Colloid Polym Sci 278:364–368 (2000)

ORIGINAL CONTRIBUTION

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Preparation and characterization of new micelle-forming cholesterol amphiphiles

Received: 7 July 1999
Accepted in revised form: 5 November 1999

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Abstract New sulfated and sulfonated cholesterol amphiphiles were synthesized and characterized by dynamic light scattering and electron microscopy. The surfactants form micelles with diameters up to 30 nm. Their critical micelle concentration values were determined by conductometry and photometric measurements. Use of the new amphiphiles enables the transfer of an asymmetric hydrogenation reaction into aqueous solution by keeping the activity and increasing the enantioselectivity. Interesting liquid-crystalline behaviour was observed.

Key words Cholesterol amphiphile · Chiral surfactant · Micelles · Liquid crystalline

Introduction

Steroid-based amphiphiles are of great interest, for instance in membrane-building processes and as drug delivery systems [1, 2]. Several methods of derivatization, such as ethoxylation [3], glycosylation [4] and esterification [5], have been used to introduce hydrophilic groups onto the cholesterol unit in order to obtain amphiphilic substances. For example, pH and concentration depends on a set of conditions, whether these surfactants form micelles or bilayer systems [6]. Some of them exhibit special enantioselective interactions which could be used for chiral discrimination methods or in asymmetric synthesis [7, 8].

Recently we reported cholesterol amphiphiles forming vesicles with diameters between 20 nm and 1 μm. Some of them were able to induce circular dichroism signals in nonchiral dyes, others were responsible for some enantiomeric excess in an olefin hydrogenation reaction [9].

Continuation of this work led to new sulfated and sulfonated cholesterol amphiphiles which surprisingly form micelles. We investigated the aggregation behaviour of the new surfactants by light-scattering and electron microscopy techniques, always comparing the behaviour with that of the vesicle-forming analogues. It was possible to determine their critical micelle concentration (cmc) values with conductometry and photometric measurements. The new substances are highly dispersible in water and support an olefin hydrogenation reaction in water by solubilization of the reactants and the catalyst. Experiments concerning enantioselective

interactions with the micellar structures were unsuccessful in contrast to the former vesicular systems. Last but not least the new compounds exhibit strongly developed liquid-crystalline behaviour.

Experimental

Materials

The chemicals were provided by Fluka (triethylene glycol, tetraethylene glycol, chlorosulfonic acid, NaH), Aldrich (1,3-propane sultone) and Sigma (5-cholesten-3β-ol 3-tosylate) and were used without further purification. Chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM).

Syntheses

8-(Cholest-5-en-3β)oxy-3,6-dioxaoctanol and 11-(cholest-5-en-3β)oxy-3,6,9-trioxaundecan-1-ol were prepared as reported elsewhere [10].

Sodium 8-(cholest-5-en-3β)oxy-3,6-dioxaoctan-1-sulfate (1)

Chlorosulfonic acid (1 ml, 15 mmol) was dissolved in 30 ml absolute dioxane. After cooling the solution to 0 °C 15 ml dry pyridine was added. In the meantime, a solution of 1.0 g (1.9 mmol) 8-(cholest-5-en-3β)oxy-3,6-dioxaoctanol in 20 ml dry pyridine and 50 ml absolute dioxane was prepared. Both mixtures were combined and the resulting mixture was stirred at room temperature under an argon atmosphere for 24 h. Removal of the solvents gave a solid residue which was taken up with 45 ml methanol; this was followed by neutralization with methanolic sodium hydroxide solution. Subsequently, the white precipitate was removed by filtration and the methanolic filtrate was concentrated to 5 ml and chromatographed over a silica gel column. The eluant used was $CHCl_3$/CH_3OH = 6:1 (v/v). Evaporation of the fractions with an R_f value of 0.22 [thin-layer chromatography (TLC) $CHCl_3/CH_3OH$ = 6:1 v/v] gave **1** as a colourless, viscous substance: yield 0.9 g; 74%. Elemental analysis calculated for $C_{33}H_{57}NaO_7S$: C, 63.87%; H, 9.19%; S, 5.16%. Found: C, 63.60%; H, 8.94%; S, 5.02%. The ^{13}C NMR spectra were recorded in CD_3OD and showed the following key signals: δ = 68.26, 68.32, 70.65, 71.42, 71.47, 71.82 (ethoxy groups), 80.91 (CH_2-CH_2-O-*C*H). The ^{13}C signals of the C atoms of the cholesterol unit were omitted [11]. Mass spectrometry (MS) *m*/*z* fast atom bombardment (FAB) 597 (M-Na+).

Sodium 11-(cholest-5-en-3β)oxy-3,6,9-trioxaundecan-1-sulfate (2)

The synthesis was performed as for **1** but using 11-(cholest-5-en-3β)oxy-3,6,9-trioxaundecan-1-ol (1.1 g; 2.0 mmol) as a reactant. The eluant used was $CHCl_3/CH_3OH$ = 9:1 (v/v). Evaporation of the fractions with an R_f value of 0.17 (TLC $CHCl_3/CH_3OH$ = 8:1 v/v) gave **2** as a colourless, viscous substance: yield 1.0 g; 75%. Elemental analysis calculated for $C_{35}H_{61}NaO_8S$: C, 63.25%; H, 9.18%; S, 4.82%. Found: C, 62.24%; H, 9.08%; S, 4.96%. The ^{13}C NMR-spectra were recorded in CD_3OD and showed the following key signals: δ = 68.12, 68.24, 70.70, 71.32, 71.32, 71.32, 71.36, 71.80 (ethoxy groups), 80.93 (CH_2-CH_2-O-*C*H). The ^{13}C signals of the C atoms of the cholesterol unit were omitted [11]. MS *m*/*z* (FAB) 641 (M-Na+).

12-(Cholest-5-en-3β)oxy-4,7,10-trioxadodecan-1-sulfonic acid sodium salt (3)

8-(Cholest-5-en-3β)oxy-3,6-dioxaoctanol (1.4 g, 2.7 mmol) was dissolved in 30 ml absolute dioxane. After addition of NaH (0.15 g, 3.7 mmol) the mixture was stirred for 4 h under an argon atmosphere at room temperature, followed by addition of 1,3-propane sultone (3.0 ml). After stirring for a further 20 h the dioxane was removed by distillation and the remaining residue was stirred with 40 ml methanol for another 30 min. The methanolic solution was then concentrated to 5 ml and chromatographed over a silica gel column. The eluant used was $CHCl_3$/CH_3OH = 8:1 (v/v). Evaporation of the fractions with an R_f value of 0.19 (TLC $CHCl_3/CH_3OH$ = 8:1 v/v) gave 3 as a colourless, viscous substance: yield 0.3; 16%. Elemental analysis calculated for $C_{36}H_{63}NaO_7S$: C, 65.25%; H, 9.51%; S, 4.83%. Found: C, 63.80%; H, 9.64%; S, 5.46%. The ^{13}C NMR spectra were recorded in $CDCl_3$ and showed the following key signals: δ = 69.65, 69.72, 70.16, 70.16, 70.34, 70.79 (ethoxy groups), 47.81 (NaO_3S-*C*H_2-CH_2-CH_2-O-), 24.60 (NaO_3S-*C*H_2-*C*H_2-CH_2-O-), 67.42 (NaO_3S-CH_2-CH_2-*C*H_2-O-), 79.47 (CH_2-CH_2-O-*C*H). The ^{13}C signals of the C atoms of the cholesterol unit were omitted [11]. MS *m*/*z* (FAB) 639 (M-Na+).

15-(Cholest-5-en-3β)oxy-4,7,10,13-tetraoxapentadecan-1-sulfonic acid sodium salt (4)

The synthesis was performed as for **3** but using 11-(cholest-5-en-3β)oxy-3,6,9-trioxaundecan-1-ol (1.25 g, 2.22 mmol) as a reactant. The eluant used was $CHCl_3/CH_3OH$ = 5:1 (v/v). Evaporation of the fractions with an R_f value of 0.24 (TLC $CHCl_3/CH_3OH$ = 5:1 v/v) gave **4** as a colourless, viscous substance: yield 0.7; 45%. Elemental analysis calculated for $C_{38}H_{67}NaO_8S$: C, 64.59%; H, 9.49%; S, 4.53%. Found: C, 63.69%; H, 9.38%; S, 4.56%. The ^{13}C NMR spectra were recorded in $CDCl_3$ and showed the following key signals: δ = 69.60, 69.81, 70.15, 70.16, 70.23, 70.29, 70.43, 70.69 (ethoxy groups), 47.88 (NaO_3S-*C*H_2-CH_2-CH_2-O-), 24.75 (NaO_3S-CH_2-*C*H_2-CH_2-O-), 67.20 (NaO_3S-CH_2-CH_2-*C*H_2-O-), 79.47 (CH_2-CH_2-O-*C*H). The ^{13}C signals of the C atoms of the cholesterol unit were omitted [11]. MS *m*/*z* (FAB) 683 (M-Na+).

Characterization

^{13}C NMR spectra were obtained on a Bruker AC 250 spectrometer. Elemental analyses were performed using a Leco CHNS-932 device. MS was performed with an Intectra AMD 402 mass spectrometer.

Dynamic light scattering

Dynamic light scattering was performed using a Coulter N4 Plus spectrometer including a size-distribution processor (SDP). The SDP analysis gives the particle size distribution. Since the N4 Plus does not count individual particles, the instrument must separate the decay times (due to differently sized particles). These decay times are mixed together in a composite autocorrelation function (ACF) that characterizes all the particles simultaneously. The composite ACF is actually a sum of decaying exponentials. The algorithm used in SDP analysis is based on a FORTRAN program called CONTIN, written by Provencher [12, 13]. The measurements were carried out at 20 °C with 7×10^{-4} M aqueous solutions of the new compounds (measuring angles 15.7°, 23.0°, 30.1°, 62.6°, and 90.0°). The result of the blank reading with the bidistilled water used for preparing the solutions was subtracted. The volume-weighted particle size distributions were used for further interpretations.

Determination of cmc values

The cmc values were determined first by conductivity measurements of a set of concentrated solutions of the surfactants. The

measurements were performed within 30 min after preparing the solutions with a WTW LF95 conductivity meter and were interpreted by a graphical method. The cmc values were determined in parallel by a photometric method measuring the absorption of the dye 1-(2-pyridylazo)-2-naphthol for concentrated solutions of the surfactants [14]. The measurements were performed with a Perkin Elmer Lambda 2 UV/VIS spectrometer and were interpreted by a graphical method.

Electron micrographs

Droplets of 7×10^{-4} M solutions of the amphiphiles were placed onto copper grids (7 μm, 400 mesh, Baltec) and frozen in liquid propane. After very slow evaporation of the water the electron micrographs were obtained using a Carl Zeiss 912 Omega transmission electron microscope.

Olefin hydrogenation reaction

Hydrogenation was performed under ambient pressure at 25 °C. Solvent (15 ml H_2O), surfactant (0.1 mmol), methyl (*Z*)-α-acetamidocinnamate substrate (1 mmol), [Rh(1,5-cyclooctadiene)$_2$]BF_4 rhodium complex (0.01 mmol) and (2*S*,4*S*)-(-)-*N*-*tert*-butoxycarbonyl-4-diphenylphosphino-2-diphenylphosphinomethyl pyrrolidine chiral phosphine ligand (0.011 mmol) were placed in a deaerated hydrogenation flask with a water jacket and were stirred for 20 h before setting the flask under a hydrogen atmosphere (0.1 Mpa). The reaction was followed by volumetric measurement. When the reaction was complete, the mixture was extracted with chloroform (5 ml). In this extract the enantioselectivity was determined by gas chromatography with a Hewlett Packard 5890 Series II gas chromatograph using a 12.5-m × 0.2-mm silica fused column coated with XE-60 L-*tert*-butyl valinamide at 165 °C (flame ionization detector).

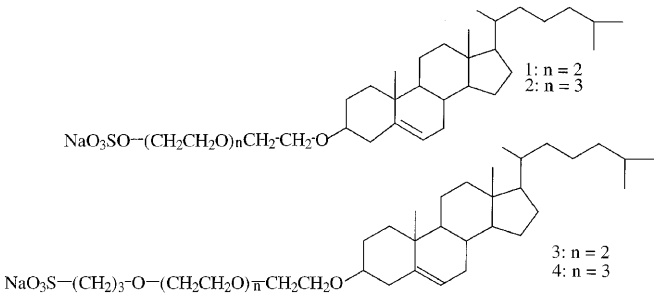
Liquid-crystalline behaviour

The mesophase behaviour was studied with an Olympus BH polarizing microscope equipped with a Mettler FP82 heating stage. Phase identification was done by the observation of characteristic textures, the miscibility with reference compounds and the contact with water.

Results and discussion

Characterization

The structures of the newly synthesized cholesterol-based amphiphiles are given in Scheme 1. The cmc values found for the new surfactants are shown in Table 1. They are quite low, around 2×10^{-4} mol/l for all; the values obtained using the photometric method are lower than those obtained from conductivity measurements. This is in agreement with the common observation that additives lower the cmc [15]. The preparation of such low concentrated standard solutions is difficult and results in a relatively large repetitive error.



Scheme 1 Structures of the new amphiphilic compounds

To summarize the results of all measuring angles of the dynamic light scattering experiments, we found a particle size range between 1.4 and 30 nm with only small differences between the individual compounds (Fig. 1). Assuming the expansion of one monomer of the surfactants between 1.5 and 2.8 nm [16, 17] with respect to a stretched or angled conformation this result demonstrated the coexistence of monomers and micelles in the solutions.

Electron micrographs of all solutions showed a uniform surface of dried material. We found no distinct aggregates as were observed for the vesicle-forming substances [9]. This could be explained by the relatively high number of monomers between the micelles compared with the vesicular solutions and by the small size of the micelles in comparison to that of the monomers.

Asymmetric hydrogenation

The asymmetric hydrogenation reaction of amino acid precursors catalyzed by rhodium complexes could be effected in water as a medium in the presence of amphiphiles [18]. The basic reaction performed in our laboratory is shown in Scheme 2. Such systems are of great interest regarding the replacement of organic solvents in organic synthesis.

Solubilization of the reactants and the catalyst causes a significant increase in the activity of the catalyst and also in the enantioselectivities. The results for the

Table 1 Critical micelle concentrations (*cmcs*) of the synthesized surfactants determined by conductometry and photometry

Compound	cmc, conductometry	cmc, photometric method
1	$2.2 \times 10^{-4} \pm 0.3 \times 10^{-4}$ mol/l	$2.0 \times 10^{-4} \pm 0.5 \times 10^{-4}$ mol/l
2	$1.5 \times 10^{-4} \pm 0.3 \times 10^{-4}$ mol/l	$1.2 \times 10^{-4} \pm 0.5 \times 10^{-4}$ mol/l
3	$1.8 \times 10^{-4} \pm 0.3 \times 10^{-4}$ mol/l	$1.3 \times 10^{-4} \pm 0.5 \times 10^{-4}$ mol/l
4	$3.0 \times 10^{-4} \pm 0.3 \times 10^{-4}$ mol/l	$1.8 \times 10^{-4} \pm 0.5 \times 10^{-4}$ mol/l

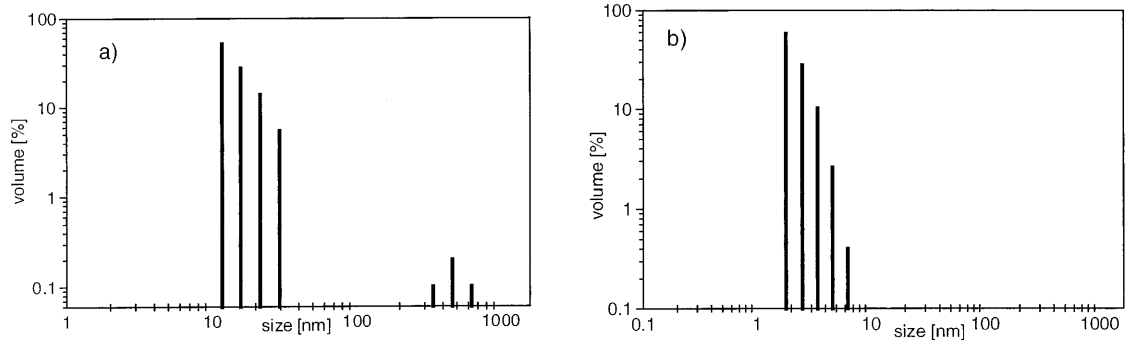
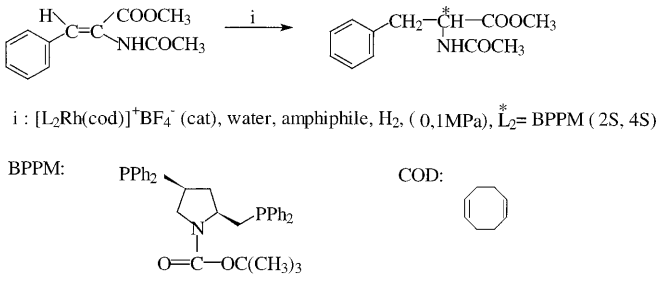


Fig. 1 Particle size distribution of **2** at measuring angles of **a** 23.0° and **b** 62.6° (volume weighted)



Scheme 2 Asymmetric hydrogenation of methyl (*Z*)-α-acetamidocinnamate. The structures of 1,5-cyclooctadiene (*cod*) and (2*S*,4*S*)-(-)-*N*-*tert*-butoxycarbonyl-4-diphenylphosphino-2-diphenylphosphinomethyl pyrrolidine (*BPPM*) are also shown

Table 2 Hydrogenation of methyl (*Z*)-α-acetamidocinnamate (1 mmol) in water and in methanol (15 ml) with the catalytic system [Rh(1,5-cyclooctadiene)$_2$]BF_4 + (2*S*,4*S*)-(-)-*N*-*tert*-butoxycarbonyl-4-diphenylphosphino-2-diphenylphosphinomethyl pyrrolidine (0.011 mmol). Effect of the synthesized surfactants (0.2 mmol). (0.1 MPa H_2, 25 °C)

Reaction conditions	$t/2$ (min)[a]	Enantiomeric excess $R\% \pm 1\%$
Pure water	90	78
Methanol	2	93
Water, addition of **1**	6	93
Water, addition of **2**	6	95
Water, addition of **3**	7	95
Water, addition of **4**	6	96

[a] $t/2$ is the time for the consumption of half the hydrogen

reaction in pure water, in methanol and in aqueous amphiphile solutions using the newly synthesized surfactants are summarized in Table 2. The amphiphile solutions show very good activities and even better enantioselectivities than in methanol. The activity of the new micelle-forming substances was twice as high as that of the previously investigated vesicular systems, but there was almost no difference concerning the enantioselectivity [9]. It seems that diffusion processes occur more slowly in the rigid vesicles than in the micellar aggregates.

Investigation of liquid crystallinity

All the amphiphilic compounds studied are liquid-crystalline compounds with interesting mesomorphous behaviour which can be classified as hydrated, thermotropic and lyotropic behaviour (Table 3). All the compounds have no tendency to crystallize. Thus, they are isolated as viscous liquid-crystalline materials after preparation. They contain small amounts of water (less than 5%) which is bonded by strong interactions. All the compounds exhibit a smectic A phase (S_A) in this "original" hydrated state.

The compounds lose their water of hydration at about 120 °C. After this, the thermotropic liquid-crystalline properties can be measured. **1**, **3** and **4** have a columnar phase from room temperature up to the clearing temperature above 200 °C, whereas **2** has a smectic A phase (lamellar phase). All the compounds decompose at the clearing temperature. This is quite

Table 3 Mesophase behaviour of the cholesterol-based amphiphiles

Compound	Thermotropic behaviour[a]	Lyotropic behaviour[b]	Hydrated behaviour[c]
1	Cr ? Col 200 dec	Col → S_A	S_A
2	Cr ? S_A 146 dec	S_A	S_A
3	Cr ? Col 240–300 dec	Col → S_A → Col → Cub	S_A
4	Cr ? Col 230–275 dec	Col → S_A → Col	S_A

[a] Cr = melting temperature, Col = columnar phase, Cub = discontinuous cubic phase, S_A = smectic A phase, dec = decomposition, ? = not observable; all temperatures are given in degrees centigrade
[b] Phase sequence with increasing amounts of water
[c] Mesophase of the freshly prepared samples before drying

usual for ionic cholesterol compounds. Amphiphilic substances with only one polar head group and only one alkyl chain normally have lamellar phases. The columnar phases of **1**, **3** and **4** can be explained by the sterically dominating nonpolar part [19]. Addition of small amounts of water enlarges the size of the polar head groups and turns the columnar phases into lamellar phases.

The lyotropic behaviour was studied at room temperature by adding water to the dry samples (contact preparation). Addition of a small amount of water to **1** leads to a lamellar S_A phase which has myelin figures at the water frontier. Excess water does not create further mesophases because of the limited hydration of this compound. **4** is similar to **1**, but has a better solubility in water; thus, higher amounts of water lead to the formation of a columnar phase and no myelin figures were observed. **3** has an even higher solubility than **4** and a discontinuous cubic phase (micellar cubic phase) occurred at higher water concentrations. **2** only has a lamellar phase in water.

Bicontinuous cubic phases are absent for all the compounds. This is quite unusual and may be explained by the stiffness of the nonpolar molecular part. Most of the amphiphiles which are studied for liquid-crystalline and/or catalytic properties have a small bicontinuous columnar phase between the lamellar and columnar phases. The absence of this phase might be an indicator of the aggregation behaviour of these molecules and could have an influence in the catalytic process.

In summary the newly synthesized compounds have complex thermotropic and lyotropic behaviour. Lamellar and columnar phases are present; bicontinuous phases are absent. The clearing temperatures are high and the compounds decompose in the isotropic phase. The substances have no tendency to crystallize.

Conclusions

The new type of cholesterol amphiphile contains an enhanced hydrophilic part in comparison to amphiphiles described recently. Surprisingly, the cmc and the size indicate micelle formation in aqueous medium, whereas the previously studied amphiphiles aggregated to form vesicles. The use of micelles in asymmetric hydrogenation of a phenylalanine precursor was very successful and caused enhancement of the rate and the enantioselectivity. All the amphiphiles synthesized are crystalline liquids and exhibit an interesting thermotropic and lyotropic behaviour.

Acknowledgements The authors thank K. Kortus, H. Baudisch and M. Michalik for analytical assistance. Thanks also go to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

References

1. Deme B, Dubois M, Zemb T, Cabane B (1997) Colloids Surf A 121:135
2. Schwarz MA, Raith K, Ruttinger HH, Dongowski G, Neubert RHH (1997) J Chromatogr 781:377
3. Decher G, Ringsdorf H (1993) Liq Cryst 13(1):57
4. Boullanger P, Chevalier Y, Crozier MC, Lafont D, Sancho MR (1995) Carbohydr Res 278:91
5. Abel E, Fedders MF, Gokel GW (1995) J Am Chem Soc 117:1265
6. Ringsdorf H, Schlarb B, Venzmer J (1988) Angew Chem 100:117
7. Dobashi A, Hamada M (1997) J Chromatogr 780:179
8. Cho I, Kim GC (1988) J Org Chem 53:5187
9. Oehme G, Meißner D, Holzhüter G, Schareina T, Grassert I (1996) J Prakt Chem 338:614
10. Patel KR, Li MP, Schuh JR, Baldeschwieler JD (1984) Biochim Biophys Acta 797:20
11. Bremser W, Hardt A, Ernst L, Faschinger W, Gerhards R, Lewis PME (1987) Carbon-13 NMR spectral data. VCH, Weinheim
12. Provencher SW (1975) J Chem Phys 64:2772
13. Provencher SW (1982) Comput Phys Commun 27:229
14. Furton KG, Norelus A (1993) J Chem Educ 70:254
15. Fendler JH (1982) Membrane mimetic chemistry. Wiley, New York
16. D'Alagni M, Galantini L, Giglio E, Gavuzzo E, Scaramuzza L (1994) J Chem Soc Faraday Trans 90:1523
17. Brockerhoff H, Ramsammy LS (1982) Biochim Biophys Acta 691:227
18. Grassert I, Paetzold E, Oehme G (1993) Tetrahedron 49:6605
19. Vill V, Kelkenberg H, Thiem J (1992) Liq Cryst 11:459