Hildebrand plots[123]. This allows the effect of guest structure upon complex stability to be studied and hence molecular recognition can be investigated. Other workers have successfully employed $^{2}H$ and $^{19}F$ NMR as probes into cyclodextrin complexes[124].

## 1.13 CYCLODEXTRINS AS CHIRAL SOLVATING AGENTS

In NMR spectroscopic analysis, cyclodextrins are mainly used as chiral NMR shift reagents. In many cases, the influence of cyclodextrin inclusion complex formation on the NMR features of the two enantiomers of a chiral compound differs in chemical shifts[125]. Diastereoisomeric inclusion complex formation between α-cyclodextrin and fluorinated amino acid derivatives has been investigated by $^{19}F$ NMR[126]. In 10% $D_2O$ the chemical shifts of the R-amino acid derivative - cyclodextrin complex are to higher frequency of the S amino acid complex for deprotonated N-(*p*-fluorobenzoyl) valine, deprotonated α-(*p*-fluorophenyl) glycine and N-acetyl-α-(*p*-fluorophenyl) glycine. The shift difference between the diastereoisomers formed with R and S enantiomers can be used for chiral analysis and enantiomeric purity determinations. Chiral discrimination in $D_2O$ solution has also been observed for the enantiomers of propanolol hydrochloride where inclusion by β- or γ- cyclodextrin yields a pair of diastereoisomers with distinct $^1H$ NMR spectra[127].

## REFERENCES


1) I. O. Sutherland Chem. Soc. Rev. (1986) 15 63; X. Wang, S. D. Erickson, T. Limori, W. C. Still J. Am. Chem. Soc. (1992) 114 4128; A. Collet Tetrahedron (1987) 43 5725; J. Rebek Angew. Chem. Int. Ed. Engl. (1990) 29 245; *'Membranes'* G. Eisenman Ed. Marcel Dekker, New York, 1975; *Synthesis of Macrocycles: The Design of Selective Complexing Agents'* R.M.Izatt, J. J. Christensen Eds. Wiley Interscience, New York 1987.

2) Y. A. Ovchinnikov, V. T. Ivanov, A. M. Skrob *'Membrane Active Complexones'* Elsevier, New York 1974.

3) D. J. Cram, J. M. Cram Acc. Chem. Res. (1978) 11 8.

4) D. J. Cram Angew. Chem. Int. Ed. Engl. (1988) 27 1009.

5) J. Rebek Acc. Chem. Res. (1990) 23 399.

6) A. C. Coxon, D. A. Laidler, R. B. Pettman, J. F. Stoddart J. Am. Chem. Soc. (1978) 100 8260.

7) D. J. Cram, T. Kaneda, R. C. Helgeson, G. M. Lein J. Am. Chem. Soc. (1979) 101 6752.

8) H. B. Burgi, J. D. Dunitz, E. Schefter Acta Cryst. Sect. B (1974) 30 1517.

9) I. Goldberg Acta Cryst. Sect. B (1975) 31 754.

10) G. Ranghino, S. Romano, J. M. Lehn, G. Wipff J. Am. Chem. Soc. (1985) 107 7873.

11) D. J. Cram, G. M. Lein J. Am. Chem. Soc. (1985) 107 3657.

12) D. J. Cram Angew. Chem. Int. Ed. Engl. (1986) 25 1039.

13) P. Holy, W. E. M. K. Seiler, W. Simon, J-P. Vigneron Helv. Chim. Acta (1990) 73 1171.

14) T. Nishi, A. Ikeda, T. Matsuda, S. Shinkai J. C. S. Chem. Commun. (1991) 339.

15) R. M. Izatt, J. S. Bradshaw, S. A. Nielson, J. D. Lamb, J. J. Christensen Chem. Rev. (1985) 85 271.

16) C. J. Pedersen J. Am. Chem. Soc. (1967) 89 7017.

17) E. Weber, F. Vögtle Top. Curr. Chem. (1981) 98 1; Y. Takeda Top. Curr. Chem. (1984) 121 1.

18) J-M. Girodeau, J-M. Lehn, J-P. Sauvage Angew. Chem. Int. Ed. Engl. (1975) 14 764.

19) P. Oggenfuss, W. E. Morf, U. Oesch, D. Ammann, E. Pretsch, W. Simon Anal. Chim. Acta. (1986) 180 299.

20) R. Kataky, P. E. Nicholson, D. Parker, A. K. Covington Analyst (1991) 116 135.

21) E. Graf, J-M. Lehn Helv. Chim. Acta (1981) 64 1040; J-M Lehn Structure Bonding (1973) 16 1.

22) F. Schmidtchen, G. Müller J. C. S. Chem. Commun. (1984) 1115.

23) D. Gehin, P. A. Kollman, G. Wipff J. Am. Chem. Soc. (1989) 111 3011.

24) M. J. Bovill, D. J. Chadwick, I. O. Sutherland, D. J. Watkin J. C. S. Perkin Trans. 2 (1980) 1529.

25) J-M.Lehn Pure Appl. Chem. (1979) 51 979.

26) J-P. Behr, J-M. Lehn, P. Verling Helv. Chim. Acta (1982) 65 1853.

27) G. W. Gokel Chem. Soc. Rev. (1992) 39; G. W. Gokel. J. E. Trafton *'Cation binding by Lariat Ethers'* in *'Cation Binding by Macrocycles'*, Y. Inoue, G. W. Gokel (Eds), Marcel Dekker, New York (1990) p. 253.

28) J. F. Stoddart Topics Stereochem. (1987) 17 207; S. T. Jolly, J. S. Bradshaw, R. M. Izatt J. Heterocyclic Chem. (1982) 19 3.

29) J. F. Stoddart Annu. Rep. Prog. Chem. Sect. B (1983) 353; D. J. Cram, K. N. Trueblood Top. Curr. Chem. (1981) 98 43.

30) J. F. Stoddart Chem. Soc. Rev (1979) 8 85.

31) V. Prelog Pure Appl. Chem (1978) 50 893.

32) J-M. Lehn Angew. Chem. Int. Ed. Engl. (1988) 27 89.

33) C. J. Pedersen Angew. Chem. Int. Ed. Engl. (1988) 27 1021.

34) S. C. Peacock, D. J. Cram J. C. S. Chem. Commun. (1976) 282.

35) V. Prelog, D. Bedekovic Helv. Chim. Acta (1979) 62 2285.

36) J-P. Behr, J-M. Girodeau, R. G. Hayward, J-M Lehn, J-P. Sauvage Helv. Chim. Acta (1980) 63 2096.

37) W. Bussmann. J-M. Lehn, U. Oesch, P. Plumere, W. Simon Helv. Chim. Acta (1981) 64 657.

38) J. F. Stoddart *'Chiral Crown Ethers'* in Topics in Stereochem. Vol 17. E. L. Eliel, S. H. Wilen (Eds.), Wiley Interscience, New York, 1988, p.207.

39) Y. Chao, D. J. Cram J. Am. Chem. Soc. (1976) 98 1015; M. Alonso-Lopez, J. Jimenez-Barbero, M. Martin-Lomas, S. Penades Tetrahedron (1988) 44 1535; T. Matsui, K. Koga Tetrahedron Letters (1978) 1115.

40) B.L. Allwood, H. Shahriari-Zavareh, J.F. Stoddart, D.J. Williams J. C. S. Chem Commun. (1984) 1461.

41) R. F. Borch, S. R. Levitan J. Org. Chem. (1972) 37 2347; S. Itsuno, K. Ito, A. Hirao, S. Nakahama J. C. S. Chem. Commun. (1983) 469.

42) G. D. Y. Sogah, D. J. Cram J. Am. Chem. Soc. (1979) 101 3035.

43) M. Lauth, P. Gramain J. Liq. Chromatogr. (1985) 8 2403; L. A. Fernando, M. L. Miles, L. H. Bowen Anal. Chem. (1980) 52 1115.

44) E. V. Zagorevakaya, N. V. Kovaleva J. Chromatogr. (1986) 365 7; A. Ono Analyst (1983) 108 1265.

45) A. Kohoutova, E. Simolkova-Keulemansova, L. Feltl J. Chromatogr. (1989) 471 139.

46) H. Bender Carbohydr. Res. (1978) 65 85.

47) A. Villers Compt. Rend. Acad. Sci. (Paris) (1891) 112 536.

48) F. Schardinger Wein. Klin. Wochenschi. (1904) 17 207.

49) A. Hybl, R. E. Rundle, D. E. Williams J. Am. Chem. Soc. (1965) 87 2779.

50) P. R. Sundararajan and V. S. R. Rao Carbohydrate Research (1970) 13 351.

51) V. G. Murphy, B. Zaslow, A. D. French Biopolymers (1975) 14 1487.

52) A. O. Pulley, D. French Biochem. Biophys. Res. Communs. (1961) 5 11.

53) B. Gillet, D. J. Nicole, J. J. Delpeuch Tetrahedron letters (1982) 23 65; K. Harata Bull. Chem. Soc. Jpn. (1977) 50 1416.

54) B. Casu, M. Reggiano, G. G. Gallo, A. Vigevani J. Chem. Soc. Special Publication 23 217.

55) W. Saenger Angew. Chem. Int. Ed. Engl. (1980) 19 344.

56) J. Szejtli *'Topics in Inclusion Science'*, Kluwer Dordrecht, Netherlands 1988; V. T. D'Souza, M. L. Bender Acc. Chem. Res. (1987) 20 146; D. French Adv. Carbohydr. Chem. (1957) 12 189.

57) K. Harata Bull. Chem. Soc. Jpn. (1979) 52 1807.

58) K. Kano, H. Matsumoto, Y. Yoshimura, S. Hashimoto J. Am. Chem. Soc. (1988) 110 204.

59) Y. Inoue, H. Hoshi, M. Sakaurai, R. Chujo J. Am. Chem. Soc. (1985) 107 2319; D. A. Alston, A. M. Z. Slawin, J. F. Stoddart, D. J. Williams, R. Zarzycki Angew. Chem. Int. Ed. Engl. (1988) 27 1184.

60) K. Harata, H. Uedaira, J. Tanaka Bull. Chem. Soc. Jpn. (1978) 51 1627; M. Komiyama, H. Hirai Bull. Chem. Soc. Jpn. (1981) 54 828.

61) K. Harata Bull. Chem. Soc. Jpn. (1976) 49 2066; W. Saenger, K. Beyer, P. C. Manor Acta Crystallogr. Sect. B (1976) 32 120; K. Harata Bull. Chem. Soc. Jpn. (1977) 50 1416.

62) T. Steiner, W. Hinrich, W. Saenger, G. A. Hoyer Carbohydrate Res. (1989) 192 43.

63) F. Djedaini, S. Z. Lin, B. Perly, D. Wouessidjewe J. Pharm. Sci. (1990) 79 643.

64) E. A. Lewis, L. D. Hansen J. Chem. Soc. Perkin Trans. 2. (1973) 2 2081.

65) F. Cramer Angew. Chem. (1967) 73 49; R. J. Bergeron, D. M. Pillor, G. Gibeity, W. P. Roberts Bioorg. Chem. (1978) 7 263.

66) F. Cramer, W. Kampe J. Am. Chem. Soc. (1965) 87 1115.

67) G. Nemethy, H. A. Scheraga J. Chem. Phys. (1962) 36 3401.

68) R. J. Bergeron, M. A. Channing, G. J. Gibeity, D. M. Pillor J. Am. Chem. Soc. (1977) 99 5146.

69) D. W. Griffiths, M. L. Bender Adv. Catal. (1973) 23 209; Y. Nozaki, C. Tanford J. Biol. Chem. (1971) 246 2211.

70) K. Lindner, W. Saenger Angew. Chem. Int. Ed. Engl. (1978) 17 694.

71) R. L. Van Etten, G. A. Clowes, J. F. Sebastian, M. L. Bender J. Am. Chem. Soc. (1967) 89 3253.

72) W. Saenger, M. Noltemeyer, P. C. Manor, B. Hingerty, B. Klar Bioorg. Chem. (1976) 5 187.

73) J. M. MacLennan, J. J. Stezowski Biochem. Biophys. Res. Commun. (1980) 92 933.

74) A. Hersey, B. H. Robinson J. Chem. Soc. Faraday Trans. (1984) 80 2039; M. Komiyama, M. L. Bender J. Am. Chem. Soc. (1978) 100 2259.

75) R. I. Gelb, L. M. Schwartz, B. Cardelino, D. A. Laufer, H. S. Fuhfman,

R. F. Johnson J. Am. Chem. Soc. (1981) 103 1750.

76) V. T. Liveri, G. Cavallaro, G. Giammonia, G. Pitarresi, G. Puglisi, G. Ventura Thermochimica Acta (1992) 199 125.

77) I. Tabushi Tetrahedron (1984) 40 269; R. Breslow Science (1982) 218 532.

78) R. M. Paton, E. T. Kaiser J. Am. Chem. Soc. (1970) 92 4723.

79) N. Henrich, F. Cramer J. Am. Chem. Soc. (1965) 87 1121.

80) R. L. Van Etten, J. F. Sebastian, G. A. Cowles, M. L. Bender J. Am. Chem. Soc. (1967) 89 3242.

81) P. A. Croft, R. A. Bartsch Tetrahedron (1983) 39 1417; J. Boger, R. Corcorn, J-M Lehn Helv. Chim. Acta (1978) 61 2190; M. L. Bender, M. Komiyama *'Cyclodextrin Chemistry'* Springer-Verlog, Berlin, 1978.

82) M. Mikojajczyk, J. Dabrowicz J. Am. Chem. Soc. (1978) 100 2510.

83) W. L. Hinze Separ. Purific. Methods (1981) 10 159.

84) F. M. Menger, M. A. Dulany Tetrahedron Letters (1985) 26 267.

85) K. Harata J. C. S. Chem. Commun. (1988) 928; K. Harata Carbohydr. Res. (1989) 192 33; W. Saenger, R. K. McMullan, J. Fayos, D. Mootz Acta Crystallog. Sect. B (1974) 30 2019.

86) K. Harata Bull. Chem. Soc. Jpn. (1982) 55 1367.

87) K. Harata, K. Uekama, M. Otagiri, F. Hirayama J. Inclusion Phenom. (1984) 1 279.

88) K. Harata, K. Uekama, M. Otagiri, F. Hirayama Bull. Chem. Soc. Jpn. (1982) 55 3904; K. Harata, K. Uekama, M. Otagiri, F. Hirayama, Y. Sugiyama Bull. Chem. Soc. Jpn. (1982) 55 3386.

89) K. Harata Bull. Chem. Soc. Jpn. (1977) 50 1416.

90) K. Harata, K. Uekama, M. Otagiri, F. Hirayama Bull. Chem. Soc. Jpn. (1987) 60 497.

91) K. Harata, F. Hirayama, T. Imai, K. Uekama, M. Otagiri Chem lett. (1984) 1549; K. Harata, K. Uekama, M. Otagiri, F. Hirayama J. Inclusion Phenom. (1985) 2 583.

92) S. Li, W. C. Purdy Chem Rev. (1992) 92 1457.

93) Y. Mizobuchi, M. Tanaka, T. Shono J. Chromatogr. (1981) 208 35; B. Zsadon, M. Szilasi, F. Tusdos, J. Szejtli J. Chromatogr. (1981) 208 109; K. Fujimura, T. Ueda, T. Ando Anal. Chem. (1983) 55 446; Y. Kawaguchi, M. Tanaka, M. Nakae, F. Funazo Anal. Chem. (1983) 55 1852; M. Tanaka, Y. Kawaguchi, M. Nakae, F. Funazo, Y. Mizobuchi, T. Shono J. Chromatogr. (1984) 229 341.

94) S. M. Han, D. W. Armstrong in *'Chiral Separation by HPLC'*, A. M. Krstulovic (Ed.), John Wiley and Sons, New York, 1989. Chapter 10; J. W. Timothy, D. W. Armstrong J. Liq. Chromatogr. (1986) 9 407.

95) T. E. Beesley Am. Lab. (1985) 17 78; D. W. Armstrong, W. DeMond, A. Alak, W. L. Hinze, T. Riehl, K. H. Bui Anal. Chem. (1985) 57 234.

96) D. W. Armstrong, X, Yang, S. M. Han, R. Menges Anal. Chem. (1987) 59 2594.

97) H. P. Nowotny, D. Schmalzig, D. Wistuba, V. Schurig J. High Resolut. Chromatogr. (1989) 12 383.

98) R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, D. A. Laufer J. Am. Chem. Soc. (1981) 103 1750; H. Schlenk, J. L. Gellerman, D. M. Sand Anal. Chem. (1962) 34 1529.

99) Y. Mizombuchi, M. Tanaka, T. Shono J. Chromatogr. (1980) 194 153.

100) V. Schurig, H-P. Nowotny Angew. Chem. Int. Ed. Engl. (1990) 29 939.

101) W. A. König, A. Kruger, D. Icheln, T. Runge J. High. Res. Chromatogr. (1992) 15 184.

102) a)W. A. König, R. Krebber, G. Wenz J. High Res. Chromatogr. (1989) 12 790;

b)W. A. König, S. Lutz, M. Hagen, R. Krebber, G. Wenz, K. Baldenius, J. Ehlers, H. tom Dieck J. High Res. Chromatogr. (1989) 12 35;

c)T. Reiher, H-J. Hamann J. High Res. Chromatogr. (1992) 15 346.

103) W. A. König, S. Lutz, G. Wenz, E. von der Bey J. High Res. Chromatogr. and Chromatogr. Communs. (1988) 11 506.

104) W. A. König, R. Krebber, G. Wenz J. High Res. Chromatogr. (1989) 12 641.

105) D. W. Armstrong, H. L. Jin J. Chromatogr. (1990) 502 154.

106) A. Berthod, W. Li, D. W. Armstrong Anal. Chem. (1992) 64 873.

107) T. Okubo, Y. Maeda, H. Kitano J. Phys. Chem. (1989) 93 3721.

108) R. Palepu, V. C. Reinsborough Can. J. Chem. (1988) 66 325; R. Palepu, J. M. Richardson, V. C. Reinsborough Langmuir (1989) 5 218; R. Palepu, V. C. Reinsborough Can. J. Chem. (1989) 67 1550.

109) C. D. Lavandier, M. P. Pelletier, V. C. Reinsborough Aust. J. Chem (1991) 44 457.

110) I. Satake, S. Yoshida, K. Hayakawa, T. Maeda, Y. Kusumoto Bull. Chem. Soc. Jpn. (1986) 59 3991.

111) C. Tanford *'The Hydrophobic Effect:Formation of micelles and Biological Membranes'* 2nd Ed. John Wiley and sons, New York, 1980.

112) W. J. James, D. French, R. E. Rundle Acta Crystallogr. (1959) 12 385.

113) V.G. Monographs in Mass Spectrometry (1992) No.3 M. R. Clench *'A Comparison of Thermospray, Plasmaspray, Electrospray and Dynamic FAB'*.

114) J. Szejtli, E. Lazzlo, B. Banky, G. Seres Hungarian Patent 175 584 (1977).

115) C. Yaozu, C. Nengyu, C. Ning, L. Haiquan, Z. Fanzhi Kexue Tongbao (1987) 32 1180; C. Yaozu, C. Nengyu, C. Ning, L. Haiquan, Z. Fanzhi Biomed. Environ. Mass Spectrom. (1987) 14 1:9.

116) P. R. Ashton, J. F. Stoddart, R. Zarzycki Tetrahedron Letters (1988) 29 2103.

117) a)J. R. Johnson, N. Shankland, I. H. Sadler Tetrahedron (1985) 41 3147.
b)M. Otagiri, T. Imai, F. Hirayama, K. Uekama, M. Yamasaki Acta. Pharm Sci. (1983) 20 11.
c)M. Komiyama, H. Hirai Bull. Chem. Soc. Jpn. (1981) 54 828.

118) Y. Yamamoto, M. Onda, Y. Takahashi, Y. Inoue, R. Chujo Carbohydr. Res. (1987) 170 229; Y. Inoue, Y. Takahashi, R. Chujo Carbohydr. Res. (1985) 144 c9-c11.

119) D. J. Wood, F. E. Hruska, W. Saenger J. Am. Chem. Soc. (1977) 99 1735; R. J. Bergeron, R. Rowan Bioorg. Chem. (1976) 5 425.

120) P. V. Damarco, A. L. Thakkar J. C. S. Chem. Commun. (1970) 2; A. L. Thakkar, P. V. Demarco J. Pharm. Sci. (1971) 60 652.

121) J. Lehmann, E. Kleinpeter, J. Krechl J. Incl. Phenom. Molec. Recognit. Chem. (1991) 10 233.

122) F. Djedaini, B. Perly J. Pharm. Sci. (1991) 80 1157.

123) J. A. Hildebrand, H. A. Benesi J. Am. Chem. Soc. (1949) 72 2703.

124) N. J. Smith, T. M. Spotwood, S. F. Lincoln Carbohydr. Res. (1989) 192 9.

125) D. D. MacNicol Tetrahedron letters (1975) 38 3325; J. J. Richards, M. L. Webb Anal. Proceed. (1992) 29 251.

126) S. E. Brown, J. H. Coates, S. F. Lincoln, D. Coghlan, C. J. Easton J. C. S. Faraday Trans. (1991) 87 2699.

127) D. Greatbanks, R. Pickford Magn. Reson. Chem. (1987) 25 208.

# CHAPTER TWO

# SYNTHESIS AND CHARACTERISATION OF HIGHLY FUNCTIONALISED CYCLODEXTRIN DERIVATIVES

## 2.1 INTRODUCTION

The aim of this synthetic investigation has been the development of highly selective molecular receptors which, after incorporation in a solvent polymeric membrane, are able to act as chemical sensors by measurement of a potentiometric response to the presence of an ionic substrate. In order for a potential receptor to be used in such a way it must conform to several well established criteria:

i) it must be soluble in a solvent such as THF in order to allow incorporation into a solvent polymeric membrane, formed by evaporation of a homogeneous solution.

ii) it should be, as far as possible, insoluble in water to prevent leeching from the membrane. This increases the lifetime of the membrane considerably.

iii)its complexation mechanism with substrates should be such that $\Delta G^{\ddagger}$ for exchange between the free and bound states is of the order of 40 to 65 kJ $mol^{-1}$ to allow good response times.

iv)ideally the receptor should be stable and charge neutral, respond to the target ion independently of pH, thus allowing possible *in vivo* applications and finally it should exhibit a Nernstian response to the analyte.

The extent to which cyclodextrins, and more especially their derivatives, have been employed as selective hosts[1] and even chemical sensors, by other workers, has already been indicated in chapter one. In particular the work of both Armstrong and König (section 1.10) demonstrates that highly alkylated, lipophilic cyclodextrin derivatives are capable of structural-, stereo-, and enantio- selective recognition of alkenes, alcohols and $\alpha$ and $\beta$ aryl amines and carboxylic acids.

This chapter describes the synthesis, purification and characterisation of several, mostly octylated, α-, β- and γ- cyclodextrin derivatives which, as chapters three and four will show, may act as efficient chemical sensors. The syntheses of several acyclic dextrin octylated derivatives are also discussed. These were studied as non macrocyclic cyclodextrin analogues to investigate the importance of the macrocyclic cavity in selective aryl ammonium ion binding.

## 2.2 CYCLODEXTRIN ALKYLATION

Alkyl derivatives of cyclodextrins appear early in the literature, mainly because per-O-methylation was used as a means of purification of the parent molecule[2]. Subsequently a large number of alkylated derivatives have been synthesized. The synthesis and certain chemical and physical properties of per-O-methylated cyclodextrins have been detailed by several workers[3].

The method of methylation initially proposed by Kuhn and Trischman[4] used $Ba(OH)_2.8H_2O/BaO$ and methyl sulphate in (1:1) DMF/DMSO at 0°C to give 2,6-di-O-methylation. However, subsequent investigation found that alkylation of cyclodextrins with long chain alkyl bromides (eg. 1-bromopentane) under such conditions was slow and incomplete. Ciucanu and Kerek[5] found that methylation of monosaccharides with MeI/NaOH in DMSO is rapid and complete. Several workers have since applied this method to the 2,6-di-O-alkylation of cyclodextrins[6]. Indeed efforts have been made to prepare symmetrically mono- and di- alkylated cyclodextrin derivatives[7], particularly for applications in capillary GLC[8]. It has been observed that regioselective derivatisation depended upon both the reaction conditions and the nature of the substituent being introduced.

Thus, pentylation can be achieved more selectively than methylation. Further alkylation of OH(3) may then be achieved using sodium hydride as base in refluxing THF.

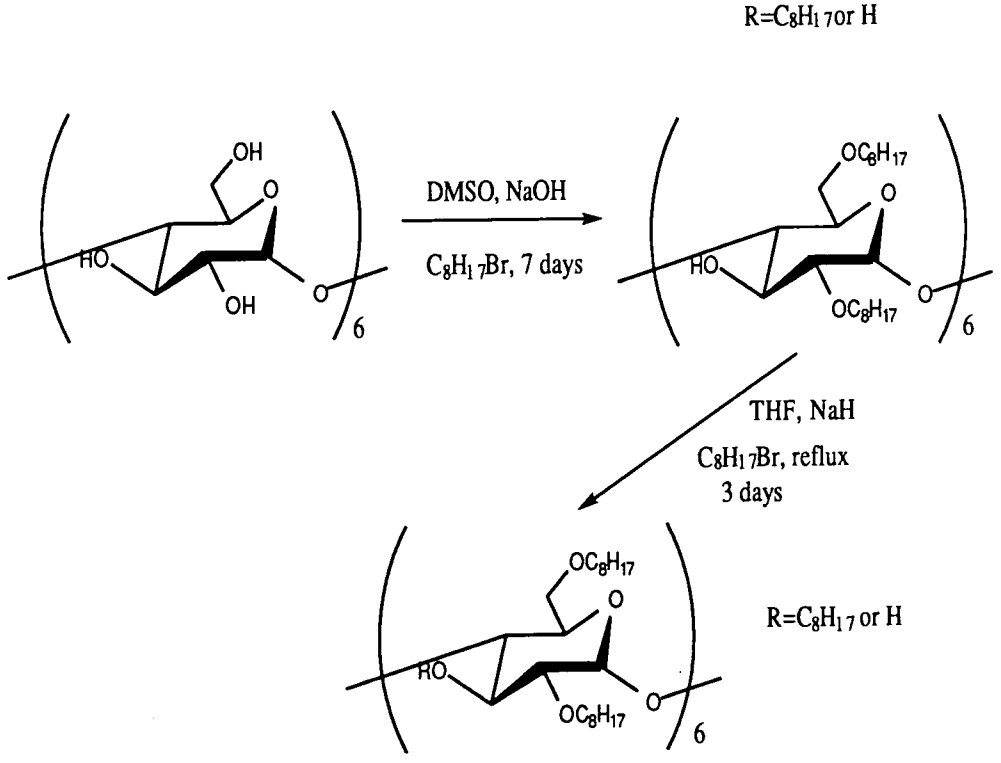



Figure 2.1 Alkylation of α-cyclodextrin

Despite the existence of an established method for cyclodextrin alkylation, based on the well known Williamson ether synthesis, several problems concerned with purification of the product, monitoring of the reaction, and reaction time were apparent when extrapolating this method to octylation. A change of solvent to THF in order to ease monitoring of the reaction was unsuccessful, even in the presence of a phase transfer catalyst, because the native cyclodextrins are not even sparingly soluble in THF. Also, elevation of the temperature to greater than 30° and/or the use of a strong base such as sodium hydride resulted in no isolable product. This may well be a result of increased reactivity of the DMSO solvent under these conditions[9].

Therefore, despite the problems of involatility associated with using DMSO and 1-bromooctane a method similar to that developed by Ciucanu and Kerek was employed. Investigations into optimization of the yield found that the molar ratio 1-bromooctane : sodium hydroxide : cyclodextrin hydroxyl group was crucial. Reproducible functionalisation was achieved with 3:3:1 ($NaOH:C_8H_{17}Br$:CD hydroxyl) and $20cm^3$ of DMSO apparently being the most effective amount of solvent to use for 1g of cyclodextrin. These findings seem quite universal for all three parent cyclodextrins. The only obvious difference on comparing α- β- and γ- cyclodextrin octylation is that γ-cyclodextrin gives markedly lower yields of alkylated product. This might be explained by cyclodextrin - 1-bromooctane complex formation in the case of α- and β- cyclodextrin leading to a reduction in the conformational space available to both the hydroxyl and $CH_2Br$ reacting sites. Such inclusion may then lead to a more favourable $\Delta G_{reaction}$. However no attempt was made to investigate this phenomenon.

### 2.2.1 Octylation With Sodium Hydroxide as Base

In the case of all three parent cyclodextrins the use of sodium hydroxide as base in DMSO results in 2,6-di-O-octylation of each glucopyranose ring to give a product which is quite homogeneous. However some inhomogeneity is apparent on investigation by NMR, reductive depolymerisation and mass spectral analysis. This is discussed at length in the later characterisation sections of this chapter.

### 2.2.2 Octylation With Sodium Hydride as Base

In order to further alkylate the 2,6-di-O-octyl cyclodextrins, more forcing conditions must be employed in order to overcome the stabilising effects of OH(3)···O(2)′ hydrogen bonding. The use of sodium hydride as base in refluxing THF for three days results in partial octylation at the 3 position of the glucopyranose subunits. A noticeable loss of the overall $C_n$ symmetry in both $^1H$ and $^{13}C$ NMR indicated that there were a number of constitutional isomers and homologues present in the product. This indicated incomplete alkylation which was confirmed by the presence of an O—H absorption in the FT-IR spectrum of all three 'per'-O-octylated cyclodextrins.

## 2.3 OTHER DERIVATIVES

Several other, especially α-, cyclodextrin derivatives were synthesized in order to probe the effects of a change in functionality at the 3(OH) position. It was envisaged that such modification of the cyclodextrins' functionality may throw some light on the mechanism(s) involved in molecular recognition by these macrocyclic receptors.

### 2.3.1 3-O-Acetyl-2,6-Di-O-Octyl-α-Cyclodextrin

In order to develop a potential selective receptor which incorporates a hydrogen bond acceptor 2,6-di-O-octyl-α-cyclodextrin was acetylated in the O-3 position of the glucopyranose subunits under standard acetylating conditions (ethanoic anhydride/triethylamine). This reaction was monitored by FT-IR which indicated the disappearance of the O-H stretch ($3300cm^{-1}$) and the appearance of an ester carbonyl stretch ($1735cm^{-1}$), as shown in figure 2.2.

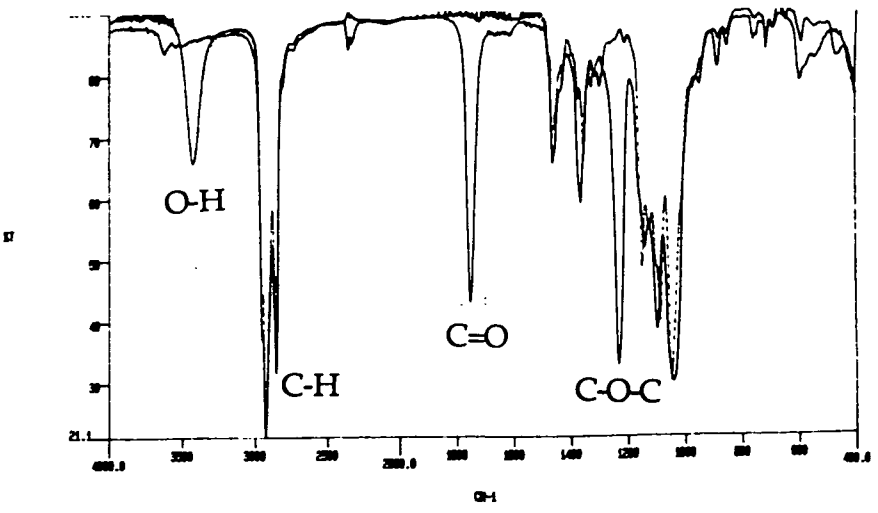



Figure 2.2 FT-IR Spectra Indicating 3-O-Acetylation of 2,6-Di-O-Octyl-α-Cyclodextrin

### 2.3.2 Methylated Cyclodextrins

Although methylation of native cyclodextrins is of little use in this investigation, because of their water solubility, methylation was employed to 'cap' residual hydroxyl groups remaining in the 'per'-O-octyl derivatives. Methylation was achieved under forcing conditions similar to those adopted in the 'per'-O-octylation reaction above. The 'per'-O-octyl cyclodextrin was heated to 35°C under the conditions of reflux in THF with iodomethane in the presence of sodium hydride as base. Methylation should permit investigation of the hydrogen bonding role of residual OH groups in the ability of the 'per'-O-octyl cyclodextrins to act as structural and enantiomer selective hosts. Such a hydrogen bonding role may involve the included guest or be intramolecular within the cyclodextrin. If the residual 3(OH) groups are involved in intramolecular hydrogen bonding then this will affect the conformational mobility of the host and therefore influence its hospitality. Methylation also proved to be a useful analytical tool when investigating the extent of octylation through quantitative $^{13}C$ NMR studies (section 2.5.2).

As with the acetyl ester derivative above, methylation was examined, quite effectively, by infra red spectroscopy. Disappearance of the O-H stretch, which had been very evident in the 'per'-O-octyl derivatives was indicative of complete methylation.

### 2.3.3 Octylation of Oligosaccharides

Glucose, maltose and maltotriose were each octylated by reactions analogous to those used for the cyclodextrins. The only discernible difference was that glucose was per-O-octylated by a one step DMSO/NaOH reaction. This may be related to the inability of glucose to form O(2)···OH(3)′ hydrogen bonds. This is because such hydrogen bonding is known to decrease the reactivity of the 3 position secondary hydroxyl group of the cyclodextrins and other oligosaccharides.

## 2.4 PURIFICATION OF OCTYLATED CYCLODEXTRINS

After initial work up, purification (to remove residual 1-bromooctane and DMSO) was achieved by silica gel chromatography using between 0 and 2 % methanol in dichloromethane as the column solvent depending on the derivative in question. This almost invariably resulted in a cyclodextrin derivative which was a clear, often slightly yellow, highly viscous oil. The slight yellow colour was attributed to a cyclodextrin - $Br_2$ complex ($Br_2$ formed by the action of light on OctBr). The only exception to this general observation was in the case of 2,6-di-O-octyl-β-cyclodextrin. With this cyclodextrin derivative column chromatography (0→1 % methanol / dichloromethane, silica) resulted in the isolation of two products $R_f$ = 0.33 and 0.25. These appeared to be indistinguishable by $^1H$ and $^{13}C$ NMR analysis. However +FD-MS, figure 2.3, revealed them to be two rather different mixtures of homologues. The compound corresponding to $R_f$ = 0.25 is rather monodisperse consisting almost

octylated cyclodextrin (m/e 2706). The compounds corresponding to an $R_f$ of 0.33 were mostly 'over-octylated' material; ie. with 15 (m/e 2818) and 16 (m/e 2930) octyl groups.

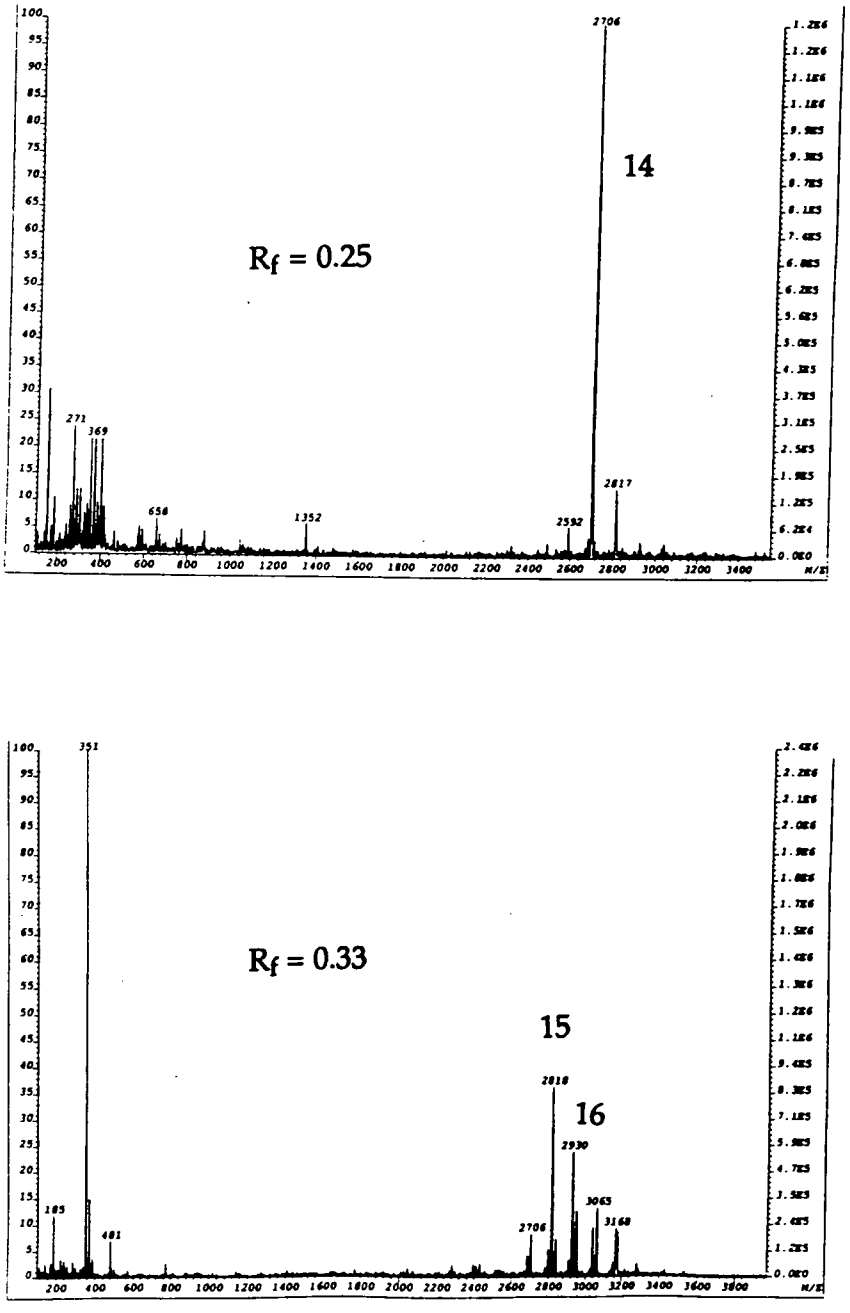



Figure 2.3 (+)-Field Desorption Spectra for 2,6-Di-O-Octyl-β-Cyclodextrin

## 2.5 CHARACTERISATION OF HIGHLY LIPOPHILIC CYCLODEXTRIN DERIVATIVES

Several methods of characterisation were employed to determine the extent of derivatisation and the homogeneity of the products. Some methods, such as proton NMR and FT-IR, were generally of only qualitative use. Other methods such as reductive depolymerisation and, in particular, mass spectral analysis were able to give a detailed analysis of the extent of derivatisation. Each technique employed is discussed in detail below.

### 2.5.1 Proton NMR

Several other workers have carried out detailed studies on cyclodextrins and their, particularly methylated, derivatives with the aid of proton NMR at various magnetic field strengths[10]. In this work $^1H$ NMR spectra were acquired in d-chloroform solution for the numerous lipophilic derivatives described above. In native cyclodextrins and symmetrically substituted derivatives (where all glucopyranose subunits have the same substitution pattern at O(2), O(3) and O(6)) all glucopyranose subunits are magnetically equivalent because of the presence of the $C_n$ (n=6,7 or 8 for α-,β- and γ- cyclodextrin respectively) symmetry axis in solution. Consequently a single set of NMR resonances is observed, as if there were only one glucopyranosyl residue[11].

Figure 2.4 compares the proton NMR spectra for di-O-octylated and 'per'-O-octylated α-cyclodextrin derivative which are representative of all the cyclodextrin derivatives under investigation here. It is evident from these spectra that there is a marked increase in complexity of the resonances upon 3-O-alkylation.

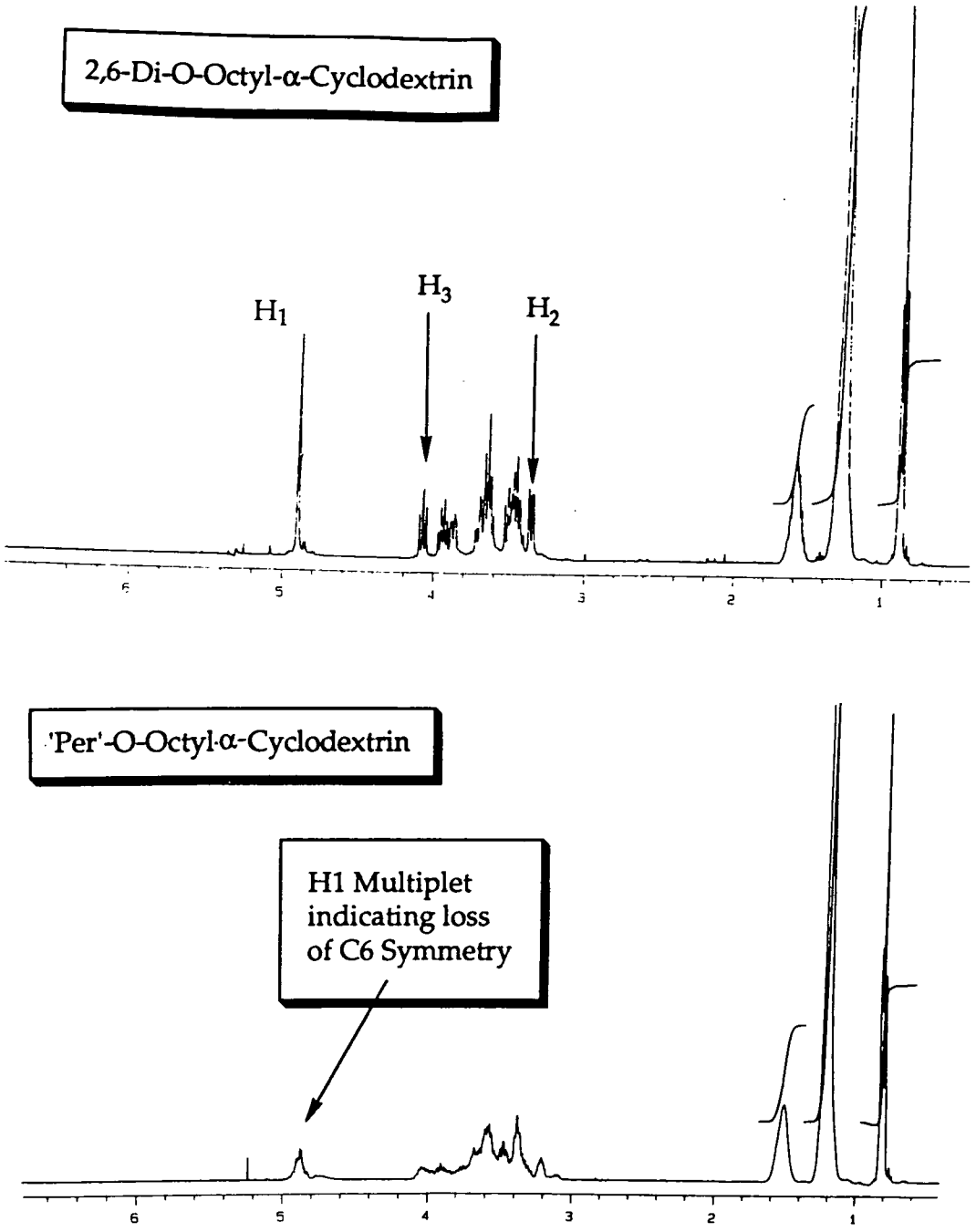



Figure 2.4 400MHz $^1$H NMR spectra of 2,6-di-O-Octyl-α-Cyclodextrin (upper) and 'per'-O-Octyl-α-Cyclodextrin (lower) in d-chloroform at 25°C

This increased complexity suggests a lower degree of symmetry for the 'per'-O-octyl derivatives, with the $C_n$ (n=6, 7 and 8 for α-, β- and γ-cyclodextrin) major axis of symmetry still more or less intact for the di-O-octyl derivatives. Such a loss of the high order of symmetry usually associated with cyclodextrins and their simple derivatives is indicative of

incomplete and non symmetrical alkylation of the hydroxyl groups. This incomplete alkylation is most probably at the 3(OH) position of the cyclodextrin as a result of intramolecular hydrogen bonding and steric crowding around an already highly functionalised secondary hydroxyl rim. The proton NMR spectra of 2,6-di-O-octyl-α-cyclodextrin and 'per-O-octyl'-α-cyclodextrin are discussed in detail below. The proton numbering scheme shown in figure 2.5 is used throughout the discussion.

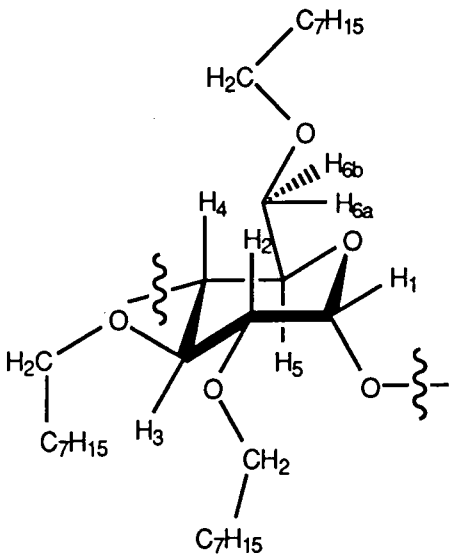



Figure 2.5 Proton Numbering Scheme.

Figure 2.6 shows the proton NMR and $^1H$-$^1H$ COSY spectrum of 2,6-di-O-octyl-α-cyclodextrin. A diagnostic resonance is that of H(1) at 4.90ppm which in this particular derivative is co-incident with OH(3). Using this diagnostic signal, the proton resonances for all five CHO protons and the $CH_2O$ protons of the glucopyranose subunit can be readily assigned from the COSY spectrum. Most noticeable from this is that H(2) and H(3) give resonances of well resolved structure at 3.35ppm and 4.07ppm respectively. This well resolved multiplet structure suggests that the cyclodextrin has been alkylated in a 'fairly symmetrical' manner, so maintaining the $C_6$ major symmetry axis.

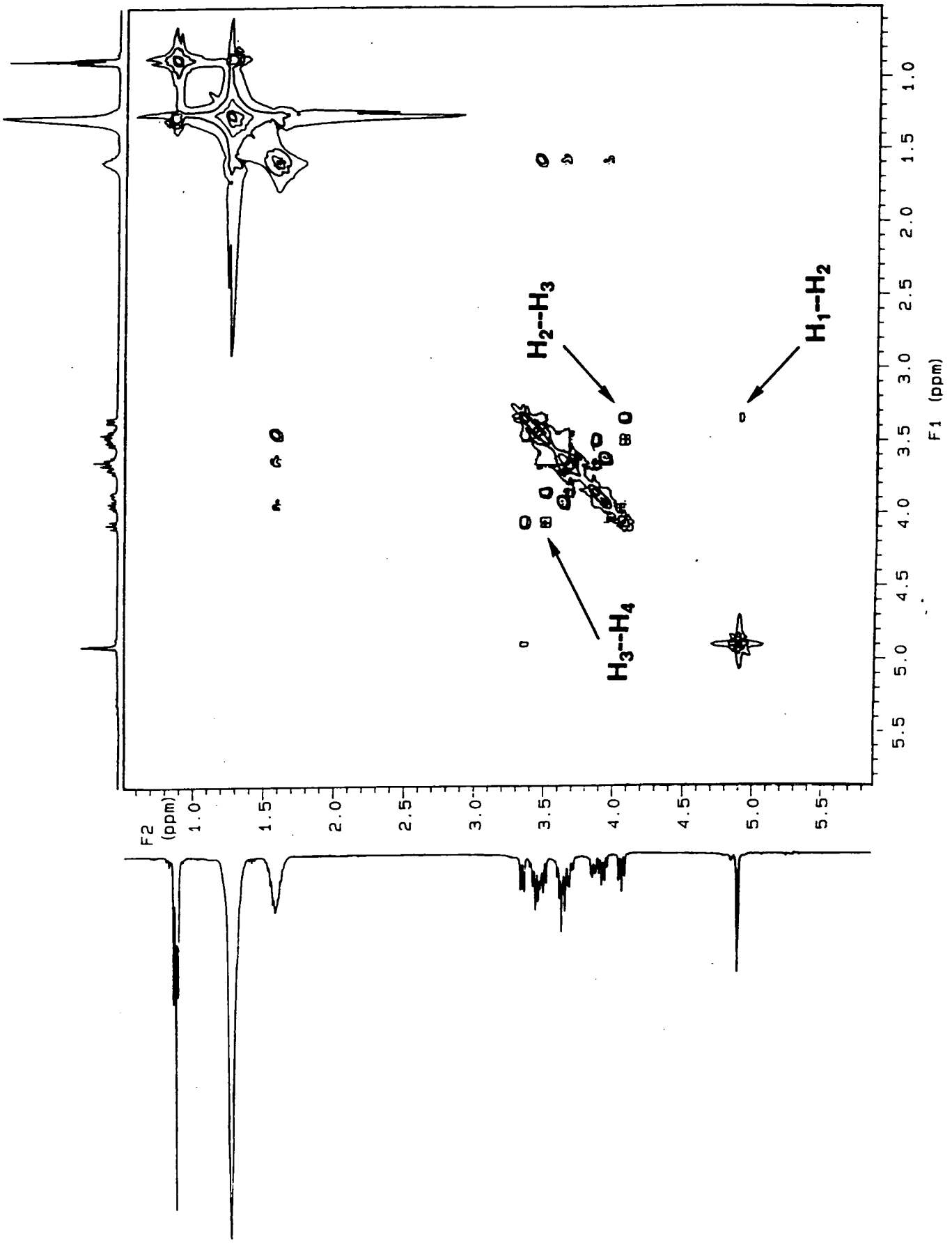



Figure 2.6 400MHz $^{1}H$-$^{1}H$ COSY Spectrum of 2,6-Di-O-Octyl-α-Cyclodextrin in d-chloroform Solution at 25°C

In the COSY spectrum, coupling of H(5) to H(6a) and H(6b), although evident, does not show the fact that H(6a) and H(6b) are anisochronous. However, this was readily detected through the use of $^{1}H$-$^{13}C$ HETCOR (section 2.5.2). $J_{H1H2}$ was observed to be 3.2 Hz which is in good agreement with the $J_{1,2}$ values for methylated cyclodextrins[10].

Three $CH_2O$ — $C\underline{H_2}CH_2O$ cross peaks are evident from the COSY spectrum in figure 2.6 which indicates that at least one of the octyl chain $CH_2O$ groups has two diastereotopic protons. Actually both octyl $CH_2O$ groups give anisochronous resonances, but again this could only be confirmed through the use of HETCOR. The fact that the octyl chain $CH_2O$ groups both give anisochronous resonances suggests that there is some preferential orientation of the octyl chains with respect to the cyclodextrin cavity. Such a preferential orientation of alkyl chains has recently been reported by Meier-Augenstein[12] for 2,6-di-O-pentyl-β-cyclodextrin. The determination of $^{2}J$ coupling constants showed that the pentyl chain attached to the 6 position oxygen of the cyclodextrin was preferentially oriented across the cavity with a gauche - trans conformation (figure 2.7) being suggested[11,12] for C(6). This type of orientation is perhaps not surprising since a hydrophobic alkyl chain may well be expected to interact with the hydrophobic cyclodextrin cavity. Both gauche - trans and gauche - gauche conformations are evident in the crystal structure of per-O-methyl cyclodextrin[13], suggesting that the preferred orientation observed with long chain alkyl ether derivatives is hydrophobically driven.

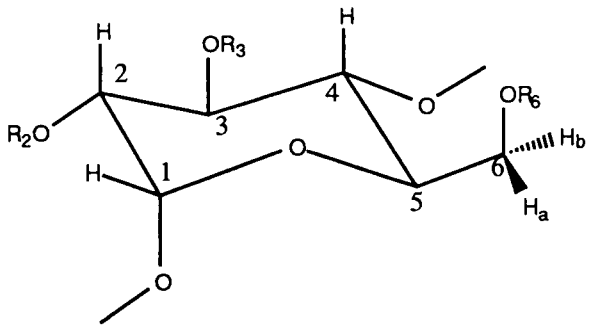



Figure 2.7 Gauche - Trans conformation at C(6) of a Glucopyranosyl Unit.

Figure 2.8 shows the proton NMR and 500MHz COSY spectra for 'per-O-octyl'-α-cyclodextrin. Comparison of this $^1H$ spectrum with that of the 2,6-di-O-octylated homologue indicates a sharp increase in complexity and a corresponding loss of resolution for, in particular, the H(1), H(2) and H(3) resonances. As a result a COSY spectrum was acquired at 500MHz to aid assignment. Exact resonance positions are difficult to assign for most of the protons in this compound, although approximate chemical shift values can be obtained from the COSY as figure 2.8 illustrates. These assignments were then further confirmed by HETCOR. Interestingly however, the overall symmetry appears somewhat more intact upon 3-O-acetylation of α-cyclodextrin suggesting that acetylation of 3(OH) may occur more readily than octylation. The observation that 3-O-acetylation is complete is further confirmed by infra red analysis and more especially by ES-MS investigation.

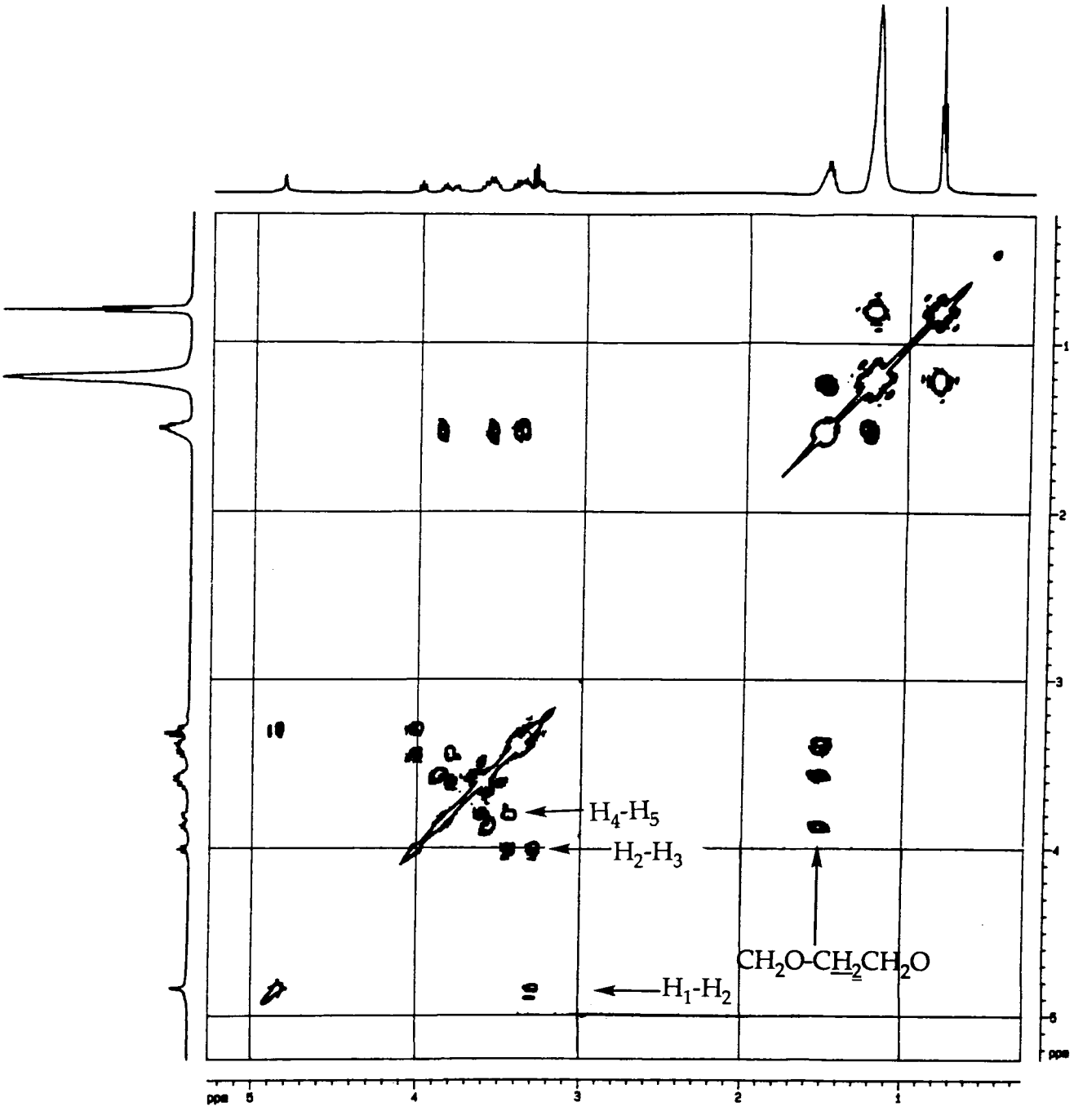



Figure 2.8 500MHz COSY spectra for 'per-O-octyl'-α-cyclodextrin in d-chloroform at 25°C

Proton integration was unsatisfactory in all cases for determining the extent of alkylation. This is because the change in the integration ratios of the peaks involved with the addition, or subtraction, of a single octyl group is well within the error involved in determining the integrals.

### 2.5.2 $^{13}C$ NMR

High resolution $^{13}C$ NMR has been employed by numerous workers as an analytical tool to investigate the homogeneity of cyclodextrin derivatives[3a,14] and was initially employed to investigate the conformations of the native cyclodextrins in aqueous solution[10c]. Figure 2.9 shows a 100MHz high resolution $^{13}C$ spectrum obtained for 2,6-di-O-octyl-α-cyclodextrin along with those of the native dextrin and the 'per'-O-octyl analogue for comparison.

In the case of proton NMR, the appearance of the resonance due to H(1) was found to be most useful for qualitative determination of homogeneity. In $^{13}C$ analysis it is the attached carbon,C(1) at approximately 101ppm that is also diagnostic. In the case of the di-O-alkylated-α-cyclodextrin a single, well resolved C(1) resonance is observed at 101.4ppm, further indicating that the symmetry of the system is conserved.

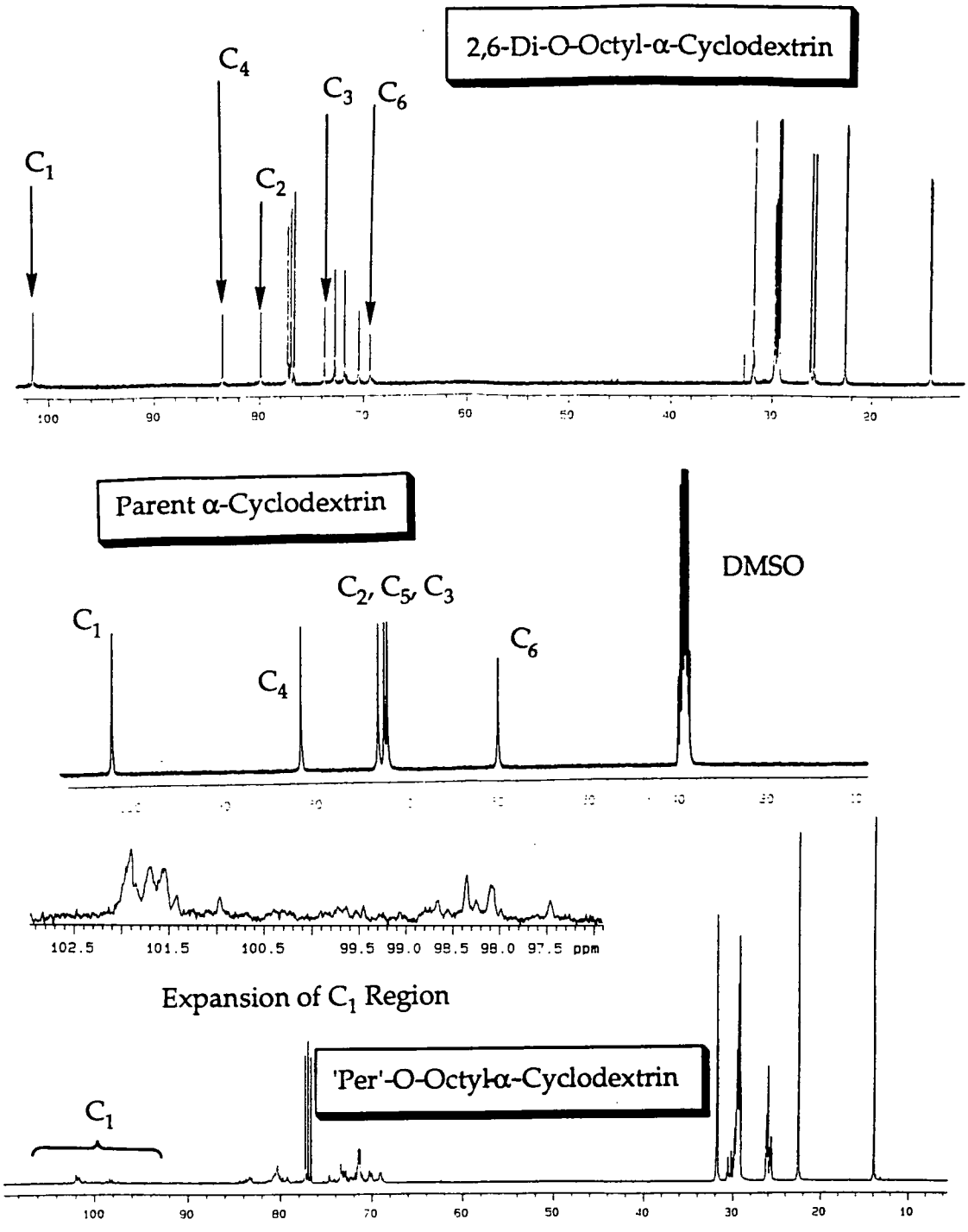



Figure 2.9 100MHz $^{13}C$ spectra for 2,6-di-O-octyl-α-cyclodextrin, native α-cyclodextrin and 'per'-O-octyl-α-cyclodextrin in d-chloroform solution at 25°C

For the 'per'-O-octyl derivative the situation is quite different. There are apparently two 'sets' of C(1) resonance evident at 101.7 and 98ppm. This suggests that the cyclodextrin derivative actually consists of several constitutional isomers and homologues. Figure 2.9 summarises the information which may be obtained by direct $^{13}C$ analysis.

As a result of the complexity of the proton NMR spectra and the similarity of several of the $^{13}C$ resonances Heteronuclear Correlation Spectra (HETCOR) were obtained, as mentioned previously, for all derivatives. In each case this allowed the assignment of virtually all the carbon and proton resonances, with the help of homonuclear proton COSY. Figure 2.10 shows the HETCOR spectra obtained for 2,6-di-O-octyl-α-cyclodextrin and its 'per'-O-octyl analogue.

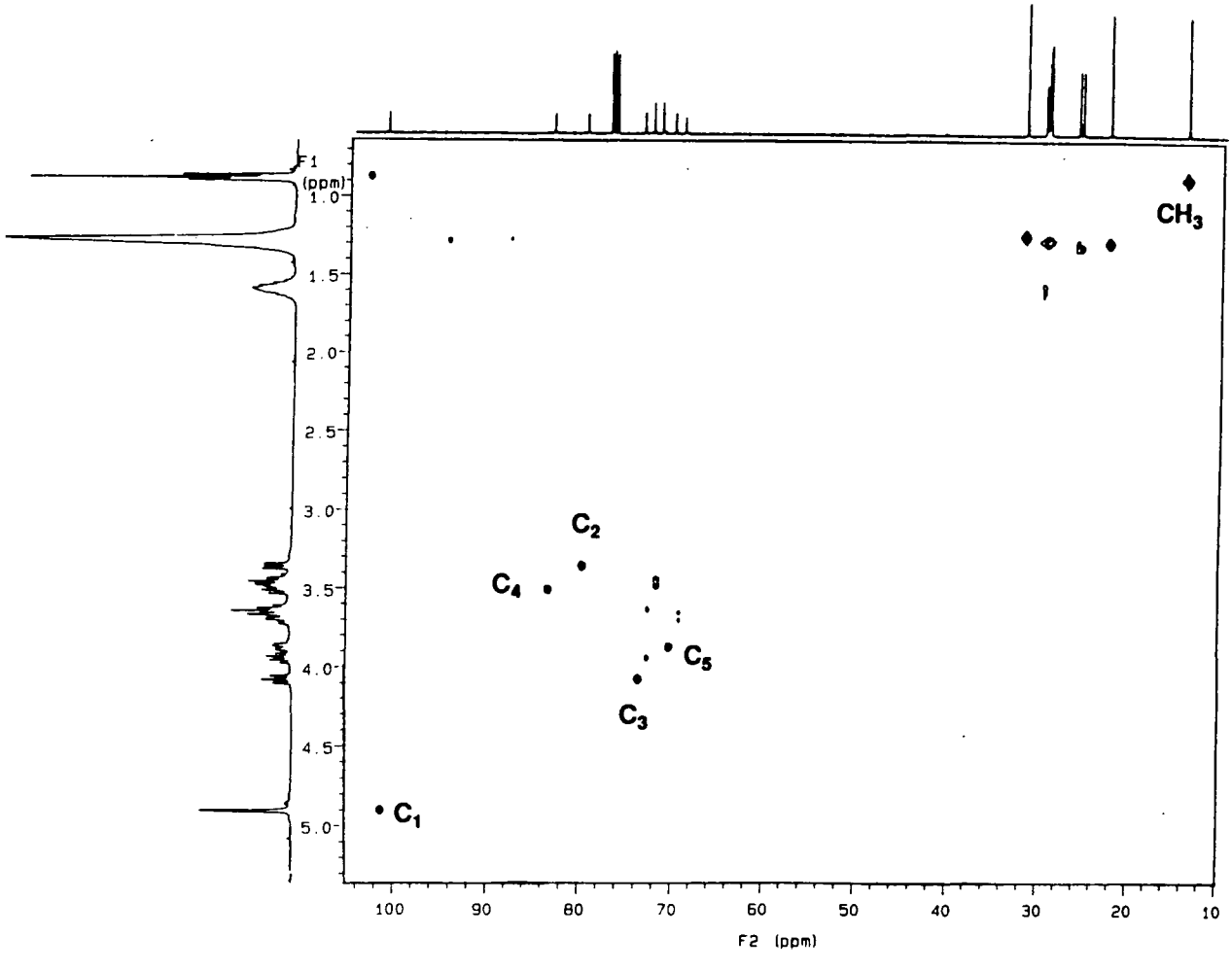



Figure 2.10 100MHz $^{1}H$-$^{13}C$ Correlation Spectrum for 2,6-di-O-octyl-α-cyclodextrin in d-chloroform solution at 25°C

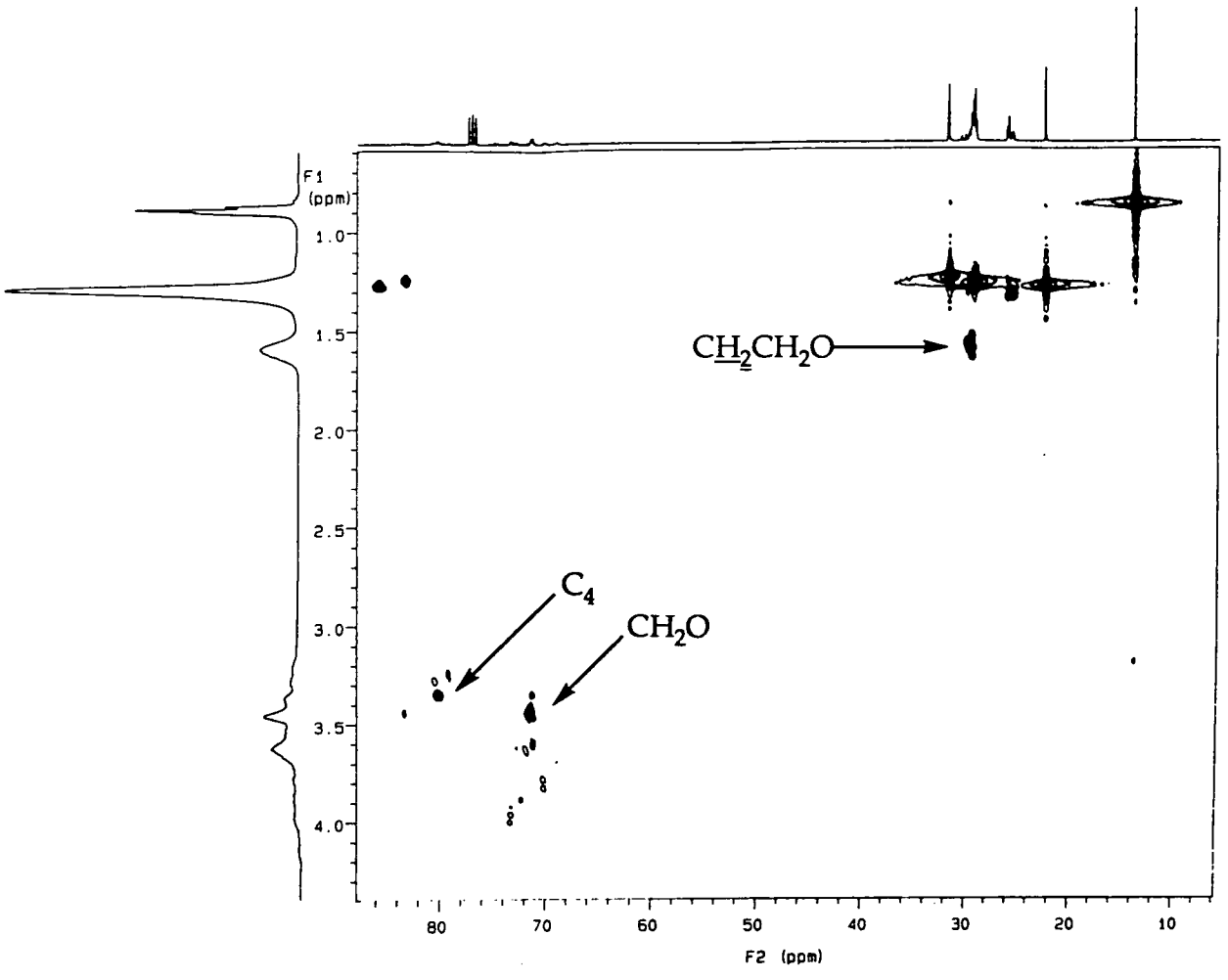



Figure 2.10 (cont.) 100MHz $^{1}H$-$^{13}C$ Correlation Spectrum for 'per'-O-octyl-α-cyclodextrin in d-chloroform solution at 25°C

The utility of HETCOR in the assignment of the $^{13}C$ and $^{1}H$ spectra for these compounds is also illustrated in figure 2.10. No attempt was made however to assign the $CH_2$ $^{13}C$ resonances of the octyl chains (other than $CH_2O$, $C\underline{H_2}CH_2O$ and $CH_3$). Meier-Augenstein attempted to assign the corresponding $CH_2$ resonances for 2,6-di-O-pentyl-β-cyclodextrin through the use of $^{13}C$ $T_1$ acquisition[12] but arrived at inconclusive results. This is presumably because alkyl chain - alkyl chain interactions remove the incremental change in $T_1$ along the alkyl chain that would otherwise be expected. Tables 2.5.2A to C indicated some of the more diagnostic $^{13}C$ resonances for octylated derivatives of all three cyclodextrins.

**Table 2.5.2A Selected $^{13}C$ Resonances of α-Cyclodextrin Derivatives**

**(100MHz d-chloroform solution 25°C)**

| | $^{13}C$ Resonance of carbon number: | | | | | | |
|---|---|---|---|---|---|---|---|
| Derivative | 1 | 2 | 3 | 4 | 5 | 6 | $CH_2O$ |
| α-cyclodextrin | 102.2 | 72.4 | 74.1 | 82.1 | 72.8 | 61.1 | — |
| 2,6-di-O-octyl- | 101.4 | 79.9 | 73.8 | 83.5 | 70.4 | 69.3 | 72.7 |
| α-cyclodextrin | | | | | | | 71.8 |
| 'per'-O-octyl- | 101.7 | 79.8 | 73.8 | 83.3* | 70.5 | 69.8 | 71.8 |
| α-cyclodextrin | 98* | | | | | | 72.8 |
| | | | | | | | 70.9 |
| [a]methylated- | 99.2* | 80.3* | 80.5 | 82.9* | 70.7 | 69.8 | 71.8 |
| 'per'-O-octyl- | | | | | | | 72.7 |
| α-cyclodextrin | | | | | | | 71.1 |
| 3-O-acetyl-α- | 100.8 | 78.6 | 77.3 | 80.4 | 71.3 | 69.1 | 71.8 |
| cyclodextrin[b] | | | | | | | 71.5 |

[a] $\delta_C$ (OMe) = 61.8.

[b] $\delta_C$ (C=O) = 170.5.

* indicates that the resonance could not be assigned with absolute certainty because of the complexity of the spectrum and should be taken as ±0.2ppm.

**Table 2.5.2B Selected $^{13}C$ Resonances of β-Cyclodextrin Derivatives (100MHz d-chloroform solution 25°C)**

| | $^{13}C$ Resonance of carbon number: | | | | | | |
|---|---|---|---|---|---|---|---|
| Derivative | 1 | 2 | 3 | 4 | 5 | 6 | $CH_2O$ |
| β-cyclodextrin | 102.8 | 72.9 | 74.1 | 82.0 | 72.9 | 61.0 | — |
| 2,6-di-O-octyl- | 101.8 | 80.3 | 74.2 | 82.9 | 71.1 | 69.4 | 72.9 |
| β-cyclodextrin | | | | | | | 71.8 |
| 'per'-O-octyl- | 101.4* | 80.5* | 74.1 | 83.1* | 70.9 | 69.5 | 71.7 |
| β-cyclodextrin | and | | | | | | 72.9 |
| | 99.1* | | | | | | 71.1 |
| [a]methylated- | 99.2* | 80.9* | 82.3 | 82.9* | 71.0 | 69.4 | 71.8 |
| 'per'-O-octyl- | | | | | | | 72.8 |
| β-cyclodextrin | | | | | | | 71.1 |

[a] $\delta_C$ (OMe) = 61.8.
* indicates that the resonance could not be assigned with absolute certainty because of the complexity of the spectrum and should be taken as ±0.2ppm.

**Table 2.5.2C Selected $^{13}C$ Resonances of γ-Cyclodextrin Derivatives (100MHz d-chloroform solution 25°C)**

| | $^{13}C$ Resonance of carbon number: | | | | | | |
|---|---|---|---|---|---|---|---|
| Derivative | 1 | 2 | 3 | 4 | 5 | 6 | $CH_2O$ |
| γ-cyclodextrin | 103.1 | 72.7 | 74.2 | 82.0 | 72.9 | 61.1 | — |
| 2,6-di-O-octyl- | 101.8 | 80.8 | 73.3 | 83.2 | 70.4 | 69.1 | 73.1 |
| γ-cyclodextrin | | | | | | | 71.6 |
| 'per'-O-octyl- | 102.3* | 80.1* | 74.1 | 82.1* | 70.8 | 70.3 | 71.8 |
| γ-cyclodextrin | | | | | | | 72.7 |
| | | | | | | | 71.2 |

* indicates that the resonance could not be assigned with absolute certainty because of the complexity of the spectrum and should be taken as ±0.2ppm.

Similarly to the results of other workers[10d], the C(2), C(3) and C(6) ring carbons show the strongest changes in chemical shift induced by octylation of the corresponding hydroxyl groups. Tables 2.5.2A, 2.5.2B and 2.5.2C clearly show that in every case, these resonances were observed to move to higher frequency upon octylation.

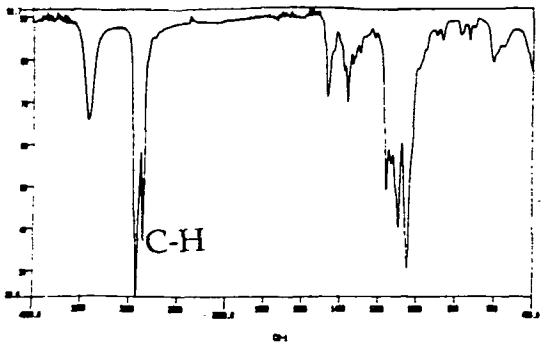
The use of high field, high resolution proton and carbon NMR and correlation spectroscopy therefore allows assignment of resonances for each lipophilic cyclodextrin derivative synthesized and gives a qualitative estimation of the degree of inhomogeneity for each compound.

## 2.6 Infra Red Spectroscopy

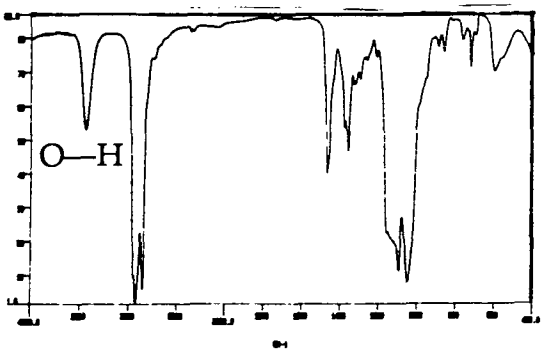
Infra red spectroscopy is a classical technique for the characterisation of organic molecules and is especially useful for the determination of the functionality present in a given system. It has previously been applied to cyclodextrins for the investigation of conformational mobility[15]. However its use as a characterisation technique is somewhat limited, largely resulting from the lack of distinctive functionality for many of the derivatives synthesized.

In this investigation Fourier transform infra red spectroscopy was used. Samples were prepared as thin films on NaCl plates by evaporation from a dichloromethane solution. Figure 2.11 shows typical spectra obtained for several cyclodextrin derivatives.

2,6-DI-O-OCTYL-α-CYCLODEXTRIN




'PER'-O-OCTYL-α-CYCLODEXTRIN




METHYLATED 'PER'-O-OCTYL-α-CYCLODEXTRIN

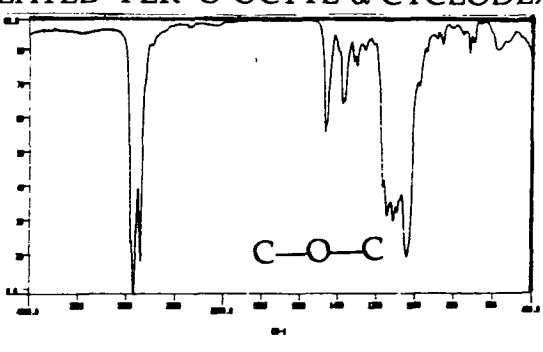



Figure 2.11 FT-IR Spectra of Several Alkylated α-Cyclodextrins as Thin Films on NaCl plates

Several conclusions can be drawn from these spectra:

i) 'per'-O- octylation results in derivatives which still contain OH groups.

ii) 'capping' of residual OH groups by methylation is effective.

iii) 3-O-acetylation is clearly evident and complete.

iv) octylation with sodium hydroxide gives derivatives with a strong O-H stretch still present.

Many qualitative conclusions can therefore be drawn from infra red spectroscopy regarding the nature of the dextrin derivatives. However, because of the macroscopic nature of this technique it is not able to investigate the homogeneity or the exact degree of functionalisation.

### 2.5.4 Reductive Depolymerisation

High resolution proton and carbon NMR spectroscopy has indicated that the products of cyclodextrin octylation are not homogeneous. Quantification of the extent of over/under alkylation of the cyclodextrin hydroxyl groups using proton NMR integrations prove ambiguous because of the complexity of the spectra. Thus NMR analysis is only able to indicate the inhomogeneity of the various alkylated cyclodextrins currently under discussion. Such findings are in agreement with those of other workers and indeed, it has been suggested that <5% of impurity cannot be detected by NMR[12].

As an analytical technique reductive depolymerisation represents a development of the methylation analysis of polysaccharides[16]. As a method of characterisation it has previously been applied to several cyclodextrin and other oligosaccharide derivatives. Several variations[17] in the method of depolymerisation have been developed since its introduction by Gray in 1982[18]. Figure 2.12 shows a schematic representation of the steps involved in the depolymerisation of a cyclodextrin by a method similar to that previously described by Mischnick-Lübbecke for the analysis of $C_4$ - $C_6$ alkylated cyclodextrins[17]. Studies by other workers with model glycosides have shown that reductive cleavage is regiospecific under these conditions. That is, selective cleavage of the glycosidic carbon - oxygen bond is observed via the formation and reduction of cyclic oxonium ions[17].

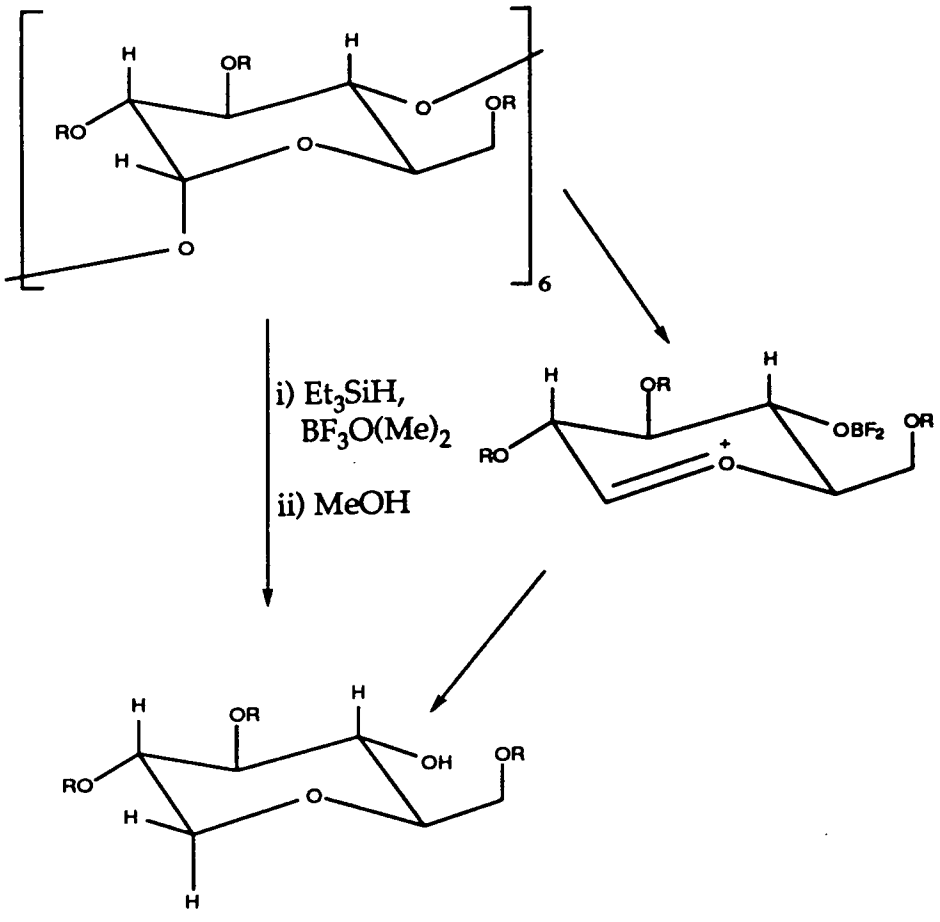



Figure 2.12 Reductive Depolymerisation

Treatment of the substituted cyclodextrin with triethylsilane in the presence of $BF_3.OMe_2$ results in Lewis acid catalysed cleavage of the glycosidic linkages to give the corresponding alkylated 1,5-anhydro-D-glucitols. This reduces the mass of the system considerably, effectively giving the individual glucose monomer subunits which, after acetylation, can be investigated by gas chromatography. GC-MS then allows quantification of each type of substituted pyranose system. This allows a mean degree of alkylation to be calculated for each derivative. For example, in the investigation of O-pentylated cyclodextrins by Mischnick-Lubbecke, over pentylation of the '2,6-di-O-pentyl' derivative was clearly evident (8.2%). However, very little under alkylation of the per-O-pentyl derivative was apparent (<0.4%) indicating virtually complete alkylation.

complete alkylation. The results obtained here for several alkylated cyclodextrin derivatives are summarised in table 2.5.4A.

**Table 2.5.4A Reductive Depolymerisation of Octylated α–, β– and γ– cyclodextrins**

| Cyclodextrin Derivative | % Disubstituted* | % Trisubstituted* | Mean No. of octyl groups |
|---|---|---|---|
| 2,6-di-O-octyl-α-cyclodextrin | 88 | 12 | 12.7 |
| 'per'-O-octyl-α-cyclodextrin | 43 | 57 | 15.4 |
| methylated 'per'-O-octyl-α-cyclodextrin | < 1 | 58‡ | 15.4 |
| 2,6-di-O-octyl-β-cyclodextrin | 95 | 5 | 14.3 |
| 'per'-O-octyl-β-cyclodextrin | 51 | 49 | 17.4 |
| 'per'-O-octyl-γ-cyclodextrin | 45 | 55 | 20.4 |

* as % areas from GC-MS.
‡ 42% 3-O-methylated.

Figure 2.13 shows the GC trace obtained for each octylated α-cyclodextrin.

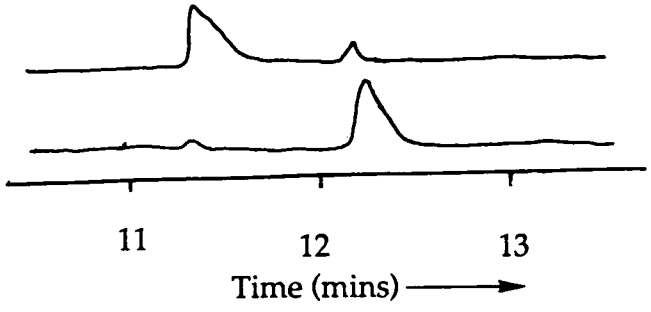



Figure 2.13 GC Traces for 2,6-Di-O-Octyl-α-Cyclodextrin (upper) and 'Per'-O-Octyl-α-Cyclodextrin (lower).

The table and GC traces above indicate that all the cyclodextrin derivatives investigated by reductive depolymerisation constitute several homologues. With the 'per'-O-octyl derivatives the incomplete octylation may well be associated with the well documented comparatively low reactivity of OH(3) as a result of intramolecular hydrogen bonding. However, steric hindrance will most likely play a part as well[20]. In that

i) OH(3) points across the face of the cavity rather than away from the cavity, as is this case with OH(2)

and

ii) the numerous octyl chains already attached to the cyclodextrin core may be expected to hinder the approach of the $CH_2Br$ group to the remaining OH(3) functionalities.

Although steric hindrance probably goes some way to explaining why octylation is universaly incomplete[21] in the 'per'-O-octyl cyclodextrins, there must be some property of the $S_N2$ reaction of octyl bromide with the cyclodextrin OH(3) group that inhibits complete alkylation. This is because, as mentioned earlier, Mischnick-Lubbecke has shown by reductive depolymerisation analysis that per-O-pentylation is apparently complete. It is likely that there is some inclusion dependent phenomenon which prevents approach of the $CH_2Br$ and OH(3) groups in the required geometry for $S_N2$ reaction. It may in fact be that one (or more) of the octyl chains already attached to the cyclodextrin core is weakly bound by the cyclodextrin. Such an alignment of the octyl chains may then 'mask' the remaining hydroxyl groups and prevent their reaction with 1-bromooctane.

In conclusion it may be said that reductive depolymerisation allows the application of classical analytical techniques to cyclodextrin derivatives with reproducible results.

### 2.5.5 The Application of Mass Spectrometry

In chapter one it was shown that,in the field of cyclodextrin chemistry, the application of mass spectral analysis is a relatively new technique. Inspired by the work of Yaozo[22] and Stoddart[23] FAB spectra were acquired for several octylated cyclodextrin products using a glycerol matrix. Figure 2.14 shows typical FAB spectra for 2,6-di-O-octyl-α-cyclodextrin and 'per'-O-octyl-α-cyclodextrin which can be seen to be of relatively poor quality, particularly in the molecular ion region. However, these spectra do quite clearly show the greater inhomogeneity of the 'per'-O-octyl cyclodextrins.

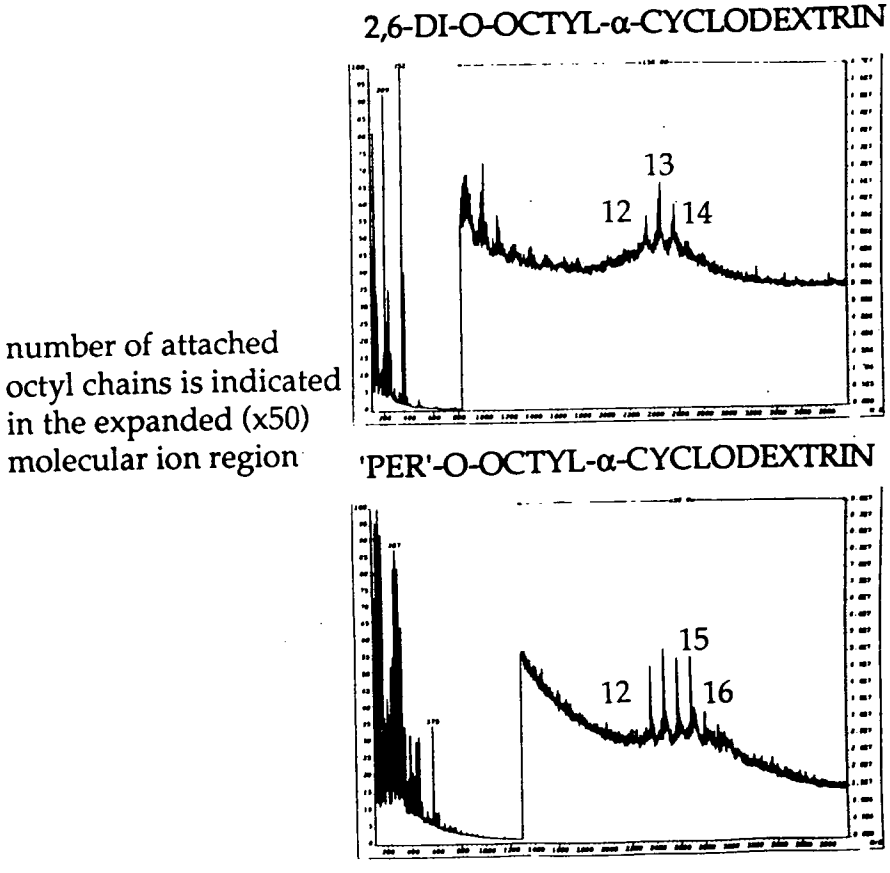
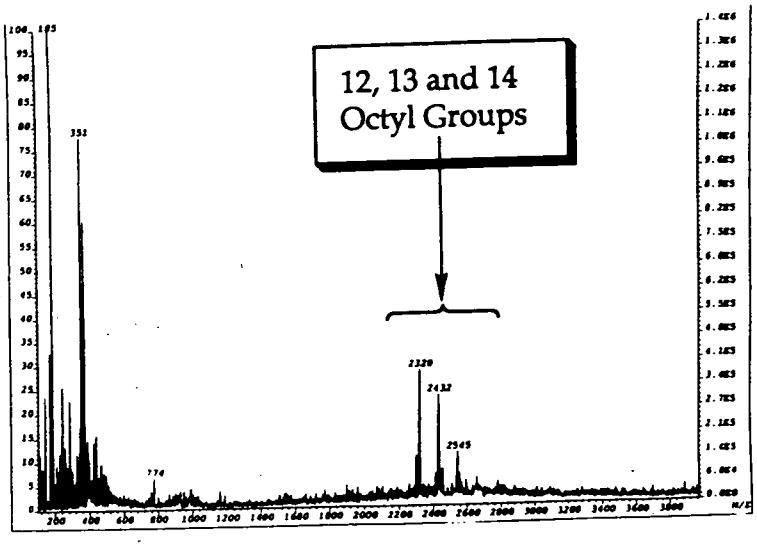



Figure 2.14 (+)-FAB Mass Spectra for 2,6-Di-O-Octyl-α-Cyclodextrin and 'Per'-O-Octyl-α-Cyclodextrin

Other ionisation techniques were employed in order to improve the ease of cyclodextrin ionisation, so increasing the molecular ion peak heights and improving the overall quality of the spectra. Figures 2.15a and 2.15b show (+) Field Desorption (FD) and Electrospray (ES) mass spectra respectively for several octylated cyclodextrin derivatives.

2,6-DI-O-OCTYL-α-CYCLODEXTRIN




'PER'-O-OCTYL-α-CYCLODEXTRIN

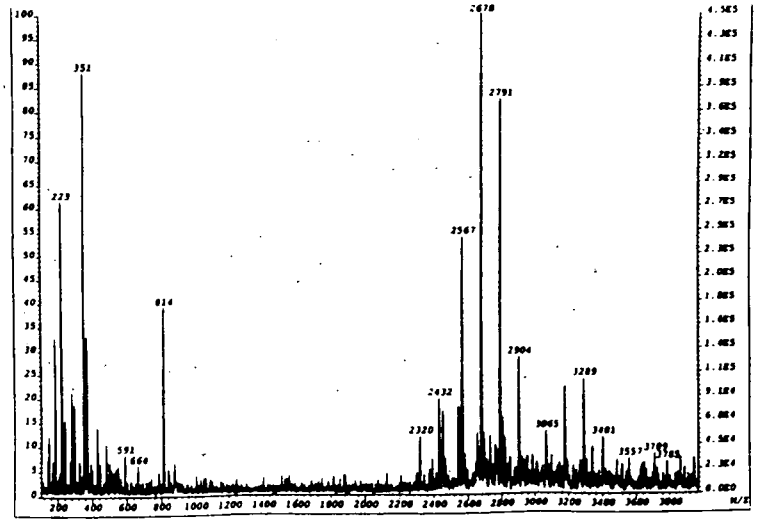


Figure 2.15a (+)-Field Desorption Spectra for 2,6-Di-O-Octyl-α-Cyclodextrin and 'Per'-O-Octyl-α-Cyclodextrin

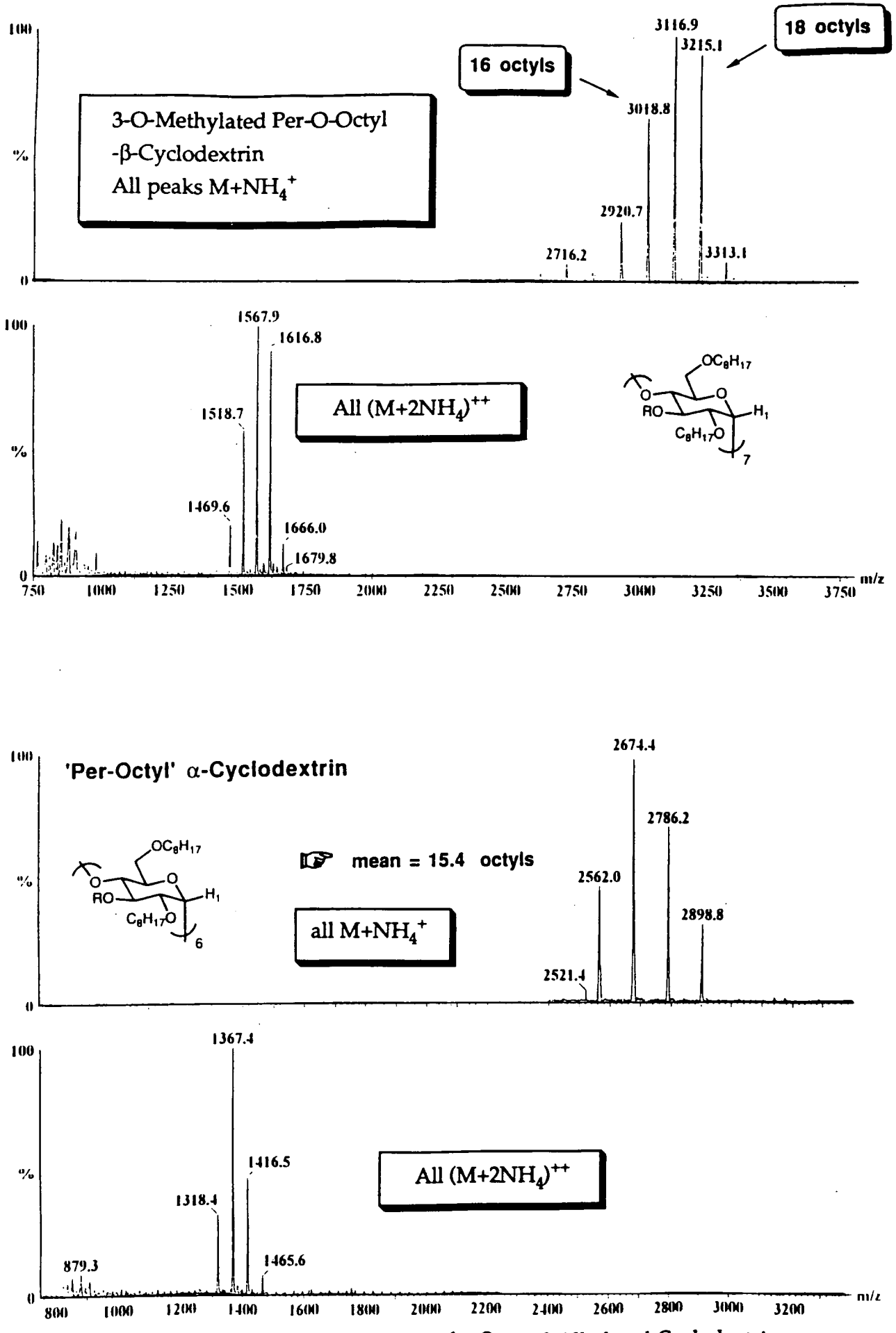



Figure 2.15b Electrospray Mass Spectra for Several Alkylated Cyclodextrins

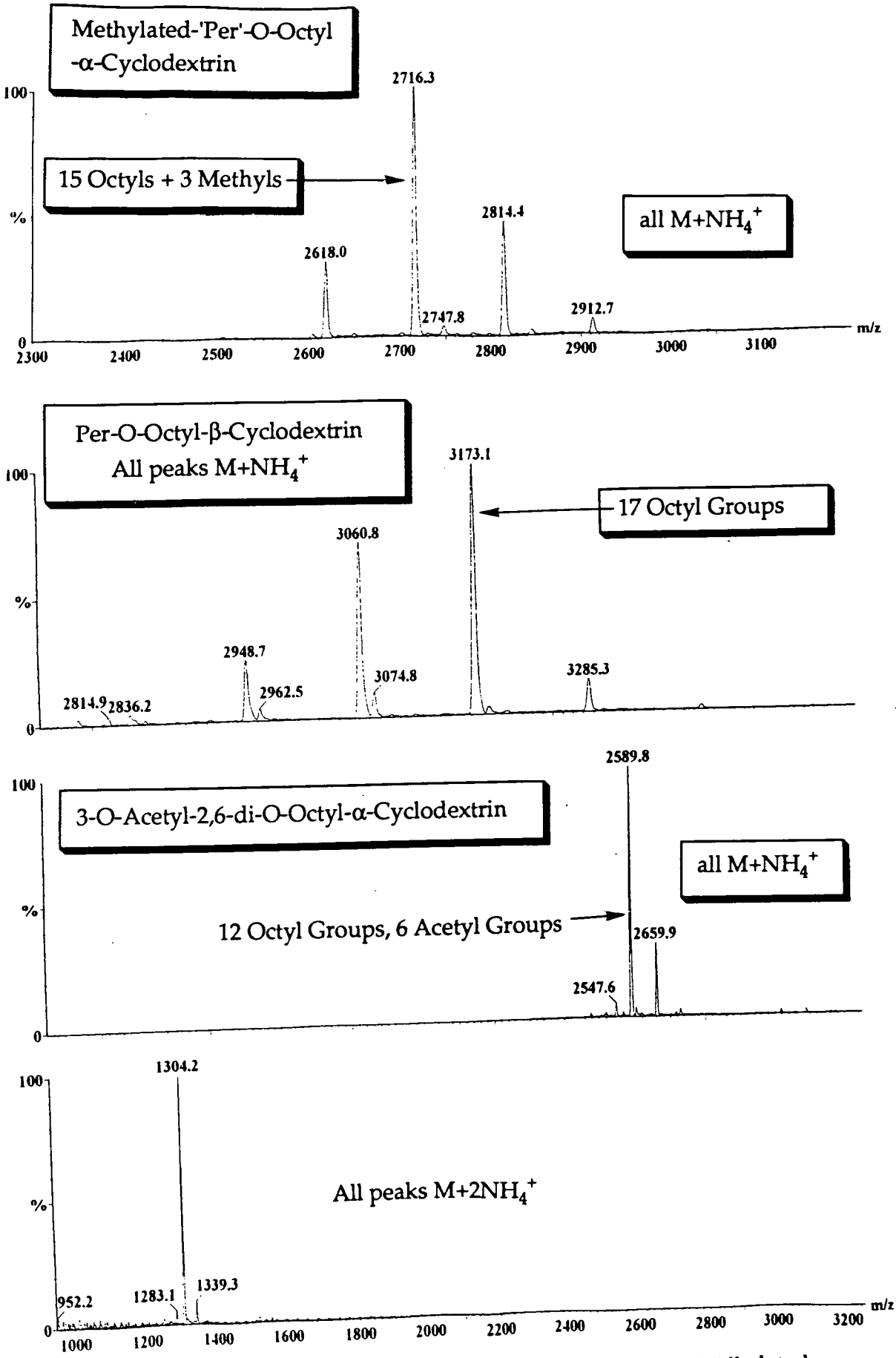



Figure 2.15b (cont.) Electrospray Mass Spectra for Several Alkylated Cyclodextrins

In both cases the resolution is a dramatic improvement on that observed by FAB-MS with the molecular ion peak heights increased substantially. Although both are an improvement upon FAB, the electrospray method affords better quality spectra with reproducible accurate molecular ion masses and a much improved signal to noise ratio. ES-MS also allows the simultaneous observation of multiply charged ions.

These benefits of ES over FD lead to the conclusion that ES is the better technique for investigation of the lipophilic cyclodextrin derivatives under discussion here.

#### 2.5.5.1 Quantitative Application of Mass Spectrometry

In general, mass spectral analysis can only be applied as a qualitative technique in molecular characterisation; ie. although the species present can be determined from molecular ion peaks and fragmentation patterns the molar ratio of the molecules present cannot be obtained. This is because ion peak height is not only dependent upon the molar ratio of a given compound in a sample but is also dependant on the ionisation potential of the molecule, since it is $M^+$ which is detected. Despite this however, in the case of cyclodextrin homologues the ionisation potentials will be approximately equal. Hence a very good estimate of the extent of functionalisation can be derived from the relative peak heights by use of a weighted mean. Figure 2.16 demonstrates the application of this 'degree of functionalisation' technique in the case of 'per'-O-octyl-α-cyclodextrin.

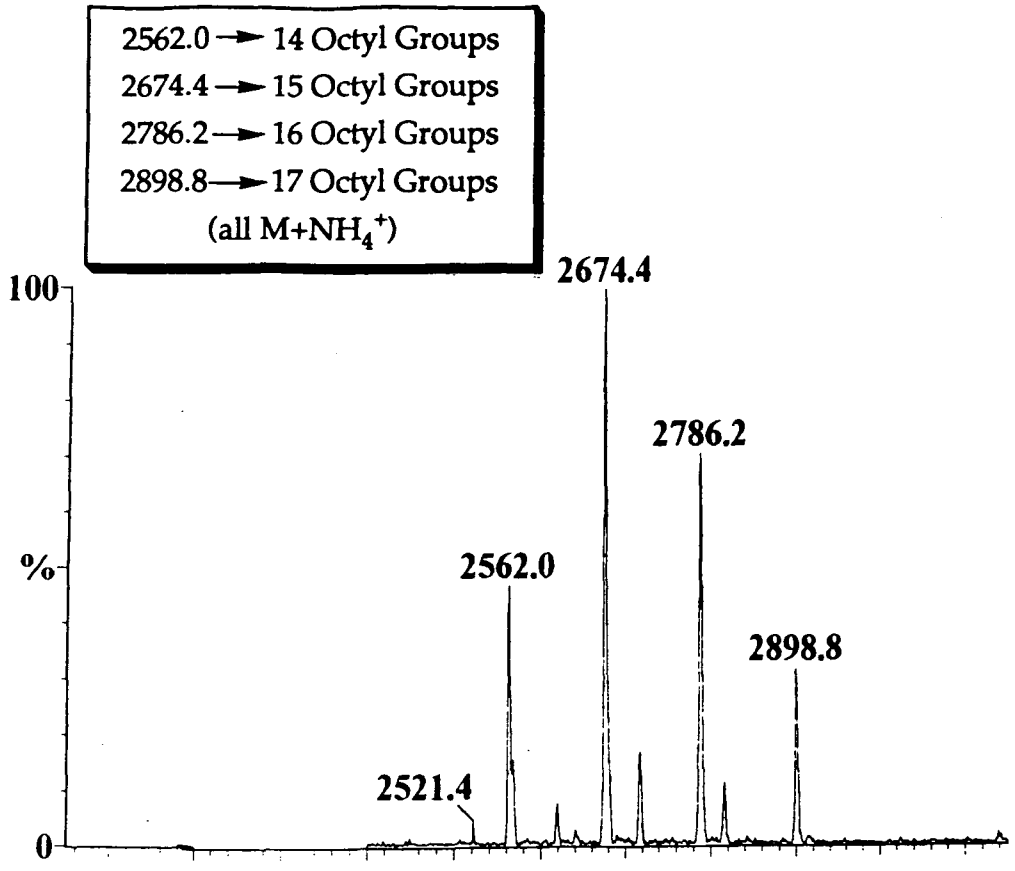



Figure 2.16 Determination of the 'Degree of Funcuonalisation' of 'Per'-O-Octyl-α-Cyclodextrin by ES-MS

Table 2.5.5.1A summarises the results of similar calculations for the various other derivatives synthesized. A similar type of mass spectral technique has previously been applied by Pitha *et al* to determine the molar degree of substitution for 2-hydroxypropyl substituted cyclodextrins[24].

**Table 2.5.5.1A Degree of Cyclodextrin Functionalisation Determined by ES-MS**

| Cyclodextrin derivative | Number of molecular ions Present. | Mean number of Octyl groups. |
|---|---|---|
| 'per'-O-octyl-α-cyclodextrin | 4 | 15.4 |
| Methylated 'per'-O-octyl-α-cyclodextrin | 4 | 15.4 |
| 3-O-acetyl-α-cyclodextrin | 3 | 12.2 |
| 'per'-O-octyl-β-cyclodextrin | 4 | 17.4 |
| Methylated 'per'-O-octyl-β-cyclodextrin | 4 | 17.4 |
| 'per'-O-octyl-γ-cyclodextrin | 5 | 20.7 |

It can be concluded from this mass spectral investigation that:

i)octylation is incomplete in each case.

ii)methylation of residual hydroxyl groups is apparently successful.

and

iii)3-O-acetylation of 2,6-di-O-octyl-α-cyclodextrin is complete.

The large number of constitutional isomers and closely related homologues which result from 'per'-O-octylation may be attributed to steric crowding around the secondary hydroxyl rim of the cavity and to the need to disrupt OH(3)···O(2)′ hydrogen bonding prior to $S_N2$ reaction at the O(3) position of the glucopyranose subunits. Since methyl capping of the hydroxyls is shown to be complete by ES-MS (ie. all peaks $(CD(Oct)_n(Me)_m)^+$, for α: n+m=18 and for β: n+m=21) it is reasonable to assume that incomplete octylation is largely a result of steric hindrance preventing approach of the $CH_2Br$ and OH(3) reacting groups in the correct orientation to allow the $S_N2$ reaction to occur. This was discussed earlier in relation to the results of the reductive depolymerisation investigation.

### 2.5.6 The use of Methylation to Investigate the Degree of Octylation

As mentioned above, incomplete octylation is probably associated with increasing steric hindrance at the secondary hydroxyl rim of the cyclodextrin as alkylation proceeds. Use of the less sterically demanding alkylating agent iodomethane allows complete 'capping' of residual hydroxyl groups. Infra red spectroscopy demonstrates this qualitatively with the disappearance of the cyclodextrin hydroxyl stretch at $3300cm^{-1}$. This is conclusively confirmed by ES-MS.

Figure 2.17 shows the $^1H$ spectrum for methylated 'per'-O-octyl-α-cyclodextrin. The use of two dimensional homo- and hetero- nuclear correlation spectroscopy allows assignment of both $^1H$ and $^{13}C$ resonances in a manner similar to that described in detail earlier for 'per'-O-octyl-α-cyclodextrin. Comparison of the proton spectrum in figure 2.17 with that of the non methylated 'per'-O-octyl-α-cyclodextrin (figure 2.8) shows a MeO singlet at 3.45ppm.

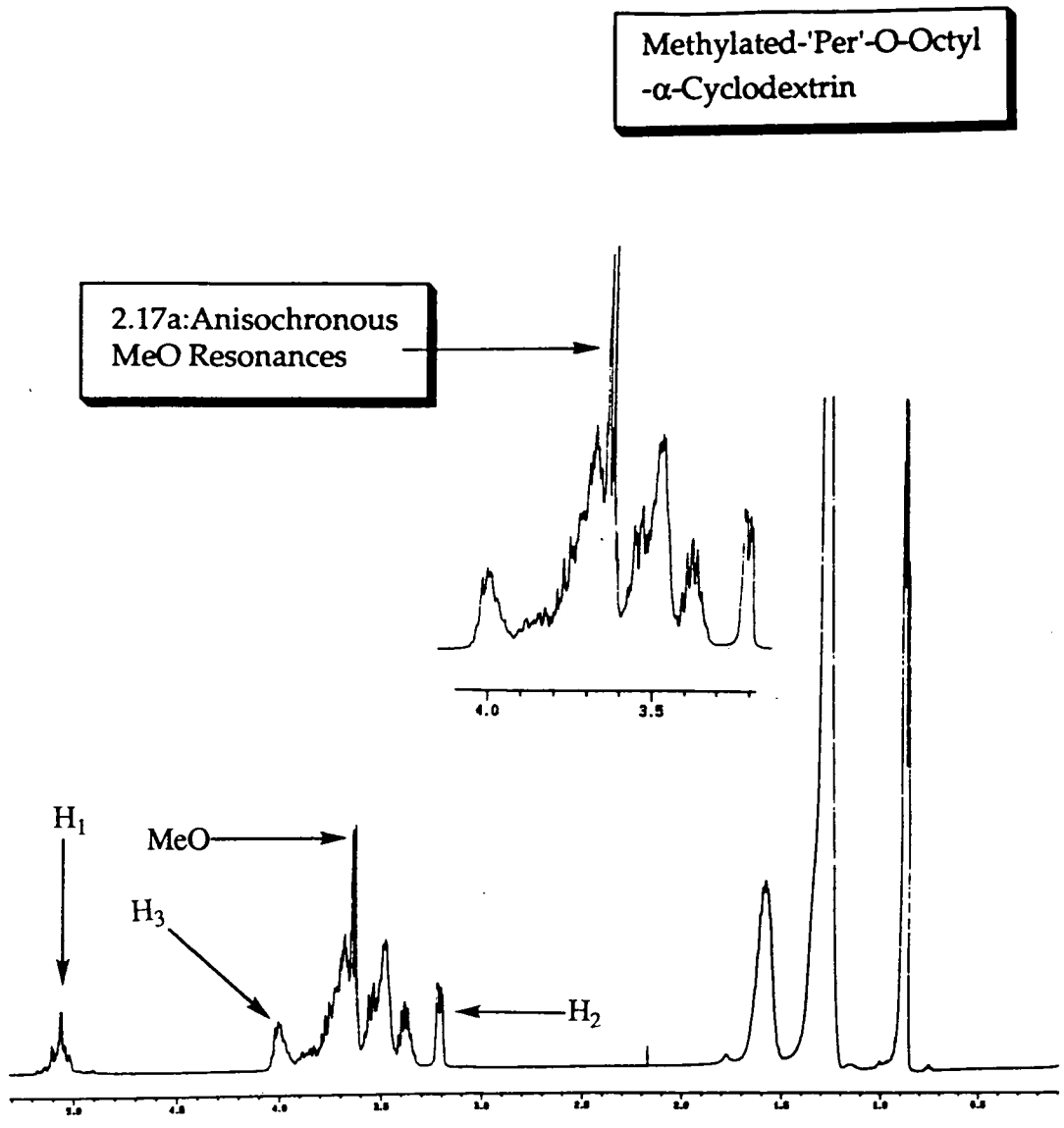



Figure 2.17 400MHz $^1H$ Spectrum for Methylated 'Per'-O-Octyl-α-Cyclodextrin in d-chloroform Solution at 25°C

The inset (figure 2.16a) shows an expanded 500 MHz spectrum of this region which shows that this resonance is in fact made up of 3 closely spaced singlets. This non-equivalence in the $^1H$ resonances of the OMe

groups is consistent with there being several constitutional isomers (and closely related homologues) present. Integration of this MeO resonance relative to the the octyl $\underline{CH_2}CH_2O$ and -$CH_3$ resonances, as well as the cyclodextrin H(1) resonance proved difficult and inconclusive. However, the number of methyl groups per cyclodextrin moiety could be roughly estimated to be 2.5 (0.3).

Carbon-13 NMR proved to be a much more informative technique, largely because (as figure 2.18 illustrates) the methyl carbon singlet is well resolved relative to the other resonances at 61.8ppm. The addition of 0.5 molar % of $Cr(acac)_3$ as a paramagnetic relaxation agent allowed acquisition of quantitative $^{13}C$ spectra for these methylated derivatives. Figure 2.18 shows a (quantitative) $^{13}C$ spectrum obtained for the α-cyclodextrin compound which could be integrated accurately to give the relative number of methyl groups in the compound (and hence the degree of octylation).

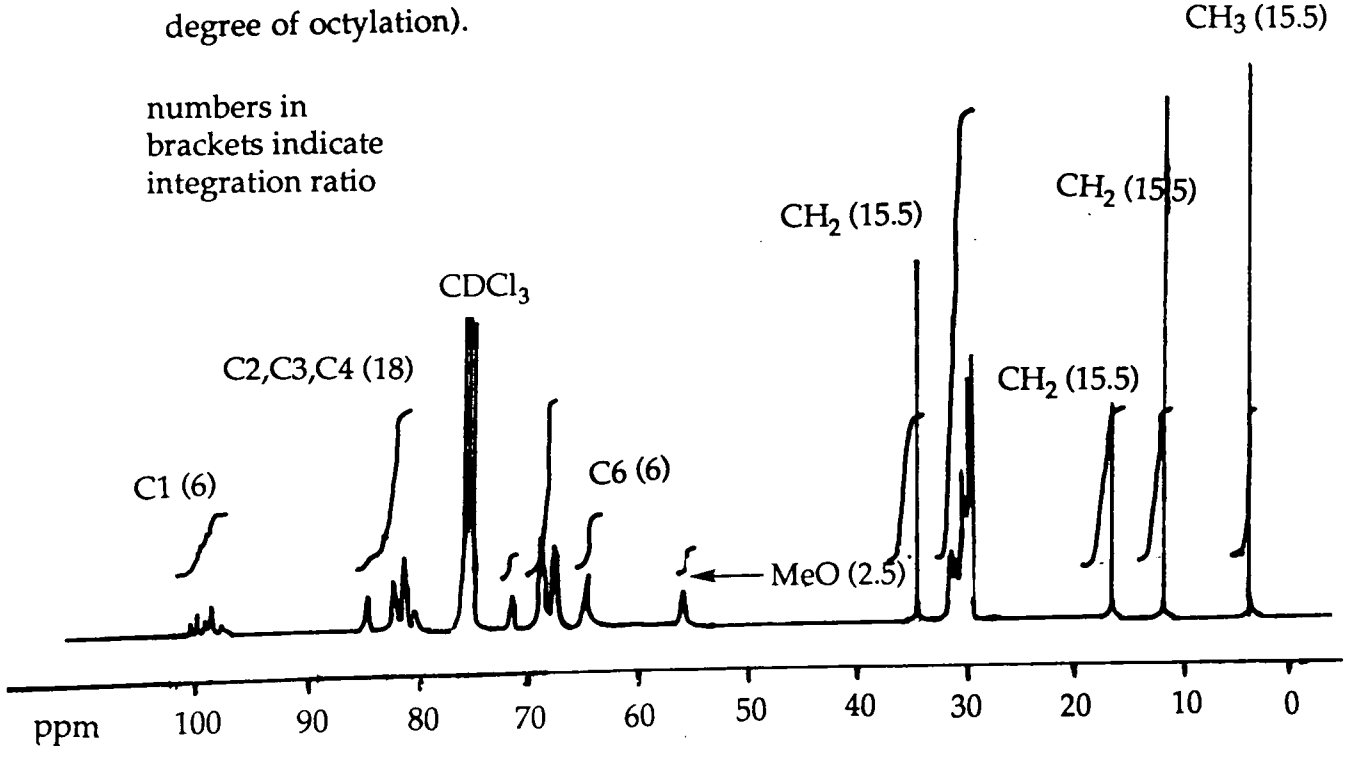



Figure 2.18 A (quantitative) 100MHz $^{13}C$ spectrum obtained for Methylated 'Per'-O-Octyl-α-cyclodextrin

Experimentally the acquisition of quantitative $^{13}C$ NMR spectra is difficult, particularly for high molecular weight molecules, such as cyclodextrin derivatives, which contain a large proportion of tertiary carbon atoms with relatively long relaxation times. However, the paramagnetic relaxation agent, $Cr(acac)_3$, dramatically reduces the relaxation time for each carbon by introduction of a rapid and dominant paramagnetic relaxation mechanism. Hence, under such conditions, reproducible spectra could be obtained with a 10s recycle and the use of gated decoupling, to remove any nuclear Overhauser effects during acquisition.

Integration revealed 2.5 methyl groups per α-cyclodextrin, which is in good agreement with mass spectral and reductive depolymerisation data. Table 2.5.6A summarises the results obtained for each methylated derivative.

**Table 2.5.6A Degree of Octylation for 'Per"-O-Octyl α-, β- and γ- Cyclodextrin.**

| | Degree of Octylation determined by: | | |
|---|---|---|---|
| Per-O-Octyl-Cyclodextrin | $^{13}C$ NMR | ES-MS | Reductive depolymerisation |
| α | 15.5 | 15.4 | 15.4 |
| β | 17.3 | 17.5 | 17.4 |
| γ | — | 20.7 | 20.4 |

## 2.6 CHARACTERISATION OF NON-MACROCYCLIC DEXTRINS

The dextrins glucose, maltose and maltotriose, which correspond to the monomer, dimer and trimer of the cyclodextrin glucopyranosyl subunit, were octylated in a similar manner to the cyclodextrins. When characterising these compounds there are several important differences to the cyclodextrin derivatives discussed in detail above, which result from lower molecular weight and different molecular symmetry. It is these differences which are discussed below.

### 2.6.1 Mass Spectrometry

The investigation of these dextrins by mass spectral analysis was found to be quite straightforward with DCI spectra being readily obtained for each' as figure 2.19 shows for maltose. In each case octylation is complete after the use of sodium hydride as base, although glucose was found to 'per'-O-octylate after the first step (NaOH, DMSO, $C_8H_{17}Br$). This complete octylation is further evidence that in the case of cyclodextrin octylation incomplete reaction is a consequence of steric crowding at the secondary hydroxyl rim. Any effects due to intramolecular hydrogen bonding would be expected to occur in maltose, and to a greater degree in maltotriose.

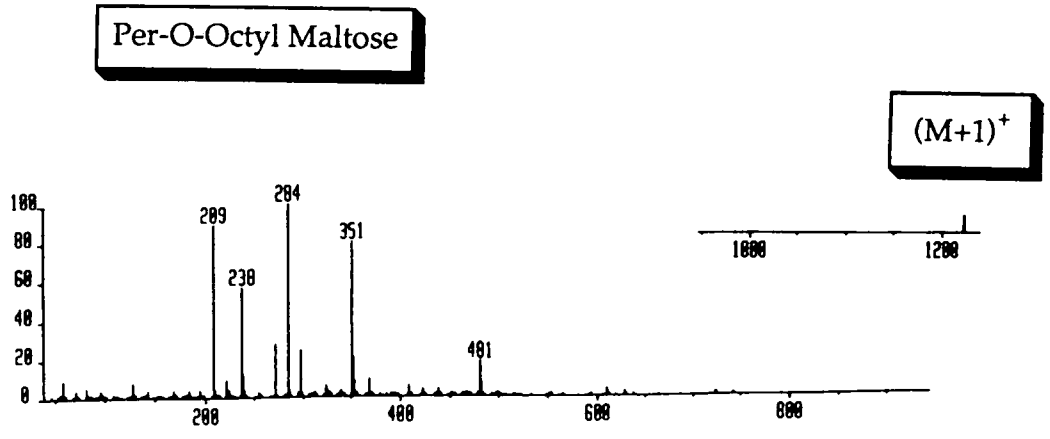



Figure 2.19 DCI Mass Spectrum of Per-O-Octyl Maltose

### 2.6.2 Proton NMR

All three dextrins were investigated by one and two dimensional proton NMR. Problems were encountered in the assignment of these complex spectra which resulted from lower molecular symmetry, although the assignments for the simplest, glucose, were rather more straightforward than for the other compounds. Figure 2.20 shows $^{1}H$ and COSY spectra for per-O-octyl maltotriose and their use in the assignment of some resonances. As with the functionalised cyclodextrins, HETCOR, in association with COSY, was found to confirm and simplify several of these assignments and its application is discussed in the $^{13}C$ section below. Despite the use of high field (400MHz) two dimensional techniques, many of the resonances in the more complex CHO and $CH_2O$ region of the per-O-octyl maltose and maltotriose spectra could not be assigned with any degree of certainty.

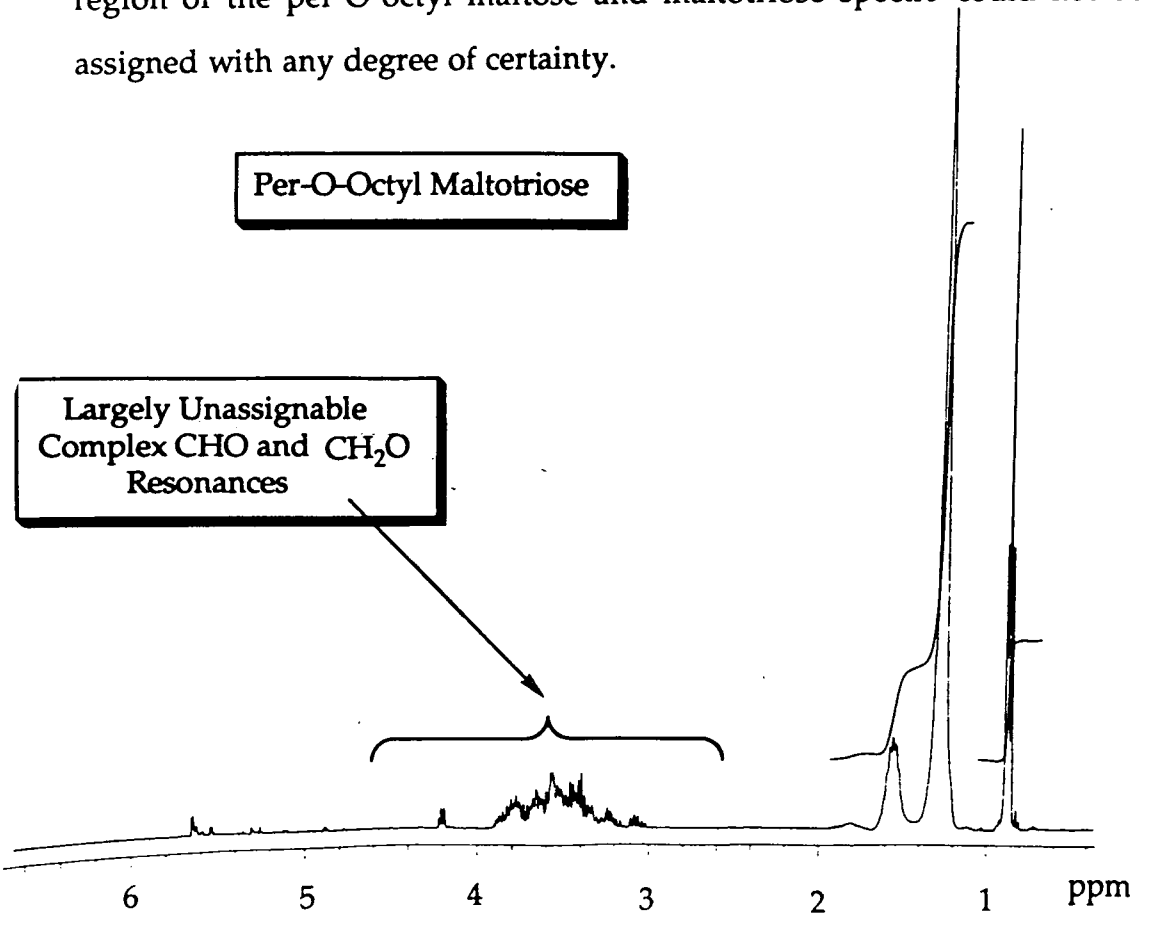



Figure 2.20 400MHz $^{1}H$ and COSY spectra for Per-O-Octyl Maltotriose in d-chloroform solution at 25°C

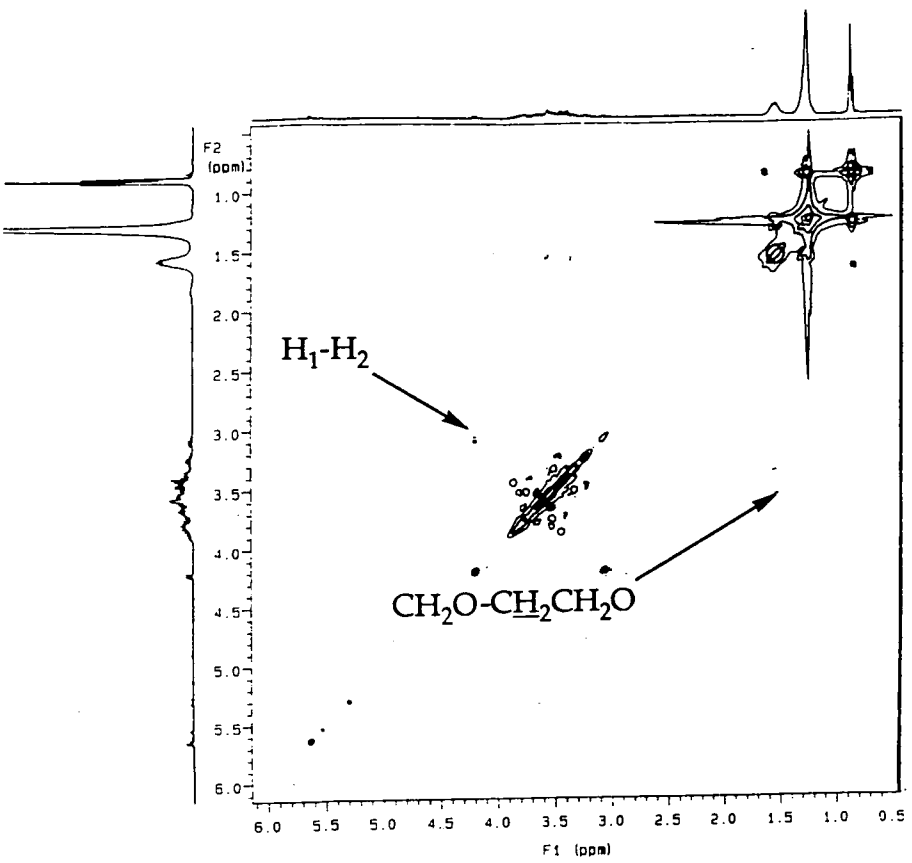



Figure 2.20 (cont.) 400MHz $^1H$ and COSY spectra for Per-O-Octyl Maltotriose in d-chloroform solution at 25°C

### 2.6.3 $^{13}C$ NMR

$^{13}C$ NMR spectra for the acyclic derivatives were found to be more complex than for the cyclodextrins as a consequence of the lower overall symmetry of the molecules in question, with the exception of glucose. Figure 2.21 shows a 90MHz $^{13}C$ spectrum for per-O-octyl maltotriose which, if it is compared with the accompanying spectrum for per-O-octyl-α-cyclodextrin, can be seen to be relatively complex. As mentioned earlier, HETCOR allows assignment of some of the resonances in these spectra and figure 2.21 also shows the application of this two dimensional technique to obtain several assignments.

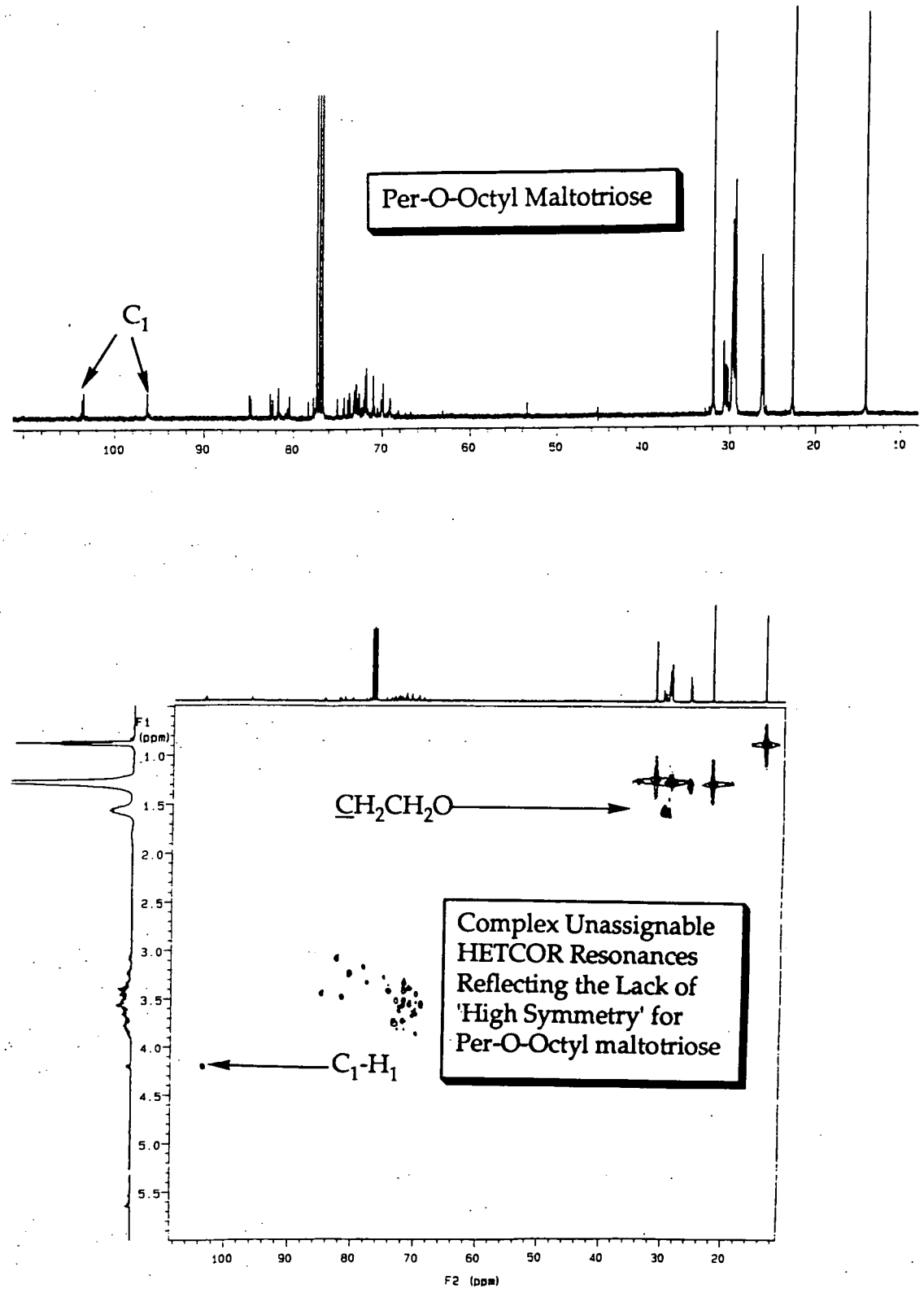



Figure 2.21 100MHz $^{13}C$ and $^{1}H$-$^{13}C$ Correlation spectraof Per-O-Octyl maltotriose in d-chloroform solution at 25°C

## 2.7 CONCLUSIONS

Investigation of octylated cyclodextrins has demonstrated the inhomogeneity of all derivatives but most especially the 'per'-O-octyl derivatives. Although direct $^{1}H$ and $^{13}C$ analysis are able to suggest incomplete and non-symmetrical alkylation they are unable to quantify the extent of alkylation. Reductive depolymerisation allows assessment of the degree of octylation of each derivative. However, the most informative technique is ES-MS which allows not only an assessment of the degree of functionalisation but also indicates the number of homologues present.

The exact reason why per-O-octylation is incomplete remains unclear. It is likely that there is a significant increase in the degree of steric crowding around the secondary hydroxyl rim as alkylation proceeds and this may well go some way to explaining the lack of complete alkylation. However, per-O-pentylation of both α- and β- cyclodextrin has previously been reported to be complete. This suggests that factors other than simply steric crowding may contribute in the prevention of complete octylation of all three cyclodextrins. It is quite probable therefore that some form of complex is formed between the cyclodextrin and octyl bromide or indeed between the cyclodextrin core and the octyl chains already attached which prevents complete reaction.

## REFERENCES


1) I. Tabushi Acc. Chem. Res. (1982) 15 66.

2) D. French Adv. Carbohydr. Chem. (1957) 12 189.

3) a)J. Boger, R. J. Corcoran, J-M. Lehn Helv. Chim. Acta. (1978) 61 2190

b)B. Casu, M. Reggiani Carbohydr. Res. (1979) 76 59

c)J. Szejtli, A. Liptak, I. Jodal, P. Fugedi, P. Nanasi, A. Neszmelyi Starch/Stürke (1980) 32 165.

4) R. Kuhn, H. Trishmann Chem. Ber. (1963) 96 284.

5) I. Ciucanu, F. Kerek Carbohydr. Res. (1984) 131 209.

6) G. Wenz Carbohydr. Res. (1991) 214 257; W. A. König, G. Wenz, S. Lutz, E. von der Bey Ger. Pat. DE 3,810,737 (1988); W. A. König, G. Wenz, S. Lutz, E. von der Bey Chem. Abstr. (1989) 113 24434x.

7) A. P. Croft, R. A. Bartsch Tetrahedron (1983) 39 1417.

8) W. A. König, S. Lutz, P. Mischnick-Lubbecke, B. Brassat, G. Wenz Carbohydr. Res. (1988) 183 11.

9) B. Casu, M. Reggiani, G. G. Gallo, A. Vigevani Tetrahedron (1966) 22 3061.

10) a) C. A. Glass Can. J. Chem. (1965) 43 2652

b) Y. Yamamoto, Y. Inoue Carbohydr. Res. (1989) 8 29

c) P. Colson, H. J. Jennings, I. C. P. Smith J. Am. Chem. Soc. (1974) 96 8081.

d) C. M. Spencer, J. F. Stoddart, R. Zarzycki J. C. S. Perkin Trans. 2 (1987) 1323.

11) D. J. Wood, F. E. Hruska, W. Saenger J. Am. Chem. Soc. (1977) 99 1735.

12) W. Meier-Augenstein, B. V. Burger, H. S. C. Spies Magn. Res. Chem. (1991) 29 681.

13) K. Harata, K. Uekama, M. Otagiri, F. Hirayama J. Incl. Phenom.

(1984) 1 279.

14) E. Bretmaier, W. Voelter '$^{13}C$ *NMR Spectroscopy*' Verlog Chemie, Weinheim/Bergstr. 1974 pp.223-242; K. Takeo, K. Hirose, T. Kugo Chem. Letts. (1973) 1233.

15) B. Casu, M. Reggiani, G. G. Gallo, A. Vigevani Tetrahedron (1968) 24 803; C. T. Greenwood, J. Rossotti J. Polymer Sci. (1958) 27 481.

16) H. Bjorndal, B. Lindberg, S. Svensson Carbohydr. Res. (1967) 5 433.

17) P. Mischnick Carbohydr. Res. (1989) 192 233; J-G. Jun, G. R. Gray Carbohydr. Res. (1987) 163 247; J. U. Bowie, P. V. Trescony, G. R. Gray Carbohydr. Res. (1984) 125 301

18) D. Rolf, G, R. Gray J. Am. Chem. Soc. (1982) 104 3539.

19) P. Mischnick-Lübbecke, R. Krebber Carbohydr. Res. (1989) 187 197.

20) F. M. Menger, M. A. Dulany Tetrahedron Letters (1985) 26 267.

21) K. Tsujihara, H. Kurita, M. Kawazu Bull. Chem. Soc. Jpn. (1977) 50 1567.

22) C. Yaozu, C. Nengyu, C. Ning, L. Haiquan, Z. Fanzhi Kexue Tongbao (1987) 32 1180.

23) P. R. Ashton, J. F. Stoddart, R. Zarzycki Tetrahedron Letters (1988) 29 2103.

24) J. Pitha, L. Szabo, H. M. Fales Carbohydr. Res. (1987) 168 191.

# CHAPTER THREE

# ENANTIOSELECTIVE CYCLODEXTRIN BASED CHEMICAL SENSORS

## 3.1 THE IMPORTANCE OF ENANTIOMERIC PURITY

For a long time it has been apparent that, in many cases, the two enantiomeric forms of a chiral molecule possess very different biological activities. For example, the S-form of Penicillamine is an antiarthritic drug whereas the R-form is extremely toxic. With this in mind analytical methods that allow determination of the enantiomeric composition‡ of a compound, both simply and effectively, are required. Several such analytical techniques have been developed for the determination of the enantiomeric purity of a chiral molecule.

### 3.1.1 Chiroptical Methods

Prior to the development of NMR techniques in the mid 1960's, chiroptical methods were applied to the determination of enantiomeric purity. This often involved measuring the optical rotation of a sample using a polarimeter under defined conditions of temperature, solvent, concentration and at a given wavelength of plane polarised light. This value was then compared to a known rotation for an enantiomerically pure sample of the same compound measured under identical conditions. There are however two major problems with this method of analysis:

i) enantiomeric purity and optical purity are not necessarily equivalent[1].

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‡ Enantiomeric purity is normally expressed in terms of the enantiomeric excess, ee:

$$\%ee = \frac{[S] - [R]}{[S] + [R]}$$

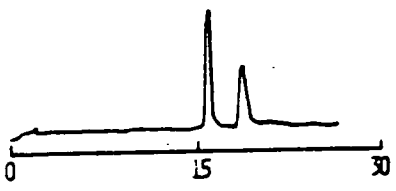
So, for example, an ee of 98% signifies an enantiomer ratio of 99:1

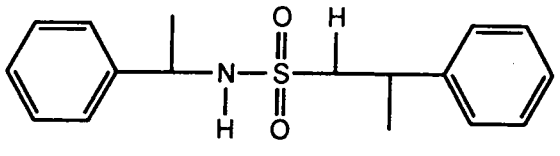
and

ii) many literature values of optical rotation for an enantiopure compound have been shown to be inaccurate through the use of NMR, HLPC and GC methods of analysis[2].

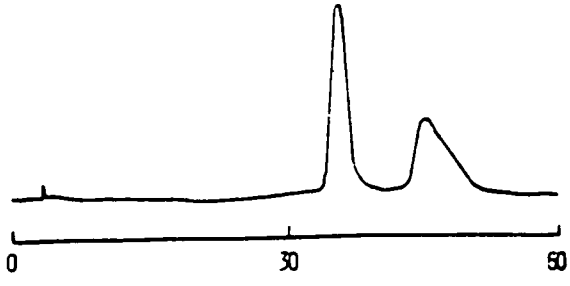
### 3.1.2 High Performance Liquid Chromatography

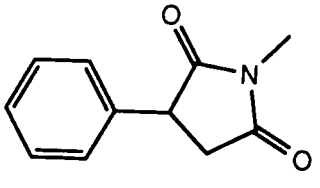
In recent years rapid progress in the application liquid chromatography to chiral analysis has occurred[3]. This is largely a result of the development of several synthetic (macrocycle based) chiral stationary and mobile phases. Although amino-acid derived chiral stationary phases have proved quite useful[4], polymer bound cyclodextrin derivatives have been amongst the most successful chiral stationary phases recently developed[5] (section 1.10). Figure 3.1 shows a typical normal phase HPLC trace obtained using a β-cyclodextrin based chiral stationary phase for the separation of (±)-phensuximide and N, N'-bis(α-methylbenzyl) Sulphamide.




N,N'-bis(α-methbenzyl) sulphamide




(±)-Phensuximide

Figure 3.1 Chiral Resolution of (±)-Phensuximide and N, N'-bis(α-methylbenzyl) Sulphamide by Normal Phase HPLC using a Per-O-acetyl β-Cyclodextrin Chiral Stationary Phase[6].

### 3.1.3 Gas Chromatography

The application of gas chromatography to chiral analysis has increased markedly over the past few years[7]. Several new chiral stationary phases have been developed, largely through the use of highly lipophilic, thermally stable cyclodextrin derivatives. Such developments have widened the application of this technique to a greater diversity of chiral analyte ranging from terpenoid hydrocarbons[8] to, the more polar, carboxylic acid and amine derivatives[9]. Figure 3.2 shows the separation of several chiral diol derivatives using a per-O-pentyl-α-cyclodextrin chiral stationary phase.

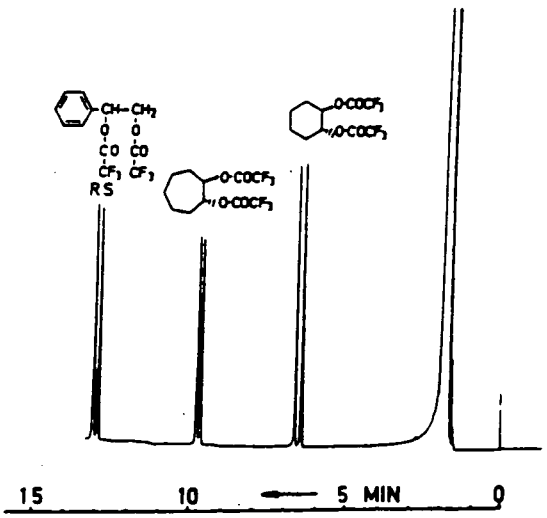
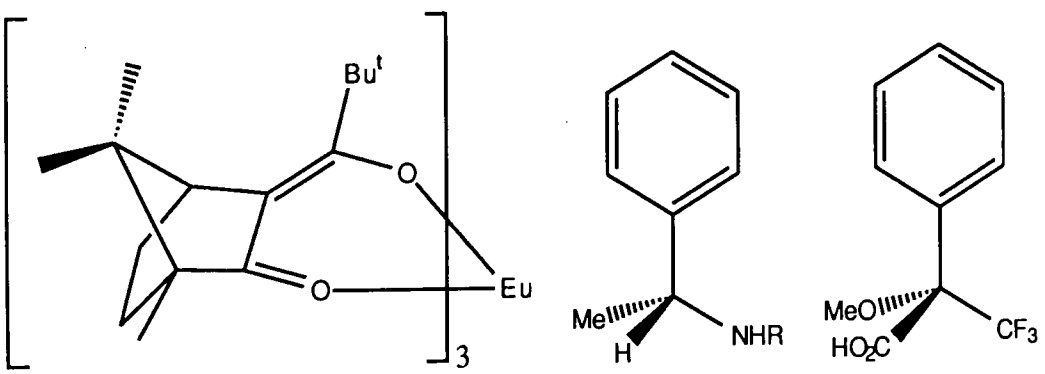



Figure 3.2 Chiral Resolution of Several Diols by Gas Chromatography using a per-O-pentyl-α-cyclodextrin chiral stationary phase.

These recent advances in gas chromatographic chiral analysis have resulted in increased separation factors, α, for a wide range of chiral compounds. However, for a substrate to be investigated by this technique it (or a derivative such as a trifluoroacetate for an alcohol) must be sufficiently volatile and be thermally stable.

### 3.1.4 NMR Methods

Although enantiomers cannot be distinguished in an achiral medium, because the resonances of enantiotopic nuclei are isochronous, diastereoisomers may be distinguished because the resonances of certain diastereotopic nuclei are anisochronous[10]. The determination of enantiomeric purity using NMR therefore requires the use of a chiral auxiliary that converts the mixture of enantiomers into a diastereoisomeric mixture. As long as there is a large enough chemical shift non-equivalence to give baseline resolution of the appropriate signals, then integration gives a direct measure of diastereoisomeric composition which can be related directly to the enantiomeric composition of the original mixture. There are three types of chiral auxiliary used[11]: lanthanide shift reagent[12] (LSR), chiral solvating agent[13] (CSA) and chiral derivatising agent[14] (CDA). An example of each is given in figure 3.3.




Tris(pivaloyl-d-camphorato) -Europium (Eu[pvc]$_3$) LSR

(R)-1-Phenyl ethylamine. CSA

(S)-MTPA

CDA

Figure 3.3 Examples of Chiral Auxiliaries for Chiral NMR Analysis.

Both LSR and CSA may be used directly however a CDA requires the separate formation of diastereoisomers prior to NMR analysis and care must be taken to prevent kinetic resolution and racemisation of the

derivatising agent. Figure 3.4 shows an example of how a chiral europium complex may resolve the methyl, methine and ortho aromatic protons of α-phenylethylamine[15]

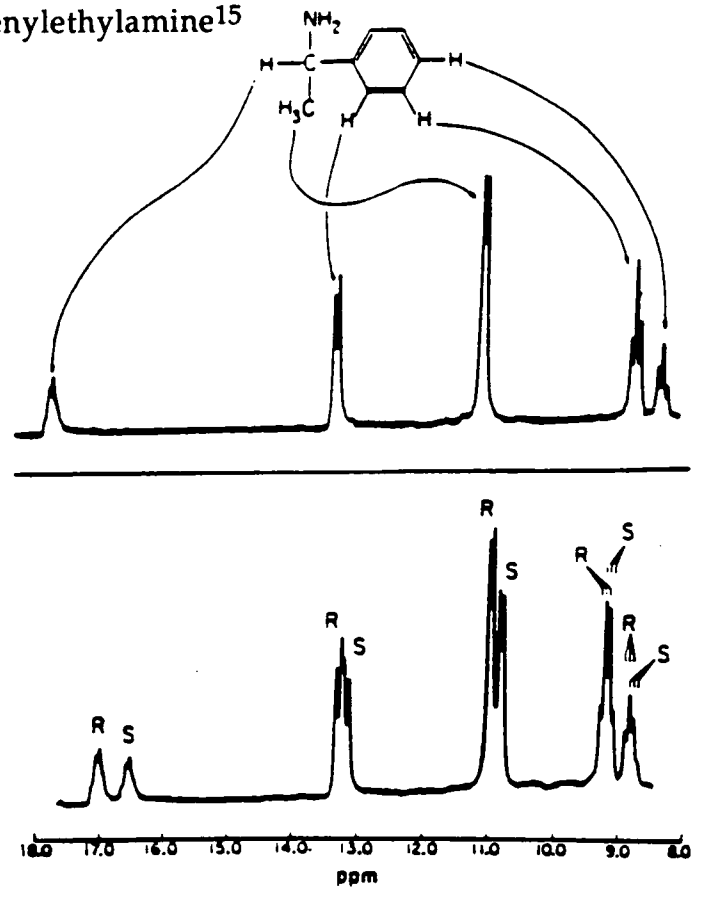



Figure 3.4 100MHz Proton NMR Spectra of a $CCl_4$ Solution of $Eu(pvc)_3$ and (S)-α-phenylethylamine (upper) and a mixture of (R)- and (S)- α-phenylethylamine (lower).

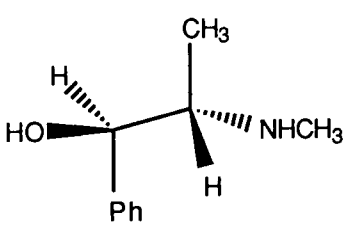
### 3.1.5 Potentiometric Methods

Several workers have developed potentiometric methods for chiral analysis[16]. Such investigations have concentrated on the discrimination of chiral α-aryl primary ammonium ions such as the α-phenylethyl ammonium ion (section 1.4). The general requirement is an enantiopure receptor molecule which is lipophilic to allow immobilisation in a solvent polymeric membrane. Such approaches have essentially concentrated on crown ether derivatives and have resulted in good enantiomer selectivity and structural selectivity over competing primary

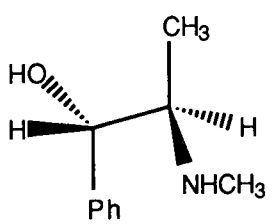
ammonium ions. However these chiral ionophores such as the bis(carboxamide) 18-crown-6 derivative developed by Lehn (section 1.4) generally form complexes of greater stability with alkali and alkaline earth metal ions. This lack of chemoselectivity over competing inorganic cations has been a major drawback in the development of macrocyclic polyether based chiral ionophores.

## 3.2 CYCLODEXTRINS AS POTENTIOMETRIC SENSORS

The primary aim of this work has been to investigate the feasibility of using the highly lipophilic cyclodextrin derivatives described in chapter two as sensing ionophores in potentiometric ion-selective electrodes for monosubstituted α- and β- aryl ammonium ions such as ephedrine, figure 3.5. It was assumed that the enantiopure host might form diastereoisomeric complexes selectively with the chiral aryl ammonium analyte, thus allowing the selective detection of one enantiomer in the presence of the other. The advantage of such a sensing mechanism is that it would allow the determination of enantiomeric purity quickly, efficiently and without the need for analyte derivatisation. An electrode system such as that described below was used for the electrochemical investigation[17].




(-)-(1R, 2S)-Ephedrine




(+)-(1S, 2R)-Ephedrine

Figure 3.5 Analyte Structures.

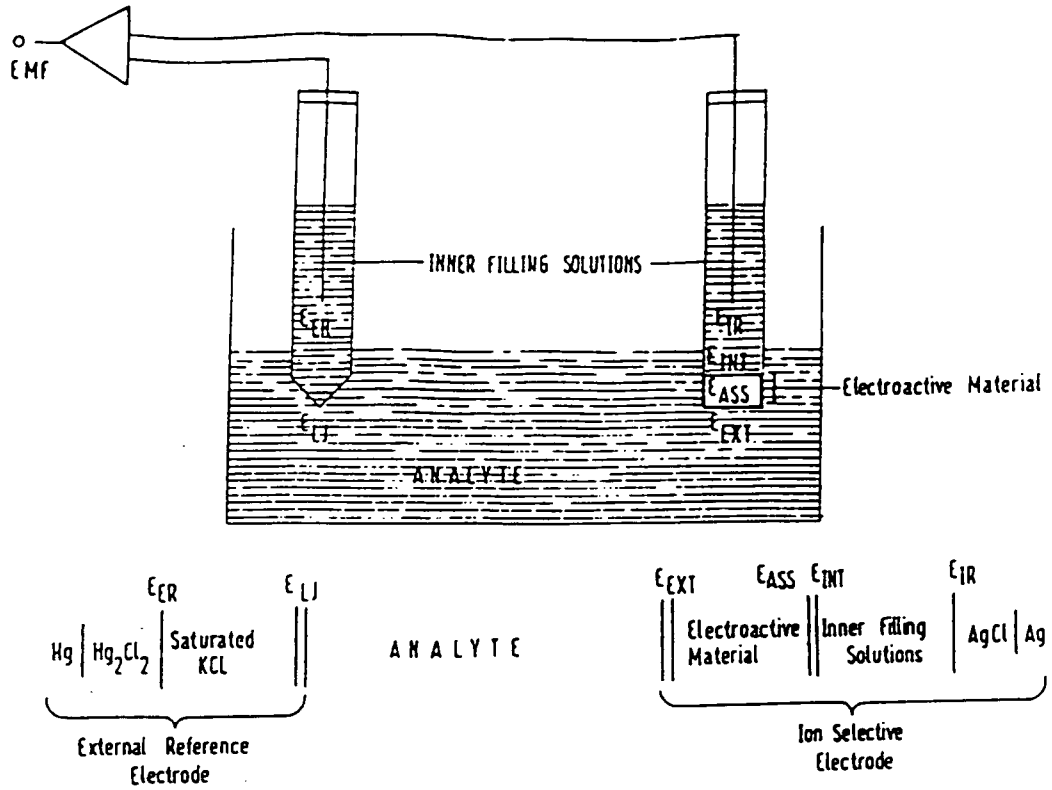
### 3.2.1 The Ion-Selective Electrode system

The general system, shown in figure 3.6, comprises:

i) The ion selective electrode, ISE, which responds more or less selectively to the activity of the particular ionic species, eg. a protonated chiral amine. The electroactive membrane is supported in an inert glass tube and the tube is filled with the appropriate inner filling solution. Electrical contact is then made via an inner $Ag^+/AgCl$ reference electrode.

ii) An external reference electrode provides a stable, reproducible and reversible potential against which variations in the potential of the ISE can be measured. The external reference electrode used here is $Ag^+/AgCl$.

iii) ISE's have high input impedance ($<10^{12}$ ohms) and hence an operational amplifier of similar impedance is needed to interface between the electrode and the voltmeter.




$E_{ER}$ = Potential of the external reference electrode.

$E_{LJ}$ = Liquid junction potential between the salt bridge and the analyte.

$E_{EXT}$ = External phase boundary potential of the membrane.

$E_{INT}$ = Internal phase boundary potential of the membrane.

$E_{ASS}$ = Asymmetry potential within the membrane.

$E_{IR}$ = Potential of the internal reference electrode.

Figure 3.6 A Typical Ion Selective Electrode System

### 3.2.2 Nernstian Response of Ion Selective Electrodes and the Effect of Interferent Ions

The potential difference, E, detected by the measuring system can be expressed as:

$$E = E_{ASS} + E_{IR} + E_{ER} + E_{LJ} + E_{INT} + E_{EXT} \quad (1)$$

Assuming certain of the above potentials (which are defined in figure 3.6) to be constant, the Nernst relationship (2) can be derived:

$$E_{EXT} = CONSTANT + \frac{2.303RT}{zF} \cdot \log a \qquad (2)$$

where, R=gas constant; T=temperature in Kelvin; F=Faraday constant;

z=charge of relevant ion.

This equation assumes ideal behaviour of the electrode towards the primary ion and may not hold in the presence of interference from other ionic species. The Nickolsky-Eisenman equation (3) is an expression of the response of an ion selective electrode to ions other than the primary ion.

$$E = E_o + 2.303 \frac{RT}{zF} \log \left[a_i + \sum_{j \neq i} K^{POT}_{ij} (a_j)^{z_i/z_j}\right] \qquad (3)$$

where $a_i$ = activity of the primary ion

$a_j$ = activities of the interferents in the analyte

$K^{POT}$ = weighting factors specifying the ion selectivity of the electro-active material and are termed selectivity coefficients.

$K^{POT}$ can be defined as the ratio between the activities of the primary ion and the interferent ion that would give an identical electrode response when present alone in the solution of the ion. Thus

$$K^{POT}_{ij} = \frac{a_i}{a_j} \qquad (4)$$

### 3.2.3 Limit of Detection

For the sake of convenience a practical limit of detection has been recommended by IUPAC. This may be taken as the activity of the primary ion at the point of intersection in the extrapolated illustration, figure 3.7. The limit of detection determines the measuring range of the electrode.

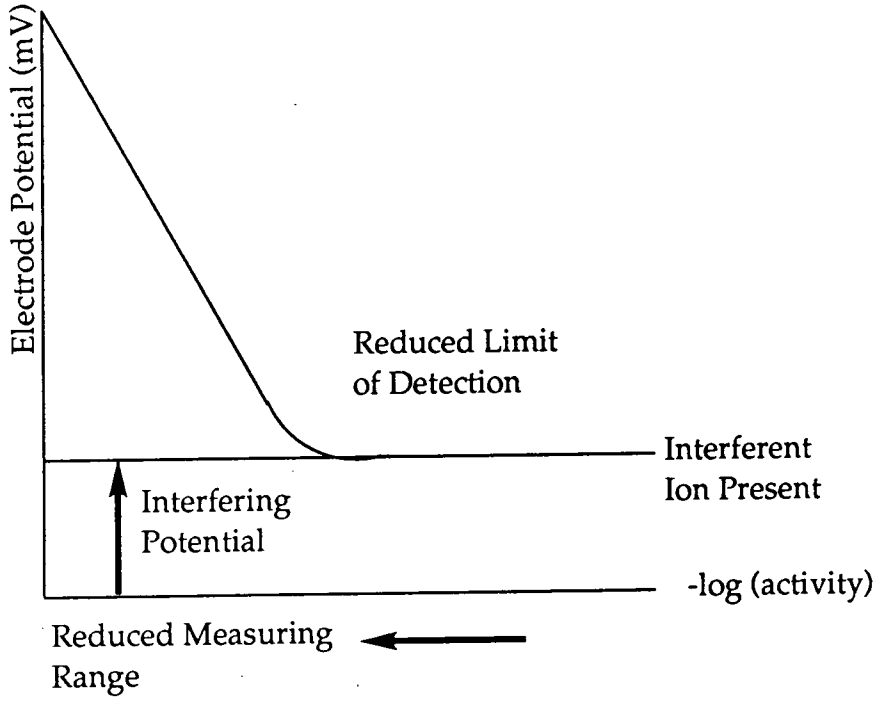



Figure 3.7 Limit of Detection

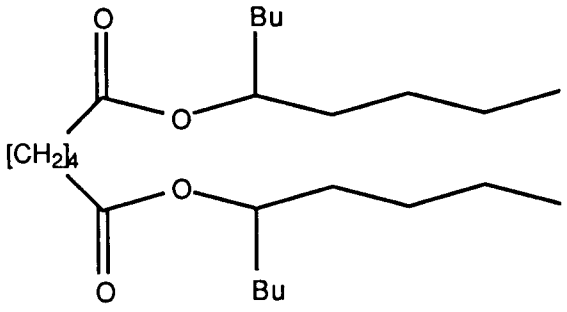
## 3.3 PER-O-OCTYL-α-CYCLODEXTRIN AS AN ENANTIOSELECTIVE EPHEDRINE SENSOR

This highly lipophilic α-cyclodextrin derivative was synthesized and characterised as detailed in chapter two. This section of work aims to describe its function as an enantioselective sensor. The effects of structural variation of the host and guest on the ability of the α-

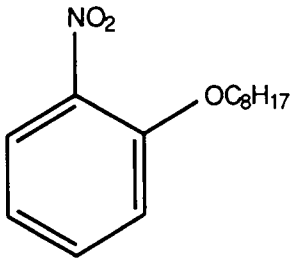
cyclodextrin based ionophore to act as an enantioselective host are also investigated. Throughout this discussion the phrase 'per-O-octyl' is used to signify the octylated cyclodextrin products produced by NaH/THF/OctBr alkylation. However, residual hydroxyl groups are present in these compounds which have been shown to be a mixture of constitutional isomers and homologues (chapter 2).

### 3.3.1 Membrane Preparation

Membranes were prepared using two quite different plasticizers. The aim of this was to determine the effect of the plasticizer upon the electrochemical behaviour of the ISE. Figure 3.8 shows the structures of the two plasticizers, *o*-NPOE and the less polar BBPA, used in this investigation. The membrane composition for the *o*-NPOE-based membranes was 1.2% ionophore, 65.6% *o*-NPOE, 32.8% PVC and 0.4% potassium tetrakis(*p*-chlorophenyl) borate in $6cm^3$ of tetrahydrofuran (THF). For the BBPA-based membranes, the composition was 2.0% ionophore, 65.6% BBPA, 32% PVC and 0.4% potassium tetrakis(*p*-chlorophenyl) borate in $10cm^3$ of THF. The membranes were cast by a controlled evaporation method[18].




Bis(1-butylpentyl)adipate.
(BBPA)




*o*-Nitrophenyloctyl ether.
(*o*-NPOE)

Figure 3.8 Membrane Plasticizers.

### 3.3.2 *o*-NPOE as Plasticizer

Initial investigations used *o*-NPOE as plasticizer and per-O-octyl-α-cyclodextrin as the sensing ionophore in an electrode set up similar to that described above. The electrodes were initially conditioned in 0.01 M solutions of the appropriate enantiomer and calibrated by continuous dilution. Table 3.3.2A shows the behaviour of these electrodes with both enantiomers and a racemic mixture of ephedrine hydrochloride (EPH.HCl)

**Table 3.3.2A Behaviour of Electrodes with Per-O-Octyl-α-Cyclodextrin using *o*-NPOE as the Plasticizer and $10^{-2}$ mol $dm^{-3}$ Eph.HCl as the Inner Filling Solution.**

| Sensor | Slope/mV decade$^{-1}$ | Limit of detection, -log[c] | Overall Selectivity, -log $K^{pot}$ |
|---|---|---|---|
| (+) EPH.HCl* | 60.0 | 4.64 | 3.82 |
| (-) EPH.HCl* | 60.0 | 4.54 | 3.68 |
| (-) EPH.HCl | NQ† | NQ† | — |
| (±) EPH.HCl | NQ† | NQ† | — |
| (+) EPH.HCl | 59.0 | 4.80 | — |

*Background of serum levels of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$(150mM $Na^+$, 4.3mM $K^+$, 1.26mM $Ca^{2+}$, 0.9mM $Mg^{2+}$).
NQ† indicates slope and limit of detection have not been quoted because of unusual electrode behaviour.

The (+) enantiomer showed a normal Nernstian response with a detection limit, -log[c]=4.8. With the (-) enantiomer of ephedrine and the racemate Nernstian behaviour was observed down to a concentration of $10^{-3}$ M. However, further dilution resulted in an unusual 'hyper-Nernstian' behaviour, as shown graphically in figure 3.9. In a background of serum levels of $Na^+$, $K^+$ and $Ca^{2+}$ this hyper Nernstian behaviour was not observed. Electrodes for both enantiomers functioned

satisfactorily under these conditions with high overall selectivity coefficients, given by equation (3), as table 3.3.2A shows. Figure 3.9 summarises this information pictorially in terms of calibration graphs for the electrode with each of the analyte solutions tested.

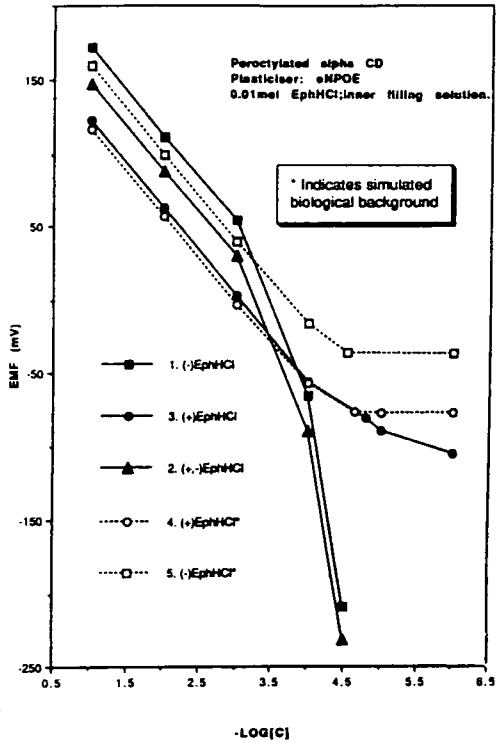



Figure 3.9 Electrode Response to the Enantiomers of Ephedrine with *o*-NPOE as Plasticizer.

### 3.3.3 The use of BBPA as a Plasticizer

Since cyclodextrins are renowned for their ability to include aromatic moieties it was felt that the aromatic nature of the *o*-NPOE may be involved in the unusual behaviour of the electrode towards (-) ephedrine, possibly through competitive inclusion. This is not unreasonable since Lewis and Hansen have reported a logK=3.7 (1.1) for the binding of *o*-nitrophenol by α-cyclodextrin in water. Furthermore, Matsue developed a cyclodextrin based regioselective electrode system for the determination of *o*-nitrophenol in the presence of *p*-nitrophenol[19]. This system was 33 times more sensitive to *o*-nitrophenol than to its para

isomer. Species - selective voltammetric determination of *o*-nitrobenzene derivatives was also successfully performed on this electrode system with α-cyclodextrin in solution[20]. A change of plasticizer to the non aromatic, less polar BBPA removed the concentration dependent 'hyper Nernstian' response. Figure 3.10 and table 3.3.3A summarise the results obtained with this plasticizer.

**Table 3.3.3A Behaviour of Electrodes with Per-O-Octyl-α-Cyclodextrin using BBPA as the Plasticizer and $10^{-2}$ mol $dm^{-3}$ Eph.HCl as the Inner Filling Solution.**

| Sensor | Slope/mV decade$^{-1}$ | Limit of detection, -log[c] | Overall Selectivity, -log $K^{pot}$ |
|---|---|---|---|
| (+) EPH.HCl | 59.0 | 5.05 | — |
| (-) EPH.HCl | 46.0 | 5.40 | — |
| (+) EPH.HCl* | 56.0 | 3.55 | 2.73 |

*Background of serum levels of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$(150mM $Na^+$, 4.3mM $K^+$, 1.26mM $Ca^{2+}$, 0.9mM $Mg^{2+}$).

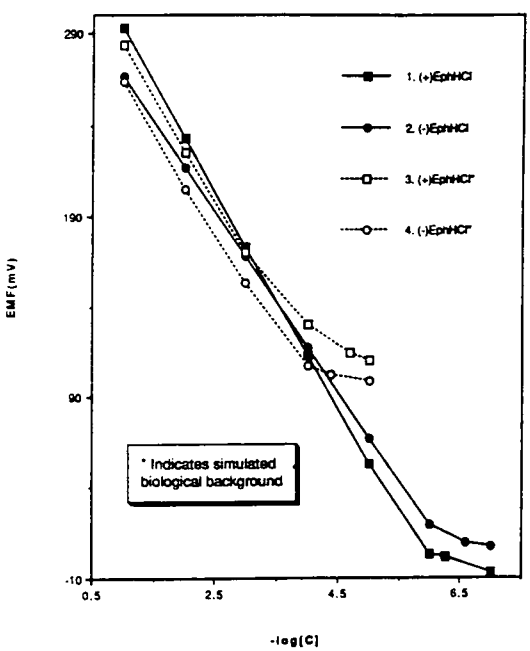



Figure 3.10 Electrode Response to the Enantiomers of Ephedrine with BBPA as Plasticizer.

Of particular importance were the observations that the (-) Eph.HCl electrode showed a slope 10mV per decade less than the (+) enantiomer sensor and that there was a difference in electrode potentials of 26 mV. The limit of detection and cation selectivity over inorganic ions were very good for both enantiomers.

The bias potential, $\Delta E_{bias}$, of the two electrodes -one conditioned in 0.1M (+) ephedrinium hydrochloride, the other with the (-) enantiomer- was measured in a cell with no liquid junctions. This was found to be 24.5 mV according to the equation

$$\Delta E_{bias} = E_{(+)} - E_{(-)} = 24.5 \pm 0.5mV \quad (5)$$

with BBPA as plasticizer, at room temperature, constant over 4 hours. This corresponds to a free energy difference, $-\Delta G_{cell}$, between the two diastereomeric complexes of 2.4 kJ $mol^{-1}$, according to the equation

$$\Delta G_{cell} = -nF\Delta E_{bias} \quad (6)$$

### 3.3.4 Development into an Enantioselective Sensor

It is clear from the above discussion that there is an enantioselective response to the ephedrinium analyte by the α-cyclodextrin based ionophore. Using solutions of predetermined enantiomeric purity the electrode could be calibrated to measure the enantiomeric purity of the ephedrinium ion directly, figure 3.11.

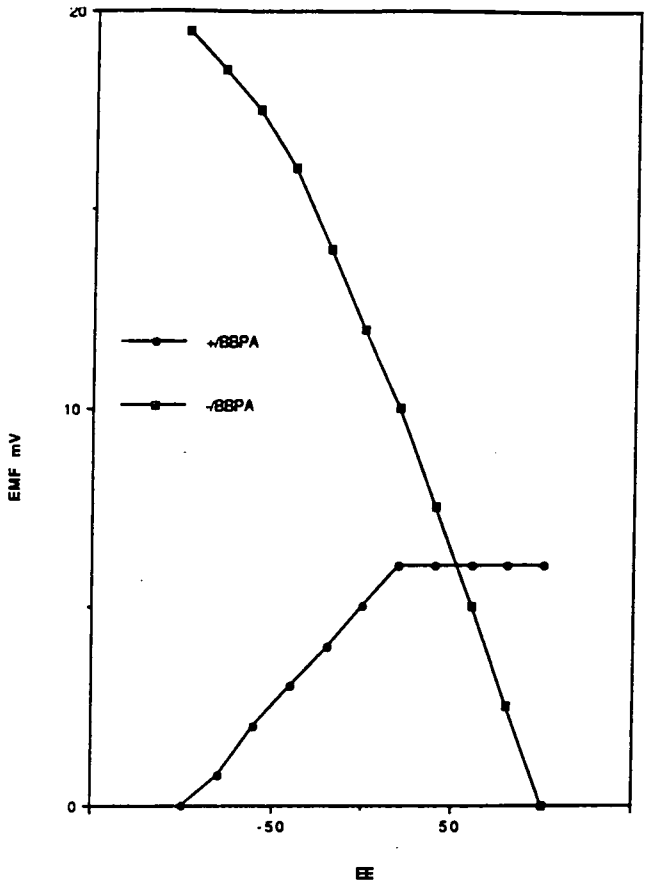



Figure 3.11 Calibration of the 'Per'-O-Octyl-α-Cyclodextrin Based Ionophore as an Enantiomer Selective Ephedrine Sensor

## 3.4 INVESTIGATION OF ENANTIOMER DISCRIMINATION

As chapter one indicates, several methods have been employed in order to investigate the mechanism of enantiomer discrimination by cyclodextrin hosts. The most reliable and informative methods use changes in enantiomer selection upon modification of the receptor and/or the substrate. Such structural variations have been employed with good effect by König and Armstrong in their gas chromatographic investigations of cyclodextrin chiral stationary phases (section 1.10).

### 3.4.1 Host Modification

Several potential, cyclodextrin and oligosaccharide, ionophores were incorporated into an electro-active membrane and used in a similar

manner to that described above for per-O-octyl-α-cyclodextrin as enantioselective ephedrinium sensors. Table 3.4.1A details the electrochemical results obtained from this investigation of molecular recognition by host modification.

**Table 3.4.1A The effect of host structure upon enantioselection with BBPA or *o*-NPOE* as plasticizer and 0.001 mol $dm^{-3}$ $NH_4Cl$ inner filling solution.**

i) **Macrocyclic Hosts**

| Electrode ionophore | Analyte | E° (mV) | Slope (mV $decade^{-1}$) | Limit of detection (-log c) |
|---|---|---|---|---|
| Methylated peroct αCD | (-) EPH.HCl | 262 | 39.5 | 2.0 |
| Methylated peroct αCD | (+) EPH.HCl | 259 | 42.5 | 2.0 |
| | | **ΔE° =-3mV** | | |
| 3-O-acetyl-2,6-dioctαCD | (-) EPH.HCl | 264 | 58 | 3.0 |
| 3-O-acetyl-2,6-dioctαCD | (+) EPH.HCl | 293 | 58 | 3.2 |
| | | **ΔE° = 29mV** | | |
| 2,6-di-O-octαCD* | (-) EPH.HCl | 207 | 59 | 2.0 |
| 2,6-di-O-octαCD* | (+) EPH.HCl | 231 | 24 | – |
| | | **ΔE° = 24mV** | | |
| peroctβCD | (-) EPH.HCl | 258 | 51 | 3.9 |
| peroctβCD | (+) EPH.HCl | 266 | 56 | 4.0 |
| | | **ΔE° = 8mV** | | |

**ii) Non Macrocyclic Hosts**

| Electrode ionophore | Analyte | E° (mV) | Slope (mV decade$^{-1}$) | Limit of detection (-log c) |
|---|---|---|---|---|
| peroct Maltotriose* | (-) EPH.HCl | 203.0 | 50 | NQ† |
| peroct Maltotriose* | (+) EPH.HCl | 213.5 | 50 | 3.7 |
| | | **ΔE° =10.5mV** | | |
| peroct Maltose | (-) EPH.HCl | 207.5 | NQ† | NQ† |
| peroct Maltose | (+) EPH.HCl | 208.5 | 60 | 5.6 |
| | | **ΔE° = 1mV** | | |
| peroct glucose* | (-) EPH.HCl | 205 | 50 | 2.0 |
| peroct glucose* | (+) EPH.HCl | 211 | 50 | 2.0 |
| | | **ΔE° = 6mV** | | |
| peroct sucrose* | EPH.HCl | No response to either enantiomer | | |

NQ†:not quoted as a result of the unusual 'super Nernstian' behaviour of the electrode.

Several important conclusions can be drawn from these electrochemical findings:

i)The cyclodextrin macrocyclic cavity is essential for well defined enantioselectivity.

ii)Methyl capping of the residual hydroxyl groups in per-O-octyl-α-cyclodextrin markedly reduces the enantioselective response of the electrode, thus indicating the requirement for some hydroxyl groups on the host.

iii)With 2,6-di-O-octyl-α-cyclodextrin as the sensing ionophore, good enantiomer selection is evident but the behaviour of the electrode is poor, with only a moderate limit of detection. This observation, in connection with that in (ii) above , indicates that hydroxyls are essential for enantiomer discrimination but that a large number of hydroxyls results in a less well defined electrode response. This may be a result of too high a binding constant removing the reversibility necessary for well-defined electrode response.

iv)Increase in cavity size to the β-cyclodextrin analogue reduces both the enantiomer discrimination and the limit of detection. This indicates that a cavity of the correct dimension is necessary for chiral recognition.

v)Comparison of the non-macrocyclic ionophores, glucose, maltose and maltotriose, indicates that a glycosidic linkage is critical for enantiomer selection and reasonable electrode characteristics. Indeed, per-O-octyl maltose is quite a good sensor for ephedrine. This glycosidic linkage may allow the receptor to form a 'molecular cleft' for guest encapsulation as figure 3.12 illustrates for maltose.

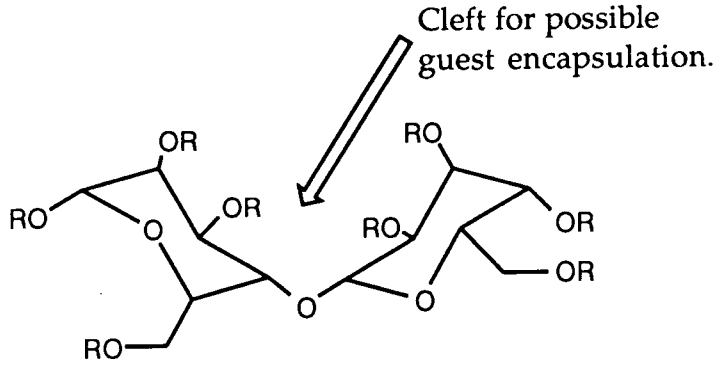
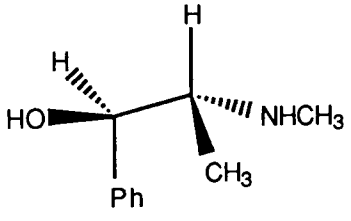



Figure 3.12 'Cleft' Formation by Maltose.

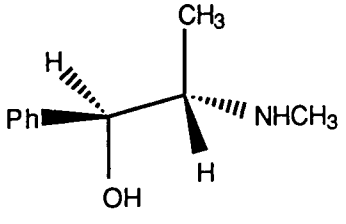
vi)Change in functionality to 3-O-acetyl-α-cyclodextrin results in a slightly enhanced enantioselective behaviour, but the electrode response was poor with only a moderate limit of detection.

### 3.4.2 Guest Modification

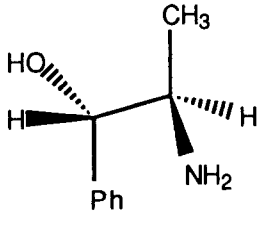
Several structurally similar β-aryl ammonium ions such as amphetamine, norephedrine and deoxyephedrine (methamphetamine) were used as modified analytes for the per-O-octyl-α-cyclodextrin based electrode. This allowed some assessment of the functionalities of the β-aminoalcohol, ephedrine, important in producing a well defined electrochemical response by the α-cyclodextrin based ionophore. The modified analytes investigated are shown in figure 3.13.



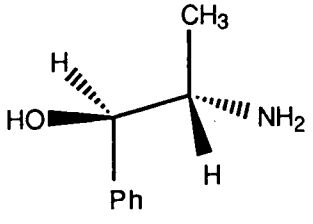

(-)-(1R, 2R)-Pseudoephedrine



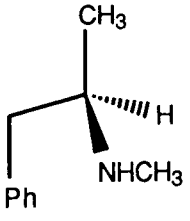

(+)-(1S, 2S)-Pseudoephedrine



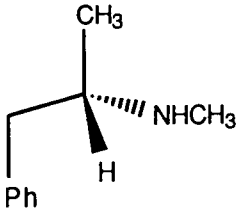

(+)-(1S, 2R)-Norephedrine




(-)-(1R, 2S)-Norephedrine

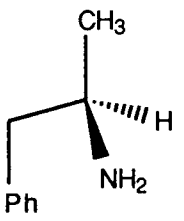



(-)-R-Deoxyephedrine

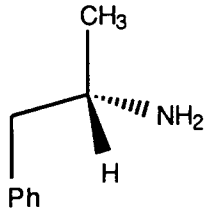



(+)-S-Deoxyephedrine

Figure 3.13 Structural Modification of Ephedrine.




(-)-R-Amphetamine




(+)-S-Amphetamine

Figure 3.13 cont. Structural Modification of Ephedrine.

### 3.4.2.1 Pseudo Ephedrine

The remaining two stereoisomers in the ephedrine series, (+) and (-) pseudoephedrine, were found to respond in a similar Nernstian manner to that of ephedrine, as table 3.4.2A shows. This therefore allows determination of the concentration of (-) ephedrine in the presence of any or all of the other three stereoisomers. It is interesting to note however that in this case it is the (-) enantiomer which shows a slightly enhanced slope and limit of detection. Both (+)ephedrine and (-)ψephedrine have the R absolute configuration at the chiral centre β to the aryl ring, the only difference between the two diastereoisomers is the absolute configuration at the other chiral centre. This perhaps suggests therefore that the configuration at the ammonium carrying chiral centre is of central importance in determining the ability of the analyte to bind with the cyclodextrin host.

### 3.4.2.2 Behaviour Towards Norephedrine

This β-amino alcohol, closely related to ephedrine, was also observed to interact with the α- cyclodextrin ionophore in an enantioselective manner. A similar electrode set-up may be therefore be used to determine the enantiomeric purity of norephedrine with the (-) enantiomer again giving a reduced slope, similar to that observed with ephedrine, as table 3.4.2A clearly shows. The similar response to the

enantiomers of norephedrine to those of ephedrine suggests that the NMe methyl group is not a pre-requisite for a well defined enantioselective response by the electrode.

**Table 3.4.2A Behaviour of Electrodes with Per-O-Octyl-α-Cyclodextrin, using BBPA as the Plasticizer, as NorEph.HCl and ψEph.HCl sensor, $10^{-2}$ mol $dm^{-3}$ analyte as the inner filling solution.**

| Sensor | Slope/mV decade$^{-1}$ | Limit of detection, -log[c] | Overall Selectivity, -log $K^{pot}$ |
|---|---|---|---|
| (+) norEPH.HCl | 58.0 | 5.05 | — |
| (-) norEPH.HCl | 46.0 | 3.80 | |
| (±) norEPH.HCl* | 58.0 | 2.90 | 2.1 |
| (+) ψEPH.HCl | 56.0 | 4.70 | |
| (-) ψEPH.HCl | 59.0 | 5.10 | — |
| (±) ψEPH.HCl | 59.0 | 5.20 | — |

*Background of serum levels of $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$(150mM $Na^+$, 4.3mM $K^+$, 1.26mM $Ca^{2+}$, 0.9mM $Mg^{2+}$).

### 3.4.2.3 Behaviour Towards Deoxyephedrine and Amphetamine

Removal of the hydroxyl group of ephedrine to give deoxyephedrine (methamphetamine) gives a marked increase in enantiomer discrimination, as measured by $\Delta E^\circ$ (table 3.4.2B). However, the response of the electrode is poor for each enantiomer. This suggests that the hydroxyl functionality of the ephedrinium ion may be important in producing a well defined electrochemical behaviour with the α-cyclodextrin based electrode. With (-) amphetamine the electrode characteristics are good which suggests that the hydroxyl group of ephedrine may be more involved in restricting the conformation of the ephedrine itself through intramolecular hydrogen bonding, rather than being involved in a host-guest interaction. Such restriction of

conformational mobility within the ephedrinium ion may pre-define the geometry in a manner 'preorganised' for interaction with the cyclodextrin. Removal of the hydroxyl group then allows the host to manipulate the guest to a greater extent resulting in more well defined enantiomer discrimination but a poorer electrode response because of a 'less dynamic' host-guest interaction. Further modification by removal of the NMe methyl group to give amphetamine may then change the binding mode, restoring the dynamics of the electrode system. The suggestion is therefore that, of the modified guests investigated, deoxyephedrine may be most complementary to the α-cyclodextrin cavity.

**Table 3.4.2B Behaviour of electrodes with per-O-octyl-α-cyclodextrin using BBPA as the plasticizer as Amph.HCl and Mamph.HCl sensor, $10^{-2}$ mol $dm^{-3}$ analyte as the inner filling solution.**

| Analyte | E° (mV) | Slope (mV $decade^{-1}$) | Limit of detection |
|---|---|---|---|
| (+)methamphetamine | 125 | 12 | — |
| (-)methamphetamine | 289 | NQ‡ | |
| | **ΔE°=164** | | |
| (-)amphetamine | 215.5 | 47.5 | 3.6 |

NQ‡ indicates slope and limit of detection have not been quoted because of unusual electrode behaviour.

## 3.5 APPLICATION OF NMR TO THE INVESTIGATION OF MOLECULAR RECOGNITION

NMR may be applied as a powerful probe into inter- and intra- molecular interactions for cylodextrin complexes[21]. In this section the application of various NMR techniques to the per-O-octyl-α-cyclodextrin-ephedrinium ion complex is described and the effects of structural modifications, similar to those adopted in the electrochemical analysis above, are demonstrated. All spectra discussed here were acquired in d-chloroform solution ($0.7cm^3$) using the trifluoroacetate salt of the chiral β-phenyl ammonium ions to give improved solubility. All NMR solutions were 90μM cyclodextrin with 2.5 molar equivalents of the chiral substrate.

### 3.5.1 Proton NMR

In chapter two the relative complexity of the proton spectra for the cyclodextrin derivatives investigated here was discussed. This complexity results from the presence of a number of constitutional isomers and homologues which together give an average of 15.4 octyl groups per macrocyclic core. Consequently it is difficult to investigate the effects of host-guest interaction with any degree of certainty for some of the more complex multiplets in the 3.3-4.1 region of the cyclodextrin spectrum. However, with this in mind, there are several important (enantiomer dependent) differences between the two diastereoisomeric complexes and these are discussed below.

#### 3.5.1.1 Effects of Complex Formation on the Ephedrine $^1H$ NMR

The $^1H$ NMR assignments for the ephedrine resonances are given in figure 3.14. It is immediately apparent from the spectrum of free ephedrine that the two diastereotopic ammonium protons are very anisochronous ($\Delta\delta$ = 1.03ppm), suggesting that, in d-chloroform solution,

the ammonium ion may be hydrogen bonded intramolecularly to the proximate hydroxyl group. This restricts free rotation about the MeC-N bond.

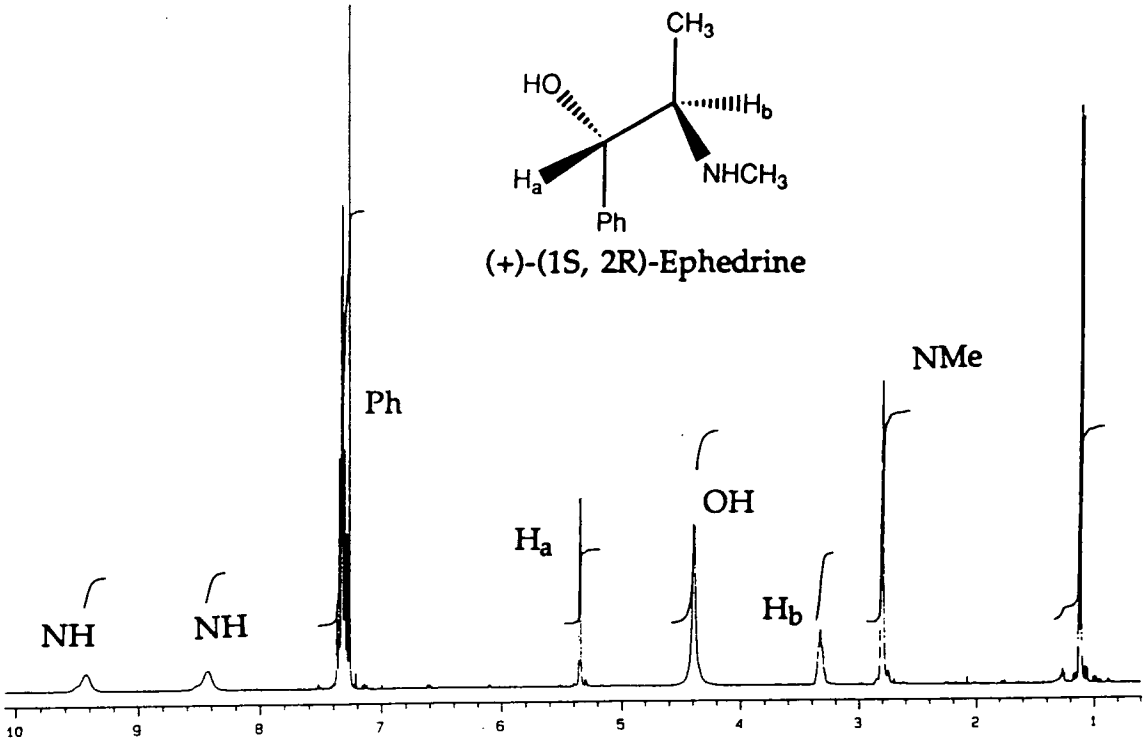
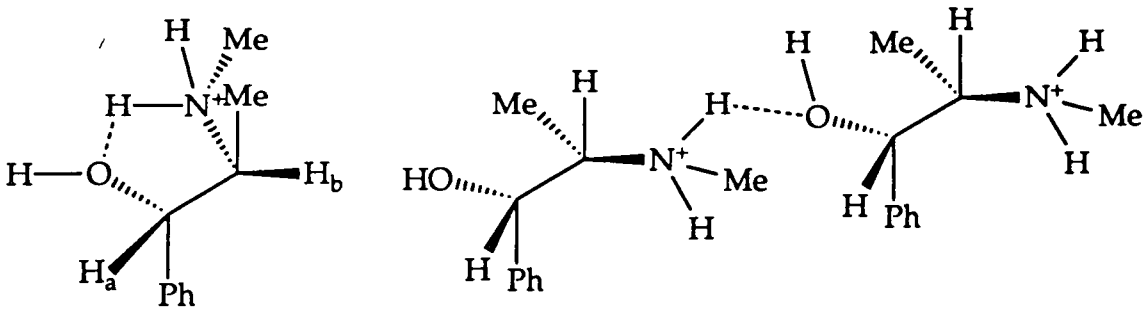



Figure 3.14 400MHz $^1$H NMR of Ephedrine in d-chloroform Solution at 25°C

Figure 3.15 shows that this is a result of $^+$N-H···O hydrogen bonding, which may be of a intra- or inter- molecular nature. Investigations into the type of hydrogen bonding by FT-IR showed it to be of a duel nature. At a concentration of 0.4M there is a broad OH stretch at 3350cm$^{-1}$ this diminishes on dilution to 0.07M indicating that this absorption is due to intermolecular hydrogen bonding. A second absorption at 3600cm$^{-1}$ is independent of concentration and is the intramolecularly hydrogen bonded OH stretch.




Intramolecular Intermolecular

Figure 3.15 intra- and inter- molecular $^{+}$N-H···O hydrogen bonding in Ephedrine

Upon complexation the chemical shift of the diastereotopic ammonium protons is sensitive to which ephedrine enantiomer is bound. There is a clear increase in $\Delta\delta$ for (+) ephedrine which is not apparent with the (-) enantiomer. This enantiomer dependent change in the chemical shift difference is further emphasised at 223K and this is illustrated in figure 3.16.

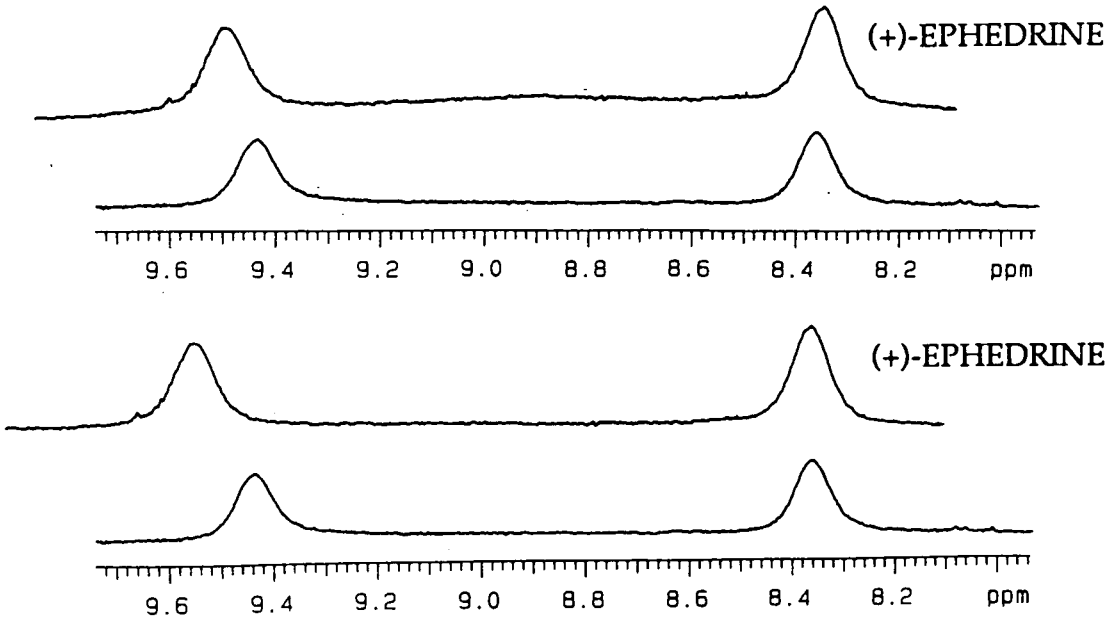



Figure 3.16 Enantiomer Dependent Changes in $\Delta\delta$ for the Ephedrinium Ammonium Protons in d-chloroform Solution at 293K (Upper) and 223K (Lower)

On cooling, the ammonium ion protons for (+) ephedrine become even more anisochronous with $\Delta\delta$= 1.17. This suggests conformational freezing is occurring with a higher population of a specific low energy conformation than at room temperature. With the (-) enantiomer no change in the chemical shift difference for the ammonium ion protons is evident. These changes in the chemical shift difference for the diastereotopic ephedrinium ion ammonium protons suggest that the interaction between the ammonium ion group of the ephedrinium guest and the cyclodextrin host plays a significant role in enantiomer discrimination.

A change in the $H_a$-$H_b$ coupling constant, $J_{Ha\text{-}Hb}$, is also apparent upon complexation of the (+) enantiomer with per-O-octyl-α-cyclodextrin in d-chloroform solution. The coupling constant $J_{Ha\text{-}Hb}$ decreases from 2.4 Hz to 1.6 Hz for the (+) ephedrinium ion but remains constant at 2.4 Hz for the other enantiomer (figure 3.17). Application of the Karplus equation to this geminal H-H coupling constant allows translation of this decrease in J into an increase in the geminal angle, φ, by approximately 6° (figure 3.17).

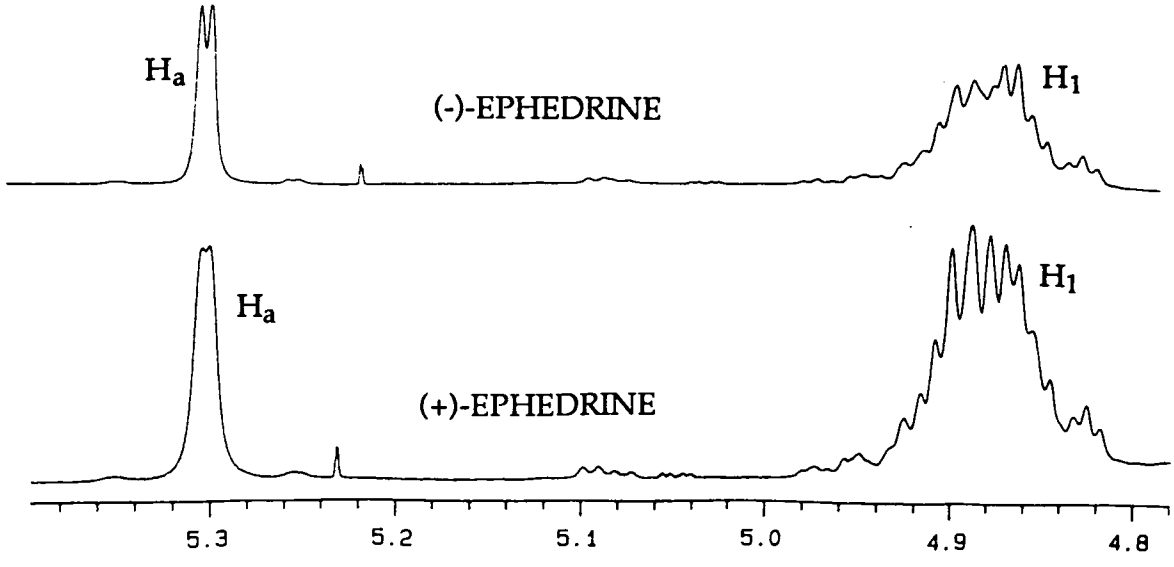



Figure 3.18 Changes in $J_{Ha\text{-}Hb}$ for Ephedrine upon Complexation with 'Per'-O-Octyl-α-Cyclodextrin in d-chloroform Solution at 25°C

This enantiomer dependent change in φ suggests a separation of the hydrogen bonding $MeNH_2^+$ and OH groups which, as well as weakening the hydrogen bond, facilitates conformational changes throughout the molecule. In particular the relative dispositions of the three major functionalities, Ph, $MeNH_2^+$ and OH, are changed markedly. Although no definitive conclusions can be drawn from this it does seem to suggest that the cyclodextrin host is influencing the shape of the whole (+) ephedrine molecule which is indicative of a closer host-guest interaction than with (-) ephedrine. This type of preference for closer association with one enantiomer would suggest that induced fit chiral recognition is occurring with a more well-defined host-guest interaction for one enantiomer, the (+), but not the other. Such induced fit chiral recognition has previously been observed by several workers when comparing the crystal structures of methylated cyclodextrins with enantiomeric guests (section 1.9.1).

Considering the phenyl group of ephedrine it is evident from figure 3.18 that there are enantiomer dependent changes in the structure of this multiplet. However, these changes could not be assigned to differences in the behaviour of any, or indeed all, of the five aromatic protons because of the inherent complexity of the multiplet structure.

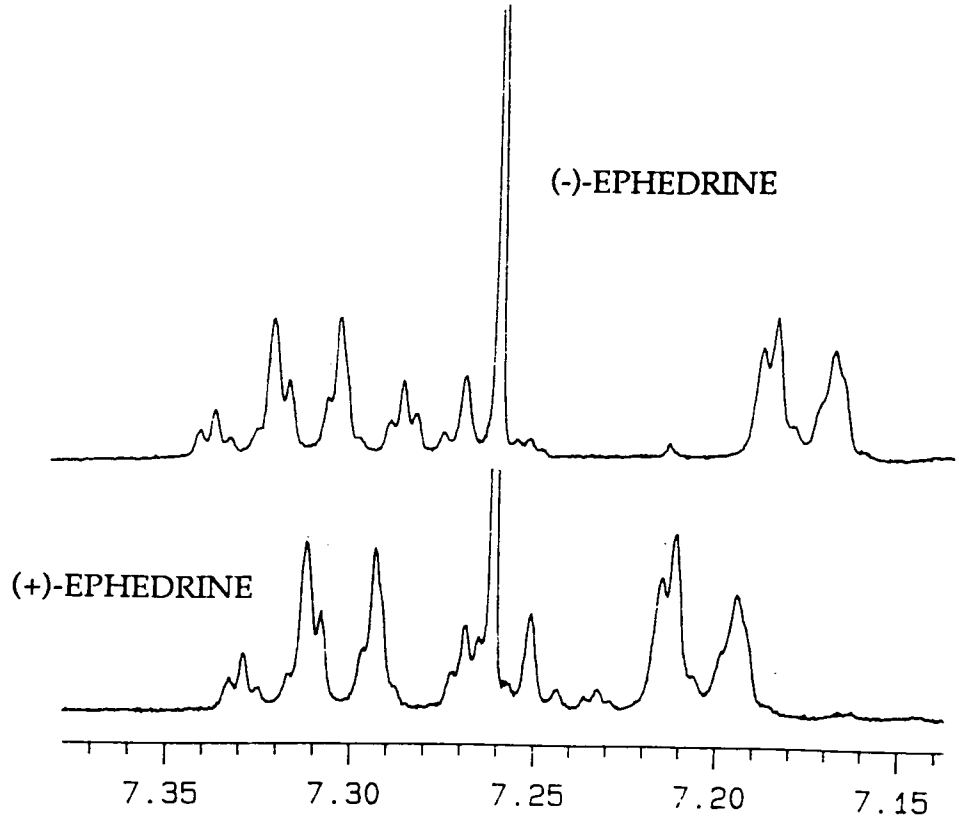



Figure 3.18 Changes in the form of the Phenyl $^1H$ resonances of Ephedrine upon Complexation with 'Per'-O-Octyl-α-Cyclodextrin in d-chloroform Solution at 25°C

Nevertheless, these enantiomer dependent changes do suggest phenyl group participation in the enantioselective host-guest interaction. Indeed, Harata showed that the orientation of the mandelic acid phenyl group within the per-O-methyl-α-cyclodextrin cavity was dependent on which enantiomer was included[22]. Other workers have observed enantiomer dependent changes in the form of the phenyl $^1H$ NMR resonances upon complexation with a parent cyclodextrin host[23], usually in $D_2O$ solution.

The hydroxyl group of ephedrine is seen to undergo a chemical shift change upon complexation which may well be allied to the changes in conformation discussed earlier, and may even be enantiomer dependent. However, it is ill advised to draw definite conclusions about the role of the hydroxyl group from changes in chemical shift because of the undoubted presence of water in all NMR samples used‡ .

### 3.5.1.2 Effects Upon Cyclodextrin Spectrum

Close inspection of figure 3.19 indicates that several unassignable, enantiomer dependent, changes in the form of the cyclodextrin multiplets at 3.3-4.1 ppm occur upon complexation with both enantiomer of ephedrine.

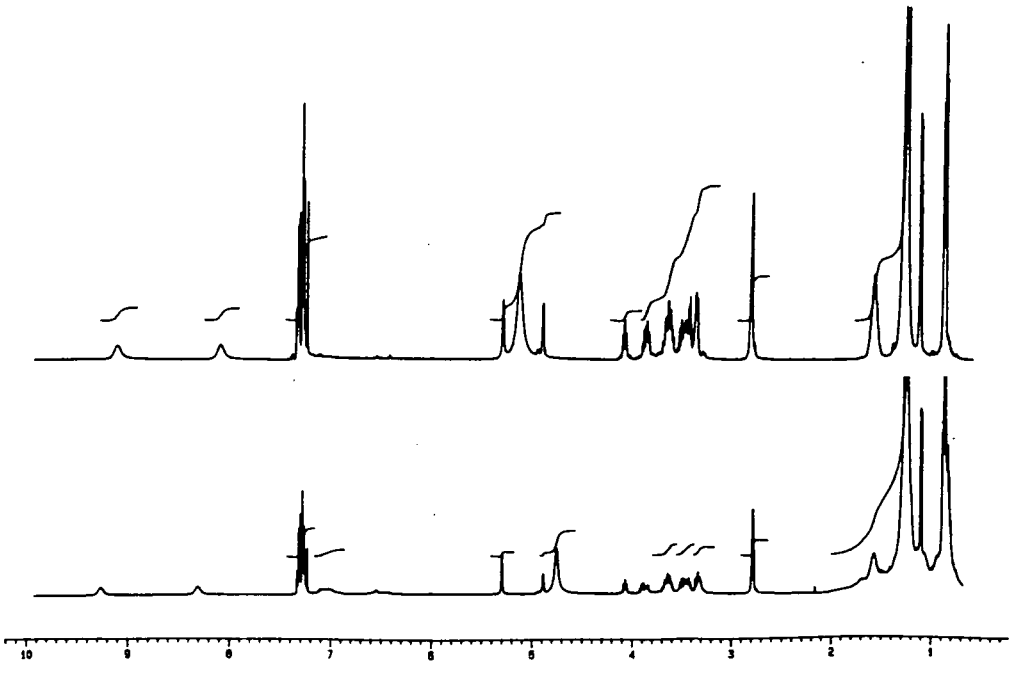


Figure 3.19 400MHz $^1$H NMR of 'Per'-O-Octyl-α-Cyclodextrin in the presence of (-)-Ephedrinium (Upper) and (+)-Ephedrinium (Lower) in d-chloroform Solution at 25°C

‡ *Despite efforts to exclude water its presence (δ=2.2ppm) will affect the chemical shift of the OH group. The effect is dependent upon the relative molar ratio of water to ephedrine hydroxyl.

In order to emphasize, and hence hopefully assign some of these changes variable temperature spectra were acquired at 25°, 0°, -25° and -50° for the two diastereoisomeric complexes. Those obtained at -50°C are shown in figure 3.20.

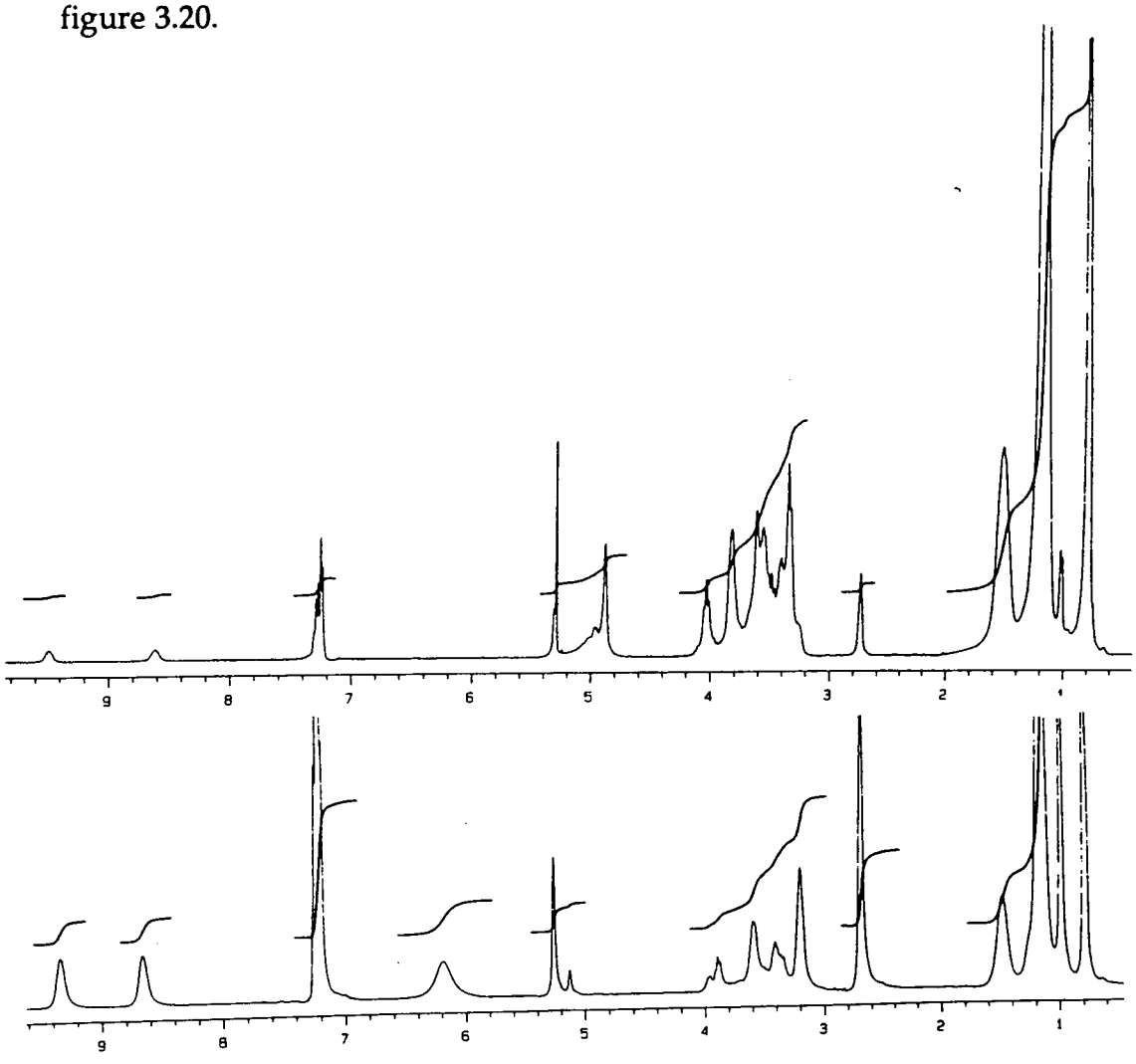



Figure 3.20 $^1H$ NMR of 'Per'-O-Octyl-α-Cyclodextrin in the presence of (-)-Ephedrinium (Upper) and (+)-Ephedrinium (Lower) in d-chloroform Solution at 223K

A reduction in temperature does, at least slightly, emphasize the differences in the CHO and $CH_2O$ resonances of the cyclodextrin. In particular, changes in the overall form of the muliplet at 3.4ppm indicates that octyl $CH_2O$ groups and perhaps H(4) of the cyclodextrin skeleton undergo changes in form which are dependent on which

enantiomer of ephedrine is included. However such changes were unassignable, even with the aid of $^1H$-$^1H$ COSY. The only distinct, quantifiable change, on cooling the complexes was an increase in the diastereotopic nature of the (+) ephedrine ammonium protons, not evident for the (-) enantiomer as detailed earlier.

Despite reports of inclusion dependent changes in $^{13}C$ resonances for cyclodextrin complexes[24], attempts to investigate complex formation by $^{13}C$ NMR proved fruitless with no inclusion dependent changes evident, even at high magnetic field strength (100MHz).

## 3.6 LONGITUDINAL RELAXATION TIMES

### 3.6.1 Longitudinal Magnetisation and Mechanisms of relaxation

Longitudinal magnetisation owes its existence entirely to the difference between the populations of the nuclear spin states $\alpha$ and $\beta$. At thermal equilibrium $N_\alpha$ and $N_\beta$ obey the Boltzmann distribution law. After disturbance of this equilibrium brought about by the action of RF irradiation, $N'_\alpha$ and $N'_\beta$ return to their original values as a result of longitudinal relaxation which is a first order process. It follows therefore that longitudinal relaxation necessarily involves population changes, so that the processes that bring it about are those that induce transitions between the spin states. The relaxation process, being first order, may be characterised by either a rate constant, $R_1$, or by its reciprocal, a relaxation time, $T_1$.

The nucleus may couple with its surroundings either magnetically or electrically, and in each case the strength of the coupling is represented by a coupling constant. In promoting relaxation, coupling is a necessary but not sufficient condition. The loss of energy by a spin system is a

stimulated emission process and as such the probability involves a frequency dependence. Frequencies arise in the coupling of the nucleus to its surroundings through time modulation of the coupling constant by rotation of some part or all of the molecule. A characteristic correlation time is the form in which this modulation enters the model for each relaxation mechanism. Hence, two factors present in every relaxation mechanism are a coupling constant, A, and a correlation time, $\tau$

$$R=\text{const. x } A^2\tau \qquad (7)$$

where R is the first order rate constant for the relaxation mechanism.

### 3.6.2 Rotational Correlation

Rotational correlation describes for molecules their orientation with respect to one another compared with their relative orientation at an earlier time. Since nuclear relaxation is a cooperative process it must be described in terms of parameters characteristic of a molecular ensemble. As such it must be described in terms of a time period during which various properties remain correlated in the face of ceaseless random particle motions. This time period is represented mathematically by an autocorrelation function F(t) whose value goes from 1 to 0 as the relative orientations go from the correlated to the randomised state. The time constant that characterises this exponential decay is defined as the rotational correlation time, $\tau_c$.

Assuming isotropic motion of an approximately spherical molecule then $\tau_c$ can be related to the volume of the molecule by the Stokes-Einstein-Debye relation[25]

$$\tau_c = \frac{4\pi\eta a^3}{3kT} \qquad (8)$$

In 1948 Bloemberg first showed that the energy transfer essential to relaxation will occur if the position vectors determining instantaneous magnetic dipolar or electric quadrupolar coupling of a nucleus with its surroundings are a function of time. In liquids these functions F(t) vary with time in a random fashion as the molecules containing the magnetic nuclei undergo Brownian motion. It is the difference between the F(t) values over short intervals of time, $\tau$, that are characterised by the correlation function k(t)

$$k(t) = \text{const.} \times e^{-\tau/\tau_c} \tag{9}$$

This equation thus defines $\tau_c$ for the ensemble as the length of time required by the kT randomising forces to reduce F(t) to $^1/_e$ of its initial value. The relationship between $\tau_c$ and $T_1$ can be represented graphically as shown in figure 3.21

### 3.6.3 Spectral Density

A magnetic or electric field fluctuating at the Lamour frequency promotes relaxation and in order to recognise the frequencies within the continuously decaying k(t) function it is converted into the frequency domain by Fourier transformation to give

$$J(w) = \text{const.} \times \frac{\tau_c}{1 + \omega^2\tau_c^2} \tag{10}$$

These frequencies, $\omega$, modulate the approach to zero of the correlation function k(t) and provide an oscillating magnetic field at the nucleus, one of which occurs at the Larmor frequency, $\omega_o$, and thus makes a contribution to the relaxation proportional to its spectral density[26] $J(\omega_o)$.

### 3.6.4 Mechanisms of Longitudinal Relaxation

Nuclear species that come under investigation by NMR differ from one another in terms of magnetic moment, nuclear quadrupole moment and bonding radius of the atom within which the nucleus is located. These values, together with the electric field gradient at the nucleus under study, determine the relative extent to which each of the five mechanisms summarised in table 3.6.4A contribute to the relaxation process.

| Mechanism | Interaction | Maximum coupling constant range | Maximum $R$ ($s^{-1}$) | Correlation time (s) |
|---|---|---|---|---|
| Quadrupolar | Electric | 0.24 MHz ($^{2}H$) to 150 MHz ($^{35}Cl$) | $10^{6}$ | $\tau_\theta$ typically $10^{-12}$–$10^{-10}$ |
| Spin–Rotation | Magnetic | 0.5 kHz ($^{13}CCl$) to 116 kHz ($^{31}PH$) | $10^{2}$ | $\tau_\theta$ typically $10^{-13}$–$10^{-11}$ |
| Dipole–dipole | Magnetic | 33 kHz ($^{195}PtH$) to 140 kHz ($^{13}CH$) | 1 | $\tau_\theta$ typically $10^{-12}$–$10^{-10}$ |
| Shielding anisotropy | Magnetic | $\sigma-\sigma_{\perp}$ = 1600 ppm minimum for 0.16 MHz and $R^{sa}$ = 0.05 $s^{-1}$ $\sigma-\sigma_{\perp} \approx$ 5000 ppm gives 0.5 MHz for $^{199}Hg$ | $10^{-1}$ | $\tau_\theta$ typically $10^{-12}$–$10^{-10}$ |
| Scalar coupling | Magnetic | $J_{IS} < 10^{4}$ Hz | $10^{-1}$ $R_2^{sc} < 10^{3}$ | $\tau_s$ typically $>10^{-6}$ |

Table 3.6.4A Summary of Relaxation Mechanisms

In the case of the molecules under investigation here, it is likely that $^{1}H$ longitudinal relaxation is mainly brought about by the dipolar and spin rotation relaxation mechanisms. However, no attempt was made to investigate the extent of the contribution of each mechanism to the observed $T_1$.

### 3.6.5 Application of Proton Longitudinal Relaxation Times

Molecular association has a profound effect upon the longitudinal relaxation times of both the host and guest. This is because association leads to changes in the reorientational mobility of the host and guest which results in corresponding changes in $\tau_c$ and hence $T_1$. The effect of changes in $\tau_c$ upon $T_1$ is dependent upon the motional mobility of the system. In the most common case $\omega_o\tau_c \ll 1.12$. This is described as the extreme motional narrowing region and, as can be seen from the graph in figure 3.22, as $\tau_c$ is inversely proportional to $T_1$ and is at a minimum when $\omega_o\tau_c=1.12$. Away from the motional narrowing region the slow molecular motions of medium to large molecules, such as some cyclodextrin derivatives, peptides and proteins, lead to relatively long $\tau_c$ values. In these cases, as figure 3.21 shows, $T_1$ is directly proportional to $\tau_c$.

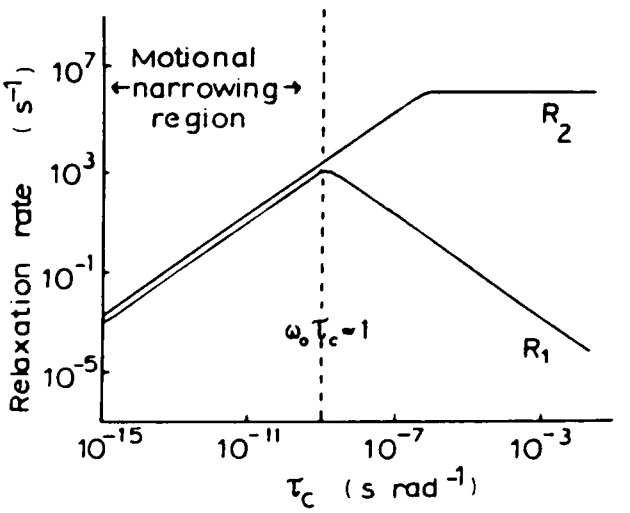



Figure 3.21 The Rate of Longitudinal Relaxation as a function of rotational correlation time.

Recently Lehn and coworkers have investigated 'bouquet-shaped' molecules based on a β-cyclodextrin core using proton and carbon relaxation times in various solvents and at various temperatures in order

to investigate the mechanisms involved in $T_1$ relaxation[27]. Such highly functionalised cyclodextrins are similar in some respects to the per-O-octyl cyclodextrins under investigation here. It was found that $^{13}C$ relaxation times were generally of the order 0.1seconds suggesting that the dipole-dipole interaction is the major component in the relaxation mechanism. Also, nuclear Overhauser effect factors (NOEF) were small indicating that such cyclodextrin derivatives, at moderate concentration in d-chloroform, were away from the extreme motional region of $\tau_c$.

In comparing experimental NOEF and $\tau_c$ values with those predicted theoretically Lehn found large experimental departures from the theoretical predictions for an isotropically tumbling molecule. This was taken to suggest that, assuming the dipole-dipole relaxation mechanism is dominant, there may be large deviations from isotropic molecular reorientation with anisotropic local motions occurring within the molecule. Such deviations from isotropy would be in line with the fact that the $C_7$ symmetry axis probably represents the major inertia axis and therefore the preferential reorientation axis of these molecules.

For both $^1H$ and $^{13}C$ longitudinal relaxation times, variation of the temperature and viscosity of the measurement indicated that $T_1$ was directly proportional to $\tau_c$. The theoretical relation between $T_1$ and $\tau_c$ has already been discussed earlier and these finding by Lehn correspond to the upward branch of $T_1 = f(\tau_c)$. This result suggests that the NMR behaviour of the 'bouquet' cyclodextrins lies outside the extreme narrowing domain ($\omega_o\tau_c>1.12$). Such findings are in accord with those of other workers and are in line with the large molecular size of highly functionalised cyclodextrin derivatives.

Proton $T_1$'s were acquired at 250 MHz in d-chloroform by the inversion recovery method[28], with ten data sets being obtained, each of 32 transients, and a variable delay of 0.1 to 3.0 seconds being used. Tables 3.6.5A, 3.6.5B and 3.6.5C show the relaxation times obtained for host, guest and the two diastereoisomeric complexes.

**Table 3.6.5A Per-O-octyl-α-cyclodextrin**

**Relaxation Times (90μM in 0.7cm$^3$ $CDCl_3$)**

| δ $^1$H | assignment | [a]$T_1$ (s) | SD(±) |
|---|---|---|---|
| 4.89 | H(1) | 0.383 | 0.003 |
| 4.06 | H(3) | 0.500 | 0.004 |
| 3.88 | H(5)* | 0.383 | 0.003 |
| 3.65 | * | 0.246 | 0.013 |
| 3.53 | * | 0.328 | 0.009 |
| 3.37 | H(2) | 0.427 | 0.014 |
| 1.57 | $\underline{CH_2}CH_2O$ | 0.407 | 0.004 |
| 1.26 | $CH_2$ | 0.880 | 0.008 |
| 0.87 | $CH_3$ | 1.98 | 0.010 |

* indicates assignment uncertain as a result of the complex nature of the resonances.
[a] mean of two independent determinations.

**Table 3.6.5B Per-O-octyl-α-cyclodextrin (90μM in 0.7cm$^3$ $CDCl_3$)**

| Relaxation Times in the presence of | | | | | | | |
|---|---|---|---|---|---|---|---|
| i) (-)Eph.trifluoroacetate[a]. | | | | ii) (+)Eph.trifluoroacetate[a]. | | | |
| δ $^1$H | assignment | [b]$T_1$ (s) | SD(±) | δ $^1$H | assignment | [b]$T_1$ (s) | SD(±) |
| 4.90 | H(1) | 0.306 | 0.005 | 4.89 | H(1) | 0.304 | 0.003 |
| **4.05** | **H(3)** | **0.487** | **0.002** | **4.06** | **H(3)** | **0.285** | **0.009** |
| 3.87 | H(5)* | 0.376 | 0.004 | 3.89 | H(5)* | 0.374 | 0.006 |
| 3.65 | * | — | — | 3.66 | * | — | — |
| 3.58 | * | 0.325 | 0.006 | 3.55 | * | 0.277 | 0.002 |
| 3.34 | H(2) | 0.424 | 0.007 | 3.37 | H(2) | 0.419 | 0.011 |
| 1.57 | $\underline{CH_2}CH_2O$ | 0.414 | 0.003 | 1.58 | $\underline{CH_2}CH_2O$ | 0.339 | 0.010 |
| 1.25 | $CH_2$ | 0.876 | 0.006 | 1.27 | $CH_2$ | 0.732 | 0.004 |
| 0.83 | $CH_3$ | 1.91 | 0.009 | 0.88 | $CH_3$ | 1.72 | 0.009 |

* indicates assignment uncertain as a result of the complex nature of the resonances.
[a] 2.5 molar equivalents
[b]mean of two independent determinations.

**Table 3.6.5C(i) Effect of Complexation[b] on (+) Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)**

| $\delta$ $^1H$ assignment | [a]$T_1$ (s) free | SD(±) | [a]$T_1$ (s) complexed | SD(±) | $\Delta T_1$ |
|---|---|---|---|---|---|
| 9.23 NH | 0.234 | 0.013 | 0.154 | 0.020 | -0.08 |
| 8.22 NH | 0.172 | 0.011 | 0.868 | 0.019 | **+0.696** |
| 7.34 Ph | 1.116 | 0.008 | 1.076 | 0.009 | -0.04 |
| 5.36 $H_a$ | 0.326 | 0.007 | 0.207 | 0.017 | -0.119 |
| 3.38 $H_b$ | 0.462 | 0.007 | — | — | — |
| 2.83 NMe | 0.401 | 0.003 | 0.316 | 0.004 | **-0.085** |
| 1.151 Me | 0.469 | 0.009 | 0.510 | 0.008 | **+0.04** |

[a] mean of two independent determinations.
[b] in the presence of 90μM cyclodextrin

**Table 3.6.5C(ii) Effect of Complexation[b] on (-) Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)**

| $\delta$ $^1H$ assignment | [a]$T_1$ (s) free | SD | [a]$T_1$ (s) complexed | SD | $\Delta T_1$ |
|---|---|---|---|---|---|
| 9.23 NH | 0.234 | 0.013 | 0.153 | 0.013 | -0.09 |
| 8.22 NH | 0.172 | 0.011 | 0.145 | 0.010 | -0.027 |
| 7.34 Ph | 1.116 | 0.008 | 1.069 | 0.008 | -0.047 |
| 5.36 $H_a$ | 0.326 | 0.007 | 0.293 | 0.006 | -0.033 |
| 3.38 $H_b$ | 0.462 | 0.007 | — | — | — |
| 2.83 NMe | 0.401 | 0.003 | 0.354 | 0.007 | -0.047 |
| 1.151 Me | 0.469 | 0.009 | 0.432 | 0.008 | -0.037 |

[a] mean of two independent determinations.
[b] in the presence of 90μM cyclodextrin

### 3.6.5.1 Changes in Guest Relaxation Times

Table 3.6.5.1A compares the changes in longitudinal relaxation time for the two ephedrine enantiomers in the presence of 'per'-O-octyl-α-cyclodextrin.

**Table 3.6.5.1A Comparison of $\Delta T_1$ for (+) and (-) Ephedrine Trifluoroacetate (6.5mg in 0.7cm$^3$ $CDCl_3$) with 'per'-O-octyl-α-cyclodextrin as host**

| Proton(s) | $\Delta T_1$(+) (secs.) | $\Delta T_1$(-) (secs.) | $\Delta\Delta T_1$= $\Delta T_1$(+)-$\Delta T_1$(-) |
|---|---|---|---|
| NH | -0.08 | -0.09 | +0.01 |
| NH | +0.696 | -0.027 | **+0.723** |
| Ph | -0.04 | -0.047 | +0.007 |
| $H_a$ | -0.119 | -0.033 | -0.086 |
| $NCH_3$ | -0.085 | -0.047 | **-0.038** |
| $CH_3$ | +0.04 | -0.037 | **+0.077** |

With (-) ephedrine the observed changes in proton longitudinal relaxation time upon complexation were not unusual for the association of a small, low molecular weight, molecule with a much larger one such as a cyclodextrin derivative. That is, a slight decrease in $T_1$ as a consequence of the decrease in molecular motion,ω, associated with a more slowly tumbling larger molecule. This suggests that although molecular association is occurring there are no well defined host-guest interactions.

For (+) ephedrine the situation is very different with marked, informative changes in $T_1$ relaxation times occurring upon complexation with the α-cyclodextrin host. There is a distinct decrease in $T_1$ for the

amine methyl group,$\underline{CH_3}N$, from 0.401 seconds to 0.316 seconds and an increase for the methyl group, $\underline{CH_3}C$, from 0.469 seconds to 0.510 seconds. The ammonium protons also behave in an interesting manner with one having a much longer relaxation time than the other, 0.154 seconds and 0.868 seconds. These results indicate the participation of the $\underline{CH_3}N$, $\underline{CH_3}C$, and $^+N\underline{H_2}$ groups of ephedrine in enantiospecific complexation between (+) ephedrine and per-O-octyl-α-cyclodextrin, in d-chloroform solution. Such findings parallel the observations made as a result of electrochemical investigation of guest modification.

These results suggest a closer host-guest interaction for the (+) enantiomer of ephedrine with the α-cyclodextrin host. This is because the large changes in $T_1$ for several groups in the case of the (+) enantiomer indicate that there may be a change in the mechanism by which longitudinal relaxation is occurring for the $\underline{CH_3}N$, $\underline{CH_3}C$, and $^+N\underline{H_2}$ groups in (+) ephedrine. It is perhaps reasonable to assume that the chiral centre β to the phenyl ring is involved to a greater extent in the enantioselective host-guest interaction. Earlier, electrochemical investigations suggested that, as in the case of (+) ephedrine, an R absolute configuration at this chiral centre gave a more well defined electrochemical response (section 3.4). From these investigations it appears that solvation of the positive charge at nitrogen through $^+N$—H···O hydrogen bonding with the ether oxygens in or around the rim of the Lewis basic cyclodextrin cavity may well be an important host-guest interaction. The increase in $T_1$ for one ammonium ion proton suggests that complexation reduces the rate of relaxation through the suppression of one (or more) mechanisms of relaxation and perhaps decreases the local re-orientational correlation time, $\tau_q$, through an increase in local motional mobility. It is likely that complexation weakens, if not inhibits

completely, intramolecular hydrogen bonding between the $^{+}NH_2$ and OH groups. This hydrogen bonding gives a rapid relaxation mechanism and it would not be surprising therefore that its inhibition would give rise to a significantly increased $T_1$ value. If one ammonium ion proton is involved in hydrogen bonding to the cyclodextrin host it will have a replacement for the mechanism lost through the suppression of intramolecular hydrogen bonding. Hence its rate of relaxation, $T_1$ would not be expected to increase significantly and, depending on the strength of the hydrogen bond(s) with the host may even decrease. Further evidence of possible differentiation of the ammonium ion protons by the host as an explanation of the rise in $T_1$ for one $^{+}NH$ but not the other is obtained from the fact that complexation increases the chemical shift non-equivalence for the diastereotopic NH's for (+) ephedrine but not (-) ephedrine. This non-equivalence is further increased on cooling to -50°C suggesting that there is a low energy conformation of the complex in which the NH protons experience very different local magnetic and electronic environments.

Further positive charge solvation may also occur through $^{+}NCH_2$—H···O hydrogen bonding interactions. This would account for the observed decrease in the $NCH_3$ proton $T_1$ upon complexation because the hydrogen bonding interaction will give an extra dipole-dipole relaxation mechanism for the longitudinal relaxation resulting in quicker relaxation. The increase in $T_1$ for the C-$CH_3$ methyl group is perhaps less easily explained. Upon inclusion of the ephedrinium ion within the cyclodextrin cavity, the rate of molecular re-orientation for the guest is expected to decrease as a result of host - guest interactions. Such a reduction in the rate of molecular re-orientation upon inclusion within a α-cyclodextrin cavity is typically by a factor of about 4 for monosubstituted

aryl groups[28]. The C—Me methyl group is expected to undergo rapid unhindered internal rotation about its threefold axis in both the free and bound ephedrinium ion. Such internal rotation will decease the local re-orientational correlation time, $\tau_\theta$, for this methyl group[29]. In the limiting case when such local re-orientation is very fast with respect to the overall re-orientation of the molecule, the local methyl motion may become decoupled[30] by a factor ca.10. The (+)-ephedrinium ion is thought to be bound more tightly by the cyclodextrin host which may lead to decoupling of the rapid internal methyl re-orientation. This would then account for the observed increase in proton $T_1$ for this methyl group upon complexation.

### 3.6.5.2 Changes in Host Relaxation Time

Determination of the proton $T_1$'s for per-O-octyl-α-cyclodextrin host in the absence and presence of ephedrine resulted in changes in the $T_1$'s which were dependent upon the enantiomer of ephedrine included within the cavity. Table 3.6.5.2A compares the $\Delta T_1$ values for per-O-octyl-α-cyclodextrin in the presence of the two ephedrine enantiomers.

**Table 3.6.5.2A Comparison of $\Delta T_1$ for 'per'-O-octyl-α-cyclodextrin with (+) and (-) Ephedrine Trifluoroacetate as guest (90μM CD, 6.5mg Eph.TFA in 0.7cm$^3$ $CDCl_3$)**

| Proton(s) | $\Delta T1_{(+)}$ (S) | $\Delta T1_{(-)}$ (S) | $\Delta\Delta T_1$= $\Delta T_1(+)-\Delta T_1(-)$ |
|---|---|---|---|
| 4.89, H(1) | -0.079 | -0.077 | -0.002 |
| 4.05, H(3) | -0.215 | -0.013 | -0.202 |
| 3.87, H(5)* | -0.009 | -0.007 | -0.002 |
| 3.65, * | — | — | — |
| 3.58, * | -0.051 | -0.003 | -0.048 |
| 3.34, H(2) | -0.008 | -0.003 | -0.005 |
| 1.57, $\underline{CH_2}CH_2O$ | -0.068 | +0.007 | -0.075 |
| 1.25, $CH_2$ | -0.148 | -0.004 | -0.144 |
| 0.88, $CH_3$ | -0.260 | -0.07 | -0.253 |

* indicates assignment uncertain as a result of the complex nature of the resonances.

It is apparent from table 3.6.5.2A that H(3), the octyl chain protons and an unassignable resonance at 3.58ppm (which is most likely a $CH_2O$ group of an octyl chain) have changes in $T_1$ which are dependant upon the enantiomer of ephedrine present. The only assignable glucopyranose subunit proton of the cyclodextrin framework which behaves in an enantiomer dependant manner is H(3) with $\Delta\Delta T_1$= -0.202 seconds. This proton points into the macrocyclic cavity near to the secondary hydroxyl rim and may be experiencing cross dipolar relaxation (NOE's) from the ephedrine phenyl group hydrogens. There is a literature precedent for this type of interaction[31]. In the case of the α-cyclodextrin-tryptophan[32] complex in $D_2O$, Lipkowitz and co-workers observed a $\Delta T_1$=-0.07seconds for H(3) with the (+) enantiomer of tryptophan as guest but with the (-)

enantiomer as guest they found $\Delta T_1$=+0.11seconds. Investigation by 1D NOE spectroscopy showed a 0.5% enhancement for H(3) upon irradiation of $H_a$ of (+) tryptophan. No NOE enhancement could be observed in the case of the (-) tryptophan enantiomer indicating an enantiomer dependent host guest interaction between H(3) and $H_a$ of the (+) tryptophan molecule.

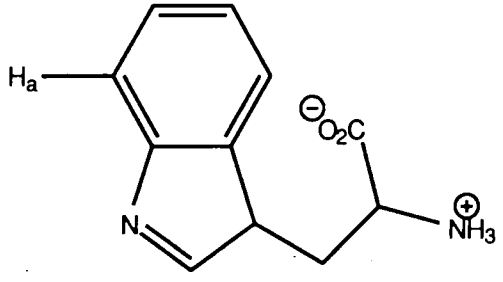



Figure 3.22 Tryptophan.

The changes in the $T_1$'s for the octyl chains attached to the cyclodextrin framework upon complexation suggest a change in environment associated with inclusion of the ephedrine guest within the cyclodextrin cavity. The extremities of the chains may well be more sensitive towards changes in the cyclodextrin core conformation than might be expected. This is because, since they describe a greater volume, they may be sensitive to changes in steric hindrance. Such a phenomenon has previously been observed for the arboral series[33]. With (+) ephedrine as guest the octyl chains show a relatively large decrease in $T_1$'s. It is probable that in the free host the octyl chains are mobile and there may well be an exchange process involving an octyl chain which is included within the hydrophobic cyclodextrin cavity and those external to the cavity that are associated with each other through alkyl chain - alkyl chain hydrophobic interaction (see section 4.5.2). Upon complexation the octyl chains will be prevented from penetrating the cavity to any appreciable extent by the competing guest. Hence, their conformations with respect to each other and the cyclodextrin core may well be

influenced by changes in the conformational mobility of the glucopyranose subunits which result from host-guest interactions. These changes in conformation and environment for the alkyl chains may well affect the rate of longitudinal relaxation for all octyl chain protons. The effects are more marked with (+) ephedrine as the guest which could well be a consequence of a more favourable $\Delta G_{complex}$ for that enantiomer. That is, the (+) enantiomer of ephedrine probably competes more effectively with the octyl chains for occupancy of the macrocyclic cavity.

A closer complementarity in binding interactions between host and guest with inclusion of (+) ephedrine may bring about significant changes in the conformation of the cyclodextrin ring. Such conformational changes for the cyclodextrin have been observed for the inclusion of various aromatic guests by per-O-methylated cyclodextrins through X-ray crystallographic analysis and have been shown to be more marked for one enantiomer of a pair (section 1.9). Such changes in conformation may then change the disposition of the octyl chains relative to the macrocycle and to each other. Inclusion dependent changes in host conformation may then lead to a corresponding change in $\tau_c$.

#### 3.6.5.3 Structural Variation of Guest

Throughout the previous NMR sections which discuss the recognition of ephedrines by α-cyclodextrin several conclusions have been drawn about the functional groups which are important for enantioselective recognition of these β-aryl ammonium ions. In an attempt to determine how essential each functional group of the ephedrine guest is for enantiomer discrimination several longitudinal relaxation time experiments were carried out on modified guests of similar structure to those investigated electrochemically.

i)N-Methyl Ephedrine

**Table 3.6.5.3A Effect of Complexation[b] on (-) N-Methyl Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)[a]**

| $\delta$ $^1H$ assignment | [a]$T_1$ (s) free | SD(±) | [a]$T_1$ (s) complexed | SD(±) | $\Delta T_1$ |
|---|---|---|---|---|---|
| 10.75 NH | 1.57 | 0.028 | 0.573 | 0.020 | -0.997 |
| 7.35 Ph | 3.05 | 0.006 | 2.48 | 0.002 | -0.57 |
| 5.53 $H_a$ | 1.22 | 0.003 | 0.083 | 0.003 | -0.39 |
| 3.44 $H_b$[c] | 1.25 | 0.008 | — | — | — |
| 2.95 $NMe_2$ | 0.942 | 0.006 | 0.759 | 0.004 | -0.187 |
| 1.15 Me | 0.911 | 0.004 | 0.838 | 0.012 | -0.07 |

[a] mean of two independent determinations.
[b] in the presence of 90µM cyclodextrin
[c] obscured by cyclodextrin resonance

**Table 3.6.5.3A Effect of Complexation[b] on (+) N-Methyl Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)**

| $\delta$ $^1H$ | [a]$T_1$ (s) | SD | [a]$T_1$ (s) | SD | $\Delta T_1$ |
|---|---|---|---|---|---|
| assignment | free | | complexed | | |
| 10.75 | 1.57 | 0.028 | 0.520 | 0.04 | -1.05 |
| NH | | | | | |
| 7.35 | 3.05 | 0.006 | 2.41 | 0.004 | -0.64 |
| Ph | | | | | |
| 5.53 | 1.22 | 0.003 | 0.78 | 0.006 | -0.44 |
| $H_a$ | | | | | |
| 3.44 | 1.25 | 0.008 | — | — | — |
| $H_b$[c] | | | | | |
| 2.95 | 0.942 | 0.006 | 0.687 | 0.004 | -0.260 |
| $NMe_2$ | | | | | |
| 1.15 | 0.911 | 0.004 | 0.822 | 0.003 | -0.09 |
| Me | | | | | |

[a] mean of two independent determinations.
[b] in the presence of 90μM cyclodextrin
[c] obscured by cyclodextrin resonance

N-methylation of ephedrine results in a much less well defined enantiomer dependent change in proton $T_1$'s upon complexation with the α-cyclodextrin host. This suggests that the ammonium ion plays a significant role in the enantioselective interaction between the complexing species. The affect of N-methylation is to increase the steric requirement of the 'onium moeity. It is perhaps reasonable to suggest therefore that $^+$N—H···O interactions play a significant role in structuring of the complex and the enantiomer selection process. The removal of one of the two ammonium hydrogens of ephedrine will also reduce the effectiveness of $^+$N charge solvation through host - guest hydrogen bonding within the cyclodextrin cavity. Thus, the effect of N-methylation

is to increase the steric requirement of the 'onium ion moeity and reduce the effectiveness of charge solvation within the cavity.

ii)Pseudo Ephedrine

**Table 3.6.5.3B Effect of Complexation[b] on (-) Pseudo Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)**

| $\delta$ $^1H$ assignment | [a]$T_1$ (s) free | SD(±) | [a]$T_1$ (s) complexed | SD(±) | $\Delta T_1$ |
|---|---|---|---|---|---|
| 8.99 NH | 0.211 | 0.009 | 0.223 | 0.013 | **+0.012** |
| 8.09 NH | 0.205 | 0.016 | 0.203 | 0.013 | -0.002 |
| 7.34 Ph | 1.581 | 0.006 | 1.375 | 0.011 | -0.206 |
| 4.66 $H_a$ | 0.485 | 0.005 | 0.409 | 0.006 | -0.076 |
| 3.30 [c]$H_b$ | 0.638 | 0.005 | — | — | — |
| 2.79 NMe | 0.597 | 0.002 | 0.483 | 0.008 | -0.114 |
| 1.11 Me | 0.586 | 0.003 | 0.567 | 0.004 | -0.019 |

[a] mean of two independent determinations.
[b] in the presence of 90μM cyclodextrin
[c] obscured by cyclodextrin resonance

**Table 3.6.5.3A Effect of Complexation[b] on (+) Pseudo Ephedrine trifluoroacetate $T_1$ Relaxation Times (6.5mg in 0.7cm$^3$ $CDCl_3$)**

| $\delta$ $^1H$ assignment | [a]$T_1$ (s) free | SD | [a]$T_1$ (s) complexed | SD | $\Delta T_1$ |
|---|---|---|---|---|---|
| 8.99 NH | 0.211 | 0.009 | 0.206 | 0.017 | -0.005 |
| 8.09 NH | 0.205 | 0.016 | 0.202 | 0.013 | -0.003 |
| 7.34 Ph | 1.581 | 0.006 | 1.463 | 0.029 | -0.118 |
| 4.66 $H_a$ | 0.485 | 0.005 | 0.423 | 0.009 | -0.062 |
| 3.30 [c]$H_b$ | 0.638 | 0.005 | — | — | — |
| 2.79 NMe | 0.597 | 0.002 | 0.517 | 0.003 | -0.080 |
| 1.11 Me | 0.586 | 0.003 | 0.548 | 0.007 | -0.038 |

[a] mean of two independent determinations.
[b] in the presence of 90μM cyclodextrin
[c] obscured by cyclodextrin resonance

From the preceding tables it is evident that the proton $T_1$'s of (+)-ψ-ephedrine behave in a manner which closely parallels those of (-)-ephedrine upon association with the α-cyclodextrin host. That is, all proton $T_1$'s reduce slightly as a result of the slower molecular motions of a larger molecule. With (-)-ψ-ephedrine the changes in proton $T_1$'s which occur on complexation are in accord with those for (+)-ephedrine, although in this case the changes are somewhat less marked. In this case, $CH_3C$, $CH_3N$ and $^+NH_2$ all show changes in $T_1$ upon complexation which cannot be explained by the slower motions of a larger molecule. Both (-)-

ψ-ephedrine and (+)-ephedrine have an R configuration at the chiral centre β to the aryl ring and it is this chiral centre which carries the functional groups which show enantiomer dependent changes of $T_1$ upon complexation. This seems to suggest therefore that this chiral centre plays a major role in the host - guest interactions which bring about enantiomer selection, and that an R configuration may allow more favourable interaction.

## 3.7 NUCLEAR OVERHAUSER EFFECT SPECTROSCOPY

The use of proton longitudinal relaxation times has allowed some assessment of the functionalities involved in the ephedrine - α-cyclodextrin complex and given an insight into how enantiomer discrimination may occur. However, in order to establish which guest functionalities interact with a specific region of the glucopyranose subunits within the cyclodextrin molecule one and two dimensional NOE spectra were acquired at 500MHz. In the two dimensional NOESY experiment[34] cross peaks arise between two nuclei A and B from longitudinal transfer $I_{AZ} \rightarrow I_{BZ}$ during the mixing time. Such homonuclear Overhauser effects have previously been widely applied to structure determination[35]. In small molecules, where magnetic dipolar relaxation of the nuclei is in the extreme narrowing limit, the maximum positive effect[36] that can be observed is 50%. The $^1H$ homonuclear NOE measurement is one of the most effective techniques available to obtain information concerned with through space distances amongst proton nuclei within 5Å.

Although some workers have reported attempts to obtain intermolecular cyclodextrin-guest NOE's, most have been unable to observe any intermolecular enhancement, particularly for highly functionalised cyclodextrin derivatives[31b]. The major problem associated with applying NOE spectroscopy to cyclodextrin derivatives is that quite often they are of such a size and mobility that the condition of extreme motional narrowing cannot be assumed. Indeed, away from motional narrowing when $\omega_o\tau_c = 1.12$ no NOE enhancement can be observed and when $\omega_o\tau_c > 1.12$ the NOE approaches -1 and specificity is lost as a result of spin diffusion[37]. Observation of the NOE at short times[38] or of transient[39] NOE's may overcome this difficulty in part, but at the price of observing very small effects.

In a number of cases, all of which involve the native cyclodextrin as the host in $D_2O$, small enhancements (ca.3%) have been observed. Such NOE effects have usually been observed through the use of 1D NOE difference spectra and often involve an enhancement between H(3) of the cyclodextrin and an aromatic proton[31a,40]. Perhaps one of the best examples is of work carried out by Lipkowitz which only showed an NOE enhancement with the (+) enantiomer of tryptophan[32], as described earlier (section 3.6.5.2). Other work by Sanchez-Ferrando has shown intermolecular NOE enhancements between β-cyclodextrin and adamantine[41].

There have been no reports, to date, of NOE enhancement being observed between a highly functionalised cyclodextrin derivative and a guest in an apolar solvent such as d-chloroform. The problems are associated with the fact that cyclodextrins, their derivatives and more especially complexes of their derivatives are of a size and mobility such that

extreme motional narrowing cannot be assumed and indeed, $\omega\tau_c \approx 1$, this means that little NOE enhancement is expected and indeed if $\omega\tau_c = 1.12$ then no enhancement at all would be evident. Hence, for the cyclodextrin-ephedrinium complexes under investigation here enhancement is likely to be very low, <1%. Experimental investigation showed that no NOE enhancement could be observed for the two diastereomeric complexes, in either mode of operation. This suggests that the enhancement is, at best, <0.3%. Such results parallel the findings of Bothner-By in his investigation of tetrasaccharides[42].

In an attempt to overcome the experimental difficulties inherent in 1D NOE and 2D NOESY a rather novel rotating frame experiment was employed, Rotating Frame Overhauser Effect Spectroscopy (ROESY)[42]. This technique has great potential for the study of peptides, oligonucleotides and cyclodextrins because it avoids the problems inherent to the conventional transient NOE experiment when the molecule of interest is not in the slow motion limit. Theoretical investigation shows that the maximum transient NOE in the rotating frame increases from 38.5% for $\omega_0\tau_c \ll 1$ to 67.5% for $\omega_0\tau_c \gg 1$. This has two important consequences:

i)the NOE is always positive and does not vanish for any value of $\omega_0\tau_c$.

and

ii)multispin effects will be minor.

The positive sign also ensures that the effect will not be confused with transfer of magnetisation by chemical exchange[43].

ROESY is a two dimensional coherence transfer experiment[44] which differs from NOESY as a result of a spin locking pulse being applied which has the effect of regaining the specificity lost as a result of spin diffusion. This often allows weak enhancement to then be seen and is particularly important in cases where $\omega\tau_c>1$. In these cases molecular motions have the effect of making a conventional NOE enhancement of indeterminant sign. Consequently most effects time average to zero on the NMR timescale. However the spin locking pulse in ROESY attempts to overcome such difficulties by making all effects positive[45], the problem then becomes one of observing the small enhancements above the $T_1$ noise and zero quantum COSY breakthrough in the baseline of the ROESY spectrum. Of particular concern is the potential danger of artifacts arising through Hartman - Hahn coherence transfer processes[46]. ROESY has been applied to structural and conformational analysis for a select number of cyclodextrin derivatives[47] and its unprecedented use in the field of cyclodextrin complexation chemistry was recently described by Perly and co-workers[48]. They experienced similar difficulties to those described above in attempts to obtain NOESY spectra for, in their case, native cyclodextrin-steroid complexes.

Several attempts were made to acquire ROESY spectra for the 'per'-O-octyl-α-cyclodextrin-ephedrine complexes using various spin locking pulses of different duration and power. Despite this however, no reproducible inter-molecular cross peaks could be observed. Figure 3.23 shows a typical ROESY spectrum with several intra-molecular cross peaks highlighted.

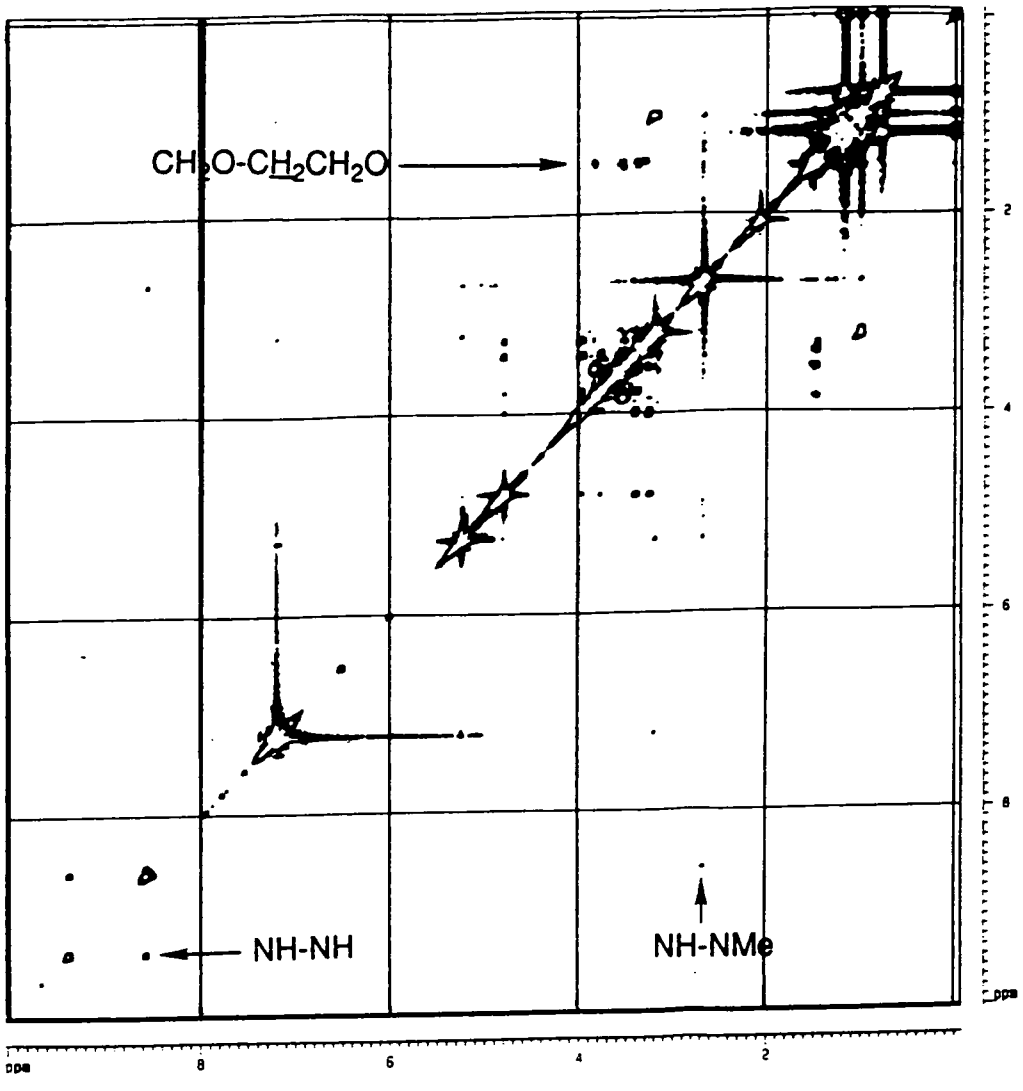



Figure 3.23 500MHz ROESY Spectrum of 'Per'-O-Octyl-α-Cyclodextrin (90μM) and (+) Ephedrine (225μM) in d-chloroform Solution at 20°C

## 3.8 APPLICATION OF MASS SPECTROMETRY

An example of the use of mass spectrometry to investigated molecular association by cyclodextrins was described by Stoddart in 1988 (section 1.12.1). In chapter two an ionisation technique amenable to highly lipophilic cyclodextrin derivatives was described, ES-MS. The formation of a per-O-octyl α-cyclodextrin - ephedrinium complex under the conditions of ES-MS was investigated by injection of a solution containing the cyclodextrin derivative (50pM) and (+)ephedrine (0.2mM) in isopropanol. Figure 3.24 shows ES-MS spectra for 'per'-O-octyl-α-cyclodextrin in the absence and presence of ephedrine.

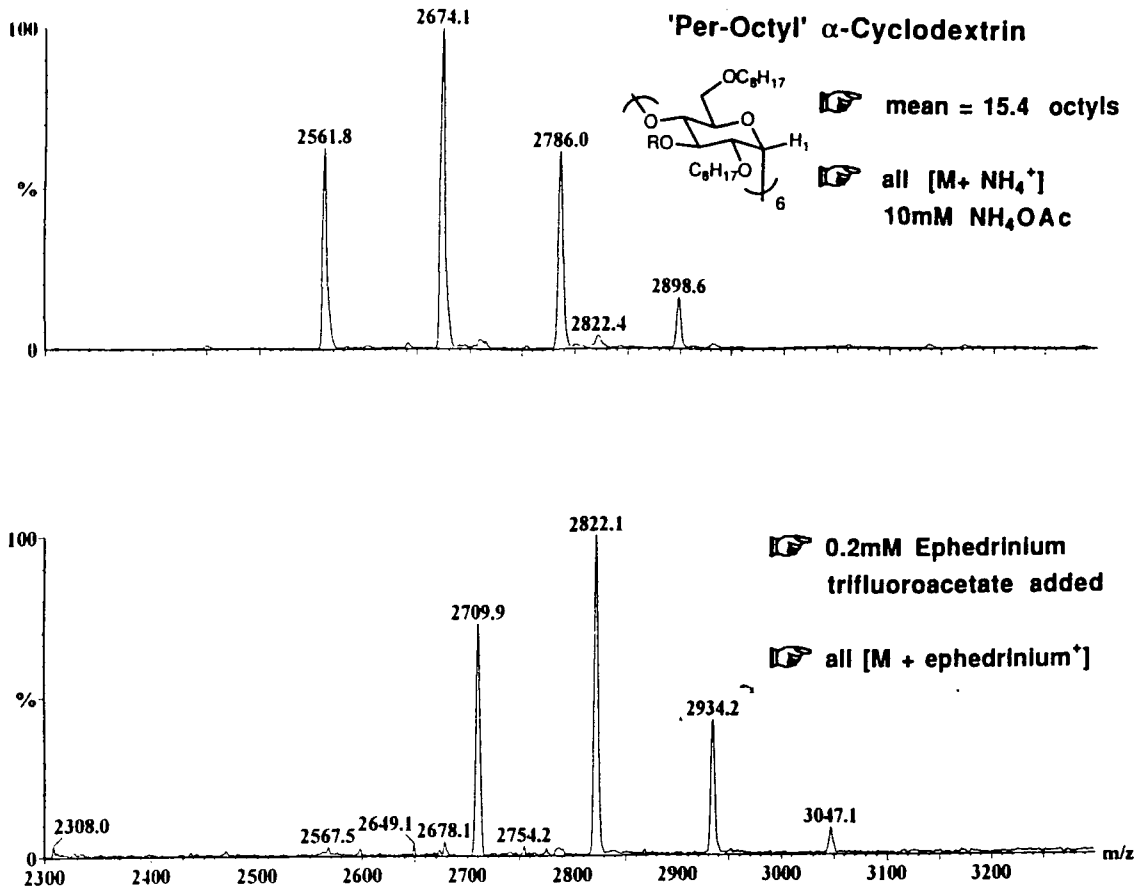



Figure 3.24 ES-MS Spectra for 'Per'-O-Octyl-α-Cyclodextrin in the Absence (upper) and Presence of Ephedrinium Trifluoroacetate (lower).

In the latter case $[CD+eph]^+$ molecular ion peaks are clearly evident, indicating adduct formation. No multiply charged ions could be observed indicating that molecular association is limited to a 1:1 stoichiometry. To demonstrate the affinity of the α-cyclodextrin cavity for the ephedrinium ion a competition experiment was performed between ephedrinium and ammonium cations as potential guests. Figure 3.25 shows the results of this competitive experiment and indicates a 250:1 selectivity for ephedrinium over ammonium.

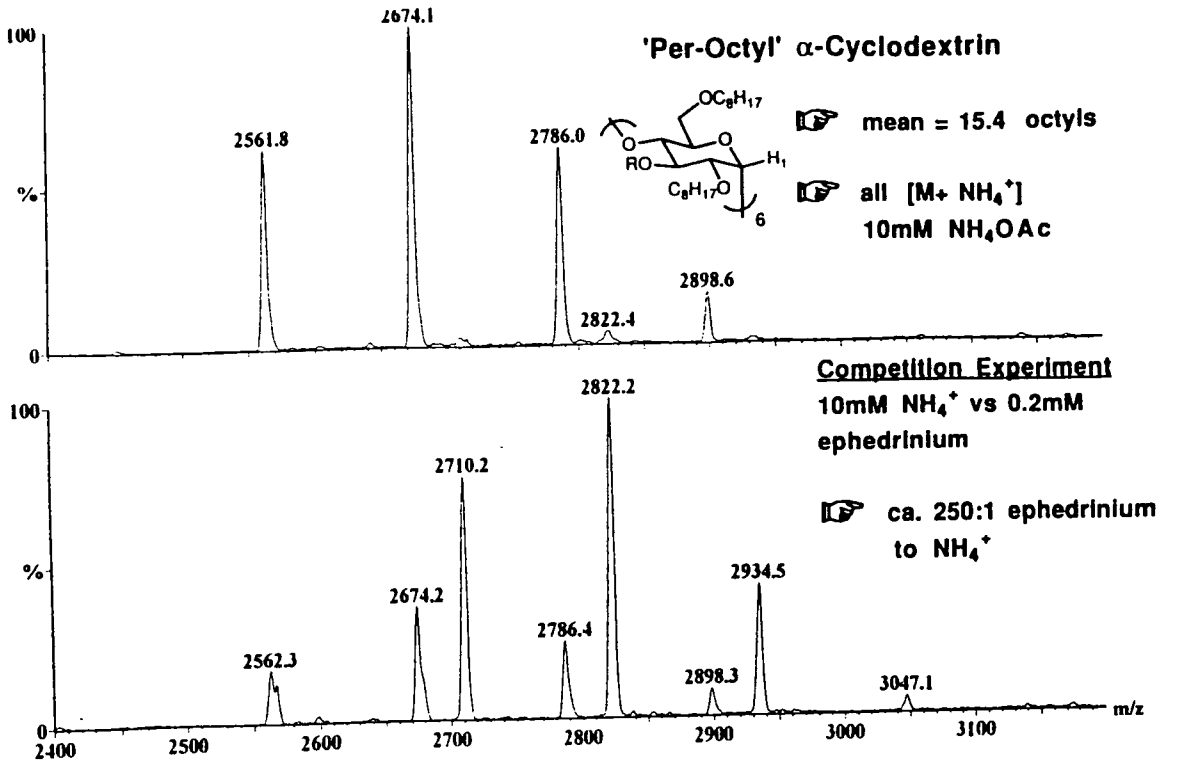



Figure 3.25 ES-MS Investigation of Competition between $^{+}NH_4$ and $^{+}EPH$ for adduct formation with 'Per'-O-Octyl-α-Cyclodextrin.

Mass spectrometry is therefore able to confirm that, as shown electrochemically, 'per'-O-octyl-α-cyclodextrin is able to form an adduct with the ephedrinium cation. It is also able to indicate that, under the condition of ES-MS, ephedrinium - cyclodextrin complexation occurs rather selectively in the presence of competing ammonium ions.

## 3.9 ENANTIOSELECTIVE BINDING OF THE EPHEDRINIUM ION BY α-CYCLODEXTRIN

The driving force of cyclodextrin complexation in general remains unclear (section 1.7.2). However, several different interactions have been proposed. Van der Waals' interactions, hydrogen bonding, a 'hydrophobic push', release of high energy water from the cavity and dipole-dipole interactions are amongst the most important. The cyclodextrin cavity may be considered as a hydrophobic space where many

small van der Waals' interactions work in a cooperative way to complex the ephedrinium ion. In this sense, these attractive van der Waals' contributions may be considered as the major driving force for the primary host-guest interaction.

The observation that methyl capping of the 'per'-O-octyl-α-cyclodextrin more or less removes the enantioselective behaviour of the ionophore suggests that the 3(OH) groups are essential for a 'well defined' electrochemical response. However, with 2,6-di-O-octyl-α-cyclodextrin as the ionophore the electrode characteristics are poor for both enantiomers. It is known that the hydrogen bond donor abilities of both OH and OR groups of cyclodextrin derivatives are approximately equal. Therefore, a change in the function of 3(O) as a hydrogen bond acceptor seems unlikely. Two other possibilities exist for the role of the residual OH groups in the enantiomer discrimination process:

i) The 3(OH) group may be involved in a O(3)—H···O hydrogen bonding interaction with the ephedrine hydroxyl. Methyl capping would remove this interaction. 2,6-di-O-octylation may leave this interaction dominant resulting in poor complexation dynamics as a consequence of the formation of a relatively stable complex.

and

ii) Residual 3(OH) groups may restrict the conformational mobility of the cyclodextrin glucopyranosyl subunits with respect to one another as a result of 3(OH)···2(O) hydrogen bonding interactions. Such interactions may well influence the hospitality of the host.

Investigation of host modification has indicated that a glycosidic linkage is essential for any enantiomer discrimination. However, although per-O-octyl maltose appears to be a good sensor for (+) ephedrine it is unable to act as a chiral sensor. Also, the β-cyclodextrin ionophore did not distinguish the enantiomers of ephedrine to any appreciable extent. This indicates that an α-cyclodextrin cavity is required for well defined enantiomer selection and good electrode characteristics.

N-methylation of ephedrine results in no detectable evidence of enantiomer discrimination, as measured by changes in proton relaxation time, $\Delta T_1$. However, norephedrine behaves well electrochemically showing good enantioselectivity and this is further supported by the proton $T_1$ investigation. Such behaviour clearly indicates the role of the ammonium ion in the enantiomer selection process, with an increase in the steric requirement of the ammonium ion leading to markedly reduced enantioselectivity. The enantiomer dependent NMR behaviour of the two diastereotopic ammonium protons, $^{+}N—H$, further indicated the role of this moiety. Upon inclusion within the cyclodextrin cavity the counter ion ($Cl^-$ or $CF_3CO_2^-$) will be prevented from close approach to the ephedrinium cation. A partial charge of -0.46 (e units) associated with each of the cyclodextrin oxygen atoms suggests that $^{+}N—H\cdots O$ interactions are not unreasonable. Also, C—H···O interactions are likely (see section 4.2). Such observations have led to the postulation of an interaction between the 'glycosidic region' of the cyclodexrtin host and the ephedrine ammonium ion as is depicted in figure 3.26

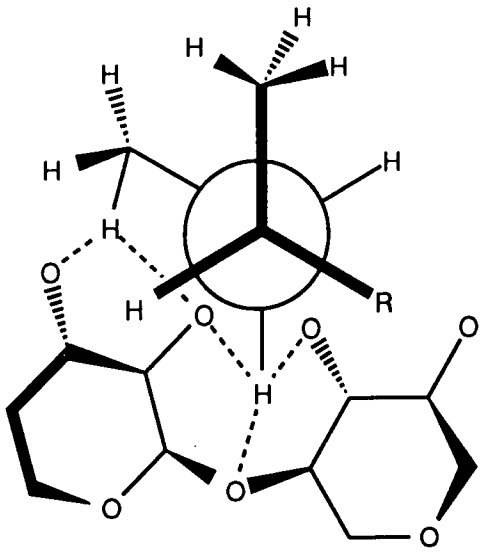



Figure 3.26 Proposed Interaction Between the (+)-Ephedrine Ammonium Ion and 'Per'-O-Octyl-α-Cyclodextrin.
View is Newmann Projection along C*—$N^+$ bond

As figure 3.26 illustrates, such a binding interaction would allow both $^+$N—H···O and C—H···O interactions between the host and guest. If such interactions were only possible with the (+) enantiomer of ephedrine then several of the electrochemical and NMR observations may be rationalised:

i)Very different $T_1$'s for the two ammonium protons (0.154 secs and 0.868 secs.) would result from one being involved in $^+$N—H···O interactions whilst the other is directed towards a less polar environment in the O(3) and O(2) octyl chain region of the host.

ii)The decrease in $T_1$ for $NCH_3$ (-0.085 secs.) may be a consequence of C—H···O hydrogen bonding offering an alternative dipolar relaxation mechanism.

iii)In order for such hydrogen bonding interactions to occur the relative orientations of the ammonium ion, hydroxyl and phenyl ring must be altered. Such changes in conformation are observed through a reduction in the $H_a$-$H_b$ $^3J$ coupling constant (from 2.4 Hz to 1.6 Hz).

iv)An increase in $\Delta\delta$ for the diastereotopic NH protons, on complexation of (+)-ephedrine only may be expected if intermolecular hydrogen bonding restricts the rate of rotation about the C(Me)—N bond.

v)Such a mode of host-guest interaction would also explain the observed enantioselection for norephedrine and the lack of enantioselectivity for N-methyl ephedrine, on the basis of the steric requirement of the ammonium ion. That is, N-methylation of ephedrine may well prevent a 'close approach' of the ammonium ion to the glycosidic region of the host.

The electrochemical and NMR investigations have suggested that the absolute configuration at the chiral centre β to the phenyl ring is of central importance for enantiomer selection. Furthermore, it is apparent that an R absolute configuration at this chiral centre allows a 'better defined' host-guest interaction. If the hydrogen bonding mode suggested above is correct then the enantiomer selective interaction may involve the orientation of the C-Me methyl group attached to this chiral centre with respect to the cyclodextrin cavity. Figure 3.27 indicates that in the case of (+)-ephedrine this methyl group is oriented away from the glycosidic region of the cyclodextrin host, if the hydrogen bonding mode suggested above is correct.

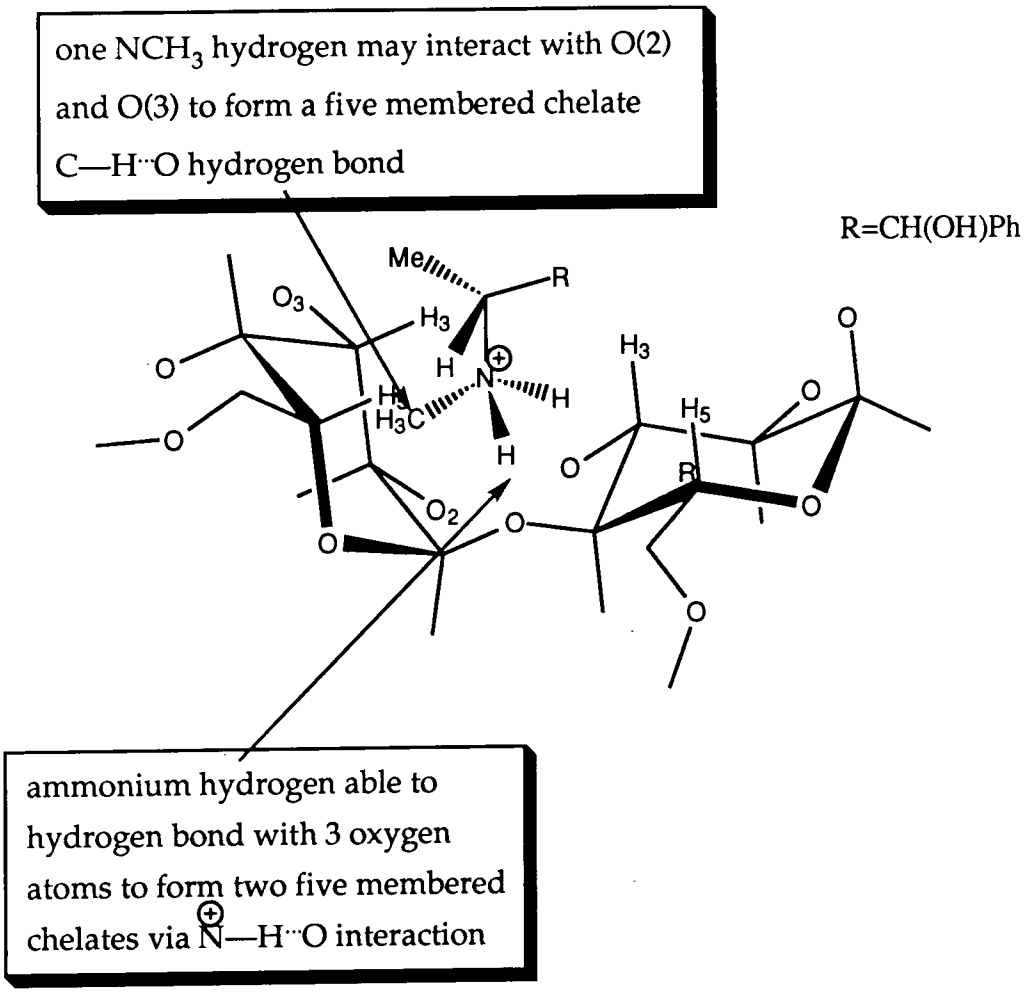



Figure 3.27 Proposed Enantioselective Binding of (+)-Ephedrine by α-Cyclodextrin

With the (-)-enantiomer of ephedrine, the 2S configuration orients the methyl group towards H(3) and between H(3) and H(5) (figure 3.28). This steric interaction may prevent close approach of the ammonium ion to the glycosidic region of the host thus inhibiting the hydrogen bonding interactions discussed above.

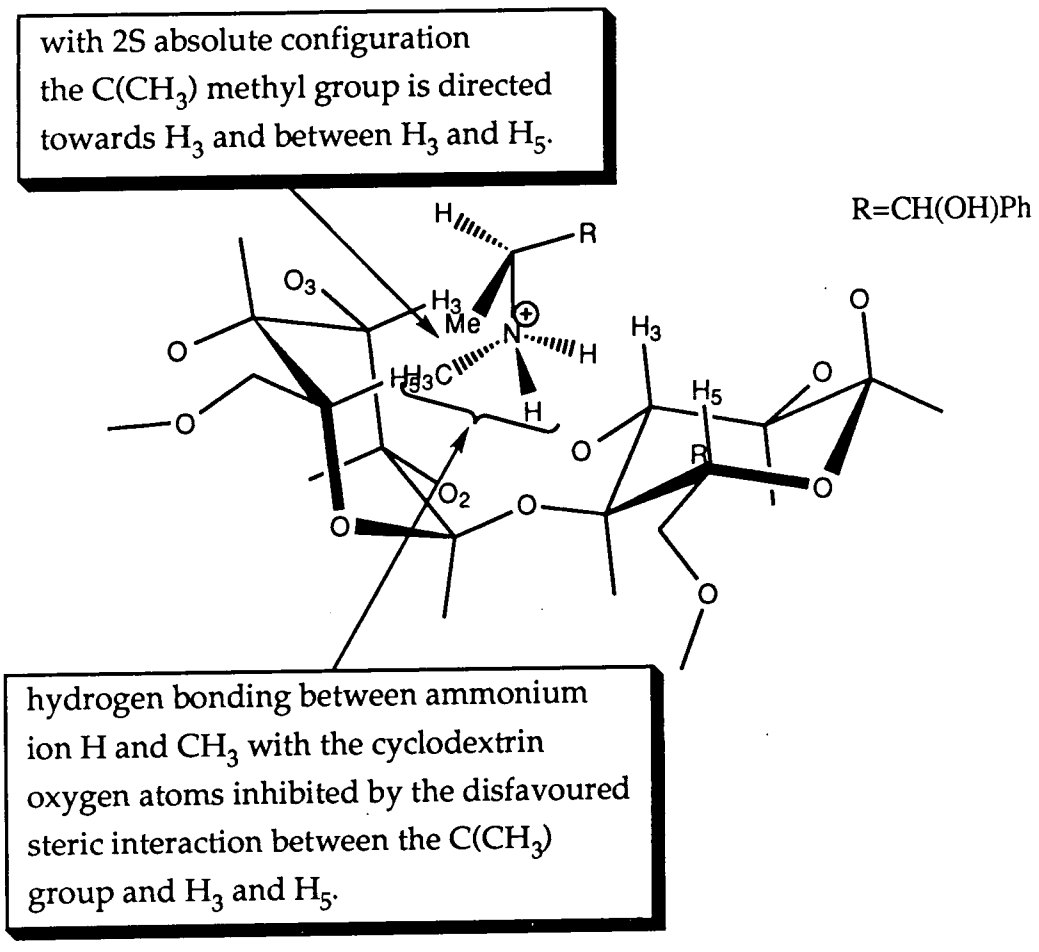



Figure 3.28 Proposed Interaction of (-)-Ephedrine with α-Cyclodextrin

Such a role for the C—Me methyl group is not unreasonable since proton $T_1$ investigations have suggested that this group is important in the enantiomer selection process. The significant increase in proton $T_1$ for this methyl group upon complexation of the (+) enantiomer (+0.04 secs.) may be associated with the fact that it would be directed into the hydrophobic cyclodextrin cavity.

Binding in this manner would require the phenyl ring to be included within the cavity yet inclined at an angle of about 20-30° to the mean z axis of the cavity, in a similar manner to that discussed for R mandelic acid in chapter one (section 1.9). This inclination of the phenyl ring, if absent for the (-) enantiomer, would also go some way to explaining the observed differences in the form of the phenyl $^1H$ NMR resonances for the two enantiomers upon complexation with the α-cyclodextrin host.

Although no definitive conclusions can be drawn about the mechanism of enantiomer discrimination for the ephedrinium - 'per'-O-octyl-α-cyclodextrin, the NMR and electrochemical evidence indicates that the mode of host-guest interaction proposed here is not unreasonable.

## REFERENCES


1) A. Horeau, J. P. Guette Tetrahedron (1974) 30 1923; J. Jurcak, A. Zamojskii Tetrahedron (1972) 28 1505.

2) V. Schurig, E. Gil-Av Isr. J. Chem. (1977) 15 96.

3) Y. Okamoto, K. Hatada J. Chromatogr. (1986) 363 173; Y. Okamoto, K. Hatada J. Chromatogr. (1987) 389 95.

4) W. H. Pirkle, T. C. Pochapsky J. Am. Chem. Soc. (1986) 108 352.

5) A. M. Stalcup, H. L. Jin, D. W. Armstrong J. Liq. Chromatogr. (1990) 13 473; T. J. Wood, D. W. Armstrong J. Liq. Chromatogr. (1986) 9 407.

6) D. W. Armstrong, A. M. Stalcup, M. L. Hilton, J. D. Duncan, J. R. Faulkner, S-C. Chang Anal. Chem. (1990) 62 1610.

7) V. Schurig, A. P. Nowotny Angew. Chem. Int. Ed. Engl. (1990) 29 939.

8) W. A. König, A. Kruger, D. Icheln, T. Runge J. High Resolut. Chromatogr. (1992) 15 184.

9) W. A. König, S, Lutz, C. Colberg, N. Schmidt, G. Wenz, E. von der Bey, A. Mosandl, C. Gunther, A. Kustermann J. High Resolut. Chromatogr. Chromatogr. Commun. (1988) 11 621.

10) D. J. Cram, J. L. Mateos J. Am. Chem. Soc. (1959) 81 5150.

11) P. L. Rinaldi Prog. Nucl. Magn. Reson. Spectrosc. (1982) 15 291.

12) *'Methods in Stereochemical Analysis'* T. C. Morrill (Ed.) VCH Publishers Inc. New York (1986) Volume 5.

13) W. H. Pirkle, D. J. Hoover Top. Stereochem. (1982) 13 263.

14) S. Yamaguchi in *'Asymmetric Synthesis'* J. D. Morrison (Ed.) Academic Press (1983) Volume 1, Chapter 7.

15) D. Parker Chem. Rev. (1991) 1441

16) W. Bussmann, J-M Lehn, U. Oesch, P. Plumere, W. Simon Helv. Chim. Acta. (1981) 64 657.

17) *'Ion Selective Electrodes'* E. Pungor (Ed.). Akademiai Kiado, Budapest (1989).

18) G. J. Moody, J. D. R. Thomas *'Selective Ion Sensitive Electrodes'* Merrow, Watford, Hertz. (1971).

19) T. Matsue Anal. Chem. (1986) 58 2096.

20) T. Matsue, U. Akiba, T. Osa, I. Uchida Stud. Org. Chem. (Amsterdam) (1987) 30 397.

21) R. Bergerson, R. Rowan Bioorg. Chem. (1976) 5 425; Y. Yamamota, Y. Kanda, Y. Inoue, R. Chujo, S. Kobayashi Chem. Letts. (1988) 495; Y. Inoue, T. Okuda, Y. Miyata, R. Chujo Carbohydr. Res. (1984) 125 65.

22) K. Harata, K. Uekama, M. Otagiri, F. Hirayama Bull. Chem. Soc. Jpn. (1987) 60 497.

23) L. Lehmann, E. Kleinpeter, J. Krechl J. Incl. Phenom. Molec. Recognit. Chem. (1991) 10 233; M. Komiyama, H. Hirai Chem. Letts. (1980) 1467 & 1471; R. J. Bergeron, M. A. Channing, G. J. Gibely, D. M. Piller J. Am. Chem. Soc. (1977) 99 5146.

24) For example: H. Dodziuk, J. Sitkowski, L. Stefanicik, J. Jureczak, D. Sybilska J. C. S. Chem. Commun. (1992) 207.

25) U. Edling, C. Holloway, G. C. Levy J. Am. Chem. Soc. (1976) 98 5069.

26) D. Doddrell, V. Glushko, A. Allerhand J. Chem. Phys. (1972) 56 3683.

27) J. Canceill, L. Jullien, L. Lacombe, J-M. Lehn Helv. Chim. Acta. (1992) 75 791.

28) J. P. Behr, J-M Lehn J. Am. Chem. Soc. (1976) 98 1743.

29) G. C. Levy, J. D. Cargioli, F. A. L. Anet. J. Am. Chem. Soc. (1973) 95 1527.

30) C. Brevard, J. P. Kintzinger, J-M. Lehn Tetrahedron (1972) 28 2447.

31) a)R. J. Bergeron, R. Rowan III Bioorg. Chem. (1976) 5 425.

b)Y. Inoue, Y. Takahashi, R. Chujo Carbohydr. Res. (1985) 144 c9-c11.

32) K. B. Lipkowitz, S. Raghothama, J. Yang J. Am. Chem. Soc. (1992) 114 1554.

33) D. A. Tomalia, A. M. Naylor, W. A. Goddard Angew. Chem. Int. Ed. Engl. (1990) 29 138.

34) J. Jeener, B. H. Meier, P. Bachmann, R. R. Ernst J. Chem. Phys. (1979) 71 4546.

35) A. A. Bothner-By in *'Biological Applications of Magnetic Resonance'* R. G. Shulman (Ed.) Academic Press, New York 1979 pp.177-219; A. Abragam *'The Principles of Nuclear Magnetism'* Clarendon Press, Oxford 1961.

36) H. J. Noggle, R. E. Schirmer *'The Nuclear Overhauser Effect'* Academic Press, New York, 1971 p.25.

37) A. Kalk, H. J. C. Berendson J. Magn. Reson. (1976) 24 343.

38) G. Wagner, K. J. Wuthrich J. Magn. Reson. (1979) 33 675.

39) S. L. Gordon, K. J. Wuthrich J. Am. Chem. Soc. (1978) 100 7094.

40) M. Watanabe, H. Nakamura, T. Matsuo Bull. Chem. Soc. Jpn. (1992) 65 164.

41) C. Jamie, J. Redondo, F. Sanchez-Ferrando, A. Virgili J. Org. Chem. (1990) 55 4773; C. Jamie, J. Redondo, F. Sanchez-Ferrando, A. Virgili J. Molec. Struct. (1991) 248 317.

42) A. A. Bothner-By, R. L. Stevens, J. Lee, C. D. Warren, R. W. Jeanloz J. Am. Chem. Soc. (1984) 106 811; A. Bax, D. G. Davis J. Magn. Reson. (1985) 63 207.

43) H. H. Limbach, J. Hennings J. Magn. Reson. (1982) 49 322.

44) L. Braunschweiler, R. R. Ernst J. Magn. Reson. (1983) 53 521.

45) M. Rance J. Magn. Reson. (1987) 68 337.

46) P. A. Bottomley, L. S. Smith, W. M. Leue, C. Charles J. Magn. Reson. (1985) 64 347.

47) W. Saka, Y. Yamamoto, Y. Inoue, R. Chujo, K. Takahashi, K. Hattor Bull. Chem. Soc. Jpn. (1990) 63 3175

48) B. Perly paper presented at *'Euchem Conference on Supramolecular Reactivity and Catalysis'* University of Padova (Italy), 8-13 September 1991

# CHAPTER FOUR

# SELECTIVE BINDING OF AMMONIUM IONS BY LIPOPHILIC, CHARGE NEUTRAL CYCLODEXTRIN DERIVATIVES

## 4.1 INTRODUCTION

The neuropharmacological properties of numerous quaternary ammonium compounds[1] such as acetyl choline have led many workers to investigate the selective binding of these ions[2]. There have been several reported examples of size selective binding of tetra-alkylammonium ions, $R_4N^+$ by macrocyclic and macropolycyclic molecules constructed from rigid non-polar subunits bearing polar functional groups[3]. Such macrocycles may function as water soluble molecular receptors capable of complexing substrate species by inclusion into their hydrophobic cavity. Their binding properties usually result from the synergistic operation of electrostatic interactions and of hydrophobic effects[4]. Since the rigid groups are usually aromatic residues these receptors belong, in most cases, to the cyclophane family and related structures[5]. The work of Schneider[6] has shown that there is a strong electrostatic interaction between the anionic cyclophane shown in figure 4.1 and 'onium ions such as $^+NMe_4$ and $^+NEt_4$.

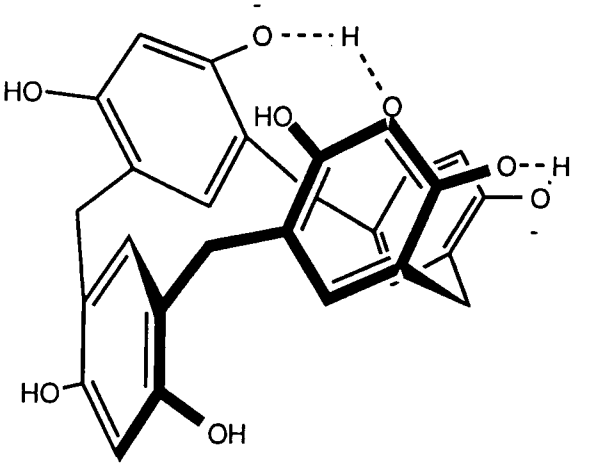



Figure 4.1 Schneider's anionic cyclophane host for 'onium ions.

Variation of pH (pD) indicated that for complexation to occur to any appreciable extent the cyclophane must be deprotonated. Such observations are indicative of a coulombic interaction between an anionic host and cationic guest being the major driving force for complex formation. Also the requirement for host deprotonation suggests that hydrophobic and ion-dipole interactions play a minor role in structuring of the complex. Table 4.1A shows the association constants that Schneider obtained from complexation-induced $^1H$ NMR shift titrations for various 'onium ions in the presence of his anionic cyclophane. These results indicate size selective binding of $^+NR_4$ in alkaline solution.

**Table 4.1A Size Selective Complexation of $^+NR_4$ by Schneider's anionic cyclophane**

| 'onium ion | proton | $\Delta\delta^a$ | K ($10^3$ mol$^{-1}$) | $\Delta G°$ (kcal mol$^{-1}$) |
|---|---|---|---|---|
| $^+NMe_4$ | $CH_3$ | 1.84 | 29±6 | 6.1 |
| $^+NEt_4$ | $CH_2$ | 1.18 | 3.3±0.5 | 4.8 |
| $^+NProp_4$ | 1-$CH_2$ | 0.42 | 0.032±0.005 | 2.0 |
| $^+NBu_4$ | all H | <0.01 | <0.002 | <0.5 |

a) From measurements in $D_2O$ (0.5M NaOD) at 298 ± 1 K.

Binding of such cationic species by essentially charge neutral ionophores has been restricted to systems involving electron rich aryl groups where there is a well defined ion-dipole interaction between the cationic 'onium ion and an aromatic π system[7]. The well documented binding of aralkylammonium ions (where charge is generally delocalised over the aryl framework) by electron rich crown ethers may also involve a certain degree of solvation of quaternary nitrogen alkyl groups by ether oxygens ($^+$N—C—H···O) although the dominant binding interaction in these cases involves edge - face and face - face π - stacking interactions[8].

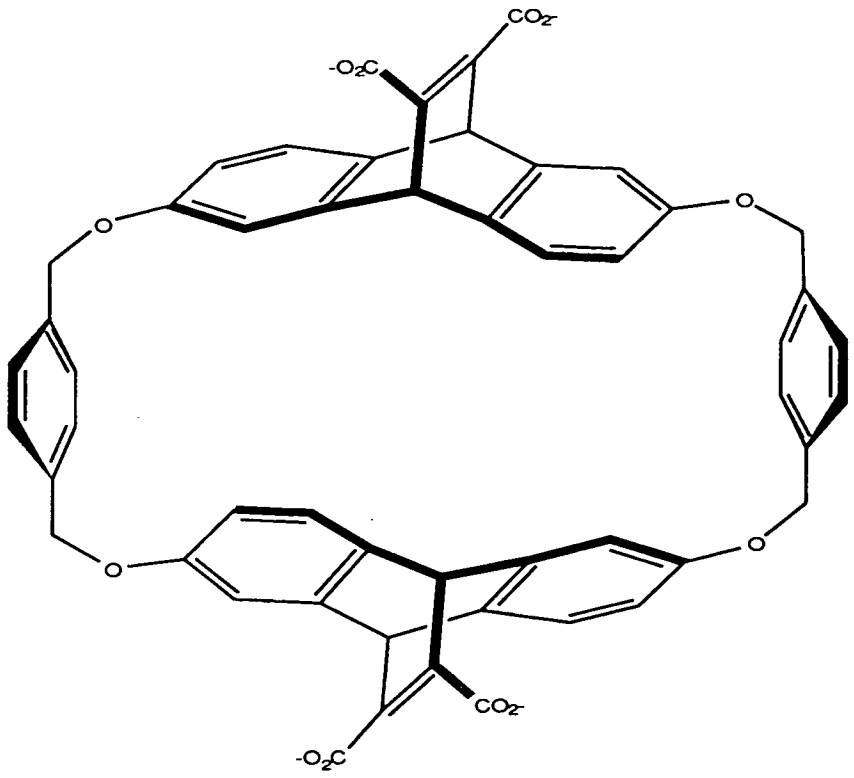



Figure 4.2 Dougherty's Anionic Cyclophane.

Dougherty found that the anionic macrocycle shown above was a general receptor for positively charged ammonium and immonium ion guests[9] such as those depicted in figure 4.3.

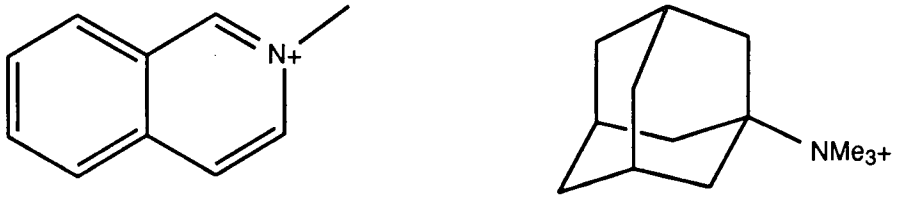



Figure 4.3 Immonium and ammonium ion guests for Dougherty's anionic cyclophane.

Comparison of the binding constants for these cationic guests with those of charge neutral guests such as quinoline indicated that positive charge solvation rather than hydrophobic effects were the discriminating factor[10]. Dougherty attributed the tight binding of the 'onium ions to an

ion-dipole effect in which the positive charge of the guest is 'solvated' by the electron rich aromatic rings of the host. Electrostatic interactions were considered unlikely because of the rigid structure of the host preventing close contact between the carboxylates and the guest charge upon inclusion. In addition, removal of the aromatic nature of the host was observed to substantially reduce the binding interaction, indicating that the role of the aromatic *p*-xylyl rings is crucial.

In this chapter the ability of the three cyclodextrin cavities ($\alpha$, $\beta$, $\gamma$) to act as 'size' selective pockets for the binding and detection of tetrahedral ammonium ions, as well as long chain surfactants will be investigated.

## 4.2 ELECTROCHEMICAL INVESTIGATION

The membrane preparation technique and the electrochemical cell arrangement used in this investigation are analagous to those described for the enantioselective investigation in chapter three. The electrode response to the different 'onium ion analytes investigated, in the absence and presence of interferent ions are given in tables 4.2A and 4.2B respectively.

**Table 4.2A Electrode Characteristics [slopes (mV per decade) with limits of detection ($10^{-n}$ mol $dm^{-3}$) in parentheses ] for 'Onium Ion Sensors[c] (310K, 0.001 mol $dm^{-3}$ $NH_4Cl$ inner filling solution)**

| | ELECTRODE | | | | | |
|---|---|---|---|---|---|---|
| | per-O-octyl-α-CD | | per-O-octyl-β-CD | | per-O-octyl-γ-CD | |
| Analyte | oNPOE | BBPA | oNPOE | BBPA | oNPOE | BBPA |
| $NH_4Cl$[a] | 57 (4.9) | — | No response | | No response | |
| $Me_2NH_2Cl$ | — | 2.0 | 52 (4.0) | 35 (3.4) | No response | |
| $Me_4NCl$[b] | — | 10 (1.9) | 61 (4.7)[d] | 48 (4.0) | 12.5 (2.0) | 2.5 (2.0) |
| $Et_4NCl$ | 22 (3.0) | — | 28 (3.6) | — | 42.0 (4.0) | 37.5 (2.9) |
| Acetyl Choline.Cl[e] | — | — | 60(5.0) | 52(3.1) | — | — |
| Choline[e] Chloride.Cl | — | — | 60(3.0) | No response. | — | — |
| MTMA.Br[f] | — | — | 58(6.5) | — | — | — |
| Dopamine.HCl | 61 (4.5) | 61 (5.4) | 60 (4.4) | — | — | — |

[a] The methyl capped analogue responded much less well.
[b] The methyl capped derivative responded equally well with similar slopes and limit of detection.
[c] A 'blank electrode' without any cyclodextrin added comprising *o*-NPOE/PVC/TKB with 0.01 mol $dm^{-3}$ $Me_4N^+Cl^-$ inner filing solution showed no response to $Me_4N^+Cl^-$.
[d] With 0.001 mol $dm^{-3}$ $Me_4N^+Cl^-$ as inner filing solution in place of $NH_4Cl$, the slope was 58mV with a limit of detection 10-5.7 mol $dm^{-3}$.
[e] inner filling solution was 0.01 mol $dm^{-3}$ of acetyl choline chloride of choline chloride.
[f] MTMA.Br = myristyltrimethylammonium bromide, $[C_{14}H_{29}NMe_3]^+Br^-$ (at concentrations below its c.m.c)

It is apparent from table 4.2A that per-O-octyl-α-cyclodextrin responds well to $NH_4^+$, although the results in table 4.2B indicate that there is severe interference from, in particular, potassium. The related β-cyclodextrin is a good ionophore for the tetramethylammonium cation showing a Nernstian response down to a concentration of $10^{-5}$ mol $dm^{-3}$, with good selectivity over $Mg^{2+}$, $Na^+$, $K^+$, $Ca^{2+}$ and $NH_4^+$ being observed.

An electrode prepared without the β-cyclodextrin ionophore present gave no response to the analyte indicating the active role of the cyclodextrin derivative as a chemical sensor. This finding is important because other workers[11], in particular Masadome[12] and coworker, have demonstrated that PVC membranes plasticized with *o*-NPOE may give a Nernstian response to long chain ($>C_{10}$) cationic and anionic surfactants and tetraalkyl ammonium ions. For example, a PVC membrane electrode plasticized with N,N-dimethyloleamide[13] showed a Nernstian response to the tetrabutylammonium cation down to a concentration of $10^{-5}$ mol $dm^{-3}$. In such cases ionic sites or ionic impurities in PVC or the plasticizer are considered to be the factors governing the response of the plasticized PVC membrane electrode[14]. For cationic surfactants variation of the plasticizer showed that the slope of the response was in accord with the dielectric constant of each plasticizer[12a]. With anionic surfactants it was demonstrated that cationic impurities in PVC rather than those in the plasticizer, whose chemical forms are not clear, may play a role in the response of the plasticized PVC membrane electrode to anionic surfactants[12a].

The methyl capping of residual hydroxyl groups in the cyclodextrin host gave no change in the response of the β-cyclodextrin host to the tetramethylammonium cation. This is in contrast to the response of the α-cyclodextrin based electrode to the ammonium cation which showed a greatly reduced response upon methyl capping. This difference in behaviour of the two host-guest systems suggests that different types of binding interaction are operative. In the case of the per-O-octyl-α-cyclodextrin-ammonium ion complex, the binding interaction may well involve $^{+}$N-H···O hydrogen bonds within the Lewis basic cyclodextrin cavity. Methyl capping of the residual hydroxyl groups could then lead to

a reduction in the binding constant through the removal of these hydroxyl groups. This would confine the interaction to $^{+}$N-H/ether oxygen hydrogen bonds with sterically inaccessible and conformationally restricted octyl ether oxygen atoms. Consequently, hydrogen bonding interactions may be at a greater distance so decreasing the extent of $^{+}$N charge solvation within the cavity and leading to a less favourable $\Delta G_{complex}$. Although this may go some way to explaining the reduced response upon methyl capping of the 'per'-O-octyl-α-cyclodextrin host, it is unlikely that it would lead to a reduction in the electrode reponse to the extent observed. This is because hydroxyl and MeO oxygens (and indeed ether oxygen atoms in general) have similar partial charges and hence will have similar hydrogen bond acceptor abilities. It is perhaps more likely that the effect of methyl capping is allied to conformational changes within the host facilitated by removal of the remaining O(2)···OH(3)' hydrogen bonding interactions. These conformational changes may then inhibit host - guest interaction. That is, changes in host conformation may have reduced the extent of host-guest complementarity (which will be relatively low initially because of the poor $^{+}NH_4$ to cavity size correlation).

In the case of the β-cyclodextrin-$Me_4N^{+}$ complex, interaction is expected to be via C-H···O hydrogen bonding[15] with perhaps both hydroxyl and ether oxygens. In this case the binding interactions may be more numerous because there is a good correlation between the size of the β-cycloextrin cavity and that of the 'onium ion guest. Each host-guest interaction would then contribute slightly to overall positive charge solvation but the extent of the overall host-guest interaction will not depend on a small number of strong binding interactions, as is likely to be the case in the α-cyclodextrin-$NH_4^{+}$ complex. Such C—H···O hydrogen

bonding involving carbohydrates has been extensively studied by neutron diffraction[16]. This is because there are abundant C—H donor and O acceptors in such systems. These interactions are primarily electrostatic in nature and therefore depend on the partial atomic charges within the carbohydrate, table 4.2C. For the $^{+}NMe_4$ ion MNDO calculations show a partial charge of +0.06 e units on each methyl hydrogen atom. The magnitude of these partial atomic charges indicates the possible significance of C—H···O interactions, from an electrostatic point of view, in cyclodextrin - 'onium ion guest association.

**Table 4.2C Magnitude of Partial Atomic Charges (in e units) in Carbohydrates as Estimated by ab initio Calculations[17]**

| Atom Type | Estimated Charge |
|---|---|
| O (both ring and hydroxyl) | -0.46 |
| anomeric C | 0.23 |
| all other C | -0.01 |
| hydroxyl H | 0.34 |
| H bonded to C | 0.13 |

Saenger has recently investigated the extent of intra- and inter- molecular C—H···O interactions for the β-cyclodextrin - ethanol/water complex by neutron diffraction studies[18]. In this investigation numerous intramolecular cyclodextrin C—H···O were evident with $d_{H...O} < 2.7$Å. Also C—H···O hydrogen bonds with $d_{H...O}$ as short as 2.39Å were observed for water molecules that cannot arrange in the preferred tetrahedral O—H···O hydrogen bonding coordination. Interestingly Saenger was also able to observe a C—H···O interaction between the methylene group of included ethanol and a glycosidic β-cyclodextrin oxygen with $d_{H...O}$ =2.63. Figure 4.4 highlights some of the more

important β-cyclodextrin ethanol octahydrate intermolecular C—H···O interaction at 15K.

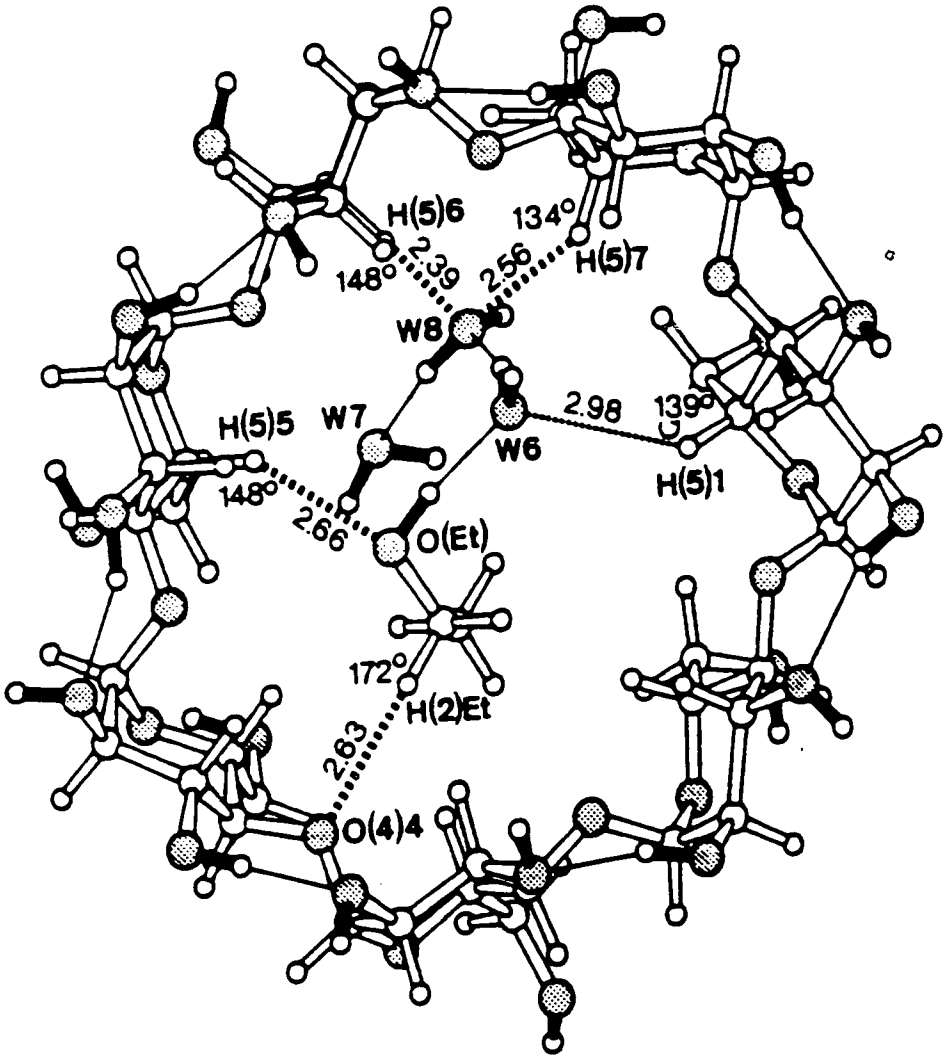



Figure 4.4 Host/Guest C—H···O Interactions in a Cyclodextrin Inclusion Complex: Crystal Structure of β-Cyclodextrin Ethanol Octahydrate at 15K; View is onto the equitorial plane of the macrocycle.

Comparing the response of the α-cyclodextrin and more especially the β-cyclodextrin based electrode to $NMe_4^+$ with that to $Me_2NH_2^+$, a reduced slope and limit of detection is evident for the dialkyl 'onium ion. This reduced response of the electrode may be associated with the lower point group symmetry of the dialkyl 'onium ion. This is because the binding interactions possible between the host and guest will now have a

dependence upon the orientation of the $Me_2NH_2^+$ ion as it approaches the cyclodextrin cavity. In this respect the $\Delta G_{complex}$ will have an orientation dependence in the case of the $Me_2NH_2^+$ ion, which is not the case for the highly symmetrical $NMe_4^+$ ion. Along with the effect of a reduction in symmetry, there will also be a size effect. The $^+NMe_2H_2$ ion is smaller than $^+NMe_4$ and, on the basis of size selective inclusion, may be expected to form a less stable complex with the β-cyclodextrin host. In other words the effect of changing the ionic radius and removing the essentially spherical nature of the guest is to lower the degree of binding site complementarity between the host and guest.

**Table 4.2B Selectivity Coefficients (-log $K^{pot}$, 0.1mol $dm^{-3}$ interferent, 310K) for Detection of Ammonium Ions**

| | | Interferent | | | | | |
|---|---|---|---|---|---|---|---|
| Electrode | Analyte* | $K^+$ | $Na^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $^+NH_4$ | Choline |
| Oct.α-CD /BBPA | dopamine | 1.5 | 1.8 | 3.3 | 3.2 | — | — |
| Oct.α-CD /oNPOE | $NH_4^+$ | 0.1 | 1.9 | — | — | — | — |
| Oct.β-CD /oNPOE | $NMe_4^+$ | 3.2 | 3.8 | 4.7 | 4.7 | 3.5 | — |
| Oct.β-CD /oNPOE | Acetyl Choline‡ | 3.5 | 4.2 | 4.5 | — | 3.2 | 1.8 |

* As chloride in MiliQ water.

‡ For choline chloride, using an electrode based on octβ-CD/*o*-NPOE, $^{-log}{}^{POT}_{clin}$ =3.4 ('clin' is a simulated backgroung of clinical ions (150mM $Na^+$; 4.3mM $K^+$; 1.26mM $Ca^{2+}$; 0.9mM $Mg^{2+}$) with acetyl choline as analyte under these conditions, $-\log K^{pot}_{clin}$ = 4.2 (60mV slope).

Table 4.2B shows that the per-O-octyl-β-cyclodextrin based electrode shows good selectivity for the $NMe_4^+$ ion in the presence of interferent group IA and IIA metal cations. Such selectivity is indicative of a high

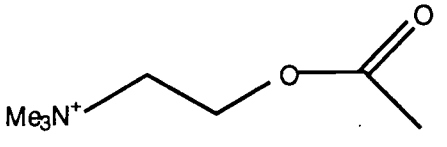
degree of complementarity between the two complexing species. This complementarity is manifested in a well defined electrochemical response and suggests that the $^{+}NMe_4$ ion, with an ionic radius of 3.5Å, is of a size similar to that of the β-cyclodextrin cavity whose diameter is approximately 7.8Å[19]. For the α-cyclodextrin - $NH_4^+$ complex severe interference is evident, particularly from potassium. This suggests that the complementarity between the α-cyclodextrin cavity and the $NH_4^+$ ion is low and that the inorganic metal ion $K^+$ even be more complementary to the size of the cavity and the arrangement of the oxygen binding sites. Indeed, $K^+$ (2.66Å) has a comparable (but slightly smaller) ionic diameter to that of $NH_4^+$ (2.86Å). Having said that however, it is very unlikely that the binding of $NH_4^+$ by α-cyclodextrin contains any element of size selectivity.

### 4.2.1 Detection of other achiral ammonium ions

In addition to the apparent size selectivity shown in the sensor towards $NH_4^+$, $NMe_4^+$ and $NEt_4^+$ other, $^{+}NR_3R'$ based, ammonium ions were found to give a good electrochemical response with, in particular, the per-O-octyl-β-cyclodextrin based chemical sensor. As table 4.2A shows, the β-cyclodextrin based sensor is able to detect the presence of the cationic surfactant myristyltrimethylammonium bromide, $[C_{14}H_{29}NMe_3]^+Br^-$, at concentrations below its critical micelle concentration (cmc). Other workers have previously[20] indicated that the cmc of such surfactants increases linearly with increasing cyclodextrin concentration in aqueous solution. This has been rationalised in terms of displacement of the micellation equilibrium as a consequence of cyclodextrin - sufactant complexation[20].

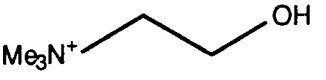
Several workers have investigated the ability of native cyclodextrins and their methylated derivatives to include cationic surfactants through conductometric[21], spectrophotometric[22], potentiometric[23] and calorimetric[24] studies (Section 1.11). These investigations have suggested that the association constant for a cyclodextrin-surfactant complex is largely dependent on the hydrophobicity of the surfactant in aqueous solution. In general the greater the hydrophobicity of the surfactant the higher the association constant, K, for the cyclodextrin complex. Despite this however, hydrophobic interactions are not solely responsible for the stability of these complexes because other interactions such as hydrogen bonding or van der Waals' forces may contribute to the overall complex stability[25]. In the present case, the per-O-octyl cyclodextrin derivative has not only a hydrophobic cavity but also hydrophobic peripheral octyl chains. It is perhaps reasonable to assume therefore that the cyclodextrin-surfactant interaction involves some alkyl chain - alkyl chain hydrophobic interaction[26], as well as inclusion within the cavity of part of the surfactant alkyl chain.

The response of the electrode based on 'per'-O-octyl-β-cyclodextrin towards the neurotransmitter acetyl choline was also evaluated.




.Cl⁻

Acetyl Choline Chloride



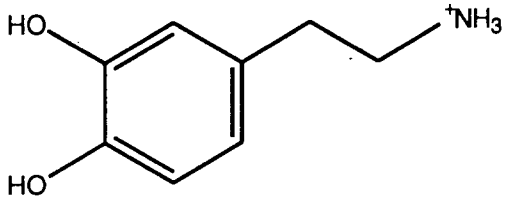

.Cl⁻

Choline Chloride

A Nernstian response was obtained down to 10μmol $dm^{-3}$ concentrations and interference from a simulated background of clinical ions (150mM $Na^+$; 4.3mM $K^+$; 1.26mM $Ca^{2+}$; 0.9mM $Mg^{2+}$) was minimal with an overall selectivity coefficient of $-\log K^{POT} = 4.2$ being observed.

Reasonable selectivity was also observed over the more hydrophilic analyte choline chloride (-log $K^{POT}$ =1.8) so that this sensor for acetyl choline could in principle be used to monitor esterase activity in biological systems[1].

The per-O-octyl-α-cyclodextrin ionophore is also able to detect the neurotransmitter dopamine, although there is more marked interference from potassium and sodium ions in this particular case.




Dopamine

## 4.3 INVESTIGATION OF SIZE SELECTIVE INCLUSION BY PROTON NMR

In order to investigate the inclusion of 'onium ions by highly lipophilic cyclodextrin derivatives using NMR methods, 'onium ion salts soluble in d-chloroform were obtained. In the case of $^{+}NMe_4$ the trifluoroacetate was prepared from tetramethylammonium hydroxide and excess trifluoroacetic acid followed by drying under reduced pressure. For $^{+}NEt_4$ the commercially available hexafluorophosphate was used. Figure 4.5 shows the 250MHz $^1H$ NMR spectra for the 'per'-O-octyl-b-cyclodextrin ionophore (90μM) with both $NMe_4^+$ and $NEt_4^+$ (2 molar equivalents) in d-chloroform. These spectra are representitive of all those for the 'onium ion - cyclodextrin complexes under investigation here.

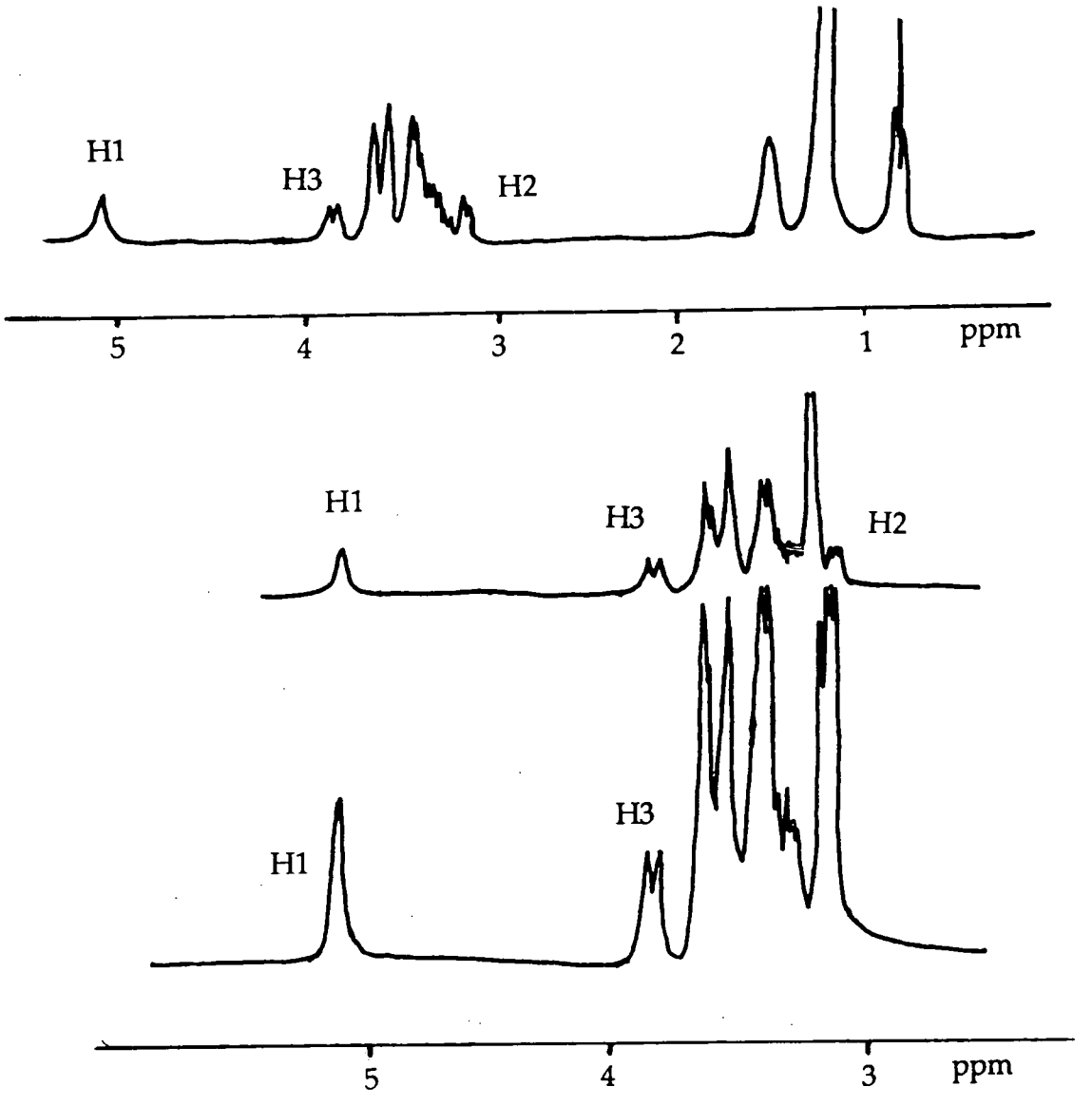



Figure 4.5 250MHz $^1H$ NMR Spectra for the Per-O-octyl-β-cyclodextrin Ionophore (90μM) (upper) and in the presence of both $NMe_4^+$ (middle) and $NEt_4^+$ (lower) (2 molar equivalents) in d-chloroform solution at 20°C.

It is apparent from these spectra that no chemical shift change for the cyclodextrins' resonances can be observed in the presence of the 'onium ion guest. This is suggestive of weak, non specific host-guest interaction and it may indicate that the driving force of complexation is hydrophobic in nature[27]. Weak methyl hydrogen to cyclodextrin oxygen hydrogen bonding may lead to solvation of the positively charged nitrogen centre (as discussed earlier in relation to the effect of methyl capping of the host).

## 4.4 APPLICATION OF MASS SPECTROMETRY

In chapter three it was shown that ES-MS can be applied successfully to the investigation of host-guest interactions and indeed to the determination of guest selectivity for, in that case, the ephedrinium cation. Here the application of ES-MS to the characterisation of 'onium ion complex formation with per-O-octyl cyclodextrin hosts is demonstrated. The ability of this mass spectral technique to show selectivity of the host for a given 'onium ion is also investigated.

Along with the distribution of octylation products, the mass spectral characterisation of per-O-octyl-α-cyclodextrin in chapter two suggested its ability to associate with $NH_4^+$ cations. Likewise the β-cyclodextrin analogue, although having a different octylation pattern, is able to form $[M+NH_4^+]$ ions. Both the α- and β- cyclodextrin derivatives are able to form $[M+2NH_4]^{2+}$ doubly charged ions. This interaction is unlikely to be size selective and merely reflects hydrogen - bonding of an indeterminate nature between the ammonium ion and the electron rich cyclodextrin cavity.

More informative results are obtained when the 'electrochemically successful' per-O-octyl-β-cyclodextrin-$NMe_4^+$ complex is investigated by ES-MS, figure 4.6. Injection of a solution containing 0.5 ng/μl per-O-octyl-β-cyclodextrin and 0.2mM $NMe_4^+$ in isopropanol results in $[M+NMe_4]^+$ ions being exclusively observed. No $[M+2NMe_4]^{2+}$ ions are evident in this case which may be related the inability of the β-cyclodextrin cavity to accommodate more than one guest ion. This observation, along with the earlier electrochemical observations, suggests that there may well be a good guest size to cavity size correlation for the β-cyclodextrin - $NMe_4^+$ complex.

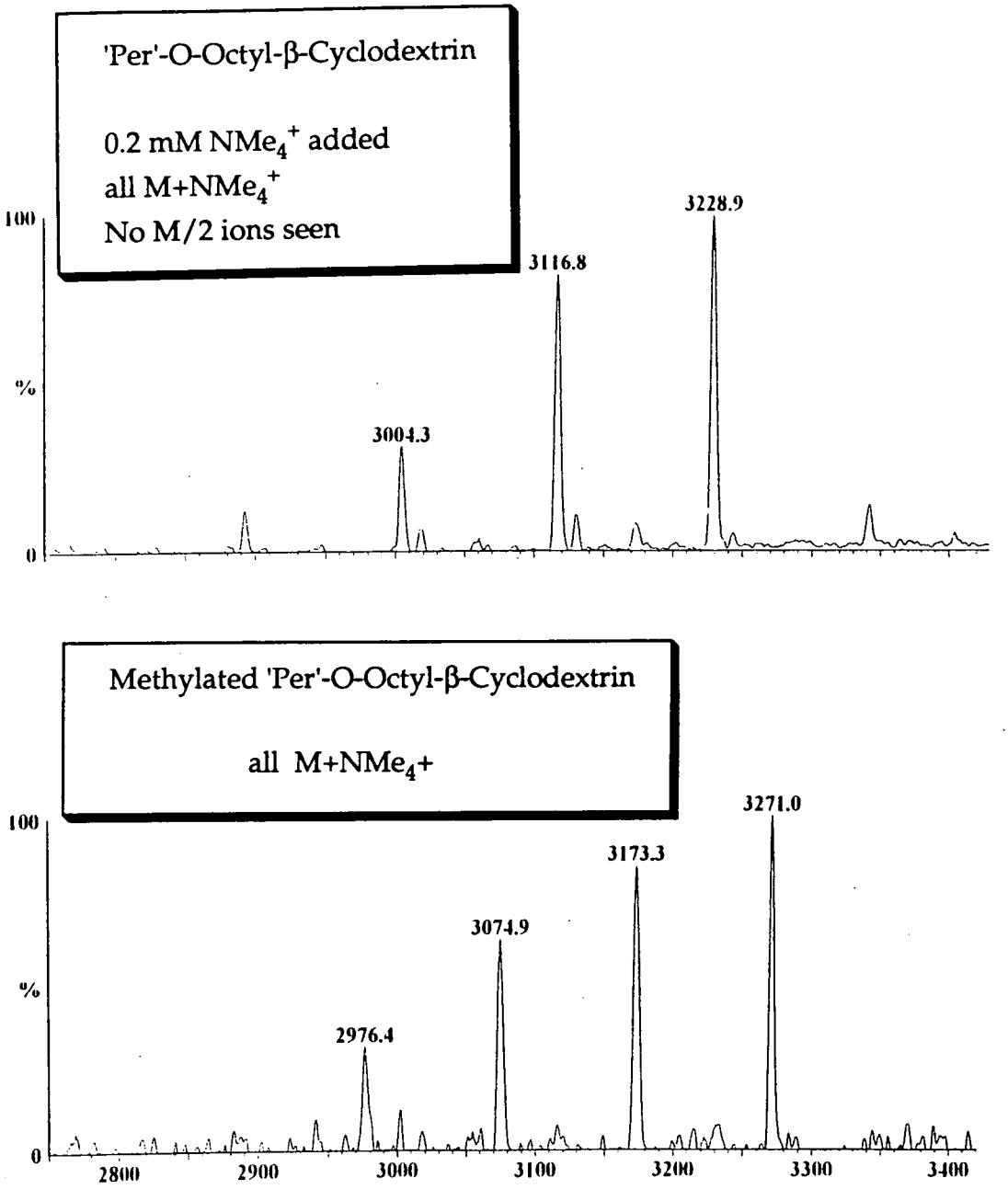



Figure 4.6 Adduct formation between 'per'-O-octyl-β-cyclodextrin and $NMe_4^+$, and between methylated 'per'-O-octyl-β-cyclodextrin and $NMe_4^+$

Methylation of residual hydroxyl groups in per-O-octyl-β-cyclodextrin was not observed to affect the host-guest selectivity electrochemically and this phenomenon was also observed by mass spectrometry. Figure 4.7 shows the ES-MS spectrum for methylated per-O-octyl-β-cyclodextrin with both $NH_4^+$ (10mM) and $NMe_4^+$ (0.1mM) ions present in the injected sample.

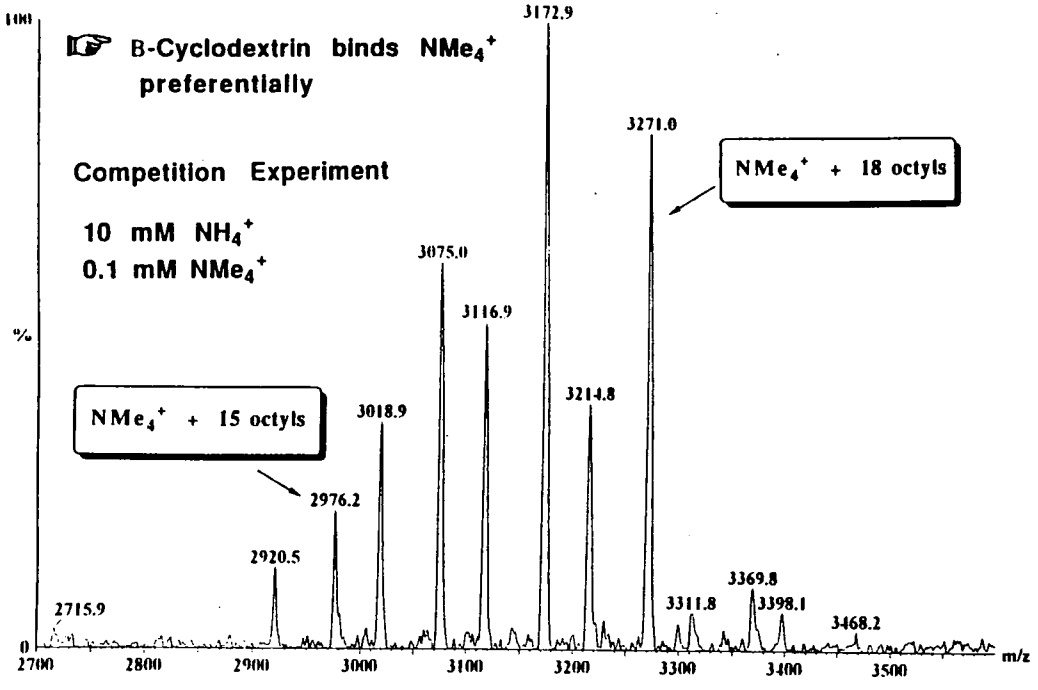



Figure 4.7 ES-MS spectrum for methylated per-O-octyl-β-cyclodextrin with both $NH_4^+$ (10mM) and $NMe_4^+$ (0.1mM) ions present in the injected sample.

This mass spectral investigation clearly demonstrates good selectivity for the tetramethylammonium ion in the presence of competing ammonium ions under the conditions of ES-MS. Assuming equivalent ionisation potentials for the two 1:1 complexes, which is not unreasonable as the discussion in chapter three indicated, then the selectivity of the ionophore towards $NMe_4^+$ can be estimated to be of order of 200:1, by comparison of peak heights.

The selective detection of the cationic surfactant $C_{14}H_{29}NMe_3^+$ by the per-O-octyl-β-cyclodextrin and methylated per-O-octyl-β-cyclodextrin has already been discussed in the previous section in relation to the electrochemical investigation of 'onium ion complex formation. Figure 4.8 shows that complexation between this surfactant and methylated per-O-octyl-β-cyclodextrin can be observed by mass spectrometry and

demonstrates 1:1 adduct formation with no doubly charged ions being evident, as was the case with the $NMe_4^+$ complex. In this case peaks at 3256.7 (3257.1) [16 octyls, 5 methyls + $C_{17}H_{38}N^+$], 3355.3 (3355.3) [17 octyls, 4 methyls + $C_{17}H_{38}N^+$] and 3453.7 (3453.7) [18 octyls,3 methyls + $C_{17}H_{38}N^+$] were quite distinct.

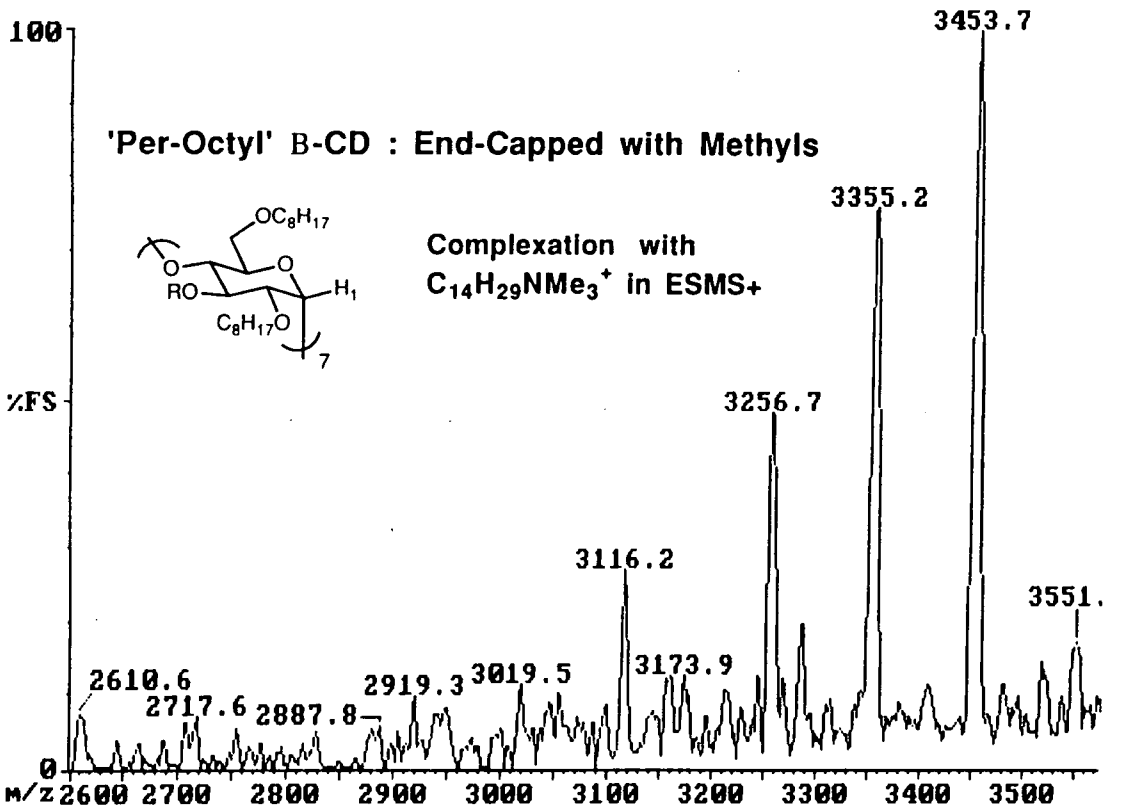



Figure 4.8 Adduct Formation Between 'Per'-O-Octyl-β-Cyclodextrin and $C_{14}H_{29}NMe_3^+$ under the conditions of ES-MS

With 'per'-O-octyl-β-cyclodextrin and acetyl choline 1:1 adduct formation was also indicated with peaks at 3077.3 (3076.7), 3189.0 (3188.9), 3301.5 (3301.1) and 3413.3 (3413.3) due to the 16, 17, 18 and 19-octylated complexes respectively being observed. Spectra due to complex with choline were weaker but discernible at the expected masses.

## 4.5 PROTON LONGITUDINAL RELAXATION TIMES, $T_1$

In the preceding chapter the ability of longitudinal relaxation time, $T_1$, determinations to aid the investigation of enantioselective binding by a cyclodextrin host was demonstrated. Here proton $T_1$ acquisition is used to investigate the electrochemically evident selective binding of 'onium cations by cyclodextrin based ionophores. Proton $T_1$'s were acquired for the cyclodextrin host, 'onium ion guest and the host-guest complex at 250 MHz by the inversion recovery method[28]:

$$180°—\tau—90°—\text{aquisition}$$

with $\tau$ = 0.1 to 3.5 seconds and a recycle delay of 10 seconds. Paramagnetic oxygen was excluded from all samples by a repetitive 'freeze-evacuate-thaw' process under an argon atmosphere. All $T_1$ determinations were made in 0.7 $cm^3$ of (9:1) d-chloroform/$d^3$-acetonitrile with 10μmol cyclodextrin and/or 2.5 molar equivalents of the 'onium ion salt.

### 4.5.1 Changes in 'onium ion $T_1$'s upon complexation

Table 4.5.1A summarises the results obtained for $^+NMe_4$ in the presence and absence of the three per-O-octyl cyclodextrin hosts.

**Table 4.5.1A Proton Longitudinal Relaxation Times for $NMe_4^+$ in the presence of 'per'-O-octyl-cyclodextrin hosts**

| guest/host[a] | $\delta_{1H}$[b] | $T_1$ (s)[c] | S.D. (±) | $\Delta T_1$ (s) |
|---|---|---|---|---|
| $NMe_4^+$ | 3.26 | 1.273 | 0.014 | — |
| $NMe_4^+$/α-CD | 3.24 | 1.120 | 0.008 | -0.153 |
| $NMe_4^+$/β-CD | 3.24 | 1.067 | 0.009 | -0.206 |
| $NMe_4^+$/γ-CD | 3.22 | 1.413 | 0.004 | +0.140 |

[a] 90μM of host and 2.5 molar equivalents of $^+NMe_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.

Upon complexation with the cyclodextrin host the rate of molecular tumbling for the 'onium ion guest is expected to decrease slightly as a result of host-guest binding interactions leading to an increase in the effective volume. This will result in an increase in $\tau_c$, the reorientational correlation time, for the guest. Hence a slight reduction in the $T_1$ value for the guest protons may be expected, assuming the condition of motional narrowing is applicable ($\omega_o\tau_c$«1.12). Considering the $NMe_4^+$ results, there is a reduction in $T_1$ ($\Delta T_1$) for the $NMe_4^+$ protons with both the α- and β- cyclodextrin hosts. However, the greater reduction with the β- host is suggestive of the intervention of host-guest interactions giving an additional dipolar relaxation pathway. Such an interaction could take the form of $^+N—C—H\cdots O$ hydrogen bonding between the 'onium ion methyl groups and the ether oxygen atoms of the cyclodextrin within the Lewis basic cavity, as discussed in section 4.2.

The increase in $T_1$ for the $NMe_4^+$ protons with γ-cyclodextrin present as the potential host is initially suprising, indicating a lower $\tau_c$ upon complexation with the host. In order to investigate this further $^{19}F$ $T_1$'s were determined for the $CF_3CO_2^-$ counter ion for each complex and the free ammonium salt in (9:1) d-chloroform/$d^3$-acetonitrile solution. As table 4.5.1B indicates, this fluorine relaxation time increases substantially in the presence of the α- and β- cyclodextrin hosts but decreases slightly in with the γ-cyclodextrin host present. Such changes in $^{19}F$ $T_1$ indicate that, in the case of the γ-CD host, the trifluoroacetate counter ion could be preferentially included within the cavity. This may then result in the $NMe_4^+$ cation experiencing a 'more symmetrical' environment in solution, allowing an increase in the rate of molecular motions as a result of charge separation. Such an increase in molecular mobility could then be associated with an increase in proton $T_1$ as a consequence of a

reduction in the reorientational correlation time. In the presence of the α- and β- cyclodextrin the reverse is true; the $NMe_4^+$ is bound within the cavity and the counter ion experiences a 'more symmetrical' environment in the bulk solution.

**Table 4.5.1B $^{19}F$ Longitudinal Relaxation Times for $CF_3CO_2^-$ in the presence of per-O-octyl-cyclodextrin hosts**

| guest/host[a] | $\delta_{19F}$[b] | $T_1$(s)[c] | S.D. (±) | $\Delta T_1$ |
|---|---|---|---|---|
| $CF_3CO_2^-$ | -119.5 | 1.45 | 0.003 | — |
| $CF_3CO_2^-$/α-CD | -119.6 | 1.85 | 0.003 | +0.40 |
| $CF_3CO_2^-$/β-CD | -119.7 | 1.91 | 0.004 | +0.46 |
| $CF_3CO_2^-$/γ-CD | -119.6 | 1.39 | 0.006 | -0.06 |

[a] 90μM of host and 2.5 molar equivalents of $^+NMe_4$ (approx 6.5 mg).
b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.

As suggested earlier, the affect of complexation upon the $NMe_4^+$ proton $T_1$'s may be related to the change in the effective volume of the $NMe_4^+$ ion upon complexation, since $\tau_c$ is dependent upon the volume of the molecule[29]:

$$\tau_c = \frac{4\pi\eta a^3}{3kT} \qquad (8)$$

Taking the gross assumption that both the hosts and guest involved in the complexation are spherical then approximate volumes can be obtained for the cyclodextrin derivative and the tetramethyl ammonium ion in the free state. This equation might also be applicable to the analysis of their molecular motions in the complex[30]. For the free guest a=3.5Å and for the complex, a≈25Å. This corresponds to a volume change by a factor of approximately 400 for the guest upon complexation

within the cyclodextrin cavity. This simple calculation suggests that complexation with a cyclodextrin host may be expected to increase the reorientational correlation time, $\tau_c$. Such increases in $\tau_c$ for a guest and cyclodextrin host upon complex formation were observed by Suzuki *et al* in their $^{13}C$ $T_1$ investigation of cyclodextrin - azo dye complexes[31]. Their investigation indicated that $\tau_c$ increases by a factor of about 1.5 for several methyl orange derivatives on complexation with 'per'-O-methylated-β-cyclodextrin.

The effects of the presence of each of per-O-octyl cyclodextrins on proton longitudinal relaxation times for $^{+}NEt_4$ are summarised in table 4.5.1C.

**Table 4.5.1C Longitudinal Relaxation Times for $NEt_4^+$ in the presence of per-O-octyl-cyclodextrin hosts**

| guest/host[a] | $\delta_{^1H}$[b] | $T_1$(s)[c] | S.D. (±) | $\Delta T_1$(s) |
|---|---|---|---|---|
| $NEt_4^+$ | 3.17 | 1.122 | 0.009 | — |
| $NEt_4^+$/α-CD | 3.17 | 1.595 | 0.009 | +0.473 |
| $NEt_4^+$/β-CD | 3.17 | 1.680 | 0.023 | +0.558 |
| $NEt_4^+$/γ-CD | 3.16 | 1.142 | 0.024 | +0.020 |

[a] 90μM of host and 2.5 molar equivalents of $^{+}NMe_4$ (approx 6.5 mg).
b] in 9:1 d-chloroform/$d^3$-acetonitrile, for the $CH_2$ resonance.
[c] mean of two independent determinations.

Considering the longitudinal relaxation times for $NEt_4^+$ present as guest, both α- and β- cyclodextrin hosts result in an increase in the $T_1$ for the $NEt_4^+$ methylene hydrogens. With per-O-octyl-γ-cyclodextrin as host no change in $T_1$ is observed,within the error of the determination.

### 4.5.2 Changes in Cyclodextrin Host $T_1$'s upon Complex Formation

For all the cyclodextrin-'onium ion complexes, the $T_1$'s of the free and bound host were also determined in an effort to investigate further the nature of any host-guest interactions. These $T_1$ determinations are discussed below. Throughout the discussion isotropic molecular reorientation is assumed in order to allow analysis of the changes in $T_1$ upon complex formation. However, the work of Lehn discussed in chapter three (section 3.6.5) indicates that the motions of a cyclodextrin derivative may well be anisotropic as a result of preferential rotation about the $C_n$ (n=6,7,8 for α,β,γ respectively) symmetry axis.

**Table 4.5.2A Longitudinal Relaxation Times for per-O-octyl-α-cyclodextrin in the presence of $NMe_4^+$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$ (proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_{1(s)}$ | S.D. (±) | $\Delta T_{1(s)}$ |
| 4.87 (H1) | 0.355 | 0.007 | 0.351 | 0.006 | -0.004 |
| 4.00 (H3) | 0.255 | 0.009 | 0.258 | 0.003 | +0.003 |
| 3.87 (H5) | 0.296 | 0.017 | 0.292 | 0.003 | -0.004 |
| 3.57 ($CH_2O$)‡ | 0.250 | 0.006 | 0.274 | 0.009 | **+0.024** |
| 3.47‡ | 0.278 | 0.003 | — | — | — |
| 3.37‡ | 0.297 | 0.023 | 0.300 | 0.007 | +0.003 |
| 3.20 (H2) | 0.386 | 0.003 | — | — | — |
| 1.51 ($\underline{CH_2}CH_2O$) | 0.336 | 0.004 | 0.369 | 0.008 | **+0.033** |
| 1.19 $(CH_2)_5$ | 0.721 | 0.010 | 0.851 | 0.002 | **+0.130** |
| 0.80 ($CH_3$) | 1.736 | 0.003 | 1.933 | 0.008 | **+0.197** |

[a] 90μM of host and 2.5 molar equivalents of $^+NMe_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.
‡indicates assignment unclear from the complex spectrum.

**Table 4.5.2B Longitudinal Relaxation Times for per-O-octyl-β-cyclodextrin in the presence of $NMe_4^{+}$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$ (proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_1$(s) | S.D. (±) | $\Delta T_{1(s)}$ |
| 5.09 (H1) | 0.357 | 0.005 | 0.362 | 0.006 | +0.005 |
| 3.87 (H3) | 0.132 | 0.080 | 0.202 | 0.008 | **+0.070** |
| 3.63(H5)‡ | 0.309 | 0.028 | 0.384 | 0.003 | **+0.075** |
| 3.57 ($CH_2O$)‡ | 0.418 | 0.005 | 0.481 | 0.012 | **+0.063** |
| 3.43‡ | 0.248 | 0.021 | 0.318 | 0.009 | +0.070 |
| 3.15 (H2) | 0.348 | 0.006 | 0.505 | 0.003 | **+0.157** |
| 1.51 ($\underline{CH_2}CH_2O$) | 0.296 | 0.023 | 0.374 | 0.003 | **+0.078** |
| 1.19 $(CH_2)_5$ | 0.772 | 0.006 | 0.836 | 0.009 | **+0.064** |
| 0.80 ($CH_3$) | 1.713 | 0.003 | 1.855 | 0.007 | **+0.142** |

[a] 90μM of host and 2.5 molar equivalents of $^{+}NMe_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.
‡indicates assignment unclear from the complex spectrum.

**Table 4.5.2C Longitudinal Relaxation Times for per-O-octyl-γ-cyclodextrin in the presence of $NMe_4^{+}$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$ (proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_{1(s)}$ | S.D. (±) | $\Delta T_{1(s)}$ |
| 5.13 (H1) | 0.378 | 0.003 | 0.381 | 0.002 | +0.003 |
| 3.85 (H3) | 0.220 | 0.005 | 0.248 | 0.009 | +0.028 |
| 3.62‡ | 0.398 | 0.002 | 0.405 | 0.003 | +0.007 |
| 3.55 ($CH_2O$) | 0.507 | 0.005 | 0.511 | 0.009 | **+0.004** |
| 3.40‡ | 0.333 | 0.009 | 0.340 | 0.008 | +0.007 |
| 3.32‡ | 0.305 | 0.002 | 0.331 | 0.009 | +0.026 |
| 3.12 (H2) | 0.462 | 0.009 | 0.491 | 0.003 | +0.029 |
| 1.49 ($\underline{CH_2}CH_2O$) | 0.360 | 0.002 | 0.368 | 0.003 | **+0.008** |
| 1.19 $(CH_2)_5$ | 0.773 | 0.007 | 0.792 | 0.007 | **+0.019** |
| 0.79 ($CH_3$) | 1.841 | 0.015 | 1.845 | 0.007 | **+0.004** |

[a] 90μM of host and 2.5 molar equivalents of $^{+}NMe_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.
‡indicates assignment unclear from the complex spectrum.

Comparison of the $\Delta T_1$ values for the three per-O-octyl cyclodextrin hosts with tetramethylammonium trifluoroacetate present (tables 4.5.2A, B and C) shows marked differences for the three host molecules.

In the case of the β-cyclodextrin host significant increases in $T_1$ are apparent for all assignable proton resonances except that of H(1). For example, there is a 50% increase in the $T_1$ observed for H(3). This perhaps suggests that complexation with $^{+}NMe_4$ brings about conformational changes within the host which lead to a reduction in the rate of molecular tumbling and a corresponding increase in $\tau_c$ leading to an

increase in the $T_1$'s for the host protons. This analysis assumes that the motions of the host are such that its NMR behaviour is away from the motional narrowing region. Such conformational changes leading to a reduction in the rate of molecular tumbling may be associated with reorientation of the octyl chains. In the free host the hydrophobic octyl chains may be partially included within the cyclodextrin cavity, with one inside the hydrophobic cyclodextrin cavity at any given time. These alkyl chains may also be associated with one another through hydrophobic interactions, with their relative dispositions being dependent upon the relative orientations of the individual glucose subunits of the cyclodextrin. Upon complexation with $^{+}NMe_4$ the 'onium ion may be envisaged as being included within the cavity with C-H···O hydrogen bonding as the major stabalizing interactions. In this case, the octyl chains will be prevented from penetrating the cavity to any appreciable extent. Furthermore, a 'well-defined' complex could bring about conformation changes within the host in order to facilitate closer, and perhaps more numerous, stabilising interactions with the 'onium ion guest. Such exclusion of the octyl chains from the cavity and conformational reorganisation of the host, as a result of binding, may extend the overall average length of the many octyl chains. This would increase the volume of the molecule and give a reduction in the rate of molecular reorientation leading to an increase in $T_1$. A more likely explaination of the observed increase in host $T_1$'s is the formation of a 'well-defined', structured complex with the 'onium ion guest leading to a more rigid structure. Such rigidity may lead to a reduction in the 'local' mobility around each hydrogen nucleus leading to an increase in $\tau_\theta$, the local reorientational correlation time and an associated increase in $T_1$.

In the case of both the α– and γ– cyclodextrins, the only apparent significant changes in $T_1$ upon complexation appear to be those of the octyl chain protons. This is probably associated with competitive inclusion of the 'onium ion guest (or its counter ion) and resulting changes in conformation as discussed above for the β-cyclodextrin host. That is, the octyl chains are reordering and going to a more mobile average position upon complexation.

**Table 4.5.2D Longitudinal Relaxation Times for per-O-octyl-α-cyclodextrin in the presence of $NEt_4^+$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$(proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_{1(s)}$ | S.D. (±) | $\Delta T_{1(s)}$ |
| 4.87 (H1) | 0.355 | 0.007 | 0.358 | 0.007 | +0.003 |
| 4.00 (H3) | 0.255 | 0.009 | 0.260 | 0.020 | +0.005 |
| 3.87 (H5) | 0.296 | 0.017 | 0.292 | 0.019 | -0.004 |
| 3.57 ($CH_2O$) | 0.250 | 0.006 | 0.299 | 0.033 | **+0.049** |
| 3.47 | 0.278 | 0.003 | 0.284 | 0.005 | +0.006 |
| 3.37 | 0.297 | 0.023 | 0.299 | 0.003 | +0.002 |
| 3.20 (H2)* | 0.386 | 0.003 | - | - | - |
| 1.51 ($\underline{CH_2}CH_2O$) | 0.336 | 0.004 | 0.349 | 0.002 | **+0.013** |
| 1.19 $(CH_2)_5$ | 0.721 | 0.010 | 0.791 | 0.002 | **+0.070** |
| 0.80 ($CH_3$) | 1.736 | 0.003 | 2.353 | 0.016 | **+0.617** |

* indicates obscured by $NEt_4^+$ resonances.

[a] 90μM of host and 2.5 molar equivalents of $^+NEt_4$ (approx 6.5 mg).

b] in 9:1 d-chloroform/$d^3$-acetonitrile.

[c] mean of two independent determinations.

**Table 4.5.2E Longitudinal Relaxation Times for per-O-octyl-β-cyclodextrin in the presence of $NEt_4^+$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$ (proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_1$(s) | S.D. (±) | $\Delta T_{1(s)}$ |
| 5.09 (H1) | 0.357 | 0.005 | 0.352 | 0.007 | -0.005 |
| 3.87 (H3) | 0.132 | 0.080 | 0.186 | 0.007 | **+0.054** |
| 3.63(H5)‡ | 0.309 | 0.028 | 0.362 | 0.003 | **+0.053** |
| 3.57 ($CH_2O$)‡ | 0.418 | 0.005 | 0.468 | 0.024 | **+0.050** |
| 3.43‡ | 0.248 | 0.021 | 0.301 | 0.008 | +0.053 |
| 3.15 (H2)* | 0.348 | 0.006 | - | - | - |
| 1.51 ($\underline{CH_2}CH_2O$) | 0.296 | 0.023 | 0.366 | 0.002 | **+0.072** |
| 1.19 $(CH_2)_5$ | 0.772 | 0.006 | 0.817 | 0.005 | **+0.045** |
| 0.80 ($CH_3$) | 1.713 | 0.003 | 2.080 | 0.020 | **+0.367** |

* indicates obscured by $NEt_4^+$ resonances.
[a] 90μM of host and 2.5 molar equivalents of $^+NEt_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.
‡indicates assignment unclear from the complex spectrum.

**Table 4.5.2F Longitudinal Relaxation Times for per-O-octyl-γ-cyclodextrin in the presence of $NEt_4^+$[a]**

| | Free | | Complexed | | |
|---|---|---|---|---|---|
| $\delta_{1H}$(proton)[b] | $T_{1(s)}$[c] | S.D. (±) | $T_1$(s) | S.D. (±) | $\Delta T_{1(s)}$ |
| 5.13 (H1) | 0.378 | 0.003 | 0.373 | 0.007 | -0.005 |
| 3.85 (H3) | 0.220 | 0.005 | 0.212 | 0.006 | -0.008 |
| 3.62‡ | 0.398 | 0.002 | 0.407 | 0.003 | +0.009 |
| 3.55 ($CH_2O$) | 0.507 | 0.005 | 0.510 | 0.008 | **+0.003** |
| 3.40‡ | 0.333 | 0.009 | 0.334 | 0.008 | +0.001 |
| 3.32‡ | 0.305 | 0.002 | 0.303 | 0.005 | -0.002 |
| 3.12 (H2)* | 0.462 | 0.009 | - | - | - |
| 1.49 ($\underline{CH_2}CH_2O$) | 0.360 | 0.002 | 0.369 | 0.002 | **+0.009** |
| 1.19 $(CH_2)_5$ | 0.773 | 0.007 | 0.843 | 0.005 | **+0.070** |
| 0.79 ($CH_3$) | 1.841 | 0.015 | 2.262 | 0.014 | **+0.421** |

* indicates obscured by $NEt_4^+$ resonances.
[a] 25mg of host and 2.5 molar equivalents of $^+NEt_4$ (approx 6.5 mg).
[b] in 9:1 d-chloroform/$d^3$-acetonitrile.
[c] mean of two independent determinations.
‡indicates assignment unclear from the complex spectrum.

With $^+NEt_4$ as guest the situation is similar to that observed with the tetramethyl analogue. That is substantial increases in the $T_1$'s for all β-cyclodextrin host protons, except H(1), occur upon complexation aaccompanied by increases in the relaxation times for the octyl chains of each of the three hosts. Using similar arguments to those developed above for $^+NMe_4$, these results suggest that the β-cyclodextrin based host forms a more well defined complex with both tetraethyl and tetramethyl ammonium ions.

## 4.6 APPLICATION OF $^{14}N$ NMR

Although $^{14}N$ is of high natural abundance (≈99%) it suffers from a low magnetogyric ratio, $\gamma$, and is quadrupolar (I=1). These two properties of the nucleus lead to a low sensitivity and line broadening respectively. Line broadening is brought about through interaction of the quadrupole moment, Q, with the electric field gradient at the nucleus but is not severe because the quadrupole moment is relatively small[32]. As a result high resolution work is still possible if the local electronic symmetry and mobility are both high. This is the case for $NR_4^+$ ions. Indeed, dynamic processes in a variety of systems with a range of timescales have been investigated by nitrogen-14 NMR[33].

### 4.6.1 Nuclear Quadrupole Coupling

Coupling between a nuclear electric quadrupole moment, Q, and an electric field gradient, $\chi$, generated by the nuclear environment provides an efficient route for londgitudinal relaxation[34]. In the case of the $^{14}N$ nucleus the quadrupolar moment is relatively small but nevertheless $^{14}N$ NMR spectroscopy is still dominated by quadrupolar relaxation. Consequently, under the conditions of extreme narrowing, the $^{14}N$ linewidth ($\omega_{1/2}$) is related to the quadrupolar relaxation time, $T_q$ and to structural and motional factors by:

$$\pi\,\omega_{1/2} = \frac{1}{T_q} = \frac{3}{8}\chi^2\left(1+\frac{\eta^2}{3}\right)T_q \qquad (11)$$

Where, $T_q = T_2 = T_1$ for mobile species and $\tau_q = \tau_c$, the effective rotational correlation time of the nuclear quadrupole assuming isotropic molecular motions and extreme motional narrowing. The electric field gradient, $\chi$, is zero for four co-ordinate nitrogen in symmetrical tetrahedral ions, $NR_4^+$. For such ions in solution quadrupolar relaxation is induced by re-

orientation of solvent dipoles and buffeting of neighbouring ions. $^{14}N$ NMR may thus be a useful probe into cyclodextrin - 'onium ion complexes since complexation should lead to changes in the rate of $^{14}N$ relaxation as a result of changes in molecular mobility and effective molecular volume upon complexation. Such changes in the rate of relaxation will be manifested by changes in both $T_1$ and $T_2$.

### 4.6.2 $^{14}N$ Longitudinal Relaxation Time and Line Width Determinations

Nitrogen-14 NMR spectra were acquired for $NMe_4^+$ with various molar ratios of per-O-octyl-β-cyclodextrin present at 36MHz. Longitudinal relaxation times were measured by the inversion recovery method and transvere relaxation times were measure as the natural line width ($\omega_{1/2}$). Paramagnetic oxygen was excluded from all NMR samples by repetitive 'freeze-evacuate-thaw' under an argon atmosphere. The solvent used for this investigation was 90% d-chloroform / 10% $d^3$-acetonitrile with the acetonitrile acting as an internal reference, as well as aiding the solubility of the ammonium salt. Each spectrum was acquired using a 10mm probe and $5cm^3$ of solvent with broad band proton decoupling being used during acquisition to increase the observed signal to noise ratio and allow the natural $^{14}N$ linewidth to be measured.

**Table 4.6.2A $^{14}N$ Longitudinal Relaxation Times for $NMe_4^+$ in the Presence of Per-O-octyl-β-cyclodextrin.**

| Molar Ratio: [a]CD/$NMe_4^+$ | $\delta_N$[b] | $T_1$ (msec.)[c] | SD(±) | $\Delta T_1$ (msec.) |
|---|---|---|---|---|
| 0 | -338.0 | 0.879 | 0.003 | — |
| 1 | -338.0 | 0.793 | 0.003 | -0.086 |
| 3 | -338.0 | 0.491 | 0.004 | -0.388 |

[a] All samples contained 0.012 mmol of $NMe_4^+CF_3CO_2^-$ (2.2mg). The molecular mass of per-O-octyl-β-cyclodextrin was taken to be 3200 in calculating the molar ratio.
[b] Relative to nitromethane (δ=0) as an external reference[35].
[c] The $^{14}N$ $T_1$ of $d^3$-acetonitrile (δ=-126.3) was constant throughout (1.94 msec.).

From the above table it is evident that the presence of per-O-octyl-β-cyclodextrin significantly influences the $T_1$ of the $^{14}N$ nucleus in $^+NMe_4$ and that the effect is dependent upon the relative molar ratio of cyclodextrin to 'onium ion. Although an increase in cyclodextrin concentration is expected to increase the viscosity, η, of the solution, which would lead to a decrease in $T_q$ itself since $Tq=T_1 \alpha (1+\eta^2)/3$ and $\eta<1$ by definition, the change in viscosity on going from no cyclodextrin present to 3 equivalents of cyclodextrin (305mg) was found to be minimal. Measurement of the viscosity for all solutions used in the NMR investigation, relative to $\eta(CDCl_3)$ =0.52, showed that only slight variation occurred. In the presence of three equivalents of the cyclodextrin $\eta$=0.54 (0.01). This lack of a viscosity effect was further confirmed by the fact that the $^{14}N$ $T_1$ of the $d^3$-acetonitrile co-solvent was independent of cyclodextrin concentration.

The molar ratio dependence of the $^{14}N$ $T_1$ is likely to be a result of more of the 'onium ion being bound by the cyclodextrin at higher cyclodextrin concentrations as the complexation equilibrium is driven to the right. The decrease in $T_1$ with increase in per-O-octyl-β-cyclodextrin

concentration suggests that complexation decreases the rate of molecular re-orientation for the $^{+}NMe_4$ ion, as already suggested in the interpretation of the proton $T_1$ results earlier. This decrease in mobility would give an associated increase in $\tau_c$, the reorientation correlation time, and a corresponding decrease in $T_1$.

**Table 4.6.2B $^{14}N$ Line Widths, $\omega_{1/2}$, for $NMe_4^+$ in the Presence of 'Per'-O-octyl-β-cyclodextrin.**

| [a]Molar Ratio: $CD/NMe_4^+$ | $\delta_N$[b] | $\omega_{1/2}$ [d](Hz) 296K | $\Delta(\omega_{1/2})$ (Hz) | $\omega_{1/2}$ [d](Hz) 313K |
|---|---|---|---|---|
| 0 | -338.0 | 0.70 | — | — |
| 1 | -338.0 | 0.75 | 0.05 | — |
| 3[c] | -338.0 | 1.15 | 0.45 | 1.1 |
| 5 | -338.0 | 1.26 | 0.56 | 1.1 |
| 8 | -338.0 | 1.42 | 0.72 | 1.4 |

[a] All samples contained 0.012 mmol of $NMe_4^+CF_3CO_2^-$ (2.2mg). The molecular mass of per-O-octyl-β-cyclodextrin was taken to be 3200 in calculating the molar ratio.
[b] Relative to nitromethane (δ=0) as an external reference.
[c] With 3 molar equivalents of 'per'-O-octyl-α-cyclodextrin present, $\omega_{1/2}$ =0.73 Hz.
[d] Digital resolution = 0.125 Hz.

The increase in linewidth on increasing the molar ratio of per-O-octyl-β-cyclodextrin indicates a decrease in the lifetimes of the spin states of the nitrogen nucleus by application of the Heisenberg uncertainty principle. This in turn suggests that the rate of transverse relaxation is increasing upon complexation of the 'onium ion with the cyclodextrin. Such observations are in good agreement with the $T_1$ measurements presented above and are to be expected for a quadrupolar nucleus under the conditions of motional narrowing, since $T_q=T_1=T_2$.

## CONCLUSIONS

Funtionalised α, β and γ cyclodextrins recognise long and short chain 'onium ions and aryl 'onium ions. Per-O-octylated β-cyclodextrin appears to be the most size selective towards alkylammonium ions. Both Per-O-octylated α- and β- cyclodextrins respond well to aryl ammonium ions with minimal interference from alkali and alkaline earth metal cations. The more polar plasticizer *o*-NPOE and the additive TKB enhance response towards alkyl ammonium ions whereas aryl ammonium ions respond equally well with either *o*-NPOE or BBPA as plasticizer. These lipophilic cyclodextrin derivatives appear well suited to sensing a wide spectrum of 'onium ions.

Comparative changes in $T_1$ seen in the proton NMR lend further support to the selective complexation of $NMe_4^+$ and $NEt_4^+$ by per-O-octyl-β-cyclodextrin. This hypothesis is substantiated by the obsevation that an increase in linewidth and decrease in $T_q$ are seen in the $^{14}N$ NMR of $NMe_4^+$ upon complexation with per-O-octyl-β-cyclodextrin.

## REFERENCES


1) D. J. Triggle *'Chemical aspects of the Autonomic Nervous System'* Academic Press, London 1965.

2) For example: M. Dhaenes, L. Lacombe, J-M. Lehn, J-P. Vigneron J. C. S. Chem. Commun. (1984) 1097.

3) R. Meric, J-P. Vigneron, J-P. Lehn J. C. S. Chem. Commun. (1993) 129; J. Frank, F. Vögtle Angew. Chem. Int. Ed. Engl. (1992) 31 528; F. Diedrich Angew. Chem. Int. Ed. Engl. (1988) 27 362.

4) J. Canceill, A. Collet, J. Gabard, F. Kotzyiba-Hibert, J-M. Lehn Helv. Chim. Acta. (1982) 65 1894.

5) F. Diederich *'Cyclophanes'* Royal Society of Chemistry, Cambridge, 1991.

6) H-J. Schneider, D. Güttes, U. Schneider J. Am. Chem. Soc. (1988) 110 6449; H-J. Schneider, D. Güttes, U. Schneider Angew. Chem. Int. Ed. Engl. (1986) 25 647.

7) A. Collet, J-P. Dutasta, B. Lozach, J-P. Chauvet Abstr. Int. Symp. Macrocyclic Chem. (1991) 16 KL21.

8) P. L. Anelli, P. R. Ashton, R. Ballardini, V. Balzaric, M. Delgado, M. T. Gandolfi, T. T. Goodnow, A. E. Kaifer, D. Philp, M. Pietrazkiewicz, L. Prodi, M. V. Reddington, A. M. Z. Slawin, N. Spencer, J. F. Stoddart, C. Vicerit, D. J. Williams J. Am. Chem. Soc. (1992) 114 193.

9) D. A. Stauffer, D. A. Dougherty Tetrahedron Letters (1988) 29 6039; M. A. Petti, T. J. Shepodd, R. E. Burrows, D. A. Dougherty J. Am. Chem. Soc. (1988) 110 6825.

10) T. J. Shepodd, M. A. Petti, D. A. Dougherty J. Am. Chem. Soc. (1988) 110 1983.

11) J. R. Luch, T. Higuchi, C. A. Sternon Anal. Chem. (1982) 54 1583.

12) a) T. Masadome, W. Wakida, Y. Kawabata, T. Imato, N. Ishibashi Anal. Sci. (1992) 8 89.

b) T. Masadome, J. Yang, T, Imato, N. Ishibashi Bunseki Kagaku. (1992) 41 231.

c)N. Ishibashi, T. Masadome, T. Imato Anal. Sci. (1986) 2 487

13) S. Srianiyata, W. R. White, T. Higuchi, L. A. Sternon Anal. Chem. (1978) 50 232.

14) E. Linder, E. Graf, Z. Neigreisz, K. Toth, E. Pungor, R. P. Buck Anal. Chem. (1988) 60 295; A. van den Berg, P. D. van del Wal, M. Skowronska-Ptasinska, E. J. R. Sudholter, D. N. Reinhoudt, P. Bergveld Anal. Chem. (1987) 59 2827.

15) R. Taylor, O. Kennard J. Am. Chem. Soc. (1982) 104 5063; R. D. Green *'Hydrogen Bonding by C-H Groups'* Wiley interscience, New York, 1974.

16) G. A. Jeffery, W. Saenger *'Hydrogen Bonding in Biological Structures'* Springer - Verlag, Berlin, 1991; C. Caccarelli, G. A. Jeffery, R. Taylor J. Mol. Struct. (1981) 70 255.

17) K. Rasmussen Acta Chem. Scand. A (1982) A36 323.

18) T. Steiner, W. Saenger J. Am. Chem. Soc. (1992) 114 10146.

19) F. Cramer, W. Saenger, H-Ch. Spatz J. Am. Chem. Soc. (1967) 89 14.

20) T. Okubo, H. Kitano, N. Ise J. Phys. Chem. (1976) 80 2661.

21) R. Palepu, V. C. Reinsborough Can. J. Chem. (1988) 66 325,

22) A. Hersey, B. H. Robinson, H. C. Kelly J. C. S. Faraday Trans. 1 (1986) 82 1271.

23) I. Satake, T. Takeshita, K. Hayakawa, T. Maeda Bull. Chem. Soc. Jpn. (1985) 58 2746.

24) V. T. Liveri, G. Cavallaro, G. Giammonia, G. Pitarresi, G. Puglisi, C. Ventura Thermochemica Acta (1992) 199 125.

25) E. S. Aman, D. Serve J. Colloid. Interface. Sci. (1990) 138 365; M. Komiyama, M. L. Bender J. Am. Chem. Soc. (1978) 100 2259.

26) N. Muller Acc. Chem. Res. (1990) 23 23.

27) K. Harata Bull. Chem. Soc. Jpn. (1976) 49 2066.

28) F. Freeman, H. D. W. Hill J. Chem. Phys. (1970) 53 4103.

29) U. Edlung, C, Holloway, G. C. Levy J. Am. Chem. Soc. (1976) 98 5069.

30) Y. Inoue, Y. Katono, R. Chujo Bull. Chem. Soc. Jpn. (1979) 52 1692.

31) M. Suzuki, J. Szejtli, L. Szente Carbohydr. Res. (1989) 192 61.

32) J. W. Jost, C. T. O'Konski J. Mol. Struct. (1983) 111 387.

33) J. Mason Chem. Rev. (1981) 81 205; W. von Philipsborn, R. Muller Angew. Chem. Int. Ed. Engl. (1986) 25 386.

34) J-M. Lehn, J-P. Kintzinger in *'Nitrogen NMR'* M. Witanowski, G. A. Webb (Eds.), Plenum, London 1973, pp 80-161.

35) P. R. Srinivasan, R. L. Lichter J. Magn. Reson. (1977) 28 227.

# CHAPTER FIVE

# TOWARDS SYNTHETIC MACROCYCLIC IONOPHORES

## 5.1 INTRODUCTION

Derivatives of crown ethers, in particular 18-crown-6, and related macrocyclic structures have been investigated by numerous workers as size, structural and enantiomer selective ionophores (see sections 1.3.1, 1.4.1 and 1.5). Such investigations have usually centred upon the ability of the 18-crown-6 framework to interact with primary ammonium ions as a result of an ion - dipole interaction between the $C_3$ related oxygens of the macrocycle and the $^{+}N$—H hydrogens of the ammonium ion. Such work was briefly reviewed in chapter one where it was indicated that enantioselectivity factors, $\alpha$, as high as 2.6 have been reported for the interaction of chiral primary ammonium ions with chiral crown ether derivatives such as the tetracarboxamide developed by Lehn (section 1.4.1).

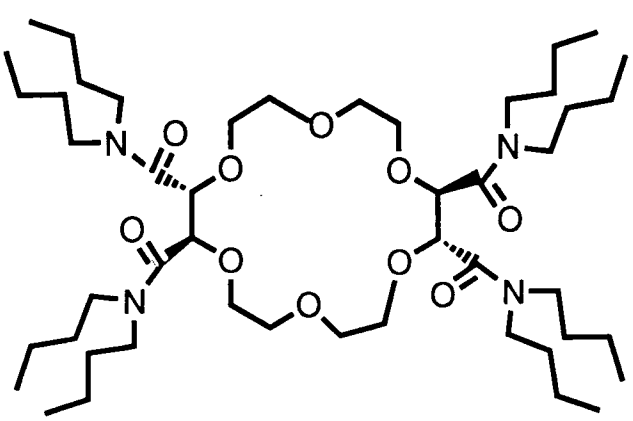


Figure 5.1 Lehn's Tetracarboxamide 18-Crown-6 Derivative

In this investigation the aim has been to synthesize chiral derivatives of 18-crown-6 and cyclam. Despite a failure to isolate pure target molecules, several important synthetic observations were made which are discussed below.

## 5.2 CHIRAL 18-CROWN-6 DERIVATIVES

Since aliphatic 18-crown-6 derivatives are known to form complexes with primary ammonium ions via three $N^+$-H···O hydrogen bonds it was envisaged that a chiral derivative with flanking electron deficient aryl groups may act as an ionophore for α-phenyl ammonium ions. The primary host - guest interaction may then involve an ion - dipole interaction between the ammonium ion and the $C_3$ related oxygen atoms of the macrocyclic polyether. It was hoped that the binding interaction may also involve some degree of charge transfer[1] between the π electron deficient aryl groups of the host and the π electron rich phenyl group of the chiral primary ammonium ion. Such π - π interactions have been used to good effect by other workers investigating molecular self assembly[2] and are a major driving force in the synthesis of [n]-pseudorotaxanes[3]. This type of π - π interaction has also been demonstrated to be the major interaction between nucleic acid base pairs in aqueous solution[4].

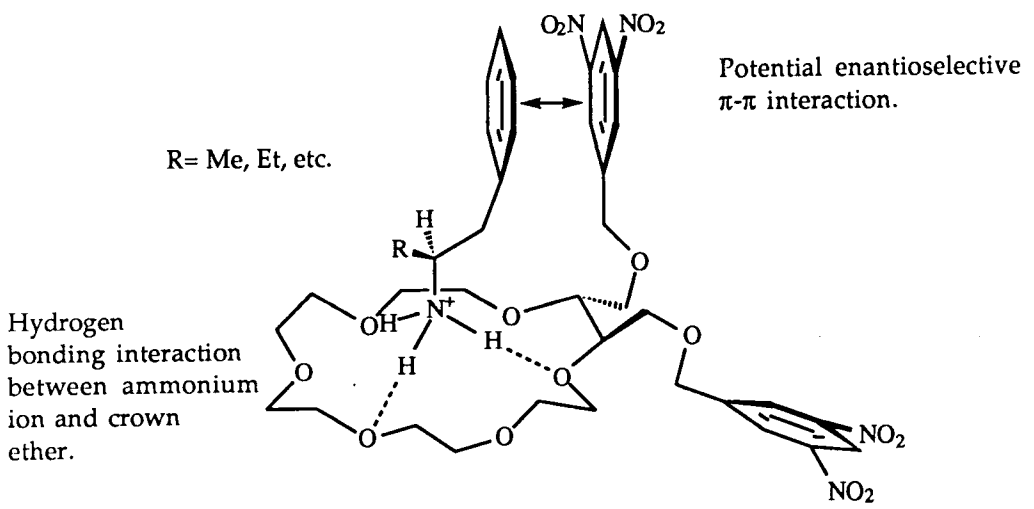



Figure 5.2 Possible mode of enantiomer discrimination by a chiral electron deficient 18-crown-6 derivative.

As an initial target, attempts were made to synthesize dibenzyl-18-crown-6[5] and N, N, N', N'-tetramethyl-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxamide, both of which have previously been prepared by other workers[6]. Also under consideration were the non-macrocyclic analogues of these compounds. The idea being to use such acyclic polyethers to investigate the influence of the 'macrocyclic effect' on complex formation and in particular on the enantiomer discriminating properties of the hosts.

### 5.2.1 Potassium Mediated Synthesis

The use of potassium ions for the template synthesis of 18-crown-6 derivatives is well documented. It relies on the ability of $K^+$ to interact with the reacting polyether chains, so limiting the conformational space available to the reacting groups and hence reducing the entropy loss upon cyclisation, leading to a more favourable $\Delta G_{reaction}$. Tartaric acid derivatives were employed to confer chirality to the macrocycle. Such compounds have been widely used as chiral building blocks in asymmetric synthesis[7] and are highly suitable for this application since they result in a substituted macrocycle which has $C_2$ symmetry and hence facial equivalence. The primary interest in macrocyclic ligands synthesized from tartaric acid has been their use as molecular catalysts for model enzyme studies[8].

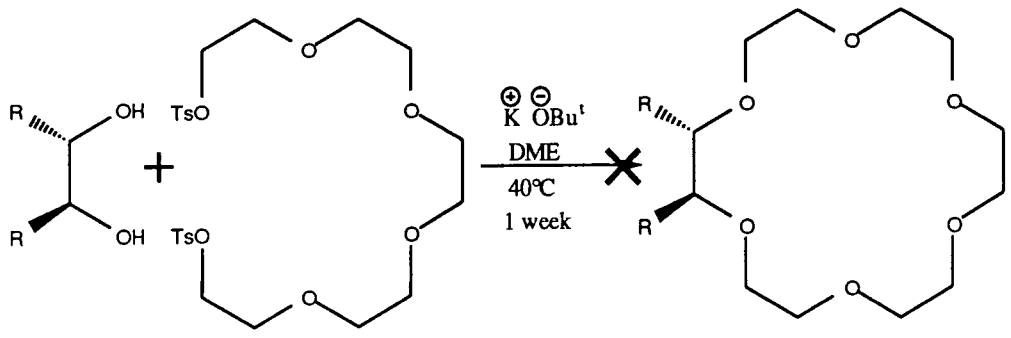



Figure 5.3 Potassium Mediated Cyclisation Reaction.

Despite the templating effect of $K^+$, problems resulted from E1 elimination competing effectively with the desired $S_N2$ cyclisation reaction, even at moderate temperature. This resulted in a multitude of side products to the reaction, increasing the difficulty of purification. Preparative thin layer chromatography allowed isolation of several products which were generally of an alkene nature. However the required macrocycle could not be isolated pure, despite the use of further purification techniques, most notably HPLC. Figure 5.4 provides mass spectral evidence of product formation by this potassium mediated route.

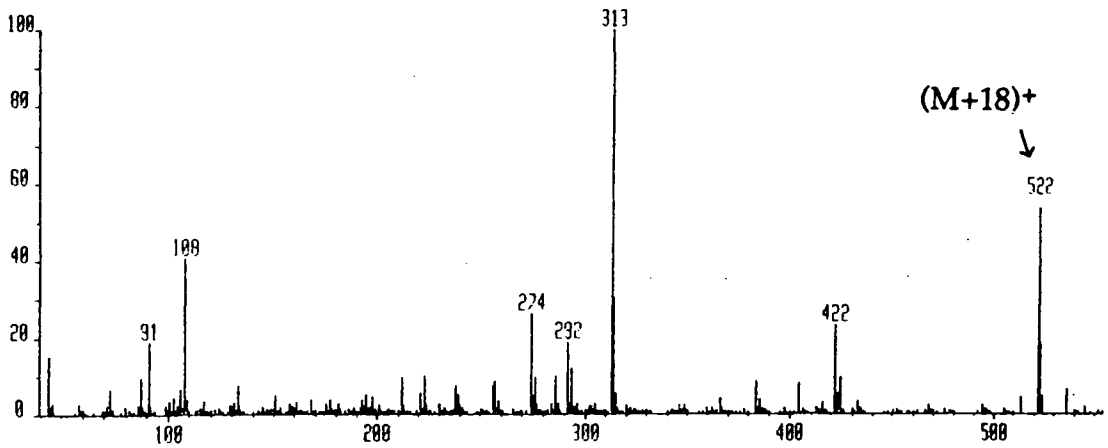
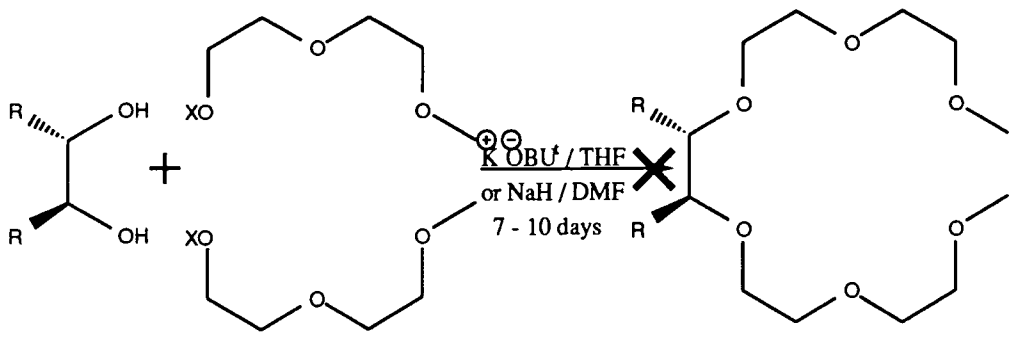



Figure 5.4 Mass Spectral Evidance of Cyclisation via a $K^+$ Mediated Route.

In the case of the acyclic 18-crown-6 analogues, no product formation could be observed by mass spectral analysis. This seemed rather surprising because such a reaction should be favoured both enthalpically and entropically over its cyclisation counterpart.




R = $C(O)NMe_2$ or $CH_2OCH_2Ph$.

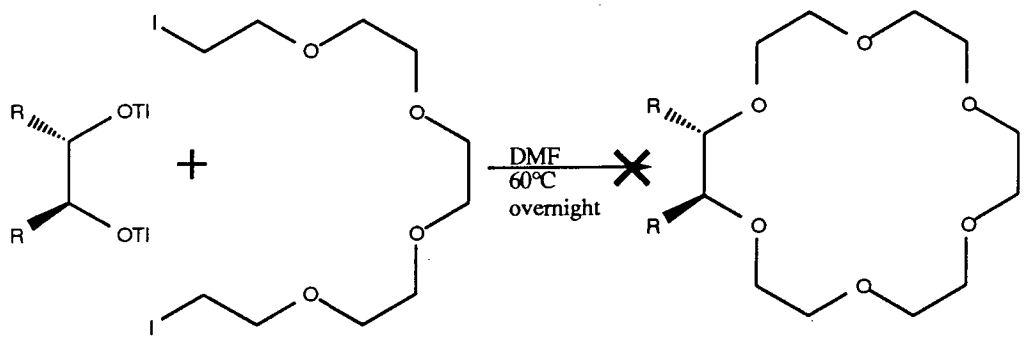
Figure 5.5 Alkali Metal Mediated Synthesis of Acyclic Crown Ether Analogues.

### 5.2.2 Thallium (I) Mediated Synthesis

Alkylation of thallium (I) alkoxides is a useful modification of the Williamson ether synthesis which is compatible with readily racemized chiral centres. It was first used as a method of preparing ethers of tartaric acid derivatives[9]. However, its application to the synthesis of crown ethers containing tartaric acid derived moieties has resulted in the production of chiral crown ether in yields of about 40%[10].

The primary interest in macrocyclic ligands synthesized from tartaric acid has been their use as molecular catalysts for model enzyme studies. So, for example, 2R, 3R-Dibenzyl-18-crown-6 is an intermediate in the synthesis of thiol appended regioselecive catalysts[5].

The method requires initial formation of the dithallous chiral tartrate. Reaction with pentaethylene glycol diiodide in DMF should then lead to cyclisation, the major driving forces being the template effect of $Tl^+$ on the ring closure reaction and the insolubility of the thallium (I) iodide in DMF or acetonitrile.




R = $C(O)NMe_2$ or $CH_2OCH_2Ph$.

Figure 5.6 Thallium Mediated Cyclisation.

Several problems were encountered in the application of this methodology, largely centred on the instability of the 1,2-diol to oxidation. Literature evidence supports the observation that $Tl^+$ is capable of acting as an oxidant[11] for such 1,2-diols, leading to two carbonyl species as shown in figure 5.7.

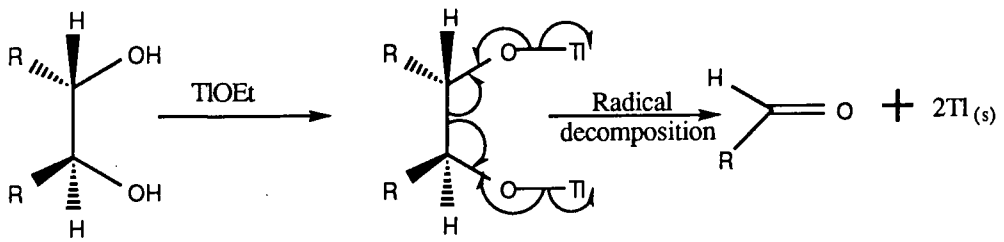



Figure 5.7 Secondary 1,2-Diol Oxidation with $Tl^+$ as the Oxidant[12].

In this investigation $^{13}C$ NMR confirmed that, within the multitude of products from this reaction, carbonyl species, C=O, were present ($\delta_{13C} \approx$ 175ppm) as shown in figure 5.8. This, along with mass spectral investigation, leads to the conclusion that diol oxidation is effectively competing with cyclisation again leading to numerous side products. Purification again proved impossible with the only direct evidence of cyclisation coming from mass spectral analysis.

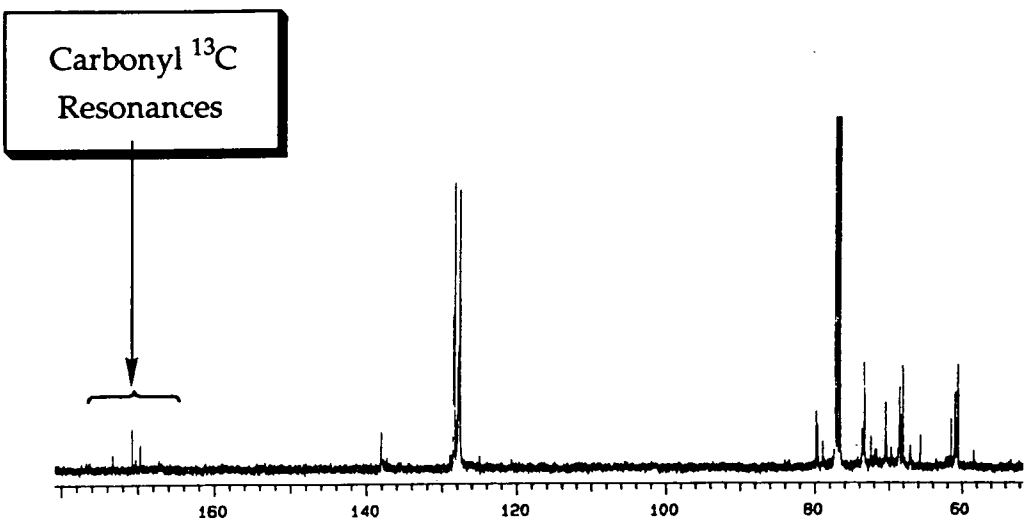
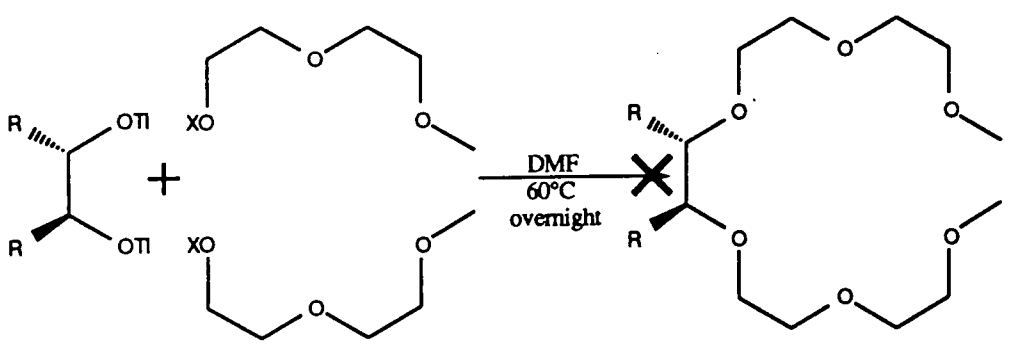



Figure 5.8 90MHz $^{13}C$ NMR Spectrum Indicating C=O Formation in the $Tl^+$ Mediated 18-Crown-6 Synthesis.

As was the case with the alkali metal mediated reaction, no formation of the acyclic crown ether analogues could be observed using this method.




R = $C(O)NMe_2$ or $CH_2OCH_2Ph$.

Figure 5.9 Thallium Mediated Acyclic Polyether Synthesis

## 5.3 TOWARDS THE DISCRIMINATION OF ARYL CARBOXYLATE ANIONS: MACROCYCLES CONTAINING NITROGEN

### 5.3.1 Introduction

In order to achieve selective binding of an anionic substrate a receptor incorporating either positively charged or electron - deficient binding sites is required[13]. Several workers have previously developed receptors containing Lewis acids[14], quaternary ammonium salts[15], protonated polyamines[16] and guanadiniums[17]. For example, quite recently Lehn and co - workers have developed a chiral receptor molecule containing a rigid guanidinium binding subunit for the chiral recognition of aromatic carboxylate anions such as the mandelate anion[18]:

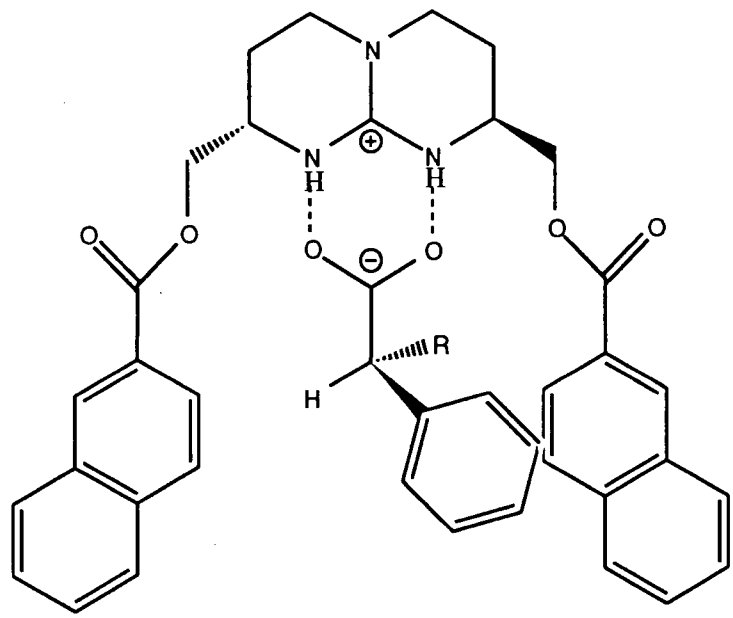



Figure 5.10 Binding of an Aryl Carboxylate by Lehn's receptor.

Such a receptor involves an electrostatic interaction between the carboxylate and the guanadinium subunit as the primary host-guest interaction. Enantioselectivity may then be facilitated by a $\pi$-$\pi$ charge transfer interaction between the naphthyl group of the receptor and the phenyl group of the carboxylate. Unfortunately this, and most other synthetic anion receptors, shows only modest enantiomer discriminating ability. For example, crystallisation of the diastereoisomeric salt of Lehn's receptor with mandelic acid results in a diastereoisomeric excess of only 17%.

In this section of research attempts were made to synthesize chiral cyclam derivatives which, after co-ordination of $Ni^{2+}$ within the macrocyclic cavity, may allow discrimination of the enantiomers of $\alpha$ and/or $\beta$ aryl carboxylate anions. The primary host - guest interaction in such a complex was envisaged as being an ion-ion electrostatic interaction between $Ni^{2+}$ and the carboxylate anion.

### 5.3.2 Synthesis of Nitrogen Analogues of Crown Ethers

Nitrogen containing macrocycles were first reported by Stetter and Roos[19]. Moderate cyclisation yields were obtained for the condensation of terminal alkyl dihalides with bis-sulphonamide salts under the conditions of high dilution for macrocycles such as 1,4,7,10-tetraazacyclododecane (cyclen) tetrachloride[20]. Subsequently Richman[21] reported that such macrocycles could be synthesized using bis-sulphonamide sodium salts and sulphonate ester leaving groups in a dipolar aprotic solvent. By variation of the counter ion of the bis-sulphonamide salt, Richman showed that yields of 70-80% could be obtained without high dilution and in the absence of metal ion template effects. Several other methods of cyclisation have since been reported[22].

### 5.3.3 The Effect of Nitrogen on the Metal Ion Binding Properties of Macrocycles.

Substitution of nitrogen into a crown framework in place of oxygen greatly influences the complexation of metal cations. The complexation of 'hard' cations such as $K^+$ is weakened and that of 'soft' cations such as transition element ions, is strengthened. This change in hospitality of the macrocycle is largely a consequence of the lower electronegativity of the nitrogen atom[23]. So, for example, 1,4,8,11-tetraazacyclotetradecane (cyclam) has been shown to exhibit strong binding towards transition element ions such as $Mn^{3+}$, $Zn^{2+}$, $Cu^{2+}$, $Ni^{2+}$ and $Co^{3+}$. Indeed, cyclam forms a thermodynamically stable and kinetically inert complex with $Ni^{2+}$ in aqueous solution[24] (log K = 20; 25°C, $H_2O$).

### 5.3.4 Cyclam Derivatives.

The co-ordination geometry of $Ni^{2+}$ can be influenced by changing the steric requirement of the coordination axis as a result of substitution on the cyclam ring[25]. A large number of cyclam derivatives with methyl substituents on the 1,4,5,7,8,11,12,13 and 14 positions of the ring have been synthesized. Such substitution has been shown to control the accessibility to the coordinated $Ni^{2+}$ by axial ligands as a result of the steric barrier offered by the methyl substituents[26]. Despite the fact that several chiral C-functionalised cyclam derivatives have appeared in the literature, their applications seem restricted to modification of the coordination geometry and metal binding properties of the macrocycle. For example, the $Mn^{3+}$ complex of the chiral, imidazole appended, macrocycle shown in figure 5.11 has been developed as a potential model for multi-electron redox catalyst[27]. Other nitrogen containing macrocycles have been made chiral by virtue of alkylation at nitrogen[28].

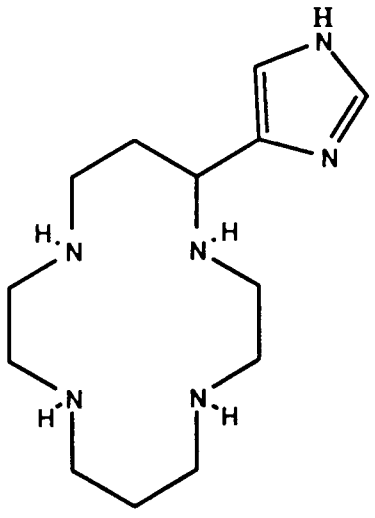


Figure 5.11 Chiral Imidazole Appended Cyclam Derivative[29]

### 5.3.5 Anion Coordination By Cyclam Derivatives

Figure 5.12 shows the crystal structure of the 2,3,11,12-tetramethyl cyclam $Ni(NO_3)_2$ complex[30]. The co-ordinated anion occupies an axial site above the trans III macrocyclic structure and is coordinated to the $Ni^{2+}$ ion within the macrocycle, and perhaps a nitrogen atom of the cycle. Such an electrostatic interaction between an anion and a $Ni^{2+}$ ion co-ordinated within the cyclam framework should be possible for other anions such as carboxylates.

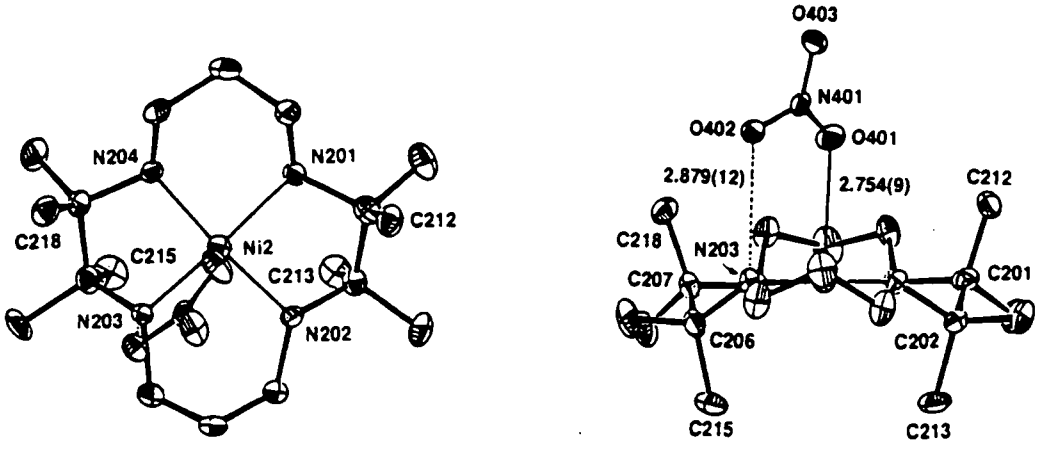



Figure 5.12 Crystal Structure of the 2,3,11,12-Tetramethyl Cyclam $Ni(NO_3)_2$ Complex

In this research the initial goal was to synthesize C-functionalized chiral cyclam derivatives. The co-ordination of $Ni^{2+}$ within such a macrocycle should then allow an ion-ion electrostatic interaction with a chiral carboxylate anion. This would then lead to a diastereoisomeric supramolecular structure.

If both the macrocycle and the co-ordinated carboxylate contain aryl subunits then π-π charge transfer between these groups may facilitate structural and enantiomeric discrimination. Such π - π interactions have already been discussed above in relation to selective binding by chiral crown ether derivatives.

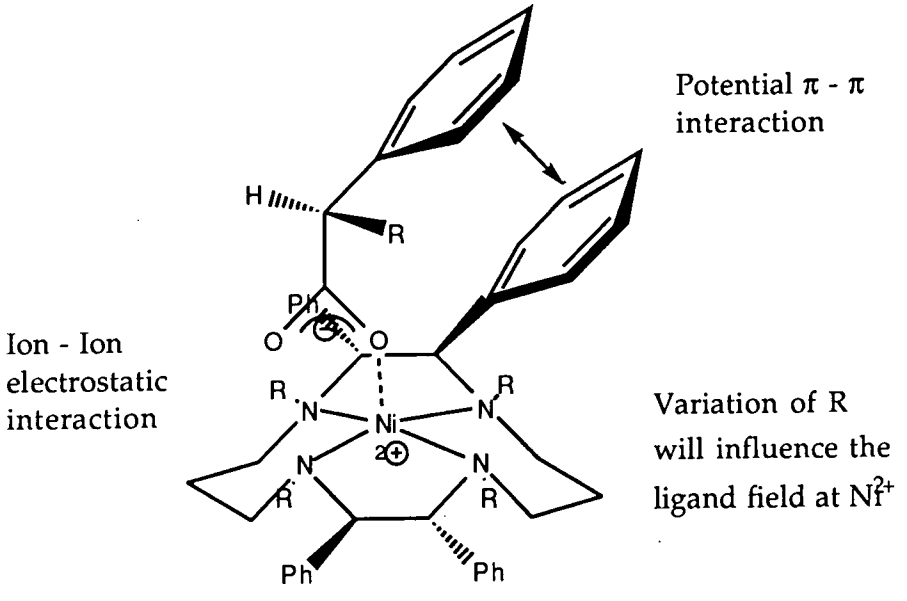



Figure 5.13 Possible Interactions between an Aryl Substituted Cyclam Derivative and a α-aryl carboxylate anion.

The primary electrostatic interaction should be reversible. Hence it should be possible to develop an enantiomer selective chemical sensor for α and/or β aryl carboxylate anions.

It may also be possible to modify the dynamics of the carboxylate-$Ni^{2+}$ interaction as a result of changing the ligand field experienced by the nickel ion. This may be achieved by N-alkylation of the macrocycle. Such alteration of the ligand field as a result of changing the conformation of the macrocycle may also allow some degree of control to be exerted over the aryl-aryl π-π interaction. Consequently the enantiomer discriminating ability of the receptor may be altered as a result of N-alkylation with alkyl groups of various steric requirement.

### 5.3.6 Introduction of Chirality

1,2-Diphenylethane-1,2-diamine (DPEDA) is a chiral subunit which can readily be obtained in an enantiopure form. Recent interest has focused on its ability to act as a chiral auxiliary in enantioselective transformations[31]. For example, it has been used by Corey in the enantioselective allylation of aldehydes to give secondary alcohols in reasonably high enantiomeric excess[32]:

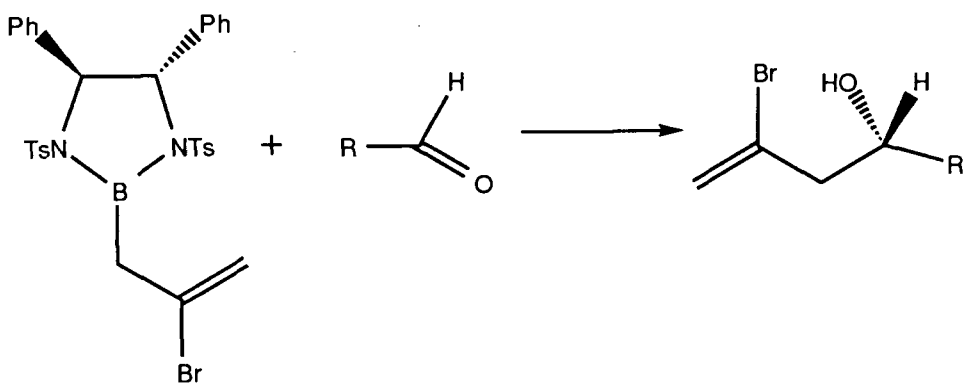



Figure 5.14 Enantioselective Aldehyde reduction using DPEDA as a Chiral Auxiliary.

**Table 5.3.6A Reaction of Aldehydes with Chiral (1S, 2S)-2-Haloallyl Borane at -78°C in Dichloromethane.**

| R of RCHO | % yield | % ee | abs. config. |
|---|---|---|---|
| $C_6H_5$ | 73 | 79 | S |
| (E)-$C_6H_5CH$=CH | 79 | 87 | S |
| n-$C_5H_{11}$ | 71 | 94 | R |

DPEDA has also been applied to the NMR analysis of chiral carboxylic acids such as mandelic acid[33].

The ability of DPEDA to discriminate the enantiomers of such compounds makes it an attractive subunit for incorporation into chiral nitrogen and nitrogen/oxygen macrocyclic ionophores.

### 5.3.7 Towards the synthesis of Chiral Cyclam Derivatives

The initial synthetic approach focused on the synthesis of a 4,7-diazadecane-1,10-diol derivative of both ethane-1,2-diamine and 1R,2R-DPEDA. The bis-sulphonamide of each of these diamines was successfully synthesized in high yield (75%) under standard conditions (TsCl / $Et_3N$ of Py / 0°C). At this point in the synthesis problems were encountered in developing a methodology suitable for N-alkylation of both the chiral and achiral bis-sulphonamides. The use of a halide leaving group on a $C_3$ alkyl chain eventually proved successful for the alkylation of the bis sulphonamide of ethane-1,2-diamine at each nitrogen atom, figure 5.15. However, the reaction time required for this reaction was inordinately long, even at elevated temperature, perhaps indicating that this reaction is kinetically disfavoured.

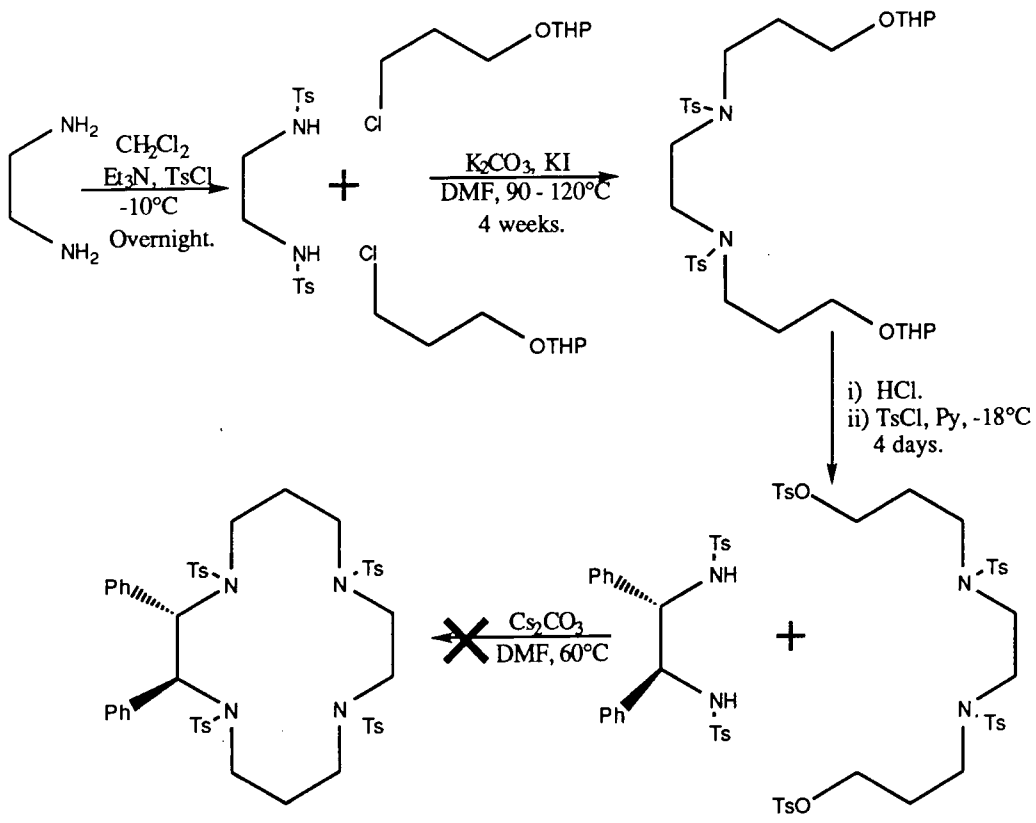



Figure 5.15 Reactions of Ethane-1,2-diamine.

Alkylation of DPEDA proved to be even more difficult. The use of a halide leaving group was eventually found to give the mono alkylated compound. The di-N-alkylated target compound could not be observed by either mass spectral or $^{1}H$ and $^{13}C$ NMR analysis of the crude reaction mixture.

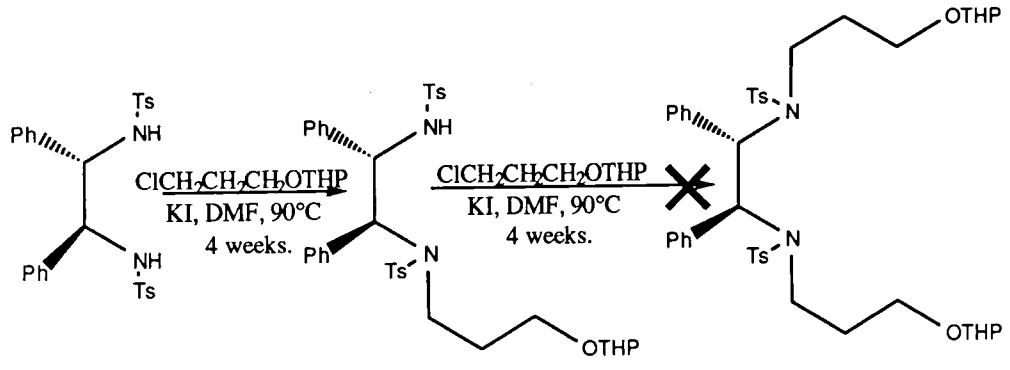



Figure 5.16 Alkylation of the Bis-sulphonamide of DPEDA using a Halide Leaving Group.

Since Richman had previously reported higher cyclisation yields for nitrogen cycles using a sulphonate ester leaving group[21], an alkylation reaction was attempted along similar lines for the bis-sulphonamide of DPEDA. In this case no reaction could be observed and elevation of the temperature resulted in consumption of the sulphonate ester, presumably as a result of E1 elimination.

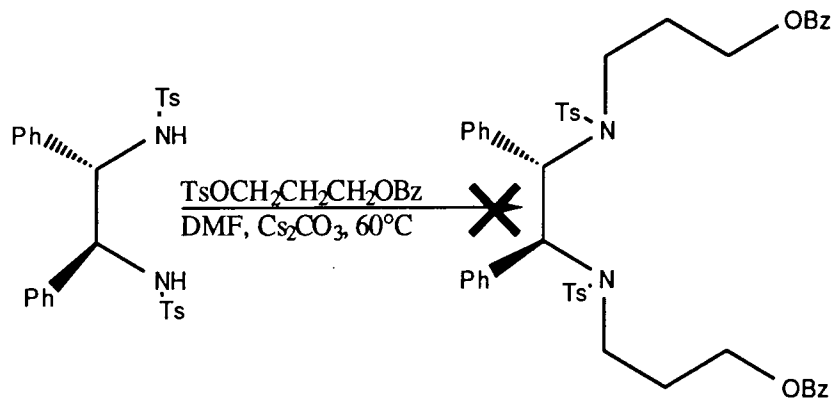



Figure 5.17 Alkylation of the Bis-sulphonamide of DPEDA using a Halide Leaving Group.

A further variation on the N-alkylation reaction was attempted, this time employing methyl acrylate in a Michael addition reaction. Again however, no alkylation of the DPEDA was evident.

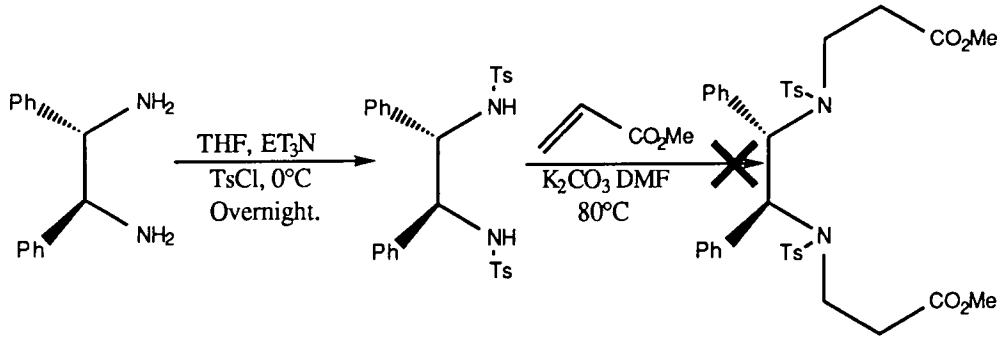



Figure 5.18 Attempted Michael Addition.

In a final attempt to alkylate the bis sulphonamide of DPEDA a cyclisation reaction using the previously prepared N,N'-bis(tolyl-*p*-suphonyl)-1,10-bis(tolyl-*p*-sulphonyloxy)-4,7-diazadecane was attempted, figure 5.15. Again however, no reaction could be observed by mass spectral analysis of the crude reaction mixture.

## 5.4 THE ATTEMPTED SYNTHESIS OF CHIRAL [14]-$N_2O_2$ DERIVATIVES

Two synthetic approaches were employed in attempts to synthesize a chiral [14]-$N_2O_2$ derivative. Such a compound should allow investigation of the effect of replacing two nitrogen atoms of the cyclam framework with oxygen atoms on the metal ion binding properties of the macrocycle. It was felt that such modification of the cycle - $Ni^{2+}$ interaction may also influence the binding of a carboxylate anion by the cationic macrocyclic structure. The first synthetic method employed a sulphonate ester leaving group in a reaction with the bis-sulphonamide of DPEDA.

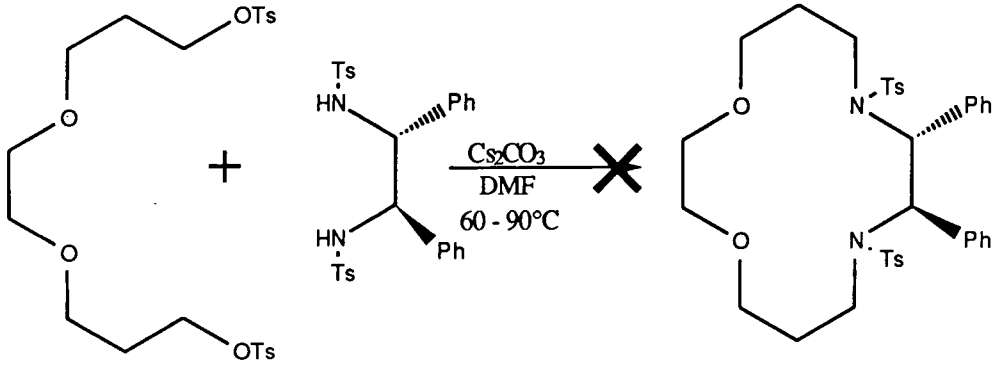



Figure 5.19 Attempted Synthesis of a Chiral [14]-$N_2O_2$ Derivative.

This reaction was unsuccessful with consumption of the sulphonate ester being quite rapid at elevated temperature (> 60°C). The second approach focused on the reaction of the free amine with a dimethyl ester, but this again proved unsuccessful.

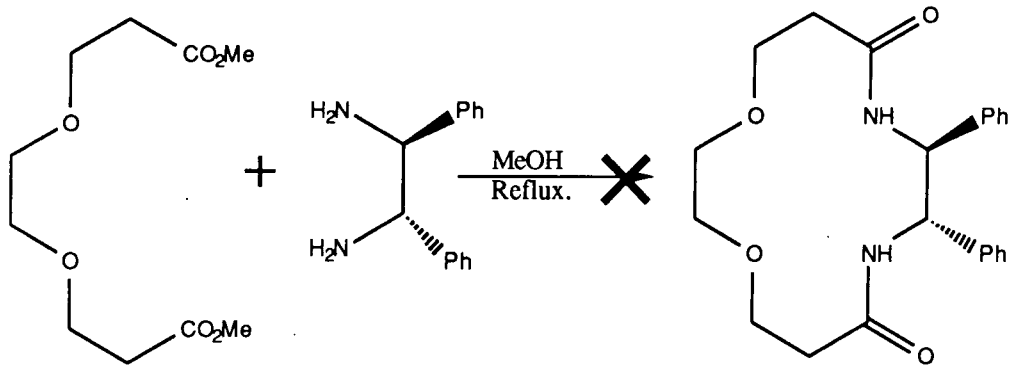



Figure 5.20 Attempted Synthesis of a Chiral [14]-$N_2O_2$ Derivative.

In order to ascertain the synthetic viability of the first attempted cyclisation reaction the achiral [14]-$N_2O_2$ framework was prepared by a similar method. This reaction proceeded smoothly and gave the macrocyclic product in high yield.

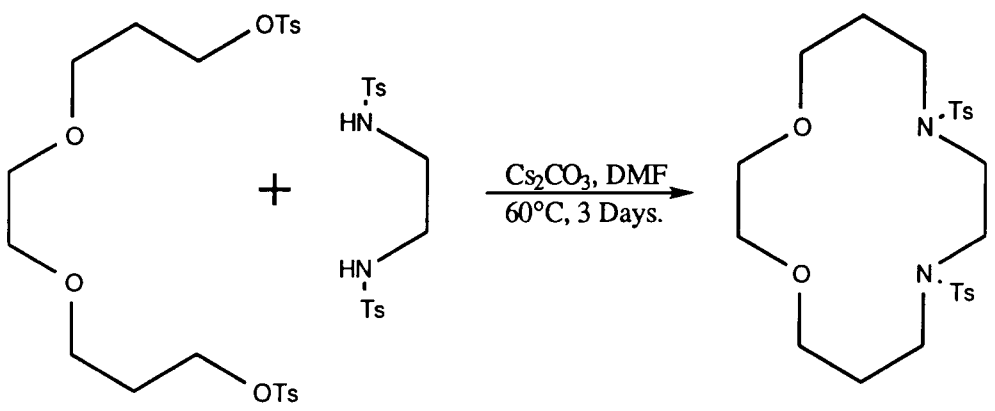



Figure 5.21 Synthesis of [14]-$N_2O_2$.

This seems to indicate that the lack of reaction with the bis-sulphonamide of DPEDA is a result of properties inherent to this diamine and perhaps more especially its bis-sulphonamide. The low reactivity of this diamide is thought to be a consequence of steric crowding in the trigonal pyramidal transition state which is associated with the $S_N2$ N-alkylation reaction.

## 5.5 CONCLUSIONS

It has been demonstrated in the above discussion that the synthesis and isolation of chiral 18-crown-6 and cyclam derivatives is a far from trivial matter despite, especially in the case of the crown ethers, a sizeable literature precedent. In the case of crown ether derivatives, synthetic investigations showed that problems aries because of the ease of E1 elimination of the polyethylene glycol leaving group (TsO-,MesO-,Cl- or I-) and from the relative ease by which 1,2-diols may be oxidized.

For the chiral nitrogen cycles, a literature precedent is much less evident, with the failure of product formation being attributed to the low reactivity of the sterically hindered nitrogen atoms of DPEDA and particularly of its bis-sulphonamide derivative.

Future attempts to synthesize chiral cyclam derivatives might employ the formation of an intermeadiate cyclic amide in high dilution reactions which parallel the syntheses of achiral derivatives by Tabushi[22a]. Alternatively, a $Ni^{2+}$ templated approach involving condensation of the free amine of DPEDA with acetone or 2,4-dioxopentane to yield a cyclic imine may prove fruitfull.

## REFERENCES


1) C. A. Hunter, J. K. M. Sanders J. Am. Chem. Soc. (1990) 112 5525

2) P. R. Aston, D. Philp, M. V. Reddington, A. M. Z. Slawin, N. Spencer, J. F. Stoddart, D. J. Williams J. Chem. Soc. Chem. Commun. (1991) 1680

3) P. R. Aston, D. Philp, N. Spencer, J. F. Stoddart J. Chem. Soc. Chem. Commun. (1991) 1677

4) N. G. Williams, L. D. Williams, B. R. Shaw J. Am. Chem. Soc. (1989) 111 7205

5) T. Matsui, K. Kogo Tetrahedron Letters (1978) 1115

6) S. T. Jolly, J. S. Bradshaw, R. M. Izatt J. Heterocyclic Chem. (1982) 19 3

7) J. D. Morrison, A. I. Scott *'Asymmetric Synthesis'* Academic Press, New York, 1984 Vol 4

8) J-P. Behr, J-M Lehn Helv. Chim. Acta (1980) 63 2112

9) H. O. Kalinowski, D. Seebach, G. Grass Angew. Chem. Int. Ed. Engl. (1975) 14 762

10) L. A. Frederick, T. M. Fyles, N. P. Gurprasad, D. M. Whitfield Can. J. Chem. (1981) 59 1724

11) E. C. Taylor, A. McKillop Acc. Chem. Res. (1970) 3 338

12) A. McKillop, R. A. Raphael J. Org. Chem. (1972) 37 4204

13) F. P. Schmidtchen Nachr. Chem. Tech. Lab. (1988) 36 8

14) M. Newcomb, M. T. Blanda Tetrahedron (1988) 29 4261

15) J. Arago. A. Bencini, A. Bianchi, A. Domenech, E. Garcia-Espana J. Chem. Soc. Dalton Trans. (1992) 319

16) E. Kimura, M. Kodama, T. Yatsunami J. Am. Chem. Soc. (1981) 103 3182

17) G. Muller, J. Riede, F. P. Schmidtchen Angew. Chem. Int. Ed. Engl. (1988) 27 1516

18) A. Echavarren, A. Galan, J-M. Lehn, J. de Mendoza J. Am. Chem. Soc. (1989) 111 4994

19) H. Stetter, E-E. Roos Chem. Ber. (1954) 87 566; H. Stetter, E-E. Roos Chem. Ber. (1955) 88 1390

20) H. Stetter, K-H. Mayer Chem. Ber. (1961) 94 1410

21) J. E. Richman, T. J. Atkins J. Am. Chem. Soc. (1974) 96 2268

22) a)I. Tabushi, H. Okino, Y. Kuroda Tetrahedron Letters (1976) 4339
b)B. K. Vriesema, J. Buter, R. M. Kellog J. Org. Chem. (1984) 49 110 and references therein

23) H. K. Frensdorff J. Am. Chem. Soc. (1971) 93 600

24) R. C. Luckay, R. D. Hancock J. Chem. Soc. Dalton Trans. (1991) 1491

25) E. Kimura, T. Koike, H. Nada, Y. Iitaka Inorg. Chem. (1988) 27 1036

26) *'Coordination Chemistry of Macrocyclic Compounds'* G. A. Melson (Ed) Plenum Press, New York, 1979

27) K. L. Brewer, M. Calvin, R. S. Lumpkin, J. W. Otuos, L. O. Spreer Inorg. Chem. (1989) 28 4446

28) J. R. Morphy, D. Parker, R. Alexander, A. Bains, A. F. Carne, M. A. W. Eaton, A. Harrison, A. Millican, A. Phipps, S. K. Rhind, R. Titmas, D. Weatherby J. Chem. Soc. Chem. Commun. (1988) 156

29) E. Kimura, M. Shionoya, T. Yamauchi, M. Shiro Chem. Lett. (1991) 1217

30) K. Kobiro, A. Nakayama, T. Hiro, M. Suma, Y. Tobe Inorg. Chem. (1992) 31 676

31) E. J. Corey, C. M. Xu, S. S. Kim J. Am. Chem. Soc. (1989) 111 5495; E. J. Corey, P. Dasilva, S. Virgil, P. W. Yuen, R. D. Connell J. Am. Chem. Soc. (1989) 111 9243

32) E. J. Corey, R. Imwinkelried, S. Pikul, X-B. Xiang J. Am. Chem. Soc. (1989) 111 5493

33) R. Fulwood, D. Parker Tetrahedron Asymm. (1992) 3 25

# CHAPTER SIX

# EXPERIMENTAL

## 6.1 INTRODUCTION

### 6.1.1 General

Optical rotations were measured with a Perkin - Elmer 141 Polarimeter. Gas Chromatography was carried out with a Hewlitt Packard HP 5890 using a SE 30 capillary column. HPLC analyses were carried out with a Varian 5500 instrument using a reverse - phase (Hypersil 5005) column for analytical work with aqueous acetonitrile gradient elution. Column chromatography on silica was effected using Merck 60 7354 or 9385 for flash chromatography and on alumina using Merck neutral alumina, previously treated with ethyl acetate. Tlc analysis was performed on silica gel 60 $F_{254}$ (MERCK).

### 6.1.2 Mass Spectral Analysis

Field desorption spectra were recorded on a ZAB-2VSE instrument operating in the positive ionisation mode and a mass range of 4000 Da. Fast atom bombardment spectra were recorded on a ZAB-2VSE instrument operating in the FAB positive ionisation mode. The matrix used was mNBA with added TFA. The mass range was 4000 and mass scale calibration employed caesium iodide as the calibration compound.

ES-MS measurements were made on a VG Quattro-BQ, a quadrupole instrument with an atmospheric pressure electrospray source and a mass range for single charged ions of 4000. Samples, as solutions in isopropanol (typically 20 - 50 pmol $\mu dm^{-3}$) were introduced into the source at 5 $\mu dm^{-3}$ $min^{-1}$. Mass scale calibration employed the ammonium adducts from polypropylene glycols 2000 and 3000 (1$\mu$g $\mu dm^{-3}$) were introduced into the source at 5 $\mu dm^{-3}$ $min^{-1}$ in 50:50 acetonitrile : water containing 1mM $NH_4OAc$. Ammonium acetate (10 mmol $dm^{-3}$), tetramethylammonium trifluoroacetate (0.2 to 2 mmol $dm^{-3}$),

ephedrinium trifluoroacetate (0.2 mmol $dm^{-3}$), or myristyltrimethylammonium bromide (0.5 mmol $dm^{-3}$) solutions in isopropanol were added to the cyclodextrin samples. Agreement between observed and calculated m/z values was typically within 0.4 Da.

### 6.1.3 NMR

NMR spectra were recorded on a Varian VXR 400, a Bruker AC 250 or a Bruker AMX 500 spectrometer. Low power (1W) WALTZ decoupling was used to acquire $^1H$ decoupled $^{13}C$ spectra. $T_1$ values were recorded by means of the inversion recovery sequence[1]. Chemical shifts were quoted to the higher frequency of $SiMe_4$ and are quoted in ppm with coupling constants in Hz.

Rotating Frame Overhauser Effect Spectra (ROESY) were recorded on a Bruker AMX 500 instrument. The applied sequence was:

$(\pi/2, {}^1H) — 10^{-5}s — (\text{spin lock } {}^1H) — (FID, t_2) — ({}^1H \text{ by decoupler})$

512 experiments were recorded. Before Fourier transformation and phase sensitive treatment the data were multiplied with cosine bell squared in each dimension[2].

1-D difference NOE spectra were obtained by taking the difference of two spectra. Low - power presaturation was effected prior to pulse. While acquiring data, accumulation was switched alternately between scans from on- to off- resonance irradiation, so that experimental errors cancel giving good difference spectra. Difference was taken after Fourier transforming the individual free induction decays using a larger line broadening function ( of the order of 1Hz). Generally several hundred accumulations were carried out for each irradiation

The $^{1}H$ - $^{1}H$ and $^{1}H$ - $^{13}C$ correlation spectra were recorded for solutions in d-chloroform with a Varian VXR-400 MHz instrument, using standard pulse sequences and procedures[3].

**HEXAKIS-2,6-DI-O-OCTYL-α-CYCLODEXTRIN (1)**

Powdered α-cyclodextrin hydrate (1g, 0.97mmol) was dried overnight under reduced pressure. Anhydrous DMSO (20cm$^3$) and fused, dried, powdered sodium hydroxide (2.2g, 55mmol) were added sequentially and the mixture stirred at room temperature for 1h. . 1-Bromooctane (14.1g, 73 mmol) was then added slowly to the vigorously stirred solution. The complete mixture was stirred at room temperature and the reaction monitored by tlc (20%methanol / 80%dichloromethane, silica, $R_f$ (α-cyclodextrin) = 0.25). After one week solvents were removed under reduced pressure. The resulting yellow solid was taken up in dichloromethane (40cm$^3$) and the solution filtered. The organic phase was washed with distilled water (2x30cm$^3$), dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure to give the crude product as a viscous yellow oil . Column chromatography (0 → 1% methanol /dichloromethane , $R_f$ (product) = 0.3) then gave the required product as a clear viscous oil (1.7g, 76%). m/z (+FD) 2320$(M+1)^+$ for 12 octyl groups, 2432$(M+1)^+$ for 13 octyl groups, 2545$(M+1)^+$ for 14 octyl groups. $\delta_H$($CDCl_3$) 4.90(12H, s, $H_1$ and OH(3)), 4.07(6H, t, J9.4Hz, part of a A'M'N' system, $H_3$), 3.93(6H, m, $H_c$), 3.86(6H, m, $H_5$), 3.66(6H, m, $H_{6a}$), 3.63(6H, m, $H_d$), 3.62(6H, m, $H_{6b}$), 3.50(6H, m, $H_4$), 3.44(6H, m, $H_e$), 3.41(6H, m, $H_f$), 3.35(6H, d of d, J3.6Hz 9.4Hz, part of an A'M'X' system, $H_2$), 1.518(12H, m, $C\underline{H_2}CH_2O$), 1.256(120H, m, $CH_2$). $\delta_C$($CDCl_3$) 101.4($C_1$), 83.5($C_4$), 79.9($C_2$), 73.7($C_3$), 72.7($CH_2O$), 71.8($CH_2O'$), 70.4($C_5$), 69.3($C_6$), 31.8($CH_2$), 29.8($CH_2$), 29.7($CH_2$), 29.5($CH_2$), 29.3($CH_2$), 29.25($C\underline{H_2}CH_2O$), 29.2($C\underline{H_2}CH_2O$), 14.1($CH_3$).

**HEXAKIS-2,3,6-'TRI'-O-OCTYL-α-CYCLODEXTRIN (2)**

1-Bromooctane (1.04g, 5.4mmol) was added to a stirred solution of sodium hydride (120mg, 5.4mmol) and the di-alkylated α-cyclodextrin (1) (1.39g, 0.6 mmol) in anhydrous tetrahydrofuran ($35cm^3$). The complete mixture was heated to reflux for 4 days. Filtration, followed by removal of the solvents under reduced pressure gave the crude product. Column chromatography (1% methanol / 99% dichloromethane, silica) then gave the required product as a highly viscous oil (1.3 g, 73%). m/z (ES+, 50ng $\mu l^{-1}$ isopropyl alcohol, 10mM ammonium acetate) 2561.9$(M+18)^+$ for 14 octyl groups, 2674.3$(M+18)^+$ for 15 octyl groups, 2786.6$(M+18)^+$ for 16 octyl groups, 2898.0$(M+18)^+$ for 17 octyl groups, 1346.2$(M+2NH_4)^{2+}$ for 15 octyl groups, 1402.5$(M+2NH_4)^{2+}$ for 16 octyl groups, 1459$(M+2NH_4)^{2+}$ for 17 octyl groups. $\delta_H(CDCl_3)$ 4.92*(6H, bs, $H_1$), 4.08*(6H, m, $H_3$), 3.94(6H, m, $H_c$), 3.88(6H, m, $H_d$), 3.86*(6H, m, $H_5$), 3.67(6H, m, $H_{6a}$), 3.61(6H, m, $H_e$), 3.62(6H, m, $H_{6b}$), 3.50*(6H, m, $H_4$), 3.48(6H, m, $H_f$), 3.45*(6H, m, $H_g$), 3.41(6H, m, $H_f$), 3.352(6H, m, $H_2$), 1.52(36H, m, $C\underline{H_2}CH_2O$), 1.24(180H, m, $CH_2$), 0.88(54H, t, $CH_3$). As a result of the low symmetry of this product, several of the proton assignments are only tentatively reported (indicated by *). Such assignments were made through the use of $^1H$-$^{13}C$ heteronuclear correlation spectroscopy. Integrals for the CHO and $CH_2O$ are only approximate and are such that the total is equal to that observed experimentally. $\delta_C(CDCl_3)$ 101.7 and 98 ($C_1$), 83.3($C_4$), 79.8($C_2$), 73.8($C_3$), 72.8($CH_2O$), 71.8($CH_2O'$), 70.9($CH_2O''$), 70.5($C_5$), 69.8($C_6$), 31.8($CH_2$), 31.8($CH_2$), 30.3($CH_2$), 30.1($CH_2$), 29.8 - 28.9‡ ($CH_2$ and $C\underline{H_2}CH_2O$), 26.1-26.0‡ ($CH_2$), 25.8-25.9‡($CH_2$), 14.1($CH_3$).

‡ indicates several resonances which were not resolved at 100MHz.

## FURTHER OCTYLATION OF 'PER'-O-OCTYL-α-CYCLODEXTRIN (2b)

Reductive depolymerisation and ES-MS analysis shows a mean of 15.4 octyl groups for each cyclodextrin core in per-O-octyl-α-cyclodextrin. This mean value is used in mole equivalence calculations in the experimental below.

Per-O-octyl-α-cyclodextrin (500mg, 0.18mmol) was dried overnight under reduced pressure at 50°C. Anhydrous tetrahydrofuran (10cm$^3$) and sodium hydride (40mg, 1.67mmol) were added sequentially under an atmosphere of nitrogen and the mixture stirred at room temperature for 1h. 1-Bromooctane (500mg, 2.6mmol) was added and the complete reaction mixture heated to reflux. After 3 days further sodium hydride (15mg, 0.6mmol) was added and reflux maintained for a further 4 days. After cooling, solid material was removed by filtration and the solvent then removed under reduced pressure. Column chromatography (0→1% methanol/dichloromethane, silica) gave the product as a clear colourless oil (410mg, 82%). NMR, mass spectral analysis and infra red spectroscopy showed this product to be indistinguishable from the per-O-octyl-α-cyclodextrin (2) starting material.

## HEXAKIS-(3-O-ACETYL)-HEXAKIS-2,6-DI-O-OCTYL-α-CYCLODEXTRIN (3)

Hexakis-2,6-di-O-octyl-α-cyclodextrin (2) (2.2g, 0.95mmol) was dried overnight under reduced pressure. Anhydrous acetic anhydride (10cm$^3$) and anhydrous triethylamine (3.0cm$^3$, 4.1g, 41mmol) were added and the complete reaction mixture heated to 60°C under conditions of reflux, under a nitrogen atmosphere. After 4 days, solvents were removed under reduced pressure to give a dark brown oil. Column chromatography (2% methanol/98% dichloromethane, silica) gave the

required product as a colourless oil (1.67g, 66%). m/z (ES, ammonia) 2589.8$(M+18)^+$, 2659.9$(M+18)^+$ for 13 octyl groups/5 acetyl groups, 1304.2$(M+2NH_4)^{++}$, 1339.3$(M+2NH4)^{++}$ for 13 octyl groups/5 acetyl groups. Infra red ($\upsilon$, $cm^{-1}$) 1765 (C=O). $\delta_H(CDCl_3)$ 5.18(6H, bs, H(3)), 4.91(6H, d, J 2.4, part of an A'M' system, H(1)), 3.97(6H, m, $H_c$ of $CH_2O$), 3.89(6H, m, $H(6)_a$), 3.76(6H, m, $H_d$ of $CH_2O$), 3.61(6H, m, $H(6)_b$), 3.57(6H, m, $H_e$ of $CH_2O'$), 3.47(6H, m, H(5)), 3.44(6H, m, H(4)), 3.37(6H, m, $H_f$ of $CH_2O'$), 3.24(6H, m, H(2)), 2.08(18H, s, $CH_3$-C(O)-O), 1.58(12H, m, $CH_2CH_2O$), 1.50(12H, m, $CH_2CH_2O'$), 1.26(120H, m, $CH_2$), 0.88(32H, m, $CH_3$). $\delta_C(CDCl_3)$ 170.5(C=O), 100.8(C(1)), 80.4(C(4)), 78.6(C(2)), 77.3(C(3)), 71.8($CH_2O$), 71.5($CH_2O'$), 71.3(C(5)), 69.1(C(6)), 31.8($CH_2$), 29.8-29.7($CH_2$ and $C\underline{H_2}CH_2O$), 26.1($CH_2$), 25.9($CH_2$), 22.3($CH_2$), 21.8($CH_2$), 14.1($CH_3$).

**3-O-METHYLATED-'PER'-O-OCTYL-α-CYCLODEXTRIN (4)**

A mean value of 15 octyl groups for each cyclodextrin core in 'per'-O-octyl-α-cyclodextrin (2) was assumed in calculating the molar quantities for this methylation.

'Per'-O-octyl-α-cyclodextrin (2) (450mg, 0.17mmol) was dried overnight under reduced pressure. Anhydrous tetrahydrofuran (10$cm^3$) and sodium hydride (80mg, 3.3mmol) were added sequentially and the mixture stirred under a nitrogen atmosphere for 1h. Iodomethane (568mg, 249μl, 4.0mmol) was added and the complete reaction mixture heated to 35°C under conditions of reflux. After 3 days, further sodium hydride (20mg, 0.83mmol) and iodomethane (568mg, 249μl, 4.0mmol) were added and stirring at 35°C continued for 4 more days. Solid material was removed by filtration under a nitrogen atmosphere and the solvents were then removed under reduced pressure. The resulting yellow oil was taken up in dichloromethane (25$cm^3$) filtered, washed with distilled

water (2x10cm$^3$) and dried over sodium hydroxide pellets (about 3g). Removal of the solvent under reduced pressure gave the product as a clear viscous oil (398mg, 87%). m/z (CI, ammonia) 2618(29%, $[M+18]^+$) for 14 octyl groups/4 methyl groups, 2716(100%, $[M+18]^+$) for 15 octyl groups/3 methyl groups, 2814(56%, $[M+18]^+$) for 16 octyl groups/2 methyl groups, 2913(16%, $[M+18]^+$) for 17 octyl groups/1 methyl group. Assuming equal ionisation potentials for each homologue, the mean number of methyl groups for each cyclodextrin core can be calculated, as a weighted mean, to be 2.7. $\delta_H(CDCl_3)$ 5.04(6H, m, H(1)), 3.96*(6H, m, H(3)), 3.95(6H, m, $H_c$ of $CH_2O$), 3.78*(6H, m, H(5)), 3.71(6H, m, $H(6)_a$), 3.70(3H, m, $H_g$ of $CH_2O''$), 3.69*(6H, m, $H_e$ of $CH_2O'$), 3.64*(6H, m, $H_d$ of $CH_2O$), 3.59*(6H, m, $H(6)_b$), 3.565(5H, m, $OCH_3$), 3.56(4H, m, $OCH_3$), 3.47(6H, m, $H_f$ of $CH_2O'$), 3.44(3H, m, $H_h$ of $CH_2O''$), 3.36*(6H, m, H(4)), 3.17(6H, m, H(2)), 1.57(30H, m, $C\underline{H_2}CH_2O$), 1.32(150H, m, $CH_2$), 0.82(45H, m, $CH_3$). As a result of the low symmetry of this product, several of the proton assignments are only tentatively reported (indicated by *). Such assignments were made through the use of $^1H$-$^{13}C$ heteronuclear correlation spectroscopy. Integrals for the CHO and $CH_2O$ are only approximate and are such that the total is equal to that observed experimentally. $\delta_C(CDCl_3)$ 99‡($C_1$), 82‡($C_4$), 80‡($C_2$), 80.5($C_3$), 72.7($CH_2O$), 71.8($CH_2O'$), 71.1($CH_2O''$), 70.7($C_5$), 69.8($C_6$), 61.8($OCH_3$), 31.9($CH_2$), 31.6-28.9‡ ($CH_2$ and $C\underline{H_2}CH_2O$), 26.1-26.0‡ ($CH_2$), 25.8-25.9‡($CH_2$), 22.4($CH_2$), 14.1($CH_3$).

‡ indicates several resonances which were not resolved at 100MHz.

**HEPTAKIS-2,6-DI-O-OCTYL-β-CYCLODEXTRIN (5)**

1-Bromooctane (16.3g, 84mmol) was added to a stirred solution of fused, dried, powdered sodium hydroxide (2.65g, 63mmol) and anhydrous β-cyclodextrin (1.2g, 1.05mmol) in DMSO (25cm$^3$). After one week, tlc

analysis (silica, 20%methanol / 80% dichloromethane, $R_f$(β-cyclodextrin) = 0.23) indicated complete reaction. The solvent was removed under reduced pressure to yield a pale yellow solid which was extracted into dichloromethane (35cm$^3$). The organic phase was washed with distilled water (2 x 25cm$^3$), dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure to give the crude product as a yellow oil (1.9 g). Column chromatography (silica, 0→1% methanol/ dichloromethane) then gave two products ($R_f$ = 0.33 and 0.25) which could only be distinguished by mass spectral analysis. m/z (+FD) for $R_f$=0.25 2592 $M^+$ for 13 octyl groups, 2706 $M^+$ for 14 octyl groups, 2817 $M^+$ for 15 octyl groups; $R_f$=0.33 2706 $M^+$ for 14 octyl groups, 2818 $M^+$ for 15 octyl groups, 2930 $M^+$ for 16 octyl groups. $\delta_H(CDCl_3)$ 5.05(7H, bs, OH), 4.87(7H, s, $H_1$, J3.6Hz part of an A'M' system), 3.89(7H, t, J9.5Hz, part of a A'M'N' system, $H_3$), 3.84(7H, m, $H_c$), 3.70(7H, m, $H_5$), 3.63(7H, m, $H_d$), 3.61(7H, m, $H_{6a}$), 3.56(7H, m, $H_{6b}$), 3.47(7H, m, $H_e$), 3.43(7H, m, $H_f$), 3.40(7H, m, $H_4$), 3.31(7H, d of d, J3.6Hz, 9.6Hz, part of an A'M'X' system, $H_2$), 1.52(14H, m, $C\underline{H}_2CH_2O$), 1.24(140H, m, $CH_2$), 0.88(42H, t, $CH_3$). $\delta_C(CDCl_3)$ 101.8($C_1$), 82.9($C_4$), 80.3($C_2$), 74.2($C_3$), 72.9($CH_2O$), 71.8($CH_2O'$), 71.1($C_5$), 69.4($C_6$), 31.8($CH_2$), 29.9($CH_2$), 29.7($CH_2$), 29.7($CH_2$), 29.3($CH_2$), 29.25($C\underline{H}_2CH_2O$), 29.2($C\underline{H}_2CH_2O$), 14.0($CH_3$).

**HEPTAKIS-2,3,6-TRI-O-OCTYl-β-CYCLODEXTRIN (6)**

1-Bromooctane (1.04g, 5.4mmol) was added to a stirred solution of sodium hydride (120mg, 5.4mmol) and the di-O-alkylated β-cyclodextrin (5) (1.39g, 0.6 mmol) in anhydrous tetrahydrofuran (35cm$^3$). The complete mixture was heated to reflux for 4 days. Filtration, followed by removal of the solvents under reduced pressure gave the crude product. Column chromatography (1% methanol / 99% dichloromethane, silica) then gave the required product as a highly viscous oil (1.3 g, 73%). m/z

(ES+, 50ng $\mu l^{-1}$ isopropyl alcohol, 10mM ammonium acetate) 2948.7$(M+18)^+$ for 16 octyl groups, 3060.8$(M+18)^+$ for 17 octyl groups, 3173.1$(M+18)^+$ for 18 octyl groups, 3285.3$(M+18)^+$ for 19 octyl groups, 1483.5$(M+2NH_4)^{2+}$ for 16 octyl groups, 1539.9$(M+2NH_4)^{2+}$ for 17 octyl groups, 1595.9$(M+2NH_4)^{2+}$ for 18 octyl groups, 1651.8$(M+2NH_4)^{2+}$ for 19 octyl groups. $\delta_H(CDCl_3)$ 4.86*(7H, s, $H_1$), 3.91*(7H, m, $H_3$), 3.90(7H, m, $H_c$), 3.86(7H, m, $H_d$), 3.71(7H, m, $H_5$), 3.69(7H, m, $H_{6a}$), 3.61(7H, m, $H_e$), 3.62(7H, m, $H_{6b}$), 3.58(7H, m, $H_f$), 3.48(7H, m, $H_g$), 3.44(7H, m, $H_4$), 3.41(7H, m, $H_f$), 3.33(7H, m, $H_2$), 1.51(36H, m, $C\underline{H_2}CH_2O$), 1.26(210H, m, $CH_2$), 0.88(63H, t, $CH_3$). As a result of the low symmetry of this product, several of the proton assignments are only tentatively reported (indicated by *). Such assignments were made through the use of $^1H$-$^{13}C$ heteronuclear correlation spectroscopy. Integrals for the CHO and $CH_2O$ are only approximate and are such that the total is equal to that observed experimentally. $\delta_C(CDCl_3)$ 101.4 and 99.1‡($C_1$), 83.1‡($C_4$), 80.5‡($C_2$), 74.1($C_3$), 72.9($CH_2O$), 71.7($CH_2O'$), 71.1($CH_2O''$), 70.9($C_5$), 69.5($C_6$), 32.1($CH_2$), 31.5-29.0‡ ($CH_2$ and $C\underline{H_2}CH_2O$), 26.3-26.1‡ ($CH_2$), 25.9-25.8‡($CH_2$), 22.1($CH_2$), 14.1($CH_3$).

‡ indicates several resonances which were not resolved at 100MHz.

**3-O-METHYLATED-'PER'-O-OCTYL-β-CYCLODEXTRIN (7)**

A mean value of 17 octyl groups for each cyclodextrin core in 'per'-O-octyl-β-cyclodextrin (6) was assumed in calculating the molar quantities for this methylation.

'Per'-O-octyl-β-cyclodextrin (6) (500mg, 0.16mmol) was dried overnight under reduced pressure. Anhydrous tetrahydrofuran ($10cm^3$) and sodium hydride (90mg, 3.7mmol) were added sequentially and the mixture stirred under a nitrogen atmosphere for 1h. Iodomethane

(568mg, 249µl, 4.0mmol) was added and the complete reaction mixture heated to 35°C under conditions of reflux. After 3 days, further sodium hydride (20mg, 0.83mmol) and iodomethane (568mg, 249µl, 4.0mmol) were added and stirring was continued at 35°C for 4 more days. Solid material was removed by filtration under a nitrogen atmosphere and the solvents were then removed under reduced pressure. The resulting yellow oil was taken up in dichloromethane ($25cm^3$) filtered, washed with distilled water ($2x10cm^3$) and dried over sodium hydroxide pellets (about 3g). Removal of the solvent under reduced pressure gave the product as a clear viscous oil (398mg, 87%). m/z (CI, ammonia) 2920.7(25%, $[M+18]^+$) for 15 octyl groups/6 methyl groups, 3018.8(68%, $[M+18]^+$) for 16 octyl groups/5 methyl groups, 3116.9(100%, $[M+18]^+$) for 17 octyl groups/4 methyl groups, 3215.1(91%, $[M+18]^+$) for 18 octyl groups/3 methyl group, 3313.1(8%, $[M+18]^+$) for 19 octyl groups/2 methyl group. Assuming equal ionisation potentials for each homologue, the mean number of methyl groups for each cyclodextrin core can be calculated, as a weighted mean, to be 3.5. $\delta_H(CDCl_3)$ 4.93(7H, m, $H_1$), 3.91(7H, m, $H_c$ of $CH_2O$), 3.87*(7H, m, $H_3$), 3.81*(7H, m, $H_5$), 3.73(7H, m, $H(6)_a$), 3.70(3H, m, $H_g$ of $CH_2O''$), 3.67*(7H, m, $H_e$ of $CH_2O'$), 3.66*(7H, m, $H_d$ of $CH_2O$), 3.56*(7H, m, $H(6)_b$), 3.565(5H, m, $OCH_3$), 3.51(7H, m, $OCH_3$), 3.48(7H, m, $H_f$ of $CH_2O'$), 3.44(3H, m, $H_h$ of $CH_2O''$), 3.41*(7H, m, $H_4$), 3.33(7H, m, $H_2$), 1.56(30H, m, $C\underline{H_2}CH_2O$), 1.25(170H, m, $CH_2$), 0.88(51H, m, $CH_3$). As a result of the low symmetry of this product, several of the proton assignments are only tentatively reported (indicated by *). Such assignments were made through the use of $^1H$-$^{13}C$ heteronuclear correlation spectroscopy. Integrals for the CHO and $CH_2O$ are only approximate and are such that the total is equal to that observed experimentally. $\delta_C(CDCl_3)$ 99‡($C_1$), 82‡($C_4$), 80‡($C_2$), 80.4($C_3$), 72.7($CH_2O$), 71.8($CH_2O'$), 71.3($CH_2O''$), 70.6($C_5$), 69.8($C_6$), 61.8($OCH_3$), 31.9($CH_2$), 31.6-

28.9‡ ($CH_2$ and $C\underline{H}_2CH_2O$), 26.2-26.0‡ ($CH_2$), 25.8-25.9‡($CH_2$), 22.4($CH_2$), 14.0($CH_3$).

‡ indicates several resonances which were not resolved at 100MHz.

**OCTAKIS-2,6-DI-O-OCTYL-γ-CYCLODEXTRIN (8)**

Powdered γ-cyclodextrin hydrate (1.0g, 0.77mmol) was dried overnight under reduced pressure. Anhydrous DMSO (20cm$^3$) and fused, dried, powdered sodium hydroxide (2.2g, 55.5mmol) were added sequentially under a nitrogen atmosphere and the mixture vigorously stirred for 1h. 1-Bromooctane (9.6cm$^3$, 10.7g, 55.7mmol) was added and the complete reaction mixture stirred at room temperature. The reaction was continued until tlc analysis (20%methanol/80%dichloromethane, silica) showed complete consumption of the γ-cyclodextrin ($R_f$=0.26). After 7 days solvents were removed under reduced pressure. Dichloromethane (50cm$^3$) and distilled water (30cm$^3$) were added. The lower organic layer was separated, washed with distilled water (2x25cm$^3$) and dried over anhydrous magnesium sulphate. Filtration and removal of the solvent under reduced pressure gave a yellow oil. Column chromatography (0→2%methanol /dichloromethane, silica) gave the required product (Rf=0.32, 2%methanol) as a viscous pale yellow oil (1.1g, 47%). $\delta_H(CDCl_3)$ 4.96(8H, d, J3.6, part of an A'M' system, H(1)), 3.96(8H, m, H(3)), 3.75(8H, m, H(5)), 3.68(8H, m, $H(6)_b$), 3.63(16H, m, $CH_2O$), 3.59(8H, m, $H(6)_a$), 3.44(16H, m, $CH_2O$), 3.40(8H, m, H(4)), 3.36(8H, d of d, J3.5, J9.6, part of an A'M'X' system, H(2)), 1.58(32H, m, $C\underline{H}_2CH_2O$), 1.27(160H, m, $CH_2$), 0.88(48H, t, $CH_3$). Integration ratios quoted are such that ΣH=80 for all the CHO and $CH_2O$ resonances. $\delta_C(CDCl_3)$ 101.8(C(1)), 83.2(C(4)), 80.8(C(2)), 73.3(C(3)), 73.1($CH_2O$), 71.6($CH_2O$), 70.4(C(5)), 69.1(C(6)), 31.9($CH_2$), 29.7($CH_2$), 29.6($CH_2$), 29.5($CH_2$), 29.4($CH_2$), 29.3($CH_2$), 29.2($C\underline{H}_2CH_2O$), 14.1($CH_3$).

**OCTAKIS-2,3,6-'TRI'-O-OCTYL-γ-CYCLODEXTRIN (9)**

1-Bromooctane (1.38g, 7.2mmol) was added to a stirred solution of sodium hydride (172mg, 7.2mmol) and the di-alkylated α-cyclodextrin (8) (1.1g, 0.3 mmol) in anhydrous tetrahydrofuran (35cm$^3$). The complete mixture was heated to reflux for 4 days. Filtration, followed by removal of the solvents under reduced pressure gave the crude product. Column chromatography (1% methanol / 99% dichloromethane, silica) then gave the required product as a highly viscous oil 0.49g, 46%). m/z (ES+, 50ng $\mu l^{-1}$ isopropyl alcohol, 10mM ammonium acetate) 3222.8$(M+18)^+$ for 17 octyl groups, 3335.0$(M+18)^+$ for 18 octyl groups, 3447.3$(M+18)^+$ for 19 octyl groups, 3559.7$(M+18)^+$ for 20 octyl groups, 3672.1$(M+18)^+$ for 21 octyl groups, 3784.1$(M+18)^+$ for 22 octyl groups, 1718.7$(M+2NH_4)^{2+}$ for 18 octyl groups, 1767.6$(M+2NH_4)^{2+}$ for 19 octyl groups, 1816.9$(M+2NH_4)^{2+}$ for 20 octyl groups, 1402.5$(M+2NH_4)^{2+}$ for 21 octyl groups, 1866.2$(M+2NH_4)^{2+}$ for 22 octyl groups. $\delta_H(CDCl_3)$ 4.96*(8H, s, $H_1$), 4.01*(8H, m, $H_3$), 3.94(8H, m, $H_c$), 3.88(8H, m, $H_d$), 3.79(8H, m, $H_5$), 3.67(8H, m, $H_{6a}$), 3.61(8H, m, $H_e$), 3.60(8H, m, $H_{6b}$), 3.42(8H, m, $H_4$), 3.48(8H, m, $H_f$), 3.45(8H, m, $H_g$), 3.41(8H, m, $H_f$), 3.34(8H, m, $H_2$), 1.57(48H, m, $C\underline{H_2}CH_2O$), 1.26(240H, m, $CH_2$), 0.88(72H, t, $CH_3$). As a result of the low symmetry of this product, several of the proton assignments are only tentatively reported (indicated by *). Such assignments were made through the use of $^1H$-$^{13}C$ heteronuclear correlation spectroscopy. Integrals for the CHO and $CH_2O$ are only approximate and are such that the total is equal to that observed experimentally. $\delta_C(CDCl_3)$ 102.3($C_1$), 82.1($C_4$), 80.1($C_2$), 74.1($C_3$), 72.7($CH_2O$), 71.8($CH_2O'$), 71.2($CH_2O''$), 70.8($C_5$), 70.3($C_6$), 31.8($CH_2$), 31.6($CH_2$), 30.5($CH_2$), 30.1($CH_2$), 30.0 - 28.9‡ ($CH_2$ and $C\underline{H_2}CH_2O$), 26.3-26.1‡ ($CH_2$), 25.8-25.9‡($CH_2$), 14.2($CH_3$).

‡ indicates several resonances which were not resolved at 100MHz.

## 6.3 REDUCTIVE DEPOLYMERISATION OF CYCLODEXTRIN DERIVATIVES

Several cyclodextrin derivatives were reductively depolymerised by a method similar to that described by Mishnick-Lübbnecke[4].

The modified cyclodextrin (17mg, 0.006mmol)) was dried overnight under reduced pressure. The flask was cooled to 0°C and anhydrous dichloromethane (80µl) added under a nitrogen atmosphere. The mixture was stirred until solution was complete. Triethylsilane (54µl, 0.34mmol) and boron trifluoride dimethylether (33µl) were added sequentially and the complete mixture was stirred under an atmosphere of nitrogen for 16h. The reaction was quenched with methanol ($1.5cm^3$) and the reddish brown solution was passed through a cation exchange column ($2cm^3$ of Dowex-50x4-400 ion exchange resin) contained inside a pipette. A further $6cm^3$ of methanol were used to elute the column. The resulting solution was dried overnight under reduced pressure to give a brown oil (16mg).

Acetic anhydride ($10cm^3$) and anhydrous triethylamine ($2cm^3$) were added under a nitrogen atmosphere. The complete mixture was heated to 75°C with stirring and the temperature maintained for 4h. Solvents were removed under reduced pressure. The resulting oil taken up in dichloromethane ($10cm^3$), washed with distilled water ($2x5cm^3$), dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure to give a viscous brown oil. This oil was then analysed by GC-MS.

The results obtained from GC-MS analysis of the cyclodextrin derivatives investigated by this method are detailed in the table below.

**Table 6.3A Results from GC-MS Analysis of Reductively Depolymerized Cyclodextrin Derivatives[a]**

| Cyclodextrin derivative | %Dioctylated, (k')[b] | %Trioctylated, (k') | mean No. of octyl groups |
|---|---|---|---|
| 2,6-di-O-octyl-α-cyclodextrin | 88, (12.45) | 12, (11.34) | 12.7 |
| 'per'-O-octyl-α-cyclodextrin | 43, (12.43) | 57, (11.35) | 15.4 |
| ‡Methylated 'per'-O-octyl-α-cyclodextrin | <1, (12.45) | 58, (11.35) | 15.4 |
| 2,6-di-O-octyl-β-cyclodextrin | 95, (12.45) | 5, (11.41) | 14.3 |
| 'per'-O-octyl-β-cyclodextrin | 51, (12.51) | 49, (11.31) | 17.4 |
| 'per'-O-octyl-γ-cyclodextrin | 45, (12.45) | 55, (11.31) | 20.4 |

[a] Numbers in parentheses refer to the retention time in minutes.
[b] k' = Retention time (minutes).
‡ 48% 3-O-methylated, k' = 14.79 minutes.

## 6.4 ATTEMPTED CYCLODEXTRIN ALKYLATIONS

Several attempts were made to synthesize alkylated cyclodextrins via modified $S_N2$ reactions (section 2.2). The synthetic methods employed are detailed in the following experimental section.

### HEXAKIS-2,6-DI-O-BUTYL-α-CYCLODEXTRIN

Powdered α-cyclodextrin hydrate (1.1g, 1.1mmol) was dried overnight under reduced pressure. Anhydrous DMF (10cm$^3$), caesium carbonate (1.0g, 3.2mmol) and 1-chlorobutane were added sequentially and the complete mixture stirred at 60°C under an atmosphere of nitrogen for 4 days. The solvents were removed under reduced pressure and the resulting solid was extracted into dichloromethane (2x25cm$^3$). Removal of the solvent under reduced pressure gave no isolable product.

### HEXAKIS-2,6-DI-O-HEXYL-α-CYCLODEXTRIN

Powdered α-cyclodextrin hydrate (100mg, 0.1mmol) was dried overnight under reduced pressure. Anhydrous DMSO (6cm$^3$), fused, dried, powdered potassium hydroxide and 1-iodohexane (100μl, 0.5mmol) were added sequentially and the complete mixture stirred at room temperature under a nitrogen atmosphere for 3 days. Removal of the solvents under reduced pressure followed by extraction into dichloromethane (2x10cm$^3$) gave no isolable product.

### HEXAKIS-2,6-DI-O-BUTYL-α-CYCLODEXTRIN

1-Chlorobutane was added to a stirred solution of α-cyclodextrin (1.2g, 1.2mmol), fused, dried powdered sodium hydroxide (0.3g, 7.2mmol) and tetrabutyl ammonium hydrogen sulphate (0.1g) in anhydrous tetrahydrofuran. The complete reaction mixture was heated to reflux under an atmosphere of nitrogen for 16h. The solvent was removed

under reduced pressure and the resulting solid was extracted into dichloromethane (2x25cm$^3$). Evaporation of the dichloromethane under reduced pressure gave no isolable product.

### HEXAKIS-2,6-DI-O-BUTYL-α-CYCLODEXTRIN

Caesium carbonate (0.1g, 0.32mmol) was added to a stirred solution of α-cyclodextrin (100mg, 0.1mmol) in 1-chlorobutane (10cm$^3$). The complete mixture was heated to 50°C under a nitrogen atmosphere for 48h. The solvent was removed under reduced pressure and the resulting solid was extracted into dichloromethane (2x25cm$^3$). Evaporation of the dichloromethane under reduced pressure gave no isolable product.

## 6.5 OLIGOSACCHARIDE ALKYLATIONS

### 2,3,4,6-TETRAKIS-(O-OCTYL)-α-METHYL-D-GLUCOSE (10)

To a stirred solution of anhydrous α-methyl-D-glucose (1.0g, 5.2 mmol) in DMSO (20 ml) was added fused, dried, powered sodium hydroxide (2.5g, 62 mmol). This mixture was stirred at room temperature for 1h after which time 1-bromooctane (11.9g, 10.7 ml, 62 mmol) was added. The complete solution was stirred under a nitrogen atmosphere until tlc analysis showed complete disappearance of glucose ($R_f$=0.3, 10%methanol/ 90%dichloromethane, silica). After five days all volatile material was removed under reduced pressure. The residue was extracted into dichloromethane (40cm$^3$) and the organic phase washed with distilled water (3 x 20cm$^3$) and dried over anhydrous magnesium sulphate to give a yellow oil. Column chromatography (100%dichloromethane, silica) then gave the required product ($R_f$ = 0.6) as a colourless oil (1.9g, 58%). m/z (CI, ammonia): 660(M+18)$^+$, 628 (M-Me)$^+$, 611 (M-MeO)$^+$, and 481, 351. $\delta_H$($CDCl_3$) 4.775(1H, d,J3.6, H(1)), 3.85(1H, m, H(3)), 3.79(2H, m, $CH_2O$), 3.77(1H, m, $H(6)_a$), 3.68(1H, m, $H(6)_b$), 3.60(2H,

m, $CH_2O$), 3.54(2H, m, $CH_2O$), 3.52(1H, m, H(5)), 3.42(2H, m, $CH_2O$), 3.38(3H, m, $OCH_3$), 3.34(1H, m, H(4)), 3.273(1H, d of d, J3.6Hz, J10Hz, H(2)), 1.57(8H, m, $C\underline{H_2}CH_2O$), 1.27(40H, m, $CH_2$), 0.88(12H, m, $CH_3$). $\delta_C(CDCl_3)$ 98.1($C_1$), 81.6($C_3$), 80.6($C_2$), 77.7($C_4$), 73.6($C_6$), 73.1($CH_2O$), 71.7($C_5$), 71.6($CH_2O$), 70.1($CH_2O$), 69.2($CH_2O$), 54.9($OCH_3$), 31.83($CH_2$), 31.82($CH_2$), 31.80($CH_2$), 31.7($CH_2$), 30.5($C\underline{H_2}CH_2O$), 30.4($C\underline{H_2}CH_2O$), 30.0($C\underline{H_2}CH_2O$), 29.6($CH_2$), 29.54($CH_2$), 29.51($CH_2$), 29.45($CH_2$), 29.37($CH_2$), 29.30($CH_2$), 29.28($CH_2$), 29.24($CH_2$), 29.23($CH_2$), 26.24($CH_2$), 26.20($CH_2$), 26.18($CH_2$), 25.9($CH_2$), 22.6($CH_2$), 14.0($CH_3$).

**2,3,4,6-TETRAKIS-(O-OCTYL)-O-α-D-GLUCOPYRANOSYL-(1→4)-1,2,3,6-TETRAKIS-(O-OCTYl)-O-α-D-GLUCOSE.**

**PER-O-OCTYL MALTOSE (11)**

D-Maltose monohydrate (1.0g, 2.78mmol) was dried under reduced pressure overnight. DMSO (20$cm^3$) and fused, dried, powdered sodium hydoxide (2.67g, 66.7mmol) were added sequentially and the mixture stirred at room temperature under nitrogen for 1h. 1-Bromooctane (13.07g, 66.7mmol) was added and the complete reaction mixture stirred at room temperature under a nitrogen atmosphere for 7 days. Solvents were removed under reduced pressure and the crude product purified by column chromatography (2%methanol/98%dichloromethane, silica) to give a mixture of partially alkylated homologues ($R_f$=0.25-0.40). The partially alkylated homologues were combined and the solvent was removed under reduced pressure to give a yellow oil (2.1g). The yellow oil was dried under reduced pressure overnight. Anhydrous tetrahydrofuran (35$cm^3$) and sodium hydride (400mg, 16.7mmol) were added under a nitrogen atmosphere and the mixture was stirred at room temperature for 1h. 1-Bromooctane (3.27g, 16.7mmol) was added and the complete mixture heated to reflux under a nitrogen atmosphere for 4

days. Tlc analysis (100% dichloromethane, silica) indicated complete alkylation ($R_f$=0.7). Solvents were removed under reduced pressure and column chromatography (100% dichloromethane, silica) gave the required product ($R_f$=0.70) as a slightly yellow oil (2.1g, 61%). m/z (CI, ammonia) 1240$(M+18)^+$, 1223$(M+1)^+$. $\delta_H(CDCl_3)$ 4.84(1H, d, $H(1)_A$), 4.17(1H, d, $H(1)_B$), 3.82 - 3.14*(26H, m, CHO and $CH_2O$), 3.05(2H, m, H(2)), 1.49(16H, m, $C\underline{H_2}CH_2O$), 1.18(80H, m, $CH_2$), 0.81(24H, m, $CH_3$). $\delta_C(CDCl_3)$ 103.4($C(1)_A$), 96.3($C(1)_B$), 31.6($CH_2$), 31.3($CH_2$), 22.8($CH_2$), 14.4($CH_3$), and 84.9, 81.7, 80.4, 77.8, 74.2, 73.6, 73.1, 72.9, 72.6, 72.5, 71.9, 71.8, 71.7, 71.0, 70.0, 69.9, 69.1, 30.6-30.2*, 29.8-29.5*, 26.4-26.2*.

* indicates several resonances which could not be distinguished from the complex spectrum.

Attempts were made to assign all the resonances for per-O-octyl maltose through the use of COSY and HETCOR. However, the spectra were still too complex for complete assignment.

**2,3,4,6-TETRAKIS-(O-OCTYL)-O-α-D-GLUCOPYRANOSYL-(1→4)-2,3,6-TRIS-(O-OCTYL)-O-α-D-GLUCOPYRANOSYL-(1→4)-1,2,3,6-TETRAKIS-(O-OCTYL)-O-α-D-GLUCOSE.**

**PER-O-OCTYL-MALTOTRIOSE (12)**

Maltotriose hydrate (1.0g, 2.0mmol) was dried overnight at 50°C under reduced pressure. Anhydrous DMSO (20$cm^3$) and fused, dried, powdered sodium hydroxide (2.70g, 67.6mmol) were added sequentially under an atmosphere of dry nitrogen. The mixture was stirred at room temperature for 1h and 1-bromooctane (13.25g, 67.6mmol) added. The complete reaction mixture was stirred at room temperature for 6 days after which time tlc analysis (15% methanol/85% dichloromethane, silica) showed total consumpation of the maltotriose ($R_f$=0.36). Solvents were removed under reduced pressure and the crude mixture purified by

column chromatography (100% dichloromethane, silica) to give a mixture of several partially alkylated homologues ($R_f$=0.4-0.6) which were re-combined for further alkylation. Removal of the solvent under reduced pressure gave a yellow oil (2.23g). The yellow oil was dried overnight under reduced pressure. Anhydrous tetrahydrofuran ($40cm^3$) and sodium hydride (400mg, 16.7mmol) were added under a nitrogen atmosphere and the mixture stirred at room temperature for 1h. 1-Bromooctane (3.27g, 16.7mmol) was added and the complete mixture heated to reflux for 4 days. After cooling, solid material was removed by filtration under an atmosphere of dry nitrogen. The solvent was removed under reduced pressure and column chromatography (10%ethyl acetate/90%hexanes, silica) gave the required product ($R_f$=0.6) as a slightly yellow clear oil (1.93g, 56%). m/z (CI, ammonia) $1738(M+18)^+$, $1721(M+1)^+$. $\delta_H(CDCl_3)$ 4.22(3H, m, H(1)), 3.86-3.16(40H, m, CHO and $CH_2O$), 3.37 and 3.55 ($CH_2O$ of $OC_8H_{17}$), 1.56(22H, m, $C\underline{H_2}CH_2O$), 1.27(110H, m, $CH_2$), 0.88(33H, t, $CH_3$). $\delta_C(CDCl_3)$ $103.6(C(1)_A)$, $103.4(C(1)_B)$, $96.3(C(1)_C)$, $82.6(C(2)_A)$, $82.4(C(2)_B)$, $82.3(C(2)_C)$, $31.9(CH_2)$, $31.8(CH_2)$, $22.7(CH_2)$, $14.1(CH_3)$, and 84.9, 84.8, 81.7, 80.4, 78.3, 77.7, 77.3, 74.8, 74.2, 73.8, 73.6, 73.2-72.87*, 72.6, 72.5, 71.9-71.7*, 70.9, 70.1-69.8*, 69.1, 30.7-30.2*, 29.8-29.3*, 26.3-26.1*.

* indicates several resonances which could not be distinguished from the complex spectrum.

Attempts were made to assign all the resonances for per-O-octyl maltotriose through the use of COSY and HETCOR. However, the spectra were still too complex for complete assignment.

## 6.6 PREPARATION OF AMMONIUM TRIFLUOROACETATES

The trifluoroacetates of several amines were prepared for the NMR analyses detailed in chapters three and four. Each trifluoroacetate was prepared from either the hydrochloride or the free amine and an example of each is given here.

### 1S, 2R-(+)-EPHEDRINE TRIFLUOROACETATE (13)

A solution of (+)-ephedrine hydrochloride (1g, 5.0mmol) in distilled water ($30cm^3$) was basified (pH=10) with 4M sodium hydroxide. The resulting solution was extracted into dichloromethane ($3x20cm^3$). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure to give the free amine as a colourless oil. Excess trifluoroacetic acid ($3cm^3$) was added and the resulting solution dried under reduced pressure overnight to give a highly viscous colourless oil in quantitative yield. $\delta_H(CDCl_3)$ 9.23 (1H, bs, NH), 8.22(1H, bs, NH), 7.34(5H, m, aromatic H), 5.36(1H, d, C<u>H</u>(OH), 3.38(1H, d of d, $CH(CH_3)$, 2.83(3H, bs, $NCH_3$), 1.51(3H, d, $CH_3$) $\delta_C(CDCl_3)$ 162.4(C=O), 139.0(aromatic C), 128.5(aromatic CH), 127.9(aromatic CH), 125.5(aromatic CH), 70.9(CH(OH)), 61.3(<u>C</u>$(CH_3)$N), 31.2($NCH_3$), 8.6($CH_3$—C).

### 1R, 2S-(-)-EPHEDRINE TRIFLUOROACETATE (14)

Excess trifluoroacetic acid ($2.5cm^3$) was added to 1R, 2S-(-)-ephedrine (1.0g, 5.4mmol). The resulting solution was dried under reduced pressure overnight to give a highly viscous colourless oil in quantitative yield. $\delta_H(CDCl_3)$ 9.23 (1H, bs, NH), 8.22(1H, bs, NH), 7.34(5H, m, aromatic H), 5.36(1H, d, C<u>H</u>(OH), 3.38(1H, d of d, $CH(CH_3)$, 2.83(3H, bs, $NCH_3$), 1.51(3H, d, $CH_3$) $\delta_C(CDCl_3)$ 162.4(C=O), 139.0(aromatic C), 128.5(aromatic CH), 127.9(aromatic CH), 125.5(aromatic CH), 70.9(CH(OH)), 61.3(<u>C</u>$(CH_3)$N), 31.2($NCH_3$), 8.6($CH_3$—C).

## 6.7 ELECTROCHEMISTRY

### 6.7.1 Membrane Preparation

The membrane composition for the *o*NPOE based membranes was 1.2% ionophore, 65.6% *o*NPOE, 32.8% PVC and 0.4% potassium tetrakis(*p*-chlorophenyl) borate in 6 $cm^3$ THF. For the BBPA based membranes, the composition was 2.0% ionophore, 65.6% BBPA and 32.4% PVC in 10 $cm^3$ in THF.

The membranes were cast according to published procedures[5].

### 6.7.2 Calibration and Selectivity Measurements

A Philips IS (561) electrode body was used to mount the electroactive membranes. The reference electrode was a Philips double junction RE3/DJ electrode. The electrochemical cells were set up using two different inner filling solutions for the ion-selective electrode:

(1) Ag,AgCl | 0.1 mol $dm^{-3}$ EPHHCl | PVC membrane | Analyte ||
0.1 mol $dm^{-3}$ Li Acetate(salt bridge) | KCl(satd) | $Hg_2Cl_2$(s);Hg.

(2) Ag,AgCl | 1.0 mmol $dm^{-3}$ $NH_4Cl$ | PVC membrane | Analyte ||
0.1 mol $dm^{-3}$ Li Acetate (salt bridge) | KCl(satd) | $Hg_2Cl_2$(s);Hg.

A constant dilution technique was used for calibration and selectivity measurements. The selectivity measurements were performed in a background of 150.0 mmol $dm^{-3}$ NaCl, 4.3 mol $dm^{-3}$ KCl and 1.26 mol $dm^{-3}$ $CaCl_2$.

All emf measurements were made at 25 (0.1)°C.

### 6.7.3 Asymmetry Potential Measurements

The bias potential between two per-O-octylated α–CD/BBPA electrodes, both containing 1.0 mmol $dm^{-3}$ $NH_4Cl$ as the inner filling solution, one conditioned in (+) EPH.HCl and the other in (-) EPH.HCl, was measured in the cell shown in Fig 2. This potential is the asymmetry potential difference between the two electroactive membranes.

### 6.7.4 Behaviour of the Electrodes in Solutions of Varying Enantiomeric Excess

A range of solutions was prepared containing 0%-100% of the (+) and (-) enantiomers of ephedrine hydrochloride. The behaviour of the electrochemical cell containing 1.0 mmol $dm^{-3}$ $NH_4Cl$ inner filling solution and with the ion selective electrode mounted with an a CD/BBPA or a CD/oNPOE electroactive membrane conditioned either in (-) EPH.HCl or (+) EPH.HCl, was observed.

## 6.8 EXPERIMENTAL TO CHAPTER FIVE

### (±)-1,2-DIPHENYLETHANE-1,2-DIAMINE (15)

This compound was synthesized in a two step method similar to that described by Corey[6].

i)5-spirocyclohexyl-2,3-diphenylisoimidazole (15a)

A mixture of benzil (105g, 0.5mol), cyclohexanone(53cm$^3$, 0.5mol), ammonium acetate (400g) and acetic acid (1L) was boiled under reflux (118°C) for one hour. The mixture was slowly poured into vigorously stirred water (1.5L), stirred for 2h and left overnight. Crystals were then collected by filtration, washed with water (4x200cm$^3$), crushed to a fine powder and dried under reduced pressure to give the product as a slightly yellow powder (138g, 94%). This was used for the next step without further purification. An analytically pure sample was obtained by recrystallisation from (4:1) methanol/water to give 15a as a white crystalline solid. Melting point 106-107°C (lit[6] 107-108°C). $\delta_H(CDCl_3)$ 7.45(10H, m, aromatic H), 2.03(4H, m, $CH_2$), 1.83(6H, m, $CH_2$). $\delta_C(CDCl_3)$ 164.3(C=N), 133.6(aromatic C), 130.4(aromatic CH), 129.4(aromatic CH), 128.8(aromatic CH), 104.5(=N-C-N=), 35.2($CH_2$), 26.1($CH_2$), 24.6($CH_2$).

ii)(±)-1,2-DIPHENYLETHANE-1,2-DIAMINE

To a magnetically stirred solution of 15a (40.0g, 138.7mmol) in anhydrous tetrahydrofuran (200cm$^3$) and ammonia (250cm$^3$) at -78°C was added lithium (3.9g, 555mmol) in small pieces. Stirring was continued for 3h during which time a blue colour persisted. Ethanol (16.4cm$^3$, 277.4mmol) was then added in three portions every 15 minutes and stirring was continued for a further hour. Ammonium chloride (40.0g) was then added at -78°C, the cooling bath was removed and the ammonia slowly evaporated. When the temperature of the reaction mixture reached 0°C,

water ($200cm^3$) was added and the phases separated. The aqueous phase was washed with ether ($3x200cm^3$) and the combined organic extracts were washed with brine ($200cm^3$), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to a volume of $150cm^3$. Complete evaporation of the solvents for a $5cm^3$ sample gave pure 5-spirocyclohexyl-2,3-diphenylimidazolidine for analysis. $\delta_H(CDCl_3)$ 7.20(10H, s, aromatic H), 4.19(2H, s, CH), 2.10(2H, bs, NH), 1.71(8H, m, $CH_2$), 1.47(2H, m, $CH_2$). $\delta_C(CDCl_3)$ 140.3(aromatic C), 127.9(aromatic CH), 126.8(aromatic CH), 126.6(aromatic CH), 77.5(-N(H)-C-N(H)-), 69.2(HC-Ar), 39.3($CH_2$), 25.1($CH_2$), 23.5($CH_2$).

The resulting solution was cooled to 0°C and treated with 2M hydrochloric acid ($200cm^3$) and the biphasic mixture was vigorously stirred at room temperature overnight. Water ($300cm^3$) and dichloromethane ($200cm^3$) were then added and the phases separated. The organic phase was washed with distilled water ($2x100cm^3$) and the combined aqueous phases were washed with dichloromethane ($2x100cm^3$). The aqueous phase was treated with 2M sodium hydroxide ($200cm^3$) and then extracted into dichloromethane ($4x100cm^3$). The organic phase was dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure to give (±)-1,2-diphenylethane-1,2-diamine as a white crystalline solid (26.5g, 90%). Melting point 82-83°C (lit[6] 83°C). m/z (CI, ammonia) $213(M+1)^+$, $196(M-NH_2)^+$, $106(M/2)^+$. $\delta_H(CDCl_3)$ 7.32(10H, m, aromatic H), 4.16(2H, s, CH), 1.69(4H, bs, $NH_2$). $\delta_C(CDCl_3)$ 143.9(aromatic C), 128.7(aromatic CH), 127.5(aromatic CH), 127.4(aromatic CH), 62.4(CH).

**OPTICAL RESOLUTION OF (±)-1,2-DIPHENYLETHANE-1,2-DIAMINE (DPEDA).**

Optical resolution of (±)-DPEDA was carried out by fractional crystallisation using mandelic acid (MA) as a chiral resolving agent in a similar manner to that described by Saigo[7].

Racemic DPEDA (1.70g, 8mmol) and (+)-mandelic acid(2.43g, 16mmol were dissolved in hot ethanol (20cm$^3$). Cooling to room temperature gave white crystals which were collected by filtration and washed with ice cooled ethanol (2x1.5cm$^3$). Two recrystallisations from ethanol (25cm$^3$ then 20cm$^3$) followed by drying under high vacuum at 30°C gave the (+)DPEDA.(+)MA (1:2) diastereoisomeric salt (1.69g, 82%). Melting point 164-165°C (lit[7] 164-165°C). % Analysis found C,69.6; H, 6.3; N, 5.3; $C_{30}H_{32}N_2O_6$ requires C, 69.8; H, 6.2; N, 5.2%.

The diastereoisomeric salt (1,68g, 3.3mmol) was treated with 4M sodium hydroxide (36cm$^3$) and extracted with diethyl ether (3x40cm$^3$). The combined organic extracts were dried over sodium hydroxide pellets and concentrated under reduced pressure. Recrystallisation from hexanes gave (+)-DPEDA (0.61g, 72%). % Analysis found C, 79.3; H, 7.4; N, 13.0; $C_{14}H_{16}N_2$ requires C, 79.2; H, 7.6; N, 13.3%. $[\alpha]^{22}$ +106.0 (c1.09, methanol) (lit[7] +106.5)

The enantiomeric excess of the resolved (+) DPEDA was determined by proton NMR (400MHz) through the use of (S)-(-)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid ((-)-MTPA) as a chiral solvating agent in d-chloroform. Racemic (±)-DPEDA (25mg, 0.13mmol) and (-)-MTPA (63mg, 0.27mmol) in d-chloroform (1cm$^3$) gave anisochronous methine resonances in the $^1$H spectrum of (±)-DPEDA (δ=4.54(9) for (-)DPEDA and

δ=4.51(8) for (+)-DPEDA). The integration ratio of these signals was 1:1 and Δδ=0.31. For the resolved, (+), DPEDA (30mg, 0.14mmol) the use of (-)-MTPA (68mg, 0.29mmol) revealed only one methine resonance (δ=4.51(9)). No (-)-DPEDA signal could be observed despite repetition of the NMR experiment. This indicated an enantiomeric excess ≥99.5% for the resolved (+)-DPEDA.

**1R,2R-BIS(TOLYL-*p*-SULPHONYL)-1,2-DIPHENYL ETHANE-1,2-DIAMINE (16)**

A solution of 1R,2R-1,2-diphenylethane-1,2-diamine (15) (1.0g, 4.7mmol) in anhydrous tetrahydrofuran (15 $cm^3$) was stirred at 0°C in an ice bath under an atmosphere of dry nitrogen. Anhydrous (freshly distilled) triethylamine (1.16g, 1.6 $cm^3$, 11.5mmol) and then toluene-*p*-sulphonyl chloride (2.0g, 10.5mmol) were added sequentially. After addition was complete the reaction was kept at 0°C for 4h and then stored at 4°C overnight. The solvent was removed under reduced pressure to give a pale yellow solid. Recrystallisation from (10:1) methanol/chloroform then gave the required product as a white crystalline solid (1.91g, 78%). Melting point 209 - 209.6°C (lit[6] 202°C). % Analysis found C, 64.5; H, 5.5; N, 5.4; $C_{28}H_{28}N_2S_2O_4$ requires C, 64.6; H, 5.4; N, 5.4%. m/z (CI, ammonia) 538$(M+18)^+$, 521$(M+1)^+$, 350$(M\text{-}NHTs)^+$, 260$(M/2)^+$. $\delta_H(CDCl_3)$ 7.47(4H, d,J 8.1Hz, part of AA'XX' system, aromatic H), 6.83(10H, m, aromatic H), 6.68(4H, d, J8.1Hz, part of AA'XX' system, aromatic H), 5.77(2H, d, NH), 4.49(2H, d of d, CH), 2.31(6H, s, $CH_3$). $\delta_C(CDCl_3)$ 141.6(aromatic C), 138.3(aromatic C), 137.9(aromatic C), 128.8(aromatic CH), 127.4(aromatic CH), 127.3(aromatic CH), 126.6(aromatic CH), 126.1(aromatic CH), 62.4(CH), 20.8($CH_3$).

## 1-CHLORO-3-TETRAHYDROPYRANYLOXY PROPANE (17)

A solution of 3,4-dihydro-2H-pyran (6.0cm$^3$, 5.5g, 65.7mmol) and toluene-*p*-sulphonic acid (50mg) in anhydrous tetrahydrofuran (10cm$^3$) was stirred at 0°C in an ice bath under an atmosphere of nitrogen. 1-Chloropropan-3-ol (5cm$^3$, 5.66g, 60mmol) was added dropwise over a period of 30 minutes. After the addition was complete the resultant solution was stirred at 0°C for a further 1h then allowed to warm to room temperature before stirring overnight. The solvents were removed under reduced pressure to give a brown oil which was purified by short path distillation (50°C/0.5mmHg) to give the required product as a clear colourless oil (10.3g, 96.2%). Gas chromatographic analysis 95% (k′=12.6mins for 40→270°C at 10°min$^{-1}$). m/z (CI, ammonia) 196(M+18)$^+$, 179(M+1)$^+$. $\delta_H$($CDCl_3$) 4.61(1H, t, OC(H)O), 3.87(2H, m, $CH_2O$), 3.67(2H, t, $CH_2Cl$), 3.53(2H, m, $CH_2O$), 2.05(2H, m, $CH_2$), 1.67(6H, m, $CH_2$). $\delta_C$($CDCl_3$) 98.6(OC(H)O), 63.6($CH_2O$), 61.9($CH_2O$), 41.74($CH_2Cl$), 33.2($CH_2$), 31.0($CH_2$), 25.9($CH_2$), 19.9($CH_2$).

## 3-(TOLYL-*p*-SULPHONYLOXY))-1-(O-BENZYL)-PROPANE (18)

A mixture of propan-1,3-diol (70cm$^3$), sodium hydroxide (7.0g, 175mmol) and tetrabutylammonium hydrogensulphate (1.0g) was vigorously stirred and heated to 60°C under an atmosphere of nitrogen. A solution of benzyl chloride (20cm$^3$, 22g, 174mmol) in anhydrous tetrahydrofuran (30cm$^3$) was added dropwise over a 4h period. After complete addition the resultant reaction mixture was maintained at 60°C for a further 48h. The solvent was removed under reduced pressure, distilled water added (50cm$^3$) and the resultant mixture extracted into diethyl ether (4x50cm$^3$). The combined organic extracts were dried over anhydrous potassium carbonate, filtered and the solvent removed under reduced pressure. Excess propan-1,3-diol was removed by short path distillation and the

remaining 3-O-(benzyl ether)-propan-1-ol (18a) was used for the next step without further purification. $\delta_H(CDCl_3)$ 7.32(5H, s, aromatic H), 5.67(1H, bs, OH), 5.14(2H, s, Ar-C$\underline{H_2}$), 4.06(4H, m, $CH_2O$), 1.93(2H, quint., $CH_2$)

A stirred solution of (18a) (29.0g, 174mmol) in dry pyridine (100cm$^3$) was cooled to 0°C in an ice bath. Toluene-*p*-sulphonyl chloride (36.5g, 191mmol) was then added and the complete solution stored at -18°C for 4 days under a sealed atmosphere of nitrogen. The mixture was poured into ice (400cm$^3$) and the resultant oil extracted into diethyl ether (4x100cm$^3$). The combined organic extracts were washed with 2M hydrochloric acid (2x100cm$^3$), brine (100cm$^3$), distilled water(75cm$^3$) and dried over anhydrous magnesium sulphate. Filtration and removal of the solvents under reduced pressure gave the crude product as a pale yellow oil. Crystallisation at -78°C from a minimum volume of (1:3) ethyl acetate/hexanes gave the required product (18) as a light brown solid (37.9g, 68%). Melting point 33.4-34.1°C. % Analysis found C, 63.6; H, 6.3; $C_{17}H_{20}SO_4$ requires C, 63.8; H, 6.3 %. $\delta_H(CDCl_3)$ 7.78(2H, d, J7.6Hz,part of AA'XX' system, aromatic H), 7.31(2H, d, J7.6Hz,part of AA'XX' system, aromatic H), 7.24(5H, m, aromatic H), 4.40(2H, s, Ar-$CH_2$), 4.16(2H, t, J5.7Hz, part of an A'M' system, $CH_2$-OTs), 3.49(2H, t, J5.8Hz, part of an A'N' system, $CH_2$-OBz), 2.40(3H, s, $CH_3$), 1.93(2H, quint.,J5.8Hz, part of an A'M'N' system). $\delta_C(CDCl_3)$ 144.7(aromatic C), 138.1(aromatic C), 133.0(aromatic C), 129.8(aromatic CH), 128.8(aromatic CH), 127.9(aromatic CH), 127.6(aromatic CH), 127.5(aromatic CH), 73.0($\underline{C}H_2$-$C_6H_5$), 67.7($CH_2O$), 65.6($CH_2O$), 29.3($CH_3$), 21.6($CH_2$).

## N,N'-BIS(TOLYL-*p*-SULPHONYL)-ETHANE-1,2-DIAMINE (19)

Ethane-1,2-diamine ($10cm^3$, 9.0g, 149.6mmol) was stirred in anhydrous dichloromethane ($100cm^3$) with freshly distilled triethylamine ($50cm^3$, 36.4g, 359mmol) at -10°C in an ice salt slush bath under an atmosphere of nitrogen. A solution of toluene-*p*-sulphonyl chloride (63.0g, 330mmol) in anhydrous dichloromethane ($100cm^3$) was added dropwise, keeping the temperature below 0°C throughout the addition. After the addition was complete the reaction mixture was allowed to warm to room temperature and stirred overnight. Distilled water ($100cm^3$) was added and the organic layer separated. The organic layer was washed with 3M hydrochloric acid ($2x40cm^3$), distilled water ($2x80cm^3$), dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure. Recrystallisation from (10:1) methanol/chloroform gave (19) as a white crystalline solid (41.8g, 76%). Melting point 118 - 119 °C. % Analysis found C, 52.5; H, 5.5; N, 7.6; $C_{16}H_{20}N_2O_4S_2$ requires C, 52.5; H, 5.5; N, 7.7 %. m/z (CI, ammonia) $386(M+18)^+$, $369(M+1)^+$, $215(M\text{-}Ts)^+$. $\delta_H(CDCl_3)$ 7.06(4H, d, J7.8Hz, part of AA'XX' system, aromatic H), 6.72(4H, d, J7.8Hz, part of AA'XX' system, aromatic H), 5.83(2H, brs, NH), 2.34(4H, m, $CH_2$), 1.79(3H, s, $CH_3$). $\delta_C(CDCl_3)$ 141.6(aromatic C), 136.5(aromatic C), 128.5(aromatic CH), 125.8(aromatic CH), 41.7($CH_2$), 19.7($CH_3$).

## N,N'-BIS(TOLYL-*p*-SULPHONYL)-4,7-DIAZADECANE-1,10-DIOL (20)

A solution of N,N'-bis(tolyl-*p*-sulphonyl)ethane-1,2-diamine (19) (5.0g, 13.6mmol), potassium iodide (0.3g) and potassium carbonate (4.13g, 29.9mmol) in anhydrous DMF ($50cm^3$) was stirred under a nitrogen atmosphere. 1-Chloro-3-tetrahydropyranyloxypropane (17) (5.35g, 29.9mmol) was added and the complete mixture heated to 90°C. The reaction was monitored by tlc analysis (2% methanol/98% dichloromethane, silica; $R_f(19)=0.34$, $R_f(20a)=0.50$). After one week

further 1-chloro-3-tetrahydropyranyloxypropane (17) (1.0g, 5.6mmol) was added and the temperature increased to 120°C. After four weeks the solvent was removed under reduced pressure and the resulting dark brown oil partitioned between chloroform ($100cm^3$) and distilled water ($50cm^3$). The organic layer was separated, washed with distilled water, dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure. Column chromatography (2% methanol/98% dichloromethane, silica; $R_f$(20a)=0.50) gave the intermediate N,N'-bis(tolyl-*p*-sulphonyl)-1,10-bis(tetrahydropyranyloxy)-4,7-diazadecane (20a) as a clear colourless oil (3.71g, 42%). m/z (CI, ammonia) $670(M+18)^+$, $551(M\text{-}OTHP)^+$, $497(M\text{-}Ts)^+$, $485(M\text{-}2THP)^+$, $343(M\text{-}2Ts)^+$. $\delta_H(CDCl_3)$ 7.13(4H, d, J8.0Hz, part of AA'XX' system, aromatic H), 6.78(4H, d, J8.0Hz, part of AA'XX' system, aromatic H), 4.55(2H, brs, -O-C(H)-O-), 3.79(4H, m, $CH_2O$), 3.46(4H, m, $CH_2O$), 3.29(4H, s, N-$CH_2$-$CH_2$-N), 3.25(4H, m, $CH_2N$), 2.40(6H, s, $CH_3$), 1.85-1.49(16H, m, $CH_2$). $\delta_C(CDCl_3)$ 142.9(aromatic C), 135.3(aromatic C), 129.2(aromatic CH), 126.6(aromatic CH), 98.3(O-C(H)-O), 64.9($CH_2O$), 63.8($CH_2O$), 47.8($CH_2N$), 46.7($CH_2N$), 30.1($CH_3$), 28.4($CH_2$), 24.9($CH_2$), 20.9($CH_2$), 19.1($CH_2$).

To a stirred solution of N,N'-bis(tolyl-*p*-sulphonyl)-1,10-bis(tetrahydropyranyloxy)-4,7-diazadecane (20a) (3.7g, 5.7mmol) in methanol ($100cm^3$) was added 6M hydrochloric acid ($40cm^3$). The resultant mixture was refluxed for 1h, basified with 4M sodium hydroxide solution and then extracted into dichloromethane ($2x80cm^3$). Drying over anhydrous magnesium sulphate and removal of the solvents under reduced pressure then afforded N,N'-bis(tolyl-*p*-sulphonyl)-4,7-diazadecane-1,10-diol (20) as a pale yellow crystalline solid. % Analysis found C, 54.4; H, 6.7; N, 5.6; $C_{22}H_{32}N_2S_2O_6$ requires C, 54.5; H, 6.6; N, 5.8 %. m/z (CI, ammonia) 485 $(M+1)^+$, $427(M\text{-}(CH_2)_3OH)^+$, 331(M-

Ts), 242(M/2)$^+$. $\delta_H$($CDCl_3$) 7.68(4H, d, J7.5Hz, part of AA'XX' system, aromatic H), 7.34(4H, d, J7.5Hz, part of AA'XX' system, aromatic H), 3.69(4H, t, $CH_2OH$), 3.28(4H, s, N-$CH_2CH_2$-N), 3.23(4H, t, $CH_2N$), 3.15(2H, brs, OH), 2.43(6H, s, $CH_3$), 1.79(4H, quint., $CH_2$). $\delta_C$($CDCl_3$) 141.4(aromatic C), 133.2(aromatic C), 128.7(aromatic CH), 127.3(aromatic CH), 73.1($CH_2OH$), 48.1($CH_2N$), 47.3($CH_2N$), 29.8($CH_3$), 24.6($CH_2$).

**N,N'-BIS(TOLYL-*p*-SULPHONYL)-1,10-BIS(TOLYL-*p*-SULPHONYLOXY)-4,7-DIAZADECANE (21)**

A stirred solution of N,N'-bis(tolyl-*p*-sulphonyl)-4,7-diazadecane-1,10-diol (20) (2.5g, 5,2mmol) in anhydrous pyridine (30$cm^3$) was cooled to 0°C in an ice bath under an atmosphere of nitrogen. Solid toluene-*p*-sulphonyl chloride (3.0g, 15.7mmol) was added portionwise and the complete mixture stored at -18°C for 4 days. The mixture was poured into ice (100$cm^3$) with vigorous stirring. The resulting off white crystals were filtered, washed with distilled water (2x15$cm^3$) and dried *in vacuo* at room temperature (3.19g, 78%). Melting point 87.5-88.5°C. m/z (CI, ammonia) 810(M+18)$^+$, 793(M+1)$^+$, 637(M-Ts)$^+$, 621(M-OTs)$^+$, 450(M-2OTs)$^+$. $\delta_H$($CDCl_3$) 7.77(4H, d, J11.0Hz, part of AA'XX' system, aromatic H), 7.66(4H, d, J7.6Hz, part of AA'XX' system, aromatic H), 7.35(4H, d, J11.0Hz, part of AA'XX' system, aromatic H), 7.33(4H, d, J7.6Hz, part of AA'XX' system, aromatic H), 4.07(4H, t, $CH_2O$), 3.20(4H, s, $NCH_2CH_2N$), 3.14(4H, t, $CH_2N$), 2.44(12H, s, $CH_3$), 1.94(4H, quint., $CH_2$). $\delta_C$($CDCl_3$) 145.0(aromatic C), 143.9(aromatic C), 135.0(aromatic C), 132.7(aromatic C), 130.0(aromatic CH), 129.9(aromatic CH), 127.9(aromatic CH), 127.3(aromatic CH), 67.7($CH_2O$), 49.0($CH_2N$), 46.7($CH_2N$), 28.5($CH_3$), 21.6($CH_3$ or $CH_2$), 21.5($CH_3$ or $CH_2$).

**1,8-DICYANO-3,6-DIOXAOCTANE (22)**

A solution of aqueous sodium hydroxide ($40cm^3$, $2\%^w/_v$) and ethane-1,2-diol (74.4g, 1.2mol) was stirred at 0°C in an ice bath. Acrylonitrile was added dropwise over 1h and the complete reaction mixture was stirred for a further 2h at 0°C and then overnight at room temperature. The lower organic layer was separated, dried over anhydrous potassium carbonate and filtered. Purification by short path distillation (90-90°C, 0.1mmHg) then gave the product as a colourless oil which was identical with an authentic sample (170.5g, 85%). Gas chromatographic analysis 97%, k'=16.6mins (40°-270°C at $10°min^{-1}$). $\nu_{max}$ (thin film) ($cm^{-1}$) 2225(-CN). m/z (ammonia) $169(M+1)^+$, $98(M-CH_2CH_2CN)$. $\delta_H(CDCl_3)$ 3.58(4H, t, $CH_2O$), 3.53(4H, s, $CH_2CN$). $\delta_C(CDCl_3)$ 117.7(CN), 69.4($CH_2O$), 64.9($CH_2O$), 17.9($CH_2$).

**DIETHYL-4,7-DIOXADECAN-1,10-DIOATE (23)**

Concentrated sulphuric acid was added with care to a solution of ethanol (100ml) at 0°C. 1,8-Dicyano-3,6-dioxadecane (22) (50.2g, 0.3mol) was added and the mixture stirred under the conditions of reflux for 24h. The mixture was allowed to cool and evaporated to dryness under reduced pressure. Distilled water ($100cm^3$) was added to the residue and the mixture was extracted with dichloromethane ($3x20cm^3$) and then diethyl ether ($3x20cm^3$). The combined organic extracts were washed with brine ($2x20cm^3$), dried over anhydrous magnesium sulphate and concentrated under reduced pressure to give the crude product. The product was purified by short path distillation (40°C, 0.1mmHg) to give a clear oil (65.6g, 84%). Infra Red ($\upsilon_{max}$ $cm^{-1}$) 1735(C=O), 1110(C—O—C). m/z (CI, ammonia) $280(M+18)^+$, $263(M+1)^+$. $\delta_H(CDCl_3)$ 4.15(4H, q, J 7.0Hz, part of an $A_2'X_3'$ system, $C\underline{H_2}CH_3$), 3.75(4H, t, J6.4Hz, part of an $A_2'X_2'$ system, $CH_2O$), 3.61(4H, s, $CH_2O$), 2.60(4H, t, J6.4Hz, part of an $A_2'X_2'$ system,

$CH_2CO$), 1.25(6H, t, J 7.0Hz, part of an $A_2'X_3'$ system, $CH_2C\underline{H}_3$). $\delta_C(CDCl_3)$ 171.3(C=O), 68.7($CH_2O$), 66.3($CH_2O$), 60.1($\underline{C}H_2CH_3$), 34.8($\underline{C}H_2CO$), 13.9($CH_3$).

**4,7-DIOXADECAN-1,10-DIOL (24)**

A suspension of lithium aluminium chloride (4 molar equivalents, 20.0g) in anhydrous diethyl ether (50$cm^3$) was mechanically stirred at 0°C in an ice bath under a nitrogen atmosphere. A solution of diethyl-4,7-dioxa-1,10-dioate (23) (38.8g, 148mmol) in anhydrous diethyl ether was added portionwise at such a rate as to maintain gentle reflux of the system. After complete addition the mixture was stirred at 0°C for a further 2h before warming to room temperature and stirring overnight. The reaction mixture was then cooled to 0°C and distilled water (20$cm^3$) added <u>slowly</u> to the vigorously stirred solution. This was followed by addition of 15% sodium hydroxide solution (40$cm^3$) and distilled water (20$cm^3$). The resultant white precipitate was filtered, washed with diethyl ether (5x100$cm^3$). The combined organic extracts and the filtrate were then concentrated under reduced pressure to give a viscous oil. Short path distillation (90°C, 0.05mmHg) then gave the required product as a clear oil (14.1g, 56%). Infra Red ($\upsilon_{max}$ $cm^{-1}$) 3550-3210(OH), 1105(C—O—C). m/z (CI, ammonia) 197$(M+18)^+$, 179$(M+1)^+$. $\delta_H(CDCl_3)$ 3.60(4H, t, J 6.0Hz, part of an $A_2'X_2'$ system, $C\underline{H}_2OH$), 3.52(4H, t, J6.0Hz, part of an $A_2'X_2'$ system, $CH_2O$), 3.46(4H, s, $CH_2O$), 1.70(4H, q, J 6.0Hz, part of an $A_2'X_2'$ system, $CH_2C\underline{H}_3$). $\delta_C(CDCl_3)$ 69.4($CH_2O$), 68.4($CH_2O$), 59.1($CH_2OH$), 31.8($CH_2\underline{C}H_2CH_2$).

**1,10-BIS(TOLYL-*p*-SULPHONYLOXY)-4,7-DIOXADECANE (25)**

4,7-dioxa-decane-1,10-diol (24) (10.0g, 56.2mmol) was stirred in anhydrous pyridine (200cm$^3$) at 0°C in an ice bath under an atmosphere of nitrogen. Solid toluene-*p*-sulphonyl chloride (32.1g, 168.5mmol) was added and stirring was continued for 1h. The reaction mixture was stored at -18°C for 3 days. The mixture was poured into ice (400cm$^3$), with stirring and the resultant crystals were collected by filtration. The white crystals were washed with distilled water (2x50cm$^3$) and dried under reduced pressure at room temperature (23.2g, 85%). % Analysis found C, 54.0; H, 6.2; $C_{22}H_{30}S_2O_8$ requires C, 54.3; H, 6.2%. m/z (CI, ammonia) 504$(M+18)^+$, 487$(M+1)^+$, 331$(M\text{-}Ts)^+$. $\delta_H(CDCl_3)$ 7.70(4H, d, J8.8Hz, part of AA'XX' system, aromatic H), 7.27(4H, d, J8.8Hz, part of AA'XX' system, aromatic H), 4.04(4H, t, $CH_2O$), 3.35(8H, m, $CH_2O$), 2.38(6H, s, $CH_3$), 1.81(4H, quint., $CH_2$). $\delta_C(CDCl_3)$ 144.6(aromatic C), 132.9(aromatic C), 129.8(aromatic CH), 127.7(aromatic CH), 70.0($CH_2O$), 66.6($CH_2O$), 66.4($CH_2O$), 29.1($CH_3$), 21.5($CH_2$).

**1,14-BIS(TOLYL-*p*-SULPHONYLOXY)-3,6,9,12-TETRAOXA TETRADECANE (26)**

A stirred solution of 3,6,9,12-tetraoxatetradecane-1,14-diol (6.0g, 25mmol) in anhydrous pyridine (60cm$^3$) was cooled to 0°C in an ice bath under an atmosphere of nitrogen. Solid toluene-*p*-sulphonyl chloride (19.1g, 100mmol) was added portionwise and the complete mixture stirred at 0°C for two hours and the stored at -18°C for 3 days. The mixture was poured into ice with stirring and the resulting oil extracted into diethyl ether (3x100cm$^3$). The combined organic extracts were washed with 2M hydrochloric acid (3x50cm$^3$), brine (100cm$^3$), distilled water (75cm$^3$) and then dried over anhydrous magnesium suphate. Solvents were removed under reduced pressure to give a pale yellow oil (9.58g, 69%) which could

not be recrystallised. m/z (CI, ammonia) 564$(M+18)^+$, 391$(M-Ts)^+$. $\delta_H(CDCl_3)$ 7.79(4H, d, J9.9Hz, part of AA'XX' system, aromatic H), 7.34(4H, d, J9.8Hz, part of AA'XX' system, aromatic H), 4.16(4H, t, $CH_2O$), 3.68(4H, t, $CH_2O$), 3.59(12H, m, $CH_2O$), 2.45(6H, s, $CH_3$).

**1-(METHANE SULPHONYL)-5-METHOXY-3-OXAPENTANE (27)**

A solution of 2-(2-methoxyethoxy) ethanol (15.0g, 125mmol) in anhydrous tetrahydrofuran (200$cm^3$) was stirred at -10°C in an ice salt slush bath under an atmosphere of nitrogen. Anhydrous (freshly distilled) triethylamine (34.8$cm^3$, 250mmol) was added slowly and the mixture stirred for 30 minutes. Methane sulphonyl chloride (16.3g, 142mmol) was added dropwise over a 1h period. The complete reaction mixture was allowed to warm to room temperature and then stirred overnight. As the reaction proceeded a white precipitate of triethylamine hydrochloride formed in a pale yellow solution. The mixture was extracted into dichloromethane (3x100$cm^3$) and the combined organic extracts were washed with 3M hydrochloric acid until the washings were acidic. The organic phase was dried over anhydrous magnesium sulphate and the solvents removed under reduced pressure to give the product as a pale yellow oil (18.3g, 80%). m/z (CI, ammonia) 216$(M+18)^+$, 199$(M+1)^+$, 138$(M-SO_2CH_3)^+$. $\delta_H(CDCl_3)$ 4.37(2H, m, $CH_2O$), 3.74(2H, m, $CH_2O$), 3.64(2H, m, $CH_2O$), 3.56(2H, m, $CH_2O$), 3.36(3H, s, $CH_3O$), 3.07(3H, s, $CH_3SO_2$).

**1-IODO-5-METHOXY-3-OXAPENTANE (28)**

Potassium iodide was added to a stirred solution of 1-(methane sulphonyl)-5-methoxy-3-oxapentane (27) (10.0g, 50mmol) in anhydrous DMF (70$cm^3$) under a nitrogen atmosphere. The mixture was heated to 50°C overnight. Solvents were removed under reduced pressure

(50°C/0.25mmHg) and the crude mixture extracted into dichloromethane ($3x50cm^3$). The combined organic extracts were washed with water ($2x25cm^3$), dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure to give the product (27) as a orange oil (9.8g, 68%). This product was used without further purification. $\delta_H(CDCl_3)$ 3.69(6H, m, $CH_2O$), 3.32(3H, s, $CH_3$), 3.20(2H, t, $CH_2I$).

**1-(TOLYL-*p*-SULPHONYL)-5-METHOXY-3-OXAPENTANE (29)**

A stirred solution of 2-(2-methoxyethoxy) ethanol (6.0g, 50mmol) in anhydrous pyridine ($70cm^3$) was cooled to 0°C in an ice bath under an atmosphere of nitrogen. Solid toluene-*p*-sulphonyl chloride was added (19.1g, 100mmol) and the complete reaction mixture stored at -18°C for 3 days. The mixture was poured into ice ($300cm^3$) and the resulting oil extracted into diethyl ether ($3x100cm^3$). The combined organic extracts were washed with 2M hydrochloric acid ($2x75cm^3$), brine ($100cm^3$), distilled water ($75cm^3$) and then dried over anhydrous magnesium sulphate. Removal of solvents under reduced pressure gave a pale yellow oil (9.4g, 69%) which could not be recrystallised. m/z (CI, ammonia) $292(M+18)^+$, $137(M-Ts)^+$. $\delta_H(CDCl_3)$ 7.79(2H, d, J9.0Hz, part of AA'XX' system, aromatic H), 7.27(2H, d, J9.0Hz, part of AA'XX' system, aromatic H), 4.17(2H, t, $CH_2O$), 3.68(2H, t, $CH_2O$), 3.51(4H, m, $CH_2O$) 3.33(3H, s, $CH_3$), 2.43(3H, s, $CH_3$). $\delta_C(CDCl_3)$ 143.9(aromatic C), 132.4(aromatic C), 129.2(aromatic CH), 127.1(aromatic CH), 70.8($CH_2O$), 69.6($CH_2O$), 68.6($CH_2O$), 67.5($CH_2O$), 57.9($CH_3O$), 20.57($CH_3$).

**1-CHLORO-5-METHOXY-3-OXAPENTANE (30)**

A stirred solution of 2-(2-methoxyethoxy)ethanol (6.2g, 52mmol) and anhydrous pyridine (4.7g, 60mmol) in anhydrous toluene ($35cm^3$) was cooled to 0°C under a nitrogen atmosphere. Thionyl chloride (3.2g, 75mmol) was added dropwise over a 2h period and the complete mixture heated to 50°C for 24h. The mixture was allowed to cool and 2M hydrochloric acid ($5cm^3$) was added dropwise with vigorous stirring. On standing two layers separated and the lower, aqueous, layer was discarded. The organic layer was washed with distilled water ($2x15cm^3$), dried over anhydrous magnesium sulphate and the solvents removed under reduced pressure to give a pale brown oil. Short path distillation (65°C/0.1mmHg) gave the required product as a colourless oil. m/z (CI, ammonia) $158(M+18)^+$, $156(M+18)^+$, $123(M-CH_3)^+$. $\delta_H(CDCl_3)$ 3.72(6H, m, $CH_2O$), 3.43(3H, s, $CH_3$).

**1,4-DI-O-BENZYL-L-THREITOL (31)**

This compound was synthesized in a similar manner to that described by Marsh et al[8]

A stirred solution of 2,3-di-isopropylidene-L-threitol (5.0g, 31mmol), sodium hydroxide (3.0g, 75mmol), and a catalytic amount of tetrabutylammonium hydrogen sulphate (0.3g) in anhydrous tetrhydrofuran ($75cm^3$) was heated to reflux under a nitrogen atmosphere. Benzyl chloride (9.0g, 71mmol) was added dropwise over a 1h period and the complete mixture stirred under reflux for 24h. The reaction mixture was allowed to cool, filtered and the solvent removed under reduced pressure to give a pale yellow oil. Short path distillation (148°-153°C/0.1mmHg) gave the required ketal as a colourless oil (9.4g, 89%). $\delta_H(CDCl_3)$ 7.32(10H, s, aromatic H), 4.64(4H, s, Ar-$CH_2O$-), 4.18(2H, s, CH), 3.58(4H, s, $CH_2O$), 1.62(6H, s, $CH_3$).

The ketal (9,4g, 27.5mmol) was dissolved in methanol ($50cm^3$) and 0.5M hydrochloric acid was added ($5cm^3$). Acetone and methanol were slowly distilled off and the reaction monitored by tlc ( 50% ethyl acetate/50% hexanes, silica; $R_f$(ketal=0.6), $R_f$(diol)=0.2). After 4h additional methanol ($10cm^3$) and 0.5M hydrochloric acid were added ($3cm^3$) and the mixture stored overnight. The reaction mixture was diluted with saturated sodium hydrogencarbonate solution ($80cm^3$) and extracted into diethyl ether ($3x80cm^3$). The combined organic extracts were dried over anhydrous magnesium sulphate, filtered and concentrated under reduced pressure to give a pale yellow solid. Recrystallisation from (1:1) chloroform/hexanes gave the product as a white waxy solid. Melting point 56°-57°C (lit[8] 56°-57°C). m/z (CI, ammonia) $320(M+18)^+$, $211(M-Bz)^+$, $150(M/2)^+$. $\delta_H(CDCl_3)$ 7.30(10H, s, aromatic H), 4.50(4H, s, Ar-$CH_2O$-), 3.83(2H, s, CH), 3.57(4H, s, $CH_2O$), 2.92(2H, brs, OH). $\delta_C(CDCl_3)$ 137.7(aromatic C), 128.4(aromatic CH), 127.7(aromatic CH), 126.8(aromatic CH), 73.5(Ar-$CH_2O$), 70.9($CH_2O$), 70.5(CH).

**S,S-(-)-N,N,N',N'-TETRAMETHYLTARTRAMIDE (32)**

This compound was synthesized in a similar manner to that described by Seebach[9].

A solution of S,S-(-)-diethyltartrate (10.3g, 50.1mmol) in anhydrous methanol ($50cm^3$) was stirred at -10°C in an ice salt slush bath under an atmosphere of nitrogen. Dimethylamine (101mmol, $18cm^3$ of a 5.6M solution in ethanol) was added dropwise over a 1h period. The complete reaction mixture was allowed to warm to room temperature and stirred overnight under a nitrogen atmosphere. As the reaction proceeded the solution darkened to an orange viscous mixture and the extent of reaction was monitored by infra red ($\nu_{max}(cm^{-1})$,nujol mull, 1730(ester

C=O), 1638(amide C=O)). The solvents were removed under reduced pressure and the crude product was recrystallised from (1:1) ethanol/hexanes to afford a white crystalline solid (6.9g, 58%). % Analysis found C, 23.0; H, 3.2; N, 1.3; $C_8H_{16}N_2O_4$ requires C, 23.2; H, 3.3; N, 1.3%. Melting point 185°-186.5°C (lit[9]. 187°C). m/z (CI, ammonia) 222$(M+18)^+$, 160$(M-N(CH_3)_2)^+$. $\delta_H(CDCl_3)$ 4.66(2H, s, CH), 4.20(2H, s, OH), 3.10(3H, s, $CH_3N$), 2.90(3H, s, $CH_3N$).

**1,14-DIIODO-3,6,9,12-TETRAOXATETRADECANE (33)**

Potassium iodide (7.1g, 43mmol) was added to a stirred solution of 1,14-bis(tolyl-*p*-sulphonyloxy)-3,6,9,12-tetraoxatetradecane (26) (9.6g, 17mmol) in anhydrous DMF (55$cm^3$). The complete mixture was heated to 50°C and stirred overnight under a nitrogen atmosphere. Solvents were removed under reduced pressure (50°C/0.25mmHg) and the resulting crude mixture extracted into dichloromethane (2x50$cm^3$). The combined organic extracts were washed with distilled water (2x30$cm^3$), dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure to give the product as an orange oil (5.1g, 68%). m/z (CI, ammonia) 476$(M+18)^+$, 331$(M-I)^+$. $\delta_H(CDCl_3)$ 3.76(4H, t, $CH_2O$), 3.67(12H, brs, $CH_2O$), 3.28(2H, t, $CH_2I$).

**N,N'-BIS(TOLYL-*p*-SULPHONYL)-1,12-DIOXA-5,8-DIAZA CYCLOTETRADECANE (34)**

Caesium carbonate (5.81g, 17.9mmol) was added to a solution of 4,7-dioxa-1,10-bis(tolyl-*p*-sulphonyloxy)decane (25) (3.96g, 8.2mmol) in anhydrous DMF (40$cm^3$) under a nitrogen atmosphere. A solution of N,N'-bis(tolyl-*p*-sulphonyl) ethane-1,2-diamine (19) (3.0g, 8.2mmol) in anhydrous DMF (40$cm^3$) was added dropwise over a 4h period with vigorous stirring. The reaction was stirred at room temperature for 12h and heated to 60°C for

4h. The solvent was removed under reduced pressure and the residue taken up in dichloromethane (100cm$^3$) and washed with distilled water (2x100cm$^3$). The organic layer was dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure to give an off white solid. Recrystallisation from hot toluene (35cm$^3$) gave the product on cooling (2.41g, 58%). % Analysis found C, 56.2; H, 6.6; N, 5.5; $C_{24}H_{34}N_2S_2O_6$ requires C,56.4; H, 6.7; N, 5.5%. m/z (CI, ammonia) 528(M+18)$^+$, 511(M+1)$^+$, 355(M-Ts)$^+$, 200(M-2Ts)$^+$. $\delta_H(CDCl_3)$ 7.64(4H, d, J8.1Hz, part of AA'XX' system, aromatic H), 7.27(4H, d, J8.1Hz, part of AA'XX' system, aromatic H), 3.49(4H, s, $NCH_2CH_2N$), 3.40(4H, t, J5.6Hz, part of an A'X' system, $CH_2O$), 3.24(4H, t, J5.1Hz, part of an A'X' system, $CH_2N$), 2.45(6H, s, $CH_3$), 1.83(4H, m, $CH_2$). $\delta_C(CDCl_3)$ 143.8(aromatic C), 136.8(aromatic C), 130.2(aromatic CH), 127.8(aromatic CH), 69.7($CH_2O$), 66.3($CH_2O$), 48.7($CH_2N$), 46.6($CH_2N$), 30.8($CH_3$), 22.0($CH_2$).

## 6.9 ATTEMPTED SYNTHESES

### 5R,6R-N,N'-BIS(TOLYL-*p*-SULPHONYL)-5,6-DIPHENYL-1,10-BIS(O-BENZYL)-4,7-DIAZADECANE

Caesium carbonate (1.37g, 4.2mmol) was added to a stirred solution of 1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenylethane-1,2-diamine (16) (1g, 1.9mmol) in anhydrous DMF (15cm$^3$) under a nitrogen atmosphere. A solution of 1-(tolyl-*p*-sulphonyloxy)-3-(O-benzyl)-propane (18) (1.6g, 5.0mmol) in anhydrous DMF (15cm$^3$) was added dropwise over a period of 4h with vigorous stirring. The complete reaction mixture was heated to 60°C and the reaction monitored by tlc ($R_f$(16)= 0.28, $R_f$(18)= 0.45; 2% methanol/98% dichloromethane). After one week further alkylating agent (18) was added (0.8g, 2.5mmol) and the reaction temperature increased to 90°C for 7 days. Further (18) was added (0.8g, 2.5mmol) and

the reaction temperature increased to 110°C for two weeks. Solvents were removed under reduced pressure and the crude mixture partitioned between dichloromethane (100cm$^3$) and distilled water (100cm$^3$). The organic layer was separated, dried over anhydrous magnesium sulphate and the solvents removed under reduced pressure. Proton and carbon-13 NMR showed no diagnostic evidance of product formation (only one $CH_2N$ signal). This was further confimed by mass spectral analysis.

**5R,6R-N,N'-BIS(TOLYL-*p*-SULPHONYL)-1,10-BIS (TETRAHYDROPYRANYLOXY)-5,6-DI-PHENYL-4,7-DIAZADECANE AND**

**2R,3R-N,N'-BIS(TOLYL-*p*-SULPHONYL)-7-TETRAHYDROPYRANYLOXY-2,3-DIPHENYL-1,4-DIAZAHEPTANE (35)**

To a solution of caesium carbonate (3.0g, 9.23mmol) and 1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenylethane-1,2-diamine (16) (2.0g, 3.85mmol) in anhydrous DMF (20cm$^3$) was added 1-chloro-3-tetrahydropyranyloxy propane (17) (2.75g, 15.4mmol) and a catalytic amount of potassium iodide (0.5g), under a nitrogen atmosphere. The complete reaction mixture was heated to 90°C and monitored by tlc ($R_f$(ditosylamide)=0.30, 2%methanol/98%dichloromethane; silica). After one week tlc analysis indicated complete disappearance of (16) and the solvents were removed under reduced pressure. The crude reaction mixture was extracted into dichloromethane (2x50cm$^3$). The organic phase was washed with distilled water (2x25cm$^3$), dried over anhyrous magnesium sulphate, filtered and the solvent removed under reduced pressure to give a pale yellow oil. Mass spectral analysis (CI, ammonia) on this crude product indicated the presence of (35) [680(M+18$^+$] but showed no diagnostic ion for the dialkylated species. Column chromatography (2%methanol / 98% dichloromethane, silica) gave (35)

as a clear colourless oil (1.39g, 55%). m/z (CI, ammonia) 680(M+18)$^+$, 663(M+1)$^+$, 577(M-THP)$^+$. $\delta_H(CDCl_3)$ 7.66(2H, d, J7.5Hz, part of AA'XX' system, aromatic H), 7.28(2H, d, J7.4Hz, part of AA'XX' system, aromatic H), 7.25(2H, d, J7.5Hz, part of AA'XX' system, aromatic H), 6.95(10H, m, aromatic H), 6.71(2H, d, J7.5Hz, part of AA'XX' system, aromatic H), 6.31(1H, d, J6.4Hz, part of an A'M' system, NH), 5.16(1H, d of d, J11.5Hz,6.4Hz, part of an A'M'X' system, C<u>H</u>NHTs), 4.92(1H, d, J11.5Hz, part of an A'X' system, C<u>H</u>N(Ts)$CH_2$), 4.63(1H, t, OC(H)O), 3.81(2H, t, $CH_2O$), 3.53(2H, m, $CH_2N$), 2.41(3H, s, $CH_3$), 2.26(3H, s, $CH_3$), 1.96(2H, m, C<u>H$_2$</u>$CH_2O$), 1.65(6H, m, $CH_2$). $\delta_C(CDCl_3)$ 143.6(aromatic C), 142.1(aromatic C), 138.1(aromatic C), 137.9(aromatic C), 137.2(aromatic C), 133.5(aromatic C), 129.67(aromatic CH), 129.64(aromatic CH), 129.56(aromatic CH), 129.54(aromatic CH), 129.51(aromatic CH), 129.32(aromatic CH), 129.26(aromatic CH), 128.95(aromatic CH), 128.87(aromatic CH), 128.81(aromatic CH), 128.76(aromatic CH), 128.57(aromatic CH), 127.68(aromatic CH), 127.54(aromatic CH) 127.48(aromatic CH), 127.38(aromatic CH), 126.92(aromatic CH), 126.61(aromatic CH) 98.4(OC(H)O), 64.5($CH_2O$), 63.7($CH_2O$), 62.4(CHN), 57.7(CHN), 45.2($CH_2N$), 30.5($CH_3$), 29.9($CH_3$), 25.2($CH_2$), 21.2($CH_2$), 21.0($CH_2$), 13.9($CH_2$).

**9R,10R-N,N'-BIS(TOLYL-*p*-SULPHONYL)-9,10-DIPHENYL-1,4-DIOXA-8,11-DIAZACYCLOTETRADECANE**

Caesium carbonate (1.4g, 4.3mmol) was added to a solution of 1,10-bis(tolyl-*p*-sulphonyloxy)-4,7-dioxadecane (25) (1.0g, 2.06mmol) in anhydrous DMF (10cm$^3$) under a nitrogen atmosphere. A solution of 1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenylethane-1,2-diamine (16) (1.0g, 1.92mmol) in anhydrous DMF (10cm$^3$) was added dropwise over a 2h period with vigorous stirring. The reaction mixture was stirred at room temperature for 24h and then heated to 60°C for 3 days and

monitored by tlc ($R_f$(ditosylamide)=0.30, 2%methanol/98%dichloromethane; silica). Further (25) was added (0.2g, 0.4mmol) and the temperature increased to 90°C for 7 days. The solvent was removed under reduced pressure and the residue taken up in dichloromethane (50cm$^3$) and washed with distilled water (2x30cm$^3$). The organic phase was dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure to give a pale yellow solid. Recrystallisation from hot toluene (10cm$^3$) gave pure starting material (16) (0.32g). Mass spectral and proton NMR analysis of the filterate showed no evidence of product formation.

**9R,10R-9,10-DIPHENYL-7,12-DIOXO-1,4-DIOXA-8,11-DIAZACYCLOTETRADECANE**

To a stirred solution of diethyl-4,7-dioxadecane-1,10-methanoate (23) (1.05g, 4.5mmol) in anhydrous methanol (45cm$^3$) under an atmosphere of nitrogen was added solid 1R,2R-1,2-diphenyl ethane-1,2-diamine (16) (0.95g, 4.48mmol). The complete reaction mixture was stirred at room temperature for 7 days after which time proton NMR analysis (solvent removed under reduced pressure for a 2cm$^3$ sample followed by addition of 1cm$^3$ of d-chloroform and filtration) showed no appreciable reaction. The reaction temperature was increased to reflux for 7 days after which time mass spectral, proton NMR and carbon-13 NMR analysis showed only starting materials present in the mixture.

**2R,3R-N,N',N'',N'''-TETRAKIS(TOLYL-*p*-SULPHONYL)-1,4,8,11-TETRAAZA-2,3-DIPHENYL CYCLOTETRADECANE**

Caesium carbonate (1.52g, 4.66mmol) was added to a solution of N,N'-bis(tolyl-*p*-sulphonyl)-1,10-bis(tolyl-*p*-sulphonyloxy)-4,7-diazadecane (21) (1.71g, 2.17mmol) in anhydrous DMF (15cm$^3$) under a nitrogen

atmosphere. A solution of 1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenyl ethane-1,2-diamine (16) (1.1g, 2.12mmol) in anhydrous DMF ($10cm^3$) was added dropwise over a period of 2h with vigorous stirring. The reaction mixture was stirred at room temperature overnight and heated to 60°C for 3 days. Tlc analysis (2%methanol/98%dichloromethane; silica) showed total consumption of N,N'-bis(tolyl-*p*-sulphonyl)-1,10-bis(tolyl-*p*-sulphonyloxy)-4,7-diazadecane (21) ($R_f$=0.30) and a further 0.5g (0.63mmol) was added. Stirring was continued at 80°C for 7 days. Solvents were removed under reduced pressure and the residue taken up in dichloromethane ($60cm^3$) and washed with distilled water ($2x40cm^3$). The organic layer was dried over anhydrous magnesium sulphate, filtered and the solvents removed under reduced pressure to give a pale yellow solid. Mass spectral, proton NMR and carbon-13 NMR analysis gave no evidence of product formation.

1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenylethane-1,2-diamine (16) was recovered by recrystallisation from hot toluene (0.6g, 55%)

**DIMETHYL-5R,6R-N,N'-BIS(TOLYL-*p*-SULPHONYL)-4,7-DIAZA-5,6-DIPHENYLDECANE-1,10-DIOATE**

A mixture of 1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenyl ethane-1,2-diamine (16) (1.0g, 1.9mmol), methyl acrylate (1.0g, 11.5mmol), potassium carbonate (0.55g, 4.0mmol) and anhydrous DMF ($20cm^3$) was stirred and heated to 80°C under reflux for 48h. The solvents were removed under reduced pressure to give a dark brown oil. Mass spectral, proton NMR and carbon-13 NMR analysis gave no evidence of product formation.

1R,2R-N,N'-bis(tolyl-*p*-sulphonyl)-1,2-diphenyl ethane-1,2-diamine (16) was recovered by recrystallisation from hot toluene (0.3g, 30%).

**1,14-DIMETHOXY-7S,8S-N,N,N',N'-TETRAMETHYLTARTRAMIDE-3,6,9,12-TETRAOXATETRADECANE**

1-Chloro-5-methoxy-3-oxapentane (30) (1.0g, 7.2mmol) and a catalytic quantity of tetrabutylammonium hydrogensulphate (0.1g) were added to a stirred solution of 1S,2S-N,N,N',N'-tetramethyltartramide (32) (0.85g, 4.4mmol) in anhydrous tetrahydrofuran under a nitrogen atmosphere. The complete reaction mixture was heated to reflux and the reaction monitored by tlc (100% dichloromethane, alumina; $R_f$(30)=0.75). After 5 days further 1-chloro-5-methoxy-3-oxapentane (30) (0.5g, 3.6mmol) was added and reflux continued for 1 week. Solvents were evaporated under reduced pressure. Mass spectral, proton NMR and carbon-13 NMR analysis gave no evidence of product formation.

**1,14-DIMETHOXY-7S,8S-N,N,N',N'-TETRAMETHYLTARTRAMIDE-3,6,9,12-TETRAOXATETRADECANE**

1-(tolyl-*p*-sulphonyloxy)-5-methoxy-3-oxapentane (29) (2.0g, 7.3mmol) was added to a stirred solution of sodium hydride (340mg, 14.2mmol) and 1S,2S-N,N,N',N'-tetramethyltartramide (32) (0.70g, 3.5mmol) in anhydrous DMF (15cm$^3$) under a nitrogen atmosphere. The complete reaction mixture was heated to 55°C for 5 days. Solvents were removed under reduced pressure and the resulting brown cake extracted into chloroform (2x30cm$^3$). The combined organic extracts were washed with distilled water (2x20cm$^3$), dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure to give a brown viscous oil. Mass spectral, proton NMR and carbon-13 NMR analysis gave no evidence of product formation.

**2R,3R-2,3-BENZOYL-1,4,7,10,13,16-HEXAOXACYCLOOCTADECANE**

Potassium-t-butoxide (10mmol) and 1,14-bis(tolyl-*p*-sulphonyloxy)-3,6,9,12-tetraoxadecane (26) as 0.25M solutions in dimethoxy ethane were added dropwise to a stirred solution of 2R,3R-1,4-bis(benzoyl)-threitol (31) (3.0g, 9,9mmol) and Potassium-t-butoxide (10mmol) in dimethoxy ethane ($200cm^3$) under a nitrogen atmosphere. The complete reaction mixture was stirred at room temperature and the reaction monitored by tlc (1%methanol/99%dichloromethane, alumina, $R_f(31)=0.28$). After 10 days solvents were removed under reduced pressure to give the crude product as a yellow oil. Partial purification was achieved by column chromatography ( 3→8% methanol in dichloromethane, alumina, Rf(product)=0.3 with 8% methanol) to give a pale yellow oil. Further purification attempts by recrystallisation, distillation,preparative thin layer chromatography and reversed phase HPLC proved fruitless. m/z (CI, ammonia) $522(M+18)^+$.

**2S,3S-N,N,N',N'-TETRAMETHYLAMIDE-1,4,7,10,13,16-HEXAOXA CYCLOOCTADECANE**

Thallium (I) ethoxide (750µl, 10.4mmol) was added to a vigorously stirred solution of 1S,2S-N,N,N',N'-tetramethyl tartramide (32) (1.0g, 5.2mmol) in anhydrous acetonitrile ($20cm^3$). This mixture was stirred for 15 minutes and then solvents were removed under reduced pressure to give an off white paste. Anhydrous DMF ($20cm^3$) was added and the mixtured stirred for 30 minutes before addition of 1,14-diiodo-3,6,9,12-tetraoxatetradecane (33) (2.4g, 5.2mmol). The complete mixture was stirred at room temperature for 4h and then heated to 60°C overnight. After cooling distilled water ($20cm^3$) was added and an orange solid (TlI) removed by filtration. Column chromatography (100% dichloromethane, alumina) gave initial purification. However, further purification

attempts by recrystallisation, distillation and preparative thin layer chromatography proved fruitless. m/z (CI, ammonia) 407$(M+1)^+$.

## REFERENCES


1) G. C. Levy, I. R. Peat J. Magn. Reson. (1975) 18 500

2) A. B. Bax D. G. Davis J. Magn. Res. (1985) 63 207

3) A. Bax, R. Freeman, G. Morris J. Magn. Reson. (1981) 42 164; J. A. Wilde, P. H. Bolton J. Magn. Reson. (1984) 59 343

4) P. Mischnick-Lübbecke, R. Krebber Carbohydr. Res. (1989) 187 197

5) A. Craggs, G. J. Moody, J. D. R. Thomas J. Chem. Educ. (1974) 51 541

6) E. J. Corey, R. Imwinkleried, S. Pikul, Y. B. Xiang J. Am. Chem. Soc. (1989) 111 5493 (supplementary material)

7) K. Saigo, N. Kubota, S. Takebayshi, M. Hagegawa Bull. Chem. Soc. Jpn. (1986) 59 931

8) E. A. Marsh, S. van Deusen, S. B. Hemperly Organic Synthesis 68 92

9) D. Seebach, H. Dörr, B. Bastani, V. Shrig Angew. Chem. Int. Ed. Engl. (1975) 14 762

# APPENDICES

# COLLOQUIA, CONFERENCES AND PUBLICATIONS

UNIVERSITY OF DURHAM

*Board of Studies in Chemistry*

COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

12 February 1990 to 31 July 1990

**Lunazzi**, Prof. L. (University of Bologna) 12.02.90

*Application of Dynamic NMR to the Study of Conformational Enantiomerism*

**Sutton**, Prof. D. (Simon Fraser University, Vancouver) 14.02.90

*Synthesis and Applications of Dinitrogen and Diazo Compounds of Rhenium and Iridium* *

**Crombie**, Prof. L. (University of Nottingham) 15.02.90

*The Chemistry of Cannabis and Khat*

**Bleasdale**, Dr. C. (University of Newcastle upon Tyne) 21.02.90

*The Mode of Action of some Anti - Tumour Agents* *

**Clark**, Prof. D.T. (ICI Wilton) 22.02.90

*Spatially Resolved Chemistry (using Nature's Paradigm in the Advanced Materials Arena)* *

**Thomas**, Dr. R. K. (University of Oxford) 28.02.90

*Neutron Reflectometry from Surfaces*

**Stoddart**, Dr. J. F. (University of Sheffield) 01.03.90

*Molecular Lego* *

**Cheetham**, Dr. A. K. (University of Oxford) 08.03.90

*Chemistry of Zeolite Cages*

**Powis**, Dr. I. (University of Nottingham) 21.03.90

*Spinning Off in a Huff : Photodissociation of Methyl Iodide*

**Bowman**, Prof. J. M. (Emory University) 23.03.90

*Fitting Experiment with Theory in Ar-OH*

**German**, Prof. L. S. (Soviet Academy of Sciences) 09.07.90 *

*New Syntheses in Fluoroaliphatic Chemistry : Recent Advances in the Chemistry of Fluorinated Oxiranes*

**Platanov**, Prof. V.E. (Soviet Academy of Sciences, Novosibirsk) 09.07.90

*Polyfluoroindanes : Synthesis and Transformation*

**Rozhkov**, Prof. I. N. (Soviet Academy of Sciences, Moscow) 09.07.90

*Reactivity of Perfluoroalkyl Bromides*

(* indicates lectures attended by the author)

**UNIVERSITY OF DURHAM**

*Board of Studies in Chemistry*

COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1 August 1990 to 31 July 1991

**Macdonald**, Dr. W.A. (ICI Wilton) 11.10.90

*Materials for the Space Age* *

**Bochmann**, Dr. M. (University of East Anglia) 24.10.90

*Synthesis, Reactions and Catalytic Activity of Cationic Titanium Alkyls*

**Soulen**, Prof. R. (South Western University, Texas) 26.10.90

*Preparation and Reactions of Bicycloalkenes* *

**Jackson**, Dr. R.F.W. (University of Newcastle upon Tyne) 31.10.90

*New Synthetic Methods : a-Amino Acids and Small Rings* *

**Logan**, Dr. N. (University of Nottingham) 01.11.90

*Rocket Propellants* *

**Kocovsky**, Dr. P. (University of Uppsala) 06.11.90

*Stereo-Controlled Reactions Mediated by Transition and Non-Transition Metals* *

**Gerrard**, Dr. D. (British Petroleum) 07.11.90

*Raman Spectroscopy for Industrial Analysis*

**Scott**, Dr. S.K. (University of Leeds) 08.11.90

*Clocks, Oscillations and Chaos*

**Bell**, Prof. T. (SUNY, Stoney Brook, USA) 14.11.90

*Functional Molecular Architecture and Molecular Recognition* *

**Pritchard**, Prof. J. (Queen Mary & Westfield College) 21.11.90

*Copper Surfaces and Catalysts*

**Whitaker**, Dr. B.J. (University of Leeds) 28.11.90

*Two-Dimensional Velocity Imaging of State-Selected Reaction Products*

**Crout**, Prof. D. (University of Warwick) 29.11.90

*Enzymes in Organic Synthesis* *

**Pringle**, Dr. P.G. (University of Bristol) 05.12.90

*Metal Complexes with Functionalised Phosphines* *

**Cowley**, Prof. A.H. (University of Texas) 13.12.90

*New Organometallic Routes to Electronic Materials*

**Alder**, Dr. B.J. (Lawrence Livermore Labs., California) 15.01.91

*Hydrogen in all its Glory*

**Sarre**, Dr. P. (University of Nottingham) 17.01.91

*Comet Chemistry*

**Sadler**, Dr. P.J. (Birkbeck College London) 24.01.91

*Design of Inorganic Drugs : Precious Metals, Hypertension & HIV* *

**Sinn**, Prof. E. (University of Hull) 30.01.91

*Coupling of Little Electrons in Big Molecules : Implications for the Active Sites of Metalloproteins and other Macromolecules* *

**Lacey**, Dr. D. (University of Hull) 31.01.91

*Liquid Crystals* *

**Bushby**, Dr. R. (University of Leeds) 06.02.91

*Biradicals and Organic Magnets*

**Petty**, Dr. M.C. (Durham University) 14.02.91

*Molecular Electronics*

**Shaw**, Prof. B.L. (University of Leeds) 20.02.91

*Syntheses with Coordinated, Unsaturated Phosphine Ligands* *

**Brown**, Dr. J. (University of Oxford) 28.02.91

*Can Chemistry Provide Catalysts Superior to Enzymes?* *

**Dobson**, Dr. C.M. (University of Oxford) 06.03.91

*NMR Studies of Dynamics in Molecular Crystals*

**Markam**, Dr. J. (ICI Pharmaceuticals) 07.03.91

*DNA Fingerprinting* *

**Schrock**, Prof. R.R. (M.I.T.) 24.04.91

*Metal-Ligand Multiple Bonds and Metathesis Initiators* *

**Hudlicky**, Prof. T. (Virginia Polytechnic Institute) 25.04.91

*Biocatalysis and Symmetry Based Approaches to the Efficient Synthesis of Complex Natural Products*

**Brookhart**, Prof. M.S. (University of North Carolina) 20.06.91

*Olefin Polymerizations, Oligomerizations and Dimerizations Using Electrophilic Late Transition Metal Catalysts*

**Brimble**, Dr. M.A. (Massey University, New Zealand) 29.07.91

*Synthetic Studies Towards the Antibiotic Griseusin-A*

(* indicates lectures attended by the author)

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COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1st October 1991 to 31st July 1992

**Burton**, Prof. D.J. (University of Iowa, USA) 12.09.91

*Fluorinated Organometallic Reagents*

**Adcock**, Prof. J.L. (University of Tennessee, USA), 12.09.91

*Aerosol Direct Fluorination*

**Salthouse** ,Dr. J.A. (Manchester University), 17.10.91

*Son et Lumiere - a Demonstration Lecture* *

**Keeley**, Dr. R. (Metropolitan Police Forensic Science), 03.10.91

*Modern Forensic Science* *

**Johnson**, Dr. B.F.G. (Edinburgh University), 06.11.91

*Cluster-Surface Analogies*

**Butler**, Dr. A.R. (St. Andrews University), 07.11.91

*Traditional Chinese Herbal Drugs: a Different Way of Treating Disease*

**Koch**, Prof. H. F. (Ithaca College, USA), 08.11.91

*Relative Leaving Abilities of fluoride Ion Versus Proton Transfer, in the Neutralisation of Carbanions, Generated in Alcohols*

**Gani**, Prof. D. (St. Andrews University), 13.11.91

*The Chemistry of PLP-Dependant Enzymes* *

**More O'Ferrall**, Dr. R. (University College, Dublin), 20.11.91

*Some Acid-Catalysed Rearrangements in Organic Chemistry*

**Ward**, Prof. I.M. (Leeds University), 28.11.91

*The Science & Technology of Orientated Polymers* *

**Grigg**, Prof. R. (Leeds University), 04.12.91

*Palladium Catalysed Cyclisation and Ion Capture Processes* *

**Smith**, Prof. A.L. (ex-Unilever), 05.12.91

*Soap, Detergents and Black Puddings*

**Cooper**, Dr. W.D. (Shell Research), 11.12.91

*Colloid Science, Theory, and Practice*

**Snyder**, Mr. C.E. (U.S. Air Force, Ohio), 09.01.92

*Perfluoropolyethers*

**Long**, Dr. N.J. (Exeter University), 16.01.92

*Metallocenophanes-Chemical Sugar-tongs*

**Harris**, Dr. K.D.M. (St Andrews University), 22.01.92

*Understanding the Properties of Solid Inclusion Compounds* *

**Holmes**, Dr. A. (Cambridge University), 29.01.92

*Cycloaddition Reactions in the Service of the Synthesis of Piperidine and Indolizidine Natural Products* *

**Anderson**, Dr. M. (Shell Research, Sittingbourne), 30.01.92

*Recent Advances in the Safe and Selective Chemical Control of Insect Pests*

**Fenton**, Dr. D.E. (Sheffield University), 12.02.92

*Polynuclear Complexes of Molecular Clefts as Models for Copper Biosites*

**Saunders**, Dr. J. (Glaxo Group Research Limited), 13.02.92

*Molecular Modelling in Drug Discovery* *

**Thomas**, Prof. E.J. (Manchester University), 19.02.92

*Application of Organo-Stannanes to Organic Synthesis* *

**Vogel**, Prof. E. (University of Cologne), 20.02.92

*Porphyrins: Molecules of Interdisciplinary Interest* *

**Nixon**, Prof. J.F. (University of Sussex), 25.02.92

*Phosphaalkynes, New Building Blocks in Inorganic and Organometallic Chemistry*

**Hitchman**, Prof. M.L. (Strathclyde University), 26.02.92

*Chemical Vapour Deposition*

**Billingham**, Dr. N.C. (University of Sussex), 05.03.92

*Degradable Plastics - Myth or Magic*

**Fielding**, Dr. H.C. (ICI, Chemicals & Polymers), 10.03.92

*Fluoropolymer Membranes*

**Thomas**, Dr. S.E. (Imperial College, London), 11.03.92 *

*Recent Advances in Organoiron Chemistry*

**Hann**, Dr. R.A. (ICI Imagedata), 12.03.92

*Electronic Photography - An Image of the Future.*

**Maskill**, Dr. H. (Newcastle University), 18.03.92 *

*Mechanistic Studies of Organic Group Transfer Reactions*

**Knight**, Prof. D.M. (Durham University), 07.04.92

*Interpreting Experiments: The Beginning of Electrochemistry*

**Marhold**, Dr. A. (Bayer Co., Leverkusen), 30.04.92

*Fluorine Chemistry in the Bayer Company*

**Gehert**, Dr. J-C. (Ciba Geigy, Basel), 13.05.92

*Some Aspects of Industrial Agrochemical Research*

(* indicates lectures attended by the author)

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COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1st October 1992 to 11 February 1993

**Bryndza,** Dr. H. E. (Du Pont Central Research) 20.10.92

*Synthesis, Reactions and Thermochemistry of Metal(alkyl)cyanide Complexes and Their Impact on Olefin Hydrocarbon Catalysis*

**Davis,** Prof. A. G. (University College, London) 22.10.92

*The Behaviour of Hydrogen as a Pseudometal*

**Cockroft,** Dr. J. K. (University of Durham) 28.10.92

*Recent Developments in Powder Diffraction*

**Kee,** Dr. T. (University of Leeds) 04.11.92

*Synthesis and Coordination Chemistry of Silylated Phosphites*

**Robins,** Prof. D. (Glasgow University) 11.11.92 *

*Pyrrolizidine Alkaloids: Biological Activity, Biosynthesis and Benefits*

**Nix,** Dr. R. (Queen Mary College, London) 18.11.92

*Characterisation of Heterogeneous Catalysts,*

**Vallee,** Prof. Y. (University of Caen) 25.11.92

*Reactive Thiocarbonyl Compounds*

**Quin,** Prof. L. D. (University of Massachusetts, Amherst) 25.11.92

*Fragmentation of Phosphorus Heterocycles as a Route to Phosphoryl Species with Uncommon Bonding*

**Hegarty,** Prof. A. F. (University College Dublin) 02.12.92

*Highly Reactive Enols Stabalised by Steric Protection*

**Burgess,** Dr. A. N. (ICI, Runcorn) 09.12.92

*The Structure of Perfluorinated Ionomer Membranes*

**Clary,** Dr. D. C. (University of Cambridge) 20.01.93

*Energy Flow in Chemical Reactions*

**Kerr,** Dr. W. (University of Strathclyde) 27.01.93

*Development of the Pauson-Khand Annulation Reaction. Organocobalt Mediated Synthesis of Natural and Unnatural Products*

**Roberts,** Prof. S. M. (University of Exeter) 03.02.93

*Enzymes in Organic Synthesis* *

**Gillies,** Dr. D. (University of Surrey) 10.02.93

*NMR and Molecular Motion in Solution*

**Knox,** Prof. S. A. R. (Bristol University) 11.02.93

*Organic Chemistry at Polynuclear Metal Centres*

# RESEARCH CONFERENCES

*North East Graduate Symposium*

University of Newcastle-Upon-Tyne 2 April 1990

*Autumn Meeting of the Royal Society of Chemistry*

University of York, 24-26 September 1991

*Euchem Conference on Supramolecular Reactivity and Catalysis**

University of Padova (Italy), 8-13 September 1991

*North East Graduate Symposium*

University of Newcastle-Upon-Tyne 3 April 1992

(* indicates poster presented by the author)

# PUBLICATIONS

1) *A Chiral Sensor Based on Peroctylated α-cyclodextrin*
Paul S. Bates, R. Kataky and David Parker.
Journal of the Chemical Society Chemical Communications
(1992) pages 153-155.

2) *Functionalised α-Cyclodextrins as Potentiometric Chiral Sensors.*
R. Kataky, Paul S. Bates and David Parker.
Analyst (1992) 17 pages 1313-1317.

3) *Selective Binding and Detection of 'Onium Ions by Lipophilic Neutral Cyclodextrins.*
Paul S. Bates, R. Kataky and David Parker.
Journal of the Chemical Society Chemical Communications.
(1993) pages 691-693

4) *Characterisation of the Complexation Behaviour of Lipophilic Cyclodextrins by Electrospray Mass Spectrometry.*
Paul S. Bates, David Parker and Brian N. Green
Journal of the Chemical Society Chemical Communications.
(1993) pages 693-696

5) *Functionalised Cyclodextrins as Potentiometric Sensors for 'Onium Ions.*
Paul S. Bates, R. Kataky and David Parker.
Analyst. Accepted for Publication.

6) Part of the work described in this thesis was the subject of an article in *Chemistry in Britain* March 1992 page 211.