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Cationic β-cyclodextrin bilayer vesicles†

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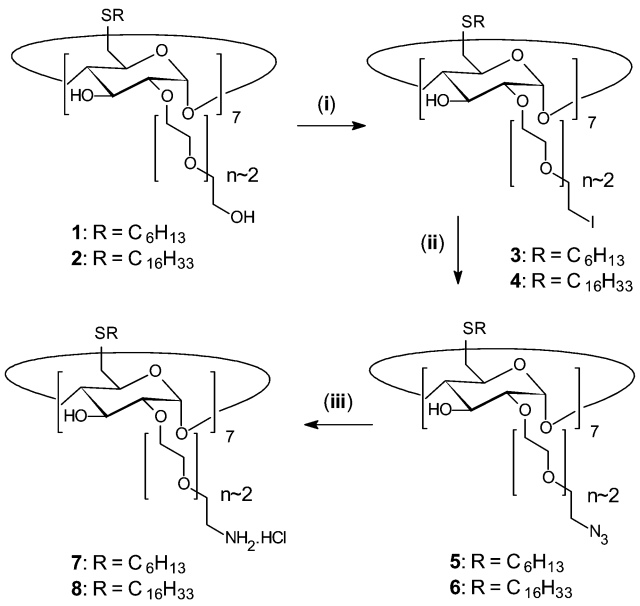
Cationic amphiphilic β-cyclodextrins, substituted with hydrophobic *n*-alkylthio chains at the primary hydroxyl side and hydrophilic ω-amino-oligo(ethylene glycol) units at the secondary side, form bilayer vesicles with a diameter of 30–35 nm (when alkyl = hexadecyl) or nanoparticles with a diameter of *ca*. 120 nm (when alkyl = hexyl) in water.

The ability of cyclodextrins (CDs) to act as hosts to apolar guest molecules by including them in their hydrophobic cavities is well established. Increasingly, the native CDs now serve as scaffolds on which multiple functional groups can be assembled with controlled geometry. This has opened new areas of supramolecular chemistry. Hydrophobically modified CDs are macrocyclic amphiphiles which can form a variety of assemblies including monolayers,[1] micelles[2] and nanoparticles.[3] The amphiphilic CDs can be non-ionic, anionic or cationic. CDs with lipophilic alkylthio 'tails' and oligo(ethylene glycol) 'head groups' form non-ionic vesicles in water.[4] Recently, it has been reported that amphiphilic CD sulfates form anionic vesicles.[5] Here we describe cationic amphiphilic CDs which are the first CDs to form cationic bilayer vesicles in water. Previously, only micelles and monolayers of cationic CDs had been reported.[1b,c,f,g,2a] Vesicle formation by cationic amphiphilic macrocycles has been reported for calix[4]arenes and cryptands.[6]

Scheme 1 shows the synthetic route to the cationic β-CD amphiphiles **7** and **8**. The alkylthio β-CDs **1** and **2** were prepared as described, with seven identical alkylthio groups per CD (one per glucose) and about 3 units of ethylene glycol per glucose for CD **1** and about 2 units of ethylene glycol per glucose for CD **2**.[7] The ω-iodo CD derivative **4** was synthesised as reported for **3**,[7] but with higher excesses of reagents. Isolation and purification were easier due to its higher lipophilicity, resulting in a higher yield. Complete halogenation of the oligo(ethylene glycol) substituents was obtained for both. The ^{13}C-NMR spectra show replacement of the terminal alcohol carbon of the oligo(ethylene glycol) units at 61.2 ppm by the iodide carbon at 3.0 ppm. The azides **5** and **6** (with the azide carbon at 51 ppm) were prepared by treatment of the ω-iodo CD derivatives with NaN_3. To obtain the target ω-amino CDs **7** and **8**, the azides were reduced using PPh_3 in DMF, followed by aqueous ammonia. The amines were isolated as the hydrochloride salts at pH 2. All compounds were characterised by ^{1}H and ^{13}C-NMR as well as ^{1}H–^{13}C HSQC NMR spectroscopy (500 MHz, $CDCl_3$ or d_6-DMSO), MALDI-TOF MS and elemental analysis. The Electronic Supplementary Information show a detailed description of the synthesis and characterisation of **4**–**8**.†



Scheme 1 Synthetic procedures: (i) DMF, PPh_3 (**1**: 14 equiv., **2**: 20 equiv.), NIS (**1**: 14 equiv., **2**: 20 equiv.), 100 °C, 4–5 h, 45–70%. (ii) DMF, NaN_3 (50 equiv.), 100 °C, 4–5 d, 65–70%. (iii) (a) DMF, PPh_3 (14 equiv.), 2 h; (b) 10% NH_3(aq), **7**: 10 h, **8**: 18 h at 45 °C; (c) 1 M HCl, 50–65%.

Self-assembly properties of these cationic CD amphiphiles in water at neutral pH were investigated by transmission electron microscopy (TEM) and dynamic light scattering (DLS).‡ Fig. 1 shows micrographs of the particles formed by the cationic CD amphiphiles. CD **8** forms relatively small bilayer vesicles with a diameter of 30–35 nm and a narrow size distribution. In comparison, its non-ionic precursor **2** forms much larger vesicles (average diameter 100–300 nm) under the same conditions.[4] The smaller size of the cationic vesicles can be explained in terms of electrostatic repulsion and hydration of the cationic head groups, which will cause greater membrane curvature.[8] The DLS results are consistent with TEM, indicating an average hydrodynamic diameter of 35 nm for vesicles of **8**. In contrast, TEM of CD **7** shows nanoparticles of dispersed size distribution, instead of vesicles. The average hydrodynamic diameter of the nanoparticles of **7** was 120 nm according to DLS. This demonstrates how the mode of aggregation of these amphiphilic macrocycles is determined by the balance between

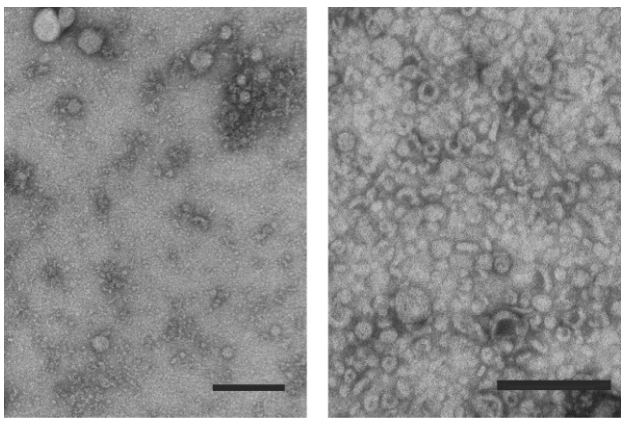


Fig. 1 Electron micrographs of ω-amino CDs **7** (left) and **8** (right) in water (0.1 mg mL^{-1}). Negative staining with UO_2Ac. Scale bars = 200 nm.

† Electronic supplementary information (ESI) available: experimental data. See http://www.rsc.org/suppdata/cc/b2/b207238f/

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hydrophobic alkyl chains and hydrophilic ω-amino-oligo-(ethylene glycol) head groups, as previously observed for non-ionic CD **1**.[9]

Further evidence that **8** forms cationic vesicles was obtained by encapsulation of the hydrophilic fluorescent dye Rhodamine B in the aqueous interior of the vesicles. Vesicles of **8** were prepared in a solution of Rhodamine B (5.0 mM Rhodamine B, 10 mM Hepes, 140 mM NaCl, pH 7.4). Encapsulated Rhodamine B was separated from the free dye by gel filtration on a 20 × 1 cm column of cationic DEAE Sephadex A25–120, using 10 mM Hepes/140 mM NaCl as eluent. (We observed a strong aspecific absorption of CD **8** on neutral Sephadex G25.) The CD vesicles eluted at 7–8 mL, whereas the free dye eluted beyond 12 mL. Coincidence of entrapped Rhodamine B with the elution of the vesicles confirms the existence of an aqueous interior (Fig. 2A). Addition of Triton X-100 to the vesicles leads to a marked increase in Rhodamine B fluorescence, indicating relief of self-quenching upon dilution of the dye from the encapsulated volume into the bulk solution. The amount of entrapped Rhodamine B is small, as may be expected for such small vesicles which have only a minimal internal aqueous compartment.

Attempts were made to determine the L_β–L_α bilayer phase-transition temperature of vesicles of CD **8** using differential scanning calorimetry. In contrast to CD **2**,[4] no thermal transition was detected for vesicles of CD **8**. However, it is well established for liposomes that this phase transition becomes athermic upon reduction of the vesicle diameter.[10]

Finally, we prepared vesicles from mixtures of cationic CD **8** and non-ionic CD **2** to investigate the effect of the CD composition on the size of vesicles prepared under identical conditions‡ (Fig. 2B). The large vesicle size (about 300 nm) for the neutral CD **2** decreases rapidly with addition of the cationic CD **8**. At approximately equimolar composition, the head group repulsion is already significant enough to reduce the average vesicle size to 30–40 nm, where it remains constant up to 100 mol% of CD **8**. Rhodamine B was also encapsulated in vesicles composed of 50 mol% each of CD **8** and CD **2**, and in vesicles composed entirely of CD **2** (results similar to Fig. 2A, see ESI†). Encapsulation in vesicles of CD **2** differed in that the Rhodamine B solution was prepared in 10 mM Hepes buffer and Sephadex G25 was used for separation. Again, coincidence of the entrapped Rhodamine B with elution of the vesicles confirms the existence of an aqueous compartment inside the vesicles.

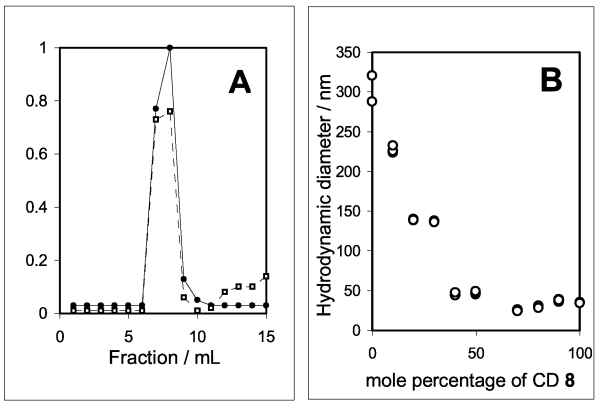

Fig. 2 A Encapsulation of Rhodamine B in vesicles of CD **8**: elution profile of DEAE Sephadex column. Closed symbols, solid line: vesicles (normalised light scattering at 400 nm). Open symbols, dashed line: Rhodamine B (normalised fluorescence intensity at 550 nm). **B** Average hydrodynamic diameter obtained from DLS (as a function of mole percentage of **8**) for vesicles composed of cationic CD **8** and non-ionic CD **2**.

In summary, we have synthesised a cationic amphiphilic β-cyclodextrin that forms bilayer vesicles in aqueous solution. Its potential as a vector in gene delivery—by analogy with non-viral carriers such as cationic lipids and polymers[11]—will be reported in due course.

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Notes and references

‡ CD solutions (0.1 mg mL^{-1}) were prepared from stock solutions (1 mg mL^{-1}) of CDs **7** and **8** in $CHCl_3$ which was slowly evaporated under a constant stream of N_2 to form a thin film. The CD film was hydrated with Hepes buffer (10 mM, pH 7.4), maintained at 60 °C for 1 h, then sonicated for 1.5 h at 60 °C. The solutions were investigated by TEM using a JEOL2000 electron microscope after negative staining with uranyl acetate and by DLS using Malvern instrumentation. Synthesis and analytical data of CDs **4**–**8** are available as ESI.† *WARNING:* Cyclodextrin **4** was air-dried at ambient temperature and should not be dried in vacuum since it then becomes insoluble in all organic solvents.

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