Mimicking Protein Environments with Bioinorganic Models.

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Abstract

Through the design and application of a novel modular synthetic strategy, a diverse family of phosphate receptors has been synthesised. The receptors were based around a complexed metal centre such as zinc (II) or copper (II) within a polyazamacrocycle framework. The initial design was to couple the polyazamacrocycle directly to a crown ether system to give a heteroditopic scaffold where the two components were in close proximity to each other. Due to the synthetic difficulties encountered, a novel approach was developed, whereby the use of an amino acid linker was employed to couple the two species.

The modular approach successfully allowed the variation of the metal centre through incorporation of different sized polyazamacrocycles. In the first instance we used a four-nitrogen donor host, cylen, to chelate zinc and in the second we attached a smaller triazamacrocycle, tacn to complex copper metal.

The linker was changed to allow variations in polarity, functionality and structure through the selection of specific backbone residues. The synthesis and characterisation of glycine, aminodiacetic acid and glutamic acid derivatives proved that the methodology was effective and viable for a number of different linkers with varying structure. The use of iminodiacetic acid enabled the coupling of two cyclen metal hosts which enabled the synthesis of a bimetallic zinc complex and a novel tritopic system.

Most importantly, the artificial receptors that we have created were shown to be excellent hosts for phosphate moieties. The proposed mode of binding in our systems was entropically driven through the efficient desolvation of the crown ether cavity upon phosphate binding. The interactions were in general, endothermic ($+\Delta H°$) which was assigned to the reorganisation of solvent molecules upon expulsion which is in agreement with a growing body of evidence in the literature.

Through isothermal titration calorimetry, ^1H NMR and UV/vis titration experiments, we were able to determine that the ditopic systems acted as ion pair receptors. The binding of one ion affected the affinity of the ion partner. In the case of $ZnL_1.(OTf)_2$ it was shown that the individual binding of the cation is negligible but in the presence of phosphate the cation exerts a positive influence on the binding of the anion. We were able to calculate through simple control experiments that the binding of inorganic phosphate with a number of different metal counter ions exhibited positive cooperativity. This is one of the first

examples of positive cooperativity between ion pairs in an aqueous environment at physiological pH. This thesis describes in detail the thermodynamic binding events of the different ligand systems synthesised and attempts to correlate the binding results to the structures of the host.

"The most beautiful thing we can experience is the mysterious. It is the source of all true art and all science. He to whom this emotion is a stranger, who can no longer pause to wonder and stand rapt in awe, is as good as dead: his eyes are closed."

Albert Einstein

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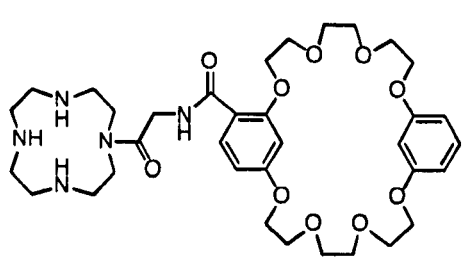
General

ATP	adenosine triphosphate
Boc	*tert*-butyloxycarbonyl
Cbz	carboxybenzyl
d	doublet
DCC	N,N-dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EI	electron impact
equiv	equivalents
ESMS	electrospray mass spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
FAB	fast atom bombardment
Fmoc	fluorenylmethyloxycarbonyl
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfronic acid
HOBt	1-Hydroxybenzotriazole
IR	infrared
ITC	isothermal titration calorimetry
m	multiplet
MOPS	(3-[N-morpholino]propanesulfonic acid)
MeCN	acetonitrile
MeOH	methanol
mmol	millimoles
m.p.	melting point
MS	mass spectrometry
NMR	nuclear magnetic resonance
NBS	*N*-bromosuccinamide
P_i	inorganic phosphate
ppm	parts per million
s	singlet
t	triplet
TBAF	*tert*-butyl ammonium fluoride
TBDMS	*tert*-butyl dimethyl sulphoxide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
tosyl, Ts	*p*-tolyl sulphonyl
TRIS	(tris[hydroxymethyl]amino-methane)
UV	ultra-violet
Vis	visible
18-benzocrown-6	6,7,9,10,12,13,15,16,18,19-Decahydro-5,8,11,14,17,20-hexaoxa-benzocyclooctadecene

Ligands

cyclam	1,4,8,11-tetrazacyclotetradecane
cyclen	1,4,7,10-tetraazacyclododecane
tacn	1,4,7-triazacyclononane
tren	tris(2-aminoethyl)amine
L_1	2,5,8,11,18,21,24,27-octaoxa-tricyclo[26.3.1.$0^{12,17}$]dotriaconta-1(31),12(17), 13,15, 28(32),29 - hexaene-29-carboxylic acid [2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-amide
L_2	2,5,8,11,17,20,23,26-octaoxa-tricyclo[25.3.1.$1^{12,16}$]dotriaconta-1(30),12,14,16(32),27(31),28-hexaene-13-carboxylic acid (2-oxo-2-[1,4,7]triazecan-4-yl-ethyl)-amide
L_3	2,5,8,11,17,20,23,26-octaoxa-tricyclo[25.3.1.$1^{12,16}$]dotriaconta-1(30),12,14,16(32),27(31),28-hexaene-13-carboxylic acid ({bis-[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-carbamoyl}-methyl)-amide
L_4	2,5,8,11,14,17-hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carboxylic acid[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-amide
cyclen-gly-Cbz	[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-carbamic acid benzyl ester.



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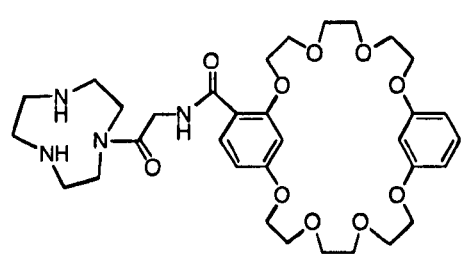

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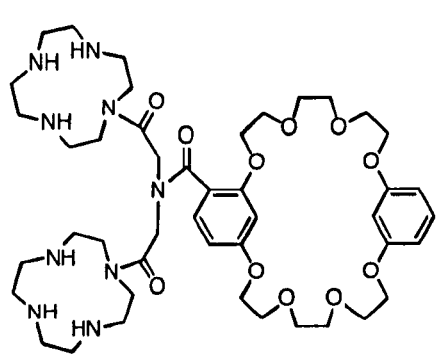

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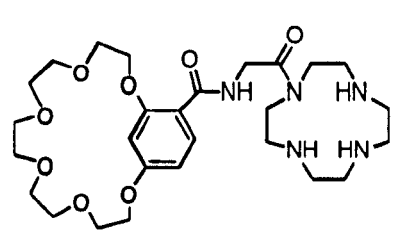

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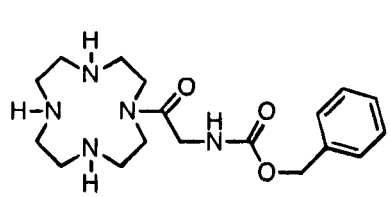


cyclen-gly-Cbz

Chapter 1

1.1.0 Introduction

1.1.1 Bioinorganic chemistry

Bioinorganic chemistry was reawakened and came to prominence when protein crystallography uncovered the huge significance of metal ions in the mechanisms of enzymes, proteins and the origins of life itself.[1] The crystal structures defined exactly the cavities, reaction centres and complex binding sites situated within enzyme systems capable of catalysing reactions to many orders of magnitude greater than that achieved by chemists. The understanding of how these systems operated under physiological conditions and the mechanisms by which the transformations occurred became the target of much research within the scientific community. Shown in Figure 1 is the crystal structure of a complex structural architecture of a light harvesting protein. The ultimate ambition of the biomimetic chemist was to create model systems which could mimic, to a high degree of accuracy the function of the enzyme or, indeed, to develop models capable of disrupting key transformations to hinder the progress of disorders promoted by these pathways.[2]

Figure 1. Bacterial light harvesting protein LH1.[3]

In order to design such systems, it is necessary to grasp the complexity, intricacy and beauty of nature's achievements. The replication of massive protein scaffolds was obviously not an option, even for the most gifted organic chemist. Replication of important structural motifs and binding sites was the key to developing relevant model systems.[4] Consequently, it was necessary to appreciably understand and gauge the relative importance of the chemical biology involved in the target protein or enzyme.

Our work was focussed on the search for a structural motif that would bind the phosphate moiety to high levels of magnitude. The importance of phosphate and its role in protein phosphorylation will be explained further in chapter 2 and the reasons for developing new methodologies elucidated upon.

This chapter gives a brief introduction to bioinorganic chemistry and chemical biology. It discusses the benefits of intricate superstructures and how crystallographic chemists have elucidated the binding sites and metallo-chemistry present. Examples of phosphate binding enzymes are described and the nature of the phosphate binding explored.

1.1.2 Introduction to amino acids and proteins

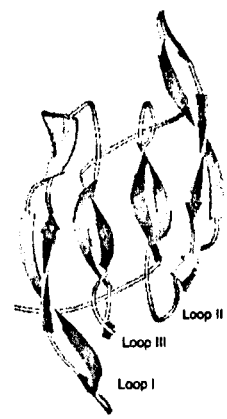


Figure 2. Protein schematic showing α-helix and β-pleated sheet conformations.

The name protein was derived from the Greek *Proteios*, by Berzelius,[5] which means "of the first rank" denoting the significant importance of these molecules. Proteins are biological polymers that play important roles in virtually all the chemical processes of life. Proteins play crucial roles in nearly all biological processes - in catalysis, transport, coordinated motion, excitability and control of growth and differentiation.

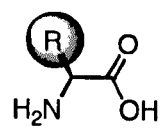
1. *Enzyme Catalysis*. Enzymes are macromolecules which catalyse nearly all chemical reactions in biological systems. Enzymes exhibit enormous catalytic power and specificity. Nearly all known enzymes are proteins thus are involved in determining the chemical transformations that occur in nature.[6]

2. *Transports and Storage*. Many small molecules and ions are transported by specific proteins. Dioxygen transportation occurs through haemoglobin in erythrocytes whereas myoglobin, a related protein, transports oxygen in muscle.[6]

3. *Generation and transmission of nerve impulses.* The response of nerve cells to specific stimuli is mediated by receptor proteins. Receptor proteins can be activated by the small molecules such as acetylcholine which are responsible for transmitting nerve impulses at synapses (junctions between nerve cells).[6]

4. *Control of growth and differentiation.* Controlled sequential expression of genetic information is essential for the orderly growth and differentiation of cells. In higher organisms, growth factor proteins such as platelet derived, vascular endolithial and fibroblast growth factors control differentiation. Indeed proteins serve in all cells as sensors that control the flow of energy and matter.[7]

Proteins are abundant in all cells representing approximately 15% of the total cell mass. There are thousands of different types of proteins in even simple cells, but all proteins are derived from the same simple building blocks: a set of amino acids. Amino acids are the basic structural units of proteins. An amino acid consists of a carboxyl group, an amine group, a hydrogen atom and an R group all of which are bonded to an α-carbon atom.



Glycine R = H
Glutamic acid R = CH_2CH_2COOH
Serine R =CH_2OH
Cysteine R = CH_2SH

Figure 3. Some examples of amino acid side chain residues.

There are twenty R side chains which vary in size, shape, hydrogen-bonding capacity and chemical reactivity. Indeed all proteins from every type of species are derived from the same twenty amino acids. It is, therefore, understandable why there is such a huge variety in characteristics in protein structures. The functionality and properties of the three-dimensional structures that are formed are derived from these building blocks and reflect the amino acid combinations or code.

Amino acids range from the simplest, glycine, where the R group is a hydrogen atom and the functionality is limited to larger R groups, such as phenylalanine, where the substituent group is a bulky phenyl group. Other amino acids, such as glutamic acid and serine, have more diverse functionality where the side chains are a carboxylic acid and an aliphatic hydroxyl. There are polar amino acids with hydrophilic side chains. Lysine and arganine are positively charged at neutral pH. Histidine can be charged or uncharged depending on the local environment. Indeed histidine is often found in the active sites of enzymes where the imadazole can readily switch between the two states to catalyze the making and breaking of bonds. The set of amino acids also contain two acidic side chains, aspartic

acid and glutamic acid. Their side chains are almost always negatively charged at physiological pH. Seven of the twenty amino acids have readily ionisable side chains. In proteins the amino acids are conjugated through reaction of the amine of one residue with carboxyl group of another.

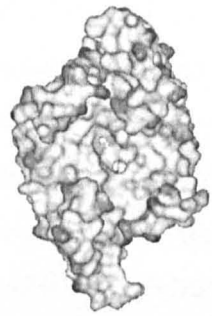


Figure 4. The different colours denote the electrostatic potential surfaces of a growth factor.

Many amino acids can be joined via peptide bonds to form large polypeptide chains. A polypeptide chain consists of a regularly repeating part, called the main chain and a variable part comprising of the distinctive side chains. The main chain is sometimes termed the backbone. Most natural polypeptide chains contain between 50 and 2000 amino acid residues. In this way huge scaffolds can be assembled with varying regions of hydrophobic and hydrophilic domains within the same structure. Shown in Figure 4. is the structure of a growth factor. It is possible to see the diverse topology of the protein and the different areas of hydrophobicity and polarity.

In 1953 Fredrick Sanger[8] determined the amino acid sequence of insulin. It showed for the first time that a protein has a precisely defined amino acid sequence. In each protein the amino acid sequence is specific for the function, and the code is essential for the determination of the function and mechanism of action. A science has developed around the ability to manipulate the sequence of amino acids and to enhance the action of the enzyme or to vary its function. It is therefore possible to manipulate the polarity of the protein by replacing polar residues with more lipophilic fatty acid side chains.

In the same way model systems must have varying regions of polarity and hydrophobicity. This must be achieved in much smaller dimensions than in a macromolecular protein structure through specific functional groups appended to the superstructure of the model system.

1.1.3 Protein structure

A protein is described by its sequence or its primary structure which indicates the order of amino acids in the chain. Amino acid sequences are important because they specify the conformation of the proteins through folding. The remarkable diversity of function displayed by proteins lies in their ability to fold into 3-dimensional structures that bind highly diverse molecules. A stretched out or random polypeptide chain has no biological function. The function arises from conformation. Proteins are well-defined structures due to the rigidity of the peptide bond and the rotational freedom about the single bonds on either side of the peptide bond. This enables a variety of diverse structures with well-defined three-dimensional forms. The freedom of rotation around the peptide bond enables the multiple folding pathways which are determined by the side chain interactions and therefore the amino acid sequence. In the same way artificial systems must be designed to fold in a preordained way which will be accessible to substrates in an aqueous environment. Intermolecular interactions are key to having successful biological mimics.

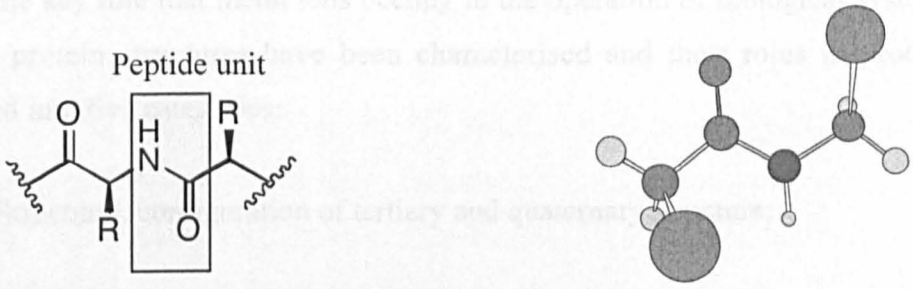


Figure 5. The planar geometry of the amide bond gives proteins structural rigidity.

The folding of polypeptide chains into regular structures was determined by Pauling and Corey[9] who proposed two periodic peptide structures, called the α-helix and the β-pleated sheet (Figure 6.). The structures are stabilised via hydrogen bonding through the NH and CO peptide bond in the main chain. The reason for the significant difference in structure is that in the β-pleated sheet the H-bonding interactions take place between the NH and the CO groups in different polypeptide chains as opposed to the α-helix where the Van-der Waals interactions are intramolecular.

In summary, there are many forces which help to define the structure of proteins: steric interactions, hydrogen bonding, electrostatic interactions and hydrophobic effects where non-polar residues are buried within the core of the protein to avoid unfavourable interactions with the aqueous environment. It is also well established that the tertiary and

quaternary structure of proteins are stabilized by covalent cross-linking between cysteine residues to form disulphide bridges.

The tertiary structure relates to the overall three-dimensional structure of the polypeptide chain and the quaternary structure describes the nature of the interactions between two or more polypeptide chains held together in a precise conformation by non-covalent interactions.

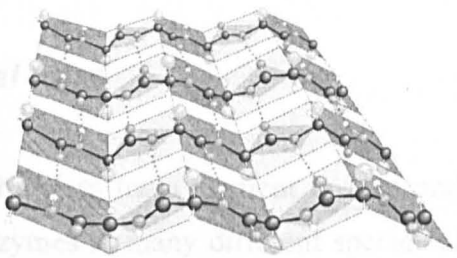


Figure 6. Schematic diagram illustrating the structure of the β-pleated sheet.

Having established the structure of proteins from their primary units it is important to realise the key role that metal ions occupy in the operation of biological systems.[10] Metal sites in protein structures have been characterised and their roles in proteins can be classified into five categories:

(1) Structural, configuration of tertiary and quaternary structure;

(2) Storage-uptake, binding and release of metals in soluble form;

(3) Electron transfer, uptake, release and storage of electrons;

(4) Dioxygen binding, metal-O_2 coordination and decoordination;

(5) Catalytic, substrate binding, activation and turnover.

Our area of work focuses on the binding, activation and turnover of substrates. In particular we are interested in utilising metal ions in our biomimetic systems. We were particularly interested in the operations of zinc enzymes and the direct interactions occurring between the metal binding site and the phosphate substrates in a number of different proteins.

1.2.0 Enzymes

Enzymes are globular proteins whose function is determined by their shape and the nature of the protein structure. The most striking characteristics of enzymes are their immense catalytic power and specificity. By utilising the full range of intermolecular interactions they are able to bring the substrates together with optimal orientation and positioning for chemical bond formation or cleavage. By stabilizing the precise transition state the enzyme determines the reaction pathway that occurs.

1.2.1 Zinc metal ions

Zinc is the second most abundant trace element in man and is required as an integral component of over 100 enzymes in many different species of all phyla. Zinc ions have been found to play structural and active catalytic roles, generally as a strong Lewis acid in its role in biological systems.[11,12] It is known that enzymes which are responsible for the hydrolysis of phosphate esters, carboxylic esters and peptides have one or more Zn^{2+} ions present almost exclusively at the active catalytic site.[13] Research into zinc containing enzymes by Bertini[14] concluded that zinc(II) is preferentially used in nature above other transition metals due to its unique adaptability in forming tetra-, penta- and hexa-coordinated species. Zinc's unique compatability allows the catalytic site to bind and dissociate multiple and varying substrates easily. Two prominent examples of zinc ion binding and activation are illustrated below.

1.2.2 Alkaline Phosphatase

Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups in the 5- and 3- positions from many types of molecules including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation.* As the name suggests, alkaline phosphatases are most effective in an alkaline environment.[15] As is shown in Figure 7, it contains two Zn^{2+} and one Mg^{2+} in its active site.[16] The two zinc ions are about 4.0 Å apart and were found to be essential to enzyme activity. Although in close proximity, the Mg^{2+} ion does not participate directly in the reactivity. Zn1 is coordinated to two histidine units, an aspartagine and oxygen from the phosphate monoester. Zn2 is coordinated to two Asp, one His and oxygen from the phosphate species. Both metal centres are involved in the reaction mechanism and accommodates both phosphoryl transfer reactions.[17] The zinc ions are not only involved in the activation of the nucleophile but also in the leaving group and stabilization of the transition state.

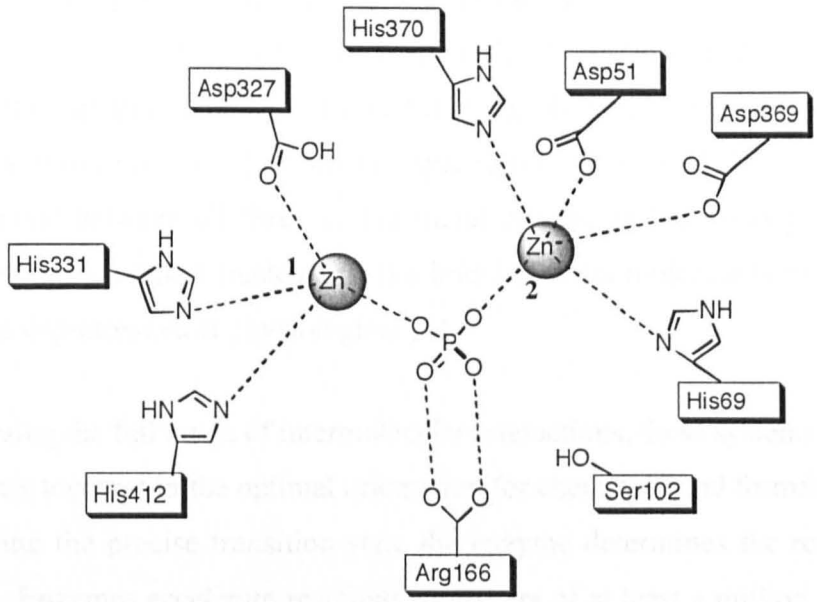


Figure 7. Illustration depicting the binding site of the two zinc metal sites. The phosphate species is held in position by position through a number of electrostatic interactions with both metals and amino acid interactions.

Examples of trinuclear zinc centred enzymes exist. A good example of such a structural motif is Phopholipase C.

1.2.3 Phospholipase C

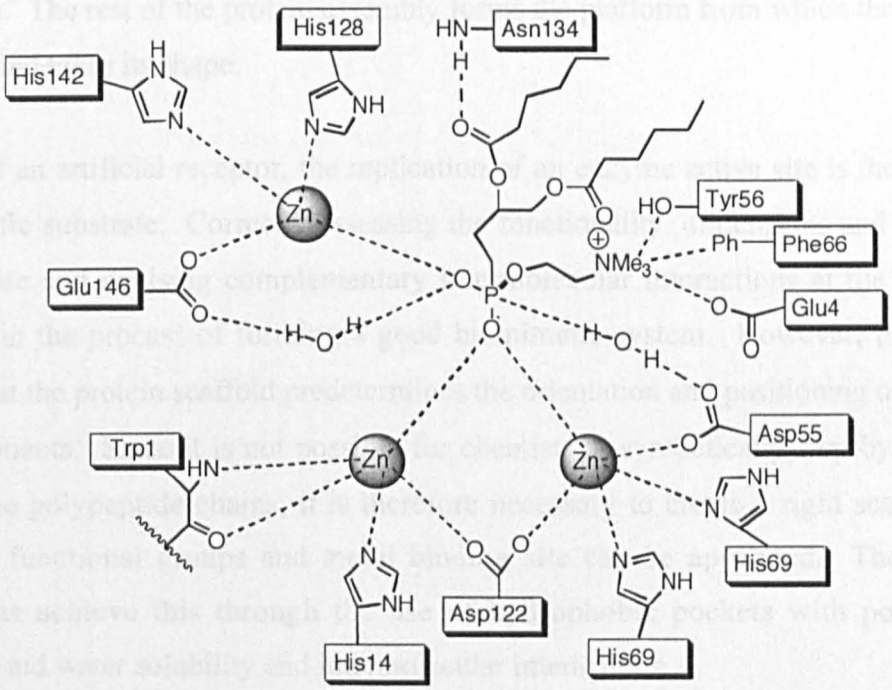


Figure 8. Diagram illustrating the binding of a phosphate species to the Phospholipase C.

Phospholipase C from *Bacillus Cereus* hydrolyses phosphatidylinositol and phosphatidylcholine and is involved in second messenger generation.[18] The active site is

illustrated in Figure 8. Phospholipase C has three Zn^{2+} ions, two of which are strongly bound and in close proximity to each other (3.3 Å) and the third is more distant, 6 Å. X-ray crystallographic data has revealed that the binding of a substrate analogue, 3(S), 4-bis[(hex-anoyloxy)butyl]-1-phosphonylcholine to phospholipase C shows binding interactions between all three of the metal centres and the phosphate species.[19] It is proposed that the active nucleophile is a bridging water molecule between the Zn1 and Zn3 which is deprotonated at physiological pH.

By utilising the full range of intermolecular interactions, these systems are able to bring the substrates together in the optimal orientation for chemical bond formation or cleavage. By stabilizing the precise transition state the enzyme determines the reaction pathway that occurs. Enzymes accelerate reactions by factors of at least a million. An example of the power and importance of enzymes is the role of carbonic anhydrase in the hydration of carbon dioxide. Carbonic anhydrase is one of the fastest enzymes known, hydrating 10^5 molecules of CO_2 per second. This reaction is 10^7 times faster than the uncatalysed one. Enzymes are also highly specific both in terms of the reaction type catalyzed and the substrate selection.

The active site of these enzymes is normally thought of as a pocket shaped cavity in the molecular assembly. It is in this region of the protein that the catalytic action is undertaken. The rest of the protein assembly forms the platform from which the active site is derived and takes its shape.

In terms of an artificial receptor, the replication of an enzyme active site is the ideal host for a specific substrate. Correctly assessing the functionality, dimensions and polarity of the substrate and devising complementary intermolecular interactions at the active site would begin the process of forming a good biomimetic system. However, the problem remains that the protein scaffold predetermines the orientation and positioning of the active site components. Since it is not possible for chemists to synthetically step-by-step piece together the polypeptide chains, it is therefore necessary to create a rigid scaffold upon which the functional groups and metal binding site can be appended. The synthetic chemist has achieve this through the use of hydrophobic pockets with polar groups attached to aid water solubility and intermolecular interactions.

The next chapter gives a very broad overview of the synthetic receptors that have been created to bind anions and in particular the biologically important phosphate species. The nature of binding ionic substrates in a polar environment will also be discussed.

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Chapter 2

2.1.0 Biomimetic systems

Over the last 30 years chemists have endeavoured to replicate enzyme activity in small molecule artificial systems. The massive strides in protein crystallography and the consequent structure elucidation of many proteins and enzymes have allowed chemists to view the active sites of these superstructures. We are now able to see the components that constitute the reaction centre and give enzymes their function.

What are the benefits gained from building and mimicking the function of a particular enzyme reaction centre, when the crystal structure has been obtained? Why is it then necessary to replicate the active site? There are several answers to these questions. Although the crystal structure gives an accurate picture of the metal ions, amino acid side chains, steric interactions and intermolecular bonding, it does not elucidate the mechanism of action. It is possible to surmise the mechanistic route but this cannot be proved without the use of a model system where numerous tests can be undertaken and the chemistry determined. The other problem with X-ray crystallography is that the structure calculated represents the protein in a solid state, and not in its natural aqueous environment. Therefore, it is not possible to accurately know whether the crystalline structure reflects the aqueous situation. In terms of creating artificial receptors, the modelling of certain aspects of the active centre is crucial to the binding and stabilisation of the substrate species.

This chapter will attempt to look at artificial host systems which have been created to act as host sites for both cations and anions. We were particularly interested in the binding of the phosphate species.

2.1.1 Protein phosphorylation

Phosphate is a polyatomic ion consisting of one phosphorous atom and four oxygens. In oxo acids of phosphorous, all the POH bonds are ionisable. Orthophosphoric acid H_3PO_4 is one of the oldest known and most important phosphorus compounds. The acid is tribasic: at 25 °C, $pK_1 = 2.15$, $pK_2 = 7.1$, $pK_3 = 12.4$.[1] The phosphate ions, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} are well known as ligands, of monodentate, chelating or bridging types. Many of the most essential chemicals in life processes are phosphate esters. These include the genetic substances DNA and RNA, as well as cyclic AMP (adenosine monophosphate). In

addition, the transfer of phosphate groups between ATP (Figure 9) and ADP is of fundamental importance to the energetics of biological systems.

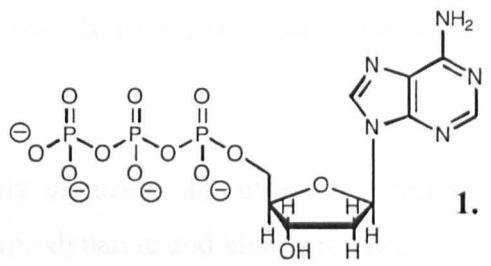


Figure 9. Molecular structure of adenosine triphosphate, (ATP).

The activity of many enzymes, membrane channels and other target proteins are regulated by phosphorylation, the most common type of reversible covalent modification.[2]

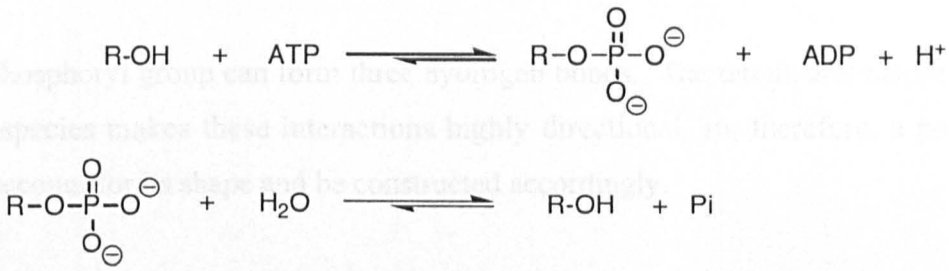


Figure 10. Phosphorylation and dephosphorylation of hydroxyl containing side chain

There are a set of enzymes which catalyze these transformations which are known as protein kinases. These promote the transfer of a terminal phosphoryl group on ATP to specific serine and threonine residues by one class of protein and to a tyrosine residue by another.

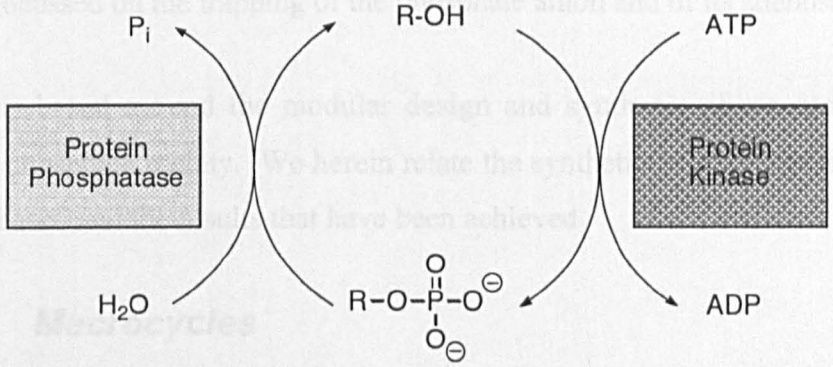


Figure 11. The reactions catalysed by protein kinases and protein phosphatases.

In order to reverse the effects of kinase, catalysts known as protein phosphatases hydrolyse the phosphoryl groups attached to the protein. Phosphorylation and dephosphorylation are irreversible under physiological conditions. It has been found that the hydrolysis of ATP

to ADP and P_i results in a $\Delta G°$ of -12 kcal/mol.[2] This implies that under these conditions the transfomation is highly favourable thermodynamically and ensures that the target proteins cycle unidirectionally between unphosphorylated and phosphorylated form. The rate of the cycling therefore is dependant upon the activity of the kinases and the phosphatases.

Phosphorylation is a highly important and effective means of controlling the activity of proteins for structural, thermodynamic and kinetic reasons.

(1) The addition of a phosphoryl group adds a two negative charge to the modified protein. Electrostatic interactions in the unmodified protein can be disrupted and new ones can be formed. Structural changes such as these can markedly alter substrate binding and catalytic ability.

(2) A phosphoryl group can form three hydrogen bonds. The tetrahedral nature of the phosphate species makes these interactions highly directional, so, therefore, a potential host must account for its shape and be constructed accordingly.

(3) Phosphorylation often causes highly amplified effects. A single activated kinase can phosphorylate hundreds of target proteins in a short space of time. Furthermore, a single target enzyme can transform a large number of substrate molecules.

It can therefore be seen that there is great potential for exploiting the kinase pathways. Small molecule disrupters could be used to modulate the effects of phosphorylation through specifically targeting either the phosphate moiety, ADP or ATP. There has been a lot of work focussed on the trapping of the phosphate anion and of its adenosine esters.

This thesis is based around the modular design and synthesis of receptors capable of trapping the phosphate moiety. We herein relate the synthetic background from which the project was based and the results that have been achieved.

2.1.2 Macrocycles

It is fair to say that the majority of biomimetic chemistry has been based on the utilisation of macrocycles as hydrophobic pockets, or enzyme clefts. So, what are macrocycles and why are they so popular in this field? Why are acyclic chains with the same donor atoms not used instead of the synthetically more difficult to obtain, cyclic derivatives? In

general, it has been found that macrocycles have far more interesting properties than their straight chain derivatives. The majority of macrocyclic metal complexes are both kinetically and thermodynamically more stable. The high stability of macrocyclic metal complexes has led to their utilisation in the construction of model metalloproteins. Macrocycles have been defined as cyclic molecules with three or more potential donor atoms in a heteroatom ring of at least nine atoms.[3] Biomimetic chemistry has focussed around the synthesis of macrocycles as host systems to mimic receptors because of their general rigidity and pre-organisation. Cram[4] noted that a rigid host having all of its binding sites pre-organised in complementarity to the respective guest functions should show the strongest binding. Schmidtchen[5] surmised that since most interactions were cumulative, one must expect that the total interaction energy will increase with increasing numbers of functional groups. Therefore the binding constants should increase the larger and more diversely structured the guest and the better its functional surface and size.

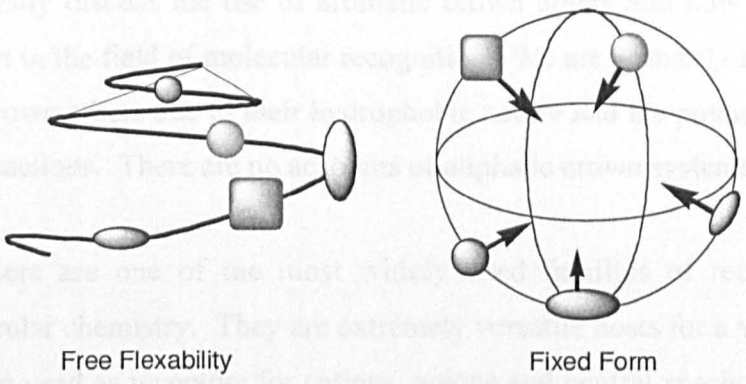


Figure 12. Two different approaches toward guest design. Functional groups arranged in a linear fashion which require folding to define the structure or a preformed molecular scaffold

To this end, there has been a huge focus on having a basic superstructure scaffold which has functionality to partake in binding and upon which separate functionalities can be appended to increase binding enthalpy. This has not however been easily achieved and as yet there are no real examples of artificial hosts which bind to guests with binding constants comparable to that of enzymes. The reasons are due to the difficulty in constructing 3-D arrays of functionality, with correct orientation and positioning within a large framework to maximise binding.

Indeed it is interesting to relate the thinking of Schmidtchen to this problem and understand the unusual way in which synthetic chemists have addressed the problem of mimicking enzymes. Rigid host design is in complete contrast to the modular linear approach adopted by nature albeit through millions of years of evolution. The protein

structure is defined by the placement of functionality at specific positions throughout the protein chain and the consequent folding determines the shape and orientation of the binding features. Schmidtchen argues that the adoption of such a strategy may yield fruits that have not been achieved through the rigid host strategy. None the less the successes and understanding brought through the synthesis of macrocyclic frameworks to mimic biological function has been invaluable.

This chapter aims to give a very brief overview of the history of host-guest chemistry and the attempts by chemists to create functional receptors. In particular we will look at the role of crown ethers and polyazamacrocyles in biomimetic chemistry and the utilisation of cyclodextrin basins.

2.1.3 Crown ethers

We will firstly discuss the use of aromatic crown ethers and how they have formed an integral part in the field of molecular recognition. We are primarily interested in the use of aromatic crown ethers due to their hydrophobic nature and the potential structural benefits of π-π interactions. There are no accounts of aliphatic crown systems in this discussion.

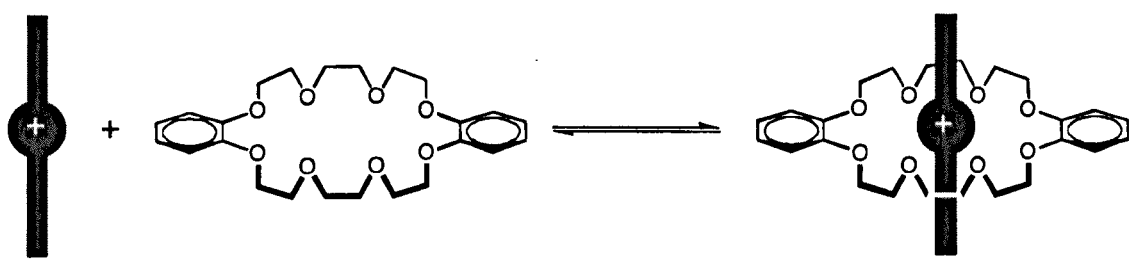
Crown ethers are one of the most widely used families of receptor compounds in supramolecular chemistry. They are extremely versatile hosts for a wide variety of guests, having been used as receptors for cations, anions and neutral species.[6] Crown ethers have most recently come to prominence as synthetic organic models for transmembrane channel ion transport carriers. The synthesis of more varied functionalised crown ethers has thus become an ever-expanding field of research.[7]

The seminal work by Pederson, who discovered aromatic crown ethers in 1967,[8] when he was trying to synthesise a multidentate phenolic ligand, but instead formed 18-dibenzocrown-6, was a cornerstone in the building of supramolecular chemistry. Pederson showed that there was significant binding to cations such as Na^+ and K^+. Indeed, he noted that the choice of base was crucial to the formation of the cyclised material. He realised that there was an element of pre-organisation/self-organisation involved and concluded that the ring closing step, either by a second molecule of bis(2-chloroethyl) ether or catechol, was facilitated by the sodium ion acting as some form of template. Pederson postulated that the compounds appeared to form salt-polyether complexes formed by interactions between the cation and the oxygens. These compounds were also shown to form stable ammonium complexes.

It was discovered that a stable complex formation required complementary crown and metal sizes, if the ion was too big the complex did not occur. The number and symmetry of the oxygens were important to the stabilisation of the host-guest complexation. Complexes were more stable with increasing oxygen atoms, provided that their distribution was symmetrical and the ring was co-planar. An oxygen atom was considered to be co-planar if it lies in the same plane as all the other oxygens in the ring.

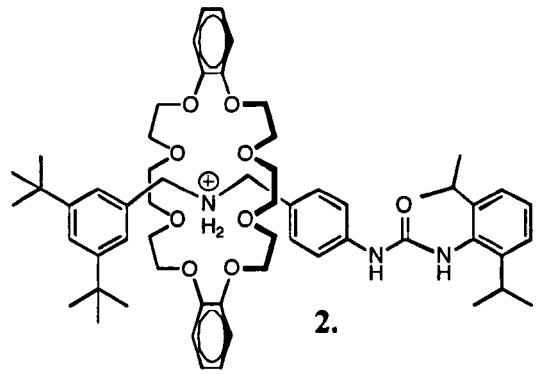
The formation of stable salt complexes in methanol with 18-dibenzocrown-6 was observed with all the alkali metals with the exception of lithium. This was attributed to the strong solvation of its ions. Pederson listed the factors necessary for the formation of a stable complex: the relative sizes of the ion and the hole in the polyether; the number of oxygen atoms in the crown; the co-planarity of the oxygen atoms; the symmetrical placement of the oxygen atoms; the basicity of the oxygen atoms; steric hindrance in the polyether ring; the tendency of the ion to associate with the solvent and the electrical charge on the ion.

2.1.4 *Unfunctionalised crown ethers*



Scheme 1. A schematic representation of a crown ether binding a dialkyl ammonium ion.

The discovery that crown ethers complexed cations so strongly led to much work in the field.[9] Cram[10] and Stoddart[11] have both investigated the properties of crown ether binding with Stoddart looking in particular at secondary alkyl ammonium cation complexation (Scheme 1). It has been shown that the $R_2NH_2^+$ ions are complexed in a threaded, rather than a face to face manner.[12] Stoddart used a variety of dibenzocrown ethers to look at the binding of dialkylated ammonium cations in organic solvents. His studies showed that the size of the ring and the substitution pattern of the aromatic linkers were of prime importance to successful complexation. On going from a catechol (1,2-dihydroxybenzene) substituted 24-dibenzocrown-8 to resorcinol (1,3-dihydroxybenzene) based crown ether resulted in the loss of a stable complex.

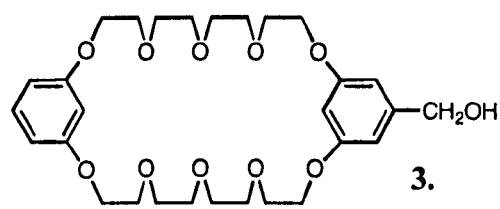


2.

Figure 13. Threaded manner with which substrate binds 24-dibenzocrown-8.

Shown in Figure 13 is an example of a threaded crown ether binding through electrostatic interactions with a secondary ammonium cation. It has been shown in many examples in the literature that crown ethers must be tailored to suit the guest and obtain good complexation. More recently the work of Leigh[13] and Stoddart has reflected this lesson, their crown ethers are specifically designed to cater for a specific substrate. Indeed both groups have developed substrates that can interchange between binding sites dependant upon the solvent conditions through optimal binding interactions. These molecular machines are just one of the current applications with which crown ethers are an integral part.

2.1.5 *Functionalised crown ethers*



3.

Figure 14. Gibson's benzyl alcohol functionalised crown ether.

The functionalisation of these compounds, providing a pendant arm through which a second coordination sphere could be attached was not as well covered in the literature. Indeed it is only in the last five to six years that chemists have approached this problem. The use of covalently linked crown ethers to create function goes back to the seminal work of Gokel,[14] who used a tethered acid unit to provide a linker for multiple crown ether assemblies. More recently Gibson[15] and Beer[16] have constructed benzocrown ether systems with pendant methyl ester functionality and benzyl alcohol derivatives. Shown in Figure 15 is the macrocyclic system created by Gibson to observe the binding of paraquat.

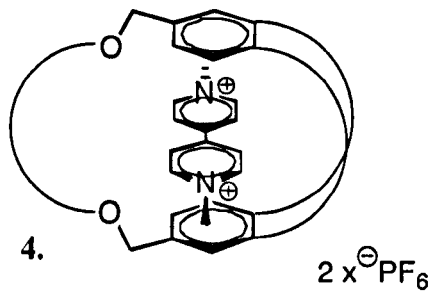


Figure 15. Gibson's difunctionalised crown ether was shown to encapsulate the substrate through complementary π-π bonding.

Crown ethers, used as receptors, can either encircle or fold around a guest molecule. Gibson's group synthesised a difunctionalised dibenzocrown ether which was then covalently linked via a spacer to form a bicyclic system. The unit was shown (Figure 15) to be stronger and more pre-organised than the traditional crown ether system and improved the association constants of a paraquat guest by a 100 times over that of previous rotaxane structures. In this work, favourable binding effects were achievable through the confinement of the guest species within a highly ordered pre-organised scaffold. Gibson's chemistry illustrated the obvious benefits of constructing a structure which fully encapsulated the substrate.

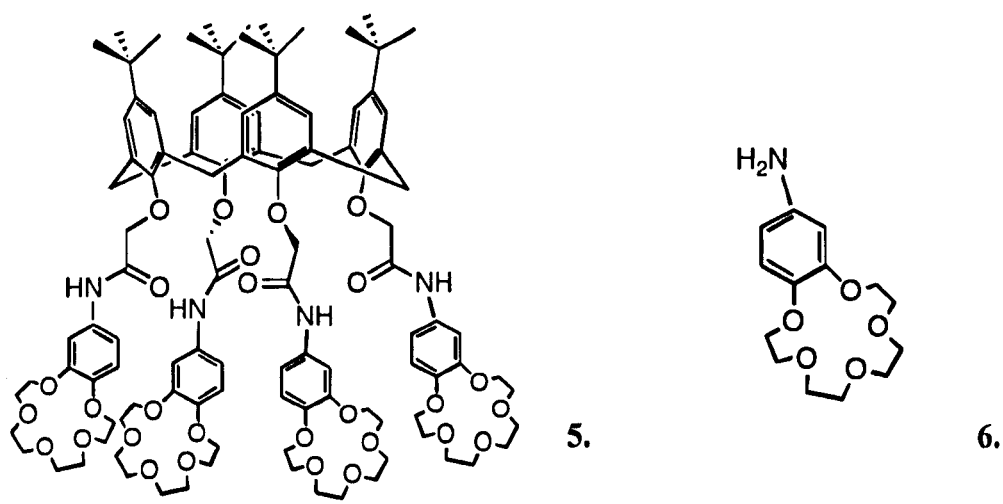
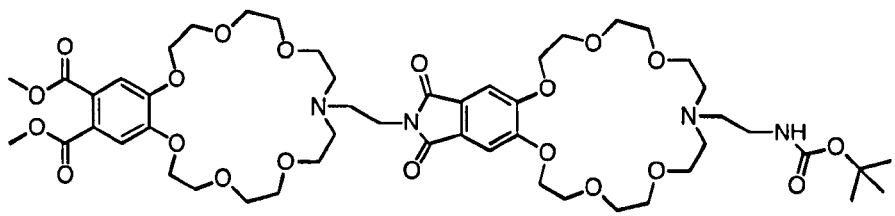


Figure 16. Beer's calixarene functionalised crown ether molecular scaffold and its substituent group.

Beer synthesised a bis(15-benzocrown-5) substituted calix[4]arene that simultaneously complexed cations and anions (shown in Figure 16). The 15-benzocrown-5 derivative **6** was attached to the calixarene via an amide bond. The crown ether was substituted with a primary benzyl amine which was coupled through an amide bond to a calixarene equipped with pendant carboxyl groups. Beer showed in one of the first examples of an artificial ion pair receptor, that ligand **5** could bind a K^+ cation and phosphate anion cooperatively.

The concept of ion pair phenomena and the thermodynamic principles of cooperative binding will be discussed in the next chapter.



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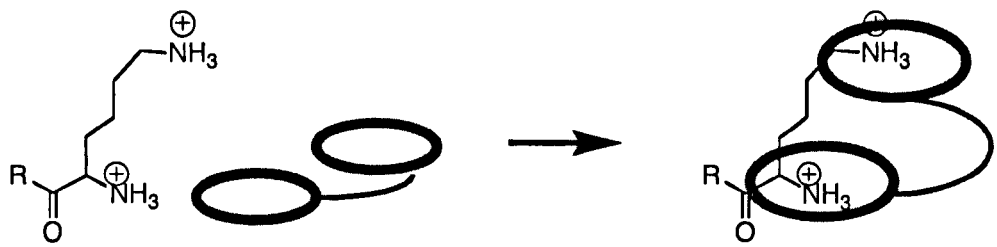


Figure 17. Köenig's substituted benzo-crown receptor system and the proposed mechanism of binding to N-terminal lysines.

Most recently Köenig[17] has covalently coupled two crown ether systems via a short tether. Both crown ethers are functionalised by incorporated methyl ester groups and a phthalic ester or phthalimide. The ditopic system was used to examine the binding of N-terminal lysines in peptides. Due to the differences in each crown ether it was possible to monitor independently, changes to their specific emission properties. The affinity of the ligand to bis-ammonium ions was shown to be distance dependent which allowed the author to distinguish between isomeric small peptides containing a lysine residue in different positions. Köenig's work highlighted the importance of the relative positions of the two components in ditopic receptors to each other. With the correct spacing and geometry the ideal complementarity between host and guest can be achieved.

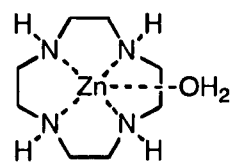
It is of course, vitally important that synthetic receptors selectively bind the desired substrate and be able to differentiate between two similar substrates. To achieve perfect complementarity the synthetic design must allow for manipulation of pocket sizes and incorporate additional functionality which will help to recognize a specific substrate. For this reason, crown ethers have been used frequently in chelation chemistry and in molecular recognition. It is not synthetically difficult to modify the ring size through additional polyether links or aromatic spacers.

The next section of this chapter details the use of polyaza-macrocycles and their importance in metal complexation and the unique properties exhibited in their role in biomimetic chemistry.

2.2.0 Polyazamacrocycles

In the same period that Pederson discovered the oxa-crown ether motif, a range of polyazamacrocyles were becoming available, including van Alphen's[18] 1,4,8,11-tetraazatetracyclodecane or trivially named cyclam.

This section looks in particular at the chemistry of the azacrown macrocycles. Metal complexes of azacrowns have been widely used in biomimetic chemistry for molecular recognition and catalysis. We were interested in this family of compounds because of their excellent reputation as metal binding sites. The polyazamacrocycle moiety is particularly well known for its high binding affinity and stability to metal ions.



8.

Figure 18. The Lewis acidic zinc cyclen complex.

Zinc complexes of polyazamacrocycles have been the focus of a lot of work due to zinc's key roll in many enzymatic transformations. The impetus to discover the role of the zinc ion at the active sites led to a number of model studies which showed that the hydrolysis mechanism of carboxypeptidase A was due to the Lewis acidity of the zinc(II) metal centre.

Kimura[19] showed that it was possible to replicate this function to a certain degree by synthesising the zinc complex of 1,5,9,11 -triazacyclododecane. He demonstrated the potential of his system by calculating the pK_a of the water molecule which occupies the vacant coordination on the zinc ion and showed its greatly reduced pK_a from that of free water. The pK_a was found to be 7.3,[20] nearly two orders of magnitude lower than the value for free solvated zinc ions in aqueous solution. Through a series of model studies involving functional pendant arms, Kimura demonstrated that it was possible to recreate the catalytic action of Carboxypeptidase A on simple phosphate esters. It has not thus far been possible to replicate the mechanism on the stronger peptide bond.

Kimura applied the zinc complex of cyclen in the role of a phosphate receptor. His work was developed after Zn(II)-macrocyclic polyamine complexes had emerged as a novel family of host molecules.

2.2.1 Cationic hosts

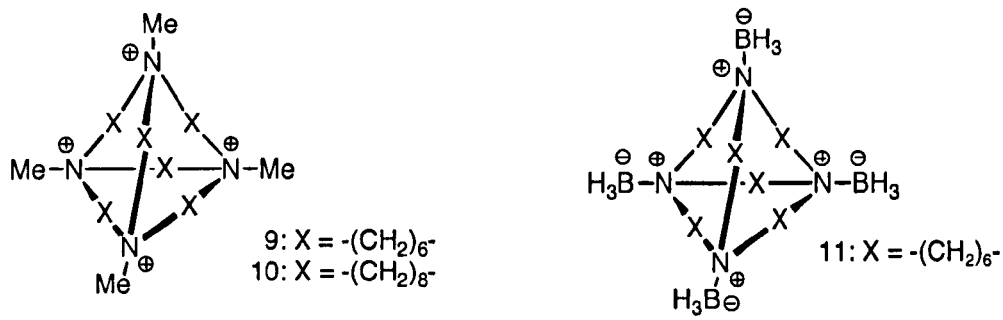
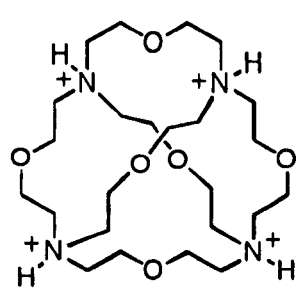


Figure 19. Schmidtchen's macrotricyclic quaternary ammonium hosts.

The earliest examples of cyclic polyamines were by Schmidtchen[21] in 1968. He synthesised macrotricyclic quaternary ammonium hosts **9** and **10**, demonstrating that they could be used to form stable complexes with a variety of anionic guests in an aqueous environment. The theory was to create a three dimensional array of H-bond donating moieties.

Due to the fact that these receptors were positively charged, the binding affinity of the desired substrate was in direct competition with the counter anion. To overcome this problem, zwitterionic host, **11** was synthesised and the binding affinity was shown to be much stronger. These compounds demonstrated size selective binding for Cl^- ions. The majority of synthesised hosts are equipped with acidic hydrogens at complementary positions to hydrogen bond with their guest phosphate oxyanions.



12.

Figure 20. Graf and Lehn's[22] protonated cryptate.

The field further developed when Graf and Lehn synthesised the protonated cryptate which was shown to encapsulate F^-, Br^- and Cl^- anions.[22] There have been numerous examples of positively charged anion receptors that contain protonated nitrogen atoms or metal ions.

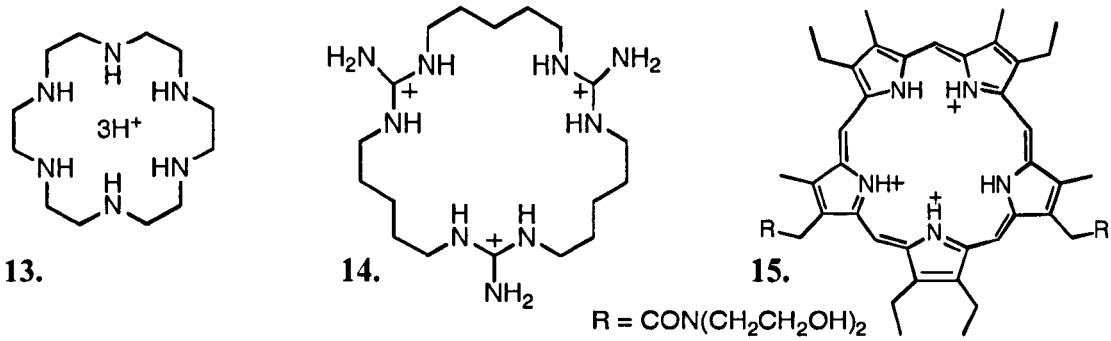


Figure 21. Examples of polycationic polyamines,[24] polyguanidinium cations,[26] sapphyrins.[27]

The 1:1 affinity constants {K_a = [phosphate]/[host][phosphate] (M^{-1})} can sometimes be as high as 10^2 and 10^6 M^{-1} in non-aqueous solutions such as $CHCl_3$, CH_3CN and DMSO.[23] Examples of such compounds are polycationic polyamines,[24] polyquaternery ammonium cations,[25] polyguanidinium cations[26] and sapphyrins.[27] These systems depend upon strong coulombic forces of attraction between the protonated phosphates and the cationic host at neutral pH.

Lehn reported the synthesis of the first macrocyclic guanidinium based receptor. These were shown to have only weak interactions with phosphate (K_a = 50 M^{-1}). Further development by Hamilton,[28] who synthesised bisguanidinium compounds showed markedly improved binding constants (K_a = 5 x 10^4 M^{-1}). Hamilton's work showed the importance of encapsulating the phosphate species with the larger framework.

Sessler[27] looked at the binding power of the porphyrin ring system and it was found that the cavity was too small to incorporate a suitable guest through coulombic interactions with the H-bond donor N-H dipoles. To counter this problem he expanded the ring system to include more pyrrolic spacer groups and found that the sapphyrin group of compounds processed anion-binding affinity. It has a penta-pyrrolic frame which is planar with three convergent N-H dipoles pointing toward the centre of the cavity. These compounds were shown to exhibit great stability and binding strength for the fluoride anion.

These compounds are not however, suitable for operating in a hostile competitive solvent such as water. These convergent clusters of site directed hydrogen bonds couldn't compete against the hydration of phosphate by water which ultimately resulted in poor binding affinities and dissociation of the host-phosphate complex. There are only a number of phosphate receptors capable of operating in a hostile aqueous environment.

2.2.2 Neutral receptors

16.

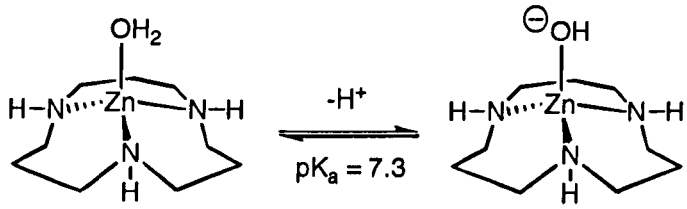
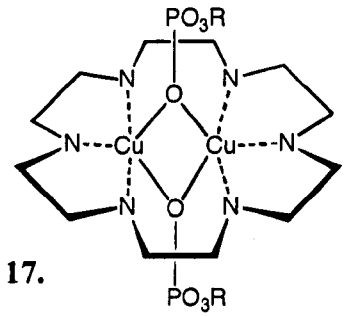


Figure 22. Deprotonation of the Zn^{2+}-[12]aneN$_3$ complex.

The model systems that have been described thus far have been positively charged species which rely upon operation in uncompetitive solvents. In nature, the transport of sulfate or phosphate anions through cell membranes is regulated by neutral anion binding proteins.[29] Their high specificity is due to a recognition site in which the anion is completely desolvated and bound exclusively via hydrogen bonds and Lewis acidic metal centres. To this end we were interested in synthesising a ligand system which was neutral, contained a metal binding site and an encapsulating hydrophobic region which would maximise the ionic interacts through the removal of the disruptive water molecules. We felt that the metal binding site would be best replicated by using Kimura's zinc cyclen complex. It has been shown to be an incredibly useful species in a number of biologically relevant areas.

The development of Zn^{II}-macrocyclic polyamine complexes have attracted a lot of interest. It was found that Zn(II)-1,5,9-triazacyclododecane, **16** binds hydroxide anions very strongly with affinity constants of $K_a = 2.5 \times 10^6\ M^{-1}$ through potentiometric titrations.[20] Other anions such as HCO_3^-, SCN^-, halides and deprotonated sulphonamide were shown to bind to **16** aswell but not to the same degree of strength. The mechanism of action is due to the displacement of the coordinated water molecule to form stable 1:1 complexes. More basic anions show a higher binding constant affinity for the Lewis-acidic zinc binding site. The anion affinity correlates well with the pK_a values of the corresponding conjugated acids of the anions.[30]

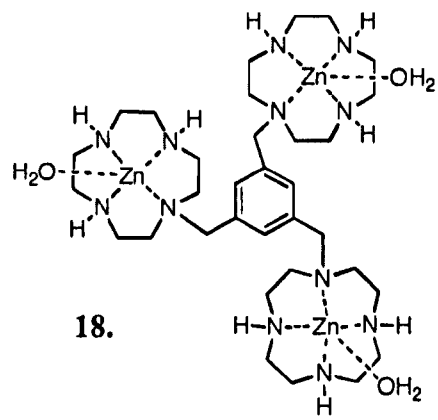


17.

Figure 23. Ren's simple dicopper-[18]ane-N_6 complex bound to phosphate monoester species.

Ren has synthesised a dicopper[18]ane-N_6 complex **17** as the platform for phosphate monoester binding. The dicopper motif was shown to bind phosphate monoesters in high affinity and selectivity. Other anions association constants were at least two orders of magnitude less than a series of phosphate monoesters. The acetate counter anions are displaced with the addidition of the phosphate species to give a 2:1 phosphate to ligand species.

2.2.3 *Multi-metallic zinc-cyclen complexes*



18.

Figure 24. Kimura's phosphate host tris-cyclen model constructed around a benzene scaffold.

Since trinuclear zinc (II) complexes are found at active sites in zinc enzymes such as phospholipase C and P1 nuclease,[31] Kimura[32] constructed a three dimensional molecular scaffold based on a tri-zinc cyclen species **18**. It was shown to bind phosphate dianions in aqueous solution with high affinity dependant on pH. The tripodal ligand was a good receptor for C_3 symmetric organic phosphates in slightly acidic solution with high 1:1 affinity constants, resulting from the cooperative recognition with three zinc(II) ions. By carrying out the experiments in an acidic environment Kimura was eliminating the competitive hydroxide species. This does not truly reflect the physiological environment experienced by enzymes and so to realistically mimic the function, experiments must be carried out under physiological conditions, pH 7.4.

Kimura has also synthesised mono-metallic systems[19] which incorporate one zinc cyclen moiety and pendant arm amine substitution to model the effects of carbonic anhydrase and alkaline phosphatase. They reversibly bind phosphate dianions such as $H_2PO_4^-$, phenyl phosphate (PP^{2-}) and 4-nitrophenyl phosphate in 1:1 complexes with binding affinities of $K_a = 1 \times 10^3\ M^{-1}$. It was observed that the metal free ligand showed no binding affinity with the phosphate dianions.

19.

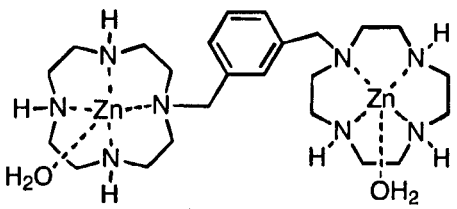


Figure 25. Dicyclen zinc complex tethered by a xylene spacer group.

Dicyclen zinc complexes have been constructed which are linked via a simple xylene spacer.[33] It binds two phosphate dianions in higher affinity ($K_a = 1 \times 10^4\ M^{-1}$) than the mono derivative. In all these interactions it has been shown that the P-O^- moiety becomes a mono-dentate donor. This is in agreement with the binding of phosphate in alkaline phosphatase, carboxypeptidase A and phospholipase C.[34]

In most of Kimura's receptor work the focus has been to replicate the active metal centre and to largly ignore the surrounding environment. There has however been a substantial body of work aimed at mimicking the secondary and tertiary structures of proteins to improve binding through multiple interactions, increasing the enthalpy contribution. Large hydrophobic pockets have been included in the structures to give a positive entropic contribution through the desolvation of the cavity.

2.2.4 *Cyclodextrin host sites*

One of the most interesting and well-studied hydrophobic binding pockets is the naturally occurring cyclodextrins.[35] Cyclodextrins (CDs) are cyclic oligosaccarahides consisting of a-1,4-linked D-(+)-glucopyranose units. The hydrophobic cavity is a distorted bowl shape with primary alcohols located at the narrow rim and the secondary alcohols around the wider rim. Shown in Figure 26 are the basic structures of cyclodextrin basins.

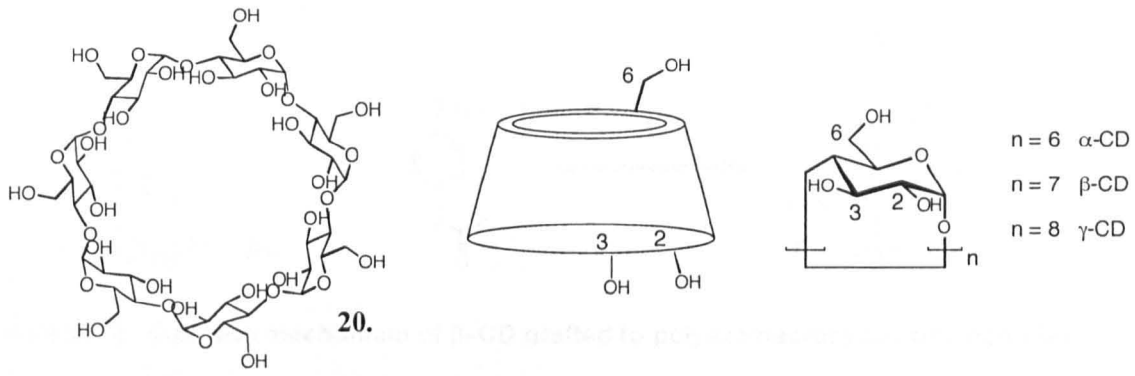
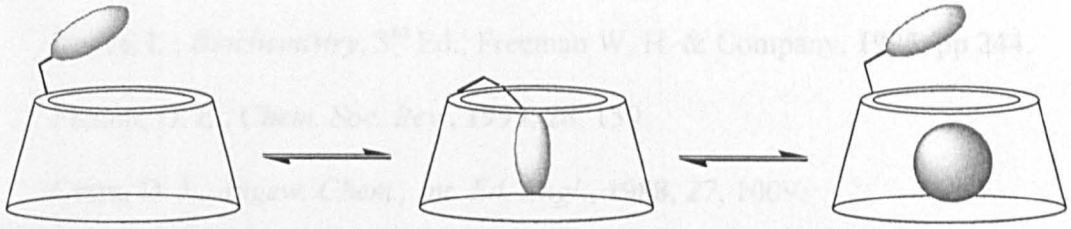


Figure 26. Cyclodextrin basin and the respective positioning of the hydroxyl groups.

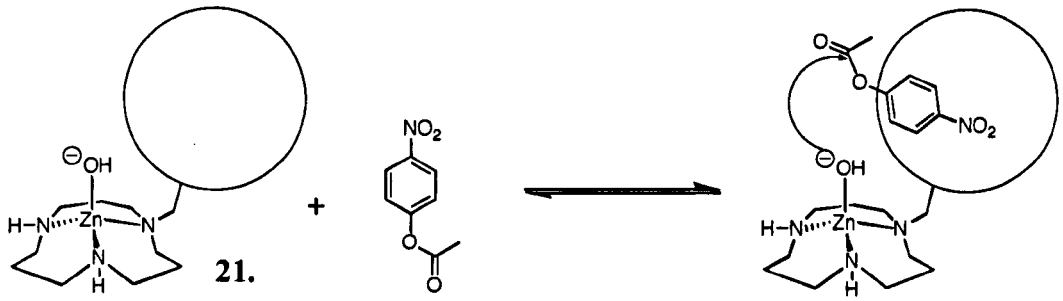
Due to the hydrophobic nature of the CD cavity it is ideally suited toward a hydrophobic guest in an aqueous environment. The primary alcohols surrounding the upper rim and the lower secondary alcohols can be modified to include different functionality to modify the receptors polarity and selectivity toward guest species. There have been many such examples of modified CD's being used to incorporate guests such as creatine by Parker.[36] Kean and co workers have synthesised receptors to cater for the inclusion of carboxylic acids,[37] Yoshida and Ichikawa have prepared systems which selectively bind anilinonaphthalenesulfonate anions.[39]

2.3.0 Lariat-type receptors



Scheme 2. Proposed mechanism for lariat-type cyclodextrin receptors.

An interesting development in the development of CD's was the construction of lariat-type receptors, whereby functional groups were appended to the hydrophobic core. CD synthetic receptors were constructed where the covalently linked group was encapsulated within the pocket upon solvation in an aqueous environment. It was shown that systems could be created whereby the pendant arm could be selectively displaced from the pocket by the desired substrate. Liu *et al.*[39] developed systems which were able to trap chiral aliphatic alcohols using organoselenium and L-tryptophan lariats. α-Amino acid recognition has also been studied at length by Inoue[41] using lariat based CDs.



Scheme 3. Catalytic mechanism of β-CD grafted to polyazamacrocyclic zinc complex.

Of most interest to us was the use of a cyclen or cyclam lariat being grafted into the cyclodextrin system. Our own concept was to incorporate the crown ether to a polyaza-macrocycle. Kim and Lee[40] prepared β-CD with a pendant aza-macrocyle through the reaction of 6-deoxy-*O*-tosyl-β-CD to one of the nitrogens on the macrocycle to form the coupled species. The zinc complex of the aza-crown was formed and hydrolysis experiments were undertaken with simple ester substrates to determine its ability as catalyst and host. It was shown that the substrate did indeed bind to the CD and the ligand gave a 291 fold acceleration of hydrolysis compared with the unsubstituted zinc complex.

2.4.0 References

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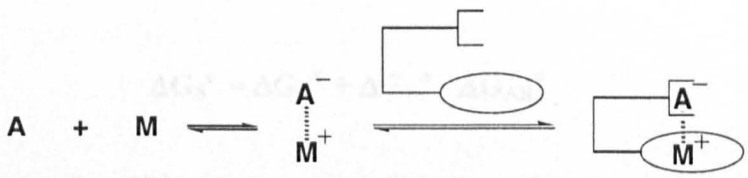
Chapter 3

3.1.0 Ion-pair receptors and cooperativity

Most binding studies examining the effects of anion and cation binding are carried out in organic solvents where ionic bonds have maximal strength and specificity. Furthermore, most experiments have looked at the effect of one ion partner and not the other. Experiments have been designed so that with a non-coordinating cation or anion the observer could examine the individual binding of a specific ion. In the case of anion binding, experiments with *tert*-butyl ammonium salts were performed and with cation binding, the perchlorate or triflate anion used. This methodology should, providing the receptor is well designed, improve the binding of the ion partner, as there is no longer a tether or association between the cation and the anion.



Scheme 4. Ion-pair recognition using heteroditopic salt-binding host.[1]

Unfortunately nature is not as selective and the particular cation or anion rarely has a non-coordinating counterion. Biological systems have to incorporate both ions which have a bond or tether to each other. Indeed it has been shown in many researcher's work that biological systems often exhibit a phenomena known as ion pair binding or cooperativity. Where the binding of one ion is not regarded as independent of the other, it is known as cooperativity.[2] In host systems that bind both the anion and cation cooperatively, the bond or tether existing between the two ions can either hinder or increase the binding affinity. This is known as positive or negative cooperativity.

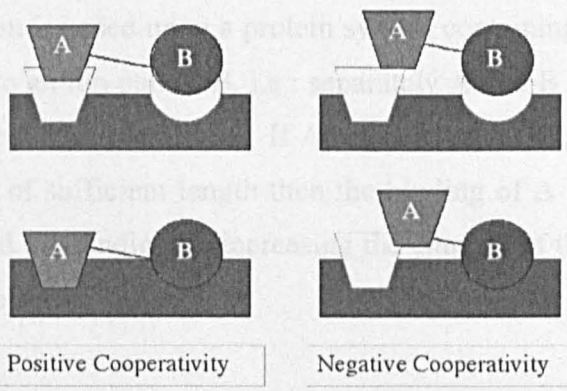


Figure 27. Jencks' model to explain negative and positive cooperativity.[2] **In positive cooperativity the tether is of exact length to allow correct binding, whereas in negative cooperativity the shorter "bond" prevents good docking of ion A.**

In terms of a simple model system shown in Figure 27; where the tether between the ion pair is of ideal strength and size to allow the docking of both ions in their respective "pockets" is known as positive cooperativity. Negative cooperativity follows that the electrostatic interaction between the ions is detrimental to the binding of one ion and therefore the ion pair. In molecular terms the binding of one ion could have either positive conformation effects upon the host and promote binding or counter-wise its binding might negatively affect the conformational complementarity.

3.1.1 Thermodynamics of ion pair binding

In terms of a thermodynamic model, both Jencks[3] and Williams[4] have examined the nature of ion pair cooperativity. In 1981 Jencks formulated an equation which looked at the free energies of the binding event and took into consideration the free energy of the tether that exists between ions, $\Delta G_S°$ and where A-B is the ion pair.

$$\Delta G_S° = \Delta G_A° + \Delta G_B° - \Delta G_{AB}°$$

Jencks proposed that the Gibbs' free energy of binding of the ion pair, A-B ($\Delta G_{AB}°$) is the summation of the free energies of binding for the individual parts A ($\Delta G_A°$) and B ($\Delta G_B°$) and the Gibbs' free energy of connection ($\Delta G_S°$).

Positive cooperativity is observed when the overall energy of binding is greater than the summation of the Gibbs' free energies of binding for the individual components. When the overall binding energy is weaker than the summation of the Gibbs' free energy for individual interactions this is known as negative cooperativity. Therefore, a positive $\Delta G_S°$ represents positive cooperativity and a negative $\Delta G_S°$ represents negative cooperativity.

Jencks proposed that the biggest contributing factor toward positive cooperativity is entropy. His equation is based upon a protein system containing two binding pockets that are complementary to an ion pair A-B, i.e.: separately A and B would bind ideally, but as an ion pair the outcome is not as clear. If A binds to its respective pocket and the tether between A and B is of sufficient length then the binding of A will increase the effective molarity of B and aid the binding by increasing the entropy of the ion pair.[4] Thus positive cooperativity is entropically driven.

Jencks also postulated that negative cooperativity was due to decreased enthalpy. If the tether between A and B is too short to allow the binding components to realize their full enthalpic potential, negative cooperativity will result.

Another view by Williams and Westwell,[3] was that the enhanced binding is due to the increased enthalpy caused by the tether. Williams proposed that positive cooperativity will have a significant enthalpic component and that negative cooperativity can arise from either entropy or enthalpy, dependent on the extent to which they compensate each other. Williams' model relies on high affinity binding having a large exothermic value, ($-\Delta H°$) and less residual motion (small $T\Delta S°$). In simple terms increased contacts between host and guest provide a larger enthalpy component. As a consequence of the increased interactions, the tethering of A-B leads to a more tightly held complex, due to shorter contacts, reducing residual motion and the entropy contribution.

3.1.2 Experimental modelling

In aqueous models and biological systems it has been shown that negative cooperativity is the norm. Practical experimentation by Anslyn [5] to examine the thermodynamic nature of negative cooperativity was done using a tripodal ligand system. Anslyn used isothermal titration calorimetry to examine the thermodynamic effects of the separate ion components, to see whether entropy was the main contributing factor toward negative cooperativity. The ligand design was a simple cryptand scaffold with three pendant ammonium binding groups and a copper metal binding site at the centre.[6] This gave four potential binding sites and so he used four different carboxylic acid complementary guests: 1,2,3,4-butanetetracarboxylate; tricarballate; glutarate and acetate.

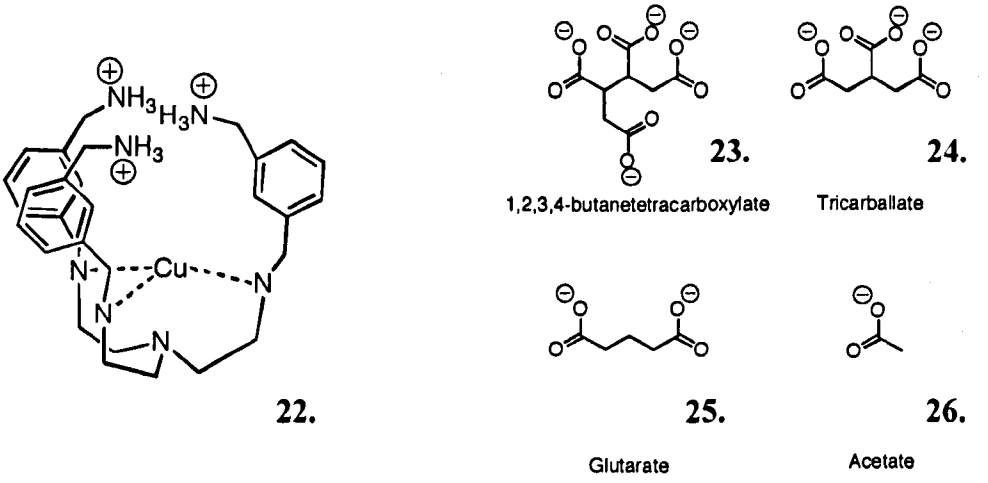


Figure 28. Anslyn's cryptand and the structures of the various model binding components.

In terms of an ion pairing model system, two glutarates are equivalent to one 1,2,3,4-butanetetracarboxylate. Anslyn postulated that he could then measure to a certain degree of accuracy the $\Delta G_{AB}°$ (1,2,3,4-butanetetracarboxylate) and the Gibbs' free energies of the component parts, $\Delta G_A°$ (glutarate) + $\Delta G_B°$ (glutarate). In the same way tricarballate (ΔG_{AB}) is equivalent to the binding of glutarate ($\Delta G_A°$) and acetate ($\Delta G_B°$).

Anslyn found in every combination of experiments, that the cooperativity was negative, i.e.: when the 1,2,3,4-butanetetracarboxylate is considered as the combination of two glutarates, or even one tricarballate and one acetate, negative cooperativity is found, ($-\Delta G_s°$). Of thermodynamic interest were the differences in binding of the four different species. Acetate and glutarate were found to be endothermic and entropy driven, as the main means of binding were directly to the Cu(II) metal centre. The entropic gain was assigned to the desolvation of the cavity, a point which has been verified in the literature.[7] On the other hand the binding of tricarballate and 1,2,3,4-butanetetracarboxylate was shown to be primarily entropic but the additional carboxylate interactions offered favourable enthalpy to enhance the binding through increased electrostatic interactions. Thus the increasing carboxylate interactions convert the endothermic binding into an exothermic event. This was in agreement with Williams' theory, as the number of binding interactions increase they become more exothermic because there is less residual motion and the contacts are tighter.[4]

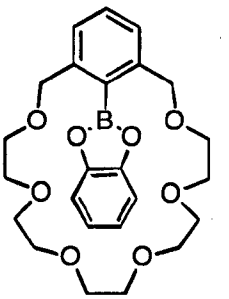
Another interesting point that was examined in these experiments was whether the entropy factor would increase with the increasing size of the substrate due to the desolvation of the cavity. It was found that the entropy did indeed increase when going from acetate to glutarate but decreased on going to the tricarballate and 1,2,3,4-butanetetracarboxylate. On going from acetate to glutarate there was larger desolvation, but on going to the bigger substrates the improved solvent expulsion was countered by the formation of a more ordered and rigid structure. Lower entropy was the result of the decreased residual motion within the complex.

Anslyn concluded that entropy was the origin of negative cooperativity. The loss in entropy for A-B was associated with the loss of residual motion and decreased solvent/counter ion release upon binding A-B versus A and B. Anslyn determined that the primary contribution to the decreasing entropy was the lower release of solvent.

The next section discusses the models which have been specifically synthesised to look at the ion pair phenomena.

3.2.0 Ion pair receptors

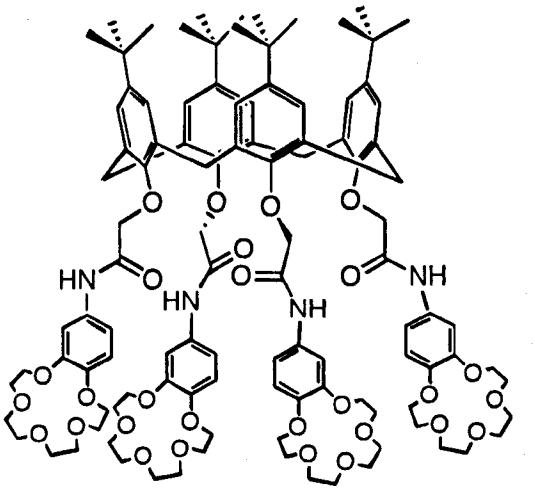
Simultaneous anion and cation recognition has opened up a new field in the already well-developed cation and anion coordination chemistry.[8] The synthesis of heteroditopic receptors with the ability to coordinate both ion partners has become the goal of many bioinorganic chemists. These systems have potential as new selective extraction and transportation models for ion pair species of biological importance and for zwitterion recognition.[9] In order to bind both ions chemists have looked to combine anion and cation coordination chemistry. The fusing together of two different hosts has become the key to establishing a good system. The most popular choices of hosts were macrocyclic crown ethers, calixarenes, cavitands, cyclodextrins and pyrrolic-based cages.



27.

Figure 29. Reetz's crown ether complex simultaneously binds F^- and K^+ ions.

One of the first examples of an ion pair receptor was by Reetz,[10,11] who synthesised a 21-benzo crown-6 with a pendant boronic ester. It was shown that this system could bind a fluoride anion as well as a potassium cation. The system was stabilised through electrostatic interactions and orbital overlap with the Lewis acidic boron.



5.

Figure 30. Chemical and schematic diagram of ditopic calixarene based receptor.

Beer has synthesised a number of novel ditopic bis(benzocrown ether) substituted calix[4]arenes which simultaneously complex cations and anions.[12] As has been discussed in chapter 2, Beer used the calixarene hydrophobic pocket as a host site for the anion trapping. By substituting the lower ring with four benzo-15-crown-5 units attached via amide bonds, he formed an ideal cation binding site. Through the combination of all these functional groups it was possible to construct a multidentate scaffold. Using proton NMR titrations, metal binding could be observed by monitoring the shift of the amide proton during ion-pair complexation. Beer's experiments identified the existence of simultaneous 1:1 stoichiometries for the binding of the anion in the lower rim of the calixarene and the cation bound in the crown ether domain. Interestingly in the absence of an alkali metal cation there is no affinity for anions. So the binding of one ion is dependant on the association of the other through ion pair cooperativity.

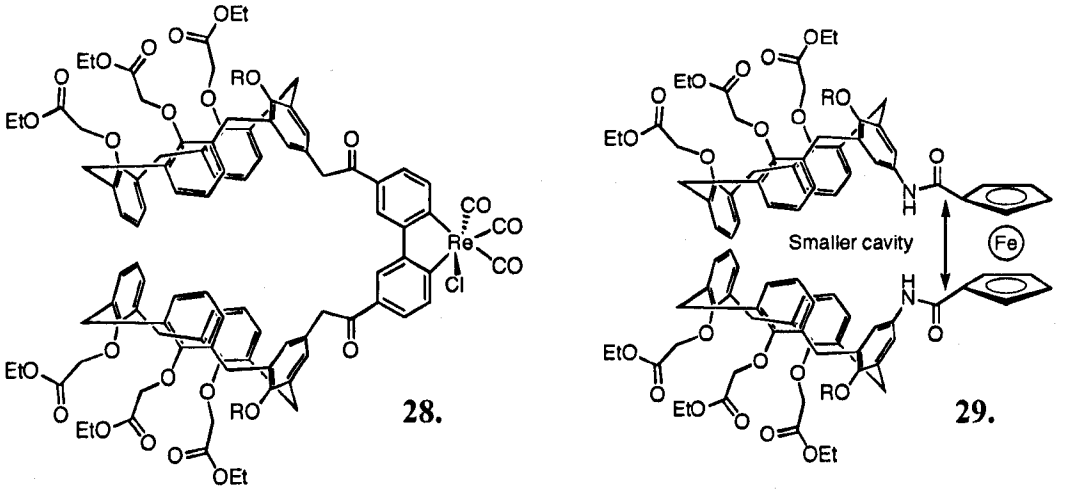
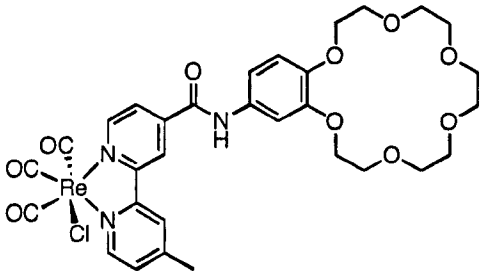


Figure 31. Beer's rhenium centred and ferrocene centred bis calixarenes.

Beer has since made a family of these cooperative binding ligands.[9] Complex systems which are based around rhenium[13] and iron[14] metals have been synthesised. The structures of two of these compounds are shown above in Figure 31. The bis(calixarene) rhenium(I) receptor **28** has been shown to bind alkali metal ions and iodine anions with positive cooperativity in organic media. In an analogous experiment the central bridging core was replaced by a substituted ferrocene derivative. However the lack of binding for halides in this system was associated with the free rotation around the iron (II) metal axis. The subsequent decrease in pre-organisation of the scaffold resulted in a reduction in binding affinity for the anion. Beer also noted that the distances between the amide bonds on the adjacent cyclopentadienes might be too distant to promote stabilisation of the structure through H-bonding and coordination of the anion.



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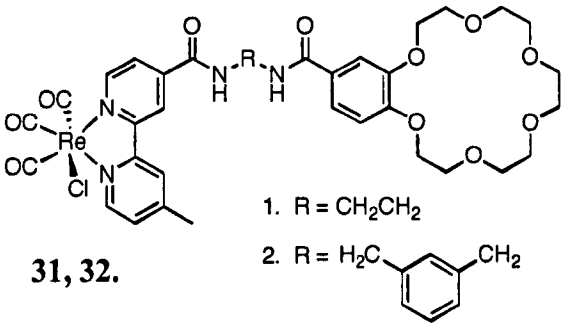
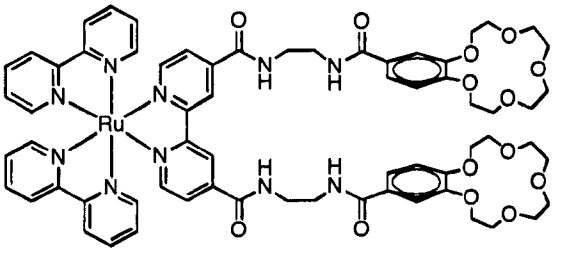


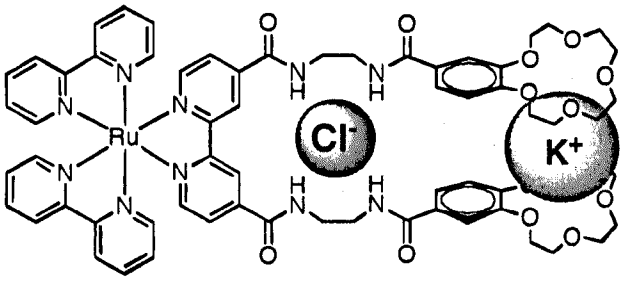
31, 32.



33.

Figure 32. Rhenium(I) and ruthenium(II) based ion-pair receptors.

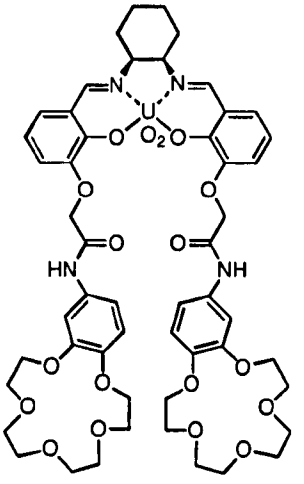
Shown above in Figure 32 are some of the rhenium(I) and ruthenium(II) based ion pair receptors synthesised by Beer and Dent.[2] X-ray crystallographic data showed that the cation binding was through ion-dipole interactions with the crown ether(s). Anion binding was achieved through hydrogen bonding to the bipyridine protons and bipyridine amide NH protons. It has been shown through a series of studies[15] that the diameter of K^+ (2.66 Å), is complementary for binding to 18-benzocrown-6 moieties (2.6-3.2 Å). Since 15-benzocrown-5 has a smaller diameter (1.7-2.2 Å), it was found for compound **33** that an intermolecular sandwich complex was formed. There is precedent in the literature for the formation of K^+ based intermolecular sandwich complexes with crown ethers which are smaller than the cation.[16] The 18-benzocrown-6 derivative was found to bind Cl^- and AcO^- anions. Beer wanted to look at the cooperative affect of using the K^+ salt and to determine whether the inclusion of the K^+ aided or hindered the association of the anion. In all cases the ligands displayed preferential binding for acetate over chloride which was assigned to the different basicities of the anions.



34.

Figure 33. Ruthenium(II) based ion pair receptors with pendant 15-benzocrown-5.

In the presence of the coordinated K^+ ion, positive cooperative binding was dependant upon the distances between the two binding sites. In the case of receptors **30** and **31** where the spacer groups are smaller, this promoted through space electrostatic interactions between the ions, the result was positive cooperativity and improved anion binding affinity. However receptor **32** did not experience this affect due to the longer *m*-xylyl spacer groups between anion and cation binding sites. The tether between the two ions was not long enough to allow the cooperative binding to occur. Beer showed that it was possible to dictate, through well-designed scaffolds, systems capable of ion pair cooperativity which display high affinity for a specific ion pair. His structures also reiterated the importance of the tether between ions.



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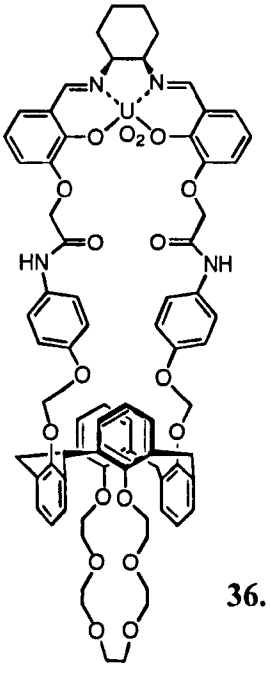


36.

Figure 34. Reinhoudt's uranyl salophen based ion pair receptors.

Reinhoudt[17] used a similar approach, but chose anion binding uranyl salophen based groups instead of the calixarene host. The cation binding sites were constructed from crown ethers (**35**) and an amalgamation of calixarene and crown ether species (**36**). The structures of two of the scaffolds are shown in Figure 34. Using mass spectrometry (FAB-MS), Reinhoudt was able to identify a signal which corresponded to the [**35** + K^+ + $H_2PO_4^-$]$^-$ complex.[18] By replacing the crown ethers with 1,3-alternate calix[4]arene crown 6 he was able to selectivity bind CsCl.[19] Further replacement of the 1,3-alternate calix[4]arene crown 6 with the Na^+ selective calix[4]arene tetra(ethyl ester) showed intense peaks for the binding of **36** + $H_2PO_4^-$, and also for **36** + Na^+.[20]

Smith used functionalised crown ethers to examine the effect of ion pairing.[1] Smith showed that anion binding by neutral hosts in organic solvents could be inhibited by the presence of alkali metal cations. Smith constructed a set of four ligands; a monotopic urea derived ligand and three ditopic ligands with the same urea functionality, but with different covalently linked crown ethers of varying sizes to allow selective cation binding. The four different structures are detailed in Figure 35.

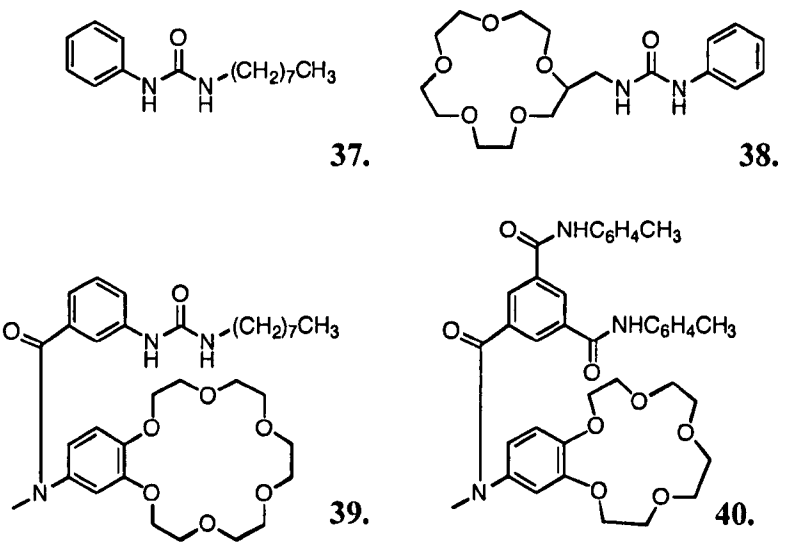


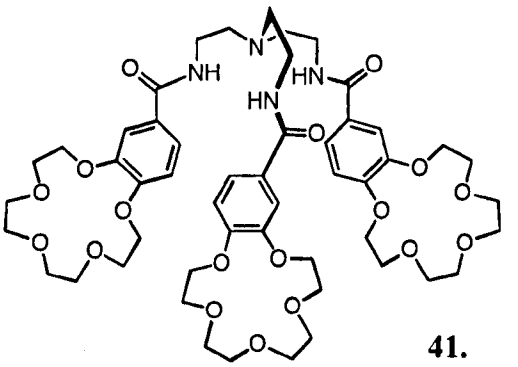
Figure 35. 4 different ligands synthesised by Smith to investigate cation cooperativity.

Smith found that in the case of monotopic compound **37**, that there was no positive cooperativity exhibited by the cation upon anion binding affinity. When the anion was coordinated in the presence of a non-coordinating counter ion strong binding affinity was observed. In the presence of a number of alkali metal cations the binding was dramatically reduced. Smith showed that this trend could be reversed by using heteroditopic receptors which catered for the cation binding.[21] It was postulated that cooperativity was dependent upon two major factors; the size of the cation and the basity of the anion.

Firstly, the cooperativity factor for a specific host/anion system increases with decreasing metal cation charge to surface area ratio, i.e., $Na^+ < K^+ < Cs^+$. Secondly, the cooperativity factor for Smith's host/cation system increased with decreasing anion basicity, i.e., $Cl^- < Br^- < I^-$ and $CH_3CO_2^- < NO_3^-$. In certain cases (when one or both of the ions are small and charge-localized), this inhibition effect was still observed with the crown containing hosts **38-39** which suggested that the crown-encapsulated cation was still able to sequester the anion away from the host's NH residues. This is in agreement with the abundant crystallographic literature showing anions axially coordinated to metal cations that are simultaneously complexed inside crown ethers. [22,23]

However it is important to note that these experiments were carried out in organic solvents. The effects seen within an organic media, are severely hindered in a competitive aqueous environment. We wished to look at the binding of ions at physiological pH in an aqueous environment. There have been a few recent examples of ion pair receptors which have been operational in physiological conditions. Anslyn, Beer and others have created systems which are ion receptors at pH 7.4.

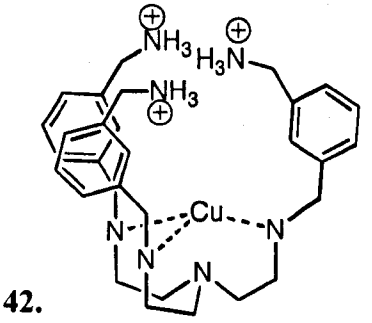
3.2.1 Anion receptors in an aqueous environment



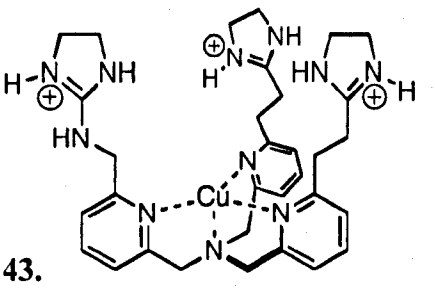
41.

Figure 36. Tren based 15-benzocrown-5 radioactive pertechnetate sequestering reagent.

Beer and co-workers[24] have synthesised a tren (tris(2-aminoethyl)amine) based scaffold with 15-benzocrown-5 pendant amide arms. The system was designed to sequester radioactive pertechnetate anions from aqueous solution which are produced during the nuclear fuel reprocessing process. It was shown to efficiently chelate the anion in an aqueous enviroment but was less effective without the presence of a co-bound cation. Importantly, Beer had employed a polyamine structure with crown ether functionality which was water soluble and operational within an aqueous environment.



42.



43.

Figure 37. Ammonium and guanidinium copper based phosphate receptor.

Anslyn [25] synthesised two similar receptors based on a copper centered cryptand structure with varying pendant arms. One receptor was functionalised with three ammonium groups

and the other with guanidinium functionality. Both receptors were shown to have high affinity with the phosphate anion at physiological pH in a 98: 2 water: methanol solution (K_a = 1 x 10^4 M^{-1}). Anslyn proposed that the binding occurs through ion pairing between the charged functional groups on the host and substrate. Through the use of ITC Anslyn was able to determine that the binding of phosphate to **42** was primarily entropy driven through the desolvation of the cryptand cavity. Receptor **43** was shown to display favourable enthalpy and entropy changes. The primary differences in thermodynamic data were related to the different solvation spheres of the receptors. The larger and more organised solvation of the ammonium groups meant that on binding, larger quantities of solvent are released hence the increased entropy. In the case of guanidinium ions it has been shown that the solvation sphere is considerably less organised[26] so the desolvation did not have the same entropic driving force.

The studies showed that the largest binding free energy was due to phosphate complexation to the copper metal centre. Anslyn demonstrated that although the metal centre dominated the affinity, the reasons for such strong selectivity for the phosphate tetrahedral oxyanion were the appended functional groups. The increased ion pair contacts, preorganization and size gave the desired complementarity which induced such strong binding.

3.3.0 Conclusions

It can be seen that the field of ion pair receptors is a growing area of bioinorganic chemistry. The seminal work by Anslyn,[5] Beer,[12] Inoue[8] and others have helped to gain an understanding of how to best incorporate the anion and cation in an artificial receptor. The complementary use of anion and cation binding sites incorporated within the same host has been the most effective format for successful binding affinity. We must also consider the thermodynamic issue involved in a binding event. Anslyn's work has shown that the promotion of positive entropy through desolvation is a key factor in successful binding.

Both Anslyn and Beer, have shown that the length of the tether between the ion pair is of crucial importance, have substantiated Jencks' findings through their work. If a heteroditopic system can be constructed which has binding sites complementary to the ion pair and the distance between the ions then high affinity binding can be achieved with positive cooperativity.

3.4.0 References

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Chapter 4

4.1.0 Synthetic strategies

The search for the unifying structural motif that selectively and strongly binds the phosphate moiety has remained elusive to chemists.[1] As has been established in the introduction, many systems have been developed to achieve this goal but enzymatic binding constants have not yet been realised. A common theme in such systems has been the inclusion of a hydrophobic pocket through the use of cryptands,[2] calixarenes,[3] cyclodextrins[4] and cyclic polyamine structures.[5] Equally, the use of metal template scaffolds have been used to similar effect in forming a large cavity with a metal based centre.[6] In almost all examples of these systems, zinc or copper is utilised for this purpose, templating cyclic or acyclic poly-aza/oxa compounds into an ordered complex. In order to improve upon the current artificial phosphate binding systems, a new approach had to be developed. The compound design, tried to incorporate many facets of organic and bioinorganic chemistry, to best mimic the phosphate-metal binding environment. It is obviously apparent that in our model systems we cannot hope to construct a highly ordered macromolecular superstructure such as a protein, but we can incorporate key features of proteins to mimic their function on a smaller scale. In an ideal situation we could establish multiple metal binding sites and regions of hydrophobicity and polarity which would complement different phosphate containing moieties within our model compound.

4.1.1 Ligand design and template

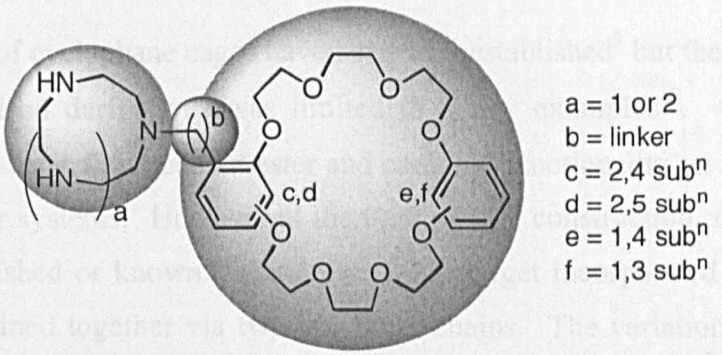


Figure 38. Schematic diagram illustrating the ligand design which incorporates a number of different aromatic substitution patterns and polyazamacrocycle sizes.

The design motif (Figure 38) was based upon incorporating both a metal binding site and a hydrophobic region. The overall structure was hydrophilic to enable the compounds to

operate in an aqueous environment. The ligand system involved the coupling to a polyazamacrocycle which could be altered in size to incorporate different metal centres. The polyazamacrocycle has a dual function, acting as both a metal binding site and providing a polar component. Both cyclen and 1,4,7-triazocyclononane were employed to co-ordinate a metal ion, either copper or zinc. The chemistry of these metals when coordinated to the macrocycles has been well established and has been described in the introduction.[7] The polyazamacrocycle can then be coupled to an aromatic crown ether which has been shown to be an excellent ampiphilic binding pocket through the work of Stoddart.[8] There were obviously many methods by which these compounds could be assembled and the challenge was to find the most appropriate and optimal methodology. From the outset, the two main synthetic challenges were the formation of the functionalised crown ether ring and the coupling of the crown ether to the polyazamacrocycle. We shall firstly address the challenges to the synthetic chemist, confronted by the construction of a functionalised crown ether and discuss how the difficulties faced were eventually overcome.

4.1.2 Cyclophane synthetic strategy

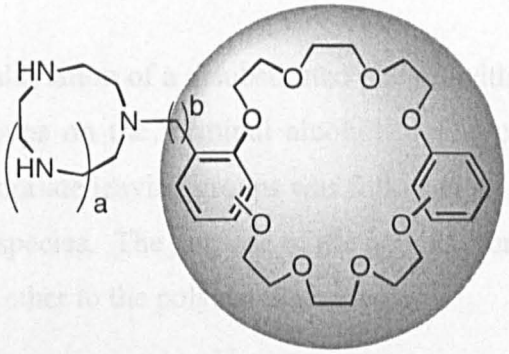


Figure 39. The aromatic crown ether structure in the ligand structure.

The synthesis of cyclophane cages have been well established[9] but the literature precedent for functionalised derivatives was limited to a few examples[10]. Gibson[11] has since constructed systems with pendant ester and carboxyl functionality on a 32-dibenzocrown-10 crown ether systems. However at the time of ring construction, these methodologies were not published or known. The crown ether target incorporated two aromatic rings which were joined together via two polyether chains. The variation of the substitution pattern of the aromatic rings, allowed strict control of cavity folding and the rotational freedom of the macrocycle. The aromatic linkers in the polyether cyclophane pocket were included to promote beneficial folding by π–π interactions, forming a three dimensional hydrophobic scaffold.

The design incorporated a metal binding site which was encapsulated/shielded by the close proximity of the hydrophobic pocket and it was rationalised that this effect was beneficial for the efficient trapping of the phosphate moiety.

Retrosynthetically, there are **two** possible routes by which we could approach the cyclophane synthesis.

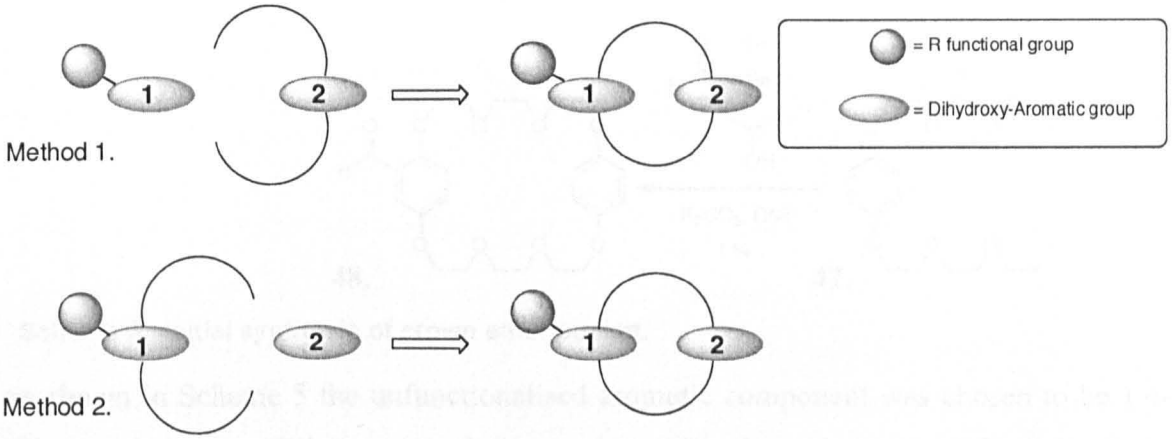


Figure 40. Schematic diagram illustrating different methods of crown ether construction.

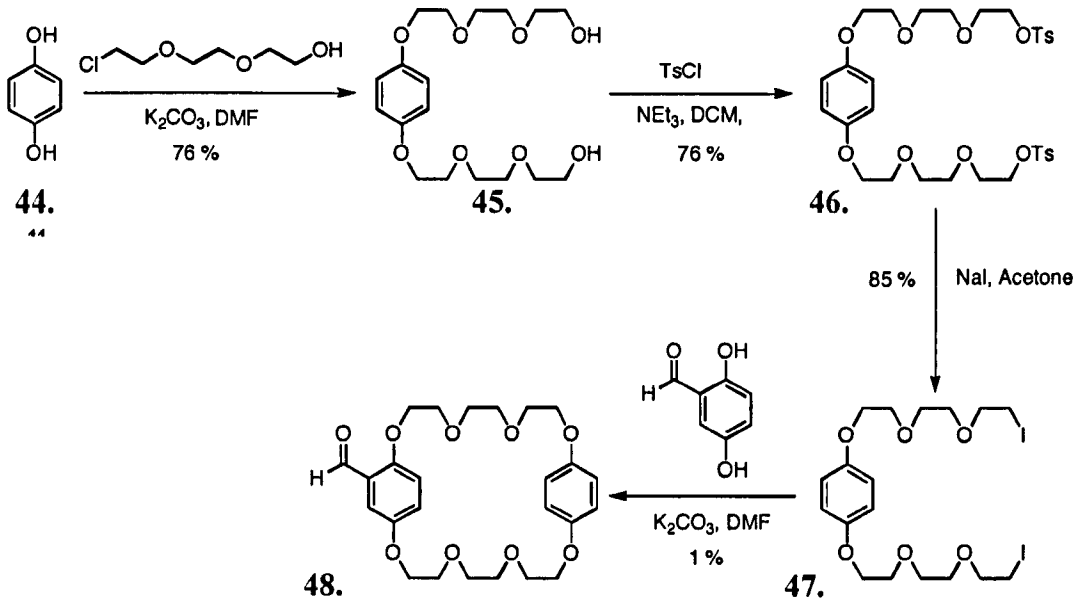
Method 1.

Method 1 involved the alkylation of a disubstituted phenol with two polyether chains with appropriate leaving groups on the terminal alcohols. The conversion of the terminal hydroxyl groups to appropriate leaving groups was followed by ring closure with a suitable functionalised phenolic species. The purpose of the pendant functionality was to enable to attachment of the crown ether to the polyaza macrocycle.

Method 2.

The alternative method was to incorporate the functionalised aromatic ring in the first step. The reason for this modification in ring construction was due to the electronic effects that the functional group may induce. For example, if the pendant group were electron withdrawing, the nucleophilicity of the hydroxyls on the benzene ring would be reduced. The loss of reactivity during the low yielding cyclisation step was not desirable and inclusion of the electron withdrawing aromatic linker at an earlier stage was preferable.

4.1.3 *Electron withdrawing cyclophane synthesis*



Scheme 5. Initial synthesis of crown ether pocket.

As shown in Scheme 5 the unfunctionalised aromatic component was chosen to be 1,4-dihydroxybenzene. This gave maximum cavity size and rotation around the linker axis. The di-alkylation of resorcinol with 2-[2-(2-chloroethoxy)ethoxy]ethanol in the presence of K_2CO_3 in DMF yielded the desired product **45** in reasonable yield.[12] The terminal alcohol units were converted to tosylated leaving groups **46** in 75 % yield. Cyclisation to form the macrocycle using high dilution techniques with 2,5-dihydroxybenzaldehyde as the functionalised linker were unsuccessful. Cyclised product was never obtained despite the use of a variety of conditions and varying bases. This was attributed to the tosyl groups not being labile enough to initiate ring cyclisation. In such a sterically unconstrained "floppy" system, a more reactive leaving group was required. Substitution of the tosylate groups to the more labile iodo-species **47** using the Finckelstein reaction[13] was achieved quantitatively.

Attempts to cyclise with 2,5-dihydroxybenzaldehyde with a series of bases (K_2CO_3, NaH, Cs_2CO_3), solvents (THF, DMF, CH_2Cl_2) and temperature ranges were ultimately unsuccessful. Product **48** was only observed with the use of K_2CO_3 in DMF at high temperatures. With yields of less than 1 %, marginally improved by the increased length of injection time, it became apparent that a different synthetic approach was required.

The poor conversion can be associated with two specific factors. Firstly, in both aromatic units the hydroxyl groups were *para* to each other, therefore the maximum expansion of the ring size was achieved. The yields were consequently lower than when compared with

the synthesis of smaller crown ethers.[14] The other variable which needed to be addressed, was the order in which the components were assembled. The dihydroxybenzaldehyde derivative is an electron withdrawing species, consequently reducing the nucleophilicity of the deprotonated hydroxyl groups and therefore, the overall reactivity of the cyclisation step. Inclusion of the electron-withdrawing dihydroxybenzaldehyde at an earlier, less significant step in the synthesis would improve yields and increase the cyclisation yield.

Base	Solvent	Temp. °C	Yield %
NaH	THF	78	0
Cs_2CO_3	DMF	90	0
K_2CO_3	DMF	90	0.5

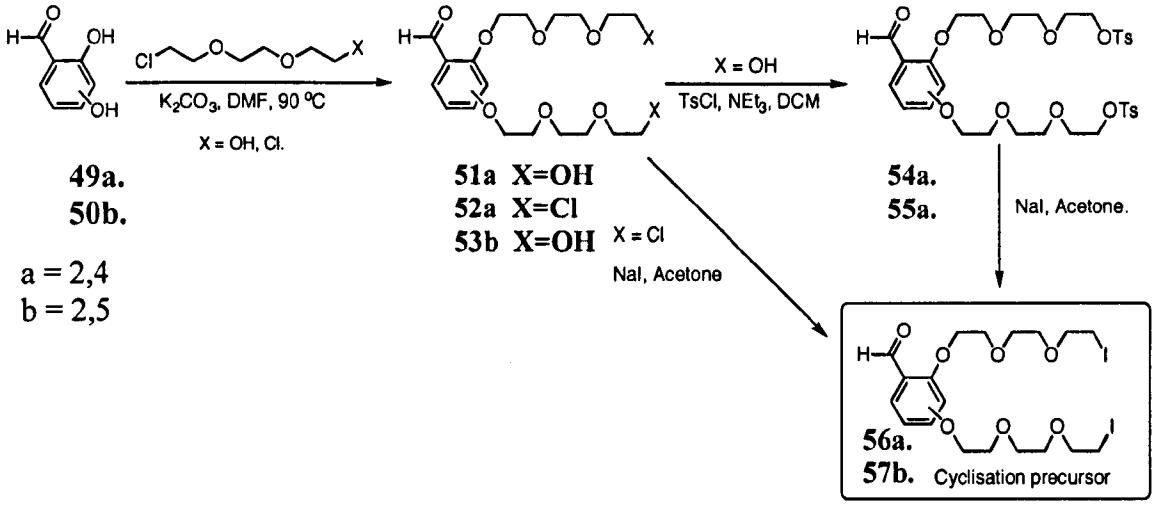
Table 1. Temperatures and conditions used in ring cyclisation.

Based upon these observations a new strategy was developed which incorporated the dihydroxybenzaldehyde derivative at an earlier stage of the synthesis. The preparation of the 2,4-dihydroxybenzaldehyde derivative offered the opportunity to observe the effects on yield by a smaller cavity size.

4.1.4 Aldehyde functionality

The inclusion of the aldehyde functionality was judged to be the best possible pendant arm. Variation of this functional group meant that numerous routes toward the ligand system were possible. For example, the conversion to the corresponding alcohol or carboxylic acid meant that different approaches to the fused crown ether and polyazamacrocycle were possible. The linker strategy and the methods employed are explained later in this chapter. It is worth mentioning that the methyl ester derivative was also considered. After ring cyclisation it was proposed that ester hydrolysis would leave the carboxylic acid derivative for further reaction. The problem however, was the unknown solubility of the carboxylic acid derivative and the potential loss of the compound in an aqueous environment. It was decided that the aldehyde derivative was much more suited for the pendant arm functionality.

4.1.5 Method 2. Synthesis of cyclisation precursor



Scheme 6. Synthesis of cyclisation precursor.

To overcome the problems experienced by the early synthetic efforts, method 2 was adopted. The other potential problem regarding ring cyclisation yields, was the size of the cavity and the substitution pattern of the aromatic rings. The use of 2,4 and 2,5-dihydroxybenzaldehyde enabled the comparison between the different cyclisation yields obtained with the varying precursors. The systems were then optimised accordingly. The two isomers of the ligand precursors were prepared in good yield adapting the methodologies from the initial synthetic efforts. There was much precedent for the alkylation of phenols using a variety of conditions and bases in the literature.[15] The most efficient synthesis was obtained through the use of K_2CO_3 and DMF. Tosylation of the terminal alcohols with tosyl chloride and triethylamine was achieved in quantitative yield. Cyclisation attempts were made at this juncture, but were unsuccessful, resulting in the replacement of the tosylate units with the more labile iodine leaving groups.

Indeed the only unsatisfactory step in our macrocyclic procedure was the formation of the dihydroxy-species (**51a, 53b**), created after the first step in the scheme. Due to the polarity and consequent water solubility, aqueous work-up was not feasible. This resulted in the removal of the DMF solvent via arduous azeotroping with toluene. The polarity of the compound meant that further purification through silica gel chromatography was not possible and the crude material was carried through to the next step. In order to eliminate this problematic and time-consuming step, we used a different alkylating agent. 2-[2-(2-chloroethoxy) ethoxy] ethoxy-chloride.[16] The reaction furnished the dichlorinated cyclisation precursor (**52a**) in reasonable yield, but also in high purity after aqueous work-up. Attempts to cyclise at this stage gave poor yields and so the iodine precursor was preferred. High dilution techniques were applied to improve the quantity of desired

product. Reduction of the polymeric side product was achieved by the slow drop-wise addition of 2,4 or (2,5)-dihydroxybenzaldehyde to a solution of the alklating agent over a 48 hr period and obtained 75 % yield of the desired product.

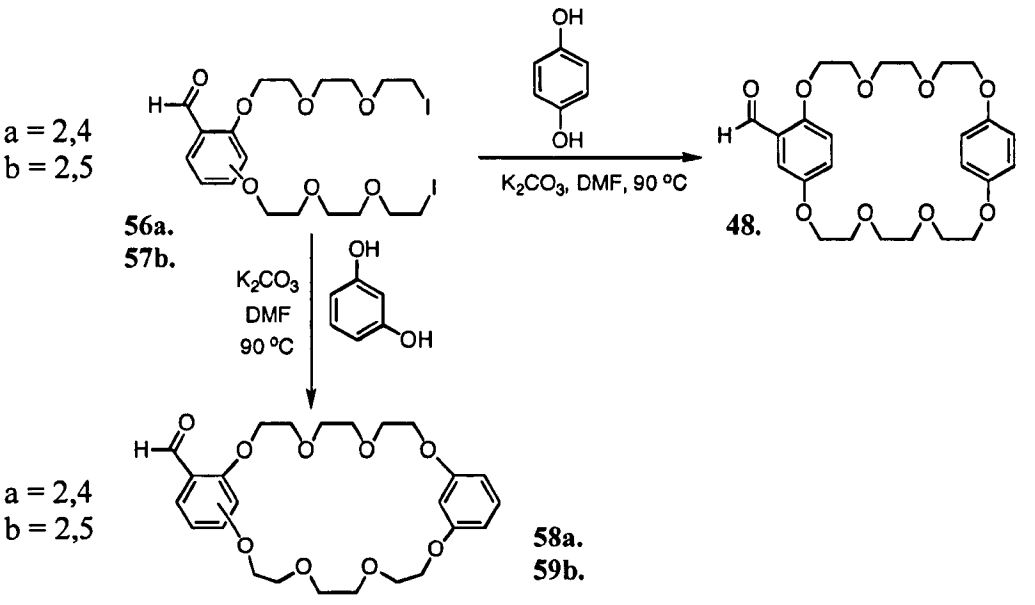
We had managed to optimise the route toward a satisfactory cyclisation precursor which could be produced in high yield and purity over a short space of time. The next challenge was to improve the cyclisation yields to obtain viable yields.

4.1.6 *Synthesis and optimisation of cyclisation protocols*

Having developed a viable and efficient route to the cyclisation precursor we then attempted to cyclise with 1,3-dihydroxybenzene (resorcinol) and 1,4-dihydroxybenzene. The use of the tighter 1,3 substitution pattern would promote a more compact structure and hence a less sterically constrained structure.

Through the seminal work of Pederson[9] and the subsequent examples in the literature[10] regarding the synthesis of macrocyclic crown ethers, it was evident that cyclisation yields were directly related to favourable template effects. The selection of the appropriate metal, with regards to cavity size, had a profound effect upon the cyclisation yields. It was understood that the metal reduces the activation energy of the cyclisation step by stabilising the favourable geometry required for the transformation to occur. For example, in order to form a five oxygen donor crown linked with ethyl groups, the efficiency of the reaction is directly related to the metal counter ion of the base. The use of NaOH in preference to K_2CO_3 results in as much as a 200 % increase in product obtained. To this end, a number of bases and a variety of solvents were tested to obtain the most efficient yield of product.

4.1.6 Cyclisation using different aromatic linkers

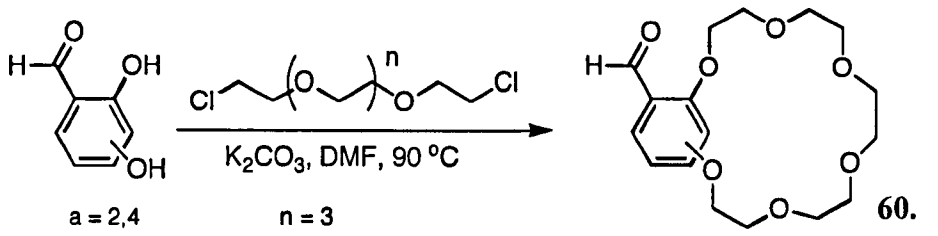


Scheme 7. Synthesis of cyclisation products.

Cyclisation to form the product **48** with the second methodology gave only 2 % conversion to the *para*-substituted conformation. The further use of the *para* substituted linker was terminated due to the consistently low yields obtained. The subsequent use of 1,3-dihydroxybenzene had a beneficial effect on both the 2,5 (**59**) and especially on the 2,4 isomer (**58**) cyclisation yields. The yields of cyclisation, with regards to the 2,5 derivative were still low, with maximum 5 % yields being obtained. A 15 % yield for the cyclisation of the resorcinol and the 2,4-dihydroxybenzaldehyde aromatic linker motifs was a substantial improvement. Further optimisation through increased reaction temperature and reduced rate of reagent addition, resulted in an excellent 25 % yield of **58** being obtained. High dilution techniques where both reactants were slowly added over a 48 hr period to a stirred mixture of K_2CO_3 in DMF coupled with high temperatures (<100 °C) were the key to high yields for the cyclisation step. The substantial gains in cyclised product were essential to having a viable synthetic strategy.

4.1.7 Summary

We have successfully developed a viable and efficient synthetic route toward substituted aromatic crown ethers with optimisation achieved at every step to the best of our abilities. The high dilution techniques were successfully employed using a syringe pump at both the initial and final alkylation steps. Necessary optimisation of reagents and cofactors has reduced the size of the cavity, but has not compromised the design of our ligand.



a = 2,4 n = 3 **60.**

Scheme 8. Synthesis of 19-benzocrown-6.

4.1.8 *Synthetic strategy-smaller crown ether derivatives*

The need for varied ring size was important and the synthesis of multiple ring dimensions was necessary. Development of a smaller crown ether was required to fulfil the potential of the ligand design. The synthesis was accomplished by the removal of the second unfunctionalised aromatic ring and replaced by an ether linkage. It was possible to form the cyclised species in a one-pot reaction. By varying the length of the di-alkylating agent and the substitution pattern of the dihydroxy-benzaldehyde it was possible to alter the cavity size. The commercially available 1,2-bis(2-chloroethoxy-)ethane chain containing four oxygen donors was used to couple the two hydroxyl groups on the aromatic ring to give a six oxygen membered ring system. In recognition of the proposed cavity size, K_2CO_3 was utilised as a base and template in the cyclisation step. It has been shown in the literature that the K^+ ion is strongly complexed to similar sized ring systems with six oxygen donors. Initial yields of 10-15 % were optimized by adjusting the dilution and rates of addition to give the product in excellent yields of 35-40 %.

In conclusion, we have developed a system where crown ethers of varying ring size can be obtained. By simply reducing or increasing the number of ether links in the alkylating agent we can manipulate the ring dimensions to meet the substrate demands. In particular, the work focussed on the 19-benzocrown-6 ring system due to its complementarity for the potassium cation.

4.2.0 Synthesis of polyaza-macrocyles and their protection strategies

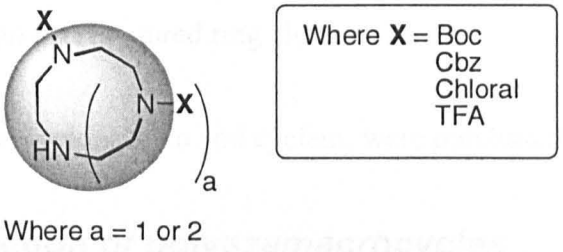
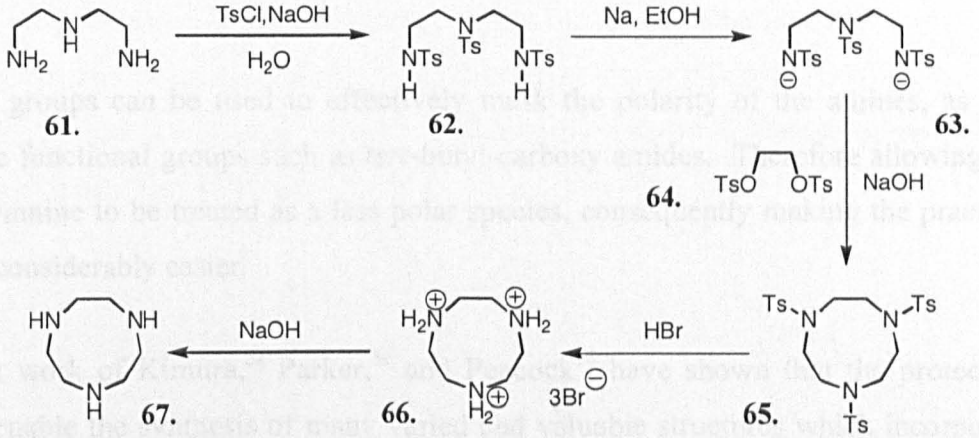


Figure 41. Schematic diagram of protecting strategies available in the literature.

Having established the synthetic methodology toward the crown ether systems, selection of the appropriate polyazamacrocycles (discussed in the introduction) and suitable protecting group strategies were required. In most cases the polyazamacrocycles were commercially available, however with regard to TACN, the purchase of the compound was prohibitive to the viability of the synthesis. It was therefore decided that the synthesis of the tri-aza-macrocycle was the only alternative.

4.2.1 Synthesis of triazacyclononane (tacn)



Scheme 9. Synthesis of triazacyclononane.

The synthesis of tacn was first developed by Richmond & Atkins[17] in the 1970's and the Richmond Atkins strategy has remained the most popular method by which to construct the ring structure. The Richmond & Atkins synthesis provides a simple route for 9 to 24 membered macrocycles containing 3 to 8 nitrogen atoms. The scale up of this methodology gave a substantial amount of product to carry out all the reactions required. The synthesis (Scheme 9) proceeded via a tosylated intermediate to yield the final product as a pertosyl macrocycle. The tosyl groups were then removed by treatment with HBr. In comparison with the crown ether cyclisation, Richmond & Atkins showed that the

successful synthesis did not depend on the template effect. When sodium counter cations were replaced with tetra methyl ammonium ions there was no significant change in yield. It was believed that the high yields obtained in the tacn synthesis were due to favourable steric interactions involving the tosyl groups which causeed the open chain intermediate to fold into a conformation that favoured ring closure.

The other polyazamacrocycle, cyclen and cyclam, were purchased from Aldrich.

4.2.1 Protection of polyazamacrocycles

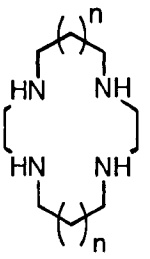
When working with these highly polar and reactive species, protecting groups were required for several reasons. The ability to selectively mono-substitute a poly-aza-macrocycle was of the utmost importance to the synthesis. Through the use of protecting groups, multiple substitution reactions can be negated.

There are also inherent difficulties associated with compounds containing multiple amines. The highly polar nature of these compounds makes working with and purification with aqueous media extremely difficult. Problems with simple extractions, separations and purification on silica gel chromatography make the practical chemistry considerably more difficult.

Protecting groups can be used to effectively mask the polarity of the amines, as less hydrophilic functional groups such as *tert*-butyl-carboxy amides. Therefore allowing the cyclic polyamine to be treated as a less polar species, consequently making the practical chemistry considerably easier.

The recent work of Kimura,[5a] Parker,[5c] and Peacock[18] have shown that the protection strategies enable the synthesis of many varied and valuable structures which incorporate the cyclic polyamine motif. There have been many diverse methods by which chemists have approached this problem. Indeed there is not sufficient space to relate in detail the quantity of work focussed on resolving this problem. We will look briefly at the three main macrocycles that are studied within this work; tacn, cyclen and cyclam and relate the methods we used to selectively mono-substitute. The protection of the tetra-amine macrocycles, cyclen and cyclam are addressed first.

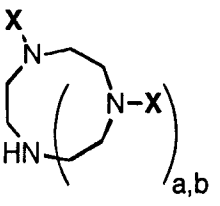
4.2.2 Cyclen and cyclam protection strategies



n = 0, cyclen
n = 1, cyclam

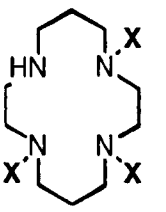
Figure 42. Structure of cyclen and cyclam..

Since cyclen and cyclam are polyamines, the standard amine protection groups have been utilised. There are numerous examples of Boc and Cbz protected cyclic polyamines where the authors have managed to synthesise and isolate in very good yield the tri-protected cyclic polyamine species.[19] This has generally been achieved with the use of high dilution techniques and mechanical syringe pumps which allows the slow addition of a protecting group into a dilute concentration of macrocycle. There have been examples of the use of trifluoroacetate (TFA) protecting groups[20] which are resistant to most chemical transformations and can be removed with relative ease under hydrolysis conditions. The reaction goes cleanly toward the tri-protected cyclen species without formation of the other three possible derivatives. This methodology negates the need for time-consuming column chromatography and gives the product in excellent yield. Most recently, there has been the development of chloral protecting groups which have been shown to furnish tri-substituted cyclen in good yield.[21]

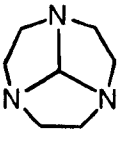


Where a = 1
b = 2

68a, X = Boc
69b, X = Boc
70b, X = TFA
71b, X = Chloral



72, X = Boc



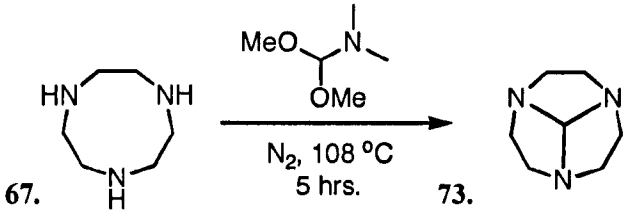
73.

Figure 43. Synthesised protected polyazamacrocycles

The synthesis all the derivatives were undertaken in order to have multiple protecting group options in the synthetic strategy. It was important to choose correctly the

appropriate protecting group for a specific synthesis. In each case the ramifications of steric interactions, stereoelectronics and the possible side effects of the deprotection strategies would have to be considered. To this end, the synthesis of the Boc, TFA and chloral derivatives of the cyclen were achieved. Only the Boc derivative of the cyclam unit was produced. The synthesis of the Boc protected cyclen involved reaction with $(Boc)_2$ in the presence of NEt_3, under high dilution conditions which gave the desired product in 75 % yield. The TFA protected derivative was obtained by reaction with three equivalents of ethyl trifluoroacetate, giving pure product in 89 % yield with no major purification issues. The chloral-substituted derivative was obtained through the reaction of cyclen with chloral chloride and obtained in good yield, 76 %.

4.2.2 *Tacn protection strategies*



67. 73.

Scheme 10. Synthesis of "capped" triazacyclononane.

The use of the smaller triazamacrocycle, trivially named tacn (triazacyclononane), was required for the chelation of a copper ion. The chemistry and properties of tacn have been well established, as well as the methods of preparing mono-substituted derivatives. Due to the reactivity of the tacn species it is not possible to simply mono-alkylate and obtain cleanly, single pendant arm substitution. The reaction results in a mixture of mono-, di and tri substituted species which are extremely difficult to separate. An old, yet highly effective and still broadly used technique to avoid these problems is used, called "capping". The theory and experimental details were carried out by Richmond & Atkins who showed that reaction of tacn with N,N-dimethylformamide-dimethylacetal produced the capped structure, **73** (Scheme 10). The capped structure allows only mono-substitution with a halogenated alkylating agent to form the quaternary amine salt. Decapping of the ligand was achieved using simple basic hydrolysis conditions and the product isolated via Dean-Stark procedures.

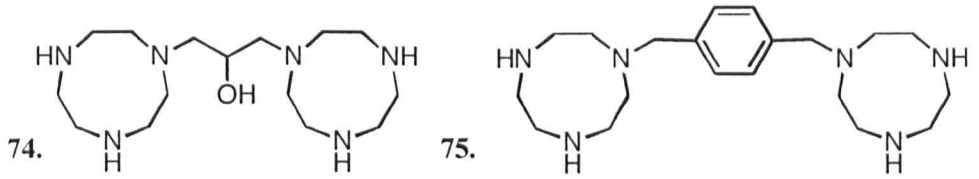


Figure 44. Examples of successful syntheses using protecting groups.

Peacock[22] and others[23] have shown the reliability of this methodology with the synthesis of multiple mono-alkylated cyclic polyamine structures (Figure 44). We were able to synthesise the capped TACN in 86 % and in good purity after Kugelröhr distillation.

4.2.4 *Boc and TFA protection strategy*

TFA and Boc protection of tacn was also applicable. This was achieved through the use of high dilution conditions with the polyamine and $(Boc)_2$ in the presence of triethylamine. Interestingly it was found that the Boc protected tacn was substantially more polar, than that of the other tetra-amines, cyclen and cyclam. The synthesis of the trifluoroacetate derivative of tacn was not undertaken. The reasons are given later in the discussion.

The next stage of development was to devise a method by which the crown ether was attached to the cyclic polyamine.

4.3.0 Pendant arm linker strategy

4.3.1 *Benzyl bromide strategy*

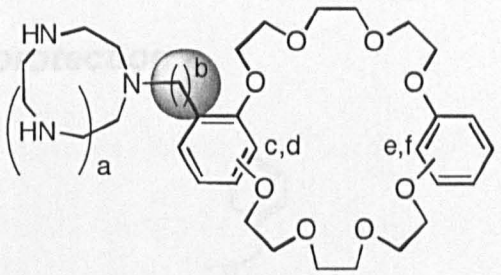
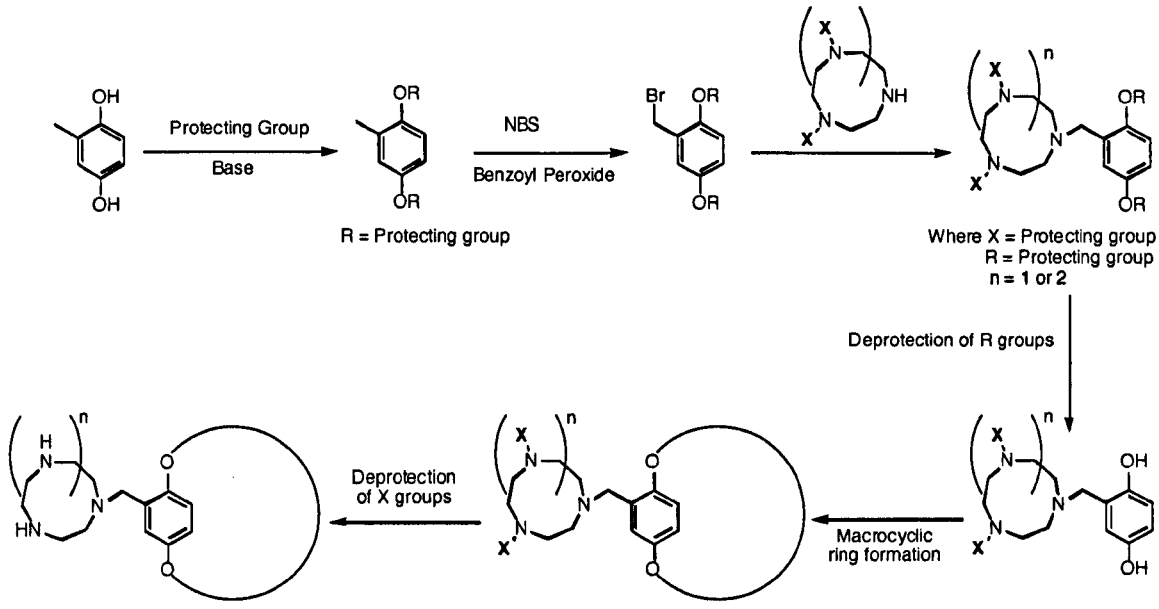


Figure 45. Development of linker strategy.

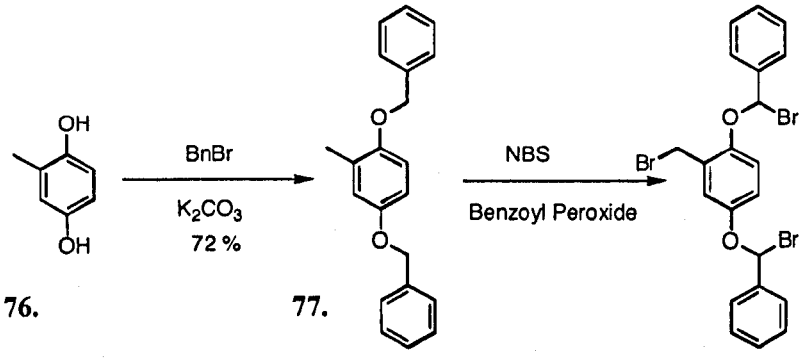
Our initial strategy was to graft the polycyclic amine to the crown ether via simple nucleophilic substitution, whereby the secondary amine would displace a benzylic bromine attached to the benzene linker. Shown in Scheme **11** overleaf is the general strategy employed to create the ligand.



Scheme 11. General strategy toward benzyl bromide linker strategy.

The starting material was methyl hydroquinone, a cheap commercially available material. Our general strategy involved the protection of the two reactive phenols with standard protection reagents. This would be followed by the radical bromination of the methyl group using *N*-bromosuccinamide and the radical initiator benzoyl peroxide. The addition of the polyazamacrocycle via nucleophilic substitution would furnish the coupled species. Deprotection of the phenol would give the precursor for the crown ether macrocycle formation. The aim was then to construct the crown ether around the two phenolic positions. The synthesis of the crown ether precursor has already been discussed and reported in the experimental section, compound **47**. The protection strategies employed are discussed in the following section.

4.3.2 Benzyl protection

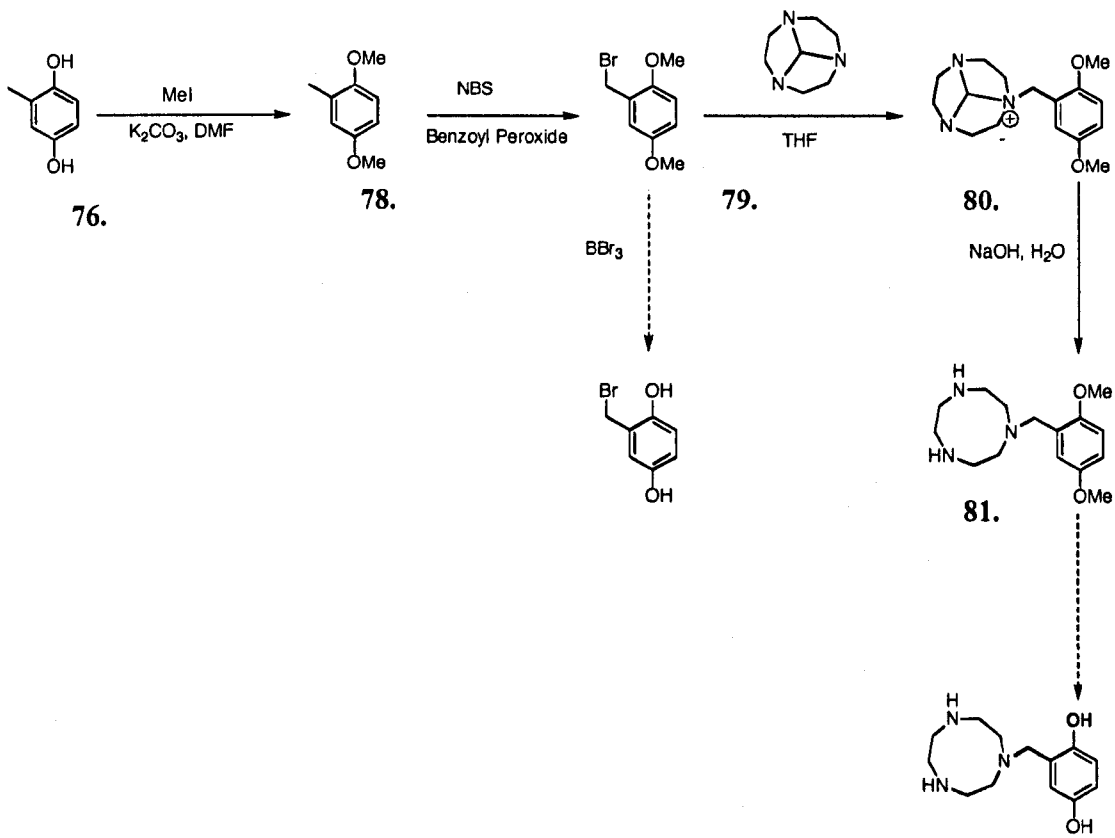


Scheme 12. Synthesis of benzyl bromide adduct.

Benzyl protection of the phenols was adopted, as it was believed they could be easily removed using mild hydrogenation conditions. Protection was achieved in high yield and

purity using K_2CO_3 and benzyl bromide in DMF to give **77** (see Scheme 12). Bromination using NBS and a catalytic amount of benzoyl peroxide resulted in the addition of bromine on all the benzylic positions. The stabilisation of the radical species on the benzylic positions through the conjugated benzene system, proved to be a major stumbling block. It was not possible to isolate any of the desired product using silica gel chromatography due to the instability of the benzyl bromide species. We required a strategy that yielded the brominated species as a clean compound

4.3.3 *Methyl ethers*



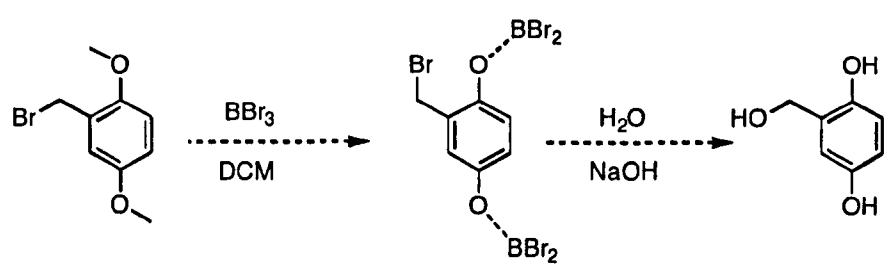
Scheme 13. Failed route toward cyclisation precursor through methyl protection.

In order to overcome the initial problems associated with the bromination of the benzylic positions of the benzyl groups, another protecting group was needed. Methylation of the two-hydroxyl groups was chosen as the desired replacement. Methyl ethers are essentially inert to very strongly acidic or basic conditions with the corollary being that rather forcing conditions are often needed to remove them again. The methyl cation is insufficiently stable to allow cleavage via an Sn_1 pathway; therefore, Lewis acid activation is required to facilitate an Sn_2 pathway. Lewis acids (BBr_3, TMSBr, $AlBr_3$) liberate the halide ion on complexation which completes the cleavage process.[24] The intermediate metal alkoxides are covalently bound which reduces their tendency to participate in the reverse process-

methylation by the liberated metal halide. We were therefore convinced that such a strategy would be beneficial to our approach.

Methyl protection using MeI and K_2CO_3 in DMF was achieved successfully in good yield and high purity. The benzyl bromide derivative was synthesised in CCl_4 with NBS in excellent yield, providing the leaving group for addition to capped 1,4,7-triazacyclononane. 1,4,7-triazatricyclodecane's nucleophilic displacement of the bromine atom in THF furnished the bromide salt **80**, of the coupled groups in good yield. Reflux in H_2O followed by the addition of NaOH, removed the cap and afforded the free base **81**. We failed however, in our attempts to remove the methyl protecting groups. The simple explanation for the lack of success was understood after careful monitoring of the reaction. When the BBr_3 was slowly added to the reaction solution, there was an instantaneous precipitation in the vessel. Indeed by the time the entire reagent had been added to the flask there were large quantities of insoluble material. Since BBr_3 was a homogeneous catalyst, a heterogeneous reaction environment was not beneficial and helped explain the lack of reaction. It was evident that the BBr_3 strongly coordinated to the triazamacrocycle and 'crashed' out of the organic solvent.

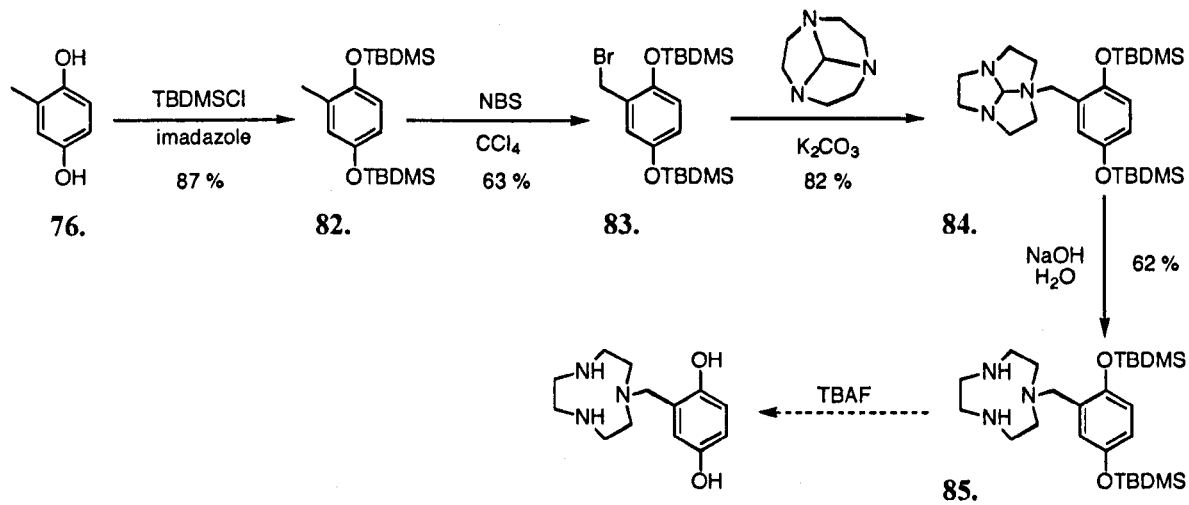
In theory, reaction of the uncoupled 2,5-dimethoxybenzylbromide with the boron reagent should remove this problem and furnish the dihydroxybenzyl bromide for reaction with the capped tacn species. Unfortunately it was found that even at low temperatures we were unable to isolate the unstable deprotected benzyl bromide species. Underestimation of the reactivity of the aromatic quinone species and its tendency to oxidise meant that it was not possible to isolate the desired bromide product. It is probably due to the nucleophilic displacement of the bromine atom upon aqueous work up with NaOH. The bromine atom would be readily displaced. Shown below in Scheme 14 is the intermediate complex which is broken up on work up.



Scheme 14. Proposed mechanism for the removal of bromine through nucleophilic displacement of the bromine upon aqueous workup.

4.3.3 TBDMS protection

The next protecting group that was then employed was TBDMS, a silyl moiety which is easily deprotected using either HF or TBAF.[25] We believed that the bulky tertiary butyl groups would limit bromination of the protecting groups. Successful protection of phenols in good yield was followed by the selective bromination the methyl group in high yield, avoiding unwanted isomer formation. The nucleophilic addition of the capped 1,4,7-triazacyclononane was achieved with ease. However, the same problems experienced with the methoxy protection, reoccurred. Deprotection was not feasible at this stage, or on the TBDMS brominated species **83**. All attempts resulted in uninterruptible ^{1}H NMR that could not be purified or carried through to the next step of the synthesis. A new synthetic strategy had to be designed which eliminated the formation of intermediate compounds which were unstable and too polar.



Scheme 15. Failed route toward cyclisation precursor through TBDMS protection.

The alternative strategy developed, was the utilisation of the pendant aldehyde which was available on the synthesised crown ether. Coupling of the polyazamacrocycle directly to the pre-formed crown ether would eliminate the formation of the problematic intermediates

4.4.0 Functionalisation of the pendant arm

4.4.1 Methodology

The pendant aldehyde functionality meant that there were three methods by which we could directly couple the two separate groups. Reaction between the aldehyde and the secondary amine of the protected polyazamacrocycle, through reductive amination to form

the imine and subsequent reduction to the amine with a mild reducing agent was one option.

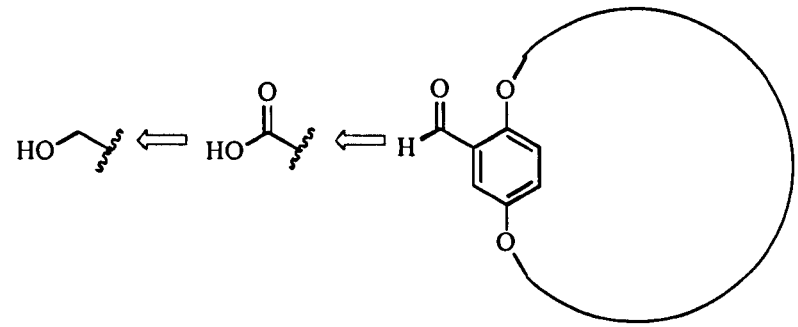
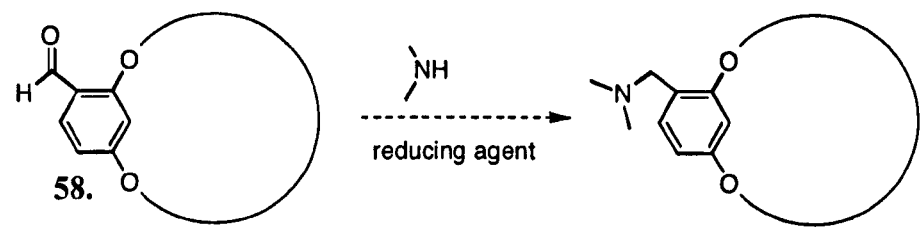


Figure 46. Proposed functional group interchanges between alcohol and acid.

Alternatively, reduction of the aldehyde to the benzyl alcohol would allow the formation of a good leaving group. Coupling via displacement of the labile group would give the desired linker. The final option was the oxidiation of the aldehyde to the carboxylic acid. Subsequent amide coupling of the two groups would furnish the coupled units linked by an amide bond.

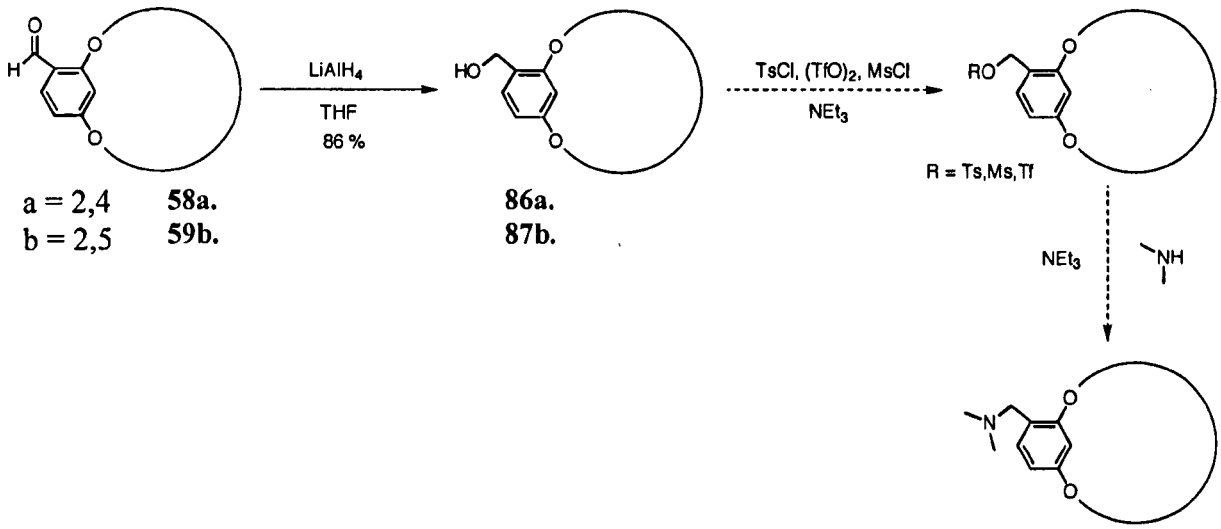
4.4.2 *Reductive amination*



Scheme 16. Coupling through reductive amination.

Due to the minimal number of steps required, reductive amination was the method of preference. The procedure was well documented in the literature with many reagents catering specifically for this transformation.[26] However, there are very few examples of coupling involving a polyazamacrocycle with reductive amination. All our attempts to bridge the two groups were unsuccessful and led to the recovery of starting material. A wide variety of reducing agents, solvents and reaction conditions were used. These included sodium cyanoborohydride, sodium triacetoxyborohydride and hydrogenation conditions with H_2 gas and Pd/C (both at atmospheric pressure and 256 bar). All the solvents were freshly distilled and the additional use of drying agents such as $MgSO_4$ and 4Å molecular sieves all failed to deliver the desired product. Despite our best efforts we were unable to isolate the product

4.4.3 Nucleophilic substitution



Scheme 17. Reduction methodology toward coupled macrocycles.

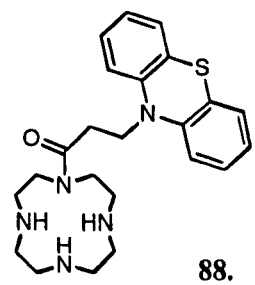
In order to utilise the nucleophilicity of the amine, the synthesis of an electrophilic derivative of the crown ether was required. The best method to achieve this was to reduce the pendant aldehyde to the corresponding alcohol and then convert to a suitable leaving group such as a tosylate, mesylate or triflate. Reduction of the aldehyde to the alcohol was achieved without note in respectable yield (86 %). However the formation of a stable leaving group and ligand was extremely problematic. Attempts to isolate the reactive species were unsuccessful due to its instability. It was believed that the triethylamine displaced the leaving group and was then removed by the activity of the benzylic position.

The activated benzylic position was electron rich and would stabilise the loss of the leaving group. To counter this problem we used a variety of non-nucleophilic bases, such as 2,6-lutidine and 2,6-di-*tert*-butyl-4-methylpyridine but this proved to be unsuccessful. Efforts to isolate the compound were abandoned. The work instead focussed on forming the leaving group derivative and reacting *in situ* with the cyclic polyamine. This was unsuccessful due to the instability of the leaving group, even at extremely low temperatures, -78 °C.

We confirmed this by trying to convert the alcohol to a bromine using PBr_3, but without success. An unresolvable mixture of compounds was obtained.

4.4.4 Amide coupling

Finally, we attempted to form the carboxylic acid derivative by using well-established amide coupling protocols to obtain the desired product. There has been extensive use of peptide coupling reagents in forming substituted cyclen rings. These techniques have been used in parallel with Boc and Cbz protecting group strategies. Köenig's phenathazine substituted cyclen (Figure 47), showed the benefits of both our proposed methodologies[19]. Köenig successfully employed the tri-protected cyclen moiety to monosubstitute the free amine and showed the relative ease with which the amide bridge could be made.



88.

Figure 47. Mono-substituted cyclen derivative coupled through acid coupling.

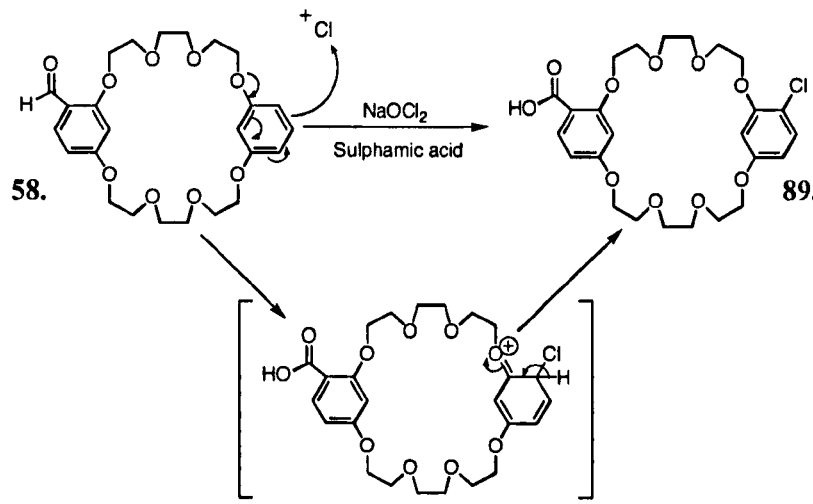
4.4.5 Synthesis of the carboxylic acid

It was hoped that the carboxylic acid coupling would have removed the problems that were associated with the nucleophilic substitution strategy. Reaction between the carboxylic acid and the secondary amine were attempted using peptide-coupling reagents such as DCC and the water-soluble EDCI derivative. The use of a catalytic amount of DMAP was utilized to try and improve the procedure. Conversion of the aldehyde to the carboxylic acid was problematic. We initially anticipated that the simple oxidation of the aldehyde to the carboxylic acid, using one of the many oxidising agents available, would be straightforward and unproblematic.

$$RCHO + HClO_2 \longrightarrow RCO_2H + HOCl$$

The use of sodium chlorite to oxidise the aldehyde to the corresponding acid was employed. Sodium chlorite is a mild oxidising agent which is commonly used on substituted aromatic systems. It was also necessary to use a scavenger such as sulphamic acid or resorcinol to mop up the reactive hypochlorite species (HOCl) released by the oxidant. This eliminates the various unwanted positive Cl species present.[27] Analysis of

the TLC plate showed the presence of two products that were inseparable on silica gel chromatography, due to the minimal difference in R_F values. It was found that during the course of oxidation to the carboxylic acid, regio-isomeric chlorination had occurred at the resorcinol "end" of the crown ether. Mono-chlorination had occurred at both the 2 and 4 position of the resorcinol. Using a solvent mixture of CH_2Cl_2 and petroleum ether it was possible to crystallise and separate one of the isomers. We were unfortunately unable to obtain X-ray crystallographic quality crystals to determine which isomer had crystallised



Scheme 18. Proposed mechanism for the formation of chlorinated regioisomers.

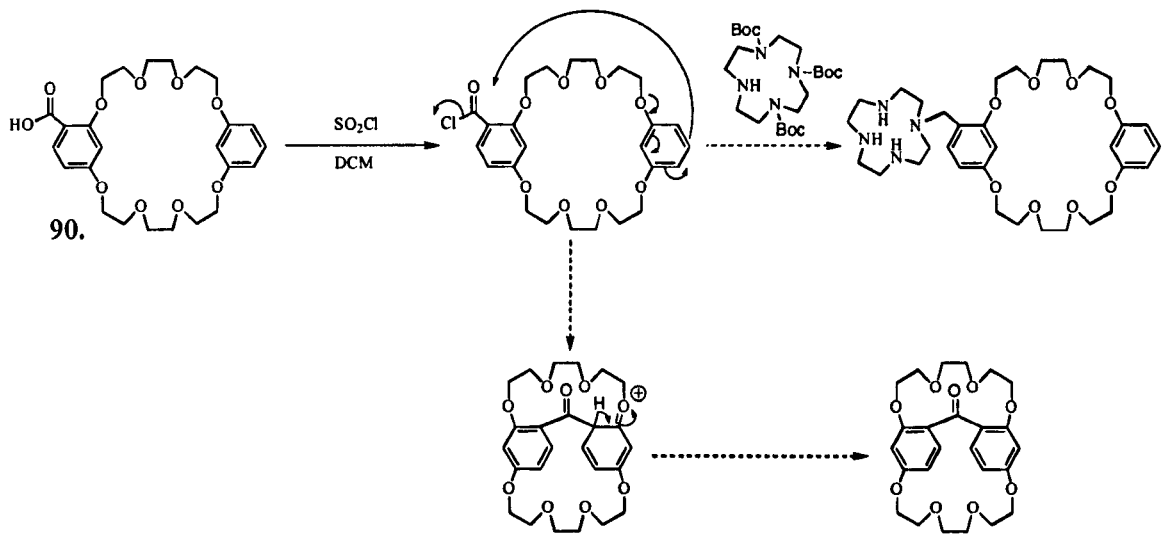
In order to eliminate this side reaction, increased equivalents of sulphamic acid were used, but to no effect. This methodology might be beneficial for the selective functionalisation of the second aromatic ring. Removing the need for problematic cyclisation steps with two functionalised aromatic rings which form inespararble regio-isomers. However, functionalisation of the regio-specific chlorinated species would not be trivial. Repeating the same experiment with sodium bromite might allow the functionalisation through Suzuki, Stille, Negishi and many other palladium based cross coupling reactions. We were not able to pursue this avenue of work given time constraints.

Silver oxide, a mild oxidising agent, was unsuccessfully employed to remove the unwanted side reaction. The return of unreacted starting material signalled the need for a more reactive reagent. The eventual application of $KMnO_4$ was used to solve the reactivity problem, giving the desired carboxylic acid in moderate yield.

The direct coupling of the two moieties via the Boc protected cyclen proved unsuccessful. Attempts to couple the TFA derivative to the crown ether were equally unsuccessful. Classical DCC coupling as well as modern reagents such as EDCI with HOBt were tried but found to be unreactive. A more aggressive strategy was adopted and endeavours to

join the two fragments via a more reactive species were tried. The formation of the mixed anhydride and then acid chloride all failed to produce the desired product. Comprehensive scanning of different reagents and reaction conditions to form the acid chloride was undertaken with the use of thionyl chloride and oxalyl chloride. Despite scrupulously dry solvents and distilled reagents we were unable to form our coupled material.

4.4.6 *Macrocyclic ketone bowl*

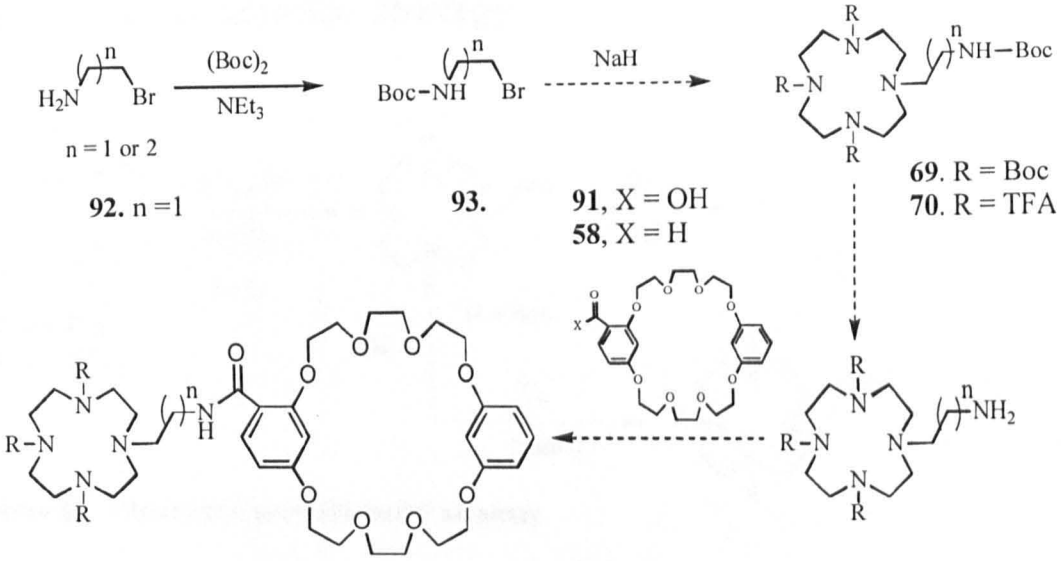


Scheme 19. Proposed mechanism for the formation of the ketone bowl.

It was proposed that the lack of reaction between the two components could be rationalized by the formation of a short-lived novel macrocyclic bowl. We have already established the nucleophilic reactivity of the resorcinol "tail" of our macrocyclic system. It might be feasible that nucleophilic attack occurred at the carbonyl of the acid chloride where the chloride was displaced and forms a macrocyclic ketone bowl structure. Indeed, the aromatic systems might encourage π–π interactions between the adjacent aromatic groups, suggesting that the two positions could be in close proximity. Sn_2 substitution could well be possible through the favourable geometry of the crown ether-folding pattern. Unfortunately it has not, thus far, been possible to isolate and characterize this compound, presumably, due to its instability at higher temperatures.

This does not, however, explain the lack of reaction between the two components under milder conditions. It was possible that the grafting of the two large components at this stage was sterically disfavoured. The bulky Boc or TFA groups may hinder the desired union. In order to overcome this problem, it was proposed that the use of a small sterically undemanding linker molecule might overcome this problem. The linker molecule needed to be commercially available and synthetically easy to manipulate.

4.4.7 Short chain alkyl linker strategy



Scheme 20. Failed short chain alkyl linker strategy.

The use of a short alkyl chain with a functional group at each terminal end would allow reaction at either side to form the coupled product. The selection of bromoethylamine was adopted. Doherty and co. workers[28] have shown that it was possible to couple the Boc protected linker molecule to a secondary amine group. The alkyl bromide segment was used to couple to the amine group and the carboxylic acid of the crown ether was to be fused to the amine of the linker, via amide coupling. The first step was to sheath the reactive primary amine with a Boc protecting group. We then tried to join the linker to the Boc and TFA protected poly-aza-macrocycle using standard alkylation conditions. The unreactivity of the bromosubstrate could be due to the close proximity of the electron withdrawing amine, thus reducing the reactivity of the C-Br bond. In order to combat this effect, the use of a longer chain, bromopropylamine was attempted. This method was also unsuccessful with no product being formed. The other reason for the lack of alkylation product might be due to steric interactions between the alkyl chain and the Boc groups. Molecular modelling studies using the energy minimisation program CS Chem3D Pro® showed significant steric interactions. The conformation of the cyclen resulted in the

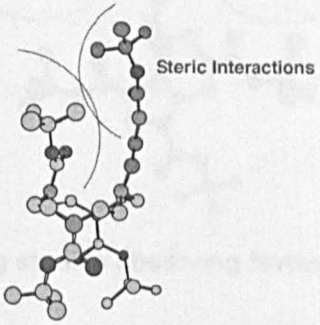
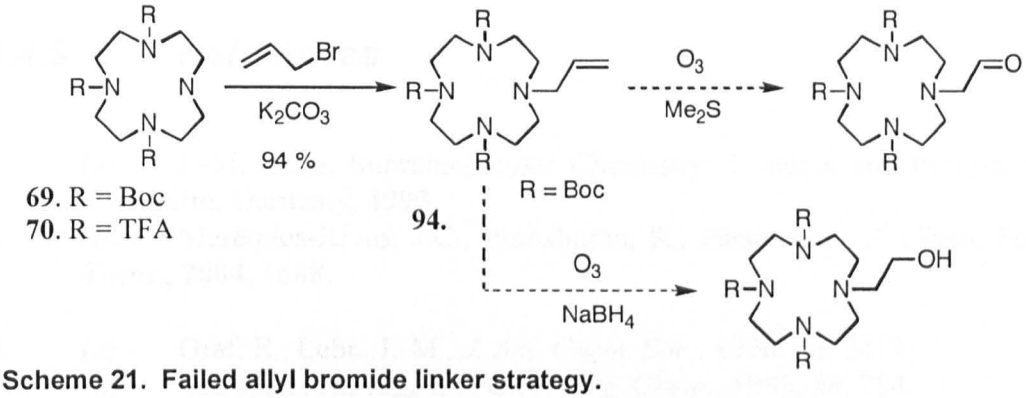


Figure 48. Molecular modelling study of unfavourable steric interactions.

unfavourable proximity of a tertiary butyl group with the alkyl chain.

4.4.8 Allyl bromide strategy



Scheme 21. Failed allyl bromide linker strategy.

An alkylating agent reactive enough to alkylate the sterically hindered amine was desired. Allyl bromide was chosen for its electrophilicity and reactivity. Molecular modelling studies identified that there would not be the same conformatonal difficulties experienced with the sterically hindered Boc protected bromoethylamine. We were confident that we could successfully alkylate the cyclic polyamine and then convert the relatively labile allyl group into either the alcohol or the aldehyde. The alkylation of the Boc and TFA protected cyclen was carried out in excellent yield and purification was straightforward. Through the ozonolysis procedure, it was hoped that the interconversion of the allyl group to the aldehyde could be achieved through work-up with dimethyl sulphide.[29] Similarly, it was hoped that work-up with $NaBH_4$ would furnish the terminal alcohol.[30] Progress was again hindered by the return of an uninterruptible mixture of compounds after work-up with both reaction procedures. Exhaustive efforts to overcome these teething problems all resulted in failure.

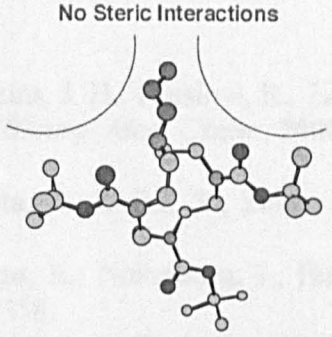


Figure 49. Molecular modelling studies observing favourable steric interactions.

The development of a new synthetic strategy which would overcome the persistant problems that were experienced was required. Ideally a multi-functional linker which would enable the successful coupling of the two groups, without the teething problems was needed.

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Chapter 5

5.1.0 Modular synthetic strategy

Modification in the ligand design and synthetic approach resulted in a new modular synthesis. Our objective was to develop a synthetic route that could be easily adapted to build a large range of compounds. These compounds would have varying physical properties, but the same structural motif. To synthesise such a family of compounds, an efficient and replicable route had to be developed. The methodology needed to be flexible to incorporate different structural components. The ligand design comprised three separate variables that could be altered with minimal changes to the core synthetic route. The three component parts were: the cyclic polyamine, the linker unit and the crown ether. The synthetic methodology needed to be robust enough to tolerate changes to all three parts. It was decided that an amino acid linker would be the ideal candidate.

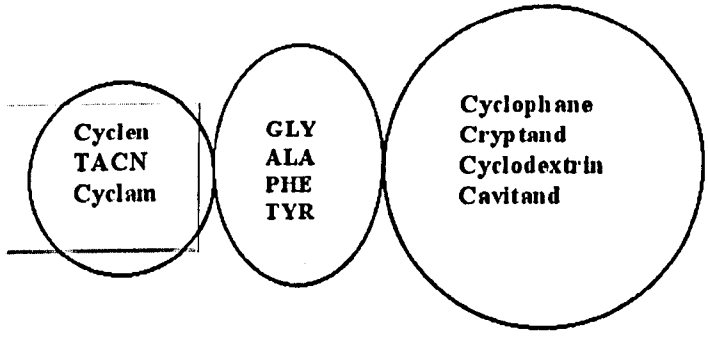


Figure 50. Possible combinations of component parts.

5.1.1 Amino acid linker

Structurally, an amino acid had the required dual functionality required. With terminal carboxylic acid and primary amine groups, it would be possible to couple the two molecules together through amide bonds. Based upon many years of development and optimization, peptide-coupling chemistry was considered a safe synthetic strategy.[1] In terms of flexibility and size, a simple glycine unit was chosen for the bridging of the two entities. Of additional benefit to the amino acid strategy was the massive diversity available through the R group on the α carbon. Variation in the R-group would enable the introduction of many different facets: size, polarity and functionality. The use of a glutamic acid or a histidine amino acid linker would provide a novel tri-topic receptor with multiple functionality and potentially exciting chemical properties. The schematic diagram (Figure 50) illustrates the synthetic possibilities for a small diverse library of receptors. The coupling of a specific cyclic polyamine would allow chelation, with high affinity, to

the desired metal ion. The size of the cyclophane would be adjusted by synthetic means to cater for the dimensions of the proposed guest. Figure 51 shows a compartmental view of the ligand design.

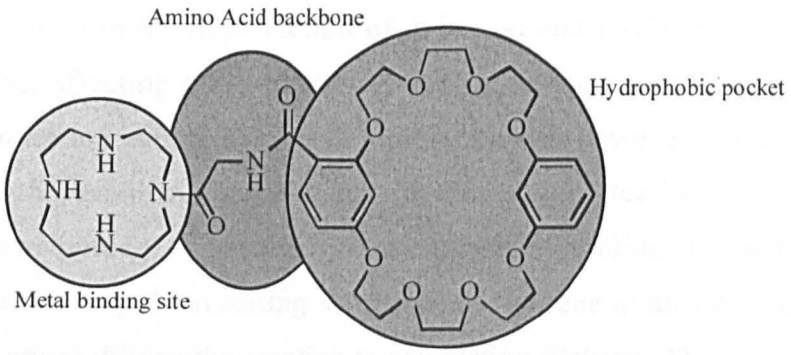
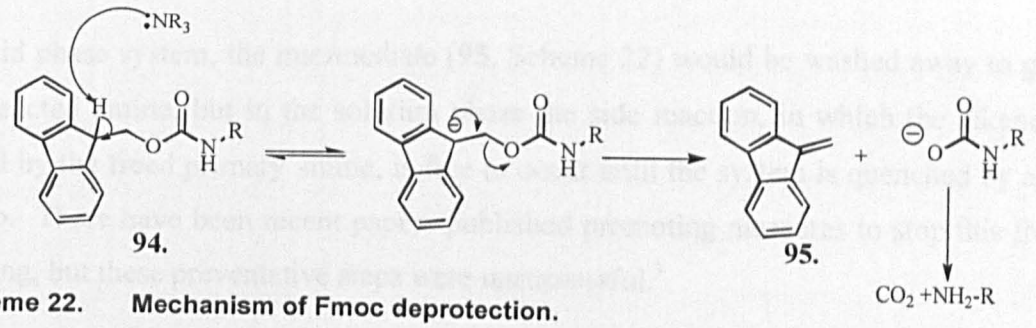


Figure 51. Schematic diagram depicting a potential target receptor.

5.1.2 General synthetic strategy

The general synthetic strategy was to couple an N-protected amino acid to the cyclic polyamine, followed by the deprotection of the amino acid to expose the reactive primary amine. This would then be coupled to either the aldehyde or acid derivative of the crown ether cage. This route allowed changes to every component. The main challenges associated with amino acid coupling chemistry were the selection and application of multiple protecting groups. The suitable protection of the polyamine and amino acid and their subsequent deprotection was of crucial importance. The protection strategy of the cyclic polyamine and the amino acid would have to be different to enable selective deprotection.

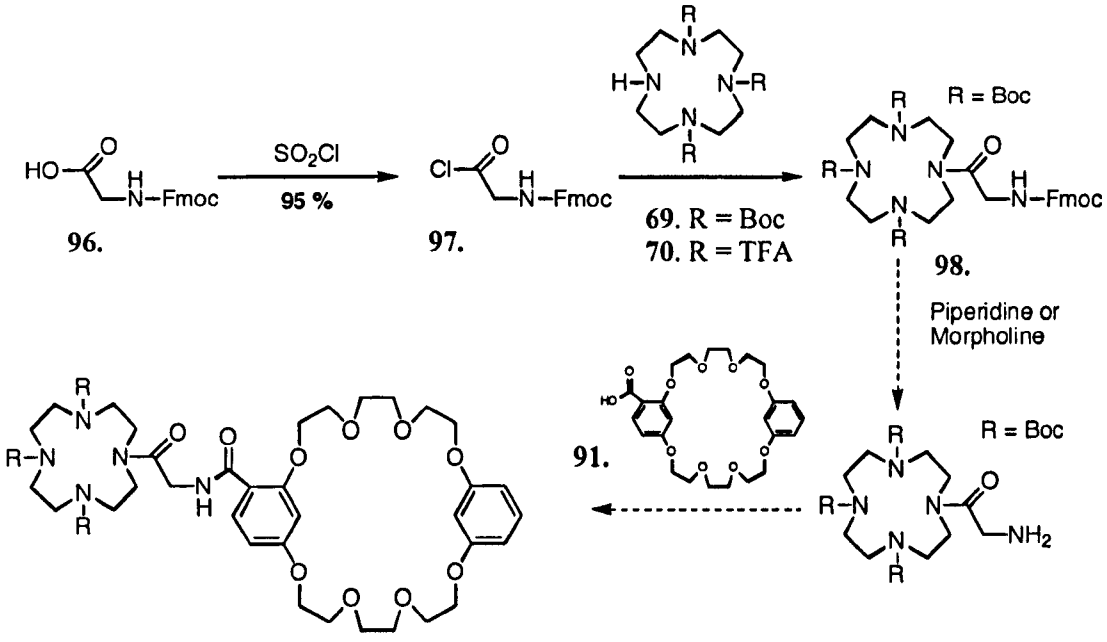


Scheme 22. Mechanism of Fmoc deprotection.

5.1.3 Route 1: Fmoc protection group strategy

The initial attempts involved the coupling of the TFA protected cyclen to Fmoc-glycine. However, no product was ever formed. The experiment was then repeated with the Boc protected cyclen. It was possible to form and isolate the acid chloride of the Fmoc-glycine

unit before successfully coupling to the polyamine in quantitative yield. Fluorenylmethoxycarbonyl (Fmoc) is a well-established protecting group,[2] used widely in the N-protection of amino acids for solid-phase peptide synthesis. It is stable to the acidic conditions that cleave Boc groups from amines and quite resistant to catalytic hydrogenolysis used in the deprotection of N-benzyl and N-Cbz groups. It can also be released without affecting other amine protecting groups under basic conditions. The attempted deprotection with piperidine or morpholine was however unsuccessful. In order to understand the problems associated with Fmoc deprotection a closer look at its chemistry was required. A secondary amine (e.g. piperidine) is used both to effect deprotection and to trap the resulting 9-methylene fluorene in an essentially irreversible addition of the amine driving the reaction to completion (Scheme 22).[3]



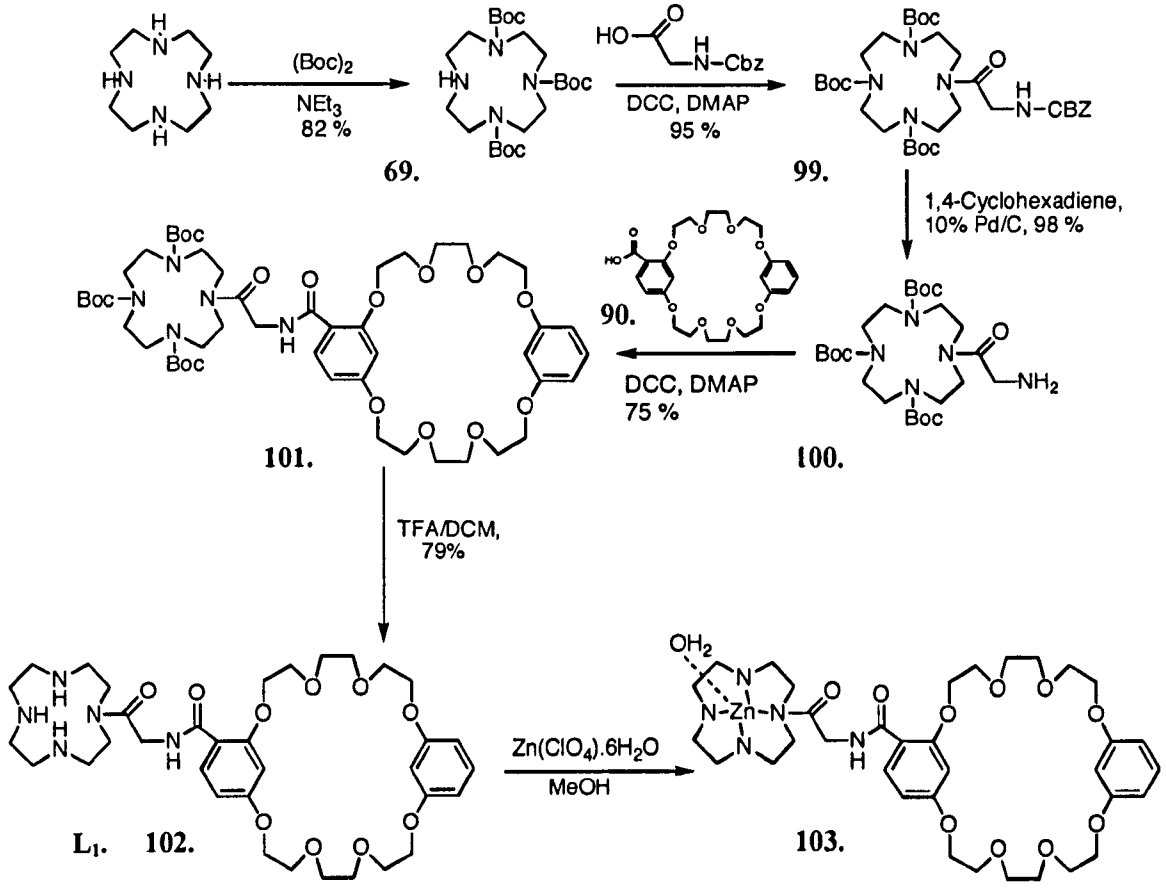
Scheme 23. The failed Fmoc synthetic strategy.

In a solid phase system, the intermediate (**95**, Scheme 22) would be washed away to give the unreacted amine, but in the solution phase the side reaction, in which the alkene is attacked by the freed primary amine, is free to occur until the system is quenched by acid work up. There have been recent papers published promoting measures to stop this from happening, but these preventative steps were unsuccessful.[3]

5.1.4 Route 2: Cbz-protecting group strategy

The protection strategy was changed and we adopted the use of N-benzyloxycarbonyl-glycine. Carboxybenzyl or Cbz, a carbamate protecting group like Boc, was put on by treating with benzyl chloroformate (BnOCOCl) and weak base. Cbz-protected amines

behave like amides, they are no longer nucleophilic, because the nitrogen's lone pair is "tied up" in conjugation with the carbonyl group.[4] They are resilient to both aqueous acid and base but removed by hydrogenolysis or HBr. The reaction of the Boc protected cyclen with Cbz-glycine using DCC and DMAP furnished our combined unit in good yield. The application of a non-polar protection strategy was beneficial as purification was uneventful and achieved with minimal difficulty by silica gel column chromatography.



Scheme 24. Synthetic methodology toward $ZnL_1.(OTf)_2$.

The next step in the synthesis was to deprotect the Cbz group whilst leaving the acid labile Boc groups in position. The procedure was taken from recent literature examples[5] where transfer hydrogenenation using, 1,4-cyclohexadiene with Pd/C in MeOH was used to cleave the carbamate group cleanly and quantitatively to furnish **100**. The primary amine of the coupled glycine was revealed in quantitative yield without need for further purification. We believed that reaction of the secondary amine with the aldehyde or the corresponding acid derivative of the cyclophane cage could achieve the next stage of the ligand synthesis. In terms of efficiency, reductive amination of the aldehyde and amine was preferred, as it reduced the number of synthetic steps in the procedure.

Unfortunately, it was found that were was very little product being produced and the separation of the mixture was non-trivial. The alternative strategy of grafting the

macrocyclic acid to glycine derivative using the peptide coupling reagents, DCC and DMAP, finally gave us the desired compound **101** in 75% yield. Boc deprotection of the ligand precursor, using acid conditions (TFA/DCM) gave the desired hybrid ligand $\mathbf{L_1}$ in good yield. Shown below in Figure **52** is the ^{1}H NMR spectrum for $\mathbf{L_1}$.

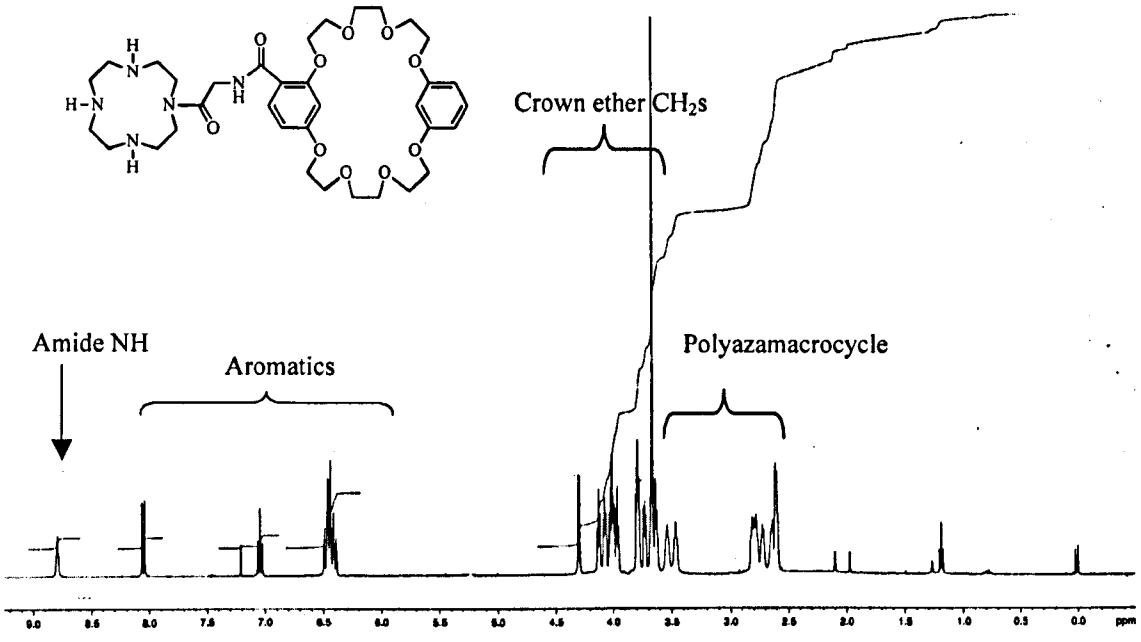


Figure 52. 1H NMR of L_1. It can be seen that it was possible to obtain these polar compounds in extremely high purity.

In order to analyze the binding constants for phosphates a non-coordinating counter anion for the zinc source was required. $Zn(CF_3SO_3)_2$ was selected as a preferable option to the less amenable zinc perchlorate. The zinc(II) complex was prepared by reacting $\mathbf{L_1}$ with one molar equivalent of $Zn(CF_3SO_3)_2$ in anhydrous methanol. Potential difficulties lay in the site-specific coordination of the metal in the cyclic polyamine. In order to determine the metal ion's location, ^{1}H NMR titrations were carried out to examine where proton shifts occurred during metal chelation. As can be seen from our titrations in Figure **53**, the zinc metal was bound solely to the cyclic polyamine and not in the crown ether. There was a definite loss of definition in the protons of the polyazamacrocycle and little or no effect imposed upon the crown ether protons. The complex $[Zn\mathbf{L_1}(OH_2)](CF_3SO_3)_2$ was readily soluble in water as well as protic solvents.

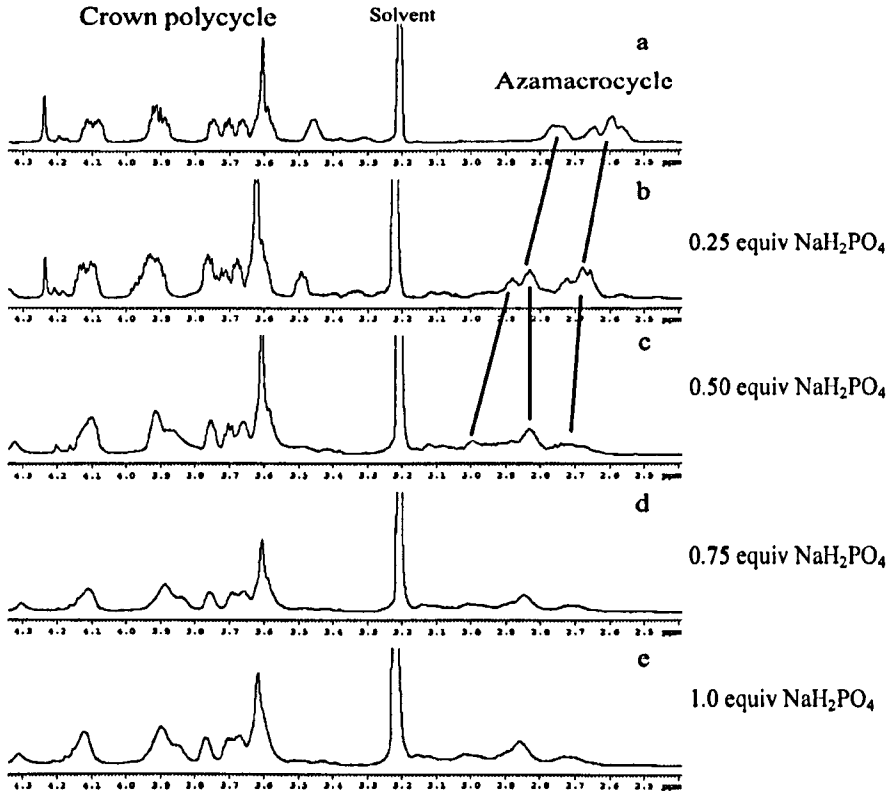


Figure 53. ^{1}HNMR titration of $Zn(CF_3SO_3)_2$ into solution of L_1 in MeOD. The titration involves the step-wise addition of four 0.25 molar equivalents of $Zn(OTf)_2$ into a solution of L_1. Spectrum e represents the 1:1 molar ratio of $Zn(Of)_2$ to L_1. It can be seen that there is almost no shifts in the crown ether signals. This represented the successful complexation of the Zn metal in the polyazamacrocycle.

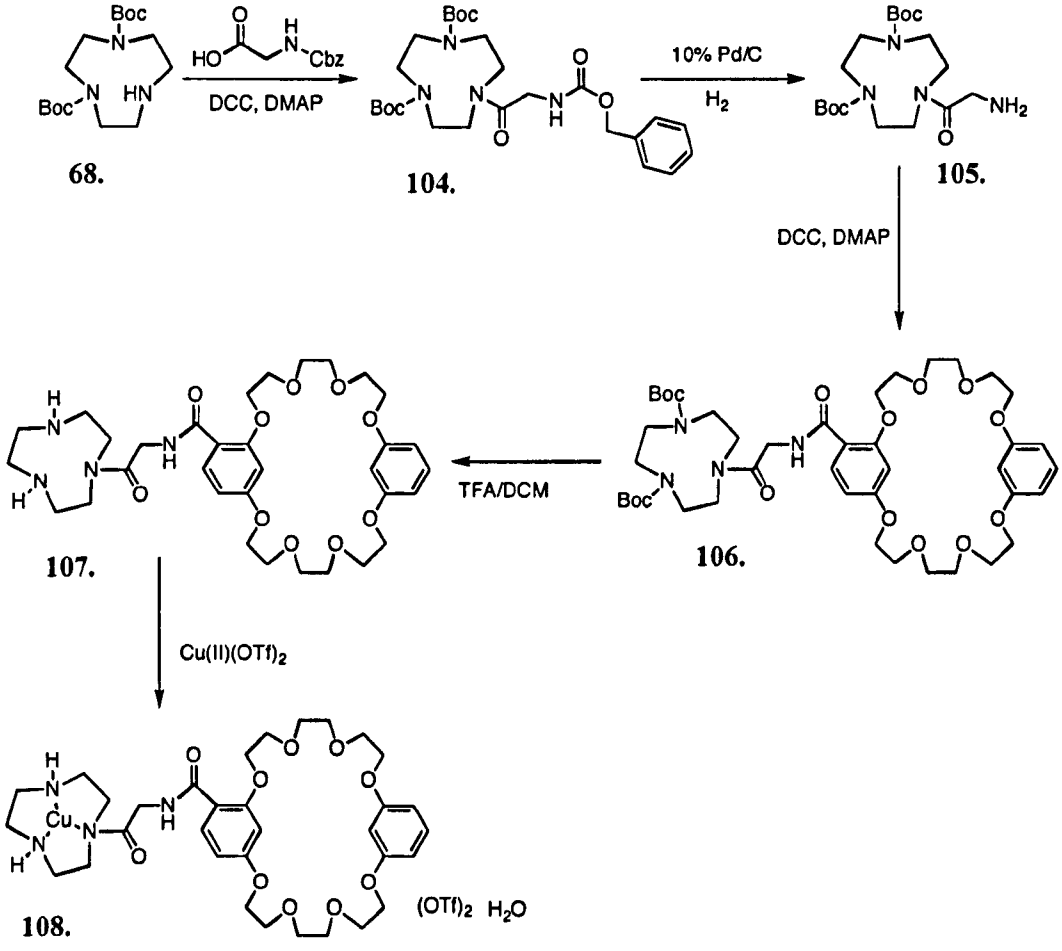
5.2.0 Variation of the cyclic polyamine

5.2.1 Copper based receptor mimics

Having established the synthetic methodology toward building the primary zinc ligand, the feasibility of the modular system was tested by altering the cyclic polyamine. Variation in the size of the metal host component would allow the preferential binding of a different metal ion. Of particular interest were the chelation of a copper ion and the construction of a copper based receptor. Comparisons could be drawn against the large number of examples of artificial copper based receptors in the literature.[6,7] Our efforts focused around the synthesis of a tacn derivative which binds copper with high affinity. The tacn basin is essentially an ideal fit for the copper ion which sits just above the plane in a tetrahedral geometry with a coordinated water molecule occupying the free site. The design was based on the original synthesis of $\mathbf{L_1}$ with the amino acid and crown ether components remaining constant. The two significant differences were the inclusion of tacn as a direct

replacement for cyclen and the chelation of copper triflate in place of the corresponding zinc source.

5.2.2 *Synthesis of tacn ligand derivative*



Scheme 25. Synthesis of $CuL_2.(OTf)_2$ tacn derivative ligand.

The determining step in the procedure was the selective Boc protection of two amines on the triazamacrocycle to give **68**. With the use of high dilution conditions and mechanical syringe pumps, we were able to manipulate the reaction to obtain very satisfactory yields of **68** in 74 %. Subsequent acid coupling of the Boc protected tacn to the Cbz-glycine was carried out in good yield to give **104**, then deprotected with standard hydrogenation conditions, H_2 and Pd/C in MeOH, to furnish the primary amine **105**. Reaction with the crown ether acid gave the receptor precursor **106**. Acid deprotection of the Boc groups with TFA revealed the free base **107**. The addition of 1 molar equivalent of $Cu(II)(OTf)_2$ gave the desired copper derivative **108**.

The synthesis of the copper ligand showed that the incomplete Boc protection of different sized polyazamacrocycles could be universally applied. The importance of being able to include different metal hosts in our ligand design is that it enables the building of multiple

systems which could mimic a number of enzymes with varying metal centres. Shown below is the ^{1}H NMR spectrum for the ligand precursor L_2.

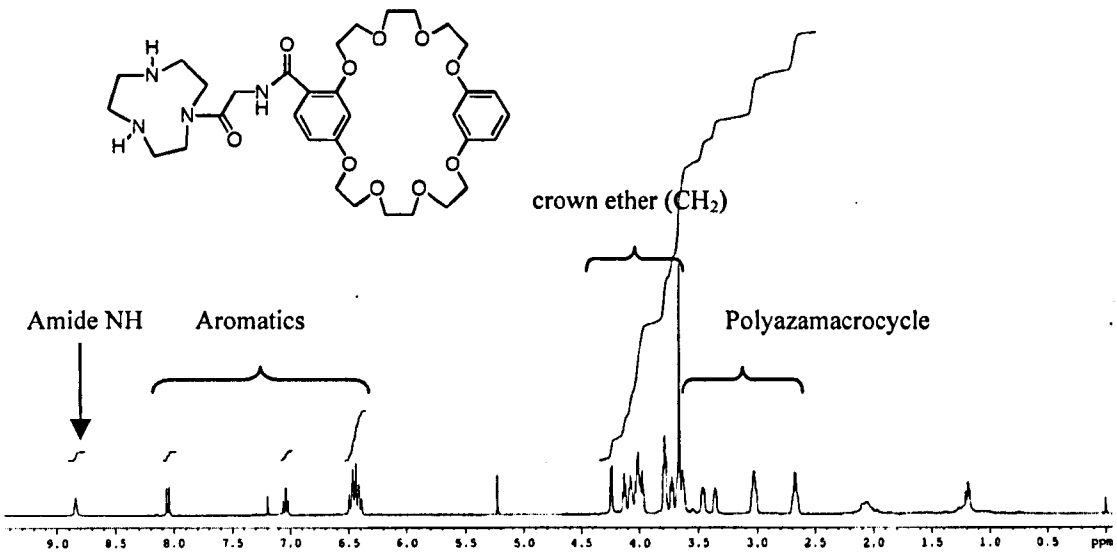


Figure 54. ^{1}H NMR spectrum for the ligand precursor L_2.

5.3.0 Synthesis of a bimetallic receptor system

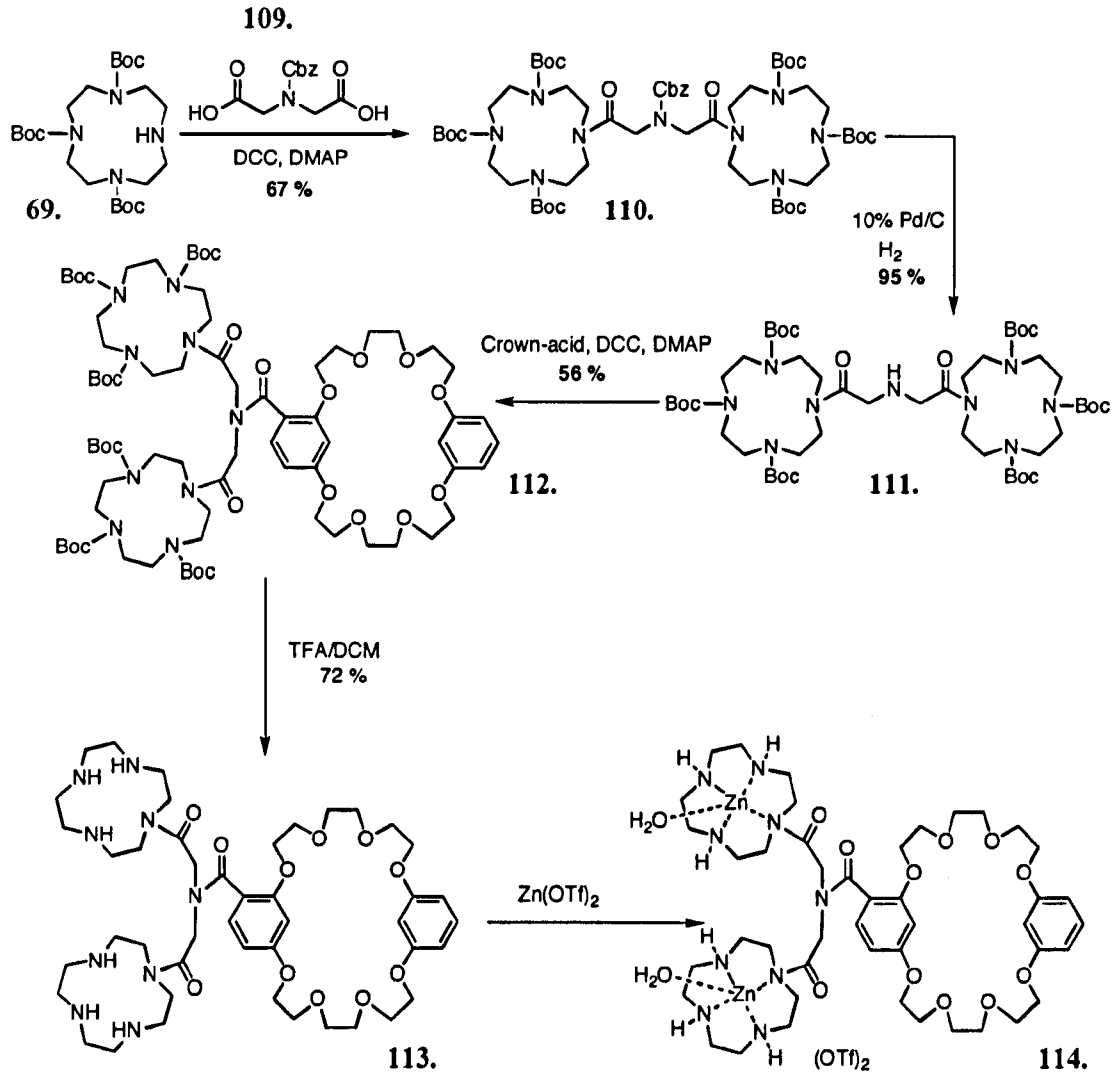
5.3.1 Di-zinc ligand derivative

As has been discussed in the introduction, many enzyme systems such as superoxide dismutase, ribonucleotide reductase and alcohol dehydrogenase contain bimetallic reactive sites for the activation and reaction of substrates. In particular, bimetallic reaction centres are key to the transformations found in protein phosphorylation[8] and phosphate ester hydrolysis.[9] It was therefore important that our systems could imitate the multimetallic motifs found in enzymes. The imitation of phosphorylases, such as carboxypeptidase A, with a two zinc metal centred ligand was of particular interest. Direct comparisons between the mono and di-metallic ligands could be made and conclusions drawn about the merits of both structures.

Synthetically, the incorporation of two cyclic polyamines would give excellent host sites for the construction of a bimetallic system. To achieve this goal the linker unit needed to contain a bridging amine which coupled directly with the macrocyclic crown and two pendant carboxylic acid sites for the addition to the polyazamacrocycles. Since no such natural α-amino acid exists, a suitable replacement was found in aminodiacetic acid. It fulfilled the required functional specifications: a central secondary amine and two pendant

carboxylic acid groups. The general strategy was therefore the same, but with the addition of a second cyclen unit.

5.3.2 Synthesis of bimetallic ligand derivative



Scheme 26. Synthesis of bimetallic $Zn_2L_3.(OTf)_4$ derivative ligand.

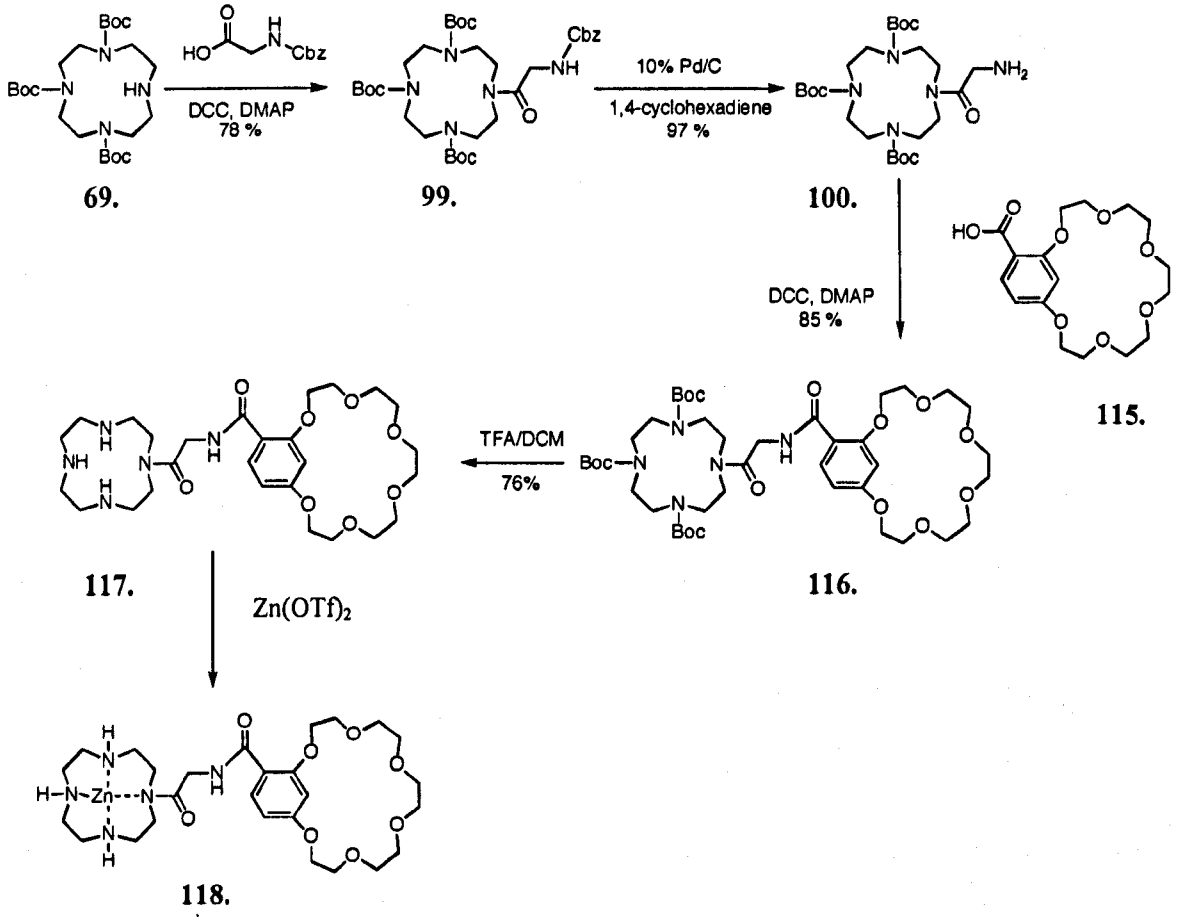
Shown in Scheme 26 is the procedure used in the preparation of the tritopic ligand L_3. The first step involved the Cbz protection of the linker's secondary amine using BnOCOCl and NaOH. Coupling of the two cyclen units to the protected aminodiacetic acid produced the desired compound **110**, in 67 % yield. Deprotection of the Cbz group with the transfer hydrogenation using 1,4-cyclohexadiene and Pd/C was unsuccessful, returning unreacted starting material. Having carried out molecular modelling using the program Hyperchem 4.0 the possible reasons for the failure could be the extremely hindered position of the central amine. Standard hydrogenation conditions, H_2 and Pd/C, were preferable reagents, removing the protecting group to afford the secondary amine **111**, in 95 % yield. Coupling of the dicyclen species with the crown ether afforded the Boc-protected derivative of our second-generation ligand in a 56 % yield. The low yield can be explained by the lower

reactivity of the secondary amine and by unfavourable steric interactions. Deprotection under acidic conditions (TFA) yielded the ligand $\mathbf{L_3}$ in an unoptimised 72 %. Reaction of $\mathbf{L_3}$ with two equivalents of $Zn(CF_3SO_3)_2$ in methanol gave the corresponding bimetallic complex $Zn_2L_3(OH_2)_2(CF_3SO_3)_4$ in quantitative yield.

The synthesis of the bimetallic complex showed that our modular approach could be adapted to cater for a variety of design features. The most exciting system envisaged was the construction of a ligand with two different sized cyclic polyamines, where each host site selectively binds a specific metal and creates an artificial multi-metal complex. This would offer an ideal opportunity to mimic some of the most important enzymes such as superoxide dismutase and alcohol dehydrogenase which include zinc and copper at the metal centre.

5.4.0 Variation of the macrocyclic crown ether

5.4.1 Synthesis of 19-benzocrown-6 derivative



Scheme 27. Synthesis of 19-benzocrown-6 $ZnL_4.(OTf)_2$ ligand derivative.

In order to cater for both large and small phosphate species, a compact crown ether ligand derivative was synthesised. The synthesis of the aldehyde 19-benzocrown-6 was described

in chapter 5. Attempts to oxidise the pendant aldehyde to the carboxylic acid with $KMnO_4$ and then $NaMnO_4$ was found to be problematic, giving very low yields. The use of sodium chlorite overcame these problems affording the desired acid in almost quantitative yield.[12] The removal of the resorcinol group from the crown ether removed the possibility of adverse chlorination, with the added benefit of minimum purification.

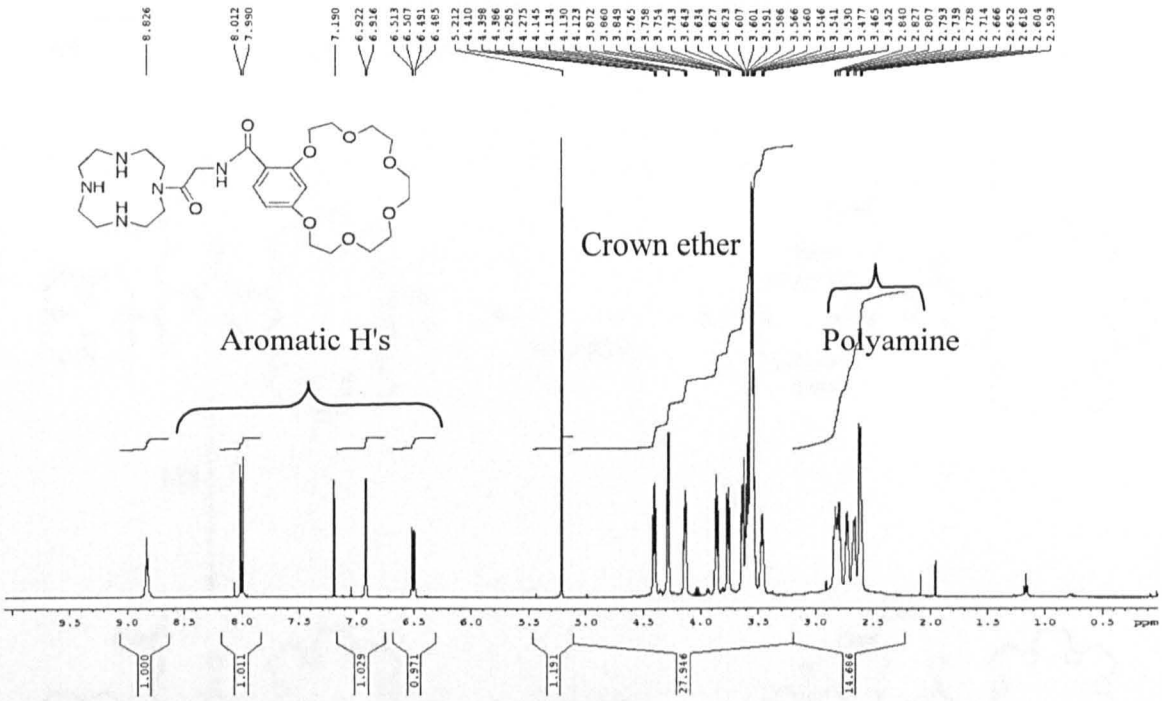


Figure 55. ^{1}H NMR spectrum of L_5 ligand.

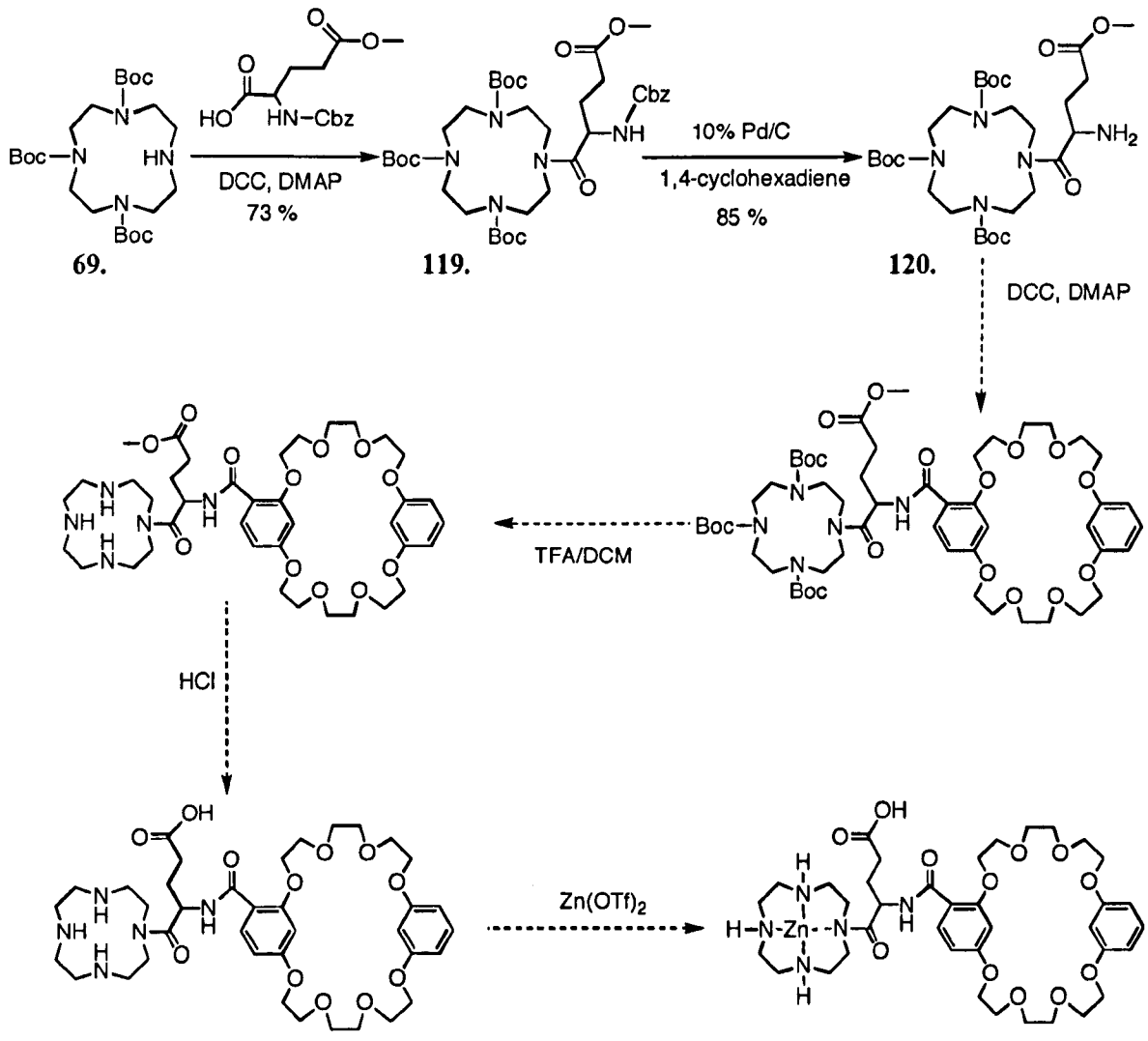
The ligand design was based on the original zinc $\mathbf{L_1}$ motif, with only the replacement of the large cyclophane structure by the 19-benzocrown-6. The modular methodology was then applied, providing the zinc triflate complex of $\mathbf{L_4}$ in excellent yield.

5.5.0 Variation of the amino acid linker

5.5.1 Synthesis of the glutamic acid derivative ligand

The final examination of the modular strategies' viability was to determine whether or not an amino acid with a functionalised side chain could be incorporated into a ligand system. The effect of an interacting side chain with the metal centre was an appealing prospect which had to be addressed. The amino acids which were of particular interest: glutamic acid, histidine and arginine, are key residues, seen at the sites of phosphate reaction centres.[11] Molecular modelling investigations with Hyperchem 4.0 determined that of the three candidates, only glutamic acid had a sterically favoured opportunity to interact with the zinc metal centre.

5.5.2. Glutamic acid derivative synthetic protocols



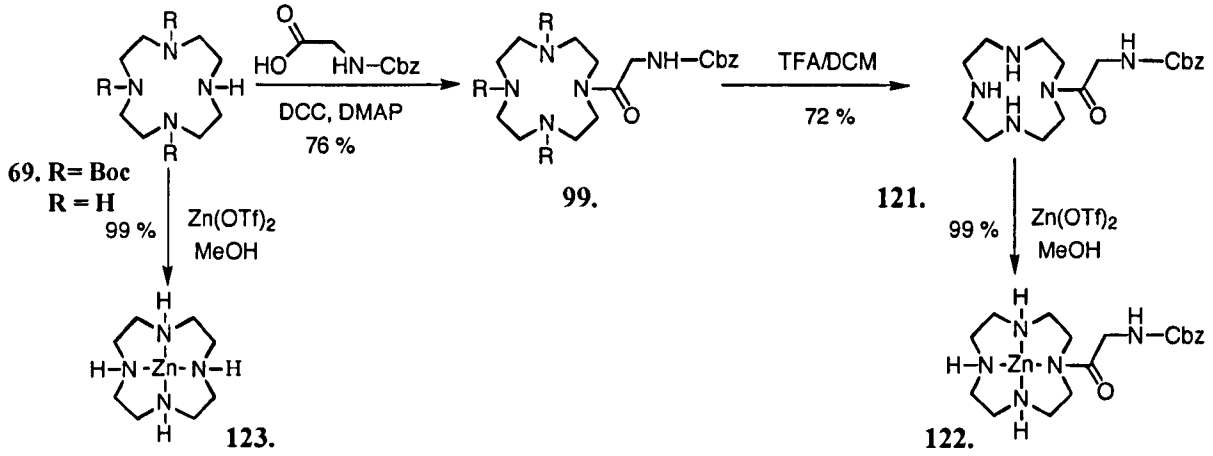
Scheme 28. Synthetic progress toward L_5.

The use of glutamic acid meant that a dual protecting group strategy was employed. The amine was protected by a Cbz group and the acid converted to a methyl ester. The coupling of the glutamic acid to the **69** with peptide coupling reagents was achieved in good yield to form **119**, before the selective deprotection of the Cbz moiety gave the exposed primary amine, **120**. The bridging amide formation to link the glutamic acid to the 26-dibenzocrown-8 was equally successful. However time constraints meant that the completion of this ligand was not possible. Completion should be a matter of removing the Boc protecting groups, before methyl ester hydrolysis reveals the carboxylic acid functionality.

The inclusion of glutamic acid showed that it was possible to incorporate a wide variety of functionality and diversity into the methodology. The addition of di or tripeptides with a specific backbone sequence can allow the synthesis of receptors with multiple recognition

sites. The potential exists to build larger more complex structures to interact with biologically important superstructures like DNA and RNA.

5.6.0 Synthesis of control systems



Scheme 29. Synthesis of the zinc complex of control 1 and 2.

In order to gauge the effectiveness of our ligands, appropriate control experiments were carried out to understand the mechanics of the systems. The control compounds were synthesised fragments of the ligand components. The first control was simply the zinc complex of cyclen. This allowed the direct comparison of phosphate binding to the cyclen complex versus the ligand system. In order to determine the effect of the amino acid, a zinc cyclen complex coupled to a glycine unit was synthesised. This involved the coupling of the Boc protected cyclen with glycine-Cbz, followed by the acid mediated deprotection of the Boc groups giving the pure compound in excellent yield and high purity. The addition of an equimolar equivalent of zinc triflate gave the desired zinc complex quantitatively **123**.

5.7.0 References

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Chapter 6

6.1.0 Phosphate binding experiments

The binding of our systems to phosphate moieties was measured principally by isothermal titration calorimetry and verified via UV/vis titration experiments, ^{1}H NMR, electrospray mass spectrometry and potentiometric pH titration. A typical ITC spectrum is illustrated below in Figure **56**, showing the binding isotherm produced from the interaction of a host and guest.

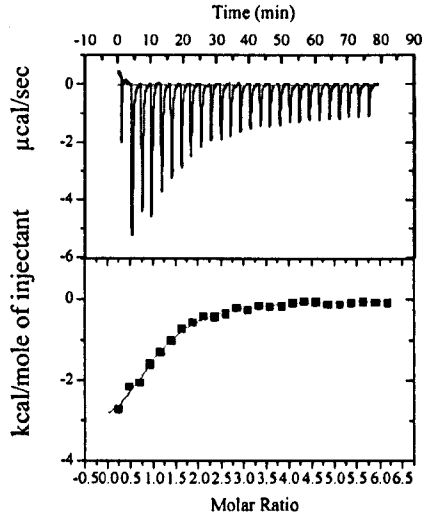

Figure 56. Typical isothermal titration calorimetry curve.

6.1.1 Buffered solutions

In each experimental procedure it was necessary to monitor the binding at physiological pH 7.4. There are a number of non-coordinating buffers such as HEPES, TRIS and MOPS. However, previous research has shown that HEPES was the preferred buffer to TRIS which exhibited competitive binding with phosphate.[1] However work by Ren[2] also showed that HEPES was weakly bound to metal complexes through presumed interaction with the sulphonate group tail. To minimise heats of mixing a good non-coordinating buffer was required.

Simple ^{1}H NMR control experiments were undertaken to explore the interactions between the ligand and HEPES at low and high concentrations of the buffer. Preparation of NMR samples of ligand ($ZnL_1.(OTf)_2$) in D_2O with varying concentrations of buffer (0 equiv, 1

equiv, 10 equiv and 100 equiv), showed that there was no interaction with either the metal or the crown ether on the NMR timescale.

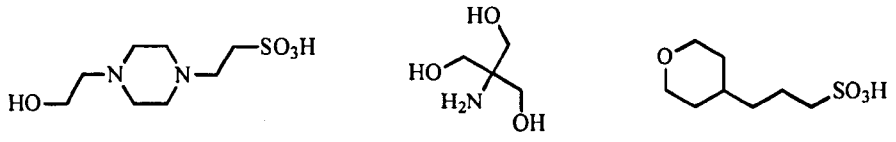

Figure 57. Structure of buffer compounds.

6.1.2 *Isothermal titration calorimetry (ITC)*

Every molecular interaction either generates or absorbs small amounts of heat; ultra-sensitive ITC can detect these small changes in heat. Each ITC experiment provides a complete thermodynamic profile of the interaction including the binding constant (K_a), the number of binding sites(n), enthalpy (ΔH), entropy (ΔS) and free energy (ΔG). Using ITC it was possible to examine the thermodynamic interactions between a variety of substrates and the ligands. Each experiment consists of a number of equal-volume injections of substrate solution into the macromolecule solution (ligand) contained in the sample cell. Each peak represents the energy released from binding, resulting from a single injection of substrate into ligand.

The experimental results were analysed using Origin data analysis software, the area of each injection peak is automatically determined and the results are plotted. This plot is referred to as a binding isotherm, where the total heat per injection (kcal per mole of substrate injected) is plotted against the molar ratio of substrate and ligand. The precise shape of the binding isotherm contains all the information required to completely characterize the binding reaction.

For every experiment, subsequent controls on buffer-buffer and buffer-phosphate interactions were carried out and the interactions subtracted from the final binding isotherm to give an accurate profile of the ligand-phosphate binding interaction.

A variety of phosphates were used to examine the potential of the compounds to act as receptors in an aqueous environment. The ligand systems were required to be active at physiological pH and so experiments were carried out in buffered water solution at pH 7.4.

Different metal salts of the phosphates were used to observe the effect of the cation on the binding strength.

In a typical ITC experiment a long-needle syringe, with a paddle fastened to its end was filled with the phosphate solution (~5.00 mmol) and inserted into the sample cell which was loaded with ligand solution (0.25 mmol). After the syringe insertion, the assembly rotates at a pre-selected speed (240 rotations a minute) and injects sample at selected intervals. Each experiment consisted of 25 x 10μ L titration injections os substrate.

6.1.3 UV/visible titration

Binding experiments were also carried out using UV/vis titration experiments. Since the ligand derivatives have both aromatic chromophores and metal to ligand transitions, UV/vis binding analysis was ideal. The monitoring of the variation in absorbance at a specific wavelength, with the addition of phosphate, allowed the correct identification of binding stoichiometries. For UV/Vis titrations, a solution of the phosphate (10 mmol) in HEPES buffer at pH 7.4 was added to the ligand solution (0.125 mmol) and the UV/vis spectrum was recorded for each titration point between 0 and 5 equivalent of phosphate guest. The data was then plotted to determine binding stoichiometries.

6.1.4 ^{1}H NMR titrations

The use of ^{1}H NMR as a tool for monitoring binding in aqueous solution was extremely beneficial. Observations to identify the exact location at which phosphate binding was occuring was carried out, as well as calculations to determine the binding constants using the change in shift of a specific nucleus on the host for each addition from 0 to 3 equivalents of phosphate. The program EQNMR[2] analyzed the resulting data.

6.1.5 Electrospray mass spectrometry

Electrospray mass spectrometry was used to identify the binding products in aqueous solution. General mass spectrometry techniques such as FAB^+, CI^+ and EI^+ generate numerous fragment products and there are several instances where these techniques are not suitable. The mass determination of biological samples (e.g. peptides, proteins and oligonucleotides) and for inorganic and/or organometallic compounds are much more difficult to obtain. In these cases the sample is either involatile or too fragile to be

transferred to the gas phase intact through EI mass spectrometric techniques, though FAB has addressed these limitations to a certain degree.

The other major flaw with traditional mass spectrometric techniques is that they are only applicable to solid or gaseous samples and not for aqueous state chemistry. Solution state chemistry is a key importance to biological chemistry and to our research of binding in an aqueous environment.

The technique of electrospray (ionisation) mass spectrometry (ESMS) addresses these limitations by allowing the transfer of ions within a solution directly to the gas phase through a 'soft' method, allowing fragile samples to remain intact. This technique has been exploited in the study of biological systems but is now being applied to inorganic samples.

6.2.0 Properties and binding experiments for $ZnL_1.(OTf)_2$.

6.2.1 *Properties of $ZnL_1.(OTf)_2$.*

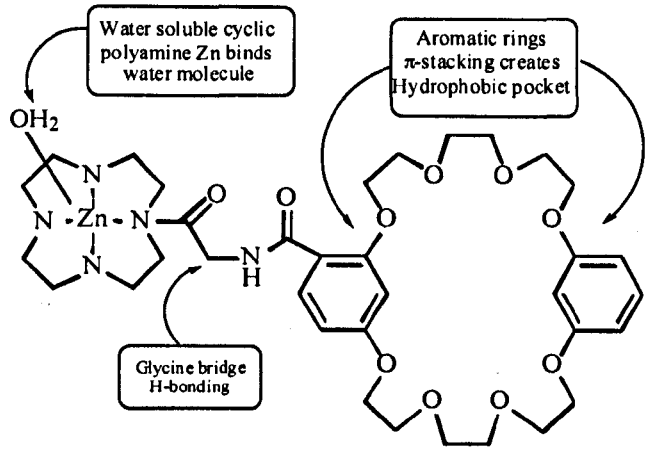

Figure 58. Differing regions of polarity and function.

In order to quantify the water solubility of the ligand, determination of the partition coefficient was necessary. The logP value was obtained experimentally for $ZnL_1.(OTf)_2$ by taking the UV spectrum of an aqueous solution of the ligand, of known concentration and calculating the extinction coefficient from the observed absorption and concentration profile. The solution was then added to an equivalent volume of octanol and shaken vigorously before being left overnight to allow separation of the two layers. The two phases were separated and the differential concentration of $ZnL_1.(OTf)_2$ partitioned between octanol and water was measured using the Beer-Lambert law. The experimentally

obtained logP value was verified by comparison with the program ClogP.4.0 obtained from the website www.biobyte.com.

The ClogP.4.0 program estimated the log octanol/water partition coefficient of all ligands using atom/fragment contribution calculations. Both values obtained were in close approximation to each other giving values of 0.23. This was representative of a system containing both hydrophobic and hydrophilic regions, indicating that L_1 was ampiphilic. Natural enzymes contain within short distances, domains that are hydrophilic and hydrophobic in nature. This unique blend of polarity and reactivity makes enzymes such powerful catalysts. In order to mimic artificial systems, the ligand needed to contain diverse physiochemical properties.

Having established that $ZnL_1.(OTf)_2$ was operational in an aqueous environment, it was then necessary to test the binding ability.

6.2.2 *Binding results for $ZnL_1.(OTf)_2$ and Zn(cyclen).$(OTf)_2$.*

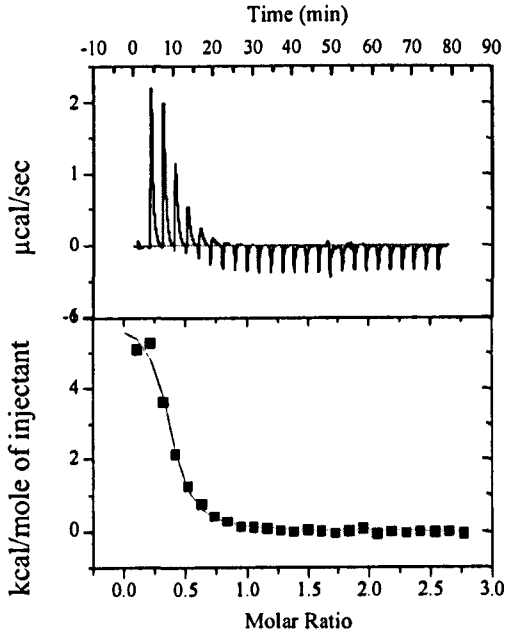

Figure 59. ITC graph for the binding of $ZnL_1.(OTf)_2$ to LiH_2PO_4

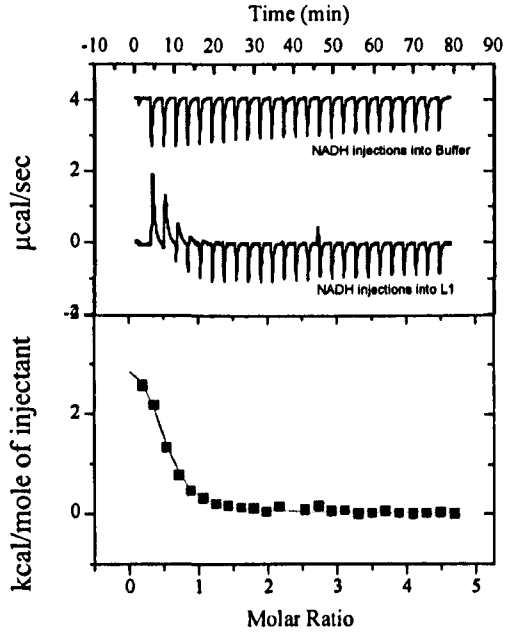

Figure 60. ITC graph for the binding of $ZnL_1.(OTf)_2$ to NaH_2PO_4

The phosphates selected for testing were $Na[H_2PO_4]$, $K[H_2PO_4]$, $Li[H_2PO_4]$, sodium-glycerophosphate and sodium-nitrophenylphosphate. This allowed comprehensive analysis of the phosphate size on binding constants and examination of ion pair cooperativity.

Reported in Table 2 are the binding results obtained for $ZnL_1(OTf)_2$, and Zn(cyclen).$(OTf)_2$. The initial comparison between $ZnL_1(OTf)_2$ and the Zn(cyclen).$(OTf)_2$ control was to determine whether the ligand system was beneficial to the primary metal binding site.

Ligand	Substrate	Ligand:Substrate binding ratio	K x 10^4	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$-T\Delta S°$/kcal mol^{-1}
$Zn(H_2O)L_1$	$Na[H_2PO_4]$	2:1	4.93±0.72[a]	-6.39	3.47±0.17	9.86
$Zn(H_2O)L_1$	$K[H_2PO_4]$	2:1	9.32±1.69[a]	-6.78	2.44±0.09	9.22
$Zn(H_2O)L_1$	$Li[H_2PO_4]$	2:1	25.4±5.40[a]	-7.39	5.97±0.24	13.33
$Zn(H_2O)$cyclen	$Li[H_2PO_4]$	1:1	1.34±0.03[b]	-5.13	-3.56±0.05	1.57
$Zn(H_2O)$cyclen	$Na[H_2PO_4]$	1:1	1.60±0.09[b]	-5.74	-3.25±0.08	2.49
$Zn(H_2O)$cyclen	$K[H_2PO_4]$	1:1	1.52±0.19[b]	-5.70	-3.89±0.24	1.81
$Zn(H_2O)L_1$	Sodium glycerophosphate	1:1	0.48±0.06[b]	-5.02	0.48± 0.02	5.50
$Zn(H_2O)$cyclen	Sodium glycerophosphate	1:1	0.87±0.04[b]	-5.38	-1.63±0.06	3.75
$Zn(H_2O)L_1$	Disodium4-nitrophenylphosphate	1:1	0.39±0.04[b]	-4.90	0.58± 0.04	5.48
$Zn(H_2O)$cyclen	Disodium4 nitrophenylphosphate	1:1	0.51±0.21[b]	-5.05	-0.45±0.19	4.60

Table 2. Thermodynamic data for the binding of various substrates to $ZnL_1.(OTf)_2$ and Zn(cyclen).$(OTf)_2$ in HEPES buffer at pH 7.4 at 25 °C. [a] mol^{-2} dm^6. [b] mol^{-1} dm^3.

The first point to be taken from the table was the large binding constant for the $[H_2PO_4]$ anion in aqueous solution with various counter cations. The results compare favourably with the binding constants recently reported by Anslyn ($2.5x10^4$)[3] and Ren ($4.2x10^3$ buffered; 2.4×10^4 unbuffered).[4]

Secondly we have obtained high binding constants for the $Na[H_2PO_4]$ phosphate moiety in aqueous solution. Variation of the cation led to substantial increases in binding affinity. Potassium phosphate was bound approximately twice as strongly to $ZnL_1.(OTf)_2$ compared with the sodium salt. Furthermore, the corresponding Li^+ salt was three times more strongly bound than the potassium salt. In comparison, the binding of the sodium, lithium and potassium phosphate to Zn(cyclen).$(OTf)_2$ was essentially identical. Since the crown ether was of such a size as to favour the binding of K^+ over Na^+ and Li^+, this result

strongly suggested that the crown was coordinating the cation and thus that $ZnL_1.(OTf)_2$ was an ion pair receptor. The lithium phosphate was an interesting result in that its intention had been to provide a negative control experiment. The predicted low complexation of the lithium metal was to show negative cooperativity. The large dimensions of the crown ether made the tight binding of the small $Li[H_2PO_4]$ quite unexpected. The high affinity is ascribed to conformational optimisation through hydrophobic interactions, forming a compact crown ether structure, suitable for the smaller cation binding. It is interesting to note that higher binding is accompanied by an increased entropy value. Lithium has a highly ordered solvation sphere which upon binding is presumably disrupted as well as the desolvation of the crown ether cavity. This "double" desolvation could be responsible for the increased binding through an increase in entropy.

6.2.3 *Ion pair cooperative binding of $ZnL_1.(OTf)_2$*

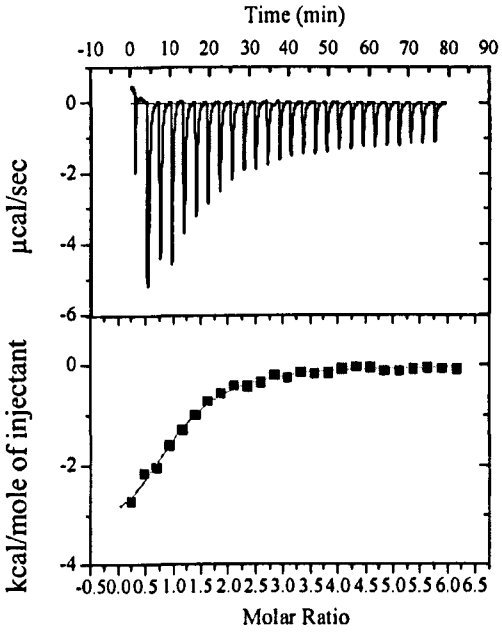

Figure 61. ITC graph for the binding of Zn(cyclen).$(OTf)_2$ to KH_2PO_4

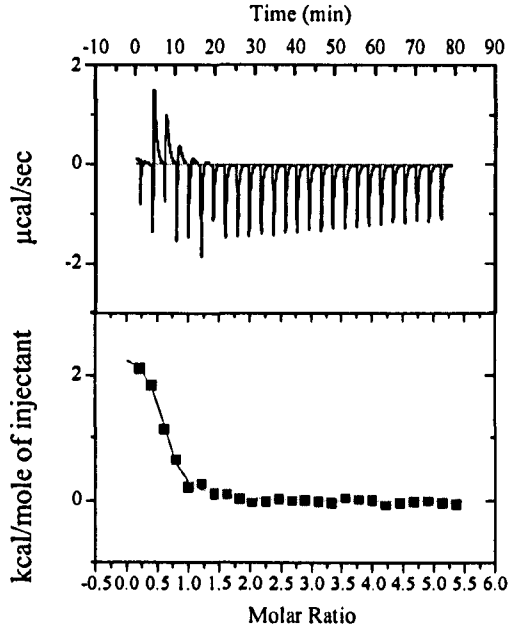

Figure 62. ITC graph for the binding of $ZnL_1.(OTf)_2$ to KH_2PO_4.

In order to prove that the $ZnL_1.(OTf)_2$ was an ion pair receptor, it was necessary to show that the association of one ion altered the binding affinity of the counterion.[5] Using ITC we were able to observe the binding of Li^+, K^+ or Na^+ cations by the use of a non-coordinating anion such as perchlorate. Under identical experimental conditions, there was no evidence of monotopic interactions to $ZnL_1.(OTf)_2$ by either $NaClO_4$, $LiClO_4$ or $KClO_4$. It must therefore be assumed that anion binding was essential for cation complexation, ie: positive heterotropic allostery.[6] Cooperative and allosteric phenomena have been well documented in the literature by Reinhoudt,[6] Beer[7] and Anslyn.[3]

Importantly our initial ligand system $ZnL_1.(OTf)_2$ binds $[H_2PO_4]$ salts between three and seventeen times more strongly than the Zn(cyclen).$(OTf)_2$ control, demonstrating the enhanced binding provided by the crown arm. Shown in Figures **61** and **62** is the comparitive binding of $K[H_2PO_4]$ by Zn(cyclen).$(OTf)_2$ and $ZnL_1.(OTf)_2$. The sodium salt of *p*-nitrophenyl phosphate binds to essentially the same extent to the ligand and the control within experimental error. The substituted aromatic phosphate ester was expected to bind tightly to the ligand's hydrophobic regions through complementary π-π interactions. However the binding constants obtained were disappointingly low. It was assumed that the cavity of the crown ether was folded in such a manner which prevented the inclusion of larger substrates.

As shown in Figures **63** and **64**, the bulky glycerophosphate was bound more strongly by Zn(cyclen) than by $ZnL_1.(OTf)_2$ The same conclusions were drawn for the lack of substantial complexation.

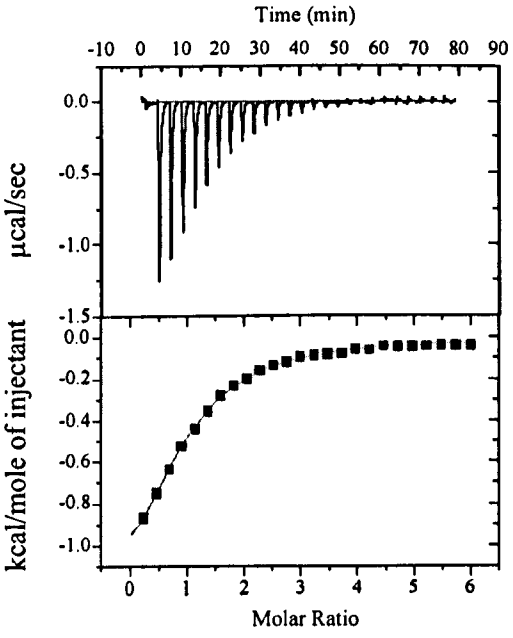

Figure 63. ITC graph for the binding of Zn(cyclen).$(OTf)_2$ to Na-glycerophosphate.

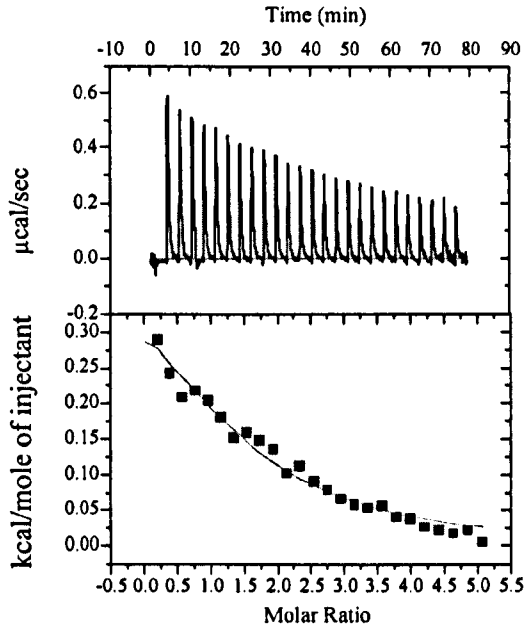

Figure 64. ITC graph for the binding of $ZnL_1.(OTf)_2$ to Na-glycerophosphate

6.2.4 *Thermodynamic profile*

Of particular interest to the work was the nature of binding between the ligand and the ion pair, and to what extent the interaction or tether between the ions exacted upon binding strengths. Where the effect of binding of one ion is not regarded as independent of the other is known as cooperativity.[1b] Cooperative binding can be either positive or negative,

dependant upon the Gibbs' free energy of binding and its constituent parts. Positive cooperativity is observed when the overall energy of binding is greater than the summation of the Gibbs' free energies of binding for the individual components. When the overall binding energy is weaker than the summation of the Gibbs' free energy for individual interactions this is known as negative cooperativity.[8]

The thermodynamic aspects of ion pair cooperativity were discussed by Jencks[8] and Williams[9] and later modelled by Anslyn.[3] Jencks defined negative and positive cooperativity in a mathematical equation, where A-B are the two components of the ion pair.

$$\Delta G_s^\circ = \Delta G_A^\circ + \Delta G_B^\circ - \Delta G_{AB}^\circ$$

Jencks' proposed that the Gibbs' free energy of binding A-B (ΔG_{AB}°) is the summation of the free energies of binding for the individual parts A (ΔG_A°) and B (ΔG_B°) plus an additional term, the Gibbs' free energy of connection (ΔG_S°).

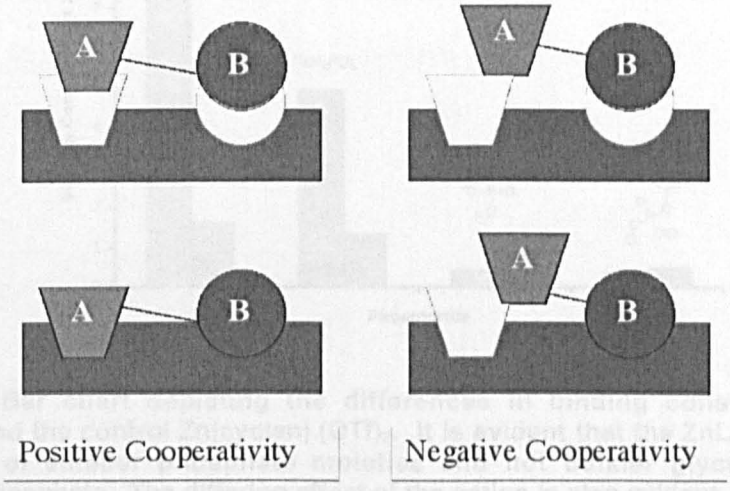

Figure 65. Depiction of positive and negative cooperativity of binding of ion pair A-B.[8]

This related to the bond or tether between the two ions. We wished to explore the nature of the cooperativity within our system using ITC. In order to do so it was necessary to determine the Gibbs' free energy for the individual binding partners. As has been discussed previously in this chapter, the binding of the metal cation was not observed with ITC experiments. In order to measure the binding of the phosphate anion we synthesised the non-coordinating cation, *tert*-butyl ammonium salt of phosphate. However it was found that this phosphate salt was insoluble in aqueous solution at the concentrations required and it was therefore impossible to determine the anion binding constant and ΔG_B°. In terms of a model system, it was known that the principal mechanism of phosphate

binding to the ligand was through the zinc metal. Due to the negligible involvement of the cation in phosphate binding to the zinc cyclen amino acid control it might be possible to use this binding data to represent the binding of the independent phosphate anion to Zn**L**$_1$.(OTf)$_2$ and obtain a rough estimate of $\Delta G_B°$. Using these values for the binding of Na[H_2PO_4] it was possible to determine $\Delta G_S°$ to be 0.65 kcal mol^{-1}. By convention a positive $\Delta G_S°$ represents positive cooperativity. For K[H_2PO_4] the $\Delta G_S°$ value was 1.08 kcal mol^{-1} and Li[H_2PO_4] was found to be 2.26 kcal mol^{-1}. It is apparent therefore that entropy was the largest contributing factor for positive cooperativity which is in agreement with the findings of Jencks'.[8] The larger entropy value for Li[H_2PO_4] gave rise to a larger positive $\Delta G_S°$ component. Crucially, the values obtained were calculated for a model system which does not take into account the crown ether, but does include the principle binding mechanism of the phosphate to the metal centre.

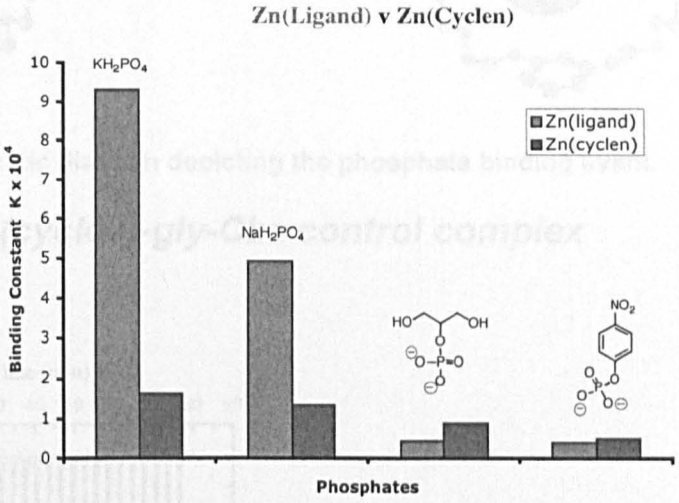

Figure 66. Bar chart depicting the differences in binding constants K_a, between ZnL_1.(OTf)$_2$ and the control Zn(cyclen).(OTf)$_2$. It is evident that the ZnL_1.(OTf)$_2$ is suited to the binding of smaller phosphate moieties and not bulkier glycerophosphate and nitrophenylphosphate. The differing affect of the cation is also evident.

With regard to enthalpy terms, the zinc cyclen amino acid control was exothermic (-$\Delta H°$), whereas the binding with Zn**L**$_1$.(OTf)$_2$ was endothermic (+$\Delta H°$). The endothermic binding event can be assigned to the reorganisation of solvent molecules upon expulsion. This has been documented in the literature for the binding of carboxylate to a Cu(II) centre.[1a] If a simple subtraction was made between the parameters for Na[H_2PO_4] bound to Zn**L**$_1$.(OTf)$_2$ and to Zn(cyclen).(OTf)$_2$ it was apparent that on going from the simple aza macrocycle to the receptor, this resulted in a $\Delta\Delta H°$ of +6.72 kcal mol^{-1} and a $\Delta T\Delta S°$ of +7.37 kcal mol^{-1} producing an overall $\Delta\Delta G°$ of -0.65 kcal mol^{-1}. We propose that the explanation for this effect was the presence of a pocket of hydrogen-bonded water trapped between the [OH]$^-$

ion (at pH 7.4) attached to the zinc ion, and the crown ether. The binding of the anion to the zinc resulted in the expulsion of the water, requiring an input of energy but generating a large amount of entropy. The $\Delta T\Delta S°$ for binding sodium and potassium phosphate to both receptors were essentially the same within experimental error. $Li[H_2PO_4]$ gave the highest $\Delta H°$ value which was attributed to the solvent molecule reorganisation, and the largest $T\Delta S°$ factor presumably resulting from the efficient expulsion of solvent and counter ions. Shown in the Figure **67** above is a bar chart highlighting the differences in binding constants between our ligand and the cyclen control.

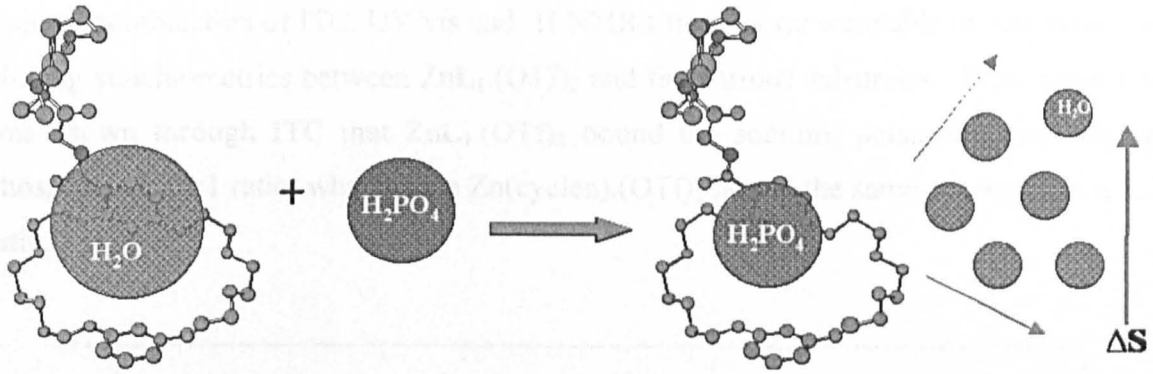

Figure 67. Schematic diagram depicting the phosphate binding event.

6.2.5 *Zn(cyclen)-gly-Cbz control complex*

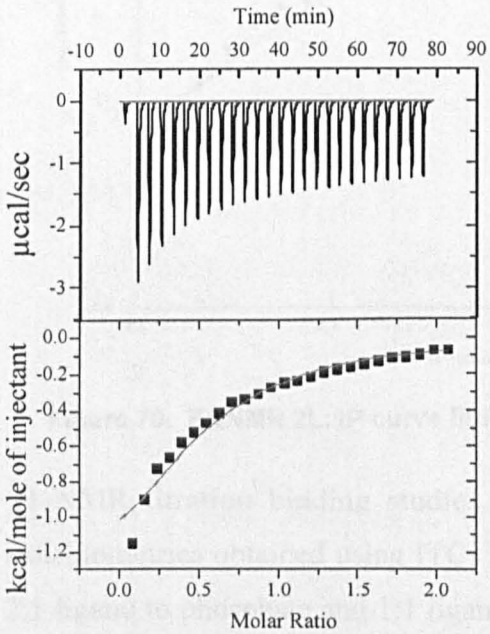

Figure 68. ITC graph for the binding of $Na[H_2PO_4]$ to Zn(cyclen)-gly-Cbz.

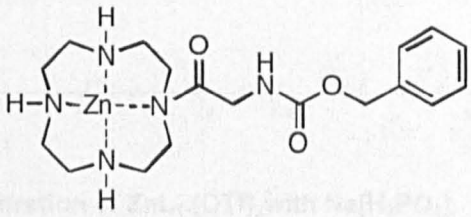

Figure 69. Structure of Zn(cyclen)-gly-Cbz.

The same experiments were repeated with the Zn(cyclen)-glycine control to establish the role of the amino acid bridge and whether the crown adversely affected the binding. It was

found that the glycine unit had an adverse affect with moderate binding constants obtained for the control ($K = 0.5 \times 10^4\ M^{-1}$). The thermodynamic profile of the interactions were consistent with the zinc cyclen control. The binding event was exothermic with a $\Delta H°$ value of -1.76 kcal mol^{-1} and a small positive $T\Delta S°$ value of 3.36 kcal mol^{-1}. The smaller entropy factor clearly proved that $ZnL_1.(OTf)_2$ bound the substrates through the entropically favoured desolvation of the crown ether cavity.

6.2.6 *Binding stiochiometries*

Using a combination of ITC, UV/vis and ^1H NMR titrations we were able to determine the binding stoichiometries between $ZnL_1.(OTf)_2$ and the various substrates. From table 1 it was shown through ITC that $ZnL_1.(OTf)_2$ bound the sodium, potassium and lithium phosphate in a 2:1 ratio, whereas the Zn(cyclen).$(OTf)_2$ bound the same substrates in a 1:1 ratio.

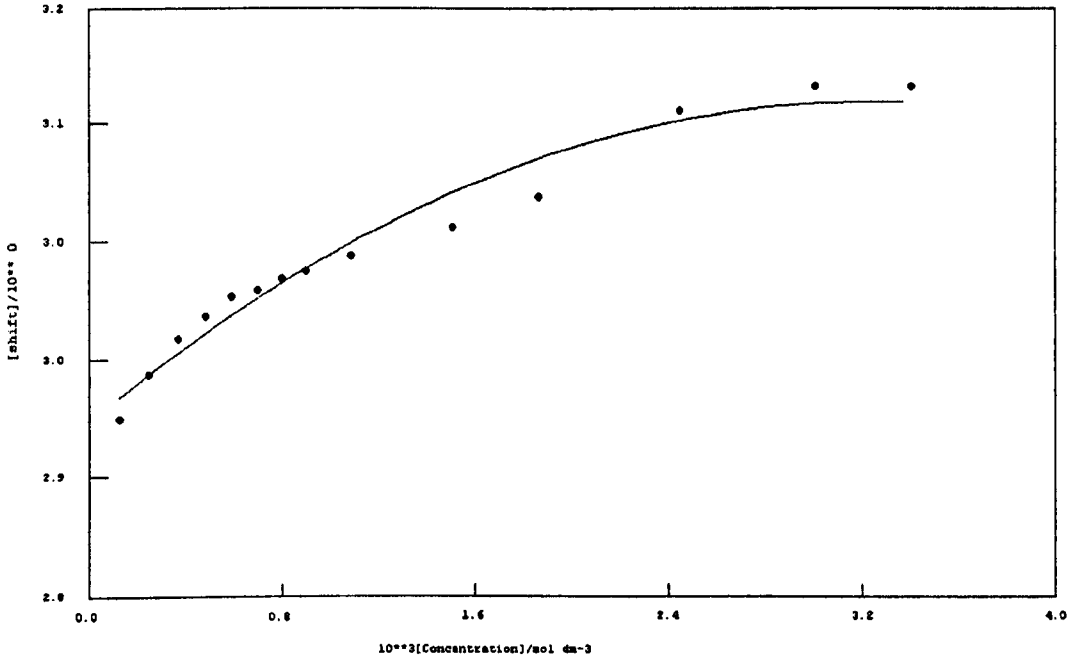

Figure 70. EQNMR 2L:1P curve fitting for the titration of $ZnL_1.(OTf)_2$with $Na[H_2PO_4]$.

^1H NMR titration binding studies were carried out in D_2O to confirm the binding stoichiometries obtained using ITC. The curve was plotted in EQNMR and fitted using a 2:1 ligand to phosphate and 1:1 ligand to phosphate ratio to determine the best fit. It was found that both systems fitted the curve but the 2:1 ratio had a much lower error value and therefore a more accurate fitting. The poor quality of this spectrum was associated with the error in assigning a specific proton on the NMR.

The differing binding ratios were understood by the hydrophilicity of the two species. Through the calculation of partition coefficients the Zn(cyclen).$(OTf)_2$ complex was shown to be substantially more water soluble than the ampiphilic $ZnL_1.(OTf)_2$ which required vigorous stirring to obtain a homogenous solution.

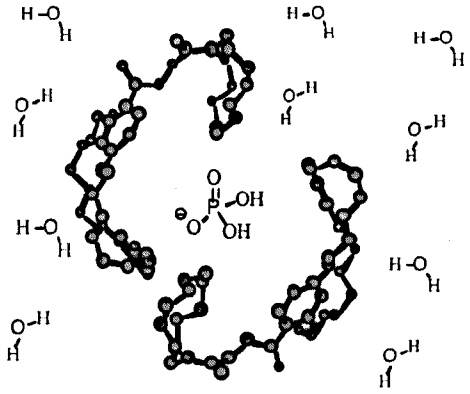

Figure 71. Schematic diagram illustrating the 2:1 binding ratio between $ZnL_1.(OTf)_2$ and $H_2PO_4^-$.

The efficient solvation of Zn(cyclen).$(OTf)_2$ was clearly related to the formation of a 1:1 complex. Therefore, hydrophobic effects were singled out as the principle reason for the 2:1, $ZnL_1.(OTf)_2$:phosphate ratio. The hydrophobic aggregation of two $ZnL_1.(OTf)_2$ to form a tight binding pocket that encapsulated a phosphate species, provided a suitable model that reflected the experimental findings.

6.2.7 *$ZnL_1.(OTf)_2$ summary*

$ZnL_1.(OTf)_2$ was shown to be a successful phosphate binding host which displayed ion pair cooperative binding properties. Optimal interactions were achieved with specific phosphate salts and in particular with lithium phosphate which was bound with exceptional strength. The poor binding of larger phosphate monoesters was presumed to be due to the small cavity created between the polyazamacrocycle and the crown ether.

6.3.0 Binding results for $CuL_2.(OTf)_2$

6.3.1 *ITC binding experiment*

The synthesis of $CuL_2.(OTf)_2$ offered an interesting opportunity to directly compare the effect of a different metal on phosphate binding in an identical system. Previous work by Anslyn,[1a] who constructed a copper centred crytpand scaffold with pendant guanidinium arms, showed the potential of copper based artificial receptors. We wished to explore the potential of a copper based motif within the ligand system.

The results for the binding of $CuL_2.(OTf)_2$ with the phosphate species were interesting. A complete lack of any substantial binding to any of the phosphate moieties was unexpected. We observed negligible binding of the phosphate and no binding of the phosphate esters. In order to ascertain the reasons for the lack of complexation, further experimentation was carried out using UV/vis and potentiometric pH titrations

6.3.2 *UV/vis titration experiments*

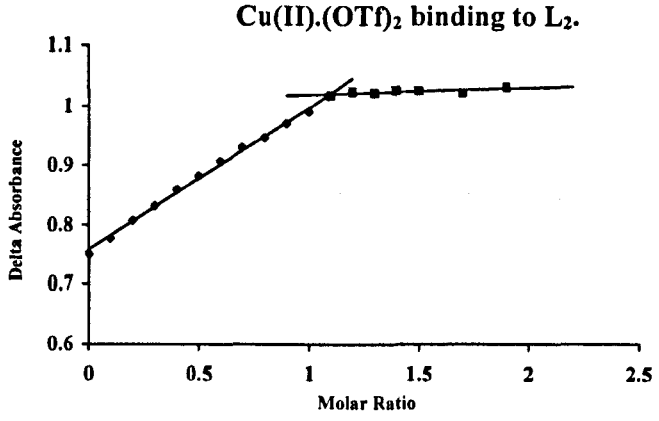

Figure 72. UV/Vis Job's plot for the complexation of $Cu.(OTf)_2$ to L_2.

UV/vis experiments were initially carried out to examine the complexation of copper triflate to L_2 in an aqueous environment. The possibility remained that the formation of copper hydroxide would be favoured over ligand chelation. A simple Job's plot experiment revealed precisely that a 1:1 ligand:copper complex was established in aqueous buffered conditions. The attempts to examine binding through potentiometric pH titrations were hampered by solubility problems with the ligand precursor. However, titration experiments with $CuL_2.(OTf)_2$ to $Na[H_2PO_4]$ were carried out successfully and produced a straight line graph confirming that weak binding was occurring. Extensive crystallisation procedures with numerous solvents and conditions were used to try and obtain X-ray crystallographic quality crystals. Crystals were obtained but due to incoherent layering, suitable diffraction was not acquired for structural elucidation.

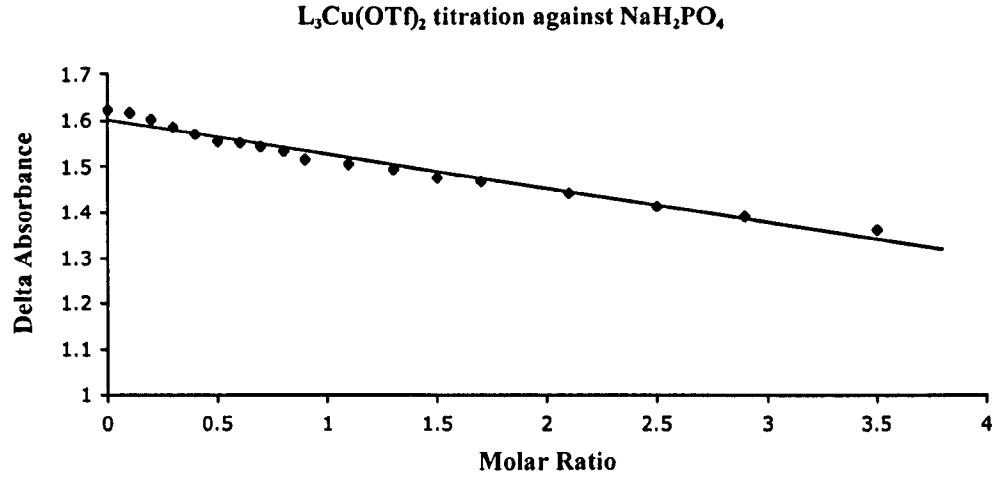

Figure 73. Titration graph for the addition of NaH_2PO_4 into $CuL_2.(OTf)_2$.

The failure to bind phosphate could be due to the coordination of the copper ion to the adjacent carbonyl group of the amino acid. This blocking of the active binding site would explain the negative results. In order to prove this theory, the logical step would be to reduce the amide group with $LiAlH_4$ and compare the binding results. Time constraints however, prevented exploration of this aspect of the research.

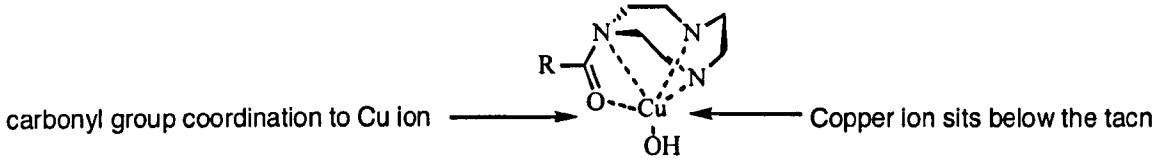

Figure 74. Depiction of possible Cu-amide coordination.

6.4.0 Binding results for bimetallic $Zn_2L_3.(OTf)_4$

The bimetallic zinc complex of L_3 was tested for its ability to bind phosphate via ITC, UV/vis, ^1H NMR and electrospray mass spectrometry. The partition coefficient was calculated to be -1.26, highlighting the increased hydrophilicity obtained by the addition of the second polyazamacrocycle and its polar amine groups.

6.4.1 ITC binding experiments

The same experimental parameters were used to determine the binding constants. The unusual binding isotherm obtained for both $Na[H_2PO_4]$ and $K[H_2PO_4]$ meant that it was not possible to fit the curve using the models that were available to us. The first segment of the ITC trace reveals an exothermic process occurs which was followed by an

endothermic reaction. The traces are reminiscent of the curves obtained by Anslyn when studying the formation of 2:1 complexes of citrate with a tris guanidinium host.[10]

Ligand	Substrate	Ligand: Substrate binding ratio	K x 10^4	ΔG^0/kcal mol^{-1}	ΔH^0/kcal mol^{-1}	$-T\Delta S^0$/kcal mol^{-1}
Zn_2L_3	$Na[H_2PO_4]$	1:2	na	na	na	na
Zn_2L_3	$K[H_2PO_4]$	1:2	na	na	na	na
Zn_2L_3	sodium glycerophosphate	1:1	1.78±0.21[a]	-5.79±0.68	3.89±0.38	9.68
Zn_2L_3	disodium4-nitrophenylphosphate	1:1	1.23±0.34[a]	-5.57±1.54	0.63±0.11	6.21

Table 3. Thermodynamic data for the binding of various substrates to $Zn_2L_3.(OTf)_4$ in HEPES buffer at pH 7.4 at 25 °C. [a] mol^{-1} dm^3.

One possible argument to account for the data was that at high ligand:substrate ratios (*i.e.* beginning of the titration) the phosphate anion acted as a template for cluster formation of two $[Zn_2L_3(OH_2)_2]^{4+}$ moieties. Indeed there was literature precedent for the templating action of anions especially in helicate formation around a spherical chloride ion.[11]

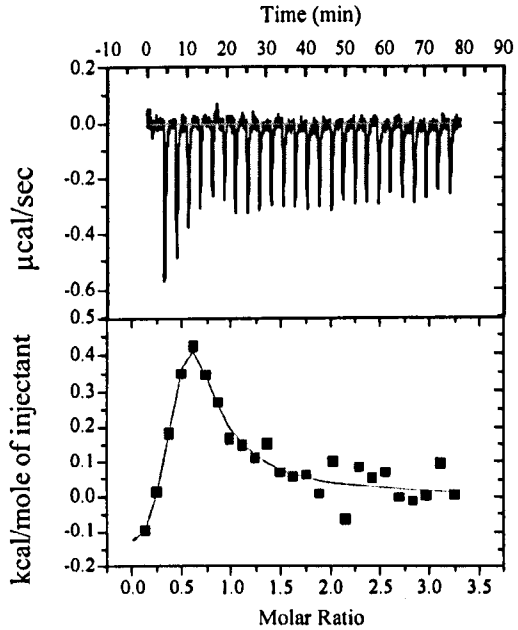

Figure 75. ITC Graph for the binding of NaH_2PO_4 to $Zn_2L_3.(OTf)_4$ displaying unusual binding isotherm.

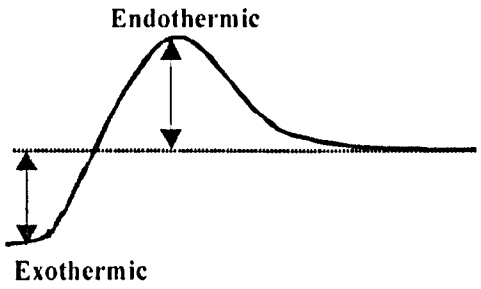

Figure 76. Schematic diagram illustrating the thermodynamics of the binding curvebinding curve

As the ligand:substrate ratio increased the cluster was broken up leading to a 1:2 complex formation. Unfortunately, electrospray mass spectrometry (ES-MS) experiments carried

out on solutions of varying $[Zn_2L_3(OH_2)_2]^{4+}$: HPO_4^{2-} ratios all displayed similar features. All ES-MS results revealed an intense cluster of peaks centred at *m/z* = 701 corresponding to $[Zn_2L_3(HPO_4)_2Na_2]^{2+}$.

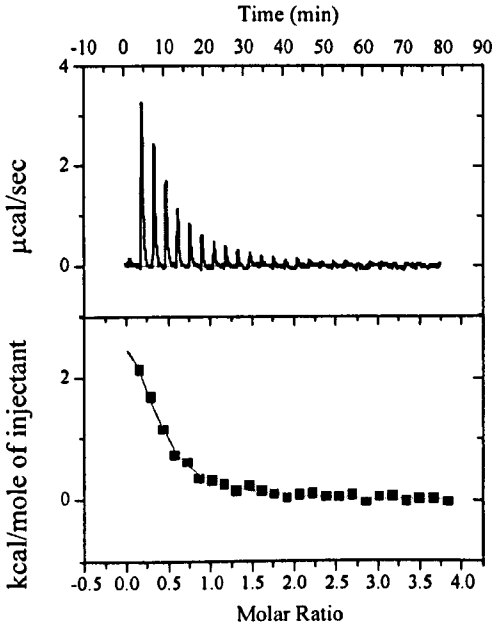

Figure 77. ITC graph for binding of $Zn_2L_3.(OTf)_4$ to Na-glycerophosphatephosphate.

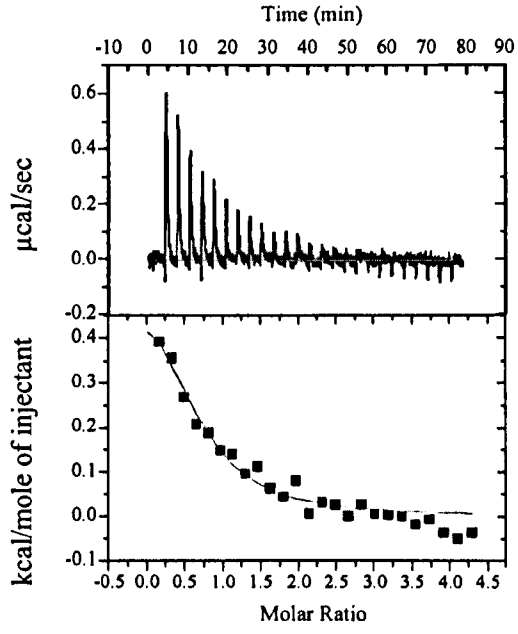

Figure 78. ITC graph for binding of $Zn_2L_3.(OTf)_4$ to Na-4-nitrophenylphosphate

6.4.2 *UV/vis titrations*

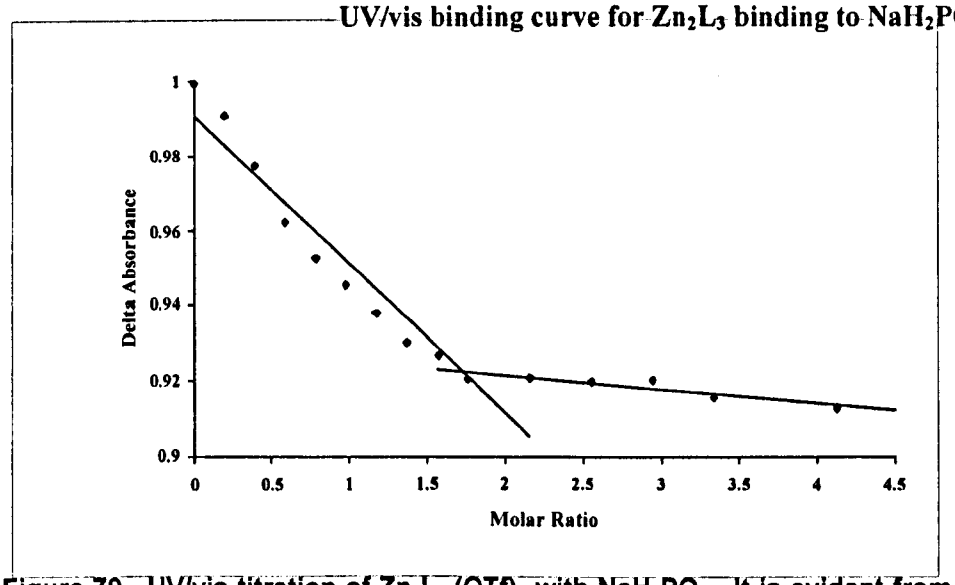

Figure 79. UV/vis titration of $Zn_2L_3.(OTf)_4$ with NaH_2PO_4. It is evident from the shape of the initial curve that there is something more complex than a simple 2:1 binding isotherm. The UV/vis spectrum reflects the findings of the ITC trace for Na^+ and K^+ phosphate.

In order to confirm the stoichiometry of binding components, UV/vis titration experiments were undertaken. The titration experiments showed the binding of two phosphates to one ligand. This equated to a phosphate anion for each zinc metal centre. This ratio of zinc complex to phosphate is in agreement with the ESMS titration results (*i.e.*, 1:2 (ligand:substrate) complexation).

6.4.3 Binding of phosphate esters

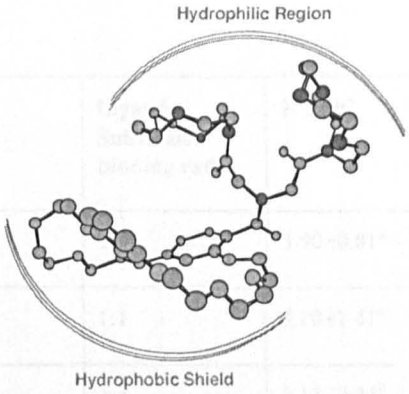

Figure 80. Molecular model of L_3 showing clearly the hydrophobic and hydrophilic regions of L_3.

The results of the experiments are illustrated in Table **3**. The binding of the glycerophosphate and 4-nitro-phenyl phosphate was markedly increased from the initial zinc monometallic $\mathbf{L_1}$ derivative. The binding stoichiometries showed that the $Zn_2\mathbf{L_3}.(OTf)_4$ was forming a 1:1 ratio with both the phosphate esters tested. Molecular modelling studies demonstrated that the binding cavity created between the adjacent crown ether and polycyclic amine scaffold was much larger and therefore could incorporate bulkier phosphates.

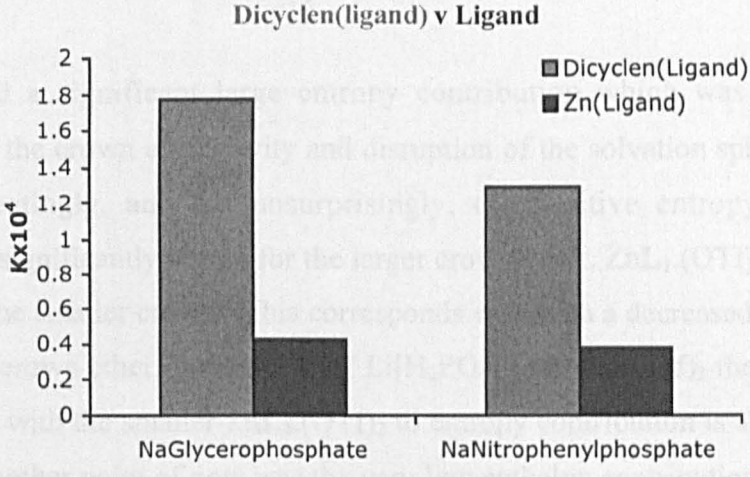

Figure 81. Improvements in binding constants for sodium glycerophosphate and 4-nitro phenyl phosphate.

6.5.0 Binding results for $ZnL_4.(OTf)_2$.

The development of different sized cyclophane cages allowed both the formation of larger structurally loose scaffolds and the synthesis of smaller well-defined ring systems. Our work focused around the synthesis of the smaller cage, promoting the binding of smaller phosphate species. Indeed previous identification of optimal crown-metal binding sizes[12] allowed the selection of a specific crown ether for the potassium cation. The principal cations investigated were potassium, sodium and lithium.

Ligand	Substrate	Ligand: Substrate binding ratio	K x 10^4	ΔG^0/kcal mol^{-1}	ΔH^0/kcal mol^{-1}	$-T\Delta S^0$/kcal mol^{-1}
ZnL_4	$Na[H_2PO_4]$	1:1	3.90±0.81[a]	-6.57	0.39±0.02	6.57
ZnL_4	$K[H_2PO_4]$	1:1	5.10±1.41[a]	-6.42	0.29±0.02	6.71
ZnL_4	$Li[H_2PO_4]$	2:1	3.13±0.21[b]	-6.16	1.71±0.01	7.84

Table 4. . Thermodynamic data for the binding of various substrates to $ZnL_4.(OTf)_2$ in HEPES buffer at pH 7.4 at 25 °C. [a] mol^{-1} dm^3. [b] mol^{-2} dm^6

The use of 19-benzocrown-6 specifically targets the complexation of a potassium counter ion. It can be seen from Table 4 that the highest binding affinity was indeed for $K[H_2PO_4]$ with a K_a value of 5.1 x 10^4 M^{-1}. It was observed that all the interactions were endothermic (+ΔH°). $K[H_2PO_4]$ had the lowest unfavourable enthalpic contribution, presumably through some form of electrostatic interactions with the crown ether which resulted in the largest $\Delta G_{AB}°$ and hence the highest binding constant. The diameter of K^+ (2.66 Å) provides a good match for the cavity size of 19-benzocrown-6 (2.6-3.2 Å) and hence the lowest unfavourable enthalpy.

$Li[H_2PO_4]$ had a significant large entropy contribution which was attributed to the desolvation of the crown ether cavity and disruption of the solvation sphere of the lithium metal. Interestingly, and not unsurprisingly, the positive entropy gained through desolvation is significantly higher for the larger crown ether, $ZnL_1.(OTf)_2$, when compared to the that of the smaller crown. This corresponds well with a decreased solvent expulsion in the smaller crown ether. In the case of $Li[H_2PO_4]$ in $ZnL_1.(OTf)_2$ the entropy was 13.3 kcal mol^{-1} and with the smaller $ZnL_4.(OTf)_2$ to entropy contribution is almost halved, 7.84 kcal mol^{-1}. Another point of note was the very low enthalpy contributions (the interactions

are almost thermally neutral) which suggest that the binding is almost entirely entropically driven.

In terms of determining cooperativity, the same model was applied in the case of $ZnL_1.(OTf)_2$, whereby the assumption is made that the primary binding mode of phosphate is to the zinc metal centre and this can be represented by the zinc cyclen thermodynamic profile. Further control experiments were carried out on sodium, potassium and lithium perchlorate salts which showed that there was negligible cation binding to the ligand in an aqueous environment. The $\Delta G_s°$ values were calculated for all the different metal phosphates, establishing that in all three examples there was positive cooperative binding, $+\Delta G_s°$.

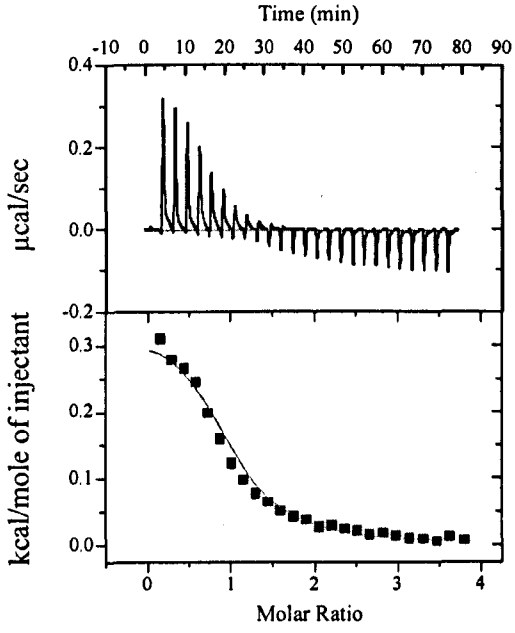

Figure 82. ITC graph for the binding of $Na[H_2PO_4]$ by $ZnL_4.(OTf)_2$.

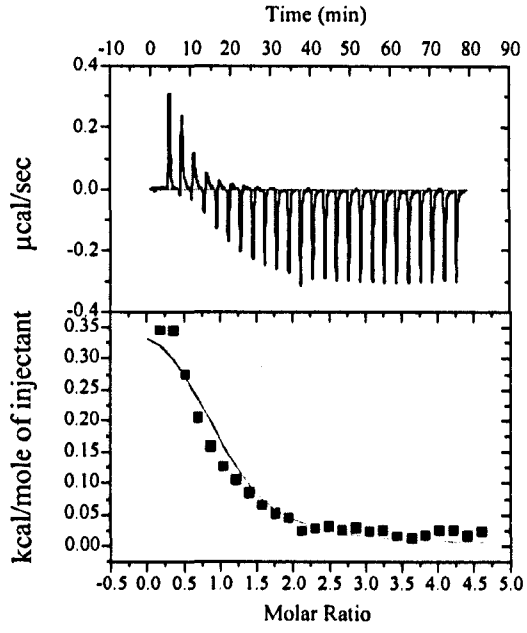

Figure 83. ITC graph for the binding of $K[H_2PO_4]$ by $ZnL_4.(OTf)_2$.

The highest cooperativity was exhibited by $Li[H_2PO_4]$ which gave a $\Delta G_s°$ value of +1.03 kcal/mol^{-1} in comparison to +0.72 and +0.83 for K^+ and Na^+ respectively. There is higher positive cooperativity in the case of $Li[H_2PO_4]$ but despite this fact the binding is not particularly strong. This due to the unfavourable enthalpy contributions associated with lack of binding to the crown ether.

There was no binding observed for the bulkier sodium glycerophosphate and 4-nitro-phenylphosphate derivatives. It was predicted that such drops in binding would be observed due to the reduction in cavity size and therefore hydrophobic pocket.

6.6.0 Glutamic acid derivative ligand L_5

The final ligand design was the synthesis of a zinc cyclen coupled to the 26-dibenzocrown-8 via a glutamic acid linker group. Time constraints meant that we were unable to complete the synthesis and subsequent testing. It can be seen from the progress achieved that the modular approach was successfully applied and should furnish the target compound. It would be interesting to observe the effect of the carboxylic acid on the zinc metal centre and how it could be used in the mimicking of carboxypeptidase A as a hydrolysis catalyst.

6.7.0 Conclusions

We have successfully developed a modular strategy for the synthesis of artificial phosphate receptors which operate in an aqueous environment. The synthetic methodology was thoroughly tested and proven by the extensive synthetic work toward our five target compounds. The premise behind the strategy and its advantages were the three simple components. Variation of each unit individually showed the potential of the system and the number of derivatives that are synthetically possible. The variation of the polyaza macrocycle unit allowed the chelation of different metal species, e.g. cyclen-zinc, tacn-copper, cyclam-nickel. The manipulation of the amino acid linker was highly advantageous allowing variation in the functionality, polarity and the metal centre environment.

Our studies have also added to the understanding of the ion pair phenomenon. We have shown that the binding of the phosphate moiety was intricately bound to the binding of its cation through chelation to the crown ether. The association of the cation significantly alters the binding affinity of the phosphate anion. The ligands exhibit positive cooperative binding compared to most artificial systems which display negative cooperativity.

The future development of this work should focus around the improvement of larger hydrophobic frameworks. The replacement of the crown ether with either the macromolecular scaffolds of cryptands or calixarenes offers the binding of the phosphate groups on a macromolecular scale with species such as RNA and DNA. The ability to selectively substitute calixarenes and crytpands is well established and should not prove too problematic for incorporation into the modular strategy.

6.8.0 References

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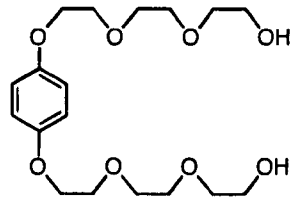
   (b) Pflugrath, J. W., Quiocho, F. A., *Nature*, **1985**, *314*, 257.

Chapter 7

7.1.0 Experimental procedures



(45) Preparation of 1, 4-Bis [2-{2-(2-hydroxyethoxy) ethoxy} ethoxy] benzene

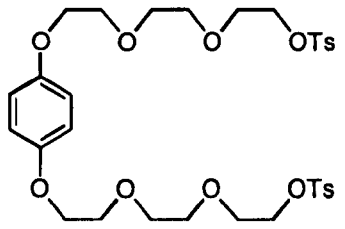
Method adapted from Benniston *et al*[1]. A solution of 1,4-dihydroxybenzene (3.00 g, 27 mmol) in dry *t*-butyl alcohol (30 mL) was added to a solution of potassium *t*-butoxide (6.12 g, 54.0 mmol) in dry *t*-butyl alcohol (30 mL) under nitrogen. The mixture was refluxed for 2 hrs and then the 2-[2-(2-chloroethoxy) ethoxy]ethanol] (9.19g, 54.0 mmol) was added over 15 mins. The reaction mixture was refluxed for 65 hrs. After this time the reaction was cooled to room temperature and filtered. The solid residue was washed with CH_2Cl_2 (3 x 50 mL) and the combined organic solutions evaporated under reduced pressure and the residue dissolved in CH_2Cl_2 (100 mL). The organic solution was washed with 2M HCl (30 mL) and distilled water (2 x 30 mL) and then dried over $MgSO_4$. The solution was concentrated *in vacuo* and the residue purified by column chromatography (ethyl acetate: 10% petroleum ether) to give pure product as a colourless viscous oil (7.45 g, 76 %):

HRMS (EI^+), calcd. for $C_{18}H_{30}O_8$ [M^+] m/z = 374.1941, fnd. 374.1943;

ν_{max} (KBr)/cm^{-1}; 3345, 2873, 2361, 1507, 1454, 1349, 1228, 1111, 1064, 942, 829, 731, 698, 679,659;

^{1}H NMR (d_6-DMSO) δ 2.39 (2H, s (broad), 2 x OH), 3.55 (4H, t, J = 5 Hz, CH_2), 3.60-3.70 (12H, m, CH_2) 3.77 (4H, t, J = 5 Hz, CH_2), 4.02 (4H, t, J = 5 Hz, CH_2), 6.77 (4H, s, Ar-H);

^{13}C NMR ($CDCl_3$) δ 61.5 (2 x CH_2), 67.8 (2 x CH_2), 69.8 (2 x CH_2), 70.4 (2 x CH_2), 71.2 (2 x CH_2), 72.5 (2 x CH_2), 115.1 (CH), 115.5 (CH), 115.6 (CH), 116.0 (CH), 152.9 (2 x C). Consistent with literature data.[1]



(46) Preparationn of 1, 4-Bis [2-{2-(2-tosyloxyethoxy) ethoxy} ethoxy] benzene.

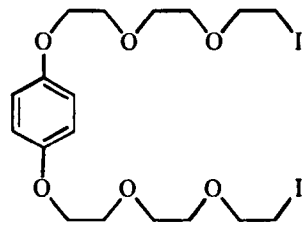
To a solution of 1, 4-Bis [2-{2-(2-hydroxyethoxy) ethoxy} ethoxy] benzene (10.0 g, 26 mmol) dissolved in dry pyridine (50 mL) was added 5 equivalents tosyl chloride (21.9 g, 130 mmol) at 0 °C. The solution was refridgerated overnight, poured into ice cold 1M hydrochloric acid (50 mL), filtered then washed with copious volumes of water (4 x 100 mL), and then extracted with CH_2Cl_2 (3 x 30 mL). The organic layers were combined and dried over $MgSO_4$, before the solvent was removed under reduced pressure to yield crude product which was purified using silica gel chromatography (5:1, ethyl acetate:petroleum ether) to give pure product as yellow oil (13.4 g, 76 %):

HRMS (EI+), calcd. for $C_{32}H_{42}O_{12}S_2$ [M^+] m/z = 682.2118, fnd. 682.2119;

ν_{max} (KBr)/cm^{-1}; 2871, 2135, 1709, 1596, 1506, 1452, 1352, 1293, 1228, 1173, 1109, 1061, 1013, 916, 817, 768, 704, 661;

^{1}H NMR ($CDCl_3$) δ 2.36 (6H, s, 2 x CH_3), 3.55 (8H, m, CH_2), 3.62 (4H, t, J = 4.5 Hz, CH_2), 3.72 (4H, t, J = 4.5 Hz, CH_2), 3.98 (4H, t, J = 4.5 Hz, CH_2), 4.08 (4H, t, J = 4.5 Hz, CH_2), 6.81 (4H, s, Ar-H), 7.27 (4H, d, J = 8 Hz, Ar-H), 7.73, (4H, d, J = 8 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 21.6 (2 x CH_3), 67.7 (CH_2), 67.9 (CH_2), 68.7 (CH_2), 69.3 (CH_2), 69.6 (CH_2), 69.9 (2 x CH_2), 70.5 (CH_2), 70.7 (CH_2), 70.78 (CH_2), 71.36 (2 x CH_2), 115.1 (4 x CH), 127.9 (4 x CH), 129.7 (2 x CH), 129.8 (2 x CH), 144.8 (4 x C), 153.05 (2 x C). Consistent with literature data.[1]



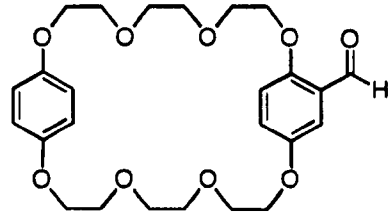
(47) Preparation of 1,4-Bis-{2-[2-(2-iodo-ethoxy)-ethoxy]-ethoxy} benzene.

To a solution of 1, 4-Bis [2-{2-(2-tosyloxyethoxy) ethoxy} ethoxy] benzene (2.06 g, 3.02 mmol) in dry acetone (20 mL) was added potassium iodide (2.26 g, 15.1 mmol) and the mixture was refluxed for 14 hrs at 78 °C. After this period of time, CH_2Cl_2 (20 mL) was added, the mixture was filtered and the solvent removed under reduced pressure to yield a mixture of oil and solid. To the mixture was added chloroform (30 mL) which dissolved the oil but not the solid which was filtered off and the solvent removed *in vacuo* to yield the product as yellow crystals (1.49 g, 85 %):

HRMS (EI+), calcd. for $C_{18}H_{28}O_6I_2$ [M^+] m/z = 593.9980, fnd. 593.9976;

^{1}H NMR ($CDCl_3$) δ 3.18 (4H, t, J = 5 Hz, CH_2), 3.60-3.72 (12H, m, CH_2), 3.77 (4H, t, J = 5 Hz, CH_2), 4.03 (4H, t, J = 5 Hz, CH_2), 6.79 (4H, s, Ar-H);

^{13}C NMR ($CDCl_3$) δ 68.4 (2 x CH_2), 70.3 (2 x CH_2), 70.5 (2 x CH_2), 71.1 (2 x CH_2), 72.3 (2 x CH_2), 115.9 (4 x CH), 153.4 (2 x C).



(48) Preparation of 2,5,8,11,16,19,22,25-Octaoxa-tricyclo[24.2.2.2^{12,15}]-dotriaconta-1(29),12,14,26(30),27,31-hexaene-13-carbaldehyde.

A solution of 1, 4-Bis [2-{2-(2-iodo-oxyethoxy) ethoxy} ethoxy] benzene (1.57 g, 2.66 mmol) in dry DMF (10 mL) and a solution of 2,5-dihydroxybenzaldehyde (0.35 g, 2.66 mmol) in dry DMF (10 mL) were added simultaneously over a 5 hr period to a stirred suspension of K_2CO_3 (1.10 g, 7.98 mmol) in dry DMF (15 mL) under N_2 using a syringe pump driver. The mixture was refluxed for five days before being cooled to room temperature. Excess K_2CO_3 was quenched by the addition of distilled water and the

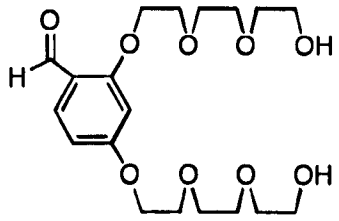
solvent removed under reduced pressure. The residue was then partitioned between CH_2Cl_2 (150 mL) and water (300 mL). Sodium chloride was added to break up the emulsion that formed during the partitioning process. The organic phase was washed with 2M HCl (50 mL) and distilled water (3 x 150 mL), dried over $MgSO_4$ and finally concentrated *in vacuo* to give crude product which was purified using silica gel chromatography (5:1 ethyl acetate:petroleum ether) to furnish the product as a colourless oil (0.055 g, 19 %):

HRMS (FAB+), calcd. for $C_{25}H_{32}O_9$ $[M^+]$ m/z = 476.2046, fnd. 476.2050;

ν_{max} (KBr)/cm^{-1}; 2877, 2361, 2092, 1598, 1507, 1352, 1231, 1174, 1121, 1016, 916, 821, 768, 743, 722;

^{1}H NMR ($CDCl_3$) δ 3.66 (6H, m, CH_2), 3.70-3.77 (4H, m, CH_2), 3.80 (2H, t, J = 5.0 Hz, 2H, CH_2), 3.88-3.97 (4H, m, CH_2), 4.04 (4H, m, CH_2), 6.64 (4H, d, J = 4.2 Hz, Ar-H), 6.73 (1H, d, J = 11.9 Hz, Ar-H), 6.89 (1H, dd, J = 6.4 and 3.2 Hz, Ar-H), 7.18 (1H, d, J = 3.2 Hz, Ar-H), 10.32, (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 66.1 (CH_2), 66.15 (CH_2), 67.4 (2 x CH_2), 68.6 (CH_2), 68.65 (CH_2), 68.7 (CH_2), 68.9 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.03 (CH_2), 100.2 (CH), 105.8 (CH), 105.9 (CH), 110.4 (CH), 115.0 (CH), 123.6 (CH), 124.6 (C), 128.7 (CH), 152.1 (C), 155.4 (C), 158.9 (2 x C), 188.8 (CHO).



(51) Preparation of 2,4-Bis-{2-[2-(2-hydroxyethoxy)ethoxy]-ethoxy}-benzaldehyde.

Solid 2,4-dihydroxybenzaldehyde (10 g, 72 mmol) was added to a stirred mixture of K_2CO_3 (29.8 g, 216 mmol) in dry DMF (60 mL), at 90 °C under N_2. The mixture was stirred at this temperature for 1 hr before addition of 2-[2-(2-chloroethoxy) ethoxy] ethanol (24.2 g, 144 mmol) and KI (22.6 g, 158 mmol). The reaction mixture was refluxed for 48 hrs and cooled to room temperature. The DMF was removed by azeotropic distillation with toluene (3 x 250 mL) to leave a red oil which was dissolved in CH_2Cl_2 (200 mL) and

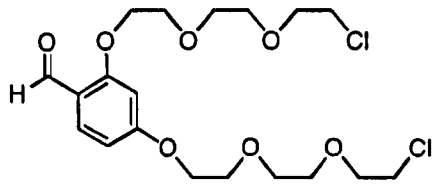
filtered to remove potassium salts. The organics were dried over $MgSO_4$ and concentrated under reduced pressure to yield a viscous red oil (23.1 g, 79.9 %):

HRMS (FAB$^+$), calcd. for $C_{19}H_{31}O_9$ [M$^+$] *m/z* = 403.1968, fnd. 403.1959;

ν_{max} (KBr)/cm^{-1}; 3406, 2868, 1657, 1596, 1435, 1386, 1258, 1188, 1094, 932, 829, 754, 740, 700, 686, 660;

^{1}H NMR ($CDCl_3$) δ 2.56 (1H, t, *J* = 6.0 Hz, OH), 2.68 (1H, t, *J* = 6.0 Hz, OH), 3.60-3.63 (4H, m, CH_2), 3.68-3.75 (12H, m, CH_2), 3.86-3.92 (4H, m, CH_2), 4.21 (2H, t, *J* = 5 Hz, CH_2), 4.24 (2H, t, *J* = 5 Hz, CH_2), 6.53-6.58 (2H, m, Ar-H), 7.80 (1H, d, *J* = 8 Hz, Ar-H), 10.35 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 68.2 (CH_2), 68.8 (CH_2), 69.75 (CH_2), 69.78 (CH_2), 70.1 (CH_2), 70.4 (CH_2), 70.5 (CH_2), 71.0 (CH_2), 71.1 (CH_2), 72.5 (CH_2), 72.7 (CH_2), 72.8 (CH_2), 100.3 (CH), 107.4 (CH), 119.6 (C), 131.5 (CH), 163.1 (C), 165.5 (C), 189.1 (CHO). Note: the crude compound was carried through to the next stage as a result of purification difficulties.



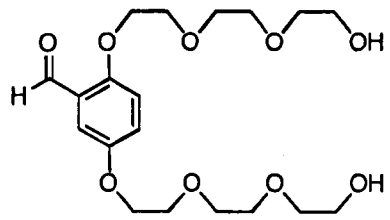
(52) Preparation of 2,4-Bis-{2-[2-(2-chloro-ethoxy)ethoxy]-ethoxy}-benzaldehyde.[2]

Solid 2,4-dihydroxybenzaldehyde (15.0 g, 0.10 mmol) was added to a stirred mixture of K_2CO_3 (33.02 g, 0.23 mmol) in dry DMF (200 mL), at 90 °C under N_2. The mixture was stirred at this temperature for 1 hr before addition of 2-[2-(2-chloroethoxy) ethoxy] ethoxy-chloride (44.69 g, 0.23 mmol) over a 48 hr period using a syringe driver. The reaction mixture was refluxed for 48 hrs and cooled to room temperature. H_2O (100 mL) was then added and the product extracted using Et_2O (3 x 150 mL), the organic layers combined and washed with brine (3 x 200 mL). The organics were then dried over $MgSO_4$ and concentrated under reduced pressure to give crude material which was purified using silica gel chromatography to yield pure product as a yellow oil (21.5 g, 45 %):

HRMS (FAB+), calcd. for $C_{19}H_{28}O_7Cl_2$ [M$^+$] *m/z* = 438.1212, fnd. 438.1216;

^{1}H NMR ($CDCl_3$) δ 3.56 (4H, dt, J = 6 and 4 Hz, CH_2), 3.61-3.71 (12H, m, CH_2), 3.84 (2H, t, J = 4.4 Hz, CH_2), 3.89 (2H, t, J = 4.4 Hz, CH_2), 4.15 (2H, t, J = 4.4 Hz, CH_2), 4.19 (2H, t, J = 4.4 Hz, CH_2), 6.43 (1H, d, J = 2.1 Hz, Ar-H), 6.49 (1H, dd, J = 8.0 and 2.1 Hz, Ar-H), 7.73 (1H, d, J = 8.0 Hz, Ar-H), 10.29 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 68.1 (CH_2), 68.6 (2 x CH_2), 69.9 (2 x CH_2), 71.1 (2 x CH_2), 71.2 (CH_2), 71.3 (2 x CH_2), 71.7 (2 x CH_2), 100.0 (CH), 107.0 (CH), 119.7 (C), 130.7 (CH), 163.2 (C), 165.6 (C), 188.7 (CHO).



(53) Preparation of 1,4-Bis[2-{2-(2-Hydroxyethoxy)ethoxy}ethoxy]benzyl-aldehyde.

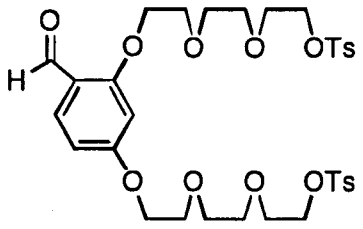
To a stirred mixture of Chloroethoxyethoxyethanol (30.0 g, 180 mmol), K_2CO_3 (39.8 g, 280 mmol) and KI (22.6 g, 158 mmol) in DMF (500 mL) under N_2 at 90 °C was added 2,4-dihydroxybenzaldehyde (10.0 g, 72.0 mmol) via a syringe pump over 4 hrs. The reaction solution was then stirred overnight before quenching with H_2O (500 mL). The product was extracted with CH_2Cl_2 (3 x 100 mL), the organic layers combined and washed thoroughly with brine (3 x 200 mL) to remove all traces of DMF. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure to give crude product which was further purified by silica gel chromatography (5:1, ethyl acetate:petroleum ether) to furnish pure product (14.4 g, 49 %):

HRMS (EI+), calcd. for $C_{19}H_{30}O_9$ [M^+] m/z = 402.1890, fnd. 402.1887;

ν_{max} (KBr)/cm^{-1}; 3345, 2873, 2361, 1507, 1454, 1349, 1228, 1111, 1064, 942, 829, 731, 698, 679;

^{1}H NMR ($CDCl_3$) δ 2.43 (1H, s (broad), OH), 2.55 (1H, s (broad), OH), 3.52-3.65 (12H, m, CH_2), 3.76-3.80 (4H, m, CH_2), 4.06 (2H, t, J = 4.4 Hz, CH_2), 4.15 (2H, t, J = 4.4 Hz, CH_2), 6.89 (1H, d, J = 9.0 Hz, Ar-H), 7.10 (1H, dd, J = 9.0 and 3.2 Hz, Ar-H), 7.20 (1H, s, Ar-H), 7.25 (1H, d, J = 3.2 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 66.9 (2 x CH_2), 68.2 (2 x CH_2), 68.5 (CH_2), 68.6 (CH_2), 69.3 (CH_2), 69.38 (CH_2), 69.7 (CH_2), 69.9 (CH_2), 71.4 (CH_2), 71.5 (CH_2), 109.9 (CH), 114.5 (CH), 123.3 (CH), 124.6 (C), 152.1 (C), 155.1 (C), 189.0 (CHO).



(54) Preparation of 2,4-Bis-{2-[2-(2-tosyl-ethoxy)-ethoxy]-ethoxy} benzaldehyde.

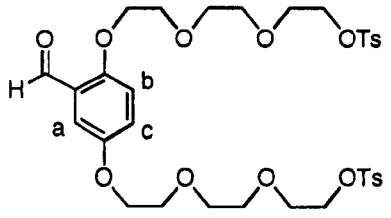
To a stirred solution of **51** (22.0 g, 54 mmol) and Et_3N (17.3 mL, 162 mmol) in dry CH_2Cl_2 (50 mL) cooled to 0 °C under an Ar atmosphere were slowly added portions of *p*-toluenesufonyl chloride (27.31 g, 162 mmol). The mixture was allowed to warm to room temperature and the reaction monitored by TLC (silica gel, 5:1 ethyl acetate/petrol). After disappearance of the starting material, water (150 mL) was added to the reaction mixture. The product was extracted with CH_2Cl_2 (200 mL) and washed with brine (3 x 100 mL). The separated organic layer was dried over $MgSO_4$, and the solvent removed to afford a viscous brown oil which was purified using flash chromatography (silica gel 5:1 ethyl acetate/petroleum ether) to yield a yellow oil (20 g, 52 %):

Elemental analysis calcd. for $C_{33}H_{42}O_{13}S_2$: C, 55.76; H, 5.95%, fnd. C, 55.75; H, 5.81%;

ν_{max} (KBr)/cm^{-1}; 2861, 1731 (C=O), 1671, 1596, 1575, 1500 (Ar), 993, 933, 873, 815;

^{1}H NMR ($CDCl_3$) δ 2.36 (6H, s, CH_3), 3.51-3.56 (4H, m, CH_2), 3.58-3.63 (8H, m, CH_2), 3.77 (2H, t, J = 5 Hz, CH_2), 3.80 (2H, t, J = 5 Hz, CH_2), 4.10 (8H, m, CH_2), 6.42 (1H, d, J = 2 Hz, Ar-H), 6.47 (1H, dd, J = 8 and 2 Hz, Ar-H), 7.25 (4H, d, J = 8 Hz, Ar-H), 7.71 (5H, d, J = 8 Hz, Ar-H), 10.27 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 22.0 (2 x CH_3), 68.1 (CH_2), 68.6 (CH_2), 69.1 (CH_2), 69.6 (CH_2), 69.8 (CH_2), 70.6 (2 x CH_2), 71.1 (CH_2), 71.17 (CH_2), 71.2 (CH_2), 72.3 (2 x CH_2), 99.9 (CH), 107.1 (CH), 119.6 (C), 128.3 (4 x CH), 130.2 (4 x CH), 130.6 (CH), 133.3 (C), 145.23 (C), 145.26 (C), 163.3 (C), 165.6 (C), 188.6 (CHO).



(55) Preparation of 1, 4-Bis [2-{2-(2-tosyloxyethoxy) ethoxy} ethoxy] benzyl-aldehyde.

The diol **53** (10 g, 26 mmol) was dissolved in dry pyridine (50 mL) followed by the addition of 5 equivalents tosyl chloride (21.9 g, 130 mmol) at 0 °C. The solution was refrigerated overnight, poured into ice-cold 1M hydrochloric acid (50 mL), filtered and washed with copious volumes of water (4 x 100 mL) and then extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was dried with $MgSO_4$ and solvent removed to yield crude product which was further purified using silica gel chromatography (5:1, ethyl acetate:petroleum) to yield pure product (13.4 g, 76 %):

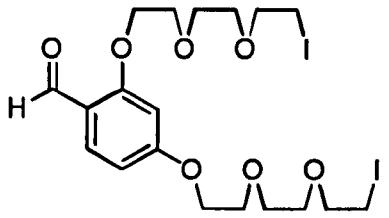
Elemental analysis calcd. for $C_{33}H_{42}O_{13}S_2$: C, 55.77; H, 5.91; S, 9.01 %, fnd. C, 55.75; H, 5.99; S, 8.97;

HRMS (FAB+), m/z 711.1 $[M+H]^+$, 710.1 $[M^+]$;

ν_{max} (KBr)/cm^{-1}; 2871, 2135, 1709, 1596, 1506, 1452, 1352, 1293, 1228, 1173, 1109, 1061, 1013, 916, 817, 768, 704, 661, 628;

^{1}H NMR ($CDCl_3$) δ 2.41 (6H, s, 2xCH_3), 3.55-3.69, (12H, m, CH_2), 3.79 (2H, t, J = 4.5 Hz, CH_2), 3.84 (2H, t, J = 4.5 Hz, CH_2), 4.07-4.22, (8H, m, CH_2), 6.55, (1H, d, J = 9.0 Hz, Ar-H), 7.13 (1H, d, J = 3.2 Hz, Ar-H), 7.15 (1H, d, J = 3.2 Hz, 1H, Ar-H), 7.27 (5H, m, Ar-H), 7.28-7.34 (4H, s, Ar-H), 10.4 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 68.4 (2 x CH_2), 69.1 (2 x CH_2), 69.3 (2 x CH_2), 69.6 (2 x CH_2), 70.0 (2 x CH_2), 71.0 (2 x CH_2) 111.5 (CH), 115.4 (CH), 124.4 (CH), 125.8 (C) 128.3 (CH), 130.2 (CH) 133.3 (C), 145.2 (2 x CH), 153.4 (2 x CH), 156.5 (2 x CH), 189.9 (CHO).



(56) Preparation of 2,4-Bis-{2-[2-(2-iodo-ethoxy)-ethoxy]-ethoxy} benzaldehyde.

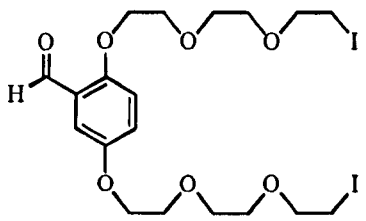
To a solution of the above compound 4-bis [2-{2-(2-tosyloxyethoxy) ethoxy} ethoxy] benzaldehyde (20.0 g, 28 mmol) in dry acetone (350 mL), at reflux, was added NaI (16.93 g, 113 mmol) over a 30 min. period. The mixture was further refluxed for 14 hrs under an N_2 atmosphere. The solution was cooled to room temperature and the solvent removed under reduced pressure. To the mixture was added CH_2Cl_2 (150 mL) and the resultant solid residue filtered. Removal of the solvent afforded a yellow oil which was further purified by silica gel column chromatography (5:1 ethyl acetate/petrol) to give pure product as a colouless oil (13.77 g, 77.7 %):

Elemental analysis calcd for $C_{19}H_{28}O_7I_2$: C, 36.65; H, 4.50; I, 40.84%, fnd. C, 36.65; H, 4.61; I, 40.89%;

HRMS (EI^+), calcd for $C_{19}H_{28}O_7I_2$ [M^+] m/z = 621.9925, fnd. 621.9926;

^{1}H NMR ($CDCl_3$) δ 3.19 (4H, dt, J = 7.0 and 2.0 Hz, CH_2), 3.60-3.72 (12H, m, CH_2), 3.82 (2H, t, J = 4.6 Hz, CH_2), 3.86 (2H, t, J = 4.6 Hz, CH_2), 4.11-4.16 (4H, m, CH_2), 6.43 (1H, d, J = 2.2 Hz, Ar-H), 6.50 (1H, dd, J = 8.7 and 1.8 Hz, Ar-H), 7.73 (1H, d, J = 8.7 Hz, Ar-H), 10.28, (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 68.1 (2 x CH_2), 68.6 (2 x CH_2), 69.9 (2 x CH_2), 70.6 (2 x CH_2), 71.2 (CH_2), 71.3 (CH_2), 72.3 (2 x CH_2), 100.0 (CH), 107.0 (CH), 119.7 (C), 130.7 (CH), 163.2 (C), 165.6 (C), 188.6 (CHO).



(57) Preparation of 1,4-bis [2-{2-Iodo-oxyethoxy) ethoxy} ethoxy] benzyl-aldehyde.

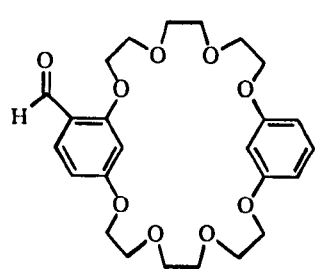
To a solution of 1,4-Bis [2-{2-tosyloxyethoxy) ethoxy} ethoxy] benzyl-aldehyde (0.50 g, 0.70 mmol) in dry acetone (20 mL) was added potassium iodide (0.52 g, 3.52 mmol) and the mixture was refluxed for 14 hrs. After which time the reaction mixture was filtered, the solvent removed under reduced pressure and dissolved in CH_2Cl_2 (20 mL) and refiltered before the solvent was removed to give a yellow oil which was purified using silica gel chromatography (5:1, ethyl acetate:petroleum ether) to furnish pure product as a colourless oil (0.30 g, 70 %):

Elemental analysis calcd. for $C_{19}H_{28}O_7I_2$: C, 36.65; H, 4.50; I, 40.94 %, fnd. C, 36.71; H, 4.58; I, 40.94;

HRMS (EI+), calcd. for $C_{19}H_{28}O_7I_2$ [M^+] *m/z* =621.9925, fnd. 621.9923;

^{1}H NMR ($CDCl_3$) δ 3.18 (4H, t, *J* = 6.7 Hz, CH_2), 3.60-3.72 (12H, m, CH_2), 3.77 (4H, t, *J* = 5 Hz, CH_2), 4.03 (4H, t, *J* = 5 Hz, CH_2), 6.91 (1H, d, *J* = 9 Hz, Ar-H), 7.12 (1H, dd, *J* = 9 and 3.2 Hz, Ar-H), 7.25 (1H, d, *J* = 3.2 Hz, Ar-H), 10.41 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 68.4 (CH_2), 69.4 (CH_2), 70.1 (2 x CH_2), 70.6 (2 x CH_2), 71.2 (2 x CH_2), 72.3 (2 x CH_2) 72.4 (2 x CH_2), 111.5 (CH), 115.4 (CH), 124.5 (CH), 125.8 (C), 153.4 (C), 156.5 (C), 189.9 (CHO).



(58) Preparation of 2,5,8,11,17,20,23,26-Octaoxatricyclo [25.3.1.1^{12,16}] dotriaconta 1(30), 12(32), 13,15, 27(31), 28-hexaene-13-carbaldehyde.

To a stirred suspension of K_2CO_3 (10.7 g, 77 mmol) in dry DMF (30 mL) under N_2 were added simultaneoulsy over a 28 hr. period separate solutions of 2,4-bis [2-{2-(2-iodo-oxyethoxy)ethoxy}ethoxy]benzaldehyde (6.08 g, 9.7 mmol) in dry DMF (40 mL) and 1,3-dihydroxybenzene (1.0 g, 9.7 mmol) in dry DMF (40 mL). The mixture was heated at 90 °C for five days before being cooled to room temperature. The residue was dissolved in distilled water (100 mL) and the solution extracted with CH_2Cl_2 (3 x 50 mL). The organic washings were combined and washed with 2M HCl (100 mL) and distilled water (2 L). Sodium chloride was added to break up the emulsion that formed during the partitioning process. The organic phase was isolated, dried over $MgSO_4$ and concentrated under reduced pressure to yield a crude product which was chromatographed on silica gel (5:1 ethyl acetate/petrol) to give the target compound as a pale yellow viscous oil (1.03 g, 22.5 %):

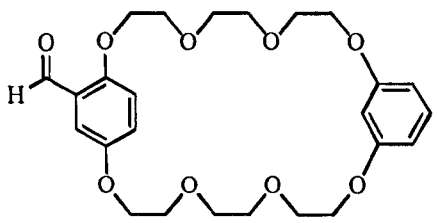
Elemental analysis calcd. for $C_{25}H_{32}O_9$: C, 63.01; H, 6.76%, fnd. C, 62.60; H, 6.78 %;

HRMS (EI^+), calcd. for $C_{25}H_{32}O_9$ [M^+], m/z = 476.2046, fnd; 476.2046;

ν_{max} (KBr)/cm^{-1}; 2867, 1671 (C=O), 1594 and 1490, (Ar), 840, 815 (*meta*-disubstituted Ar);

^{1}H NMR ($CDCl_3$) δ 3.61-3.67 (8H, m, CH_2), 3.72-3.83 (8H, m, CH_2), 3.99 (2H, t, J = 6 Hz, CH_2), 3.99 (2H, t, J = 6 Hz, CH_2), 4.06-4.10 (4H, m, CH_2), 6.38-6.47 (5H, m, Ar-H), 7.05 (1H, t, J = 7.3 Hz, Ar-H), 7.68 (1H, d, J = 8.6 Hz, Ar-H), 10.34 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 67.6 (CH_2), 67.8 (CH_2), 68.0 (CH_2), 68.5 (CH_2), 69.7 (CH_2), 69.8 (CH_2), 70.0 (CH_2), 70.1 (CH_2), 71.0 (CH_2), 71.2 (CH_2), 71.4 (CH_2), 71.6 (CH_2), 100.0 (CH), 102.4 (CH), 106.9 (CH), 107.0 (CH), 107.7 (CH), 119.6 (C), 130.1 (CH), 130.5 (CH), 160.3 (C), 160.36 (C), 163.4 (C), 165.7 (C), 188.7 (CHO).



(59) Preparaation of 2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.2.2.1^{12,16}] dotriaconta-1(30),12(32),13,15,27(31),28-hexaene-28-carbaldehyde.

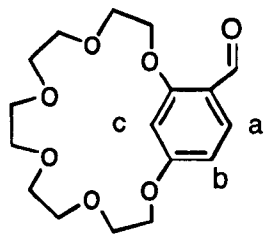
To a stirred suspension of K_2CO_3 (7.47 g, 54.1 mmol) in dry DMF (80 mL) under N_2 were added simultaneoulsy over a 28 hr. period separate solutions of 2,5-bis [2-{2-(2-iodo-oxyethoxy)ethoxy}ethoxy]benzaldehyde (9.62 g, 15.4 mmol) in dry DMF (45 mL) and 1,3-dihydroxybenzene (1.70 g, 15.4 mmol) in dry DMF (45 mL). The mixture was heated at 90 °C for five days before being cooled to room temperature. The residue was dissolved in distilled water (100 mL) and the solution extracted with CH_2Cl_2 (3 x 50 mL). The organic washings were combined and washed with 2M HCl (100 mL) and distilled water (2 L). Sodium chloride was added to break up the emulsion that formed during the partitioning process. The organic phase was isolated, dried over $MgSO_4$ and concentrated under reduced pressure to yield a crude product which was chromatographed on silica gel (5:1 ethyl acetate/petrol) to give the target compound as a yellow viscous oil (0.32 g, 4.36 %):

Elemental analysis calcd. for $C_{25}H_{32}O_9$: C, 63.01; H, 6.76%, fnd. C, 62.89; H, 6.81 %;

HRMS (EI^+), calcd. for $C_{25}H_{32}O_9$ [M^+], m/z = 476.2046, fnd. 476.2045;

^{1}H NMR ($CDCl_3$) δ 3.60-3.68 (8H, m, CH_2), 3.72 (2H, t, J = 4.3 Hz, CH_2), 3.745-3.78 (4H, m, CH_2), 3.80 (2H, t, J = 4.7 Hz, CH_2), 3.92 (2H, t, J = 4.9 Hz, CH_2), 3.94 (2H, t, J = 4.5 Hz, CH_2), 4.03 (2H, t, J = 4.4 Hz, CH_2), 4.12 (2H, t, J = 4.5 Hz, CH_2), 6.33 (1H, t, J = 2.3 Hz, Ar-H), 6.43 (2H, dd, J = 8.2 and 2.2 Hz, Ar-H), 6.87 (1H, d, J = 9.0 Hz, Ar-H), 7.02 (1H, dd, J = 9.0 and 3.2 Hz, Ar-H), 7.07 (1H, t, J = 8.2 Hz, Ar-H), 7.19 (1H, s, Ar-H), 7.25 (1H, d, J = 3.21 Hz, Ar-H), 10.41 (1H, s, CHO);

^{13}C NMR ($CDCl_3$) δ 67.5 (CH_2), 67.6 (CH_2), 68.9 (CH_2), 70.0 (CH_2), 70.1 (2 x CH_2), 70.3 (2 x CH_2), 71.3 (CH_2), 71.40 (CH_2), 71.46 (CH_2), 71.48 (CH_2), 101.7 (CH), 107.3 (2 x CH), 111.9 (CH), 116.5 (CH), 125.0 (CH), 126.1 (C), 130.1 (CH), 153.6 (C), 156.8 (C), 160.4 (2 x C), 190.2 (CHO).



(60) Preparation of 2,5,8,11,14,17-hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22), 19-triene-19-carbaldehyde.[3]

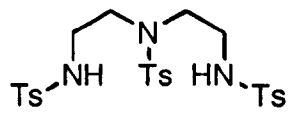
To a stirred mixture of K_2CO_3 (3.15 g, 22.1 mmol) in DMF (30 mL) was added penta(ethylene glycol)di-*p*-toluene sulphonate (5.00 g, 9.11 mmol) in DMF (45 mL) and 2,4-dihydroxybenzaldehyde (1.26 g, 9.11 mmol) in DMF (45 mL) at 90 °C under an N_2 atmosphere via a twin drive syringe pump over 48 hrs and then left for a further 24hrs. The reaction solution was left to cool for 1 hr before it was quenched with water (100 mL) and extracted with diethyl ether repeatedly (3 x 100 mL). The organic solutions were then combined and washed with brine (3 x 150 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure to yield crude product which was further purified using silica gel chromatography (5:1, ethyl acetate: petroleum ether) to give pure product as a colourless oil (0.78 g, 25 %):

HRMS (FAB^+), calcd. for $C_{17}H_{24}O_7$ [M^+] *m/z* = 340.1522, fnd. 340.1523;

ν_{max} (KBr)/cm^{-1}; 2983, 2884, 2260, 1668 (C=O), 1607, 1579, 1353, 1257, 1184, 1106, 1056, 941, 800, 767;

^{1}H NMR ($CDCl_3$) δ 3.62-3.78 (12H, m, CH_2), 3.87-3.90 (4H, m, CH_2), 4.27 (2H, t, *J* = 4.4 Hz, CH_2), 4.42 (2H, t, *J* = 4.4 Hz, CH_2), 6.57 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.97 (1H, d, *J* = 2.4 Hz, Ar-H_c), 7.78 (1H, d, *J* = 8.4 Hz, Ar-H_a), 10.32 (1H, s, CHO);

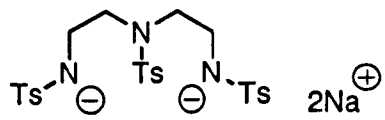
^{13}C NMR ($CDCl_3$) δ 68.0 (CH_2), 69.2 (CH_2), 69.7 (CH_2), 70.5 (CH_2), 70.6 (CH_2), 70.8 (2 x CH_2), 70.9 (CH_2), 71.2 (CH_2), 71.4 (CH_2), 101.3 (CH), 108.0 (CH), 119.5 (C), 129.9 (CH), 163.7 (C), 165.5 (C), 188.6 (CHO).



(62) Preparation of N.N'.N"-tris(2-tolylsulphonyl)-1,4,7-triazacyclononane.[4]

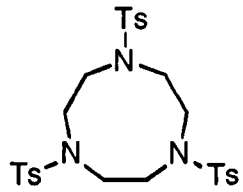
61 was dissolved in DMF (700 mL) under an N_2 atmosphere. The solution was heated to 90-110 °C whilst 1,2-bis(2-tolylsulphonyl)oxyethane (74 g, 0.2 mol) dissolved in DMF (300 mL) was added drop-wise over 4-12 hrs. The solution was stirred overnight after complete addition of the 1,2-bis(2-tolylsulphonyl)oxyethane. The solution was allowed to cool and the DMF removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (400 mL) and washed with water (3 x 400 mL). The organic layer was dried over $MgSO_4$ and the solvent removed to give a white powder which was recrystallised with EtOH (68%):

^{1}H NMR ($CDCl_3$) δ 2.40 (9H, s, CH_3), 3.40 (12H, s, CH_2), 7.23-7.74 (12H, dd, Ar-CH). Corresponds with literature values.



(63) Preparation of N,N',N"-tris(4-tolylsulphonyl)diethylene triamine disodium salt.[4]

A 2L Buchi flask was equipped with a large stirring bar, a condenser and a nitrogen inlet/outlet. The flask was flushed with nitrogen gas and then charged with **62** (113 g, 0.2 mol) suspended in commercial EtOH (900 mL). Sodium metal (9.2 g, 0.4 mol) was added in one portion. The solution was then gradually brought to reflux and maintained at this temperature for 1 hr after the dissolution of the sodium metal was complete. The colourless solution was then allowed to cool under an N_2 atmosphere and the flask was transferred to a rotary evaporator. The solvent was removed and kept under reduced pressure, to ensure that the product was dry. The material was carried through to the next stage.



(65) N,N',N''-tris(4-tolylsulphonyl)diethylene triamine.[4]

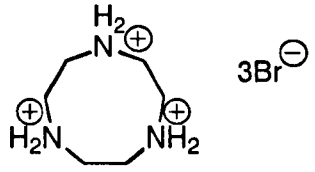
Finely ground TsCl (544 g, 2.98 mol) in diethyl ether (4 L) was added to a 10 L flange flask equipped with a mechanical stirrer. Diethylene tramine (100 g, 0.96 mol) and NaOH (120 g, 3.0 mol) were dissolved in 4 L of water and added to the flask in one portion. The resulting mixture was stirred vigorously overnight. Methanol (1 L) was added to the stirred reaction mixture and the solid product was filtered off and washed with copious volumes of warm and then cold water. The material was washed further with a small volume of MeOH (500 mL) and air dried (356 g, 65 %):

^{1}H NMR ($CDCl_3$) δ 2.41 (3H, s, CH_3), 2.58 (6H, s, CH_3), 2.95 (4H, m, CH_2), 3.10 (4H, m, CH_2), 7.54 (12H, m, CH). Corresponded with literature values.

(64) 1,2-bis(4-tolylsulphonyl)Oxyethane.[4]

Ethane-1,2-diol(40g, 0.64 mol) was dissolved in dry pyridine (400 mL) and 5 equiv of TsCl (309 g, 1.61 mol) were added with vigorous shaking at 0 °C. After refrigeration overnight, the reaction mixture was added to crushed ice, quenched with concentrated HCl (150 mL). Once the ice has melted the precipitate which formed was filtered off, washed with large volumes of water and dried. Recrystallisation by dissolution in a minimum of hot $CHCl_3$ and subsequent addition of 4 volumes of MeOH afforded the product as a crystalline white solid (174 g, 73 %):

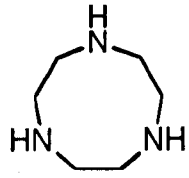
^{1}H NMR ($CDCl_3$) δ 2.50 (6H, s, CH_3), 4.42 (4H, s, CH_2), 7.58 (8H, dd, Ar-CH). Corresponds with literature values.



(66) Preparation of 1,4,7-Triazacyclononane trihydrobromide.[4]

Concentrated sulphuric acid (120 mL) was heated to 165 °C in a 500 mL conical flask. N.N'.N"-tris(2-tolylsulphonyl)-1,4,7-triazacyclononane **65**, (50 g, 0.085 mol) was added in one portion. The solution was stirred rapidly for 6 minutes. After being allowed to cool, the black solution was transferred to a dropping funnel and added drop-wise with stirring to dry EtOH (400 mL) yielding the polyhydrosulphate salt as white floccules. Dry ether (300 mL) was added and the solution cooled to 0 °C. The solid was then filtered off using glass fibre filter paper. The hydrosulphate salt was then dissolved in the minimum volume of hot water and an equivalent volume of conc. HBr (48 %) was added to precipitate the hydrobromide salt of the product. This was filtered off, washed with small volumes of conc. HBr and EtOH and dried *in vacuo* (23 g, 74 %):

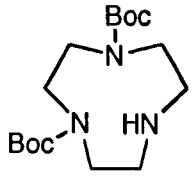
^{1}H NMR (D_2O) δ 3.69 (12H, s, CH_2). Corresponds with literature values.



(67) Preparation of 1,4,7-triazacyclononane.[4]

1,4,7-Triazacyclononane-trihydrobromide (20 g, 0.05 mol), toluene (200 mL), water (20 mL) and NaOH (6.80 g, 0.17 mol) were placed in a 500 mL round-bottomed flask fitted with a Dean-Stark trap. The mixture was then refluxed for 24 hrs. The toluene was decanted off, and a further 50 mL of toluene was added and heated to 50 °C to extract any remaining product from the NaBr crust which had formed. This was also decanted and the toluene removed under reduced pressure. The product was a clear pale oil which crystallized under refrigerated conditions (5.59 g, 81.8 %):

^{1}H NMR ($CDCl_3$) δ 2.28 (12H, s, CH_2). Corresponds with literature values.

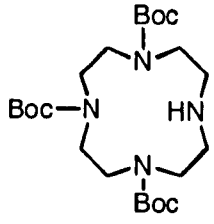


(68) Preparation of [1,4,7]Triazecane-1,7-dicarboxylic acid di-*tert*-butyl ester. To a solution of tacn (0.16 g, 1.3 mmol) and NEt_3 (0.23 mL, 1.8 mmol) in $CHCl_3$ (10 mL) was added $(Boc)_2$ (0.50 g, 2.3 mmol) in $CHCl_3$ (5 mL) via a syringe pump over a 4h period and left to stir overnight at room temp under an N_2 atmosphere. The solvent was removed under reduced pressure to yield a white solid which was purified by silica gel chromatography (10:1, ethyl acetate:methanol) to give pure product as a colourless oil (0.27 g, 62 %):

HRMS (FAB^+), calcd. for $C_{16}H_{31}N_3O_4$ $[M^+]$ *m/z* = 329.2315, fnd. 329.2315;

^{1}H NMR ($CDCl_3$) δ 1.41 (18H, s, CH_3), 2.85-2.87 (4H, m, CH_2), 3.15-3.22 (4H, m, CH_2) 3.35-3.42 (4H, m, CH_2);

^{13}C NMR ($CDCl_3$)(rotamers) δ 28.9 (6 x CH_3), 47.7 (CH_2), 48.1 (CH_2), 48.5 (CH_2), 48.6 (CH_2), 49.9 (CH_2), 50.2 (CH_2), 50.8 (2 x CH_2), 52.0 (CH_2), 52.7 (CH_2), 52.8 (CH_2), 53.4 (CH_2), 80.0 (C), 80.1 (C), 156.1 (C), 156.4 (C).



(69) Preparation of 1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.[5]

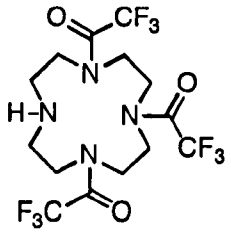
Under an N_2 atmosphere at room temperature, a solution of di-*tert*-butyl dicarbonate (3.42 g, 15.6 mmol) in $CHCl_3$ (10 mL) was added slowly over 3 hrs to a solution of cyclen (1.0 g, 5.8 mmol) and Et_3N (1.76 g, 16.1 mmol) in $CHCl_3$. The reaction mixture was stirred for a further 24 h, and the solvent was removed under vacuum to give crude product which was purified by column chromatography on silica gel (5:1 ethyl acetate:petrol) to afford the pure product as a white foam (2.25 g, 82 %): mp 54-55 °C;

HRMS (EI^+) calcd. for $C_{23}H_{45}N_4O_6$ [MH^+] *m/z* = 473.3339, fnd. 473.3338;

ν_{max} (KBr)/cm^{-1}; 3313, 2975, 2802, 1666 (C=O), 1463, 1413, 1386, 1363, 1319, 1288, 1247, 1149, 1116, 1087, 1058, 983, 943, 892, 858, 821, 773;

^{1}H NMR ($CDCl_3$) δ 1.37 (18H, s, CH_3), 1.39 (9H, s, CH_3), 2.77 (4H, s (broad), CH_2), 3.21-3.31 (8H, m, CH_2), 3.54 (4H, s (broad), CH_2);

^{13}C NMR ($CDCl_3$) δ 27.5 (9 x CH_3), 43.9 (CH_2), 44.9 (CH_2), 47.8 (CH_2), 48.4 (2 x CH_2), 48.8 (CH_2), 49.9 (2 x CH_2), 78.1 (2 x C), 78.4 (C), 154.4 (C=O), 154.6 (2 x C=O).

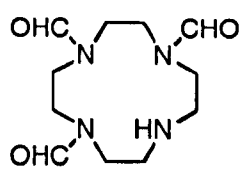


(70) Preparation of 1-[4,7-Bis-(2,2,2-trifluoro-acetyl)-1,4,7,10teraaza-cyclododec-1-yl]-2,2,2-trifluoro-ethanone.[6]

To a stirred solution of cyclen (2 g, 11 mmol) and NEt_3 (1.53 mL, 11.0 mmol) in MeOH (5 mL) under an N_2 atmosphere at room temperature was added ethyltrifluoroacetate (5.54 mL, 46.0 mmol) via a syringe pump over a 3 hr period. The reaction solution was allowed to stir continuously overnight before the solvent was removed under reduced pressure and purified using silica gel chromatography (5:1, ethyl acetate:petroleum ether) to give pure product as a light white foam (4.21 g, 83 %):

HRMS (EI^+), calcd. for $C_{14}H_{17}O_3N_4F_9$ [M^+], *m/z* =460.1157, fnd. 460.1157;

^{1}H NMR ($CDCl_3$) δ 2.70-3.10 (4H, m, CH_2), 3.20-3.70 (8H, m, CH_2), 3.80-3.87 (4H, m, CH_2). ^{13}CNMR was uninterpretable.



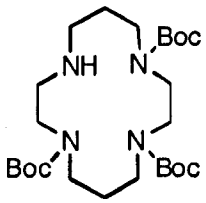
(71) Preparation of 1,4,7,10tetraaza-Cyclododecane-1,4,7-tricarbaldehyde.[7]

To a stirred solution of cyclen (2.0 g, 11 mmol) in EtOH (50 mL) at room temperature was added slowly chloral hydrate (7.27 g, 44 mmol) and left to stir overnight. The reaction was quenched with H_2O (100 mL) and the product extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were then washed with bine and dried over $NaSO_4$ before the solvent was removed under reduced pressure to yield crude product. Purification using silica gel chromatography furnished the pure compound as a colourless oil (2.30 g, 82 %): Due to the restricted rotation around the N-CHO bond the ^1H NMR and ^{13}C NMR spectra of the compound were broad and complicated, so characterisation was accomplished using MS and elemental analysis:

Elemental analysis calcd. for $C_{11}H_{20}N_4O_3$: C, 51.55; H, 7.87; N, 21.86 %, fnd. C, 55.64; H, 7.91; N, 21.93 %;

HRMS (FAB^+), calcd. for $C_{45}H_{74}N_9O_{11}$ $[M^+]$ *m/z* =256.1535, fnd. 256.1537;

ν_{max} (KBr)/cm^{-1}; 3498, 3297, 2827, 2379, 2157, 1708, 1643, 1423, 1398, 1363, 1305, 1222, 1160, 1052, 1025, 1002, 971, 929, 902, 796, 750.



(72) Preparation of 1,4,8,11tetraaza-Cyclotetradecane-1,4,8-tricarboxylic acid tri-tert-butyl ester.[8]

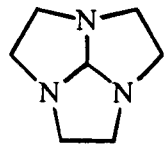
Under an N_2 atmosphere at room temperature, a solution of di-*tert*-butyl dicarbonate (4.33 g, 19.8 mmol) in $CHCl_3$ (50 mL) was added slowly over 3 hrs to a solution of cyclam (1.42 g, 7.0 mmol) and Et_3N (2.02 g, 19.8 mmol) in $CHCl_3$ (40 mL). The reaction mixture was stirred for a further 24 hrs, and the solvent was removed under vacuum to give crude product which was purified by column chromatography on silica gel (5:1 ethyl acetate:petrol) to afford the pure product as a white foam (1.68 g, 47 %):

HRMS (FAB^+) calcd. for $C_{25}H_{49}N_4O_6$ $[M^+]$ *m/z* = 501.3652, fnd. 501.3650;

ν_{max} (KBr)/cm^{-1}; 3495, 2929, 2251, 1681 (C=O), 1613, 1462, 1413, 1366, 1248, 1163, 918, 772, 730;

^{1}H NMR ($CDCl_3$) δ 1.47 (27H, s, CH_3), 1.63 (2H, s (broad), CH_2), 1.95 (2H, s (broad), CH_2), 2.63 (2H, s (broad), CH_2), 2.80 (2H,d, J = 5.2 Hz, CH_2), 3.30-3.47 (12H, m, CH_2),

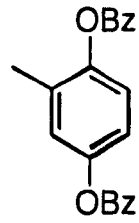
^{13}C NMR ($CDCl_3$) δ 28.5 (9 x CH_3), 45.0-55.0 (10 x CH_2), 79.5 (3 x C), 154-157 (3xC).



(73) Preparation of 1, 4,7-triazatricyclodecane.[9]

Tacn (8.61 g, 0.068 mol) was added to *N,N*-dimethylformamide-dimethylacetal (7.37 g, 0.062 mmol) and refluxed at 110 °C for 5 hours under an N_2 atmosphere. Excess acetal and methanol that formed during the reaction were removed at 40-50 °C under reduced pressure. The remaining yellow oil was distilled using a semi-micro distillation set at 108 °C at 18 mm Hg. The product, a colourless oil was protected from the sunlight and stored at -20 °C where it form crystallised to form white needles (8.43 g, 88 %):

^{1}H NMR ($CDCl_3$) δ 2.15-2.59 (12H, m, CH_2), 4.41 (1H, s, CH). Consistent with literature values.



(77) Preparation of 1,4-bis-benzyloxy-2-methyl-benzene.[10]

To methyl-hydroquinone (1.24 g, 0.01 mol) dissolved in DMF (150 mL), was added K_2CO_3 (9.16 g, 0.04 mol) and benzyl bromide (6.84 g, 0.04 mol). The reaction was refluxed for 16 hrs at 120 °C and stirred continuously, the reaction termination point was determined using TLC (1:1, petroleum ether:CH_2Cl_2). The solution was then cooled to room temperature and water added (100 mL). The solution was washed with ammonia (1 M, 3 x 50 mL), then H_2O (3 x 100 mL). The product was then extracted with CH_2Cl_2 (3 x 50 mL), combined and dried over $MgSO_4$. The solvent was removed under reduced

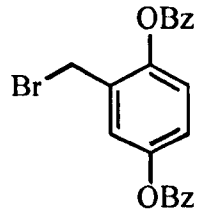
pressure to give crude product as a brown oil which was purified using silica gel chromatography (1:1, ethyl acetate:petroleum ether) to yield pure 1,4-Bis-benzyloxy-2-methyl-benzene as white needles (4.13 g, 72 %):

Elemental analysis calcd. for $C_{21}H_{16}O_4$: C, 75.89; H, 4.85 %, fnd. C, 75.94; H, 4.91 %;

HRMS (FAB^+), calcd. for $C_{21}H_{16}O_4$ $[M^+]$ *m/z* =332.1049, fnd. 332.1051;

ν_{max} (KBr)/cm^{-1}; 3741, 2927, 1974, 1646, 1542, 1461, 1361, 114, 817;

^{1}H NMR (DMSO) δ 2.10 (3H, s, CH_3), 4.90 (4H, d, J = 8.0 Hz, CH_2), 6.64 (1H, dd, J = 8.9 and 2.9 Hz, Ar-H), 6.71 (1H, d, J = 8.8 Hz, Ar-H), 6.77 (1H, d, J = 2.9 Hz, Ar-H), 7.30-7.51 (10H, m, Ar-H).

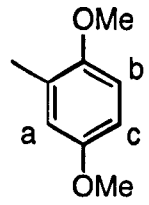


Preparation of 1,4-Bis-benzyloxy-2-bromomethyl-benzene

Method 1: Method adapted from Rao *et al.*[11] A solution of 2,5-dibenzyoxy-toluene (1.00 g, 3.20 mmol) in CCl_4 (100 mL) was added to a suspension of N-bromosuccinimide (0.54 g, 3.30 mmol) in CCl_4 (30 mL) in the presence of a catalytic amount of benzoyl peroxide (10 mg). The solution was mechanically stirred and refluxed for 2.5 hrs. The cooled reaction vessel was filtered to remove the succinimide, the residue was washed with carbon tetrachloride (30 mL) and the combined organic solution was evaporated off. This yielded a yellow/brown oil, ^{1}H NMR of which has shown to be a mixture of brominated compounds, the major component was the brominated benzyl group.

Method 2: A solution of 2,5-dibenzyoxy-toluene (1.0 g, 3.2 mmol) in CCl_4 (100 mL) was added to a suspension of N-bromosuccinimide (0.54 g, 3.30 mmol) in CCl_4 (30 mL) in the presence of a catalytic amount of benzoyl peroxide (10 mg). The solution was mechanically stirred and irradiated with a 200 W lamp for 5 hrs. The mixture was filtered to remove the succinimide then washed with carbon tetrachloride (30 mL). The combined organic solutions were removed under reduced pressure. This gave a yellow/brown oil.

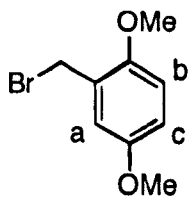
The ^{1}H NMR showed the oil to be a complex mixture of brominated compounds, the major component was the brominated benzyl group.



(77) Preparation of 1,4-Dimethoxy-2-methyl-benzene.

Method was adapted from Sembiring *et al*[12]. Iodomethane (3.13 g, 0.02 mol) was added dropwise by syringe to a mechanically stirred mixture of methylhydroquinone (1.24 g, 0.01 mol) and K_2CO_3 (4.14 g, 0.03 mol) in degassed DMF (100 mL) under an N_2 atmosphere. The mixture was stirred for 16 hrs overnight at 100 °C. Cooled water (75 mL) was added to the resulting pink solution until all the K_2CO_3 had dissolved. The solution was extracted with diethyl ether several times (3 x 50 mL) and the combined extracts washed successively with 2.5 M sodium hydroxide (100 mL) and water (3x100 mL). The ether phase was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a red liquid. Distillation under 20 mm Hg pressure at 120 °C gave a bright red liquid, dimethoxytoluene (1.15 g, 75 %):

^{1}H NMR ($CDCl_3$) δ 2.27 (3H, s, CH_3), 3.79 (3H, s, CH_3) 3.84 (3H, s, CH_3), 6.71 (1H, dd, J = 9 and 2 Hz, Ar-H_c), 6.77, (1H, d, J = 9 Hz, Ar-H_b), 6.80, (1H, s, Ar-H_a). Consistent with literature.

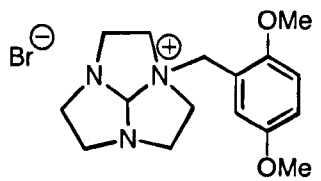


(79) Preparation of Dimethoxybenzyl bromide.[12]

A solution of 2, 5-dimethoxytoluene (1.00 g, 6.57 mmol) in CCl_4 (100 mL) was added to a suspension of *N*-bromosuccinimide (1.08 g, 6.60 mmol) in the presence of a catalytic amount of benzoyl peroxide (10 mg). The mixture was mechanically stirred and refluxed for 2.5 hrs. The cooled mixture was filtered to remove the succinimide, then washed with carbon tetrachloride (40 mL) and the combined organic solution distilled off. The resulting

yellow solid was recrystallised from hexane containing a few drops of benzene to give 2, 5-dimethoxybenzylbromide as colourless needles (1.08 g, 71 %):

^{1}H NMR ($CDCl_3$) δ 3.71 (3H, s, CH_3), 3.78 (3H, s, CH_3) 4.46 (2H, s, CH_2), 6.82 (1H, s, Ar-H_a), 6.86, (1H, dd, J = 8.9 and 2.4 Hz, Ar-H_c), 6.92 (1H, d, J = 2.4 Hz, Ar-H_b). Consistent with literature.[12]



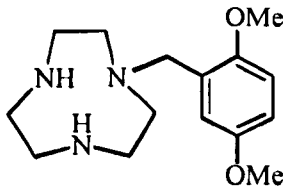
(80) Preparation of Dimethoxybenzyl-1,4,7-triazacyclo[5.2.1.0]decane.

Method adapted from Peacock *et al.*[13] To a one-necked flask flushed with N_2 and charged with 1,4,7-triazacyclodecane (0.59 g, 4.00 mmol) in dry THF (40 mL), was slowly added dimethoxybenzyl bromide (1.00 g, 4.00 mmol) in dry THF (20 mL). The reaction mixture was stirred overnight and the resultant white precipitate filtered, then washed with THF (40 mL) and dried (0.87 g, 75 %):

Elemental analysis calcd. for $C_{16}H_{24}O_2N_3Br$: C, 51.90; H, 6.53; N, 11.35 %, fnd. C, 51.92; H, 6.55; N, 11.41 %;

^{1}H NMR (D_2O) δ 3.21 (2H, dd, J = 10.3 and 5.6 Hz, CH_2), 3.35 (2H, dd, J = 11.3 and 6.3 Hz, CH_2), 3.41 (1H, dd, J = 10.0 and 5.7 Hz, CH_2), 3.43 (1H, dd, J = 10.0 and 5.7 Hz, 1H, CH_2), 3.55 (2H, dq, J = 13.3 and 2.7 Hz, CH_2), 3.75 (3H, s, CH_3), 3.78 (3H, s, CH_3), 3.89 (2H, dq, J = 12.6 and 2.7 Hz, CH_2), 4.13 (2H, dd, J = 10.0 and 5.7 Hz, CH_2), 4.17 (1H, dd, J = 10.0 and 5.7 Hz, CH_2), 4.61 (2H, s, CH_2), 6.86 (1H, d, J = 9 Hz, Ar-H), 6.95 (1H, dd, J = 9.0 and 3.0 Hz, Ar-H), 7.18 (1H, d, J = 3.0 Hz, Ar-H);

^{13}C NMR (D_2O) δ 41.8 (2 x CH_2), 43.5 (CH_2), 46.5 (CH_3), 46.6 (CH_3), 47.2 (CH_2), 54.7 (CH_2), 55.8 (CH_2), 55.87 (CH_2), 112.6 (CH), 114.2 (CH), 117.9 (CH), 125.7 (C), 128.1 (CH), 151.6 (C), 152.6 (CH_2).

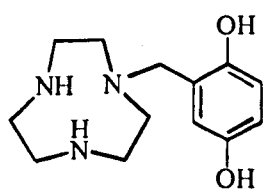


(81) Preparation of (2,5-Dimethoxy-benzyl)-[1,4,7]triazonane.

Dimethoxybenzyl triazacyclodecane (0.85 g, 2.90 mmol) was refluxed in water (40 mL) for 4 hours followed by the addition of NaOH (0.34 g, 8.50 mmol). The resulting basic solution was refluxed for 24 hours and the water removed with toluene using a Dean-Stark apparatus. Removal of the toluene afforded an oil which was sufficiently pure (0.51 g, 59 %):

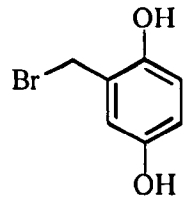
Elemental analysis calcd. for $C_{15}H_{25}O_2N_3$: C, 64.49; H, 9.02; N, 15.04 %, fnd. C, 64.41; H, 8.97; N, 14.96 %;

^{1}H NMR ($CDCl_3$) δ 2.55 (8H, s, CH_2), 2.68 (4H, s, CH_2), 3.62 (2H, s, CH_2), 3.68 (3H, s, CH_3), 3.72, (3H, s, CH_3), 6.68 (1H, dd, J = 8.1 and 2.3 Hz, Ar-H), 6.73 (1H, d, J = 8.8 Hz, Ar-H), 6.84 (1H, d, J = 2.5 Hz, Ar-H).



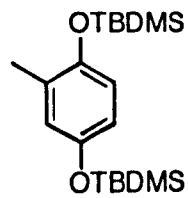
Preparation of [1,4,7]Triazonan-1-ylmethyl-benzene-1,4-diol.

Method was adapted from Gamble *et al.*[14] BBr_3 (0.49 g, 1.90 mmol) was added drop-wise to a solution of dimethoxybenzyl triazacyclononane (0.50 g, 1.7 mmol) in CH_2Cl_2 (50 mL), upon which the solution turned dark. The resulting mixture was stirred overnight, quenched with water (30 mL), and stirred for a further 15 mins. The solid was filtered off, the water layer neutralised with aqueous 2M NaOH (50 mL) and extracted with CH_2Cl_2 (2 x 100 mL). The CH_2Cl_2 extracts were stirred with aqueous 10% HCl (20 mL) and the resulting solid filtered off. The CH_2Cl_2 extracts were dried over $MgSO_4$, and concentrated under reduced pressure to yield a complex mixture of compounds which could not be separated or purified.



Preparation of 2-Bromomethyl-benzene-1,4-diol

BBr_3 (6.51 g, 25.9 mmol) was added dropwise to a solution of dimethoxybenzylbromide (2.0 g, 8.6 mmol) in CH_2Cl_2 (30 mL), upon which the solution turned dark. The reaction was slightly exothermic. The resulting mixture was stirred overnight, quenched with water (30 mL), and stirred for a further 15 mins. The resulting solid was filtered off and the water layer neutralised with aqueous 2M NaOH (50 mL) and extracted with CH_2Cl_2 (2 x 50 mL). The CH_2Cl_2 extracts were acidified with aqueous 10% HCl (50 mL), then dried over $MgSO_4$, and concentrated *in vacuo*, yielding crude product. The ^1H NMR showed that there was a complex mixture of compounds. These could not be separated, as the molecule was too polar to separate by silica gel chromatography.



(82) Preparation of 1,4-Bis-(tert-butyl-dimethyl-silanyloxy)-2-methyl-benzene.

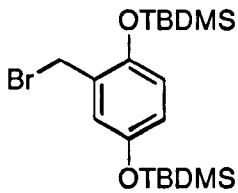
TBDMSCl (24.2 g, 161 mmol) was added drop-wise to a solution of methylhydroquinone (5.0 g, 40 mmol) in DMF (80 mL) with imadazole (11.1 g, 161 mmol). The resulting mixture was stirred vigorously overnight at room temperature under N_2. The solution was poured into water (300 mL) and the crude product extracted with ether (3 x 100 mL). The combined organic extracts were washed with saturated brine (2 x 100 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a colourless oil which formed large crystals when cooled (12.3 g, 87%):

Elemental analysis calcd. for $C_{19}H_{36}O_2Si_2$: C, 64.71; H, 10.29 %, fnd. C, 64.65; H,. 10.25; N, 9.51 %;

ν_{max} (KBr)/cm^{-1}; 2950, 2147, 1492, 1250, 1217, 1003, 912, 837, 797, 774, 679, 654, 636, 620;

^{1}H NMR ($CDCl_3$) δ 0.01 (12H, d, J = 2.8 Hz, CH_3), 0.85 (18H, d, J = 14.0 Hz, CH_3), 1.97 (3H, s, CH_3), 6.35 (1H, dd, J = 8.6 and 2.9 Hz, Ar-H), 6.42 (1H, s, Ar-H), 6.44 (1H, d, J = 1.6 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ -4.45 (2 x CH_3), -4.23 (2 x CH_3), 17.0 (C), 18.1 (C), 18.2 (C), 25.7 (CH_3), 25.8 (CH_3), 117.4 (CH), 118.9 (CH), 122.5 (CH), 129.5 (C), 148.0 (C), 149.2 (C).



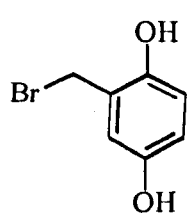
(83) Preparation of 2-Bromomethyl-1,4-bis-(tert-butyl-dimethyl-silanyloxy)-benzene.

A solution of TBDMS protected methyl hydroquinone (1.07 g, 3.04 mmol) in CCl_4 (30 mL) was added to a suspension of *N*-bromosuccinimide (0.54 g, 3.04 mmol) in the presence of a catalytic amount of benzoyl peroxide (10 mg). The mixture was mechanically stirred and refluxed for 2.5 hrs. The cooled mixture was filtered to remove the succinimide, then washed with carbon tetrachloride (40 mL). The combined organic solutions were distilled off, resulting in a yellow solid (0.82 g, 62 %):

Elemental analysis calcd. for $C_{19}H_{35}O_2Br_2Si_2$: C, 52.88; H, 8.17 %, fnd. C, 52.92; H, 8.21 %;

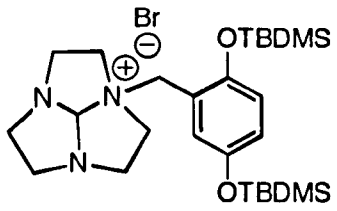
^{1}H NMR ($CDCl_3$) δ 0.01 (6H, s, CH_3), 0.03 (6H, s, CH_3), 0.80 (9H, s, CH_3), 0.86 (9H, s, CH_3), 4.25 (2H, s, CH_2), 6.49 (2H, s, Ar-H), 6.64 (1H, t, J = 1.1 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ -4.2 (2 x CH_3), -4.1 (2 x CH_3), 18.1 (C), 18.2 (C), 25.72 (3 x CH_3), 25.75 (3 x CH_3), 25.8 (3 x CH_3), 29.2 (CH_2-Br), 119.2 (CH), 121.1 (CH), 122.2 (CH), 128.5 (C), 148.0 (C), 149.3 (C).



Preparation of 2-Bromomethyl-benzene-1,4-diol.[15]

To 2,5-DiTBDMSO benzylbromide (0.14 g, 3.25 mmol) in anhydrous THF (20 mL) was added tetrabutylammonium fluoride (TBAF) 1 M in THF (0.204 g, 7.81 mmol) at room temperature, and the mixture was stirred for 30 min. The mixture was filtered through silica gel; the silica was rinsed with Et_2O (100 mL). The organic layers were combined and the solvent evaporated. This method was unsuccessful due to the complex mixture obtained on work up and the inability to isolate any product from the mixture.

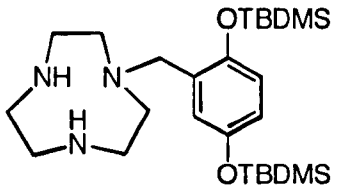


(84) Preparation of 84.

To a one-necked flask flushed with N_2 and charged with 1,4,7-triazacyclodecane (0.26 g, 1.90 mmol) in dry THF (30 mL), was slowly added 2-Bromomethyl-1,4-bis-(tert-butyl-dimethyl-silanyloxy)-benzene (0.82 g, 1.90 mmol) in dry THF (20 mL). The reaction mixture was stirred overnight and the resultant white precipitate filtered then washed with THF (40 mL) and dried (0.51 g, 54%):

Elemental analysis calcd. for $C_{26}H_{48}O_2N_3Si_2Br$: C, 54.71; H, 8.48; N, 7.36 %, fnd. C, 54.75; H, 8.51; N, 7.40 %;

^{1}H NMR ($CDCl_3$) δ 0.01 (6H, s, CH_3), 0.05 (6H, s, CH_3), 0.77 (9H, s, CH_3), 0.79 (9H, s, CH_3), 3.05 (2H, dd, J = 11 and 7 Hz, CH_2), 3.15 (2H, m, CH_2), 3.25 (2H, dd, J = 11 and 7 Hz, CH_2), 3.40 (1H, dd, J = 11 and 7 Hz, CH_2), 3.73 (1H, dd, J = 11 and 7 Hz, CH_2), 3.75 (1H, dd, J 10 and 7 = Hz, CH_2), 4.15 (1H, dd, J = 10 and 7 Hz, CH_2), 4.23 (2H, m, Ar-H), 4.34 (2H, s, CH_2), 5.69 (1H, s, CH), 6.60-6.70 (2H, m, Ar-H), 6.80 (1H, d, J = 2.7 Hz, Ar-H).

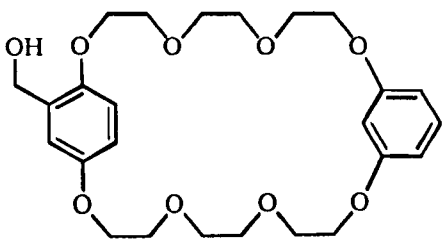


(85) Preparation of 85.

The same procedure for the synthesis of **81** was applied for the decapping of the tacn, 63%;

Elemental analysis calcd. for $C_{25}H_{49}O_2N_3Si_2$: C, 62.58; H, 16.29; N, 8.76 %, fnd. C, 62.67; H, 16.39; N, 8.82 %;

^{1}H NMR ($CDCl_3$) δ 0.01 (6H, s, CH_3), 0.04 (6H, s, CH_3), 0.80 (9H, s, CH_3), 0.85 (9H, s, CH_3), 2.66 (8H, s, CH_2), 2.78 (4H, s, CH_2), 3.68 (2H, s, CH_2), 6.65-6.72 (2H, m, Ar-Hs), 6.83 (1H, d, J = 2.9 Hz, Ar-H).



(86) Preparation of (2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.2.2.1^{12,16}] dotriaconta-1(30),12,14,16(32),27(31),28-hexaen-28-yl)-methanol.[17]

A solution of **15** (0.10 g, 0.20 mmol) in dry THF (2 mL) was added drop-wise to a 25 mL, round-bottomed flask containing a stirred solution of $LiAlH_4$ (0.011 g, 0.31 mmol) in THF (5 mL) at 0 °C under an argon atmosphere. The reaction was allowed to warm to an ambient temperature and then heated at reflux (78 °C) for 18 hrs. The mixture was cooled to 0 °C and the excess $LiAlH_4$ was decomposed by slow addition of $Na_2SO_4.H_2O$ crystals. The resulting suspension was filtered through Celite, and the solid material was washed with THF (40 mL) and the filtrate was concentrated under reduced pressure to furnish the crude product as thick white oil. The oil was purified by chromatography on silica gel using a ethyl acetate:petroleum ether mixture (5:1) to give pure product (0.091 g, 91 %):

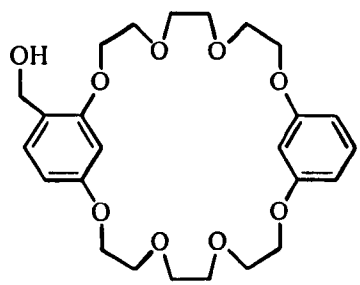
Elemental Analysis calcd. for $C_{25}H_{34}O_9$: C, 62.75; H, 7.31%, fnd. C, 62.47; H, 7.31 %;

HRMS (EI^+), calcd. for $C_{25}H_{34}O_9$ [M^+], m/z = 478.2203, fnd. 478.2205;

ν_{max} (KBr)/cm^{-1}; 3434, 2867, 2161, 1589, 1492, 1450, 1353, 1334, 1280, 1207, 1184, 1124, 1056, 987, 921, 844, 763;

^{1}H NMR ($CDCl_3$) δ 3.15 (1H, t, J = 6.6 Hz, OH), 3.61-3.67 (8H, m, CH_2), 3.70 (2H, t, J = 5 Hz, CH_2), 3.76 (6H, dt, J = 9 and 5 Hz, CH_2), 3.93 (4H, dt, J = 9 and 5 Hz, CH_2), 4.04 (4H, m, CH_2), 4.54 (2H, s, CH_2), 6.37-6.45 (3H, m, Ar-H), 6.70 (2H, s, Ar-H), 6.86 (1H, s, Ar-H), 7.05 (1H, t, J = 8.1 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 62.3 (CH_2), 67.4 (CH_2), 67.6 (CH_2), 69.1 (CH_2), 69.7 (CH_2), 70.0 (CH_2), 70.1 (CH_2), 70.3 (CH_2), 70.4 (CH_2), 71.1 (CH_2), 71.3 (CH_2), 71.4 (CH_2), 101.7 (CH), 107.4 (CH), 107.5 (CH), 114.8 (CH), 115.4 (CH), 116.7 (CH), 130.0 (CH), 132.3 (C), 151.8 (C), 153.7 (C), 153.7 (C), 160.3 (C), 160.4 (C).



(87) Preparation of (2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.3.1.1^{12,16}] dotriaconta-1(31), 12 (32), 13, 15, 27, 29-hexaen-13-yl)-methanol.

A solution of **14** (0.43 g, 0.91 mmol) in dry THF (2 mL) was added drop-wise to a 25 mL, round-bottomed flask containing a stirred solution of $LiAlH_4$ (0.05 g, 1.36 mmol) in THF (5 mL) at 0 °C under an argon atmosphere. The reaction was allowed to warm to an ambient temperature and then heated at reflux (78 °C) for 18 h. The mixture was cooled to 0 °C and the excess $LiAlH_4$ was decomposed by slow addition of $Na_2SO_4.H_2O$ crystals. The resulting suspension was filtered through Celite, and the solid material was washed with THF (60 mL) and the filtrate was concentrated under reduced pressure to furnish the crude product as thick whitish oil. The oil was purified by chromatography on silica gel using an ethyl acetate-petroleum ether mixture (5:1) to give pure product as an opaque thick oil (0.428 g, 98.6 %):

Elemental analysis calcd. for $C_{25}H_{34}O_9$: C, 63.01; H, 6.76%, fnd. C, 62.74; H, 7.16 %;

HRMS (EI^+), calcd. for $C_{25}H_{34}O_9$ [M^+], m/z = 478.2203, fnd. 478.2202;

ν_{max} (KBr)/cm^{-1}; 3421, 2869, 1587, 1504, 1490, 1450, 1357, 1332, 1286, 1261, 1180, 1120, 1054, 995, 944, 840, 763;

^{1}H NMR ($CDCl_3$) δ 2.92 (1H, t, J = 6.4 Hz, OH), 3.59-3.70 (8H, m, CH_2), 3.76-3.82 (8H, m, CH_2), 3.91-3.97 (8H, m, CH_2), 4.02-4.09 (4H, m, CH_2), 4.52 (2H, d, J = 5 Hz, CH_2), 6.34-6.45 (5H, m, Ar-H), 7.02 (1H, d, J = 2.6 Hz, Ar-H), 7.04 (1H, d, J = 2.6 Hz, Ar-H);

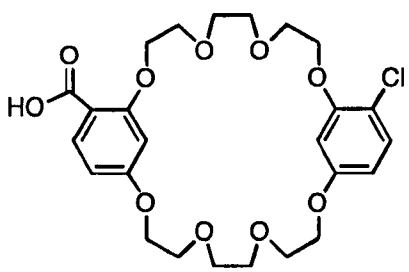
^{13}C NMR δ 62.2 (CH_2), 67.5 (CH_2), 67.7 (CH_2), 67.9 (CH_2), 68.4 (CH_2), 70.01 (CH_2), 70.07 (CH_2), 70.1 (CH_2), 70.8 (CH_2), 71.2 (CH_2), 71.4 (CH_2), 71.9 (CH_2), 101.3 (CH), 102.2 (CH), 105.8 (CH), 106.8 (CH), 107.7 (CH), 123.3 (C), 130.0 (CH), 130.1 (CH), 158.4 (C), 160.1 (C), 160.3 (2 x C).

General Procedure for the Mesylation of Benzyl alcohol.

To a stirred solution of the alcohol (0.20 mmol) in CH_2Cl_2 (5 mL) was added NEt_3 (0.24 mmol) and cooled to -15 °C. Methanesulphonyl chloride (0.25 mmol) was added drop wise to the stirred solution. After complete addition the mixture was allowed to warm to ambient room temperature and left stirring overnight. The reaction was quenched with saturated $NaHCO_3$ (20 mL) and the product extracted in CH_2Cl_2 (3 x 30 mL) which was dried over $MgSO_4$ and the solvent removed under reduced pressure to furnish the product.

General Procedure for the formation of Benzyl Triflate.

NEt_3 (0.043 mmol) was added drop wise to a solution of the alcohol (0.037 mmol) in CH_2Cl_2 (2 mL). The solution was cooled to -45 °C and left for 1h. Triflic Anhydride (0.056 mmol) was added drop-wise to the stirred solution. After complete addition the mixture was allowed to warm to ambient room temperature and left stirring overnight. The reaction was quenched with 1 M $NaHCO_3$ (10 mL) and H_2O (10 mL) and then extracted with CH_2Cl_2 (3 x 30 mL). The organic fractions were combined and washed with brine (30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the product.



(89) Preparation of 30-Chloro-2,5,8,11,17,20,23,26-octaoxa-tricyclo [25.3.1.112,16]dotriaconta-1(31),12(32),13,15,27,29-hexaene-13-carboxylic acid.[18a,b]

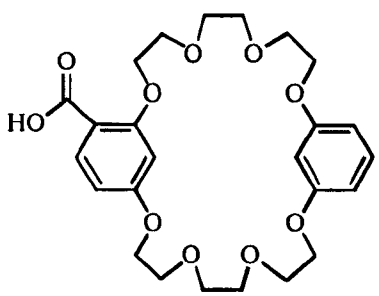
To a stirred solution of **58** (0.59 g, 1.23 mmol), in acetone/H_2O (mL) was added sulphamic acid (0.26 g, 2.72 mmol) at 0 °C over a 20 min period. $NaClO_2$ was then added over 30 min period at 0 °C and allowed to heat to room temperature and left to stir for 2 hrs. After this period of time the solvent was concentrated under reduced pressure, extracted with CH_2Cl_2 (20 mL), washed with brine (3 x 10 mL) and dried over $MgSO_4$ to yield crude product which was further purified to using silica gel chromatography to give a mixture of the two isomers. The separation of the two regioisomers was achieved through the slow crystallisation of one isomer using CH_2Cl_2 and petroleum ether (36 %):

HRMS (FAB^+) calcd. for $C_{25}H_{31}O_{10}ClNa$ [M^+Na], *m/z* = 549.1503, fnd. 549.1514;

ν_{max} (KBr)/cm^{-1}; 2360, 2129, 2110, 1725, 1606, 1258, 1190, 1135, 921, 846, 786, 754, 736, 695;

^{1}H NMR ($CDCl_3$) δ 3.73 (4H, s, CH_2), 3.75 (4H, s, CH_2), 3.80 (2H, t, *J* = 4.4 Hz, CH_2), 3.84 (2H, t, *J* = 4.4 Hz, CH_2), 3.89 (4H, t, *J* = 4.4 Hz, CH_2), 4.04 (2H, t, *J* = 5.0 Hz, CH_2), 4.07 (2H, t, *J* = 4.4 Hz, CH_2), 4.20 (2H, t, *J* = 4.4 Hz, CH_2), 4.26 (2H, t, *J* = 4.4 Hz, CH_2), 6.45 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H), 6.56 (1H, d, *J* = 2.8 Hz, Ar-H), 6.61 (1H, d, *J* = 2.4 Hz), 6.67 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H), 7.20 (1H, d, *J* = 8.4 Hz, Ar-H), 8.10 (1H, d, *J* = 8.8 Hz, Ar-H), 10.8 (1H, s (broad), CHO);

^{13}C NMR ($CDCl_3$) δ 67.8 (CH_2), 68.0 (CH_2), 68.7 (CH_2), 69.0 (CH_2), 69.2 (CH_2), 69.7 (CH_2), 69.8 (CH_2), 70.8 (CH_2), 71.0 (CH_2), 71.5 (2 x CH_2), 100.6 (CH), 102.4 (CH), 106.7 (CH), 108.2 (CH), 111.0 (C), 115.5 (C), 130.1 (CH), 135.1 (CH), 155.0 (CH), 158.5 (CH), 158.9 (CH), 164.2 (C), 165.4 (C).



(90) Preparation of 2,5,8,11,17,20,23,26-Octaoxatricyclo [25.3.1.1^{12,16}] dotriaconta 1(30), 12(32), 13,15, 27(31), 28-hexaene-13-carboxylic acid.[19]

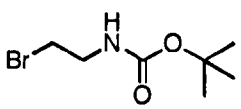
The macrocyclic aldehyde **58** (0.05 g, 1.05 mmol) was dissolved in acetone (2 mL) and treated with a solution of $KMnO_4$ (0.033 g, 2.10 mmol) in acetone: H_2O (1:1) (2 mL). The solution was stirred at room temperature for 6 hrs. The insoluble inorganic salts were removed by filtration through a short silica plug (10 g) and elution with ethyl acetate (100 mL). The filtrate was concentrated, redisssolved in CH_2Cl_2 (20 mL) which was washed with brine (2 x 30 mL), separated and dried over $MgSO_4$. Removal of the CH_2Cl_2 afforded a crude product which was purified by silica gel chromatography using ethyl acetate/petrol (5:1) as eluant to yield the pure product (0.032 g, 62 %):

HRMS (EI^+) calcd. for $C_{25}H_{32}O_{10}$ [M^+], *m/z* = 492.1995, fnd. 492.1997;

ν_{max} (KBr)/cm^{-1}; 3272, 2869, 2360, 1720 (C=O), 1604, 1490,1438, 1361, 1284, 1255, 1182, 1120, 1056, 987, 943, 836, 763, 682, 634;

^{1}H NMR ($CDCl_3$) δ 3.62-3.66 (8H, m, CH_2), 3.67-3.71 (2H, m, CH_2), 3.72 (2H, t, *J* = 4.0 Hz, CH_2), 3.77 (2H, t, *J* = 4.0 Hz, CH_2), 3.83 (2H, t, *J* = 4.0 Hz, CH_2), 3.88 (2H, t, *J* = 4.0 Hz, CH_2), 4.08 (2H, t, *J* = 4.0 Hz, CH_2), 4.12 (2H, t, *J* = 4.0 Hz, CH_2), 4.27 (2H, t, *J* = 4.0 Hz, CH_2), 6.29 (1H, t, *J* = 2.4 Hz, Ar-H), 6.36 (1H, t, *J* = 2.04 Hz, Ar-H), 6.38 (1H, t, *J* = 2.16 Hz), 6.45 (1H, d, *J* = 2.4 Hz, Ar-H), 6.55 (1H, dd, *J* = 10.4 and 2.0 Hz, Ar-H), 6.99 (1H, t, *J* = 8.0 Hz, Ar-H), 7.92 (1H, d, *J* = 8.0 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 67.3 (CH_2), 67.7 (CH_2), 68.2 (CH_2), 69.1 (CH_2), 69.6 (CH_2), 69.8 (CH_2), 70.0 (CH_2), 70.1 (CH_2), 70.7 (CH_2), 71.2 (CH_2), 71.4 (CH_2), 71.8 (CH_2), 101.0 (CH), 102.3 (CH), 106.4 (CH), 107.6 (CH), 108.0 (CH), 111.5 (C), 130.2 (CH), 135.5 (CH), 159.1 (C), 160.2 (C), 160.3 (C), 164.5 (C), 165.8 (C).



(92) Preparation of (2-bromo-ethyl)-carbamic acid *tert*-butyl ester.[20]

To a stirred solution of bromo ethylamine (1.0 g, 4.8 mmol) and NEt_3 (0.58 g, 5.7 mmol), in CH_2Cl_2 (15 mL), was slowly added $(Boc)_2$ (1.17 g, 5.3 mmol) dissolved in CH_2Cl_2 (20 mL) over a 5 min period under an N_2 atmosphere. The reaction was left overnight before quenching with water (50 mL). The organic layer was separated and dried over $MgSO_4$ and the solvent removed under reduced pressure to give crude product which was purified

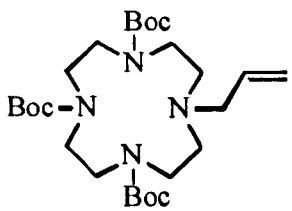
using silica gel chromatography (1:1, ethyl acetate:petroleum ether) to give pure compound as a white foam (0.89 g, 83 %):

HRMS (CI^+), calcd. for $C_7H_{15}O_2NBr$ [M^+H] *m/z* = 224.0286, fnd. 224.0282;

ν_{max} (KBr)/cm^{-1}; 3340, 2975, 1687, 1506, 1454, 1392, 1365, 1249, 1160, 1043, 950, 862, 779;

^{1}H NMR ($CDCl_3$) δ 1.38 (9H, s, CH_3), 3.38 (2H, t, *J* = 8.3 Hz, CH_2), 3.46 (2H, t, *J* = 5.6 Hz, CH_2), 4.88 (1H, s, NH);

^{13}C NMR ($CDCl_3$) δ 28.7 (3 x CH_3), 33.2 (CH_2), 42.7 (CH_2), 80.7 (C), 157.5 (C).



(93) 10-Allyl-1,4,7,10yetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-tert-butyl ester.[21]

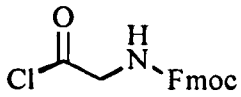
To a stirred mixture of **69** (0.32 g, 0.67 mmol) and K_2CO_3 (0.10 g, 0.75 mmol) in anhydrous DMF (30 mL) at 0 °C under N_2, was slowly added allyl bromide (0.06 mL, 0.68 mmol) and left to stir overnight. The solvent was removed and the solid residue dissolved in $CHCl_3$. The mixture was passed through a celite column and the pad was washed with $CHCl_3$ (50 mL). The filtrate was concentrated and the residue purified by silica gel chromatography (1:1, CH_2Cl_2:hexane) to give pure product as a colourless oil (0.33 g, 96 %):

HRMS (EI^+), calcd. for $C_{26}H_{48}O_6N_4$ [M^+], *m/z* = 512.3574, fnd. 512.3578;

ν_{max} (KBr)/cm^{-1}; 2974, 2106, 1680 (C=O), 1458, 1411, 1362, 1314, 1245, 1150, 1106, 1032, 974, 924, 862, 820, 806, 772, 676;

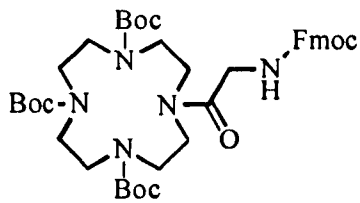
^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s, CH_3), 1.48 (9H, s, CH_3), 1.49 (9H, s, CH_3), 2.60-2.71 (4H, m, CH_2), 3.21-3.58 (12H, m, CH_2), 5.20 (1H, d, *J* = 2.4 Hz, CH), 5.24 (1H, s, CH), 5.87-5.94 (1H, m, CH);

^{13}C NMR ($CDCl_3$) δ 28.8 (9 x CH_3), 47.4 (CH), 49.9-51.7 (8 x CH_2), 60.7 (CH_2), 67.4 (CH_2), 80.9 (3 x C), 120.3 (2 x CH), 125.4 (2 x CH), 127.4 (2 x CH), 128.0 (2 x CH), 141.6 (C), 144.2 (C), 155.9 (2 x C), 156.5 (2 x C).



(97) Preparation of Chlorocarbonylmethyl-carbamic acid 9H-fluoren-9-ylmethyl ester.[22]

To a stirred mixture of gly-Fmoc (0.20 g, 0.67 mmol) in CH_2Cl_2 (4 mL) under an N_2 atmosphere at 0 °C was added slowly drop-wise thionyl chloride (0.15 mL, 2.00 mmol). The reaction was allowed to warm to room temperature and stir for 2 hrs. The end-point was determined when all the insoluble gly-Fmoc had dissolved into solution. The solvent was then removed under reduced pressure and kept under high vacuum to remove all the excess thionyl chloride for 1 hr to give a white crystalline solid which was air stable (0.21, 98 %). This material was carried through to the next stage of the synthesis.



(98) Preparation of Preparation of 7-(3,3-Dimethyl-butyryl)-10-[2-(9H-Fluoren-9-ylmethoxycarbonylamino)-acetyl]-1,4,7,10tetraza-cyclododecane-1,4-dicarboxylic acid di-tert-butyl ester.[22]

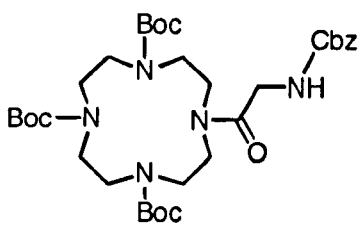
To a stirred solution of **97** (0.21 g, 0.67 mmol) and NEt_3 (0.074 g, 0.72 mmol) in CH_2Cl_2 (4 mL) was added drop-wise 3-boc-cyclen (0.31 g, 0.67 mmol) at 0 °C under an N_2 atmosphere. The reaction was allowed to stir continuously for a further 1 hr before quenching with H_2O (10 mL). The product was extracted with CH_2Cl_2 (3 x 15 mL) washed with brine (3 x 15 mL), dried over Na_2SO_4 and the solvent removed under reduced pressure to furnish the crude material which was further purified using silica gel chromatography to yield the pure product (0.48 g, 96 %):

Elemental analysis calcd. for $C_{40}H_{57}O_9N_5$: C, 63.89; H, 7.64; N, 9.31 %, fnd. C, 63.84; H, 7.53; N, 9.51 %;

ν_{max} (KBr)/cm^{-1}; 2975,1683, 1465, 1409, 1365, 1245, 1157, 1106, 1043, 910, 854, 775, 759, 738;

^{1}H NMR ($CDCl_3$) δ 1.38 (9H, s, CH_3), 1.39 (9H, s, CH_3), 1.41 (9H, s, CH_3), 3.30-3.60 (16H, m, CH_2), 3.95 (2H, d, J = 4.2 Hz, CH_2), 4.15 (1H, t, J = 7.0 Hz, CH), 4.29 (2H, d, J = 7.0 Hz, CH_2), 5.74 (1H, s, NH), 7.25 (2H, dd, J = 14.8 and 6.27 Hz, ArH), 7.32 (2H, t, J = 7.0 Hz, Ar-H), 7.52 (2H, d, J = 7.5 Hz, Ar-H), 7.68 (2H, d, J = 7.5 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 28.8 (9 x CH_3), 47.4 (CH), 49.9-51.7 (8 x CH_2), 60.7 (CH_2), 67.4 (CH_2), 80.9 (3 x C), 120.3 (2 x CH), 125.4 (2 x CH), 127.4 (2 x CH), 128.0 (2 x CH), 141.6 (C), 144.2 (C), 155.9 (2 x C), 156.5 (2 x C).



(99) Preparation of 10-(2-Phenoxycarbonylamino-acetyl)-1,4,7,10-tetraazacyclododecane-1,4,7-*tert*-butylcarbamate.[23]

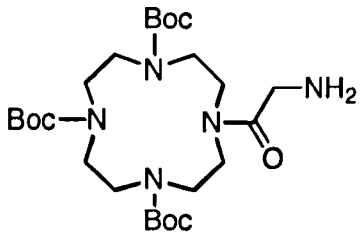
To a stirred solution of Cbz-gly (0.27 g, 1.3 mmol) in CH_2Cl_2 (2 mL) under an N_2 atmosphere was added drop-wise neat DCC (0.26 g, 1.3 mmol), followed by slow addition of 1,4,7,10-tetraazacyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester (0.61 g, 1.3 mmol) and DMAP (0.16 g, 1.3 mmol). The solution was left to stir overnight at room temperature, and then filtered to remove the urea side product. The solvent was removed under reduced pressure to give a crude material which was purified by silica gel column chromatography (5:1 ethyl acetate:petrol) to yield the pure product as a white solid (0.64 g, 75 %); mp 66-67 °C;

Elemental analysis calcd. for $C_{33}H_{53}N_5O_9$: C, 59.71; H, 8.05; N, 10.55%, fnd. C, 59.80; H, 8.24; N, 10.49%;

HRMS (EI$^+$), calcd. for $C_{33}H_{53}N_5O_9$ [M$^+$] m/z = 663.3843), fnd 663.3841;

ν_{max} (KBr)/cm^{-1}; 2973, 1685 (amide), 1465, 1409, 1363, 1245, 1157, 1106, 1043, 971, 856, 775;

^{1}H NMR ($CDCl_3$) δ 1.31-1.45 (27H, m, CH_3), 3.10-3.60 (16H, m, CH_2), 3.95 (2H, d, J = 4.4 Hz, CH_2), 5.05 (2H, s, CH_2), 5.65 (1H, s (broad), NH), 7.20-7.30 (5H, m, Ar-H).



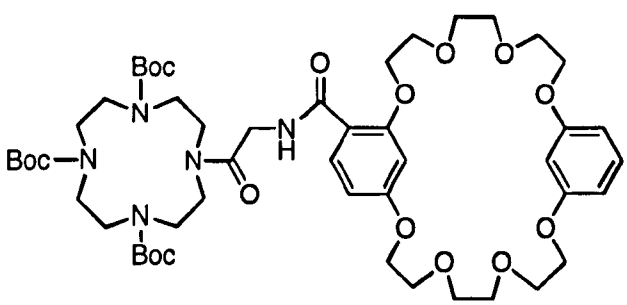
(100) Preparation of 10-(2-Amino-acetyl)-1,4,7,10 tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.[24]

To a stirred solution of **99** (0.34 g, 51 mmol) in absolute EtOH (5 mL) at room temperature, was slowly added 10% Pd/C (0.34 g, 1 equiv) followed by 1,4-cyclohexadiene (0.41 g, 5.1 mmol). The solution was stirred for 2 hrs and filtered through a celite pad and the solvents removed under reduced pressure to yield pure product as a white foam which required no further purification (0.25 g, 91 %): mp 96-97 °C;

HRMS (EI^+), calcd. for $C_{25}H_{47}N_5O_7$ [M^+] m/z = 529.3475, fnd. 529.3473;

ν_{max} (KBr)/cm^{-1}; 2975, 1683 (C=O), 1467, 1409, 1363, 1247, 1159, 1106, 970, 856, 777;

^{1}H NMR: ($CDCl_3$) δ 1.39-1.41 (27H, m, CH_3), 3.2-3.65 (18H, m, CH_2).



(101) Preparation of 10-{2-[(2,5,8,11,18,21,24,27-Octaoxa-tricyclo [26.3.1.0^{12,17}] dotriaconta-1(31),12(17),13,15,28(32),29-hexaene-29-carbonyl)-amino]-acetyl}-1,4,7,10 tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.

To a stirred solution of **90** (60 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) under N_2 atmosphere, was added drop-wise DCC (0.25 g, 0.13 mmol), followed by the addition of 10-(2-aminoacetyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester

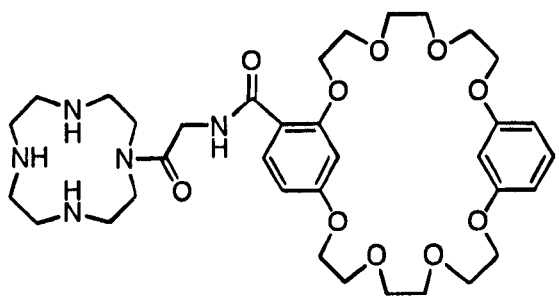
100 (0.064 g, 0.13 mmol) and DMAP (0.014 g, 0.13 mmol). The solution was left to stir overnight at room temperature and filtered to remove the urea side product. The solvent was removed under reduced pressure to give a crude material which was purified by silica gel column chromatography (5:1 ethyl acetetate:petroleum ether) to yield pure product as a white foam (0.74 g, 61 %):

HRMS (EI^+), calcd. for $C_{50}H_{78}N_5O_{16}$ $[MH]^+$ *m/z* =1004.5444, fnd. 1004.5446;

ν_{max} (KBr)/cm^{-1}; 2931, 1685, 1641, 1604, 1465, 1409, 1365, 1249, 1159, 1106, 973, 848, 775;

^{1}H NMR ($CDCl_3$) δ 1.40-1.60 (27H,m, CH_3), 3.40-3.65 (16H, m, CH_2), 3.70-3.75 (8H, m, CH_2), 3.81 (2H, t, *J* = 2.4 Hz, CH_2), 3.87 (4H, t, *J* = 5 Hz, CH_2), 4.03 (2H,t, *J* = 5 Hz, CH_2), 4.07 (2H, t, *J* = 5 Hz, CH_2), 4.10 (2H, t, *J* = 5 Hz, CH_2), 4.16 (2H, t, *J* = 5 Hz, CH_2), 4.21 (2H, t, *J* = 5 Hz, CH_2), 4.28 (2H, d, *J* = 5 Hz, CH_2), 6.47-6.58 (5H, m, Ar-H), 7.12 (1H, t, *J* = 8 Hz, Ar-H), 8.12 (1H, d, *J* = 8 Hz, Ar-H), 8.80 (1H, s(broad), NH);

^{13}C NMR ($CDCl_3$) δ 28.8 (9 x CH_3), 49.9-51.5 (m, 8 x CH_2), 67.5 (CH_2), 67.8 (CH_2), 67.9 (CH_2), 68.8 (CH_2), 69.4 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.1 (CH_2), 70.9 (CH_2), 71.2 (CH_2), 71.3 (CH_2), 71.4 (CH_2), 80.7 (C), 80.8 (C), 80.9 (C), 100.7 (CH), 102.4 (CH), 106.4 (CH), 107.1 (CH), 107.6 (CH), 114.8 (C), 130.1 (CH), 133.8 (CH), 156.0 (C), 157.1 (C), 158.9 (C), 160.3 (C), 162.9 (C), 165.2 (C).



(102) Preparation of 2,5,8,11,18,21,24,27-Octaoxa-tricyclo [26.3.1.0^{12,17}] dotriaconta-1(31),12(17), 13,15, 28(32),29 - hexaene-29-carboxylic acid [2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-amide (L_1).[25]

To a stirred solution of **101** (0.26 g, 0.25 mmol) in CH_2Cl_2 (3mL) at room temperature under an N_2 atmosphere was added dropwise TFA (1mL). The solution was stirred for 3 h, then quenched by addition of 3M NaOH (10 mL). The organic layer was washed with

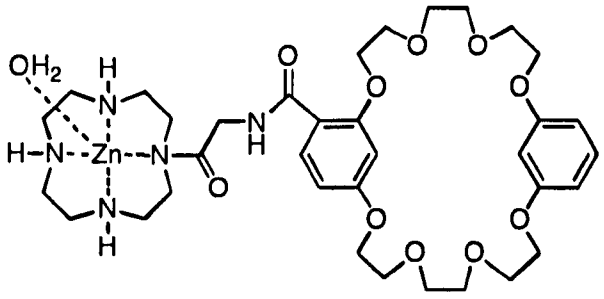
brine (3 x 20 mL), separated and dried over Na_2SO_4. Removal of the solvent afforded the pure product as a colourless-oil (0.13 g, 72 %):

HRMS (FAB^+), calcd. for $C_{35}H_{54}N_5O_{10}$ [MH^+]; *m/z* = 704.3871, fnd. 704.3866;

ν_{max} (KBr)/cm^{-1}; 3361, 2867, 1631 (C=O tertiary amide), 1600 (C=O) secondary amide), 1521, 1452, 1353, 1257, 1184, 1106, 1056, 941, 800, 767;

^{1}H NMR ($CDCl_3$) δ 2.55-2.90 (12H, m, CH_2), 3.45-3.48 (2H, m, CH_2), 3.52-3.55 (2H, m, CH_2), 3.61-3.69 (8H, m, CH_2), 3.72 (2H, t, *J* = 4.8 Hz, CH_2), 3.78 (4H, t, *J* = 5.2 Hz, CH_2), 3.95 (2H, t, *J* = 5.2 Hz, CH_2), 3.99-4.03 (4H, m, CH_2), 4.07 (2H, t, *J* = 4.4 Hz, CH_2), 4.13 (2H, t, *J* = 5.2 Hz, CH_2), 4.30 (2H, d, *J* = 4.0 Hz, CH_2), 6.39 (5H, m, Ar-H), 7.05 (1H, t, *J* = 8.0 Hz, Ar-H), 8.02 (1H, d, *J* = 8.4 Hz, Ar-H), 8.78 (1H, s, NH);

^{13}C NMR ($CDCl_3$) δ 43.1 (CH_2), 45.3 (CH_2), 46.8 (CH_2), 47.8 (CH_2), 48.5 (CH_2), 48.6 (CH_2), 49.0 (CH_2), 49.8 (CH_2), 67.5 (CH_2), 67.8 (CH_2), 67.87 (CH_2), 68.9 (CH_2), 69.4 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.1 (CH_2), 70.9 (CH_2), 71.1 (CH_2), 71.3(CH_2), 71.4 (CH_2), 100.6 (CH), 102.3 (CH), 106.3 (CH), 107.0 (CH), 107.7 (CH), 114.8 (C), 130.1 (CH), 133.9 (CH), 158.9 (C), 160.3 (2 x C), 162.9 (C), 165.2 (C), 170.6 (CO).



(103) Preparation of $[ZnL_1(OH_2)](CF_3SO_3)_2$

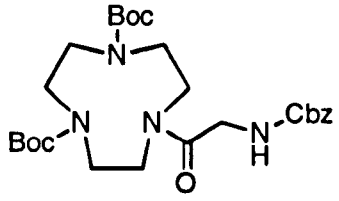
To a stirred solution of L_1 (0.06 g, 0.08 mmol) in MeOH (1 mL) at room temperature under an N_2 atmosphere was added zinc(II) triflate (0.034 g, 0.08 mmol). The mixture was stirred for 2 hrs and filtered through glass fibre paper. The solution was concentrated under reduced pressure to yield the product as a white solid (0.062 g, 78 %): mp 76-77 °C;

HRMS (FAB^+), calcd. for $[ZnL(CF_3SO_3)]^+$ *m/z* = 916.2557, fnd. 916.2492;

ν_{max} (KBr)/cm^{-1}; 3397, 2009, 1957, 1598 (C=O amide), 1452, 1222, 1166, 1025, 831, 761;

^{1}H NMR (MeOD) δ 2.76-3.25 (16H, m, CH_2), 3.60-3.71 (12H, m, CH_2), 3.75 (2H, t, J = 4.6 Hz, CH_2), 3.86 (t, 2H, J = 4.9 Hz, CH_2), 3.94 (4H, t, J = 4.4 Hz, CH_2), 4.10 (2H, t, J = 4.3 Hz, CH_2), 4.16 (2H, t, J = 4.5 Hz,CH_2), 4.31 (2H, s, CH_2(Gly)), 6.31-6.41 (3H, m, Ar-H), 6.52-6.61 (2H, m, Ar-H), 6.98 (1H, t, J = 8.0 Hz, Ar-H), 7.86 (1H, d, J = 8.8 Hz, Ar-H);

^{13}C NMR (MeOH-d_4) δ 45.1 (CH_2), 45.3 (CH_2), 45.5 (CH_2), 46.8 (2 x CH_2), 47.8 (3 x CH_2), 68.8 (CH_2), 69.0 (CH_2), 69.4 (CH_2), 70.4 (CH_2), 70.6 (CH_2), 70.9 (CH_2), 71.2 (CH_2), 71.8 (CH_2), 72.1 (CH_2), 72.2 (2 x CH_2), 72.3 (CH_2), 102.1 (CH), 103.6 (CH), 108.0 (CH), 108.4 (CH), 108.5 (CH), 117.5 (C), 120.5 (C), 123.7 (C), 127.1 (C), 131.3 (CH), 134.7 (CH), 161.0 (C), 161.83 (C), 161.85 (C).



(104) Preparation of 4-(2-Benzyloxycarbonylamino-acetyl)-[1,4,7]triazecane-1,7-dicarboxylic acid di-*tert*-butyl ester.

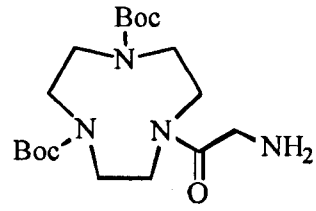
To a solution of Cbz-gly (0.13 g, 0.63 mmol) in CH_2Cl_2 (3 mL) were added sequentially DCC (0.14 g, 0.69 mmol), **68** (0.21 g, 0.63 mmol) in CH_2Cl_2 (3 mL) and DMAP (0.076 g, 0.63 mmol) under an N_2 atmosphere at room temperature, and stirred overnight. The reaction solution was filtered to remove excess urea and solvent removed under reduced pressure before purification by silica gel chromatography (5:1, ethyl acetate: petroleum ether) to give pure product as a white solid (0.26 g, 78 %): 85-87 °C;

HRMS (EI^+), calcd. for $C_{26}H_{40}N_4O_7$ [M^+] m/z =520.2896, fnd. 520.2897;

ν_{max} (KBr)/cm^{-1}; 3050, 2360, 2341, 1739, 1364, 1265, 1156, 1109, 901, 759;

^{1}H NMR ($CDCl_3$) δ 1.30-1.50 (18H, m, CH_3), 3.10-3.60 (12H, m, CH_2), 3.95 (2H, s, CH_2), 5.04 (2H, s, CH_2), 5.57-5.73 (1H, m, NH), 7.25 (5H, m, Ar-H);

^{13}C NMR ($CDCl_3$) (rotamers) δ 28.8 (6 x CH_3), 43.1 (CH_2), 43.3 (CH_2), 47.3 (CH_2), 47.6 (CH_2), 48.7 (CH_2), 49.0 (CH_2), 49.4 (CH_2), 49.8 (CH_2), 50.1 (CH_2), 50.4 (CH_2), 50.7 (CH_2), 52.2 (CH_2), 52.9 (CH_2), 67.1 (CH_2), 80.5 (C), 80.7 (C), 80.8 (C), 128.3 (2xCH), 128.4 (2 x CH), 128.8 (CH), 155.7 (C), 156.1 (C), 156.4 (2 x C).



(105) Preparation of 4-(2-Amino-acetyl)-[1,4,7]triazecane-1,7-dicarboxylic acid di-*tert*-butyl ester.

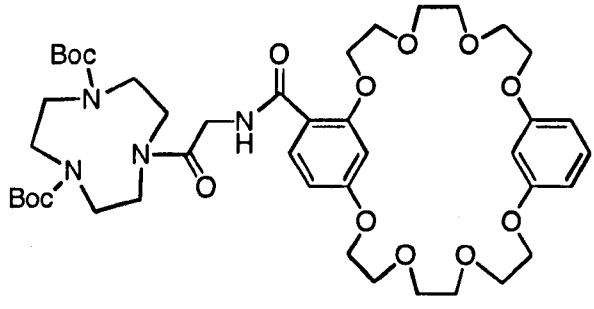
To solution of **104** (0.25 g, 0.49 mmol) in MeOH (3 mL) was added slowly Pd/C 10 % (0.20 g) under an N_2 atmosphere. The vessel was then evacuated and filled with H_2 and left to stir overnight. After this period, the H_2 was expelled and the solution filtered through a celite pad and the solvent removed under reduced pressure to yield pure product as a colourless oil (0.14 g, 73 %): mp 93-94 °C;

HRMS (FAB^+), calcd. for $C_{18}H_{34}N_4O_5$ [M^+] *m/z* =386.2529, fnd. 386.2534;

ν_{max} (KBr)/cm^{-1}; 3366, 2924, 2855, 2363, 2340, 1693, 1515, 1461, 1409, 1364, 1320, 1246, 1032, 987, 821;

^{1}H NMR ($CDCl_3$) δ 1.30-1.50 (18H, m, CH_3), 1.59 (2H, s, NH_2), 3.10-3.60 (14H, m, CH_2);

^{13}C NMR Due to different rotamers, it was not possible to interpret the spectrum.



(106) Preparation of 4-{2-[(2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.3.1.1^{12,16}] dotriaconta-1(30),12,14,16(32),27(31),28-hexaene-13-carbonyl)-amino]-acetyl}-[1,4,7]triazecane-1,7-dicarboxylic acid di-*tert*-butyl ester.

To a solution of **90** (0.07 g, 0.15 mmol) in CH_2Cl_2 (2 mL) was added dropwise DCC (0.03 g, 0.15 mmol) followed by the addition of **105** (0.060 g, 0.15 mmol) in CH_2Cl_2 (2 mL) and DMAP (0.02 g, 0.30 mmol) at room temperature under an N_2 atmosphere. The reaction solution was stirred for 24 hrs before being filtered through glass fibre paper under suction to remove the urea side product. The reaction solution was then washed with 1M NaOH

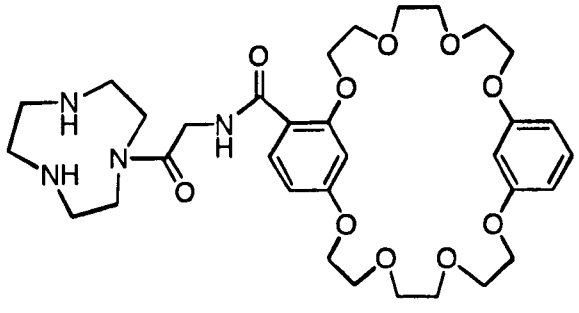
(20 mL) and brine (50 mL) before the solvent was removed under reduced pressure and purified by silica gel column chromatography (10:1, ethyl acetate:methanol) to yield pure product **106** as a colourless oil (0.080 g, 61 %):

HRMS (FAB^+), calcd. for $C_{43}H_{64}N_4O_{14}Na$ [M+Na] *m/z* =883.4333, fnd. 883.4317;

ν_{max} (KBr)/cm^{-1}; 3753, 2935, 1711, 1651, 1535, 1461, 1365, 1226, 1087, 821, 653, 543;

^{1}H NMR ($CDCl_3$) δ 1.30-1.50 (18H, m, CH_3), 3.10-3.57 (12H, m, CH_2), 3.61 (2H, d, *J* = 5.6 Hz, CH_2), 3.66 (6H, s (broad), CH_2), 3.72 (2H, t, *J* = 4.0 Hz, CH_2), 3.77 (4H, dd, *J* = 4.0 and 2.4 Hz, CH_2), 3.96-4.04 (6H, m, CH_2), 4.07 (2H, t, *J* = 5.0 Hz, CH_2), 4.13 (2H, t, *J* = 4.8 Hz, CH_2), 4.19 (2H, s(broad), CH_2), 6.39-6.50 (5H, m, Ar-H), 7.04 (1H,t, *J* = 8.0 Hz, Ar-H), 8.02 (1H, t, *J* = 6.8 Hz, Ar-H), 8.68-8.82 (1H, m, NH);

^{13}C NMR ($CDCl_3$) δ 28.4 (6xCH_3), 42.4-51.1 (12xCH_2 isomers of TACN), 67.2 (CH_2), 67.5 (CH_2), 68.5 (CH_2), 68.6 (CH_2), 69.1 (CH_2), 69.52 (CH_2), 69.58 (CH_2), 69.6 (CH_2), 69.7 (CH_2), 70.4 (CH_2), 70.6 (CH_2), 70.8 (CH_2), 71.0 (CH_2), 80.0 (C), 80.3 (C), 100.3 (CH), 102.0 (CH), 106.0 (CH), 106.8 (CH), 107.3 (CH), 129.7 (2xCH), 133.5 (CH), 155.5 (C), 155.7 (C), 158.5 (C), 160.0 (2 x C), 162.5 (C), 164.8 (C), 169.4 (C).



(107) Preparation of 2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.3.1.1^{12,16}]dotriaconta-1(30),12,14,16(32),27(31),28-hexaene-13-carboxylic acid (2-oxo-2-[1,4,7]triazecan-4-yl-ethyl)-amide (L_2).

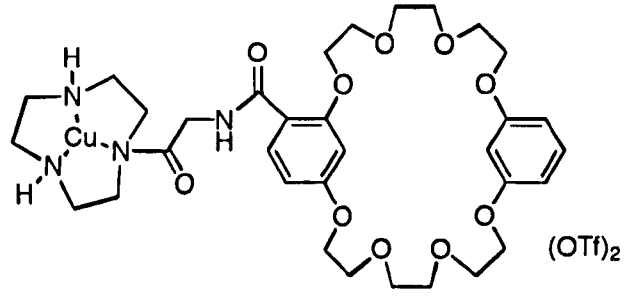
To a solution of **106** (0.059 g, mmol), in CH_2Cl_2 (2 mL) under N_2 at room temperature was slowly added TFA (1 mL) and the solution stirred for 4 hrs. The reaction was quenched with 3M NaOH (10 mL) and the organics extracted with CH_2Cl_2 (3 x 10 mL) and further washed with brine (30 mL) before being dried over $NaSO_4$. The solvent was removed under reduced pressure to yield pure product as white foam (0.033g, 75 %): mp 76-77 °C;

HRMS (FAB^+), calcd. for $C_{33}H_{49}N_4O_{10}$ [MH^+] *m/z* = 661.3449, fnd. 661.3455;

ν_{max} (KBr)/cm^{-1}; 3741, 2927, 1708, 1646, 1538, 1457, 1365, 1226, 1087, 821;

^{1}H NMR ($CDCl_3$) δ 2.10 (2H, s, NH), 2.65 (4H, m, CH_2), 3.01 (4H, m, CH_2), 3.36 (2H, t, *J* = 4.8 Hz, CH_2), 3.46 (2H, t, *J* = 4.8 Hz, CH_2), 3.62-3.69 (8H, m, CH_2), 3.72 (2H, t, *J* = 4.8 Hz, CH_2), 3.78 (4H, t, *J* = 4.4 Hz, CH_2), 3.96- 4.06 (6H, m, CH_2), 4.07 (2H, dd, *J* = 8.8 and 4.4 Hz, CH_2), 4.14 (2H, t, *J* = 4.5 Hz, CH_2), 4.22 (2H, d, *J* = 4.0 Hz, CH_2) 6.39-6.50 (5H, m, Ar-H), 7.04 (1H, t, *J* = 8.0 Hz, Ar-H), 8.05 (1H, d, *J* = 8.4 Hz, Ar-H), 8.84 (1H, s (broad), NH);

^{13}C NMR ($CDCl_3$) δ 42.7 (CH_2), 47.1 (CH_2), 48.0 (CH_2), 49.6 (CH_2), 49.7 (CH_2), 53.0 (CH_2), 53.6 (CH_2), 67.2 (CH_2), 67.5 (CH_2), 67.57 (CH_2), 68.6 (CH_2), 69.1 (CH_2), 69.54 (CH_2), 69.59 (CH_2), 69.7 (CH_2), 70.6 (CH_2), 70.8 (CH_2), 71.0 (CH_2), 71.1 (CH_2), 100.3 (CH), 102.0 (CH), 106.0 (CH), 106.7 (CH), 107.3 (CH), 114.5 (C), 129.7 (CH), 133.5 (CH), 158.7 (C), 160.0 (2 x C), 162.6 (C), 164.9 (C), 169.2 (C).



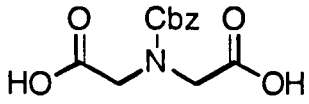
(108) Preparation of $[Cu(L_2)(OH_2)_2](CF_3SO_3)_2$.

To a solution of $Cu(CF_3SO_3)_2$ (0.03 g, 0.83 mmol) in MeOH (1.0 mL) was added **L_2** (0.05 g, 0.75 mmol) in MeOH (0.5 mL) at room temperature under an N_2 atmosphere. The reaction solution was stirred for 1hr before an insoluble blue solid precipitated out of solution. The solid was filtered off and washed with MeOH before being slowly recrystallized from nitromethane over 24 hrs to form pure product (0.06 g, 92 %): mp 110-111 °C;

Elemental analysis calcd. for $C_{35}H_{48}N_4O_{16}F_6S_2Cu$: C, 41.11; H, 4.73 N, 5.48 %, fnd. C, 40.95; H, 4.81; N, 5.41 %;

HRMS (FAB$^+$), calcd. for $C_{33}H_{48}N_4O_{10}Cu$ [M$^+$] *m/z* =723.2666, fnd. 723.2621.

ν_{max} (KBr)/cm^{-1}; 3569 (OH stretch), 3476 (N-H stretch), 2927, 1654, 1604, 1492, 1458, 1260, 1162, 1124, 1031, 639, 575;



(109) Preparation of [(2-Hydroxy-acetyl)-phenoxycarbonyl-amino]-acetic acid.[26]

To a solution of aminodiacetic acid (0.30 g, 2.2 mmol) in 2M NaOH (10 mL) was added dropwise benzyl chloroformate (0.35 mL, 2.4 mmol) and a further 2M NaOH (5 mL) at 5 °C. The mixture was stirred at room temperature for 2 hrs, washed with diethyl ether (2 x 10 mL), acidified to pH 2 with 1 M HCl and extracted with ether (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent removed under reduced pressure to yield pure product as a colourless oil (0.42 g, 70 %):

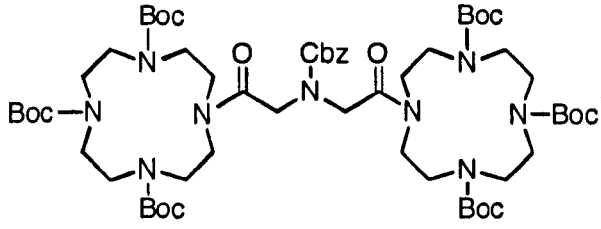
Elemental analysis calcd. for $C_{12}H_{13}N_5O_6$: C, 53.93; H, 4.87; N, 5.24 %, fnd. C, 53.78; H, 4.90; N, 5.27 %;

HRMS (Cl^+), calcd. for $C_{12}H_{14}N_3O_6$ [MH^+] *m/z* =268.0821, fnd. 268.0820;

ν_{max} (KBr)/cm^{-1}; 3570, 2565, 1687, 1474, 1406, 1303, 1194 1081;

^{1}H NMR ($CDCl_3$) δ 4.04 (2H, s, CH_2), 4.10 (2H, s, CH_2), 5.08 (2H, s, CH_2-Ar), 7.18-7.32 (5H, m, Ar-H);

^{13}C NMR ($CDCl_3$) δ 50.2 (CH_2), 50.4 (CH_2), 69.0 (CH_2), 128.3 (2xCH), 128.8 (CH), 129.0 (2xCH), 135.9 (C), 156.5 (C), 174.1 (C), 174.8 (C);



(110) Preparation of 110.

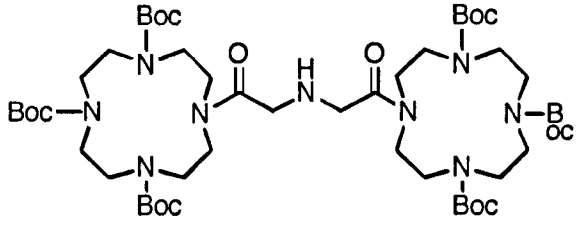
To a solution of **109** (0.20 g, 0.70 mmol) in CH_2Cl_2 (5 mL) was added dropwise DCC (0.32 g, 1.5 mmol) followed by **69** (0.92 g, 1.9 mmol) in CH_2Cl_2 (10 mL) and DMAP (0.19 g, 1.5 mmol) at room temperature under an N_2 atmosphere. The reaction solution was stirred for 24 hrs before being filtered through glass fibre paper under suction to remove the urea side product. The solvent was removed under reduced pressure and purified by silica gel column chromatography (5:1, ethyl acetate:petrol) to yield pure product as a white solid (1.85 g, 82 %): mp 97-98 °C;

Elemental analysis calcd. for $C_{58}H_{97}N_9O_{16}$; C, 59.21; H, 8.31; N, 10.72 %, fnd. C, 59.27; H, 8.41; N, 10.60 %;

HRMS (FAB^+), calcd. for $C_{58}H_{98}N_9O_{16}$ $[MH]^+$ *m/z* =1175.7053, fnd. 1175.7050;

ν_{max} (KBr)/cm^{-1}; 3054, 2986, 2305, 1686, 1421, 1265, 1160, 896, 746, 705, 585, 429;

^{1}H NMR ($CDCl_3$) δ 1.30-1.50 (54H, m, CH_3), 3.10-3.60 (32H, m, CH_2), 4.31 (4H, s, CH_2), 5.07 (2H, s, CH_2), 7.20-7.30 (5H, m, Ar-H).



(111) Preparation of 111.

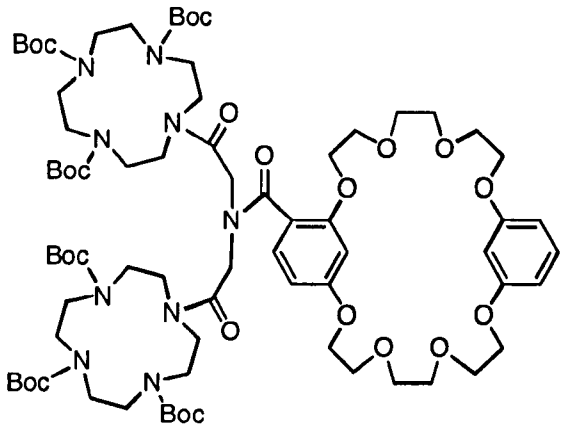
To solution of **110** (0.34 g, 0.28 mmol) in MeOH (4 mL) was added slowly Pd/C 10 % (0.34 g) under an N_2 atmosphere at room temperature. The vessel was evacuated and filled with H_2 and left to stir overnight. After this period, the H_2 was expelled and the solution filtered through a celite pad and the solvent removed under reduced pressure to yield pure product as a colourless oil (0.24 g, 79 %):

Elemental analysis calcd. for $C_{50}H_{91}N_9O_{14}$: C, 57.62; H, 8.80; N, 12.09 %, fnd. C, 57.15; H, 8.75; N, 11.84 %;

ν_{max} (KBr)/cm^{-1}; 3054, 2985, 1690, 1466, 1420, 1367, 1265, 1162, 896, 748, 705, 584, 435;

^{1}H NMR ($CDCl_3$) δ 1.41 (54H, s, CH_3), 3.10-4.00 (37H, m, CH_2);

^{13}C NMR ($CDCl_3$) δ 28.45 (6 x CH_3), 28.48 (6 x CH_3), 28.6 (6 x CH_3), 49.4-52.0 (broad multiplet, 34 x CH_2), 80.1 (2 x C), 80.2 (2 x C), 80.3 (2 x C), 155-158 (8 x C).



(112) Preparation of 112.

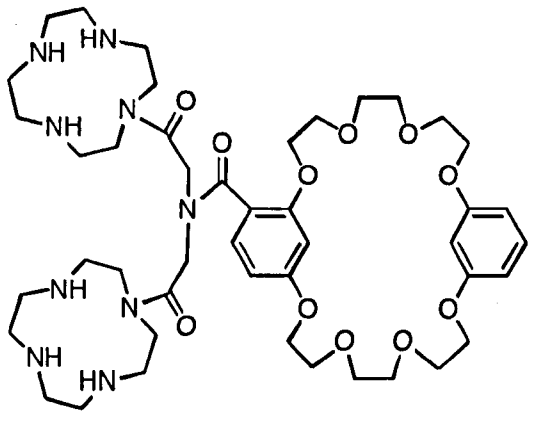
To a solution of **90** (0.06 g, 0.13 mmol) in CH_2Cl_2 (2 mL) was added dropwise DCC (0.30 g, 1.3 mmol) followed by the addition of **111** (0.14 g, 0.13 mmol) in CH_2Cl_2 (3 mL) and DMAP (0.16 g, 0.13 mmol) at room temperature under an N_2 atmosphere. The reaction solution was stirred for 24 h before being filtered through glass fibre paper under suction to remove the urea side product. The reaction solution was then washed with 1M NaOH (20 mL) and brine (50 mL) before the solvent was removed under reduced pressure and purified by silica gel column chromatography (10:1, ethyl acetate: methanol) to yield pure product as a white foam (0.09 g, 45 %): mp 72-73 °C;

HRMS (FAB^+), calcd. for $C_{75}H_{122}N_9O_{23}$ [MH^+] *m/z* =1516.8643, fnd. 1516.8654;

ν_{max} (KBr)/cm^{-1}; 2984, 1751, 1465, 1447, 1374, 1240, 1097, 1047, 938, 847, 634, 608, 443,434, 410, 405;

^{1}H NMR ($CDCl_3$) δ 1.35-1.50 (54H, m, CH_2), 2.95-3.55 (32H, m, CH_2), 3.58-3.65 (8H, m, CH_2), 3.71-3.81 (8H, m, CH_2), 3.98-4.07 (8H, m, CH_2), 4.19 (2H, s, CH_2), 4.51 (2H, s, CH_2), 6.39-6.44 (3H, m, Ar-H), 6.45 (1H, d, J = 2.0 Hz, Ar-H), 6.48 (1H, t, J = 2.4 Hz, Ar-H), 7.04 (1H, t, J = 8.4 Hz, Ar-H), 7.11 (1H, d, J = 8.4 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 28.3 (6 x CH_3), 28.4 (6 x CH_3), 28.5 (6 x CH_3), 48.5-50.11 (16 x CH_2), 51.2 (CH_2), 53.4 (CH_2), 60.3 (CH_2), 67.3 (CH_2), 67.5 (CH_2), 67. 6 (CH_2), 68.8 (CH_2), 69.2 (CH_2), 69.5 (CH_2), 69.6 (CH_2), 69.7 (CH_2), 70.73 (CH_2), 70.76 (CH_2), 70.9 (CH_2), 80.2 (2 x C), 80.3 (2 x C), 80.4 (2 x C), 101.6 (CH), 102.1 (2 x CH), 107.0 (CH), 107.2 (2 x CH), 118.47 (C), 129.7 (CH), 155.4 (C), 155.7 (C), 156.3 (2 x C), 156.7 (C), 157.1 (C), 159.9 (2 x C), 160.0 (2 x C), 161.0 (2 x C), 169.7 (C).



(113) Preparation of 2,5,8,11,17,20,23,26-Octaoxa-tricyclo[25.3.1.1^{12,16}] dotriaconta-1(30),12,14,16(32),27(31),28-hexaene-13-carboxylic acid ({bis-[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-carbamoyl}-methyl)-amide (L_3).

To a stirred solution of **112** (0.05 g, 0.038 mmol) in CH_2Cl_2 (2 mL) under N_2 at room temperature was slowly added TFA (1 mL) and the solution stirred for 4 hrs. The reaction was quenched with 3M NaOH (10 mL) and the organics extracted with CH_2Cl_2 (3 x 10 mL) and further washed with brine (30 mL) before being dried over Na_2SO_4. The solvent was removed under reduced pressure to yield pure product as white foam (0.025g, 75 %): mp 83-84 °C;

Elemental analysis calcd. for $C_{45}H_{73}N_9O_{11}$: C, 59.00; H, 8.03; N, 13.76 %, fnd. C, 58.79; H, 8.25; N, 13.67%;

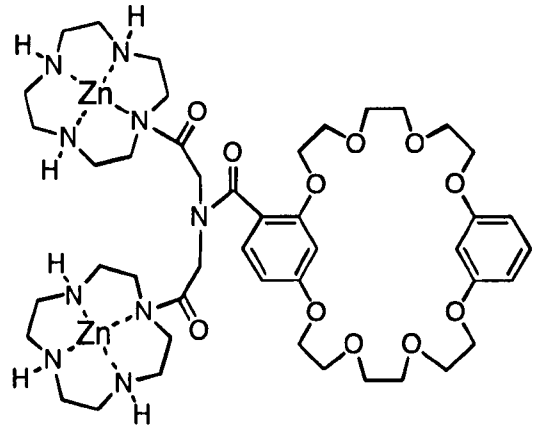
HRMS (FAB^+), calcd. for $C_{45}H_{74}N_9O_{11}$ [MH^+] *m/z* =916.5463, fnd. 916.5457;

ν_{max} (KBr)/cm^{-1}; 3741, 2927, 1974, 1646, 1542, 1461, 1361, 114, 817;

^{1}H NMR ($CDCl_3$) δ 2.40-3.54 (38H, m, NHs and CH_2), 3.58 (4H, s, CH_2), 3.62 (4H, s, CH_2), 3.71 (4H, t, *J* = 4.0 Hz, CH_2), 3.74 (4H, t, *J* = 4.8 Hz, CH_2), 3.99 (4H, t, *J* = 4.8 Hz, CH_2), 4.02 (4H, t, *J* = 4.8 Hz, CH_2), 4.22 (2H, d, *J* = 13.6 Hz, CH_2), 4.40 (1H, s (broad), CH_2), 4.46 (1H, s (broad), CH_2), 6.35-6.50 (5H, m, Ar-H), 7.04 (1H, t, *J* = 8.0 Hz, Ar-H), 7.12 (1H, d, *J* = 8.0 Hz, Ar-H);

^{13}C NMR ($CDCl_3$) δ 45.2 (CH_2), 45.4 (CH_2), 45.8 (CH_2), 46.4 (CH_2), 47.1 (CH_2), 47.2 (CH_2), 47.5 (CH_2), 47.7 (CH_2), 48.1 (2 x CH_2), 48.2 (CH_2), 48.3 (CH_2), 48.7 (CH_2), 48.7 (CH_2), 49.0 (CH_2), 49.4 (CH_2), 49.8 (CH_2), 51.4 (2 x CH_2), 67.3 (CH_2), 67.5 (CH_2), 67.59 (CH_2), 68.7 (CH_2), 69.3 (CH_2), 69.5 (CH_2), 69.7 (CH_2), 70.5 (CH_2), 70.8 (CH_2), 70.91 (CH_2), 70.92 (CH_2),101.2 (CH), 102.3 (CH), 106.6 (CH), 106.9 (CH), 107.21 (CH), 118.4

(C), 129.8 (CH), 129.88 (CH), 155.9 (C), 160.01 (C), 160.02 (C), 160.9 (C), 169.9 (C), 170.2 (C), 170.25 (C).



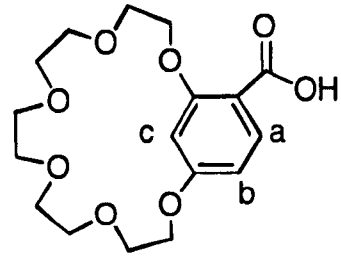
(114) Preparation of $[Zn_2L_3(OH_2)_2](CF_3SO_3)_4$.

To a stirred solution of L_3 (0.012 g, 0.011 mmol) in anhydrous MeOH (0.5 mL) under an Ar atmosphere was added zinc triflate (0.01 g, 0.024mmol). The reaction was stirred for 4 hrs at room temperature before the excess zinc triflate was filtered off through glass fibre paper and the solvent removed to yield pure product as a white foam (0.020 g, 91 % yield): mp 82-83 °C;

Elemental analysis calcd. for $C_{49}H_{73}F_{12}N_9O_{23}S_4Zn_2$: C, 35.82; H, 4.48; N, 7.67 %, fnd. C, 36.01; H, 4.59; N, 7.90 %;

HRMS (FAB^+), calcd. for $C_{49}H_{74}N_9O_{11}Zn_2$ $[M^+]$ *m/z* =1045.3981, fnd. 1045.3976;

ν_{max} (KBr)/cm^{-1}; 3752, 3397, 2927, 1977, 1716, 1691, 1658, 1495, 1365, 121, 830, 764.



(115) 2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carboxylic acid.

To a solution of **60** (0.17 g, 0.52 mmol) in an acetone/ water solution (3:1 ratio, 2.5 mL), was added slowly sulphamic acid (0.152 g, 1.56 mmol) followed by sodium chlorite (0.052 g, 0.57 mmol) at room temperature. The reaction solution was allowed to stir continuously

for 3 hrs before being quenched with the addition of CH_2Cl_2 (40 mL) and washed several times with brine (3 x 40 mL). The organic layer was then dried over $MgSO_4$ and the solvent removed under reduced pressure to yield impure compound that was purified by column chromatography (5:1, ethyl acetate: petroleum ether) to give pure product as a colourless oil (0.17 g, 91 %):

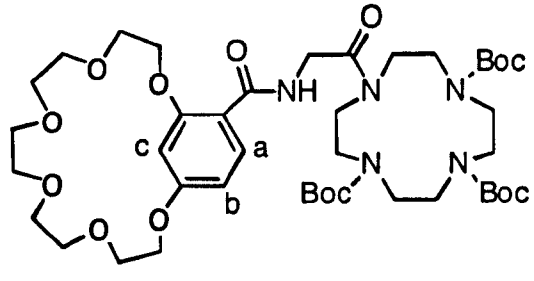
Elemental analysis calcd. for $C_{17}H_{24}O_8$: C, 57.30; H, 6.79 %, fnd. C, 57.40; H, 6.85 %;

HRMS (FAB^+), calcd. for $C_{17}H_{24}O_8$ [M^+] *m/z* = 356.1471, fnd. 356.1473;

ν_{max} (KBr)/cm^{-1}; 3531, 3312, 2920, 2359, 2094, 1697, 1606, 1541, 1502, 1461, 1294, 1236, 1184, 1144, 1105, 1091, 983, 887, 801;

^{1}H NMR ($CDCl_3$) δ 3.45-3.65 (12H, m, CH_2), 3.78 (2H, t, *J* = 4.4 Hz, CH_2), 3.84 (2H, t, *J* = 4.8 Hz, CH_2), 4.20 (2H, t, *J* = 4.4 Hz, CH_2), 4.45 (2H, t, *J* = 4.4 Hz, CH_2), 6.60 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 6.95 (1H, d, *J* = 2.0 Hz, Ar-H_c), 7.99 (1H, d, *J* = 8.8 Hz, Ar-H_a), 10.75 (1H, s (broad), COOH);

^{13}C NMR ($CDCl_3$) d 68.0 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.5 (2 x CH_2), 70.7 (CH_2), 70.8 (CH_2), 71.1 (CH_2), 71.2 (CH_2), 71.5 (CH_2), 102.0 (CH), 109.0 (CH), 110.9 (C), 134.6 (CH), 159.4 (C), 164.5 (C), 165.5 (COOH).



(116) Preparation of 10-{2-[(2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carbonyl)-amino]-acetyl}-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.

To a stirred solution of **115** (0.18 g, 0.50 mmol) in CH_2Cl_2 (20 mL) under an N_2 atmosphere at room temperature was added slowly DCC (0.11 g, 0.55 mmol) followed by the drop-wise addition of **100** (0.11 g, 0.50 mmol) in CH_2Cl_2 (10 mL) and DMAP (0.07 g, 0.55 mmol). The solution was left to stir overnight at room temperature before being filtered through glass fibre paper and the solvent removed under reduced pressure to give a

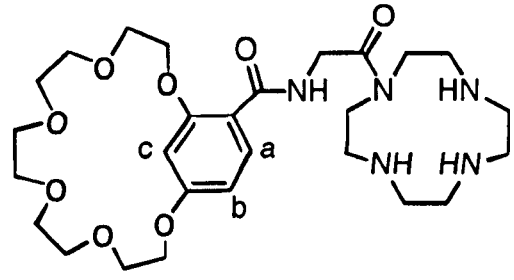
yellow oil which was purified using silica gel chromatography (5:1, ethyl acetate:petroleum ether) to furnish pure product as a white oil (0.31 g, 79 %): mp 95-97 °C;

HRMS (FAB^+), calcd. for $C_{42}H_{70}O_{14}N_5$ $[M^+]$ *m/z* = 868.4919, fnd. 868.4908;

ν_{max} (KBr)/cm^{-1}; 3361, 3256, 2971, 2109, 1684, 1643, 1601, 1463, 1365, 1243, 1159, 1109, 937, 855, 777;

^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, CH_3), 1.48 (9H, s, CH_3), 1.53 (9H, s, CH_3), 3.35-3.78 (28H, m, CH_2) 3.86 (2H, t, *J* = 4.4 Hz, CH_2), 3.96 (2H, t, *J* = 4.8 Hz, CH_2), 4.24 (2H, t, *J* = 4.4 Hz, CH_2), 4.28 (2H, d, *J* = 4.0 Hz, GLY-CH_2), 4.49 (2H, t, *J* = 4.4 Hz, CH_2), 6.60 (1H, dd, *J* = 8.8 and 2.4 Hz, Ar-H_b), 7.01 (1H, d, *J* = 2.0 Hz, Ar-H_c), 8.10 (1H, d, *J* = 8.8 Hz, Ar-H_a), 8.86 (1H, s (broad), NH);

^{13}C NMR ($CDCl_3$) δ 68.0 (CH_2), 69.9 (CH_2), 70.0 (CH_2), 70.5 (2 x CH_2), 70.7 (CH_2), 70.8 (CH_2), 71.1 (CH_2), 71.2 (CH_2), 71.5 (CH_2), 102.0 (CH), 109.0 (CH), 110.9 (C), 134.6 (CH), 159.4 (C), 164.5 (C), 165.5 (COOH).



(117) 2,5,8,11,14,17-Hexaoxa-bicyclo[16.3.1]docosa-1(21),18(22),19-triene-19-carboxylic acid[2-oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-amide(L_4).

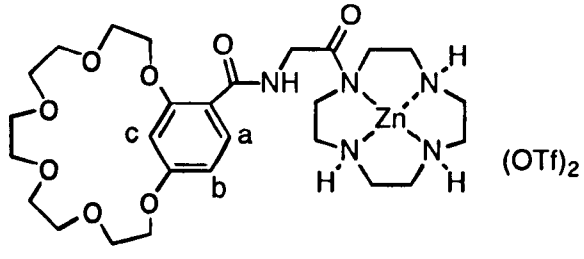
To a stirred solution of **116** (0.17 g, 0.22 mmol) in CH_2Cl_2 (20 mL) was added TFA (3 mL) under an N_2 atmosphere and was left to stir at room temperature for 4 hrs before the solution was quenched with 3M NaOH (20 mL) and washed several times with brine (3 x 30 mL). The organic layer was then dried over $NaSO_4$ and the solvent removed under reduced pressure to yield the pure compound as a colourless oil (0.095 g, 75 %):

HRMS (FAB^+), calcd. for $C_{27}H_{45}O_8N_5$ $[M^+]$ *m/z* = 567.3268, fnd. 567.3275;

ν_{max} (KBr)/cm^{-1}; 3381, 2923, 2347, 2245, 1633, 1604, 1531, 1469, 1355, 1294, 1256, 1184, 1121, 989, 911, 827;

^{1}H NMR ($CDCl_3$) δ 2.60 (t, 4H, J = 5.6 Hz, CH_2), 2.66 (t, 2H, J = 5.6 Hz, CH_2), 2.72 (t, 2H, J = 4.4 Hz, CH_2), 2.79 (t, 2H, J = 5.6 Hz, CH_2), 2.82 (t, 2H, J = 5.2 Hz, CH_2), 3.46 (t, 2H, J = 4.8 Hz, CH_2), 3.52-3.66 (m, 14H, CH_2), 3.75 (t, 2H, J = 4.4 Hz, CH_2), 3.86 (t, 2H, J = 4.8 Hz, CH_2), 4.13 (t, 2H, J = 4.4 Hz, CH_2), 4.28 (d, 2H, J = 4.0 Hz, CH_2), 4.39 (t, 2H, J = 4.8 Hz, CH_2), 6.50 (dd, 1H, J = 8.8 and 2.4 Hz, Ar-H_b), 6.92 (d, 1H, J = 2.4 Hz, Ar-H_c), 8.00 (d, 1H, J = 8.8 Hz, Ar-H_a), 8.82 (t, 1H, J = 4.0 Hz, NH);

^{13}C NMR ($CDCl_3$) δ 42.6 (CH_2), 44.9 (CH_2), 46.5 (CH_2), 47.4 (CH_2), 47.9 (CH_2), 48.1 (CH_2), 48.3 (CH_2), 49.1 (CH_2), 49.4 (CH_2), 67.9 (CH_2), 69.6 (CH_2), 69.8 (CH_2), 70.5 (2 x CH_2), 70.8 (3 x CH_2), 71.2 (CH_2), 71.3 (CH_2), 102.3 (CH), 107.6 (CH), 114.7 (C), 133.0 (CH), 159.2 (C), 162.7 (C), 165.0 (C=O), 170.3 (C=O).



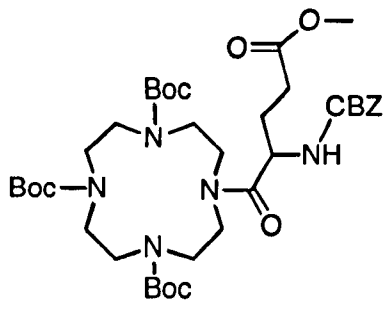
(118) $Zn(H_2O)L_5.(OTf)_2$.

To a stirred solution of **117** (0.05 g, 0.088 mmol) in anhydrous MeOH (1 mL) was added $Zn(OTf)_2$ (0.032 g, 0.088 mmol) under an N_2 atmosphere and was left to stir at room temperature for 4 hrs. The solution was filtered and the solvent removed under reduced pressure to yield the pure compound as a colourless oil (0.08g, 97 %);

HRMS (FAB^+), calcd. for $C_{27}H_{44}O_8Zn$ [M^+] m/z = 630.2481, fnd. 630.2448;

ν_{max} (KBr)/cm^{-1}; 3222, 2870, 2356, 1603 (C=O), 1545, 1456, 1361, 1242, 1152, 1025, 978, 844, 815, 716, 663 cm^{-1};

^{1}H NMR ($CDCl_3$) δ 2.60-3.30 (16H, m, CH_2), 3.45-3.53 (6H, m, CH_2), 3.57 (4H, t, J = 4.4 Hz, CH_2), 3.73 (2H, t, J = 4.4 Hz, CH_2), 3.82 (4H, t, J = 4.4 Hz, CH_2), 4.18 (2H, t, J = 4.4 Hz, CH_2), 4.29 (2H, s, CH_2), 4.41 (2H, t, J = 4.4 Hz, CH_2), 6.55 (dd, 1H, J = 8.8 and 2.4 Hz, Ar-H_b), 6.95 (d, 1H, J = 2.0 Hz, Ar-H_c), 7.87 (1H, d, J = 8.8 Hz, Ar-H_a).



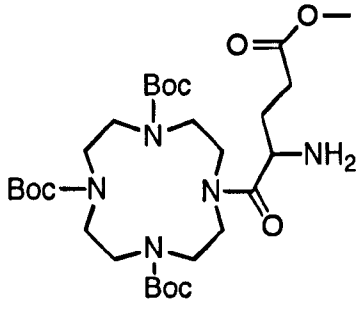
(119) 10-(2-Benzyloxycarbonylamino-4-methoxycarbonyl-butyryl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acid tri-*tert*-butyl ester.

To a solution of Cbz-GLU-OMe (0.50 g, 1.60 mmol) in CH_2Cl_2 (3 mL) were added sequentially DCC (0.37 g, 1.80 mmol), **69** (0.80 g, 1.60 mmol) in CH_2Cl_2 (3 mL) and DMAP (0.19 g, 1.60 mmol) under an N_2 atmosphere at room temperature, and stirred overnight. The reaction solution was filtered to remove excess urea and solvent removed under reduced pressure before purification by silica gel chromatography (5:1, ethyl acetate:petrol) to give pure product as a white solid (0.87 g, 72 %):

HRMS (EI^+), calcd. for $C_{37}H_{59}N_5O_{11}$ [M^+] *m/z* =749.4211, fnd. 749.4219;

ν_{max} (KBr)/cm^{-1}; 2357, 2253, 2107, 1674, 1645, 1519, 1470, 1402, 1366, 1240, 1137, 1053, 912, 846, 820, 777;

^{1}H NMR ($CDCl_3$) δ 1.36-1.39 (27H, m, CH_3), 1.90-2.40 (4H, m, CH_2), 3.20-3.50 (16H, m, CH_2), 3.67 (2H, s, CH_2), 4.25 (1H, dd, *J* = 12 and 8 Hz, CH), 5.01 (2H, d, *J* = 2.8 Hz, CH_2), 5.87 (1H, s (broad), NH), 7.21-7.32 (5H, m, Ar-H).



(120) Preparation of 10-(2-Amino-4-methoxycarbonyl-butyryl)-1,4,7,10tetraaza-cyclododecane-1,4,7-tricarboxylic acidtri-tert-butyl ester.

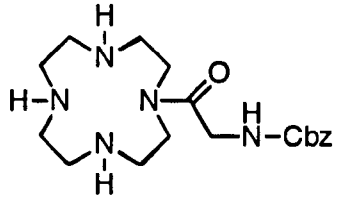
To solution of **119** (0.50 g, 0.66 mmol) in MeOH (5 mL) was added slowly Pd/C 10 % (0.25 g) under an N_2 atmosphere. The vessel was then evacuated and filled with H_2 and

left to stir overnight. After this period, the H_2 was expelled and the solution filtered through a celite pad. The solvent was removed under reduced pressure to yield pure product as a colourless oil (0.39 g, 96 %):

HRMS (FAB^+), calcd. for $C_{29}H_{53}N_5O_9$ $[M^+]$ *m/z* =615.3843, fnd. 615.3841;

ν_{max} (KBr)/cm^{-1}; 2950, 2147, 1492, 1250, 1217, 1003, 912, 837, 797, 774, 679, 654;

^{1}H NMR ($CDCl_3$) δ 1.35-1.45 (27H, m, CH_3), 2.21-2.50 (4H, m, CH_2), 3.10 (1H, dd, *J* = 8.4 and 5.2 Hz, CH_2), 3.25-3.54 (16H, m, CH_2), 6.65 (3H, s, CH_3).



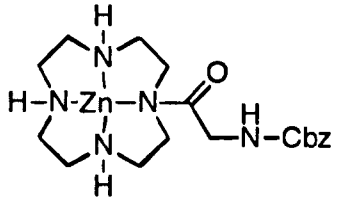
(121) Preparation of [2-Oxo-2-(1,4,7,10tetraaza-cyclododec-1-yl)-ethyl]-carbamic acid benzyl ester.

To a solution of **46** (0.14 g, 0.21 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added TFA (1 mL), allowed to warm up to room temperture and stirred overnight. To the reaction solution was added 3M NaOH (10 mL) and the product extracted with CH_2Cl_2 (3 x 10 mL), washed with brine (3 x 15 mL), dried over $NaSO_4$ and the solvent removed under reduced pressure to yield pure product as an amorphous white solid (0.07 g, 91 %):

HRMS (EI^+), calcd. for $[M]^+$ *m/z* = 363.2270, fnd. 363.2271;

ν_{max} (KBr)/cm^{-1}; 3370, 2361, 2106, 1667 (C=O amide), 1539, 1454, 1219, 1165, 1024, 843, 757, 697, 668;

^{1}H NMR ($CDCl_3$) δ 2.07 (3H, s (broad), NH), 2.58 (6H, s (broad), CH_2), 2.75 (6H, m, CH_2), 3.39 (2H, t, *J* = 6.8 Hz, CH_2), 3.94 (2H, t, *J* = 6.2 Hz, CH_2), 4.04 (2H, d, *J* = 4.0 Hz, CH_2), 5.02 (2H, s (broad), CH_2), 5.80 (1H, s (broad), NH), 7.17-7.26 (5H, m, Ar-H).



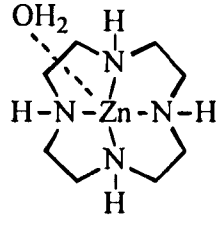
(122) Preparation of Zn(cyclen-gly-Cbz).$(OTf)_2$.

To a stirred solution of **121** (0.07 g, 0.191 mmol) in MeOH (1 mL) at room temperature under an N_2 atmosphere was added zinc(II) triflate (0.07 g, 0.19 mmol). The mixture was stirred for 2 hrs and filtered through glass fibre paper. The solution was concentrated under reduced pressure to yield the product as a white solid (0.13 g, 92 %):

Elemental analysis calcd. for $C_{20}H_{29}N_5O_9F_6S_2Zn$: C, 33.04; H, 4.02; N, 9.63 %, fnd. C, 33.14; H, 4.12; N, 9.71 %;

HRMS (EI^+), calcd. for $[M]^+$ *m/z* = 427.1562, fnd. 427.1568;

^{1}H NMR (D_2O) δ 2.71-3.10 (10H, m, CH_2), 3.60-3.71 (4H, d, *J* = 12.1 Hz, CH_2), 3.74 (2H, m, CH_2), 4.03 (2H, s, CH_2), 5.17 (2H, s (broad), CH_2), 7.30-7.40 (5H, m, Ar-H);



(123) Preparation of Zn(cyclen).$(OTf)_2$

To a stirred solution of cyclen (0.10 g, 0.58 mmol) in anhydrous MeOH (1 mL) under an N_2 atmosphere was added $Zn(OTf)_2$ (0.21 g, 0.58 mmol) and stirred for 1 hr until all the $Zn(OTf)_2$ had dissolved into solution. The solution was then filtered through glass fibre paper and the solvent removed under reduced pressure to give product which was a white foam (0.30 g, 96 %):

HRMS (FAB^+), calcd. for $[Zn(cyclen)(CF_3SO_3)]^+$ *m/z* = 385.0500, fnd. 385.0509;

ν_{max} (KBr)/cm^{-1}; 3270, 2887, 2095, 1450, 1222, 1158, 1089, 1024, 970, 866, 808, 761, 723, 686;

^{1}H NMR (MeOD) δ 2.66-2.73 (8H, m, CH_2), 2.79-2.86 (8H, m, CH_2).

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Appendix

Publications

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