THE DESIGN OF SYNTHETIC TRANSMEMBRANE CARRIERS
FOR ANIONS

by

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This thesis reports a number of novel receptors found to facilitate the transmembrane transport of anions in synthetic vesicle systems.

A series of structurally simple thioureas were found to function as efficient Cl⁻/NO₃⁻ and Cl⁻/HCO₃⁻ antiporters while the corresponding ureas were inactive. Of these receptors, a simple indolylthiourea was found to be an extremely potent transporter which could function at concentrations as low as 1:25 000 (molar ratio with respect to lipid).

Subsequently, a series of bipodal bis-alkyl-indolylureas were found to mediate Cl⁻/NO₃⁻ antiport, with the observed anion transport found to be highly dependent on the length of the central alkyl chain bridging the two indolylurea moieties. The mechanism of transport and the structure-activity relationships were extensively investigated using a wide range of vesicle-based techniques. The solution phase anion binding properties of these receptors was found to be complex as a result of the flexibility of the receptors and the distance between the binding sites. The binding of dihydrogen phosphate and oxalate by receptors in this series was also demonstrated in the solid state.

A series of bipodal bis-phenylthioureas were also found to mediate Cl⁻/NO₃⁻ and Cl⁻/HCO₃⁻ antiport which was again found to be dependent on the alkyl chain length. The effect of substituent variation on transport activity was also examined, while the solution phase binding properties were found to be similarly complex to the bis-indolylurea receptors.

The anion transport activity of a series of strapped calix[4]pyrroles provided by J. L. Sessler and C.-H. Lee was investigated. It was found that the straps were able to encourage transport mechanisms not exhibited by the parent macrocycle, while a series of control compounds were inactive.
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DECLARATION OF AUTHORSHIP

I, **Cally Jo Elizabeth Haynes**, declare that the thesis entitled

**The design of synthetic transmembrane carriers for anions**

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;

- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

- where I have consulted the published work of others, this is always clearly attributed;

- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

- I have acknowledged all main sources of help;

- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

- parts of this work have been published as:


Signed: ................................................................................................................

Date: ......................................................................................................................
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>AE</td>
<td>Anion exchange protein</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>bpy</td>
<td>2,2’-Bipyridine</td>
</tr>
<tr>
<td>br.</td>
<td>Broad resonance (NMR)</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyl</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Centigrade</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CDI</td>
<td>1,1’-Carbonyldiimidazole</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane regulatory protein</td>
</tr>
<tr>
<td>CIC</td>
<td>Chloride channel proteins</td>
</tr>
<tr>
<td>clogP</td>
<td>Calculated logP (Octanol:water partition coefficient)</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOPC</td>
<td>1,2-dioleoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>DPPC</td>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Eq.</td>
<td>Equivalents</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>EYPC</td>
<td>Egg yolk phosphocholine</td>
</tr>
<tr>
<td>FCCP</td>
<td>Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infra-red spectroscopy</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>h</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>HPTS</td>
<td>8-Hydroxypyrene-1.3.6-trisulfonic acid trisodium salts</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
</tbody>
</table>

8
ISE  Ion selective electrode
ITC  Isothermal titration calorimetry
J  Coupling constant (NMR)
K  Kelvin
$K_a$  Association constant
KCC  K-Cl co-transporter
LRMS  Low resolution mass spectrometry
m  Multiplet (NMR)
M  Molarity
Me  Methyl
MeCN  Acetonitrile
MDCK  Madin Darby canine kidney
MeOH  Methanol
min  Minutes
mol  Mole(s)
mmol  Millimole(s)
$M_p$  Melting point
MS  Electrospray (mass spectrometry)
m/z  Mass to charge ratio
NCC  Na-Cl co-transporter
NKCC  Na-K-Cl co-transporter
NDI  Naphthalenediimide
NMR  Nuclear magnetic resonance spectroscopy
PC  Phosphocholine
Pd/C  Palladium on carbon catalyst
PDI  Phenylenediimide
Ph  Phenyl
POPC  1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
ppm  Parts per million
Sat.  Saturated
SLC  Sodium lithium co-transporter protein
SPQ  6-Methoxy-N-(3-sulfopropyl)quinolinium
TBA  Tetrabutylammonium
TEA  Tetraethylammonium
Tren Tris(2-aminoethyl)amine
UV-vis Ultraviolet-visible spectroscopy
w.r.t. With respect to

**Contributions from other researchers**

All of the work presented in this thesis was completed by the author (CJEH) unless otherwise stated. On these occasions, the work is attributed to the contributing researcher by the initials as abbreviated below:

SJM Stephen J. Moore
NA Nathalie Andrews
CCT Dr. Christine C. Tong

The work described in Chapter 4 was contributed to by Jennifer L. Sutton, an undergraduate project student working under my supervision.
The development of synthetic transmembrane anion transporters is a relatively new and rapidly expanding area of supramolecular chemistry.\textsuperscript{1-3} The transport of anions such as chloride and bicarbonate is an important research target due to the critical biological roles that these anions play and the diseases that result from the misregulation of their transport in the cellular environment. Over recent years a wide variety of synthetic anion transporters have been reported, from membrane spanning channels to simple amphiphilic anion receptors which function as anion carriers through the lipid bilayer.

1.1 Biological regulation of ion transport

The hydrophobic interior of the eukaryotic cell membrane enables its function as a barrier between intra- and extra-cellular media.\textsuperscript{4} Cell organelles such as mitochondria and the Golgi apparatus are also membrane bound. The membrane is permeable to small, neutral molecules such as water and to gases such as oxygen and carbon dioxide. However, ionic species are unable to cross this barrier unassisted.

The transport of ions across cell membranes is important in many cellular processes. Ion gradients created by ion pumps (“active” membrane transporters which utilize ATP hydrolysis as an energy source) provide the electrochemical gradient to drive the action of passive ion channels and other transport proteins. These processes are used to regulate cell volume, transport nutrients into cells, to produce electrical signals, and to regulate secretions across epithelial layers.\textsuperscript{5,6}

The transport of ions in biological systems is enabled by membrane spanning proteins, which provide a pathway for ions to cross the bilayer. The movement of an ion into the hydrophobic interior of the membrane is highly unfavourable- the energy penalty
can be considered as equivalent to the desolvation energy of the ion. The transporter provides favourable interactions with the ion to compensate for this penalty during the translocation process, thus reducing the energy barrier. Fyles states that an ion transporter may be viewed as “a catalyst of translocation”. The most biologically prevalent anions are chloride, bicarbonate and phosphate, of which chloride is the least hydrophilic; thus, anion transporters are likely to be inherently selective for chloride.

Membrane transport may be via one of three mechanisms: uniport, in which a single species is transported; antiport, in which two species are transported in opposite directions, or symport, in which two species are transported in the same direction. These processes are represented in Figure 1.1. If considered as an isolated process, for the passive transport of an ion to occur, to maintain charge neutrality the ion may be transported by a symport mechanism, with an oppositely charged ion, or transported by an antiport mechanism, in the opposite direction to a similarly charged ion. However, natural transport proteins can passively uniport ions providing the necessary electrochemical gradient has been established by the action of other proteins.

![Figure 1.1](image)

**Figure 1.1** Transmembrane transport by uniport, antiport and symport processes.

In the cellular environment, the transmembrane transport of anions is crucial in the maintenance of vital concentration gradients used for signalling and cellular regulation. Of particular biological relevance is the transmembrane transport of chloride.
Biologically, chloride can be transported by a number of different membrane proteins.\(^1\) CFTR (cystic fibrosis transmembrane conductance regulator), is a chloride uniport channel found in epithelial cells. It belongs to the family of cAMP activated channels. The open-close behaviour of this family of channels is regulated by ATP hydrolysis; however, once open they allow the transmembrane flow of anions down an electrochemical gradient.\(^8\) The largest family of Cl\(^-\) channels are the CIC proteins. Some CIC proteins function as voltage-gated channels; others facilitate H\(^+\)/Cl\(^-\) co-transport. Ca\(^{2+}\) activated Cl\(^-\) channels are also known, which are regulated by an intracellular increase in free Ca\(^{2+}\) concentration. Other classes of Cl\(^-\) channels are regulated by cell volume, and are vital for the control of osmotic regulation, and by the interaction selectively with neurotransmitters such as glycine and \(\gamma\)-aminobutyric acid (GABA).

Additionally, there are anion transport proteins that do not form channels but instead bind the ions and facilitate their movement across the membrane by a conformational change. The co-transporter SLC12 proteins are responsible for the symport of chloride. Within this family, the NKCC (Na-K-Cl co-transporter) proteins facilitate the symport of 2 Cl\(^-\) anions with 1 Na\(^+\) and 1 K\(^+\) cation, while the NCC (Na-Cl co-transporter) proteins are responsible for Na\(^+\)/Cl\(^-\) symport and the KCC (K-Cl co-transporter) proteins facilitate K\(^+\)/Cl\(^-\) symport. These proteins are found in a range of tissues and are responsible for renal reabsorption of these ions. Cl\(^-\)/HCO\(_3\)^- anion exchangers (AEs) are proteins that facilitate the antiport of chloride and bicarbonate. The transport of bicarbonate is important for regulation of cellular pH as it forms a buffer in aqueous equilibrium with CO\(_2\). In this way it is also crucial for cellular respiration, as the majority of CO\(_2\) is transported to the lungs from respiring tissue in aqueous solution in the blood as HCO\(_3\)^-.

Biological misregulation of anion transport is associated with a number of diseases (channelopathies).\(^6\),\(^9\) Malfunction of Cl\(^-\) channels has been linked with Bartter’s syndrome, Dent’s disease, Best disease, nephrolithiasis (kidney stones), osteopetrosis, and most notably, cystic fibrosis (CF). Misregulation of HCO\(_3\)^- transport is also associated with CF, along with diseases affecting the brain, heart and lungs.
1.2 Cystic fibrosis and anion transport

Cystic fibrosis (CF) is a genetic disorder associated with mutation of the gene responsible for CFTR production.\textsuperscript{10} CF affects the epithelial cells of secretory organs including the lungs, pancreas, intestinal tract and sweat glands, causing the production of thick, sticky mucus. This mucus obstructs ducts and airways. The most common cause of mortality in CF patients is airway disease. In 2009 the median survival age for patients with CF was 35.9, although this is improving.\textsuperscript{11} Children born with CF in the UK in the 2000s are now predicted to have a median survival into their 50s.\textsuperscript{12}

When the gene responsible for CF was first identified, it was expected that it would code for a chloride channel because CF epithelia had been previously shown to be impermeable to chloride.\textsuperscript{13} There are references in medieval folklore which state that infants who tasted “salty” were hexed as they were expected to die at an early age.\textsuperscript{14, 15} Today, a common diagnostic test for CF is the measurement of chloride levels in sweat, with abnormally high chloride levels being indicative of CF.\textsuperscript{16} These observations indicate the important role that homeostatic regulation of chloride levels plays in the disease.

Mutation of the gene which encodes for CFTR results in the production of CFTR which is defective, inhibiting transmembrane chloride transport via this pathway.\textsuperscript{10} Individual cellular transport processes are often closely linked, with one transport process providing an electrochemical gradient to drive another. An example of this, in the epithelial cells of the pancreatic duct, is represented in Scheme 1.1.\textsuperscript{6}
CFTR mediates the excretion of Cl\(^-\) from the cell, which in turn drives the action of the Cl\(^-\)/HCO\(_3^-\) AE protein, causing HCO\(_3^-\) efflux. The consequences of this process then drive a variety of other ion transport processes. The net result of this combination of processes is the secretion of water due to the osmotic imbalance that is established; in CF sufferers, the defective nature of CFTR partially inhibits this transport cycle. The interlinked nature of these processes reveals that the disturbance of a single transport event (the transport of Cl\(^-\) by CFTR) can cause large disruptions in the regulation of a number of other electrochemical gradients. It is commonly thought that these disruptions lead to the secretion of less water and causes thicker, stickier mucus to be produced.\(^{17}\) However, Quinton has hypothesized that the abnormal nature of the mucus is also due to reduced HCO\(_3^-\) levels affecting the structure of gel forming mucins which make up the mucus.\(^{18}\) Both of these hypotheses indicate that the development of synthetic transporters for Cl\(^-\) and HCO\(_3^-\) is of interest due to their potential therapeutic application for CF sufferers; to replace the action of abnormal CFTR and to repair the damaged anion gradients.

**Scheme 1.1** A representation of the ion transport processes in the epithelial cells of the pancreatic duct.
1.3 Naturally occurring anion transporters

There are multiple examples of natural products which have been found to mediate the transmembrane transport of cations, of which valinomycin (Figure 1.2) and gramicidin A are widely known.\(^{19}\)

![Valinomycin](image)

Figure 1.2 Valinomycin, a naturally occurring potassium ionophore and carrier.

However, there are comparatively few examples of naturally occurring, non-protein based anion transporters. The most intensively studied of these is the prodigine family of natural products, of which the prodigiosins such as 1 (Figure 1.3) are members.

Prodigiosins are a family of tripyrrolic red pigments isolated from microorganisms such as *Streptomyces* and *Serratia*.\(^{20}\) These molecules have been found to induce a range of biological effects including antibiotic, antitumor and immunosuppressive activity.\(^{21, 22}\) They have also been shown to bind H\(^+\)Cl\(^-\) by a combination of electrostatic interactions and hydrogen bonding as shown in Figure 1.3. Parr *et al.* have reported the crystal structure of the H\(^+\)Cl\(^-\) salt of a structurally similar synthetic prodigiosin shown in Figure 1.3.\(^{23}\) This crystal structure shows the Cl\(^-\) anion coordinated by three hydrogen-bonding interactions from the protonated tripyrrolic scaffold. Prodigiosin 1 has also been shown to mediate efflux of chloride from phospholipid vesicles in the presence of a pH gradient.\(^{24, 25}\)
Figure 1.3 The complexation of $\text{H}^+/\text{Cl}^-$ by prodigiosin 1 and the crystal structure of the $\text{H}^+/\text{Cl}^-$ salt of a structurally similar synthetic prodigiosin.

There are conflicting theories as to how prodigiosins cause the observed biological effects. In particular, it has been proposed that the observed anticancer activity is due to prodigiosin inducing copper mediated cleavage of double stranded DNA.\textsuperscript{26-29} However, there is also evidence to suggest that the anticancer activity is related to anion transport capabilities. In particular, there is mechanistic evidence that anticancer treatments which have been found to enhance the drug sensitivity of multidrug resistant cancer cells and to cause their apoptosis function by causing the deacidification of cell organelles by $\text{H}^+/\text{Cl}^-$ co-transport.\textsuperscript{30}

Work by Sessler et al. investigated the $\text{H}^+/\text{Cl}^-$ binding and transport ability of a series of synthetic prodigiosin analogues (compounds 2-6), by comparison to their \textit{in vitro} anticancer activity.

Binding studies performed by isothermal titration calorimetry (ITC) in MeCN indicated that chloride (added as the tetrabutylammonium salt) was bound only to a detectable
degree if the pyrrolic skeleton was protonated. X-ray crystal structure analysis of the HCl
salts of these receptors indicated that Cl⁻ was bound in a 1:1 manner in the solid state by a
combination of electrostatics and hydrogen bonding, as shown in Figure 1.4. The ability
of 2-6 to mediate H⁺Cl⁻ efflux from vesicles was investigated, and compound 2, the most
structurally similar to naturally occurring prodigiosins, was found to be the most effective
carrier. The structurally simpler dipyrrromethanes 3 and 4 were also found to be highly
effective carriers. The in vitro anticancer activity of these receptors was assessed using a
cell proliferation assay with human lung and prostate cancer cell lines. The order of
anticancer activity was found to correlate with the observed relative H⁺Cl⁻ transport
activities. This significant finding supports the proposed mechanism of anticancer activity
and indicates that anion transporters may have a future application as new anticancer
therapies.

Figure 1.4 The crystal structure of 6.H⁺Cl⁻.

J. T. Davis and co-workers have also demonstrated that prodigiosin 1 functions as an
efficient Cl⁻/NO₃⁻ antiporter in liposomal systems using a combination of fluorescence
based vesicle studies with either HPTS to monitor the intra-vesicular pH or lucigenin to
monitor the intra-vesicular chloride concentration. In each case the external anion was
chloride. It was found that if the internal anion was sulfate, a small amount of chloride
influx occurred accompanied by a decrease in internal pH, consistent with the influx of
H⁺Cl⁻. However, if the internal anion was NO₃⁻ a larger amount of chloride influx was
observed and the internal pH remained constant, indicating a preference for Cl-/NO$_3^-$ antiport. Following on from this work, collaborative research from J. T. Davis and Gale found that prodigiosin 1 functions as a potent Cl-/HCO$_3^-$ antiporter. This high HCO$_3^-$ transport activity was postulated to be a result of the proposed binding mode shown in Figure 1.5, utilizing the hydrogen bond donors and acceptors provided by the tripyrrolic skeleton.

![Figure 1.5](image)

**Figure 1.5** The proposed mode of prodigiosin 1 binding to HCO$_3^-$.

Structurally related to the prodigiosins is the tambjamine alkaloid family of natural products, which include compounds 7-10. Recent work from Quesada and co-workers describes the chloride binding and transport properties of these molecules and synthetic analogues 11 and 12. $^3$H NMR titration of the hyperchlorate salts of 7-12 with TBACl in DMSO-$d_6$ resulted in significant shifts of the resonances associated with the NH protons involved in the binding event up to the addition of 1 equivalent of anion. This indicated strong, 1:1 binding of chloride by these receptors in their protonated state. Compounds 7-12 were also found to function as efficient Cl-/NO$_3^-$ and Cl-/HCO$_3^-$ antiporters. The highest anion antiport activity was mediated by synthetic analogue 12.

![Compounds](image)

7 $R = $\text{CH}_2\text{CH(CH}_3)_2$
8 $R = $\text{Et}$
9 $R = $\text{CH}_2\text{CH}_2\text{Ph}$
10 $R = $\text{C}_2\text{H}_5$
11 $R = $\text{H}$
12 $R = $\text{tBu}$

J. T. Davis and co-workers have investigated the anion transport activity of the sphingolipid C2-ceramide 13 (Figure 1.6). $^{32}$ This ceramide is known to form membrane
spanning pore assemblies when added to a lipid bilayer at concentrations above 10 mol% (with respect to lipid).\textsuperscript{33} Davis \textit{et al.} demonstrated that at concentrations lower than this, 13 can still facilitate anion transport. Compound 13 was found to mediate Cl\(^-\)/NO\(_3\)\(^-\) and Cl\(^-\)/HCO\(_3\)\(^-\) antiport in EYPC vesicle systems at a concentration of 1 mol\%, well below the concentration required for pore formation. Additionally, this concentration of 13 was found not to facilitate the release of carboxyfluorescein from vesicles. Carboxyfluorescein is an anionic fluorescent dye that can be transported across lipid bilayers through large, membrane spanning pores. However, higher concentrations of 13 were found to mediate carboxyfluorescein transport. This indicates that at lower concentrations, 13 does not form pores and must therefore function as a mobile carrier. The 1,3-diol unit of ceramide 13 was found to be essential for its transport activity, as an analogous ceramide in which these OH groups were protected was found to be inactive. Evidence obtained from the \(^1\)H NMR titration of 13 with TBACl in CD\(_2\)Cl\(_2\) indicated that these OH groups were involved in hydrogen bond formation to Cl\(^-\) anions, in addition to the amide NH. This work highlights that predicting the mode of action of anion transporters within the bilayer is not always straightforward.

\begin{center}
\includegraphics[width=0.5\textwidth]{ceramide13.png}
\end{center}

\textbf{Figure 1.6} Ceramide 13.

### 1.4 The design of synthetic anion transporters

While ceramide 13 is capable of transporting anions as both a pore and a mobile carrier, in general only one of these two possible transmembrane transport routes are utilized in transporter design. There are many examples of synthetic species that span the bilayer and form anion channels, thus mimicking the action of naturally occurring membrane proteins such as CFTR or gramicidin. Alternatively, smaller molecules have been found to function as mobile carriers. Carrier molecules are designed to bind the ion
and allow its passage across the bilayer by diffusion of the less lipophobic receptor-ion complex. Prodigiosins and tambjamines are naturally occurring examples of mobile carriers, while valinomycin is a well-known example of a potassium carrier. Ion channels may be formed by small molecules which can self assemble within the bilayer; thus, initial prediction of channel formation vs carrier activity is not always straightforward. However, there are design principles that are distinct to the development of each class of transporter.

The mode of action of mobile carriers requires that they are able to form a complex with the anion of interest. It is therefore unsurprising that many anion carriers draw inspiration from the well-established field of anion complexation chemistry.

### 1.5 Anion receptor chemistry

The design of selective hosts for anions is a vast and ever expanding area of supramolecular chemistry.\textsuperscript{34-42} The most common means of complexing an anion through non-covalent interactions is by hydrogen bonding. Convergent hydrogen bond donor motifs can be used to selectively bind anions of interest. The most commonly utilized hydrogen bond donors for this purpose are NHs, including amides, ureas, pyrroles, indoles and carbazoles, and combinations of these functionalities. Anion binding by CH hydrogen bond donors is also known; in particular, the CH bond in the 5-position of 1,2,3-triazoles have been reported to form hydrogen bonds of similar strength to amides.\textsuperscript{43} Other principles of host-guest supramolecular chemistry can also be applied, including binding site preorganization, chelate and macrocyclic effects. There is now a vast library of literature anion receptors based on hydrogen bond donor motifs which may be of use in the design of new anion carriers. The synthetic prodigiosin and tambjamine analogues described above utilize pyrrole and amide hydrogen bond donors, which are supplemented at acidic pH by an additional hydrogen bond donor resulting from protonation of a basic pyrrolic nitrogen and the corresponding electrostatic attraction to the now cationic receptor.
1.6 From anion receptors to anion carriers

The challenge of designing anion carriers relies not just on complexation of the anion, but also on the lipid-solubilization of the resulting host-guest complex. In addition to a convergent array of hydrogen bond donors for binding the anion, it is desirable for potential anion carriers to possess a certain degree of lipophilicity in order to aid the partitioning of the receptor and the receptor-anion complex within the lipid bilayer. Hydrogen bond donors make the anion binding site polar and hydrophilic - thus, successful carriers have been developed by choosing a scaffold for this site lipophilic enough to counter the strong interactions between the binding site with the aqueous phase, or by effectively screening the binding site, and by extension, the anion from the bilayer.

Some of the first and amongst the most effective anion carriers reported to date are the series of cholapods developed by A. P. Davis and co-workers. These cholic acid derivatives, of the general form shown in Figure 1.7, combine a lipophilic steroid skeleton with pendant urea or thiourea arms for anion complexation.

![Figure 1.7 The general structure of the cholapods reported by A. P. Davis et al.](image)

Earlier work from the same authors had shown carbamoyl-functionalized analogues to form extremely strong complexes with Cl\(^-\) and Br\(^-\) in chloroform solution.\(^{44, 45}\) Bis(phenyl)urea derivatives had also been shown to interact strongly with phospholipids and to facilitate flippase activity - the translocation of phospholipid molecules between leaflets of a lipid bilayer - due to strong interaction of the phosphate head groups of the phospholipid molecule.\(^{46, 47}\) The intrinsic lipophilicity of the cholapod skeleton prompted the authors to assess the possibility that this group of receptors could “shuttle” anions across phospholipid bilayers. Initial investigations found that cholapod 14 (shown in
Figure 1.8) facilitated Cl\(^{-}/NO_3^{-}\) exchange across vesicle bilayers, while 15-18 were less effective.\(^{48}\) This correlated with the strength of anion binding by these receptors in wet chloroform (measured by Cram’s extraction technique\(^{49, 50}\) using TBA\(^+\) salts), where compound 14 showed the strongest binding of Cl\(^{-}\) and NO\(_3^{-}\). The chloride efflux mediated by these receptors was almost entirely inhibited if the external NO\(_3^{-}\) was replaced with SO\(_4^{2-}\), providing evidence for an anion antiport mechanism. A fluorescence transport assay using the potential sensitive dye Safranin-O indicated that addition of receptor 19 to a suspension of Cl\(^{-}\) containing vesicles in which the external anion was SO\(_4^{2-}\) induced a transmembrane electric potential due to a small amount of chloride efflux with no corresponding influx of an alternative anion. Cl\(^{-}\) transport was also demonstrated using \(^{35}\)Cl NMR techniques. A mobile carrier mechanism was supported by the observation that chloride efflux in vesicles composed of DPPC was inhibited below the gel/liquid phase transition temperature. Finally, receptor 14 was found to mediate Cl\(^{-}\) transport in Madin Darby canine kidney (MDCK) epithelia using the Ussing chamber technique, in which a layer of cells is grown on a support and the transepithelial current due to ion transport is monitored. The observed current was, as expected, highly anion dependent.

Further investigation revealed that the anion transport properties of this design could be enhanced by further improving the anion binding strength. Thioureas have been found to bind anions more strongly than analogous ureas due to the greater acidity of the hydrogen bond donors.\(^{51, 52}\) Compound 19 was found to have a chloride affinity 200 times greater than 15 under the same conditions, and was also found to mediate Cl\(^{-}/NO_3^{-}\) exchange more effectively.\(^{53}\) However, the high lipophilicity of this compound prevented
the investigation of its anion transport unless it was preincorporated into the vesicle membranes, as addition of a THF solution of 19 to an aqueous suspension of vesicles resulted in its precipitation.

Building on this work, A. P. Davis et al. went on to study in detail a larger series of 12 new cholapods in order to further investigate the effect of small structural changes on the cholapod transport efficiency.\textsuperscript{54} The most efficient carrier investigated in this work was compound 20 which, when preincorporated into the lipid bilayer, was remarkably potent, displaying activity at loadings as low as 1:250 000 (carrier:lipid). In general, increasing the anion affinity of the carrier resulted in higher carrier efficiency. For example, examining the effect of modifying the urea groups in positions 7 and 12 from phenyl urea (compound 16) to $p$-nitrophenyl urea (compound 14) to $p$-nitrophenyl thiourea (compound 20) resulted in an observed increase in transport efficiency as expected from the increased anion affinities. However, modifying the substituent in the 3-position resulted in variation of transport efficiency which did not correlate with anion binding strength. Cationic cholapods such as compound 21 were found to be largely inactive. This observation was attributed to the formation of strong, electroneutral chloride complexes that only slowly release anions back into the aqueous phases. Experiments with eicosyl esters such as 22 did not show enhanced transport efficiency from their methyl ester analogues, thus implying that variation of carrier lipophilicity was not a contributing factor to the observed transport trends. Due to the intrinsically high lipophilicity of these carriers, it is likely that all of these receptors are fully contained within the vesicle bilayer.

![Figure 1.9 Structural variations on the cholapod scaffold.](image)
More recently, work from the same authors has described the cyclization of the cholapod scaffold into so-called “cholaphanes” such as 23.\textsuperscript{55} These molecules were found to exhibit higher transport efficiencies than their acyclic analogues. This was attributed to greater binding site encapsulation to better shield the anion from the apolar interior of the vesicle membrane. The cholaphanes were found to bind chloride slightly more strongly than acyclic analogues, but nitrate was bound more weakly; this is most likely a result of size selectivity. Simplification of the cholic acid skeleton to a \textit{trans}-decalin scaffold (compounds 24) has also resulted in highly potent carriers capable of transporting chloride at a 1:25 000 (carrier:lipid) ratio.\textsuperscript{56}

![Figure 1.10](image.png)

\textbf{Figure 1.10} The recently published cholaphane and \textit{trans}-decalin, inspired by the cholapod design.

The rigidity of the cholapod framework results in a well preorganised binding site for anions. It is a well established concept within host-guest chemistry that preorganisation of the binding site leads to more favourable guest binding.\textsuperscript{57} The benefits of binding site preorganization in anion carriers has also been explored by J. T. Davis, Gale, Quesada and co-workers. They reported that simple hydroxyisophthalamide 25 functioned as a highly efficient Cl\textsuperscript{-}/NO\textsubscript{3}\textsuperscript{-} antiporters in vesicle systems.\textsuperscript{58} The binding site of these receptors is preorganised by intramolecular hydrogen bonding interactions, as shown in Figure 1.11, as evidenced by the X-ray crystal structure of the free receptor. Conversely, receptors 26 and 27 are not preorganised in this way. The crystal structure of 27 indicates that intramolecular hydrogen bonding interactions cause the free receptor to adopt a conformation which disfavours anion binding. Consequently, 23 was found to interact more strongly with TBACl in CD\textsubscript{3}CN (demonstrated by \textsuperscript{1}H NMR titration). 26 and 27 also exhibited no anion transport activity. Building on this work, 26 was reported by the same authors as an effective Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} antiporter.\textsuperscript{59}
Meso-octamethylcalix[4]pyrrole 28 was first synthesized by Baeyer in 1886 by the acid catalyzed condensation of acetone and pyrrole. In 1996, J. L. Sessler and co-workers first described the use of 28 and analogue 32 as receptors for F\(^-\) and Cl\(^-\). The interaction of these anions with the calix[4]pyrroles was investigated by \(^1\)H NMR titration with the anion tetrabutylammonium salts in CD\(_2\)Cl\(_2\), and by X-ray crystallography of the complexes formed on addition of the TBA\(^+\) salts. They found that in the solid state, free calix[4]pyrrole 28 adopts a 1,3-alternate conformation, but on binding Cl\(^-\) the ligand adopts a cone-like conformation with all of the pyrrole NHs converging to complex the anion.

Further investigation revealed that calix[4]pyrrole 28 can function as an ion pair receptor for cesium halides, as Cs\(^+\) cations may be bound in the cone-like cavity formed when the calix[4]pyrrole complexes the anion, both in solution and in the solid state. The crystal structure of 28.Cs\(^+\)Cl\(^-\) is shown in Figure 1.13. This ion pair complexation prompted a collaborative project between Sessler, and Gale to study the ability of calix[4]pyrroles 28-32 to transport ion pairs across lipid bilayers. Compound 28 was
found to efficiently mediate the efflux of chloride from CsCl containing vesicles. This activity was mostly independent of the external anion; only a small increase in transport activity was observed by exchanging the external anion from $\text{SO}_4^{2-}$ to $\text{NO}_3^-$. This implied a predominant Cs$^+$/Cl$^-$ symport mechanism. The transport was highly cation dependent; negligible transport was observed in the absence of Cs$^+$. This is unsurprising as the smaller group I cations are not bound in the cone-like cavity of calix[4]pyrroles as they are of a less complementary size and are more hydrophilic. The inability of calix[4]pyrroles to facilitate a Cl$^-$/NO$_3^-$ antiport mechanism in the absence of Cs$^+$ most likely reflects the poor binding of NO$_3^-$ and the lower lipophilicity of the anion-receptor complex when a cation is not coordinated to provide charge neutrality. Interestingly, calix[4]pyrroles 29-31 showed no Cs$^+$/Cl$^-$ symport activity, while 32 mediated only small amounts of CsCl flux. This may be due to poorer solubility of these compounds under the conditions of the assay.

![Figure 1.13](image)

**Figure 1.13** The crystal structure of 28.Cs$^+\text{Cl}^-$. The Cs$^+$ cation has been drawn in space filling mode for clarity.

There has been intensive study of the synthesis and anion binding properties of strapped calix[4]pyrroles. Many groups have found that strapped calix[4]pyrroles exhibit stronger chloride binding. This is attributed to greater anion encapsulation and, if applicable, the formation of additional hydrogen bonds from the strap. Gale and co-workers report the synthesis of 1,2,3-triazole strapped calix[4]pyrrole 33 (**Figure 1.14**). The binding of TBACl by 33 was investigated by ITC in MeCN and DCM. In both cases, Cl$^-$ was found to be complexed more strongly by 33 than by unmodified calix[4]pyrrole
Like compound 28, compound 30 was found to mediate Cs\(^+\)/Cl\(^-\) symport. However, it was also found to promote Cl\(^-\) efflux from vesicles containing other group I metal chloride salts. In the presence of larger cations such as Cs\(^+\) and Rb\(^+\), the transport rates were less dependent on the nature of the external anion; however, when the internal cations were smaller (K\(^+\) or Na\(^+\)) there was a larger reduction in transport rate observed on exchanging the external NO\(_3\)\(^-\) for SO\(_4\)\(^2-\). These observations indicated that both M\(^+\)/Cl\(^-\) symport and Cl\(^-\)/NO\(_3\)\(^-\) antiport processes could be facilitated by 33. Larger, more charge diffuse cations were preferentially transported by a symport mechanism, thus the rate was less dependent on the external anion; more hydrophilic cations were energetically more difficult to transport, thus a predominant antiport mechanism was observed in these cases. The ability to facilitate an anion antiport mechanism may be due to the stronger anion binding and greater anion encapsulation by strapped calix[4]pyrroles, therefore more effectively screening the charge of the anion from the hydrophobic interior of the bilayer. Importantly, it also demonstrated that functionalization of the calix[4]pyrrole scaffold can result in modulation of transport properties and facilitate alternative transport mechanisms.

Further to this work, P. A. Gale et al. reported the synthesis and anion binding and transport properties of strapped calix[4]pyrroles 34-36, containing two 1,2,3-triazole groups to aid anion complexation joined with an alkyl chain of varying length (Figure 1.14). Binding studies conducted by ITC in MeCN with TBACl indicated that all of these receptors bind Cl\(^-\) similarly strongly. The crystal structure of the chloride complex of 36 is shown in Figure 1.15 and shows that the calix[4]pyrrole has adapted the “cone”
conformation on binding to the anion. In this structure, the 1,2,3-triazole CH\(-\cdot\)Cl\(^-\) bond lengths are 2.788 Å and 2.753 Å, with both of these CH donors orientated towards the anion. Chloride transport studies revealed that a preference for either a Cs\(^+\)/Cl\(^-\) symport or a Cl\(^-\)/NO\(_3\)\(^-\) antiport mechanism varied across the series, although each receptor seemed to be capable of both. Compounds 34 and 35 operated predominantly by a Cs\(^+\)/Cl\(^-\) symport system but were able to facilitate low levels of anion antiport. However, the chloride efflux mediated by compound 35 was found to be far less dependent on the encapsulated M\(^+\) cation implying a shift towards a favoured anion antiport mechanism. Chloride transport by compound 35 was also inhibited the most when the external anion was SO\(_4\)\(^{2-}\).

It was concluded that the observed trends in transport efficiency could be due to the partitioning of the receptor or receptor-anion complex with the aqueous or lipid phase, or the varying mobility of the receptors in the lipid phase, or a combination of these factors. The results did demonstrate that transport efficiency is not only related to anion binding strength.

Fluorination of aromatic structures is thought to increase their lipophilicity.\(^{66}\) Additionally, fluorination of the scaffold of an anion receptor can lead to stronger binding due to the electron withdrawing nature of the fluorine substituents. The anion transport activity of octafluorocalix[4]pyrrole 36 was investigated by Sessler and Gale.\(^{67}\) They found that Cl\(^-\) efflux mediated by 36 from vesicles suspended in NaNO\(_3\) was almost

![Figure 1.15](image.png)
completely independent of the encapsulated anion, implying a purely anion antiport mechanism. Compound 36 was also found to mediate Cl⁻/HCO₃⁻ exchange; the first example of a calix[4]pyrrole to facilitate this mechanism.

![Figure 1.16 Octafluorocalix[4]pyrrole 36.](image)

Gale and co-workers have investigated the anion transport properties of a series of ureas and thioureas based on the tren (tris(2-aminoethyl)amine) scaffold. They found that urea was inactive for Cl⁻ transport, while 38 mediated only low levels of Cl⁻/NO₃⁻ and Cl⁻/HCO₃⁻ exchange. However, thioureas 39 and 40 were effective Cl⁻/NO₃⁻ and Cl⁻/HCO₃⁻ antiporters. As mentioned previously, thioureas have been found to bind anions more strongly than their urea analogues; however, in this case the urea based receptors were found to form stronger 1:1 complexes with TBACl (binding constants of 658, 830, 447 and 191 M⁻¹ respectively, calculated by ¹H NMR titration in DMSO-d₆/H₂O (0.5 %)). Thus, the better transport activity of the thiourea analogues could not be explained by stronger Cl⁻ binding. The authors concluded that the greater lipophilicity of the thiourea carriers resulted in better partitioning of the anion-carrier complex with the lipid bilayer.

![Figure 1.17 Urea and thiourea receptors based on the tren scaffold.](image)

The crystal structure of the carbonate complex of receptor 40 (grown from a DMSO solution of the receptor with TEA/HCO₃) shows a 2:1 host:guest stoichiometry in the solid state, although the solution phase testing indicated a 1:1 complex formation with
bicarbonate. This structure shows that the anion is almost fully encapsulated by the receptors and is bound by 12 hydrogen bonds and a number of longer range interactions. The anion, which was added as HCO$_3^-$ has presumably been deprotonated by free anion in solution to give the CO$_3^{2-}$ complex. This effect has been previously observed by Gale et al.$^{69-71}$

![Figure 1.18 The carbonate complex of tren receptor 40.](image)

One approach to ensure the effective partitioning of a receptor within the lipid bilayer is to choose a naturally occurring scaffold with desirable partitioning properties. B. D. Smith and co-workers have synthesized the urea functionalized phospholipid 41 which can be preincorporated into the vesicle bilayers and facilitate anion antiport via a novel “relay” mechanism as represented in Figure 1.19.$^{72}$ The authors found that Cl$^-$/NO$_3^-$ antiport was only observed if the receptor was preincorporated into the vesicle membrane, due to the necessity of the receptor locating within both leaflets of the bilayer for this mechanism to occur. This design combines a stationary scaffold with a mobile “arm” which can complex and transport the anion, thus employing effective strategies from both channel and carrier design.
Figure 1.19 A representation of the proposed relay mechanism of Cl⁻ transport by phospholipid based receptor 41: (a) Coordination of the anion from the source phase by the urea functionalised arm of 41; (b) transfer of the anion to a second receptor molecule located in the opposite leaflet of the bilayer; (c) release of the anion into the receiving phase.

Anion receptors which facilitate an anion antiport mechanism are able to bind and transport both components of the antiport process. Similarly, receptors designed to facilitate a symport process often provide a binding site for both the anion and the cation of interest. Calix[4]pyrroles are ditopic Cs⁺Cl⁻ receptors and facilitate a Cs⁺/Cl⁻ symport mechanism. Similarly, B. D. Smith et al. have investigated the ion pair binding and transport properties of ditopic macrobicycle 42. This receptor contains an isophthalamide unit for anion complexation and a crown ether unit for binding an alkali metal cation. It was found to bind K⁺Cl⁻ and Na⁺Cl⁻ as contact ion pairs in DMSO solution; additionally, the binding of Cl⁻ to the receptor was enhanced if K⁺ or Na⁺ was already complexed by the receptor. 42 was found to mediate K⁺/Cl⁻ and Na⁺/Cl⁻ symport from vesicles; this process was not dependent on the nature of the external anion. However, no transport was observed in the presence of Cs⁺Cl⁻, as Cs⁺ has been found to be too large to bind within the crown ether unit. Interestingly, addition of the individual cation and anion binding components of this receptor 43 and 44 did not mediate Cl⁻ transport; thus the ditopic nature of this receptor was shown to be highly important.
Figure 1.20 Ditopic receptor 42 transports NaCl and KCl as ion pairs.

However, recent work from Gale and co-workers has described a “dual host” approach to salt transport, in which the individual components of a symport pair can be transported by different ionophores in two complementary uniport processes.\textsuperscript{74} Valinomycin was chosen as a highly potent $K^+$ carrier; in combination with known $Cl^-$ transporters, $Cl^-$ efflux from KCl containing vesicles was observed. A mixture of valinomycin and strapped calix[4]pyrroles 33 and 36 was shown to promote $K^+$/Cl$^-$ more effectively than the sum of the activity of the individual receptors; however, unmodified calix[4]pyrrole 28 was found not to promote this mechanism. The authors reasoned that the transport activity of calix[4]pyrrole 28 is highly dependent on the presence of Cs$^+$, thus it could not function as a Cl$^-$ uniporter; however, as 33 and 36 are able to facilitate anion antiport, they were thought to be more able to facilitate the movement of a single anion across the bilayer at any one time.
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Figure 1.21 Valinomycin and the calix[4]pyrroles used for the dual host transport of K⁺/Cl⁻.

More recently, Gale et al. have applied the dual host approach to the Cl⁻/HCO₃⁻ antiport process. In this work, 33 and 36 were once again employed to mediate Cl⁻ uniport, although these receptors were not capable of HCO₃⁻ transport. Based on the previous finding that simple thioureas are effective Cl⁻/HCO₃⁻ antiporters, 45 and 46 were chosen to mediate the transport of HCO₃⁻. The Cl⁻/HCO₃⁻ antiport activity of 45 and 46 was enhanced in the presence of 33 and 36, with the combined action of these receptors mediating more Cl⁻ flux than the summed activity of the individual receptors. Again, no such enhancement was observed on addition of calix[4]pyrrole 28.

Figure 1.22 The simple thioureas examined as part of a dual host system for Cl⁻/HCO₃⁻ antiport.
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The concept of designing symporters which contain a binding site for both components of the transport process can be extended to H⁺/Cl⁻ co-transport. Prodigiosin 1 contains a basic pyrrolic N (a binding site for H⁺). This inspired collaborative work from Gale, B. D. Smith and co-workers to investigate the H⁺/Cl⁻ co-transport activity of some receptors containing a methylimidazole group as a basic site for H⁺ binding to mimic the action of prodigiosin. Initially, the authors discovered by ¹H NMR titration in CD₃CN that the binding of Cl⁻ by 47 was enhanced when the receptor was protonated in the presence of 1 equivalent of HPF₆ (Kₐ was increased from 60 to 397 M⁻¹). However, no such enhancement was observed for receptor 48, which lacked a basic site. Correspondingly, of these two receptors only 47 was found to mediate Cl⁻ efflux from vesicles containing NaCl suspended in NaNO₃ using an ISE method. This efflux was highly pH dependent. The greatest Cl⁻ efflux was observed in the presence of a pH gradient (inside = pH 4, outside = pH 6.7), indicating that a H⁺/Cl⁻ co-transport mechanism was operating. However, in the absence of a pH gradient (inside = pH 7.2, outside = pH 7.2) reduced Cl⁻ efflux was observed, indicating that a Cl⁻/NO₃⁻ mechanism was also possible. When the intra- and extra- vesicular solutions were acidic, no transport was observed. The authors reasoned that, when the external solution was acidic no transport occurred because the externally added receptor would become protonated in the extravesicular solution and become too hydrophilic to pass through the vesicle membrane.
Further to this work, the same authors investigated the transport activity of receptors 49 – 51. They found that receptor 49 exhibited an enhanced affinity for Cl\(^-\) in the presence of HPF\(_6\) (in DMSO-\(d_6\)/H\(_2\)O (0.5 %)). The stronger binding of H\(^+\)/Cl\(^-\) by 49 over 50 was attributed to the preorganizational effect inferred over the binding site by intramolecular hydrogen bonding as shown in Figure 1.24. In the presence of a pH gradient, 49 was found to be the most effective Cl\(^-\) transporter from vesicles containing NaCl suspended in NaNO\(_3\). 50 was also found to mediate H\(^+\)/Cl\(^-\) co-transport to a lesser degree, while 51, which does not contain a protonatable site, did not mediate Cl\(^-\) transport under these conditions. In the absence of pH gradient, only 49 was found to mediate Cl\(^-\) efflux from vesicles, but less effectively than in the presence of a pH gradient; thus, 49 was the only receptor to also mediate Cl\(^-\)/NO\(_3^-\) antiport.
D. K. Smith et al. have reported that anion receptors based on the tren scaffold can facilitate the transport of H⁺/Cl⁻ through a non-polar phase using a U-tube experiment. The carrier efficiency was found to correlate with the binding site encapsulation, indicating that the most effective carriers could screen the charge of the anion from the hydrophobic organic phase. J. T. Davis and co-workers have also utilized tren as a scaffold for H⁺/Cl⁻ co-transport. They used two convergent catechol groups as hydrogen bond donors to complex the anion. The role and substitution pattern of these OH hydrogen bond donors were found to be highly important, as analogues 55 and 56 exhibited no anion transport activity. The positions of the OH hydrogen bond donors in analogue 56 are less convergent than those in receptor 52, thus anion binding is less favourable. Receptor 52 was found to mediate a variety of H⁺/X⁻ co-transport processes in the presence of a pH gradient, using vesicles containing HPTS. Analogues 53 and 54 with shorter and longer alkyl substituents were found to be less active. This was attributed to poorer partitioning with the bilayer. The anion selectivity was found to follow the Hofmeister series. The Hofmeister series orders ions according to their ability to cause proteins to precipitate from aqueous solution. The precipitation of the protein is caused by the strong competing interactions between the ion and the water, thus increasing the effective concentration of the protein. Thus, the Hofmeister series can be viewed as ranking the ions according to the strength of their interaction with water. This is a highly relevant concept when considering the transmembrane transport of ionic species, as to cross the lipid bilayer the ion must be dehydrated; the stronger the interaction of the ion with water, the less favourable this process will be.
1.7 Synthetic anion channels

The design of ion channels requires a different approach to that of mobile carrier design. In contrast to mobile carriers, the ability of a synthetic channel to bind the anion of interest is less important. The key function of a channel is to form a structure which will span the membrane, either as a single entity or a self assembled pore, and allow the passage of the anion by a lower energy pathway. CFTR is an example of a membrane spanning chloride channel.

The determination of the crystal structures of several naturally occurring protein based channels has provided insight into their action, as reviewed by Gouaux and MacKinnon. Transport proteins have been found to contain water filled pores which function as an entrance to the channel for hydrated ions. The ions must subsequently pass a narrower portion of the channel interior causing dehydration. This also functions as an ion selectivity filter on the basis of size exclusion.

One approach to channel design is to try to mimic the function and structure of naturally occurring peptide based ion channels. The first reported development of a series
of synthetic chloride channels was reported by Tomich et al., who have extensively investigated the chloride conducting properties of various membrane spanning amino acid sequences derived from the second transmembrane segment of the brain glycine receptor, M2GlyR (compound 57, Figure 1.26). The unmodified sequence was known to form anion channels in lipid bilayers, single cells and epithelial monolayers; however, the poor aqueous solubility of this species and its propensity to aggregate in solution limited its effectiveness. This prompted Tomich et al. to investigate the application of some synthetic analogues of this sequence. Over 200 peptides based on this sequence were synthesized by systematic modification of individual amino acids within the sequence, one of which is shown in Figure 1.26. These synthetic peptides were analyzed for increased water solubility, decreased aggregation and anion transport capabilities. For example, C-K₄-M2GlyR (compound 58, Figure 1.26) was found to have increased water solubility due to the presence of the additional lysine subunits.

![Figure 1.26](image)

The peptides were found to promote an anion dependent current in lipid bilayers, in whole cell patches and in epithelial monolayers. They found that the water soluble peptides exist as monomers in solution and form supramolecular assemblies within the bilayer to form bioactive structures. Aggregation of the monomers was found to be inhibited by the addition of an aromatic residue at the C-terminus, thus reducing the formation of high molecular weight associations of the peptide which had no activity. The placement of aromatic and charged amino acids within the acyl core was crucial to the final channel geometry; for example, placement of a tryptophan C-terminally to an arginine resulted in distortion of the helix due the guanido group orientating towards the lipid/water interface. This in depth analysis of structure-activity relationships for a series of synthetic anion transporters highlights that the behavior of a target molecule within a bilayer can be readily modulated synthetically. It also reveals that synthetic systems can
be applied to function effectively in natural systems- an important consideration for the therapeutic application of these species.

Gokel and co-workers have also reported a synthetic peptide for chloride transport based on a naturally occurring amino acid sequence, which they named SCMTR (synthetic chloride membrane transporter, compound 59).\textsuperscript{95} Based on the observation that all members of the C1C family of channel proteins contain the amino acid sequence GKxGPxxH in the anion pathway, the authors chose to synthesize a heptapeptide; this was combined with a proline residue to introduce an “arch” (the presence of proline in channel forming peptides is known to cause a “hinge-bend” shape\textsuperscript{96}), flanked by glycine residues. A long chain dialkylamide terminus was chosen to function as a membrane anchor. The structure of SCMTR is shown in Figure 1.27.

\[(C_{18}H_{37})_2NCOCH_2OCH_2CO-GGPGGG-OCH_2Ph\]

Figure 1.27 The structure of SCMTR 59.

Peptide 59 was found to cause rapid efflux of Cl\textsuperscript{-} from vesicle systems. However, replacement of the proline in the sequence with leucine resulted in diminished Cl\textsuperscript{-} transport, thus demonstrating the importance of the structural constraints the proline residue has on peptide shape. A truncated analogue containing a 4-residue peptide sequence, again not containing proline, was also found to be inactive. An anion antiport mechanism was confirmed by the inhibition of Cl\textsuperscript{-} transport by 59 when the external anion was SO\textsubscript{4}\textsuperscript{2-}, and channel activity was demonstrated using voltage clamp techniques. Interestingly, the channel formation by 59 was found to be voltage gated between -3 and 10 mV, as indicated by the transmitted current dependence on membrane potential. Voltage gating is a key feature of naturally occurring ion channels which is challenging to replicate in synthetic systems but crucial for biological control of the open and close behaviour. Further investigation by the same authors revealed that the Cl\textsuperscript{-} conductance of this type of peptide was enhanced if the chain length of the anchor was decreased.\textsuperscript{97} This was attributed to the simpler aggregation equilibria of the shorter analogue formed in the aqueous phase prior to partitioning with the lipid bilayer- thus, insertion of the active dimeric species is faster.
It is not only peptides that can form membrane spanning ion channels. Amphotericin B (Figure 1.28) is a natural product isolated from *Streptomyces nodosus*. It has antifungal activity and has been shown to form channels in sterol-containing bilayers which transmit both anions and cations. This indicates that the design of ion channels need not focus purely on synthetic peptides. Additionally, amphotericin B is not long enough to span the bilayer, thus the active channel state is an aggregate formed within the bilayer. This demonstrates that synthetic channels do not need to be large enough to span the bilayer as a single entity.

![Figure 1.28 Amphotericin B](image)

The rigid polyene backbone of amphotericin B inspired Matile and co-workers to investigate the ion channel forming ability of some “rigid-rod” molecules. They found that a rigid rod composed of an octa(p-phenylene) backbone with protruding glycerol residues functioned as a unimolecular H⁺ conducting channel in EYPC vesicles (Figure 1.29). They found that this octaphenylene rod was more active than analogous tetraphenyl and hexaphenyl rods. These were speculated to be too short to span the bilayer (the apolar interior of an EYPC bilayer was estimated to be 36 Å). Metal cations were not transported, although H⁺/K⁺ antiport was observed in the presence of valinomycin. Interestingly, the authors reported that these channels could also mediate OH⁻/Cl⁻ exchange in vesicle systems- a process which can be viewed as analogous to H⁺/Cl⁻ symport. Further work by the same authors explored the effect of adding hydrophobic sidechains to these octaphenyleneles in order to compensate the unfavourable interactions between the hydroxyl groups and the apolar membrane interior. This was found to improve proton conductance, but the anion transport properties of these molecules were not investigated.
Further to this work, Matile and co-workers reported the synthesis and anion channel formation of oligo-(p-phenylene)-$N,N$-naphthalenediimide (O-NDI) rods of type 61. The NDI units were selected due to their $\pi$-acidity. It was hoped that anions could be conducted through a bilayer by these species guided by anion-$\pi$ interactions, thus stabilizing the anion within the bilayer as shown in Figure 1.30 (a). The rods were named anion-$\pi$ slides due to this proposed mode of action. These species were found to mediate OH$^-$/$X^-$ exchange in EYPC vesicles containing the pH sensitive fluorescent dye HPTS. The observed transport was not dependent on the nature of any of the metal cations present, but was found to be highly anion dependent- thus, O-NDI rods function as anion selective channels. O-NDI rods with one cationic terminus were found to be the most active for anion transport. Importantly, this work demonstrates that a transmembrane anion channel need not resemble a classical pore like structure, but should simply provide stabilizing interactions to the anion as it crosses the lipid bilayer. Interestingly, the neutral O-NDI rods showed an anti-Hofmeister selectivity ($\text{Cl}^- > \text{F}^- > \text{Br}^- > \text{I}^-$). As an extension of this work, the authors synthetically tethered two O-NDI rods together to form a hairpin structure; this resulted in an even greater Cl$^-$ selectivity.
Figure 1.30 (a): O-NDI rods (61) reported by Matile to function as anion-π slides, and (b): O-PDI rods (62) which facilitate the movement of anions and electrons.

More recent work from Matile et al. describes the structure and function of some oligo-p-phenyl-\(N,N\)-perylenediimides (O-PDI rods, 62), shown in Figure 1.30 (b).\(^{101}\) PDIs are π-acidic semiconductors. The rods were designed to be long enough to span the membrane, with one anionic terminus to ensure membrane orientation. The O-PDI rods were found to facilitate Cl/OH\(^-\) exchange and to display the same anion selectivity as the O-NDI rods. The semiconducting ability of PDIs also prompted the authors to examine the ability of these receptors to transport electrons- photosynthetic activity- using the Hurst assay. In this experiment, vesicles are prepared containing [Co(bpy)\(_3\)]\(^{3+}\), the photoreduction of which can be detected by a change in absorption around 320 nm. The extra-vesicular solution contained EDTA, used as an electron donor. Thus, observed reduction of the [Co(bpy)\(_3\)]\(^{3+}\) is evidence of the passage of electrons across the bilayer. The O-PDIs were found to exhibit photosynthetic activity under these conditions.\(^{101}\)
addition of a proton carrier, FCCP, to the system did not alter this observed activity; this supports the theory that the active transport of an electron is coupled to the passive exchange of an anion. This work represents a novel application for adapted anion transport systems.

Schmitzer and co-workers have described the Cl⁻ transport activity of a series of imidazolium salts 63-65. Inspired by the work of Matile, the authors proposed that potential anion-π interactions between these molecules and Cl⁻ should be strong due to the positively charged imidazolium substituent close to the aromatic system. Only compound 65 was found to mediate Cl⁻/NO₃⁻ exchange in lucigenin containing vesicles. Emission spectra of 64 and 65 indicated the existence of eximers in liposomal membranes, thus implying aromatic-aromatic interactions are present. A computational study predicted that compound 65 should form an aggregate of the correct length to span the bilayer. Additionally, the observed transport activity was inhibited by the addition of α-cyclodextrin or cucurbit[7]uril. These species are known to form complexes with imidazolium salts. Their addition was assumed to disrupt channel formation and enhance the partitioning of the transporter in the aqueous phase over the lipid phase.

![Figure 1.31 The imidazolium salts investigated by Schmitzer et al.](image)

The self assembly of channels from smaller constituent units has also been demonstrated by Yang and co-workers, who report that compound 66 forms chloride selective channels. The design of 66 was chosen to incorporate α-aminooxy hydrogen
bond donors, as this had been previously shown to be effective for the complexation of Cl$^-$.\textsuperscript{104, 105} Cl$^-$/NO$_3^-$ antiport was demonstrated in unilamellar vesicles containing the halide sensitive dye 6-Methoxy-N-(3-sulfopropyl)quinolinium (SPQ). Using patch clamp techniques, single channel measurements were recorded which proved the presence of channels. Additionally, MDCK cells which had been loaded with SPQ were used to demonstrate Cl$^-$ transport in cells. It is remarkable that such a small compound is able to assemble into membrane spanning channels, and notable that a small scaffold designed to complex Cl$^-$ was found to function as a channel rather than a mobile carrier. The biological potential of this work was further demonstrated when the authors reported that 66 could regulate cell membrane potentials and the action of voltage gated Ca$^{2+}$ channels.\textsuperscript{106} This indicates that the action of synthetic anion transporters can regulate other physiological effects and be incorporated into natural processes.

![Figure 1.32](image)

Figure 1.32 Some simple molecules known to assemble into anion channels.

Similarly, Gokel has reported that certain dianilides of picolinic or isophthallic acid are able to assemble into membrane spanning channels.\textsuperscript{107} Aromatic substituents containing electron withdrawing groups were found to the most effective anion transporters. 69, 70 and 72 were shown to efficiently mediate Cl$^-$ influx into DOPC vesicles containing lucigenin as a fluorescent Cl$^-$ sensitive probe, while 67, 68 and 71 were less active. The planar bilayer conductance technique was used as proof of channel assembly; the authors reasoned that such a membrane spanning structure would require aggregation within the bilayer. Evidence of stacking was obtained by fluorescence measurements in both DMSO and within lipid bilayers, leading the authors to hypothesize that the stacking of multiple monomer units is responsible for the formation
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of a pore. This work demonstrates that even small molecules can assemble to form pores, and that prediction of channel vs. carrier activity may not always be straightforward as self assembly can be unpredictable.

J. T. Davis and co-workers explored ion transport activity of a calix[4]arene tetrabutylamide 74. They found that 74 could mediate H⁺/Cl⁻ efflux from HPTS containing vesicles in the presence of a pH gradient. However, when the internal solution was Na₂SO₄ no transport was observed, indicating that HSO₄⁻ could not be transported in this way. Channel formation was proved using voltage clamp experiments. This transport was not cation dependent. The secondary amide NH groups were found to be essential for anion transport activity. Calix[4]arene 75, which does not contain amide NH groups to aid anion binding, was found to bind Na⁺ and to mediate ion transport which was dependent on the nature of the encapsulated cation but not anion dependent.

The authors failed to crystallize active calix[4]arene 74 but were able to grow crystals of 73.H⁺/Cl⁻.3H₂O, shown in Figure 1.33. Calix[4]arene 73 does not promote membrane ion transport, presumably because it is less lipophilic than 74 and 75; however, the authors reasoned that it should bind H⁺/Cl⁻ analogously to 74. The crystal structure indicates that individual calix[4]arenes are bridged by hydrogen bonding to Cl⁻ and water filled pores.
This solid state structure, while not necessarily representative of channel formation in vesicle bilayers, indicated that the formation of this hydrogen bonded array did not involve complexation of the Cl\(^-\) within the cavity of the calix[4]arene and that as such, the calix[4]arene macrocycle may not be directly involved in the anion transport process. This prompted the authors to investigate the ion transport activity of some acyclic oligophenoxyacetamides 76-81.\(^{109}\) Using vesicles containing HPTS, they found that trimer 78 was an order of magnitude more active for H\(^+\)/Cl\(^-\) co-transport than calix[4]arene 74; meanwhile, analogues 76, 77 and 79-81 exhibited limited activity. No ion transport was observed if the Cl\(^-\) was exchanged for SO\(_4\)^{2-}, thus indicating the highly anion dependent nature of this transport process. The transport of Cl\(^-\) by 78 was also observed directly using \(^{35}\)Cl\(^-\) techniques.
J. T. Davis and co-workers have also found that tetraamide substituted calix[4]arenes fixed in a partial cone or a cone conformation can facilitate Cl⁻/NO₃⁻ antiport. They found that partial cone 82 and cone 83 could facilitate Cl⁻/NO₃⁻ antiport under neutral conditions in vesicles containing lucigenin. 82 was found to be twice as active as 83 under the same conditions. The substitution pattern of the calix[4]arene was found to be critical, as 85, in which one of the amide arms was replaced by an ester, was found to be much less active than the analogous tetramide 83. However, calixarene 84, which contained only 3 amide arms was more active than the tetramide. The pH sensitive nature of the free phenolic group prompted the authors to examine 84 as a pH tunable transporter. They found that increasing the pH of the system resulted in a decrease in observed transport activity corresponding to deprotonation of the phenolic OH group. This pH dependence was not observed with tetraamide 83. Thus, 84 was found to function as a pH gated Cl⁻ transporter.
Gin and co-workers have explored the ion channel formation by a modified β-cyclodextrin 86.\textsuperscript{110, 111} The oligoether chains were thought to be hydrophobic enough to penetrate the bilayer and long enough to span the bilayer; thus, this design comprises a water soluble head group, presumed to position near the surface of the bilayer, coupled with hydrophobic chains to provide a transmembrane ion pathway. In vesicles containing HPTS they demonstrated that 86 could facilitate Na\textsuperscript{+}/OH\textsuperscript{−} influx (or Na\textsuperscript{+}/H\textsuperscript{+} antiport). The observed pH changes were not found to be cation selective; however, the influx of Na\textsuperscript{+} was observed directly using \textsuperscript{23}Na NMR. The anion selectivity of the channel was probed by variation of the counteranion (Cl\textsuperscript{−}, Br\textsuperscript{−} and I\textsuperscript{−}) using Na\textsuperscript{+} salts. In this case, a more complex process was found to occur. On addition of NaBr or NaI, the intra-vesicular pH was observed to sharply fall; this was attributed to rapid X\textsuperscript{−} influx (H\textsuperscript{+}/X\textsuperscript{−} symport or X\textsuperscript{−}/OH\textsuperscript{−} antiport). The internal pH was then observed to steadily increase, indicative of a
slower Na\(^+\) transport process. The rate of influx of Cl\(^-\) was more comparable with the rate of efflux of Na\(^+\) thus producing a gradual decrease in intra-vesicular pH with no observable re-equilibration process. The anion selectivity followed the Hofmeister sequence (I\(^-\) > Br\(^-\) > Cl\(^-\)). Thus, 86 was found to function as a highly selective anion channel.

![Figure 1.36 A functionalized β-cyclodextrin reported by Gin et al. to function as an anion channel.](image)

This design inspired collaborative work from Montesarchio and Tecilla to investigate the ion transport properties of some cyclic phosphate-linked oligosaccharides (CyPLOS) 87-91.\(^{112}\) The authors hypothesized that the anionic macrocycle could form a pore at the polar membrane surface, with the amphiphilic chains penetrating the inner core of the bilayer in a manner analogous to Gins cyclodextrin based channel.\(^{110, 111}\) They found using vesicles containing HPTS (internal solution = pH 7.0) that spiking in NaOH after addition of the ionophore resulted in an increase in intra-vesicular pH. The biggest response was exhibited by CyPLOS 87; analogues 88 and 89 were found to be much less active, indicating the importance of the tetraethylene glycol chains, potentially as these form the polar transmembrane pathway for the ions. CyPLOS 90 and 91 also exhibited ion transport activity. The behaviour of these compounds was similar to that exhibited by Gins β-CD; the observed transport was found not to be cation selective, (although Na\(^+\) transport was confirmed using \(^{23}\)Na\(^+\) techniques), but highly dependent on the nature of the counteranion, displaying Hofmeister selectivity.
The self-assembly of cyclic D,L-α-peptides has been used to produce self assembled ion channels. Matile et al. have investigated the transport activity of peptidomimetic oligourea/amide macrocycles 92-97. Solution phase aggregation studies indicated that 32 formed hydrogen bonded dimers in CDCl₃ or CD₂Cl₂, whereas 92 and 95 formed tubular stacks. The stacks associate via dipole-dipole interactions between the macrocycles to form nanotubes. The dipole of the macrocycles is represented in Figure 1.38.

Figure 1.37 CyPLOS macrocycles which form a pore at the surface of the bilayer.

Figure 1.38 The oligourea/amide macrocycles reported by Matile which assemble to form stacks by dipole-dipole interactions.
Using a HPTS assay, the $\text{H}^+$ co-transport of anions including $\text{Cl}^-$ was demonstrated. As the macrocycles are too small to complex the ions of interest within the central cavity, a “Jacobs-Ladder” mechanism was proposed. The macrocycles are assumed to stack with their macrodipoles aligned to provide a pathway for ions through the bilayer. Anions may be bound to one face of a macrocycle by the urea and amide hydrogen bond donors. The macrocycles are theorized to rotate $180^\circ$ and thus facilitate the movement of anions along the ladder. This mechanism is represented in Figure 1.39. Additionally, macrocycle 92 showed an anti-Hofmister bias, whereas 93 and 94 exhibited anion selectivity according to the Hofmeister sequence; however, both 92 and 93 showed extra selectivity for $\text{Cl}^-$. Macrocycles 92-94 were found to be an order of magnitude more active than alkyl substituted receptors 95-97.

Figure 1.39 The proposed “Jacobs-ladder” mechanism of anion transport mediated by Matile’s oligourea/amide macrocycles.
1.8 Experimental techniques to assess anion transport

1.8.1 Vesicles

Laboratory made, unilamellar vesicles serve as a simple and easily reproducible model of a cell membrane. The techniques described within this thesis were developed by B. D. Smith and co-workers. The preparation of the vesicles used in this work is versatile, allowing for careful control of the intra- and extra-vesicular solutions. The vesicles are formed by a freeze thaw and extrusion technique, described in detail within Chapter 6.2. There is a criteria that the intra- and extra-vesicular solutions should be isotonic, as transmembrane osmotic imbalances can result in membrane rupture. Solutions are buffered to prevent the build up of undesirable pH gradients. However, it is possible to vary the salt composition of the intra- and extra-vesicular solution within these limits. By systematically varying these salt compositions, it is possible not only to observe anion transport but also to gain important mechanistic insight into the process.

1.8.2 Monitoring the movement of anions

It is possible to observe the transport of various anions by a number of methods. Almost exclusively in this work, the transport of chloride has been monitored using a chloride selective electrode (ISE) as depicted in Figure 1.40(a). The intra-vesicular solution is “invisible” to the ISE; thus, an increase in chloride concentration of the extra-vesicular solution can be monitored and equated to chloride efflux.
Figure 1.40 The movement of anions across vesicle membranes can be tracked using (a) an ISE technique, or (b) using fluorescent dyes encapsulated within the vesicle.

Alternatively, a number of fluorescent probes may encapsulated within the vesicles and utilized to track the movement of anions (Figure 1.40(b)). The structures of some commonly used dyes are shown in Figure 1.41. Halide sensitive dyes such as lucigenin and 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ) may be used to monitor changes in the intra-vesicular chloride concentration as their fluorescence is quenched by chloride. 53 8-Hydroxypyrene-1,3,6-trisulfonate (HPTS, also known as pyranine) is a pH sensitive dye, which has been used to great effect in monitoring the changes in intra-vesicular pH associated with H⁺/Cl⁻ co-transport. 116 As this technique is independent of the nature of the anion, it has also been utilized to demonstrate the co-transport of anions other than chloride with protons. Safranin-O is a potential sensitive dye that may be used to identify changes in the membrane potential induced by the transport of ions. 117 Meanwhile, J. T. Davis and co-workers have pioneered the use of ¹³C NMR to monitor the transport of H¹³CO₃⁻. 59 Distinct resonances may be observed for intra- and extra-vesicular H¹³CO₃, allowing the movement of this anion to be assessed. Addition of Mn²⁺ causes broadening of the signal associated with the extra-vesicular H¹³CO₃ but not the intra-vesicular H¹³CO₃, allowing the identity of each resonance to be established. Carboxyfluorescein is an anionic fluorescent dye which is self-quenching; thus, changes in its intra-vesicular concentration can be easily monitored. Anion transporters that can facilitate the transport of carboxyfluorescein anions are often hypothesized to be forming
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large, non-selective pores, due to the greater anion selectivity inferred by binding site design in mobile carriers. However, it should be noted that Gokel et al. have observed the transport of carboxyfluorescein in a U-tube experiment, thus it is not impossible that the transport of carboxyfluorescein may occur by a mobile carrier mechanism.

Figure 1.41 The structure of various fluorescent dyes that can be used to track the movement of ions in vesicle studies.

1.8.3 Variation of salt composition

Variation of the intra- and extra-vesicular salt solutions can distinguish between and characterise an antiport or symport mechanism. An initial test for chloride antiport processes uses vesicles containing NaCl suspended in NaNO₃. If chloride efflux is promoted by addition of a carrier, it may be via a Cl⁻/NO₃⁻ antiport mechanism or a Na⁺/Cl⁻ symport mechanism. Anion antiport mechanisms are dependent on the nature of both the intra- and extra-vesicular anion as both are involved in the transport process. Substituting the external anion for SO₄²⁻ should inhibit an antiport mechanism, as SO₄²⁻ is divalent and highly hydrophillic, and to date there are no known synthetic receptors able to facilitate its transport. If a Cl⁻/NO₃⁻ antiport mechanism is proved, it is then desirable to
establish if the carrier is able to facilitate the antiport of Cl\(^-\) and HCO\(_3\)\(^-\) due to the greater biological relevance of this process. This is investigated using vesicles containing NaCl suspended in Na\(_2\)SO\(_4\). The receptor is added and, as the receptor is an antiporter, no chloride efflux should be observed. A pulse of NaHCO\(_3\) is then added, and any resulting chloride efflux may be attributed to a Cl\(^-\)/HCO\(_3\)\(^-\) antiport process. HCO\(_3\)\(^-\) transport may also be directly observed using the \(^{13}\)C NMR technique as detailed above.

Alternatively, if the carrier is able to facilitate M\(^+\)/Cl\(^-\) symport, the transport will not be dependent on the nature of the extra-vesicular anion. It will however be dependent on the nature of the intra-vesicular cation. Using vesicles suspended in Na\(_2\)SO\(_4\) to rule out any antiport activity, the alkali metal cation in the intra-vesicular MCl solution can be varied to establish the preferred cation for the symport process. Meanwhile, a H\(^+\)/Cl\(^-\) symport process can be evaluated by varying the relative starting pH of the intra- and extra-vesicular solutions. For example, for a solution of vesicles containing NaCl suspended in Na\(_2\)SO\(_4\), H\(^+\)/Cl\(^-\) efflux should be encouraged if the intra-vesicular solution is more acidic than the external solution, and inhibited if the opposite pH gradient is applied.

### 1.8.4 Choice of lipid

There are a number of different phospholipids commonly utilized for transport experiments. Phosphotidylcholines (PCs) are a major component of cell membranes. POPC is commonly used for transport studies, has been used by Gale and co-workers in all publications to date, and as such has been used most commonly within this thesis. The structure of POPC and some other phospholipids used in similar studies are shown in Figure 1.42. EYPC (egg yolk PC) is a phospholipid derived from egg yolks which is predominantly composed of POPC and thus the properties are similar. DOPC and related di-unsaturated PCs have also been used for vesicle formation. In particular, using lipids from this family with different chain lengths has been used as a tool to probe the effect of bilayer thickness on transporter efficiency,\(^{54}\) as changing this variable should influence the observed activity of a channel and a mobile carrier in different ways. Additionally, the saturated phospholipid DPPC has been used in mobility assays as described below.
1.8.5 Mobility assays

There are various proofs that have been employed to demonstrate mobile carrier activity over channel formation. It was previously common to use the lipid DPPC in mobility assays. DPPC undergoes a phase transition from the solid gel phase to a liquid crystalline (fluid) phase at 41 °C. The action of a channel- which is relatively stationary once established- should be undisturbed by this transition once the channel has been inserted into the bilayer; however, the action of mobile carriers is dependent on diffusion through the bilayer, and thus should be inhibited if the system is cooled below 41 °C. Inhibition of transport on cooling a system of DPPC vesicles was therefore taken as proof of mobile carrier activity.\textsuperscript{118} However, Regen has described the so-called “squeezing out” of an ionophore;\textsuperscript{119} that is, that on cooling a system of DPPC vesicles below the transition temperature, the transporter was expelled from the bilayer. Thus, it is now not necessarily accepted that a loss of transport on cooling in this system is proof of a mobile carrier mechanism.

For small molecules which are not large enough to span the bilayer, it has been argued that a first order concentration dependence on observed transport activity is proof that no cooperativity is occurring and that the active species cannot be an aggregate.\textsuperscript{48}
Additionally, the inclusion of cholesterol within a membrane reduces its fluidity and thus the rate of transport by mobile carriers should be reduced.\textsuperscript{58, 119}

1.8.6 Voltage clamp experiments

While the formation of channels may be implied by various means including carboxyfluorescein transport and variation of the lipid bilayer thickness, according to Fyles, the voltage clamp technique “is the sole method to unequivocally establish the presence of channel-like activity”.\textsuperscript{120} It is certainly the only known method which can directly observe the action of ion channels. The term “voltage clamp” may refer to planar lipid bilayer studies, in which the current flowing across a painted bilayer is monitored, or patch clamp measurements,\textsuperscript{121} which monitor the transmembrane current across a patch of a cell or vesicle membrane. Using these techniques, it is possible to observe a transmembrane current as a result of ion transport. Crucially, it is possible to record “single channel” measurements- the observation of ion transport due to one or a small number of discrete channels.\textsuperscript{122} As such, it is possible to observe characteristic open-close behaviour of individual channels as step-wise changes in conductance. In contrast, the action of a mobile carrier might be expected to produce an increase in membrane current but with no such open-close behaviour.

1.8.7 The Hill equation

The Hill equation was first introduced by A. V. Hill in 1910 to describe the cooperativity of oxygen binding to haemoglobin.\textsuperscript{123} More specifically, the equation described the relationship between the concentration of a substrate (in this case oxygen), and an observed effect (the % of saturated haemoglobin binding sites). Since then, the Hill equation has been applied to a wide range of systems.\textsuperscript{124} It has been often utilized in pharmacodynamics to describe the relationship between drug concentration (C) and observed drug effect (E).\textsuperscript{125, 126} A form of the Hill equation that is useful in this context is shown below (Equation 1.1), where EC\textsubscript{50} is the drug concentration for which 50 % of maximum effect is obtained and n is the Hill coefficient of sigmoidality. The Hill
coefficient has been interpreted to represent the number of ligand binding patterns required to mediate the observed effect.

\[ E = \frac{E_{\text{max}}C^n}{(EC_{50}^n + C^n)} \]

**Equation 1.1** A form of the Hill equation frequently applied to pharmadynamics.

The Hill equation can be applied to ion transport systems by examining the effect of varying the concentration of transporter (C) on the observed ion flux (E). In this way, a value of EC\(_{50}\) can be calculated and used to quantify transport activity— the lower the value of EC\(_{50}\), the more potent the transporter. Calculation of the Hill coefficient can provide mechanistic insight into the transport process, representing the number of transporter molecules required to transport a single ion; either as an aggregate mobile carrier complex, or as a self-assembled channel species. However, Matile reports that the use of the Hill coefficient to study supramolecular self assembly is not always straightforward. The formation of highly stable assemblies may give a Hill coefficient of 1, presumably because the assembly “behaves” as a single entity and can be misinterpreted as a monomer. It has also been suggested that the Hill analysis is only compatible with endergonic self-assembly, thus inconsistencies may arise if the assembly is exergonic.

### 1.8.8 Testing in real cells

While testing in synthetic bilayers is more commonly used due to the ease at which such bilayers can be produced, there are also examples of synthetic transporters which have been tested in real cells by a number of techniques. The patch clamp technique can be applied to monitor the whole-cell current.\(^{121}\) This gives no insight into channel vs carrier activity but can be used to quantify anion transport. The Ussing chamber technique measures the current across a monolayer of epithelial cells grown on a permeable support.\(^{127}\) It is also possible to incubate cells with fluorescent dyes such as SPQ; changes in the internal fluorescence of the cells can then be observed as indicative of ion transport.\(^{128}\)
Experiments using real cells have been reported far more commonly in the testing of synthetic chloride channels. To the authors’ knowledge, the only reported example of testing a mobile carrier in real cells to date is a cholapod published by A. P. Davis which has shown activity using the Ussing chamber technique.

1.8.9 Comparison of experimental techniques

Little attempt has been made within this introduction to distinguish the relative effectiveness of different transport systems. The small variations in the transport experiments utilized by different research groups makes this an extremely difficult task. Such variations may include:

- The concentrations of salts. The effectiveness of a transporter is linked to the magnitude of the electrochemical gradient that drives the transport process.
- The choice of phospholipid. Commonly used lipids in vesicle-based transport studies include POPC, EYPC, and DOPC. The composition of the lipid bilayer directly affects the ease of transport through it. Additionally, the activity of several channels has been found to be dependent on the thickness of the bilayer, and thus the structure of the phospholipid.
- The use of cholesterol. The addition of cholesterol to a bilayer causes the lipids to become more ordered and the bilayer to become more viscous. This effect has been used to probe the action of mobile carriers that are diffusion controlled; however, some research groups use vesicles containing cholesterol as standard procedure. Clearly, in the testing of mobile carriers this is a significant factor.
- Preincorporation. If a receptor is too lipophilic to pass through an aqueous phase in order to partition with the vesicle bilayer, its transport activity can still be evaluated by preparing vesicles in which the receptor is already contained within the vesicle membrane. However, these results are incomparable with the results obtained without preincorporation.
Within the experiment reported in this thesis, these factors have been kept constant unless otherwise stated so that the experiments are comparable with each other and with previous publications by the Gale group.

### 1.9 Aims of this thesis

This chapter has covered the major advances in the development of synthetic anion transporters over recent years. The work in this thesis focuses on the development of new mobile carriers for anions. There are a large variety of structural motifs that have been previously used for this purpose. This work aims not only to identify new anion carriers but also to gain insight into the structure-activity relationships. In short, this work attempts to determine—“what makes a good anion carrier?”

In order to address this challenge, this thesis has been divided such that each chapter examines a different series of structurally related receptors and analyzes the trends that become apparent in their anion binding and transport properties. The anion transport properties of the receptors discussed in this thesis have been investigated using vesicle-based methods. The solution phase anion binding properties of new receptors has also been investigated, along with the solid state anion binding properties where possible.

*Chapters 2, 3 and 4* examine a progression in receptor design from structurally simple ureas and thioureas (*Chapter 2*) to a series of bipodal bis-alkyl ureas (*Chapter 3*) and bipodal bis-alkyl thioureas (*Chapter 4*). *Chapter 5* reports the anion transport studies relating to a number of strapped calix[4]pyrroles which were provided by J. L. Sessler and C.- H. Lee.
Chapter 2
Structurally simple transmembrane anion transporters

2.1 Introduction

The use of ureas as hydrogen bond donors for anion complexation was first explored by Wilcox\(^{129}\) and since then urea and thiourea groups have been successfully incorporated into a vast number of neutral anion receptors.\(^{130}\) The directionality of the N-H hydrogen bond donors facilitates the effective binding of Y-shaped anions such as carboxylates and the chelation of spherical anions such as Cl\(^-\) (Figure 2.1).

![Figure 2.1 The binding of Y-shaped and spherical anions by (thio)urea receptors.](image)

2.1.1 Ureas vs. thioureas for anion binding

Thioureas are more acidic than analogous ureas.\(^{52}\) Steiner has stated that hydrogen bonding can be simplistically viewed as partial proton transfer between the hydrogen bond donor and acceptor.\(^{131}\) In any case, considering the greater polarization of the N-H bond, it is reasonable to expect that thioureas will form stronger hydrogen bonds with anions, thus making them more potent anion receptors, although this is not always the case.\(^{69, 132}\) Work by Umezawa and co-workers investigated the anion binding properties
of bis-urea 98 and bis-thiourea 99 (Figure 2.2).\textsuperscript{133} Binding studies were carried out by \textsuperscript{1}H NMR titration of the ligands with tetrabutylammonium anion salts in DMSO-$d_6$. Both receptors were found to be selective for $\text{H}_2\text{PO}_4^-$ ($K_a = 110$ and $820$ M$^{-1}$ respectively) The interaction of thiourea 99 with $\text{CH}_3\text{CO}_2^-$ ($K_a = 470$ M$^{-1}$) was also found to be stronger than receptor 98 ($K_a = 43$ M$^{-1}$). This observation was directly attributed to the formation of stronger hydrogen bonds to the anion by the more acidic thiourea N-H donors. It has also been reported that thioureas have a lower propensity to self associate through intermolecular hydrogen bonding than ureas due to the lower hydrogen bond basicity of the sulfur,\textsuperscript{134} so there is less competition for hydrogen bond formation to the anion.

![Figure 2.2](image)

Figure 2.2 The urea and thiourea receptors reported by Umezawa.

However, the higher acidity of thioureas can result in their deprotonation by basic anions. The deprotonation of neutral NH groups in anion receptors by F$^-$ was first reported by Gunlaugsson\textsuperscript{135, 136} and Gale.\textsuperscript{137-139} Subsequently, Fabbrizzi et al. investigated this effect in detail, comparing the interaction of a urea and a thiourea with various anions.\textsuperscript{140} Receptors 100 and 101 (Figure 2.3) contain a phthalimide substituent as a chromophore to allow the interactions of the receptors with anions to be monitored using UV-vis and NMR techniques in DMSO solution. They found that urea 100 was only deprotonated by F$^-$, while compound 101 undergoes deprotonation in the presence of F$^-$, $\text{CH}_3\text{CO}_2^-$, $\text{PhCO}_2^-$ and $\text{H}_2\text{PO}_4^-$. The extent of deprotonation of 101 correlated with the stability of the corresponding hydrogen bridged dianion [X-H-X]$^-$ complex. Only Cl$^-$ failed to deprotonate 101 to any extent. Thus, the authors concluded that, while hydrogen bond donor acidity is a desirable property for anion recognition that can lead to strong anion binding, receptors which are too acidic can lead to the formation of complexes which are unstable with respect to the release of HX.
Figure 2.3 Fabbrizzi et al. have studied the deprotonation of (thio)ureas containing a chromophore.

Interesting work from Hayashita and Teramae has investigated binding anions in aqueous solution using thioureas 102-109, shown in Figure 2.4, in cationic vesicles. The authors noted that binding anions in aqueous solution is highly challenging due to the hydration of the anion. Therefore, they proposed that anion recognition in water could be achieved by incorporating the receptor into a vesicle bilayer in order to shield the binding site from water. The receptors were preincorporated into vesicles composed of didodecyldimethylammonium bromide (DDAB), a simple amphiphile.

Figure 2.4 Thioureas with a lipophilic alkyl substituent can complex anions within a vesicle bilayer.

The UV-vis absorption spectra of the compounds in DDAB indicate that the positioning of the receptor binding site was highly dependent on the length of the receptors alkyl substituent. The $\lambda_{\text{max}}$ of the receptors shifts to higher wavelengths (by comparison to the value in acetonitrile) progressively as the alkyl chain length increases. The authors explain that, for the receptors with a longer alkyl chain, the binding site is located on the surface of the vesicle whereas the receptors with shorter chain lengths position their binding site in the hydrophobic interior of the bilayer. This is because the longer chain analogue can adequately position within the bilayer such that the
hydrophobic alkyl chain resides in the apolar bilayer interior, with the polar binding site located on the vesicle surface. However, the shorter chain analogues are not long enough to facilitate this positioning, resulting in the whole molecule positioning within the hydrophobic bilayer interior. Further evidence was obtained by comparison of the $^1$H NMR spectra of 102-109 in DMSO-$d_6$ and in DDAB-D$_2$O. In DMSO-$d_6$ the vicinal protons of each receptor appear at similar chemical shift, but in DDAB-D$_2$O solution the chemical shifts range from 8.00 ppm (compound 1) to 8.22 ppm (compound 8). UV-vis titration of the receptor 102 in DDAB vesicles with various anions resulted in a bathochromic shift in $\lambda_{\text{max}}$ with an enhanced molar absorptivity. These observations were attributed to the formation of the receptor-anion complex and a change in the positioning of the receptor within the vesicle membrane as a result of the hydrophilicity of the anion. The observed anion selectivity followed the Hofmeister bias ($\text{Br}^- > \text{H}_2\text{PO}_4^- > \text{Cl}^- >> \text{HCO}_3^-, \text{CH}_3\text{CO}_2^-$). However, compound 109 showed little selectivity and was found to interact weakly with more hydrophilic anions such as acetate, which did not interact with compound 102. Therefore it was assumed that anion binding by 109 takes place on the surface of the vesicle resulting in weaker binding due to contact with the aqueous phase, while compound 102 binds the anions within the bilayer resulting in a strength of interaction highly dependent on the lipophilicity of the anion. Compounds 103-108 showed a progressive intermediate response between these two extremes. This study demonstrates that thioureas can membrane-solubilize anions including chloride, which is highly relevant to the challenge of transporting the anions across the bilayer. Presumably, this chain length selectivity would be dependent on the thickness of the bilayer relative to the length of the receptor.

2.1.2 Indole substituted ureas and thioureas

Heterocycles such as indole, pyrrole and carbazole are useful functional groups for anion recognition$^{142}$ as they contribute an NH hydrogen bond donor which is not accompanied by a hydrogen bond acceptor (as in the case of other functional groups such as amides and ureas which contain a carbonyl C=O). This is an advantageous property for anion complexation as there are consequently no competing inter- or intramolecular
hydrogen bonding interactions associated with the use of indoles. This property is also useful in the design of anion transporters as additional hydrogen bond acceptors make a scaffold less lipophilic, thus making their partitioning with the bilayer less favourable.

As they contribute only one hydrogen bond donor, indoles are frequently used in combination with adjacent urea moieties to produce a convergent hydrogen bonding cleft. Pfeffer et al. have reported receptors 109-111 (Figure 2.5)\textsuperscript{143} The anion binding properties of these receptors were investigated by $^1$H NMR titration with the tetrabutylammonium salts of interest. By examining the change in chemical shifts of each hydrogen bond donor on the addition of aliquots of anion, the authors were able to determine the involvement of each NH proton in anion complexation. Titration of host 110 with TBA$^+$AcO$^-$ revealed that only the thiourea NHs were found to significantly shift, thus implying that the indole and amide NHs did not contribute to the binding process. This may be due to the strong binding of Y-shaped anions by the (thio)urea motif. Titration of host 109 with TBA$^+$H$_2$PO$_4^-$ revealed that all of the hydrogen bond donors were involved in anion complexation. Binding constants were determined, revealing that smaller receptors 109 and 110 were selective for H$_2$PO$_4^-$, most likely due to the more convergent binding sites thus allowing all of the hydrogen bond donors to contribute anion binding. Receptor 111 was selective for AcO$^-$, as the longer spacer between the thiourea and the indole and amide moieties reduced the favourability of the cooperative binding of H$_2$PO$_4^-$.

![Figure 2.5 The indole containing receptors reported by Pfeffer et al.](image)
Gale and co-workers have also used a combination of indoles and ureas to yield highly effective scaffolds for anion recognition. Di-indolylurea 112 (Figure 2.6) was found to selectively bind H$_2$PO$_4^-$ (by $^1$H NMR titration with the TBA$^+$ salt).$^{69}$ A 1:1 binding stoichiometry was confirmed for this process by Job plot analysis. Remarkably, interaction was observable in DMSO-$d_6$/H$_2$O mixture of increasing polarity; binding constants were calculated as 5170 M$^{-1}$ in DMSO-$d_6$/H$_2$O-10 % and 160 M$^{-1}$ in DMSO-$d_6$/H$_2$O-25 %. The strength of this interaction in highly competitive solvent systems indicates that the diindolylurea cleft is well suited to binding the H$_2$PO$_4^-$ anion. Further to this work, Gale $et$ $al.$ found that increasing the number of hydrogen bond donors on the diindolyl urea scaffold (receptor 113, Figure 2.6) resulted in hosts which could complex a range of anions extremely strongly in DMSO-$d_6$/H$_2$O mixtures.$^{70}$ Interestingly, the titration of 113 with TBAH$_2$PO$_4$ in DMSO-$d_6$/H$_2$O-0.5 % resulted in the appearance of new peaks, apparently in slow exchange, after the addition of 1.4 eq. anion. The authors found that these peaks indicated the deprotonation of the bound H$_2$PO$_4^-$ anion to HPO$_4^{2-}$. They reasoned that the strong complexation of the anion by 6 hydrogen bond donors could modify the pK$_a$ of the bound anion by stabilizing the deprotonated form. The deprotonation of a bound anion had been previously observed in the solid state;$^{69}$ however, this was the first reported example of this effect in solution.

Further to this work, the same authors described the synthetic modification of the diindolylurea scaffold to incorporate pendant indole substituents (receptor 114, Figure 2.6).$^{71}$ This receptor contains 8 hydrogen bond donors which could potentially complex a single anion. In DMSO-$d_6$/H$_2$O 0.5%, receptor 114 facilitated the deprotonation of bound H$_2$PO$_4^-$ and HCO$_3^-$ by free anion in solution. The interactions towards TBA$_2$SO$_4$ were also investigated by $^1$H NMR titration under the same conditions. The binding of SO$_4^{2-}$ was judged to be strong based on the shape of the binding curves; however, the binding curves could not be fitted to a 1:1 or 2:1 profile. The data indicated initial strong 1:1 complexation followed by the formation of higher order complexes at higher SO$_4^{2-}$ concentrations. In the solid state, the crystal structure of 114.SO$_4^{2-}$ showed a 1:1 stoichiometry in which the anion was bound by 8 hydrogen bonds. However, other anions including benzoate were bound in a 3:1 manner in which the pendant indole groups
rotated away from the binding cleft to facilitate the binding of further equivalents of anion.

![Di-indolylureas](image)

**Figure 2.6** Gale and co-workers have extensively studied a progression of di-indolylureas.

Prior to this work, Gale and *et al.* had reported that receptors of the form 115 (Figure 2.7) bound Cl, H$_2$PO$_4$, AcO and PhCO$_2$ in DMSO-$_d_6$/H$_2$O 0.5 %. On binding to oxoanions, the $^1$H NMR titration showed that all of the NH hydrogen bond donors underwent a downfield shift consistent with hydrogen bonding to the anion. This prompted Plavec and co-workers to investigate the conformational changes that occur when these receptors interact with anions by a combination of heteronuclear NMR spectroscopy and *ab initio* quantum mechanical calculations. They found that the free receptors preferentially adopt an *anti-anti* conformation (Figure 2.7); however, interaction with anions such as AcO and Cl causes a conformational change to the *syn-syn* conformation, allowing the NH hydrogen bond donors to converge and contribute to binding the anion.
Figure 2.7 The different conformations that receptors of type 115 can adopt.

Jeong and co-workers have investigated the anion binding properties of bis-indolylurea receptor 117 by comparison to the analogous bis-amide 116, shown in Figure 2.8.\textsuperscript{146} UV-vis titration with the tetrabutylammonium salts in DMSO revealed that 116 and 117 bind H$_2$PO$_4^-$ ($K_a = 1.4 \times 10^5$ M$^{-1}$ and 3.9 $\times$ 10$^5$ M$^{-1}$ respectively) more strongly than AcO$^-$ ($K_a = 4.3 \times 10^4$ M$^{-1}$ and 7.5 $\times$ 10$^4$ M$^{-1}$ respectively). These anions were bound similarly strongly by 116 and 117, indicating that the additionally hydrogen bond donors in 117 contribute little to the binding of these anions. However, bis-urea 117 was found to bind HP$_2$O$_7^{3-}$ much more strongly than bis-amide 116 ($K_a > 5 \times 10^6$ M$^{-1}$ and 5.2 $\times$ 10$^5$ M$^{-1}$ respectively). This indicates that the additional hydrogen bond donors were beneficial in the complexation of a larger anion, possibly due to a better fit to the binding cleft. Additionally, the authors found that a number of dicarboxylates were also bound strongly by 117 (UV-vis titration in 10 % MeOH/DMSO), which showed the greatest affinity for adipate ($K_a = 8.1 \times 10^5$ M$^{-1}$).
Jurczak and co-workers have also utilized a combination of indole and urea NH hydrogen bond donors in their design of receptors 118-120 (Figure 2.9). They found by $^1$H NMR titration that these receptors interacted with various anions in MeOH-$d_3$, a highly competitive and protic solvent. All of the anions tested were complexed in a 1:1 binding mode except for the larger HP$_2$O$_7^{\text{3-}}$ facilitated a 2:1 host:guest binding mode. Receptors 118-120 were found to interact most strongly with tetrahedral oxoanions such as H$_2$PO$_4^{-}$ ($K_a = 135$ M$^{-1}$, 535 M$^{-1}$ and 265 M$^{-1}$ respectively following the urea NH signal). Interestingly, these ligands interact as strongly with Cl$^-$ as they do with benzoate, a much more basic anion. This may be due to the steric hinderance of binding benzoate. The stronger anion binding by 119 over 118 was attributed to the greater acidity of the hydrogen bond donors due to the enhanced electron withdrawing effect of the phenyl substituents. The additional indole group in 120 does not result in increased binding of the anions tested; however, the selectivity is improved. Receptor 119 binds HSO$_4^-$ similarly strongly to H$_2$PO$_4^-$( $K_a = 280$ M$^{-1}$ and 535 M$^{-1}$), whereas the affinity of 120 for HSO$_4^-$ ($K_a = 90$ M$^{-1}$) is greatly reduced by comparison to H$_2$PO$_4^-$( $K_a = 265$ M$^{-1}$).
2.1.3 Ureas vs. thioureas for anion transport

Amongst the most successful anion carriers reported to date are the cholapods developed by A. P. Davis and co-workers.\textsuperscript{48, 53-56} The key features of these carriers are the lipophilic cholic acid scaffold and, most commonly, the use of ureas as an anion binding motif. However, within the same work, A. P. Davis et al. have found that analogous thiourea substituted cholapods function as more efficient anion carriers. This observation was attributed to the stronger Cl\textsuperscript{-} binding by the thiourea receptors. For example, compound 20 binds chloride more strongly than compound 14 ($K_a (14) = 5.2 \times 10^8$ M\textsuperscript{-1}, $K_a (20) = 2.0 \times 10^9$ M\textsuperscript{-1}) and is also a more effective anion carrier. However, these two cholapods could only be tested comparatively by pre-incorporating the receptors into the vesicle membrane, as the thiourea analogue was found to precipitate if added externally. This highlights the greater hydrophobicity of the thiourea group if compared to the urea group.
A. P. Davis and co-workers have used urea and thiourea derivatised cholapods.

A. P. Davis et al. also investigated the anion transport activity of urea 121, shown in Figure 2.11, as a simple analogue of the cholapods. When preincorporated into the lipid bilayer, 121 was found to mediate Cl/NO$_3^-$ exchange although it was, unsurprisingly, much less active than cholapod 14. Additionally, B. D. Smith and co-workers have described a functionalised phospholipid which facilitates anion transport by a relay mechanism. The anion binding domain of this transporter is a $p$-nitrophenyl urea.

Gale and co-workers have also investigated the anion transport properties of a series of tripodal ureas and thioureas based on the tren scaffold 37-40 (Figure 2.12) In this work, the urea substituted ionophores were inactive for anion transport, while the thioureas were found to be efficient Cl/NO$_3^-$ and Cl/HCO$_3^-$ antiporters. In this work, binding studies were carried out by $^1$H NMR titration in DMSO-$d_6$/H$_2$O (0.5 %) and little correlation between anion binding strengths and transport efficiencies was observed. The greater anion transport activity of the thiourea based molecules was attributed to their greater lipophilicity, thus better enabling the partitioning of the anion-receptor complex within the bilayer.
2.2 Structurally simple anion transporters for chloride

Inspired by the excellent transport activity exhibited by the bis-urea or thiourea functionalised cholapods and by Gale’s tripodal thioureas, a series of structurally simple ureas and thioureas 122-127 (Figure 2.13) were synthesized to allow their comparative anion transport properties to be investigated.68 The simplicity of the structures was chosen to allow for the straightforward assessment of substituent effects, and to allow comparison to the simple $p$-nitrophenyl urea 121. The investigation of the tren based carriers had studied the effect of alkyl vs. aromatic (thio)urea substitution and found them to be similarly effective. Drawing on this, butyl- and phenyl- substituted ureas and thioureas 122-125 were synthesized. Indolyl- substituted urea and thiourea 126 and 127 were synthesized to investigate the effect of incorporating an additional indole hydrogen bond donor into the receptor design. The $i$-pentyl substituent common to all of the carriers was chosen as a general alkyl chain that had been previously incorporated into successful carrier design.59
The work discussed in this chapter was published in 2011.\textsuperscript{76}

2.2.1 Synthesis

Compounds 122 and 126 were synthesized by SJM. Compounds 124 and 125 were synthesized by NA. Compound 123 was synthesized by CCT. Details of these synthetic procedures can be found elsewhere.\textsuperscript{76} (A list of the full names of any contributing authors may be found in the abbreviations section. Where applicable, the contributions of these authors have been highlighted in the text.)

Thiourea 127 was synthesized as shown in Scheme 2.1. 7-Nitroindole was reduced using a Pd/C catalyst under an atmosphere of H\textsubscript{2} in EtOH, and subsequently the amine was converted to the corresponding isothiocyanate by reaction with thiophosgene in a two phase mixture of DCM and sat. NaHCO\textsubscript{3} \textsuperscript{(aq)}.\textsuperscript{69} Finally, the isothiocyanate was reacted with \textsuperscript{i}pentylamine in DCM to give compound 127 in 25 % yield after purification. Full experimental details may be found in Chapter 6.4.

\begin{center}
\textbf{Scheme 2.1} The synthesis of thiourea 6. Reagents and conditions: (i) H\textsubscript{2}, Pd/C, EtOH, 2 h, RT, assumed 100 % yield; (ii) thiophosgene, DCM/ sat. NaHCO\textsubscript{3} \textsuperscript{(aq)}, overnight, RT, assumed 100 % yield; (iii) \textsuperscript{i}pentylamine, DCM, overnight, RT, 25 % overall yield.
\end{center}

Crystals of compound 127 suitable for X-ray crystallography were grown from a DCM/ Et\textsubscript{2}O (50:50) solution by slow evaporation. The structure is shown in Figure 2.14. Full details of the X-ray diffraction analysis can be found in the appendix section A3.1.
Figure 2.14 The crystal structure of (a) a single molecule of receptor 127 and (b) intermolecular hydrogen bonding between two receptor molecules. Non-acidic hydrogen atoms have been omitted for clarity.

The structure shows that in the solid state the receptor is not preorganised for anion binding, as the thiourea NH adjacent to the indole group is orientated away from the binding cleft. However, this is not a reliable indication of the favoured conformation of this receptor in solution. In the solid state, a hydrogen bonding interaction between the NH and the S atom of another receptor molecule dictates this conformation (Figure 2.14(b)).
2.2.2 Chloride transport studies

The ability of compounds 122-127 to facilitate Cl⁻/NO₃⁻ antiport was investigated as follows. A solution of the receptor in DMSO (final receptor concentration 0.02 mM, or 2 mol% with respect to lipid) was added to a suspension of unilamellar POPC vesicles containing 489 mM NaCl suspended in 489 mM NaNO₃. All of the solutions were buffered to pH 7.2 with 5 mM sodium phosphate salts. The lipid concentration in the sample was 1 mM. The chloride concentration of the external solution was monitored using a chloride selective electrode. After 300 s the vesicles were lysed by the addition of octaethylene glycol monodecyl ether solution (H₂O/DMSO 7:1 v/v) to calibrate 100 % chloride efflux. Full details of the vesicle preparation and transport experiments may be found in the Chapter 6.2. The results are shown in Figure 2.15.

Figure 2.15 Chloride efflux promoted by receptors 122-127 (2 mol% with respect to lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.

Thioureas 123, 125 and 127 were found to facilitate Cl⁻/NO₃⁻ antiport, while the corresponding ureas were completely inactive. Compounds 123 and 127 were the most active, and under these conditions they mediate a similarly high level of Cl⁻ efflux. Work
by SJM showed that the activity diminished in vesicles composed of POPC:cholesterol (7:3), thus implying a mobile carrier mechanism for these receptors. To confirm that the observed chloride efflux was due to an anion antiport mechanism, the chloride efflux mediated by 122-127 was monitored from vesicles containing NaCl suspended in Na₂SO₄. The results are shown in Figure 2.16.

![Figure 2.16](image)

**Figure 2.16** Chloride efflux promoted by receptors 122-127 (2 mol% with respect to lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials.

When the external anion was exchanged for SO₄²⁻ the observed Cl⁻ transport was inhibited. This dependence on the external anion indicates that the thiourea receptors 123, 125 and 127 mediate Cl⁻ transport by an antiport mechanism.

### 2.2.3 The Hill analysis

The data in Figure 2.15 indicates that n-butyl thiourea 123 and 7-indolyl thiourea 127 are similarly effective anion transporters. However, at lower carrier concentrations it becomes apparent that receptor 127 is more potent. For example, the chloride efflux
mediated by each receptor at a concentration of 0.05 mol% (with respect to lipid) is shown in Figure 2.17.

This data clearly indicates that receptor 127 is a more efficient carrier at lower concentrations. This highlights that testing at a single concentration is not sufficient to fully examine the relative efficiencies of two carriers.

In order to further investigate and quantify the observed Cl/NO$_3^-$ antiport activity, a Hill analysis was performed for this process. The full analysis for compound 123 is shown below as an example of this procedure; all other Hill analyses may be found in the Appendix (section A1.1). A more detailed explanation of this analysis may be found in Chapter 1.

\[ y = \frac{V_{\text{max}} x^n}{(k^n + x^n)} \]

**Equation 2.1** A form of the Hill equation where $y$ is the % chloride efflux at 270 s, $V_{\text{max}}$ is the maximum chloride efflux (100%), $x$ is the carrier loading, $k$ is the EC$_{50}$ (the carrier loading required to promote 50 % total chloride efflux at 270 s, measured in mol% with respect to lipid) and $n$ is the Hill coefficient, representing the number of carriers required to transport a single anion.
The Cl/NO₃⁻ antiport promoted by different concentrations of compound 123 was investigated. The results are shown below. The corresponding graphs for 125 and 127 may be found in the appendix (section A1.1).

![Graph showing chloride efflux promoted by various concentrations of 123 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials.](image)

**Figure 2.18** Chloride efflux promoted by various concentrations of 123 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials.

The total chloride efflux after 270 s was plotted against the carrier concentration and this data was fitted to the Hill equation (Equation 2.1) using Origin® 8.1. This analysis is shown below.
The Hill analysis for Cl-/NO$_3^-$ antiport mediated by compound 123. The data was fitted to Equation 2.1 using Origin. $R^2 = 0.99977$, $k = 0.110 \pm 0.006$, $n = 1.05 \pm 0.04$.

The relevant constants from the Hill analyses are shown in Table 2.1.

Table 2.1 The EC$_{50}$ at 270 s for the Cl-/NO$_3^-$ antiport by 123, 125 and 127 and the Hill coefficient (n) calculated by the Hill analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC$_{50}$ at 270 s (Cl-/NO$_3^-$)/mol%</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>0.11</td>
<td>1.0</td>
</tr>
<tr>
<td>125</td>
<td>3.05</td>
<td>1.36</td>
</tr>
<tr>
<td>127</td>
<td>0.03</td>
<td>1.52</td>
</tr>
</tbody>
</table>

The EC$_{50}$ 270 s values demonstrate that the most potent receptor is indole substituted thiourea 127. This receptor exhibited similar transport activity to compound 123 at 2 mol% loading (Figure 2.15); however, it mediates chloride transport at much lower concentrations than compound 123 which is reflected in the significantly lower EC$_{50}$ 270 s value. In fact, compound 127 is able to facilitate Cl-/NO$_3^-$ exchange at a loading of 0.004 mol% or 1:25 000 (carrier to lipid). The most active carrier which had been reported prior to this work was a cholapod reported by A. P. Davis to function at a loading of 1:250 000 (carrier to lipid), but only if preincorporated into the lipid bilayer.$^{54}$ The potency of receptor 127 is remarkable considering its simplicity. Compound 123 also
functions as an efficient Cl\(^{-}/\NO_3^{-}\) antiporter, while compound 125 is the least efficient of the thioureas tested. The Hill coefficient (n) values indicate that the transport process by each receptor is unimolecular, i.e that each anion is transported by a single receptor. This offers further evidence of a mobile carrier process rather than the possible assembly of pores.

### 2.2.4 Bicarbonate transport and binding studies

Work by SJM demonstrated that compounds 123, 125 and 127 can also mediate Cl\(^{-}/\HCO_3^{-}\) exchange. EC\(_{50}\) values for this process are shown in Table 2.2, along with binding constants for 122-127 with relevant anions (determined by SJM by titration with tetrabutylammonium or tetraethylammonium salts in DMSO-\(d_6/\H_2\O\) 0.5 %) and the clogP (calculated octanol-water partition coefficient, a measure of lipophilicity) and polar surface area (PSA) of the receptors (calculated using Spartan '08 for Macintosh).

**Table 2.2** EC\(_{50}\) values for Cl\(^{-}/\HCO_3^{-}\) antiport calculated by Hill analysis by SJM. Anion binding constants determined by \(^1\H\) NMR titration with the tetrabutylammonium or tetraethylammonium anion salts in DMSO-\(d_6/\H_2\O\) (0.5 %), performed by SJM. Errors on binding constants are < 10 %. clogP and PSA values were calculated using Spartan '08 for Macintosh. In the case of compounds 126-127 a range of PSA values is quoted, representing the different rotational conformations that the indole can adopt.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC(_{50}) at 270 s (Cl(^{-}/\HCO_3^{-}))</th>
<th>(K_a) (Cl(^{-}))/M(^{-1})</th>
<th>(K_a) (NO(_3)^{-})/M(^{-1})</th>
<th>(K_a) (HCO(_3)^{-})/M(^{-1})</th>
<th>clogP</th>
<th>PSA/Å(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>122</td>
<td>-</td>
<td>&lt;10</td>
<td>a</td>
<td>18</td>
<td>1.99</td>
<td>37.0</td>
</tr>
<tr>
<td>123</td>
<td>0.30</td>
<td>10</td>
<td>a</td>
<td>58</td>
<td>3.14</td>
<td>22.2</td>
</tr>
<tr>
<td>124</td>
<td>-</td>
<td>21</td>
<td>a</td>
<td>135</td>
<td>2.42</td>
<td>34.8</td>
</tr>
<tr>
<td>125</td>
<td>2.08</td>
<td>22</td>
<td>a</td>
<td>343</td>
<td>3.57</td>
<td>21.5</td>
</tr>
<tr>
<td>126</td>
<td>-</td>
<td>96</td>
<td>a</td>
<td>1170</td>
<td>2.02</td>
<td>44.6-47.7</td>
</tr>
<tr>
<td>127</td>
<td>0.04</td>
<td>28</td>
<td>a</td>
<td>516</td>
<td>3.16</td>
<td>31.3-35.5</td>
</tr>
</tbody>
</table>
Again, receptor 127 is also the most potent carrier for Cl⁻/HCO₃⁻ exchange, while 123 and 125 are also active. The values of EC₅₀ for this exchange process are higher than for Cl⁻/NO₃⁻ antiport. This is commonly observed for 2 reasons: (i) HCO₃⁻ is more hydrophilic than NO₃⁻ and is therefore expected to be more difficult to transport across a bilayer;³ (ii) the nature of the Cl⁻/HCO₃⁻ assay provides a smaller HCO₃⁻ gradient (40 mM external HCO₃⁻) than the NO₃⁻ gradient present in the Cl⁻/NO₃⁻ assay (489 mM external NO₃⁻) thus there is a smaller electrochemical gradient to drive the transport process.

The binding constants obtained do not reveal a strong correlation between anion affinity and the transport activity. The greater transport activity of the thiourea receptors by comparison to their urea analogues is attributed to their greater lipophilicity, as demonstrated by their higher clogP and lower PSA values. However, it is postulated that the indolyl thiourea 127 is the most active carrier as it combines the lipophilic thiourea scaffold with an additional NH hydrogen bond donor for anion complexation.

2.2.5 Amides and thioamides

A series of amides and thioamides 128-133 were synthesized as analogues of 122-127 in order to investigate the importance of the (thio)urea functionality compared to a receptor containing only one hydrogen bond donor.

![Figure 2.20 The amides and thioamides studied as analogues of receptors 122-127.](image)

Compound 130 was synthesized by the activation of 4-methylvaleric acid with 1,1’-carbonyldiimidazole (CDI) in CHCl₃, followed by reaction with aniline.
**Chapter 2 Structurally simple anion transporters**

![Scheme 2.2](image)

**Scheme 2.2** Reagents and conditions: (i) CDI, CHCl₃, reflux, 2 h; (ii) aniline, CHCl₃, reflux, overnight, 76 % yield.

Thioamides 129 and 131 were synthesized by reaction of the corresponding amide with Lawesson’s reagent in THF.

![Scheme 2.3](image)

**Scheme 2.3** Reagents and conditions: (i) Lawesson’s reagent, THF, reflux, overnight.

Compounds 128, 132 and 133 were synthesized by SJM by similar procedures. The Cl⁻/NO₃⁻ antiport activity of 128-133 was investigated by SJM. Unsurprisingly, amides 128, 130 and 132 were found to be inactive. The transport activity of the thioamides 129, 131 and 133 was found to be greatly diminished by comparison to the thioureas. This highlights the importance of the chelating ability of the thiourea NHs to anion transport.

### 2.3 Conclusions

The work discussed in this chapter demonstrates that structurally simple thiourea based anion receptors function as highly potent anion antiporters. The key feature of the thiourea scaffold which makes it more effective than an analogous urea is proposed to be the enhanced lipophilicity and reduced PSA, both of which factors aid the partitioning of the receptor and the formed receptor-anion complex within the lipid bilayer. Indolyl thiourea 127 was found to be a remarkably potent anion carrier considering the simplicity of its structure. At the time when this work was completed, it was the most potent synthetic anion carrier that had been developed by the Gale group. This indicates that 7-indoles are a useful structural motif in the design of anion carriers, and that the reduction
in lipophilicity associated with the inclusion of an additional hydrogen bond donor into a receptor scaffold does not necessarily cause a corresponding decrease in transport activity if the hydrogen bond donor is of use in anion complexation. Additionally, this work shows that even very simple anion receptors can function as highly potent transporters and gives evidence that encapsulation of the anion is not necessary for efficient transport.

The development of anion carriers which function at low concentrations is an important target as the target of this research is to produce carriers with potential application as drugs for the treatment of various diseases. High potency is a desirable target in the synthesis of new pharmaceuticals as it allows the administration of lower concentrations and potentially reduces the risk of other harmful side effects.
Chapter 2 Structurally simple anion transporters
Chapter 3
Bis-indolylureas as tunable chloride transport agents

3.1 Introduction

It is well established that anion receptors based on urea and thiourea hydrogen bond donors can also function as highly efficient anion antiporters. In the studies reported in Chapter 2, it was demonstrated that structurally simple thiourea carriers were more active than their urea analogues. This observation was attributed to the higher lipophilicity of these species. The most active compound studied was indolyl thiourea 127, implying that inclusion of the indole moiety in transporter scaffolds may be a useful design strategy. However, A. P. Davis et al. have effectively demonstrated that urea based molecules can also function as extremely active transporters. The key features of the cholapod design seem to be the lipophilicity of the cholic acid skeleton and the chelating ability of the pendant urea arms.

3.1.1 Bis-ureas for anion binding

Lin and co-workers have investigated the anion binding properties of the series of (bis)-p-nitrophenylureas 134-136 shown in Figure 3.1. Investigation of the anion binding properties of these receptors by UV-vis titration in DMSO indicated that compound 134 was selective for F\(^-\) (log \(K_a = 5.31\)) over \(\text{H}_2\text{PO}_4^-\) and AcO\(^-\), while the addition of other halides resulted in little or no spectral response. Receptor 135 was found to be selective for \(\text{H}_2\text{PO}_4^-\) with log \(K_a = 4.35\), while receptor 136 displayed little selectivity. Job plot analyses showed that the anions were bound in a 1:1 receptor:anion
stoichiometry. The trends in binding selectivity were explained as a result of increasing the length of the central alkyl chain. Receptor 134 (with the shortest chain) was selective for the comparatively small F\(^-\) anion, whereas receptor 135 was selective for the larger H\(_2\)PO\(_4\)\(^-\) as a result of the longer alkyl linker between the urea groups. Upon further extension of this central alkyl chain (compound 136), any selectivity due to size complementarity was lost.

![Figure 3.1](image.png)

**Figure 3.1** The bis-ureas 134-136 synthesized by Lin and co-workers.

Beer and co-workers have investigated the anion binding properties of some mono- and bis- urea substituted ferrocenes 137-140 as shown in **Figure 3.2**\(^{149}\). \(^1\)H NMR titrations in CD\(_3\)CN were used to demonstrate that the bis-urea substituted receptors 139 and 140 bound Cl\(^-\) and H\(_2\)PO\(_4\)\(^-\) more strongly than their monosubstituted analogues. A 1:1 binding stoichiometry for these binding events was confirmed by Job plot analyses, indicating that the bis-urea receptors were chelating the anion- a likely cause of the observed higher binding constants by comparison to the mono-urea receptors. Receptors 137 and 139 were found to be selective for H\(_2\)PO\(_4\)\(^-\) over Cl\(^-\), while receptors 138 and 140 displayed the opposite binding preference. This observation was attributed to the bulky substituents of receptors 138 and 140, which sterically disfavoured the binding of the larger H\(_2\)PO\(_4\)\(^-\) anion.

![Figure 3.2](image.png)

**Figure 3.2** The mono- and bis- urea substituted ferrocenes reported by Beer.
Gale, Loeb and co-workers have investigated the anion complexation behaviour of a Pt(II) complex bearing urea containing ligands.\textsuperscript{150} They used $^1$H NMR titrations to investigate the binding mode of receptor \textbf{141 (Scheme 3.1)} with TBACl and K$_2$SO$_4$ in DMSO-$d_6$. They found that Cl$^-$ was complexed in a 1:2 receptor to anion stoichiometry, with each Cl$^-$ coordinated \textit{via} 4 hydrogen bond donors from 2 urea groups. However, SO$_4^{2-}$, as a larger anion with a higher charge, was bound in a 1:1 stoichiometry and complexed by all 8 hydrogen bond donors simultaneously. These binding modes are shown in \textbf{Scheme 3.1}, and were confirmed in the solid state by X-ray crystallography. This demonstrates that for a multi-urea containing receptor, if conformational flexibility exists, the binding mode will be dependent on the nature of the anion.

\textbf{Scheme 3.1} Tetra-urea receptor \textbf{141} adopts different receptor:anion stoichiometries depending on the anion.
3.1.2 Self assembly of bis-ureas

The propensity of bis-ureas to self assemble is well documented as urea moieties are often found to form a network of hydrogen bonding interactions as shown in Figure 3.3. As such, bis-ureas have commonly been found to act as gelators for organic solvents.

![Figure 3.3](image_url) Hydrogen bonding interactions between urea moieties.

Work by Steed and co-workers has utilized this behaviour in their studies on the series of bis-ureas 142-148. They found that compounds 142, 144, 146 and 148, in which \( n \) = even, functioned as low molecular weight gelators (LMWGs) forming organogels in a variety of solvents, whereas the remaining compounds (143, 145 and 147) in which \( n \) = odd exhibited no gelation behaviour. This was attributed to the relative orientations of the two urea groups in these sets of molecules; if \( n \) = odd, the urea groups should be orientated in the same direction, while if \( n \) = even, the urea groups should be orientated in opposite directions, which was found to be preferable for gelation. Interestingly, the gel strength of compounds 142, 144, 146 and 148 was found to be reduced and in some cases completely inhibited in the presence of small amounts \( \text{AcO}^- \), \( \text{Cl}^- \) and \( \text{Br}^- \) and \( \text{NO}_3^- \) (added as their TBA\(^+\) salts). This modulation of gel strength was not observed upon addition of \( \text{BF}_4^- \). These observations were attributed to disruption of the urea hydrogen bonding networks in the presence of anions that could compete for hydrogen bonding interactions.
3.1.3 Self assembly of ureas for ion transport

Fyles et al. report the dynamic self assembly of a series of crown ether substituted ureas such as 149 into alkali metal cation conducting pores. Individually, these molecules function as ditopic receptors in which an anion is bound by the urea moiety and a cation may be bound within the crown ether. On first appearances then, these molecules may be expected to function as discrete ion carriers. However, their self assembly within liquid membrane phases led to consideration of the possibility that they could form potentially membrane spanning superstructures. Planar bilayer conductance studies proved single channel behaviour of the assembled structures. It was proposed that the assembled structures were formed by intermolecular hydrogen bonding interactions between urea groups as depicted in Figure 3.5 which would bring the crown ether moieties into close proximity and allow the passage of a cation through the assembled network.
3.1.4 Variation of ion transporter length

The optimum design of transmembrane ion channels relies on the channel being of a complementary size to span the membrane. Fyles and co-workers report the cation channel formation of compound 151, while compounds 150 and 152 with shorter and longer alkyl chains respectively were found to be almost completely inactive under the same conditions (Figure 3.6). Voltage clamp techniques were used to demonstrate single channel activity. It was reasoned to be unlikely that a single molecule of 151 could form a pore and therefore assumed that an aggregate structure must assemble within the bilayer. As the alkyl chains of compound 151 are significantly shorter than the fatty acid components of phospholipids (on average C\textsubscript{16}) it was reasoned that the aromatic groups would most likely reside in the midpolar region of the bilayer, allowing interaction with the fatty acid-phosphocholine carbonyl groups.
Regen and co-workers have reported a Na\(^+\) channel forming derivative of spermine and cholic acid, 153.\(^{159}\) The ion transport activity of this channel was found to be strongly dependent on the thickness of the bilayer, providing evidence that the molecule was spanning the membrane. This compound was studied in comparison to compound 154 (a pre-existing Na\(^+\) channel). It was found that 153 showed the highest Na\(^+\) conductance in membranes containing lipids with fatty acid residues that were 2 carbons longer than the membranes in which 154 promoted the highest Na\(^+\) flux. This was attributed to the fact that 153 is longer than 154 by 2 methylene units.

This principle has also been extensively explored by Gokel and co-workers in their studies of the series of peptides shown in Figure 3.8.\(^{160}\) Systematic variation of the substitution of both the C- and N-terminus (R’ and R respectively) of these peptides allowed the optimum channel length to be determined.
Chapter 3 Bis-indolylureas

![Figure 3.8](#) The basic structure of the series of heptapeptides studied by Gokel et al. with various alkyl substitution at the N- and C-terminus.

It was found that variation of the structure of these molecules had a large effect on their chloride transport efficiency. Variation of the length of the alkyl chain at the N-terminus (compounds 155-158) revealed that the optimum N-substituent was C8 (156). In general, increasing the chain length up to C8 resulted in an increase in transport activity; however, after this point the activity was found to decrease with increasing chain length. Similarly, an optimum chain length for the C-terminus substituent was also observed. The authors attributed this finding to a balance between the hydrophilicity and the lipophilicity of the molecule, and that pore formation by dimeric pairs might be inhibited if the chains were too long.

3.2 Bis-indolylureas

The series of receptors discussed in the following chapter are shown in Figure 3.9.

![Figure 3.9](#) The bis-indolyl ureas discussed in this chapter.

The design of these carriers was based on the following principles:

- Thioureas have been shown to be excellent anion transporters. However, thioureas are often found to be toxic\(^\text{161}\) which may limit their applicability for biological application. Ureas are less commonly toxic and thus might be more suited to this goal.
• However, in Chapter 2 simple mono-ureas were found to be inactive for anion transport, thus the complexity of the system must be increased. There are many literature examples in which the use of multiple urea units has lead to enhanced anion recognition properties. This principle could be applied to anion transport.
• The indolyl (thio)urea hydrogen bond donor motif has been incorporated into a highly successful anion carrier (receptor 127). Therefore, indole was chosen as a substituent in this series of carriers.
• The lipophilicity of anion carriers has been implicated as an important structural consideration in the design of new targets. The bis-alkylurea scaffold allows the straightforward modification of the lipophilicity of these carriers in a stepwise manner by simply increasing the length of the central alkyl chain.

3.3 Synthesis

The series of symmetrical bis-indolylureas 159-167 were synthesized as shown in Scheme 1. The reduction of 7-nitroindole was achieved by stirring in EtOH under an atmosphere of H₂ using catalytic Pd/C (10%). The corresponding amine was reacted with CDI in DCM; the intermediate thus formed (3.1) was converted to the desired product by reflux with the corresponding diamine in a DCM:DMF mixture (99:1) to give compounds 159-167 in 24-82 % overall yield. Full synthetic details can be found in Chapter 6.4.

Scheme 3.2 Reagents and conditions: (i) H₂, Pd/ C, EtOH, 3 h, RT; (ii) CDI, DCM, N₂, RT, overnight; (iii) ½ equivalent diamine, DCM:DMF (99:1), N₂, reflux, overnight. The yields quoted are overall yields starting from 7-nitroindole.
3.4  Anion transport studies

3.4.1  Chloride transport activity

Compounds 159-167 were tested for Cl⁻/NO₃⁻ antiport activity. The results are shown in Figure 3.10.

![Figure 3.10](image)

Figure 3.10 Chloride efflux promoted by compounds 159-167 (2 mol% with respect to lipid) from unilamellar vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed by addition of detergent to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.

The results show that compounds 159-167 exhibit a range of chloride transport activity. The maximum activity under these conditions was observed for compounds 164 and 165 with n = 9 and 10 respectively. Between compounds 159-165, transport activity was found to increase with the increasing length of the central alkyl chain; after this point, transport activity is reduced with increasing chain length.

Chloride transport was demonstrated to occur via a Cl⁻/NO₃⁻ antiport mechanism as chloride efflux was inhibited when the external solution was replaced with Na₂SO₄.
Compounds 159-167 were also found to mediate only low levels of Cl⁻/HCO₃⁻ antiport (see Section A1.2 in the appendix).

A Hill analysis was performed for each compound in order to examine the mediated Cl⁻/NO₃⁻ exchange process in more detail. The relevant constants from the Hill analysis are shown in Table 3.1. The full Hill analyses can be found in the appendix (section A1.2).

Table 3.1 EC⁵₀ values and Hill coefficients (n) were derived from the Hill analyses of mediated chloride efflux by compounds 159-167 from unilamellar vesicles in a Cl⁻/NO₃⁻ exchange process. Values of clogP and PSA were calculated using Spartan '10 for Macintosh. The range of PSA values given reflects the different conformations of the indolyl urea unit. [a] These compounds were not active enough to perform the Hill analysis (EC⁵₀ > 4 mol%).

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC⁵₀ (mol% carrier:lipid)</th>
<th>n</th>
<th>clogP</th>
<th>PSA/Å²</th>
</tr>
</thead>
<tbody>
<tr>
<td>159</td>
<td>[a]</td>
<td>-</td>
<td>-0.97</td>
<td>89.2-94.7</td>
</tr>
<tr>
<td>160</td>
<td>[a]</td>
<td>-</td>
<td>-0.55</td>
<td>89.2-94.7</td>
</tr>
<tr>
<td>161</td>
<td>[a]</td>
<td>-</td>
<td>-0.13</td>
<td>89.1-94.5</td>
</tr>
<tr>
<td>162</td>
<td>2.78</td>
<td>1.93</td>
<td>0.29</td>
<td>90.0-94.0</td>
</tr>
<tr>
<td>163</td>
<td>2.53</td>
<td>1.56</td>
<td>0.70</td>
<td>89.3-94.8</td>
</tr>
<tr>
<td>164</td>
<td>1.33</td>
<td>2.41</td>
<td>1.12</td>
<td>89.2-94.8</td>
</tr>
<tr>
<td>165</td>
<td>1.46</td>
<td>2.26</td>
<td>1.54</td>
<td>89.2-94.7</td>
</tr>
<tr>
<td>166</td>
<td>2.07</td>
<td>2.14</td>
<td>1.95</td>
<td>89.9-94.7</td>
</tr>
<tr>
<td>167</td>
<td>3.37</td>
<td>2.36</td>
<td>2.37</td>
<td>89.9-95.5</td>
</tr>
</tbody>
</table>
The results of the Hill analysis confirm the order of transporter efficiency observed in Figure 3.10. Increasing the length of the central alkyl chain from receptors 159 (n = 4) and 164 (n = 9) results in an increase in chloride transport activity. This correlates with an increase in the lipophilicity of the receptors, as evidenced by the increasing clogP values, thus enhancing the interaction of the receptor and the receptor-anion complex with the hydrophobic interior of the lipid bilayer. However, between receptors 164 (n = 9) and 167 (n = 12) a decrease in transport activity is observed on increasing the alkyl chain length. In order to investigate this effect, the differences in the initial rate of chloride efflux promoted by these receptors was investigated.

3.4.2 Partitioning kinetics

Closer analysis of the data from the Cl⁻/NO₃⁻ antiport assays revealed that compound 167 (n = 12) promotes slower initial chloride efflux than its shorter chain analogues. However, after a short period of time an increased transport rate is observed. For example, a comparison between compound 167 (n = 12) and compound 162 (n = 7) is shown in Figure 3.11. The same trend is observed at all carrier loadings; the loadings shown below were selected, as the trend is more pronounced at higher loadings.
Figure 3.11 Chloride efflux promoted by receptors 162 and 167 (4 mol% w.r.t. lipid) from unilamellar vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed by addition of detergent to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.

By the end of the experiment (300 s) these receptors have achieved a comparable % chloride efflux; however, it is clear that the initial rate of chloride efflux is very different. This demonstrates that the initial rate of chloride efflux mediated by the longer chain compounds is slower. This may be an explanation for their reduced overall transport activity.

A reason for the slower initial transport rate might be that the longer chain analogues take longer to partition into the lipid bilayer and begin mediating chloride flux. In order to investigate this hypothesis, an experiment was designed to allow the receptors to partition with the vesicle bilayer before the anion transport was initiated. Receptors 162 and 167 (4 mol% with respect to lipid) were added to a suspension of vesicles containing NaCl suspended in Na$_2$SO$_4$. Little chloride efflux was observed, as these conditions do not support an antiport mechanism. After 2 minutes, a pulse of NaNO$_3$ was
added and the resulting chloride efflux was monitored using a chloride ISE. The results are shown in Figure 3.12.

![Graph showing chloride efflux promoted by receptors 162 and 167](image)

**Figure 3.12** Chloride efflux promoted by receptors 162 and 167 (4 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 167 mM Na_2SO_4 buffered to pH 7.2 with 5 mM sodium phosphate salts. At t = 120 s a pulse of NaNO_3 was added such that the final NO_3^- concentration was 40 mM. At the end of the experiment the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.

In this experiment, the chloride efflux promoted by receptors 162 and 167 appears to start at a comparable rate. There is no lag phase for the chloride transport by 167, which indicates that in the previous experiment poor partitioning kinetics are responsible for this behaviour. By the end of the experiment, compound 167 has mediated a greater amount of chloride efflux than 162, whereas in the previous experiment these values were similar. This indicates that the partitioning kinetics may be partially responsible for the observed reduction in transport activity on increasing the chain length > n = 9.

A value of the initial rate of chloride efflux was calculated for each receptor based on the chloride efflux mediated within the first 30 s of the experiment (2 mol% carrier loading with respect to lipid). The graph in **Figure 3.13** shows the correlation between this initial rate of transport and the total chloride efflux at 270 s mediated by each of the
receptors according to the central alkyl chain length. These values are tabulated in the appendix.

![Graph](image)

**Figure 3.13** The chloride efflux at 270 s (black line and axis) and the initial rate of chloride efflux in the first 30 s (blue line and axis) mediated by compounds 159-167 (2 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts.

In general, there is good correlation between the amount of chloride efflux mediated in 270 s (a measure of the overall activity of the transporter) and the initial rate of chloride efflux. However, the biggest discrepancy in this trend is observed for compound 165 (n = 10). The transport efficiency of 165 is only slightly reduced compared to 164 (n = 9); however, there is a large reduction in the initial rate of transport. This implies that it is at this point that the partitioning kinetics becomes a limiting factor in the mediated chloride efflux. Correspondingly, this is also the point at which transport efficiency begins to decrease with increasing chain length. Compound 164 is the most efficient carrier as it represents the best balance between lipophilicity and mobility through the aqueous phase.

### 3.4.3 Water solubility

The clogP values listed in Table 3.1 imply that compounds 159-161 are hydrophilic. For compounds with clogP > 0, it is expected that in a water:octanol system the majority
of the compound will reside in the water phase. However, injection of a DMSO solution of receptor 159 into water resulted in the receptor visibly precipitating. To investigate this further, a sample of 159 (theoretically the most hydrophilic receptor) in DMSO-\(d_6\) (10 µl, 10 mM) was added to 0.5 ml D\(_2\)O. The sample was sonicated for 30 mins, after which a \(^1\)H NMR spectrum of the sample was recorded with \(~4000\) scans in order to attempt to detect low concentrations of dissolved 159. The spectrum recorded is shown in Figure 3.14.

![Figure 3.14](image)

**Figure 3.14** The \(^1\)H NMR spectrum recorded of a sample of receptor 159 (10 µl of a 10 mM solution in DMSO-\(d_6\)) injected into 0.5 ml D\(_2\)O after \(~4000\) scans.

There are no visible resonances in the aromatic region of this spectrum. This implies that the amount of dissolved receptor is too low to be detected by this means, and that receptor 159 is highly insoluble in water. This shows that, while the clogP values may be useful to investigate a relative trend in the lipophilicity across a series of molecules, they should not be taken as an absolute value. In this perspective, compound 159 is more hydrophilic than compound 160 but it is not soluble in water.
This finding is significant when considering the partitioning of receptors 159-167 between the aqueous and lipid phases. Clearly, the level of receptor contained within the water phase will be small. Therefore the observed trends in anion transport activity are not due to the increased localization of the receptor within the membrane as the chain length increases, as all of these receptors would be expected to be fully contained within the lipid bilayer. However, the increasing chain length would also result in the increased lipophilicity of the receptor-anion complex. It is likely that the increased lipophilicity of the receptor-anion complex is responsible for the enhanced transport activity as the chain length increases.

This finding also indicates that the poorer partitioning kinetics displayed by the longer chain analogues is not a result of their aqueous insolubility, as all of these compounds are insoluble in water. However, it is possible that these compounds have a higher tendency to aggregate on passing through the aqueous phase which may limit their activity.

3.4.4 Analogous mono-indolylureas

A series of analogous mono-indolylureas 168-176 shown in Figure 3.15 were synthesized by SJM.

![Figure 3.15](image)

Figure 3.15 The series of analogous mono-indolylureas 10-18, synthesized by SJM.

Work by SJM demonstrated that these compounds mediate low levels of Cl\(^-\)/NO\(_3^-\) antiport, but that this is greatly reduced compared to compounds 160-167. Additionally, di-indolylurea 112, which has been previously reported as an anion receptor by the Gale group, was found to mediate very low levels of Cl\(^-\)/NO\(_3^-\) exchange. This highlights the importance of the bis-urea scaffold.
3.4.5 Transporter mechanism-mobility assays

To investigate if 159-167 were transporting anions via a mobile carrier mechanism, their Cl/NO$_3^-$ antiport ability was tested in unilamellar vesicles composed of POPC:cholesterol (7:3). This is a commonly used proof of mobile carrier activity. Cholesterol organises the membrane resulting in higher viscosity; this will significantly reduce the rate of transport of a mobile carrier as this activity is controlled by the diffusion of the receptor-anion complex through the lipid bilayer.

However, a reduction in the rate of chloride transport by 159-167 was not observed in this experiment. As shown in the comparative graph for compound 164 (Figure 3.16), a comparable rate is observed in vesicles containing cholesterol. The corresponding graphs for the other compounds in this series may be found in the appendix (Section A1.2).

![Graph](image.png)

**Figure 3.16** Chloride efflux mediated by receptor 164 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials.

This finding is not proof of channel formation by these compounds; however, channel formation could not be disproved by this method. Zhao has reported increased glucose transport by synthetic receptors in the presence of cholesterol; this observation
was rationalized as follows. POPC bilayers with comparable levels of cholesterol are known to phase separate into cholesterol-rich and cholesterol-deficient domains. This may cause greater aggregation of receptors that interact more favourably with one of these domains as they are more likely to accumulate in these areas. The action of mobile carriers has been found in the past to be unperturbed by the addition of cholesterol in the membrane— for example, Gale et al. found that fluorocalix[4]pyrrole 36 exhibited comparable transport rates in both POPC and POPC:cholesterol (7:3) membranes. The mobile carrier activity of fluorocalix[4]pyrrole was implied by its observed Cl⁻ transport in a U-tube experiment. However, the poor solubility of compounds 159-167 in solvents that are immiscible with water prevented the use of similar experiments.

It has previously been inferred that a first order, or linear dependence of transport activity on receptor concentration is indicative of mobile carrier activity if the molecule is too small to span the bilayer. The Hill analyses for Cl⁻/NO₃⁻ antiport by 162-165 demonstrate a linear relationship between receptor concentration and % chloride efflux at 270 s. The data for compound 163 is shown below.

![Graph](image)

**Figure 3.17** The chloride efflux after 270 s promoted by receptor 163 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were dispersed in 489 mMNaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts.
This observation implies that a mobile carrier mechanism is operational. However, given the well-documented propensity of ureas to self associate, with examples of this known within the bilayer,\textsuperscript{158} further proof of mechanism was sought.

A. P. Davis \textit{et al.} have reported that examining the behaviour of a series of compounds in bilayers of varying thickness can be used to distinguish between anion channel or carrier activity.\textsuperscript{54} When testing a series of cholapods, they found a simple correlation between membrane thickness and transport rates. A similar relationship has been demonstrated by Läuger for K\textsuperscript{+} transport by valinomycin.\textsuperscript{163} As the membrane thickness increases, the rate of transport by a mobile carrier will decrease as there is a greater distance across which the ion must be transported. However, as discussed in section 3.1.3, the correlation between the activity of a channel and the bilayer thickness is more complex. Similar rates should be observed for a range of bilayer thicknesses, but this should reduce dramatically once the channel can no longer span the membrane.

Additionally, considering the Hill coefficients for the Cl\textsuperscript{−} transport by \textbf{161-167} are \textasciitilde2 for each transporter, therefore it is unlikely that channels are formed by large aggregates of these receptors. These receptors are not large enough to span the bilayer as a single entity, but a structure composed of two receptors may be long enough. Matile has reported that the thickness of the hydrophobic interior of an EYPC bilayer (mainly composed of POPC) is approximately 36 Å.\textsuperscript{99} The crystal structure of \textbf{159} (Figure 3.30, discussed in section 3.5) indicates that the length of this receptor is 17.825 Å from C2 to C10 (shown in Figure 3.18).
Figure 3.18 A comparison of (a) the approximate length of receptor 159 by comparison to the thickness of the hydrophobic interior of a POPC bilayer, and (b) a possible 2-receptor assembly which could theoretically span the bilayer. The crystal structure image was taken from the 159\(\text{K}_2[\text{oxalate}][18\text{-crown-6}]\) structure depicted in Figure 3.30. Non-acidic protons and both anions and cations have been removed for clarity.

Although the alkyl chain of the receptor is kinked in this structure, this can be used as an estimate of the dimensions of this molecule. Thus, it may be possible that as the chain length increases, the receptors may be able to form membrane spanning assemblies as shown in Figure 3.18(b). The propensity of ureas to self assemble via hydrogen bonding interactions could provide a route to this assembly. This would also explain the observed trend in Cl\(^-\) transport activity across the series, as it may be possible that compound 164 is of the optimum length to form this type of structure in a bilayer of this thickness. Therefore, an experiment was undertaken to examine the effect of changing the bilayer thickness on the trends in transport activity across the series.

The lipids used for this experiment were C\(_{18}(1)\text{PC (DOPC)}\) and C\(_{16}(1)\text{PC, shown in Figure 3.19. These lipids were previously utilized in a similar assay by A. P. Davis et al.}^{54}\) and were selected as they contain unsaturated acyl chains, which maintain the fluid
phase at room temperature. Like POPC they contain a phosphocholine (PC) head group, but the two appending acyl chains are of the same length and are both mono-unsaturated. The lipid bilayer thickness is known to vary linearly with acyl chain length in PC vesicles. The bilayer thickness of C<sub>18</sub>(1)PC is reported to be 32 Å. As C<sub>16</sub>(1)PC is a less commonly used lipid, an experimental value for this parameter is not available, although it will be smaller than for C<sub>18</sub>(1)PC. The effect of reducing the length of a phospholipid acyl chain by 2 methylene units may be estimated by the difference in thickness of bilayers composed of the saturated acyl chain lipids C<sub>16</sub>(0)PC and C<sub>14</sub>(0)PC, (7.6 Å); a similar difference in bilayer thickness may be expected for C<sub>18</sub>(1)PC and C<sub>16</sub>(1)PC.

![Diagram of phospholipids](image)

**Figure 3.19** The structures of the phospholipids used in these studies.

The Cl⁻/NO₃⁻ antiport activity of 159-167 was investigated in vesicles composed of C<sub>16</sub>(1)PC and C<sub>18</sub>(1)PC. The results are shown in **Figure 3.20**.
As expected, the transport activity of 159-167 was reduced in vesicles composed of C\textsubscript{18}(1)PC by comparison to C\textsubscript{16}(1)PC. This is due to the increasing bilayer thickness and thus a larger transmembrane distance for the receptor-anion complex to travel. As a
measure of the anion transport activity of 159-167 in these experiments, the chloride efflux after 270 s is shown in Figure 3.21.

![Figure 3.21](image)

**Figure 3.21** The chloride efflux after 270 s promoted by receptors 159-167 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of C_{16}PC or C_{18}PC containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO\(_3\) buffered to pH 7.2 with 5 mM sodium phosphate salts.

The data in Figure 3.21 demonstrates that the trend in anion transport activity across the series 159-167 is the same in both sets of vesicles, despite the variation in bilayer thickness. This trend is also the same as observed in POPC vesicles. If these receptors were forming membrane spanning structures, it would be expected that reducing the thickness of the bilayer should favour channel formation by a shorter chain receptor. As this is not the case, these results indicates that a mobile carrier mechanism is operational.

### 3.4.6 Summary of anion transport results

Compounds 159-167 show a range of chloride transport activity, which is dependent on the length of the central alkyl chain. The best Cl/NO\(_3\)\(^-\) antiporter is compound 164 (n = 9). This optimum chain length may be explained as a balance between the lipophilicity of the carrier, a favourable characteristic for ion transport which increases with increasing
chain length, and the kinetics of the receptor passing through the aqueous phase and partitioning within the bilayer, which appears to decrease for the longest chain lengths. Mobile carrier activity has been demonstrated by comparing the transport activity in bilayers of varying thickness. Additionally, the greatly reduced Cl⁻/NO₃⁻ antiport activity of mono-ureas 168-176 demonstrates that the bis-urea scaffold facilitates anion transport by a mechanism in which the two urea groups are not functioning independently. This implies that their transport capability is not solely due to lipophilicity arguments and that the inclusion of two urea moieties in receptor design is advantageous for transmembrane anion transport.

3.5 Solution phase anion binding

3.5.1 ¹H NMR titrations

The solution phase anion binding properties of compounds 159-167 with a selection of anions relevant to the transport experiments and in biological systems were investigated by ¹H NMR titration in DMSO-d₆/ 0.5 % H₂O. Figure 3.22 shows the change in chemical shift of the urea NH proton adjacent to the indole moiety on titration with various anions. Across the series 159-167, similar binding profiles were observed for each anion tested, therefore each analysis is representative of all of the receptors in this series. The full analysis for each receptor can be found in the appendix (section A2.1).
Figure 3.22 The change in chemical shift of the indolyl urea NH proton (starting at ~ 8.3 ppm) of receptor 159 during the $^1$H NMR titration with various anions (added as the tetrabutylammonium or tetraethylammonium salts) in DMSO-$d_6$/H$_2$O 0.5 %.

Across the series 159-167, none of the receptors were observed to interact with TBANO$_3$. On titration with TBACl, the indolyl urea NH resonance at ~8.3 ppm is observed to gradually shift downfield for the duration of the experiment, consistent with hydrogen bond formation to the anion. This is observed for all of the NH hydrogen bond donors in this receptor, as shown in Figure 3.23(a), which indicates that all of the NH groups participate in the anion binding.

Figure 3.23 (a) The change in chemical shift of the NH hydrogen bond donors of receptor 159 on titration with TBACl in DMSO-$d_6$/H$_2$O 0.5 %; (b) The Job plot analysis for this process following the indolyl urea NH resonance at ~8.3 ppm.
A Job plot analysis, shown in Figure 3.23(b), confirmed a 1:1 binding mode for this process. This implies that a weak 1:1 complex with chloride is formed. However, it should be noted that the formation of a 2:2 complex, as shown in Figure 3.24, is also theoretically possible and would be indistinguishable from a 1:1 complex by these means.

![Figure 3.24](image)

Figure 3.24 A representation of the possible 1:1 and 2:2 binding modes for the complexation of Cl⁻ by bis-indolylureas receptors 159-167.

The titration data was fitted to a 1:1 binding model using WinEQNMR 2. The binding constants obtained are shown in Table 3.2. These results show that increasing the length of the alkyl chain across the series has no observable effect on the Cl⁻ binding strengths under these conditions. Thus, there is no observable correlation between the Cl⁻ binding strengths of these receptors and their Cl⁻ transport activity.

Table 3.2 Binding constants (M⁻¹) obtained from the ¹H NMR titration of 159-167 with TBACl in DMSO-d₆/H₂O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. All errors are < 10 %.

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On titration with TBA₂SO₄, the urea NH resonance is observed to rapidly shift downfield until approximately 1 equivalent of anion. After this point, the curve plateaus briefly, followed by a gradual downfield shift. As shown in Figure 3.25(a), similar behaviour is observed for the other NH hydrogen bond donors of these receptors. This profile is indicative of strong binding to 1 equivalent of SO₄²⁻, followed by a much weaker complexation of a second equivalent of anion. The high negative charge of SO₄²⁻
results in strong binding of the first equivalent, but also makes the binding of a second anion electrostatically unfavourable. The Job plot analysis for this process, shown in Figure 3.25(b), indicates that the 1:1 complex was the most prevalent. This reflects the much stronger binding of the first equivalent. The titration data was fitted to a 1:2 binding model using WinEQNMR 2, as the data could not be adequately fit to a 1:1 binding model. The binding constants calculated are shown in Table 3.3. Although the errors associated with the 1:2 model were large in some cases, this is a consequence of the shape of the binding curves, in particular the plateau after the addition of 1 equivalent of anion. This in itself is likely to be a consequence of the much stronger binding of the first equivalent of anion. There is a general trend across the series that $K_1$ decreases and $K_2$ increases. This reflects the decreasing favourability of the formation of a 1:1 complex of the form shown for Cl$^-$ in Figure 3.24 due to increasing the chain length. The binding of the second equivalent is therefore more favourable as dissociation of the 1:1 complex is less unfavourable.

![Figure 3.25](image_url)

**Figure 3.25** (a) The change in chemical shift of the NH hydrogen bond donors of receptor 159 on titration with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5 %; (b) The Job plot analysis for this process following the indolyl urea NH resonance at ~ 8.3 ppm.
Table 3.3 Binding constants (M⁻¹) obtained from the ¹H NMR titration of 159-167 with TBA₂SO₄ in DMSO-d₆/H₂O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value.

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<td>(14 %)</td>
<td>(23 %)</td>
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<td>(35 %)</td>
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The binding profiles from the titration of 159-167 with TEAHCO₃ and TBAH₂PO₄ were similar in appearance. The continuous downfield shift of the urea NH resonances until approximately 2.4 equivalents of anion is indicative of a predominantly 1:2 binding mode (as shown in Figure 3.26(a) and Figure 3.27(b)). The data in these figures indicates that HCO₃⁻ is bound most strongly by the urea NH hydrogen bond donors, as expected for a Y-shaped anion. In contrast, H₂PO₄⁻ is bound most strongly by the indole and indolyl urea NH donors. When the data was fitted to a 1:2 binding model using WinEQNMR 2, in some cases the calculated binding constants had large errors. These binding constants are shown in Table 3.4 and Table 3.5. Further to this, a Job plot analysis indicated that the binding of HCO₃⁻ does not proceed by a purely 1:2 binding mode as the Job plot peaked at 0.4 (Figure 3.26(b)). This could be a result of a mixture of 1:1 and 1:2 binding, or it could alternatively be representative of the presence of multiple equilibria. The Job plot analysis for the binding of H₂PO₄⁻ does peak at approximately 0.33 (as expected for a 1:2 binding model, shown in Figure 3.27(b)). However, the large errors associated with the binding constants calculated using this model, and the similarity to the HCO₃⁻ binding profile may indicate that a purely 1:2 binding mode does not exist under these conditions.
Figure 3.26 (a) The change in chemical shift of the NH hydrogen bond donors of receptor 159 on titration with TEA\(\text{HCO}_3\) in DMSO-\(d_6\)/H\(_2\)O 0.5 %; (b) The Job plot analysis for this process following the indolyl urea NH resonance at ~ 8.3 ppm.

Figure 3.27 (a) The change in chemical shift of the NH hydrogen bond donors of receptor 159 on titration with TBA\(\text{H}_2\text{PO}_4\) in DMSO-\(d_6\)/H\(_2\)O 0.5 %; (b) The Job plot analysis for this process following the indolyl urea NH resonance at ~ 8.3 ppm.
Table 3.4 Binding constants (M⁻¹) obtained from the 'H NMR titration of 159-167 with TEAHCO₃ in DMSO-d₆/H₂O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value.

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Table 3.5 Binding constants (M⁻¹) obtained from the 'H NMR titration of 159-167 with TBAH₂PO₄ in DMSO-d₆/H₂O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value.

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<td>(19 %)</td>
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The binding of the second equivalent of HCO₃⁻ or H₂PO₄⁻ is generally of similar strength to the binding of the first equivalent. This indicates that the two anion binding sites are able to bind anions almost independently if the anions are only singly charged and thus the electrostatic repulsions are not too strong. There is no observable trend in binding strengths across the series. In some cases, the value of $K_2$ is observed to be higher than $K_1$. This result would normally be indicative of a cooperative binding effect. However, in this case it is more likely that this unusual observation is merely an artefact of the poor fit of the data to the binding model, as the cooperative binding of two anionic species is improbable.

### 3.5.2 Bis-urea receptors and the 1:2 binding model

While the binding curves from the titrations of receptors 159-167 with TEAHCO₃ and TBAH₂PO₄ indicate that a predominately 1:2 binding mode is operational, the
imperfect fit of the titration data to a simple 1:2 binding model implies the existence of multiple equilibria. In order to investigate whether the binding of H$_2$PO$_4^-$ by these receptors could facilitate its deprotonation by free anions in solution, as has been previously observed for other indolylurea receptors reported by the Gale group,$^{70, 71}$ a sample of 161 was titrated with 1.4 equivalents of H$_2$PO$_4^-$ followed by up to 2 equivalents of TBAOH (1M in MeOH). The results were unlike any of the previously reported titration data (see the appendix for details), thus indicating that this process is not occurring. However, this does not rule out multiple binding equilibria.

A simple 1:2 binding model between a receptor L and an anion X examines the general equilibria as shown below.

\[
L \rightleftharpoons LX \rightleftharpoons LX_2
\]

However, the binding of 1 or 2 equivalents of anion (X) to a bis-urea receptor (L) may result in multiple equilibria as there are two forms that the 1:1 complex can adopt: one in which the anion interacts with a single binding site, and one in which the receptor curves and both urea groups interact with the anion. As mentioned previously, a 2:2 complex (L$_2$X$_2$) is also theoretically possible. These equilibria are represented in Figure 3.28.
If the most stable form of the 1:1 complex is LX(II), there is an additional equilibrium to consider on moving from the 1:1 complex to the 1:2 complex as one of the urea groups must dissociate from the first anion in order to bind the second. If a 2:2 complex is possible, this further complicates the system. This additional equilibrium may explain the imperfect fit of this data to a 1:2 binding model.

### 3.6 X-ray crystallography

Crystals of compounds 159-167 proved to be extremely difficult to grow, as did crystals of their anion complexes. This is most likely due to the high flexibility of the receptors, indicating that the molecules are likely to be disordered in the solid state. Additionally, the poor solubility of these receptors in the majority of organic solvents prevented the straightforward control of crystallization by variation of the mother liquor. Often, any crystalline material obtained was not of suitable quality for single crystal X-ray analysis.

Crystals of the H$_2$PO$_4^-$ complex of 161 were grown by slow evaporation of a DMSO solution of the receptor with an excess of TBAH$_2$PO$_4$. The structure was
elucidated by single crystal X-ray diffraction and is shown in Figure 3.29. Full details of the X-ray diffraction analysis can be found in the appendix (section A3.2).

**Figure 3.29(a)** The X-ray structure of the crystals grown from a DMSO solution of 161 and TBAH$_2$PO$_4$ adopts a pseudo 1:2 stoichiometry due to oligomerisation of the phosphate anions. Counterions and non-interacting hydrogen atoms have been removed for clarity.
Figure 3.29(b) Two views of the expanded crystal structure of 161 binding to an oligomerised phosphate rod. The receptors wrap around the rod in a helical fashion. Counterions and non-interacting hydrogen atoms have been removed for clarity.

The structure shows that the phosphate anions have oligomerised into a rigid rod via hydrogen bonding interactions between the anions. The receptor-anion complex adopts a pseudo 1:2 stoichiometry, with each indolylurea unit coordinating 1 anion via two urea NH\textsuperscript{-}O hydrogen bonds (Figure 3.29(a)). The indole NHs are orientated out of the binding cleft due to intramolecular hydrogen bonding to the urea C=O (as shown in Figure 3.29(a)), thus are not contributing to binding the anion. This is in contrast to the solution phase binding studies, in which the downfield shift of the indole NH resonance indicates hydrogen bonding to the H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}. The receptors wrap around the oligomerised
phosphate rod in a helical manner, as illustrated in Figure 3.29(b). Some bond lengths and angles of interest are shown in Table 3.6.

Table 3.6 Some bond lengths and angles of interest relating to the crystal structure of 161-TBAH₂PO₄.

<table>
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<th>Bond Length/Angle</th>
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<td>d(NH···O₂⁻)</td>
<td>1.912-2.260 Å</td>
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<tr>
<td>d(N···O₂⁻)</td>
<td>2.750-2.989 Å</td>
</tr>
<tr>
<td>∠(N-H-O₂⁻)</td>
<td>141-158 °</td>
</tr>
<tr>
<td>d(NHindole···Ourea)</td>
<td>1.961 Å</td>
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<tr>
<td>∠(Nindole-H-Ourea)</td>
<td>135 °</td>
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<tr>
<td>d(P···P)</td>
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<tr>
<td>d(OHphosphate···Ophosphate₂)</td>
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<tr>
<td>d(Ophosphate···Ophosphate₂)</td>
<td>2.474-2.553 Å</td>
</tr>
<tr>
<td>∠(Nindole-H-Ourea)</td>
<td>157-159 °</td>
</tr>
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Single crystals of the oxalate complex of compound 159 were grown by slow evaporation of a DMSO solution of 159 containing 18-crown-6 and potassium oxalate. 18-crown-6 was included in this experiment to enhance the low solubility of potassium oxalate in DMSO. Compound 159 and 18-crown-6 (1:1) were dissolved in hot DMSO, and potassium oxalate was added. The solution was agitated with further heating and filtered while still hot. The sample was allowed to stand for 6 weeks. The structure of the resulting crystals is shown in Figure 3.30. Full details of the X-ray diffraction analysis can be found in the appendix (section A3.3).
Figure 3.30(a) The crystal structure of $159 \cdot K_2[\text{oxalate}][\text{18-crown-6}]_2$. Non-interacting hydrogen atoms and additional $K'[\text{18-crown-6}]$ units have been omitted for clarity.
The oxalate anions are coordinated in a 2:2 host:guest stoichiometry as shown in Figure 3.30(a). Each oxalate anion is coordinated by two indolylurea units from two different receptors by a total of 9 hydrogen bonding interactions. The K\(^+\) counterions are bound by 18-crown-6. Two of the K\(^+\)[18-crown-6] units are coordinated to the oxygen donors of the urea groups; the other two K\(^+\)[18-crown-6] required for complex neutrality can be found coordinated to the oxalate anions as shown in Figure 3.30(b). The NH···O\(_{\text{oxalate}}\) bond lengths are in the range of 1.876 – 2.703 Å. While this structure cannot be interpreted as evidence of the mode of binding any anions in solution, it is evidence that a discrete 2:2 complex is possible in the solid state using receptors of this type.
3.7 Conclusions

The work in this chapter demonstrates that the bis-urea scaffold has been used to yield effective anion transporters, while variation of the central alkyl chain length allows for the straightforward modulation of transport activity. The bis-urea scaffold has also been demonstrated to be more effective for carrier design that the equivalent mono-urea scaffold. Carriers 159-167 contain anion binding clefts inspired by literature anion receptor designs. However, it is worth noting that from a purely anion complexation perspective, the design of these receptors may be considered to be relatively poor. The increasing length and flexibility of the central alkyl chain which separates the two distinct binding sites results in the binding sites becoming increasingly likely to function individually. The lack of rigidity in the receptor design means that there is little preorganization towards anion complexation, leading to multiple possible binding modes and complex binding equilibria. There is also little difference between the binding properties of the receptors across the whole series. However, the increasing carrier efficiency across the series serves to highlight that a theoretically poor anion receptor can effectively facilitate anion transport. Thus, the design of future carriers should consider both the interactions between the host and the guest and the interactions between the resulting complex and the lipid bilayer.
Chapter 3 Bis-indolylureas
Chapter 4

Bis-phenylthioureas as tunable transporters for chloride and bicarbonate

4.1 Introduction

The receptors described in Chapter 3 were found to function as Cl⁻/NO₃⁻ antiporters with varying anion transport activity that was highly dependent on the length of the central alkyl chain. This observed trend allows for the straightforward control of transport activity. The receptors were also found to be significantly more active than their analogous monosubstituted indolylureas, thus implying that the bis-urea scaffold facilitates anion transport not just by providing two discrete urea subunits instead of one, but that the two urea groups function complementarily.

The bis-(alkyl)-urea scaffold is easily synthesized and thus can be used as a model for improved systems. One particular area for improvement is the lack of Cl⁻/HCO₃⁻ antiport activity. While the observed Cl⁻/NO₃⁻ antiport trends can be partially attributed to the variations in lipophilicity across the series, this lack of HCO₃⁻ transport implies that either the receptors are not inherently lipophilic enough to facilitate the transmembrane transport of the more hydrophilic HCO₃⁻ anion, or that the receptors do not interact with HCO₃⁻ sufficiently strongly to transport it. Considering the stronger binding of HCO₃⁻ than Cl⁻ or NO₃⁻ by these receptors in polar DMSO-d₆/H₂O (0.5 %) solution, the first explanation is the most likely. Therefore, inspired by the generally greater Cl⁻/HCO₃⁻ antiport activity of thioureas over ureas (as described in Chapter 2 and documented elsewhere), a series of bis-phenylthioureas 177-182 were synthesized in order to investigate whether the dependency of the length of the central alkyl chain to transport...
efficiency could be applied to the transmembrane transport of $\text{HCO}_3^-$, and if the bis-thiourea scaffold could mediate more efficient anion transport than the analogous mono-thioureas. For comparison, the transport activity of bis-indolylthiourea 183 and bis-phenylurea 184 were also investigated. The carriers described in this chapter are shown in Figure 4.1.

![Figure 4.1 The bis-(thio)urea carriers described in this chapter.](image)

### 4.2 Synthesis

Thioureas 177-182 were synthesized by refluxing a solution of phenylisothiocyanate with the corresponding diamine in DCM/DMF (1%) as shown in scheme 3.1.

![Scheme 4.1 Reagents and conditions: ½ eq. diamine, DCM/DMF (1%), reflux, N$_2$, overnight.](image)

Compound 183 was synthesized by the reflux of 7-isothiocyanato-$1H$-indole (synthesized by a literature procedure as described in Chapter 2$^{69}$) with 1,8-diaminoctane in DCM/DMF (1%).

![Scheme 4.2 Reagents and conditions: (i) ½ eq. 1,8-diaminoctane, DCM/DMF (1%), reflux, N$_2$, overnight.](image)
Compound **184** was synthesized by the room temperature reaction of phenylisocyanate with 1,8-diaminoctane in DCM.

![Scheme 4.3](image)

**Scheme 4.3** Reagents and conditions: (i) ½ eq. 1,8-diaminoctane, DCM, RT, 2 h.

Full synthetic procedures can be found in **Chapter 6.4**.

### 4.3 Anion transport studies

#### 4.3.1 Results

Initially, the Cl$^-$/NO$_3^-$ antiport activity of **177-182** was investigated. A solution of each receptor in DMSO was added to a suspension of unilamellar vesicles containing NaCl suspended in NaNO$_3$. The results are shown below.

![Figure 4.2](image)

**Figure 4.2** Chloride efflux promoted by receptors **177-182** (2 mol% w.r.t lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials.
The results showed that under these conditions, 177-182 exhibited a range of transport activities and that increasing the length of the alkyl chain resulted in an increase in the total chloride efflux. Interestingly, the highest transport activity was observed for 182 (n = 12); unlike the series of receptors described in Chapter 3, there is no maximum chain length for increased efficiency observed within this series.

The Cl⁻/HCO₃⁻ antiport activity of 177-182 was also investigated by addition of a DMSO solution of the receptor to vesicles containing NaCl suspended in Na₂SO₄. After 120 s, a solution of NaHCO₃ was added such that the total HCO₃⁻ concentration was 40 mM. The results are shown in Figure 4.3.

*Figure 4.3* Chloride efflux mediated by 177-182 (2 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.

These results show that bis-thioureas 177-182 are able to mediate Cl⁻/HCO₃⁻ antiport. Under these conditions, a similar trend is observed as for the Cl⁻/NO₃⁻ antiport by these receptors. The longest receptors 181 and 182 (n = 11 and 12) are the most active anion carriers.
Hill analyses were performed for the Cl/NO$_3^-$ and Cl/HCO$_3^-$ antiport by 177-182 and for bis-indolylthiourea 183. Bis-phenylurea 184 was found to be inactive for both transport modes and was observed to precipitate during the experiment. Table 4.1 contains the relevant constants from the Hill analyses and the clogP and PSA values for each compound (calculated using Spartan '10 for Macintosh). The full Hill analyses can be found in the appendix (section A1.3). The values determined for compound 125 and 127 (as discussed in chapter 2) and compound 163 (discussed in chapter 3) have been included for comparison. The structures of these carriers is shown in Figure 4.4.

![Figure 4.4](image_url)
Table 4.1 Values of EC$_{50}$ for Cl$^{−}$/NO$_3^{−}$ and Cl$^{−}$/HCO$_3^{−}$ exchange and clogP and PSA values for compounds 177-182 and some structurally related analogues for comparison. Values of EC$_{50}$s for Cl$^{−}$/NO$_3^{−}$ and Cl$^{−}$/HCO$_3^{−}$ antiport were calculated by a Hill analysis. clogP values were calculated using Spartan ‘10 for Macintosh [Ghose Crippen model]). The receptors were minimized using AMI semi-emperical methods with the two urea or thiourea NH groups parallel and the clogP and PSA values calculated. For the indole containing species, two conformations were minimized—one with the indole NH forming a convergent array with the urea NH groups and the other with the indole NH orientated towards the urea or thiourea O or S atom (hence a range of values for PSA is given). [a] The observed transport activity was too low to allow the determination of EC$_{50}$ by a Hill analysis (EC$_{50}$ > 4 mol%); [b] determined by SJM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC$_{50}$ (Cl$^{−}$/NO$_3^{−}$) / mol%</th>
<th>EC$_{50}$ (Cl$^{−}$/HCO$_3^{−}$) / mol%</th>
<th>clogP</th>
<th>PSA/ Å</th>
</tr>
</thead>
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<td>[a]</td>
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<td>1.13</td>
<td>6.04</td>
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<td>182</td>
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<td>0.52</td>
<td>[a]</td>
<td>2.99</td>
<td>62.0-67.3</td>
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<tr>
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<td>Precipitates</td>
<td>Precipitates</td>
<td>2.50</td>
<td>69.2</td>
</tr>
<tr>
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<td>0.70</td>
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</table>

These results confirm that the most effective bis-thiourea for Cl$^{−}$/NO$_3^{−}$ and Cl$^{−}$/HCO$_3^{−}$ antiport agent is compound 182, and that in general, increasing the length of the alkyl chain results in improved transport activity. This correlates with the increased clogP value and hence higher lipophilicity associated with increasing the length of the central aliphatic chain. Across the series 177-182 there is little change in the PSA. This is because the most polar regions of the molecule are the binding sites and increasing the length of the aliphatic chain has little bearing on this.
4.3.2 Analysis of structure-activity relationships

4.3.2.1 Substituent effects

The anion antiport properties of bis-indolylthiourea 183 are shown by comparison to the analogous bis-phenylthiourea 178 in Figure 4.5(a) and (b).

**Figure 4.5(a)** Chloride efflux promoted by 178 (blue) and 183 (red) (2 mol% with respect to lipid) from unilamellar POPC vesicles containing NaCl suspended in NaNO₃.

**Figure 5(b)** Chloride efflux promoted by 178 (blue) and 183 (red) (2 mol% with respect to lipid) from unilamellar POPC vesicles containing NaCl suspended in Na₂SO₄. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM.

Compound 183 was more active for Cl⁻/NO₃⁻ antiport than 178 as represented by the EC₅₀ values and as shown in Figure 4.5(a). This correlates with the finding from Chapter 2 that a mono-indolylthiourea 127 was more active than a mono-phenylthiourea.
125, possibly due to the better anion binding by a receptor with more hydrogen bond donors. However, at 2 mol\% loading, the Cl⁻/HCO₃⁻ antiport activity of 178 and 183 appear to be similar (Figure 4.5(b)). While these activities were too low to be quantified by a Hill analysis, it seems that in this case there is little advantage to the indolyl substituent by comparison to a phenyl substituent.

Bis-indolylurea 163 was more active for Cl⁻/NO₃⁻ antiport than bis-phenylurea 184, which precipitated during the experiment. Compound 163 is more hydrophilic than 184 (as judged by the clogP values) and also has a higher PSA, which may aid its passage through the aqueous phase. However, it is unlikely that lipophilicity is the sole reason that 184 precipitates, as it is less lipophilic than all of the listed thiourea compounds which are active. It is possible that 184 forms very strong intermolecular hydrogen bonds with itself, thus making its precipitation energetically favourable. Thioureas are known to be less likely to self associate by hydrogen bonding due to the lesser H-bonding basicity of the C=S group by comparison to C=O.¹³⁴

4.3.2.2 Ureas vs thioureas

As mentioned above, bis-phenylurea 184 is completely inactive for anion transport, thus it is less active than the analogous thiourea 178. Compound 183 was also found to be more active for Cl⁻/NO₃⁻ antiport than the analogous bis-urea 163, as evidenced by the values of EC₅₀. These observations correlate with the finding discussed in Chapter 2 that thioureas tend to be more active than their urea analogues, previously argued on the basis of their greater lipophilicity.

4.3.2.3 The advantage of the bis-thiourea scaffold

Figure 4.6 A comparison of the Cl⁻/NO₃⁻ antiport activity of the phenylthiourea based carriers.
Compound 177 (n = 7) was found to be less active for Cl\(/\)NO$_3^-$ exchange than mono-phenylthiourea 125 despite containing two thiourea binding sites. The presence of a second binding site makes the carrier less lipophilic as it contains a second polar region. The clogP values are significantly reduced, and the PSA approximately doubles. Thus, the activity is reduced compared to the mono-thiourea. Increasing the central alkyl chain length compensates this, as the longer chain bis-thioureas (compounds 178-182, n = 8-12) were found to be more active than mono-thiourea 125. Compounds 177-180 (n = 7-10) were also found to be less active for Cl\(/\)HCO$_3^-$ antiport than 125; however, the longest and most active analogues 181 and 182 (n = 11 and 12) were more active than 125 for both transport modes. These trends are summarised in Figure 4.6.

![Figure 4.7 A comparison of the Cl\(/\)NO$_3^-$ antiport activity of the indoly(thio)urea based carriers.](image)

In Chapter 3, it was found that bis-indolyurea 163 was more active than analogous mono-ureas such as 126 (which exhibits no anion transport activity). However, when considering bis-thiourea 183 this trend is reversed, as it is significantly less active than 127. The trends for the indole containing carriers are illustrated in Figure 4.7.

### 4.3.3 Mobility Assay

A mobile carrier mechanism for carriers 177-183 was confirmed by examining their Cl\(/\)NO$_3^-$ antiport activity in vesicles composed of POPC:cholesterol (7:3). The comparative graph for receptor 182 is shown in Figure 4.8. The other analyses may be found in the appendix (section A1.3).
A reduction in transport rate was observed for all of 177-183 compounds in membranes containing 30 % cholesterol; this indicates that the transport is diffusion controlled and confirms that these compounds function as mobile carriers rather than channels.

### 4.4 Solution phase anion binding studies

The solution phase anion binding properties of 177-184 with various anions was investigated by $^1$H NMR titration in DMSO-$d_6$/H$_2$O 0.5 %. The H-atoms discussed in this section are assigned as shown in Figure 4.9.

**Figure 4.9** The phenylthiourea moiety of receptors 177-182 with the protons relevant to the $^1$H NMR titrations labelled.
None of the series 177-184 were observed to interact with TBANO₃ in these experiments. The receptors were found to interact weakly with TBACl under these conditions. The binding curves were similar across the series. As shown in Figure 4.10(a), both of the thiourea NH protons (Hₐ and Hₜ) participate in hydrogen bonding. A doublet centered at ~7.4 ppm, which corresponds to aromatic CHₜ, was also observed to undergo a small downfield shift. This implies that there may be a small hydrogen bonding contribution from this CH donor. A Job plot analysis (Figure 4.10(b)) indicated that the binding stoichiometry was predominantly 1:1, and the data was fitted to a 1:1 binding model using WinEQNMR 2. The binding constants are shown in Table 4.2. This data indicates that the binding of Cl⁻ by these receptors is weak under these conditions, and that there is no trend across the series. Attempts to fit the binding curves from aromatic CHₜ to a 1:1 model gave binding constants which were below the lower limit of detection by this method (< 10 M⁻¹). This trend is similar to that observed for receptors 159-167, discussed in Chapter 3, although the binding is comparatively slightly weaker. Again, there is no correlation between the Cl⁻ binding strengths and transport activity.

![Figure 4.10](image)

**Figure 4.10** (a) The change in chemical shift of Hₐ, Hₜ and Hₜ of receptor 180 on titration with TBACl in DMSO-d₆/H₂O 0.5%; (b) The Job plot analysis for this process following Hₐ.

**Table 4.2** Binding constants (M⁻¹) obtained from the ¹H NMR titration of 177-181 with TEACl in DMSO-d₆/H₂O 0.5% following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value. All errors are < 10%.

<table>
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<tr>
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<th>182</th>
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<td>14</td>
<td>9</td>
<td>11</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>
During the titration of receptors 177-182 with TEAHCO$_3$, the thiourea NH resonances were observed to immediately broaden. This prevented the investigation of HCO$_3^-$ binding by following these resonances. However, the aromatic CH$_c$ resonance was observed to shift downfield. This shift is shown in Figure 4.11(a). The shift of the CH$_c$ resonance indicates hydrogen bond formation to the anion, and the shape of the curve indicates that a 1:2 complex is likely to be the predominant species in solution. The Job plot analysis for this process, shown in Figure 4.11(b) indicated that the stoichiometry was potentially a mixture of 1:1 and 1:2 binding.

![Figure 4.11](a) The change in chemical shift of the aromatic CH$_c$ resonance of receptor 180 on titration with TEAHCO$_3$ in DMSO-$d_6$/H$_2$O 0.5%; (b) The Job plot analysis for this process following H$_c$.

The binding curves from the CH$_c$ resonance were fitted to a 1:2 binding model using WinEQNMR 2. The binding constants obtained are shown in Table 4.3.

<table>
<thead>
<tr>
<th></th>
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<tr>
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<td>(28 %)</td>
<td>(14 %)</td>
<td>(18 %)</td>
<td>(7 %)</td>
<td>(26 %)</td>
<td>(17 %)</td>
</tr>
</tbody>
</table>

The large errors associated with some of these binding constants implies that, as with receptors 159-167 (Chapter 3), the simple 1:2 binding model does not perfectly
describe the equilibria present in these systems. There is no observable trend in binding strength across the series.

During the titration of 177-182 with TBAH$_2$PO$_4$, the thiourea NH$_b$ was observed to broaden throughout the experiment and therefore could not be tracked for the entire titration. The change in chemical shift of thiourea NH$_a$ and aromatic CH$_c$ is shown in Figure 4.12.

![Figure 4.12](image)

**Figure 4.12** (a) The change in chemical shift of $H_a$ and $H_c$ of receptor 180 on titration with TBAH$_2$PO$_4$ in DMSO-$d_6$/H$_2$O 0.5 %; (b) The Job plot analysis for this process following $H_a$.

These binding curves were fitted to a 1:2 binding model using WinEQNMR 2. The calculated binding constants are shown in Table 4.4 and Table 4.5.

<table>
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<td>(23 %)</td>
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<td>(12 %)</td>
<td>(11 %)</td>
<td>(41 %)</td>
<td>(24 %)</td>
<td>(18 %)</td>
<td>(37 %)</td>
</tr>
</tbody>
</table>
Table 4.5 Binding constants (M⁻¹) obtained from the ¹H NMR titration of 177-181 with TBAH₂PO₄ in DMSO-d₆/H₂O 0.5% following the aromatic CH₆ resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value.

<table>
<thead>
<tr>
<th></th>
<th>177</th>
<th>178</th>
<th>179</th>
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<th>181</th>
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</tr>
</thead>
<tbody>
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<td>(15 %)</td>
<td>(4 %)</td>
</tr>
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<td>(5 %)</td>
<td>(13 %)</td>
<td>(14 %)</td>
<td>(17 %)</td>
<td>(7 %)</td>
</tr>
</tbody>
</table>

Again, there is no observable trend in binding strengths across the series. The binding of \(\text{H}_2\text{PO}_4^-\) seems to be of similar strength to the binding of \(\text{HCO}_3^-\). In some cases the errors on these values are large, implying that a simple 1:2 model does not accurately describe the equilibria that are present. The binding constants obtained from following \(\text{H}_a\) and \(\text{H}_c\) are similar, and can therefore be compared to the \(\text{HCO}_3^-\) binding constants. The binding of \(\text{HCO}_3^-\) by these receptors is similarly strong as the binding of \(\text{H}_2\text{PO}_4^-\).

In order to investigate whether the observed peak broadening effects were due to the receptor being deprotonated by basic anions, a control titration was performed with receptor 180 and TBAOH (1 M in MeOH) under the same conditions. The stack plot for this titration is shown in Figure 4.13.
Figure 4.13 Stack plot showing the titration of receptor 180 with a methanolic solution of TBAOH in DMSO-\textit{d}_6/\text{H}_2\text{O} 0.5 \%).

This titration shows that when these receptors are deprotonated, the aromatic CH resonances shift upfield. As this behaviour is not observed in the titrations with HCO$_3^-$ and H$_2$PO$_4^-$, this suggests that deprotonation of the receptor by these anions is not occurring.

During the titration of 177-182 with TBA$_2$SO$_4$, the H$_a$, H$_b$ and H$_c$ resonances were observed to shift downfield until the addition of 1 equivalent of anion, as shown in Figure 4.14(a); at this point, the downfield shift slowed and a clear turning point was observed, but the resonances continued to slowly shift throughout the rest of the experiment. This behaviour is analogous to the SO$_4^{2-}$ binding by receptors 159-167, which were found to strongly complex one equivalent of SO$_4^{2-}$ and to weakly complex the second equivalent of anion. The Job plot analysis shown in Figure 4.14(b) indicates that the binding stoichiometry is potentially a mixture of 1:1 and 1:2. This is in contrast to the Job plot obtained from the SO$_4^{2-}$ binding by bis-indolylureas 159-167, which appeared to show 1:1 binding as the second binding event was so weak. The binding curves following H$_a$ were fitted to a 1:2 binding model using WinEQNM R 2. The binding
constants thus obtained are shown in Table 4.6. The binding curves following the aromatic CH$_c$ fitted best to a 1:1 binding model as the change in chemical shift throughout the titration was much less, and the change in chemical shift after the addition of 1 equivalent of anion was almost negligible. The binding constants obtained from this analysis are shown in Table 4.7.

![Figure 4.14](image)

**Figure 4.14** (a) The change in chemical shift of H$_a$, H$_b$ and H$_c$ of receptor 180 on titration with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5%; (b) The Job plot analysis for this process following H$_a$.

**Table 4.6** Binding constants (M$^{-1}$) obtained from the $^1$H NMR titration of 177-181 with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5% following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQQNMR 2. The errors are reported in brackets as a percentage of the value.

<table>
<thead>
<tr>
<th></th>
<th>177</th>
<th>178</th>
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**Table 4.7** Binding constants (M$^{-1}$) obtained from the $^1$H NMR titration of 177-181 with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5% following the aromatic CH resonance at ~7.4 ppm (centre of a doublet). The data was fitted to a 1:1 binding model using WinEQQNMR 2. The errors are reported in brackets as a percentage of the value.

<table>
<thead>
<tr>
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<th>178</th>
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<td>(14 %)</td>
<td>(13 %)</td>
<td>(4 %)</td>
<td>(12 %)</td>
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</tr>
</tbody>
</table>
The anion binding by compounds 183 and 184 was also investigated by $^1$H NMR titration in DMSO-$d_6$/H$_2$O 0.5%. The change in chemical shift of the thiourea NH resonance of receptor 183 and the urea NH resonance of receptor 184 on titration with a variety of anions is shown in Figure 4.15.

![Figure 4.15](image)

Figure 4.15 The change in chemical shift of (a) the thiourea NH resonance of 183 at ~7.5 ppm, and (b) the urea NH resonance of 184 at ~6.1 ppm during the $^1$H NMR titration with various anions (added as he tetrabutylammonium or tetraethylammonium salts) in DMSO-$d_6$/H$_2$O.

Like the previously discussed receptors, 183 and 184 do not interact with TBANO$_3$ under these conditions. The binding profiles for the interaction of 183 and 184 with Cl$^-$, HCO$_3^-$ and H$_2$PO$_4^-$ are similar to those obtained from bis-indolylureas 159-167 (Chapter 3) and bis-phenylthioureas 177-182 which implies that they exhibit similar binding modes. The shape of the binding curve from the titration of 184 with TBA$_2$SO$_4$ seems to increase only very slightly after the addition of 1 equivalent of anion. This implies that either a 1:1 binding mode is prevalent, or that the binding of the second equivalent of anion is extremely weak. In contrast, the binding curve from the titration of 183 with TBA$_2$SO$_4$ indicates that a second equivalent of anion is bound, resulting in a two-step binding curve. This implies that the binding of the second SO$_4^{2-}$ anion by 183 is correspondingly stronger than by 184. The binding curves obtained were fitted to an appropriate binding model. The corresponding Job plot analyses may be found in the appendix (section A2.2) The binding constants obtained are shown in Table 4.8.
Table 4.8 Binding constants (M$^{-1}$) obtained from the $^1$H NMR titration of 183 and 184 with various anions (added as their tetrabutylammonium or tetraethylammonium salt) in DMSO-$d_6$/H$_2$O 0.5% following the thiourea NH resonance of 183 at ~7.4 ppm and the urea NH resonance of 184 at ~6.1 ppm. The data was fitted to a 1:1 or 1:2 binding model using WinEQNMR 2. The errors are reported in brackets as a percentage of the value.

<table>
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<tr>
<td></td>
<td>(9 %)</td>
<td>(12 %)</td>
<td></td>
</tr>
<tr>
<td>H$_2$PO$_4^-$</td>
<td>2281</td>
<td>3584</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7 %)</td>
<td>(7 %)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>344</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7 %)</td>
<td>(8 %)</td>
<td></td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>5632</td>
<td>$&gt; 10^4$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(7 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>217</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(11 %)</td>
<td>(19 %)</td>
<td></td>
</tr>
</tbody>
</table>

This data shows that, like 159-167 and 177-182, Cl$^-$ is weakly complexed in a 1:1 binding mode. HCO$_3^-$ and H$_2$PO$_4^-$ are complexed in a predominantly 1:2 fashion, and the binding strengths of the first and second equivalent of anion for each receptor is similar. Receptors 183 and 184 bind SO$_4^{2-}$ in a 1:2 binding mode; however, the second equivalent of SO$_4^{2-}$ is bound more strongly by 183.
4.5 Conclusions

The work in this chapter demonstrates that the bis-urea scaffold reported in Chapter 3 can be readily modified to yield new successful anion carriers. The bis-phenylthiourea scaffold was found to be more active for anion transport than an analogous mono-thiourea at longer chain lengths. This shows that the bis-thiourea scaffold becomes more advantageous as the lipophilicity of the carrier increases. An analogous bis-phenylurea 184 was found to be completely inactive for anion transport. Unlike the series of bis-indolylthioureas 159-167 reported in Chapter 3, the greatest transport activity is observed for compound 182, the longest receptor in the series. This implies that the bis-phenylthiourea receptors do not suffer reduced mobility through the aqueous phase as the longer chain analogues do not mediate reduced transport activity as a result of their slower membrane partitioning. This may be a result of the lesser hydrogen bond basicity of the C=S group, meaning that the receptors are less likely to aggregate in the water phase, despite their greater lipophilicity. This greater lipophilicity of the bis-thiourea scaffold may be the cause of their Cl/HCO$_3^-$ antiport activity, a transport mode which the bis-indolylureas 159-167 can not facilitate. Additionally, a bis-indolylthiourea 183 was found to be more active for anion transport than its phenyl-substituted analogue, again highlighting the advantages of including the indole moiety in receptor design.

As anion receptors, the anion complexation behaviour of the carriers reported in this chapter is similar to the bis-indolylureas 159-167. This demonstrates that the observed complex anion binding behaviour is not specific to a single group of bis-(thio)ureas but is a general consequence of the flexibility of this type of scaffold. These results further confirm that more active anion carriers may be synthesised without consideration of improving their anion binding strengths. These results also reiterate that it is becoming increasingly evident that the interaction of the receptor-anion complex within the hydrophobic interior of the lipid bilayer is a more important parameter in the design of new transporters than the anion binding strength. The design of new anion carriers could be based on structures with predicted clogP values in an optimum range for anion transport, providing they contain a suitable anion binding site.
Chapter 4 Bis-phenylthioureas
Chapter 5
Anion transport by strapped calix[4]pyrroles

5.1 Introduction

In 2008 Gale and co-workers first described the use of calix[4]pyrrole 28 (Figure 5.1) as a Cs⁺/Cl⁻ symporter. This behaviour was attributed to the function of 28 as an ion pair receptor for CsCl, in which binding of the Cl⁻ organises the macrocycle into a cone conformation, allowing the Cs⁺ to be complexed within the cup-like cavity thus created. Cs⁺ is a large, charge diffuse cation and is bound by the calix[4]pyrrole cup due to effective size complemetarity. Cs⁺ is also the least hydrophilic of the stable alkali metal cations, and as such would be expected to be the least energetically unfavourable cation in this group to transport across a lipid bilayer. However, Cs⁺ is not a biologically relevant cation, so the potential application of 28 as a biological chloride transport agent is limited as no transport can occur in the absence of Cs⁺. There is currently much academic interest in the synthetic modification of the calix[4]pyrrole framework in order to create receptors to facilitate anion transport under biological conditions.

5.1.1 Anion binding by modified calixpyrroles

Since Sessler et al. first reported the use of calix[4]pyrroles (such as 28) as anion receptors in 1996, there has been extensive work dedicated to the modification of this scaffold to yield alternative anion selectivites and enhanced anion binding strengths. Early efforts focused on functionalizing the β-pyrrolic positions or the meso-substituents as these changes are synthetically the most straightforward.
β-fluorination of the calix[4]pyrrole scaffold leads to enhanced anion binding due to the increased acidity of the pyrrole NHs. Sessler and co-workers have reported the synthesis and anion binding studies of some fluorinated calix[n]pyrroles 36 and 185-188 as shown in Figure 5.2. Binding studies were undertaken using ITC in MeCN (2 % H2O v/v) and revealed that fluorocalix[4]pyrrole 36 bound TBACL more strongly than unmodified calix[4]pyrrole 28 (K_a = 31000 M^{-1} and 5400 M^{-1} respectively). A number of binding studies were completed by ITC in MeCN and DMSO. The results showed that increasing the size of the cavity resulted in an increased selectivity for Br^− over Cl^−. The binding of larger anions such as H2PO4^− was also enhanced as the cavity size increased across the series.

Sessler and co-workers have also reported the expansion of the calix[4]pyrrole cavity by employing bipyrrole in place of pyrrole, yielding calix[n]bipyrrroles such as 189 (Figure 5.3). By a combination of ^1H NMR titration and ITC in MeCN and DMSO (or deuterated equivalents) they found that 189, like calix[4]pyrrole 28, displayed selectivity for Cl^− over Br^− and I^− (F^− was not tested), although 28 was the stronger chloride receptor.
However, 189 was found to bind Br\(^-\) and I\(^-\) more strongly than 28 as a result of the expanded cavity. Subsequently, the same authors reported that calix[2]bipyrrole[2]furan 190 and calix[2]bipyrrole[2]thiophene 191 (Figure 5.3) were weaker receptors for all of the anions tested than 28 and 189.\(^{169}\) However, 190 and 191 were found to be selective for carboxylates such as benzoate \((K_a = 63000 \text{ M}^{-1} \text{ and } 139000 \text{ M}^{-1} \text{ respectively})\), while 28 was found to complex benzoate similarly strongly to Cl\(^-\) \((115000 \text{ M}^{-1} \text{ and } 140000 \text{ M}^{-1} \text{ respectively})\) and was thus less selective. The relatively weaker binding of the spherical Cl\(^-\) anion by these receptors with a larger, non-symmetrical binding cleft was attributed to the poorer size complementarity of the cavity.

![Figure 5.3 The expanded calixbipyrroles reported by Sessler et al.](image)

Modification of the binding properties of the calix[4]pyrrole scaffold can also be achieved by the so-called “strapping” of the macrocycle to give receptors of the general form shown in Figure 5.4.\(^ {170}\) This structural modification should yield a receptor with a more preorganized binding site, as the calix[4]pyrrole cleft will be better shielded from solvent molecules. Variation of the length of the strap can allow control of size selectivity, and inclusion of additional hydrogen bond donors into the strap can also contribute to the binding of the anion.
The first example of a strapped calix[4]pyrrole (compound 192) was reported by Lee and co-workers in 2002. Subsequently, the same authors described the synthesis and comparative anion binding studies of 193 and 194. These receptors are shown in Figure 5.5.

Attempts to determine the chloride binding constants of these receptors by $^1$H NMR were unsuccessful as slow exchange kinetics were observed. Consequently, binding constants were determined by ITC in dry MeCN for 192-194 with TBACl. By comparison to un-strapped calix[4]pyrrole 28, ($K_a = 2.2 \times 10^5$ M$^{-1}$), compound 192 bound chloride 10 times more strongly ($K_a = 2.2 \times 10^6$ M$^{-1}$). The strongest chloride binding was observed with compound 193 ($K_a = 1.8 \times 10^7$ M$^{-1}$), while compound 194 ($K_a = 1.9 \times 10^5$ M$^{-1}$) did not exhibit enhanced chloride binding compared to 28. This suggests that additional hydrogen bonding interactions from the pyrrole NH of 193 and the phenyl CH of 192 contributed to stronger binding. Single crystal X-ray diffraction studies of the chloride complexes of 192 and 193 provided evidence of these hydrogen bonding interactions in the solid state, whilst evidence for this in solution was obtained from the corresponding downfield shift of the resonances of these protons in the $^1$H NMR titration.
of these molecules with TBACl in DMSO-d$_6$. This highlights the advantages of inclusion of additional hydrogen bond donors into the strap.

There are a large variety of functional groups that can be incorporated into the strap in order to achieve desirable properties. For example, compounds 195$^{173}$ and 196$^{174}$ contain a chromophore and a fluorophore respectively, which is of use for anion sensing. Meanwhile, the strap of calix[4]pyrrole 197 contains a Ni(II) capped porphyrin which can enhance anion binding via Lewis acid interaction with the bound metal cation.$^{175}$ Receptors 197R and 197S contain a chiral strap.$^{176}$ Correspondingly, they were found to bind chiral carboxylates with high affinity in MeCN, with the strongest complexes formed between the (S)-guest-(S)-host pair and the (R)-guest-(R)-host pair.

![Figure 5.6](image.png)

Figure 5.6 The incorporation of a wide variety of structural motifs can result in novel binding and sensing properties.

### 5.1.2 Modified calix[4]pyrroles as anion transport agents

Calix[4]pyrrole based carriers may be of use biologically if their transport activity can function in the absence of Cs$^+$. This has been achieved in the past by synthetic modification of the calix[4]pyrrole scaffold. For example fluorocalix[4]pyrrole 36 functions as a Cl$^-$/NO$_3^-$ and Cl$^-$/HCO$_3^-$ antiporter but is unable to facilitate any symport mechanisms.$^{67}$ In general, anions are bound more strongly by 36 in comparison to 28. It is possible that a stronger interaction with the secondary anion (NO$_3^-$ or HCO$_3^-$) is the route of the observed antiport activity of 36. Unlike 28, compound 36 does not mediate
Chapter 5 Strapped calix[4]pyrroles

Cs⁺/Cl⁻ symport. This may be due to the electron withdrawing fluorine substituents resulting in a reduction in electron density of the pyrrole-π-system, reducing the affinity of the macrocycle for cesium. Additionally, fluorinated aromatic structures are known to be more lipophilic than non-fluorinated analogues;⁶⁶ this may contribute to the effective partitioning of the receptor-anion complex within the bilayer.

Figure 5.7 The structurally modified calix[4]pyrroles which have been reported to facilitate Cl⁻ transport in the absence of Cs⁺.

Strapping the calix[4]pyrrole framework has also led to anion carriers which can function both in the presence and in the absence of Cs⁺. Gale and co-workers have reported a series of 1,2,3-triazole strapped calix[4]pyrroles 33-36.⁶⁴, ⁶⁵ These carriers can function as Cs⁺/Cl⁻ symporters, but can also facilitate Cl⁻/NO₃⁻ exchange. The preferred transport pathway differs across the series, but this is further proof that strapping the calix[4]pyrrole framework can encourage an antiport mechanism. Additionally, carriers 31 and 36 have also been shown to function as part of dual host system, in which they are though to facilitate the uniport of Cl⁻, which is coupled to a second transport event mediated by a different carrier.⁷⁴, ⁷⁵

5.2 New calix[4]pyrrole based carriers

The design of new calix[4]pyrrole based transporters can utilize the chloride complexation behaviour of the calix[4]pyrrole core. An anion antiport mechanism may be encouraged by the inclusion of additional hydrogen bond donors into the strap, thus
providing greater stabilization of the receptor with the second anion of interest. Alternatively, it is hoped that if the strap facilitates the binding of a metal cation other than Cs\(^+\), the symport of a biologically relevant ion pair may be encouraged. In particular, the groups of J. L. Sessler and C.-H. Lee have been at the forefront of this research and, in collaboration with the Gale group, have been working towards the synthesis of calix[4]pyrroles for biological applications. In particular, the goal of synthesizing new K\(^+/Cl^-\) symporters has been highlighted by these authors. Some typical intra- and extracellular ion concentrations are shown in Table 5.1.\(^4\) It is hoped that the passive transport of Cl\(^-\) out of cells (against the electrochemical gradient) may be achieved by co-transport with K\(^+\), for which there is a favourable gradient.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Cell (mM)</th>
<th>Blood (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl(^-)</td>
<td>4</td>
<td>116</td>
</tr>
<tr>
<td>HCO(_3^-)</td>
<td>12</td>
<td>29</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>12</td>
<td>145</td>
</tr>
<tr>
<td>K(^+)</td>
<td>139</td>
<td>4</td>
</tr>
</tbody>
</table>

5.3 Calix[4]pyrrole-crown-6: anion transport by an ion pair receptor

Calix[4]pyrrole 200 was provided by the groups of J. L. Sessler and C.-H. Lee. It was designed as an ion pair receptor to facilitate the binding of a metal cation by the polyethyleneglycol strap, based on the well-documented complexation of group (I) metal cations by crown ethers.\(^{177}\) In unpublished work, 200 was found to complex Cl\(^-\) and F\(^-\) in MeCN. Treatment of the chloride complex of 200 with group (I) metal cations revealed that no significant complexation of Na\(^+\) or K\(^+\) occurred. Treatment with Li\(^+\) resulted in a tightly bound ion pair in which the Li\(^+\) was complexed by the crown ether strap. Conversely, Cs\(^+\) was bound in the cup-like cavity of the calix[4]pyrrole analogously to parent macrocycle 28, giving rise to a host separated ion pair.
5.3.1 Anion transport studies

Due to the metal cation coordination properties of 200, initial anion transport studies were conducted to investigate its $M^+/\text{Cl}^-$ symport activity. The chloride efflux from vesicles containing MCl suspended in Na$_2$SO$_4$ mediated by 200 was monitored. The intra- and extra-vesicular solutions were chosen to ensure that the observed chloride efflux could only occur via a symport mechanism. The results are shown in Figure 5.9.

Figure 5.9 Chloride efflux mediated by receptor 200 (4 mol% w.r.t. lipid) from POPC vesicles containing 489 mM MCl buffered to pH 7.2 with 5 mM sodium phosphate salts ($M =$ group (I) cations). The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.
It was found that 200 can mediate Cs⁺/Cl⁻ symport; however, the symport of other group (I) cations was greatly reduced (and generally mirrors the increasing lipophilicity of the cations with increasing ionic radius). This is unsurprising, as the binding studies conducted by Sessler et al. revealed that Na⁺ and K⁺ are not complexed as part of a chloride ion pair. Li⁺ is small and highly charge dense, thus its transport across a lipid phase is highly unfavourable.

The Cl⁻/NO₃⁻ antiport activity of 200 was investigated by monitoring chloride efflux mediated by 200 from vesicles containing NaCl or CsCl suspended in NaNO₃. A comparative graph showing the effect of replacing the external SO₄²⁻ with NO₃⁻ is shown in Figure 5.10.
Figure 5.10 Chloride efflux mediated by receptor 200 from vesicles containing 489 mM NaCl (graph (a), 4 mol% carrier loading) or 489 mM CsCl (graph (b), 2 mol% carrier loading) buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ or 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.
The results show that receptor 200 can mediate Cl/NO$_3^-$ antiport, although this is less efficient than the Cs$^+/Cl^-$ symport pathway. When both pathways are available (CsCl/NaNO$_3$) the highest chloride efflux is observed, indicating a likely combination of these transport pathways. Hill analyses for these processes were performed. While the Cl/NO$_3^-$ antiport activity of 200 is too low to yield a reliable EC$_{50}$ value, the EC$_{50}$ value for Cs$^+/Cl^-$ symport is 1.94 mol%, while when both pathways are possible the observed EC$_{50}$ value is lower at 1.17 mol%, thus reflecting this enhancement. (The full Hill analyses can be found in section A1.4 in the appendix).

In order to verify whether 200 was functioning as a mobile carrier, chloride efflux from CsCl containing vesicles composed of POPC:cholesterol (7:3) suspended in Na$_2$SO$_4$ was measured. Calix[4]pyrroles have only been reported to function as mobile carriers in the past. However, no reduction in transport rate was observed by comparison to vesicles which were composed of 100 % POPC. Despite this finding, in light of U-tube experiments conducted by J. L. Sessler et al. which showed that 200 is capable of transporting various ions across an apolar phase, it was concluded that it most likely functions as a mobile carrier.

5.3.2 Conclusions

Receptor 200 was designed as a ditopic ion pair receptor. However, it fails to transport any ion pairs except for CsCl. This is most likely because the size of the crown ether cavity is too small to facilitate the binding of any cation except Li$^+$, which is extremely hydrophilic and thus difficult to transport. CsCl may be bound as a host separated ion pair and is most likely transported in this manner, analogously to unmodified calix[4]pyrrole 28. A promising route towards the development of a similar receptor for the symport of NaCl or KCl would be to expand the crown ether moiety in order to favour the binding of these larger cations.
5.4 Pyridyl strapped calix[4]pyrroles

Compounds 201-203 were provided by the groups of J. L. Sessler and C.-H. Lee. Calix[4]pyrrole 201 contains an amide linked pyridyl strap, providing additional hydrogen bond donors for anion complexation. In particular, unpublished work by J. L. Sessler et al. has demonstrated that this receptor is capable of binding HCO$_3^-$ utilizing these hydrogen bond donors in combination with the calix[4]pyrrole core. By contrast, calix[4]pyrrole 202 contains an analogous ester linked strap, which does not provide these additional hydrogen bond donors. Compound 203 was also investigated as a control to determine if any observed results for compound 201 could be attributed to the strap alone. Significantly, compound 201 has been recently found to exhibit in vitro anticancer activity, while control compounds 202 and 203 are not effective. Previous work from Sessler et al. has found correlation between the H$^+$/Cl$^-$ co-transport activity of a series of synthetic prodigiosins and their anticancer activity.$^{178}$ It was hoped that in this study, the anticancer activity of 201 could be correlated to a biologically relevant transport process.

![Chemical structures](image)

Figure 5.11 Pyridyl strapped calix[4]pyrroles 201 and 202, and control compound 203.

5.4.1 Anion transport studies

The design of receptor 201 facilitates stronger anion binding due to hydrogen bonding contributions from the strap. Therefore, the anion antiport activity of these compounds was initially investigated. In particular, the HCO$_3^-$ affinity of 201 prompted the investigation of it’s Cl$^-$/HCO$_3^-$ antiport activity. The results are shown below.
Figure 5.12 Chloride efflux mediated by receptors 201-203 (4 mol% w.r.t. lipid) from unilamellar POPC vesicles containing NaCl buffered to pH 7.2 with (a) 5 mM sodium phosphate salts, or (b) 20 mM sodium phosphate salts. (a) The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts; (b) the vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts, at t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of each experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.
Compound 201 was found to mediate only extremely low levels of Cl/NO$_3^-$ exchange and Cl/HCO$_3^-$ exchange. Control compounds 202 and 203 were also found to be largely inactive for these transport modes.

The M$^+$/Cl$^-$ symport activity of compound 201 was investigated for a range of group (I) metal cations. The results are shown in Figure 5.13.

![Figure 5.13 Chloride efflux promoted by receptor 201 (4 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM MCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials.](image)

Calix[4]pyrrole 201 was found to function as an efficient Cs$^+$/Cl$^-$ symporter, but was found to have significantly lower activity in the presence of other alkali metal cations. In this way, calix[4]pyrrole 201 has similar transport activity to unmodified calix[4]pyrrole 28. A Hill analysis was performed for the Cs$^+$/Cl$^-$ transport mediated by 201 which yielded an EC$_{50}$ 270 s value of 1.01 mol%. The full Hill analysis may be found in the appendix (section A1.4). The M$^+$/Cl$^-$ symport activity of 202 and 203 were also investigated under the same conditions. The results are shown in Figure 5.14.
5.4.2 Conclusions

The results of the anion transport assays do not give insight into the observed biological activity of compound 201 by comparison to 202 and 203. While compound 201 is significantly more active for the symport of Cs⁺/Cl⁻ than the control compounds, the absence of Cs⁺ in the cellular environment makes this process biologically irrelevant and thus it cannot be the cause of the observed anticancer activity. Compound 201 cannot symport chloride with a biologically relevant cation or antiport chloride with a biologically relevant anion; thus, it cannot facilitate charge neutral transport of biologically prevalent species. However, as discussed in Chapter 1, ion transport
processes in cells are complicated and are often closely linked. It is possible that in the cellular environment, the uniport of chloride by 201 is electrochemically mediated by the action of a naturally occurring membrane protein.
6.1 General remarks

$^1$H NMR (300 MHz) and $^{13}$C{$^1$H} NMR (75 MHz) were determined on a Bruker AV300 spectrometer. Chemical shifts for $^1$H NMR are reported in parts per million (ppm), calibrated to the solvent peak set. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. Chemical shifts for $^{13}$C{$^1$H} NMR are reported in ppm, relative to the central line of a septet at $\delta = 39.52$ ppm for DMSO-$d_6$. Infrared (IR) spectra were recorded on a Matterson Satellite (ATR). FTIR are reported in wavenumbers ($\text{cm}^{-1}$). HRMS(ES) spectra were recorded using a Bruker Apex III spectrometer and reported as $m/z$ (relative intensity). All solvents and starting materials were purchased from commercial sources and used without further purification unless otherwise stated. Dry DCM was obtained by distillation over CaH$_2$ and degassed for 15 min prior to use. Aniline was distilled prior to use. POPC was supplied by Genzyme. Other phospholipids were supplied by Avanti. Chloride concentrations during transport experiments were determined using an Accumet or Cole-Parmer chloride selective electrode.

6.2 Vesicle studies

6.2.1 General remarks

The vesicles used in the transport studies were prepared by a standard literature procedure.$^{115}$ The ionic strengths of the intra- and extra-vesicular solutions were chosen
to be isotonic during vesicle preparation to avoid the rupturing of the vesicle membranes. All solutions were buffered to pH 7.2 with 5 mM or 20 mM (in the case of the HCO₃⁻ assays) sodium phosphate salts unless otherwise stated. The ionic strength of the solutions was controlled at 500 mM with the desired internal salt (most commonly NaCl) or external salt (most commonly NaNO₃ or Na₂SO₄) as applicable.

### 6.2.2 Preparation of vesicles

A lipid film was prepared by evaporation of a chloroform solution of POPC (1 ml containing ~ 28 mg POPC) was evaporated using a rotary evaporator and dried under high vacuum overnight. The lipid was suspended in the required internal solution (1 ml) using a lab dancer. 9 freeze-thaw cycles were completed on the suspension-freezing using liquid nitrogen followed by warming to room temperature-after which the suspension was allowed to stand for 30 minutes. The vesicles thus formed were extruded 25 times through 200 nm polycarbonate membranes. The vesicles were subjected to dialysis in the desired external solution for a minimum of 2 h to remove unencapsulated internal salts. The lipid was then diluted to a concentration of 1 mM using the desired external solution.

### 6.2.3 Transport experiments

Unilamellar POPC vesicles, prepared as described above, were suspended in 489 mM NaNO₃ or 167 mM Na₂SO₄ solution buffered to pH 7.2 with sodium phosphate salts. The lipid concentration per sample was 1 mM. A DMSO solution of the carrier molecule (10 mM) was added to start the experiment and the chloride efflux was monitored using a chloride sensitive electrode. At 5 min, the vesicles were lysed with 50 µl of polyoxyethylene(8)lauryl ether (0.232 mM in 7:1 water:DMSO v/v) and a total chloride reading was taken at 7 min. In the case of the Cl⁻/HCO₃⁻ antiport assays, after 2 min a solution of NaHCO₃ in 167 mM Na₂SO₄ was added such that the total HCO₃⁻ concentration was 40 mM. The vesicles were then lysed after 7 min.
6.3  Titration and Job plot methods

6.3.1  $^1$H NMR titrations

1.5 ml of a 0.01 M solution of the receptor was prepared. Of this solution, 0.5 ml was added to an NMR tube which was then sealed with an air tight suber seal. The remaining 1 ml of the receptor solution was used to make a 0.15 M solution of the desired guest. The anion/receptor solution was titrated into the NMR tube in small aliquots and a $^1$H NMR spectrum was recorded after each addition. This resulted in an increasing concentration of guest throughout the experiment while the receptor concentration was kept constant. Chemical shifts for each peak were calibrated to the solvent peak. The data was fitted to a relevant binding model using WinEQNMR $^2$ in order to generate values for the binding constant(s).

6.3.2  $^1$H NMR Job plots

Two solutions were prepared; the first was a 3 ml, 0.01 M solution of the receptor and the second was a 3 ml, 0.01 M of the guest. 0.5 ml of the receptor solution was added to an NMR tube. The volume of receptor solution was then decreased by 0.05 ml and the amount of guest solution was increased by 0.05 ml for each successive NMR tube until a 9:1 anion:receptor ratio was reached. A $^1$H NMR spectrum was recorded for each of the ten samples, and calibrated to the solvent peak. This data was used to produce a Job plot in accordance with the methods described by Job.$^{179}$ The molar fraction of the receptor ($\chi_r$) was plotted against the values given by the formula given in Equation 1.

$$y = \frac{\delta_{\text{obs}} - \delta_{\text{int}} \times \chi_r}{\delta_{\text{fin}} - \delta_{\text{int}}}$$

*Equation 6.1* The formula used to generate a Job plot, where $\delta_{\text{obs}}$ is the observed chemical shift, $\delta_{\text{int}}$ is the initial chemical shift and $\delta_{\text{fin}}$ is the final chemical shift.
6.4 Synthetic procedures

6.4.1 Synthesis for chapter 2

7-aminoindole

7-aminoindole was prepared according to a literature procedure as follows. 69 7-nitroindole (1.5 mmol) was dissolved in EtOH and Pd/C (10 % by mass, catalytic) was added. The solution was stirred under atmosphere of H₂ for 3 h at RT until the colour had changed from yellow to colourless. The Pd/C was removed by filtration through celite, and the solvent was removed to yield 7-aminoindole as a white solid.

Assumed 100 % yield. This compound was used without characterisation due to its assumed instability.

7-isothiocyanato-1H-indole

7-isothiocyanato-1H-indole was prepared according to a literature procedure. 69 7-aminoindole (prepared as described above, 1.5 mmol) was dissolved in a 2-phase mixture of DCM (75 ml) and sat. NaHCO₃ (aq) (75 ml) and stirred vigorously. Thiophosgene, (0.171 g, 0.114 ml, 1.5 mmol) was added and the reaction was stirred overnight at room temperature. The organic layer was isolated and washed with 2 x 100 ml water. The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure to leave a brown residue. The residue was triturated in hexane to afford 7-isothiocyanato-1H-indole as a brown solid which was isolated by filtration.

Assumed 100 % yield. This intermediate was not characterized due to its assumed high reactivity.
1-(1H-indol-7-yl)-3-isopentylthiourea 127

7-isothiocyanato-1H-indole (1.5 mmol) was dissolved in 100 ml DCM and i-pentylamine (0.131 g, 1.5 mmol) was added. The reaction was stirred at room temperature overnight under nitrogen. The solution was washed with 2 x 100 ml water and the organic layer was dried over MgSO₄. The crude mixture was subjected to column chromatography on silica (elucent DCM/MeOH 4%). The solvent was removed to leave an orange residue. Hexane (10 ml) was added causing an off white solid to form which was isolated by filtration and recrystallized from DCM to give compound 127 as a white solid.

Total yield (from 7-nitroindole): 89 mg (24 %); ¹H NMR (300 MHz, DMSO-dma): δ= 10.88 (br s, 1H, NH), 9.20 (br s, 1H, NH), 7.42 (m, 2H, 2 x overlapping aromatic CH), 7.28 (t, 1H, J=2.8 Hz, aromatic CH), 6.97 (m, 2H, 2 x overlapping aromatic CH), 6.46 (dd, 1H, J₁=2.6 Hz, J₂=1.9 Hz, NH), 3.48 (m, 2H, CH₂), 1.56 (m, 1H, alkyl CH), 1.42 (q, 2H, J=6.9 Hz, CH₂); ¹³C NMR (75 MHz, DMSO-dma): δ= 180.6 (thiourea C=S), 129.4 (aromatic CH), 125.6 (aromatic CH), 119.0 (aromatic CH), 118.0 (aromatic CH), 42.6 (CH₂), 25.6 (alkyl), 22.5 (alkyl); LRMS(ESI-): m/z = 260.2 ([M-H]), 274.2 ([M.MeOH-H]); HRMS(ES): for C₁₄H₂₀N₃S [M + H]⁺ m/z = 262.1378 (calculated), 262.1378 (found); for C₁₄H₁₉N₃SNa [M + Na]⁺ m/z = 284.1197 (calculated), 284.1195 (found); for C₂₈H₃₈N₆S₂Na [2M + Na]⁺ m/z = 545.2497 (calculated), 545.2509 (found); IR (film): ν= 3370 (indole NH stretching), 3310 (urea NH stretching), 3180 (urea NH stretching); M_p: 88-90 °C.

N-butyl-4-methylpentanethioamide 129

128 (prepared by SJM, 300 mg, 1.75 mmol) was dissolved in THF and Lawesson’s reagent (710 mg, 1.76 mmol) was added. The reaction was refluxed overnight. On cooling, the solvent was removed in situ and the oily residue obtained was re-dissolved in
DCM. The product was washed with 2 x 100 ml of brine followed by 2 x 100 ml of 0.1 M HCl and 2 x 100 ml sat. NaHCO₃. The product was further purified by column chromatography on silica (elution with DCM). This afforded the product as a colourless oil.

Yield: 245 mg (75 %); ¹H NMR (300 MHz, DMSO-d₆): δ = 7.15 (br.s, 1H, NH), 3.66 (td, 2H, J₁=7.3 Hz, J₂=5.5 Hz, CH₂), 2.66 (m, 2H, CH₂), 1.65 (m, 5H, alkyl CH + 2 x CH₂), 1.41 (m, 2H, CH₂), 0.95 (m, 9H, 3 x CH₃); ¹³C NMR (75 MHz, DMSO-d₆): δ=203.8 (carbonyl CO), 44.8 (alkyl), 43.2 (alkyl), 38.2 (alkyl), 29.2 (alkyl), 27.0 (alkyl), 22.3 (alkyl), 19.6 (alkyl), 13.6 (alkyl); LRMS(ESI+): m/z = 188.3 ([M + H]⁺); HRMS(ES): for C₉H₁₉NSNa [M + Na]⁺ m/z= 210.1292 (calculated), 210.1287 (found); IR (film): ν= 3240 (amide NH stretching).

4-methyl-N-phenylpentanamide 130

This compound has been previously reported.¹⁸⁰⁻¹⁸⁴

4-methyl valeric acid (500 mg, 4.30 mmol) was activated by reflux in chloroform (100 ml) with CDI (700 mg, 4.30 mmol). After 2 hours, aniline (440 mg, 4.73 mmol) was added and the reaction was refluxed overnight. On cooling the product was washed with 2 x 100 ml water followed by 2 x 100 ml 0.1 M HCl and 2 x 100 ml sat. NaHCO₃. The combined organic layers were dried over MgSO₄ and the solvent was removed to give an off-white solid. This was triturated in hexane to afford the product as a white solid.

Yield: 623 mg (76%); ¹H NMR (300 MHz, DMSO-d₆): δ = 9.86 (s, 1H, NH), 7.59 (d, 2H, J=8.7 Hz, aromatic CH), 7.28 (t, 2H, J=7.9 Hz, aromatic CH), 7.01 (m, 1H, aromatic CH), 2.30 (m, 2H, CH₂), 1.52 (m, 3H, Alkyl CH + CH₂); 0.90 (d, 6H, J=6.4 Hz, 2 x CH₃); ¹³C NMR (75 MHz, DMSO-d₆): δ= 171.4 (carbonyl CO), 139.3 (aromatic CH), 128.6 (aromatic CH), 122.8 (aromatic CH), 119.0 (aromatic CH), 34.5 (aromatic CH), 34.0 (aromatic CH), 27.2 (aromatic CH), 22.2 (aromatic CH); LRMS(ESI-): m/z = 190.2 ([M – H]⁻); HRMS(ES): for C₁₁H₁₆NONa [M + Na]⁺ m/z= 214.1208 (calculated), 214.1202
4-methyl-N-phenypentanethioamide 131

This compound has been previously reported.\textsuperscript{183}

130 (300 mg, 1.57 mmol) was dissolved in THF and Lawesson’s reagent (634 mg, 1.57 mmol) was added. The reaction was refluxed overnight. On cooling, the solvent was removed \textit{in situ} and the oily residue obtained was re-dissolved in DCM. The product was washed with 2 x 100 ml of brine followed by 2 x 100 ml of 0.1 M HCl and 2 x 100 ml sat. NaHCO\textsubscript{3}. The organic layer was dried over MgSO\textsubscript{4} and the solvent removed to give compound 131 as an off white solid.

Yield: 201 mg (62%); \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 11.49\) (s, 1H, NH), 7.77 (d, 2H, J=8.1 Hz, aromatic CH), 7.39 (t, 2H, J=7.9 Hz, aromatic CH), 7.22 (m, 1H, aromatic CH), 2.75 (m, 2H, CH\textsubscript{2}), 1.64 (m, 3H, alkyl CH + CH\textsubscript{2}), 0.92 (d, 6H, J=6.2 Hz, 2 x CH\textsubscript{3}); \textsuperscript{13}C NMR (75 MHz, DMSO-\textit{d}_6): \(\delta = 204.3\) (thiourea C=S), 139.6 (aromatic CH), 128.4 (aromatic CH), 125.8 (aromatic CH), 123.3 (aromatic CH), 45.1 (alkyl CH\textsubscript{2}), 27.1 (alkyl), 22.4 (alkyl); LRMS(ESI-): \(m/z = 206.2\) ([M – H]); HRMS(ES): for C\textsubscript{11}H\textsubscript{17}N \([M + H]^+\) \(m/z=\) 208.1160 (calculated), 208.1154 (found); IR (film): \(\nu = 3180\) (amide NH stretching); \(M_p: 61-62\) °C.

6.4.2 Synthesis for chapter 3

\textit{N}-(1\textit{H}-indol-7-yl)-1\textit{H}-imidazole-1-carboxamide (Intermediate 3.1)

Intermediate 3.1 was prepared according to a literature procedure.\textsuperscript{76} 7-aminoindole\textsuperscript{69} (1.5 mmol) was dissolved in dry DCM (50 ml) and CDI (0.75 g, 4.5 mmol) was added. The
reaction was stirred under N\textsubscript{2} at RT overnight. The white precipitate thus formed was isolated by filtration and used without further purification.

The bis-indolylureas 159-167 were synthesized according to the same general procedure as follows:

\textit{1,1'-(butane-1,4-diyl)bis(3-(1H-indol-7-yl)urea) 159}

Intermediate 3.1 (200 mg, \sim 0.8 mmol) was dissolved in dry DCM:DMF (50:1, DCM dried over molecular sieves prior to use). 1,4-Diaminobutane (7.1 mg, 0.44 mmol) was added and the mixture was heated to reflux under N\textsubscript{2} and stirred overnight. The mixture was cooled and the white precipitate was collected by filtration. The solid was washed with 3 x 10 ml of water and 3 x 10 ml diethyl ether and dried under vacuum to give 159 as a white solid.

Yield: 43 mg (24 %); \textsuperscript{1}H NMR (300 MHz, DMSO-\textit{d}_6): \ce{\delta = 10.68} (s, 2H, NH), 8.35 (s, 2H, NH), 7.29 (t, 2H, J=2.56 Hz, aromatic CH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.07 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.26 (t, 2H, J=5.49 Hz, aromatic CH), 3.18 (br m, 4H, 2x eq CH\textsubscript{2}), 1.53 (br m, 4H, 2x eq CH\textsubscript{2}); \textsuperscript{13}C NMR (75 MHz, DMSO-\textit{d}_6): \ce{\delta = 155.7} (CO), 129.1 (aromatic CH), 127.9 (aromatic CH), 124.9 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.8 (aromatic CH), 101.4 (aromatic CH), 27.4 (multiple overlapping CH\textsubscript{2}); LRMS (ESI-): \textit{m/z} = 403.1 ([M-H]); HRMS (ES): for C\textsubscript{22}H\textsubscript{25}N\textsubscript{6}O\textsubscript{2} [M + H]\textsuperscript{+} \textit{m/z} = 405.2039 (calculated), 405.2032 (found); IR (film): \nu = 3390 (indole NH stretching), 3310 (urea NH stretching); decomposes above 240 °C.
1,1'-(pentane-1,5-diyl)bis(3-(1H-indol-7-yl)urea) 160

Yield: 63 mg (34 %); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta= 10.67\) (s, 2H, NH), 8.32 (s, 2H, NH), 7.29 (t, 2H, J=2.56 Hz, NH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.06 (d, 2H, J=6.95 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.24 (t, 2H, J=5.49 Hz, aromatic CH), 3.16 (q, 4H, J\(_1\)=6.59 Hz, J\(_2\)=6.22 Hz, 2x eq CH\(_2\)), 1.52 (m, 4H, 2x eq CH\(_2\)), 1.38 (m, 2H, CH\(_2\)); \(^13\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta= 155.6\) (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 101.4 (aromatic CH), 29.6 (CH\(_2\)), 23.8 (CH\(_2\)); LRMS (ESI\(^+\)): \(m/z = 419.1\) ([M + H]\(^+\)), 441.2 ([M + Na]\(^+\)); HRMS (ES): for C\(_{23}\)H\(_{27}\)N\(_6\)O\(_2\) [M + H]\(^+\) \(m/z = 419.2195\) (calculated), 419.2190 (found); IR (film): \(\nu= 3390\) (indole NH stretching), 3390 (urea NH stretching); decomposes above 240 °C.

1,1'-(hexane-1,6-diyl)bis(3-(1H-indol-7-yl)urea) 161

Yield: 75 mg (39 %); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta= 10.67\) (s, 2H, NH), 8.31 (s, 2H, NH), 7.29 (t, 2H, J= 2.93 Hz, NH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.06 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.40 (m, 2H, aromatic CH), 6.22 (t, 2H, J=5.49 Hz, aromatic CH), 3.15 (q, 4H, J\(_1\)=6.59 Hz, J\(_2\)=6.22 Hz, 2x eq CH\(_2\)), 1.50 (br m, 4H, 2x eq CH\(_2\)), 1.36 (br m, 4H, 2x eq CH\(_2\)); \(^13\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta= 155.6\) (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.8 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH\(_2\)), 26.2 (CH\(_2\)); LRMS (ESI\(^+\)): \(m/z = 455.2\) ([M + Na]\(^+\)); HRMS (ES): for C\(_{24}\)H\(_{30}\)N\(_6\)O\(_2\) [M + H]\(^+\) \(m/z = 433.2352\) (calculated), 433.2348 (found); IR (film): \(\nu= 3390\) (indole NH stretching), 3310 (urea NH stretching); decomposes above 240 °C.
1,1'-(septane-1,7-diyl)bis(3-(1H-indol-7-yl)urea) 162

Yield: 73 mg (37 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$= 10.67 (br s, 2H, NH), 8.30 (s, 2H, NH), 7.29 (t, 2H, J=2.93 Hz, NH), 7.20 (d, 2H, J=8.05 Hz, aromatic CH), 7.05 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.21 (t, 2H, J=5.85 Hz, aromatic CH), 3.14 (q, 4H, J$^1$= 6.59 Hz, J$^2$=6.22 Hz, 2x eq CH$_2$), 1.49 (m, 4H, 2x eq CH$_3$), 1.34 (br m, 6H, 3 x CH$_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$= 155.6 (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 29.8 (CH$_2$), 28.6 (CH$_2$), 26.4 (CH$_2$); LRMS (ESI+): $m/z$ = 470.3 ([M + Na]$^+$); HRMS (ES+): for C$_{25}$H$_{31}$N$_6$O$_2$ [M + H]$^+$ $m/z$ = calculated 447.2508, found 447.2498; IR (film): v= 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 240 °C.

1,1'-(octane-1,8-diyl)bis(3-(1H-indol-7-yl)urea) 163

Yield: 70 mg (34 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$= 10.67 (br s, 2H, NH), 8.29 (s, 2H, NH), 7.30 (t, 2H, J=2.56 Hz, NH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.04 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.20 (t, 2H, J= 5.85 Hz, aromatic CH), 3.13 (q, 4H, J$^1$=6.59 Hz, J$^2$=5.85 Hz, 2x eq CH$_2$), 1.48 (m, 4H, 2x eq CH$_3$), 1.32 (br m, 8H, 4x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$= 155.6 (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 124.8 (aromatic CH), 119.2 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 108.7 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH$_2$), 28.8 (CH$_2$), 26.40 (CH$_2$); LRMS (ESI+): $m/z$ = 483.2 ( [M + Na]$^+$); HRMS (ES): for C$_{26}$H$_{33}$N$_6$O$_2$ [M + H]$^+$ 461.2665 (calculated), 461.2659 (found); IR (film): v= 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 240 °C.
1,1'-[(nonane-1,9-diyl)bis(3-(1H-indol-7-yl)urea)] 164

Yield: 104 mg (66 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 10.68 (s, 2H, NH), 8.35 (s, 2H, NH), 7.29 (t, 2H, $J$=2.56 Hz, aromatic CH), 7.20 (d, 2H, $J$=7.68 Hz, aromatic CH), 7.07 (d, 2H, $J$=7.32 Hz, aromatic CH), 6.88 (t, 2H, $J$=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.26 (t, 2H, $J$=5.49 Hz, aromatic CH), 3.14 (q, $J$= 6.6 Hz, 4H, 2x eq CH$_2$), 1.46 (br m, 4H, 2x eq CH$_2$), 1.31 (br s, 10 H, 5x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 155.6 (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.715 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH$_2$), 29.1 (CH$_2$), 28.8 (CH$_2$), 26.4 (CH$_2$); LRMS (ESI+): $m/z$ = 497.2 ([M + Na]$^+$); HRMS (ES): for C$_{27}$H$_{34}$N$_6$O$_2$ $m/z$ = [M + H]$^+$ calculated 475.2821, found 475.2820; IR (film): $\nu$ = 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 227 °C.

1,1'-[(decane-1,10-diyl)bis(3-(1H-indol-7-yl)urea)] 165

Yield: 97 mg (60 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 10.67 (s, 2H, NH), 8.35 (s, 2H, NH), 7.29 (t, 2H, $J$=2.56 Hz, aromatic CH), 7.20 (d, 2H, $J$=7.68 Hz, aromatic CH), 7.07 (d, 2H, $J$=7.32 Hz, aromatic CH), 6.88 (t, 2H, $J$=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.26 (t, 2H, $J$=5.49 Hz, aromatic CH), 3.14 (q, $J$= 6.6 Hz, 4H, 2x eq CH$_2$), 1.46 (br m, 4H, 2x eq CH$_2$), 1.31 (br s, 12 H, 6x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 155.6 (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 124.8 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH$_2$), 29.0 (CH$_2$), 28.8 (CH$_2$), 26.4 (CH$_2$); LRMS (ESI+): $m/z$ = 511.2 ([M + Na]$^+$); HRMS (ES): for C$_{28}$H$_{37}$N$_6$O$_2$ $m/z$ = [M + H]$^+$ calculated 489.2978, found 489.2982; IR (film): $\nu$ = 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 225 °C.
Chapter 6 Experimental methods

1,1'-(undecane-1,11-diyl)bis(3-(1H-indol-7-yl)urea) 166

Yield; 137 mg (82%); $^1$H NMR (300 MHz, DMSO-$d_6$): δ = 10.67 (s, 2H, NH), 8.35 (s, 2H, NH), 7.29 (t, 2H, J=2.56 Hz, aromatic CH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.07 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.26 (t, 2H, J=5.49 Hz, aromatic CH), 3.14 (q, J= 6.6 Hz, 4H, 2x eq CH$_2$), 1.46 (br m, 4H, 2x eq CH$_2$), 1.31 (br s, 14 H, 7x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ = 155.6, (CO), 129.1 (aromatic CH), 127.8, (aromatic CH), 125.0 ((aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH$_2$), 29.0 (CH$_2$), 28.8 (CH$_2$), 26.4 (CH$_2$); LRMS (ESI+) m/z = 525.3 ([M + Na]$^+$, 16.99%), 503.3 ([M + H]$^+$, 4.97%); LRMS (ESI-): m/z = 501.7 ([M-H]$^-$); HRMS (ES+): for C$_{29}$H$_{38}$N$_6$O$_2$Na [M + Na]$^+$ m/z = calculated 525.2954, found 525.2957; IR (film): ν = 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 215 °C.

1,1'-(dodecane-1,12-diyl)bis(3-(1H-indol-7-yl)urea) 167

Yield: 125 mg (77%); $^1$H NMR (300 MHz, DMSO-$d_6$): δ = 10.67 (s, 2H, NH), 8.35 (s, 2H, NH), 7.29 (t, 2H, J=2.56 Hz, aromatic CH), 7.20 (d, 2H, J=7.68 Hz, aromatic CH), 7.07 (d, 2H, J=7.32 Hz, aromatic CH), 6.88 (t, 2H, J=7.68 Hz, aromatic CH), 6.39 (m, 2H, aromatic CH), 6.26 (t, 2H, J=5.49 Hz, aromatic CH), 3.14 (q, J= 6.6 Hz, 4H, 2x eq CH$_2$), 1.46 (br m, 4H, 2x eq CH$_2$), 1.31 (br s, 14 H, 7x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ = 155.6 (CO), 129.1 (aromatic CH), 127.8 (aromatic CH), 125.0 (aromatic CH), 119.1 (aromatic CH), 114.5 (aromatic CH), 111.7 (aromatic CH), 101.4 (aromatic CH), 29.8 (CH$_2$), 29.0 (CH$_2$), 28.8 (CH$_2$), 26.4 (CH$_2$); LRMS (ESI-) m/z = 515.2 ([M-H]$^-$); HRMS (ES): for C$_{30}$H$_{41}$N$_6$O$_2$ [M + H]$^+$ m/z = calculated 517.3291, found 517.3281; IR (film): ν = 3390 (indole NH stretching), 3320 (urea NH stretching); decomposes above 221 °C.
6.4.3 Synthesis for chapter 4

The bis-phenylthioureas 177-182 were synthesized according to the same general procedure as follows:

1,1'-(septane-1,7-diyl)bis(3-phenylthiourea) 177

Phenyl isothiocyanate (0.5 g, 3.7 mmol) was dissolved in 75 ml DCM/DMF (1%) and 1, 7-diaminoheptane (241 mg, 1.8 mmol) was added. The mixture was refluxed overnight under nitrogen. On cooling, the mixture was washed with water (150 ml), dilute HCl (100 ml) and sat. NaHCO₃ (aq) (100 ml). The organic layer was dried over MgSO₄ and concentrated to approximately 5 ml in volume. The DCM solution was cooled to -5 ºC to induce recrystallisation. The resulting precipitate was isolated by filtration and washed with cold DCM to give compound 177 as an off white solid.

Yield: 291 mg (39 %); ¹H NMR (300 MHz, DMSO-d₆): δ= 9.41 (br s, 2H, NH), 7.71 (br s, 2H, NH), 7.41 (m, 4H, aromatic CH), 7.31 (m, 4H, aromatic CH), 7.09 (m, 2H, aromatic CH), 3.45 (br m, 4H, CH₂), 1.54 (br m, 4H, CH₂), 1.32 (br m, 6H, CH₂); ¹³C NMR (75 MHz, DMSO-d₆): δ= 180.2 (CS), 139.3 (aromatic CH), 128.5 (aromatic CH), 124.0 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH₂), 28.5 (CH₂), 28.4 (CH₂), 26.4 (CH₂); LRMS(ESI⁺): m/z = 401.2 ([M + H]⁺); HRMS(ES): for C₂₁H₂₉N₄S₂ [M + H]⁺ m/z = 401.1234 (calculated), 401.1813 (found); IR (film): ν= 3240 (NH stretching), 3060 (NH stretching), 2920 (alkyl CH stretching), 2850 (alkyl CH stretching); M_p: 82-85 ºC.
1,1'-(octane-1,8-diyl)bis(3-phenylthiourea) 178

Yield: 268 mg (35 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 9.40 (br s, 1H, NH), 7.71 (br s, 1H, NH), 7.41 (m, 2H, aromatic CH), 7.30 (m, 2H, aromatic CH), 7.09 (m, 1H, aromatic CH), 3.45 (br m, 2H, CH$_2$), 1.54 (br m, 2H, CH$_2$), 1.31 (br m, 4H, 2 x CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 180.2 (CO), 139.3 (aromatic CH), 128.5 (aromatic CH), 124.0 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH$_2$), 28.7 (CH$_2$), 28.4 (CH$_2$), 26.4 (CH$_2$); LRMS(ESI-): $m/z$ = 413.1 ([M - H]); HRMS(ES): for C$_{22}$H$_{31}$N$_4$S$_2$ [M + H]$^+$ $m/z$ = 415.1990 (calculated), 415.1992 (found); IR (film): $\nu$ = 3240 (NH stretching), 3060 (NH stretching), 2920 (alkyl CH stretching), 2850 (alkyl CH stretching); $M_p$: 115-117 °C.

1,1'-(nonane-1,9-diyl)bis(3-phenylthiourea) 179

Yield: 272 mg (35 %); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 9.48 (br s, 2H, NH), 7.77 (br s, 2H, NH), 7.45 (m, 4H, aromatic CH), 7.37 (m, 4H, aromatic CH), 7.15 (m, 2H, aromatic CH), 3.50 (br m, 4H, CH$_2$), 1.59 (br m, 4H, CH$_2$), 1.36 (br m, 10H, CH$_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 180.2 (CS), 139.3 (aromatic CH), 128.5 (aromatic CH), 124.0 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH$_2$), 28.9 (CH$_2$), 28.7 (CH$_2$), 28.4 (CH$_2$), 26.4 (CH$_2$); LRMS(ESI+): $m/z$ = 429.2 ([M + H]$^+$); HRMS(ES): for C$_{23}$H$_{33}$N$_4$S$_2$ [M + H]$^+$ $m/z$ = 429.2147 (calculated), 429.2146 (found); IR (film): $\nu$ = 3250 (NH stretching), 3060 (NH stretching), 2920 (alkyl CH stretching), 2850 (alkyl CH stretching); $M_p$: 82-85 °C.
1,1'-(decane-1,10-diyl)bis(3-phenylthiourea) 180

Yield: 792 mg (98 %); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta = 9.40\) (br s, 2H, NH), 7.70 (br s, 2H, NH), 7.40 (m, 4H, aromatic CH), 7.31 (m, 4H, aromatic CH), 7.09 (m, 2H, aromatic CH), 3.44 (br m, 4H, CH\(_2\)), 1.53 (br m, 4H, CH\(_2\)), 1.29 (br m, 12H, CH\(_2\)); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta = 180.2\) (CS), 139.3 (aromatic CH), 128.5 (aromatic CH), 124.0 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH\(_2\)), 28.9 (CH\(_2\)), 28.7 (CH\(_2\)), 28.4 (CH\(_2\)), 26.4 (CH\(_2\)); LRMS(ESI+): \(m/z = 443.2\) ([M + H]\(^+\)); HRMS(ES): for C\(_{24}\)H\(_{37}\)N\(_4\)S\(_2\) [M + H]\(^+\) \(m/z = 443.2303\) (calculated), 443.2298 (found); IR (film): \(\nu = 3240\) (NH stretching), 3070 (NH stretching), 2920 (alkyl CH stretching), 2850 (alkyl CH stretching); \(M_p\): 91-93 °C.

1,1'-(undecane-1,11-diyl)bis(3-phenylthiourea) 181

Yield: 807 mg (95 %); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta = 9.40\) (br s, 2H, NH), 7.70 (br s, 2H, NH), 7.40 (m, 4H, aromatic CH), 7.31 (m, 4H, aromatic CH), 7.09 (m, 2H, aromatic CH), 3.44 (br m, 4H, CH\(_2\)), 1.53 (br m, 4H, CH\(_2\)), 1.29 (br m, 14H, CH\(_2\)); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta = 180.2\) (CS), 139.3 (aromatic CH), 128.5 (aromatic CH), 124.0 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH\(_2\)), 29.0 (CH\(_2\)), 28.8 (CH\(_2\)), 28.4 (CH\(_2\)), 26.4 (CH\(_2\)); LRMS(ESI+): \(m/z = 457.2\) ([M + H]\(^+\)); HRMS(ES): for C\(_{25}\)H\(_{37}\)N\(_4\)S\(_2\) [M + H]\(^+\) \(m/z = 457.2460\) (calculated), 457.2457 (found); IR (film): \(\nu = 3240\) (NH stretching), 3060 (NH stretching), 2910 (alkyl CH stretching), 2850 (alkyl CH stretching); \(M_p\): 120-124 °C.
1,1'- (dodecane-1,12-diyl) bis(3-phenylthiourea) 182

182 was prepared by the general procedure described above. The reaction mixture was washed with 100 ml water which resulted in precipitation of the product in the organic phase. The precipitate was isolated by filtration and washed with cold DCM to give the product as an off white solid.

Yield: 813 mg (77 %); $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ = 9.40 (br s, 2H, NH), 7.70 (br s, 2H, NH), 7.40 (m, 4H, aromatic CH), 7.31 (m, 4H, aromatic CH), 7.09 (m, 2H, aromatic CH), 3.45 (br m, 4H, CH$_2$), 1.54 (br m, 4H, CH$_2$), 1.28 (br s, 16H, CH$_2$); $^{13}$C NMR (75 MHz, DMSO-d$_6$): $\delta$ = 180.2 (CS), 139.3 (aromatic CH), 128.5 (aromatic CH), 123.96 (aromatic CH), 122.9 (aromatic CH), 43.8 (CH$_2$), 29.0 (CH$_2$), 28.7 (CH$_2$), 28.4 (CH$_2$), 26.4 (CH$_2$); LRMS(ESI+): $m/z$ = 471.3 ([M + H]$^+$); HRMS(ES): for C$_{26}$H$_{39}$N$_4$S$_2$ [M + H]$^+$ $m/z$= 471.2616 (calculated), 471.2621 (found); IR (film): $\nu$ = 3240 (NH stretching), 3070 (NH stretching), 2910 (alkyl CH stretching), 2850 (alkyl CH stretching); $M_p$: 118-120 °C.

1,1'- (octane-1,8-diyl) bis (3-(1H-indol-7-yl)thiourea) 183

7-isothiocyanato-1H-indole$^{69}$ was dissolved in 75 ml DCM/DMF (1%) and 1,8-diaminoctane (111 mg, 7.7 x 10$^{-4}$ mol) was added and the mixture was refluxed overnight. On cooling, the organic layer was washed with 2 x 100 ml water and the organic layer was dried over MgSO$_4$. The DCM solution was concentrated and cooled to -5 °C to induce recrystallization. The resulting precipitate was isolated by filtration and washed with cold DCM. Compound 183 was isolated as an off white solid.

Yield: 170 mg (22 %); $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ = 10.89 (br s, 1H, NH), 9.23 (br s, 1H, NH), 7.43 (m, 2H, aromatic CH), 7.28 (m, 1H, aromatic CH), 6.98 (m, 2H,
aromatic CH), 6.46 (m, 1H, NH), 3.45 (m, 2H, CH

13C NMR (75 MHz, DMSO-d6): \( \delta = 180.6 \) (CO), 131.2 (aromatic CH), 129.4 (aromatic CH), 125.6 (aromatic CH), 123.0 (aromatic CH), 119.0 (aromatic CH), 118.0 (aromatic CH), 101.6 (aromatic CH), 44.3 (CH2), 28.7 (CH2), 28.5 (CH2), 26.4 (CH2); LRMS(ESI-): \( m/z = 491.2 \) ([M - H]); HRMS(ES): for C26H33N6S2 [M + H]\(^+\) \( m/z = 493.2208 \) (calculated), 493.2201 (found); IR (film): \( \nu = 3370 \) (NH stretching), 3340 (NH stretching), 3170, (NH stretching), 2990 (alkyl CH stretching), 2930 (alkyl CH stretching), 2860 (alkyl CH stretching); M\(_p\): 170-172 °C.

1,1'-(octane-1,8-diyl)bis(3-phenylurea)184

Phenyl isocyanate (0.826 g, 6.94 mmol) was dissolved in 75 ml DCM and 1,8-diaminooctane (0.5g, 3.47 mmol) was added. The mixture was stirred for 2 hours under N\(_2\) during which time a white precipitate formed. The precipitate was isolated by filtration and the solid was washed with 10 ml water and 10 ml Et\(_2\)O. Compound 11 was isolated as a white solid (1.36 g, 51% yield).

Yield: 1.36 g (51 %); \(^1\)H NMR (300 MHz, DMSO-d6): \( \delta = 8.34 \) (s, 1H, NH), 7.37 (d, 2H, J= 8.7 Hz, aromatic CH), 7.20 (t, 2H, J= 7.5 Hz, aromatic CH), 6.87 (m, 1H, aromatic CH), 6.09 (t, 1H, J= 5.7 Hz, NH), 3.06 (q, 2H, J= 6.4 Hz, CH2), 1.42 (m, 2H, CH2), 1.29 (br m, 4H, 2 x CH2) \(^13\)C NMR (75 MHz, DMSO-d6): \( \delta = 155.2 \) (CO), 140.6 (aromatic CH), 128.6 (aromatic CH), 120.8 (aromatic CH), 117.5 (aromatic CH), 29.3 (CH2), 28.8 (CH2), 26.3 (CH2); LRMS(ESI-): \( m/z = 381.2 \) ([M - H]); HRMS(ES): for C22H31N4O2 [M + H]\(^+\) \( m/z = 383.2447 \) (calculated), 383.2443 (found); for C22H30N4O2 \( m/z = 405.2267 \) (calculated), 405.2262 (found); IR (film): \( \nu = 3330 \) (NH stretching), 3310, (NH stretching) 2930 (alkyl CH stretching), 2850 (alkyl CH stretching); M\(_p\): 215-217 °C.
References

42. P. A. Gale, Chem. Commun., 2011, 47, 82-86.
References

References

References

References


Appendix

A1 Anion transport studies

In this section, the additional vesicles studies relevant to the results reported in Chapters 2-5 are listed. The vesicles were prepared by the procedure reported in Chapter 6.2. Transport studies were performed using an ISE method monitoring chloride efflux from unilamellar vesicles composed of POPC unless otherwise stated. The Hill analysis is described in Chapter 1.8.7, and the general procedure is outlined in Chapter 2.2.3.
Appendix 1 Anion transport studies

A1.1 Additional transport studies from Chapter 2

A1.1.1 Hill analyses

Figure A1.1 Chloride efflux promoted by various concentrations receptor 123 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.2 Hill plot for the Cl⁻/NO₃⁻ antiport promoted by receptor 123. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

Figure A1.3 Chloride efflux promoted by various concentrations receptor 125 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.4 Hill plot for the Cl/NO₃⁻ antiport promoted by receptor 125. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

Figure A1.5 Chloride efflux promoted by various concentrations receptor 127 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.6 Hill plot for the Cl$^-$/NO$_3^-$ antiport promoted by receptor 127. The data was fitted to the Hill equation using Origin® 8.1.
A1.2 Additional transport studies from Chapter 3

A1.2.1 Mechanistic studies

Figure A1.7 Chloride efflux promoted by receptors 159-167 (4 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.
Appendix 1 Anion transport studies

Figure A1.8 Chloride efflux promoted by receptors 159-167 (4 mol% w.r.t. lipid) from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 20 mM sodium phosphate salts. At $t = 120$ s, a pulse of NaHCO$_3$ was added such that the final HCO$_3^-$ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are $< 5 \%$. 

NaHCO$_3$
A1.2.2 Hill analyses

Receptors which failed to mediate > 50 % total chloride efflux after 270 s at a loading of 4 mol% (w.r.t. lipid) were deemed not active enough to warrant a full Hill analysis.

![Figure A1.9](image)

**Figure A1.9** Chloride efflux promoted by various concentrations receptor 159 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.10 Chloride efflux promoted by various concentrations receptor 160 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.11 Chloride efflux promoted by various concentrations receptor 161 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.12 Chloride efflux promoted by various concentrations receptor 162 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.

Figure A1.13 Hill plot for the Cl⁻/NO₃⁻ antiport promoted by receptor 162. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

Figure A1.14 Chloride efflux promoted by various concentrations receptor 163 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.15 Hill plot for the Cl⁻/NO₃⁻ antiport promoted by receptor 163. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

Figure A1.16 Chloride efflux promoted by various concentrations receptor 164 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.17 Hill plot for the Cl⁻/NO₃⁻ antiport promoted by receptor 164. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

**Figure A1.18** Chloride efflux promoted by various concentrations receptor 165 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO_3_ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.

**Figure A1.19** Hill plot for the Cl/NO_3 <sup>-</sup> antiport promoted by receptor 165. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

Figure A1.20 Chloride efflux promoted by various concentrations receptor 166 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.21 Hill plot for the Cl⁻/NO₃⁻ antiport promoted by receptor 166. The data was fitted to the Hill equation using Origin® 8.1.
Appendix 1 Anion transport studies

**Figure A1.22** Chloride efflux promoted by various concentrations receptor 167 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

**Figure A1.23** Hill plot for the Cl$^-$/NO$_3^-$ antiport promoted by receptor 167. The data was fitted to the Hill equation using Origin® 8.1.
A1.2.3 Mobility assays- cholesterol

**Figure A1.24** Chloride efflux promoted by receptor 159 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

**Figure A1.25** Chloride efflux promoted by receptor 160 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.26 Chloride efflux promoted by receptor 161 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.27 Chloride efflux promoted by receptor 162 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.28 Chloride efflux promoted by receptor 163 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.29 Chloride efflux promoted by receptor 164 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.30 Chloride efflux promoted by receptor 165 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.31 Chloride efflux promoted by receptor 166 (2 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.32 Chloride efflux promoted by receptor 167 (2 mol% w.r.t lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

A1.3 Additional transport studies from Chapter 4

A1.3.1 Mechanistic studies

Figure A1.33 Chloride efflux promoted by receptors 177-183 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na_2SO_4 buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
A1.3.2 Hill analyses: Cl⁻/NO₃⁻ antiport

Figure A1.34 Chloride efflux promoted by various concentrations receptor 177 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.

Figure A1.35 Chloride efflux promoted by various concentrations receptor 178 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.
Appendix 1 Anion transport studies

Figure A1.36 Hill plot for the Cl/NO$_3^-$ antiport promoted by receptor 178. The data was fitted to the Hill equation using Origin$^\text{®}$ 8.1.

![Hill plot for Cl/NO$_3^-$ antiport](image)

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Figure A1.37 Chloride efflux promoted by various concentrations receptor 179 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

![Chloride efflux](image)
Appendix 1 Anion transport studies

Figure A1.38 Hill plot for the Cl/NO$_3^-$ antiport promoted by receptor 179. The data was fitted to the Hill equation using Origin® 8.1.

Figure A1.39 Chloride efflux promoted by various concentrations receptor 180 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %. 
Appendix 1 Anion transport studies

Figure A1.40 Hill plot for the Cl/NO$_3^-$ antiport promoted by receptor 180. The data was fitted to the Hill equation using Origin® 8.1.

Figure A1.41 Chloride efflux promoted by various concentrations receptor 181 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.42 Hill plot for the Cl/NO$_3^-$ antiport promoted by receptor 181. The data was fitted to the Hill equation using Origin® 8.1.

Figure A1.43 Chloride efflux promoted by various concentrations receptor 182 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO$_3$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.44 Hill plot for the Cl/NO₃⁻ antiport promoted by receptor 182. The data was fitted to the Hill equation using Origin® 8.1.

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Figure A1.45 Chloride efflux promoted by various concentrations receptor 183 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.46 Hill plot for the Cl/NO$_3^-$ antiport promoted by receptor 183. The data was fitted to the Hill equation using Origin$^\text{®}$ 8.1.
A1.3.3  Hill analyses: Cl⁻/HCO₃⁻ antiport

**Figure A1.47** Chloride efflux promoted by various concentrations of receptor 177 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

**Figure A1.48** Chloride efflux promoted by various concentrations of receptor 178 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.49 Chloride efflux promoted by various concentrations of receptor 179 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.50 Chloride efflux promoted by various concentrations of receptor 180 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.51 Hill plot for the Cl/HCO₃⁻ antiport promoted by receptor 180. The data was fitted to the Hill equation using Origin® 8.1.

Figure A1.52 Chloride efflux promoted by various concentrations of receptor 181 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100% chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5%.
Appendix 1 Anion transport studies

Figure A1.53 Hill plot for the Cl⁻/HCO₃⁻ antiport promoted by receptor 181. The data was fitted to the Hill equation using Origin® 8.1.

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<th>Model</th>
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<th>Adj. R-Sqr</th>
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<td>n</td>
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Figure A1.54 Chloride efflux promoted by various concentrations of receptor 182 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO₃ was added such that the final HCO₃⁻ concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.55 Hill plot for the Cl/HCO\textsubscript{3} antiport promoted by receptor 182. The data was fitted to the Hill equation using Origin\textsuperscript{®} 8.1.

Figure A1.56 Chloride efflux promoted by various concentrations of receptor 183 from unilamellar POPC vesicles containing 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na\textsubscript{2}SO\textsubscript{4} buffered to pH 7.2 with 20 mM sodium phosphate salts. At t = 120 s, a pulse of NaHCO\textsubscript{3} was added such that the final HCO\textsubscript{3} concentration was 40 mM. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
A1.3.4 Mobility assays - cholesterol

Figure A1.57 Chloride efflux promoted by receptor 177 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na\textsubscript{2}SO\textsubscript{4} buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.58 Chloride efflux promoted by receptor 178 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na\textsubscript{2}SO\textsubscript{4} buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.59 Chloride efflux promoted by receptor 179 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.60 Chloride efflux promoted by receptor 180 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Figure A1.61 Chloride efflux promoted by receptor 181 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.62 Chloride efflux promoted by receptor 182 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
Appendix 1 Anion transport studies

Figure A1.63 Chloride efflux promoted by receptor 183 (1 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
A1.4 Additional transport studies from Chapter 5

A1.4.1 Mobility assay

Figure A1.64 Chloride efflux promoted by receptor 200 (4 mol% w.r.t. lipid) from unilamellar vesicles composed of POPC or POPC:cholesterol (7:3) containing 489 mM NaCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na₂SO₄ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.
A1.4.2 Hill analysis

Figure A1.65 Chloride efflux promoted by various concentrations receptor 200 from unilamellar POPC vesicles containing 489 mM CsCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 167 mM Na$_2$SO$_4$ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.66 Hill plot for the Cs$^+$/Cl$^-$ symport promoted by receptor 200. The data was fitted to the Hill equation using Origin® 8.1.
Figure A1.67 Chloride efflux promoted by various concentrations receptor 200 from unilamellar POPC vesicles containing 489 mM CsCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.68 Hill plot for the chloride efflux promoted by receptor 200 from unilamellar POPC vesicles containing 489 mM CsCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM NaNO₃ buffered to pH 7.2 with 5 mM sodium phosphate salts. The data was fitted to the Hill equation using Origin® 8.1.
Figure A1.69 Chloride efflux promoted by various concentrations receptor 201 from unilamellar POPC vesicles containing 489 mM CsCl buffered to pH 7.2 with 5 mM sodium phosphate salts. The vesicles were suspended in 489 mM Na₂SO₄ buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, the vesicles were lysed to calibrate 100 % chloride efflux. Each point represents the average of 3 trials. All standard deviations are < 5 %.

Figure A1.70 Hill plot for the Cs⁺/Cl⁻ symport promoted by receptor 201. The data was fitted to the Hill equation using Origin® 8.1.
A2  $^1$H NMR titrations

Where reported, anion stability constants have been elucidated by $^1$H NMR titrations with the anions added as the tetrabutylammonium or tetraethylammonium salts in DMSO-$d_6$/H$_2$O 0.5% at 298 K. The resulting data was fitted to a 1:1 or 1:2 binding model using WinEQNMR 2. The resulting titration profiles are reported in this section, with the relevant Job plot analyses used to ascertain the most suitable binding model. Also included are the stack plots from titrations used to investigate potential deprotonation events.
Appendix 2 $^1$H NMR titrations

A2.1 Chapter 3

Figure A2.1 Binding curve from the $^1$H NMR titration of receptor 159 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 biding model using WinEQNMR 2. $K_d = 37$ M$^{-1}$ (0.857).

Figure A2.2 Job plot analysis for the interaction of receptor 159 with TBACl following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.3 Binding curve from the $^1$H NMR titration of receptor 159 with TBANO$_3$ in DMSO-$d_5$/$H_2$O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR. $K_a < 10 \text{ M}^{-1}$. 
Appendix 2 \(^1\)H NMR titrations

**Figure A2.4** Binding curve from the \(^1\)H NMR titration of receptor 159 with TEAHCO\(_3\) in DMSO-\(d_6\)/H\(_2\)O 0.5 \% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 802\ \text{M}^{-1}\) (105.4), \(\beta_2 = 660442\ \text{M}^{-2}\) (20610).

**Figure A2.5** Job plot analysis for the interaction of receptor 159 with TEAHCO\(_3\) following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.6 Binding curve from the $^1$H NMR titration of receptor 159 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 722$ M$^{-1}$ (105.2), $\beta_2 = 752348$ M$^{-2}$ (31800).

Figure A2.7 Job plot analysis for the interaction of receptor 159 with TBAH$_2$PO$_4$ following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.8 Binding curve from the $^1$H NMR titration of receptor 159 with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 > 10^4$ M$^{-1}$, $\beta_2 = 178800$ M$^{-2}$ (40880).

Figure A2.9 Job plot analysis for the interaction of receptor 159 with TBA$_2$SO$_4$ following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.10 Binding curve from the $^1$H NMR titration of receptor 160 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 35 \text{ M}^{-1} \times 0.6944$.

Figure A2.11 Binding curve from the $^1$H NMR titration of receptor 160 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10 \text{ M}^{-1}$. 
Appendix 2 \(^1\)H NMR titrations

Figure A2.12 Binding curve from the \(^1\)H NMR titration of receptor 160 with TEA\(\text{HCO}_3\) in DMSO-\(d_5/\)\(H_2O\) 0.5 % following the urea NH resonance at \(~8.3\) ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 1178\) \(M^{-1}\) (304.4), \(\beta_2 = 1067700\) \(M^{-2}\) (45340).

Figure A2.13 Binding curve from the \(^1\)H NMR titration of receptor 160 with TBA\(\text{H}_2\text{PO}_4\) in DMSO-\(d_5/\)\(H_2O\) 0.5 % following the urea NH resonance at \(~8.3\) ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 1542\) \(M^{-1}\) (131.5), \(\beta_2 = 702727\) \(M^{-2}\) (19250).
Appendix 2 $^1$H NMR titrations

Figure A2.14 Binding curve from the $^1$H NMR titration of receptor 160 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 \% following the urea NH resonance at $\sim$8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_i > 10^4$ M$^{-1}$, $\beta_2 = 567017$ M$^{-2}$ (99590).

Figure A2.15 Binding curve from the $^1$H NMR titration of receptor 161 with TBACl in DMSO-$d_5$/H$_2$O 0.5 \% following the urea NH resonance at $\sim$8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_d = 37$ M$^{-1}$ (1.953).
**Appendix 2** $^1$H NMR titrations

Figure A2.16 Binding curve from the $^1$H NMR titration of receptor 161 with TBANO$_3$ in DMSO-$_d_5$/H$_2$O 0.5 % following the urea NH resonance at $\sim$8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$.

Figure A2.17 Binding curve from the $^1$H NMR titration of receptor 161 with TEAHCO$_3$ in DMSO-$_d_5$/H$_2$O 0.5 % following the urea NH resonance at $\sim$8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_f = 8131$ M$^{-1}$ (176.2), $\beta_2 = 862682$ M$^{-2}$ (45590).
Appendix 2 $^1$H NMR titrations

**Figure A2.18** Binding curve from the $^1$H NMR titration of receptor 161 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_l = 386$ M$^{-1}$ (108.2), $\beta_2 = 627030$ M$^2$ (80940).

**Figure A2.19** Binding curve from the $^1$H NMR titration of receptor 161 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_l = 9768$ M$^{-1}$ (1932), $\beta_2 = 192435$ M$^2$ (48660).
Appendix 2 $^1$H NMR titrations

Figure A2.20 Binding curve from the $^1$H NMR titration of receptor 162 with TBACl in DMSO-$d_6$/ H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 32$ M$^{-1}$ (0.8869).

Figure A2.21 Binding curve from the $^1$H NMR titration of receptor 162 with TBANO$_3$ in DMSO-$d_6$/ H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 $^1$H NMR titrations

**Figure A2.22** Binding curve from the $^1$H NMR titration of receptor 162 with TEAHCO$_3$ in DMSO-$_d$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 584$ M$^{-1}$ (61.44), $\beta_2 = 465949$ M$^2$ (16290).

![Figure A2.22](image1.png)

**Figure A2.23** Binding curve from the $^1$H NMR titration of receptor 162 with TBAH$_2$PO$_4$ in DMSO-$_d$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 341$ M$^{-1}$ (66.77), $\beta_2 = 789322$ M$^2$ (17010).

![Figure A2.23](image2.png)
Figure A2.24 Binding curve from the $^1$H NMR titration of receptor 162 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 7170$ M$^{-1}$ (1216), $\beta_2 = 512992$ M$^{-2}$ (59230).

Figure A2.25 Binding curve from the $^1$H NMR titration of receptor 163 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 39$ M$^{-1}$ (1.806).
Appendix 2 $^1$H NMR titrations

Figure A2.26 Binding curve from the $^1$H NMR titration of receptor 163 with TBANO$_3$ in DMSO-$d_5$/$H_2O$ 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$.

Figure A2.27 Binding curve from the $^1$H NMR titration of receptor 163 with TEAHCO$_3$ in DMSO-$d_5$/$H_2O$ 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 1101$ M$^{-1}$ (169), $\beta_2 = 837798$ M$^{-2}$ (35830).
Appendix 2 $^1$H NMR titrations

Figure A2.28 Binding curve from the $^1$H NMR titration of receptor 163 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 420 \text{ M}^{-1} (99.67), \beta_2 = 542492 \text{ M}^2 (27860)$.

Figure A2.29 Binding curve from the $^1$H NMR titration of receptor 163 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 8560 \text{ M}^{-1} (1125), \beta_2 = 623547 \text{ M}^2 (36340)$. 
Appendix 2 $^1$H NMR titrations

Figure A2.30 Binding curve from the $^1$H NMR titration of receptor 164 with TBACl in DMSO-d$_5$ / H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 38$ M$^{-1}$ (1.309).

Figure A2.31 Binding curve from the $^1$H NMR titration of receptor 164 with TBANO$_3$ in DMSO-d$_5$ / H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 $^1$H NMR titrations

Figure A2.32 Binding curve from the $^1$H NMR titration of receptor 164 with TEA$\text{HCO}_3$ in DMSO-$d_5$/$\text{H}_2\text{O}$ 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 560 \text{ M}^{-1} (60.5)$, $\beta_2 = 583357 \text{ M}^2 (21680)$.

Figure A2.33 Binding curve from the $^1$H NMR titration of receptor 164 with TBA$\text{H}_2\text{PO}_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 572 \text{ M}^{-1} (103.6)$, $\beta_2 = 585002 \text{ M}^2 (32430)$. 
Appendix 2 $^1H$ NMR titrations

Figure A2.34 Binding curve from the $^1H$ NMR titration of receptor 164 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_i = 3280$ M$^{-1}$ (643.9), $\beta_2 = 360871$ M$^{-2}$ (45020).

Figure A2.35 Binding curve from the $^1H$ NMR titration of receptor 165 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 37$ M$^{-1}$ (0.6931).
Appendix 2 $^1$H NMR titrations

**Figure A2.36** Binding curve from the $^1$H NMR titration of receptor 165 with TBANO$_3$ in DMSO-$d_6$/H$_2$O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$.

**Figure A2.37** Binding curve from the $^1$H NMR titration of receptor 165 with TEAHC$_3$ in DMSO-$d_6$/H$_2$O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_f = 1328$ M$^{-1}$ (283.6), $\beta_2 = 870364$ M$^{-2}$ (46370).
Appendix 2 \(^1\)H NMR titrations

**Figure A2.38** Binding curve from the \(^1\)H NMR titration of receptor 165 with TBAH\(_2\)PO\(_4\) in DMSO-\(d_5\)/H\(_2\)O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 631 \text{ M}^{-1} (65.27), \beta_2 = 52739 \text{ M}^2 (14270).\)

**Figure A2.39** Binding curve from the \(^1\)H NMR titration of receptor 165 with TBA\(_2\)SO\(_4\) in DMSO-\(d_5\)/H\(_2\)O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 3320 \text{ M}^{-1} (651.3), \beta_2 = 411641 \text{ M}^2 (66940).\)
Appendix 2 $^1$H NMR titrations

Figure A2.40 Binding curve from the $^1$H NMR titration of receptor 166 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 biding model using WinEQNMR 2. $K_a = 32$ M$^{-1}$ (0.5787).

Figure A2.41 Binding curve from the $^1$H NMR titration of receptor 166 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 biding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 $^1$H NMR titrations

**Figure A2.42** Binding curve from the $^1$H NMR titration of receptor 166 with TEAHCO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 290$ M$^{-1}$ (28.62), $\beta_2 = 246354$ M$^{-2}$ (14300).

**Figure A2.43** Binding curve from the $^1$H NMR titration of receptor 166 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 508$ M$^{-1}$ (118), $\beta_2 = 206422$ M$^{-2}$ (22410).
Appendix 2 $^1$H NMR titrations

Figure A2.44 Binding curve from the $^1$H NMR titration of receptor 166 with TBA$_2$SO$_4$ in DMSO-$d_6$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 1267$ M$^{-1}$ (345.3), $\beta_2 = 207037$ M$^{-2}$ (47340).
Appendix 2 $^1$H NMR titrations

Figure A2.45 Binding curve from the $^1$H NMR titration of receptor 167 with TBACl in DMSO-$d_6$/H$_2$O 0.5% following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 44$ M$^{-1}$ (1.097).

Figure A2.46 Job plot analysis for the interaction of receptor 167 with TBACl following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.47 Binding curve from the $^1$H NMR titration of receptor 167 with TBANO in DMSO-$d_6$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Figure A2.48 Binding curve from the $^1$H NMR titration of receptor 167 with TEAHCO$_3$ in DMSO-$d_5/$H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_i = 1026$ M$^{-1}$ (708.1), $\beta_2 = 740068$ M$^{-2}$ (12680).

Figure A2.49 Job plot analysis for the interaction of receptor 167 with TEAHCO$_3$ following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.50 Binding curve from the $^1$H NMR titration of receptor 167 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~ 8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 896$ M$^{-1}$ (79.49), $\beta_2 = 447317$ M$^{-2}$ (21800).

Figure A2.51 Job plot analysis for the interaction of receptor 167 with TBAH$_2$PO$_4$ following the urea NH resonance at ~ 8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.52 Binding curve from the $^1$H NMR titration of receptor 167 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the urea NH resonance at ~8.3 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_i = 2296 \text{ M}^{-1} (202), \beta_2 = 407788 \text{ M}^{-2} (82750)$.

Figure A2.53 Job plot analysis for the interaction of receptor 167 with TBA$_2$SO$_4$ following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

A2.2 Chapter 4

Figure A2.54 Binding curve from the $^1$H NMR titration of receptor 177 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 16$ M$^{-1}$ (0.8447).

Figure A2.55 Binding curve from the $^1$H NMR titration of receptor 177 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 ¹H NMR titrations

Figure A2.56 Binding curve from the ¹H NMR titration of receptor 177 with TEAHCO₃ in DMSO-d₅/H₂O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. β₁ = K₁ = 384 M⁻¹ (104.6), β₂ = 159015 M⁻² (11340).

Figure A2.57 Binding curve from the ¹H NMR titration of receptor 177 with TBAH₂PO₄ in DMSO-d₅/H₂O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. β₁ = K₁ = 718 M⁻¹ (53.63), β₂ = 196699 M⁻² (19920).
Appendix 2 $^1$H NMR titrations

Figure A2.58 Binding curve from the $^1$H NMR titration of receptor 177 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 715$ M$^{-1}$ (24.31), $\beta_2 = 184464$ M$^2$ (61910).

Figure A2.59 Binding curve from the $^1$H NMR titration of receptor 177 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 4612$ M$^{-1}$ (139.7), $\beta_2 = 124601$ M$^2$ (76010).
Appendix 2 $^1$H NMR titrations

Figure A2.60 Binding curve from the $^1$H NMR titration of receptor 177 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 1293$ M$^{-1}$ (87.8).

Figure A2.61 Binding curve from the $^1$H NMR titration of receptor 178 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 15$ M$^{-1}$ (1.209).
Appendix 2 $^1$H NMR titrations

**Figure A2.62** Binding curve from the $^1$H NMR titration of receptor 178 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$.

**Figure A2.63** Binding curve from the $^1$H NMR titration of receptor 178 with TEAHCO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_f = 1280$ M$^{-1}$ (170.2), $\beta_2 = 232547$ M$^2$ (10900).
Appendix 2 $^1H$ NMR titrations

Figure A2.64 Binding curve from the $^1H$ NMR titration of receptor 178 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 806 \text{ M}^{-1}$ (75.17), $\beta_2 = 167388 \text{ M}^2$ (11170).

Figure A2.65 Binding curve from the $^1H$ NMR titration of receptor 178 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 716 \text{ M}^{-1}$ (37.01), $\beta_2 = 155321 \text{ M}^2$ (25230).
Appendix 2 $^1$H NMR titrations

Figure A2.66 Binding curve from the $^1$H NMR titration of receptor 178 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 5248$ M$^{-1}$ (163.8), $\beta_2 = 191265$ M$^2$ (97750).

Figure A2.67 Binding curve from the $^1$H NMR titration of receptor 178 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_0 = 3379$ M$^{-1}$ (487.1).
Appendix 2 $^1$H NMR titrations

**Figure A2.68** Binding curve from the $^1$H NMR titration of receptor 179 with TBACl in DMSO-$d_5$/H$_2$O 0.5% following the thiourea NH resonance at $\sim$7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 10 \text{ M}^{-1}$ (0.512).

**Figure A2.69** Binding curve from the $^1$H NMR titration of receptor 179 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5% following the thiourea NH resonance at $\sim$7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10 \text{ M}^{-1}$. 
Appendix 2 $^1$H NMR titrations

**Figure A2.70** Binding curve from the $^1$H NMR titration of receptor 179 with TEAHCO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_f = 114 \text{ M}^{-1} (20.65)$, $\beta_2 = 21730 \text{ M}^2 (491)$.

**Figure A2.71** Binding curve from the $^1$H NMR titration of receptor 179 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_f = 157 \text{ M}^{-1} (64.53)$, $\beta_2 = 34612 \text{ M}^2 (11710)$. 
Figure A2.72 Binding curve from the $^1$H NMR titration of receptor 179 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 346$ M$^{-1}$ (37.69), $\beta_2 = 88311$ M$^{-2}$ (521.4).
Appendix 2 $^1$H NMR titrations

**Figure A2.73** Binding curve from the $^1$H NMR titration of receptor 179 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 6339 \text{ M}^{-1} (1119), \beta_2 = 444065 \text{ M}^{-2} (48400)$.

**Figure A2.74** Binding curve from the $^1$H NMR titration of receptor 179 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_v = 2091 \text{ M}^{-1} (273.5)$. 
Figure A2.75 Binding curve from the $^1$H NMR titration of receptor 180 with TBACl in DMSO-$d_6$/H$_2$O 0.5% following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 11 \text{ M}^{-1}$ (0.5375).

Figure A2.76 Job plot analysis for the interaction of receptor 180 with TBACl following the thiourea NH resonance at ~7.7 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.77 Binding curve from the $^1$H NMR titration of receptor 180 with TBANO in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_d < 10$ M$^{-1}$. 

\[ \text{Concentration, M} \]
Appendix 2 $^1H$ NMR titrations

Figure A2.78 Binding curve from the $^1H$ NMR titration of receptor 180 with TEAHCO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 264 \text{ M}^{-1}$ (4.811), $\beta_2 = 59925 \text{ M}^{-2}$ (430.2).

Figure A2.79 Job plot analysis for the interaction of receptor 170 with TEAHCO$_3$ following the aromatic CH resonance at ~7.4 ppm.
Appendix 2 \(^1\)H NMR titrations

**Figure A2.80** Binding curve from the \(^1\)H NMR titration of receptor 180 with TBAH\(_2\)PO\(_4\) in DMSO-\(_d_5\)/H\(_2\)O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_j = 343\) M\(^{-1}\) (23.34), \(\beta_2 = 87795\) M\(^2\) (591.9).

**Figure A2.81** Binding curve from the \(^1\)H NMR titration of receptor 180 with TBAH\(_2\)PO\(_4\) in DMSO-\(_d_5\)/H\(_2\)O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_j = 308\) M\(^{-1}\) (38.7), \(\beta_2 = 96231\) M\(^2\) (3777).

**Figure A2.82** Job plot analysis for the interaction of receptor 180 with TBAH\(_2\)PO\(_4\) following the thiourea NH resonance at ~7.7 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.83 Binding curve from the $^1$H NMR titration of receptor 180 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 3195$ M$^{-1}$ (766.9), $\beta_2 = 122175$ M$^{-2}$ (73710).

Figure A2.84 Binding curve from the $^1$H NMR titration of receptor 180 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 1764$ M$^{-1}$ (65.37).

Figure A2.85 Job plot analysis for the interaction of receptor 180 with TBA$_2$SO$_4$ following the urea NH resonance at ~8.3 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.86 Binding curve from the $^1$H NMR titration of receptor 181 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 16 \text{ M}^{-1} (1.182)$.

Figure A2.87 Binding curve from the $^1$H NMR titration of receptor 181 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10 \text{ M}^{-1}$. 
Appendix 2 $^1$H NMR titrations

Figure A2.88 Binding curve from the $^1$H NMR titration of receptor 181 with TEAHCO$_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 189$ M$^{-1}$ (45.47), $\beta_2 = 54817$ M$^{-2}$ (4834).
Appendix 2 \( ^1H \) NMR titrations

**Figure A2.89** Binding curve from the \( ^1H \) NMR titration of receptor 181 with TBAH\(_2\)PO\(_4\) in DMSO-\(d_5\)/H\(_2\)O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \( \beta_1 = K_1 = 458 \text{ M}^{-1} \) (44.48), \( \beta_2 = 50590 \text{ M}^2 \) (7370).

**Figure A2.90** Binding curve from the \( ^1H \) NMR titration of receptor 181 with TBAH\(_2\)PO\(_4\) in DMSO-\(d_5\)/H\(_2\)O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \( \beta_1 = K_1 = 516 \text{ M}^{-1} \) (75.21), \( \beta_2 = 105518 \text{ M}^2 \) (7700).
Appendix 2 $^1$H NMR titrations

Figure A2.91 Binding curve from the $^1$H NMR titration of receptor 181 with TBA$_2$SO$_4$ in DMSO-$_d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 5179$ M$^{-1}$ (208.9), $\beta_2 = 316833$ M$^{-2}$ (34190).

Figure A2.92 Binding curve from the $^1$H NMR titration of receptor 181 with TBA$_2$SO$_4$ in DMSO-$_d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 2593$ M$^{-1}$ (300.1).
Appendix 2 $^1$H NMR titrations

Figure A2.93 Binding curve from the $^1$H NMR titration of receptor 182 with TBACl in DMSO-$d_5$/H$_2$O 0.5% following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 11$ M$^{-1}$ (0.6014).

Figure A2.94 Binding curve from the $^1$H NMR titration of receptor 182 with TBANO$_3$ in DMSO-$d_5$/H$_2$O 0.5% following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 $^1$H NMR titrations

Figure A2.95 Binding curve from the $^1$H NMR titration of receptor 182 with TEAHC$\text{O}_3$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 247$ M$^{-1}$ (31.26), $\beta_2 = 33963$ M$^{-2}$ (3818).

Figure A2.96 Binding curve from the $^1$H NMR titration of receptor 182 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 676$ M$^{-1}$ (153.5), $\beta_2 = 69062$ M$^{-2}$ (11810).
Appendix 2 $^1$H NMR titrations

Figure A2.97 Binding curve from the $^1$H NMR titration of receptor 182 with TBAH$_2$PO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 473$ M$^{-1}$ (21.05), $\beta_2 = 48132$ M$^{-2}$ (2978).

Figure A2.98 Binding curve from the $^1$H NMR titration of receptor 182 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.7 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 3427$ M$^{-1}$ (541), $\beta_2 = 308496$ M$^{-2}$ (45540).
Figure A2.99 Binding curve from the $^1$H NMR titration of receptor 182 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the aromatic CH doublet centered at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_d = 1525$ M$^{-1}$ (161.5).
Appendix 2 $^1$H NMR titrations

Figure A2.100 Binding curve from the $^1$H NMR titration of receptor 183 with TBACl in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a = 17$ M$^{-1}$ (0.8565).

Figure A2.101 Job plot analysis for the interaction of receptor 183 with TBACl following the thiourea NH resonance at ~7.4 ppm.
Figure A2.102 Binding curve from the $^1$H NMR titration of receptor 183 with TBANO$_3$ in DMSO-$d_6$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$. 
Appendix 2 $^1$H NMR titrations

Figure A2.103 Binding curve from the $^1$H NMR titration of receptor 183 with TEAHCO$_3$ in DMSO-$d_6$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNM 2. $\beta_1 = K_1 = 2907$ M$^{-1}$ (252.8), $\beta_2 = 982512$ M$^2$ (33830).

Figure A2.104 Job plot analysis for the interaction of receptor 183 with TEAHCO$_3$ following the thiourea NH resonance at ~7.4 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.105 Binding curve from the $^1$H NMR titration of receptor 183 with TBAH$_2$PO$_4$ in DMSO-$d_5$ / H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 2281$ M$^{-1}$ (167.7), $\beta_2 = 785442$ M$^{2}$ (19430).

Figure A2.106 Job plot analysis for the interaction of receptor 183 with TBAH$_2$PO$_4$ following the thiourea NH resonance at ~7.4 ppm.
Appendix 2 $^1$H NMR titrations

**Figure A2.107** Binding curve from the $^1$H NMR titration of receptor 183 with TBA$_2$SO$_4$ in DMSO-$d_6$ / H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 5632 M^{-1} (392.4)$, $\beta_2 = 1219980 M^{-2} (107300)$.

**Figure A2.108** Job plot analysis for the interaction of receptor 183 with TBA$_2$SO$_4$ following the thiourea NH resonance at ~7.4 ppm.
Appendix 2 \(^1\)H NMR titrations

Figure A2.109 Binding curve from the \(^1\)H NMR titration of receptor 184 with TBACl in DMSO-\(d_6\)/H\(_2\)O 0.5 % following the urea NH resonance at ~6.1 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. \(K_a = 14\) M\(^{-1}\) (0.3389).

Figure A2.110 Job plot analysis for the interaction of receptor 184 with TBACl following the thiourea NH resonance at ~6.1 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.111 Binding curve from the $^1$H NMR titration of receptor 184 with TBANO$_3$ in DMSO-$d_6$/H$_2$O 0.5 % following the urea NH resonance at ~6.1 ppm. The data was fitted to a 1:1 binding model using WinEQNMR 2. $K_a < 10$ M$^{-1}$.
Appendix 2 \(^1\)H NMR titrations

**Figure A2.112** Binding curve from the \(^1\)H NMR titration of receptor 184 with TEA-HCO\(_3\) in DMSO-\(d_5\)/H\(_2\)O 0.5 % following the urea NH resonance at ~6.1 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. \(\beta_1 = K_1 = 3882 \text{ M}^{-1} \) (345.7), \(\beta_2 = 215025 \text{ M}^{-2}\) (18020).

**Figure A2.113** Job plot analysis for the interaction of receptor 184 with TEA-HCO\(_3\) following the thiourea NH resonance at ~6.1 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.114 Binding curve from the $^1$H NMR titration of receptor 184 with TBAH$_2$PO$_4$ in DMSO-$d_6$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_1 = 3584$ M$^{-1}$ (239.7), $\beta_2 = 4832$ M$^{-2}$ (13770).

Figure A2.115 Job plot analysis for the interaction of receptor 184 with TBAH$_2$PO$_4$ following the thiourea NH resonance at ~6.1 ppm.
Appendix 2 $^1$H NMR titrations

Figure A2.116 Binding curve from the $^1$H NMR titration of receptor 184 with TBA$_2$SO$_4$ in DMSO-$d_5$/H$_2$O 0.5 % following the thiourea NH resonance at ~7.4 ppm. The data was fitted to a 1:2 binding model using WinEQNMR 2. $\beta_1 = K_i > 10^4$ M$^{-1}$, $\beta_2 = 419700$ M$^{-2}$ (44060).

Figure A2.117 Job plot analysis for the interaction of receptor 184 with TBA$_2$SO$_4$ following the thiourea NH resonance at ~6.1 ppm.
Appendix 2 $^1$H NMR titrations

A2.3 Stack plots

A2.3.1 Chapter 3

Figure A2.118 Stack plot showing the titration of receptor 161 with TBAH$_2$PO$_4$ in DMSO-$d_6$/H$_2$O 0.5 %.

Figure A2.119 Stack plot showing the titration of receptor 161 with up to 1.1 eq. TBAH$_2$PO$_4$ in DMSO-$d_6$/H$_2$O 0.5 % followed by the addition of TBAOH (1 M in MeOH).
Appendix 2 $^1$H NMR titrations

A2.3.2 Stack plots from Chapter 4

Figure A2.120 Stack plot showing the titration of receptor 180 with TEAHCO$_3$ in DMSO-$d_6$/H$_2$O 0.5%.

Figure A2.121 Stack plot showing the titration of receptor 180 with TBAOH (1 M in MeOH) in DMSO-$d_6$/H$_2$O 0.5%.
Appendix 2 $^1$H NMR titrations

Figure A2.122 Stack plot showing the titration of receptor 183 with TEAHCO$_3$ in DMSO-$d_6$/H$_2$O 0.5 %.

Receptor + 2.5 eq TBAOH

Receptor + 2.0 eq TBAOH

Receptor + 1.5 eq TBAOH

Receptor + 1.0 eq TBAOH

Receptor + 0.5 eq TBAOH

Receptor

Figure A2.123 Stack plot showing the titration of receptor 183 with TBAOH (1 M in MeOH) in DMSO-$d_6$/H$_2$O 0.5 %.
Appendix 3 X-ray crystal structure data

A3  X-ray Crystal Structure Data

The crystal structures presented in Chapters 2 and 3 were solved by the EPSRC National Crystallography Service (Dr M. E. Light). The refinement of the structures and the fractional coordinates are reported for the sake of completeness and so that the structure may be regenerated from the text of this thesis if necessary.
## A3.1 Chapter 2

### A3.1.1 Receptor 127

**Table A3.1** Crystal data and structure refinement details.

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<td>0.592 and $-0.314$ e Å$^{-3}$</td>
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</table>


**Special details:** All hydrogens were identified in the difference map and subsequently placed in idealised positions and refined using a riding model. An unidentified (probably Et$_2$O) disordered solvent is present in channels running along the c direction. This was treated using the Squeeze algorithm (SQUEEZE - Sluis, P. v.d. & Spek, A. L. (1990) Acta Cryst. A46, 194-201.). This has left a void of 252.00 Å$^3$. 
Table A3.2 Atomic coordinates \([\times 10^4]\), equivalent isotropic displacement parameters \([\text{Å}^2 \times 10^3]\) and site occupancy factors. \(U_{eq}\) is defined as one third of the trace of the orthogonalized \(U_{ij}\) tensor.

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<th>(y)</th>
<th>(z)</th>
<th>(U_{eq})</th>
<th>S.o.f.</th>
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Appendix 3 X-ray crystal structure data

Chapter 3

Receptor 161 (TBAH₂PO₄ complex)

Table A3.3 Crystal data and structure refinement details.

<table>
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<tr>
<th>Identification code</th>
<th>2011sot0537(CHC6 TBAH2PO4)</th>
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<tr>
<td>Empirical formula</td>
<td>C₅₆H₁₀₄N₈O₁₀P₂</td>
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<tr>
<td></td>
<td>C₂₂H₃₈N₆O₂₂, 2(C₁₆H₁₆N), 2(H₂PO₄)</td>
</tr>
<tr>
<td>Formula weight</td>
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<tr>
<td>Temperature</td>
<td>120(2) K</td>
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<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
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<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>C2/c</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 19.3998(17) Å</td>
</tr>
<tr>
<td></td>
<td>b = 20.670(2) Å</td>
</tr>
<tr>
<td></td>
<td>c = 16.7096(11) Å</td>
</tr>
<tr>
<td>Volume</td>
<td>6211.3(9) Å³</td>
</tr>
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<td>Z</td>
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<tr>
<td>Density (calculated)</td>
<td>1.188 Mg / m³</td>
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<tr>
<td>Absorption coefficient</td>
<td>0.129 mm⁻¹</td>
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<tr>
<td>F(000)</td>
<td>2424</td>
</tr>
<tr>
<td>Crystal size</td>
<td>Block; Colourless</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.25 × 0.20 × 0.20 mm³</td>
</tr>
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<td>θ range for data collection</td>
<td>3.00 – 25.03°</td>
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</tr>
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<td>Max. and min. transmission</td>
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<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
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<td>Data / restraints / parameters</td>
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</tr>
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<td>0.428 and −0.319 e Å⁻³</td>
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Special details: All hydrogen atoms were identified in the difference map, the torsion angle of CH₃ and O-H groups was allowed to refine. Due to symmetry constraints the occupancies of H2B and H3A were fixed at 0.5
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### Table A3.5. Hydrogen bonds [Å and °].

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<th>$d(H⋯A)$</th>
<th>$d(D⋯A)$</th>
<th>$\angle(DH4)$</th>
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Symmetry transformations used to generate equivalent atoms:

(i) $-x,y,-z+1/2$  (ii) $-x,-y+1,-z+1$
A3.2.2 Receptor 159 (Potassium oxalate/18-crown-6 complex)

Table A3.6 Crystal data and structure refinement details.

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<th>Value</th>
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</tr>
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<td>Formula weight</td>
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<td>Temperature</td>
<td>120(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.68890 Å</td>
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<td>Crystal system</td>
<td>Monoclinic</td>
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<td>Space group</td>
<td>P21/n</td>
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</tr>
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<td>Volume</td>
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<td>Z</td>
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<td>Density (calculated)</td>
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<td>-27 ≤ h ≤ 25, -6 ≤ k ≤ 8, -37 ≤ l ≤ 20</td>
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<tr>
<td>Reflections collected</td>
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<tr>
<td>Independent reflections</td>
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<td>Completeness to θ = 24.30°</td>
<td>92.0 %</td>
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<td>Absorption correction</td>
<td>Semi–empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>1.000 and 0.277</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
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<tr>
<td>Data / restraints / parameters</td>
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<tr>
<td>Goodness-of-fit on F²</td>
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<td>Final R indices [F² &gt; 2α(F²)]</td>
<td>R1 = 0.1183, wR2 = 0.2544</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.3426, wR2 = 0.4094</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.368 and -0.404 e Å⁻³</td>
</tr>
</tbody>
</table>


Special details: All hydrogen atoms were placed in idealised positions and refined using a riding model.
Appendix 3 X-ray crystal structure data

Table A3.7 Atomic coordinates \([\times 10^4]\), equivalent isotropic displacement parameters \([\text{Å}^2 \times 10^3]\) and site occupancy factors. \(U_{eq}\) is defined as one third of the trace of the orthogonalized \(U^t\) tensor.

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Appendix 3 X-ray crystal structure data

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Symmetry transformations used to generate equivalent atoms:
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(iv) x,y−1,z  (v) −x,−y+1,−z+1