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From Anion Receptors to Transporters

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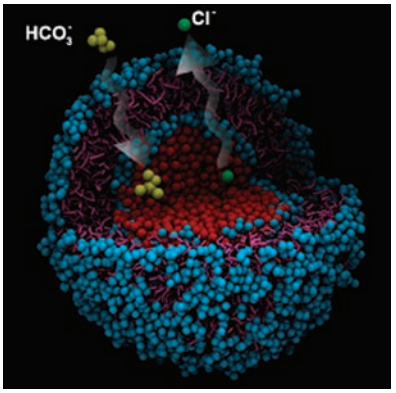
CONSPECTUS

Cystic fibrosis is the most well-known of a variety of diseases termed channelopathies, in which the regulation of ion transport across cell membranes is so disrupted that the threshold of a pathology is passed. The human toll exacted by these diseases has led a number of research groups, including our own, to create compounds that mediate ion transport across lipid bilayers.



In this Account, we discuss three classes of synthetic compounds that were refined to bind and transport anions across lipid bilayer membranes. All of the compounds were originally designed as anion receptors, that is, species that would simply create stable complexes with anions, but were then further developed as transporters. By studying structurally simple systems and varying their properties to change the degree of preorganization, the affinity for anions, or the lipophilicity, we have begun to rationalize why particular anion transport mechanisms (cotransport or antiport processes) occur in particular cases. For example, we have studied the chloride transport properties of receptors based on the closely related structures of isophthalamide and pyridine-2,6-dicarboxamide: the central ring in each case was augmented with pendant methylimidazole groups designed to cotransport H^+ and Cl^-. We observed that the more preorganized pyridine-based receptor was the more efficient transporter, a finding replicated with a series of isophthalamides in which one contained hydroxyl groups designed to preorganize the receptor. This latter class of compound, together with the natural product prodigiosin, can transport bicarbonate (as part of a chloride/bicarbonate antiport process) across lipid bilayer membranes.

We have also studied the membrane transport properties of calix[4]pyrroles. Although the parent *meso*-octamethylcalix[4]-pyrrole functions solely as a Cs^+/Cl^- cotransporter, other compounds with increased anion affinities can function through an antiport process. One example is octafluoro-*meso*-octamethylcalix[4]pyrrole; with its electron-withdrawing substituents, it can operate through a chloride/bicarbonate antiport process. Moreover, calix[4]pyrroles with additional hydrogen bond donors can operate through a chloride/nitrate antiport process. Thus, increasing the affinity of the receptor in these cases allows the compound to transport an anion in the absence of a cation.

Finally, we have studied the transport properties of simple thioureas and shown that these compounds are highly potent chloride/bicarbonate antiport agents that function at low concentrations. In contrast, the urea analogues are inactive. The higher hydrophobicity (reflected in higher values for the logarithm of the water−octanol partition constant, or log *P*) and lower polar surface areas of the thiourea compounds compared to their urea analogues may provide a clue to the high potency of these compounds. This observation might serve as a basis for designing future small-molecule transporters.

1. Introduction

Anion complexation chemistry has developed significantly in the last 20 years.[1,2] The anion complexation community can now design neutral receptors to complex anions in aqueous solution, sensors that can be used to image phosphates in vivo and compounds that can selectively bind and crystallize one anion from a complex mixture of anions.[3] An important application of anion receptor chemistry is the development of compounds that can bind and transport anions across lipid bilayer membranes.[4−7] This is because a variety of diseases or "channelopathies", most notably cystic fibrosis, arise when anion transport across biological membranes is disrupted.[8] A number of research groups have made great progress in this area and have

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developed a range of compounds that function as discrete molecular carriers or as synthetic channels allowing anions to pass through a lipid bilayer. However, it is true to say that our understanding of how to design an anion transporter is not yet as fully developed as our understanding of how to design a selective anion receptor. Here we show how our work on anion receptor design[9] has allowed us to develop several new series of membrane transport agents for anions and to rationalize why a particular anion transport mechanism is employed. Rather than synthesizing compounds with increasing complexity, we have limited our studies to compounds that are relatively structurally simple, allowing us to systematically vary the properties of these compounds and hence gain an insight into the molecular properties that are key for the production of an efficient transporter.

2. Isophthalamides and Analogues: The Role of Preorganization

The prodigiosins (e.g., **1**−**3**) are a class of natural products that have been shown to possess HCl cotransport and chloride/nitrate antiport properties.[10−12] They have attracted much attention recently because of their range of biological activity from functioning as proton-pump inhibitors to triggering apoptosis.[12] In 2005, in collaboration with Bradley D. Smith at the University of Notre Dame, we reported the synthesis and anion transport properties of compound **4**.[13] Like prodigiosin, the compound contains two hydrogen bond donors and a basic site, and it was designed to bind HCl by protonation of the methylimidazole ring and then subsequent formation of a complex with chloride via the formation of three hydrogen bonds and an electrostatic interaction. We demonstrated that compound **4** transports chloride across a lipid bilayer membrane composed of a mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)/cholesterol (7:3 molar ratio) via a combination of HCl cotransport and chloride/nitrate antiport. In a continuation of the collaboration, we wished to simplify the design of this receptor but retain the membrane transport properties. Isophthalamides and pyridine-2,6-dicarboxamides have been employed in a variety of receptors for charged and neutral species.[14,15] We designed and synthesized compounds **5** and **6** based on the pyridine-2,6-dicarboxamide and isophthalamide skeletons and compared their transport properties to model compound **7**.[16] Like compound **4**, compounds **5** and **6** both contain two hydrogen bond donors and a protonatable imidazole ring while the model compound **7** lacks the imidazole group. The X-ray crystal structure of the HCl complex of compound **5** is shown in Figure 1.

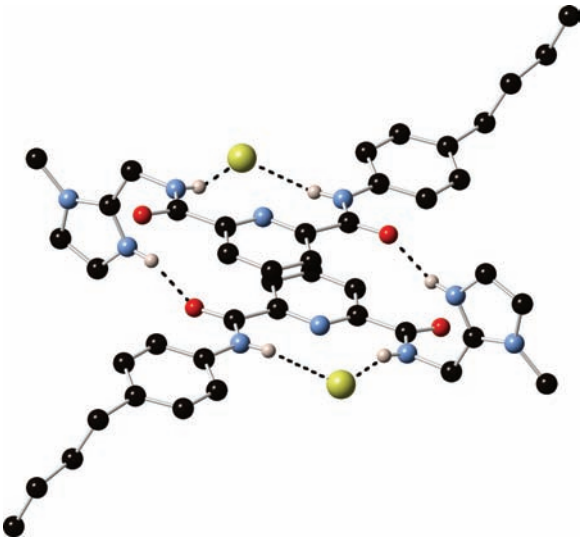


FIGURE 1. X-ray crystal structure of the HCl complex of receptor **5** illustrating the formation of a dimeric complex in the solid state.

The membrane transport properties of these compounds were investigated using POPC/cholesterol vesicles (7:3 molar ratio). The vesicles were prepared in buffered sodium chloride solution and were subjected to dialysis with sodium nitrate solution producing a suspension of vesicles containing chloride in a sodium nitrate solution. The receptors were introduced to the suspension in a small quantity of dimethyl sulfoxide (DMSO). If the receptor functions as a chloride transporter, then chloride will be released from the vesicles and can be detected using a chloride selective electrode. At the end of the experiment, detergent is added to lyse the vesicles and calibrate the electrode to 100% chloride release. As this is a passive transport process, a charge gradient cannot build up during the efflux of chloride. Consequently, for chloride to exit the vesicle, either a metal cation or a proton must leave with it (a symport or cotransport process) or a nitrate anion must be transported from the external solution to the interior of the vesicle (an antiport process). We found that, under neutral conditions, compound **5** released chloride most efficiently, with compound **6** showing a much reduced rate of chloride transport relative to compound **5** and compound **7** showing no chloride transport properties under these conditions. In the presence of a pH gradient (pH 4.0 inside the vesicles and pH 7.0 outside), the rate of chloride efflux was found to increase significantly for compounds **5** and **6** (Figure 2). Fluorescence experiments using the pH

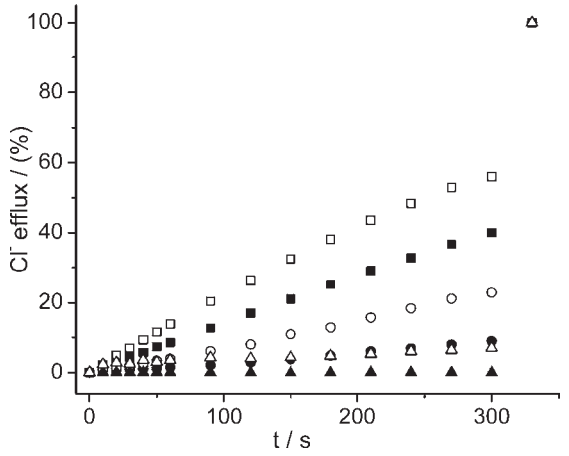
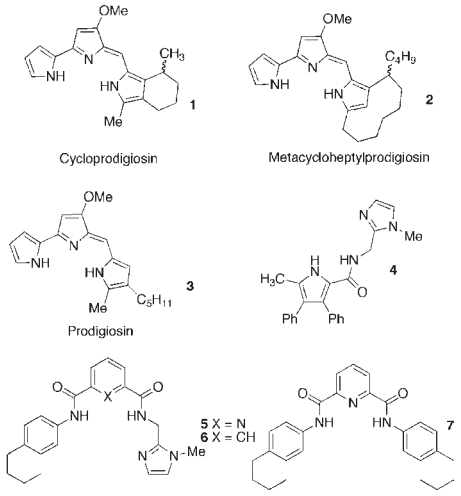


FIGURE 2. Chloride efflux upon addition of **5** (■/□), **6** (●/○), or **7** (▲/△) (8 μM in each case) to vesicles composed of POPC/cholesterol (7/3 molar ratio) under neutral (filled symbols) and pH gradient (empty symbols) conditions. The vesicles contained neutral, NaCl (500 mM), pH 7.0, or pH gradient, NaCl (500 mM), citric acid (5 mM), pH 4.0 solutions, and were immersed in $NaNO_3$ (500 mM), pH 7.0, or $NaNO_3$ (500 mM), sodium phosphate (5 mM), pH 7.0 solutions, respectively. At 300 s, they were lysed to obtain 100% chloride release. Reproduced with permission from ref 16. Copyright 2007 Royal Society of Chemistry.

sensitive fluorescent dye Lysosensor Blue showed that compounds **5** and **6** could deacidify the acidic interior of vesicles. This is evidence that under pH gradient conditions the compounds function as H^+/Cl^- symporters. However, in the absence of a pH gradient, chloride efflux was still observed. To probe the mechanism of this process further, the experiments were repeated with sulfate as the external anion rather than nitrate. Sulfate is highly hydrophilic and cannot pass through a lipid bilayer membrane. We found that upon addition of compound **5** under these conditions no chloride efflux was observed. This is evidence that leads us to suggest that compound **5** and to a lesser extent compound **6** function as chloride/nitrate antiporters under neutral conditions. Hence, in the presence of a pH gradient, the enhanced rate of chloride transport may be due to a combination of both HCl symport and Cl^-/NO_3^- antiport. Further experiments in pure POPC vesicles resulted in a faster release of chloride under identical conditions. Cholesterol reduces the fluidity of POPC membranes, hence increasing their viscosity. The decrease in the rate of transport in the POPC/cholesterol membrane is evidence that the compound is functioning as a discrete molecular carrier rather than forming a channel. So, in this series of compounds, the "champion" transporter is compound **5**. Stability constant determinations in DMSO-d_6 showed that under neutral conditions both receptors have only a low affinity for chloride and in the presence of 1 equiv of HPF_6 compound **5** exhibits an increased affinity for chloride ($K_a = 59\ M^{-1}$). Under the same conditions, compound **6** still interacts only weakly ($K_a < 10\ M^{-1}$). Receptors based on the 2,6-dicarboxamidopyridine skeleton are preorganized into the *syn-syn* conformation (due to intramolecular NH···N hydrogen bonds), whereas the predominant conformation of isophthalamides in solution is the *syn-anti* conformation. Hence, the pyridine system is more highly preorganized which corresponds to the champion transporter from this series of compounds.



In a further exploration of the membrane transport properties of isophthalamides and the role of preorganization, in collaboration with Jeffery T. Davis at the University of Maryland and Roberto Quesada at the Univeristy of Burgos, a series of *n*-butylamide derivatives were prepared.[17] Compound **8** is a simple isophthalamide, while compound **9** contains hydroxy groups in the 4- and 6- position. In this compound, the hydroxy groups form intramolecular hydrogen bonds to the amide oxygen atoms so preorganizing the receptor into the *syn-syn* conformation suitable for anion complexation. Model compound **10** was also prepared which contains methoxy groups in the 4- and 6- position. In this case, the methoxy groups accept hydrogen bonds from the amide NH groups (so adopting an *anti-anti* conformation) which are therefore no longer available to form bonds with anions. The conformations are illustrated in Figure 3 which shows the X-ray crystal structures of **9** and **10**. The stability constants of compounds **8** and **9** with chloride and bromide measured in acetonitrile are shown in Table 1. The results show that compound **9** has a higher affinity for halide anions than compound **8**, presumably as a consequence of the preorganizing effect of the OH groups. An

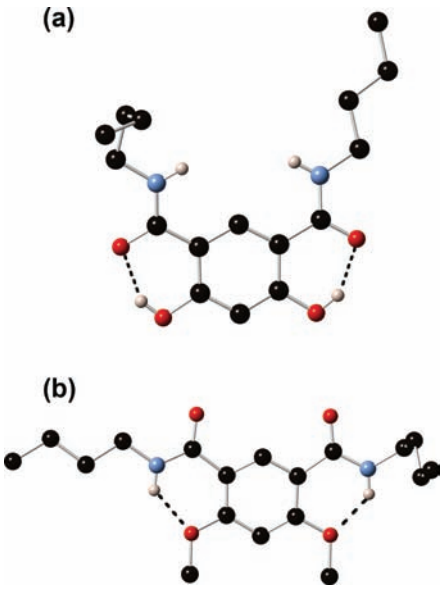


FIGURE 3. X-ray crystal structures of (a) compound **9** and (b) compound **10**. In the case of compound **9**, only one of the three molecules in the asymmetric unit is shown. Two of the molecules adopt the *syn-syn* conformation with each bound to one of the amide oxygen atoms of the third molecule which consequently adopts the *anti-anti* conformation. Nonacidic hydrogen atoms have been omitted for clarity.

TABLE 1. Stability Constants K_a (M^{-1}) for **8** and **9** Binding Cl^-, Br^-, and I^- (n-Bu_4N^+ Salts) Measured at 298 K in CD_3CN[a]

cmpd	Cl^-	Br^-	I^-
8	195	60	15
9	5230	716	152

[a]Errors < 10%.

additional factor which favors anion complexation in this case is that the intramolecularly bound OH groups will polarize the amide groups, increasing the acidity of the NH hydrogen bond donors. Compound **10** does not interact with anions under these conditions. The membrane transport properties of the series of compounds were studied using the chloride sensitive dye lucigenin. The dye was encapuslated within vesicles prepared in sodium nitrate solution composed of egg yolk phosphatidylcholine buffered to pH 6.4. The potential carriers were added in DMSO to the vesicle solution (to make a concentration of 2 mol % carrier to lipid) and sodium chloride added to the extravesicular solution. Transport of chloride into the vesicles quenches the fluorescence of lucigenin. It was found that at pH 6.4 compound **9** possesses significant chloride transport ability yet compounds **8** and **10** are completely inactive (Figure 4). It was also found that deprotonation of the hydroxy groups resulted in a loss of transport ability with

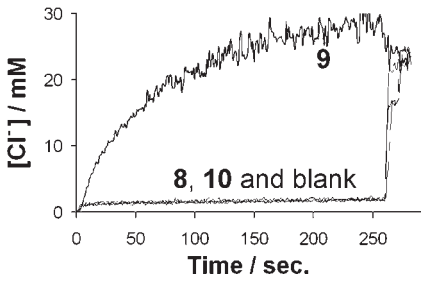
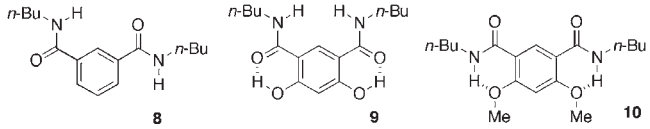


FIGURE 4. Chloride transport across EYPC liposomes (0.5 mM in lipid) containing lucigenin in a 100 mM $NaNO_3$, 10 mM sodium phosphate buffer (pH 6.4) at 25 °C. Compounds **8**–**10** were added to give a 2:100 ligand/lipid ratio. At $t = 0$ s, NaCl was added to give an external Cl^- concentration of 25 mM. Lucigenin fluorescence was converted to Cl^- concentration using the Stern–Volmer constant determined under the assay conditions. Reproduced with permission from ref 17. Copyright 2007 American Chemical Society.

compound **9**, showing minimal chloride transport ability at pH 9.1. Presumably, when deprotonated at the hydroxyl positions, the amide NH groups will form intramolecular hydrogen bonds with the phenolate oxygen atoms and hence not be available for anion complexation.



In 2009, the Southampton, Maryland and Burgos groups decided to investigate the membrane transport of the bicarbonate anion by both natural products and synthetic carriers.[18] Bicarbonate is more hydrophilic than chloride and is another important biological anion involved in a wide range of processes including respiration and photosynthesis. Additionally, misregulation of bicarbonate transport may also play a role in cystic fibrosis. We chose to employ potent chloride transport agents prodigiosin **3**, 4,6-dihydroxyiosphthalamide **9**, and new dihydroxyisophthalamides **11** and **12** as putative bicarbonate transporters. Initial studies were conducted using a chloride selective electrode in a similar manner to the experiments described previously. POPC vesicles loaded with chloride were suspended in sodium sulfate solution, and a solution of potential transporter added in a small amount of DMSO. No chloride efflux was observed, as sulfate is too hydrophilic to cross the lipid bilayer. A pulse of bicarbonate was then added to the extravesicular solution (at 120 s; see Figure 5). At this point, chloride efflux commenced. This was attributed to a chloride/bicarbonate antiport process with bicarbonate entering the vesicles and chloride leaving (Figure 5). Thus, using an ion-selective electrode, we can detect one component of the Cl^-/HCO_3^- antiport process by monitoring the efflux of

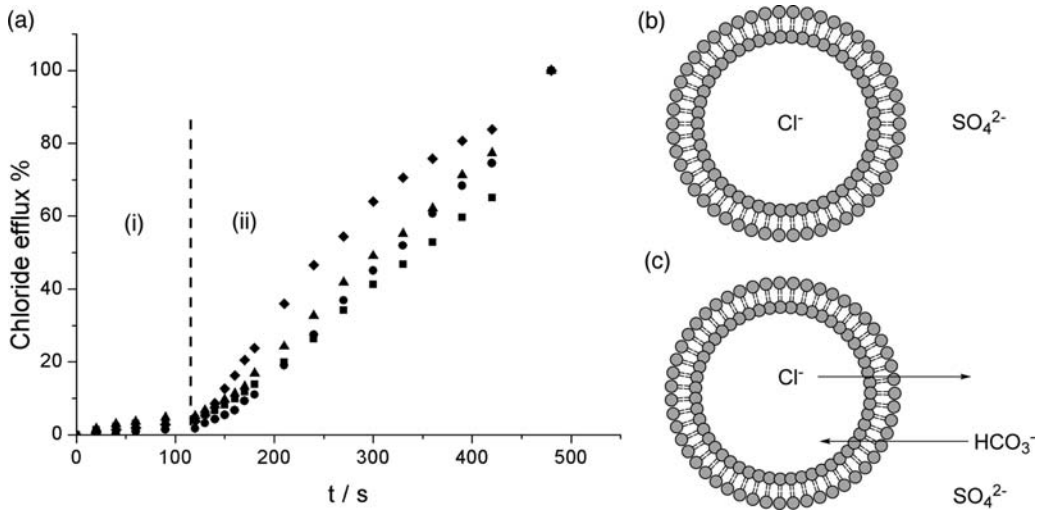
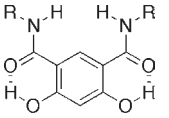


FIGURE 5. Experiment showing chloride transport measured using a Cl^- selective electrode commencing upon addition of a bicarbonate pulse to the external solution. (a) (i) Chloride efflux promoted upon addition of **3** (◆) (0.04% molar carrier to lipid) and **9** (■), **11** (▲), **12** (●) (1% molar carrier to lipid) to unilamellar POPC vesicles loaded with 451 mM NaCl and 20 mM phosphate buffer pH 7.2 dispersed in 150 mM Na_2SO_4 20 mM phosphate buffer pH 7.2. (ii) At $t = 120$ s, a solution of $NaHCO_3$ was added to give a 40 mM external concentration. At $t = 420$ s, the vesicles were lysed by addition of detergent, and the final reading at $t = 540$ s was considered to equal 100% chloride efflux. (b) In the presence of the carrier compounds **3**, **9**, **10**, or **12**, chloride was not released from the vesicles when suspended in a sulfate solution. (c) Upon introduction of bicarbonate to the solution, chloride efflux began as one component of the chloride/bicarbonate antiport mechanism. Reproduced with permission from ref 18. Copyright 2009 Nature Publishing Group.

chloride form the vesicles. Bicarbonate transport could be monitored directly using ^{13}C NMR techniques. Carbon-13 labeled $H^{13}CO_3^-$ was used in these experiments. A typical procedure is shown in Figure 6. EYPC vesicles were prepared in $NaH^{13}CO_3$ solution buffered to pH 7.3 and suspended in Na_2SO_4 solution also buffered to pH 7.3. The ^{13}C NMR spectrum of the vesicles shows two peaks corresponding to encapsulated bicarbonate with a second smaller peak corresponding to a small quantity of bicarbonate in the extravesicular solution. A pulse of chloride was then added to the extravesicular solution, resulting in no change to the NMR spectrum. Carrier was then added at 0.1% molar carrier to lipid for prodigiosin and 1% molar carrier to lipid for the synthetic transporters in a small amount of DMSO, resulting in bicarbonate efflux and chloride influx; this can be observed in the ^{13}C NMR spectrum with the resonance corresponding to encapsulated bicarbonate disappearing and the upfield resonance corresponding to extravesicular bicarbonate increasing in intensity. In a control experiment, DMSO, but no carrier, was added to confirm that the DMSO was not disrupting the lipid bilayer and allowing bicarbonate to leave. Finally, manganese chloride was added to the NMR tube. This paramagentic transition metal will interact with extravesicular bicarbonate, resulting in the loss of the NMR signal. However, the Mn^{2+} cation cannot cross the lipid bilayer and hence it does not effect the signal from encapsulated bicarbonate. As can be seen in Figure 6, the bicarbonate signal vanishes upon addition of $MnCl_2$ when either the prodigiosin or 4,6-dihydroxyisophthalamide is present but not in their absence, suggesting that in this latter case the bicarbonate remains encapulsated within the vesicle. The experiment was repeated starting with chloride encapsulated within the vesicles. Manganese chloride was added, resulting in the loss of the ^{13}C NMR resonance from extravesicular bicarbonate which then recovered when a carrier was added and bicarbonate influx occurred.



11 R = *iso*-pentyl
12 R = n-octyl

3. Calixpyrroles as Lipid Bilayer Anion Transporters: Ion Pair Transport versus Anion Antiport

Calixpyrroles are macrocyclic species consisting of pyrrole rings linked via sp^3 hybridized carbon atoms in the 2- and 5- positions.[19] In 1996, Sessler and co-workers at the University of Texas at Austin reported that compounds **13** and **17** bind anions both in solution and in the solid state, forming four hydrogen bonds from the pyrrole NH groups to an anionic guest. These compounds bind anions via NH···anion hydrogen bonds and when doing so adopt a

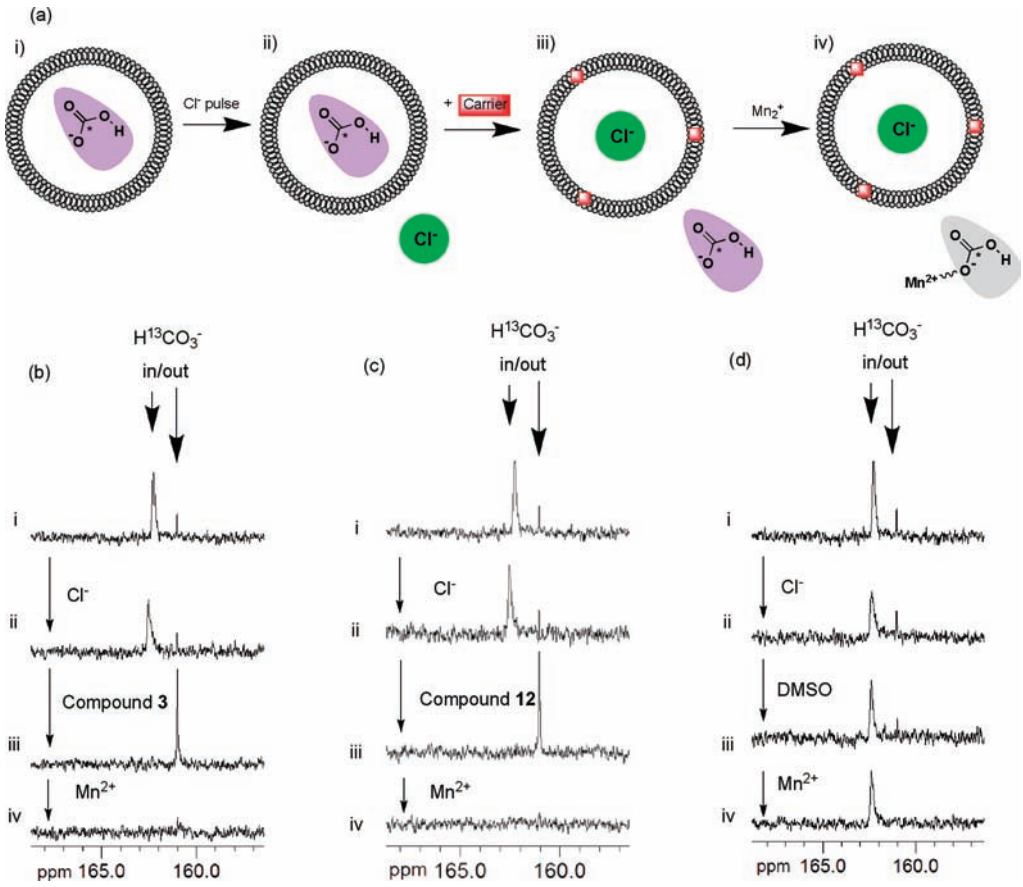


FIGURE 6. ^{13}C NMR experiments demonstrate that both natural products and synthetic receptors are capable of chloride/bicarbonate antiport by releasing encapsulated bicarbonate as evidenced by the HCO_3^- ^{13}C resonance disappearing in the presence of paramagnetic $MnCl_2$ in the external solution. Representation of the titration sequence (a) and NMR stack plots (b–d) for monitoring the transmembrane transport of HCO_3^- ions in $H^{13}CO_3^-$-loaded EYPC liposomes by **3** and **12**. A 50 mM NaCl pulse was added to EYPC vesicles loaded with 100 mM $NaH^{13}CO_3$, 20 mM HEPES buffer (pH 7.5) and dispersed in 75 mM Na_2SO_4, 20 mM HEPES buffer (pH 7.5), and ^{13}C NMR data was acquired before (i) and after (ii) the Cl^- pulse. NMR spectra were also collected after the addition of transporter or DMSO (**3**, 0.1 mol %; **12**, 1 mol %; or DMSO, 870 mol % (10 μL)) (iii) followed by addition of 0.5 mM Mn^{2+} (1:100 Mn^{2+}/Cl^- ratio; iv). Reproduced with permission from ref 18. Copyright 2009 Nature Publishing Group.

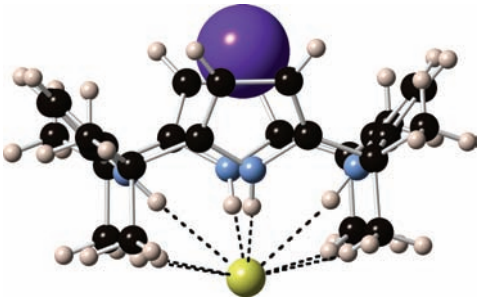


FIGURE 7. X-ray crystal structure of the cesium chloride complex of *meso*-octamethylcalix[4]pyrrole **13**.

cone or cuplike conformation allowing cations to complex in the cavity formed by the pyrrole rings (Figure 7).[20–23] We tested whether calix[4]pyrroles could transport chloride across lipid bilayer membranes by preparing POPC vesicles loaded with cesium chloride and suspending them in sodium nitrate solution at pH 7.2.[24] We then added a variety of different calix[4]pyrroles **13**–**17** in DMSO to the vesicular suspension at 2% molar carrier to lipid concentration and monitored chloride release using a chloride selective electrode (Figure 8). Compounds **14**–**16** were inactive, compound **17** released slightly less than 20% of the encapsulated chloride over 300s, while *meso*-octamethylcalix[4]pyrrole **13** proved to be an efficient chloride transporter under these conditions. We then prepared vesicles loaded with NaCl, KCl, or RbCl and repeated the experiments with compound **13**; no release of chloride was observed, giving evidence that the cesium cation is involved in the transport process. Next, we repeated the experiment with CsCl loaded vesicles suspended in sodium sulfate solution. Under these conditions, we observed that chloride was released from the vesicles (Figure 9). As sulfate cannot pass through the lipid bilayer membrane, we therefore believe that compound **13** transports chloride

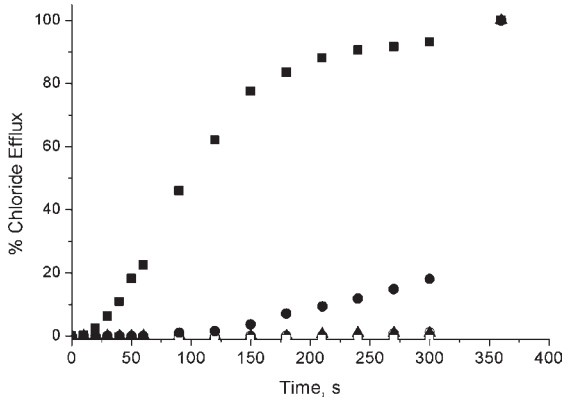


FIGURE 8. Chloride efflux promoted upon addition of **13** (■), **14** (○), **15** (▲), **16** (□), and **17** (●) (2% molar carrier to lipid) to unilamellar POPC vesicles loaded with 488 mM CsCl 5 mM phosphate buffer pH 7.2 dispersed in 488 mM $NaNO_3$ 5 mM phosphate buffer pH 7.2. Reproduced with permission from ref 24. Copyright 2008 Royal Society of Chemistry.

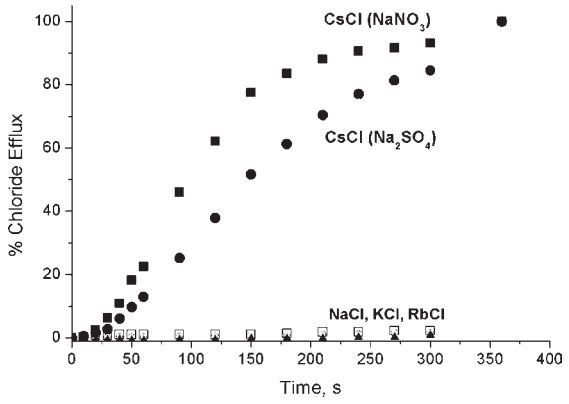
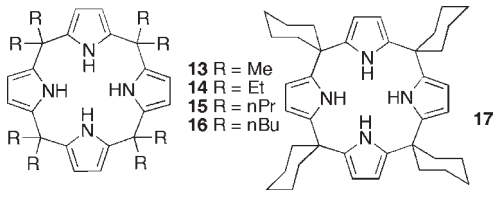
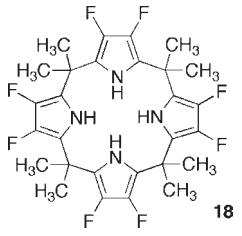


FIGURE 9. Chloride efflux promoted upon addition of **13** (2% molar carrier to lipid) to unilamellar POPC vesicles loaded with 488 mM CsCl (■), RbCl (○), KCl (▲), or NaCl (□), 5 mM phosphate buffer pH 7.2 dispersed in 488 mM $NaNO_3$ 5 mM phosphate buffer pH 7.2 and unillamellar POPC vesicles loaded with 488 mM CsCl (●) 5 mM phosphate buffer pH 7.2 dispersed in 145 mM Na_2SO_4. Reproduced with permission from ref 24. Copyright 2008 Royal Society of Chemistry.

through the bilayer membrane as a cesium chloride ion pair.



We also examined the anion transport properties of octafluoro-*meso*-octamethylcalix[4]pyrrole **18** prepared by the Austin group.[25] Fluorinated calixpyrroles form stronger complexes with anions than nonfluorinated analogues due to the electron withdrawing effect of the fluorine substituents and have been observed to reduce Hofmeister bias in anion extraction processes.[26] We repeated the membrane transport experiments performed with compound **13** and found there was little dependence on the nature of the countercation (Figure 10). This suggests that compound **18** does not transport an ion pair but rather transports chloride as part of a chloride/nitrate antiport process. This was confirmed by changing the extravesicular solution to sodium sulfate; under these conditions, no chloride efflux was observed. It may be in this case that the higher affinity of the fluorinated macrocycle allows both chloride and nitrate to be bound and transported in the absence of a metal cation. The fact that chloride was not transported from CsCl containing vesicles in Na_2SO_4 solution also suggests that compound **18** is less effective at binding cesium due to the presence of the fluorine substituents. We also conducted bicarbonate transport experiments using the ion selective electrode method described above and found that compound **18** can facilitate chloride/bicarbonate antiport. Thus, by changing the substituents (and hence enhancing the affinity of the receptor for anions), we can switch the transport mechanism from CsCl cotransport only (compound **13**) to Cl^-/NO_3^- or Cl^-/HCO_3^- antiport (compound **18**).



An alternative method of enhancing the affinity of calix[4]pyrroles for anionic guests is to introduce additional hydrogen bond groups.[27] Flood et al. and other groups have shown that the CH group in the triazole is polarized and functions effectively as a hydrogen bond donor.[28] We therefore decided to synthesize a strapped calix[4]pyrrole **19** containing a 1,2,3-triazole group with the aim of enhancing the affinity and possibly the anion transport properties (by creating a more encapsulated or enclosed anion binding site).[29] A ^1H NMR titration with tetrabutylammonium chloride in deuterated acetonitrile showed that the receptor forms a complex with chloride with slow exchange on the NMR time scale (Figure 11). Therefore, isothermal titration calorimetry was used to determine the affinity of receptor **19** for chloride. In acetonitrile, compound **13** binds chloride (added as the tetraethylammonioum salt) with a stability

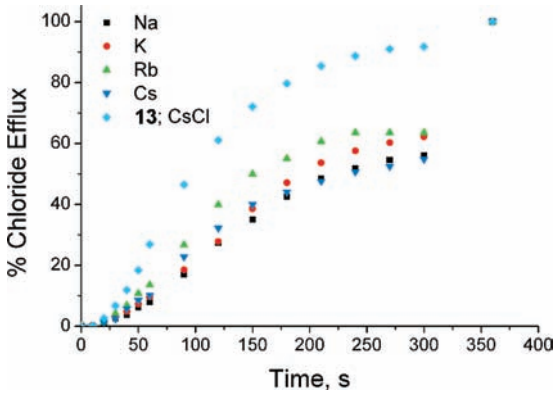


FIGURE 10. Chloride efflux promoted by 0.04 mol equiv of **13** (◆) across unilamellar POPC vesicles loaded with 489 mM cesium chloride and by **18** in unilamellar POPC vesicles loaded with 489 mM sodium (■), potassium (●), rubidium (▲), and cesium(▼) chloride salts buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Reproduced with permission from ref 25. Copyright 2010 American Chemical Society.

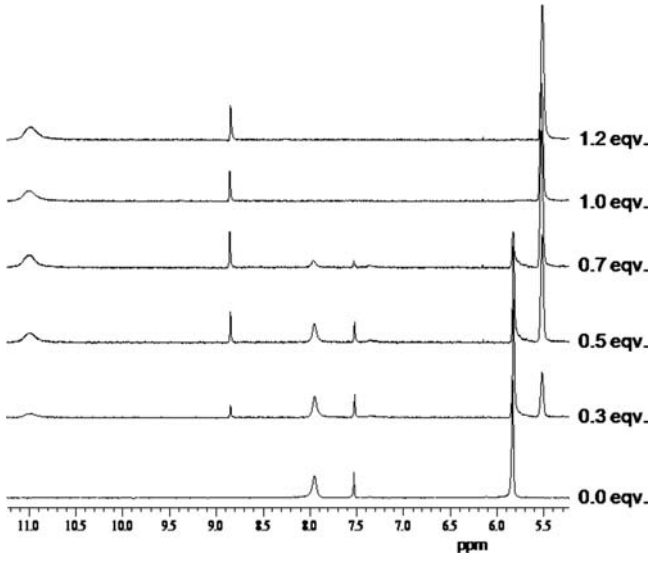


FIGURE 11. Partial ^{1}H NMR spectrum (300 MHz) of compound **19** upon addition of tetrabutylammonium chloride in acetonitrile-d_3 solution at 298K. Reproduced with permission from ref 29. Copyright 2008 Royal Society of Chemistry.

constant of 1.9×10^5 M^{-1} while compound **19** binds chloride with a stability constant of 2.6×10^6 M^{-1}. Membrane transport experiments were conducted with a variety of group 1 metal chlorides encapsulated within the vesicles suspended in either sodium nitrate or sodium sulfate solution with all solutions buffered to pH 7.2. It was found that there was very little change in the rate of efflux of chloride from vesicles containing cesium chloride when measured in $NaNO_3$ or Na_2SO_4 solution, suggesting that the compounds cotransport the Cs^+/Cl^- ion pair in a similar

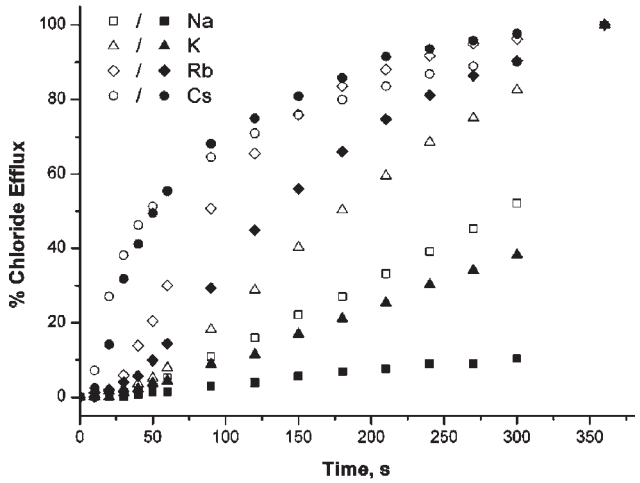
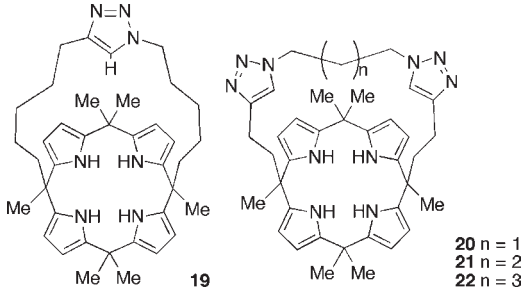


FIGURE 12. Chloride efflux promoted by **19** at 0.04 mol equiv of carrier to lipid, across unilamellar POPC vesicles loaded with 489 mM NaCl (□/■), KCl (△/▲), RbCl (◇/◆), and CsCl (○/●) buffered to pH 7.2 with 5 mM phosphate dispersed in 489 mM $NaNO_3$ (open symbols) or 167 mM Na_2SO_4 (closed symbols) buffered to pH 7.2 with 5 mM phosphate.

fashion to compound **13** (Figure 12). However, when the experiments were repeated with vesicles containing NaCl, a much faster rate of chloride efflux was observed in $NaNO_3$ solution than in Na_2SO_4 solution, evidence in support of a Cl^-/NO_3^- antiport process predominating in this case. Again by increasing the affinity of the receptor as compared to parent compound **13** (and in this case encapsulating the binding site and so shielding it from the environment), a different transport mechanism has been accessed to drive chloride transport. Compounds containing two triazole rings in the strap **20**–**22** have also been studied. We have shown in these cases that the mechanism of transport is dependent on the length of the linker between the two rings.[30,31]



4. Thiourea Based Transporters: Simple Yet Effective Transporters

Urea and thiourea groups have been used in a wide variety of anion receptor systems by ourseleves and others.[32,33] We have investigated the use of urea and thiourea containing molecules as simple transmembrane anion transporters for

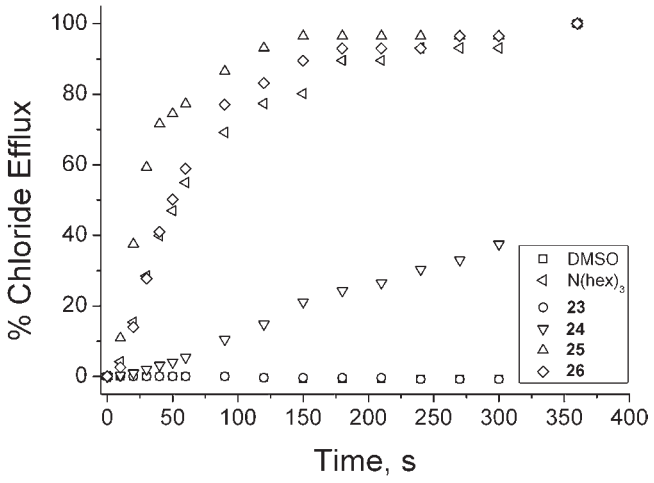


FIGURE 13. Chloride efflux promoted by 2% molar carrier to lipid concentrations of compounds **23**−**26** and trihexylamine (N(hex)$_3$) from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with sodium phosphate salts. The vesicles were dispersed in 489 mM NaNO3 buffered to pH 7.2 with 5 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride release.

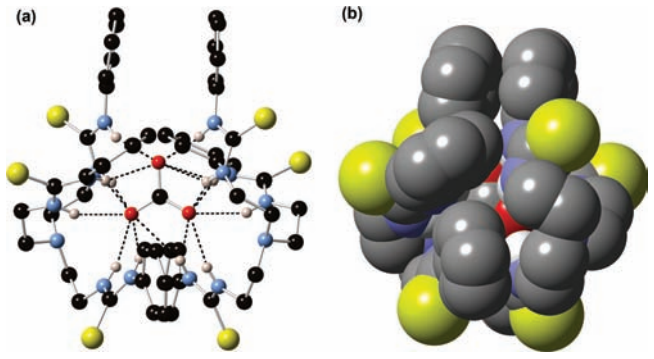


FIGURE 14. (a) Ball and stick and (b) space-filling views of the X-ray crystal structure of the tetraethylammonium carbonate complex of receptor **26**.

chloride and bicarbonate.[34] We prepared a series of compounds based on tris(2-aminoethyl)amine (tren) and studied their anion transport properties (together with trihexylamine as a model compound) in POPC vesicles. We found that while the urea containing compounds were either inactive (**23**) or poor to moderate (**24**) chloride/nitrate antiport agents, the thiourea compounds were very active (Figure 13). This led us on to examine the bicarbonate transport properties of these receptors. Studies using ion-selective electrodes to detect chloride efflux and ^{13}C NMR experiments similar to those described earlier with $H^{13}CO_3^-$ conducted by Jeff Davis and Will Harrell showed that the thiourea compounds are also potent chloride/bicarbonate transport agents.

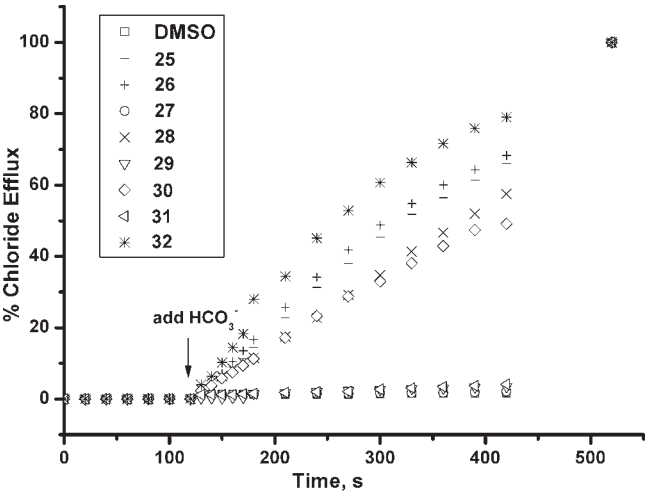
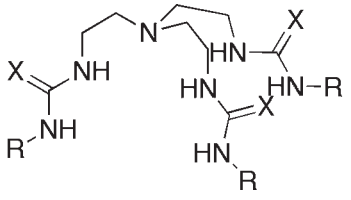


FIGURE 15. Chloride efflux promoted by 0.02 molar equiv of receptors **25**−**32** from unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 20 mM sodium phosphate salts upon addition of a $NaHCO_3$ pulse to make the extravesicular bicarbonate concentration 40 mM. The vesicles were dispersed in 167 mM Na_2SO_4 buffered at pH 7.2 with 20 mM sodium phosphate salts. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride release.

Crystals of the carbonate complex of receptor **26** were obtained by crystallization from a DMSO/water solution containing the receptors and tetraethylammonium bicarbonate. The X-ray crystal structure revealed that 2 equiv of receptor **26** bind a single carbonate anion in the solid state via 12 hydrogen bonds in the range 2.824(6)−3.070(7) Å with several other longer range NH···O interactions (Figure 14). This is similar structurally to a tren-based tris-urea carbonate complex previously reported by Ghosh and Ravikumar.[35]



23 R = n-Bu ; X = O
24 R = Ph; X = O
25 R = n-Bu; X = S
26 R = Ph; X = S

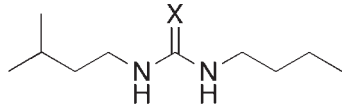
The potency of the tren-based tris-thioureas led us on to study simple molecules containing a single thiourea group (**28**, **30**, **32**) and compare the transport activity of these species to their urea analogues (**27**, **29**, **31**).[36] The thiourea compounds were shown to be capable of chloride/bicarbonate antiport (Figure 15) by ion-selective electrode and ^{13}C NMR techniques, with compound **32**, which contains an indole group linked to the thiourea via the 7-position, showing the fastest rate of chloride/bicarbonate antiport among this series of compounds (and tren-based tris thioureas **25** and **26**). Our

TABLE 2. EC_{50} Values at 270 s and Initial Rate of Chloride Release (% Chloride Efflux per Second) for Compounds **28**, **30**, and **32** for Release of Chloride in Chloride/Nitrate and Chloride/Bicarbonate Systems[a]

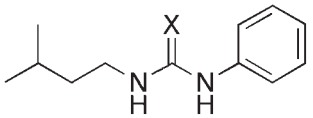
cmpd	EC_{50} at 270 s (Cl^--NO_3^-)[b]	initial rate of chloride release (Cl^--NO_3^-) % Cl^- efflux/s at 2% carrier loading	EC_{50} at 270s (Cl^--HCO_3^-)[b]	initial rate of chloride release (Cl^-−HCO_3^-) % Cl^- efflux/s at 2% carrier loading	*c* log *P*[c]	PSA (Å^2)[c]
27					1.99	37.0
28	0.109%	0.431	0.304%	0.227	3.14	22.2
29					2.42	34.8
30	3.05%	0.074	2.08%	0.188	3.57	21.5
31					2.02	44.6−47.7
32	0.029%	0.614	0.0356%	0.386	3.16	31.3−35.5

[a]Calculated log *P* and TPSA (Å^2) are also presented for compounds **27**−**32**. [b]EC_{50} values calculated from a Hill analysis of the transport data expressed in mol % carrier to lipid. [c]Calculated log *P* and polar surface area (PSA) calculated using Spartan '08 for Macintosh.

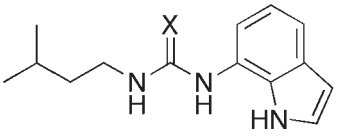
group and others have previously shown that the indole group is an effective hydrogen bond donor in a variety of anion receptors.[37] The urea compounds were not active. Calculations showed that the thiourea compounds have higher log *P* values (log of the water−octanol partition constant) and lower total polar surface areas than the urea analogues (Table 2). Among the thiourea compounds, compound **32** has the highest affinity for both chloride and bicarbonate. It is nonetheless remarkable that such simple compounds function as potent chloride/bicarbonate (and chloride/nitrate) antiporters.



27 X = O
28 X = S



29 X = O
30 X = S



31 X = O
32 X = S

5. Conclusions

We have seen that simple compounds not only can form complexes with anions selectively but also can function to mediate the transport of anionic species including chloride and bicarbonate across lipid bilayer membranes. Crystallographic analysis has often provided clues to next generation receptor design. However, the design of new anion transporters from first principles is more challenging and an area in which we are currently directing our efforts. One clue might be to take lessons here from drug design.[38] The goal of using small molecules as treatments for diseases caused by misregulation of chloride transport, such as cystic fibrosis, will be brought closer when compounds designed as membrane transporters have optimized absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. However, by optimizing ADMET properties, we may at the same time optimize the parameters (for example, these might include log *P*, total polar surface area, and other parameters) upon which efficient anion transport in lipid bilayer membranes depends. However, unlike small molecule drugs, we wish our transporters to remain predominantly in the membrane and not diffuse out of it.

My thanks go to all of my co-workers past and present in Southampton (their names appear in the references). I'd also like to thank Bradley Smith at the University of Notre Dame for our collaboration on HCl cotransport that sparked our continuing interest in membrane transport. I'd also like to thank Jeffrey Davis at the University of Maryland and Roberto Quesada at the University of Burgos for our collaboration on membrane transport, Jonathan Sessler at the University of Texas at Austin for our collaboration on calix[4]pyrrole, and Franz Schmidtchen at the Techische Universität München for the isothermal titration calorimetry studies presented in this Account. Additionally, I thank the EPSRC for access to the crystallographic facilities at the University of Southampton and the EPSRC together with the NSF and European Union for funding.

BIOGRAPHICAL INFORMATION

Philip A. Gale received a B.A. (Honors) in chemistry in 1992, a M.A. in 1995, and a D.Phil. in 1995 from the University of Oxford. He then moved as a Fulbright Postdoctoral Fellow to the University of Texas at Austin in 1995, returning to the University of Oxford in 1997 as a Royal Society University Research Fellow and moving in 1999 to the University of Southampton, where he is now Professor of Supramolecular Chemistry and Head of the School of Chemistry. A major focus of his research is the supramolecular chemistry of anionic species. He was won a number of research awards including the RSC's Corday-Morgan Medal and Prize.

FOOTNOTES

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