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Calix[4]pyrrole-based anion transporters with tuneable transport properties†

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Three new bis-1,2,3-triazole strapped calix[4]pyrroles have been prepared *via* 'click' chemistry and their anion complexation and lipid bilayer transport properties studied by a combination of single crystal X-ray diffraction studies, ^{1}H NMR titration techniques, isothermal titration calorimetry and lipid bilayer anion transport studies in POPC vesicles. Bilayer transport efficiency for transmembrane chloride transport was found to directly depend on the length of the alkyl chain present in the bis-triazole strap.

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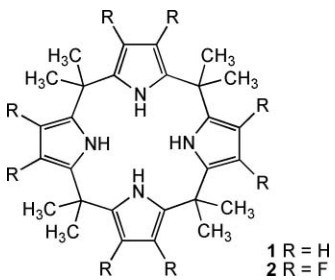
Introduction

The development of new compounds to facilitate anion transport through lipid bilayer membranes[1] is an area of intense interest and research activity due to the potential future application of these species to diseases such as cystic fibrosis that result from dysregulation of anion transport through cell membranes.[2]

The calix[4]pyrroles (such as *meso*-octamethylcalix[4]pyrrole **1**) are a class of tetrapyrrolic macrocycle, known for over a century,[3] whose application to anion complexation was first reported in 1996 by Sessler and co-workers.[4] Since then these compounds and their derivatives have been shown to be effective extractants for anions from aqueous to organic solution showing in some cases anti-Hofmeister bias.[5] Additionally compound **1** has been shown to be capable of binding ion pairs with the anion bound to the pyrrole NH groups and large charge diffuse cations such as caesium occupying the cup shaped cavity formed by the four pyrrole rings.[6] Strapped calix[4]pyrroles have emerged more recently as a series of derivatives which possess significantly higher affinities for anions than the parent macrocycles.[7] These compounds contain a linker between distal *meso*-positions which may contain additional hydrogen bond donor groups that contribute to the stability of anion complexes.



In 2008, we reported the first evidence that *meso*-octamethylcalix[4]pyrrole **1** could facilitate the diffusion of anions across a lipid bilayer.[8] Specifically, we demonstrated that compound **1** could bind and transport caesium chloride across a POPC lipid bilayer membrane. The limited transport properties of this parent system were expanded upon by increasing the affinity of receptor for anionic species. For example octafluorocalix[4]pyrrole **2**, a derivative with enhanced anion affinity due to the presence of electron withdrawing fluorine substituents, functions as a bicarbonate transporter.[9]

In 2009, we reported that compound **3**, a calix[4]pyrrole strapped by an alkyl chain containing a single 1,2,3-triazole group, also has higher affinity for chloride than the parent macrocycle and functions as a caesium chloride co-transporter.[10] 1,2,3-Triazoles have recently been shown by Flood and others to be effective hydrogen bond donor groups with the polarization of the heterocycle resulting in the CH group forming strong hydrogen bonding interactions with halides.[11] We found that the introduction of the triazole strap to compound **3** resulted in a new mode of transport across POPC membranes (chloride/nitrate antiport) that was not observed with parent compound **1**. We wished to examine more closely the role played by the strap in modulating the transport efficiency and mechanism of a calixpyrrole. We therefore synthesised three new compounds **4–6** (Scheme 1) containing a strap composed of two 1,2,3-triazole groups linked by an alkyl chain of variable length (3–5 carbon atoms) and studied their anion complexation and transport properties by a combination of NMR, X-ray crystallography, isothermal titration calorimetry and membrane transport studies. The length of the linker is shown to have a profound effect on the ability of these species to transport anions across POPC lipid bilayer membranes and the mechanism of transport.

Results and discussion

Synthesis of strapped calix[4]pyrroles

The synthesis of the strapped calix[4]pyrroles **4–6** is shown in Scheme 1. The key compounds, diketones (**14–16**) containing two

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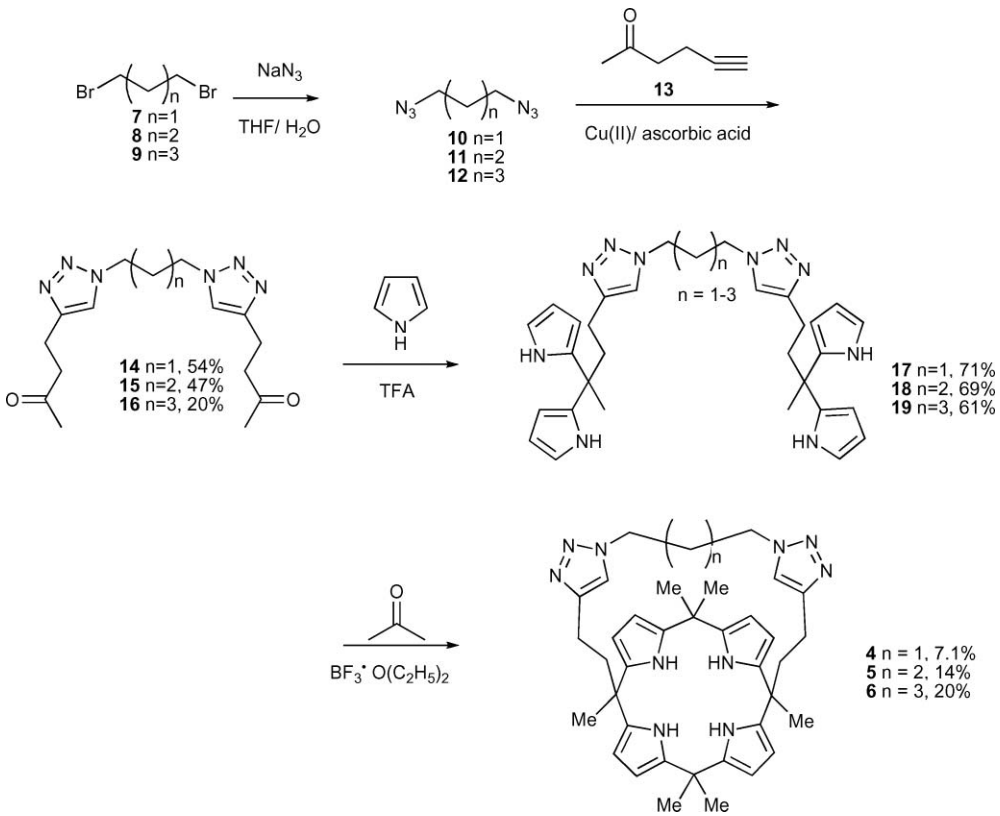
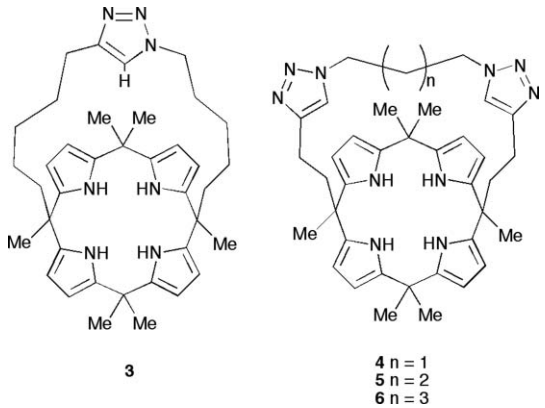


Scheme 1 Synthesis of strapped calix[4]pyrroles **4–6**



triazole rings, were successfully prepared *via* a copper catalysed azide–alkyne condensation ('click' reaction).[12] Dibromoalkanes (**7–9**) were reacted with sodium azide to prepare bis azides (**10–12**) *in situ*, which were subsequently coupled with 5-hexyn-2-one (**13**)[13] to afford the diketones (**14–16**). The diketones were reacted with freshly distilled pyrrole in the presence of a catalytic amount of trifluoroacetic acid to afford **17–19**, respectively. The acid catalyzed condensation of **17–19** with acetone resulted in the formation of desired strapped calix[4]pyrroles (**4–6**). All new compounds were fully characterized by ^{1}H NMR, ^{13}C {^{1}H}NMR and mass spectrometry.

X-Ray crystallography

Single crystals of compounds **5**‡ and **6**§‡ were obtained by slow evaporation of methanol solutions (containing a small amount of dichloromethane) of the receptors and the structures obtained by single crystal X-ray diffraction techniques. The structures shown in Fig. 1 and 2 respectively reveal that the calixpyrrole macrocycles adopt the 1,2-alternate conformation in the solid state binding two equivalents of methanol.[14] A further molecule of methanol is bound by a single hydrogen bond to one of the complexed methanol molecules in both cases. Additionally, the structure of a mixed methanol–acetonitrile solvate of compound **5** has been elucidated (see ESI†).

‡ Crystal data for compound **5**·3CH$_3$OH, $C_{38}H_{48}N_{10}$·3(CH_4O), M = 740.99, Monoclinic, a = 10.0844(2), b = 12.6130(3), c = 32.3426(7) Å, α = 90.00°, β = 98.7700(10)°, γ = 90.00°, U = 4065.70(15) Å^3, T = 120(2) K, space group $P2_1/n$, Z = 4, 38876 reflections measured, 9262 unique reflections (R_{int} = 0.0600). The final R_1 values were 0.0745 ($I > 2\sigma(I)$). The final w$R(F_2)$ values were 0.1336 ($I > 2\sigma(I)$). The final R_1 values were 0.1074 (all data). The final w$R(F_2)$ values were 0.1495 (all data). CCDC Deposition number: 769114.

§ Crystal data for compound **6**·3MeOH, $C_{39}H_{50}N_{10}$·3(CH_4O), M = 755.02, Monoclinic, a = 10.0381(5), b = 13.1722(6), c = 31.7659(15) Å, α = 90.00°, β = 96.894(2)°, γ = 90.00°, U = 4169.8(3) Å^3, T = 120(2) K, space group $P2_1/n$, Z = 4, 33744 reflections measured, 7352 unique reflections (R_{int} = 0.1267). The final R_1 values were 0.1101 ($I > 2\sigma(I)$). The final w$R(F_2)$ values were 0.2636 ($I > 2\sigma(I)$). The final R_1 values were 0.1709 (all data). The final w$R(F_2)$ values were 0.2949 (all data). CCDC Deposition number: 769115.

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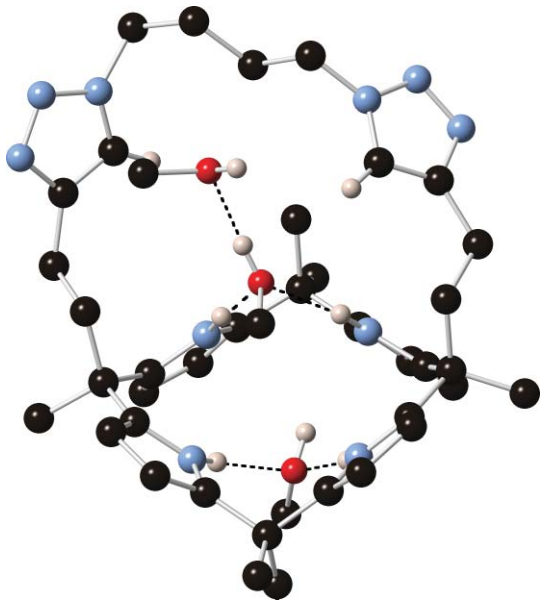

Fig. 1 The X-ray crystal structure of the methanol solvate of compound **5**. Non-acidic hydrogen atoms have been omitted for clarity.

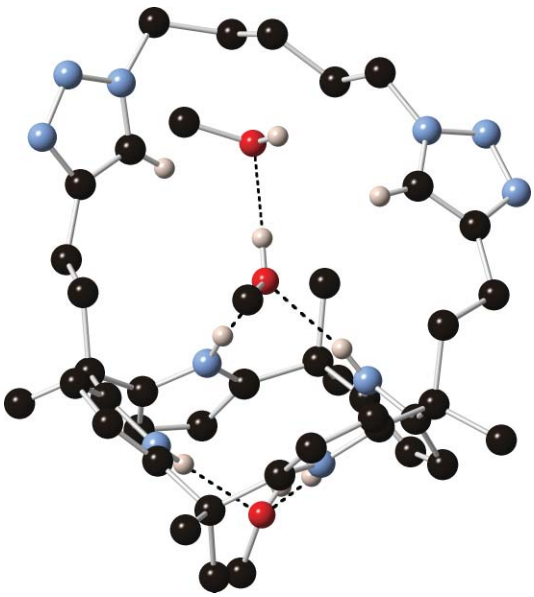

Fig. 2 The X-ray crystal structure of the methanol solvate of compound **6**. Non-acidic hydrogen atoms have been omitted for clarity.

Anion complexation studies

Initial anion complexation studies focused upon obtaining single crystals of anion complexes of the strapped calixpyrroles.

Crystals of the tetrabutylammonium chloride complex of compound **5** were obtained by slow evaporation of a methanol–dichloromethane solution of the receptor in the presence of five equivalents of tetrabutylammonium chloride.¶§ The structure shows the chloride anion bound to the four pyrrole rings *via* N···Cl interactions in the range 3.273(3)–3.315(2) Å, one triazole C···Cl$^-$ hydrogen bond 3.625(3) Å. The other triazole group does not bind the chloride guest in the solid state. The axial CH_2 groups attached to the *meso*-positions of the calixpyrrole in the complex are within hydrogen bonding distance of the chloride[15] with C···Cl$^-$ distances of 3.663(3) and 3.660(3) Å (not shown in Fig. 3).

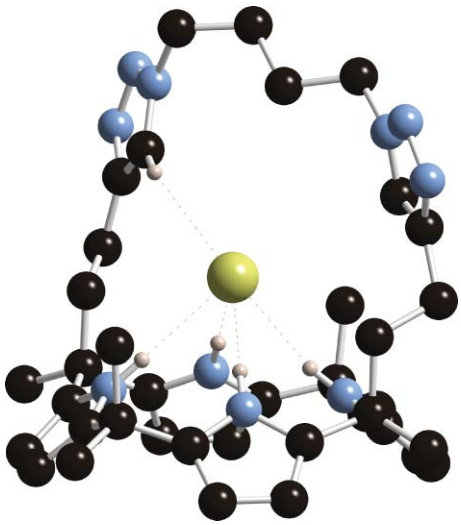

Fig. 3 The X-ray crystal structure of the chloride complex of compound **5**. Non-acidic hydrogen atoms, disorder, methanol and the tetrabutylammonium counter cation have been omitted for clarity.

Crystals of the tetrabutylammonium chloride complex of compound **6** were obtained by slow evaporation of a methanol–dichloromethane solution of the receptor in the presence of five equivalents of tetrabutylammonium chloride.‖ The structure shown in Fig. 4 reveals the chloride anion bound by six hydrogen bonds, four from the pyrrole NH group in the range 3.275(4)–3.295(4) Å and two from the triazole CH groups of 3.676(8) and 3.666(9) Å in one of the orientations of the disordered strap. The axial CH_2 or CH_3 groups attached to the calixpyrrole *meso*-positions in the complex have four C···Cl contacts in the range 3.715(6)–3.831(5) Å (not shown in Fig. 4). Similar contacts occur in other calixpyrrole anion complexes.[4,6]

The structure of the tetrabutylammonium bromide complex of compound **5** has also been elucidated (see the ESI†).

The anion binding properties of compounds **4–6** were studied by ^{1}H NMR titration techniques in CD_2Cl_2 solution. In all three cases under these conditions, addition of tetrabutylammonium chloride resulted in slow exchange on the NMR timescale between the free ligand and chloride complex with downfield shifts of the pyrrole NH groups and triazole CH indicative of hydrogen bond formation. The ^{1}H NMR spectra upon addition of chloride to compound **5** are shown in Fig. 5.

¶ Crystal data for compound **5**.$C_{16}H_{36}N.Cl.MeOH$, $C_{16}H_{36}N$ $C_{38}H_{48}N_{10}.CH_4O.Cl$, M = 954.81, Monoclinic, a = 10.55010(10), b = 27.0192(6), c = 19.2592(4) Å, α = 90.00°, β = 92.7850(10)°, γ = 90.00°, U = 5483.45(17) Å^3, T = 120(2) K, space group $P2_1/m$, Z = 4, 48202 reflections measured, 9884 unique reflections (R_{int} = 0.0792). The final R_1 values were 0.0889 ($I > 2\sigma(I)$). The final $wwR(F_2)$ values were 0.1581 ($I > 2\sigma(I)$). The final R_1 values were 0.1274 (all data). The final $wR(F_2)$ values were 0.1784 (all data). CCDC Deposition number: 769116.

‖ Crystal data for compound **6**.$C_{16}H_{36}N.Cl$, $C_{39}H_{50}N_{10}.C_{16}H_{36}N.Cl$, M = 936.80, Monoclinic, a = 10.5941(2), b = 15.6673(3), c = 31.9512(5) Å, α = 90.00°, β = 96.9750(10)°, γ = 90.00°, U = 5264.04(16) Å^3, T = 120(2) K, space group $P21/c$, Z = 4, 41611 reflections measured, 9255 unique reflections (R_{int} = 0.0442). The final R_1 values were 0.1182 ($I > 2\sigma(I)$). The final $wR(F_2)$ values were 0.2964 ($I > 2\sigma(I)$). The final R_1 values were 0.1410 (all data). The final $wR(F_2)$ values were 0.3195 (all data). CCDC Deposition number: 769117.

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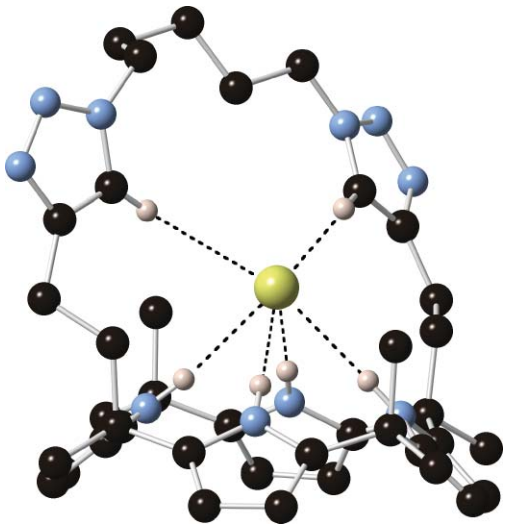

Fig. 4 The X-ray crystal structure of the chloride complex of compound **6**. Non-acidic hydrogen atoms, disorder and the tetrabutylammonium counter cation have been omitted for clarity.

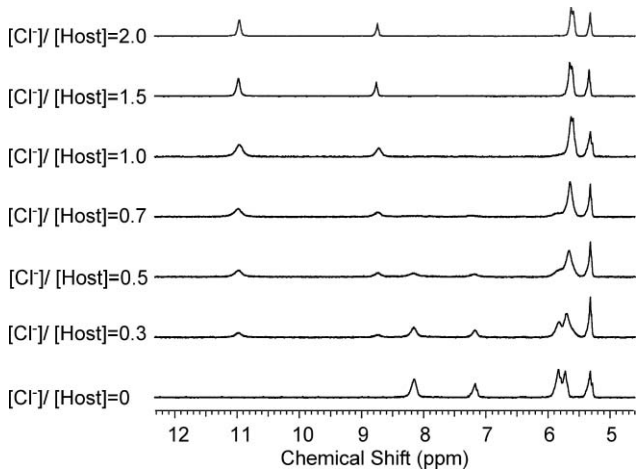

Fig. 5 Proton NMR titration of compound **5** with tetrabutylammonium chloride.

Investigations of the binding energetics by ITC

Receptor design depends upon balancing all the various energetic and structural contributions of the system. The standard host–guest binding free energy ΔG° and the transport properties of membrane carriers are not related to the number and type of attractive interaction within the receptor binding sites. With a series of related receptors in hand (**1**, **3**, **4**, **5**, **6**), it is possible to elucidate the effect the triazole straps have on the stability of the complex. In order to allow for a meaningful trend analysis we investigated a variety of anionic guests, distinguished by their chemical nature and size (halides, oxoanions, cyanide; all introduced as tetraalkylammonium salts) by isothermal titration calorimetry (ITC) in acetonitrile at 303 K. The solvent was chosen for solubility reasons and also to compare with previous results from the literature. Although acetonitrile is a good solvent for salts, it does not interfere with the hydrogen bonding interactions in these systems. The results of the calorimetric studies using receptor **1** is shown in Table 1 and the novel receptors **3–6** are shown in Tables 2–5.

Table 1 Thermodynamic state functions of the complexation of tetraalkylammonium salts by calixpyrrole **1** as measured by ITC in acetonitrile at 303 K; TEA = tetraethylammonium., TBA = tetrabutylammonium

entry	salt	K_{ass}/M^{-1}	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^\circ$/kcal mol^{-1}
1[16]	TEA(Cl)	1.9×10^5	−7.2	−10.1	−3.07
2[17]	TEA(Br)	2.8×10^3	−4.77	−8.34	−3.56
3[17]	TBA(H_2PO_4)	1.5×10^4	−5.79	−11.6	−5.8

In all cases we observed exothermic guest complexation that is compensated to an appreciable extent by an unfavourable entropy contribution. Except for the hydrogencarbonate and dihydrogenphosphate that show indications of more complex binding equilibria at high anion : receptor molar ratios, all other titrations follow a 1 : 1 stoichiometric binding scheme quite closely. Strapping the calixpyrrole macrocycle with triazole-containing bridges invariably boosts the binding free energy and thus turns out to be a profitable general way for affinity enhancement. Chloride, bromide and dihydrogen phosphate are bound up to 50 times more strongly; however, the individual stepwise increases within the series of receptors follow more subtle trends. In terms of the magnitude of the absolute stability constants, chloride is always bound most strongly. The introduction of one triazole hydrogen bond donor group into the strap results in an improvement of the affinity by a factor of 10 (entries 1, 4) however the incorporation of a second yields only a rather marginal gain (entry 13). Surprisingly the second triazole moiety scarcely contributes to the overall enthalpy of binding (entries 4 *versus* 13, 19, 25). Lengthening the

Table 2 Thermodynamic state functions of the 1 : 1 complexation of tetraalkylammonium salts by triazolo-calixpyrrole **3** as measured by ITC in acetonitrile at 303 K.[10]

entry	salt	K_{ass}/M^{-1}	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^\circ$/kcal mol^{-1}
4	TEA(Cl)[a]	$2.6 \times 10^6 \pm 3.2 \times 10^5$	−8.89	−11.88 ± 0.037	−2.9
5	TEA(Cl)[b]	$1.3 \times 10^6 \pm 3.2 \times 10^5$	−8.47	−12.0 ± 0.037	−3.5
6	TEA(benzoate)[b]	$6.0 \times 10^3 \pm 3.2 \times 10^5$	−5.23	−9.90 ± 0.037	−4.7
7	TBA(p-nitrobenzoate)[a]	$1.3 \times 10^3 \pm 3.2 \times 10^5$	−4.32	−8.54 ± 0.037	−4.2
8	TBA(AcO)[a]	$1.6 \times 10^5 \pm 3.2 \times 10^5$	−7.23	−13.1 ± 0.037	−5.9
9	TBA(F)·3H_2O[a]	$1.5 \times 10^5 \pm 3.2 \times 10^5$	−7.19	−11.34 ± 0.037	−4.1
10	TEA(NCO)[b]	$1.1 \times 10^6 \pm 3.2 \times 10^5$	−8.38	−13.3 ± 0.037	−4.9
11	TBA(H_2PO_4)	$7.8 \times 10^3 \pm 3.2 \times 10^5$	−5.39	−5.14 ± 0.037	+0.2
12	TEA(HCO_3)[b]	$2.0 \times 10^5 \pm 3.2 \times 10^5$	−7.36	−11.4 ± 0.037	−4.0

[a] Host solution titrated into guest solution. [b] Guest solution added into host solution.

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Table 3 Thermodynamic state functions of the 1 : 1 complexation of tetraalkylammonium salts by bis-triazolo-calix[4]pyrrole **4** as measured by ITC in acetonitrile at 303 K (host added to guest)

entry	salt	K_{ass}/M^{-1}	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^{\circ}$/kcal mol^{-1}
13	TEA(Cl)	$3.5 \times 10^6 \pm 3.2 \times 10^5$	–9.07	–12.6 ± 0.037	–3.5
14	TEA(Br)	$1.6 \times 10^4 \pm 4.9 \times 10^2$	–5.82	–9.1 ± 0.10	–3.2
15	TEA(HCO_3)	$1.3 \times 10^5 \pm 6.9 \times 10^4$	–7.08	–12.1 ± 0.10	–5.0
16	TEA(benzoate)	$2.7 \times 10^3 \pm 2.8 \times 10^2$	–4.75	–8.6 ± 0.76	–3.8
17	TBA(H_2PO_4)	$7.7 \times 10^3 \pm 2.9 \times 10^2$	–5.4	–16.7 ± 0.38	–11.2
18	TEA(CN)	$8.4 \times 10^4 \pm 1.7 \times 10^4$	–6.82	–14.1 ± 0.037	–7.3

Table 4 Thermodynamic state functions of the 1 : 1 complexation of tetraalkylammonium salts by bis-triazolo-calix[4]pyrrole **5** as measured by ITC in acetonitrile at 303 K (host added to guest)

entry	salt	K_{ass}/M^{-1}	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^{\circ}$/kcal mol^{-1}
19	TEA(Cl)	$8.7 \times 10^6 \pm 4.9 \times 10^5$	–9.61	–12.05 ± 0.037	–2.4
20	TEA(Br)	$1.7 \times 10^5 \pm 4.0 \times 10^4$	–7.25	–8.5 ± 0.037	–1.3
21	TEA(HCO_3)	$2.2 \times 10^5 \pm 8.6 \times 10^4$	–7.40	–12.4 ± 0.037	–5.0
22	TEA(benzoate)	$5.5 \times 10^4 \pm 1.1 \times 10^4$	–6.57	–12.16 ± 0.037	–5.6
23	TBA(H_2PO_4)	$5.0 \times 10^4 \pm 1.5 \times 10^4$	–6.55	–12.35 ± 0.074	–5.8
24	TEA(CN)	$3.8 \times 10^5 \pm 1.9 \times 10^4$	–7.71	–12.14 ± 0.037	–4.4

Table 5 Thermodynamic state functions of the 1 : 1 complexation of tetraalkylammonium salts by bis-triazolo-calix[4]pyrrole **6** as measured by ITC in acetonitrile at 303 K (host added to guest)

entry	salt	K_{ass}/M^{-1}	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^{\circ}$/kcal mol^{-1}
25	TEA(Cl)	$4.0 \times 10^6 \pm 4.7 \times 10^5$	–9.15	–11.56 ± 0.067	–2.4
26	TEA(Br)	$1.0 \times 10^5 \pm 2.5 \times 10^4$	–6.93	–9.22 ± 0.030	–2.3
27	TEA(I)	no significant heat effect			
28	TEA(HCO_3)	$1.5 \times 10^5 \pm 1.0 \times 10^4$	–7.17	–14.93 ± 0.16	–7.72
29	TEA(benzoate)	$5.3 \times 10^4 \pm 1.1 \times 10^4$	–6.55	–11.09 ± 0.041	–4.5
30	TBA(H_2PO_4)	$2.0 \times 10^4 \pm 9.3 \times 10^2$	–5.96	–12.12 ± 0.15	–6.2
31	TEA(CN)	$1.4 \times 10^5 \pm 2.6 \times 10^4$	–7.13	–16.84 ± 0.035	–9.7
32	TEA(AcO)	$2.8 \times 10^5 \pm 2.3 \times 10^4$	–7.55	–13.57 ± 0.11	–6.0
33	TEA(HSO_4)	no significant heat effect			

distance between the triazole modules diminishes the enthalpic interaction, whilst concomitantly raising the entropic component to more positive values by an equivalent amount. This almost ideal compensation is reminiscent of general solvent interactions and thus suggests the absence of a specific role of the second triazole unit in chloride complexation.

Interestingly, the larger bromide anion, despite its well established weaker hydrogen-bonding capacity, experiences a dramatic increase in complexation strength caused by the additional triazole hydrogen-bond donors. In this case both energetic components, the enthalpy and entropy, change favourably relative to the changes observed with parent calixpyrrole **1**, with the highest affinity for bromide found with strapped calixpyrrole **5** entirely on the basis of the entropic contribution. Iodide as the largest halide and poorest hydrogen bond acceptor does not show any sign of binding to these receptors at all. In contrast, basic anions like carboxylates and in particular cyanide form rather stable host–guest complexes in acetonitrile. The small and potent hydrogen bond acceptor cyanide shows a peculiarity in the series of bis-triazolo hosts: the intermediate-sized receptor **5** possesses the same enthalpic contribution for this anion as chloride, yet the more negative entropy renders the cyanide complex weaker than the chloride complex by a factor of 20. Nevertheless, it is the least negative entropy contribution in the series making **5** the best cyanide receptor. The hosts with larger or shorter straps both have substantially stronger binding enthalpies for cyanide, but their entropies are even more negative and largely wipe out the enthalpic advantage.

Most likely, the anions capable of bidentate hydrogen bonding (cyanide, isocyanate, oxoanions) exert an organizing effect on the host that encompasses both triazole moieties in cooperative fashion and leads to a better-defined structure of the host–guest complexes.

Amongst the simple carboxylates, we observe the anticipated trend in binding enthalpy with the hydrogen bond acceptor capacity that is tentatively correlated to the pK_a value of the conjugate acid.[18] Thus, for the monotriazole receptor **3**, acetate (pK_a 4.7) is more strongly bound than benzoate (pK_a 4.2) and *p*-nitrobenzoate (pK_a 3.4, entries 6, 7, 8). Host **6** shows a similar trend (entries 29, 32). Again, this clear-cut dependence is compromised by entropy compensation rendering the trend barely visible in the stability constants. A similar mechanism operates with the bicarbonate anion and in this case although the enthalpy of binding changes by more than 3.5 kcal within the series of triazolo-hosts (entry 12 *versus* 28) which is equivalent to a maximum ratio of binding constants of 350, the differences are compensated by the entropy contribution and are not apparent in the free energy of binding. Presumably, the amphoteric bicarbonate guest imposes an intense and specific influence on complex structure which is most apparent in the titration curve with receptor **6** depicted in Fig. 6.

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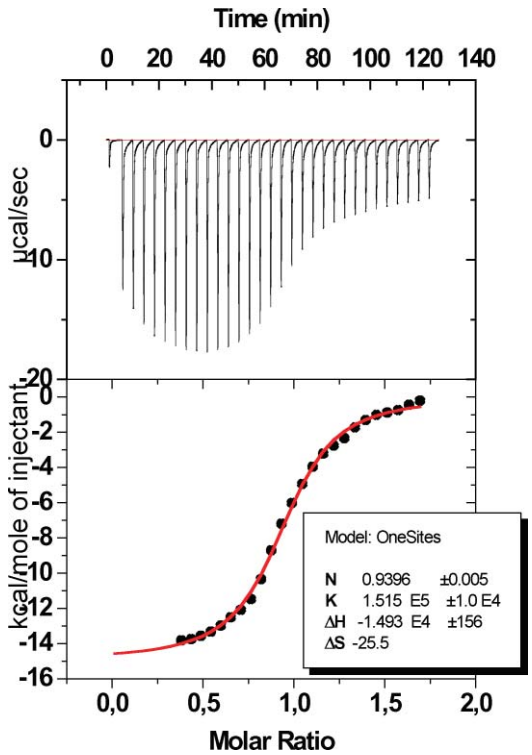

Fig. 6 ITC-titration in acetonitrile of tetraethylammonium bicarbonate (0.282 mM) with bis-triazolo-strapped host compound **6** at 303 K.

In addition to the dominant sigmoidal shape indicative of ordinary 1 : 1 host–guest binding, strong deviations from the ideal form occur at the beginning and towards the end of the titration suggesting more supramolecular or even covalent processes occurring in these regimes. One possible complication might arise from the acidity of such oxoanions in that the anionic guest may act as a hydrogen-bond donor towards acceptor sites on the host. The triazolyl groups of the hosts **3–6** are possible candidates although the conjugate heterocyclic cations are quite strong acids (pK_a(*N*-methyl-1,2,3-triazolium) = 1.2). Trans-protonation between host and guest is therefore less likely which is also corroborated by the lack of a significant heat effect upon titration of **6** with the most acidic anion hydrogensulfate (pK_a 1.99, entry 33). Another possibility is that trans-protonation occurs between bound and free anion as has been observed previously in neutral hydrogen bond donor complexes with dihydrogen phosphate and bicarbonate.[19]

If an interwoven network of hydrogen bonds is established upon binding acidic anions by compounds **4–6** then the dihydrogen-phosphate anion may form such a network. It is likely that subtle differences in the layout and spacing of the hydrogen bond donor and acceptor groups may result in profound effects on the stability of the complex as the number of optimally aligned structural arrangements of one host and one guest species are severely limited. In principle, such a scenario is experimentally observed. Bridging the parent calix[4]pyrrole with a mono-triazole strap dramatically hampers the formation of a satisfactory complex structure with $H_2PO_4^-$ as indicated by the loss of more than 6 kcal in binding enthalpy (entries 3 *versus* 11). Embedding another triazole unit into a strap of the same length, using host **4** as a probe, then increases the enthalpic interaction by almost 12 kcal (entries 11, 17). As such a massive boost cannot be sensibly accounted for by the formation of at best two novel and dedicated hydrogen bonds in acetonitrile solvent (ε = 36; the triazole module may donate and accept just one hydrogen bond each) we are led to conclude that the covalent connectivity of the bis-triazolo host **4** creates the correct and necessary environment for $H_2PO_4^-$ anion to act as the missing piece in a mosaic of hydrogen bonding. The newly formed structure, however, is characterized by the most negative entropy in the entire host series, evidence that this represents a unique fit of the participating receptor and anion. Even a small incremental elongation of the strap to give hosts **5** and **6** progressively decreases this optimal complex structure (entries 23, 30).

Establishing a subtly knitted network of hydrogen bonds in host–guest binding should require some time and is certainly also subject to further environmental influences in particular in organized assemblies like membranes. We therefore anticipate significant differential kinetic effects in anion transport across membranes that emerge not so much from the variant extraction equilibria, but rather from limiting build-up rates of the anion transposing complexes.

Transport studies

The transport properties of compounds **4–6** were measured using POPC vesicles loaded with CsCl, RbCl, KCl and NaCl and suspended in an external $NaNO_3$ solution. A sample of carrier (4% molar carrier to lipid) was added as a DMSO solution and the resultant Cl^- efflux monitored using a chloride selective electrode.[20] 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 show that all the compounds transport chloride from vesicles loaded with caesium chloride and suspended in $NaNO_3$ solution, but that chloride release from vesicles loaded with other group 1 metal cation chloride salts occurs more slowly for compounds **4**, **5** and **6** (Fig. 7, 8 and 9). We wished to investigate the influence of the composition of the external media on the transport activity shown by **4–6**. For this purpose caesium chloride loaded vesicles were suspended in a Na_2SO_4 solution. The results (Fig. 10) show that carrier activity is essentially maintained for compound **4** but when going to compounds **5** and **6** (increasing the length of the strap) the rate of chloride release becomes more dependent on the nature of the external electrolyte and increases by 1.7 times for compound **5** and 2.8 times for compound **6** when changing from sulfate to nitrate as the external anion (measured at 300 s). Sulfate is a highly hydrophilic (kosmotropic) anion and we assume that it cannot be transported across the lipid bilayer. Thus chloride release in these systems occurs either by caesium chloride co-transport or by chloride/nitrate antiport. The finding that with compound **4** the rate of release of chloride is essentially independent of the nature of the external anion is evidence that the compound is functioning as a caesium chloride co-transporter. However, with compounds **5** and **6** the increasing difference in transport rate is indicative of an increasing propensity to operate *via* an anion antiport process (potentially in addition of caesium chloride co-transport). Thus the length of the strap influences the transport mechanism significantly.

Moving back to vesicle transport studies with nitrate as the external anion, we observe that the rate of chloride efflux increases

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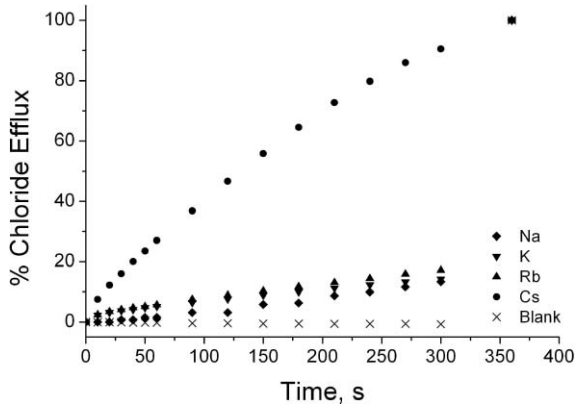

Fig. 7 Chloride efflux promoted by 0.04 molar equivalents of **4** in unilamellar POPC vesicles loaded with 489 mM Na (♦), K (▼), Rb (▲) and Cs (●) chloride salts buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. DMSO was used as a blank (×). Each point represents the average of three trials.

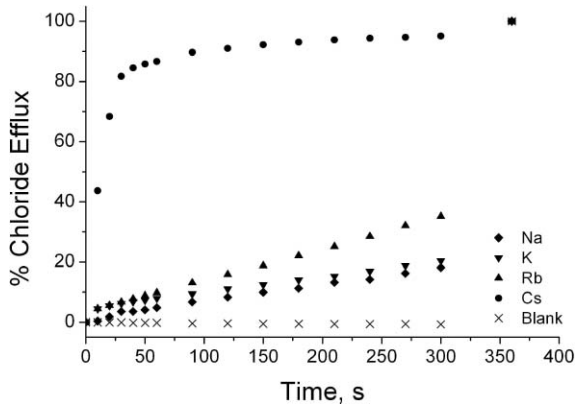

Fig. 8 Chloride efflux promoted by 0.04 molar equivalents of **5** in unilamellar POPC vesicles loaded with 489 mM Na (♦), K (▼), Rb (▲) and Cs (●) chloride salts buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. DMSO was used as a blank (×). Each point represents the average of three trials.

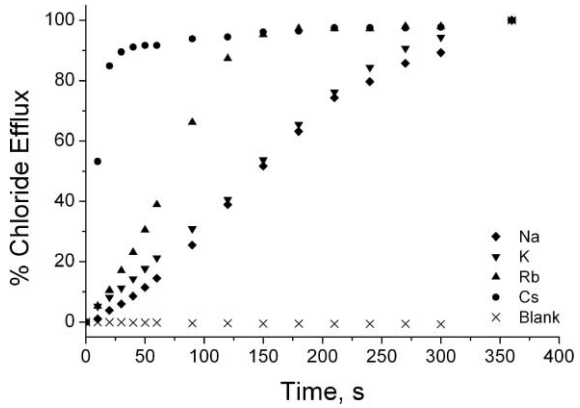

Fig. 9 Chloride efflux promoted by 0.04 molar equivalents of **6** in unilamellar POPC vesicles loaded with 489 mM Na (♦), K (▼), Rb (▲) and Cs (●) chloride salts buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. DMSO was used as a blank (×). Each point represents the average of three trials.

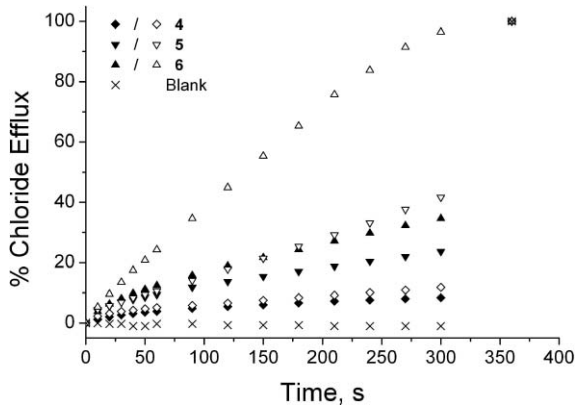

Fig. 10 Chloride efflux promoted by 0.01 molar equivalents of **4–6** in unilamellar POPC vesicles loaded with 489 mM CsCl salts buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 162 mM Na_2SO_4 buffered (closed symbols) or 489 mM $NaNO_3$ (open symbols) to pH 7.2 with 5 mM phosphate. DMSO was used as a blank (×). Each point represents the average of three trials.

on going from compound **4** to compound **6** regardless of the internal counter-cation (Fig. 7–9). This trend in transporter efficiency may be an influence associated with partitioning of the carrier from the aqueous phase to the lipid bilayer, mobility within the membrane, 'solubilising' the anion in the lipophilic portion of the membrane or a combination of all these factors.[21] The observed chloride transport activity for compounds **4–6** with vesicles loaded with NaCl suspended in a sulfate medium is low and therefore we suggest that the compounds function as chloride-nitrate antiport agents and not sodium chloride co-transporters.

The chloride transport efficiency of compounds **4–6** is reduced in bilayers containing 30% cholesterol (Figure S30†), which leads us to suggest that the compounds are functioning as discrete molecular carriers and not as channels.[22] The effective concentration required to induce 50% total chloride efflux (EC_{50}) may be used as a measure of carrier efficiency. The EC_{50} values for compounds **4**, **5** and **6** observed for chloride release from CsCl encapsulated vesicles are 0.060, 0.012 and 0.0069 molar equivalents with respect to lipid at 270 s.

Conclusions

By increasing the length of the chain between two triazole groups in a strapped calix[4]pyrrole we have produced a transporter that surpasses the chloride transport efficiency of the previous generation system **3** (Fig. 11) which contained a single triazole group[10] and switched the mechanism of transport from a predominant co-transport process in the case of compound **4** and CsCl to an chloride/nitrate antiport process with compound **6**. These studies show that the rate of chloride transport in these systems does not depend directly on the chloride affinity of the strapped calixpyrrole systems. The link between chloride affinity and transport efficiency is not clear and we must look at other factors including the ability of the receptor to partition into the membrane and the mobility of the carrier. These findings contribute to the emerging picture in the

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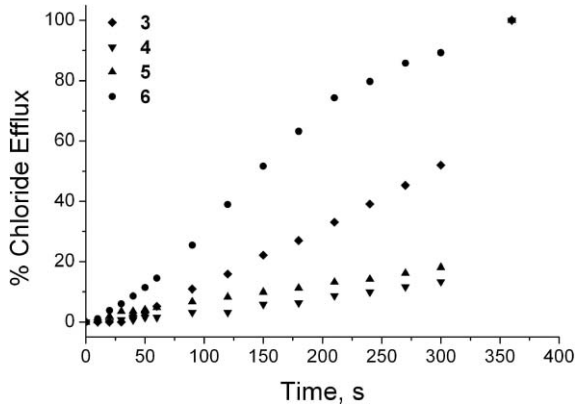

Fig. 11 Chloride efflux promoted by 0.04 molar equivalents of **3** (♦), **4** (▼), **5**(▲) and **6** (●) in unilamellar POPC vesicles loaded with 489 mM NaCl buffered to pH 7.2 with 5 mM phosphate. The vesicles were dispersed in 489 mM $NaNO_3$ buffered to pH 7.2 with 5 mM phosphate. Each point represents the average of three trials.

design of discrete molecular carriers for chloride. They illustrate that very subtle changes in structure and hydrophobicity which are not related to affinity have profound effects on both the mechanism and efficiency of transport.

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