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The Synthesis of Steroidal-Based Anion Receptors and Analysis of their Binding Properties

by

Shay Broderick

A Thesis Presented to the University of Dublin for the Degree of Doctor of Philosophy.

Department of Chemistry,
Trinity College,
University of Dublin.

October 1999.

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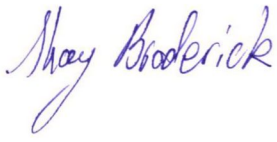

For my Parents

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Abbreviations

BOC	*t*-butoxycarbonyl
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone
DMSO	dimethyl sulfoxide
H-bond	hydrogen bond
IR	infra red
NMR	nuclear magnetic resonance
OAc^-	Acetate anion
R_f	distance travelled by material relative to solvent front
rt	room temperature
TBA	tetrabutylammonium
TBABr	tetrabutylammonium bromide
TBACl	tetrabutylammonium chloride
TBATs	tetrabutylammonium toluenesulfonate
TCE	1,1,2,2-tetrachloroethane
TLC	Thin layer chromatography
TMABr	tetramethylammonium bromide
TMACl	tetramethylammonium chloride
TMATs	tetramethylammonium toluenesulfonate
THF	tetrahydrofuran
TMSCl	chlorotrimethylsilane
UV	ultra violet

Summary

Chapter one is an introduction to molecular recognition and supramolecular chemistry; their origin and the history of their progress in recent years. It includes a brief account of the factors that must be considered when designing a receptor for anion recognition. This is followed by a discussion of the most important advances in this area published to date. The discussion is presented in four sections divided according to the type of bonding interactions predominantly exploited by the receptor. This chapter concludes with a description of the aims of the project.

Chapter two outlines the first synthesis of a sulfonamido *bis*-carbamate derivative of methyl cholate synthesised by Justin Perry. This is followed by an alternative method of synthesis for this type of compound that can result in higher yields and allows easier to purification. The lipophilicity of these compounds is increased by derivatising the ester side chain of the host to ensure a full recovery of the receptor after analysis.

Chapter three discusses the various receptors made using this novel route outlined in chapter two. Their potency as anion receptors is analysed using the technique of NMR titration with a range of tetrabutylammonium halides.

Chapter four discusses new methods for analysing the ability of the receptors to bind anions. Three different techniques are discussed and they all give an insight into the strength of host-guest binding.

Chapter five deals with the *tris*-amino analogue of methyl cholate synthesized by Robert Williams. Some of the physical and chemical properties of this compound are investigated here. Finally, the synthesis of the *tris*-sulfonamide analogues and their selectivity for chloride over bromide is discussed.

Chapter six presents an overall conclusion of the work done in this thesis.

Chapter seven contains the experimental details.

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Chapter One : Introduction to Supramolecular Chemistry

1.1 Supramolecular chemistry and molecular recognition

Supramolecular chemistry was described by Jean-Marie Lehn as the "chemistry beyond the molecule";[1] *i.e.* the chemistry of the intermolecular bond, covering the structure and functions of entities formed by the association of two or more chemical species. In contrast to intramolecular bonding, supramolecular assemblies are not formed using covalent interactions to link their constituent parts, but must rely on weaker binding interactions *e.g.* hydrogen bonding, electrostatic forces, π-π interactions or hydrophobic interactions. The field of supramolecular chemistry may be loosely divided into two areas: molecular self-assembly and molecular recognition.

Molecular self-assembly is concerned with the aggregation of a relatively large number of molecules into a large structure. Examples in biological systems include the formation of lipid bi-layer membranes and micelle formation.[2] Molecular recognition has been defined as a process involving both bonding and selection of a substrate (guest) by a given receptor (host). It implies a structurally well-defined pattern of intermolecular interactions and may involve a specific function. A simple analogy is that of the lock and key hypothesis as proposed by Emil Fischer over 100 years ago. By understanding the way molecular recognition works, one can try to design a synthetic receptor with the aim of emulating a process in nature. Some of the objectives of molecular recognition studies include : the imitation of biological processes (biomimesis), illumination of some of the principles governing molecular interactions and the creation of new systems useful in catalysis, separation technology and ion selective transport. Molecular interactions form the basis of highly specific processes that occur in nature *e.g.* enzymatic reactions, immunological antibody-antigen association, transcription and translation of genetic code, cellular recognition *etc.*

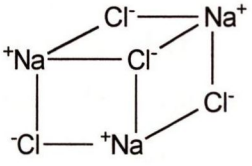
The design of artificial receptor molecules capable of displaying high efficiency and selectivity requires the correct manipulation of the energetic and stereochemical features of the intermolecular forces within the molecular architecture. A more accurate refinement of Fischer's lock and key theory is a hand and glove analogy which accounts for conformational changes that occur in the active site of a receptor before and after complexation.[3]

1.2 Types of bonding interactions

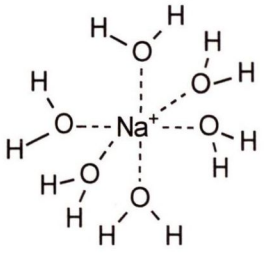
Non-covalent bonds are weaker than covalent bonds (*e.g.* hydrogen bonding is of the order of 20 $kJmol^{-1}$ compared to 350 $kJmol^{-1}$ for a carbon- carbon single bond) and are therefore easily disrupted.[4] Consequently host-guest complexes are short-lived in solution (hydrogen bonds in solution exist for approximately 10^{-10} s). Non-covalent interactions which govern supramolecular systems are all fundamentally electrostatic in nature (due to interactions between electric charges). They are discussed under the various sections described below.

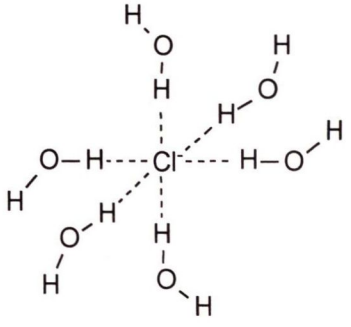
1. **Ion - ion interactions**

These involve the interaction between two charged species. These forces are non-directional in nature and have significant bond energy in the solid state. For example sodium chloride in an ionic lattice has a bond energy of 494 $kJmol^{-1}$, but in water this energy diminishes to approximately one percent of that value.[6]



Solid NaCl
Lattice energy = 494 $kJmol^{-1}$





NaCl in aqueous solution
Solvation energy = 4.5 $kJmol^{-1}$

2. **Ion - dipole and dipole - dipole interactions**

In polar molecules a permanent electric dipole exists. It exists as a result of differences in electronegativity which induce an uneven distribution of electron density. The forces between a permanent dipole and a point charge (or ion) are the strongest interaction in this class, but are similar in nature to dipole - dipole interactions. Compared to charge - charge interactions these forces are much weaker.

3. **Induced dipole - induced dipole interactions and dispersive forces**

These interactions are important for non-polar molecules. The attraction arises due to an instantaneous uneven electron distribution. This induced dipole generates an electric field, polarising any non-polar molecules in close proximity. These transient fluctuating forces are weak in water due the low atom density together with the low polarizability of oxygen. Hydrocarbons however, have a high atom density and the polarizability of a CH_2 group is greater than that of an oxygen. These are the factors that contribute to the association of hydrophobic molecules.

4. **Hydrogen bonds**

Figure 1.1 a hydrogen bond formed between AH to B.

A = oxygen, nitrogen or sulfur *etc.*

B = anion or atom containing a spare pair of electrons.

The hydrogen bond has been defined as the attractive force between a hydrogen atom attached to an electronegative atom A and a second atom B, bearing a lone pair of electrons. The hydrogen bond is among the most important interactions involved in molecular recognition of biological systems.[6] In biological systems the hydrogen bond determines the tertiary shape of protein molecules. DNA consists of two polymer strands twisted into a helix and held that way by hydrogen bonds. Molecular recognition based chemical systems also favour hydrogen bonds due to their :

a) relative strength. In a typical system where A and B are oxygen or nitrogen, the bond strength is about 20 $kJmol^{-1}$. For neutral molecules the thermodynamic strength of a hydrogen bond normally lies in the range 10 - 65 $kJmol^{-1}$. [5] However, if one of the components is ionic, the strength increases to 40 - 190 $kJmol^{-1}$.

b) bond directionality. Maximum interactions occur when the A-H-B bond angle is close to 180^{o} as seen in figure 1.1. Typically bond lengths are in the region of 1.5 - 3.0 Å long.[6]

5. **Aromatic interactions**

Strong, attractive interactions between π systems have been known for over sixty years. They control the intercalation of drugs into DNA, the packing of aromatic molecules in crystals and the binding properties of polyaromatic macrocycles. Complexes of π-deficient aromatics, for example picric acid and 1,3,5-*tris*-aminobenzene, a π-rich aromatic first suggested this interaction type (*i.e.* an electron donor and an electron acceptor attract one another $\pi \rightarrow \pi^*$). Hunter and Sanders suggested that this concept can be misleading. They believe that the properties of the atoms at the point of intermolecular contact are important rather than the overall molecular properties.[6] In other words, the strong geometrical requirements for interactions between aromatic molecules, can be explained by accounting for π - σ as well as π - π* interactions. The two different types of geometries are shown below.

i) Co-facial interactions (Face-to-face)

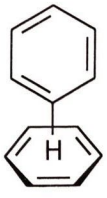
Figure 1.2

Face to face interaction

In this system the favoured orientation is where one aromatic ring is offset with respect to the second aromatic ring in order to minimise π -electron repulsion forces.

ii) Edge to face interactions

Figure 1.3

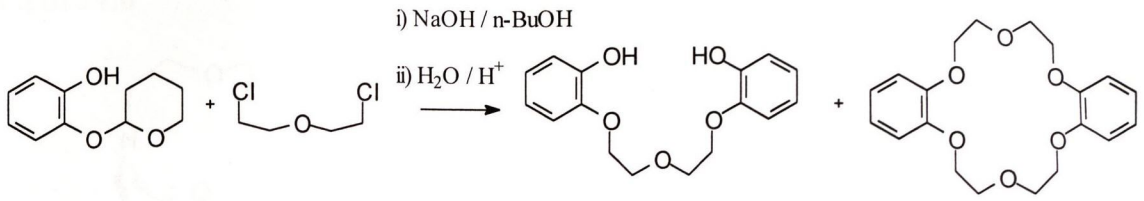


Edge to face interaction

In this system the C - H bond of one aromatic ring is directly above and perpendicular to the other aromatic ring. However it has been observed experimentally that a mixture of the two geometries predominates.[8]

1.3 Discovery of crown ethers and other artificial hosts.

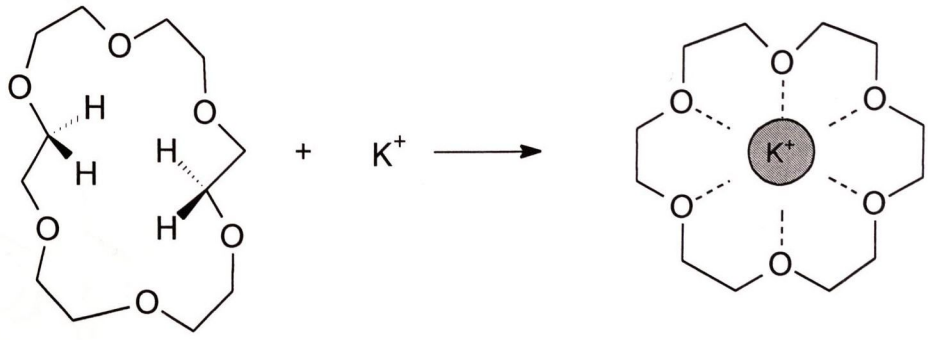
Figure 1.4



1.1 **1.2**

Supramolecular chemistry was born from the discovery of crown ethers and cryptands. C. J. Pedersen's serendipitous discovery occurred in his effort to prepare *bis*-phenol **1.1** from mono-protected catechol and *bis*(2-chloroethyl) ether (figure 1.4). As it turned out the mono-protected catechol was not pure. The impurity was catechol and so a small amount of crown ether **1.2** was formed. Pedersen found that the crystalline hexaether was only slightly soluble in methanol, but on addition of a sodium salts it dissolved surprisingly easily. Many derivatives of this type were developed, most notably 18-crown-6 (**1.3**). These compounds complex cations through ion - dipole attractive forces, to such an effective degree that inorganic salts become soluble in organic solvents such as chloroform.[7] The number of ethylene glycol bridges in the ring has a profound effect on the selectivity of the complexation with metal cations. *e.g.* 12-crown-4, 15-crown-5 and 18-crown-6 preferentially bind Li^+, Na^+ and K^+ respectively.

Figure 1.5



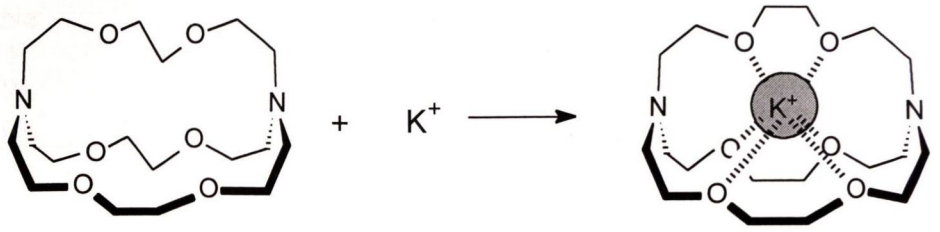
1.3
(No cavity)

K^+ Complex
(K^+ Induced cavity)

An important feature of the crown ethers is that in the uncomplexed state, two of the methylene groups are contorted into the centre of the molecule blocking the binding cavity. It is only upon complexation with an alkali metal ion that a cavity is concertedly organised and filled (figure 1.5 and 1.6).

Similarly, Lehn's cryptand **1.4** consisted of a three-dimensional cage that was only organised and filled upon complexation of an alkali metal ion.[8]

Figure 1.6



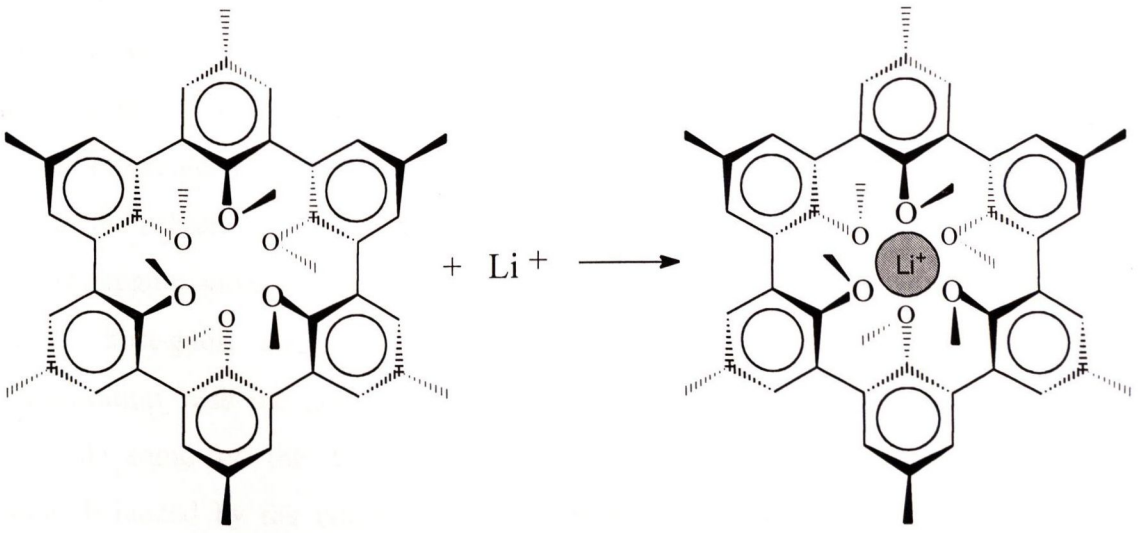
Lehn's cryptand

1.4

(K^+ Induced cavity)

In 1983 D. J. Cram introduced the cavitands[9] (figure 1.7) as a new class of cation receptor. In these systems the donor atoms are part of intraannular substituents which point into the centre of a rigid ring. Their synthesis was achieved in high yield due to a template effect. The key feature of this class of receptor is that the uncomplexed state contains an empty cavity. The cavity is preorganised during synthesis and not during complexation as seen earlier in the cases of the crown ethers and cryptands. Cram described this preorganisation as 'the ability of a molecule to maintain complementarity for a guest even in its absence'. The importance of this preorganisation is reflected in the high binding free energy shown for lithium with the spherand shown in figure 1.7. Cram's spherand is the strongest known binder to the smaller alkali metal ions Li^+ and Na^+ (Table 1).[10]

Figure 1.7



1.5

Cram's spherand with enforced cavity

Li^+ complex

Table 1. Binding free energy ($-\Delta G^{o}$ [kcal mol^{-1}]) of complexes as their picrate salts.

Guest \ Host	Pedersen's Crown ether	Lehn's Cryptand	Cram's Spherand
	1.3	**1.4**	**1.5**
Li^+	6.3	-	>23
Na^+	8.4	14.4	19.2
K^+	11.4	18.0	<<6
Rb^+	9.9	18.8	-
Cs^+	8.5	10.3	-

1.4 Thermodynamics of complex formation

As with any thermodynamic process, for complexation to be favourable, the free energy of complexation (ΔG^{o}) must be negative. The free energy may be defined mathematically by equation **1.1.**

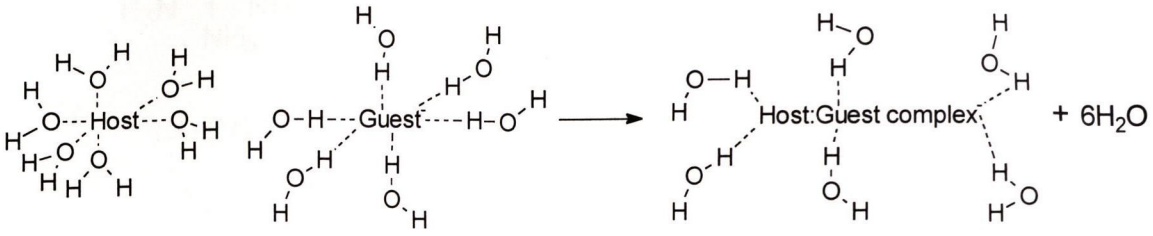
$$\Delta G^{o} = \Delta H^{o} - T\Delta S^{o} \qquad \textbf{(Equation 1.1)}$$

ΔH^{o} = enthalpy of binding, T= temperature and ΔS^{o} = entropy of binding.

Usually host-guest complexation is enthalpically driven (ΔH^{o} = negative) and entropically (ΔS^{o} = negative) disfavoured.[11] Maximum enthalpy change occurs when there is greatest complementarity between host and guest. Complementarity is not just the number of bonds between host and guest but also the strength of the bonds.

Entropy change is much more difficult to predict. It can be dependent on a wide range of factors such as the loss of vibrational, translational and rotational degrees of freedom upon complexation. This is the inevitable entropic disadvantage with host-guest complexation. If the solvent used is a hydroxylic solvent, a favourable entropic effect may be observed. For example in scheme 1.1, if the host, the guest and the complex are each solvated by 6 solvent molecules, the size of the total solvent shell decreases by 6 solvent molecules upon complexation. These solvent molecules can be liberated to the bulk of the solvent with a consequent gain in entropy. This solvophobic effect is rarely seen in organic solvents *e.g.* chloroform. The principle of preorganisation does not just include host-guest complementarity but also low solvation of the host prior to complexation with the guest. If the binding site of both the host and the guest are solvated, some of the favourable enthalpy due to the host-guest interaction is counterbalanced by the enthalpy required to remove the solvent molecules from the binding site.

Scheme 1.1



Solvated free host **Solvated free guest** **Solvated complex**

The efficiency of binding is commonly quoted as an association constant K_{ass}. For a simple complex with a stoichiometry of 1:1 host/guest ratio K_{ass} is derived as :

$$[H] + [G] \underset{}{\overset{K_{ass}}{\rightleftharpoons}} [HG] \qquad \textbf{(equation 1.2)}$$

[H] = concentration of free host

[G] = concentration of free guest

[HG] = concentration of complex.

or

$$K_{ass} = \frac{[HG]}{[H][G]}$$

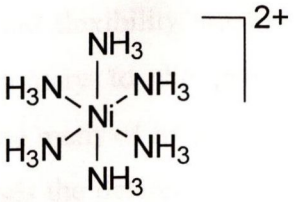
The free energy of association can be calculated from K_{ass} at a given temperature (T) by :

$$K_{ass} = e^{\frac{(-\Delta G^{O})}{RT}} \qquad \textbf{(equation 1.3)}$$

Values for free energy of complexation and association constants are both commonly used to describe a receptor's potency for binding a guest.

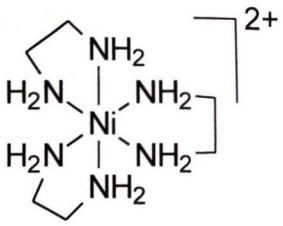
The chelate effect

The chelate effect describes the way a guest with two or more binding sites complexes with a host receptor. For example, the two nickel complexes shown below contain the same number of donor centres but their complexation constants (K_{ass}) are considerably different. This chelate effect is a result of entropy gain in the system. The entropy or disorder in a system grows after coordination of a bidentate ligand because two solvent molecules are displaced, thus the number of unbound mobile molecules increases. If a guest molecule in a host-guest system contains more than one binding site, *e.g.* a *bis*-ammonium salt, a similar chelate effect would be observed.



1.6 $[Ni(NH_3)_6]^{2+}$

$K_{ass} = 10^{8.7}$



1.7 $[Ni(NH_2CH_2CH_2NH_2)_3]^{2+}$

$K_{ass} = 10^{18.7}$

1.5 Factors to be considered when designing a receptor

In general, the term host describes a molecule with the ability to bind a particular guest molecule with greater strength than is commonly found as a result of unspecific molecular interactions. The host may show a selectivity based on many different chemical aspects *e.g.* chemo-, regio- or stereoselectivity. A host that complexes a guest in one particular solvent may completely fail to bind the same guest under different solvent conditions. It is only insight and prudent engineering of the host structure that may lead us to optimal host-guest complexation. Since molecular interactions alone govern the selectivity of a guest to a host, account must be taken of a number of factors before design and synthesis of a host receptor.

1. The chemical nature of the guest to be bound.

The chemical nature of a guest may be described under the following subheadings :

a) Size (radius if spherical)

In order to attain strong complexation between the host and the guest, the dimensions of the binding site of the host must be complementary in size to the guest. Rigid receptors with binding sites complementary to the guest (*e.g.* Cram's spherand for Li^+) often form very strong complexes, with good guest selectivity since there is no need to adapt their binding cavities upon complexation. However if a rigid receptor's binding site is not complementary to the guest, poor binding will result due to an inability to adapt to the guest size. If the receptor is flexible, it may adapt its binding site to suit the guest size. However, flexible receptors generally form complexes with mediocre selectivity, because of the ability to adapt the binding site to suit the accommodation of an undesired guest. Design and synthesis of rigid receptors with complementary binding sites for the desired guests can often be tedious and difficult synthetically, therefore a compromise between

rigidity and flexibility must be reached. If the receptor is too rigid it may not be fully complementary to the guest and the risk of slow guest exchange kinetics could undermine many of its potential applications. Therefore a balance must be one where the host senses the desired guest's size and shape and can then wrap itself around the guest to establish several binding interactions.

b) Shape

Cations, anions and neutral guest molecules have many different shapes and geometries. *e.g.* an anion may be spherical, planar, trigonal, tetrahedral or octahedral. In order to design a receptor, the geometry of the guest must be considered before designing the receptor.

c) The electrostatic charge of the guest

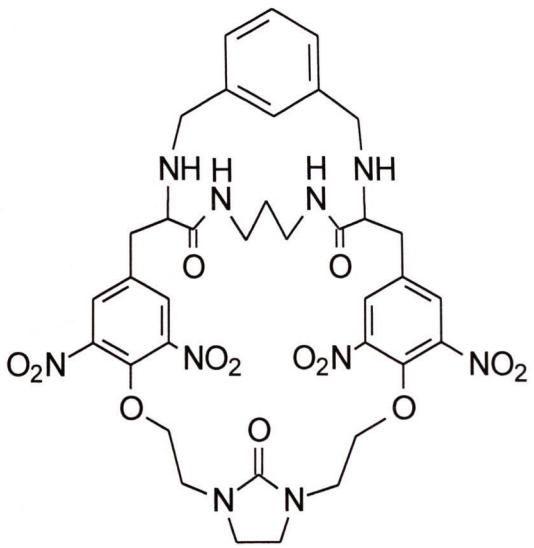
If the guest has a positive charge (*e.g.* an ammonium group), the receptor's cavity may be designed to include a negative charge in order to improve the strength of complexation. Conversely if the guest is negatively charged (*e.g.* halides), the receptor binding site may be furnished with a positive charge to achieve strong complexation. As with all existing receptors, undesired guest complexation is an inevitable problem. This problem can only be minimised by exploitation of the different chemical properties of the guests and by careful engineering of the receptor.

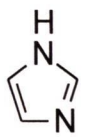
2. In which solvent is the host-guest binding to take place ?

The receptor may be designed to be either water-soluble or soluble in organic solvents. If the receptor is to be water-soluble, lipophilic organic substituents are to be avoided in the synthesis. If the receptor is to be soluble in organic solvents, functional groups bearing a charge are to be avoided. There are then many different types of organic solvents that may be used, *e.g.* polar aprotic solvents (dimethyl formamide), polar protic solvents (methanol), or apolar aprotic solvents like chloroform.

Solvation problems are less problematic in apolar aprotic solvents but are not completely diminished. There is no doubt that the solvent structure unavoidably changes on complexation. The net free energy of this process may either favour or hamper host-guest association irrespective of the direct mutual interactions of host and guest. Experiment has shown that complex stability is even subject to the size of the solvent molecules and a difference in orders of magnitude has been documented in a homologous

series of chemically similar solvents. Still *et al* reported an almost linear relationship between solvent size and binding energy for the imadazole receptor **1.8,** with greater association occurring with increasing solvent molecule size.[12] They suggest that the binding cavity is sensitive to the size and shape of the solvent molecules. Entry to, and solvation of the binding cavity is not as energetically favourable for larger solvent molecules as for smaller solvent molecules. Therefore in large molecular size solvents there is less solvation of the binding site and hence increased binding energy.





1.8 Still's imadazole receptor **1.9 Imadazole**

3. Is there a special purpose that the complexation must serve ?

Host-guest complexation systems serve many different functions *e.g.* catalysis, phase transfer catalysis, ion selective electrodes and membrane transport. Not all of these functions involve maximisation of the binding energy between host and guest, in fact, it is well recognised that strong substrate binding is detrimental to catalysis.[13] In the cases of phase transfer catalysis and membrane transport, it is accepted that strong complexation is needed to transfer the guest from one phase into another or across an organic lipid bi-layer. Similarly for ion selective electrodes, host-guest complexation must be strong enough to induce a response in the electrode even when the guest is present in very low concentrations. Recently it has been generally accepted that anion receptors used in ion selective electrodes should be electrically neutral.[14] Therefore, maximum binding energy is a requisite in their application.

1.6 The nature of anions

Anions are different from all other types of compound by virtue of their negative charge. Therefore, it is expected that coulombic forces largely dominate any long range non-covalent interactions they experience. Anions are all different in nature, some more subtly than others. Their difference may be in size, shape, hardness or lipophilicity. It is these differences that the supramolecular chemist tries to exploit to achieve selectivity in their systems.

The halides F^-, Cl^-, Br^-, and I^- are all monatomic and therefore spherical in nature. As seen in Table 2 their radii increase in size from fluoride to iodide. Compared to the cationic alkali metal ions, they generally show diminished electrostatic interactions with their environment down the series, while their dispersion interactions are greatly enhanced. This results in the easier transfer of anions from aqueous media to organic media. In a two phase system, the water phase has the higher polarizability and can thus stabilise the softer anionic species to a greater extent. Table 2 shows some properties of the halides compared to the alkali metal cations. Anions are considerably larger than cations, and if subjected to the same host-guest binding concept would require larger host structures. Except for anions such as AlH_4^-, $B^-(C_6H_5)_4$ and *closo*-$B_{12}H_{12}^{2-}$, all anions have a lone pair of electrons. This Lewis basicity is the second most important feature

Table 2. Ionic size, experimental enthalpies, and Gibbs enthalpies of hydration for selected ions.[15]

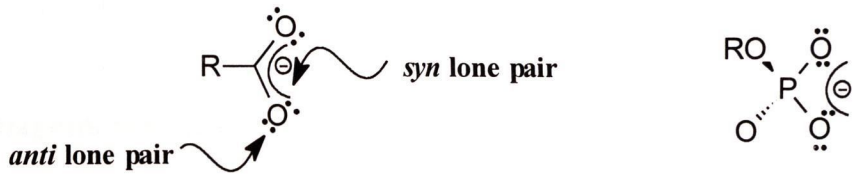
Ion type	Ionic radius [pm]	ΔH_{hyd} [kJ mol^{-1}]	ΔG_{hyd} [kJ mol^{-1}]
F^-	133	-510	-465
Cl^-	181	-367	-340
Br^-	196	-336	-315
I^-	220	-291	-275
Li^+	69	-531	-475
Na^+	102	-416	-365
K^+	138	-334	-295
Cs^+	170	-283	-250
$N^+(C_2H_5)_4$	337	-73	0

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 of binding groups. This is an indispensible tool to differentiate between anions of very similar size structure and charge, namely the biological distinction between phosphate and sulfate

anions.[16] As the halides are spherical, their lone electron pairs do not add directionality to the system and are they are thus difficult to exploit in receptor design.

The oxyanions, phosphates, sulfates and carboxylates are of particular biological interest. They may contain structural elements that modify the properties of the negatively charged substructure. The carboxylate has a flat bonding motif containing two oxygen atoms with two distinct types of lone pair electrons as seen in figure 1.8.

Figure 1.8



Tetrahedral oxyanions (*e.g.* phosphate), can be oriented so that they have an isosteric arrangement of electron lone pairs to the carboxylate anion and can therefore be bound in a similar fashion. Sulfate anions, although far less studied than their phosphate counterparts, are beginning to reveal an important role *in vivo*. For example, Vos *et al* showed that replacing a sulfate group with a phosphate led to a loss of biological activity in the essential oligosaccharide Heparin.

Anion recognition and reasons for its late progress

It is often said that Nature abhors a vacuum. A consequence of this is that a non-preorganised receptor will not contain an empty binding cavity in the absence of the guest. There are two different processes that may occur :

a) The receptor's binding site is solvated (figure 1.9).

One or more solvent molecules may occupy the space of the binding site until the guest is introduced and the solvent is displaced as an unwanted guest.

Figure 1.9

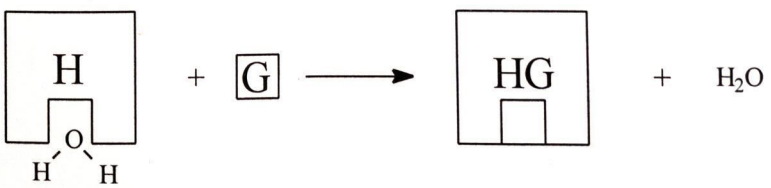


Diagram of a solvated host binding cavity becoming de-solvated on addition of guest.

b) The binding site is non-preorganised (figure 1.10).

In this case, the receptor is non-preorganised for the guest molecule. On introduction of the guest, a binding site is concertedly organised and filled by the guest. The two cases shown are over-simplified but demonstrate the unfavourable thermodynamic effect due to the necessity of large binding sites.

Figure 1.10

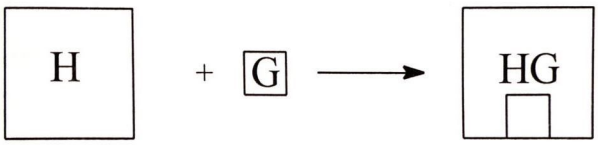
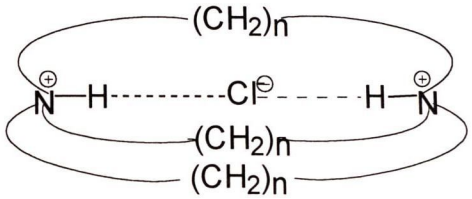


Diagram of an unsolvated host with a non-preorganised binding site

Design and synthesis of receptors for anions is invariably more difficult than that of cation recognition. Construction of hosts for cations evolved rapidly owing to better established concepts of ligand design and the need for less effort in construction for generally smaller and topologically less demanding cations. Designing anion receptors with larger binding cavities possessing well-defined geometrical binding features is not a trivial task. The incorporation of suitable functional groups into the binding site must be carefully designed to avoid any undesirable processes such as self-association.

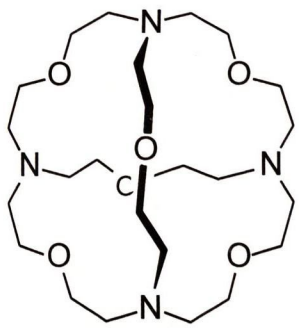
1.7 Introduction to anion binding

The field of anion recognition began in 1968 when Park and Simmons synthesised **1.10** and serendipitously discovered their potencies in binding chloride over all other anions.[17] The encapsulation-type process rested on the correlation of the sizes of the host and guest. Association required the crossing of an activation barrier in accordance with a process in which the guest invades a collapsed molecular cavity. This was later confirmed by an X-ray crystal structure of the complex. The spherical anion is non-covalently encapsulated in a pre-formed molecular cage and held there by an array of hydrogen bonds within the cavity.



1.10 n = 9 or 10

It was eight years later that Graf and Lehn made their discovery of the of the macrocycle **1.11**. The macrocycle was first designed to bind alkali metal cations.[18] On protonation of the four nitrogen atoms, they discovered that 1.11 complexed fluoride, chloride and bromide.[19] X-ray crystallographic analysis showed that the chloride anion is bound in the centre of the cavity by four convergent hydrogen bonds. The fluoride and bromide can be encapsulated in a similar mode but iodide, or any of the other polyatomic anions cannot. The unprecedented selectivity shown for chloride over bromide ($>10^5$) is due to the size of the binding site in combination with an almost undistortable molecular skeleton.



1.11

Graf and Lehn's discovery broadened the interest of many chemists in anion recognition and over the last thirty years, a large number of different binding motifs have been

published. This introduction will attempt to classify these systems according to their guest selectivity, (*e.g.* halides, phosphates *etc*). These classes will then be further divided into subclasses based on the host's primary binding principles (*e.g.* positively charged, neutral receptors and lastly a mixture of both types).

1.8 Contributions in the literature to sulfate and phosphate based guests

A brief introduction to sulfate and phosphate anions together is presented as their size and topology are quite similar. These ions may be mono-anionic (**i** and **ii**) or di-anionic(iii and iv) in nature depending on the pH of their environment. Tri-anionic phosphate (PO_4^{3-}) is not found at moderate pH conditions, and so will not be discussed in this thesis.

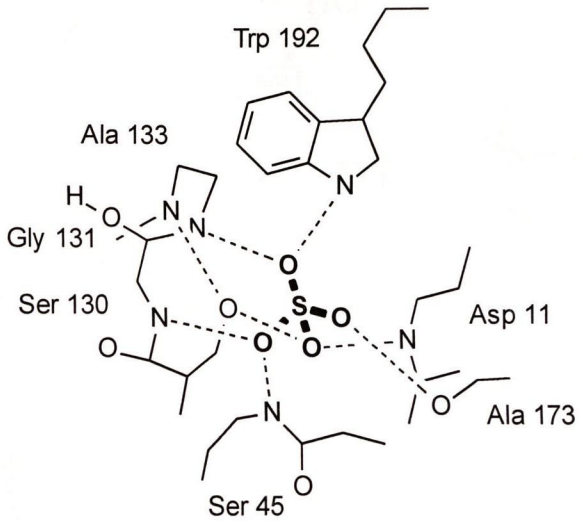
(i) (ii) (iii) (iv)

In recent years, considerable effort has been spent on the development of receptors capable of the specific recognition of phosphate and phosphate-bearing entities, ATP, and nucleic acids.[20] These guests play a key role in critical processes in nature ranging from energy storage, transduction and information processing. As a result, nature has developed a very specific method to manipulate the phosphate group over the sulfate group by using phosphate binding proteins (PBP) and sulfate binding proteins (SBP).

Quiocho *et al* first described the binding properties of SBP's isolated from *Salmonella typhimurium* by X-ray crystallography.[21] Despite the similarities between the phosphate and sulfate anions, the phosphate anion was bound with an affinity of five orders of magnitude lower than the sulfate anion. The ligand binding site is exquisitely designed to bind sulfate but not the weak acid phosphate. When bound, the sulfate is buried deep in the cleft of the protein and is held tightly in place by a total of seven strong hydrogen bonds, five of them donated by the main chain peptide NH groups, a serine hydroxyl and the NH group of a tryptophan residue (figure 1.11). There are no counter charges near the sulfate anion, but it has been proposed that the charges on the sulfate are stabilised by dipole interactions, especially those through hydrogen bonds formed with polarised peptide units. The protein's ability to discriminate against phosphate was explained by

the latter's ability to act as a hydrogen bond donor in a delicately balanced framework of hydrogen bonds buried deep in the protein's interior.

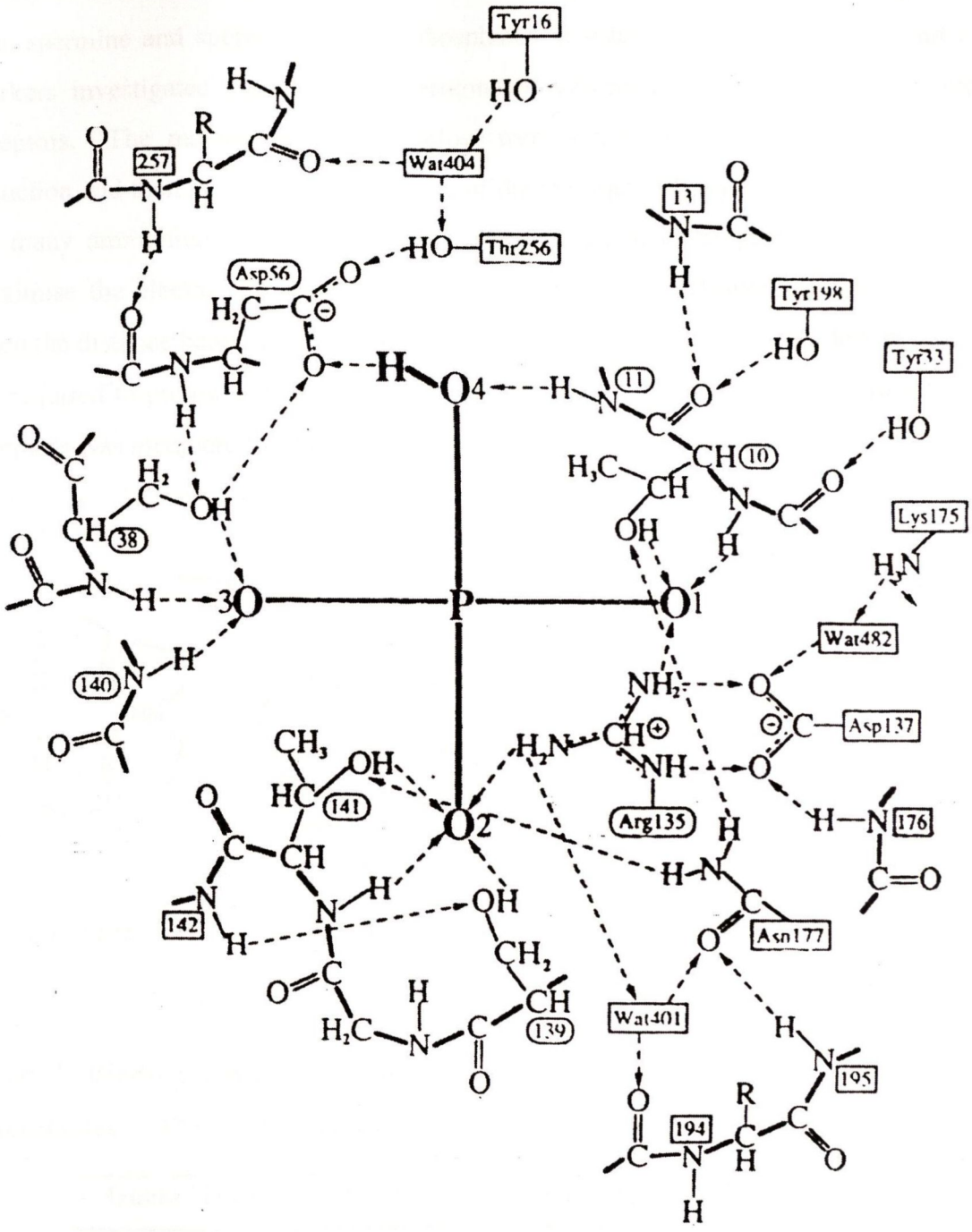
Figure 1.11



S. *typhimurium* SBP-sulfate complex showing the hydrogen bonding interactions based on X-ray crystallography. (Hydrogen atoms are omitted for clarity)

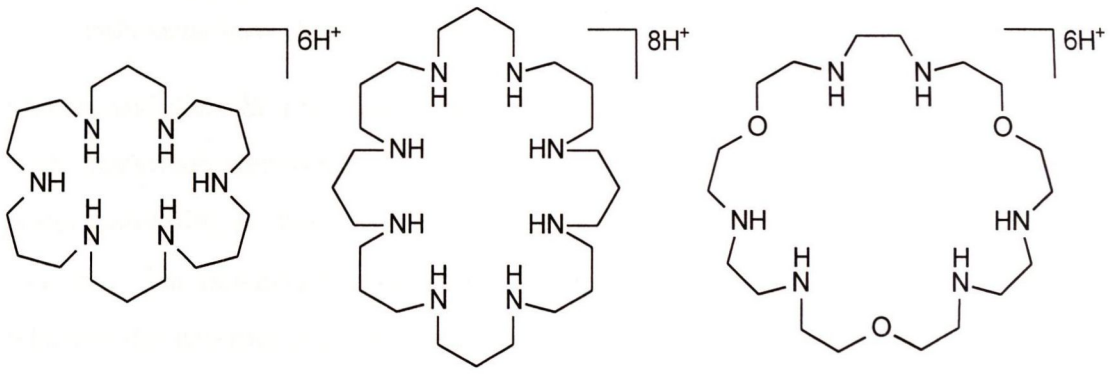
It was not much later that Quiocho published his findings on PBP's.[22] These proteins, being electrically neutral at their binding site, are similar to SBP's by relying on hydrogen bonds to bind the phosphate anion. As seen in figure 1.12, when bound, the phosphate group is held tightly by twelve strong hydrogen bonds all formed with protein groups. The protein has a slight preference for the di-basic phosphate over the mono-basic anion. This preference can be explained from figure 1.12. The O4 accepts an H-bond from an NH of a backbone peptide unit whose carbonyl oxygen is in turn the recipient of two hydrogen bonds. It is also in close contact with $O_{\delta 2}$ of the Asp 56 carboxylate side chain. This close association demands that the two oxygens share a proton on the O4 phosphate, resulting in a H-bond between the phosphate and the carboxylate side chain. This distinctive interaction is the key to molecular understanding of the exquisite specificity of phosphate binding proteins.

Figure 1.12



The crucial difference between SBP's and PBP's is the absence of this carboxylate side chain in the SBP's which is suited to be a hydrogen bond acceptor. Therefore substrate recognition critically hinges on the protonated or deprotonated state of the oxyanions and on the presence or absence of H-bond acceptor groups in the binding site. These biological examples demonstrate the power of H-bonds in enabling proteins to distinguish between phosphate and sulfate.

Approaches to the design of synthetic receptors to date have been based on a large variety of motifs to date. Born from the knowledge that biological linear polyamines (*e.g.* spermine and spermidine), bind phosphates in water at neutral pH, Lehn and co-workers investigated the use of polyprotonated azacrown ethers (**1.12i-iii**) as anion receptors. The macrocycles shown below were synthesised by stepwise alkylation, reduction and then subsequent ring closure of the appropriately substituted tosylamides.[23] As many ammonium groups as possible were placed in close proximity in order to maximise the electrostatic attraction for the anionic guest. However limitations arise when the distance between the cationic centres becomes too narrow. Very low pH values are required to protonate all of the ammonium sites in (**1.12.ii**). Selectivity for all three receptors was mediocre for the anionic guests shown in Table 3.



1.12.i.6H$^+$ **1.12.ii.8H$^+$** **1.12.iii.6H$^+$**

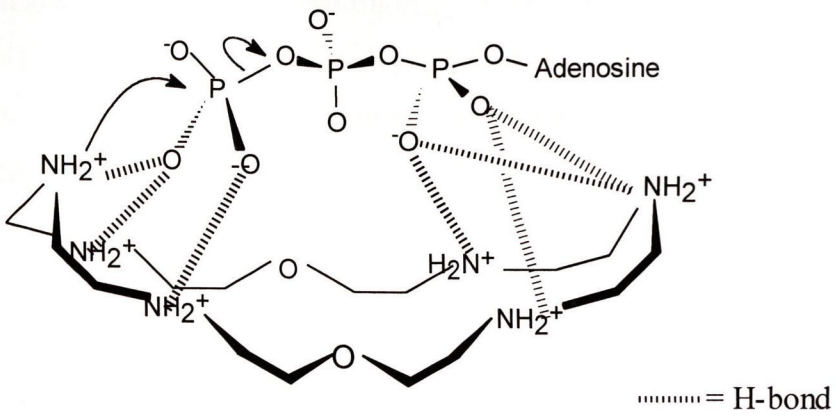
Table 3. Binding constants log K_{ass} (±0.2) for anion binding by polyammonium macrocycles (1.12.i -1.12.iii) in water.[24]

Guest / Host	1.12i.6H$^+$	1.12ii.8H$^+$	1.12iii.6H$^+$
SO_4^{2-}	4.0	4.0	4.5
AMP^{2-}	3.4	4.1	4.7
ADP^{3-}	6.5	7.5	7.7
ATP^{4-}	8.9	8.5	9.1

The oxazocrown ether (**1.12.iv**) was shown to hydrolyse ATP by binding the triphosphate by a subtle array of hydrogen-bonding sites (figure 1.13).[25] Compared to ATPase, the

activity shown by this synthetic enzyme in terms of catalytic turnover is minuscule (10^2; ATPases reach acceleration factors of 10^{10}).

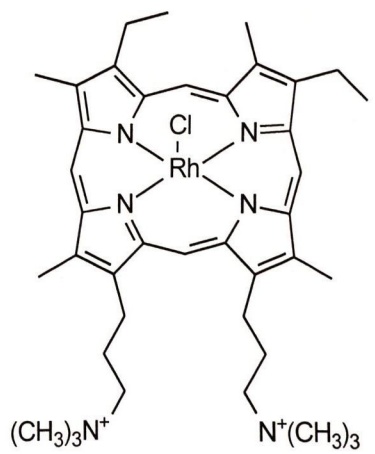
Figure 1.13



(1.12.iv)

One possible binding mode of Lehn's ATP-[24]N_6O_2 complex indicating how cleavage of the terminal phosphoryl group might take place.

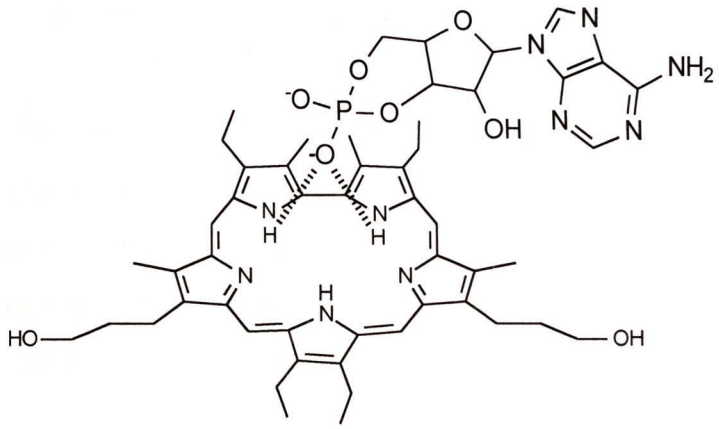
Ogoshi and Kuroda investigated the use of Rhodium (III) porphyrins **1.13** supplemented with quaternary ammonium sites in binding ATP. The high positive charge conveys water solubility to the receptor and prevents the flat molecule from dimerising by stacking. The phosphate group of AMP^{2-} electrically interacts with the ammonium sites, whereas the heterocyclic nucleotide base coordinates to the metal to form the complex. The anion binding capacity of the porphyrins was shown to be completely dependent on the presence of the metal ion. The free tetra-pyrrolic ligand has no binding power at all because the cavity is too small to allow the use of the convergent NH bonding for anion stabilisation.



1.13

Expansion of the porphyrin cavity by incorporating more pyrrole moieties was first achieved by Sessler *et al.* when he showed recently that although these sapphyrins form very stable complexes in methanol with the fluoride anion they also interact with nucleic acids (Figure 1.14).[26] Sapphyrins, unlike porphyrins have a cavity that is large and basic in nature. They remain protonated and positively charged at neutral pH, allowing them to bind nucleic acids *via* "phosphate chelation".

Figure 1.14

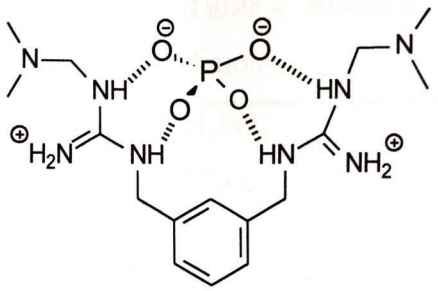


1.14

Sessler's monobasic cAMP-sapphyrin complex.

The guanidinium functional group has been widely used to bind anions and phosphate is no exception. The Hamilton group synthesised a *bis*-guanidinium group based receptor **1.15** which was found to complex phosphodiesters strongly in acetonitrile (figure 1.15) with a K_{ass} of 5 x 10^4 M^{-1}.[27] The receptor was found to increase the reaction rate of transesterifications by 300 compared to a mono-guanidinium receptor with only a 2.5-fold rate increase.[28] Unfortunately the receptor did not discriminate against dicarboxylate anions.

Figure 1.15

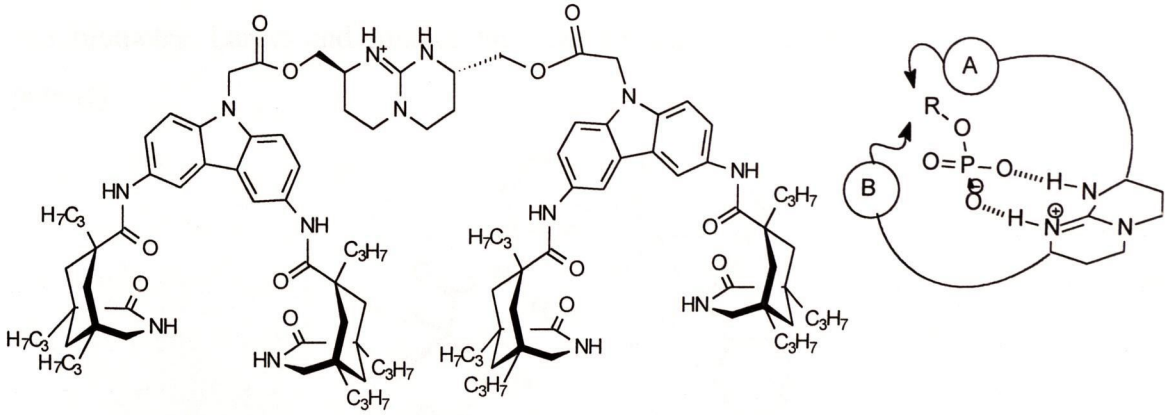


1.15

Binding geometry of Hamilton's bis-guanidinium based receptor complexed with phosphate.

Rebek and de Mendoza used a guanidinium based receptor **1.16** to extract oligonucleotides (dAA) into dichloromethane.[29] Extraction was shown to be achieved not just by phosphate binding but also by aromatic stacking effects and hydrogen bonding. The receptor was shown to be remarkably effective in removing oligonucleotides from dilute aqueous solutions in a two-phase single extraction experiment.

Figure 1.16

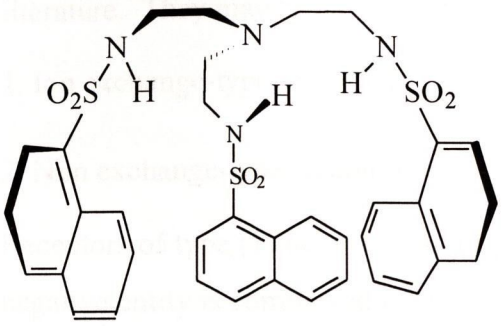


1.16

Rebek and Mendoza's guanidinium-phosphate complex showing the binding concept of the chiral bicyclic guanidinium group. Polytopic guest recognition results from the attachment of additional anchor groups A and B.

Reinhoudt *et al* synthesised a neutral receptor that bound phosphates purely by hydrogen bonding.[30] Surprisingly, the simple neutral host **1.17** was found to form rather more stable complexes with dihydrogen phosphate than sulfates or chloride. The potency of the receptor is believed to be due to the electrophilicity of the sulfonamido NH groups and the preorganisation of the binding site by π-stacking interaction of the naphthyl

groups. Reinhoudt describes this compound as a useful lead in the application of membrane transport and ion selective electrodes because of its electroneutrality.



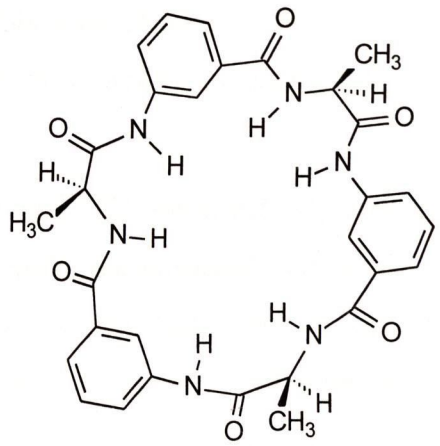
1.17

Table 4. Binding constants for Receptor 1.17

Anions	Stability constants (M^{-1})
$H_2PO_4^-$	14,200
HSO_4^-	38
Cl^-	1,600

The countercation in all cases was TBA. Binding constants were determined from conductommetric studies in acetonitrile at 298K. Errors were reported to be 5% for K_{ass} $<10^2$ and 10% for $K_{ass} < 10^2$.

Ishiba *et al* synthesised a cyclic peptide **1.18**, composed of dipeptide building blocks containing *meta*-aminobenzoic acid as a rigid structure-enforcing unit that ensures an organised array of hydrogen bond donating functional groups, which converge on the centre of the macrocycle.[31] UV spectroscopic analysis shows that the receptor binds *p*-nitrophenylphosphate very strongly (K_{ass} = 1.2 x 10^6 M^{-1}) in DMSO with a 1:1 host-guest stoichiometry. Larger and smaller ring size analogues of **1.18** were of inferior binding potency.



1.18

1.9 Contributions in the literature to halide guests

Park and Simmons' chloride receptor **1.10**, brought about considerable interest in halide recognition due to its biomedical and environmental significance. In recent years there have been many different host systems mentioned with various binding motifs in the literature. They may be all divided into two main classes.

1. Ion exchange-type receptors.

2. Non exchange-type receptors.

Receptors of type (1) have an overall positive charge. It may be logical to suggest that a negative entity is complexed by, a positively charged host, but unfortunately the problem is not that straight forward. In order to conserve electroneutrality, a positive host must be accompanied by a counteranion. Therefore selectivity for the desired anion is not easily achieved as all anions will experience the same coulombic attractive forces of the receptor. This is the reason why selectivity for this class of receptors is mediocre. The great majority of type (1) receptors published to date show a selectivity pattern in accordance with the Hofmeister series.[32] Most of the early work on anion recognition involved positively charged receptors. *e.g.* polyamines are relatively simple to synthesise and on protonation or quaternisation give polycationic structures. Repulsion between the positive charges ensures that they remain separated and organised around a central cavity and the need for elaborate rigid frameworks is eliminated. Halide selectivity has met with mediocre success in this class of receptor and will not be discussed any further in this thesis because of their unsuitability for phase transfer catalysis and ion selective elcectrode development.

Non-ion exchange-type receptors (2) are effectively neutral in nature. Due to the fact that positively charged receptors are unsuitable for phase transfer applications and ion selective electrode chemistry, interest has grown in recent years in the field of neutral anion receptors. Electroneutrality is of prime virtue for three reasons :

i) The internal competition established with counteranions unavoidably present in cationic hosts and frequently responsible for weak binding and poor selectivity is non-existent in this class of receptor.

ii) Neutral receptors are lipophilic in nature and can therefore be studied in organic solvents.

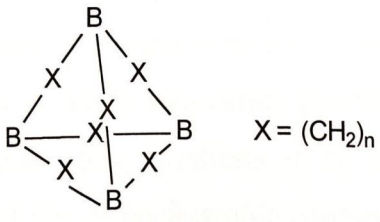
iii) While pure coulombic forces just scan size, density and distance of charge, Lewis acidic/Lewis basic interactions depend on more subtle interactions such as stereoelectronics, hardness/softness of bonds and back-bonding ability *etc.* Neutral receptors are sub-classed here according to the anion binding motif used.

1. Lewis acidic systems
2. Hydrogen-bonding systems
3. Miscellaneous systems
4. Difunctional and zwitterionic systems.

Lewis acidic systems

As in the crown ethers and cryptands, the presence of a number of Lewis basic moieties in a molecular framework furnishes host molecules with a strong cation binding ability. Adopting the same concept for binding anionic species requires the incorporation of a number of Lewis acids into a molecular skeleton with electron deficient sites exposed for interaction with the anion guest. The synthetic concept most widely used for transition metal-based receptors involves the design of an organic ligand that is predisposed to take up a Lewis acidic metal cation in a straight-forward complexation step, leaving the cation co-ordinatively unsaturated. However main groups (III) and (IV) and mercury have been well reputed to form stable covalent bonds and therefore can be embedded into molecular frameworks using covalent connections.

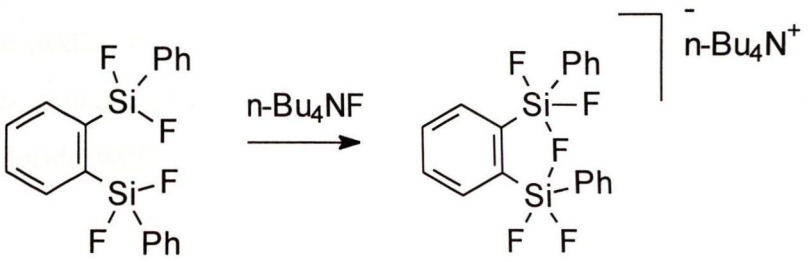
Jacobson *et al* performed semi-emperical molecular orbital calculations (AM1) on the tetracyclic borane **1.19**. On the basis of calculated standard enthalpies of reaction, fluoride and chloride were found to favour the host when n = 3.[33] When free of anion guests, the receptor existed with all boron atoms sp^2 hybridised. On anion inclusion, the boron-boron distances substantially reduced and were accompanied by partial hybridisation from sp^2 to sp^3 of one or more of the boron centres.



1.19 n = 2 - 4

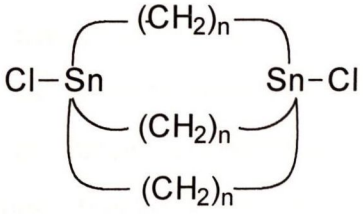
Ito Tamao and Mayashi *et al* found that the *bis-ortho*-silyl substituted benzene **1.20** complexed fluoride in acetone at low temperatures (193 K) with a binding constant K_{ass} = 10^9 M^{-1}.[34] The binding constants were determined by ^1H and ^{19}F NMR spectroscopy by comparative studies. The Lewis acidity of the rigidly held *bis*-silyl substructure sufficed to dissolve potassium fluoride in acetone and tetrahydrofuran even without the help of crown ethers.

Figure 1.17



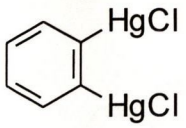
1.20

Newcomb *et al* constructed the stanna (IV) macrocycles **1.21a** and **1.21b** which bound fluoride with a K_{ass} = 1 x 10^4 and 2 x 10^4 M^{-1} in chloroform respectively. **1.21a** was found to discriminate against chloride by a factor of 10^5. The crystal structure of the complex revealed the fluoride anion to occupy the cavity in contact with both tin atoms. Increasing the cavity size by using a longer hydrocarbon link between the tin atoms resulted in the formation of a chloride inclusion complex **1.21b**, however, the chloride in this case was coordinated to only one tin atom.



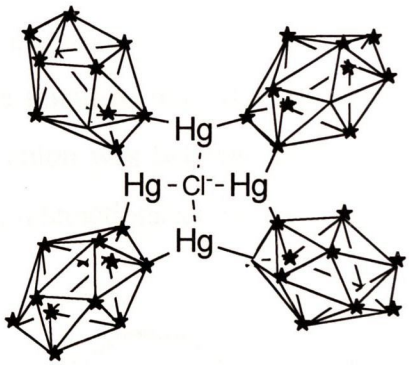
1.21a n = 6
1.21b n = 8

Beauchamp *et al* first exploited the unusual, but favourable ability of mercury to form stable bonds to carbon extending colinearly from the metal.[35] The Lewis acid character of organomercury compounds is due to two empty p-orbitals oriented perpendicular to the metal-carbon axis and to each other. Synthesis of the **1.22** revealed that on binding a chloride anion, no distortion of the stereoelectronic configuration of the system occurred and the anion was simply surrounded by four mercury centres.



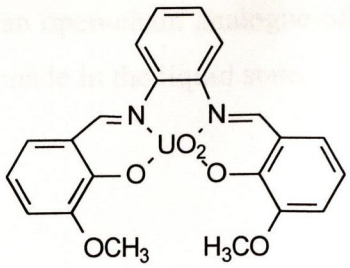
1.22

This favourable phenomenon was elaborated by Hawthorne when he reacted dilithio-*O*-carborane with mercuric dichloride to form the cyclic tetramer **1.23** as a chloride in high yield.[36] X-ray crystal structures confirmed NMR investigations of the chloride anion oriented in the middle of a square, with four mercury atoms forming the corners. The high yield was explained by a template macrocyclisation step, because replacement of mercuric dichloride with a mercuric acetate salt, resulted in the formation of a cyclic trimer.[37]

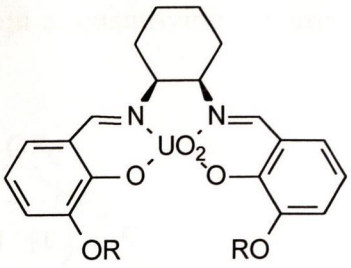


1.23 ★ = BH

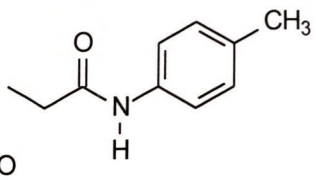
Reinhoudt *et al* elegantly exploited the properties of the uranyl cation in the construction of **1.24, 1.25 and 1.26**.[38] The distinct coordination polyhedron, allows the anchoring of the guest in an organic polychelate, while still one coordination site is open for additional binding of a Lewis base. The attachment of additional anchoring groups recruited from the rich variety of conventional functional groups used in molecular recognition promoted guest selectivity in **1.25** and **1.26**. Hydrogen bonds, a reliable tool for boosting the intrinsic selectivity of uranylsalens, provided the selectivity for $H_2PO_4^-$ over Cl^- by up to a factor of 100 in these systems. X-ray crystal structures reveal the anion to be coordinated to the uranyl Lewis acidic centre with supplementary hydrogen bonds from the amido functional groups of the ligands. It was found that small amounts of water in DMSO destroyed the optimal host-guest configuration of **1.26**, leading to a dramatic drop in the affinity of $H_2PO_4^-$ (K_{ass} = 40 M^{-1} in DMSO/H_2O 9:1 v/v). $H_2PO_4^-$ transport across a supported liquid membrane was achieved using **1.26** as a carrier.[39]



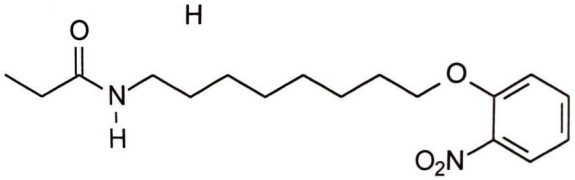
1.24



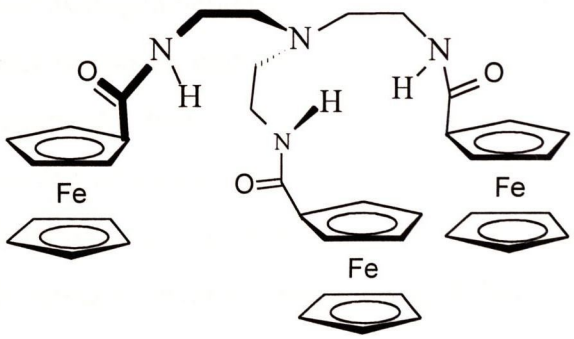
1.25 R =



1.26 R =



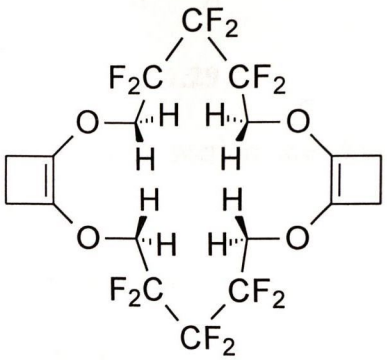
Beer *et al* used the favourable properties of ferrocene when he synthesised the phosphate receptor **1.27**.[40] Using an electrochemical detection technique, the neutral tripodal ferrocene-based receptor was found to bind $H_2PO_4^-$ over Cl^- even in the presence of a ten-fold excess of Cl^-. The anion was believed to be complexed *via* a combination of favorable Lewis acidic-anion interactions as well as amide (-CON-H) anion to hydrogen interactions.



1.27

Farnham *et al* synthesised the fluorocrown ether **1.28**, a novel fluoride ion nesting complex.[41] X-ray crystal structure analysis shows that the central fluoride ion is held within the cavity by the interaction with the four CH_2 groups.[42] Apparently the vicinal CF_2 groups polarise the adjacent C-H bond sufficiently to enable this unusual binding mode. NMR showed that complexation was strong and resulted in the 18-membered ring undergoing substantial conformational change to accommodate the fluoride guest. Preorganisation appears to be essential as fluoride complexation was not observed with

an open-chain analogue of **1.28**. No attempt at quantifying the strength of binding was made in the liquid state.

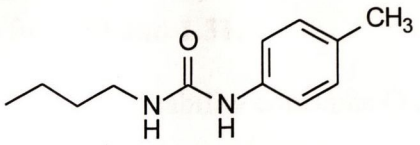


1.28

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 donor moieties. The main virtue of incorporating H-bond donator groups into a receptor is to conserve electroneutrality. Electroneutrality is a desirable property in the applications of membrane transport and potentiometric ion-sensing. The versatility of construction offers many options in receptor design, but the effectiveness largely depends on the extent of solvation. It is a common observation that hydrogen bonding which holds a host and guest together in less polar solvents may totally vanish on switching to water as the solvent. This is due to the low enthalpy of formation of a hydrogen bond in water. The dielectric permittivity, combined with the presence of a delicately balanced network of H-bonds requires only reorientation and rarely *de novo* formation to satisfy all the H-bonding needs of the host and guest. 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.

Kelly *et al* reported the relative binding affinities of oxoanions with a urea-based host **1.29,** discovering unsurprisingly that greater Brönsted basicity and higher charge of the guest led to a higher complex stability.[43] In an analogous system Hamilton *et al* discovered another correlation of improved complex stability, the higher acidity of the H-bond donor host.[44] It is now well known that the dependence on solvent of host-guest association is most important.



1.29

The neutral receptors discussed in this section are divided into four main sections according to their skeletal structure :

1. Calixpyrrole and calixarene-based systems.

2. Sulfonamido, amido and urea-based systems.

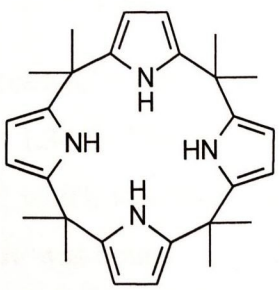
3. Difunctional-based systems.

4. Cholic acid-based systems.

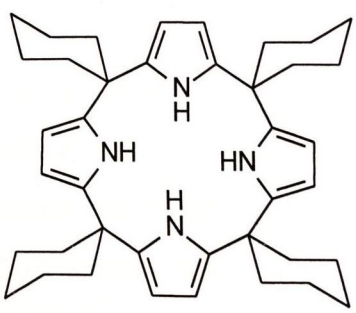
Due to the interest shown in this area in recent years, these divisions have become less distinct and overlap in many areas has arisen.

1. Calixpyrrole and Calixarene-based systems.

In 1886, Baeyer published his work on the condensation of pyrrole and acetone.[45] Although he did not realise it, the white crystalline solid was an octamethyl substituted form of porphyrinogen **1.30**. It was only in the 1960's that Brown *et al.* reported a refined procedure that permitted them to obtain **1.31** in high yield.[46] Sessler *et al*, inspired by Woodward's work on di-protonated saphyrins binding fluoride, studied the properties of **1.30** and **1.31**. His findings not only revealed that they were effective 1:1 anion binding agents in solution, but they were also selective.[47] Specifically they showed a marked preference for fluoride over the other putative anionic guests (*viz.* chloride, bromide, iodide, $H_2PO_4^-$ and HSO_4^- as seen in Table 5).



1.30



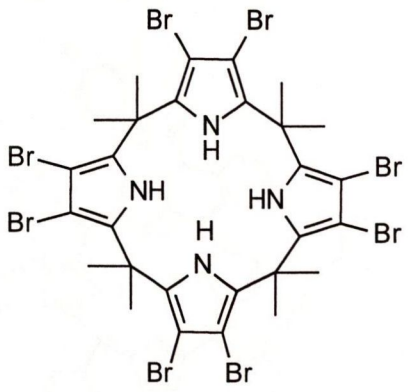
1.31

Table 5. Binding constants for 1.30 and 1.31.

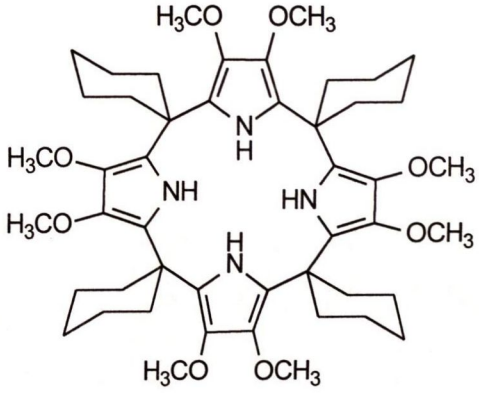
	Stability constants (M^{-1})	
Anion	**1.30**	**1.31**
F^-	17,170 (±900)	3,600 (±395)
Cl^-	350 (±5.5)	117 (±4.0)
Br^-	10 (±0.5)	not determined
I^-	<10	not determined
$H_2PO_4^-$	97 (±3.9)	<10
HSO_4^-	<10	not determined

The counterion TBA was used in all cases. Studies were determined by ^1H NMR titration studies in CD_2Cl_2 at 298 K.[48]

Further work involved the synthesis of calix[4]pyrroles containing functional groups appended to the C-rim of the macrocycle bowl namely **1.32** and **1.33**.[48] Receptors **1.32** and **1.33** were studied using ^1H NMR titration techniques, with **1.33** showing lower stability constants than the β-free substituent analogue **1.31**, presumably due to the electron-donating effects of the methoxy groups. **1.32** shows higher stability constants with anions than its β-free substituent control **1.30**, presumably due to the electron-withdrawing effects of the bromine substituents increasing the acidity of the pyrrole NH protons.



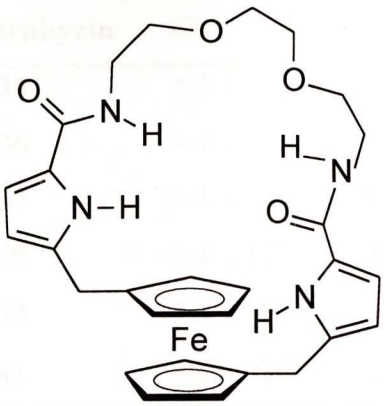
1.32



1.33

Sessler *et al* successfully incorporated pyrrole units into a ferrocene-based system when they synthesised **1.34**.[49] The X-ray crystal structure of **1.34** revealed the presence of an internal 'pocket' which was shown to accommodate either one or two anions depending on size. Fluoride was bound in a 1:2 ratio of host to guest while all other anions tested were found to be bound in a 1:1 stoichiometry. As summarised in Table 6, the host binds $H_2PO_4^-$ and chloride anions with high affinty in acetonitrile. The reduced affinity observed for bromide and HSO_4^- was ascribed to the lower basicity of these anions and

correspondingly weaker hydrogen bond interactions. The system was shown to be a useful precursor for redox-based sensors for $H_2PO_4^-$ and fluoride anions.



1.34

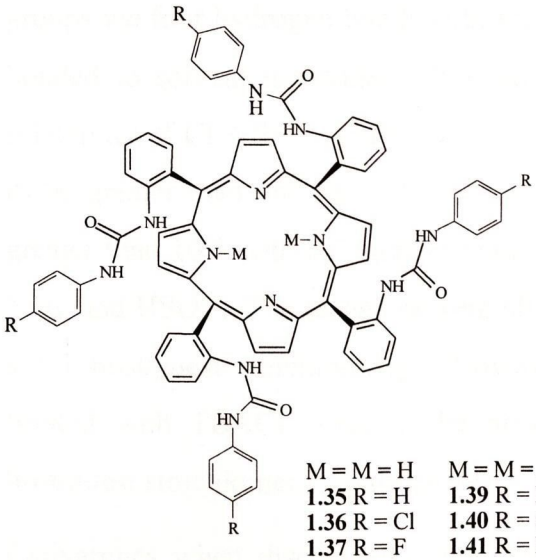
Table 6. Stability constants for 1.34.

Anion	Stability constants (M^{-1})
F^-	$>10^5$
Cl^-	9031
Br^-	857
HSO_4^-	889
$H_2PO_4^-$	11305

TBA salts were used in all titrations. Errors were reported to be <15%. Studies were determined by ^1H NMR titration studies in CD_3CN at 298 K.

Burns *et al* synthesised a series of neutral, urea-appended, free base porphrins along with their Zn(II) complexes.[50] The Zn(II) free porphyrins were found to bind chloride strongly in DMSO-d_6, even in DMSO-d_6/D_2O (88:12 v/v), a much more competitive solvent.

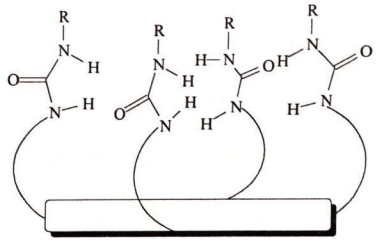
Figure 1.18



M = M = H
1.35 R = H
1.36 R = Cl
1.37 R = F
1.38 R = NO_2

M = M = Zn(II)
1.39 R = H
1.40 R = Cl
1.41 R = F

Cf.



Schematic drawing of Burns' porphyrin skeletal structure.

Table 7. Association constants (K_{ass}) for porphyrins 1.35-1.39, and 1.41 with Cl^-, Br^-, NO_3^-, HSO_4^- and $H_2PO_4^-$.

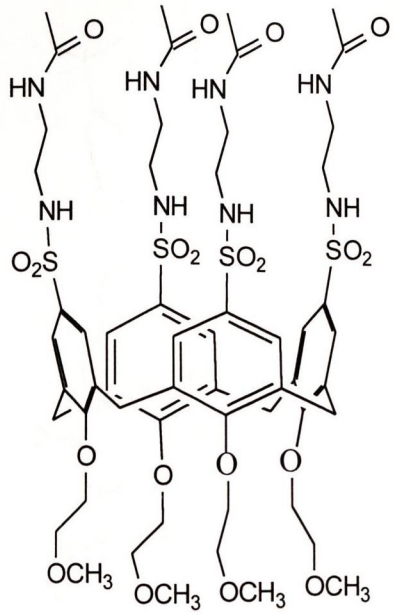
	Stability constant (M^{-1})				
porphyrin	Cl^-	Br^-	HSO_4^-	NO_3^-	$H_2PO_4^-$
1.35	$> 1 \times 10^5$	1.01×10^4	115	90	400
1.36	$> 1 \times 10^5$	1.004×10^4	137	60	300
1.37	$> 1 \times 10^5$	9.99×10^3	147	55	1.4×10^3
1.38	$> 1 \times 10^5$	1.005×10^4	226	163	9.56×10^3
1.39	9.5×10^3	1.51×10^3	*a*	23	49
1.41	9.82×10^3	1.1×10^3	*a*	*a*	489

Errors were found to range from ±10 to 20%. [a] undetermined due to receptor signals broadening in solution. Studies were done in DMSO-d_6.

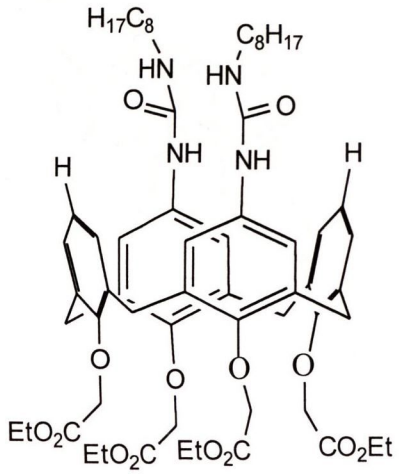
The series was found to exhibit a significant binding selectivity for the spherical halides over the tetrahedral anions $H_2PO_4^-$, HSO_4^- and the trigonal NO_3^- anion in DMSO-d_6. The corresponding metalloporphyrins were found to exhibit a decrease in binding strength and selectivity. X-ray crystal structures of the **1.36**/Cl^- and **1.36**/Br^- complexes revealed the guest bound deep within the porphyrin pocket bound by two adjacent urea functional groups *via* four hydrogen bonds with the two remaining urea functional groups hydrogen bonded to solvent molecules. Receptors **1.35-1.38** were found to exhibit the unique selectivity of $Cl^- > Br^- >> H_2PO_4^- > HSO_4^- > NO_3^-$ in DMSO. K_{ass} for chloride was found to be greater than 10^5 M^{-1}. All of the receptors exhibited a Cl^- binding selectivity of greater than 10:1 with Br^-, greater than 280:1 with $H_2PO_4^-$ and greater than 1000:1 with NO_3^- and HSO_4^-. The receptors were all reported to bind the guests shown in Table 7 in a 1:1 host/guest stoichiometry. However, the binding data described for **1.37** when titrated with TBACl suggests the presence of some self-association or some other host/guest stoichiometry taking place.

Calixarenes when discovered, were first used as cation receptors.[51] They were later found to be a suitable skeletal structure for anion binding when derivatised with H-bond donor substituents. Reinhoudt *et al.* demonstrated the selectivity with which they may be functionalised both at the phenolic (lower rim) and at the *para*-positions of the phenolic rings (upper rim). Functionalisation at the upper rim with sulfonamido substituents leads

to a three dimensional cavity **1.42**, which complexes tetrahedral HSO_4^- better than spherical anions such as the halides, or planar anions like NO_3^- (Table 8).[52]



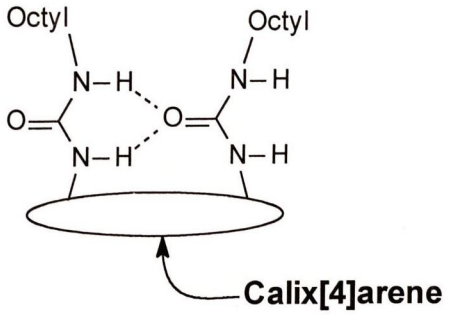
1.42



1.43

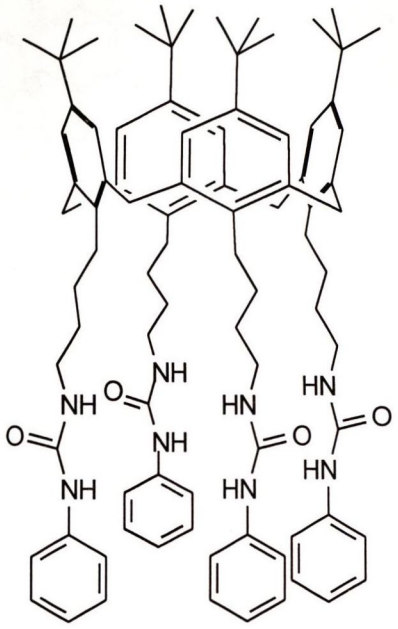
When octylurea moieties were introduced at the upper rim to yield **1.43,** no signs of halide complexation were observed.[53] This was proved to be due to strong intra-molecular hydrogen bonding between the diametrically positioned urea moieties (Figure **1.19**) stabilising **1.43** in a pinched cone conformation that is stable up to 120 °C in $C_2D_2Cl_4$.

Figure 1.19

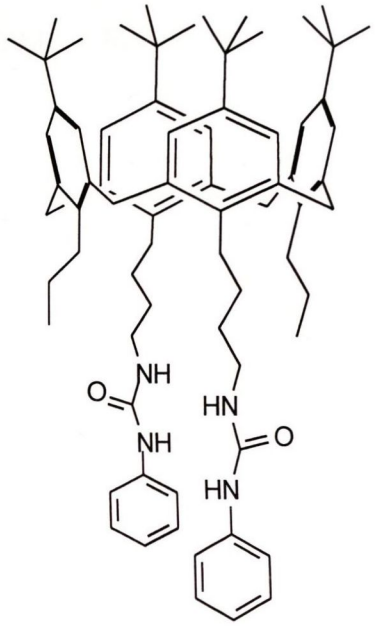


In contrast, calix[4]arenes functionalised at the lower rim with urea substituents namely **1.44** and **1.45**, bind specifically halide ions. The *tetra-kis*-(phenylurea) derivative **1.44** was found to bind chloride and bromide, but not the stronger H-bond acceptors fluoride and $H_2PO_4^-$.[54] Although the bidentate phenylurea derivative **1.45** has four H-bond donor sites fewer than **1.44,** the association constants are significantly higher than those of **1.44**

and the selectivity for chloride over bromide is enhanced (Table 8). This is presumably due to less inter- and intramolecular hydrogen bond formation in **1.45**, than in **1.44**.



1.44



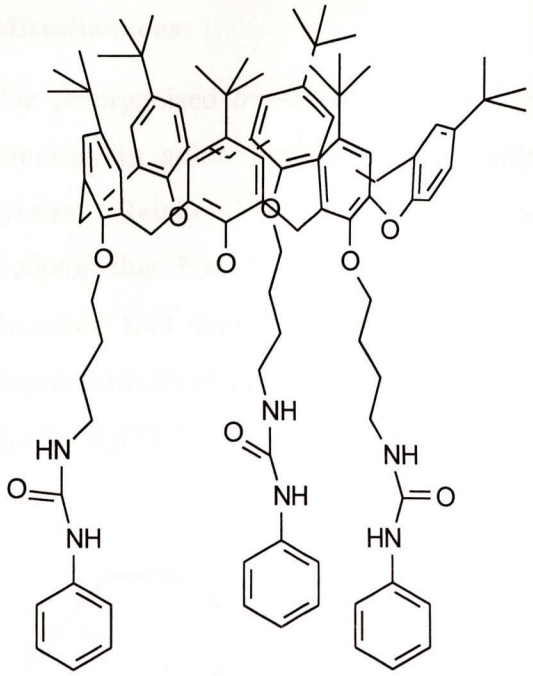
1.45

Table 8. Binding constants for 1.42, 1.44 and 1.45.

	Stability constants(M^{-1})		
Anions	**1.42**	**1.44**	**1.45**
$H_2PO_4^-$	[a]	<1	[b]
HSO_4^-	103,400	[b]	[b]
F^-	[b]	<1	[b]
Cl^-	1250	2015	7105
Br^-	[b]	1225	2555
NO_3^-	513	[b]	[b]
ClO_4^-	<1	[b]	[b]

[a] = mixed complexation, [b] = not determined, Studies were determined by ^1H NMR titration studies in $CDCl_3$ at 298 K. Errors were reported to be < 5%.[54]

The larger binding cavity in *tris*(phenylurea) calix[6]arene derivative **1.46** reverses the guest selectivity as seen in Table 9, bromide binding being favoured over chloride.[55] Apparently this better fit dominates the expected higher H-bonding affinity of the hard chloride anion for the hard urea hydrogens. Admittedly size selectivity is rather unexpected for this type of host, having flexible ligating sites.



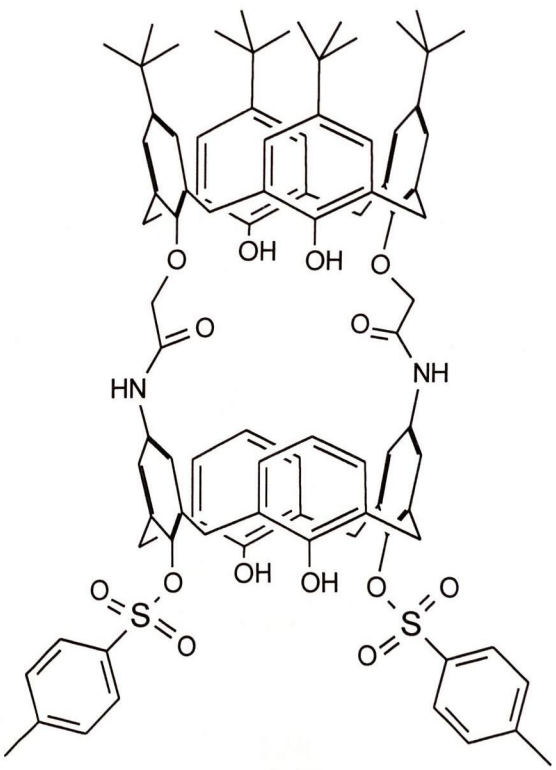
1.46

Table 9. Binding constants for 1.46.

	Stability constants (M^{-1})
Anions	**1.46**
Cl^-	480
Br^-	1,450

1:1 host/guest stoichiometry was observed in both titration studies.
Studies were determined by ^1H NMR titration studies in $CDCl_3$ at 298 K.

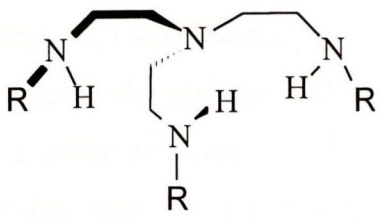
Beer *et al* synthesised a novel *bis*-calixarene **1.47** in which the upper rim of one calix[4]arene is covalently linked *via* amide bonds to the lower rim of another in a head to tail fashion.[56] The resulting cavity was found to be too small for encapsulation of $H_2PO_4^-$ or HSO_4^- but chloride and fluoride are bound, being favoured by the latter with one order of magnitude.



1.47

Miscellaneous

The preorganised *tris*-amino-functional systems **1.48** and **1.49** have attracted a lot of attention in anion binding since Hamilton's work on phosphate binding using this system. Reinhoudt *et al* later discovered that derivatisation of the system led to a receptor that bound not just phosphate but also the chloride anion equally well.[57] Receptor **1.49** showed a higher affinity for $H_2PO_4^-$, presumably due to the increased electrophilicity of the sulfonamide NH groups and higher preorganisation of the binding site for $H_2PO_4^-$.



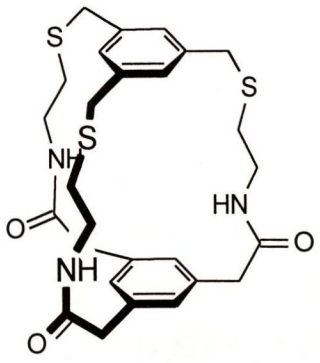
1.48 R = pentyl
1.49 R = tosyl

Table 10. Binding constants for 1.49.

	Stability constants (M^{-1})	
Anions	**1.48**	**1.49**
$H_2PO_4^-$	280	3,500
HSO_4^-	31	38
Cl^-	290	1,600

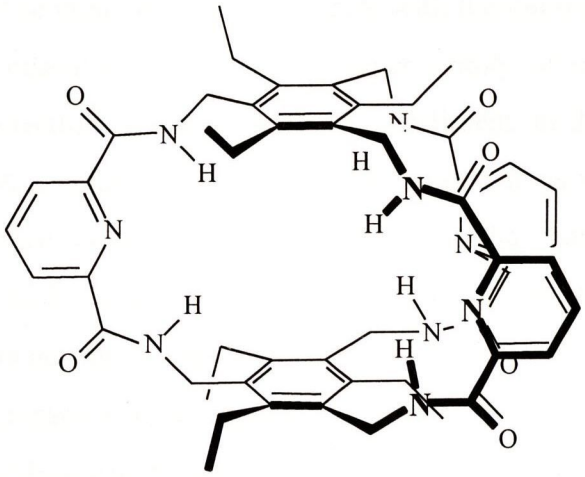
TBA was used as the counterion in all titration studies. Studies were determined by ^1H NMR titration studies in CD_3CN at 298 K.

Pascal Jr. *et al* synthesised a neutral organic macrocycle cyclophane **1.50** with three amide N-H groups directed towards the central cavity.[58] From X-ray crystallographic evidence the cavity size is such that small anions (*e.g.* fluoride and hydroxide) should be easily accommodated by forming three strong H-bonds with the amide NH groups. Preliminary studies show complexation with fluoride in DMSO, but it is uncertain whether the fluoride is encapsulated within the cyclophane. No attempt was made to estimate the binding values for fluoride.



1.50

Anslyn *et al* reported the synthesis of a neutral bicyclic cyclophane **1.51**, similar in cavity size to Pascal Jr.'s cyclophane **1.50** (both are extended in a rigid conformation in which the capping benzene rings are 7Å apart in the solid phase).[59] Anslyn's cyclophane consists of six amide N-H bonds hydrogen bonded to the pyridine nitrogens, forcing a convergent geometry. The alternating steric interactions in the starting material 1,3,5-*tris*-aminomethyl-2,4,6-*tris*-ethylbenzene, ensures that the amine groups are preorganised on the same face for reaction with 2,6-pyridine dicarbonyl dichloride to form the cyclophane in 40% yield. Crystals of the free host and the chloride complex were grown from a CH_2Cl_2/CH_3CN solution for X-ray analysis. The free receptor is shown to be occupied by two molecules of water and one molecule of CH_3CN. The chloride complex shows a 2:1 stoichiometry of guest to host. Each chloride forms hydrogen bonds with the amides of one acyl-pyrimidine unit. The remaining space within the cavity is occupied by a water molecule, which is hydrogen bonded to each chloride and to the last acyl-pyridine unit, forming a cooperative network of eight hydrogen bonds. The host was found to exhibit enhanced binding of acetate and nitrate over the other anions, most likely due to encapsulation of these geometrically equivalent guests causing the formation of several hydrogen bonds to the anion.



1.51

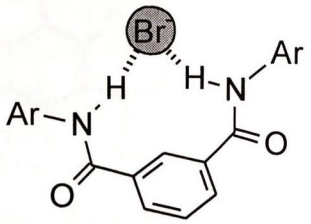
Table 11. Binding constants for 1.51.

Anions	Stability constants (M^{-1})
Acetate	770 ± 120
Nitrate	300 ± 30
Cyanide	115 ± 10
Chloride	40 ± 8
$H_2PO_4^-$	25 ± 5
Bromide	15 ± 8

Studies were determined from ^1H NMR titration studies in 25 % CD_2Cl_2/CD_3CN at 298 K

Crabtree *et al*, inspired by Hamilton and Still's work on nucleotide base receptors based on an isopthalamide skeleton, designed a simple non-preorganised acyclic halide receptor **1.52**.[60] The X-ray crystal structure of [**1.52**•Br]$^-P^+Ph_4$ showed a 1:1 complexation stoichiometry. On complexation, the receptor adopts an unusual *syn-syn* conformation

and forms two hydrogen bonds with the anion. Solubility limitations of **1.52** required a more soluble derivative **1.53**, for determining the strength of binding (Table 12).



1.52 Ar = C_6H_5

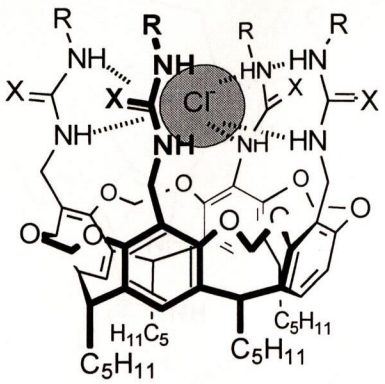
1.53 Ar = *p*-n-BuC_6H_4

Table 12. Binding study results of 1.53.

Anions	Stability constants (M^{-1})
Cl^-	$6.1 \times 10^4 \pm 12\%$
Br^-	$7.1 \times 10^4 \pm 3\%$
I^-	$4.6 \times 10^2 \pm 7\%$

The counterion, Tetraphenylphosphonium was used in all of the above titrations. Binding constants were determined from ^1H NMR titration studies in CD_2Cl_2 at 298 K.[60]

Reinhoudt *et al* synthesised a novel class of neutral anion receptors he named resorcinarene cavitands **1.54a-d**, bearing multiple (thio)urea hydrogen bond donor groups.[61] When compared to calix[4]arenes, resorcinarenes are more rigid platforms, suitable for further functionalisation and they are unable to form intramolecular hydrogen bonds. In chloroform however, they were found to form intermolecular hydrogen bonds. The complexation of chloride with the thioureacavitand is slightly stronger than with the ureacavitand due to the higher acidity of the thiourea hydrogens. The effect of the electron-withdrawing fluoro substituent on the phenyl ring is clear from the increase in K_{ass} value. The bromide and iodide anions were found to be bound weaker because of their "softer" nature. Surprisingly, the "harder" fluoride anion was also bound more weakly due to either its unsatisfactory fit, or the stronger hydration of the anion and hence more weakly association. The receptor **1.54d** was also shown to be effective in the transport of tetrapropylammonium chloride, tetrapropylammonium bromide, potassium chloride and potassium bromide across a lipid membrane.



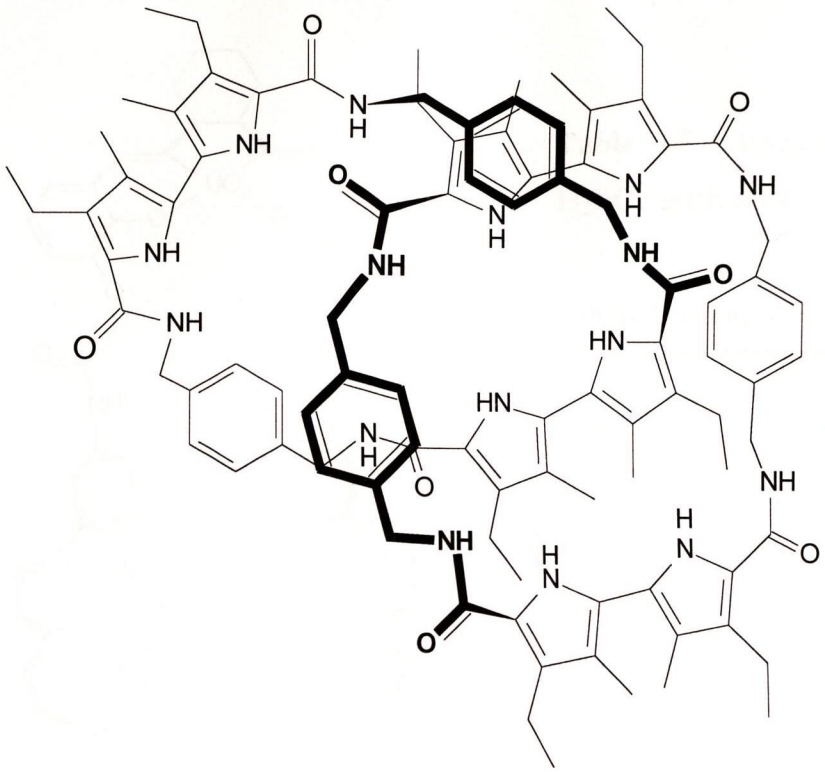
1.54a : X = S, R= *t*-octyl
1.54b : X = O, R= *p*-F-C_6H_4
1.54c : X = O, R= octadecyl
1.54d : X = O, R= CH_2CH_2-*o*-NO_2-C_6H_4

Table 13. Association constants for 1:1 complexes of receptors 1.54a, 1.54b, 1.54c, and 1.54d.

	Stability constants (M^{-1})			
Anion:	**1.54a**	**1.54b**	**1.54c**	**1.54d**
F^-		1.2×10^5		
Cl^-	1.5×10^5	$>2 \times 10^5$	3×10^4	8.6×10^4
Br^-	1.2×10^4	1.8×10^5		1.3×10^4
I^-	1.0×10^4	1.5×10^5		1.4×10^4

TBA salts were used as guest counterion in all the above titrations. Studies were done with halide ions in $CHCl_3$ determined by Infrared spectroscopy.

Sessler *et al* synthesised a bipyrrole-based [2]catenane **1.55**, a novel type of neutral anion receptor.[62] The catenane contains a wealth of H-bond donor groups (pyrrole and amide NH functionalities), and H-bond acceptor sites (amide C=O groups), whose mutual interactions provide at least in part the template effects considered necessary to facilitate catenane synthesis. It was found that the conformational and dynamic behaviour of **1.55** was effected by anion complexation. On addition of TBAF, the catenane changed conformation to accommodate the anion in a 1:2 host:guest ratio. Other anions showed a 1:1 stoichiometry with the receptor showing a high selectivity for chloride.



1.55

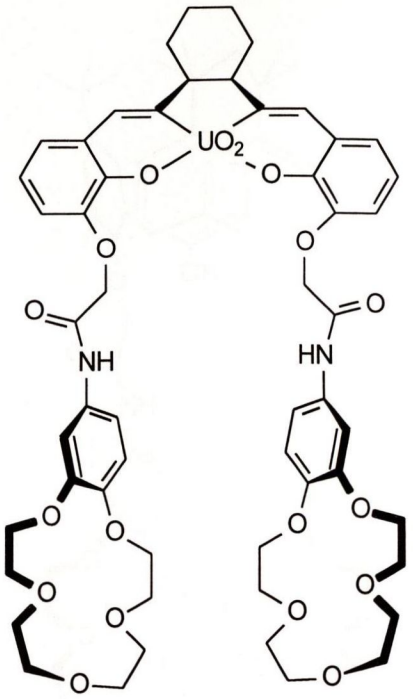
Table 14. Binding constants for 1.55.

Guest	Stability constant
F^-	1.48×10^5
Cl^-	3.55×10^6
Br^-	not determined
$H_2PO_4^-$	$>1 \times 10^7$
AcO^-	9.63×10^5

Binding constants were determined from ^1H NMR titration studies in 1,1,2,2-*tetra*-chloro-ethane -d_2 at 298 K.

Difunctional and/or Zwitterionic receptors

Difunctional receptors are receptors which bind anions and cations simultaneously. Receptors of this type are of interest in the selective extraction and/or transport of metal salts across lipophilic membranes. This area of molecular recognition has only recently attracted attention. It was only in 1994 when Reinhoudt *et al* synthesised a receptor with a well known anion binding site covalently attached to a crown ether to form the neutral ditopic receptor **1.56**.[63] The Lewis-acidic UO_2^+ unit together with the amido functionalities are effective anion receptors with a high selectivity for $H_2PO_4^-$. The [15]Crown-5 ether is a well known cation binder with a high selectivity for K^+. The electrochemical behaviour of **1.56** did not change on the addition of potassium tetraphenylborate, probably since the crown ether moieties in **1.56** are situated far away from the redox-active UO_2 centre. FAB mass spectra of the 1:1 complex of **1.56** and $KH_2PO_4^-$, prepared by mixing the host and guest in acetonitrile with 1% water and evaporating the solvent, gave an intense peak corresponding to [**1.56** + K^+] and [**1.56** + $H_2PO_4^-$] in the FAB^+ and FAB^- spectra respectively.



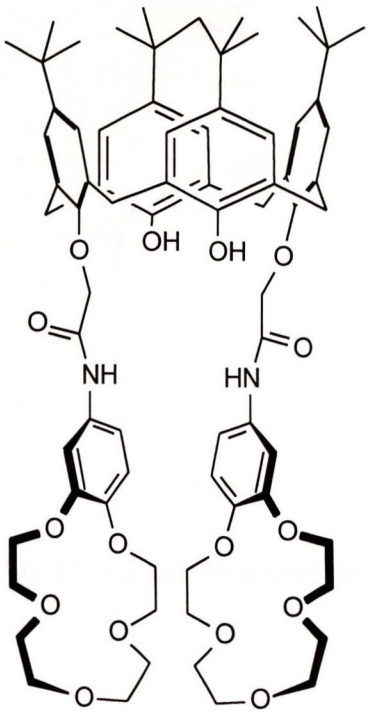
1.56

Table 15. Association constants K_{ass} of $H_2PO_4^-$ with host 1.56.

Anion/Technique:	Stability constants (^{1}H NMR)	(Voltammetry)
$H_2PO_4^-$	1.1×10^3	1.3×10^3

TBA was used as the counterion in both titrations. Studies were determined from ^{1}H NMR in DMSO-d_6, and Cyclic voltametry in 2% water in DMSO.

Beer *et al* used a calix[4]arene, substituted at the lower rim by two benzo-15-crown-5 groups to make **1.57,** used to complex both anions and cations simultaneously.[64] The idea was similar in principle to Reinhoudt's uranyl ditopic receptor **1.56**. ^{1}H NMR titration experiments with TBACl and $TBAHSO_4$ gave little indication of complexation. However, anion titration experiments with potassium and ammonium complexes of **1.57** in CD_3CN gave evidence of 1:1 stoichiometric anion recognition of Cl^-, NO_3^- HSO_4^- and $H_2PO_4^-$ as summarised in Table 16.



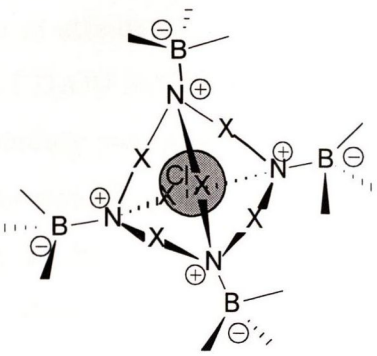
1.57

Table 16. Anion stability constant (K_{ass}) data for potassium and ammonia complexes of 1.57.

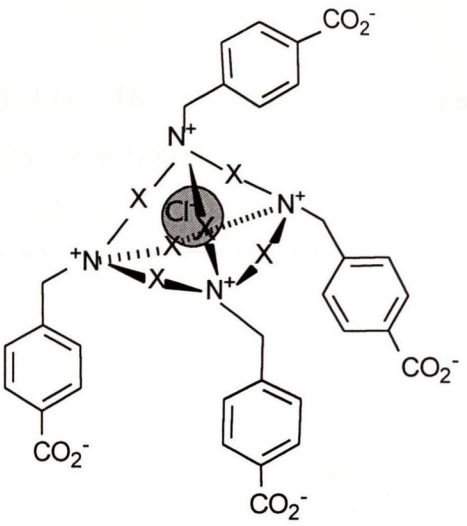
Complex	Anion	Stability constants (M^{-1})
[K**1.57**]PF_6	Cl^-	3,500
[K**1.57**]PF_6	NO_3^-	1,300
[NH_4**1.57**]PF_6	NO_3^-	1,800
[K**1.57**]PF_6	HSO_4^-	5,600
[NH_4**1.57**]PF_6	HSO_4^-	6,400
[K**1.57**]PF_6	$H_2PO_4^-$	$>10^4$
[NH_4**1.57**]PF_6	$H_2PO_4^-$	$>10^4$

Stability constants were determined in CD_3CN with errors ≥15%. Complexes with $H_2PO_4^-$ were too strong to be accurately measured, so an estimate was given. The K_{ass} values were determined by ^1H NMR titrations.

Schmidtchen *et al* synthesised zwitterionic macrotricyclic tertiary amines of high connectivity and rigidity (*ie.* **1.58** and **1.59**).[65] The receptor **1.58** contains four borane-amine dative bonds in a fixed orientation with their positive ends securely pointing towards the central cavity as confirmed by X-ray crystal structure. A large number of inorganic anions were complexed in chloroform solution by the borane-amine adduct **1.58**, with discrimination according to the anion size. A strictly 1:1 host:guest ratio was observed for all the anions bound. The zwitterionic receptor **1.59** showed extraordinary solubilisation in water, testifying indirectly that, intramolecular ion pairing is negligible.[66] ^1H and ^{35}Cl NMR titration data revealed the formation of stable complexes of halide and cyanide in water (K_{ass} = 300-6 x 10^3 M^{-1}). Van't Hoff plots characterised bromide and iodide binding as enthalpically driven, as expected for an inclusion process.



1.58 X = $(CH_2)_6$



1.59 X = $(CH_2)_6$

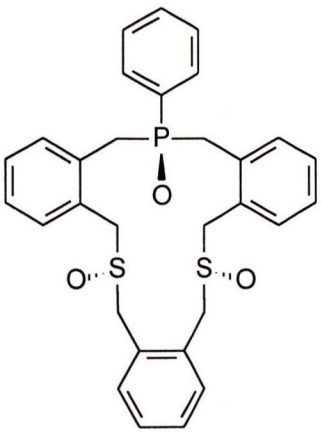
Table 17. Association constants K_{ass} (M^{-1}) for 1.58.

	Association constants (M^{-1})	
Anion	**1.58**	**1.59**
Cl^-		270
Br-	12.5 ± 1.25	2,150
I^-		6,480
CN^-		950

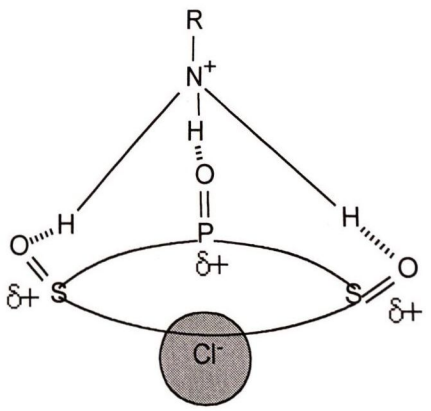
[a] TBA salts were used in NMR titration. Studies were done for **1.58** and **1.59** in 20 vol.% $CD_2Cl_2/CDCl_3$ at 297 K, and D_2O at 327 K respectively.

Gellman *et al* reported the synthesis of macrocycle **1.60**, a phosphine oxide disulfoxide that was found to bind ammonium ions in methanol-chloroform solutions.[67] He later

Figure 1.20



1.60



Diagrammatic representation of Gellman's phosphine oxide disulfoxide chloride receptor showing one possible mode of binding of an ammonium chloride

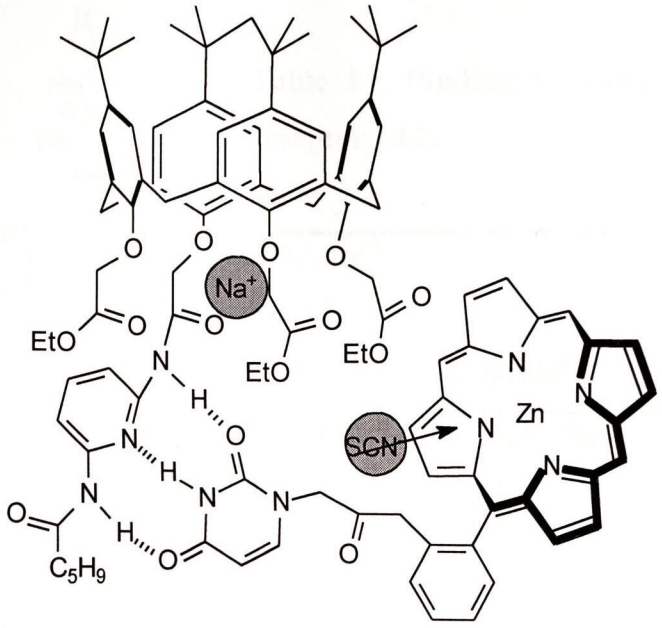
reported that it also bound halides with the order of affinity $Cl^- \approx Br^- > I^- > F^-$ in 2 vol % CD_3OD in $CDCl_3$ (Table 18).[68] Guest binding was found to be due to the sulfur and phosphine oxide dipoles pointing to the same face due to the peculiar stereochemistry of the sulfur centres, therefore presenting a parallel alignment. The opposite hemisphere, the macrocycle then exposes a surface of high positive potential available for anion association. Addition of fluoride, the anion of highest charge density, resulted in no signs of binding. However **1.60** readily formed complexes with the other halide anions. The low affinity of **1.60** for fluoride was explained to be due to the solvation of this anion by the methanol. The anion binding simultaneously resulted in an array of oxide functions on the opposite face of the macrocycle, which are known to be good hydrogen bond acceptors, thus binding the cation too.

Table 18. Binding constants K_{ass} (M^{-1}) for 1.60.

Anion	Stability constants (M^{-1})
F^-	(very weak)
Cl^-	65
Br^-	65
I^-	40
HSO_4^-	(very weak)
$H_2PO_4^-$	(very weak)

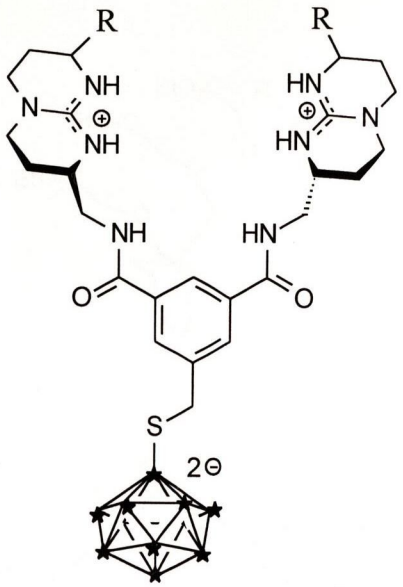
Studies were done in 2 vol. % CD_3OD in $CDCl_3$.

Reinhoudt *et al* synthesised a self-assembling bifunctional receptor **1.61**.[69] The strategy behind this was the simultaneous complexation of anion and cation by a cationic and an anionic binder non-covalently held in solution. The cation receptor component, the calixarene, is a well known receptor with a high selectivity for sodium. The zinc porphyrin was expected to bind anions in aprotic solution. The cation and anion receptors are functionalised with complementary diamidopyridine and thymine units respectively to ensure self-assembly. Addition of the porphyrin component to a toluene-d_8 solution of the [calixarene•Na^+SCN^-] a pronounced interaction resulted, with a stability constant (K_{ass}) of 2.8×10^4 M^{-1}, with a 1:1 host :guest stoichiometry.



1.61

Schmidtchen *et al* synthesized a novel neutral zwitterionic receptor that was found to bind sulfate in DMSO.[70] The foldable *bis*(guanidinium) component of **1.62** is of proven utility in oxoanion complexation and the covalent attachment of the anionic *closo*-borane cluster moiety annihilates the net charge, conserving electroneutrality and hence hydrophobicity of the resulting zwitterion. The icosahedral borane cluster was chosen for conservation of electroneutrality in the system because of its chemical stability, and its lipophilic periphary with very poor hydrogen bond and Lewis acid acceptor properties, *ie*. its two-fold negative charges cannot be screened by protonation. The covalent network assures the segregation of charge, prohibiting internal collapse of the oppositely charged moieties. Dimerisation of the receptor occurred in DMSO however (K_{dim} = 250 M^{-1}). On addition of singly charged anions (*e.g.* Br^- and NO_3^-) NMR studies revealed no effect on the receptor. With sulfate however, several protons showed marked shifts, indicating strong host-guest interactions with fast exchange kinetics.



1.62 ★ = BH
R = $CH_2OSiPh_2{}^tBu$

Table 19. Binding constants K_{ass} (M^{-1})[a] for receptor 1.62.

anion	Stability constant (M^{-1})
NO_3^-, Br^- [b]	no effect
SO_4^{2-} [b]	1.0×10^3
p-nitrophenyl phosphate[c]	7.0×10^3

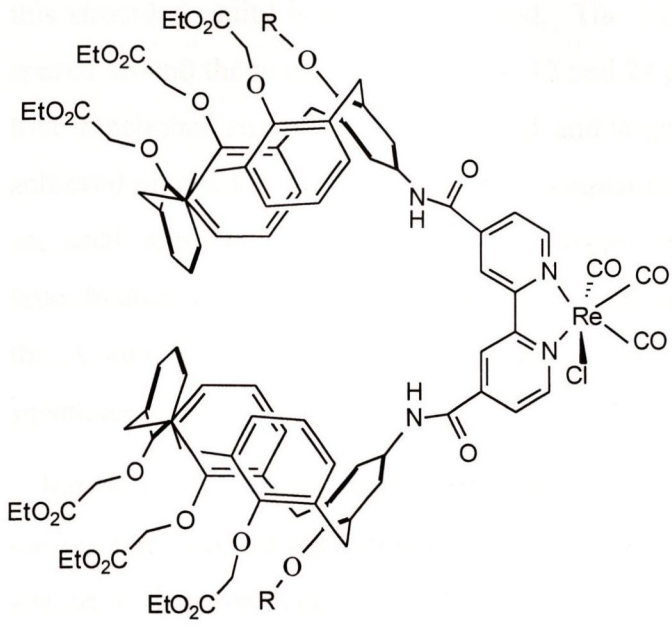
[a]Tetraethyl-ammonium salts.

[b]Monosodium[2.2.2]cryptates.

Disodium[2.2.2]cryptates.

Studies were done in DMSO at 298 K.

Beer *et al* synthesised heteroditopic *bis*(calix[4]arene) rhenium(I) dipyridyl receptors **1.63** and **1.64** which simultaneously bind alkali metal cations and an iodide anion with positive cooperativity.[71] Similar to his work published earlier, in covalently bonding a well known cation binder (in this case a lower rim ester functionalised calix[4]arene) to a well known anion binder (in this case a rhenium organometallic combined with amide ligands) alkali metal/iodide recognition is achieved. Upon alkali metal cation complexation at the lower rim in **1.63**, the upper rim is concertedly organised for iodide binding. The addition of $LiClO_4$, $NaClO_4$, and KF_6 salts caused the ^1H NMR to broaden, and sharpen again after two equivalents were added, suggesting a $2M^+$:L stoichiometry for binding. TBA chloride and iodide addition resulted in 1:1 stoichiometry with Cl^- complexation too strong ($K_{ass} > 10^4$ M^{-1}) to estimate in acetonitrile. The iodide titration experiments were repeated with two equivalents of alkali metal salt (Table 20).



1.63 R = H
1.64 R = CH_2CO_2Et

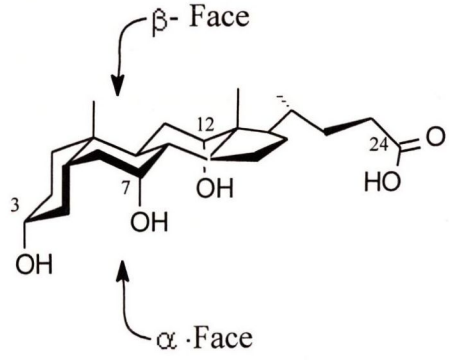
Table 20. Binding constants for 1.63 and 1.64 with iodide in the presence and absence of alkali metal cations.

Receptor	Metal cation	$K_{ass}(M^{-1})$
1.63	None	67
1.63	Li^+	294
1.63	Na^+	202
1.63	K^+	100
1.64	None	40
1.64	Li^+	305
1.64	Na^+	322
1.64	K^+	209

Errors were estimated to be ≤5%. Titrations were carried out in the presence of 2 equivalents of alkali metal cation salt, perchlorates for lithium, sodium and hexa-fluorophosphate for potassium. Studies were done in CD_3CN.

Cholic acid-based receptors

Bile acids have attracted the attention of a number of research groups recently due to their ready availability and their unique structural features. The Davis group in this department has been utilising cholic acid **1.65**, primarily due to its rich functionality.



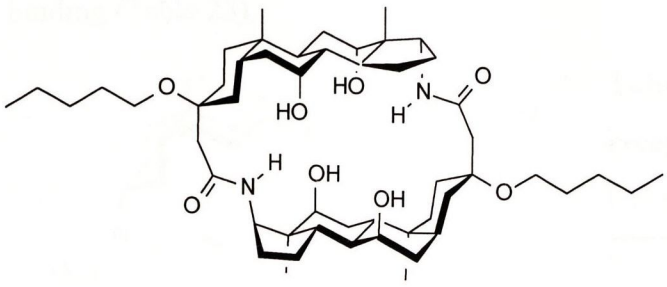
1.65

Cholic acid may be described as a facial amphiphile with a concave hydrophilic α-face and a convex hydrophobic β-face. The array of hydroxyl groups lining one surface of the bile acid provides opportunities for the attachment of appropriate molecular units to generate structures of supramolecular interest. The steroid nucleus is one of the largest

rigid units readily available with multiple chiral centres. The biological importance of this structural entity is well documented. The four functional groups are fairly evenly spaced around the molecule (at the 3, 7, 12 and 24 position). Differentiation between the four functional groups can be achieved and a great deal of synthetic control can be achieved in transformations. The C_3-OH is equatorial whereas the C_7 and C_{12}-OH groups are axial, allowing the former to be selectively derivatised. Differentiation between the latter hydroxyl groups while possible, is not a trivial task. The *cis*-ring junction between the A and B, rings imparts a curvature to the skeleton, resulting in the equatorial substituent at the C_3 position directed at *ca.* 90° to the main portion of the skeleton.

It has been well documented that the bile acids fulfill various physiological functions during digestion and resorption of fat and cholesterol.[72] Outside of medical interest, the steroid nucleus has been viewed as a 'rigid lump of grease'. It is valuable for promoting liquid crystallinity and also in the study of hydrophobic aggregates such as lipid membranes. Because of the unique disposition of functional groups around the nucleus, the Davis group has synthesised receptors for the recognition of polar organic molecules, N-acetyl protected amino acids, and inorganic anions.

The discovery of the crown ethers and cryptands, *neutral organic molecules* capable of surrounding inorganic cations and promoting their solubility in non-polar organic media, prompted the study of "anion equivalents" in that they bind anions using convergent, preorganised functionality mounted on a macrocyclic framework. The Davis group reported the synthesis of a rigid, neutral macrocyclic organic receptor **1.66,** preorganised for halide binding.[73] The rigid framework of the cholaphane maintains a binding cavity and limits the possibility of intramolecular hydrogen bonding. The binding cavity is bordered by a high density of convergent, polar functional groups preorganised to bind spherical anions by multiple H-bond donation. The hydroxyl groups were intended to create an environment mimicking aqueous solvation of the halides, but they are surrounded by a lipophilic envelope. As outlined in Table 21, the host affinity increased in chloroform with smaller and more basic halides.



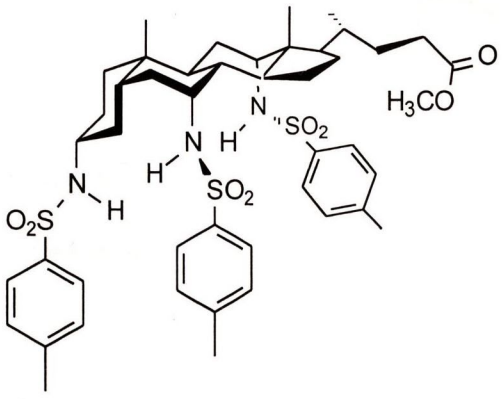
1.66

Table 21. Binding constants for 1.66.

Halide/K_{ass} (M^{-1})		$-\Delta G^{o}$ ($kJmol^{-1}$)
F^-	3220 ± 350	20.01
Cl^-	990 ± 80	17.09
Br^-	250 ± 20	13.68

Binding study results from ^1H titrations of **1.66** with TBA halides in $CDCl_3$ at 298 K.

Recently, a more "tunable" anionophore strategy using cholic acid as a starting material was presented by this group, which yielded a receptor with exceptional affinity for the chloride anion in a non-polar organic solvent.[74] The monomeric receptors **1.67**, **1.68**, **1.69** and **2.6** were based on a podand type architecture, with three NH-containing groups attached to the steroid. From molecular modelling studies, the three NH protons can be positioned to form hydrogen bonds of *ca.* 2.5Å to a centrally located guest. The receptor formed 1:1 complexes with the anions shown in Table 22, with a ten-fold selectivity for Cl^- over the other anions tested. The receptor was originally made *via* a somewhat lengthy synthesis involving 18 steps.



1.67

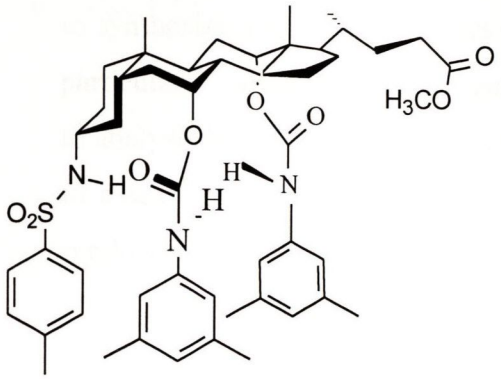
Table 22. Binding constants for 1.67.

anion	Stability constants
Cl^-	92,000 (±28,000)
Br^-	9,200 (±700)
I^-	525 (±45)
TsO^-	950 (±80)

Association constants were determined by ^1H NMR titrations with tetrabutylammonium salts in $CDCl_3$ at 298 K.

An alternative podand-type structure based on cholic acid, accessible *via* an easier synthesis, was the *bis*-carbamoyl sulfonamide **1.68**.[74] Although less preorganised than **1.67**, it can form a slightly larger binding pocket with potential NH · substrate distances of *ca.* 2.7Å. The addition of TBA salts to the receptors **1.68**, **1.69** and **1.70** in $CDCl_3$

caused changes in the receptor's ^{1}H NMR spectra, indicative of 1:1 host guest:anion binding (Table 23).



1.68

Table 23. Binding constants for receptor 1.68.

Guest	Stability constants (M^{-1})
F^-	15,400 (±1500)
Cl^-	7,200 (±660)
Br^-	7,200 (±760)
I^-	930 (± 70)
TsO^-	865 (± 120)

TBA salts were used in $CDCl_3$ as solvent.

Control experiments with **1.69** and **1.70** established that the generally high binding constants were due to cooperative action of the three H-bond donor groups in each receptor, with the major binding contribution coming from the 3α- NH proton. This is presumably due to its higher acidity and hence better H-bonding ability. All of the receptors reported showed a 1:1 host/guest stoichiometry for the anions shown in Tables 23 and 24 with no signs of dimerisation or self association.

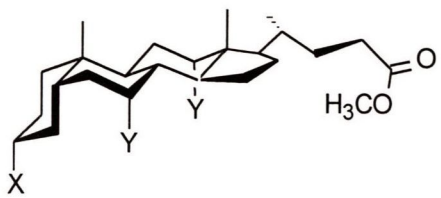


Table 24. Binding study control experiments with 1.69 and 1.70.

Receptor	Substituent X	Substituent Y	Binding constant K_{ass} (M^{-1}) for TBABr.
1.69	TsNH	OCHO	40 (± 1)
1.70	EtO(CO)O	O(CO)NHar (ar = 3,5-dimethylphenyl)	17 (± 3)

TBA salts were used in all titrations. Binding constants were determined from ^{1}H NMR titration studies in $CDCl_3$ at 298 K.

1.10 Aims of the project

The aims of this project were :

1) to synthesise a range of receptors derived from cholic acid for halide binding, in particular, a series based on the same skeletal structure as **1.68**.
2) to analyse the receptors' ability to bind anions in chloroform.
3) to assess the binding ability of receptors published in the literature, receptors synthesised by other research groups and receptors synthesised by other members of our research group using the same conditions for comparative reasons.

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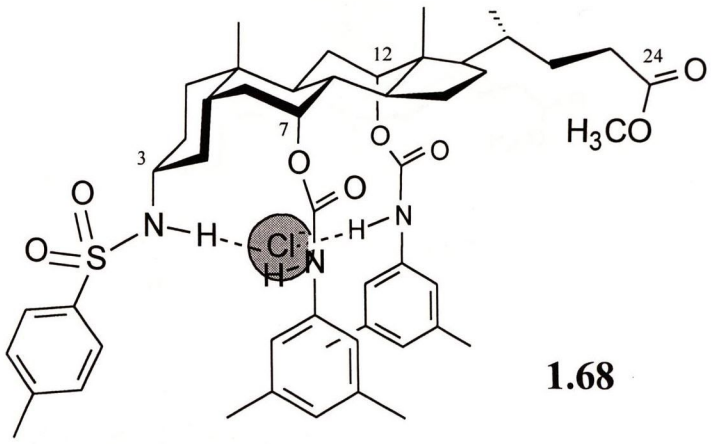
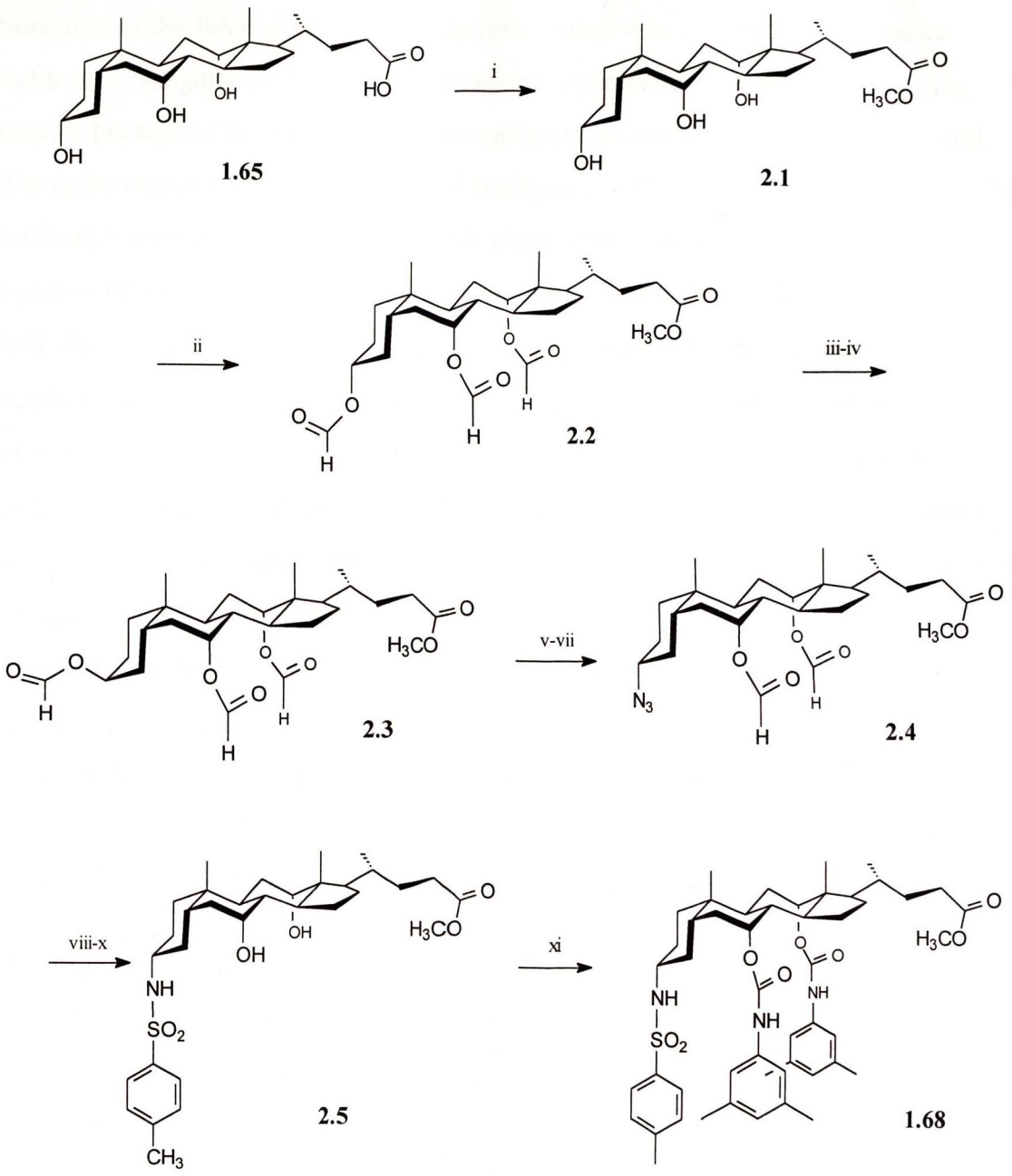
Chapter Two : Discussion on the synthesis of the receptors analysed for anion binding in this thesis.



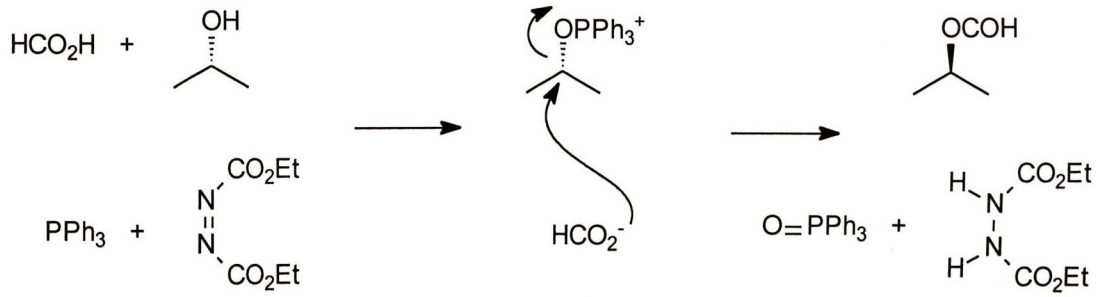
1.68

The synthesis of Perry's receptor **1.68** is described in scheme 2.0. Firstly, the carboxylic acid group of cholic acid was protected by esterification. Of the three hydroxyl groups present in cholic acid, the 3α-hydroxyl is distinguished by its equatorial orientation. Therefore, its conversion to a 3α-amine could be achieved selectively. *p*-Toluenesulfonyl chloride was chosen to react with the 3α-amine for a number of reasons. The product is UV active and can therefore be easily visualised by TLC. The *para*-substituted benzene displays an NMR signal easily discernible from the other protons on the receptor. Above all, it was chosen for its chemical stability and relatively high acidity. The 7 and 12-hydroxyl groups were converted to carbamates to lower the hydrogen-bond donor moieties with respect to the steroidal framework so that they are closer in space to the 3α-NH group. The carbamates are also easy to prepare (differentiation between the 7 and 12-hydroxyl groups was not necessary, because both react with isocyanate in the last step yielding the receptor **1.68**). The hydrophobic aromatic rings of the carbamate groups promote the solubility of the receptor in chloroform. The protons of the 3,5-dimethyl substituted benzene are distinctive by ^{1}H NMR and so can be easily followed during binding studies.



Scheme 2.1 *Reagents, conditions (and yields)* reported by Perry[1]: i, *conc.* H_2SO_4 (cat.), dry MeOH, room temp, 24 h (93%); ii, HCO_2H, cat. $HClO_4$, 50 °C, 2 h, (78%); iii, NaOAc.$3H_20$, MeOH, room temp, 4 days, (87%); iv, DEAD, PPh_3, HCO_2H, dry THF, rt, 24 h, (90%); v, NaOAc, MeOH, room temp, 5 days, (91%); vi, CH_3SO_2Cl, pyridine, dry CH_2Cl_2, 0 °C, 15 mins, (87%); vii, NaN_3, DMPU, room temp, 24 h, (88%); viii, Zn dust, AcOH, room temp, 18 h, (94%); ix, base wash, dry DCM, TsCl, cat. Et_3N, (95%); x, NaOMe, dry MeOH, room temp, 6 h, (95%); xi, 3,5-$(Me)_2C_6H_3NCO$, dry DCM, cat. TMSCl , 4 days, (94%).

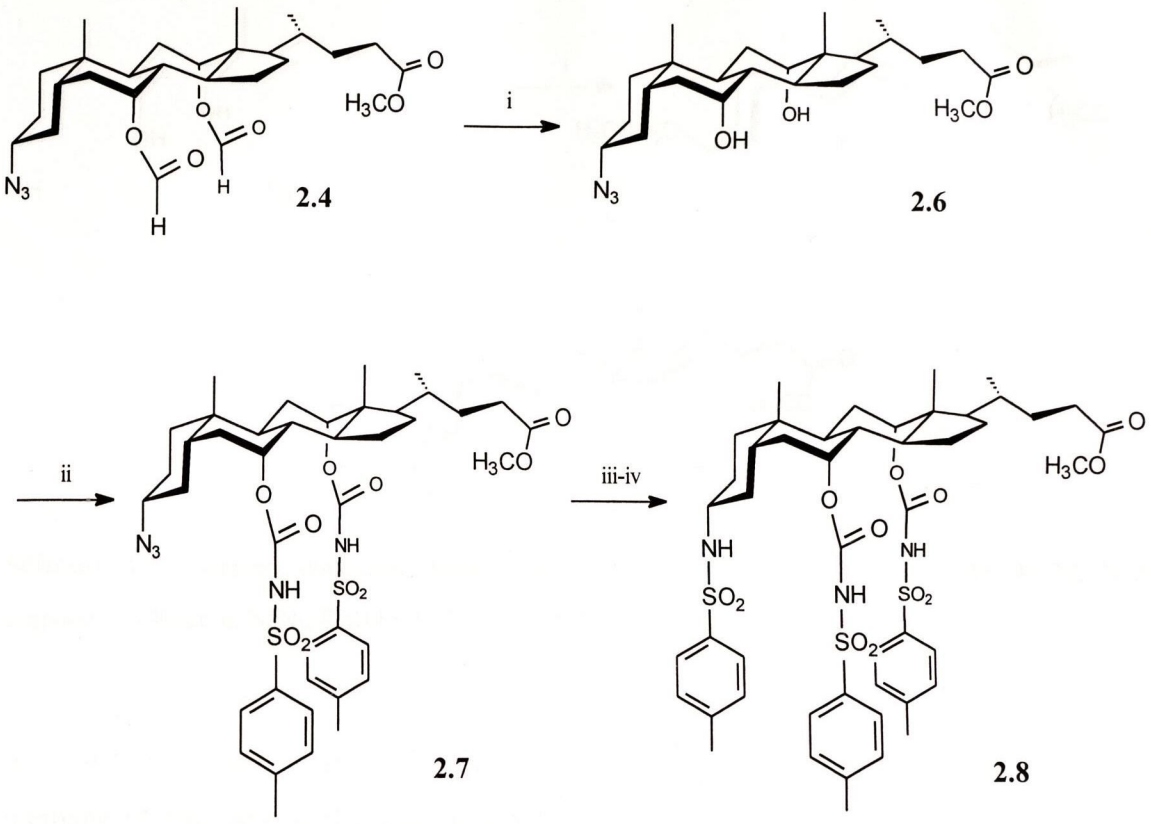
Protection of the three alcohol groups by formylation was achieved using a procedure of Webb *et al.*, (slightly modified).[2] The selective deprotection of the 3α-formyl group was possible because of its unique equatorial configuration and was achieved by Bhattarai.[3] A Mitsunobu reaction, using the method of Hoffman *et al*,[4] yielded the 3β-formyl 7α, 12α-*bis*(formyl) derivative in 90% yield with clean inversion at the 3 position. Selective deprotection of the 3β-formyl group in the presence of the 7α and 12α-formyl groups to yield the 3β-free hydroxyl was achieved in high yields by hydrolysis using sodium acetate in methanol. The 3β-hydroxyl group was then reacted with methanesulfonyl chloride to yield the mesylate. Azide S_N2 substitution of the 3β-mesylate gave the 3α-azide **2.4**. This double inversion method employing a Mitsunobu-type reaction followed by a nucleophilic substitution reaction yielded **2.4** with absolute retention of configuration. **2.4** was then reduced to the 3α-amine using zinc/acetic acid, followed by an aqueous sodium bicarbonate wash. Reaction with one molar equivalent of *p*-toluenesulfonyl chloride yielded the 3α-sulfonamido-7α,12α-*bis*(formate). Deprotection of the 7α and 12α-formate groups was carried out using sodium methoxide to afford the diol **2.5**. Then using a procedure of Vaultier *et al.* (slightly modified)[5] **2.5** was reacted with 2 molar equivalents of isocyanate to yield the *bis*(carbamoyl) sulfonamide **1.68** with an overall yield of 29%.



Scheme 2.2 The Mitsunobu reaction mechanism.

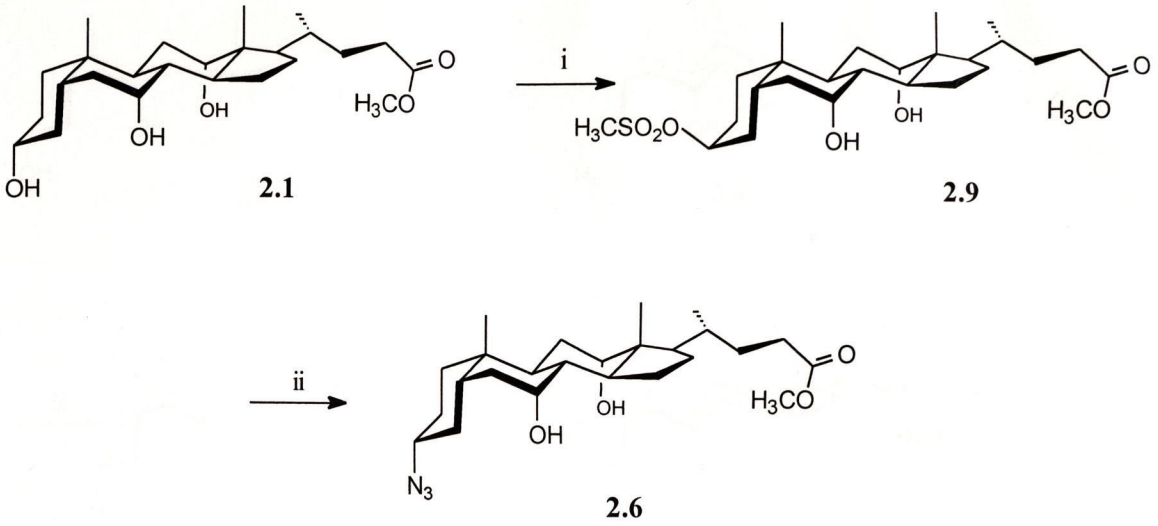
On repeating this procedure it was found that some of the steps took longer than expected to go to completion. For example hydrolysis of the 3α-formyl group took seven days rather than four days as reported by Perry.[1] The final step of the synthesis was found to proceed very slowly under Perry's conditions. It was found that the reaction could be accelerated by adding a large excess of isocyanate and heating the reaction to 40°C. However, purification of the product was difficult due to the large excess of isocyanate

used. It was felt desirable at this stage to modify the procedure of Perry for easier purification of the final product. The modified synthesis was used in the synthesis of **2.8.** It involved deprotection of the 7α and 12α-formyate groups in the presence of the 3α-azide to yield **2.6** as shown in scheme 2.3. The azido-diol **2.6** was then converted to the azido-*bis*-carbamate **2.7** with three-fold excess of the isocyanate added to yield 87% product using TMSCl as a source of dry HCl *in situ.* The 3α-azide group was then reduced with zinc/acetic acid yielding the ammonium acetate salt in 90% yield. The impure ammonium salt was difficult to purify by column chromatography because of its polarity. The salt was shaken with saturated aqueous $NaHCO_3$ to liberate the free amine and was then allowed to react with one equivalent of tosyl chloride to yield the final receptor **2.8** with an overall yield of 31%. Purification of **2.8** by column chromatography also proved to be difficult due to partial decomposition of the carbamate groups on the silica gel. Prolonged exposure of the receptor to silica caused almost complete decomposition of the receptor. However the receptor could be purified by slow recrystallisation from a 2:1:1 chloroform/hexane/ether mixture to yield a clean white powder.



Scheme 2.3 *Reagents, conditions, (and yields)* : i, NaOMe, MeOH, rt, 5 h, (95%); ii, *p*-$(CH_3)C_6H_4SO_2NCO$, cat. TMSCl, dry CH_2Cl_2, 2 days (87%); iii, Zn dust, AcOH, 1 day (90%); iv, base wash, *p*-$(CH_3)C_6H_4SO_2Cl$, cat. Et_3N, dry CH_2Cl_2 2 h (92%).

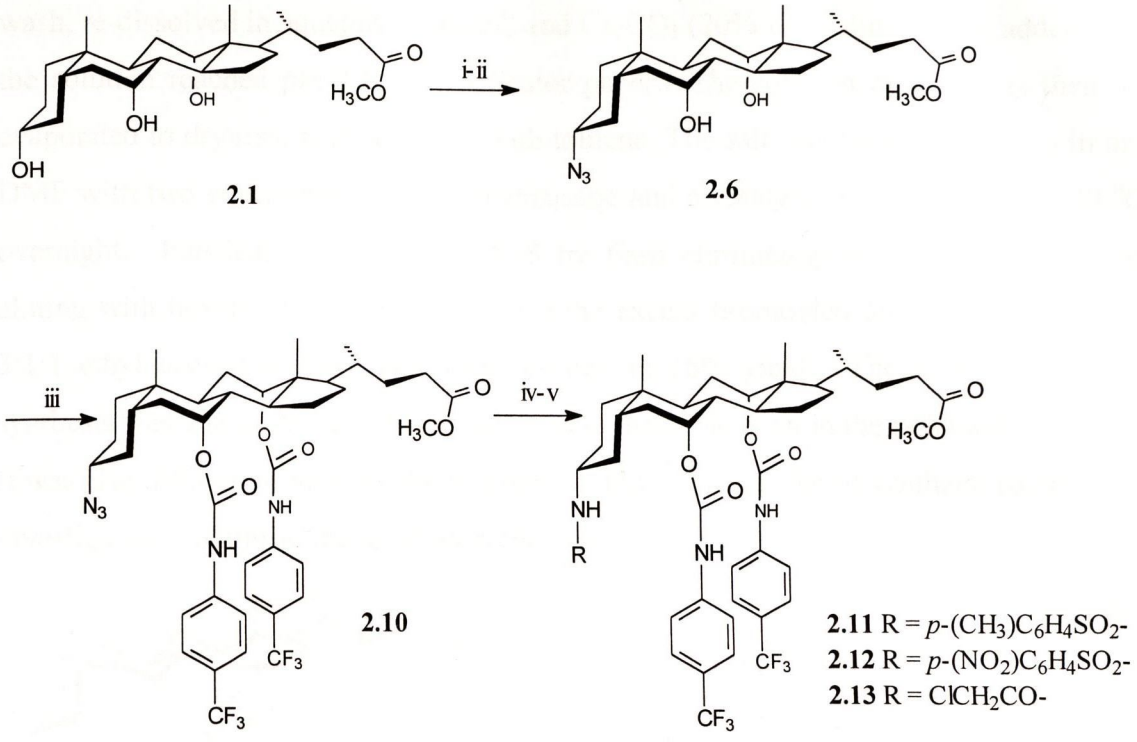
Although this process was workable and high yielding it was desirable to seek a shorter route to the 3α-azido-7α, 12α-diol **2.6**. Lawless and Dresen, both former members of the Davis group, developed an alternative route involving the use of methanesulfonic acid as the acid/nucleophile in the Mitsunobu reaction, yielding the 3β-mesylate selectively in the presence of 7 and 12-hydroxyl groups in high yields (scheme 2.3).[6] For economy of operations, the 3β-mesylate was not isolated in high purity but was so on azide substitution. The selectivity of the Mitsunobu reaction for the 3α-hydroxyl over the other alcohol groups was presumably due to the equatorial nature of this substituent. This improved synthetic route yielded the 3α-azido-7α, 12α-diol **2.6** in three steps from cholic acid with an overall yield of 69%. The procedure was also less time consuming than the former method of Perry (scheme 2.1) that involved five extra steps. On repeating this procedure it was soon noticed that although a high yield was obtained the majority of the time, a noticeable amount of failure occurred also. Methyl cholate was recovered from these failed reactions.



Scheme 2.4 *Reagents, conditions, (and yields)* : i, DEAD, PPh_3, CH_3SO_3H, dry THF, 40 °C, 24 h, evaporation (90%); ii, NaN_3, DMPU, 45 °C, 24 h, (83%).

In 1996 Anderson *et al.* published their work on the Mitsunobu reaction using methanesulfonic acid as the acid/nucleophile.[7] The conditions they used were similar to those of Lawless,[6] except for an addition of 0.4 equivalents of triethylamine to the reaction and obtaining similar yields. On modifying the procedure of Lawless, by the addition of 0.4 equivalents of triethylamine, the reaction was found to go successfully overnight in consistently good yield.

This new procedure was then used to prepare the key intermediate **2.6** for the synthesis of the receptors in this chapter. Scheme 2.5 shows synthetic route for the receptors synthesised using this method. These receptors were all found to be stable on silica gel and were purified by column chromatography with an overall yield of approximately 50%. The receptors were all found to be soluble in chloroform. However, **2.10** was found to go into solution only slowly. This was probably due to the polar groups present on the aromatic rings making it less soluble in organic media.

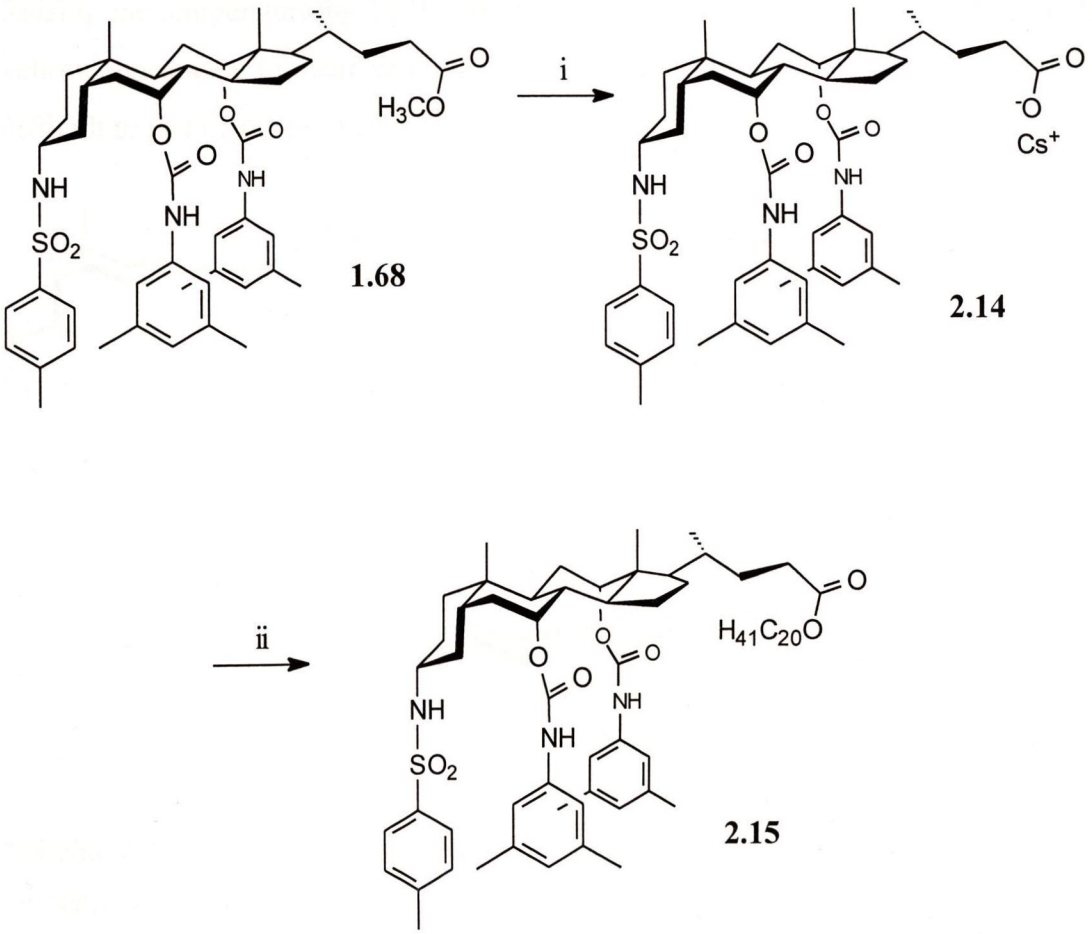


Scheme 2.5 *Reagents, conditions, (and yields)* : i, DEAD, PPh_3, CH_3SO_3H, Et_3N, dry THF, 24 h, evaporation, (90%); ii, NaN_3, DMPU, 45 °C, 24 h, (83%); iii, *p*-$(CF_3)C_6H_4NCO$, TMSCl (cat.), dry CH_2Cl_2, 2 days, (73%); iv, Zn dust, AcOH, (90%); v, base wash, *p*-RSO_2Cl, cat. Et_3N, dry CH_2Cl_2, 2 h, (~92%).

The lipophilicity of receptors **2.11**, **2.12** and **2.13** was assessed by measuring the amount of receptor that was distributed into the aqueous layer in a single extraction of a chloroform solution of the receptor in water. Approximately 30% of the receptor in all cases was unrecovered on extraction.

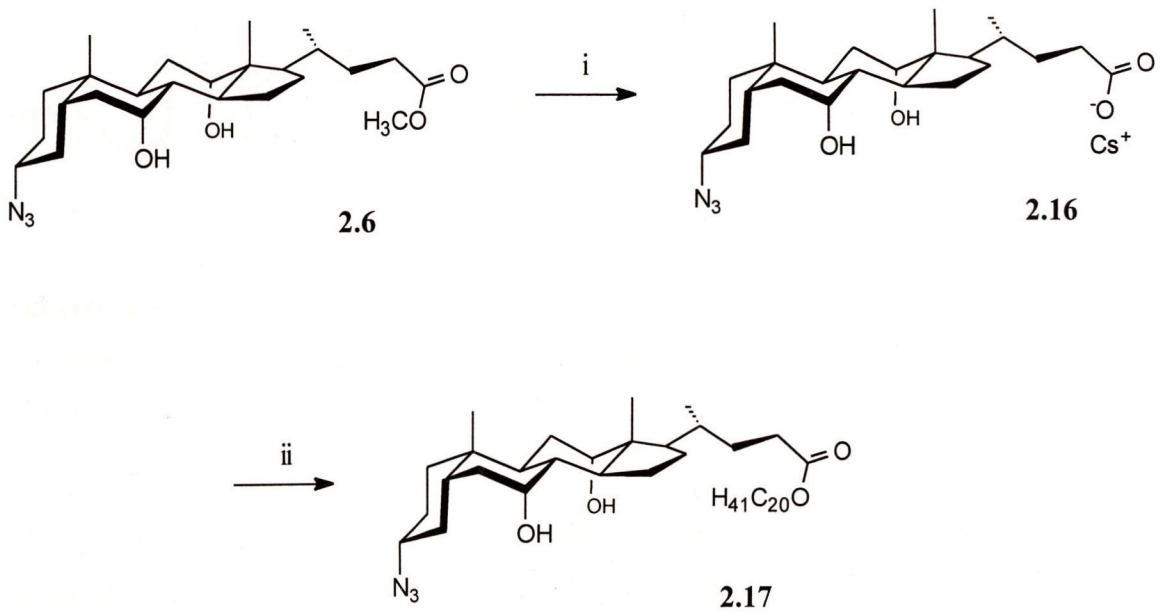
A danger of making the receptors more potent and consequently more polar and less lipophilic; is that on anion complexation, the complex may be even less soluble in chloroform. It was felt sensible at this stage to make the receptors more lipophilic by incorporating a longer chain ester and so not affecting the binding site in any major way. Scheme 2.6 shows one of the synthetic pathways for the more lipophilic receptors. The receptor **1.68** was synthesised by the method of Perry[1] (scheme 2.1). When subjected to KOH in aqueous methanol the methyl ester was hydrolysed leaving the carbamates and the 3α-sulfonamide intact. After hydrolysis appeared complete by TLC (decrease in R_f), the solution was neutralised with HCl. The cesium salt was then isolated by organic

wash, re-dissolved in aqueous methanol, and Cs_2CO_3 (20% w/v solution) was added until the solution reached pH 8 by pH indicator paper. The solution of **2.14** was then re-evaporated to dryness, azeotropically with toluene. The salt was then re-dissolved in dry DMF with two equivalents of 1-bromoeicosane and a catalytic amount of NaI at 40 °C overnight. Purification of receptor **2.15** by flash chromatography on silica gel first eluting with hexane/chloroform to remove the excess bromoeicosane then eluting with 3:1:1 ethyl acetate/hexane/chloroform resulted in 16% yield. The low yield of this synthesis was due to the cesium salt being quite insoluble even in the polar solvent DMF. It was also difficult to monitor the reaction by TLC. Two different synthetic routes were investigated to optimise the reaction at this stage.



Scheme 2.6 *Reagents, conditions, (and yields)* : i, KOH, MeOH/H_2O (10/1), 24 h, neutralised with HCl, Cs_2CO_3 (20%) until pH 8 then evaporated to dryness; ii, $C_{20}H_{41}Br$, cat. NaI, dry DMF, 40 °C, 24 h (16%).

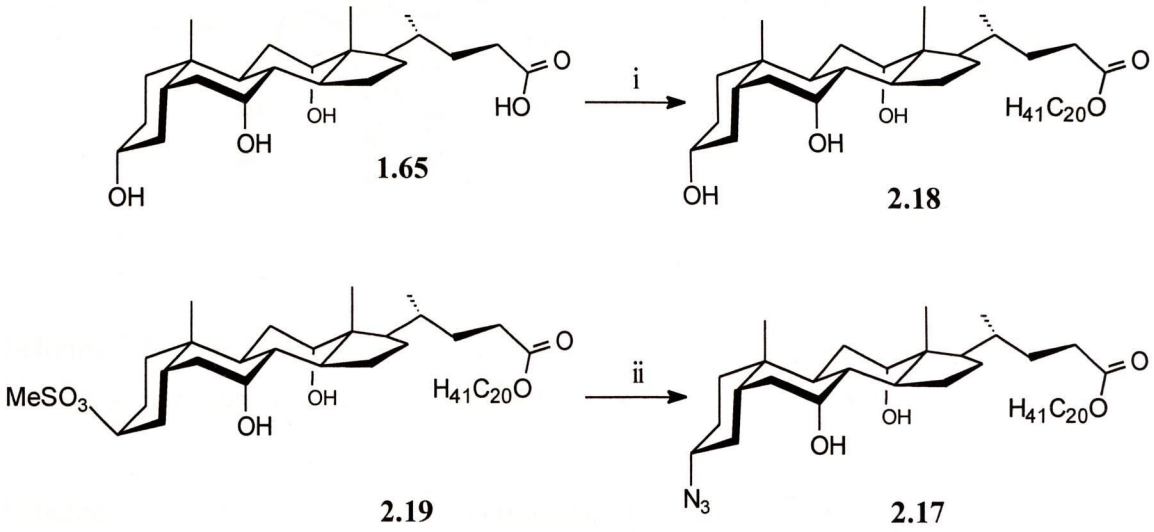
Scheme 2.7 shows the first of these routes. The key intermediate, 3α-azido-7α, 12α-diol **2.6,** was seen as a desirable stage for the conversion of the methyl ester to a more lipophilic ester. The methyl ester was hydrolysed by raising the pH to approx. 11-12 with Cs_2CO_3. On completion of the reaction by TLC analysis, the solution was adjusted to pH 8 with 1M HCl, evaporated to dryness with toluene and re-dissolved in dry DMF. Three molar equivalents of 1-bromoeicosane were added with catalytic NaI and the solution was stirred at 40°C for three days. The lipophilic azido-diol **2.17** was purified by extraction with ether followed by column chromatography giving a yield of 62%. The azide functional group was found to survive the reaction conditions used. Attempts to optimise this synthesis by increasing the reaction temperature met with little success. Raising the temperature by 10 °C resulted in the reaction solution going from a cloudy yellow appearance to clear yellow. The reaction was lower yielding and it was more difficult to isolate the product pure.



Scheme 2.7 *Reagents, conditions, (and yields)* : I, Cs_2CO_3, MeOH/H_2O (10/1), 1 M HCl; ii. $C_{20}H_{41}Br$, cat. NaI, dry DMF 40 °C (62%).

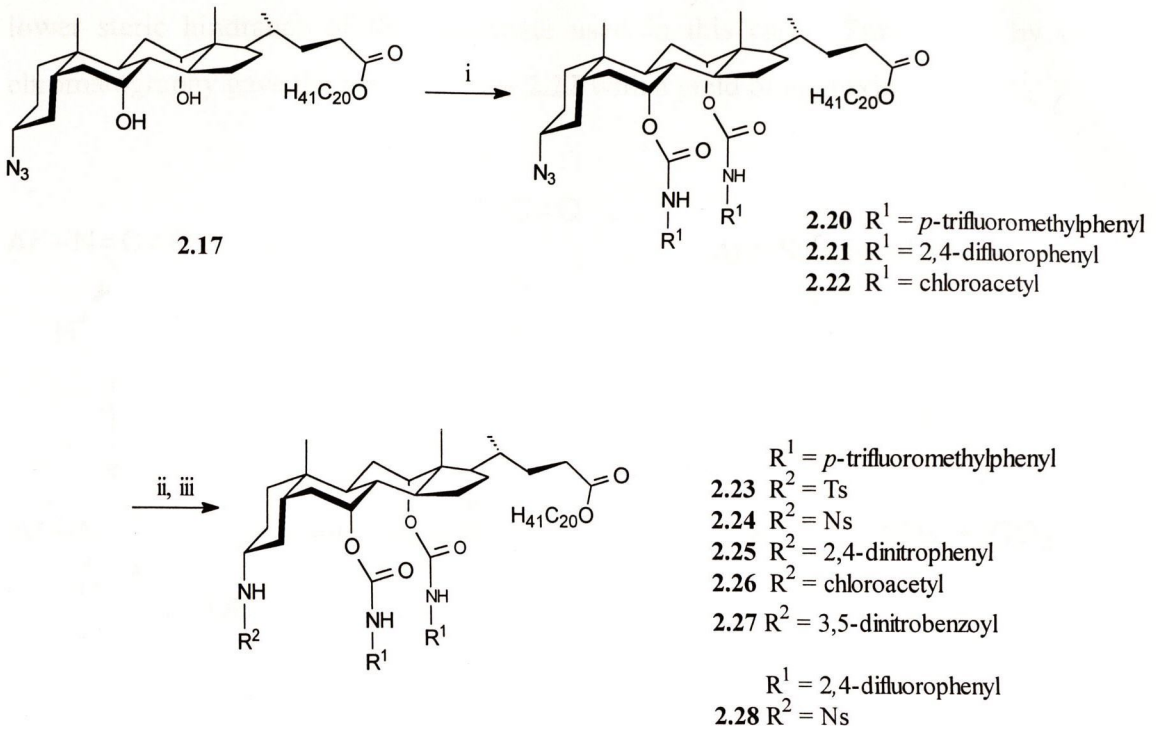
The second route used to synthesise the lipophilic azido-diol **2.17** is shown in scheme 2.8. A solution of cholic acid in methanol/water was brought to pH 7-8 by adding an aqueous solution of Cs_2CO_3 and then it was evaporated to dryness by forming an azeotrope with toluene. The white salt was then esterified with 1-bromoeicosane in dry

DMF with a yield of 73% after vigorous stirring for 4 days. The eicosyl cholate was purified using an organic/aqueous wash. The organic layer was evaporated and purified by column chromatography by first flushing the column with hexane/chloroform (2:1) to remove the excess bromoeicosane and then eluting with ethyl acetate/hexane/chloroform (5:1:1) to yield **2.18** in 73% yield. A Mitsunobu reaction on **2.18**, using the modified Lawless procedure, followed by azide substitution (see Scheme 2.8) yielded the lipophilic azido-diol **2.17** with an overall yield of 49%. This procedure was found to be the least troublesome for obtaining **2.17** from cholic acid.



Scheme 2.8 *Reagents, conditions, (and yields) :* i, Cs_2CO_3, $MeOH/H_2O$ (10/1), 1M HCl to pH 8 then evaporated, $C_{20}H_{41}Br$, cat. NaI, dry DMF, 40 °C, water wash, (73%); ii, DEAD, PPh_3, CH_3SO_3H, Et_3N, dry THF, 40 °C, 24 h, (65%); iii, NaN_3, DMPU, 40 °C, 16 h, (67%).

The azido-diol **2.17,** as synthesised in scheme 2.8, was used as a key intermediate for the synthesis of a range of receptors for anion binding. The procedure for their synthesis is described in scheme 2.9. Carbamoylation was accelerated by increasing the reaction temperature. The solvent was changed to tetrachloroethane, to allow higher reaction temperatures to be reached. The reaction went to completion in all cases after 3 days by TLC analysis. The isocyanates used in all cases of scheme 2.9 were found to be moisture sensitive. During the synthesis of **2.20**, the liquid isocyanate was found to contain a sediment after exposure to the atmosphere. The reaction mixture was partially purified by cooling to 0 °C and filtering off the white solid impurity, believed to be the decomposed isocyanate.



2.17

2.20 R^1 = *p*-trifluoromethylphenyl
2.21 R^1 = 2,4-difluorophenyl
2.22 R^1 = chloroacetyl

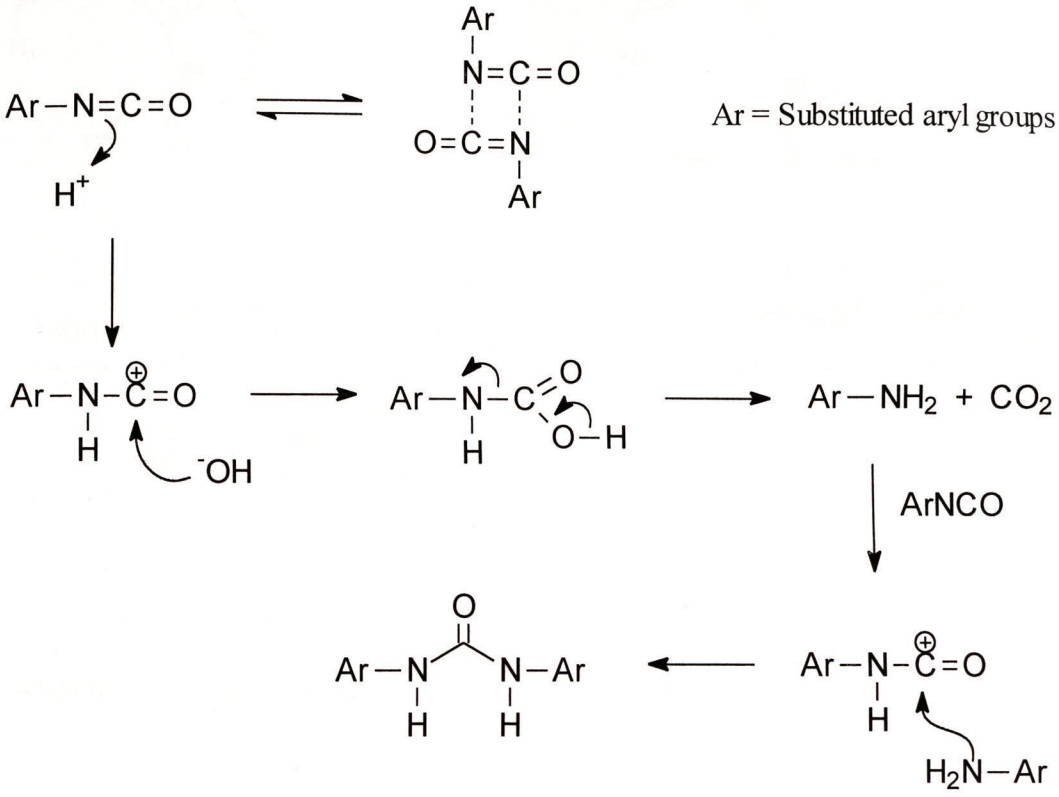
R^1 = *p*-trifluoromethylphenyl
2.23 R^2 = Ts
2.24 R^2 = Ns
2.25 R^2 = 2,4-dinitrophenyl
2.26 R^2 = chloroacetyl
2.27 R^2 = 3,5-dinitrobenzoyl

R^1 = 2,4-difluorophenyl
2.28 R^2 = Ns

Scheme 2.9 *Reagents, conditions, (and yields) :* i, R^1NCO, dry 1,1,2,2-tetrachloroethane, cat. TMSCl, 1-3 days, 60 °C (70-90%); ii, Zn dust, AcOH, (90%); iii, R^2Cl, cat. Et_3N, dry CH_2Cl_2, (65-92%).

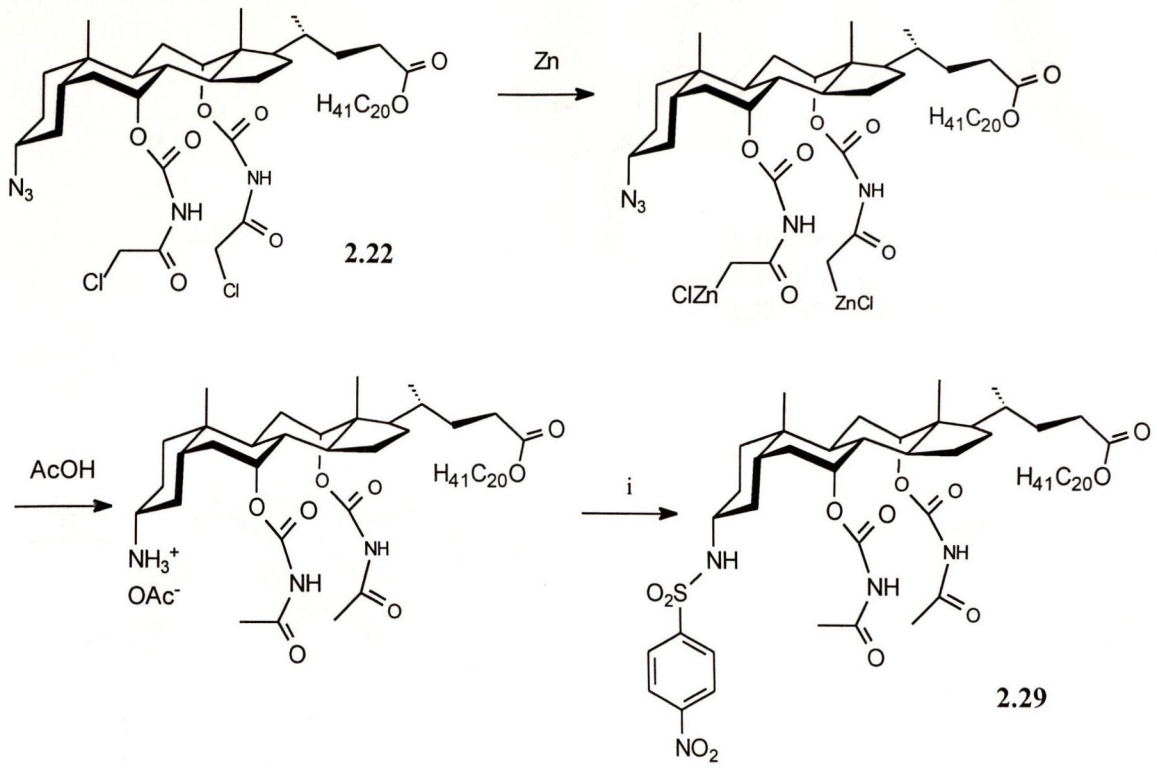
Scheme 2.10 shows the mode of decomposition of the isocyanate to the corresponding urea. According to Kogon[8], many substituted aryl isocyanates oligomerise in solution while some are stable to increased temperature, *e.g.* *p*-nitrophenyl isocyanate. On exposure to moisture, the isocyanate forms a carbamic acid which readily decarboxylates to the corresponding aniline derivative. It is this aniline that further reacts with another molecule of isocyanate to form the urea impurity present in significant quantities in the first step of scheme 2.9. On synthesising **2.20**, the majority of the urea impurity was removed by cold filtration, resulting in approximately 80% pure product in the mother liquor. In the case of **2.34** and **2.35** (scheme 2.12), the *p*-nitrophenyl isocyanate was found to be semi-soluble in tetrachloroethane. The insoluble material was presumed to be predominantly the decomposed isocyanate (nitrophenylurea). The reaction however, was found to go to completion after 3 days at 60 °C in the presence of a four-fold excess of the isocyanate. The products **2.34** and **2.35**, were isolated almost pure by filtering the reaction mixture when cold to remove the solid impurities. Further purification required column chromatography in all cases. Synthesis of the azide *bis*-carbamate **2.22** was found to be complete after 1 day at room temperature. This was presumably due to the

lower steric hindrance of the isocyanate used in this case. Purification by column chromatography gave the *bis*-carbamate **2.22** with a yield of approximately 80%.



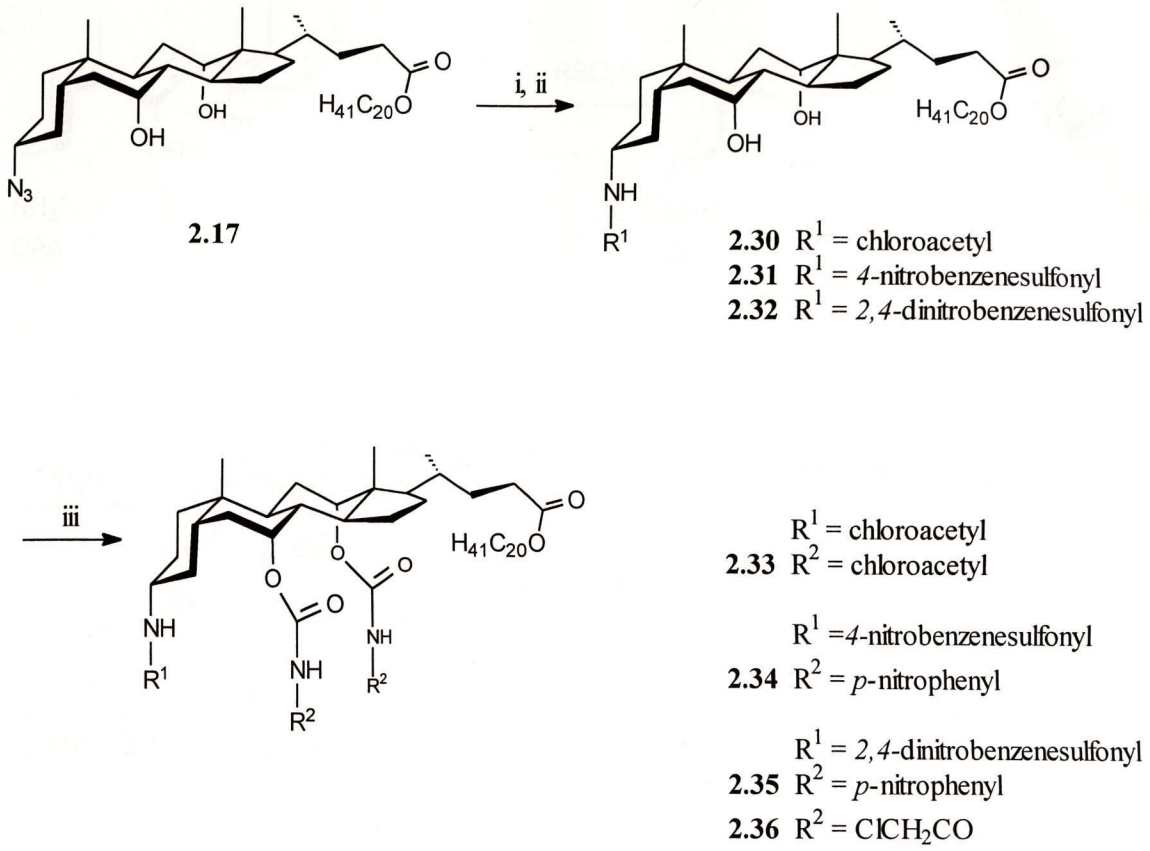
Scheme 2.10

Reduction of the azide group using zinc and acetic acid in Scheme 2.9 proved successful in the presence of the carbamate groups, except in the case of **2.22**. In this case the chloroacetyl groups were reduced in a Reformatsky-type reaction yielding the 3α-amido *bis*-acetyl-carbamate as shown in scheme 2.11. Zinc/acetic acid conditions were also expected to reduce nitro aromatic substituents if already present.



Scheme 2.11 *Reagents, conditions, (and yields) :* i, TsCl, cat. Et_3N, DCM, RT, 2 h, (67%).

An alternative synthetic sequence was required to synthesise receptors with these types of carbamates. The sequence involved functionalisation first at the 3 position, followed by carbamoylation of the 7 and 12 positions as shown in Scheme 2.12. In the case of **2.30** and **2.31**, the NH substituents at the 3 position were stable to the conditions for carbamoylation of the 7 and 12 positions.



2.17

2.30 R^1 = chloroacetyl
2.31 R^1 = *4*-nitrobenzenesulfonyl
2.32 R^1 = *2,4*-dinitrobenzenesulfonyl

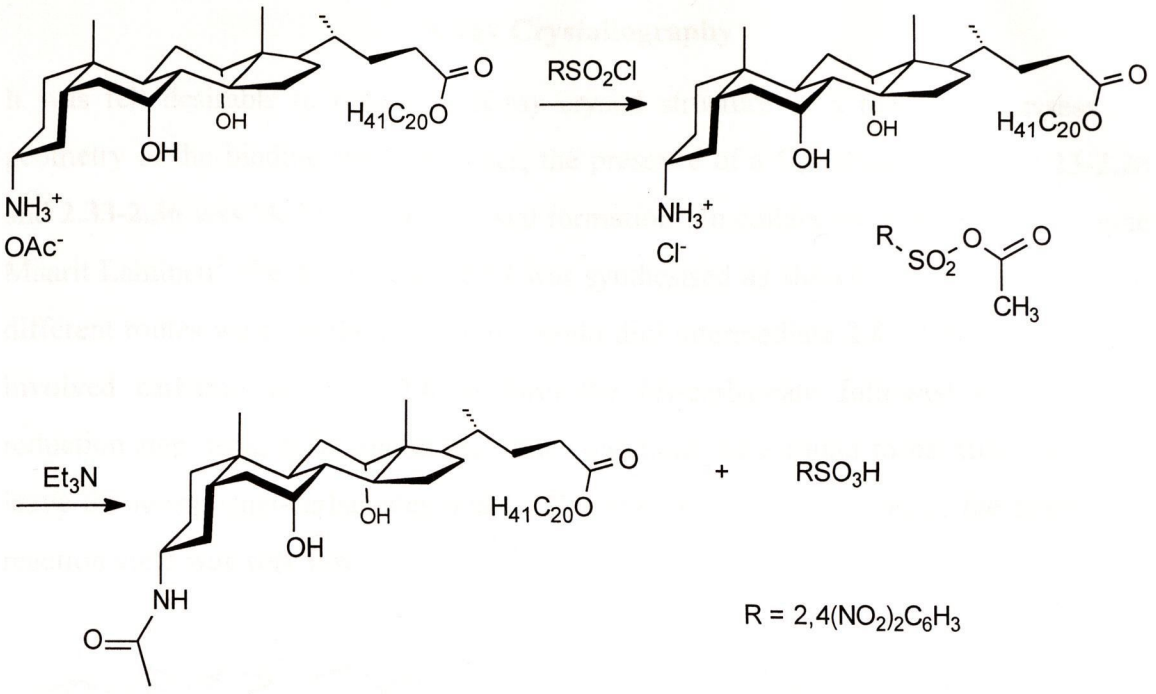
R^1 = chloroacetyl
2.33 R^2 = chloroacetyl

R^1 =*4*-nitrobenzenesulfonyl
2.34 R^2 = *p*-nitrophenyl

R^1 = *2,4*-dinitrobenzenesulfonyl
2.35 R^2 = *p*-nitrophenyl
2.36 R^2 = $ClCH_2CO$

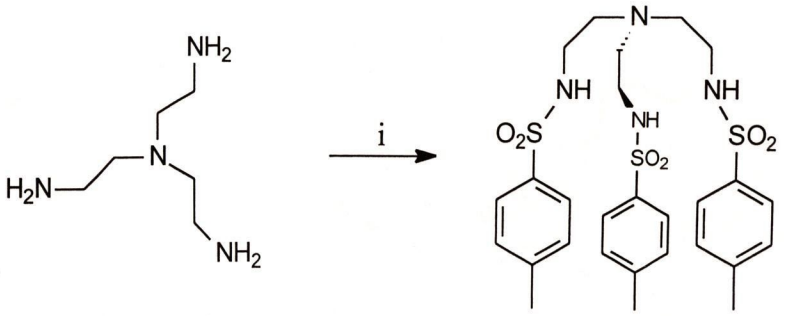
Scheme 2.12 *Reagents, conditions, (and yields) :* i, Zn dust, AcOH, 1 day, (90%); ii, base wash, R^1Cl, cat. Et_3N, dry CH_2Cl_2, 1-2 days, 0 °C-room temp, (52-92%); iii, R^2NCO, cat. TMSCl, dry DCM, 40 °C, (80-90%).

However, in the case of **2.32**, the reaction was done at 0 °C to avoid reaction of the sulfonyl chloride with the 7 and 12 alcohol groups. The sulfonyl chloride was not used in excess in this case. The base wash was imperative after the Zn/AcOH reduction of the azide to the ammonium salt to render it as the free amine. Without a thorough base wash an undesired side reaction occurred as seen in scheme 2.13. A control reaction, in which no base wash was performed on the ammonium salt, resulted in the formation of the 3α-acetamide shown in Scheme 2.13 with a yield of 94%.



Scheme 2.13

It was decided to synthesise Reinhoudts' receptor **1.49** for a comparative study for potency in anion recognition, under the same conditions used in this thesis. The receptor was synthesised according to the procedure of Reinhoudt and co-workers, shown in scheme 2.14.

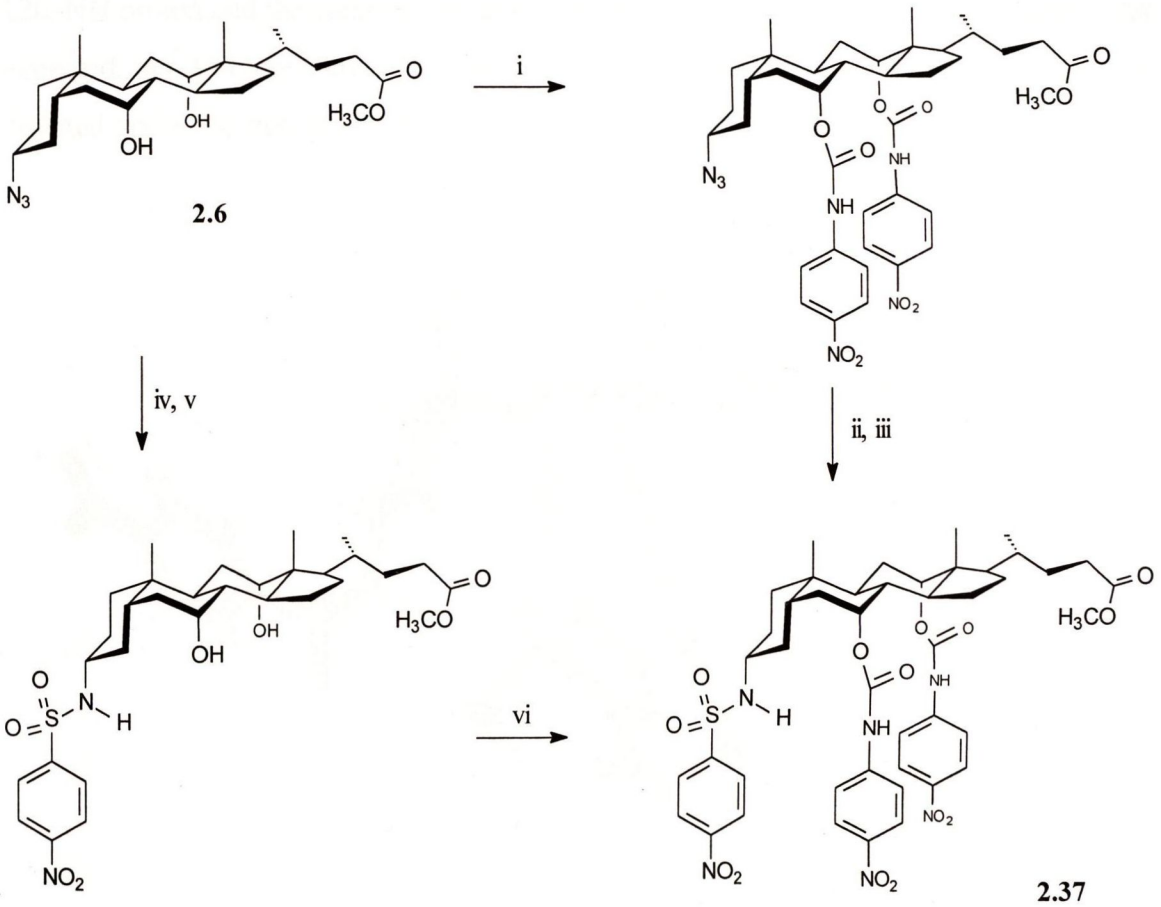


1.49

Scheme 2.14 *Reagents, conditions, (and yields) :* i, TsCl, dry CH_2Cl_2, cat. Et_3N, 10 °C, 3 h, then room temp for 3 h, base wash, recrystallised from MeOH (84%).

X-ray Crystallography

It was felt desirable to obtain an X-ray crystal structure of a receptor to assess the geometry of the binding site. However, the presence of a C_{20} alkyl chain, in **2.23-2.28** and **2.33-2.36** was likely to hinder crystal formation. In collaboration with my colleague Maarit Lahtinen[9], the methyl ester **2.37** was synthesised as shown in scheme 2.15. Two different routes were employed from the azido-diol intermediate **2.6**. The first sequence involved carbamoylation of **2.6** to form the *bis*-carbamate followed by the azide reduction step using mild conditions. The conditions were found to be mild enough to leave the nitrophenyl-carbamates intact. However after sulfonylation of the amine, the reaction yield was very low.



Scheme 2.15 *Reagents, conditions, (and yields) :* i, *p*-$NO_2C_6H_4NCO$, TMSCl, DCM, rt, 2 d, (77%); ii, PPh_3, THF, 60 °C, 24 h, H_2O, 4 h; iii, *p*-$(NO_2)C_6H_4SO_2Cl$, TEA cat, DCM, rt, 2 d, (12% from **2.6**); iv, Zn/AcOH; v, *p*-$(NO_2)C_6H_4SO_2Cl$, DCM, 0 °C, (40% from **2.6**); vi, *p*-$(NO_2)C_6H_4NCO$, TMSCl, DCM, 30°C

PPh_3, THF, 60 °C, 24 h, H_2O, 4 h; iii, *p*-$(NO_2)C_6H_4SO_2Cl$, TEA cat, DCM, rt, 2 d, (12% from **2.6**); iv, Zn/AcOH; v, *p*-$(NO_2)C_6H_4SO_2Cl$, DCM, 0 °C, (40% from **2.6**); vi, *p*-$(NO_2)C_6H_4NCO$, TMSCl, DCM, 30°C, 2 d, (8%).

The second sequence involved reducing **2.6** to the ammonium salt, followed by reacting with one equivalent of tosyl chloride. The product was then treated with 2 equivalents of *p*-nitrophenyl isocyanate to yield **2.37** in low yield. Both sequences proved difficult and very low yielding predominantly due to solubility problems in suitable solvents for reaction. The receptor **2.37** was found only to be soluble in acetone, ethyl acetate, THF and DMSO.

Crystals were grown from chloroform/acetone and were found to contain one equivalent of acetone and 1/3 equivalent chloroform. The X-ray structure shows the coordination of the 7α-NH proton to the carbonyl oxygen of acetone with a N-H· ·O distance and angle of 2.09 Å and 149.5 ° (N-H-O) respectively. A second H-bond was found between the 12α-NH proton and the carbonyl oxygen of the methyl ester of a neighbour molecule. As expected, the structure shows the hydrophilic binding cavity created by three NH protons directed under the steroid and available for binding.

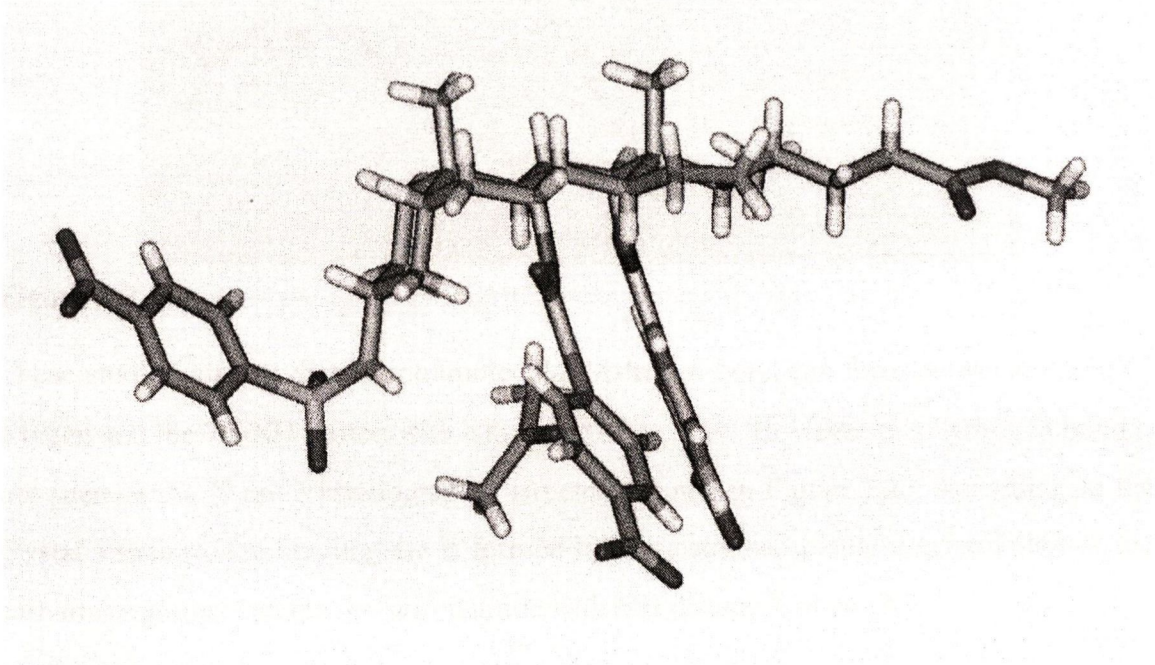


Figure 2.1. X-Ray crystal structure of **2.37** complexed with one acetone solvent molecule, one of the co-solvents used in the crystallisation.

Molecular Modelling Studies

A computer-based molecular modelling structure of receptor **2.37** is shown below. The modelling studies employed MacroModel V5.0 on a Silicon Graphics O_2 workstation. The structure shown in Figure 2.2 is the lowest energy conformer resulting from a 10,000 step Monte-Carlo search. The calculation was initialised from a randomly chosen minimised starting geometry. The force field used in all cases was MM2 with a chloroform solvent model.

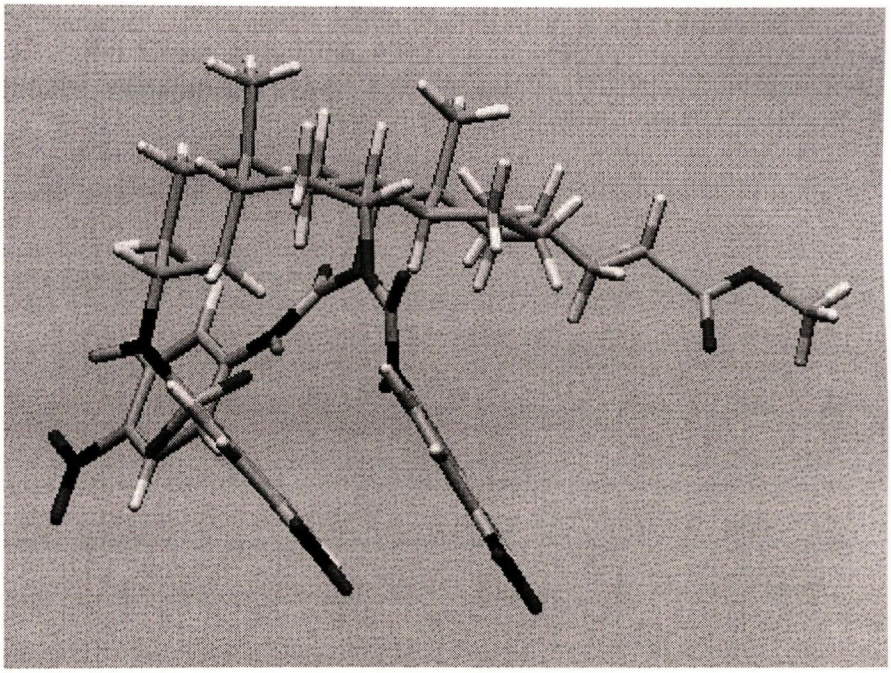


Figure 2.2.

These studies suggest that an intramolecular hydrogen bond can form between a 3α-SO_2 oxygen and the 7α-NH proton with a distance of *ca.* 2 Å. However this hydrogen bond is not seen in the X-ray crystallographic structure shown in Figure 2.2. According to the crystal structure, the binding site is formed by an unstrained planar conformation of the carbamate groups and the 3α-sulfonamide with NH distances of *ca.* 2.7 Å.

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[9] Maarit Lahtinen, *unpublished work.*

Chapter Three : Binding Studies

Anion binding properties in the solution state are discussed in this chapter for the receptors synthesised in the previous chapter.

3.0 Theoretical background to binding studies

When two molecules bind together to form a 1:1 complex, there are generally changes in measurable properties. Binding studies involve the addition of aliquots of a guest solution to a solution of the host while 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, NMR, IR and UV spectra. NMR is a convenient probe because it provides information about every atom in the host and guest complex. This information includes details of the rate of association/dissociation, conformational changes occurring, hydrogen bonding and any other changes attendant on binding. In this thesis, 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' only 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.

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. Departure from this stoichiometry can be detected by poor fitting binding curves (see later). Alternatively a strict 1:1 stoichiometry can be proven using a Job plot analysis.[1] 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 the work described in this thesis receptors **2.8**, **2.12** and **2.34** were subjected to NMR dilution studies ranging in host concentration from approximately 6 mM to 1.2 mM. Spectra for all the receptors essentially remained unchanged over this range. Binding

curves consistent to good accuracy with 1:1 host/guest stoichiometry were observed (see later).

Determination of Association Constant (K_{ass})

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

$$[H] + [G] \underset{}{\overset{K_{ass}}{\rightleftharpoons}} [HG]$$

initial :	$[H]_i$	$[G]_i$	0
at equilibrium :	$[H]_i$-$[HG]$	$[G]_i$-$[HG]$	$[HG]$

$$K_{ASS} = \frac{[HG]}{\{[H]_i - [HG]\}\{[G]_i - [HG]\}}$$

$$K_{ass} = \frac{[HG]}{[H]_i[G]_i - [H]_i[HG] - [G]_i[HG] + [HG]^2}$$

by rearrangement :

$$[HG]^2 - \left\{[H]_i + [G]_i + \frac{1}{K_{ass}}\right\}[HG] + [H]_i[G]_i = 0$$

solving for [HG] gives :

$$[HG] = \frac{[H]_i + [G]_i + \frac{1}{K_{ass}} - \sqrt{\left\{[H]_i + [G]_i + \frac{1}{K_{ass}}\right\}^2 - 4[H]_i[G]_i}}{2} \qquad \textbf{(1)}$$

The observed NMR shift δ_{obs} is linked to the concentration of the complex by the equation :

$$\delta_{obs} = \frac{\{[H]\delta_H + [HG]\delta_{HG}\}}{[H]_i}$$

$$\delta_{obs} = \frac{\{[H]_i - [HG]\}\delta_H + [HG]\delta_{HG}}{[H]_i}$$

$$\delta_{obs} = \delta_H + \frac{(\delta_{HG} - \delta_H)[HG]}{[H]_i}$$

Therefore :

$$[HG] = \frac{[H]_i(\delta_{obs} - \delta_H)}{(\delta_{HG} - \delta_H)} \qquad \textbf{(2)}$$

Substituting (2) into (1) and cross multiplying gives :

$$\frac{2\{[H]_i(\delta_{obs} - \delta_H)\}}{(\delta_{HG} - \delta_H)} = [H]_i + [G]_i + \frac{1}{K_{ass}} - \sqrt{\left\{[H]_i + [G]_i + \frac{1}{K_{ass}}\right\}^2 - 4[H]_i[G]_i} \qquad \textbf{(3)}$$

Equation (3) has three variables ($[H]_i$, $[G]_i$ and δ_{obs}) and three parameters (δ_H, δ_{HG} and K_{ass}). δ_H can be determined from a spectrum of the host in the absence of guest.

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_{HG} are known. In practice δ_{HG} is not accurately known and cannot be directly observed, but only approached asymptopically. Therefore, an estimation of δ_{HG} allows one to calculate K_{ass} from a measured value. In principle there is a unique δ_{HG} which 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_{HG} until some statistical measure of the data spread (standard deviation) is minimised.

Association constants were calculated using a Microsoft Excel spreadsheet designed by Murray.[2] Error analyses were performed on the statistics package Systat 5.2. Both methods gave essentially the same value for δ_{HG} and K_{ass}. The errors were quoted to show the agreement of the observed data with the calculated best fit data.

In this thesis, two types of conditions were used for binding studies. They differ in the degree of solvation of the TBA salt and the receptor used in the study.

1) Dry conditions.

The receptor was dried under high vacuum at room temperature for 3 hours prior to use. The deuterated chloroform used in host and guest solution preparation was dried and de-acidified over anhydrous potassium carbonate before use.

2) Wet conditions

In this case the deuterated chloroform was shaken vigorously with de-ionised water, separated and then filtered through phase separation paper (Aldrich catalogue number : Z.13346-9). The resultant $CDCl_3$ was assumed to be saturated with dissolved water and was used to prepare the host and guest solutions for the binding study.

All NMR titrations were performed on either a Bruker MSL-300 (300.13 MHz) or a Bruker DPX-400 (400.13 MHz) instrument at 298 K and 303 K respectively. In all the experiments, the TBA salts were used as received from Aldrich to prepare guest solutions.

3.1 Binding Studies Results

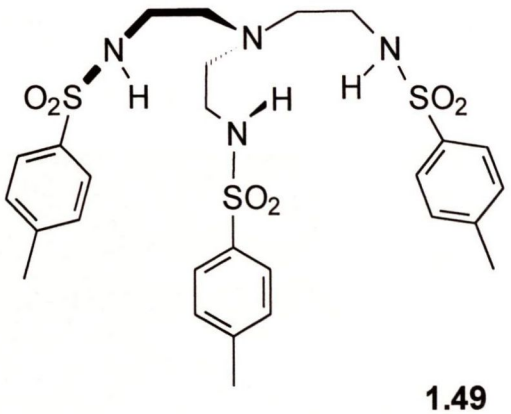
The lack of neutral anion receptors published to date has prompted a number of groups to consider the use of hydrogen bond donor functionality in anion recognition. A simple and flexible molecule **1.49**, synthesised by Reinhoudt and co-workers, has shown encouraging results in binding anions in organic solvents. The strength of anion binding K_{ass} was determined in acetonitrile with tetrabutylammonium$^+$ salts as guests, using a conductometric technique where X = Cl^-, HSO_4^- and $H_2PO_4^-$. In the present work **1.49** was considered a useful comparison for the steroidal receptors and was studied by NMR in $CDCl_3$. Binding curves 1A and 1B show the NMR titration data for Reinhoudt's receptor **1.49** titrated with tetrabutylammonium chloride.

On successive additions of aliquots of the chloride salt to the receptor solution, substantial changes occurred to the ^1H NMR spectrum of the receptor indicative of chloride complexation. Throughout the titration, only one set of host resonances was observed indicating that the complexed and the uncomplexed forms of the receptor are in fast exchange on the NMR timescale.

Binding curve 1A shows the NMR data of the NH resonance throughout the titration and a graph of actual data with the best computer fit based on the iterative non-linear curve fitting program.

Among the receptor resonances, the NH signal was found to undergo the strongest downfield shift ($\Delta\delta$ = 2.6 ppm). The signal was found to broaden with increasing guest concentrations but it sharpened again on chloride saturation. The measured data were found to be in good agreement with the computer fit data (see graph) giving a binding constant K_{ass} of 4,400 ± 470 M^{-1}. The errors reported were calculated from standard deviations of the binding constant values calculated from each point on the curve.

Binding curve 1B shows the ^1H NMR data for the aryl protons *ortho* to the SO_2 group of the tosyl aromatic groups from the same binding study. The aromatic proton signals did not shift to the same extent as the NH resonance, however, the signals remained sharp throughout the titration giving a binding constant K_{ass} of 4,000 ± 222 M^{-1}.



1.49

Binding curve 1A. NMR Binding Study of Association of Reinhoudt's Receptor **1.49** with Tetrabutylammonium chloride.

Dry Conditions were used during this study.

NMR used : MSL 300 at 298 K.

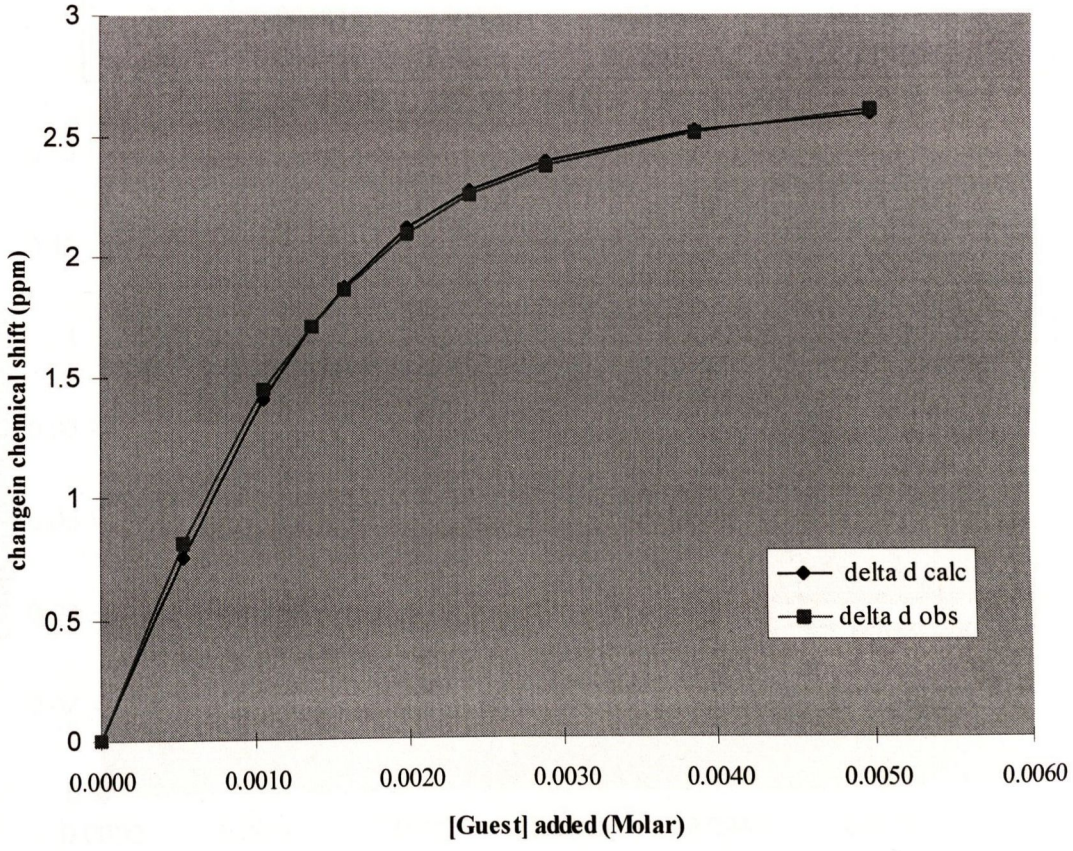
The chemical shift of the NH protons was followed throughout this titration.

Solution concentrations : Host = 1.645 mM Guest = 64.4 mM.

Initial volume of host = 600 μL. Initial shift (δ) of the NH proton = 5.9334 ppm

μL of Guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000532	6.5199	0.8105	0.75422
10	0.001055	7.1512	1.4418	1.398784
13	0.001365	7.4226	1.7132	1.705507
15	0.00157	7.5747	1.8653	1.87123
19	0.001976	7.8007	2.0913	2.116299
23	0.002377	7.9666	2.2572	2.273286
28	0.00287	8.0837	2.3743	2.39453
38	0.003835	8.2256	2.5162	2.519568
50	0.004952	8.3148	2.6054	2.589272

K_{ass} = 4,400 ± 470 M^{-1} δHG = 8.4656 ppm.



Binding curve 1B. NMR Binding Study of Association of Reinhoudt's Receptor **1.49** with Tetrabutylammonium chloride.

Dry Conditions were used during this study.

NMR used : MSL 300 at 298 K.

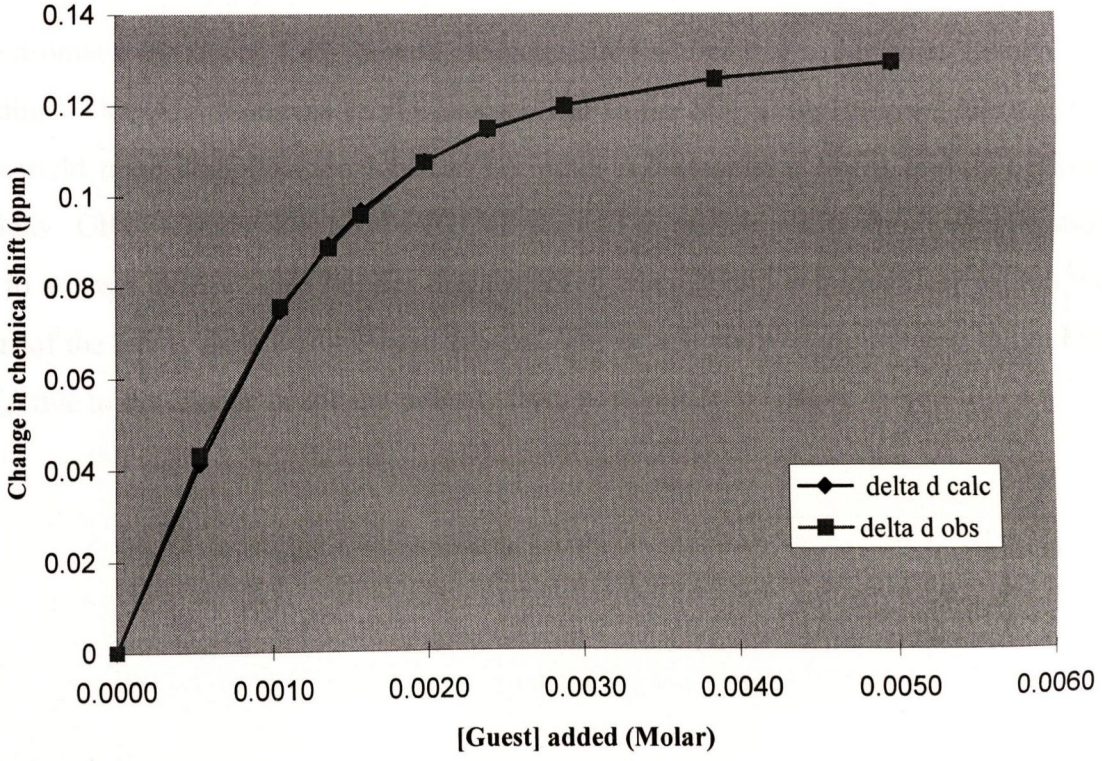
The chemical shift of the aryl proton *ortho* to the SO_2 group was followed throughout this study.

Solution concentrations : Host = 1.645 mM Guest = 64.4 mM.

Initial volume of host = 600 μL. Initial shift (δ) of the aryl proton = 7.8337ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000532	7.8769	0.0432	0.0412981
10	0.001055	7.9091	0.0754	0.0745321
13	0.001365	7.9222	0.0885	0.0892093
15	0.001570	7.9295	0.0958	0.0967899
19	0.001976	7.9412	0.1075	0.1076537
23	0.002377	7.9486	0.1149	0.1145422
28	0.002870	7.9535	0.1198	0.1199488
38	0.003835	7.9593	0.1256	0.1257315
50	0.004952	7.9630	0.1293	0.1291003

K_{ass} = 4,000 ± 222 M^{-1} δHG = 7.9715 ppm.



Binding curve 2A shows the ^1H NMR titration of Reinhoudt's receptor **1.49** with tetrabutylammonium bromide following the tosylamino NH signal. The receptor's ^1H NMR spectrum underwent similar changes upon TBABr additions as in the case of TBACl, however, the degree of movement varied considerably. The limiting chemical shift upon saturation of the host with bromide was only 1.3 ppm (2.6 ppm for chloride). This smaller change suggested that the bromide was bound less strongly by **1.49**. The computer fit data were found to be in good agreement with the actual data with K_{ass} of $340 \pm 12\ M^{-1}$.

Binding curve 2B shows the NMR data following the aryl proton *ortho* to the SO_2 group of the tosyl group in the same experiment. The change in chemical shift was also less than in the case of TBACl (binding curve 1B) suggesting a weaker complex. The association constant K_{ass} was found to be $470 \pm 15\ M^{-1}$.

The receptor was found to bind chloride selectively over bromide by a factor of ten. This selectivity for chloride may be due to :

(i) higher charge density of chloride over bromide

(ii) preorganisation of the host for chloride.

As the ligand is not expected to exhibit the rigidity about the binding site necessary for preorganisation, we are inclined to the view that (i) is more important.

The aromatic signals of **1.49** showed characteristic features that occur when involved in binding. One AB resonance (aryl protons *ortho* to the SO_2 group) moved substantially downfield upon complexation whereas the other AB resonance (*meta* protons) moved upfield. Other signals (*e.g.* tosyl CH_3), all showed downfield shifts upon complexation but to a lesser extent. This implies that the anion when bound is buried deep within the heart of the cavity distant from these groups. The results showed an increase in binding (K_{ass}) due to the change in solvent polarity from acetonitrile to $CDCl_3$.

Binding curve 2A. NMR Binding Study of Association of Reinhoudt's Receptor **1.49** with Tetrabutylammonium bromide.

Dry Conditions were used during this study.

NMR used : MSL 300 at 298 K.

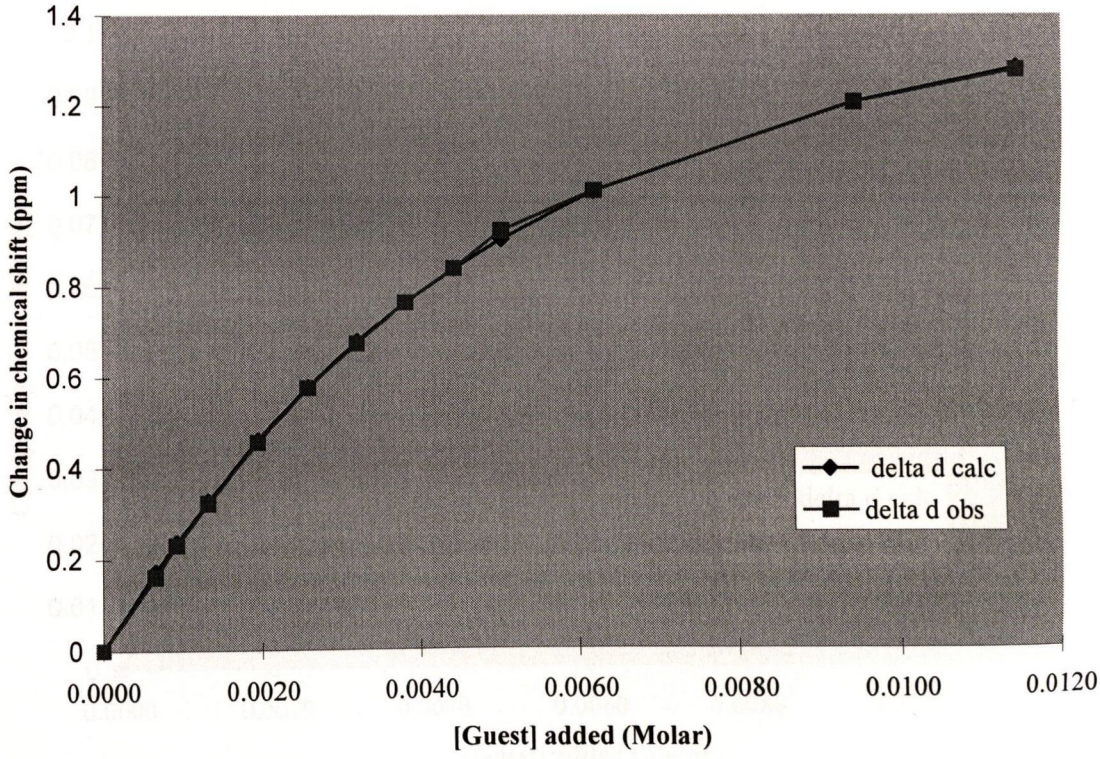
The chemical shift of NH protons was followed throughout this titration.

Solution concentrations : Host = 3.3 mM Guest = 79.5 mM.

Initial volume of host = 600 μL. Initial shift (δ) of NH = 5.8021

μL of Guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.00066	5.9638	0.1617	0.172828
7	0.00092	6.0325	0.2304	0.236291
10	0.00131	6.1232	0.3211	0.325569
15	0.00195	6.2564	0.4543	0.459260
20	0.00258	6.3756	0.5735	0.575463
25	0.00320	6.4744	0.6723	0.676043
30	0.00381	6.5651	0.763	0.762962
35	0.00441	6.6404	0.8383	0.838116
40	0.00500	6.7245	0.9224	0.903244
50	0.00615	6.8123	1.0102	1.009328
80	0.00941	7.0076	1.2055	1.206251
100	0.01143	7.0793	1.2772	1.282464

K_{ass} = 340 ± 12 M^{-1} δHG = 7.4200 ppm.



Binding curve 2B. NMR Binding Study of Association of Reinhoudt's Receptor **1.49** with Tetrabutylammonium bromide.

Dry Conditions were used during this study.

NMR used : MSL 300 at 298 K.

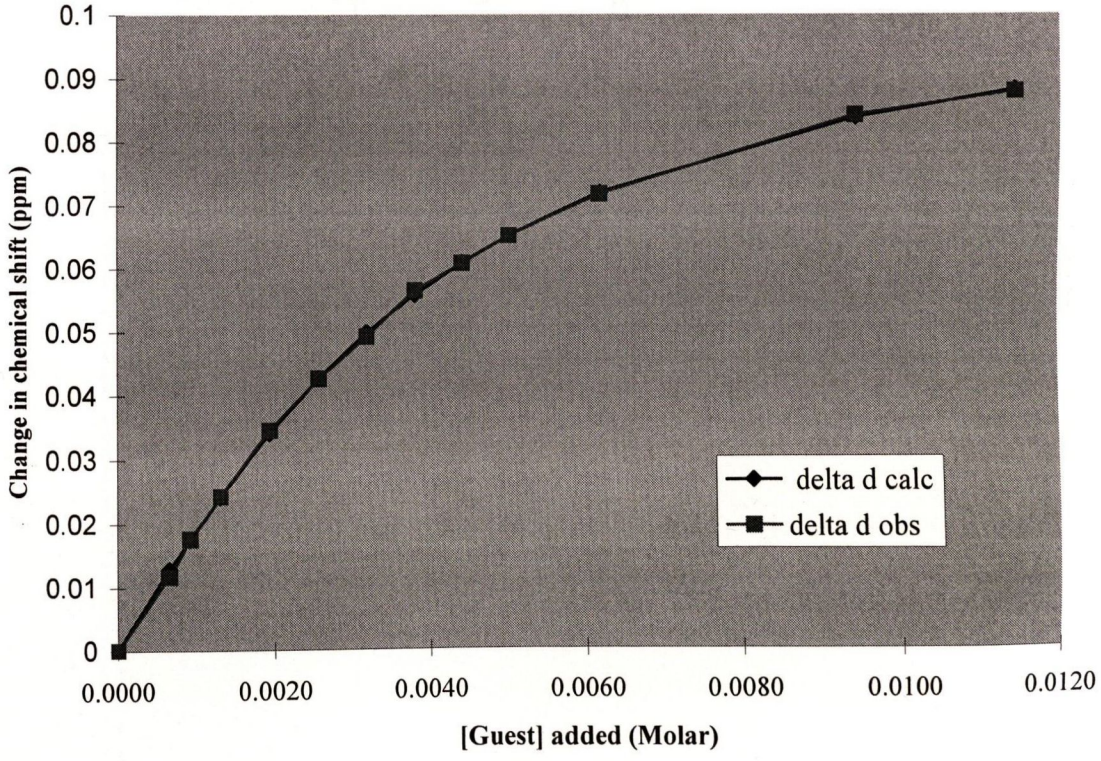
The chemical shift of the aryl proton *ortho* to the SO_2 group was followed throughout this titration.

Solution concentrations : Host = 3.3 mM Guest = 79.5 mM.

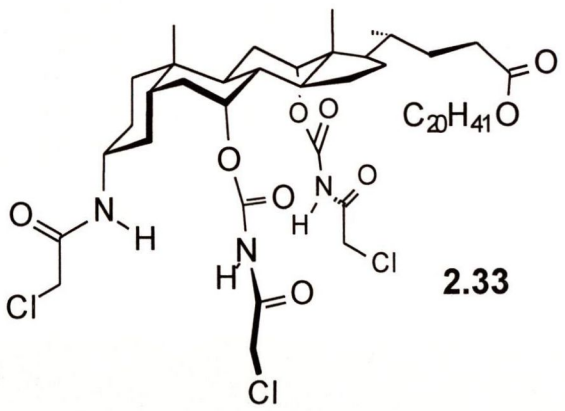
Initial volume of host = 600 μL. Initial shift (δ) of aromatic proton = 7.8291

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000661	7.8408	0.0117	0.012827
7	0.000923	7.8467	0.0176	0.017538
10	0.001311	7.8533	0.0242	0.024153
15	0.001951	7.8635	0.0344	0.033991
20	0.002581	7.8715	0.0424	0.042425
25	0.003200	7.8781	0.049	0.049589
30	0.003810	7.8854	0.0563	0.055645
35	0.004409	7.8898	0.0607	0.060760
40	0.005000	7.8942	0.0651	0.065088
50	0.006154	7.9008	0.0717	0.071907
80	0.009412	7.9132	0.0841	0.083742
100	0.011429	7.9169	0.0878	0.088029

$K_{ass} = 470 \pm 15\ M^{-1}$ δHG = 7.9390 ppm.



Binding curves 3A, 3B and 3C show the ^{1}H NMR data of a binding study of receptor **2.33** titrated with tetrabutylammonium chloride in dry $CDCl_3$. Throughout the titration, only one set of host resonances was observed for the chloride complex and the free host indicating that they are in fast exchange on the NMR timescale. The host was found to undergo substantial changes in its ^{1}H NMR spectra on chloride addition. The 3α-NH proton of the host were found to undergo the most downfield shifts (Δδ of the 3α-NH and the carbamate NH protons is 1.8, 2.5 and 2.7 ppm respectively) throughout the titration. The basicity of the chloride anion promoted the proton exchange kinetics to cause the NH signals to broaden with increased guest concentration. The signals however did not vanish into the baseline at any stage during the titration. In addition, the reduction in signal broadening observed upon addition of one equivalent of guest to host, is consistent with the host becoming 'frozen out' as a result of anion binding. The 3α–NH and carbamate NH protons were followed throughout the titration in binding curves 3A, 3B and 3C and they are used to calculate the strength of binding K_{ass} of 46,600 ± 4,700, 46,900 ± 5,690 and 47,700 ± 5,460 M^{-1} respectively.



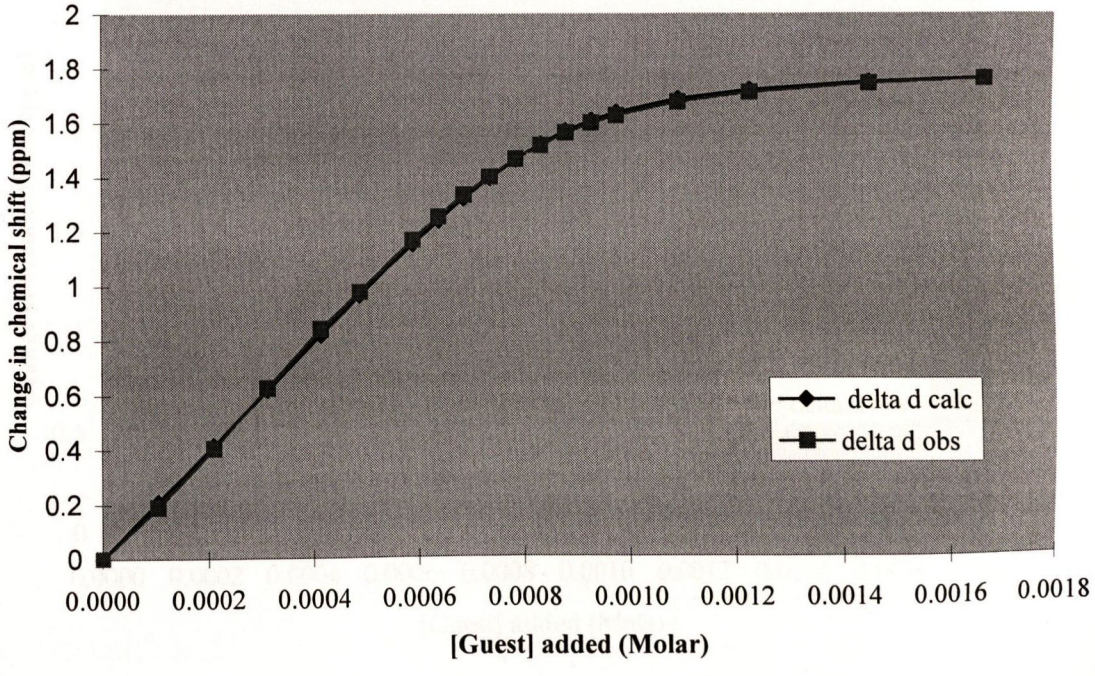
2.33

Binding curve 3A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α–NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 16 mM.
Initial volume of host = 600 μL. Initial shift (δ) of 3α–NH = 6.3736 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000106	6.5630	0.1894	0.207355
8	0.000211	6.7775	0.4039	0.413005
12	0.000314	6.9958	0.6222	0.616138
16	0.000416	7.2116	0.838	0.815379
19	0.000491	7.3446	0.971	0.960853
23	0.000591	7.5352	1.1616	1.146255
25	0.000640	7.6205	1.2469	1.233424
27	0.000689	7.7008	1.3272	1.315320
29	0.000738	7.7685	1.3949	1.390445
31	0.000786	7.8338	1.4602	1.457264
33	0.000834	7.8839	1.5103	1.514593
35	0.000882	7.9304	1.5568	1.562040
37	0.000929	7.9668	1.5932	1.600173
39	0.000977	7.9931	1.6195	1.630278
44	0.001093	8.0433	1.6697	1.680340
50	0.001231	8.0784	1.7048	1.713218
60	0.001455	8.1098	1.7362	1.740607
70	0.001672	8.1273	1.7537	1.754479

K_{ass} = 46,600 ± 4,700 M^{-1} δHG = 8.1707 ppm.

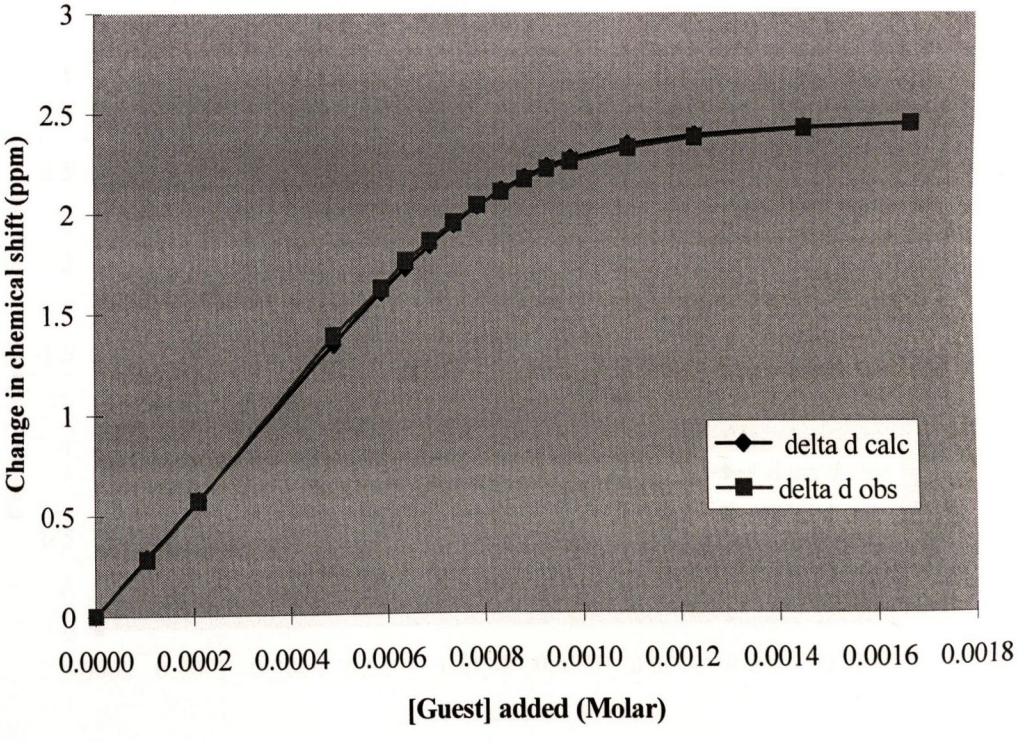


Binding curve 3B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 16 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.5362 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000106	8.8160	0.2798	0.289044
8	0.000211	9.1070	0.5708	0.575727
19	0.000491	9.9149	1.3787	1.339587
23	0.000591	10.1532	1.617	1.598182
25	0.000640	10.2900	1.7538	1.719788
27	0.000689	10.3915	1.8553	1.834052
29	0.000738	10.4844	1.9482	1.938876
31	0.000786	10.5722	2.036	2.032104
33	0.000834	10.6387	2.1025	2.112067
35	0.000882	10.7027	2.1665	2.178210
37	0.000929	10.7554	2.2192	2.231328
39	0.000977	10.7930	2.2568	2.273226
44	0.001093	10.8594	2.3232	2.342808
50	0.001231	10.9134	2.3772	2.388432
60	0.001455	10.9573	2.4211	2.426398
70	0.001672	10.9799	2.4437	2.445614

K_{ass} = 46,900 ± 5,690 M^{-1} δHG = 11.0408 ppm.

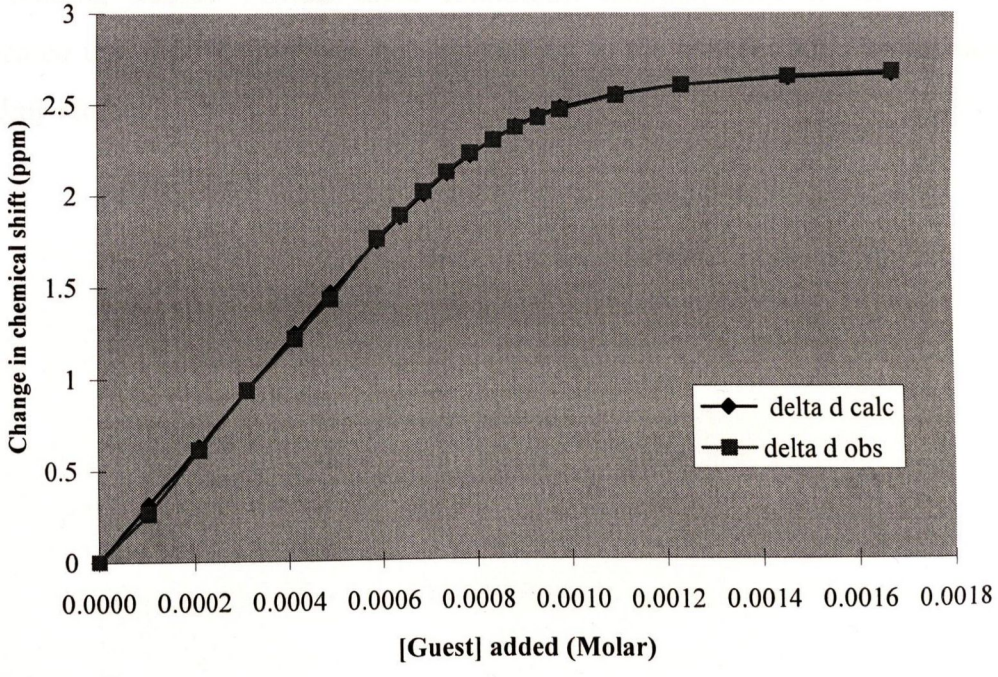


Binding curve 3C. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 16 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.6190 ppm

μL of guest added	[guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000106	8.8837	0.2647	0.314286
8	0.000211	9.2300	0.611	0.626048
12	0.000314	9.5548	0.9358	0.934078
16	0.000416	9.8308	1.2118	1.236331
19	0.000491	10.0428	1.4238	1.457132
23	0.000591	10.3715	1.7525	1.738736
25	0.000640	10.4995	1.8805	1.871233
27	0.000689	10.6299	2.0109	1.995772
29	0.000738	10.7390	2.12	2.110042
31	0.000786	10.8419	2.2229	2.211652
33	0.000834	10.9146	2.2956	2.298739
35	0.000882	10.9849	2.3659	2.370669
37	0.000929	11.0389	2.4199	2.428318
39	0.000977	11.0827	2.4637	2.473686
44	0.001093	11.1618	2.5428	2.548770
50	0.001231	11.2170	2.598	2.597800
60	0.001455	11.2672	2.6482	2.638482
70	0.001672	11.2922	2.6732	2.659036

K_{ass} = 47,700 ± 5,460 M^{-1} δHG = 11.3410 ppm



Binding curves 4A, 4B and 4C are for receptor **2.33** titrated with tetrabutylammonium bromide. The ^{1}H NMR spectrum of the host underwent similar changes to those when titrated with chloride. However the degree of shift of the NH protons varied considerably. The 3α-NH and carbamate NH protons signals were followed throughout the titration giving a K_{ass} of 15,500 ± 917, 16,200 ± 800 and 15,500 ±866 M^{-1} respectively. The measured data appear to be in good agreement with the calculated data (see binding curves) giving consistent K_{ass} values for chloride and bromide complexation.

Table 3.1 Complex Induced Shifts (CIS)

	3α-NH	carbamate NH	carbamate NH
Cl-	1.8	2.5	2.7
Br-	1.6	2.0	2.3

The role of the imido-carbamates is evident in the presence of guest anions. The NH resonances shift significantly downfield (> 2 ppm) whereas the 3α-amido NH is perturbed to a lesser extent (< 2 ppm). Considering that the 1:1 stoichiometry appears to remain fixed even in the presence of a large excess of guest, one is forced to assume the penetration of the guest into the molecular cavity of the host and consequently the formation of a stable complex.

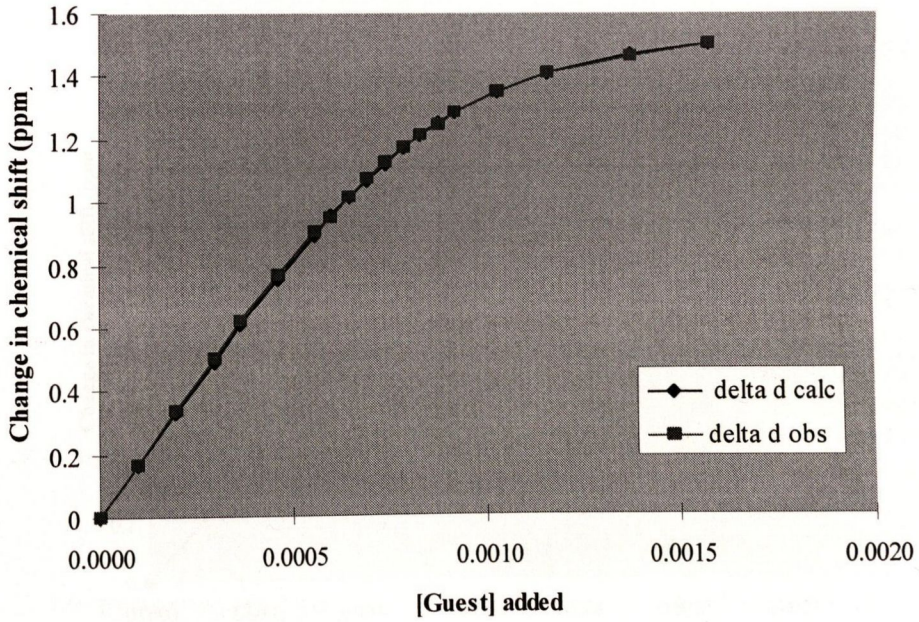
The possibility of the host self-associating was discounted by dilution studies of the receptor in $CDCl_3$ in the range of 10^{-2} - 10^{-3} M which includes the concentrations used in the binding studies. Under these conditions, minimal shift of the NH resonances indicated that the receptor was not aggregating in the concentration range used for the binding studies.

Binding curve 4A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α–NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 15 mM.
Initial volume of host = 600 μL. Initial shift (δ) of 3α–NH = 6.3500 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	9.93E-05	6.5179	0.1679	0.166750
8	1.97E-04	6.6872	0.3372	0.330204
12	2.94E-04	6.8515	0.5015	0.489252
15	3.66E-04	6.9657	0.6157	0.604739
19	4.60E-04	7.1125	0.7625	0.752035
23	5.54E-04	7.25	0.9	0.889314
25	6.00E-04	7.3	0.95	0.953259
27	6.46E-04	7.3621	1.0121	1.013549
29	6.92E-04	7.4223	1.0723	1.069822
31	7.37E-04	7.4737	1.1237	1.121781
33	7.82E-04	7.5189	1.1689	1.169232
35	8.27E-04	7.5628	1.2128	1.212107
37	8.71E-04	7.5942	1.2442	1.250477
39	9.15E-04	7.6318	1.2818	1.284542
44	1.02E-03	7.6995	1.3495	1.352982
50	1.15E-03	7.7573	1.4073	1.410415
60	1.36E-03	7.815	1.465	1.469147
70	1.57E-03	7.8488	1.4988	1.503196

K_{ass} = 15,500 ± 917 M^{-1} δHG = 7.9715 ppm

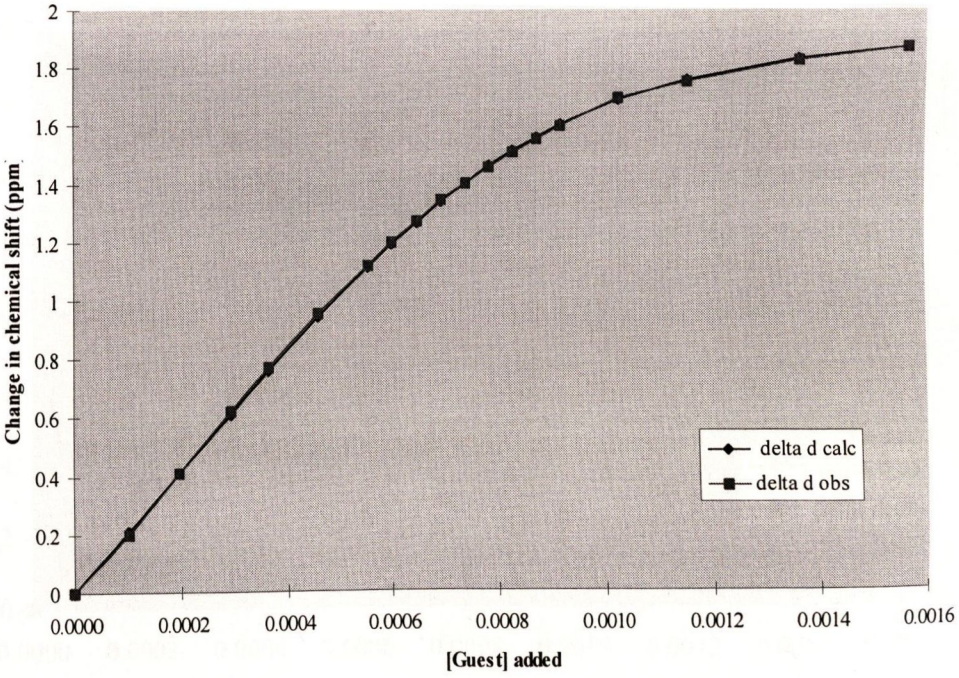


Binding curve 4B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 15 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.5187 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	9.934E-05	8.7181	0.1994	0.207395
8	1.974E-04	8.9264	0.4077	0.410832
12	2.941E-04	9.1384	0.6197	0.608958
15	3.659E-04	9.2850	0.7663	0.752952
19	4.604E-04	9.4670	0.9483	0.936796
23	5.538E-04	9.6300	1.1113	1.108338
25	6.000E-04	9.7091	1.1904	1.188298
27	6.459E-04	9.7869	1.2682	1.263704
29	6.916E-04	9.8572	1.3385	1.334078
31	7.369E-04	9.9149	1.3962	1.399025
33	7.820E-04	9.9751	1.4564	1.458277
35	8.268E-04	10.0265	1.5078	1.511734
37	8.713E-04	10.0717	1.553	1.559478
39	9.155E-04	10.1170	1.5983	1.601762
44	1.025E-03	10.2100	1.6913	1.686317
50	1.154E-03	10.2711	1.7524	1.756728
60	1.364E-03	10.3414	1.8227	1.828101
70	1.567E-03	10.3855	1.8668	1.869167

K_{ass} = 16,200 ± 800 M^{-1} δHG = 10.5291 ppm

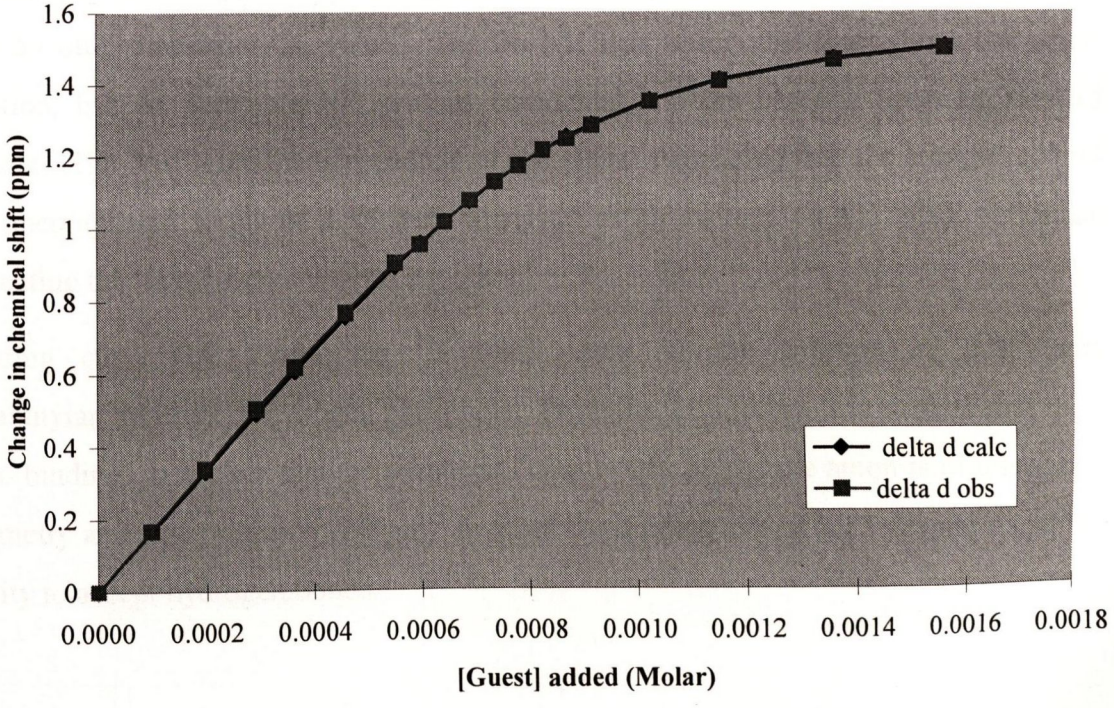


Binding curve 4C. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 0.9 mM Guest = 15 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.5914 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	9.934E-05	8.8085	0.2171	0.234083
8	1.974E-04	9.0380	0.4466	0.463539
12	2.941E-04	9.2626	0.6712	0.686810
15	3.659E-04	9.4407	0.8493	0.848930
19	4.604E-04	9.6615	1.0701	1.055704
23	5.538E-04	9.8396	1.2482	1.248416
25	6.000E-04	9.9274	1.336	1.338181
27	6.459E-04	10.0140	1.4226	1.422816
29	6.916E-04	10.0942	1.5028	1.501812
31	7.369E-04	10.1670	1.5756	1.574752
33	7.820E-04	10.2335	1.6421	1.641363
35	8.268E-04	10.2912	1.6998	1.701551
37	8.713E-04	10.3451	1.7537	1.755415
39	9.155E-04	10.4004	1.809	1.803235
44	1.025E-03	10.4869	1.8955	1.899311
50	1.154E-03	10.5710	1.9796	1.979935
60	1.364E-03	10.6537	2.0623	2.062383
70	1.567E-03	10.7064	2.115	2.110181

K_{ass} = 15,500 ± 866 M^{-1} δHG = 10.8677 ppm



Receptor **2.33** was investigated for its ability to bind other anions, namely iodide, acetate, dihydrogen phosphate and nitrate. Binding curves 5A and 5B show the ^{1}H NMR data for the titration of **2.33** with tetrabutylammonium iodide. Binding curve 5A shows the data from the chemical shift of the carbamate NH proton throughout the titration giving a K_{ass} of 3,658 ± 125 M^{-1}. Binding curve 5B shows the data for the other carbamate NH proton giving a K_{ass} of 3,853 ± 132 M^{-1} in good agreement with the carbamate NH proton followed in the binding curve 5A. The receptor's ^{1}H NMR spectra underwent changes similar to those for the addition of the other halides, chloride and bromide. However the degree of shift of the NH protons was less ($\Delta\delta$ for the carbamate NH protons is 1.5 and 1.72 ppm respectively for iodide binding). This moderate binding of iodide by **2.33** is most likely due to the unsuitability of the binding cavity size for the large 'soft' iodide anion compared to that of the small 'hard' chloride anion. Contrary to what might be expected, no signs of iodoacetyl formation was evident, on addition of iodide to the chloroacetyl functional groups of **2.33**.

Binding curve 6A shows the NMR data for the titration of **2.33** with tetrabutylammonium acetate. The 3α-NH proton was followed throughout this titration giving a K_{ass} of 18,410 ± 1,720 M^{-1}. Although the acetate anion is known for its strong ability to accept hydrogen bonds, its inferior binding by **2.33** when compared to that of chloride is evident from the binding study. The planar geometry of the acetate anion is not suitable to fit the binding cavity of **2.33** and therefore the hydrogen bonds formed are not of optimal strength. This host/guest unsuitability of **2.33** and acetate is translated into an only moderate K_{ass} value. The 3α-NH was observable throughout the whole titration, but the carbmate NH protons broadened into the baseline upon addition of acetate. The host resonances appeared as one signal suggesting that the complexed and the uncomplexed forms of **2.33** were observed as an average on the NMR timescale indicating that fast kinetics was taking place.

Binding curve 7A shows the ^{1}H NMR data for the titration of **2.33** with tetrabutylammonium dihydrogen phosphate. From the data shown it is evident that there is no binding. It is clear that the tetrahedral dihydrogen phosphate anion is of unsuitable geometry and size (radius = 200 pm)[3] to enter the binding site of **2.33** despite its strong ability to accept hydrogen bonds.

Binding curves 8A, 8B and 8C show the ^{1}H NMR data for the titration of **2.33** with tetrabutylammonium nitrate. Binding curve 8A follows the 3α-NH proton throughout the titration giving a K_{ass} of 17,627 ± 620 M^{-1}. Binding curve 8B follows a carbamate NH proton giving a K_{ass} of 19,368 ± 1,173 M^{-1} and 8C follows the other carbamate NH giving 18,173 ± 1,982 M^{-1}. The three NH protons gave K_{ass} values in close agreement with each other. The binding strength of **2.33** for nitrate is comparable to that of binding bromide and acetate despite the planar-trigonal geometry of the anion. The strong binding of nitrate is perhaps due to the size of the anion being comparable to that of chloride (Cl^- : radius = 181 pm, NO_3^- : radius = 176 pm)[2], and so it is capable of entering the binding site of **2.33** and forming three moderately strong hydrogen bonds to the three NH protons available.

Anslyn's receptor **1.51** discussed in chapter one, was described in the literature for its selectivy for NO_3^- over Cl^-, Br^-, $H_2PO_4^-$ and HSO_4^-. However, the receptor was found to bind OAc^- over NO_3^- by a factor of 2.6. Comparing the results of **2.33** to **1.51**, the former binds NO_3^- and OAc^- to the same extent although OAc^- is 10^6 times more basic. Further, $H_2PO_4^-$ is also significantly more basic than NO_3^-, yet it is precluded from the cavity presumably due to size and therefore a much lower affinity.

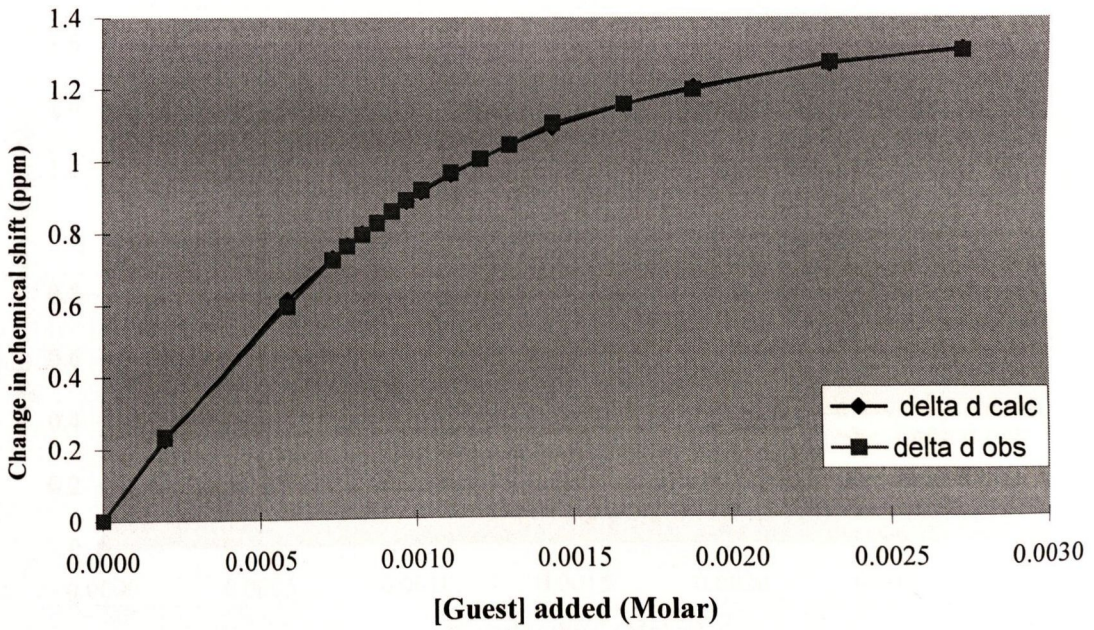
In conclusion, receptor **2.33** was found to bind chloride selectively over bromide, acetate and nitrate by a factor of three and iodide by a factor of eleven. This selectivity for chloride is due to the suitability of the receptor's binding site towards a spherical anion of comparable size to chloride.

Binding curve 5A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium iodide.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 30 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.6153 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000198675	8.8473	0.232	0.225863
12	0.000588235	9.2074	0.5921	0.609973
15	0.000731707	9.3366	0.7213	0.726073
16	0.000779221	9.3742	0.7589	0.761185
17	0.00082658	9.4068	0.7915	0.794522
18	0.000873786	9.4394	0.8241	0.826113
19	0.00092084	9.4720	0.8567	0.855998
20	0.000967742	9.5034	0.8881	0.884225
21	0.001014493	9.5310	0.9157	0.910852
23	0.001107544	9.5787	0.9634	0.959559
25	0.0012	9.6176	1.0023	1.002672
27	0.001291866	9.6564	1.0411	1.040774
30	0.001428571	9.7179	1.1026	1.089787
35	0.001653543	9.7706	1.1553	1.154070
40	0.001875	9.8107	1.1954	1.202269
50	0.002307692	9.8873	1.272	1.268068
60	0.002727273	9.9211	1.3058	1.309938

K_{ass} = 3,658 ± 125 M^{-1} δHG = 10.1107 ppm

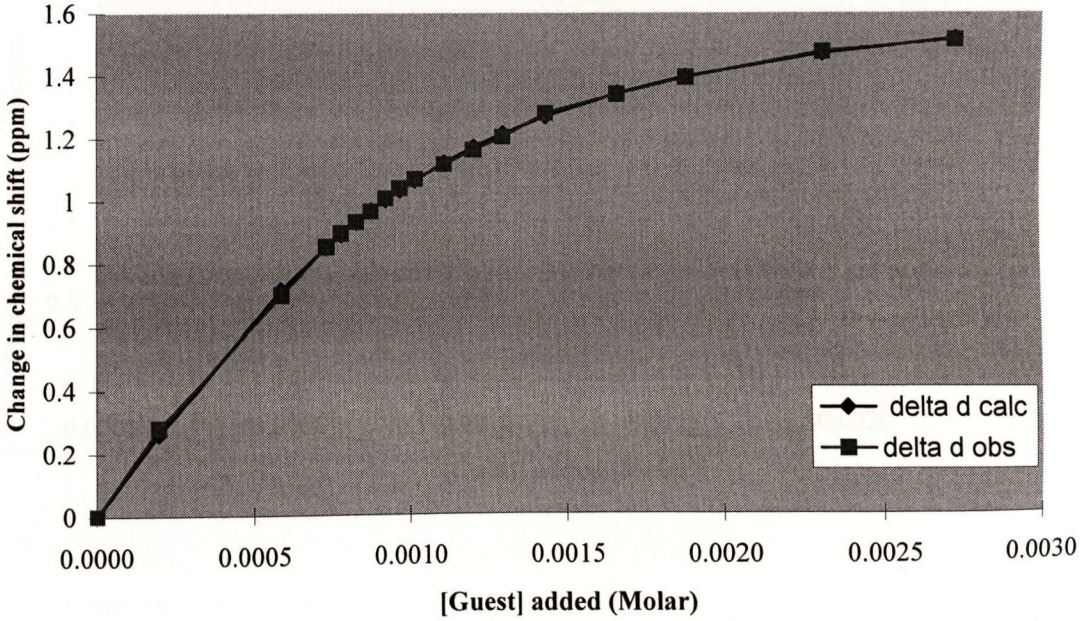


Binding curve 5B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium iodide.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 30 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.7357 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000199	9.0154	0.2797	0.2627
12	0.000588	9.4319	0.6962	0.7105
15	0.000732	9.5837	0.848	0.8459
16	0.000779	9.6263	0.8906	0.8868
17	0.000827	9.6627	0.927	0.9256
18	0.000874	9.6966	0.9609	0.9623
19	0.000921	9.7380	1.0023	0.9970
20	0.000968	9.7706	1.0349	1.0297
21	0.001014	9.8007	1.065	1.0606
23	0.001108	9.8484	1.1127	1.1169
25	0.001200	9.8948	1.1591	1.1666
27	0.001292	9.9362	1.2005	1.2103
30	0.001429	10.0127	1.277	1.2664
35	0.001654	10.0729	1.3372	1.3396
40	0.001875	10.1269	1.3912	1.3941
50	0.002308	10.2097	1.474	1.4679
60	0.002727	10.2473	1.5116	1.5146

K_{ass} = 3,853 ± 132 M^{-1} δHG = 10.4544 ppm

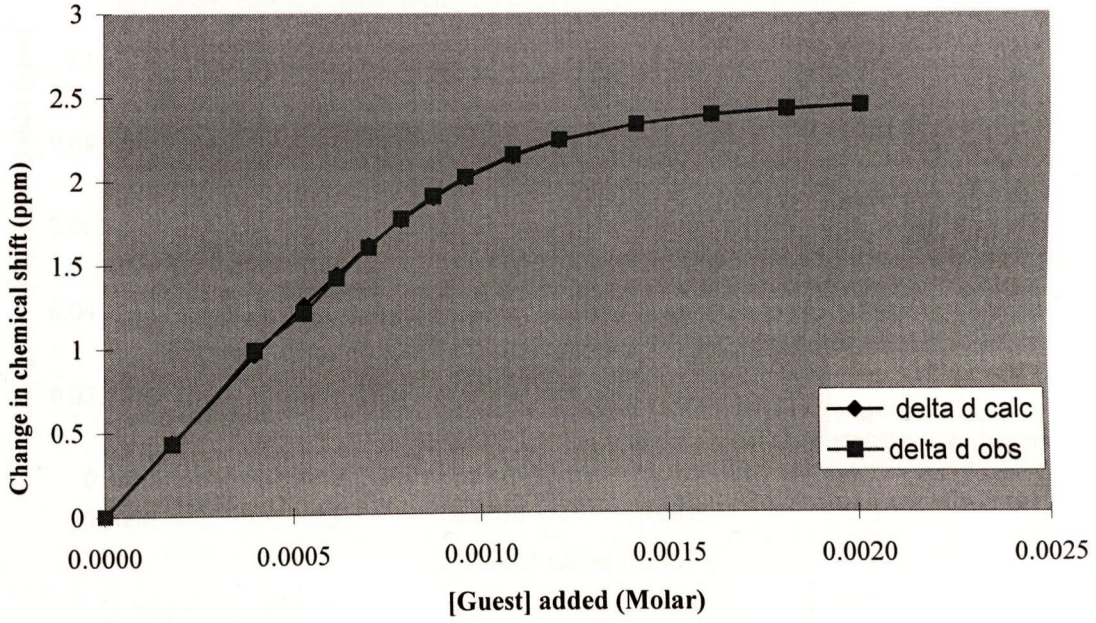


Binding curve 6A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium acetate.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α–NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the 3α–NH = 6.4363 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in Shift relative To δ (ppm)	Computer calculated change in shift (ppm)
4	0.000179	6.8641	0.4278	0.433750
9	0.000399	7.4173	0.981	0.956163
12	0.000529	7.6418	1.2055	1.251599
14	0.000616	7.8501	1.4138	1.436738
16	0.000701	8.0370	1.6007	1.608938
18	0.000786	8.2088	1.7725	1.764723
20	0.000871	8.3443	1.908	1.900861
22	0.000955	8.4585	2.0222	2.015393
25	0.001080	8.5902	2.1539	2.147539
28	0.001204	8.6792	2.2429	2.239918
33	0.001408	8.7721	2.3358	2.335703
38	0.001608	8.8298	2.3935	2.390989
43	0.001806	8.8662	2.4299	2.425706
48	0.002000	8.8875	2.4512	2.449164

K_{ass} = 18,410 ± 1,720 M^{-1} δHG = 9.0046 ppm



Binding curve 7A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium dihydrogen phosphate.

Dry Conditions were used.

NMR used : DPX 400 at 303 K.

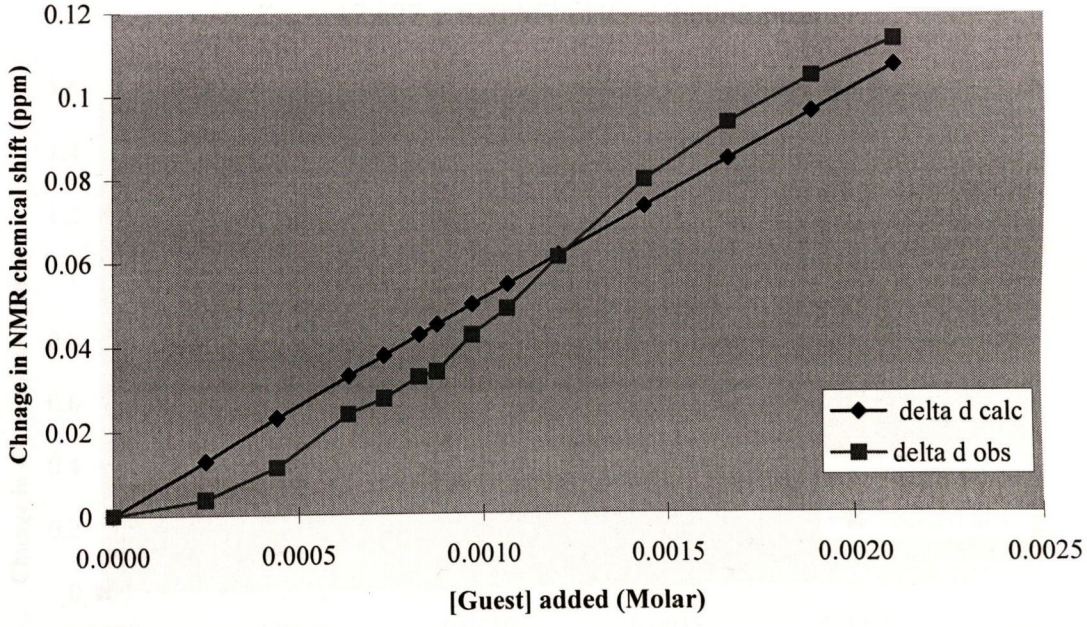
The chemical shift of the 3α–CH_2Cl protons was followed throughout this titration.

Solution concentrations : Host = 1.0 mM Guest = 30 mM.

Initial volume of host = 600 μL. Initial shift (δ) of the 3α–CH_2Cl = 4.0329 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000248	4.0366	0.0037	0.012804
9	0.000443	4.0441	0.0112	0.022891
13	0.000636	4.0567	0.0238	0.032843
15	0.000732	4.0604	0.0275	0.037769
17	0.000827	4.0655	0.0326	0.042662
18	0.000874	4.0667	0.0338	0.045097
20	0.000968	4.0755	0.0426	0.049941
22	0.001061	4.0818	0.0489	0.054754
25	0.001200	4.0943	0.0614	0.061913
30	0.001429	4.1131	0.0802	0.073690
35	0.001654	4.1269	0.094	0.085276
40	0.001875	4.1382	0.1053	0.096677
45	0.002093	4.1470	0.1141	0.107896

$K_{ass} = 0\ M^{-1}$

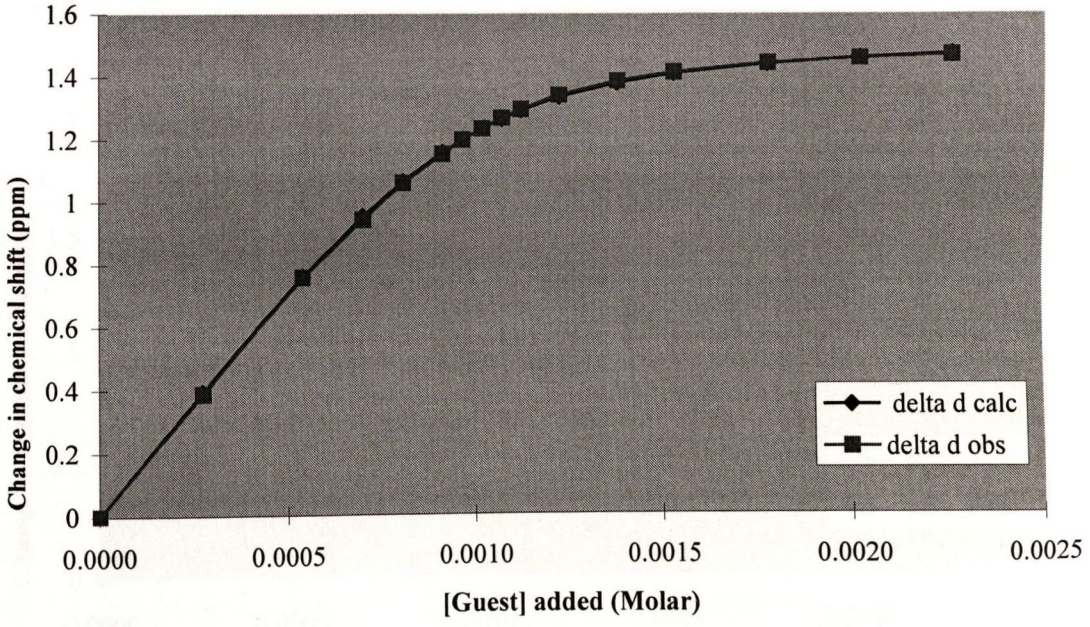


Binding curve 8A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium nitrate.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of the 3α–NH proton was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 33 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the 3α–NH = 6.4276 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.0002727	6.8127	0.3851	0.391525
10	0.0005410	7.1827	0.7551	0.756966
13	0.0006998	7.3684	0.9408	0.950998
15	0.0008049	7.4863	1.0587	1.063140
17	0.0009092	7.5804	1.1528	1.157666
18	0.0009612	7.6255	1.1979	1.197779
19	0.0010129	7.6607	1.2331	1.233172
20	0.0010645	7.6945	1.2669	1.264093
21	0.0011159	7.7221	1.2945	1.290915
23	0.0012183	7.7673	1.3397	1.334067
26	0.0013706	7.8131	1.3855	1.379191
29	0.0015215	7.8400	1.4124	1.409109
34	0.0017697	7.8695	1.4419	1.440315
39	0.0020141	7.8852	1.4576	1.459213
44	0.0022547	7.8959	1.4683	1.471723

K_{ass} = 17,627 ± 620 M^{-1} δHG = 9.0046 ppm

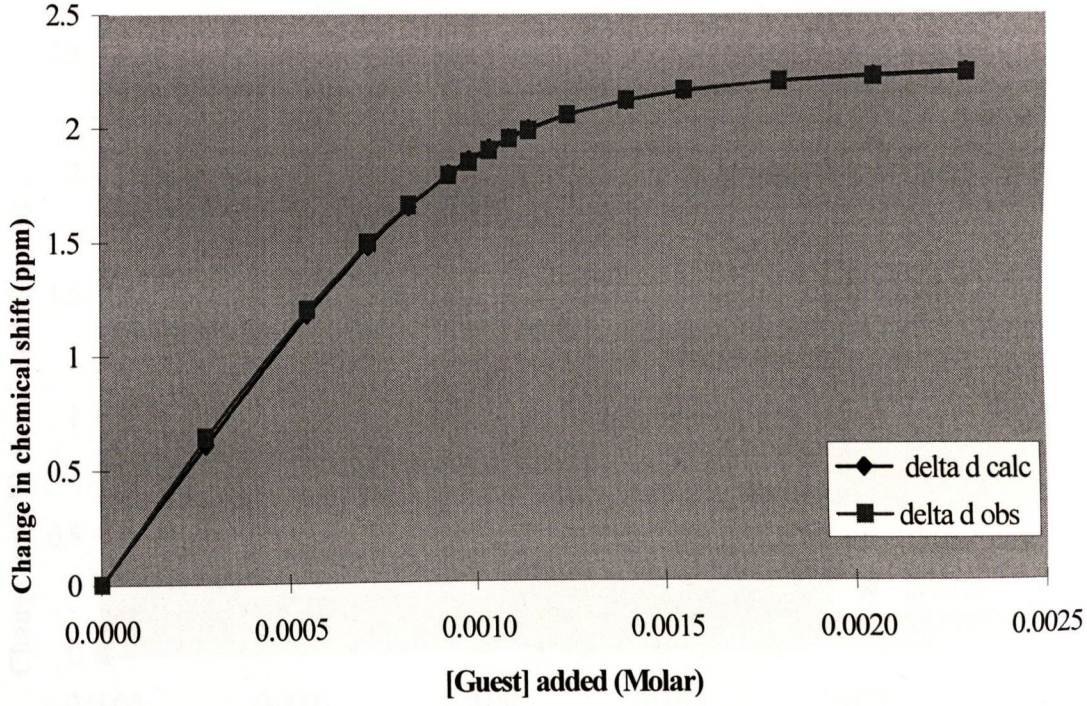


Binding curve 8B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium nitrate.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH proton was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 33 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.6090 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000277	9.2500	0.641	0.607504
10	0.000549	9.8057	1.1967	1.175927
13	0.000710	10.1018	1.4928	1.477604
15	0.000817	10.2686	1.6596	1.650913
17	0.000923	10.3991	1.7901	1.795282
18	0.000976	10.4568	1.8478	1.855726
19	0.001028	10.5082	1.8992	1.908497
20	0.001081	10.5596	1.9506	1.954091
21	0.001133	10.5960	1.987	1.993209
23	0.001237	10.6638	2.0548	2.055226
26	0.001391	10.7290	2.12	2.118835
29	0.001545	10.7716	2.1626	2.160356
34	0.001797	10.8118	2.2028	2.203206
39	0.002045	10.8331	2.2241	2.228985
44	0.002289	10.8482	2.2392	2.246002

K_{ass} = 19,368 ± 1,173 M^{-1} δHG = 10.9384 ppm

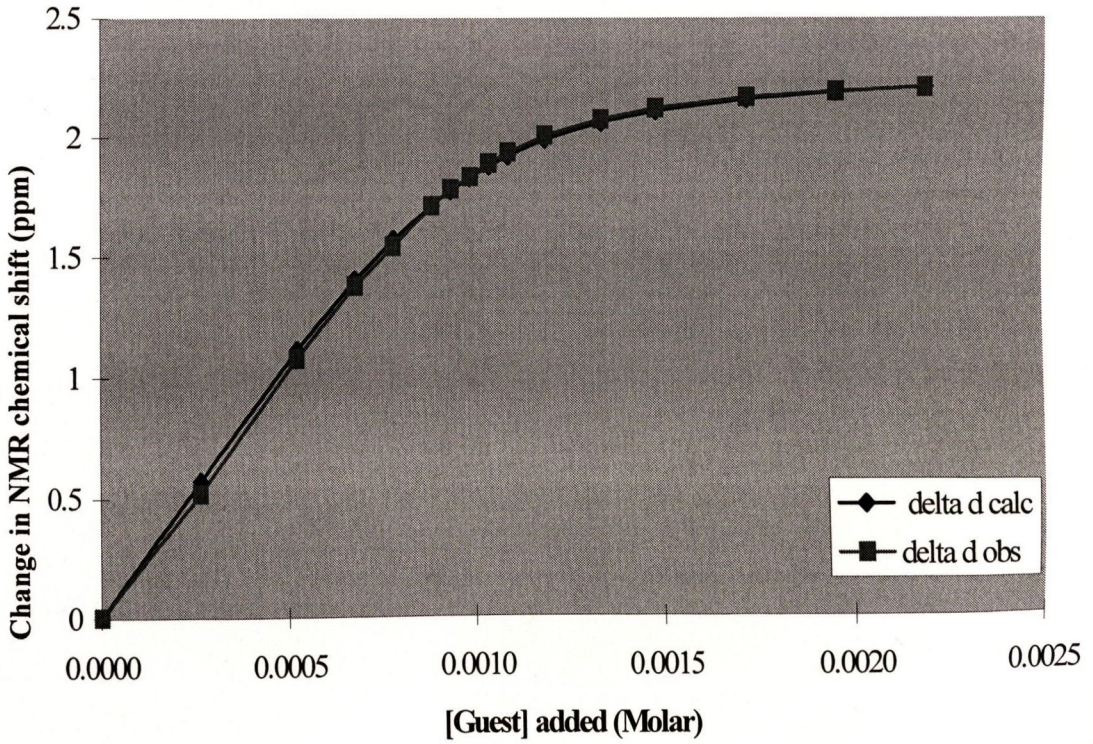


Binding curve 8C. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium nitrate.

Dry Conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH proton was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 33 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.7307 ppm

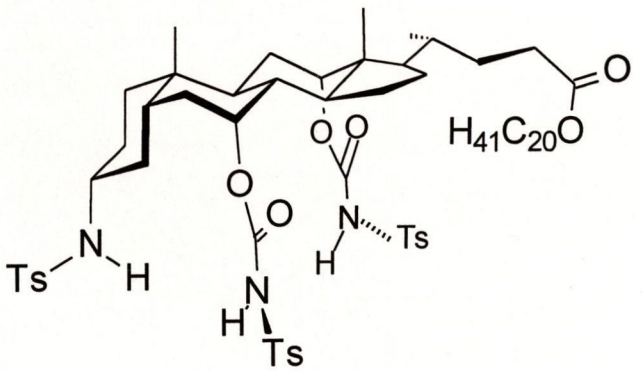
μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.0002645	9.2500	0.5193	0.571566
10	0.0005246	9.8057	1.075	1.107989
13	0.0006786	10.1018	1.3711	1.396223
15	0.0007805	10.2686	1.5379	1.565296
17	0.0008817	10.4367	1.706	1.710173
18	0.0009320	10.5082	1.7775	1.772506
19	0.0009822	10.5609	1.8302	1.827978
20	0.0010323	10.6173	1.8866	1.876807
21	0.0010821	10.6638	1.9331	1.919424
23	0.0011814	10.7353	2.0046	1.988395
26	0.0013291	10.8042	2.0735	2.060774
29	0.0014754	10.8494	2.1187	2.108658
34	0.0017161	10.8958	2.1651	2.158266
39	0.0019531	10.9209	2.1902	2.188044
44	0.0021863	10.9360	2.2053	2.207614

K_{ass} = 18,173 ± 1,982 M^{-1} δHG = 10.9384 ppm



Binding curves 9A and 10A show the details of binding of receptor **2.8** with tetrabutylammonium chloride. The NH protons were found to undergo the most significant downfield shifts throughout the experiments, ranging from 2 ppm for the 3α-NH to 1 ppm for the 7 and 12α-NH protons. However, these signals broadened with increased guest concentrations before eventually vanishing into the baseline. It was only upon saturation of the receptor with chloride that the NH protons reappeared and sharpened. Small perturbations were observed for the aromatic CH protons which underwent characteristic changes upon anion complexation. Three AB resonances (*ortho*-protons) were found downfield at the end of the titration whereas the *meta*-protons remained upfield and second order in nature. Although the aromatic signals were sharp, their second order nature precluded us from using them to obtain K_{ass}.

The 19 methyl group was found to remain sharp and observable throughout the titration and therefore easy to follow. The binding study was performed twice and the same signal was followed as shown in binding curves 9A and 10A. As one can see from both curves, the actual data, when plotted against the theoretical data, gave a poor fit and so a large error was seen in both cases. The poor fit was due to the small change in chemical shift of the 19 methyl protons upon chloride complexation. This small chemical shift (<0.1 ppm) is due to the fact that this methyl group, on the β-face of the steroid, is far removed from the binding site and undergoes minimal change on chloride complexation.



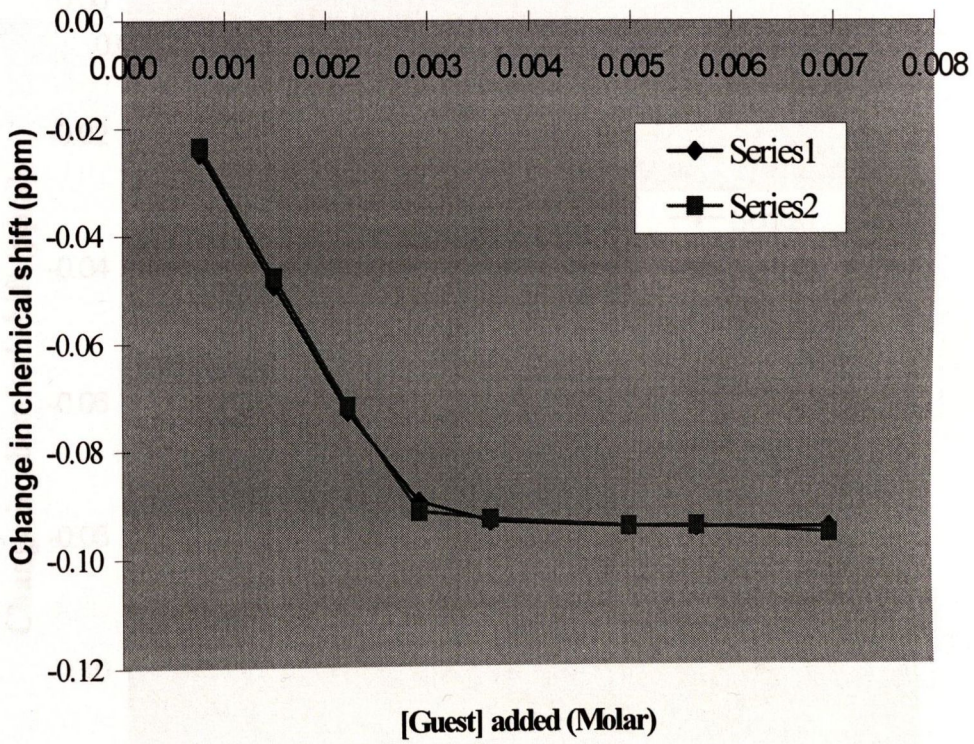
2.8

Binding curve 9A. NMR Binding Study of Association of Receptor **2.8** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : MSL 300 at 298 K.
The chemical shift of $19CH_3$ was followed throughout this titration.
Solution concentrations : Host = 0.29 mM Guest = 9.1 mM.
Initial volume of host = 600 μL. Initial shift (δ) of $19CH_3$ = 0.7869 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000752	0.7634	-0.0235	-0.024692
10	0.001492	0.7393	-0.0476	-0.049163
15	0.002220	0.7152	-0.0717	-0.072756
20	0.002935	0.6954	-0.0915	-0.089726
25	0.003640	0.6940	-0.0929	-0.093389
35	0.005016	0.6925	-0.0944	-0.094485
40	0.005688	0.6925	-0.0944	-0.094653
50	0.007000	0.6910	-0.0959	-0.094828

K_{ass} = 56,000 ± 31,000 M^{-1} δHG =0.6917 ppm



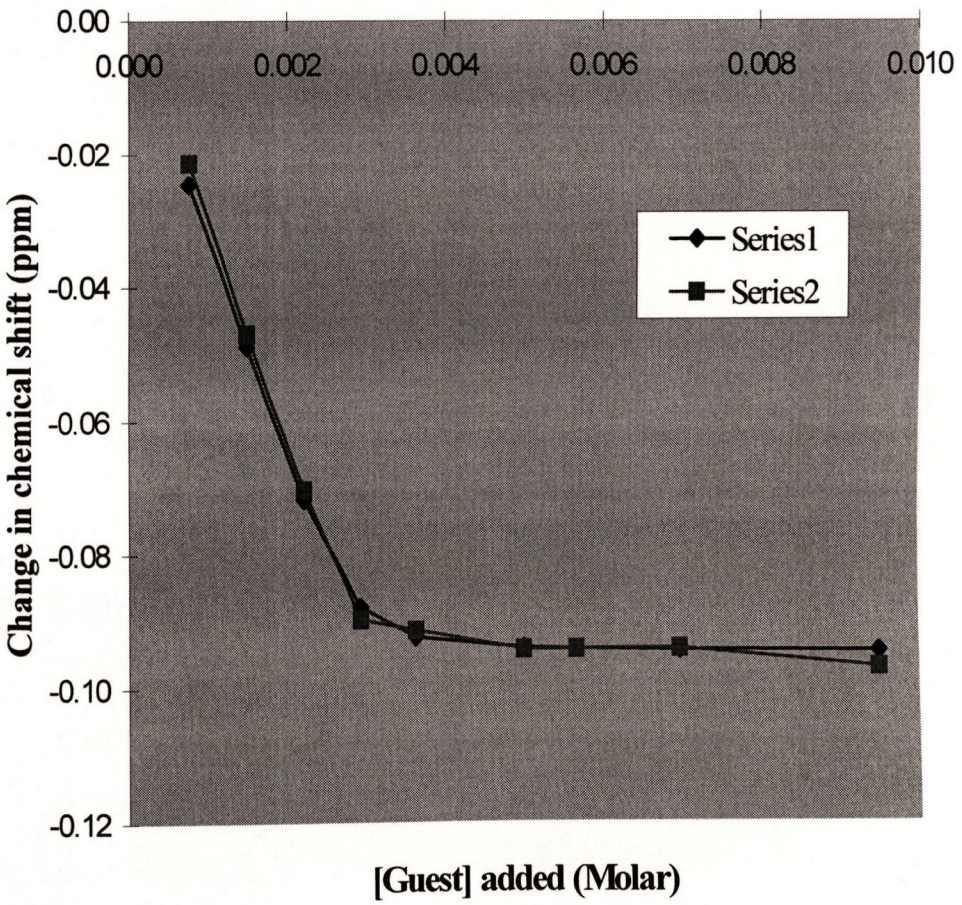
series 1 = delta d calc series 2 = delta d obs

Binding curve 10A. NMR Binding Study of Association of Receptor **2.8** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : MSL 300 at 298 K.
The chemical shift of $19CH_3$ was followed throughout this titration.
Solution concentrations : Host = 0.29 mM Guest = 9.1 mM.
Initial volume of host = 600 μL. Initial shift (δ) of $19CH_3$ = 0.7869 ppm.

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000752	0.7656	-0.0213	-0.024595
10	0.001492	0.7400	-0.0469	-0.048853
15	0.002220	0.7166	-0.0703	-0.071872
20	0.002935	0.6970	-0.0899	-0.088095
25	0.003640	0.6954	-0.0915	-0.092533
35	0.005016	0.6925	-0.0944	-0.094145
40	0.005688	0.6925	-0.0944	-0.094402
50	0.007000	0.6925	-0.0944	-0.094672
70	0.009507	0.6896	-0.0973	-0.094897

K_{ass} = 36,000 ± 21,000 M^{-1} δHG =0.6916 ppm.



series 1 = delta d calc series 2 = delta d obs

Binding curves 11A and 12A show the data of the NMR titration studies of receptor **2.8** with tetrabutylammonium bromide. The ^{1}H NMR spectrum of the receptor underwent similar changes as in the TBACl binding studies. The 19 methyl group was followed in both cases to obtain K_{ass} of 41,600 ± 30,000 and 40,000 ± 22,000 M^{-1} respectively. Although the errors are large in both cases, the consistency between both curves is an indication of the accuracy of K_{ass}.

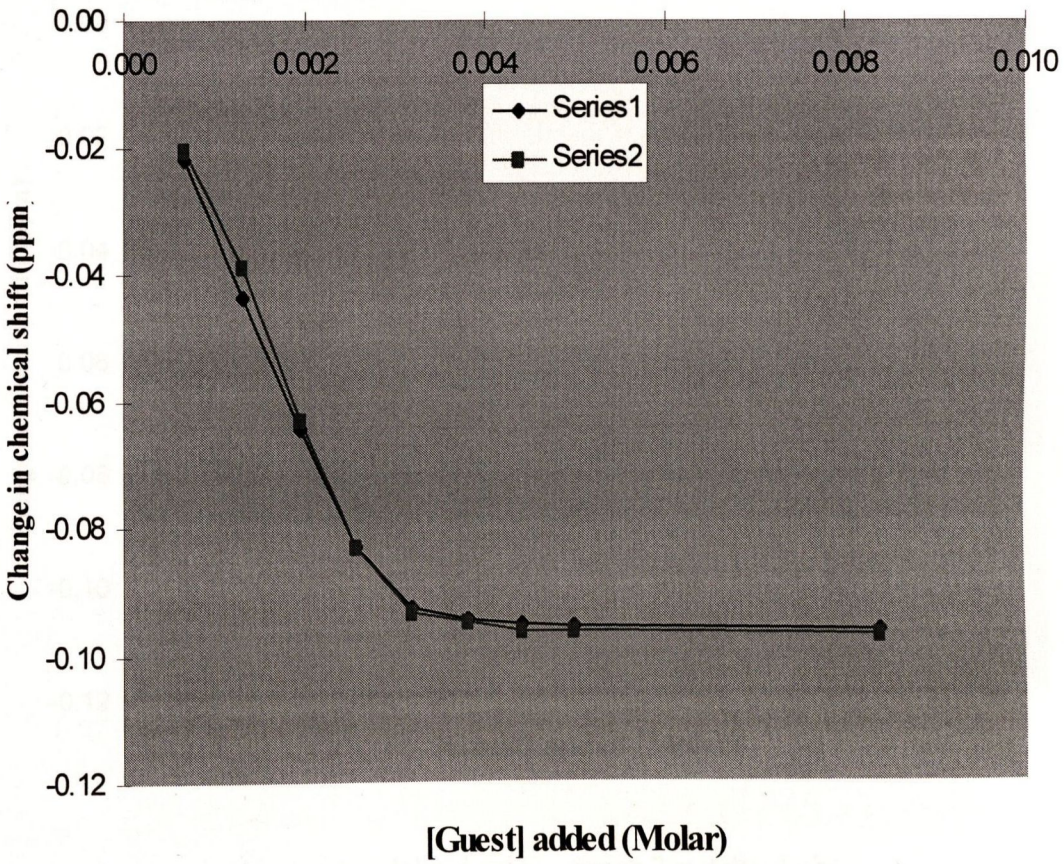
The 21 methyl group also underwent significant changes during the titrations for chloride and bromide. The resonance initially appeared as a doublet but upon addition of a halide the resonance broadened into the baseline until re-emerging 0.3 ppm downfield when one equivalent of halide had been added. This significant perturbation was unexpected as the 21 methyl group is believed to be far removed from the binding cavity in the receptor. A much lower degree of shift of this methyl group was observed for the other receptors examined under similar conditions. All other protons on the β-face of **2.8** underwent only small changes in the ^{1}H NMR spectrum as expected. The sulfonyl-carbamates were not used as functional groups again because of their instability in acidic media was undesirable in anionophore design.

Binding curve 11A. NMR Binding Study of Association of Receptor **2.8** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : MSL 300 at 298K.
The chemical shift of $19CH_3$ was followed throughout this titration.
Solution concentrations : Host = 0.29 mM Guest = 9.1 mM.
Initial volume of host = 600 μL. Initial shift (δ) of $19CH_3$ = 0.7869 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000665	0.7664	-0.0205	-0.021896614
10	0.001320	0.7481	-0.0388	-0.043600578
15	0.001963	0.7239	-0.063	-0.064753136
20	0.002597	0.7035	-0.0834	-0.083444587
25	0.003220	0.6932	-0.0937	-0.092667351
30	0.003833	0.6918	-0.0951	-0.094844307
35	0.004437	0.6903	-0.0966	-0.095574881
40	0.005031	0.6903	-0.0966	-0.095926718
70	0.008410	0.6896	-0.0973	-0.096526893

K_{ass} = 41,600 ± 30,000 M^{-1} δHG =0.6917 ppm



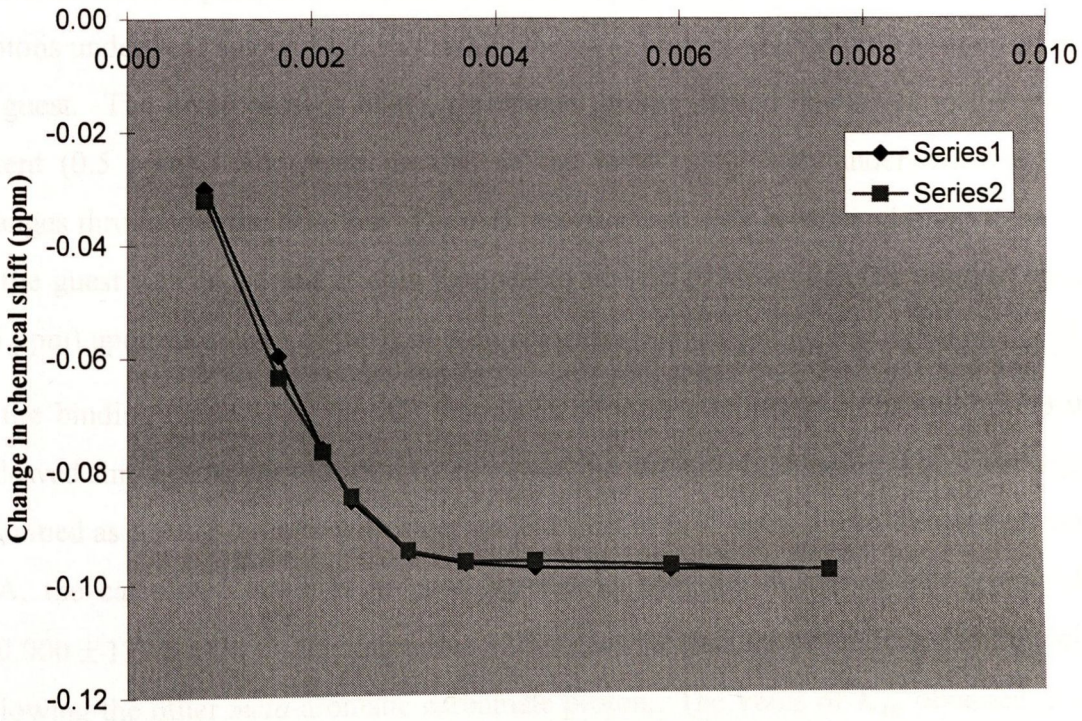
series 1 = delta d calc series 2 = delta d obs

Binding curve 12A. NMR Binding Study of Association of Receptor **2.8** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : MSL 300 at 298K.
The chemical shift of $19CH_3$ was followed throughout this titration.
Solution concentrations : Host = 0.27 mM Guest = 10 mM.
Initial volume of host = 600 μL. Initial shift (δ) of $19CH_3$ = 0.7876 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000826	0.7554	-0.0322	-0.030121
10	0.001639	0.7241	-0.0635	-0.059636
13	0.002121	0.7109	-0.0767	-0.076221
15	0.002439	0.7029	-0.0847	-0.085557
19	0.003069	0.6932	-0.0944	-0.094700
23	0.003692	0.6912	-0.0964	-0.096792
28	0.004459	0.6912	-0.0964	-0.097625
38	0.005956	0.6902	-0.0974	-0.098187
50	0.007692	0.6891	-0.0985	-0.098429

K_{ass} = 40,000 ± 22,000 M^{-1} δHG =0.6887 ppm



series 1 = delta d calc series 2 = delta d obs

Equipped with the knowledge that the 3α-toluenesulfonamide is a major contributor to halide binding (shown by the work of Perry *et al*), it was decided to modify receptor **2.8** by using more stable carbamate groups at the 7/12 positions while leaving the 3 position unchanged. The carbamate chosen in this case contained a trifluoromethyl group *para* substituted on a benzene ring. The resulting receptor **2.11** was found to be stable to washing with mildly acidic (2M HCl) or basic media (1M $NaHCO_3$).

Binding curves 13A and 13B show the NMR data for receptor **2.11** with tetrabutylammonium chloride. The addition of TBACl to **2.11** in $CDCl_3$ caused changes to the receptor's ^1H NMR spectra indicative of anion binding (*vide infra*). The NH resonances experienced the strongest downfield shifts but broadened rapidly with increased guest concentrations. The basicity of the guest anion probably promoted the proton exchange kinetics to cause the signals to disappear completely. Upon saturation of the host with one equivalent of chloride the NH proton signals re-appeared as sharp singlets far downfield (change in shift of 3α-NH and the carbamate NH protons was 1.2, 2.27 and 2.66 ppm respectively).

The aromatic CH protons also underwent shifts upon complexation. The *ortho* aromatic protons underwent a downfield shift while the *meta* protons shifted upfield upon addition of guest. The *ortho* protons of the carbamate groups shifted downfield to the greatest extent (0.5 ppm). The *meta* protons of the tosyl group also underwent significant changes throughout the titration. The AB resonance quickly broadened into the baseline as the guest was added and it only reappeared upfield (with a complex induced shift of 0.6 ppm) upon saturation of the host with chloride.

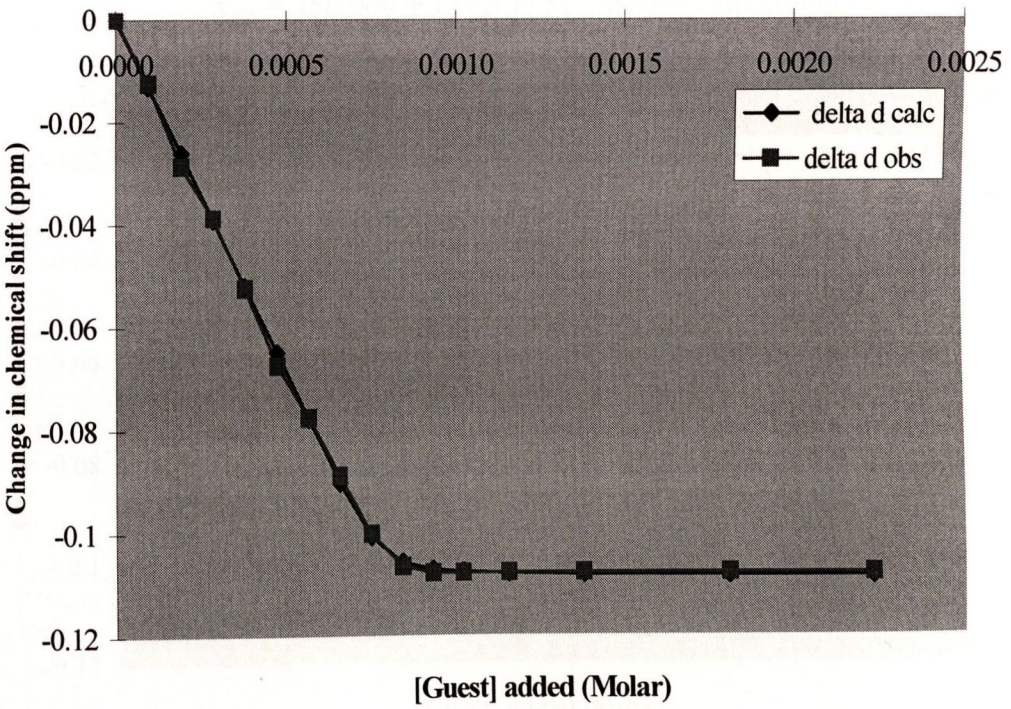
In the binding curve 13A, the AB resonance of a *meta*-aromatic carbamate group was followed throughout the titration to calculate the strength of binding K_{ass}. The signal remained as a sharp doublet with a net upfield shift of 0.11 ppm. From the binding curve 13A, the calculated curve is in good agreement with the measured data giving K_{ass} 350,000 ± 137,500 M^{-1}. Binding curve 13B shows the data from the same binding study following the other *meta*-aromatic carbamate proton. The value of K_{ass} obtained in this case was 434,000 ± 147,000 M^{-1}. Even though the errors are large, the moderately close agreement between the different proton signals provided some measure of their accuracy.

Binding curve 13A. NMR Binding Study of Association of Receptor **2.11** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *meta* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 29 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *m*-aromatic proton = 7.7911 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
2	9.635E-05	7.7786	-0.0125	-0.013095
4	1.921E-04	7.7623	-0.0288	-0.026175
6	2.871E-04	7.7522	-0.0389	-0.039232
8	3.816E-04	7.7384	-0.0527	-0.052253
10	4.754E-04	7.7234	-0.0677	-0.065202
12	5.686E-04	7.7133	-0.0778	-0.077996
14	6.612E-04	7.7021	-0.089	-0.090346
16	7.532E-04	7.6908	-0.1003	-0.100858
18	8.447E-04	7.6845	-0.1066	-0.105883
20	9.355E-04	7.6832	-0.1079	-0.107251
22	1.026E-03	7.6832	-0.1079	-0.107753
25	1.160E-03	7.6832	-0.1079	-0.108087
30	1.381E-03	7.6832	-0.1079	-0.108322
40	1.813E-03	7.6832	-0.1079	-0.108494
50	2.231E-03	7.6832	-0.1079	-0.108563

K_{ass} = 434,000 ± 147,000 M^{-1} δHG = 7.6824 ppm

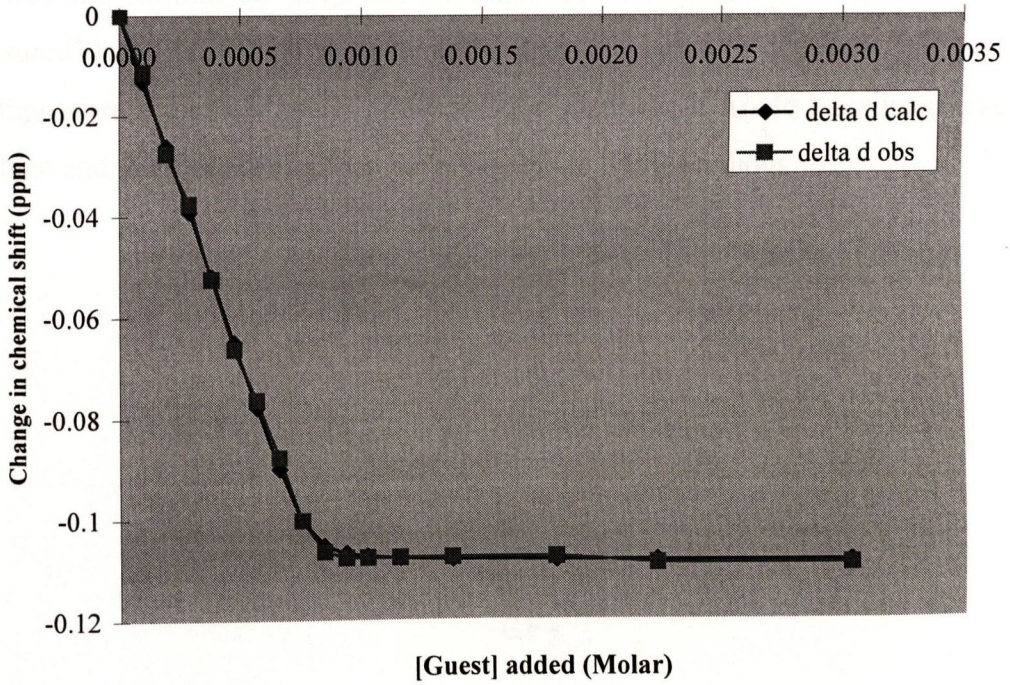


Binding curve 13B. NMR Binding Study of Association of Receptor **2.11** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *meta* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 29 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *m*-aromatic proton = 7.771 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
2	0.000096	7.7598	-0.0112	-0.0131
4	0.000192	7.7434	-0.0276	-0.0261
6	0.000287	7.7334	-0.0376	-0.0392
8	0.000382	7.7184	-0.0526	-0.0522
10	0.000475	7.7046	-0.0664	-0.0651
12	0.000569	7.6945	-0.0765	-0.0778
14	0.000661	7.6832	-0.0878	-0.0900
16	0.000753	7.6707	-0.1003	-0.1003
18	0.000845	7.6644	-0.1066	-0.1054
20	0.000935	7.6632	-0.1078	-0.1069
22	0.001026	7.6632	-0.1078	-0.1075
25	0.001160	7.6632	-0.1078	-0.1079
30	0.001381	7.6632	-0.1078	-0.1082
40	0.001813	7.6632	-0.1078	-0.1084
50	0.002231	7.6619	-0.1091	-0.1085
70	0.003030	7.6619	-0.1091	-0.1086

K_{ass} = 350,000 ± 137,500 M^{-1} δHG = 7.6623 ppm



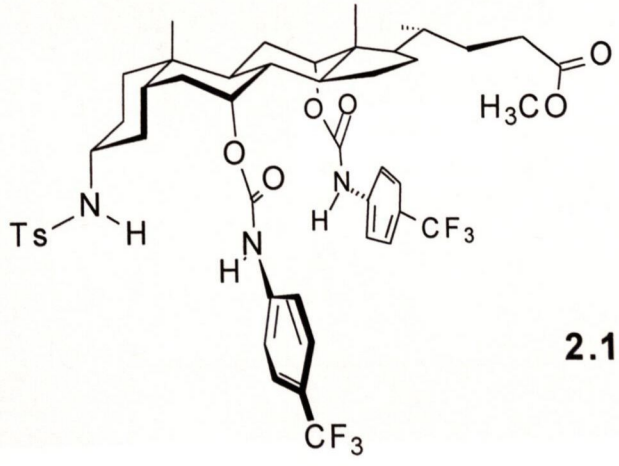
Binding curves 14A 14B and 15A show the NMR data for receptor **2.11** with tetrabutylammonium bromide. The addition of bromide to the receptor in $CDCl_3$ caused changes in the host's ^1H NMR spectra similar to those caused by the chloride anion. The changes were indicative of strong anion binding with a 1:1 host-guest stoichiometry, however the degree of NH movement varied significantly from that of chloride binding.

Table 3.2

	Anion	
Complexation induced shift (CIS)	Chloride (ppm)	Bromide (ppm)
carbamate NH protons :	2.82	2.33
	3.12	2.66

From Table 3.2 it is apparent that the 7α and 12α-NH protons interact with the bromide to a lesser extent than the chloride. Unfortunately it was not possible to determine the CIS of the 3α-NH proton due to overlap with the aromatic signals.

Binding curves 14A and 14B show the NMR data of the *meta*-aromatic protons belonging to a carbamate group of **2.11** titrated with bromide, the same signal that was used for chloride analysis. The curves show that the calculated data is in good agreement with the actual data giving K_{ass} values of 400,000 ± 118,000 and 420,000 ± 178,000 M^{-1} respectively. Binding curve 15A shows a repeat study following the same signal as in the binding curve 14A giving K_{ass} of 395,000 ± 149,000 M^{-1}. The binding constants for chloride and bromide are large and the errors are correspondingly large even though the measured data fits well to the calculated data. In order to determine the strength of binding more accurately by ^1H NMR titration methods, it would have been essential to use host and guest concentrations not amenable to NMR methods.



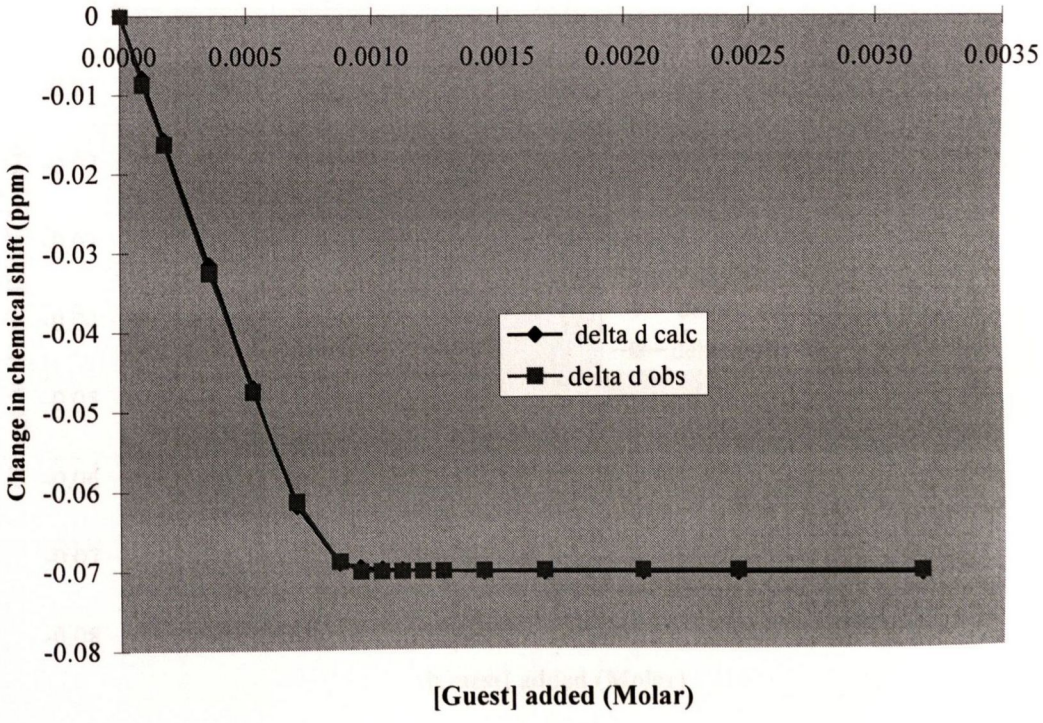
2.11

Binding curve 14A. NMR Binding Study of Association of Receptor **2.11** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *meta* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *m*-aromatic proton = 7.771 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
2	8.970E-05	7.7623	-0.0087	-0.007912
4	1.788E-04	7.7547	-0.0163	-0.015817
8	3.553E-04	7.7384	-0.0326	-0.031581
12	5.294E-04	7.7234	-0.0476	-0.047189
16	7.013E-04	7.7096	-0.0614	-0.061907
20	8.710E-04	7.7021	-0.0689	-0.069060
22	9.550E-04	7.7008	-0.0702	-0.069680
24	1.038E-03	7.7008	-0.0702	-0.069948
26	1.121E-03	7.7008	-0.0702	-0.070095
28	1.204E-03	7.7008	-0.0702	-0.070186
30	1.286E-03	7.7008	-0.0702	-0.070249
34	1.448E-03	7.7008	-0.0702	-0.070328
40	1.688E-03	7.7008	-0.0702	-0.070394
50	2.077E-03	7.7008	-0.0702	-0.070450
60	2.455E-03	7.7008	-0.0702	-0.070480
80	3.176E-03	7.7008	-0.0702	-0.070510

K_{ass} = 400,000 ± 118,000 M^{-1} δHG = 7.7004 ppm

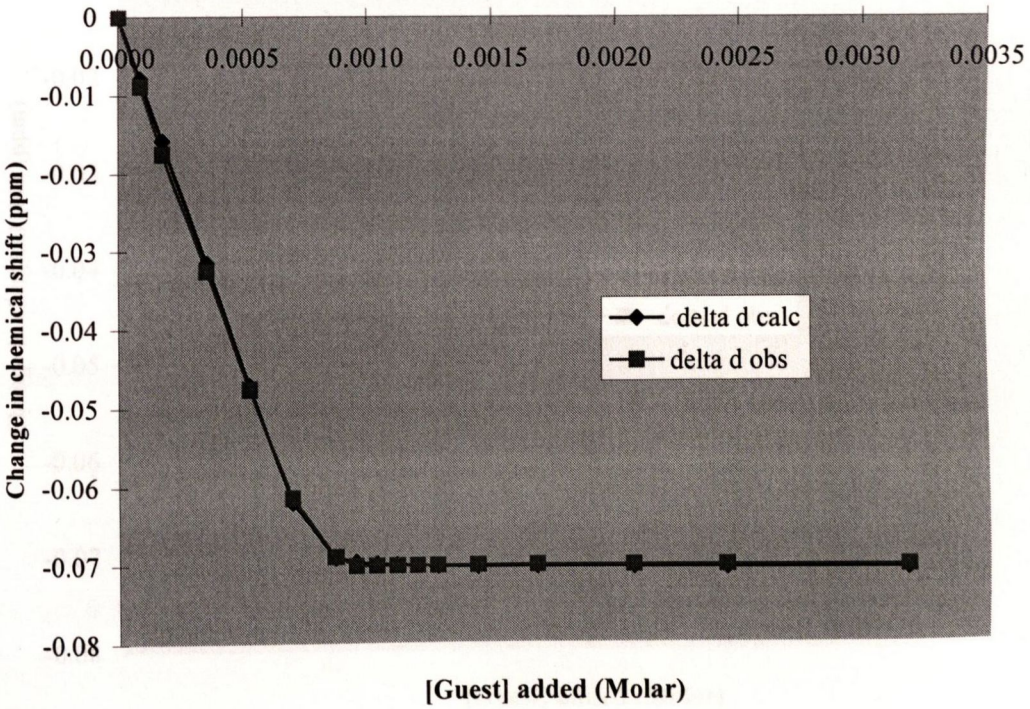


Binding curve 14B. NMR Binding Study of Association of Receptor **2.11** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *meta* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *m*-aromatic proton = 7.7911 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
2	8.970E-05	7.7823	-0.0088	-0.0088
4	1.788E-04	7.7736	-0.0175	-0.0175
8	3.553E-04	7.7585	-0.0326	-0.0326
12	5.294E-04	7.7434	-0.0477	-0.0477
16	7.013E-04	7.7296	-0.0615	-0.0615
20	8.710E-04	7.7221	-0.069	-0.069
22	9.550E-04	7.7209	-0.0702	-0.0702
24	1.038E-03	7.7209	-0.0702	-0.0702
26	1.121E-03	7.7209	-0.0702	-0.0702
28	1.204E-03	7.7209	-0.0702	-0.0702
30	1.286E-03	7.7209	-0.0702	-0.0702
34	1.448E-03	7.7209	-0.0702	-0.0702
40	1.688E-03	7.7209	-0.0702	-0.0702
50	2.077E-03	7.7209	-0.0702	-0.0702
60	2.455E-03	7.7209	-0.0702	-0.0702
80	3.176E-03	7.7209	-0.0702	-0.0702

K_{ass} = 420,000 ± 178,000 M^{-1} δHG = 7.7205 ppm

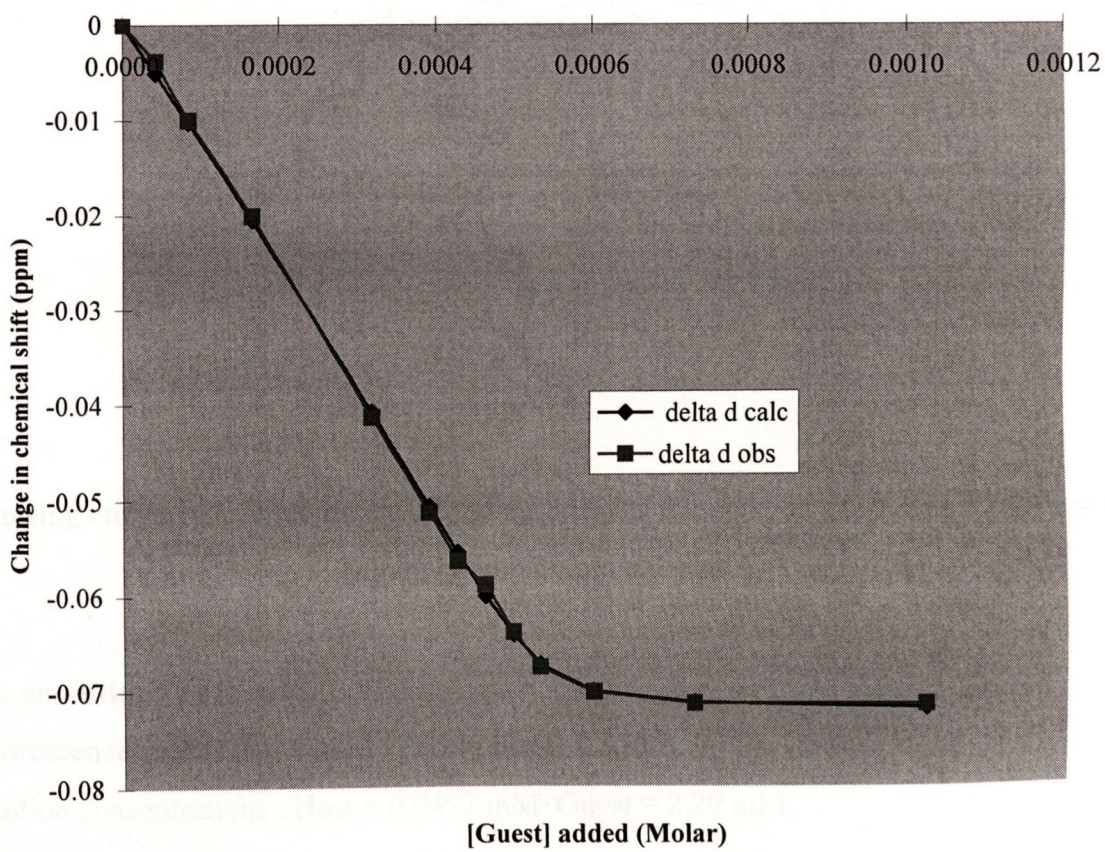


Binding curve 15A. NMR Binding Study of Association of Receptor **2.11** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *meta* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 0.6 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *m*-aromatic proton = 7.771 ppm

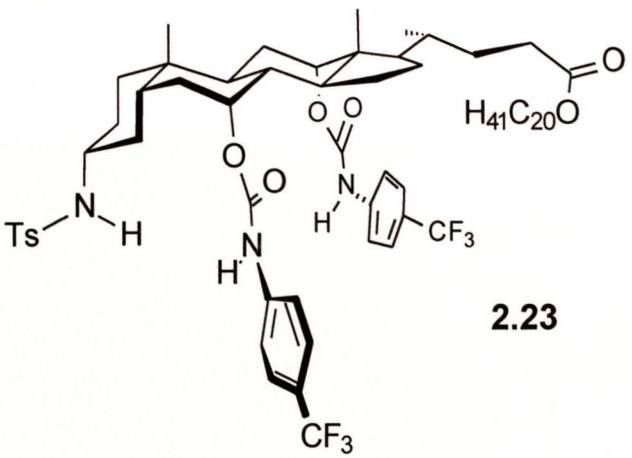
μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	4.215E-05	7.7655	-0.0038	-0.005134153
10	8.361E-05	7.7593	-0.01	-0.010263976
20	1.645E-04	7.7492	-0.0201	-0.020505553
40	3.188E-04	7.7279	-0.0414	-0.040836358
50	3.923E-04	7.7179	-0.0514	-0.050789463
55	4.282E-04	7.7128	-0.0565	-0.055612684
60	4.636E-04	7.7103	-0.059	-0.060200298
65	4.985E-04	7.7053	-0.064	-0.064288625
70	5.328E-04	7.7016	-0.0677	-0.067401314
80	6.000E-04	7.6990	-0.0703	-0.070222657
100	7.286E-04	7.6978	-0.0715	-0.071404072
150	1.020E-03	7.6978	-0.0715	-0.071876975

K_{ass} = 395,000 ± 149,000 M^{-1} δHG = 7.7004 ppm



A more lipophilic derivative of receptor **2.11** was synthesised (see scheme 2.9) with a C_{20} chain ester. This new long aliphatic side chain was not expected to interfere significantly with the anion binding ability of the receptor because of its distance from the binding site. Upon investigation, the new receptor **2.23** was found to fluoresce. The receptor was investigated for its anion binding properties by a fluorescence titration technique. Binding curves 16A and 17A shows the data from the fluorometric titration studies for tetrabutylammonium chloride and bromide respectively. On excitation at 272 nm, **2.23** emits a fluorescence between 285 and 350 nm (maximum at 306.56 nm).

For the case of chloride addition in the binding curve 16A, a strong enhancement of the fluorescence was observed with increasing concentration of the guest species, until saturation is reached at 1:1 host-guest stoichiometry. From the data in the binding curve 17A, the strength of binding K_{ass} was calculated using specially written non-linear least squares curve-fitting program implemented with Excel version 5.0 and Systat. The programs made allowances for Stern-Vollmer quenching of both **2.23** and complex by the chloride anion giving a K_{ass} of 374,100 ± 30,000 M^{-1}. The error was calculated from the Systat program.



2.23

Binding curve 16A. Fluorescence Binding Study of Association of Receptor **2.23** with Tetrabutylammonium chloride.

Dry conditions were used.

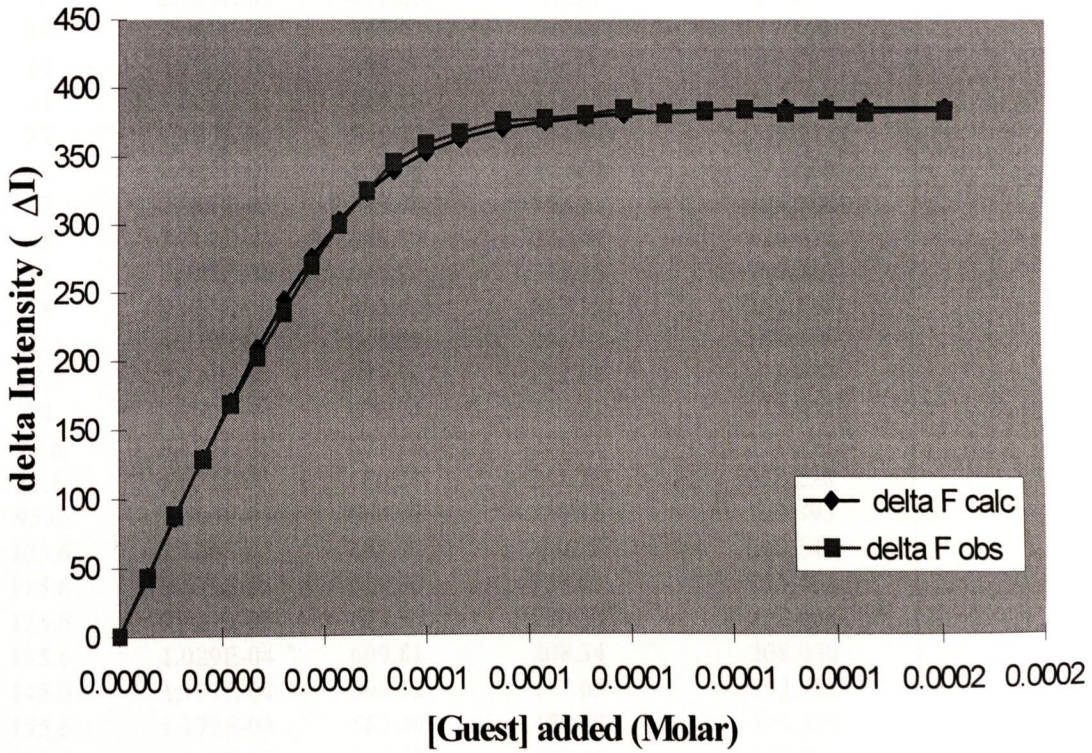
Fluorescence spectrometer used : Perkin Elmer LS50 B.

Solution concentrations : Host = 0.0477 mM Guest = 2.20 mM.

Initial volume of host = 2.5 mL. Initial intensity of the emission signal (I)= 362.26

µL of guest added	[Guest] added	Observed intensity (I)	Change in intensity relative to FH (I)	Computer calculated change in intensity (ΔI)
6.25	5.486E-06	405.2700	43.01	43.8507
12.5	1.095E-05	450.2900	88.03	86.9778
18.75	1.638E-05	490.5600	128.3	129.1028
25	2.178E-05	530.5000	168.24	169.8119
31.25	2.716E-05	564.6500	202.39	208.4952
37.5	3.251E-05	597.4800	235.22	244.2907
43.75	3.784E-05	632.3300	270.07	276.1093
50	4.314E-05	662.8500	300.59	302.8834
56.25	4.841E-05	687.2500	324.99	324.0564
62.5	5.366E-05	708.2700	346.01	339.9334
70	5.992E-05	721.5100	359.25	353.3728
78	6.656E-05	729.7300	367.47	362.9779
88	7.481E-05	738.2100	375.95	370.7169
98	8.299E-05	739.9600	377.7	375.6430
108	9.110E-05	742.7000	380.44	378.9032
117	9.836E-05	747.2100	384.95	380.9365
127	1.064E-04	743.5500	381.29	382.5307
137	1.143E-04	745.4400	383.18	383.6404
147	1.222E-04	746.5300	384.27	384.4076
157	1.300E-04	743.9500	381.69	384.9255
167	1.378E-04	745.7300	383.47	385.2574
177	1.455E-04	744.5200	382.26	385.4476
197	1.607E-04	745.6200	383.36	385.5224

K_{ass} = 374,100 ± 30,000 M^{-1} FHG = 766.806



Binding curve 17A shows the titration data of receptor **2.23** with tetrabutylammonium bromide. As with chloride, the bromide anion caused a strong enhancement in the fluorescence of **2.23** until 1:1 host-guest stoichiometry. Upon further addition of bromide the fluorescence intensity decreased. This fall off in fluorescence was caused by the quenching effects of the halide added. The efficiency of quenching is known to increase with the mass of the halide added, hence the greater quenching in binding curve 17A over binding curves 16A. The quenching factor of the receptor and complex of bromide was accounted for in calculating the strength of binding giving a K_{ass} of 360,150 ± 14,400 M^{-1}. The good fitting curve and the low error value using fluorescence spectroscopy was obtainable due to the low concentrations of host and guest required using this method.

Binding curve 17A. Fluorescence Binding Study of Association of Receptor **2.23** with Tetrabutylammonium bromide.

Dry conditions were used.

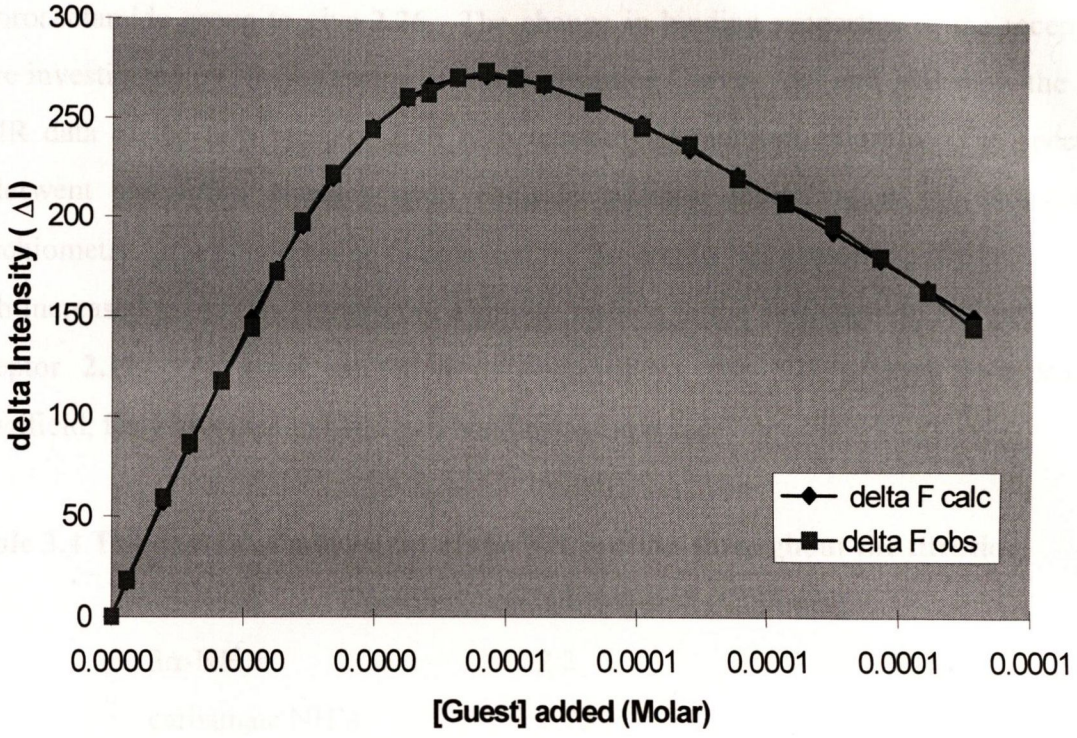
Fluorescence spectrometer used : Perkin Elmer LS50 B.

Solution concentrations : Host = 0.0462 mM Guest = 2.0 mM.

Initial volume of host = 2.5 mL. Initial intensity of the emission signal (I) = 401.67

µL of guest added	[Guest] added	Observed intensity (I)	Change in intensity relative to FH(I)	Computer calculated change in intensity (ΔI)
3	2.397E-06	419.65	18.18	17.459
10	7.968E-06	460.91	59.44	57.569
15	1.193E-05	488.20	86.73	85.540
21	1.666E-05	519.58	118.11	118.104
27	2.137E-05	546.30	144.83	149.211
32	2.528E-05	574.09	172.62	173.640
37	2.917E-05	599.38	197.91	196.288
43	3.382E-05	623.89	222.42	220.433
51	3.998E-05	646.92	245.45	246.056
58	4.535E-05	663.62	262.15	261.310
62.4	4.870E-05	665.58	264.11	267.456
68.2	5.311E-05	673.58	272.11	271.950
74	5.750E-05	676.74	275.27	273.064
79.8	6.187E-05	673.38	271.91	271.597
85.6	6.621E-05	670.25	268.78	268.228
95.6	7.366E-05	660.98	259.51	259.393
105.6	8.106E-05	647.97	246.5	248.147
115.6	8.839E-05	639.30	237.83	235.493
125.6	9.567E-05	622.30	220.83	222.006
135.6	1.029E-04	609.81	208.34	208.030
145.6	1.101E-04	599.12	197.65	193.781
155.6	1.172E-04	582.34	180.87	179.396
165.6	1.242E-04	565.32	163.85	164.967
175.6	1.313E-04	547.09	145.62	150.556

K_{ass} = 360,150 ± 14,400 M^{-1} FHG = 766.122



The results of the binding studies of receptor **2.11** and its lipophilic derivative **2.23** are summarised in table 3.3.

Table 3.3 Results of NMR and fluorescence titration studies of 2.11 and 2.23 respectively.

		Binding constants K_{ass} (M^{-1})	
Receptor	Technique	Cl^-	Br^-
2.11	NMR	434,000 ± 147,000	395,000 ± 149,000
		350,000 ± 137,500	400,000 ± 118,000
			420,000 ± 178,000
2.23	Fluorescence	374,000 ± 30,000	360,000 ± 14,400

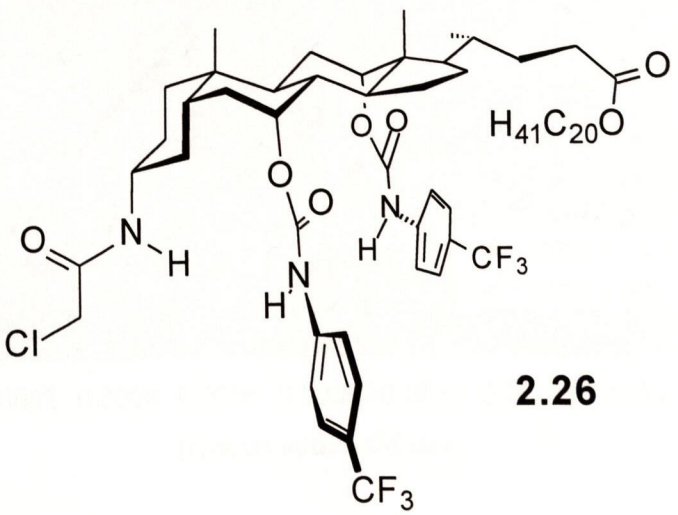
From the two different analytical tools used, the results correlate within the errors specified. It appears that any effects on binding caused by the long chain aliphatic ester are of minor significance to the binding constant K_{ass} values observed. It is clear from the results of the ^1H NMR titration studies that the technique has reached its limit of sensitivity with K_{ass} values $>10^5$ M^{-1}.

Receptor **2.23** was modified at the 3 position by replacing the toluenesulfonamide with a chloroacetamide group to give **2.26**. The change in binding properties of the receptor were investigated by NMR titration methods. Binding Curves 18A and 18B show the ^1H NMR data of the new receptor **2.26** with tetrabutylammonium chloride. The spectra underwent significant changes upon chloride addition indicating a 1:1 host-guest stoichiometry. It was interesting to note that the 3α-amido NH proton rapidly broadened with increased guest concentration in a similar fashion to the sulfonamido NH proton in receptor **2.11**. At guest saturation (~1 equivalent), the NH protons re-appeared downfield, fully involved in hydrogen bonding as expected.

Table 3.4 The degree of movement of the NH protons throughout the titration.

	Complexation induced shift (CIS/ppm)
3α-NH	2.2
carbamate NH's	2.75
	2.78

One can see from Table 3.4 that the carbamate NH protons underwent stronger interactions with the chloride anion than the 3α-NH proton. Additional motions of up to 0.5 ppm for the *ortho* aromatic CH signals and 0.06 ppm for the steroidal 19 methyl group were used for determining the binding constants (K_{ass} = 132,000 ± 20,200 and 145,000 ± 27,600 M^{-1} in the binding curves 18A and 18B respectively). The amido-CH_2 protons also underwent a marginal downfield shift throughout the titration (0.16 ppm) leading one to assume the penetration of the guest into the molecular cavity of the host and consequently the formation of a well structured complex.



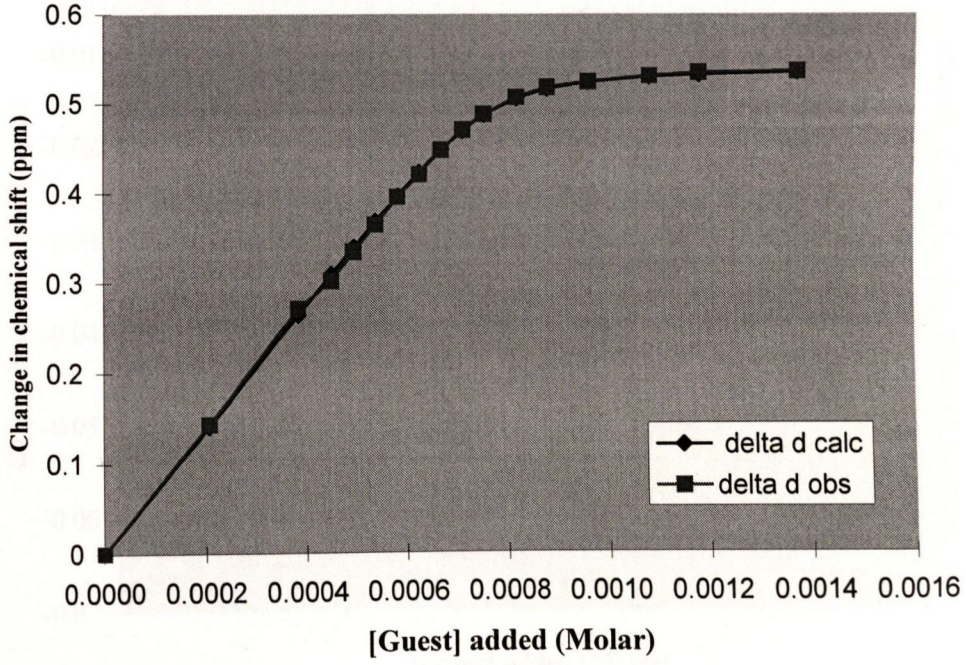
2.26

Binding Curve 18A. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of aryl proton *ortho* to the NH group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 14 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *o*-aromatic CH = 7.6168 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
9	0.000207	7.7598	0.143	0.14019294
17	0.000386	7.8865	0.2697	0.263250529
20	0.000452	7.9178	0.301	0.308553258
22	0.000495	7.9504	0.3336	0.338275863
24	0.000538	7.9805	0.3637	0.367417598
26	0.000581	8.0119	0.3951	0.395696275
28	0.000624	8.0370	0.4202	0.4226577
30	0.000667	8.0646	0.4478	0.447588913
32	0.000709	8.0872	0.4704	0.469489671
34	0.000751	8.1047	0.4879	0.487323346
37	0.000813	8.1236	0.5068	0.505752925
40	0.000875	8.1349	0.5181	0.516400943
44	0.000957	8.1411	0.5243	0.524063543
50	0.001077	8.1474	0.5306	0.529658553
55	0.001176	8.1512	0.5344	0.532131708
65	0.001368	8.1524	0.5356	0.534810674

K_{ass} = 132,000 ± 20,200 M^{-1} δHG =8.1578 ppm

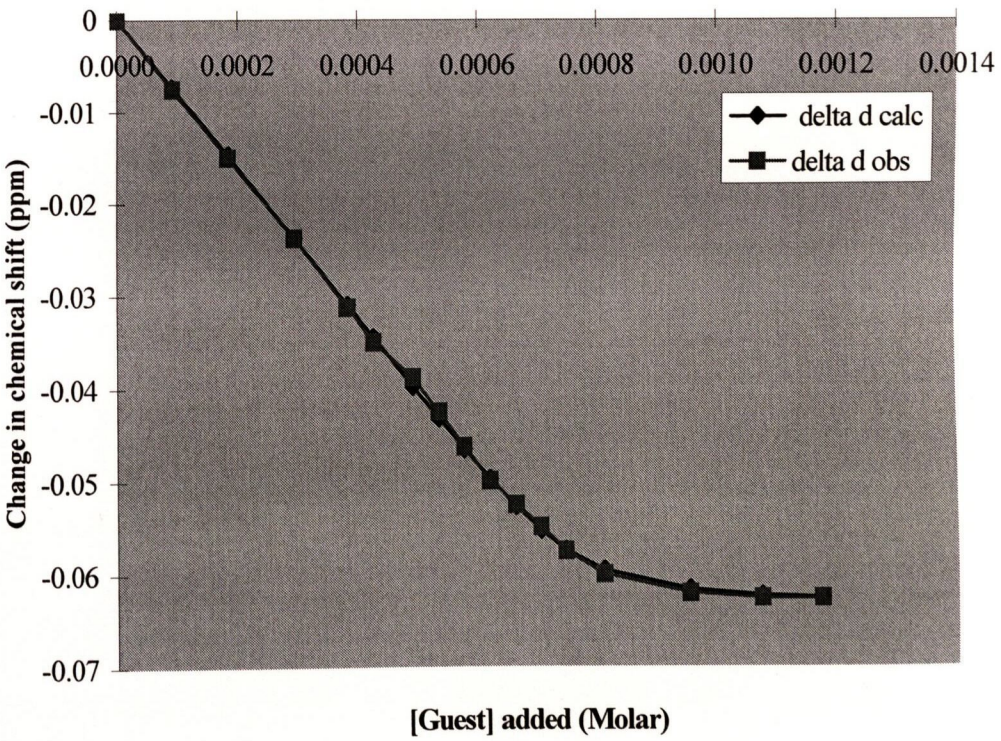


Binding Curve 18B. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 19 methyl group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 14 mM.
Initial volume of host = 600 μL. Initial shift (δ) of $19CH_3$ = 0.9946 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	9.272E-05	0.9871	-0.0075	-0.00735
8	1.842E-04	0.9796	-0.015	-0.01468
13	2.969E-04	0.9708	-0.0238	-0.02380
17	3.857E-04	0.9633	-0.0313	-0.03102
19	4.297E-04	0.9595	-0.0351	-0.03460
22	4.952E-04	0.9557	-0.0389	-0.03989
24	5.385E-04	0.9520	-0.0426	-0.04334
26	5.815E-04	0.9482	-0.0464	-0.04669
28	6.242E-04	0.9445	-0.0501	-0.04990
30	6.667E-04	0.9419	-0.0527	-0.05287
32	7.089E-04	0.9395	-0.0551	-0.05548
34	7.508E-04	0.9369	-0.0577	-0.05760
37	8.132E-04	0.9344	-0.0602	-0.05975
44	9.565E-04	0.9324	-0.0622	-0.06182
50	1.077E-03	0.9319	-0.0627	-0.06243
55	1.176E-03	0.9319	-0.0627	-0.06270

K_{ass} = 145,000 ± 27,600 M^{-1} δHG =0.9310 ppm



Binding curves 19A and 20A show the ^{1}H NMR data of receptor **2.26** with tetrabutylammonium bromide. The spectra of **2.26** underwent similar changes to TBACl addition, however, the degree of movement of the NH and aromatic CH protons was less. In both binding studies 19A and 20A the aryl CH protons signals *ortho* to the NH groups of the carbamates were monitored to calculate the binding constant for bromide giving K_{ass} of 52,000 ± 3,700 and 49,000 ± 4,000 M^{-1} respectively. It is clear from the curves in both cases that the receptor did not reach guest saturation as quickly as in the case when chloride was the guest, indicating a weaker complex was formed with bromide. The measured data and the calculated data in both cases for bromide gave a good fit with acceptable errors. The consistency in the binding constants from both signals provides a measure of their accuracy.

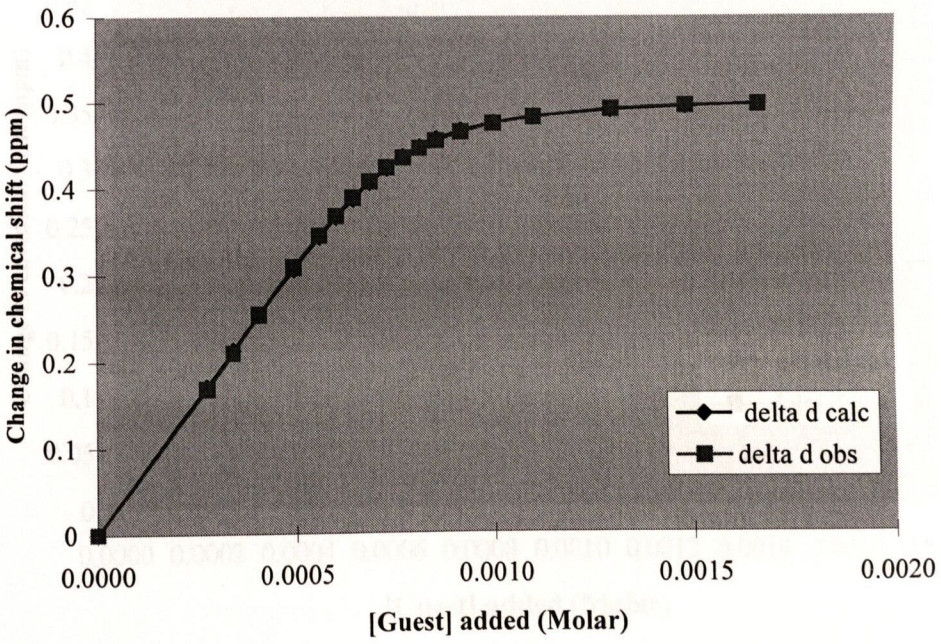
The reduced affinity of **2.26** for bromide over chloride is ascribed to the lower basicity of this anion and therefore a set of correspondingly weaker interactions with the NH protons. The anion binding studies showed that **2.26** exhibited a loss in binding strength compared to the 3α-sulfonamide-based receptor **2.11**. This decrease in binding strength may be attributed to the acidity of the 3α-NH proton. Sulfonamides (pK_a 9-10) are about 5 pK_a units more acidic than functionally equivalent amides (pK_a 14-15), a factor that is expected to increase their hydrogen bond donor ability and their binding constants dramatically.

Binding Curve 19A. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *ortho* to the NH group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 14 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *o*-aromatic CH = 7.6193 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
12	0.000275	7.7874	0.1681	0.170612
15	0.000341	7.8287	0.2094	0.212137
18	0.000408	7.8727	0.2534	0.252825
22	0.000495	7.9266	0.3073	0.305088
25	0.000560	7.9630	0.3437	0.341958
27	0.000603	7.9856	0.3663	0.364893
29	0.000645	8.0069	0.3876	0.386072
31	0.000688	8.0257	0.4064	0.405086
33	0.000730	8.0420	0.4227	0.421596
35	0.000772	8.0533	0.434	0.435443
37	0.000813	8.0646	0.4453	0.446713
39	0.000854	8.0734	0.4541	0.455701
42	0.000916	8.0834	0.4641	0.465780
46	0.000997	8.0935	0.4742	0.474811
51	0.001097	8.1010	0.4817	0.481911
61	0.001292	8.1098	0.4905	0.489551
71	0.001481	8.1135	0.4942	0.493476
81	0.001665	8.1148	0.4955	0.495837

K_{ass} = 52,000 ± 3,700 M^{-1} δHG =8.1251 ppm

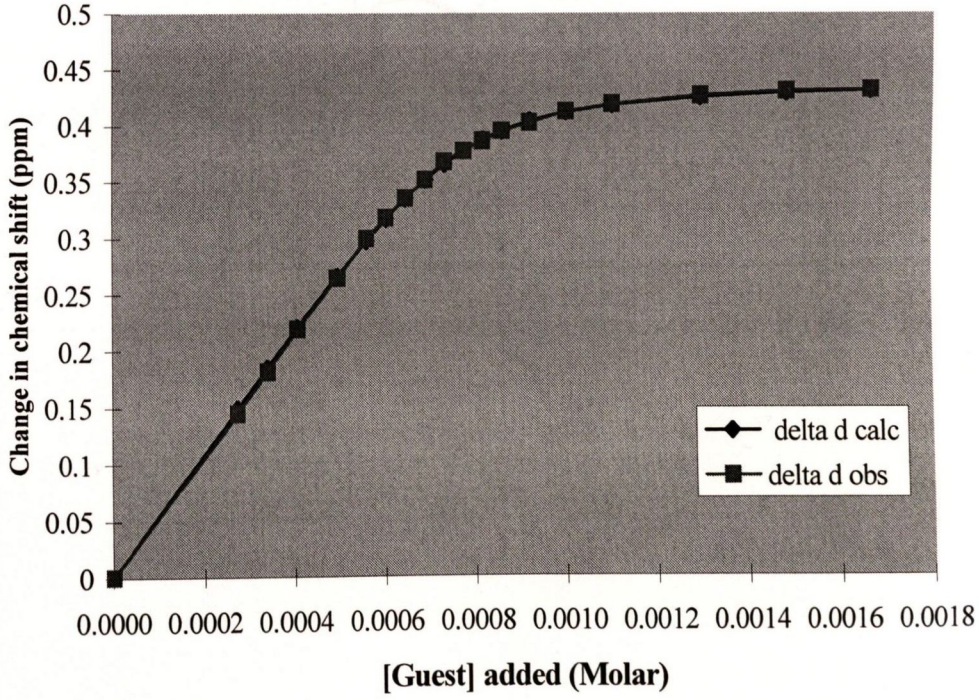


Binding Curve 20A. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *ortho* to the NH group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 14 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *o*-aromatic CH = 7.6193 ppm

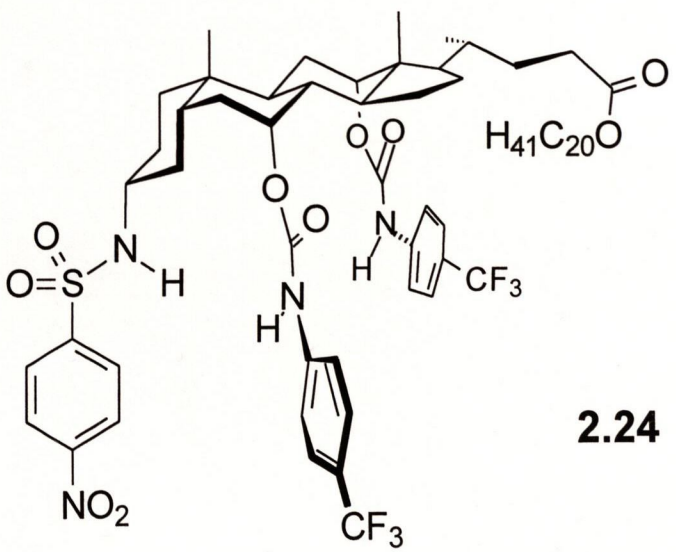
μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
12	0.000275	7.7635	0.1442	0.148242
15	0.000341	7.7999	0.1806	0.184280
18	0.000408	7.8375	0.2182	0.219564
22	0.000495	7.8827	0.2634	0.264829
25	0.000560	7.9178	0.2985	0.296712
27	0.000603	7.9366	0.3173	0.316523
29	0.000645	7.9542	0.3349	0.334808
31	0.000688	7.9705	0.3512	0.351228
33	0.000730	7.9868	0.3675	0.365509
35	0.000772	7.9968	0.3775	0.377526
37	0.000813	8.0056	0.3863	0.387354
39	0.000854	8.0144	0.3951	0.395237
42	0.000916	8.0219	0.4026	0.404140
46	0.000997	8.032	0.4127	0.412185
51	0.001097	8.0383	0.419	0.418558
61	0.001292	8.0458	0.4265	0.425462
71	0.001481	8.0496	0.4303	0.429027
81	0.001665	8.0508	0.4315	0.431175

K_{ass} = 49,000 ± 4,000 M^{-1} δHG =8.1251 ppm



With the knowledge that the sulfonamide group is more potent in anion binding than the amido group at the 3 position, it was decided to modify receptor **2.23** by introducing a more electron withdrawing sulfonamide group to improve its anion binding ability further. A. V. Willi reported the pK_a values of various sulfonamide groups claiming the *para*-nitrobenzene sulfonamide (pK_a= 9.14) to be the most acidic in water (*p*-toluenesulfonamide pK_a = 10.17).[4] Receptor **2.24** was therefore synthesised. The more electron withdrawing group was expected to render the NH proton more acidic and therefore more available for hydrogen bonding. The carbamate groups were left unchanged.

Equipped with a 3α-*p*-nitrobenzenesulfonamide group and two acidic carbamate (*p*-$CF_3C_6H_4NH$) groups at the 7 and 12 positions, **2.24** was expected to show extremely potent anion binding properties. Binding curves 21A and 22A show the NMR data for receptor **2.24** titrated with tetrabutylammonium chloride and bromide respectively. In both cases the ^1H NMR spectra underwent significant changes on addition of one equivalent of guest. On further addition of guest, practically no change in the host's spectra occurred. From the data it was immediately obvious that the binding was too strong to use the NMR titration technique to analyse this receptor. A different technique was needed to calculate K_{ass}.



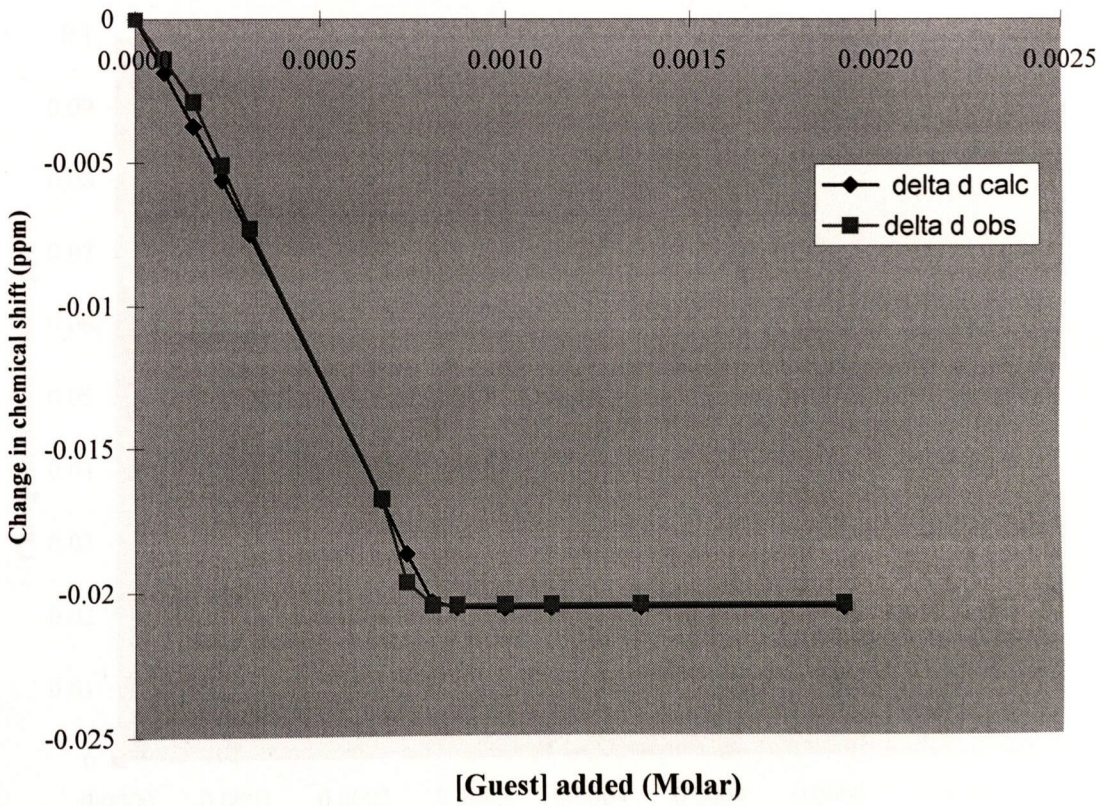
2.24

Binding Curve 21A. NMR Binding Study of Association of Receptor **2.24** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of the 19 methyl group was followed throughout this titration.
Solution concentrations : Host = 0.8 mM Guest = 9.6 mM.
Initial volume of host = 600 μL. Initial shift (δ) of 19 methyl group = 0.7585 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	7.934E-05	0.7569	-0.0014	-0.001874
10	1.574E-04	0.7554	-0.0029	-0.003748
15	2.341E-04	0.7532	-0.0051	-0.005622
20	3.097E-04	0.7510	-0.0073	-0.007496
45	6.698E-04	0.7415	-0.0168	-0.016858
50	7.385E-04	0.7386	-0.0197	-0.018719
55	8.061E-04	0.7378	-0.0205	-0.020390
60	8.727E-04	0.7378	-0.0205	-0.020590
70	1.003E-03	0.7378	-0.0205	-0.020608
80	1.129E-03	0.7378	-0.0205	-0.020612
100	1.371E-03	0.7378	-0.0205	-0.020615
150	1.920E-03	0.7378	-0.0205	-0.020616

$K_{ass} > 10^6\ M^{-1}$ δHG =0.7377 ppm

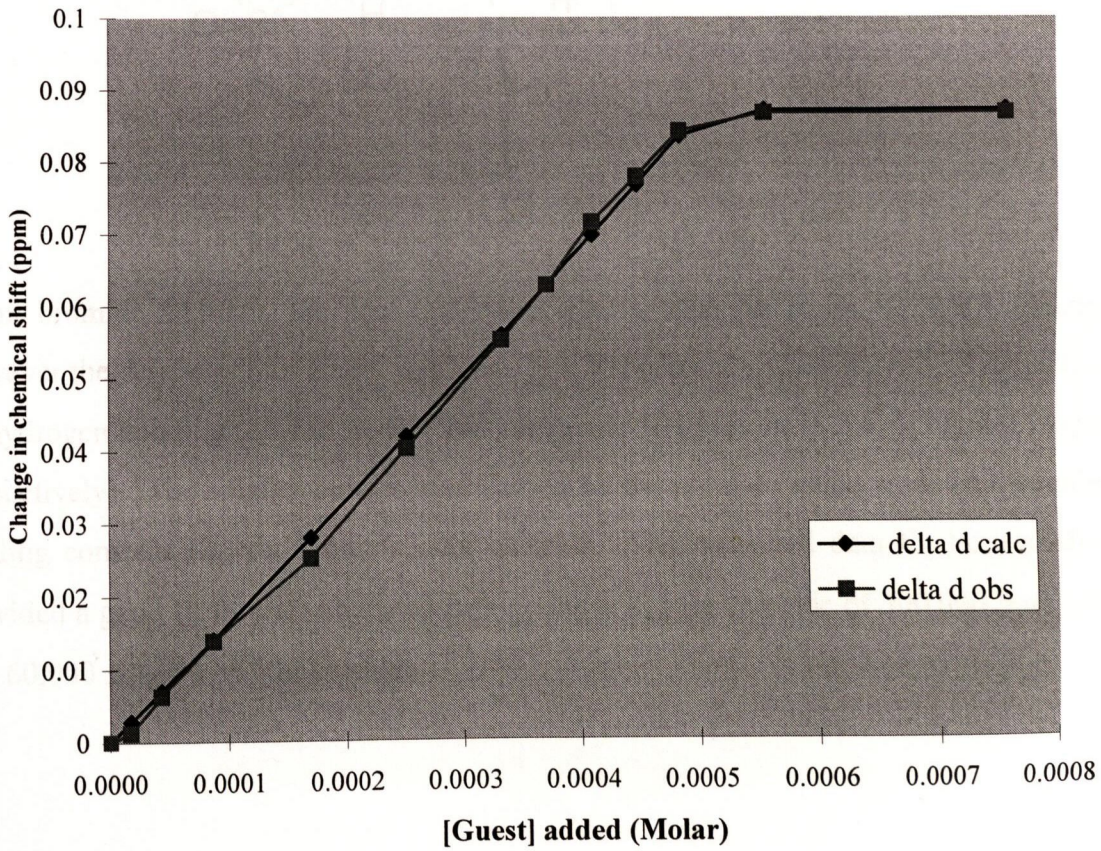


Binding Curve 22A. NMR Binding Study of Association of Receptor **2.24** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 12β-H was followed throughout this titration.
Solution concentrations : Host = 0.55 mM Guest = 5.3 mM.
Initial volume of host = 600 μL. Initial shift (δ) of 12β-H = 5.1312 ppm

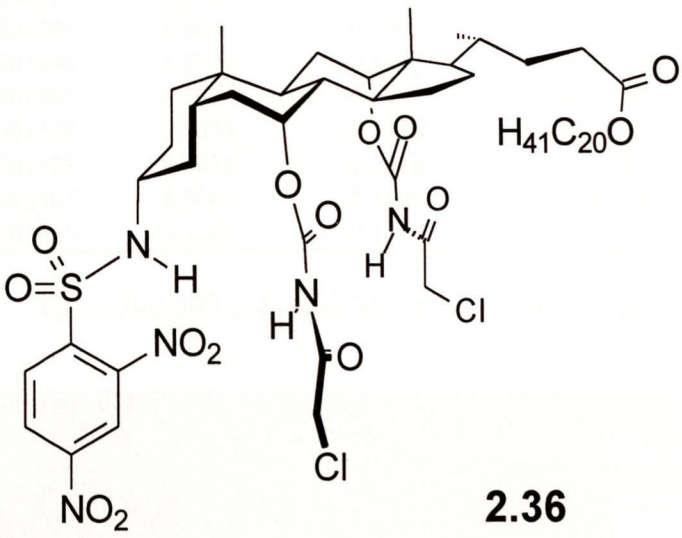
μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
2	1.761E-05	5.1325	0.0013	0.002792
5	4.380E-05	5.1375	0.0063	0.006981
10	8.689E-05	5.1450	0.0138	0.013961
20	1.710E-04	5.1563	0.0251	0.027920
30	2.524E-04	5.1714	0.0402	0.041876
40	3.313E-04	5.1864	0.0552	0.055826
45	3.698E-04	5.1940	0.0628	0.062794
50	4.077E-04	5.2027	0.0715	0.069751
55	4.450E-04	5.2090	0.0778	0.076675
60	4.818E-04	5.2153	0.0841	0.083393
70	5.537E-04	5.2178	0.0866	0.086961
100	7.571E-04	5.2178	0.0866	0.087073

$K_{ass} > 10^6\ M^{-1}$ δHG =5.2183 ppm



The results of the binding studies of receptor **2.24** with tetrabutylammonium chloride and bromide, although not quantitative, confirm the hypothesis that on making the NH protons on the receptor at the cavity more acidic, they are rendered more available for hydrogen-bonding.

Receptor **2.36** was synthesised according to scheme 2.12 and analysed for its anion binding ability. Binding Studies 23A and 24A show the NMR data of **2.36** titrated with TBACl and TBABr respectively. As with all of the receptors in this series, the NH resonances experienced the strongest downfield shifts for chloride ($\Delta\delta$ for 3α–NH and the carbamate NH protons was 2.81, 2.42 and 2.66 ppm respectively). The aromatic signals underwent smaller changes upon anion addition, *e.g.* the aromatic CH proton *ortho* to the nitro groups broadened upon guest addition before sharpening again 0.15 ppm upfield upon guest saturation.



2.36

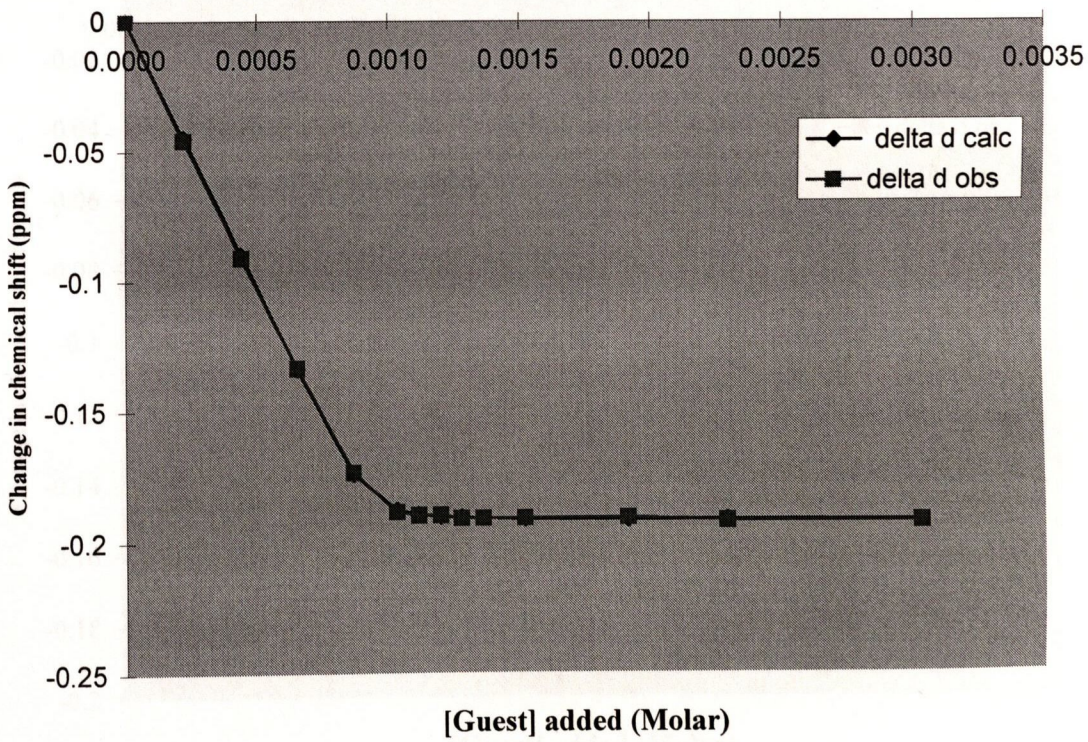
Upon bromide addition, the host underwent similar changes in its ^{1}H NMR spectra, however the degree of movement was less. The NH protons shifted downfield indicative of hydrogen bonding ($\Delta\delta$ for 3α-NH and carbamate NH protons is 2.6, 1.9 and 2.2 ppm respectively). The smaller perturbation caused by the bromide anion leads to a smaller binding constant K_{ass} for bromide over chloride. The measured data for both studies provided a good fit for the calculated data giving a K_{ass} for chloride of 300,000 ± 41,000 and 60,000 ± 4,000 M^{-1} for bromide.

Binding Curve 23A. NMR Binding Study of Association of Receptor **2.36** with Tetrabutylammonium chloride.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of aromatic CH proton *ortho* to both NO_2 groups was followed throughout this titration.
Solution concentrations : Host = 0.96 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *o*-CH proton = 8.6987 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000223	8.6529	-0.0458	-0.044966
10	0.000443	8.6077	-0.091	-0.089749
15	0.000659	8.5651	-0.1336	-0.133950
20	0.000871	8.5250	-0.1737	-0.174188
24	0.001038	8.5099	-0.1888	-0.188161
26	0.001121	8.5086	-0.1901	-0.189791
28	0.001204	8.5086	-0.1901	-0.190589
30	0.001286	8.5074	-0.1913	-0.191051
32	0.001367	8.5074	-0.1913	-0.191350
36	0.001528	8.5074	-0.1913	-0.191711
46	0.001923	8.5074	-0.1913	-0.192110
56	0.002305	8.5061	-0.1926	-0.192281
76	0.003036	8.5061	-0.1926	-0.192436

K_{ass} = 300,000 ± 41,000 M^{-1} δHG = 8.5060 ppm

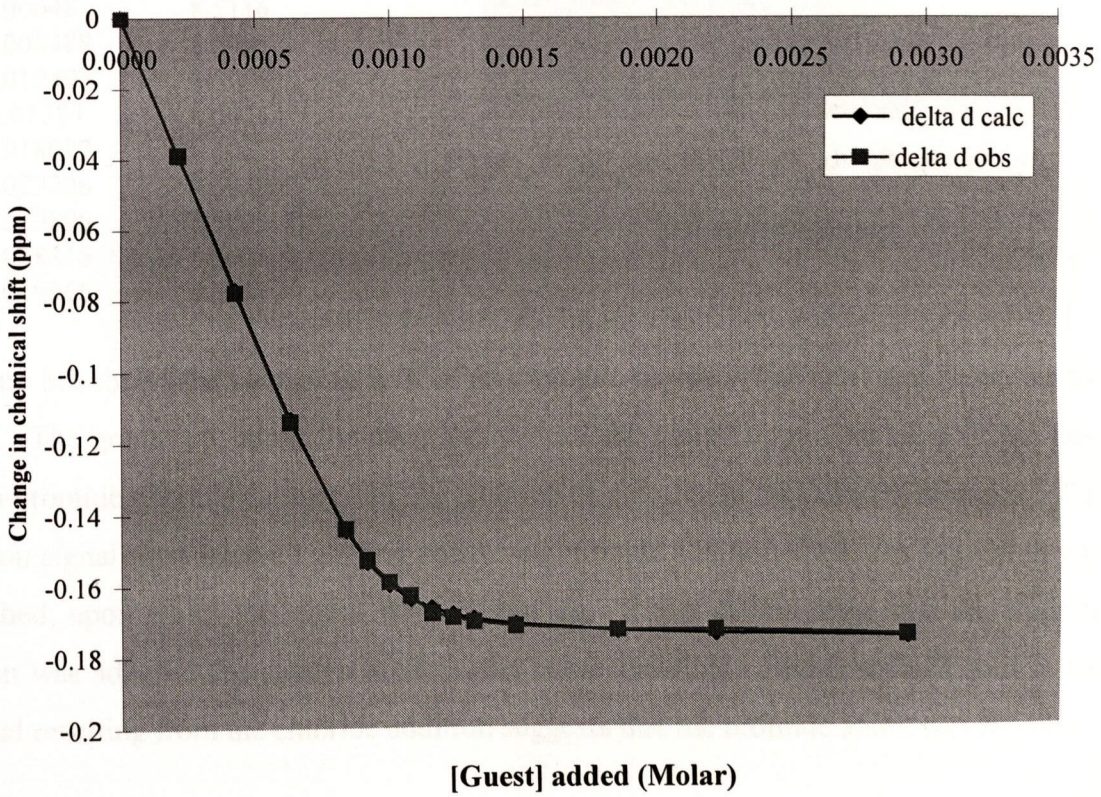


Binding Curve 24A. NMR Binding Study of Association of Receptor **2.36** with Tetrabutylammonium bromide.

Dry conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of aromatic CH proton *ortho* to both NO_2 groups was followed throughout this titration.
Solution concentrations : Host = 0.96 mM Guest = 27 mM.
Initial volume of host = 600 μL. Initial shift (δ) of *o*-CH proton = 8.6981 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000215	8.6592	-0.0389	-0.038912
10	0.000426	8.6203	-0.0778	-0.077151
15	0.000634	8.5839	-0.1142	-0.113665
20	0.000839	8.5538	-0.1443	-0.144675
22	0.000920	8.5450	-0.1531	-0.153592
24	0.001000	8.5388	-0.1593	-0.159907
26	0.001080	8.5350	-0.1631	-0.164057
28	0.001159	8.5300	-0.1681	-0.166755
30	0.001238	8.5287	-0.1694	-0.168563
32	0.001316	8.5275	-0.1706	-0.169828
36	0.001472	8.5262	-0.1719	-0.171446
46	0.001851	8.5250	-0.1731	-0.173317
56	0.002220	8.5250	-0.1731	-0.174134
76	0.002923	8.5237	-0.1744	-0.174877

K_{ass} = 60,000 ± 4,000 M^{-1} δHG =8.5218 ppm



The selectivity for chloride was confirmed in a separate experiment, in which **2.36** was first saturated with bromide guest. Upon chloride addition to the host/bromide complex, significant changes in the NMR spectra of the complex were observed.

Figure 3.1 Competition binding study of receptor 2.36.

series 1 = ^{1}H NMR chemical shift of aryl proton.

[G]	δ (ppm)
Br^- addition	
0.000215	8.6592
0.000426	8.6203
0.000634	8.5839
0.000839	8.5538
0.00092	8.545
0.001	8.5388
0.00108	8.535
0.001159	8.53
0.001238	8.5287
0.001316	8.5275
0.001472	8.5262
0.001851	8.525
Cl^- addition	
0.00222	8.525
0.002923	8.5237
0.003146	8.525
0.003805	8.5162
0.00492	8.5124
0.006481	8.5116
0.008488	8.5099
0.010495	8.5086
0.01384	8.5074
0.018077	8.5061
0.023206	8.5049
0.029896	8.5036
0.038816	8.5024
0.049966	8.5017

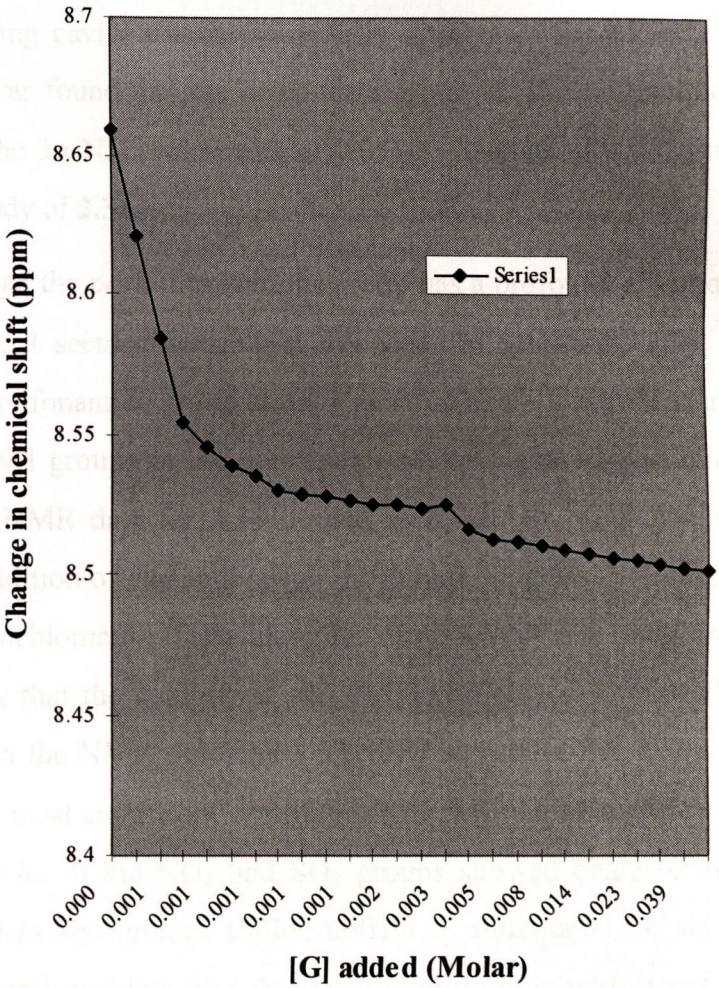
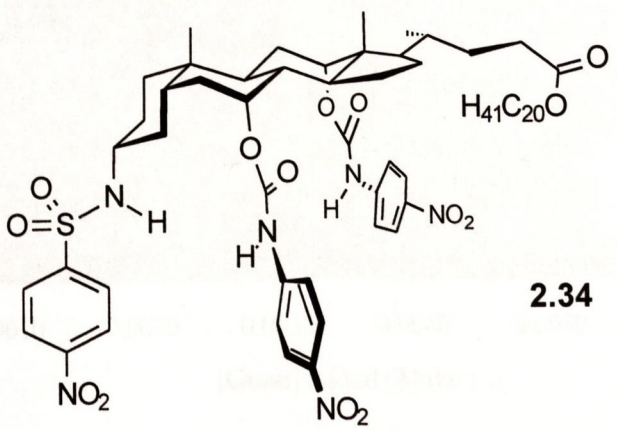


Figure 3.1 shows the change in shift of an aromatic proton *ortho* to a nitro group on the ring. The graph 3.1 shows the chemical shift of the proton upon saturation of the host with bromide, directly followed by the addition of chloride to the **2.36**/Br^- complex. The proton signal was observed moving upfield on bromide addition until host saturation was reached, upon which the signal stopped moving. It was at this stage that the chloride anion was added. The sudden break in the curve showing a further upfield shift in the signal resulting from the chloride addition suggests that the bromide anion was expelled

from the binding site of the host in the presence of the preferred chloride anion. The graph confirms the receptor's binding preference for chloride over bromide.

This selectivity for chloride over bromide by **2.36** was unexpected given the similarity of the receptor's binding cavity to the other receptors in this series. However the presence of the nitro group in the *ortho* position of the sulfonamide appears to have a significant effect on the cavity in the presence of the imido carbamates at the 7 and 12 positions. This subtle change in the binding cavity was not obviously apparent from molecular modelling studies, however it was found that the *ortho*-nitro group on the sulfonamide may form a hydrogen bond to the 3α-NH proton with a NH O_2N distance of 1.861 pm, from an energy minimisation study of **2.36**.

Equipped with the knowledge that the *para*-nitrobenzene group has a profound effect on the acidity of an α-NH proton, it seemed sensible at this stage to synthesise **2.34,** a receptor with a *p*-nitrobenzene sulfonamide group at the 3 position and a *p*-nitrobenzene group directly attached to the NH groups of the carbamates at the 7 and 12 positions. Binding curve 25A shows the NMR data for **2.34** titrated with TBABr. The sudden break in the binding curve on addition of one equivalent of bromide suggests very strong binding with a 1:1 host/guest stoichiometry. Throughout the titration only one set of host signals was observed indicating that the complexed and the uncomplexed form of the receptor are in fast exchange on the NMR timescale. The NH signals of the 3, 7 and 12α-NH protons underwent the most significant downfield shift on bromide addition as expected. The aryl protons *ortho* to the NO_2 and SO_2 groups showed characteristic downfield shifts whereas the *meta* aryl protons shifted upfield as anticipated for anion binding. The 7α-NH proton signal was followed throughout the titration with bromide giving a K_{ass} of $> 10^6$ M^{-1}. In order to calculate K_{ass} more accurately a new technique was needed.



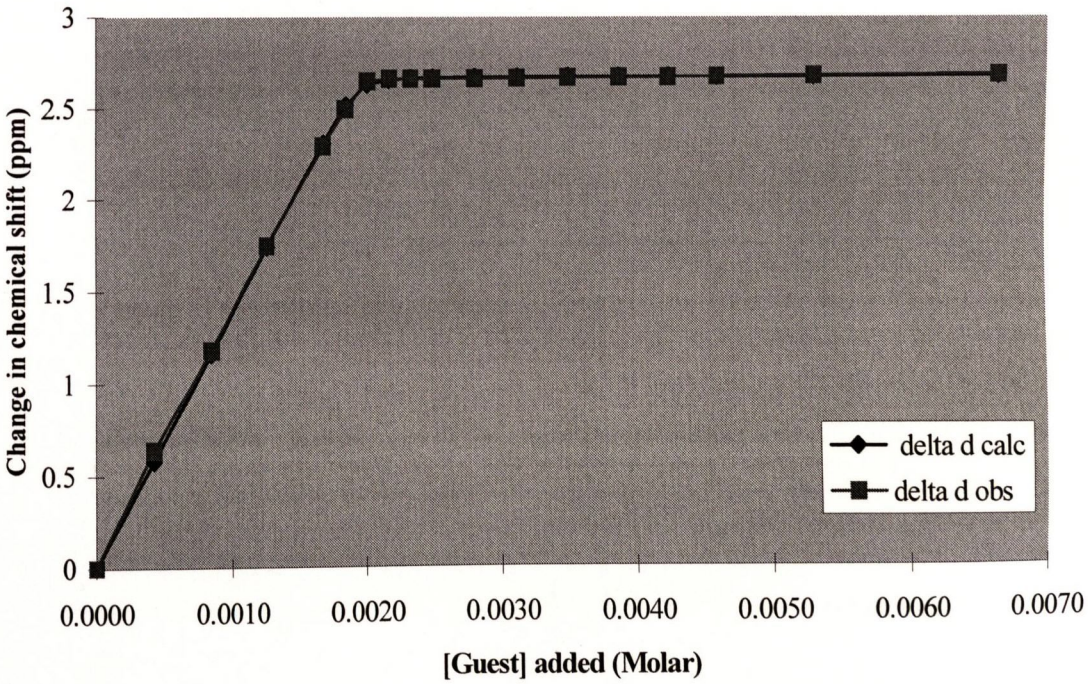
2.34

Binding curve 25A. NMR Binding Study of Association of Receptor **2.34** with Tetrabutylammonium bromide.

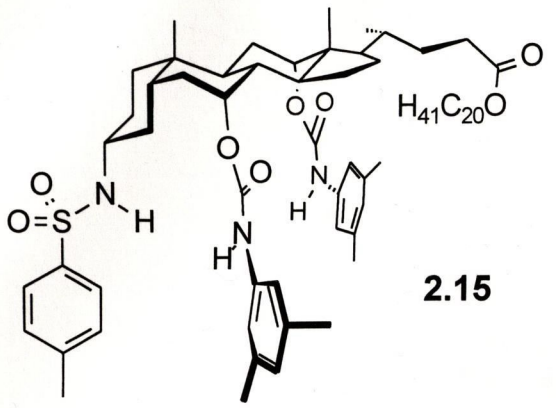
Dry conditions were used.
NMR used : MSL 400 at 303 K.
The chemical shift of 7α–NH was followed throughout this titration.
Solution concentrations : Host = 2.0 mM Guest = 57 mM.
Initial volume of host = 600 μL. Initial shift (δ) of 7α–NH = 7.4223ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000430	8.0621	0.6398	0.579112
10	0.000852	8.5977	1.1754	1.157738
15	0.001268	9.1635	1.7412	1.734985
20	0.001677	9.7129	2.2906	2.304623
22	0.001839	9.9186	2.4963	2.514717
24	0.002000	10.0729	2.6506	2.634481
26	0.002160	10.0855	2.6632	2.658334
28	0.002318	10.0855	2.6632	2.664997
30	0.002476	10.0867	2.6644	2.667985
34	0.002789	10.0880	2.6657	2.670743
38	0.003097	10.0892	2.6669	2.672039
43	0.003477	10.0917	2.6694	2.672931
48	0.003852	10.0930	2.6707	2.673466
53	0.004221	10.0942	2.6719	2.673823
58	0.004584	10.0955	2.6732	2.674078
68	0.005293	10.0980	2.6757	2.674418
88	0.006651	10.1043	2.682	2.674784

K_{ass} = > 10^6 M^{-1} δHG = 10.0980 ppm



[1] Connors, K. A. Binding Constants, John Wiley & Sons **1987**.
[2] Murray, B. A. Ph.D. Thesis, University of Dublin, 1993.
[3] Schmidtchen, F. P.;Chem. Rev., **1997**, *97*, 1609.
[4] Willi, A. V. *Helv. Chim. Acta.* **1956**, *39*, 46.



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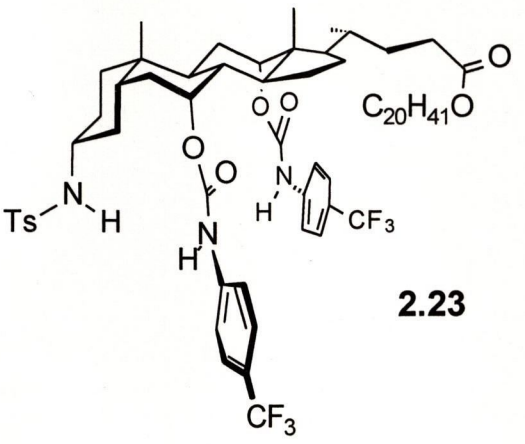

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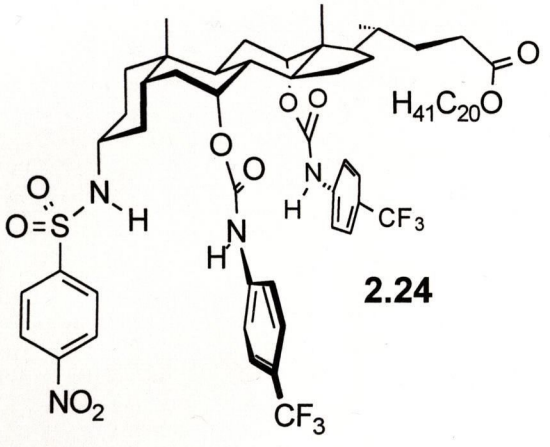

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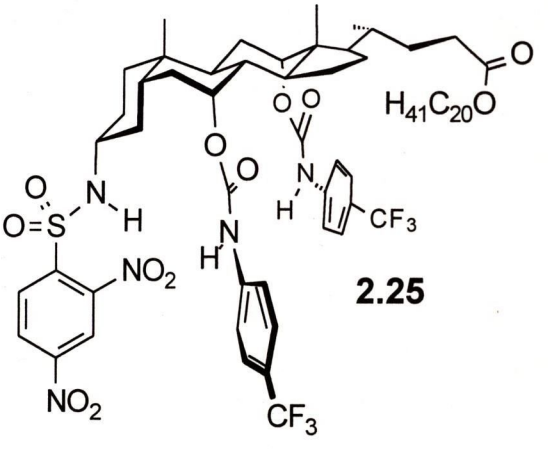

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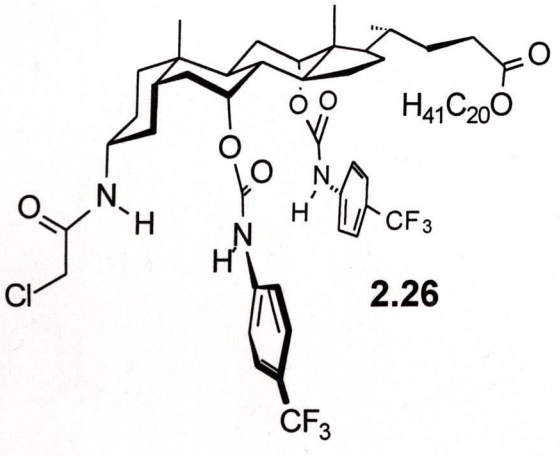

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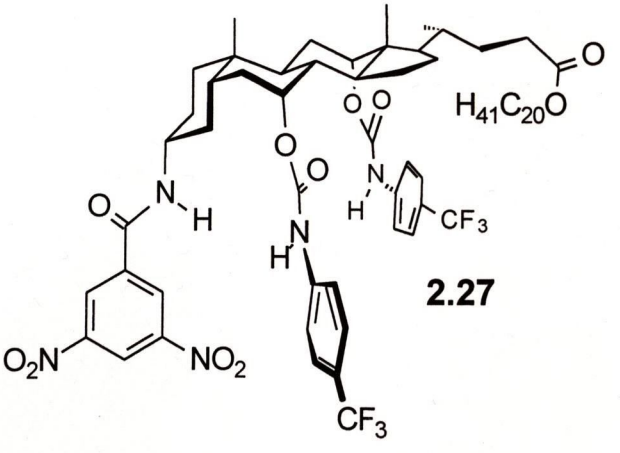

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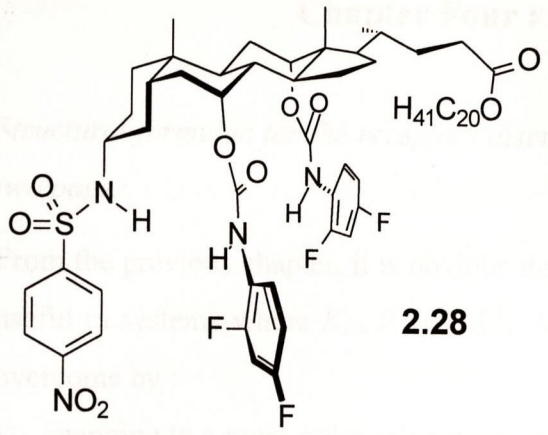

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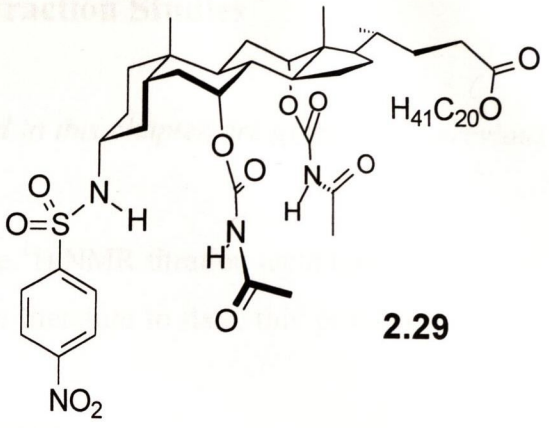

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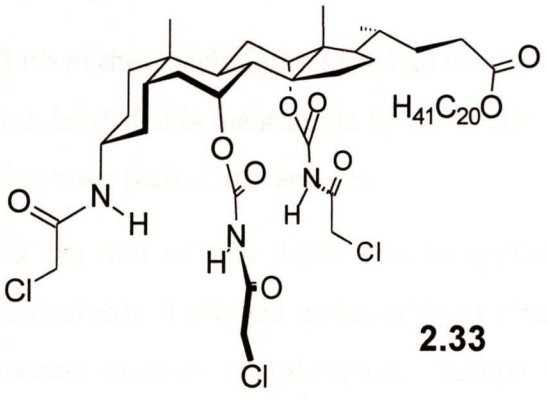

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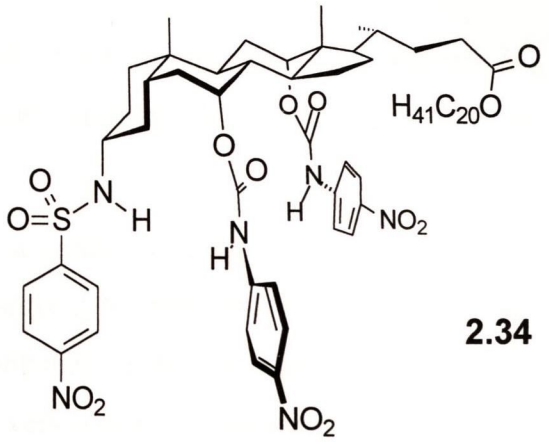

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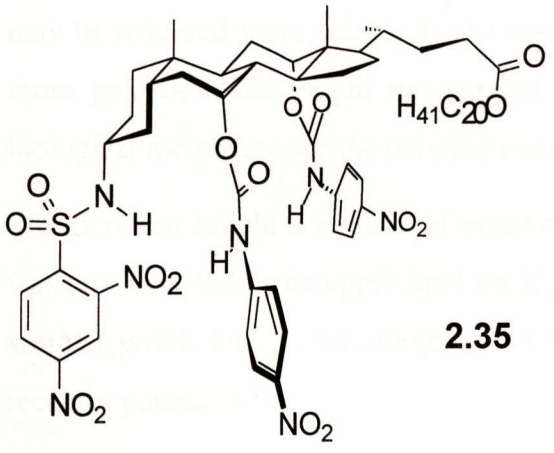

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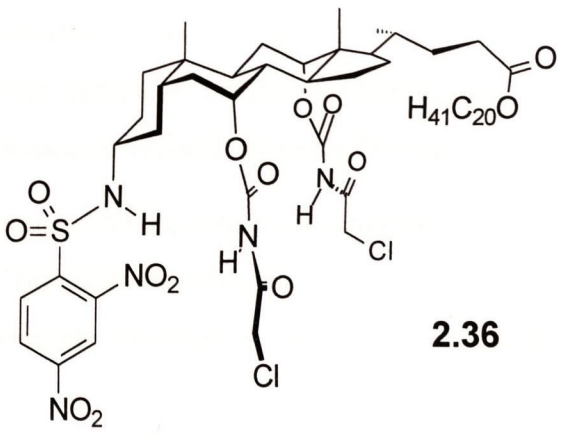


2.36

Chapter Four : Extraction Studies

Structural formulae for the receptors discussed in this chapter are given in the previous two pages.

From the previous chapter, it is obvious that the ^{1}H NMR titration technique is no longer useful in systems where $K_{ass} > 10^5$ M^{-1}. In the literature to date, this problem has been overcome by :

1) changing to a more polar solvent *e.g.* acetonitrile.
2) the addition of a polar solvent to increase the polarity of the medium *e.g.* 5 % CD_3OD in $CDCl_3$.

Both of these techniques have had the desired effect of lowering the binding constant K_{ass} to a level that is measurable by the NMR titration technique. However, these techniques also have their disadvantages.

As the aim of this thesis was to synthesise a series of receptors to bind anions in chloroform, it seemed undesirable to change solvent for their analysis because of their success in anion complexation. Receptors published in the literature to date with K_{ass} values quoted in acetonitrile or DMSO may be very potent, but the results may not relate directly to their ability to bind in chloroform. The receptors may even be only sparingly soluble in chloroform. On addition of a more polar solvent to the system, the receptor may be rendered more soluble in the solvent mixture. The performance of receptors in more polar solvents might not parallel that in non-polar media of interest, such as biological membranes or the polymer materials used in ion-selective membranes.

We therefore sought a method of measuring anion binding ability in chloroform which was not restricted by an upper limit for K_{ass}. Two different extraction methods examined seemed promising, as conditions could in principle be tuned to account for different receptor potencies :

i) Solubilisation of a salt in chloroform in the presence of a host.

ii) Extraction of a salt from an aqueous solution into chloroform containing host.

The first method (i) involved the solubilisation of a hydrophilic salt in a chloroform solution of host. The salts used in these experiments were found to be only poorly soluble in pure chloroform. However, in the presence of host, the salts were found to be

soluble in significant quantities. The idea behind this method was to compare the molar % extraction of the salt to receptor, for a series of receptors, to gain an insight of the anion binding ability of the receptors for the anions examined.

The second method (ii) involved the extraction of a hydrophilic salt from an aqueous phase into a chloroform solution of host. The idea behind this experiment was to establish if a receptor can extract any of the salt from the aqueous layer into the host/chloroform layer. In theory, the more salt extracted, the more potent the receptor for anion binding.

The solubilisation of anions as quaternary ammonium and phosphonium salts have been extensively studied. Selectivities were generally based on the Hofmeister series[1], : ClO_4^- > SCN^- > I^- > salicylate$^-$ > NO_3^- > Br^- > Cl^- > HCO_3^- > AcO^- > SO_4^{2-} > HPO_4^{2-} *i.e.* a preference for lipophilic and a rejection of hydrophilic anions. In these cases, the free energy of transfer of the anions from the aqueous phase to the organic phase and therefore their selectivity is governed by the free energy of hydration of these anions. This is in contrast to neutral receptor systems described in this thesis where the selectivity also depends on the free energy of the interaction of the ions with the ligand. In the latter case, a wide variety of ion selectivities is made accessible with selectivity controlled by a combination of ligand-ion complementarity of size, shape and functionality. The mechanistic studies of anion receptor interactions in nature have been limited, hampered at least in part by the unavailability of suitable model systems for anion binding and extraction from aqueous media. Although numerous elegant anion binding receptors have been reported in recent years, the number of synthetic systems capable of achieving both anion binding and extraction remains predominantly limited to systems based on the classical ion exchangers involving a net positive charge on the host. To the best of our knowledge, there are few exceptions to this rule.[2]

This chapter is divided into three separate sections based on the different extraction methods used :

a) Solubilisation of TMA salts in chloroform in the presence of a host, and extraction of TMACl, TMABr, TMATs and $TMAH_2PO_4$ from an aqueous solution into chloroform containing host.
b) Extraction of TEA salts from an aqueous solution into chloroform containing host.
c) Solubilisation of alkali earth salts in chloroform.

a) Solubilisation and extraction of TMA salts.

In the first experiment a qualitative indication of the potency of the receptors for anion binding was obtained by solubilisation of solid salt in chloroform. As expected, the more potent the receptor, the more salt was expected to dissolve. In the second experiment, a measurable amount of salt was extracted from the salt/aqueous phase into the host/chloroform phase. The higher the extraction into the latter phase, the stronger the anion binding by the receptor present.

Procedure for the solubilisation of tetramethylammonium salts in chloroform in the presence of a receptor.

A deuterated chloroform solution of host (0.25 mmol in 1 mL) was sonicated for 10 minutes with TMAX (0.4 mmol) added. The solution was then filtered through a hydrophobic filter tip syringe to remove the undissolved salt. A ^{1}H NMR spectrum of the resulting filtrate was obtained. The α-CH_2 of the ester side chain of the host was integrated against the CH_3 signal of the TMAX salt dissolved to obtain the percentage extraction efficiency.

Table 4.1 Results of sonication of an excess of the TMA salts in a chloroform solution of host.

	Mol. % extraction	
Receptor	TMACl	TMABr
2.23	100	50
2.26	100	36
2.28	100	17
2.33	100	30
2.36	100	75

The receptors shown were all found to be capable of solubilising 1 mole equivalent of tetramethylammonium chloride in chloroform and less than one mole equivalent of TMABr (the solubility of the free salts TMACl and TMABr are very low in chloroform). The electrostatic lattice energy that holds the ions together in its crystal form must be overcome in order for the salt to dissolve and form a dilute solution where the ions are well separated. The tetramethylammonium salts of chloride and bromide were chosen

because of their hydrophilicity-lipophilicity balance, which strongly disfavours their solubility in chloroform. The four methyl groups provide an intense singlet in the ^1H NMR spectra that allows the determination of the salts' concentration in the organic phase.

Comparison of the liquid-solid extraction ^1H NMR spectra with that of the free host reveals large downfield shifts of both the NH protons involved in anion binding, and the aryl protons *ortho* to the NH in the carbamate groups and SO_2 in the sulfonamide groups. In conclusion, the receptors appear to bind the anions chloride and bromide in the liquid state presumably with 1:1 guest-host stoichiometry.

Crown ethers are known for their ability to extract cations from aqueous into non-polar media.[3] It was felt that a similar technique for anions could allow estimation of a receptor's ability to bind and extract anions into the organic phase. Table 4.2 shows the results of a two-phase extraction of tetramethylammonium salts from an aqueous phase into a host organic phase by the procedure described below.

Procedure for the extraction of tetramethylammonium salts from an aqueous phase into a chloroform phase in the presence of host.

An aqueous solution of TMAX (0.25 mmol in 0.5 mL) was stirred vigorously with a host solution (0.1 mmol in 1 mL of chloroform) for 15 minutes at room temperature. The two layers were then allowed to settle and the organic layer was filtered through hydrophobic filter paper and the filtrate was evaporated to dryness. The resulting solid was re-dissolved in $CDCl_3$ and a ^1H NMR spectrum of the mixture was obtained. The α-CH_2 of the ester side chain of the host was integrated against the CH_3 signal of the TMAX salt extracted to obtain the percentage extraction efficiency.

Table 4.2 Two-phase extraction results.

	Mol. % extraction of salt			
Receptor	TMACl	TMABr	TMATs	$TMAH_2PO_4$
2.15	0.0	0.0	1.9	0.0
2.23	0.5	2.0	9.0	0.0
2.24	2.5	27.0	84.0	0.0
2.25	2.0	14.0	55.5	0.0
2.27	4.0	25.0	88.0	nd
2.28	0.0	0.0	2.0	0.0
2.29	1.0	3.5	6.5	0.5
2.33	1.0	2.0	3.0	0.0
2.34	17.0	72.5	95.5	1.8
2.35	4.0	43.0	90.0	nd
2.36	2.5	6.5	6.0	0.5

nd = not determined.

The long ester chain was present on all the receptors used for extractions to ensure that the receptor is not lost to the aqueous layer during an extraction. The ester was not expected to interfere strongly with the binding site of the receptors. From Table 4.2, **2.15**, the 'C20 analogue of Perry's receptor **1.68** was found to be completely ineffective at extracting TMACl, TMABr and $TMAH_2PO_4$ from an aqueous solution into chloroform. A trace of TMATs (1.9%) was extracted by the receptor into chloroform presumably due to the higher lipophilicity of this anion over the others examined.

Receptor **2.33** was also found to extract a minimal amount of TMACl and TMABr but a slightly larger percentage of TMATs than **2.15**. The small percentage extraction can be explained by the moderate acidity of the NH protons in the cavity of this receptor. The small but significant extraction of TMACl and TMABr from the aqueous solution into the chloroform can be explained by the increase in the acidity of the carbamate NH protons at the 7 and 12 positions compared to receptor **2.15**. The receptor was found to be completely ineffective at extracting $TMAH_2PO_4$ at the concentration used in the extraction.

Receptor **2.23** was also found to extract a minimal amount of TMACl and TMABr but a significantly larger amount of TMATs (9.0%) than receptor **2.15**. This increase in extraction of TMATs is most likely due to an increase in the acidity of the carbamate NH protons at the 7 and 12 positions of **2.23**. The *para*-CF_3 substituted aromatic ring was

expected to have a strong electron withdrawing effect on the NH protons rendering them more available for hydrogen bonding.

2.24, with a *p*-nitrobenzenesulfonamide at the three position and *p*-trifluoromethylphenyl carbamates at the 7 and 12, was found to show a significant increase in extraction of all three salts examined namely TMACl, TMABr and TMATs. The large increase in extraction by **2.24** compared to **2.23** was due to the increase in acidity of the 3α-NH caused by the nosyl group

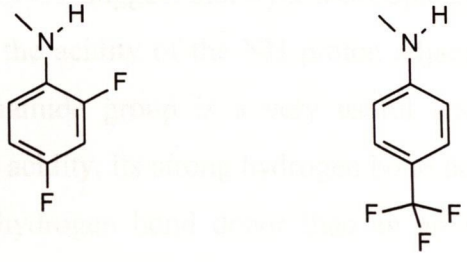
However from Table 4.2, the receptor **2.25** was found to be less potent in its ability to extract anions from the aqueous phase compared to **2.24**. One possible and likely explanation for this receptor's poor performance is that the *ortho*-nitro group on the aryl ring is hindering the anion binding site, being involved in *intra*-molecular hydrogen bonding to the NH proton that is spatially close to it. This quenching of the NH proton by the *ortho*-nitro group explains the lack of potency of **2.25** in anion extraction efficiency.

In receptor **2.27** the 3α amine was substituted with a 3,5-dinitrobenzoyl group. The receptor was found to be comparable to **2.24** in terms of anion extraction efficiency. From molecular modelling, the *meta* substituted nitro groups were far removed from the binding site and were not expected to interfere with the binding of the receptor. The similarity in the extraction results of **2.24** and **2.27** suggests that the 3α NH protons in both receptors are of comparable acidity. The expected higher acidity of the sulfonamido NH over the amido NH was offset by the fact that the amido group consisted of a di-nitro substituted aromatic ring.

Receptor **2.28** is found to be very poor at binding and extracting anions from the aqueous phase into the organic phase. From molecular modelling the *ortho*-fluorine does not appear to participate in *intra*-molecular hydrogen bonding (the nearest interaction of the 7α-*ortho*-fluorine to an NH is *ca.* 2.15 Å to the 7α-NH proton) as in the case of the *ortho*-nitro group in **2.25**. The reason for the poor anion extraction performance of **2.28** is probably due to substituent effects of the fluorine. Substituent effects can be broadly divided into steric effects and electronic effects. Electronic effects can be further divided into σ inductive effects and π conjugative (resonance) effects. Although it is not a rigid rule, because of the small size of a fluorine substituent, its influence on structure and reactivity is usually considered to derive largely from fluorine's electronic nature.

Fluorine is the most electronegative atom, and it thus exhibits a potent σ inductive electron-withdrawing effect in all situations. It is also a potentially strong π electron donor to carbon π systems *etc.* (due to the close match in size of the lone pair 2p orbitals of fluorine with those of carbon). The net impact of a fluorine substituent will result from a complex interplay of these disparate interactions. To make matters even more complicated, the combined influences of multiple fluorine substituents is not additive and cannot be readily derived from an understanding of the effect of a single fluorine substituent.

Figure 4.1



(i) (ii)

Differences in fluorine substituent effects is particularly noticeable between receptors **2.23** and **2.28**. The difference between the two receptors is the carbamate groups shown in figure 4.1. From table 4.2, one can deduce that the *para*-trifluoromethyl substituent in receptor **2.23** is far more electron withdrawing than the *ortho-para*-difluoro substituent in **2.28**. This is probably due to the lack of π interaction of the fluorine atoms with the aromatic ring in (ii) due to the carbon spacer, and so only σ inductive electron-withdrawing effect are noticeable in **2.23**.

The new receptor **2.34** showed the most impressive extraction results of all the salts examined. On comparing the results of **2.34** and **2.23**, the advantage of the *para*-nitrophenyl groups over the *para*-trifluoromethylphenyl group on the carbamates were immediately obvious. **2.34** was found to extract almost 1 equivalent of TMATs from an aqueous solution and over 70 mol % of TMABr. The moderate extraction of TMACl of 17% was due to the higher hydrophilicity of this anion over the other anions examined.

Equipped with the knowledge that the *para*-nitrophenyl carbamates gave very impressive results compared to the other substituents used, receptor **2.35** was synthesised for comparative reasons. **2.35** was found to be a powerful receptor for anion extraction, but not to the same scale as **2.34** (see Table 4.2). One reason for the lower anion extraction

values of **2.35** against **2.34** is due to the presence of an *intra* molecular hydrogen bond between the *ortho*-nitro group to the 3α-NH as in the case of the receptor **2.25**.

Receptors **2.29** and **2.36** were synthesised with non-aromatic carbamates. The receptors were examined for their anion extraction abilities. From Table 4.2 it is clear that their potency for anion extraction is by far inferior to the ability of receptor **2.34**.

In conclusion, it is clear that electron withdrawing groups have a strong effect on the NH protons in the binding cavity by rendering them more acidic and therefore more available for hydrogen bonding. The results of this systematic study of substituent effects in binding Cl^-, Br^-, Ts^- and $H_2PO_4^-$ suggest that a *para*-nitrophenyl substituent provides the strongest enhancement of the acidity of the NH proton adjacent to it. The results also demonstrate that a sulfonamide group is a very useful and potent group for anion recognition due to its high acidity, its strong hydrogen bond donor ability (a sulfonamide is known to be a better hydrogen bond donor than an amide) and its low ability to participate in *intra* molecular hydrogen bonds (*e.g.* O=S=O••H-N-R). It has been well documented in the literature that amides are much better hydrogen bond acceptors than sulfonamides.[4] In all of the receptors, only minimal $H_2PO_4^-$ extraction was observed. This result reflects the lack of complementarity of the binding site for this anion and the anion's stronger hydrophilicity. The high extraction values of TMATs was due to the anions' higher lipophilicity over the other anions. The extent of extraction in this series does not quantify the strength of anion binding by the receptor, but a qualitative insight can be obtained on a comparative scale between the receptors from Table 4.2.

b) Extraction of TEA salts from aqueous media into chloroform containing a host.

The previous section reported the effects of electronic tuning of the binding cavity on the extraction efficiency of the host for tetramethylammonium salts. This section describes the extension of the organic/aqueous phase extraction experiment to give a quantitative measurement of the potency of the receptors in this series. A major advantage of this relatively novel method* was that the method of assessing K_{ass} was not restricted to an upper limit. As illustrated below this method should be able to measure binding

* This procedure was previously used by Cram, D. J. for examining the extraction ability of various cationophores. To the best of our knowledge this method has not been used for anionophores to date.

constants of, ideally, any magnitude in a non-polar medium such as chloroform. Due to the low percentage of tetramethylammonium chloride and bromide extraction by the majority of receptors, a more lipophilic counterion in the quaternary ammonium series (Quats) was required for these quantitative experiments. The tetraethylammonium counterion was found to have the appropriate lipophilicity. It was also chosen because the four methylene groups of the salt provide a distinctive quartet signal in an area of the ^{1}H NMR spectrum with little overlap of the host signal (in some cases the signal of the salt overlaps with the 3β proton belonging to the steroidal receptor). The signal allows the determination of the salt's concentration with respect to the host, in the chloroform layer.

From the ^{1}H NMR spectrum, integration values of the salt against host, it is possible to estimate the extraction constant K_e in chloroform according to equation (**1**) where $[H]_{CHCl_3}$ is the concentration of free host in the chloroform phase, $[TEA^+HX^-]_{CHCl_3}$ is the concentration of complex in the chloroform phase, $[TEA^+]_{H_2O}$ is the concentration of tetraethylammonium cation in the aqueous phase and $[X^-]_{H_2O}$ is the concentration of anion in the aqueous phase (X^- is the anion concerned *e.g.* Cl^-). In equation (**2**), K_e is expressed in terms of measurable quantities by NMR analysis by the fact that $[TEA^+]_{H_2O} = [Cl^-]_{H_2O}$ in order to conserve electroneutrality in both phases.

$$[H]_{CHCl_3} + [TEA^+]_{H_2O} + [X]_{H_2O} \overset{K_e}{\rightleftharpoons} [TEA^+HX]_{CHCl_3} \qquad (1)$$

$$K_e = \frac{[TEA^+HX]_{CHCl_3}}{[H]_{CHCl_3}\,[TEA^+]_{H_2O}\,[X]_{H_2O}} \qquad (2)$$

At equilibrium there will also be a concentration of free $[TEA^+][X^-]$ in the organic phase, governed by equations 3 and 4 (K_d = distribution constant).

$$[TEA^+]_{H_2O} + [X]_{H_2O} \overset{K_d}{\rightleftharpoons} [TEA^+X]_{CHCl_3} \qquad (3)$$

$$K_d = \frac{[TEA^+X]_{CHCl_3}}{[TEA^+]_{H_2O}\,[X]_{H_2O}} \qquad (4)$$

Combining equations (3) and (4)

$$K_{ass} = \frac{K_e}{K_d} . \qquad (5)$$

K_{ass} may therefore be determined provided $\frac{[TEA^+HX^-]_{CHCl_3}}{[H]_{CHCl_3}}$ and K_d can be measured. This method is especially useful for powerful receptors, because quantitative complex formation can be avoided by adjusting the aqueous concentration of TEA^+X^-. This measure keeps $[TEA^+X^-]_{CHCl_3}$ small, which also simplifies the measurement of :

$$\frac{[TEA^+HX^-]_{CHCl_3}}{[H]_{CHCl_3}} .$$

As shown by Cram K_e can be related to R (mole fraction of guest to host in the organic phase) and the volumes of organic and aqueous media (V_{CHCl3} and V_{H2O}) used in the extraction [3d] by equation (6).

$$K_e = \frac{R}{(1-R)\{[TEA^+]_{H_2O} - R\,[H]_{CHCl_3}(V_{CHCl_3}/V_{H_2O})\}^2} \qquad (6)$$

The mathematical model used in the above analysis is based on the assumptions that :

1) in water, the salt remains completely dissociated, while in chloroform the salt forms a tight monomeric ion pair.
2) all the guest in the chloroform layer formed a 1:1 complex with the host.

(This model is later given strong support by varying the host concentration and the guest concentrations and obtaining the same K_{ass} values).

Procedure for the determination of the extraction coefficient K_e for each receptor, for TEACl and TEABr.

The experiment was carried out by vigorously stirring an aqueous solution of TEAX of known concentration with a chloroform solution of host, at 30 °C in a water bath for 15 minutes. The stirring was then stopped and the two layers were allowed to separate for 5 minutes. The organic layer was then passed through hydrophobic filter paper and

evaporated to dryness. The host-salt complex was re-dissolved in $CDCl_3$ (500 μL) and a ^1H NMR spectrum was acquired. The methylene signal of the TEAX salt was integrated against the α-CH_2 of the ester side chain of the steroid to obtain the relative concentration of guest to host, (R in equation 6 above).

The concentration of the host in most experiments was 0.01 M in chloroform and the volume was 1.0 mL. The differences in salt lipophilicity between TEACl and TEABr were too great for determination of K_e at one set of guest concentrations. So too was the difference in binding abilities of the hosts examined. Accordingly the guest concentration was varied by the addition of more or less water in order to obtain K_e values between 0.05 and 0.7 for each extraction experiment for each host.

Analysis of the aqueous layers revealed that none of the host was distributed into this layer during equilibration. All of the receptor used in the extraction was found in the chloroform layer at equilibrium.

Table 4.3 Extractions results with the receptors shown in terms of R, the mole fraction of guest to host at a given salt concentration [TEAX].

Salt/Host	2.23	2.24	2.25	2.28	2.34	2.35	2.36
[TEACl]	0.5	1.0	1.0	2.0	0.0125	0.05	0.5
R :	0.221	0.406	0.604	0.190	0.058	0.188	04405
[TEABr]	0.2	0.025	0.05	0.5	0.01	0.01	0.2
R :	0.471	0.460	0.515	0.193	0.367	0.102	0.536

In separate extraction experiments, the salts TEACl and TEABr were distributed between equal volumes of chloroform and water in the absence of a host, at 2 M aqueous concentration. No sign of either salt ions was detectable in the ^1H NMR spectra of the organic layers

From the values of R determined for each receptor from the ^1H NMR spectra, it was possible to calculate K_e, the extraction coefficient from (**2**).

Table 4.4. Extraction coefficient K_e for each extraction experiment at a given salt concentration [TEACl].

Salt/Host	2.23	2.24	2.25	2.28	2.34	2.35	2.36
[TEACl]	0.5	0.1	0.2	2.0	0.0125	0.05	0.5
K_e	1.1449	69.4448	40.5084	0.0589	401.6186	94.0196	3.2055
[TEABr]	0.2	0.025	0.05	0.5	0.01	0.01	0.2
K_e	23.3456	1497.571	448.0430	0.9641	6247.974	1159.388	30.7951

Determination of the distribution coefficient K_d for TEACl and TEABr.

In order to determine the association constant K_{ass} for each receptor for TEACl and TEABr the distribution coefficient K_d, for the salts must be determined first. The distribution coefficient was estimated using two different procedures, involving (i) direct measurements and (ii) indirect measurements using binding study data.

Estimation of K_d for TEACl.

(i) *Direct measurements using large scale partition experiments.*

In this extraction 50 mL of aqueous TEACl (0.5 M) was stirred with 500 mL of chloroform pre-saturated with water* [1]at 30 °C. The stirring was stopped after 30 minutes and the two layers were allowed to settle. The organic layer was separated, passed through hydrophobic filter paper and evaporated to dryness. The mixture was re-dissolved in $CDCl_3$ with an added standard (1,1,2,2-tetrachloroethane) of a known concentration and a ^1H NMR spectrum was acquired. From integration of methylene signal of the TEA salt against the TCE standard added in the ^1H NMR spectrum, there appeared to be 1.31 x 10^{-6} mol TEACl extracted into the chloroform layer. The TCE integration standard was found to be 83% relaxed after the time allowed (1 second) on the NMR ^1H programme used. This factor was accounted for in the calculations of K_d. The TEA cation was 100 % relaxed on the timescale used.

*$CHCl_3$ was distilled and filtered through basic alumina, shaken with distilled water and finally passed through hydrophobic filter paper and stored in a dark bottle out of light. Presaturation with water was necessary in order to ensure that the volume of the aqueous phase was not altered by dissolution of water in the chloroform.

$$[TEA^+]_{H2O} + [Cl^-]_{H2O} \underset{}{\overset{K_d}{\rightleftharpoons}} [TEACl]_{CHCl3}$$

start:	0.5 M	0.5 M	0 M
at equilibrium :	0.499 M	0.499 M	2.62×10^{-6} M

From equation (**4**) :

$$K_d = 1.05 \times 10^{-5} \text{ M}^{-1}.$$

Repeated extractions could not be done on aqueous phases of lower salt concentrations because of sensitivity problems, while integrating the ^{1}H NMR spectra. However, a repeat extraction at the same aqueous salt concentration gave $K_d = 8.3 \times 10^{-6}$ M^{-1}.

(ii) *Indirect measurements using binding study data.*

From equation (**3**), K_d can be determined if K_e and K_{ass} are known. K_e is directly measurable from extraction experiments. K_{ass} may be obtained from accurate and reliable binding studies of various receptors under the same conditions as extractions *ie.* water-saturated chloroform.

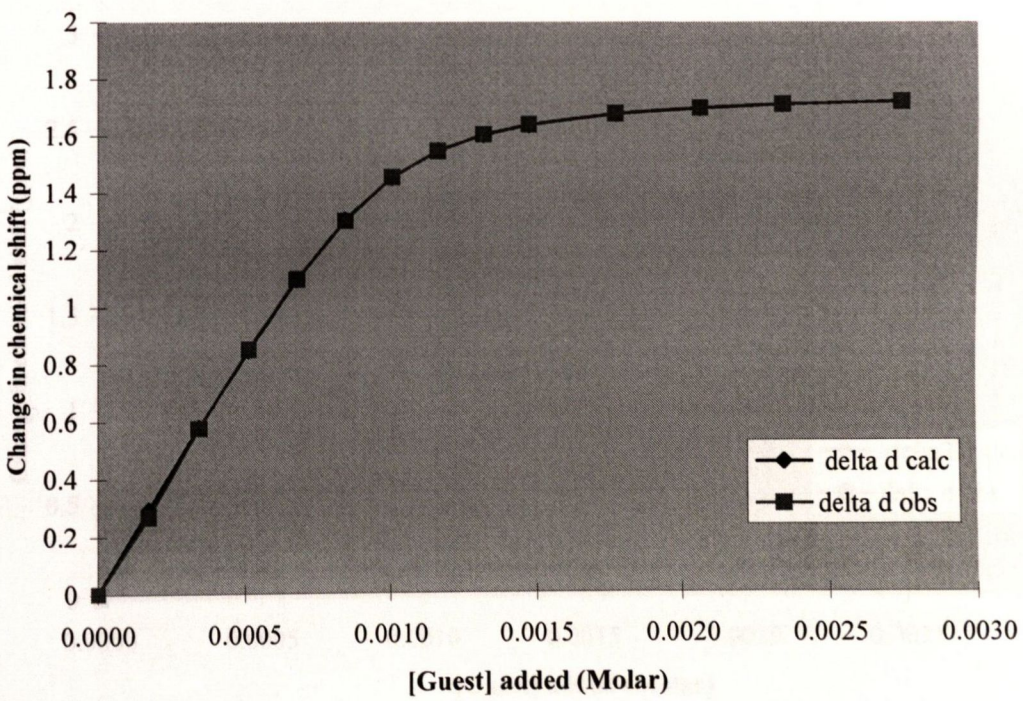
Receptor **2.33** was titrated with tetrabutylammonium chloride in water-saturated chloroform at 30 °C. It has been demonstrated that the difference in binding strength of TEACl and TBACl by **2.15** was negligible.[5] Binding study 26 shows the data for the NMR titration study.

Binding curve 26A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α–NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 21 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the 3α–NH = 6.4276 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000174	6.6960	0.2684	0.292370
10	0.000344	7.0021	0.5745	0.577207
15	0.000512	7.2800	0.8524	0.848988
20	0.000677	7.5265	1.0989	1.097210
25	0.000840	7.7297	1.3021	1.304488
30	0.001000	7.8828	1.4552	1.454051
35	0.001157	7.9743	1.5467	1.547793
40	0.001313	8.0320	1.6044	1.603803
45	0.001465	8.0671	1.6395	1.638436
55	0.001763	8.1060	1.6784	1.677006
65	0.002053	8.1260	1.6984	1.697311
75	0.002333	8.1380	1.7104	1.709660
90	0.002739	8.1480	1.7204	1.721094

K_{ass} = 21,000 ± 1,260 M^{-1} δHG = 8.1901 ppm

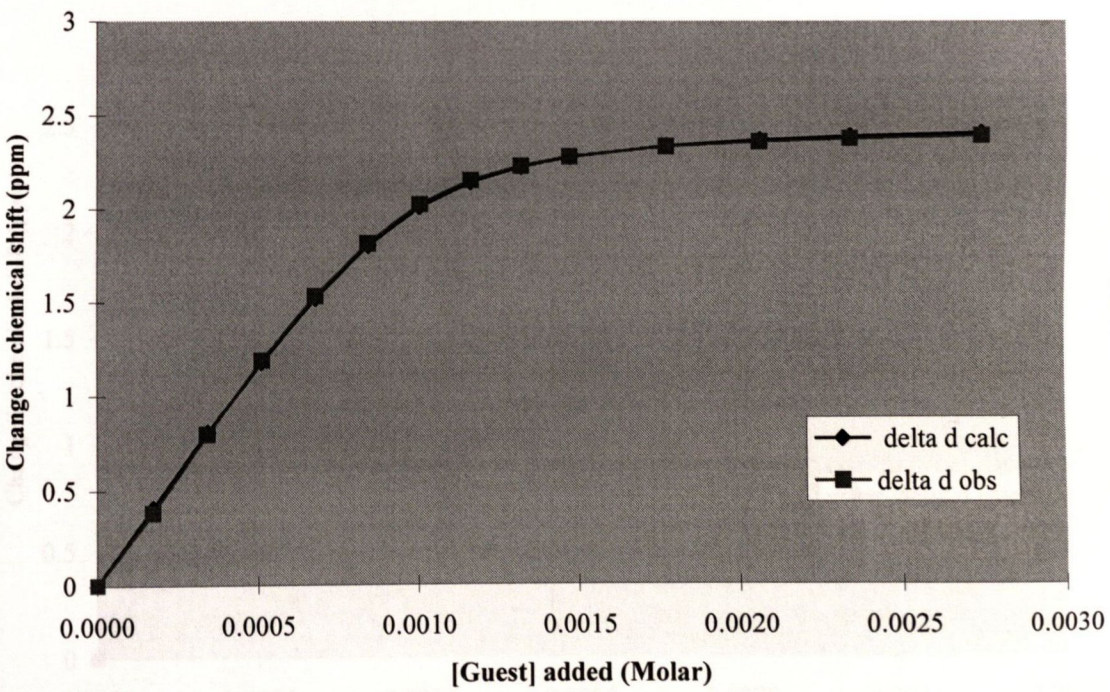


Binding curve 26B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 21 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.6115 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000174	8.9954	0.3839	0.406812
10	0.000344	9.4068	0.7953	0.802291
15	0.000512	9.7982	1.1867	1.178419
20	0.000677	10.1444	1.5329	1.520485
25	0.000840	10.4279	1.8164	1.805446
30	0.001000	10.6362	2.0247	2.012579
35	0.001157	10.7641	2.1526	2.145073
40	0.001313	10.8406	2.2291	2.226089
45	0.001465	10.8908	2.2793	2.277071
55	0.001763	10.9435	2.332	2.334689
65	0.002053	10.9686	2.3571	2.365360
75	0.002333	10.9849	2.3734	2.384115
90	0.002739	10.9987	2.3872	2.401541

K_{ass} = 20,000 ± 900 M^{-1} δHG = 11.0765 ppm

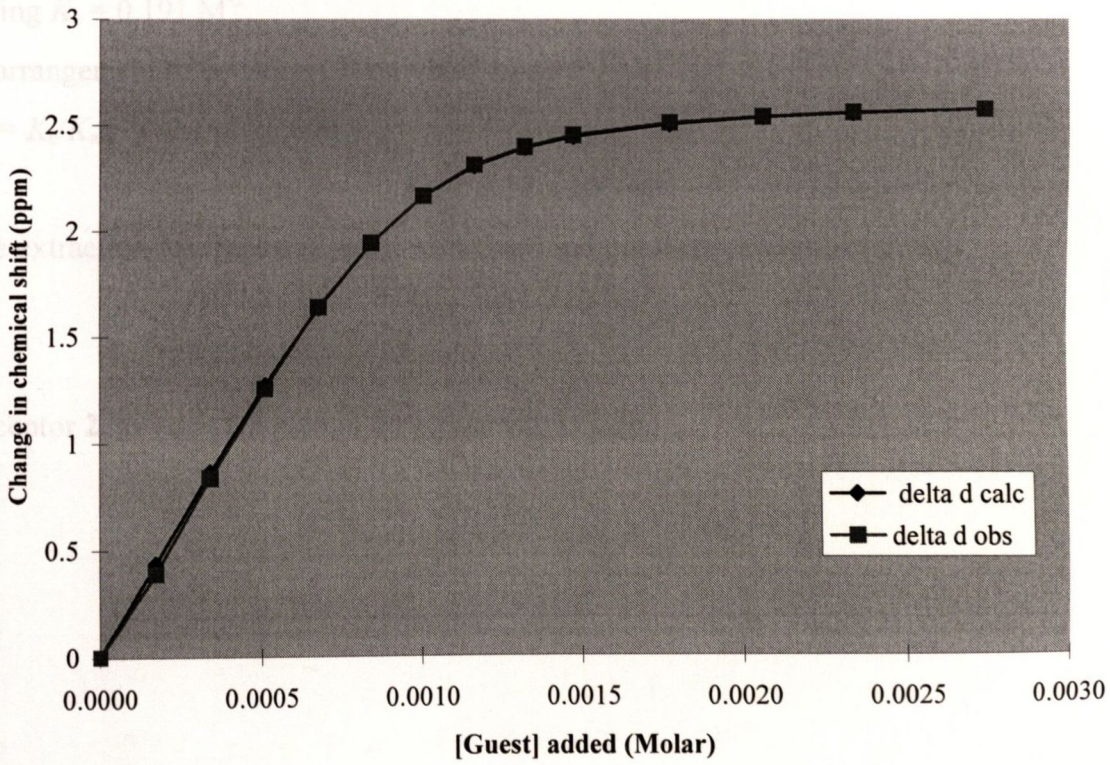


Binding curve 26C. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium chloride.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 21 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH = 8.7282 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000174	9.1183	0.3901	0.436872667
10	0.000344	9.5636	0.8354	0.862049459
15	0.000512	9.9801	1.2519	1.267101874
20	0.000677	10.3677	1.6395	1.636272593
25	0.000840	10.6726	1.9444	1.944175945
30	0.001000	10.8946	2.1664	2.167142339
35	0.001157	11.0414	2.3132	2.308302565
40	0.001313	11.1254	2.3972	2.393611696
45	0.001465	11.1806	2.4524	2.446816561
55	0.001763	11.2395	2.5113	2.506493436
65	0.002053	11.2684	2.5402	2.538078693
75	0.002333	11.2884	2.5602	2.557338424
90	0.002739	11.3035	2.5753	2.575200471

K_{ass} = 21,000 ± 1,000 M^{-1} δHG = 11.3683 ppm



During the titration study, the 3α-NH and the carbamate NH protons were observable throughout the experiment. Analysis of the three signals gave K_{ass} values in good agreement with each other (K_{ass} for the 3α-NH and the carbamate NH protons was 21,000 ± 1,260, 20,000 ± 900 and 21,000 ± 1,000 M^{-1} respectively). The carbamate NH followed in binding curve 26B provided the most accurate value of the signals. From the ^1H NMR spectrum of the pure host at the start of the binding study, integration of the water signal against the host signal gives an indication of the amount of water dissolved in the chloroform. Analysis shows that 27-30 equivalents of water to host are present in the $CDCl_3$. The host was found to undergo similar changes in shift as when the titration was done in dry chloroform however, the degree of shift was less. This considerable reduction in binding strength is due to solvation of the anion and the receptors binding site by the dissolved water in the chloroform.

Receptor **2.33** was tested in an extraction experiment of TEACl according to the conditions below. According to the ^1H NMR spectrum, integration of the salt to the complex shows that 4.56 mol % were extracted into the chloroform by **2.33**.

$$[H]_{CHCl3} + [TEA^+]_{H2O} + [Cl^-]_{H2O} \overset{K_e}{\rightleftharpoons} [TEA^+HCl^-]_{CHCl3} \qquad (1)$$

start :	0.01 M	0.5 M	0.5 M	0 M
at equilibrium :	0.009544 M	0.499544 M	0.499544 M	0.000456 M

giving $K_e = 0.191\ M^{-2}$.

Rearrangement of equation (**3**) provides K_d as :

$K_d = K_e/K_{ass} = 0.191/20{,}000$

$$K_d = 9.57 \times 10^{-6}\ M^{-1}.$$

The extraction was repeated at the same host and guest concentrations giving

$$K_d = 1.05 \times 10^{-5}\ M^{-1}.$$

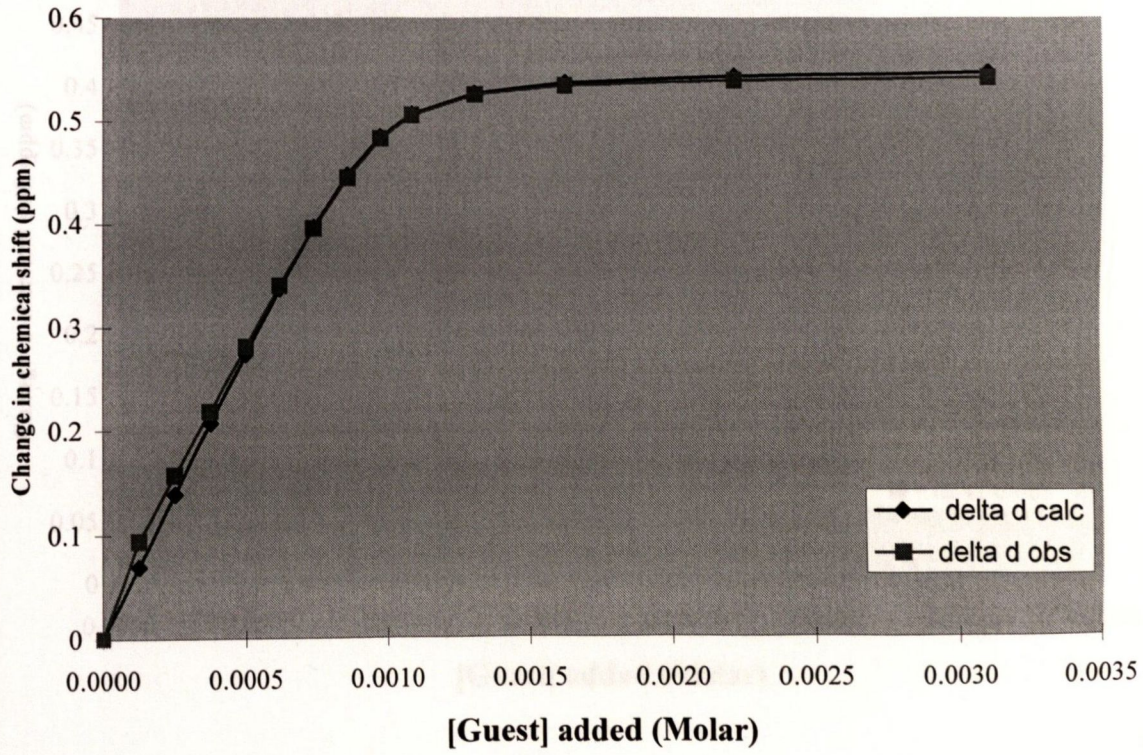
Receptor **2.26** was exploited in the same way as **2.33**.

Binding curve 27A. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium chloride.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *ortho* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 15.5 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the *ortho*-aryl proton = 7.6055 ppm.

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000128	7.6995	0.094	0.069607
10	0.000254	7.7623	0.1568	0.138661
15	0.000378	7.8237	0.2182	0.206855
20	0.000500	7.8865	0.281	0.273629
25	0.000620	7.9465	0.341	0.337888
30	0.000738	8.0019	0.3964	0.397396
35	0.000854	8.0508	0.4453	0.447940
40	0.000969	8.0884	0.4829	0.484479
45	0.001081	8.1110	0.5055	0.506621
55	0.001302	8.1311	0.5256	0.526640
70	0.001619	8.1398	0.5343	0.536962
100	0.002214	8.1449	0.5394	0.543399
150	0.003100	8.1474	0.5419	0.546596

K_{ass} = 50,000 ± 14,550 M^{-1} δHG = 8.1568 ppm

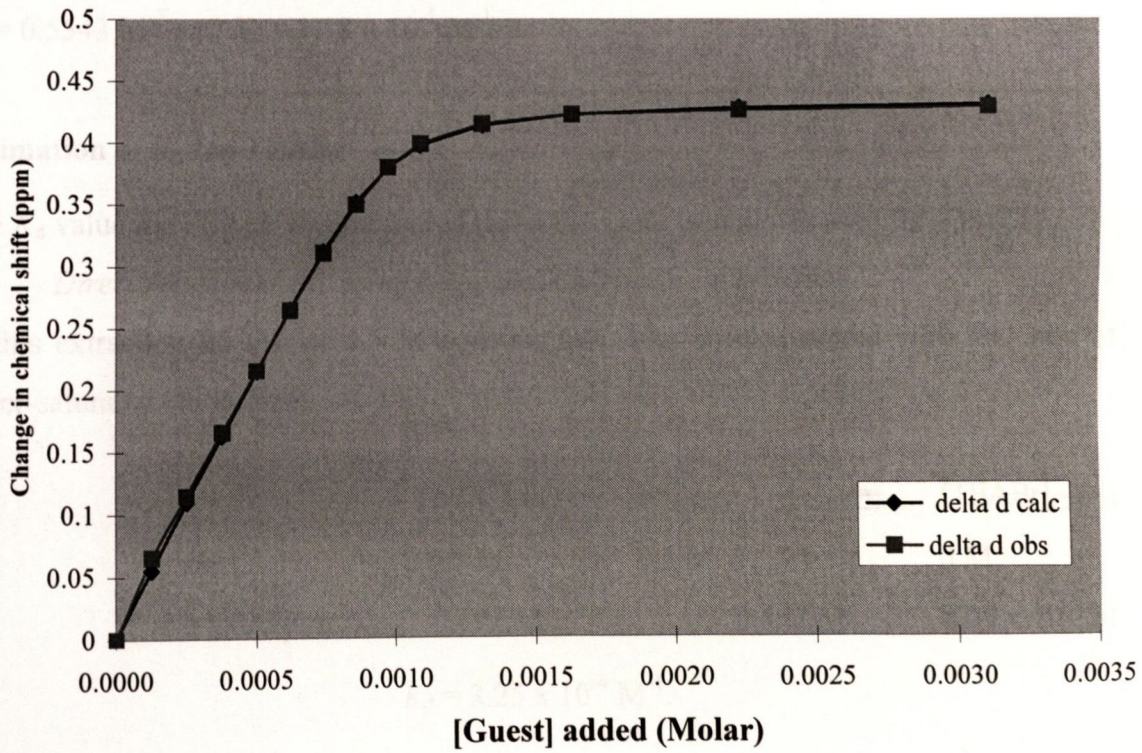


Binding curve 27B. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium chloride.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *ortho* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 15.5 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the *o*-aromatic proton = 7.6055 ppm.

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000128	7.6707	0.0652	0.054797
10	0.000254098	7.7196	0.1141	0.109132
15	0.000378049	7.7710	0.1655	0.162752
20	0.0005	7.8212	0.2157	0.215197
25	0.00062	7.8701	0.2646	0.265577
30	0.000738095	7.9166	0.3111	0.312117
35	0.000854331	7.9554	0.3499	0.351573
40	0.00096875	7.9856	0.3801	0.380221
45	0.001081395	8.0044	0.3989	0.397835
55	0.001301527	8.0207	0.4152	0.414099
70	0.001619403	8.0282	0.4227	0.422635
100	0.002214286	8.0320	0.4265	0.428001
150	0.0031	8.0345	0.429	0.430675

K_{ass} = 47,000 ± 6,266 M^{-1} δHG = 8.0401 ppm



Receptor **2.26** showed appreciable binding of TBACl even in water saturated chloroform as shown above. In both cases, an aryl proton *ortho* to an NH carbamate group was followed throughout the titration giving a K_{ass} of 50,000 ± 14,000 and 47,000 ± 6,266M^{-1} respectively. The two signals were in close agreement and provided a good measure of their accuracy. The ^1H NMR spectrum of the pure host showed 27- 30 molar equivalents of dissolved water present in the $CDCl_3$. As in the case of receptor **2.33**, the K_{ass} value was considerably reduced in water saturated chloroform than in dry chloroform due to solvation of the anion and the host binding site by the dissolved water present.

Extraction experiments on **2.26** were performed according to the conditions given below:

$$[H]_{CHCl3} + [TEA^+]_{H2O} + [Cl^-]_{H2O} \overset{K_e}{\rightleftharpoons} [TEA^+HCl^-]_{CHCl3}$$

at start :	0.01 M	0.5 M	0.5 M	0 M
at equilibrium :	0.00872 M	0.49872 M	0.49872 M	0.00128 M

giving $K_e = 0.5902\ M^{-2}$.

From equation (**3**) : $K_d = K_e / K_{ass} = 0.5902 / 47{,}000$

$$K_d = 1.26 \times 10^{-5}\ M^{-1}.$$

The extraction was repeated with similar host and guest concentrations giving :
$K_e = 0.5543\ M^{-2}$ and $K_d = 1.18 \times 10^{-5}\ M^{-1}$.

Estimation of K_d for TEABr

The K_d value for TEABr was measured using the same techniques used for TEACl.

(i) *Direct measurements using large scale partition experiments.*

In this extraction 20 mL of 0.5 M aqueous salt solution was stirred with 500 mls of water-saturated chloroform.

$$[TEA^+]_{H2O} + [Br^-]_{H2O} \overset{K_d}{\rightleftharpoons} [TEABr]_{CHCl3}$$

at start :	0.5 M	0.5 M	0 M
at equilibrium :	0.497 M	0.497 M	8.05×10^{-5} M

$$K_d = 3.25 \times 10^{-4}\ M^{-1}.$$

The extraction was repeated with a salt concentration of 0.2 M. The volume of aqueous salt phase was 50 mL while the organic volume was kept at 500 mL.

$$[TEA^+]_{H2O} + [Br^-]_{H2O} \overset{K_d}{\rightleftharpoons} [TEABr]_{CHCl3}$$

at start :	0.2 M	0.2 M	0 M
at equilibrium :	0.199 M	0.199 M	9.13×10^{-6} M

$K_d = 2.30 \times 10^{-4}$ M^{-1}.

The extraction was repeated a second time with a salt concentration of 0.1 M. The volume of aqueous salt phase was 50 mL while the organic volume was kept at 500 mL.

$$[TEA^+]_{H2O} + [Br^-]_{H2O} \overset{K_d}{\rightleftharpoons} [TEABr]_{CHCl3}$$

at start :	0.1	0.1	0 M
at equilibrium :	0.099 M	0.099 M	1.94×10^{-6} M

$K_d = 1.94 \times 10^{-4}$ M^{-1}.

(ii) *Binding studies using water-saturated chloroform.*

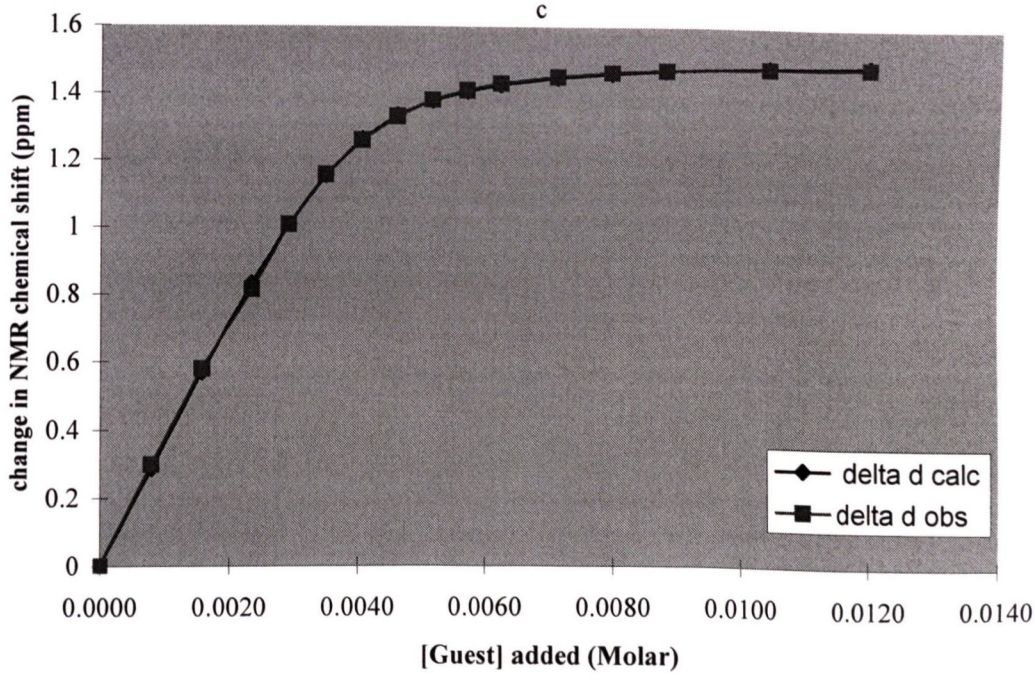
Receptor **2.33** was titrated with TBABr in water saturated chloroform and studied by ^1H NMR spectroscopy. The data for the study is given below.

Binding curve 28A. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α-NH proton was followed throughout this titration.
Solution concentrations : Host = 4.0 mM Guest = 100 mM.
Initial volume of host = 500 μL. Initial shift (δ) of the 3α-NH proton = 6.4771 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.0007937	6.7763	0.2992	0.288313
8	0.0015748	7.0560	0.5789	0.568074
12	0.0023438	7.2900	0.8129	0.831286
15	0.0029126	7.4850	1.0079	1.008143
18	0.0034749	7.6337	1.1566	1.155261
21	0.0040307	7.7372	1.2601	1.263356
24	0.0045802	7.8087	1.3316	1.334281
27	0.0051233	7.8576	1.3805	1.378893
30	0.0056604	7.8877	1.4106	1.407583
33	0.0061914	7.9084	1.4313	1.426925
38	0.0070632	7.9279	1.4508	1.447663
43	0.0079190	7.9404	1.4633	1.460629
48	0.0087591	7.9479	1.4708	1.469428
58	0.0103943	7.9555	1.4784	1.480534
68	0.0119718	7.9592	1.4821	1.487224

K_{ass} = 6,000 ± 304 M^{-1} δHG = 7.9934 ppm

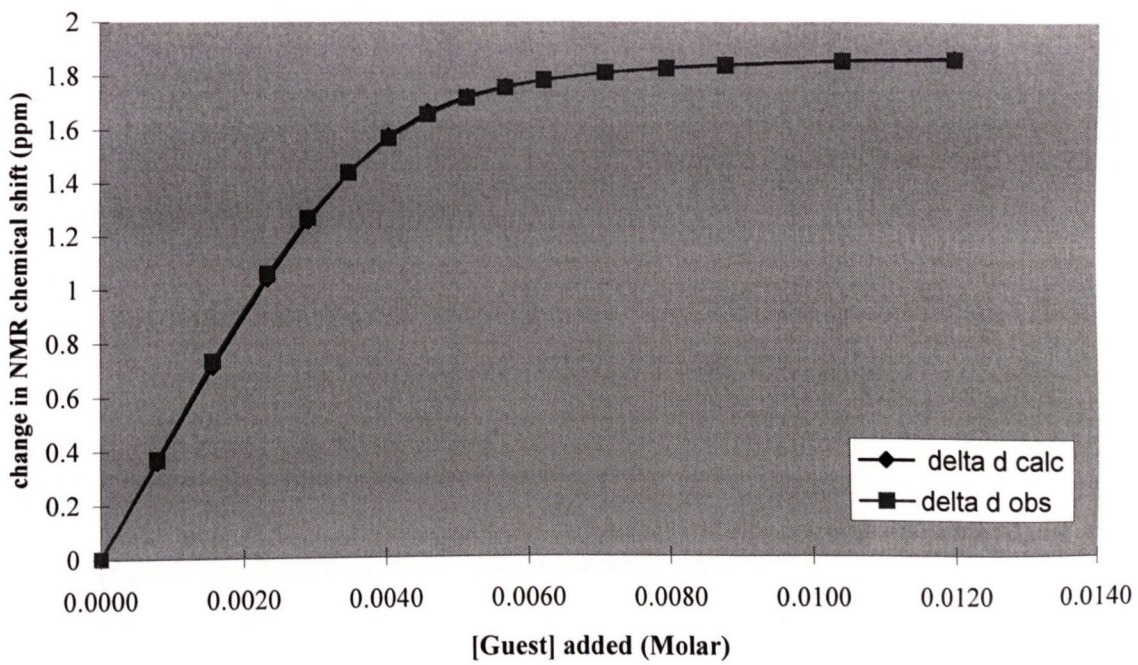


Binding curve 28B. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH proton was followed throughout this titration.
Solution concentrations : Host = 4 mM Guest = 100 mM.
Initial volume of host = 500 μL. Initial shift (δ) of the carbamate NH proton = 8.6517 ppm.

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000794	9.0205	0.3688	0.360551
8	0.001575	9.3822	0.7305	0.709951
12	0.002344	9.7091	1.0574	1.037984
15	0.002913	9.9199	1.2682	1.257879
18	0.003475	10.0905	1.4388	1.440692
21	0.004031	10.2197	1.568	1.575602
24	0.004580	10.3088	1.6571	1.665027
27	0.005123	10.3690	1.7173	1.721926
30	0.005660	10.4079	1.7562	1.758854
33	0.006191	10.4354	1.7837	1.783907
38	0.007063	10.4630	1.8113	1.810905
43	0.007919	10.4794	1.8277	1.827854
48	0.008759	10.4906	1.8389	1.839382
58	0.010394	10.5044	1.8527	1.853961
68	0.011972	10.5107	1.859	1.862758

K_{ass} = 5,700 ± 301 M^{-1} δHG = 10.5528 ppm

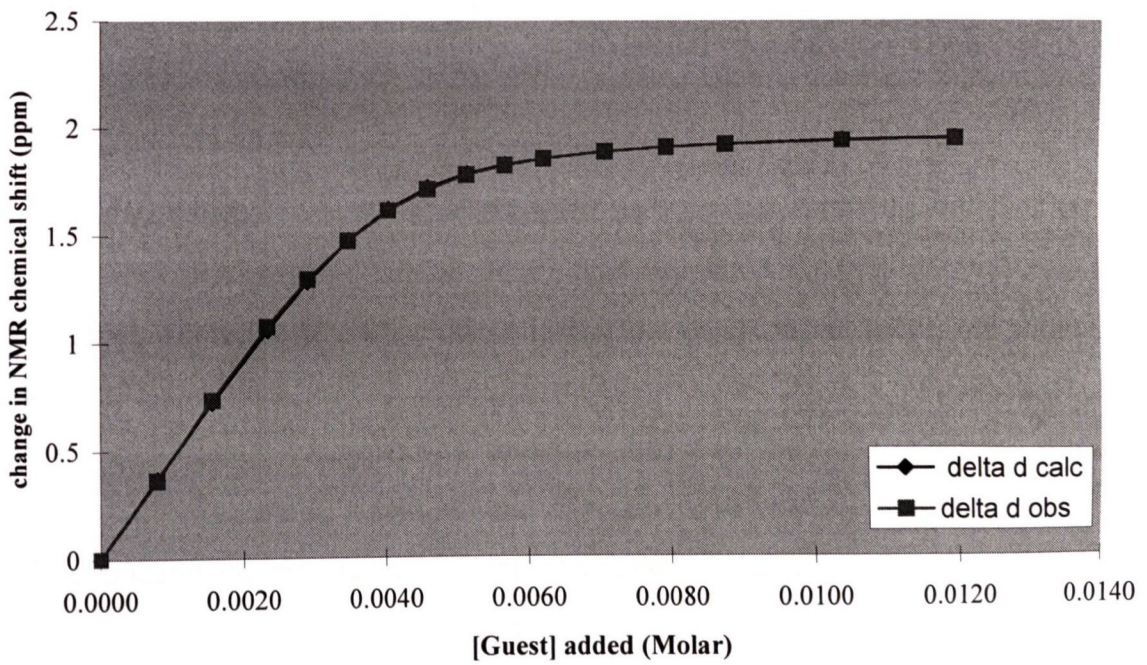


Binding curve 28C. NMR Binding Study of Association of Receptor **2.33** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carabamte NH proton was followed throughout this titration.
Solution concentrations : Host = 4 mM Guest = 100 mM.
Initial volume of host = 500 μL. Initial shift (δ) of the carbamate NH proton = 8.9088 ppm.

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
4	0.000794	9.2713	0.3625	0.366994
8	0.001575	9.6414	0.7326	0.722828
12	0.002344	9.9813	1.0725	1.057788
15	0.002913	10.2021	1.2933	1.284059
18	0.003475	10.3828	1.474	1.475246
21	0.004031	10.5245	1.6157	1.620213
24	0.004580	10.6224	1.7136	1.719135
27	0.005123	10.6888	1.78	1.783312
30	0.005660	10.7327	1.8239	1.825323
33	0.006191	10.7616	1.8528	1.853890
38	0.007063	10.7930	1.8842	1.884642
43	0.007919	10.8130	1.9042	1.903887
48	0.008759	10.8256	1.9168	1.916935
58	0.010394	10.8419	1.9331	1.933374
68	0.011972	10.8507	1.9419	1.943253

K_{ass} = 5,400 ± 178 M^{-1} δHG = 10.8947 ppm



During the titration study, the 3, 7 and 12α-NH protons were observable throughout the experiment. Analysis of the three signals gave K_{ass} values in good agreement with each other (K_{ass} for the 3α-NH and the carbamate NH protons was 6,000 ± 304, 5,700 ± 301 and 5,400 ± 178 M^{-1} respectively). The carbamate NH in binding curve 28C provided the most accurate K_{ass} of the signals examined. From the ^1H NMR spectrum of the pure host at the start of the binding study, integration of the water signal against the host signal gives an indication of the amount of water dissolved in the chloroform. There appeared to be a consistent amount of water dissolved in the chloroform in all the ^1H NMR titration studies (27 molar equivalents to every mole of host). The host was found to undergo similar changes in shift as when the titration was done in dry chloroform however, as expected the degree of shift was less. This considerable reduction in binding strength is again due to solvation of the anion and the receptors binding site by the dissolved water in the chloroform.

Receptor **2.33** was tested in an extraction experiment of TEABr according to the conditions below. Integration of the salt to the complex in the ^1H NMR spectrum shows that 27.70 mol % of TEABr was extracted into the chloroform by **2.33**.

$$[H]_{CHCl3} + [TEA^+]_{H2O} + [Br^-]_{H2O} \overset{K_e}{\rightleftharpoons} [TEA^+HBr^-]_{CHCl3} \quad (1)$$

start :	0.01 M	0.5 M	0.5 M	0 M
at equilibrium :	0.00708 M	0.49708 M	0.49708 M	0.00292 M

Using equation (**2**) gives $K_e = 1.67\ M^{-2}$.

Rearrangement of equation (**3**) provides K_d as :

$K_d = K_e/K_{ass} = 1.55 / 5{,}400$

$$K_d = 3.1 \times 10^{-4}\ M^{-1}.$$

The extraction was repeated twice at the same host and guest concentrations and volumes giving

$$K_e = 1.55\ M^{-2} \text{ and } K_d = 2.87 \times 10^{-4}\ M^{-1}$$

$$\text{and } K_e = 1.43\ M^{-2} \text{ and } K_d = 2.65 \times 10^{-4}\ M^{-1}$$

The extraction was repeated a third time at a guest concentration of 0.4 M giving

$$K_e = 1.83\ M^{-2} \text{ and } K_d = 3.4 \times 10^{-4}\ M^{-1}$$

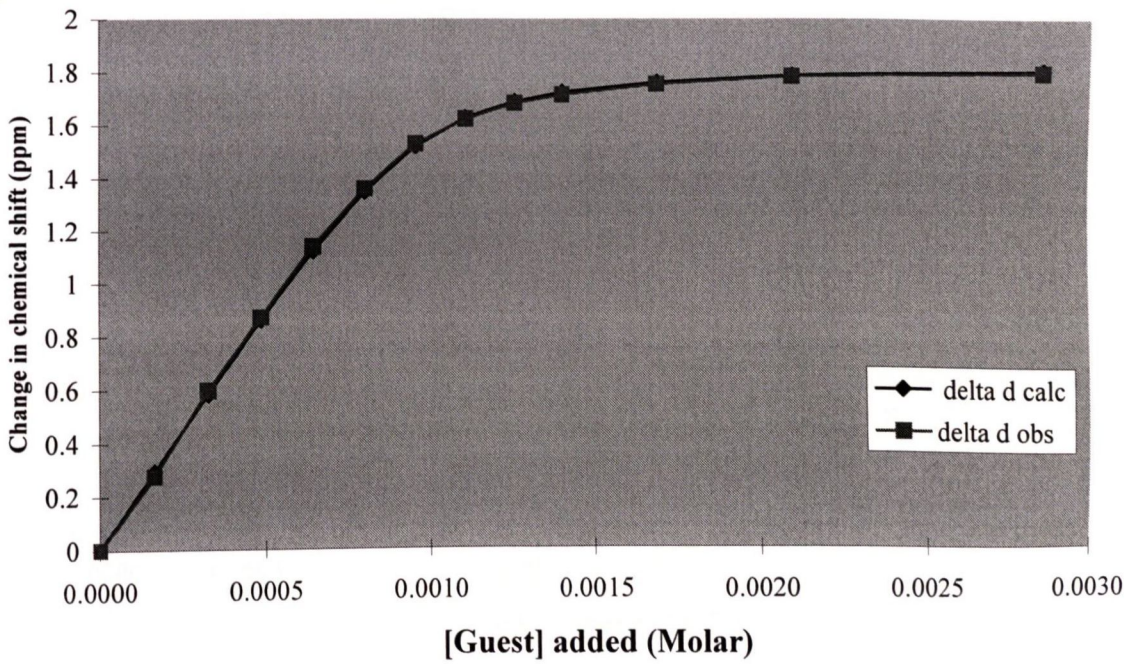
Receptor **2.26** was titrated with TBABr in water saturated chloroform and studied by ^1H NMR spectroscopy. The data for the study is given below.

Binding curve 29A. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of 3α-NH proton was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 20 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the 3α-NH proton = 6.5700 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000165	6.8465	0.2765	0.293755
10	0.000328	7.1664	0.5964	0.582119
15	0.000488	7.4411	0.8711	0.861058
20	0.000645	7.7096	1.1396	1.122346
25	0.000800	7.9300	1.36	1.349796
30	0.000952	8.1000	1.53	1.520722
35	0.001102	8.1938	1.6238	1.627274
40	0.001250	8.2527	1.6827	1.687666
45	0.001395	8.2841	1.7141	1.722861
55	0.001679	8.3230	1.753	1.759755
70	0.002090	8.3518	1.7818	1.784244
100	0.002857	8.3656	1.7956	1.803232

K_{ass} = 30,000 ± 2,868 M^{-1} δHG = 8.4031 ppm

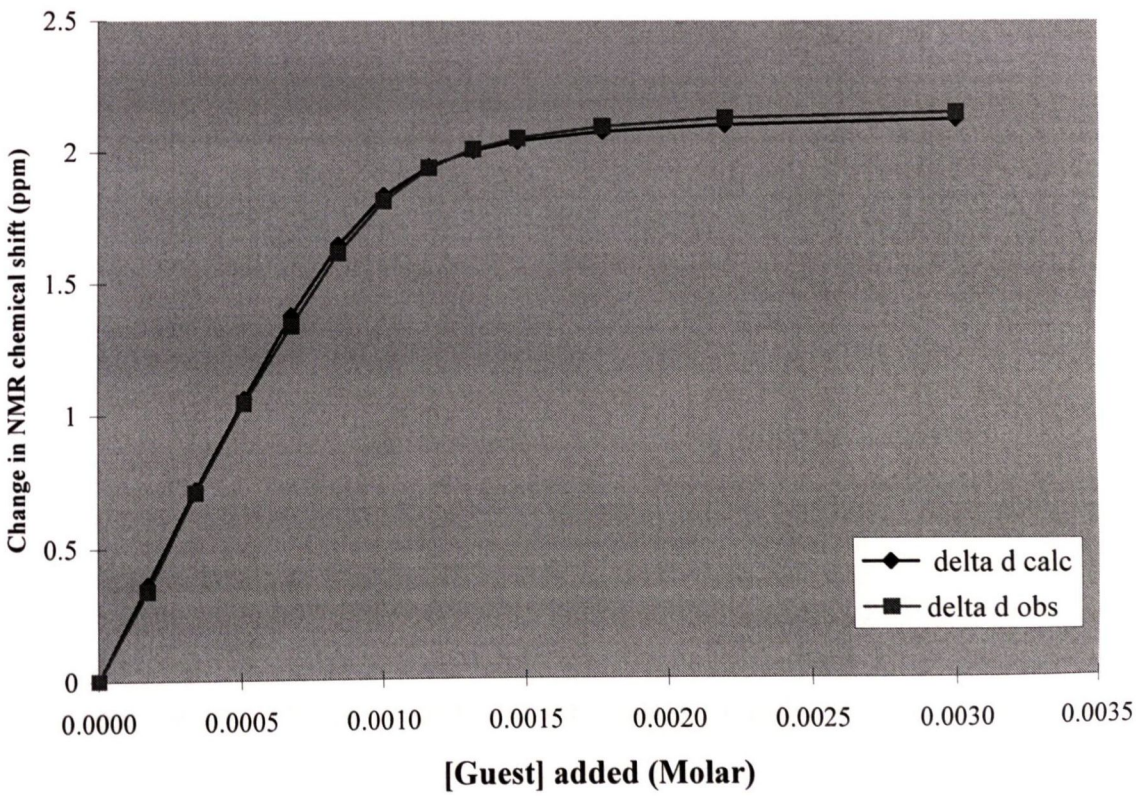


Binding curve 29B. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of a carbamate NH proton was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 20 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the carbamate NH proton = 7.4386 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000174	7.7773	0.3387	0.362894
10	0.000344	8.1461	0.7075	0.718600
15	0.000512	8.4836	1.045	1.061304
20	0.000677	8.7783	1.3397	1.378677
25	0.000840	9.0556	1.617	1.646438
30	0.001000	9.2538	1.8152	1.835254
35	0.001157	9.3792	1.9406	1.945253
40	0.001313	9.4495	2.0109	2.005824
45	0.001465	9.4921	2.0535	2.041172
55	0.001763	9.5360	2.0974	2.078795
70	0.002194	9.5686	2.13	2.104354
100	0.003000	9.5887	2.1501	2.124622

K_{ass} = 30,000 ± 5,950 M^{-1} δHG = 9.5961 ppm

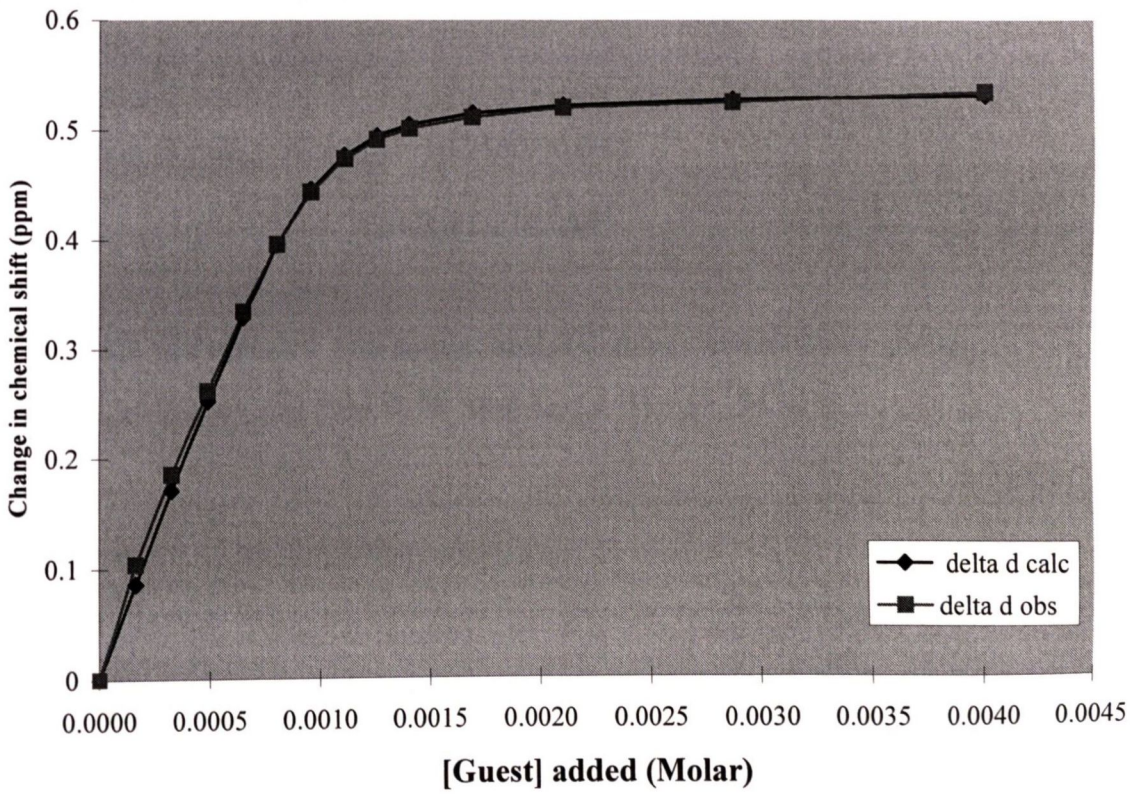


Binding curve 29C. NMR Binding Study of Association of Receptor **2.26** with Tetrabutylammonium bromide.

Wet conditions were used.
NMR used : DPX 400 at 303 K.
The chemical shift of an aryl proton *ortho* to the NH carbamate group was followed throughout this titration.
Solution concentrations : Host = 1.0 mM Guest = 20 mM.
Initial volume of host = 600 μL. Initial shift (δ) of the *o*-aromatic proton = 7.6130 ppm

μL of guest added	[Guest] added	Observed shift (ppm)	Change in shift relative to δ (ppm)	Computer calculated change in shift (ppm)
5	0.000165	7.7171	0.1041	0.086078
10	0.000328	7.7986	0.1856	0.170621
15	0.000488	7.8752	0.2622	0.252470
20	0.000645	7.9479	0.3349	0.329241
25	0.000800	8.0094	0.3964	0.396166
30	0.000952	8.0571	0.4441	0.446412
35	0.001102	8.0872	0.4742	0.477527
40	0.001250	8.1047	0.4917	0.495001
45	0.001395	8.1148	0.5018	0.505113
55	0.001679	8.1248	0.5118	0.515656
70	0.002090	8.1336	0.5206	0.522626
100	0.002857	8.1386	0.5256	0.528018
150	0.004000	8.1474	0.5344	0.531137

K_{ass} = 31,200 ± 5,490 M^{-1} δHG = 8.1495 ppm



Receptor **2.26** showed appreciable binding of TBABr even in water saturated chloroform as demonstrated above. In two of the cases above, the 3α-NH and the carbamate NH protons were observable throughout the titration giving a K_{ass} of 30,000 ± 2,868 and 30,000 ± 5,950 M^{-1} respectively. An aryl proton *ortho* to the NH carbamate group was also followed throughout the titration giving a K_{ass} of 31,200 ± 5,490 M^{-1}. The three signals were in close agreement and provided a good measure of their accuracy. The 3α-NH proton signal provided the most accurate value for K_{ass}. The ^1H NMR spectrum of the pure host showed 27-30 molar equivalents of dissolved water present in the $CDCl_3$. As in the case of receptor **2.33**, the K_{ass} value was considerably reduced in water-saturated chloroform from that in dry chloroform, due to solvation of the anion and the host binding site by the dissolved water present.

Extraction experiments on **2.26** were performed according to the condition given below: 1 mL of host (0.01 M) and 1 mL of TEABr (0.2 M) was used in the extraction. The ^1H NMR spectrum shows that 28.50 mol % of TEABr was extracted into the chloroform by **2.26**.

$$[H]_{CHCl3} + [TEA^+]_{H2O} + [Br^-]_{H2O} \overset{K_e}{\rightleftharpoons} [TEA^+HBr^-]_{CHCl3}$$

at start :	0.01 M	0.2 M	0.2 M	0 M
at equilibrium :	0.00715 M	0.19715 M	0.19715 M	0.00285 M

giving $K_e = 10.2550\ M^{-2}$.

From equation (**3**) : $K_d = K_e / K_{ass} = 10.2550/30,000$

$$K_d = 3.42 \times 10^{-4}\ M^{-1}.$$

The extraction was repeated with similar host and guest concentrations giving :

$$K_e = 9.151\ M^{-2} \text{ and } K_d = 3.05 \times 10^{-4}\ M^{-1}.$$

The results obtained for K_d using the two procedures described, for both TEACl and TEABr are summarised in Table 4.5. An average value of K_d for TEACl and TEABr was calculated using both receptor results along with both techniques (1.055×10^{-5} M^{-1} and 2.887×10^{-4} M^{-1} respectively) for the water/chloroform biphasic mixture used in this thesis.

Table 4.5

	K_d (M^{-1})			
Technique	**K_d (M^{-1})**	**[Et_4NCl]**	**K_d (M^{-1})**	**[Et_4NBr]**
Partition experiment	1.05×10^{-5}	0.5 M	3.25×10^{-4}	0.5 M
	8.30×10^{-6}	0.5 M	2.30×10^{-4}	0.2 M
			1.94×10^{-4}	0.1 M
Binding studies + extractions				
Receptor 2.33	1.05×10^{-5}	0.5 M	2.87×10^{-4}	0.5 M
	9.6×10^{-6}	0.5 M	2.65×10^{-4}	0.5 M
			3.10×10^{-4}	0.5 M
			3.40×10^{-4}	0.4 M
Receptor 2.26	1.26×10^{-5}	0.5 M	3.40×10^{-4}	0.2 M
	1.18×10^{-5}	0.5 M	3.05×10^{-4}	0.2 M
Overall average K_d :	**1.055×10^{-5}**		**2.887×10^{-4}**	

The above values for K_d were used to calculate association constants K_{ass} corresponding to the K_e values in Table 4.4. These figures, and the results of additional extraction experiments are discussed below

Receptor **2.23** was found, on examination by this extraction technique, to bind chloride and bromide anions moderately strongly. The receptor was found to extract 22.1 mol % of TEACl from an aqueous phase of 0.5 M concentration into the chloroform phase. According to equation (**2**), K_e was calculated as 1.145 M^{-2}. The binding constant K_{ass} was derived from equation (**5**) as 109,000 M^{-1}. The receptor was also found to extract 47.1 mol % of TEABr from an aqueous phase of 0.2 M concentration. In this case K_{ass} was calculated at 81,000 M^{-1}.

Table 4.6 Results of extractions for receptor 2.23.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.23	0.221	0.01	0.5	1.1450	109,00	Cl^-
2.23	0.471	0.01	0.2	23.3456	81,000	Br^-

Receptor **2.24** showed a more efficient extraction of both salts into the chloroform phase. The experiments for both anions required a significant decrease in salt concentration in the aqueous phase to attain a reasonable R value ($0.05 < R > 0.7$). **2.24** was found to extract 4.06 mol % of TEACl from an aqueous phase of 0.1 M concentration, giving a K_e of 69.4450 M^{-2} and a K_{ass} of 6,582,000 M^{-1}. The receptor was also found to extract 46 mol % of TEABr from an aqueous phase of 0.025 M concentration. In this case K_e was calculated as 1497.5710 M^{-2} and a K_{ass} of 5,187,000 M^{-1}. This large increase in binding for both anions was due to replacement of a 3α-tosyl group for a more acidic 3α-nosyl group rendering the NH α to the group more available for hydrogen bonding, even in water-saturated chloroform.

Table 4.7 Results of extractions for receptor 2.24.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.24	0.4059	0.01	0.1	69.4450	6,582,000	Cl^-
	0.46	0.01	0.025	1497.5710	5,187,000	Br^-

Receptor **2.25** was shown to be less potent at anion extraction than **2.24** by requiring a more concentrated salt aqueous phase for quantitative extraction determination. The receptor was found to extract 60 mol % of TEACl from an aqueous phase of 0.2 M concentration and 51 mol % of TEABr from an aqueous phase of 0.05 M concentration giving K_{ass} values for TEACl and TEABr of 3,605,000 M^{-1} and 1,537,000 M^{-1} respectively. This poorer binding shown by receptor **2.25** compared to **2.24** was due to the intramolecular hydrogen bonding of the *ortho*-NO_2 group on the 3α-sulfonamido aromatic ring to the 3α-NH proton significantly depreciating the anion binding strength of the cavity.

Table 4.8 Results of extractions for receptor 2.25.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.25	0.6038	0.01	0.2	40.5084	3,840,000	**Cl^-**
	0.573	0.01	0.2	35.5561	3,370,000	
	0.5153	0.01	0.05	448.0430	1,552,000	**Br^-**
	0.5106	0.01	0.05	439.4808	1,522,000	

Receptor **2.28** was shown to be one of the weakest anion binding receptors in this series. The receptor required very high salt concentrations in the aqueous phase to show appreciable extraction mole ratios. From a TEACl solution (2 M), the receptor extracted 19 mol.% of the salt into the chloroform phase giving a K_e of 0.0589 M^{-2} and a K_{ass} of 5,600 M^{-1}. The receptor extracted 19.3 mol % of TEABr from an aqueous phase of 0.5 M concentration giving a K_e of 0.9641 M^{-2} and a K_{ass} of 3,300 M^{-1}. This poor performance shown by **2.28** at anion binding and extraction is most likely due to the back-bonding (π-donation) of the fluorine substituent to the aromatic ring rendering the carbamate NH protons less acidic than in the case of **2.24** where the fluorine substituents are unable to π-donate to the aromatic ring.

Table 4.9 Results of extractions for receptor 2.28.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.28	0.19	0.01	2.0	0.0589	5,600	**Cl^-**
	0.193	0.01	0.5	0.9641	3,300	**Br^-**

Receptor **2.34** was shown in the last section to be the most powerful anion binder in this series. In TEACl and TEABr extraction experiments the receptor showed exceptional binding strength. A number of extractions were performed on **2.34** for TEACl at various host and guest concentrations. A number of extractions were also done for different concentrations of TEABr. The results are summarised in Table 4.10. From Table 4.10 above for chloride extraction, varying the aqueous phase salt concentration has a large effect on the amount of salt extracted into the chloroform phase. However, it does not have a dramatic effect on the K_e and K_{ass} values calculated according to the mathematical model used. Similarly, varying the host concentration in the chloroform phase has only a slight effect on the K_e and K_{ass} values calculated. From these details we can confirm that:

1. host association *e.g.* dimerisation is not a significant contributing factor in the K_{ass} calculation.
2. The salts TEACl and TEABr are completely monomeric and dissociated in the aqueous phase and tightly associated to the receptor in the chloroform phase at the concentrations used in Table 4.10.
3. The host is binding the anion in a strict stoichiometry, most likely 1:1 as confirmed from binding studies in the previous chapter and TMA salt solubilisation studies in the previous section.

The K_{ass} values quoted in Table 4.10 are by far the largest quoted to date in the literature for chloride and bromide complexation by neutral organic anionophores.

Table 4.10 Extraction results of 2.34 with TEACl and TEABr at various host and guest concentrations.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.34	0.785	0.01	0.1	429.9723	40,756,000	Cl^-
	0.47	0.01	0.05	390.5676	37,020,000	
	0.2957	0.01	0.0333	401.2515	35,431,000	
	0.18375	0.01	0.025	373.7945	38,033,000	
	0.0584	0.01	0.0125	401.6186	38,068,000	
	0.136	0.005	0.02	398.9255	37,813,000	
	0.1445	0.0025	0.02	425.3352	40,316,000	
2.34	0.52	0.01	0.0125	7714.8130	21,642,000	Br^-
	0.367	0.01	0.01	6247.9740	20,126,000	
	0.276	0.01	0.00833	5810.3260	26,723,000	

Receptor **2.35** underwent extractions as shown in Table 4.11. The K_e and K_{ass} values shown although large, were not as large as those shown by receptor **2.34**. **2.35** was

Table 4.11 Extraction results for receptor 2.35.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.35	0.188	0.01	0.05	94.0196	8,912,000	Cl^-
	0.1853	0.01	0.05	92.3421	8,753,000	
	0.102	0.01	0.01	1159.3880	4,016,000	Br^-
	0.094	0.01	0.01	1057.3120	3,662,000	

Receptor **2.36** was shown to be a moderate chloride and bromide binder in table 4.12. shown to be the second most potent receptor for chloride and bromide complexation in the series. The strength of anion binding shown by this receptor is due to the high acidity of the carbamate NH protons along with the acidic 3α-sulfonamido NH proton. The lack of potency when compared to **2.34** is due to an *intra* molecular hydrogen bond of the *ortho*-nitro group on the 3α aromatic ring to the NH proton spatially close to it, disrupting the binding cavity for anion inclusion. This disruption in the binding site is transcribed in the association constant K_{ass} of 8.8 x 10^6 M^{-1} for TEACl and 3.8 x 10^6 M^{-1} for TEABr.

Table 4.12 Extraction results for receptor 2.36.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
2.36	0.4405	0.01	0.5	3.2055	304,000	Cl^-
	0.441	0.01	0.5	3.2120	304,000	
	0.5384	0.01	0.2	30.7951	107,000	Br^-
	0.536	0.01	0.2	30.4918	106,000	
	0.2437	0.01	0.1	33.0225	114,000	

The moderate extraction efficiency of **2.36** is due mainly to two reasons :

1. The *intra* molecular hydrogen bond of the *ortho*-nitro group to the 3α-sulfonamido NH proton as in receptor **2.36** disrupting the binding site for anion complexation.
2. The carbamate groups used in this receptor are not strong electron withdrawing substituents and so do not render the NH protons very acidic and hence less available for hydrogen bonding.

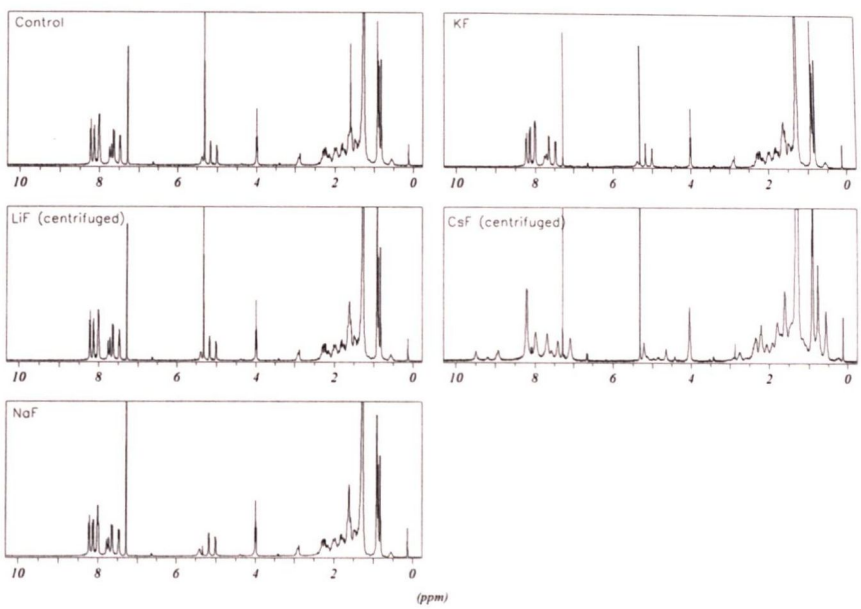
c) Solubilisation of alkali earth salts in chloroform in the presence of 2.34.

From the results of the first section in this chapter on TMA salt solubility tests in chloroform in the presence of receptor **2.34**, it was felt that the receptor may be able to solubilise some hydrophilic salts such as NaCl in chloroform. A range of hydrophilic salts were investigated for their solubility in chloroform in the presence of receptor **2.34** and also in its absence. The solutions were examined firstly by the change in the receptors' ^1H NMR spectra upon addition of the salt to the host solution.

Procedure

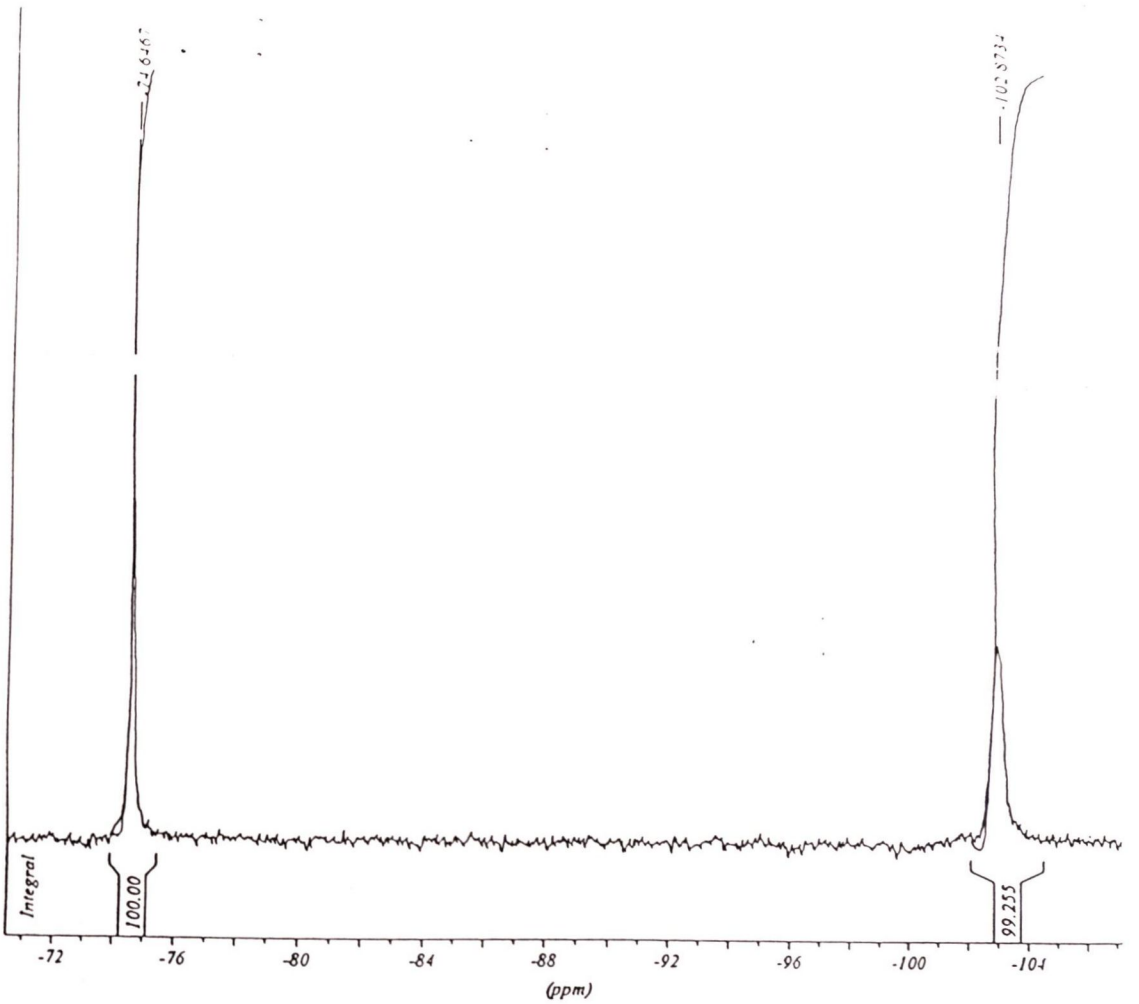
The alkali earth halide (approximately 0.06 mmol) was sonicated in $CDCl_3$ (600 μL) with **2.34** (0.035 mmol) for 2 hours in a 1 mL micro-tube. The solution was then allowed to settle for 15 minutes outside the sonic bath. The solution was then filtered through a hydrophobic PTFE filtered tip syringe (0.45 μm pore size). The syringe was further rinsed with $CDCl_3$ (500 μL) to ensure all of the complex was removed from the syringe and the samples were analysed for changes in the hosts' ^1H NMR spectrum. The salts examined were divided according to the anion present.

Figure 4.1 ^1H NMR spectra of the free host 2.34 (control) and the host having undergone various salt extractions, *i.e.* LiF, NaF, KF, and CsF.



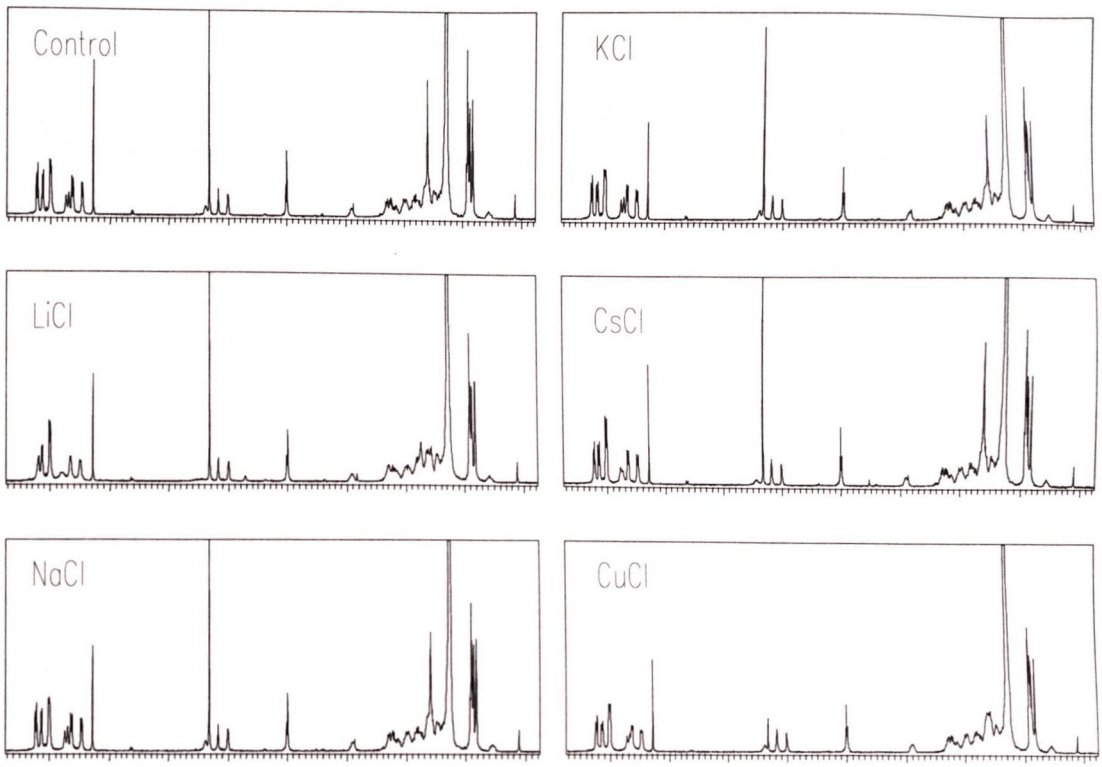
From the spectra shown the receptor **2.34** underwent no significant changes on addition of LiF, NaF and KF, but on addition of CsF, the receptor underwent considerable changes in its ^1H NMR spectrum. The 3, 7 and 12α-NH protons all shifted downfield on addition of the salt (the 3 and one carbamate-NH proton moved from 5.4 and 7.75 to 745 and 8.95 ppm respectively, the initial shift of the other carabamate NH proton could not be determined). The aromatic proton signals of the host broadened in nature, also indicative of anion binding, hence salt solubilisation in chloroform. The salt solubility was confirmed by fluorine spectroscopy shown below.

Figure 4.2 Fluorine NMR spectrum of 2.34 with cesium fluoride and trifluoroacetic acid added to standardise the integration of the salt dissolved.



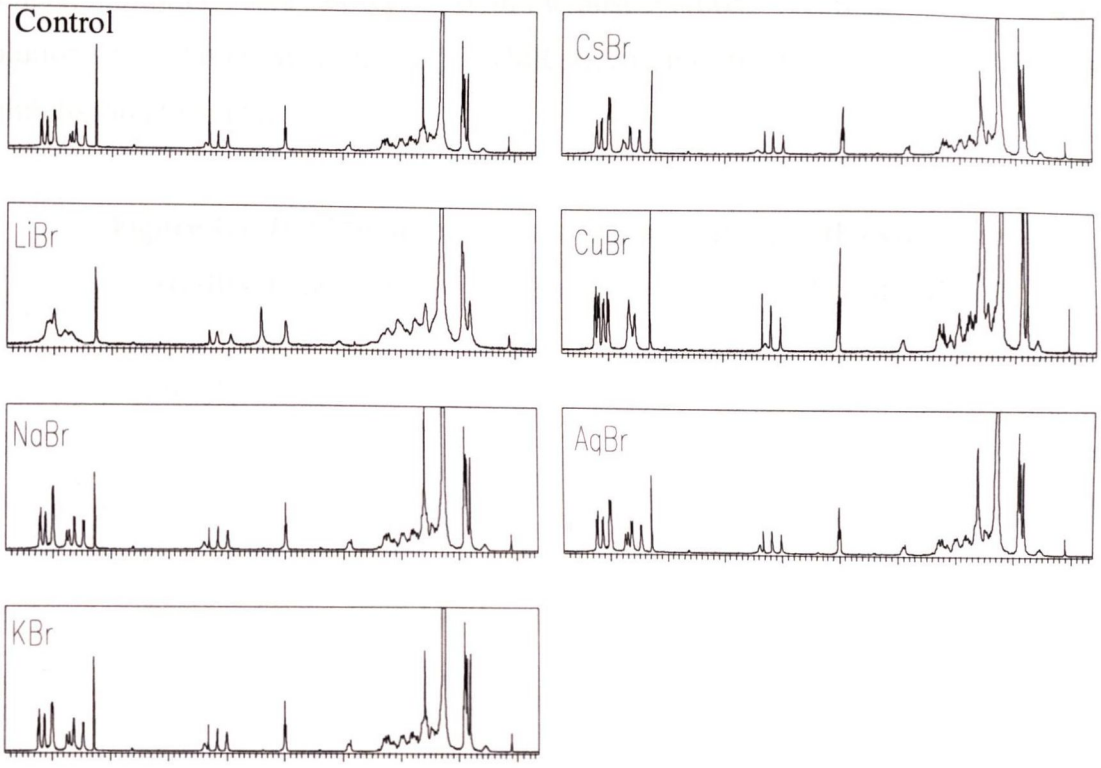
The fluorine spectrum shown above shows the ^{19}F signal of CsF at -102.87 ppm and a known concentration of trifluoroacetic acid at -74.65 ppm. The integration of the two signals showed that 87 mol % of CsF dissolved in the chloroform in the presence of the receptor. Addition of the standard, TFA caused no changes in the fluorine NMR spectrum of CsF. A control experiment was done by performing an extraction of CsF in the absence of **2.34** and obtaining a ^{19}F spectrum. The spectrum showed no signal, and therefore no sign of salt solubilisation in this case.

Figure 4.3 ^{1}H NMR spectra of the free host 2.34, with the results of solid extraction of LiCl, NaCl, CsCl and CuCl salts.



In the case of LiCl extraction, the host NH protons all broadened but did not undergo any change in chemical shift. The spectrum suggests that only a small amount, if any of the salt dissolved in the presence of the receptor **2.34**. In the cases of NaCl and KCl, the ^{1}H NMR spectra of the host showed no changes on addition of the salt, suggesting that no extraction was occurring. In the case of CsCl, the ^{1}H NMR spectrum was broad, but again no sign of any chemical shift movement of any of the hosts protons suggested minimal anion binding or salt solubilisation. On addition of CuCl to the host solution the solution changed from a pale yellow colour to green, even after the solution was centrifuged. The host NH protons were all broader than those in the free host, but similar in chemical shift suggesting minimal anion binding.

Figure 4.4 ^{1}H NMR spectra of the free host 2.34 along with extraction studies with LiBr, NaBr, KBr, CsBr, CuBr and AgBr.



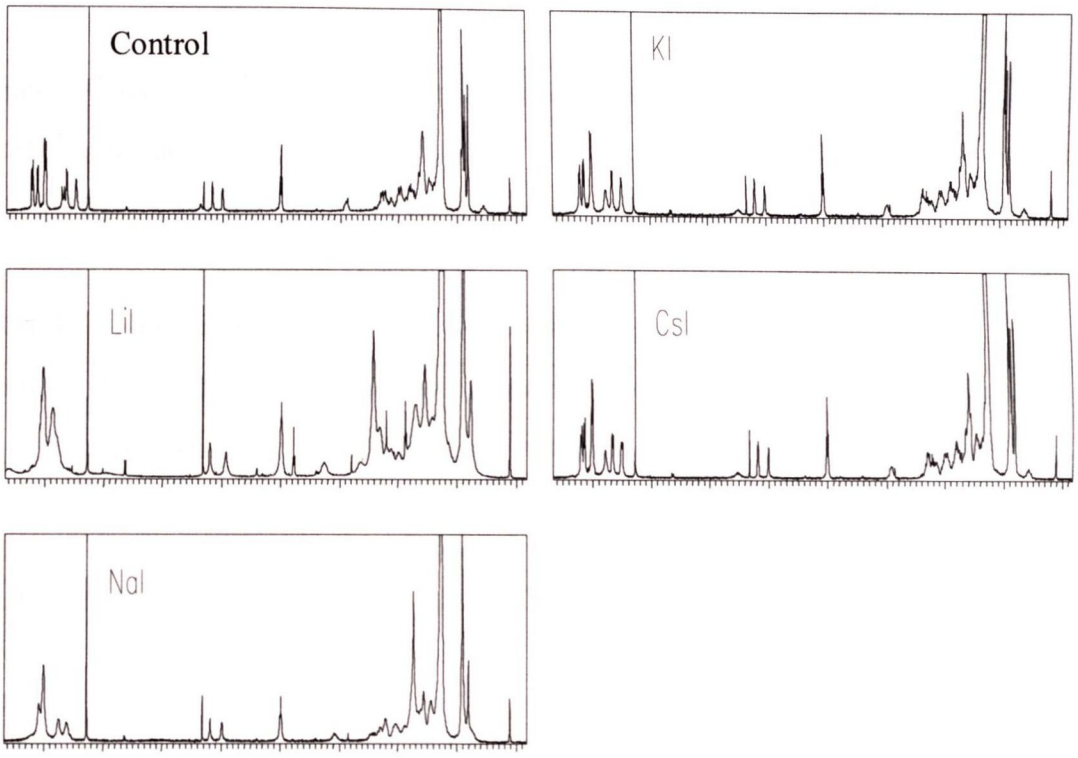
On addition of LiBr to the host solution and sonication, the solution formed a gelatinous precipitate at the top of the solution. A small amount of solid remained in the bottom of the tube. The gel was sufficiently solid to prevent the passage of the liquid at the bottom of the micro-test tube when inverted. Deviating from the normal procedure, further chloroform (1 mL) was added causing the gel to dissolve. The mixture was centrifuged and filtered to remove solids in the liquid. The solution was re-evaporated to dryness and then re-dissolved in $CDCl_3$ (600 μL). The solution was found to solidify again over a 2 hour period. The spectrum showed that the signals of the host were all broadened in nature, particularly the 3α-NH proton, which disappeared from the spectrum, either becoming broad or, moving under the aromatic signals.

In the case of NaBr and KBr extractions, the host underwent no changes in its ^{1}H NMR spectrum indicating no sign of salt solubilisation taking place.

On addition of CsBr salt to the host solution, the NH protons of **2.34** broadened in nature but did not shift downfield. The aromatic proton signals also broadened to some degree indicating that a small amount of anion binding was taking place. On addition of CuBr, the colour went from a pale yellow to black. ^{1}H NMR analysis showed no change in the

3α-NH proton however, the 7 and 12α-NH protons clearly shifted downfield under the aromatic proton signals. The black colour persisted upon centrifugating and filtering the sample, indicating some salt solubility in the host solution. Addition of AgBr to a host solution showed no changes in the ^{1}H NMR spectrum of the host suggesting that no salt solubilisation took place.

Figure 4.5 ^{1}H NMR spectra of the free host along with extraction studies done with 2.34 with the salts LiI, NaI, KI, and CsI.



Addition of LiI to the host solution brought about similar actions as LiBr in the mixture *i.e.* Formation of a gel. Again the solution was re-dissolved in chloroform (1 mL), then centrifuged and filtered to remove solids in the liquid. The solution was re-evaporated to dryness and then re-dissolved in $CDCl_3$ (600 μL). The spectrum of the host was very broad. One of the NH protons of the host was observable far downfield indicative of anion complexation. None of the other NH protons could be observed upon extraction. On addition of NaI to the host solution, the NH protons broadened into the baseline and were not visible in the spectrum. The aromatic proton signals were also broad in nature suggesting some anion binding was taken place. The addition of KI to the host solution showed only minor changes to the host spectrum, namely NH broadening. Similarly, the

addition of CsI produced NH broadening of the hosts' ^{1}H NMR spectrum, however, the addition of CuI produced no changes in the hosts' ^{1}H spectrum.

The amount of salt extracted into the liquid phase in the presence of receptor **2.34** was quantified by ICP-AE analysis of the countercations.

Procedure

The salt (0.06 mmol) was sonicated in $CHCl_3$ (600 μL) in the presence of the receptor **2.34** (0.06 g, 0.05 mmol) for 2 hours. The solution was then filtered through a hydrophobic PTFE filter tip syringe (0.45 μm pore size). The syringe was further rinsed with $CHCl_3$ (500 μL) to recover the complex fully and the filtrate was then evaporated to dryness. Conc. HCl (1 mL) was then added to the complex followed by H_20 (9 mL) and glacial acetic acid (10 mL) to solubilise the mixture fully. The samples were then analysed by ICP-atomic emission spectroscopy. The results are given in the table below.

Table 4.13 Results from salt extraction by ICP Analysis.

Salt	Control (ppm)	Solubilised salt concentration (ppm) for 1mL sample	mol % extraction of guest to host
LiCl	0.42	4.2	0.12
LiBr	0	36.1	10.4
LiI	0.16	29.6	8.5
NaF	0	8.4	0.73
NaCl	0	4.7	0.41
NaBr	0	4.4	0.38
NaI	0	61.4	5.3
CuCl	3.09	53.0	1.72
CuI	0.03	0.63	0.02

In the cases where a gel was formed, excess chloroform was added to redissolve the solid. The solution was then filtered, evaporated to dryness and re-dissolved according to the procedure above. Control experiments involved extraction of the salts into chloroform in the absence of **2.34**. Analysis of the solvent mixture gave background values of 0.32 ppm for Cu samples, 0 ppm for Na samples and 0 ppm for Li samples. (Background values were taken into account in calculating the percentage mole extraction of salt)

One can see from Table 4.13 the significant extraction of LiBr and LiI by receptor **2.34** compared to the other salts examined. This result is confirmed by the significant changes in the host ^{1}H NMR spectra in the presence of these salts. A smaller amount of the sodium salts was also extracted into the liquid phase. The colour change of the host on

addition of CuCl was due to the small percentage extraction of the salt into the chloroform in the presence of the receptor. Potassium salts were not determined due to argon interference bands on either side of the K wavelength. Detection was tried at a number of different wavelengths without success.

[1] Hofmeister, F. *Arch. Exp. Pathol. Pharmakol.* **1888**, *24*, 247.

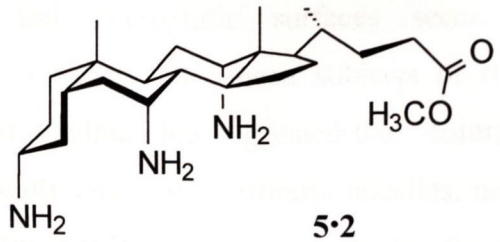
[2] Visser, H.C.; Rudkevich, D.M.; Verboom, W.; de Jong, F.; Reinhoudt, D.N. *J. Am. Chem. Soc.*, **1994**, *116*, 11554 and references therin.

[3] (a) Kyba, E. P.; Helgeson, R. C.; Madan, K.; Gokel, G. W.; Tarnowski, T. L.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.*, **1977**, *99*, 2564. (b) Timko, J. M.; Moore, S. S.; Walba, D. M.; Hiberty, P. C.; Cram, D. J. *J. Am. Chem. Soc.*, **1977**, *99*, 4207. (c) Newcomb, M.; Timko, J. M.; Walba, D. N.; Cram, D. J. *J. Am. Chem. Soc.*, **1977**, *99*, 6392. (d) Moore, S. S.; Tarnowski, T. L.; Newcomb, M.; Cram, D. J. *J. Am. Chem. Soc.*, **1977**, *99*, 6398. (e) Newcomb, M.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.*, **1977**, *99*, 6405. (f) Koenig, K. E.; Lein, G. M.; Stuckler, P.; Kaneda, T.; Cram, D. J. *J. Am. Chem. Soc.*, **1979**, *101*, 3553.

[4] Gennari, C.; Gude, M.; Potenza, D.; Piarulli, U. *Chem. Eur. J.* **1998**, *4*, 1924.

[5] Triona Walshe, personnal communication.

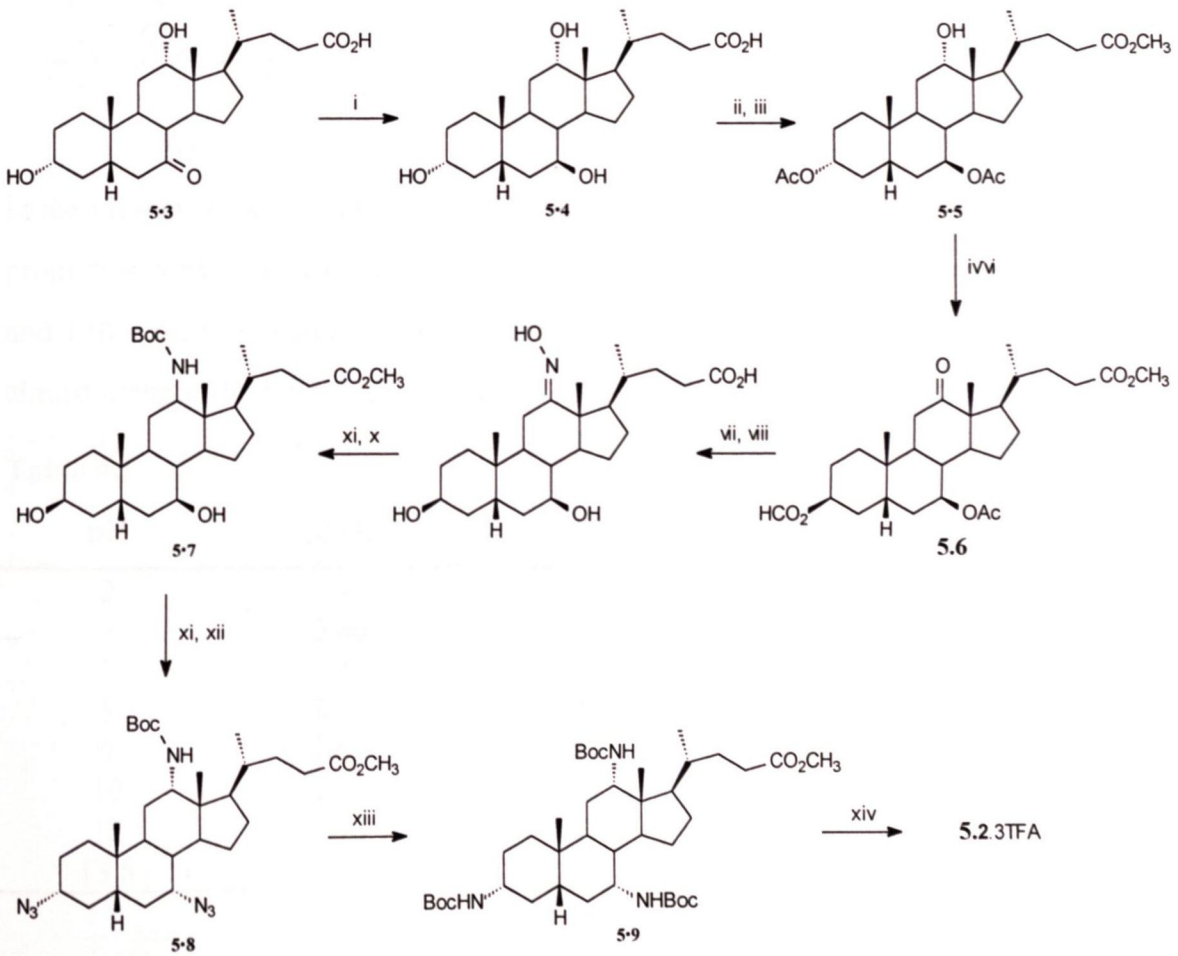
Chapter Five : Discussion of the *tris*-amino analogue of methyl cholate.



5·2

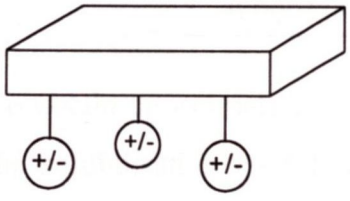
As exemplified in the previous chapters, cholic acid and its deoxy analogues have found various uses in supramolecular chemistry. In many of these applications there are significant advantages in the replacement of the hydroxyl by amino functionality. Amino groups can be derivatised rapidly and quantitatively while retaining hydrogen bond donor capabilities. With this in mind, Robert Williams, a former member of this group,[3] developed a synthesis of **5.2**, the *tris*-amino analogue of methyl cholate as shown in Scheme 1. The availability of **5.2** presented a number of interesting opportunities.

Scheme 5.1



Scheme 5.1. Reagents and conditions : i, K, *tert*-amyl alcohol, reflux; ii, MeOH, H_2SO_4; iii, Ac_2O, py, room temp., 8 h; iv, $Na_2Cr_2O_7$, AcOH; v, MeOH, AcCl; vi, Ph_3P, DEAD, HCO_2H, THF; vii, H_2NOH, NaOAc, MeOH; viii, NaOH, MeOH; ix, Na, EtOH, then $(Boc)_2O$; x, CsF, MeI, DMF; xi, MsCl, cat. Et_3N, CH_2Cl_2; xii, NaN_3, DMPU, 80 °C, 10 d; xiii, PtO_2, H_2, $(Boc)_2O$; xiv TFA, CH_2Cl_2.

For example, the potential of **5.2** in acid conditions as a "facial amphiphile", with enforced hydrophobic and hydrophilic surfaces seemed worth investigating.[1] Amphiphiles of unusual geometry have been subjects of recent research in several laboratories. In particular, Gellman has suggested that "contrafacial amphiphiles" such as **5.10** may be geometrically unsuited to forming micelles, unlikely to self-associate in aqueous solution, and thus available to act as vehicles for drug delivery across cell membranes.[2] Though sometimes considered a natural prototype for facial amphiphiles, cholic acid is considered imperfect in this respect. While the steroidal faces are clearly differentiated, its most hydrophilic centre is the side-chain carboxylate, on which it relies for water solubility. In contrast, **5.2** lacks a highly polar side chain, but should possess far greater hydrophilicity on the α-face of the steroid at acidic or neutral pH.



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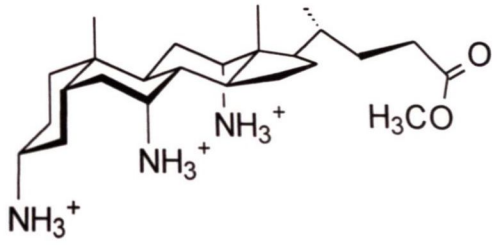


5.2.3H$^+$

In the present work preliminary studies on **5.2** in aqueous solution suggested aggregation properties consistent with Gellman's proposal. A ^{1}H NMR study following the 3β, 7β, and 12β protons at variable pH (2-13.5) confirmed that all three nitrogen atoms remain almost completely protonated up to a pH ~ 7 as shown in Table 5.1.

Table 5.1.

pH	3β-H	7β-H	12β-H
2	2.81	3.27	3.51
5	2.80	3.27	3.51
7	2.80	3.23	3.46
8	2.73	3.01	3.26
9	2.65	2.84	3.03
10	2.19	2.58	2.74
12	2.13	2.55	2.72
13.5	2.12	2.55	2.72

At pH 5-6, the triamine was soluble to at least 1 M. ^{1}H NMR spectra taken at a range of concentrations at this pH were well resolved and essentially similar, suggesting that tri-protonated **5.2** is monomeric over at least this range. Table 5.2 shows the NMR chemical shift of the 7β-proton at different concentrations. On moving from 80mM to

0.3mM the change in chemical shift was only 0.01 ppm. Also, the signal did not appear to shift consistently in any direction. This data suggests that no signs of self association took place at the concentrations used.

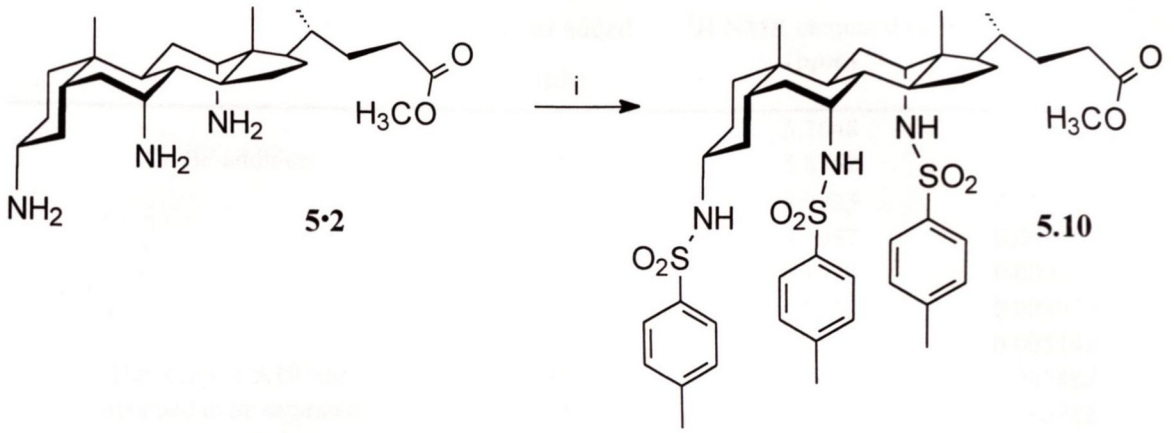
Table 5.2 NMR chemical shift data for a dilution study of 5.2.

concentration of 5.2	NMR chemical shift of 7β proton
0.08	3.6691
0.067	3.6803
0.05	3.6829
0.033	3.6778
0.025	3.6716
0.01	3.6778
0.005	3.6766
0.0017	3.6829
0.0007	3.6703
0.000333	3.6791

To obtain an estimate of the critical aggregation concentration (CAC), we investigated the solubilisation by **5.2** of the hydrophobic dye Orange OT again at pH 5-6. This method had previously been applied to cholic acid at pH 7, yielding CAC's of ~ 0.01 M. In the case of **5.2**, solutions in contact with Orange OT remained colourless at concentrations up to ~ 0.45 M, after which small amounts of the dye began to dissolve. An accurate determination was precluded by the high concentrations required and the limited quantity of amphiphile available. However, the data clearly suggest a CAC in the same region (0.35- 0.5 M) as those reported by Gellman for *bis*-anionic dibenzobarrelene facial amphiphiles, and well above any concentration which might be attained *in vivo*. The unusual amphiphilic profile of **5.2** may find use in drug delivery systems, especially for anionic guests.

In work by Williams and Perry, **5.2** was also shown to be a starting point for anionophores. Tosylation of the 3 amino groups gave **5.10**, which was found to exhibit good selectivity for chloride over the other anions examined for binding strength. The receptor was found to bind the chloride with remarkable affinity as shown in Table 5.3.

Scheme 5.2



Scheme 5.2. Reagents and conditions : i, $NaHCO_3$ wash, TsCl, cat.Et_3N, dry CH_2Cl_2.

Table 5.3 K_{ass} values of 5.10 for various anions under dry conditions.

Anion	K_{ass} (M^{-1})
Cl^-	92,000 ± 28,000
Br^-	9,200 ± 700
I^-	525 ± 45
TsO^-	950 ± 80

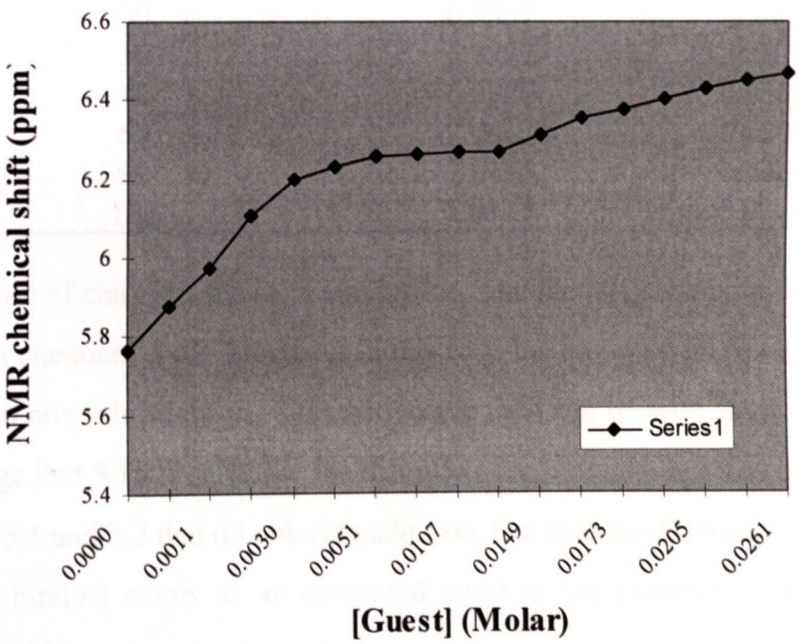
The degree of NH proton shift observed by **5.10** on binding chloride was markedly different than when binding the other anions, *e.g.* bromide. The NH protons underwent the largest shift on chloride addition.

The selectivity of **5.10** for chloride versus bromide implied that the former should be able to displace the latter from the binding site. In the present work it was decided to investigate this possibility. A competitive binding study involving the saturation of **5.10** with bromide followed by the addition of aliquots of chloride was performed. Dry conditions were used in this study. The results of this competition study are shown below. Receptor **5.10** was first saturated with almost 5 equivalents of TBABr. Saturation was confirmed by the lack of movement of the NH protons downfield on any further bromide addition. It was at this stage that the host/bromide complex was titrated with TBACl. On addition the NH protons underwent substantial changes in the ^1H NMR spectrum as shown in Table 5.4 and figure 5.1.

Table 5.4 NMR titration data of the 3α-NH proton for the competition study of 5.10 with TBABr followed by TBACl.

	Guest added (μL)	^{1}H NMR chemical shift (ppm)	[G] (M)
		5.7648	0
On Br^- addition	4	5.8752	0.000545
	8	5.9725	0.001084
	15	6.1057	0.002009
	23	6.1978	0.003041
	30	6.2322	0.003922
	40	6.2571	0.005148
The receptor **5.10** was	60	6.2630	0.007487
assumed to be saturated	90	6.2681	0.010743
with Br^- at this stage.	120	6.2659	0.013727
The Br^-/**5.10** complex was then	10	6.3105	0.014943
titrated with Cl^-	20	6.3522	0.01612
	30	6.3749	0.01726
	40	6.4005	0.018364
	60	6.4284	0.020472
	90	6.4517	0.023405
	120	6.4656	0.026093

Figure 5.1 chemical shift of the 3α-NH proton throughout the competition binding study.



At the start of the titration TBABr caused a dramatic downfield shift of the NH proton. Upon saturation of **5.10** with bromide (~5 equivalents) the NH proton levelled off at~ 6.26 ppm and showed no more downfield shift on bromide addition. On addition of chloride at this stage, the NH proton underwent further downfield shifts as shown in the latter stage in the graph.

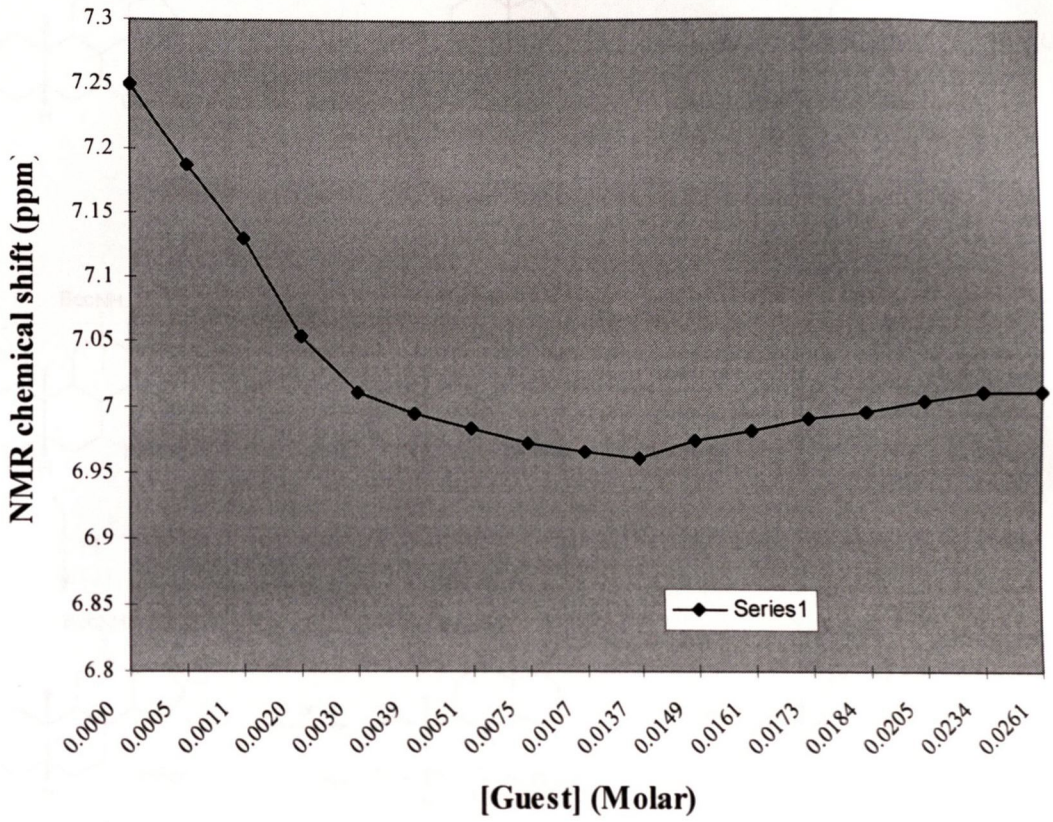
The aryl protons *meta* to the CH_3 of the tosyl groups also underwent significant change upon bromide and chloride addition as shown below.

Table 5.5 NMR titration data of the aryl protons *meta* to the CH_3 of a tosyl group for the competition study of 5.10 with TBABr followed by TBACl.

Guest added (μL)	^{1}H NMR chemical shift	[G] (M)
Br^- addition	7.2498	0
4	7.1868	0.000545
8	7.1298	0.001084
15	7.0544	0.002009
23	7.0120	0.003041
30	6.9952	0.003922
40	6.9850	0.005148
60	6.9747	0.007487
90	6.9681	0.010743
120	6.9637	0.013727
Cl^- addition		
10	6.9769	0.014943
20	6.9857	0.01612
30	6.9937	0.01726
40	6.9989	0.018364
60	7.0063	0.020472
90	7.0135	0.023405
120	7.0127	0.026093

On addition of chloride, the aromatic proton, like the NH proton, underwent considerable change in chemical shift. However in this case the aromatic proton shifted upfield, more so than on bromide addition. This noticeable shift can be seen from figure 5.3. With the knowledge that **5.10** is selective for chloride over bromide, one can deduce from the data in figure 5.1 and 5.2 that on chloride addition, the receptor **5.10** expels the bromide anion from the binding cavity as an unwanted guest in the presence of the more favourable guest chloride.

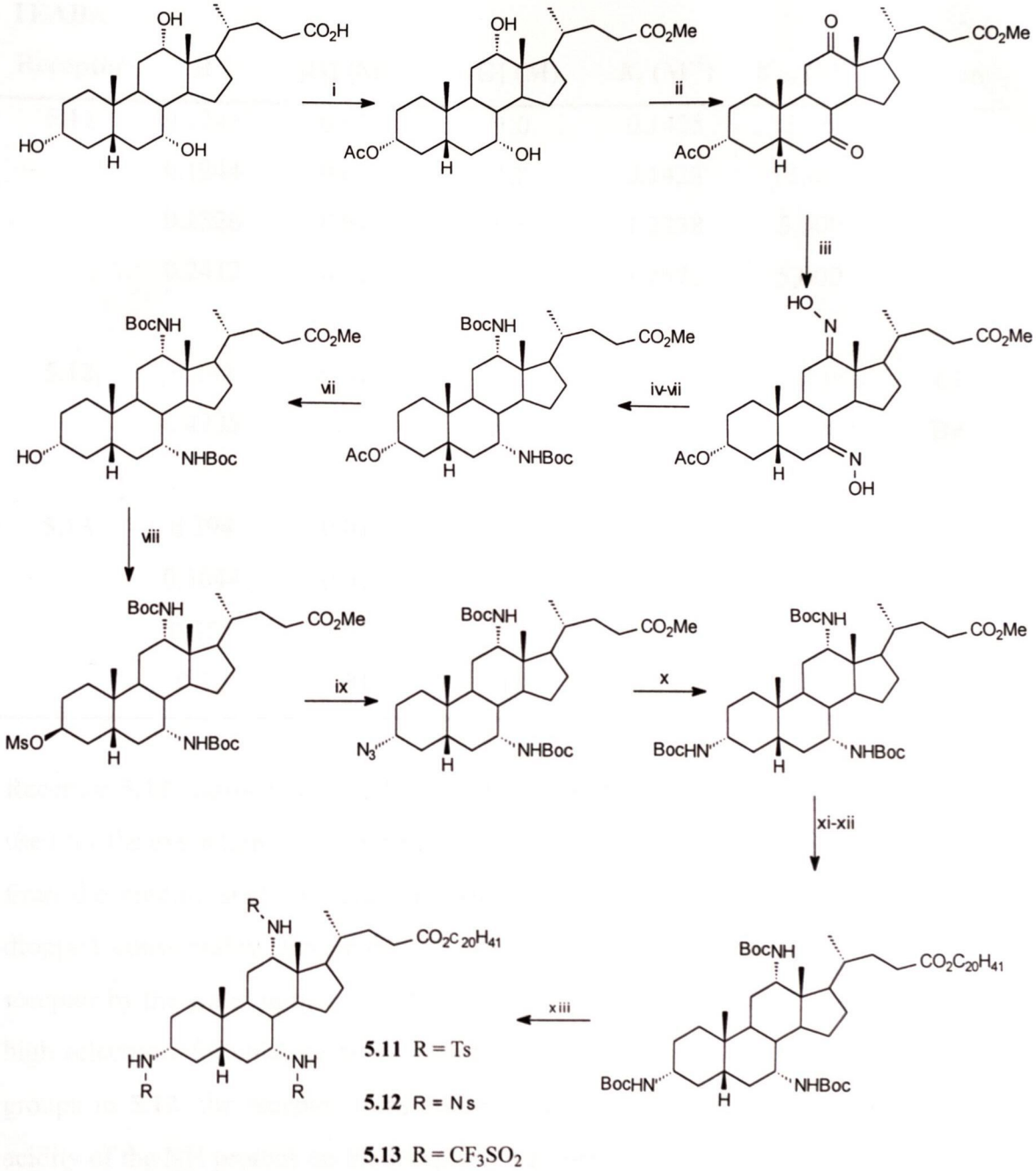
Figure 5.2 Upfield shift of the *meta*-aromatic proton on addition of bromide until saturation is reached at ~7 ppm.



In conclusion, the host/bromide complex recognises the chloride anion on its addition and the host displaces the bromide anion as an unwanted guest in preference for the chloride anion.

The investigation of analogues of **5.10** was clearly desirable, given the high binding constant for chloride. Initially the work was delayed by the length and low yield of the original route to **5.9** (Scheme 5.1). However an improved route (Scheme 5.3) was developed by Nieves Perez-Payan, who then proceeded to prepare the lipophilic anionophores **5.11-5.13**.

Scheme 5.3



Scheme 5.3 Reagents and conditions : i, *p*-TsOH, AcOMe, 23 h, reflux; ii, $K_2Cr_2O_4$, AcOH, 48 h, rt: iii, $H_2NOH.HCl$, AcONa, MeOH, 4.5 h, reflux; iv, H_2, $PtO_2.xH_2O$, AcOH, 6 d, rt; v, Zn, AcOH, 12 h, rt; vi, $(Boc)_2O$, $NaHCO_3$ aq., THF, 3 d, rt; vii, Na_2CO_3, MeOH, 12 h, rt; viii, Ph_3P, Et_3N, $MeSO_3H$, DEAD, THF, 1 d, 45 °C; ix, NaN_3, DMF, 2 d, 45 °C; x, H_2, Pd/C (10%), $(Boc)_2O$, EtOH, 24 h, rt; xii, CsOH, $MeOH/H_2O$ (10:1), pH 11; xii, $C_{20}H_{41}Br$, dry DMF, cat. NaI; xiii, TFA, 0 °C, 3 h, DCM, RCl.

In the present work, receptors **5.11**, **5.12** and **5.13** were examined for their anion binding properties by extraction methods in water-saturated chloroform, the results of which are shown below.

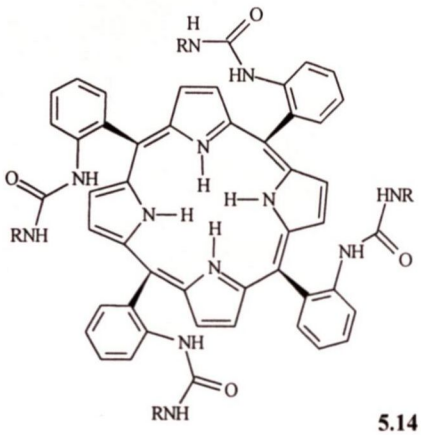
Table 5.5 Results of extraction studies of 5.11, 5.12 and 5.13 with TEACl and TEABr.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
5.11	0.1242	0.01	1.0	0.1425	13,000	Cl^-
	0.1244	0.01	1.0	0.1428	13,000	
	0.2326	0.01	0.5	1.2238	5,000	Br^-
	0.2413	0.01	0.5	1.2971	5,000	
5.12	0.384	0.01	0.125	41.1505	3,714,000	Cl^-
	0.4735	0.01	0.0625	239.205	986,000	Br^-
5.13	0.394	0.01	2.0	0.1638	15,000	Cl^-
	0.3644	0.01	2.0	0.1444	13,000	
	0.355	0.01	1.0	0.5583	2,000	Br^-
	0.30	0.01	1.0	0.4338	2,000	

Receptor **5.11** shows moderate binding for chloride and bromide under the conditions used for the extractions *i.e.* water-saturated chloroform. On comparing the values of K_{ass} from the binding study of receptor **5.10** and extractions of **5.11**, the binding constant dropped considerably presumably due to the solvation of the anion, cation and the receptor by the water in the chloroform. From Table 5.5 the receptor appears to lose its high selectivity for chloride over bromide. On replacement of the tosyl groups by nosyl groups in **5.12**, the receptor increased in anion binding ability due to the increase in acidity of the NH protons on the receptor. **5.12** was found to bind chloride by a factor of > 250 stronger than **5.11,** and bromide by a factor of > 200 due to the replacement of three tosyl groups, each amplifying the anion binding strength by a considerable factor. Replacement of the tosyl groups of **5.11** by the trifluoromethanesulfonamido groups in **5.13** was expected to have a large effect on the anion binding ability of the receptor, because of their well known acidity. However, examination of the anion binding potency of **5.13** by the extraction method, revealed a large decrease in anion binding. The poor performance of **5.13** in anion binding may be due to a large increase in the solvation of the binding site of the host due to the absence of the bulky hydrophobic aromatic groups that are present in **5.11** and **5.12**.

Bonar-Law *et al.* synthesised a series of receptors with a similar porphyrin skeletal structure to those of Burns *et al.* shown in the introduction.[3] It was of interest to see how this class of receptor, previously studied in DMSO, would behave in chloroform. Receptor **5.14** was analysed for its chloride and bromide binding ability using the same extraction method.

Scheme 5.4



5.14 R = $C_{12}H_{25}$

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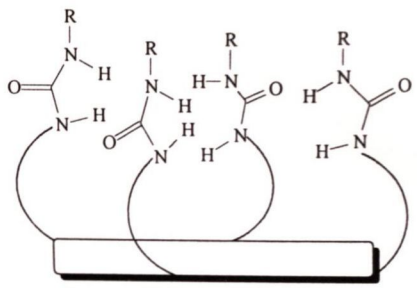


The anion binding ability of receptor **5.14** in chloroform is shown in Table 5.6. This receptor proved roughly comparable to **5.11** for chloride and bromide binding.

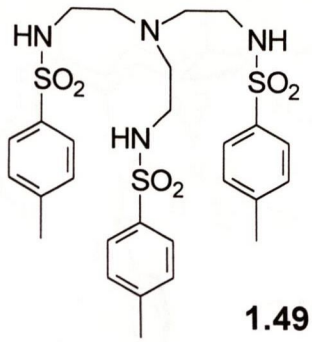
Table 5.6 Results of the liquid-liquid extraction studies of Bonar-Law's receptors.

Receptor	R	[H] (M)	[G] (M)	K_e (M^{-2})	K_{ass} (M^{-1})	Anion
5.14	0.52	0.01	2	0.273672	26,000	Cl^-
	0.53	0.005	2	0.283415	27,000	
	0.2	0.005	1	0.251003	24,000	
	0.24	0.01	0.5	1.275372	4,000	Br^-
	0.25	0.01	0.5	1.346767	5,000	
	0.17	0.005	0.3945	1.321753	5,000	

[1] Broderick, S.; Davis, A. P.; Williams, R. J. *Tetrahedron lett.* **1998**, *39*, 6083.

[2] Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3943.

[3] Bonar-Law, R. P.; *personnal communication.*



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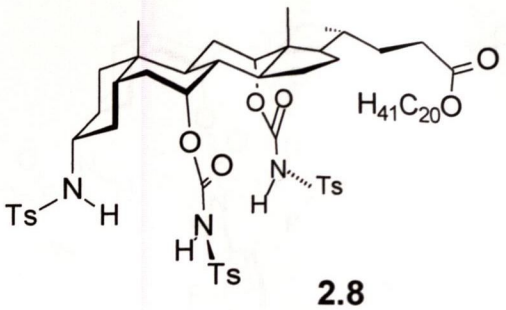

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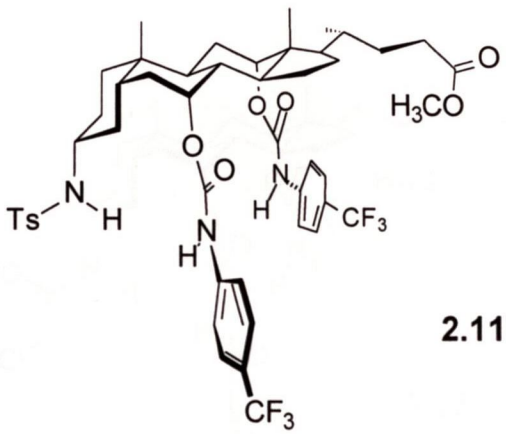

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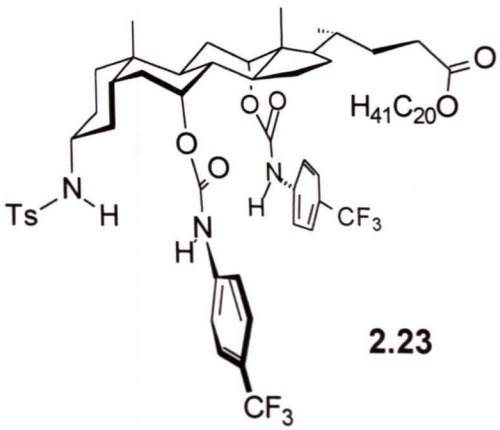

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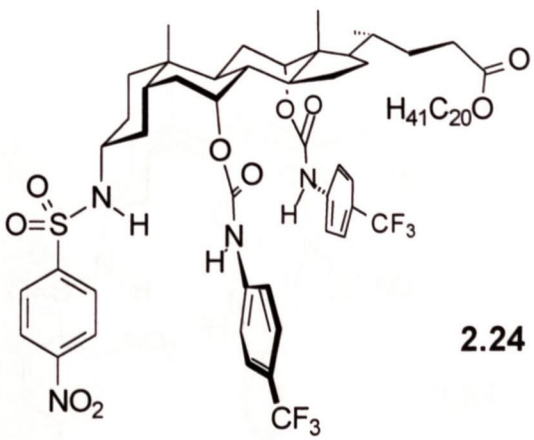

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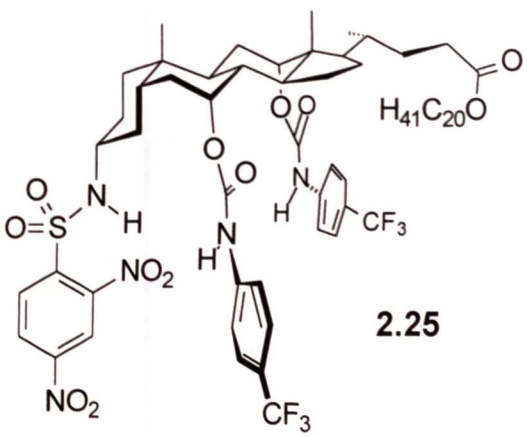

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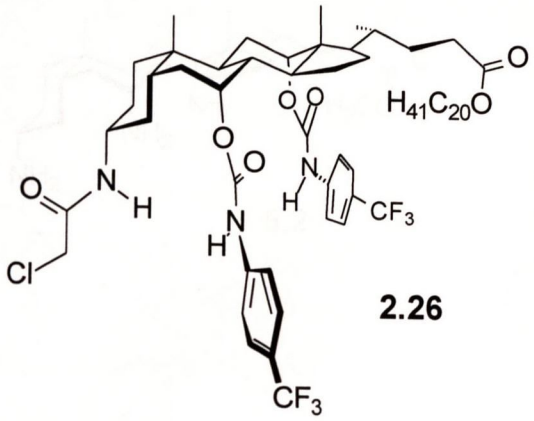

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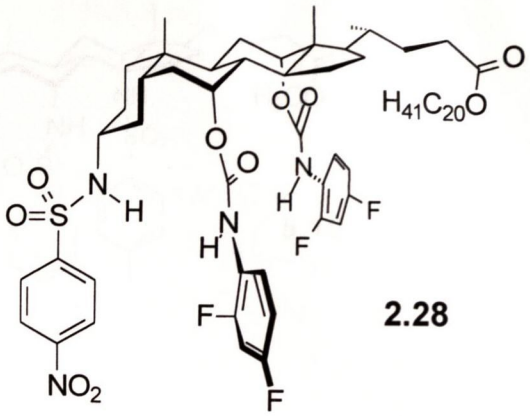

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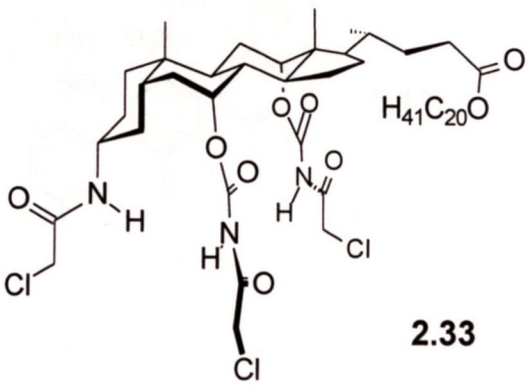

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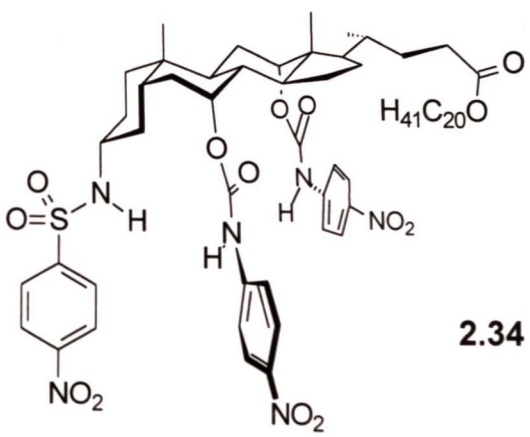

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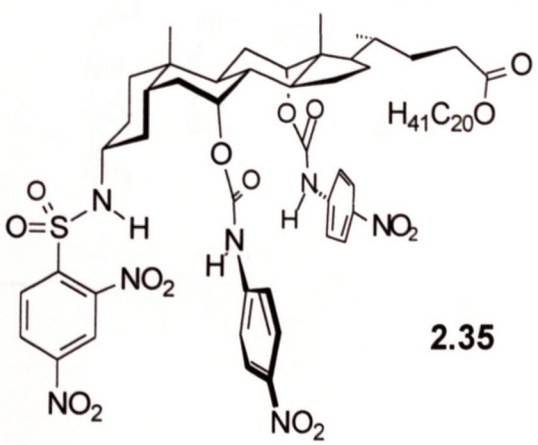

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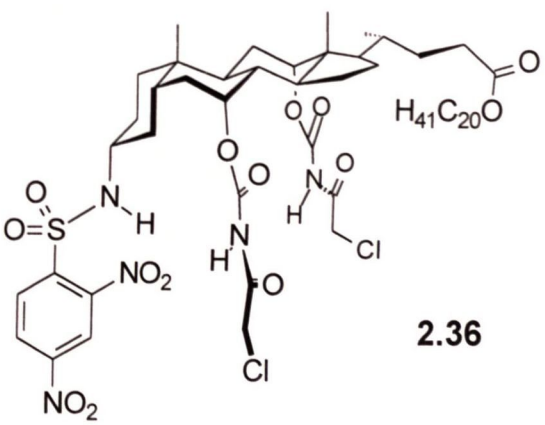

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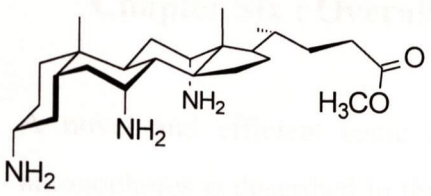

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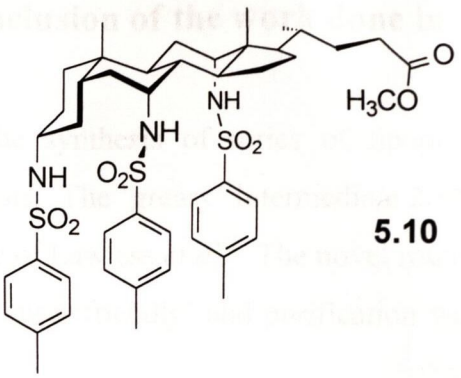

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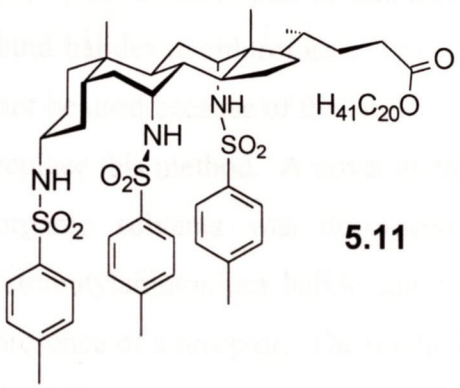

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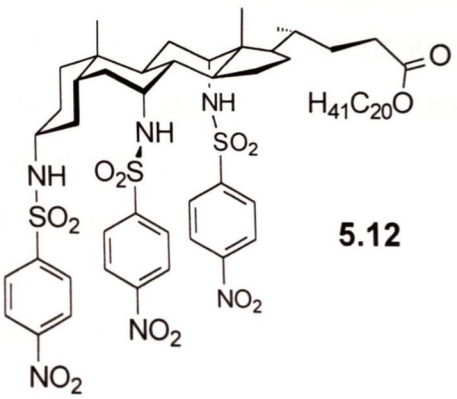

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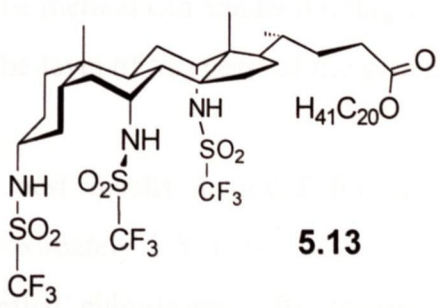

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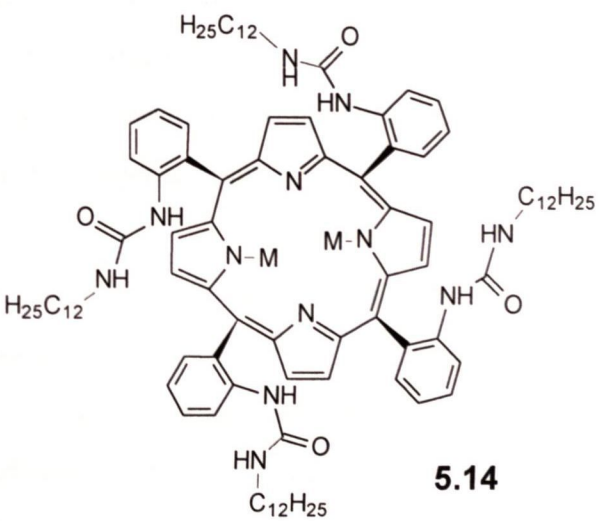

5.14

Chapter Six : Overall conclusion of the work done in this thesis

A novel and efficient route for the synthesis of series of lipophilic steroid-based anionophores is described in this thesis. The 'greasy' intermediate **2.17** was prepared in high yield from a modified procedure of Lawless *et al.*[1] The novel route from **2.17** to the final receptor was found to be more 'user friendly' and purification was easier than the previous method used in our group.

The second major part of this thesis involved the analysis of each receptor's ability to bind halides in chloroform. The usual procedure for this task (^{1}H NMR titration) could not be used because of the large K_{ass} values obtained. Another procedure was required to replace this method. A novel method of measuring a receptor's ability to bind anions in organic solvents was developed. The procedure involved the extraction of a tetrabutylammonium halide salt from an aqueous phase into an organic phase in the presence of a receptor. The method was advantageous over ^{1}H NMR titration studies for the following reasons:

1) The method is easy to repeat and takes much less time.
2) The method requires little time on an NMR machine.
3) The method can handle binding values with essentially no upper limit.
4) The level of 'dryness' of the system is no longer an issue.

The best results achieved for anion binding were for **2.34** with a K_{ass} value of approximately 3.8 x 10^7 M^{-1} for chloride and 2.3 x 10^7 M^{-1} for bromide in water saturated chloroform. By conjecture, **2.34** would be even more powerful binding chloride and bromide under the dry conditions used for ^{1}H NMR titration studies.

An X-ray crystal structure is presented of **2.37**, the methyl ester analogue of **2.34**. The results shown in Table 6.1 illustrate the fact that as the receptors become more powerful at binding anions, the ^{1}H NMR titration technique becomes inapplicable.

Tables 6.1 to 6.3 summarise the binding data obtained during this work. For convenience, the structures of the receptors are repeated on the three preceding pages.

Table 6.1 Results of the ^{1}H NMR binding studies done under dry conditions.

	K_{ass} (M^{-1})	
Receptor	**Cl^-**	**Br^-**
1.49	4,000 ± 222	340 ± 12
2.33	46,000 ± 4,700	16,200 ± 800
2.8	36,000 ± 21,000	40,000 ± 22,000
2.11	350,000 ± 137,500	400,000 ± 118,000
2.23	374,100 ± 30,000	360,150 ± 14,400
2.26	132,000 ± 20,200	52,000 ± 3,700
2.24	$> 10^6$	$> 10^6$
2.36	300,000 ± 41,000	60,000 ± 4,000
2.34		$> 10^6$

Table 6.2 shows the selectivity of this class of receptor for the small, spherical chloride anion.

Table 6.2 Results of the ^{1}H NMR titration studies of 2.33 with a range other anions.

Receptor	**I^-**	**OAc^-**	**$H_2PO_4^-$**	**NO_3^-**
2.33	3,658 ± 125	18,410 ± 1,720	~ 0	17,627 ± 620

Table 6.3 summarises the results from extraction studies from receptors presented in chapter 4 and 5.

Table 6.3 Results of the novel extraction technique for measuring K_{ass} for the receptors with tetraethylammonium chloride and bromide.

R	**[H]**	**[G]**	**V_{org}**	**V_{aq}**	**K_e**	**K_{ass}**	**Receptor**	**Anion**
0.221	0.01	0.5	1	1	1.144887	109,000	**2.23**	**Cl^-**
0.471	0.01	0.2	1	1	23.34561	81,000		**Br^-**
0.4059	0.01	0.1	1	5	69.44476	6,582,000	**2.24**	**Cl^-**
0.46	0.01	0.025	1	4	1497.571	5,187,000		**Br^-**
0.6038	0.01	0.2	1	1	40.50842	3,840,000	**2.25**	**Cl^-**
0.573	0.01	0.2	1	1	35.55619	3,370,000		
0.5153	0.01	0.05	1	4	448.043	1,552,000		**Br^-**
0.5106	0.01	0.05	1	4	439.4808	1,522,000		
0.19	0.01	2	1	0.5	0.058865	6,000	**2.28**	**Cl^-**

R	[H]	[G]	V_{org}	V_{aq}	K_e	K_{ass}	Receptor	Anion
0.193	0.01	0.5	1	1	0.964058	3,000	**2.28**	**Br^-**
0.0584	0.01	0.0125	1	8	401.6186	38,068,000	**2.34**	**Cl^-**
0.785	0.01	0.1	1	1	429.9723	40,756,000		
0.47	0.01	0.05	1	2	390.5676	37,021,000		
0.18375	0.01	0.025	1	4	373.7945	35,431,000		
0.2957	0.01	0.033333	1	3	401.2515	38,033,000		
0.136	0.005	0.02	1	5	398.9255	37,813,000		
0.1445	0.0025	0.02	1	5	425.3352	40,316,000		
0.367	0.01	0.01	1	10	6247.974	21,642,000		**Br^-**
0.276	0.01	0.00833	1	12	5810.326	20,126,000		
0.52	0.01	0.0125	1	8	7714.813	26,723,000		
0.188	0.01	0.05	1	5	94.01958	8,912,000	**2.35**	**Cl^-**
0.1853	0.01	0.05	1	5	92.34208	8753,000		
0.102	0.01	0.01	1	10	1159.388	4,016,000		**Br^-**
0.094	0.01	0.01	1	10	1057.312	3,662,000		
0.4405	0.01	0.5	1	1	3.205472	304,000	**2.36**	**Cl^-**
0.441	0.01	0.5	1	1	3.212046	304,000		
0.5384	0.01	0.2	1	1	30.79514	107,000		**Br^-**
0.2437	0.01	0.1	1	2	33.02252	114,000		
0.1242	0.01	1	1	0.5	0.14252	14,000	**5.11**	**Cl^-**
0.1244	0.01	1	1	0.5	0.142784	14,000		
0.2326	0.01	0.5	1	1	1.223765	4,000		**Br^-**
0.2413	0.01	0.5	1	0.5	1.297094	4,000		
0.384	0.01	0.125	1	2	41.15054	3901,000	**5.12**	**Cl^-**
0.4735	0.01	0.0625	1	4	239.2051	829,000		**Br^-**
0.394	0.01	2	1	0.5	0.16383	16,000	**5.13**	**Cl^-**
0.3644	0.01	2	1	0.5	0.144379	14,000		
0.355	0.01	1	1	0.5	0.558287	2,000		**Br^-**
0.3	0.01	1	1	0.5	0.433761	1,500		
0.52	0.01	2	1	0.5	0.273672	2,600	**5.14**	**Cl^-**
0.53	0.005	2	1	0.5	0.283415	2,700		
0.2	0.005	1	1	0.5	0.251003	24,000		
0.24	0.01	0.5	1	1	1.275372	4,000	**5.14**	**Br^-**
0.25	0.01	0.5	1	1	1.346767	5,000		
0.17	0.005	0.3945	1	1	1.321753	5,000		

R = ratio of substrate/receptor in organic phase, as measured *e.g.* by NMR integration, [H] = total concentration of receptor in organic phase, [G] = initial concentration of guest in aqueous phase, V_{org} = volume of organic phase, V_{aq} = volume of aqueous phase, K_e = extraction constant, K_d = distribution constant of substrate between aqueous and organic phases (in absence of receptor) and K_{ass} = Association constant.

References

[1] Lawless, L. J. Ph.D. Thesis, University of Dublin, 1998.

Chapter Seven : Experimental Details

General

^{1}H and ^{13}C NMR spectra were run on Bruker MSL-300 or DPX-400 spectrometers using deuterated chloroform as solvent (unless otherwise noted) with tetramethylsilane as internal standard. Elemental analysis were carried out in the microanalytical laboratory, Department of Chemistry, Iniversity College Dublin. Mass spectra were recorded by Chris Cryer at the University of Bath. Melting points were recorded on a Griffin melting point apparatus and are uncorrected. Infra-red spectra were recorded on a Perkin-Elmer 883 spectrophotometer. 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 n-butanol. Chromophoric spots were visualised in Ultra-violet light. Other compounds were visualised with 5% Phosphomolybdic acid in ethanol.

Cholic acid was obtained as a gift from Diamalt GmbH and used without further purification. Solvents were distilled before use and dried using standard techniques described in "Purification of laboratory chemicals" by Perrin & Perrin.[1] Flash chromatography of reaction products was carried out using Kieselgel 60 (Merck) 400-230 mesh, using the method of Still *et al.*[2] NMR and IR spectra were assigned by comparison with literature values for similar compounds.[3,4,5, 6, 7] NH protons were identified in ^{1}H NMR spectra by disappearance on shaking with D_2O.

Binding Studies

All NMR titration studies were performed using the Bruker MSL-300 (300.13 MHz) or DPX400 (400 MHz) spectrometers at 298 K and 303 K respectively. They were initially dried under high vacuum and stored in a desiccator. TBATs, TBACl, *etc.* were obtained from Aldrich and stored in a desiccator. All solutions used under dry conditions were prepared using deuterated chloroform which had been partially dried and de-acidified by storage over anhydrous potassium carbonate. All compounds used as either host or guest were dried under high vacuum for at least 1 hour prior to solution preparation.

Methyl cholate 2.1

procedure of Fieser & Rajagopalan[3] (slightly modified)

Cholic acid **2.0** (36 g, 0.088 mol) was dissolved in dry methanol (270 mL). The solution was allowed to stir overnight with a catalytic amount of *conc.* sulphuric acid (4.7 mL) under an atmosphere of argon. The mixture was filtered cold and washed with cold methanol. The resulting white powder was dried *in vacuo*. (34.23 g, 92% yield), mp 155-157 °C (lit., 156-157 °C)[3] from ether-hexane; TLC R_f 0.33 in ethyl acetate; δ_H (300 MHz, $CDCl_3$) 0.69 (3H, s, $18CH_3$), 0.89 (3H, s, $19CH_3$), 0.97 (3H, d, $21CH_3$), 3.46 (1H, br m, 3β-H), 3.65 (3H, s, OCH_3), 3.86 (1H, m, 7β-H), 3.98 (1H, m, 12β-H).

Methyl 3α,7α,12α-trisformyloxy-5β-cholan-24-oate 2.2

(modified procedure of Hughs, Smith & Webb)[4]

Methyl cholate **2.1** (3.0 g, 7.1 mmol) was dissolved in formic acid (12 mL) and a catalytic amount of perchloric acid (70%, 0.2 mL) was added. The solution was stirred at 65 °C for 2 h then cooled to 40 °C, followed by the dropwise addition of acetic anhydride (12 mL). The reaction was cooled to room temperature and slowly poured into ice-cold water (120 mL) while vigorously stirring. The white solid precipitate was collected by filtration and dried under vacuum. The product was re-dissolved in $CHCl_3$, dried over $MgSO_4$, filtered, washed, evaporated and dried under high vacuum at 90°C for 4 h to yield the product **2.2** (3.3 g, 84%); TLC R_f 0.21 in hexane-ethyl acetate (1:1); mp 150-152 °C (lit., 150-152 °C)[4] from ethyl acetate-hexane; δ_H (300 MHz, $CDCl_3$) 0.75 (3H, s, $18CH_3$), 0.83 (3H, d, $21CH_3$), 0.94 (3H, s, $19CH_3$), 3.65 (3H, s, OCH_3), 4.71 (1H, br m, 3β-H), 5.07 (1H, m, 7β-H), 5.26 (1H, m, 12β-H), 8.02 (1H, s, 3α-formyl H), 8.10 (1H, s, 7α-formyl H), 8.16 (1H, s, 12α-formyl H).

Methyl 3α-hydroxy-7α,12α-diformyloxy-5β-cholan-24-oate

procedure of Bhattarai[5] (slightly modified)

The triformate **2.2** (3 g, 5.4 mmol) was dissolved in methanol (40 mL). Sodium acetate (12 g, 0.146 mol) was added and the mixture was allowed to stir at room temperature until the reaction was complete by TLC analysis (7 days). The reaction mixture was dried *in vacuo*, and redissolved in $CHCl_3$. The solution was washed with water and the organic

layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Chromatography using ethyl acetate/hexane (1:1) as eluant yielded the di-protected steroid (2.53 g, 92%) as a colourless gum; TLC R_f 0.60 in hexane/ethyl acetate (1:1); δ_H (300 MHz, $CDCl_3$) 0.75 (3H, s, $18CH_3$), 0.82 (3H, d, J 6.3 Hz, $21CH_3$), 0.91 (3H, s, $19CH_3$), 3.49 (1H, m, 3β-H), 3.64 (3H, s, OCH_3), 5.05 (1H, m, 7β-H), 5.25 (1H, m, 12β-H), 8.09 (1H, s, 7α-formyl H), 8.13 (1H, s, 12α-formyl H); δ_C (75.5 MHz, $CDCl_3$) 12.07 (C18), 14.15 (C21), 17.42, 21.03 (C19), 22.33, 22.74, 25.46, 27.12, 28.52, 30.29, 30.61, 30.84, 31.43, 34.20, 34.71, 34.82, 37.70, 38.50, 40.92, 42.91, 44.93, 47.14, 51.51, 60.37, 70.81, 71.45, 75.26, 160.55 (OCOH), 160.72 (OCHO), 174.47 (C24).

Methyl 3β,7α,12α-tri(formyloxy)-5β-cholan-24-oate 2.3

procedure of Perry[6] (slightly modified)

A solution of the above 3α-hydroxy *bis*-formyloxy steroid (4 g, 8.4 mmol), triphenylphosphine (4.4 g, 16.8 mmol), and 95-97% formic acid (0.63 mL, 16.8 mmol),in dry THF (50 mL) was stirred at room temperature. DEAD (2.9 g, 16.8 mmol) in dry THF (20 mL) was added dropwise to the to the reaction solution and stirring continued at room temperature for 18 h under an atmosphere of argon. The solvent was then removed at reduced pressure and the product was extracted with ether (30 mL). Filtration and removal of solvent followed by column chromatography using hexane/ethyl acetate (3:1) as eluant afforded **2.3** (3.8 g, 90%); TLC R_f 0.6 in hexane/ethyl acetate (1:1); δ_H (300 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.84 (3H, d, J 6.0 Hz, $21CH_3$), 0.98 (3H, s, $19CH_3$), 3.66 (3H, s, OCH_3), 5.07 (1H, m, 7β-H), 5.15 (1H, m, 3α–H), 5.27 (1H, m, 12β-H), 8.05 (1H, s, 3α formyl H), 8.09 (1H, s, 7α formyl H), 8.16 (1H, s, 12α formyl H); δ_C (75.5 MHz, $CDCl_3$) 12.01 (C18), 17.34 (C21), 22.66 (C19), 22.66, 24.63, 25.71, 27.04, 27.89, 29.98, 30.52, 30.75, 30.82, 32.64, 34.41, 34.63, 36.17, 37.56, 42.87, 44.90, 47.08, 51.42 (OCH_3), 70.00 (C3), 70.88 (C7), 75.22 (C12), 160.44-160.57 (3 x OCHO), 174.35 (C24).

Methyl 3β-hydroxy-7α,12α-di(formyloxy)-5β-cholan-24-oate

(procedure of Perry)[6]

To a solution of the 3β-formate **2.3** (2.38 g, 4.7 mmol) in methanol (28 mL) was added sodium bicarbonate (1.48 g, 17.6 mmol). The reaction was stirred at room temperature until complete by TLC analysis. Removal of methanol gave a white solid which was partitioned between ether (50 mL) and water (50 mL). The aqueous layer was extracted once with ether (50 mL). The organic extracts were combined and dried with $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by column chromatography using hexane/ethyl acetate (1:1) to yield a colourless gum (1.93 g, 86%); TLC R_f 0.4 in 1:1 hexane/ethyl acetate; δ_H (300 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.84 (3H, d, J 6.3 Hz, $21CH_3$), 0.96 (3H, s, $19CH_3$), 3.66 (3H, s, OCH_3), 4.05 (1H, m, 3α–H), 5.05 (1H, m, 7β-H), 5.26 (1H, m, 12β-H), 8.10 (1H, s, 7α formyl H), 8.15 (1H, s, 12α formyl H); δ_C (75.5 MHz, $CDCl_3$) 11.99 (C18), 17.30 (C21), 22.64 (C19), 22.67, 25.68, 27.04, 27.43, 27.67, 29.27, 30.51, 30.75, 31.03, 34.62 (2 x C), 35.38, 35.58, 37.54, 42.88, 44.89, 47.05, 51.42 (OCH_3), 66.18 (C3), 71.21 (C7), 75.40 (C12), 160.47-160.66 (2 x OCHO), 174.41 (C24).

Methyl 3β-methanesulfonyl-7α,12α-di(formyloxy)-5β-cholan-24-oate

(procedure of Perry)[6]

Pyridine (2.7 mL, 31 mmol) and methanesulfonyl chloride (1.1 g, 14.4 mmol) were added to a solution of the 3β-hydroxy steroid (3.2 g, 6.3 mmol) in dry DCM (100 mL) and the reaction was stirred at 0 °C under an atmosphere of argon for 15 minutes before DIPEA (2.9 mL, 16 mmol) was added. After a further 40 minutes, a saturated aqueous solution of sodium bicarbonate (100 mL) was added and the reaction mixture was allowed to warm to room temperature while stirring vigorously. The organic phase was separated and washed with 2M aqueous HCl (100 mL) and water (100 mL). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The product was purified by column chromatography using hexane/ethyl acetate (1:1) as eluant affording the 3β-mesylate (3.21 g, 88%); TLC R_f 0.5 in hexane/ethyl acetate (1:1); ν_{max} (film from $CDCl_3$) 1750-1720 cm^{-1}(C=O), 1200 cm^{-1}(sulfonate); δ_H (300 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.84 (3H, d, J 6.3 Hz, $21CH_3$), 0.99 (3H, s, $19CH_3$), 3.00

(3H, s, mesylate CH_3), 3.66 (3H, s, OCH_3), 4.98 (1H, m, 3α-H), 5.08 (1H, m, 7β-H), 5.27 (1H, m, 12β-H), 8.09 (1H, s, 12α formyl H), 8.15 (1H, s, 12α formyl H); δ_C (75.5 MHz, $CDCl_3$) 12.13 (C18), 17.48 (C21), 22.63 (C19), 22.77, 25.85, 25.94, 27.14, 28.12, 29.61, 30.65, 30.85, 30.89, 33.97, 34.46, 34.74, 35.89, 37.70, 38.58, 42.97, 45.05, 47.23, 51.49 (OCH_3), 70.93 (C7), 75.34 (C12), 79.93 (C3), 160.40 (2 x OCHO), 174.41 (C24).

Methyl 3α-azido-7α,12α-di(formyloxy)-5β-cholan-24-oate 2.4

(procedure of Lawless)[7]

To a solution of the 3β-methanesulfonyloxy steroid (3.5 g, 6.3 mmol) in DMPU (50 mL) was added sodium azide (10 g, 15.4 mmol) and the solution was allowed to stir for 1 day. The reaction was partitioned between ether (200 mL) and water (200 mL). The organic fraction was washed once with water (200 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by flash chromatography using hexane/ethyl acetate (2:1) as eluant affording **2.4** (2.83 g, 85%), TLC R_f 0.5 in hexane-ethyl acetate (5:3); mp 112-113 °C (lit., 112-113 °C)[7] from ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1740 cm^{-1}(C=O); δ_H (300 MHz, $CDCl_3$) 0.75 (3H, s, $18CH_3$), 0.83 (3H, d, J 6.2 Hz, $21CH_3$), 0.94 (3H, s, CH_3), 3.23 (1H, m, 3β-H), 3.65 (3H, s, OCH_3), 5.07 (1H, m, 7β-H), 5.26 (1H, m, 12β-H), 8.12 (1H, s,7α formyl H), 8.16 (1H, s, 12α formyl H); δ_C (75.5 MHz, $CDCl_3$) 12.07 (C18), 17.42 (C21), 22.41 (C19), 22.72, 25.46, 26.66, 27.09, 28.52, 30.61, 30.83, 31.36, 34.28, 34.69, 34.74, 35.06, 37.68, 41.26, 42.89, 44.95, 47.16, 51.43 (OCH_3), 61.06 (C3), 70.49 (C7), 75.12 (C12), 160.43 (OCHO), 160.53 (OCHO), 174.20 (C24).

Methyl 3α-(*p*-toluenesulfonyl)amino-7α,12α-*bis*formyloxy-cholanoate

(procedure of Perry)[6]

To acetic acid (20 mL) was added **2.4** (0.200 g, 0.398 mmol) and an excess of powdered zinc (0.5 g). After 18 h of vigorous stirring, the solution was filtered through a Büchner funnel and the solid residue was washed with acetic acid (2 x 10 mL). The filtrate and the washings were combined and the acetic acid was removed under reduced pressure. The crude product was re-dissolved in DCM (25 mL) and washed with saturated aqueous sodium hydrogen carbonate solution (25 mL). The solution was concentrated *in vacuo* and the residue was then re-dissolved in DCM (20 mL) and approximately 1.1

equivalents of toluenesulfonyl chloride (0.085 g, 0.44 mmol) and excess TEA (0.4 mL) were added. After stirring for 15 minutes, the solution was washed with 2M aqueous HCl (20 mL), the solvent was removed at reduced pressure and chromatographed using 2:1 ethyl acetate/hexane as eluant to give the 3α-tosyl steroid as a white solid (0.234 g, 93% from **2.4**); mp 198 °C (Lit. mp 196-198 °C recrystallised from ether/hexane);[6] TLC R_f 0.5 ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1715 cm^{-1} br (C=O), 1385 cm^{-1}(SO_2N); δ_H (300 MHz, $CDCl_3$) 0.72 (3H, s, $18CH_3$), 0.81 (3H, d, J 6.3 Hz, $21CH_3$), 0.87 (3H, s, $19CH_3$), 2.43 (3H, s, aryl CH_3), 2.98 (1H, m, 3β–H), 3.65 (3H, s, OCH_3), 4.33 (1H, d, J 7.5 Hz, NH), 5.01 (1H, m, 7β–H), 5.23 (1H, m, 12β–H), 7.29 (2H, d, J 8.2 Hz, aryl H), 7.73 (2H, d, J 8.2 Hz, aryl H), 8.02 (1H, s, HCO_2), 8.13 (1H, s, HCO_2); δ_C (75.5 MHz, $CDCl_3$) 12.11 (C18), 17.46 (C21), 21.51 (C19), 22.42, 22.75, 25.52, 27.14, 27.14, 28.52, 28.74, 30.64, 30.89, 31.29, 34.04, 34.74, 35.41, 36.92, 37.65, 41.48, 42.94, 44.98, 47.20, 51.52 (OCH_3), 53.72 (C3), 70.70 (C7), 75.26 (C12), 126.89 (aryl C), 129.69 (aryl C), 138.33 (aryl C), 143.32 (aryl C), 160.47 (2 x OCHO), 174.42 (C24); Found C, 64.63; H, 7.82; N, 2.20. $C_{34}H_{49}NO_8S$ requires C, 64.63; H, 7.82; N, 2.22

Methyl 3α -(*p*-toluenesulfonyl)amino-7α,12α-*bis*-hydroxy-cholanoate 2.5
(modified procedure of Perry)[6]

The 3α-tosyl *bis*-formyloxy steroid (0.202 g, 0.320 mmol) was dissolved in dry methanol (20 mL) and excess sodium methoxide (0.10 g, 1.85 mmol) was added. After 24 h, the solution was neutralised with 2M aqueous HCl and the solvent was removed at reduced pressure. The residue was washed twice with chloroform (2 x 10 mL), the washings were dried ($MgSO_4$) and the solvent was removed at reduced pressure. The residue was chromatographed with 1:1 hexane/ethyl acetate to give **2.5** as a white solid (0.175 g, 95%); mp 216 °C (lit. mp 216-218 °C);[6] TLC R_f 0.25 in 1:1 hexane/ethyl acetate; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH/OH), 1720 cm^{-1} br (C=O), 1380 cm^{-1}(SO_2N); δ_H (300 MHz, $CDCl_3$) 0.67 (3H, s, $18CH_3$), 0.85 (3H, s, $19CH_3$), 0.97 (3H, d, J 6.2 Hz, $21CH_3$), 2.42 (3H, s, aryl CH_3), 2.95 (1H, m, 3β–H), 3.67 (3H, s, OCH_3), 3.82 (1H, m, 7β–H), 3.96 (1H, m, 12β–H), 4.54 (1H, d, J 7.8 Hz, NH), 7.28 (2H, d, J 8.3 Hz, aryl H), 7.75 (2H, d, J 8.3 Hz, aryl H); δ_C (75.5 MHz, $CDCl_3$) 12.53 (C18), 17.33 (C21), 21.51 (C19), 22.60, 23.09, 26.67, 27.40, 28.29, 28.97, 30.86, 31.02, 34.32, 34.40, 35.10, 35.75,

37.52, 39.46, 42.01 x 2, 46.48, 47.18, 51.52 (OCH_3), 54.03 (C3), 68.18 (C7), 72.81 (C12), 126.92 (aryl C), 129.58 (aryl C), 138.60 (aryl C), 142.98 (aryl C), 174.69 (C24).

Methyl 3α-(p-toluenesulfonyl)amino-7α,12α-*bis*(3,5-dimethylphenyl-aminocarbonyloxy)-cholanoate 1.68

(modified procedure of Perry)[6]

To a solution of **2.5** (0.100 g, 0.174 mmol) in dry DCM (10 mL) was added approximately 4.4 equivalents of 3,5-dimethylphenyl isocyanate (0.1 mL, 0.76 mmol) and a catalytic amount of chlorotrimethylsilane. After stirring for 1 day at 40 °C, the reaction mixture was stirred for 2 h with water (5 mL). The organic phase was separated, dried (with $MgSO_4$) and the solvent was removed at reduced pressure. The residue was chromatographed with 2:1 ethyl acetate/hexane yielding **1.68** as white needles (0.142 g, 94%); mp 240 °C (Lit. mp 240-244 °C from ether-hexane);[6] TLC R_f 0.4 in 2:1 hexane/ethyl acetate; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1715 cm^{-1} br (C=O), 1385 cm^{-1}(SO_2N); δ_H (300 MHz, $CDCl_3$) -0.26 (1H, m, 1β-H), 0.66 (3H, s, 19CH_3), 0.72 (3H, s, 18CH_3), 0.86 (3H, d, J 5.5 Hz, 21CH_3), 2.07 (6H, s, carbamate CH_3), 2.24 (6H, s, carbamate CH3), 2.38 (tosylamido CH_3), 2.60 (1H, m, 3β–H), 3.61 (3H, s, OCH_3), 4.82 (1H, m, 7β–H), 5.15 (1H, m, 12β–H), 5.67 (1H, d, J 6.8 Hz, NH), 6.60 (1H, s, aryl H *para* to NH), 6.64 (1H, s, aryl H *para* to NH), 6.99 (2H, s, aryl H *ortho* to NH), 7.17 (2H, d, J 8.1 Hz, *meta* to SO_2), 7.24 (2H, s, aryl H *ortho* to NH), 7.59 (1H, s, carbamate NH), 7.67 (1H, s, carbamate NH), 7.86 (2H, d, J 8.1 Hz, *ortho* to SO_2); δ_C (75.5 MHz, $CDCl_3$) 12.46 (C18), 17.68 (C21), 21.18 (carbamate CH_3), 21.39 (carbamate CH_3), 21.47 (tosylamido CH_3), 22.20 (C19), 22.84, 26.28, 27.23, 28.68, 29.63, 30.75, 30.96, 31.32, 33.34, 33.92, 34.74, 35.80, 37.73, 41.32, 43.42, 45.22, 47.31, 51.40 (OCH_3), 53.11 (C3), 70.87 (C7), 74.93 (C12), 116.36, 115.77, 124.17, 126.70, 130.04, 137.40, 138.27, 138.36, 138.80, 138.85, 144.13, 153.02 (NHCO), 174.63 (C24); Found C, 69.05; H, 7.59; N, 4.81; S, 3.92. $C_{50}H_{67}N_3O_8S$ requires C, 69.02; H, 7.76; N, 4.83; S, 3.68.

Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate 2.6

To a solution of the 3α-azido steroid **2.4** (3.35 g, 6.6 mmol) in dry methanol (10mL) was added 2M sodium methoxide (10 mL). The reaction was stirred at room temperature under an atmosphere of argon for 5 h. The reaction solution neutralised with 2M aqueous

HCl and the solvent was removed under reduced pressure. The residue was washed twice with $CHCl_3$ (2 x 40 mL) the washings were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by flash chromatography using hexane/ethyl acetate (1:1) as eluant affording **2.6** (2.89 g, 95%); TLC R_f 0.80 in hexane/ethyl acetate (1:1); mp 105-106 °C from ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH/OH), 2150 cm^{-1}(azide); δ_H (300 MHz, $CDCl_3$) 0.68 (3H, s, $18CH_3$), 0.90 (3H, d, $19CH_3$), 0.96 (3H, s, $21CH_3$), 3.13 (1H, br, 3β-H), 3.70 (3H, s, OCH_3), 3.85 (1H, m, 7β-H), 3.98 (1H, m, 12β-H); δ_C (75.5 MHz, $CDCl_3$) 12.45 (C18), 17.29 (C19), 22.53 (C21), 23.16 (C15), 26.50 (C9), 26.78 (C11), 27.48 (C16), 28.15 (C2), 30.78 (C6), 31.02 (C22), 34.53, (C23), 34.74, (C10), 35.26 (C20), 35.33 (C4), 35.43 (C1), 39.35 (C8), 41.78 (C5), 41.85 (C14), 46.51 (C13), 47.17 (C17), 51.52 (OCH_3), 61.28 (C3), 68.24 (C7), 72.00 (C12), 174.85 (C24).

Methyl 3α-azido-7α,12α-*bis*(*p*-toluenesulfonylaminocarbonyloxy)-5β-cholan-24-oate 2.7

To a solution of the azido-dihydroxy steroid **2.7** (2 g, 4.46 mmol) in dry DCM (40mL) was added *p*-toluenesulfonyl isocyanate (2.0 mL, 13.4 mmol) with a catalytic amount of chlorotrimethylsilane (0.05 mL, 4.8 mmol) under an atmosphere of argon. The solution was allowed to stir at room temperature for 2 days. The solvent was removed *in vacuo,* and the product was purified by flash chromatography using hexane/ethyl acetate (4:1) as eluant affording **2.7** (2.78 g, 72%), mp 162 °C; ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N); TLC R_f 0.61 in hexane/ethyl acetate (4:1); δ_H (300 MHz, $CDCl_3$) 0.54 (3H, d, $21CH_3$), 0.62 (3H, s, $18CH_3$), 0.84 (3H, s, $19CH_3$), 2.40 (6H, m, aryl CH_3), 3.14 (1H, m, 3β–H), 3.68 (3H, s, OCH_3), 4.75 (1H, m, 7β–H), 4.97 (1H, m, 12β–H), 3.14 (4H, m, aryl H), 7.90 (6H, m, aryl H, 7α, 12α-NH); δ_C (75.5 MHz, $CDCl_3$) 11.57, 13.54, 16.80, 21.02 (aryl CH_3), 21.09, 21.93, 25.05, 26.22, 28.12, 30.50, 30.60, 33.63, 33.88, 33.97, 34.64, 37.37, 40.59, 42.38, 44.59, 46.56, 51.04, 52.99, 60.89, 62.58, 73.28, 77.80, 125.98, 127.45, 127.63, 127.87, 129.09, 129.22, 135.49, 138.67, 143.01, 144.25, 149.84 (OCONH), 149.99 (OCONH), 174.06 (C24).

Methyl 3α-(*p*-toluenesulfonylamino)-7α,12α-*bis*(*p*-toluenesulfonyl-aminocarbonyloxy)-5β-cholan-24-oate 2.8

To the azido-*bis*-carbamate steroid **2.7** (2.5 g, 18.7 mmol) and Zn dust (5 g) was added glacial acetic acid (25 mL). The reaction mixture was stirred at room temperature for 18 h. The reaction was then filtered and the Zn was washed with acetic acid (3 x 5 mL). The filtrate was concentrated by forming an azeotrope with toluene under reduced pressure. The residue was purified by passing through a silica gel plug using DCM/methanol (40:1) as eluant. Then the product was partitioned between DCM and saturated aqueous $NaHCO_3$ solution. The aqueous fraction was washed once with DCM (20 mL). The organic layers were combined and dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the free ammonium acetate salt. To this salt (1.06 g, 14.2 mmol) in dry DCM was added *p*-toluenesulfonyl chloride (0.3 g, 15.6 mmol) with a catalytic amount of TEA. The solution was allowed to stir for 2 h under an atmosphere of argon. The solution was then washed with 2M aqueous HCl (20 mL).The aqueous fraction was washed once with DCM (20 mL). The organic layers were combined and dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by flash chromatography using ethyl acetate/hexane (2:1) as eluant affording a white solid. The product was slowly recrystallised from $CDCl_3$/diethyl ether/hexane (2:1:1) to yield **2.8** (0.66 g, 16%); mp 139 °C; TLC R_f 0.55 in hexane/ethyl acetate (2:1); ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1715 cm^{-1} br (C=O), 1385 cm^{-1}(SO_2N); δ_H (300 MHz, $CDCl_3$) 0.42 (3H, d, J 5.7 Hz, 21CH_3), 0.54 (3H, s, 18CH_3), 0.73 (3H, s, 19CH_3), 2.41 (3H, s, aryl CH_3), 2.42 (3H, s, aryl CH_3), 2.44 (3H, s, aryl CH_3), 3.05 (1H, m, 3β-H), 3.63 (3H, s, OCH_3), 4.70 (1H, m, 7β-H), 4.97 (1H, m, 12β-H), 5.59 (1H, br s, 3α-NH), 7.24 (6H, m, aryl H), 7.82 (6H, m, aryl H), 9.21 (1H, s, carbamate NH), 9.30 (1H, s, carbamate NH); δ_C (75.5 MHz, $CDCl_3$) 12.13 (C18), 17.36 (C21), 21.51, 21.53, 21.55, 22.27, 22.47, 25.89, 26.91, 28.75, 28.94, 30.60, 31.07, 31.10, 34.36, 35.26, 35.92, 37.66, 41.15, 43.10, 45.09, 47.10, 51.45 (OCH_3), 53.43 (C3), 73.58 (C7), 77.88 (C12), 126.55, 127.12, 128.06, 128.16, 129.52, 129.57, 129.68, 129.79,129.93, 135.92, 136.08, 143.58, 144.55, 144.90, 150.12, 150.39, 174.37 (C24).

Methyl 3β-methanesulfonoxy-7α,12α-dihydroxy-5β-cholan-24-oate 2.9

(*via* procedure of Lawless)[5]

Methanesulfonic acid (1.6 mL, 24.7 mmol, 2.1 equiv) was added to a solution of methyl cholate **2.1** (5g, 11.8mmol) and triphenylphosphine (9.4 g, 36 mmol, 3 equiv) in dry THF (38 mL) under argon. The temperature was raised to 40 °C and DEAD (5.7 mL, 36 mmol, 3 equiv) was added dropwise over a 12 minute period with vigorous stirring and careful exclusion of moisture. The mixture was stirred vigorously for 24 h at 40 °C under argon. The volatiles were then removed under reduced pressure and the residue re-dissolved in chloroform. The crude product was purified by flash chromatography on silica gel eluting with ethyl acetate/hexane/dichloromethane (7:1:0.05) to give the mesylate **2.9** as an off-white solid (5.3 g, *ca.* 90%); TLC R_f 0.6 in ethyl acetate; δ_H (400 MHz, $CDCl_3$) 0.67 (3H, s, 18CH_3), 0.89 (3H, s, l9CH_3), 0.94 (3H, d, 21CH_3), 2.82 (1H, d, 7/12-OH), 2.96 (3H, s, 3β-OSO_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 (100.62 MHz; $CDCl_3$) 12.44 (C18), 17.25 (C21), 22.63 (C19), 23.13 (CH_2), 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, 46.47 (C13), 47.19, 51.49 ($CO_2\underline{C}H_3$), 68.29 (C7), 70.91 (C12), 80.72 (C3), 174.73 (C24).

Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate 2.6

(procedure of Lawless)[7]

To a solution of 3β-methanesulfonyloxy steroid **2.9** (3.5 g, 6.3 mmol) in DMPU (20 mL) was added sodium azide (10 g, 15.4 mmol) and the solution was allowed to stir for 3 days at 45 °C. The reaction 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 flash chromatography using hexane/ethyl acetate (2:1) as eluant affording **2.6** (2.83 g, 85%); TLC R_f 0.5 in hexane/ethyl acetate (5:3); mp 112-113 °C (lit., 105-106 °C)[7] from ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1740 cm^{-1}(C=O); δ_H (300 MHz, $CDCl_3$) 0.75 (3H, s, 18CH_3), 0.83 (3H, d, J 6.2 Hz, 21CH_3), 0.94 (3H, s, 19CH_3), 3.23 (1H, m, 3β–H), 3.65 (3H, s, OCH_3), 5.07 (1H, m, 7β–H), 5.26 (1H, m, 12β–H), 8.12 (1H, s,7α formyl H), 8.16 (1H, s, 12α formyl H).

Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate 2.6

(*via* new procedure)

Diethyl azodicarboxylate (1.10 mL, 6.99 mmol) was added dropwise *via* a syringe to a stirred solution of methyl cholate **2.1** (1.00 g, 2.37 mmol), triphenylphosphine (1.86 g, 7.09 mmol), triethylamine (0.14 mL, 1.0 mmol) and 99% methanesulfonic acid (0.31 mL, 4.78 mmol) in dry THF (10 mL) under an atmosphere of argon at room temperature. Maintaining the blanket of argon, the resulting pale yellow suspension was stirred at this temperature for 24 h. The suspension was evaporated under reduced pressure (maintaining the temperature below 40 °C). The resulting residue was purified by flash chromatography eluting with hexane/EtOAc/DCM (1:7:0.05) to afford the 3β-methanesulfonyloxy steroid **2.9**. The product was re-dissolved in DMPU (20 mL) and sodium azide (10 g, 15.4 mmol) was added and the mixture was allowed to stir at 40 °C 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 eluant affording **2.6** (0.87 g, 72%). (data same as above).

Methyl 3α-azido-7α,12α-*bis*[(*p*-trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate 2.10

To the azido-dihydroxy steroid **2.6** (1.0 g, 2.23 mmol) in dry DCE was added (ααα-*p*-trifluoromethyl)phenyl isocyanate (0.96 mL, 6.69 mmol) with a catalytic amount of chlorotrimethylsilane under an atmosphere of argon. The solution was allowed to stir at 40 °C for 1 day. The solvent was removed and the product was purified by flash chromatography using hexane/ethyl acetate (1:1) as eluant affording **2.10** (1.36 g, 73%); mp 158 °C TLC R_f 0.67 in 1:1 hexane/ethyl acetate; ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1740 cm^{-1}(C=O); δ_H (400 MHz, $CDCl_3$) 0.77 (3H, s, $18CH_3$), 0.86 (3H, d, $21CH_3$), 0.95 (3H, s, $19CH_3$), 3.18 (1H, m, 3β–H), 3.61 (3H, s, OCH_3), 4.98 (1H, m, 7β–H), 5.13 (1H, m, 12β–H), 6.94 (1H, d, carbamate NH), 7.07 (1H, d, carbamate NH), 7.58 (8H, s, aryl H); δ_C (100 MHz, $CDCl_3$) 12.24 (C18), 17.50 (C21), 22.44 (C19), 22.80, 25.75, 27.06, 29.03, 30.65,30.88, 31.34, 34.26, 34.45, 34.60, 34.93, 37.78, 41.00, 43.62,

45.33, 47.40, 51.45, 61.06, 72.32, 126.27, 126.32, 141.08, 152.42 (OCONH), 152.65 (OCONH), 174.58 (C24).

Methyl 3α-amino-7α,12α-*bis*[(*p*-trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate

To the azido *bis*-carbamate **2.10** (0.923 g, 1.123 mmol) dissolved in glacial acetic acid (8mL) was added Zn dust (1.5 g). The reaction mixture was stirred at room temperature for 18 h. The reaction was then filtered and the Zn was washed with acetic acid (3 x 5 mL). The combined filtrate solvent was removed by forming an azeotrope with toluene under reduced pressure. The ammonium acetate product was purified by passing through a silica gel plug using DCM/methanol (40:1) as eluant. The product was then partitioned between DCM and saturated aqueous $NaHCO_3$ solution. The aqueous fraction was washed once with DCM (20mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound as an acetate salt (0.7 g 71%); mp 174 °C; ν_{max} (film from $CDCl_3$) 2155 cm^{-1}(azide), 1730 cm^{-1}(C=O); δ_H (400 MHz, $CDCl_3$) 0.79 (3H, s, $18CH_3$), 0.94 (6H, m, $19CH_3$, $21CH_3$), 2.74 (1H, m, 3β-H), 3.59 (3H, s, OCH_3), 4.94 (1H, m, 7β-H), 5.21 (1H, m, 12β-H), 7.42 (4H, d, J 7.2 Hz, aryl H *ortho* to CF_3), 7.56 (4H, d, J 7.2 Hz, aryl H *ortho* to CF_3), 8.11 (1H, br s, carbamate NH), 8.60 (1H, br s, carbamate NH); δ_C (75.5 MHz, $CDCl_3$) 12.27 (C18), 17.60 (C21), 22.44 (C19), 22.90, 25.82, 27.12, 28.84, 29.19, 30.67, 30.94, 31.51, 34.32, 34.66, 35.03, 37.97, 40.80, 43.43, 45.31, 47.47, 51.21, 51.41, 72.18, 117.89, 118.06, 126.16, 141.46, 141.58, 152.66,152.74, 174.53 (C24).

Methyl 3α-(*p*-toluenesulfonylamino)-7α,12α-*bis*[(*p*-trifluoromethyl)phenyl-aminocarbonyloxy]-5β-cholan-24-oate 2.11

To the above salt (0.1 g, 0.126 mmol) in dry DCM (10 mL) was added *p*-toluenesulfonyl chloride (0.0264 g, 0.139 mmol) with a catalytic amount of TEA. The solution was allowed to stir under an atmosphere of argon for 2 h. 2M aqueous HCl (10 mL) was then added and the solution was allowed to stir for a further hour. The organic layer was then separated, dried over $MgSO_4$ and evaporated to dryness. The product was purified by chromatography using ethyl acetate/hexane (1:1) as eluant affording **2.11** as a white foam

(0.114 g, 94%); mp 216 °C; TLC R_f 0.22 in 1:1 hexane/ethyl acetate; ν_{max} (film from $CDCl_3$) 1715 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N); δ_H (400 MHz, $CDCl_3$) -0.20 (1H, m, 4α–H), 0.66 (3H, s, 18CH_3), 0.74 (3H, s, 19CH_3), 0.88 (3H, d, J 6.0 Hz, 21CH_3), 2.42 (3H, s, tosylamido CH_3), 2.58 (1H, m, 3β-H), 3.60 (3H, s, OCH_3), 4.83 (1H, m, 7β-H), 5.18 (1H, m, 12β-H), 6.49 (1H, br s, tosylamido NH), 7.16 (2H, d, J 7.5 Hz, tosylamido aryl H *meta* to SO_2), 7.29 (4H, m aryl H *ortho* to CF_3), 7.51 (2H, d, aryl *meta* to CF_3), 7.70 (2H, d, J 8.1 Hz, aryl H *meta* to CF_3), 7.79 (2H, d, J 8.4 Hz, tosylamido aryl H *ortho* to SO_2), 8.22 (1H, br s, carbamate NH), 8.55 (1H, br s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 12.28 (C18), 17.62 (C21), 21.27 (tosylamido CH_3), 21.85 (C19), 22.80, 25.77, 27.22, 28.32, 28.82, 30.67, 30.90, 31.07, 33.44, 34.73, 35.38, 37.76, 41.03, 43.08, 45.05, 45.98, 47.25, 51.35 (OCH_3), 53.19 (C3), 71.49 (C7), 75.69 (C12), 117.81, 118.5, 125.99, 126.35, 126.94, 129.52, 130.16, 137.98, 142.34, 143.94, 152.82 (NHCO), 153.04 (NHCO), 174.49 (C24).

Methyl 3α-(*p*-nitrobenzenesulfonylamino)-7α,12α-*bis*[(*p*-trifluoromethyl)-phenylaminocarbonyloxy]-5β-cholan-24-oate 2.12

To the salt from the reaction of **2.6** (0.05 g, 0.63 mmol) in dry DCM (10 mL) was added *p*-nitrobenzenesulfonyl chloride (0.0154 g, 0.693 mmol) with a catalytic amount of TEA. The solution was allowed to stir under an atmosphere of argon for 4 h. 2M aqueous HCl (10 mL) was then added and allowed to stir for a further hour. The organic layer was then separated, dried over $MgSO_4$ and evaporated to dryness. The product was purified by chromatography using ethyl acetate/hexane (1:1) as eluant affording **2.12** as a pale yellow foam (0.577 g, 91%); mp 170 °C; R_f 0.37 in ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH),1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N), 1530 cm^{-1}(NO_2); δ_H (400 MHz, $CDCl_3$) 0.18 (1H, m, H), 0.74 (3H, s, 18CH_3), 0.75 (3H, s, l9CH_3), 0.86 (3H, d, 21CH_3), 2.73 (1H, m, 3β-H), 3.61 (3H, s, OCH_3), 4.90 (1H, m, 7β-H), 5.15 1H, m, 12β-H), 5.79 (1H, br m, 3α-NH). 7.33 (1H, m, carbamate NH), 7.47 (4H, m, aryl H *ortho* to CF_3), 7.51 (2H, d, aryl H *ortho* to CF_3), 7.59 (2H, d, aryl H *ortho* to NO_2), 8.00 (2H, d, aryl H *meta* to CF_3), 8.23 (2H, d, aryl H *meta* to NO_2); δ_C (100.13 MHz, $CDCl_3$) 10.92, 11.83, 13.60, 17.15, 21.49, 22.12, 22.16, 22.37, 25.38, 26.75, 28.15, 28.58, 28.73, 29.21, 30.26, 30.42, 30.73, 31.11, 33.14, 34.21, 35.97, 37.26, 40.59, 40.89, 42.93, 44.81, 46.94, 51.00, 53.64, 71.58, 117.44, 117.63, 124.38, 124.92, 125.69, 127.25, 141.33, 145.38,

150.04, 151.95, 152.20, 174.06; Found C, 57.44; H, 5.40; N, 5.31. $C_{47}H_{54}F_6N_4O_{10}S$ requires C, 57.54; H, 5.55; N, 5.71.

Methyl 3α-(chloroacetyl)amino-7α,12α-*bis*[(*p*-trifluoromethyl)phenyl-aminocarbonyloxy]-5β-cholan-24-oate 2.13

To the salt from the reaction of **2.6** (0.14 g, 0.163 mmol) in dry DCM (10 mL) was added chloroacetyl chloride (0.014 mL, 0.18 mmol) with a catalytic amount of TEA. The solution was allowed to stir under an atmosphere of argon for 2 h. 2 M aqueous HCl (10 mL) was then added and allowed to stir for a further hour. The organic layer was then separated, dried over $MgSO_4$ and evaporated to dryness. The product was purified by flash chromatography using hexane/ethyl acetate (3:1) as eluant affording **2.13** as a white foam (0.156 g, 94%); mp 132 °C; TLC R_f 0.51 in ethyl acetate/hexane; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N); δ_H (400.13 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.86 (3H, d, $21CH_3$), 0.92 (3H, s, $19CH_3$), 3.35 (1H, m, 3β-H), 3.61 (3H, s, OCH_3), 3.95 (2H, s, CH_2Cl), 4.94 (1H, m, 7β-H), 5.11 (1H, m, 12β-H), 6.53 (1H, d, 3α-NH), 7.45 (1H, s, carbamate NH), 7.54 (1H, s, carbamate NH), 7.58 (8H, s, aryl H); δ_C (100.13 MHz, CDCl3) 12.13 (C18), 17.44 (C21), 22.46, 22.82 (C19), 25.71, 26.72, 27.13, 28.96, 30.67, 30.86, 31.34, 34.20, 34.45, 34.61, 35.45, 37.82, 41.25, 42.66 (CH_2Cl), 43.57, 45.28, 47.24, 51.50 (C3), 72.45, 118.02, 122.31, 124.80, 124.86, 125.23, 125.30, 125.90, 126.23, 141.36, 141.49, 152.62, 152.81, 165.49, ($COCH_2Cl$), 174.83 (C24); Found C, 59.71; H, 6.13; N, 4.55. $C_{43}H_{52}ClF_6N_3O_7$ requires C, 59.21; H, 6.01; N, 4.82.

Eicosyl 3α-(*p*-toluenesulfonylamino)-7α,12α-*bis*(3,5-dimethylphenylamino-carbonyloxy)-5β-cholan-24-oate 2.15

To a solution of **1.68** (0.098 g, 0.113 mmol) in MeOH/water (10:1) (5 mL) was added KOH (0.125 g, 0.223 mmol) to adjust the pH to 10. The reaction mixture was allowed to stir for 15 h. The solution was neutralised using aqueous HCl (1 molar) and DCM (5 mL) was added to the mixture. The organic layer was then separated. The aqueous fraction was washed once with DCM (10 mL). The organic layers were combined and dried over $MgSO_4$ and the volatiles were removed under reduced pressure. The white

salt was re-dissolved in MeOH/water (10:1) (3 mL) with aqueous cesium carbonate (20%) added dropwise until the pH of the solution was 7-8. The solution was re-evaporated to dryness by forming an azeotrope with toluene. To the resulting solid salt in dry DMF (3 mL) was added 1-bromoeicosane (0.1634 g, 0.452 mmol), sodium iodide (0.0075 g, 0.05 mmol) and allowed to stir at 40 °C for 24 h. The solution was washed with water (5 mL) and the organic phase was evaporated to a viscous oil. The product was purified by flash chromatography using hexane/ethyl acetate/chloroform (1:3:1) as eluant affording **2.15** as a colourless oil (0.025 g, 16%); mp 134 °C; TLC R_f 0.4 in 1:3:1 hexane/ethyl acetate/chloroform; ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1715 cm^{-1} br (C=O), 1385 cm^{-1}(SO_2N); δ_H (400 MHz, $CDCl_3$) -0.20 (1H, m, 4α-H), 0.69 (3H, s, 18CH_3), 0.75 (3H, s, 19CH_3), 0.89 (3H, d, 21CH_3), 0.90 (3H, t, ester CH_3), 1.29 (41H, m, ester CH_2's), 2.05 (6H, s, 2 x xylene CH_3), 2.67 (6H, s, 2 x xylene CH_3), 2.40 (3H, s, sulfonamido CH_3), 2.62 (1H, m, 3β-H), 4.03 (2H, t, ester CH_2), 4.83 (1H, m, 7β-H), 5.19 (1H, m, 12β-H), 5.71 (1H, d, J 6.8 Hz, 3α-NH), 6.62 (1H, s, xylene H *para* to NH), 6.66 (1H, s, xylene H *para* to NH), 7.02 (2H, s, xylene H *ortho* to NH), 7.19 (2H, d, J 8.1 Hz, *meta* to SO_2), 7.27 (2H, s, xylene H *ortho* to NH), 7.60 (1H, br s, carbamate NH), 7.69 (1H, br s, carbamate NH), 7.89 (2H, d, J 8.1 Hz, *ortho* to SO_2); δ_C (100.13 MHz, $CDCl_3$) 12.01 (C18), 13.62 (ester CH_3), 17.24 (C21), 20.70 (carbamate CH_3), 20.94 (carbamate CH_3), 21.01 (tosylamido CH_3); 21.77 (C19), 22.21, 26.28, 22.41, 25.45, 25.83, 26.77, 28.19, 28.78, 28.88, 29.04, 29.12, 29.22, 30.37, 30.82, 30.89, 31.45, 32.91, 33.55, 34.28, 35.39, 37.35, 40.89, 42.98, 44.81, 46.97, 52.72, 63.91, 70.42, 74.53, 115.42, 115.98, 123.77, 126.30, 129.60, 137.07, 137.84, 137.92, 138.43, 138.46, 143.64, 152.57 (NH<u>C</u>O), 152.63 (NH<u>C</u>O), 173.78 (C24).

Eicosyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate 2.17

To a solution of azido-diol **2.6** (4.0 g, 8.92 mmol) in MeOH/water (10:1) (10 mL) was added Cs_2CO_3 until the pH of the solution was 11-12 by litmus paper. The reaction mixture was allowed to stir overnight for reaction to go to completion. The reaction mixture was partially acidified to a pH 8 by by slow addition of aqueous HCl (1M). The solution was evaporated to dryness by forming an azeotrope with toluene. The product was re-dissolved in dry DMF (20 mL) with bromoeicosane (4.84 g, 13.4 mmol) with a catalytic amount of sodium iodide and allowed stir at 40 °C for 3 days. The solution was

then added to ether (60 mL) and water (200 mL) and the organic layer was further washed with water (100 mL) three times to remove the DMF solvent. The combined organic layers were dried (Na_2SO_4), evaporated to dryness and the product was purified by column chromatography using hexane/ethyl acetate/chloroform (1:3:1) as eluant yielding **2.17** in 67% yield (4.3 g); mp 59 °C; (HRMS: found: M^+, 713.607058, $C_{44}H_{79}N_3O_4Na_1$, requires m/z 713.607058); δ_H (300 MHz, $CDCl_3$) 0.69 (3H, s, $18CH_3$), 0.86 (3H, t, ester CH_3), 0.89 (3H, s, $19CH_3$), 0.98 (3H, d, $21CH_3$), 1.26 (38H, m, CH_2's of ester), 3.14 (1H, m, 3β-H), 3.87 (1H, m, 7β-H), 4.01 (1H, m, 12β-H), 4.06 (2H, t, OCH_2); δ_C (100.13 MHz, $CDCl_3$) 11.90, 13.60, 16.80, 21.98, 22.20, 22.80, 25.48, 25.92, 26.40, 27.14, 27.58, 28.19, 28.80, 28.87, 29.22, 30.36, 30.76, 31.44, 34.32, 34.87, 34.94, 35.08, 38.83, 41.33, 41.49, 46.08, 46.72, 60.84, 63.96, 67.85, 72.73, 174.06 (C24).

Eicosyl cholate 2.18

Aqueous caesium carbonate was added dropwise to a solution of cholic acid **1.65** (10 g, 23.81 mmol) in MeOH/H_2O (10:1) until the pH was 7/8. The solvent was removed by forming an azeotrope with toluene under reduced pressure and re-dissolved in dry DMF (60 mL). 1-Bromoeicosane(14 g, 39 mmol) with a catalytic amount of sodium iodide was added and the reaction was allowed to stir at 45 °C for 16 h. The solution was poured into cold water (200 mL) which was extracted with chloroform (100 mL). The extract was dried over $MgSO_4$ and re-evaporated to dryness. Flash chromatography on silica gel with chloroform/hexane(1:1) then chloroform/ethyl acetate/hexane (1:5:1) as the eluants yielded the ester **2.18** (11.8 g 72%); mp 81 °C; TLC R_f 0.52 in ethyl acetate/hexane (1:1); δ_H (300 MHz, $CDCl_3$) 0.66 (3H, s, $18CH_3$), 0.87 (6H, m, $19CH_3$, ester CH_3), 0.97 (3H, d, $21CH_3$), 1.26 (38H, m, CH_2's of ester), 3.42 (1H, m, 3β-H), 3.82 (1H, m, 7β-H), 3.94 (1H, m, 12β-H), 4.03 (2H, t, OCH_2), δ_C (100.13 MHz, $CDCl_3$) 12.47, 14.07, 17.31, 22.45, 22.65, 23.21, 25.94, 26.42, 27.47, 28.21, 28.67, 29.26, 29.33, 29.52, 29.58, 29.68, 30.44, 30.96, 31.36, 31.90, 34.69, 34.75, 35.22, 35.32, 39.57, 41.52, 41.69, 46.46, 47.09, 64.42, 68.44, 71.90, 73.05, 174.39 (C24).

Eicosyl 3β-methanesulfonyloxy-7α,12α-*bis*-hydroxy-5β-cholan-24-oate 2.19

Eicosyl Cholate **2.18** (7.1 g, 10.3 mmol), triphenylphosphine (8 g) methanesulfonic acid (1.4 mL) and TEA (0.57 mL, 4.12 mmol) were dissolved in dry THF (50 mL). DEAD (5.3 mL) was added dropwise over 15 mins while stirring at 40 °C under an atmosphere of argon. The mixture was evaporated to dryness and partially purified by flash chromatography on silica gel with ethyl acetate/chloroform (10:1) as the eluant yielding the partially pure mesylate **2.19** (5.1 g, 65%); mp 56 °C; TLC R_f 0.60 in ethyl acetate/hexane (1:1); δ_H (400 MHz, $CDCl_3$) 0.68 (3H, s, 18CH_3), 0.87 (3H, s, 19CH_3), 0.96 (3H, d, 21CH_3), 1.25 (41H, m, ester CH_2's), 2.63 (1H, t, 4α-H), 3.85 (1H, m, 7β-H), 3.97 (1H, m, 12β-H), 4.04 (2H, t, ester CH_2), 4.96 (1H, m, 3β-H).

Eicosyl 3α-azido -7α,12α-*bis*-hydroxy-5β-cholan-24-oate 2.17

Sodium azide (5 g) was added to a solution of the eicosyl 3β-mesylate **2.19** in DMPU (28 mL) and allowed to stir at 40 °C for 16 h. The solution was poured into cold water (100 mL) and extracted with chloroform (60 mL). The extract was dried over $MgSO_4$ and evaporated to dryness. Column chromatography on silica gel with chloroform/hexane/ethyl acetate (1:1:1) as the eluants yielded the azide **2.17** (4.18 g, 90%); mp 59 °C; TLC R_f 0.71 in ethyl acetate/hexane (1:1); δ_H (300 MHz, $CDCl_3$) 0.69 (3H, s, 18CH_3), 0.86 (3H, t, ester CH_3), 0.89 (3H, s, 19CH_3), 0.98 (3H, d, 21CH_3), 1.26 (38H, m, CH_2's of ester), 3.14 (1H, m, 3β-H), 3.87 (1H, m, 7β-H), 4.01 (1H, m, 12β-H), 4.06 (2H, t, OCH_2); δ_C (100.13 MHz, $CDCl_3$) 11.90, 13.60, 16.80, 21.98, 22.20, 22.80, 25.48, 25.92, 26.40, 27.14, 27.58, 28.19, 28.80, 28.87, 29.22, 30.36, 30.76, 31.44, 34.32, 34.87, 34.94, 35.08, 38.83, 41.33, 41.49, 46.08, 46.72, 60.84, 63.96, 67.85, 72.73, 174.06 (C24).

Eicosyl 3α-azido-7α,12α-*bis*(*p*-trifluoromethyl)phenylaminocarbonyloxy-5β-cholan-24-oate 2.20

To the azido-diol **2.17** (2.0 g, 2.8 mmol) in dry DCE (20 mL) was added (ααα-*p*-trifluoromethyl)phenyl isocyanate (0.96 mL, 6.69 mmol) with a catalytic amount of chlorotrimethylsilane under an atmosphere of argon. The solution was allowed to stir at 40 °C for 24 h. The solvent was removed and the product was purified by flash

chromatography using hexane/ethyl acetate (1:1:1) as eluant affording the *bis*-carbamate **2.20** (1.51 g, 62%); mp 165 °C; TLC R_f 0.81 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1740 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.75 (3H, s, $18CH_3$), 0.90 (3H, t, ester CH_3), 0.94 (3H, d, $21CH_3$), 0.97 (3H, s, $19CH_3$), 1.25 (38H, m, CH_2's of ester), 3.18 (1H, m, 3β-H), 4.01 (2H, t, OCH_2), 4.97 (1H, m, 7β-H), 5.13 (1H, m, 12β-H), 6.94 (1H, br s, carbamate NH), 7.06 (1H, br s, carbamate NH), 7.67 (8H, s, aryl H); δ_C (100.13 MHz, $CDCl_3$) 12.33, 14.10, 17.60, 22.53, 22.68, 22.87, 25.84, 25.90, 26.83, 27.14, 28.61, 29.13, 29.22, 29.35, 29.49, 29.56, 29.68, 30.74, 31,19, 31.46, 31.91, 34.38, 34.50, 34.65, 35.04, 37.86, 41.08, 43.73, 45.44, 47.47, 61.20, 64.58, 72.38, 118.18, 118.20, 126.37, 141.07, 152.43, 152.68, 174.22.

Eicosyl 3α-azido-7α,12α-*bis*(2,4-difluorophenylaminocarbonyloxy)-5β-cholan-24-oate 2.21

To the azido-diol **2.17** (1.0 g, 1.4 mmol) in dry DCE (10 mL) was added 2,4-difluorophenyl isocyanate (0.36 mL, 3.08 mmol) with a catalytic amount of chlorotrimethylsilane under an atmosphere of argon. The solution was allowed to stir at 40 °C for 24 h. The solvent was removed and the product was taken up in dichloromethane and filtered when cold after 24 h. The product was then dissolved in hexane and filtered cold after a further 24 h. The partially pure product was flash columned on silica gel with hexane/ethyl acetate (3:1) as the eluant yielding the *bis*-carbamate **2.21** (0.88 g, 63%); mp 171 °C; TLC R_f 0.44 in ethyl acetate/hexane/chloroform (3:1:1); ν_{max} (film from $CDCl_3$) 2160 cm^{-1}(azide), 1715 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.86 (3H, t, ester CH_3), 0.88 (3H, d, $21CH_3$), 0.93 (3H, s, $19CH_3$), 1.24 (38H, m, CH_2's of ester), 3.16 (1H, m, 3β-H), 4.01 (2H, t, OCH_2), 4.94 (1H, m, 7β-H), 5.10 (1H, m, 12β-H), 6.89 (7H, m, aryl H, carbamate NH), 8.01 (1H, br s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 11.87, 13.60, 17.06, 22.09, 22.19, 22.37, 25.39, 25.45, 26.29, 26.64, 28.18, 28.60, 28.77, 28.86, 29.03, 29.10, 29.16, 29.21, 29.27, 30.29, 30.72, 30.94, 31.43, 33.88, 34.10, 34.18, 34.62, 37.43, 40.72, 43.11, 44.93, 47.04, 60.57, 64.00, 64.05, 71.97, 102.94, 103.01, 103.21, 103.45, 103.51, 110.59, 110.63, 110.81, 110.84, 121.78, 122.04, 122.14, 152.41 (NHCO), 152.61 (NHCO), 159.19 (C24).

Eicosyl 3α-azido-7α,12α-*bis*(chloroacetylaminocarbonyloxy)-5β-cholan-24-oate 2.22

To the azido-diol **2.17** (1.0 g, 1.4 mmol) in dry DCE (10 mL) was added chloroacetyl isocyanate (0.24 mL, 2.8 mmol) with a catalytic amount of chlorotrimethylsilane under an atmosphere of argon. The solution was allowed to stir at room temperature for 24 h. The solvent was removed and the product was purified by chromatography using hexane/ethyl acetate/chloroform (3:1:1) as eluant affording the *bis*-carbamate **2.22** (1.09 g, 82%); mp 122 °C; TLC R_f 0.28 in ethyl acetate/hexane (1:3); ν_{max} (film from $CDCl_3$) 2150 cm^{-1}(azide), 1730 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.78 (3H, s, $18CH_3$), 0.90 (6H, m, $19CH_3$, ester CH_3), 0.94 (3H, d, $21CH_3$,), 0.97 (3H, s, ester CH_3), 1.28 (38H, m, CH_2's of ester), 3.00 (1H, m, 3β-H), 4.06 (2H, t, OCH_2), 4.71 (2H, s, CH_2Cl), 4.49 (2H, s, CH_2Cl), 4.94 (1H, m, 7β-H), 5.27 (1H, m, 12β-H), 8.65 (1H, s, carbamate NH), 8.83 (1H, s, carbamate NH); δ_C(100.13 MHz, $CDCl_3$) 11.35, 13.58, 17.17, 21.79, 22.16, 22.58, 24.44, 25.43, 26.12, 26.68, 27.21, 28.16, 28.75, 28.84, 29.02, 29.08, 29.18, 30.15, 30.58, 30.67, 31.41, 33.89, 33.94, 34.15, 34.88, 37.80, 40.61, 41.45, 43.48, 43.76, 44.55, 46.81, 60.20, 63.92, 73.68, 77.54, 150.52 (OCONH), 150.64 (OCONH), 167.55 ($COCH_2Cl$), 168.12 ($COCH_2Cl$), 173.51 (C24).

Eicosyl 3α-(*p*-toluenesulfonylamino)-7α,12α-*bis*(*p*-trifluoromethyl)phenyl-aminocarbonyloxy-5β-cholan-24-oate 2.23

To the *bis*-carbamate steroid **2.10** (0.89 g, 0.87 mmol) in glacial acetic acid (16 mL) was added zinc dust (1.07 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (20 mL) with toluenesulfonyl chloride (0.1666 g, 0.873 mmol), a catalytic amount of TEA and the reaction was allowed to stir for 2 h under an atmosphere of argon. The solution was washed with aqueous HCl (2 M) and water, before being dried, and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluants yielded the tosylamido steroid **2.23** (0.485 g, 67%); mp 146 °C; (HRMS: found: M^+, 1215.674409, $C_{67}H_{95}N_3O_8F_6S_1$, requires m/z

1215.674408); TLC R_f 0.44 in ethyl acetate/hexane (1:1); ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 2140 cm^{-1}(azide), 1720 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N); δ_H (400.13 MHz, $CDCl_3$) -0.13 (1H, m, 4α-H), 0.69 (3H, s, 18CH_3), 0.76 (3H, s, 19CH_3), 0.89 (3H, d, J 4.2 Hz, 21CH_3), 0.90 (3H, t, ester CH_3), 1.28 (38H, m, CH_2's of ester), 2.45 (3H, s, tosylamido CH_3), 2.62 (1H, m, 3β-H), 4.02 (2H, t, OCH_2), 4.87 (1H, m, 7β-H), 5.21 (1H, m, 12β-H), 5.89 (1H, d, J 6 Hz, 3α-NH), 7.19 (2H, d, tosylamido aryl H *meta* to SO_2), 7.30 (2H, d, aryl H *ortho* to CF_3), 7.36 (2H, d, aryl H *meta* to CF_3), 7.51 (2H, d, aryl H *ortho* to CF_3), 7.69 (2H, d, aryl H *meta* to CF_3), 7.70 (1H, s, carbamate NH), 7.7961 (2H, d, tosylamido aryl H *ortho* to SO_2), 7.87 (1H, s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 12.41 (C18), 14.07, 17.63 (C21), 21.42 (tosylamido CH_3), 21.82 (C19), 22.66, 22.81, 25.88, 26.04, 27.24, 28.61, 29.21, 29.33, 29.47, 29.54, 29.67, 30.77, 31.11, 31.26, 31.90, 33.36, 34.70, 35.70, 37.68, 40.98, 43.37, 45.23, 47.49, 53.01 (C3), 64.47, 71.71 (C7), 75.80 (C12), 117.6, 118.21, 118.29, 125.86, 126.02, 126.5, 130.25, 137.18, 141.9, 144.80, 152.60 (NHCO), 152.79 (NHCO), 174.20 (C24).

Eicosyl 3α-(*p*-nitrobenzenesulfonylamino)-7α,12α-*bis* (*p*-trifluoromethyl)-phenylaminocarbonyloxy-5β-cholan-24-oate 2.24

To the *bis*-carbamate steroid **2.20** (0.74 g, 0.73 mmol) in glacial acetic acid(16 mL) was added zinc dust (1.0 g) while vigorously stirring. After 8 h the mixture was filtered, the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The solution was washed with saturated aqueous sodium hydrogen carbonate and water, then dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (25 mL) with nitrobenzenesulfonyl chloride (0.193 g, 0.87 mmol), a catalytic amount of TEA and the reaction was allowed to stir for 2 h under an atmosphere of argon. The solution was washed with aqueous HCl (2M) and water, dried (Na_2SO_4), and the solvent was removed. Column chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluants yielded the nosylamido steroid **2.24** (0.55 g, 65%); mp 161 °C; TLC R_f 0.30 in ethyl acetate/hexane (1:1); ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 2145 cm^{-1}(azide), 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1540 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.10 (1H, m, 4α-H), 0.73 (3H, s, 18CH_3), 0.77 (3H, s, 19CH_3), 0.88 (3H, d, 21CH_3), 0.90 (3H, t, ester CH_3), 1.27 (38H, m, CH_2's of ester), 2.71 (1H, m, 3β-H), 4.01 (2H, t, OCH_2), 4.91 (1H, m, 7β-H), 5.18 (1H,

m, 12β-H), 5.89 (1H, d, 3α-NH), 7.34 (1H, s, carbamate NH), 7.42 (9H, m, carbamato aryl H, carbamate NH), 8.04 (2H, d, J 8.8 Hz, nosylamido aryl H *ortho* to NO_2), 8.2843 (2H, d, J 8.8 Hz, nosylamido aryl H *ortho* to SO_2); δ_C (100.13 MHz, $CDCl_3$) 13.60 (C18), 17.16 (C21), 21.47, 22.20, 22.38, 25.42, 28.19, 28.75, 28.87, 29.02, 29.21, 30.30, 30.72, 31.44, 33.09, 34.19, 37.27, 42.91, 44.81, 46.98, 53.63 (OCH_3), 64.08 (C3), 71.57 (C7), 75.88 (C12), 117.41, 117.61, 124.84, 125.69, 127.24, 141.10, 144.13, 145.33, 150.06, 151.91 (NHCO), 152.17 (NHCO), 173.71 (C24); m/z (FAB) 1269.5 (m + Na, 100%), 1064 (15), 837 (30), 635 (20), 455 (10).

Eicosyl 3α-(2,4-dinitrobenzenesulfonylamido)-7α,12α-*bis*(*p*-trifluoro-methyl)-phenylaminocarbonyloxy-5β-cholan-24-oate 2.25

To the azido *bis*-carbamate steroid **2.20** (0.79 g, 0.731 mmol) in glacial acetic acid (14 mL) was added zinc dust (0.8 g) and the reaction was allowed to stir vigorously. After 8 h the mixture was filtered and the filtrate was evaporated to dryness by forming an azeotrope with toluene. The acetate salt was re-dissolved in DCM (40 mL) and shaken with aqueous sodium hydrogen carbonate then water. The organic layer was dried over $MgSO_4$ and the solution was again evaporated to dryness. The salt was re-dissolved in dry DCM (20 mL) with 2,4-dinitrobenzenesulfonyl chloride (0.260 g, 0.740 mmol), and a catalytic amount of triethylamine and allowed to stir at 0 °C for 3 h under an atmosphere of argon, followed by stirring overnight at room temperature. The resulting solution was washed with aqueous HCL (2 M), water, dried ($MgSO_4$) and evaporated to dryness. Column chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as eluant yielded **2.25** in 76% yield (0.58 g); mp 182 °C; (HRMS: found: M^+, 1291.628916, $C_{66}H_{91}N_5O_{12}F_6S$, requires m/z 1291.628915); TLC R_f 0.41 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 1725 cm^{-1}(C=O), 1370 cm^{-1}(SO_2N) 1520 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.37 (1H, m, 4α-H), 0.75 (3H, s, $18CH_3$), 0.77 (3H, s, $19CH_3$), 0.87 (6H, m, $21CH_3$, ester CH_3), 1.27 (38H, m, CH_2's of ester), 2.96 (1H, m, 3β-H), 4.02 (2H, t, OCH_2), 4.89 (1H, m, 7β-H), 5.10 (1H, m, 12β-H), 5.89 (1H, d, 3α-NH), 7.25 (1H, s, 7α-NH), 7.48 (8H, m, carbamato aryl H), 8.29 (1H, d, nosylamido aryl H *meta* to SO_2), 8.42 (2H, m, nosylamido aryl H *ortho* to SO_2, and NO_2, nosylamido aryl H *ortho* to NO_2); δ_C (100.13 MHz, $CDCl_3$) 11.80, 13.61, 17.08, 21.76, 22.20, 22.34, 25.40, 25.43, 26.69.28.14, 28.39, 28.56, 28.75,

28.87, 29.02, 29.09, 29.21, 30.28, 30.68, 30.81, 31.44, 33.38, 34.16, 34.47, 36.22, 37.28, 40.82, 43.06, 44.88, 46.86, 54.79 (OCH_3), 64.12 (C3), 71.66 (C7), 117.55, 117.65, 117.69, 120.13, 122.24, 122.27, 124.68, 124.78, 124.93, 124.96, 125.00, 125.11, 125.79, 125.82, 125.85, 126.75, 131.38, 138.86, 140.78, 140.85, 147.43, 149.52, 151.97 (NHCO), 152.29 (NHCO), 174.11 (C24).

Eicosyl 3α-(chloroacetyl)amino-7α,12α-*bis*[(*p*-trifluoromethyl)phenyl-amino-carbonyloxy]-5β-cholan-24-oate 2.26

To the *bis*-carbamate **2.20** (0.3 g, 0.28 mmol) in glacial acetic acid (6 mL) was added zinc dust (0.3 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (15 mL) with chloroacetyl chloride (0.025 mL, 0.31 mmol), a catalytic amount of TEA and it was allowed to stir for 24 h under an atmosphere of argon. The solution was washed with aqueous HCl (2 M) and water, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (3:2:1) as the eluant yielded **2.26** (0.25 g, 92%); mp 141 °C; TLC R_f 0.52 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 1740 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.82 (3H, s, $18CH_3$), 0.89 (6H, m, ester CH_3, $21CH_3$), 0.99 (3H, s, $19CH_3$), 1.28 (38H, m, ester CH_2), 3.42 (1H, m, 3β-H), 3.97 (2H, s, CH_2Cl), 4.02 (2H, t, ester CH_2), 5.00 (1H, m, 7β-H), 5.16 (1H, m, 12β-H), 6.47 (1H, br s, 3α-NH), 7.1 (1H, s, carbamate NH), 7.25 (1H, s, carbamate NH), 7.61 (8H, s, aryl H); δ_C (100.13 MHz, $CDCl_3$) 11.79, 13.61, 17.11, 22.11, 22.20, 22.43, 25.34, 25.44, 26.18, 26.75, 28.14, 28.59, 28.75, 28.87, 29.02, 29.09, 29.21, 30.30, 30.72, 30.91, 31.44, 33.83, 34.24, 35.12, 37.49, 40.92, 42.36, 43.20, 44.89, 46.88, 64.12, 72.16, 117.57, 117.66, 124.64, 124.98, 125.87, 140.90, 152.07, 152.37, 164.94,173.90; (HRMS: found: 1137.637150, $C_{61}H_{90}N_3O_7$, requires m/z 1137.637150).

Eicosyl 3α-(*p*-nitrobenzenesulfonylamino)-7α,12α-*bis*-(2,4-difluorophenyl-aminocarbonyloxy)-5β-cholan-24-oate 2.28

To the *bis*-carbamate **2.21** (0.89 g, 0.87 mmol) in glacial acetic acid (14 mL) was added zinc dust (1.1 g) while vigorously stirring. After 8 h, the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (20 mL) with *p*-nitrobenzenesulfonyl chloride (0.23 g, 1.04 mmol), a catalytic amount of TEA and the reaction was allowed to stir for 24 h under an atmosphere of argon. The solution went from yellow to orange overnight. The solution was washed with aqueous HCl (2 M) and water, dried, and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluant yielded the dinitrobenzoyl steroid **2.28** (0.73 g, 74%); mp 176 °C; (HRMS: found: M+, 1182.631381 $C_{64}H_{90}N_4O_{10}F_4S$, requires m/z 1182.631380; TLC R_f 0.44 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1530 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.57 (1H, m, 4α-H), 0.76 (3H, s, 18CH_3), 0.84 (3H, s, 19CH_3), (6H, m, 21CH_3, ester CH_3), 1.27 (38H, m, CH_2's of ester), 2.87 (1H, m, 3β-H), 4.03 (2H, t, OCH_2), 4.92 (1H, m, 7β-H), 5.13 (1H, m, 12β-H), 6.78 (6H, m, carbamato aryl H), 7.06 (1H, s, carbamate NH), 7.97 (1H, s, carbamate NH), 8.00 (2H, d, nosylamido aryl H *ortho* to NO_2), 8.30 (2H, d, nosylamido aryl H *ortho* to SO_2); δ_C (100.13 MHz, $CDCl_3$) 11.90, 13.60, 13.63, 17.08, 21.96, 22.20, 22.36, 25.44, 25.51, 26.68, 28.17, 28.43, 28.76, 28.87, 29.03, 29.09, 30.30, 30.77, 30.85, 31.43, 33.41, 34.20, 34.61, 35.98, 37.34, 40.90, 41.71, 43.01, 44.92, 47.02, 53.58 (OCH_3), 64.03 (C3), 71.72 (C7), 102.80, 103.05, 103.31, 110.77, 111.04, 122.16, 122.32, 122.41, 123.83, 127.51, 127.63, 146.14, 149.59 (NHCO), 152.55 (NHCO), 173.68 (C24).

Eicosyl 3α-(3,5-dinitrobenzoylamino)-7α,12α-*bis*(*p*-trifluoromethyl)phenyl-aminocarbonyloxy-5β-cholan-24-oate 2.27

To the *bis*-carbamate **2.20** (0.375 g, 0.348 mmol) in glacial acetic acid (7 mL) was added zinc dust (0.4 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and the solid was

redissolved in $CHCl_3$. The liquor was washed with aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCE (20 mL) with 3,5-dinitrobenzoyl chloride (0.088 g, 0.383 mmol), a catalytic amount of TEA and allowed to stir for 24 h under an atmosphere of argon. The solution went from yellow to deep red overnight. The solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (3:2:1) as the eluant yielded the dinitrobenzoyl steroid **2.27** (0.25 g, 58%); mp 178 °C; (HRMS: found: m^+, 1256.661927 $C_{67}H_{92}N_5F_6O_{11}Na$, requires m/z 1256.661928); TLC R_f 0.44 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N), 1525 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.83 (3H, s, $18CH_3$), 0.90 (6H, m, $21CH_3$, ester CH_3), 1.03 (3H, s, $19CH_3$), 1.28 (38H, m, CH_2's of ester), 3.65 (1H, m, 3β-H), 4.01 (2H, t, OCH_2), 5.03 (1H, m, 7β-H), 5.15 (1H, m, 12β-H), 6.41 (1H, d, 3α-NH), 7.17 (1H, s, carbamate NH), 7.32 (1H, s, carbamate NH), 7.55 (8H, m, carbamato aryl H), 8.81 (2H, s, aryl H *ortho* to carbonyl), 9.09 (1H, t, aryl H para to carbonyl); δ_C (100.13 MHz, CDCl3) 12.19, 14.07 (C18), 15.52 (C21), 22.62, 22.65, 22.90, 25.77, 25.88, 26.94, 27.19, 28.56, 29.12, 29.19, 29.32, 29.46, 29.54, 29.60, 29.67, 30.75, 31.16, 31.44, 31.90, 34.41, 34.50, 34.67, 35.59, 37.97, 41.51, 43.69, 45.37, 47.30, 52.52 (C7), 64.65, 117.99, 120.88, 126.28, 126.81, 138.31, 141.27, 148.56, 152.52 (NHCO), 152.85 (NHCO), 162.20, 174.53 (C24).

Eicosyl 3α-(*p*-nitrobenzenesulfonylamino)-7α,12α-*bis*(acetylaminocarbonyloxy)-5β-cholan-24-oate 2.29

To the *bis*-carbamate steroid **2.22** (0.95 g, 1.00 mmol) in glacial acetic acid (16 mL) was added zinc dust (1.0 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (25 mL) with nitrobenzenesulfonyl chloride (0.266 g, 1.2 mmol), a catalytic amount of TEA and allowed to stir for 2 hrs under an atmosphere of argon. The solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated to dryness. Flash chromatography on silica gel with ethyl acetate/hexane/chloroform (1:1:1)

as the eluants yielded the nosylamido steroid **2.29** (0.68 g, 67%); mp 138 °C; TLC R_f 0.80 in dichloromethane/methanol (10:1); ν_{max} (film from $CDCl_3$) 3600-3200 cm^{-1}(NH), 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1520 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.75 (3H, s, 18CH_3), 0.88 (12H, m, 19CH_3, 21CH_3, 2 x acetyl CH_3), 1.26 (38H, m, CH_2's of ester), 3.07 (1H, m, 3β-H), 4.03 (2H, t, OCH_2), 4.90 (1H, m, 7β-H), 5.13 (1H, m, 12β-H), 6.01 (1H, d, 3α-NH), 8.12 (2H, d, nosylamido aryl H *meta* to SO_2), 8.21 (2H, d, nosylamido aryl H *ortho* to SO_2), 8.61 (1H, s, carbamate NH), 8.75 (1H, s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 11.71 (C18), 13.61, 17.23 (C21), 21.95 (C19), 22.19, 22.43, 23.77, 25.44, 27.93, 28.18, 28.77, 28.87, 28.91, 29.04, 29.11, 29.20, 29.31, 30.24, 30.76, 31.43, 33.59, 34.14, 34.66, 35.62, 37.44, 40.79, 42.28, 44.74, 46.94, 53.39, 64.04, 72.56,123.97 (aryl C *meta* to SO_2), 127.94 (aryl C *ortho* to SO_2), 145.97, 149.66, 151.01 (NHCO), 151.25 (NHCO), 173.08, 174.61 (C24). m/z (FAB) 1087.5 (m +2Na-H, 100%), 923.5 (55), 843.5 (15), 717.5 (10).

Eicosyl 3α-(chloroacetylamido)-7α,12α-dihydroxy-5β-cholan-24-oate 2.30

To the azido-diol **2.17** (2.55 g, 3.57 mmol) in glacial acetic acid (30 mL) was added zinc dust (2.6 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (30 mL) with chloroacetyl chloride (0.31 mL, 3.8 mmol), a catalytic amount of TEA and allowed to stir for 2 h under an atmosphere of argon at room temperature. The solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluants yielded **2.30** (2.47 g, 92%); mp 74 °C; TLC R_f 0.28 in ethyl acetate/hexane (1:1); ν_{max} (film from $CDCl_3$) 1740 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.68 (3H, s, 18CH_3), 0.88 (3H, t, ester CH_3), 0.90 (3H, m, 19CH_3,), 0.96 (3H, d, 21CH_3), 1.28 (38H, m, CH_2's of ester), 2.82 (2H, br s, 2 x OH), 3.65 (1H, m, 3β-H), 3.84 (1H,m, 7β-H), 4.00 (1H, m, 12β-H), 4.02 (2H, t, OCH_2), 4.03 (2H, s, CH_2Cl), 6.48 (1H, d, 3α-NH); δ_C (100.13 MHz, $CDCl_3$) 12.42, 13.98, 17.23, 22.44, 22.57, 23.10, 25.85, 26.52, 27.45, 28.29, 28.57, 29.16, 29.23, 29.44, 29.48, 29.58, 30.78, 31.24, 31.80, 34.44, 34.51,

35.20, 35.58, 35.96, 39.31, 41.67, 41.90, 42.60, 46.48, 47.21, 49.96, 64.39, 68.26, 73.07, 164.96, 174.32 (C24).

Eicosyl 3α-(4-nitrobenzenesulfonylamido)-7α,12α-dihydroxy-5β-cholan-24-oate 2.31

To the azido-diol **2.17** (2.55 g, 3.57 mmol) in glacial acetic acid (30 mL) was added zinc dust (2.6 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and redissolved in $CHCl_3$. The liquor was washed with saturated aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCM (30 mL) with *p*-nitrobenzenesulfonyl chloride (0.84 g, 3.8 mmol), and a catalytic amount of TEA and allowed to stir for 2 h under an atmosphere of argon at 0 °C. The solution was allowed to heat to room temperature and the solution was washed with aqueous HCl (2 M) and water, dried ($MgSO_4$), and evaporated to dryness. Column chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluants yielded **2.31** (2.47 g, 92%); mp 93 °C; TLC R_f 0.28 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 1730 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1530 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.67 (3H, s, 18CH_3), 0.88 (6H, m,19CH_3, ester CH_3), 0.97 (3H, d, 21CH_3), 1.28 (38H, m, CH_2's of ester), 2.74 (2H, br s, 2 x OH), 3.00 (1H, m, 3β-H), 3.84 (1H,m, 7β-H), 4.00 (1H, m, 12β-H), 4.05 (2H, t, OCH_2), 6.10 (1H, d, 3α-NH), 8.09 (2H, d, aryl H *ortho* to NO_2), 8.33 (2H, d, aryl H *meta* to NO_2); δ_C (100.13 MHz, $CDCl_3$) 11.42, 13.05, 16.31, 21.43, 21.64, 22.11, 24.91, 25.50, 26.43, 27.09, 27.63, 27.81, 28.23, 28.30, 28.50, 28.55, 28.65, 28.72, 29.88, 30.28, 30.88, 33.40, 33.43, 34.14, 34.77, 36.44, 38.34, 70.87, 40.98, 45.40, 46.14, 53.36, 63.52, 67.32, 71.95, 123.25, 127.13, 146.63, 148.83, 173.42 (C24).

Eicosyl 3α-(chloroacetylamido)-7α,12α-*bis*(chloroacetylaminocarbonyloxy)-5β-cholan-24-oate 2.33

To the chloroacetyl-diol **2.30** (1.0 g, 1.4 mmol) in dry DCE (20 mL) was added chloroacetyl isocyanate (0.24 mL, 2.8 mmol) with a catalytic amount of chlorotrimethylsilane under an atmosphere of argon. The solution was allowed to stir at

40 °C for 24 hrs. The solvent was removed and the product was purified by chromatography using hexane/ethyl acetate/chloroform (3:1:1) as eluant affording the *bis*-carbamate **2.33** (1.09 g, 82%); mp 118 °C; TLC R_f 0.28 in ethyl acetate/hexane (1:3); ν_{max} (film from $CDCl_3$) 1740 cm^{-1}(C=O); δ_H (400.13 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.88 (6H, m, 21 CH_3, ester CH_3), 0.97 (3H, s, $19CH_3$), 1.26 (38H, m, CH_2's of ester), 3.65 (1H, m, 3β-H), 3.40 (2H, s, 3α-CH_2Cl), 4.04 (2H, t, OCH_2), 4.62 (2H, s, CH_2Cl), 4.70 (2H, s, CH_2Cl), 4.96 (1H, m, 7β-H), 5.16 (1H, m, 12β-H), 6.46 (1H, d, 3α-NH), 8.60 (1H, s, carbamate NH), 9.27 (1H, s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 11.37, 13.57, 17.20, 21.98, 21.15, 22.65, 24.51, 25.41, 26.65, 27.26, 28.14, 28.73, 28.82,29.00, 29.16, 30.14, 30.56, 30.87, 31.39, 34.05, 35.10, 35.17, 37.78, 40.69, 41.67, 42.35, 44.03, 44.65, 46.87, 49.53, 52.99 (C3), 63.95, 74.00 (C7), 77.92 (C12), 150.46 (NHCO), 150.62 (NHCO), 164.53, 167.40, 168.24, 169.54, 173.45 (C24); m/z (FAB) 1084.4 (m +2Na -2H, 75%), 909.5 (40), 750.5 (100%).

Eicosyl 3α-(2,4-dinitrobenzenesulfonylamido)-7α,12α-dihydroxy-5β-cholan-24-oate 2.32

To the azido-diol **2.17** (5.1 g, 7.14 mmol) in glacial acetic acid (60 mL) was added zinc dust (5.2 g) while vigorously stirring. After 8 h the mixture was filtered and the reaction solvent was removed by forming an azeotrope with toluene and the solid was redissolved in $CHCl_3$. The liquor was washed with aqueous sodium hydrogen carbonate and water, dried over $MgSO_4$ and re-evaporated to dryness. The white solid was re-dissolved in dry DCE (60 mL) at 0 °C with 2,4-dinitrobenzenesulfonyl chloride (0.193g, 0.87mmol), a catalytic amount of TEA and the reaction was allowed to stir for 2 h under an atmosphere of argon. The solution was allowed to heat to room temperature. The solution was washed with aqueous HCl (2M) and water, dried ($MgSO_4$), and evaporated to dryness. Flash chromatography on silica gel with hexane/ethyl acetate/chloroform (1:1:1) as the eluants yielded the dinitrobenzene-sulfonamide **2.32** (0.55 g, 52%); mp 93 °C; TLC R_f 0.28 in ethyl acetate/hexane (1:1); ν_{max} (film from $CDCl_3$) 1740 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N); δ_H (400.13 MHz, $CDCl_3$) 0.70 (3H, s, $18CH_3$), 0.90 (6H, m, $19CH_3$, ester CH_3,), 0.98 (3H, s, $21CH_3$), 1.28 (38H, m, CH_2's of ester), 3.24 (1H, m, 3β), 4.01 (1H, m, 12β), 4.07 (2H, t, OCH_2), 5.47 (1H, d, 3α-NH), 8.38 (1H, d, aryl H *meta* to SO_2),8.54 (1H, d, aryl H *ortho* to SO_2), 8.68 (1H, s, aryl H *ortho* to NO_2); δ_C (100.13 MHz, $CDCl_3$)

12.05, 13.62, 16.85, 22.10, 22.20, 25.48, 28.21, 28.80, 28.87, 29.07, 29.12, 29.18, 29.22, 30.38, 31.45, 34.00 34.71, 38.99, 46.10, 46.88, 55.08, 64.05, 67.69, 72.49, 76.22, 76.54, 76.86, 120.22, 126.66, 131.66, 173.92 (C24).

Eicosyl 3α-(2,4-dinitrobenzenesulfonylamido)-7α,12α-*bis*(chloro-acetylaminocarbonyloxy)-5β-cholan-24-oate 2.36

To the dinitrobenzenesulfonamide steroid **2.32** (0.66 g, 0.72 mmol) in dry DCE (10 mL) was added chloroacetyl isocyanate (0.13 mL, 1.51 mmol) with a catalytic amount of chlorotrimethylsilane, and the solution was allowed to stir at room temperature under an atmosphere of argon for 1 day. The solvent was removed and the product was purified by flash chromatography using hexane/ethyl acetate/chloroform (3:1:1) as eluant affording the *bis*-carbamate **2.36** (0.51 g, 76%); mp 148 °C; TLC R_f 0.35 in ethyl acetate/hexane (1:3); ν_{max} (film from $CDCl_3$) 1725 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1530 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.76 (3H, s, $18CH_3$), 0.89 (6H, m, 21 CH_3, ester CH_3), 0.93 (3H, s, $19CH_3$), 1.26 (38H, m, CH_2's of ester), 3.21 (1H, m, 3β-H), 4.03 (2H, t, OCH_2), 4.63 (2H, s, $COCH_2Cl$), 4.68 (2H, s, $COCH_2Cl$), 4.94 (1H, m, 7β-H), 5.15 (1H, m, 12β-H), 5.76 (1H, d, 3α-NH), 8.34 (1H, d, aryl H *meta* to SO_2), 8.51 (1H, d, aryl H *ortho* to SO_2), 8.62 (1H, s, carbamate NH), 8.66 (1H, s, aryl H *ortho* to NO_2), 8.94 (1H, s, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 11.94, 14.05, 17.62, 22.19, 22.63, 22.96, 24.97, 25.88, 27.11, 27.89, 28.61, 28.71, 29.21, 29.29, 29.64, 30.58, 31.11, 31.87, 34.05, 34.61, 35.37, 36.30, 38.02, 41.09, 42.32, 44.21, 44.61, 45.09, 47.36, 54.47 (C3), 64.45, 74.05 (7), 78.19 (12), 120.73, 127.06, 132.09, 140.21, 147.83, 149.73, 150.87 (OCONH), 150.93 (OCONH), 168.55 ($COCH_2Cl$), 169.39 ($COCH_2Cl$), 173.99 (C24); m/z (FAB) 1200.4 (M +2Na -2H, 90%), 991.5 (35), 904.5 (100), 717.5 (35), 635.5 (45).

Eicosyl 3α-(*p*-nitrobenzenesulfonylamido)-7α,12α-(*p*-nitrophenylamino-carbonyloxy)-5β-cholan-24-oate 2.34

To the *p*-nitrobenzenesulfonamide **2.31** (0.61 g, 0.66 mmol) in dry DCE (15 mL) was added *p*-nitrophenyl isocyanate (0.43 g, 2.65 mmol) with a catalytic amount of chlorotrimethylsilane, and the solution was allowed to stir at 60 °C under an atmosphere of argon for 3 days. The reaction mixture was cooled to 0 °C and filtered to remove solid impurities. The mother liquor was evaporated to dryness and the product was purified by

flash chromatography using hexane/ethyl acetate/chloroform (1:1:1) as eluant affording the *bis*-carbamate **2.34** (0.59 g, 75%); mp 168 °C; TLC R_f 0.22 in ethyl acetate/hexane (1:1); ν_{max} (film from $CDCl_3$) 1730 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1525 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.56 (1H, m, 4α-H), 0.81 (3H, s, 18CH_3), 0.87 (6H, m, 19CH_3, ester CH_3), 0.90 (3H, d, 21CH_3), 1.27 (38H, m, ester CH_2's), 2.93 (1H, m, 3β-H), 3.98 (2H, t, ester CH_2), 5.03 (1H, m, 7β-H), 5.17 (1H, m, 12β-H), 5.43 (1H, br s, 3α-NH), 7.45 (2H, d, carbamato aryl H *ortho* to NO_2), 7.61 (2H, d, carbamato aryl H *meta* to NO_2), 7.81 (1H, br s, carbamate NH), 7.97 (5H, m, aryl H, carbamate NH), 8.00 (2H, d, aryl H), 8.14 (2H, d, sulfonamido aryl H *ortho* to NO_2); δ_C (100.13 MHz, $CDCl_3$) 11.66, 13.61, 13.71, 17.11, 22.01, 22.19, 25.41, 26.88, 28.11, 28.73, 28.87, 29.01, 29.10, 29.20, 30.25, 30.80, 30.91, 31.43, 33.78, 34.23, 37.55, 41.10, 42.44, 45.00, 47.10, 54.10, 64.03, 72.18, 112.88, 117.00, 124.05, 124.39, 125.90, 127.36, 141.35, 141.62, 144.78, 146.10, 149.52, 151.77, 152.17, 173.70; (HRMS: found:1200.639224, $C_{64}H_{92}N_6O_{14}S$, requires m/z 1200.639224).

Eicosyl 3α-(2,4-nitrobenzenesulfonylamido)-7α,12α-(*p*-nitrophenyl-amino-carbonyloxy)-5β-cholan-24-oate 2.35

To the dinitrobenzenesulfonamide **2.32** (0.61 g, 0.66 mmol) in dry DCE (10 mL) was added *p*-nitrophenyl isocyanate (0.43 g, 2.65 mmol) with a catalytic amount of chlorotrimethylsilane and the reaction was allowed to stir at room temperature under an atmosphere of argon for 3 days. The reaction mixture was cooled to 0 °C and filtered to remove solid impurities. The mother liquor was evaporated to dryness and the product was purified by flash chromatography using hexane/ethyl acetate/chloroform (1:1:1) as eluant affording the *bis*-carbamate **2.35** (0.51 g, 72%); mp 168 °C; TLC R_f 0.20 in ethyl acetate/hexane/chloroform (1:1:1); ν_{max} (film from $CDCl_3$) 1725 cm^{-1}(C=O), 1380 cm^{-1}(SO_2N) 1530 cm^{-1}(NO_2); δ_H (400.13 MHz, $CDCl_3$) 0.83 (3H, s, 18CH_3), 0.89 (6H, m 19CH_3, ester CH_3), 0.91 (3H, d, 21CH_3), 1.26 (38H, m, ester CH_2's), 3.32 (1H, m, 3β-H), 3.97 (2H, t, ester CH_2), 5.08 (2H, m, 7β-H, 12β-H), 5.43 (1H, br s, 3α–NH), 7.50-8.34 (14H, m, aryl H, carbamate NH, carbamate NH); δ_C (100.13 MHz, $CDCl_3$) 11.48, 13.60, 17.09, 22.06, 22.19, 25.40, 28.11, 28.74, 28.86, 29.01, 29.09, 29.20, 30.26, 30.81, 31.42, 34.21, 45.05, 63.97, 110.76, 117.03, 124.31, 130.30, 141.27, 145.02, 148.95,

151.84, 173.68, 177.14; (HRMS: found:1245.624303, $C_{64}H_{91}N_7O_{16}S$, requires m/z 1245.624303).

Methyl 3α,7α,12α-*tris*-amino-cholanoate-5β-cholan-24-oate 5.2
(Procedure of Williams)[8]

To a solution of *tris*-boc protected amine **5.9** (10.0 mg, 1.14 x 10^{-5} mol) in dry DCM (0.25 mL) at 0 °C under argon was added trifluoroacetic acid (0.50 mL) and the resulting solution stirred at 0 °C for 3 h. The solvent was removed *in vacuo* and the residue was dried under high vacuum for 1 h yielding **5.2** (93%). δ_H (400.13 MHz, D_2O) 0.90 (3H, s, 18CH_3), 0.99 (3H, d, 21CH_3), 1.05 (3H, m 19CH_3), 3.65 (3H, s, ester CH_3), 3.21 (1H, m, 3β-H), 3.67 (1H, m, 7β-H), 3.90 (1H, m, 12β-H).

Methyl 3α,7α,12α-*tris*-(*p*-toluenesulfonamido)-5β-cholan-24-oate 5.10
(Procedure of Williams)[8]

To a solution of *tris*-boc protected amine **5.9** (0.0044 g, 6.12 10^{-6} mol) in dry DCM (0.2 mL) at 0 °C under argon was added trifluoroacetic acid (0.21 mL) and the resulting solution stirred at 0 °C for 3 h. The solvent was removed *in vacuo* and the residue was dissolved in dry DCM (0.2 mL). Triethylamine (5 μL, 3.7 10^{-5} mol) and *p*-toluenesulfonyl chloride (0.0034 g, 1.8 10^{-5} mol) were added and the resulting solution stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue was purified by column chromatography (eluting with 2:1 hexane/ethyl acetate) to give **5.10** (0.0018 g, 33%) as a colourless gum. δ_H (400.13 MHz, $CDCl_3$) 0.63 (3H, s, 18CH_3), 0.75 (3H, s, 19CH_3), 0.79 (3H, d, 21CH_3), 2.44 (9H, br s, aryl CH_3), 2.86 (1H, m, 3β-H), 3.32 (1H, m, 7β-H), 3.65 (3H, s, OCH_3), 3.80 (1H, m, 12β-H), 5.76 (3H, m 3 x NH), 7.29 (6H, m aryl H), 7.70 (4H, m, ortho aryl H), 7.81 (2H, d, ortho aryl H).

General procedure for the preparation of TMATs and $TMAHPO_4$ salts.

The relevant acid (0.01 mol) was dissolved in water (25 mL) and titrated with an aqueous solution of tetramethylammonium hydroxide (0.01 M) until an equilivance point was observed by pH meter. The resulting solution was evaporated to dryness by forming an azeotrope with toluene. The resulting solid salt was dried under high vacuum overnight at 40 °C.

[1] Purification of Laboratory Chemicals 3rd ed; D.D. Perrin, W. L. Armarego, Eds., Pergammon Press, New York, NY.
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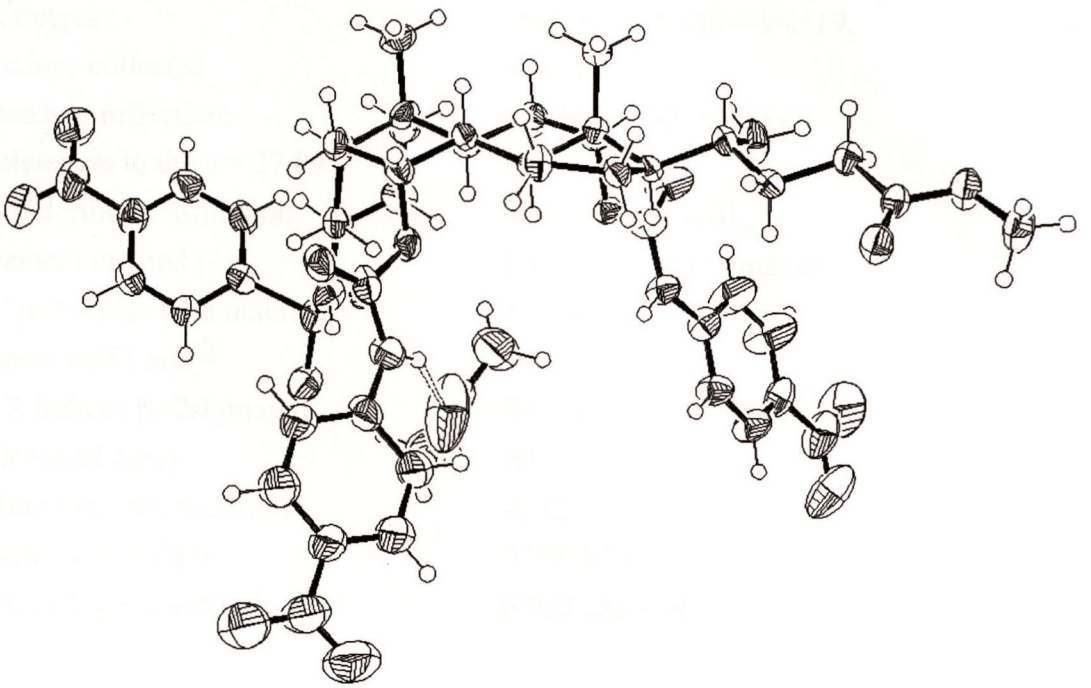
[5] Bhattarai, K. M.; Davis, A. P.; Perry, J. J.; Walter, C. J.; Menzer S.; Williams, D. J. *J. Org. Chem.* **1997**, *62*, 8463.
[6] Perry, J. Ph.D. Thesis, University of Dublin 1995.
[7] Lawless, L. J. Ph.D. Thesis, University of Dublin 1998.
[8] Williams R. P. Ph.D. Thesis, University of Dublin 1995.

Appendix



Crystal data and structure refinement for mas001.

Identification code	mas001	
Empirical formula	C48.50 H60.50 Cl1.50 N6 O15 S	
Formula weight	1052.76	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	orthorhompic	
Space group	P $2\ 2_1\ 2_1$	
Unit cell dimensions	a = 13.8297(4) Å	a= 90°.
	b = 14.6246(3) Å	b= 90°.
	c = 25.6877(7) Å	g = 90°.
Volume	5195.4(2) $Å^3$	
Z	4	
Density (calculated)	1.346 Mg/m^3	

Absorption coefficient	0.212 mm^{-1}
F(000)	2220
Crystal size	0.40 x 0.35 x 0.10 mm^3
Theta range for data collection	2.95 to 27.89°.
Index ranges	-18<=h<=18, -19<=k<=19, -33<=l<=33
Reflections collected	12338
Independent reflections	12338 [R(int) = 0.0000]
Completeness to theta = 27.89°	99.6 %
Max. and min. transmission	0.9791 and 0.9201
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	12338 / 3 / 662
Goodness-of-fit on F^2	1.028
Final R indices [I>2sigma(I)]	R1 = 0.0931, wR2 = 0.2116
R indices (all data)	R1 = 0.1805, wR2 = 0.2571
Absolute structure parameter	-0.12(14)
Extinction coefficient	0.0003(7)
Largest diff. peak and hole	0.923 and -0.416 $e.Å^{-3}$