Cationic cyclocholamides; toroidal facial amphiphiles with potential for anion transport†

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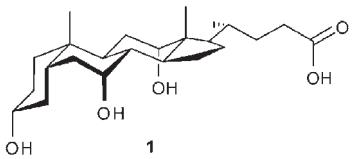
Received (in Cambridge, UK) 7th April 2008, Accepted 8th May 2008
First published as an Advance Article on the web 17th June 2008
DOI: 10.1039/b805777j

Cholic acid has been transformed into cyclotrimeric and cyclotetrameric toroidal amphiphiles with inward-directed ammonium substituents; the cyclotrimer 3b effects the transport of chloride anions across vesicle bilayer membranes.

Amphiphiles are molecules with both hydrophilic and hydrophobic moieties. The more common amphiphiles possess small polar head-groups and long hydrocarbon tails, but other geometries are possible. For example, there is widespread interest in "facial amphiphiles" (FAs),[1,2] rigid molecules in which one face is lipophilic and the other polar. These systems have potential applications in controlled self-assembly,[1b,2a–d] anti-microbial chemotherapy,[1d,e,2e–g] gene delivery,[1f,g] vesicle fusion[1g] and the stabilisation of membrane proteins.[1h] We have previously shown that cholic acid **1** can be converted to triamino esters which, after tris-protonation, exist as the cationic, high-contrast facial amphiphiles **2**.[1g,3] We have now used **2** to extend the FA concept, by preparing macrocyclic oligomers (cyclocholamides)[4] **3**. These "toroidal facial amphiphiles" combine lipophilic exteriors with highly hydrophilic, polycationic interiors, and show potential for anion transport across phospholipid membranes.



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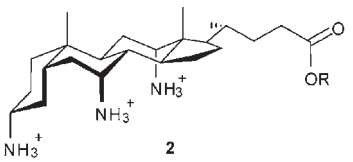

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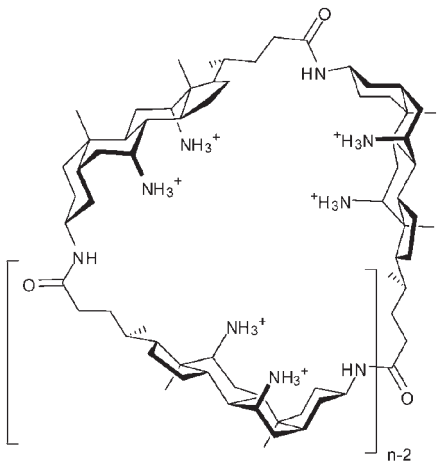
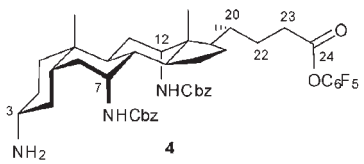
Our synthetic approach to **3** involved cyclo-oligomerisation of intermediate **4** at high dilution, followed by 7,12-*N*-deprotection. Molecular modelling and precedent[5] suggested that control over ring size should be possible through choice of the 7,12-protecting groups. By using the moderately bulky Cbz, we hoped to avoid dimerisation to **3a** (of less interest due to its small cavity) while allowing cyclotrimerisation and the formation of higher oligomers. The benzyl chromophores would also aid separation by HPLC.



3a n = 2 **3b** n = 3 **3c** n = 4



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Steroidal azide **5** was available as starting material *via* our previously reported large-scale procedure.[6] As shown in Scheme 1,† the *N*-protection was first adjusted by installing Cbz at positions 7 and 12, and Boc at position 3. Ester hydrolysis and treatment with pentafluorophenol–DIC gave activated ester **7**. The Boc group was removed with TFA, and the resulting salt was added slowly to DMAP in THF (final concentration 0.8 mM). Under these high dilution conditions cyclisation took place to give a mixture of **8b–d**,[7] separable by HPLC. Typical yields were 34%, 16% and 2%, respectively. Removal of the Cbz groups by hydrogenolysis proved difficult, probably due to steric congestion, but cleavage with HBr–AcOH proceeded smoothly. Toroidal facial amphiphiles **3b**·$6Br^-$ and **3c**·$8Br^-$ were produced in almost quantitative yields.

The geometries and conformational properties of **3b** and **3c** were assessed using molecular modelling (see ESI†). Both macrocycles possess flexibility due to the steroidal C20–C24 side chain (for numbering, see **4**). However, the calculations suggested that cyclotrimer **3b** is strongly biased towards conformations with inward directed NH_3^+. This may be

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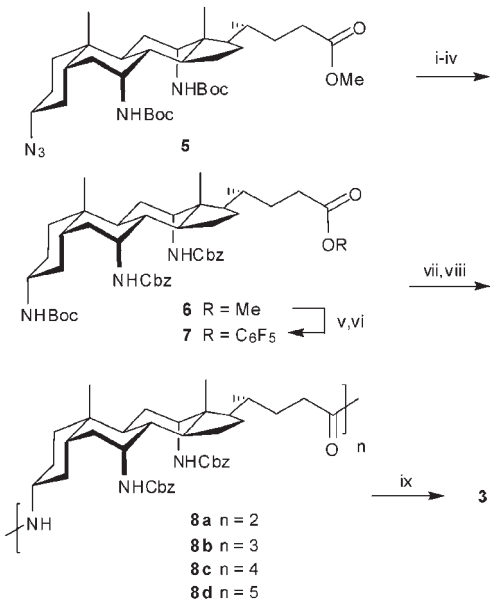
† Electronic supplementary information (ESI) available: Further details concerning the syntheses of macrocycles **3**, molecular modelling and electron microscopy of **3b/c**, and studies of ion transport by **3b**. See DOI: 10.1039/b805777j



Scheme 1 Synthetic route to macrocycles **3**. *Reagents and conditions*: (i) TFA, DCM; (ii) BnOCOCl, $NaHCO_3$ aq., THF; (iii) PMe_3, THF, then H_2O; (iv) $(Boc)_2O$, $NaHCO_3$ aq., THF; (v) NaOH, MeOH, H_2O; (vi) C_6F_5OH, *N*,*N*′-diisopropylcarbodiimide (DIC), THF; (vii) TFA, DCM; (viii) DMAP, THF, high dilution; (ix) HBr, AcOH.

attributed to repulsion between NH_3^+ groups; conformations with outward-directed NH_3^+ possess smaller inter-cation distances.† The global minimum was a C_3-symmetric structure with a cavity ~9 Å in diameter (see Fig. 1a). Cyclotetramer **3c** was found to possess greater freedom of movement, but open structures with inward-directed NH_3^+ predominated at low energies (see Fig. 1b). The global minimum structure surrounded a cavity with an average internal diameter of ~14 Å.

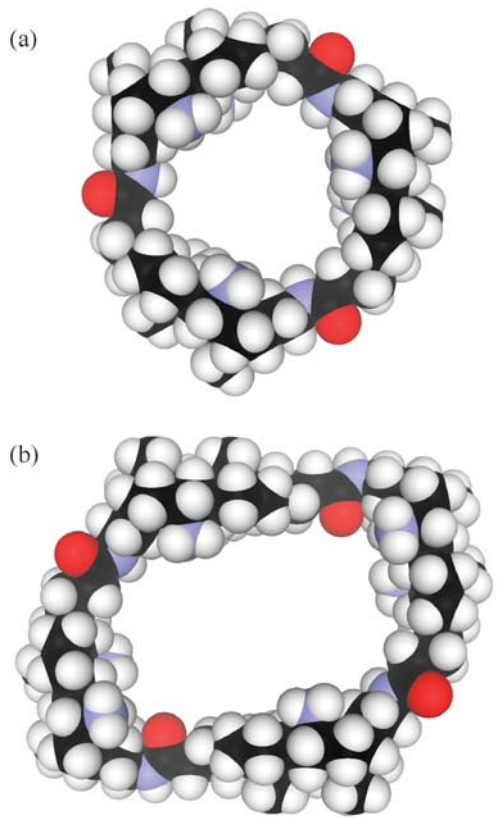


Fig. 1 Global minima for (a) cyclotrimer **3b**, and (b) cyclotetramer **3c** as predicted by Monte Carlo molecular mechanics [Macromodel 9.1, MMFFs force field, GB/SA (water) continuum solvation].

Amphiphile geometry has a major influence on self-assembly in aqueous media.[2a] The aggregation properties of **3b**·$6Br^-$ and **3c**·$8Br^-$ were studied in water–methanol mixtures. Both macrocycles were freely soluble in methanol and gave well-resolved ^1H NMR spectra in CD_3OD, suggesting minimal aggregation. Addition of water caused no visible effect up to H_2O–MeOH (2 : 1), but when the resulting mixtures were left to stand (presumably with loss of some MeOH) the solutions turned cloudy. The aggregates were analysed by transmission electron microscopy (TEM). Cyclotrimer **3b**·$6Br^-$ produced elongated cone-shaped structures, often nested or clustered (see Fig. 2). The origin of these shapes is obscure, but they demonstrate that the toroidal FA architecture can lead to distinctive morphologies. Cyclotetramer **3c**·$8Br^-$ yielded simple spheres,† possibly reflecting its greater flexibility and, correspondingly, a lesser inclination towards organised self-assembly.

Toroidal FAs with hydrophobic exteriors have potential for membrane transport. Locating within the bilayer, they can self-assemble to form channels or act as carriers for polar species. In the case of **3**, the cationic interiors should give selectivity for anionic substrates.[8] To test this possibility we studied the ability of **3b** to promote chloride efflux from unilamellar vesicles, using the chloride electrode method previously employed for neutral steroid-based transporters.[9] In a typical experiment, vesicles were formed from egg yolk phosphatidylcholine (EYPC) and aqueous NaCl (500 mM) by extrusion through a filter membrane (pore size 0.2 μm). External NaCl was exchanged for $NaNO_3$ (500 mM) by dialysis. Macrocycle **3b** was added as the hexakis-trifluoroacetate salt (final concentration 11 μM), and the solution external to the vesicles was monitored using a chloride-selective electrode. The experiment was terminated by addition of detergent (octaethylene glycol monodecyl ether) which

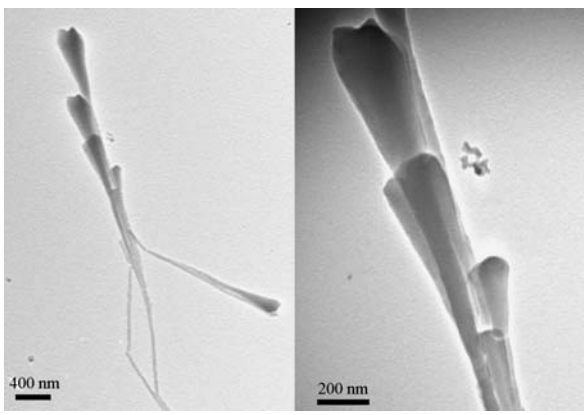
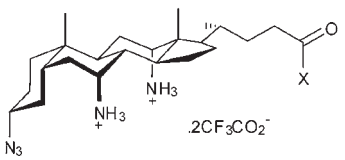


Fig. 2 Aggregates formed from **3b**·$6Br^-$ in methanol–water, as observed by TEM. The same assembly is shown at two levels of magnification. These elongated, cone-shaped structures were found throughout the sample, sometimes singly and sometimes clustered as shown.

liberated all remaining chloride. The results showed that the addition of **3b** initiated chloride efflux, and that *ca.* 80% of the chloride was released within 5 minutes.†

Further experiments with **3b** revealed that the rate of transport increased roughly linearly with concentration, and that the use of less fluid phospholipid–cholesterol (7 : 3) vesicle membranes lowered transport rates.† Both results tend to suggest that transport is due to a carrier mechanism, as opposed to a static, multicomponent channel. Addition of phosphate buffer (pH = 6.5–8) produced a further lowering of transport rates, possibly due to competition for the binding site. Monomeric control compounds **9** were tested to confirm the importance of the toroidal architecture. Neither promoted chloride transport to a measurable extent, suggesting that the macrocyclic structure is necessary to shield the chloride from the membrane hydrocarbon. **3b** was also tested for bromide transport, by replacing the KCl in the vesicles by KBr and employing a bromide-selective electrode. Interestingly, rates were lower by a factor of ~2.4 at steady state,† perhaps due to slow release of the lipophilic bromide anion. Finally, anion *vs.* cation selectivity was tested by encapsulating KCl in the vesicles, suspending in $NaNO_3$, adding **3b**, then following both Cl^- and K^+ efflux using appropriate ion-selective electrodes. While Cl^- emerged from the vesicles as expected, the efflux of K^+ was negligible (Fig. 3). This experiment confirms the expected anion-selectivity, and also shows that the macrocycle does not disrupt the vesicles.



9a X = OMe
9b X = NHc-C_6H_{11}

In conclusion we have shown that cholic acid **1** can be used to prepare a new class of amphiphilic molecules, by enhancing facial amphiphilicity (converting –OH to $–NH_3^+$) then cyclo-oligomerising. The resulting systems **3** are toroidal facial amphiphiles with hydrophobic outer surfaces and strongly hydrophilic interiors. This combination of properties points to membrane-based applications, and we have shown that cyclotrimer **3b** can transport chloride ions across phospholipid bilayers. There is potential for the recognition and transport of other anionic species, and this will be the subject of future research.

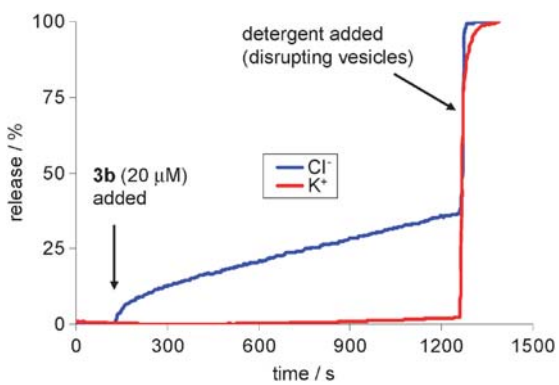


Fig. 3 Anion-selective transport by **3b**·$(CF_3CO_2^-)_6$ through vesicle membranes.†[10] Vesicles: EYPC–cholesterol, 7 : 3. Inside: KCl (500 mM)–phosphate buffer (pH = 7, 10 mM). Outside: $NaNO_3$ (500 mM)–phosphate buffer. Changes to Cl^- and K^+ external concentrations were monitored simultaneously using ion-selective electrodes.

Financial support from the BBSRC (BBS/B/11044), the EPSRC (EP/C528859/1) and the University of Bristol is gratefully acknowledged. Dr Sean A. Davis and Jon A. Jones are thanked for help with TEM imaging.

Notes and references

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10 Chloride efflux was slower than in some other experiments, due to the presence of buffer and the incorporation of cholesterol in the membranes (see discussion in text, and Fig. S7 and S8 in the ESI†).