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Erythrocyte-like liposomes prepared by means of amphiphilic cyclodextrin sulfates†

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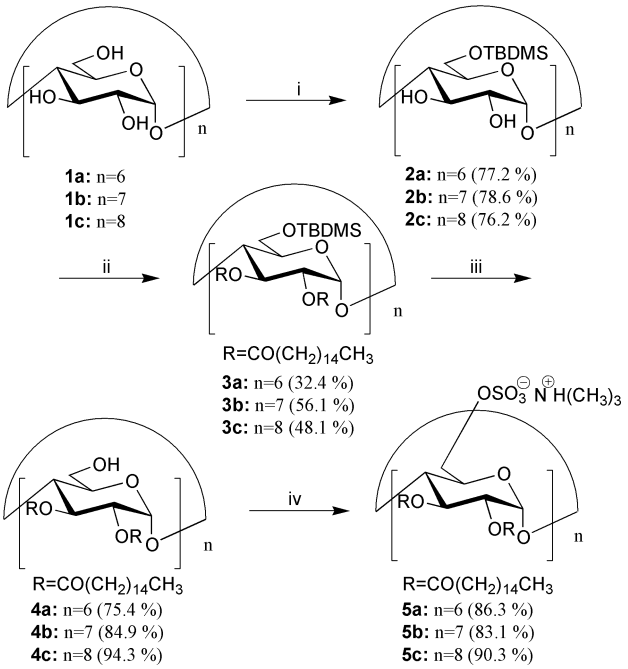
A novel class of sulfated glycolipids with excellent self-assembling capacity to form stable monolayers at an air–water interface and specific erythrocyte-like liposomes was synthesised from α, β, and γ-cyclodextrins as starting materials.

Liposomes are artificial cell-like materials useful both as biomembrane models and as potential drug carriers.[1] Since they are closed vesicles consisting of unilamellar or multilamellar membranes, they can encapsulate various molecules in their internal aqueous phase or their phospholipid membranes.[2] In fact, liposomes carrying antibodies or oligosaccharide chains have also been reported as effective and specific reagents in antibacterial, antitumor,[3] and anti-Human Immunodeficiency Virus (HIV) therapies.[4] Carbohydrates in particular, which are biodegradable and have low immunogenicity, seem to be suited as signal molecules for the modulation of both the physical properties and functions of liposomes. Indeed, glycolipids specifically recognized by proteins displayed on cell surfaces can be used for as specific and efficient ligands in liposome-based drug delivery systems.[5]

Cyclodextrins (CDs) have been reported not only as the host moiety for small guest molecules but as appropriate scaffolds for the design of potent glycoligands that possess efficient 'glycoside cluster effects'.[6,7] Since anionic sugars such as sialic acid, glucuronic acid, and some sulfated sugars found in many glycoconjugates have crucial roles in a variety of biological recognition systems, our attention was directed toward the feasibility of CDs as scaffolds in the synthesis of a novel type of clustered glycolipid composed of anionic sugar residues. Although many amphiphilic CD derivatives for encapsulation, solubilization and transport of drugs have been reported and shown to exhibit excellent self-assembly ,[8–15] we establish herein an efficient synthetic route to 6-*O*-sulfated CD amphiphiles—the first examples of CD-based anionic amphiphiles that can form specific erythrocyte-like vesicles.

Scheme 1 shows the synthetic strategy to sulfated CD amphiphiles **5a–c**. First, 6-*O*-*tert*-butyldimethylsilyl (TBDMS) derivatives **2a–c** prepared from α, β, and γ-CDs (**1a–c**)[16] were treated with palmitoyl anhydride and 4-dimethylaminopyridine (DMAP) in dry pyridine according to Lesieur's condition[13] to afford intermediates **3a–c** in 32–51% yield. Next, compounds **4a–c** prepared by the selective removal of TBDMS groups at C-6 positions of **3a–c** were treated with sulfur trioxide–trimethylamine complex in DMF and toluene to give the desired sulfated CD amphiphiles **5a–c** in 83–90% yield. All new compounds synthesized in this communication were characterized by NMR spectroscopy (600MHz, $CDCl_3$), MALDI-TOF mass spectroscopy, differential scanning calorimetry, and elemental analysis.†

The monolayer behavior of non-sulfated CD amphiphiles **4a–c** and sulfated CD amphiphiles **5a–c** at the air–water interface



Scheme 1 (i) *tert*-Butyldimethyl chloride, pyridine, rt, 12 h; (ii) palmitoyl anhydride, 4-dimethylaminopyridine, pyridine, 80 °C, 48 h; (iii) $Bf_3{\cdot}Et_2O$, dichloromethane, r.t., 3 h; (iv) sulfur trioxide–trimethylamine complex–DMF and toluene, 80 °C 48 h.

was investigated. Solutions of these derivatives dissolved in pure chloroform (0.2 mg mL^{-1}) were first deposited on a Langmuir film balance and the compression (mN m^{-1})–*A* (nm^2) isotherms were recorded at a constant rate of 60 mm^2 min^{-1} at 23 °C. As shown in Fig. 1, compounds **5a–c** formed stable monolayers with high collapse pressures and the desired area per CD residue. The collapse pressure values for all sulfated CD amphiphiles (**5a–c**) were found to be much higher than those of

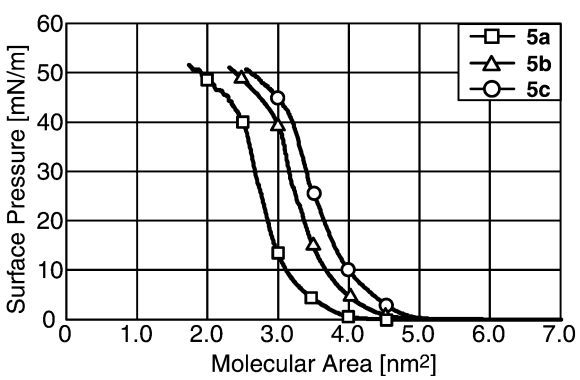


Fig. 1 Surface pressure (π) – molecular area (*A*) isotherms for sulfated CD amphiphiles at the air–water interface.

† Electronic supplementary information available: experimental details and selected analytical and spectroscopic data for compounds **5a–c**. See http://www.rsc.org/suppdata/cc/b1/b110673b/

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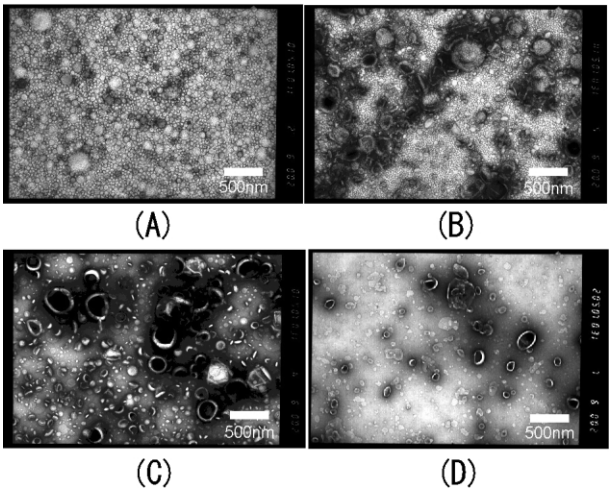

Fig. 2 Electron micrographs of vesicles after negative staining with uranyl acetate. Vesicles composed of DPPC, cholesterol and amphiphilic CD **5b**. (A) 100:10:0, (B) 100:10:1, (C) 100:10:5 and (D) 0:0:100 by molar ratio.

the neutral CD amphiphiles **4a–c**, suggesting that much improved self-assembly of these sulfated CD amphiphiles at the air–water interface results from the introduction of 6-*O*-sulfate groups into the precursors **4a–c**.

A mixture of compound **5b** with dipalmitoyl phosphatidylcholine (DPPC, Wako Pure Chemicals) and cholesterol dissolved in dichloromethane was dispersed in water by using a sonication bath at 50 °C for 20 min. The emulsion was concentrated to a half volume solution by spraying N_2 gas, a small amount of pure water was added and followed by treatment in a sonication bath for 10 min. The residue was similarly concentrated by spraying N_2 gas to obtain a gel of the lipid mixture. After diluting the gel with a suitable volume of pure water, the mixture was treated by voltex mixer for 3 min and the obtained suspension was chromatographed on a column of Sephadex G-50 with pure water to afford the fraction containing CD nanoparticles. Morphologies of the liposomes prepared from **5b**, DPPC, and cholesterol were investigated by using a transmission electron microscope (TEM) (HITACHI H-800, accelerating voltage 75 kV) after negative staining with uranyl acetate solution. TEM views of liposomes prepared from the different ratio of lipids are shown in Fig. 2. As indicated in Fig. 2(B)–(D), it appears that vesicles containing compound **5b** exhibited much larger diameters (100–300 nm) than those of the liposomes composed of DPPC and cholesterol [DPPC:cholesterol = 10:1, Fig. 2(A)]. Addition of the sulfated CD amphiphiles seemed to induce the formation of interesting erythrocyte-like liposomes having controlled encapsulation capacity for fluorescent-labelled compounds. Since some sulfated derivatives of β-CD show potent inhibitory effects on the replication of HIV-1,[17] sulfated CD amphiphiles synthesised here might greatly contribute to the creation of new CD-based carrier molecules having significant biological functions.

In summary, we have designed novel amphiphilic CD sulfates having excellent capacity to form stable monolayers at the air–water interface and interesting erythrocyte-like liposomes (Fig. 3). We are currently investigating the feasibility of these compounds as drug delivery systems and looking at their biological activity; the results will be reported as soon as possible.

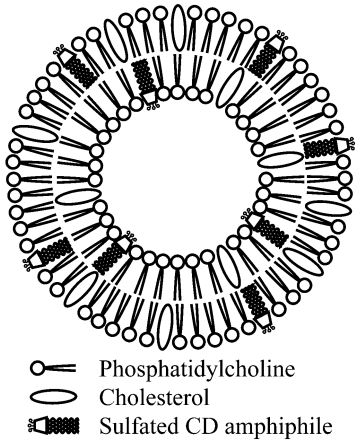

Fig. 3 Schematic structure of liposome containing DPPC, cholesterol and sulfated CD amphiphiles.

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