1,2,3-Triazole-strapped calix[4]pyrrole: a new membrane transporter for chloride†

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A new triazole strapped calix[4]pyrrole synthesised *via* 'click' chemistry shows high affinity for chloride and lipid bilayer chloride transport properties.

Calix[4]pyrroles such as *meso*-octamethylcalix[4]pyrrole **1** have been extensively studied as anion and ion-pair receptors over recent years.[1] Subsequently 'strapped' calix[4]pyrroles, pioneered by Lee and Sessler, have been synthesised that contain a single linker between distal *meso*-positions. These compounds have increased affinity for anions relative to the parent macrocycle.[2] Recently there has been increased interest in CH hydrogen bond donors[3] and in particular in the use of 1,2,3-triazoles formed *via* 'click' chemistry[4] as hydrogen bond donor groups for anion complexation.[5] We decided to use 'click' chemistry to introduce a triazole strap into a calix[4]pyrrole and measure the anion binding[6] and transport properties[7] of the resulting hybrid pyrrole/triazole anion receptor **2** (Figure 1).

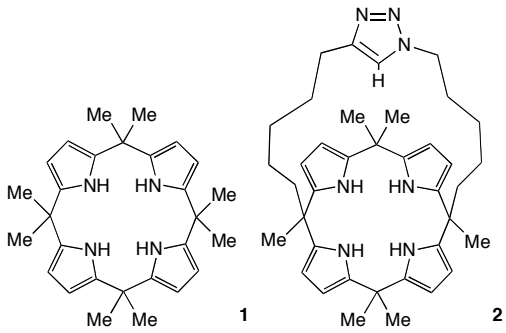

Fig. 1 Structure of *meso*-octamethylcalix[4]pyrrole **1** and strapped calix[4]pyrrole derivative **2**.

Full details of the synthesis of compound **2** are are provided in the ESI. Single crystals of compound **2** were prepared by recrystallisation from methanol. ¶ The structure (shown in Figure 2) reveals the calix[4]pyrrole adopting a 1,2-alternate conformation in which two methanol molecules are each bound to two pyrrole NH groups. Crystal structures of two DMSO solvates of compound **2** have also been elucidated (see ESI).

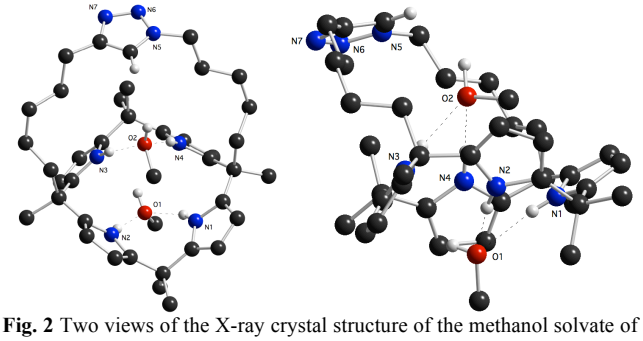

Fig. 2 Two views of the X-ray crystal structure of the methanol solvate of **1** showing the calixpyrrole macrocycle adopting the 1,2-alternate conformation.

The anion binding properties of compound **2** were studied by ^{1}H NMR titration techniques in acetonitrile-d_3 solution. Under these conditions addition of chloride resulted in slow exchange on the NMR timescale (Figure 3) with downfield shifts of the pyrrole NH groups and triazole CH indicative of hydrogen bond formation. The slow exchange properties led us to study the anion binding properties of compound **2** by isothermal calorimetry.

In acetonitrile, the host-guest interaction of **2** and tetraethylammonium chloride appears as a clean, exothermic 1:1 stoichiometric binding process that is symmetric at millimolar concentrations (the result is independent of the sequence of addition of the binding partners; Figure 4). In comparison to the parent calixpyrrole **1** under the same conditions[1b] the enthalpy of complexation with **2** is substantially more negative as is expected from the participation of an extra triazole-chloride hydrogen bond. However, this improved stickiness of **2** over **1** is again counteracted by an, on average, a slightly more negative entropy component resulting in an outcome that improves affinity due to the triazole strap by about an order of magnitude in K_{ass} only.

The strap also levels off the differences in complex stability depending on the solvent. Whilst in the case of the parent calixpyrrole **1** chloride binding in dichloromethane is weaker than in the more polar solvent acetonitrile because of an unfavorable complexation entropy overcompensating the more exothermic enthalpy output there is almost an ideal balance of the enthalpic/entropic gains and losses in the strapped host **2** attesting to the less significant role of solvation in the latter case. Even under the most severe hydrogen bonding competition in methanol, **2** shows an exothermic response in chloride binding which, however, did not suffice to derive an association constant.

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† Electronic Supplementary Information (ESI) available: Synthesis and characterization data of new compounds, vesicle preparation and transport assays details. See http://dx.doi.org/10.1039/b000000x/

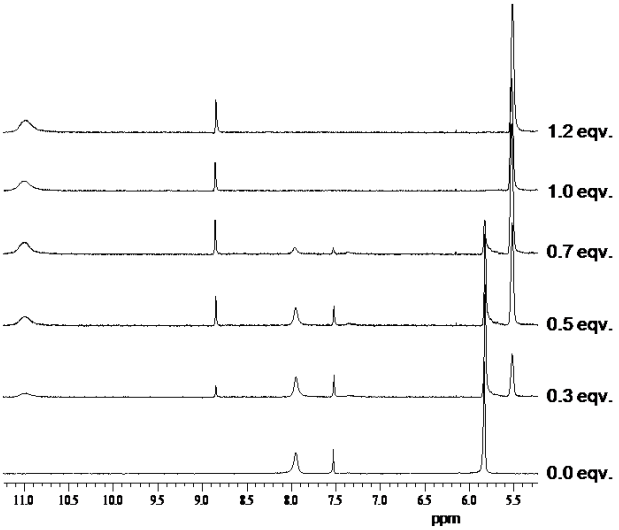

Figure 3 Partial ^{1}H NMR spectrum (300 MHz) of compound **2** upon addition of tetrabutylammonium chloride in acetonitrile-d_3 solution at 298K.

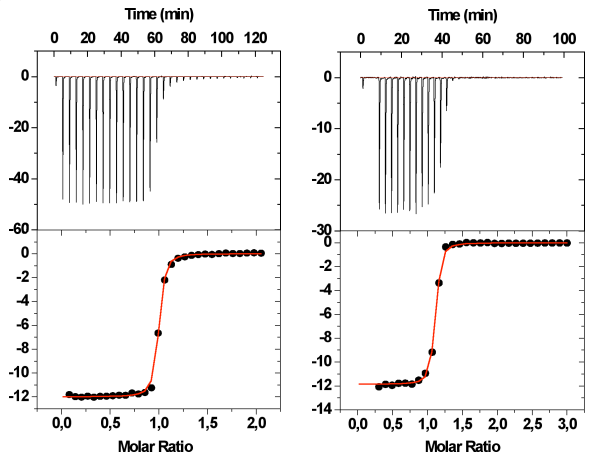

Figure 4 Isothermal calorimetric titration in acetonitrile at 303 K of tetraethylammonium chloride added into the solution of host **2** at 0.94 mM (left) or host **2** titrated into tetraetylammonium chloride solution at 0.327 mM (right)

[Please insert Table 1 at the bottom of this page – see below]

We have previously shown that compound **1** functions as a CsCl co-transporter across lipid bilayer membranes.[8] However, this compound showed no transport activity for other group 1 metal chloride salts. The selectivity was attributed to the ability of the macrocycle to bind chloride to the NH hydrogen bonding array and caesium within the cavity formed by the pyrrole rings upon anion complexation.[9] In order to study the transport properties of compound **2**, we prepared separate samples of unilamellar 1-palmitoyl-2-oleoylphophatidylcholine (POPC) vesicles loaded with CsCl, RbCl, KCl and NaCl and suspended them in an external $NaNO_3$ solution. A sample of calixpyrrole **2** (4% molar carrier to lipid) was added as a DMSO solution and the resultant Cl^- efflux monitored using a chloride selective electrode.[10] 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.

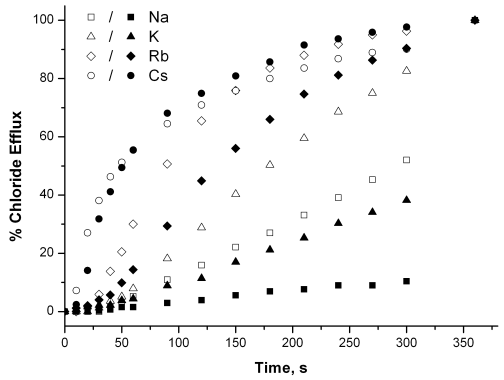

Figure 5 Chloride efflux promoted by **2** at 0.04 molar equivalents of carrier to lipid, across unilamellar POPC vesicles loaded with 489 mM NaCl (□/■), KCl (△/▲), RbCl (◇/◆) and CsCl (○/●) buffered to pH 7.2 with 5mM phosphate dispersed in 489 mM $NaNO_3$ (open symbols) or 167 mM Na_2SO_4 (closed symbols) buffered to pH 7.2 with 5 mM phosphate.

The results show that compound **2** (Figure 5), in contrast to compound **1**,[10] is capable of transporting chloride from sodium, potassium and rubidium chloride containing vesicles with fastest release of the larger group 1 metal cation salts.

We wished to investigate the influence of the composition of the external media on the transport activity shown by **2**. For this purpose group 1 metal chloride loaded vesicles were suspended in a Na_2SO_4 buffer. The results (Figure 5) show that carrier activity is essentially maintained although there is an increasing difference in the efficiency of transport as the metal ion size decreases. Sulfate is an extremely hydrophilic anion and is not normally possible to extract this anion from an aqueous phase into a lipid bilayer membrane. In the case of the release of chloride mediated by compound **2** from caesium chloride containing vesicles there is little difference between the rate of chloride release as the exterior anion is changed from nitrate to sulfate. However, as the cation size decreases there is an increasing difference in rate of release with, in the case of potassium and sodium, a considerably faster release of chloride from vesicles suspended in nitrate solution compared to those suspended in sulfate solution. This evidence leads us to suggest that both ion-pair co-transport and chloride-nitrate antiport mechanisms are responsible for the release of chloride. As the cation size decreases, the transport mechanism changes from a predominantly ion-pair transport mechanism in the case of caesium chloride to a predominanty chloride – nitrate antiport process in the cases of sodium and potassium.

In order to provide evidence on the mechanism of the transport process we performed transport assays using vesicles composed of a 70:30 mixture of POPC and cholesterol. The results shown in the Figure 6 clearly indicate a significant reduction of the transport activity of **2** when the vesicle bilayer composition includes cholesterol. Addition of cholesterol into the bilayer membrane significantly reduces its fluidity and this result is fully consistent with the process in question being governed by a carrier mechanism, rather than, for instance, channel formation.[11]

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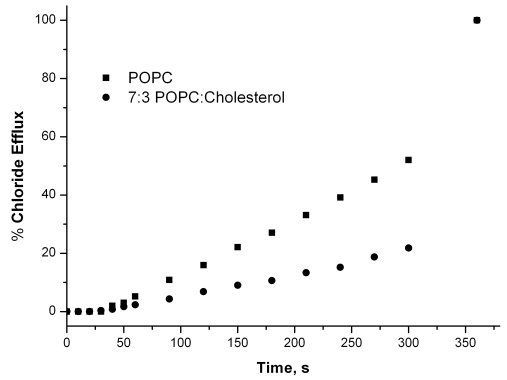

Figure 6 Chloride efflux promoted by **2**, at 0.04 molar equivalents of carrier to lipid, across unilamellar vesicles made of POPC (■) or POPC with 30 mol% of cholestrol (●) loaded with 489 mM NaCl buffered to pH 7.2 5mM phosphate buffer. The vesicles were dispersed in 489 mM $NaNO_3$.

Strapped calix[4]pyrrole **2** functions as a chloride transporter in synthetic POPC and POPC/cholesterol vesicles. We propose that two mechanisms may be responsible for the release of chloride dependent upon the nature of the counter cation, namely ion-pair co-transport and chloride-nitrate antiport. This is in contrast to the parent macrocycle **1** which functions as a caesium chloride co-transporter only. We are conducting studies to further probe anion transport mechanisms mediated by calix[4]pyrroles. The results of these studies will be reported in due course.

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Notes and references

¶ Crystal data for compound **2**·2MeOH $C_{40}H_{59}N_7O_2$, Mr = 669.94, T = 120(2) K, Orthorhombic space group *Pbca*, a = 10.2826(2), b = 20.5801(6), c = 35.6410(10) Å, V = 7542.2(3) Å^3, ρ_{calc} = 1.180 Mg m^{-3}, μ = 0.074 mm^{-1}, Z = 8, reflections collected: 43094, independent reflections: 6648 (R_{int} = 0.1269), final R indices [$I > 2\sigma I$]: $R1$ = 0.0656, $wR2$ = 0.1445, R indices (all data): $R1$ = 0.1318, $wR2$ = 0.1737.

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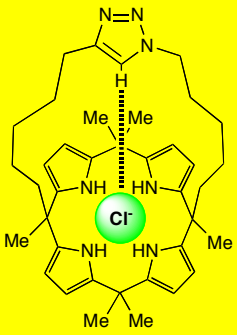
Graphical abstract



A new 1,2,3-triazole strapped calix[4]pyrrole functions as a chloride transporter in lipid bilayer membranes via both ion-pair transport and anion antiport processes.

		acetonitrile				dichloromethane			
host	mode of titration	K_{ass} [M^{-1}]	ΔG^o/ kcal mol^{-1}	ΔH^o/ kcal mol^{-1}	$T\Delta S^o$/ kcal mol^{-1}	K_{ass} /M^{-1}	ΔG^o/ kcal mol^{-1}	ΔH^o/ kcal mol^{-1}	$T\Delta S^o$/ kcal mol^{-1}
1[1b]		1.9 e5	-7.19	-10.1	-3.07	4.9 e4	-6.33	-10.96	-4.63
2	host⇨guest	2.6 e6	-8.89	-11.9	-2.96	2.7 e6	-8.91	-13.2	-4.3
2	guest⇨host	1.3 e6	-8.47	-12.0	-3.52	not determined			

Table 1: Energetics of tetraethylammonium chloride binding to calixpyrroles **1** and **2** at 303 K as determined by isothermal titration calorimetry

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