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Octafluorocalix[4]pyrrole: a chloride/bicarbonate antiport agent

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There is intense current interest in the development of synthetic transmembrane transporters for biologically relevant ionic species. Approaches to this challenge have included the development of channels[1] that span the lipid bilayer and ionophores[2] capable of binding ions and facilitating their diffusion across the membrane. There has been particular interest recently in anion transport since misregulation of this process is a hallmark of diseases such as cystic fibrosis.[3] Our groups together with our collaborators have developed transmembrane chloride transporters that function by HCl co-transport[4] and by chloride-nitrate antiport processes.[5] We recently discovered that *meso*-octamethylcalix[4]pyrrole **1** functions as a membrane transport agent for cesium chloride ion pairs but not sodium, potassium or rubidium chloride, presumably due to the ability of calixpyrrole anion complexes to bind large charge diffuse cations such as cesium in the calixpyrrole cup shaped cavity formed by the pyrrole rings when binding chloride.[6] Here, we report the anion transport properties of *meso*-octamethyl-octafluorocalix[4]pyrrole **2** and show, in contradistinction to the limited anion transport properties of the parent macrocycle, that the fluorinated system (which has a higher affinity for anionic guests than the parent macrocycle **1** due to the presence of electron withdrawing fluorine substituents)[7] is an effective chloride anion transporter that functions with a variety of monovalent counter cations. It operates *via* an anion antiport mechanism that allows for the exchange of *inter alia* 1) chloride for nitrate and, more importantly, 2) chloride for the more hydrophilic (and physiologically relevant) bicarbonate anion. To the best of our knowledge, this is not something that has yet proved possible using simple synthetic pyrrole-based anion receptors,.

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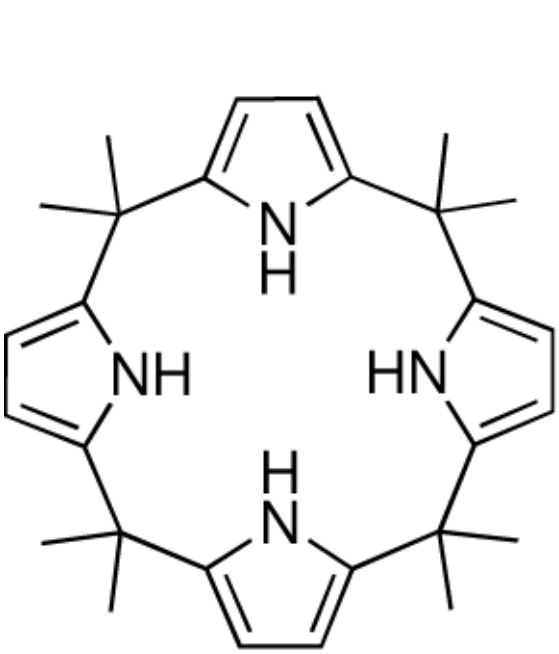
Supporting Information Available: Details of vesicle preparation and transport studies, including DPPC mobility studies.



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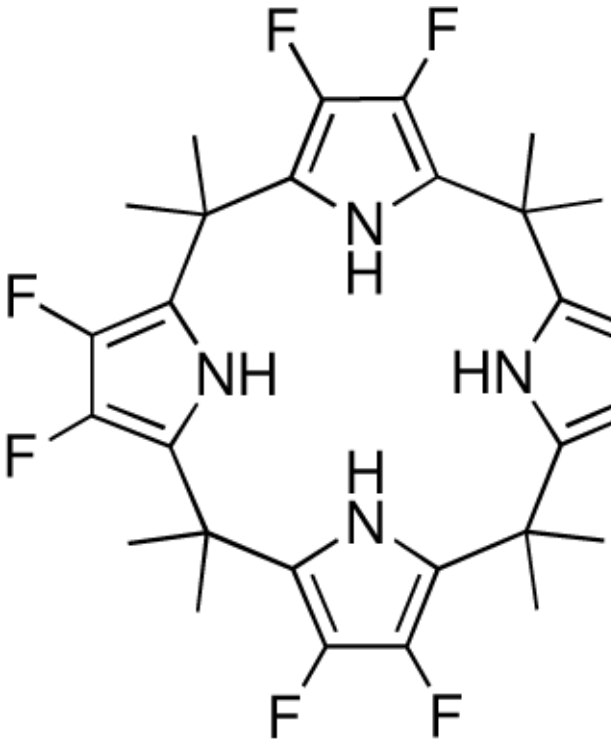

Compounds **1** and **2** were prepared by literature methods.[7] In order to study the transport properties of compound **2** we prepared a series of unilamellar 1-palmitoyl-2-oleoylphophatidylcholine (POPC) vesicles loaded with group one metal chloride salts and suspended them in an external $NaNO_3$ solution. A sample of calix[4]pyrrole **2** (4% molar carrier to lipid) was added as a DMSO solution and the resultant Cl^- efflux monitored using a chloride selective electrode.[8] After five minutes, the vesicles were lysed by addition of detergent and the final reading of the electrode used to calibrate 100% release of chloride. The results are shown in Figure 1.

The results show little cation dependence on the rate of chloride efflux from the vesicles in the case of compound **2**. We have previously shown in analogous experiments made with compound **1** that no chloride efflux occurs from vesicles containing NaCl, KCl or RbCl under the same conditions. On the other hand, chloride is released by compound **1** from vesicles containing CsCl *via* an ion-pair transport mechanism (Figure 1).[6] The lack of cation dependence on the rate of release of chloride by compound **2** is evidence consistent with an anion antiport process in which chloride and nitrate anions are exchanged across the lipid bilayer membrane by the fluorinated calixpyrrole. DPPC mobility assays at 37°C and 45°C provide support for the notion that compound **2** functions as a discrete molecular carrier (see ESI).[9] The EC_{50} value at 270s was measured for compound **2** and was found to be 3.1% molar carrier to lipid with nitrate as the external anion.

To investigate the nature of this mechanism further, the above series of experiments was repeated with the vesicles suspended in sodium sulfate solution rather nitrate. Sulfate is significantly more hydrophilic than nitrate ($\Delta G_h(SO^{2-}{}_4)$ −1080 $kJmol^{-1}$; $\Delta G_h(NO_3^-)$ −300 $kJmol^{-1}$)[10] and cannot pass through the lipid bilayer membrane. The results (shown in Figure 2) reveal that compound **2** does not release chloride under these conditions independent of whether the vesicles are made up using sodium, potassium, rubidium or cesium chloride. Such a finding supports the hypothesis that receptor **2** is functioning as a chloride/nitrate anion antiport agent.

Recently, it has been shown by Davis, Gale and Quesada that the natural product prodigiosin and pre-organised 4,6-dihydroxyisophthalamides function as chloride/bicarbonate antiport agents.[11] Bicarbonate is more hydrophilic than nitrate ($\Delta G_h(HCO^-_3)$ −335 kJmol^{-1})[8] and hence is a greater challenge to transport through a lipid bilayer. To the best of our knowledge, it is not something that has been achieved using simple synthetic pyrrolic receptors. In order to test whether compound **2** could achieve the counter transport of bicarbonate, vesicles containing NaCl were prepared and suspended initially in a solution of Na_2SO_4. Compound **2** was added to this suspension in DMSO solution. At this point no evidence of chloride release is seen. After 120s, $NaHCO_3$ was added to the solution, at which point chloride efflux from the vesicles was observed to commence. We take this as evidence that this compound functions as a chloride/bicarbonate antiport agent (Figure 3). A model study with compound **1** under identical experimental conditions demonstrated that this compound does not function as a chloride/bicarbonate antiporter. DMSO was added without calixpyrrole, demonstrating that the solvent does not disrupt the structure of the vesicles as no chloride was released (Figure 3). The EC_{50} value for at 600s for compound **2** was found to be 5.7% molar carrier to lipid with bicarbonate as the external anion (see ESI for more details).

In 2008, Moyer, Sessler and Bowman-James demonstrated that compound **2** can overcome Hofmeister bias in liquid-liquid extraction processes.[12] In this study we have shown that this receptor can function not just as an extractant, but is an effective chloride transporter capable of effecting antiport against the highly hydrophilic bicarbonate anion. Such behavior stands in marked contrast to what was seen with **1**. This structure-based disparity leads us to predict that it should be possible to design yet-improved anion extractants and carriers based on the calixpyrrole framework. Work along these latter lines is currently in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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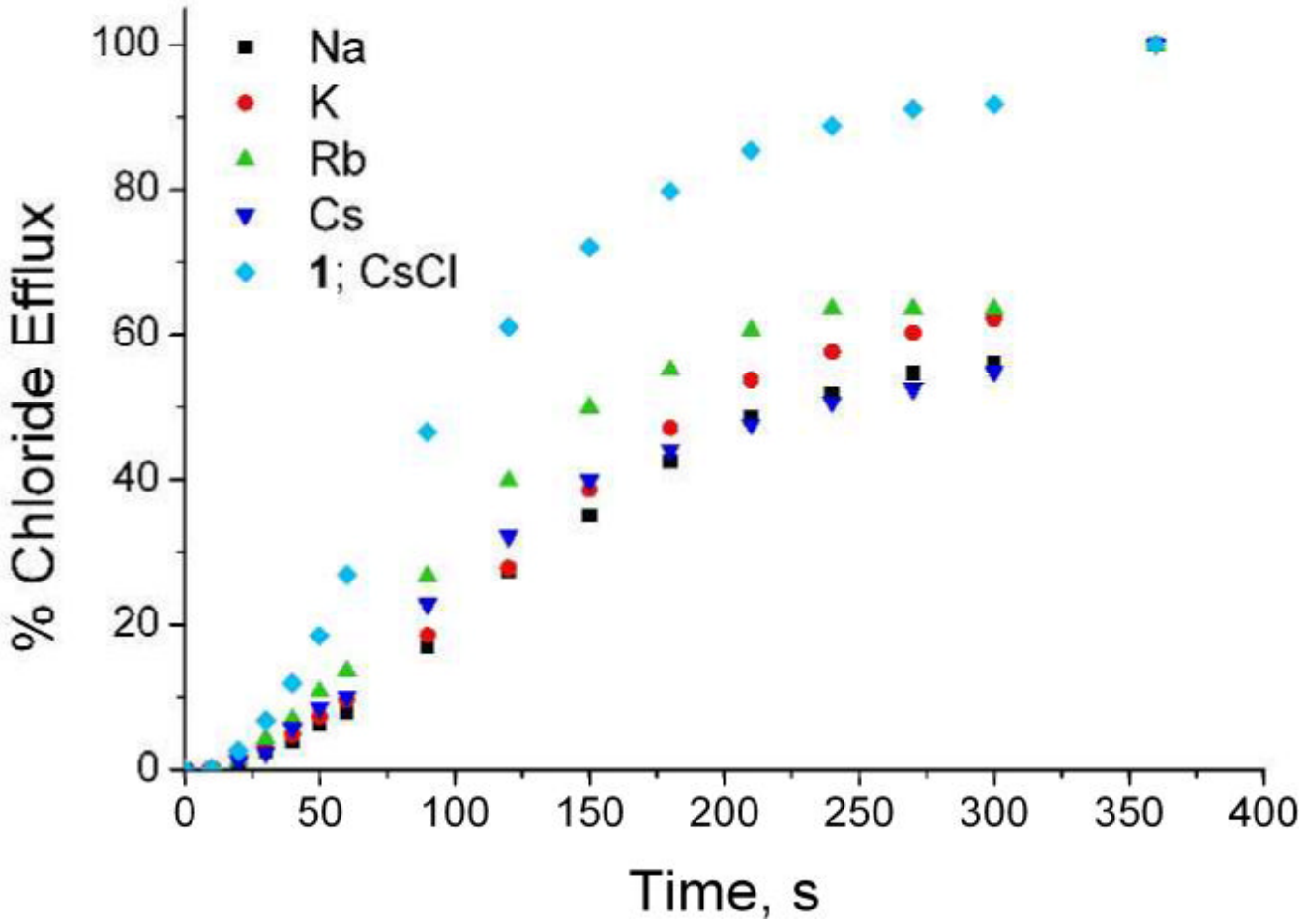

Figure 1.

Chloride efflux promoted by 0.04 molar equivalents of **1** (◆) across unilamellar POPC vesicles loaded with 489 mM cesium chloride and by **2** in unilamellar POPC vesicles loaded with 489 mM sodium (■), potassium (●), rubidium (▲) and cesium(▼) chloride salts buffered to pH 7.2 with 5mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials.

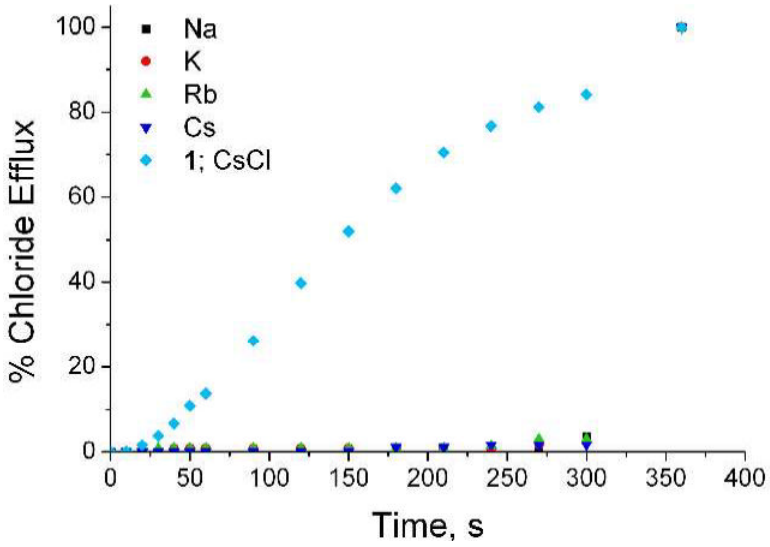

Figure 2.

Chloride efflux promoted by 0.04 molar equivalents of **1** (◆) across unilamellar POPC vesicles loaded with 489 mM cesium chloride and by **2** in unilamellar POPC vesicles loaded with 489 mM sodium (■), potassium (●), rubidium (▲) and cesium (▼) chloride salts buffered to pH 7.2 with 5mM phosphate. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials.

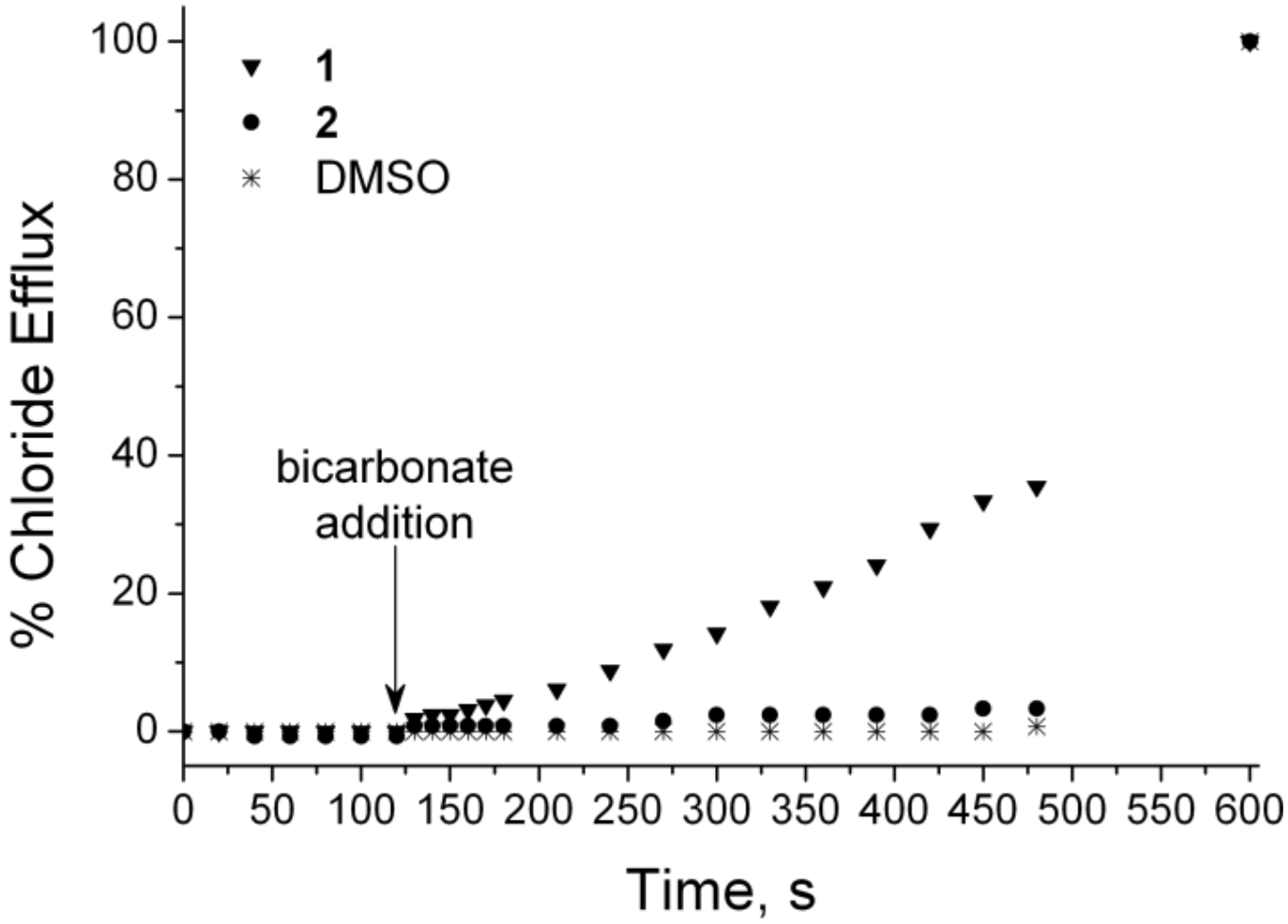

Figure 3.

Chloride efflux promoted by 0.04 molar equivalents of **1** (●) and **2** (▼) across unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 20 mM phosphate upon addition of a $NaHCO_3$ pulse, to make the extravesicular bicarbonate concentration 40 mM. The vesicles were dispersed in 162 mM Na_2SO_4 buffered to pH 7.2 with 20 mM phosphate. Each point represents the average of three trials.