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Anionic Facial Amphiphiles from Cholic Acid

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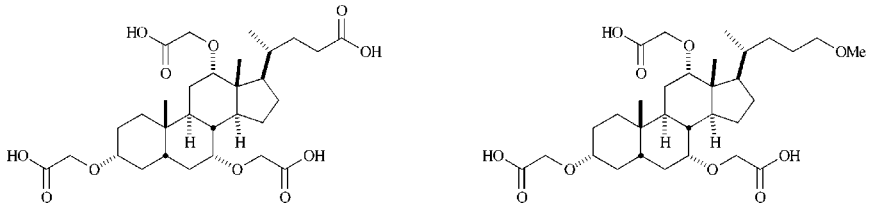
ABSTRACT



Anionic facial amphiphiles have been prepared from cholic acid. These compounds offer antipodes of recently reported cationic amphiphiles derived from cholic acid. The synthesis of the anionic amphiphiles was accomplished in few steps from a common intermediate. In contrast to many other anionic facial amphiphiles, the cholic acid derived amphiphiles appeared to aggregate at relatively low concentration.

Typical amphiphile morphology (e.g., that of diacylglycerolphosphates) offers a polar headgroup and a hydrophobic tail. Multiple examples of nontypical amphiphiles have been reported, including tripodal[1] and facial amphiphiles,[2] which display a number of properties that differ from those of typical amphiphiles including enhanced abilities to solubilize specific types of molecules[1] and lack of critical aggregation behavior.[2b] Interactions of facially amphiphilic compounds with lipid bilayers are of particular interest because facial amphiphiles can permeabilize membranes[3,4] and act as potent antibacterial agents.[4−6] For example, multiple cationic peptide antibiotics, including many isolated from natural sources[5] and those developed from β-peptides,[6] adopt facially amphiphilic helical conformations.

Cholic acid is a common bile acid which displays a degree of facial amphiphilicity due to the orientation of its three hydroxyl groups on one face of the molecule. This steroid has been used extensively in the development of compounds used in molecular recognition studies[7] and has been used in the preparation of nondenaturing detergents.[8] Nevertheless, cholic acid cannot be considered strongly facially amphiphilic because it does not bear charged groups on its polar face. In an effort to increase the amphiphilicity of cholic acid, triamino analoges of cholic acid (**1a**[2d] and **1b**,[9] Figure 1) have been prepared. In aqueous solution, these compounds

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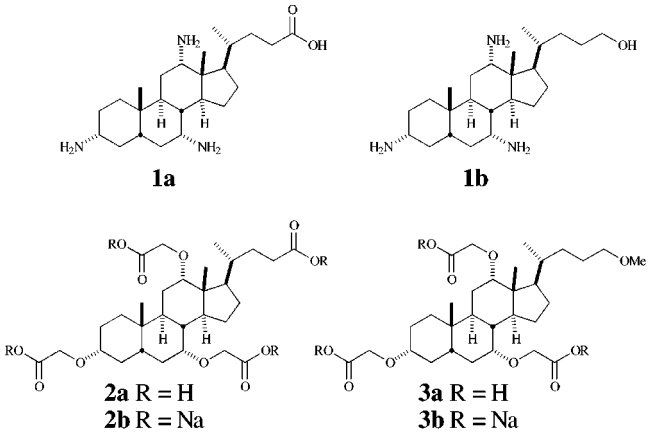


Figure 1. Structures of facial amphiphiles **1a** **1b**, **2**, and **3**.

offer a cationic face opposite to the hydrophobic face of the steroid, and studies with **1a** show no evidence of aggregation below 100 mM.[2d]

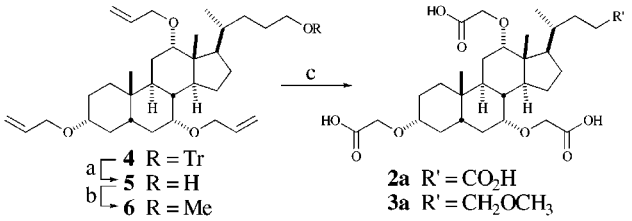
Another means of increasing the amphiphilicity of cholic acid would be to incorporate negatively charged groups onto a cholic acid scaffolding yielding antipodes of **1a** and **1b**. We have prepared anionic facial amphiphiles **2** and **3** from cholic acid, and in these molecules three carboxyl groups are oriented on or near one face of the cholic acid backbone.

In **2a** and **3a**, the carboxyl groups are linked to the steroid as ethers of glycolic acid. The short methylene tethers were used to limit the number of orientations of the carboxylate groups and provide an acid- and base-stable linkage to the steroid backbone.

To understand the types of conformations available to the carboxylates of **2a** and **3a**, we did molecular modeling using semiempirical calculations (AM1-SM2,[10] a method which accounts for aqueous solvation). It was expected that conformations of these compounds might influence their amphiphilicity. To simplify the calculations, only the A, B, and C rings, with their appended groups, were used in the calculations (the D ring was later added to the structures and was minimized using molecular mechanics). The effects of rotations about the carbon−ether oxygen bonds were calculated. If the C−O−C−C torsion angles were ∼180° (anti) (A in Figure 2), the carboxylates were much closer together than when the same torsional relationships were gauche (B, in Figure 2). These two conformations, A and B, were calculated to be within 1 kcal/mol of one another with A calculated to be the lowest in energy. Because of the low difference in energy between these two forms, it is likely that both are present in aqueous solution among other forms in which mixtures of anti and gauche torsional relationships about the C−O−C−C bonds are present. As shown in Figure 2, both conformers are facially amphiphilic, although because the carboxylates are oriented closer together on one face of the steroid, conformation A would be expected to more facially amphiphilic.

Compounds **2a** and **3a** were prepared beginning with **4** (Scheme 1). As noted previously,[4a,b] to form the allyl ether bonds in **4**, it was necessary to use a reduced (C-24) form of cholic acid. Removal of the trityl group gave **5**, and methyl ether formation at C-24 yielded **6**. Oxidation of **5** and **6** with ruthenium trichloride and periodate effected carbon−carbon bond cleavage and oxidation to glycolic acids **2a** and **3a** in a single step in 30−33% yields. Considering the number of reactions that occur in this step, we deemed the yields adequate. Purification of **2a** and **3a** as their ammonium salts was relatively facile using an eluent of dichloromethane, methanol, and ammonium hydroxide. Salts **2b** and **3b** were prepared by adding a very small excess of sodium hydroxide to the amphiphiles followed by lyophilization.

Scheme 1. Preparation of **2a** and **2b**



Reagents (yields in parentheses): a) TsOH, MeOH, CH_2Cl_2 (92%). b) MeI, NaH, DMF (90%). c) $RuCl_3 \cdot H_2O$, $NaIO_4$, MeCN, CCl_4, H_2O (30% for **2a** from **5**; 33% for **3a** from **6**).

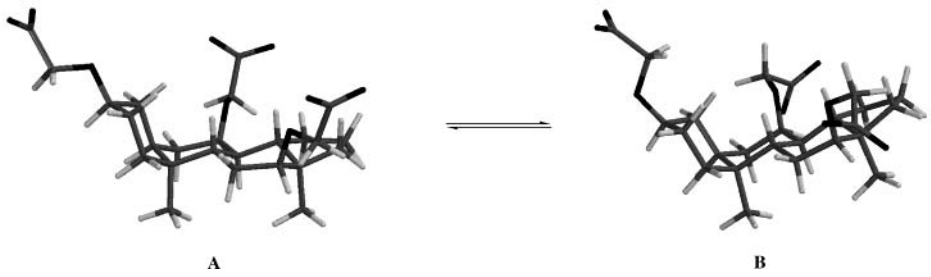


Figure 2. Modeled structures (AM1-SM2 semiempirical calculations) of the A, B, and C rings of **2b** (the D ring was added after the calculation to facilitate visual orientation of the structures).

To determine if the aggregation properties of **2b** and **3b** were similar to those of other anionic facial amphiphiles, we measured the concentrations at which they aggregated. We were especially interested to learn if the compounds demonstrated critical aggregation phenomena. There are multiple means of observing aggregation including changes in NMR spectra,[2b,11] dye solubilization,[12] and alterations in the luminescent properties of fluorescent probes.[13] In these experiments, it is important that the protonation states of the carboxyl groups be known. Consequently, we measured the pK_a values of the carboxylic acids in **3a** to find at what pH the tricarboxylate would form. Because of the proximity of the carboxylates in **2b** and **3b**, there was concern the tricarboxylate might form at pHs higher than expected for isolated carboxylic acids. Separate pK_a values for each of the carboxylic acids were not obtained from titrations of **3a** with NaOH solutions;[14] only a single pK_a value (5.7) was measured, even after pH values of >12 were reached in the titrations. Consequently, aggregation experiments were performed with solutions at pH ≥11.

Because amphiphiles **2b** and **3b** do not contain aromatic groups, it was expected that ^{1}H NMR chemical shift changes concomitant with aggregation would be minimal. Over a large concentration range (0.01−100 mM) in D_2O no significant chemical shift changes were observed in the ^{1}H NMR spectrum of the salt of **3b**.

Separate experiments with dye solubilization and a fluorescent probe were much more illuminating. We used the water-insoluble dye orange OT for the solubilization experiments. In the presence of the amphiphiles, solubility of the dye in water increased markedly above concentrations of ca. 0.5 and 0.8 mM of salts **2b** and **3b**, respectively (Figure 3).

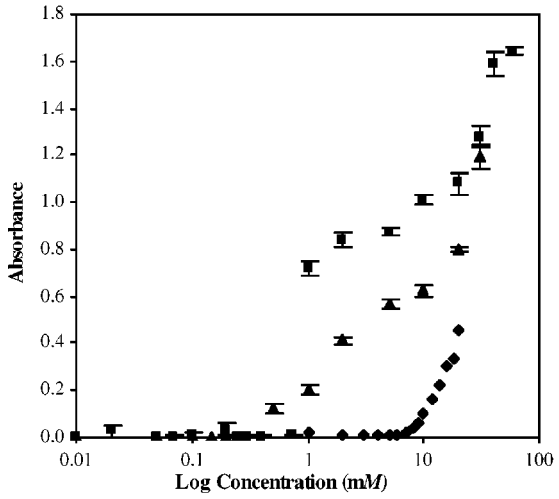


Figure 3. Results from orange OT solubilization experiments. See Supporting Information for experimental details: ▲ data for **2b**; ■, data for **3b**; ♦, data for sodium dodecyl sulfate. Error bars indicate ±1 standard deviation.

The aggregation concentration of sodium dodecyl sulfate (SDS) was also measured to verify the experimental method. On the basis of the behavior of other facially amphiphilic compounds, it was unexpected that **2b** and **3b** would solubilize the dye at relatively low concentrations and that apparent critical behavior would be exhibited. In particular, the very sharp increase in dye solubilization caused by **3b** just below 1 mM was surprising. To verify our results, the experiments were performed > 5 times with independently prepared solutions. Error bars are included in Figure 3 for data from compounds **2b** and **3b**.

We used the fluorescent probe Prodan in an attempt to corroborate the dye solubilization results, and data from experiments with Prodan were in very good agreement with our aggregation concentrations measured via dye solubilization. The wavelength of Prodan fluorescence changes in response to its surroundings; in a low dielectric environment, Prodan exhibits an emission maximum from 435 to 485 nm.[13a] Alone, in aqueous solution, Prodan fluoresces at approximately 510 nm. In the presence of incrementally increasing concentrations of each amphiphile, the fluorescence of the probe at 435 nm increased beginning at concentrations of approximately 1 and 2 mM of **2b** and **3b**, respectively (Figure 4).

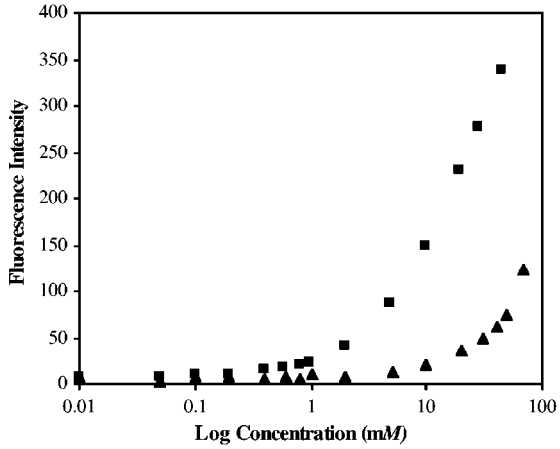


Figure 4. Results from Prodan fluorescence experiments (emission measured at 435 nm). See Supporting Information for experimental details: ■, data for **2b**; ▲, data for **3b**.

The wavelengths at which Prodan fluoresces have been correlated to the nature of its hydrophobic environment. For example, in well-ordered lipid bilayers (gel phase), Prodan gives an emission maximum at ca. 435 nm. In less well ordered bilayers (liquid-crystalline phase) the emission maximum is observed at ca. 485 nm.[13b] In control experi-

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ments with SDS, aggregation resulted in an increase in Prodan fluorescence at approximately 485 nm without emission at 435 nm (Figure 5). The fact that **2b** and **3b**

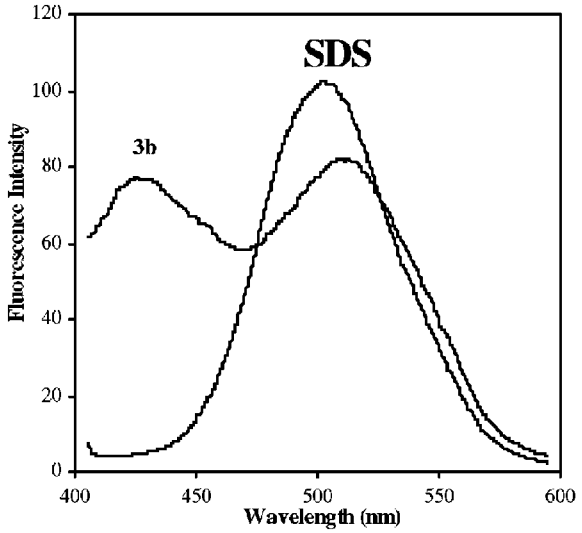

Figure 5. Emission spectra (λ_{ex} = 351 nm) of Prodan (0.45 μM) with **3b** and SDS above their critical aggregation concentrations.

caused an increase in fluorescence of the probe at 435 nm (Figure 5) suggests that the aggregates of SDS and **2b** and **3b** are inherently different. Prodan fluorescence at 435 nm has been correlated with aprotic environments, while fluorescence at 485 occurs in more polar, protic environments.[13] Consequently, aggregates of **2b** and **3b** appear to provide a much less polar environment than the micelles formed from SDS.

The results from both dye solubilization and Prodan fluorescence experiments suggest that **2b** and **3b** aggregate at relatively low concentrations and that a critical concentration is required before the onset of aggregation. These results contrast those found with the cationic amphiphile **1a**, which does not appear to aggregate at low concentration (<100 mM). In addition, the behaviors of **2b** and **3b** are different from those of other reported anionic facial amphiphiles which do not display critical aggregation properties.[2b] The data suggest that there may be more of a driving force for sequestering the aliphatic hydrophobic faces of **2a** and **2b** than for other anionic amphiphiles which present aromatic faces. Apparently aggregation of **2b** and **3b** results in formation of a very hydrophobic environment, although due to the topology of these amphiphiles it is unlikely that they form typical micellar aggregates.

We attempted to characterize the size of the aggregates formed by **2b** and **3b** using light scattering and differential scanning calorimetry (DSC).[15] Above the aggregation concentration of **3b**, no light scattering was observed. In DSC experiments with **2b** and **3b**, no heats of aggregate dissociation were measured. Apparently, the aggregates formed by these amphiphiles are inufficiently large to be measured with these techniques.

By modifying the hydroxyl groups of cholic acid, we have prepared novel facial amphiphiles. The sodium salts **2b** and **3b** are highly soluble in water (>100 mM) yet retain the ability to aggregate at much lower concentrations. These compounds may find use in solubilizing hydrophobic molecules, in permeabilizing membranes, and in the design of novel macromolecular architectures.

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Supporting Information Available: Experimental details for the preparation of **2a**, **2b**, **3a,** and **3b** and descriptions of pK_a, aggregation concentration measurements, and DSC experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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