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meso-Octamethylcalix[4]pyrrole: an old yet new transmembrane ion-pair transporter†,‡

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The first example of lipid bilayer membrane transport of a salt by a calix[4]pyrrole is reported.

The development of ditopic ion-pair receptors has recently attracted much interest.[1] Ditopic receptors that simultaneously bind anion and cation pairs have advantages in terms of substrate affinity and selectivity over monotopic hosts. Moreover, in systems where the bound cation and anion carry the same charge, the supramolecular host–salt complex formed will be neutral. This gives the complex optimal solubility in organic solvents, an important advantage when applying these systems in extraction processes or as transmembrane carriers for charged species. On the other hand, to the best of our knowledge there is only one example of a synthetic molecule capable of simultaneous transmembrane ion-pair transport across a lipid bilayer,[2] and no examples of natural products capable of salt binding and transport, although related formal H^+/Cl^- co-transport is a common mechanism in both natural (prodigiosins)[3] and synthetic chloride carriers.[4]

Calix[4]pyrrole derivatives have been extensively studied as anion receptors over the past few years.[5] These compounds can be made in high yield in one step and are easy to functionalise, making them attractive binding motifs for an-ionic guests. More recently their potential as ion-pair receptors has been recognised.[6] Preorganisation of the macrocycle into a cone conformation upon anion binding allows the inclusion of large, charge diffuse cations such as caesium or organic cations such as imidazolium or pyridinium into the aromatic “cup”, as demonstrated both in solution and solid state studies.[7] These findings led to the application of *meso*-octamethylcalix[4]pyrrole as an extractant for halide salts from aqueous to organic solutions.[8,9] Herein we report the first example of transmembrane transport in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and POPC–cholesterol vesicles of a salt by *meso*-octamethylcalix[4]pyrrole.

†This *communication* is dedicated to an inspiring leader in the supramolecular chemistry arena, Professor Seiji Shinkai, on the occasion of his 65th birthday.

‡Electronic supplementary information (ESI) available: Synthesis and characterisation data of compound **4**. See DOI: 10.1039/b814988g

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Calix[4]pyrrole derivatives **1–5** (Fig. 1) were synthesised by condensation of pyrrole and the appropriate ketone according to literature procedures.[10‡] In order to study the transport properties of these macrocycles we prepared unilamellar POPC vesicles[11] loaded with CsCl and suspended them in an external $NaNO_3$ solution. A sample of the respective calix[4]pyrrole **1–5** (2% molar carrier to lipid) was added as a DMSO solution and the resultant Cl^- efflux monitored using a chloride selective electrode.[12] 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 Fig. 2.

Under these conditions *meso*-octamethylcalix[4]pyrrole **1** showed excellent transport activity inducing the release of virtually all of the encapsulated chloride within 5 min. Different substitution at the *meso*-position of the macrocycle resulted in a dramatic loss of transport activity with only the tetraspirocyclohexyl derivative **5** showing detectable activity as a carrier. There is no immediate explanation for the lack of activity of compounds **2–4**, although the possibility that poor solubilities of these derivatives under the conditions of the assay hamper the incorporation of the macrocycles into the phospholipid bilayer cannot be ruled out. We then explored the cation selectivity of the transport process and similar assays using NaCl, KCl and RbCl loaded vesicles were carried out (Fig. 3). Lack of chloride efflux in these experiments is evidence that supports the hypothesis that chloride is transported selectively as a calixpyrrole-bound ion-pair with caesium. These assays also rule out the possibility of Cl^- efflux being promoted by simple disruption of the vesicles by the macrocycle. We also wished to investigate the influence of the composition of the external medium on the transport activity shown by **1**. For this purpose CsCl loaded vesicles were suspended in buffered Na_2SO_4 solution (Fig. 3). The result shows that carrier activity is essentially maintained and is independent of the external anion, further supporting a CsCl co-transport mechanism for this process (Scheme 1).

We screened the transport activity of **1** through a range of carrier concentrations (20–0.1 μM, 2–0.01 mol% carrier to lipid) repeating the transport assays using different amounts of macrocycle (Fig. 4). Almost complete chloride efflux is obtained with loadings of 2–0.1% molar carrier to lipid within the first five minutes of the experiment; meanwhile the transport activity clearly decreases with lower carrier concentrations, although detectable activity is observed even at 0.01% molar carrier to lipid (0.1 μM) concentrations.

Finally, in order to shed light into the transport mechanism of the process we performed transport assays using vesicles composed of phospholipid and cholesterol. The results, shown in Fig. 5, clearly indicate a significant reduction in the transport activity of **1** when the vesicle bilayer composition includes cholesterol. Incorporation of the steroid to the bilayer membrane significantly reduces its fluidity and this result indicates that a carrier mechanism is likely for this process.[13]

In conclusion, we have demonstrated that *meso*-octamethylcalix[4]pyrrole mediates the selective transport of an ion pair (CsCl) through model phospholipid bilayers. Efforts aimed towards producing efficient ion-pair carriers with different selectivities are currently underway in our laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

‡Electronic supplementary information (ESI) available: Synthesis and characterisation data of compound **4**. See DOI: 10.1039/b814988g

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11. A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (20 mg mL^{-1}) (Genzyme) or a 70: 30 POPC–cholesterol mixture was evaporated using a rotary evaporator and the lipid film obtained was dried under high vacuum for at least 2 h. The lipid film was rehydrated by addition of a metal chloride (MCl) salt solution (488 mM MCl and 5 mM phosphate buffer, pH 7.2) and careful vortexing. The lipid suspension was then subjected to nine freeze–thaw cycles and twenty-nine extrusions through a 200 nm polycarbonate nucleopore membrane using a LiposoFast Basic extruder (Avestin, Inc.) to obtain unilamellar vesicles. The vesicles were dialysed against a $NaNO_3$ solution (488 mM $NaNO_3$ and 5 mM phosphate buffer, pH 7.2) or Na_2SO_4 (162 mM Na_2SO_4 and 5 mM phosphate buffer, pH 7.2) to remove unencapsulated MCl salts.

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was used: unilamellar vesicles (200 nm mean diameter) composed of POPC or POPC–cholesterol containing an encapsulated solution of 488 mM MCl and 5 mM phosphate buffer pH 7.2, were suspended in a solution of 488 mM $NaNO_3$ and 5 mM phosphate buffer pH 7.2, or 162 mM Na_2SO_4 and 5 mM phosphate buffer pH 7.2, for a final lipid concentration of 1 mM. A DMSO solution of the carrier molecule, typically 10 μL to avoid influence of the solvent molecules in the assay, was added and the chloride release from vesicles was monitored using an Accumet chloride selective electrode for 7 min. At a time (t) = 5 min the vesicles were lysed with detergent (polyoxyethylene (8) lauryl ether) to release all chloride ions; the resulting value was considered to represent 100% release and used as such.

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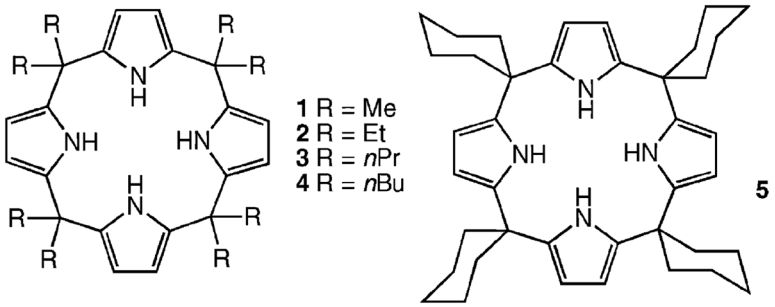


Fig. 1.
Structures of calix[4]pyrroles **1**–**5**.

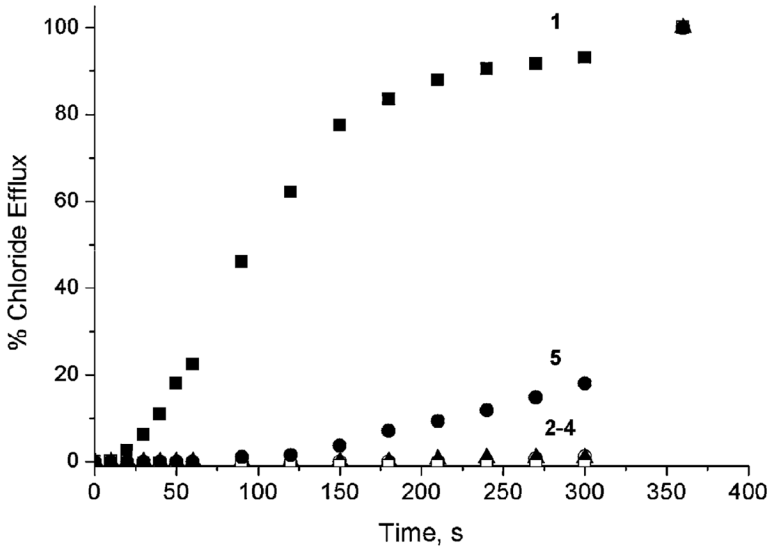


Fig. 2.

Chloride efflux promoted upon addition of **1** (■), **2** (○), **3** (▲), **4** (□), and **5** (●) (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.

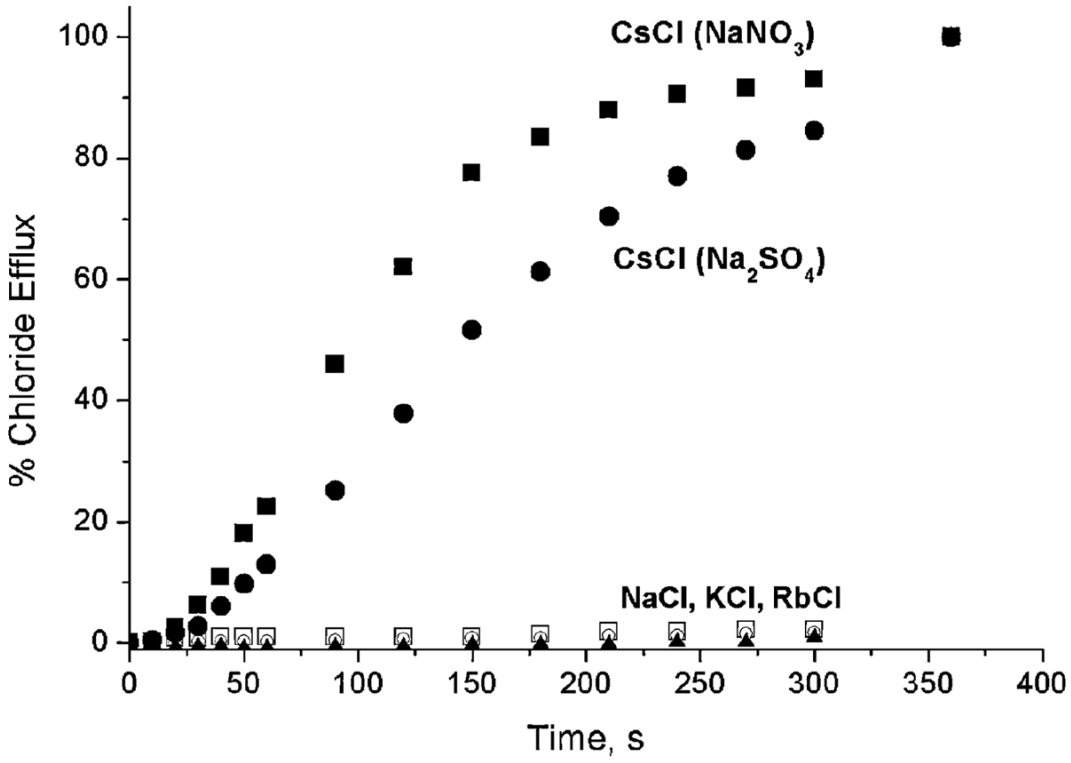


Fig. 3.

Chloride efflux promoted upon addition of **1** (2% molar carrier to lipid) to unilamellar POPC vesicles loaded with 488 mM CsCl (■), or RbCl (○), or KCl (▲), or NaCl (□), 5 mM phosphate buffer, pH 7.2, dispersed in 488 mM $NaNO_3$, 5 mM phosphate buffer, pH 7.2, and unilamellar POPC vesicles loaded with 488mMCsCl (●), 5 mM phosphate buffer, pH 7.2, dispersed in 162 mM Na_2SO_4.

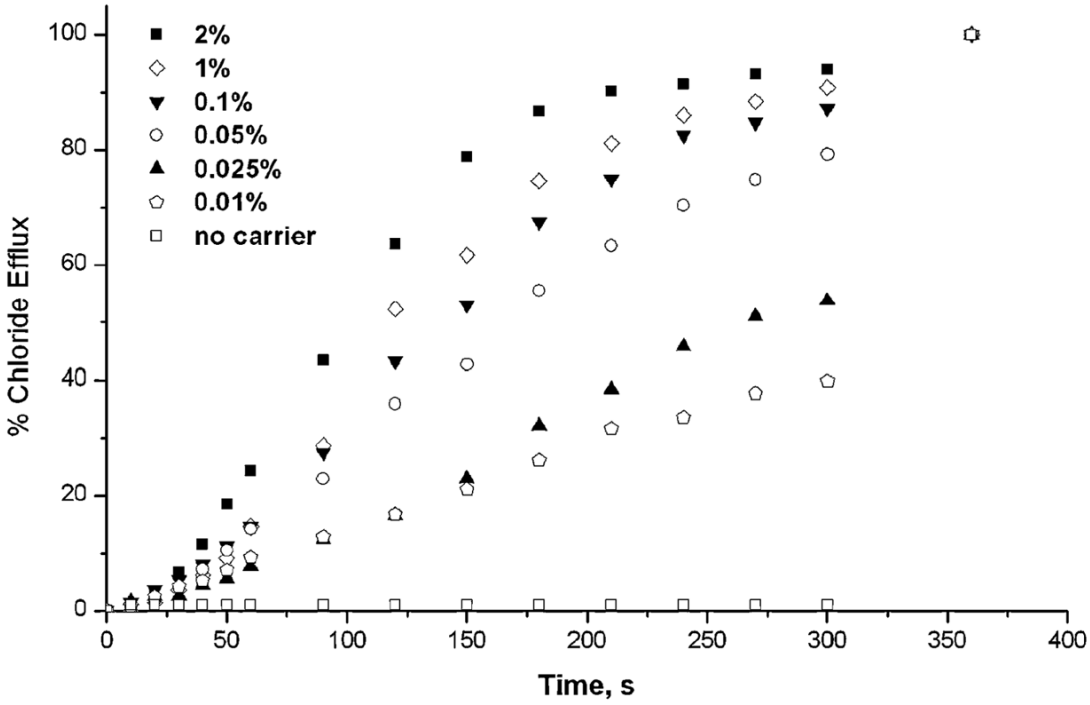


Fig. 4.

Chloride efflux promoted upon addition of **1** (20–0.1 μM; 2–0.01% 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.

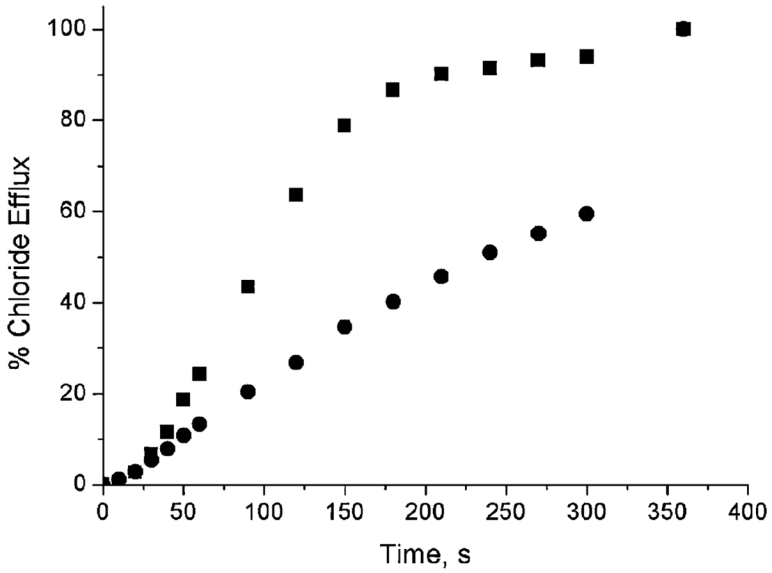
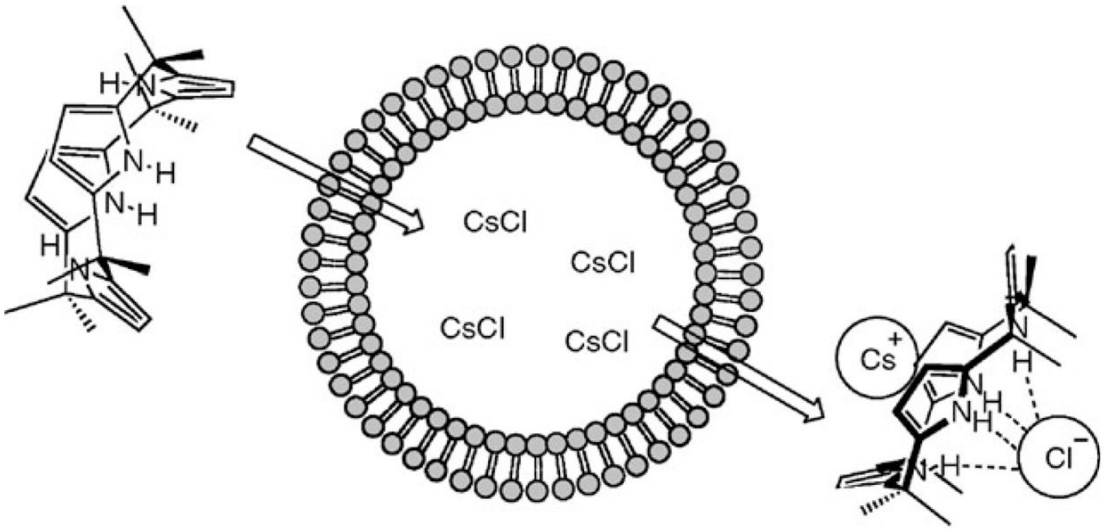


Fig. 5.

Chloride efflux promoted upon addition of **1** (2% molar carrier to lipid) to unilamellar POPC vesicles (■), or POPC–cholesterol (70: 30 molar ratio) (●) 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.



Scheme 1.
Proposed mechanism for chloride efflux promoted by calix[4]pyrrole **1**.