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10.1016/j.tet.2017.04.064

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A folding decalin tetraurea for transmembrane anion transport

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Abstract:

A new approach to anionophore design combines a trans-decalin scaffold with flexible o-phenylene bis-urea side-arms. The scaffold preorganises the side-arms, which can fold to encompass anionic substrates. A prototype has been synthesised by coupling an o-aminophenyl urea to a decalin bis-isocyanate, and has been shown to be active for anion binding and transport. Modelling and NMR studies show that the unbound receptor is capable of intramolecular hydrogen bonding but can reorganise to form up to eight hydrogen bonds to a chloride anion.

Keywords:
supramolecular chemistry; anion binding; anion transport; host-guest systems; urea; foldamer

1. Introduction

Transmembrane ion carriers were among the earliest dynamic supramolecular systems, achieving their effects through a sequence of molecular-scale movements.¹ For many years the focus was almost exclusively on cation transport,² but more recently there has been growing interest in anionic substrates.³ Anion transport through cell membranes is a key step in many biological processes, from maintaining homeostasis to the conversion of energy. As cell membranes are impermeable to most ions, transmembrane transport of ions is performed by proteins embedded in the lipid bilayers. The absence or malfunction of such membrane proteins is linked to various diseases, of which cystic fibrosis is the best known.⁴ This raises the possibility that anion carriers (anionophores) could be used as treatments for these conditions, replacing the dysfunctional natural systems.³a,⁵ Certain anion receptors⁶ have indeed been shown to act as carriers, transporting anions such as chloride across phospholipid bilayer models and cell membranes.³,⁷

We have previously reported a series of anion carriers based on the 2,7-diaxially substituted trans-decalin scaffold (for numbering, see 1). The framework is available as a protected 2,7-diamine with a 4a-ester substituent through an efficient 6-step synthesis.⁸ In prototypes 1 the amines are derivatised as ureas, creating a binding site with four NH groups capable of acting as H-bond donors to a bound chloride ion.⁸ The axial disposition of the urea groups decreases flexibility and increases preorganisation; there is limited scope for rotation about the C2-N and C7-N bonds, and this helps to position the NH for binding while preventing intramolecular H-bond formation. It was later found
that replacement of the ureas with thioureas increased effectiveness considerably, especially when the anionophores were preincorporated in synthetic lipid membranes. The bis-thiourea 2 displayed the highest rates of chloride transport in vesicles reported thus far for a synthetic carrier (850 Cl⁻/s).⁹,¹⁰ Meanwhile, decalin bis-ureas performed well in a test for activity in cells. Transporter 1b proved especially effective, while showing almost no toxicity.⁷b

Another motif which has been successful in anion binding and transport is ortho-phenylene bis-urea (OPBU).¹¹ As shown by Gale et al., simple prototypes such as 3 are powerful anionophores,¹² and extended variants such as 4 are also effective.¹³ The OPBU unit has also been deployed multiply in a variety of receptors, such as chloride-binding foldamers 5¹⁴ and tripods 6.¹⁵ Given the effectiveness of both decalin bis-(thio)ureas and OPBUs as anion carriers, we were interested to discover the result of combining the two families. A structure such as 7 would incorporate two OPBU units in a preorganised fashion, potentially capable of binding anions such as chloride through eight H-bond donors. The receptor would presumably be capable of forming intramolecular H-bonds, stabilising non-bonding conformations. However, the creation of so many NH···Cl⁻ bonds on binding might compensate, so that useful affinities could be achieved. Moreover, the OPBU units should be able to wrap around the chloride, in a folding motion. This dynamic behaviour could retain fast binding kinetics (by avoiding rigidity) while resulting in effective anion encapsulation. Surrounding the target anion had been found to increase transport activities¹⁶ as well as to moderate selectivities.¹⁷

Here we report the synthesis of tetra-urea 7, structural and binding studies, and preliminary transport experiments. We find that 7 can be organised to create an effective chloride binding site, and is active as both receptor and transporter. Tetra-urea 7 serves as a prototype for foldamer transporters which could make valuable contributions to anionophore development.
2. Results and Discussion

2.1 Synthesis

To synthesise tetra-urea 7 we chose a convergent strategy in which preformed pendant groups are coupled to the decalin scaffold. Initially we sought an electrophilic reagent for reaction with amino groups on the decalin units, following the strategy employed for compounds 1.8 Unfortunately, efforts to synthesize bis-urea precursors 9 and 13 (Scheme 1) were unsuccessful. Amine 8 was readily prepared from 1,2-phenylenediamine and 4-trifluoromethylphenyl isocyanate in THF. However, after treatment of 8 in pyridine with 4-nitrophenyl chloroformate in DCM,18 the subsequent addition of cyclohexylamine (as a model compound for the decalin bisamine) did not give the desired urea. Instead benzimidazolone 1019 (main product) and trisurea 4 (trace) were found, as confirmed by 1H NMR and mass spectrometry. In attempts to prepare isocyanate 13 via a Curtius rearrangement, carboxylic acid 1120 was treated first with diphenylphosphoryl azide (DPPA) and triethylamine in THF. However, instead of acyl azide 12 or isocyanate 13, the 2,4-quinazolinedione 14 was obtained, via an intramolecular cyclisation reaction.21 Similar intramolecular reactions have previously been observed in the presence of base or upon heating the reaction mixture.22 These observations suggested that attempting to introduce a urea precursor (carbamate, isocyanate, or acyl azide) at the ortho position of a phenyleneurea gives rise to intramolecular reactions which are much faster than reactions with other amines.
Scheme 1. Attempts to synthesise bis-urea precursors 9 (a) and 13 (b), and observed products 10 and 14.

We therefore adopted the alternative approach in which the urea carbonyl is first connected to the decalin scaffold, as in bis-isocyanate 16 (Scheme 2). Spyropoulos and Kokotos recently published a convenient isocyanate synthesis involving treatment of a Boc-protected amine with triflic anhydride and 2-chloropyridine in DCM. This method could be successfully applied to our decalin scaffold 15. After formation of the bis-isocyanate 16 was complete, as indicated by TLC, the resulting solution was added to an excess of amine 8 in pyridine. Target tetra-urea 7 was obtained with a yield of 79% (from 15) after purification by column chromatography (see Experimental Section for details).

Scheme 2. Synthesis of target tetra-urea 7 from Boc-protected bis-amine 15 and amine 8, via the intermediate bis-isocyanate 16.
2.2 Conformational properties of tetra-urea 7

Fig. 1. Molecular modelling of 7 and conformations of side-arms. (a) Energy minimised structure of 7.Cl⁻. NH···Cl⁻ hydrogen bonds are shown as yellow broken lines. (b) Predicted ground state structure of 7, and (c) a more extended conformation ~3 kJ/mol above ground state. (d) Schematics of conformations I-III of an o-phenylene bis-urea arm. The interactions between protons of 7 as detected by NMR spectroscopy (ROESY) are indicated with red arrows and the NH···O interactions that stabilise conformations II and III are indicated in blue.

Molecular modelling of receptor 7 with chloride confirmed the possibility of a folded structure with efficient anion encapsulation. As shown in Fig. 1a, all four urea groups were able to make contact with the substrate, forming eight hydrogen bonds with lengths of 2.4-2.6 Å. As expected, a conformational search of the free receptor revealed a flexible structure favouring conformations...
with intramolecular hydrogen bonds. The ground state structure and an alternative which is \(~3\) kJ/mol higher in energy are shown in Figs. 1b and 1c respectively. Starting from the conformation of 7 in the complex with Cl\textsuperscript{−}, both conformations require rotations around an o-phenylene to N bond, to obtain a conformation in which one urea group can form a H-bond to the carbonyl of the other urea in the same OPBU side-arm. The two possible options (II and III) are drawn schematically in Fig. 1d.

To study the presence of these conformations experimentally, four 1D-ROESY NMR experiments were performed on a solution of 7 in DMSO-d\textsubscript{6}, in which the different NH signals were inverted (Fig. 2). As expected, strong enhancements between NH\textsuperscript{a}–NH\textsuperscript{b} and between NH\textsuperscript{c}–NH\textsuperscript{d} were observed in all of the ROE experiments, indicating that the two NH groups within each urea are co-directed. Enhancements were also observed between NH\textsuperscript{b} and NH\textsuperscript{c}, between NH\textsuperscript{b} and CH\textsubscript{12}, and between NH\textsuperscript{c} and CH\textsubscript{15}, suggesting contributions from conformations I, II and III (Fig. 1d). The observed ROEs can be converted into interproton distances which represent the ensemble of conformers present\textsuperscript{24} and the populations required to fulfil these time-averaged distances suggest that in DMSO, conformations I, II, and III are present in roughly a 1:1:1 ratio.

![Diagram](image_url)

**Fig. 2.** (a) \(^1\)H NMR spectrum of 7 in DMSO-d\textsubscript{6}. (b-d) 1D ROESY spectra of 7 with NH\textsuperscript{b} (b), NH\textsuperscript{c} (c), or NH\textsuperscript{d} (d) inverted.
2.3 Binding to chloride

Binding of tetra-urea 7 to chloride was tested through $^1$H NMR titration with $n$-Bu$_4$NCl in DMSO-d$_6$ with 0.5% H$_2$O added (Fig. 3a). All four NH signals shifted by 0.22-0.32 ppm upon addition of 7.5 equivalents of chloride, consistent with the structure in Fig. 1a where all urea protons are involved in binding. The movements of the NH$^a$, NH$^b$, and NH$^d$ signals were each fitted to a 1:1 binding isotherm yielding $K_a = 8.7 \times 10^2 \pm 30$ M$^{-1}$ (Fig. 3b). The high quality of the fit indicates that 1:1 binding is likely to be the predominant process. As might have been expected, this value is not especially high compared to the earlier decalin bis-ureas. For example, the affinity measured for 1b under these conditions was only slightly lower ($K_a = 6.8 \times 10^2$ M$^{-1}$) while that for bis-urea 17 was almost identical ($K_a = 8.8 \times 10^2$ M$^{-1}$). It thus seems that the ability of 7 to form eight hydrogen bonds to chloride is almost exactly counterbalanced by the negative effect of intramolecular hydrogen bonding promoting non-binding conformations. It should be noted that the presence of conformation I (Fig. 1d) in unbound receptor, as revealed by the ROESY spectra, does not necessarily imply that the binding conformation is significantly populated. The conformations of the two bis-urea arms of 7 are presumably coupled, and it is possible that structures in which both arms take up conformation I are disfavoured.

Fig. 3a. $^1$H NMR spectra of receptor 7 (1 mM) in DMSO-d$_6$/H$_2$O (200:1), with various amounts of $n$-Bu$_4$NCl added, at 298 K. b. Graph showing the observed binding curve (blue) and calculated fitting (red) for the NH$^d$ signal of 7 when titrated against chloride.
2.4 Transmembrane transport of anions

Chloride transport by 7 was studied in large unilamellar vesicles (200 nm) composed from POPC and cholesterol (7:3 ratio). Tetra-urea 7 was preincorporated into the membrane during vesicle production, at loadings of 1:1000 (transporter:lipid) down to 1:10,000. The vesicles contained the fluorescent and halide sensitive dye lucigenin, while NaNO$_3$ was present on both sides of the membranes. To measure transport activity, a pulse of NaCl was added to the liposomes while the fluorescence was monitored.$^{25}$ Influx of Cl$^-$ was detected through quenching of the lucigenin emission. Electroneutrality was maintained by counter-transport of NO$_3^-$ out of the vesicles. Fluorescence decay curves from experiments at three loadings are presented in Fig. 4, and confirm that 7 possesses significant transport activity. To quantify its effectiveness, we calculated a specific initial rate $[I]$ for chloride/nitrate exchange by 7, as outlined in earlier work.$^9$ The value of $[I] = 13 \text{ s}^{-1}$ is close to those previously measured for decalin bis-ureas 1b and 1c ([I] = 16 and 19 s$^{-1}$ respectively), while significantly higher than that for 1a ([I] = 3 s$^{-1}$). As an initial probe of selectivity, the NO$_3^-$ was replaced by SO$_4^{2-}$ and HCO$_3^-$; these strongly solvated anions are difficult to extract from water, so that counter-transport is likely to become the rate-determining step. In the case of SO$_4^{2-}$ anion exchange was prevented, while for HCO$_3^-$ some activity was observed (Fig. S16). Again, this behaviour is similar to that observed previously for decalin bis-ureas 1.$^{8,26}$ Overall, these results suggest that the elaboration of 1 into tetra-urea 7 has had surprisingly little effect on the transport properties, given the major change in binding site geometry and dynamics. However, the assays employed in this work provide limited information on mechanism or selectivity. In the future we hope to apply more discriminatory techniques such as planar lipid bilayer (PLB) conductance measurements, which are more likely to reveal distinctive properties of the tetra-urea system.

![Fluorescence decay curves](image)

**Fig. 4.** Chloride/nitrate exchange by 7 in 200 nm POPC/cholesterol (7:3) vesicles, as followed by the lucigenin method.
3. Conclusion

In tetra-urea 7 we have combined the disubstituted trans-decalin scaffold with o-phenylene bis-ureas, yielding a receptor which can bind anions through programmed folding and encapsulation. Although the bis-urea arms show intramolecular hydrogen bonding in the unbound state, the conformation can change to bind a chloride ion with an array of up to eight H-bonds. When 7 is compared to bis-ureas 1, it seems that the energy required to overcome intramolecular H-bonding is nearly equal to the gain in binding from the additional two urea groups. Affinity constants for chloride in DMSO are thus very similar, and preliminary studies on anion transport also show little difference between 7 and 1. However, it seems likely that the foldamer-type architecture prototyped in 7 will show distinctive properties on further study and development, and could make a significant contribution to future anionophore design.
4. Experimental Section

4.1 Synthesis and Characterisation
Boc-protected diamine 15 was synthesised according to our previously reported procedures.\textsuperscript{8,9} Commercially available compounds were used without further purification. All reactions were performed under N\textsubscript{2}. Flash column chromatography was performed using silica gel (Aldrich, pore size 60 Å, particle size 40-63 µm) as the absorbent. Routine monitoring of reactions was performed using precoated silica gel TLC plates (Merck silica gel 60 F\textsubscript{254}). Spots were visualised by UV light or with phosphomolybdic acid in ethanol (PMA) upon heating.

\( ^1\text{H}, \ ^{13}\text{C}, \text{ and } ^{19}\text{F} \) NMR spectra were recorded using Varian 400 MHz and Bruker Avance III HD 500 MHz cryoprobe (carbon sensitive) spectrometers. All chemical shifts (\( \delta \)) are quoted in parts per million (ppm) and the residual solvent signal was used to reference the spectrum. Signal splittings are described as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet peaks (m). For the assignments of the NMR signals of 8 the same numbering system is used as is indicated for 7. Mass spectra by electrospray ionisation (positive mode) were recorded on a Bruker MicrOTOF. Infrared spectra were recorded on a Perkin Elmer Spectrum Two FT-IR.

4.1.1 1-(2-Aminophenyl)-3-[4-(trifluoromethyl)phenyl]urea 8\textsuperscript{27}
1,2-Phenylenediamine (541 mg, 5.00 mmol) was dissolved in dry THF (15 mL) upon stirring for 40 minutes and 4-trifluoromethylphenyl isocyanate (690 µL, 904 mg, 4.96 mmol) was added slowly. The reaction mixture was stirred for 1.5 hours and the precipitate was filtered off and washed with diethyl ether to yield 1.37 g crude product. This was recrystallized from EtOH (65 mL) to obtain 1.13 g (3.8 mmol, 77\%) of compound 8 as off-white flakes.

\( ^1\text{H} \) NMR (400 MHz, DMSO-\textsubscript{d6}) \( \delta \) 9.19 (s, 1H; NH\textsuperscript{f}), 7.83 (s, 1H; NH\textsuperscript{f}), 7.70 – 7.54 (m, 4H; ArH\textsuperscript{16,17}), 7.32 (dd, \( J = 7.9, 1.5 \) Hz, 1H; ArH\textsuperscript{15}), 6.86 (td, \( J = 7.6, 1.5 \) Hz, 1H; ArH\textsuperscript{14}), 6.75 (dd, \( J = 7.9, 1.5 \) Hz, 1H; ArH\textsuperscript{12}), 6.58 (td, \( J = 7.5, 1.5 \) Hz, 1H; ArH\textsuperscript{13}), 4.81 (s, 2H; NH\textsubscript{2}); \(^{13}\text{C} \) NMR (101 MHz, DMSO-\textsubscript{d6}) \( \delta \) 152.9 (O=C\textsuperscript{19}), 143.9 (ArC\textsuperscript{16}), 141.2 (ArC\textsuperscript{15}), 126.1 (q, \( J = 3.8 \) Hz; ArC\textsuperscript{17}), 124.8 (ArC\textsuperscript{14}), 124.6 (q, \( J = 271 \) Hz; CF\textsubscript{3}19), 124.2 (ArC\textsuperscript{12}), 124.1 (ArC\textsuperscript{13}), 121.4 (q, \( J = 31.9 \) Hz; ArC\textsuperscript{17}), 117.5 (ArC\textsuperscript{16}), 116.8 (ArC\textsuperscript{15}), 115.9 (ArC\textsuperscript{12}); \(^{19}\text{F} \) NMR (377 MHz, DMSO-\textsubscript{d6}) \( \delta \) -59.87 (CF\textsubscript{3}3), IR: 3416, 3339, 3298, 1667, 1605, 1552, 1501, 1410, 1319, 1244, 1100, 1070, 839, 757, 654 cm\textsuperscript{-1}; MS(ESI): \( m/z \) calculated for \( C_{14}H_{13}F_2N_3O^+ \) [M + H\textsuperscript{+}]: 296.1, found 296.1.

4.1.2 Decalin tetra-urea 7
Boc-protected diamine 15 (49.8 mg, 113 µmol) was dissolved in dry dichloromethane (7.5 mL) and 2-chloropyridine (64 µL, 77.4 mg, 6.0 eqv) and triflic anhydride (56 µL, 95.2 mg, 337 µmol, 3.0 eqv) were added. This solution was stirred for 1 hour to form the isocyanate as monitored by TLC (\( R_t = 0.7 \) in 30% EtOAc in petroleum spirit (40-60); PMA). The isocyanate solution was then transferred into a flask containing a solution of 8 (122 mg, 413 µmol, 3.66 eqv) in dry pyridine (10 mL). The resulting dark orange solution was stirred for 18 hours, concentrated, dissolved in ethyl acetate (25 mL), washed with H\textsubscript{2}SO\textsubscript{4} (0.5 M, 3 x 20 mL) and water (20 mL), dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated to give a red sediment (172 mg). This was purified by column chromatography over silica gel eluted with 3% methanol in dichloromethane, yielding 78.5 mg (89 µmol, 79\%) of target compound 7 as a very light yellow solid.

\( R_t = 0.47 \) (7% MeOH in DCM); \(^1\text{H} \) NMR (500 MHz, DMSO-\textsubscript{d6}) \( \delta \) 9.46 (s, 2H; NH\textsuperscript{f}), 8.11 (s, 2H; NH\textsuperscript{f}), 7.87 (s, 2H; NH\textsuperscript{f}), 7.64 (d, \( J = 8.6 \) Hz, 4H; ArH\textsuperscript{16}), 7.61 – 7.52 (m, 6H; ArH\textsuperscript{12,17}), 7.44 (dd, \( J = 7.9, 1.7 \) Hz, 2H; ArH\textsuperscript{15}), 7.07 – 7.00 (m, 2H; ArH\textsuperscript{13}), 7.02 – 6.95 (m, 2H; ArH\textsuperscript{17}), 6.79 (d, \( J = 6.0 \) Hz, 2H; NH\textsuperscript{f}), 4.11 (q, \( J = 7.1 \) Hz, 2H; CH\textsubscript{3}10), 3.90 (s, 3H; CH\textsubscript{3}eq\textsuperscript{2,7}), 2.05 (td, \( J = 13.6, 4.3 \) Hz, 2H; CH\textsubscript{2}1.8), 1.85 – 1.67 (m, 5H;
$\text{CH}_\text{eq}^{4,5}$, $\text{CH}^{8a}$, $\text{CH}_\text{ox}^{3,6}$), 1.51 – 1.33 (m, 6H; $\text{CH}_\text{eq}^{4,5}$, $\text{CH}_\text{eq}^{1,8}$, $\text{CH}_\text{eq}^{3,6}$), 1.19 (t, $J = 7.1$ Hz, 3H; $\text{CH}^{11}$); $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 174.1 (O=C$^9$), 155.5 (O=C$^{18}$), 153.1 (O=C$^{19}$), 143.7 (ArC$^{16a}$), 133.2 (ArC$^{15a}$), 129.3 (ArC$^{12a}$), 126.0 (q, $J = 3.9$ Hz; ArC$^{17}$), 125.4 (ArC$^{13}$), 124.7 (ArC$^{12}$), 124.5 (q, $J = 270$ Hz; CF$_3^{20}$), 122.8 (ArC$^{14}$), 122.3 (ArC$^{11}$), 121.6 (q, $J = 320$ Hz; ArC$^{17a}$), 117.7 (ArC$^{16}$), 59.6 (CH$_2^{10}$), 47.5 (C$^{19}$), 44.9 (CHN$^{2,7}$), 33.3 (CH$_2^{1-8}$), 33.3 (CH$_3^{8a}$), 32.0 (CH$_2^{4,5}$), 27.5 (CH$_3^{3,6}$), 14.1 (CH$_3^{11}$); $^{19}$F NMR (377 MHz, DMSO-$d_6$) δ -59.98 (CF$_3$); IR: 3286, 2937, 1639, 1604, 1533, 1451, 1322, 1162, 1110, 1068, 1016, 841, 739 cm$^{-1}$; HRMS(ESI): m/z calculated for $\text{C}_{43}\text{H}_{48}\text{F}_6\text{N}_8\text{NaO}_6$ $[^{\text{[M + Na]}^+}$: 905.3180, found 905.3173.

![Chemical structure](image)

4.2 ROESY Experiments

ROESY Experiments were performed on a solution of 7 (4 mM) in DMSO-$d_6$ on a Varian 600 MHz VNMRS NMR spectrometer equipped with a triple resonance HCN cryogenic probe, running VNMRJ4.2 software. ROESY spectra were acquired in 64 scans with a 200 ms mixing time, 1 second relaxation delay, 6600 Hz spectrum width and an 80 Hz selection inversion bandwidth. Internuclear distances and the contributing conformer populations were calculated from the PANIC-corrected ROE intensities using the methodology described in reference 24. The results are summarised in the table below:

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<th>PANIC ROE Intensity</th>
<th>ROE Distance / Å</th>
<th>Conformer I</th>
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<th>Conformer III</th>
<th>Conformer Ensemble$^b$</th>
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<tr>
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<td>1.98$^c$</td>
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<td>2.03</td>
<td>2.03</td>
<td>2.03</td>
</tr>
<tr>
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<td>2.36</td>
<td>1.9</td>
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<td>2.27</td>
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<tr>
<td>H$^c$-H$^d$</td>
<td>82.2$^d$</td>
<td>1.87</td>
<td>2.03</td>
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<tr>
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<td>3.7</td>
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<td>2.55</td>
</tr>
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<td>2.23</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
<td>2.30</td>
</tr>
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</table>

$^a$ See section 4.5 on Molecular Modelling $^b$ Based on a 1:1:1 ratio of Conformers I-III; $^c$ NH$^a$-NH$^b$ used as ROE reference distance as per reference 24; $^d$ ROE Intensity reported as the average from the two 1D-ROESY spectra for NH$^c$ and NH$^d$.

4.3 Binding study by $^1$H NMR spectroscopy

The binding constant of 7 to chloride was determined by a $^1$H NMR titration against n-Bu$_4$N$^+$Cl$^-$ in DMSO-$d_6$/H$_2$O (200:1). The hygroscopic guest n-Bu$_4$N$^+$Cl$^-$ was dried under high vacuum to remove residual solvents or water prior to solution preparation. Aliquots of 1-10 µL guest solution (n-Bu$_4$N$^+$Cl$^-$ dissolved at 70 mM in the host solution) were added to a NMR tube containing 500 µL 1.0 mM solution of host 7 in DMSO-$d_6$/H$_2$O (200:1). The $^1$H NMR spectra (8 scans) were measured on a Varian 500 MHz NMR spectrometer, equipped with a proton sensitive probe, at 298 K. The shifts of NH...
signals b, c, and d were fitted to 1:1 binding model using a least-squares fitting procedure in a custom-made Excel spreadsheet.

4.4 Transport studies in liposomes
1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol solutions in deacidified chloroform were combined with a solution of 7 in methanol to obtain a POPC to cholesterol ratio of 7:3, and a transporter to lipid (POPC + cholesterol) ratio as specified in the experiments. The solvents from the lipid/receptor mixture were evaporated under a stream of N₂ and the lipid film was dried under high vacuum for 1 h. The residue was hydrated with 500 µL of an aqueous solution of 10,10'-dimethyl-9,9'-biacridinium nitrate (lucigenin, 0.8 mM) in NaNO₃ (225 mM), sonicated for 30 s and stirred for 1 h to give heterogeneous vesicles. Multilamellar vesicles were disrupted by 10 freeze-thaw cycles and then the solution was diluted to 1 mL (by adding 0.5 mL of 225 mM NaNO₃) and carefully extruded (29 times) through a polycarbonate membrane (200 nm pore size). The external lucigenin was removed by passing the solution though a size exclusion column (Sephadex 50G, eluted with 225 mM NaNO₃). The collected vesicles were further diluted with NaNO₃ solution (225 mM) to obtain a vesicle solution with 0.4 mM lipid concentration.

3.00 mL of this vesicle solution was placed in a quartz cuvette with a small stir bar and the fluorescence intensity (excitation at 450 nm, emission at 535 nm) was measured over time at 25 °C, using a PerkinElmer LS45 fluorescence spectrometer. 75 µL of aqueous NaCl (1.0 M in 225 mM NaNO₃, to give an overall exterior chloride concentration of 25 mM Cl⁻) was added ~30 seconds after the start of the measurement and the fluorescence intensity was measured for another 14 minutes. Fluorescence data were collected for at least four of these runs. The plateau (before addition of chloride) and the vertical drop (the first 1-2.5 seconds after chloride addition, due to quenching of external lucigenin) were removed. The averaged and normalised data are quantified as detailed previously.⁹

4.5 Molecular Modelling
Modelling of 7 and 7.Cl⁻ was performed using Maestro 9.7/MacroModel 10.3 (Schrödinger Inc.) employing the MMFFs force field, with chloroform GBSA solvation. A Monte Carlo Molecular Mechanics (MCMM) conformational search, in which all acyclic bonds were allowed to rotate, yielded the structures given in Fig. 1.

Acknowledgements
The authors wish to thank Paul Lawrence for his help with the NMR experiments. This work was supported by the EPSRC through the Bristol Chemical Synthesis Centre for Doctoral Training (EP/G036764/1) and research grant number EP/J00961X/1 and by the FNRS through a Chargée de Recherche grant to HV.

Appendix A. Supplementary data
NMR spectra of compounds 7 and 8 and additional anion transport data by 7 are given in the Supplementary file.
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