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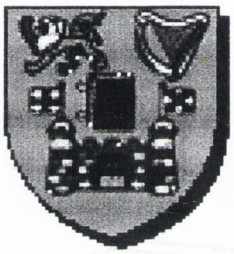
FLUORESCENT PHOTOINDUCED ELECTRON TRANSFER SENSING OF ANIONS USING CHARGE NEUTRAL CHEMOSENSORS

By

Mark Glynn



A Thesis Submitted to the Department of Chemistry, University of Dublin, Trinity College in the Fulfilment of the Requirement for the Degree of Doctor of Philosophy.

Department of Chemistry
Trinity College
University of Dublin

January 2003

DECLARATION

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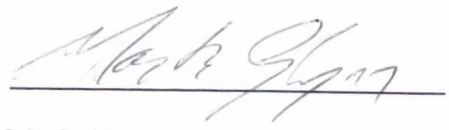


Mark Glynn

ABSTRACT,

The primary aim of this research was to design, synthesise and analyse anion sensors using fluorescence detectors. The thesis is spread over five chapters. Chapter one, the introduction begins with a brief back round to the area of supra molecular chemistry followed by an in depth analysis of the current research in the field of anion binding. Initially the examples, illustrations are of different host/guest systems developed, positively charged. Both metal free hosts and metal based hosts. This chapter also outlined the brief history of anion binding using fluorescence detector up to and including the most recent advances in this area.

Chapter two deals with the thiourea and area based PET sensors designed with one binding site. This chapter outlines the various synthetic routes investigated in attempt to synthesis these hosts. The second and third chapter outlines the synthetic routes investigated in the attempts to synthesis mono and big based receptors respectfully. This synthesis is followed by an in depth discussion on the analytical techniques used to determine the sensing ability of these read molecules. Chapter two specifically deals with sensors containing one bind site and measures the affinity and selectivity of these of these sensors towards biologically significant mono valent anions. Chapter three discusses the synthesis and analysis of sensors containing two binding sites. The affinity of these sensors towards both monovalent and divalent anions are discussed with this chapter. The conclusions obtained from the fluorescence spectroscopy are then substantiated using NMR measurements.

Chapter four discusses the work conducted in conjunction with professor Davis on cholic acid derivatives. The synthetic routes investigated in effort to synthesis fluorescent derivatives of cholic acid based anion binders is discussed here in detail.

Chapter five contains the experimental detail.

Acknowledgements

I would like to thank my supervisors Dr Gunnlaugsson and Prof. Davis for giving me the opportunity to undertake my PhD in Trinity. I would especially like to thank Thorri for his unlimited support throughout my PhD. From a professional and personal point of view Thorri will always hold a place deep in my heart. His enthusiasm towards chemistry and his love for research was infectious. I must admit initially there were several times I cursed this enthusiasm couple with his persistency in making sure I was constantly working. However not long into my research I realised the decision to work for Thorri was one of the best decisions that I had ever made. I would now like to consider Thorri a friend as well as a former supervisor.

Next there is the group in the lab, not least Claire and Caroline. From the very first day when we scrubbed the lab from top to bottom up until today there has been a great atmosphere amongst the group. The different personalities merged very well to make the group as successful as it is today. Long may it continue. From a personal viewpoint I believe this is why the time passed so quickly. Without naming everybody in the group I would like to extend special thanks to Jilly and Julie who had the unenviable task of proof reading the chapters. I only wish that you could also take the viva for me as well. It would have been impossible to complete the PhD without the help of people outside the lab as well. My sincere thanks to all of the technicians, Fred and Eddie in stores, John, Brendan and Paul, and of course Mr NMR himself John O'Brien. For constantly helping in every which way you could - thank you all. A special note of thanks also goes to Michael and Karen for keeping me sane while I was doing measurements on the fluorimeter.

Outside Trinity I have been fortunate enough to have a constant support from a large pool of friends. All of which put up with my "moans and groans" over the last couple of years. Jan, Philly, Ais and Dean have been there from the start. Tobin lent an ear on more than one occasion and it definitely helps to have somebody in your corner who has "been there done that." One group of people that stick out more than most is the entire Colgan family. Not only did Dee buy me a couple of pints on more than a few occasions, her support and encouragement was endless. The rest of the Colgans not only encouraged me but also made "411" a place I always felt welcome, and for many a time a refuge from the research.

Next there is my family; they have been a source of non-stop support throughout my entire education, my PhD being no exception. My parents have offered help at every turn and

I only hope that I can live to your aspirations. As for Paul, Paula, Gary, Fran, Jess, Darragh and Alex; all I will say is that when this thesis is finally over, the apparently eternal student has finally got a job. Finally on my family the focal point of my college career has been my Gran. Easily one of the most influential people that will feature in my life. If I have one regret it is that she did not live to see me graduate with my PhD. One thing is certain: nobody will go hungry in heaven with plenty of Lasagne and apple tart to go around.

My last acknowledgment extends to Sue, though you have only been with me a short time. I already feel like you are a major part of my life. In completing this thesis I close a chapter of my life. I thank you for been so understanding and giving me all the time to complete this thesis with no hassle or arguments from your side. I just only hope it is the last chapter of my life that does not totally involve you.

In Memory of Fitzer

CHAPTER 1

INTRODUCTION

1.1 PREAMBLE

Supramolecular chemistry is one of the fastest growing areas of experimental chemistry.[1] The combination of supramolecular chemistry and photochemistry represents a novel and powerful area of current research in the field of host guest interactions. This thesis is based on the concept of the partnership where the aim is to design novel sensing systems where the recognition of an ion can be detected by a change in luminescence properties. This chapter will give a general introduction to the basic concepts of both areas and then concentrate on the recent advances in the specific area of supramolecular photochemistry with respect to binding of anionic species.

1.2 INTRODUCTION TO SUPRAMOLECULAR CHEMISTRY

Supramolecular chemistry is highly interdisciplinary in nature, and as a result it attracts not just chemists, but biochemists, biologists, environmental scientists, and a whole other range of researchers. In general terms, supramolecular molecules are based on the association of two or more building blocks, which are usually but not always held together by intermolecular interactions. These interactions are the foundations for highly specific biological processes, for example base pairing in the DNA double helix. Two anti-parallel strands are held together by complementary hydrogen bonding between pairs of bases in addition to other forces such as π - π interactions and dipole interactions. These forces play a major role in the overall stability of such naturally occurring supramolecular systems.

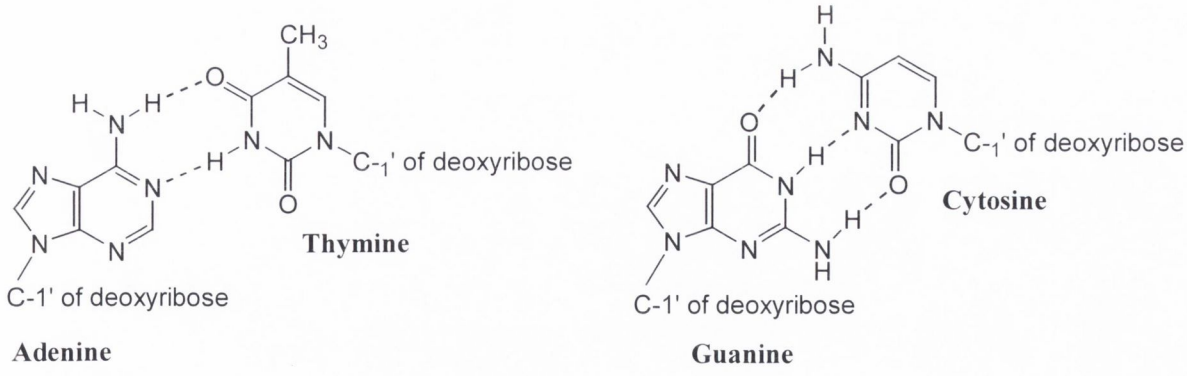


Figure 1:1 Complementary hydrogen bonding between base pairs

Both the hydrogen bond donors and acceptors on the nucleic acid bases are arranged in such a way that adenine forms two hydrogen bonds with thymine, and guanine forms three hydrogen bonds with cytosine. This complementarity extends throughout the double helical structure where one strand of DNA is complementary to the other.

Another example includes substrate binding of enzymes and receptors. Binding of a substrate to a receptor involves molecular recognition, which is defined by the energy involved in the binding and selection of a substrate by a given receptor molecule. Particular emphasis has been placed on the design of a receptor that can mimic biological systems and achieve significant recognition, catalytic activities or potential therapeutics.

Much of the emphasis today in the construction of supramolecular host molecules concerns multiple interaction sites between the host and guest molecule due to the weakness of the non-covalent interactions employed, i.e. the greater the number of binding sites the higher the binding affinity achieved. This means that we can construct a stable host-guest assembly or complex using relatively weak non-covalent interactions while ensuring that there are as many as possible of these interactions operating simultaneously. Such interactions are seen in nature for instance in enzymes, as discussed above. An important feature to note is that the total strength of these interactions is greater than the strength of the sum of the individual interactions. In addition when designing a supramolecular host it is important that such a host molecule exhibits selectivity in at least one of the following areas:

- substrate selectivity,
- chemoselectivity,
- regioselectivity or
- stereoselectivity.

With the aim of affinity, high selectivity, and sensitivity, in mind it is necessary to carefully design the receptor. For instance the host may be either designed to be flexible or rigid. A flexible host is potentially capable of engulfing or surrounding the guest molecule, while a rigid host is entropically more favourable due to a low ΔS (change in entropy) upon complexation when compared to that of a flexible host.[2] On the other hand, a rigid host having all its recognition groups preorganised in a complementary configuration to the respective guest shows strongest binding.[3] This is represented in *Figure 1.2*. The various shapes attached to the line represent different functional groups on a compound. A rigid host forces these groups to be preorganised, therefore only allowing binding if the host and substrate structure are complementary to one another. In spite of this an inherent flaw associated with rigid hosts is the risk of having slow guest exchange kinetics, which would rule out many applications for the host guest system such as catalysis. The host molecule must be therefore capable of forming a kinetically labile complex with the guest, which allows

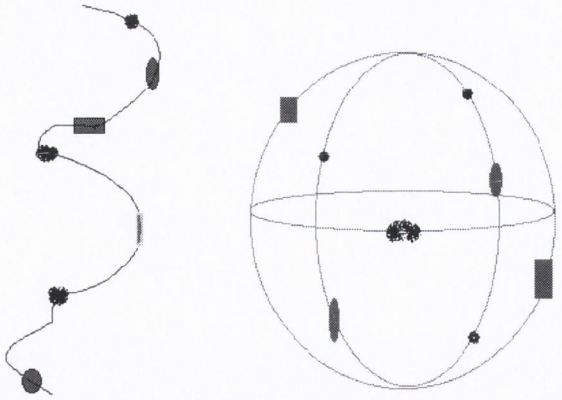


Figure 1.2: Potential structure of the host molecule

rapid guest exchange. In nature there are many examples of flexible yet highly specific host guest systems *e.g.* the DNA binding discussed earlier. Whether the receptor is rigid and complex or structurally simple, recognition and discrimination between different anions can only be successful if the receptor can provide suitable coordination sites. To date, the majority of efficient artificial receptors are all rigid in structure. However in order to mimic the actions and interactions of nature, optimisation of both rigid and flexible systems is an active area of current research.[4]

This thesis concentrates solely on anion sensor research, for that reason minimal time shall be given to discussing their cation counterparts. However numerous reviews are available discussing cation sensing. [5] A variety of synthetic receptors have been designed and studied for their binding strength and selectivity towards different anions. These have included Lewis acids[6], protonated poly-ammonium macrocycles[7,8], pyroles[4,] and guanidiniums.[9] Nature provides us with many examples of proteins using hydrogen bonding as a means of binding substrates. In numerous crystal structures of enzyme-substrate complexes the protein backbones are aligned with their amino terminus directed towards negatively charged groups in the substrate. This so-called macro dipole effect also contributes to the stability of sulphate and phosphate anion-binding proteins.[10] Arginine is an anion binding amino acid. The arginine residue contains a guanidine unit. Guanidinium, the protonated form of guanidine, is an excellent anion binding site as it remains protonated over an extremely wide range of pH and can participate in double hydrogen bonds with carboxylates, phosphate and sulphate etc. (*Figure 1.3*).

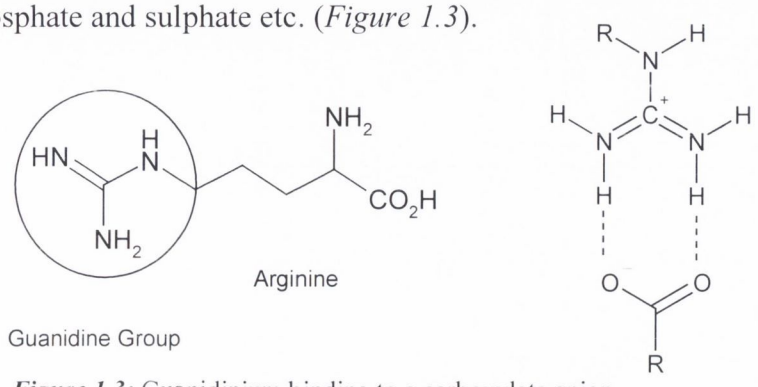


Figure 1.3: Guanidinium binding to a carboxylate anion

1.3 DISTINGUISHING ANIONS FROM OTHER GUEST SPECIES

The obvious distinction between anions and other guest species is their negative charge. This property is the most important feature that is taken into consideration when designing anion receptors. Except for the anions AlH_4^-, $B^-(C_6H_5)$ and *closo*-$B_{12}H_{12}^{2-}$, all anions have lone pairs of electrons. This Lewis basicity is the second most important feature of anions to be exploited in the construction of molecular hosts. It may add directionality to the system and therefore render it sensitive to the spatial arrangement and orientation of binding groups. This is an indispensable screen to differentiate between anions of similar size structure and charge, such as the biological relevant phosphate and sulphate anions.[11] The shape of the anion can be used advantageously in the design of a potent yet selective receptor. Anions exhibit a wide range of geometries, which challenge the molecular designer to create a complementary binding site. For instance the halides are spherical, their lone electron pairs do not introduce directionality to the system and are thus difficult to exploit in receptor design (*Figure 1.4*). However the cavity size of a halide receptor may be instead manipulated, to

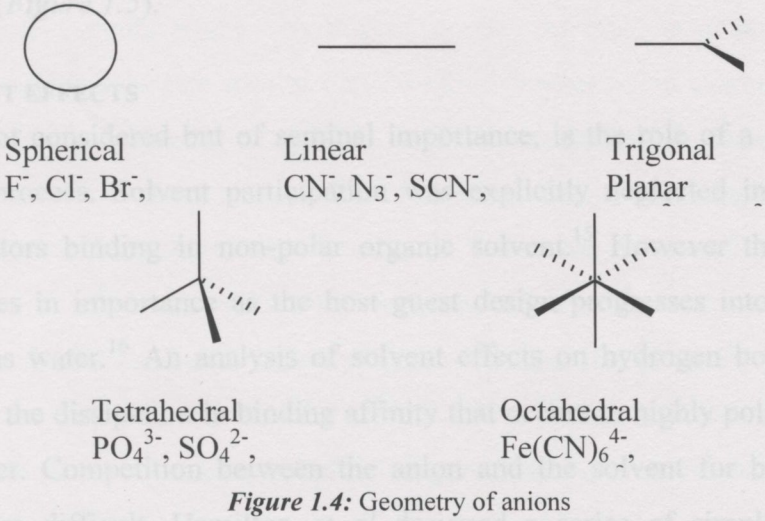


Figure 1.4: Geometry of anions

introduce selectivity. The halides F^-, Cl^-, Br^-, and I^- are all monatomic and spherical in nature. Compared to the cationic alkali metal ions, they generally exhibit diminished electrostatic interactions with their environment, while their dispersion interactions are greatly enhanced. This results in easier transfer of anions from an aqueous media to an organic media. While few molecular details are known about anion specific ion pumps, a passive antiport system HCO_3^-/Cl^- important for respiration (CO_2 disposal) was identified in erythrocytes. The relatively common hereditary disease Cystic Fibrosis is known to stem from a genetically caused mis-regulation of chloride channels.[12] This example illustrates the medicinal incentives for developing a sensor selective for chloride.

The oxyanions: carboxylates, phosphates, and sulphates are of particular biological interest. The carboxylate has a flat trigonal planar in structure, containing two oxygen atoms with two distinct types of lone pair electrons (*Figure 1.5*). Di- and tri-carboxylates are critical components of numerous metabolic processes including for instance, the citric acid and glyoxylate cycles.[13] They also play an important role in the generation of high energy phosphate bonds.[13] In addition nucleotide polyphosphates are the basic components in the bioenergetics of all living organisms.[14]

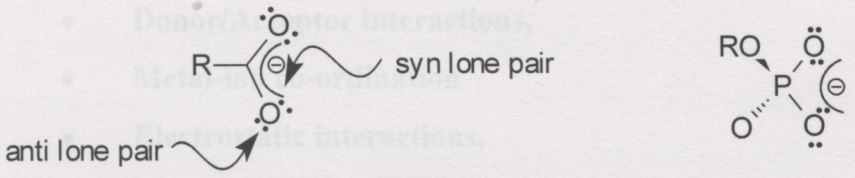


Figure 1.5: Illustration of the carboxylate and phosphate oxyanion functional group

Tetrahedral oxyanions *e.g.* phosphate, can be orientated so that they have an isosteric arrangement of lone pair electrons to the carboxylate anion and can therefore be bound in a similar fashion (*Figure 1.5*).

1.31 SOLVENT EFFECTS

Often not considered but of seminal importance, is the role of a solvent in the host guest binding process. Solvent participation was explicitly neglected in the earliest small molecule receptors binding in non-polar organic solvent.[15] However the influence of the solvent increases in importance as the host guest design progresses into more competitive solvents such as water.[16] An analysis of solvent effects on hydrogen bonded complexes is complicated by the dissipation in binding affinity that occurs in highly polar solvents, such as alcohol or water. Competition between the anion and the solvent for binding sites makes association more difficult. Hamilton *et al* designed a series of simple receptors, which complex dicarboxylate derivatives.[17] A vigorous analysis of solvent effects on the thermodynamics of the binding process for these systems was carried out.[18] They determined that in less polar solvents i.e. DMSO, complexation is enthalpically driven. However in methanol and methanol/water mixtures association becomes endothermic with favourable entropy providing the driving force for association.

1.4 ANION RECOGNITION

The design of artificial receptor molecules, capable of providing specific properties of highest efficiency and selectivity requires the correct manipulation of the energetic and stereochemical features of the non covalent intermolecular forces. These non-covalent forces are:

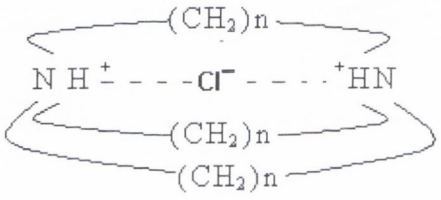
- **Hydrogen Bonding,**
- **Donor/Acceptor interactions,**
- **Metal-ion co-ordination**
- **Electrostatic interactions,**
- **π-π stacking interactions,**
- **Van der Waals.**

Their strengths range from weak (Van der Waals) to moderate (hydrogen bonds), or very strong (metal-ion co-ordination). Hydrogen bonds provide associations of stabilities comparable to enzyme substrate species (4–120 $kjmol^{-1}$) whereas an example of metal-ion co-ordination lies in the domain of antigen antibody complexes, where many individual interactions are involved. Numerous artificial receptors used in the recognition of neutral and cationic species have been previously investigated,[19] whereas the coordination chemistry and recognition of anions,[20] despite their very important roles in chemistry and in biology have not received much attention until recently. Anion binding has generally proved to be more challenging (with respect to cations) due to their lower charge to radius ratio, pH sensitivity and range of geometries as discussed before (*Figure 1.4*). As a result there are still very few anion receptors, which can be said to parallel the cation binding crown ethers and spherands. In this and the next section selected hosts and sensors developed over the past few years will be discussed. Due to the wide variety of hosts and sensors that have been developed, this thesis is divided into distinct sections. It will be split in two sections; **Positively Charged** and **Neutrally Charged** hosts. Within each section both metal free and metal containing receptors are discussed. Any hosts/sensors that incorporate fluorescence detection are discussed separately.

1.41 Positively Charged Anion Receptors

Using electrostatic interactions is probably the most obvious way to bind anions. Ammonium and guanidinium units that form $^{+}N\text{-}H\cdots X^{-}$ bonds have most frequently been used.

The bulk of the early anion receptors synthesised were polyammonium compounds such as **1** and **2**. Park and Simmons synthesised **1,** with the specific aim of anion binding in mind, are said to have synthesised the first anion host.[21] This receptor was aimed for halides where the halide is encapsulated in the cavity of the preformed molecular cage. In this case the halide is held by the array of hydrogen bonds within the bicyclic framework as well as by electrostatic interaction. This discovery should have started a landslide of investigations into the whole area of anion binding. Despite this initial discovery by Simmons *et al*, the research into anion binding did not grow as quickly as that of its cation counterpart. The field of anion binding only began to develop in 1976 when Graf and Lehn reported the protonated cryptand, **2**, which demonstrated a high affinity for Br^- and Cl^- ions.[22] Polyammonium compounds have been designed where a combination of electrostatic interactions and hydrogen bonding are



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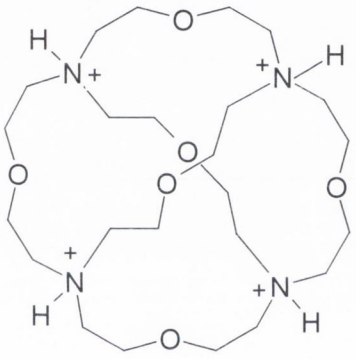

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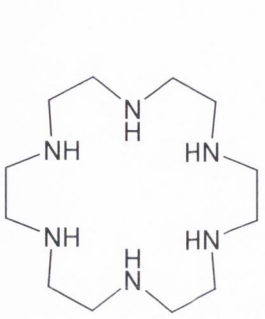
employed to bind anions. Similar to its cation counterpart it was found that simply by changing the size of the cavity (e.g. from **3** to **4**) both selectivity towards certain anions and sensitivity (different concentrations) could be modulated. For example Lehn *et al* studied the binding ability of several macrocycles with a variety of anions. Citrate^{3-} was bound 1000 times more strongly by simply increasing the cavity size from a hexa-protonated **3** to octaprotonated **4**. However since protonation requires low pH, polyammonium compounds are unsuitable for physiological applications where the recognition would take place at neutral pH. Under physiological conditions, the guanidine group is protonated (pK_a = 13.5) and therefore able to bind strongly to a substrate, ligand, or a receptor *via* electrostatic interactions. On the other hand, the exploitation in host-guest chemistry is hampered by the very effective solvation of the guanidinium function in water along with the lower charge density as compared to that of ammonium based receptors, leading to weaker electrostatic interactions. In spite of this the attractive features of the guanidinium group have led to the development of an appreciable number of artificial guanidinium-based receptors for anions.[23]

Many compounds containing guanidine as a functional group exhibit potent anti-viral or neurotoxic bioactivities, *e.g.* **5**, Ptilomycalin A.[24]



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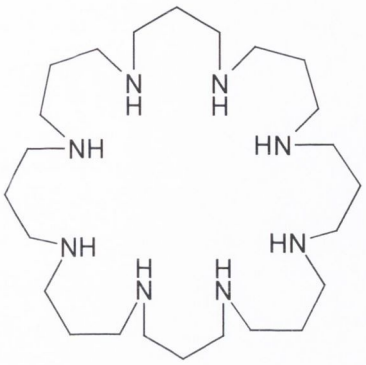

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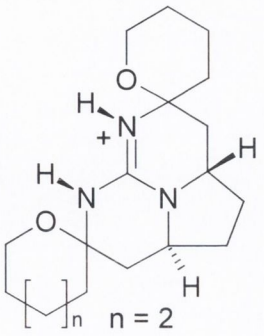

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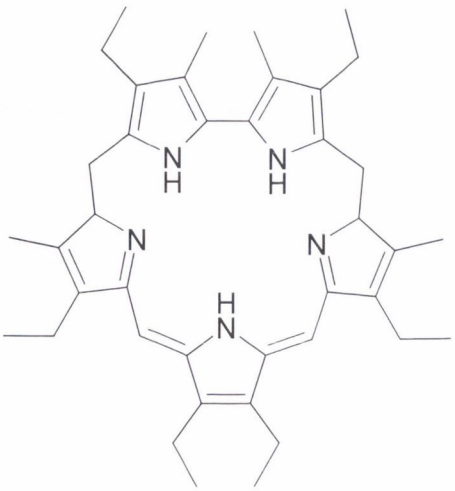
Sessler *et al* designed a pentapyrrolic unit anion receptor in 1990.[25] Increasing the cavity size by adding one more pyrole to the porphyrin unit leads to a class of compounds known as sapphyrins *e.g.* **6** and **7**. These macrocycles take advantage of the converging array of amine and iminium protons to achieve anion recognition. Sessler found that the diprotonated form of **6** binds the fluoride anion in the solid state as its mixed PF_6^- salt and acts as an effective carrier for the through transport of fluoride anion in a model three phase [aqueous 1]-[CH_2Cl_2]-[aqueous 2] membrane system.[26] Binding studies in solution were also carried out in methanol (K_a for F^- = 9.6 +/- 2.0 x 10^4 M^{-1} relative to $K_a(Cl^-)$ and $K_a(Br^-)$ estimated at ~10^2 and <10^2 respectively). Sessler also stated that a classic expanded porphyrin, acted as a carrier for nucleotide monophosphates at neutral pH. These compounds were also shown to have high affinities for various oligonucleotides, including DNA. It was shown that this type of receptor binds the anion using a combination of electrostatic, hydrogen bonding and π stacking interactions. Sessler also initiated the investigation into dimeric



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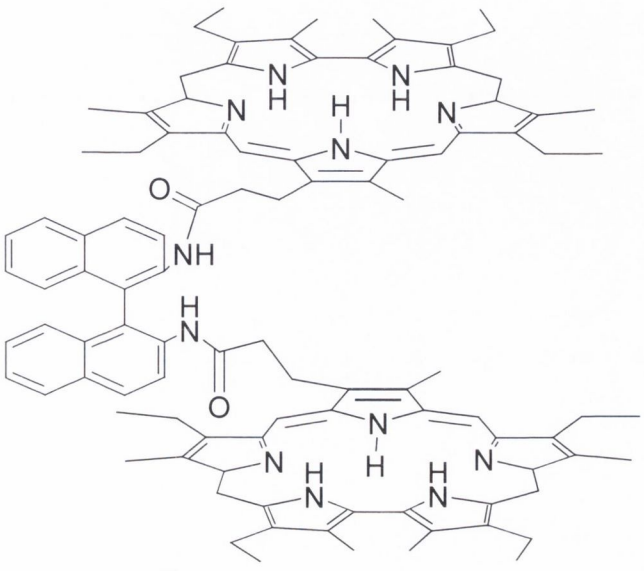

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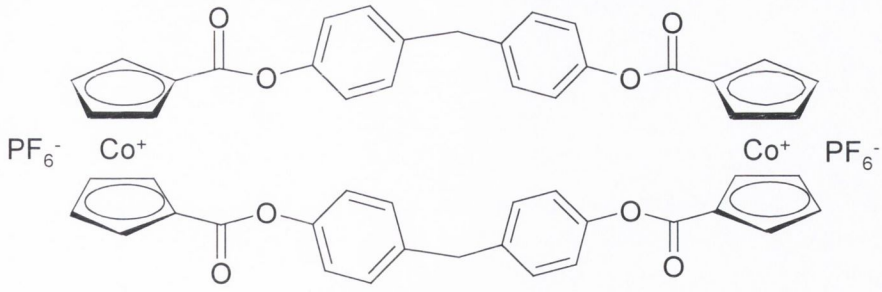
sapphyrins, **7**, and discovered that they are effective receptors for dicarboxylate anions.[27] **7** formed strong complexes with *N*-carbobenzyloxy-protected aspartate and glutamate anions. K_a values were in the order of 10^4-10^5 M^{-1} in 19:1 (v/v) DCM:MeOH. **7** also displayed preference for glutamate over aspartate with a modest level of enantiomeric selectivity.

An intrinsic feature of positively charged receptors is that hosts with more than one binding site have geometric restrictions. In addition, as a result of both the non-directional electrostatic binding and the interference from the counter anion it is relatively more difficult to incorporate selectivity into charged hosts. This has lead to the synthesis of metal-based anion receptors. The metal ions in these receptors play a number of different roles:

- the metal may act as a coordination site for the anion. The metal centre has to be co-ordinatively unsaturated thus leaving one or more sites vacant and available for the incoming anion. Metal-ligand interactions are usually stronger than electrostatic interactions. In addition if the metal belongs to the d-block it has directionality. As a consequence of this directionality selectivity is greatly increased.
- the metal may act as a non-coordinating reporter group that signals the presence of an anion by perturbation in its physical properties (e.g. changes in its redox and/or spectroscopic properties).
- the metal may be an element in the receptor designed to withdraw electron density away from a π electron system therefore increasing the affinity of a hydrophobic receptor for anions.

Many elegant examples exist in literature of metal-based anion receptors.[28] A crude metal ion, in a solvated form, cannot be used as an anion receptor, because it possesses too many binding sites and will bind indiscriminately to any anion present. Thus it is necessary to occupy some of the co-ordination sites therefore introducing the possibility of selectivity. Beer and co-workers published the first cobalt based receptor **8**, in 1989.[29] This organometallic macrocycle possesses a dipositive charge arising from the Co (III) centres and is therefore capable of binding bromide *via* electrostatic interactions. Unfortunately the generally poor solubility of these types of macrocyclic ligands coupled with their arduous syntheses and lability to ester hydrolysis forced Beer to investigate alternative receptors. By appending secondary amide arms to the cobaltocenium moiety neutral groups capable of coordinating anions such as Cl^- and $H_2PO_4^-$ are introduced into the receptor.[30]



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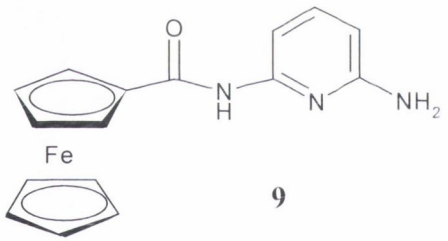
The binding process was measured by monitoring the NMR shifts of the amide protons upon introduction of an anion to the system. This class of receptor binds through a combination of hydrogen bonding via the amide protons and electrostatic interaction to the metal centre.

Along with ^1H NMR, the binding could also be monitored via cyclic voltametry measurements. The redox potential of the reversibly reducible cobaltocenium moiety was significantly perturbed upon complexation. The complexation of the anion stabilizes the positive charge making the cobaltocenium unit more difficult to reduce. The larger the "perturbation" the larger the stability constant. Ferrocene analogues of these receptors were also reported (**9** - **11**). Receptors **9** and **11** are capable of detecting $H_2PO_4^-$ anions in the



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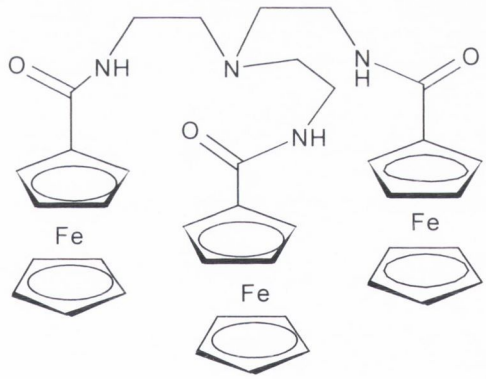

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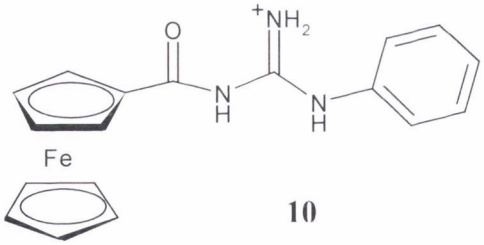

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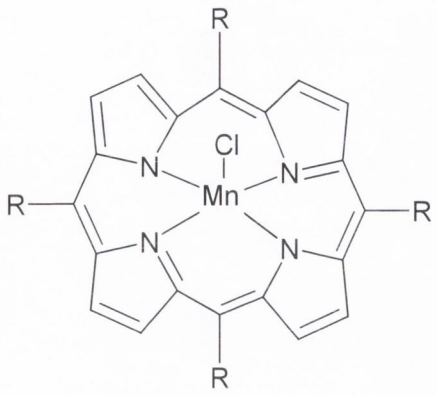
presence of 10 fold excess of HSO_4^- and Cl^- ions. On the other hand due to their neutrality the electrostatic interactions were lost and consequently the potency is greatly reduced. However electrostatic interaction may be activated by oxidation of the ferrocene to ferrocenium. Ferrocene has also recently been combined with the guanidinium moiety to produce receptor **10**. This molecule has been electrochemically shown to recognise the biologically important pyrophosphate anion in 50:50 water/methanol.

Metalloporphyrins are another type of metal based anion receptor. These compounds may be either positively charged or charge neutral hosts. Mn (III) porphyrin complexes such



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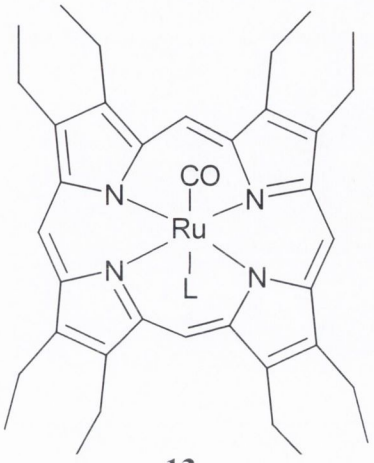

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as **12** act as positively charged hosts whereas the Ru (II) porphyrin **13** complex is an electroneutral host. Positively charged receptors are unsuitable for phase transfer applications and ion selective electrode chemistry, therefore interest has grown in recent years in the development of neutral anion receptors.[2]

1.42 Neutral Receptors

Neutral anion hosts have an added advantage as they should exhibit better membrane transport properties due to their increased lipophilicity. Neutral molecules are of particular interest for medicinal chemistry as they are also thought to be considerably less toxic than charged molecules. There are three main reasons why electroneutrality is of prime virtue:

- the internal competition established with the counteranions unavoidably present in cationic hosts is non-existent in this class of receptors. This competition is frequently responsible for weak binding and poor selectivity.
- the neutral receptors are lipophilic in nature and can therefore be studied in organic solvents.
- while pure coulombic forces just scan size, density and distance of charge, Lewis acidic/ Lewis basic interactions depend on more subtle interactions such as stereoelectronics, hardness/ softness of bonds

Neutral receptors are divided into a number of groups according to the anion binding motif used. There are:

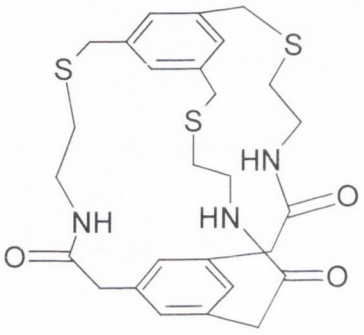
- Lewis acidic systems
- Hydrogen-bonding type systems
- Difunctional and zwitterionic type systems
- Miscellaneous type systems

This introduction will concentrate on host guest systems that incorporate hydrogen bonding as one of, if not the only, non-covalent interaction used to attract anions.

1.421 Hydrogen bonding systems

In an attempt to mimic nature in its high binding selectivity, several anion receptors have been developed with three-dimensional hydrogen bond donating moieties *e.g.* **14**. Considerable effort has been recently directed toward the development of synthetic receptors that depend solely on hydrogen bond arrays.[31] The main virtue of incorporating H-bond donor groups into a receptor is to conserve electroneutrality. The range of hydrogen bond donor groups available (*e.g.* carbamates, ureas, thioureas) offers extreme versatility of construction. This versatility offers limitless options in receptor design, but the effectiveness largely depends on the extent of solvation. Anion recognition in biological systems is achieved *via* hydrogen bonding by highly preorganised proteins containing sterically well-defined complexation sites in the interior of the protein. The main challenge in the field of anion complexation has been the design of receptors with a high selectivity for biologically important anions for example; phosphates, (poly) carboxylates, and halides (especially chloride). Chemically sophisticated macrocyclic hosts with preorganised binding sites such as receptor **15** can mimic the complexation properties of receptor proteins for anions. Good selectivities in anion binding have also been achieved with structurally less complicated acyclic receptors, due to the suitable orientation of the H-donor functions employed. It is a common observation that H-bonding responsible for the interaction between the host and the guest in relatively non-polar solvents may cease on switching the solvent to polar protic solvent. This is due to the low enthalpy of formation of a H-bond in water, methanol *etc.* The main obstacle in host design using H-bonds is the suppression of interference from competing H-bond acceptor molecules in the presence of the desired guest. In 1986, Pascal *et al.* prepared **14**. It was the first receptor to bind an anion solely through hydrogen bonds, with three convergent amide protons pointing into a cavity.[32] **16** showed evidence of binding fluoride ions in DMSO-δ_6. Kelly *et al.* reported the relative binding affinities of oxoanions

such as phenyl nitrate, phenyl sulphonate, with a urea-based host **16** discovering the obvious sequence of greater Bronsted basicity and higher charge of the guest, led to a higher complex stability[33] (K_a for nitrate = 150 M^{-1}, whereas K_a = 3600 M^{-1} for sulphonate, both in DMSO). Teramae *et al* produced a series of structurally simple ureas and thioureas (*e.g.* **17**) that displayed significant changes in their UV/Vis spectra upon introduction of acetate to their environment.[34] This simple thiourea conjugated with one *p*-nitrophenyl unit binds anions exclusively via a formation of hydrogen bonds in MeCN with selectivity of $AcO^- > H_2PO_4^- > Cl^- >> ClO_4^-$.



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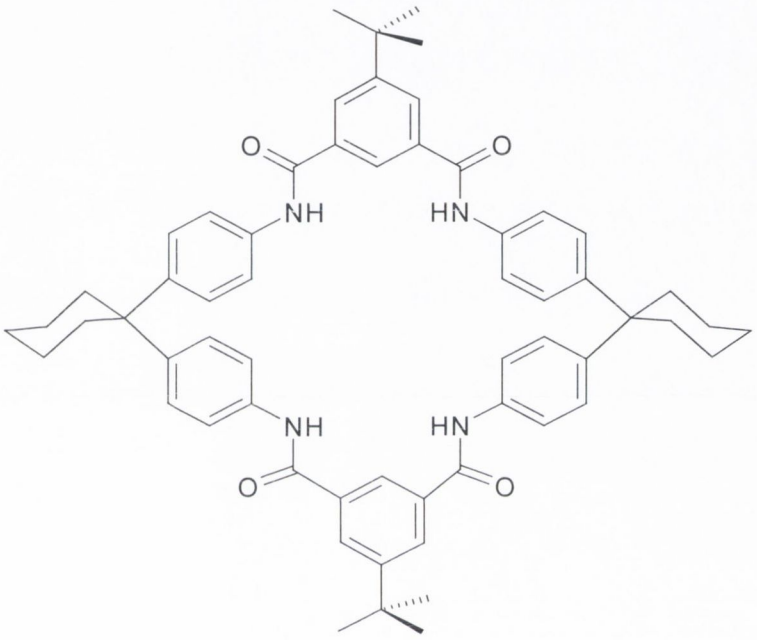

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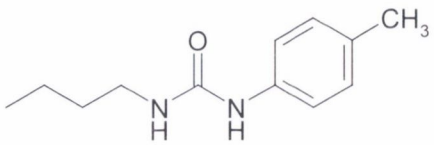

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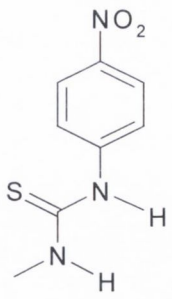

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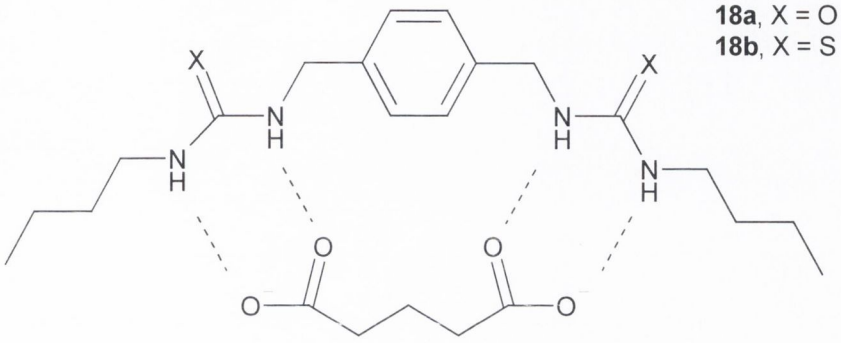
Table 1.1: Basicity and Stability constants in DMSO of various bidentate anions with receptor **16**

Guest	pK_b	$K[M^{-1}]$
Ph—OPO_3H^-	13	30
Ph—PO_3H^-	12	140
Ph—CO_2^-	10	150
Ph—PO_3^-	7	2500

In an analogous system **18**, Hamilton *et al* discovered a correlation between improved complex stability, and acidity of the H-bond donor host.[35] Binding constants for **18a** with the TBA salt of glutaric acid in DMSO-δ_6 was 6.4 x 10^2 M^{-1} for urea whereas thiourea **18b** showed a fifteen-fold increase for the same TBA salt of glutartic acid (K_a = 1.0 x 10^4 M^{-1}). It is well known that the dependence of host-guest association on solvent is most important.



18

Among a variety of possible H-bond donor groups, the amide, thiourea and urea motifs have proven to be very useful in neutral anion binding receptors.[36] Thiourea derivatives have proven particularly useful in the construction of these anion binding receptors. The relatively acidic thiourea NH protons with strong hydrogen-bond donor capability can establish multipoint hydrogen bond patterns with complementary acceptor groups in a specific predictable manner. Moreover the ability of the electronic charge in the lone pair of the sulphur to diffuse leads to the thiocarbonyl group being a weak hydrogen bond acceptor, and therefore unlikely to interfere in conformational or complexation studies involving strong acceptor centres (e.g. carboxylates). Vögtle and co-workers have incorporated anion coordinating urea groups into the interior of lipophilic dendrimers.[37] These organic soluble dendrimers are suitable for performing liquid-liquid extraction of anions from an aqueous solution. The dendrimers were found to be capable of binding and transferring biologically important anions such as pertechenate, AMP, ADP and ATP into an organic phase. The stability of the pertechenate complexes formed suggests that these systems have potential applications as imaging agents.

Another area of increasing interest in the last decade is the anion coordination ability of receptors containing pyrrole groups. This area of chemistry has been driven by Sessler and co-workers who have produced a variety of expanded porphyrins and polypyrrole macrocycles capable of binding anions.[25,27] Unlike the urea moiety, these receptors form a convergent binding site as in the case of **19** and **20** *(Figure 1.6)*.

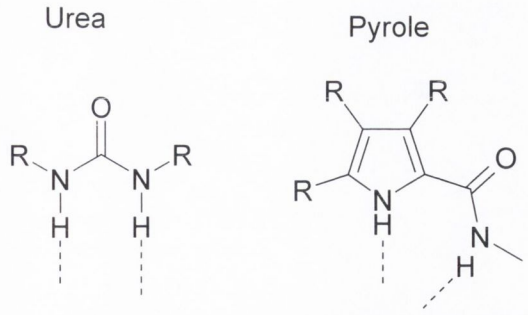
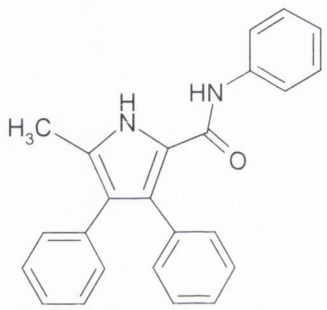


Figure 1.6: Binding structure of Ureas versus that of Pyrole

Gale *et al* developed simple pyrrole-amide ligands **19** and **20**, to assess the anion complexation ability of this moiety alone. [38]



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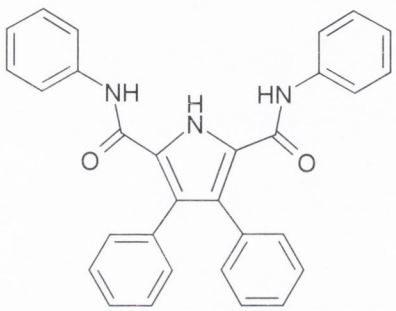

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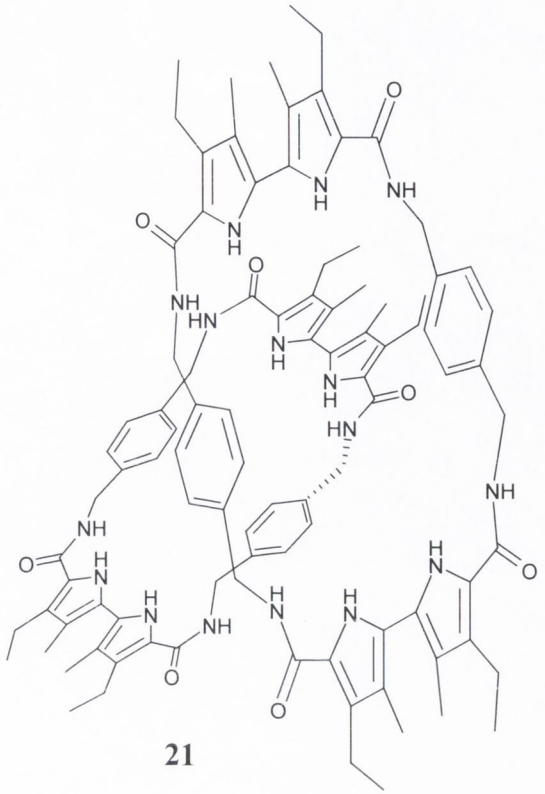
It has been previously shown that a single pyrrole ring is ineffective at complexing anions in solutions.[39] Therefore, the ability of these receptors to act as anion complexation agents relies on the formation of a cleft like conformation involving either two or three hydrogen bond donors. Sessler and Vögtle have reported the synthesis of a bipyrrole based [2]catenane, **21** that forms extremely stable complexes with anions such as $H_2PO_4^-$ in 1,1,2,2-tetrachloroethane.[40] Table 1.2 displays the binding constants for other anions tested with **21**.

Table 1.2:Anion binding constants for **21** as determined by ^{1}H NMR titration techniques

Anion	Ka (M^{-1})
$H_2PO_4^-$	$>1x10^7$
F^-	$1.48x10^5$
Cl^-	$3.55x10^6$
AcO^-	$9.63x10^5$



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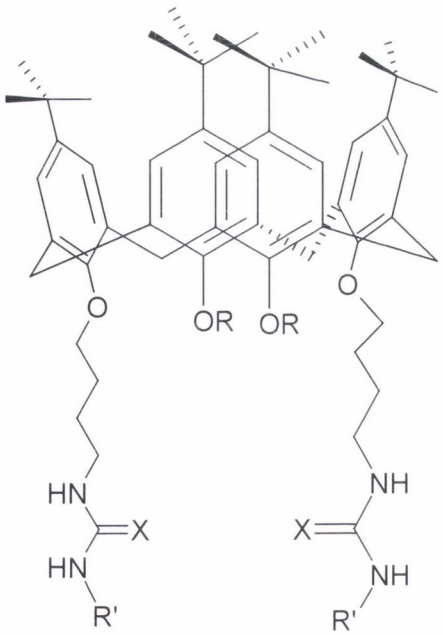
Anion binding studies using ^{1}H NMR methods cannot be discussed without mentioning calixarenes. As with cation binding hosts it is also possible to use calixarene frameworks to organise binding moieties. A calixarene is a cyclooligomer formed via phenol formaldehyde reactions. The upper rim is usually polar in character whereas the lower rim contains non-polar groups. Therefore calixarenes can form inclusion complexes with a wide variety guest species, depending on the binding groups attached to each rim. McKervey and

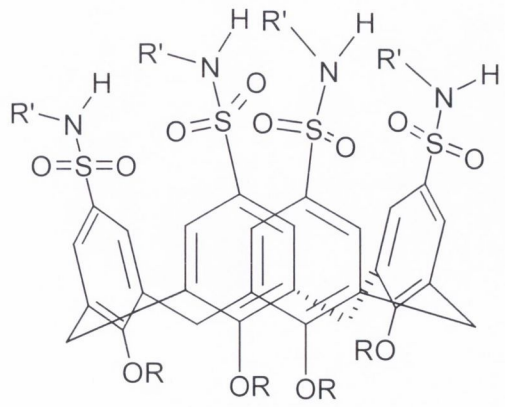


X = O or S

R= $(CH_2)_4NHC(X)NHR'$

R' = phenyl, n-propyl, n-octyl, ter-butyl

22



R= ethers, esters or amides

R' = H, n-propyl, tert-butyl, $(CH_2)_2NHC(O)Me$

23

Svehla were first to discover that calixarenes with cation-complexing groups attached to the lower rim could be used in ion selective electrodes (ISE's).[41]

In 1994 Reinhoudt and co-workers produced a series of calix[4]arene anion receptors functionalised at the lower rim, **22**, with two and four (thio)urea moieties.[42] These receptors exhibited selectivity for Cl^- over Br^-, I^- and CN^-. It should also be noted that these receptors exhibited no complexation to $H_2PO_4^-$ anions. Reinhoudt also produced several calixarenes functionalised with four sulphonamide groups at the upper rim selectively, **23**, which recognise HSO_4^-.[43]

The interaction of the (thio)urea and pyrrole based hosts with the anionic guest has generally been studied by ^1H NMR methods. However this method suffers from low sensitivity, as in order to get appreciable NMR signals there must be relatively high concentrations of the host and guest present. In addition the NH peaks monitored have a tendency to be very broad when complexed with anions, therefore making it more difficult to accurately determine the binding constant. As a consequence the attachment of reporter groups (fluorescent or electrochemical) to hydrogen bonding moieties is currently being investigated thoroughly.[44] This introduction will now discuss sensors incorporating fluorescent reporter groups only.

1.5 Luminescence

Incorporating luminescent moieties into an anion binding framework is the main aim of this thesis. Before discussing selected examples from the literature it is necessary to give a brief introduction to luminescence itself followed by its implication to anion sensing. Besides electrochemical sensor molecules, a method of great practical relevance to the monitoring of successful complexation is the use of chromoionophores or luminescent devices. Here, anion complexation induces changes in the spectroscopic properties of the host molecule or the receptor assembly, ideally leading to an analyte specific visible colour change (i.e. change in absorption spectra), and/or a spectral shift in the emission spectrum and/or a significant change of the emission intensity (quantum yield, lifetime etc.,). Photon emitting i.e. luminescent molecules are detectable with extreme sensitivity at the single molecule level. Thus luminescence is a natural approach to the operation of devices at molecular level.

In 1935 Jablonski interpreted the various fates of an excited species on an electronic level. He portrayed them on what is now known as a Jablonski diagram.[45] When the molecule absorbs light it is excited from the lowest vibrational level in its ground state (S^0) to a range of vibrational levels in the singlet first excited state (S*). During the time the molecule spends in

the excited state, energy is dissipated from the higher vibrational levels, and the lowest vibrational level is attained. Fluorescence occurs if the molecule then emits light as it reverts from this level to various vibrational levels in the ground state. Non-radiative processes, the most important of which is generally collisional deactivation, also gives rise to dissipation of energy from the excited state. As a result, there will be a reduction in the intensity of fluorescence and in many cases it will be absent altogether. The other process that may occur is intersystem crossing to a triplet state. Emission of light from the triplet state is termed phosphorescence, a phenomenon that is longer-lived than fluorescence. As a consequence of the loss of vibrational energy in the excited state, fluorescent emission occurs at longer wavelengths than absorption, the difference between the wavelengths of maximum emission and maximum absorption for a fluorescent compound being referred to as the Stokes' shift.

Fluorescence in particular is an attractive way of obtaining accurate sensing information since this technique is highly sensitive and it involves short response times. In addition it is a non-toxic technique involving non-destructive methods. As a result fluorescent chemosensors have found practical application in cellular imaging, environmental monitoring and biological assays.[46] A fluorescent chemosensor is a compound that incorporates a binding site, a fluorophore and a mechanism of communication between the two. There are certain criteria a fluorescent chemosensor must fulfil:

- the binding domain must have sufficient selectivity for the analyte of interest, compared to others present.
- the binding must be reversible.
- there must be a signal transduction mechanism between the binding site and the fluorescence domain.
- the signal should not be substantially affected by potential fluorescent quenchers within the environment.

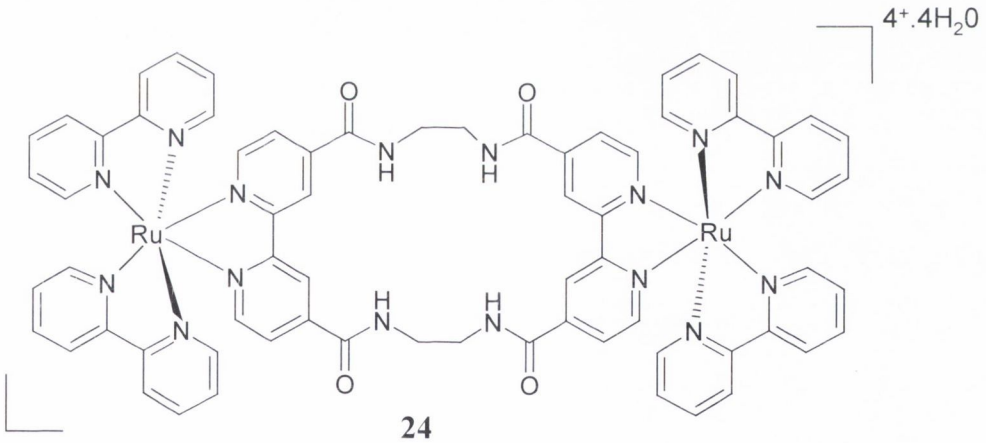
The synthesis of efficient chemosensors requires a thorough knowledge of the principles governing the processes of molecular recognition and signal transduction. That is, the mechanism by which the complexation of the sensor with the analyte causes a change in the physical properties of the sensor itself. Fluorescent chemosensors have many advantages over the wide range of chemosensors available. Fluorescent measurements are usually very sensitive, are inexpensive, easily performed and are versatile. The fluorescence detection of complexation may be accomplished through incorporating a fluorescent chromophore in the

receptor. A change in conformation on binding alters the physical properties of the chromophore and thus alters the fluorescence output.

Changes in fluorescence can be introduced via any number of factors. Metal Ligand to Charge Transfer (MLCT) in metal based systems, Energy transfer (ET), and Photon induced Electron Transfer (PET). Several elegant examples of each of these factors can be found throughout the literature.[47] Since the bulk of this thesis is concerned with aspects of PET it is worthwhile considering a few of its features. While selected examples of sensors that detect ions using the PET mechanism are discussed in the introduction, the principle of luminescent PET sensing is discussed in detail in Chapter 2. As previously mentioned there are a large number luminescent cation sensors, nonetheless the main focus of this thesis is on anion sensors so cation sensors will not be discussed.

1.51 Metal Ligand to Charge Transfer (MLCT)

In an effort to exploit the discoveries of metal-based cation receptors, anion complexing through second sphere coordination has been investigated. Until recently the majority of metal-based anion sensors had centred around cobalt and ferrocene amine and



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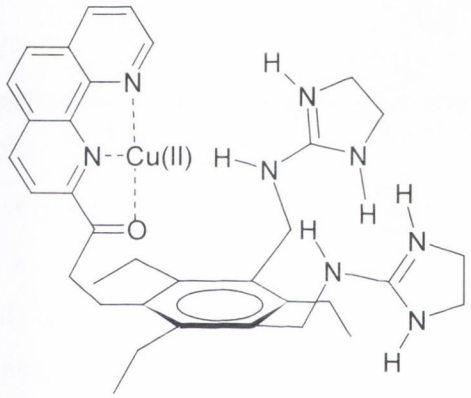
amide complexes[48]. Beer and co-workers designed sensors containing a ruthenium(II) bipyridyl moiety.[49] By combining this redox and photoactive moiety into already proven systems, a new class of anion receptors was developed. **24** forms an extremely stable 1:1 stoichiometric complex with chloride in DMSO solutions with K_a = 40,000 M^{-1}. This is two orders of magnitude greater than its acyclic analogue. Both ^1H NMR and ^{31}P NMR proved that there was very little binding of $H_2PO_4^-$. This dramatic selectivity is attributed to the rigid structure of the macrocycle, as well as the complementary structure of the cavity. Fluorescence studies indicated a blue shift in the metal-ligand transfer emission band upon addition of chloride with significant intensity enhancement, but no shift was observed upon

addition of $H_2PO_4^-$. This is believed to be due to the fact that during binding the complex becomes extremely rigid, thus inhibiting vibrational and rotational modes of non-radiative decay. When considering using MCLT systems for a method of fluorescence detection it should be noted that the sensing proficiency of each receptor is dependant upon the nature of the photoactive transition metal and the signalling unit's proximity to the anion binding site.

25 was designed using a metal centre to create an additional binding site for biscarboxylates such as citrate.[50] Furthermore Cu(II) quenches a photo-excited state of the 1,10-phenathroline fluorophore. When citrate was added to a solution of the copper complex **25,** the copper could no longer quench the phenathroline resulting in a significant increase in fluorescence being observed (K_a for citrate = 3.4 x 10^4 M^{-1} in 85:15 MeOH/H_2O @ pH = 7.4).

Parker and co-workers synthesised complexes of **26a** and **26b** with a lanthanide metal centre Eu(III) and Tb(III) respectively. [51] In buffered water both complexes were coordinated



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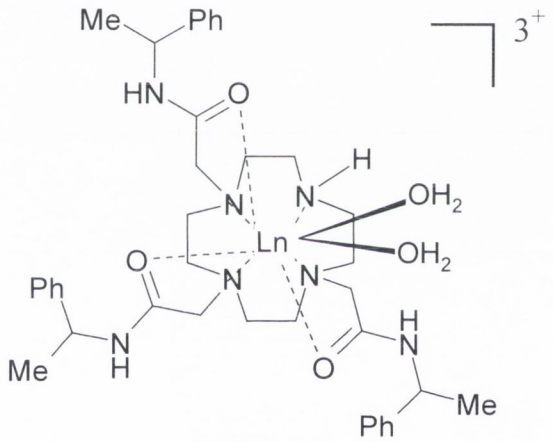
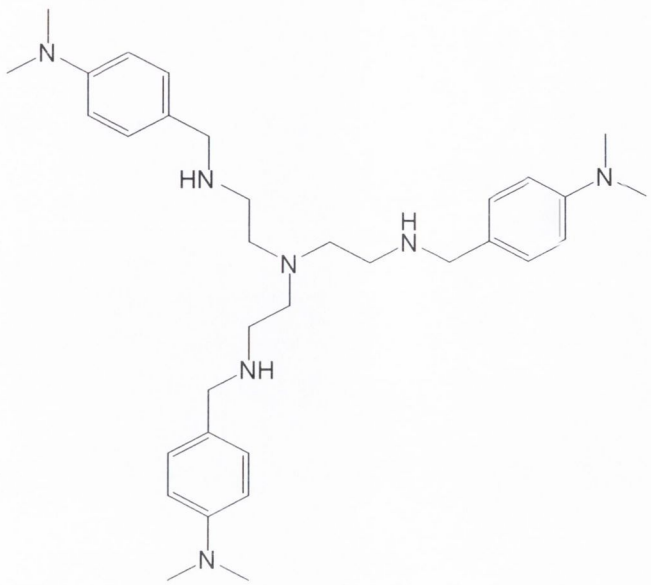


26a Eu(III)
26b Tb(III).

to two water molecules as shown in **26a** and **26b**. In the presence of certain anions, they are displaced therefore increasing the luminescence of the metal. Chloride, bromide and iodide do not displace either water molecule resulting in no change in luminescence. However fluoride, acetate and sulphate displaced one water molecule leading to a significant increase in the luminescent lifetimes. Hydrogen carbonate showed the most significant change in Tb luminescence as this anion displaced both water molecules.

Fabbrizzi and co-workers designed several sensors based around the tripodal tetraamine (tren) backbone such as **27**. This backbone tends to form five-coordinate metal complexes of trigonal bipyramid stereochemistry e.g. $[M^{II}(tren)]^{2+}$, in which one of the two axial positions is left available for coordination to another monodentate ligand, either a



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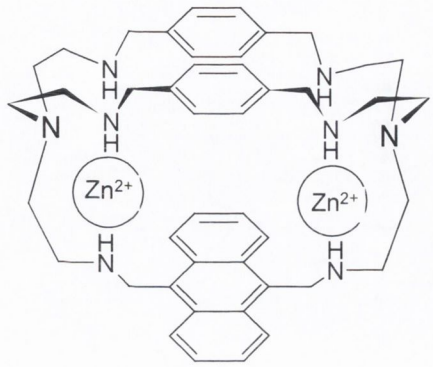
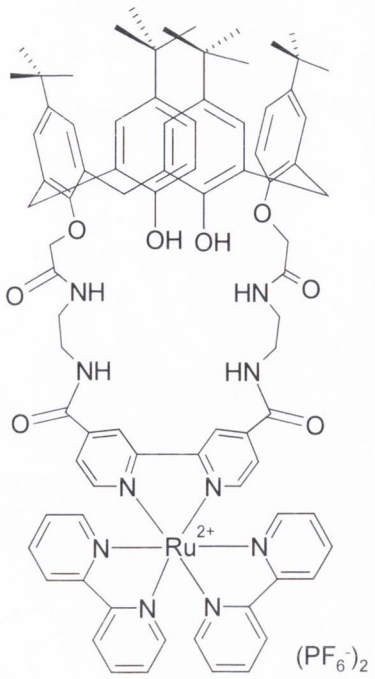


28

solvent molecule or an anion.[52] Zn(II) can form fairly stable complexes with nitrogen bearing ligands and cannot deactivate any nearby excited lumophore as it does not show any one-electron redox activity and possess a completely filled *d* shell. **27** has an N,N-dimethlaniline (DMA) subunit appended to each terminal amine nitrogen of the atom tren.[53] Carboxylates give stable 1:1 adducts with the $[Zn^{II}(\mathbf{27})]^{2+}$ receptor in MeOH, with log K values ranging from 4 to 5. However only carboxylates bearing an aromatic residue such as benzoate, are able to quench the emission of the DMA fluorophore (to 10-20% of its original value, log K = 4.69). Fabbrizzi and co-workers also synthesised an anthracene bis-tren cage system containing two Zn^{2+} ions (**28**) as a potential sensor in aqueous systems for ambidentate anions, such as N_3^-, and NCO^-. **28** is fluorescent when no anion is present. However upon introduction of N_3^- ion the fluorescence is quenched. It is speculated that the quenching occurs due to electron transfer from the electron rich azide anion to the anthracene moiety. [54]

In 1999 Beer and co-workers reported the synthesis of new ruthenium(II) and rhenium(I)bipyridyl calix[4]diquinone and calix[4]arene receptors (*e.g.* **29** and **30**), which selectively bind and sense the acetate anion. ^{1}H NMR titrations of these sensors with TBA salts of acetate, chloride and hydrogen phosphate in DMSO-δ_6 suggested a 1:1 receptor:anion complex.



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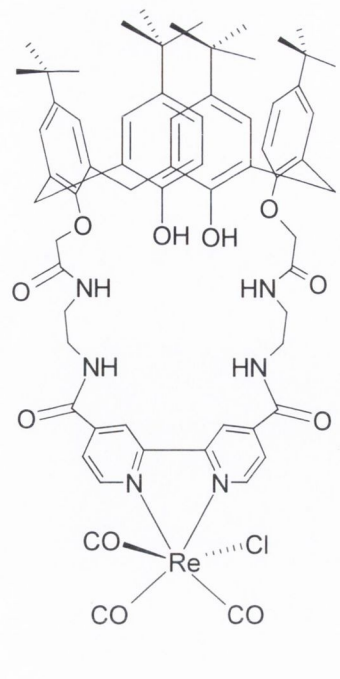

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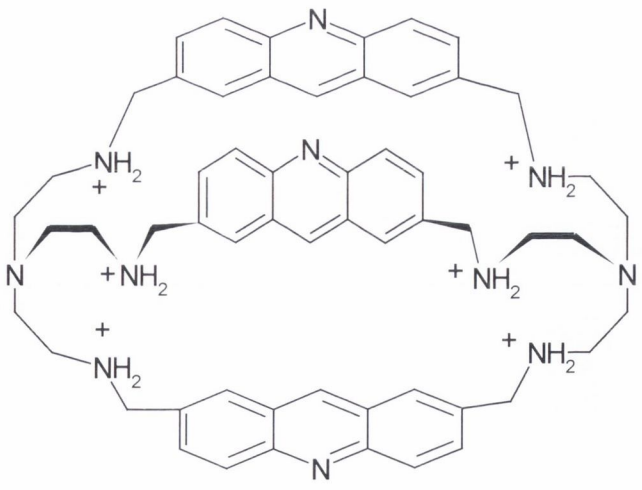
Table 1:3: Stability Constants of receptors **29** and **30** with anions in DMSO-δ_6

Receptor	Cl^-	AcO^-	$H_2PO_4^-$
29	840	4060	240
30	435	760	185

1.52 METAL FREE SENSORS

Lehn *et al.* designed an octamine cage, **31**.[55] At pH 6 all but the apical tertiary nitrogen atoms are protonated. The hexa-protonated cage is poorly fluorescent when void, the acridine



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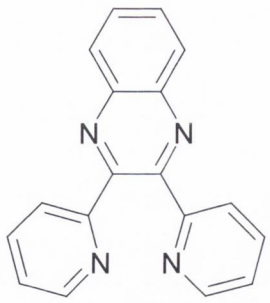

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monomer emission is almost completely quenched due to the formation of an excimer band. The introduction of a substrate into the cage prevents excimer formation resulting in a revival of monomer emission. The revival of the monomer emission band which arises from both

conformational changes and specific electronic interactions with the substrate, allowed the determination of high stability constants (K_a ranging from 10^3 to 10^7 M^{-1}).

Table 1.4. Anion binding constants for **31** in water at pH 6 in 1mmol dm^{-3} cacodylate buffer, 4 mmol dm^{-3} NaCl

Anion	Log K_a
Acetate	-
$Succinate^{2-}$	-
$Glutarate^{2-}$	2.7
$Adipate^{2-}$	3.6
BTC^{3-}	7.0
ATP^{4-}	7.0

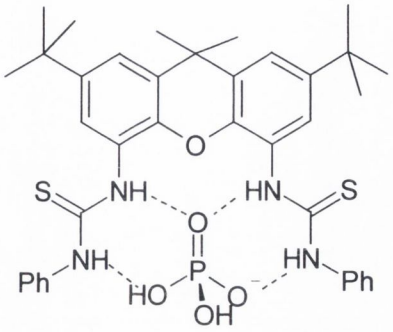
Kruger *et al.*[56] produced a novel quinoxaline-based anion sensor, **32**. Protonation of **32** results in a significant change in the conformation of the molecule. This conformational change induces luminescence. This luminescence was quenched by a variety of biologically and commercially significant anions such as dihydrogen phosphate. The binding values displayed in *Table 1.5* show the order for overall binding affinity to be $H_2PO_4^- \approx F^- > Cl^- > Br^- > I^-$.

Table 1.5: Anion binding constants for **32** as determined by luminescence quenching

Anion	Ka (M^{-1})
$H_2PO_4^-$	21500
F^-	19400
Cl^-	13400
Br^-	530
I^-	140

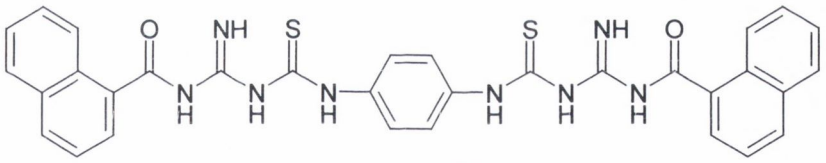
These results rule out quenching due to the heavy atom affect. This pyridium based receptor is capable of forming hydrogen bonds with anions through quaternised NH groups in addition to utilising coulombic interactions between the charged receptor and the anion guest. The overall order for binding affinity in MeCN was $H_2PO_4^- \approx F^- > Cl^- >> Br^- > I^- > PF_6^-$. Sessler & co-workers synthesised a derivative of **32** that acts as a simple colourmetric anion receptors **51** that will be discussed later in the introduction.

Umezawa and co-workers produced a series of thiourea receptors.[57] **33** is an example of one of these receptors. It binds anions such as AcO^- and Cl^- very strongly with stability constants up to 195,000 M^{-1} for $H_2PO_4^-$.



33

Hennrich and co-workers developed a number of structurally simple fluorescent sensor molecules based on the iminothiourea/1,2,4-thiadiazole unit. They exhibited extraordinarily strong fluorescent enhancement selectivity upon complexation of HCO_3^-, CO_3^{2-} and HPO_4^{2-}.[58] The fluoroionphore system **34**, consists of two receptor side arms connected *via* a phenylene group. The reduced form contains eight potential hydrogen bond donating NH groups whereas the oxidised form contains only four. In order to estimate the cooperative effect of the two side arms the mono equivalents, **36** and **37** were also synthesised, as a comparison.



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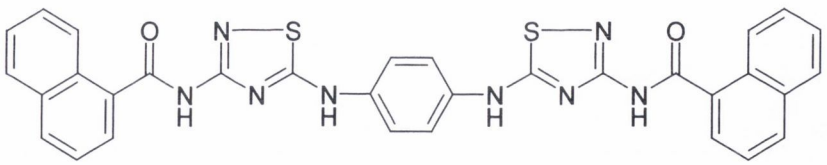

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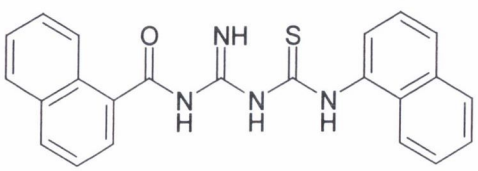
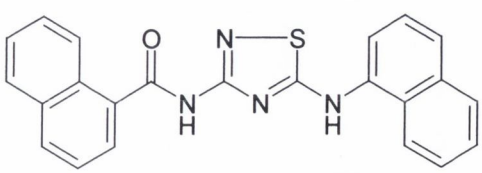


36

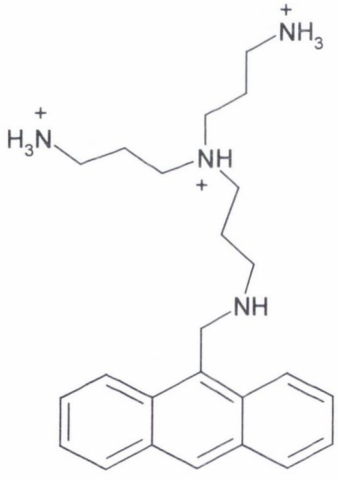


37

As expected the reduced forms **35** and **37** bound the anions significantly better. No changes in the emission properties were observed on addition of Cl^-, I^-, Br^-, ClO_4^- or NO_3^-. Whereas strong fluorescent enhancement was observed on addition of HCO_3^- and CO_3^{2-} salts, with a slight hypsochromic shift of the emission wavelength. The behaviour of the mono systems are similar but to a lesser extent to the bis systems indicating that there is no involvement of the naphthyl NH in the binding process. The extremely strong enhancement of the fluorescence intensities observed is most likely the result of an increase in rigidity of the receptor molecule upon anion complexation studies. Protonation experiments were carried out to prove only minor contributions from a complexation controlled photoinduced electron transfer (PET effect) seem to be involved.

1.53 PHOTO INDUCED ELECTRON TRANSFER (PET)

As previously mentioned numerous PET sensors for cations have been developed. However since very few PET anion sensors have been developed selected examples of both metal free and metal based sensors will be contained within this section. Czarnik *et al.* have



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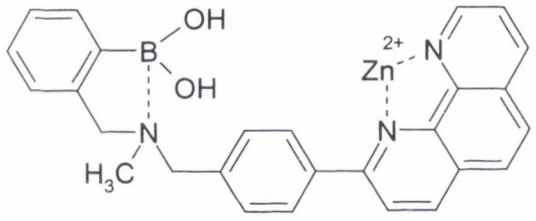
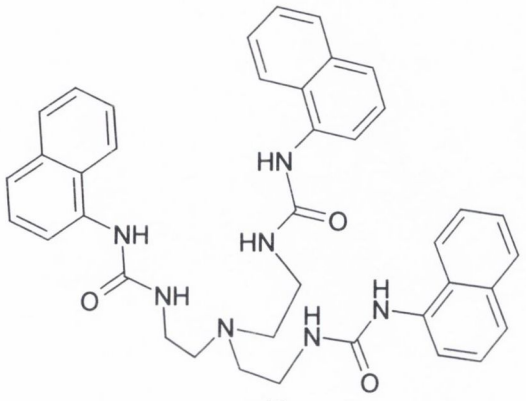


39

developed one of the first examples of a fluorescent anion sensing system, **38** utilising the PET process.[59] In aqueous solution at pH 6 the amines of **38** are protonated except for the benzylic amine next to the anthracene fragment. This amine behaves as a donor and transfers an electron to the photo-excited anthracene therefore quenching the fluorescence. On addition of HPO_4^{2-} the three ammonium groups form hydrogen bonds with three oxygen groups from the anion. In addition the benzylic amine forms a hydrogen bond with the anion. This interaction prevents the lone pair from the nitrogen atom being available for electron transfer therefore fluorescence is increased.

In 1997, Shinkai and co-workers demonstrated two-point binding of sialic acid using receptor **39**, which features a Zn(II)-carboxylate coordination and a boronic acid diol complex in the host guest structure.[60] The fluorescence modulation is the result of a PET mechanism in which the use of a nitrogen-boron interaction modulates the HOMO-LUMO interaction.



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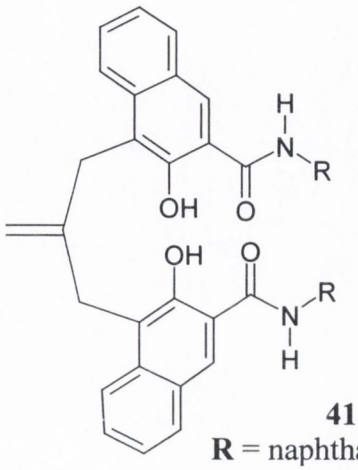


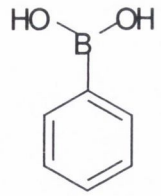
41
R = naphthalene

Wu *et al* developed a thiourea based receptor **40**, containing three naphthylene units.[61] The bis and mono equivalents of this sensor were also examined. **40** was described as a PET sensor with the quaternary nitrogen acting as the donor, the receptor acting as a spacer and the fluorophore being the naphthalene unit. However **40** senses anions *via* a combination of PET and Energy Transfer. Binding of the anion to the NH directly attached to the naphthalene unit allows energy transfer to occur between the receptor and the fluorophore therefore affecting the emission properties of the compound. In addition the absorption spectra significantly changes upon addition of the anion to the system, again breaking one of the criteria specified in order to be classified as an ideal PET sensor. **40** exhibits highly selective complexation of tetrahedral oxoanions, especially $H_2PO_4^-$, whereas it does not bind well with spherical halide anions such as Br^- and I^- and planar AcO^-.

Hiratani *et al* developed a novel anion sensing luminescent compound **41**.[62] Few fluoride selective receptors have been reported relative to their chloride and bromide counterparts. A range of anions were analysed including F^-, Cl^-, Br^- and I^- as well as non-spherical anions *e.g.* $H_2PO_4^-$ and the compound was found to have significant F^- selectivity. K_a (F^-) = 2.6 x 10^5 M^{-1}, K_a (Cl^-) = 4.5 x 10^2 M^{-1}, K_a (Br^-) = 1.2 x 10^1 M^{-1} in CH_3CN.

When boron binds with certain anions the hybridisation changes from sp^2 to sp^3. Boron centred fluoride receptors were first studied by Katz, who trapped fluoride ions between two electron accepting boron atoms in 1,8-naphalthalenediylbis (dimethylborane).[63]

More recently Reetz combined a Lewis acid boron and a crown ether to create a ditopic host for F^- and metal ions.[64] James *et al* produced a series of fluorescent PET sensor with boronic acid receptor units that displayed F^- selective fluorescent quenching in aqueous solution at pH



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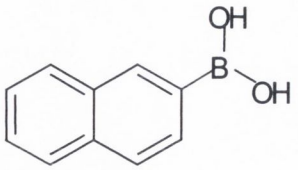

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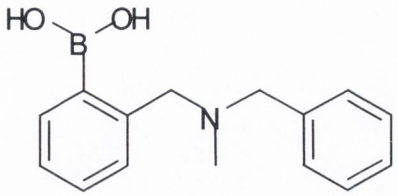
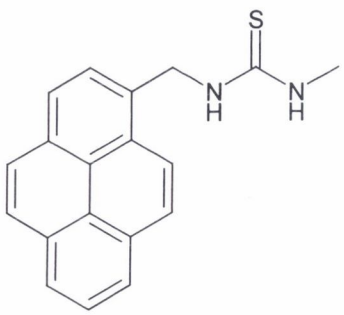


44

5.5 (**42-44**).[65] When phenylboronic acid **42** and 2-naphthylboronic acid **43** are titrated with KF in a 50% (w/w) methanol water buffer at pH 5.5 the fluorescence of both fluorophores decreases. The stability constants for F^- are 1.04×10^4 and 1.08×10^4 M^{-1} respectively. **44** was specifically designed to increase the strength of F^- binding relative to **42** by virtue of an additional hydrogen bonding site, which is available when the amine is protonated. Upon protonation the fluorescence is high because PET from the nitrogen is reduced. The F^- stability constant is 101 M^{-1}. **44** can effectively detect concentrations of F^- in the range of 5 – 30 mM. The single fluoride adduct of compound **42** is selectively stabilised by the additional hydrogen bonding from the protoanted amine from **44**. Similar titrations were carried out on **42-44** using KCl and KBr but no change in fluorescence was observed until very high concentrations of the salts were used.

Teramae *et al* recently synthesised a thio-urea based anion receptor linked to a pyrene moiety via a methylene spacer, **45**. [66] Binding studies of **45** with TBA acetate were conducted in CH_3CN. ^1H NMR could prove that binding was occurring *via* the N-H bonds however the signal was too broad to quantify the binding constant. A concurrent analysis of the fluorescent and UV/vis properties showed that upon addition of various anions to the system the



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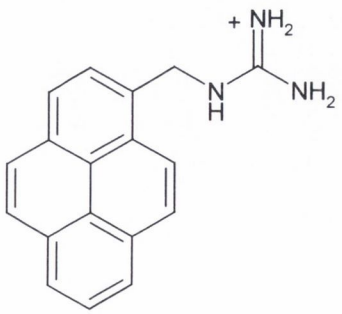

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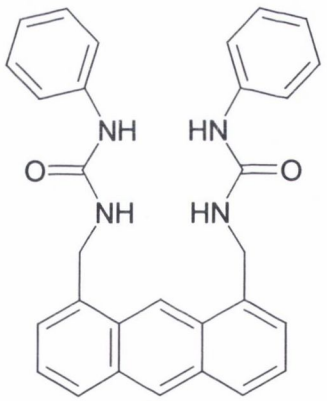
monomer emission reduced dramatically with little change in the absorption spectra being observed. The association constant for AcO^- was determined to be $7.0 \times 10^3\ M^{-1}$, whereas K_a for $H_2PO_4^-$ was $5.2 \times 10^3\ M^{-1}$ and Cl^- was $1.0 \times 10^3\ M^{-1}$ respectively. This compound does not exhibit *ideal* PET behaviour since the monomer emission quenching was followed by the formation of an intramolecular exciplex emission (see *Section 2.1*). Teramae also reported another particularly simple self assembly system for sensing anions, with a pyrene functionalised mono-guanidinium receptor, **46**. This did not show any obvious changes in the fluorescence spectra upon addition of $H_2PO_4^-$ as well as other monovalent anions such as AcO^-, Cl^- and Br^-. However in the presence of pyrophosphate a structureless band appeared with an emission maximum @ 476nm appeared, and there was quenching of the monomer emission ($K_a = 3.4 \times 10^4\ M^{-1}$).[67]

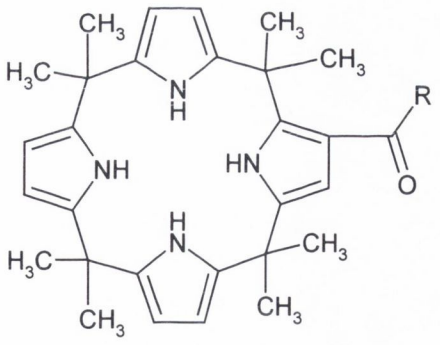
The 9,10-dimethyl anthracene spine has been manipulated before notably by de Silva[68] and Fabrizzi[69] to detect cations. Nevertheless it has not yet been used to detect anions. Czarnik and Vance did however exploit the 1,8-dimethyl anthracene spine in sensing pyrophosphate.[70] Recently Yoon *et al* synthesised a new anthracene derivative bearing two phenylurea groups at the 1,8 position of anthracene, **47**. This sensor shows a selective fluorescence quenching effect with F^- via a PET mechanism (K_a = 71270 M^{-1} in CH_3CN:DMSO 9:1 with K_a (Cl^-) = 614 M^{-1}, K_a (Br^-) = 121 M^{-1}, and K_a (I^-) = 30 M^{-1}).



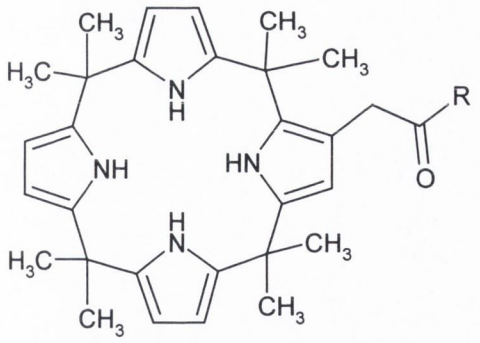
47

1.54 Optical Calix [4] pyrrole systems

Sessler *et al* initially attempted to make redox active calix[4]pyrrole sensors by attaching ferrocene moieties.[71] However the substantial changes that did occur in the electrochemical properties of these materials were unpredictable and could not be rationalised. Therefore the group investigated the feasibility of attaching fluorescent reporter groups to the calix[4]pyrrole binding site.[72] **48**, **49** and **50** were examined for the binding



48 R = NH-1-anthryl



49 R = NH-1-anthryl
50 R = $NHCH_2$-9-anthryl

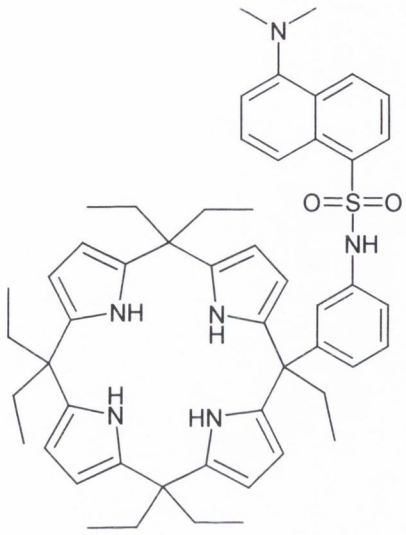
capability of a variety of anions. These systems were studied by NMR and fluorescence. ^1H NMR spectroscopy can determine the binding constant by monitoring the position of the calix[4]pyrrole NH protons as a function of anion added. However this system is a prime example of why fluorescence techniques are more advantageous than NMR spectroscopy. NH resonances broaden upon binding. The high concentrations required to carry out NMR titrations also add to NH broadening. This broadening makes it difficult to determine the binding constant accurately. However fluorescence spectroscopy shows significant quenching in varying degrees upon anion addition. The stability constants determined using fluorescence techniques are displayed in *Table 1.6*.

Table 1.6: Stability constants from compounds **48**, **49** and **50** with various anions[71]

	Log K in CH_2Cl_2			Log K in CH_3CN		
Anion	**48**	**49**	**50**	**48**	**49**	**50**
F^-	4.94	4.52	4.49	5.17	4.69	4.69
Cl^-	3.69	2.96	2.79	4.87	3.81	3.71
Br^-	3.01	a	a	3.98	2.86	a
$H_2PO_4^-$	4.2	3.56	a	4.96	3.9	a

[a] Quenching insufficient to provide an accurate stability value

Sessler *et al* continued to develop more potent and selective anion binding calixpyrrole units such as **51** and **52**. A rigid spacer was used so as to fix the distance between the quencher (anion) and the signalling moiety (Dansyl and Fluorescein respectively). The spacer contained either a sulphonamide group or a thiourea group. These linker moieties were introduced with the expectation that they might provide additional binding sites for the anion and they would work cooperatively with the calix[4]pyrrole moiety to enhance overall binding. Dansyl and fluorescein were used as reporter groups because they are water soluble and therefore the sensor would function in water. Secondly interference from fluorescent impurities is avoided.



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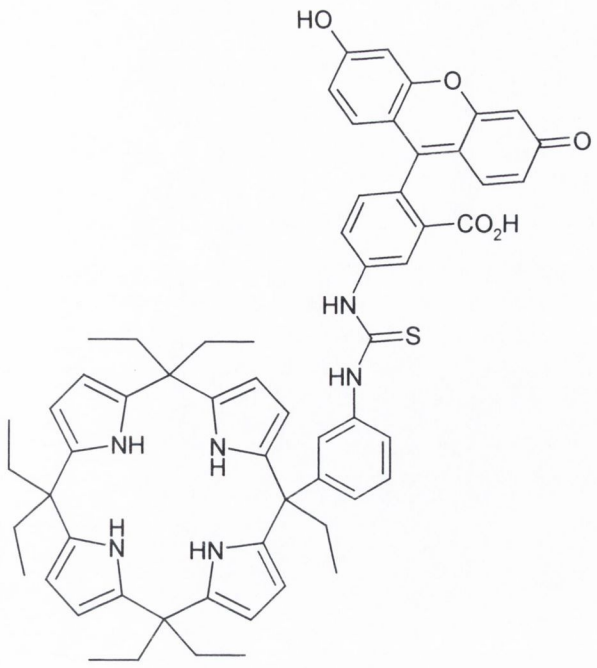


52

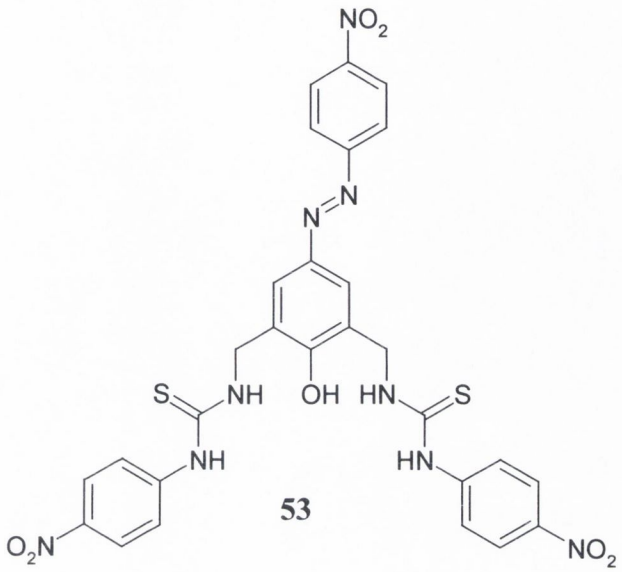
Sensors **51** and **52** displayed the highest binding affinities for anions recorded for calix[4]pyrrole type receptors to date. They are also the first to show high phosphate/chloride selectivity (2 orders of magnitude). Additionally **52** is the first sensor to operate successfully in the presence of water at physiological pH.

1.55 COLORIMETRIC SENSORS

All of the sensors mentioned above have considerable medical and industrial applications. However the field of colorimetric sensing is particularly attractive since visual detection can give immediate qualitative information while absorption spectroscopy gives quantitative information. Compounds are classed as colorimetric sensors when perturbation of the electronic properties of reporter groups upon ion complexation produces a response detectable by visual means. Several examples of this type of sensor have recently been published. [73]

Jong-In Hong and co-workers developed a new anion sensor with a dual chromophore approach, **53**.[74] The anion recognition via hydrogen bond interactions is easily monitored by anion complexation induced changes in UV/Vis absorption spectra and with the naked eye. Binding occurs *via* the four thiourea NH protons. Additional hydrogen bonding occurs via the OH of the azophenol unit, which also acts as a colour monitoring unit. The *p*-nitrophenyl group to the thiourea moiety acts as another chromophore enabling colour differentiation of

anions in a cooperative manner along with the azophenol group. $H_2PO_4^-$, F^- and AcO^- give stronger complexes and hence notable colour changes. $H_2PO_4^-$, with four oxygens affects both

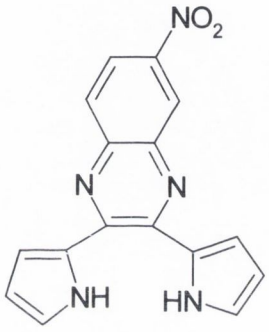


53

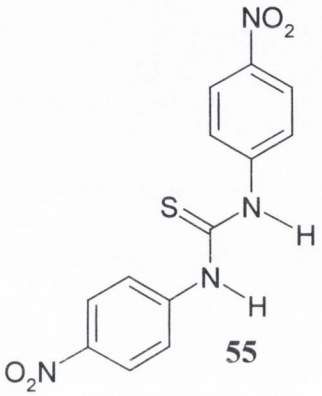
chromophores *via* multiple H Bonds giving rise to a pronounced colour change, while F^- and AcO^- have relatively weaker effect on the p-nitrophenyl group with respect to inducing colour changes. This enables colour discrimination between $H_2PO_4^-$, F^- and AcO^-. Upon addition of the $H_2PO_4^-$ the colour of the solution changes from light yellow to violet. The same colour change did not occur when appreciable amounts of HSO_4^-, Cl^- and Br^- solutions were added. The bathochromic shift may be explained by the increased stability of the anion complex of **53** relative to the free ligand. When only phenyl groups are attached to the thiourea moieties λ_{max} values upon complexation are the same for $H_2PO_4^-$, F^- and AcO^-, therefore no distinction between the anions could be determined using this system. Hong and co-workers also developed a colorimetric sensor by making *para*-nitro phenyl aza derivatives of porphyrin-based ureas.[75] These compounds showed a dramatic colour change upon addition of F^- due to increased interaction with the nitrophenylazo phenolic OH group.

Sessler & co-workers synthesised simple colourmetric anion receptors such as **54**. **54** undergoes a clear yellow to purple colour change on addition of fluoride ions in DMSO. The colour change is due to the binding of the anion via the pyrolic NH's. This compound shows strong selectivity for fluoride over chloride and phosphate.

Teramae synthesised a derivative of one of his own compounds **17** mentioned earlier in this section.[76] This compound **55** showed improved complex stability and optical response when compared to **17**. The effect of various anions as TBA salts on the spectroscopic properties of **55** were analysed. Negligible effects were observed upon the addition of Cl^-, Br^- $HI_2SO_4^-$ and NO_3^-. Significant changes were observed in the presence of 1 equivalent of AcO^-. The charge transfer absorption band shifts by 50 nm resulting in a visible colour change from colourless to yellow.

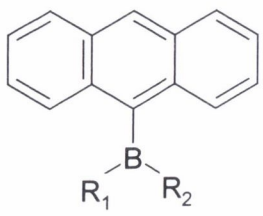


54

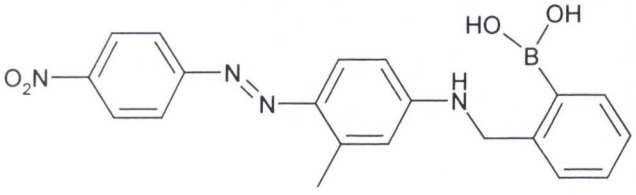


55

Tamao *et al* also exploited the Lewis acid nature of boron in synthesising several boron containing π-electron systems (**56-58**).[77] When TBAF as a fluoride source was added to a THF solution of each borane a dramatic colour change was observed from orange to colourless for **56** or from yellow to colourless for **57** and **58**. **56** showed good selectivity for the fluoride ion. The compound showed smaller binding constants for AcO^- and OH^- ($1.7 \times 10^3\ M^{-1}$ and $1.1 \times 10^3\ M^{-1}$) respectively by titration with their TBA salts in THF. Furthermore no complexation was observed upon addition of excess TBA salts of Cl^-, Br^- or ClO_4^- by UV-vis spectra. The colourmetric sensing is due to the turning off of the π-conjugation extended through the vacant p-orbital on the boron atom by formation of the fluoroborates (ICT). In contrast the silicon analogue of **56** displays a fluorescence change due to disturbance in the through space interaction among the anthracene moieties. This is due to the structural change from the tetra coordinate fluorosilane to the penta-coordinate difluorosilicate.



56 (R1 = R2 = 9-anthryl)
57 (R1 = 9-anthryl, R2 = mesityl)
58 (R1 = R2 = mesityl)

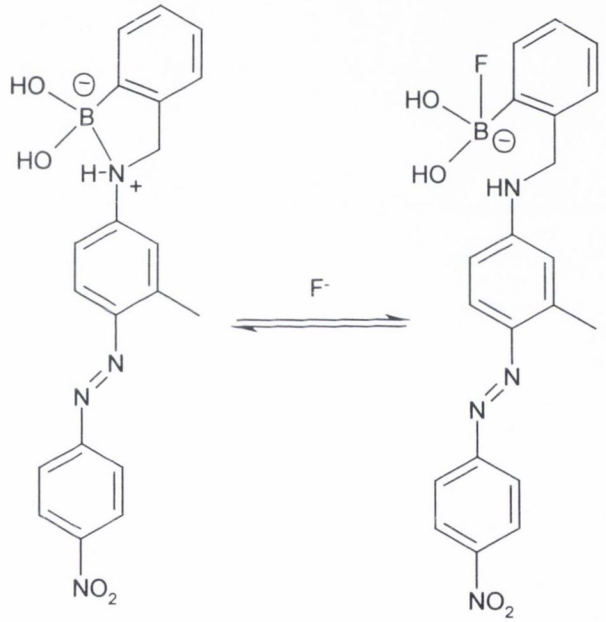


59

Table 1.6: UV-Vis Absorption Spectral Data for Tamao's compounds[77]

Free Borane		Borane with TBAF		
	λ max (nm)		λ max (nm)	K_a/M^{-1}
56	470	**56.F**	406	2.8x10^5
57	448	**57.F**	406	2.6x10^5
58	420	**58.F**	403	2.9x10^5

James *et al.* also developed a colourmetric anion sensor selective for fluoride, **59**.[78] This sensor works on the same basis as **42** and **43**, *i.e.* through Lewis acid base interaction between boron and the anions. When potassium halides (F^-, Cl^-, Br^- and I^-) are added to a solution of **59** the intensity of absorbance at 450nm increases. This is due to ICT. However it is also due to an increase in the dielectric constant of the solution. This effect has been well

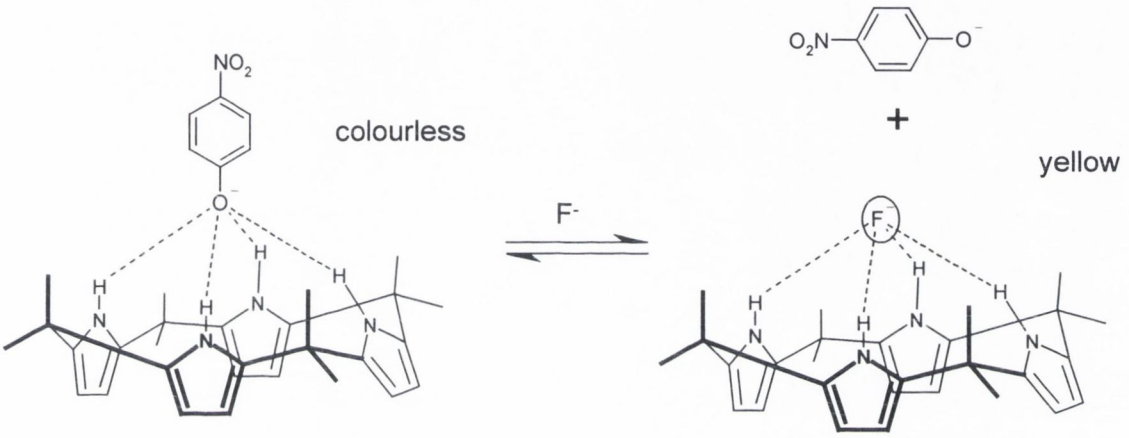


59a **59b**

documented.[79] When **59** is titrated with potassium fluoride the colour changes from orange (450nm) to claret (563nm). This colour change is also observed for pH titrations of **59**. The colour change with the pH change is associated with the formation of a tetrahedral boronate anion. Therefore the addition of potassium fluoride must also produce a tetrahedral boronate anion. Formation of the boronate anion can only be achieved if the B-N bond of an orange coloured species is broken to give the claret coloured species.

Another version of colourmetric sensor is the use of the displacement technique. It is possible that even though a synthetic receptor does not have any attached fluorophores or chromophores, anions can still be quantified from a modulation of an absorbance or emission

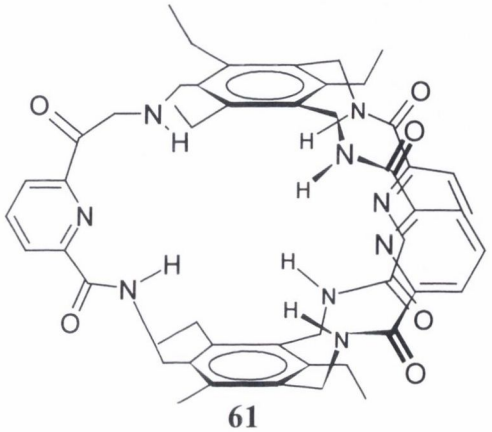
of indicators, using a competition assay with colourmetric or fluorescent molecules as indicators. The advantages of this method are: a) it can be applied to a receptor without a covalent attachment of a chromophore, (b) it is more sensitive than NMR or electrochemical measurements and (c) it is applicable in both aqueous and organic solvents. Gale and co-workers have developed a colourmetric displacement assay for anions based on the calix[4]pyrrole (**60**).[80] In this scenario, an initial calix [4] pyrrole complex involving a coloured anion is used that becomes dissociated upon the addition of a more strongly



60

coordinating anionic analyte. The 4-nitrophenolate anion looses its intense yellow colour when bound to meso-octamethyl-calix [4] pyrrole. In this case anions such as fluoride, displace the 4-nitrophenolate anion from the complex thus enhancing the absorbance of the 4-nitrophenolate anion. This was observed as a colourless to yellow colour change.

Anslyn *et al.* also designed a system utilising the displacement technique.[81] **61** is a neutral receptor that forms a complex with anionic dyes such as Resorufin or Methyl Red in organic solvents. The equilibrium between the receptor and dye is perturbed by the addition of nitrate ion, resulting in nitrate-receptor complex (K_a = 380 M^{-1}). This displacement of the dye results in a change in UV/vis spectroscopy of the indicators.

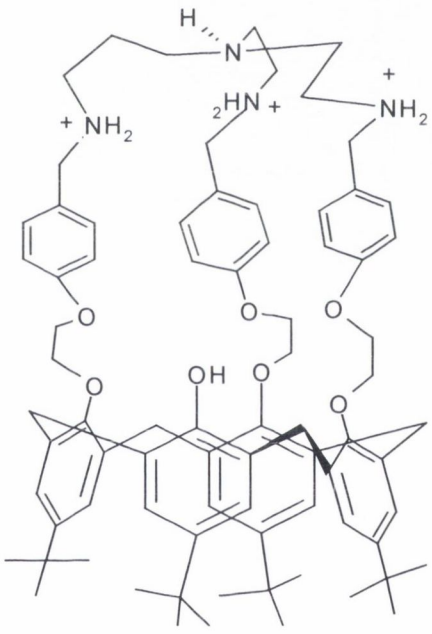


61

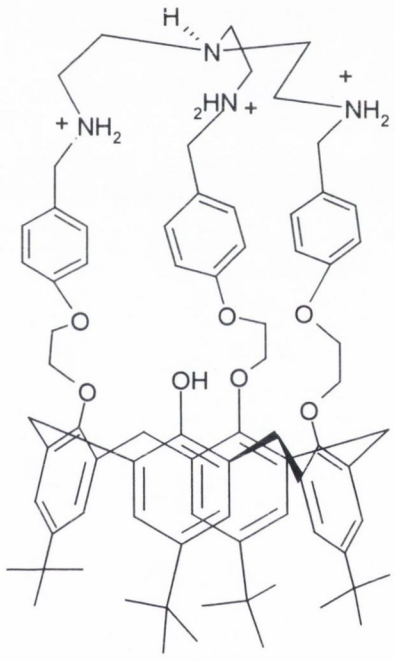
1..56 DITOPIC RECEPTORS

The natural evolution of the field of both cation and anion binding is the amalgamation of the findings of both areas to develop a receptor that will cooperatively bind both ions. Such a receptor is known as a ditopic receptor. There are many potential applications for this new class of reagents such as membrane transport, ISE's as well as reaction catalysts. Reinhoudt and co-workers have elegantly demonstrated that a calix[4]arene derivative with cation binding ester groups on the lower rim and anion binding ureas on the upper rim can efficiently bind Cl^- only in the presence of Na^+.[82]

Tuntulani *et al.* synthesised a receptor combining the calix[4]arene framework with tris(2-amino)ethylamine, (tren) and glycolic chains enabling the molecules to bind anions and



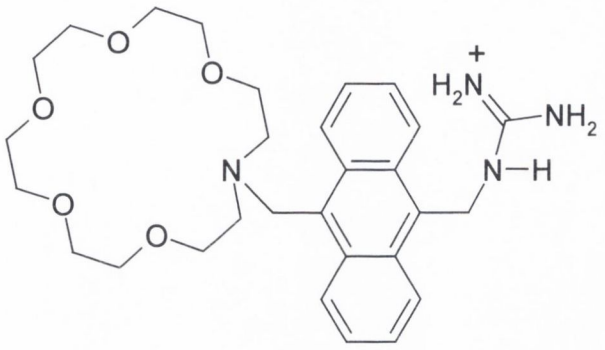
62a



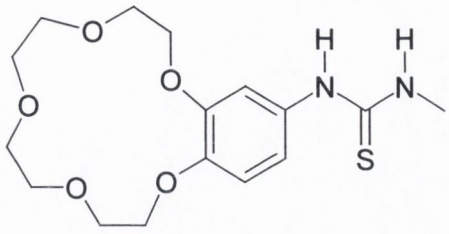
62b

cations cooperatively. The *para* and *ortho* isomers (**62a** and **62b** respectively) of this compound were made and their relative binding abilities compared. The binding studies were conducted using ^1H NMR titrations. The affinities for the *ortho* and *para* isomers of **62** for the sodium, potassium and TBA halide salts were examined. The studies showed that the tripodal ammonium cavities of both of the isomers are not suitable for binding F^-. The *para* isomer showed a high affinity for I^- relative to its *ortho* counterpart specifically when TBA^+ was the cation present. The *ortho* isomer binds both Br^- and I^- much stronger when potassium is used relative to when TBA^+ or Na^+ is used.

de Silva *et al* have synthesised molecule **63** designed to sense the neurotransmitter GABA ($H_3N^+CH_2CH_2CH_2CO_2^-$).[83] The azacrown ether unit binds the ammonium unit terminal of GABA whereas the guanidinium group binds the carboxylate end. The anthracene



63



64

part of the molecule has two important roles in the sensor design, it serves as a fluorophore and also acts as a rigid backbone for the molecule, which confers linear recognition capability on the molecule. The rigidity of the molecule due to the presence of the anthracene ring also makes the binding process entropically more favourable.

Teramae *et al* also synthesised a bifunctional receptor on the basis of benzo-15-crown-5 functionalised with a thiourea as an anion binding site **64**.[84] When Na^+ is bound in the crown ether, the anion binding in CD_3CN is significantly increased as compared with free **64**. An analogous host phenylthiourea (*i.e.* lacking the crown ether moiety) was also analysed under similar conditions. ^1H NMR titrations were conducted using TBA^+ salts of various

Table 1.7: Association constants of **64** and Ph-TU with Anions[84] in the absence and presence of NaBPh4

	64		**Ph-TU**	
Anion	no Na^+	Na^+	no Na^+	Na^+
NO_3^-	6	66	20	20
I^-	4.3	20	6.6	6.4
Br^-	25	260	75	45

anions. Sodium was introduced by adding two equivalents of $NaBPh_4$. The difference in binding of the anions with and without Na^+ present is indicative of the stronger acidity of the thiourea hydrogens when the Na^+ is complexed. An intramolecular electrostatic interaction between Na^+ ion and the anion being bound to the crown and the thiourea moieties respectively may be another reason for the higher binding values.

Another emerging field in supramolecular chemistry is chiral recognition. Determination of enantiomeric purity has become important in recent years due to increasing

restrictions on the composition of pharmaceuticals whose efficacy is dependent upon a chiral moiety. The behaviour of the enantiomers of a chiral drug may show striking differences in terms of biological activity, potency, toxicity and routes of metabolism.[85] Application of chiral recognition has been studied for over two decades.[86] Recently, the development of fluorescence-based enantioselective sensors for application in chiral catalyst screening has begun to attract interest.[87]

1.6 AIMS OF THIS PROJECT

The principal aim of this project is to develop and analyse a range of neutral fluorescent anion receptors. This chapter has outlined the progress within the area to date. We have set out to develop systems not only to compare with, but to exceed the potency and selectivity of the sensors to date. This thesis is comprised of two main sections followed by a smaller project each developed with the ultimate goal of obtaining a practical efficient selective anion sensors with biological or commercial benefit. The first approach was to develop flexible structurally non-complex sensors. These sensors are discussed in Chapters 2 and 3. Chapter 2 will show the synthesis, characterisation and analysis of various fluorescent sensors containing one binding site. The binding ability of these sensors were analysed with numerous biologically important mono-valent anions. Chapter 3 examines the introduction of a second binding site to the systems discussed in Chapter 2. Here the binding affinity of this new family of sensors are tested towards the same mono-valent anions analysed in Chapter 2. In addition the ability of this new family of sensors to selectivity sense important bi-valent anions is also discussed. The second avenue explored was the synthesis of a fluorescent analogue of a well known anion binding skeleton, cholic acid. This work was carried out in conjunction with Prof. Tony Davis (formerly of Chemistry Department, TCD).

CHAPTER 2

MONO THIOUREA AND UREA BASED ANION SENSORS

2.1 INTRODUCTION

As discussed in the chapter 1, luminescent anion sensing has recently been achieved[88] through the use of anion receptors composed, from amine[89] or polyamine moieties,[90] metal based Lewis acid centres,[91] calix[4]pyrroles[92], thiouronium[93] and protonated quinoxaline,[94] but the use of simple and easily synthesised electroneutral anion receptors for such sensing has been less investigated.[95] Nature sometimes uses structurally simple yet highly effective molecules to selectively bind one guest in preference to another. Synthetic research has endlessly tried to mimic the potency of these natural receptors. However strong selectivity in addition to high binding values, has only been obtained whilst using complex rigid molecules that are difficult and sometimes costly to synthesise. Intrigued by this fact, we set out to develop the charge neutral chemosensors **65 - 67**, employing the criteria of PET sensing using the *fluorophore-spacer-receptor* model developed by de Silva for the detection of cations.[96] A few research groups have attempted to develop PET anion sensors. [89,90,95,97]

We set out to use our knowledge of photochemistry and in particular Photoinduced Electron Transfer (PET) coupled with our knowledge of supramolecular host guest chemistry to develop the first ever neutral PET sensor for anions that would exhibit an ideal response. In order for a compound to have ideal PET behaviour only the quantum yield (intensity) and lifetime of the fluorescence should be modulated upon ion recognition due to changes in the free energy of electron transfer (ΔG PET) between the excited state of the fluorophore and the

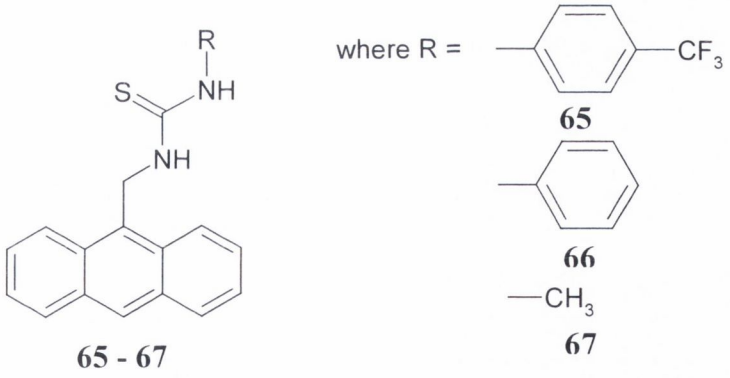


Figure 2.1: Initial thiourea sensors designed, synthesised and analysed

receptor upon ion recognition. Furthermore no changes should be observed in the absorption spectra of the fluorophore since it is separated from the receptor by a spacer.[96] PET is discussed in more detail in section 2.2. We chose anthracene as the fluorophore since it has been used by de Silva and Fabbrizzi for PET sensors in cation sensors and consequently its photophysical properties are well known. We chose a thiourea moiety as the receptor unit. These moieties were separated by a methylene unit. The thiourea moiety has been exploited in

several types of anion receptors as shown in examples **18**, **52** and **64**. This hydrogen bonding moiety has been used in conjunction with electrostatic and/or Lewis base interactions, in addition to being the sole interactive force with the anion in other receptor systems. We chose the thiourea moiety because of its neutral properties and also because of the ability to "tune" the selectivity of the receptor by changing the skeleton groups attached to the thiourea functional group. In this instance we chose 4-(trifluoromethyl)phenyl, phenyl and a methyl group to illustrate the selectivity obtained by changing the skeleton groups attached to the thiourea. The electron withdrawing ability of the skeleton groups going from **65** – **67** decreases and consequently the acidity of the thiourea protons decreases. This decrease in acidity results in reduced binding affinity for the sensors for the various anions. The synthesis and spectroscopic investigations of our sensors are discussed in depth following the explanation of PET.

2.2 PHOTOINDUCED ELECTRON TRANSFER (PET)

The principal of luminescent PET sensing is summarised in *Figure 2.2*. Using the model *Fluorophore-Spacer-Receptor* developed by de Silva for detection of cations such as H^+, Ca^{2+}, Na^+ etc.

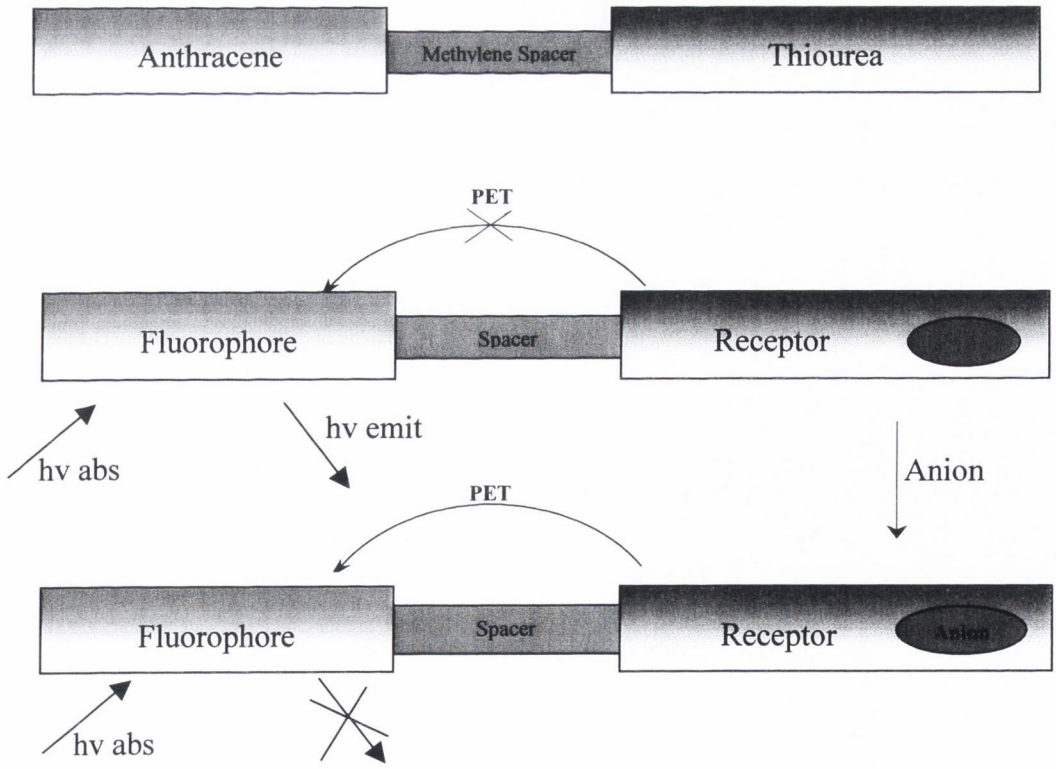


Figure 2-2 Explanation of PET based on de Silva model

As discussed in Chapter 1, one of the key mechanisms for the dynamic quenching of the excited states is electron transfer. PET describes the movement of an electron rich species (donor) to an electron poor species (acceptor). The mechanism for electron transfer depends upon the enhancement or reduction of redox reactivity upon photoexcitation, and its direction depends upon the relative energies of the highest occupied molecular orbitals (HOMOs) and lowest unoccupied molecular orbitals (LUMOs) of the species involved. For cation sensors, the emission is usually switched on upon recognition, since the oxidation potential of the sensor is increased. We propose that the opposite effect would be seen for anion systems, due to an increase in the reduction potential upon anion binding, since the electron density of the receptor is increased. This would lead the quenching of the anthracene fluorescence. This theory is best explained by use of the energy diagrams displayed in *Figure 2.3*. We propose two possible mechanisms using these energy diagrams:

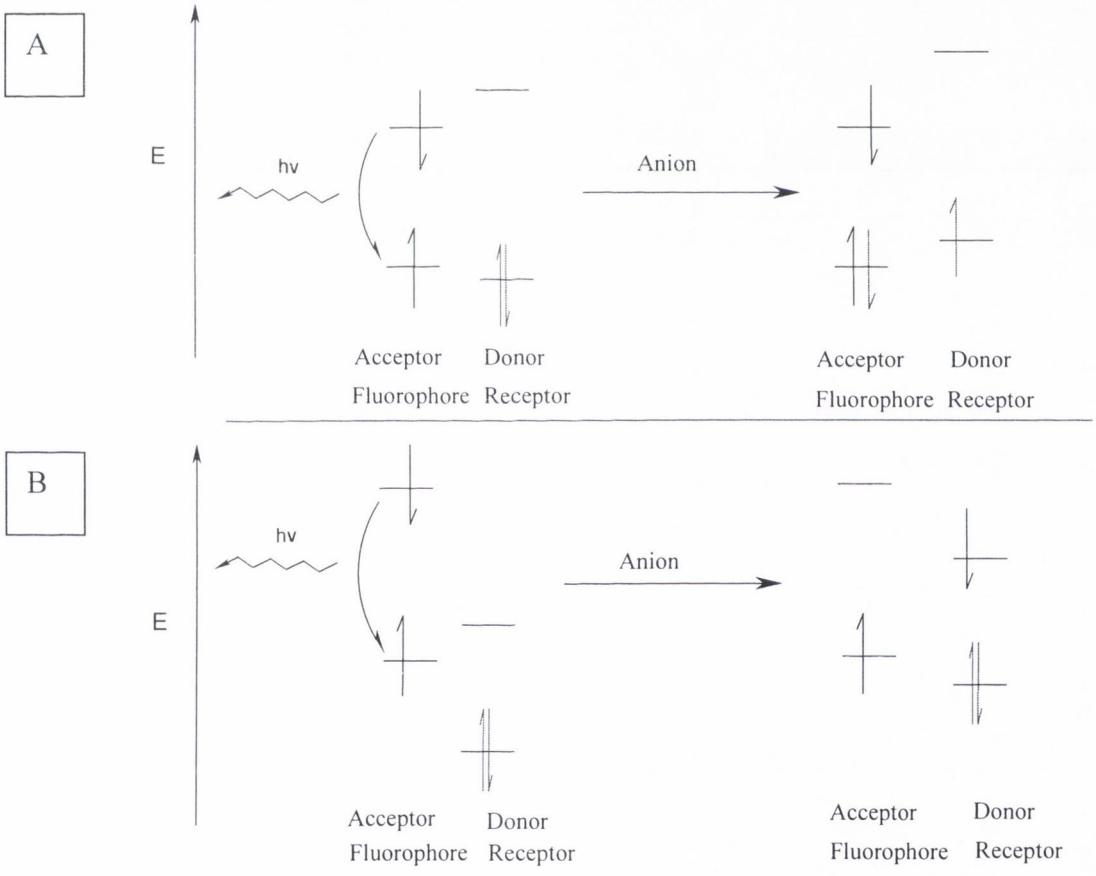


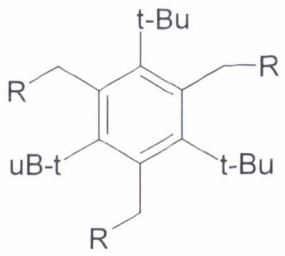
Figure 2-3: Oxidation potentials of a sensor with and without an anion present

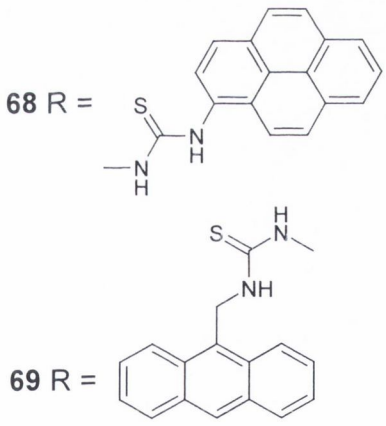
A) In the free state the oxidation potential of the receptor is higher than that of the lumophore herefore PET is thermodynamically unfavourable. However introduction of an anion to the ystem lowers the oxidation potential of the receptor allowing PET to occur, therefore causing

the emission to be quenched with a concomitant reduction in the quantum yield. The HOMOs and LUMOs here refer to ground state configurations.

B) In the free state the LUMO of the receptor is lies between the HOMO and LUMO of the fluorophore. Electron transfer can occur from the LUMO of the fluorophore to the LUMO of the receptor. However this is unfavourable which is why most of the energy deactivates through fluorescence when the electron returns from the excited state to the ground state. However introduction of an anion to the system lowers the oxidation potential of the receptor making PET more favourable to occur, therefore causing the emission to be quenched with a concomitant reduction in the quantum yield. We could not verify which mechanism actually occurred as the thioureas were found to be irreversibly oxidised and hence the value of the electron transfer ΔG_{ET} could not be determined.

We realised from experience and from the literature, that if these criteria were to be met and the optical response is to be optimised, it is necessary to have the shortest possible spacer unit between the receptor and the fluorophore since electron transfer is a function of $1/r^6$. Shortly after our work was published on **65-67**[98] Suzuki *et al* published work on a preorganized tripodal fluorescent sensor based on hydrogen bonding of thiourea groups for



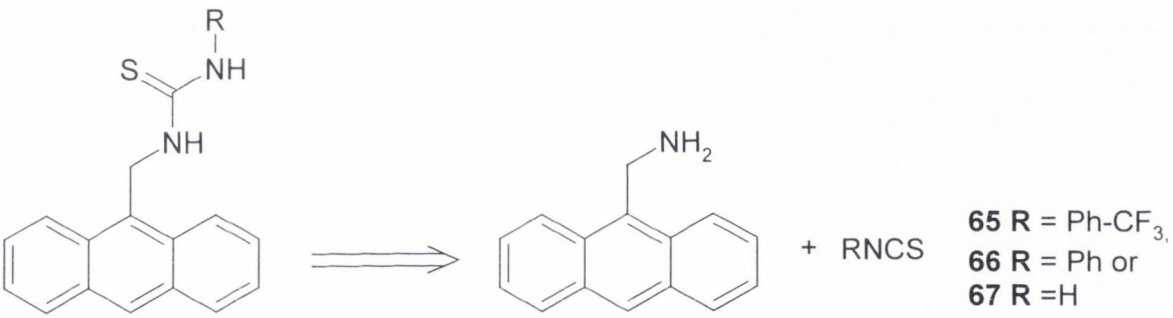


optical phosphate ion sensing (**68** and **69**).[99] The two sensors were synthesised and their binding selectivity toward several guest anions were. **68** showed an increase in fluorescence intersity upon introduction of anions such as AcO^-, $H_2PO_4^-$ and Cl^- to the environment. The fluorescence intensity increased in the order of $H_2PO_4^- > AcO^- > Cl^-$, which is different from the basicity of the anions. It should be noted that the reference compound used in this analysis, **45** showed a reverse in selectivity for acetate over phosphate. A slight excimer emission was also observed due to intramolecular interaction of the pyrene rings. The absorption spectra also increased with increasing anion concentration resulting in two clear isobestic points. Conversely when anions were added to a solution of **69** the fluorescence

decreases and the absorption spectra changes were neglible. This indicates that **69** acts as a PET sensor. The degree of intensity changes for **69** showed quenching in the order $H_2PO_4^-$ > AcO^- > Cl^- >> ClO_4^-. Both compounds were found to be selective for dihydrogen phosphate, with binding constants of 3.7 x 10^5M^{-1} and 1.9 x 10^4M^{-1} in CH_3CN for **68** and **69** respectively. The reversal in selectivity between phosphate and acetate was also observed when comparing binding ability of **68** to the 9-butyl thiourea methyl anthracene. Therefore it was concluded that the anion selectivity between the two oxyanions is caused by the tripodal structure of the receptors **68** and **69**. Like **69** our thiourea sensors also use anthracene as the fluorphore. The following section outlines the various synthetic routes investigated in order to obtain the desired sensors.

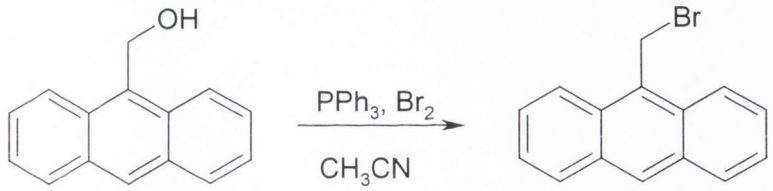
2.3 Synthesis

A key advantage to this family of sensors is the quick and easy synthetic route available. In order to find the most efficient synthetic route it is necessary to conduct a retro-synthetic analysis on **65**. Several derivatives of anthracene are commercially available and



Scheme 2.1: Retrosynthetic analysis of **65-67**

therefore several synthetic avenues were open to exploration. 9-Carboxyanthracenealdehyde was used as our starting point. Sodium borahydride ($NaBH_4$) was used in slight excess to reduce the aldehyde to 9-hydroxylmethyl anthracene in high yields (>92%). This method produces high yields of pure product in an extremely simple yet efficient manner. Once isolated, the alcohol was converted into the corresponding halide. The traditional method is to convert the alcohol, **71** to the chloromethyl anthracene by refluxing in thionyl chloride. This method, though synthetically simple was not ideal, since the reagent is toxic and can be difficult to completely separate from the product. In addition, we also found that the reaction needs to be left on for at least 12 hours to obtain a good yield (>70%). Therefore, two separate investigations were carried out. Firstly, the bromide derivative, **72** was made as this is known to be more reactive due to bromide being a better leaving group than chloride. Secondly an alternative, the use of a more efficient chlorinating agent was investigated. Both methods

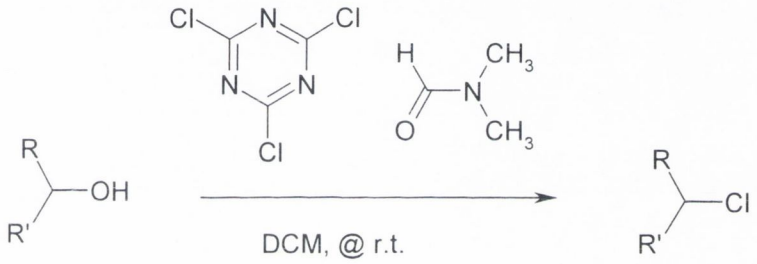


71 **72**

Scheme 2.2: Synthesis of 9-bromo methyl anthracene

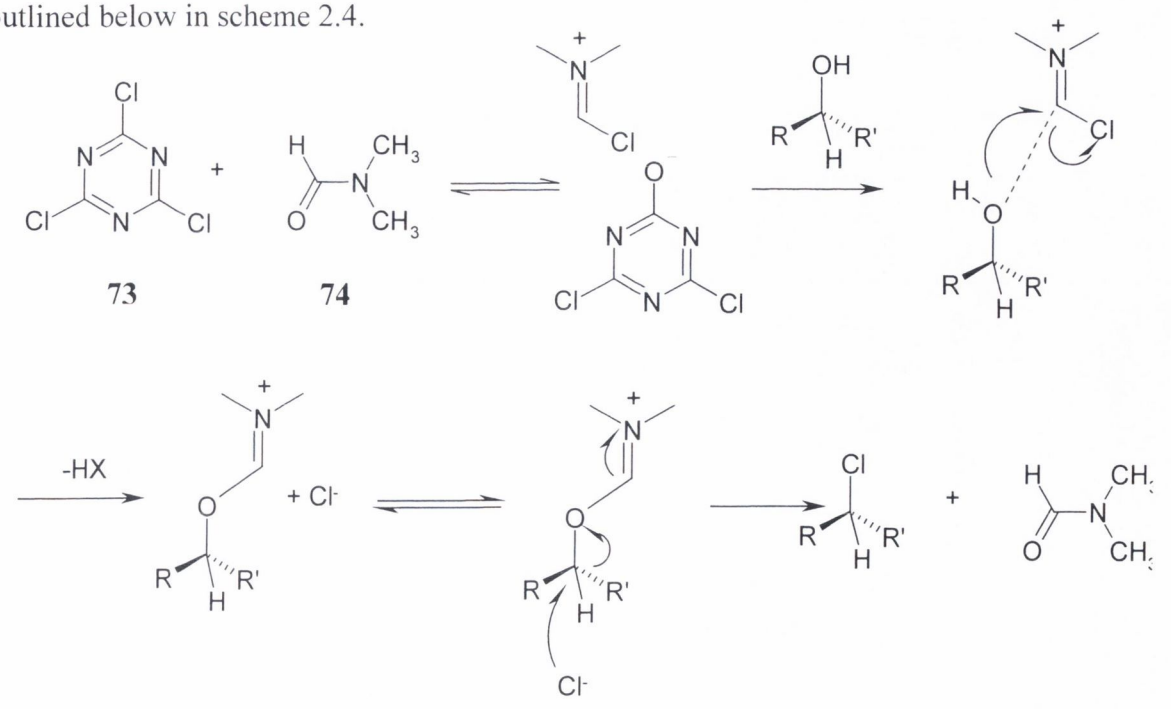
investigated were very successful. **72** was synthesised using a method developed by Kitchling *et al* who successfully converted 9-Hydroxymethylanthracene to 9 bromomethylanthracene[100]. One equivalent of bromine was added slowly to a solution of PPh_3 in dry CH_3CN. This was followed by the slow addition of 9-Hydroxymethylanthracene to this solution. The product precipitated out and was easily recrystalized in chloroform in high yields (86%). **72** resulted in better yields and shorter reaction times of subsequent reactions.

Concurrently a second chlorinating method was also investigated using cyanuric



Scheme 2.3: An alternative chlorinating agent to thionyl

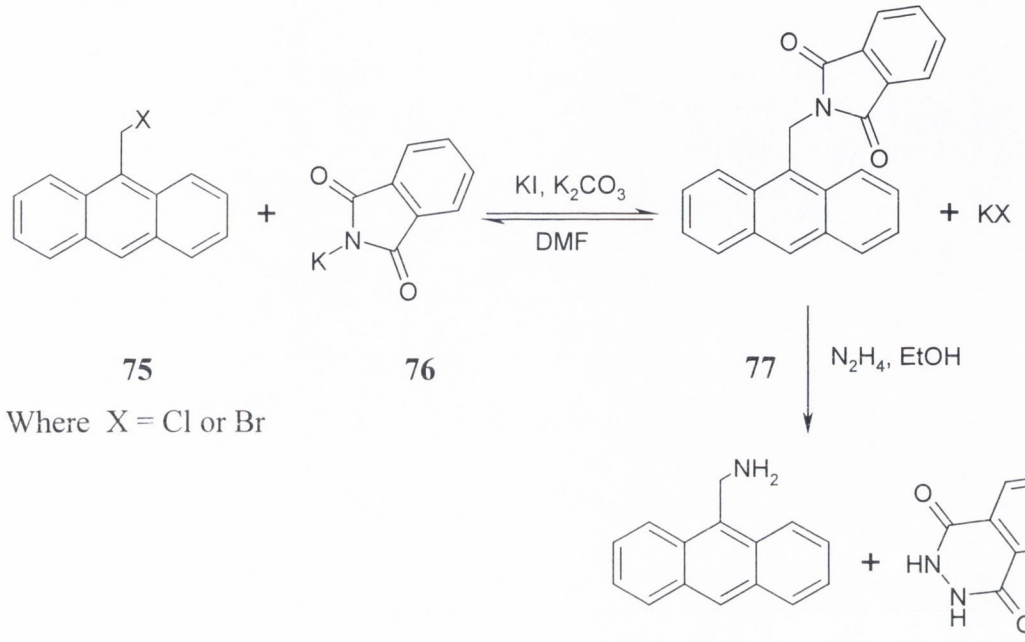
chloride, **73**.[101] This alternative was investigated to check if higher yields or purity could be obtained. **73** is cheaper and cleaner than thionyl chloride. It also results in higher yields (>90%) in addition to shorter reaction times. The suggested mechanism for this reaction is outlined below in scheme 2.4.



73 **74**

Scheme 2.4: Suggested mechanism for chlorination of an alcohol using cyanuric chloride

The resulting higher yields and cleaner process make this alternative reagent an attractive replacement to "tried and tested" procedures. The next stage involved the amination of the halide. Like the chlorination step, known methods were first used, then alternative more efficient methods were sought. The first method tried was the Gabriel synthesis[102].

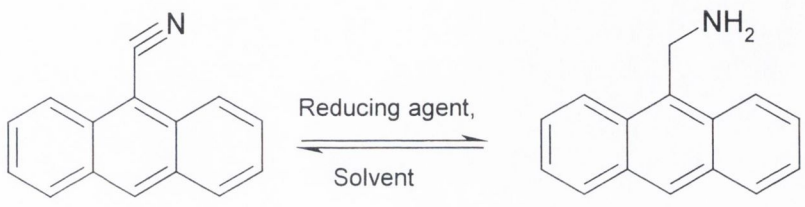


Scheme 2.5: Gabriel Synthesis

This involves reacting the halide, **75** with potassium phthalimide, **76** to obtain 9-methyl phthalimide anthracene, **77**. This intermediate was then reacted with hydrazine to obtain the desired amine. This procedure, though well known, is not documented very well. Difficulties were encountered with the solubility of the **77**. Therefore converting **77** to the amine resulted in very poor yields. Considering that **70** was only one product in a series of desired target molecules, an alternative method was required.

Initially the Gabriel synthesis was conducted on the bromine derivative of the anthracene moiety. However, again, solubility problems were encountered with the phthalimide intermediate – essentially the yield of the intermediate was improved (by almost 15% to 43%). However, the solubility problem remained the same. Therefore two alternative methods are investigated. Firstly numerous attempts were made to reduce 9-cyanoanthracene, a commercially available raw material. Sodium borahydride, lithium aluminium hydride and borane were three reducing agents that were separately investigated under different conditions. The reaction time, temperature and solvent were varied. The most severe conditions for each reducing agent was refluxing for 5 days in DMF. The most successful reducing reagent in terms of percentage yield and reaction time, was the borane THF complex

in THF using 2 equivalents of BH_3 (*scheme 2.6*). The reaction was monitored by TLC over several days. The resulting amine was identified on TLC plate by using ninhydrin. TLC showed at least on a qualitative basis, that the reaction was completed over 24 hours. Despite the fact that a significant amount of starting material still remained, this method was vastly more successful than the Gabriel synthesis. The product was then easily purified by acid base extraction. The crude product was dissolved in $CHCl_3$ and washed with 1M HCl. The aqueous



78 **70**

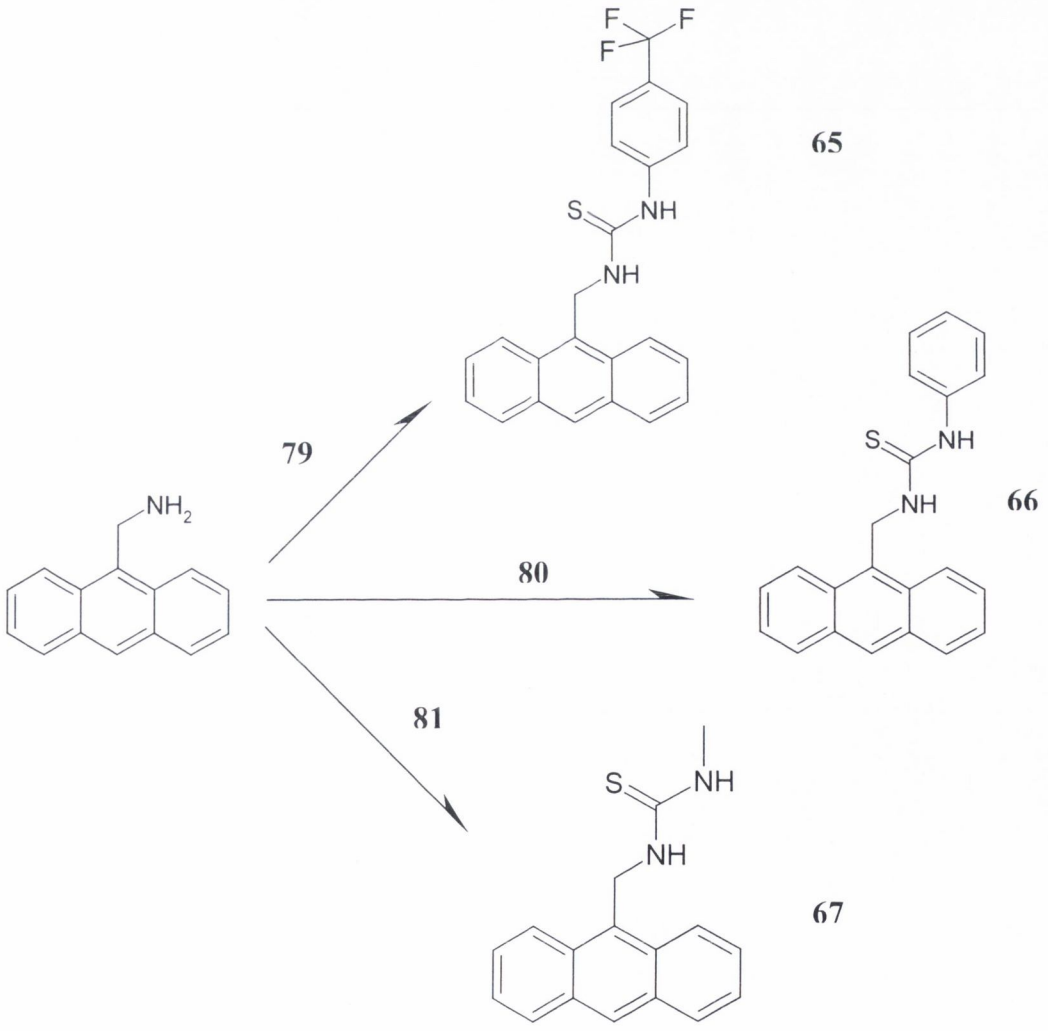
Reagent	Solvent	Temperature	% Yield
$NaBH_4$	THF	RT	n/a
$LiAlH_4$	THF	RT	n/a
B_2H_6	THF	Reflux	68
$NaBH_4$	THF	Reflux	~25
$LiAlH_4$	THF	Reflux	~25
B_2H_6	DMF	Reflux	65
$NaBH_4$	DMF	Reflux	40
$LiAlH_4$	DMF	Reflux	43

Scheme 2.6: Reduction of 9-cyano-anthracene

layer was reduced under vacuum, then the resulting residue was dispersed into 10% $NaHCO_3$ and extracted with $CHCl_3$. The presence of the target compound was easily determined by ^1H NMR due to the presence of the new peak at 5.6ppm (from the CH_2 spacer group). The pure compound was obtained in good yields (~70%). However, reproducibility of yield was not good. While this method produced the raw material for the first sensors, the lack of reproducibility was disappointing.

As a result of this a third method was investigated involving the use of hexamethylenetetramine. This reagent is cheap, efficient and easy to use. It reacted readily with alkyl halides in $CHCl_3$ to yield an insoluble amine complex. This insoluble complex was then filtered and dispersed in a solution of ethanol, water and concentrated HCl in a ratio of 20:4:5. The complex dissolved in the solution is heated at 70°C for three hours. The solution was then removed from the heat and left in darkness overnight to recrystalise. The amount of resulting precipitate was dramatically increased by reducing the solvent with slight heating. This amine was then pivotal in the synthesis of the three main PET chemosensors (*scheme*

2.7). The 9-aminomethyl anthracene was reacted in dry DCM at room temperature, under inert atmosphere with an equimolar amount of 4-(trifluoromethyl)phenyl-, phenyl and methyl isothiocyanate respectively,**79 – 81** respectively, yielding **65 - 67** as off white solids that were purified by crystallisation from chloroform. Numerous commercially available isocyanates could have been chosen. The three different isothiocyanates were chosen with the aim of being able to modulate or tune the acidity of the thiourea receptor moiety, which would lead to different receptor-analyte complex stability and hence different binding constants. All products were analysed by conventional methods i.e. NMR, IR, Mass spectrometry, CHN, etc. As with the monosensors **65-67** the sensors dissociated while using the Electrospray Mass Spectrometer preventing MS analysis. However elemental analysis and NMR spectroscopy were sufficient to prove the sensors identity and purity. The next section outlines the spectroscopic analysis used to determine the sensing ability of each of the sensors. *Figure 2.4* shows the NMR spectra of **66**. This spectra is the most complicated ^{1}H NMR of the three



Scheme 2.7: Synthetic route of sensors **65-67:** All reactions were carried out in DCM at RT

the three sensors synthesised, yet it easily interpreted. The thiourea protons are easily identified due to their broad appearance and their position. The aromatic protons are easily identified due their position and their splitting and coupling constants. Consequently using NMR techniques to determine the binding constant was very easy. NMR was used as a confirmational tool for the results obtained using UV and Fluorescence spectroscopy. NMR can also give useful structural information about the anion/ sensor complex that cannot be determined by UV and Fluorescence spectroscopy alone. The NMR analysis shall be discussed in full in *section 2.3.7*. The next section outlines the other spectroscopic analysis conducted and discussion the results obtained.

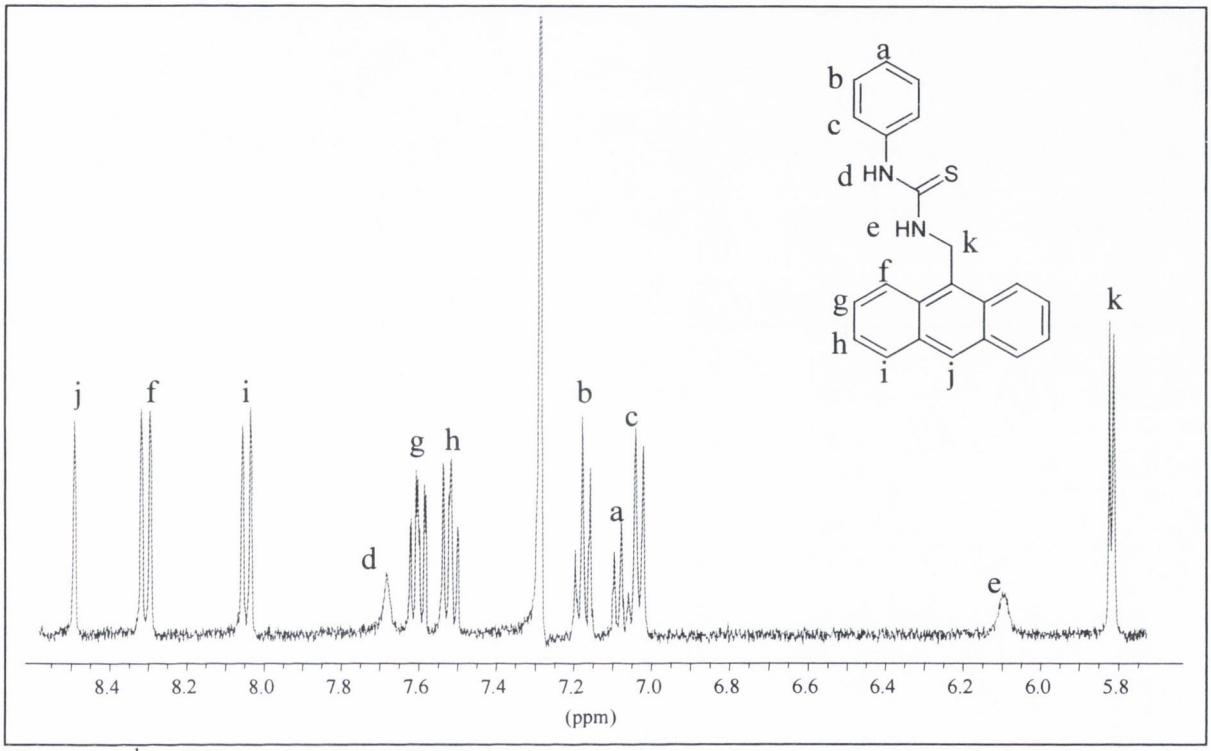


Figure 2.4: ^{1}H NMR spectra of **66** in $CDCl_3$

2.4 SPECTROSCOPIC INVESTIGATIONS

Having synthesised **65 – 67** successfully we turned to investigate the sensing and selective recognition ability of these sensors. All of the investigations were carried using HPLC grade DMSO. This solvent was chosen because it is a highly polar aprotic solvent. The UV/Vis and fluorescence spectra of these compounds, taken in DMSO are illustrated in *Figure 2.5* and *2.6*. The characteristic three peaks of anthracene observed in both the fluorescence and UV spectra were monitored upon incremental additions of tetrabutylammonium salts of certain anion solutions (concentrations ranging from $1x10^{-5}$ – 1M) to a solution of known concentration of the sensor in DMSO. *Figure 2.5* shows the

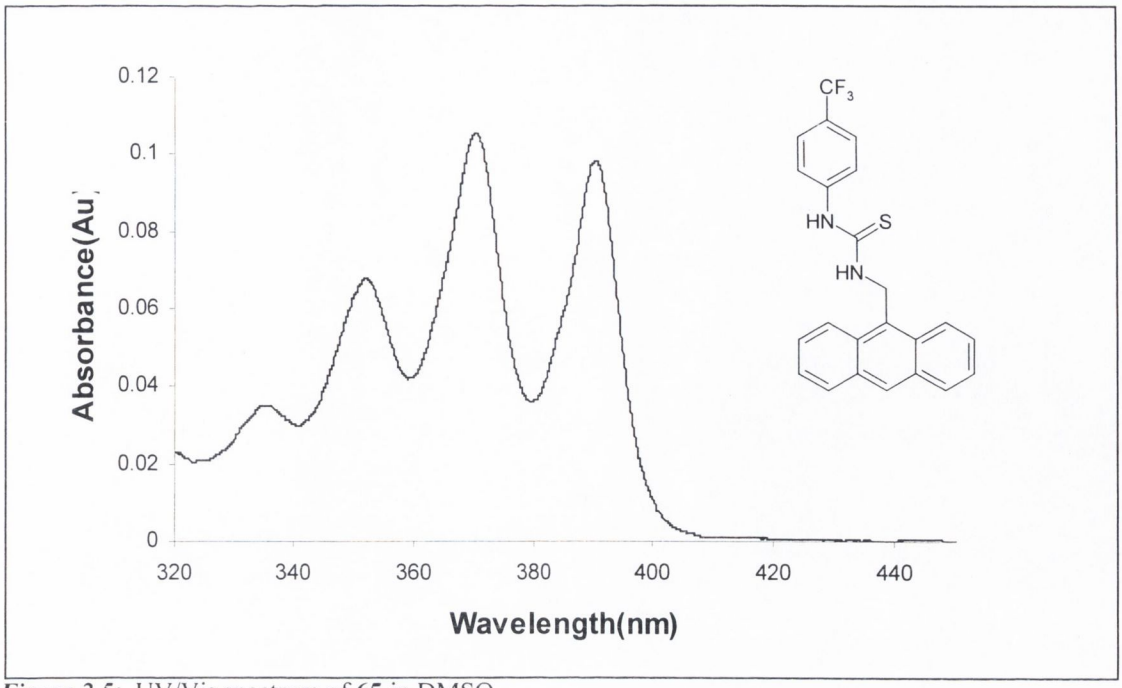


Figure 2.5: UV/Vis spectrum of **65** in DMSO

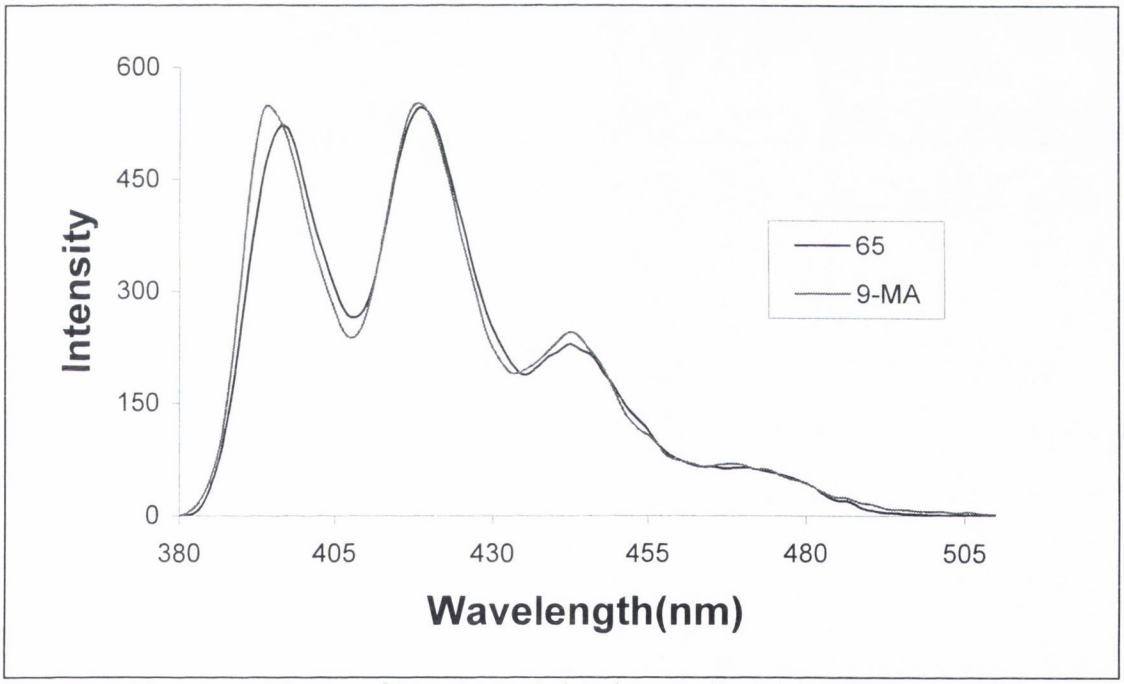


Figure 2.6: Fluorescence Spectra of **65** and 9 Methyl Anthracene (9-MA) in DMSO

absorption spectra of the monosensor **65** (3 x 10^{-4} M), consisting of bands at 390, 370, 352, and 336nm. The corresponding fluorescence spectra of **65**, (*figure 2.6*) displayed a mirror like reflection of the UV peaks. The fluorescence emission spectra consisted of three sharp bands at 443, 419 and 397nm with Φ_F =0.1080. The lifetimes, τ of the 65 –67 were too short to measure. The quantum yields are discussed in detail in *section 2.36*. The characteristics of the UV and fluorescence peaks (i.e. the position, intensity and shape) were monitored while performing a titration with various anions. We initially investigated the binding of **65** using

$(C_4H_9)_4N(O_2CCH_3)$, since AcO^- is known to form strong directional hydrogen bonding with thiourea, as well as having a functional group of great biological relevance, as previously discussed. The changes observed in the UV spectrum (*figure 2.8*) are minimal. In contrast the corresponding changes in the fluorescence spectra are very significant (*figure 2.7*). The position and shape of the peaks did not change. But the intensity was dramatically affected upon addition of the anions. As already mentioned in the absence of AcO^- the fluorescence

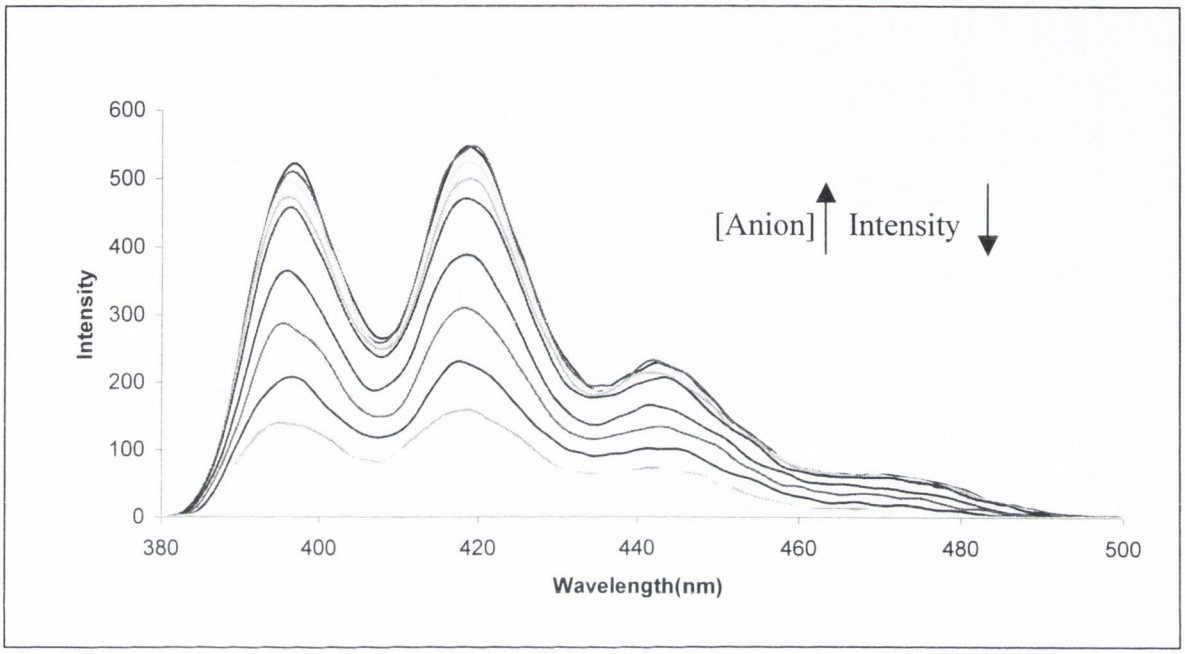


Figure 2.7: Fluorescence titration of **65** with TBA.AcO (0 –32 mM)

emission spectra consisted of three sharp bands. Upon addition of the AcO^- (0-32mM), the intensity of these bands gradually decreased with no other spectral changes being observed (*i.e.* no spectral shifts or formation of new emission bands), *Figure 2.7*. Using PET nomenclature, the emission can be said to being approximately 70% (at 443nm) '*switched off*'

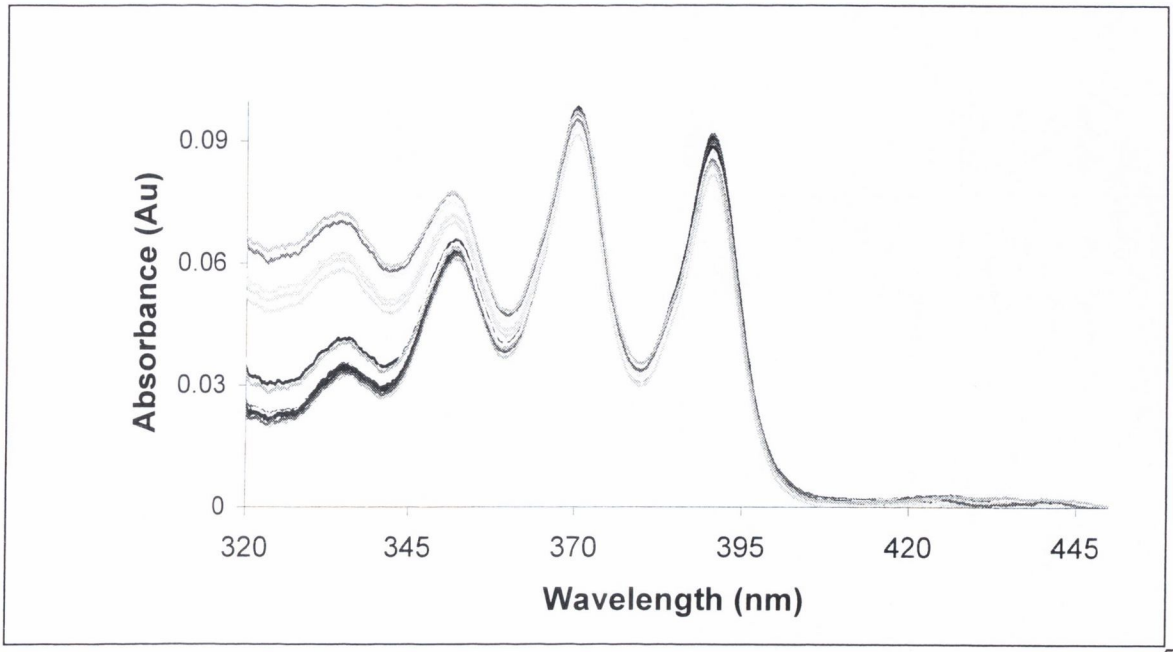


Figure 2.8: UV/Vis Titration of **65** with TBA AcO (0 – 32 mM)

with ΦF=0.0070. Concurrently, the absorption spectra of **65**, consisting of bands at 390, 370, 352, and 336 nm, was hardly affected by the addition of AcO^-. The change in absorption observed at 336nm is caused by the increase in absorbance at the wavelength corresponding to the 4-trifluoromethyl phenyl unit. The fact that the absorbance due to the anthracene peaks do not change significantly confirms the insulating role of the methylene spacer, which minimises any ground state interactions between the fluorophore and the anion receptor. This insulating role of the methylene spacer is also evident by the similarity of the UV and fluorescence spectra of **65 – 67** with that of 9-methylanthracene, the standard used for the quantum yields (*Figure 2.6*). The shapes of the peaks obviously are similar, however the positions of the peaks of the 9-methylanthracene are only marginally different to the corresponding peaks of **65 – 67**. Data from the spectra from the fluorescence titration was then used to establish a relationship between the intensity and the concentration of the anion and essentially determine the binding constant. Initially a plot of intensity (at 419nm) versus concentration of the anion was plotted (*figure 2.9a*). This relationship though not linear is very significant. Simple mathematical manipulation of the data allows the quick determination of the binding constant. The equation used to determine the binding constant is explained in section 2.22. In addition to this equation, an alternative graph (*figure 2.9b*): Intensity versus –log[anion] results in a graph that allows a quick visual approximation of the binding constant. The sigmoidal shaped graph obtained illustrating that the quenching occurs over two log concentration units, which is consistent with 1:1 binding and simple equilibrium.

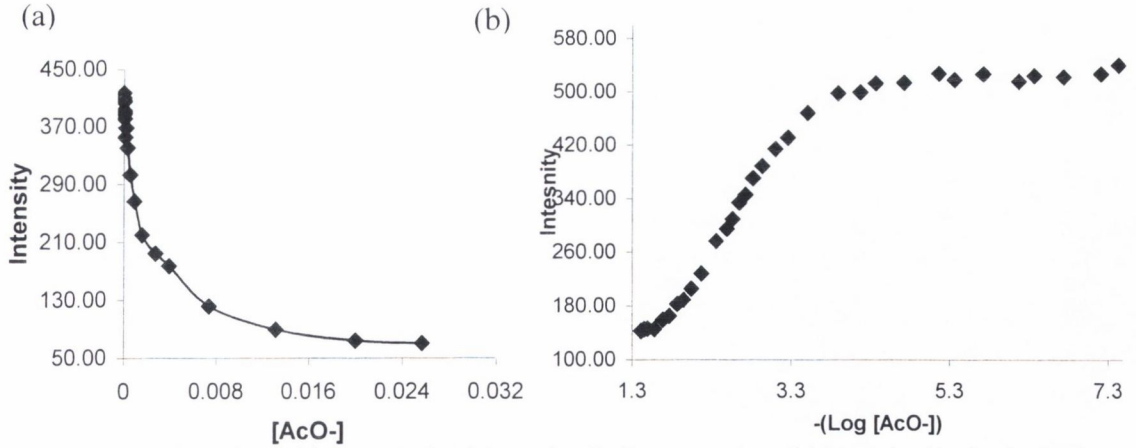


Figure 2.9: Titration of **65** with TBA.AcO (a) Intensity *Vs* Concentration; (b) Intensity *Vs* –log[anion]

A quick approximation of the binding constant may be obtained from this graph by drawing tangents to the two plateaus of the graph and extrapolating them to the *y* axis. The binding constant is then obtained by measuring the mid-point between the two tangents and obtaining

the corresponding value from the *x* axis. The mathematical determination of the binding constant using fluorescence techniques is explained in the next section.

2.41 Binding Studies

When two molecules bind together to form a 1:1 complex, the nature of the molecules change *i.e.* their properties change. Binding studies involve the addition of aliquots of a guest solution to a solution of the host and recording the change in a property of the host. There are many different properties that can be measured but the most usual ones are conductivity, ^{1}H NMR, IR, UV and Fluorescence spectra.[103] We have chosen fluorescence spectroscopy as the primary technique in the binding study. We also confirmed our results by conducting ^{1}H NMR titrations as well. Before attempting to calculate the strength of host-guest binding, there are two important experimental precautions that must be observed.

a) Check for self-association of the host or guest. This possibility can usually be confirmed or eliminated by taking spectra at various concentration ranges to be used in the binding experiment. These effects are minimised by using the relevant species in as dilute a range as possible.

b) Check for stoichiometries other than 1:1 host:guest. A strict 1:1 stoichiometry can be proven using a Job plot analysis.[103] The presence of other stoichiometries *e.g.* 2:1 or 1:2 must be known and quantified before calculating the strength of 1:1 binding.

In this thesis, screening experiments established that there are no signs of host or guest self-association at the concentrations used in the binding studies. A strict 1:1 stoichiometry was observed in all cases unless otherwise mentioned. The determination of the binding constant from the changes in the absorption or fluorescence emission spectra may be mathematically described by the equations below. The equilibrium for the binding of the anion to the sensor may be written as:

$$X^- + L \underset{}{\overset{\beta}{\rightleftharpoons}} LX^- \qquad \textbf{I}$$

The binding constant may be given by:

$$\beta = \frac{[LX^-]}{[X^-][L]} \qquad \textbf{II}$$

The total anion free concentration in solution $[X^-]_{total}$ can be expressed by conservation equation:

$$[X^-]_{total} = [X^-] + [LX^-] \quad \textbf{III}$$

where $[X^-]$ is the free anion concentration in solution and $[LX^-]$ is the bound anion concentration in the solution. In the same way, the total concentration of the sensor $[L]_{total}$ can be expressed;

$$[L^-]_{total} = [L] + [LX^-] \quad \textbf{IV}$$

where $[L]$ is the free sensor concentration in the solution and $[LX^-]$ is the bound sensor concentration in solution. The fluorescence intensity I_F is proportional to the concentration of the fluorophore and can be expressed by equation **V**:

$$I_F = k[LX^-] + k'[L] \quad \textbf{V}$$

$$I_{Fmin} = k'[L]_{total} \quad \textbf{VI}$$

$$I_{Fmax} = k[LX^-]_{max} = k[L]_{total} \quad \textbf{VII}$$

Where I_{Fmin} is the fluorescence emission when the sensor is not bound and I_{Fmax} is the maximum intensity. k and k' are proportional factors. From these equations the binding constants can be derived. By substituting for $[L]_{total}$ and $[LX^-]$ in equation and with some arrangements, **VIII** is obtained:

$$I_{Fmax} - I_F = (k-k')[L] \quad \textbf{VIII}$$

By substituting for k and k' using **V** and **VI**, **IX** can be obtained:

$$\frac{(I_{Fmax} - I_{Fmin})}{(I_{Fmax} - I_F)} = 1 + \frac{[ML^+]}{[L^+]} \quad \textbf{IX}$$

Finally equation **X** is found to be the relationship between the fluorescence intensity and the anion concentration. The log β is isolated and consequently the binding constant, β is determined.

$$\log\left(\frac{(I_{Fmax} - I_{Fmin})}{(I_{Fmax} - I_F)}\right) = \log ß + \log\left[[X^-]_{total} - \left(\frac{(I_{Fmax} - I_{Fmin})}{(I_{Fmax} - I_F)}\right)[L]_{total}\right] \quad \textbf{X}$$

Using equation **X** the binding constant for **65** with AcO- is $-\log \beta = 2.54$ units at 319nm. This value is in "good agreement" with the approximated value, 2.65 obtained by drawing tangents to the sigmoidal curve (section 2.2). Once the binding ability of the sensor was established it was then necessary to investigate the selectivity and the sensitivity of the sensor towards biologically important anions. We carried out a series of titrations using $N(C_4H_9)_4^+$ salts of $H_2PO_4^-$, F^-, Cl^- and Br- in DMSO. The changes in fluorescence spectra vary significantly. In the case of $H_2PO_4^-$ and F^- (Figure 2.10 and 2.11 respectively) the fluorescence emission was quenched by approximately 50% (Φ_F =0.0156) and 90% (Φ_F =0.0011) respectively (at 443 nm) but only minor quenching (<7%) was observed when the titrated with Cl^- (Φ_F=0.108) or

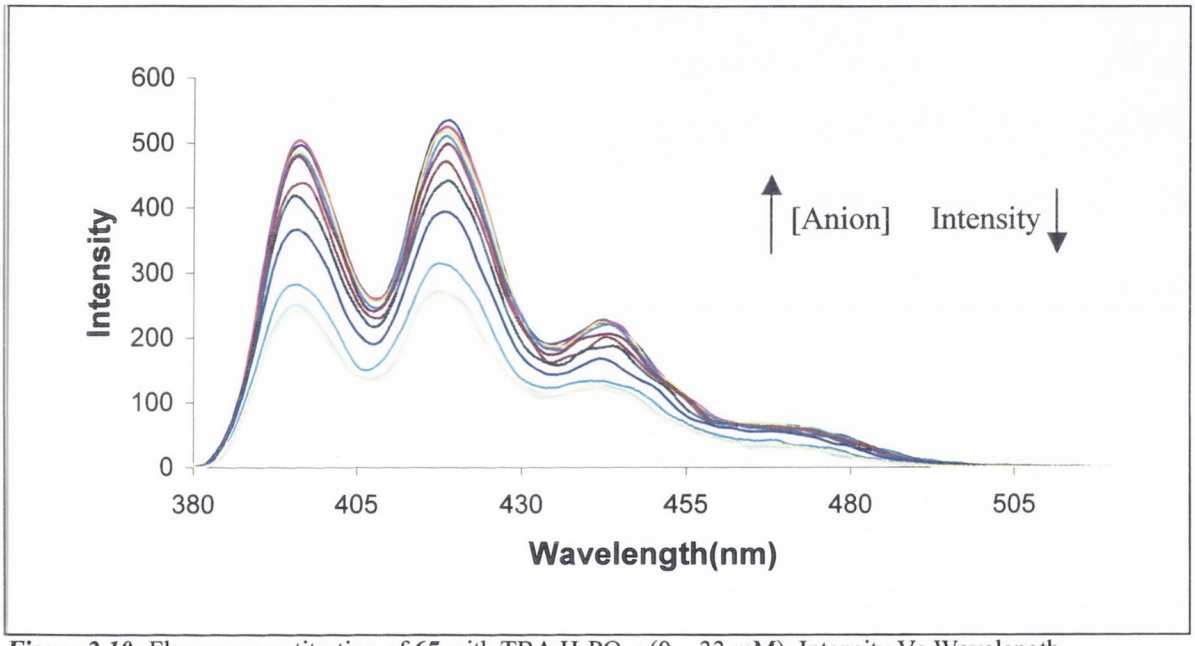


Figure 2.10: Fluorescence titration of **65** with TBA.H_2PO_4 : (0 – 33 mM) Intensity Vs Wavelength

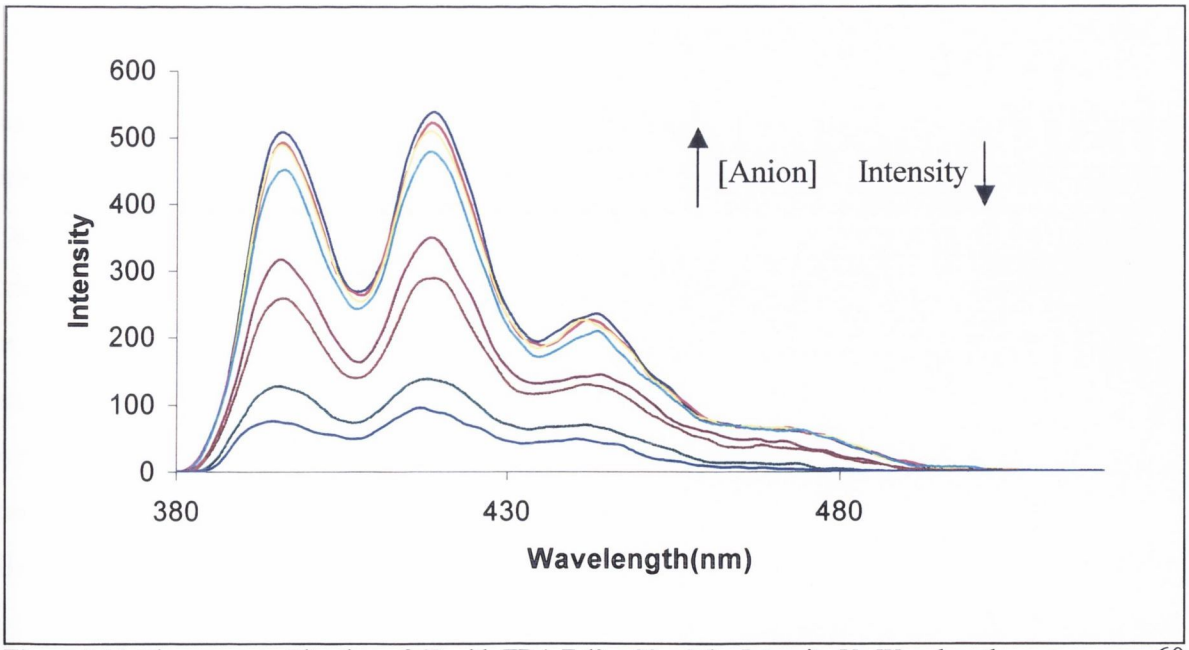


Figure 2.11: Fluorescence Titration of **65** with TBA.F (0 – 33 mM) : Intensity Vs Wavelength

Br^- (Φ_F = 0.088), ruling out a quenching by heavy atom effect. All of the values obtained are summarised in *Table 2.1*. In the case of the $H_2PO_4^-$ and the AcO^- the binding was expected due the complementary structure of the anion with respect to the binding site. The results obtained for the halides are simply explained due to the size exclusion. The chloride and bromide ion are too big to fit inside the binding site of the thiourea. Whereas the F^- fits in due to its smaller size. Binding is also increased due to its higher charge to radius ratio compared to the other halides. After all the data was collated for the various different anions it then could be collated and illustrated together to visibly display the different affinities that **65** has for the different anions (*Figure 2.12*). Since comparisons were being drawn with regards to the selectivity of the sensor to the various anions (previously mentioned). It is necessary to use the same

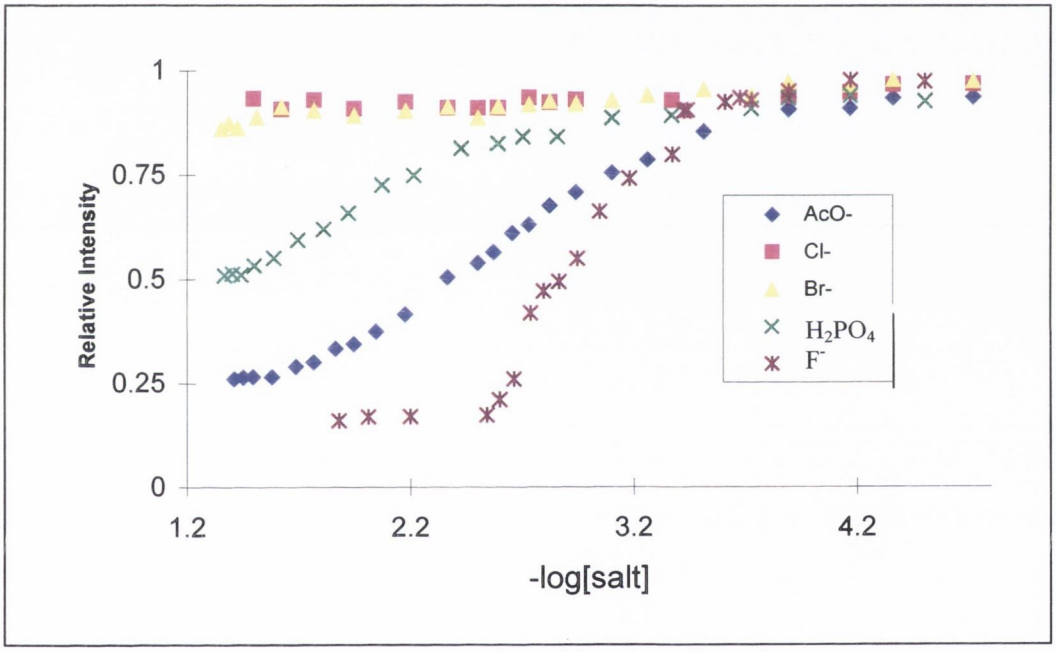


Figure 2.12: Comparison of titration results of **65** with various TBA salts

conditions and where possible the same solution for every titration. Each titration took approximately three hours to complete from start to finish. In addition each titration was repeated several times to confirm the results obtained. Therefore to complete all of the titrations using the one solution was not possible. Every precaution was taken in order to have the exact same conditions every time. It was also necessary to verify that the intensity of the solution did not degrade over time. To investigate this UV and fluorescence readings were taken every 10 minutes for a period of twelve hours. Neither the absorbance nor intensity changed significantly over time (*Figure 2.13*). The sensor was also tested in the presence of TBA.AcO (10mM). The same procedure was repeated for the other anions. Again there was

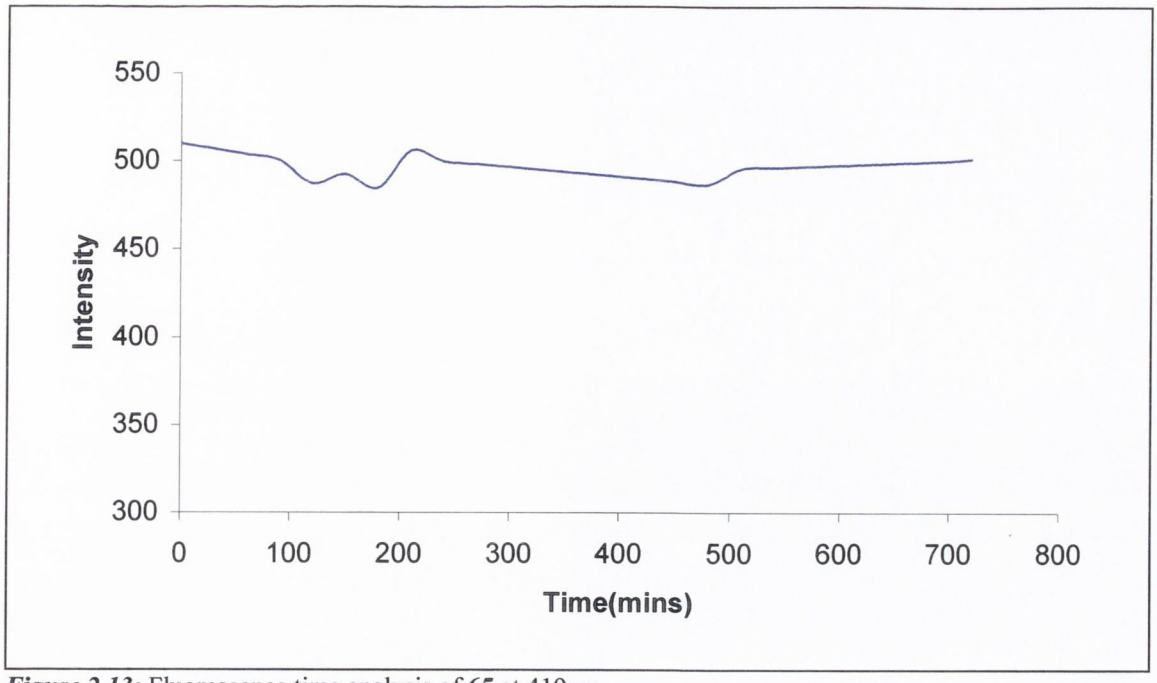


Figure 2.13: Fluorescence time analysis of **65** at 419nm

no significant change over time. Once it was confirmed that any drop in the intensity *was* due to the binding of the sensor with the anion then the results for each anion was compared. In order to correctly compare the values obtained for each titration it is necessary to normalise the data. This involves obtained for each titration it is necessary to normalise the data. This involves plotting relative intensity against –log[anion]. Relative intensity is calculated by dividing the intensity of the solution by the initial intensity in the absence of the anion. This method not only allows comparisons to be made with different anions it also allows the results from various different sensors on the one anion to be compared e.g. results for titrations of **65** – **67** with TBA.AcO. Now the parameters have been established the next step was the analysis of the different sensors and the comparison of the binding affinities.

2.42 Comparison of Thiourea Sensors

The two other sensors **66** and **67** were subjected to titrations with the same anions. As expected the binding affinity for the anions was not as strong relative to **65** due to the lower electron withdrawing ability of the skeleton groups **of 66** and **67**. The UV and fluorescence spectra were similar to that of **65** due to the common anthracene ring with only a slight shift λ_{max} down to 419nm and 418.5nm for **66** and **67** respectively. Similar emission and absorption effects were observed for **66** and **67** during the titrations with the various TBA salts i.e. both sensors behaved like *ideal* PET sensors. The results obtained for these sensors are illustrated in *Figures 2.14* and *2.15.* The binding values for all three sensors are summarised in *Table 2.1.* As with **65** the Cl^- and Br^- titrations do not show significant ion-induced spectral changes to allow calculation of log β values. For **66**, the same selectivity trend was observed as for **65**, with smaller binding constants due to the reduced acidity of the thiourea protons. For **67** the

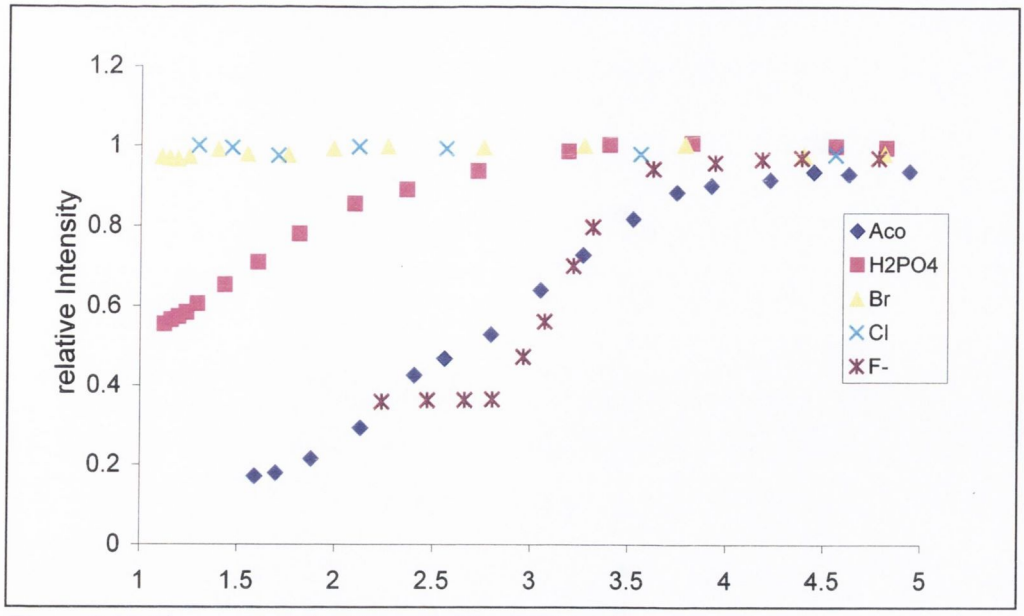


Figure 2.14: Fluorescence Titration with **66** and TBA salts

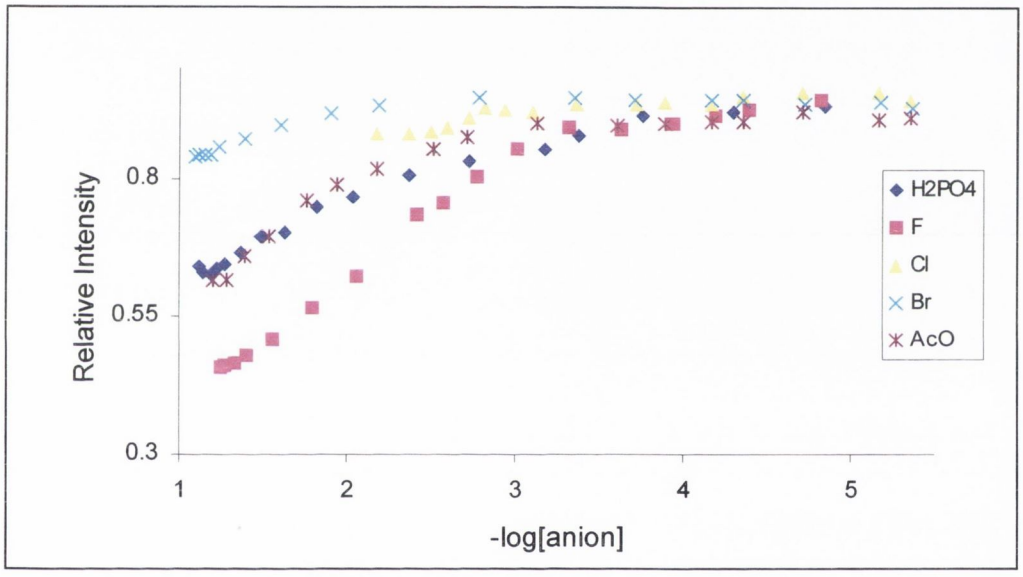
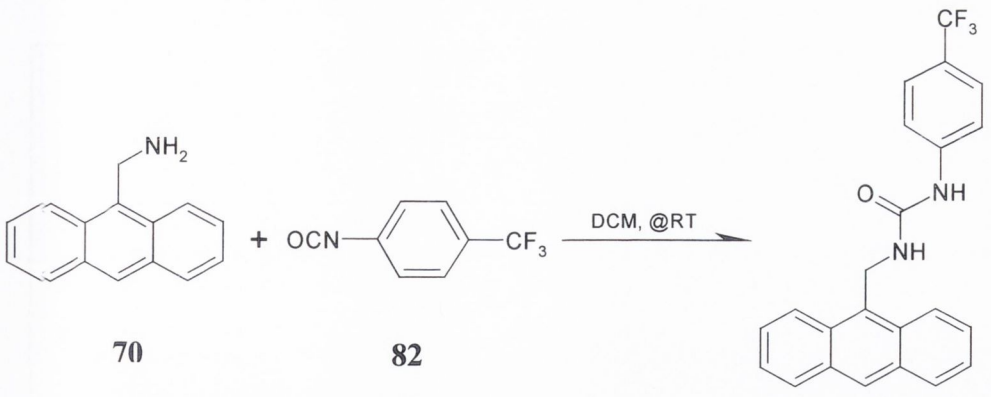


Figure 2.15: Fluorescence Anion Titration with **67**

order of selectivity and the sensitivity was somewhat different with $H_2PO_4^-$ (log = 2.05 (± 0.05)) being selectively detected over AcO^- (log = 1.75 (± 0.05)). These results show that the anion sensor's affinity can be controlled by simple design. It was then decided to further investigate this family of sensors, not by changing the skeleton group but by analysing the urea equivalent of **65**. As before, the synthesis is very straight forward; 4(trifluoromethyl)phenyl-isocyante, **82** was added to a solution of the 9-amionmethylanthracene in dry CH_2Cl_2 (*scheme 2.8*). The resulting solution was stirred for 15



70 82 83

Scheme 2.8: Synthesis of **83**

minutes and the sensor precipitated out of solution. The sensor was recrystalised from CH_2Cl_2. Again the binding ability of this sensor was first tested against TBA.AcO. Once the sensing ability of the urea was confirmed the remainder of the anions were tested. The order of

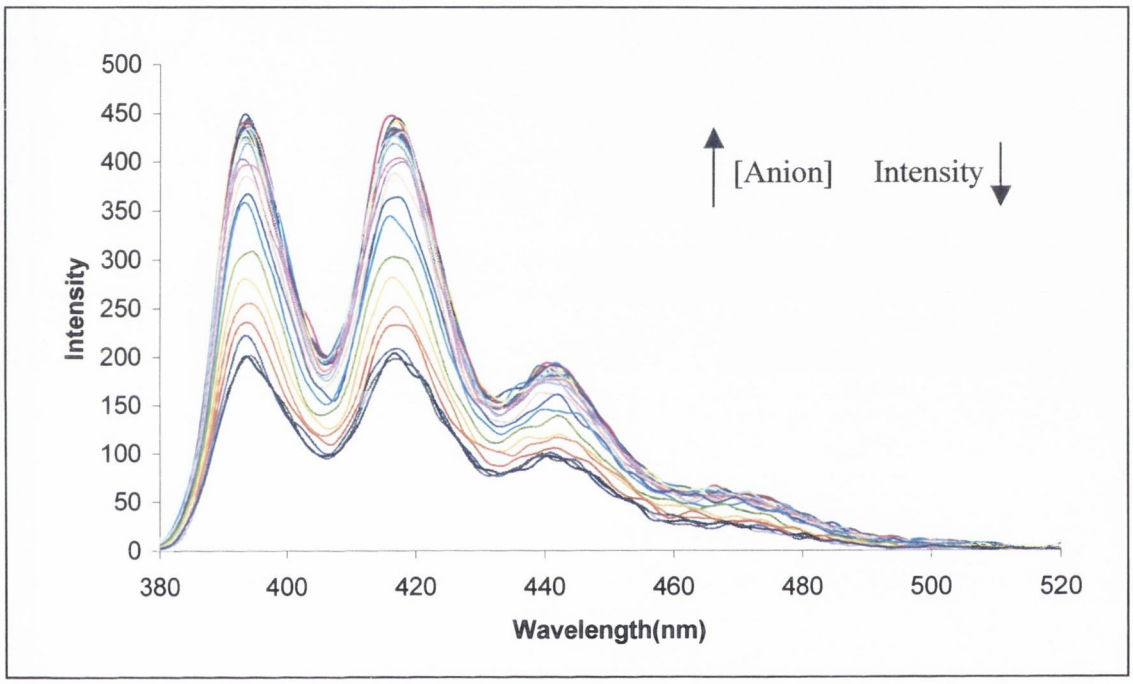


Figure 2.16: Fluorescence Titration of **83** with $TBAH_2PO_4$ (0 – 32 mM)

selectivity for **83** versus **67** is similar for the anions tested i.e. $F^- > AcO^- > H_2PO_4^- >> Cl^-$ and Br^-. The binding constants for the anions were F^- is –log (2.5), AcO^- is –log (2.31) and -log (2.07) for $H_2PO_4^-$. Yet again there was no changes in the UV spectrum during the titrations indicating that the urea receptor also acts as an ideal PET sensor. It should be noted though that the % reduction in the fluorescence titrations for **83** was not as significant with a 74% reduction for AcO^- as opposed to 83% reduction observed for **65**. The comparison of the affinities of the different sensors each individual anion may be seen in *Figure 2.18 – 2.20.*

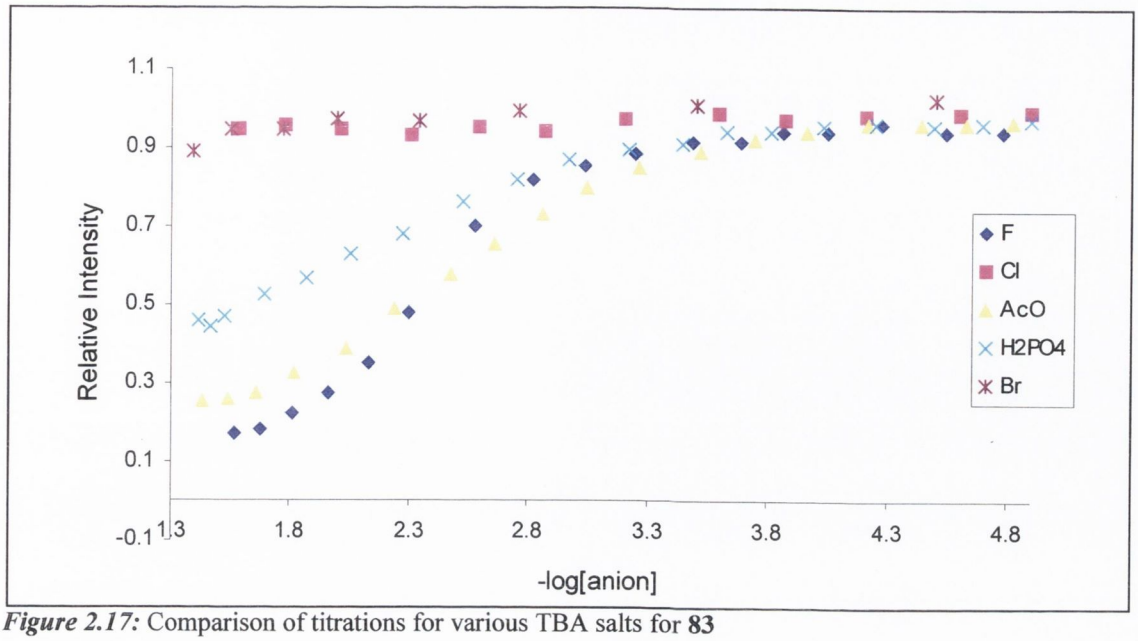


Figure 2.17: Comparison of titrations for various TBA salts for **83**

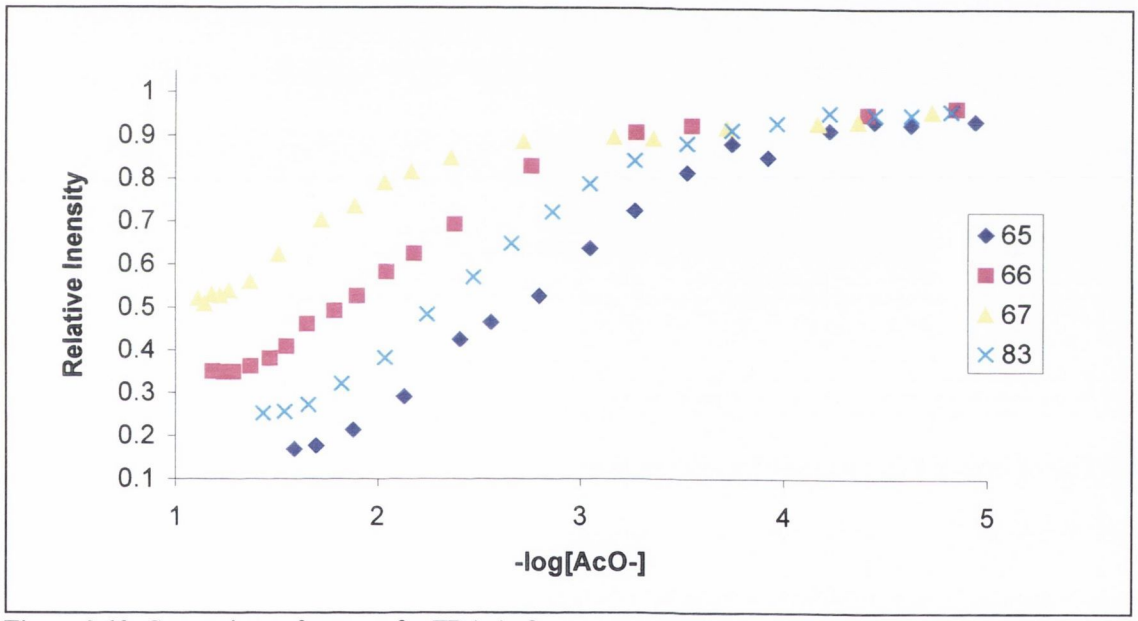


Figure 2.18: Comparison of sensors for TBA.AcO

The affinity of the sensors for AcO^- was as anticipated in so far as the order of selectivity is **65** > **83** > **66** > **67**. This order of affinity corresponds to the relative acidity of the thiourea and urea protons i.e. the sensor with the most acidic protons exhibits the strongest binding. The orderaffinity of the sensors varies slightly for F^- relative to those of AcO^-. As expected **65** exhibits the strongest binding for F^- and **67** the weakest. However the affinity of **66** and **8**3 are reversed for selectivity. With regards to affinities of the sensors towards $H_2PO_4^-$ **83** binds the $H_2PO_4^-$ most strongly followed closely by **65**. The halides Cl^- and Br^- do not show significant binding to list the order of affinity for the sensors.

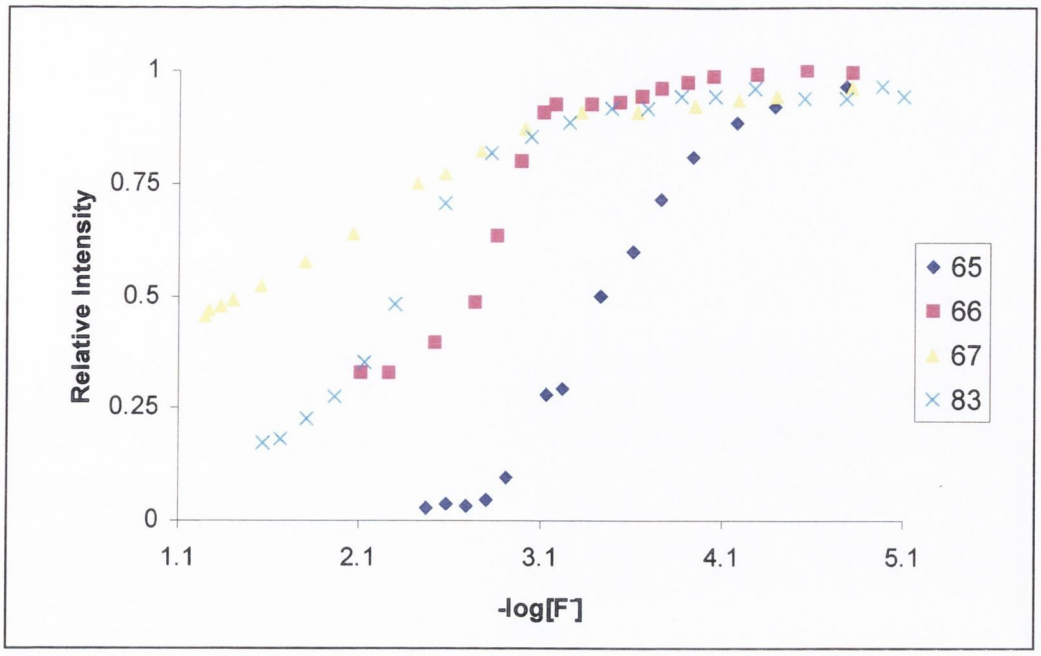


Figure 2.19: Comparison of sensors for TBA.F

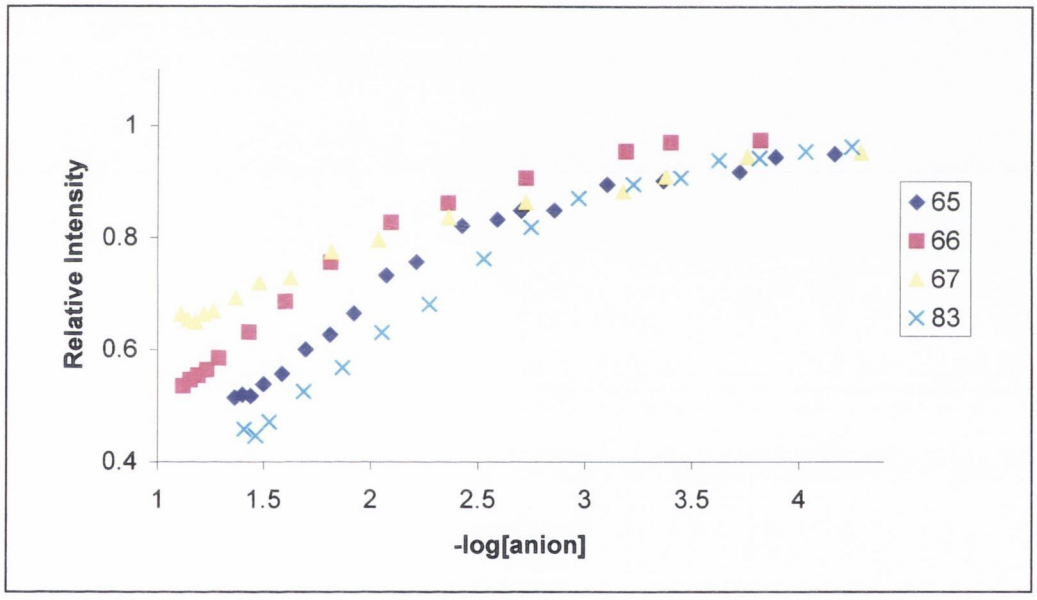
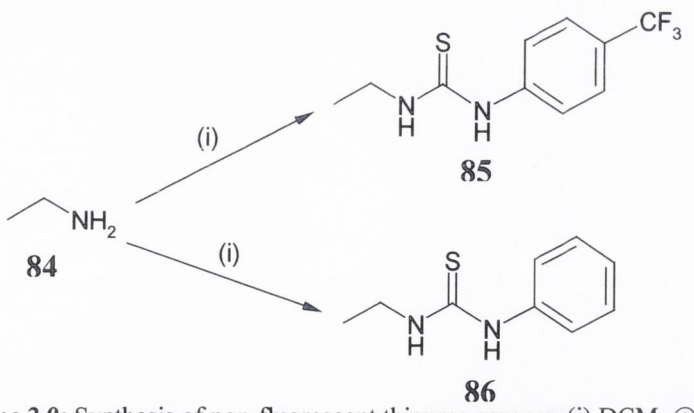


Figure 2.20: Comparison of sensors for TBA.H_2PO_4

These results prove the hypothesis that the affinities of the binding process can be manipulated by changing the acidity of the thiourea / urea protons.[35] The next step is analyse the affect that the fluorophore has (if any) on the binding process. In order to investigate this it was necessary to synthesise the receptor unit as a separate moiety. **85** and **86** were non-fluorescent receptors corresponding to their fluorescent counterparts, **65** and **66**. These receptors were made by simply adding one equivalent of **79** and **80** to separate solutions of ethylamine, **84**. Both of theses sensors were subject to titrations with TBA.AcO under similar conditions to those used for the fluorescent analogs. The UV of **85** is illustrated in *Figure 2.21*. As can be seen from the graph the fine structure obtained with anthracene is lost,



Scheme 2.9: Synthesis of non-fluorescent thiourea sensors, (i) DCM, @RT,

replaced by one broad band with a λmax of 286 nm in DMSO. The binding process was monitored by measuring the absorbance at 286nm during incremental additions of the known concentrations of the anion to a solution of known concentration of the sensor. As with the fluorescent analogs the resulting titrations yielded sigmoidal shaped curves when absorbance was plotted against –log[anion]. The key difference being it was absorbance and not fluorescence intensity that was used for the y-axis. The resulting sigmoidal shape stretched over 2 pA units insinuating that a simple equilbria is formed with a 1:1 binding ratio of host :guest. Similar binding constants were found for **85** by measuring the changes in its absorption spectra at 286 nm relative to the binding values obtained for **65**.

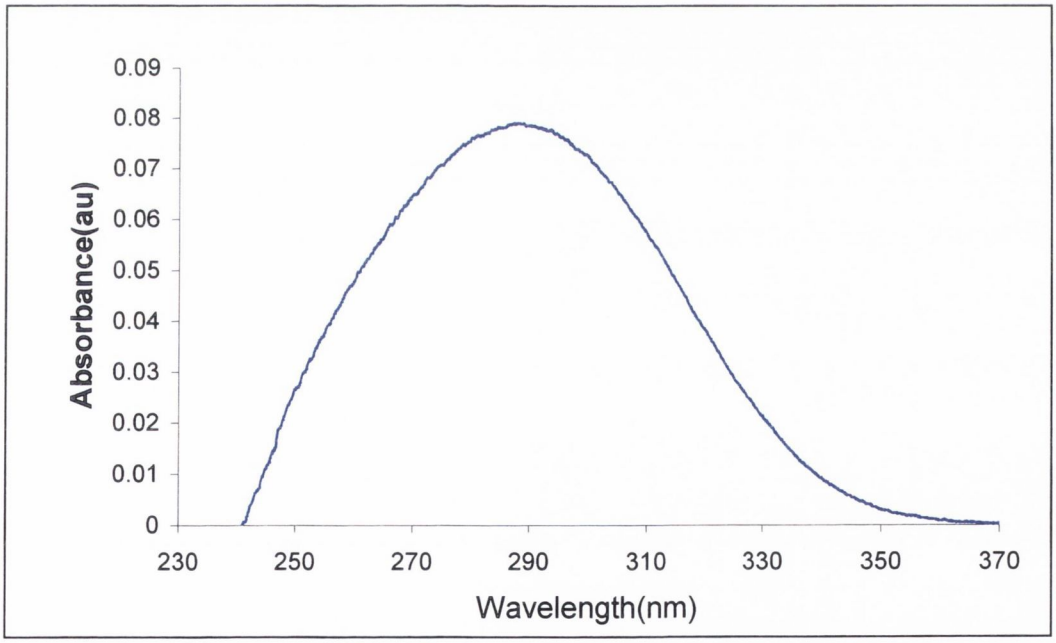


Figure 2.21: UV/vis spectra of **85** in DMSO

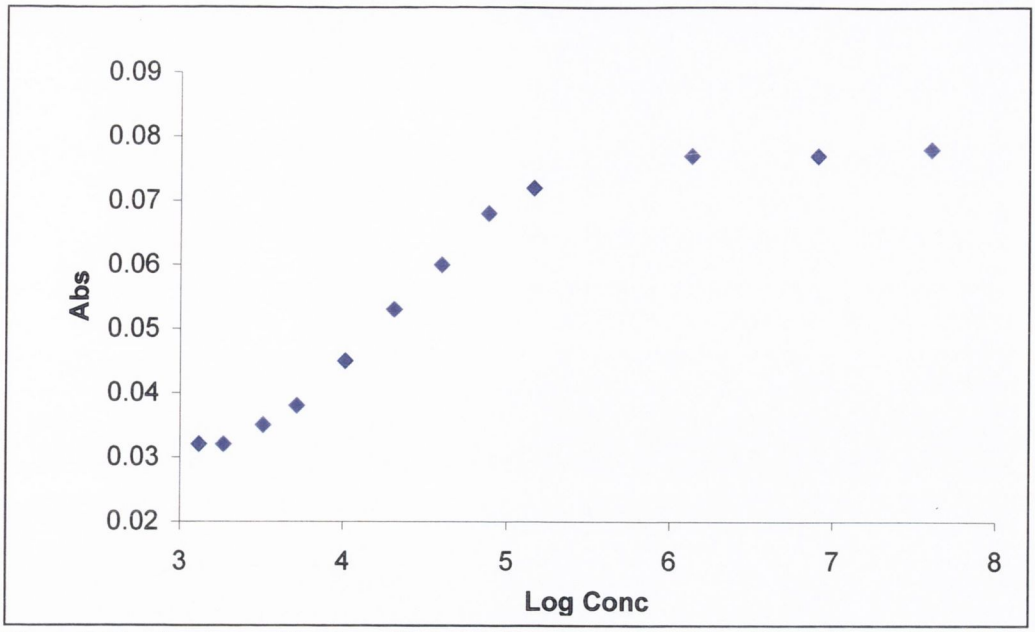


Figure 2.22: Absorbance Titration of **85** with TBA.AcO: Absorbance Vs –log[anion]

These results were confirmed by ^{1}H NMR. This implies that we should get similar binding constants and selectivity independent of the fluorophore used. We decided to test this hypothesis by attaching the thiourea binding site to a different fluorophore: 1,8-naphthalimide. The next section outlines the results of the subsequent studies and the conclusions determined.

2.43 NAPHTHALIMDES

The fluorescence properties of naphthalimide derivatives are different to that of their anthracene counterparts as they emit fluorescence in the green region. This is a significant advantage when designing sensors for biological applications as anthracene emits at wavelengths similar to that of biological systems. 1,8-naphthalimides fluoresces due to an internal charge transfer excited state within the naphthalimide molecule, creating a separated partial positive and negative charge on excitation, (*Figure 2.23*). Here the amino has a partial positive charge while the imide function is partially negatively charged.

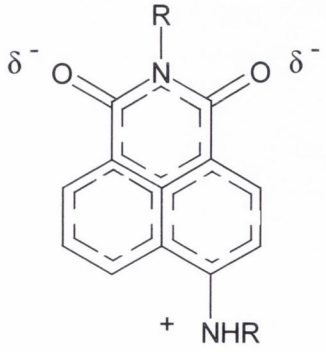
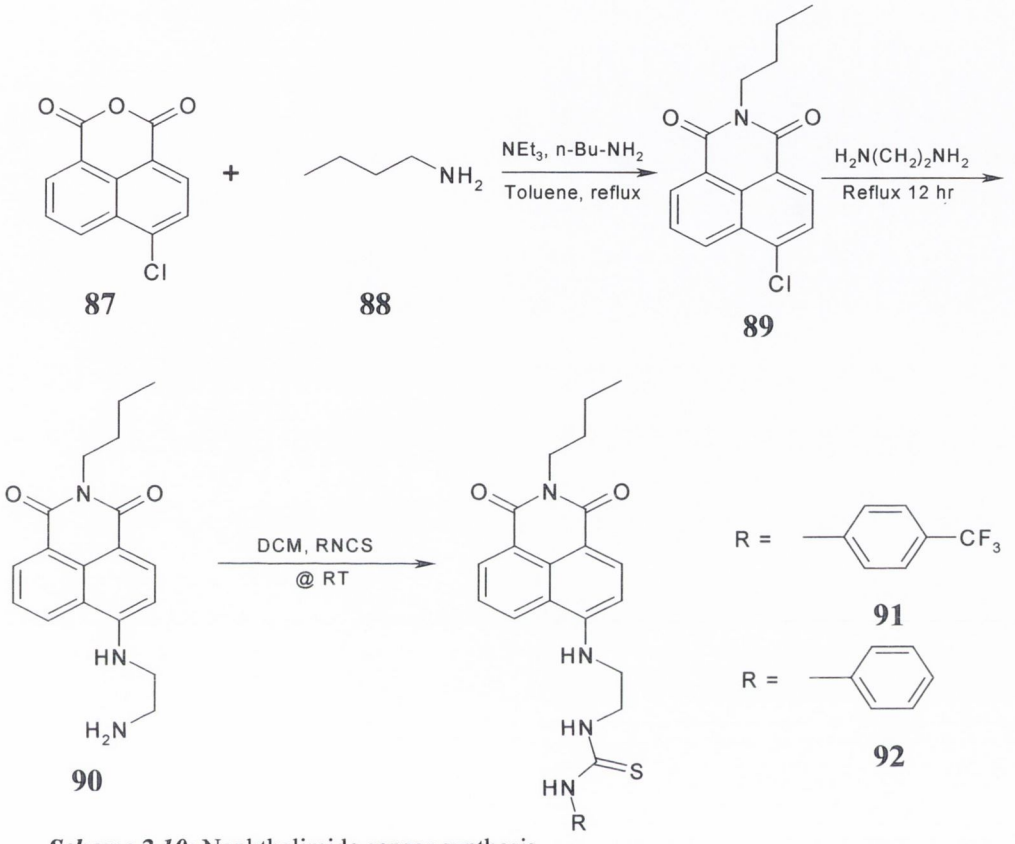


Figure 2.23: The charge transfer nature of 1,8 naphthalimide

This results in a so-called "push-pull" excited state. In the final stage, deactivation results in loss of the excited state energy through the emission of light as fluorescence. To the best of our knowledge no fluorescent anion sensors have been developed that fluoresce in this area. The synthesis is straight forward and due to the fact that the products precipitate out of



Scheme 2.10: Naphthalimide sensor synthesis

solution when the reaction is complete the materials are easily isolated and purified. The reaction scheme is illustrated above in *Scheme 2.10*. N-butyl-4-(4'-aminoethyl)amino-1,8-naphthalimide, **89** was easily synthesised by refluxing 4 Chloro-1,8-naphthalimide, **87** with n-butylamine in toluene. The product precipitated out of solution within 30 minutes but a significant increase in yield (from 57% - 85%) was obtained by leaving the reaction for 24 hours. Once isolated **87** was refluxed in ethylenediamine overnight. The solution was allowed to cool then the product was forced out of solution by adding ice to the solution. The resulting precipitate was dried over P_2O_5 before reacting with **79** to yield **91** and **80** to yield **92**. The resulting products were recrystalised from DCM. Consistent with the titrations involving the other sensors, **65 – 67** and **83** the first anion tested with the sensor was AcO-. The change in the fluorescence properties of the sensor upon incremental additions of TBA.AcO to a solution of the sensor were monitored. Concurrently the UV spectra (*Figure 2.24*) were also monitored closely. These sensors again exhibited ideal PET sensor behaviour, *i.e.* only the intensity of the

fluorescence changed. There were no shifts in emission bands nor significant changes in the UV spectrum. Using the same analysis as before i.e. plotting the intensity versus –log[anion]

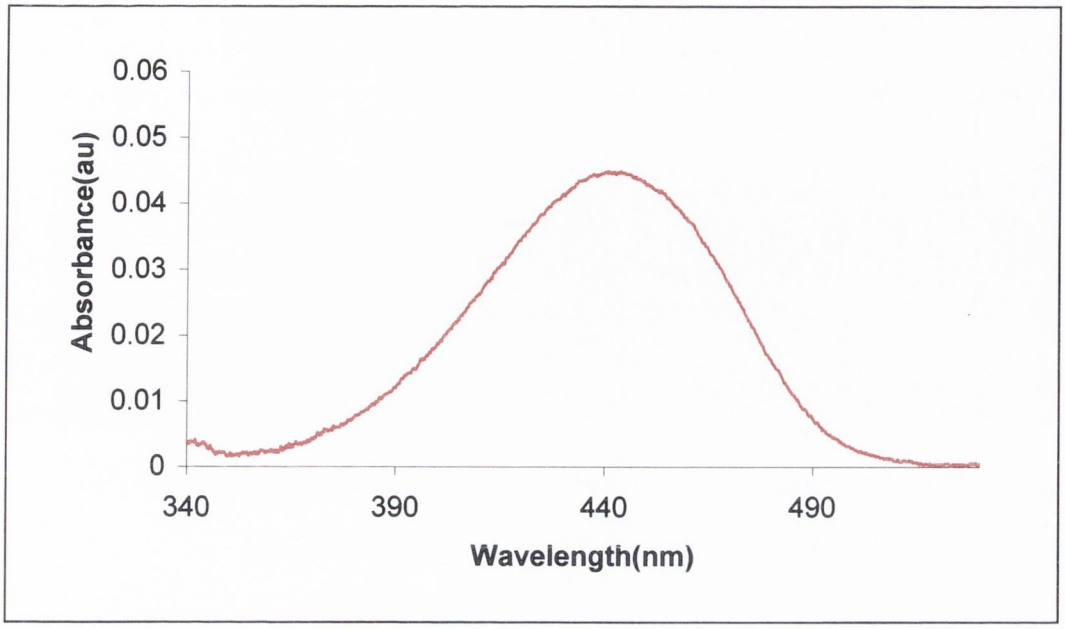


Figure 2.24: UV/vis Spectra of **91** in $CHCl_3$

the sensor showed a simple 1:1 equilibrium binding. Whilst the synthesis of this sensor was relatively short and straight forward there was less than 20% reduction in fluorescence for the AcO- titrations. As a result of this small reduction in fluorescence it would be very difficult to confidently determine the affinity and selectivity of the sensor towards each anion tested using fluorescence techniques. *Figure 2.25* compares the relative drop in fluorescence of **65** with the

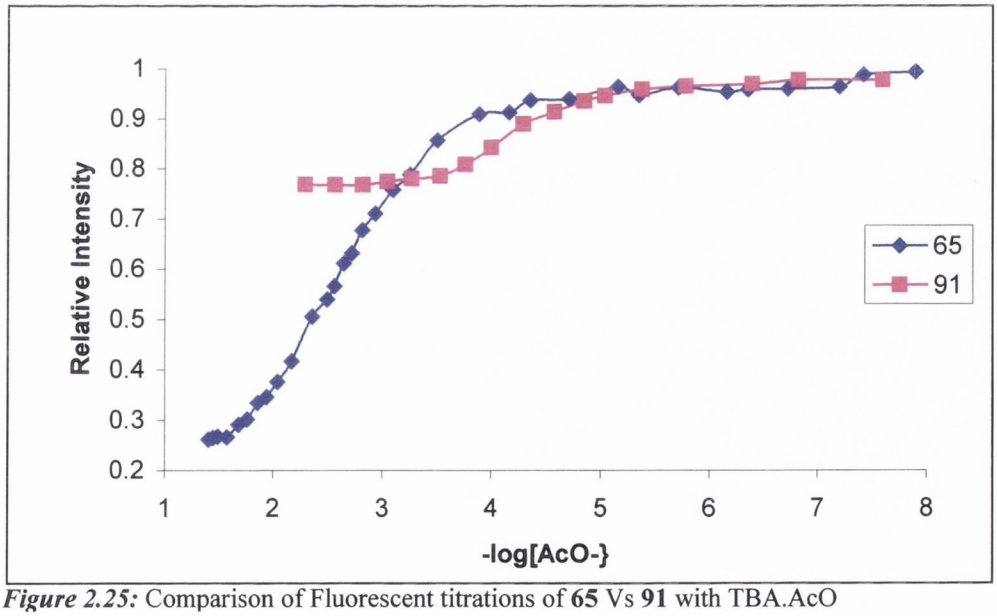
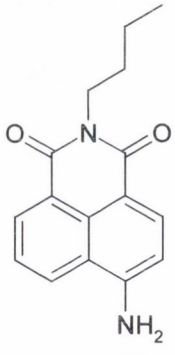


Figure 2.25: Comparison of Fluorescent titrations of **65** Vs **91** with TBA.AcO

corresponding naphthalimde based sensor upon addition of the anion to the solution of the sensor. This graph illustrates the dramatic difference between the two systems. At the same time this graph also shows that the binding is significantly stronger using **91**. The binding unit in both sensors are the same i.e. 4-trimethylfluoro phenyl thiourea. This implies that the fluorophore of **91** is involved in the binding process, due to the electron withdrawing nature of the fluorophore. This finding is significant, however the low reduction in fluorescence intensity relative to that observed for the AcO- titration with **65** is not promising. If the percentage reduction in fluorescence intensity is low it makes it difficult to establish the degree of selectivity that the **91** exhibits towards the various anions tested. Therefore no further investigations were conducted using these sensors. Nevertheless further attempts were made to incorporate the naphthalimide derivative as a fluorophore into an anion sensor. Subsequent investigation carried out by colleagues in the Gunnlaugsson group have discovered different naphthalimide derivatives that act as more efficient sensors whilst still using the thiourea binding site. This area is presently under further investigation and is expected to be suitable for publication very shortly.



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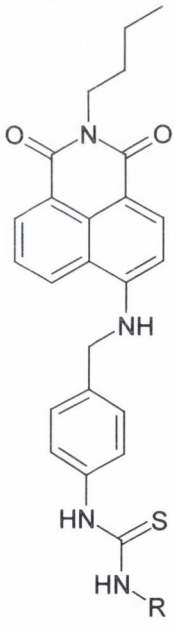
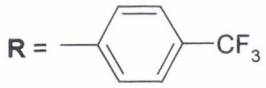




94

2.44 SOLVENT EFFECTS

When the fluorescence titrations of **65 - 67** were carried out in CH_3CN, CH_3CO_2Et or THF, the emission was also quenched upon addition of AcO^- but the degree of quenching was somewhat smaller. In ethanol, which is a highly competitive hydrogen bonding solvent, no binding was observed between **65** and AcO^- (*Figure 2.26*). Whereas there was a notable degree

of quenching observed when CH_3CN was used(*Figure 2.27*). This binding was found to be reversible by simply adding ethanol to the solution. $CHCl_3$ was not tested as the sensor was only soluble upon heating. It is also important to note that *no* exciplex emission was observed in any of these solvents: in contrast, Teramae *et al.* have recently shown that a pyrene analogue of **67**, **45** is a ratiometric anion indicator based on the control of intramolecular exciplex emission.[66] However this compound will be discussed in more detail in Chapter 4.

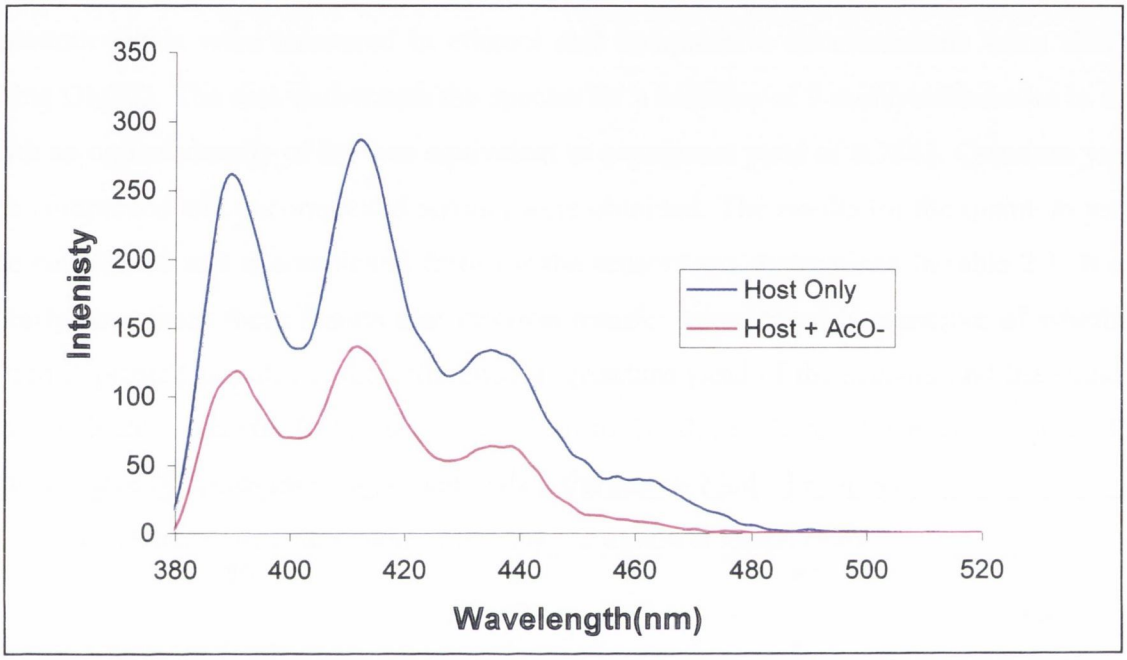


Figure 2.27: Fluorescence spectra of **65** in CH_3CN (i)host only (ii) host + 40mM of AcO

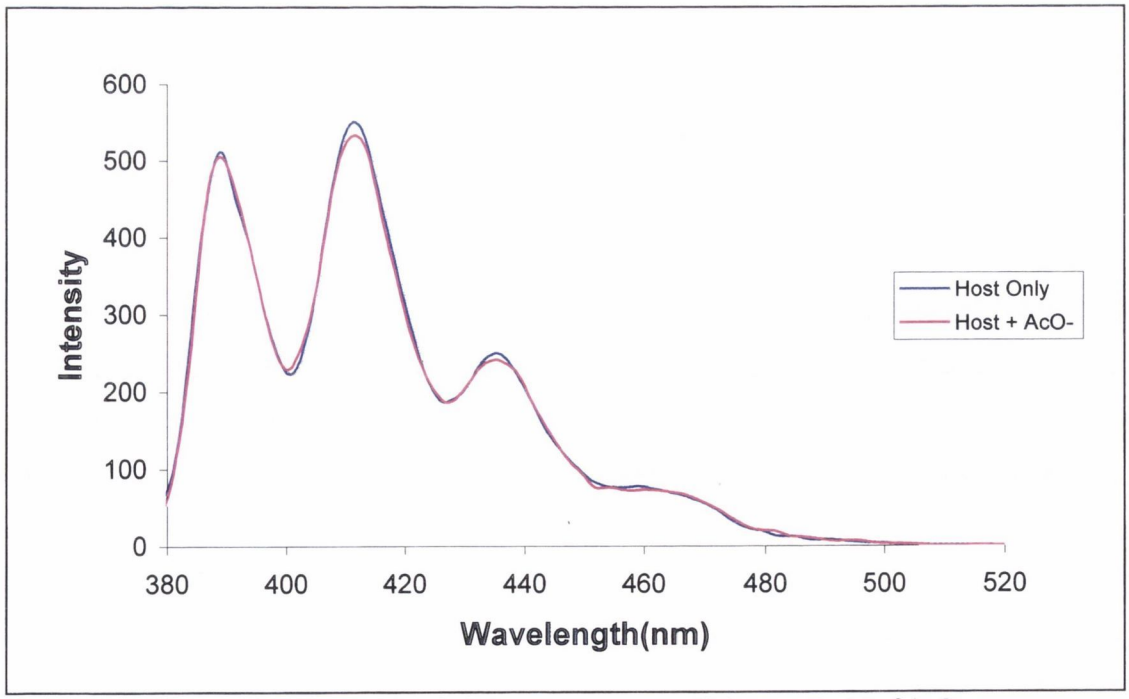


Figure 2.26: Fluorescence spectra of **65** in Ethanol (i)host only (ii) host + 40mM of AcO^-

2.45 Quantum Yield measurements

The overall efficiency of an excited state process is usually described by its quantum yield Φ. This is the number of events divided by the number of photons absorbed by the system. The event can be either photophysical or photochemical. 9 methyl anthracene in ethanol serves as a standard for the determination of the fluorescence quantum yields for all anion sensors. The quantum yields were measured in ethanol and comparative measurements were also taken using DMSO. The area underneath the spectra for a solution of 9 methylanthracene in ethanol with an optical density of 0.1 was equivalent to a quantum yield of 0.2843. Quantum yields of the complexed and uncomplexed sensors were obtained. The results for the quantum yields in the complexed and uncomplexed form for the sensors are summarised in table 2.1. It can be clearly seen from these results that electron transfer takes place irrespective of whether the anion is present or not, *i.e.* the difference in quantum yield of the sensors and the standard is due to electron transfer from the receptor unit to the fluorophore. However the quenching of fluorescence becomes more significant when the sensor binds the anion.

Table 2.1: Ion induced Fluorescence and binding parameters of sensors **65-67**

	65			**66**			**67**		
	Φ_F	$\%F_{Red}$	Log β	Φ_F	$\%F_{Red}$	Log β	Φ_F	$\%F_{Red}$	Log β
Uncomplexed	0.1080			0.187			0.34		
with AcO-	0.0070	75	2.33	0.016	73	2.15	0.20	43	1.75
with H2PO4-	0.0156	50	2.05	0.101	46	1.82	0.11	38	2.05
with F-	0.0011	90	3.35	0.067	64	2.90	0.23	55	2.37
with Cl-	0.1037	7	a	0.173	8	a	0.30	12	a
with Br-	0.096	12	a	0.172	7	a	0.25	14	a

ΦF = Quatum yield in DMSO; % F_{red} = % reduction in fluorescence; log β = log of binding constant; a – too low to determine

2.46 NMR Titrations

In this thesis, the binding constants were confirmed by conducting ^{1}H NMR titrations in DMSO-$_{\delta 6}$. The titrations were carried out by adding known amounts of an anion solution to a solution of the sensor and monitoring the changes in the ^{1}H NMR. We believe the binding of the anion occurs through the NH's of the thiourea. NMR would prove this hypothesis since upon complexation the position of the NH peaks will shift downfield relative to their positions in the uncomplexed sensor. NMR is a convenient probe because it provides information about every atom in the host and guest substrate. Therefore not only was NMR used to confirm the binding constants obtained through Fluorescence techniques but it also gave useful information on the structure of the complex of the sensor with the anion. The information

within the NMR spectra contains the details of the rate of association/dissociation, conformational changes occurring, H-bonding and any other changes attendant on binding. The receptors synthesised were analysed by ^{1}H NMR spectroscopy and all showed fast binding (on the NMR time-scale). In other words the spectrometer does not 'see' isolated bound or free molecules of host or guest but rather an average of both, *i.e.* the residence time in each state is too short to be observed and resolved by NMR.

Determination of Association Constant (K_{ass})

On addition of a guest (G) to a host (H) in solution the following equilibrium process occurs:

$[H]_u + [G]_u \underset{}{\overset{K_{ass}}{\rightleftharpoons}} [HG]$ where $[H]_u$ = uncomplexed host
$[G]_u$ = uncomplexed guest
K_{ass} = binding (or association) constant **XI**

The equilibrium constant may be calculated by using equation **XII**

$K_{ass} = [HG]/ [H]_u . [G]_u$ **XII**

The fraction of the total host concentration that is complexed with guest may be expressed by the equation **XIII**:

$f_c = [HG]/[H] + [HG]$ where f_c = fraction of host complexed **XIII**

therefore the fraction of host uncomplexed may be expressed as

$(1- f_c) = [H]/[H] + [HG]$ $(1- f_c)$ = Fraction of host uncomplexed **XIV**

$\delta = f_c\delta_c + (1-f_c)\delta_H$ or $fc = \Delta\delta / \Delta\delta_c$ $\Delta\delta$ = observed change in shift
$\Delta\delta_c$ = total change in shift **XIV**

From **XI** $[HG] = K_{ass}[H][G]$

Therefore $f_c = [HG] / [H] + [HG] = K_{ass}[H][G]/[H] + K_{ass}[H][G]$

$f_c = K_{ass}[G] / 1 + K|_{ass}[G]$

Therefore from **XIV**
$f_c = \Delta\delta / \Delta\delta_c = K_{ass}[G] / 1 + K_{ass}[G]$ **XV**

For fast host guest complexation δ (the observed shift) is the average of δ_H (pure host shift) Equation **XV** describes a hyperbola. When f_c is plotted against [G] the origin $f_c = 0$ corresponds to the situation of pure host. The asymptote corresponds to pure complex. The

concentration of guest required to achieve half complexation of the host is equal to the reciprocal of K_{ass}, the binding constant. The association constant can in principle be calculated from any signal in the NMR spectrum of the host, at any host and guest concentrations if, δ, δ_H, and δ_C are known. In practice δ_C is not accurately known and cannot be directly observed, but only approached asymptopically. Therefore, an estimation of δ_C allows one to calculate K_{ass} from a measured value. In principle there is a unique δ_C that will give the same value of K_{ass} when applied to other δ values measured under different conditions. *i.e.* different [H] and [G]. However, in practice (experimental error) all data sets do not give the same K_{ass}. The best approach is to iterate δ_C until some statistical measure of the data spread (standard deviation) is minimised.

An algebraic manipulation (Eadie-Hofstee method) was carried out in order to produce a linear relationship yielding K_{ass}, the binding constant as in *Figure 2.28*. The Eadie Hofstee linearisation equation was derived by taking the reciprocal of each side of **XV** and then multiplying across by $\Delta\delta$ and rearranging resulting in equation **XVI**

$$\Delta\delta_c = (\Delta\delta + \Delta\delta.\, k_{ass}[G]).(1/k_{ass}[G]) \qquad \textbf{XVI}$$

Multiplying out the right hand side of equation gives equation **XVII**

$$\Delta\delta_c = (\Delta\delta/k_{ass}[G]) + \Delta\delta \qquad \textbf{XVII}$$

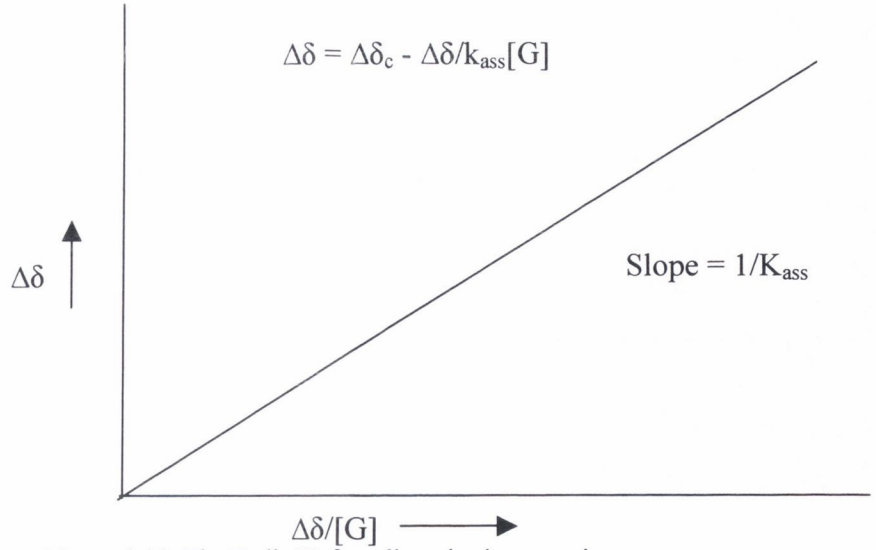


Figure 2.28: The Eadie Hofstee linearisation equation.

2.5 NMR Results

The ^{1}H NMR of **65** in DMSO-d6, showed two sharp signals at 7.93 ppm and 6.20 ppm for the thiourea hydrogens (*Figure 2.29*). The peaks due to the anthracene backbone of the sensor are located between 7.2 and 8.5ppm with no overlapping of the peaks observed. The splitting of each peak is very distinct allowing easy identification of the various protons. During titrations the position and shape of these peaks were monitored very closely. The two peaks due to the thiourea protons were substantially shifted downfield upon addition of 0.1 – 2 equivalents of $(C_4H_9)_4N(O_2CCH_3)$ (+1.92 and 1.66 ppm respectively after 1eq.). Simultaneously minimal

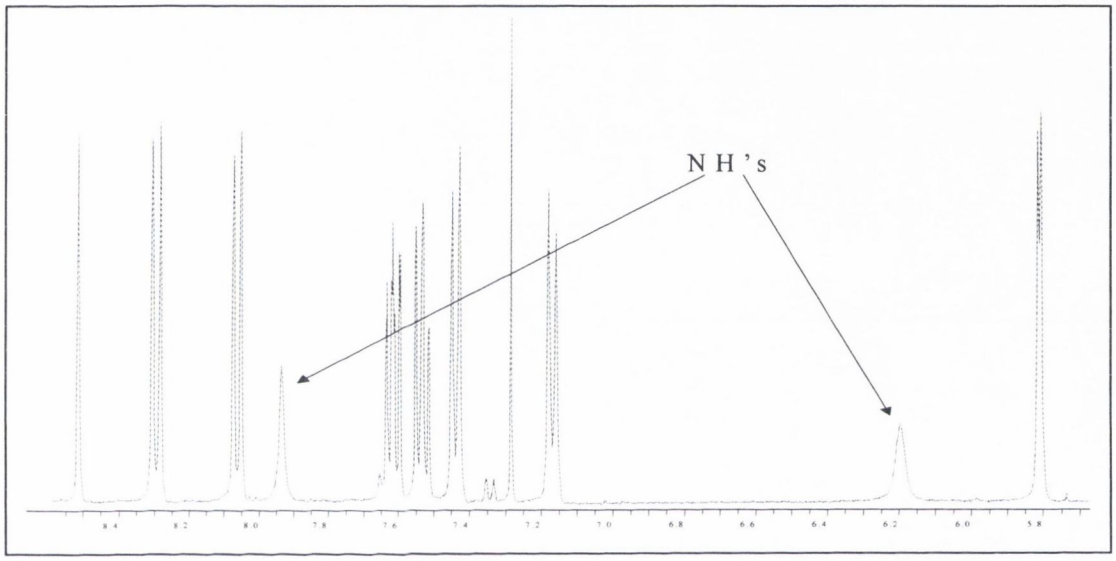


Figure 2.29: NMR spectra of **65** in DMSO-$_{\delta 6}$

change was observed for the peaks of aromatic protons and the peaks for the methylene spacer protons upon addition of anions throughout the titration. This indicates that there is little or no interaction between the aromatic electron rich anthracene and the anion upon complexation. As mentioned earlier ^{1}H NMR was used to confirm the binding constant obtained using fluoresence analysis. Association constants were calculated using a microsoft Excel spreadsheet designed by Murray.[104] The binding studies were carried out using DMSO–$_{\delta 6}$. Throughout the titration, only one set of host resonnances was observed indicating that the complexed and uncomplexed form of the receptor are in fast exchange on the NMR timescale. In addition to confirming the binding constant value, information was obtained with regards to the strucutre of the host guest complex. As can be seen from the spectra in *Figure 2.32* there are substantial changes in the complexed spectra relative to the uncomplexed sensor.

The protons for thioureas moved from 9.63 and 8.36 ppm to 12.36 and 11.05 ppm respectively in the presence of 2 equivalents of tetrabutylammonium acetate. This very large shift (nearly three units) is significant but just as significant is the fact that the position of the methylene protons and the peaks from the aromatic protons do not change. The peaks broaden as the guest solution is added but the positions of the aromatic protons due not change. This signifies that the anion does not interact with the anthracene ring (confirmation of the results obtained from the UV spectra). *Figure 2.30* binding curves illustrates the actual shift versus the calculated shift. The measured data was found to be in good agreement with the computer fit data giving a binding constant of K_{ass} of 1507 M^{-1} in DMSO-$_{\delta 6}$. It is also important to note that change in shift was mainly up to the addition of 1 equivalent of the anion. This break in the

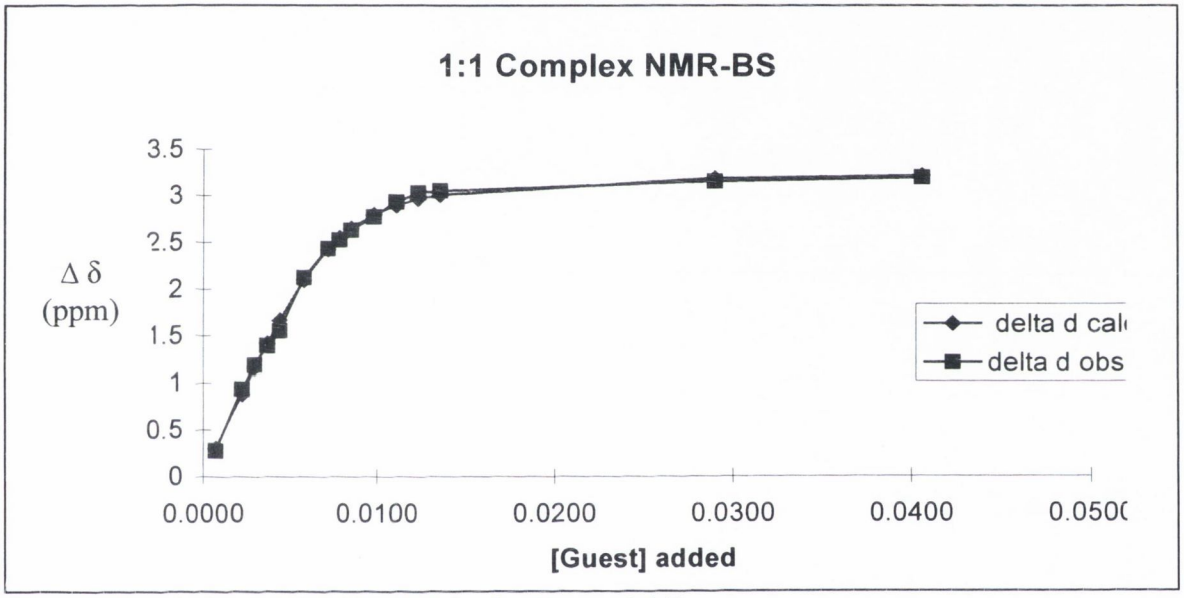


Figure 2.30: Calculated shift in1H NMR titration Vs actual shift

saturation curve at the 0.01M shows that the host forms a 1:1 complex. This is clearly evident when a graph of Δppm against anion equivalents is plotted (*Figure 2.32*). Addition of 0.1

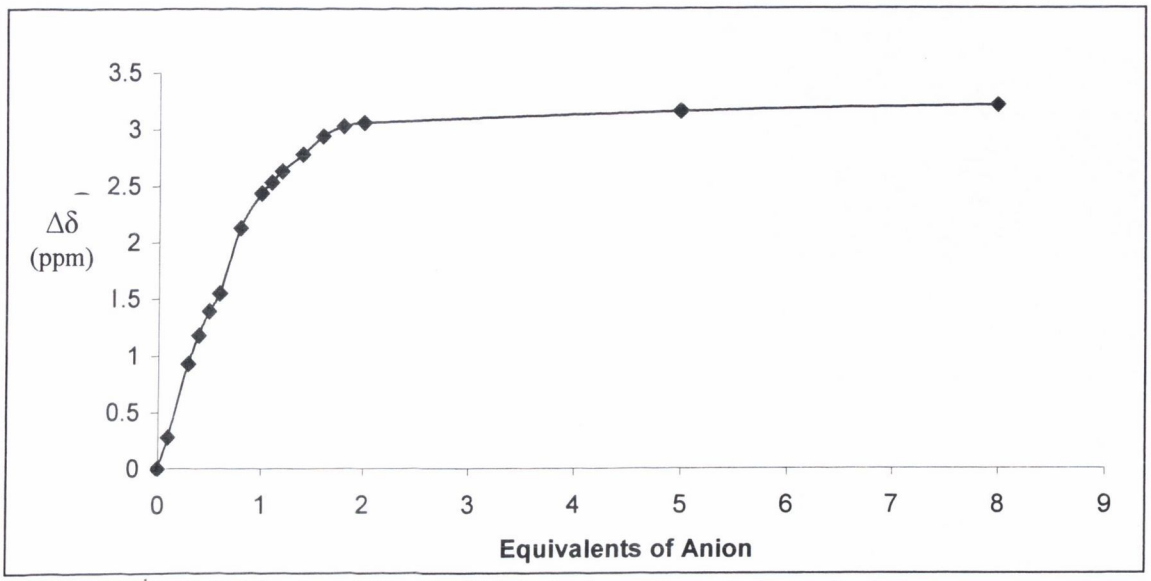
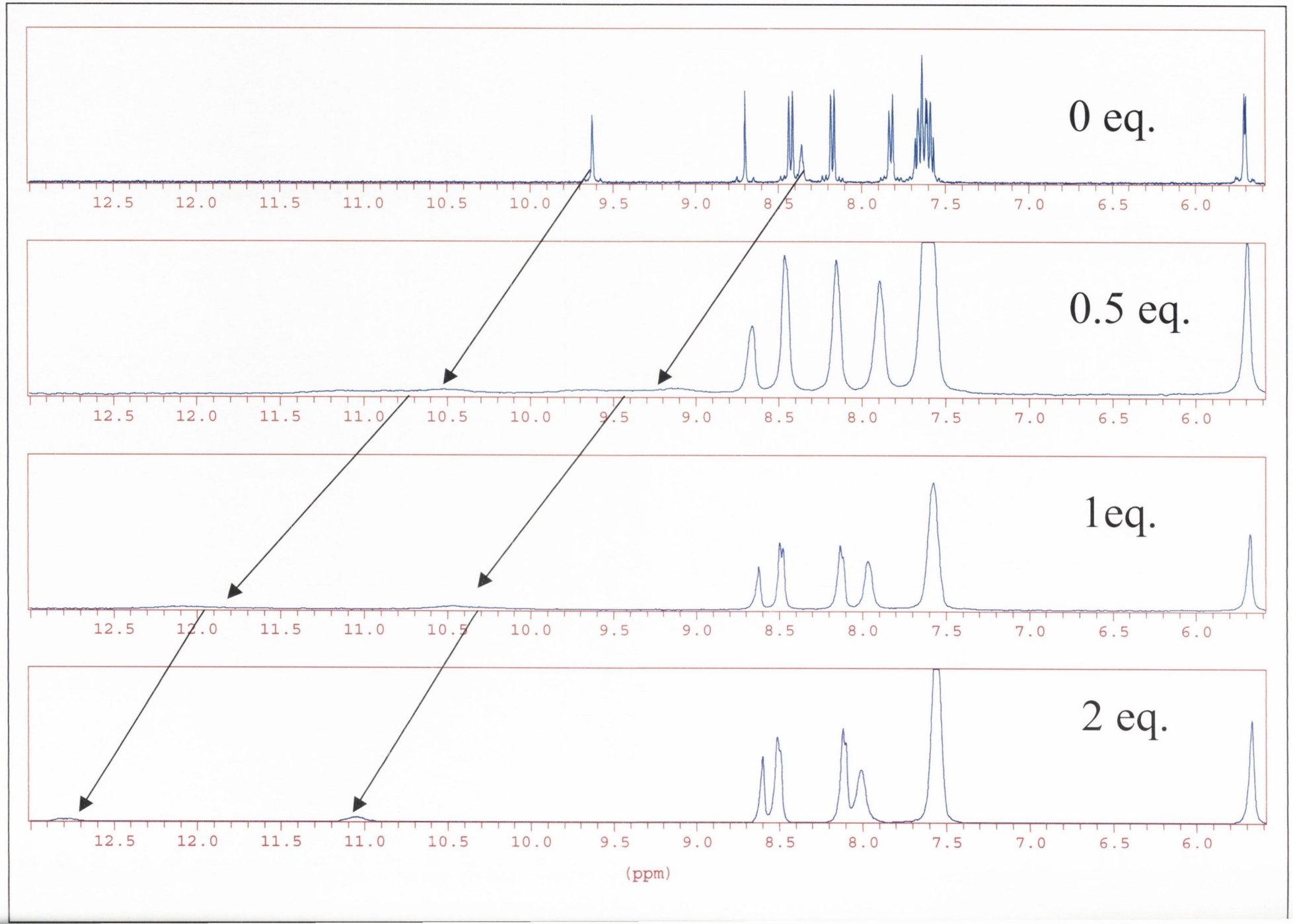


Figure 2.32: ^{1}H NMR Titration of **65** with TBA.AcO: Δppm *Vs* equivalents of anion

equivalents of anion were made to the solution and the observed Δδppm was plotted against equivalents of anion. *Figure 2.33* shows selected spectra from this titration illustrating the changes in the ^{1}H NMR upon formation of the host guest complex. These spectra clearly illustrate the fact that the positions of the peaks due to the anthracene protons are not changed. The broadening of these peaks that is observed is due to the addition of the TBA slats. The protons of the methyl group of the TBA are "drowning" out the anthracene proton peaks. The NH peaks monitored throughout the titration also broaden and blend into the baseline as the concentration of the TBA slat increases. Nevertheless the NMR software incorporated into the Bruker DPX 400 spectrometer allows the position of this peaks to accurately determined.


0 eq.
0.5 eq.
1eq.
2 eq.
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0
(ppm)

2.6 Conclusions

Obtaining crystals of the sensor: anion complex proved very difficult. Nevertheless collating all of the results obtained so far enables us to propose a structure for the complex. The protons of the thiourea moiety are facing away from the anthracene ring. This prevents the anion interacting with the electron density associated with the aromatic ring. If interaction did occur upon complexation there would be significant changes in the UV titration spectra. There also would be substantially shifts in the aromatic peaks of the NMR spectra. This indicates that the binding results are not influenced in any way by the fluorophore. In summary our initial hypothesis is that the binding site is isolated from the fluorophore. Results from each of the analytical techniques: Fluorescence, UV, and NMR were combined to prove this hypothesis. We propose that the quenching is likely to be due to the modulations of G_{PET} upon anion sensing. This can be regarded as an enhancement in the rate of electron transfer from the HOMO of the thiourea-anion complex to the anthracene excited state, upon anion recognition *i.e,* the reduction potential of the thiourea is increased causing PET to become competitively more viable, which causes the fluorescence emission to be quenched or *switched off'*. In conclusion, the simple fluorescent PET anion chemosensors **65-67** and **83** show *ideal* PET sensing behaviour upon ion recognition, *e.g.* only the fluorescence emission is *'switched off'* in the presence of AcO^-, $H_2PO_4^-$ and F^-, **65-67** and **83** are a very important contribution to the fast growing field of supramolecular anion recognition and sensing. The next logical step is to further investigate the area by designing new sensors of similar structure and also add more binding sites to the existing structure. This area of investigation is thoroughly discussed in Chapter 3.

CHAPTER 3

BIS THIOUREA AND UREA BASED ANION SENSORS

3.1 INTRODUCTION

In this chapter we demonstrate PET fluorescent sensing of anions flanked with ***two*** binding sites.[105] Such fluorescence sensing is both exceptional and of great physiological relevance since many dicarboxylates are components of various metabolic processes, and pyrophosphate is the product of ATP hydrolysis under cellular conditions.[106] However, it has up to now been difficult to achieve without the use of structurally complicated hosts.[107] With this in mind we wanted to use our simple design discussed in Chapter 2 we developed **95** and **96**, which have two thiourea moieties that can form hydrogen bonding complexes with bis-anions. We proved in the last chapter that the thiourea moiety is a successful binding unit that can be incorporated into a PET sensor. To the best of our knowledge, these chemosensors are the first examples of charge neutral fluorescent PET sensors that show ideal PET behaviour for bis-anions.

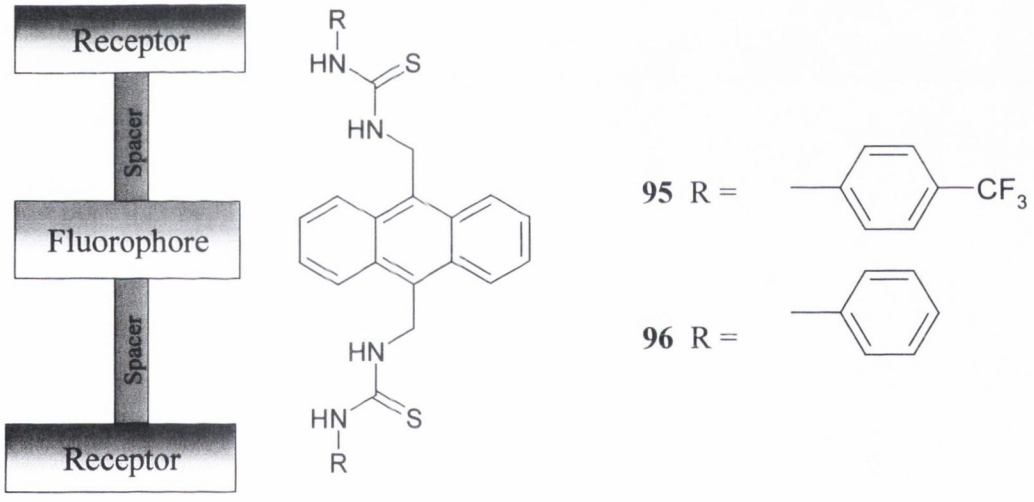


Figure 3.1: de Silva Model representation of bis thiourea sensors[108]

Sensors **95** and **96** (*Figure 3.1*) can be described as being designed as *"receptor-spacer-fluorophore-spacer-receptor"* conjugates[109] where the anion recognition takes place at the two-thiourea moieties. The fluorescent photoinduced electron transfer (PET) chemosensors **95** and **96** were designed for the recognition of anions possessing two binding sides such as dicarboxylates and pyrophosphate; the anion recognition in DMSO takes place through the two charge neutral thiourea receptor sites with concomitant PET quenching of the anthracene moiety. The second binding site must be strategically positioned in order to optimise the sensing ability of the sensor. As can be seen in *Figure 3.2* in order for binding to occur simultaneously at both sites the anion has to "bridge" across the fluorophore. This is very

different to the binding process observed with the mono systems in the last chapter. This chapter begins with the synthesis of this new family of sensors followed by an in depth discussion and analysis of the spectroscopic investigations conducted on this new family of sensors.

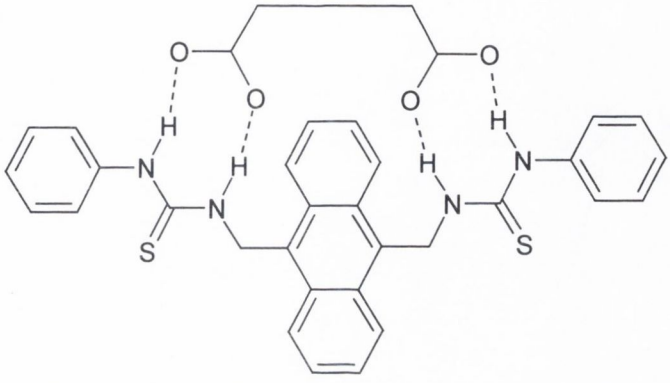
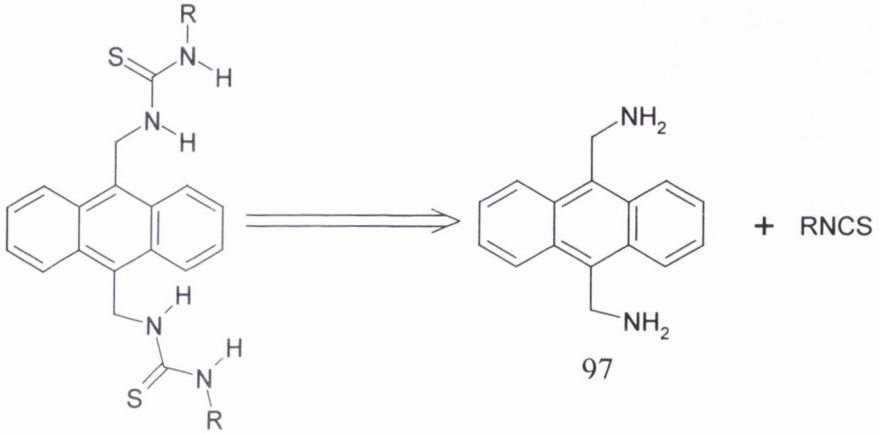


Figure 3.2: Illustration of potential binding between **96** and a bis acetate anion

As with the mono systems discussed in the previous chapter the key to **95** and **96** is that they are very easy to synthesise and, simple modification to the thiourea moiety (by incorporating aromatic or aliphatic electron withdrawing groups) can be used to *"tune"* the anion sensitivity and selectivity, as the acidity of the thiourea hydrogens is modulated. The selectivity may also be *"tuned"* by simply synthesising the urea counterparts of **95** and **96**. The next section describes in detail the synthetic route used in order to obtain this new family of sensors for bis anions.

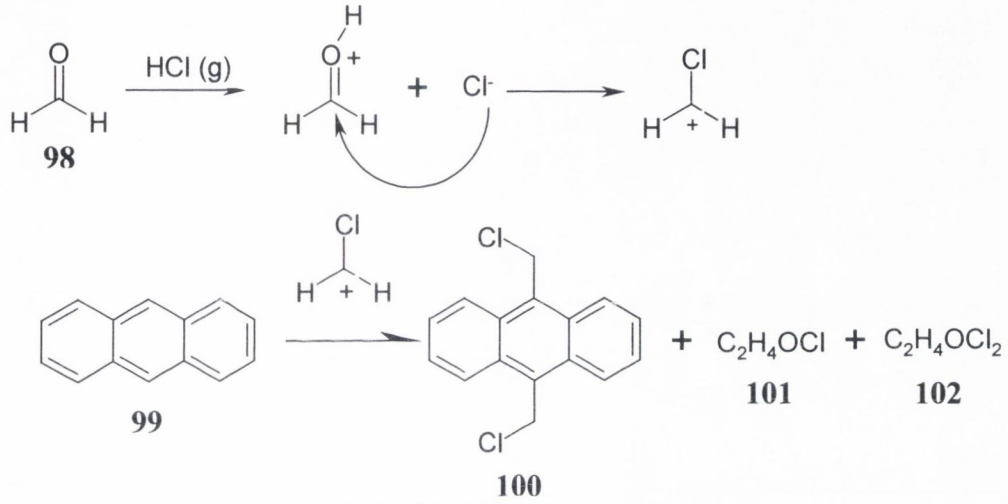
3.2 SYNTHESIS

Retro-synthetic analysis indicates, similar to the mono systems, the key precursor to the sensors is the anthryl amine, except in this case a bis methyl amine. The synthetic route with the mono systems involved starting with the anthracene aldehyde, which was reduced to the corresponding methyl alcohol. This alcohol was in turn converted to the halide before obtaining the amine using hexamethylenetetraamine. **95** and **96** were synthesized in good yield from 9,10-diamino-methylanthracene (**97**). **97** was synthesised *via* the 9,10 bis halide



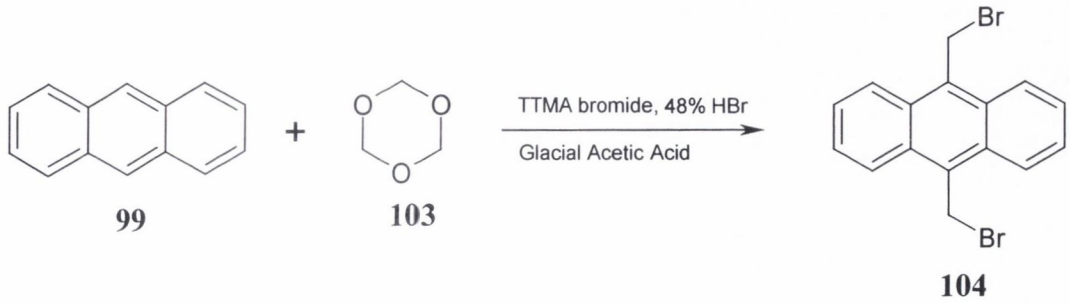
Scheme 3.1: Retrosynthesis of bis-amino sensors

derivative. Fortunately, it is possible to synthesise the bis halide directly from anthracene, therefore reducing the number of steps previously used. Furthermore we also used cheaper materials than before using anthracene, paraformaldehyde and HCl gas. This gave the chloride derivative, **100** in one step. Initially **100** was made by reacting anthracene with a suspension of paraformaldehyde in glacial acetic acid in the presence of HCl gas. The mechanism is



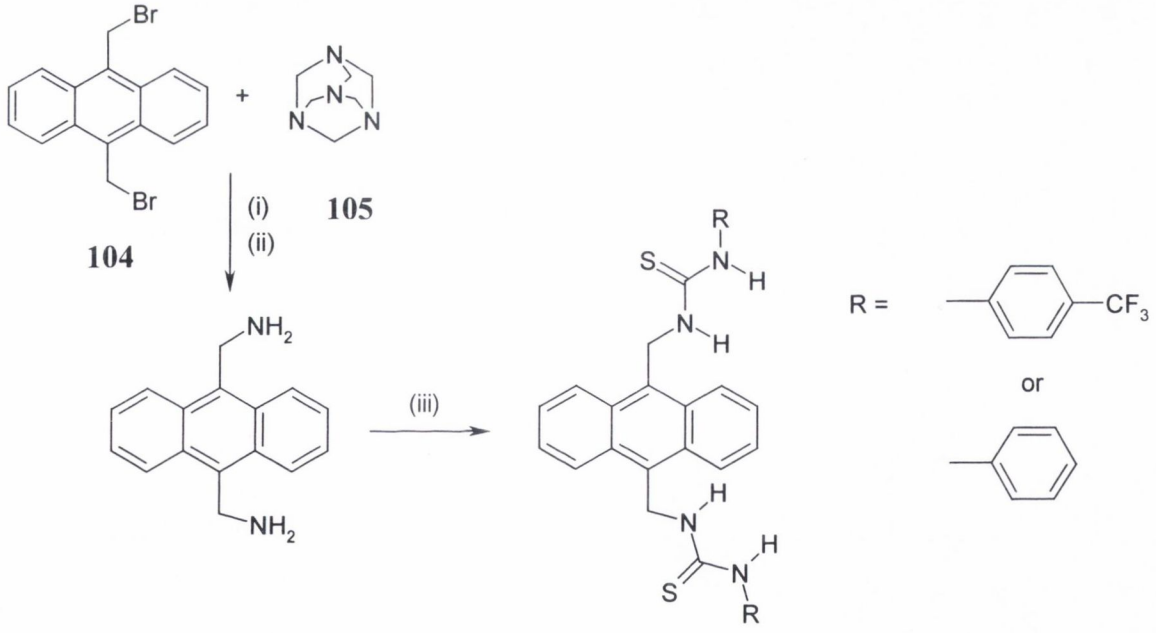
Scheme 3.2: Synthesis of 9,10 bis chloromethylanthracene

displayed in *Scheme 3.2* The anthracene ring is subject to electrophillic aromatic substitution. Positions 9 and 10 are the most reactive positions on the anthracene ring. Despite the good yields (80%) obtained using this method alternative procedures were sought due to the toxic by products (**101** and **102**) produced in this reaction. Because of this we decided that we shall synthesise the bis bromo-analogue **104**, using a method derived by Altava *et al.* This derivative was synthesised by reacting anthracene with 48% HBr and 1,3,5 trioxane, **103**, in glacial acetic acid in the presence of a catalytic amount of tetradecyltrimethyl ammonium bromide.[110] The desired product was removed by filtration and dried over P_2O_5 followed by recrystalisation from toluene.



Scheme 3.3: Synthesis of 9,10 (Bis)bromomethylanthracene

The method was very straight forward and produced **104** in high yield of pure product (75%). Since this method was very efficient attempts were made to synthesise 9-bromomethylanthracene a compound required for the mono sensors discussed in Chapter 2, using the similar conditions. However despite the fact that only half the equivalents of **103** were used the majority of the product obtained was **104**, with a very low overall yield. ^{1}H NMR showed that very little 9-bromomethyl anthracene was produced relative to **104**. The synthesis of 9,10-diamino-methylanthracene has previously been described in the literature, using Gabriel synthesis.[111] However, due to the insolubility of the bis-phthalimide intermediate the yield of **97** was found to be rather poor, less than 35%. With this in mind we synthesized **97** using the same method used for **70** in Chapter 2, *i.e.* hexamethylenetetramine, **105** in anhydrous $CHCl_3$ under inert atmosphere.[112] This method gave **97** in 85% yield as a crude product that could be used without any further purification. The two sensors **95** and **96** were subsequently made by reacting **97** with phenyl and 4-(trifluoromethyl)-phenylisothiocyanate, respectively, in dry CH_2Cl_2 under argon at room temperature. The resulting light-yellow precipitate was collected by filtration, washed several times with cold CH_2Cl_2, and recrystallized from with hot CH_2Cl_2 or $CHCl_3$.



Scheme 3.4: Synthesis of bis sensors using **104,** (i) $CHCl_3$, reflux (ii)EtOH, H_2O, HCl, 12 hours at 70°C (iii) DCM, at RT for 12 hours

All products were analyzed by conventional methods. As with the monosensors **65-67** the sensors **95** and **96** dissociated while using the Electrospray Mass Spectrometer thus preventing MS analysis. However both CHN and NMR techniques proved that the sensors

were synthesised and pure for analysis. Due to the inherent symmetry of the bis sensors the NMR is very simple (*Figure 3.3*). The two broad peaks at 8.06 and 9.39 ppm represent the two thiourea protons. The doublet at 5.75 ppm represents the singlet and the various aromatic protons are easily assigned by their position and splitting pattern. The ^{13}C NMR is also very simple and through the use of COSY experiments the individual peaks were easily assigned. As with the monosensors discussed in Chapter 2 ^{1}H NMR is used to confirm the binding constants obtained using fluorescence spectroscopy. In addition NMR also provides vital information on the structure of the host guest complex. Therefore NMR analysis is discussed in depth in *section 3.4.* Before discussing the results from the NMR analysis we will examine in detail the findings from the Fluorescent and UV spectroscopic investigations.

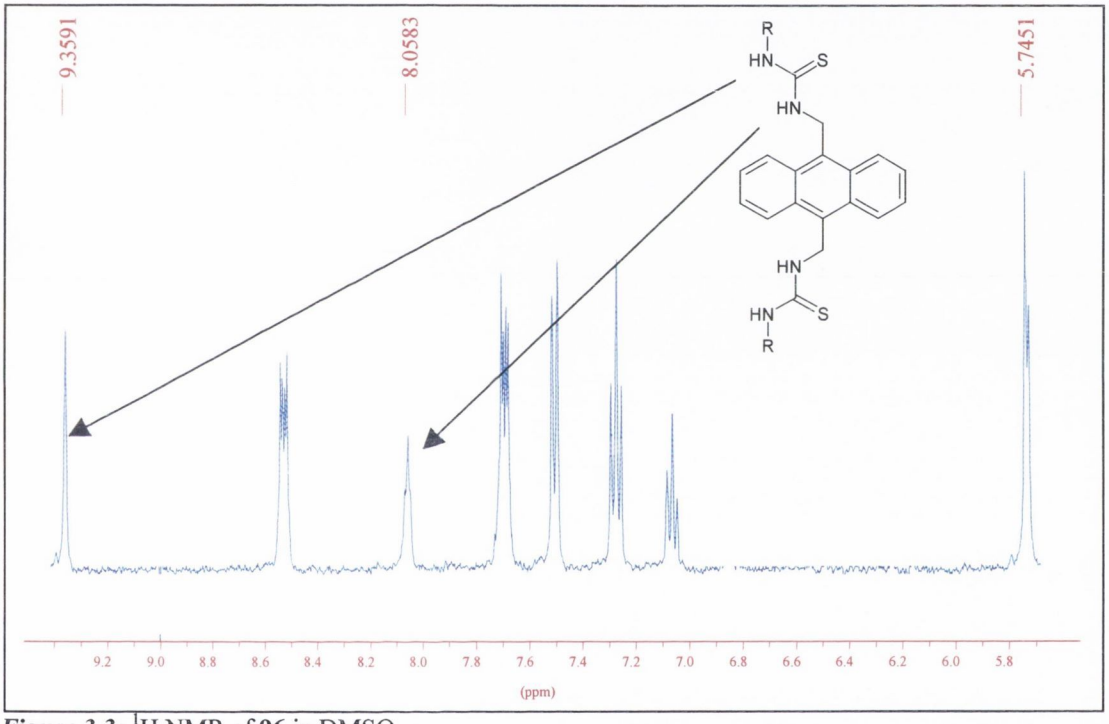


Figure 3.3: ^{1}H NMR of **96** in $DMSO_{-d6}$

3.3 Spectroscopic Investigations

Due to its close relation to the **65** and **66** it was suspected that the fluorescence properties of an anthracene system containing two binding sites should be affected in a similar fashion to the mono system when an anion is introduced to the system. The UV spectra for **95** shown in *Figure 3.4* exhibits the same fine structure of the peaks observed for mono systems due to the common anthracene ring, with just a slight shift in the peaks (400, 379, 359 nm). For comparative purposes the UV spectra for 9,10-dimethyl-anthracene (DMA) is superimposed on the UV spectra for **95**. DMA has peaks at 397, 375, 356 nm. This illustrates

the fact that the receptor has only a slight but very significant influence on the absorption bands of the bis-sensor compared to that of 9,10 dimethylanthracene. The fluorescence emission spectra of **95** compared to that of 9,10 dimethylanthracene. The fluorescence emission spectra of **95** consisted of three bands at 409, 430 and 455 nm when excited at 379

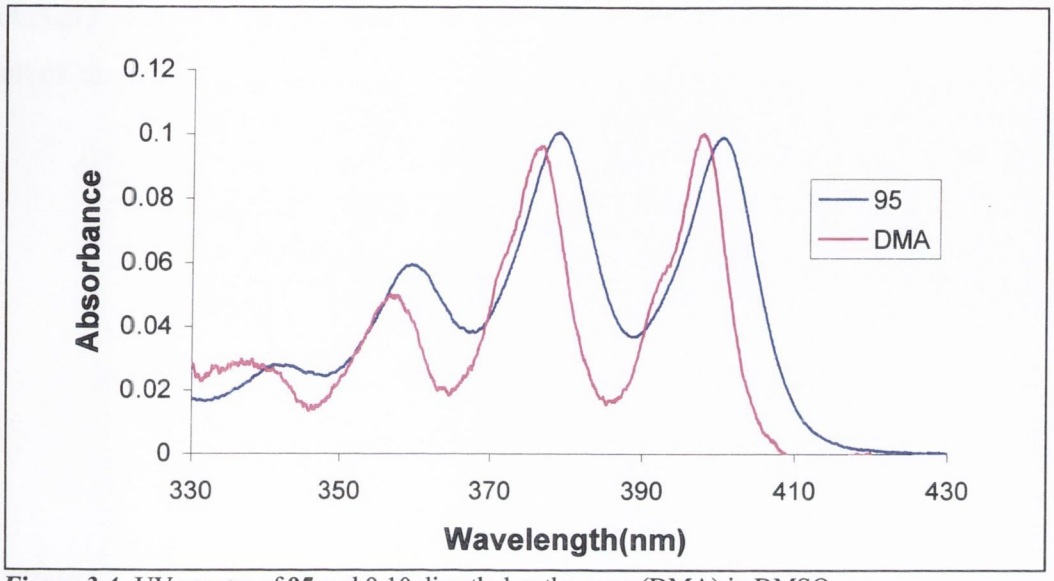


Figure 3.4: UV spectra of **95** and 9,10 dimethyl-anthracene (DMA) in DMSO

nm with Φ_F = 0.047 (*Figure 3.5*). The shift in wavelength observed in the fluorescence spectra of 9, 10 DMA relative to that of **95** is significant. The shorter wavelengths (395, 419, 443 nm) indicates a bigger energy gap between the HOMO and LUMO's of 9, 10 DMA relative to **95**. The comparison of the fluorescent properties of our bis sensors relative to 9, 10 DMA is necessary. The reasons shall become more apparent in *section 3.3.1.*

In order to be consistent with the analysis of the mono-sensors we initially analysed the sensing ability of the bis systems towards AcO- then analysed the affinity of the sensors for the other anions previously tested for **65 -67** and **83** in DMSO. The position shape and

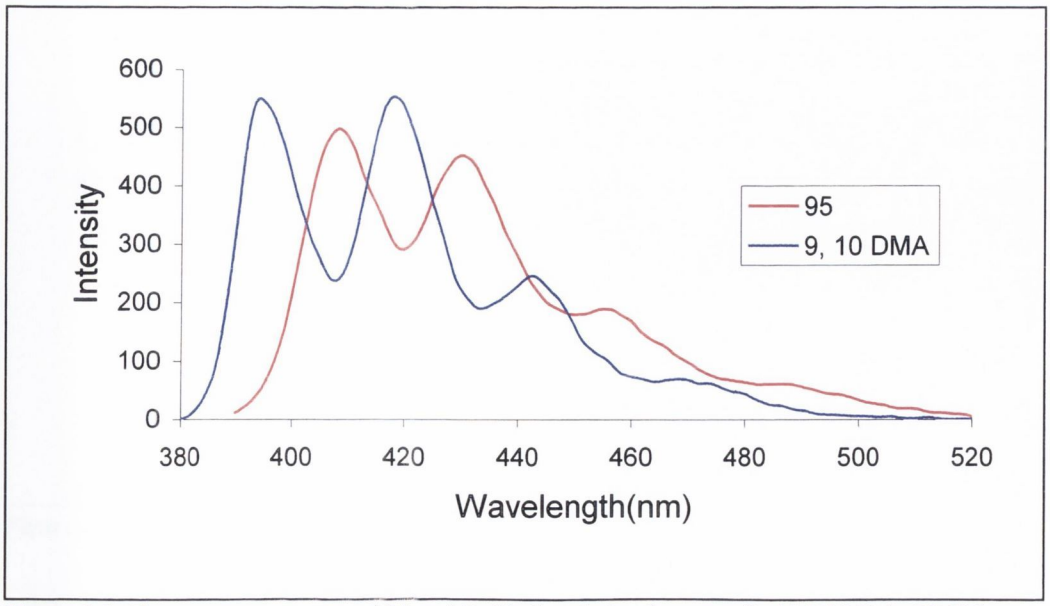


Figure 3.5: Fluorescence spectra of **95** and 9, 10 dimethylanthracene (9, 10 DMA) in DMSO

intensity of the UV and fluorescence peaks were monitored throughout the titration of the sensor with various mono-valent anions tested in Chapter 2. As with the mono systems **65-67** and **83** no significant change occurred in the UV spectrum yet concurrently there was significant changes in the fluorescent spectra. Both sets of spectra for the titration with TBA.AcO (the UV and fluorescence) are displayed in *Figures 3.6* and *3.7* respectively. As a result of the changes in the UV spectra throughout the titration being minimal the sensitivity

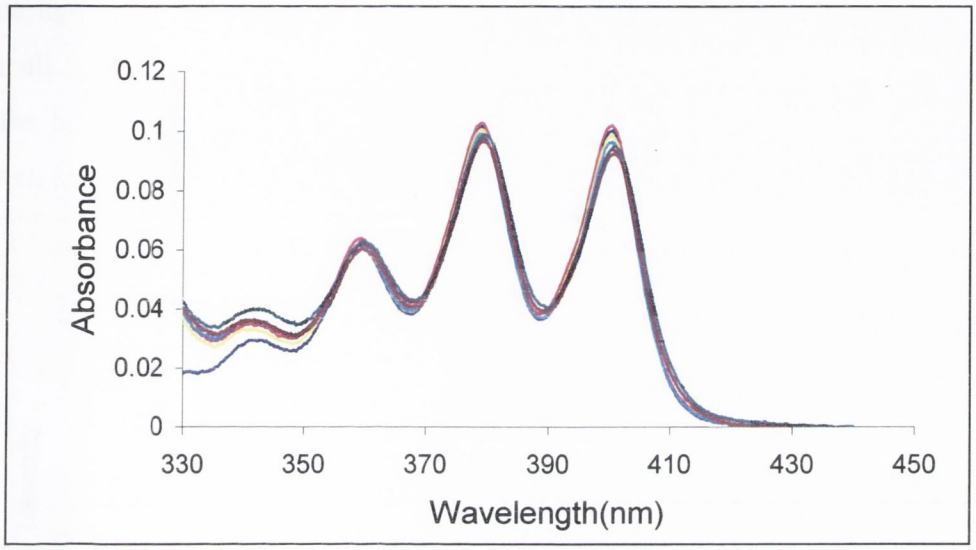


Figure 3.6: UV titration of **95** with TBA.AcO: (0-58mM) Absorbance *vs* Wavelength (nm)

and the selectivity of these sensors toward a series of mono- and bis-anions was evaluated by observing the changes in their fluorescence emission spectra in DMSO upon anion titration with $(C_4H_9)_4N^+$ salts in DMSO-$_{\delta 6}$. Upon addition of monodentate anions such as AcO^- the emission at 430 nm was approximately 70% "*switched off*" or quenched which must be

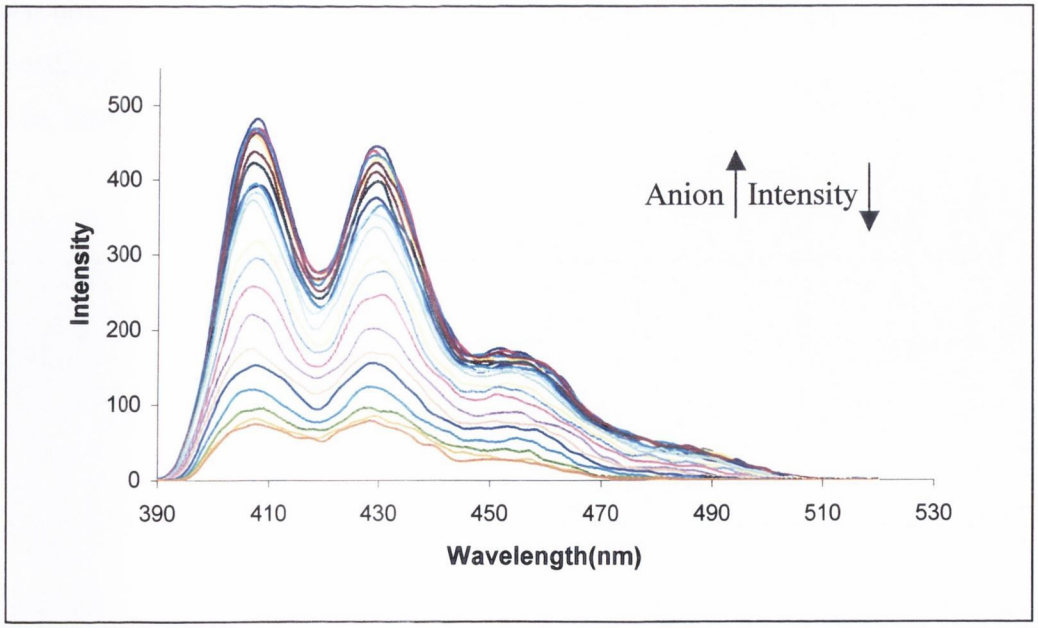


Figure 3.7: Fluorescence Titration of **95** with TBA.AcO: (0-56mM) Intensity vs Wavelength (nm)

due to the formation of the anion-receptor complex as can be seen in *Figure 3.7*. No other spectral changes were observed in the emission spectra, *i.e.*, there was no evidence of either exciplex or excimer emissions.[113] As already stated, concurrently the changes in the absorption spectra (peaks at 358, 378 and 400 nm) of the anthracene moiety were only minor. These results coincided with the results found for the sensor with one binding site *e.g.* **65 –67** and **83**. However there was one distinct difference. When plotting the changes of the fluorescence intensity at 430 nm as a function of pA (-log[anion]) it was observed that as before, in all cases, sigmoidal profiles as shown in *Figure 3.8* for AcO^- were obtained. However, for both **95** and **96**, these profiles changed over ca. 3-4 pA units. This can be regarded as an indication of a possible 2:1 binding. This was expected as each anion can

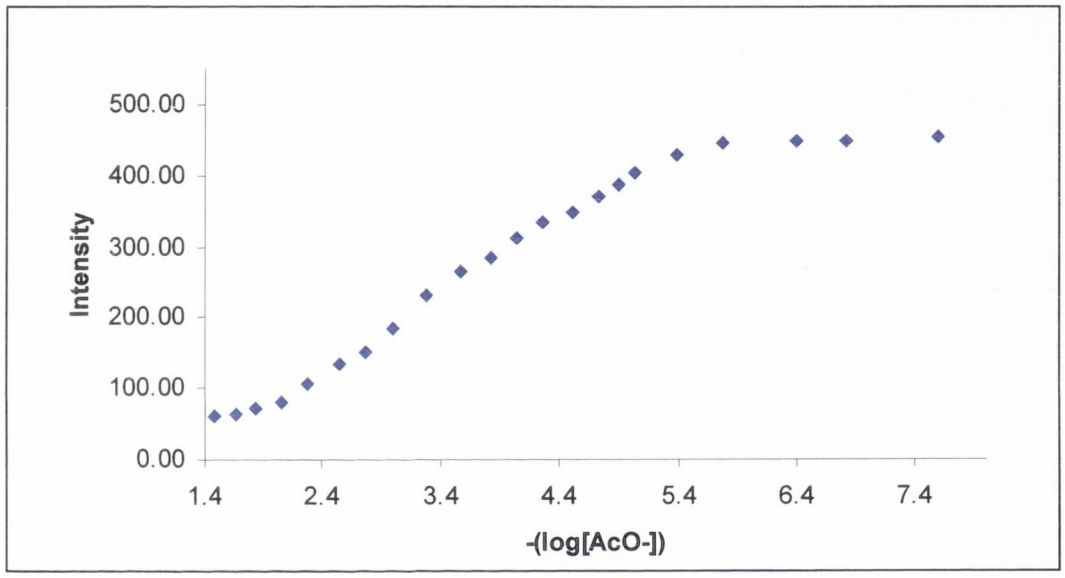


Figure 3.8: Intensity Vs –log[AcO-] for **95** with TBA.AcO

separately bind to each of the binding sites, *i.e.* two anions for every one sensor. This was further confirmed by observing the changes in ^1H NMR of the thiourea protons upon titration that will be discussed later. Similar results were observed for $H_2PO_4^-$.

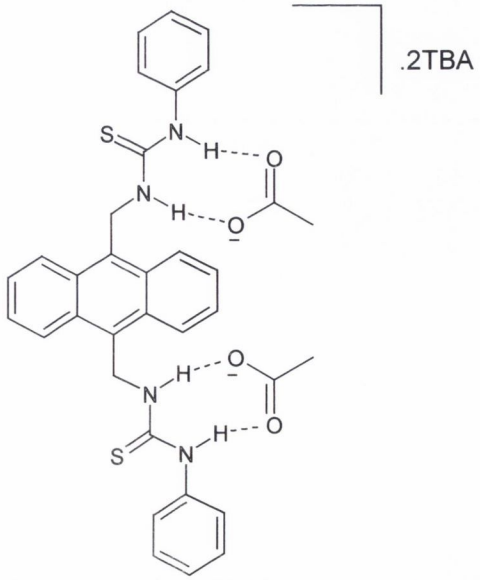


Figure 3.9: Schematic representation of 2:1 binding of AcO^- with **96**

What is being observed here is a typical PET behaviour, since the receptors are separated from the fluorophore by the two -CH_2- spacers; the only interaction between the two moieties is *via* electron transfer, as previously described for **65 – 67** and **83** in Chapter 2. Furthermore upon addition of spherical anions such as Cl^- and Br^- no significant quenching was observed, ruling out quenching by the heavy atom effect. Like the mono-sensors, F^- quenched the emission effectively ~98% due to its small size and high charge density. There was also no quenching observed upon titration of **95** or **96** with TBA.ClO_4 (*Figure 3.10*). All of these results are of the same nature as shown for **65-67** in Chapter 2. Therefore similar conclusions can be drawn for these results.

To investigate the selectivity of the **95** for some anions over others and to fully demonstrate that Cl^- did not bind to the sensor a known amount of AcO^- was added to a solution of the sensor containing a known amount of TBA.Cl. The addition of AcO^- to a solution of **95** with a 40 mM background concentration of Cl^- quenched the emission to the same degree as seen previously for AcO^- with no Cl^- present, clearly indicating that **95** was selectively binding AcO^- over Cl^-. The same results were obtained when **96** was subjected to the same conditions.

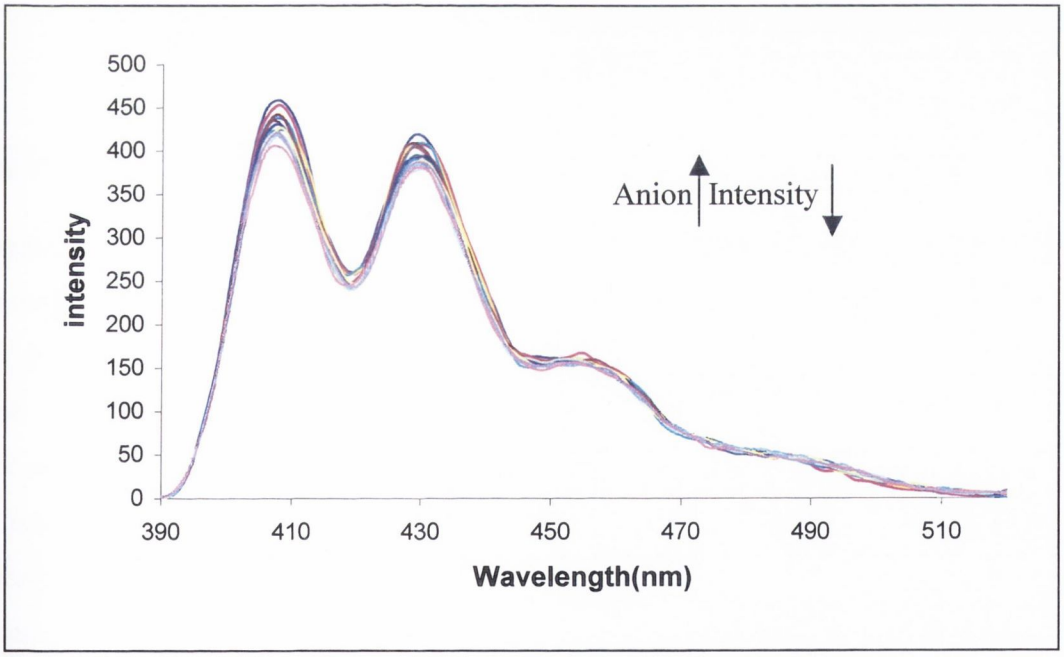


Figure 3.10: Fluorescence Titration of **95** with TBA.ClO_4; Intensity vs –log[anion]

Once it was established that the sensors with the two binding sites had similar properties to their mono binding site counterparts it was necessary to conduct further tests on the new family of sensors. The next step is to determine whether **95** and **96** can selectively sense biologically important bis-anions such as glutarate, malonate, and pyrophosphate (as tris(tetrabutylammonium) hydrogen pyrophosphate). If the binding occurs in a 1:1 ratio

utilising both binding sites simultaneously it is predicted that the binding constant should be significantly higher which may give rise to better quenching.

3.31 Fluorescence Investigation of 95 and 96 using bis anions

In order for comparisons to be made the bis anions were investigated in a similar manner to the mono anions. The first bis anion to be analysed was pyrophosphate. The fluorescence reduced by almost 95% by the end of the titration. As before, no other significant spectral changes were observed in the emission spectra *i.e.* no change in emission wavelength or

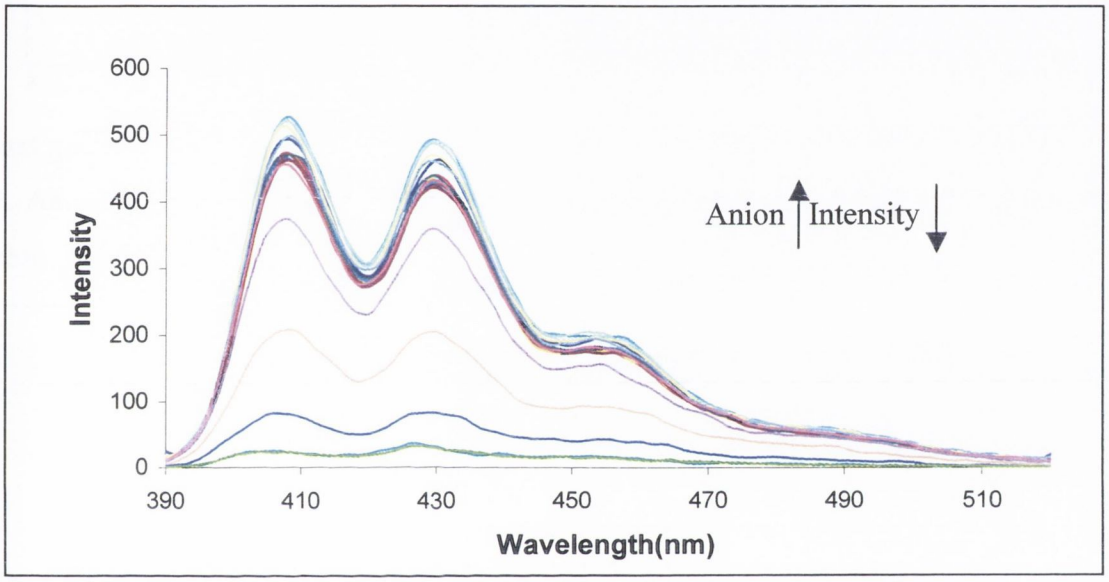


Figure 3.11: Fluorescence titration of **95** with TBA.HP_2O_7; Intensity Vs –log[anion]

or formation of excimer emission bands (*Figure 3.11*). A plot of "Intensity versus – log[anion]" shows that the binding occurs over two p[A] units (2.9 to 4.9 units), indicating 1:1 binding and a simple equilibrium with log $\beta = 4.34$. This is very significant, as in order for the anion to simultaneously bind to both sites it would have to bridge across the anthracene ring as depicted in *Figure 3.2*. If this were the case there would potentially be some interaction between the anion and the electron density of the anthracene. Therefore concurrently with the fluorescence titration, the corresponding UV/Vis spectra were also analysed.

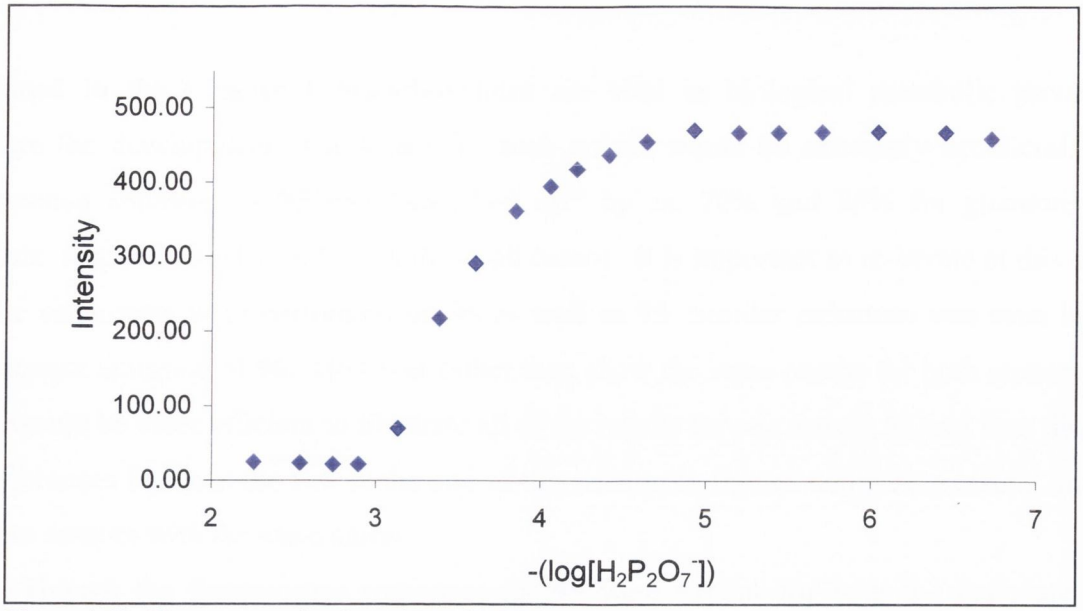


Figure 3.12: Fluorescence titration of **95** with $HP_2O_7^-$

At first glance the there is no significant change in UV/vis at longer wavelengths as in *Figure 3.13*. As with the mono systems any change that has occurred can be potentially due to dilution. However upon closer examination an isosbestic point was found at 406 nm. This

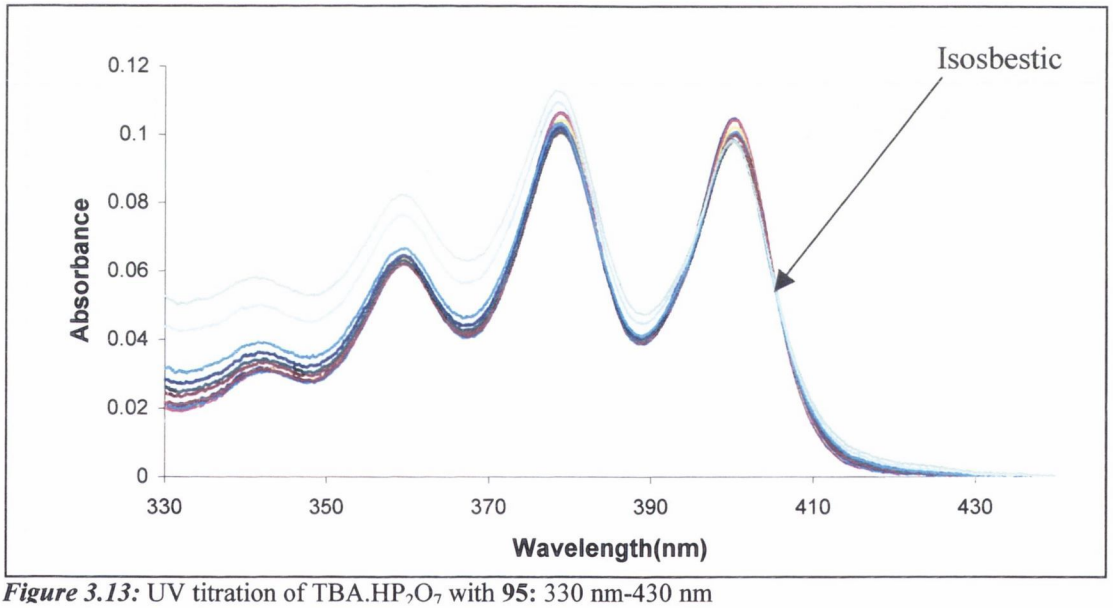


Figure 3.13: UV titration of TBA.HP_2O_7 with **95:** 330 nm-430 nm

indicates that there is some level of interaction between the bis anion and the anthracene ring. This finding is potentially due to the anion bridging between the two binding sites over the anthracene ring. This interaction may also be observed in the anthracene peaks of the ^1H NMR of the complex during a titration of **95** with pyrophosphate which shall be discussed in *section 3.4*. The formation of this isosbestic point was verified by repeating the titration several times. The hypothesis that the anion "bridges across" the anthracene of the sensor was further tested by analysing other bis anions of varying size. Two organic anions, malonate and glutarate were chosen, as their TBA salts are commercially available. More importantly as

mentioned in the Chapter 1 biscarboxylates are vital in biological metabolic processes therefore the development of a sensor for such anions would be extremely beneficial. The fluorescence emission of **95** was "switched off" by ca. 70% and 86% for glutarate and malonate, respectively ([anion] = 40mM in all cases). It is important to re-iterate at this stage that the same tests were performed on **96** as well as **95**. Similar reduction was seen in the fluorescence emission of **96**. However rather than show the same results for both sensors, we feel it would be more efficient to illustrate all of the results for one sensor, **95** and then display the differences between the two at the end of this section in figures comparing results for the different sensors with the same anion.

Though the fluorescence responses for **95** were similar for both the malonate and glutarate salts, in so far as the reduction in fluorescence upon incremental addition of an anion to the environment. A distinct difference is observed when relative intensity is plotted against –log [anion]; Malonate exhibits a 1:1 binding ratio whereas glutarate has a binding ratio of 2:1 (*Figure 3.14*). The changes in the quantum yield of fluorescence (Φ_F) of **95** upon anion

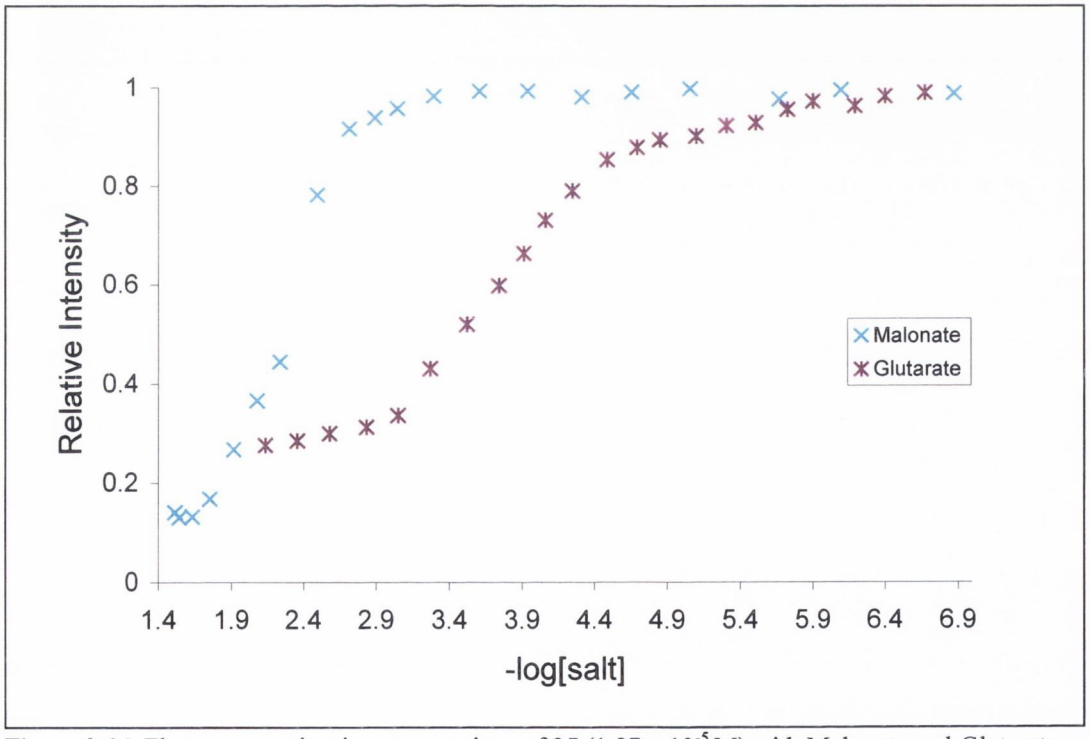


Figure 3.14: Fluorescence titrations comparison of **95** (1.87×10^{-5} M) with Malonate and Glutarate

sensing was measured in comparison with that of 9,10-dimethylanthracene (9,10-DMA) in DMSO. There is a range of standards that could have been chosen when determining the quantum yields of this new family of sensors. 9,10-DMA was chosen because not only was it used as a standard to determine the quantum yield of the sensors but since 9,10-DMA forms

the backbone of our new bis sensors, the fluorescence response of the fluorophore independent of the thiourea binding sites could be determined. Φ_F measurements of 9,10-DMA, which lacks the two receptors sites, gave a $\Phi_F = 0.87$ in DMSO, which is substantially larger than that of **95**, $\Phi_F = 0.047$, and **96**, $\Phi_F = 0.11$, in DMSO. This implies that PET is active prior to the anion recognition, but becomes even more so *after* anion recognition. In order to confirm that the presence of the anions tested have no effect on the fluorescence of 9,10-DMA it was necessary to measure the fluorescence of 9,10-DMA in the presence of both mono and bidenatate anions. Firstly 10 mM of AcO^- was added to a solution of known concentration of 9,10-DMA and the fluorescence of the resulting solution was analysed: the Φ_F of 9,10-DMA was not affected. This result was confirmed by repeating the procedure using a bis anion, pyrophosphate and again the same result was obtained. All of the fluorescence data for the sensors discussed so far is summarised in *Table 3.1* and *3.2* respectively.

Table 3.1: Fluorescence data obtained for titrations of TBA salts with **95**

Anion	AcO-	F-	$H_2P_2O_7$	Glutarate	Malonate	H_2PO_4
-logKa	3.56	4.13	3.4	3.74	2.34	3.1
Ka	3630.78	13489.63	2511.89	5495.41	218.78	1258.93
%Reduction	84%	95%	95%	70%	80%	77%
Range	4	3.5	2	3	2	3.5
Qyield	0.006	0.003	0.001	0.012	0.011	0.008

Table 3.2: Fluorescence data obtained for titrations of TBA salts with **96**

Anion	AcO-	F-	$H_2P_2O_7$	Glutarate	Malonate	H_2PO_4
-logKa	2.77	3.03	3.07	3.15	2.02	3.44
Ka	588.84	1071.52	1174.90	1412.54	104.71	2754.23
%Reduction	69%	91%	90%	68%	76%	63%
Range	4	3.2	2	3.27	2	3.6
Qyield	0.017	0.014	0.017	0.039	0.035	0.025

We believe that this relatively high degree of fluorescence quenching observed for all the anions above is due to the increase in the reduction potential of the thiourea receptor moieties *after* anion recognition. The increase in the reduction potential reduces the difference between the HOMO of the receptor and the LUMO of the fluorophore making PET more favourable. Consequently the rate of the electron transfer from the HOMO of the receptor to the excited state of the fluorophore is affected, i.e., ΔG_{et} becomes more negative upon anion recognition and hence more thermodynamically favourable. As a result the emission is *"switched off"*. This is represented in *Figure 2.3*. As mentioned in Chapter 2, this hypothesis cannot be confirmed as we were unable to measure the changes in the redox

potential for the receptor since the thiourea was irreversibly oxidized. In addition it is important to note that no other spectral changes were seen in the emission spectra, eliminating other photophysical routes that would lead to a reduction in fluorescence.

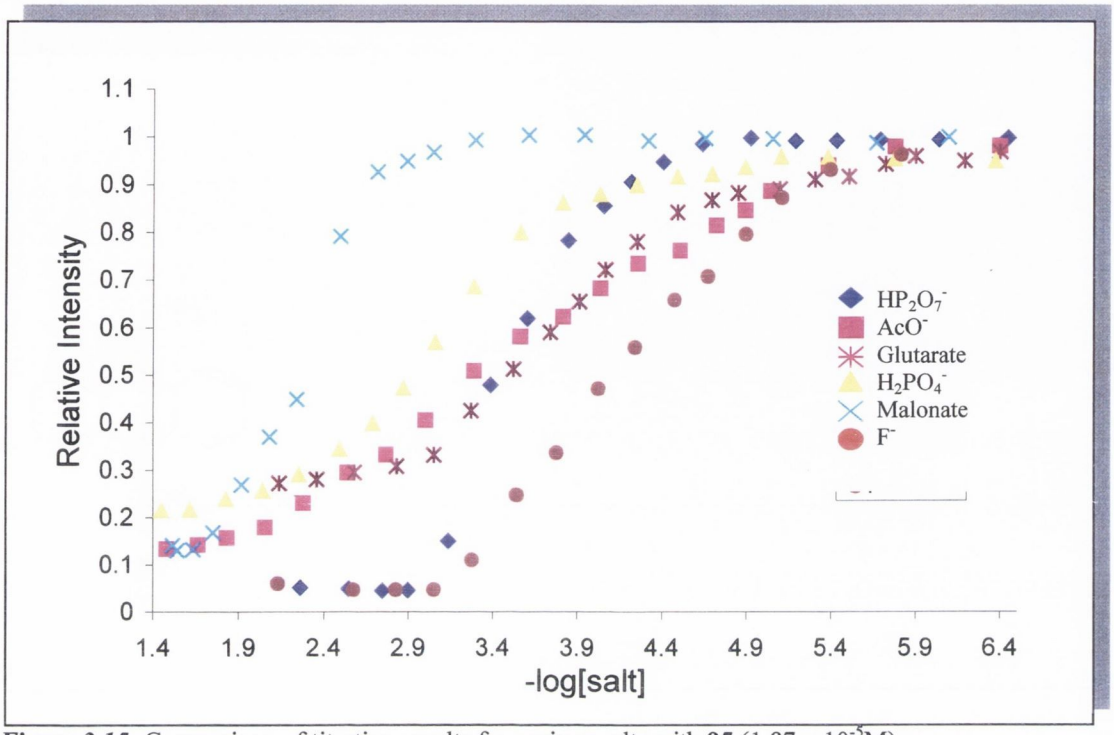
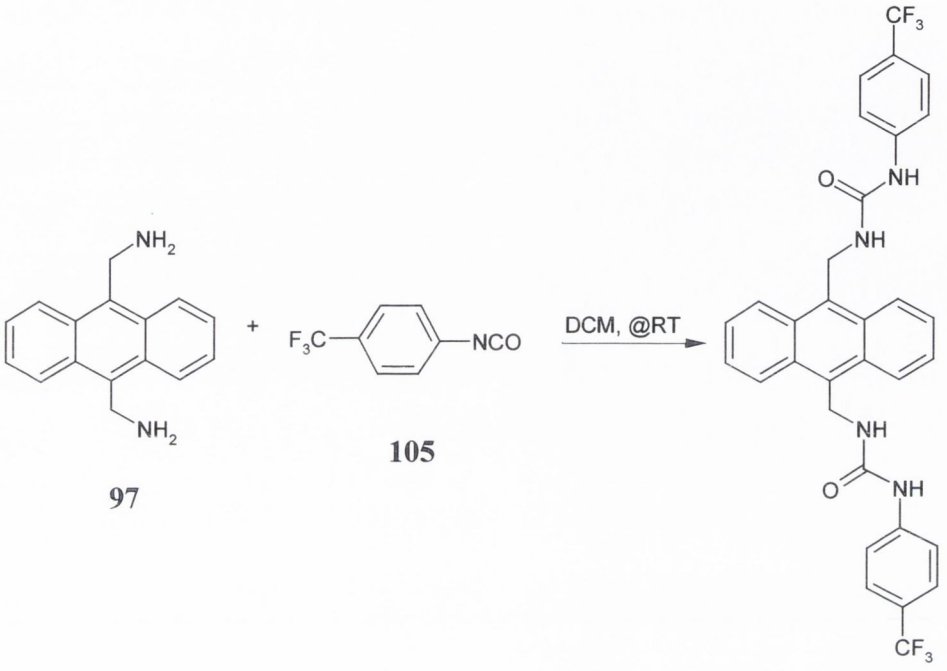


Figure 3.15: Comparison of titration results for various salts with **95** (1.87×10^{-5}M)

Plotting the emission changes at 430 nm vs. pA gave sigmoidal curves for all the bis-anions (*Figure 3.15*). Importantly, the emission is *"switched off"* over two pA units,for pyrophosphate and malonate, indicating 1:1 binding and simple equilibrium. For the larger glutarate anion, the emission was *"switched off"* over ca. 3pA units. From these changes using *Equation X* in Chapter 2 the binding constant log β of 3.74 (± 0.05), 2.34 (± 0.05), and 3.40 (± 0.05) was determined for glutarate, malonate and pyrophosphate, respectively, for **95**. *Figure 3.15* displays the titration curves obtained for all of the anion titrations for **95**. These graphs illustrate the different affinities that **95** has for the various anions displayed. The sigmoidal curves representing the titration of **95** with Malonate and HP_2O_7 respectively each stretch over 2pA units indicating 1:1 binding ratio and a simple equilibrium. However the remainder of the anions were "switched off" over more than 2pA units indicating a binding ratio greater than 1:1. The binding of malonate is significantly less relative to the binding of HP_2O_7. All of the values obtained are summarised in *Table 3.1*.

For **96**, these values were found to be 3.07 (±0.05), 3.15 (±0.05), and 2.02 (±0.05) for pyrophosphate, glutarate, and malonate, respectively. The order of selectivity shown by **96** is relatively the same as that exhibited by **95**, with the exception of H_2PO_4, which exhibits stronger affinity for **96** relative to **95**.

It was at this stage we decided to expand our investigation to include the urea equivalent of **95**, therefore further "tuning" the selectivity of this family of sensors. The synthesis of the urea is relatively straightforward. Similar to **95** and **96** the precursor to this sensor is the bis amine, **97**. 4-trifluoromethylphenylisocyanate, **105** was added to a solution of **97** in DCM. The product



97 **105** **106**

precipitated out of solution after 15 minutes and was isolated by filtration. The product was then recrystalised from DCM before analysis. The binding affinity of **106** was tested with the same anions as tested for **95** and **96**. Identical conditions and techniques were used so as direct comparisons could be made. As expected **106** exhibited the same fluorescence behaviour as **95**

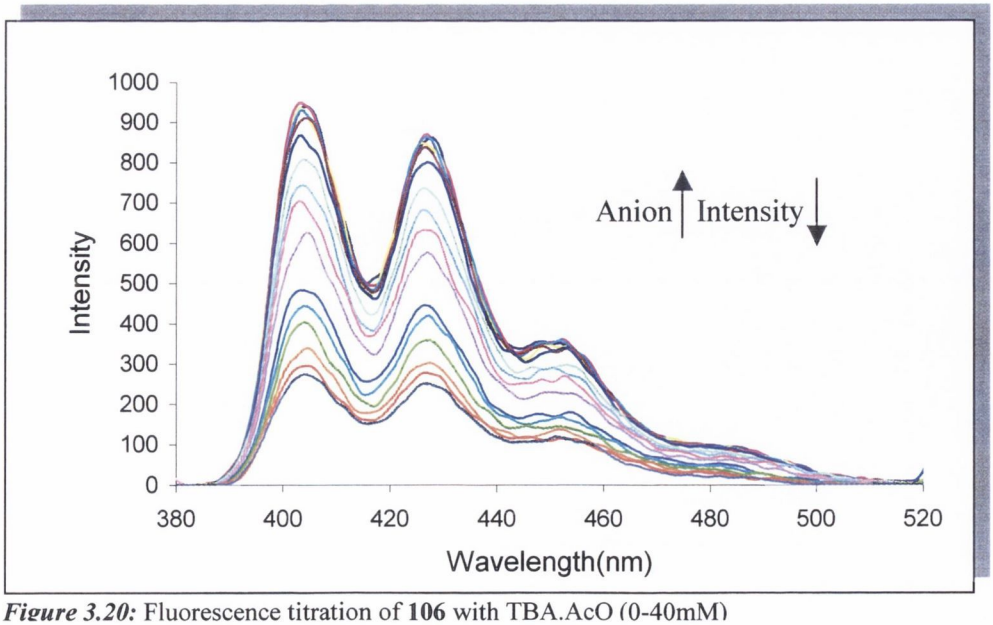


Figure 3.20: Fluorescence titration of **106** with TBA.AcO (0-40mM)

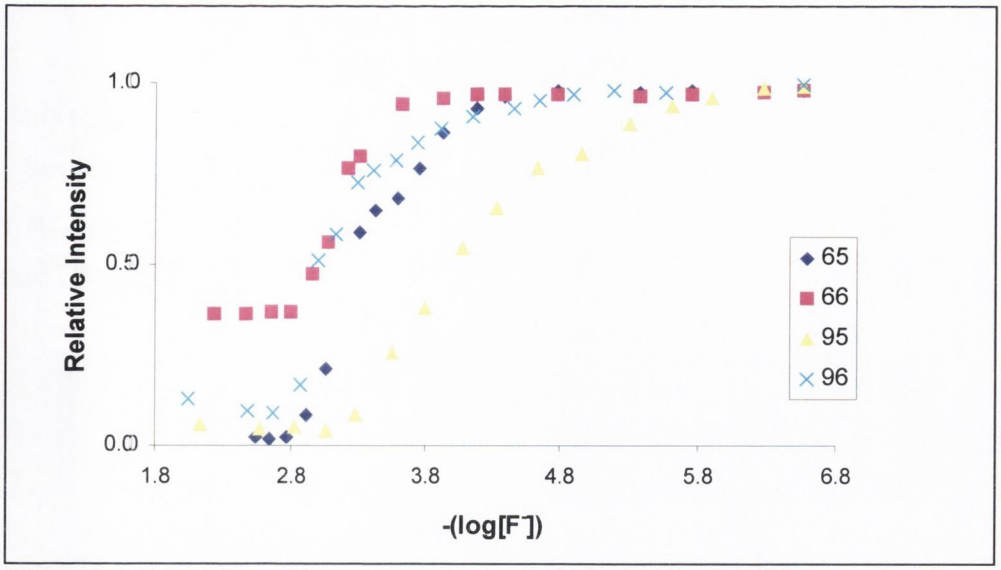


Figure 3.17 Comparison of fluorescence titrations of thiourea sensors with F^-

By comparing the results obtained for the titrations of the individual anions with the various sensors, a clearer picture may be developed of the "tunability" of these sensors.

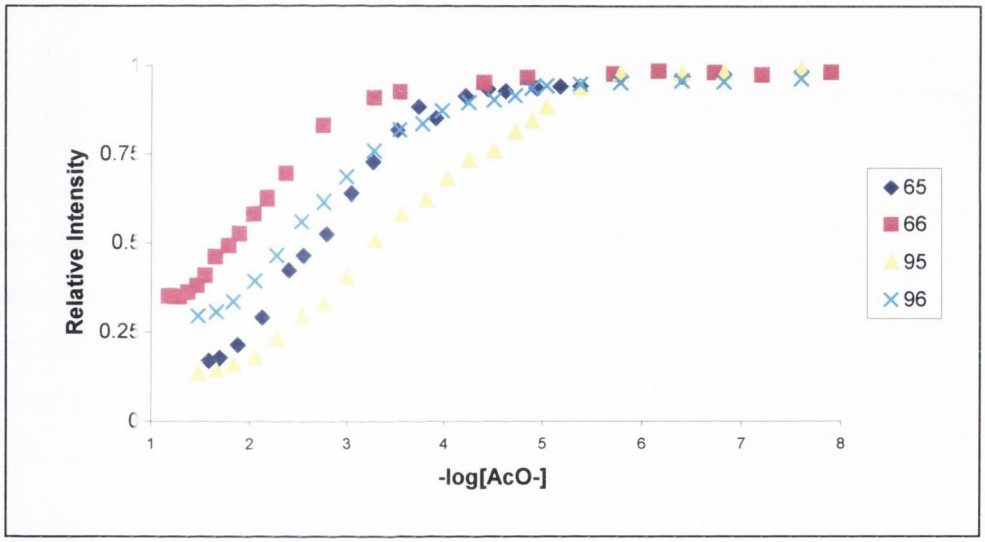


Figure 3.18: Comparison of fluorescence titrations of thiourea sensors with AcO^-

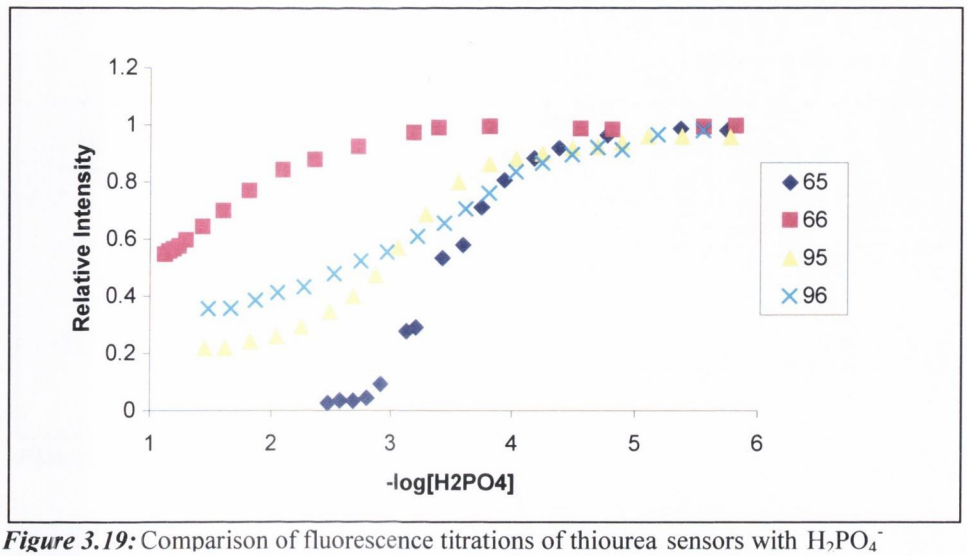
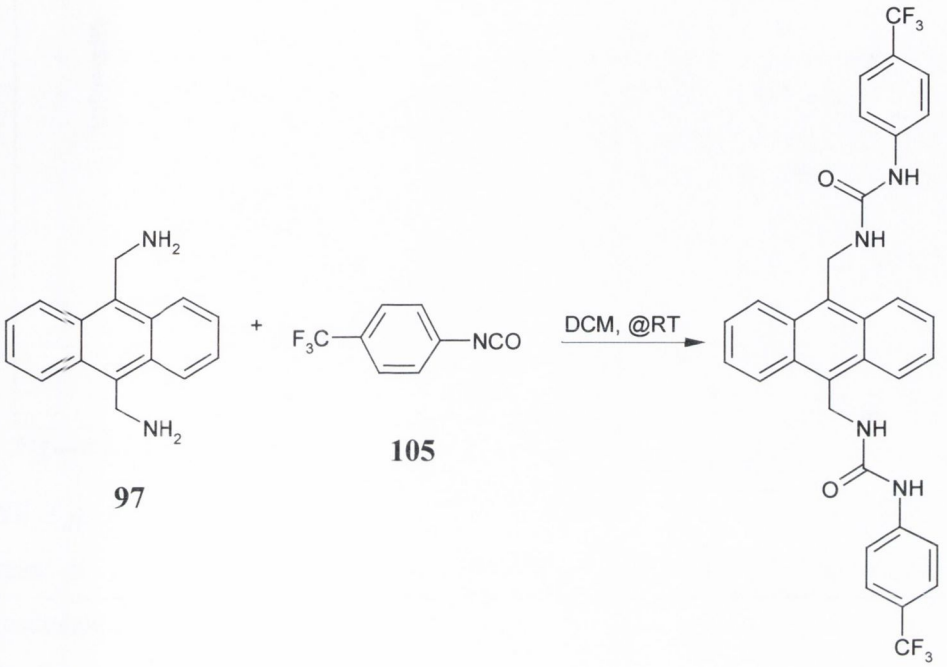


Figure 3.19: Comparison of fluorescence titrations of thiourea sensors with $H_2PO_4^-$

It was at this stage we decided to expand our investigation to include the urea equivalent of **95,** therefore further "tuning" the selectivity of this family of sensors. The synthesis of the urea is relatively straightforward. Similar to **95** and **96** the precursor to this sensor is the bis amine, **97**. 4-trifluoromethylphenylisocyanate, **105** was added to a solution of **97** in DCM. The product



97 **105** **106**

precipitated out of solution after 15 minutes and was isolated by filtration. The product was then recrystalised from DCM before analysis. The binding affinity of **106** was tested with the same anions as tested for **95** and **96**. Identical conditions and techniques were used so as direct comparisons could be made. As expected **106** exhibited the same fluorescence behaviour as **95**

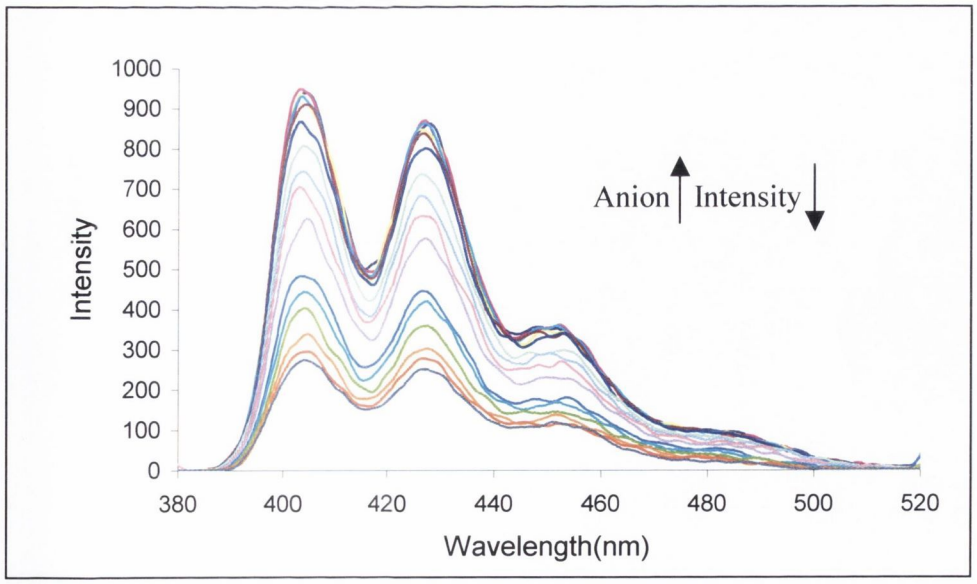


Figure 3.20: Fluorescence titration of **106** with TBA.AcO (0-40mM)

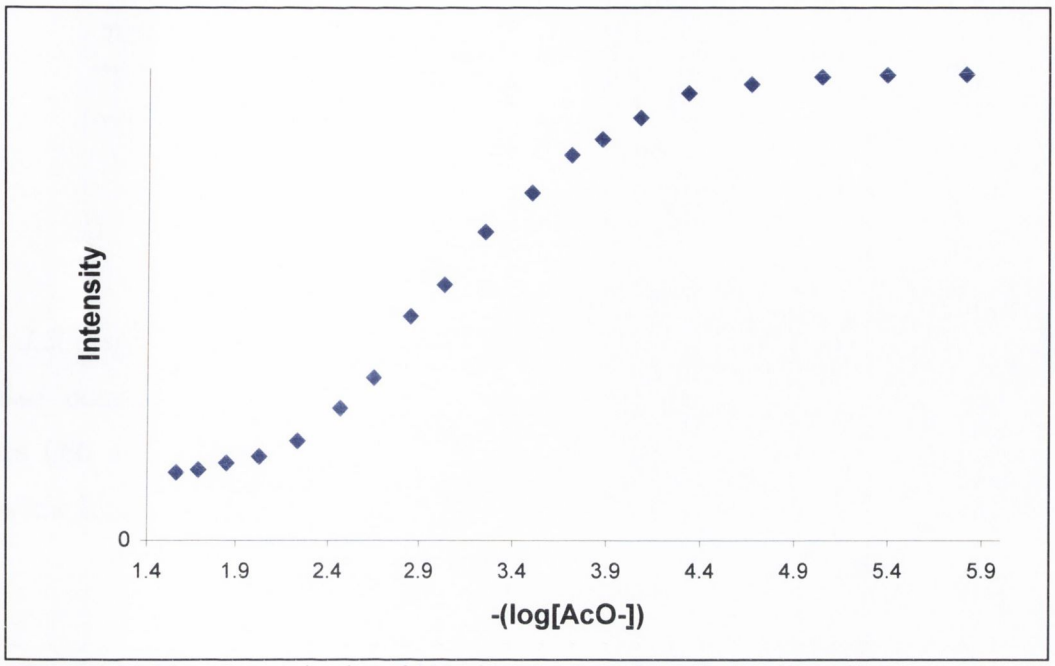


Figure 3.21: Fluorescence titration of **106** with TBA.AcO: Intensity vs –log[anion]

and **96** *e.g. Figure 3.20*. **106** exhibits 2:1 binding for the mono valent anions and glutarate whereas it exhibits 1:1 binding for malonate and $HP_2O_7^{2-}$. *Figure 3.22* displays the fluorescence response of **106** towards all of the anions tested. **106** exhibits relatively stronger binding towards glutarate and malonate when compared to **95** and **96**. However **106** does not show strong affinity towards $HP_2O_7^{2-}$. All of the binding values for the bis sensors are summarised in *Table 3.3*.

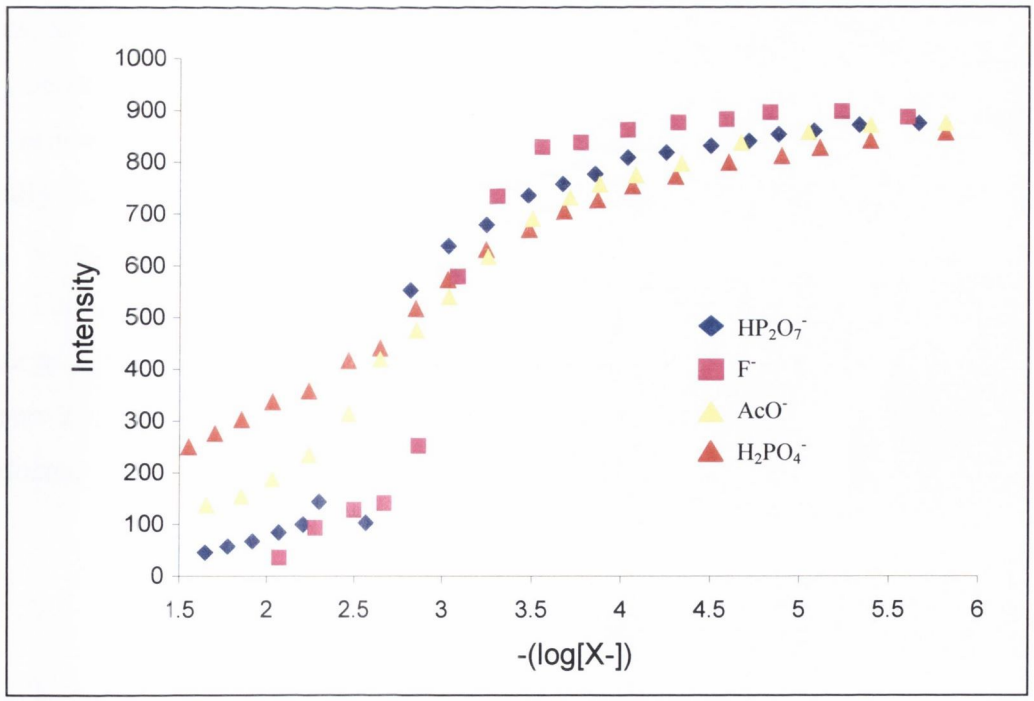


Figure 3.22: Comparison of titration results for various salts with **106**

Table 3.3: Binding constants obtained using fluorescence titrations for bis sensors

Anion	AcO	H_2PO_4	F	Glutarate	Malonate	HP_2O_7
Sensor						
95	3.56	3	4.07	3.74	2.34	3.45
96	2.77	3.45	3.03	3.15	2.02	3.07
106	3	2.2	3.3	3.73	2.66	2.72

Table 3.3 displays the binding values obtained for the bis sensors analysed. At this stage we can also compare the binding ability of the mono and bis thiourea (**65** and **95**) and urea sensors (**66** and **96**). The binding results are displayed in *Table 3.4* whereas the titration curves are illustrated in *Figures 3.23-3.25*.

Table 3.4: Binding constants obtained using fluorescence titrations for thiourea and urea sensors

Anion	AcO	H_2PO_4	F
Sensor			
65	2.55	2.05	3.35
83	2.49	2.1	2.52
95	3.56	3.4	4.07
106	3	2.2	3.3

Table 3.4 clearly illustrates that all the sensors bind fluoride strongest relative to the other anions. The table also illustrates that the thiourea based sensors binds AcO^- stronger than their urea equivalents. It also indicates that **95** is significantly stronger than the other sensors when it comes to sensing $H_2PO_4^-$. More significantly there is a difference of a factor of 10 in binding constants for AcO^- and $H_2PO_4^-$ between **65** and **95**. Even though the binding ratio of sensor: anion is 2:1 for **95** the affinity for the bis sensor is significantly higher. This is potentially due to the fact that by adding an extra binding site the change in entropy upon binding would be smaller relative to change in entropy observed upon binding with the mono sensors. This lower change in entropy would lead to stronger binding according to the Gibbs free energy equation. The next section discusses the results obtained using NMR analysis. As in chapter 2 this technique was used to support the results obtained so far and also provide vital information on the structure of the complex.

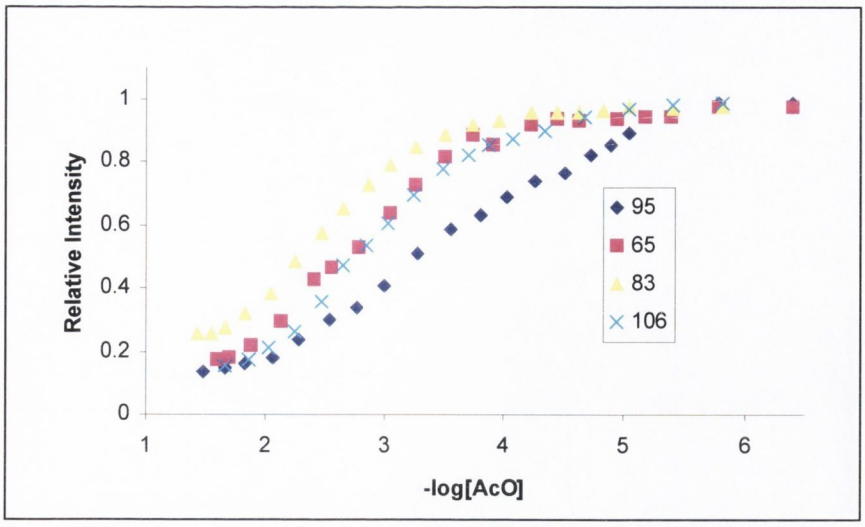


Figure 3.23: Fluorescence titrations of thiourea and urea sensors with AcO^-

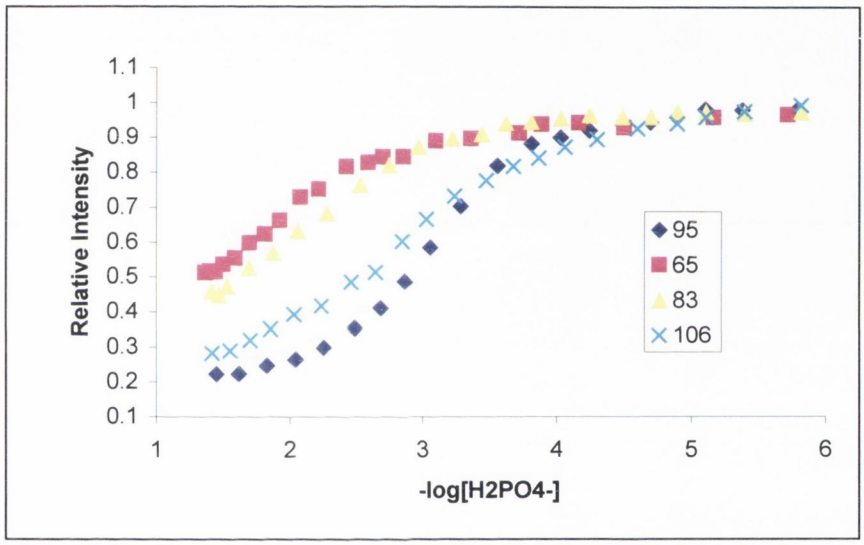


Figure 3.24: Fluorescence titrations of thiourea and urea sensors with $H_2PO_4^-$

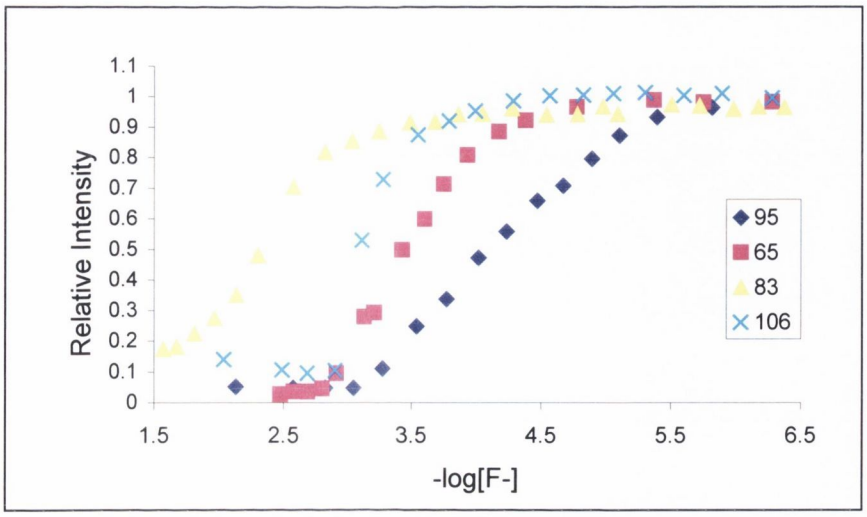


Figure 3.25: Fluorescence titrations of thiourea and urea sensors with F^-

3.4 NMR STUDIES

To investigate the binding interactions in greater detail the anion binding of acetate, phosphate, and pyrophosphate to **95** and **96** was also evaluated by using ^{1}H NMR in DMSO-$_{d6}$. As mentioned earlier the high level of symmetry makes the ^{1}H NMR of **95** and **96** in DMSO-$_{d6}$ very simple, with only two sets of aromatic signals and a single resonance for the –CH_2- spacer. For **95**, the thiourea protons appeared as two resonances at 9.68 and 8.38 ppm,

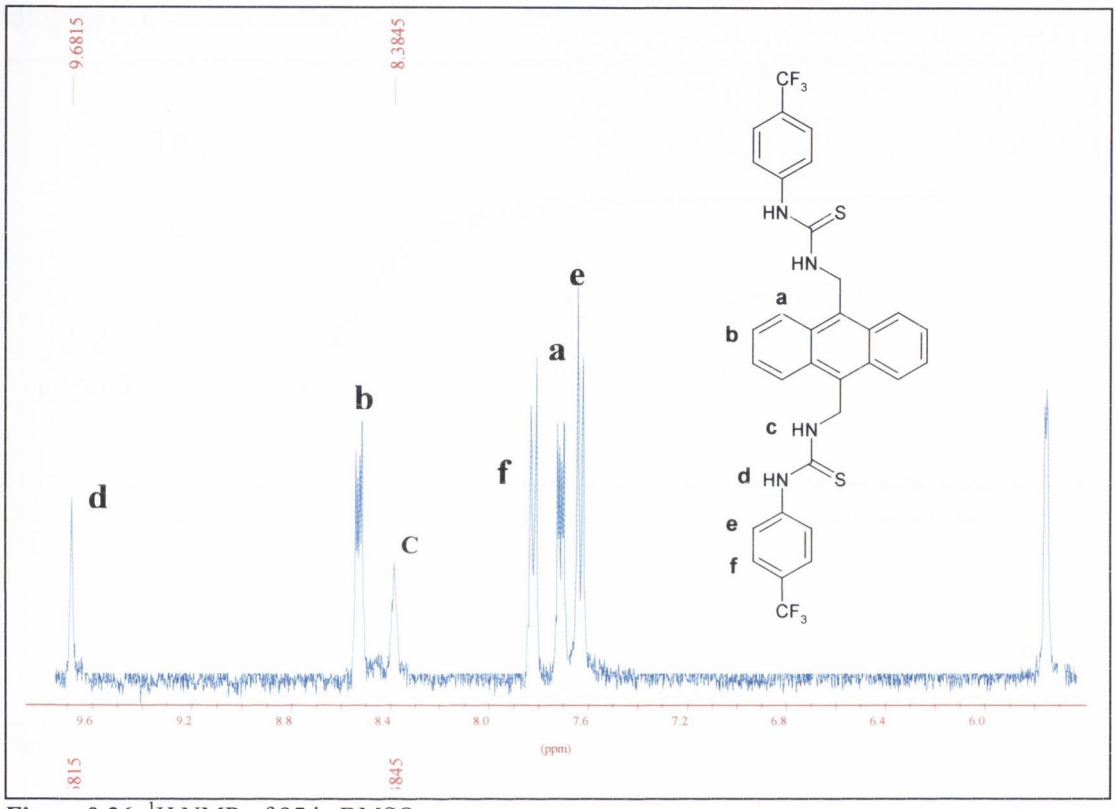


Figure 3.26: ^{1}H NMR of **95** in DMSO-$_{\delta 6}$

respectively (*Figure 3.26*). The position of the thiourea protons changed dramatically upon addition (greater than 3 ppm) of certain anions to a solution of the sensors in DMSO-$_{\delta 6}$. In addition the position and characteristics of the peaks due to the aromatic protons were also monitored. These peaks did not change during titrations of the sensors with the monodentate anions such as AcO^- and $H_2PO_4^-$ (*Figures 3.32* and *3.33* respectively). However significant change was observed upon titration of **95** or **96** with the bis anions *e.g.* $HP_2O_7^{2-}$. Values observed for the sensors using ^{1}H NMR spectroscopy correlate well with log β values obtained using fluorescence data. From these changes, a log β of 3.61(± 0.05) was determined, with a clear 1:1 binding, which is in good agreement with that seen using fluorescence techniques (log β of 3.45). For such recognition to take place, the bis anion would have to bridge the anthracene moiety. This bridging is evident due to the presence of the isosbestic point was observed at 406 nm in the absorption spectra of **95**(*Figures 3.13*).

The ^1H NMR spectra of **95** showed somewhat greater changes for the anthracene resonance's upon titration with pyrophosphate than that seen in the ^1H NMR titration of $H_2PO_4^-$, therefore supporting the theory that the anion bridges across the anthracene ruling out the possibility obtaining the 1:1 ratio *via* two sensors binding to two anions simultaneously. *Figure 3.27* clearly show that the change observed in resonance of the thiourea protons is in good agreement with the theoretical change in resonance and *Figures 3.28* displays a clear 1:1 binding ratio of sensor:anion.

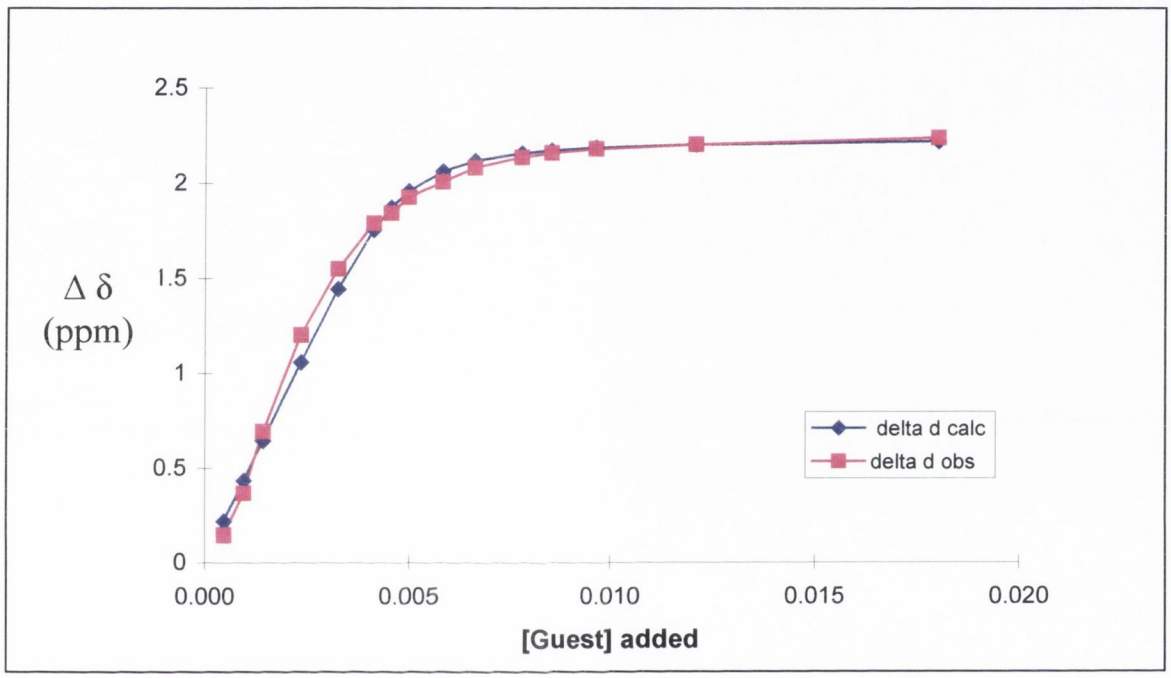


Figure 3.27: NMR Titration of **95** with $HP_2O_7^-$: Calculated fit compared to observed fit

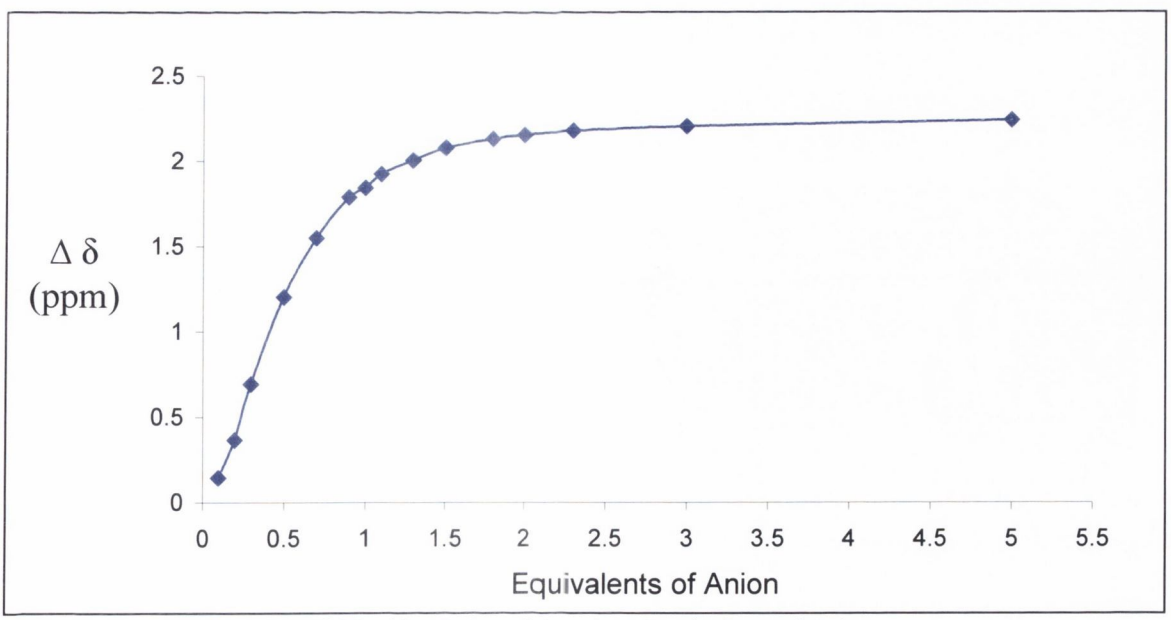
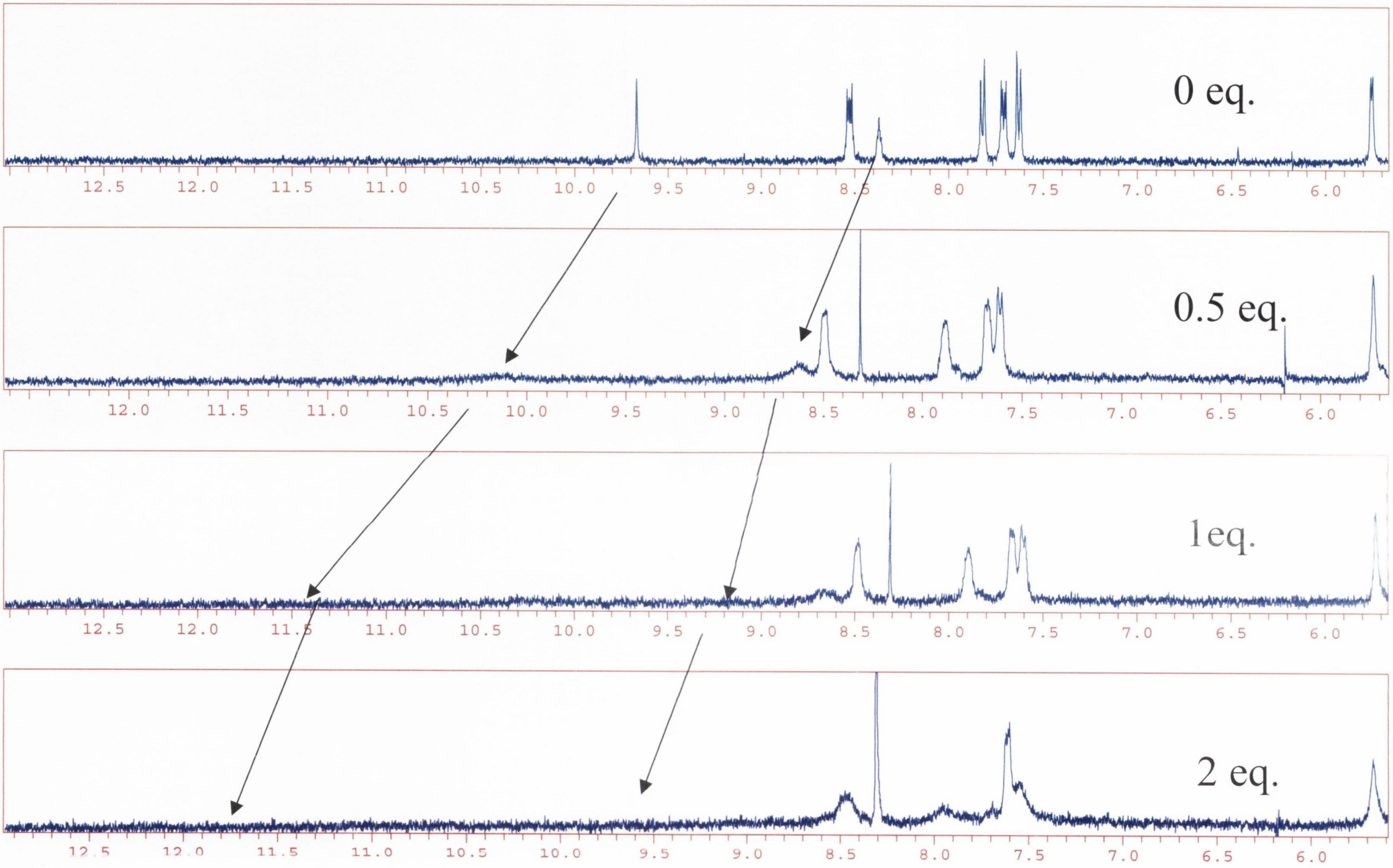


Figure 3.28: NMR Titration of **95** with $HP_2O_7^-$: Δδ (ppm) Vs Equivalents of Anion


0 eq.
0.5 eq.
1eq.
2 eq.
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0
(ppm)

With either $H_2PO_4^-$ or AcO^- (0.5-5 equiv of TBA salts), the thiourea resonances were gradually shifted downfield by >2.5ppm, confirming the formation of anion-receptor complexes. Analysis of the change in the "inner proton" (8.38 ppm) *vs* concentration showed 1:2 binding for both of these anions as seen for AcO^- and $H_2PO_4^-$ in *Figure 3.30 and 3.31* respectively. In contrast to the ^1H NMR spectra obtained for the titration of **95** with pyrophosphate the anthracene peaks and the doublet due to the methylene protons were barely affected during the titration (*Figure 3.32* and *3.33* respectively). These results in conjunction with the results from the fluorescence titrations indicate that the binding of the occurs via bridging of the anion across the anthracene ring whereas binding of the mono valent anion occurs separately at each binding site.

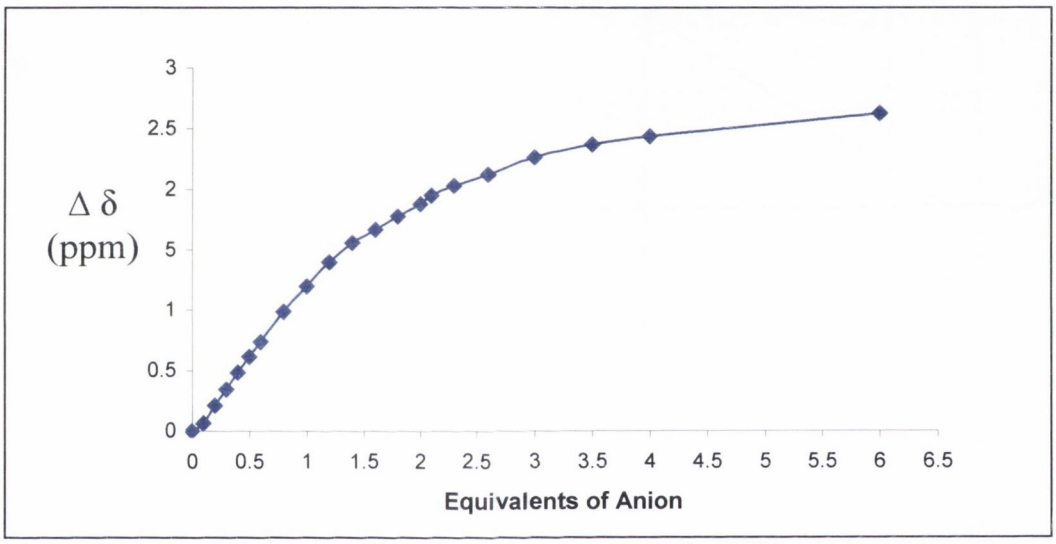


Figure 3.30: NMR Titration of **95** with AcO^-: Δδppm vs Equivalents of Anion

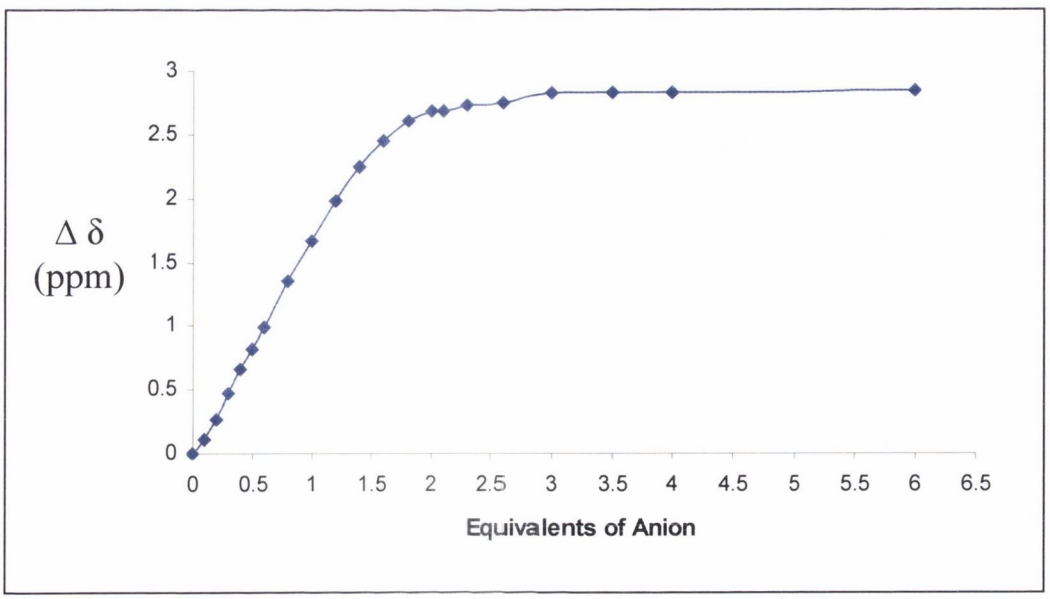
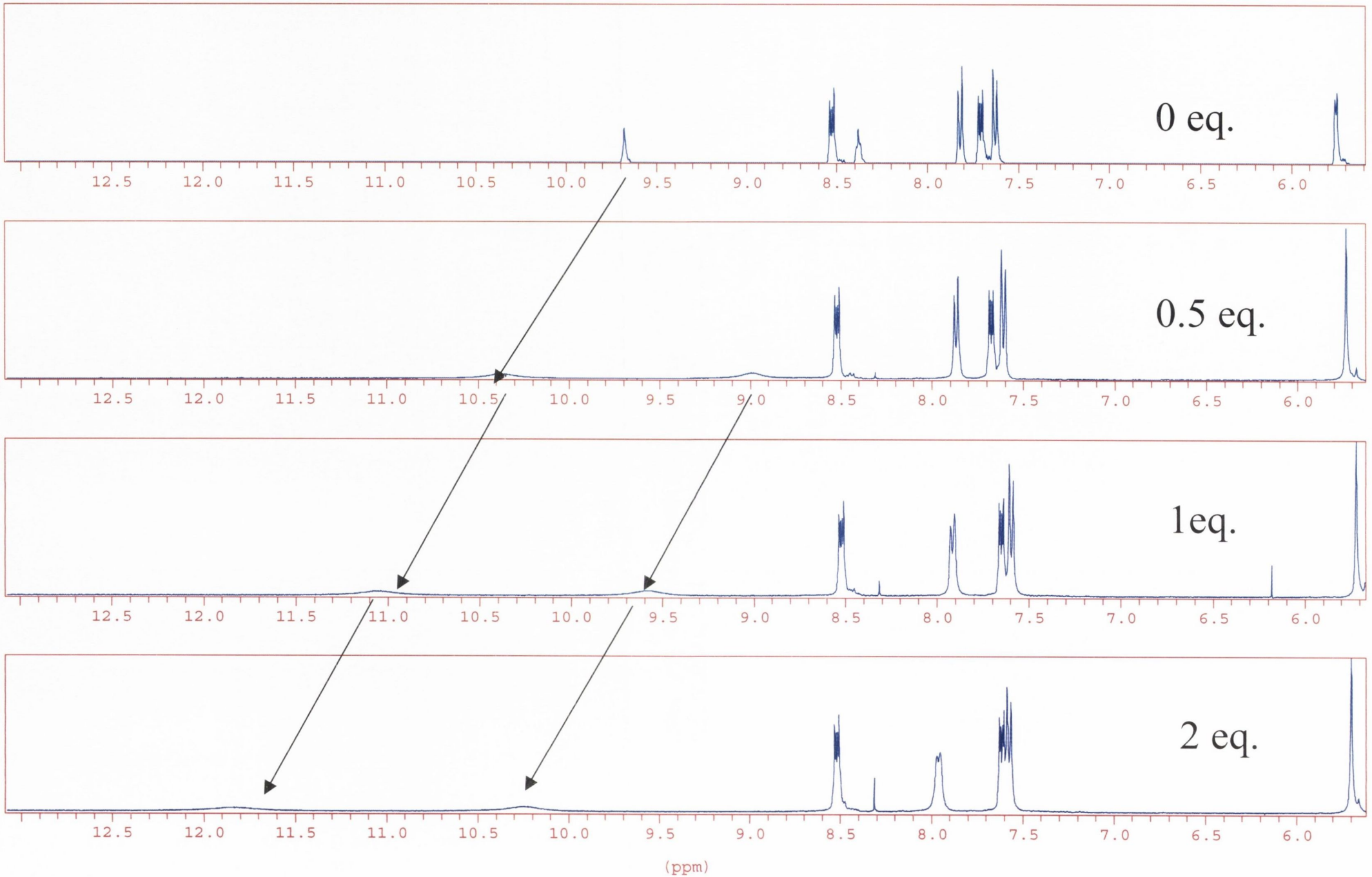
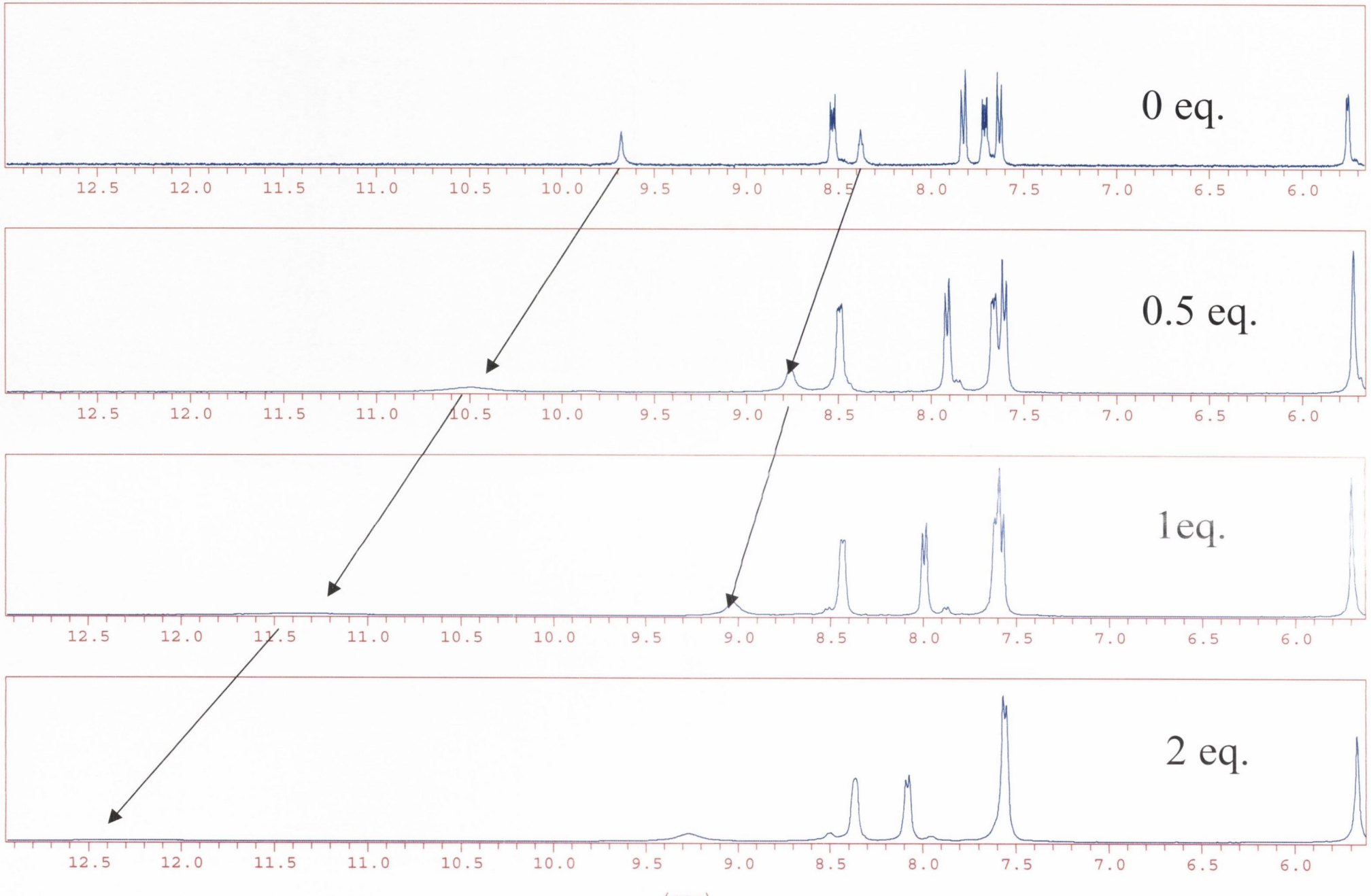
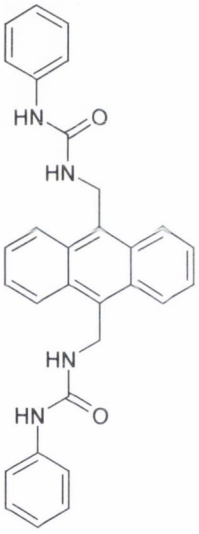


Figure 3.31: NMR Titration of **95** with $H_2PO_4^-$: Δδppm vs Equivalents of Anion


0 eq.
0.5 eq.
1eq.
2 eq.
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0
(ppm)


0 eq.
0.5 eq.
1eq.
2 eq.
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0
(ppm)

The goal of this chapter was to diversify the compounds discussed in Chapter 2 by adding an extra binding site to these sensors with the hope of increasing the binding ability for mono valent anions already tested and also sense biologically important bis anions such as $H_2P_2O_7^{2-}$ and bis carboxylates. In achieving this goal the novel anthracene based bis sensors **95**, **96** and **106** were developed. The results obtained met all expectations in so far as they all exhibited selective sensing of bis anions. Malonate and $HP_2O_7^{2-}$ exhibited a 1:1 binding and a simple equilibrium whereas glutarate, AcO^-, $H_2PO_4^-$ and F^- exhibited a 2:1 binding for all of the sensors. NMR titrations not only confirmed the binding constants but gave significant information on the structure of the anion/sensor complex. This on-going work has been published already and is subject to future publication based on continual research by the Gunnlaugsson *et al.* into the area. As with the compounds in Chapter 2 there is a library of sensors that can be developed in an effort to optimise the affinity and selectivity of a sensor



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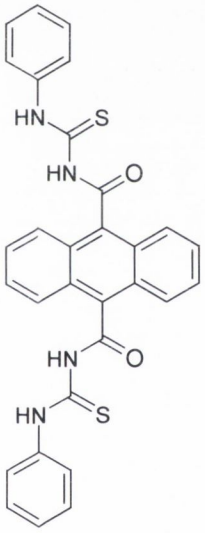


108

towards biologically important anions. **107** and **108** are examples of compounds that have been synthesised are awaiting analysis by fellow members of the Gunnlaugsson group. It is believed that several more sensors are close to development that we hope will be better able to selectively sense some of numerous biologically important bis anions.

CHAPTER 4

CHOLIC ACID BASED ANION SENSORS

4.1 INTRODUCTION

As mentioned earlier in the Chapter 1, numerous attempts have been made to mimic the high binding constants that occur in nature.[114] To date the most successful receptors have been rigid structures. This is explained by the smaller change in entropy exhibited by a rigid host when changing from a free host to a host guest complex. The work in this thesis mentioned so far involves the synthesis and analysis of structurally simple and flexible PET sensors for the detection of anions. The next stage in this project was to try and utilize the findings obtained so far within this thesis and try to syntheise a rigid PET sensor. AP Davis *et al.* have extensive experience in the field of anion binding. They have adopted a strategy based on the steroid cholic acid [115, 116], which provides rigid scaffolding for pre-organised arrays of H-bording functionality. This project is the natural progression of the continuous research conducted by A.P. Davis *et al.* It involves converting an already successful binding system to a fluorescent chemosensor.

4.2 CHOLIC ACID AS A RECEPTOR[117]

Several highly successful receptors designed by Davis *et al* are outlined below. Before discussing these receptors, it is necessary to discuss the structure of cholic acid in a little detail, therefore highlighting the usefulness of such a structure as a potential receptor. Cholic acid has four main functional groups at positions C3, C7, C12 and C24. The carboxylic acid group at C24 and the 3 hydroxyl groups at C3, C7 and C12. The hydroxyl groups are ca. 5.9-6.2 A^{o} apart.[118] These three functional groups are chemically similar, secondary hydroxyl units, but the asymmetric nature of the steroid nucleus allows them to be distinguished. The 3α-OH, being equatorial, may be picked out in a variety of methods, and the two axial hydroxyl groups at C7 and C12 can be differentiated by selective acylation. The acid itself has distinct combination of flexibility and rigidity. The tetracyclic centre gives rigidity and the side chain allows flexibility. A further attraction of cholic acid is its curved profile, which makes it ideal for developing concave receptors.

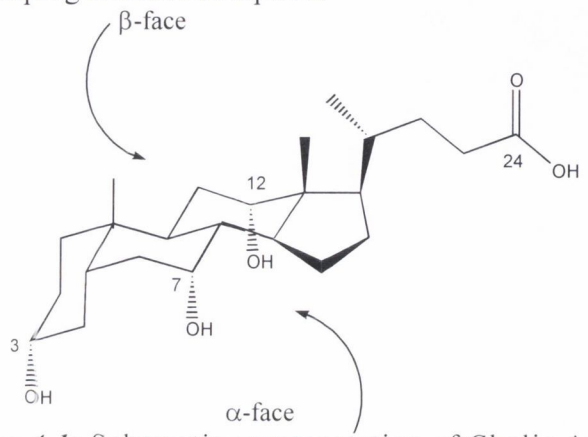
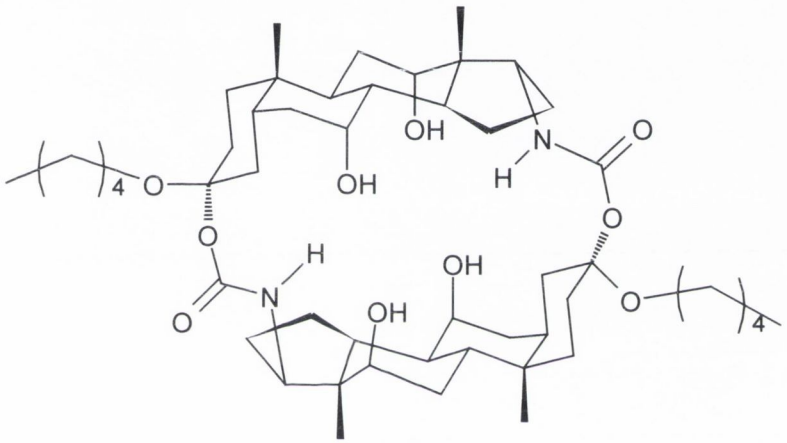


Figure 4.1: Schematic representation of Cholic Acid

Earlier work demonstrated that the methyl ester of the acid can act as a receptor for tridentate oxoanions in hydrocarbon solvents *e.g.* CH_3Cl.[119] Based on these results and molecular modeling studies conducted within the Davis group it was predicted that the codirected hydroxyl groups present in cholic acid may be manipulated to optimise selectivity for binding of anions. These features make the cholic acid structure useful for a variety of applications. Davis *et al.* developed a number of receptors based on the cholic acid unit, for sugars and anionic species. For example, the host **109**, a macrodilactam derived from two molecules of cholic acid. The recognition properties of the receptor towards fluoride, chloride and bromide were analysed using ^1H NMR titrations. This receptor showed selectivity, favouring the smaller more basic anions, such as F^- (K_a = 3220 M^{-1} in $CDCl_3$)[120]



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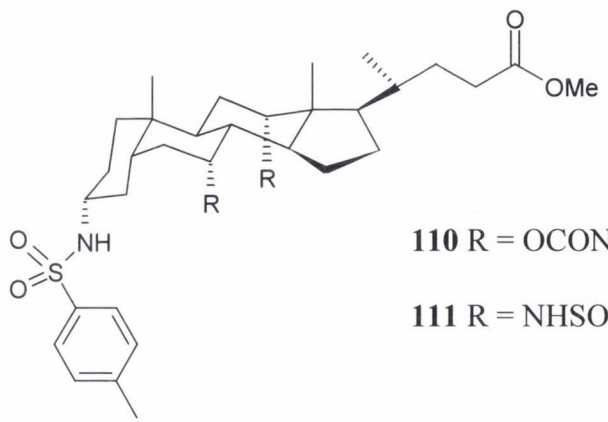
Tripodal receptors have also been developed, **110** and **111**. Anion recognition is achieved in these receptors through, at least three NH- groups containing groups, either sulphonamides or carbamates, attached through steroidal positions C3, C7 and C12.



110 R = $OCONH(3,5\text{-}Me_2C_6H_3)$

111 R = $NHSO_2(4\text{-}MeC_6H_4)$

Table 4.1: Binding constants (M^{-1}) in $CHCl_3$ for Cyclic and tripoidal derivatives of Cholic acid

Receptor	F^-	Cl^-	Br^-	I^-	TsO^-
109	3220 +/- 350	990+/- 80	250+/- 20		
110	15400 +/- 1500	7200+/- 660	7200 +/- 760	930 +/- 70	865 +/- 120
111		92000 +/-280000	9200 +/- 720	525 +/- 45	950 +/- 80

Unlike **109**, receptor **110** and **111** are unable to encapsulate the guest. However this system has the advantage of added versatility. The H-bond donor properties of these receptors may be optimised by tuning the NH acidity as demonstrated in Chapters 2 and 3. Over the last few years Davis *et al.* have synthesised numerous cholic acid derivatives by chemically modifying the functional groups located at positions C3, C7, and C12. Each derivative has obtained higher binding constants and selectivity for different anions *e.g.* **113** for Cl^- and Br^-. Broderick[121] synthesised various derivatives of cholic acid as illustrated below by replacing the three hydroxyl groups with a range of different functional groups with varying electron withdrawing ability with the aim of improving the selectivity of the different sensors towards different anions *e.g.* the halides.

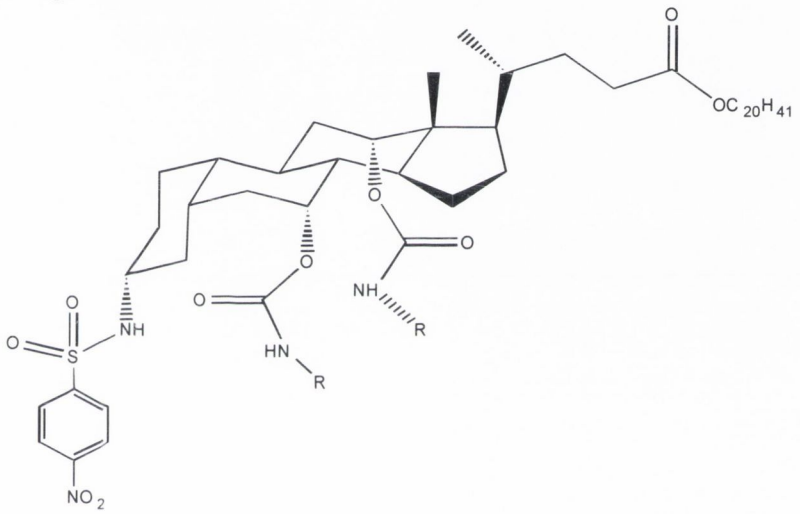


Table 4.2: Binding constants for Brodericks compounds in $CHCl_3$

Compound	R Group	Binding studies (M^{-1})	
		$Et_4N^+Cl^-$	$Et_4N^+Br^-$
112	-Ph-CF_3	$7.4x10^6$	$6.0x10^6$
113	-Ph-NO_2	$4.29x10^7$	$2.64x10^7$
114	$C(O)CH_2Cl$	341,000	128,000

These results show selectivity for chloride over the other anions e.g AcO^-. The binding constant for fluoride was too high to be determined. As mentioned earlier they have synthesized cholic acid derivatives and have studied the interactions of these receptors by NMR spectroscopy. However as their research progressed they produced compounds whose binding constants were too high for NMR to quantify. As described in Chapter 2, NMR spectroscopy is an invaluable technique that is valuable in that it can provide detailed structural information as well as binding constants. However it is limited in two respects. Firstly it relies on expensive equipment. This limits development in certain directions, notably the use of the systems as a basis for anion sensors. Secondly it is relatively insensitive, which

causes difficulties when measuring very high binding constants (*Table 4.2*). This lead to two alternatives;

- Use an efficient extraction method allowing more efficient NMR measurements for the compounds that exhibit very high binding constants
- Incorporate fluorescent moieties onto the deriviatized cholic acid structure which allows fluorescent spectroscopy to be employed as an alternative method for determining the binding constants.

A.Ayling, a co-worker within the Davis group investigated an alternative extraction method using a technique developed by Cram *et al.*[122] This method may be briefly summarised as;
An organic phase containing a liophillic receptor (H) is stirred or shaken with an aqueous phase containing the substrate (M^+X^-). The extraction constant K_{et} is determined from the quantity of substrate extracted into the organic phase at equilibrium. This is expressed in equation the below:

$$\mathbf{K_{ex} = [HMX]_{org} / [H]_{aq}[M^+]_{aq}[X^-]_{aq}} \qquad \mathbf{I}$$

The association constant can then be calculated from the equation:

$$\mathbf{K_a = K_{ex}/K_d,} \qquad \mathbf{II}$$

where $$\mathbf{K_d = [MX]_{org} / [M^+]_{aq}[X^-]_{aq}} \qquad \mathbf{III}$$

In simpler terms, K_d is the distribution constant of the substrate between the two phases in the absence of the receptor. This method is especially useful for the determination of high binding constants because the degree of complexation in the organic phase can be controlled by varying the substrate concentration in the aqueous phase.

However, the aim of this project was to investigate the possibility of attaching fluorescent moieties to the cholic acid structure. It involves converting an already successful binding system to fluorescent chemosensor, therefore providing one with a more sensitive alternative method for calculating the binding constant of a compound. As mentioned in the introduction a fluorescent chemosensor is a compound that incorporates a binding site, a fluorophore and a mechanism of communication between the two. It was suspected that if chromophores are attached to positions C7 and C12 that there would be sufficient orbital overlap of the two chromophores to yield distinct optical properties. This orbital interaction leads to what is known as excimer or exicplex formation. An excimer is orbital interaction of the excited state and ground state of two identical chromophores. Whereas an exciplex is

interaction of the excited state and ground state of two different chromophores. Since our initial investigations were conducted using identical chromophores (*Figure 4.3*) on positions C7 and C12 this project is solely concerned with excimers. Exciplex formation using different chromophores was not investigated in this project. The theory of excimers is best illustrated below (*Figure 4.2*). The excited state of one fluorophore interacts with the ground state of another. Since energy is inversely proportional to wavelength, the lower energy emitted by the excimer will appear at a longer wavelength.

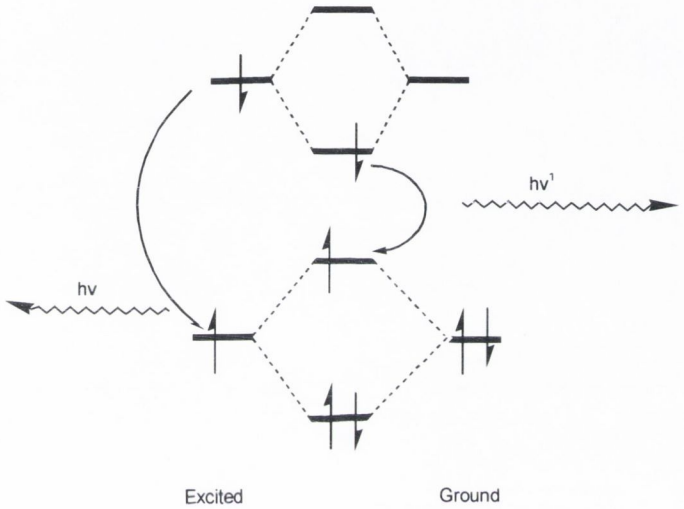
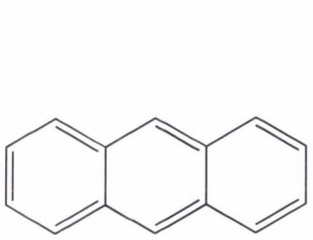


Figure 4.2: Schematic representation of excimer formation

The anthracene ring is an efficient fluorophore and the dual fluorescence emission (monomer and excimer) is particularly suited to reflect intramolecular interactions in the excited state.[123] There are two potential outcomes of synthesising the cholic acid derivatives containing two chromophores: the excimer may only be formed upon binding or it may already exist in the free host. If the excimer was only formed upon complexation it should have been relatively easy to identify and quantify the binding. However if the excimer exists with the free host it would be possible to quantify binding by determining the change in intensity of the emission spectra.



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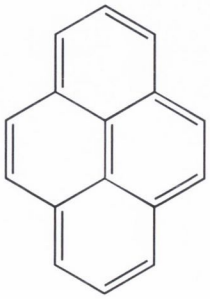


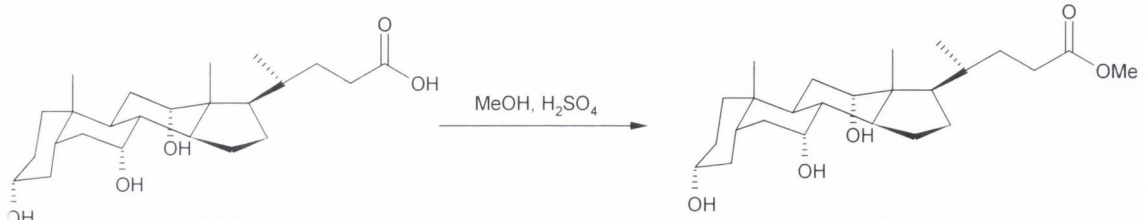
116

Figure 4.3: The chromophores to be attached to the steroidal backbone: **115** (Anthracene), **116** (Pyrene)

Additionally, another possibility is to attach a chromophore to the C3 position of the steroid and monitor the effects on fluorescence upon binding. This moiety may result in the formation of excimers if the distance between the two chromophores (position C3 and position C7 or C12) was small enough to allow orbital interaction. Lehn *et al* developed an octamine cage, **31**.[124] The hexa-protonated cage was poorly fluorescent when void, the acridine monomer emission was almost completely quenched due to the formation of an excimer band. In contrast, the addition of anions, caused a dramatic increase in the fluorescence (by a factor of up to 27 depending on the anion present). The introduction of a substrate into the cage prevented excimer formation resulting in a revival of monomer emission. The revival of the monomer emission band which arises from both conformational changes and specific electronic interactions with the substrate, allowed the determination of high stability constants (K_a ranging from 10^3 to 10^7 M^{-1}) displayed in *Table 1-4,* Chapter 1. Teramae also reported another particularly simple self assembly system for sensing anions, with a pyrene functionalised mono-guanidinium receptor, **46**. This did not show any obvious changes in the fluorescence spectra upon addition of $H_2PO_4^-$ as well as other monovalent anions such as AcO^-, Cl^- and Br^-. However in the presence of pyrophosphate a structureless band appeared with an emission maximum @ 476 nm appeared, and there was quenching of the monomer emission ($K_a = 3.4 \times 10^4\ M^{-1}$).[125]

4.3 Synthesis

The initial synthetic work with the cholic acid steroid is very similar to the synthesis carried out by colleagues within the Davis group. However each researcher within the group explored many different synthetic avenues and applications of cholic acid derivatives. It is the introduction of the chromophores to position 7 and 12 that distinguish the research. Within this research it was set out to optimise the existing procedures or derive alternative, more efficient methods for producing key intermediates, as well as designing novel fluorescent chemosensors. Our first approach to synthesise these compounds is the protection of the carboxylic acid group. This involves esterfication of the acid moiety to yield methyl cholate **118**. This compound was the backbone of all the derivatives that were synthesised. Simply by



117 **118**

Scheme 4.1: Synthesis of Methyl Cholate

stirring a solution of cholic acid, **117** and a catalytic amount of sulphuric acid in methanol the carboxylic acid was protected as an ester. The average percentage yield for this reaction was 92%. The resulting product, **118** contained three hydroxyl groups at positions C3, C7, and C12 that can be individually manipulated to introduce the desired selectivity. Due to the high molecular weight of each of the steroid derivatives , they char light brown on TLC plates. This enabled the progress of each of the reactions to be monitored very easily. In addition though the ^{1}H NMR of these compounds are very complex relative to the compounds sythesises in Chapters 2 and 3, ^{1}H NMR can also be used relatively easily to determine whether a reaction worked or not. The spectra of **118** (*Figure 4.4*) is used as a reference for the other compounds synthesised and the key intermediates. The ^{1}H NMR of each compound displays a steroidal backbone of peaks ranging from 0.7-2.2ppm. There is no significant change within this region for all the derivatives synthesised. The main distinguishing feature of each derivative is the location of the C3, C7 and C12 β hydrogens. The location and integral value of these peaks gives an indication of whether the reaction was successful or not. The C3β H is most upfield and normally in the form of a multiplet. This peak is followed by a singlet at 3.68 ppm, due to the presence of the methyl protons from the methyl ester. Finally the C7 and C12 βH's are at 3.88 and 4.01 ppm respectively. The position of the aforementioned peaks are monitoring closely after every reaction.

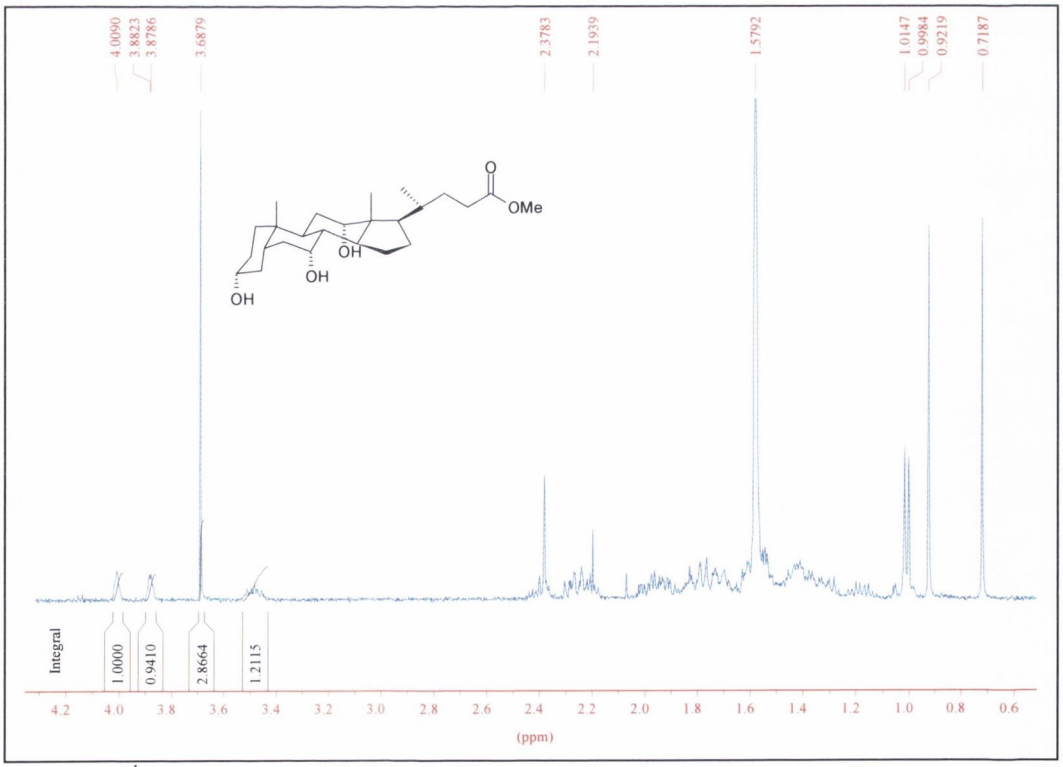
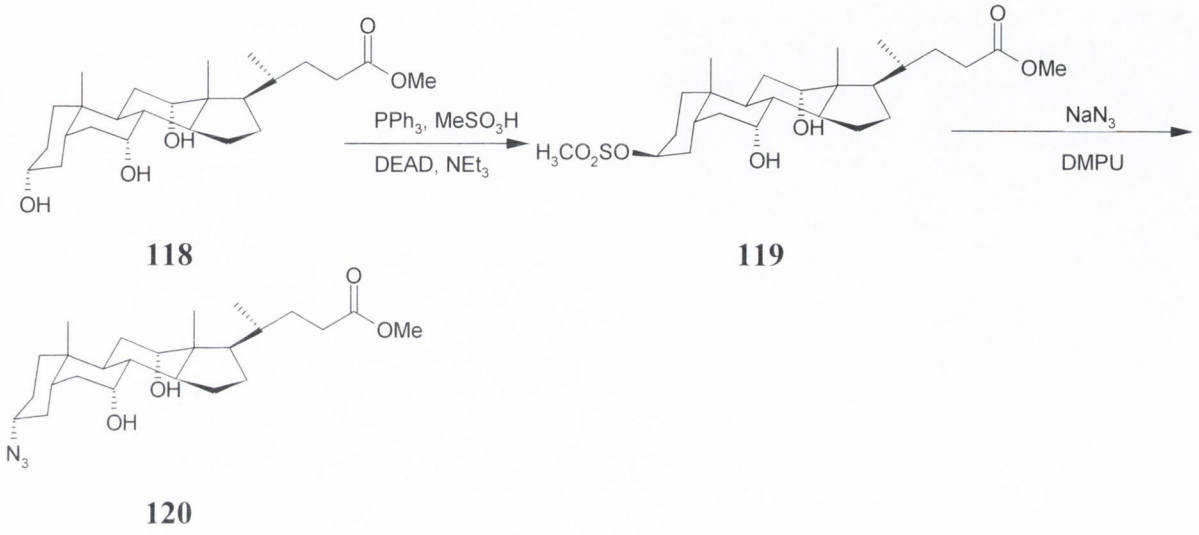


Figure 4.4: ^{1}H NMR spectra of **118** in $CDCl_3$

Once **118** is obtained the synthetic procedure can take one of two routes (scheme 4.2 and scheme 4.11). Scheme 4.2 involves reacting the C3 position to form the desired functional group while scheme 4.11 involves protecting C3 and reacting the C7 and C12 positions preferentially. Scheme 2 is discussed towards the end of this chapter.



118 **119**

120

Scheme 4.2: Synthesis of the azide derivative of the cholic acid steroid, **120**

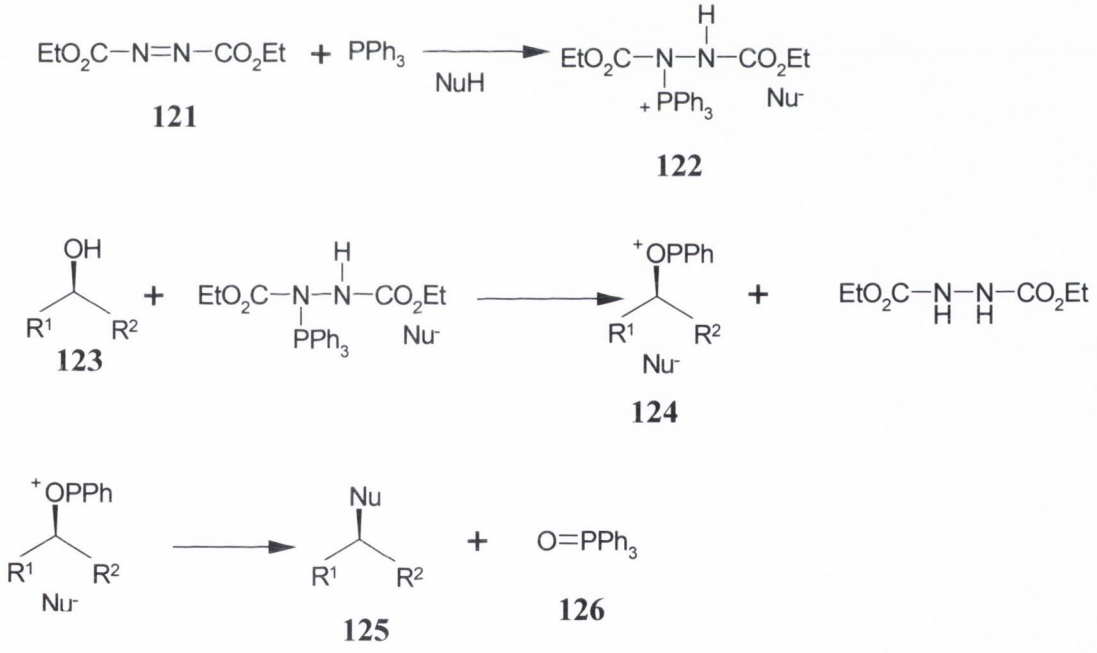
4.31 SCHEME 4.2

The Mitsunobu step was carried out according to the method of Davis *et al.*. However the reproducibility of this reaction was found to be problematic. Therefore each of the parameters was examined in detail so that this pivotal step would be more reliable and efficient. The parameters examined were:

- order of addition,
- rate of addition,
- temperature and
- reaction time.

Due to the fact that the slightest amount of moisture present would inhibit the reaction, all of the raw materials were vigorously pre-dried.[126] A series of experiments were designed varying each parameter. The optimum conditions were based on the average percentage yield for each parameter. The optimum conditions found were, stir at 44° C for 48 hours.

The best order of addition appeared to be dissolving the steroid and the triphenylphosphine in THF, then adding the methane sulfonic acid and triethylamine. The addition of materials was completed by the dropwise addition of DEAD. The rate of addition was found only to be significant for DEAD. If the material was added to fast the reaction did not work at all. Despite the rate of addition appearing to be insignificant for the other raw materials it was noted that a significant increase in percentage yield (>10%) was obtained if there was a delayed time period (approximately 15 minutes) between the addition of triethylamine and the DEAD. It also should be noted that when this time delay before the addition of DEAD was in place the reaction *always* worked. The only possible explanation for this is to allow a pre-equilibrium to be established between the other raw materials. These results at first glance do not coincide with the mechanism proposed within the literature.[127] The mechanism proposed involves DEAD, **121** first forming an equilibrium with PPH_3 and the methane sulphonic acid. The resulting complex, **122** then reacts with the secondary alcohol **123**, in our case the hydroxy group of **118**. The product of this reaction quickly undergoes

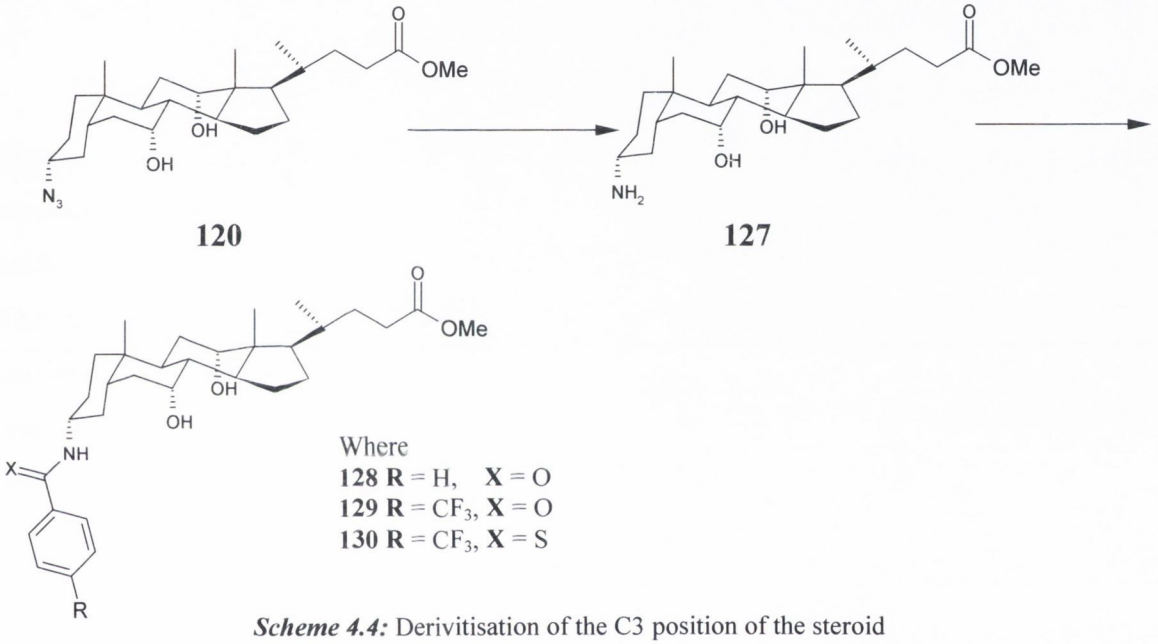


Scheme 4.3: Mitsunobu mechanism

a nucleophillic substitution with methane sulphonic acid to produce the desired product. However we found that the DEAD must be added last in order for the reaction to work. However Iimori and co-workers published work that supports both the mechanism above and the findings from our research. Iimorri *et al* found that when no nucleophile is added to Mtsunobu reactions, elimination can occur to produce alkenes.[128]

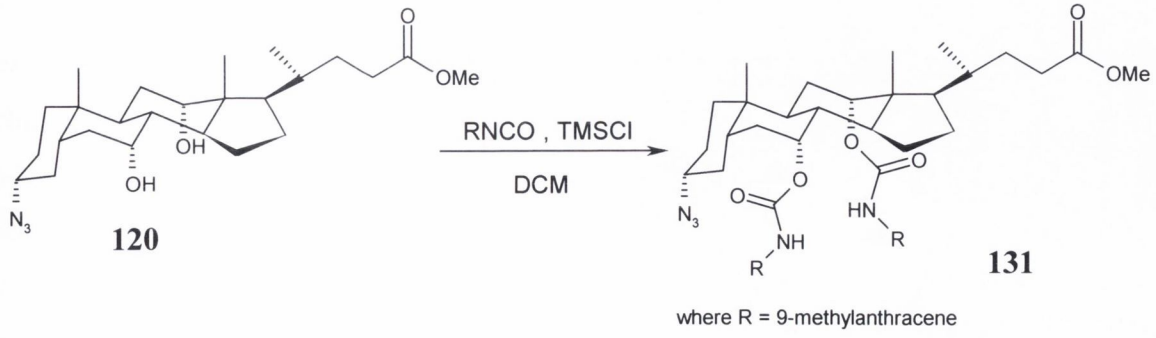
The second inversion involves the formation of the azide, **120.** This step is straight forward in terms of the reaction mechanism. To obtain the desired stereochemistry the mesylate, **119** is

subject to an S_N2 reaction resulting in a 3α azide, **120** being produced. The yield of the reaction was found to be dependent on the reaction temperature and on the moisture content of the raw materials. To avoid the mesylate reverting to methyl cholate all raw materials were pre-dried. Since the azide can act as a protecting group for the C3 position the reaction pathway can now follow one of two routes; a) further derivitisation of the C3 position as outlined in the scheme below or b) develop position C7 and C12. It is thought that the introduction of further groups at position C3 would inhibit reactions at positions C7 and C12 by increasing the steric hindrance factor. Therefore it



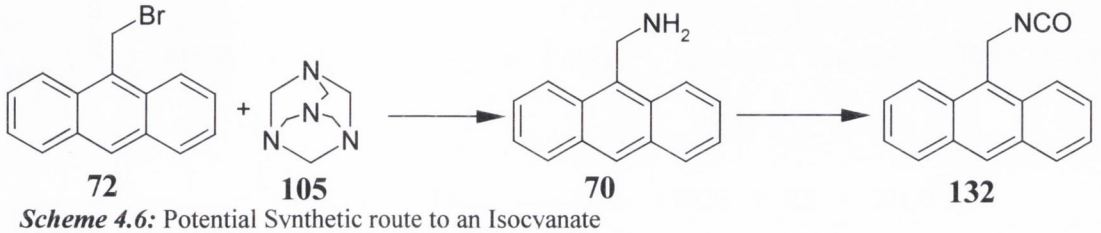
Scheme 4.4: Derivitisation of the C3 position of the steroid

was decided to react positions C7 and C12 at this stage. The carbamates group at the C7 and C12 position may be introduced by one of two ways. Firstly through a reaction of **120** with 9-anthracene methyl isocyanate or secondly by reaction of 9-aminomethylanthracene with a



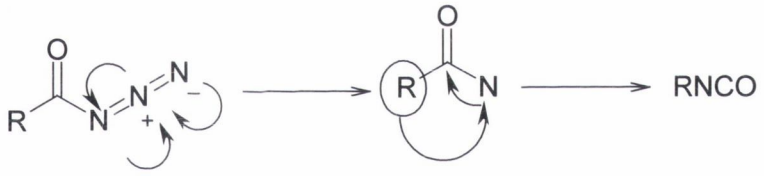
Scheme 4.5: Carbamate sensor via Isocyanate route

carbonates derivative of cholic acid. Firstly we shall discuss the feasibility of the isocyante route. Previous systems have been developed by the Davis group using the 4-nitrophenylisocyanate and 4 trifluoromethylphenylisocyante. However as the size of the substituent increases the steric effect became more dominant and the yields were reduced. [129] Since the 9-methylanthracene isocyanate was not commercially available, it was necessary to design a synthetic route. Several synthetic routes were investigated.



Scheme 4.6: Potential Synthetic route to an Isocyanate

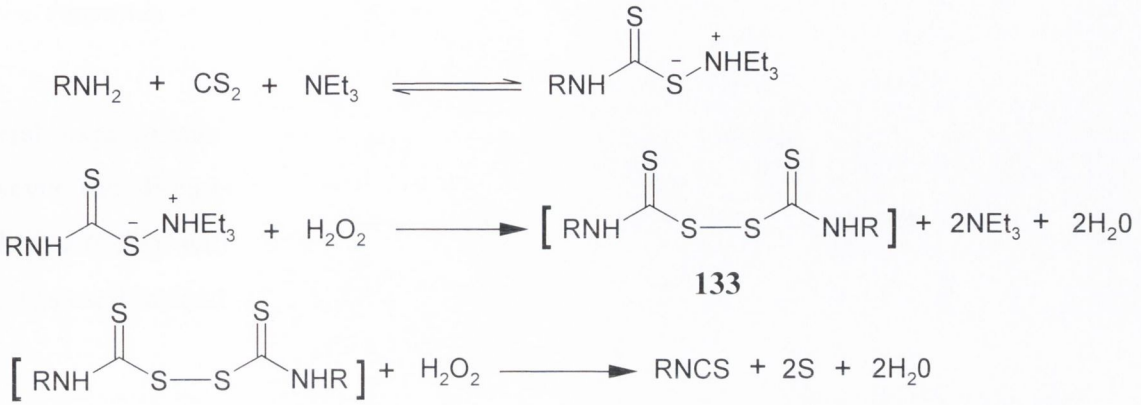
Firstly, the Curtius rearrangement was investigated. The Curtius rearrangement involves the rearrangement of acyl azides with heat as illustrated in *scheme 4.7*. The synthesis of the acyl azide was attempted by stirring the 9-anthracenecarboxylic acid with NaN_3 and CF_3COOH. The resulting product was then heated in toluene over night to yield the isocyanate. This method was repeated several times but with no success. The Schmidt and Lossen rearrangements react via a similar mechanism but the difficulty in isolating the isocyanate is well documented.[130]



Scheme 4.7: Mechanism for the curtius rearrangement

Attempts were also made to synthesise pyrene isothiocyanate, as 1-pyrene methylamine is commercially available. The use of hydrogen peroxide as a dehydrosulfurization reagent in the preparation of alkyl isothiocyanate was first reported by Johar *et al.* in 1970.[131] In their experiments, isothiocyante was made in one step by reacting hydrogen peroxide with a mixture of primary amine and carbon disulfide in the prescence of a secondary aliphatic amine, acting as a base. No heating was required and the total reaction time was less than 10 minutes. Although it was simple and very economical the yield was only moderate (~40%). The yields were even lower for aromatic isothiocyanates. Li *et al* developed a modified method that was suitable for aromatic isothiocyanates.[132] The isothiocyanation is proposed to pass through thiuram disulfide, **133** as shown in the equations

below. We used this method to synthesise the 1 pyrene-methyl-isothiocyanate. To a stirred suspension of the 1-pyrenemethylamine hydrochloride in dry THF was added 2 equivalents of Et_3N. The mixture was cooled in ice cold water and 1 equivalent of CS_2 was added. After 30



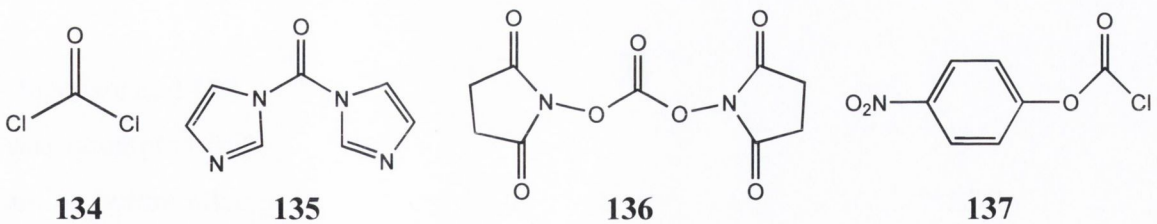
Scheme 4.8: Synthesis of 4-phenol isothiocyanate *Where R = p-HOC_6H_5*

minutes of stirring at 0 °C H_2O_2 was added dropwise. The ensuing solution was neutralised with HCl and evaporated to dryness. The resulting solid was washed with ethyl acetate and dried under vacuum. However this product proved to be insoluble in most solvents. Consequently a complete analysis of this product and indeed further use of this product was very restricted. Currently co-workers within the Gunnlaugsson group are investigating whether this method is suitable to use with fluorescent amines *i.e.* **70** and **93**. Concurrently to the isothiocyanate experiments, alternative routes to the carbamate sensors via the synthesis of carbonate derivatives of cholic acid were investigated.

The rigidity and size of the cholic acid back bone to the steroid introduces the problem of steric hinderance. Reaction of this derivative with the chromophoric isocyanate magnifies the steric hinderance of the reaction system. In order to overcome this potential problem an alternative synthetic route was investigated. This route involved the formation of a reactive carbonate intermediate.

4.32 Carbonate Derivatives of Cholic acid

Initial attempts to synthesise the di-carbonate were made by using a matrix of experiments. This matrix was designed in order to obtain the optimum starting materials and conditions to synthesise a key intermediate product. The matrix consisted of four solvents (Tetrahydrofuran, acetonitrile, toluene and dichloromethane), three bases (NEt_3, DMAP, and DBU) and three phosgene related compounds (*Figure 4.5*).

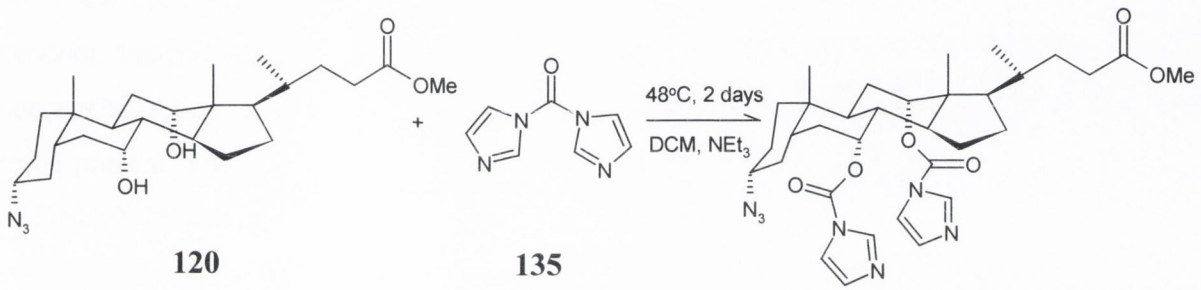


134 **135** **136** **137**

Figure 4.5; **134**) phosgene, **135**) Carbonyl Di-Imidazole

136) N,N' Di-succimidyl Carbonate , **137**) 4 p-nitrophenylchloroformate

Several experiments resulted in the mono-substitued carbonate with varying efficiency. However the di-carbonate derivative of cholic acid was made by refluxing carbonyl di-imidazole (CDI), with the diol steroid **120**, in DCM for 48 hours in the presence of DMAP. CDI was used instead of phosgene for practical safety reasons



120 **135** **138**

Scheme 4.9: Azido bis carbamate derivative of the steroid

The ^{1}H NMR spectra shows little change in the skeleton of the steroid located between 0.7 and 2.4 ppm. However the dicarbonate is characterised by the significant downfield shift of the C7 and C12 β hydrogens (*Figure 4.6*). In addition to this dramatic shift there is also new peaks due to the imidazole protons of the product at 7.21, 7.24 and 8.20 ppm.

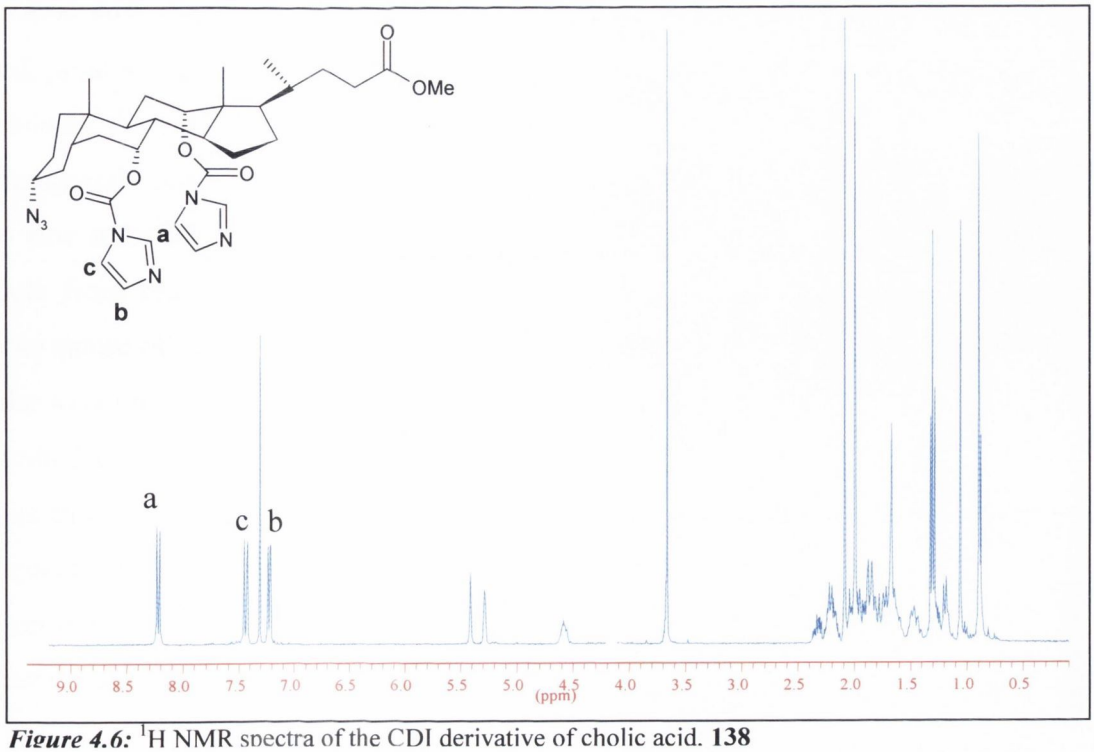
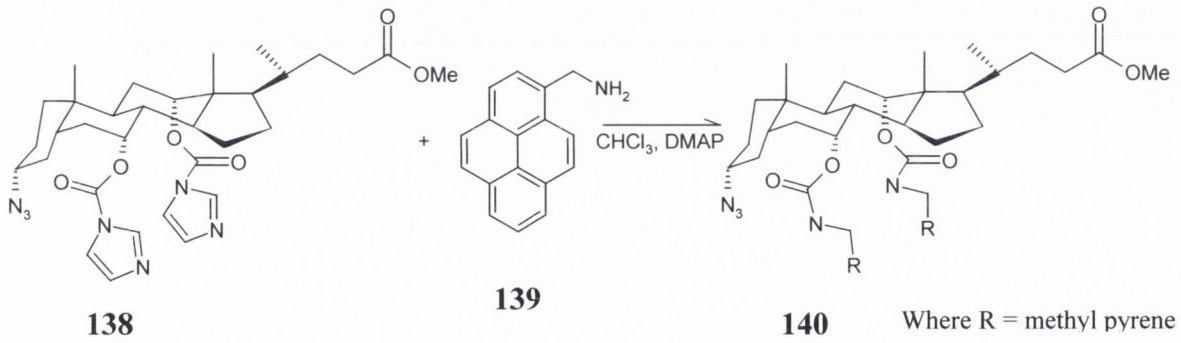


Figure 4.6: ^{1}H NMR spectra of the CDI derivative of cholic acid. **138**

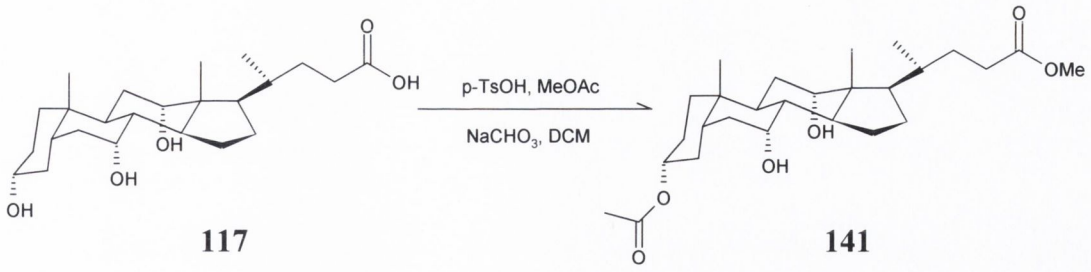
Once isolated **138** was reacted with 2 equivalents of benzyl amine in dry $CHCl_3$. This reaction was attempted initially in order to verify whether **138** would react with a relatively simple amine before attempting the synthesis with 1-pyrenemethylamine, a more sterically hindered amine. This reaction was used as a model system. The reaction mixture was refluxed over night in dry $CHCl_3$. Excess solvent was removed under reduced pressure. The resulting product was analysed by TLC and ^1H NMR. Both TLC and NMR showed at least 3 products, possibly mono substituted carbamates at positions 7 and 12 respectively and some of the desired bis carbamate product. Isolation of the individual fractions proved to be difficult and not too much time was spent on this isolation procedure as this product was insignifcant once we knew the reaction occurred. Therefore no further attempts were made to synthesise this product. Instead **138** was reacted with 1-pyrenemethylamine. However this reaction was not successful. After 48 hours of reflux in dry $CHCl_3$ TLC analysis only showed the raw materials to be present. The procedure was also repeated in toluene, CH_3CN and DCM in the prescence of DMAP. However the product was not



138 **139** **140** Where R = methyl pyrene

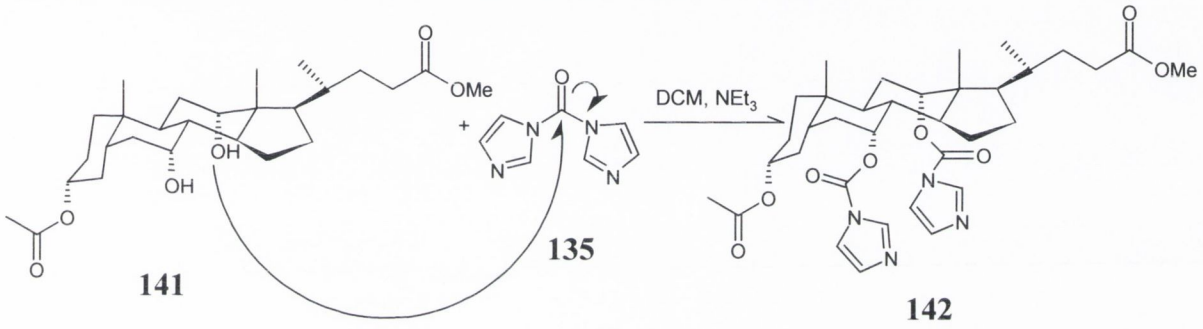
Scheme 4.10: Attempted Reaction of carbamate derivative of the steroid, **138** with 1-pyrene methyl

formed, possibly due to the steric nature of both the steroid and the amine. This procedure for synthesising the carbamate was novel and still must be optimised. In an effort to find the optimum conditions for this compound allot of very valuable raw materials were consumed. These raw materials were valuable not in monetary terms but in the fact that they were products from reactions themselves, taking over 7 days to make and purify. Due to the unstable nature of the mesylate product **119**, it was only made on a small scale at anyone time. In order to counteract this restriction synthetic routes were investigated where this step could be avoided until after the production of the carbamate derivative of the steroid. The most obvious choice was to protect the C3 position of methyl cholate with acetic acid. This allowed subsequent reactions to be carried out on positions C7 and C12 without the initial preparatory steps on the steroid. The same conditions were used to synthesise the carbamate derivative whether the azide or acetoxy group was at position C3 of the steroid.



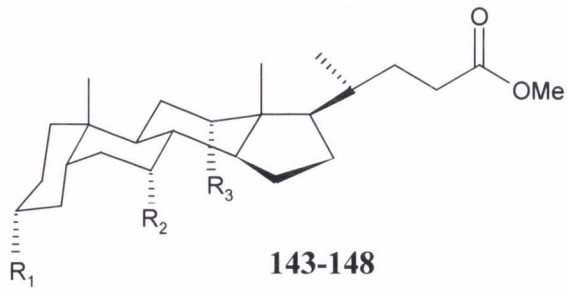
Scheme 4.11: Synthesis of **141**

It should be noted that significantly better yields (>10% difference on average) were obtained when the acetate diol, **141**was used as a starting material in preference to the azide, **120**. The carbonate reaction follows the mechanism outlined below in *scheme 4.12*.



Scheme 4.12: Mechanism of synthesis for **142**

Nevertheless the subsequent reaction of the steroid, **35** with 1-pyrenemethyl amine was again unsuccessful. Simultaneously to the aforementioned investigations Davis *et al* developed numerous other derivatives utilising the cholic acid backbone. As mentioned earlier every researcher in the Davis group working on the cholic acid developed numerous derivatives each for different applications. Whilst undertaking this work, colleagues within the Davis group investigated the binding ability of urea and thiourea derivatives of cholic acid. [133]

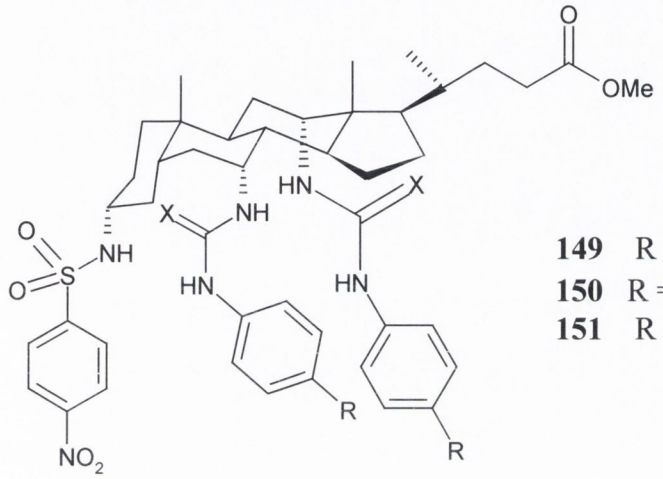


143-148

143	R_1=	NH_2	R_2=	OH	R_3=	OH
144	R_1=	OH	R_2=	NH_2	R_3=	OH
145	R_1=	OH	R_2=	OH	R_3=	NH_2
146	R_1=	NH_2	R_2=	OH	R_3=	NH_2
147	R_1=	OH	R_2=	NH_2	R_3=	NH_2
148	R_1=	NH_2	R_2=	NH_2	R_3=	NH_2

These receptors exhibited a remarkably stronger affinity for halides especially chlorides and bromides. The binding values obtained for the urea and thiourea receptors were significantly larger than those obtained for those receptors containing the carbamate groups (from 100 to

10,000 times larger). This dramatic difference in binding constants may be explained due to the prescence of the urea and thiourea receptor sites which contain two H-bonds which can be formed at positions C7 and C12. The higher acidity of the urea and thiourea protons with respect to their carbamate counterpart would also account for some of the difference in values. This dramatic difference in binding constants forced a change in direction of this project.

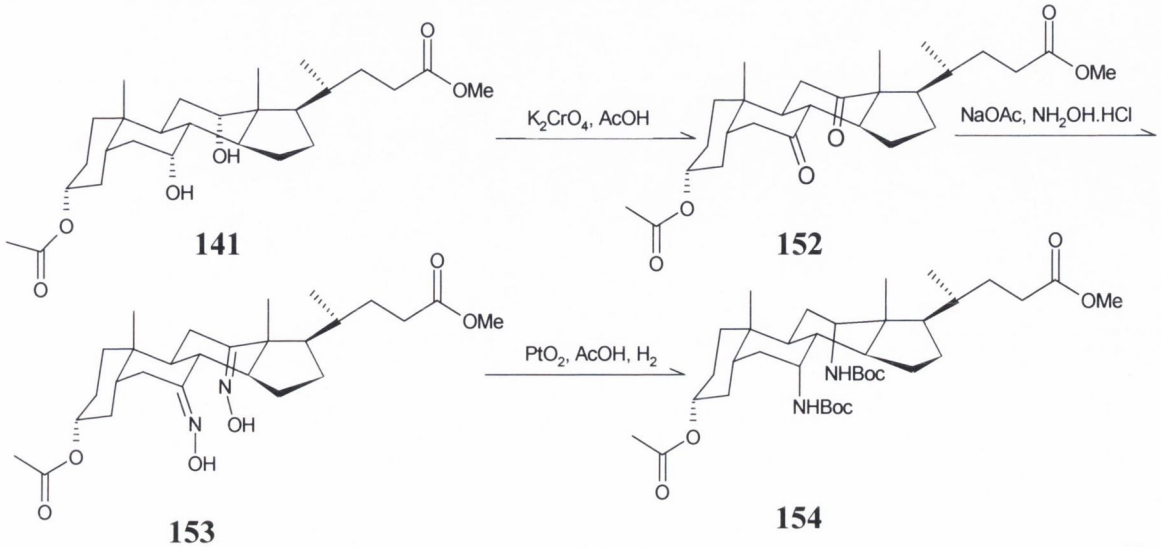


149 R = CF_3 and X = O
150 R = NO_2 and X = O
151 R = NO_2 and X = S

Table 4.3: Binding constants for thiourea receptors in $CHCl_3$

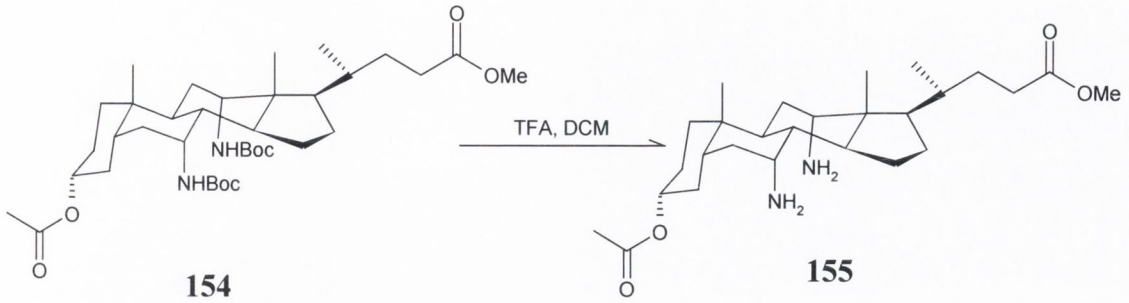
Compound	*Binding studies (M^{-1})	
	$Et_4N^+Cl^-$	$Et_4N^+Br^-$
149	$4.58x10^9$	$2.63x10^9$
150	$6.60x10^{10}$	$1.68x10^{10}$
151	$1.03x10^{11}$	$2.59x10^{10}$
112	$7.4x10^6$	$6.0x10^6$
113	$4.29x10^7$	$2.64x10^7$

Instead of trying to synthesise fluorescent carbamate derivatives of cholic acid it was decided to focus on the design and synthesis of fluorescent urea and thiourea derivatives of cholic acid. It was decided to protect the C3 position from the start and concentrate our attention to placing fluorescent thioureas at positions C7 and C12 with the idea of still utlising the phenomenon of excimer emission, mentioned earlier.



Scheme 4.13: Synthesis of bis NHBoc intermediate of the steroid

The methods used to synthesise **152 – 154** were developed by a co-worker in the Davis group.[134] **154** was reduced to the bis-amine **155,** using trifluoroacetic acid, a well known method for BOC deprotection. Once isolated **155** was then to be reacted with fluorescent isocyanates and isothiocyanates.

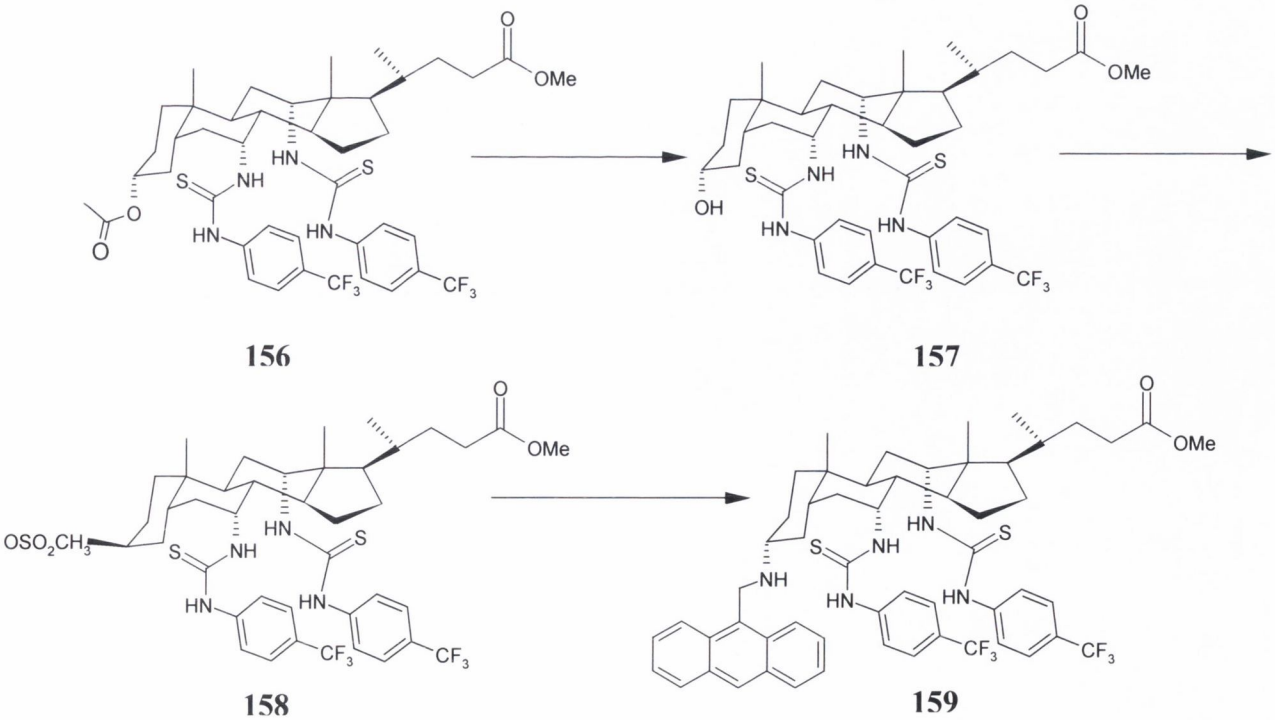


154 **155**

Scheme 4.14: Deprotection of bis NHBoc intermediate of the steroid

Therefore the final step with the carbamate and with the thiourea sensors involve the use of fluorescent isothiocyanates or isocyanates. This is why, as mentioned earlier, continual research is being conducted to synthesize fluorescent isocyanates and isothiocyanates. Once synthesized the mechanism of the final step is the same regardless of whether the target molecule is a urea, thiourea or carbamate. Due to the presence of the amine groups **155** is much more reactive than **120** and therefore overall yield of the reaction should be higher for the thiourea and urea reactions.

In addition the option was also available of attaching a fluorophore to position C3 via isocyanates or isothiocyanates, whilst placing already successful binding sites as functional groups at positions C7 and C12, *e.g.* **159**.



156 **157**

158 **159**

Scheme 4.15: Potential future research

In conclusion, though the aim of producing a fluorescent chemosensor was not obtained. It is believed that several synthetic sensors should be completed shortly once a procedure for developing the fluorescent isocyanates and isothiocyanates is developed. As with the systems developed in Chapter 2 and 3 the affinity and selectivity may be tuned by choosing urea or thiourea as part of the binding site. These hosts will be a direct conversion of successful binding systems developed by Davis *et al.* into fluorescent chemosensors.

CHAPTER 5

EXPERIMENTAL

5.1 General

5.11 NMR

^{1}H, ^{13}C NMR and ^{19}F NMR spectra were run on a BRUKER MSL-300 or a BRUKER DPX-400 spectrometer using deuterated chloroform as solvent (or otherwise noted) with tetramethylsilane as internal standard. The chemical shifts are reported in parts per million (ppm or δ), followed by the number of protons e.g. 1H, splitting pattern e.g. doublet, coupling constant (where applicable) e.g. J = 3.4 Hz, and assignment of proton e.g. Ar-H2 in brackets,. A doublet splitting pattern is represented by **d**, a double doublet as **dd**, as singlet as **s**, a multiplet as **m**, a quartet as **q**, and a broad singlet as **br s** pattern. NMR and IR spectra were assigned by comparison with literate values for similar compounds. Elemental Analyses was carried out at the Micro-analytical Laboratory of the Chemistry department in University College of Dublin. Mass spectrometry analysis was conducted using Mass Lynx NT V3.4 on a Waters 600 controller connected to a 996 photodiode array detector. The solvent systems used were either 50:50 CH_3CN: H_20 or 50:50 MeOH:H_2O. The anthracene thiourea and urea sensors ionised on the column and therefore an accurate total mass could not be obtained. Mass spec analysis was conducted on every other compound.

Melting points were recorded on a gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 883 spectrophotometer and on a Mattson Genesis II FTIP spectrometer equipped with a Gateway 2000 4DX2-66 workstation. Oils were analysed using NaCl plates, solid samples were dispersed in KBr and recorded as clear pressed discs. Only peaks due to the main functional groups are listed for identification purposes. Thin layer chromatography was carried out by using DC-Alufolien Kieselgel $60F_{254}$ 0.2mm plates. Spots due to high molecular weights were visualised by charring over a Bunsen burner. Amines were stained using 5% ninhydrin in methanol. Chromophoric spots were visualised in ultra-violet light.

5.12 Materials

Cholic acid was obtained as a gift from DiamaltgmbH and used without further purification. Solvents were distilled before use and dried using standard techniques described by Perrin.[135] All air and moisture sensitive liquids were transferred via a cannula or syringe into the reaction vessel through a rubber septa. All untreated solvents were HPLC grade. All reagents used were purchased from Aldrich, Sigma, or Lancaster and were used without further purification. Flash chromatography of reaction products was carried out using Kiesgel 60 (Merck) 400-230 mesh, by method of Still *et al.*[136]

5.13 Fluorescence

Fluorescence studies were carried out on the Perkin Elmer LS50B Luminescence spectrometer. The instrument had a Xenon discharge source lamp with agated photo-multiplier detector. Instrumental parameters were controlled by "Fluorescence Data Manager" software supplied with the spectrometer. Data analysis was conducted using this software and Microsoft "Excel 2000". The quantum yields for each sensor were measured by comparison with known standards (Section 2.6, Chapter 2).

5.14 UV

All UV measurements were conducted at 20-25°C using UNI-CAM (UV 2) spectrometer and on a Shimadzu UV-2401PC. The optical density of each sample was kept the same by maintaining a constant optical density of 0.1 units +/- 4%.

5.15 Titrations

The salts used for both the NMR and Fluorescence titrations were the tetrabutylammonium derivatives of the various anions. They were all of spectroscopic grade purchased from Aldrich. They were all dried over phosphorous pentoxide under vacuum. The anion solutions were made using spectroscopic grade solvent obtained from Aldrich. An exact amount of the TBA salt was weighed out that corresponds to 1M in 10 mls of solvent. A series of solutions of decreasing strengths ranging from 10^{-5}M to 10^{-1}M were made up from this stock solution by using the serial dilution technique. Each solution was made up to a total volume of 10mls. The reference solution for the fluorescence titrations was the solvent used to dissolve the sensor (spectroscopic DMSO unless otherwise stated). Initially a spectra of the sensor was taken. The guest solution was the added in exact increments to the solution of the sensor with

fluorescence spectra obtained after each addition. Before taking each spectra the resulting solution was mixed thoroughly and cuvettes and pipettes were rinsed thoroughly with the new solution. These steps were vital as fluorescence spectroscopy is so sensitive and contaminations would distort the results. The titration additions were made using a "Socorex" micropipette. The total of all of the additions did not exceed 10% of the original volume. When conducting a titration with an anion for the first time relatively large additions were made (i.e. large changes in concentration). This allowed a rough response to be determined. Once the solution was saturated with anion a second titration was completed with new solutions using very small concentration changes each time. Once the concentration range in which the sensor is active was determined the titrations were repeated at least twice in order to validate the results. For the fluorescence titrations the concentration of the sensor was kept constant by ensuring the solution had an optical density of 0.1. For NMR titrations the concentration of the sensor was $\approx 2 \times 10^{-2}$M. A solution of the sensor of known concentration and volume was placed in the NMR tube. This solution was analysed by ^{1}H NMR. The peaks obtained in the spectra where assigned to the various protons of the sensor. Then 5µl of a guest solution of known concentration was added to the NMR tube. The NMR tube was shaken vigorously and inverted several times to ensure uniform mixing of the sensor and anion solutions. Further additions 5µl of the anion solution (of known concentration) were made and the resulting ^{1}H NMR spectra obtained. The shift in position of the peaks for the protons of the NH urea and thiourea groups for these spectra were plotted as a function of [G] (concentration of guest added).

The aromatic protons and carbons of anthracene derivatives are identified using the following numbering system:

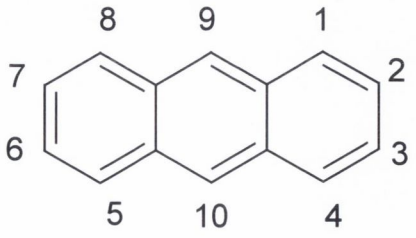
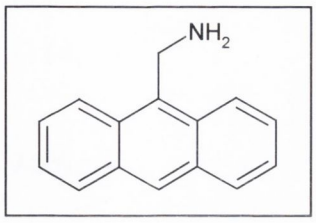


Figure 5.1: Numbering system of Anthracene

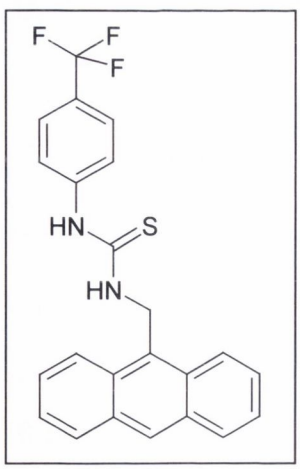
5.2 EXPERIMENTAL PROCEDURE

9-Aminomethyl anthracene[137], 70



9-Bromomethylanthracene[138], **72** (1 g, 3.68 mmol) was dissolved in anhydrous $CHCl_3$ (15 mL). This solution was added drop-wise to a solution of hexamethylenetetramine (0.5152 g, 3.68 mmol) in 10 mL of anhydrous $CHCl_3$. The resulting solution was refluxed for 5 hours with vigorous stirring. The precipitate was removed by filtration and washed several times with water. The precipitate was added to a mixture of ethanol, water and concentrated HCl (20:4:5) 29 mL. This mixture was heated at 70 ^{o}C , after 1 hour the precipitate had completely dissolved, the solution was kept stirring at 70^{o}C overnight. Once removed from the heat the solution was then allowed stand at room temperature overnight and the product precipitated out of solution as a HCl salt. This salt was washed with 10% $KHCO_3$ (10 mL) and extracted into $CHCl_3$ (35 mL). The organic layer was dried over $MgSO_4$. Excess solvent was removed under reduced pressure and the residue was dried over P_2O_5 overnight to give a pale yellow solid. (0.708 g, 93%). mp 102 ^{0}C (dec.); δ_H (400 MHz, $CDCl_3$): 8.43 (s, 1H, Ar-10H) 8.39, (d, 2H, , J = 9.0 Hz, Ar-8H, Ar-1H), 8.06(s, 2H, J = 8.0 Hz, Ar-4H, Ar-5H,), 7.58 (d, 2H, J_1 = 7.5 Hz, J_2 = 7.5 Hz, Ar-2H, Ar-7H) 7.50(d, 2H, J_1 = 7.5 Hz, J_2 = 8.0 Hz, Ar-3H, Ar-6H), 4.88 (s, 2H, -15H); δ_C(400 MHz, $CDCl_3$): 35.09, 122.94, 123.68, 125.98, 127.66, 129.66, 130.23, 130.87, 131.98; MS *m/z* (ES): 207 ($[M]^+$).

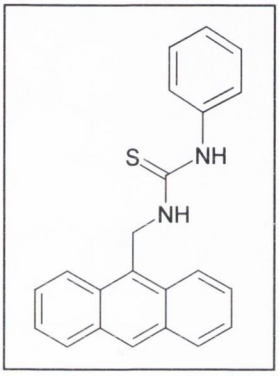
9-Trifluoromethylphenyl-thioureamethyl anthracene, 65



9-Aminomethyl anthracene, **70** (0. 1 g, 0.483 mM) was dissolved in 20 mL of dry CH_2Cl_2. To this solution was added 4-trifluoromethylphenylisothiocyanate, **79** (0.098 g, 0.485 mM, 1.01eq). A creamy yellow precipitate was immediately formed upon addition of the isothiocyanate. The reaction was allowed to stir vigorously overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from chloroform. (0.166 g, 84% yield). mp 197-198^{o}C; CHN calculated for $C_{23}H_{17}F_3N_2S$:C, 67.30%; H, 4.17%; N 6.82%; found C, 67.28%; H, 4.17%; N, 6.86%; δ_H (400 MHz, $CDCl_3$): 8.53(s, 1H, 10-H), 8.32(d, 2H, J = 8.5 Hz, Ar-8H, Ar-1H), 8.09(s, 2H, Ar-4H, Ar-

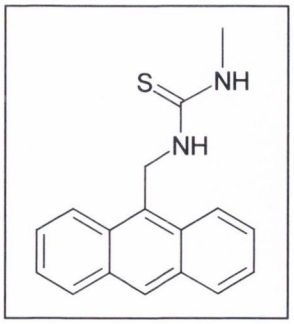
5H), 7.63(d, 2H, J_1 = 6.5 Hz, J_2 = 8.5 Hz, Ar-2H, Ar-7H) 7.54(d, 2H, J_1 = 7.5 Hz, J_2 = 7.5 Hz, Ar-3H, Ar-6H), 7.45(d, 2H, J = 8.5 Hz, 19-H, 20-H,), 7.16(d, 2H, J = 8.0 Hz, 18-H,21-H), 5.83(s, 2H, 15-H); δ_C(400 MHz, $CDCl_3$): 179.6, 139.56, 131.5, 130.5, 129.5, 127.2, 127.0, 126.61, 123.5, 123.2, 42.7 ; ^{19}F NMR: 63.16; IR (KBr) cm^{-1} 3241, 3056, 1542, 1333, 1114, 728;

9-Phenyl-thioureamethyl anthracene, 66



9-aminomethyl anthracene, **70** (0.1 g, 0.483 mM) was dissolved in 20 mL of dry CH_2Cl_2. To this solution was added phenylisothiocyanate, **80** (59ul, 0.483 mM, 1eq). The resulting solution was stirred overnight at room temperature. The resulting precipitate was removed by filtration washed with cold CH_2Cl_2 and dried over P_2O_5 and was then recrystalised from $CHCl_3$ (0.127 g, 77% yield). mp 205-207°C; CHN calculated for $C_{22}H_{18}N_2S$: C, 77.16%; H, 5.30%; N 8.18%; found C, 77.20%; H, 5.29%; N, 8.18%; δ_H (400 MHz, $CDCl_3$): 8.49 (s, 1H, Ar-10H), 8.31(d, 2H, J = 9.0 Hz Ar-1H, Ar-8H), 8.05 (d, 2H, J = 8.5 Hz Ar-4H, Ar-5H), 7.61(t, 2H, J_1 = 7.5 Hz, J_2 = 7.5 Hz, Ar-2H, Ar-7H), 7.5 (t, 2H, J_1 = 8.5 Hz, J_2 = 7.5 Hz, Ar-3H, Ar-6H), 7.18 (t, 1H, J_1 = 7.5 Hz, J_2 = 8.0 Hz, 20H), 7.10 (d, 1H, J = 7.5 Hz, 18H), 7.04 (d, 1H, J = 7.5 Hz, 19H,), 5.82 (d, 2H, J_1 = 4 Hz, 15-H); δ_C(400 MHz, $CDCl_3$): 180.05(C=S), 139.53, 131.61, 130.26, 129.44, 128.67, 128.43, 126.42, 125.15, 123.92, 129.50, 67.41(CH_2); IR (KBr) cm^{-1} 3412, 2922, 1509, 1495, 1345, 1256, 717;

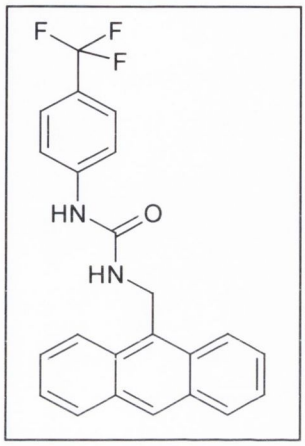
Methyl-thioureamethyl anthracene, 67



9-aminomethyl anthracene, **70** (0. 3 g, 1.45 mM) was dissolved in 20 mL of dry CH_2Cl_2. To this solution was added methyl-isothiocyanate, **81** (0.18 g, 2.6 mM, 1.8eq). The reaction was allowed to stir vigorously overnight at room temperature. The excess solvent was removed under reduced pressure. The residue was purified by flash chromatography with 100% ethyl acetate as the eluent (R_f 0.67). The product was dried over P_2O_5 followed by recrystalisation from CH_3Cl. (0.155 g, 67% yield). mp 190°C dec; CHN calculated for $C_{17}H_{16}N_2S$:C, 72.82%; H, 5.75%; N, 9.99%; found C, 72.79%; H, 10.01%; N, 9.98%; δ_H (400 MHz, $CDCl_3$): 8.49 (s, 1H, 10-H), 8.30(d, 2H, J = 8.5 Hz , Ar-1H, Ar-8H), 8.05 (d, 2H, J = 8.0 Hz, Ar-4H,

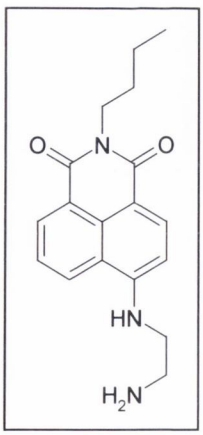
Ar-5H), 7.59(t, 2H, J_1 = 6.5 Hz, J_2 = 8.5 Hz, Ar-2H, Ar-7H), 7.51 (t, 2H, J_1 = 7.0 Hz, J_2 = 8.0 Hz, Ar-3H, Ar-6H), 5.66(s, 2H, 15-H), 2.85(s, 3H, 17-H); δ_C(400 MHz, $CDCl_3$): 129.11, 128.5, 126.8, 125.16, 123.63, 42.0, 30.2; IR (KBr) cm^{-1} 3204, 3056, 1545, 1333, 1114, 738;

9-Trifluoromethylphenyl-ureamethyl anthracene, 83



9-aminomethyl anthracene, **70** (0. 1 g, $4.83x10^{-4}$mol) was dissolved in 20 mL of dry DCM. To this solution was added 4-trifluoromethylphenylisocyanate, **82** (0.091 g, $4.85x10^{-4}$mol, 1.01eq). A creamy yellow precipitate was immediately formed upon addition of the isothiocyanate. The reaction was allowed to stir vigorously overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from chloroform. (0.160 g, 84% yield). mp 215-218°C; CHN calculated for $C_{17}H_{16}N_2S$:C, 72.82%; H, 5.75%; N, 9.99%; found C, 70.01%; H, 4.30%; N, 7.05%; δ_H (400 MHz, $CDCl_3$): 8.68 (s, 1H, Ar-10H), 8.64 (s, 2H, Ar-4H, Ar-5H), 8.49 (d, 2H, J = 9.0 Hz, Ar-1H, Ar-8H), 8.15 (d, 2H, J = 8.5 Hz, Ar-3H, Ar-6H), 7.65 (q, 2H, J_1 = 6.5 Hz, J_2 = 7.5 Hz, Ar-18H, Ar-22H), 7.56 (d, 2H, J = 7.0 Hz, Ar-3H, Ar-6H), 5.35 (s, 2H, 15-H, J = 3 Hz); δ_C(400 MHz, $CDCl_3$):160(C=O), 138.57, 131.85, 131.29, 130.55, 129.76, 128.88, 128.76, 126.99, 125.32, 125.08, 124.01, 123.92, 120.50, 57.43(CH_2); ^{19}F NMR: 66.96; IR (KBr) cm^{-1} 3427, 3342, 2922, 1641, 1550, 1330, 729;

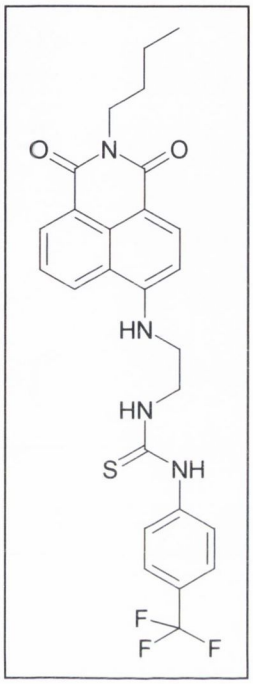
N-*butyl-4-(4'-aminoethyl)amino-1,8-naphthalimide, 90*



N-butyl-4-chloro-1,8-naphthalimide (2.0 g, 6.9 mmol) was refluxed in a large excess of ethylenediamine (30 mL). After 12 hours the solution was poured into a 250 mL beaker full of ice. This new solution was stirred vigorously overnight in darkness. The resulting red-brown precipitate was removed by filtration. This compound was dried overnight under vacuum in the presence of P_2O_5. (1.56 g, 78%) mp: 142-143°C; δ_H (400 MHz, $CDCl_3$): 8.6 (d, 1H, J = 7.5Hz, Ar-H), 8.5 (d, 1H, J = 8.5Hz, Ar-H), 8.17 (d, 1H, J = 8.5Hz, Ar-H), 7.61 (t, 1H, J = 7.5Hz, Ar-H), 6.7 (d, 1H, J = 8.5Hz, Ar-H), 6.16 (br s, 1H,), 4.16 (t,2H,), 3.42 (d, 2H, J = 5.0Hz,), 3.10 (d, 2H, J = 5.0Hz,), 1.72 (m, 2H,) 1.44 (m, 2H,), 0.96 (d, 2H, J = 7.5Hz,); δ_c(300 MHz; $CDCl_3$) 13.9, 20.4, 30.3, 39.9, 40.1, 43.8, 44.8, 104.3, 110.2, 120.4, 123.0, 124.6, 126.2,

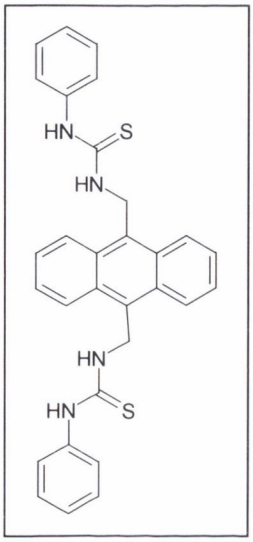
128.6, 129.7, 131.0, 134.4, 149.6, 164.2, 164.7; IR (film from KBr) ν_{max} (cm^{-1}) 3436, 2960, 1677, 1633, 1612, 1579, 1467, 1430, 1357, 1304, 1245, 1124, 1124, 773, 758, 670; MS *m/z* (ES+) 312 ([M^+])

N-*Butyl-4-(4'-triflouromethylphenylthiourea-ethyl)amino-1,8-naphthalimide, 91*



The *N*-butyl-4-(4'-aminoethyl)amino-1,8-naphthalimide, **90** (0.2 g, 0.643 mM) was dissolved in 10 mL of dry CH_2Cl_2. To this was added a solution of 4-trifluoromethylphenylisothiocyanate, **79** (0.1305 g, 0.643 mM, 1 eq). The flask was covered in tin foil and the reaction was stirred for 48 hours at room temperature. The precipitate was removed by vacuum filtration and washed with CH_3CN and dried over P_2O_5 followed by recrystalisation from chloroform (0.311 g, 94 % yield); CHN calculated for $C_{26}H_{25}F_3N_4O_2S$:C, 60.69%; H, 4.90%; N 10.89%; found C, 60.67%; H, 4.90%; N, 10.86%; δ_H (400 MHz, $CDCl_3$): 8.59 (d, 1H, *J* = 7.5Hz, Ar-H), 8.44 (d, 1H, *J* = 8.5Hz, Ar-H), 7.91 (d, 1H, *J* = 8.5Hz, Ar-H), 7.70 (t, 1H, *J* = 7.5Hz, Ar-H), 7.39 (m, 1H), 7.17 (m, 1H), 6.58 (d, 1H, *J* = 8.5Hz, Ar-H), 6.16 (br s, 1H,), 4.33 (m, 4.17 (t,2H,), 3.42 (d, 2H, *J* =5Hz,), 3.59(m, 2H), 1.72 (m, 2H,) 1.47 (m, 2H), 0.98 (d, 2H, *J* =7.5Hz,); δ_c(300 MHz; $CDCl_3$) 14.0, 22.5, 22.8, 29.3, 51.8, 53.6, 62.6, 123.3, 125.6, 125.9, 127, 127.2, 128.5, 130.5, 131.5, 133.2, 142.7, 147.2,. 183.6 IR (film from KBr) ν_{max} (cm^{-1}) 3436, 2960, 1677, 1633, 1612, 1579, 1467, 1430, 1357, 1304, 1245, 1124, 1124, 773, 758, 670;

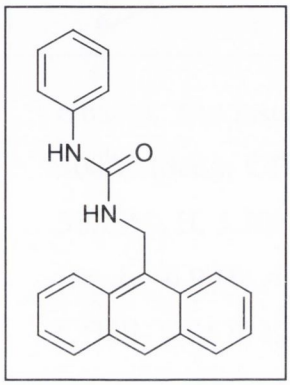
9,10-Bis-phenyl-thioureamethyl anthracene, 96



9,10-bis-aminomethyl anthracene (0.36 g, 1.53 mM) was dissolved in 30 mL of dry CH_2Cl_2. To this solution was added phenylisothiocyanate (370ul, 3.05 mM, 1eq). The resulting solution was stirred overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from $CHCl_3$ (0.774 g, 85% yield). CHN calculated for $C_{30}H_{26}N_4S_2$: C, 71.11%; H, 5.17%; N, 11.06%; found C, 71.14%; H, 5.19%; N, 11.05%; δ_H (400 MHz, $CDCl_3$): 5.73 (d, J_1 = 6.5 Hz, 4H, 15-H, 24-H), 7.06 (dd, J_1 = 7.0 Hz, J_2 = 7.4 Hz, 4 H, Ar-H), 7.27 (dd, J_1 = 7.5 Hz, J_2 = 8.0 Hz, 4 H, Ar-H), 7.50 (d, J_1=7.4 Hz, 4 H,

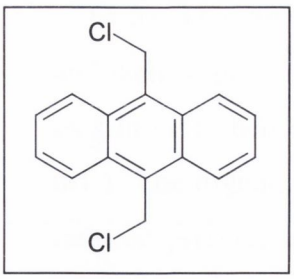
Ar-H), 7.69 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H), 8.06 (s, 2 H, NH), 8.52 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H), 9.36 (s, 2 H, NH); δ_C(400 MHz, $CDCl_3$): 180.1, 139.5, 131.6, 130.3, 129.9, 128.7, 126.4, 125.2, 123.9, 122.5, 67.4; IR (KBr) cm^{-1} 3412, 2922, 1542, 1509, 1345, 717;

9-Phenyl-ureamethyl anthracene,



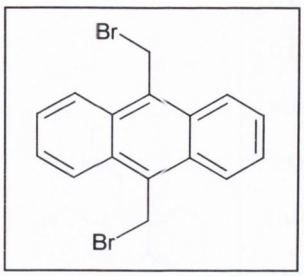
9-aminomethyl anthracene, **70** (0.1 g, 0.483 mM) was dissolved in 10 mL of dry CH_2Cl_2. To this solution was added phenylisocyanate, (0.0575 mg, 0.483 mM, 1eq). The resulting solution was stirred overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from chloroform (0.176 g, 77% yield). mp 209-210°C; CHN calculated for $C_{22}H_{17}N_2O$:C, 80.96%; H, 5.56%; N, 8.58%; found C, 80.96%; H, 5.55%; N, 8.59%; δ_H (400 MHz, $CDCl_3$): 8.15 (s, 1H, Ar-10H), 7.96(d, 2H, J = 9.0 Hz, Ar-1H, Ar-8H), 7.89 (s, 2H, , Ar-4H, Ar-5H), 7.64 (s, 2H, Ar-18H, Ar-22H), 7.38 (d, 2H, J = 9.0 Hz, Ar-2H, Ar-7H), 7.37(d, 2H, J = 8.0 Hz, Ar-3H, Ar-6H), 7.24 (s, 2H, Ar-19H, Ar-21H), 7.09 (s, 1H, Ar-20H), 4.86 (s, 2H, Ar-15H); δ_C(400 MHz, $CDCl_3$): 54.41, 120.50, 123.92, 124.01, 125.06, 125.35, 126.81, 128.67, 128.94, 129.76, 130.56, 131.26, 131.91, 138.53, 160(C=O);

9,10-Bis-chloromethylanthracene, 100



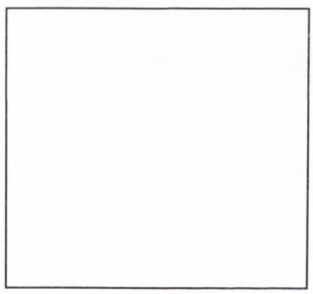
A suspension of formaldehyde (3.2 g, 0.105mol, 2.6eq) in glacial acetic acid (35 mL) was dissolved by passing HCl gas through it. To this solution was added a suspension of anthracene (7.15 g, 0.04mol) in glacial acetic acid (40 mL). This solution was heated at 60°C for 20 hours. The solid precipitate was removed under vacuum filtration. The solid was kept under vacuum filtration for 4 hours in order ensure the complete removal of toxic side products of the reaction. This solid was washed with ethanol (2x20 mL) and then dried under vacuum. Caution this experiment was completed carried out in the fumehood due to the toxicity of the side products of this reaction. (80%) mp. 277°C; δ_H (400 MHz, $CDCl_3$): 5.64 (s, 4H, 15-H, 16-H), 7.69 (dd, J_1= 3.5Hz, J_2= 4.0Hz, 4 H, Ar-H), 8.41 (dd, J_1 = 3.0Hz, J_2 = 3.5Hz, 4 H, Ar-H); δ_C(400 MHz, $CDCl_3$):: 38.33 , 123.88, 126.25, 129.33, 129.78; MS *m/z* (ES): 276 ($[M]^+$).

9,10-Bis (bromomethyl) anthracene[139], 104



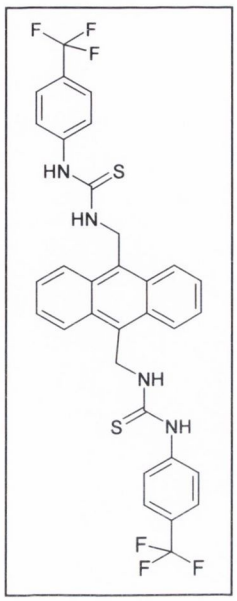
Anthracene (10 g, 56 mmol) was dissolved in a mixture of 48% aqueous hydrobromic acid (200 mL) and glacial acetic acid (50 mL). To this solution 1,3,5-trioxane (10 g, 128 mmol, 1 equivalent) and tetradecyltrimethylammonium bromide (0.4 g, catalytic amount) were added. The mixture was stirred and refluxed for 24 h. After cooling, the green solid formed was filtered and washed with water and ethanol. The resulting crude product was recrystalized from toluene (15.32 g, 75%). mp 300^{0}C (dec.); CHN calculated for $C_{16}H_{12}Br_2$:C, 52.78%; H, 3.32%; found C, 52.79%; H, 3.30%; δ_H (400 MHz, $CDCl_3$): 5.50 (s, 4H, 15-H, 16-H), 7.68 (dd, J_1 =3.4 Hz, J_2 =6.9 Hz, 4 H, Ar-H), 8.38 (dd, J_1 =3.4 Hz, J_2 = 6.9 Hz, 4 H, Ar-H); δ_C(400 MHz, $CDCl_3$): 26.6, 124.4, 126.7; IR (KB_r) 3048, 1953,1622, 1528, 1442, 1195 cm^{-1};

9,10-Bis (aminomethyl) anthracene, 97



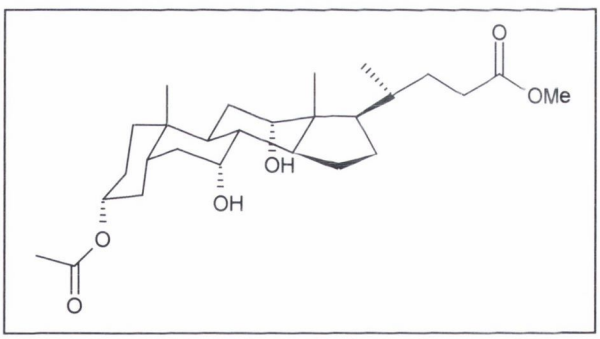
9,10 – bis bromomethylanthracene, **104** (0.37 g, 1.02 mmol) was dissolved in anhydrous $CHCl_3$ (15 mL). This solution was added drop-wise to a solution of hexamethylenetetramine (0.2848 g, 2.03 mmol) in 10 mL of anhydrous $CHCl_3$, and the resulting solution was refluxed for 5 hours with vigorous stirring. The precipitate was removed by filtration and washed several times with water. The precipitate was added to a mixture of ethanol, water and concentrated hydrochloric acid (20:4:5). This mixture was heated at 70^{o}C overnight. The precipitate dissolved initially and then re-precipitated out of solution again after 1 hour. This precipitate was removed by filtration then dispersed in 10% $KHCO_3$ (20 mL) and extracted into $CHCl_3$ (2x20 mL). The organic layer was then dried over $MgSO_4$. Excess solvent was removed under reduced pressure and the residue was dried over P_2O_5 overnight. (0.2005 g, 83%). mp. 238^{0}C; CHN calculated for $C_{16}H_{16}N_2$:C, 81.32%; H, 6.32%; N, 11.85%; found C, 81.29%; H, 6.33%; N, 11.82%; δ_H (400 MHz, $CDCl_3$): 4.43 (s, 4H, 15-H, 16-H), 7.55 (dd, J_1 =3.0 Hz, J_2=2.5 Hz, 4 H, Ar-H), 8.53 (dd, J_1 =3.0 Hz, J_2=2.5 Hz, 4 H, Ar-H); δ_C (400 MHz, $CDCl_3$): 45.6, 124.7, 126.2, 130.4, 131.7; IR (KB_r) 3458, 3048, 1953,1622, 1528, 1195 cm^{-1};

9,10-Bis-trifluoromethylphenyl-thioureamethyl anthracene, 95



9,10-bis-aminomethyl anthracene (0. 6 g, 2.54 mM) was dissolved in 30 mL of dry DCM. To this solution was added 4-trifluoromethylphenylisothiocyanate (1.03 g, 5.09 mM, 1.01eq). A creamy yellow precipitate was immediately formed upon addition of the isothiocyanate. However it is believed that this precipitate was the mono-thiourea. Therefore the reaction was allowed to stir vigorously overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from chloroform (0.999 g, 89% yield). mp. 256^{0}C; CHN calculated for $C_{32}H_{24}F_6N_4S_2$: C, 59.80%; H, 3.76%; N, 8.72%; found C, 59.81%; H, 3.74%; N, 8.75%; δ_H (400 MHz, $CDCl_3$): 5.75 (s, 2H, 15-H, 24-H), 7.63 (d, J_1=8.5 Hz, 4 H, Ar-H), 7.70 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H), 7.82 (d, J_1 = 8.5 Hz, 4 H, Ar-H), 8.53 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H); δ_C(400 MHz, $CDCl_3$): 179.9, 139.6, 132.1, 130.0, 128.7, 126.5, 125.5, 125.1, 121.5, 122.5, 67.4; ^{19}F NMR: 60.9; MS *m/z* (ES): 643 ($[M]^+$); IR (KBr) cm^{-1} 3400, 2922, 1533, 1328, 835;

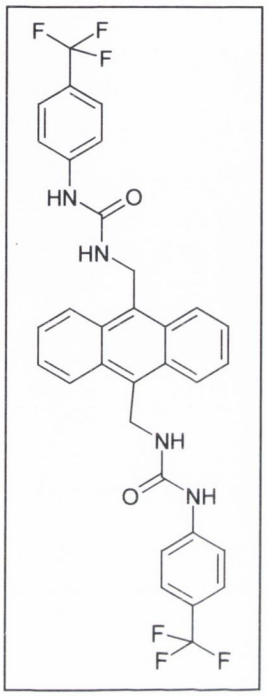
Methyl 3α-acetoxy-7α,12α-dihydroxy-5β-cholan-24-oate, 104



Cholic acid (30 g, 0.073moles) and *p*-toluenesulfonic acid were added to methyl acetate. The resulting mixture was refluxed for 24 hours. To the reaction mixture was added $NaCHO_3$ and then evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with 4 % aqueous $NaCHO_3$, dried ($MgSO_4$) and evaporated under reduced pressure, the residue was redissolved in Hexane/Ethyl acetate and filtered through flash silica eluting with Hexane/Ethyl acetate: 6.25/3.75 to get the desired product as a white solid, (29.47 g, 87%): mp 150-151^{0}C (DCM-Hexane); R_f (Ethyl acetate:Hexene/3.5:6.5) 0.26; CHN calculated for $C_{27}H_{44}O_6$:C, 69.79%; H, 9.55%; found C, 69.74%; H, 9.51%; δ_H (400 MHz, $CDCl_3$): 0.70 (s, 3H, 18-H_3), 0.91 (s, 3H, 19-H_3), 0.99 (d, 3H, J = 6.5 Hz, 21-H_3), 2.00 (s, 3H, CH_3COO), 3.72 (broad s, 1H, 7β-H), 3.85 (broad s, 1H, 12β-H), 4.56 (m,1H, 3β-H); δ_c(300 MHz; $CDCl_3$) 12.52, 17.33, 21.40, 22.48, 23.14, 26.65, 27.40, 28.83, 30.87,

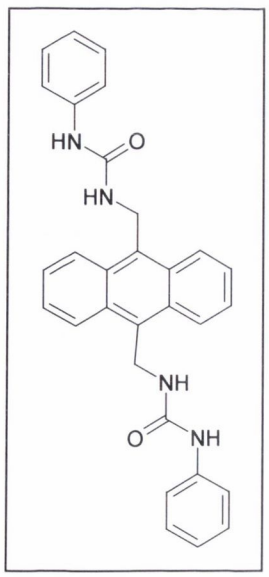
31.04, 34.41, 34.67, 34.88, 35.14, 35.18, 39.56, 41.20, 41.97, 46.54, 47.22, 51.44, 68.24, 72.88, 74.29, 170.69, 174.64; ν_{max} (KBR)/cm^{-1} 3509, 2941, 1735, 1248, 1024;

9,10-Bis-trifluoromethylphenyl-ureamethyl anthracene, 106



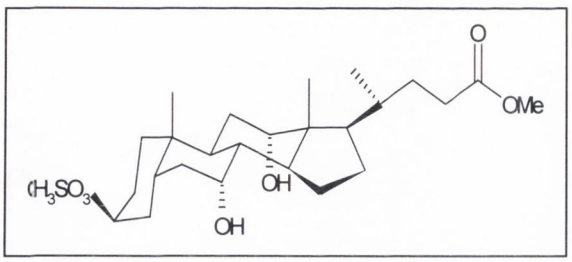
9,10-bis-aminomethyl anthracene (0. 6 g, 2.54x10-^{3}mol) was dissolved in 20 mL of dry DCM. To this solution was added 4-trifluoromethylphenylisocyanate (0.95 g, 5.09x10^{-3}mol, 1.01eq). A creamy yellow precipitate was immediately formed upon addition of the isocyanate. However it is believed that this precipitate was the mono-thiourea. Therefore the reaction was allowed to stir vigorously overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from $CHCl_3$ (1.379 g, 89% yield). CHN calculated for $C_{32}H_{24}F_6N_4O_2$:C, 62.95%; H, 3.96%; N, 9.18%; found C, 63.01%; H, 3.98%; N, 9.17% δ_H (400 MHz, $CDCl_3$): 5.75 (d, J_1 = 3.0 Hz, 4H, 15-H, 24-H), 7.63 (d, J_1=8.5 Hz, 4 H, Ar-H), 7.70 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H), 7.82 (d, J_1 = 8.5 Hz, 4 H, Ar-H), 8.53 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H); δ_C(400 MHz, $CDCl_3$): 47.6, 119.3, 120.4, 124.7, 125.6, 126.2, 126.6, 129.4, 131.2, 140.4, 158.1; IR (KBr) cm^{-1} 3332, 2923, 1641, 1544, 1328;

9,10-Bis-phenyl-ureamethyl anthracene



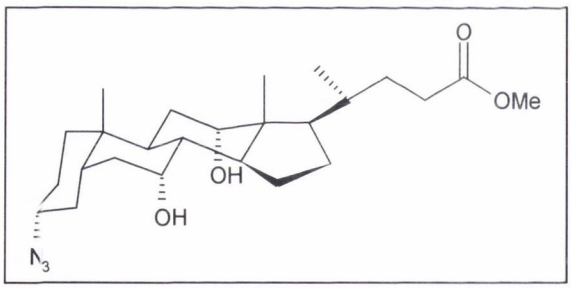
9,10-bis-aminomethyl anthracene (0.36 g, 1.53 mM) was dissolved in 20 mL of dry CH_2Cl_2. To this solution was added phenylisocyanate (362ul, 3.05 mM, 1eq). The resulting solution was stirred overnight at room temperature. The resulting precipitate was removed by filtration washed with dry $CHCl_3$ and dried over P_2O_5 followed by recrystalisation from $CHCl_3$ (0.595 g, 82% yield). CHN calculated for $C_{30}H_{26}N_4O_2$: C, 75.93%; H, 5.52%; N, 11.81%; found C, 75.86%; H, 5.50%; N, 11.77% δ_H (400 MHz, $CDCl_3$): 5.75 (s, J_1 = 3.0 Hz, 4H), 7.63 (d, J_1=8.5 Hz, 4 H, 15-H, 24-H), 7.70 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H), 7.82 (d, J_1 = 8.5 Hz, 4 H, Ar-H), 8.53 (dd, J_1 = 3.5 Hz, J_2 = 3.0 Hz, 4 H, Ar-H); δ_C(400 MHz, $CDCl_3$): 47.2, 120.4, 124.2, 124.7, 126.2, 128.2, 129.9, 131.3, 138.2, 161; IR (KBr) cm^{-1} 3410, 2929, 1543, 1321, 835;

Methyl 3β-methanesulfonoxy-7α, 12α–bis(hydroxy)-5β–cholan-24-oate, 119



Methanesulfonic acid (1.6 mL, 24.7 mM, 2.1 equiv) was added to a solution of methyl cholate, **118**(5 g, 11.8 mM) and PPH_3 (9.4 g, 36 mM, 3 equiv) in dry THF (38 mL) under argon. The temperature was raised to 40 °C and the mixture was allowed to stir for 10 minutes. DEAD (5.7 mL, 36 mM, 3 equiv) was added dropwise over 12 minute period with vigorous stirring, and careful exclusion of water. The mixture was stirred vigorously for 36 hours at 40°C under argon. The volatiles were then removed under reduced pressure and the residue was dissolved in $CHCl_3$. The crude product was purified by flash chromatography on silica gel using ethyl acetate/hexane/chloroform (7:1:0.05) to give the mesylate as an off-white solid (*4.2 g, 75%*) R_f in ethyl acetate 0.53; δ_H (400 MHz, $CDCl_3$): 0.67 (3H, s, 18 CH_3), 0.89 (3H, s, 19CH_3), 0.94 (3H, d, 21CH_3), 2.82 (1H, d, 7/12-OH), 2.96 (3H, s, 3βOSO_2CH_3), 3.64 (3H, s, OCH_3), 3.85 (1H, m, 7β–H), 3.99 (1H, m, 12β-H), 4.92 (1H, br m, 3α-H). δ_C(400 MHz, $CDCl_3$): 12.44, 17.25, 22.63, 23.13, 25.91, 27.43, 28.41, 29.77, 30.75, 31.01, 33.76, 34.51, 34.82, 35.18, 36.17, 38.43, 39.28, 41.76, 46.87, 47.19, 51.49, 68.29, 70.91, 80.72, 174.73.

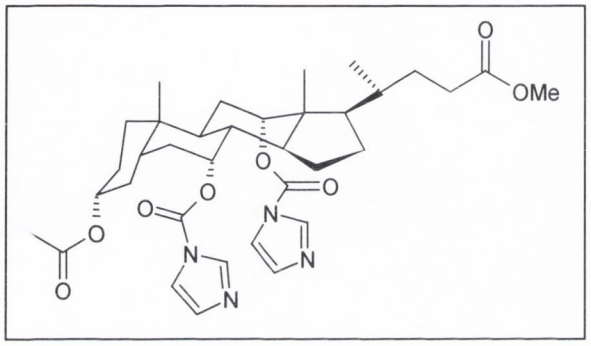
Methyl 3α–azido-7α,12α–bis(hydroxy)-5β–cholan-24-oate, 120



To a solution of 3β-methanesulfonloxy steroid, **119** (3.5 g, 6.3 mM) in dry DMPU (30 mL) was added dry sodium azide (10 g, 0.0154 mM) and allowed to stir for 1 day. The reaction solution was partitioned between ether (50 mL) and water (50 mL). The organic fraction was washed once with water (50 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by chromatography using hexane/ethyl acetate (2:1) as eluent affording the azide (2.38 g, ca.85%): R_f 0.74 in ethyl acetate:hexane (1:1). mp 110-111 °C. CHN calculated for $C_{25}H_{41}N_3O_4$:C, 67.08%; H, 9.23%; N, 9.39%; found C, 67.10%; H, 9.20%; N, 9.37% δ_H (400 MHz, $CDCl_3$): 0.68 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.97 (d, 3H, J = 6.5 Hz , 21-CH_3), 3.14 (m, 1H, 3β-H), 3.66 (s, 3H, OCH_3), 3.85 (m, 1H, 7β-H), 3.98 (m, 1H, 12β-

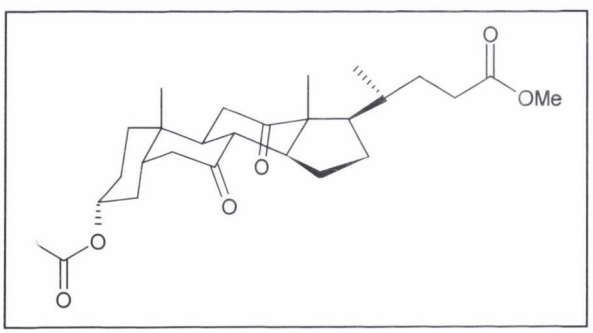
H); δ_C(400 MHz, $CDCl_3$): 12.46, 17.30, 22.56, 23.17, 26.59, 26.82, 27.46, 28.24, 30.84, 31.04, 34.56, 34.77, 35.25, 35.39, 35.47, 39.45, 41.86, 41.91, 46.55, 47.22, 51.46, 61.34, 68.23, 72.98 , 174.76.; V_{max} (film from $CDCl_3$) 2093 (azide), 1732 (C=O).

Methyl 3α–acetoxy-7α,12α–bis(imidazolecarbonate)-5β–cholan-24-oate, 142



To a solution of Methyl 3α–azido-7α,12α–bis(hydroxy)-5β–cholan-24-oate, **104** (0.15 g, 0.335 mM) in dry CH_2Cl_2 (30 mL) was added dry carbonyldi-imidazole (0.217 g, 1.34 mM, 4 eq.) and NEt_3 (0.186 mL, 0.67 mM, 2 eq). The reaction solution was refluxed for 48 hours. Excess solvent was then removed under reduced pressure. The product was then purified using flash chromatography using $CHCl_3$/EtOAc (7:5) as the eluent affording the carbonate (0.168 g, 77%): R_f 0.56 in $CHCl_3$. mp 132-134 °C. CHN calculated for $C_{35}H_{48}N_4O_8$: C, 64.40%; H, 7.41%; N, 8.58%; found C, 64.49%; H, 7.37%; N, 8.52% δ_H (400 MHz, $CDCl_3$): 0.84 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 1.02 (d, 3H, 21-CH_3), 3.63 (s, 3H, OCH_3), 4.55 (m, 1H, 3β-H), 5.25 (s, 1H, 7β-H), 5.38 (s, 1H, 12β-H), 7.21 (d, 2H, Imidazole H, J = 8.0 Hz) 7.43 (d, 2H, Imidazole H, J = 9.0 Hz), 8.23 (d, 2H, Imidazole H, J = 9.0 Hz); δ_C(400 MHz, $CDCl_3$): 11.77, 17.14, 20.85, 21.96, 22.44, 24.94, 26.25, 26.48, 28.54, 30.05, 30.30, 30.85, 33.94, 35.39, 33.98, 34.57, 37.61, 39.82, 43.26, 45.18, 47.63, 50.99, 72.56 , 75.56, 79.95, 116.21, 130.57, 170.76, 173.66; V_{max} (film from $CDCl_3$) 3549, 2941, 1732, 1680, 1300.

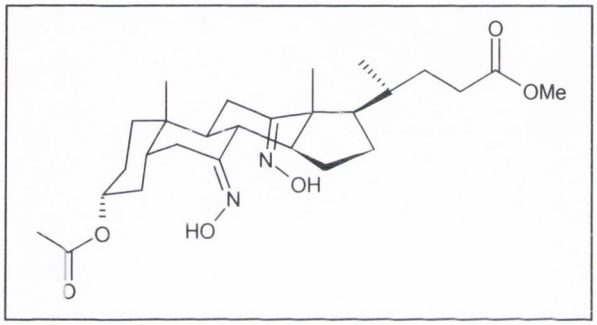
Methyl 3α-acetoxy-7, 12-dioxo-5β-cholan-24-oate, 152



Potassium chromate (3.61 g, 18.6 mmol, 4.5eq) was added to a solution of the diol (2 g, 4.3 mmol) in glacial acetic acid (75 mL). The resulting mixture was stirred for two days under argon. Water was added and a precipitate was immediately formed. This white precipitate was removed by filtration and the compound was dried over P_2O_5 overnight (1.72 g, 86.4%). mp. 159-160 °C; R_f (EtOAc-Hexane, 7:13) 0.43; CHN calculated for $C_{27}H_{40}O_6$:C, 70.41%; H, 8.75%; found

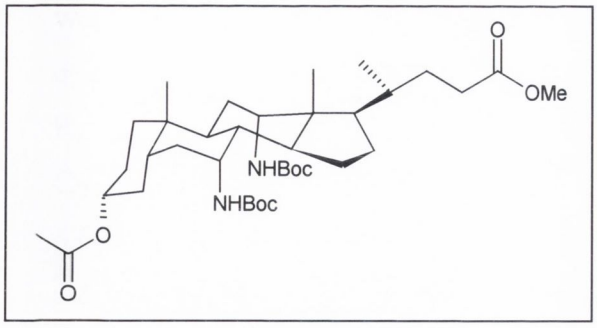
C, 70.37%; H, 8.73%; δ_H (400 MHz, $CDCl_3$): 0.85 (d, 3H, J = 6.5 Hz, 21-H_3), 1.04 (s, 3H, 18-H_3), 1.31 (s, 3H, 19-H_3), 1.99 (s, 3H, CH_3COO), 2.21-2.32(m, 3H), 2.35-2.43(m, 1H), 2.71 (t, 1H, J = 12.8 Hz), 2.79 (t, 1H, J = 11.4 Hz), 2.89 (q, 1H, J = 6.6 Hz) 3.66 (s, 3H, $COOCH_3$), 4.66 (m, 1H, 3β-H); δ_c(300 MHz; $CDCl_3$) 11.73, 18.56, 21.14, 22.36, 25.09, 25.75, 27.58, 30.44, 31.23, 33.09, 33.70, 35.47, 38.27, 44.89, 45.41, 45.41, 45.58, 48.87, 51.37, 51.77, 56.78, 72.29, 170.35, 174.43, 209.07, 212.30; ν_{max} (Nujol)/cm-1 1168,1242, 1259, 1698, 1714, 1735;

Methyl 3α-acetoxy-7, 12-dioximino-5β-cholan-24-oate, 153



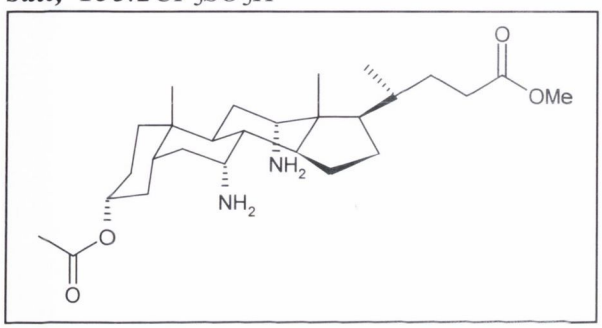
The diketone (5.85 g, 12.7mmol), sodium acetate (11.66 g, 142.33, 11eq.) and hydroxylamine hydrochloride (3.25 g, 46.66mmol. 3.7eq) were dissolved in methanol (115mls) and refluxed for 4.5 hours. The reaction mixture was evaporated under reduced pressure, re-dissolved in DCM, washed with water, dried ($MgSO_4$), evaporated under educed pressure. The residue was crystallised in chloroform-hex and filtered to obtain the title compound as a white solid, (18.01 g, 96.8%): mp>261 °C (decomp.); R_f (EtOAc: Hex/1:1) 0.65; CHN calculated for $C_{27}H_{42}N_2O_6$: C, 66.10%; H, 8.63%; N 5.71%; found C, 66.01%; H, 8.63%; N, 5.69%; δ_H (400 MHz, $CDCl_3$): 0.93 (d, 3H, J = 6.5, 21-H_3), 0.94 (s, 3H, 18-H_3), 1.16 (s, 3H, 19-H_3), 2.01 (s, 3H, CH_3COO), 2.46 (t, 1H, J = 11.0, 8α-H), 3.12 (dd, 1H, J_1 = 13.3 Hz, J_2 = 1.5 Hz), 3.28 (dd, 1H, J_1 = 13.1 Hz, J_2 = 4.5 Hz), 3.66 (s, 3H, $COOCH_3$), 4.72 (m, 1H, 3β-H), 7.66 (s, 1H, 7-C=NOH), 7.86 (s, 1H, 12-C=NOH); δ_c(300 MHz; $CDCl_3$) 12.18, 19.11, 20.06, 21.34, 22.55, 25.34, 26.06, 27.46, 27.94, 30.63, 31.48, 32.58, 34.33, 35.81, 36.01, 42.05, 43.99, 44.01, 46.03, 49.44, 51.43, 52.73, 73.01, 159.52, 164.52, 170.61, 174.74; IR ν_{max} (Nujol)/cm^{-1} 921, 1170, 1245, 1666, 1738, 3258:

Methyl 3α-acetoxy-7α, 12α-di-[n-(t-butyloxycarbonyl)amino]5β-cholan-24-oate, 154



A mixture of the dioximino compound **153** (9.4g, 17.6mmol) and platinum oxide hydrate (0.94g, 10% by weight) in glacial AcOH (40 mLs) was stirred under 1 atmosphere of H_2 for 6 days, the reaction mixture was filtered (washing with glacial AcOH). The volume of filtrates was concentrated under reduced pressure to approx. 18-20 mL. To this solution was added zinc on powder. The resulting mixture was stirred overnight at room temperature. The mixture was filtered, and the filtrand was washed with glacial acetic acid. The filtrate was evaporated under reduced pressure. The crude product was redissolved in THF and saturated. aqueous. $NaHCO_3$, to this solution was added di-$_t$-butyl dicarbonate and stirred for 3 days. The reaction mixture was evaporated under reduced pressure, redissolved in DCM, washed with dilute aqueous solution of HCl, dried (using $MgSO_4$) and evaporated under reduced pressure. The white foamy crude product was purified by flash chromatography (Hexane:EtOAc/ 3:1) and crystallised in DCM/hexane to give the desired compound **154** as a white solid, (10.6g, 84%): mp 217-222 ^{0}C (DCM-Hex); R_f (EtOAc:Hex1:1) 0.71, (EtOAc:Hex/1:2) 0.55; CHN calculated for $C_{37}H_{62}N_2O_8$: C, 67.04%; H, 9.43%; N 4.23%; found C 67.09% H 9.47% N 4.17%; δ_H (400 MHz, $CDCl_3$): 0.81 (s, 3H, 18-H_3), 0.92 (d, 3H, J = 6.0, 21-H_3), 0.95 (s, 3H, 19-H_3), 1.46 (s, 18H, $(CH_3)_3C$), 2.04 (s, 3H, CH_3COO), 3.70 (s, 4H, $COOCH_3$17β-H), 3.97 (broad s, 1H, 12β-H), 4.58 (m, 1H, 3β-H), 4.94 (broad s, 1H, 7-CH-NHR), 5.12 (broad s, 1H, 12-CH-NHR); δ_c(300 MHz; $CDCl_3$) 13.5, 17.5, 21.3, 22.8, 22.9, 26.7, 27.1, 27.8, 28.5, 30.5, 31.8, 32.2, 34.8, 34.9, 35.8, 37.1, 41.1, 44.3, 44.7, 47.2, 49.5, 52.3, 53.3, 74.4, 155.0, 155.5, 170.1, 174.7; IR n_{max} (Nujol)/cm^{-1} 3386, 3370, 1709, 1246, 1167;

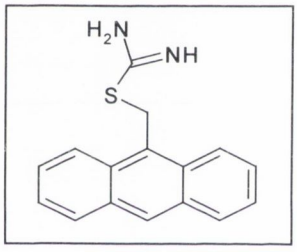
Methyl 3α-acetoxy-7α,12α-diamino-5β-cholan-24-oate, bis(trifluoromethanesulphonate) salt, 155*.2CF$_3$SO$_3$H*



To a solution of biscarbamate **154** (0.2186 g, 0.33 mmol) in DCM (5 mL) was added trifluoroacetic acid (TFA) (7.4 g, 64 mmol) at 0^{0}C. The reaction mixture was stirred overnight under argon at room temperature.

The solvents were removed and the residue was re-dissolved in DCM and evaporated for three times to obtain a white foam. The white foam was dissolved again in DCM and washed with $NaHCO_3$. The two phases were separated and the organic layer was dried over $MgSO_4$. The solvents were removed to obtain 16 as a white solid, mp. 181-182 °C; R_f=0.31 (Ethyl acetate/methanol/Et_3N 9/1/0.09); CHN calculated for $C_{27}H_{46}N_2O_4$:C, 70.04%; H, 10.02%; N 6.05%; found C, 70.01%; H, 10.03%; N, 6.06%; δ_H (400 MHz, $CDCl_3$): 0.73 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.98 (d, J = 6.5 Hz, 3H, 21-CH_3), 1.03-1.93 (m, 20H), 2.01 (s, 3H, CH_3CO_2), 2.13-2.27 (m, 2H), 2.36-2.49 (m, 2H), 3.10 (broad s, 1H, 7α-H), 3.16 (broad s, 1H, 12α-H), 3.67 (s, 3H, CO_2CH_3), 4.53-4.58 (m, 1H, 3α-H), ppm; IR (film from $CDCl_3$) ν_{max}2938, 2869, 1736, 1438, 1379, 1259, 1166, 1034 cm^{-1},

9 Thiouronium methyl anthracene



A solution of 9-bromomethyl anthracene (0.5 g, 1.868 mmol) and thiourea (0.14 g, 1.868 mmol, 1eq) was refluxed in HPLC grade ethanol (25 mL) for 24 hours. The solvent was removed under reduced pressure. The residue was dried under vacuum over phosphorous pent-oxide. CHN calculated for $C_{16}H_{14}N_2S$:C, 72.15%; H, 5.30%; N 10.52%; found C, 72.11%; H, 5.29%; N, 10.49%; δ_H (400 MHz, $CDCl_3$): 8.73 (s, 1H, 10-H) 8.45, (d, 2H, 1-H,8H), 8.19(d, 2H, 4-H,5-H), 7.70 (dd, 2H, 2-H,7H) 7.60 (dd, 2H, 3-H,6H), 5.57 (s, 2H, 15-H); δ_c(300 MHz; $CDCl_3$) 26.2, 124.4, 124.9, 125.2, 126.0, 128.2, 131.5, 132.0, 132.2, 163 ;

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APPENDIX

Appendix 1

ABBREVIATIONS

brs	Broad singlet
CDCl3	Chloroform-*d*
CDI	1,1'-Carbonyldiimidazole
d	Doublet
dd	Double doublet
δ	Chemical shift
DCM	Dichloromethane
D_2O	Deuterated Water
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
Eq	Equivalents
EtOAc	Ethyl acetate
EtOH	Ethanol
H	Hydrogen
HBr	Hydrogen bromide
HCl	Hydrochloric acid
Hex	Hexane
HPLC	High Performance Liquid Chromatography
Hz	Hertz
I_F	Intensity of fluorescence
IR	Infra red
J	Coupling constant
K_2CO_3	Potassium carbonate

$KHCO_3$	Potassium Hydrogen Carbonate
MeCN	Acetonitrile
MeOH	Methanol
MgSO4	Manganese sulphate
m.p.	Melting point
m/z	Mass charge ratio
$NaHCO_3$	Sodium Hydrogen Carbonate
NMR	Nuclear magnetic resonance
Ph	Phenyl group
q	Quartet
R_f	Retention factor
RT	Room temperature
s	Singlet
TFA	Trifluoracetic acid
UV	Ultraviolet

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Fluorescent photoinduced electron transfer (PET) sensing of anions using charge neutral chemosensors†

Thorfinnur Gunnlaugsson,*[a] Anthony P. Davis[ab] and Mark Glynn[a]

[a] *Department of Chemistry, Trinity College Dublin, Dublin 2, Ireland. E-mail: gunnlaut@tcd.ie*
[b] *School of Chemistry, University of Bristol, Cantock's Close, Bristol, UK BS8 1TS*

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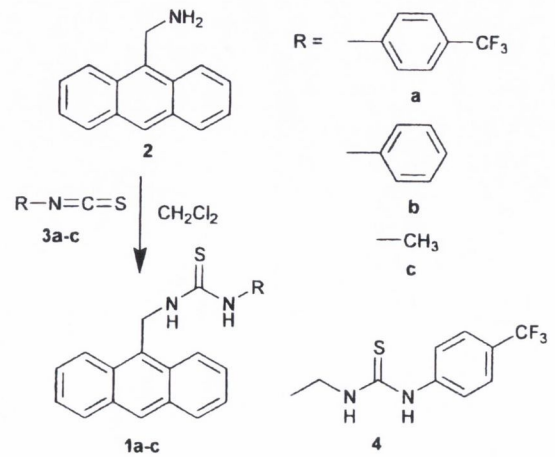
We demonstrate for the first time that the charge neutral anthracene based fluorescent sensors 1a–c, having an aromatic or aliphatic thiourea moiety as an anion receptor, show *ideal* PET sensor behaviour where the anthracene fluorescence emission is selectively quenched upon titration with AcO^-, $H_2PO_4^-$ and F^- but not by Cl^- and Br^- in DMSO.

There is great interest in the design and synthesis of luminescent based chemosensors for on-line and real time detection of physiologically important ions and molecules,[1] and for environmental monitoring of harmful pollutants.[2] While numerous fluorescent and metal based delayed luminescent sensors for cations and organic molecules have emerged from the fields of supramolecular and coordination chemistry,[3] sensors for selective detection of anions are still relatively rare, despite the fact that several elegant examples of anion receptors have been reported over the years.[4] These, however, often involve the synthesis of complex and challenging organic hosts from scaffolds such as cholic acid,[5] calixarenes and peptides.[6] Luminescent anion sensing has recently been achieved[4] through the use of anion receptors composed, for example, from metal based Lewis acid centres,[7] calix[4]pyrroles,[8] thiouronium[9] and protonated quinoxaline,[10] amine[11] or polyamine moieties,[12] but the use of simple and easily synthesised electroneutral anion receptors for such sensing has been less investigated.[13] Intrigued by this fact, we set out to develop the charge neutral chemosensors **1a–c**, employing the criteria of PET sensing using the *fluorophore–spacer–receptor* model developed by de Silva for the detection of cations.[14] A few research groups have attempted to develop PET anion sensors.[11–13,15] But, to the best of our knowledge no such systems, employing neutral anion receptors, have yet been reported that show *ideal* PET behaviour, *i.e.* (*i*) only the quantum yield (intensity) and lifetime of the fluorescence emission should be modulated upon ion recognition due to (*ii*) changes in the free energy of electron transfer (ΔG_{PET}) between the excited state of the fluorophore and the receptor upon ion recognition, and (*iii*) no changes should be observed in the absorption spectra of the fluorophore.[14]

The three PET chemosensors **1a–c**, were easily made in good yield from readily available starting materials, Scheme 1. The 9-aminomethyl anthracene **2**, synthesised by reducing 9-cyanoanthracene using B_2H_6 in THF, was reacted in dry CH_2Cl_2 at room temperature, under an inert atmosphere with an equimolar amount of 4-(trifluoromethyl)phenyl-, phenyl- and methyl isothiocyanate respectively, **3a–c**, yielding **1a–c** as off-white solids that were purified by crystallisation from CH_2Cl_2. For comparable UV-Vis binding studies, the thiourea receptor **4** was prepared in an analogous way from ethylamine. All products were analysed by conventional methods.† The three different isothiocyanates **3a–c**, were chosen with the aim of being able to modulate or tune the acidity of the thiourea receptor moiety, which would lead to different *receptor–analyte* complex stability and hence different binding constants. Of the three chemosensors, **1a** was expected to show the strongest binding due to this effect, and **1c** the least. We initially investigated the binding of **1a** using $(C_4H_9)_4N(O_2CCH_3)$, since AcO^- is known to form strong directional hydrogen bonding with thiourea, as well as having a functional group of great biological relevance.[16] The ^1H NMR of **1a** in DMSO-d_6, showed two sharp signals at 9.62 ppm and 8.36 ppm for the thiourea hydrogens. These were substantially shifted downfield upon addition of 0.1 → 2 equivalents of $(C_4H_9)_4N(O_2CCH_3)$ ($\Delta\delta$ = 1.92 and 1.66 ppm respectively after 1 eq.) signifying the formation of a 1:1 binding through hydrogen bonding with a log β = 3.2.†

The fluorescence emission spectra of **1a** when titrated with AcO^- in DMSO displayed typical PET behaviour. In the absence of AcO^- the fluorescence emission spectra consisted of three sharp bands at 443, 419 and 397 nm, with a shoulder at 473 nm, when excited at 370 nm with Φ_F = 0.1037.‡ Upon addition of the AcO^- (0 → 32 mM), the intensity of these bands gradually decreased with no other spectral changes being observed (*i.e.* no spectral shifts or formation of new emission bands), Fig. 1a. Using PET nomenclature, the emission can be said to being *ca.* 70% (at 443 nm) *'switched off'*, with Φ_F = 0.0070. Concurrently, the absorption spectra of **1a**, consisting of bands at 390, 370, 352 and 336 nm, was hardly affected by the addition of AcO^-.† This confirms the insulating role of the methylene spacer, which minimises any ground state interactions between the fluorophore and the anion receptor. Similar emission and absorption effects were observed for **1b** and **1c**. When the fluorescence titrations of **1a–c** were carried out in CH_3CN, CH_3CO_2Et or THF, the emission was also quenched upon addition of AcO^- but the degree of quenching was somewhat smaller. In EtOH, which is a highly competitive hydrogen bonding solvent, no binding was observed between **1a** and AcO^-. Furthermore, *no* exciplex emission was observed in any of these solvents; in contrast, Teramae *et al.* have recently shown that a pyrene analogue of **1c**, is a ratiometric anion



Scheme 1 The synthesis of **1a–c**. **4** was made in a similar way.

† Electronic supplementary data (ESI) available: ^1H, ^{13}C NMR for **1a–c** and UV-Vis and NMR titration results for **1a** are available as electronic supplementary information (ESI). See http://www.rsc.org/suppdata/cc/b1/b107608f/

 DOI: 10.1039/b107608f

indicator based on the control of intramolecular exciplex emission.[13]

To investigate the selectivity and the sensitivity of the sensor towards biologically important anions, we carried out a series of titrations using $N(C_4H_9)_4^+$ salts of F^-, Cl^-, Br^- and $H_2PO_4^-$ in DMSO. In the case of $H_2PO_4^-$ and F^- the fluorescence emission was quenched by *ca.* 50 (Φ_F = 0.0156) and 90% (Φ_F = 0.0011) respectively (at 443 nm), but only minor quenching (<7%) was observed when titrated with Cl^- (Φ_F = 0.108) or Br^- (Φ_F = 0.088), ruling out a quenching by heavy atom effect. We propose that the quenching is likely to be due to the modulations of ΔG_{PET} upon anion sensing. This can be regarded as an enhancement in the rate of electron transfer from the HOMO of the thiourea–anion complex to the anthracene excited state, upon anion recognition *i.e*, the reduction potential of the thiourea is increased causing PET to become competitively more viable, which causes the fluorescence emission to be quenched or *'switched off'*.§ Plotting the fluorescence intensity changes (at 443 nm) as a function of log [anion] further supports this view. Fig. 1b, shows several features commonly seen for PET cation sensors *e.g.* the profiles for AcO^-, $H_2PO_4^-$ and F^- are all sigmoidal, the quenching occurs over two log concentration units, which is consistent with 1 : 1 binding and simple equilibrium. From these changes the binding constant log β for **1a** was measured to be 3.35 (±0.05) for F^-, 2.55 (±0.05) for AcO^- and 2.05 (±0.05) for $H_2PO_4^-$.‡ Similar binding constants were found for **4a** by measuring the changes in its absorption spectra at 286 nm. Importantly, **1a** shows good anion selectivity with AcO^- being recognised over $H_2PO_4^-$, but both represent families of biological important anions. The fact that **1a** shows higher affinity and more efficient quenching for F^- than AcO^- is not surprising, since its high charge density and small size enables it to form strong hydrogen bonding with the thiourea receptor. Measurements using **1b** and **1c** and the same anions showed similar results. For **1b**, the same selectivity trend was observed as for **1a**, with smaller binding constants due to the reduced acidity of the thiourea protons. For **1c** the order of selectivity and the sensitivity was somewhat different with $H_2PO_4^-$ (log β = 2.05 (±0.05)) being selectively detected over AcO^- (log β = 1.75 (±0.05)). These results show that the anion sensor's affinity can be controlled by simple design.[16]

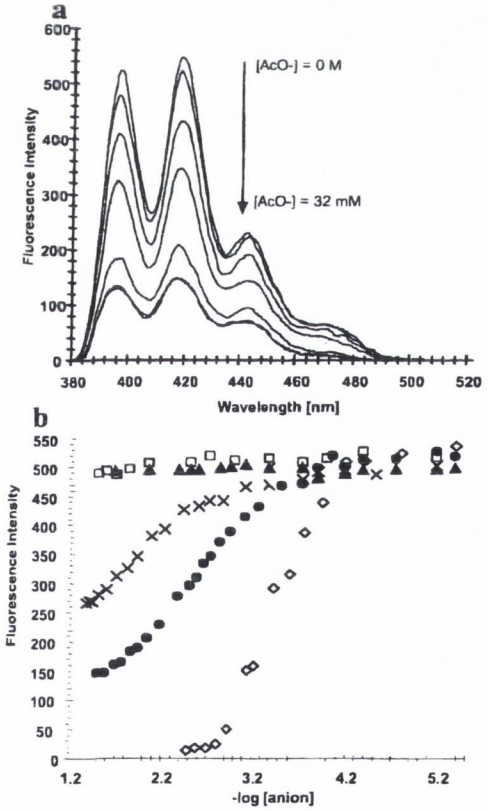


Fig. 1 (a) The changes in the fluorescence spectra of **1a** in DMSO upon addition of acetate. From top: $[AcO^-]$ = 0, 92 μM, 550 μM, 1.8 mM, 8.9 mM, 26 mM, 32 mM. (b) Titration profile for **1a** showing the changes in the fluorescence emission as a function of added anion: ◇ = F^-, ● = AcO^-, ▲ = $H_2PO_4^-$, □ = Cl^-, ▲ = Br^-, when measured at 443 nm. All titrations were repeated two to three times to ensure reproducibility.

In conclusion, the simple fluorescent PET anion chemosensors **1a–c** show *ideal* PET sensing behaviour upon ion recognition, *e.g.* only the fluorescence emission is *'switched off'* in the presence of AcO^-, $H_2PO_4^-$ and F^-. **1a–c** are a very important contribution to the fast growing field of supramolecular anion recognition and sensing.

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Notes and references

‡ **1a–c** Φ_F were measured by comparison with anthracene (Φ_F = 0.27 in EtOH); D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107. log β was determined from the equation:
$\log [(I_{max} - I_F)/(I_F - I_{min})] = \log [anion] - \log \beta$.
§ CV measurements on **4** showed two irreversible oxidative waves. Accurate ΔG_{ET} could not be determined from these measurements. We investigated the PET dependence of **1a** by comparing the Φ_F of **1a** with that of 9-methylanthracene (9MA), which lacks the anion receptor. In the absence of AcO^- the Φ_F of 9MA was found to be 0.284 in DMSO. This suggests that PET is active in **1a** prior to the anion recognition, but becomes even more efficient after anion recognition. In contrast, the addition of 40 mM of AcO^- to 9MA did not affect the Φ_F.

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Fluorescent Sensing of Pyrophosphate and Bis-carboxylates with Charge Neutral PET Chemosensors

Thorfinnur Gunnlaugsson, Anthony P. Davis, John E. O'Brien, and Mark Glynn

Department of Chemistry, Trinity College Dublin, Dublin 2, Ireland, and School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

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Fluorescent Sensing of Pyrophosphate and Bis-carboxylates with Charge Neutral PET Chemosensors†

Thorfinnur Gunnlaugsson,*,‡ Anthony P. Davis,‡,§ John E. O'Brien,‡ and Mark Glynn‡

Department of Chemistry, Trinity College Dublin, Dublin 2, Ireland, and School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK

gunnlaut@tcd.ie

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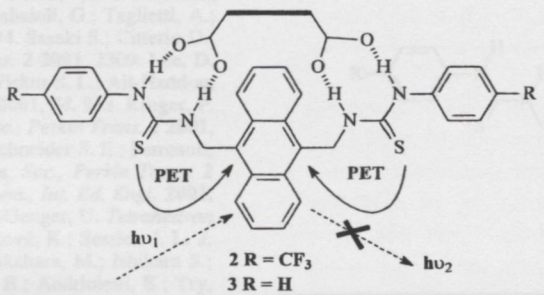
ABSTRACT



The fluorescent photoinduced electron transfer (PET) chemosensors 2 and 3 were designed for the recognition of anions possessing two binding sides such as dicarboxylates and pyrophosphate; the anion recognition in DMSO takes place through the two charge neutral thiourea receptor sites with concomitant PET quenching of the anthracene moiety. The anion binding of acetate, phosphate, and pyrophosphate to 2 and 3 was also evaluated by using ^{1}H NMR in DMSO-d_6.

Over the past few years, fluorescent and luminescent chemosensors for the detection of cations have been successfully developed.[1,2] Conversely, the development of optically based anion sensors has been less successful. Given the important role of anions in biology, clinical diagnostics, and environmental monitoring, the need for easily synthesized fluorescent anion chemosensors is of great importance.[3] A variety of anion receptors have been reported,[4,5] including many capable of luminescent sensing.[6] However, rather few have the simplicity and accessibility which is ideally required for practical devices.

We have been interested in the development of luminescent chemosensors for the detection of cations, anions, and neutral molecules.[7] We are currently particularly interested in developing luminescent anion chemosensors where the anion recognition takes place at charge neutral recognition

† Affectionately dedicated to Sigrúnar Ingibjargar Gísladóttur on the occasion of her 70th birthday.

‡ Department of Chemistry, Trinity College Dublin.

§ School of Chemistry, University of Bristol.

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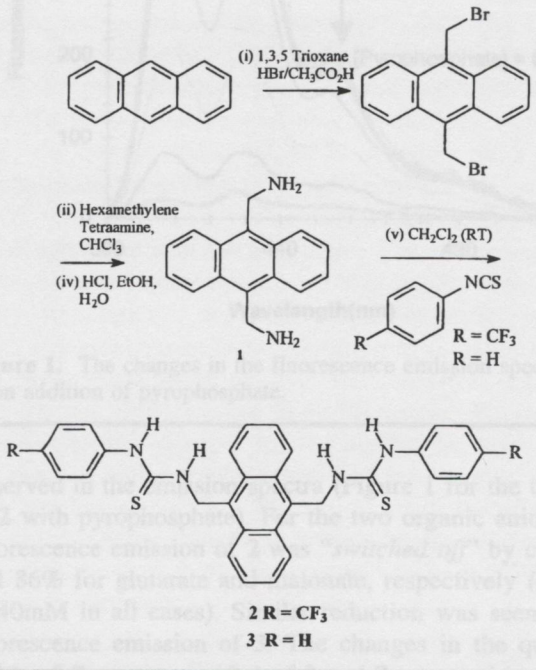
sites with concomitant changes in the photophysical properties of a lumophore by modulation of a Photoinduced Electron Transfer (PET) mechanism.[8] We have chosen to demonstrate such anion recognition of biologically relevant anions such as $H_2PO_4^-$ and AcO^- in DMSO by employing simple thiourea and urea recognition sites, connected to an anthracene fluorescent moiety by a covalent spacer. Here the anion recognition takes place through hydrogen bonding between the thiourea hydrogens and the anion.[9] Such chemosensors should in principle show *ideal* PET behavior upon anion recognition, i.e., only the quantum yield and the lifetime of the excited-state emission should be modulated upon anion recognition.[10] In this letter we demonstrate such PET fluorescent sensing of anions flanked with *two binding sites*.[11] Such fluorescence sensing is both exceptional and of great physiological relevance since many dicarboxylates are components of various metabolic processes, and pyrophosphate is the product of ATP hydrolysis under cellular conditions.[12] However, it has up to now been difficult to achieve without the use of structurally complicated hosts.[4,5] With this in mind we developed **2** and **3**, which have two thiourea moieties that can form hydrogen bonding complexes with bis-anions. To the best of our knowledge, these chemosensors are the first examples of charge neutral fluorescent PET sensors that show ideal PET behavior for bis-anions.

Sensors **2** and **3** (Scheme 1) can be described as being designed as "*receptor–spacer–fluorophore–spacer–receptor*" conjugates[13] where the anion recognition takes place at the two-thiourea moieties. They are easily synthesized, and simple modification to the thiourea moiety (by incorporating aromatic or aliphatic electron withdrawing groups) can be used to "*tune*" the anion sensitivity and selectivity, as the acidity of the thiourea hydrogens is modulated.[4] **2** and **3** were synthesized in good yield (Scheme 1) from 9,10-diaminomethylanthracene (**1**). The synthesis of this starting material has been described previously in the literature, using Gabriel synthesis.[14] However, due to the insolubility of the bis-phthalimide intermediate the yield of **1** was found to be extremely poor. With this in mind we synthesized **1** using an alternative method that involved the initial synthesis of 9,10-bis-bromomethylanthracene in one step[15] in 75% yield. Accordingly, **1** was synthesized from this bis-bromide intermediate with hexamethylenetetramine in anhydrous $CHCl_3$ under inert atmosphere.[16] This method gave **1** in 85% yield as a crude product that could be used without further purification. The two sensors **2** and **3** were subsequently

Scheme 1. The Synthesis of PET Anion Chemosensors **2** and **3**



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made by reacting **1** with phenyl and 4-(trifluoromethyl)-phenylisothiocyanate, respectively, in dry CH_2Cl_2 under argon at room temperature. The resulting light-yellow precipitate was collected by filtration, washed several times with cold CH_2Cl_2, and recrystallized from either hot CH_2Cl_2 or $CHCl_3$. All products were analyzed with conventional methods.[17] The ^1H NMR of **2** and **3** in DMSO-d_6 indicated the high symmetry of the sensors, with only two sets of aromatic signals and a single resonance for the $-CH_2-$ spacer. For **2**, the thiourea protons appeared as two resonances at 9.68 and 8.38 ppm, respectively.

The sensitivity and the selectivity of these sensors toward a series of mono- and bis-anions was evaluated by observing the changes in their fluorescence emission spectra in DMSO and in the ^1H NMR upon anion titration (with $(C_4H_9)_4N^+$ (TBA) salts) in DMSO-d_6. The fluorescence emission spectra of **2** consisted of three bands at 409, 430, and 455 nm when excited at 378 nm. Upon addition of monodentate anions such as AcO^- and $H_2PO_4^-$ the emission was ca. 70−95% "*switched off*" or quenched due to the formation of the anion−receptor complex. No other spectral changes were observed in the emission spectra, i.e., there was no evidence of either exciplex or excimer emissions.[10] Concurrently the changes in the absorption spectra (peaks at 358, 378, and 400 nm) of the anthracene moiety were only minor. Similar results were observed for **3**. This is a typical PET behavior since the receptors are separated from the fluorophore by the two $-CH_2-$ spacers; the only interaction between the two moieties is via electron transfer. Upon addition of spherical anions such as Cl^- and Br^- no significant quenching was observed, ruling out quenching by the heavy atom effect. However, F^- quenched the emission effectively (~98%) due to its small size and high charge density. The addition of AcO^- to a solution of **2** with a 40 mM background concentration of Cl^- quenched the emission to the same degree as seen previously for AcO^-, indicating that the two receptors were selectively binding AcO^- over Cl^-. Plotting the changes of the fluorescence intensity at 430 nm as a function of p*A* (−log[anion]) gave, in all cases, sigmoidal profiles (see Figure 2 for AcO^-). However, for both **2** and **3**, these profiles changed over ca. 3−4 pA units. This can be regarded as an indication of a possible 2:1 binding. This was further confirmed by observing the changes in ^1H NMR of the thiourea protons upon titration.[5] With either $H_2PO_4^-$ or AcO^- (0 → 5−6 equiv of TBA salts), the thiourea resonances were gradually shifted downfield by >2.5 ppm, confirming the formation of anion−receptor complexes. Analysis of the changes in the "inner proton" (8.38 ppm) vs concentration showed 1:2 binding for both of these anions as seen for $H_2PO_4^-$ in Figure 3.

When **2** and **3** were titrated with TBA salts of the biologically important bis-anions such as glutarate, malonate, and pyrophosphate (with tris(tetrabutylammonium) hydrogen pyrophosphate), the emission spectra were also quenched. As before, no other significant spectral changes were

(17) Calculated for **2** ($C_{32}H_{24}N_4S_2F_6$): C, 59.80; H, 3.76; N, 8.72. Found: C, 59.82; H, 3.76; N, 8.70. Calculated for **3** ($C_{30}H_{26}N_4S_2$): C, 71.12; H, 5.17; N, 11.06. Found: C, 71.10; H, 5.17; N, 11.10. ^1H NMR (400 MH, DMSO-d_6)for **2** and **3** are available as Supporting Information.

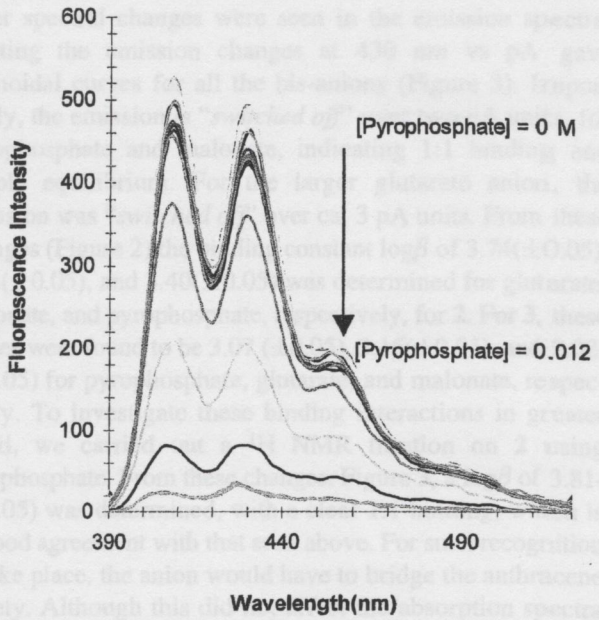

Figure 1. The changes in the fluorescence emission spectra of **2** upon addition of pyrophosphate.

observed in the emission spectra (Figure 1 for the titration of **2** with pyrophosphate). For the two organic anions, the fluorescence emission of **2** was "*switched off*" by ca. 70% and 86% for glutarate and malonate, respectively ([anion] = 40mM in all cases). Similar reduction was seen in the fluorescence emission of **3**. The changes in the quantum yields of fluorescence (Φ_F) of **2** and **3** upon anion sensing were measured in comparison with that of 9,10-dimethylanthracene (9,10-DMA). For **2**, these were measured to be

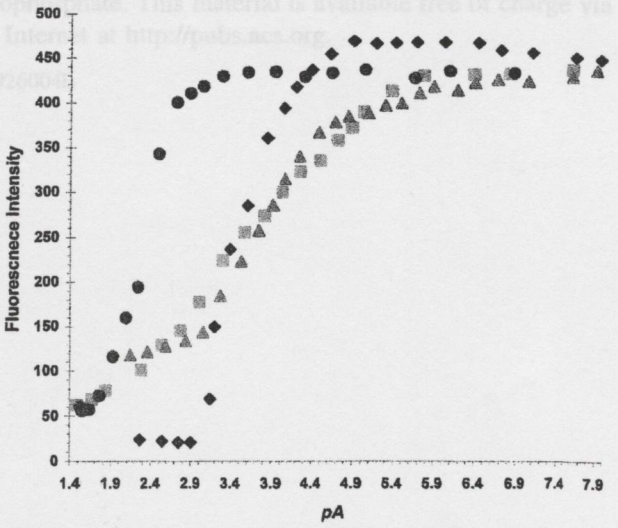

Figure 2. Fluorescence titration curve for **2** when measured at 430 nm (OD = 0.1) in DMSO: malonate (●), pyrophosphate (◆), glutarate (▲), and acetate (■) .

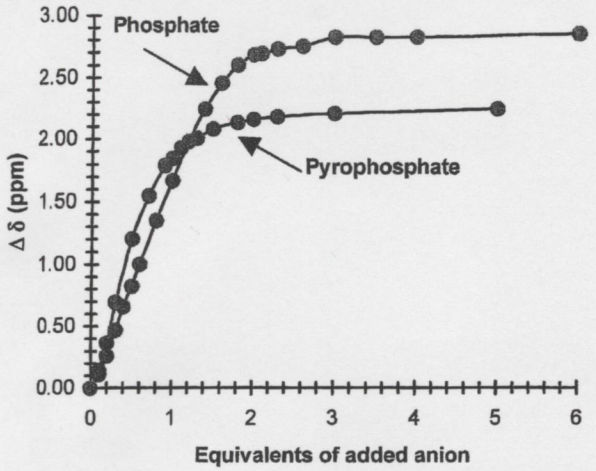

Figure 3. ^{1}H NMR titration of **2** with phosphate (red) and pyrophosphate (blue) in DMSO-d_6.

0.012 and 0.011 for glutarate and malonate, respectively.[18] We believe that this relatively high degree of fluorescence quenching is due to the increase in the reduction potential of the thiourea receptor moieties *after* anion recognition. This affects the rate of the electron transfer from the HOMO of the receptor to the excited state of the fluorophore, i.e., ΔG_{ET} becomes more negative upon anion recognition and hence more thermodynamically favorable. This causes the emission to be "*switched off*". We were unable to demonstrate this by measuring the changes in the redox potential for the receptor since the thiourea was irreversibly oxidized. However, Φ_F measurements of 9,10-DMA, which lacks the two receptor sites, gave a $\Phi_F = 0.87$ in DMSO, which is substantially larger than that of **2**, $\Phi_F = 0.047$, and **3**, $\Phi_F = 0.11$, in DMSO. This implies that PET is active prior to the anion recognition, but becomes even more so *after* anion recognition. The addition of 10 mM of AcO^- or pyrophosphate did not affect the Φ_F of 9,10-DMA. For pyrophosphate the quenching was even more efficient, being ca. 95% for **2** (Figure 2) ($\Phi_F = 0.001$) and 90% ($\Phi_F = 0.017$) for **3**. No other spectral changes were seen in the emission spectra. Plotting the emission changes at 430 nm vs pA gave sigmoidal curves for all the bis-anions (Figure 3). Importantly, the emission is "*switched off*" over two pA units, for pyrophosphate and malonate, indicating 1:1 binding and simple equilibrium. For the larger glutarate anion, the emission was "*switched off*" over ca. 3 pA units. From these changes (Figure 2) the binding constant logβ of 3.74(±0.05), 2.34(±0.05), and 3.40(±0.05) was determined for glutarate, malonate, and pyrophosphate, respectively, for **2**. For **3**, these values were found to be 3.07 (±0.05), 3.15(±0.05), and 2.02-(±0.05) for pyrophosphate, glutarate, and malonate, respectively. To investigate these binding interactions in greater detail, we carried out a ^{1}H NMR titration on **2** using pyrophosphate. From these changes, Figure 3, a logβ of 3.81-(±0.05) was determined, with a clear 1:1 binding, which is in good agreement with that seen above. For such recognition to take place, the anion would have to bridge the anthracene moiety. Although this did not affect the absorption spectra of **2** to any great extent (see Supporting Information), then upon closer examination an isosbestic point was observed at ca. 406 nm. However, the ^{1}H NMR spectra of **2** showed somewhat greater changes for the anthracene resonances upon titration with pyrophosphate than that seen in the ^{1}H NMR titration of $H_2PO_4^-$. We are currently investigating these anion-binding features in greater detail, and carrying out modifications on these chemosensors to further enhance the bis-ion selectivity and sensitivity.

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Supporting Information Available: Synthesis of **1**, **2**, and **3** and absorption spectrum of **2** upon titration with pyrophosphate. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Barnes, R. L.; Briks, J. B. *Proc. R. Soc. London, Ser. A* **1966**, *291*, 570.