Metal Ion Activated Anion Sensors

A Thesis Presented for the Degree of

Doctor of Philosophy

By

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Abstract

A series of new, octadentate, fluorescent, macrocyclic ligands have been prepared with a view to using them to study aromatic anion sequestration. The eight-coordinate Cd(II) complexes of the ligands have been shown capable of acting as receptors for a range of aromatic oxoanions. This has been demonstrated by perturbation of both ^{1}H NMR chemical shift values and the anthracene derived fluorescence emission intensity as the potential guest anion and the host are combined. Non-linear least squares regression analysis of the resulting titration curves leads to the determination of binding constants in 20% aqueous 1,4-dioxane which lie in the range $10^{2.3}$ M^{-1} (benzoate) to $10^{7.5}$ M^{-1} (2,6-dihydroxybenzoate). By reference to the X-ray determined structures of related, but non-fluorescent inclusion complexes, the primary anion retention force is known to arise from hydrogen bonding between the anion and four convergent hydroxy groups that exist at the base of a cavity that develops in the complexes as their aromatic groups juxtapose upon coordination. This work reveals significant stability enhancement when hydroxy groups are positioned on the anion at points where O-H...π hydrogen bonding to the aromatic rings that constitute the walls of the cavity becomes geometrically possible.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

A. J. Bradbury

2007

Acknowledgements

Firstly, I would like to thank my supervisor, Professor Kevin P. Wainwright for all the help and support he has given me throughout these studies, and most of all for his patience. I would also like to thank Professor S. F. Lincoln of the University of Adelaide for many of the same reasons. I would also like to thank Dr Martin Johnson for his help and also his wealth of information regarding the NMR. I would also like to thank Professor Sadegh Salehzadeh for his modeling work. My thanks also go out to everyone who I ever shared room 337 with, for all the discussions that we held about our work, and the help and support everyone has provided, so many thanks go out to Rebecca Campbell, Akhmad Damsyik, Professor Hassan Keypour, Hamid Khanmohammadi, Melissa Latter, Tim Robinson, Jozef Hodyl, Yakub, Professor Xu Xingyou, La Ode Kadidae and Rachel King. My family and friends I would like to thank for putting up with me these past few years, for both the times when I was there, and those times when I wasn't. Finally my love and thanks go out to my wife Barbara, without whom I would not have been able to survive this PhD, who has been so very patient and encouraging throughout the years.

Thank you.

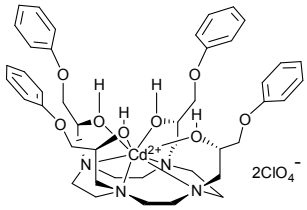
Abbreviations

AcOH	acetic acid
Å	Ångstrom (10^{-10} m)
antac-12	*N*-(2-(-9-anthracenylmethyl)aminoethyl) -1,4,7,10-tetraazacyclododecane
bp	boiling point
Bn	benzyl
Bu	butyl
CBn	benzyloxycarbonyl
cyclen	1,4,7,10-tetraazacyclododecane
δ	chemical shift
d	doublet
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
eT	Electron transfer
ET	Energy transfer
EtOH	ethanol
ε	molar extinction coefficient or molar absorptivity
ε’	molar fluorescence
I	ionic strength
IR	infra red
J	coupling constant
K	apparent stability constant
L	unspecified ligand
υ_{max}	maximum infrared absorbance (cm^{-1})
λ_{ex}	fluorescence excitation wavelength (nm)
λ_{max}	maximum wavelength
m	multiplet
M^{2+}	unspecified divalent metal ion
Me	methyl
MeCN	acetonitrile
MHz	megahertz (10^6 s^{-1})
mp	melting point
NMR	Nuclear magnetic resonance (spectroscopy)

PeT	Photoinduced electron transfer
pK_a	-log10[Ka]
q	quartet
RT	room temperature
s	singlet
SD	standard deviation
t	triplet
tert-	tertiary
TLC	Thin layer chromatography
UV-vis	Ultraviolet-visible (spectroscopy)

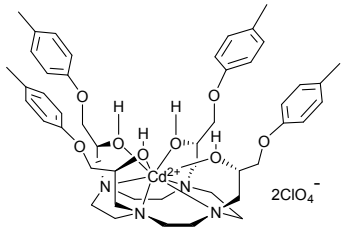
Summary of molecular receptors described in this thesis

First Generation Receptors



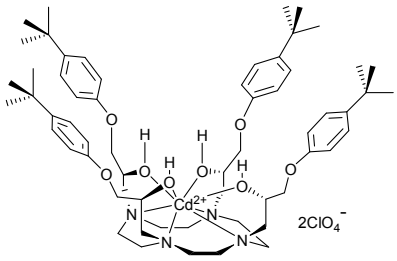
[Cd((*S*)-thphpc12)](ClO$_4$)$_2$

1



[Cd((*S*)-thmppc)](ClO$_4$)$_2$

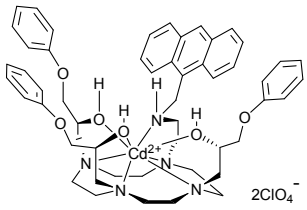
2



[Cd((*S*)-thbppc)](ClO$_4$)$_2$

3

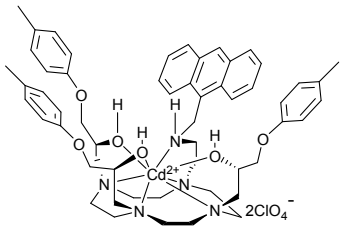
Second Generation Fluorescent Receptors



[Cd((*S*)-athppc)](ClO$_4$)$_2$

4

derived from **146**

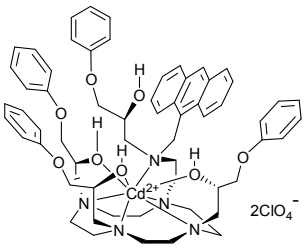


[Cd((*S*)-athmppc)](ClO$_4$)$_2$

5

derived from **170**

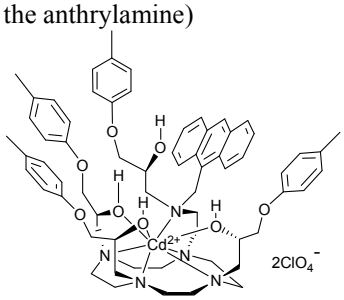
Third Generation Fluorescent Receptors (substituted at the anthrylamine)



[Cd((*S*)-apthppc)](ClO$_4$)$_2$

6

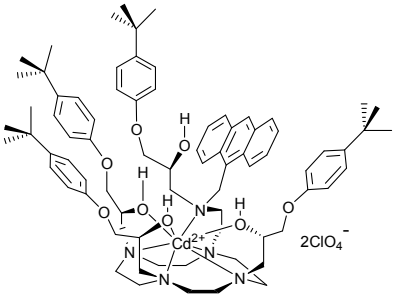
derived from **171**



[Cd((*S*)-amthmppc)](ClO$_4$)$_2$

7

derived from **172**



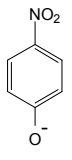
[Cd((*S*)-abthbppc)](ClO$_4$)$_2$

8

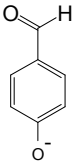
derived from **173**

Summary of guest anions utilised in this work

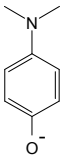
Phenolate Guests



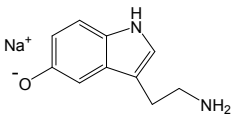
p-nitrophenolate (**9**)



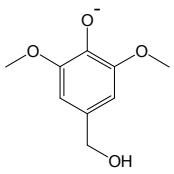
p-formylphenolate (**10**)



p-dimethylaminophenolate (**11**)

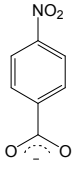


sodium salt of serotonin (**12**)

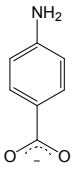


4-hydroxymethyl-2,6-methoxyphenolate (**13**)

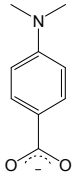
Benzoate Guests



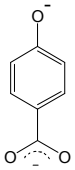
p-nitrobenzoate (**14**)



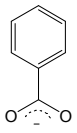
p-aminobenzoate (**15**)



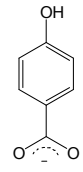
p-dimethylaminobenzoate (**16**)



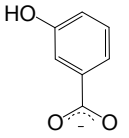
p-carboxyphenolate (**17**)



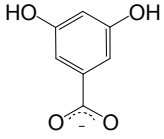
benzoate (**18**)



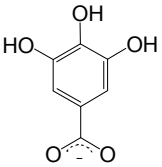
p-hydroxybenzoate (**19**)



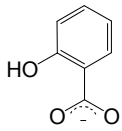
m-hydroxybenzoate (**20**)



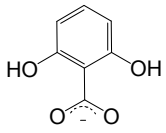
3,5-dihydroxybenzoate (**21**)



gallate (**22**)

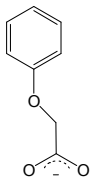


o-hydroxybenzoate (**23**)



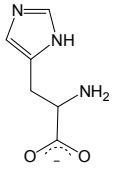
2,6-dihydroxybenzoate (**24**)

Acetate Guests

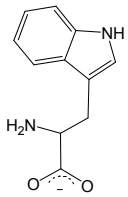


phenoxyacetate (**25**)

Amino Acid Guests

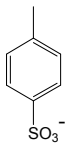


histidinate (*D*)- (**26**) (*L*)- (**27**)

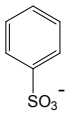


tryptophanate (*D*)- (**28**) (*L*)- (**29**)

Sulfonate Guests

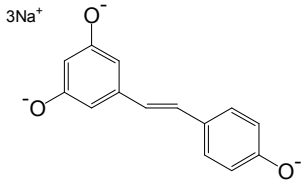


p-toluenesulfonate (**30**)

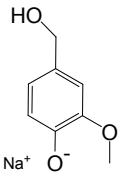


benzenesulfonate (**31**)

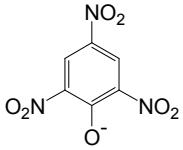
Other Potential Guests of Interest



sodium salt of resveratrol (**32**)



sodium salt of vanillyl alcohol (**33**)



picrate (**34**)

Chapter 1

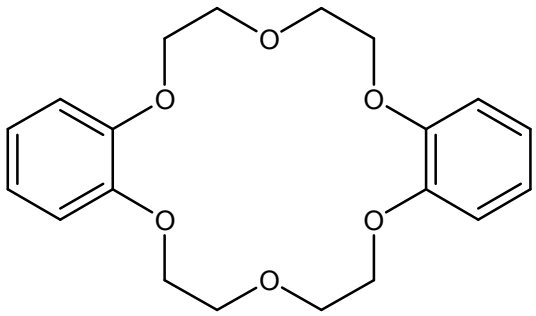
Introduction

1. Introduction

The non-covalent capture of anionic guest molecules by synthetic host molecules has remained relatively unexplored until recently, but has now become an increasingly topical subject.[1,2] When this is taken with the occurrence of molecular recognition being conveniently signalled by the emission of light from a specifically designed molecular device, it leads to the possibility of developing molecular sensors for quite specific classes of anionic guests. This is an exciting possibility since anions are ubiquitous species, and therefore their sensing is of considerable interest.[3] Anions are involved in numerous biological and chemical processes, including pollution by nitrates and phosphates that interrupt aquatic life-cycles,[3] or the plethora of enzyme substrates or co-factors that are anionic.[3]

1.1. Molecular recognition

Molecular recognition is a cornerstone of supramolecular chemistry. Given any substrate, it is almost certain that a receptor, possessing suitable structural and chemical features for substrate retention, can be designed.[4,5] Pedersen was the first to form stable, highly structured complexes of cyclic polyethers (crown ethers) which acted as receptors for cations (alkali and alkaline earth metals as well as ammonium complexes). The synthesis of 2,3,11,12-dibenzo-1,4,7,10,13,16-hexaoxacyclooctadeca-2,11-diene, **35**, and its complexation, created the first synthetic host-guest system.[6,7] Pedersen's compounds were salt-crown ether



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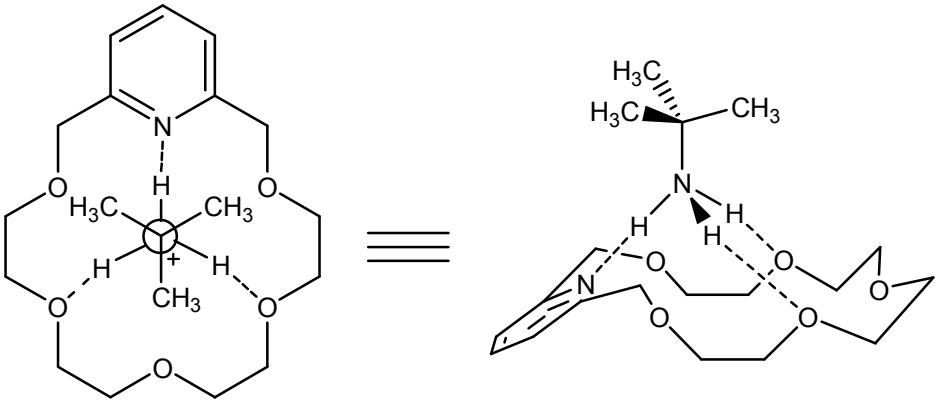
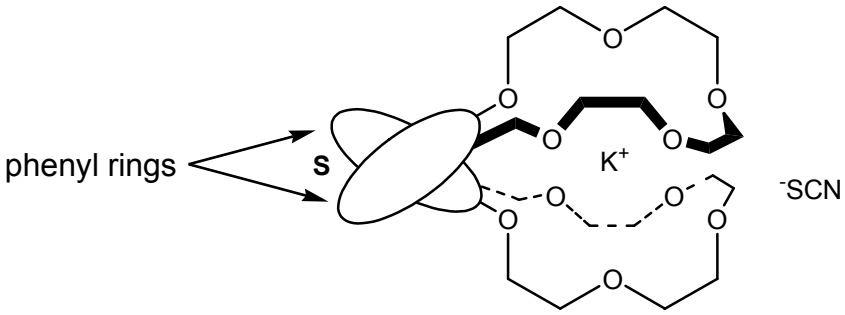
complexes held together by ion-dipole interactions between the cation and the slightly negatively charged oxygen atoms.[6] In its uncomplexed state Pedersen's 18-crown-6, **35**, contains neither a cavity nor convergent binding sites. The act of complexation is accompanied by host reorganisation to form the required cavity.[7]

Inspired by the highly selective molecular complexation between organic compounds in biological systems, Cram[7] used Pedersen's work as a jumping off point in the synthesis and study of highly structured organic molecular complexes, termed host-guest systems.[8] A general definition of host-guest complexes states that both host and guest interact within the complex through non-covalent interactions, and that the receptor and host binding sites are complementary to each other. In the host molecule the binding sites converge, and in the guest molecule the binding sites diverge.[8] The complexation of organic compounds requires high structural organization, which is produced through multiple binding sites.[8] Host-guest complexes are visualized as having three common shapes: (i) perching complexes, similar to icecream in a cone, **36**; (ii) nesting complexes, similar to eggs in a nest, **37**; or (iii) capsular complexes, like a nut in its shell, **38**.[7]



36



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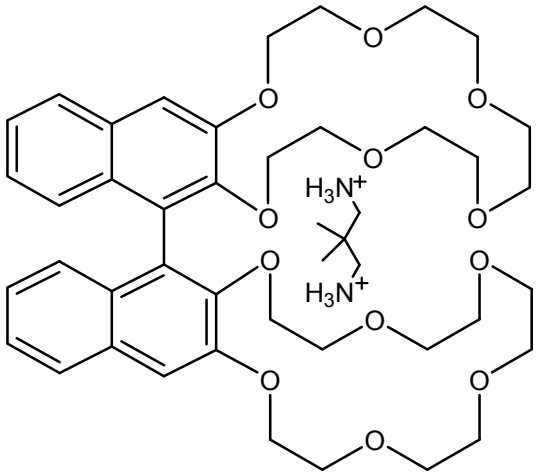
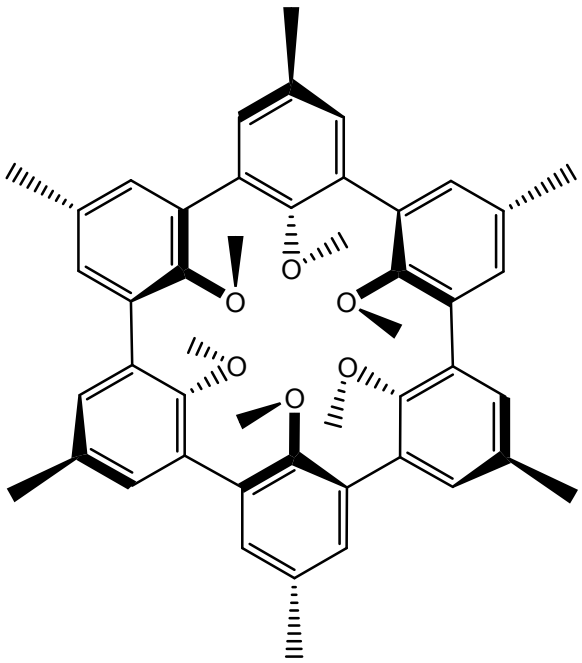


38

As an example of the nesting type, Cram and co-workers designed ligands that contain oxygen atoms octahedrally arranged around a fixed spherical cavity that is complementary to Li^+ and Na^+ ions, **39**. These completely preorganised ligands were named spherands.[7]



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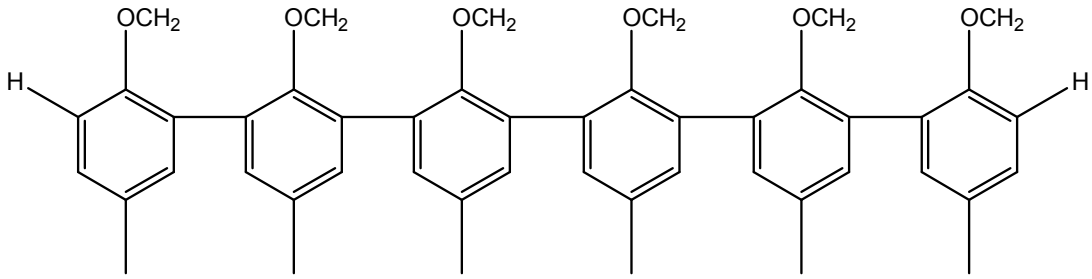
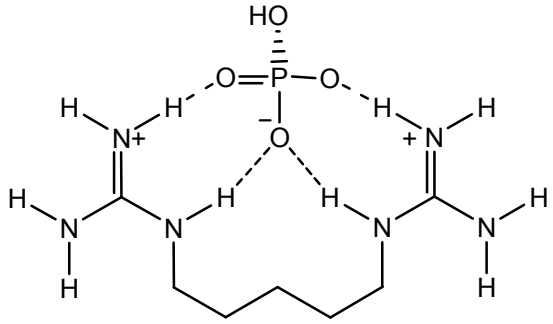
The corresponding acyclic compound is a podand, **40**. Unlike the spherand, which has a single conformation ideal for binding alkali metal ions, with unsolvated oxygens ready for binding, a podand can exist in a plethora of conformations, only two of which are able to bind metal ions octahedrally.[7] Podands are not preorganised for binding.



40

Acyclic receptors have also been designed for monohydrogenphosphate, **41**.[9] In this case the NH groups retain the anion by multiple hydrogen bonds.

Cyclic receptors have enhanced binding tendencies over non-cyclic receptors. The thermodynamic stability of a cyclic receptor-substrate complex is substantially higher than that of the complex of the corresponding non-cyclic receptor.



41

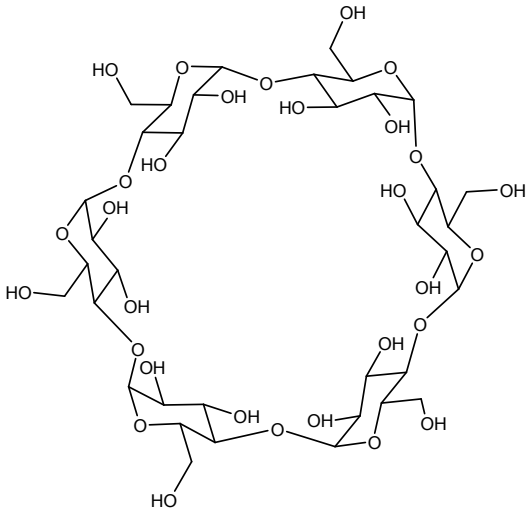
This thermodynamic macrocyclic effect[10] is due to the receptor already being preorganised for binding and thus, not having to use energy to wrap around the substrate.[11,12]

1.2. Cavity shaped receptors

1.2.1. Cyclodextrins.

Cyclodextrins, such as **42**, include various kinds of compounds within their cyclic hydrophobic cavities in water.[13-16] The binding force has been suggested as being either hydrophobic interaction, London dispersion force, hydrogen bonding, or a combination of these, depending on the substrate.[13] The three commonly available cyclodextrins (α- (**42**), β-, γ-cyclodextrins) possess cavities with different inner diameters (~5, ~7, ~9 Å respectively).[14]

Cyclodextrins can also act as reaction vessels, bringing small molecules together so that they can react.[16] Modified cyclodextrins have also been used in light harvesting antenna systems[17], as well as being connected by organic or organometallic linkers to include larger guests, such as the fluorescent dyes methyl orange and brilliant green.[18,19]



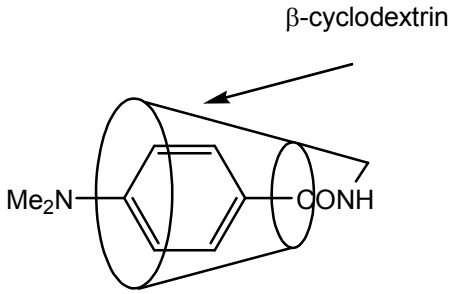
42

Fluorescent probes have been employed to examine the cyclodextrin host-guest complexes[14,20,21] and to determine the environmental polarity of a probe that is included in a cyclodextrin cavity, as environmental polarity effects the nature of fluorescence.[20,22]

Aromatic molecules complexed in cyclodextrins often have very different properties from those of the uncomplexed species. These include fluorescence enhancement, intramolecular excimer/exciplex formation, and lifetime shortening.[15,23] It has been shown that γ-cyclodextrins can form 1:2 host:guest complexes in aqueous solution,[13,23-25] which is in marked contrast to α- and β-cyclodextrins, both of which form only 1:1 host:guest complexes. When two guests are incorporated in the cyclodextrin cavity they must necessarily be held together in close proximity. Thus, the inclusion of two molecules of 2-anthracenesulfonate showed a decrease in fluorescence that was attributed to self-quenching in the intimate pair.[24] However the 1:2 complex of γ-cyclodextrin with pyrene showed that the excimer emission of pyrene was enhanced upon addition of γ-cyclodextrin.[13,26]

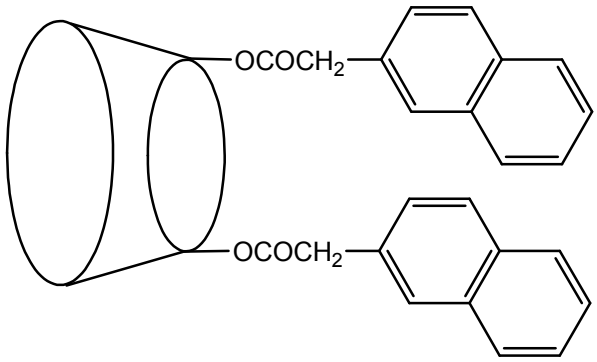
Various cyclodextrin derivatives have been prepared as receptors/sensors for organic guests in aqueous media, by appending fluorophores,[27-30] an example of

which is the addition of *p*-dimethylaminobenzonitrile to the smaller edge of a *β*-cyclodextrin *via* an amide linkage, **43**.[28]



43

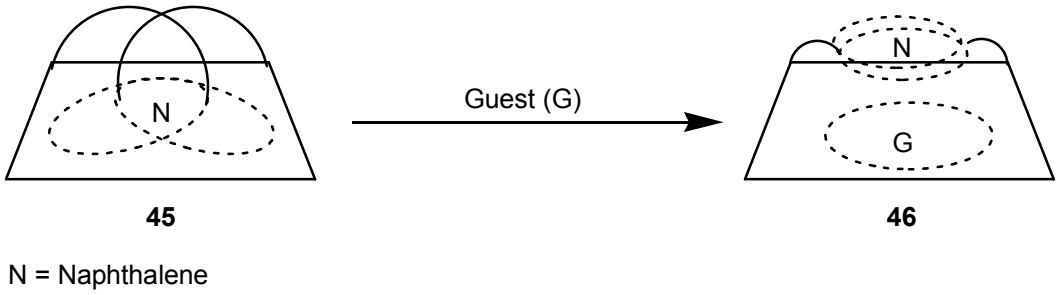
Ueno and co-workers prepared various γ-cyclodextrin derivatives with an appended covalently attached aromatic moiety, observing that the moiety acts as a spacer allowing γ-cyclodextrins to form 1:1 host-guest complexes by narrowing the large entrance of γ-cyclodextrin.[31,32] Ueno and co-workers prepared a γ-cyclodextrin bearing two 2-naphthyl moieties, **44**, and showed that upon guest binding the



44

orientation and location of the appended naphthyl moieties changed, **Figure 1.1**[25] The two naphthyl moieties act as flexible caps to the included guest molecule, contributing to the formation of the 1:1 complex.[25]

Various systems involving γ-cyclodextrin or its derivatives have since been formed in which two aromatic rings form excimers in the cavity. The naphthalene

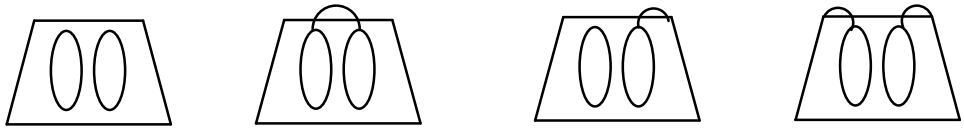


45 **46**

N = Naphthalene

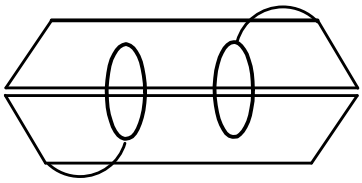
Figure 1.1. The inclusion of the guest molecule no longer allows the two naphthyl moieties to reside in the cavity. These naphthyl units now act as a cap, locking the guest inside the cavity.

moiety has been shown to form excimers in generalised systems such as **47**,[33] **48**,[34] **49**,[31] **50**.[35]



47 **48** **49** **50**

As well as 1:2 complexes, γ-cyclodextrin derivatives can form 2:2 complexes, **51**.[23]



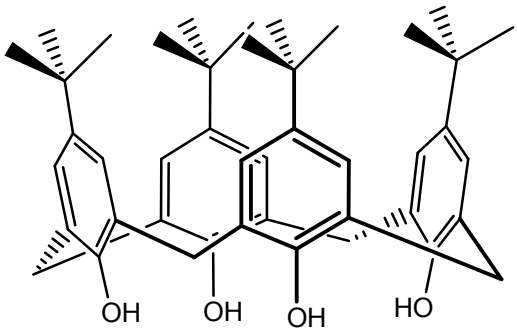
51

1.2.2. Calixarenes.

The calixarenes are a class of phenolic macrocycles consisting of four or more phenolic units bridged by methylene spacer groups.[1] As phenol-formaldehyde condensation products, calixarenes are similar in structure to certain crown ethers, which are noted for their size related selectivity in binding cations.[36-39] Calixarenes were first reported by Zinke and co-workers in 1952,[39-42] and have the ability to form host-guest complexes by trapping organic compounds, small ions, and gases in their

'torus like' cavities.[43] Other useful features are that the synthesis can be promoted by a template effect,[41] they have a well defined cavity, defined sizes, and conformations which are preorganised for molecular inclusion.[44] They can form complexes with small molecules, both in the solid-state and in solution, they are weak Brønsted-Lowry acids and can act as anionic carriers in the liquid membrane transport of alkali cations.[45] It has been shown that calixarenes generally do not bind alkali metal ions as strongly as do crown ethers or cryptands.[45,46] Because of their easy large-scale preparation and excellent capability of derivatisation, calixarenes have emerged as the third generation of supramolecules, behind cyclodextrins and crown ethers.[44] Calixarenes represent a synthetically malleable framework upon which charged, Lewis-acidic or hydrogen-bond donor or acceptor functionalities may be placed in order to design hosts of specific dimensions and selectivities.[1] As such calixarenes are useful as molecular receptors, sensors and even reaction vessels.[47]

Because calixarenes, such as **52**, are composed of phenolic units attached by



52

flexible linkages, they can change their conformations,[38] **Figure 1.2**.

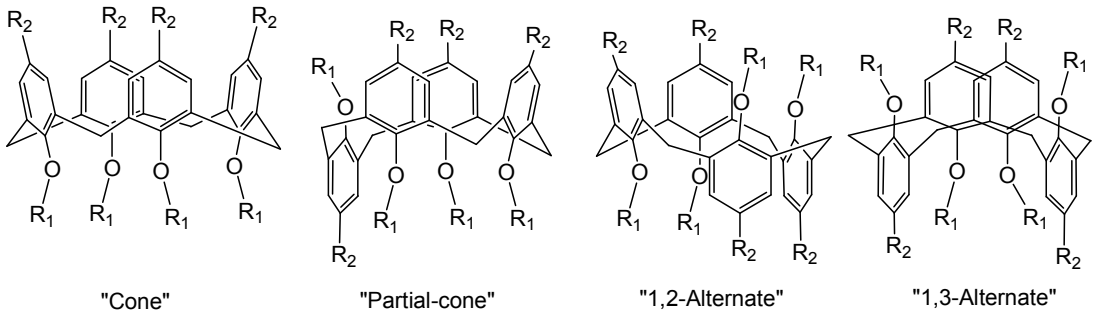


Figure 1.2. The different calixarene conformations.

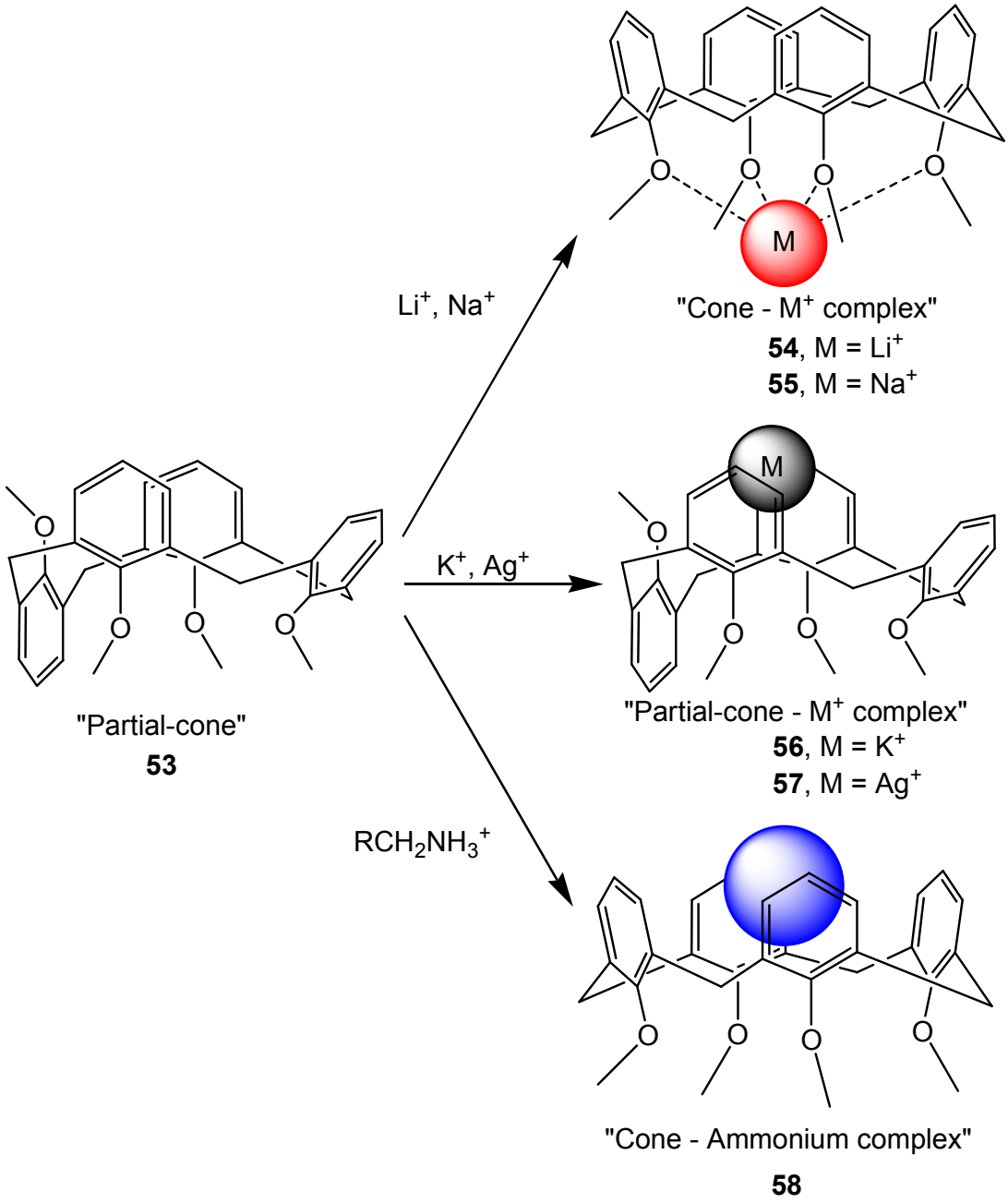


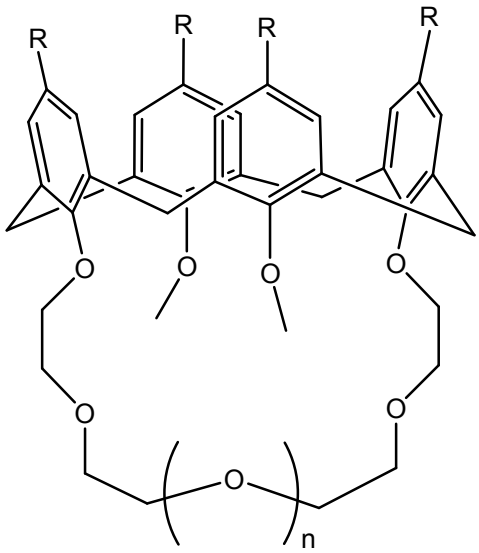
Figure 1.3. Calixarenes have an upper and lower rim, both of which may become involved in guest inclusion.

Calixarenes exist in four different conformations which are the cone, partial cone, 1,2-alternate and 1,3-alternate. From these conformations the most common is the cone conformation, which makes the molecule very suitable for utilisation as a molecular receptor as it provides a hydrophobic binding cavity.

Calixarenes and their derivatives are able to act as 'induced-fit' hosts,[48] as the cavity is flexible enough to undergo slight changes to mould itself around the guest

for a more effective fit. Calixarenes can act as hosts using either the cavity formed by the benzene rings, or by the ring of oxygen atoms at the lower rim.

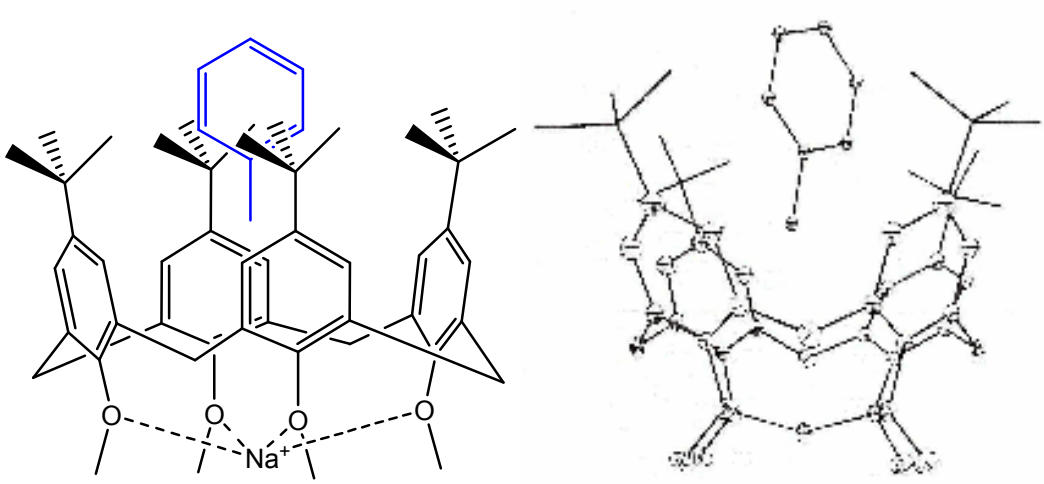
The structure of calixarenes can be arranged into the most suitable structure to complex guest molecules, by restricting ring flipping. This is done either by placing bulky groups on the rims, or by bridging the benzene rings inter or intramolecularly. Conveniently calixarenes are easily modified on either the upper or lower rim, e.g. by appending a crown ether, **59**.[38,48]



R = *tert*-Bu, n = 1

59

The first definitive inclusion of a small neutral molecule into a calixarene was observed by Andreetti.[49] In that work a toluene molecule was included in *p-tert*-butylcalix[4]arene, **52**. Later the crystal structure of [*p-tert*-butylmethoxycalix[4]arene] complexing a alkali metal ion, **60**,[46] showed that a molecule of the solvent had included within the cavity formed by the *t*-butyl cavity Thus, the ability of the calixarene to include neutral molecules is not lost by the complexation of metal ions at the lower rim.[46,50]



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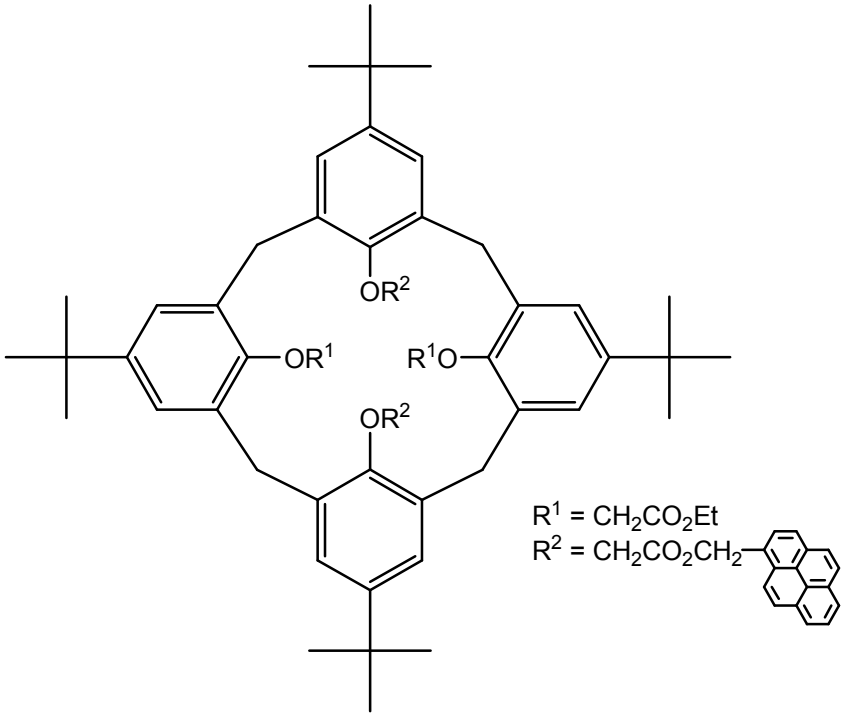
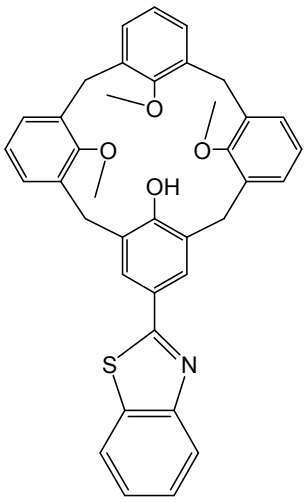
The fluorescent Na^+ sensor designed by Jin and co-workers,[51] **61**, exhibits a large shift in fluorescence on binding to Na^+. It has fluorescent pyrene moieties appended to its lower rim.



61

A sensor for Li^+, **62**, has been formed by incorporating a benzothiazole moiety as a modification to the upper ring of a calix[4]arene.[52] A related crown bis-

structure, **63**, was found to have pronounced selective fluoroionic behaviour towards Ca^{2+}.[52,53]



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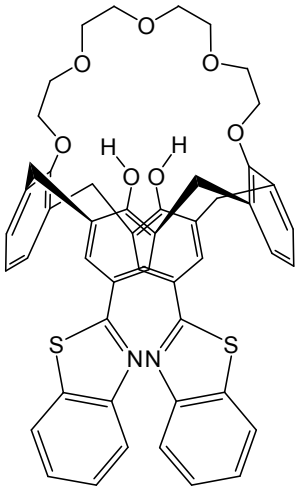


63

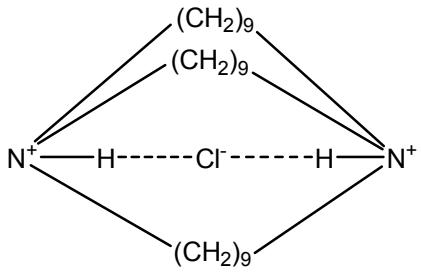
1.3. The importance of anion recognition

Most receptors mentioned so far have been for cations or neutral molecules, however anionic guests are becoming increasingly of interest. Many biological phenomena have been intensively investigated in order to gain a better understanding of the underlying chemistry happening within a living organism. In the course of this it has been noted that many biological chemical reactions involve substrate retention at the active site of an enzyme, or a similarly selective transport protein. Substrates can be neutral, cationic or, frequently, anionic molecules and the individual enzyme or protein that operates with each has a specific way of recognizing its own substrate.

The design and synthesis of molecular receptors that both recognise and retain anionic guest molecules is a challenge for the synthetic chemist. Generally speaking anions are more difficult to sequester than cationic or neutral molecules.[54] This is because, compared to isoelectronic cations, anions are relatively large. For

example Br^- has an ionic radius of 196 pm whilst that of Rb^+ is only 149 pm.[55] Multi-atom anions such as SO_4^{2-} (230 pm) and PO_4^{3-} (238 pm) are considerably larger. This means that an anion has a lower charge to radius ratio than an isoelectronic cation that will make its electrostatic interactions with a potential host weaker. However, the capture of anions in organic solvents can still be accomplished.

Synthetic receptors specifically for anions were first reported by Park and Simmons in 1968,[56] with the *in*,*in*-1,11-diazabicyclo[9.9.9]nonacosane-bisammonium dication, **64**, shown through NMR monitoring to incorporate halide ions into its cavity, through hydrogen bonding and electrostatic attraction. The encapsulation of a Cl^- ion was later confirmed using X-ray crystallography,[57] as shown diagrammatically in **64**.

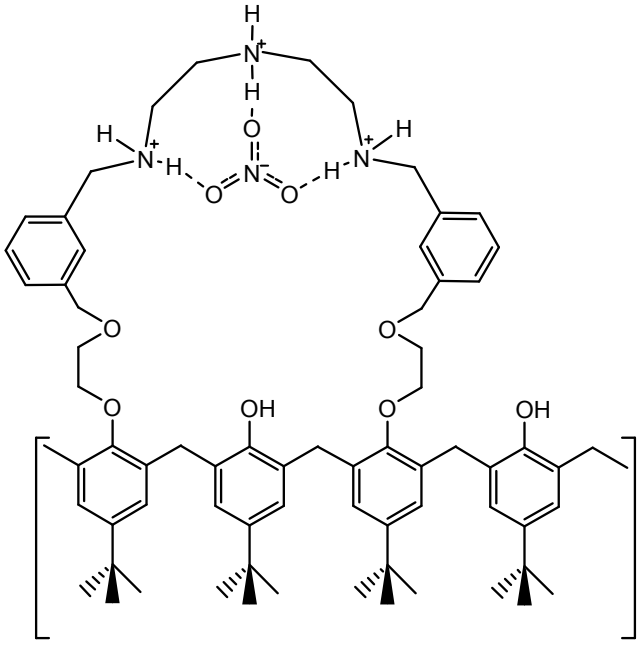


64

Subsequently a large variety of synthetic anion receptors have been investigated, including calixarenes, amides, amines, porphyrins and cyclen derivatives. Cyclen derivatives and calixarenes are of special interest to this work.

Substituted and metallated calixarenes have been used in the detection of anions[2,58,59] and as catalysts.[47] Hydrogen bonding between substituents and the guest is important in the complexation of NO_3^- by **65**. Similarly hydrogen bonding by the

calixarene's –OH groups has been shown to be important to the overall complexation of the anion $H_2PO_4^-$ by the calixarene derivative **66**.[58]



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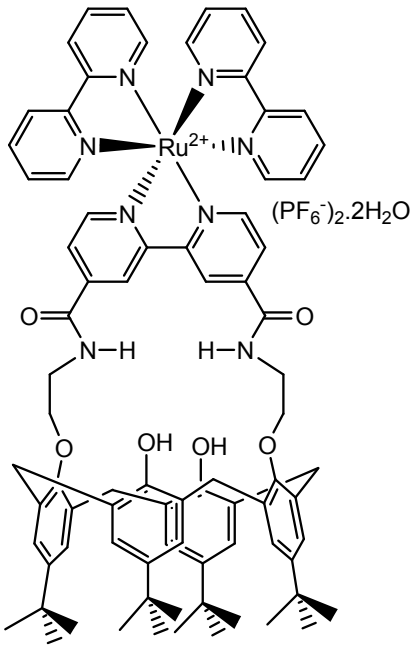
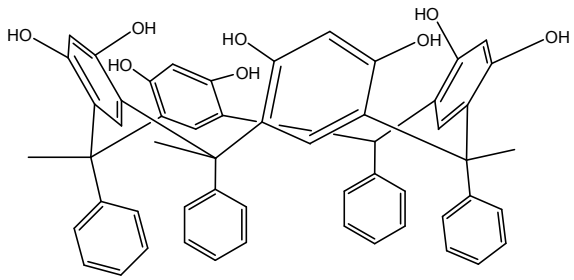


66

Calix[4]arenes functionalised at the 1,3-positions of the upper rim with amido groups act as neutral receptors for a variety of anion guests, such as hydrogensulfate, dihydrogen phosphate, acetate, benzoate, nicotinate, oxalate, terephthalate, isophthalate and fumarate.[60]

Resorcinarene-based hosts have also been shown to include guest molecules, such as C_{60}. Resorcinarenes are similar to calixarenes, and are the products of the acid catalysed condensation of resorcinol with aldehydes such as benzaldehyde and salicylaldehyde.[61] Niederl and Vogel first studied the reaction of resorcinol with a few aliphatic aldehydes in 1940.[62] The acid-catalyzed condensation of resorcinol and benzaldehyde gave rise to **67**, which is shown in its crown conformation.



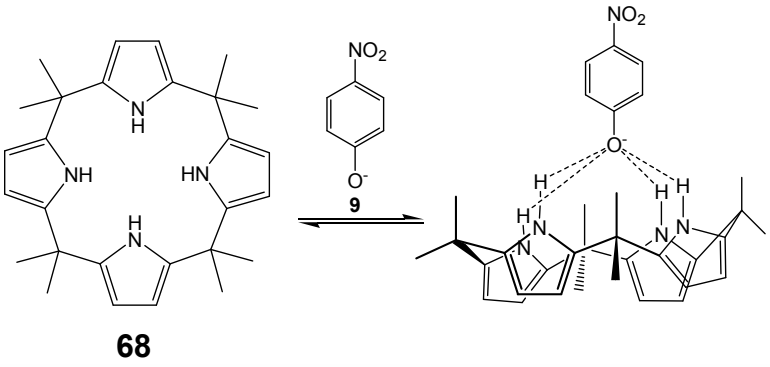
67

Resorcinarenes,[63] like calixarenes, have a crown conformer with a preformed bowl shape cavity that may be locked in place by substitution on the lower rim. This cavity allows resorcinarene based molecules to capture from aqueous solutions organic guest molecules such as benzene, hexane, chlorobutane, butanol, ethyl acetate,[64] and even ferrocene.[65]

Similarly calixpyrroles, **68**, in the crown conformer have a preformed shallow bowl shaped cavity. This cavity may likewise include guest molecules. Sessler and co-workers showed a pronounced downfield shifting of the NMR resonance due to the NH protons of calix[4]pyrroles[66] on addition of *p*-nitrophenolate, **9**, as in **Figure 1.4.** The binding between the *p*-nitrophenolate and a calix[4]pyrrole, **68**, (K = 290 ± 9.7 dm^3mol^{-1}) was stabilised through multiple N-H---$^-$O hydrogen bonding.[66]

1.4. Effect of metal ion binding on anion receptors

As anions can behave as ligands towards metal ions, metal complexes can be used as versatile anion receptors.[11,12,67] Tripodal tetraamine receptors tend to form five-coordinate metal complexes of trigonal bipyramidal stereochemistry, thus the Cu(II) complex of tris-(N-benzyl-2-ethylamino)-amine, **69**, possesses a vacant axial coordination site that is available for anion binding. However, anion retention



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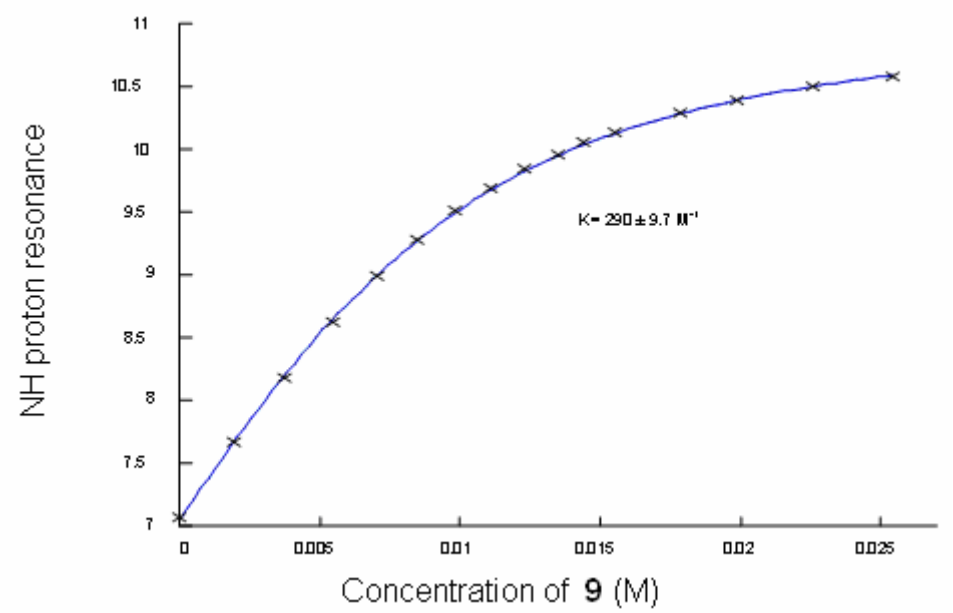
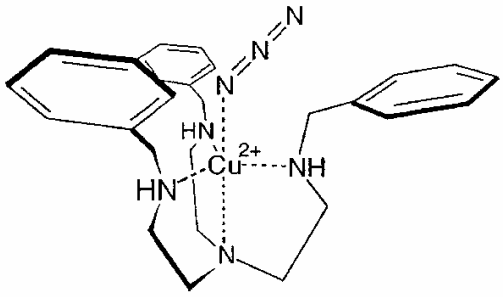
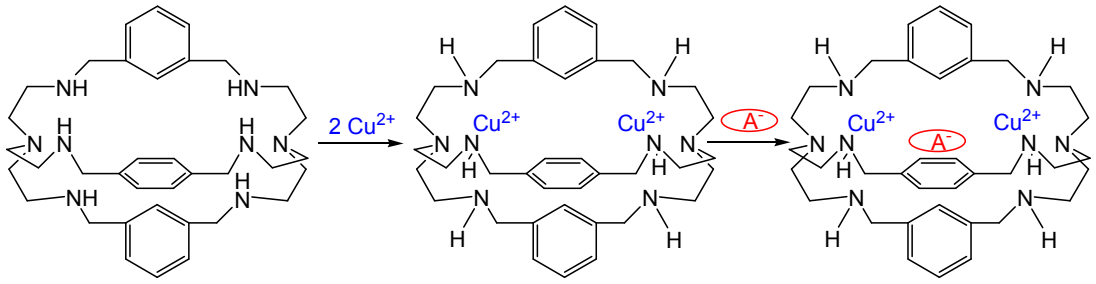


Figure 1.4. Experimental (crosses) and calculated (curve) NMR titration data for *meso*-octamethylcalix[4]pyrrole, **68**, (0.01 M) and tetrabutylammonium 4-nitrophenolate in dichloromethane-d_2 at 25 °C, taken from the supplementary material of reference.[66]



69

can be strengthened considerably if the anion is bound to the metal through multiple points of attachment. For example ambidentate anions can be encapsulated by dicopper(II) cryptates by the cascade mechanism, shown in **Figure 1.5**.[67,68]

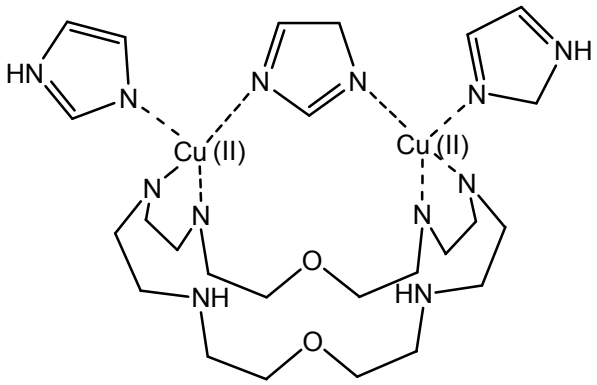


70 **71** **72**

Figure 1.5. In the cascade mechanism the ligand initially binds two copper(II) ions, creating the dicopper(II) complex that is able to encapsulate the anionic guest.

In the cascade mechanism the bis-tren cryptand sequentially includes first two equivalent metal ions, and then an ambidentate anion such as N_3^-, **Figure 1.5**.[67] Other bis-tren dicopper(II) cryptates can recognise halides such as Br^-.[67]

Imidazole has a definite tendency to bridge two Cu(II) ions with the simultaneous loss of a proton. In **73** the two Cu(II) ions encircled by a bisdien macrocycle, tightly bind a bridged imidazolate anion, whilst also binding two neutral imidazole molecules on the terminal coordination sites of the Cu(II) ions.[69]



73

Fabbrizzi and co-workers designed a specific receptor for imidazolate taking advantage of this coordination of the imidazolate ion to bind a pair of Cu(II) ions,[4,5] **Figure 1.6**. To avoid further imidazole binding, the fifth coordination site on each Cu(II) is blocked by the 2-picolyl arms appended to the middle nitrogen of each

triamine moiety.[4,5] The formation of the Cu(II)-imidazolate-Cu(II) complex is indicated by an absorption band at 690 nm.

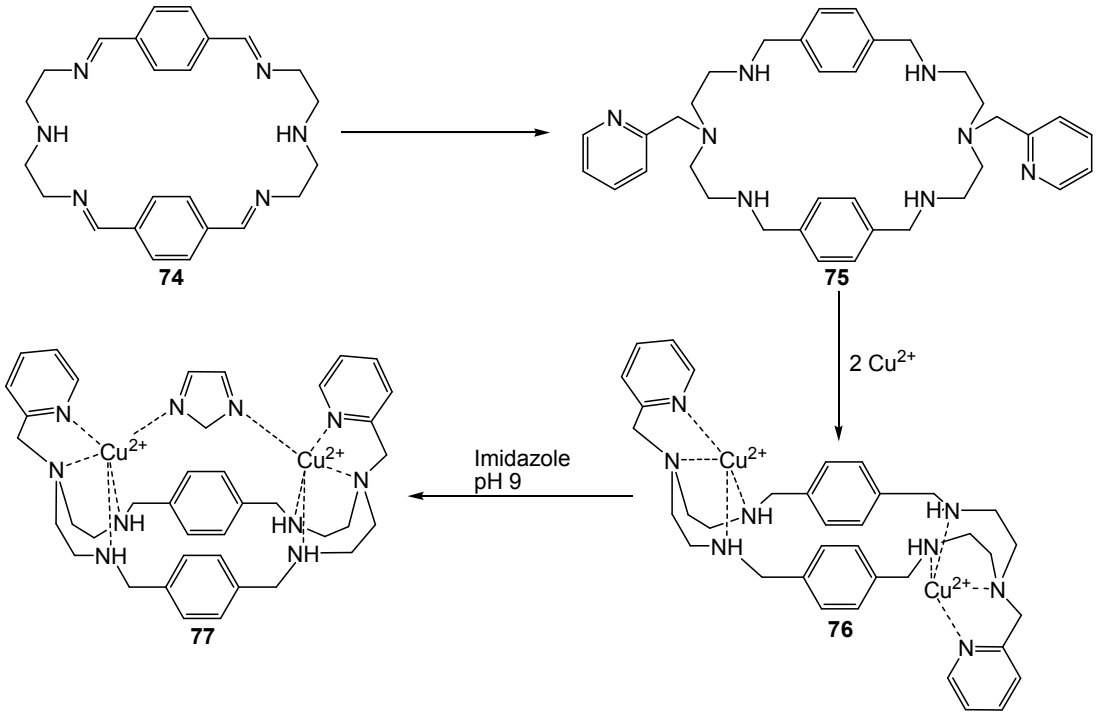


Figure 1.6. Imidazolate has a definite tendency to bridge two Cu(II) ions, the fifth coordination site on each Cu(II) centre is blocked by the appended 2-picolyl arms.

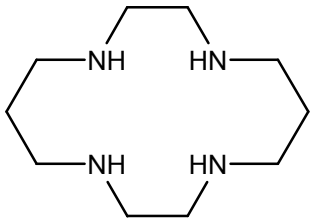
1.5. Tetraaza macrocycles and their use in anion binding

Lately there has been a growing interest in tetraazamacrocycles, not only due to their remarkable complexing properties, but also because their functionality can be widely extended by introducing one or more side-chains.[70]

One disadvantage of tetraazamacrocycles is that they are not able to completely 'wrap up' transition metal ions, which usually have a preferred coordination number of six. This has been overcome by the appending of coordinating groups to the periphery of the macrocycle. These may then coordinate to the metal ion, completing its coordination sphere.[71] These are known as pendant donor groups,[71] and many such macrocycles have been prepared and their metal ion

chemistry investigated.[72-79] These derivatives often exhibit the properties of both the open chain analogues of the arms as well as those of the parent macrocycle.[80] The pendant arms can be appended to either the nitrogen atoms of the azamacrocycle, or to carbon atoms. The synthesis of *N*-substituted macrocycles is the simpler,[71,81] and the syntheses of complexes of *N*-functionalised tetraazamacrocycles are well understood.[71]

1,4,8,11-tetracyclotetradecane (cyclam), **78**, first synthesised by Van Alphen[82], is a tetraazamacrocyclic ligand that neither differs greatly in electronic effect from amines, nor does it contain organic side chains.[83] The introduction of four pendant arms generally leads to complexes having the trans-III



78

geometry in which two arms project from one side of the macrocyclic plane and two from the other, as shown in **Figure 1.7**.[84]

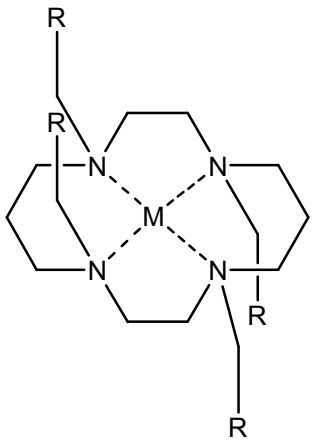
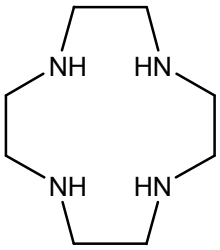


Figure 1.7. The addition of pendant arms to cyclam leads to the trans-III geometry for its complexes.

This prevents pendant arm cyclam complexes being used as a framework within which a molecular cavity suitable for guest retention might be formed.

However, 1,4,7,10-tetraazacyclododecane, cyclen, **79**, tends to have arms that group to one side. These can be brought together by an eight-coordinating metal ion to create a molecular cavity.[84] If a single metal doesn't utilise the entire coordination capability of a ligand, there is a tendency to form bimetallic complexes.[84]



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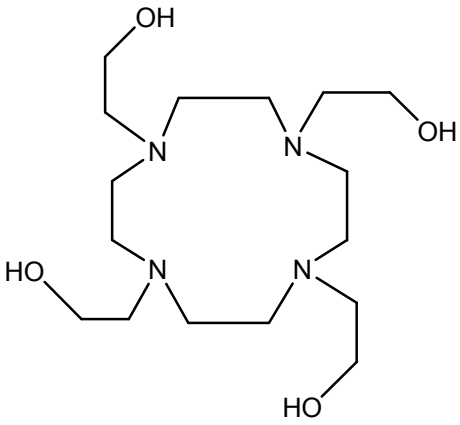
In 1982 Dale and co-workers isolated a cyclen derivative with four pendant arms as shown in structure **80**[85]. It was isolated as a monohydrate using the very simple method, conducted in water, of reacting free cyclen with ethylene oxide.



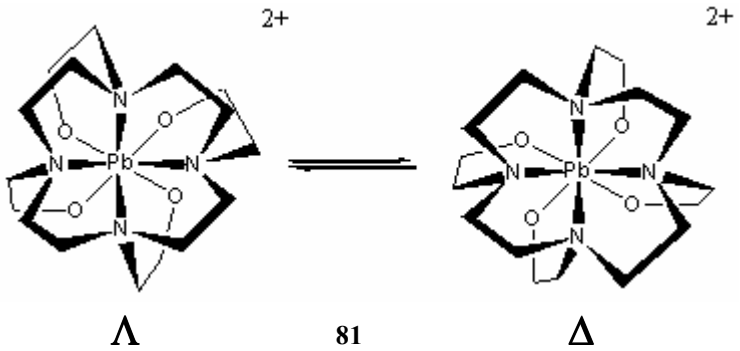
80

The complexing properties of this macrocyle were studied through the formation of its complexes with the alkali metal ions, Li^+, Na^+, and K^+. As the size of the cation increases from Li^+ to K^+, the binding modes shown by this ligand differ: With Li^+ it forms a five-coordinate complex, with Na^+ a seven-coordinate complex

and with K^+ an eight-coordinate complex, in which the potassium is bound to all nitrogen atoms and all oxygen atoms from the pendant arms. In all three complexes the pendant arms of the ligand project away from the same face of the macrocycle. In time this was to be recognized as an almost invariant characteristic of complexes of cyclen derivatives.

Selectivity of macrocyclic ligands towards metal ions also depends on the fit of the cation in the ring, and the ligand's ability to adjust itself to the electronic and geometrical requirements of the metal.[86] Amino-ether ligands designed to fit approximately cubic octa-coordination of cations the size of Na^+, Ca^{2+} and La^{3+} should include at least one twelve membered ring, with its predictable and well-defined 'square' conformation.[85] Thus, compounds based on cyclen are well suited as potential receptors and sensors for a variety of alkali, alkaline earth metal, transition metal and lanthanide cations.[3,73,75-77,87,90-137]

The attachment of a coordinating pendant arm to each nitrogen of cyclen results in ligands that form chiral eight-coordinate metal ion complexes of C_4 symmetry.[84,87] This is seen in the ligand 1,4,7,10-tetrakis(2-hydroxyethyl)-cyclen, **80**, which like most cyclen based ligands, is a strong cation complexer.[88,89] In some of its metal complexes, such as **81**, rapid intramolecular exchange between Λ and Δ enantiomers has been observed, **Figure 1.8**.



Λ **81** Δ

Figure 1.8. Rapid intramolecular exchanges between the Λ from where the arms spiral anticlockwise (as drawn) and the Δ form where the arms spiral clockwise (as drawn).

Synthetic routes towards incompletely alkylated tetraazamacrocycles are more complicated than those towards their tetra-substituted relatives.[71,72,84,90-106] They either require excess of the parent macrocycle, or lengthy protection and deprotection steps. One method of mono-functionalization of cyclic tetraamines is via their tridentate tricarbonylchromium complexes, **Figure 1.9**.[90]

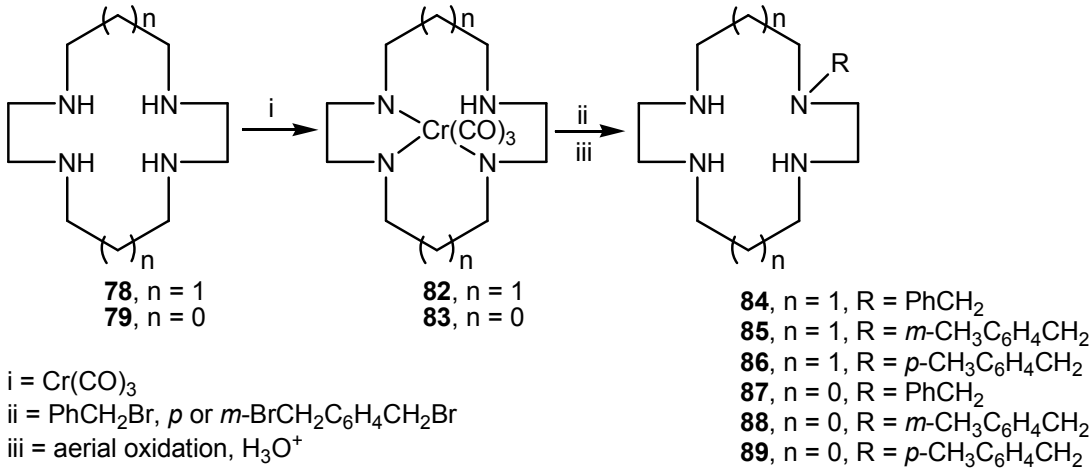
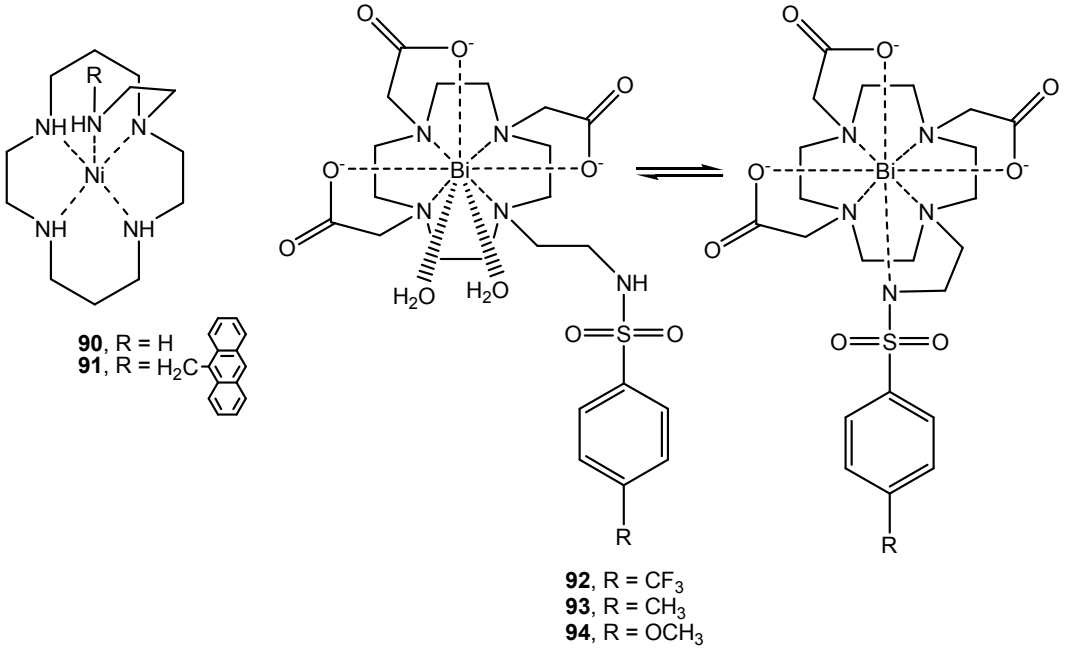
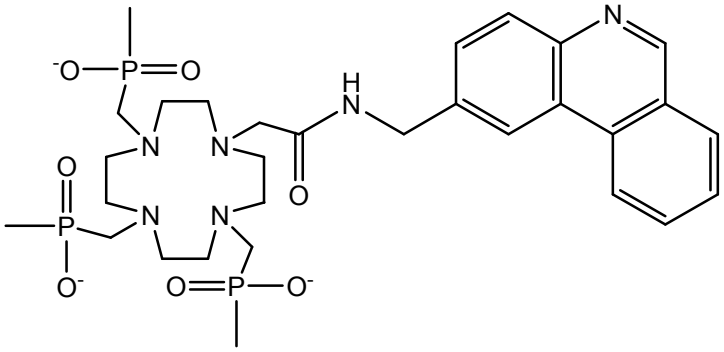


Figure 1.9. triprotection of cyclen, **79**, and cyclam, **78**.

It is reported that corresponding N^1,N^7-dialkylated cyclen derivatives can be achieved by using alkyl iodides rather than the alkyl bromides.[97] Mono-substitution of tetraazamacrocycles has given rise to various cyclen based scorpiands, which show a pH dependent colour change, **90**,[71,107,108], or luminescence, **92**.[109]

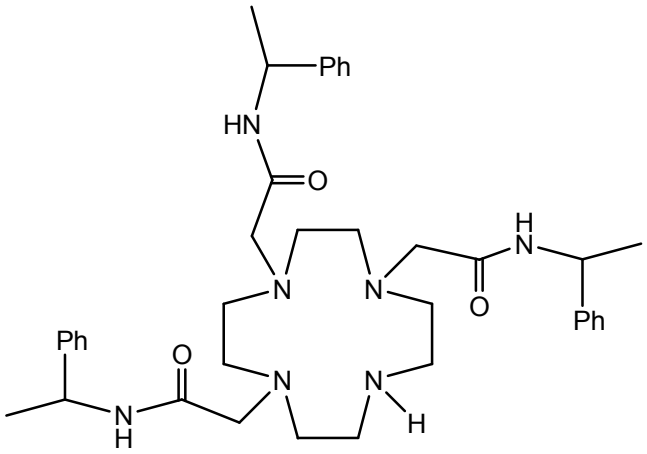


Parker and co-workers developed luminescent chemosensors for pH, halide and hydroxide ions based on macrocyclic europium-phenanthridinium conjugates, **95**.[110,111]



95

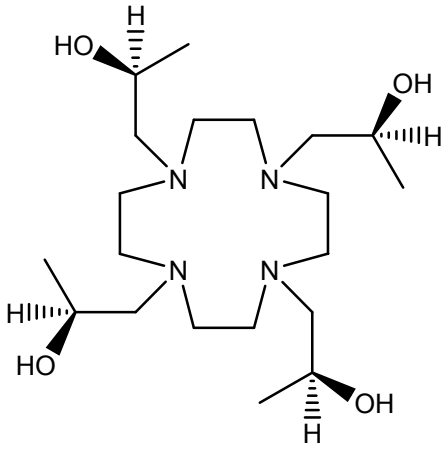
Reversible anion binding of F^-, acetate, sulfate, HCO_3^- and CO_3^{2-} in aqueous solution to a cationic nonacoordinate lanthanide centre, signalled by luminescence, was achieved by Parker and co-workers, using the cyclen-based ligand, **96**.[112,113] Bicarbonate displaced both waters at the metal centre, leading to a large change in the luminescence lifetime.[123]



96

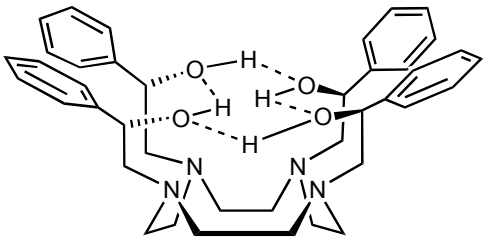
There are also cyclen-based molecular receptors for anionic guest molecules not based on ionic forces. It has already been noted that pendant donor macrocycles derived from cyclen almost invariably coordinate to a metal ion in such a way that

the four pendant arms project in the same direction. This can lead to the formation of a cavity.[106,114,115] The size and complexity of these cavities have increased over time, as racemic arms, such as those formed from racemic propylene oxide, [116-118] were replaced by the enantiomerically pure (*S*)-(-)-propylene oxide to give the homochiral (*S*,*S*,*S*,*S*)-enantiomer, **97**.[116-118]



97

Increasing the complexity of the pendant arms created molecular receptors with aromatic cavities, similar to those of calixarenes.[114] Thus the use of (*S*)-(-)-styrene oxide, formed a cavity of phenyl groups that was much more enclosed than that formed with methyl groups.[116-118] Molecular modeling predicted that complexation of this ligand, **98**, by an eight-coordinating metal ion would pull it into a robust conformation, **99**, that had a pronounced hydrophobic cavity with four convergent hydroxy groups situated at its base that might be suitable for molecular inclusion work.[119,120] Unlike the reactions of cyclen with most epoxides, which give a quantitative yield of the pendant donor macrocycle, the reaction with styrene oxide only ever gives a yield of *ca* 40%, for reasons that are still not understood. Because of this, molecular inclusion work with **98** has not been pursued to any great extent, despite the high rigidity of the cavity.[121]



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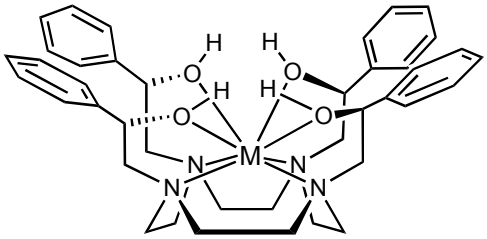
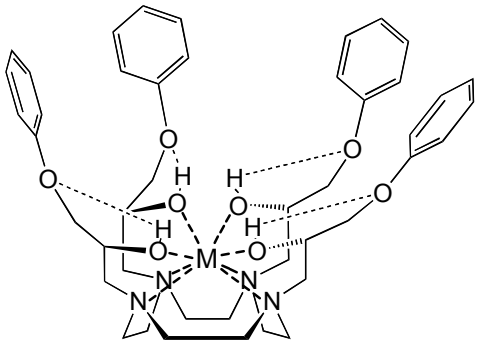


99, M = Cd^{2+}
100, M = Pb^{2+}

Smith *et al.*[114,115,122-124] synthesized the related, but much higher yielding, organic soluble macrocyclic ligand 1,4,7,10-tetrakis((2*S*)-2-hydroxy-3-phenoxypropyl)-1,4,7,10-tetraazacyclododecane, and its Cd(II) complex, **1**. The cavity of both **99** and **1** has the four aromatic moieties juxtaposed.[114] This calixarene-like cavity allows the complex to act as a molecular receptor for aromatic anions. The use of enantiomerically pure arms ensures that the cavity formed upon metal complexation is a chiral environment for potential guest inclusion.

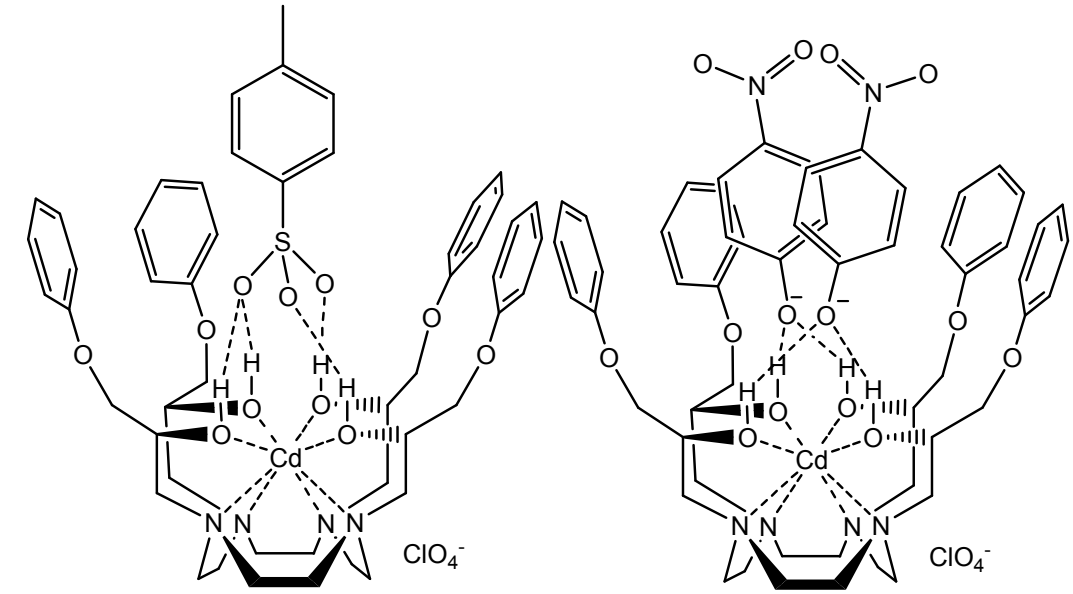
The inclusion chemistry of the Cd(II) complex, **1**, and its Pb(II) complex, **101**, has been intensively investigated and it has been established that they are excellent host compounds for the inclusion of aromatic anions such as phenolates,



1, M = Cd^{2+}
101, M = Pb^{2+}

benzoates, amino acids, aromatic sulfonates and phosphate esters. They also bind non-aromatic anions such as acetate, with lower stability, and neutral species such as acetonitrile.[124] Crystal structures of many of these inclusion complexes have been

published and these demonstrate that the anion or neutral compound is retained by multiple hydrogen bonding to the set of hydroxy hydrogen bond donors at the base of the cavity.[121,124-126] These hydroxy groups converge towards the guest anion and are quite potent hydrogen bond donors because of the electron withdrawing effect of the metal ion to which they are each coordinated. The inclusion of aromatic anions within the cavity of the Cd(II) complex, **1**, of *p*-toluenesulfonate (**102**) *p*-nitrophenolate (**103**), and (*L*)-phenylalaninate (**104**) and *p*-aminobenzoate (**105**) are shown below.[124]



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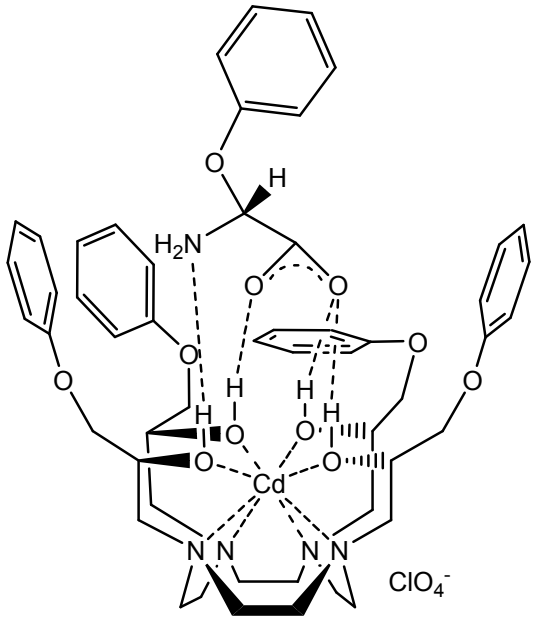

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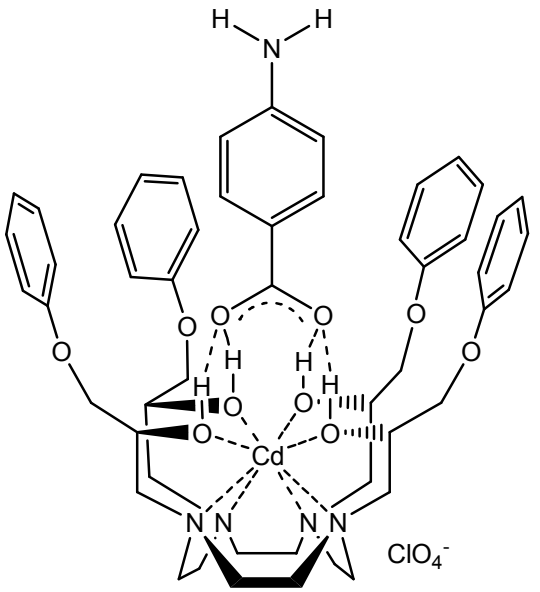
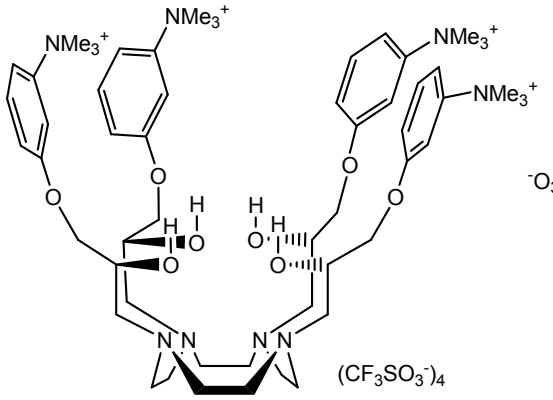


105

Although crystal structures show that inclusion of these, and similar, host-guest systems occur in the solid state, the behaviour of these receptors in solution is of greater interest. Electrical conductivity measurements have shown that these host-guest complexes have a conductivity lower than that of the host. The magnitude of the lowering depends on the strength of binding of the guest anion.[114,125] Solution ^{1}H NMR studies have been used to determine binding constants for these host-guest associations (ranging from $10^{3.3}$ for the inclusion of acetate with **1**, to $>10^5$ for the inclusion of phenoxyacetate) using DMSO-d_6 as the solvent.[125] Recently NMR binding studies in D_2O for the cadmium(II) complex of the water soluble cationic analogue, 1,4,7,10-tetrakis((2*S*)-(-)-2-hydroxy-3-[3'-(*N*,*N*,*N*-trimethylammonium)-phenoxy]-propyl)-1,4,7,10-tetraazacyclododecane tetratriflate, **106**, gave binding constants ranging from $10^{1.6}$ (D-tryptophanate) to $>10^{4.5}$ (*p*-dimethylaminobenzoate).[127] The cadmium(II) complex of the anionic analogue, 1,4,7,10-tetrakis((2*S*)-(-)-2-hydroxy-3-[2'-sulfo-4'-methylphenoxy]-propyl)-1,4,7,10-tetraazacyclododecane, **107**, gave binding constants ranging from $10^{0.4}$ (*p*-nitrobenzoate) to $10^{2.0}$ (*p*-aminobenzoate).[127]



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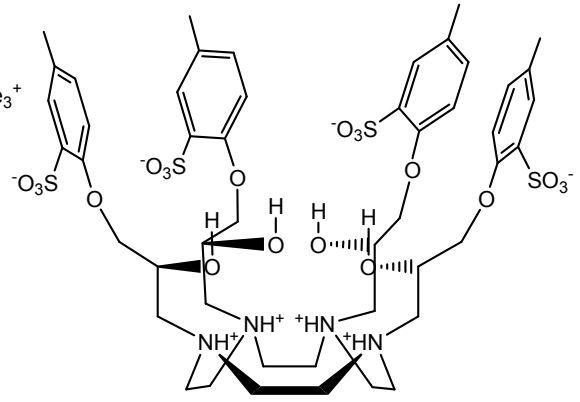


107

Crystal structures, conductivity measurements, and NMR derived binding constants, all indicate that **1** and its derivatives are molecular receptors for aromatic

anions. To convert these receptors to molecular sensors a signaling unit must be attached that will indicate the act of guest inclusion, *in situ*, in real time.

1.6. Signalling Molecular Recognition

Even the most sophisticated and specific host molecule is of limited utility if it is not able to communicate that a host-guest interaction is taking place. Molecular recognition, such as anion inclusion, can readily be detected using X-ray crystallography, but this method cannot be performed *in situ* and contemporaneously with the event. While NMR Spectroscopy can be used for *in situ* detection, it is not as sensitive as other methods.

For practical purposes fluorescence perturbation has become one of the more highly favoured means of signalling a molecular recognition event:[3,12,128] It employs relatively cheap apparatus, that can provide real time *in situ* measurements, over a range of concentrations down to the sub-micromolar level.[4] Since this technique is of higher sensitivity than routine NMR spectroscopy it allows work down to lower host and guest concentrations and consequently the acquisition of binding constant data for host-guest complexes over a wider stability range. This in turn presents an opportunity to gain a fuller understanding of the principles of molecular recognition that are operative for these hosts and of the selectivity of the receptors over a wider range of guest molecules.

Fluorescent sensors for metal cations have been developed by several groups in the past decades,[43,99,108,129-147,149a-e] and many of these operate on the basis of the metal ion (or proton) perturbing a fluorescence quenching process known as photoinduced electron transfer (PeT).

PeT perturbation is an efficient mechanism within the sensor for either quenching or inducing fluorescence in the potential fluorophore upon substrate binding.[108,137,138]

PeT is one of the two main mechanisms of fluorescence quenching in molecular sensors, with the other mechanism being energy transfer (ET). Both are described schematically in **Figure 1.10**.

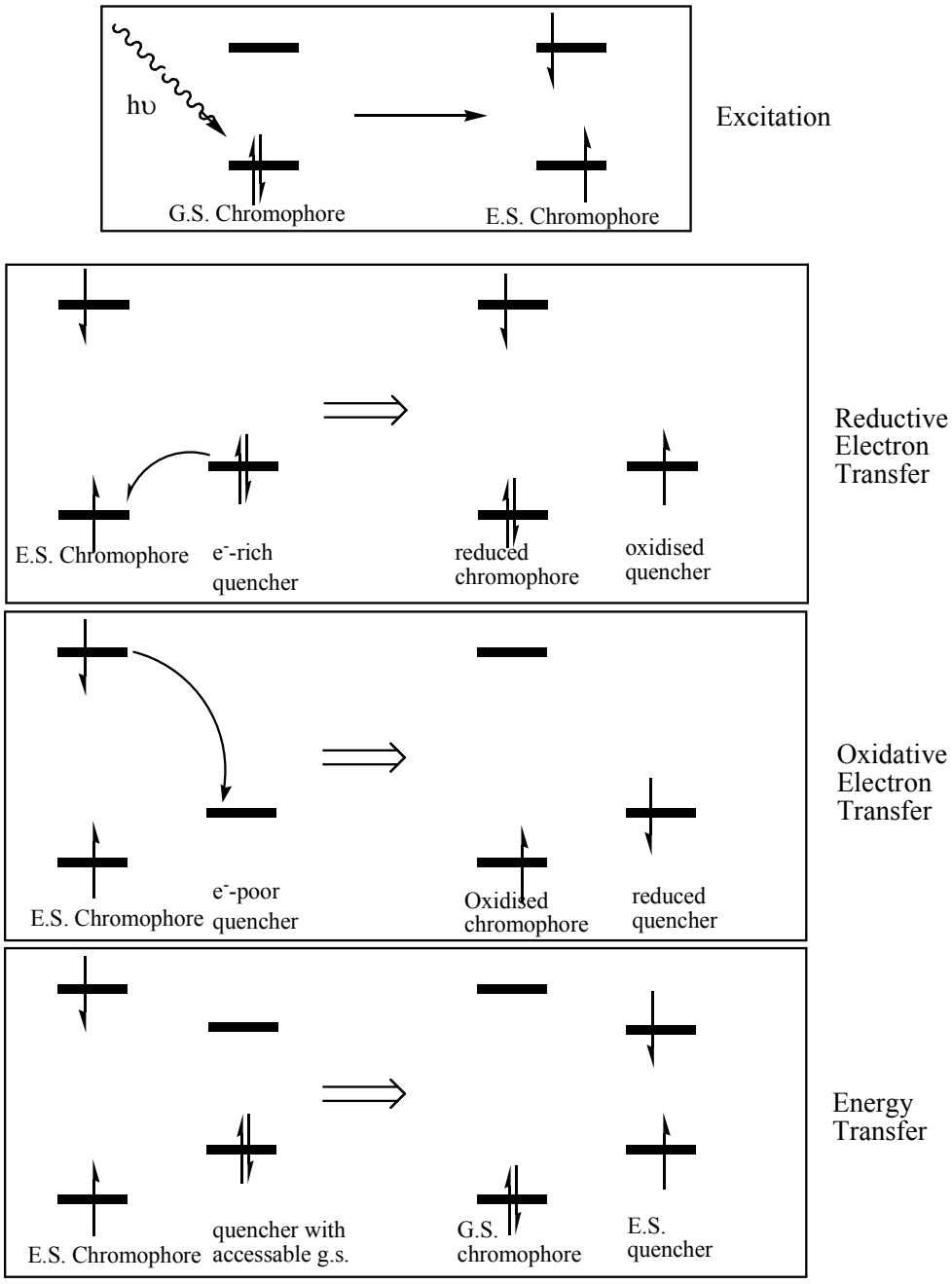


Figure 1.10. Electron transfer and energy transfer quenching of a chromophore excited state.[148]

Promotion of an electron from a filled orbital to a higher-energy empty orbital results in the excited state of that molecule being both a stronger oxidising agent and reducing agent than it was in the ground state. The promoted electron is in a high-energy orbital and can reduce an electron-poor quencher. Or the low-energy hole left by the promotion of the electron may be filled through oxidation of an electron-rich quencher. Which of these electron transfer pathways occurs depends on the species that is interacting with the fluorophore.[148,149]

In the case of anthrylamine[149(a)] moieties, there is a lone pair of electrons on the nitrogen atom that is available to the PeT process, resulting in a diminution of potential fluorescence intensity. However, this lone pair may become involved in either protonation or metal complexation, which then allows fluorescence, as the lone pair is no longer available to the PeT process.

The use of a PeT process as the desired fluorescence perturbation mechanism, in a situation where the electron transfer involves an amino moiety adjacent to the fluorophore, allows both proton (pH) and metal ion binding to be studied. The ligand at high pH (deprotonated) would have a low natural fluorescence, while protonation or metal complexation would cause an increase of the fluorescence, enabling controlled PeT switching of fluorescence.[150,151] Thus, further possible perturbation by guest inclusion within a metal complex of the fluorescent ligand could be studied using the fluorescence of the metal complex as a reference point.

The other main mechanism for quenching an excited molecule, energy transfer (ET), involves no net electron transfer. Rather, the excited-state energy of the fluorophore is transferred to the quencher, which is then itself excited.[148,149]

Another means by which an excited molecule may relieve itself of its excited-state energy is by the formation of an excimer, as shown in **Figure 1.11**.[149]

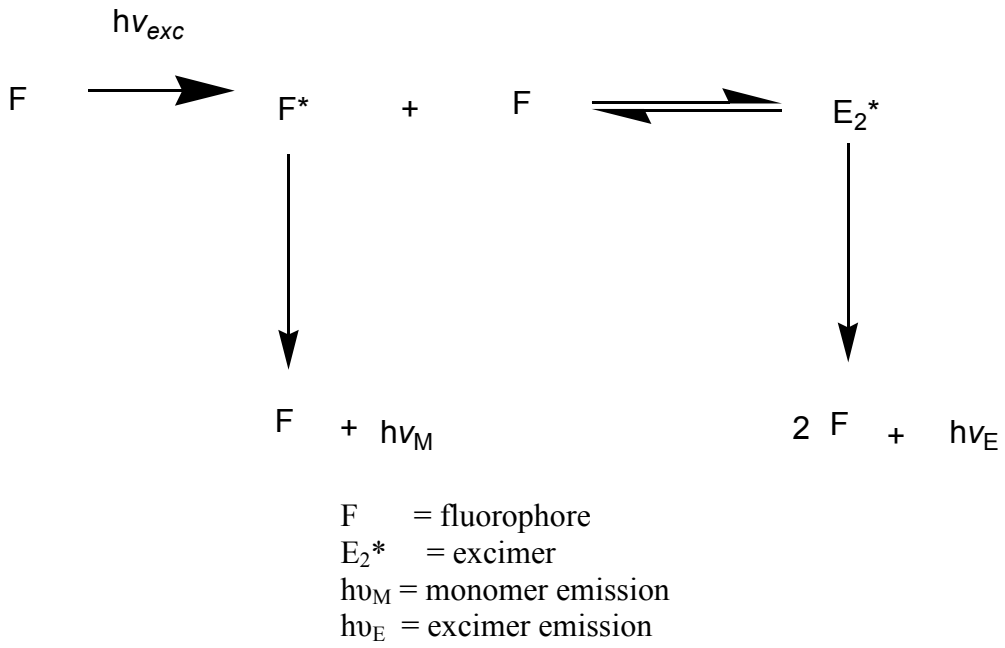


Figure 1.11. Excimer formation and emission

An excimer, as defined by Birks,[152] is a dimer which is associated in an electronic excited state and which is dissociated in its ground state. The radiative relaxation of the photo-associated species E_2* to a dissociated ground state is generally associated with a broad structureless emission band at longer wavelengths than the corresponding molecular fluorescence.[153] Such a structureless emission was first reported by Forster and Kasper[154] as a broad, blue, band, which replaced the violet structured fluorescence of dissolved pyrene on increase of concentration.[153,155] This was the first reported instance of an excimer emission. These complexes are called excimers[153,156] if the two molecules are identical and heteroexcimers or exciplexes[156] if they are different. These complexes exist only in the excited state and they dissociate into monomers upon deactivation.[156] Excimer emissions increase in intensity with increasing concentration, at expense of the fluorescence intensity of the monomer emission.[157] Ten years after the discovery of excimer fluorescence by Forster and Kasper, intramolecular excimer emission was observed by Hirayama[158] in 1,3-diphenylpropane. It was concluded that the aromatic moieties exist in a

conformation in which the aromatic rings lie approximately parallel to one another close enough for excimer formation.[159] This sandwich arrangement has to be reached during the lifetime of the excited state.[156] Thus the discovery of intramolecular excimer fluorescence has provided a powerful tool for the study of conformational changes with relaxation times in the range of 10^{-9} to 10^{-7} s.[160] Two features of pyrene account for intramolecular excimer formation in dipyrenylalkanes.[161] The stabilization energy of pyrene excimers is large (0.34 eV in cyclohexane). This means that if an excited- and ground-state pyrene can achieve the proper relative configuration, the excimer readily forms. Secondly, the long fluorescence lifetime of pyrene makes the attainment of the correct relative configuration highly probable.[161]

The first example of a fluorescent molecular sensor for anions was due to Czarnik and co-workers[162], and was a two-component system shown in **Figure 1.12**,

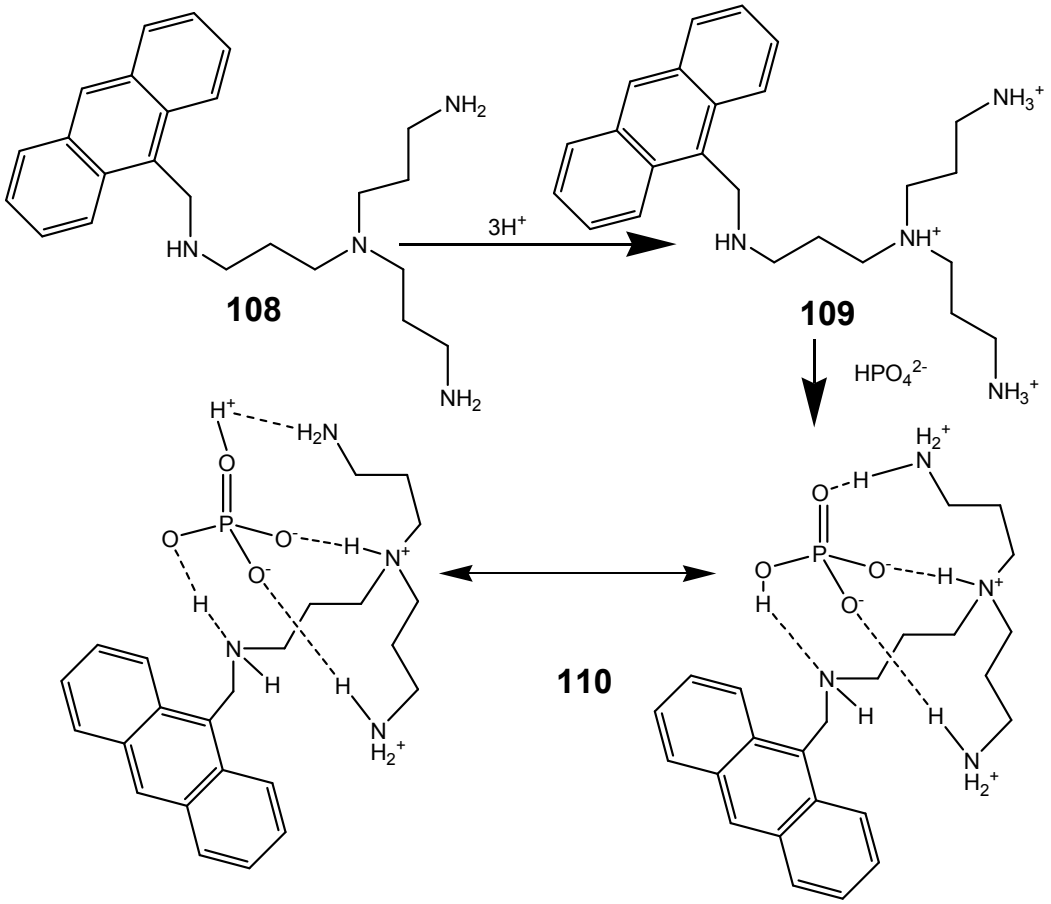
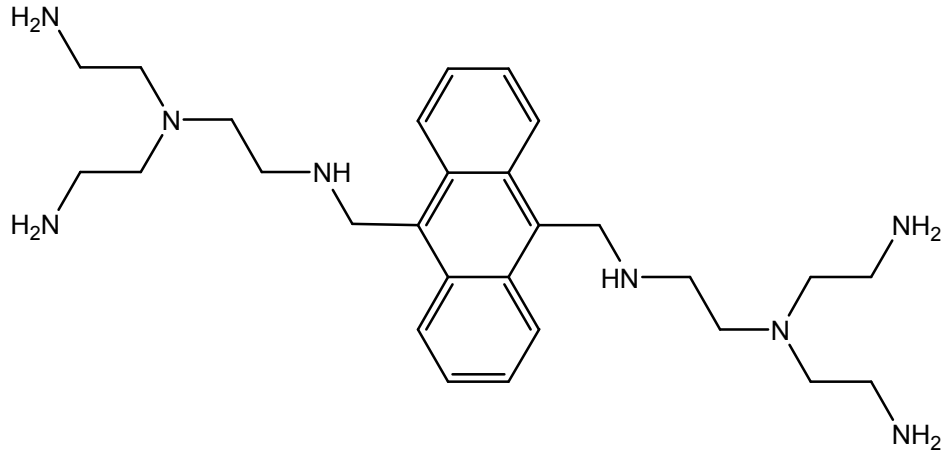


Figure 1.12. An early fluorescent molecular sensor.

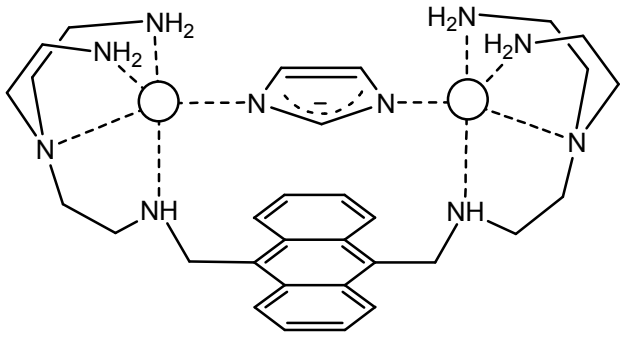
where an anthracene fragment is linked through a methylene group to a branched tetraamine, which sensed HPO_4^{2-}.[11,12,163]

Fabbrizzi and co-workers designed a fluorosensor for histidinate, **26**[164], similar to their molecular receptor for imidazole, **73**. The sensor, **111**, incorporates



111

two quadridentate binding sites for Zn(II) ions, leaving both metal ions a coordination site free to bind a further ligand (a nitrogen of an imidazolate subunit of histidine), as shown in **112**. The anthracene fragment linking the tetraamine subunits



112

gives an intense characteristically structured fluorescence emission, suitable for the sensing of the host-guest interaction.[164] The fluorescence is partially quenched in the free ligand, revived upon complexation to Zn(II), and then quenched when the imidazolate bridged complex is formed. The quenching in the bridged complex was

ascribed to eT from a π-orbital of the electron rich im$^-$ moiety, to a π-orbital of the photo-excited anthracene fragment.[164]

The anthracene functionalised ligand, **113**, can bind to Zn(II). This Zn(II) complex was tested for recognition and sensing of aromatic carboxylates, **Figure 1.13**.[165] When titrated with 4-*N*,*N*-dimethylaminobenzoate, **16**, the

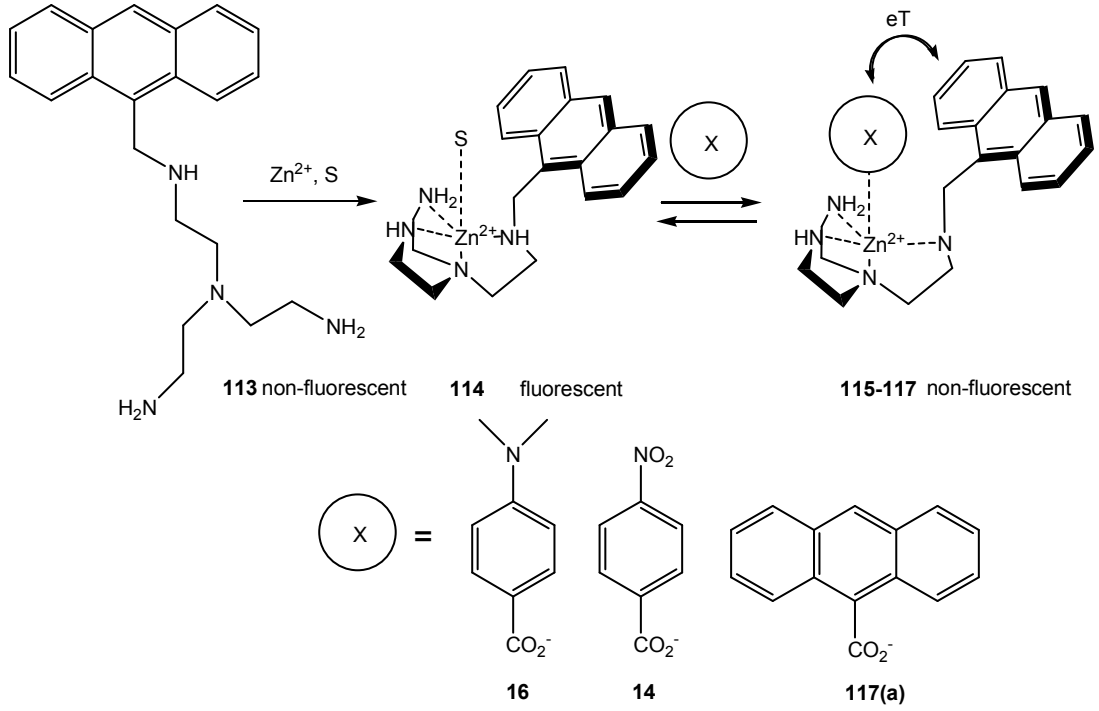


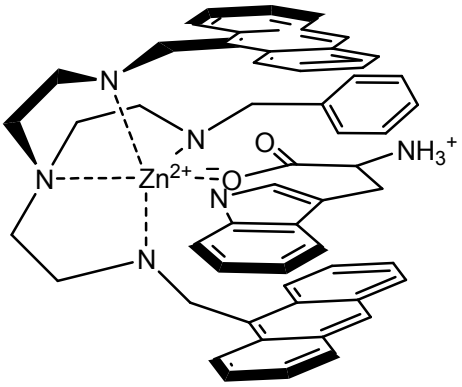
Figure 1.13. The fluorescence of the anthracene moiety changes with guest inclusion.

anthracene fluorescent emission was progressively quenched, with complete quenching at a 1:1 sensor/analyte ratio.[11,12] Quenching was ascribed to the thermodynamically favoured photoinduced electron transfer (PeT) process, from the dimethylamine donor group to the excited anthracene moiety.[11,12] Similar effects were observed when the benzoate bears an electron acceptor substituent (NO_2), **14**, but in the reverse direction, and when the anion was the fluorescent 9-anthracenoate anion, **117**.[11,12]

For the detection of specific amino acids, the receptor needs to address more than just a carboxylate group, which is common to all amino acids. The receptor

needs to be equipped with further binding sites capable of establishing a specific interaction with the side chain of the desired substrate.

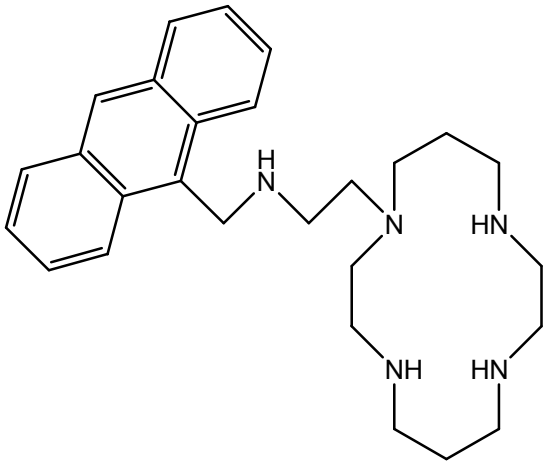
An example of such a receptor is **118**, which has a high affinity towards



118

natural amino acids bearing aromatic substituents such as phenylalanine (phe) and tryptophane (trp).[163] Titration of the Zn^{II} complex with phe resulted in no change of the anthracene emission, contrary to the behaviour with tryptophane, whose recognition is signalled by fluorescence quenching. Throughspace PeT from the indole fragment to the photoexcited anthracene induces fluorescence quenching.[12]

Systems capable of displaying a change of fluorescence on variation of pH have also been studied.[137,138,166,167] Fabbrizzi and co-workers[137,138] developed a multicomponent, covalently linked system, **119**, containing a light-emitting tail



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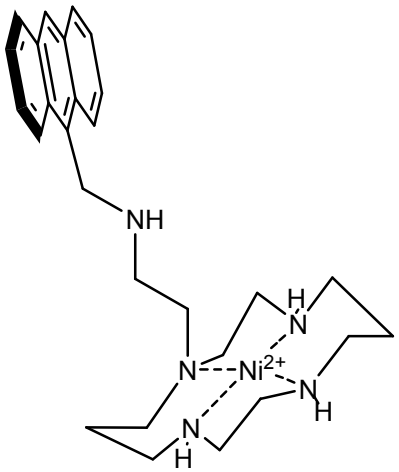


120

that can be reversibly moved to two defined positions by changes in the pH. The nickel(II) complex, **120**, in acidic conditions, has the tail protonated. Protonation of the anthrylamine means that there is no lone pair of electrons on the nitrogen to facilitate a PeT process, hence the high level of fluorescence. Protonation of this side chain also means it is forced away from the cyclen ring due to charge repulsion. However, on increasing the pH, this nitrogen atom is deprotonated, which releases the lone pair on the nitrogen to take part in a PeT process, resulting in a reduction of fluorescence intensity. However, deprotonation also removes the repulsive effects, allowing the side chain to coordinate to the metal centre in an axial position, **Figure 1.14**, which in itself will reduce the PeT effect, and partially revives the fluorescence. This deprotonated form is a pale blue violet, as opposed to the protonated yellow colour, allowing the molecular motion to be followed visibly.[108,137,138,141] Further deprotonation of a coordinated water molecule increases the PeT effect, and causes the fluorescence to become almost entirely quenched.

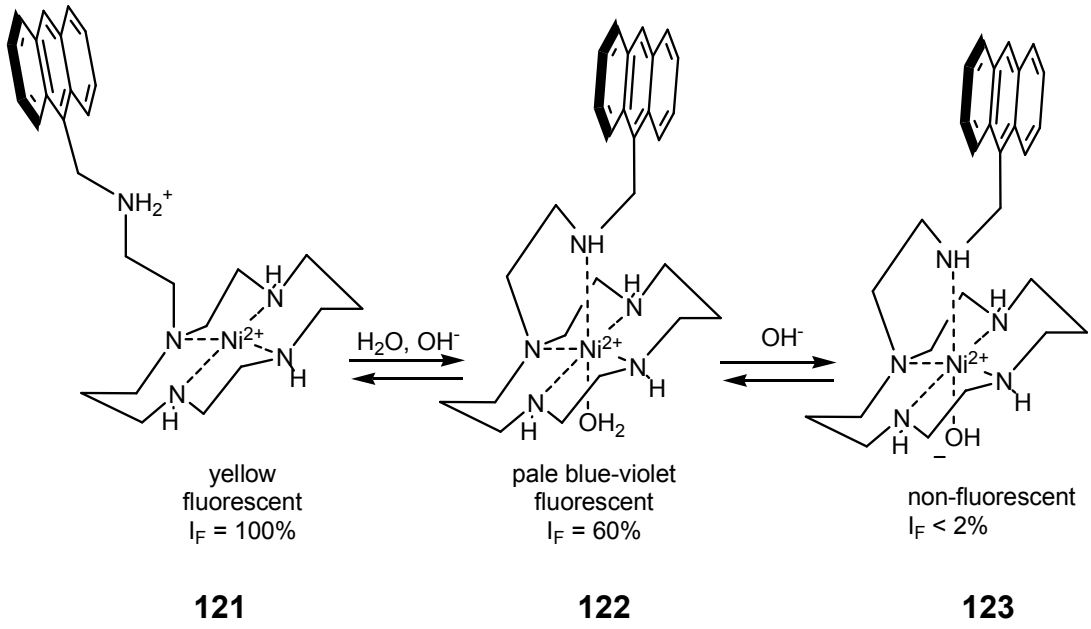


Figure 1.14. Protonation and metal complexation has significant effects on fluorescence. Deprotonation of the arm and complexation to the metal centre decreases fluorescence emission intensity by 40%, and is also accompanied by a colour change from yellow to a pale blue violet.[156]

Anion recognition is in most cases based on electrostatic interactions, although other interactions (e.g. hydrogen bonding) can be important or even dominant. In general, the low energy of electrostatic interactions does not compete successfully with the anion hydration energy, requiring recognition studies to be performed in non-aqueous, non-polar solvents.[108,137,138]

A fluorescent cage for anion sensing in aqueous solutions, **124**, was designed by Fabbrizzi and co-workers for the sensing of N_3^- (and other ambidentate anions) using the dizinc(II) complex, **125**.[137,138] The formation of the $[Zn^{2+}{}_2L(N_3)]^{3+}$ inclusion complex, **126**, is signalled by complete quenching of the anthracene fluorescence, which was ascribed to an electron transfer process from the electron rich N_3^- ion to the nearby excited fluorophore, **Figure 1.15**.[11,12,67,137,138]

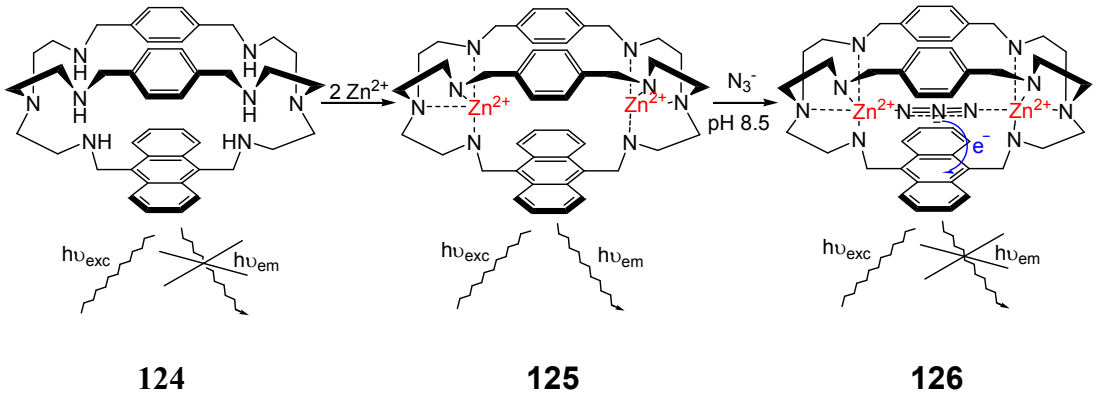
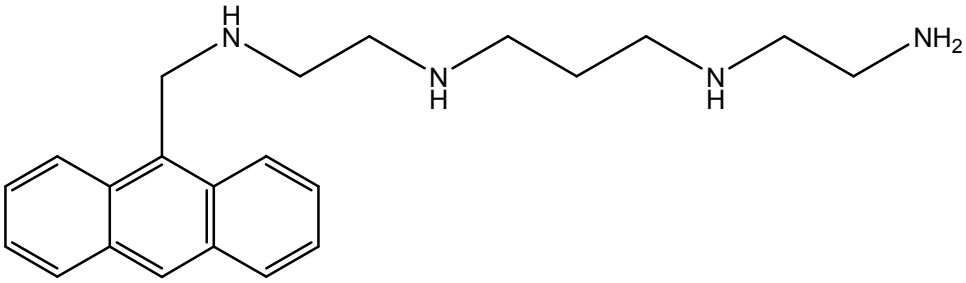


Figure 1.15. The inclusion of N_3^- within the cage completely quenches the fluorescence signal.

Probably the most commonly encountered PeT modulated fluorophore is the 9-aminomethylanthracene subunit, which has already been seen in several of the examples above. As the free amine it is only weakly fluorescent, because PeT of the lone electron pair on the nitrogen atom (anthrylamine) to the electronic vacancy in the photoexcited anthracene provides an efficient quenching mechanism, however, protonation or metal ion coordination at the amine blocks the PeT and revives the fluorescence.[149,168,169] Thus, 9-aminomethylanthracene derivatives have already found applications as pH sensors and metal ion sensors, such as **127**[149,170] and

128,[149,171] but in addition some applications as anion sensors have also been devised, such as **129**,[149,172] which contains a guanidinium unit that binds, *via* hydrogen bonding, the carboxylate end of γ–aminobutyric acid, GABA. The monoaza-18-crown-6-ether unit acts as a receptor for the ammonium terminal of GABA. The fluorescence of this dialkylaminomethyl anthracene is known to be switched on by both protonation and guest complexation, which block PeT.[175]



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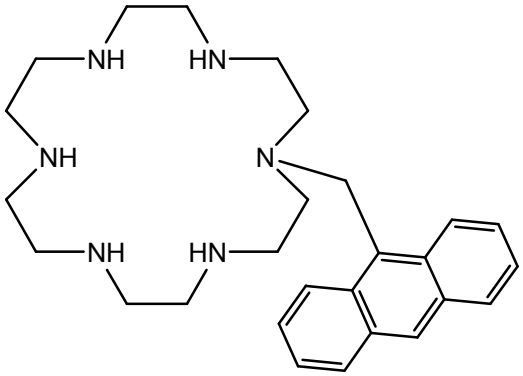

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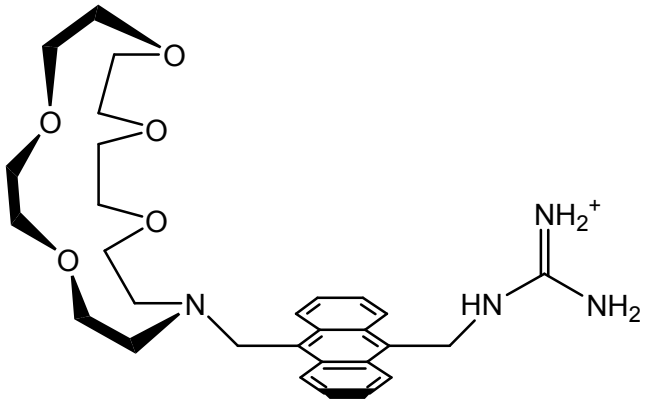
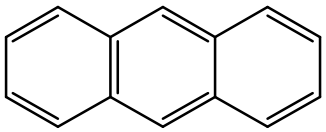


129

In many cases the anion can either perturb the electron density at the anthrylamine through hydrogen bonding,[171] or, where π-stacking is possible, exchanges electrons with the photoexcited anthracene via a through space PeT effect.[12,163,165,173]



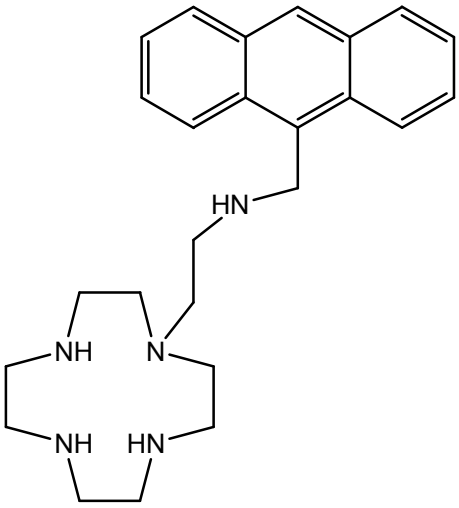
130

Anthracene, **130**, was chosen as the fluorophore in the work of Fabbrizzi and co-workers,[108,136-139,173-175] as well as the work of other groups,[132,133,149-151,176-187] as described previously, because of its well established fluorescence characteristics (fluorescence spectra of 9-substituted anthracene derivatives have a characteristic emission spectra[151,188,189] containing three emission bands and a small shoulder. It was anticipated that similar emission spectra could be obtained for the cyclen based anion receptor complexes to be used in this study if an anthracence moiety were to be covalently attached to them. Because preliminary work both in our laboratory and that of Kimura and co-workers,[151] had elucidated a way for attaching anthracene to cyclen whilst at the same time retaining the eight-coordinating and hydrogen bonding characteristics of the prototype receptor ligands such as **1,**[125] it was proposed to synthesise a series of receptor complexes in which one of the pendant arms forming the receptor cavity was ethylanthrylamine, as seen in structure **131**.

1.7. Tetraazamacrocycles with attached fluorophores

The nearest precursor to the planned series of cyclen derived receptor ligands was **antac-12, 131**.[151,190] This shows the fluorescence spectrum displayed in **Figure**

1.16, with three emission maxima bands, at 398, 418 and 443 nm and a small shoulder at 470 nm.



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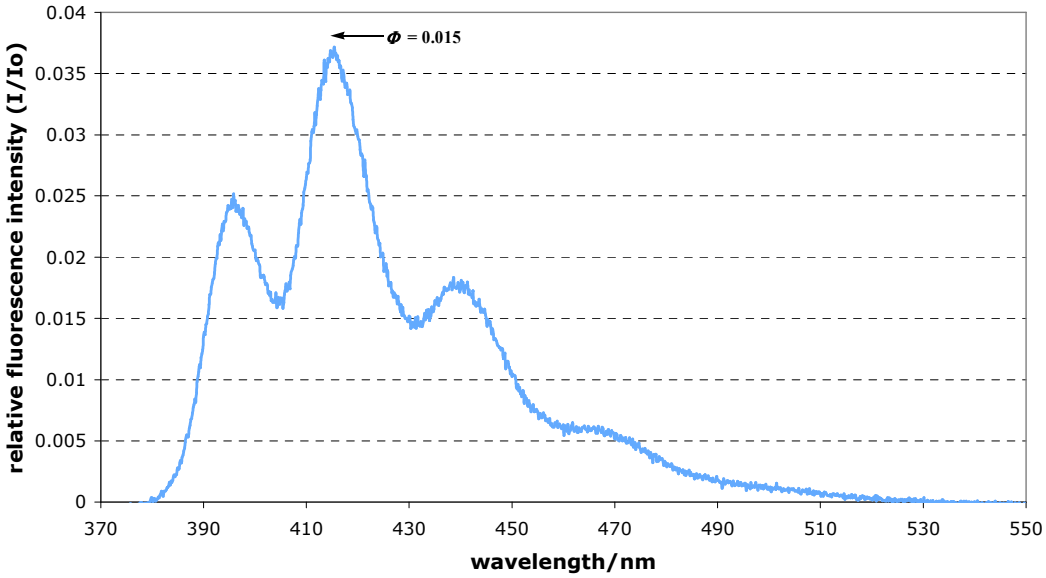


Figure 1.16 Fluorescence spectrum of **131** [1 x 10^{-6} mol dm^{-3}] at pH 12.2 in 20% aqueous 1,4-dioxane (I = 0.1 mol dm^{-3}, NEt_4ClO_4) at 298 K when excited at 350 nm.

The intensity of the fluorescence is quite weak (Φ = 0.015) due to the PeT effect. The transfer of electrons by PeT can be hindered by metal ion complexation or protonation of the amino group, which results in a more intense fluorescence.[151] Hence at low pH or in the presence of metal ions, particularly Cd(II) or Zn(II) the fluorescence spectrum of **131** changes in the way shown in **Figure 1.17.**

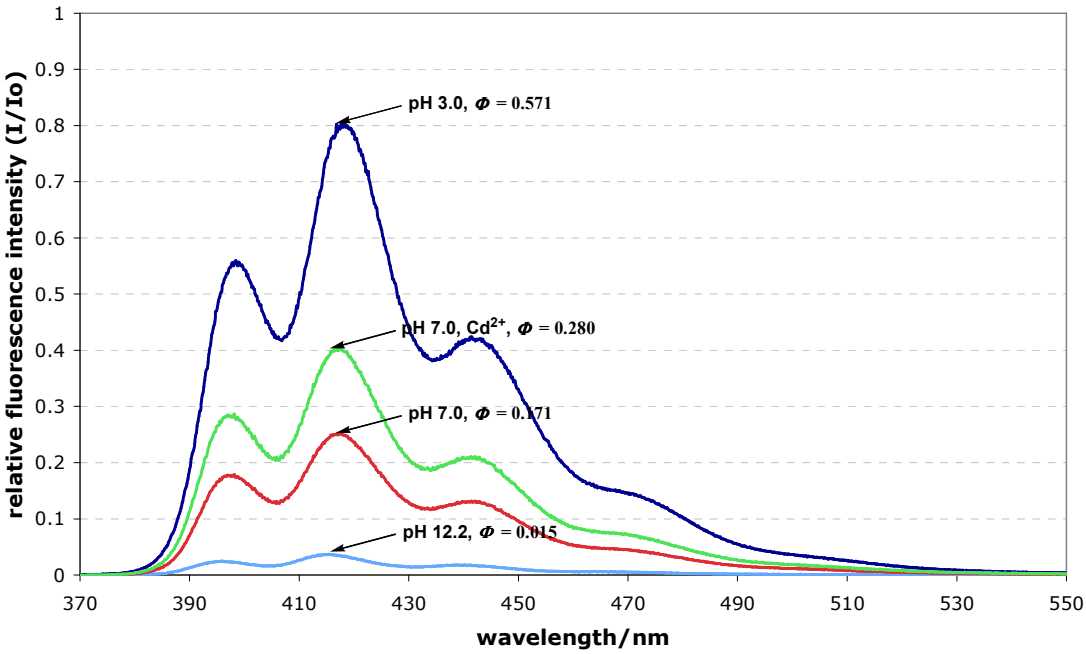


Figure 1.17. Fluorescence spectrum of **131** [1 x 10^{-6} mol dm^{-3}] upon Cd(II) complexation and protonation in 20% aqueous 1,4-dioxane (I = 0.1 mol dm^{-3}, NEt_4ClO_4) at 298 K when excited at 350 nm.

The emission bands undergo a small hypsochromic shift (of *ca* 1.3 nm) on titration of the protonated ligand (**antacH$_5$**) with NEt_4OH. More apparent is the massive decrease in fluorescence intensity, with an almost 30-fold reduction of maximum fluorescence intensity when the pH is changed from pH 3.0 (0.8) to pH 12.2 (0.03). This is almost twice that shown for **131** in a purely aqueous solvent, which showed an 11-fold increase in fluorescence when the pH was changed from pH 10 to pH 4.[151] As water is able to quench fluorescence,[24,108,139,140,191-196] it seems reasonable that a fully aqueous system would have lower fluorescence than a partially aqueous (20 %) solvent. Kimura and co-workers determined the a log K_a value of 7.2 ± 0.2 for the deprotonation of the protonated anthrylamine in **131** in water from a sigmoidal curve of the fluorescence intensities at varying pH, which was in agreement with value obtained by NMR (7.0 ± 0.3) and potentiometric pH titration (7.15).[151] In both the water[151] and 20% aqueous 1,4-dioxane systems, **131** remained weakly fluorescent even when fully deprotonated, which indicates that the

fluorescence of the ligand is not fully quenched by PeT. A small amount of quenching was observed under strongly acidic conditions (pH < 2.5) for **131**, in 20% aqueous 1,4-dioxane. This type of quenching has been reported previously in other anthracene conjugated macrocycles, and has been attributed to both protonation of the anthracene substituent, and to an acid-catalysed photochemically induced decompostion.[171,197]

A problem associated with the employment of anthracene as the attached fluorophore is that compounds containing it must be handled in the dark, and in an oxygen free environment. The presence of light and oxygen results in the formation and subsequent decomposition of the photooxidation products shown in **Figure 1.18**.[198]

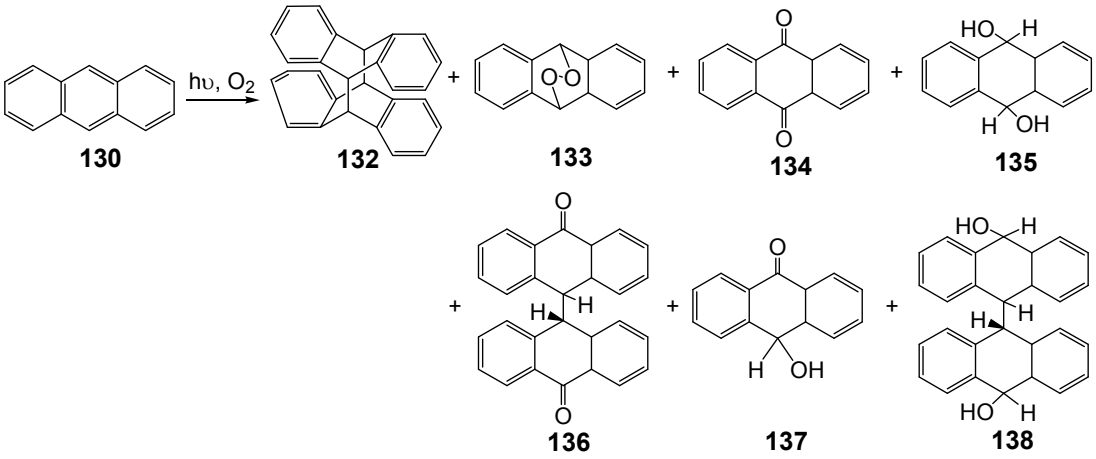
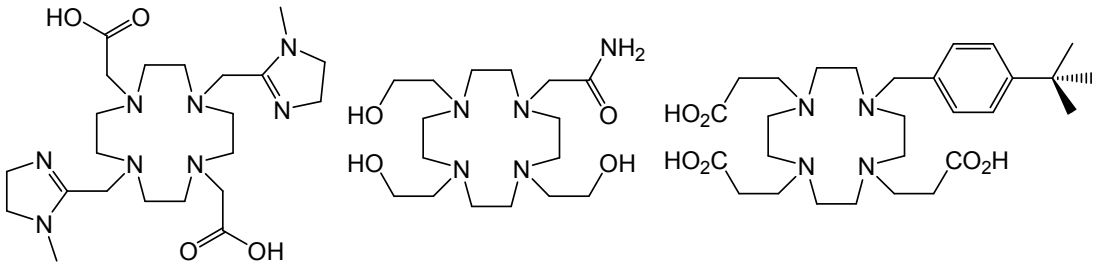


Figure 1.18. The photooxidation products of anthracene.[198]

With anthracene chosen as the fluorophore, there now came the task of attaching the fluorophore to the chosen type of molecular receptor, **1**, which had four identical pendant arms, one of which would be substituted by the fluorophore. What was required was a mixed pendant armed system, where three of the pendant arms formed the walls of the cavity, and the final arm had a dual purpose of forming the final side of the wall of the cavity and also of acting as the fluorophore.

1.7.1. Mixed Pendant Arm Macrocyclic Ligands

As the addition of pendant groups to an azamacrocycle combines the properties of both the pendant group and the macrocycle, then the use of two (or more) different pendant donor groups would be beneficial, as a range different properties could be given to the macrocyclic derivative. Some examples of cyclen-based receptor systems with mixed pendant groups are 1,7-bis(carboxymethyl)-4,10-bis(1-methylimidazol-2-ylmethyl)-1,4,7,10-tetraazacyclododecane, **139**,[199,200] 1-(carbamoylmethyl)-4,7,10-tris(hydroxyethyl)-1,4,7,10-tetraazacyclododecane, **140**, [201] and the DO3A derivative, **141**.[202]



139 **140** **141**

Figure 1.19. Cyclen-based mixed pendant ligands.

For a long time tetrasubstituted cyclen derivatives with different pendant groups received limited attention, owing to difficulties with their synthesis.[104] The main method for synthesizing heterosubstituted derivatives has utilised two successive steps, the first to obtain a partially, homo-substituted macrocycle, and the second step to produce the hetero-tetrasubstituted macrocycle.[104] It is the first step, that requires efficient, regioselective derivatisation, that is the more difficult to perform.[104] To obtain a partially, homo-substituted macrocycle requires the selective protection of one (or more) of the nitrogens,[202,203] followed by the removal of those protecting groups.

However, recent developments in the synthesis of tetraazamacrocycle based receptor complexes are such that by the time this project commenced it had become relatively straightforward to attach just three of the 2-hydroxyethyl pendant arms in **1**, allowing the opportunity to attach a signalling unit as the fourth arm.

However, not any signaling unit would do. Although anthracene had been chosen as the fluorophore, there were other structural considerations that needed to be considered. For the sensor to be a true analogue of the receptor complex **1**, it required either an OH or NH group on the fluorophore pendant arm. This would enable the arm be positioned by metal ion coordination and also contribute to anion retention in a similar way to the other three pendant arms, as shown in section 1.5.

Fortunately an anthracene based fluorophore with an adjacent methylamino group has been very well developed, as has already been shown in the work of Fabbrizzi and co-workers[108,136,137] and of Kimura and co-workers.[151] The means by which the ligand, antac-12, **131**, has been formed is of special relevance to this project.

1.8. Guest molecules effecting the PeT mechanism

Fabbrizzi and co-workers have recently discovered that a through space intramolecular photoinduced electron transfer that takes place in a Zn(II) polyamine complex, can be interrupted through coordination of a bulky carboxylate anion, which acts a curtain, **Figure 1.20**.[173] Complexation of **142** to Zn(II) gave a non-fluorescent complex where fluorescence was revived upon association with triphenylacetate. This behaviour was ascribed to the metal complexation bringing

the photoexcited anthracene fragment and the DMA subunits close enough together to allow an intra-complex through-space PeT process to occur.

Thus, An* behaves as an electron acceptor and DMA as a donor. Similar electron transfer processes were previously seen in the work of De Santis,[165] **114-118**. In this case the presence of the bulky triphenylacetate anion then blocks the eT process between the excited anthracene and the DMA subunit.[173] This use of bulky groups to block intramolecular processes is reminiscent of the use of coumarin to interrupt an electronic energy transfer (EET) process in similar Zn(II) complexes reported by Fabbrizzi and co-workers in 2001.[204]

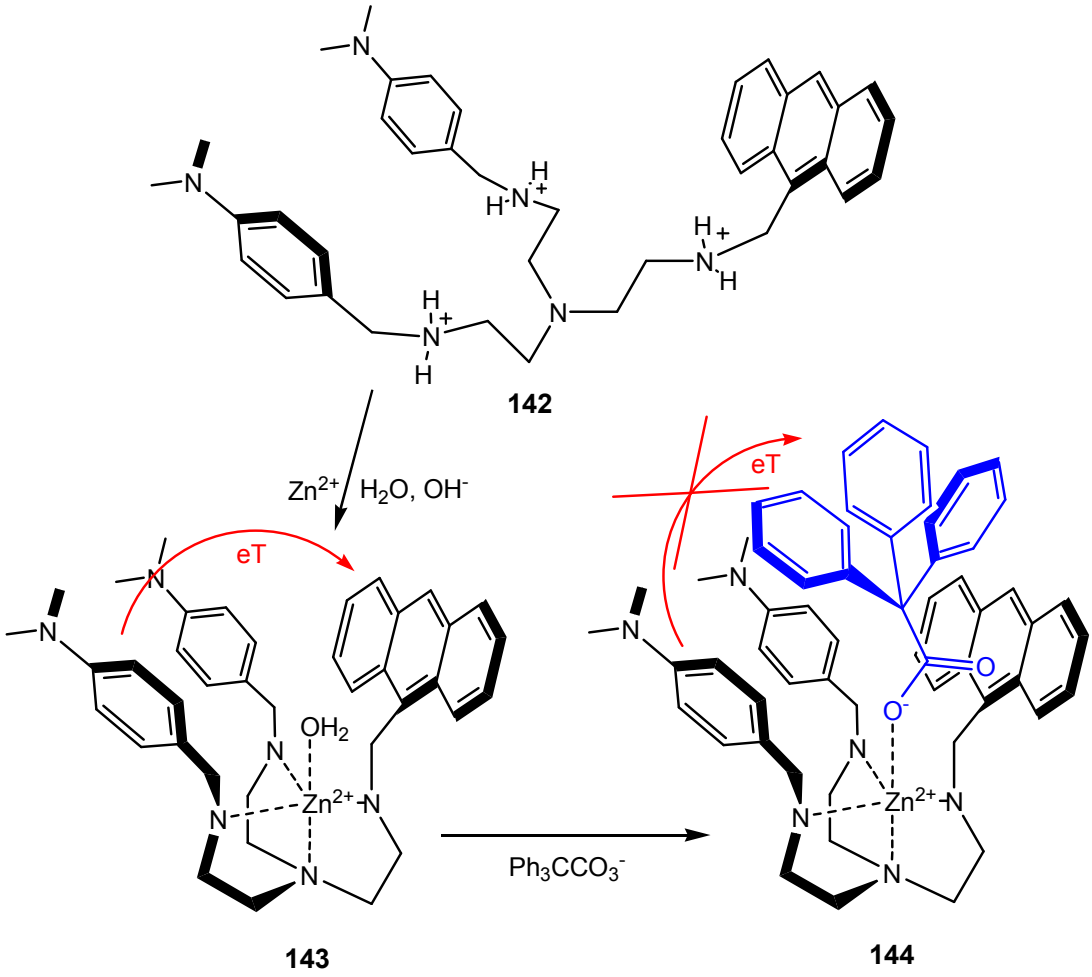
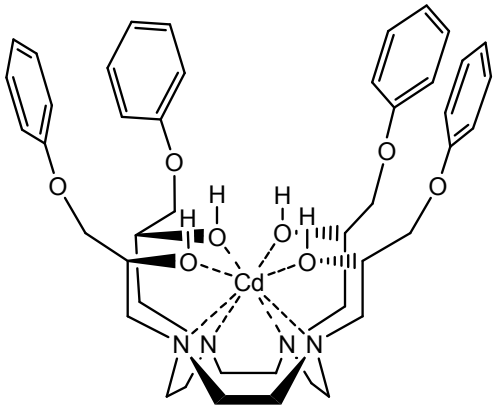


Figure 1.20. The inclusion of the guest within the cavity blocks the PeT process, enhancing fluorescence.

1.9. Aims and Significance of the Project

The PeT responsive fluorophore chosen for the work described in this thesis is the 9-anthrylmethylamino moiety and the overall plan is to incorporate it as one of the pendant arms of **1** to produce a family of sensors based on **4**. To preserve the



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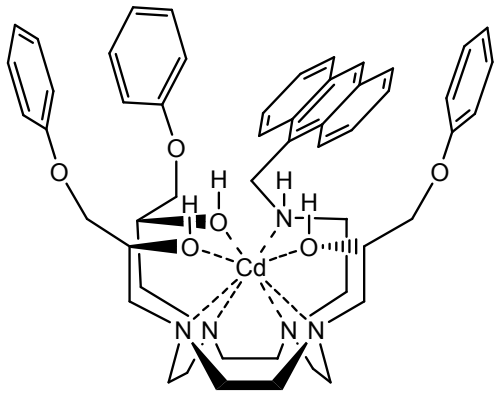


4

aromatic anion retaining capabilities of **1** the replacement arm, besides being potentially fluorescent, must also contain an electron donor group that is capable of binding to the metal ion and simultaneously acting as an hydrogen bond donor for the guest. It must also have an aromatic moiety capable of forming a part of the cavity wall. The pendant group 2-(9-anthrylmethylamino)ethyl meets these requirements.

The aims of this project are therefore: (i) the synthesis of cyclen-based macrocycles with pendant arms containing fluorescent aromatic moieties suitable for the purpose of forming molecular receptors after metal ion complexation; (ii) the inclusion of anionic aromatic guest molecules within the cavity of the metal complexes; (iii) the determination of the potential of these receptor complexes to act as sensors for anionic guest molecules by examining the changes in fluorescence that occur upon guest molecule uptake.

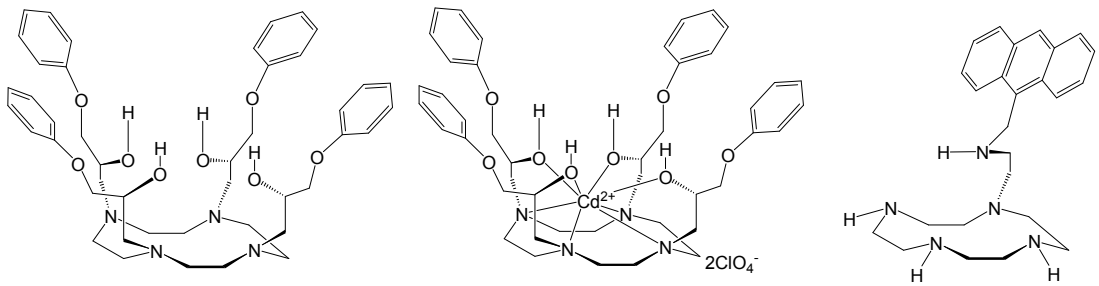
Chapter 2

Synthesis

2. Synthesis

2.1. The development of fluorescent pendant-armed cyclen-based receptor ligands

The objective of this project was to convert one of the pendant arms of the cyclen-based receptor ligand, **145**, to a similar, but potentially fluorescent, arm with minimal disruption to the including properties of the receptor complexes that would be formed from it. The use of anthracene as a fluorophore in this type of situation has been thoroughly discussed in the Introduction. Also, the synthesis of **131**, by Kimura and co-workers has demonstrated how it can be attached to cyclen resulting in a pendant arm not dissimilar to those employed in **1**, but having an N-H coordinating group rather than an O-H group. It was decided therefore to synthesise the potential receptor ligand, **146**, and to investigate whether or not its metal complexes would act as receptor complexes. If so, the fluorescence perturbation, if any, brought about by the act of molecular inclusion would be studied with a view to assessing the suitability of these cyclen based receptors to act as molecular sensors.

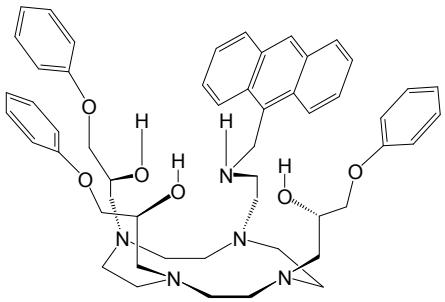


145 **1** **131**

Figure 2.1. The ligands **145**, **131** and the receptor complex **1**.

The synthetic strategy to be employed would follow three principal phases: The synthesis of the fluorescent pendant arm. The synthesis of a suitably protected cyclen derivative and the attachment of the pendant arms utilised in **1** to three of the

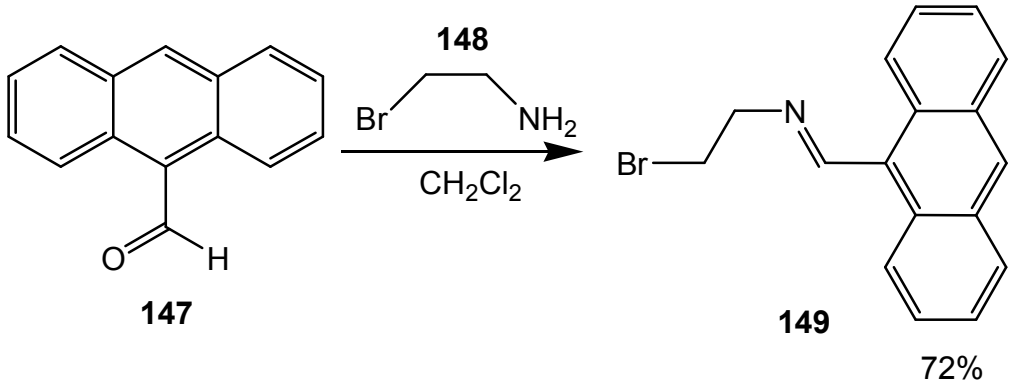
nitrogen atoms. The attachment of the fluorescent pendant arm to the remaining nitrogen atom.



146

2.2. Synthesis of the fluorescent pendant arm

The fluorescent signalling precursor unit 9-(2-bromoethyliminomethyl) anthracene, **149**, was synthesized in 72% yield using a modification of the method devised by Fabbrizzi and co-workers.[108] This involves a Schiff's base condensation between 9-anthraldehyde, **147**, and 2-bromoethylamine, **148**, as shown in **Scheme 2.1**.



147 **149** 72%

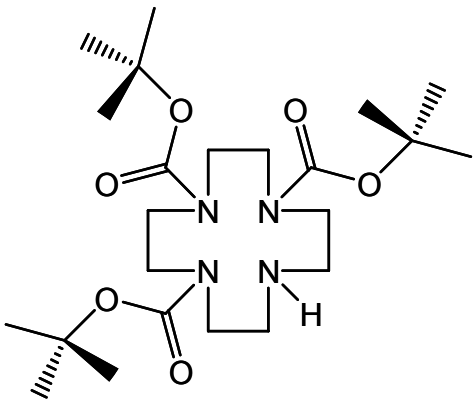
Scheme 2.1: Synthesis of the anthrylimine fluorophore.

The 2-bromoethylamine, **148**, was purchased as the hydrobromide salt, thus neutralisation with sodium hydroxide and extraction of the free base into dichloromethane prior to its use in the reaction was required. The resultant 9-(2-bromoethyliminomethyl)anthracene, **149**, was recrystallised from ether/hexane

giving the product as a fluffy yellow crystalline solid. All physical and spectral properties agreed with those in the literature.[108]

2.3. Synthesis of protected cyclen and attachment of the non-fluorescent pendant arms

There are many different methods in the literature for the protection of amines.[205-224] There are fewer methods for the protection of either one or three nitrogen atoms of a cyclen ring.[97,202,225-231] However, this is what was required for the synthesis of the desired fluorescent molecular receptors **4** and **5**. The best known method of tri-protection of cyclen is the addition of three *tert*-butyloxycarbonyl (BOC) groups, as described by Kimura and co-workers.[227]

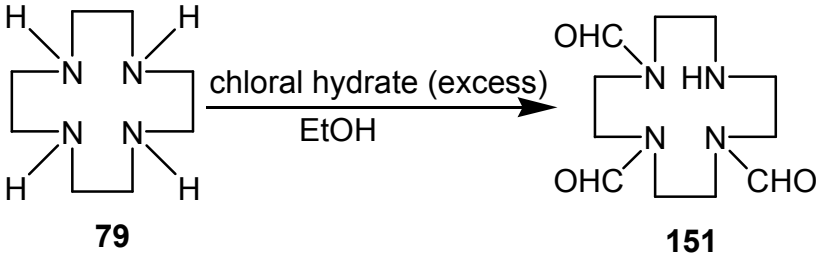


150

The synthesis of triBoc-cyclen, **150**, was investigated using the literature method,[227] however, the difficulty in forming **150** is that the addition of three BOC groups is a statistical addition, in that the products are a mixture of mono-, bi-, tri- and tetra-substituted products, with the tri-BOC-substituted cyclen as, at best, the major product. Separation of the products requires lengthy chromatography, and results in a low yield of **150**. A more recent, and much more elegant, protection method is the exclusive introduction of three formyl groups, using chloral

hydrate.[202,225] Subsequent, differential, protection of the remaining amine and removal of the formyl groups then allows the preparation of a mono-protected cyclen derivative. In this work this enabled the addition of the three identical pendant arms before the addition of the fluorophore containing arm as the last step. This was important as synthetic pathways investigated involving an earlier addition of the fluorophore resulted in decomposition, due to the photosensitivity of the anthracene.

The tri-formyl protected cyclen derivative, **151**, was synthesized from the reaction of cyclen, **79**, with an excess of chlorate hydrate, in quantitative yield and high purity, as depicted in Scheme **2.2**. The by-products of this reaction,



Scheme 2.2: Synthesis of 1,4,7-triformyl-1,4,7,10-tetraazacyclododecane, **151**.

chloroform and water, are easily removed *in vacuo* giving the pure product. Characterisation of the triformyl product **151**, was performed by ^{1}H and ^{13}C NMR. Both spectra exhibit broad peaks in the carbonyl and methylene areas. This is due to the restricted rotation around the amide carbon-nitrogen bond, which has partial double bond character, giving rise to the three distinct isomers (**Figure 2.3**) which have slightly different, but superimposed spectra.

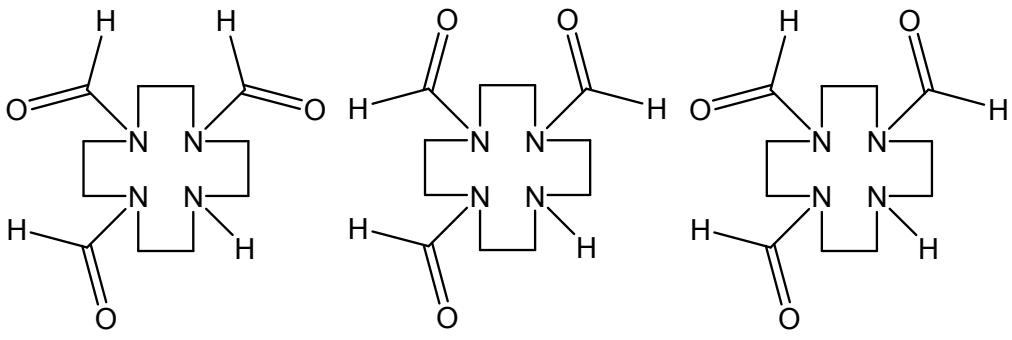
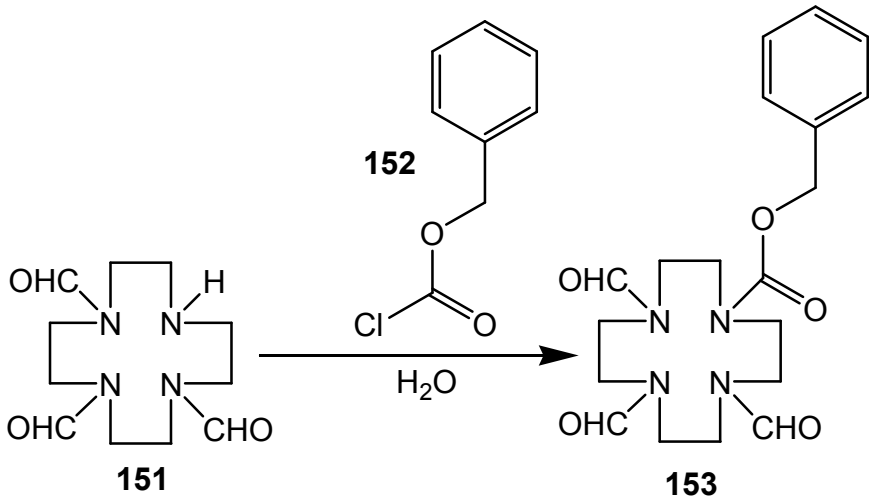


Figure 2.3. Representation of the isomers that result from the restricted rotation around the amide C-N bond.

The tri-formyl cyclen, **151**, was then reacted, without purification, with benzyl chloroformate, **152**, according to the procedure reported by Yoo and co-workers,[202] to give **153**, as shown in **Scheme 2.3**.

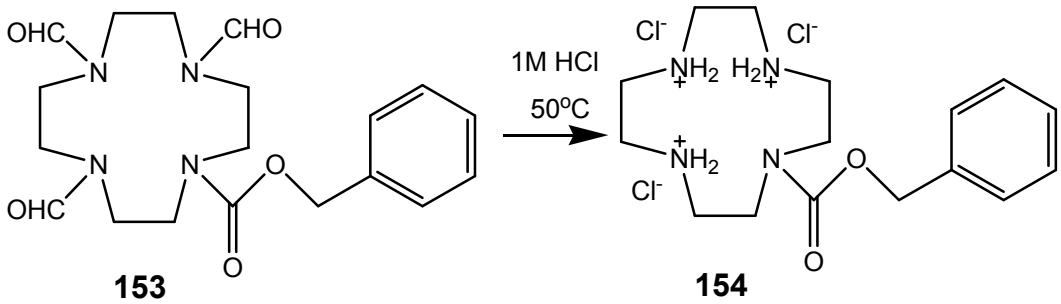


Scheme 2.3: Formation of 1,4,7-triformyl-10-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane, **153**.

To reach completion this reaction requires that the pH be maintained between pH 4 and pH 10. If the pH drops too low, removal of the formyl protecting groups may occur by hydrolysis,[225] giving rise to the possibility of multiple products with a varying amount of benzyloxycarbonyl substitution. If the reaction conditions are allowed to become even more acidic then the CBn group may also be removed, potentially giving cyclen as the product.[225] After the addition of the specified 1.5 molar equivalents of benzyl chloroformate, **152**, to the aqueous solution of **151**, the pH was found to be 9 (pH paper). The reaction was stirred for 1 h, after which time the pH of the reaction mixture was measured as *ca* pH 4 and adjusted to pH 10 by the addition of saturated sodium carbonate solution. The readjustment of pH was followed by the further addition of 1.5 molar equivalents of benzyl chloroformate. The reaction was again stirred for 1 h, after which time the pH of the reaction mixture was measured as *ca* pH 6 and readjusted to pH 10. A final 1.5 molar equivalents of benzyl chloroformate was then added, and the reaction mixture

allowed to stir overnight. The reasons for using a large excess of benzylchloroformate over multiple additions are two-fold. Firstly there is the potential for the competition between the triformyl protected macrocycle and water for the reaction with benzyl chloroformate, resulting in the production of HCl and carbonic acid monobenzyl ester.[215,216,224] This results in a lowering of the pH as well as consumption of the starting materials. Secondly work conducted in the Wainwright laboratory has shown that the addition of a fourth arm is slow in mixed pendant armed systems. In mixed arm syntheses the addition of the fourth arm may take over a week at equimolar amounts. The use of a large excess of benzyl chloroformate in the reaction with triformylcyclen speeds up the reaction so that it is completed overnight in 97% yield.

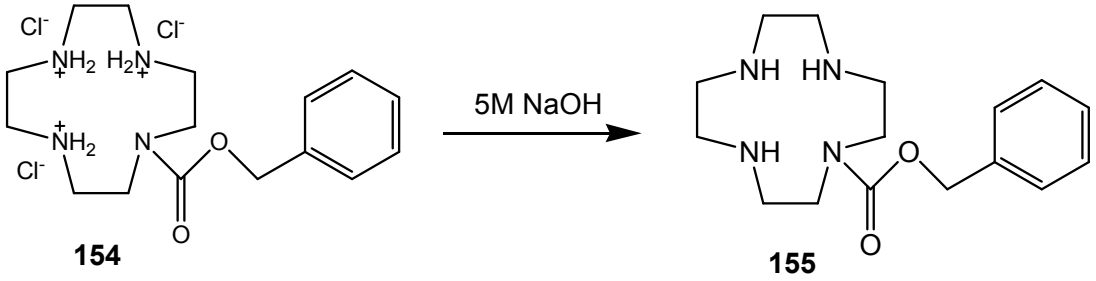
Deprotection of the formyl groups from **153** was performed in quantitative yield following the literature method.[202] Mild acidic treatment (1M HCl at 50°C) achieved selective cleavage of the three formyl groups without attacking the CBn group. This produced the hydrochloride salt of 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane, **154, Scheme 2.4**.



Scheme 2.4: Synthesis of 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane.3HCl.2H_2O, **154**.

Removal of the formyl groups was straightforward as long as the temperature of the reaction was kept at 50°C. Initial attempts at this reaction showed that if the temperature was allowed to rise above 80°C the N-CBn bond was broken resulting in cyclen being produced.[202,225]

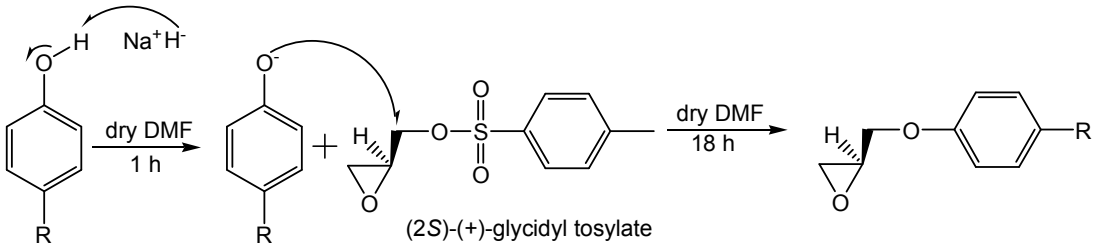
Conversion of the mono-protected macrocyclic ammonium salt to the free macrocyclic amine was simply achieved by treatment of **154** with 5M NaOH until pH 13 was achieved, giving the free amine, **155**, in 92% yield, after extraction into chloroform and removal of the solvent, Scheme **2.5**.



154 **155**

Scheme 2.5: Synthesis of 1-(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane, **155**.

With the mono-protected free amine prepared with a high overall yield, the next stage of the synthesis of **4**, **5** was the addition of the three identical pendant arms. This was achieved by the reaction of **155** with the epoxides **160**, **161** and **162**, respectively. Each epoxide was synthesized by reacting the desired phenol with (2*S*)-(+)-glycidyl tosylate. This is a reaction that proceeds with retention of stereochemistry, as shown in **Scheme 2.6**.[114,232]



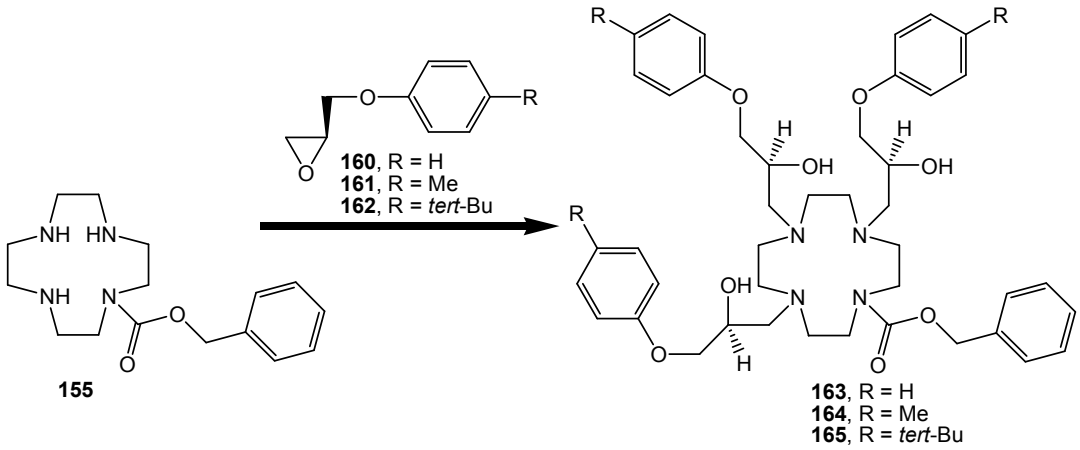
156, R = H
157, R = Me
158, R = *tert*-Bu

159

160, R = H
161, R = Me
162, R = *tert*-Bu

Scheme 2.6: The synthesis of epoxides, **160-162**.

The reactions of the mono-protected cyclen, **155**, with three equivalents of the desired chiral epoxides, either **160**, **161** or **162**, were performed by refluxing the appropriate combination in dry ethanol for 5 days. All three products, **163**, **164** and **165**, were obtained in quantitative yield, as viscous yellow oils, as shown in **Scheme 2.7**.

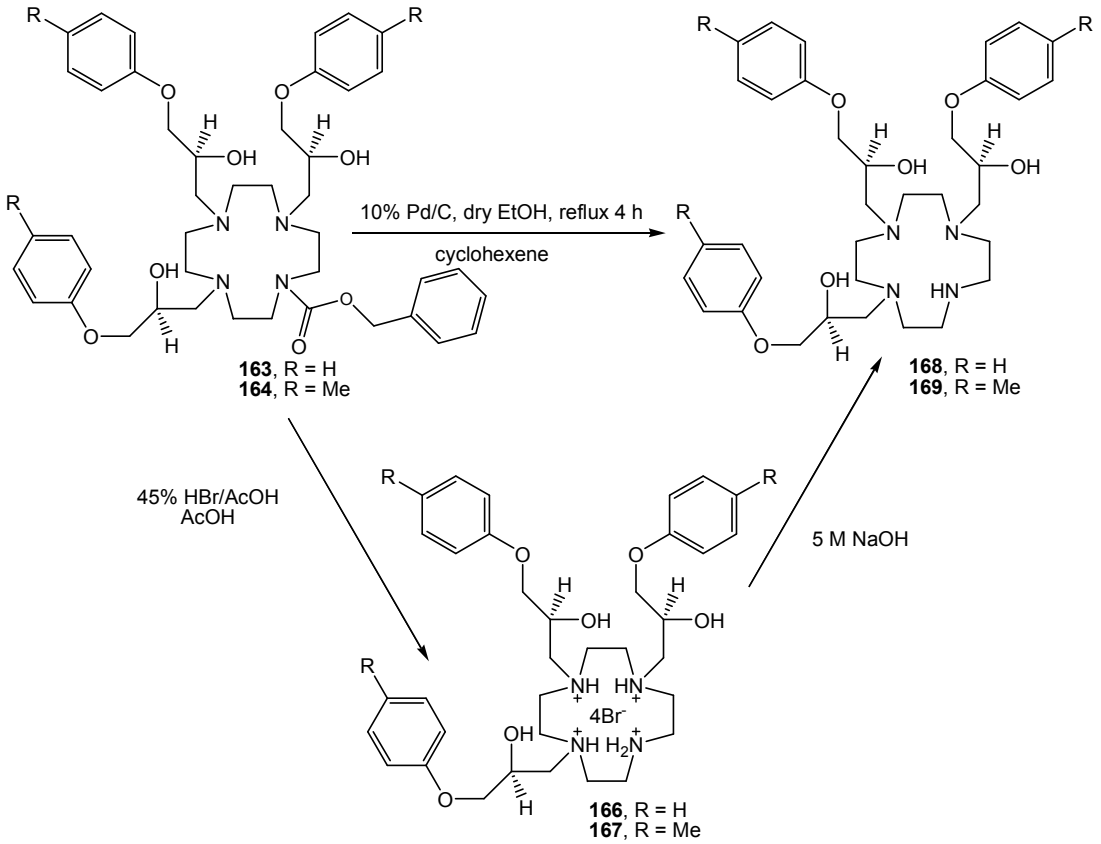


Scheme 2.7: The attachment of pendant arms to **155**.

The ^{13}C NMR spectrum of each showed the peaks expected from just a single diastereomer, confirming that racemisation of the stereogenic carbon atom had not occurred during the reaction and that the products were homochiral. The peaks for the phenoxy arms and the CBn protecting group were visible, as were the four resonances for the cyclen methylene carbons. Due to symmetry there are two sets of resonances for the phenoxy-pendant arms that are very close together, the more intense of which was for the *trans*-related pendant arms.

The removal of the CBn protecting group was accomplished by either acid hydrolysis[233,234] or catalytic transfer hydrogenation, Scheme **2.8**.[235]

Acid hydrolysis is a well-known procedure generally used in the removal of CBn groups during the synthesis of peptides.[233,234] This process was initially used successfully to remove the protecting group of the tri-phenoxy armed mono-CBn-protected cyclen derivatives **163**, and **164**. The procedure involved dissolving the protected macrocycle in acetic acid then treating the solution with 45% HBr in acetic acid for 5 h, after which time diethyl ether was added to cause the product to precipitate. The precipitated tetraprotonated product was filtered off and found to be



Scheme 2.8: Removal of the CBn protection.

extremely hygroscopic. Conversion to the free ligand was by treatment with 5 M NaOH solution. However, as the overall yield for this pathway is low (*ca* 35%), the much higher yielding and faster catalytic transfer hydrogenation process[215,216,235-237] was preferred.

Deprotection of the CBn group by catalytic transfer hydrogenation proceeded to give **168** in 97% yield, and **169,** in 60% yield. The CBn group fragments into a benzyl group and a carboxy fragment, which form toluene and formic acid respectively.[236-238] As well as protonating the nitrogen of the ring, this accounts for the four hydrogen atoms that cyclohexene is able to donate as it dehydrogenates to form benzene. The ^{13}C NMR spectra for both these compounds exhibit chemical shifts for the phenoxy pendant arm carbons at frequencies similar to those of the CBn-protected precursor, as well as the disappearance of all CBn resonances.

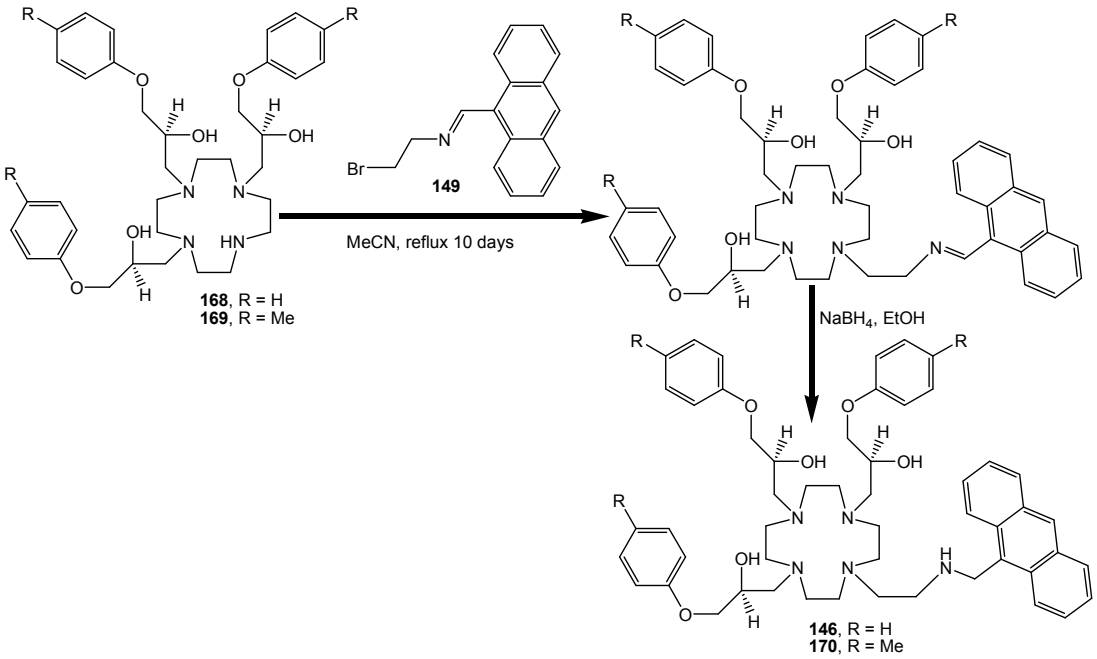
2.4. Synthesis of the fluorescent ligands

Two series of fluorescent ligands, with an anthrylamine containing fluorophore as a pendant arm, were developed. **146** and **170** were formed from the reaction of the three-pendant arm ligands, just described, with **149**, whereas **171**-**172** were formed directly from **131**.

2.4.1. Synthesis of the ligands 146 and 170.

Once the cyclen derivatives with three pendant arms had been obtained, the next stage of the reaction sequence was the addition of the fluorophore containing arm **149**, as shown in **Scheme 2.9**. Care was taken for all reactions involving the fluorophore, due to the ease of photodecomposition.

Receptor ligands **146** and **170** were prepared by the reaction of the bromoethylanthrylimine, **149**, with the macrocyclic amine **168** or **169**. The reactions



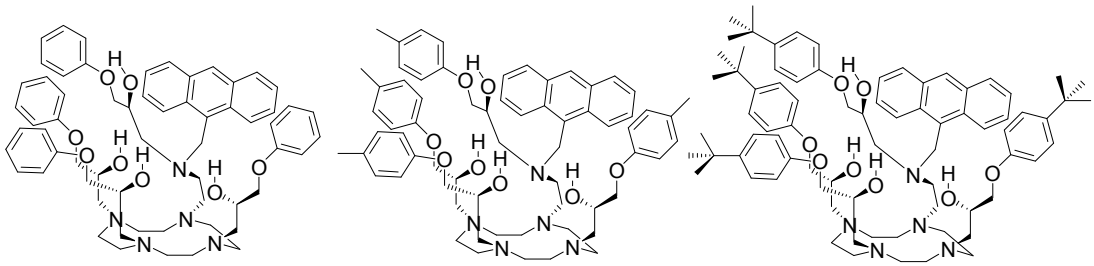
Scheme 2.9. Synthesis of the fluorescent ligands **146** and **170**.

were monitored by TLC and complete consumption of the starting materials was observed after 10 days. Analysis of the resulting red oils by NMR showed the presence of a small amount of an impurity, which could not be removed at this stage. The imines were then reduced with $NaBH_4$ giving the crude amines as red oils. Purification was achieved by column chromatography (basic alumina, 41% and 45% yields, respectively). The UV-visible spectra of the pure compounds showed the usual anthracene absorptions, which were seen for all species containing this fluorophore. The 0-0 transition at ~387 nm, the 0-1 transition at ~367 nm, the 0-2 transition (~349 nm) and the 0-3 transition (~333 nm). The ^{1}H NMR spectra were confused by the superimposition of peaks in the mass of signals that ran together in the broad region δ 5.0-2.5. However, the usual anthracene signals were all observed (a 2 H doublet, J = 8.3 Hz, at δ 8.54, a 1 H singlet at δ 8.44, a 2 H doublet, J = 8.3, at δ 8.00 and a 4 H multiplet at δ 7.47). The anthracene proton to phenoxy proton ratio of integrations was correct at 9:15 for **146** and 9:12 for **170**. The ^{13}C NMR spectra showed the expected phenoxy and anthryl aromatic peaks. Microanalysis of the acid salts confirmed the proposed formulation.

2.4.2. Synthesis of antac-12, 131

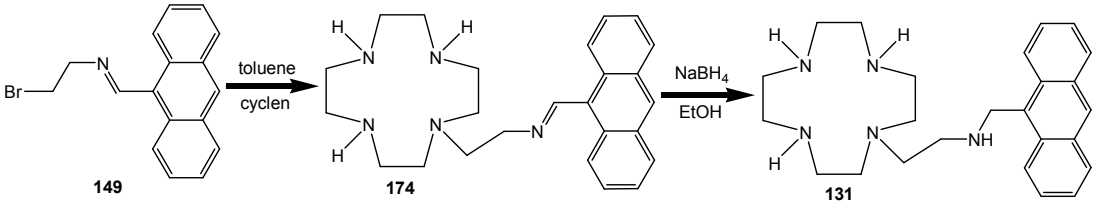
Mono-*N*-alkylation of 1,4,7,10-tetraazacyclododecane, cyclen, **79**, with the fluorogenic arm, **149**, to give **antac12**, **131**, was the first step in the synthetic pathway to the receptor ligands **171-173**.

A number of synthetic approaches for the mono-*N*-functionalisation of cyclen exist. The approach utilized here was that of direct alkylation using an excess of cyclen.[239,240] Optimum conditions required refluxing with a fivefold excess of



171 **172** **173**

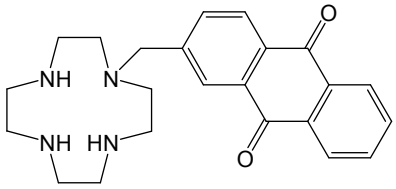
cyclen, **79**, over that of the alkylating agent **149**, as shown in **Scheme 2.10.** The cyclen acts as both the azacrown being alkylated as well as the base to neutralise the HCl produced. The cyclen hydrobromide salt formed was filtered off and recycled. This gave the imine derivative, **174**, in 86% yield, which was used without further purification.



149 **174** **131**

Scheme 2.10: The synthesis of **antac-12**, **131**.

Reduction of the imine bond, by $NaBH_4$, yielded **131**, which was purified by either the use of a basified silica column, or a basic alumina column. Pre-treating a silica column with base overcomes the tendencies of amines to become protonated (and hence bind) on the column. Thus, Kimura and co-workers[241] used an eluant system of CH_2Cl_2/MeOH/28% aq. NH_3 (95:4:1) for the related structure **175** with a yield of 35%. In this work pre-treating the silica with the stronger base



175

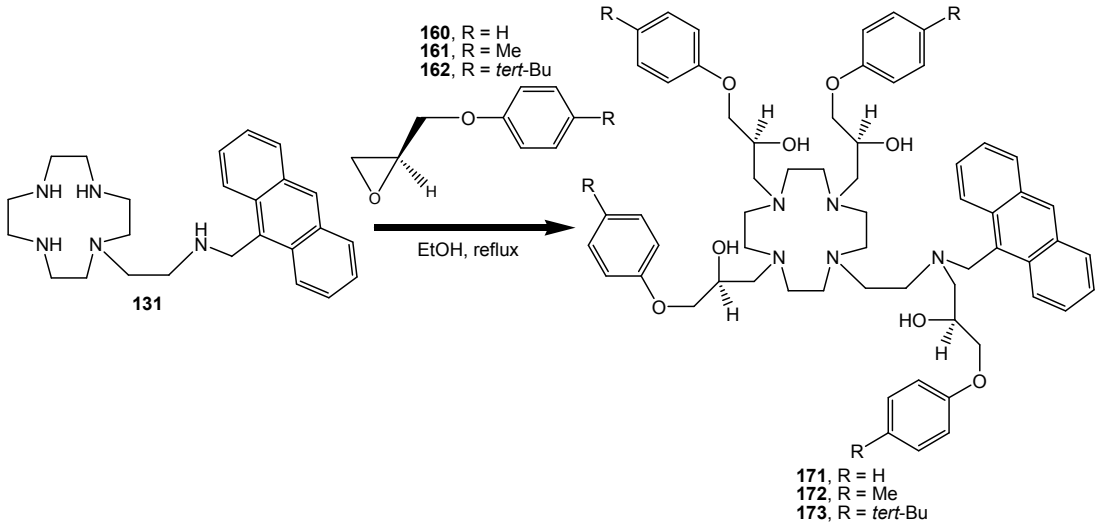
triethylamine, followed by rinsing the column with CH_2Cl_2/MeOH/28% aq. NH_3 (70:24:6) created a suitable medium for the purification of **131**, resulting in a red/gold coloured oil in 48% yield. Purification was improved by using a basic

alumina column (Fluka basic alumina oxide, pH 10±0.5, Brockman II (100-290 mesh)) with 2% MeOH/CH_2Cl_2 as the solvent system, resulting in a pale reddish oil in 55% yield. The ^1H and ^{13}C NMR spectra for the purified product were consistent with those reported for **131**.[151] The UV-Vis spectrum of the product, **131**, in acetonitrile, showed the usual anthracene-like vibrational fine-structure. Formation of the hydrochloride and hydrobromide salts by treatment of **131** with acid in ethanol produced pale brown solids that were microanalytically pure and gave ^1H and ^{13}C NMR spectra in agreement with those reported in the literature.[151]

The addition of the epoxides **160** and **161** to **131** had been initially attempted for the synthesis of **146** and **170** prior to the use of the protection and deprotection pathways. However, the problem of reaction at the anthrylamine as well as the secondary amines of the cyclen residue could not be controlled and so the pathway described earlier had to be followed. For the synthesis of the per-*N*-alkylated receptor ligands **171-173**, however, this was not an issue.

2.4.3. Synthesis of the *N*-alkylated fluorescent ligands 171-173.

Four equivalents of the appropriate enantiomerically pure epoxide were refluxed in ethanol along with **131**, as shown in scheme **2.11**. Monitoring with TLC showed that all of the initial starting materials were not completely consumed until over one week into the reaction. Purification by column chromatography gave the desired products, **171-173** as reddish/gold viscous oils in 75-85% yield. Each had the expected UV-visible, ^1H and ^{13}C NMR spectra. Microanalysis of the hydrochloride salts corresponded to the proposed structures.



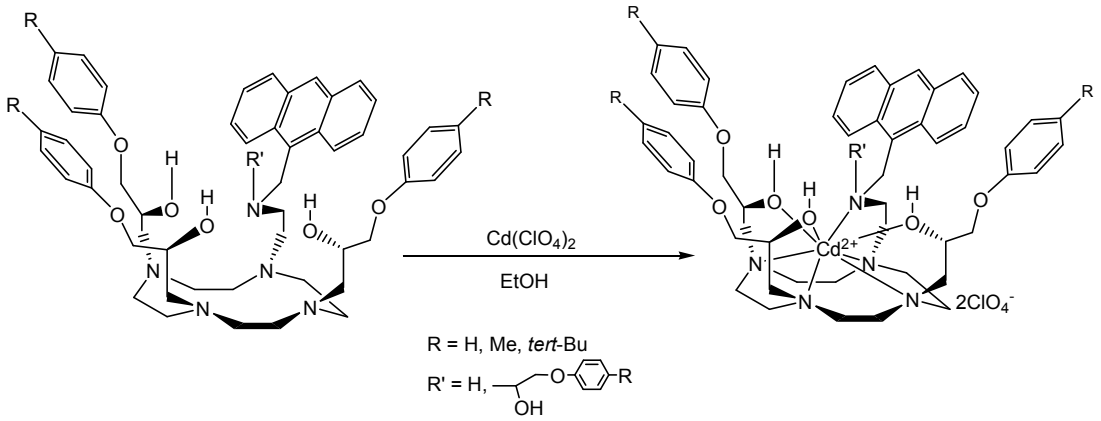
Scheme 2.11: Synthesis of the N-alkylated ligands **171-173**.

2.5. Synthesis of metal(II) complexes

Safety Note: Perchlorate salts are potentially explosive. Although no problems were encountered in this work, extreme care should be taken when handling these substances.

Cd(II) was chosen for the initial series of complexation experiments as it is well known to form eight-coordinate complexes, with the ligands under discussion, that are conformationally rigid and of high stability.[114,115,123,125,242,243]

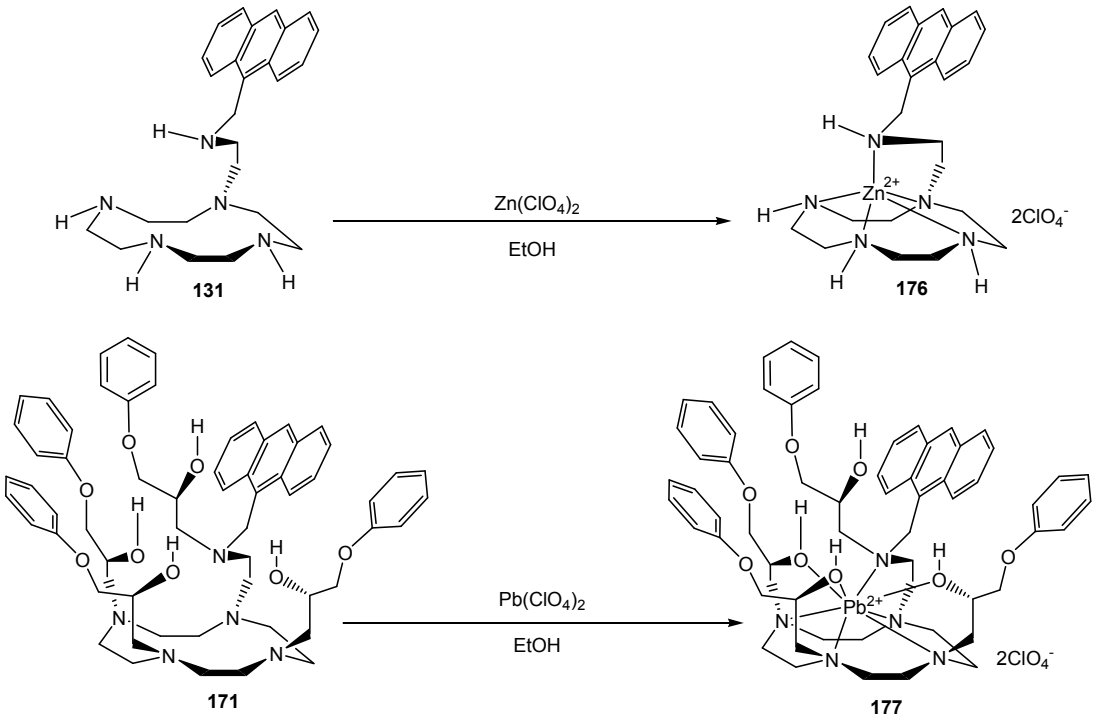
The Cd(II) complexes, **4-8**, were formed by adding a solution of cadmium(II) perchlorate hexahydrate to a solution of the desired ligand (**146, 170-173**) in boiling ethanol, as shown in Scheme **2.12**. The products precipitated and were collected by filtration as light cream coloured powders, in 45-83% yield, and were characterised by microanalysis, UV-visible spectroscopy, ^{1}H NMR and ^{13}C NMR spectroscopy.



Scheme 2.12: Preparation of Cd(II) receptor complexes, **4-8**.

The complexes were very soluble in a range of solvents including DMF, acetonitrile, 20% aqueous acetonitrile, 20% aqueous 1,4-dioxane and DMSO. The complex was also mildly soluble in MeOH, however use of MeOH as a solvent resulted in slow decomposition of the product. Knowledge of complex solubility and stability were important for the later investigation of **4-8** as potential anion sensors. The isolation of **4-8** as perchlorate salts was important as perchlorate ions generally have low affinities for coordination with cations, and thus do not compete with the donor atoms of the receptor ligand, which could result in a collapse of its conical conformation. The ionic nature of the perchlorate complexes was confirmed in the case of **4** by measuring its molar electrical conductivity and recording a value of 175 Ω^{-1} cm^2 mol^{-1} in DMF which is indicative of a 1:2 electrolyte in this solvent.[244] This allows the replacement of one perchlorate ion with a guest anion that has the potential to include in the receptor binding cavity. Unfortunately all attempts at crystallisation of these metal complexes were unsuccessful due to the decomposition of the product.

The Zn(II) complex of **131** and the Pb(II) complex of **171** were produced similarly, as shown in scheme **2.13**.

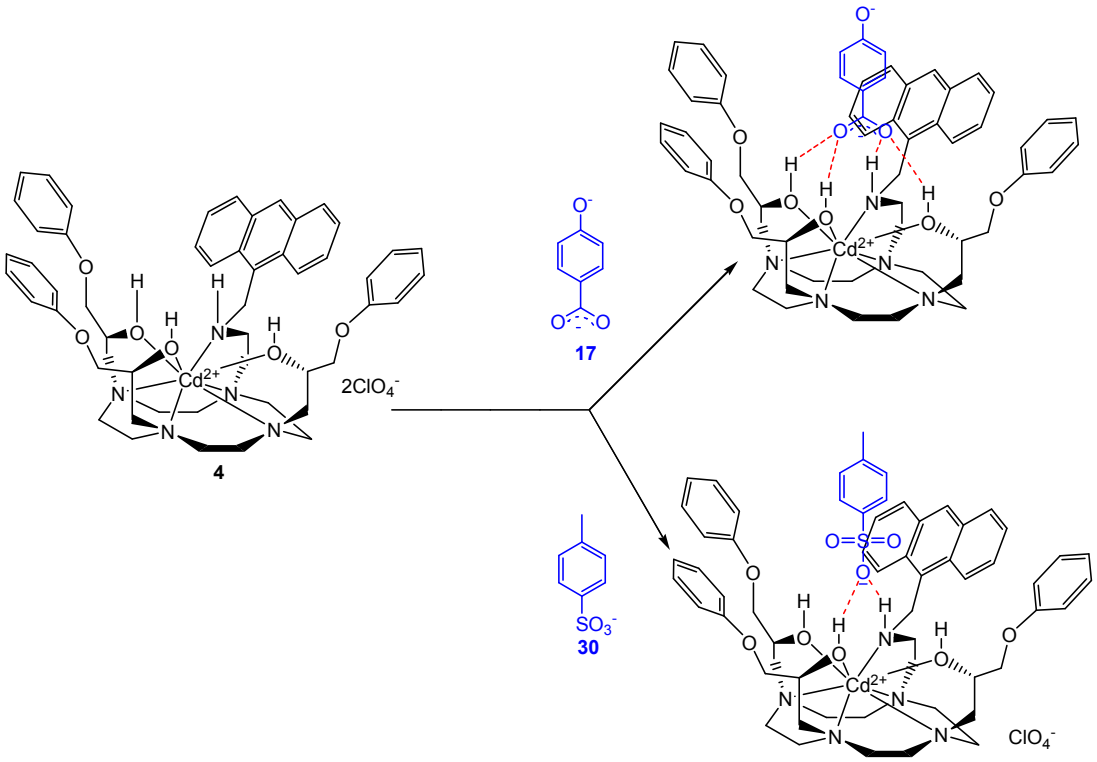


Scheme 2.13: Synthesis of [Zn(**antac-12**)]$(ClO_4)_2$, **176**, and [Pb(**171**)]$(ClO_4)_2$, **177**.

2.6. Isolation of host-guest inclusion complexes

The inclusion complexes corresponding to the inclusion of the dianion of *p*-hydroxybenzoic acid, **17**, and *p*-toluenesulfonate, **30**, within receptor complex **4** were able to be isolated. This was achieved by the addition of the sodium salt of the appropriate guest anion to a boiling solution of **4** in acetonitrile, which was refluxed for 2 h (Scheme **2.14**). Removal of solvent, followed by trituration with ether, gave light brown powders. Boiling in EtOH removed the occluded sodium perchlorate. The inclusion complexes were isolated as pale brown powders in 70-79% yield. ^{1}H and ^{13}C NMR spectra revealed the presence of signals for both the host and the guests in a 1:1 ratio and microanalysis supported this formulation. Conductivity measurements in DMF showed a transformation from the 1:2 electrolyte behaviour, seen with **4**, to approximate 1:1 electrolyte behaviour consistent with *ca* 50%

retention of **17** and a higher level of retention of **30** within the structure of **4** in this solvent.



Scheme 2.14: Schematic representation of the inclusion of the dianion of *p*-hydroxybenzoic acid and *p*-toluenesulfonate within the structure of receptor complex **4**.

Chapter 3

Initial Measurement of Binding Constants for Host-Guest Interactions

3. Initial measurement of binding constants for host-guest interactions

Prior to embarking on a study of the fluorescence of presumed host-guest complexes of the new host complexes **4**-**8**, it was important to verify, using previously tested methods, that **4**-**8** do in fact form host-guest complexes, analagous to those formed with receptors **1**-**3**. The simplest way to do this was to set out to measure some binding constants for potential host-guest combinations using the well established ^{1}H NMR method, which had previously been used to cross check with crystal structures for host-guest complexes of **1**-**3**.

3.1. The measurement of host-guest binding constants using ^{1}H NMR

The use of ^{1}H NMR titration experiments for the investigation of host-guest interaction is widespread.[58,66,114,125,245,246] In particular, these titrations can be used to calculate the binding constants for host-guest interactions, which enable quantitative comparisons of the strength of the association to be made. This in turn provides knowledge about the selectivity of each host that is studied. The principle of the method is that changes in the chemical shift of the ^{1}H NMR resonances (if any) belonging to the host or guest can be monitored and related to the amount of bound and free host or guest, respectively, in the sample. There are two possible cases that can be encountered in ^{1}H NMR when dealing with host-guest systems, slow exchange and fast exchange. If the exchange of the complexed and uncomplexed guest is slow on the NMR time scale then both free and bound species have separate distinguishable resonances, as shown in **Figure 3.1**, and the binding constant may be determined by simple integration of the NMR signals for bound and unbound host or

guest. As most host-guest equilibria are fast in the NMR timescale, the chemical shift observed for a resonance that is sensitive to complexation is the weighted average between the chemical shift of the free and bound species, as shown in **Figure 3.1**.[247]

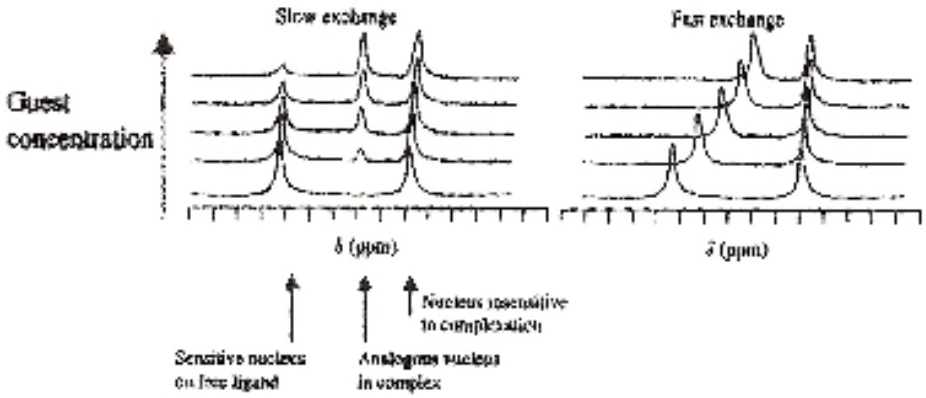


Figure 3.1. A schematic of the observed resonances in the NMR spectra of equilibrating mixtures of free host, guest and host-guest complex. This diagram was reproduced from Steed and Atwood.[247]

The shape of the titration curve (a plot of the δ of the monitored species against the increasing concentration of the other species, **Figure 3.2**) can then be analysed to give the binding constant, as described in **Appendix A.1**. During the course of this investigation only fast exchange situations were observed.

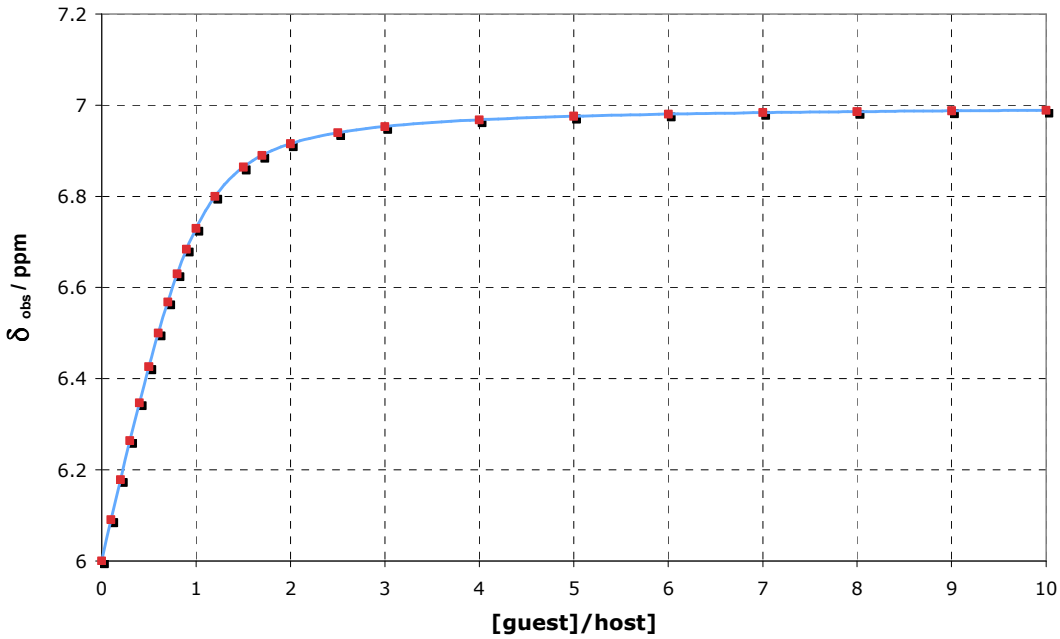


Figure 3.2. Usually for cases of fast exchange the log$K$ is determined from the plot of the chemical shift (δ) of the observed host against the concentration of the guest.[247]

3.2. Strategy for obtaining binding constant values

In this work 10^{-3} mol dm^{-3} solutions of the guest species in DMSO-d_6 were titrated with a variety of receptor complexes. This slightly unconventional approach (it is usual for the host species to be titrated with the guest species, as shown in **Figure 3.2**) was adopted because the majority of guests, like the host, were aromatic species. At high concentrations of host, the guest resonances are usually at least partly obscured by those of the host (due to the larger concentration of aromatic protons found within the hosts compared to the guest). It has been found previously[114,125] that the guest resonances undergo considerably greater chemical shift change than the host resonances and that it is these guest resonances, therefore, that must be monitored with minimal interference from host resonances. Irrespective of the approach, this method is only useful for measuring log*K* values up to *ca* $10^{4.5}$ mol^{-1} dm^3 when the concentrations of the host and guest are in the millimolar regime, as they must be for normal NMR work. Above this value the shape of the 'curve' is largely independent of *K*, as shown in **Figure 3.3.**

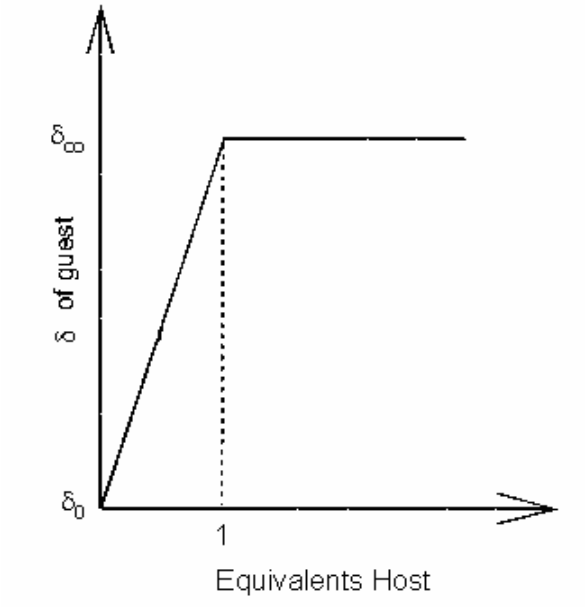


Figure 3.3. A schematic of a potential titration plot where log*K* > *ca* 4.5, and [guest] is *ca* 10^{-3} mol dm^{-3}. The entire plot is virtually linear, dependent only on δ_∞ and δ_0, rather than log*K*.

The aromatic guests chosen for study were mostly the conjugate bases of *para*-substituted aromatic acids. These have C_2 symmetry, giving rise to an AA'BB' pattern of resonances in the ^{1}H NMR spectrum, when the *para*-substituents are different (**Figure 3.4**). The pattern of resonances is quite distinctive, appearing as two distorted doublets, and allows the guest resonances to be located and monitored easily.

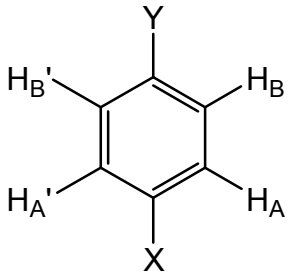


Figure 3.4. A *para*-disubstituted aromatic ring with differing substituents X and Y. H_A and H_A' are chemically equivalent, but magnetically non-equivalent, as are H_B and H_B'.

3.3. An overview of titrations of anionic guests with receptors

The detailed experimental procedure utilised in the ^{1}H NMR titration of guest anions with receptor complexes is outlined in Chapter 6. Generally speaking it involved the addition of aliquots of a solution of the receptor complex (the host) in DMSO-d_6, to a solution of the guest anion, at known concentration, in the same solvent. The chemical shift of a suitable ^{1}H NMR resonance originating from the guest anion was monitored throughout the titration process. The receptor : guest ratio progressed from 0:1 up to a large excess of receptor (typically 5:1 or greater). The binding constant (log*K*) value for the association of the guest with the host complex was determined though a non-linear regression analysis of the chemical shift data, as described in Appendix A.1.

An example is the titration of the guest anion *p*-nitrophenolate, **9**, with the receptor [Cd((*S*)-athppc)]$(ClO_4)_2$, **4**, in DMSO-d_6. Both aromatic guest protons experienced a downfield chemical shift change on addition of host. The downfield shifts in the ^{1}H NMR spectra are shown in **Figure 3.5**, and are taken to be indicative

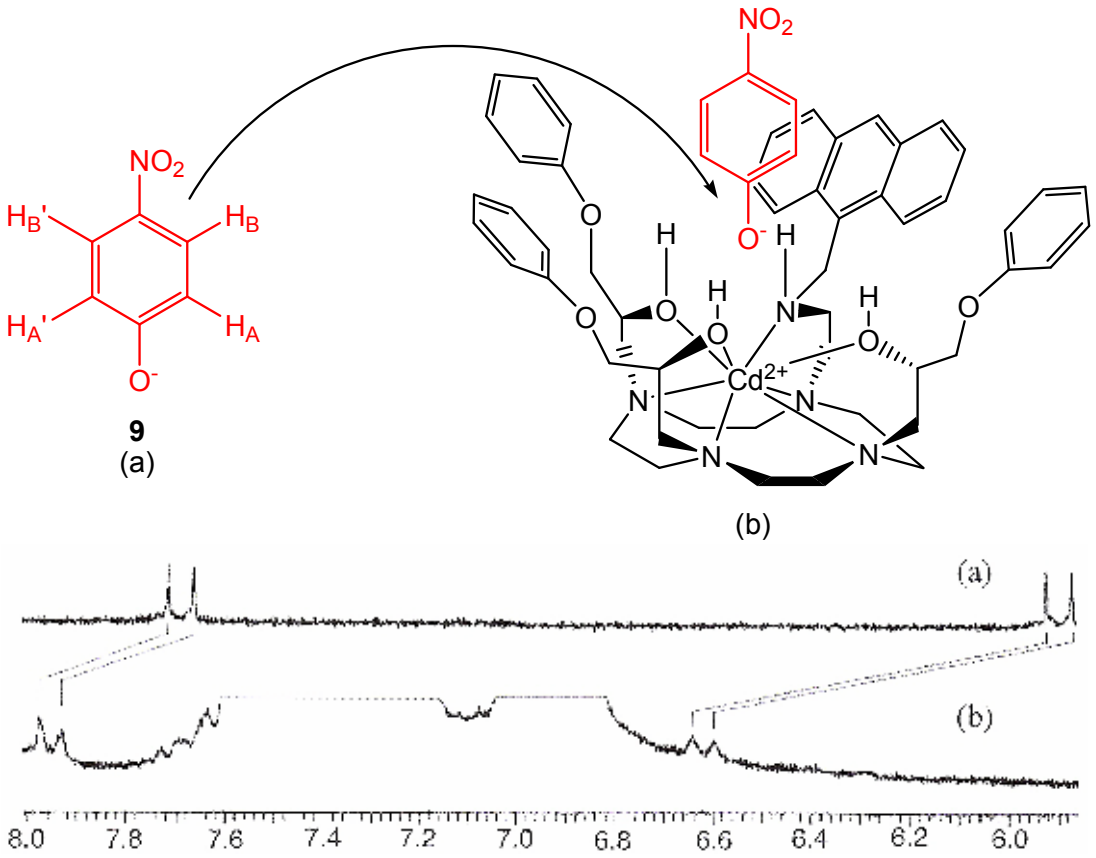


Figure 3.5. ^{1}H NMR spectra of (a), 10^{-3} mol dm^{-3} sodium *p*-nitrophenolate, **9**, and (b) 10^{-3} mol dm^{-3} sodium *p*-nitrophenolate, **9**, plus 5 x 10^{-3} mol dm^{-3} receptor **4**, in DMSO-d_6.

of an inclusion interaction. The titration curve obtained from monitoring the change in chemical shift of H_A is shown in **Figure 3.6.** Proton H_B shows a similar behaviour, although the smaller downfield chemical shift change (*ca* 0.25 ppm, compared to *ca* 0.7 ppm) is indicative of its greater distance from the phenolate binding site. The titration curve obtained from monitoring the change in chemical shift of H_B is shown in **Figure 3.7.** Both curves show an approximately linear relationship from the region of free guest to a region approximating the addition of

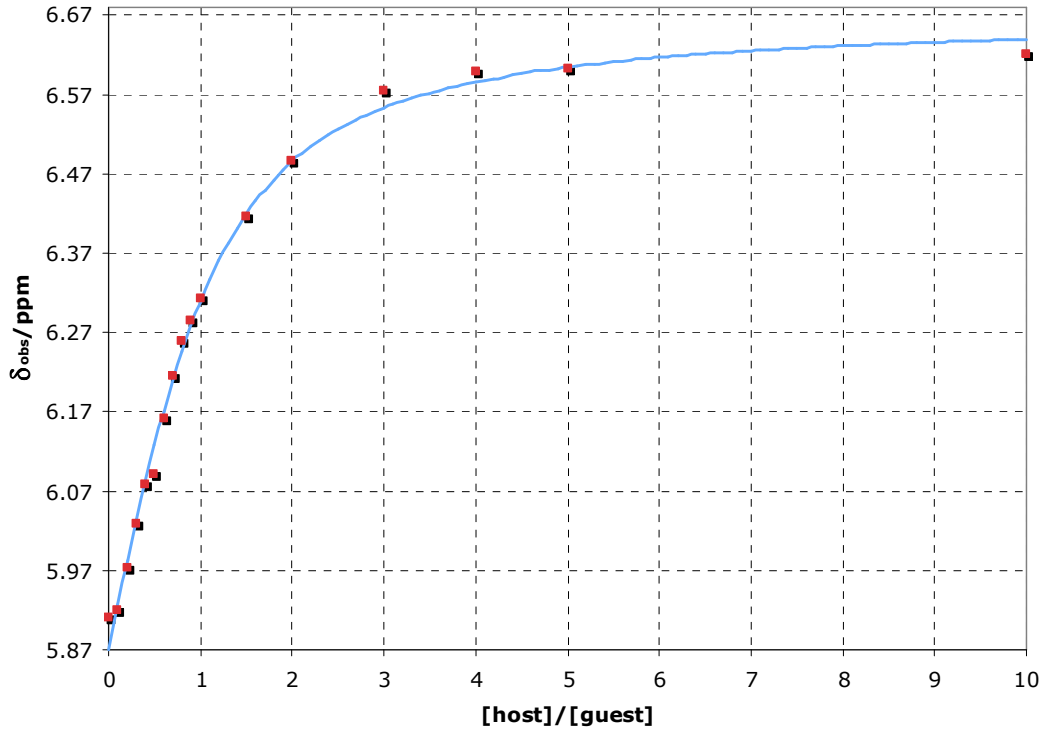


Figure 3.6. Titration curve showing chemical shift change in guest proton H_A of sodium *p*-nitrophenolate, **9**, on variation of receptor **4** : guest ratio. Squares indicate the experimental data points and the curve indicates the theoretical δ values for the calculated values of *K* and δ_{HG}.

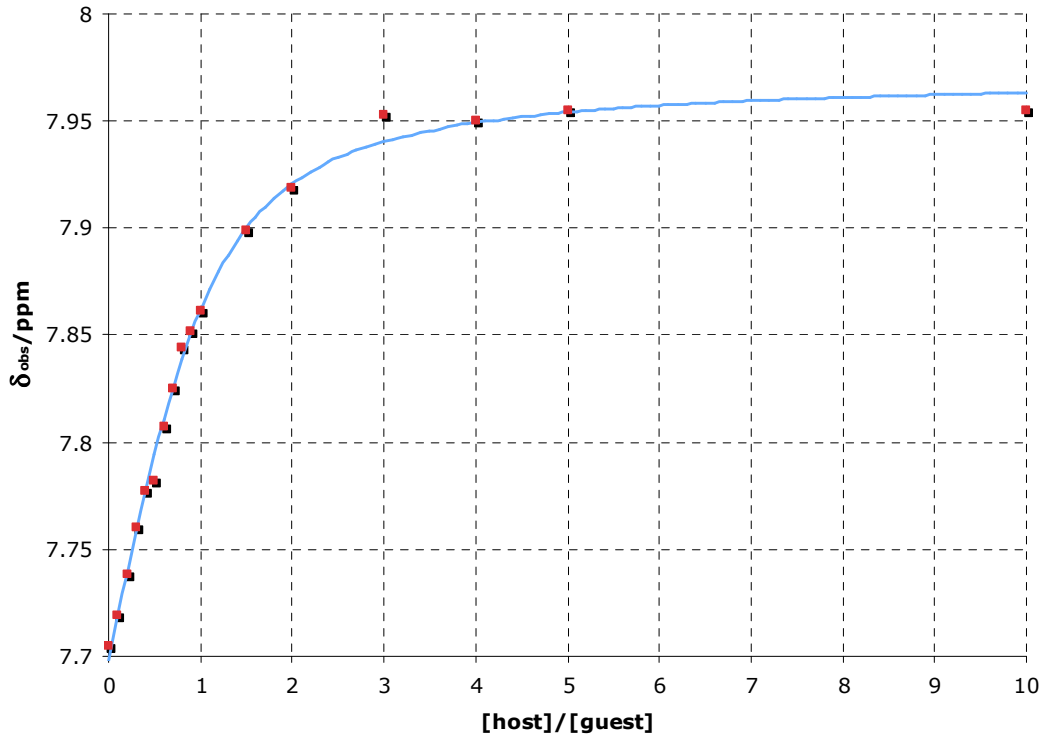


Figure 3.7. Titration curve showing chemical shift change in guest proton H_B of sodium *p*-nitrophenolate, **9**, on variation of receptor **4** : guest ratio. Squares indicate the experimental data points and the curve indicates the theoretical δ values for the calculated values of *K* and δ_{HG}.

one molar equivalent of receptor **4**, which was consistent with the formation of a 1:1 host-guest complex, with a log*K* of *ca* 4, as may be seen schematically in **Figure A.1** of Appendix A.1.

Values for the binding constant (*K*) were calculated from both sets of chemical shift data, and were the same within experimental error (log*K* = 3.5 ± 0.1). Therefore, in later cases, only the *K* value calculated from one set of aromatic resonances is reported. Indeed it was not possible to do otherwise, for many of the host-guest systems investigated, since the host peaks often swamped one of the sets of resonances for the guest.

3.4. Binding constants of anionic guests with receptors

3.4.1. Selection of potential guest species.

A series of potential guests were previously identified by Smith[114,115,120-123,125,242] as suitable for host-guest inclusion, involving receptors of the type under study here. These guests were *p*-nitrophenolate, **9**, *p*-nitrobenzoate, **14**, *p*-aminobenzoate, **15**, phenoxyacetate, **25**, acetate, **178**, histidinate (both (*D*)-, **26**, and (*L*)-, **27**) and toluenesulfonate, **30**. These guests can be divided up into five classes; phenolates, benzoates, acetates, amino acids and sulfonates. These five classes of guests encompass a plethora of potential compounds for investigation of host-guest complexation with the receptor complexes. Whilst many compounds investigated were merely chosen due to their availability, the five classes allow for many interesting guests to be examined. Examples of interesting guests are the biologically active neurotransmitter serotonin, **12**, which amongst other things

controls mood and the sleep cycle, and is the target of most antidepressant drugs. The parent amino acid, tryptophan, is also examined in this work, as is *p*-dimethylaminophenolate, **11**, which is a drug used to treat cyanide poisoning. Also of interest is the sodium salt of syringic alcohol, **13**, which is an undesirable compound in wine, and which was also found to bind.

Not all potential guests examined showed a change in chemical shift in the ^{1}H NMR spectra. These included resveratrol, **32**, (*trans*-3,5,4'-trihydroxystilbene, which has long been a part of oriental medicine but which has only attracted scientific attention in the past two decades),[248-251] and a series of other compounds found within wine; *p*-ethylphenolate, **179**, guaiacole, **180**, *p*-ethylguaiacole, **181**, vanillyl alcohol, **33**, and gallic acid, **22**. It was not fully clear, however, whether or not a lack of change in the chemical shift should always be taken as being indicative of a lack of binding, since measurements for the inclusion of some other guests, such as *p*-toluenesulfonate, **30**, showed an absence of guest chemical shift changes during the titration, even though inclusion complexes with both **1**[114] and **4** can be isolated, as mentioned in **Chapters 1 and 2**, and the guest, **30**, has been shown by X-ray diffraction to bind within the receptor cavity of **1** in the solid state.[114] Other guests, such as *p*-aminobenzoate, **15**, showed no chemical shift change with some hosts, **1-3**, but did show shift change with hosts **4**, **6** and **7**.

Establishing the reasons why some potential guests bind, whilst other compounds do not, was one objective of this work. Since hydrogen bonding is known to be involved in the retention process the ability of the guest anion to act as a hydrogen bond acceptor, as gauged by its pK_a value, was a matter of interest. A table of all investigated potential guest compounds, and their pK_a values, is shown as **Table 3.1.** Whilst there are some similarities between the pK_a values of the guests

Table 3.1. The pK_a values for the potential guest molecules examined, and whether there was an observed change in chemical shift upon titration with receptors.

Guest Class	Name:	pK_a values[a]	$\Delta\delta_{obs}$
Phenolates	*p*-nitrophenol (**9**)	7.15	Yes
	picric acid (**34**)[b]	0.38	No
	p-formylphenol (**10**)	7.72	Yes
	p-dimethylaminophenol (**11**)	5.93, 10.11	Yes
	m-dimethylaminophenol	4.79, 10.16	No
	serotonin (**12**)[b]	9.69, 10.31	Yes
	syringic alcohol (**13**)[b]	9.77	Yes
	p-cresol (**157**)	10.26	No
	p-ethylphenol (**179**)	10.2	No
	p-chlorophenol	9.43	No
	p-*tert*-butylphenol (**158**)[b]	10.2	No
	p-fluorophenol[b]	9.95	No
	p-trimethylammoniumphenol[b]	~8	No
	m-trimethylammoniumphenol[b]	~8	No
	guaiacole (2-methoxyphenolate) (**180**)[b]	9.90	No
	p-ethylguaiacole (**181**)[b]	10.31	No
	resveratrol (**32**)[b]	9.15	No
	vanillyl alcohol (**33**)[b]	9.75	No
Benzoates	*p*-nitrobenzoic acid (**14**)	3.44	Yes
	p-aminobenzoic acid (**15**)	4.70	Yes
	p-dimethylaminobenzoic acid (**16**)	5.03	Yes
	p-hydroxybenzoic acid (**17**)	4.58, 9.23	No
	o-hydroxybenzoic acid (**23**)	2.98, 13.44[c]	No
	m-hydroxybenzoic acid (**20**)	4.08, 9.92	No
	3,5-dihydroxybenzoic acid (**21**)	4.04, 10	No
	2,6-dihydroxybenzoic acid (**24**)[c]	1.05, 13.1, >14	No
	gallic acid (**22**)[b]	4.41, 10	No
Acetates	acetic acid (**178**)	4.76	Yes
	phenoxyacetic acid (**25**)	3.17	Yes
	phenylacetic acid	4.31	No
Amino Acids	histidine (*D*-), (*L*)- (**26**), (**27**)	1.82, ~6, 9.17	Yes
	tryptophan (*D*-), (*L*)- (**28**), (**29**)	2.43, 9.44	Yes
	tyrosine	2.2, 9.11, 10.13	No
	phenylalanine	2.16, 9.18	No
	alanine	2.34, 9.87	No
Sulfonates	toluenesulfonic acid (**30**)[b]	-0.43	No
	benzenesulfonic acid (**31**)	0.70	No
	naphthalenesulfonic acid	0.27	No
	methylsulfonic acid[b]	1.75	No

[a]In H_2O at 25°C, data has been taken from the NIST database.[252] [b]In H_2O, data taken from VINITI.[253] [c]In H_2O at 25°C, data has been taken from IUPAC[254]

that do show a change in the chemical shift (Δδ) during the titrations (and hence that the guests are indeed included), the pK_a values alone do not explain why other potential guests do not show a change. For example, the benzoates, **14-16**, show significant ^{1}H chemical shift changes, and have pK_a values ranging from 3.44 to 5.03, yet neither gallate, **22**, (pK_a 4.41) nor *p*-carboxyphenolate, **17**, (pK_a 4.58) show a change in chemical shift when titrated with any of the receptors. Whilst pK_a values (in water at 25°C) may provide only a little help in predicting whether a guest will demonstrate binding through a ^{1}H NMR perturbation, they do offer some explanation as to why some guests bind more strongly than others. Sodium *p*-nitrophenolate, **9**, with a pK_a of 7.15, was found to have binding constants consistently lower than *p*-formylphenolate, **10**, which has a pK_a of 7.72. Similarly *p*-nitrobenzoate, **14**, which has a pK_a of 3.44, has consistently lower binding constants than *p*-dimethylaminobenzoate, **16**, which has a pK_a of 5.03. All receptors investigated contained hydroxy groups at the base of the cavity. These are available to form strong hydrogen bonds with the anionic guests of the form O-H---O$^-$, as described by Jeffrey.[255] The high strength of the hydrogen bonding is due to the negative charge on the oxygen of the guest, causing it to act as an acceptor with an excess of electron density, while the complexation of the Cd(II) by the hydroxy-groups makes them act as relatively strongly polarized donor groups. As a higher pK_a value for the guest indicates that protons are more tightly held by the oxygen, a higher pK_a would also indicate that the guest anion has a higher hydrogen bonding acceptor strength, which would stabilise the inclusion complex. Hydrogen bond strength is probably not the only factor influencing the strength of binding, the number of hydrogen bonds, the symmetry complementarity between the donor and acceptor, and the closeness of approach, governed by steric bulk, must all play a part.

3.4.2. Titrations of anionic guests with non-fluorescent receptors having functionalised phenoxy derived cavities.

Just as the size and shape of the aromatic guest anions almost certainly have an important effect on how they are included in the receptor complex, the cavity of the host receptor molecule may also have an effect. The sensitivity of the binding constant to structural perturbations within the host was investigated first, by functionalising the upper rim[37,41] at a position *para*- to the alkoxy moiety on the aromatic rings of **1**. Methylation gave [(Cd((*S*)-thmppc)]$(ClO_4)_2$, **2**, and *tert*-butylation gave [Cd((*S*)-thbppc)]$(ClO_4)_2$, **3**. To investigate whether having these *para*-substitutions influences the guest binding, the inclusion of a guest into the receptor compounds **2** and **3**, were studied by ^{1}H NMR, with the resultant log*K* values shown in **Table 3.2.**

In general the substituents have a slightly destabilising effect, particularly for the longer guests phenoxyacetate, **25**, and histidinate (**Figure 3.8**), however the origin of the reduced stability is not clear.

3.4.3. Binding Constants of anionic guests with fluorescent receptors.

Titration of *p*-nitrophenolate, **9**, with [Cd((*S*)-athppc)]$(ClO_4)_2$, **4**, in DMSO-d_6 demonstrated immediately that this receptor still had the capacity to act as a host for guest anions, despite the replacement of one of the phenoxy arms. The binding constant (log*K*) for the 1:1 inclusion complex was found to be 3.5 ± 0.1 (as shown in Section 3.3). This is *ca* one order of magnitude smaller than the binding constant determined by Smith[114,125] between this guest and [Cd((*S*)-thphpc12)]$(ClO_4)_2$, **1**, (log*K* = 4.2 ± 0.2). This decrease in binding constant can be associated with the

Table 3.2. Binding constants (log*K*) for the binding of guest anions with receptor complexes as determined by ^{1}H NMR titration.

Guest anion	Receptor Complex (log *K*)[a]		
	[Cd((*S*)-thphpc12)], (**1**)[b]	[Cd((*S*)-thmppc)], (**2**)[c]	[Cd((*S*)-thbppc)], (**3**)[c]
p-nitrophenolate, **9**	4.2 ± 0.2	3.6 ± 0.2	3.9 ± 0.2
p-formylphenolate, **10**	>4.8[c]	4.6 ± 0.2	>4.8
p-nitrobenzoate, **14**	4.5 ± 0.8	3.7 ± 0.4	4.3 ± 0.4
phenoxyacetate, **25**	>4.5	4.0 ± 0.2	4.7 ± 0.4
(*D*)-histidinate, **26**[d]	4.2 ± 0.4	2.9 ± 0.2	3.0 ± 0.2
(*L*)-histidinate, **27**[d]	4.2 ± 0.4	2.8 ± 0.2	3.1 ± 0.2
(*D*)-tryptophanate, **28**[d]	3.3 ± 0.4 [c]	**e**	3.5 ± 0.4

[a]Measured in DMSO-d_6 at 295 K. Uncertainties are taken as two SD. [b]Data taken from Smith *et al.*[125,243] [c]This work. [d]Guest stock solution 10% (v/v) D_2O in DMSO-d_6. [e]Not attempted.

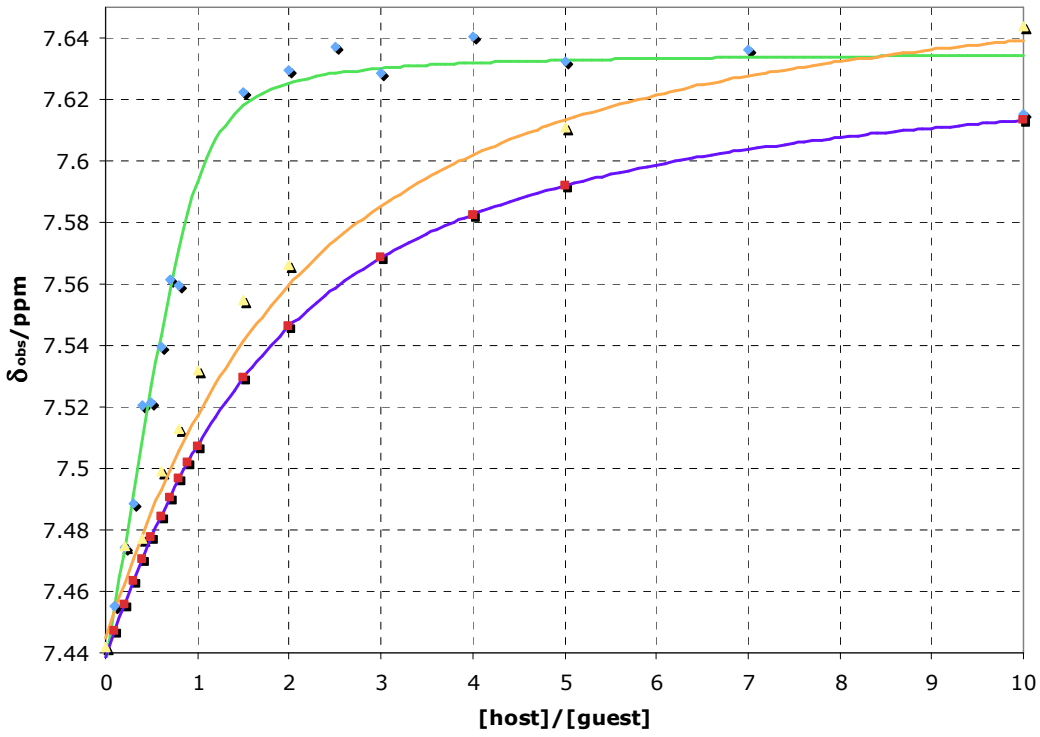


Figure 3.8. The titration curves obtained for chemical shift changes in downfield aromatic proton of sodium (*D*)-histidinate **26** (10^{-3} mol dm^{-3}) for increasing amounts of receptors [Cd((*S*)-thphpc12)], **1**, [Cd((*S*)-thmppc)], **2** and [Cd((*S*)-thbppc)], **3**. The values for **1** are taken from reference [243]. Blue diamonds indicate the experimental data points for **1**, red squares indicate the experimental data points for **2**, and yellow triangles indicate the experimental data points for **3**. The green curve indicates the theoretical δ values for the calculated values of *K* and δ_{HG} for **26** with **1**, the violet curve indicates the theoretical δ values for the calculated values of *K* and δ_{HG} for **26** with **2** and the orange curve indicates the theoretical δ values for the calculated values of *K* and δ_{HG} **26** with for **3**.

structural change in the receptor. [Cd((*S*)-thphpc12)]$(ClO_4)_2$, **1**, has four identical phenoxy-derived pendant-arms, whereas [Cd((*S*)-athppc]$(ClO_4)_2$, **4**, has one of these pendant-arms replaced by the anthracene-containing arm, which changes the shape of the cavity. Another difference between receptors **1** and **4** is that whilst all four hydrogen bond donor groups at the base of the cavity of **1** are –O-H, in **4** one of the four donors is now an –N-H group. Due to the difference in electronegativity N-H, with typical N-H---O^- hydrogen bond energy of *ca* 70 kJ mol^{-1}, is a weaker hydrogen bond donor than –O-H, which has typical O-H---O^- hydrogen bond energy of *ca* 100 kJ mol^{-1},[255] thus a decrease in binding strength is expected for guests that require four hydrogen bonds and need to hydrogen bond to the –N-H. However, this may not be the case for *p*-nitrophenolate, which has been seen previously in an X-ray diffraction determined structure to bind, in a similar situation, to just one *cis*-related pair of hydroxy groups (receptor **1** has been shown to include two *p*-nitrophenolate anions in the solid state[125]). Thus, phenolates in general may only need to form hydrogen bonds with two of the remaining three –O-H groups. If the cavity shape of receptors **1** and **4** had had no effect on guest binding, then it could be expected that the binding constants for phenolate inclusion in both cavities would have been more similar. A table of log*K* values for complexation of various guest anions with the receptor complexes, [Cd((*S*)-thphpc12)]$(ClO_4)_2$, **1**, and [Cd((*S*)-athppc]$(ClO_4)_2$, **4**, appears in **Table 3.3.** This shows that substitution of one phenoxy- pendant arm with the fluorophore, when the receptor is changed from [Cd((*S*)-thphpc12)], **1**, to [Cd((*S*)-athppc)], **4**, generally causes very little change in the log*K* values, even for non-phenolate guests that bind to all four hydrogen bond donors. The slight increase in chiral selectivity in favour of (*L*)-histidinate, **27**, shown by **4** is interesting, whilst receptor **1** has identical log*K* values for (*D*)- and (*L*)-enantiomers, receptor **4** shows

Table 3.3. Binding constants (log*K*) for the binding of guest anions with receptor complexes as determined by ^{1}H NMR titration.

Guest anion	Receptor Complex (log *K*)[a]	
	[Cd((*S*)-thphpc12)](ClO$_4$)$_2$ **1**[b]	[Cd((*S*)-athppc)](ClO$_4$)$_2$ **4**[c]
p-nitrophenolate, **9**	4.2 ± 0.2	3.5 ± 0.1
p-formylphenolate, **10**	>4.8[c]	>4.8
p-nitrobenzoate, **14**	4.5 ± 0.8	4.4 ± 0.3
p-aminobenzoate, **15**	**e**	4.3 ± 0.2
phenoxyacetate, **25**	>4.5	4.8 ± 0.2
(*D*)-histidinate, **26**[d]	4.2 ± 0.4	4.5 ± 0.4
(*L*)-histidinate ,**27**[d]	4.2 ± 0.4	>4.8
(*D*)-tryptophanate, **28**[d]	3.3 ± 0.4[c]	3.7 ± 0.4

[a]Measured in DMSO-d$_6$ at 295 K. Uncertainties are taken as two SD. [b]Taken from Smith *et al*.[125,243] [c]This work. [d]Guest stock solution 10% (v/v) D$_2$O in DMSO-d$_6$. [e]No significant changes in proton chemical shift occurred.

slight preference for the (*L*)-enantiomer. It should not be forgotten that the binding cavity of all the receptors under discussion is a chiral environment due to both the stereogenic centres in the phenoxy arms and the spiralling of the arms, induced by the metal ion, as discussed in the Introduction. It is perhaps therefore more remarkable that receptors studied to date have shown no evidence of chiral discrimination.

3.4.4. Binding constants for anionic guests with fluorescent receptors having functionalised phenoxy derived cavities.

With the upper rim functionalization of the aromatics of the cavity having limited effect on the binding of guests in the receptors containing four identical pendant arms, and with the substitution of one of the phenoxy- arms also having only a limited effect on binding, investigations were undertaken to see whether differences in binding strengths would be observed for the receptor in which both alterations had been performed. To do this inclusion of guests into the receptor compound [Cd((*S*)-athmppc)], **5**, was studied by ^{1}H NMR. The usual series of

titrations was run leading to the data in **Table 3.4**. Clearly upper rim methylation of **4** has little or no effect on the strength of the associations except for the longer guests **25**-**27**, where there is a slight decrease. This is similar behaviour to that noted earlier with **1**, **2** and **3**.

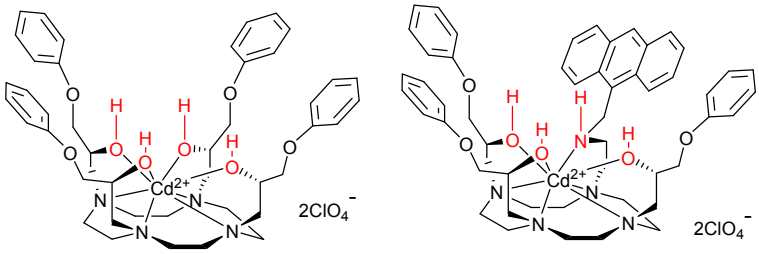
Table 3.4. Binding constants (log*K*) for binding of guest anions with receptor complexes as determined by ^{1}H NMR titration

Guest anion	Receptor Complex (log *K*)[a]	
	[Cd((*S*)-athppc)], **4**[b]	[Cd((*S*)-athmppc)], **5**[b]
p-nitrophenolate, **9**	3.5 ± 0.1	3.3 ± 0.2
p-formylphenolate, **10**	>4.8	>4.8
p-nitrobenzoate, **14**	4.4 ± 0.3	4.0 ± 0.2
phenoxyacetate, **25**	4.8 ± 0.2	4.4 ± 0.2
(*D*)-histidine,[c] **26**	4.5 ± 0.4	3.6 ± 0.2
(*L*)-histidine,[c] **27**	>4.8	4.1 ± 0.4

[a]Measured in DMSO-d_6 at 295 K. Uncertainties are taken as two SD. [b]This work. [c]Guest stock solution 10% (v/v) D_2O in DMSO-d_6.

3.4.5. Titrations of anionic guests with receptors having a substituted fluorophore-containing arm.

It is known from previous work in the area that the main interaction involved in guest inclusion within the cavity is hydrogen bonding.[114,126,243] The receptors discussed so far have had four hydrogen bond donor groups. These are the four hydroxy groups of the receptors **1**-**3** and three hydroxy groups and one NH-group of the fluorophore arm of receptors **4**-**5**, as shown in **Figure 3.9**. The effect of



[Cd((*S*)-thphpc12)](ClO$_4$)$_2$ (**1**)

[Cd((*S*)-athppc)](ClO$_4$)$_2$ (**4**)

Figure 3.9. Receptors **1** and **4**, which both have four hydrogen bond donor groups.

removing the NH-group as a potential hydrogen bond donor was investigated by appending a pendant arm at this position in **4** and **5** to produce **6** and **7**, as shown in **Figure 3.10.**

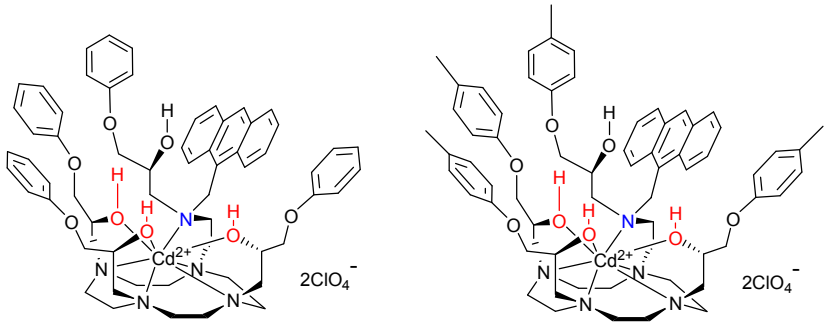


Figure 3.10. Showing receptors **6** and **7**, which have been derived from **4** and **5** by substitution at the anthrylamine.

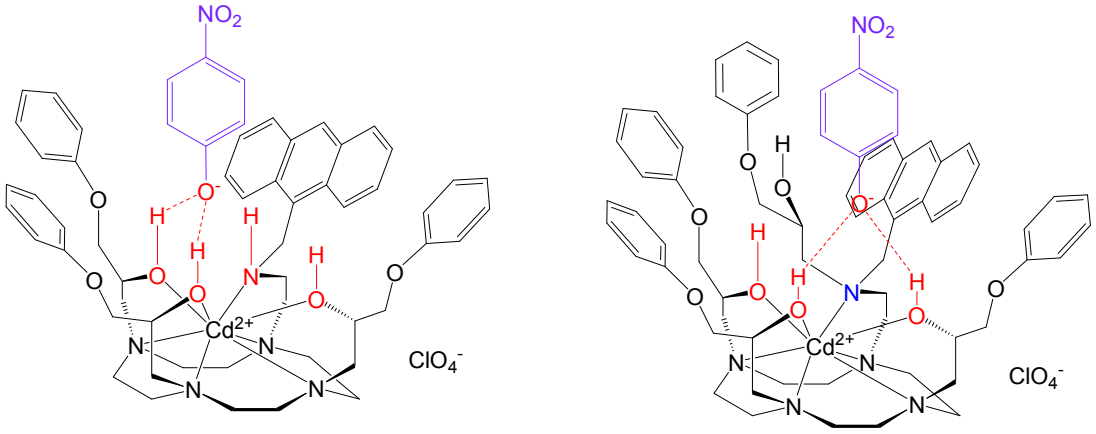
The binding constant for the *p*-nitrophenolate inclusion complex with receptor **6** was 3.2 ± 0.2, as shown in **Table 3.5**, which is identical, within the experimental error, to that found with **4**, **5** and **7**. This indicates that neither upper rim methylation of the cavity, nor the loss of the fourth hydrogen bond donor

Table 3.5. Binding constants (log*K*) for binding of guest anions with receptor complexes as determined by ^{1}H NMR titration

Guest anion	Receptor	Complex	(log *K*)[a]
	4	**6**	**7**
p-nitrophenolate, **9**	3.5 ± 0.1	3.2 ± 0.2	3.2 ± 0.2
p-formylphenolate, **10**	>4.8	>4.8	4.3 ± 0.2
p-dimethylaminophenolate, **11**	3.8 ± 0.2	3.9 ± 0.6	>4.8
sodium salt of serotonin, **12**	3.0 ± 0.4	3.2 ± 0.4	>4.8
sodium salt of syringic alcohol, **13**	2.6 ± 0.6	3.3 ± 0.4	>4.8
p-nitrobenzoate, **14**	4.3 ± 0.3	3.2 ± 0.2	3.3 ± 0.2
p-aminobenzoate, **15**	4.3 ± 0.2	3.8 ± 0.2	4.1 ± 0.2
p-dimethylaminobenzoate, **16**	4.6 ± 0.4	4.2 ± 0.2	4.0 ± 0.4
phenoxyacetate, **25**	4.8 ± 0.2	3.9 ± 0.2	4.5 ± 0.2
(*D*)-histidine,[b] **26**	4.5 ± 0.4	3.8 ± 0.2	4.3 ± 0.4
(*L*)-histidine,[b] **27**	>4.8	4.8 ± 0.4	4.3 ± 0.2
(*D*)-tryptophanate,[b] **28**	3.7 ± 0.4	4.4 ± 0.6	4.0 ± 0.8
(*L*)-tryptophanate,[b] **29**	3.5 ± 0.1	4.0 ± 0.2	3.8 ± 0.6

[a]Measured in DMSO-d_6 at 295 K. Uncertainties are taken as two SD. [b]Guest stock solution 10% (v/v) D_2O in DMSO-d_6. [c]Not attempted.

position, has much effect on *p*-nitrophenolate binding as a guest. This is consistent with *p*-nitrophenolate forming hydrogen bonds with only two hydrogen bond donors when included within the cavity.[125] The availability of hydrogen bond donor groups in receptors **4** and **6** is shown in **Figure 3.11.** Similarly, the binding of the other phenolate guests (*p*-formylphenolate, **10**, *p*-dimethylaminophenolate, **11**, and the sodium salt of serotonin, **12**), all of which need only two hydrogen bond donor groups within the cavity, shows no change in log*K* when the receptor is changed from **4** to **6.**

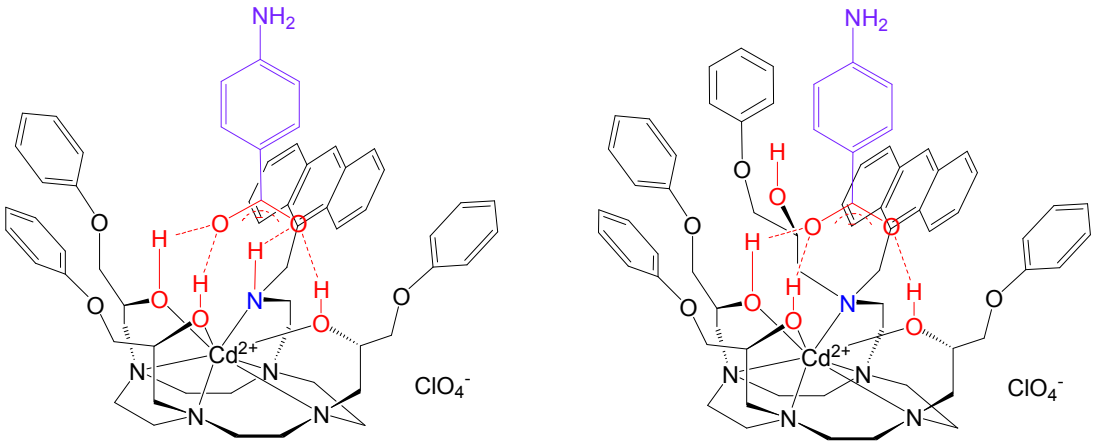


[Cd((*S*)-athppc)(*p*-nitrophenolate)](ClO$_4$) [Cd((*S*)-apthppc)(*p*-nitrophenolate)](ClO$_4$)

Figure 3.11. There is availability in both receptors of two hydrogen bond donors, which are required to bind *p*-nitrophenolate.

Whilst substitution at the anthrylamine does not have an effect on the binding of phenolates, the same cannot be said for other guests investigated. Sodium *p*-aminobenzoate, **15**, has a binding constant half an order of magnitude greater for inclusion in receptor **4** (log*K* = 4.3 ± 0.2) than for inclusion in the substituted receptor, **6**, (log*K* = 3.8 ± 0.2). As mentioned previously, *p*-aminobenzoate, **15**, as a dioxoanion, would ideally form hydrogen bonds with all four of the hydrogen bond donors within the cavity of receptor **4**, to form a strong inclusion complex, as shown in **Figure 3.12.**

With only three hydrogen bond donors within the cavity of the substituted receptor, **6**, the strength of binding with dioxoanions would naturally be decreased. However, the decrease in log*K* (of half an order of magnitude) for the inclusion of *p*-aminobenzoate, **15**, within receptor **6** compared to receptor **4**, is only half of that seen for the less basic guest *p*-nitrobenzoate, **14**, which is also a dioxoanion.



[Cd((*S*)-athppc)(*p*-aminobenzoate)](ClO$_4$)

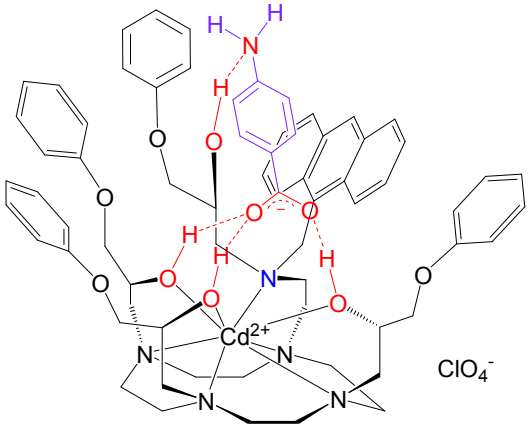
(**a**)

[Cd((*S*)-apthppc)(*p*-aminobenzoate)](ClO$_4$)

(**b**)

Figure 3.12. The inclusion of *p*-aminobenzoate, **15**, within; (a) receptor **4** and (b) receptor **6**.

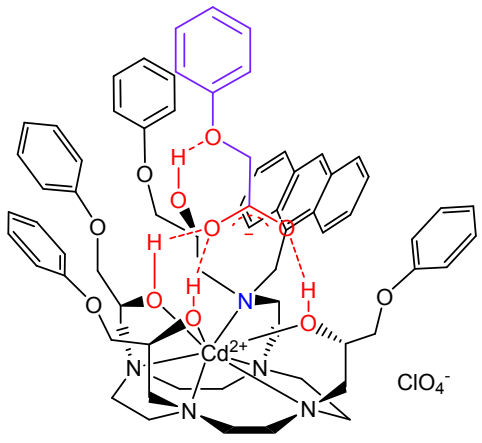
This difference between changes of log*K* can be further rationalised by considering that *p*-aminobenzoate, **15**, has a third potential hydrogen bonding group, a primary amine,[125,256,257] as shown in **Figure 3.13.**



[Cd((*S*)-apthppc)(*p*-aminobenzoate)](ClO$_4$)

Figure 3.13. Inclusion of *p*-aminobenzoate, **15**, with **6**.

The *p*-amino group of **15** can be positioned to form a hydrogen bond with the hydroxy-group of the fourth phenoxy-arm of receptor **6**. This possibly provides added stability, not enjoyed by nitrobenzoate (although O-H · · · O(nitro) bonds are also possible, these are weaker than -O-H · · · N hydrogen bonds,[258] with energies of *ca* 15 kJ mol^{-1}), which counterbalances to some extent the loss of hydrogen bonding at the base of the cavity. This reasoning is borne out by the fact that other guests investigated that contain a third potential hydrogen bonding group (a donor or acceptor group in the cases of the aminoacids **26**-**27**, and an acceptor group in *p*-dimethylaminobenzoate, **16**) and as such are potentially stabilised by an extra hydrogen bond, also show a lowering of the log*K* values, by *ca* half an order of magnitude. Whilst the oxygen of the ether linkage within phenoxyacetate has the potential to form a moderately strong hydrogen bond with the hydroxy of the fifth pendant arm, as shown in **Figure 3.14**, this is evidently not strong enough to counterbalance the stronger N-H---O$^-$ hydrogen bond lost by the appending of the fifth pendant arm.



[Cd((*S*)-apthppc)(phenoxyacetate)](ClO$_4$)

Figure 3.14. Inclusion of phenoxyacetate, **25**, with **6**.

The remaining guests examined also contain extra potential hydrogen bonding groups and all show an enhanced stability with **6** compared to **4**. Sodium

(*D*)-tryptophanate, **28**, sodium (*L*)-tryptophanate, **29**, and the sodium salt of syringic alcohol, **13**, all show a small increase in log*K* (of *ca* half an order of magnitude).

3.5. Concluding Remarks

The use of ^{1}H NMR titration experiments for the investigation of inclusion phenomena showed that the introduction of the fluorophore does not significantly impede inclusion, allowing the proposed investigation of fluorescence properties of the hosts to proceed.

From the series of binding constants obtained it was observed that derivatization of the upper rim of the cavity had only a limited effect on the strength of binding. Only the longer guest species **25**, **26** and **27** showed a slight reduction in log*K*.

When the fluorophore was introduced it was found that by comparing log*K* values for **1** versus **4** that in no case was there a destabilisation of more than one order of magnitude, and that with some guests there was a stability enhancement.

When the number of hydrogen bonding groups at the base of the cavity was reduced from four (in receptors **4** and **5**) to three (in receptor **6** and **7**) it was found that generally the log*K* values for the carboxylate containing guests were decreased by an order of magnitude, although this pattern may be disrupted if the guest has further potential hydrogen bonding sites.