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Octafluorocalix[4]pyrrole: A Chloride/Bicarbonate Antiport Agent

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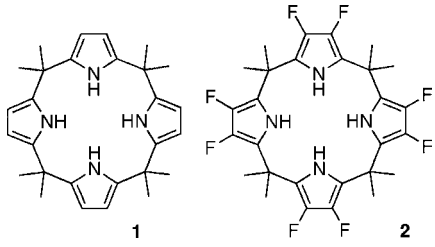
There is intense current interest in the development of synthetic transmembrane transporters for biologically relevant ionic species. Approaches to this challenge have included the development of channels[1] that span the lipid bilayer and ionophores[2] capable of binding ions and facilitating their 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 cotransport[4] and 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 because of 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*-octamethyloctafluorocalix[4]pyrrole (**2**) and show that, in contradistinction to the limited anion transport properties of the parent macrocycle, the fluorinated system (which has a higher affinity for anionic guests than the parent macrocycle **1** because of the presence of the electron-withdrawing fluorine substituents)[7] is an effective chloride anion transporter that functions with a variety of monovalent countercations. It operates via an anion antiport mechanism that allows for the exchange of, among others, (1) chloride for nitrate and, more importantly, (2) chloride for the more hydrophilic (and physiologically relevant) bicarbonate anion. To the best of our knowledge, the latter has not been achieved to date using simple synthetic pyrrole-based anion receptors.



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-oleoylphosphatidylcholine (POPC) vesicles loaded with group-1 metal chloride salts and suspended them in an external $NaNO_3$ solution. A sample of calix[4]pyrrole **2** (4 mol % carrier to lipid) was added as a dimethyl sulfoxide (DMSO) solution and the resultant Cl^- efflux monitored using a chloride-selective electrode.[8] After 5 min, the vesicles were lysed by addition of detergent, and the final reading of the electrode was used to calibrate 100% release of chloride. The results are shown in Figure 1.

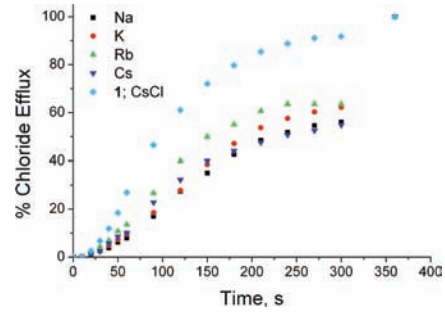


Figure 1. Chloride efflux promoted by 0.04 molar equiv of **1** (◆) across unilamellar POPC vesicles loaded with 489 mM cesium chloride and by **2** in unilamellar POPC vesicles loaded with 489 mM sodium (■), potassium (●), rubidium (▲), and cesium (▼) chloride salts buffered at pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered at pH 7.2 with 5 mM phosphate. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride release. Each point represents the average of three trials.

The results show little cation dependence of the rate of chloride efflux from the vesicles in the case of compound **2**. We have previously shown in analogous experiments performed 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 of 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. 1,2-Dipalmitoylphosphatidylcholine (DPPC) mobility assays at 37 and 45 °C and classic U-tube experiments provide support for the notion that compound **2** functions as a discrete molecular carrier (see the Supporting Information).[9] The concentration at 50% efflux (EC_{50}) at 270 s was measured for compound **2** and found to be 3.1 mol % carrier to lipid with nitrate as the external anion.

To further investigate the nature of this mechanism, the above series of experiments was repeated with the vesicles suspended in sodium sulfate solution instead of in sodium nitrate. Sulfate is significantly more hydrophilic than nitrate [$\Delta G_h(SO_4^{2-}) = -1080$ kJ mol^{-1}; $\Delta G_h(NO_3^-) = -300$ kJ mol^{-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,

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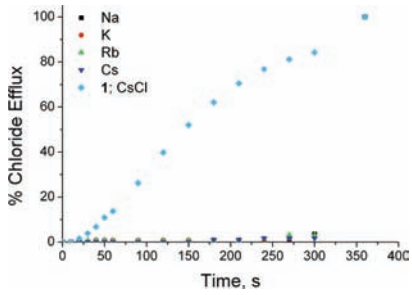

Figure 2. Chloride efflux promoted by 0.04 molar equiv of **1** (◆) across unilamellar POPC vesicles loaded with 489 mM cesium chloride and by **2** in unilamellar POPC vesicles loaded with 489 mM sodium (■), potassium (●), rubidium (▲), and cesium (▼) chloride salts buffered at pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 162 mM Na_2SO_4 buffered at pH 7.2 with 5 mM phosphate. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride release. Each point represents the average of three trials.

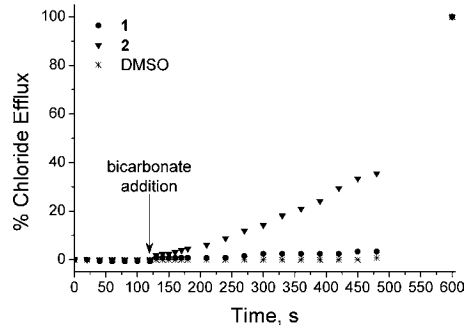

Figure 3. Chloride efflux promoted by 0.04 molar equiv of **1** (●) and **2** (▼) across unilamellar POPC vesicles loaded with 489 mM NaCl buffered at pH 7.2 with 20 mM phosphate upon addition of a $NaHCO_3$ pulse to make the extravesicular bicarbonate concentration 40 mM. The vesicles were dispersed in 162 mM Na_2SO_4 buffered at pH 7.2 with 20 mM phosphate. At the end of the experiment, detergent was added to lyse the vesicles and calibrate the ISE to 100% chloride release. Each point represents the average of three trials.

potassium, rubidium, or cesium chloride. Such a finding supports the hypothesis that receptor **2** functions as a chloride/nitrate anion antiport agent.

Recently, it has been shown by Davis, Gale, and Quesada[11] that the natural product prodigiosin and preorganized 4,6-dihydroxyisophthalamides function as chloride/bicarbonate antiport agents. Bicarbonate is more hydrophilic than nitrate [$\Delta G_h(HCO_3^-) = -335$ kJ mol^{-1}][8] and hence is a greater challenge to transport through a lipid bilayer. To the best of our knowledge, this has not been achieved to date using simple synthetic pyrrolic receptors. In order to test whether compound **2** could achieve countertransport of bicarbonate, vesicles containing NaCl were prepared and suspended initially in a solution of Na_2SO_4. Compound **2** in DMSO solution was added to this suspension. At this point, no evidence of chloride release was seen. After 120 s, $NaHCO_3$ 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, and no chloride was released (Figure 3), demonstrating that the solvent does not disrupt the structure of the vesicles. The EC_{50} value at 600 s for compound **2** was found to be 5.7 mol % carrier to lipid with bicarbonate as the external anion (see the Supporting Information 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 also as an effective chloride transporter capable of effecting antiport against the highly hydrophilic bicarbonate anion. Such behavior stands in marked contrast to that seen with **1**. This structure-based disparity leads us to predict that it should be possible to design further-improved anion extractants and carriers based on the calixpyrrole framework. Work along these latter lines is currently in progress.

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Supporting Information Available: Details of vesicle preparation and transport studies, including U-tube and DPPC mobility studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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