Synthetic receptors

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SYNTHETIC RECEPTORS

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DECLARATION

The work contained in this thesis was carried out in the Department of Chemistry at the University of Durham between October 1989 and December 1992. All the work is my own, unless otherwise indicated. It has not previously been submitted for a degree at this or any other university.

To Mum and Dad,

and to Marian.

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ABBREVIATIONS

AcCh	acetyl choline
AcChE	acetyl choline esterase
AIBN	azobisisobutyronitrile
BPA	bis-phenol-A
CI	chemical ionisation
CPK	Corey-Pauling-Koltun
COSY	correlated spectroscopy
DCE	1,2-dichloroethane
DCI	desorption chemical ionisation
DCM	dichloromethane
DMF	N,N'-dimethyl formamide
DMSO	dimethyl sulphoxide
DPM	diphenylmethane
DPP	2,2-diphenylpropane
EDA	electron donor-acceptor
FAB	fast atom bombardment
HETCOR	heteronuclear correlated spectroscopy
2-HBI	2-hydroxybenzimidazole
IR	infrared absorption
ISE	ion sensitive electrode
L	ligand
M^{n+}	mono or divalent cation
MS	mass spectroscopy
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
oNPOE	ortho-nitrophenyl octylether
PVC	poly(vinylchloride)
TFA	trifluroacetate
THF	tetrahydrofuran
THFE	tetrahydrofuranyl ether
THP	tetrahydropyran
THPE	tetrahydropyranyl ether
TLC	thin layer chromatography
TMS	tetramethylsilane
Tosyl	p-toluene sulphonyl
Tosylate	p-toluene sulphonate
VT	variable temperature

ABSTRACT

SYNTHETIC RECEPTORS

Novel, rigid cyclophanes have been designed and synthesised as receptors for the biologically important neurotransmitter, acetyl choline. The receptor cavities were less symmetrical than those of similar cyclophanes which had been previously prepared, as they incorporated two quite different functional groups on opposite sides of the cavity.

NMR experiments indicated that a cyclophane which incorporated a benzoate residue bound acetyl choline, with an exchange between the free and bound species which was slow on the NMR time scale. A second cyclophane, which incorporated both a benzoate and a pyridinium residue, also bound acetyl choline, but the exchange between the free and bound species was fast on the NMR time scale. NMR experiments also indicated that a third cyclophane, which incorporated only uncharged pyridyl and benzyl residues, did not bind acetyl choline. However, acetyl choline was efficiently transported across a PVC membrane by this neutral cyclophane with very little interference from ammonium and group I and group II metal cations.

Urea and thiourea residues were incorporated into crown-type macrocyclic frameworks. The crystal structures of two macrocycles incorporating thiourea residues ($18N_4O_2.2CS$ and $24N_4O_4.2CS$) were determined, as was the crystal structure of the $18N_4O_2.2CS$/silver(I) complex.

Preliminary experiments indicate that the bisthioureas $18N_4O_2.2CS$ and $24N_4O_4.2CS$ form quite stable complexes with the 'soft' metal cations silver (I), zinc (II), cadmium (II) and mercury (II), and that the analogous bisureas $18N_4O_2.2CO$ and $24N_4O_4.2CO$ form stable complexes with sodium and potassium, for which they are selective.

CONTENTS

CHAPTER 1. INTRODUCTION.

1.1. INTRODUCTION

The concept of receptor-substrate chemistry has been known for some considerable time, albeit as the very crude 'lock and key' picture first proposed by Emil Fischer in 1894[1].

However, an understanding of the interactions leading to the *recognition* of a particular substrate by a given receptor has only been gained in fairly recent years.

Crucial to this understanding has been the development of synthetic "macrocyclic" receptors, an area which has expanded rapidly since Charles J. Pedersen published his papers on crown ethers in 1967[2]. Many synthetic "macrocyclic" receptors have now been prepared and their differing abilities to bind various substrates have been well studied[3-7]. This has lead to a good understanding of the interactions involved in synthetic receptor-substrate chemistry and, by extension, the interactions in biochemical receptor-substrate systems such as proteins, antibodies, DNA and RNA.

The ultimate goal is the ability to tailor substrates which will specifically bind to a given receptor; or, conversely, to tailor receptors which will recognise a particular substrate. Chemists and biochemists are now on the brink of an understanding which will allow rational, targeted drug design (tailoring of a substrate) and the preparation of wholly synthetic enzymes and sensors (tailoring of a receptor). It is with the latter - synthetic receptors - that this work shall be concerned.

1.2. THE NON-COVALENT BOND.

A receptor or 'host' has been defined as having convergent binding sites[8]. In macrocyclic receptors these are arranged around a cavity in which a substrate or 'guest', having divergent binding sites, is bound, forming a host-guest or receptor-substrate complex.

This interaction arises from forces other than those in an ionic lattice or conventional covalent bond, and has been termed a "non-covalent bond". It is understood as an additive combination of relatively weak interactions[9] such as hydrogen bonding, π-stacking[10] electrostatic attraction and other dispersive forces[8]. The stability of the complex formed depends on how these interactions combine. From the studies of the many receptor-substrate complexes which have been formed, some general guidelines have been elucidated, which can help to predict or explain the relative (in)stability of complexes. These are discussed below and followed by examples of the types of macrocycles which have been prepared and complexes which have been studied.

1.2.1. Number of Binding Sites and Contact Area

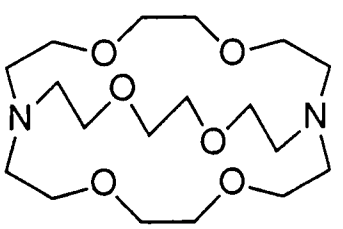
Because the non-covalent bond arises from a combination of weak interactions, the strength of the bond is obviously related to the number of the interactions between the receptor and the substrate, with more interactions giving rise to stronger bonds[11]. Van der Waals' forces should be maximised by having as large an area of contact between substrate and receptor as possible. This is done by shaping the receptor so that the cavity is concave (in order to complement the convex surface of the substrate's electron density)[8,12]. The number of binding sites, such as hydrogen bonding or electron donor-acceptor ("EDA") should also be

maximised. The receptor cavity and substrate should have complementary sizes, with a closer 'fit' between the two leading to the formation of more stable complexes.

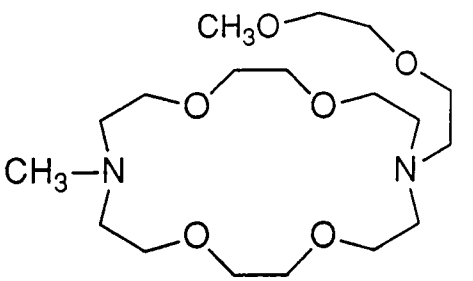
1.2.2. The Principle of Preorganisation and the Macrocyclic Effect.

It was realised very early in the study of macrocycles that their cyclic nature was of great importance to their ability to form stable complexes. For example, 18-crown-6 binds K^+ in anhydrous methanol with a logK value of 6.08. The open chain analogue, pentaethylene glycol dimethyl ether, binds K^+ in the same solvent with a logK value of only 2.3.

Similarly, it has been shown that cryptand [2.2.2] (figure 1.1) binds K^+ with a logK value of 9.75 in 95% methanol, whereas the open chain analogue binds with a logK value of 4.8[8,13,14].



cryptand[2.2.2]



'open chain' analogue

Figure 1.1

The reasons for this so called "macrocyclic" effect seem to be both entropic and enthalpic. The open chain analogue, having more degrees of freedom than its macrocyclic counterpart, requires more reorganisation in order to accommodate a guest species, and more energy to liberate it from the solvent so that binding can occur.

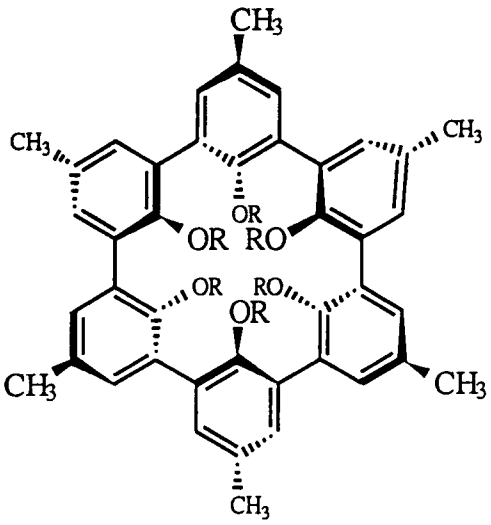
This reasoning was extended by Cram, who established the principle of preorganisation[15,16] which arises from the observation that host molecules which are flexible and require a change in conformation on complexation generally have lower stability constants than those which are rigid and have an 'open' stucture, and are consequently predisposed to the binding of a guest and require less energy to desolvate the receptor.

For example, the crown ethers and cryptands in their solution state do not possess a cavity; it is filled by inwardly turned methylene groups[12]. On formation of a complex the methylene groups turn outwards and the oxygen or nitrogen donor atoms become directed inwards in the correct orientation for binding. This reorganisation is associated with not only a detrimental entropy requirement in the formation of the complex, but also limits the favourable enthalpy change on complexation.

In macrocycles which are rigid and which possess a preformed cavity, this detrimental entropy requirement is accounted for in the synthesis of the macrocycle. For example, Cram's spherands[15,16] e.g. spherand-6, figure 1.2 are very rigid and have a cavity surrounded by oxygen donor atoms which are ideally arranged for binding spherical metal cations, leading to stability constants which are very high[17].

Another consequence of very rigid receptors is that they show enhanced size selectivity for similar potential guests. For example the spherand shown in figure 1.2[17] forms very stable complexes with Li^+ and Na^+, but does not form complexes with other cations such as K^+, Rb^+,Cs^+, Mg^{2+} or Ca^{2+}. This is in contrast to the crown ethers and cryptands which are flexible and can undergo some distortion from their

most stable conformations in order to accommodate guests of different sizes.



Spherand-6

Figure 1.2

However, it should be noted that whilst receptors which have a high degree of preorganisation form very stable complexes they have dynamic properties which, for many purposes, are far from ideal - they decomplex slowly (although acid catalysed dissociation may be fast) and so have poor transport properties. Also, where macrocycles are to be used as models for enzymes or other biologically active substances they may be required to catalyse a reaction on a bound substrate. This inevitably leads to a conformational change of the substrate which a very rigid host may be unable to accommodate.

Finally, if a complex is to form, then the receptor must have a cavity which is accessible. In the case of rigid receptors, this means that if the cavity is completely encapsulated then there are large, steric barriers which inhibit the uptake of the substrate, and complexation or decomplexation cannot occur without the breaking of covalent bonds. Unless the substrate is included during the cyclisation step in the

synthesis of the macrocycle[18] (in which case it could possibly be a *template*[19] for the reaction), complexes will not form.

For example, Cram et.al. have prepared macrocycles of the type shown in figure 1.3, which they have termed "carcerands". The compound shown forms complexes with DMF, THF, Cs^+, CsCl and H_2O. However, the free macrocycle is never isolated - the complexes are formed during the synthesis of the macrcycle as the substrates are present in the reaction medium[18]. Once the complexes are formed, the receptor and substrate cannot be separated without cleaving covalent bonds.

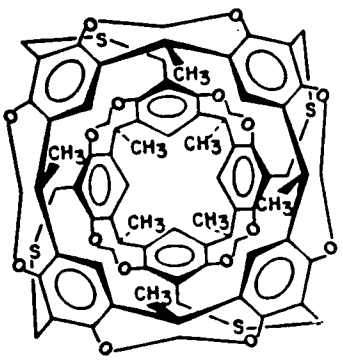


Figure 1.3

1.2.3. Solvent Effects[20,21]

Most complexation studies take place in solution, and the solvent used plays a crucial role. Its influence on the association of the receptor and substrate depends upon how well it solvates both receptor and substrate separately, the complex when formed and also upon the cohesive forces between the solvent molecules themselves.

In the solution state, scheme 1.1 can be used to describe the formation of a complex from solvated host and guest.

$$R_{(solvated)} \longrightarrow R$$
$$S_{(solvated)} \longrightarrow S$$
$$R + S \longrightarrow R.S$$

where R=receptor, S=substrate and R.S=complex

Scheme 1.1

Each step is discussed briefly below:

(i)Desolvation of Receptor.

In the simplest case, we assume that the solvation of the exterior of the receptor remains unchanged i.e. we consider only the removal of solvent molecules from the receptor cavity (a close approximation for rigid receptors - for more flexible receptors an enthalpy/entropy requirement for the rearrangement of the receptor must also be considered).

The enthalpy of the system is increased by the loss of any attractive interactions between solvent and the cavity and the cohesive attractions of the solvent molecules within the cavity. The enthalpy of the system is decreased by the association of the liberated solvent molecules with the bulk liquid.

The entropy of the system is increased by the loss of the ordered solvation of the cavity.

(ii)Desolvation of Substrate

When the substrate is removed from its solvation sphere, the enthalpy of the system is increased by the loss of guest-solvent interactions and solvent-solvent interactions within the solvation

sphere. It is decreased by the cohesive interactions of the solvent removed to the bulk liquid.

Entropy is increased by the loss of the ordered solvation shell.

(iii)Complex Formation.

Receptor and substrate associate to form a complex. The enthalpy of the system is decreased by attractive receptor-substrate interactions, but the entropy change is detrimental because of the formation of an ordered complex.

Obviously there are several terms which contribute to the overall free energy of the process and the size and sign of $\Delta G_{complexation}$ depends on the net gain or loss of entropy and enthalpy at each step. Strong solvent-solvent and weak solvent-substrate and solvent-cavity interactions lead to the formation of more stable complexes.

(Note that solvation of any counterions which may be present has not been considered).

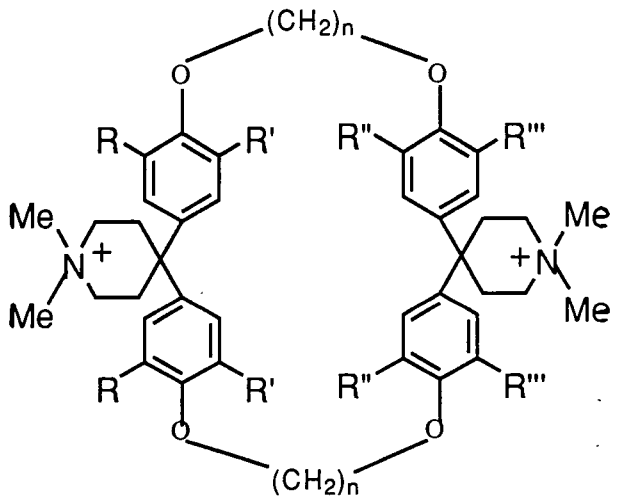


Figure 1.4

For example, the macrocycle shown in figure 1.4 forms stable complexes with para-disubstituted benzenes, but the complexes formed in water are more stable than those in methanol[22].

Table 1.1 shows some thermodynamic parameters for the complexation of this macrocycle with p-benzodinitrile and p-dimethoxybenzene.

	Ka($l mol^{-1}$)	$\Delta G(kJ mol^{-1})$	$\Delta H(kJ mol^{-1})$	$T\Delta S(kJ mol^{-1})$
complex with p-benzodinitrile				
D_2O	7.8×10^3	-21.7	-39.7	-17.9
CD_3OD	24	-7.5	-17.5	-10.0
complex with p-dimethoxybenzene				
D_20	1.0×10^4	-22.6	-42.6	-20.0
CD_3OD	8	-5.0	-18.3	-13.3

Table 1.1

In both solvents, the entropy term is unfavourable and so the complexation is enthalpy driven. However, the enthalpy term for the association of receptor and substrate must be approximately equal in the two solvents and so a large part of the difference in ΔH must arise from the desolvation terms. In fact water is very cohesive i.e. strong solvent-solvent interactions[23] and the large difference in ΔH is probably because the cohesive solvent interactions in methanol are much weaker i.e. in the desolvation of the receptor and subatrate, there is a greater net enthalpy gain in water than in methanol because of the more favourable enthalpy term gained from the association of the liberated solvent molecules with the bulk liquid.

In fact, the above demonstrates what is often called the "hydrophobic effect", which is observed in aqueous solution when the substrate is lipophilic and the receptor has a hydrophilic exterior but a lipophilic cavity. In the most extreme case, there is a negative enthalpy

term for the solvation of the substrate and the receptor cavity and both are rejected by the solvating water molecules. The substrate is 'driven' into the cavity formed by the receptor.

The hydrophobic effect is also observed for other types of receptor, such as enzymes and antibodies[24,25], and cyclodextrins[26-28].

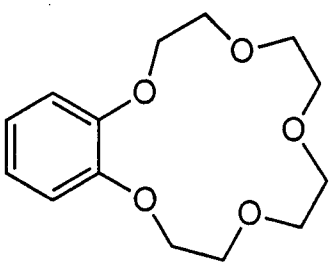
This is in contrast to, for example, the complexation of metal cations by crown ethers or cryptands in water, where the substrate (M^+) is hydrophilic and the aqueous solvent effectively competes for the substrate. This leads to a low stability constant for the complex. However, when the complexation is studied in less polar solvents, the charged substate is not so strongly solvated and stability constants of the complexes are higher

e.g. the Na^+ complex (Cl^- counter ion) with 15-crown-5 has a $\log K_S$ of 0.8 in water, and a $\log K_S$ of 3.24 in methanol, with a linear relationship to solvent concentration in methanol/water mixtures[29-31].

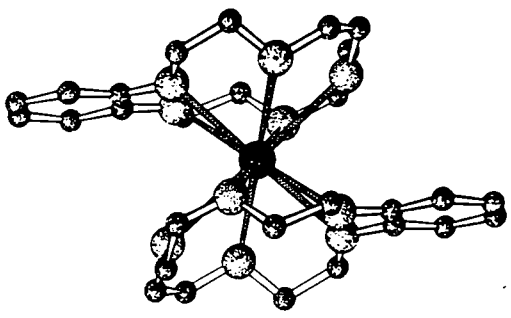
1.3. SYNTHETIC MACROCYCLIC RECEPTORS

A macrocycle can be thought of as a cyclic oligomer, the smallest macrocycles consisting of 4 or more monomer units. (Note that the monomer units are not necessarily identical). Whilst the smallest macrocycles are able to form complexes, the cavities are small and the macrocycle behaves simply as a multidentate ligand. A "sandwich" complex is formed.

e.g. Benzo-15-crown-5 is based on the cyclic tetramer of $-CH_2CH_2O-$. It forms complexes with KI, but the K^+ cation is not encapsulated. The crystal structure (hydrogen atoms omitted) is shown in figure 1.5[32].



Benzo-15-crown-5



Benzo-15-Crown-5/K^+ Complex

Figure 1.5

Larger macrocycles can form a cavity capable of encapsulating small organic molecules, or inorganic or organic ions. The macrocycle is the host or receptor, the included species is the guest or substrate.

Naturally occurring receptors (enzymes, antibodies, etc.) and macrocycles (cyclodextrins, porphyrrins, etc.) have been known for some time, but wholly synthetic macrocycles have been studied only fairly recently. However, these studies have been intense and progress has been rapid. The subject can be divided into a few broad areas, each of which is

discussed below. Crown ethers/cryptands and cyclodextrins will be mentioned only very briefly as they have both been well studied and are at least familiar to most chemists.

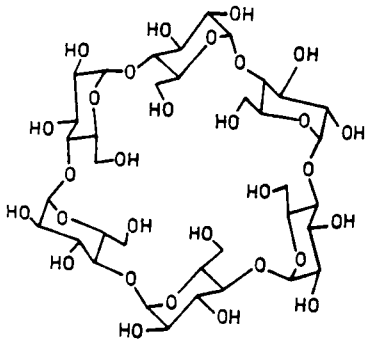
1.3.1. CROWNS AND CRYPTANDS

Crown ethers were first reported by Charles Pedersen in 1967[2] and, with the cryptands, were the earliest of the synthetic macrocycles to be properly studied. It is with these that the field of synthetic, macrocyclic chemistry is widely acknowledged to have begun. They form complexes with metal cations by EDA interactions, and with primary ammonium cations by hydrogen bonds between the ammonium N-H and the crown/cryptand ether oxygens. They have been extensively derivatised and used as phase transfer catalysts[33,34], in cation enrichment[35-40] and detection[36], as anion receptors[20,41-46],for chiral recognition of amines[47-50] and reaction catalysis[51-53]

1.3.2. CYCLODEXTRINS

Cyclodextrins, as mentioned above, are naturally occurring. They have been known since 1891[54,55] and consequently have been the subject of much study[7,56-58]. They have been extensively synthetically manipulated and have a wide range of applications.

Cyclodextrins are cyclic oligomers of saccharides, composed of 6-12 monomer units. The 6, 7 and 8 membered macrocycles are by far the most readily available, and consequently the most studied, and are respectively referred to as α, β and γ cyclodextrins. α-cyclodextrin, for example is shown in figure 1.6.



α-CYCLODEXTRIN

Figure 1.6

Cyclodextrins form complexes with a wide variety of substrates, as the rigid, hydrophobic cavity is well suited to the binding of aliphatic and aromatic substrates (Van der Waals' interactions) and the primary and secondary hydroxyl groups give hydrogen bonding potential and are able to form EDA bonds. They also impart aqueous solubility, so the hydrophobic effect is often a major contributor to the formation of stable complexes.

They can be derivatised at either or both of the primary or secondary hydroxyl faces, and cyclodextrin derivatives have been used as enzyme mimics[56,57,59], catalysts[60,61] , in chromatography[7,62] , in analytical techniques[63], for enantiomeric and conformational discrimination[64] and in many industrial applications[7], including the pharmaceutical[65], food[66], cosmetic[67] and chemical industries.

1.3.3. CYCLOPHANES

Cyclophanes were the first wholly synthetic macrocycles to be prepared[68,69], but their study as receptors did not really begin until the 1970's, when techniques such as NMR became widely available[70,71].

They are defined as macrocycles which have aromatic residues (usually 5 or 6 membered rings) making up part of the framework of the

macrocyclic ring. Orthocyclophanes have their 6 membered rings joined at the C_1 and C_2 positions. Similarly, metacyclophanes are joined via the C_1 and C_3 and paracyclophanes at the C_1 and C_4 positions[72,73].

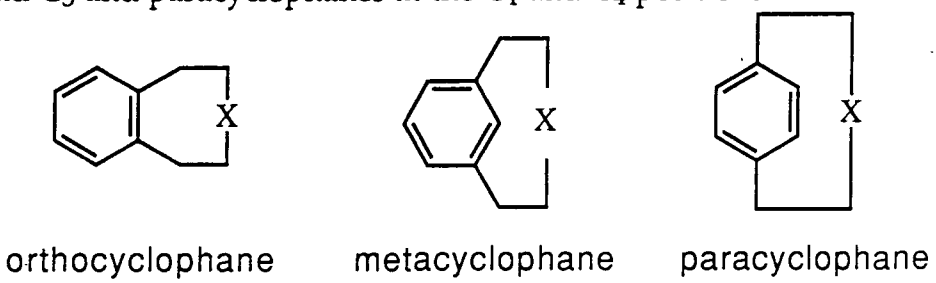


Figure 1.7

Cyclophanes lend themselves to a great deal of structural variation and so the area is further divided into those of porphyrins, calixarenes, spherands and cavitands.

1.3.3.1. Porphyrins

Porphyrins are naturally occurring cyclophanes based on cyclic tetramers of disubstituted pyrroles. They are of immense biological importance, being involved in oxygen transport in blood (haemaglobin) and storage in muscle (myoglobin), and in photosynthesis. Expanded porphyrins have found a variety of applications[74].

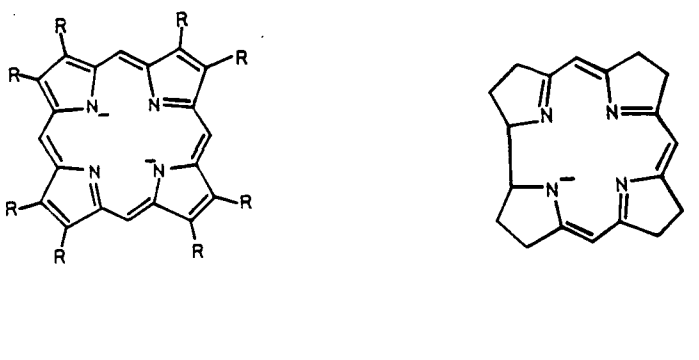
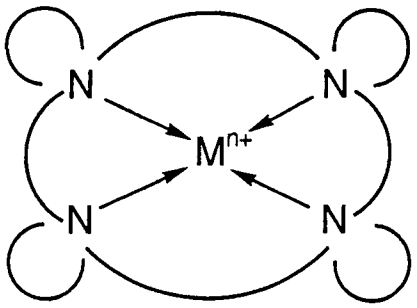


Figure 1.8

The porphyrin skeleton is shown in figure 1.8, along with the closely related corrin ring of vitamin B_{12} (involved in cobalt redox mechanisms).

Porphyrins form complexes with di and tri valent metal cations such as iron, copper, cobalt, zinc and magnesium, by EDA interactions between the porphyrin ring nitrogens and the bound cation, as represented by figure 1.9.



A shematic representation of a porphyrinate, showing the ligand donor bonds to the metal cation.

Figure 1.9

Porphyrins are extensively delocalised, and the positive charge is distributed over the whole ring.

In chlorophyll, magnesium (II) complexes of porhyrins are involved in a complex series of redox reactions which are responsible for the oxidation of water to dioxygen followed by the reduction of carbon dioxide to glucose i.e. photosynthesis.

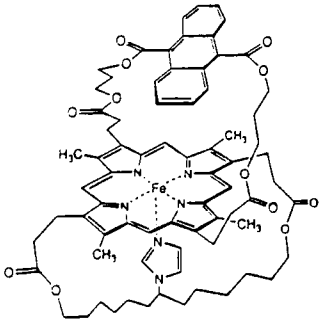
In blood and muscle, iron (II) complexes of porphyrins are responsible for the reversible binding of dioxygen.

In natural systems, the porphyrin ring is enclosed in the hydrophobic cleft of a protein. In the haem binding of iron (II), the porphyrin ring occupies four equatorial ligand sites. A histidine residue of the protein acts as a fifth, axial ligand and dioxygen is reversibly bound as a sixth ligand, also axial, at the opposite face of the porphyrin.

The complete mechanism by which porphyrins act in biochemical systems is far from understood. Most of the chemical research in this area has been concerned with the synthesis of analogues and derivatives of the biological porphyrins and the study of their ability to reversibly bind dioxygen, in the hope that this will promote a better understanding of the biological mechanisms. (For reviews see references 75 and 76).

In the earlier synthetic analogues produced, it was found that cobalt (II) complexes could be prepared which did mimic the biological iron (II) complexes. However, the synthetic iron (II) complexes were rapidly and irreversibly oxidised to iron (III) complexes[77,78]. It was later shown that oxidation to iron (III) occurrs via an intermediate, in which the binding of dioxygen is followed by the formation of an oxygen bridged dimer[79]. This irreversible oxidation can be prevented by sterically blocking the formation of the dimer, most effectively demonstrated by the bridged or 'capped' porphyrins. One face of the porphyrin has a hydrophobic bridge which is attached at opposite edges of the ring. A cavity is formed above the face of the porphyrin which is too small to allow the approach of large molecules, but large enough to allow the coordination of dioxygen to the bound iron (II).

If there is no other derivatisation, a base such as N-methylimidazole must be added to form the second axial ligand[80-83]. However, it is more efficient to attach the ligand to the porphyrin ring, as in the examples shown in figure 1.10[84,85]. Both of these show considerable stability to irreversible oxidation (with half lives of about one day in DMF and toluene respectively) and are reversible carriers of dioxygen.



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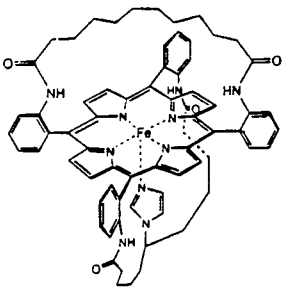

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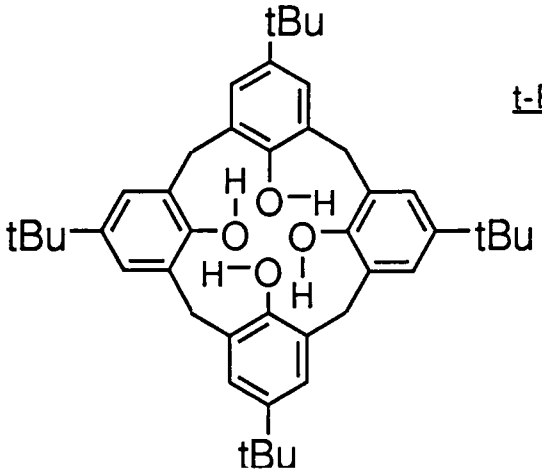
Figure 1.10

1.3.3.2. Calixarenes

Calixarenes are examples of metacyclophanes and are defined as cyclic oligomers of p-substituted phenols. The calix[n]arene is the cyclic oligomer with n monomer units. A prefix denotes the p-substituent. The calix[4-8]arenes have all been prepared, but the pentamer, calix[5]arene, has only been isolated in low yields.

The calixarenes do not show great structural diversity, but have been extensively derivatised at the hydroxyl group and at the para position. They form complexes with a wide variety of substrates, such as: metal ions[86-91], by EDA interactions with the phenolic oxygens; amines[87,92,93] by means of hydrogen bonds to the hydroxyl groups; and small organic molecules (acetone[94] and chloroform[95] , for example, are bound by Van der Waals forces; benzene[96] and other arenes[97] are bound by π-stacking attractions).

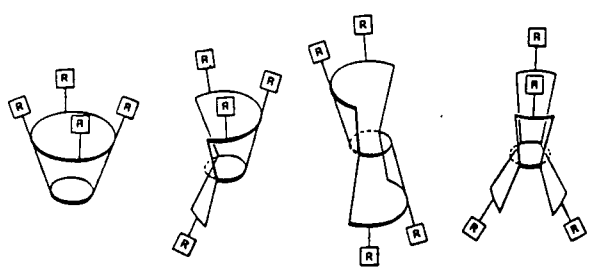
Calixarenes are conformationally mobile - the hydroxyl groups are small enough to pass through the annulus of the macrocyclic ring. In the simplest case, calix[4]arene (figure 1.11) exists as the cone, partial cone, 1,2 alternate and 1,3 alternate conformations, schematically represented by figures 1.12(i)-(iv).



t-Butyl Calix[4]arene

Figure 1.11

Replacing the hydroxyl proton by a more bulky substituent (usually an ester e.g. -OBz, -OAc[98] or ether e.g. $(CH_2CH_2O)_nH$[99]) prevents this interconversion as the cavity is too small to allow the passage of the substituent. The calixarene is 'locked' in a particular conformation. Almost always, only a single isomer is isolated - usually the cone or partial cone. However, it is not possible to predict which isomer a particular substituent will give[100], although the cone is obviously the most desirable in terms of receptor properties as it has the most complete cavity.



(i) (ii) (iii) (iv)

(i)Cone

(ii)Partial Cone

(iii)1,2-Alternate

(iv)1,3-Alternate

Calixarenes are prepared from the condensation of p-t-butyl phenol with formaldehyde. The t-butyl group, necessary to prevent polymerisation, is removed by a retro Friedel-Craft reaction[102]. It is then possible to carry out conventional aromatic substitution reactions to give para substituents such as the aminoethyl[103] or sulphonato[104] derivatives. Gutsche et. al. have developed a versatile procedure in which the hydroxyl group is allylated and a para-Claisen rearrangement then gives the p-allyl calixarene which can undergo further derivatisation[103,105].

1.3.3.3. Spherands

A spherand[12,106] has been defined as a macrocycle with a spherical cavity which is completely pre-organised prior to complexation[107]. The definition has been loosely applied to any spherical macrocycle e.g. macrotricyclic cryptand [1.1.1.1][108] , but the name has come to be almost exclusively applied to macrocycles of the type shown in figure 1.2 (page 5).

Although technically a cyclophane, the aryl groups do not actually form a part of the cavity; their function is to impart rigidity to the macrocycle. The spherical cavity is formed by the aryl oxygen atoms which, in the case of spherand-6 for example, are octahedrally arranged[106]. The cavities are generally of a size which complements the alkali and alkaline earth cations, with which the calixarenes form very stable complexes by EDA interactions between the ether oxygens and the bound cation[15]. Very similar complexes are formed by crowns and cryptands; spherands in fact have more in common with these than with most cyclophanes.

1.3.3.4. Cavitands

A cavitand has been defined as a synthetic organic compound that contains an enforced cavity large enough to accommodate small molecules or ions[109].

(Spherands are a type of cavitand, but the cavities are generally too small to accommodate anything other than the trivial M^{n+} cations).

Because of the rigid nature of the aromatic residues which, by definition, form part of their macrocyclic ring, almost all cyclophanes possess at least some structural rigidity and are therefore cavitands. However, other types of macrocycles should not necessarily be dismissed.

Cucurbituril, for example, (shown in figure 1.13) possesses a very rigid cavity but has no aromaticity. It forms very stable complexes with diammonium compounds, $^{+}H_3N\text{-}(CH_2)_n\text{-}NH_3^{+}$, with n=5,6 the optimal chain length[110]. Hydrogen bonding of ammonium N-H to the urea oxygens at both upper and lower rim is largely responsible for binding. The aliphatic chain which joins the two ammonium groups is located inside the lipophilic cavity where Van der Waals interactions also contribute to the stability of the complex.

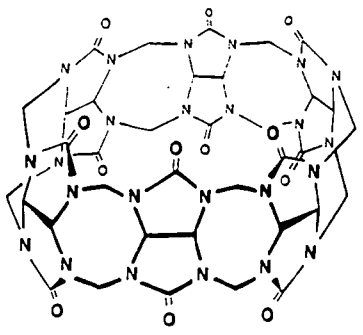
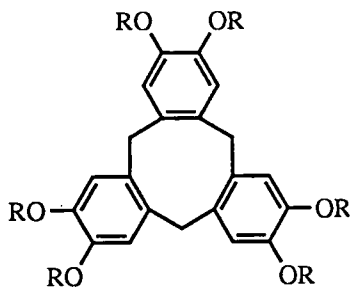


Figure 1.13

Despite this relatively rare example, almost all of the cavitands that have been prepared are cyclophanes. Relatively few structural subunits have so far dominated the literature. Each unit provides the macrocycle with rigidity, but they have different shapes which they will impart to the cavity.

Complexes are usually formed with organic guests (charged and neutral) as the cavity has a lipophilic interior inherited from the aryl "shaping groups".

Cyclotriveratrylene is an ortho-cyclophane structural unit which has been used by Collet et. al. in macrocycles such as the one shown in figure 1.14, which forms complexes with small, neutral, organic molecules such as chloroform. Macrocycles of this type show good size selectivity and a preference for aliphatic rather than aromatic substrates. sp^3 systems are bound most strongly, with sp^2 hybridised substrates bound more strongly than sp[111-113].



Cyclotriveratrylene

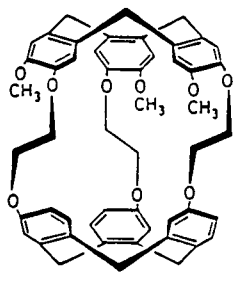
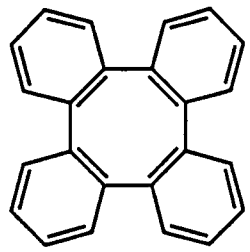


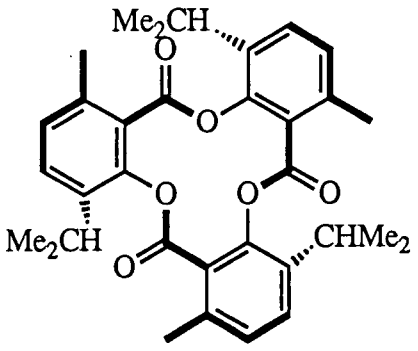
Figure 1.14

Other examples of orthocyclophane structural units include tri-o-thymotide[114] and tetraphenylene[115], shown in figure 1.15. These have not been used as building blocks for the construction of larger

macrocycles. They have been of interest for their ability to form clathrate rather than inclusion complexes.



Tetraphenylene

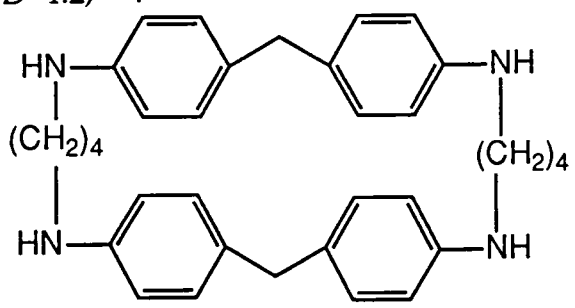


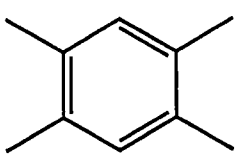
Tri-o-Thymotide

Figure 1.15

Most paracyclophanes are symmetrical, consisting of a pair of "shaping groups" joined by aliphatic or aromatic "spacers" in a 2+2 fashion.

For example, in the macrocycle shown in figure 1.16, the diphenylmethane residues are the shaping groups, and the aliphatic methylene chains are the spacing units - so called because the length of the chain determines the depth of the cavity. It forms a complex with durene both in the solid state and in water at pH<2. The crystal structure of this complex provided the first unambiguous proof of cavity inclusion complexation by a cyclophane in aqueous media. Complexes are also formed with other aromatic substrates such as 2,7-naphthalenediol (in D_2O, pD=1.2)[116].





DURENE

Figure 1.16

Since binding occurs in water, the driving force for the complexation is the hydrophobic effect, but a π-stacking interaction between the benzene rings of receptor and substrate must also contribute.

The shape of the diphenylmethane unit has been retained in other cyclophanes, but the hydrophobic nature of the cavity is improved by removing the hydrophilic tertiary nitrogen (responsible for the macrocycle's water solubility) away from the cavity.

In the example shown in figure 1.17, (R=R'=R''=R'''=OCH_3, X=-$(CH_2)_3$-, -$(CH_2)_4$-) stable complexes are formed with p-disubstuted benzenes in water; the macrocycle with C_4 spacers forming more stable complexes than the analagous macrocycle with C_3 spacers[117]. Complexation arises from a combination of π-π, Van der Waals and hydrophobic effects[22].

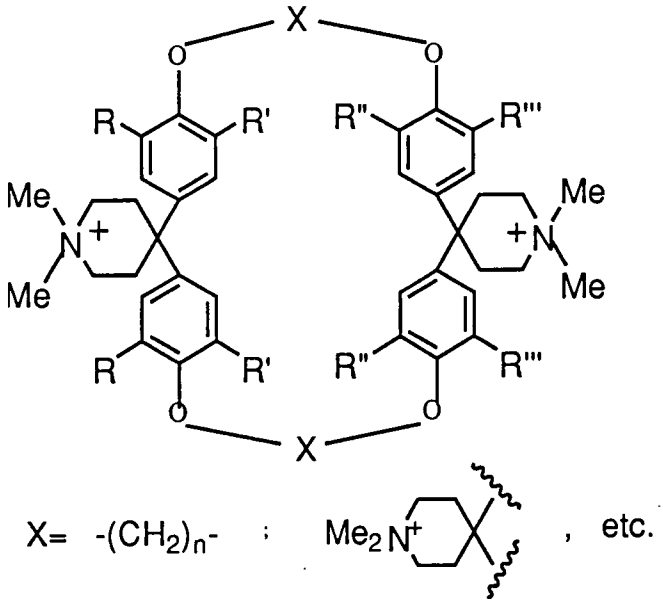
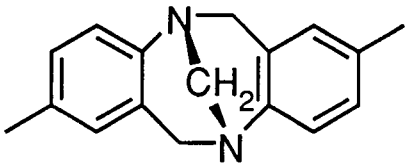


Figure 1.17

Troger's base (figure 1.18) was introduced by Wilcox et. al. as a subunit having a similar shape to diphenylmethane, but with greater rigidity and a chiral nature.



Trogers base

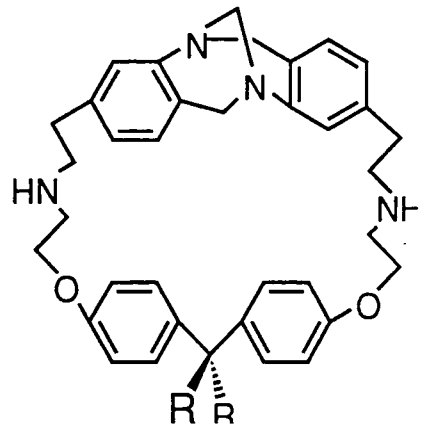
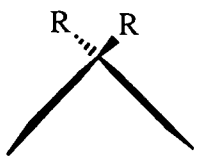
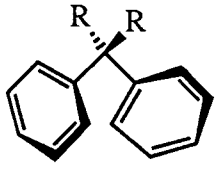


Figure 1.18

The cyclophane shown in figure 1.18[118] forms weak complexes with p-disubstituted benzenes (R=H or CH_3), more stable complexes are formed when R=H than CH_3. The methyl groups force the rings out of the gable conformation, which is preferred for binding flat, aromatic substrates, into the propeller conformation (scheme 1.2).



Gable



Propeller

R=H propeller ⇌ gable

R=Methyl propeller ⇀ gable

Scheme 1.2

Dougherty et. al. have prepared structures based on ethenoanthracene, such as the one shown in figure 1.19[119], which forms complexes with a variety of substrates, especially quaternary ammonium compounds.

Studies of the complexation properties of this receptor (X=-CO_2^- Cs+) in D_2O (pD=9) have shown that quaternary ammonium compounds (R_4N^+) bind more strongly than tertiary amines (R_3N).

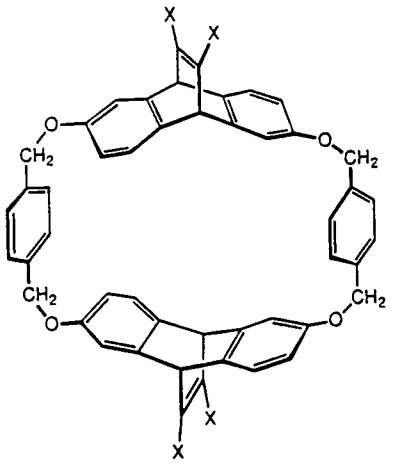


Figure 1.19

In chloroform (X=-CO_2CH_3), quaternary ammonium compounds are still bound, but stable complexes with the corresponding tertiary amines are not formed[120].

In table 1.2, some $\Delta G_{complexation}$ values (kcal mol^{-1}) are given for quinoline, isoquinoline and their N-methyl iodide derivatives (figure 1.20).

	N-methyl quinol-inium	Quinoline	N-methyl isoquin-olinium	Iso-quinoline
-ΔG D_20,pD=9	7.6	5.4	7.2	6.3
-ΔG $CDCl_3$	3.3	0.0	2.5	0.2

Table 1.2

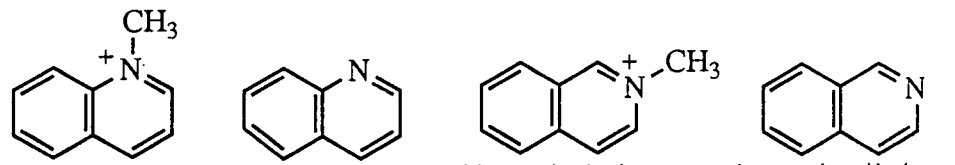


Figure 1.20

In chloroform, the only significant driving force for the complexation is the ammonium cation - aromatic π attraction[121] and so quinoline and isoquinoline are not bound[120]. In water, there is a hydrophobic effect and both the free amines and the N-methyl derivatives are bound. However, the N-methyl derivatives are bound more strongly because of the aromatic π - ammonium ion attraction. (The difference is not as large as the studies in chloroform would seem to indicate, probably because the positive charge of the quaternary ammonium must be desolvated from water).

Cation - π interactions have been shown to be of significance in amino acid - protein binding[122] and in the binding of the neurotransmitter acetyl choline to its esterase[123].

In macrocycles which have paraquat as a structural unit, such as the one shown in figure 1.21 complexes are formed with benzene derivatives by means of a charge transfer mechanism, resulting in intensely coloured complexes[124,125].

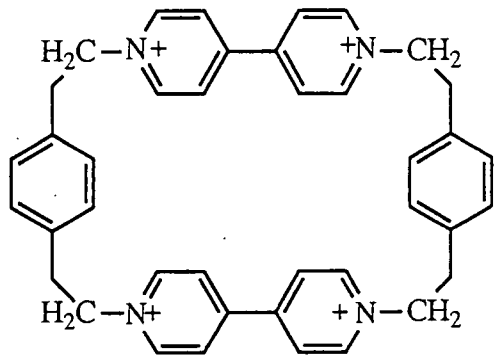
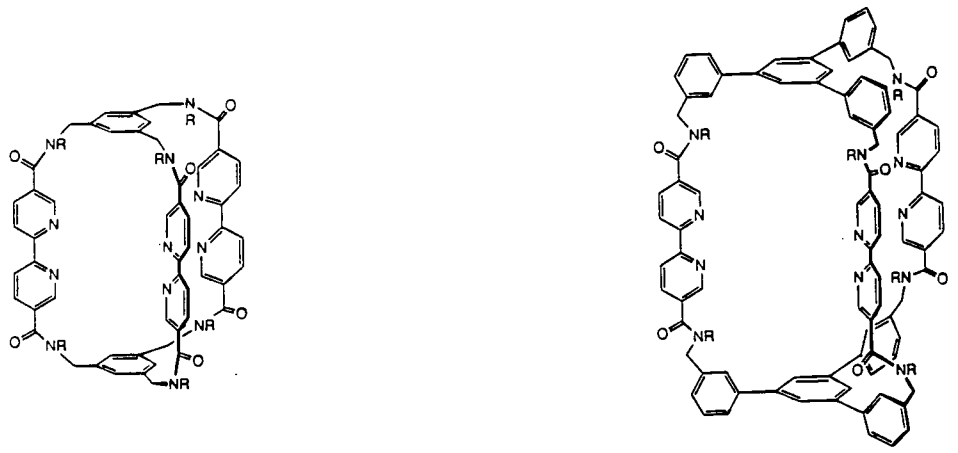


Figure 1.21

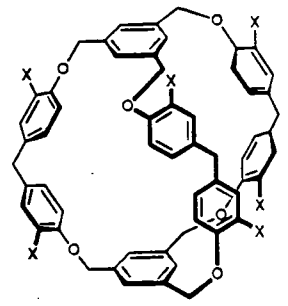
Bicyclic or "triply bridged" cyclophanes, first prepared in 1965[126] have been studied by Vogtle et. al.[127]. Of the two examples shown in figure 1.22, 1.22a is an enterobactin mimic and a very stong binder of iron (III)[128-130] and 1.22b selectively complexes with trihydroxy benzenes in dichloromethane with great sensitivity for the substituion pattern[131].



a b

Figure 1.22

More recently, Lehn et.al. have prepared the bicyclic hexacarboxylate shown in figure 1.23.



X=CO_2Na

Figure 1.23

The sodium salt of this cyclophane is water soluble and forms very stable complexes with tertiary ammonium compounds in aqueous solution[132]. Like the ethenoanthracene-based cyclophanes prepared by Dougherty (figure 1.19), both electrostatic ammonium cation - aromatic π interactions and hydrophobic effects contribute to the complex stability. If

the ammonium cation is aromatic, then π-stacking interactions may also be involved.

1.3.4. HYBRIDS AND POLYTOPIC CORECEPTORS.

When structural units from two different types of macrocycle are combined, a receptor is formed which may have interesting properties.

For instance, spherands have been combined with crowns and cryptands to give the monotopic, hybrid receptors like those shown in figure 1.24[15]. Like their parent compounds, they bind metal cations but have an intermediate degree of rigidity.

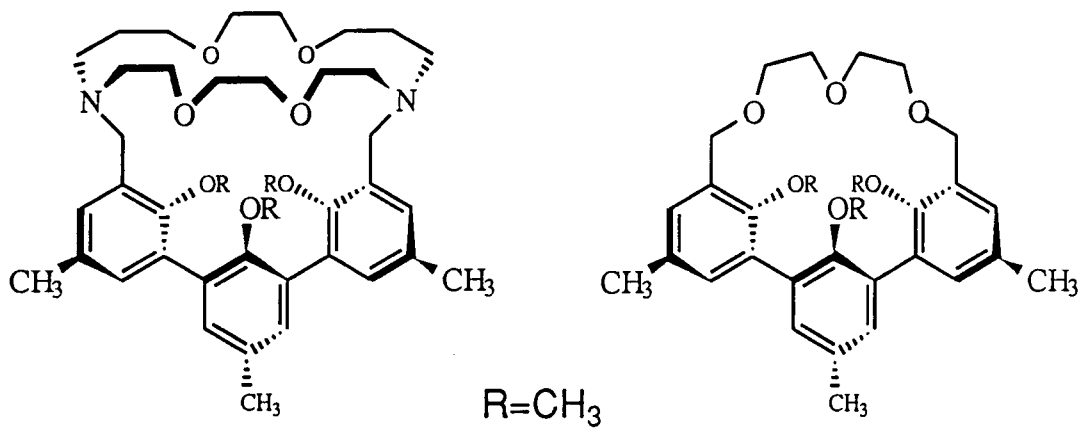


Figure 1.24

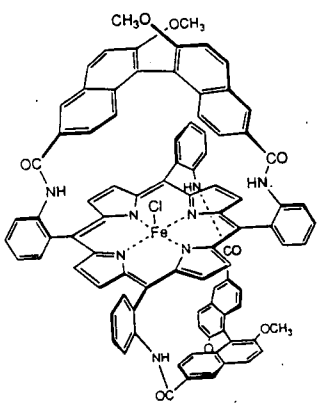
It is more interesting to combine two 'whole' macrocycles to produce a receptor which contains more than one binding site, and can bind more than one substrate simultaneously. Such a receptor is described as "polytopic". If the binding sites are not identical, then two quite different substrates may be brought into close proximity in the

receptor. This leads into a relatively unexplored area of macrocyclic chemistry, with obvious implications in catalysis[11,133-1345] and also for the preparation of models which will help the understanding of cooperative binding and allosteric effects in biological systems[136,137] In the most trivial case of a ditopic receptor, the macrocycle is simply very large (relative to substrate) so that two guests may be accomodated. This is the case in the 2:1 complex of KSCN with dibenzo-24-crown-8. Two K^+ cations are bound in the cavity, bridged by ^-SCN anions[138,139].

In a slightly more complicated case, two crown ethers are joined in a spiro fashion; each crown behaves independently of the other and can each bind one cation[140-142].

It has already been mentioned (section 1.3.3.1) that porphyrins have been derivatised such that an extra cavity is formed above the plane of the ring. There is a binding site for iron (II) and a second site for binding dioxygen. Note that the dioxygen is not bound unless iron (II) is already located in the porphyrin ring - a crude example of cooperative binding.

In the more elaborate system shown in figure 1.25, a chiral, cyclophane-type cavity is created above each face of the porphyrin. In its optically pure form (S,S), this was used as a catalyst in the hydroxylation of ethylbenzene to 1-phenylethanol, preferentially producing the R isomer (40%e.e.)[143].



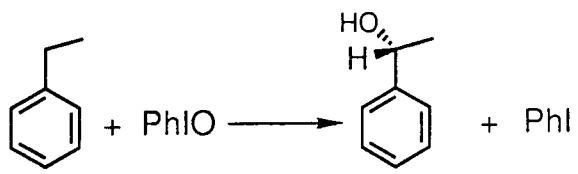
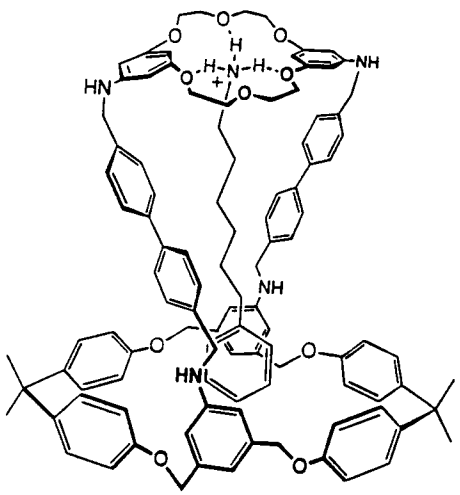


Figure 1.25

Although the enantiomeric excess is low, this shows chiral control of a free radical reaction.

In the example shown in figure 1.26, a cyclophane and a crown ether are combined. The receptor is ditopic but has not been used for binding two separate substrates; it has been used to bind phenylalkylammonium salts, $Ph\text{-}(CH_2)_n\text{-}NH_3^+$, where n=3-9 (shown with n=6)[144,145] . The main driving force for complex formation is the binding of $\text{-}NH_3^+$ to the crown ether ring. If n>4, the aliphatic chain is long enough to allow the phenyl group to bind in the cyclophane cavity (π-stacking interaction), so different binding sites in the receptor are able to bind to well separated parts of the substrate.

Figure 1.26



If n>6, the aliphatic chain is too long and must coil to allow the phenyl ring to bind in the cyclophane cavity, introducing unfavourable torsional angles in the methylene chain.

Consequently, the binding constants when n=5,6 are about a factor of 3 higher than for n=3,4,7,8,9.

1.4. SUMMARY

It has been pointed out that that the recognition of substrates by receptors occurs via a combination of weak interactions. A discussion of these interactions and the ways in which they combine has been undertaken and illustrated by examples taken from the major areas of macrocyclic chemistry. It has been shown that, if the forces leading to complex formation are well enough understood, receptors may be designed for specific tasks and specific substrates.

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CHAPTER 2. SYNTHETIC RECEPTORS FOR ACETYL CHOLINE.

2.1. INTRODUCTION: ACETYL CHOLINE AND ITS RECEPTORS.

Nerve cells interact with one-another at nerve junctions or "synapses", across which nervous impulses are transmitted. Transmission of this impulse across the synapse is performed by the diffusion of small molecules called "neurotransmitters".

Acetyl choline (AcCh, figure 2.1a) is an important neurotransmitter at the muscarinic and cholinergic synapses[1] and is stored at nerve endings in synaptic vesicles (figure 2.1b). When a nervous impulse arrives at the nerve ending, the synaptic vesicles fuse with the pre-synaptic membrane resulting in the release of AcCh into the synaptic cleft. AcCh diffuses across the synaptic cleft to the post-synaptic membrane, where it is recognised and bound by specific receptors. On binding, the receptors are thought to undergo a change in conformation i.e. they become "excited". Ion channels in the postsynaptic membrane open, which causes a change in potential across the membrane and triggers the propagation of the nervous impulse in the post-synaptic cell. AcCh is released and the receptors return to their resting states. The energy required for this synaptic transmission is provided by the mitochondria.

The AcCh released from the receptors in the post-synaptic membrane must be quickly removed so that it will not be again taken up by the receptors, preventing the transmission of further impulses by "fixing" the receptors in their excited states. This is done by hydrolysing the AcCh to choline and acetate, a reaction catalysed by the enzyme acetyl choline esterase (AcChE). Choline is taken back into the pre-synaptic membrane, where it is recycled to produce more AcCh.

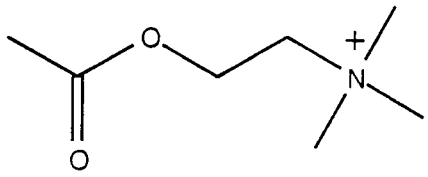


Figure 2.1a

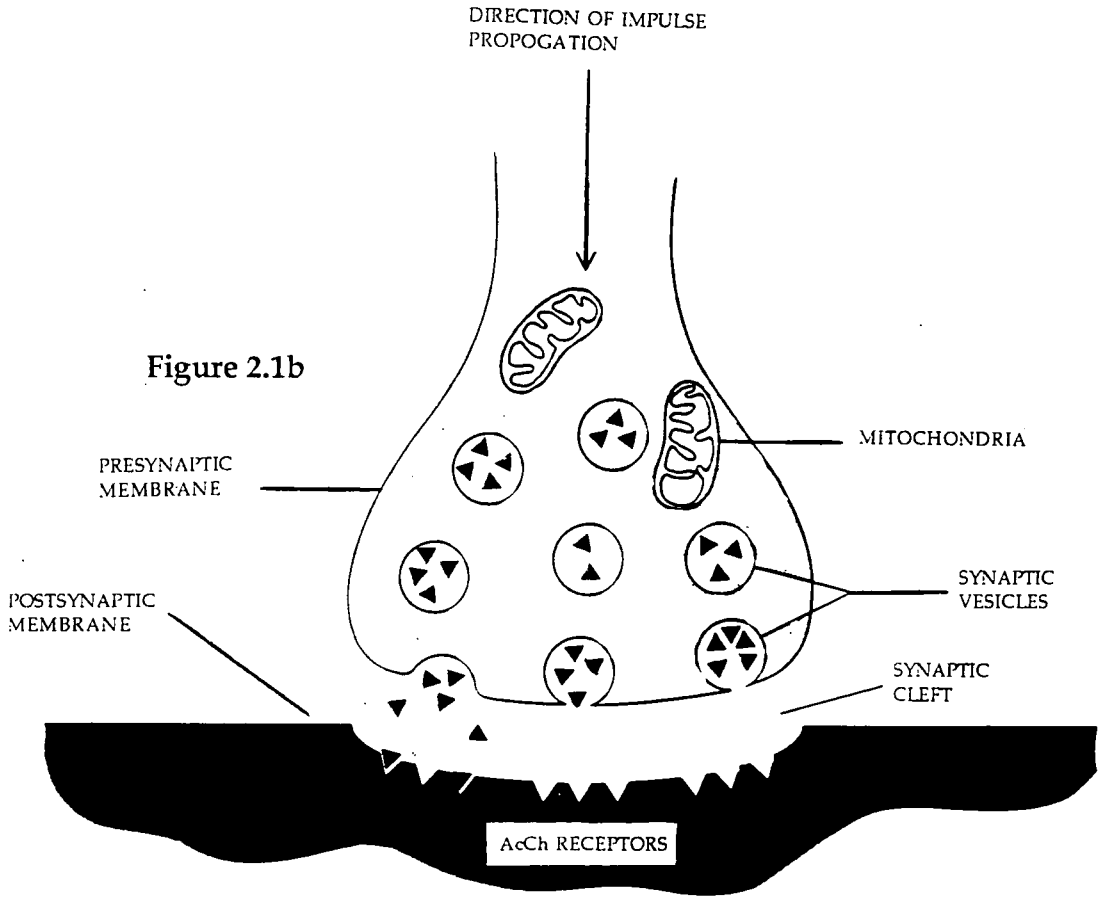
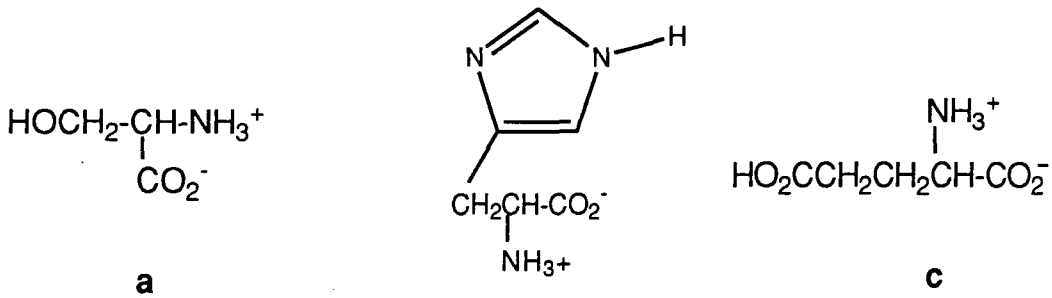


Figure 2.1b

AcChE necessarily has a very high turnover number, measured as about 25,000s^{-1} i.e. approaching diffusion control[2-4]. The mechanism of the hydrolysis (scheme 2.1) has been known for some time to involve a catalytic triad consisting of serine, histidine and one other amino acid residue known to possess a carboxylate group which was involved in the hydrolysis. This third residue was recently identified as glutamate[5] (see figure 2.2)



serine **histidine** **glutamate**

Figure 2.2

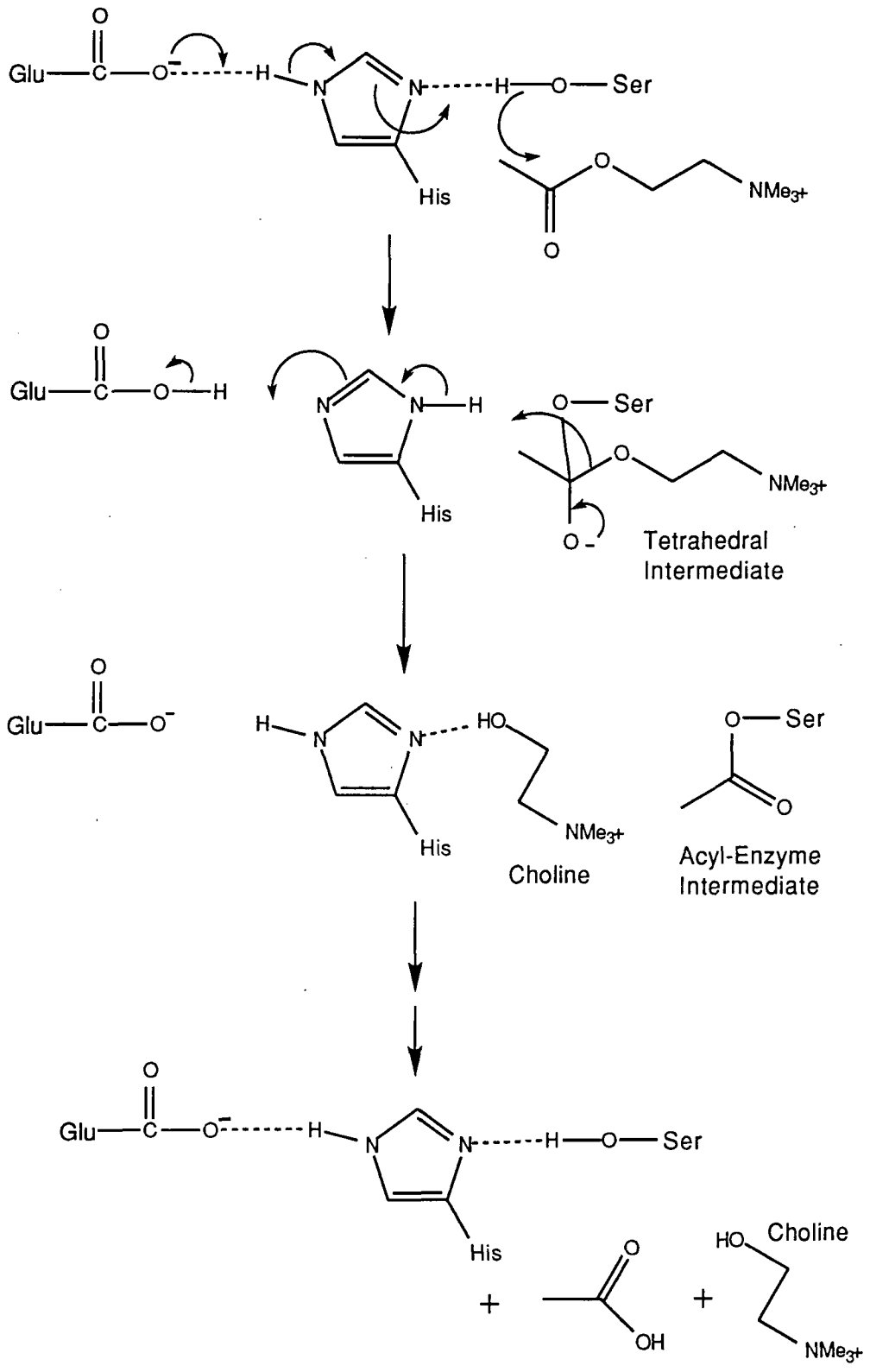
AcCh must be recognised and bound at the active site of the enzyme before hydrolysis can occur. At the outset of this work, although the mechanism of the hydrolysis was understood, very little was known about the binding site either in the AcCh receptors in the post-synaptic membrane or in the esterase. Two theories had been proposed:

either

(i) AcCh was bound near the active site of the enzyme by an ionic attraction between the positively charged ammonium head of AcCh and a specific anionic locus, probably CO_2^-, in the enzyme.

or

(ii) AcCh was located in a hydrophobic cleft in which the primary interaction contributing to binding would be an ammonium cation - aromatic π attraction. Anionic residues, remote from the cavity and the active site, were at best responsible for a minor attraction of the ammonium head of AcCh.


Glu
His
Ser
NMe3+
Tetrahedral
Intermediate
Choline
Acyl-Enzyme
Intermediate
OH

Scheme 2.1

Originally, the former case was the one which had been commonly assumed, and it was not until 1980 that the latter was proposed[6].

In order to distinguish between the two theories, a variety of experiments were designed, involving measurements of the strength of binding of various analogues of AcCh and their ability to inhibit the esterase activity[6-13]. These experiments provided valuable information but neither of the arguments could be eliminated.

It was not until 1991, two years after this work was begun, that crystals of AcChE were obtained which were of sufficient quality to allow the structure of the esterase to be determined by X-ray diffraction techniques[5]. It was shown that the active site was at the end of a narrow gorge, about 20Å in length, which was lined with some 14 aromatic moieties, but with very few acidic residues which would be capable of binding to the trimethyl ammonium head of AcCh. It was concluded that the interaction responsible for AcCh binding must be of the cation - π type.

The structures of the AcCh receptors in the post-synaptic membrane have still not been solved, but current feeling is that the interactions responsible for binding are likely to be very similar in nature to those in the esterase.

2.2 SYNTHETIC RECEPTORS FOR ACETYL CHOLINE.

At the start of this work, the argument mentioned above had not been resolved. It was thought that studies of synthetic receptors for AcCh might usefully contribute to the debate by providing models for the biological receptors.

Only one synthetic receptor had, at that time, been prepared[14] (shown in figure 2.3)

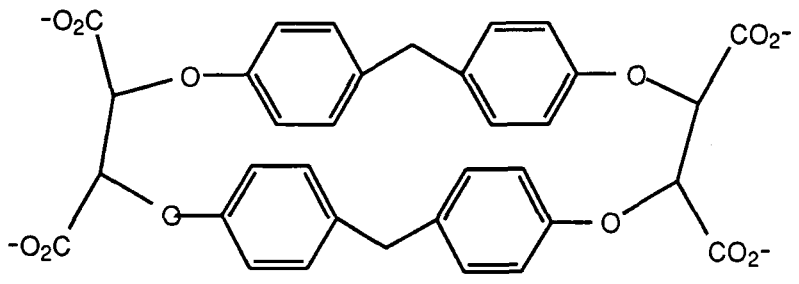


Figure 2.3

The cyclophane shown in figure 2.3 binds to acetyl choline in D_2O with a logKs of 2.7. The structure of the complex was not solved, but NMR experiments indicated that AcCh was located in the cavity of the macrocycle and bound by an electrostatic attraction to the carboxylate groups at one end of the cavity. The aliphatic chain would then lie in the cavity where there would be a hydrophobic interaction with the aromatic residues. The binding of AcCh is therefore of the type proposed in the first theory, as discussed on p41.

Since then Dougherty et. al., as mentioned in chapter 1 (p24-25), have prepared macrocycles based on the ethenoanthracene

structural unit[15]. The cyclophane shown in figure 2.4 has been shown to bind quaternary ammonium compounds; in particular, it binds AcCh in D_2O with a logKs of 4.3[15].

Despite this quite strong binding, it was shown that the negatively charged carboxylate groups are not significantly involved in the binding of AcCh ; the primary interaction is ammonium cation - aromatic π attraction (see chapter 1), demonstrating the plausibility of second theory discussed on p41.

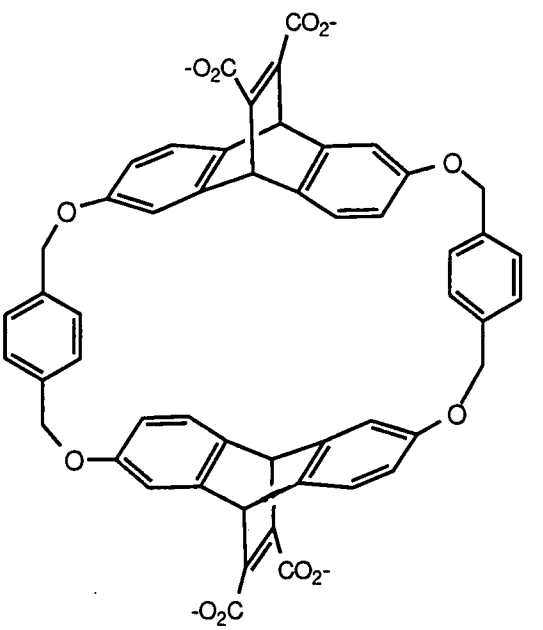


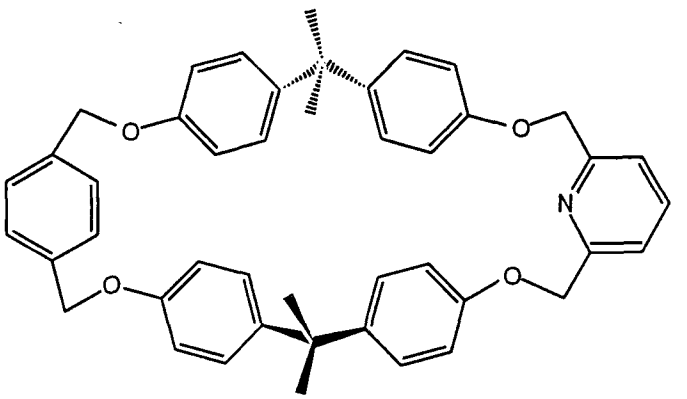
Figure 2.4

Very recently, Lehn et. al. have prepared a second receptor for AcCh, also mentioned in chapter 1 (figure 1.23, p27). The bicyclic cyclophane is shown with a hydrophobic cavity and the hydrophilic carboxylate groups facing outwards into the solvent (water), although it is conceivable that some of the carboxylate groups turn into the

cavity when in solution. Lehn proposed that AcCh, and other quaternary ammonium compounds, are bound in the cavity by a combination of cation - π and cation - anion interactions[16].

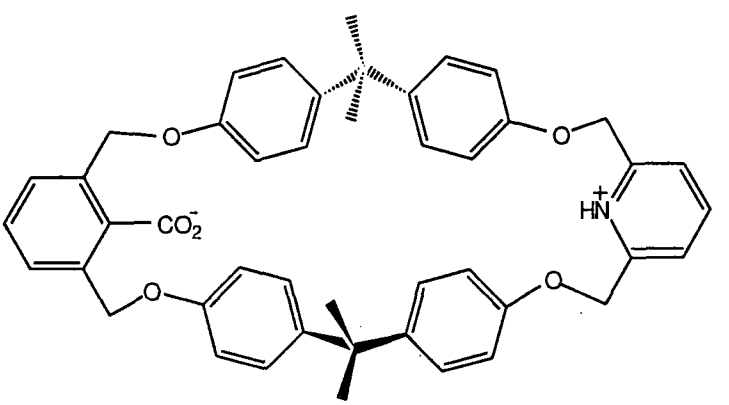
2.3. DESIGN OF NOVEL SYNTHETIC RECEPTORS FOR ACETYL CHOLINE.

That the AcChE structure would be solved could not have been known at the outset of this work, and so we sought to design and synthesise novel receptors which would bind AcCh.



a

1



b

2

Figure 2.5

Bearing in mind the only other receptor which had then been made (figure 2.3), the macrocycles **1** and **2**, shown in figure 2.5a and 2.5b, were targetted. The construction of CPK models had indicated that the cavities were of dimensions which complemented the size of AcCh, and indeed were quite similar to the cavity formed by Lehn's macrocycle (figure 2.3), in which AcCh had been presumed to occupy the cavity.

As binding sites for the trimethylammonium head of AcCh, the cavitand **1** has an aromatic "pocket" at the left hand side as shown, whereas **2** has an anionic carboxylate group. The two are otherwise very similar, which would perhaps allow a comparison of the cation - anion and cation - π interactions.

In addition to this, both **1** and **2** have a pyridine (pyridinium) residue which it was hoped might, under the right conditions of pH, promote hydrolysis of the AcCh ester group (by acyl transfer to the pyridine nitrogen) and so function as a synthetic esterase.

In contrast to Lehn's cyclophane, 2,2-diphenylpropane ("DPP") was chosen as the shaping unit in place of diphenylmethane ("DPM"). The extra methyl groups of DPP impart greater rigidity to the unit, and more rigid receptors generally imply the formation of more stable complexes (see chapter 1, section 1.2.2, especially p4). Also, DPM has a slight preference for the gable conformation, which is desirable for complexation with flat substrates (such as aromatic rings). DPP has a strong preference for the propeller conformation, which has a more "concave" surface and so should better complement the convex surface of a straight, aliphatic chain (see chapter 1, section 1.3.3.4, p24).

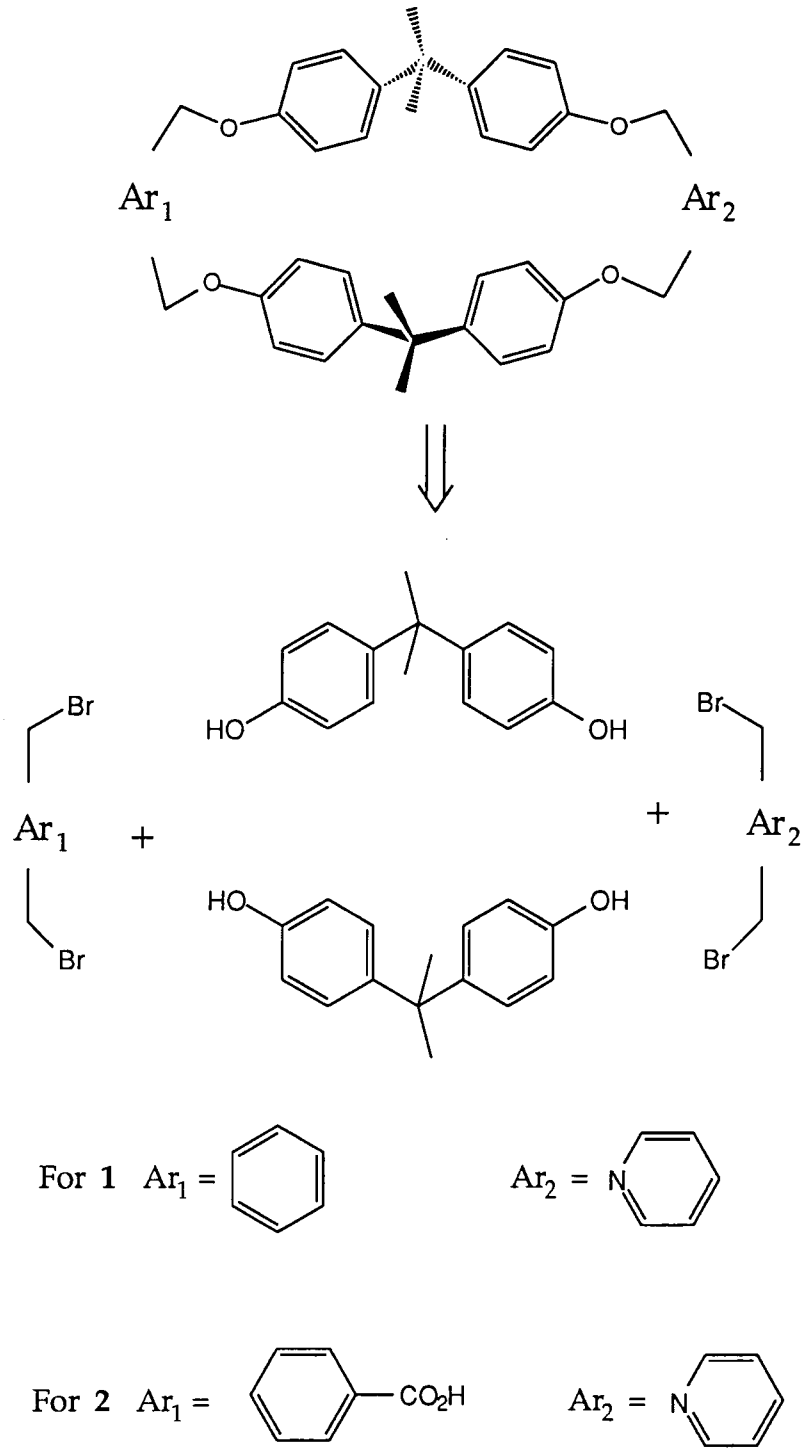
2.4. SYNTHETIC STRATEGY.

The cyclophanes **1** and **2** were subjected to simple retrosynthetic analyses, as shown in scheme 2.2 to give 2,6-bis(hydroxymethyl)pyridine, the diol 4,4-isopropylidenediphenol ("bis-phenol-A, BPA") and α,α'-dibromo-p-xylene for **1** or 2,6-bis(bromomethyl) benzoate for **2**, as readily available starting materials.

In most of the cyclophanes mentioned in chapter 1, the cyclisation is accomplished in a single step to give the "2+2" addition product. High dilution conditions[17] or templates[18] are often employed to minimise the amount of higher oligomers formed. Caesium salts, when present in the reaction medium, have often been found to lead to unusually high proportions of the cyclic product[19].

In the present case, however, a 2+2 cyclisation is obviously not practical: the macrocycle must be prepared in a stepwise fashion.

Saigo et. al. prepared the macrocycle shown in figure 2.6b not only in a 2+2 fashion, but also in a stepwise manner by first reacting 3,5-bis(bromomethyl)nitrobenzene with an excess (4 equivalents) of BPA to give the "U-shaped" pre-cursor shown in figure 2.6a in an 80% yield[20]. This was in turn reacted with a further equivalent of 3,5-bis(bromomethyl)nitrobenzene under conditions of high dilution to give the cyclic product, shown in figure 2.6b, in a yield of 35%[20].


Ar1
Ar2
O
O
O
O
Br
Br
Ar1
Ar2
HO
OH
HO
OH
Br
Br
+
+
For 1 Ar1 =
Ar2 =
N
For 2 Ar1 =
CO2H
Ar2 =
N

Scheme 2.2

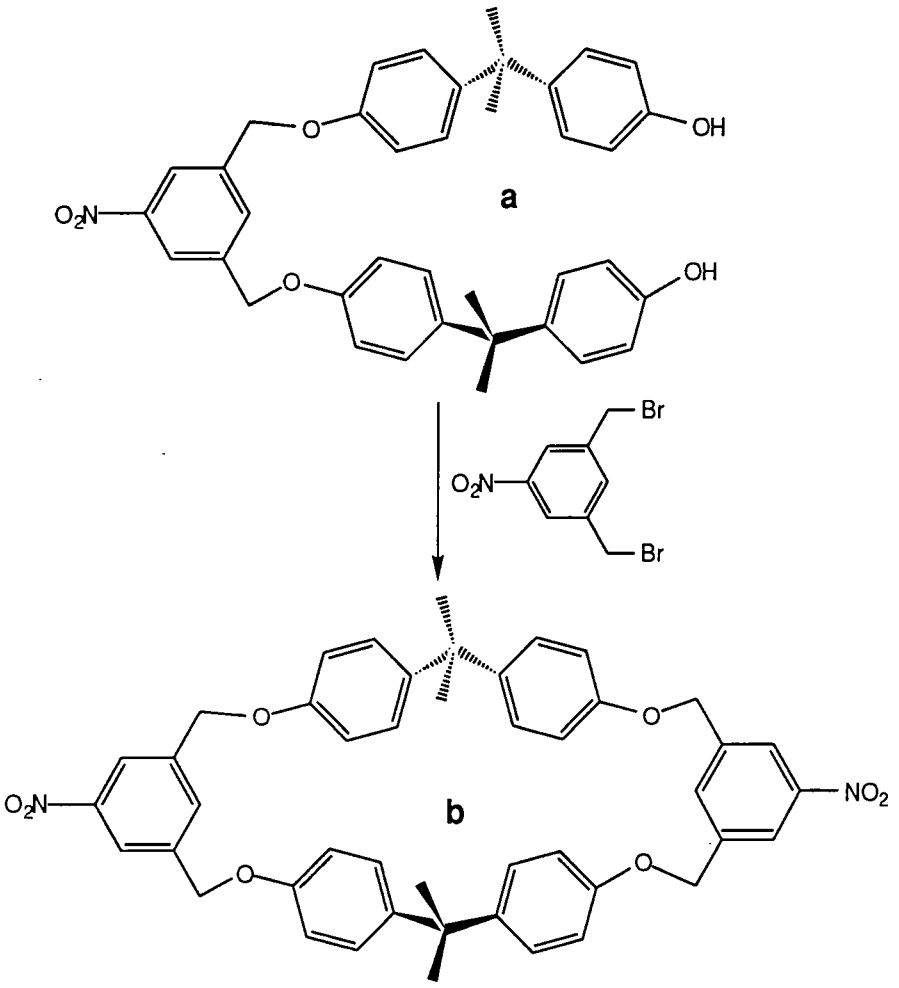
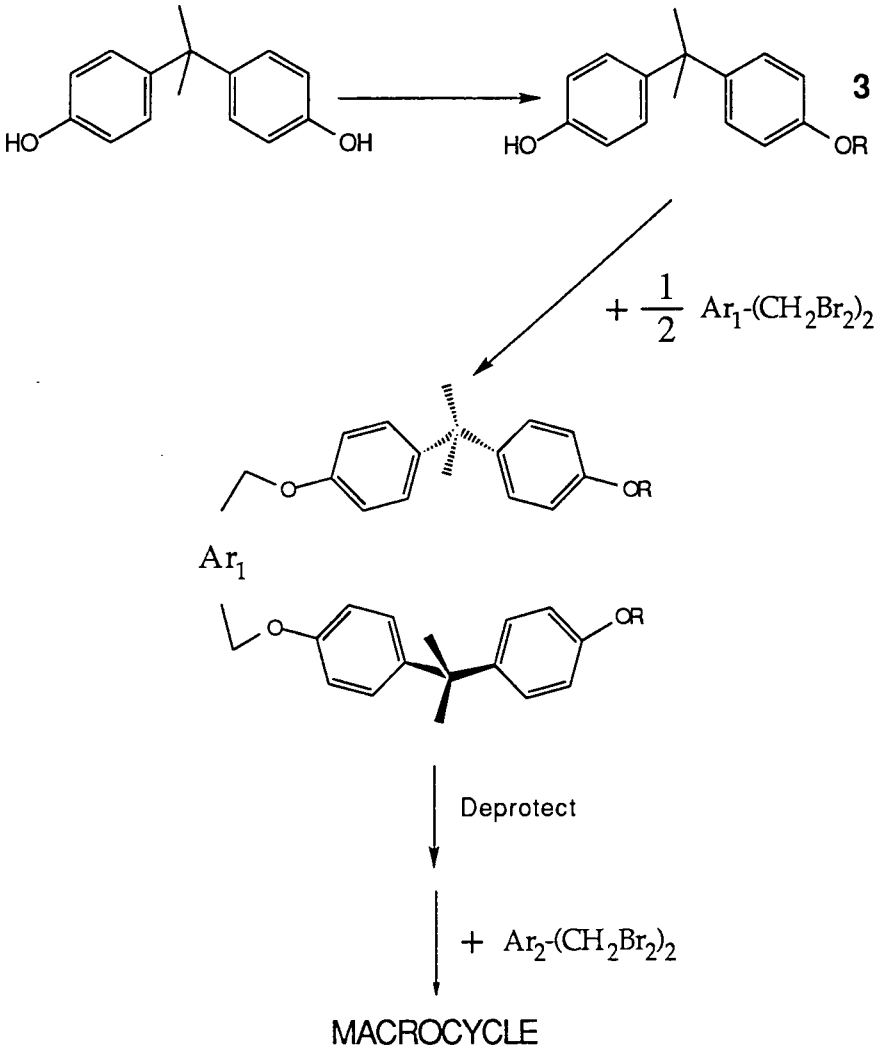


Figure 2.6

However, it was decided that for **1** and **2** a better method would be to mask one end of the BPA molecule with a protecting group before reaction with a dibromide, giving the general synthetic strategy shown in scheme 2.3.



Scheme 2.3

An efficient synthesis of the macrocycle depended on a good choice of protecting group "R". Initially, the methyl ether was chosen as the protecting group, for the following reasons:

(i) It was anticipated that the monomethyl ether **3** (R=Me) could be easily prepared by reaction of BPA with an equivalent of methyl iodide. Although a mixture of unreacted BPA and the mono- and di-methyl ethers would be obtained, they should be easily separable.

(ii) The methyl ether should survive the only required, subsequent step in the reaction scheme (alcohol + bromide in the presence of a base) to give **4** (R=Me).

(iii) Alcohol protection as methyl ethers is common, and consequently a variety of reagents exist for the cleavage of methyl ethers under a range of conditions.

2.5. SYNTHESIS OF CYCLOPHANES

2.5.1. Synthesis of Starting Materials.

The mono-methyl ether, "O-methyl-BPA", was prepared from BPA and methyl iodide by stirring as a solution in acetone in the presence of K_2CO_3 as a base. The monomethylated product **3** (R=Me) could be separated from dimethylated BPA by partitioning between aqueous sodium hydroxide and diethyl ether. However, separating it from unreacted BPA (by lowering the pH) was possible only with difficulty. Flash column chromatography gave good separation of the desired product from both starting material and the dimethylated compound.

The electrophile α,α-dibromo-p-xylene is commercially available, and 2,6-bis(bromomethyl)pyridine was prepared from 2,6-bis(hydroxymethyl)pyridine using the method of Baker et al.[21].

The synthon used for the benzoic acid residue was methyl 2,6-bis(bromomethyl)benzoate, prepared from 2,6-dimethyl benzoic acid according to the method of Cram[22].

2.5.2. Synthesis of "U-Shaped" Precursor.

O-methyl-BPA was stirred overnight with 0.5 equivalents of α,α'-dibromo-p-xylene in refluxing ethanol with K_2CO_3 as a base. The reaction was filtered hot and the product, **4** (R=Me) (see Figure 2.9) collected as the precipitate from the cooled solution in a 65% yield.

Several reagents were investigated for the demethylation step. It was found that if vigorous reagents were used e.g. BBr_3[23] then not only was the methyl ether bond cleaved, but also the benzyl ether bond. If mild reagents e.g. Sodium ethane thiolate[24] or lithium iodide in refluxing 2,6-lutidine[25] were used, then both bonds were left intact and starting material was recovered.

It therefore became necessary to consider other protecting groups, which could be removed easily under less forcing conditions.

Tetrahydrofuranyl ethers (THFE) have been used as protecting groups for alcohols[26]. They are very stable to basic conditions and would so survive the subsequent step in the reaction scheme (ethanol/K_2CO_3). However, they are very sensitive to even quite mildly acidic conditions, and it was likely that it would be necessary to use silica column chromatography in the work up of the ether. Since silica columns are mildly acidic, there was a risk that the THFE would decompose during isolation.

Tetrahydropyranyl ethers (THPE) have been more commonly used as protecting groups for alcohols[27]. They are also stable to basic conditions and are cleaved under conditions of low pH. However, they are more robust than THFE and are also easier to prepare. Consequently, THP was chosen as a potential protecting group.

The mono-THPE, 3 (R=THP), was prepared from BPA and 3,4-2H-dihydropyran at 0°C in the presence of a catalytic amount of hydrochloric acid with diethyl ether as solvent. The mono-THPE of BPA was separated from unreacted BPA and the di-THPE by flash silica chromatography.

Using HETCOR and COSY NMR experiments, the ^{1}H and ^{13}C NMR spectra of this compound were assigned. The ^{1}H and COSY spectra ($CDCl_3$, 400MHz) are shown in figure 2.7a and 2.7b.

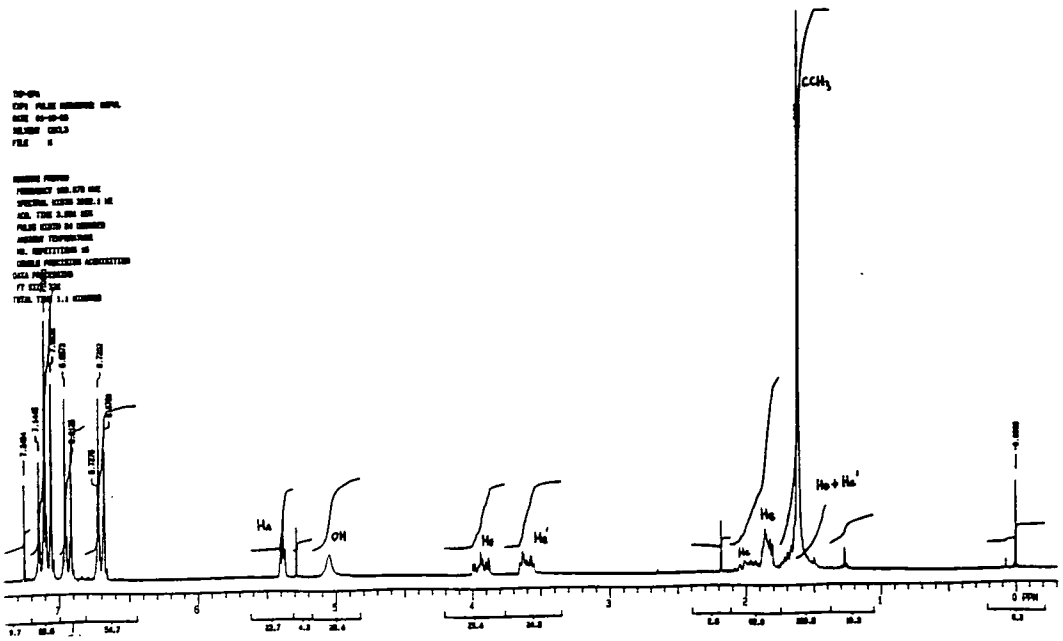


Figure 2.7a

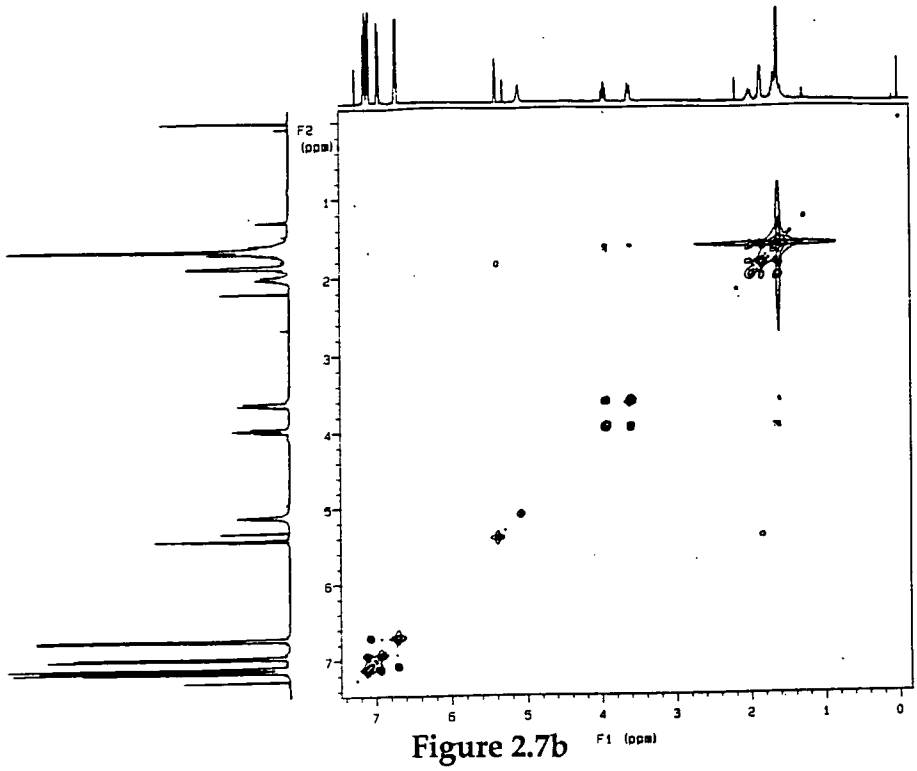


Figure 2.7b

The non-equivalence of axial and equatorial protons H_E and H_C (figure 2.8a) should be noted. This non-equivalence is not observed for H_B or H_D, implying the conformation shown in figure 2.8b, with the -OAr substituent of the THP ring in an axial position where it can interact through space with axial H_E and H_C protons.

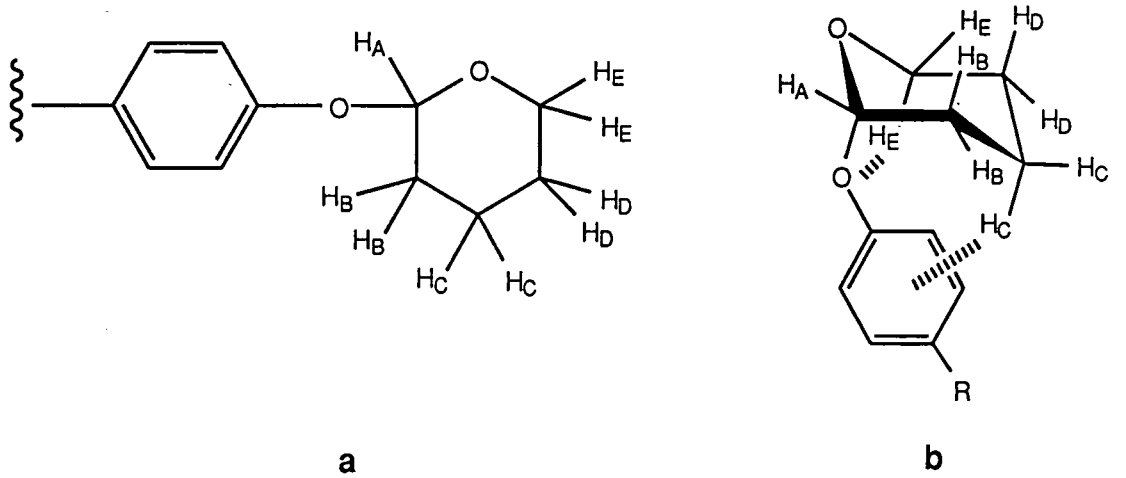


Figure 2.8

The ether O-tetrahydropyranyl BPA was then reacted with α,α'-dibromo-p-xylene in refluxing ethanol with K_2CO_3 as base. The product precipitated from the hot reaction mixture and was separated from inorganic salts to give 4 (R=THP) in about a 75% yield.

The alkyl halide 2,6-bis(bromomethyl)pyridine could be reacted with O-tetrahydropyranyl BPA under similar conditions. The product, 5 (R=THP) (figure 2.9), precipitated from the cooled, filtered reaction mixture and was also isolated in about a 75% yield.

For both **4** (R=THP) and **5** (R=THP) the non-equivalence of the H_C and H_E protons of the THP ring was again observed in the ^1H NMR spectra.

The diether was then refluxed with concentrated hydrochloric acid in a homogeneous methanol/chloroform solution to give the diols **4** (R=OH) or **5** (R=OH) in quantitative yield.

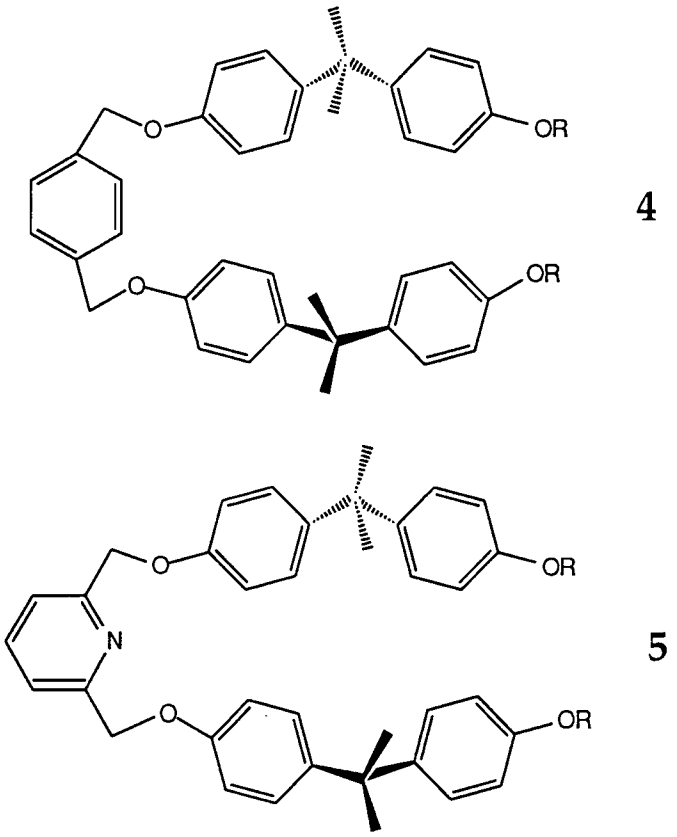


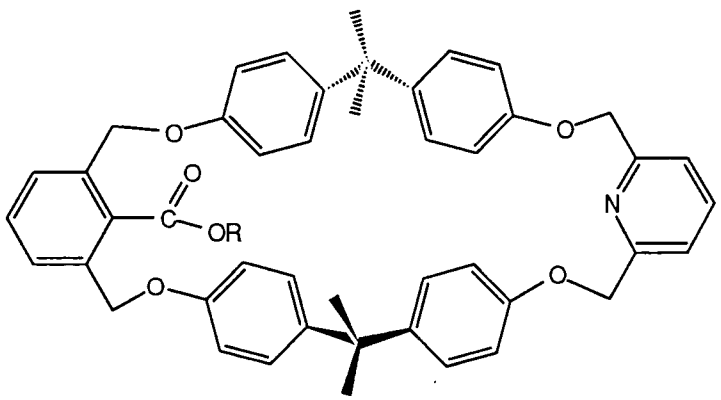
Figure 2.9

2.5.3. Macrocyclisation.

The macrocycle **1** was prepared from diol **4** (R=OH) and 2,6-bis(bromomethyl)pyridine in a 47% yield by refluxing in ethanol with K_2CO_3 as base at moderate dilution (about 10^{-2} M).

En route to the macrocycle **2**, the ester **6**, shown in figure 2.10, was prepared from diol **5** (R=OH) and methyl 2,6-bis(bromomethyl) benzoate by refluxing in ethanol with K_2CO_3 as base in a 31% yield.

It was noted that on some occasions, transesterification occurred during the cyclisation step to give the ethyl ester **6** (R=Et), whereas on other occassions the methyl ester **6** (R=Me) was obtained. The reaction was not repeated frequently enough to establish a correlation between reaction conditions and product. Mixtures of the ethyl and methyl esters were never obtained.



6

Figure 2.10

An attempt was made to perform the reaction with methanol in place of ethanol as the solvent, but a TLC analysis of the reaction

indicated that more side products had been formed, and that they ran very close to one-another and to the desired product on both silica and alumina supports with a variety of solvent systems. The next step of the synthesis requires the hydrolysis of the ester group and so the methyl and ethyl esters are of equal value. Because the reaction in ethanol gave less side products and allowed easier purification of the product, ethanol was therefore used as the solvent of choice.

As mentioned above, it has been demonstrated that for a large number of macrocyclisations the use of caesium salts in place of other bases has lead to significant improvements in yield[17]. In this case, however, replacing K_2CO_3 with Cs_2CO_3 as base resulted in a substantial drop in yield of the macrocyclic product from about 30% to less than 2%.

Many attempts were made to grow crystals of **6** which would be suitable for analysis by X-ray diffraction. Chloroform/hexane, dichloromethane/hexane and dichloromethane/methanol all gave poor quality crystals and were rejected as solvent systems for crystal growing. Crystals of a low but promising quality were precipitated from ethyl acetate by methanol and so many attempts were made using this solvent system and a variety of crystal growing techniques.

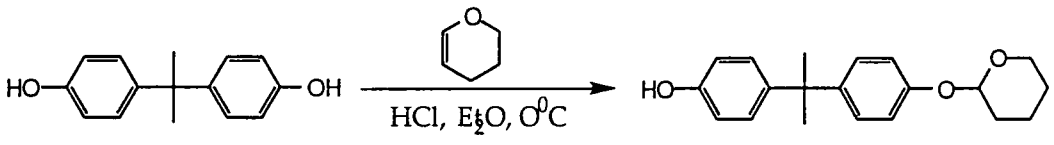
Good quality crystals of **6** were eventually obtained from an ethyl acetate solution by vapour diffusion of dry methanol through a pinhole. It had previously been observed that the crystals appear to "dry out" on standing in air and so X-ray diffraction measurements were taken at 120K under a nitrogen atmosphere. Despite these precautions, the crystals disintegrated in the X-ray beam.

Hydrolysis of the ester group of **6** was not straightforward. The macrocycle **6** was not water soluble, and molecular models had indicated that, depending on the conformation adopted, it was possible that the ester group could be "buried" in the cavity of the macrocycle leading to a significant steric barrier to the hydrolysis.

An attempted hydrolysis by refluxing the ester with hydrochloric acid in 90% aqueous methanol for 14 hours was not successful. Refluxing **6** with a 0.4M solution of LiOH in 85% aqueous THF for 3 days showed only traces of the hydrolysed product when analysed by thin layer chromatography. However, a 0.1M solution of NaOH in 90% aqueous ethanol successfully hydrolysed the ester to the sodium salt, **7a** (see scheme 2.7), after refluxing for several days.

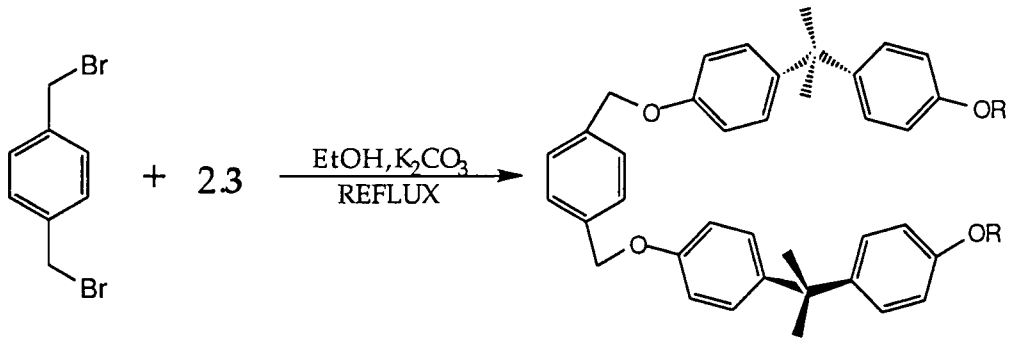
The syntheses of macrocycles **1** and **7a** are summarised in schemes 2.4 and 2.5 respectively.

Sodium salt **7a** was readily soluble in chloroform and was initially identified by ^1H NMR in deutero-chloroform (figure 2.11a). However, on standing for about 24 hours in a sealed NMR tube as a solution in $CDCl_3$, a white opaque layer began to form on the surface of the solution.



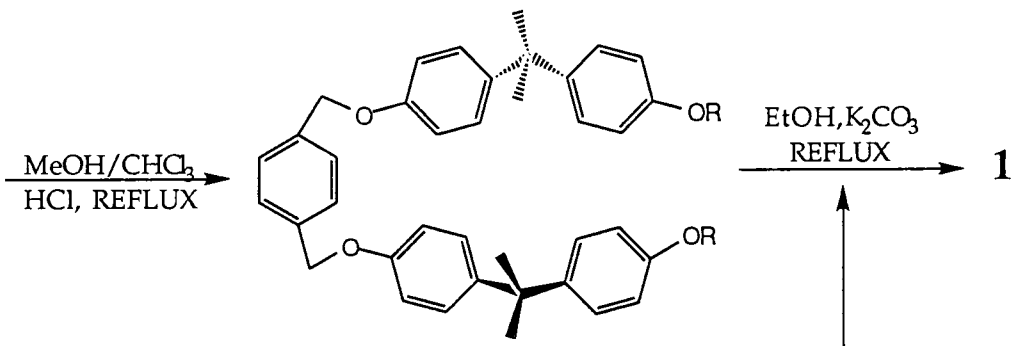
3

42%



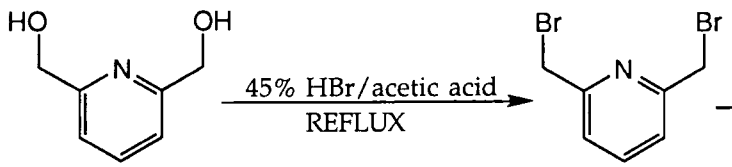
4, R=THP

78%



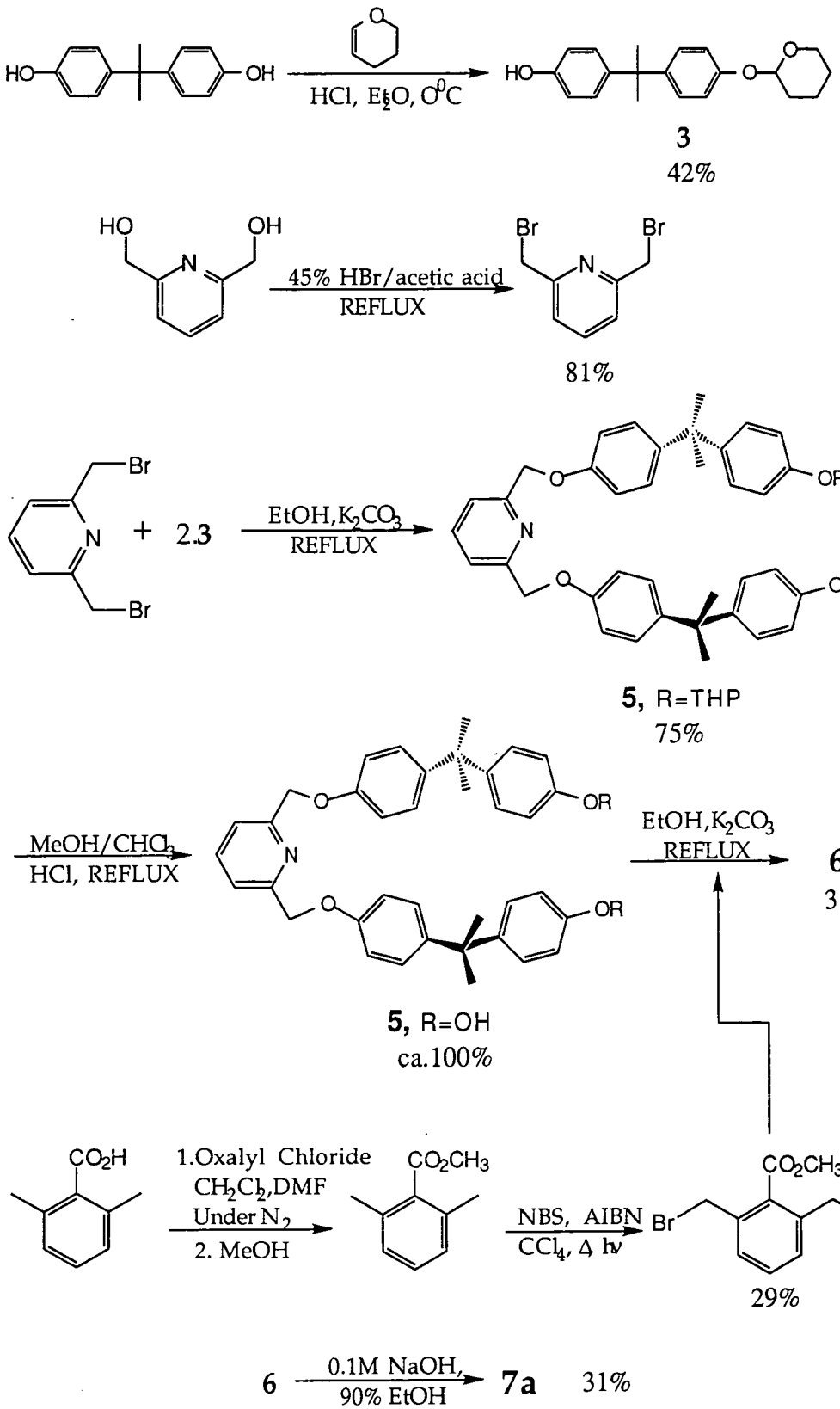
4, R=OH

ca.100%



81%

Scheme2.4


HO
OH
HCl, Et2O, 0°C
HO
O
3
42%
HO
OH
N
45% HBr/acetic acid
REFLUX
Br
Br
N
81%
Br
N
Br
+ 2.3
EtOH, K2CO3
REFLUX
O
OR
N
O
OR
5, R=THP
75%
MeOH/CHCl3
HCl, REFLUX
OR
N
OR
EtOH, K2CO3
REFLUX
6
31%
5, R=OH
ca.100%
CO2H
1.Oxalyl Chloride
CH2Cl2,DMF
Under N2
2. MeOH
CO2CH3
NBS, AIBN
CCl4, Δ hν
Br
CO2CH3
Br
29%
6
0.1M NaOH,
90% EtOH
7a
31%

Scheme 2.5

After standing for several days, $CDCl_3$ was evaporated at reduced pressure to leave a white solid which was reluctantly soluble only in an excess of chloroform. However, the solid was soluble in THF, although it was necessary to remove a small amount of insoluble material by filtration. A ^1H NMR spectrum as a solution in deutero-THF was then acquired and is shown in figure 2.11b at 250MHz and 2.11c at 400MHz. The spectrum of the freshly prepared sodium salt in $CDCl_3$, acquired within 20 minutes of making up the solution, is shown in figure 2.11a.

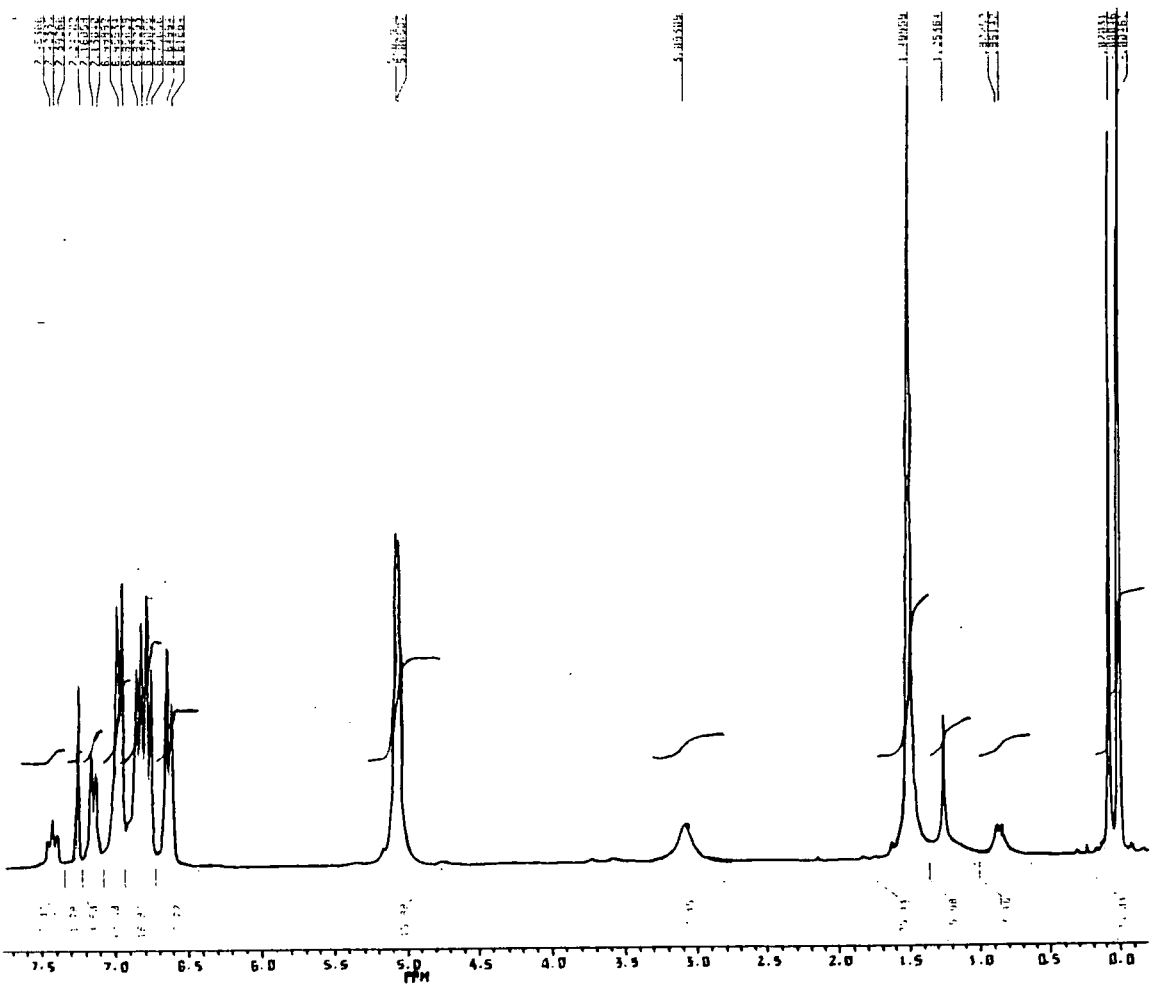


Figure 2.11a

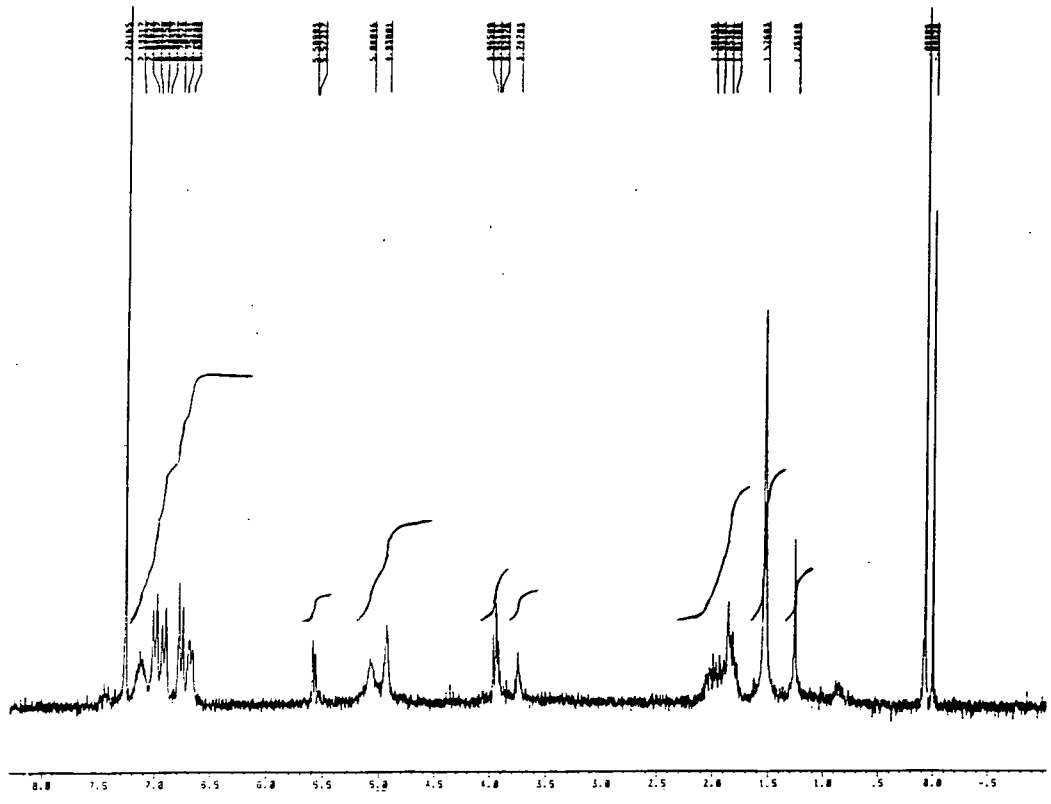


Figure 2.11b

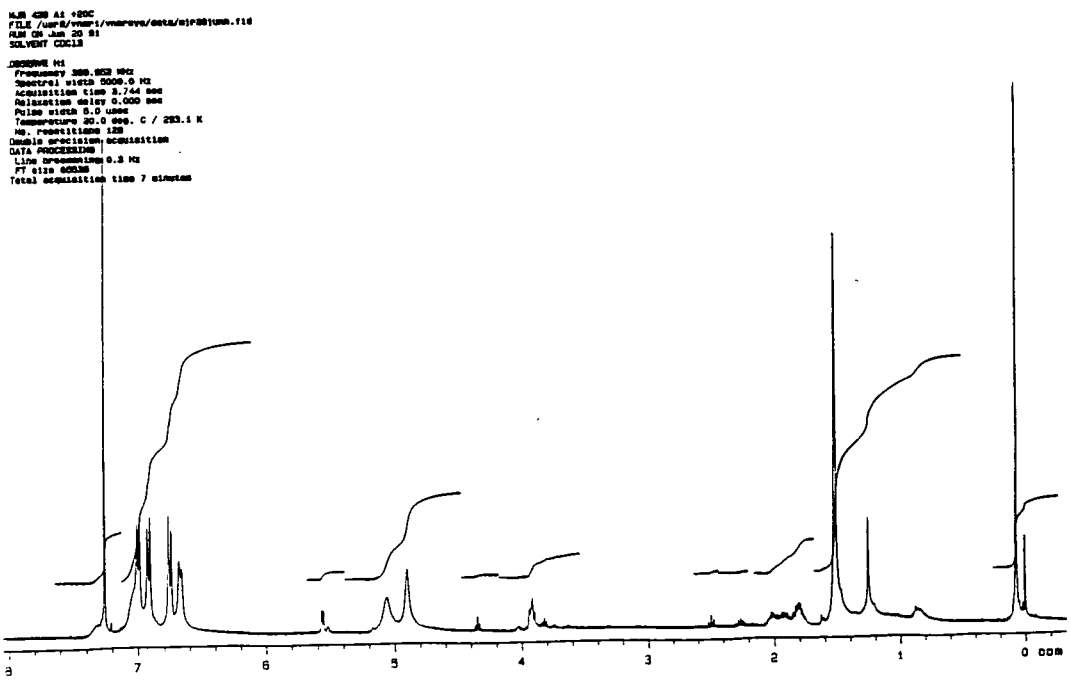


Figure 2.11c

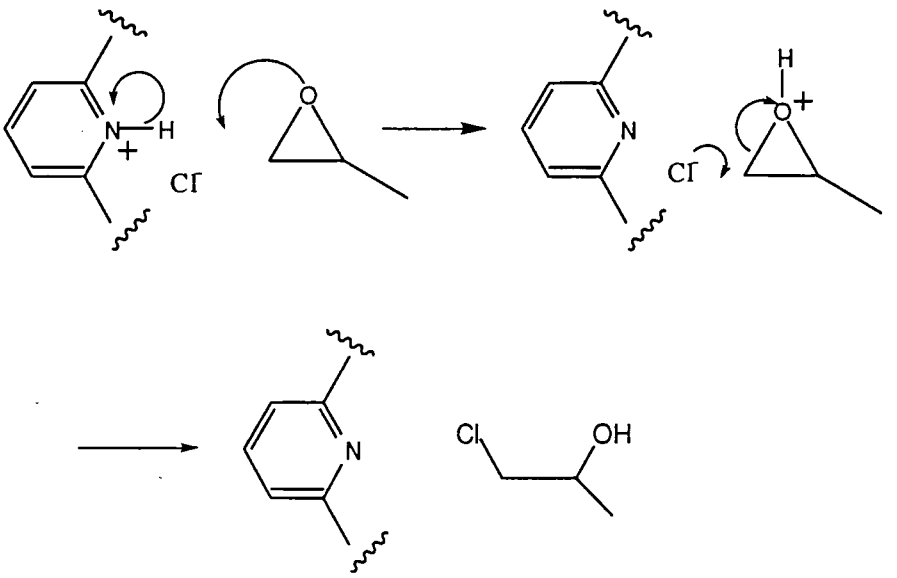
Apart from the spectra shown in figure 2.11b and 2.11c, no further characterisation of the decomposed sodium salt was obtained. However, it was obviously impossible to continue using chloroform as a solvent for the sodium salt **7a**. Freshly prepared sodium salt **7a** was found to be soluble in THF and was stable over long periods of time. Deutero-THF was consequently used for all NMR experiments involving the hydrolysed macrocycle i.e. for **7a, 7b** and **2**.

Conversion of the sodium salt to the protonated chloride salt was trivial; **7a** was stirred with hydrochloric acid as a suspension in water/THF to give **7b**.

Conversion to the neutral macrocycle **2** was less straightforward. Adjusting the pH is not a good method as the pKa's of the pyridine ring and carboxylate group were not known and a slight error in pH would lead to the formation of either the anionic (pH too high) or cationic (pH too low) species. Also, **2, 7a** and **7b** have very low aqueous solubility, so pH-metric titrations are non-trivial.

A much better method was to form the hydrochloride salt and react this with propylene oxide, a method often used in the preparation of biological zwitterions, which can be very pH sensitive[28]. The mechanism of the reaction of pyridinium with propylene oxide is shown in scheme 2.6 and the reaction "sodium salt **7a** - hydrochloride salt **7b** - neutral species **2**" is summarised in scheme 2.7.

1-Chloropropan-2-ol, the major side product of this reaction, is reasonably volatile (b.pt. 126-127°C) and can be removed under vacuum.

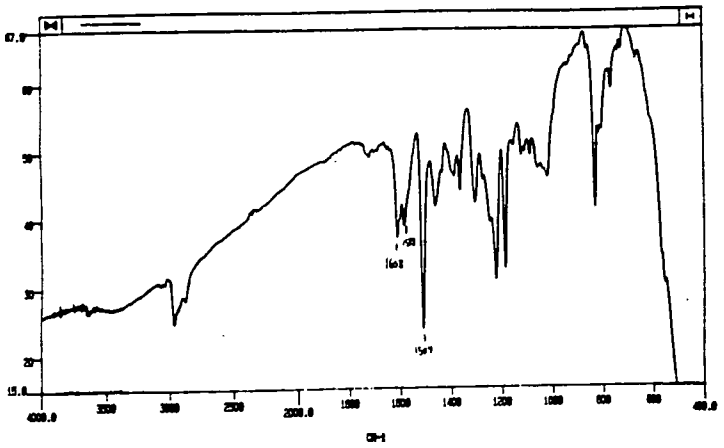


Scheme 2.6

It was difficult to predict beforehand whether the neutral macrocycle **2**, would, under the solvent conditions employed, exist as the uncharged carboxylic acid - pyridine ring or whether it would spontaneously form the charged carboxylate - pyridinium zwitterion shown in figure 2.5b.

Comparison of the infra-red spectra of **7a**, **7b** and the product of the propylene oxide reaction, shown in figures 2.12a-c, indicated that the zwitterionic species had been formed.

The IR spectrum of **7b** was observed to contain a peak at 1719cm^{-1}, consistent with an aromatic, carboxylic acid C=O stretching vibration. No carbonyl peak could be assigned in the spectrum of **7a**, but in the IR spectrum of **2** a peak at 1650cm^{-1} was attributed to a delocalised CO_2^- stretching vibration. A large, broad peak at 3435cm^{-1} was attributed to a pyridinium N-H stretch.



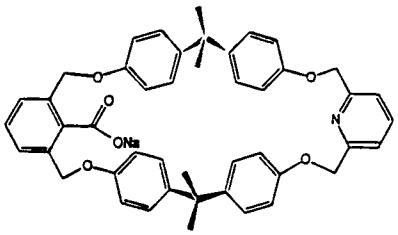
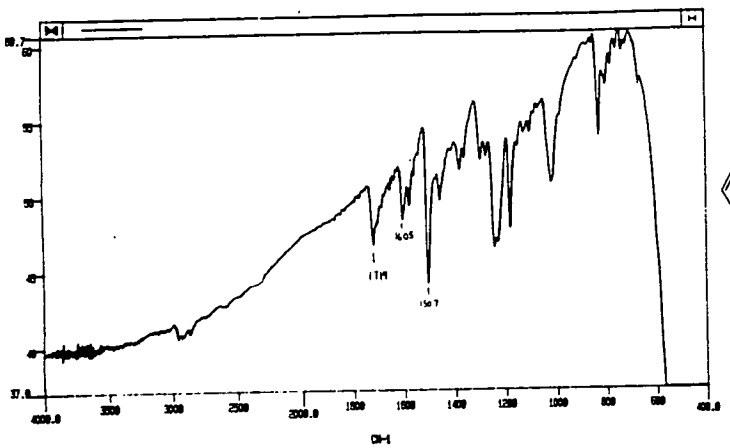


Figure 2.12a



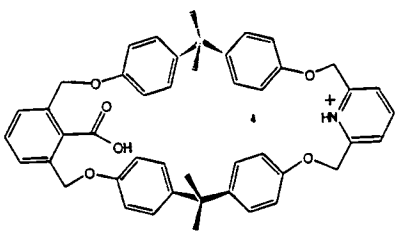
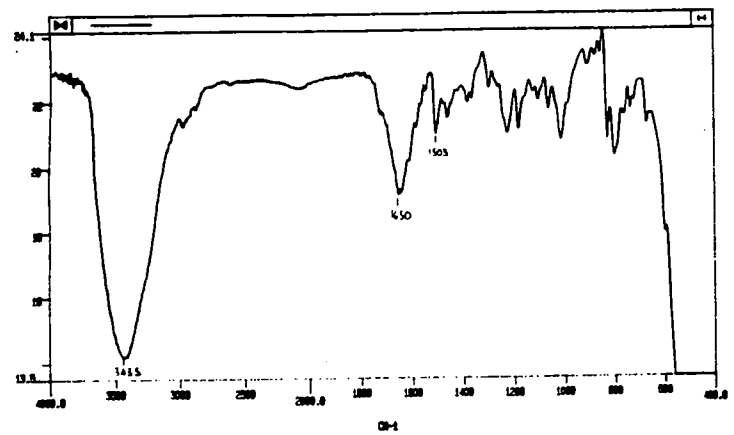


Figure 2.12b



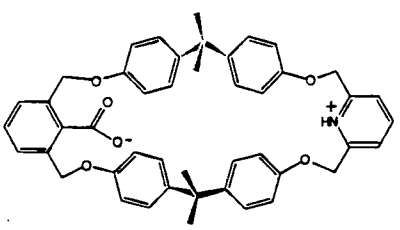
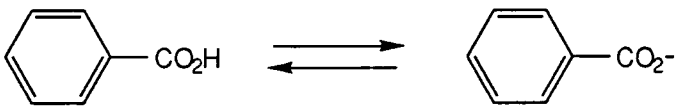
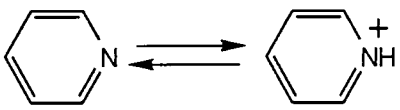
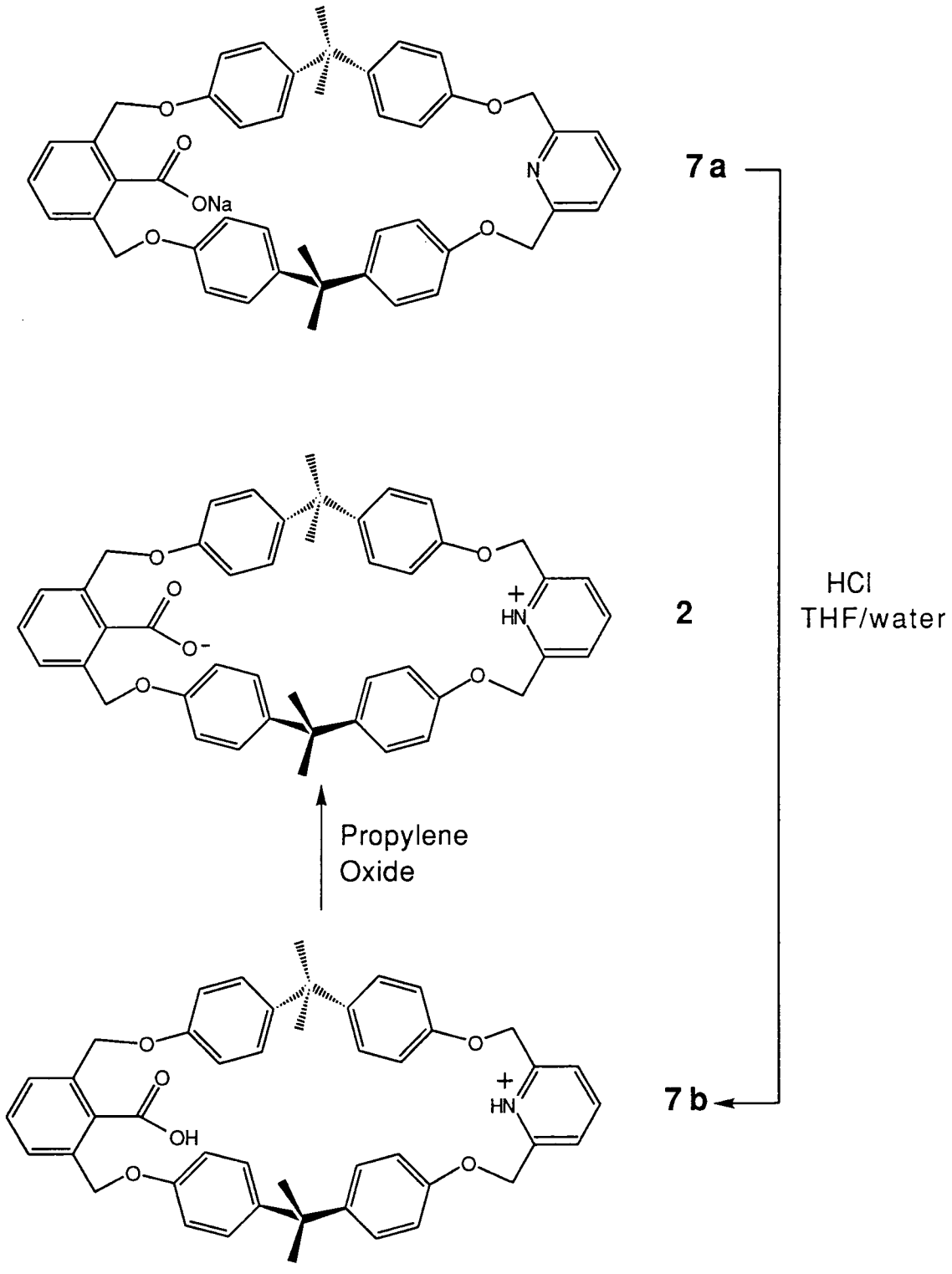


Figure 2.12c

The ^1H NMR spectra of the three compounds could also be compared. The aromatic region of **7a** is quite different from that of both **7b** and **2**, but for **7b** and **2** the aromatic regions are quite similar. This is consistent with zwitterion formation, as we would expect that changing from an aromatic carboxylic acid to carboxylate (and vice versa) would have a much smaller effect on the shift and coupling constants of the attached protons than a change from pyridine to pyridinium.

i.e. changing 

is much less significant than changing 



Scheme 2.7

2.6. BINDING STUDIES.

The use of NMR experiments in the study of receptor - sustrate binding is well established[29]. The technique depends on differences in the spectra of the free receptor and substrate and the complexed species. Differences in the chemical shift, $\Delta\delta$, of either the host or the guest at different host:guest ratios can be mathematically related to the equilibrium constant Ks. The ΔH value of the binding can be estimated from the temperature dependence of Ks using equation 2.1, the Van t'Hoff equation.

$$\frac{d\ln Ks}{dT} = \frac{\Delta H}{RT^2} \Longrightarrow \frac{d\ln Ks}{d(1/T)} = \frac{-\Delta H}{R}$$

The Van t'Hoff equation.

Equation 2.1.

A plot of lnKs vs. 1/T therefore has a slope of $-\Delta H/R$.

To measure Ks and ΔH for **1** and **2** with AcCh it was necessary to find a suitable solvent. Both the macrocycle and AcCh (commercially available as the chloride) should be soluble and the deuterated solvent should be available to enable ^1H NMR experiments to be conducted.

AcCh chloride is very hydrophilic and is insoluble in most organic solvents (except chloroform). Macrocycles **1** and **2** , however, were insoluble in water.

2.6.1. Acetyl Choline Chloride With **1**.

As AcCh chloride is reasonably soluble in chloroform, in the case of **1** chloroform was a good choice of solvent in which to study complexation phenomena. As a preliminary experiment, a solution of **1** in $CDCl_3$ was vigorously stirred with a solution of 5 equivalents of AcCh in an equal volume of D_2O for 4 days in a sealed container. After phase separation, the organic layer was dried by filtering through a plug of Na_2SO_4 and a ^1H NMR spectrum was acquired.

The signals which were assigned to the macrocycle were unshifted and no signals indicating the presence of AcCh in the chloroform solution were observed. It was concluded that AcCh chloride was too hydrophilic to be extracted into chloroform by **1**.

An experiment was conducted in the absence of water: macrocycle **1** was dissolved in chloroform and a tenfold excess of AcCh chloride added. The mixture was shaken until a homogeneous solution was formed. Solvent was evaporated at reduced pressure and the residue dried under vacuum. The mixture was then taken up in $CDCl_3$ and a ^1H NMR acquired.

Signals for both **1** and AcCh were unshifted with respect to the spectra of the separated solutions of **1** and AcCh. It was concluded that binding between macrocycle **1** and AcCh was too weak to be observed under these conditions.

Macrocycle **1** was incorporated into a PVC membrane which was then tested in an ion sensitive electrode (see Appendix 1). As a

control, an identical membrane was constructed but without incorporating **1**. This was also used in an ISE.

Using this technique, the response of **1** to a variety of substrates (electrolytes) could be quickly and easily tested using only a small quantity of the macrocycle (ca.15mg). A response of about 59mV would indicate that the test electrolyte might form stable complexes with the macrocycle in solution.

Initially, the response of the ISE's to a test solution of AcCh was measured.

For the control ISE (no macrocycle) a response of 42.5mV to a test solution of AcCh indicated that transport of AcCh was poor.

When the membrane containing **1** was used in the ISE, the electrode response improved to 59mV indicating efficient transport of AcCh by the membrane and implying that **1** can form weak complexes with AcCh.

When the test solution of 0.1M AcCh was contaminated with other electrolytes (NaCl, NH_4Cl, $CaCl_2$), also at a concentration of 0.1M, no interference was observed i.e. the electrode response was still 59mV. When the AcCh test solution was contaminated with KCl, the response decreased slightly to 55mV indicating that there may be a small amount of potassium interference.

However, when the pH of the test solution was lowered to 2.2 by adding 0.1M HCl, the response of the ISE dropped to almost 0mV. This indicates substantial pH interference, which was expected (protonation of macrocyclic pyridine ring to give a positively charged pyridinium residue, leading to repulsion of the AcCh cation).

The responses of the test ISE to 0.1M solutions of ammonium chloride, tetramethylammonium chloride and tetraethylammonium chloride were then measured. It was hoped that for tetramethylammonium chloride a response would indicate efficient transport of the electrolyte, showing that **1** might interact with N-methyl ammonium cations via an aromatic π - ammonium cation interaction (see Chapter 1).

However, very modest responses were observed for the ammonium, tetramethyl and tetraethyl ammonium chlorides (27.5mV, 38mV and 37.5mV respectively).

The response to tyramine hydrochloride ($Cl^-NH_3^+CH_2CH_2$-C_6H_5OH) was also measured, as it was thought that a π-stacking interaction with **1** might allow transport across the membrane. However, a disappointing response of only 21.5mV was observed.

2.6.2. Acetyl Choline With 7a (Sodium Salt).

The macrocycle **2** was known to be unstable in chloroform (see pages 61-66) and so it became necessary to find a second solvent system in which to study any possible complexation phenomena. Although AcCh chloride was insoluble in THF, it was found that on addition of a small amount of water, AcCh chloride was sufficiently soluble to enable NMR experiments to be conducted.

If the concentration of water was kept low then both **2** and **7a** (the zwitterion and the sodium salt) were also soluble. In practice, it

was found that a mixture of about 1% or 2% water in THF was the best compromise.

30mg of **7a** (Na^+ salt) were dissolved in 1ml of deutero-THF and 20µl of D_2O added. A ^1H NMR spectrum was acquired. Small, known amounts of AcCh chloride were added and further ^1H NMR spectra acquired after each addition so that the ratio of macrocycle : AcCh varied from 10:1 to about 1:10.

When this ratio became greater than about 1:1 i.e. in an excess of AcCh, phase separation began to occur. Small droplets of water could be seen in the NMR tube and a precipitate formed. The signals in the ^1H NMR spectrum broadened slightly.

Because the spectra of the solution did not decrease in intensity (signal : noise ratio did not decrease for a given number of acquisitions) it was assumed that the precipitate was not the macrocycle, and was taken to be sodium chloride. However, when the amount of AcCh present was calculated from the peak integrations of the spectra it was found that the weighed amount of AcCh was much greater than the amount of AcCh in solution. It was concluded that at least some of the precipitate was AcCh chloride.

When the ratio of macrocycle : AcCh (weighed amounts) reached about 1:5, most of the precipitate redissolved and the signals in the spectrum sharpened again.

The ^1H NMR spectra of **7a** and of **7a** with an excess of AcCh in deutero-THF/2% D_2O are shown in figure 2.13a and 2.13b. Although slightly greater than 10 equivalents of AcCh chloride were weighed out, the peak integrals of the spectrum indicated that the ratio **7a** : AcCh was only about 1:2.

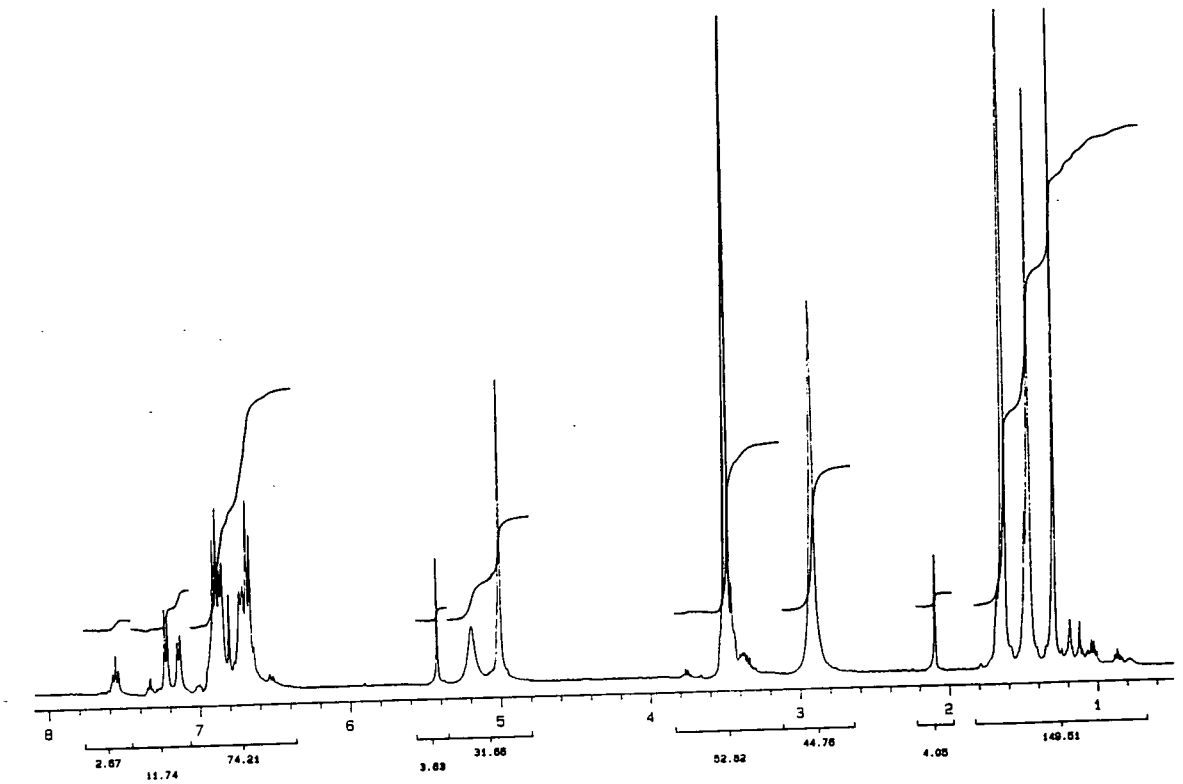


Figure 2.13a

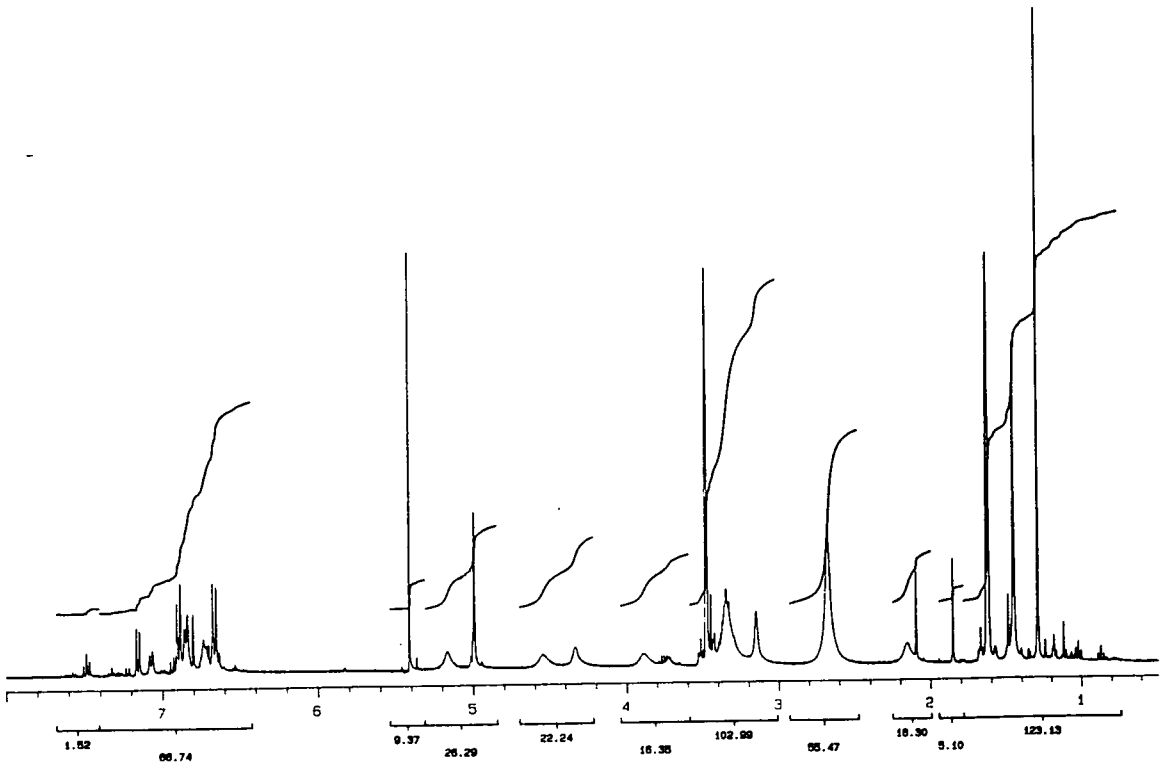


Figure 2.13b

The signals assigned to AcCh were very broad and so could not be accurately measured - even so, such a large disagreement does indicate that there was considerably less AcCh in solution than was known to be present.

As AcCh was added to **7a**, positive (upfield) shifts in the positions of all the signals associated with the macrocycle were observed, with the exception of the CCH_3 singlet. These changes are summarised in table 2.1.

The CCH_3 protons were expected to be remote from the cavity and would not therefore be influenced if AcCh were bound. Consequently, this signal was not expected to move.

Of the shifted signals, the one assigned to the pyridine triplet is the most convenient in terms of measuring the change in chemical shift, $\Delta\delta$, as it is unambiguously assigned and is well separated from other signals in the spectrum. Values of $\Delta\delta$ for the pyridine triplet at given macrocycle/AcCh ratios are given in table 2.2 and a plot of $\Delta\delta$ versus this ratio is shown in figure 2.14a. Figure 2.14b shows a plot of $\Delta\delta$ versus the log of the macrocycle/AcCh ratio. Note that the ratio was calculated using the weighed amounts of macrocycle and AcCh.

Assigned Protons	FREE MACROCYCLE Shift, ppm	Integral	MACROCYCLE + AcCh Shift, ppm	Integral
CCH_3	1.460	12	1.460	12
$ArCH_2O$	5.004	4	4.992	4
$ArCH'_2O$	5.192	3	5.165	2
	5.243	1	5.413	2
Biphenyl AA'BB' systems and Benzoate triplet	6.663-6.950	17	6.655-6.908	17
Benzoate doublet}*	7.140	2	7.074	2
Pyridine doublet}*	7.225	2	7.159	2
Pyridine triplet	7.560	1	7.487	1

*The doublets for the pyridine and benzoate rings cannot be unambiguously assigned.

Table 2.1

δ pyridine triplet (ppm)	Δδ pyridine triplet (Hz)	Ratio [7a]/[AcCh]	Log ([7a]/[AcCh])
7.560	0	1/0	1/0
7.561	-0.4	9.09	0.959
7.559	0.4	4.55	0.658
7.558	0.8	1.82	0.260
7.556	1.6	1.01 (3.47)	0.004 (0.540)
7.546	5.6	0.76 (1.72)	-0.119 (0.236)
7.533	10.8	0.46 (1.41)	-0.337 (0.149)
7.516	17.6	0.19 (0.86)	-0.721 (-0.066)
7.487	29.2	0.09 (0.53)	-1.046 (-0.276)

*The ratio is calculated from the weighed amounts of macrocycle and AcCh. Figures in parentheses give the ratio calculated from the peak integrations in the ^1H NMR spectrum.

Table 2.2.

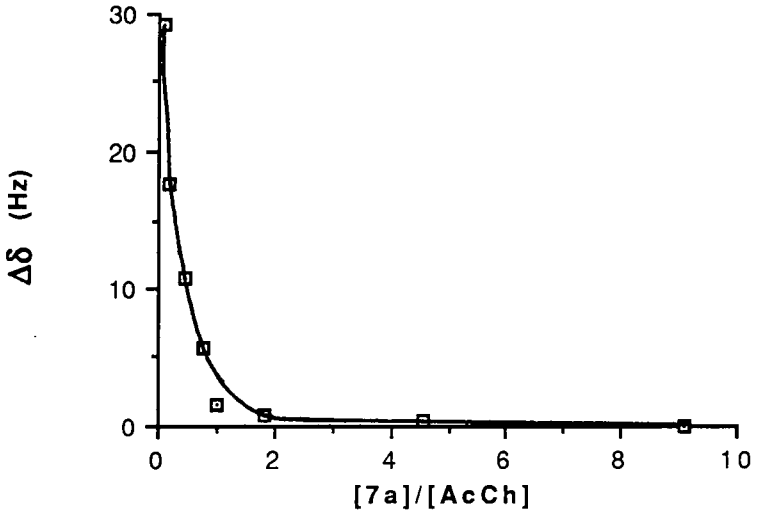


Figure 2.14a

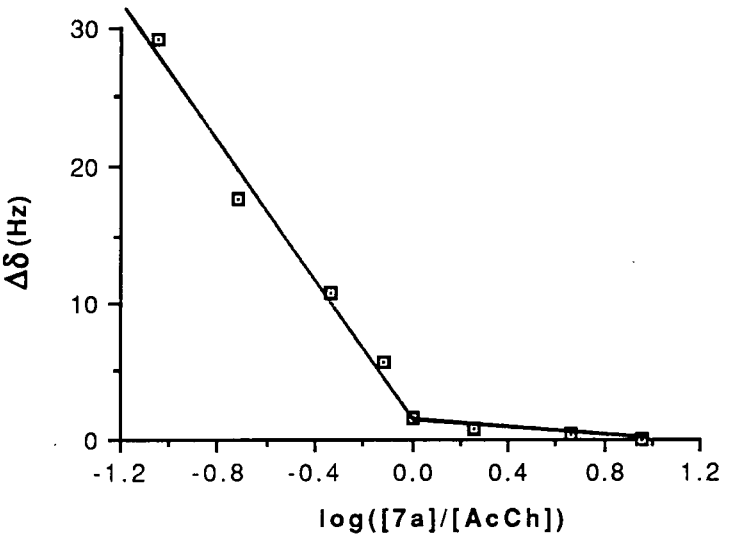


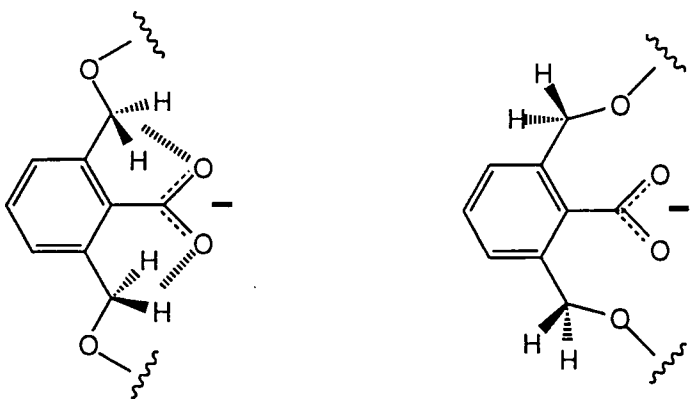
Figure 2.14b

The signals assigned to the $ArCH_2O$ protons were expected to show two singlets corresponding to the two uncoupled, chemically distinct sets of protons.

However, one of these signals is split into one sharp singlet (5.243ppm) and one quite broad singlet (5.192ppm) which are approximately in the ratio 1:3 (2H and 6H). This indicates that the macrocycle is in a conformational equilibrium, with slow exchange between the conformers on the NMR time scale.

Since this equilibrium was not observed for the methyl or ethyl esters **6**, it was assumed that the methylene protons which could be seen in two different conformations are those attached to the benzoate ring.

The larger, broad signal was assigned to the methylene protons which were turned into the cavity and influenced by the carboxylate group (figure 2.15a). The less intense, sharper signal was assigned to the methylene protons which were turned outwards, away from the carboxylate group (figure 2.15b).



a **b**

Figure 2.15

As AcCh was added, the intensity of the broad signal decreased and that of the sharp signal increased until they were approximately

in a 1:1 ratio. As the position of the conformational equilibrium was affected by AcCh, it could be inferred that the macrocycle and AcCh at least interact. Of the two conformations, the one with the methylene protons turned outwards i.e. the least favoured conformation of the free macrocycle (figure 2.15b) seems to be the one that is preferred for complexation with AcCh. If **7a** has the conformation shown in figure 2.15a and **7a'** is the macrocycle with the conformation preferred for binding, represented by figure 2.15b, then the conformational equilibrium is described by equation 2.2a and the binding equilibrium is described by equation 2.2b.

$$\mathbf{7} \underset{}{\overset{K_1}{\rightleftharpoons}} \mathbf{7a'} \qquad \textbf{Equation 2.2a}$$

$$\mathbf{7a'} + \text{AcCh} \overset{K_2}{\rightleftharpoons} \text{Complex} \qquad \textbf{Equation 2.2b}$$

Since we know from the NMR integrations that the ratio of **7a** : **7a'** is 3:1, we know that $K_1 = [\mathbf{7a'}]/[\mathbf{7a}] = 0.33$. Note, however, that this value varied considerably depending on the concentration of sodium salt **7a**, and on the amount of water (D_2O) present.

As the relative concentration of AcCh increased, new signals appeared in the NMR spectrum.

Two distinct sets of signals were seen and these were attributed to free and bound AcCh. The chemical shift values of AcCh chloride in 5% D_2O/d^8-THF (300MHz) and the signals attributed to the free and bound AcCh in the presence of **7a** are given in table 2.3. The number of protons assigned to each signal is inaccurate because of the very broad nature of the signals, although the signal which has

been assigned (questionably) to the free acetyl CH_3 group was quite sharp and this assignment is tentative.

Assigned Protons	AcCh chloride	Free AcCh in Presence of **7a**	Bound AcCh
CH_2N	4.38 (2H)	4.34 (2H)	4.54 (2H)
CH_2O	3.63 (2H)	3.73 (1H)	3.88 (2H)
NCH_3	3.17 (9H)	3.15 (3H)	3.35 (10.5H)
CH_3	1.98 (3H)	1.85 (1H) ?	2.15 (2.5H)

Table 2.3.

As mentioned above, it was thought that the two sets of signals which arose on addition of AcCh to **7a** were due to the slow exchange between free and bound AcCh.

However, phase separation occurred when an excess of AcCh had been added and it was possible that the exchange was between AcCh(aq.) and AcCh (THF). This suggestion was rejected for several reasons:

(i) Although the signals due to AcCh are weak at lower AcCh/macrocycle ratios, two distinct sets of signals can still be seen. At these lower ratios, phase separation had not occurred.

(ii) The volume of D_2O used was only 20µl and even when phase separation occurred, the depth of D_2O at the bottom of the NMR tube was very small. The NMR was acquired at a sample depth of about 2cm and so the aqueous phase would not have been seen.

(iii) That two sets of signals were seen implied that the exchange was slow on the NMR time scale. Exchange between one solvent phase and another is usually quite rapid and time averaged signals would have been seen.

It had also been suggested that while one set of signals was due to AcCh, the second set was due to choline which had been produced by the hydrolysis of AcCh via acyl transfer to the macrocyclic pyridine nitrogen.

The proposed mechanism, shown in scheme 2.8, would be analogous to that of AcChE (scheme 2.1). Note, however, that in the esterase hydrolysis of AcCh the conversion of the tetrahedral intermediate to the acyl-enzyme is assisted by the nearby histidine residue. In the proposed mechanism of hydrolysis by the macrocycle there is no assisting group for the analogous step.

It was proposed that the hydrolysis was only partially complete because the product, choline, was bound in the macrocyclic cavity more strongly than AcCh and so any catalytic activity was inhibited by the hydrolysis product.

To test this theory, two experiments were proposed:

(i) A ^{1}H NMR spectrum of **7a** with an excess of AcCh (the solution resulting from the NMR titration) could be acquired at a lower operating frequency (200MHz instead of 400MHz). If the two sets of signals arise from free and bound AcCh, which are in slow exchange on the NMR time scale, lowering the operating frequency of the NMR spectrometer should lead to an apparent increase in the exchange rate. Where two sets of signals were seen at 400MHz, each

pair should approach coalescence at 200MHz as the signals should begin to show time averaging.

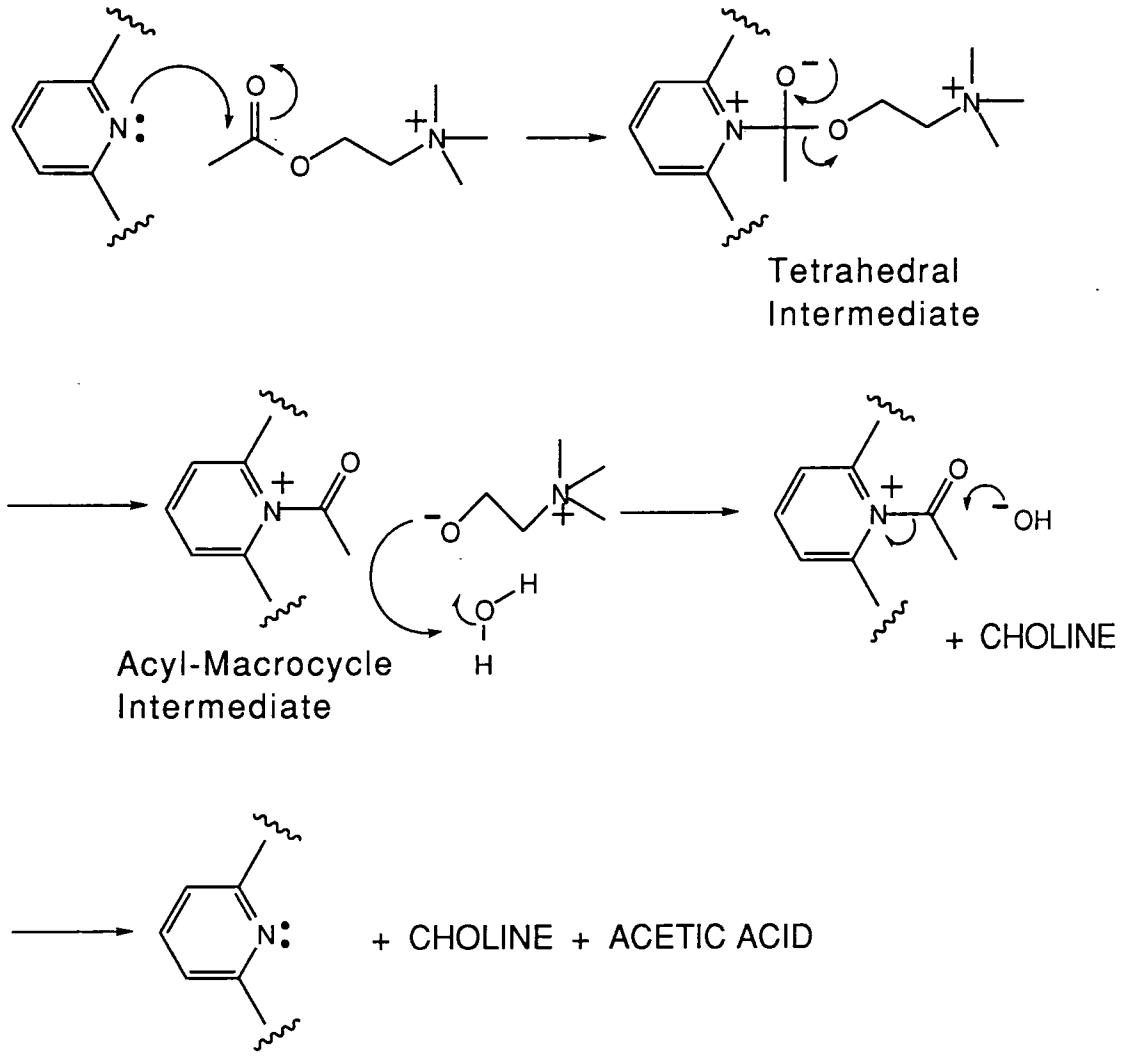
The broad nature of the signals at 400MHz was perhaps an indication that the signals, if they were due to free and bound AcCh, were already approaching coalescence.

If the two sets of signals were still seen at 200MHz, then either the exchange rate of free and bound AcCh was still too slow on the NMR time scale to show coalescence or the presence of both AcCh and choline would still be a possibility.

As it had also been suggested (see above) that the macrocycle itself was involved in a conformational equilibrium, lowering the NMR operating frequency may also provide further information about this exchange process.

(ii) To the same solution used for (i), above, choline could be added. If one set of signals was seen to undergo an increase in intensity and no new signals appeared then the presence of choline in the solution would be proved.

Conversely, if a new set of signals arose, then the absence of choline from the original solution would be proved, and the two sets of signals could be unambiguously assigned to free and bound AcCh.



Scheme 2.8.

EXPERIMENT 1. Change of NMR Operating Frequency.

The NMR titration experiment of **7a** against AcCh was conducted at 400MHz. Solvent was evaporated from the resulting solution (**7a** + excess AcCh) and the residue dried thoroughly under vacuum and stored as a solid in a sealed flask for about 2 months.

The residue was then taken up in 2ml of d^8-THF and 4 drops of D_2O added. The resulting clear solution was placed in an NMR tube and a ^1H NMR acquired at an operating frequency of 200MHz. (As in the titration experiment, phase separation occurred in the NMR tube).

The ^1H NMR spectra at 400MHz and 200MHz are shown in figures 2.16a and 2.16b respectively.

At an operating frequency of 400MHz (in the presence of macrocycle **7a**), two sets of signals could be seen for each of the AcCh N-methyl, N-methylene, O-methylene and acetyl CH_3 protons. At an operating frequency of 200MHz, coalescence of the N-methyl and N-methylene signals occured. Coalesence of the signals assigned to the AcCh O-methylene and acetyl CH_3 protons did not occur, but the separation of the signals decreased. These changes, summarised in table 2.4, suggest (but do not prove) that the two sets of signals arise from free and bound AcCh.

In order to assign one set of signals to free AcCh and the other to bound AcCh (or choline) a ^1H NMR spectrum of acetyl choline in 5%D_2O/d^8-THF was acquired at 300MHz. Obviously the chemical shift values of AcCh alone will be slightly different to the values of free AcCh in the presence of the macrocycle. However, those signals which had been assigned to AcCh in the AcCh/macrocycle mixture and had the closest chemical shift values to AcCh alone were assigned to free AcCh.

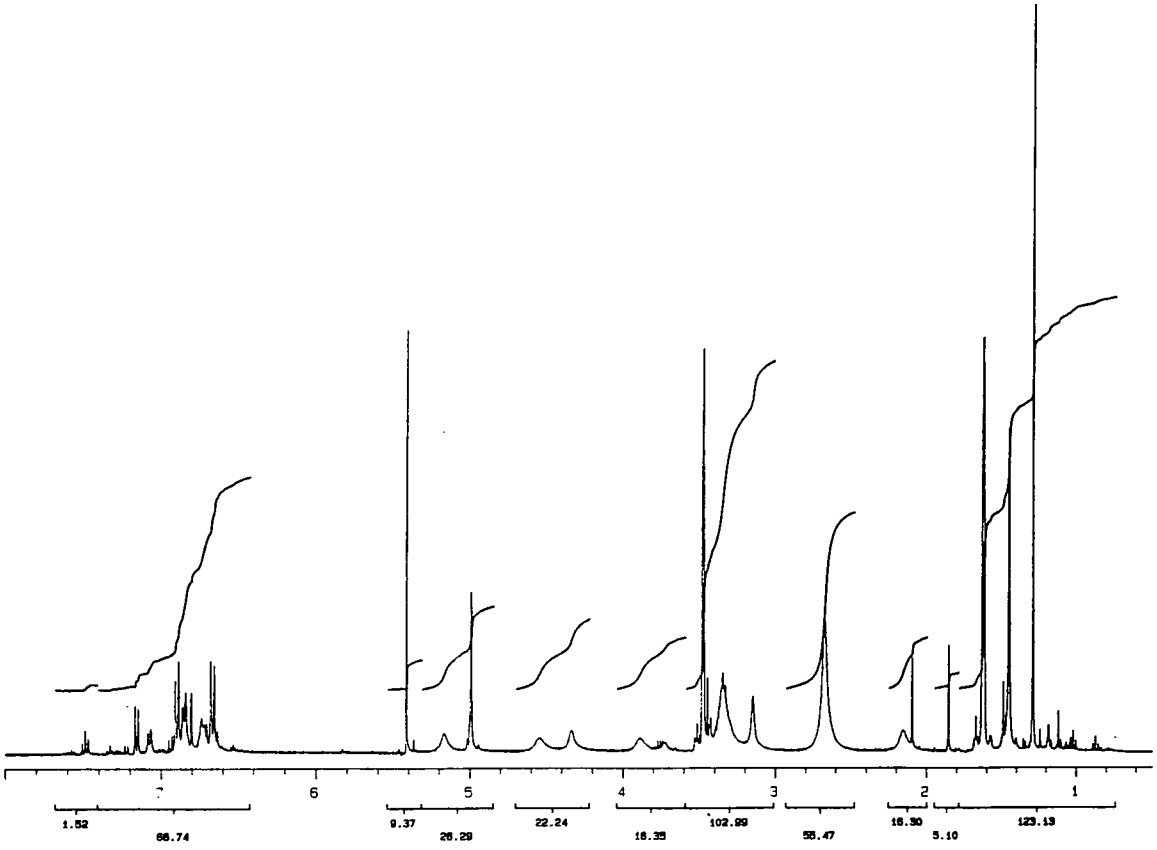


Figure 2.16a

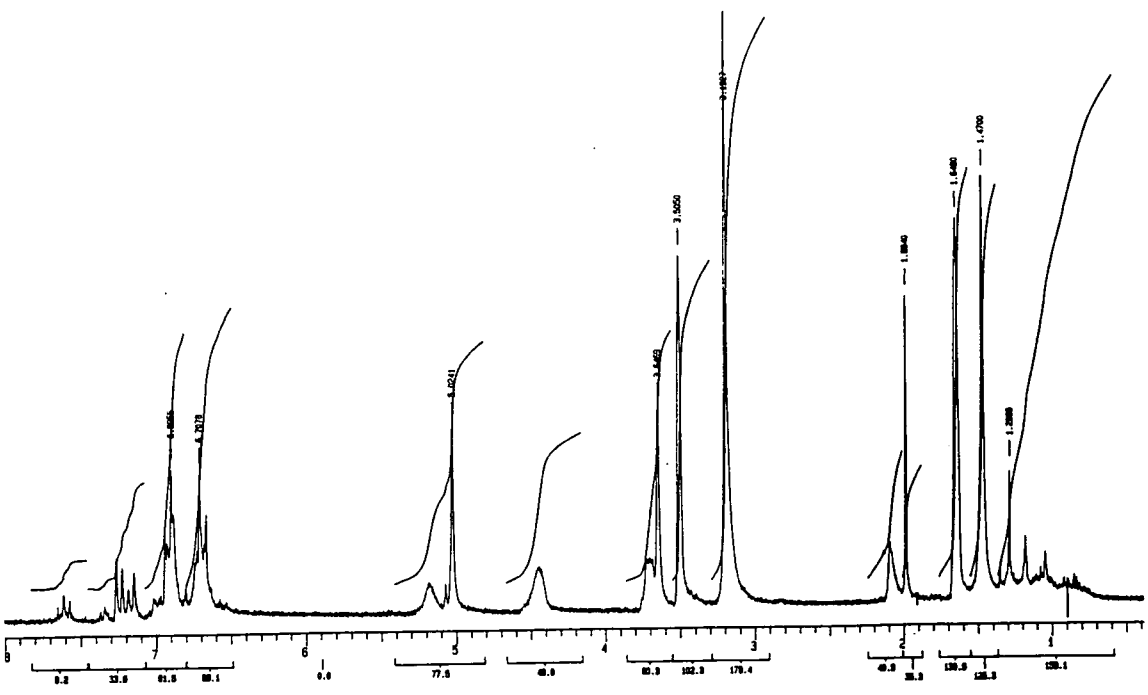


Figure 2.16b

Assigned Protons	AcCh + 7a : 400MHz		AcCh + 7a : 200MHz		AcCh : 300MHz
	Free	Bound	Free	Bound	
Acetyl CH_3	1.85?	2.15	1.98	2.09	1.89
N-methyl	3.15	3.35	3.19	3.19	3.08
O-CH_2	3.73	3.88	3.65	3.71	3.54
N-CH_2	4.34	4.54	4.45	4.45	4.29

Table 2.4.

It is interesting to note that the two pairs of signals which coalesced at 200MHz were those which were α to the nitrogen atom of AcCh. This may imply that the ammonium head was in a faster exchange than the ester end of the molecule.

For the proposed equilibrium between the macrocyclic conformations responsible for the broad $ArCH_2O$ peak at 5.17ppm and the less intense, sharp $ArCH'_2O$ peak at 5.41ppm, only the broad signal could be seen at 200MHz. However, the relative intensity of this peak (ca.2.5H) was still too low to account for the four protons which made one pair of chemically identical $ArCH_2O$ residues.

While it can be inferred from these observations that the two sets of signals seen at 400MHz are due to free and bound AcCh, the absence of choline from the solution had not been proven.

EXPERIMENT 2. Addition of Choline.

Choline is commercially available as the chloride, $Me_3N^+CH_2CH_2OH.Cl^-$. However, choline chloride was insoluble in the chosen solvent system and was converted to the trifluroacetate ("TFA") by anion exchange to the hydroxide followed by reaction with trifluroacetic acid. Choline TFA was soluble in THF and water.

A ^1H NMR spectrum of choline TFA in 5%D_2O/d^8-THF at 200MHz was acquired. A known volume of this solution, containing about 10 equivalents of choline (with respect to **7a**), was then added to the AcCh/macrocycle solution in the NMR tube. This was shaken vigorously, allowed to stand for 2 hours and then shaken again before another ^1H NMR spectrum was acquired, also at 200MHz. These spectra are shown in figures 2.17a and 2.17b respectively.

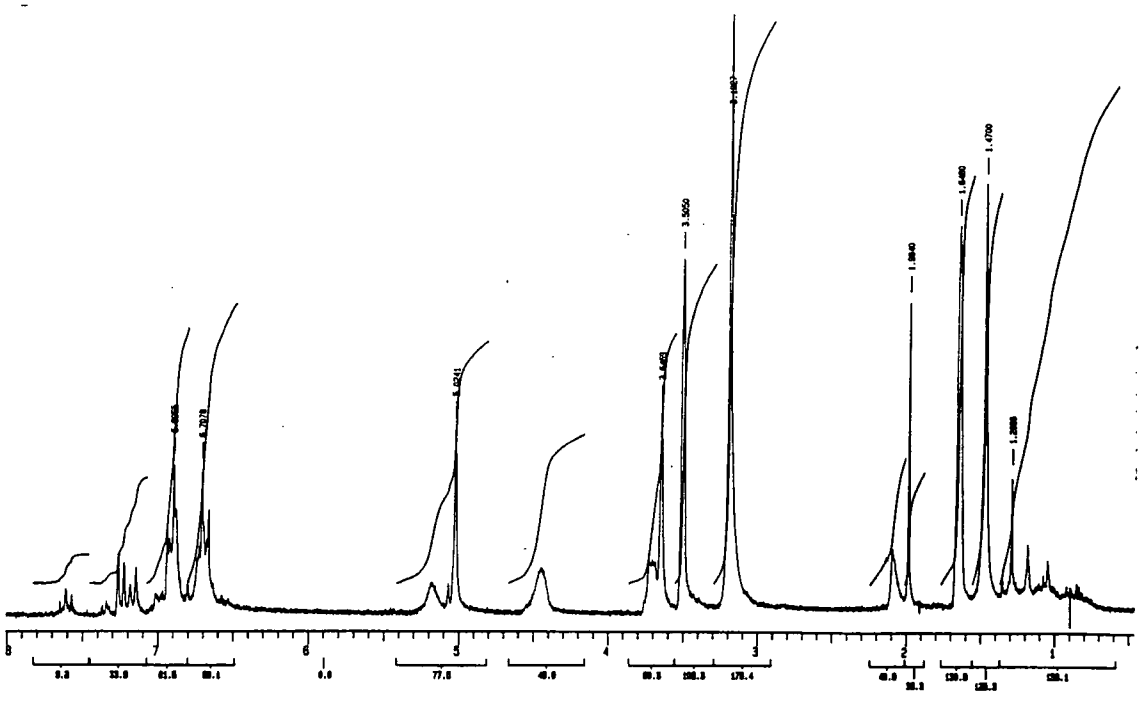


Figure 2.17a

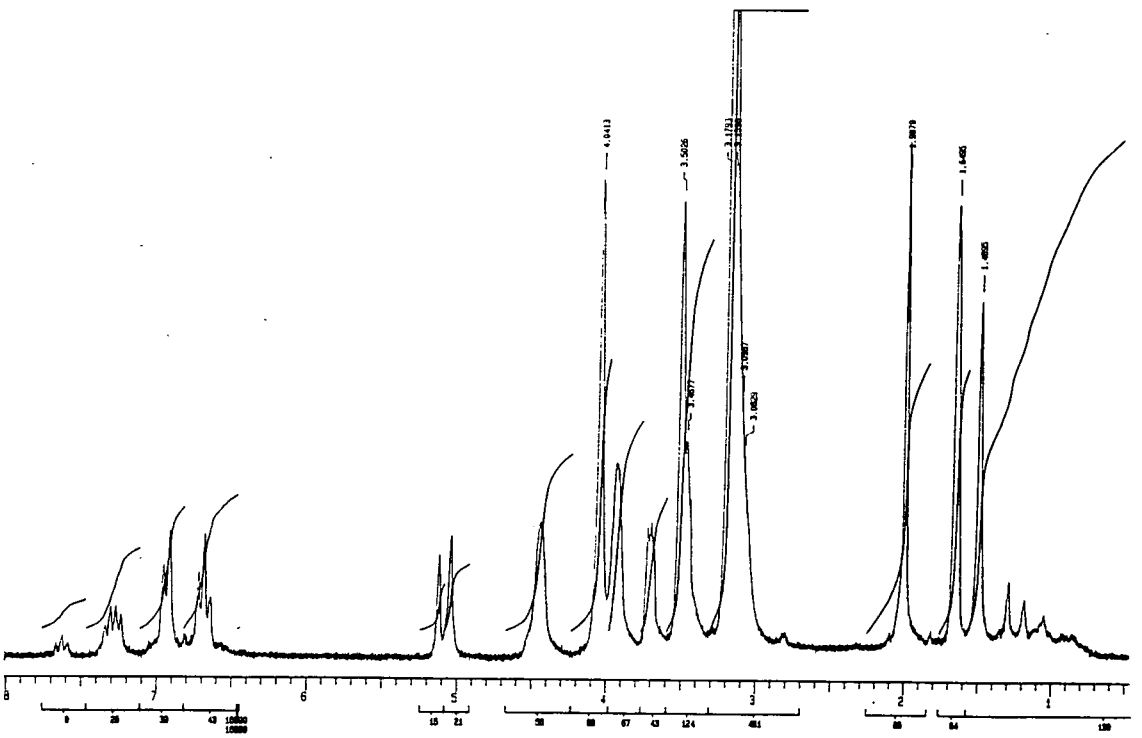


Figure 2.17b

The signal which had been assigned to the N-methyl protons of AcCh increased in intensity on addition of choline TFA. This was not surprising as the AcCh N-methyl and choline N-methyl protons should be very similar and consequently have similar chemical shift values.

For the two pairs of signals assigned to AcCh CH_2O and acetyl CH_3 protons, one signal from each pair became vanishingly small on addition of choline, while the other increased proportionally in intensity.

The peak which had been assigned to the AcCh CH_2N protons increased more than two-fold in relative intensity.

New signals arose in the spectrum, which by comparison with the choline TFA spectrum in d^8-THF/5%D_2O were assigned to choline CH_2OH (3.47ppm) and choline CH_2N (3.92ppm) protons. The chemical shifts of these peaks were not significantly different from the shifts of choline alone in d^8-THF/5%D_2O.

(A third new signal also appeared in the spectrum at 4.04ppm, which could only be assigned to some unknown impurity).

These changes show that no choline was present in the original AcCh/macrocycle solution and therefore the two sets of signals which had been observed at 400MHz were probably due to slow exchange between free and bound AcCh.

Furthermore, on addition of an excess of choline, those signals which had been assigned to bound AcCh vanished and those which had been assigned to free AcCh increased in intensity. This can be explained if both AcCh and choline are bound by macrocycle **7a**, and that AcCh is displaced from the [**7a**.AcCh] complex by choline when an excess of choline is added. Changes in the chemical shifts of the choline protons relative to choline alone were not observed, probably because the observed signals are time averaged and there was approximately a tenfold excess of choline.

Choline trifluroacetate was added to a fresh, uncontaminated sample of sodium salt **7a**. Changes in the shifts of the macrocyclic protons indicated that choline was bound, and that exchange between free and bound species was fast on the NMR time scale (200MHz). Furthermore, comparison with the spectra of **7a** with an excess of AcCh conclusively showed that no choline was present in the **7a**/AcCh mixture.

No conclusions can be drawn concerning the relative stabilities of the [**7a**.AcCh] and [**7a**.choline] complexes, but the rate of exchange between free and bound species is fast in the **7a**/choline mixture and slow in the **7a**/AcCh mixture.

In order to gain further understanding of the conformations available to the macrocycle and the influence of AcCh upon the conformational equilibria, a variable temperature ^1H NMR experiment of the sodium salt **7a** (in the absence of AcCh) was undertaken. The temperature range -50°C to +40°C was examined at an operating frequency of 400MHz.

Although the separation of two signals arising from the $ArCH_2'O$ protons decreased at higher temperatures ($\Delta\delta$ = 0.39ppm at -50°C and 0.20ppm at +40°C), no other significant changes were observed over the given temperature range.

2.6.3. Acetyl Choline with **2** (Zwitterion).

A titration similar to the one above (section 2.6.2) was conducted, using **2** in place of **7a**. ^1H NMR spectra were acquired after each addition of AcCh. A 2% solution of D_2O in d^8-THF was again used as solvent and the ratio of **2** : AcCh varied from about 10:1 to about 1:10.

Note that initially the macrocycle was not completely dissolved and a small amount of precipitate was present in the NMR tube. When sufficient AcCh was added for the macrocycle/AcCh ratio to reach about 1:1, the precipitate dissolved to give a clear, homogeneous solution.

Although the solution remained homogeneous when about 5 equivalents of AcCh had been added, when a further 5 equivalents were added it was found that some AcCh chloride remained undissolved.

The spectra of **2** and **2** with an excess of AcCh in 2%D_2O/d^8-THF are shown in figures 2.18a and 2.18b respectively.

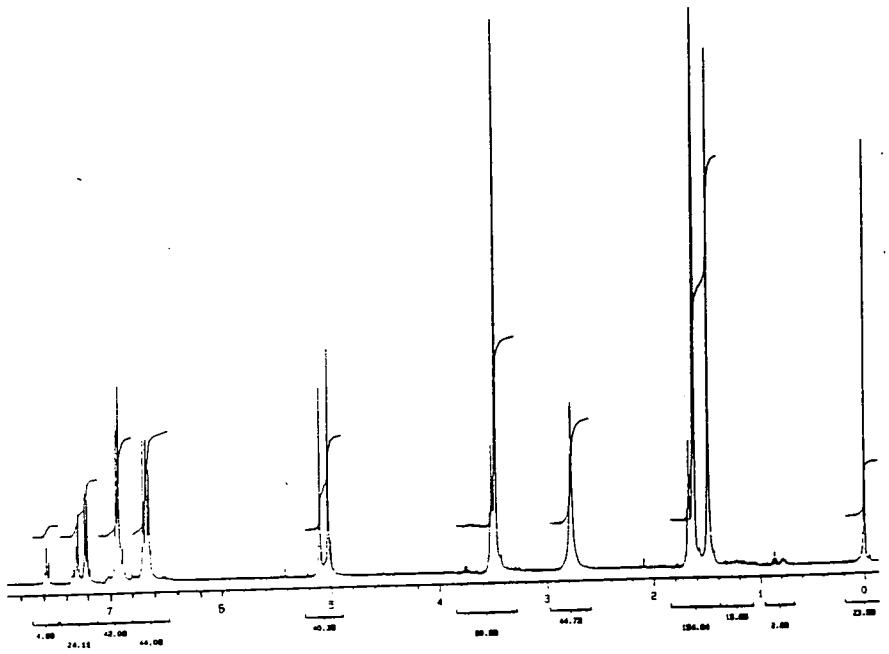


Figure 2.18a

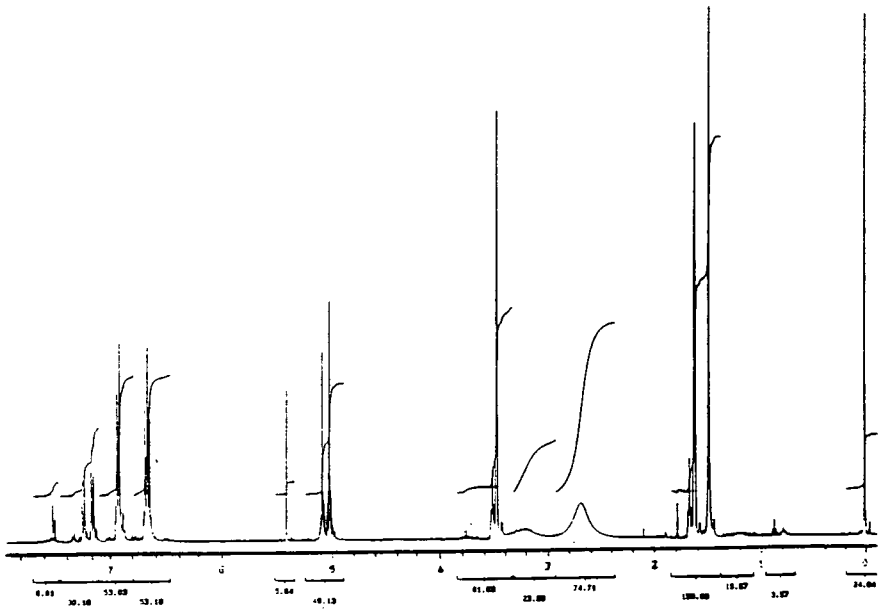


Figure 2.18b

Signals which could be assigned to the added AcCh were difficult to see (except for a broad signal at about 3.2ppm which was assigned to the NCH_3 protons) due to extreme broadening, indicating that AcCh was involved in an exchange process which was rapid on the NMR time scale. It was proposed that this exchange was between the bound and unbound states.

This proposal was in agreement with the changes in the chemical shifts of the signals which were assigned to the macrocycle, which are summarised in table 2.5.

Only the CCH_3 protons were, as expected, unaffected. They are remote from the macrocyclic cavity and should not be influenced by the inclusion of AcCh.

The protons attached to the DPP shaping groups were only slightly affected, undergoing only small changes in chemical shifts and coupling constants.

The position of the doublets arising from the pyridinium and benzoate residues changed significantly, but the two signals could not be unambiguously assigned. For the free zwitterion, the benzoate triplet coincided with one of these doublets and its presence could only be inferred from the peak integrations.

In the presence of AcCh, this triplet was split into two separate signals, one of intensity 0.3H at 7.33ppm and one of intensity 0.7H which was still obscured by the doublet at 7.17ppm.

This indicates that in the presence of AcCh the macrocycle was itself involved in an exchange process which was slow on the NMR time scale. In the absence of AcCh this exchange process was not observed.

time averaged signal is seen the observed exchange phenomena cannot be simply explained as an exchange between different conformations. It is plausible that the exchange process observed on addition of AcCh was between the free zwitterion and the [**2**.AcCh] inclusion complex.

The relative intensities of the two signals (1.7H : 0.3H) indicated that the ratio free **2** : complex was about 5:1.

Both the $ArCH_2O$ signals showed only small changes in chemical shift. One of them also decreased in intensity as AcCh was added from just less than 4H to 3.2H in the presence of excess AcCh. A signal at 5.420ppm, which could only just be seen in the spectrum of free macrocycle, was seen to undergo a proportional increase in intensity from about 0.0H to 0.8H as AcCh was added, and also moved to 5.415 in the presence of an excess of AcCh.

This also indicates that the macrocycle was involved in an exchange process, which was slow on the NMR time scale in both the presence and absence of AcCh. However, the position of the equilibrium was obviously affected by AcCh. It was proposed that the less stable conformation was the one which was most likely to form a complex with AcCh and that this interaction was responsible for the shift in the conformational equilibrium.

There was also a change in the chemical shift of the pyridinium triplet, from 7.576ppm in the free zwitterion to 7.521 in the presence of excess AcCh. This signal is the most convenient in terms of measuring changes in chemical shift as it can be unambiguously assigned, is of constant intensity and is well separated from other signals in the spectrum.

Values of Δδ for the pyridine triplet at given ratios of **2**:AcCh are shown in table 2.6 and a plot of Δδ versus this ratio and Δδ versus the log of the ratio are shown in figures 2.19a and 2.19b respectively.

δ Pyridine Triplet (ppm)	Δδ Pyridine Triplet (Hz)	Ratio [2]/[AcCh]*1	log ([2]/[AcCh])
7.576	0	1/0	1/0
7.576	0.0	10.00	1.000
7.574	0.8	5.0	0.699
7.571	2.0	1.33	0.125
7.565	4.4	0.93	-0.032
7.561	6.0	0.63	-0.201
7.557	7.6	0.48	-0.319
7.542	13.6	0.20	-0.699
7.521	22.0	0.10 (0.15)*2	-1.000

*1 Ratio is calculated from the weighed amounts of **2** and AcCh, as AcCh signals were too broad to be seen in the ^{1}H NMR spectrum and the ratio could not be calculated from peak integrations.

*2 Not all AcCh dissolved, so calculation of the ratio using the weighed amounts of **2** and AcCh gives a false value. An estimate of the amount of AcCh in solution is given in parentheses.

Table 2.6

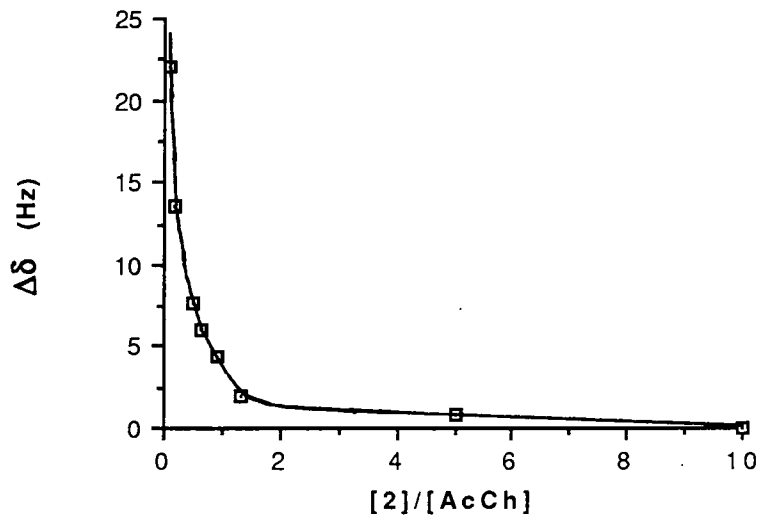


Figure 2.19a

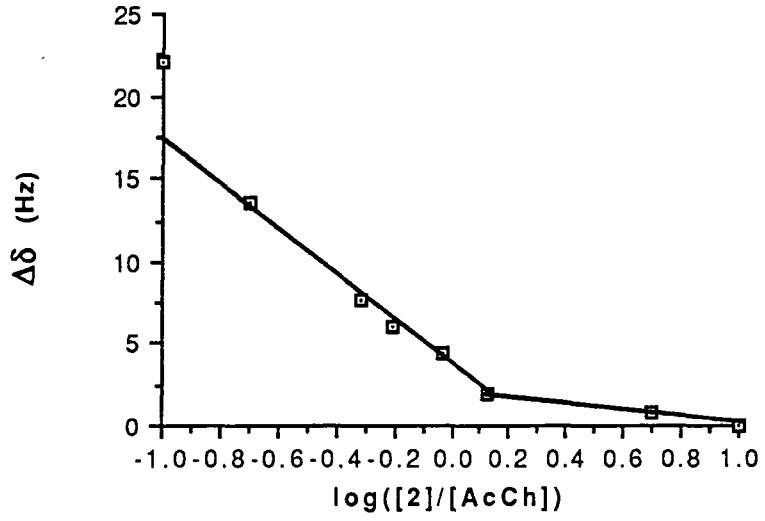


Figure 2.19b

As the AcCh signals were very broad, a variable temperature ^1H NMR experiment was performed on the solution of **2** with 10 equivalents of AcCh (but see note *2, table 2.6) in 2% D_2O/THF. Temperatures ranged from -50°C to +40°C at 10° intervals.

As a control, a similar experiment was conducted on zwitterion **2** in the absence of AcCh, in the same solvent system.

For the macrocycle/AcCh mixture it was hoped that the AcCh signals would sharpen; it was also thought likely that coalescence of the $ArCH_2O$/$ArCH'_2O$ signals at 5.091/5.415ppm and also of the benzoate triplet signals at 7.33/7.17ppm might be seen. The spectra acquired at -40°C, 0°C and +40°C are shown in figures 2.20a-c respectively.

FIGURE 2.20a

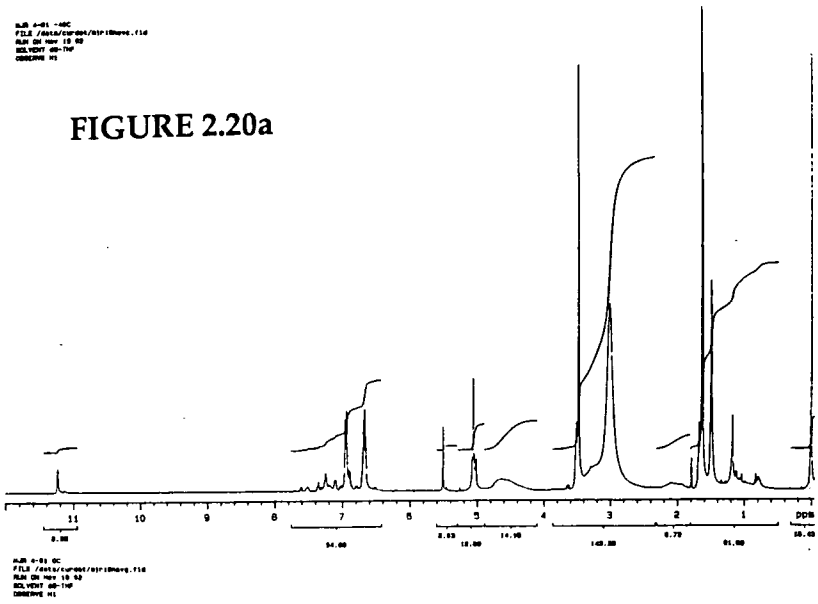


FIGURE 2.20b

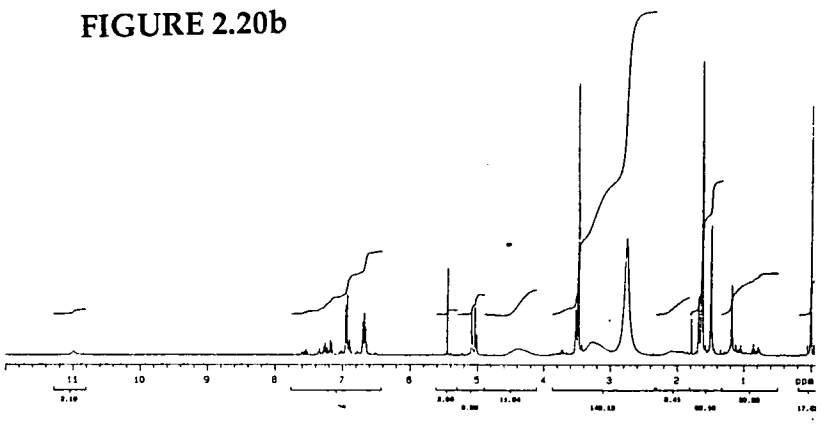
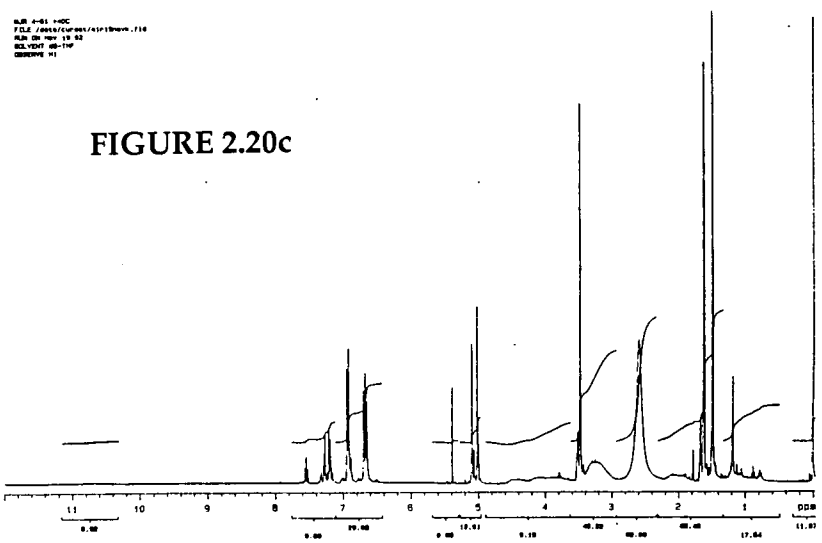


FIGURE 2.20c



At temperatures both above and below room temperature (ca.25°C), the AcCh signals sharpened sufficiently for them to be seen but they remained very broad.

At 20°C or above, two broad peaks could be seen at about 4.4 and 4.2ppm. Below 20°C, these appeared to coalesce to form a single, broad peak centred at about 4.3ppm at 10°C and about 4.7ppm at -50°C. These signals were assigned to the NCH_2 and OCH_2 protons.

A peak at about 3.2ppm could be seen at all temperatures between 0°C and +40°C. This signal was assigned to the NCH_3 protons. Below 0°C this peak and the nearby water signal (ca.2.6ppm) began to broaden even more and move towards one-another. At -50°C only a single peak could be seen, centred at about 3ppm.

At all temperatures above -50°C, a broad signal could be seen at about 2ppm. This was assigned to the acetyl CH_3 protons.

Although the broad nature of the AcCh signals made accurate measurement of peak integrals impossible, the integral ratios of the signals mentioned above approximate to the ratio 4H : 9H : 3H, consistent with their assignments.

For the macrocycle, coalesence of the signals which had been hoped for (see above) did not occur. However, several other interesting changes were observed.

In the aromatic region of the spectrum, the DPP AA'BB' protons retained their symmetry, but small changes in coupling occurred which lead to slight changes in the pattern of the splitting.

As the temperature was decreased to 10°C, the pyridinium triplet began to change and at 0°C it split into two distinct signals at

7.56ppm and 7.54ppm, indicating that the pyridinium ring existed in two different environments. Exchange between the two signals was slow on the NMR time scale below 10°C and fast at 20°C or above i.e. a coalescence temperature of about 10 °C.

At lower temperatures (0°C or below), the pyridinium doublet and the benzoate signals (doublet and triplet) were each split into "several" signals, showing that the pyridinium and benzoate residues were invoved in exchange processes. The signals coalesced at a tempersture of about 10°C. At -30°C, the signals began to broaden and below this temperature were too broad for any useful measurements to be made or for any conclusions to be drawn.

At lower temperatures, a sharp singlet could be seen at about 11ppm (11.3ppm at -50°C). As the temperature increased to -30°C this broadened considerably. This was assigned to the pyridinium NH proton, which is in fast exchange on the on the NMR time scale at all temperatures above 0°C, but was still just visible in the spectra acquired at +20°C and +30°C.

As mentioned above, coalescence of the $ArCH_2O/ArCH'_2O$ signals did not occur. Both singlets remained sharp and well separated at all temperatures, although the separation decreased as the temperature was increased.

However, the intensity of the major peak decreased as the temperature decreased until a minimum value was reached at about 0°C. As the temperature decreased further, the major peak intensity began to rise again. These changes were associated with proportional increases or decreases in the intensity of the minor peak.

Values of the major and minor peak intensities at the given temperatures are given in table 2.7. Although the significance of this change is not certain, plots of the major peak intensity versus Temperature, 1/Temperature and log (Temperature) are shown in figures 2.21a-c.

Minor Peak	Major Peak	Temp. (K)	1/Temp. ($K^{-1}x10^3$)	Log (Temp)
0.42	3.58	313	3.19	2.1955
0.74	3.26	303	3.30	2.4814
0.91	3.09	293	3.41	2.4669
1.25	2.75	283	3.53	2.4519
1.38	2.62	273	3.66	2.4362
1.27	2.73	263	3.80	2.4200
1.30	2.70	253	3.95	2.4031
0.98	3.02	243	4.12	2.3856
1.10	2.90	233	4.29	2.3674
0.77	3.23	213	4.48	2.3483

Table 2.7

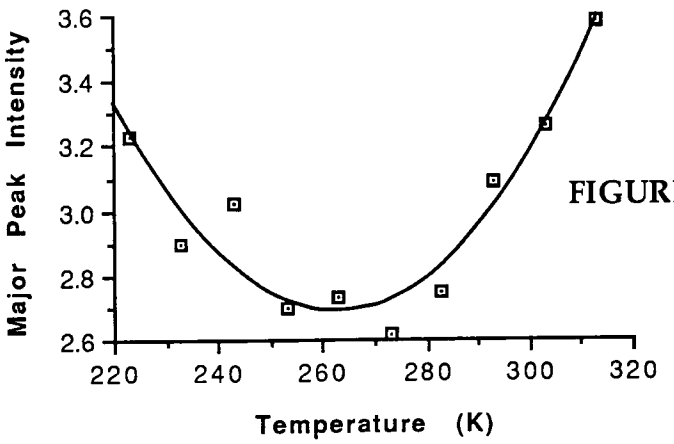


FIGURE 2.21a

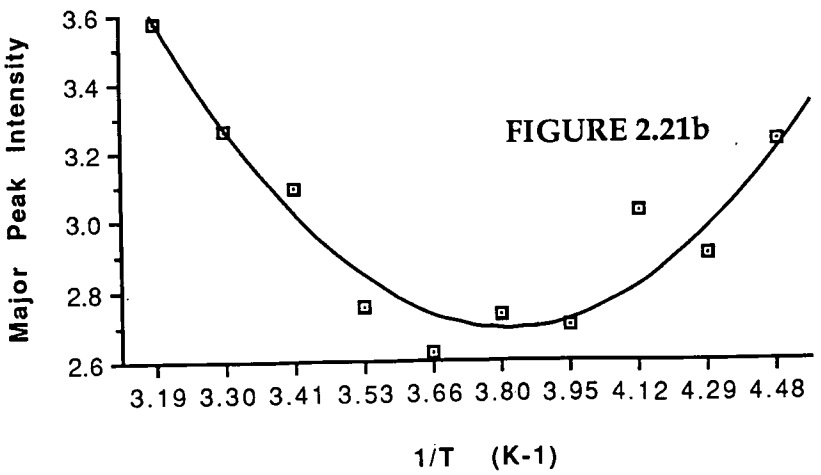


FIGURE 2.21b

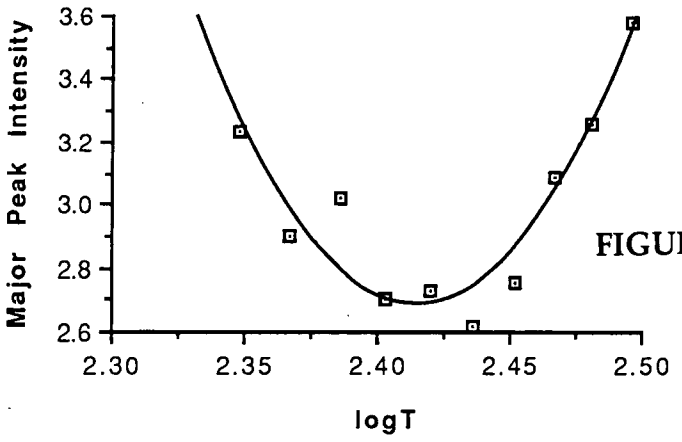


FIGURE 2.21c

As already mentioned, the signals due to AcCh were broad and so integral measurement was prone to inaccuracy. Nevertheless, the integrals were used to calculate that the ratio of **2**:AcCh was only about 1:2. This value should be compared with the weighed amounts of macrocycle **2** and AcCh chloride which indicated that at least 5 equivalents of AcCh were present in solution.

The VT NMR spectra of the macrocycle **2** in the presence of an excess of AcCh should be compared with the VT NMR of the macrocycle alone, which broadened somewhat at about 10°C or below, but otherwise showed very little change over the temperature range -50°C to +40°C. Most notably, no changes were observed which would have indicated that there were exchanges between different macrocyclic conformations.

This suggests that AcCh is responsible for the observation of the macrocyclic conformational exchange processes.

2.7. CONCLUSIONS AND FURTHER WORK.

2.7.1. CONCLUSIONS AND SUMMARY.

Three structurally similar cyclophanes (**1**, **2** and **7a**, shown on pages 47 and 70) with enforced cavities have been prepared, and their interaction with AcCh has been investigated.

The cyclophane **1** carries no formal charge and has an aromatic "pocket" as a binding site for the trimethylammonium head group of AcCh. Binding between AcCh and **1** was too weak to be observed using NMR techniques. However, it can be inferred that some complex formation does occur as AcCh is transported across a lipophilic membrane by cyclophane **1**. It is important to note that transport occurred with very little interference from ammonium or group I and group II metal cations. Transport of tetramethyl and tetraethyl ammonium cations was poor, indicating that interference from these species might also be low. Transport of the protonated tyramine cation was also poor, indicating that cyclophane **1** may show some selectivity for AcCh.

The cyclophane **7a** carries a negative charge in the form of the sodium salt of a benzoic acid residue. Using NMR experiments, it was shown that **7a** is able to bind to AcCh, presumably via an electrostatic attraction between the trimethylammonium head group of AcCh and the carboxylate binding site of cyclophane **7a**.

At all AcCh : **7a** ratios studied, two distinct sets of signals could be assigned to AcCh, and these were attributed to the free and bound species. The absence of choline from the AcCh/**7a** mixture

(and hence the lack of catalytic, "hydrolytic" activity of **7a**) was demonstrated by : (i) coalescence of the AcCh signals when the NMR operating frequency was lowered; (ii) addition of choline to the AcCh/**7a** mixture; (iii) addition of choline to fresh **7a**.

The macrocycle **7a** was itself observed in two distinct conformations which were in slow exchange on the NMR time scale. On addition of AcCh, the proportion of the less stable (higher energy) conformation was seen to increase. This indicates that the higher energy conformation is more predisposed to the binding of AcCh.

The cyclophane **2** also carried a negative charge in the form of a benzoate residue. However, the macrocyclic ring also carried a positive charge in the form of a pyridinium residue. ^{1}H NMR experiments again indicated that AcCh was bound by the cyclophane, but that exchange between the free and bound species was rapid on the NMR time scale.

Like sodium salt **7a**, zwitterion **2** was observed in two distinct conformations which were in slow exchange on the NMR time scale. The proportion of the less stable conformation again increased on addition of AcCh.

A VT NMR experiment studying the binding of **2** with AcCh showed that the benzoate and pyridinium residues were also involved in an exchange process which was rapid on the the NMR time scale at $\geq$10°C, but slow at $\leq$0°C i.e. a coalescence temperature of $\approx$10°C. No slowing of this exchange process was seen in a similar experiment with **2** alone, and the relevant signals were not time averaged (did not appear at an intermediate value). It is therefore

proposed that the exchange observed in the zwitterion/AcCh mixture was between the free macrocycle and the complexed species.

To summarise, sodium salt **7a** binds AcCh with exchange between free and bound species slow on the NMR time scale. Zwitterion **2** also binds AcCh, but the exchange between free and bound species is rapid on the NMR time scale. Complexation of cyclophane **1** with AcCh is too weak to be seen using NMR techniques. However, cyclophane **1** was able to selectively transport AcCh across a PVC membrane.

In terms of mimicking the biological receptors (see section 2.1), neither **1**, **2** nor **7a** showed any catalytic activity under the conditions studied.

The biological receptors for AcCh have both rapid rates of complexation and decomplexation, and binding constants for AcCh which are reasonably high.

Sodium salt **7a** has a binding constant which is high enough to observe complexation in NMR experiments, but has slow rates of complexation and decomplexation.

Conversely, cyclophane **1** has reasonably high exchange rates (required for efficient membrane transport), but binding is so weak that it cannot be seen using NMR methods.

However, zwitterion **2** seems to approach the criteria of both fast exchange and strong binding, as shown by NMR experiments.

Furthermore, both sodium salt **7a** and zwitterion **2** undergo conformational changes on binding to AcCh. The AcCh receptors in the postsynaptic membrane are also believed to undergo changes in conformation on binding to AcCh, resulting in the opening of ion

channels and the propagation of the nervous impulse (see section 2.1, p39).

The aims set out in Section 2.1 were therefore achieved with some degree of success.

2.7.2. FURTHER WORK.

As was mentioned earlier, measurement of the pKa's of the benzoate and pyridinium residues of **2** was not straightforward. If a reliable method of estimating them could be established, then this information may prove valuable.

In addition, although spectral evidence (IR, NMR) suggests that cyclophane **2** existed in the zwitterionic form, this case is far from proven. It seems that the exact concentration of water, and of the macrocycle itself, in the THF solution may be crucial to the equilibrium between the different states of protonation.

The elimination of water (i.e. using anhydrous THF) would simplify the study of this equilibrium. The lipophilicity of AcCh (and hence its solubility in THF) would be enhanced if the chloride counterion were replaced by trifluroacetate or hexaflurophosphate, for example.

Conversely, derivatisation of the macrocyclic skeleton with appropriate functional groups[30] in order to increase the hydrophilic nature of the macrocycle might make it possible to conduct NMR experiments in aqueous media. This would enable accurate measurement of pH and consequently pKa values.

Sodium salt **7a** seemed to form stable complexes with choline. Appropriate competition experiments (macrocycle + AcCh + choline) should reveal which substrate forms the most stable complex with macrocycles **1**, **2** and **7a**.

Invaluable structural information would be gained if crystals of the macrocycle could be grown which were suitable for analysis by X-ray diffraction. As was mentioned, crystals of ester **6** (page 59) were successfully grown, but disintegrated in the X-ray beam.

The cyclophane prepared by Saigo et. al. (figure 2.6b) formed very stable, crystalline complexes with aromatic guests such as benzene and toluene[20]. These were successfully analysed by X-ray diffraction, and it may be that cyclophane **1** or ester **6** would be induced to form more robust crystals in the presence of similar substrates.

Cyclophanes **1** and **2** both incorporate pyridine residues and can be easily protonated and isolated as, for example, the hydrochloride salt. Cyclophanes bearing such a positively charged residue may be of interest as possible receptors for anions which have a lipophilic tail group.

Biologically important anions often meet this description. However, the design and synthesis of receptors for such anions has so far been a rather neglected area.

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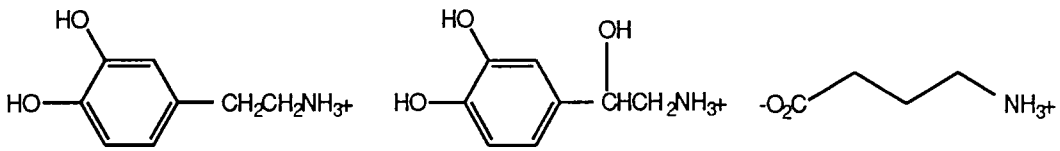
CHAPTER 3. MACROCYCLES INCORPORATING UREA AND THIOUREA RESIDUES.

3.1 INTRODUCTION

Although AcCh was the first neurotransmitter to be identified, it is now only one of many which has been discovered[1]. Where AcCh has a trimethyl ammonium "head", many other important neurotransmitters have a simple $-NH_3^+$ group. e.g. the catecholamine based neurotransmitters such as norepinephrine (noradrenaline) and dopamine, and γ-aminobutyricacid (GABA) (see figure 3.1).



DOPAMINE NOREPINEPHRINE GABA

Figure 3.1

Like the $-NMe_3^+$ head group, $-NH_3^+$ has also been found to interact favourably with π–electrons of aromatic residues[2,3]. In macrocyclic chemistry, however, crown ethers have been widely used as binding sites for $R-NH_3^+$[4].

Recently, the "rediscovery" of cucurbituril[5], figure 3.2, and its ability to form very stable complexes with alkylammonium and alkyldiammonium compounds[6], as well as with alkali and alkaline earth metal cations[7], lead us to consider urea carbonyl groups as binding sites of considerable potential.

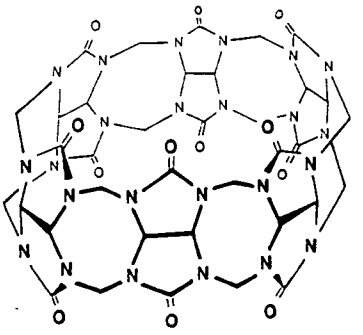
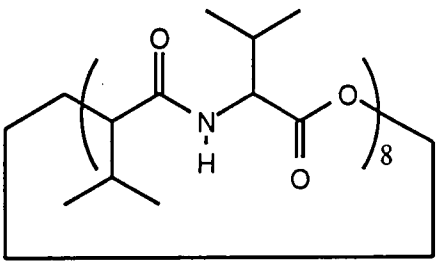
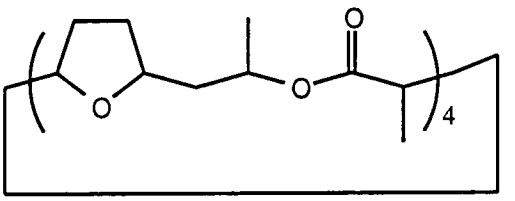


Figure 3.2

Carbonyl groups are used in the formation of metal ion binding sites in natural ionophores such as valinomycin and nonactin, figures 3.3a and 3.3b respectively.



VALINOMYCIN



NONACTIN

a **b**

Figure 3.3

However, there are very few examples of the conscious use of carbonyl groups as binding sites in synthetic macrocyclic compounds[8-11]. Consequently, we set out to synthesise macrocycles which exploited urea residues as binding sites, initially for the biologically important molecules R-NH_3^+, but also for group I and group II metal cations.

3.2. CUCURBITURIL ANALOGUES.

3.2.1. INTRODUCTION.

Only one analogue of cucurbituril has been reported to date - the cyclic pentamer decamethylcucurbit[5]uril[12], shown in figure 3.4. However, neither this analogue nor cucurbituril itself bear sites which would be suitable for simple derivitisation, allowing the attachment of functional groups. Furthermore, both cucurbituril and decamethylcucurbit[5]uril are very rigid. Although the carbonyl groups are suitably arranged for binding R-NH_3^+, they are divergent. Such an arrangement is less than ideal for binding other cations, such as the group I and group II metal cations.

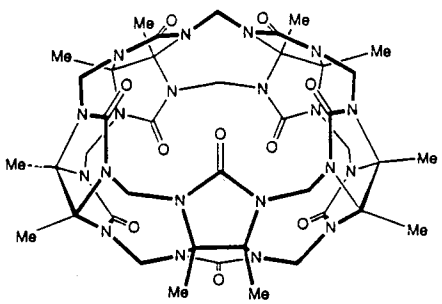
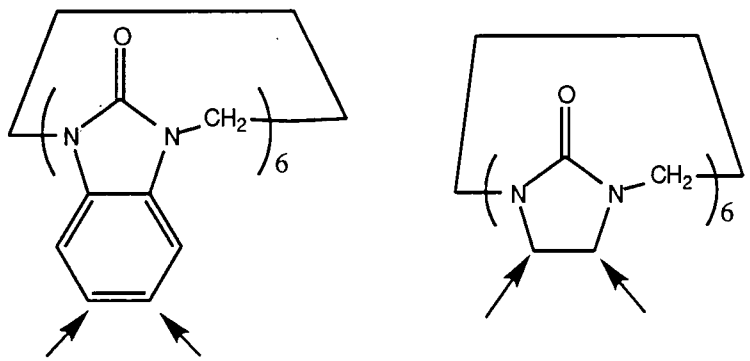


Figure 3.4

Judicious choice of the functional groups attached to a macrocycle can lead to improved selectivity or catalytic activity. If chiral functional groups are used, then the macrocycle inherits the chirality and may discriminate between optical isomers of a chiral substrate.

Consequently, the macrocycles **8** and **9**, shown in figure 3.5, based on "half-molecules" of the cucurbituril-type structures, were designed. Positions which were thought to be convenient for the attachment of functional groups are indicated.



8 **9**

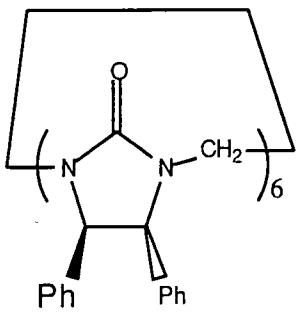
a **b**

Figure 3.5

It was anticipated that, in the case of biologically important molecules of the type R-NH_3^+, binding of the ammonium head would occur at the "upper rim" via hydrogen bonds to the urea carbonyls, as in the cucurbituril/R-NH_3^+ complexes. The alkyl tail group, R, would then have the opportunity to interact with any functional groups attached at the "lower" rim.

Also, macrocycles of this type are more flexible than the cucurbituril-type macrocycles. It was hoped that the urea oxygen atoms could arrange themselves around a substrate in an octahedral conformation.

In particular, it was thought that compound **10**, shown in figure 3.6, would show chiral recognition of optically active ammonium compounds such as the catecholamine-based nuerotransmitters mentioned above.



10

Figure 3.6

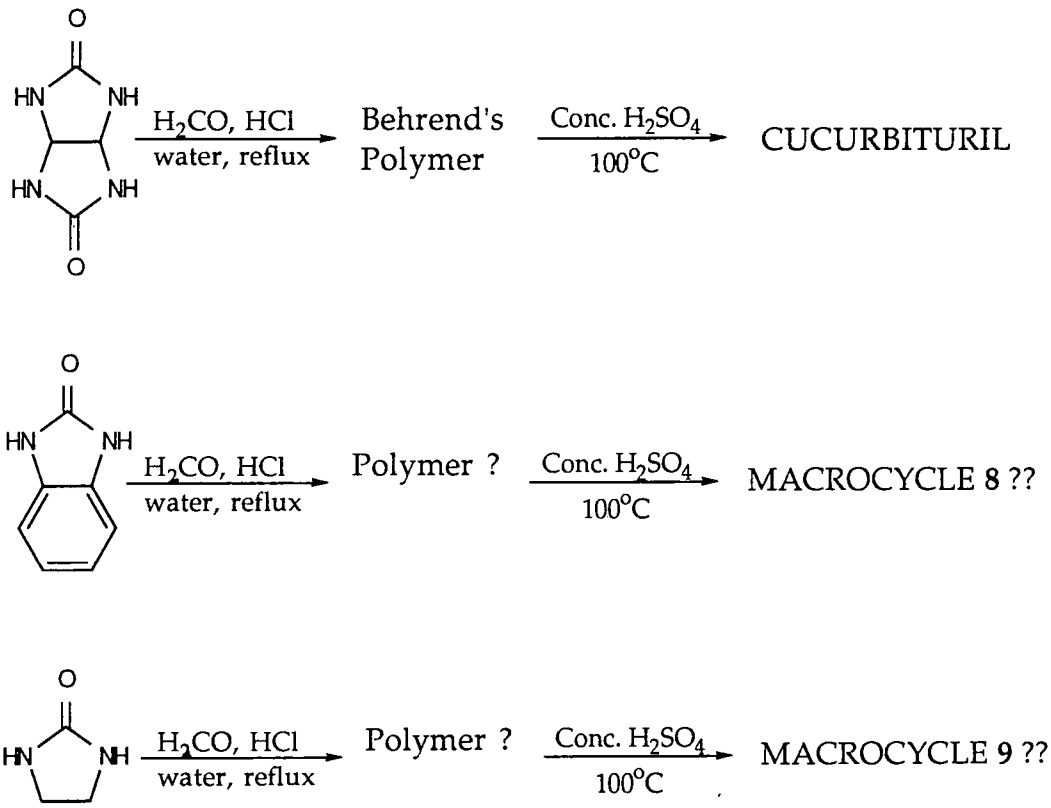
Cucurbituril is prepared by condensing glycoluril with an excess of formaldehyde in the presence of hydrochloric acid[5,13] to give a polymeric compound, called "Behrends Polymer". The polymer is then degraded with hot, concentrated sulphuric acid to give the cyclic hexamer in moderate yield (40-70%)[5].

Decamethylcucurbit[5]uril was prepared in a single step from dimethylglycoluril and formaldehyde in the presence of hydrochloric acid in a yield of 16%[12], although it had not been prepared when this work was undertaken.

The yields of both of these compounds are quite high considering the relatively straightforward syntheses and the absence of a template.

3.2.2. ATTEMPTED SYNTHESES.

As both **8** and **9** were analogous to cucurbituril, similar preparations were attempted, but using 2-hydroxybenzimidazole for **8** or 2-imidazolidone for **9** in place of glycoluril for cucurbituril. The strategy is illustrated in scheme 3.1.



Scheme 3.1

Attempted Synthesis of Macrocycle 8.

2-Hydroxybenzimidazole was refluxed in an aqueous solution of formaldehyde and hydrochloric acid to give a pale green precipitate, which was collected by filtration and dried thoroughly under vacuum to give a fine, light green powder. (On standing in sunlight or heating, the powder turned from light green to pale brown).

Because of its lack of solubility in all common solvents (water, methanol, ethanol, THF, diethyl ether, DMF, DMSO, dioxane, chloroform, dichloromethane, hexane, 60-80 petroleum ether, toluene) and its high melting point (>300°C) this was taken to be the polymeric material.

However, this product was consistently prepared in apparent yields within 0.5% of 137% and failed to give a satisfactory elemental analysis.

For the polymer, the molecular formula of the repeat unit is $C_8H_6N_2O$, which would give an analysis of C 65.75%, H 4.11%, N 19.18%. The experimental analysis obtained was C 55.98%, H 5.41%, N 13.52%.

Even when possible contamination by other substances which had been present in the reaction medium (water, formaldehyde, hydrochloric acid) was considered, a molecular formula which fitted the experimental data could not be found.

From the consistent apparent yields of about 137%, it could be calculated that the product had a molecular weight of around 200, assuming 100% reaction and a pure product.

N,N'-bis(hydroxymethyl)-2-benzimidazolidone has a molecular weight of 194 and a theoretical elemental analysis for $C_9H_{10}O_3N$ of C 55.67%, H 5.15%, N 14.43%. However, characterisation of a sample of N,N'-bis(hydroxymethyl)-2-benzimidazolidone, prepared as in the literature[14] showed that this was not the product obtained in the above reaction (see "Chapter 4 - Experimental" for details of the synthesis and characterisation of N,N'-bis(hydroxymethyl)-2-benzimidazolidone).

Furthermore, an IR spectrum of the light green powder showed a peak at 1690cm^{-1}, corresponding to a C=O absorption, but no peak which would have indicated the presence of an O-H group.

When the light green powder was added to concentrated sulphuric acid, or vice versa, at room temperature, a thick, black, tarry substance was immediately formed. Heating, or diluting with water or more concentrated sulphuric acid, did not lower the viscosity. Excess liquid could be decanted off, but the tar was insoluble in all common solvents, could not be dried under vacuum and remained completely intractable.

Refluxing the green powder with 36% aqueous hydrochloric acid or with saturated aqueous potassium hydroxide gave only recovered starting material (identified from IR spectra), although in the case of potassium hydroxide, the powder changed from light green to the light brown colour acquired on standing in sunlight.

Attempts to prepare macrocycle **8** by this route were consequently abandoned. Although no satisfactory molecular formula could be offered for the green powder, it was suggested that it was rapidly dehydrated or sulphonated (or both) by concentrated sulphuric acid.

Attempted Synthesis of Macrocycle 9.

If the polymeric condensation product of formaldehyde and 2-imidazolidone could be prepared, then degradation of the polymer by concentrated sulphuric acid should not be complicated by possible sulphonation as no aromatic ring is present.

However, refluxing a solution of 2-imidazolidone in aqueous formaldehyde/hydrochloric acid for 16hrs resulted in the formation of N,N'-dimethyl-2-imidazolidone and considerable amounts of paraformaldehyde. When the reaction time was reduced to 4hrs., much less paraformaldehyde was obtained but N,N'-dimethyl-2-imidazolidone was still the only condensation product which was identified. No evidence was found which would have indicated the formation of oligomeric or polymeric condensation products, or the intermediate N,N'-bis(hydroxymethyl)-2-imidazolidone. It was proposed that the dimethylated product was obtained by hydride transfer from formic acid (present in the formaldehyde solution as an impurity).

As the analogous polymer could not be obtained, attempts to synthesise **9** by this route were also abandoned.

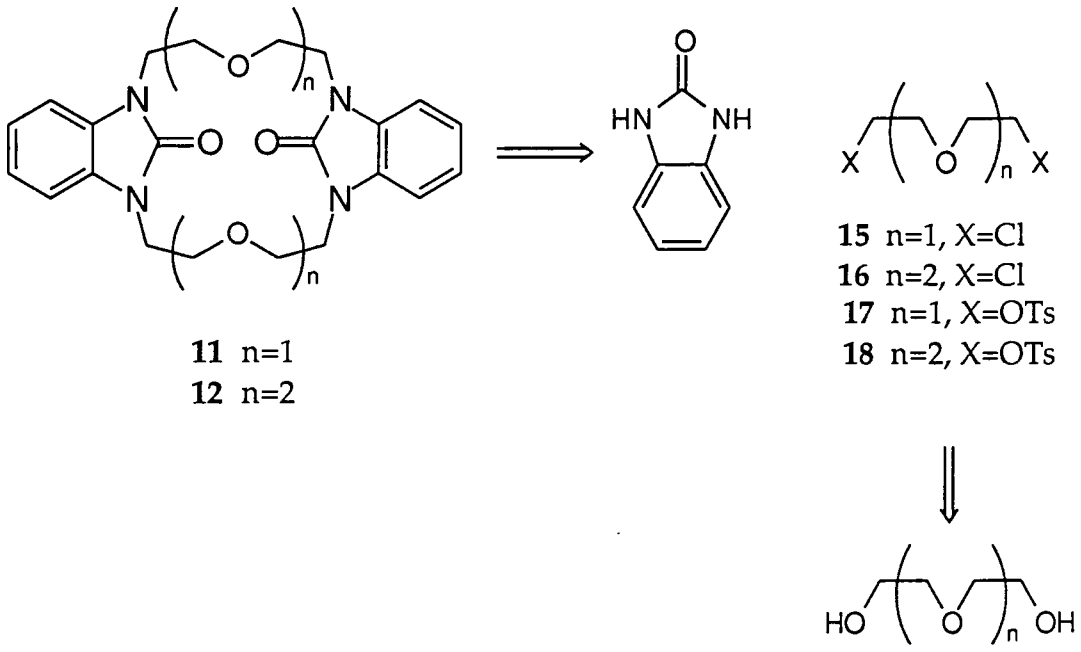
3.3.2.2. Strategy 1 - Synthesis of Crown Ether Frameworks Around Pre-Formed Ureas.

The syntheses of **11** and **12** had been described only in general terms[9]. 2-Hydroxybenzimidazole was reacted with 1,5-dichloro-3-oxapentane or 1,8-dichloro-3,6-dioxaoctane respectively, in anhydrous DMF solution in the presence of NaH or LiH. Yields of 15% and 12% and melting points of 197-99°C and 114°C were reported for **11** and **12** respectively[9].

In this strategy, retrosynthetic analysis of **11** or **12** (see scheme 3.2) gave 2-hydroxybenzimidazole and a diol as simple starting materials. In the published syntheses, the diols had been converted to the dichlorides **15** and **16** before reaction with the diamine, 2-hydroxybenzimidazole.

In our attempt to repeat the preparation of **12**, 1,8-dichloro-3,6-dioxaoctane, **16**, was replaced by 1,8-bis(toluenesulphonato)-3,6-dioxaoctane, **18**, as -OTs is widely accepted as a better leaving group than -Cl. Caesium carbonate was chosen as a base in place of sodium/lithium hydride because of the unusually high yields of macrocyclic products which have often been attributed to the presence of caesium salts in the reaction medium[15].

However, using the reagents mentioned above (2-hydroxybenzimidazole, **18**, Cs_2CO_3, DMF), 1+1 addition occurred to give the 12 membered ring, **19**, shown in figure 3.8, in a 66% yield. Even under conditions of high dilution with dropwise addition of the reagents, no evidence was found that would have suggested the formation of the desired 2+2 addition product, **12**.



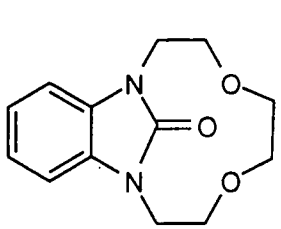
Scheme 3.2.

Using potassium carbonate in place of caesium carbonate gave a complex mixture of products (as shown by TLC analysis) which could not easily be separated. Using potassium carbonate, but with acetonitrile in place of DMF, also gave a complex mixture of products which showed the same pattern on analytical TLC.

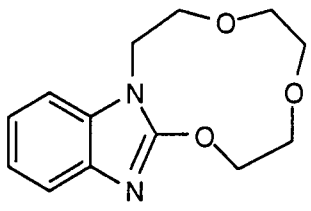
Sodium carbonate in DMF also gave a complex mixture of products. After several separations by column chromatography with an alumina support, small amounts of the 1+1 addition product, **19**, and of the O-methylated isomer, **20**, (figure 3.8) were isolated.

Although compounds **19** and **20** appeared to be identical when characterised by elemental analysis or mass spectroscopy, they could be differentiated by analytical TLC or NMR spectroscopy. However, both gave rise to complex ^{1}H NMR spectra and an unambiguous structural assignment of the two isomers could not be made. The two compounds

could be identified from their IR spectra, with compound **19** giving rise to a C=O stretching vibration at 1710cm^{-1}, which was absent from the IR spectrum of **20**. Other components of the mixture were not obtained in a pure state.



19



20

Figure 3.8

Replacing caesium carbonate with sodium hydride also gave a complex mixture of products, but no attempt to separate them was made.

These results are summarised in table 3.1.

BASE	SOLVENT	PRODUCT(S)
Cs_2CO_3	DMF	**19** (1+1 addition)
K_2CO_3	DMF	{Mixture
K_2CO_3	CH_3CN	{Mixture
Na_2CO_3	DMF	Mixture, including **19** and **20**
NaH	DMF	Mixture

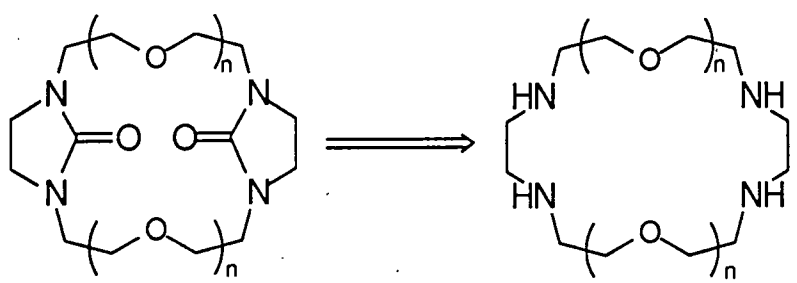
Table 3.1

Lack of success using this approach lead us to consider an alternative synthesis of the targetted macrocycles.

3.3.2.3. Strategy 2 - Insertion of (Thio)urea Residues Into Pre-Formed Aza-Crown Ether Frameworks.

3.3.2.3.1. Introduction.

Because of the difficulties encountered in obtaining good yields of the desired 2+2 macrocyclic products, an alternative approach was adopted. Where the synthetic strategy had been to build a crown ether framework around pre-formed urea residues (strategy 1, above), an approach was developed whereby a carbonyl group could be inserted between two nitrogen atoms of a pre-formed aza-crown ether, as in scheme 3.3. It was proposed that the urea-crowns **13** and **14** could be prepared from the crown ethers **21** and **22** respectively.



13 n=1

14 n=2

21 n=1

22 n=2

Scheme 3.3

As mentioned above, the synthesis of $18N_4O_2$ had been previously published. Some 24 membered aza-crown ethers ($24N_6O_2$ and $24N_2O_6$, for example) had been previously prepared, although no preparation of $24N_4O_4$ had been described.

There are several well established methods for the insertion of carbonyl groups. Phosgene, Cl_2CO, and equivalents such as dialkyl carbonates and methyl chloroformate are well known examples. "Triphosgene" (bis(trichloromethyl)chloroformate) is a very reactive phosgene equivalent which has recently been reported to give good yields in a variety of carbonyl insertion reactions[16].

However, it was recently reported that 1,2-diamines such as 1,2-diaminohexane and 1,2-diphenyl-1,2-diaminoethane react with phosgene and a variety of its equivalents to give the oligomeric products of intermolecular reactions rather than the 2-imidazolidones which would be formed by an intramolecular cyclisation[17].

In both **21** and **22** we sought to introduce urea residues by insertion of a carbonyl group between the two nitrogen atoms of a 1,2-diamine. On consideration of the results discussed above, the proposed use of phosgene equivalents was rejected.

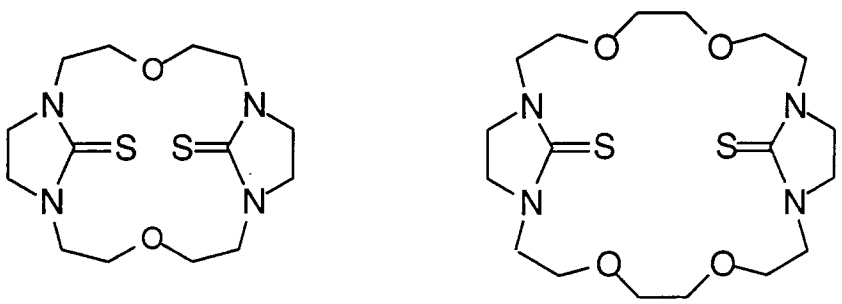
Another method of carbonyl insertion to form ureas requires the initial formation of thioureas by reaction of 1,2-diamines with carbon disulphide[18]. Thioureas can be hydrolysed with mercury (II) acetate to form ureas[17,19].

While it was hoped that this method would allow the preparation of the urea-crowns **13** and **14**, it was also thought likely that the thiourea intermediates **23** and **24** (shown in figure 3.9) would prove

to be of interest as possible ligands for "soft" cations such as Ag^{+}, Au^{+}, Hg^{2+}, Zn^{2+} and Cd^{2+}.

Mercury (II) and gold (I) both have quite strong preferrences for coordination numbers of 2 and a linear geometry; silver (I) also has some tendency towards this arrangement.

Zinc (II) and cadmium (II), however, are more accomodating, and the geometry of their complexes is largely dictated by the requirements of the solvating ligands.



23 **24**

Figure 3.9

The construction of a CPK model had indicated that the cavity of **23** would be very small and incapable of including potential substrates. However, the possibility of the formation of complexes with a stoichiometry greater than 1:1 could not be dismissed.

CPK models were also used to predict that the annulus of the ring of the bisthiourea, **23**, would be too small to allow the passage of the bulky sulphur atoms or the two five membered rings. Consequently, the molecule would be very rigid, and would be

"locked" into the conformation represented by the diagram in figure 3.10.

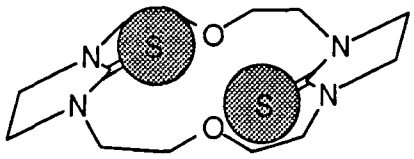


Figure 3.10

A CPK model of macrocycle **24** was also constructed, from which it was predicted that **24** would be much more conformationally mobile than **23**, as the larger ring annulus allowed the free passage of both the sulphur atoms and the five membered rings.

Furthermore, the CPK model indicated that both sulphur atoms could co-occupy the same face of the macrocycle, where they might be well placed to complex the soft metal cations mentioned above, such that the sulphur donor atoms are in a trans-orientation with respect to the cation. A representation of this conformation is shown in figure 3.11.

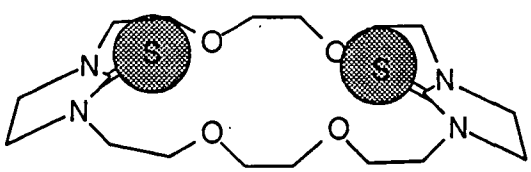


Figure 3.11

The feasibility of the proposal that **23** or especially **24** might form complexes with the soft metal cations is demonstrated by the structure of bis(ethylenethiourea) gold (I) chloride hydrate[20], shown schematically in figure 3.12.

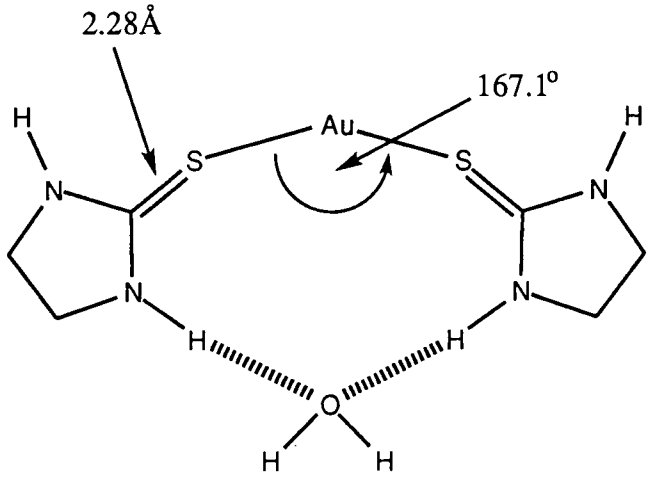


Figure 3.12

The sulphur - sulphur bond distance is about 5 Å. The S-Au-S bond angle of 167.1° is slightly strained; a bond angle closer to 180° is usually preferred by gold (I) ligands. The strain is introduced by a bridging water molecule, which also explains the cis conformations of the ethylene thiourea rings (both in the plane of the page).

For bisthiourea **24**, the conformational mobility of the molecule allows the ethylene thiourea rings to "breathe" so that the sulphur - sulphur bond distance and the S-M^{n+}-S bond angle can approach the required order of magnitude for strong binding.

3.3.2.3.2. Synthesis of $18N_4O_2.2CS$ and $18N_4O_2.2CO$.

$18N_4O_2$, **21**, was prepared from 3-oxa-1,5-bis(tolyl-p-sulphonyloxy)pentane, **17**, and N,N'-bis(tolyl-p-sulphonyl)ethane-1,2-diamine ("ethylene diamine ditosylate") as in the literature[21].

This was then reacted with an excess of carbon disulphide in 50/50-ethanol/water using conditions similar to those reported for the reaction of 1,2-ethylene diamine with carbon disulphide[18], but on a much smaller scale. A longer reaction time was also used (62hrs. total in place of 13hrs. total for 1,2-ethylene diamine). Even so, about 25% of unreacted **21** was recovered, and the yield of the desired bisthiourea, **23**, was only about 30%. This was quite low compared to a variety of other 1,2-diamines which have been converted to the corresponding thioureas in yields of between 80% and 95%[17,18].

Elemental analysis of the product indicated a molecular formula corresponding to **23**.HCl. A ^{13}C NMR spectrum of the product was consistent with the molecular structure assigned to the compound, although a relaxation delay of about 10 seconds or more was required to see the thiocarbonyl peak at 182.2ppm ($CDCl_3$, 200MHz or 400MHz).

The ^1H NMR spectrum of **23** was very complex, but showed a high degree of symmetry. As the peak at 67.8ppm in the ^{13}C NMR spectrum could be unambiguously assigned to the aliphatic CH_2O carbon, HETCOR and COSY NMR experiments could be used to fully assign both the ^1H and ^{13}C NMR spectra.

The ^1H, ^{13}C and COSY spectra are shown in figures 3.13a-c.

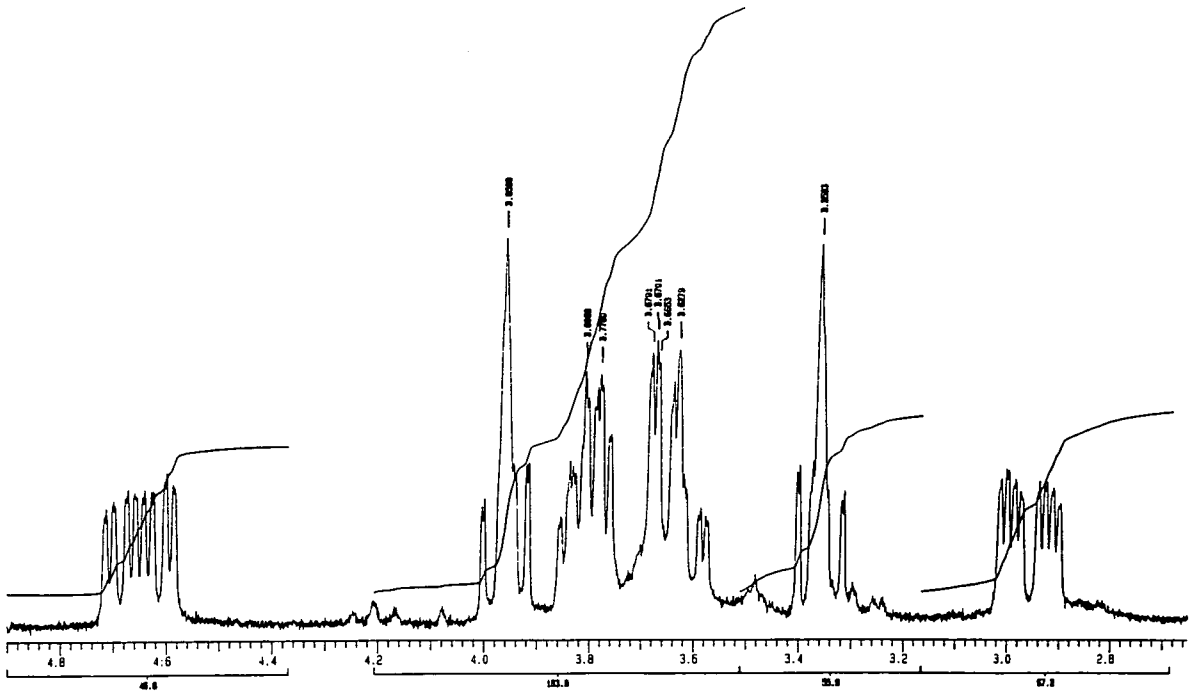


Figure 3.13a

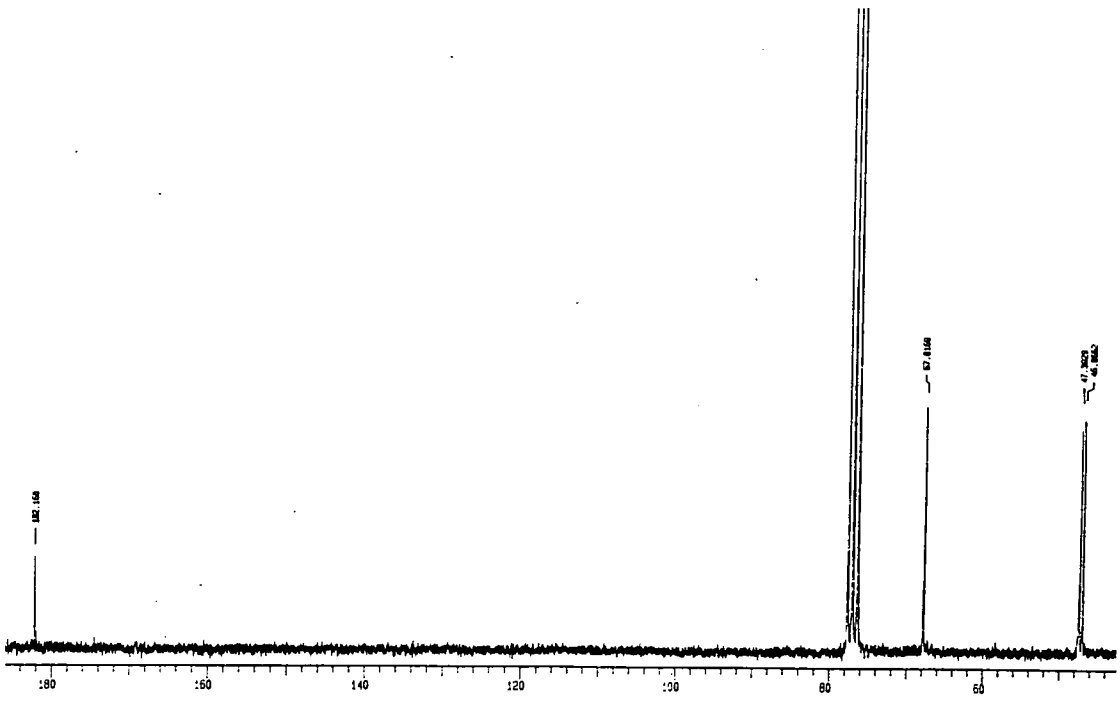


Figure 3.13b

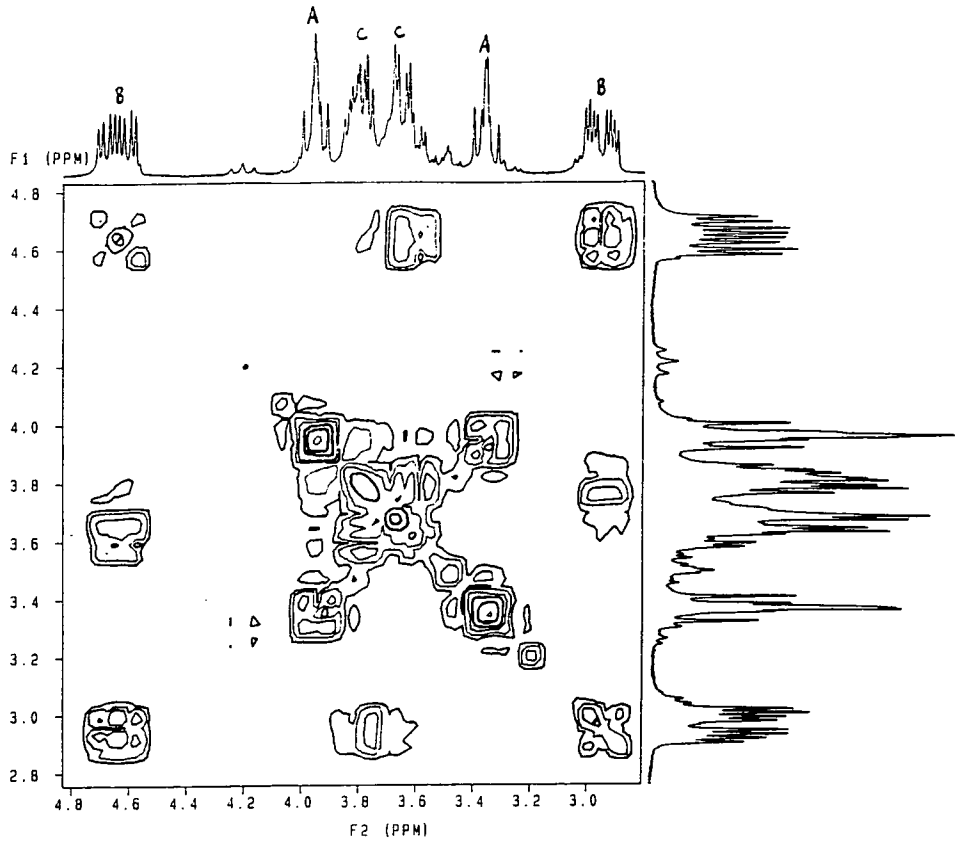


Figure 3.13c

From the structure of **23** shown in figure 3.9, we might expect that the ^1H NMR would show simply one singlet and two triplets.

However, as mentioned above, a CPK model of the molecule had indicated an extremely rigid structure. The molecule is effectively "locked" into a single conformation by the large sulphur atoms and the five membered rings - the molecule cannot alter its conformation without breaking covalent bonds. The methylene protons on each carbon (H_A, H_B, H_C in figure 3.14) are diastereotopic, leading to the apparent complexity of the spectrum.

The ^1H NMR spectrum of compound **23** was acquired at various temperatures, ranging from -50°C to +40°C. However, no significant changes occurred, indicating that no conformational exchange process was occurring at any temperature in the defined range.

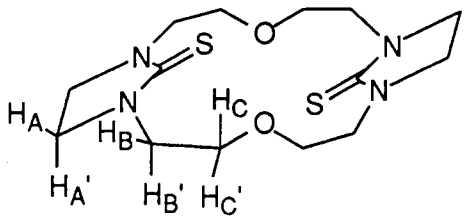


Figure 3.14

The thiourea, **23**, was then dissolved in a small volume of dichloromethane and an excess of mercury (II) acetate added. This suspension was stirred under an atmosphere of nitrogen for 24hrs. and then filtered through celite. Evaporation of the solvent left a pale yellow solid.

It had been reported that a variety of thioureas were cleanly converted to the corresponding ureas by mercury (II) acetate, and that no separate hydrolysis step was required; filtering through celite was sufficient to remove excess mercury salts.

However, the mass of the product obtained from the reaction of **23** with mercury (II) acetate indicated that considerable amounts of mercury salts might still be present.

When the solid was stirred with aqueous potassium carbonate at room temperature, a yellow colouration was immediately acquired. A brown precipitate formed after 12 hours. When the reaction was heated at reflux for 1-2hrs., considerable amounts of a dark precipitate formed.

This was removed by filtration and water evaporated from the filtrate to give the hydrolysed product and potassium carbonate. These

were separated by dissolving in anhydrous methanol and filtering through a bed of silica.

However, a mass spectrum of this product (DCI) showed only a single peak at a m/e value of 328, corresponding to the partially hydrolysed, mixed urea/thiourea compound, **25**, shown in figure 3.15.

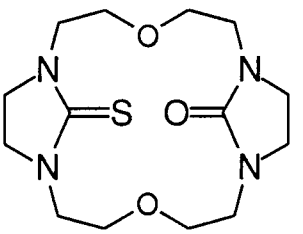


Figure 3.15

25

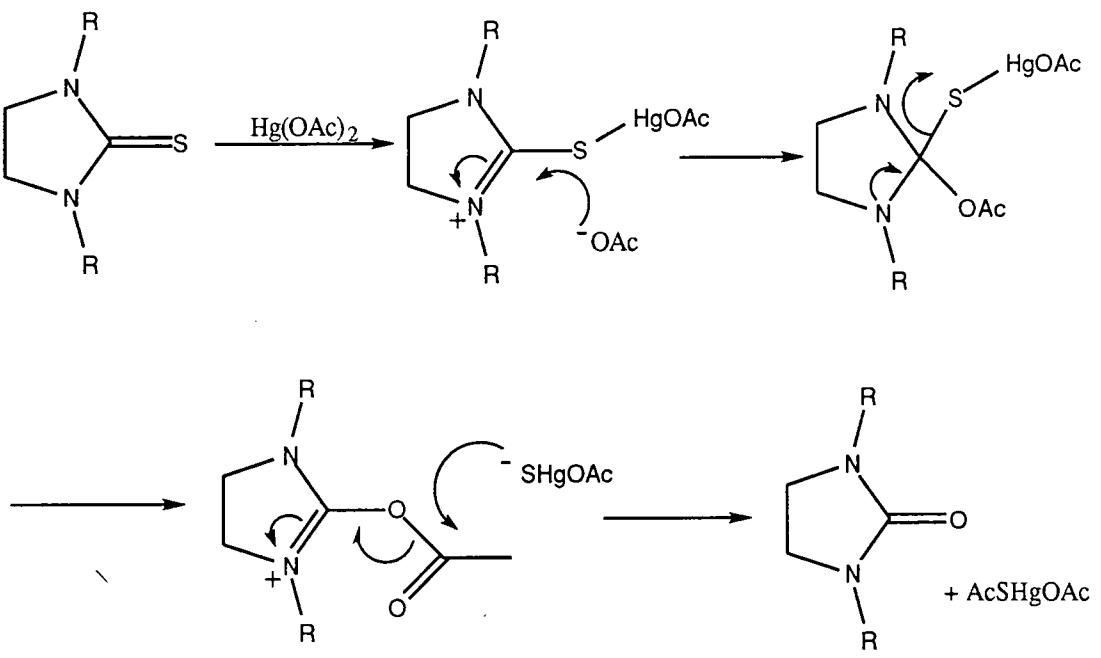
This structure was consistent with the both the ^{1}H NMR spectrum, which was complex (indicating that the molecule was still quite rigid) and showed less symmetry than the ^{1}H NMR spectrum of **23**, and with the ^{13}C NMR spectrum, which showed an extra set of four peaks compared with the ^{13}C NMR spectrum of **23**.

Even when the bisthiourea **23** was reacted with mercury (II) acetate under more forcing conditions (1,2-dichloroethane, reflux, 2 days in place of dichloromethane, room temperature, 12hrs.), the partially hydrolysed urea/thiourea **25** was still almost exclusively formed (traces of the bisurea compound, **13**, could be seen on analytical TLC).

Preparation of the bisurea **13** required a repeat of the above procedure (mercury (II) acetate/DCM or DCE followed by K_2CO_3/water). This can be explained if the proposed mechanism of the reaction[17], shown in scheme 3.4, is more closely examined.

In the case of the bisthiourea **23**, the attack of the acetate nucleophile on the thiourea carbon may be hindered because of the rigidity of the molecule, as indicated by the CPK model and ^1H NMR spectrum. Alternatively, the thiourea - mercury intermediate may be stabilised by coordination of mercury (II) to the second thiourea residue.

Either of these theories (hindered attack of the acetate or prevention of $(SHgOAc)^-$ from leaving) or a combination of both, may explain the need for a separate hydrolysis step and the fact that reaction occurs at only one thiourea group.



Scheme 3.4

Note, however, that in the second stage of the preparation (reaction of the second thiourea) a separate hydrolysis step is still required. As there is no second thiourea group present to stabilise the leaving group, it would seem that it is the steric hindrance of attack by the nucleophile that is largely responsible.

While the spectra recorded for the bisurea **13** (^{1}H and ^{13}C NMR, IR, DCI mass spectra) were consistent with the proposed structure, a satisfactory elemental analysis for carbon, hydrogen and nitrogen could not be obtained.

A mass spectrum acquired in the FAB mode showed peaks at 313 (M+1), 335 and 351. The latter two correspond to M+Na and M+K respectively, and elemental analyses for sodium and potassium showed that they were present in quantities of 3.15% and 5.54% respectively.

It was assumed that the potassium had been retained from the hydrolysis of the mercury salt. That **23** bound potassium strongly enough for the complex to pass through a short silica column with methanol eluant indicated that the **23**.K^+ complex was quite stable.

The presence of sodium seemed remarkable, as no sodium salts had been used in either the synthesis or work-up of the compound. As the water had been pre-distilled, it was proposed that sodium ions had been extracted from either the celite or silica, used in the work-up procedure. This indicates that **23** has a high affinity for sodium, and that the **23**.Na^+ complex must be quite stable.

The elemental analysis of the impure material (C 38.30%, H 5.75%, N 9.83%, Na 3.15%, K 5.54%) implied the molecular formula

$C_{18}H_{33}N_4Na_{0.8}K_{0.8}$, where a sample of pure **23** would have the molecular formula $C_{14}H_{24}N_4O_4$.

The Na^+ and K^+ cations required counterions. It is likely that acetate would be the most significant but the possible presence of inorganic anions such as carbonate and halide must also be considered.

Nevertheless, if it is assumed that each four nitrogen atoms indicates the presence of one equivalent of **23**, the ratio of **23**:M^+ is about 1:1.6, or 2:3.

3.3.2.3.3. Synthesis of $24N_4O_4.2CS$ and $24N_4O_4.2CO$.

Synthesis of the Crown Ether Framework.

Although some 24 membered aza-crown ethers had been previously prepared e.g. $24N_2O_6$[22], $24N_6O_2$[23] , pyridine[24]-N_6O_2[24], no synthesis of $24N_4O_4$, **22**, had been published.

For those which had been previously synthesised, simple 2+2 additions, analogous to the method used for the preparation of $18N_4O_2$, had not been employed.

For example, $24N_6O_2$[23] was prepared in a stepwise fashion. $TsN(CH_2CH_2NHTs)_2$ was reacted with 2-2-(chloroethoxy)ethanol to give a diol. The dioxatosylate derivative of this was then reacted with the disodium salt of $TsN(CH_2CH_2NHTs)_2$ to give the hexatosylamide of $24N_6O_2$ with about a 60% yield for the cyclisation step. The N-tosyl protecting groups were removed by refluxing a solution of the haxatosyl amide in 45% HBr/acetic acid.

However, an attempt was made to prepare the tetratosylamide of **22** in a single step by the 2+2 addition of ethylene diamine ditosylate, $TsHNCH_2CH_2NHTs$, and trigolditosylate, $TsO(CH_2CH_2O)_3Ts$ in anhydrous DMF in the presence of caesium carbonate.

Even under conditions of high dilution, the 12 membered, 1+1 addition compound was the major product. No evidence was found which would have indicated the formation of higher oligomers, cyclic or otherwise.

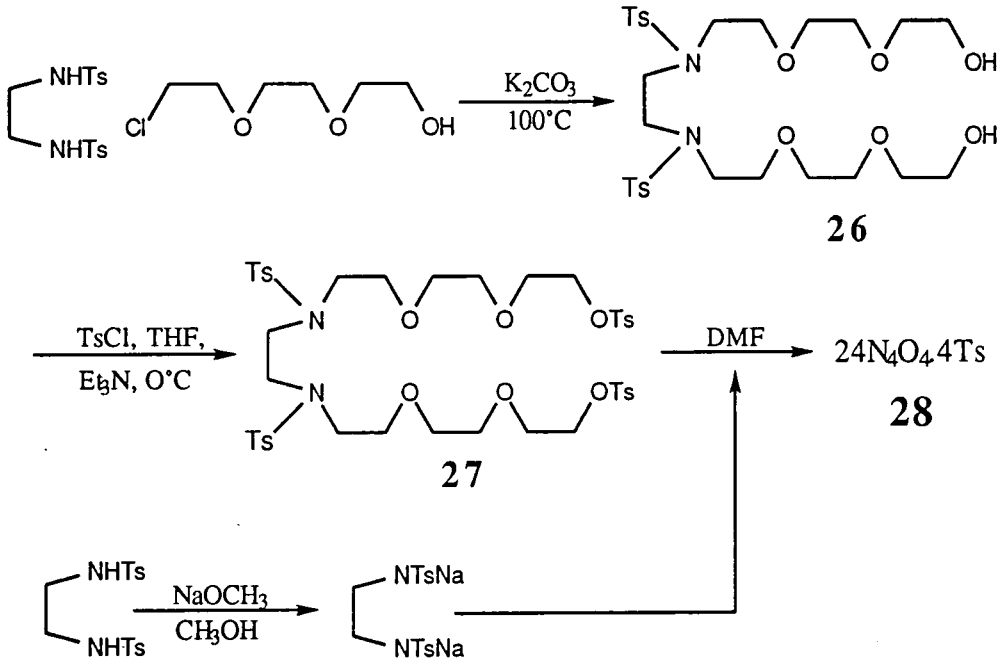
Consequently, a stepwise synthesis similar to the one used for the preparation of $24N_6O_2$ was attempted (Method 1, below). However, as will be seen below, some of the intermediate compounds formed required very laborious purification procedures, and a second method (Method 2, below) was attempted.

Synthesis of $24N_4O_4$.Tetratosylamide - Method 1.

Ethylene diamine ditosylate was dissolved in an excess of 2-{2-(2-chloroethoxy)ethoxy}ethanol in the presence of potassium carbonate. This suspension was stirred at 100°C for 20hrs., cooled to room temperature and diluted with dichloromethane before filtering. Solvent was evaporated from the filtrate at reduced pressure. Excess 2-{2-(2-chloroethoxy)ethoxy}ethanol was removed by distillation under high vacuum using a Kugelrohr (80-100°C, <0.1mmHg); considerable amounts of triethylene glycol were also removed as distillate. However, a ^1H NMR spectrum of the product diol, **26** (see scheme 3.5), showed that significant amounts of starting material were still present. Because of the very similar physical properties of 2-{2-(2-

chloroethoxy)ethoxy}ethanol and diol **26**, (solubility, column chromatography Rf values) further purification of **26** was not possible and the impure diol was used in the subsequent step.

The oxatosyl derivative of diol **26** was prepared using tosyl chloride in THF at 0°C under a nitrogen atmosphere in the presence ot triethylamine. As the starting material was impure, the crude product was contaminated with the tosyl derivative of 2-{2-(2-chloroethoxy) ethoxy}ethanol.



Scheme 3.5

Compound **27** was purified by column chromatography to give a clear, colourless oil in an overall yield of 49% from ethylene diamine ditosylate. However, separation of the product, **27**, from the contaminating tosyl derivative of 2-{2-(2-chloroethoxy)ethoxy}ethanol, was very difficult as the two compounds had similar solubilities and Rf

values. Several columns were necessary to isolate completely pure product, **27**.

A solution of the tetratosyl derivative **27** in anhydrous DMF was added to a suspension of the disodium salt of ethylene diamine ditosylate in DMF at 80°C to give the cyclic tetratosylamide $24N_4O_4.4Ts$, **28**, in a 66% yield.

Note that the heterogeneous nature of the reaction obviated the need for large solvent volumes; moderate dilution was sufficient to give reasonably high yields of the desired cyclic product.

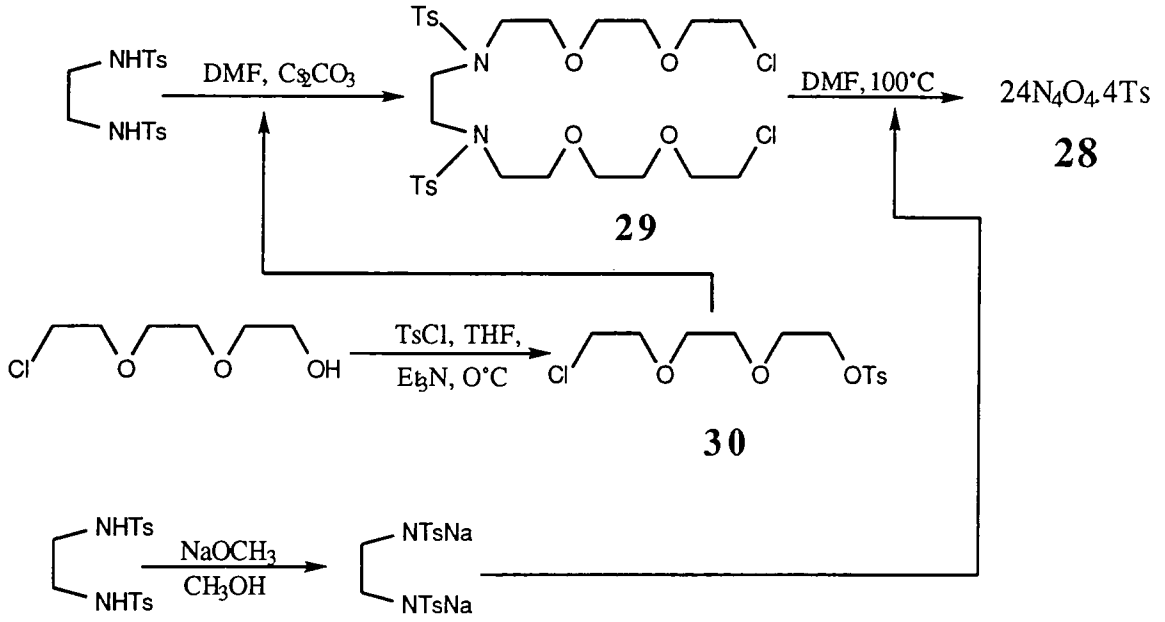
Method 1 is summarised in scheme 3.5.

Synthesis of $24N_4O_4$.Tetratosylamide - Method 2.

As mentioned above, the diol **26** could not be purified, and purification of the tetratosyl derivative **27** was extremely laborious. Consequently, a second synthetic route, similar to the one described above, was devised. This is summarised in scheme 3.6.

The tosylated derivative of 2-{2-(2-chloroethoxy)ethoxy}ethanol, **30**, was prepared using tosyl chloride in THF at 0°C under an atmosphere of nitrogen in the presence of triethylamine. Purification by column chromatography gave tosylate **30** as a clear colourless oil in a 64% yield.

Addition of tosylate **30** to a solution of ethylene diamineditosylamide in DMF in the presence of caesium carbonate at 40°C led to the formation of the dichloride **29** as a white powder in a 47% yield (after recrystallisation from methanol).



Scheme 3.6

The cyclic tetratosylamide $24N_4O_4.4Ts$, **28**, was prepared from the disodium salt of ethylene diamine ditosylate (prepared as in method 1) and dichloride **29** in anhydrous DMF at 100°C under a nitrogen atmosphere, in the presence of caesium carbonate. The crude product was recrystallised from ethanol to give **28** in a yield of 52%.

Although the overall yield for method 2 (24%) is slightly lower than for method 1 (32%), the work-up procedures for the intermediate products were far more straightforward for method 2. Consequently, method 2 was the synthetic route of choice for the preparation of the macrocyclic tetratosylamide **28**.

Detosylation of Tetratosylamide **28**.

Removal of N-tosyl protecting groups has been attempted using a variety of methods; lithium in liquid ammonia (Birch reduction), refluxing HBr/acetic acid/phenol or hot, concentrated sulphuric acid are some of the more common methods[25].

As refluxing HBr/acetic acid/phenol had been successfully used for the detosylations of 18N4O2.4Ts[21] and 24N6O2.6Ts[23] to give the free amines in good yield, this was the method which was originally tried for the detosylation of **28**.

However, when a solution of tetratosylamide **28** in 45%HBr/acetic acid (10ml per g tetratosylamide) in the presence of phenol (1.5g per g of tetratosylamide) was stirred at 100°C for 16hrs., the precipitate obtained from the cooled solution by the addition of an excess of diethyl ether was not the expected tetrahydrobromide salt of the tetraamine **22**.

A ^{1}H NMR of the tetrahydrobromide salt of the tetraamine **22** in D_2O would be expected to show two singlets and two triplets with the signals in the ratio 1:1:1:1. A ^{1}H NMR spectrum of the reaction product in D_2O was very complex with very little symmetry. A lack of signals in the aromatic region of the spectrum (6.5-7.5ppm) indicated that the N-tosyl protecting groups had been removed, but the complexity of signals in the aliphatic region (3-4ppm) indicated that the compound had lost all symmetry elements.

Similarly, a ^{13}C NMR spectrum of the tetrahydrobromide salt of the tetraamine **22** would be expected to show only 4 peaks. The ^{13}C

NMR spectrum of the product contained 17 peaks, ranging from 74ppm to 23ppm.

It seemed that the macrocycle was unstable to these reaction conditions and so it became necessary to consider other methods of removing the N-tosyl protecting groups.

Reduction of the tetratosylamide with lithium in liquid ammonia, followed by the careful addition of hydrochloric acid, successfully produced the tetrahydrochloride salt of the tetraamine **22.** This was dissolved in a small volume of water, basified with lithium hydroxide and then extracted into dichloromethane. Evaporation of solvent from the dried organic phase gave the free tetraamine **22** as a clear colourless oil, which crystallised on standing. The ^{1}H and ^{13}C NMR spectra were consistent with the assigned structure and showed the expected symmetry, as discussed above.

Incorporation of Thiourea and Urea Residues.

The bis(thiourea) $24N_4O_4.2CS$, **24**, was prepared in a yield of 25% by refluxing a solution of the tetraaza-crown ether, **22**, in 50/50-ethanol/water with an excess of carbon disulphide followed by the addition of a small amount of hydrochloric acid.

Unlike the bis(thiourea) **23**, a ^{1}H NMR of **24** in $CDCl_3$ showed the expected, simple symmetry of 2 triplets and 2 singlets in a 1:1:1:1 ratio, indicating that $24N_4O_4.2CS$ has a much more conformationally mobile structure than $18N_4O_4.2CS$. This was in agreement with the predictions made based on CPK molecular models.

Bisthiourea **24** was successfully hydrolysed to the bisurea **14** with mercury (II) acetate in refluxing 1,2-dichloroethane followed by refluxing aqueous potassium carbonate (pH=10.5). The product was separated from excess potassium carbonate by filtering an anhydrous methanolic solution through a bed of silica.

In contrast to the hydrolysis of bisthiourea **23** to bisurea **13**, both thiourea residues of **24** reacted in a single step, and no evidence was found which would have indicated the presence of of the partially hydrolysed urea/thiourea. This provides further evidence in support of the proposal that steric factors are largely responsible for the necessity of two reaction steps (one for each thiourea residue) in the hydrolysis of $18N_4O_2.2CS$ to $18N_4O_2.2CO$.

Although spectral analyses of the product (DCI mass, IR and ^1H and ^{13}C NMR) were consistent with the assigned structure, a satisfactory elemental analysis for carbon, hydrogen and nitrogen could not be obtained.

A mass spectrum acquired in the FAB mode showed peaks at 401 (M+1) and 423 (M+Na), and a small peak at 439 (M+K). Elemental analysis for sodium and potassium showed that they were present in amounts of 12.42% and 0.42% respectively.

Although the amount of potassium present was quite small, the amount of sodium was very significant, especially as no sodium salts had been used in the preparation of the bisurea. From the elemental analyses, it was calculated that the ratio of bisurea **14**:Na^+ was about 1:1, which may imply the formation of a 1:1 complex. (This assumes that every 4 equivalents of nitrogen imply the presence of 1 equivalent of **14**).

It was proposed that sodium salts had been extracted from either celite or silica, which were used in the work-up of the bisurea. As K^+ ions were known to be present before celite or silica were used, K^+ ions were presumably exchanged for Na^+ ions, accounting for the relatively low amount of potassium in the final product. This indicates that the Na^+ complex is considerably more stable than the analogous K^+ complex.

3.3.3. THIOUREA CRYSTAL STRUCTURES.

Crystals of the bisthioureas $18N_4O_2.2CS$, **23**, and $24N_4O_4.2CS$, **24**, which were suitable for analysis by X-ray diffraction experiments were grown in single attempts by the vapour diffusion of methanol, through a pinhole, into dichloromethane solutions of the relevant bisthioureas.

3.3.3.1. $18N_4O_2.2CS$, **23**.

$18N_4O_2.2CS$ crystallised in the triclinic system with space group $P\bar{1}$. Representations of the crystal structure of $18N_4O_2.2CS$ are shown in figures 3.16a and 3.16b. Values of bond angles are given in table 3.2, and values of bond lengths and selected distances are given in table 3.3.

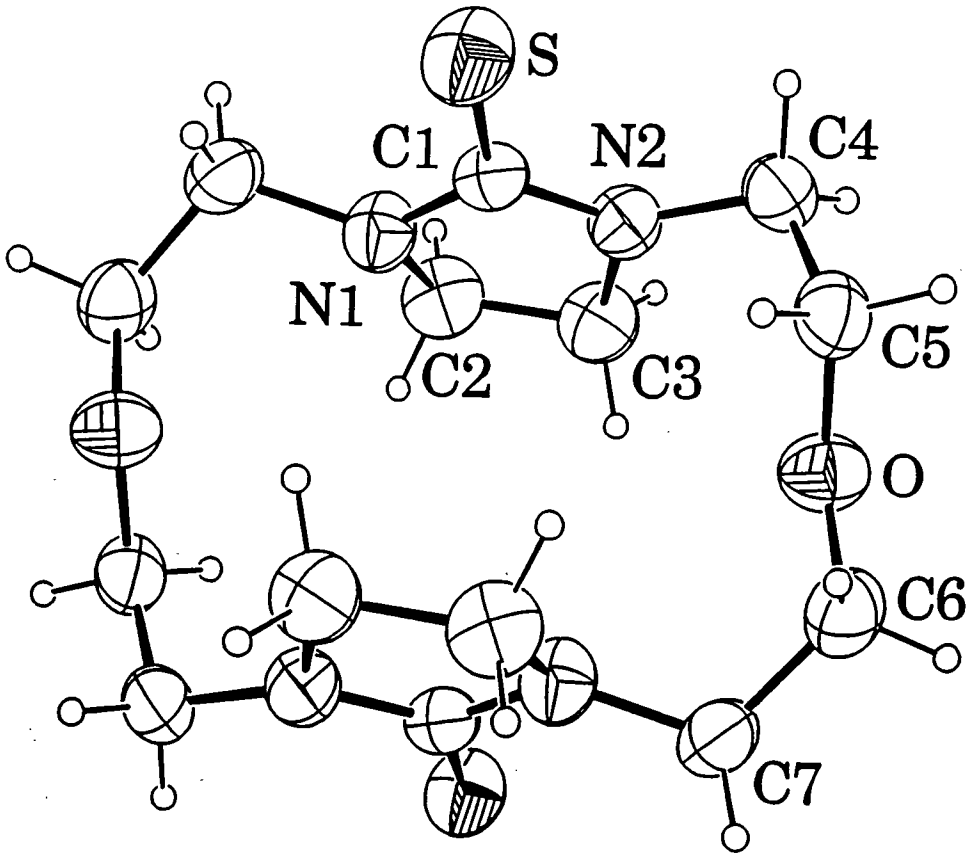


Figure 3.16a

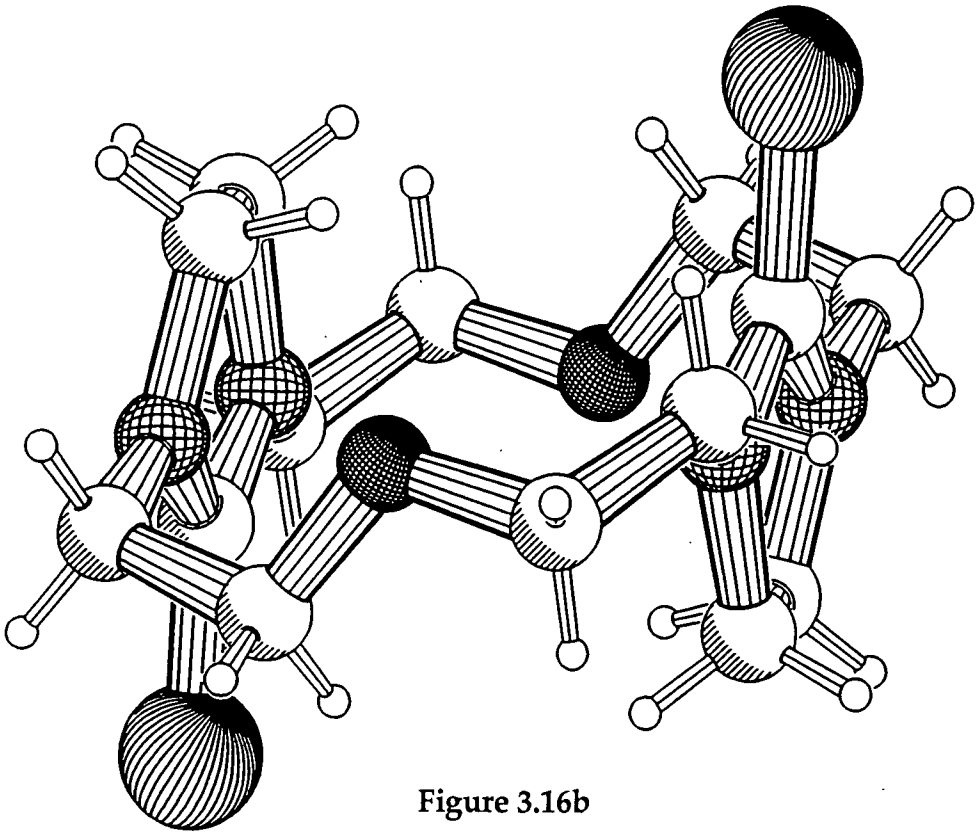


Figure 3.16b

Bond Lengths (Å).

S-C(1)	1.681(2)	O-C(5)	1.417(2)	O-C(6)	1.421(3)
N(1)-C(1)	1.349(2)	N(1)-C(2)	1.460(2)	N(1)-C(7)[a]	1.450(2)
N(2)-C(1)	1.345(2)	N(2)-C(3)	1.454(2)	N(2)-C(4)	1.450(2)
C(2)-C(3)	1.507(3)	C(4)-C(5)	1.495(3)	C(6)-C(7)	1.500(3)
		C(7)-N(1)[a]	1.450(2)		

`a' represents the symmetry related equivalent: 1-x,1-y,1-z

Table 3.2

Bond Angles (°).

C(5)-O-C(6)	114.68(15)	C(1)-N(1)-C(2)	110.60(15)
C(1)-N(1)-C(7)[a]	124.09(16)	C(2)-N(1)-C(7)[a]	121.31(16)
C(1)-N(2)-C(3)	111.33(15)	C(1)-N(2)-C(4)	125.61(15)
C(3)-N(2)-C(4)	122.28(15)	S-C(1)-N(1)	125.83(14)
S-C(1)-N(2)	125.15(14)	N(1)-C(1)-N(2)	109.03(15)
N(1)-C(2)-C(3)	102.87(15)	N(2)-C(3)-C(2)	102.45(15)
N(2)-C(4)-C(5)	113.63(16)	O-C(5)-C(4)	108.89(16)
O-C(6)-C(7)	109.80(15)	N(1)[a]-C(7)-C(6)	113.04(16)

`a' represents the symmetry related equivalent: 1-x,1-y,1-z

Table 3.3

As was predicted by a CPK model, the two C=S bonds are trans to one-another (one up, one down). However, the planes of the five membered rings are almost at 90° to the plane of the macrocyclic ring. In the CPK model, the angle was much less - about 30° (see figure 3.10), and the five membered rings could only be placed at 90° to the macrocyclic ring if considerable strain was introduced to the structure.

The macrocyclic cavity consequently has a more 'open' structure than was predicted. Although the cavity was still small, it was not as inaccessible to potential substrates (metal cations) as had been expected.

It was proposed that repulsion between the two thiourea dipoles was responsible for the apparently quite strained, relatively open conformation.

3.3.3.2. $24N_4O_4.2CS$, **24.**

$24N_4O_4.2CS$ crystallised in the orthorhombic system with space group Pbca. Representations of the crystal structure of $24N_4O_4.2CS$ are shown in figures 3.17a and 3.17b. Values of bond angles are given in table 3.4, and values of bond lengths and selected distances are given in table 3.5.

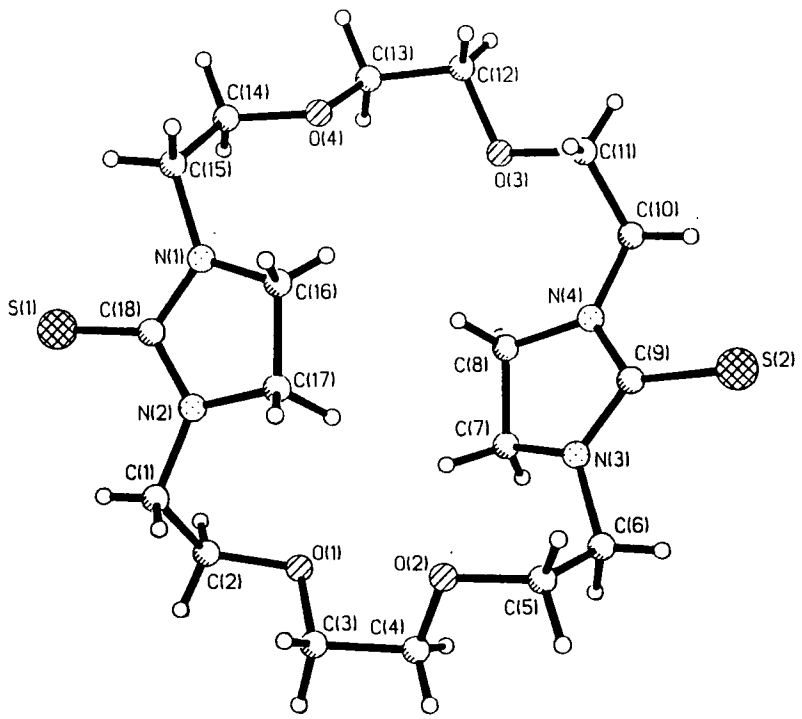


Figure 3.17a

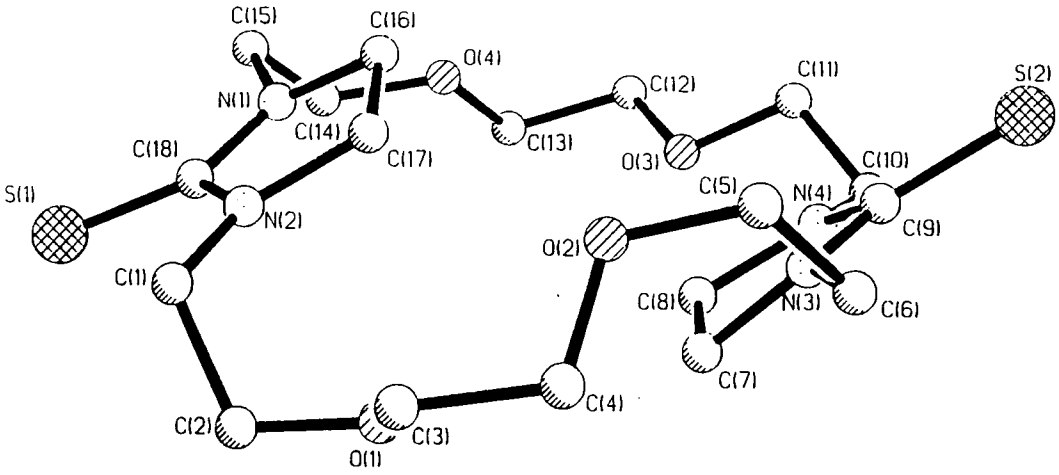


Figure 3.17b

Bond Angles (°)

C_2-O_1-C_3	113.1(3)	C_4-O_2-C_5	113.2(3)
C_{11}-O_3-C_{12}	112.1(3)	C_{13}-O_4-C_{14}	112.5(3)
C_{15}-N_1-C_{16}	121.7(3)	C_{15}-N_1-C_8	124.5(3)
C_{16}-N_1-C_{18}	111.5(3)	C_1-N_2-C_{17}	121.7(3)
C_1-N_2-C_{18}	125.4(3)	C_{17}-N_2-C_{18}	112.1(3)
C_6-N_3-C_7	120.0(3)	C_6-N_3-C_9	125.3(3)
C_7-N_3-C_9	111.3(3)	C_8-N_4-C_9	112.5(3)
C_8-N_4-C_{10}	121.7(3)	C_9-N_4-C_{10}	125.4(3)
N_2-C_1-C_2	113.5(3)	O_1-C_2-C_1	114.1(3)
O_1-C_3-C_4	108.5(4)	O_2-C_4-C_3	108.8(4)
O_2-C_5-C_6	113.7(3)	N_3-C_6-C_5	111.8(3)
N_3-C_7-C_8	102.8(3)	N_4-C_8-C_7	102.0(3)
S_2-C_9-N_3	125.6(3)	S_2-C_9-N_4	126.0(2)
N_3-C_9-N_4	108.4(3)	N_4-C_{10}-C_{11}	114.3(3)
O_3-C_{11}-C_{10}	108.4(3)	O_3-C_{12}-C_{13}	108.9(3)
O_4-C_{13}-C_{12}	108.6(3)	O_4-C_{14}-C_{15}	109.4(3)
N_1-C_{15}-C_{14}	113.8(3)	N_1-C_{16}-C_{17}	103.6(3)
N_2-C_{17}-C_{16}	103.1(3)	S_1-C_{18}-N_1	125.4(3)
S_1-C_{18}-N_2	126.0(2)	N_1-C_{18}-N_2	108.6(3)

Table 3.4

Bond Lengths (Å)

S_1-C_{18}	1.679(3)	S_2-C9	1.690(3)
O_1-C_2	1.439(4)	O1-C3	1.403(6)
O_2-C_4	1,440(6)	O_2-C_5	1.421(5)
O_3-C_{11}	1.421(4)	C_3-C_{12}	1.429(4)
O_4-C_{13}	1.428(5)	O_4-C_{14}	1.426(4)
N_1-C_{15}	1.451(5)	N_1-C_{16}	1.446(5)
N_1-C_{18}	1.354(4)	N_2-C_1	1.450(4)
N^2-C_{17}	1.458(5)	N_2-C_{18}	1.337(4)
N_3-C_6	1.455(5)	N_3-C_7	1.457(5)
N_3-C9	1.360(4)	N_4-C_8	1.467(5)
N_4-C9	1.337(4)	N_4-C_{10}	1.461(4)
C1-C2	1.499(6)	C_3-C_4	1.521(6)
C_5-C_6	1.520(6)	C_7-C_8	1.523(5)
C_{10}-C_{11}	1.505(5)	C_{12}-C_{13}	1.496(5)
C_{14}-C_{15}	1.510(6)	C_{16}-C_{17}	1.507(6)

Table 3.5

The two C=S bonds are turned away from one-another and from the centre of the macrocyclic ring i.e. they are divergent. However, molecular models have indicated that there is a relatively low energy barrier to the rotation of the macrocycle which would bring the sulphur atoms into the convergent conformation required for the binding of a substrate in the macrocyclic cavity.

It again seemed likely that repulsion between the two thiourea dipoles was responsible for the divergence of the C=S bonds. In contrast to $18N_4O_2.2CS$, very little strain is introduced by the separation of the dipoles.

3.3.4. BINDING STUDIES.

3.3.4.1. Thioureas.

3.3.4.1.1. Complexes with Silver (I).

As a preliminary investigation into the ability of bisthioureas **23** and **24** to form to complexes with soft metal cations, attempts were made to form complexes with silver (I). The bisthiourea was dissolved in a small volume of dichloromethane. An excess of a saturated methanolic solution of silver nitrate was layered on top of this and the two solutions allowed to diffuse together.

It had been hoped that crystals of the thiourea/silver (I) complex would form at the interface. Although a solid began to form almost immediately, it was not crystalline. Consequently, the two solutions were mixed together by shaking, resulting in the immediate formation of a considerable amount of precipitate.

This was collected by filtration and washed on the filter with dichloromethane and then methanol. The insolubility of the precipitate in these solvents indicated that it was neither the bisthiourea (soluble in dichloromethane) nor silver nitrate (soluble in methanol).

The precipitate was washed through the filter with water. The solution was evaporated under reduced pressure and the residue dried under vacuum to give the bisthiourea/Ag^+ complex. ^1H and ^{13}C NMR spectra of the complexes were acquired in D_2O.

Note that both the **23**.Ag^+ complex and the **24**.Ag^+ complex were photo sensitive, a black precipitate forming after standing in daylight for a few minutes. Consequently, the silver complexes were kept, as far as possible, in the dark.

$18N_4O_2.2CS/Ag^+$ Complex

The ^1H NMR spectrum of the $18N_4O_2.2CS/Ag^+$ complex was greatly simplified compared to the spectrum of free $18N_4O_2.2CS$. As discussed above, free $18N_4O_2.2CS$ is very rigid with each proton non-equivalent (see figure 3.14). The ^1H NMR spectrum of the silver (I) complex showed only a singlet and two triplets, indicating considerably more mobility (lower energy barriers to conformational interconversions).

The ^{13}C NMR spectrum showed four signals, as expected. The thiourea C=S carbon moved from 182.2ppm for the free macrocycle to 175.5ppm in the silver (I) complex, although a change in solvent from $CDCl_3$ to D_2O must be taken into account.

Most importantly, shortly after the NMR spectra of the $18N_4O_2.2CS/Ag^+$ complex had been acquired, crystals of the complex, which were suitable for analysis by X-ray diffraction, precipitated from the solution. The crystals were in the monoclinic system with space group $P2_1/n$.

As mentioned above, the complex was light sensitive, and considerable crystal decomposition occurred during the X-ray diffraction experiment. Although the data acquired was consequently of a fairly poor quality, the structure of the complex was unequivocally

solved. This is represented in figures 3.18a and 3.18b. Bond lengths and bond angles are given in tables 3.5 and 3.6 respectively.

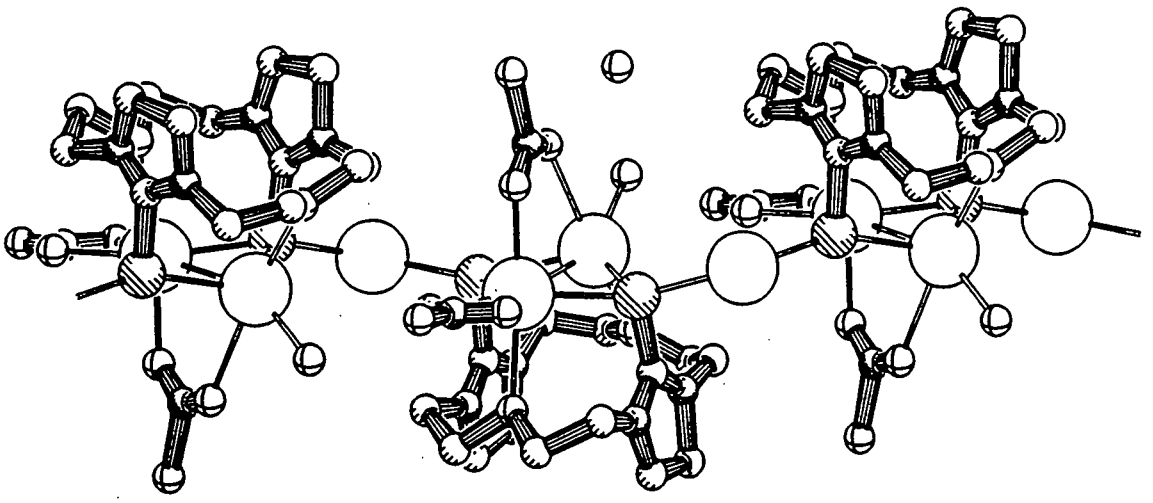


Figure 3.18a

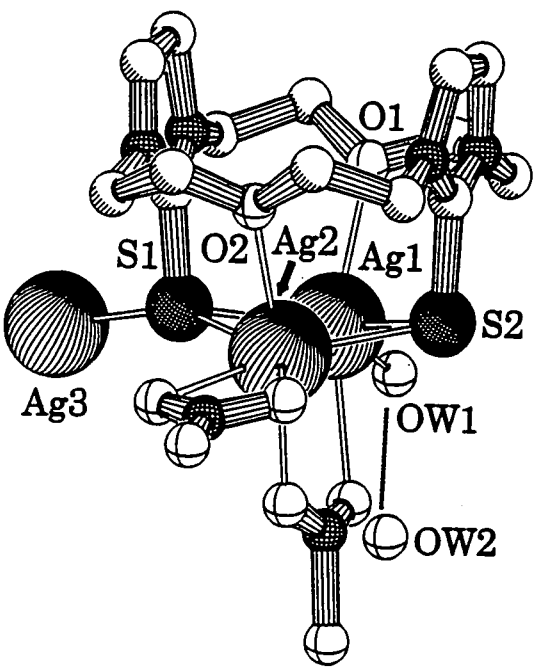


Figure 3.18b

Selected Bond Lengths and Distances (Å).

Ag(1)	Ag(2)	2.903(5)	Ag(1)	S(1)	2.53(1)	Ag(1)	S(2)	2.69(1)
Ag(1)	O(1)	2.61(3)	Ag(1)	O(1W)	2.29(3)	Ag(1)	O(11)	2.72(9)
Ag(2)	S(1)	2.67(1)	Ag(2)	S(2)	2.66(1)	Ag(2)	O(2)	2.53(2)
Ag(2)	O(13)	2.40(3)	Ag(2)	O(21)	2.43(3)	Ag(2)	O(23)	2.63(3)
Ag(2)	N(21)	2.96(4)	Ag(3)	S(1)	2.57(1)	Ag(3)	S(2)	2.54(1)
Ag(3)	O(31)	2.00(7)	Ag(3)	O(32)	2.32(5)	Ag(3)	O(41)	2.19(6)
Ag(3)	O(42)	2.45(8)	S(1)	C(1)	1.77(5)	S(2)	C(2)	1.88(5)
O(1)	C(3)	1.50(6)	O(1)	C(14)	1.39(5)	O(2)	C(8)	1.46(4)
O(2)	C(9)	1.57(4)	O(12)	N(11)	1.69(6)	O(13)	N(11)	1.23(5)
O(21)	N(21)	1.29(4)	O(22)	N(21)	1.18(4)	O(23)	N(21)	1.18(4)
O(31)	N(31)	1.24(7)	O(32)	N(31)	1.99(7)	O(32)	N(31)[a]	1.36(6)
O(41)	N(41)	1.31(5)	O(41)	N(41)[b]	1.81(7)	O(42)	N(41)[b]	1.13(9)

`a' represents the symmetry equivalent: 1-x, -y, -z

`b' represents the symmetry equivalent: 2-x, -y, -z

Table 3.5

Selected Bond Angles (°).

Ag2	Ag1	S1	58.3(2)	Ag2	Ag1	S2	56.7(2)
Ag2	Ag1	O1	118(1)	Ag2	Ag1	O1W	152(1)
S1	Ag1	S2	112.4(3)	S1	Ag1	O1	100(1)
S1	Ag1	O1W	132(1)	S1	Ag1	O11	99(2)
S2	Ag1	O1	95(1)	S2	Ag1	O1W	114(1)
S2	Ag1	O11	82(2)	O1	Ag1	O1W	87(1)
O1	Ag1	O11	160(2)	O1W	Ag1	O11	76(2)
Ag1	Ag2	S1	53.9(2)	Ag1	Ag2	S2	57.6(2)
Ag1	Ag2	O2	111.9(4)	Ag1	Ag2	O13	81(1)
Ag1	Ag2	O21	160(1)	Ag1	Ag2	O23	147(1)
Ag1	Ag2	N21	166(1)	S1	Ag2	S2	109.1(3)
S1	Ag2	O2	92.0(5)	S1	Ag2	O13	93(1)
S1	Ag2	O21	145(1)	S1	Ag2	O23	97(1)
S1	Ag2	N21	120(1)	S2	Ag2	O2	97(1)
S2	Ag2	O13	93(1)	S2	Ag2	O21	105(1)
S2	Ag2	O23	154(1)	S2	Ag2	N21	130(1)
O2	Ag2	O13	166(1)	O2	Ag2	O21	79(1)
O2	Ag2	O23	80(1)	O2	Ag2	N21	79(1)
O13	Ag2	O21	90(1)	O13	Ag2	O23	87(1)
O13	Ag2	N21	87(1)	S1	Ag3	S2	133.0(3)
S1	Ag3	O31	95(2)	S1	Ag3	O32	125(1)
S1	Ag3	O41	91(1)	S1	Ag3	O42	89(2)
S2	Ag3	O31	92(2)	S2	Ag3	O32	96(1)
S2	Ag3	O41	96(1)	S2	Ag3	O42	131(2)
O31	Ag3	O41	163(2)	O31	Ag3	O42	111(3)
O32	Ag3	O41	109(2)	O32	Ag3	O42	67(2)
O41	Ag3	O42	53(2)	Ag1	S1	Ag2	67.8(2)
Ag2	S2	Ag3	128.9(4)	Ag1	S1	Ag3	120.1(4)
Ag2	S1	Ag3	157.1(5)	Ag1	S2	Ag2	65.7(2)
Ag1	S2	Ag3	153.4(5)				

Table 3.6

As was predicted from the CPK molecular model, the cavity of the macrocycle is too small to include the Ag^+ cation. However, in contrast to the free macrocyclic ligand, the two C=S bonds of the complexed macrocyclic ligand are in a cis conformation in the complex, with a sulphur-sulphur distance of 4.34Å.

The stoichiometry of the complex is 3:1 (Ag^+ : $18N_4O_2.2CS$), with each sulphur bridging three Ag^+, and each Ag^+ bridging two sulphur atoms. All Ag^+...S bond distances are in the region 2.53Å - 2.69Å. The S...Ag(1)...S and S...Ag(2)...S bond angles are both about 110°, whereas the S...Ag(3)...S bond angle is 133°.

Ag(1) and Ag(2) are also coordinated to the ether oxygens of $18N_4O_2.2CS$ and are bridged by two oxygens of a nitrate group. A second nitrate group is coordinated to Ag(2), while Ag(1) is coordinated to a water molecule.

Ag(3) lies between two sulphur atoms of adjacent molecules of $18N_4O_2.2CS$. Ag(3) is also coordinated to two nitrate groups, which are disordered about inversion centres. These nitrates bridge symmetry related Ag(3) cations such that the two Ag(3)...Ag(3) distances are 6.22Å and 6.34Å.

Ag(1) is 5-coordinate (2 sulphurs, 1 macrocyclic ether oxygen, 1 bridging nitrate oxygen, 1 water oxygen). Ag(2) is 6-coordinate (2 sulphurs, 1 macrocyclic ether oxygen, 1 bridging nitrate oxygen, 2 oxygens of a second nitrate), as is Ag(3) (2 sulphurs of adjacent macrocycles, 2 oxygens of each of 2 nitrate groups).

$24N_4O_4.2CS/Ag^+$ Complex

For the $24N_4O_4.2CS/Ag^+$ complex, the ^1H NMR in D_2O was very broad compared to the free $24N_4O_4.2CS$ in $CDCl_3$, and the spectrum was not sufficiently resolved at 200MHz to enable peak assignment. The ^{13}C NMR spectrum of the complex in D_2O showed the expected symmetry - 4 peaks, each one corresponding to an aliphatic CH_2 carbon, and a C=S carbon at 176.4ppm (181.6ppm for the free ligand in $CDCl_3$).

Unfortunately, crystals of the $24N_4O_4.2CS/Ag^+$ complex did not form as readily as those of the $18N_4O_2.2CS/Ag^+$ complex, and so a structural analysis by X-ray diffraction was not possible. However, a FAB mass spectrum of the $24N_4O_4.2CS/Ag^+$ showed peaks at 433 (M+1), 539 ($M+^{107}Ag$) and 541 ($M+^{109}Ag$) only, indicating that stable 1:1 complexes were formed.

3.3.4.1.2. Membrane Transport Experiments.

In an effort to examine the cations with which the bisthioureas would form complexes, and to study their selectivity for different cations, the bisthioureas **23** and **24** were incorporated into PVC membranes. The cation transport properties of the membranes were then studied using an ion sensitive electrode (see Appendix 1).

0.1M aqueous solutions of Zn (II) and Cd (II) chlorides and Ag (I) nitrate were the test electrolytes. The results discussed below apply to the membranes incorporating both $18N_4O_2.2CS$ and $24N_4O_4.2CS$.

In the case of Ag(I), transport properties were very poor. A solid formed at the membrane/solution interface, and this was soon followed by the formation of a black precipitate.

For Zn (II) and Cd (II), a negative response of the electrode (of about -50mV per decade) was observed in the concentration range 10^{-2} - 10^{-3}M, indicating that anion transport across the membrane was occurring (due to the formation of [bisthiourea.MCl_3]$^-$ complexes).

These phenomena indicate that very stable complexes were formed between the bisthiourea and the relevant cation. For Ag (I), a complex formed at the membrane/solution interface. For Zn (II) and Cd (II), it was proposed that the cation was complexed by the bisthiourea in the PVC membrane, but decomplexation was very slow. The bisthiourea/M^{2+} complex in the membrane was responsible for the transport of the chloride counterions across the membrane, resulting in the observed, negative response of the electrode (see Appendix 1).

Although no quantitative measurements were gained from these experiments, the results suggest that both **23** and **24** form very stable complexes with Ag (I), Zn (II) and Cd (II).

3.3.4.1.3. Calorimetric Measurements.

A variety of direct and indirect methods have been developed to measure the thermodynamic and kinetic parameters of macrocyclic ligand - cation complex formation[26]. However, the majority of these rely on the common solubility of the macrocycle and the cation (or other substrate), usually in aqueous or methanolic solution.

In the present case, it was not possible to conduct such an experiment as no common solvent for the lipophilic bisthioureas and the hydrophilic cations M^{n+} could be found.

However, a technique which has recently been developed relies on the enhanced aqueous solubility of a complex compared to the insoluble, lipophilic ligand[7].

The concentration of a saturated aqueous solution of the macrocyclic ligand in solution is measured, by UV spectroscopy, for example. Known amounts of the test cation are added. The total concentration of the macrocycle in solution (free + complexed) is measured after each addition, and from the known concentration of the cation and the measured concentration of the macrocycle at different macocycle:cation ratios, stability constants of the complex and ΔH values for the equilibrium can be calculated.

Bisthioureas **23** and **24** have very low aqueous solubility, and as only relatively small amounts of material were required the technique seemed to be ideal. However, the method is slow as long periods of time are sometimes required for the macrocycle and cation to reach equilibrium. Although samples of both **23** and **24** have been submitted for calorimetric analyses of their complexation properties, results were not available at the time of writing.

3.3.4.2. Ureas

As has already been mentioned, the bisureas **13** and **14** could not be properly purified, probably because of the high stability of the complexes with Na^+ and K^+. Consequently, accurate measurement of

thermodynamic or kinetic parameters e.g. by NMR spectroscopy or calorimetry, was not possible.

However, it was thought possible that the selectivity of the bisureas for different cations could be measured using FAB mass spectroscopy or membrane transport techniques.

3.3.4.2.1. FAB Mass Spectroscopy Competition Experiments.

The use of FAB mass spectroscopy (FAB.MS) to determine ligand selectivities for cations has been established fairly recently[27,28].

The method relies on a mixture of cations competing for a deficiency of a ligand - a bisurea in the present case. The intensity of the signals* corresponding to m/e values of $(L+M^{n+})$, where L=ligand, is then related to the equilibrium concentrations of the $L.M^{n+}$ complexes, and hence to the selectivity of the ligand for the mixture of cations present.

The selectivity for one cation over another is then given by S, where S=log(I'/I") and I' is the intensity of the largest $(L+M^{n+})$ peak and I" is the intensity of a smaller $(L+M^{n+})$ peak. Consequently, lower S values indicate more stable $L.M^{n+}$ complexes.

The validity of this technique has been challenged[29]; it was suggested that the technique is useful only as a guide to selectivity, and could not be used to give quantitative or even semi-quantitative measurements.

* At least 20 successive spectra for each analytical solution were obtained, and scans 3-18 used to average signal intensities to give the final spectra.

However, the experiment requires very little material and is relatively quick to perform, as it requires only a single run for a given mixture of cations. Also, as it is a competition experiment which depends on an excess of cations, and gives at best only semi-quantitative selectivity values, small amounts of contamination of the ligand are not critical.

Consequently, it seemed an ideal preliminary experiment to examine the selectivity of the bisureas **13** and **14**.

A known amount of the bisurea was dissolved in a small volume of water, and one equivalent of each of either the mono- or divalent cations (NH_4^+, Li^+, Na^+, K^+, Rb^+ and Cs^+ or Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+} and Ba^{2+}) added as 0.1M aqueous solutions. The mixture was heated to about 70°C and allowed to cool to room temperature. This was repeated three times before the solution was concentrated to about 1M with respect to the bisurea and each of the cations present. The mixture was allowed to stand in a sealed sample bottle at ambient temperature for several weeks before a FAB mass spectrum was acquired, using glycerol as the solvent matrix.

The results obtained for the bisureas **13** and **14** are presented in tables 3.7-10. Note that if no entry is found for an $[L.M^{n+}]$ complex in the relevant table, then no peak was found at a m/e value indicating the presence of that complex.

$18N_4O_2.2CO$ with Monovalent Cations.

$18N_4O_2.2CO$ was contaminated with small amounts of sodium and potassium, and so these cations were in a slight excess compared to

the other cations present. Although this will almost certainly affect the apparent selectivity of the ligand, the amount of contamination was small and so the error introduced is probably not of great significance. Values of the selectivity of $18N_4O_2.2CO$ for the monovalent cations listed above are given in table 3.7.

m/e	Intensity	S^I
L+1	100	-
L+Li	4.15	0.32
L+Na	8.60	0
L+K	5.86	0.17
L+Rb	4.62	0.27
L+Cs	3.11	0.44

Table 3.7

$18N_4O_2.2CO$ with Divalent Cations.

The spectrum in this case was quite noisy, which made it difficult to see peaks of low intensity. This was especially important for metal cations which have several major isotopes e.g. zinc and especially cadmium.

Peaks corresponding to L+Na and L+K were also seen in the spectrum, with relative intensities of 6.21 and 3.27 respectively. However, the presence of Na^+ and K^+ should not affect the competition of the M^{2+} cations for the remaining ligand.

A peak at an m/e value corresponding to 2L+Ba could be seen, which seems to imply that a stable 2:1 complex (ligand : cation) was formed. Values of the selectivity of $18N_4O_2.2CO$ for the divalent cations listed above are given in Table 3.8.

m/e	Intensity	S^{II}
L+1	100	-
L+Mg	1.88	0
L+Ba	1.10	0.23
2L+Ba	1.09	

Table 3.8

These experiments indicate that $18N_4O_2.2CO$, **13**, is sodium and magnesium selective. That the peaks with m/e values corresponding to the sodium and potassium complexes are the most intense in the mixture of divalent cations, even though they are present only as ligand impurities, indicates that $18N_4O_2.2CO$ is also selective for group I cations.

The proposed selectivity of the bisurea **13** is therefore $Na^+ > K^+ > Rb^+ > Li^+ > Cs^+ \approx Mg^{2+} > Ba^{2+} >> NH_4^+, Ca^{2+}, Zn^{2+}, Cd^{2+}$.

$24N_4O_4.2CO$ with Monovalent Cations

Note that the ligand $24N_4O_4.2CO$ was contaminated with sodium (ca. 12% by weight) before addition of the chlorides listed. There is consequently an excess of sodium compared to the other cations, and this almost certainly influences the apparent selectivity. The ligand was also contaminated with potassium (<1%), but as the amount present was so small the relative peak intensity should not be noticeably affected. Values of the selectivity of $24N_4O_4.2CO$ for the monovalent cations listed above are given in Table 3.9.

m/e	Intensity	S^I
L+1	100	-
L+Li	4.42	0.37
L+Na	10.47	0
L+K	2.47	0.63
L+Rb	2.36	0.65
L+Cs	2.81	0.57

Table 3.9

$24N_4O_4.2CO$ with Divalent Cations

Peaks at m/e values corresponding to L+Na and L+K were again seen, with relative intensities of 9.75 and 1.26 respectively, but this should not affect the competition of the M^{2+} cations for the remaining ligand. Of the M^{2+} cations, only magnesium seems to form stable $L.M^{2+}$ complexes, but peaks at m/e value corresponding to the $L.(MCl)^+$ species could also be seen for magnesium, calcium and barium. The significance of these species is uncertain.

Note that only the peaks indicated in the table could be seen. It seems unusual that there was no peak which would have indicated that complexes with strontium were formed, even though species containing calcium and barium - the elements immediately above and below it in the periodic table - could be clearly seen. Values of the selectivity of $24N_4O_4.2CO$ for the monovalent cations listed above are given in table 3.10.

m/e	Intensity	S^{II}
L+1	100	-
L+Mg	2.73}	0
L+MgCl	1.62}	
L+CaCl	1.25	0.54
L+BaCl	1.38	0.50

Table 3.10

Although these experiments seem to indicate that $24N_4O_4.2CO$, **14**, is also sodium and magnesium selective, the presence of sodium as an impurity in the ligand may lead to a false reading.

The quite large peaks at m/e values corresponding to the sodium and potassium complexes in the mixture of divalent cations, even though sodium and potassium are present only as ligand impurities, indicates that $24N_4O_4.2CO$ is also selective for group I cations. As it has already been deduced that $24N_4O_4.2CO$ binds to Na^+ considerably more strongly than K^+, the selectivity of $24N_4O_4.2CO$ can be written as $Na^+ > Li^+ > Cs^+ > K^+ \approx Rb^+$ and $Na^+ >> K^+ > Mg^{2+} > Ca^{2+} \approx Ba^{2+} >> Sr^{2+}, Zn^{2+}, Cd^{2+}$.

3.3.4.2.2. Membrane Transport Experiments.

When a macrocycle is incorporated in a membrane which will be used in an ion sensitive electrode (see Appendix 1), the membrane is conditioned for 24hrs. in a 0.1M aqueous solution of the test electrolyte before the response of the membrane to the test electrolyte is measured. This allows the the cations of the test electrolyte to "saturate" the membrane so that conductivity measurements can be made.

Consequently, if the macrocycle was contaminated with relatively small amounts of cations, they would not affect the response of the membrane if it has been properly conditioned.

FAB mass spectroscopy experiments (section 3.3.4.2.1) had already indicated that both **13** and **14** would selectively bind sodium,

and so sodium would be the initial test electrolyte. Sodium contamination of **13** and **14** would not, therefore, be a problem.

If sodium was efficiently transported by the membrane (a response of about 60mV from the ISE), then the possible interference of other cations (Li^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+) would be investigated.

However, the bisureas were not sufficiently soluble in lipophilic solvents to allow the preparation of membranes. This is quite possibly because the bisureas could only be isolated as the sodium or potassium complex.

3.4. CONCLUSIONS AND FURTHER WORK.

3.4.1. CONCLUSIONS.

Urea residues have been successfully incorporated into macrocyclic frameworks, such that there are two urea residues per macrocycle. The carbonyl groups are arranged such that they can face one-another i.e. converge on a potential ionic substrate in an unusual, trans-spanning manner. Preliminary investigations into the complexes formed by the macrocycles indicated that they formed stable complexes with group I cations such as sodium and potassium.

The analogous thioureas were prepared as intermediates in the synthesis of the ureas. It was found that they form very stable complexes with soft metal cations such as silver (I), zinc (II) or cadmium (II), although the silver (I) complexes were photosensitive.

It seems likely that this method of C=X (X = S, O) insertion will prove to be quite general.

3.4.2. FURTHER WORK.

Purification and characterisation of the pure bisureas must be achieved if quantitative rather than qualitative complexation studies, using techniques such as NMR titrations, are to be made.

However, a method does exist whereby a membrane is made incorporating the charged complex rather than the free, neutral ligand. Cation transport can then be measured voltametrically[30]. Although the apparatus required for such an experiment was not available, this

method should prove to be more successful than the membrane transport experiments adopted in this work (see Appendix 1).

For the bisthioureas, qualitative data (via calorimetric methods) for the complexation reaction with a variety of early transition metal cations should be available fairly soon. For the membrane transport experiments, the voltametric method mentioned above may allow selectivity measurements of the bisthioureas.

The apparent generality of the method developed for the insertion of a C=X residue (X = O, S) suggests that it may be possible to prepare "families" of macrocycles incorporating (thio)urea residues from the relevant aza-crown ethers. The syntheses of the dibenzo compounds **11** and **12**, and the thiourea intermediates, should now be possible, given that the preparation of the aza-crown ether precursors (dibenzo-$18N_4O_2$ and dibenzo-$24N_4O_4$) should be analogous to the preparations of $18N_4O_2$ and $24N_4O_4$ as established in this work.

The method should extend to any aza-crown ether where there are two nitrogen atoms separated by two (or even three) carbon atoms. Some examples of the types of ligand which it might be possible to prepare are shown in 3.19.

Note, however, that the preparation of crown-type compounds incorporating more than about three or four (thio)urea residues is precluded by the difficulties associated with the synthesis of the larger ring sizes.

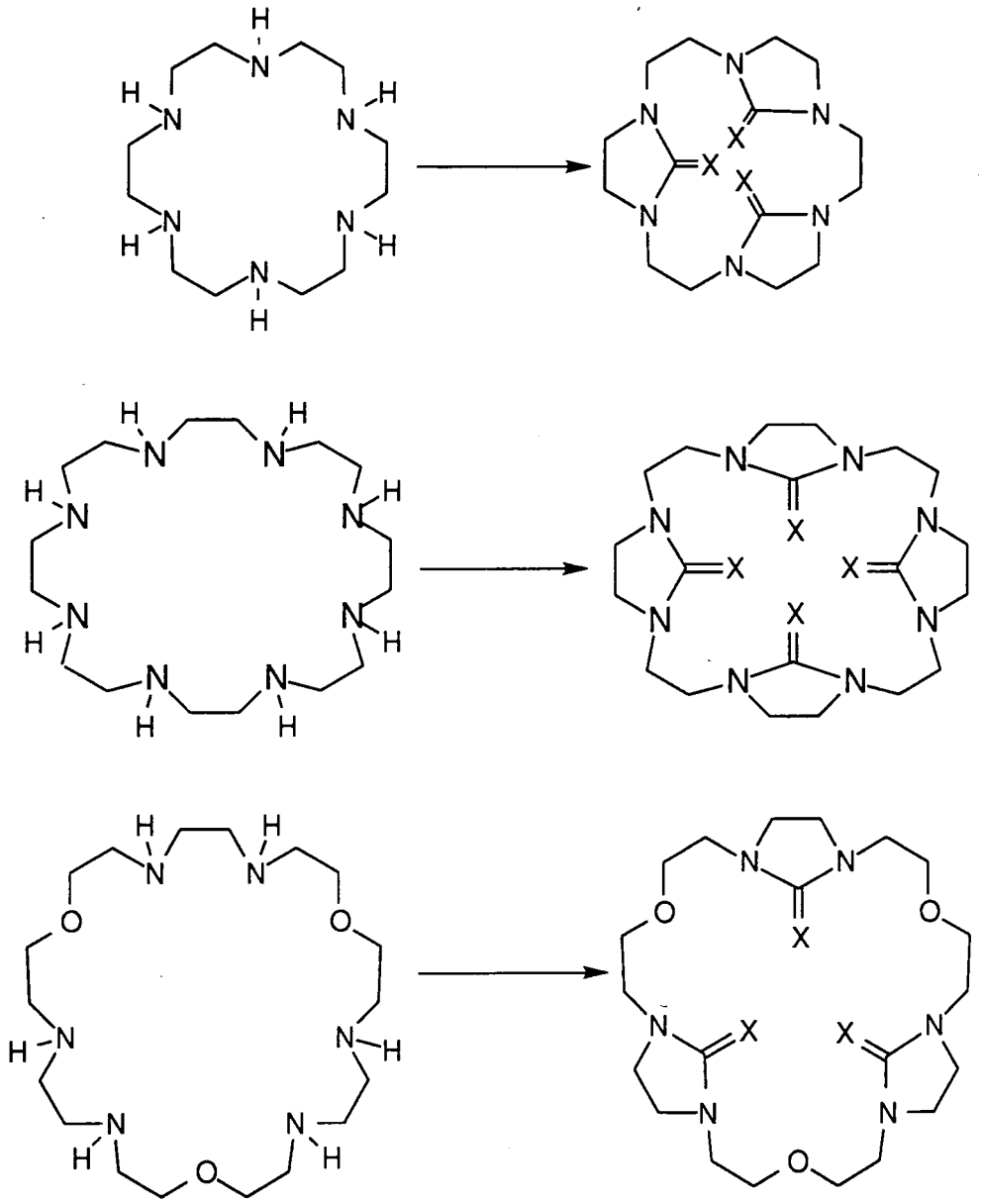


Figure 3.19

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CHAPTER 4. EXPERIMENTAL.

4.1 INTRODUCTION.

The synthetic procedures used for the preparation of the compounds mentioned in this work are described in sections 4.2 and 4.3, which refer to chapters 2 and 3 respectively. Section 4.4 details the procedures used to determine relative stability constants via FAB mass spectroscopy, while section 4.5 describes the NMR titration experiments. Section 4.6 details the experimental procedure used in the membrane transport experiments.

Rf values are given for thin layer chromatography using silica (Merck. Art. 5554, Kieselgel 60 F_{254}) or alumina (Merck. Art. 5551, aluminium oxide 150 F_{254}) supports and the solvent system specified. Column chromatography refers to either "flash" silica (Merck. Art. 9385, Kieselgel 60 0.04-0.063mm) or neutral alumina (Merck. Art. 1097, activity II-III, 0.063-0.200mm) stationary phases and the eluant system specified. Either freshly distilled or HPLC grade solvents were used as eluants.

IR spectra were recorded on a Perkin-Elmer 1720x FTIR spectrometer as thin films unless otherwise stated.

^{1}H and ^{13}C NMR spectra, unless otherwise stated, were recorded on a Varian Gemini-200 at operating frequencies of 200MHz and 50MHz respectively.

60MHz ^{1}H NMR spectra were recorded on a Hitachi-Perkin-Elmer R-24B CW spectrometer; 250MHz ^{1}H and 62.9MHz ^{13}C NMR spectra were recorded on a Bruker AC250. A Varian VXR400S was used to record 400MHz ^{1}H and 100MHz ^{13}C NMR spectra.

Chemical shift values are given in ppm and referenced to TMS at 0ppm. Coupling constants are given in Hz. For samples in D_2O, TMS was used as an external reference. For all other solvents, TMS was used as an internal reference.

4.2. SYNTHESES FOR CHAPTER 2.

O-Methyl-4,4'-Isopropylidenediphenol, (3, R=CH_3).

Bis-phenol-A (5.72g, 0.025moles) was weighed into a round bottom flask and dissolved in acetone (40ml). Potassium carbonate (3.53g, 0.026moles) was added, followed by methyl iodide (1.56ml, 0.025moles). The flask was stoppered and the suspension stirred at room temperature for 24hrs., then filtered and acetone removed from the filtrate to give a clear, colourless oil which was a mixture of unreacted starting material and the mono and dimethyl ethers. These were separated by column chromatography (flash silica, 4:1-hexane:ethyl acetate) and the monomethyl ether isolated in a yield of 2.55g (42%).

M.pt.=58-61°C. Anal. Calcd. for $C_{16}H_{18}O_2$: C 79.34%, H 7.44%. Found: C 79.44%, H 7.48%. m/e135, 227, 242(M+1), 260(M+18). ^1H NMR (CCl_4, 60MHz) δ: 7.2-6.5 (8H, m, ArH), 4.4 (1H, s, OH), 3.8 (3H, s, OCH_3), 1.7 (6H, s, CCH_3) ppm. IR: 3390 (O-H), 2960 (C-H), 1505, 1250, 1180, 830 cm^{-1}.

2,6-Bis(bromomethyl)pyridine[1].

2,6-bis(hydroxymethyl)pyridine (3.02g, 0.02moles) was dissolved in 45% w/v. HBr in acetic acid (30ml) and heated at reflux

for 10hrs. The deep, clear, red solution was allowed to cool to room temperature. On agitation of the cooled solution, considerable precipitation occured forming a pink slurry. This was carefully basified with 6M KOH causing the colour to discharge. The white precipitate was collected by filtration and washed on the filter with 0.1M KOH (3 x 10ml) and then water (4 x 20ml). The solid was dried under vacuum to leave 4.64g (81%) of a fine, white powder.

M.pt.=88°C (lit. 84-89°C). m/e 265, 266, 267. ^{1}H NMR ($CDCl_3$) δ: 7.71 (1H, t, aromatic C-H, B of A_2B system), 7.38 (2H, d, aromatic C-H, A of A_2B system), 4.54 (4H, s, $ArCH_2Br$) ppm. ^{13}C NMR ($CDCl_3$) δ: 157.2 (aromatic C-CH_2Br), 138.6 (aromatic C, para to N), 123.3 (aromatic C, meta to N), 34.0 ($ArCH_2Br$) ppm. IR (KBr disc): 1570, 1450, 1205, 815, 742, 585 cm^{-1}.

Methyl 2,6-dimethyl benzoate[2].

2,6-Dimethyl benzoic acid (20.32g, 0.14moles) was dissolved in dichloromethane (150ml) under nitrogen. Oxalyl chloride was added (12.5ml, 0.15moles), causing slight effervescence. A few drops of DMF were added to the stirred solution and effervescence became vigorous, but ceased after about 30 minutes. The solution was stirred for another 2hrs. at room temperature and solvent was then removed at reduced pressure to give a yellow oil. This was redissolved in dichloromethane (50ml) and solvent removed at reduced pressure. This procedure was repeated three times. An excess of methanol was carefully added and solvent removed to leave a yellow oil in quantitative yield (22.17g). The product was a severe lachrymator and

possessed a very unpleasant odour. This was used without further purification.

^1H NMR ($CDCl_3$) δ: 7.20-6.97 (3H, m, ArH), 3.87 (3H, s, OCH_3), 2.29 (6H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 170.92 (C=O), 135.41 (aromatic C-CO_2Me), 134.35 (aromatic C-CH3), 129.82 and 128.04 (aromatic C-H), 52.28 (OCH_3), 20.17 ($ArCH_3$) ppm.

Methyl 2,6-bis(bromomethyl) benzoate[2].

Methyl 2,6-dimethylbenzoate (22.17g, 0.14moles) was dissolved in carbon tetrachloride. NBS (48.07g, 0.27moles), freshly recrystallised from water and thoroughly dried, and a small amount of AIBN (c.50mg) was added and the mixture heated at reflux under a drying tube until all the NBS was consumed. (Occasionally, the reaction was very slow to start, in which case a tungsten bulb was used as a light source to promote radical initiation). The solution was cooled, filtered and the solvent removed under reduced pressure to give a yellow oil, crude yield 43.93g. This was purified either by column chromatography (flash silica, 2:1 - hexane:dichloromethane as eluant), or by several recrystallisations from cyclohexane to give a white, crystalline material in an average yield of 29% or 25% respectively (from 2,6-dimethyl benzoic acid).

M.pt.=68-74°C (lit.=77-79°C). TLC (silica, 2:1 - hexane: dichloromethane): R_f=0.11. Anal: calcd. for $C_{10}H_{10}Br_2O_2$: C 37.30%, H 3.13%. Found: C 37.27%, H 3.21%. ^1H NMR ($CDCl_3$) δ: 7.37 (s, 3H, ArH), 4.62 (4H, s, $ArCH_2Br$), 4.02 (3H, s, OCH_3) ppm. ^{13}C NMR ($CDCl_3$) δ: 137.36 (aromatic C-CO_2Me), 134.87 (aromatic C-CH_2Br),

131.22 and 131.12 (aromatic C-H), 53.31 (OCH_3), 31.00 (CH_2Br) ppm. IR 2358 (C-H), 1750, 1718 (C=O), 1278 cm^{-1}.

O,O'-Bis(α,α-Dimethyl-α-p-Methoxybenzene-Tolyl)-1,4-Dioxymethylene Benzene, (4, R=CH_3).

Monomethylated bis-phenol-A, (3, R=CH_3) (0.97g, 4 x 10^{-3}moles) and α,α'-dibromo-p-xylene (0.51g, 1.93 x 10^{-3}moles) were dissolved in ethanol (50ml). Potassium carbonate was added and the mixture heated at reflux for 11hrs. The reaction was filtered hot and the white solid residue dried at reduced pressure and then taken up in chloroform (100ml). The solution was filtered to remove inorganic salts. Solvent was removed from the filtrate and the residue dried under vaccuum to leave a white solid (0.72g, 65%).
M.pt.=126-128°C. Anal. Calcd. for $C_{40}H_{42}O_4$: C 81.91%, H 7.17%. Found: C 81.86%, H 7.29%. ^1H NMR ($CDCl_3$, 60MHz) δ: 7.5-6.9 (20H, m, ArH), 5.1 (4H, s, $ArCH_2O$), 3.9 (6H, s, OCH_3), 1.7 (12H, s, CCH_3). m/e 135, 149, 227, 604(M+18).

Demethylation with Boron Tribromide.

Compound 4 (R=CH_3) (0.12g, 2.1 x 10^{-4}moles), was dissolved in dichloromethane (20ml) and cooled to -78°C under nitrogen. A solution of BBr3 in dichloromethane (1M, 0.2ml, 2.0 x 10^{-4}moles) was added and the solution stirred at -78°C for 3hrs., during which a purple colouration developed. The reaction was allowed to warm to room temperature and 5% $KOH_{(aq)}$ (20ml) was carefully added (causing the colour to discharge) followed by 50ml of water. The

resulting mixture was allowed to stir for 30 minutes and was then extracted into ether (2 x 100ml).

The organic phase was dried (Na_2SO_4), filtered and solvent evaporated. The pale brown, solid residue was taken up in chloroform and filtered. Chloroform was evaporated to leave a white, solid residue. This was found to be a mixture of compounds resulting from the cleavage of both the methyl and benzyl ether bonds.

^1H NMR ($CDCl_3$, 60MHz) δ: 7.5-6.7 (20H, m, ArH), 5.2 (2H, s, $ArCH_2O$), 4.6 (2H, s, $ArCH_2Br$), 3.9 (6H, s, OCH_3), 1.8 (12H, s, CCH_3) ppm. m/e135, 149, 227, 242, 260, 289.

Demethylation with Sodium Ethane Thiolate.

Dimethyl ether 4, R=CH_3, (0.09g, 1.6 x 10^{-4}moles) was dissolved in DMF (15ml). Sodium ethanethiolate (0.07g, 7.8 x 10^{-4}moles) was added and the solution heated at 100°C for 3hrs. After cooling to room temperature, 10% $HCl_{(aq)}$ (25ml) was added, causing a white precipitate to form. This was collected by filtration, washed with $HCl_{(aq)}$ (3 x 5ml) and water (3 x 10ml) and dried under vacuum to give unreacted starting material (0.08g, 88%).

^1H NMR ($CDCl_3$, 60MHz) δ: 7.5-6.7 (20H, m, ArH), 5.2 (4H, s, $ArCH_2O$), 3.9 (6H, s, OCH_3), 1.8 (12H, s, CCH_3) ppm. m/e 135, 149, 227, 279, 604(M+18).

Demethylation with Sodium or Lithium Iodide.

Dimethyl ether 4, R=CH_3 (0.08g, 1.4 x 10^{-4}moles) was dissolved in 2,6-lutidine and the solution stirred under nitrogen. LiI (0.08g, 6.0 x 10^{-4}moles) or NaI (0.04g, 4 x 10^{-4}moles) was added and the mixture

heated at reflux under nitrogen for 43hrs., during which a red/brown colour developed. The solution was cooled to room temperature and excess dilute HCl was added, causing precipitation. The precipitate was collected by filtration, washed with dilute HCl (3 x 10ml) and then water (4 x 10ml). The pale pink solid was dried at reduced pressure to give predominantly unreacted starting material (0.08g, 100%).
^{1}H NMR ($CDCl_3$, 60MHz) δ: 7.5-6.7 (20H, m, ArH), 5.2 (4H, s, $ArCH_2O$), 3.9 (6H, s, OCH_3), 1.8 (12H, s, CCH_3) ppm. m/e135, 149, 227, 279, 604(M+18).

O-tetrahydropyranyl-4,4'-Isopropylidenediphenol, (3, R=THP).

4,4'-Isopropylidenediphenol (bis-phenol-A, 45.24g, 0.20moles) was dissolved in ether (300ml) and the solution cooled to 0°C in an ice bath. Concentrated HCl was added (10 drops) followed by 3,4-dihydro-2H-pyran (18ml, 0.20moles). The solution was stirred at 0°C for 40 minutes and then allowed to warm to room temperature and stirred for a further 2hrs. The flask was stoppered and left to stand at room temperature overnight. The solution was washed with 0.1M $KHCO_3$ (3 x 50ml), dried over magnesium sulphate, filtered, and the ether removed from the filtrate at reduced pressure to give a very viscous, clear, colourless oil. This was purified by column chromatography (flash silica, 1:4 - ethyl acetate:hexane) to give the monoether in 42% yield (26.20g) as a clear colourless oil.
TLC (silica, 1:4 - ethyl acetate:hexane) R_f=0.21. Anal: Calcd. for $C_{20}H_{24}O_3$: C 76.92%, H 7.69%. Found : C 76.80%, H 7.81%. m/e 85, 102, 135, 213, 228 (M+). ^{1}H NMR ($CDCl_3$, fully assigned using COSY NMR experiment) δ: 7.14-7.07 (4H, m, part of aromatic AA'BB'), 6.94 (2H, d,

J=4.2Hz, part of aromatic AA'BB'), 6.70 (2H, d, J=4.2Hz, part of aromatic AA'BB'), 5.39 (1H, t, J=3.2Hz, OCHO), 5.09 (1H, br.s., OH), 3.94 (1H, t of d, Jt=10.3Hz, Jd=1.4Hz, 0CHH'CH_2), 3.61 (1H, m, OCHH'CH_2), 2.00 (1H, m, aliphatic CH_2), 1.85 (2H, m, aliphatic CH_2), 1.70-1.57 (3H, m, aliphatic CH_2), 1.62 (6H, s, CCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 154.8 and 153.3 (aromatic C-OR), 144.1 and 143.1 (aromatic C-$C(CH_3)_2$), 127.9 and 127.6 (aromatic C-H), 115.8 and 114.7 (aromatic C-H), 96.5 (OCHO), 62.2 (OCH_2C), 41.7 ($Ar_2C(CH_3)_2$), 31.0 (CCH_3), 30.4 (O_2CHCH_2), 25.2 (OCH_2CH_2), 18.9 (aliphatic CH_2). IR: 3369 (O-H), 2963 (C-H), 2871 (C-H), 1610 (aromatic C-C), 1510, 1236 cm^{-1}.

O,O'-Bis(α,α-Dimethyl-α-(O-Tetrahydropyran-p-phenol)-Tolyl)-1,4-Dioxamethylene Benzene, (4, R=THP).

α,α'-Dibromo-p-xylene (0.81g, 3.1 x 10^{-3}moles) and compound 3 (R=THP) (1.90g, 6.1mmoles) were dissolved in hot ethanol (50ml) and potassium carbonate (1.87g, 14mmoles) was added. The stirred suspension was heated at reflux for 18hrs. and then filtered while hot. The white solid residue was partitioned between $CHCl_3$ and water (40ml each). The chloroform layer was washed with 0.01M HCl (15ml) and then water (2 x 40ml), dried (Na_2SO_4) and filtered. Solvent was removed at reduced pressure and the residue dried under vacuum to give a white solid, 1.76g (78%).

M.pt.=118-122°C. m/e 85, 135, 213, 331, 558 (M-2THP). ^1H NMR ($CDCl_3$, 250MHz) δ: 7.43 (4H, s, phenyl ring), 7.16-7.09 (8H, m, part of biphenyl AA'BB' system), 6.97-6.84 (8H, m, part of biphenyl AA'BB' system), 5.37 (2H, t, J=7.5Hz, OCHO), 5.02 (4H, s, $ArCH_2O$), 3.92 (2H, t of d, J_t=10Hz, J_d=3.2Hz, OCHH'), 3.59 (2H, m, OCHH'), 1.98 (2H, m,

aliphatic CH_2), 1.83 (4H, m, aliphatic CH_2), 1.63 (18H, s,m overlapping, CCH_3 and aliphatic CH_2's).

O,O'-Bis(α,α-Dimethyl-α-(O-Tetrahydropyranyl-p-phenol)-Tolyl)-2,6-Dioxymethylene Pyridine (5, R=THP).

Monoprotected bis-phenol-A, 3 (R=THP) (21.16g, 0.07moles) and 2,6-bis (bromomethyl)pyridine (9.01g, 0.034moles) were dissolved in ethanol (120ml). Potassium carbonate was added and the suspension stirred at reflux for 20hrs. On completion of the reaction (absence of starting materials on analytical TLC), the suspension was filtered while hot. Standing overnight at room temperature resulted in the formation of a white precipitate in the filtrate which was collected by filtration, washed on the filter with cold ethanol (3 x 20ml) and dried under vacuum to give a fine white powder (18.37g, 74%). M.pt.=122°C. TLC (silica, 1% methanol in dicloromethane) R_f=0.68; (silica, 4;1-hexane:ethyl acetate) R_f=0.30. Anal: calcd. for $C_{47}H_{53}O_6N$: C 77.58%, H 7.29%, N 1.93%. Found: C 77.46%, H 7.37%, N 1.91%. IR: 2944, 2864, 1518, 1254, 1190 cm^{-1}. ^1H NMR ($CDCl_3$, 400MHz) δ: 7.73 (1H, t, J=16.0Hz, B of A_2B in pyridine ring), 7.46 (2H, d, J=7.7Hz, A of A_2B in pyridine ring), 7.17-7.10 (8H, m, aromatic CH), 6.97-6.86 (8H, m, aromatic CH), 5.38 (2H, t, J=6.3Hz, OCHO), 5.17 (4H, s, $ArCH_2O$), 3.92 (2H, t of d, J_t=20.6Hz, J_d=3.2Hz, $OCHH'CH_2$), 3.63-3.56 (2H, m, $OCHH'CH_2$), 2.04-1.94 (2H, m, aliphatic CH_2), 1.84 (4H, m, aliphatic CH_2), 1.70-1.55 (18H, s,m overlapping, aliphatic CH_2's and CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ: 156.9 (aromatic, pyridine ring, ortho to N), 156.2 and 154.9 (aromatic C-O), 143.9 and 143.7 (aromatic C-

$C(CH_3)_2$), 137.6 (aromatic, pyridine ring, para to N), 127.8 and 127.6 (aromatic C-H, biphenyl), 120.0 (aromatic, pyridine ring,meta to N), 115.7 and 114.0 (aromatic C-H, biphenyl), 96.4 (OCHO), 70.5 ($ArCH_2O$), 62.1 (OCH_2), 41.7 ($Ar_2C(CH_3)_2$), 31.0 (CH_3), 30.4 (O_2CHCH_2), 25.2 (OCH_2CH_2), 18.9 aliphatic CH_2) ppm. IR 2944(C-H), 2864(C-H), 1518, 1254, 1190, 981cm^{-1}.

O,O'-Bis(α,α-Dimethyl-α-p-phenol-Tolyl)-2,6-Dioxymethylene Pyridine, (5, R=OH).

Compound **5** (R=THP) (18.25g, 0.025moles) was weighed into a round bottomed flask and 20ml of methanol was added. Chloroform (15ml) was added to the the stirred suspension in small portions until **5** dissolved. Concentrated HCl was added (30ml), causing phase separation to occur. Small amounts of methanol and $CHCl_3$ were added until the reaction was homogeneous (total volumes: methanol-60ml; $CHCl_3$-100ml; HCl-30ml), and the clear solution was heated overnight at reflux. After cooling to room temperature methanol and $CHCl_3$ were removed on a rotary evaporator, resulting in the formation of a white precipitate. This was basified with saturated KOH(aq.), causing further precipitation. The precipitate was extracted into ether (500ml), and the organic phase washed with O.1M KOH (2 x 50ml) followed by water (5 x 50ml), dried (Na_2SO_4), filtered and solvent evaporated at reduced pressure. The residue was dried under vacuum to leave a white solid, mass 13.47g (96%).

M.pt. 64-70°C. TLC: (silica, 4:1 - hexane:ethyl acetate) R_f=0.05. m/e: 334, 560(M+1). Anal: Calcd. for $C_{37}H_{37}O_4N$: C 79.43%, H 6.62%, N 2.50%. Found: C 79.61%, H 6.74%, N 2.41%. ^1H NMR ($(CD_3)_2CO$) δ: 7.85 (IH, t,

J=16Hz, B of A_2B in pyridine ring), 7.49 (2H, d, J=7.7Hz, A of A_2B in pyridine ring), 7.17 (4H, d, J=9.0Hz, aromatic C-H, part of AA'BB' system), 7.06 (4H, d, J=8.6Hz, aromatic C-H, part of AA'BB' system), 6.93 (4H, d, J=9.0Hz, aromatic C-H, part of AA'BB' system), 6.73 (4H, d, J=8.8Hz, aromatic C-H, part of AA'BB' system), 5.17 (4H, s, $ArCH_2O$), 3.05 (2H, br.s., OH), 1.60 (12H, s, CH_3). ^{13}C NMR ($(CD_3)_2CO$) δ: 158.4 (aromatic, pyridine ring, ortho to N), 157.7 and 156.3 (aromatic C-OR), 145.0 (aromatic, biphenyl, para to OH), 143.0 (aromatic, biphenyl, para to OR), 138.9 (aromatic, pyridine ring, para to N), 128.9 and 128.8 (aromatic C-H, biphenyl), 121.5 (aromatic, pyridine ring, meta to N), 115.8 and 115.3 (aromatic C-H, biphenyl), 71.7 ($ArCH_2O$), 42.5 ($ArC(CH_3)_2$), 31.8 (CH_3) ppm. IR: 3341(O-H), 2983(C-H), 1518, 1244, 1190, 842 cm^{-1}.

O,O'-Bis(α,α-Dimethyl-α-(O-Tetrahydropyranyl-p-Phenol)-Tolyl)-1,4-Dioxamethylene Benzene (4, R=OH).

This was prepared in 98% yield from the bis(tetrahydropyran) ether by acid hydrolysis using a method identical to that used for the synthesis of **5**, R=OH, substituting **4** (R=THP) for **5** (R=THP) in the method.

IR: 3340 (O-H), 2983, 2862, 1515, 1240, 1190 cm^{-1}. ^1H NMR (d6-acetone, 60MHz), δ: 7.9 (2H, s, OH), 7.3-6.4 (20H, m, ArH), 5.2 (4H, s, $ArCH_2O$), 1.5 (12H, s, CCH_3) ppm.

4-Aza-17,17,40,40-(Tetramethyl)-1,10,24,33-Tetraoxa[2,2,1,2,2,1] MetaParaParaParaParaParaCyclophane, (1).

Diol 4 (R=OH) (0.34g, 0.60mmoles) and 2,6-bis(bromomethyl)pyridine (0.16g, 0.60mmoles) were dissolved in hot ethanol (70ml). Potassium carbonate (0.34g, 2.5mmoles) was added and the stirred solution was heated at reflux for 48hrs. The hot solution was filtered and the solid residue washed with hot ethanol (3 x 15ml), dried and taken up in H_2O (35ml). This was extracted into ether (4 x 50ml) and then $CHCl_3$ (4 x 50ml). The organic phases were combined, dried (Na_2SO_4) and filtered. Solvent was removed at reduced pressure and the residue dried under vacuum to leave a white, crystalline solid, mass 0.19g (47%).

M.pt. 100-103°C. Anal. Calcd. for $C_{45}H_{44}O_4N$: C 81.69%, H 6.51%, N 2.12%. Found: C 81.59%, H 6.53%, N 2.01%. m/e 122, 135, 152, 663(M+2), 664. m/e (Cl) 652, 662 (M+1), 663, 664, 665, 696. ^1H NMR ($CDCl_3$, 300MHz) δ: 7.65 (1H, t, B of A_2B, pyridine ring), 7.38 and 7.35 (6H, d, s, overlapping, A of A_2B in pyridine ring and ArH, phenyl ring), 7.18-7.05 (8H, m, ArH, part of AA'BB' system), 6.82-6.77 (8H, m, ArH, part of AA'BB' system), 5.09 (4H, s, $ArCH_2O$), 4.95 (4H, s, $ArCH'_2O$), 1.56 (12H, s, CCH_3) ppm.

4-Aza-27-Ethoxy/Methoxycarbonyl-17,17,40,40-Tetramethyl-1,10,24,33-Tetraoxa[2,2,1,2,2,1]MetaParaParaMetaParaParaCyclophane, (6).

Potassium carbonate (0.39g, 2.83mmoles), methyl-2,6-bis(bromomethyl)benzoate (0.11g, 0.34mmoles) and compound 4 (R=OH) (0.19g, 0.34 mmoles) were dissolved in ethanol (150ml) and stirred at reflux for 48 hours. The hot solution was filtered and

ethanol removed from the filtrate at reduced pressure to leave a yellow solid. This was dried under vacuum and purified by column chromatography (flash silica; 4:1 - hexane: ethyl acetate as eluant) to give a white solid, mass 78mg (31%).

M.pt.=204-211°C (for methyl ester). TLC (silica, 4:1 - hexane:ethyl acetate): R_f=0.24. m/e (glycerol as internal reference)108, 135, 334, 734(M+1). Anal: calcd. for $C_{48}H_{47}O_6N$: C 78.58%, H 6.41%, N 1.91%. Found: C 78.36%, H 6.37%, N 1.81%. ^1H NMR ($CDCl_3$, 400MHz) δ: 7.66 (1H, t, J=15.2Hz, B of A_2B in pyridine ring), 7.41-7.38 (3H, m, (t, d overlapping), A_2B aromatic protons of benzoate), 7.33 (2H, d, J=7.6Hz, A of A_2B in pyridine ring), 7.08 (4H, m, part of AA'BB' system, diphenylmethane), 7.01 (4H, m, part of AA'BB' system, diphenylmethane), 6.81-6.77 (8H, m, overlapping AA'BB' systems of diphenylmethane), 5.18 and 5.19 (8H, s,s overlapping, $ArCH_2O$ and $ArCH'_2O$), 3.78 (2H, q, J=21.6Hz, OCH_2CH_3), 1.62 (12H, s, CCH_3), 0.92 (3H, t, J=14.0Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 100MHz) δ: 168.1 ($ArCO_2$), 157.3 (aromatic C-CH_2O, pyridine ring), 156.4 and 156.3 (aromatic C-O), 143.8 and 143.2 (aromatic C-$C(CH_3)_2$), 137.6 (aromatic C-H, pyridine ring, para to N), 136.1 (aromatic C-CO_2), 131.8 (aromatic C-CH_2O, benzoate ring), 129.9 (aromatic C-H, benzoate ring, para to ester group), 128.1 (aromatic C-H, benzoate ring, meta to ester group), 127.7 and 127.6 (aromatic C-H, diphenylmethane rings, ortho to O), 119.8 (aromatic C-H, pyridine ring, meta to N), 114.3 and 113.9 (aromatic C-H, diphenylmethane rings, meta to O), 70.9 and 68.1 ($ArCH_2O$ and $ArCH'_2O$), 61.3 (OCH_2CH_3), 41.6 ($ArC(CH_3)_2$), 30.8 (CCH_3), 13.7 (CH_2CH_3).

IR: 2970 (C-H), 2868 (C-H), 1719 (C=O), 1601 (aromatic C-C), 1588 (aromatic C-C), 1505 cm^{-1}.

4-Aza-27-Sodiumcarboxylate-17,17,40,40-Tetramethyl-1,10,24,33-Tetraoxa[2,2,1,2,2,1]MetaParaParaMetaParaParaCyclophane, (7a).

The ester **6** (170mg, 0.23mmoles) was refluxed in 0.1M NaOH in 90% ethanol (50ml) for 2 weeks. The solution was cooled to room temperature and evaporated to dryness at reduced pressure to leave a white solid. This was taken up in 15ml dichloromethane and quickly washed with water (3 x 10ml), dried (Na_2SO_4) and filtered. Solvent was immediately removed at reduced pressure to leave a white solid, which was dried under vacuum to give the sodium salt of the hydrolysed ester, **7a**, in quantitative yield.
m/e: 135, 166, 193, 334, 560, 561, 706(M+1), 707. ^1H NMR (d^8-THF/5% D_2O) δ: 7.60 (1H, t, J=15.6Hz, B of A_2B in pyridine ring), 7.26 (2H, d, J=10.0Hz, aromatic C-H, A of A_2B system), 7.12 (2H, d, J=8Hz, aromatic C-H, A of A_2B system), 6.93-6.66 (17H, m, overlapping AA'BB's of diphenyls and B of benzoate A_2B system), 5.20 (3H, br.s., $ArCH_2O$), 5.09 and 5.02 (5H, overlapping s,s, $ArCH_2O$ and $ArCH'_2O$), 1.46 (12H, s, CCH_3) ppm. IR: 2962 (C-H), 1608(aromatic C-C), 1581 aromatic C-C), 1509 cm^{-1}.

4-Amonium-27-Carboxylate-17,17,40,40-Tetramethyl-1,10,24,33-Tetraoxa[2,2,1,2,2,1]MetaParaParaMetaParaParaCyclophane, (2).

Sodium salt **7a** (44mg, 6.1 x 10^{-5}moles) was stirred in 50:50-H_2O:THF (6ml) to give a turbid solution. 6M HCl (1.5ml) was added, causing precipitation. This suspension was stirred overnight, the

precipitate collected by filtration and washed on the filter with water (4 x 5ml). The white solid residue was allowed to dry on the filter, then washed through with a minimum of THF. Solvent was removed at reduced pressure and the residue dried under vacuum to give the hydrochloride salt, **7b**.

^{1}H NMR (d^8-THF/5%D_2O) δ: 7.61 (1H, t, B of A_2B in pyridine ring), 7.32- 7.21 (4H, m, overlapping A's of A_2B in pyridine ring and benzoate), 7.00- 6.61 (17H, m, overlapping AA'BB's of diphenyls and t of benzoate), 5.41 (1.5H, s, $ArCH_2O$), 5.10 (2.5H, s, $ArCH_2O$), 5.01 (4H, s, $ArCH'_2O$), 1.26 (12H, s, CCH_3). IR: 2952 (C-H), 2857 (C-H), 1719 (C=O), 1605 (aromatic C-C), 1579 (aromatic C-C), 1507 cm^{-1}.

The hydrochloride salt was dissolved in propylene oxide (15ml) and stirred under nitrogen with precipitation occurring after about 15 minutes. The suspension was allowed to stir under nitrogen for a further 24hrs., after which solvent was removed at reduced pressure and the white solid residue dried under vacuum to give the title compound in a yield of 22.4mg (52% from Na^+ salt **7a**).

^{1}H NMR (d^8-THF) δ: 7.58 (1H, t, J=16Hz, B of A_2B in pyridine ring), 7.34- 7.20 (5H, m, A of A_2B in pyridine ring and A_2B of benzoate), 6.99-6.95 (8H, m, part of aromatic AA'BB' systems), 6.73-6.70 (8H, m, part of aromatic AA'BB' systems), 5.12 and 5.05 (8H, s,s overlapping, $ArCH_2O$'s), 1.52 (12H, s, CCH_3). IR: 3435 (N-H), 2976 (C-H), 1719 (small, contamination by starting material), 1650 ($C=O_2^-$), 1503 cm^{-1}. m/e 87, 135, 334, 560, 706 (M+1), 734 (small amount of ethyl ester contaminant).

4.3. SYNTHESES FOR CHAPTER 3.

Condensation Polymer of 2-HBI With Formaldehyde.

2-HBI (4.70g, 3.50 x 10^{-2}moles), 37% aqueous formaldehyde (8.7ml, 0.11moles of H_2CO), 37% aqueous hydrochloric acid (17ml) and water (24ml) were placed in a round bottomed flask and heated at reflux. After a few minutes, a green precipitate began to form. After 1hr., at reflux, the suspension was poured into 120ml of cold water and the mixture allowed to cool to room temperature. The solid was collected by filtration and washed with ethanol (4 x 20ml) and ether (3 x 20ml) and dried under vacuum to give a fine, light green powder (5.60g, 98%), which was observed to turn a pale brown colour on standing in bright sunlight, or on heating.

M.pt.>300°C.

N,N'-Bis(Hydroxymethyl)-2-Benzimidazolidone[3].

2-Hydroxybenzimidazole ("2-HBI") (1.00g, 7.46 x 10^{-3}moles) was stirred in 25ml hot water. 38% aqueous formaldehyde (2.1ml, 2.66 x 10^{-2}moles of H_2CO) was added, on which 2-HBI immediately dissolved. The solution was heated at reflux for 1hr. and the solution poured into 50ml of cold water. This was cooled in an ice bath and, on scratching with a glass rod, a white precipitate formed. This was collected by filtration, recrystallised from water and dried under vacuum (0.53g, 37%).

M.pt=159-161°C (lit.=164-165°C[3]). m/e (EI) 52, 79, 106, 134(M+); (DCI) 135(M+1), 152(M+18). Anal: Calcd. for $C_9H_{10}N_2O_3$: C 55.67%, H 5.15%, N 14.43%. Found: C 55.82%, H 5.16%, N 14.29%. ^1H NMR (60MHz,

D_2O) δ: 7.5 (4H, s, ArH), 5.6 (4H, s, $ArCH_2O$) ppm. IR: c.3800-2500 (v.br., Hydrogen bonded N-H and O-H; C-H), 1735 (C=O), 1620, 1540, 1420, 1090 cm^{-1}.

Condensation of 2-Imidazolidone With Formaldehyde - N,N'-Dimethy-2-Imidazolidone.

In an attempt to synthesise the condensation polymer, 2-imidazolidone (6.05g, 0.07moles) was dissolved in a mixture of 37% aqueous formaldehyde (100ml) and 37% aqueous hydrochloric acid (50ml). The solution was heated at reflux for 4 or 16hrs., during which time it acquired a pale brown colouration. After cooling to room temperature, solvent was removed at reduced pressure to leave a brown residue which was dried under vacuum and then taken up in chloroform (100ml). This was filtered to remove any solid impurity (paraformaldehyde - produced especially at longer reaction times) and solvent removed from the filtrate. The residue was dried under vacuum to leave a brown oil (6.22g, 78%).
^1H NMR ($CDCl_3$) δ: 3.29 (4H, s, NCH_2), 2.78 (6H, s, NCH_3). ^{13}C NMR ($CDCl_3$) δ: 162.5, 45.6, 31.9. m/e: 115(M+1). IR: 2950 and 2860 (aromatic and aliphatic C-H),1680 (C=O). Anal. Calcd. for $C_5H_{10}N_2O$: C 52.63%, H 8.77%, N 24.56%. Found: C 52.38%, H 8.90%, N 24.14%.

2,11 - Diaza - 5,8 - DioxaBicyclo[8,4]Hexadec - 12,14,16 - Triene (Benzo - $12N_2O_2$), (19).

In an attempt to prepare the "2+2" adduct, dibenzo-$24N_4O_4$, **12**, 1,8-bis(p-toluenesulphonato)-3,6-dioxaoctane (7.50g, 1.6 x 10^{-2}moles) was dissolved in dry DMF (130ml) under nitrogen. Caesium carbonate

was added and the suspension stirred at 60°C. To this, a solution of 2-HBI (2.19g, 1.6 x 10^{-2}moles) in dry DMF (150ml) was added dropwise over a period of 12hrs. The reaction was stirred at 60°Cfor a further 4hrs. and then allowed to cool to room temperature. DMF was evaporated at reduced pressure and the residue dried under high vacuum. It was then taken up in hot methanol (100ml) and filtered. The solid residue was washed on the filter with hot methanol (3 x 20ml), dried under vacuum and then taken up in chloroform (150ml). The chloroform was filtered to remove inorganic salts and solvent evaporated from the filtrate at reduced pressure to leave a white solid, which was dried under vacuum to give 2.61g (66%) of the title compound. No evidence was found which would have suggested that the desired 2+2 adduct had been formed.

M.pt.=128-131°C. m/e: 135, 161, 205, 249(M+1). ^{1}H NMR ($CDCl_3$, 250MHz) δ: 7.05 (4H, ArH), 3.99 (4H, t, J=10.0Hz, NCH_2CH_2O), 3.78 (4H, t, J=12.5Hz, NCH_2CH_2O), 3.51 (4H, s, OCH_2CH_2O) ppm. ^{13}C NMR (63MHz, $CDCl_3$) δ: 154.0 (C=O), 129.5 (aromatic C), 120.8 (aromatic C-H), 108.2 (aromatic C-H), 72.4 and 69.0 (CH_2O), 41.0 (CH_2N) ppm.

1,10-Dioxa-4,7,13,16-Tetraazacyclooctadecane, (21)[4].

The title compound was prepared from N,N'-bis(p-toluenesulphonyl)-1,2-diaminoethane and 1,8-bis(p-toluene sulphonato)-3,6-dioxaoctane as in the literature[4].

19,20-Dithio-4,13-Dioxa-1,7,10,16-Tetraazatricyclo[18,2,1,1,^{7,10}]Eicosane, (23).

$18N_4O_2$, **21**, (1.45g, 5.67 x 10^{-3}moles) was dissolved in 50:50-ethanol:water (4ml). CS_2 (0.67ml, 1.12 x 10^{-2}moles) was added and the solution heated to 45°C; a white precipitate began to form within a few minutes. After 2hrs., the reaction had become quite viscous and a further 6ml of solvent was added. The mixture was held at 45°C for 45hrs., with a further 1.2ml of CS_2 added in 4 aliquots during the reaction.

The temperature was then increased to 100°C for 3hrs., after which 2ml of conc. HCl was added and the reaction held at reflux for a further 14hrs.

On cooling to room temperature, the volume was reduced to about 3ml and cooled in an ice bath. The white precipitate was collected by filtration and washed with cold water (3 x 3ml), then ethanol (3 x 3ml) and dried under vacuum (0.57g, 29%).
M.pt.=246-247°C(dec.). TLC (silica, 5% methanol in DCM): Rf=0.21. m/e: 345(M+1). Anal. Calcd. for $C_{14}H_{25}N_4O_2S_2Cl$: C 44.27%, H 6.32%, N 14.76%. Found: C 44.52%, H 6.57%, N 14.75%. IR: 2920 (C-H), 2892 (C-H), 1493, 1332, 1120 cm^{-1}. ^{1}H NMR, assigned using HETCOR and COSY experiments, ($CDCl_3$, 400MHz) δ: 4.67-4.61 (4H, m, NCH_2CH_2O), 3.97-3.90 (4H, m, NCH_2CH_2N), 3.82-3.78 (4H, m, NCH_2CH_2O), 3.68-3.61 (4H, m, $NCH'_2CH'_2O$), 3.38-3.34 (4H, m, NCH'_2CH_2O), 2.98-2.93 (4H, m, $NCH'_2CH'_2N$) ppm. ^{13}C NMR, assigned using HETCOR experiment, ($CDCl_3$, 100MHz, relaxation delay=20sec.) δ: 182.2 (C=S), 67.8 (NCH_2CH_2O), 47.4 (NCH_2CH_2O), 47.0 (NCH_2CH_2N) ppm.

An excess of ethanol was added to the filtrate, causing considerable precipitation. This precipitate was also collected and identified as $18N_4O_2.4HCl$ (0.75g, accounting for 25% of starting material), which was basified and extracted into dichloromethane and could be used for the preparation of more of the bisthiourea.

19,20-Dioxo-4,13-Dioxa-1,7,10,16-Tetraazatricyclo[18,2,1,1,^{7,10}]Eicosane, (13).

Bisthiourea **23** (0.313g, 9.1 x 10^{-4}moles) was dissolved in 1,2-dichloroethane (15ml) and $Hg(OAc)_2$ (0.68g, 2.1 x 10^{-3}moles) added. The reaction was stirred at reflux for 23hrs., cooled to room temperature and filtered through celite. Solvent was removed from the filtrate to give a yellow solid with a dry mass of 0.81g.

Water (10ml) was added, and the pH adjusted to 10.5 with potassium carbonate. This mixture was heated at reflux for $2^1/_2$hrs., with a dark precipitate beginning to form after 45mins. The cooled reaction was filtered through celite and solvent evaporated from the filtrate at reduced pressure to give a white solid. This was either:

(i) taken up in methanol and filtered through a plug of silica with methanol as eluant to give the partially hydrolysed, mixed urea/thiourea, **25**.

M.pt. >145°C(dec.), 162-165°C(melts). m/e 172, 190, 329 (M+1). TLC (silica, 5% methanol in dichloromethane): R_f=0.25. IR: 2930 (C-H), 2860 (C-H), 1683 (C=O), 1495, 1456, 1265, 1125 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 4.50 (2H, d of q, J_d=14.0Hz, J_q=6Hz), 4.04 (2H, m), 3.92-3.77 (4H, m), 3.69-3.38 (10H, m), 3.16 (2H, m), 3.10-2.88 (6H, m) ppm. ^{13}C NMR ($CDCl_3$, Relaxation delay=10sec.) δ: 180.9 (C=S), 165.4 (C=O), 69.3 (OCH_2), 66.8

(OCH_2'), 48.1 (NCH_2CH_2O), 47.1 ($NCH_2'CH_2'O$), 44.1 (NCH_2CH_2N), 42.6 ($NCH_2'CH_2'N$) ppm.

or

(ii) redissolved in 1,2-dichloroethane (20ml). $Hg(OAc)_2$ (0.59g, 1.82 x 10^{-3} moles) was added and the reaction repeated as above. The solid product was taken up in methanol and filtered through a plug of silica with methanol as eluant to give the bisurea hydrolysis product (0.12g, 42%).

M.pt.=227-230°C(dec.). m/e 313(M+1). Anal. Calcd. for $C_{14}H_{24}N_4O_4$: C 55.85%, H 7.69%, N 17.95%. Found: C 38.30%, H 5.75%, N 9.83%, Na 3.15%, K 5.54%. IR: 3436, 2873 (C-H), 1683 (C=O), 1506, 1273cm^{-1}. TLC (silica, 5% methanol in dichloromethane, develop in iodine) Rf 0.07; (silica, 100% methanol, develop in iodine) Rf 0.40. ^1H NMR (D_2O, 400MHz) δ: 3.48 (12H, br.t, J=9.2Hz), 3.29 (12H, br.s). ^{13}C NMR (D_20, 100MHz) δ: 162.6, 66.4 (OCH_2), 42.8 (NCH_2), 42.4 (NCH_2) ppm.

1,4-Bis(toluenesulphonyl)-1,4-Diaza-7,10-Dioxacyclododecane.

In an attempt to make the 24 membered tetratosylamide $24N_4O_4.4Ts$, **25**, 1,8-bis(p-toluenesulphonato)-3,6-dioxaoctane (5.00g, 1.09 x 10^{-2}moles) was dissolved in dry DMF (100ml) under nitrogen and caesium carbonate (7.11g, 2.18 x 10^{-2}moles) added. To the stirred suspension a solution of N,N'-bis(p-toluenesulphonyl)-1,2-diaminoethane (4.37g, 1.13 x 10^{-2}moles) in dry DMF (100ml) was added dropwise over a period of 6hrs. The suspension was stirred for a further 2hrs. at room temperature and then 9hrs. at 55°C. After cooling to room temperature, the reaction was filtered and DMF evaporated from the filtrate at reduced pressure. The solid residue was

dried at high vacuum and then taken up in hot ethanol (150ml). Insoluble material was collected by filtration and dried under vacuum to give the 12 membered macrocyclic title product, mass 4.63g (88%). No evidence was obtained which would have indicated the formation of the desired 24 membered macrocyclic product.

M.pt.=212-215°C. m/e: 193, 327, 483(M+1). ^{1}H NMR ($CDCl_3$) δ: 7.75 (4H, d, J=8.0Hz, part of aromatic AA'BB'), 7.33 (4H, d, J=8.0Hz, part of aromatic AA'BB'), 3.66 (4H, t, J=8.0Hz, NCH_2CH_2O), 3.59 (4H, s, J=8.0Hz, OCH_2CH_2O), 3.36 (4H, s, NCH_2CH_2N), 3.29 (4H, t, J=8.0Hz, NCH_2CH_2O), 2.44 (6H, s, $ArCH_3$) ppm.

10,11-Bis(tosylaza)-3,6,15,18-Tetraoxa-Ditosyleicosane-1,20-Diol, (26).

N,N'-Bis(p-toluenesulphonyl)-1,2-diaminoethane (29.0g, 0.08moles) was dissolved in 2-[chloro(ethoxy{ethoxyethanol})] (50g). Potassium carbonate was added and the solution stirred at 100°C for 20hrs. The reaction was cooled to room temperature and dichloromethane (100ml) added to lower the viscosity. The suspension was filtered and solvent evaporated from the filtrate at reduced pressure to give a very viscous, dark oil. Excess 2-[chloro(ethoxy{ethoxyethanol})] was removed at 80-100°C under high vacuum. Considerable amounts of triethylene glycol were also obtained as distillate.

A ^{1}H NMR of the product indicated that it was still contaminated with residual amounts of 2-[chloro-(ethoxy{ethoxyethanol})] which could not be distilled off. The product was used without further purification.

m/e 133, 151, 162, 283, 633(M+1). ^{1}H NMR ($CDCl_3$) δ: 7.72 (4H, d, J=8.0Hz, part of aromatic AA'BB' system), 7.32 (4H, d, J=8.1Hz, part of aromatic AA'BB' system), 3.80-3.45 (29H, m, CH_2O of product and residual 2-[chloro-(ethoxy{ethoxyethanol})]) , 3.39 and 3.32 (8H, s,t overlapping, J_t=10.0Hz, NCH_2CH_2N and OCH_2CH_2N), 2.43 (6H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 143.9 (aromatic), 136.4 (aromatic), 130.2 (ArH), 127.7 (ArH), 73.0 (CH_2O), 71.7 (CH_2O), 71.0 (CH_2O), 70.9 (CH_2O), 70.8 (CH_2O), 70.4 (CH_2O), 62.0, 61.9, 49.8 (CH_2N),49.5 (CH_2N), 21.9 ($ArCH_3$) ppm.

10,11-Bis(tosylaza)-3,6,15,18-Tetraoxa-1,20-Bis(tosyloxy)eicosane, (27).

The diol (50.56g, 0.08moles for 100% yield from previous step) was dissolved in dry THF (150ml) and triethylamine (30ml) added. The solution was cooled to 0°C under nitrogen. To this, a solution of tosyl chloride (45.9g, 0.24moles) in THF (100ml) was added dropwise over a period of 4hrs. The reaction was allowed to stir at 0°C for a further 4hrs. before the reaction vessel was sealed and stored at 5°C for 7 days. The mixture was then partitioned between water and dichloromethane. The organic phase was dried over Na_2SO_4, filtered and solvent evaporated at reduced pressure. The residue was dried under vacuum to leave a dark brown, very viscous oil which was purified, with difficulty, by column chromatography (flash silica, solvent gradient of eluant from dichloromethane - 5% methanol in dichloromethane) to give the product as a clear, colourless oil (36.55g, 49% from N,N'-bis(p-toluenesulphonyl)-1,2-diaminoethane).
TLC (silica, 2% methanol in dichloromethane) Rf=0.26. ^{1}H NMR ($CDCl_3$) δ: 7.80-7.67 (8H, m, ArH, overlapping parts of AA'BB' systems

of OTs and NTs), 7.35-7.27 (8H, m, ArH, overlapping parts of AA'BB' systems of OTs and NTs), 4.12 (4H, t, J=8.0Hz, CH_2OTs), 3.67-3.55 (8H, m, NCH_2CH_2O and OCH_2CH_2OTs, overlapping), 3.52 (8H, s, OCH_2CH_2O), 3.35 (4H, s, NCH_2CH_2N), 3.30 (4H, t, J=8.0Hz, NCH_2CH_2O), 2.43 (12H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 144.9 (aromatic), 136.1 (aromatic), 133.9 (aromatic), 129.9 (ArH), 129.8 (ArH), 127.9 (ArH), 127.2 (ArH), 70.5 (OCH_2), 70.3 (OCH_2), 69.9 (OCH_2), 69.3 (OCH_2), 68.6 (OCH_2), 49.4 (NCH_2), 49.1 (NCH_2), 21.7 ($ArCH_3$), 21.5 ($ArCH_3$) ppm.

1,4,13,16-Tetra(p-Toluenesulphonyl)-7,10,19,22-Tetraoxa-1,4,13,16-Tetraazacyclotetraeicosane, (28).

Sodium (1.29g, 0.056 moles) was dissolved in dry methanol under nitrogen and the solution heated at reflux for 2hrs. N,N'-Bis(p-toluenesulphonyl)-1,2-Diaminoethane (10.31g, 0.028moles) was added and the solution heated at reflux for a further 2hrs. Methanol was evaporated at reduced pressure and the solid residue dried under vacuum. Dry DMF (200ml) was added and the suspension stirred under nitrogen at 80°C. To this, a solution of 10,11-bis(tosylaza)-3,6,15,18-tetraoxa-1,20-bis(tosyloxy)eicosane (26.56g, 0.02moles), in DMF (500ml) was added dropwise over a period of $3^1/_2$hrs. The mixture was stirred at 80°C for a further 24hrs. and then at allowed to cool to room temperature. Water (20ml) was carefully added and solvent removed at reduced pressure. The residue was partitioned between water and dichloromethane. The organic phase was dried (Na_2SO_4) and filtered. Solvent was evaporated from the filtrate at reduced pressure and the residue dried under vacuum to give an off-white solid. This was taken

up in hot ethanol (400ml) and the insoluble material collected by filtration and washed on the filter with hot ethanol. After grinding with a pestle and mortar, the residue was dried under vacuum to leave a white solid (17.85g, 66%).

M.pt.=178-180°C. Anal. Cald. for $C_{44}H_{60}N_{40}O_{12}S_4$: C 54.77%, H 6.22%, N 5.54%. Found: C 54.15%, H 6.28%, N 5.54%. TLC (silca, 2% methanol, in dichloromethane) Rf=0.22. ^1H NMR ($CDCl_3$) δ: 7.70 (8H, d, J=8.0Hz, ArH, part of AA'BB' system), 7.30 (8H, d, J=8.0Hz, ArH, part of AA'BB' system), 3.58-3.53 (16H, t,s overlapping, OCH_2CH_2N and OCH_2CH_2O), 3.35-3.26 (16H, s,t, overlapping, NCH_2CH_2N and NCH_2CH_2O), 2.41 (12H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 143.9 (aromatic C-S), 136.5 (aromatic C-Me), 130.2 (ArH), 127.7 (ArH), 70.9 (OCH_2), 70.6 (OCH_2), 50.1 (NCH_2), 49.6 (NCH_2) 22.0 ($ArCH_3$) ppm.

1-Chloro-3,6-Dioxa-8-Tosyloxyoctane, (30).

Tosyl chloride (33.40g, 0.18moles) and triethylamine (25ml) were dissolved in dry THF (200ml) under nitrogen. The solution was cooled to 0°C and a solution of 2-[chloro(ethoxy-{ethoxyethanol})] (20ml, 0.14moles) in dry THF (100ml) was added dropwise over a period of 2hrs. The reaction vessel was sealed and stored at 5°C for 6 days. The mixture was then filtered and solvent removed from the filtrate at reduced pressure. The residue was purified by column chromatography (flash silica, 2% methanol in dichloromethane as eluant) to give the product as a clear, colourless oil (29.03g, 64%).

TLC (silica, 1% methanol in dichloromethane) Rf=0.51. ^1H NMR ($CDCl_3$) δ: 7.80 (2H, d, J=8.0Hz, ArH, part of AA'BB' system), 7.35 (2H, d, J=8.0Hz, ArH, part of AA'BB' system), 4.17 (2H, t, J=12Hz, CH_2OTs),

3.78-3.69 (4H, m, CH_2O), 3.63-3.58 (4H, m, CH_2O and CH_2Cl), 2.45 (3H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 145.3 (aromatic C-S), 133.4 (aromatic C-Me), 130.3 (ArH), 128.4 (ArH), 71.8 (CH_2O), 71.2 (CH_2O), 71.0 (CH_2O), 69.7 (CH_2O) , 69.2 (CH_2O), 43.3 (CH_2Cl), 22.1 (ArCH) ppm.

10,11-Bis(tosylaza)-3,6,15,18-Tetraoxa-1,20-Dichloroeicosane, (29).

1-Chloro-3,6-Dioxa-8-Tosyloxyoctane (6.38g, 0.02moles) and N,N'-bis(p-toluenesulphonyl)-1,2-diaminoethane (3.64g, 0.01moles) were dissolved in dry DMF (80ml). Caesium carbonate (6.67g, 0.02moles) was added and the mixture stirred at room temperature under nitrogen for 20hrs. and then at 40°C for 2hrs. The mixture was filtered and solvent evaporated from the filtrate at reduced pressure. The solid residue was partitioned between water and dichloromethane and the organic layer dried (Na_2SO_4) and filtered.

Solvent was evaporated from the filtrate at reduced pressure and the solid residue recrystallised from methanol to give a white powder which was dried under vacuum. (6.02g, 47%).

M.pt=76-78°C. TLC (silica, 2% methanol) Rf=0.14. m/e 669(M+1, Cl=35/35), 671(M+1, Cl=35/37), 673(M+1, Cl=37/37), 686(M+18, Cl=35/35), 687(M+18, Cl=35/37), 688(M+18, Cl=37/37).^1H NMR ($CDCl_3$) δ: 7.71 (4H, d, J=8.1Hz, ArH, part of AA'BB' system), 7.32 (4H, d, J=8.1Hz, ArH, part of AA'BB' system), 3.73-3.50 (20H, (OCH_2) and CH_2Cl), 3.39 (4H, s, NCH_2CH_2N), 3.33 (4H, t, J=11.0Hz, NCH_2CH_2O), 2.43 (6H, s, $ArCH_3$) ppm. ^{13}C NMR ($CDCl_3$) δ: 143.9 (aromatic C-S), 136.6 (aromatic C-Me), 130.2 (ArH), 127.7 (ArH), 71.6 (OCH_2), 70.8 (OCH_2), 70.7 (OCH_2), 70.4 (OCH_2), 49.9 (NCH_2), 49.5 (NCH_2), 43.3 (CH_2Cl), 22.0 ($ArCH_3$) ppm.

1,4,13,16-Tetra(p-Toluenesulphonyl)-7,10,19,22-Tetraoxa-1,4,13,16-Tetraazacyclotetraeicosane, (28) - Method 2.

Sodium (0.40g, 0.018moles) was dissolved in dry methanol (50ml) and stirred at reflux under nitrogen for $1^1/_2$hrs. After cooling to room temperature, N,N'-bis(p-toluenesulphonyl)-1,2-diaminoethane (2.72g, 7.40×10^{-3}moles) was added and the solution heated at reflux for a further $2^1/_2$hrs. Methanol was then evaporated at reduced pressure and the solid residue dried under vacuum. Dry DMF (200ml) was added and the suspension stirred under nitrogen at 100°C. To this, a solution of 10,11-diaza-3,6,15,18-tetraoxa-1,20-dichloroeicosane (4.95g, 7.40×10^{-3}moles) in dry DMF (150ml) was added dropwise over a period of 7hrs. and the mixture then stirred at 100°C for a further 23hrs.

DMF was evaporated from the cooled solution at reduced pressure, and the solid residue partitioned between water and dichloromethane. The organic phase was dried over potassium carbonate, filtered and solvent removed from the filtrate under vacuum to give a yellow oil. This was recrystallised from hot ethanol to give a white solid (dry mass 3.67g, 52%).
Analysis as above.

1,4,13,16-Tetraoxa-7,10,19,22-Tetraaza-Tetraazacyclotetraeicosane, (22).

The tetratosylamide (0.49g, 5.1×10^{-4}moles) was stirred in dry THF (20ml) and ethanol (2ml). The suspension was cooled to -78°C in an acetone/dry ice bath and ammonia (about 50ml) was condensed

into the reaction vessel. Lithium (about 1g, 0.15moles) was added in small portions over a period of 25 minutes, causing an intense blue colour to develop. After 2hrs., the reaction was allowed to start warming to room temperature and excess ammonia allowed to bubble off. Ethanol (2ml) was carefully added, followed by cold water (70ml), causing most of the white solid to dissolve.

Solvent was evaporated at reduced pressure and 6M hydrochloric acid (50ml) added to the residue. The turbid solution formed was washed with diethyl ether (3 x 60ml) to give a clear aqueous phase, from which sovlent was again evaporated at reduced pressure. The white, solid residue was dried under vacuum and then redissolved in a minimum volume of water (about 2.5ml). This was basified with LiOH and extracted exhaustively into dichloromethane (several washings of about 50ml). The dichloromethane washings were combined, dried over lithium carbonate, filtered and solvent evaporated at reduced pressure. The residue was dried under vacuum to leave a clear, colourless oil, which crystallised on standing (0.13g, 74%).

M.pt.=60-63°C. Anal. Calcd. for: $C_{16}H_{36}N_4O_4$: C 55.17%, H 10.34%, N 16.09%. Found C 55.65%, H 10.45%, N 16.58%. m/e 349(M+1), 350. ^1H NMR ($CDCl_3$) δ: 3.63 (16H, s,t overlapping, OCH_2CH_2O and OCH_2CH_2N), 2.79 (16H, s,t overlapping, NCH_2CH_2N and NCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ: 70.7 (CH_2O), 70.6 (CH_2O), 49.5 (CH_2N), 49.4 (CH_2N) ppm.

4.5. ^{1}H NMR NMR TITRATION EXPERIMENTS.

A known amount of the relevant macrocycle was dissolved in a deuterated solvent (usually 2% D_2O in D^8-THF) and a ^{1}H NMR acquired (spectrum 0).

A standard solution of the test substrate (acetyl choline chloride) in water was prepared. Measured volumes of this solution were placed in sample bottles, such that the sample bottles contained the number of equivalents of AcCh (with respect to the macrocycle) indicated in the Table below. The aqueous solvent was evaporated at reduced pressure from each sample bottle and the residues dried thoroughly under vacuum.

Sample Bottle/ Spectrum Number	Equivalents of AcCh Added	Total Amount AcCh Present
(0)	0	0
(1)	0.1	0.1
(2)	0.1	0.2
(3)	0.3	0.5
(4)	0.5	1.0
(5)	0.3	1.3
(6)	0.7	2.0
(7)	3.0	5.0
(8)	5.0	10.0

The contents of the NMR tube (containing the macrocycle) were transferred to sample bottle (1) and the mixture shaken until a homogeneous solution was formed (where possible). The solution was transferred back to the NMR tube and a second spectrum acquired (spectrum 1).

This procedure was repeated for sample bottles (2)-(8). The *total* amount of AcCh present in the NMR tube, also indicated in the Table, therefore varied from 0.1 equivalents to 10 equivalents.

4.6. MEMBRANE TRANSPORT EXPERIMENTS.

4.6.1. Membrane Preparation.

The membrane composition was (by weight) ; 1.2% macrocycle, 65.6% oNPOE, 32.8%PVC, 0.4% potassium tetrakis(p-chlorophenyl) borate. Typically, between 10 and 20mg of the macrocycle were used. This mixture was dissolved in about 10ml of THF and a membrane cast by allowing solvent to evaporate in dust-free conditions, according to procedures which have been previously published[5].

4.6.2. 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 ion sensitive electrode was incorporated into an electrochemical cell described as:

Ag, AgCl | 0.001M Analyte (internal filling solution) | PVC membrane | 0.1M Analyte || 0.1M Lithium Acetate (salt bridge) | KCl (satd.) | Hg_2Cl_2(s);Hg.

A constant dilution technique was used for calibration and selectivity measurements. Selectivity measurements were performed in distilled water, or in 0.1M aqueous solutions of the interferent specified.

All emf measurements were made at 25°C (±0.1°C).

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APPENDICES.

APPENDIX 1. MEMBRANE TRANSPORT EXPERIMENTS USING ION SELECTIVE ELECTRODES.

The use of ion selective electrodes, or ISE's, to measure cation binding constants and selectivity of macrocycles is well established[1]. The technique is useful as it provides a reliable method of screening a fairly large number of substrates using a relatively small amount of material (≥10mg).

The macrocycle is dissolved, with a polymer (usually PVC) and a plasticiser in a suitable solvent, such as THF or cyclohexanone. A large anion, such as tetraphenylborane (potassium salt) is added to lower the resistance and reduce anion interference. The solution is then allowed to evaporate slowly to leave a thin flexible membrane, from which small discs are cut and incorporated into a silver/silver chloride electrode, with an internal filling of a 10^{-2}M aqueous solution of the test electrolyte.

The electrode is conditioned in an aqueous solution of the test electrolyte for 24hrs. The equilibrium potential of the cell (referenced to a Calomel electrode) is measured by the constant dilution of a 0.1M solution of the electrolyte.

The change in potential difference detected by the measuring system, E, is given by :

$$E = E^{\circ} + E_{INT} + E_{EXT} \quad \ldots\ldots \quad [1]$$

E° = potential at infinite dilution (constant)

E_{INT} = internal phase boundary potential of membrane

E_{EXT} = external phase boundary potential of membrane

E_{EXT} has a Nernstian relationship to the ionic activity of the test electrolyte.

E_{EXT} = constant + 2.303(RT/zF)log a .... [2] $R=8.314JK^{-1}mol^{-1}$

$F=9.65 \times 10^4$ $Cmol^{-1}$

T=temperature

z=No. of charges transferred i.e. charge on cation

E_{INT} has a similar Nernstian relationship to the activity of the relevant ion in the electrode internal filling solution; this solution remains unchanged and so E_{INT} is a constant. Equation [1] therefore becomes:

$$E = E' + 2.303(RT/zF)\log a \ldots\ldots [3]$$

If we assume ideality (activity=concentration) and thermostat the test electrolyte solution and the electrode to 25°C (298K), then for a 0.1M test solution of a monovalent cation such as Na^+ (z=1), equation [3] gives

$$E = E' + 59.13mV \ldots\ldots [4]$$

Consequently, a plot of the meausured potential against the concentration of the elctrolyte has a slope of about 59mV if the response is Nernstian. A slope of about 59mV therefore implies that the relevant cation is efficiently transported across the membrane by the incorporated macrocyclic ligand.

In the case of anion transport, where z=-1, the change in the measured response of the electrode (assuming efficient anion transport) becomes about -59mV.

Note that while efficient transport indicates the formation of a macrocyclic/cation complex, nothing can be concluded about the

strength of the interaction. Efficient transport does not imply strong binding, or vice versa. The qualities associated with macrocycles which have good transport properties[2] are not necessarily those associated with macrocycles which form stable complexes (see chapter 1).

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APPENDIX 2. CRYSTAL STRUCTURES.

APPENDIX 2.1. CRYSTAL STUCTURE OF LIGAND **23.**

TABLES

1. Summary of Data Collection, Structure Solution and Refinement Details
2. Bond Lengths
3. Bond Angles
4. Anisotropic Thermal Parameters
5. Deposition Data
6. Torsion Angles

Table 1. Summary of Data Collection, Structure Solution and Refinement Details

(a) **Crystal Data**

empirical formula	$C_{14}H_{24}N_4O_2S_2$
fw	344.5
colour, habit	colourless, block
crystal size, mm	0.15 x 0.25 x 0.30
cryst syst	Triclinic
a, Å	6.9554(6)
b, Å	7.8297(11)
c, Å	8.1242(13)
α, °	79.27(1)
β, °	75.50(1)
γ, °	84.37(1
V, Å3	420.2(1)
space group	*P_1*
Z	1
molecular symmetry	I
F(000)	184
d_{calc}, g cm^{-3}	1.36
μ, cm^{-1}	1.9

(b) **Data acquisition**[a]

temp, °C	21
unit-cell reflcns (2θ-range°)	25 (15 - 27)
max. 2θ (°) for reflcns	54
hkl range of reflcns	-8 8, 0 9, -9 10
variation in 3 standard reflcns	< 1%
reflcns measured	1826
unique reflcns	1826
R_{int}	-
reflcns with $I > n\,\sigma(I)$, *n*	1268, *3.0*
absorption correction type	none

(c) **Structure Solution and Refinement**

solution method	Direct methods[b]
H-atom treatment	riding, C-H 0.95
no. of variables in LS	113
k in $w = 1/(\sigma^2 Fo + kFo^2)$	0.0005
R, R_w, *gof*	0.031, 0.041, 1.29
density range in final Δ-map, e Å^{-3}	-0.15, 0.17
final shift/error ratio	< 0.001

[a] Data collection on an Enraf Nonius CAD4 diffractometer with graphite monochromatised Mo-Kα radiation (λ 0.7093 Å).
All calculations were done on a Silicon Graphics 4D-35TG computer system with the NRCVAX system of programs (E.J. Gabe, Y. Le Page, J-P. Charland, F.L. Lee and P.S. White, *J. Appl. Cryst.* (1989), **22**, 384-389)[b] or with the TEXSAN system of programs,[c] Version 5.0, (1989). Molecular Structure Corporation, The Woodlands, TX, 77381, U.S.A.

Table 2. Bond lengths (Å).

S-C(1)	1.681(2)	O-C(5)	1.417(2)	O-C(6)	1.421(3)
N(1)-C(1)	1.349(2)	N(1)-C(2)	1.460(2)	N(1)-C(7)^a	1.450(2)
N(2)-C(1)	1.345(2)	N(2)-C(3)	1.454(2)	N(2)-C(4)	1.450(2)
C(2)-C(3)	1.507(3)	C(4)-C(5)	1.495(3)	C(6)-C(7)	1.500(3)
C(7)-N(1)^a	1.450(2)				

`a' represents the symmetry related equivalent: 1-x, 1-y, 1-z

Table 3. Bond Angles (°).

C(5)-O-C(6)	114.68(15)	C(1)-N(1)-C(2)	110.60(15)
C(1)-N(1)-C(7)^a	124.09(16)	C(2)-N(1)-C(7)^a	121.31(16)
C(1)-N(2)-C(3)	111.33(15)	C(1)-N(2)-C(4)	125.61(15)
C(3)-N(2)-C(4)	122.28(15)	S-C(1)-N(1)	125.83(14)
S-C(1)-N(2)	125.15(14)	N(1)-C(1)-N(2)	109.03(15)
N(1)-C(2)-C(3)	102.87(15)	N(2)-C(3)-C(2)	102.45(15)
N(2)-C(4)-C(5)	113.63(16)	O-C(5)-C(4)	108.89(16)
O-C(6)-C(7)	109.80(15)	N(1)^a-C(7)-C(6)	113.04(16)

`a' represents the symmetry related equivalent: 1-x, 1-y, 1-z

Table 4. Positional and Thermal Parameters and Their e.s.d.'s.

Atom	x	y	z	Biso
S	0.71812(8)	0.76954(7)	0.14466(7)	4.35(2)
O	0.84950(21)	0.22761(17)	0.42342(17)	4.18(6)
N(1)	0.58907(23)	0.44309(20)	0.23198(21)	3.59(7)
N(2)	0.35121(22)	0.64877(19)	0.26287(20)	3.42(6)
C(1)	0.54914(26)	0.61674(24)	0.21495(22)	3.05(7)
C(2)	0.40531(31)	0.35138(27)	0.27609(29)	4.15(9)
C(3)	0.24876(29)	0.48868(27)	0.33491(28)	4.17(9)
C(4)	0.25052(29)	0.81926(26)	0.27137(26)	3.99(8)
C(5)	0.25069(29)	0.88765(24)	0.43126(27)	3.89(8)
C(6)	0.16928(32)	0.80528(25)	0.73788(26)	4.18(9)
C(7)	0.22184(30)	0.63829(25)	0.84557(25)	3.88(9)

B_{iso} is the Mean of the Principal Axes of the Thermal Ellipsoid.

Table 5. Deposition Data

Anisotropic thermal parameters (x 10^2 Å^2).

U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}	S
4.52(3)	4.91(3)	6.64(4)	-1.65(2)	-0.24(2)	-0.57(2)	O
6.12(9)	4.92(8)	4.68(8)	-1.36(7)	-0.95(7)	-0.25(6)	N(1)
3.91(8)	4.16(8)	5.32(10)	-0.74(6)	-0.55(7)	-0.65(7)	N(2)
3.74(8)	4.52(9)	4.48(9)	-0.56(6)	-0.69(7)	-0.29(7)	C(1)
3.85(9)	4.46(9)	3.28(9)	-0.70(7)	-0.77(7)	-0.50(7)	C(2)
4.88(11)	5.06(11)	5.97(13)	-1.69(9)	-1.12(9)	-0.81(10)	C(3)
3.90(10)	5.92(12)	5.82(14)	-1.47(9)	-0.90(9)	-0.13(10)	C(4)
4.31(11)	5.30(11)	4.87(12)	0.35(9)	-0.99(9)	0.37(9)	C(5)
4.39(11)	4.07(10)	5.63(12)	-0.15(8)	-0.50(9)	-0.07(9)	C(6)
5.84(13)	4.64(11)	5.20(13)	0.22(9)	-0.63(10)	-1.50(9)	C(7)
5.04(12)	5.42(12)	3.99(11)	-0.09(9)	-0.28(8)	-1.19(9)	

Anisotropic temperature factors are of the form:

$$\exp[-2\pi^2(h^2U_{11}a^{*2} + k^2U_{22}b^{*2} + l^2U_{33}c^{*2} + 2hkU_{12}a^*b^* + 2hlU_{13}a^*c^* + 2klU_{23}b^*c^*)].$$

Hydrogen positional and thermal parameters.

Atom	x	y	z	B_{iso}
H2A	0.3838	0.3129	0.1782	5.8(6)
H2B	0.4072	0.2547	0.3659	6.3(6)
H3A	0.2097	0.4738	0.4572	5.0(5)
H3B	0.1353	0.4865	0.2903	5.9(6)
H4A	0.3150	0.8986	0.1744	3.9(4)
H4B	0.1165	0.8119	0.2668	4.8(5)
H5A	0.1845	0.9998	0.4286	4.5(5)
H5B	0.3838	0.8953	0.4382	3.7(4)
H6A	0.0469	0.8548	0.7964	4.8(5)
H6B	0.2710	0.8842	0.7190	3.8(4)
H7A	0.2281	0.6619	0.9546	4.4(5)
H7B	0.1207	0.5596	0.8611	4.5(5)

B_{iso} is the Mean of the Principal Axes of the Thermal Ellipsoid.

Table 6. Selected Torsion Angles (°).

C2	N1	C1	S	-173.1(2)	C2	N1	C1	N2	6.7(1)
C7	N1	C1	S	-15.4(1)	C7	N1	C1	N2	164.4(3)
C1	N1	C2	C3	-16.3(1)	C7	N1	C2	C3	-174.7(3)
C1	N1	C7	C6	146.8(3)	C2	N1	C7	C6	-57.8(2)
C3	N2	C1	S	-173.7(2)	C3	N2	C1	N1	6.5(1)
C4	N2	C1	S	-3.6(1)	C4	N2	C1	N1	176.6(3)
C1	N2	C3	C2	-16.2(1)	C4	N2	C3	C2	173.4(3)
C1	N2	C4	C5	-80.3(2)	C3	N2	C4	C5	88.7(2)
N1	C2	C3	N2	18.5(1)	O	C6	C7	N1	-61.9(2)

APPENDIX 2.2. CRYSTAL STUCTURE OF LIGAND [23.Ag^+].

TABLES

1. Summary of Data Collection, Structure Solution and Refinement Details
2. Bond Lengths
3. Bond Angles
4. Anisotropic thermal parameters
5. Depostion Data
6. Torsion angles

Table 1. Summary of Data Collection, Structure Solution and Refinement Details

(a) **Crystal Data**

empirical formula	$C_{14}H_{24}N_7O_{11}S_2Ag_3$
fw	854.1
colour, habit	colourless, block
crystal size, mm	0.30 x 0.35 x 0.40
cryst syst	Monoclinic
a, Å	10.703(4)
b, Å	15.577(6)
c, Å	16.294(3)
α, °	
β, °	91.62(2)
γ, °	
V, Å3	2715(2)
space group	$P2_1/n$
Z	4
molecular symmetry	none
F(000)	1672
d_{calc}, g cm^{-3}	2.09
μ, cm^{-1}	23.3

(b) **Data acquisition**[a]

temp, °C	21
unit-cell reflcns (2θ-range°)	25 (18 - 25)
max. 2θ (°) for reflcns	40
hkl range of reflcns	-10 10, 0 15, 0 15
variation in 3 standard reflcns	75% decay (in 18hrs)
reflcns measured	2690
unique reflcns	2523
R_{int} reflcns with $I > n\,\sigma(I)$, n	1934
absorption correction type	*DIFABS*
min. max. abs. corr.	1.33

(c) **Structure Solution and Refinement**

solution method	Heavy-atom method[c]
H-atom treatment	riding, C-H 0.95 Å
no. of variables in LS	315
k in $w = 1/(\sigma^2 Fo + kFo^2)$	0.01
R, R_w, *gof*	0.126, 0.140, 14.5
density range in final Δ-map, e Å^{-3}	-2.13, 1.92
final shift/error ratio	< 0.1

[a] Data collection on an Enraf Nonius CAD4 diffractometer with graphite monochromatised Mo-Kα radiation (λ 0.7093 Å).
All calculations were done on a Silicon Graphics 4D-35TG computer system with the NRCVAX system of programs (E.J. Gabe, Y. Le Page, J-P. Charland, F.L. Lee and P.S. White, *J. Appl. Cryst.* (1989), **22**, 384-389)[b] or with the TEXSAN system of programs,[c] Version 5.0, (1989). Molecular Structure Corporation, The Woodlands, TX, 77381, U.S.A.

Table 2. Selected Bond Lengths and Distances (Å).

Ag(1)	Ag(2)	2.903(5)	Ag(1)	S(1)	2.53(1)	Ag(1)	S(2)	2.69(1)
Ag(1)	O(1)	2.61(3)	Ag(1)	O(1W)	2.29(3)	Ag(1)	O(11)	2.72(9)
Ag(2)	S(1)	2.67(1)	Ag(2)	S(2)	2.66(1)	Ag(2)	O(2)	2.53(2)
Ag(2)	O(13)	2.40(3)	Ag(2)	O(21)	2.43(3)	Ag(2)	O(23)	2.63(3)
Ag(2)	N(21)	2.96(4)	Ag(3)	S(1)	2.57(1)	Ag(3)	S(2)	2.54(1)
Ag(3)	O(31)	2.00(7)	Ag(3)	O(32)	2.32(5)	Ag(3)	O(41)	2.19(6)
Ag(3)	O(42)	2.45(8)	S(1)	C(1)	1.77(5)	S(2)	C(2)	1.88(5)
O(1)	C(3)	1.50(6)	O(1)	C(14)	1.39(5)	O(2)	C(8)	1.46(4)
O(2)	C(9)	1.57(4)	O(12)	N(11)	1.69(6)	O(13)	N(11)	1.23(5)
O(21)	N(21)	1.29(4)	O(22)	N(21)	1.18(4)	O(23)	N(21)	1.18(4)
O(31)	N(31)	1.24(7)	O(32)	N(31)	1.99(7)	O(32)	N(31)[a]	1.36(6)
O(41)	N(41)	1.31(5)	O(41)	N(41)[b]	1.81(7)	O(42)	N(41)[b]	1.13(9)

`a' represents the symmetry equivalent: 1-x, -y, -z

`b' represents the symmetry equivalent: 2-x, -y, -z

Table 3. Selected Bond Angles (°).

Ag2	Ag1	S1	58.3(2)	Ag2	Ag1	S2	56.7(2)
Ag2	Ag1	O1	118(1)	Ag2	Ag1	O1W	152(1)
S1	Ag1	S2	112.4(3)	S1	Ag1	O1	100(1)
S1	Ag1	O1W	132(1)	S1	Ag1	O11	99(2)
S2	Ag1	O1	95(1)	S2	Ag1	O1W	114(1)
S2	Ag1	O11	82(2)	O1	Ag1	O1W	87(1)
O1	Ag1	O11	160(2)	O1W	Ag1	O11	76(2)
Ag1	Ag2	S1	53.9(2)	Ag1	Ag2	S2	57.6(2)
Ag1	Ag2	O2	111.9(4)	Ag1	Ag2	O13	81(1)
Ag1	Ag2	O21	160(1)	Ag1	Ag2	O23	147(1)
Ag1	Ag2	N21	166(1)	S1	Ag2	S2	109.1(3)
S1	Ag2	O2	92.0(5)	S1	Ag2	O13	93(1)
S1	Ag2	O21	145(1)	S1	Ag2	O23	97(1)
S1	Ag2	N21	120(1)	S2	Ag2	O2	97(1)
S2	Ag2	O13	93(1)	S2	Ag2	O21	105(1)
S2	Ag2	O23	154(1)	S2	Ag2	N21	130(1)
O2	Ag2	O13	166(1)	O2	Ag2	O21	79(1)
O2	Ag2	O23	80(1)	O2	Ag2	N21	79(1)
O13	Ag2	O21	90(1)	O13	Ag2	O23	87(1)
O13	Ag2	N21	87(1)	S1	Ag3	S2	133.0(3)
S1	Ag3	O31	95(2)	S1	Ag3	O32	125(1)
S1	Ag3	O41	91(1)	S1	Ag3	O42	89(2)
S2	Ag3	O31	92(2)	S2	Ag3	O32	96(1)
S2	Ag3	O41	96(1)	S2	Ag3	O42	131(2)
O31	Ag3	O41	163(2)	O31	Ag3	O42	111(3)
O32	Ag3	O41	109(2)	O32	Ag3	O42	67(2)
O41	Ag3	O42	53(2)	Ag1	S1	Ag2	67.8(2)
Ag2	S2	Ag3	128.9(4)	Ag1	S1	Ag3	120.1(4)
Ag2	S1	Ag3	157.1(5)	Ag1	S2	Ag2	65.7(2)
Ag1	S2	Ag3	153.4(5)				

Table 4. Positional and Thermal Parameters and Their e.s.d.'s.

Atom	x	y	z	Biso	Site Occupancy
Ag(1)	0.73981(39)	0.18876(23)	0.29836(20)	6.5(2)	
Ag(2)	0.79400(38)	0.31510(21)	0.17433(18)	5.4(2)	
Ag(3)	0.75157(36)	-0.00303(21)	0.10042(20)	5.5(2)	
S(1)	0.8083(11)	0.14555(60)	0.15756(60)	3.5(5)	
S(2)	0.7915(10)	0.35378(57)	0.33251(51)	3.3(5)	
O(1)	0.9252(27)	0.1269(14)	0.3869(14)	4(1)	
O(1W)	0.6087(32)	0.1261(21)	0.3885(17)	8(2)	
O(2)	1.0271(21)	0.3326(12)	0.1563(12)	2(1)	
O(2W)	0.3787(45)	0.1370(29)	0.3519(25)	25(4)	
O(12)	0.3466(41)	0.3107(23)	0.1879(21)	11(1)	
O(13)	0.5703(28)	0.3150(16)	0.1604(15)	5(1)	
O(21)	0.8018(31)	0.4522(21)	0.1042(20)	7(2)	
O(22)	0.7945(42)	0.4826(22)	-0.0203(21)	11(3)	
O(23)	0.7969(39)	0.3526(22)	0.0179(19)	9(2)	
O(31)	0.5693(68)	0.0229(39)	0.0851(37)	7(2)	
O(32)	0.6342(53)	-0.0282(28)	-0.0183(25)	4(1)	0.5
O(41)	0.9483(54)	-0.0294(30)	0.0774(31)	15(2)	
O(42)	0.8639(82)	0.0324(50)	-0.0233(51)	29(4)	0.5
N(1)	1.0192(47)	0.0757(20)	0.2236(21)	5(2)	
N(2)	1.0573(52)	0.1585(22)	0.1332(23)	5(2)	
N(3)	1.0472(33)	0.3794(21)	0.3236(20)	3(2)	
N(4)	0.9903(37)	0.2981(20)	0.4220(21)	4(2)	
N(11)	0.4982(57)	0.2883(32)	0.2117(33)	10(1)	
N(21)	0.7952(44)	0.4275(27)	0.0291(25)	6(2)	
N(31)	0.4924(53)	0.0362(26)	0.0279(25)	8(1)	0.5
N(41)	1.0315(49)	-0.0199(23)	0.0211(22)	6(1)	0.5
C(1)	0.9699(56)	0.1250(20)	0.1745(25)	5(3)	
C(2)	0.9629(47)	0.3457(26)	0.3603(24)	4(2)	
C(3)	0.9942(57)	0.0445(29)	0.3731(28)	9(3)	
C(4)	0.9735(61)	0.0237(27)	0.2805(30)	9(4)	
C(5)	1.1638(73)	0.0707(50)	0.2230(36)	11(5)	
C(6)	1.1710(73)	0.1337(32)	0.1561(46)	7(4)	
C(7)	1.0390(49)	0.2149(38)	0.0667(30)	9(4)	
C(8)	1.0984(35)	0.3082(20)	0.0858(21)	3(2)	
C(9)	1.1023(36)	0.4145(22)	0.1829(18)	3(2)	
C(10)	1.0375(43)	0.4454(26)	0.2593(24)	5(2)	
C(11)	1.1571(60)	0.3654(31)	0.3684(28)	10(4)	
C(12)	1.1181(62)	0.3015(26)	0.4339(23)	6(3)	
C(13)	0.9123(50)	0.2452(29)	0.4785(20)	6(3)	
C(14)	0.9388(56)	0.1533(28)	0.4676(26)	8(3)	

B_{iso} is the Mean of the Principal Axes of the Thermal Ellipsoid.

Table 5. Deposition Data

a) Anisotropic thermal parameters (x 10^2 Å^2).

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Ag(1)	0.0797(36)	0.0905(27)	0.0769(22)	-0.0021(29)	0.0156(23)	0.0176(21)
Ag(2)	0.0863(35)	0.0611(21)	0.0584(19)	0.0026(26)	-0.0017(21)	0.0128(17)
Ag(3)	0.0635(31)	0.0661(21)	0.0785(22)	-0.0291(27)	0.0007(21)	0.0026(19)
S(1)	0.0295(84)	0.0370(58)	0.0669(63)	0.0054(69)	-0.0171(65)	-0.0084(53)
S(2)	0.0381(85)	0.0418(56)	0.0470(51)	0.0131(66)	-0.0018(58)	0.0010(48)
O(1)	0.072(25)	0.026(15)	0.061(17)	0.002(17)	-0.018(17)	0.011(13)
O(1W)	0.089(29)	0.120(25)	0.094(21)	-0.029(25)	0.023(21)	0.006(20)
O(2)	0.025(18)	0.017(12)	0.046(13)	0.014(14)	-0.005(14)	-0.004(11)
O(21)	0.066(28)	0.107(26)	0.089(22)	-0.005(22)	-0.024(22)	0.014(20)
O(22)	0.193(47)	0.111(28)	0.123(28)	0.032(32)	0.051(29)	0.083(25)
O(23)	0.160(42)	0.060(21)	0.113(25)	-0.020(28)	-0.024(27)	0.013(20)
N(1)	0.130(50)	-0.006(15)	0.062(23)	-0.031(23)	0.019(28)	0.010(18)
N(2)	0.088(41)	0.036(23)	0.061(23)	0.049(29)	0.001(29)	0.040(20)
N(3)	0.010(26)	0.060(23)	0.056(23)	-0.026(22)	0.027(22)	0.002(19)
N(4)	0.038(29)	0.046(22)	0.051(22)	0.047(24)	-0.014(23)	0.021(18)
N(21)	0.103(42)	0.053(26)	0.082(29)	-0.008(30)	-0.036(31)	0.041(28)
C(1)	0.190(73)	-0.034(17)	0.024(21)	-0.035(30)	-0.026(34)	0.004(16)
C(2)	0.062(43)	0.036(25)	0.033(24)	0.044(30)	-0.035(28)	-0.041(22)
C(3)	0.177(65)	0.060(30)	0.097(35)	-0.025(40)	-0.116(40)	0.019(28)
C(4)	0.195(71)	0.044(30)	0.097(37)	-0.030(38)	-0.014(42)	-0.049(29)
C(5)	0.084(58)	0.216(77)	0.115(43)	0.146(57)	-0.052(44)	0.018(48)
C(6)	0.084(56)	0.050(32)	0.155(56)	0.004(38)	0.089(49)	-0.035(36)
C(7)	0.071(45)	0.189(60)	0.096(36)	-0.082(46)	0.074(37)	-0.121(42)
C(8)	0.023(29)	0.027(20)	0.071(25)	-0.019(24)	0.009(24)	-0.009(20)
C(9)	0.020(29)	0.060(27)	0.031(19)	0.007(24)	-0.010(21)	0.004(19)
C(10)	0.056(40)	0.069(28)	0.073(28)	0.055(30)	-0.027(29)	0.007(27)
C(11)	0.207(75)	0.071(36)	0.084(33)	0.055(46)	-0.142(46)	-0.057(31)
C(12)	0.142(60)	0.047(27)	0.035(24)	0.085(38)	-0.063(32	-0.022(22)
C(13)	0.133(52)	0.086(34)	0.023(21)	0.096(36)	0.016(28)	0.017(23)
C(14)	0.170(62)	0.067(31)	0.067(31)	-0.012(39)	0.056(37)	0.041(27)

Anisotropic temperature factors are of the form:

$$\exp[-2\pi^2(h^2U_{11}a^{*2} + k^2U_{22}b^{*2} + l^2U_{33}c^{*2} + 2hkU_{12}a^*b^* + 2hlU_{13}a^*c^* + 2klU_{23}b^*c^*)].$$

The bridging nitrate group {N(11), O(11), O(12), O(13)}, the disordered nitrate groups and the water molecule O2W were refined isotropically.

b) Hydrogen Positional and Thermal Parameters.

Atom	x	y	z	B_{iso}
H(1)	1.07	0.05	0.39	8.0
H(2)	0.94	0.00	0.41	8.0
H(3)	0.88	0.02	0.27	7.3
H(4)	1.01	-0.03	0.28	7.3
H(5)	1.20	0.08	0.27	8.7
H(6)	1.18	0.01	0.20	8.7
H(7)	1.22	0.11	0.12	6.7
H(8)	1.22	0.18	0.18	6.7
H(9)	1.07	0.20	0.01	5.5
H(10)	0.95	0.23	0.06	5.5
H(11)	1.09	0.35	0.04	6.2
H(12)	1.19	0.30	0.10	6.2
H(13)	1.19	0.41	0.19	4.9
H(14)	1.09	0.46	0.14	4.9
H(15)	0.95	0.45	0.25	6.0
H(16)	1.07	0.49	0.28	6.0
H(17)	1.21	0.34	0.33	7.7
H(18)	1.19	0.42	0.39	7.7
H(19)	1.15	0.32	0.48	5.5
H(20)	1.16	0.25	0.42	5.5
H(21)	0.93	0.26	0.53	6.0
H(22)	0.83	0.25	0.46	6.0
H(23)	0.89	0.12	0.51	5.8
H(24)	1.03	0.14	0.49	5.8

B_{iso} is the Mean of the Principal Axes of the Thermal Ellipsoid.

Table 6. Selected Torsion Angles (°).

Ag1	Ag2	S1	Ag3	115(1)	Ag1	Ag2	S1	N1	-79.8(8)
Ag1	Ag2	S1	N2	-117.5(9)	Ag1	Ag2	S1	C1	-98(2)
Ag1	Ag2	S2	Ag3	-153.7(7)	Ag1	Ag2	S2	N3	112.5(8)
Ag1	Ag2	S2	N4	74.7(9)	Ag1	Ag2	S2	C2	97(2)
Ag1	Ag2	O2	N2	53.1(9)	Ag1	Ag2	O2	N3	-59.2(8)
Ag1	Ag2	O2	C7	84(2)	Ag1	Ag2	O2	C8	112(2)
Ag1	Ag2	O2	C9	-110(2)	Ag1	Ag2	O2	C10	-90(1)
Ag1	Ag2	O13	O11	14(3)	Ag1	Ag2	O13	O12	38(5)
Ag1	Ag2	O13	N11	19(4)	Ag1	Ag2	O21	O1W	62(4)
Ag1	Ag2	O21	O22	-146(2)	Ag1	Ag2	O21	O23	-150(2)
Ag1	Ag2	O21	N21	-147(2)	Ag1	Ag2	O23	O2W	-82(6)
Ag1	Ag2	O23	O21	161(1)	Ag1	Ag2	O23	O22	158(2)
Ag1	Ag2	O23	N21	159(3)	Ag1	Ag2	N21	O21	127(4)
Ag1	Ag2	N21	O22	120(21)	Ag1	Ag2	N21	O23	-58(6)
Ag1	S1	Ag2	S2	17.3(4)	Ag1	S1	Ag2	O2	115.9(5)
Ag1	S1	Ag2	O13	-76.8(7)	Ag1	S1	Ag2	O21	-171(1)
Ag1	S1	Ag2	O23	-164.3(9)	Ag1	S1	Ag2	N21	-165(1)
Ag1	S1	Ag3	S2	-27.3(8)	Ag1	S1	Ag3	O31	70(2)
Ag1	S1	Ag3	O32	120(2)	Ag1	S1	Ag3	O41	-126(1)
Ag1	S1	Ag3	O42	-179(2)	Ag1	S2	Ag2	S1	-16.6(4)
Ag1	S2	Ag2	O2	-111.3(5)	Ag1	S2	Ag2	O13	77.5(7)
Ag1	S2	Ag2	O21	168.6(8)	Ag1	S2	Ag2	O23	167(2)
Ag1	S2	Ag2	N21	167(1)	Ag2	Ag1	S1	Ag3	-156.0(6)
Ag2	Ag1	S1	N1	116.1(7)	Ag2	Ag1	S1	N2	79.0(8)
Ag2	Ag1	S1	C1	99(1)	Ag2	Ag1	S2	Ag3	130(1)
Ag2	Ag1	S2	N3	-77.1(8)	Ag2	Ag1	S2	N4	-119.1(8)
Ag2	Ag1	S2	C2	-97(2)	Ag2	Ag1	O1	N1	-57.9(8)
Ag2	Ag1	O1	N4	53(1)	Ag2	Ag1	O1	C3	-96(3)
Ag2	Ag1	O1	C4	-83(2)	Ag2	Ag1	O1	C13	85(1)
Ag2	Ag1	O1	C14	107(3)	Ag2	Ag1	O1W	O2W	27(3)
Ag2	Ag1	O1W	O21	151(1)	Ag2	Ag1	O11	O2W	173(2)
Ag2	Ag1	O11	O12	38(13)	Ag2	Ag1	O11	O13	12(2)
Ag2	Ag1	O11	N11	14(9)	Ag2	S1	Ag1	S2	-17.5(4)
Ag2	S1	Ag1	O1	-117.5(6)	Ag2	S1	Ag1	O1W	147(1)
Ag2	S1	Ag1	O11	68(2)	Ag2	S1	Ag3	S2	-132(1)
Ag2	S1	Ag3	O31	-34(2)	Ag2	S1	Ag3	O32	16(2)
Ag2	S1	Ag3	O41	130(2)	Ag2	S1	Ag3	O42	77(2)
Ag2	S2	Ag1	S1	17.9(4)	Ag2	S2	Ag1	O1	120.8(6)
Ag2	S2	Ag1	O1W	-150(1)	Ag2	S2	Ag1	O11	-79(2)
Ag3	S1	Ag1	S2	-173.5(5)	Ag3	S1	Ag1	O1	86.6(7)
Ag3	S1	Ag1	O1W	-9(1)	Ag3	S1	Ag1	O11	-88(2)
Ag3	S1	Ag2	S2	132(1)	Ag3	S1	Ag2	O2	-129(1)
Ag3	S1	Ag2	O13	38(1)	Ag3	S1	Ag2	O21	-56(2)
Ag3	S1	Ag2	O23	-49(1)	Ag3	S1	Ag2	N21	-50(2)
S1	Ag1	Ag2	S2	-160.5(4)	S1	Ag1	Ag2	O2	-75.7(6)
S1	Ag1	Ag2	O13	100.2(7)	S1	Ag1	Ag2	O21	166(2)
S1	Ag1	Ag2	O23	30(2)	S1	Ag1	Ag2	N21	69(4)
S1	Ag2	Ag1	S2	160.5(4)	S1	Ag2	Ag1	O1	83.9(7)
S1	Ag2	Ag1	O1W	-122(2)	S1	Ag2	Ag1	O11	-110(2)

APPENDIX 2.3. CRYSTAL STRUCTURE OF LIGAND **24**.

TABLES

1. Summary of Data Collection, Structure Solution and Refinement Details
2. Bond Lengths
3. Bond Angles
4. Anisotropic Thermal Parameters
5. Calculated Hydrogen Atom Coordinates

Table 1. Summary of Data Collection, Structure Solution and Refinement Details

(a) **Crystal Data**

empirical formula	$C_{18}H_{32}N_4O_4S_2$
fw	432.6
colour, habit	colourless, block
crystal size, mm	0.2 x 0.3 x 0.4
cryst syst	orthorhombic
a, Å	22.531(4)
b, Å	21.502(3)
c, Å	8.961(2)
V, Å3	4341(1)
space group	Pbca
Z	8
molecular symmetry	None
F(000)	1856
d_{calc}, g cm^{-3}	1.324
μ, mm^{-1}	0.276

(b) **Data acquisition**[a]

temp, °C	-123
unit-cell reflcns (2θ-range°)	23-25
max. 2θ (°) for reflcns	4-60
hkl range of reflcns	0<h<29, 0<k<30, 0<l<12
variation in 3 standard reflcns	<0.5%
reflcns measured	5093
unique reflcns	5043
reflcns with $I > n\,\sigma(I)$, *n*	2622
absorption correction type	None
min. max. abs. corr.	-

(c) **Structure Solution and Refinement**

solution method	Direct methods[b]
H-atom treatment	Refined Isotropically
no. of variables in LS	381
k in $w = 1/(\sigma^2 Fo + kFo^2)$	0
R, R_w, gof	0.044, 0.039, 1.58
density range in final Δ-map, e Å^{-3}	-0.25, 0.31
final shift/error ratio	<0.08

[a] Data collection on a Rigaku AFC6S diffractometer with graphite monochromatised Mo-Kα radiation (l 0.71073 Å). [b] SHEXTL PLUS

Table 2. Bond Lengths (Å)

S(1)-C(18)	1.679 (3)	S(2)-C(9)	1.690 (3)
O(1)-C(2)	1.439 (4)	O(1)-C(3)	1.403 (6)
O(2)-C(4)	1.440 (6)	O(2)-C(5)	1.421 (5)
O(3)-C(11)	1.421 (4)	O(3)-C(12)	1.429 (4)
O(4)-C(13)	1.428 (5)	O(4)-C(14)	1.426 (4)
N(1)-C(15)	1.451 (5)	N(1)-C(16)	1.446 (5)
N(1)-C(18)	1.354 (4)	N(2)-C(1)	1.450 (4)
N(2)-C(17)	1.458 (5)	N(2)-C(18)	1.337 (4)
N(3)-C(6)	1.455 (5)	N(3)-C(7)	1.457 (5)
N(3)-C(9)	1.360 (4)	N(4)-C(8)	1.467 (5)
N(4)-C(9)	1.337 (4)	N(4)-C(10)	1.461 (4)
C(1)-C(2)	1.499 (6)	C(3)-C(4)	1.521 (6)
C(5)-C(6)	1.520 (6)	C(7)-C(8)	1.523 (5)
C(10)-C(11)	1.505 (5)	C(12)-C(13)	1.496 (5)
C(14)-C(15)	1.510 (6)	C(16)-C(17)	1.507 (6)

Table 3. Bond Angles (°)

C(2)-O(1)-C(3)	113.1(3)	C(4)-O(2)-C(5)	113.2(3)
C(11)-O(3)-C(12)	112.1(3)	C(13)-O(4)-C(14)	112.5(3)
C(15)-N(1)-C(16)	121.7(3)	C(15)-N(1)-C(18)	124.5(3)
C(16)-N(1)-C(18)	111.5(3)	C(1)-N(2)-C(17)	121.7(3)
C(1)-N(2)-C(18)	125.4(3)	C(17)-N(2)-C(18)	112.1(3)
C(6)-N(3)-C(7)	120.0(3)	C(6)-N(3)-C(9)	125.3(3)
C(7)-N(3)-C(9)	111.3(3)	C(8)-N(4)-C(9)	112.5(3)
C(8)-N(4)-C(10)	121.7(3)	C(9)-N(4)-C(10)	125.4(3)
N(2)-C(1)-C(2)	113.5(3)	O(1)-C(2)-C(1)	114.1(3)
O(1)-C(3)-C(4)	108.5(4)	O(2)-C(4)-C(3)	108.8(4)
O(2)-C(5)-C(6)	113.7(3)	N(3)-C(6)-C(5)	111.8(3)
N(3)-C(7)-C(8)	102.8(3)	N(4)-C(8)-C(7)	102.0(3)
S(2)-C(9)-N(3)	125.6(3)	S(2)-C(9)-N(4)	126.0(2)
N(3)-C(9)-N(4)	108.4(3)	N(4)-C(10)-C(11)	114.3(3)
O(3)-C(11)-C(10)	108.4(3)	O(3)-C(12)-C(13)	108.9(3)
O(4)-C(13)-C(12)	108.6(3)	O(4)-C(14)-C(15)	109.4(3)
N(1)-C(15)-C(14)	113.8(3)	N(1)-C(16)-C(17)	103.6(3)
N(2)-C(17)-C(16)	103.1(3)	S(1)-C(18)-N(1)	125.4(3)
S(1)-C(18)-N(2)	126.0(2)	N(1)-C(18)-N(2)	108.6(3)

Table 4. Positional and Thermal Parameters (x 10^4) and Their e.s.d.'s (Å^2 x 10^3)

	x	y	z	U(eq)
S(1)	2948(1)	6839(1)	959(1)	34(1)
S(2)	-684(1)	9543(1)	2721(1)	31(1)
O(1)	2355(1)	9156(1)	592(3)	35(1)
O(2)	1576(1)	9753(1)	2737(3)	34(1)
O(3)	-231(1)	7329(1)	659(3)	30(1)
O(4)	722(1)	6502(1)	1508(3)	27(1)
N(1)	1874(1)	7042(1)	2202(3)	29(1)
N(2)	2410(1)	7882(1)	1922(3)	27(1)
N(3)	414(1)	9474(1)	1473(3)	27(1)
N(4)	-164(1)	8687(1)	941(3)	24(1)
C(1)	2918(2)	8282(2)	1674(5)	27(1)
C(2)	2837(2)	8725(2)	398(5)	32(1)
C(3)	2511(2)	9677(2)	1449(7)	52(2)
C(4)	1959(2)	10070(2)	1697(7)	49(2)
C(5)	1060(2)	10100(2)	3108(5)	33(1)
C(6)	591(2)	10102(2)	1887(5)	30(1)
C(7)	740(2)	9139(2)	323(4)	24(1)
C(8)	396(2)	8531(2)	195(5)	28(1)
C(9)	-135(1)	9226(2)	1682(4)	23(1)
C(10)	-700(2)	8317(2)	721(5)	28(1)
C(11)	-675(2)	7678(2)	1409(4)	28(1)
C(12)	-293(2)	6676(2)	914(5)	28(1)
C(13)	268(2)	6357(2)	450(5)	30(1)
C(14)	1260(2)	6175(2)	1228(5)	28(1)
C(15)	1729(2)	6386(2)	2321(5)	35(1)
C(16)	1539(2)	7513(2)	2991(5)	33(1)
C(17)	1878(2)	8103(2)	2681(6)	37(1)
C(18)	2401(1)	7266(2)	1714(4)	23(1)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 5. Deposition Data

a) Anisotropic Thermal Parameters ($Å^2 \times 10^3$)

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
S(1)	25(1)	30(1)	47(1)	5(1)	12(1)	-1(1)
S(2)	28(1)	30(1)	35(1)	8(1)	9(1)	-2(1)
O(1)	19(1)	30(1)	54(2)	-2(1)	-6(1)	3(1)
O(2)	24(1)	29(1)	50(2)	-3(1)	-8(1)	-1(1)
O(3)	24(1)	20(1)	45(2)	-1(1)	9(1)	0(1)
O(4)	22(1)	29(1)	32(1)	2(1)	-1(1)	-5(1)
N(1)	15(1)	28(2)	45(2)	2(1)	7(1)	3(2)
N(2)	17(1)	26(2)	39(2)	-1(1)	9(1)	-4(1)
N(3)	19(1)	26(2)	36(2)	-2(1)	6(1)	-9(1)
N(4)	16(1)	22(1)	35(2)	0(1)	3(1)	-5(1)
C(1)	21(2)	24(2)	36(2)	-5(2)	1(2)	-3(2)
C(2)	21(2)	40(2)	35(2)	-3(2)	5(2)	-4(2)
C(3)	27(2)	32(2)	96(5)	-5(2)	-3(3)	-12(3)
C(4)	32(2)	22(2)	92(4)	-8(2)	8(3)	-1(2)
C(5)	32(2)	26(2)	41(3)	-5(2)	2(2)	-9(2)
C(6)	25(2)	22(2)	42(2)	1(2)	-4(2)	-7(2)
C(7)	18(2)	28(2)	25(2)	2(1)	2(2)	-2(2)
C(8)	19(2)	34(2)	30(2)	-1(2)	5(2)	-10(2)
C(9)	22(2)	27(2)	19(2)	5(1)	-1(1)	1(2)
C(10)	13(2)	31(2)	41(2)	1(1)	0(2)	-2(2)
C(11)	23(2)	28(2)	33(2)	-6(2)	4(2)	-1(2)
C(12)	23(2)	25(2)	36(2)	-7(1)	-6(2)	-3(2)
C(13)	36(2)	23(2)	30(2)	-3(2)	-5(2)	-5(2)
C(14)	23(2)	23(2)	39(2)	3(2)	10(2)	1(2)
C(15)	23(2)	27(2)	55(3)	1(2)	3(2)	11(2)
C(16)	28(2)	40(2)	32(2)	-1(2)	6(2)	-4(2)
C(17)	23(2)	35(2)	53(3)	3(2)	11(2)	-5(2)
C(18)	18(2)	30(2)	20(2)	-1(1)	0(1)	3(2)

The anisotropic displacement factor exponent takes the form:

$-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2hka^*b^*U_{12})$

b) Hydrogen Atom Positions and Thermal Parameters ($Å^2 \times 10^3$)

	x	y	z	U
H(11)	3240(15)	8040(16)	1483(43)	46(12)
H(12)	2957(13)	8511(14)	2512(35)	14(8)
H(21)	3188(14)	8945(15)	260(37)	28(10)
H(22)	2747(15)	8474(17)	-584(42)	52(12)
H(31)	2773(17)	9985(19)	834(49)	64(14)
H(32)	2657(15)	9570(16)	2407(40)	29(11)
H(41)	2070(16)	10478(18)	2029(44)	56(13)
H(42)	1734(19)	10150(21)	565(56)	92(18)
H(51)	1156(13)	10516(15)	3352(34)	23(9)
H(52)	907(13)	9940(14)	4070(38)	21(9)
H(61)	736(13)	10352(13)	865(35)	20(9)
H(62)	268(13)	10306(14)	2201(36)	22(9)
H(71)	720(14)	9349(15)	-619(37)	29(10)
H(72)	1171(14)	9033(15)	647(36)	34(10)
H(81)	603(13)	8223(14)	840(34)	21(9)
H(82)	333(14)	8384(15)	-770(38)	27(10)
H(101)	-1027(13)	8558(14)	1110(36)	23(9)
H(102)	-789(15)	8257(16)	-459(42)	49(12)
H(111)	-1052(13)	7460(15)	1321(37)	24(9)
H(112)	-559(13)	7678(15)	2474(38)	28(10)
H(121)	-364(12)	6575(13)	1980(35)	14(9)
H(122)	-605(15)	6522(17)	283(40)	43(11)
H(131)	213(13)	5887(15)	483(34)	25(9)
H(132)	388(14)	6497(15)	-524(37)	29(10)
H(141)	1360(12)	6241(13)	164(32)	8(8)
H(142)	1192(14)	5707(16)	1250(41)	46(11)
H(151)	1610(14)	6286(15)	3417(41)	35(11)
H(152)	2060(15)	6151(16)	2113(41)	44(12)
H(161)	1516(15)	7415(16)	4016(44)	39(11)
H(162)	1163(15)	7507(18)	2586(46)	54(13)
H(171)	1988(17)	8298(19)	3543(48)	66(15)
H(172)	1644(17)	8415(18)	1961(45)	63(14)

APPENDIX 3. LECTURES AND RESEARCH COLLOQUIA.

UNIVERSITY OF DURHAM

Board of Studies in Chemistry

COLLOQUIA, LECTURES AND SEMINARS GIVEN BY INVITED SPEAKERS

1st October 1989 to 31st July 1992

(* indicates lectures attended by the author)

Palmer, Dr. F. (University of Nottingham) 17.10.89
**Thunder and Lightning*

Floriani, Prof. C. (University of Lausanne) 25.10.89
Molecular Aggregates - A Bridge Between Homogeneous and Heterogeneous Systems

Badyal, Dr. J. P. S. (University of Durham) 01.11.89
Breakthroughs in Heterogeneous Catalysis

Greenwood, Prof. N. N. (University of Leeds) 09.11.89
Novel Cluster Geometries in Metalloborane Chemistry

Bercaw, Prof. J. E. (California Institute of Technology) 10.11.89
Synthetic and Mechanistic Approaches to Ziegler - Natta Polymerization of Olefins

Becher, Dr. J. (University of Odense) 13.11.89
**Synthesis of New Macrocyclic Systems Using Heterocyclic Building Blocks*

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