Cholic Acid-Derived Facial Amphiphiles with Different Ionic Characteristics

Zhenqi Zhong, Jie Yan, and Yan Zhao

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

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A cholate derivative with three epoxide groups was synthesized from cholic acid by allylation followed by epoxidation. Ring opening of the epoxides by various nucleophiles yielded facial amphiphiles with anionic, cationic, or nonionic functional groups. The critical micelle concentrations of these amphiphiles largely depend on the number of charged groups on the molecule. A facial amphiphile with pH-tunable micellization was prepared. Its aggregation behavior changes abruptly at pH = 7.6–6.6 as a result of protonation of its amino groups.

Introduction

Cholic acid (1) has an unusual structure. Its α face is hydrophilic with three hydroxyl groups pointing to the same direction; consisting entirely of hydrocarbon, its β face is hydrophobic and faces the opposite direction. Surfactants derived from cholic acid have important physiological and biological functions. They are the main ingredients of bile salts, which form mixed micelles with lecithin to solubilize fats and cholesterol.1 Also, due to their rigid structures, cholate-based surfactants do not denature proteins easily, making them very useful in the handling of membrane proteins.2–4 They are potential therapeutic agents useful in a range of applications including lowering cholesterol, dissolving gallstones, and assisting the absorption of polar drugs.5 In recent years, cationic cholates were found to mimic antimicrobial peptides6 as antibiotics not susceptible to bacterial resistance.7,8

![β-face and α-face](Image)

1. Cholic acid

Because of its unique structure and commercial availability, cholic acid is a popular building block in supramolecular chemistry.9 More recently, it became an attractive subunit in the construction of novel amphiphiles that are sensitive to environmental stimuli. Regen and co-workers synthesized “molecular umbrellas” to shield hydrophilic agents and assist their permeation across lipid bilayers.10 Ariga et al. prepared novel supramolecular hosts whose molecular recognition properties can be controlled by surface pressure at the air–water interface.11 We recently reported amphiphilic molecular baskets, which undergo transitions between micellelike and reversed-micellelike conformations depending on the solvent polarity.12

The applications mentioned above for cholate derivatives are directly related to their facial amphiphility13 that is, their polar and nonpolar groups are located on


opposite faces instead of at opposite ends as in conventional surfactants. However, the facial amphiphilicity of cholic acid is weak with only three hydroxyl groups on the α face. To increase the facial amphiphilicity, various groups including sugars, sulfates, ammoniums and carboxylates have been introduced to the α face of cholic acid. Different linkages including acetals, sulfate esters, ethers and esters are employed in the functionalization. The level of difficulty varies widely in these syntheses. In this article, we report facile syntheses of these facial amphiphiles by the nucleophilic ring opening of an epoxide derivative of cholic acid. We use ether linkages exclusively in the synthesis because of their excellent stability in aqueous solutions. Our method functionalizes the α face of cholic acid with a wide range of polar groups with different ionic characteristics. As a result, we can tune the aggregation behavior of these facial amphiphiles systematically.

Experimental Section

General. Anhydrous tetrahydrofuran (THF) and methylene chloride were dried by passage through a column of activated alumina under compressed nitrogen. Cholic acid was crystallized from 95% ethanol and dried at 90 °C under vacuum. Orange OT (purchased from TCI, Ltd.) was precipitated from an acetone solution into water. The crude product was purified by chromatography over silica gel using CHCl3/MeOH (2:1) and CHCl3/MeOH/Et3N (3:1:0.1) as the eluents to give a colorless oil (1.67 g, 74% yield). 1H NMR (300 MHz, CD3OD, 99.98; 13C NMR (75 MHz, CD3OD): 175.20, 81.07, 81.02, 82.04, 82.74, 79.97, 76.90, 71.60, 71.54, 70.97, 70.92, 70.61, 67.43, 67.40, 67.36, 67.30, 67.12, 65.21, 65.42, 62.09, 62.06, 61.84, 61.81, 50.95, 46.32, 46.22, 46.43, 46.46, 44.54, 44.37, 42.73, 42.69, 42.65, 41.92, 39.78, 39.72, 35.47, 35.37, 35.30, 35.06, 94.81, 31.07, 30.55, 28.76, 27.94, 27.45, 22.63, 21.47, 14.17, 11.76. ESI-MS (m/z): [M + H]+ cided for C29H57NaO11, 563.80; found, 563.79.

Synthesis of Compound 5. A mixture of compound 4 (1.28 g, 2.2 mmol), dimethylaminopropylcarbamate (1.78 mL, 13.97 mmol), and MeOH (15 mL) were stirred at 50 °C under N2 for 24 h. The solvent was evaporated in vacuo. The residue was purified by column chromatography over silica gel using CHCl3/MeOH (3:1) and CHCl3/MeOH/Et3N (3:1:0.1) as the eluents to give a colorless oil (424 mg, 63% yield). 1H NMR (300 MHz, CDCl3, 99.98; 13C NMR (75 MHz, CDCl3): 175.00, 79.95, 81.02, 81.04, 81.06, 81.08, 80.88, 81.23, 77.82, 77.29, 73.53, 72.53, 72.75, 70.97, 70.92, 70.61, 67.43, 67.40, 67.36, 67.30, 67.12, 65.21, 65.42, 62.09, 62.06, 61.84, 61.81, 50.95, 46.32, 46.22, 46.43, 46.46, 44.54, 44.37, 42.73, 42.69, 42.65, 41.92, 39.78, 39.72, 35.47, 35.37, 35.30, 35.06, 94.81, 31.07, 30.55, 28.76, 27.94, 27.45, 22.63, 21.47, 14.17, 11.76. ESI-MS (m/z): [M + H]+ cided for C29H57N3O14Na, 929.0; found, 929.0; [M + Na]+ cided for C29H57N3O14Na, 929.0; found, 929.0; [M + K]+ cided for C29H57N3O14K, 945.0; found, 945.0.

Orange OT Solubilization. Aliquots of 1.5 mL of the amphiphiles at different concentrations in water were placed in screw-capped vials and gently rocked in the presence of excess solid Orange OT for 3 days. The excess dye was removed by filtration through syringe filters [Millipore Millex hydrophilic poly(tetrafluoroethylene) filters, 0.45 μm]. An aliquot of 300 μL of each solution was diluted with 1.20 mL of absolute ethanol. The absorbance of each sample was measured at 486 nm with a 1-cm path length cell. Each experiment was repeated three times with separately prepared solutions.

Pyrene Fluorescence. The excitation wavelength was set at 332 nm. The emission spectrum was recorded from 360 to 400 nm at a scan rate of 30 nm/min. The excitation slit width was 20 nm, and the emission slit width was 1.5 nm. For the study of the effect of concentration of 8 on pyrene fluorescence, a 50 mM stock solution of 8 containing 1 μM of pyrene was prepared. Aliquots of this solution were then diluted with an aqueous 1 M solution of pyrene to afford solutions of 8 at different concentrations. For the study of the pH effect, a 3.0 mM solution (10.0 mL) of 8 containing 1 μM of pyrene was prepared. Ten aliquots of 10 μL of 1 M HCl were added sequentially to the 10.0 mL of solution of 8. Fluorescence and pH measurements were recorded after each addition.

Results and Discussion

Synthesis. We choose the epoxide derivative (4) of cholic acid as the key intermediate because epoxides can be opened by many nucleophiles under mild conditions. The
ring opening reaction is compatible with many functional groups including ester, hydroxyl, and tertiary amine. Retrosynthetically, 4 can be prepared from the triallyl derivative 3 by epoxidation using meta-chloroperbenzoic acid (MCPBA). Compound 3 may be prepared by alklylation of methyl cholate using allyl bromide and sodium hydride. However, under this standard alklylation condition, the ester group may potentially undergo many side reactions including ester condensation, transesterification, and α-alkylation. Therefore, in a literature procedure, trialylation of cholic acid was performed with the carboxyl group reduced to alcohol and protected by triphenylmethyl ether. Nevertheless, it is highly desirable to retain the carboxyl group of cholic acid, for it can be used for ligation via amide coupling in the presence of other functional groups such as hydroxyl, sulfonate, or tertiary amine. If the carboxyl group is reduced to alcohol, this option is no longer available, as several more hydroxyl groups will be generated during the ring opening of epoxides. After many unsuccessful attempts, we found that the combination of allyl iodide (which is much more reactive than allyl bromide used in previous procedures), sodium hydride, and tetrabutylammonium iodide (Bu4NI) affords triallylation effectively. Most side reactions are suppressed as several more hydroxyl groups will be generated during the ring opening of epoxides. After many unsuccessful attempts, we found that the combination of allyl iodide (which is much more reactive than allyl bromide used in previous procedures), sodium hydride, and tetrabutylammonium iodide (Bu4NI) affords triallylation effectively. Most side reactions are suppressed at low temperatures (<40 °C).

With methyl cholate as the starting material, transesterification occurred during the reaction and the methyl group is partially exchanged into the allyl group. In addition, when the excess of sodium hydride was quenched by water (or aqueous hydrochloric acid) at the end of the allylation, extensive hydrolysis of the methyl ester happened. Hydrolysis may have been facilitated by the tetrabutylammonium cation, which can transfer anions such as hydroxide into the organic phase. We solved both problems by directly alklylating cholic acid using an excess of allyl iodide in the presence of sodium hydride and Bu4NI. The tetrallylated product 2 (together with any hydrolyzed product) was then converted to 3 by heating the crude mixture in methanol with sulfuric acid as the catalyst. The overall yield from 1 to 3 was 70%. Epoxidation of 3 by MCPBA produced the desired 4 in 80% yield.

Ring opening of 4 was straightforward. Its hydrolysis gave carboxylate 5. Nucleophilic attack of 4 by sodium sulfite afforded sulfonate derivative 6. Ring opening by dimethylamine generated cationic facial amphiphile 7 after protonation of the amino groups. Finally, nonionic amphiphile 8 was obtained by opening epoxide 4 with 1-(N-methylamino)-2,3-propanediol (Scheme 1).

**Characterization of Aggregation Behavior.** Amphiphiles 5–7 are soluble in water and form translucent solutions. Similar to cholic acid, 5 is only water-soluble in the carboxylate form. On the other hand, 7 is only watersoluble in the protonated, ammonium form. The main difference between the three amphiphiles is the number and the nature of charged groups: 5 has one negatively charged carboxylate, 6 has three negatively charged sulfonates, and 7 has three ammonium cations.

We used a dye solubilization method to characterize the aggregation behavior of these facial amphiphiles. This method has been widely used in the characterization of surfactants including facial amphiphiles. In this procedure, a hydrophobic dye, Orange OT, is shaken with different concentrations of an amphiphile in water. Dissolution of the dye only happens above the CMC of the amphiphile. As seen in Figure 1, amphiphile 5 begins to solubilize the hydrophobic dye above 1 (10^0) mM concentration and becomes more effective above 4–5 (10^1–10^2) mM. With three charged groups, amphiphiles 6 and 7 do not solubilize Orange OT significantly until the concentration is above 10 (10^1) and 25 (10^1.4) mM, respectively.

We also performed dye solubilization by sodium cholate, which is the parent amphiphile (data shown as in Figure 1). Sodium cholate does not solubilize Orange OT until its concentration reaches about 20 (10^1.5) mM. This number is slightly larger than the literature value (16 mM) obtained by the same method for sodium cholate in the 150 mM sodium chloride solution. Such a difference is expected because the CMC of sodium cholate is known to decrease with increasing ionic strength. What is surprising is that this CMC is higher than that of the closely

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solution pH. Dye solubilization clearly shows such a behavior. In Millipore water, solubilization of Orange OT begins at about 1 (10^{-9}) mM and becomes more significant above 3–5 (i.e., 10^{0.5}–10^{0.7}) mM, as indicated by Figure 2. At pH ≈ 4, however, significant solubilization of the dye does not occur until the concentration of 8 is above 20 (10^{1.3}) mM. Note that 8 and 7 have similar aggregation properties in the protonated form; the latter does not solubilize the dye until about 25 mM (Figure 1). The difference is that unprotonated 7 is completely insoluble in water and does not show such a pH-sensitive micellization.

Because 8 has three amino groups, protonation can take place in several stages and affects micellization differently. We were interested in such a pH effect because cationic cholates are promising antimicrobial and transfection agents but nonionic ones are not effective. A pH-sensitive cholate amphiphile, thus, may be useful in drug targeting or controlled release applications. The dye solubilization method, however, is a “batch” process and does not offer information on the micellization process in a continuous fashion. To gain such information, we used pyrene as an environmentally sensitive probe. Pyrene has five vibronic bands in its fluorescence. The first vibronic band (I_1) at 372 nm becomes more intense as its environment becomes more polar. The third band (I_3) at 384 nm is rather insensitive to the polarity of the environment. Therefore, the I_3/I_1 ratio can be used to determine whether pyrene is located in a hydrophobic or hydrophilic environment. Pyrene has been used in the characterization of both conventional surfactants and bile salts because it is highly nonpolar and prefers to reside within the hydrophobic interior of micelles. One advantage of this method is that pyrene is used at concentrations (e.g., 1 μM) far below those of the amphiphiles and, thus, its presence should have a negligible effect on the micellization process.

We first studied the effect of different concentrations of 8 on the pyrene fluorescence in water. This is an independent method to determine the CMC of the amphiphile. As shown by Figure 3, the I_3/I_1 ratio is about 0.53 at low concentrations of 8, which is the same as that of pyrene in pure water. The ratio starts to increase above 0.2 (10^{-9}) mM of the amphiphile and reaches a plateau solution pH.
at 6 (10^{0.8}) mM. This result again suggests that the aggregation of 8, like that of sodium cholate, is a graduate process. The CMC determined by the dye solubilization is about 1 (100) mM, which is the midpoint in Figure 3. Therefore, the two methods give slightly different values of the CMC. It is known that the CMC values measured for bile salts are sensitive to the techniques used, probably as a result of the stepwise aggregation of these amphiphiles.\(^{17}\) At 10 mM of 8, the \(I_3/I_1\) ratio reaches 1.2, which is significantly higher than those for common surfactant micelles (\(I_3/I_1 \approx 0.7 - 1.0\)).\(^{21}\) Therefore, the micelles formed by 8 have interiors more hydrophobic than those of conventional surfactants. Similar findings were observed for other bile salt micelles.\(^{22}\)

When aqueous hydrochloric acid was added to a 3.0 M solution of 8 in water, the amino groups on the molecule are protonated. As shown by Figure 4, the \(I_3/I_1\) ratio starts out high (pH = 9) initially because the concentration is above the CMC. A sharp decrease in \(I_3/I_1\) occurs in a narrow range of pHs from 7.6 to 6.6. The three amino groups apparently are successively protonated within this pH range. As a result, the micelles quickly break up due to electrostatic repulsion among the cholates. It is interesting that even when the pH is 2.4, at which the amphiphile is expected to be fully protonated, the \(I_3/I_1\) ratio (≈0.92) is still fairly high, suggesting that pyrene is in a somewhat hydrophobic environment. Because 8 does not solubilize Orange OT below 20 mM at pH = 4 (Figure 2), it is unlikely that any multimeric aggregates of 8 exist at pH = 2.4 at 3 mM. One possibility is that the monomer binds with pyrene under this condition. At 3 mM, monomeric 8 has a strong preference to hide its hydrophobic face but cannot aggregate due to unfavorable electrostatic interactions. Association between 8 and pyrene, however, does not have to overcome any electrostatic repulsion and, thus, could happen readily. This type of association between pyrene and the amphiphile below the CMC was also reported in other bile salts.\(^{25}\)

Conclusions

A rapid synthesis of facial amphiphiles from cholic acid is described. The method is attractive because a common epoxide intermediate is used to prepare several amphiphiles with different polarities and ionic characteristics. Such a synthesis offers a systematic way to vary the hydrophile–lipophile balance of cholate derivatives. The CMC of our cholate derivatives can be easily tuned between 1 and 25 mM by changing the number of charged groups on the structure. A facial amphiphile sensitive to pH was prepared using this synthesis. Its CMC can be tuned nearly 20-fold by a change in the solution pH. The most sensitive change occurs around pH = 7. These molecules are potentially useful in a range of applications as novel surfactants, therapeutic agents, and building blocks in supramolecular chemistry.

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