Structure and antimicrobial activity of new bile acid-based gemini surfactants

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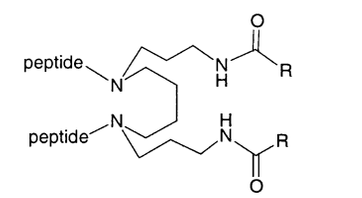
Three novel bile acid-based gemini surfactants containing a polycationic head group have been prepared using standard synthetic procedures from readily available starting materials. These compounds show good activity against both Gram-positive and Gram-negative bacteria. Antibacterial activity increases with the number of primary amino groups in the head group.

The increasing prevalence of pathogenic bacteria that are resistant to many commonly used antibiotics, such as β-lactams and macrolides, is of major clinical importance. For example, therapeutic options for the treatment of hospital infections caused by multi-antibiotic-resistant strains of *Staphylococcus aureus* and Enterococci are essentially limited to vancomycin, a cyclic peptide which has a relatively poor side effect profile, and linezolid, an oxazolidinone which is already demonstrating clinical resistance. In the community, increasing resistance in *S. pneumoniae* has reduced the utility of many of the established oral antibiotics. Consequently there is an urgent need for new antibiotics that will be effective against these bacteria.

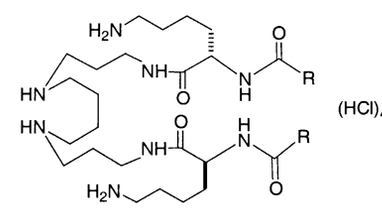
The antibacterial activity of anionic and cationic surfactants is well known.[1,2] Bile salts, anionic surfactants that result from the metabolism of cholesterol, have been shown to inhibit the growth of both Gram-positive and Gram-negative bacteria. Electron microscopy studies have revealed that treatment of bacteria with the more hydrophobic bile salts leads to changes in the morphology of cells, and ultimately leads to the breakdown of the bacterial cell wall and cell death.[3]

Cationic surfactants have also been shown to interfere with bacterial growth, and have been used as antibiotics in biomedical applications. Dilute solutions of cetyltrimethylammonium bromide (CTAB) have been included in eye-drop formulations because of its mild anti-bacterial properties,[4] and the use of low concentrations of dioctadecyldimethylammonium bromide (DODAB) to reduce bacterial contamination of water has been suggested.[2] More recently, overall positively charged cholic acid-derived antimicrobial agents have been reported to show potent antibacterial activity.[5] It is thought that the bacterial action of cationic surfactants is due to their interaction with the negatively-charged phosphate groups on the surface of the bacterial cell wall, leading to disruption of the cell division and cell–cell interaction processes.

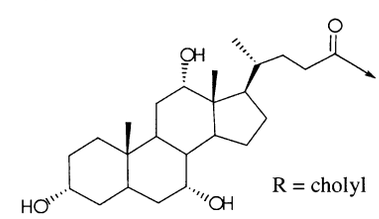
We recently reported on the synthesis and gene transfection efficacy of a number of gemini surfactants.[6–9] These cationic surfactants contain two saturated hydrocarbon 'tails' 12 to 18 carbon atoms in length, linked together by a spacer, and two head-groups, each consisting of an overall positively-charged peptide. As an extension of these studies we now report the synthesis of the three novel gemini surfactants **1**–**3** (Scheme 1) from readily available starting materials. Compared with the gemini surfactants we have reported in previous studies, the hydrocarbon 'tails' in compounds **1**–**3** have been replaced by cholic acid moieties. The linker in these molecules is spermine, with its four symmetrically-spaced amine groups. The presence of one or more lysine groups in the head-group gives these surfactants a net positive charge. These cationic gemini surfactants show low gene-transfection activity, but they turn out to be effective anti-bacterial agents.



1, RCO = cholyl, peptide = KKKS.4HCl
2, RCO = cholyl, peptide = K-ε-KKS.4HCl



3, R = cholyl.



Scheme 1

Experimental

Synthesis of 1–3

All chemicals used were of reagent grade and were used without further purification. Compounds were generally purified by column chromatography on silica gel Merck Kieselgel 60

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(230–400 mesh). TLC was performed on silica plates Merck Kieselgel 60F-254-0.25 mm. Solvents used for chromatographic separations were of Analar grade. ^{1}H NMR spectra were recorded at ambient probe temperature on a Bruker DPX 400 (400 MHz) spectrometer using the solvent as an internal deuterium lock. Coupling constants are given in hertz. ^{13}C NMR spectra were recorded on a Bruker DPX 400 (100 MHz) instrument using internal lock and proton decoupling. Moisture-sensitive reactions were performed under argon and all glassware was oven-dried before use. Mass spectrometry data were obtained on a Micromass Quattro II low-resolution triple quadrupole mass spectrometer or a Finnigan MAT 900 XLT high resolution double focusing instrument.

***N*-Hydroxysuccinimide ester of cholic acid.** To a solution of cholic acid (6.13 g, 15.0 mmol) in dry THF (100 mL) was added *N*-hydroxysuccinimide (1.78 g, 15.3 mmol, 1.03 eq.) and after 5 min a solution of DCC (3.2 g, 1.53 mmol, 1.03 eq.) in THF (20 mL). The reaction mixture was stirred for 28 h at room temperature and the dicyclohexyl urea formed was filtered and washed with EtOAc. The solvent was removed and the residue was dissolved in EtOAc, filtered and concentrated to dryness. The resulting oil was dissolved in a small amount of $CHCl_3$ and Et_2O was added to induce crystallisation. The solid was collected and dried to give the cholyl *N*-hydroxysuccinimide ester as a white powder. Yield: 7.13 g (94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (br s, 1 H), 3.81 (br s, 1 H), 3.40 (m, 2 H), 2.81 (s, 4 H, 2 CH_2^{Su}), 2.62 (m, 1 H), 2.54 (m, 1 H), 2.18 (m, 2 H), 0.88–1.88 (m, 30 H), 1.00 (d, J = 6.4, 3 H), 0.86 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.6, 77.7, 73.4, 72.2, 68.8, 47.1, 46.9, 42.0, 41.9, 39.9, 35.7, 35.5, 35.2, 35.1, 31.3, 31.0, 30.8, 28.6, 28.4, 27.8, 26.7, 26.0, 23.6, 23.0, 22.8, 17.6, 14.5, 12.8; HRMS (+ES): [M + Na]$^+$: calculated for $C_{28}H_{43}O_7N$ 528.29250, found 528.3039.

***N*^{1},*N*^{12}-Dicholyl-*N*^{4},*N*^{9}-bis(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (5).** To a solution of containing *N*^{4},*N*^{9}-bis(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane **4** (0.923 g, 2.3 mmol)† in THF (60 mL) were added cholyl *N*-hydroxysuccinimide ester (2.33 g, 4.6 mmol, 2 eq.) and Et_3N (0.705 mL, 5.06 mmol, 2.2 eq.). The reaction mixture was stirred for 48 h at RT. Most of the THF was removed and the residue diluted with water (10 mL) and $CHCl_3$ (40 mL). The organic layer was decanted and washed successively with water (10 mL), 0.1 M HCl (30 mL), water (10 mL) and brine (25 mL). The organic layer was dried over sodium sulfate, filtered and evaporated. The resulting oil was purified by chromatography on silica gel (R_f = 0.2, $CHCl_3$–MeOH, 17 : 3) to give **5** as a white solid. Yield: 1.24 g (46%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (m, 2 H, 2 NH), 3.93 (s, 2 H), 3.80 (s, 2 H), 3.42 (m, 2 H), 3.25–2.95 (m, 18 H), 2.20 (m, 6 H), 2.10 (m, 2 H), 1.90–1.32 (m, 66 H), 0.98 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.87 (s, 6 H, 2 CH_3^{19}), 0.65 (m, 6 H, 2 CH_3^{18}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7 (CONH), 156.6 (COOtBu), 82.6, 73.4, 72.3, 68.8, 66.2, 47.3, 46.9, 46.8, 39.9, 36.5, 35.2, 33.7, 32.2, 32.0, 30.8, 28.9, 28.6, 28.0, 26.7, 23.7, 23.0, 17.9, 14.5, 12.9.

***N*^{1},*N*^{12}-Dicholyl-1,12-diamino-4,9-diazadodecane·HCl (6).** To a solution of **5** (500 mg, 0.422 mmol) in MeOH (10 mL) was added conc. HCl (10 mL). The reaction mixture was stirred for 1 h and then concentrated to dryness to give **6** as a white solid. Yield: 400 mg (100%). The NMR spectrum corresponds to the free base. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.75 (m, 2 H, 2 $NHC^{1'}$), 3.95 (m, 2 H, OH), 3.74 (s, 2 H), 3.58 (s, 2 H), 3.58 (m, 4 H, 4 OH), 3.15 (m, 2 H), 3.00 (m, 4 H, 2 $CH_2^{1'}$), 2.42 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.22–1.88 (m, 16 H), 1.80–1.67 (m, 8 H), 1.65–1.55 (m, 8 H), 1.52–1.08 (m, 36 H), 0.91 (m, 2 H), 0.89 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.80 (m, 2 H), 0.77 (s, 6 H, 2 CH_3^{19}), 0.54 (m, 6 H, 2 CH_3^{18}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.9 (CONH), 71.4, 70.8, 66.6, 49.6, 48.9, 47.1, 46.5, 46.1, 41.9, 41.7, 39.9, 39.7, 37.0, 36.1, 35.7, 35.5, 35.2, 34.7, 33.0, 31.1, 30.7, 29.7, 27.7, 26.6, 23.2, 22.9 (C^{19}), 17.4 (C^{21}), 12.7 (C^{18}); HRMS (+ES): [M + H]$^+$ calculated for $C_{58}H_{102}O_8N_4$ 983.7770, found 983.7692.

***N*^{1},*N*^{12}-Dicholyl-*N*^{4},*N*^{9}-bis[*N*-(*N*-α-{*N*-α-[*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-lysyl]-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysyl}-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysyl)-L-seryl]-1,12-diamino-4,9-diazadodecane (7).** To a solution of **6** (211 mg, 0.20 mmol) in THF (40 mL) were added successively a solution of potassium carbonate (58 mg, 0.42 mmol, 2.1 eq.) in water (10 mL) and a solution of $(Boc)_4$-lysyl-lysyl-lysyl-serine *N*-hydroxysuccinimide ester (394 mg, 0.40 mmol, 2.0 eq.) in THF (15 mL). The reaction mixture was stirred at RT for 48 h. Most of the THF was evaporated and the residue was dissolved in sodium carbonate (20 mL) and chloroform (40 mL). The organic layer was separated and washed successively with water (10 mL), 0.1 M HCl (20 mL, water (10 mL) and brine (25 mL), dried (sodium sulfate), filtered and evaporated to give **7** as a white powder. Yield: 318 mg (58%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.60 (m, 2 H, 2 $NHC^{1'}$), 5.30–5.00 (m, 14 H), 5.05 (m, 2 H), 4.50 (m, 2 H), 4.40 (m, 2 H), 4.10 (m, 2 H), 4.08 (m, 2 H), 3.95 (m, 4 H, 2 $CH_2^{\beta ser}$), 3.80 (m, 2 H), 3.46 (m, 4 H), 3.06 (m, 24 H), 2.25 (m, 4 H), 2.11 (m, 2 H), 1.95–1.08 (m, 160 H), 0.99 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.88 (s, 6 H, 2 CH_3^{19}), 0.80 (m, 4 H), 0.67 (m, 6 H, 2 CH_3^{18}).

***N*^{1},*N*^{12}-Dicholyl-*N*^{4},*N*^{9}-bis(*N*-{*N*-α-[*N*-α-(L-lysyl)-L-lysyl]-L-lysyl}seryl)-1,12-diamino-4,9-diazadodecane octakis(hydrochloride) (1).** To a solution of **7** (310 mg, 0.114 mmol) in MeOH (10 mL) was added conc. HCl (10 mL). The reaction mixture was stirred at RT for 1 h, then the solvents were evaporated off. The residue was dissolved in pure water (80 mL), filtered through a sinter frit no. 3 and evaporated. The oily residue was dissolved in the minimum amount of MeOH and precipitated with Et_2O and **1** was collected as a white solid. Yield: 240 mg (95%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.62 (m, 2 H), 8.54 (m, 2 H), 8.31 (m, 8 H, 2 NH and 2 $N^{\alpha}H_3^+$), 8.17 (m, 4 H), 8.01 (m, 18 H, 2 × 3 $N^{\varepsilon}H_3^+$), 4.45 (m, 2 H), 4.31 (m, 4 H), 3.81 (m, 4 H), 3.69 (m, 2 H), 3.58 (m, 4 H, 2 $CH^{\beta ser}$), 3.25 (m, 4 H, 2 $CH_2^{1'}$), 2.75 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.70 (m, 12 H, 2 × 3 CH_2^{ε}), 2.20–1.90 (m, 16 H), 1.80–1.10 (m, 72 H), 0.90 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.85 (m, 4 H), 0.78 (s, 6 H, 2 CH_3^{19}), 0.55 (m, 6 H, 2 CH_3^{18}). HRMS (+ES): [M + 2H]$^{2+}$: calculated for $C_{50}H_{93}O_9N_9$ 963.2018, found 963.2208.

***N*^{1},*N*^{12}-Dicholyl-*N*^{4},*N*^{9}-bis[*N*-(*N*-α-{*N*-α-(*tert*-butyloxycarbonyl)-*N*-ε-[*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-lysyl]-L-lysyl}-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysyl)-L-seryl]-1,12-diamino-4,9-diazadodecane (8).** To a solution of **6** (264 mg, 0.25 mmol) in THF (40 mL) were added successively a solution of potassium carbonate (90 mg, 0.65 mmol, 2.6 eq.) in water (10 mL) and a solution of $(Boc)_4$-lysyl-ε-lysyl-lysyl-serine *N*-hydroxysuccinimide ester (550 mg, 0.506 mmol, 2.03 eq.) in THF (15 mL). The reaction mixture was stirred at RT for 48 h. Most of the THF was evaporated and the residue was dissolved in 5% sodium carbonate (20 mL) and chloroform (40 mL). The organic layer was separated and washed successively with water (10 mL), 0.1 M HCl (20 mL), water (10 mL) and brine (25 mL), dried (sodium sulfate), filtered and evaporated. The residue was dissolved in a small volume of chloroform and precipitated with Et_2O and isolated to give **8** as a white powder. Yield: 540 mg (79%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.35 (m, 2 H, 2 $NHC^{1'}$), 5.70 (m, 4 H), 5.05 (m, 2 H), 4.77 (m, 4 H), 4.38 (m, 4 H), 4.10 (m, 2 H), 3.97 (m, 4 H, 2 $CH_2^{\beta ser}$), 3.84 (m, 2 H),

† The primary amino groups of spermine were selectively protected with ethyl trifluoroacetate in acetonitrile,[10] the secondary amines then protected with *tert*-butoxycarbonyl groups, and the trifluoroacetyl groups removed using aqueous ammonia in methanol.

3.47 (m, 4 H), 3.20–2.90 (m, 24 H), 2.50 (m, 2 H), 2.25 (m, 2 H), 2.11 (m, 2 H), 1.95–1.08 (m, 168 H), 0.98 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.90 (s, 6 H, 2 CH_3^{19}), 0.80 (m, 4 H), 0.54 (m, 6 H, 2 CH_3^{18}). MS (+ES): [M + H]+ :2726.8.

N^1,N^{12}-Dicholyl-N^4,N^9-bis(N-{N-α-[N-ε-(L-lysyl)-L-lysyl]-L-lysyl}-L-seryl)-1,12-diamino-4,9-diazadodecane octakis(hydrochloride) (2). To a solution of **8** (530 mg, 0.194 mmol) in MeOH (10 mL) was added conc. HCl (10 mL). The reaction mixture was stirred at RT for 1 h and the solvents were evaporated. The residue was dissolved in pure water (80 mL), filtered through a sinter frit no. 3 and evaporated. The oily residue was dissolved in the minimum amount of MeOH and precipitated with Et_2O and **2** was collected as a white solid. Yield: 381 mg (88%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.75 (m, 4 H), 8.38–8.27 (m, 14 H, 2 NH and 2 × 2 $N^{\alpha}H_3^+$), 8.17 (m, 4 H), 8.08 (m, 12 H, 2 × 2 $N^{\varepsilon}H_3^+$), 4.20 (m, 6 H), 4.01 (m, 4 H), 3.85 (m, 8 H, 2 $CH^{\beta ser}$), 3.75 (m, 2 H), 3.08 (m, 4 H, 2 $CH_2^{1'}$), 3.00 (m, 8 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.80–2.70 (m, 12 H, 2 × 3 CH_2^{ε}), 2.20–1.90 (m, 16 H), 1.80–1.10 (m, 68 H), 0.90 (d, 6 H, J = 6.4, 2 CH_3^{21}), 0.80 (m, 4 H), 0.77 (s, 6 H, 2 CH_3^{19}), 0.55 (m, 6 H, 2 CH_3^{18}). HRMS (+ES): $[M + Na]^+$: calculated for $C_{100}H_{194}O_{18}N_{18}Na$ 1948.4036, found 1948.4228.

***N*-α-Cholyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysine (10).** To a solution of *N*-ε-(*tert*-butyloxycarbonyl)-L-lysine **9** (297 mg, 1.2 mmol) in THF (80 mL) were added successively a solution of potassium carbonate (175 mg, 1.2 mmol, 1.05 eq.) in water (10 mL) and cholyl *N*-hydroxysuccinimide ester (610 mg, 1.2 mmol, 1 eq.) in THF (10 mL). The reaction mixture was stirred overnight at room temperature. Most of THF was evaporated and water (35 mL) was added. The aqueous layer was acidified to pH 2 and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with water (20 mL) and brine (40 mL), dried over sodium sulfate, filtered and evaporated to give **10** as a white solid. Yield: 714 mg (93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (br s, 1 H, NHCO), 5.10 (br s, 1 H, NHCO), 4.50 (br s, 1 H, CH^{α}), 3.96 (br s, 1 H), 3.80 (br s, 1 H), 3.40 (br s, 1 H), 3.06 (m, 2 H, CH_2^{ε}), 2.20–1.30 (m, 42 H), 0.97 (d, 3 H, J = 3.0, CH_3^{21}), 0.85 (s, 3 H, CH_3^{19}), 0.64 (s, 3 H, CH_3^{18}). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.4, 175.3, 156.8, 79.5, 73.6, 72.3, 68.9, 66.3, 52.6, 46.7, 41.9, 40.7, 39.9, 39.8, 38.5, 35.7, 35.2, 32.0, 31.2, 32.0, 30.0, 28.9, 28.4, 26.7, 23.0, 22.8, 17.8, 15.7, 17.5, 12.8.

α-*N*-Cholyl-ε-*N*-(*tert*-butyloxycarbonyl)-L-lysine *N*-hydroxysuccinimide ester (11). To a solution of **10** (651 mg, 1.02 mmol) in THF (80 mL) were added successively *N*-hydroxysuccinimide (121 mg, 1.05 mmol, 1.03 eq.) and DCC (217 mg, 1.05 mmol, 1.03 eq.). The reaction mixture was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give **11** as a white solid. Yield: 720 mg (96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (br s, 1 H, NHCO), 5.10 (br s, 1 H, NHCO), 4.69 (br s, 1 H, CH^{α}), 3.95 (br s, 1 H), 3.82 (br s, 1 H), 3.38 (br s, 1 H), 3.02 (m, 2 H, CH_2^{ε}), 2.80 (s, 4 H, 2 CH_2^{Su}), 2.23–1.29 (m, 42 H), 0.95 (d, 3 H, J = 3.0, CH_3^{21}), 0.83 (s, 3 H, CH_3^{19}), 0.61 (s, 3 H, CH_3^{18}).

N^1,N^{12}-Bis[*N*-α-cholyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysyl]-N^4,N^9-bis(*tert*-butyloxycarbonyl)-1,12-diamino-4,9-diazadodecane (12). To a 1 : 1 mixture of water and THF (30 mL) containing **4** (210 mg, 0.5 mmol) and potassium carbonate (162 mg, 1.12 mmol, 2.2 eq.) was added *N*-α-cholyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysyl succinimidate **11** (740 mg, 1.0 mmol, 2 eq.). The reaction mixture was stirred for 20 h at RT. Most of THF was removed and the residue diluted with water (10 mL) and $CHCl_3$ (40 mL). The organic layer was separated and washed successively with water (10 mL), 0.1M HCl (20 mL), water (10 mL) and brine (25 mL). The organic layer was dried over sodium sulfate, filtered and evaporated. The resulting oil was purified by chromatography on silica gel with $CHCl_3$: MeOH (9 : 1 to 17 : 3). R_f = 0.34 ($CHCl_3$: MeOH, 9 : 1) to yield **12** as a white solid. Yield: 0.48 g (31%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (m, 2 H, 2 NHCO), 5.08 (m, 2 H, 2 $NHC^{1'}$), 4.37 (m, 2 H, 2 CH^{α}), 4.18 (m, 2 H, 2 OH), 3.94 (m, 2 H), 3.80 (m, 2 H), 3.37 (m, 6 H, 2 CH and 2 $CH_2^{3'}$), 3.18 (m, 8 H, 2 $CH_2^{1'}$ and 2 $CH_2^{4'}$), 3.05 (m, 4 H, 2 CH_2^{ε}), 2.18 (m, 4 H), 2.05 (m, 2 H), 1.90 (m, 4 H), 1.80–1.30 (m, 96 H), 1.05–0.9 (m, 10 H, 2 CH_3 and 2 × 2 CH), 0.85 (s, 6 H, 2 CH_3), 0.63 (s, 6 H, 2 CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.9, 173.1, 156.7, 156.0, 80.1, 79.5, 73.5, 68.7, 66.3, 53.8, 47.3, 46.1, 45.1, 42.0, 40.6, 40.1, 39.0, 35.9, 35.1, 34.1, 32.6, 31.4, 30.8, 30.0, 28.9, 28.1, 26.6, 24.2, 23.6, 23.3, 22.8, 17.7, 12.7.

N^1,N^{12}-Bis(*N*-α-cholyl-L-lysyl)-1,12-diamino-4,9-diazadodecane·4 HCl (3). To a solution of **12** (380 mg, 0.23 mmol) in methanol (10 mL) was added concentrated HCl (10 mL). The reaction mixture was stirred for 1 h, then evaporated to dryness. The residue was then dissolved in water (60 mL), filtered on a sintered frit funnel (no. 3), and evaporated to dryness once again. The residue was dissolved in a small amount of methanol and precipitated with Et_2O to give **3** as a white solid. Yield: 284 mg (88%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.05 (m, 4 H, 2 NH_2^+), 8.17 (t, 2 H, J = 5.2, 2 $NHC^{1'}$), 8.00 (m, 8 H, 2 $N^{\varepsilon}H_3^+$ and 2 $N^{\alpha}H$), 7.60 (m, 2 H, 2 NHCO), 4.09 (m, 2 H, 2 CH^{α}), 3.75 (m, 2 H), 3.57 (m, 2 H), 3.37 (m, 6 H, 2 CH and 2 $CH_2^{3'}$), 3.18 (m, 8 H, 2 $CH_2^{1'}$ and 2 $CH_2^{4'}$), 3.05 (m, 4 H, 2 CH_2^{ε}), 2.15 (m, 6 H), 1.97 (m, 2 H), 1.90 (m, 4 H), 1.80–1.15 (m, 52 H), 1.05–0.9 (m, 8 H, 2 CH_3 and 2 CH), 0.85 (m, 8 H, 2 CH_3 and 2 CH), 0.63 (s, 6 H, 2 CH_3). MS (+ES): $[M + H]^+$: 1240.0.

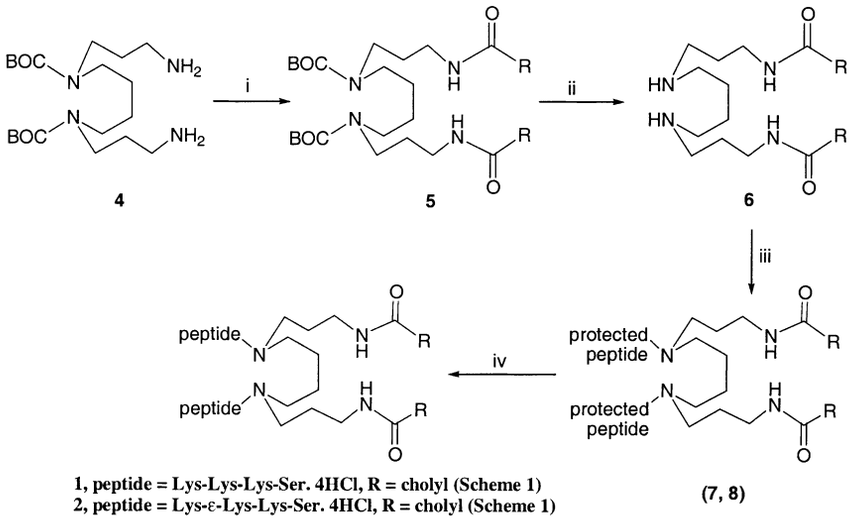
Antibacterial activity

Whole-cell antibacterial activity of gemini surfactants **1**–**3** was determined by broth microdilution against a panel of twelve aerobic, prokaryotic organisms. Compounds were tested using serial dilutions ranging from 0.06 to 64 μg mL^{-1}. Test isolates were maintained as frozen stocks which were thawed and adjusted to inoculum density of approximately 5 × 10^5 cfu mL^{-1}. Following inoculation, microtitre assay plates were incubated at 35 °C for 24 hours. The minimum inhibitory concentration (MIC) was then determined as the lowest concentration of compound that inhibited visible growth of the test isolates.

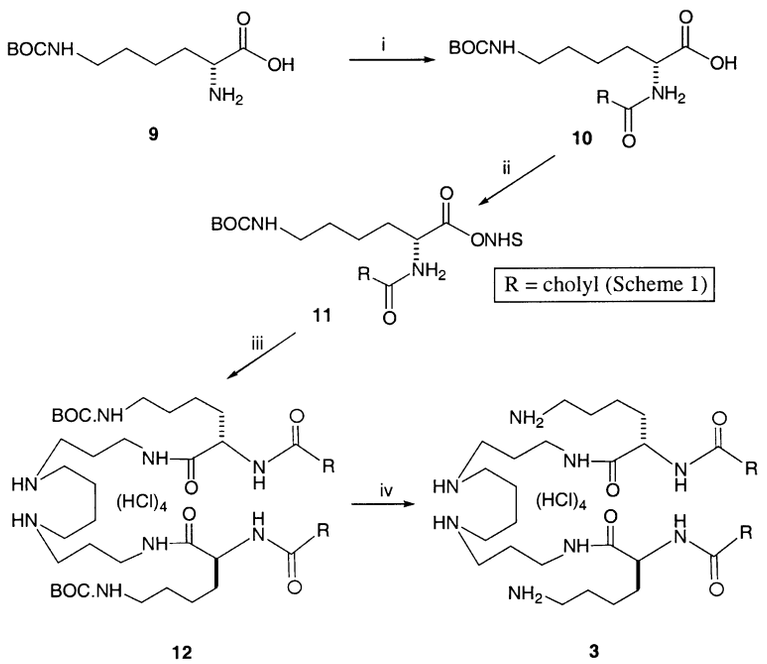
Results and discussion

The ability of bile salts to inhibit the growth of both Gram-positive and Gram-negative bacteria is well documented.[3,11] Bile acids have also been included in formulations aimed at inhibiting microorganisms such as salmonella and shigella.[12] Antibacterial activity has been reported to increase substantially with an increase of hydrophobic nature of the bile acid,[3] so that it has been claimed that the detergent effect of these biomolecules is important for their antibacterial activity. More recent studies have shown that the attachment of positively charged groups to cholic acid leads to compounds with minimum inhibition concentration (MIC) values of the order of 1 to 7 μg mL^{-1} against *S. aureus* and *E. coli* bacteria.[5]

We synthesised compounds **1**–**3** as part of our project involving the synthesis of a variety of gemini surfactants as non-viral vehicles for gene transfection.[6–9] These molecules contain two cholic acid residues as the hydrophobic 'tails'. Unlike a number of other gemini surfactants where the 'tails' were long aliphatic hydrocarbon chains, **1**–**3** failed to carry DNA to cell nuclei. However, these gemini surfactants showed various levels of efficacy as antibacterial agents.



Scheme 2 *Reagents and conditions*: (i) cholic acid *N*-hydroxysuccinimide ester, K_2CO_3, aq. THF, 18 h, RT; (ii) conc. HCl, MeOH, 1 h, RT; (iii) $(Boc)_4$-peptide hydroxysuccinimide ester, K_2CO_3, aq. THF, 18 h, RT; (iv) conc. HCl, MeOH, 1 h, RT.



Scheme 3 *Reagents and conditions*: (i) cholic acid *N*-hydroxysuccinimide ester, K_2CO_3, aq. THF, 16 h, RT; (ii) *N*-hydroxysuccinimide, DCC, 18 h, RT; (iii) **4**, K_2CO_3, aq. THF, 18 h, RT; (iv) conc. HCl, MeOH, 1 h, RT.

The unexceptional synthetic procedures used are outlined in Schemes 2 and 3. The two terminal NH_2 groups of spermine, with the two internal N selectively protected by *tert*-butoxycarbonyl groups, were acylated using the *N*-hydroxysuccinimide ester of cholic acid. Removal of the t-Boc groups made the two internal amino-nitrogens available for acylation by *N*-protected carboxy-activated lysine derivatives.

The antibacterial activity of **1**–**3** was tested against twelve aerobic, prokaryotic organisms. Results are presented in Table 1. The minimum inhibitory concentration (MIC) values range between 4 and 64 μg mL^{-1} (equivalent to 2 to 32 μM) for both Gram-positive and Gram-negative bacteria. These levels of activity of **1** against *E. Coli* and *S. Aureus* are at least an order of magnitude higher than those reported for DODAB.[2] The critical micelle concentration (cmc) for these surfactants is of the order of 1.0 mM so that antibacterial activity is observed at concentrations well below micelle formation.

A common structural feature of molecules **1**–**3** is the four to eight primary amino groups, a number of which will be positively charged under physiological conditions. From the limited data presented it is not possible to obtain conclusive structure–activity information, but it is apparent that the number of positive charges of these surfactants can have a

Table 1 Antimicrobial activities of compounds **1**–**3**

Organism	Compound (MIC)/μg mL^{-1}		
	1	**2**	**3**
S. aureus Oxford	8	16	32
S. aureus WCUH29	8	8	32
E. faecalis 1	16	32	>64
E. faecalis 7	16	16	64
H. influenzae Q1	>64	>64	>64
H. influenzae NEMC1	>64	>64	>64
M. catarrhalis 1502	4	8	8
S. pneumoniae 1629	16	32	>64
S. pneumoniae N1387	16	16	>64
S. pneumoniae ERY2	16	16	>64
E. coli 7623 AcrABEFD+	8	16	32
E. coli 120 AcrAB−	8	8	32

profound influence on their ability to prevent bacterial growth. Gemini surfactants **1** and **2**, each of which carries up to eight positive charges, show the highest overall antibacterial activity. Surfactant **3**, which shows the lowest activity, has only two primary amino groups. Closer inspection of the data for **1** and **2** shows that an increase in the flexibility of the hydrocarbon chain containing the primary amino may also lead to an improvement in antibacterial activity. These results are consistent with studies where the antibacterial activity of teicoplanin was found to increase when derivatives were prepared containing a polycationic side chain.[13]

Although the mode of antibacterial action of cationic amphiphiles **1**–**3** is not known, electrostatic interactions between these molecules and the negative charges on the bacterial outer membrane are likely to play an important role. These surfactants have structural characteristics similar to other known antimicrobial peptides, many of which also have an amphipathic nature. The hydrophobic moieties of these peptides are thought to perturb the hydrophobic fatty acid portion of the bacterial membrane, while positively charged arginine and lysine residues interact with the negatively charged phosphodiester groups.[14] It has also been suggested that the antibacterial action of these peptides is a direct result of their ability to displace inorganic divalent cations, leading to destabilisation and serious disruption of the outer bacterial membrane.[15,16]

In conclusion, we have shown that bile acid-based gemini surfactants **1**–**3** have interesting, wide-spectrum bactericidal activity. In future work electron microscopy studies will be carried out to identify morphological changes of bacteria treated with **1**–**3**, thus providing information related to a possible mechanism of action. Further compounds will be prepared with the objective of improving the level and the spectrum of bactericidal activity of these bile acid-based gemini surfactants. The therapeutic potential of these compounds and others of this type will also be explored, especially in combination with other well known antibiotics. These latter studies will evaluate any synergistic interactions due to improved penetration of these simpler antibiotics in the treatment of topical or respiratory infections.

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