Synthesis and Antibacterial Properties of Novel Hydrolyzable Cationic Amphiphiles. Incorporation of Multiple Head Groups Leads to Impressive Antibacterial Activity

Jayanta Haldar,[†] Paturu Kondaiah,[‡] and Santanu Bhattacharya*[,†]

Departments of Organic Chemistry and of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560012, India

Received November 5, 2004

Two sets of novel multiheaded cationic amphiphiles bearing one, two, and three trimethylammonium headgroups (**T1**, **T2**, and **T3**) and pyridinium headgroups (**P1**, **P2**, and **P3**), have been synthesized and tested for antimicrobial activities against both Gram-positive and Gram-negative bacteria. The multicationic headgroups in these amphiphiles were attached covalently via scissile ester-type linkages. The results were compared with those for known surface-active, nonhydrolyzable compounds cetyltrimethylammonium bromide (CTAB) and cetylpyridinium bromide (CPB). The killing effects of the new single-headed amphiphiles (**T1** and **P1**) were lower than those of CTAB and CPB, but with an increase in the number of headgroups in the amphiphiles, the killing effects increased for both sets of compounds. It was found that amphiphiles with triple headgroups (**T3** and **P3**) were most active among all the amphiphiles, whereas amphiphile **P1** had a very poor killing effect on both types of bacteria. The multiheaded pyridinium amphiphiles were more active compared to their trimethylammonium counterparts. The time needed to kill bacteria with multiheaded amphiphiles was significantly less than that of single-headed amphiphiles. Owing to the presence of a cleavable ester moiety, these new amphiphiles are hydrolyzed spontaneously at physiological conditions. This property enables them to be readily metabolized and therefore have the potential to be superior disinfectants and antiseptics for food and body surfaces.

Introduction

Amphiphilic cationic compounds have been known to exhibit strong antimicrobial activity.[1,2] They exhibit rapid activity against a broad range of microorganisms such as bacteria (both Gram-positive and Gram-negative),[3] fungi,[4] and certain viruses.[5,6] But because of the high affinity of the amphiphilic compounds toward biological membranes, it has been shown that these compounds are not quite suitable for certain applications, as they lead to skin irritation and hypersensitivity. To overcome these side effects, the concept of "soft" antimicrobial agents,[7] which possess a cleavable moiety in the molecule, such as an ester of betaine or choline, have been introduced. These esters when subjected to base- or enzyme-catalyzed hydrolysis, produce significantly less toxic components.[8,9] The antimicrobial activity of quaternary ammonium compounds bearing betaine esters is quite similar to that of the corresponding nonhydrolyzable quaternary ammonium compound cetyltrimethylammonium bromide (CTAB), but they hydrolyze spontaneously into betaine and fatty alcohol, which are readily metabolized. The hydrolysis of betaine esters is enhanced at higher pH and in cationic micellar aggregates[8,10] and reduced at higher ionic strength and in aggregates with fatty acids and hydrophobic alcohol.[11]

The ester bond in such cationic compounds is hydrolyzable at higher pH, and the pH of the sex organ vagina is low, within the range of 2−4. Therefore, these ester bonds are quite stable within this pH range and hence are able to kill bacteria and viruses in the vaginal lumen. At the vaginal wall, however, the pH value increases significantly, and when the cationic compounds come into contact with the tissues of the wall, the ester bond is likely to be hydrolyzed into less toxic substances which can be easily metabolized. Therefore, these types of compounds do not cause irritation if used as prophylactic agents, and they are the better choice than stable quaternary compounds.[12] Covalent attachment of multiple headgroups into an amphiphilic molecule[13] allows fine control of the molecular architecture and hence aggregate morphology. When such linkages are based on scissile ester bonds, then the resulting systems become attractive choices as potential anti-

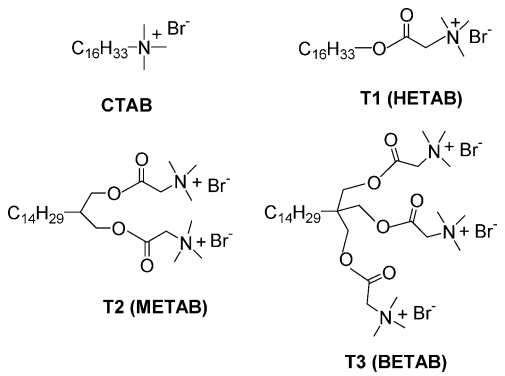


Figure 1. Molecular structures of various cationic surfactants bearing trimethylammonium headgroups.

* To whom correspondence should be addressed. Phone: 91 80 2293 2664. Fax: 91 80 2360 0529. E-mail: sb@orgchem.iisc.ernet.in. Also a recipient of the National Bioscience Career Development Award, DBT.
† Department of Organic Chemistry.
‡ Department of Molecular Reproduction, Development and Genetics.

10.1021/jm049106l CCC: $30.25
Published on Web 05/06/2005

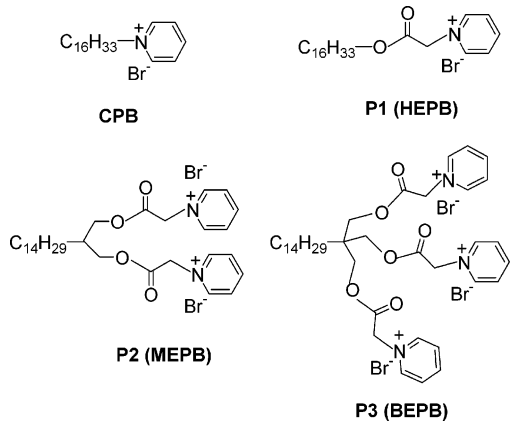
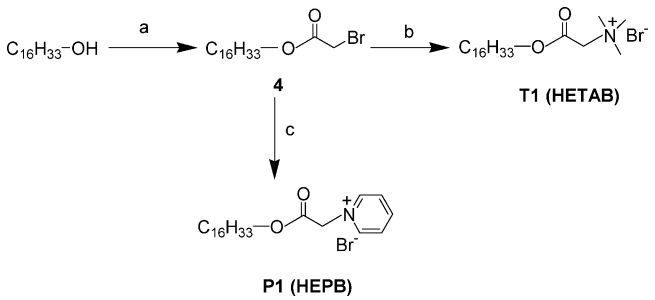


Figure 2. Molecular structures of various cationic surfactants bearing pyridinium headgroups.

Scheme 1[a]



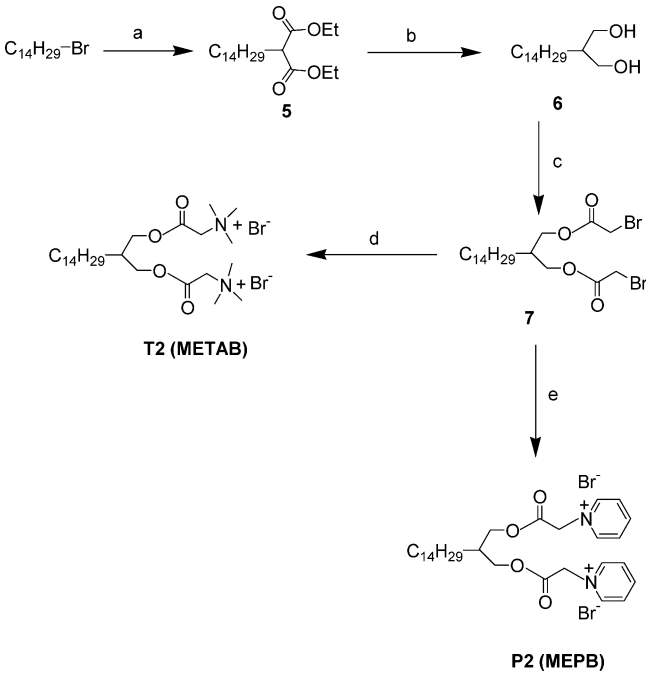
[a] Reagents, conditions, and yields: (a) $BrCH_2COBr$, DMAP, Et_3N, CH_2Cl_2, 0 °C (10 min) and room temperature (rt) (1 h), 91%; (b) NMe_3, acetone, pressure tube, rt (12 h), 98%; (c) pyridine, acetone, pressure tube, 50 °C (12 h), 90%.

microbial agents. Therefore, it is worthwhile to develop novel soft antimicrobial agents bearing esters, which can be easily hydrolyzed to produce less toxic compounds, and also have multiple charges, enabling them to interact with the bacterial cell surface more strongly than their monomeric counterparts. Hence, they are expected to have better antimicrobial properties. Herein we present the synthesis of multiheaded cationic amphiphiles. Since the main objective of the present study is the elucidation of how structural modification of cationic amphiphilic systems influences their antibacterial properties, we also present the results of antimicrobial studies of two novel sets of multiheaded amphiphiles possessing cleavable ester moieties, against both Gram-positive and Gram-negative bacteria.

Results

Synthesis. The synthesis of *single-headed amphiphiles* **T1** (HETAB) and **P1** (HEPB) began with the esterification of 1-hexadecanol with bromoacetyl bromide in the presence of Et_3N and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in CH_2Cl_2 to afford hexadecyl bromoacetate (**4**) in 91% isolated yield. After purification by column chromatography over silica gel, **4** was quaternized using trimethylamine in dry acetone in a screw-top pressure tube with stirring at room temperature (rt) for 12 h (Scheme 1). A white solid was formed which was filtered and washed repeatedly with dry acetone. The pure amphiphile **T1** (98%) was obtained upon several recrystallizations from a mixture of $CHCl_3$/*n*-hexane. To synthesize **P1**, **4** was reacted

Scheme 2[a]



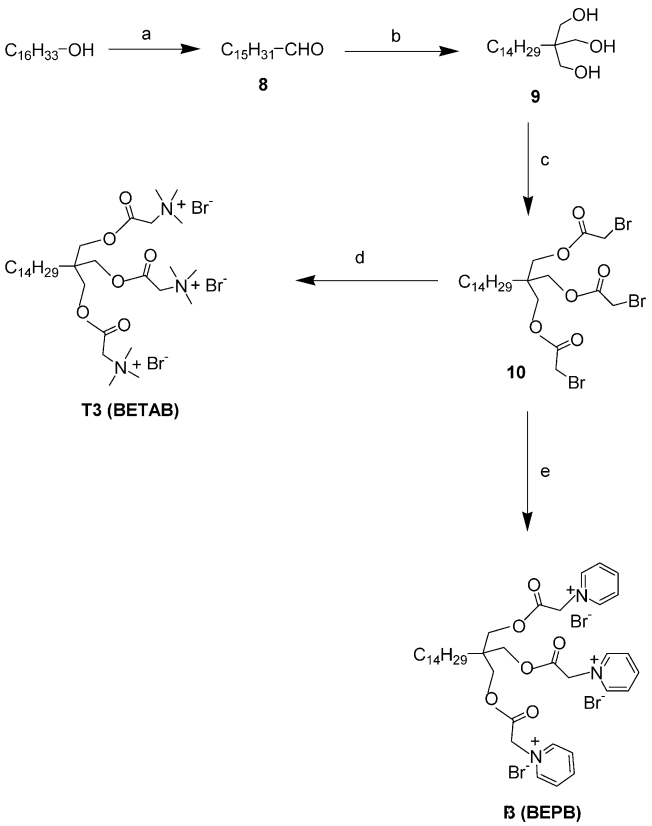
[a] Reagents, conditions, and yields: (a) diethyl malonate, NaH, THF, 0 °C (100 min), rt (2 h) and 70 °C (15 h), 71%; (b) $LiAlH_4$, $AlCl_3$, Et_2O, 0 °C (20 min) and rt (1 h), 96%; (c) $BrCH_2COBr$, DMAP, Et_3N, CH_2Cl_2, 0 °C (10 min) and rt (3 h), 64%; (d) NMe_3, acetone, pressure tube, rt (24 h), 87%; (e) pyridine, acetone, pressure tube, 50 °C (24 h), 78%.

with pyridine in dry acetone at 50 °C in a screw-top pressure tube with heating for 12 h (Scheme 1). A white solid was formed which was filtered and washed repeatedly with dry acetone, and finally pure **P1** (90%) was obtained upon repeated recrystallizations from $CHCl_3$/*n*-hexane.

For the synthesis of the *double-headed amphiphiles* **T2** (METAB) and **P2** (MEPB), diethyl malonate was monalkylated with 1-bromotetradecane in dry THF in the presence of NaH. This afforded diethyl *n*-tetradecylmalonate (**5**) in 71% yield upon purification by gravity-driven column chromatography over silica gel. Compound **5** was then reduced using $LiAlH_4$ and $AlCl_3$ in dry Et_2O to obtain the corresponding diol **6** in 96% yield. The diol was then reacted with >2 equiv of bromoacetyl bromide in the presence of dry Et_3N and a catalytic amount of DMAP in CH_2Cl_2 first at 0 °C for 10 min and then at room temperature for 3 h. Workup followed by purification over a silica gel column afforded the diester **7** in 64% yield. Pure ester **7** was finally quaternized with trimethylamine in dry acetone in a screw-top pressure tube with stirring at room temperature for 24 h (Scheme 2). A white solid formed which was filtered and washed repeatedly with dry acetone. Pure **T2** (87%) was obtained upon several recrystallizations from a mixture of $CHCl_3$/EtOAc.

For the synthesis of **P2**, pure ester **7** was reacted with pyridine in dry acetone in a screw-top pressure tube with heating at 50 °C for 24 h (Scheme 2). A gummy solid was formed on the walls of the pressure tube. The required compound **P2** (78%) was purified by repetitive precipitations upon solubilization of the gummy material using a 1:1 mixture of $CHCl_3$/acetone and then upon dropwise addition of *n*-hexane. Both **T2** and **P2** were found to be highly hygroscopic in nature.

Scheme 3[a]



[a] Reagents, conditions, and yields: (a) PCC, CH_2Cl_2, rt (5 h), 95%; (b) formaldehyde solution, KOH, 50% aqueous EtOH, rt (4 h) and 50 °C (2 h), 41%; (c) $BrCH_2COBr$, DMAP, Et_3N, CH_2Cl_2, 0 °C (10 min) and rt (5 h), 53%; (d) NMe_3, acetone, pressure tube, rt (48 h), 65%; (e) pyridine, acetone, pressure tube, 50 °C (48 h), 62%.

The synthesis of the *triple-headed amphiphiles* **T3** (BETAB) and **P3** (BEPB) began with the oxidation of 1-hexadecanol to the corresponding aldehyde **8** (95%) by pyridinium chlorochromate (PCC) in CH_2Cl_2. 1-Hexadecanal (**8**) was then converted to 2,2-bis(hydroxymethyl)hexadecanol (**9**) by reaction with formaldehyde solution in the presence of KOH and 50% aqueous EtOH initially at room temperature for 4 h and then at 50 °C for 2 h. After removal of EtOH under vacuum, the resulting mixture was taken up in Et_2O and allowed to settle. This resulted in the separation of the organic layer from this mixture, which was dried over anhydrous Na_2SO_4 and concentrated to produce a white residue. After column chromatography over silica gel, the alcohol **9** (41%) was isolated as a pure white solid. The required compound was obtained first by two aldol reactions at room temperature followed by a cross-Cannizzaro reaction at 50 °C with formaldehyde. Then the triol **9** was fully esterified to the corresponding triester **10** (53%) upon reaction with 3.6 equiv of bromoacetyl bromide in the presence of dry Et_3N and a catalytic amount of DMAP in CH_2Cl_2 first at 0 °C for 10 min and then at room temperature for 5 h. After workup and purification by column chromatography over silica gel, pure triester **10** was isolated. Finally **10** was quaternized with trimethylamine in dry acetone in a screw-top pressure tube with stirring at room temperature for 48 h (Scheme 3). A sticky solid formed on the walls of the pressure tube. The required compound **T3** (65%) was purified by repetitive precipitations by dissolving the sticky material in a 1:1 mixture of $CHCl_3$/EtOAc and then upon dropwise addition of *n*-hexane. To synthesize **P3**, **10** was reacted with pyridine in dry acetone in a screw-top pressure tube with heating at 50 °C for 48 h (Scheme 3). A brown sticky solid formed on the walls of the pressure tube and was dissolved in $CHCl_3$ and precipitated several times upon addition of *n*-hexane. During the process of precipitation the compound initially came out of the solution as a dense yellow liquid. The two phases could be separated from this by careful decantation to afford the dense material, which eventually solidified into a sticky mass, **P3** (62%). Both the surfactants were found to be highly hygroscopic in nature. All the final products and key intermediates were appropriately characterized as given in the Experimental Section.

Table 1. MBCs (μM) of Cleavable Multiheaded Cationic Amphiphiles[a]

amphiphile	*E. coli*	*S. sonnei*	*S. aureus*	*E. faecalis*
CTAB	16.9	18.3	18.9	18.5
T1	20.6	33.8	24.5	22.5
T2	9.7	14.9	15.1	12.0
T3	7.3	9.6	11.3	9.0
CPB	10.4	12.4	15.6	16.3
P1				
P2	8.2	8.9	13.9	10.4
P3	6.5	3.5	5.2	4.9

[a] MBC (μM) determined by the spread plate method. The average values are reported on the basis of three separate experiments where the variance was <10%. Amphiphile **P1** was not able to kill the bacteria completely even at concentrations as high as 300 μM.

Antibacterial Property. Antibacterial activity of the multiheaded amphiphiles was investigated, and the results were compared with those of the nonhydrolyzable surfactants CTAB and cetylpyridinium bromide (CPB). The solubility of both single-headed surfactants (**T1** and **P1**) was less in both water and Luria−Burtani (LB) medium compared to those of other amphiphiles. Between the two single-headed amphiphiles, **P1** was even less soluble than **T1**. The stock solution of all the amphiphiles was made in sterile water. The facile solubility of amphiphiles with multiple headgroups in both water and LB medium could be a consequence of the increased hydrophilicity of the resulting amphiphiles with a larger number of headgroups.

To investigate the influence of systematically enhancing the number of headgroup units incorporated at one end of a single hydrocarbon chain of the amphiphilic molecule and also the nature of the headgroups on the bactericidal effect, we have tested these two sets of amphiphiles against both Gram-positive and Gram-negative bacteria. The minimum bactericidal concentrations (MBCs) were determined by the spread plate method.[14] The MBC data for the amphiphiles against both Gram-negative and Gram-positive bacteria are shown in Table 1. The MBC value was considered as the lowest amphiphile concentration at which there was no viable cell present. The relevant data for CTAB and CPB are also included in Table 1 for comparison. The killing effect was similar for both Gram-positive and Gram-negative bacteria and increased with an increase in the number of headgroups on the amphiphile for both sets of compounds (Figure 3). In general, the amphiphiles bearing multiheaded pyridinium headgroups

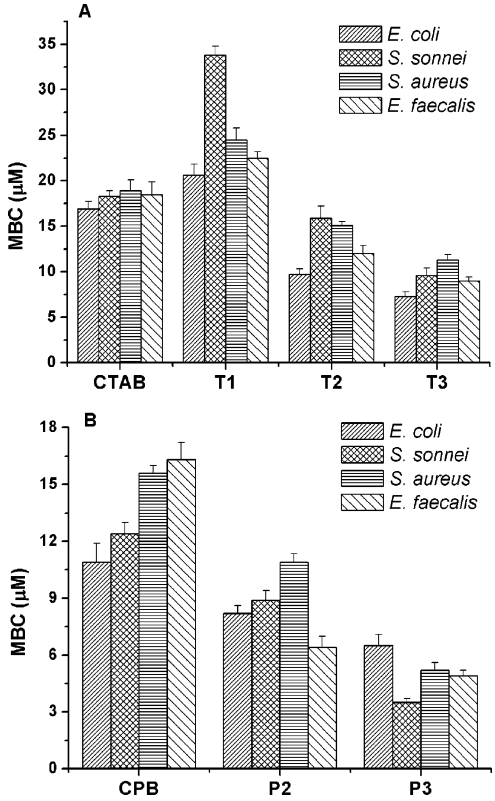


Figure 3. Comparison of bactericidal activity of various cationic amphiphiles with (A) trimethylammonium headgroups and (B) pyridinium headgroups against both Gram-positive and Gram-negative bacteria.

were found to be more active than the amphiphiles bearing trimethylammonium headgroups against either type of bacteria.

The MBC values for **T1** against *Escherichia coli* and *Shigella sonnei* were 20.6 and 33.8 μM, respectively, and 7.3 and 9.6 μM for **T3**. Against *Staphylococcus aureus* and *Enterococcus faecalis*, the MBC values were 24.5 and 22.5 μM, respectively, for **T1** and 11.3 and 9.0 μM, respectively, for **T3**. The MBC values for double-headed amphiphile **T2** were between those of the single- and triple-headed amphiphiles against all the bacteria. In the case of single-headed amphiphiles, however, CTAB was more effective than **T1** (Figure 3A).

Among the series of amphiphiles with pyridinium headgroups, **P1** had a very poor killing effect on both types of bacteria. Even at concentrations as high as 300 μM, it merely reduced the cell count for *E. coli* from 5.7 $\times$ 10^9 to 1.6 $\times$ 10^4 cells/mL, for *S. sonnei* from 3.2 $\times$ 10^7 to 2.4 $\times$ 10^2 cells/mL, for *S. aureus* from 4.9 $\times$ 10^9 to 8.3 $\times$ 10^2 cells/mL, and for *E. faecalis* from 1.2 $\times$ 10^8 to 4.5 $\times$ 10^3 cells/mL. The MBC values of amphiphile **P3** against *E. coli* and *S. sonnei* were 6.5 and 3.5 μM, respectively, and against *S. aureus* and *E. faecalis*, they were 5.2 and 4.9 μM, respectively. The MBC values for double-headed pyridinium amphiphile **P2** ranged between those of of CPB and **P3** against both Gram-negative and Gram-positive bacteria (Figure 3B).

Figures 4 and 5 show the log(survivors) versus exposure time plots for the multiheaded amphiphiles bearing trimethylammonium headgroups and pyridinium headgroups, respectively, against both Gram-negative and Gram-positive bacteria. Exposure of the amphiphiles to bacterial cells was carried out in LB medium. The concentration of **T1** and **P1** was kept at 50 μM, whereas the concentration of the other amphiphiles was maintained at 20 μM against all organisms.

Among the amphiphiles with trimethylammonium headgroups, **T3** was found to be the most active one against both types of bacteria (Figure 4). When exposed to the triple-headed amphiphile **T3**, all bacterial cells were killed within 20−30 min for both types of bacteria. In the case of **T2** and CTAB, the killing of bacteria required a longer time compared to that of **T3**. Therefore, the time taken for killing of the bacterial cells became progressively less with an increase in the number of headgroups. From parts C and D of Figure 4, which show the killing effect on Gram-positive bacteria *S. aureus* and *E. faecalis*, respectively, it is evident that with these compounds the time required to kill the Gram-positive bacteria is a little less than that for Gram-negative bacteria.

Figure 5 shows the log(survivors) versus exposure time plots for the multiheaded amphiphiles with pyridinium headgroups against both types of bacteria. In this set of compounds, it was observed that the amphiphile with triple headgroups, **P3**, was also the most active compound. The activity of **P1** was very poor, which was also found during the estimation of the MBC value (Table 1). When 20 μM **P3** was treated against both types of bacteria, all bacterial cells were killed within ~10 min. Thus, with an increase in the number of headgroups of pyridinium amphiphiles, the killing effect became even faster. In this case, it was also found that the compounds killed the Gram-positive bacteria faster than the Gram-negative bacteria (Figure 5).

Discussion

The sequence of elementary events in the lethal action of the cationic disinfectants[15] may be summarized as follows: (i) adsorption onto the bacterial cell surface, (ii) diffusion through the cell wall, (iii) binding to the cytoplasmic membrane, (iv) disruption of the cytoplasmic membrane, (v) release of K^+ ions and other cytoplasmic constituents, and (vi) precipitation of the cell contents and the death of the cell. Electrophoretic measurements have clearly demonstrated that the bacterial cell surface is usually negatively charged, and hence, the adsorption of cationic amphiphiles onto the negatively charged cell surface is facilitated by electrostatic interaction, along with the hydrophobic interaction.[16]

The MBC values decrease with an increase in the number of headgroups in the amphiphile, implying that the antibacterial activity of amphiphiles with multiple headgroups is higher than that of single-headed amphiphiles. This might be explained by taking into consideration each of the elementary processes involved in the lethal action.[15] The adsorption of amphiphiles with multicationic headgroups onto the negatively charged bacterial cell surface is expected to take place at a greater extent than that of a monocationic amphiphile through electrostatic interaction because of the much higher charge density carried by multiheaded cationic amphiphiles.[16] Furthermore, binding to the cytoplasmic membrane is also expected to be facilitated by the multiheaded cationic amphiphiles, compared to

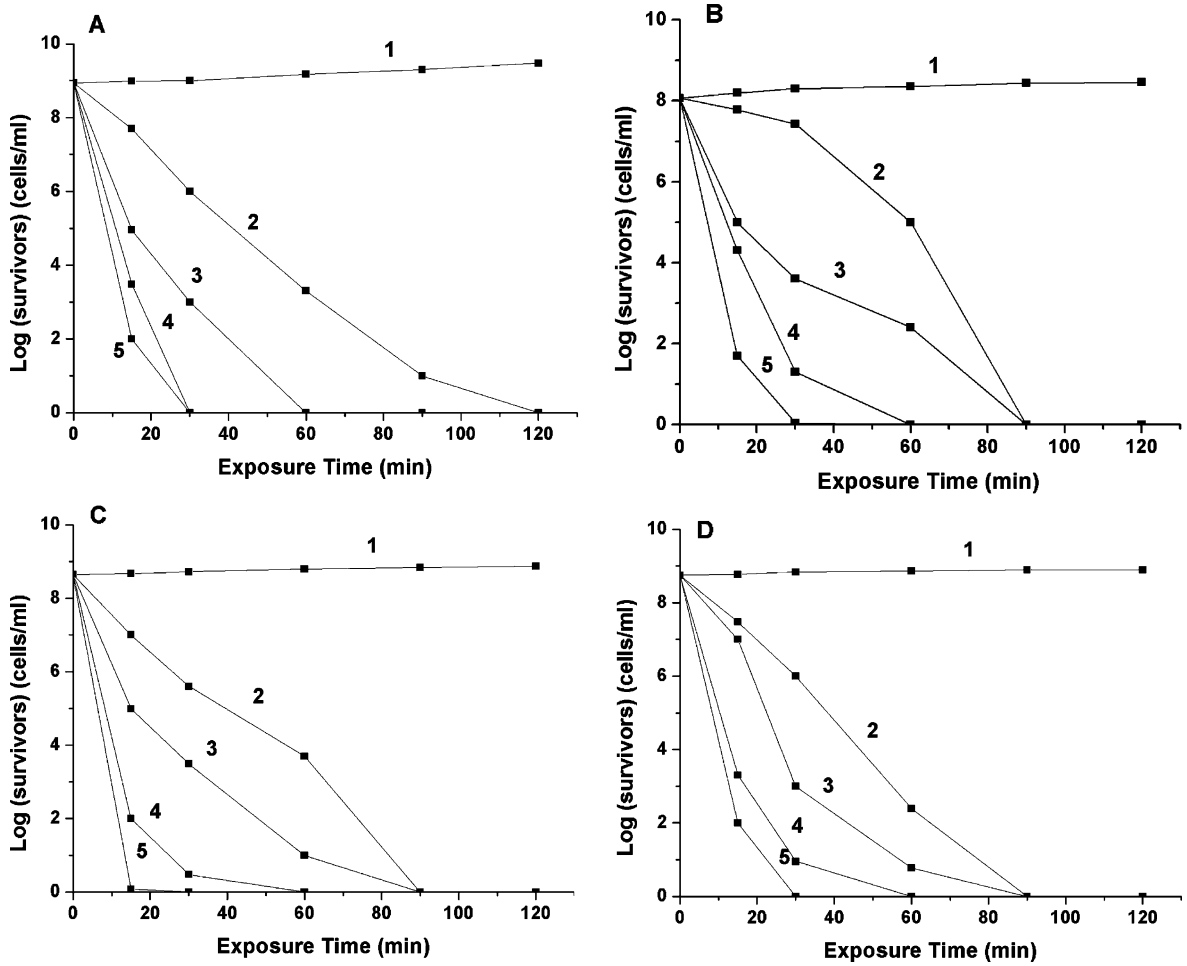


Figure 4. Log(survivors) versus exposure time plots for different amphiphiles with trimethylammonium headgroups against (A) *E. coli*, (B) *S. sonnei*, (C) *S. aureus*, and (D) *E. faecalis*: 1, control (without any amphiphile); 2, **T1** (50 μM); 3, CTAB (20 μM); 4, **T2** (20 μM); 5, **T3** (20 μM).

single-headed amphiphiles, because of the presence of a large number of negatively charged species (such as acidic phospholipids and some membrane proteins) in the membrane.[17,18] Thus, the disruption of the membrane and the subsequent leakage of K^+ ions and other cytoplasmic constituents would be enhanced by the multiheaded cationic amphiphiles.

All the amphiphiles are almost equally active for both Gram-positive and Gram-negative bacteria. It is known that the Gram-positive strains have a rather simple cell wall composed of a rigid peptidoglycan layer, which allows foreign molecules to come inside without much difficulty.[19] Thus, it is expected that any foreign substances diffuse easily through the cell walls of Gram-positive bacteria. On the other hand, in the case of Gram-negative bacteria, there is another bilayer membrane outside the peptidoglycan layer (outer membrane). Because of this outer membrane, foreign molecules cannot easily diffuse through the cell walls of Gram-negative bacteria.[19] But these types of low molecular mass amphiphiles because of their lipidlike properties can easily diffuse through both the outer membrane and the cell wall to reach the cytoplasmic membrane of the Gram-negative species and hence are able to kill the bacteria with equal efficiency.

The activity of single-headed surfactants **T1** and **P1** is lower than that of CTAB and CPB. This could be due to the fact that single-headed amphiphiles **T1** and **P1** have lower solubility in water compared to CTAB and CPB. From our previous studies it was found that they have a greater tendency to form open lamellar or other larger aggregates.[13,20] Therefore, they have a greater binding affinity in excessive amounts to a lesser number of cells. This in turn enhances their tendency to associate with compounds released from the cells. In contrast, the multiheaded amphiphiles that are highly soluble in water and form much smaller and more dynamic aggregates[13] can bind to a greater number of cells. They can also easily diffuse through the cell wall and interact with the cytoplasmic membrane to kill the bacteria more efficiently.

Conclusion

Two sets of novel multiheaded cationic amphiphiles bearing one, two, and three trimethylammonium headgroups and pyridinium headgroups were synthesized and characterized. The antibacterial activity of multiheaded amphiphiles against both Gram-negative and Gram-positive bacteria is significantly enhanced compared to that of single-headed amphiphiles. This could be due to their higher solubility in water and greater positive charge density per molecule, which enable them to interact better with the bacterial cell surface, leading to more efficient killing of the bacteria. These compounds have their cationic headgroups connected to a hydrocarbon chain via cleavable ester linkages and

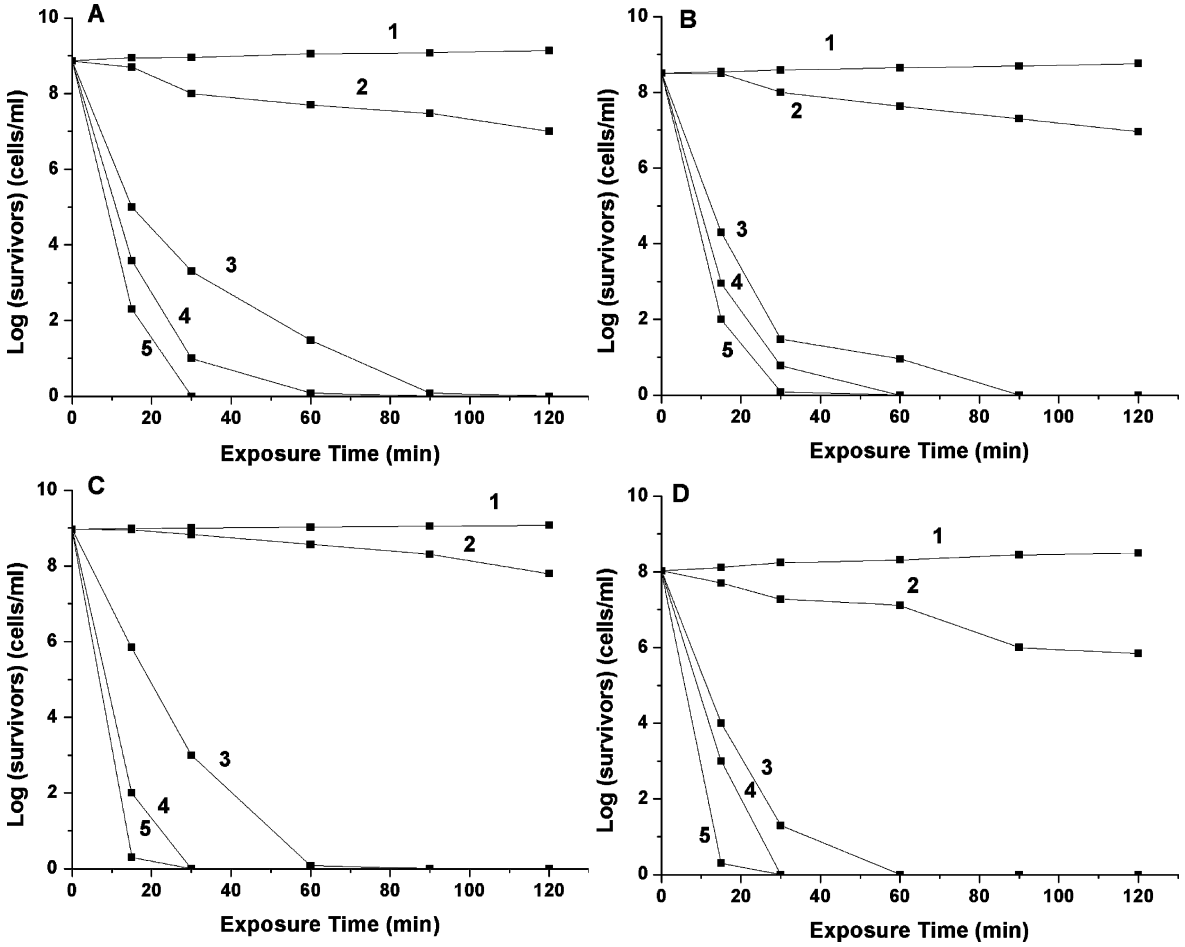


Figure 5. Log(survivors) versus exposure time plots for different amphiphiles with pyridinium headgroups against (A) *E. coli*, (B) *S. sonnei*, (C) *S. aureus*, and (D) *E. faecalis*: 1, control (without any amphiphile); 2, **P1** (50 μM); 3, CPB (20 μM); 4, **P2** (20 μM); 5, **P3** (20 μM).

can therefore be hydrolyzed to form readily metabolized substances.[8,9] This property renders this class of multiheaded amphiphiles potent candidates to replace the stable cationic compounds.

Experimental Section

All the starting materials were obtained from Aldrich or Fluka and used as received. PCC was synthesized as described by Corey et al.[21] CTAB and CPB used in this study were purchased from Aldrich. All the solvents were reagent grade and dried prior to use. Column chromatography was performed using 60−120 mesh silica gel. NMR spectra were recorded using a JEOL JNM λ-300 (300 MHz for ^{1}H) or Bruker AMX-400 (400 MHz for ^{1}H and 133.3 MHz for ^{13}C) spectrometer. The chemical shifts (δ) are reported in parts per million downfield from the peak for the internal standard TMS for ^{1}H NMR and ^{13}C NMR. Mass spectra were recorded on a Kratos PCKompact SEQ V1.2.2 MALDI-TOF spectrometer, a MicroMass ESI-TOF spectrometer, or a Shimadzu tabletop GC−MS or ESI-MS (HP1100LC-MSD) instrument. Infrared (IR) spectra were recorded on a Jasco FT-IR 410 spectrometer using KBr pellets or neat.

Hexadecyl Bromoethanoate (4). 1-Hexadecanol (1.0 g, 4.13 mmol) was dissolved in dry CH_2Cl_2 (8 mL). DMAP (0.068 g, 0.56 mmol) and dry Et_3N (0.74 mL) were added to the reaction mixture, and the resulting mixture was cooled in an ice−water bath. Bromoacetyl bromide (0.43 mL, 4.95 mmol) was added dropwise to the reaction mixture, after 10 min the ice bath was removed, and the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with $CHCl_3$ (50 mL) and successively washed with dilute HCl solution (2 M, 10 mL) and with water (10 mL). Finally, it was washed with $NaHCO_3$ (0.5 M, 10 mL) and saturated brine solution (20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to afford a residue which was purified by column chromatography over silica gel using an EtOAc/hexane (1:99) solvent mixture. A low-melting-point, white compound ($R_f \approx 0.8$ in an EtOAc/hexane (1:99) solvent mixture) was isolated in 91% yield. FT-IR (neat): 1740 cm^{-1} (C=O str). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, terminal $-CH_3$, 3H), 1.25 (br m, $(-CH_2-)_{13}$, 26H), 1.59−1.7 (m, $-CH_2-CH_2O-$, 2H), 3.85 (s, $-OC(O)CH_2-$, 2H), 4.17 (t, $-CH_2CH_2O-$, 2H). MALDI-TOF: *m/z* 386.4 $[M + Na]^+$.

Hexadecyl *N*-Ethanoate *N,N,N*-Trimethylammonium Bromide (HETAB, T1). Dry NMe_3 gas was passed into dry acetone (5.0 mL) in a screw-top pressure tube at 0 °C till the volume of the resulting solution was 15 mL. Then **4** (1.06 g, 2.92 mmol) was dissolved in dry acetone (3.0 mL) and added to the pressure tube at 0 °C. Immediately after the addition of the bromide, a white precipitate was formed. The reaction mixture was stirred at room temperature for 3−4 h for completion of the reaction. The precipitate was filtered and washed several times with dry acetone to give a white solid. Pure surfactant **T1** (98%) was obtained upon repeated recrystallizations from $CHCl_3$/hexane. FTIR (KBr): 1751 cm^{-1} (C=O str). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.25 (br s, $(CH_2)_{13}$, 26H), 1.63−1.70 (m, $-CH_2-CH_2O-$, 2H), 3.67 (*s*, $-^+N(CH_3)_3$, 9H), 4.18 (*t*, $-CH_2O-$, 2H), 4.99 (*s*, $-CH_2N^{+}-$, 2H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ 14.05, 22.66, 25.66, 28.31, 29.16, 29.32, 29.45, 29.68, 31.91, 54.34, 63.22, 66.915, 164.71. ESI-MS: *m/z* M^+ $[C_{21}H_{44}NO_2]^+$, calcd 342.3, found 342.2. Anal. Calcd for $C_{21}H_{44}NO_2Br$: C, 59.7; H, 10.5; N, 3.31. Found: C, 59.54; H, 10.63; N, 2.95.

Hexadecyl *N*-Ethanoate Pyridinium Bromide (HEPB, P1). **4** (1.45 g, 3.99 mmol) was dissolved in dry acetone (10

mL) and added to a screw-top pressure tube. Dry pyridine (0.96 mL, 11.97 mmol) was added to the pressure tube, and the mixture was heated at 50 °C for 12 h. A white solid was formed which was filtered and washed repeatedly with dry acetone. Pure surfactant **P1** (1.59 g, 90%) was obtained upon several recrystallizations from $CHCl_3$/*n*-hexane. FT-IR (KBr): 1748 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.25 (br m, $(-CH_2-)_{13}$, 26H), 1.64−1.69 (m, $-CH_2$-CH_2O-, 2H), 4.21 (t, $-CH_2O-$, 2H), 6.3 (s, $-COCH_2-$, 2H), 8.08 (t, *m-H*, 2H), 8.53 (t, *p-H*, 1H), 9.42 (d, $J = 6.6$ Hz, *o-H*, 2H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ 14.07, 22.65, 25.68, 28.40, 29.34, 29.50, 29.69, 31.91, 61.28, 127.75, 146.11, 146.78, 165.81. ESI-MS: *m/z* M^+ $[C_{23}H_{40}NO_2]^+$, calcd 362.5, found 362.5. Anal. Calcd for $C_{23}H_{40}NO_2Br$: C, 62.4; H, 9.1; N, 3.16. Found: C, 62.2; H, 9.2; N, 3.05.

Ethyl 2-(Ethoxycarbonyl)hexadecanoate (5).[22] NaH (1.25 g, 26 mmol, 50% dispersion in oil) was added to a two-necked round-bottom flask closed with a septum to which dry *n*-hexane (5 mL) was added, and the mixture was stirred for 5 min to dissolve the mineral oil. The supernatant in *n*-hexane was removed by a syringe, dry THF (10 mL) was added, and the resulting mixture was stirred at 0 °C for 15 min. Diethyl malonate (5.66 g, 35.3 mmol) in dry THF (10 mL) was then slowly added over 30 min at 0 °C to the resulting suspension. The solution became clear after 10 min of stirring. The stirring was continued for another 30 min. 1-Bromotetradecane (4 g, 14.4 mmol) in dry THF (15 mL) was added dropwise over 30 min to the stirred solution, resulting in the formation of a white viscous residue which was diluted with dry THF (10 mL), and this mixture was stirred at room temperature for 2 h, followed by refluxing for 15 h at 70 °C to ensure the completion of the reaction. The mixture was then taken up in $CHCl_3$ (200 mL) and washed first with water (100 mL × 3) and then with 10% (w/v) brine solution (150 mL). The $CHCl_3$ layer was separated and dried using anhydrous Na_2SO_4. The solvent was evaporated to leave a gumlike residue which was adsorbed on silica gel, and the required compound ($R_f \approx 0.6$ in an EtOAc/hexane (4:96) solvent mixture) was purified by column chromatography. Pure compound was isolated in 71% (3.6 g) yield. FT-IR (neat): 1735 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.2−1.3 (br m, $(-CH_2-)_{12}$, $-OCH_2CH_3 \times 2$, 30H), 1.8 (m, $-CH_2CH-$, 2H), 3.3 (t, $-CH_2CH-$, 1H), 4.15 (q, $-OCH_2CH_3 \times 2$, 4H). LR-MS: *m/z* 357 $[M + H]^+$.

2-(Hydroxymethyl)hexadecanol (6). Anhydrous $AlCl_3$ (0.634 g, 4.76 mmol) was added slowly to a solution of $LiAlH_4$ (0.639 g, 16.8 mmol) in dry diethyl ether (20 mL) at 0 °C. The color of the mixture became white, and **5** (1 g, 2.8 mmol) in the form of a solution in dry diethyl ether (10 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 20 min and then at room temperature for 1 h. At the end of this period, moist Et_2O (10 mL) was added slowly, and the mixture was stirred for 10 min. The reaction mixture was then washed with HCl solution (2 N, 10 mL), and the aqueous layer was extracted with EtOAc (50 mL × 2). All the organic layers were obtained and dried over anhydrous Na_2SO_4, and the solvent was evaporated to leave a white solid, which was separated by column chromatography using silica gel. A white solid was obtained (0.73 g, 96%) toward the end. FT-IR (Nujol): 3300 cm^{-1} (O−H str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, terminal $-CH_3$, 3H), 1.23 (br m, $(CH_2)_{13}$, 26 H), 1.7−1.8 (m, $-CHCH_2-$, 1H), 3.6−3.8 (m, $-CHCH_2OH \times 2$, 4H). LR-MS: *m/z* 273 $[M + H]^+$.

2-(Methyl bromoethanoate)hexadecyl Bromoethanoate (7). DMAP (0.05 g, 0.41 mmol) and Et_3N (0.53 mL, 3.82 mmol) were added to **6** (0.4 g, 1.47 mmol) dissolved in CH_2Cl_2 (6.0 mL), which was cooled at 0 °C. Bromoacetyl bromide (0.3 mL, 3.52 mmol) was then added dropwise at this temperature. After ∼10 min the ice bath was removed, and stirring was continued at room temperature for 3 h. The solvent from the reaction mixture was evaporated, and the required compound was purified by column chromatography over silica gel using an EtOAc/hexane (4:96) solvent mixture ($R_f \approx 0.5$ in an EtOAc/hexane (6:94) solvent mixture). A low-melting-point white solid was obtained (0.48 g, 64%). FT-IR (neat): 1743 and 1730 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.25 (br m, $(CH_2)_{13}$, 26 H), 2.06−2.17 (m, $-CHCH_2O-$, 1H), 3.85 (s, $-OC(O)CH_2- \times 2$, 4H), 4.15 (dd, $J_1 = 11.25$ Hz, $J_2 = 6.3$ Hz, $-CH(CH_aH_bO-)_2$, 2H), 4.22 (dd, $J_1 = 11.1$ Hz, $J_2 = 5.4$ Hz, $-CH(CH_aH_bO-)_2$, 2H). MALDI-TOF: *m/z* 537.8 $[M + Na]^+$.

2-(Methyl *N*-ethanoate *N,N,N*-trimethylammonium bromide)hexadecyl *N*-ethanoate *N,N,N*-Trimethylammonium Bromide (METAB, T2). Dry NMe_3 gas was passed into dry acetone (2.0 mL) at 0 °C in a screw-top pressure tube till the volume of the resulting solution was 8.0 mL. Then **7** (0.26 g, 0.506 mmol) was dissolved in dry acetone (2.0 mL) and added to the pressure tube at 0 °C. Immediately after addition of the bromide, a white precipitate was formed, and the reaction mixture was stirred at rt for 12 h for completion. The precipitate was filtered and washed several times with dry acetone to give a white solid. NMe_3 was removed from the filtrate by heating with hot water, and then the solvent was removed to give a white solid also. Pure surfactant **T2** (87%) was obtained upon repeated recrystallizations from $CHCl_3$/EtOAc. FTIR (KBr): 1749 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.26 (s, $(CH_2)_{13}$, 26H), 2.12−2.18 (m, $-CHCH_2O-$, 1H), 3.62 (s, $-^+N(CH_3)_3 \times 2$, 18H), 4.03 (dd, $J_1 = 7.2$ Hz, $J_2 = 10.8$ Hz, $-CH(CH_aH_bO-)_2$, 2H), 4.44 (dd, $J_1 = 3.4$ Hz, $J_2 = 10.8$ Hz, $-CH(CH_aH_bO-)_2$, 2H), 5.51 (ABq, $J = 17.7$ Hz, $-^+NCH_aH_b \times 2$, 4H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ 14.05, 22.65, 26.87, 28.05, 29.33, 29.50, 29.67, 31.91, 36.94, 54.27, 62.98, 68.12, 165.06. ESI-MS: *m/z* M^{2+} $[C_{27}H_{56}N_2O_4]^{2+}$ Br^- (78.91), calcd 551.3, found 551.3; M^{2+} Br^- (80.91), calcd 553.3, found 553.4; $M^{2+}/2$, calcd 236.2, found 236.1. Anal. Calcd for $C_{27}H_{56}N_2O_4Br_2,H_2O$: C, 49.85; H, 8.99; N, 4.3. Found: C, 49.53; H, 8.97; N, 3.9.

2-(Methyl *N*-ethanoate pyridinium bromide)hexadecyl *N*-Ethanoate Pyridinium Bromide (MEPB, P2). Inside a screw-top pressure tube, **7** (0.77 g, 1.49 mmol) was dissolved in dry acetone (8 mL). Dry pyridine (0.72 mL, 8.94 mmol) was added to the pressure tube, and the mixture was heated at 50 °C for 24 h. A gummy solid was formed on the wall of the pressure tube. $CHCl_3$ was added to the reaction mixture, and the resulting mixture was transferred into a round-bottom flask. The solvent and extra pyridine were removed under vacuum. The required compound **P2** (78%) was purified by repetitive precipitations by dissolving the solid in a 1:1 mixture of $CHCl_3$/acetone and then upon dropwise addition of *n*-hexane. The compound was found to be a hygroscopic solid. FT-IR (KBr): 1750 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.26 (br m, $(-CH_2-)_{13}$, 26H), 2.02−2.17 (*m*, $-CHCH_2O-$, 1H), 4.15 (dd, $J_1 = 7.2$ Hz, $J_2 = 10.8$ Hz, $-CH(CH_aH_bO-)_2$, 2H), 4.37 (dd, $J_1 = 3.3$ Hz, $J_2 = 10.8$ Hz, $-CH(CH_aH_bO-)_2$, 2H), 6.43 (ABq, $J = 17.4$ Hz, $-^+NCH_aH_b \times 2$, 4H), 8.12 (t, *m-H*, 4H), 8.59 (t, *p-H*, 2H), 9.55 (*d*, $J = 6$ Hz, *o-H*, 4H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ: 14.07, 22.68, 27.145, 27.305, 27.90, 28.08, 29.34, 29.71, 29.94, 31.93, 39.82, 42.24, 61.56, 61.78, 65.86, 67.45, 128.035, 146.36, 146.765, 165.97. ESI-MS: *m/z* M^{2+} $[C_{31}H_{48}N_2O_4]^{2+}$ Br^- (78.91), calcd 591.3, found 591.5; M^{2+} Br^- (80.91), calcd 593.3, found 593.5; $M^{2+}/2$, calcd 256.2, found 256.2. Anal. Calcd for $C_{31}H_{48}N_2O_4Br_2,H_2O$: C, 53.9; H, 7.2; N, 4.1. Found: C, 53.8; H, 7.0; N, 3.9.

1-Hexadecanal (8).[21] PCC (6.68 g, 30.99 mmol) was taken up in dry CH_2Cl_2 (100 mL) and the mixture stirred at room temperature for 5 min. After addition of hexadecanol (5 g, 20.66 mmol) to the reaction mixture, it was again stirred for 3 h at room temperature. The reaction mixture was diluted with dry ether, and the upper portion of the solvent was decanted. The insoluble residue was washed 3−4 times with dry ether, and the solvent from all the washings along with the main Et_2O fraction was evaporated to give a solid residue. The compound from this residue was purified by column chromatography over silica gel using an EtOAc/hexane (2:98) solvent mixture ($R_f \approx 0.7$ in an EtOAc/hexane (2:98) solvent mixture). A white solid compound (4.68 g, 95%) was obtained.

FT-IR (Nujol): 1720 cm^{-1} (C═O str), 3370 cm^{-1} (C−H ald str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, terminal $-CH_3$, 3H), 1.23−1.26 (br m, $(-CH_2-)_{13}$, 26H), 2.36−2.42 (m, $-CH_2CHO-$, 2H), 9.74 (br s, $-CH_2CHO-$, 1H). LR-MS: *m/z* 241 $[M + H]^+$.

2,2-Bis(hydroxymethyl)hexadecanol (9).[23] To a solution of 1-hexadecanal (4.09 g, 17.04 mmol) were added formaldehyde solution (3.54 mL, 1.0 mL contains 1.075 g) and 50% aqueous ethanol (50 mL), and the resulting mixture was cooled at 0 °C. KOH (0.954 g, 17.04 mmol) dissolved in 50% aqueous ethanol (50 mL) was added to the reaction mixture dropwise. The reaction mixture was stirred at room temperature for 4 h, after which it was heated at 50 °C for 2 h. Ethanol was evaporated, and the reaction mixture was extracted with Et_2O 4−5 times. The organic layers were collected together and dried over anhydrous Na_2SO_4. A colorless gumlike material was obtained after evaporation of the solvent. The required compound ($R_f \approx 0.3$ in an EtOAc/hexane mixture (3:2)) was purified by column chromatography using silica gel. A white solid compound (2.1 g, 41%) was isolated. FT-IR (Nujol): 3340 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, terminal $-CH_3$, 3H), 1.15−1.22 (br m, $(-CH_2-)_{13}$, 26H), 2.43 (br s, $-C(CH_2OH)_3$, 3H), 3.72 (*s*, $-C(CH_2OH)_3$, 6H). LR-MS: *m/z* 303 $[M + H]^+$.

2,2-Bis(methyl bromoethanoate)hexadecyl Bromoethanoate (10). 9 (0.302 g, 1.22 mmol) was dissolved in CH_2Cl_2 (7.0 mL). DMAP (0.089 g, 0.73 mmol) and Et_3N (0.66 mL, 4.76 mmol) were added, and the reaction mixture was cooled at 0 °C. Bromoacetyl bromide (0.38 mL, 4.4 mmol) was then added dropwise at this temperature. The ice bath was removed after 10 min, and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was diluted with $CHCl_3$ (30 mL) and successively washed with dilute HCl solution (2 M, 10 mL) and with water (10 mL). Finally it was washed carefully with $NaHCO_3$ (0.5 M, 10 mL) and saturated brine solution. The organic layer was separated and dried over anhydrous Na_2SO_4. The desired product was isolated by column chromatography over silica gel using an EtOAc−hexane solvent mixture. A colorless liquid ($R_f \approx 0.4$ in an EtOAc/hexane (8:92) solvent mixture) was obtained (0.43 g, 53%). FT-IR (neat): 1741 cm^{-1} (br C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, 3H), 1.26 (br m, $(-CH_2-)_{12}$, 24H), 1.47 (br m, $-CH_2C-$, 2H), 3.85 (s, $(-COCH_2-)_3$, 6H), 4.2 (s, $(-CH_2O-)_3$, 6H). MALDI-TOF: *m/z* 689.7 $[M + H]^+$.

2,2-Bis(methyl *N*-ethanoate *N,N,N*-trimethylammonium bromide)hexadecyl *N*-Ethanoate *N,N,N*-Trimethylammonium bromide (BETAB, T3). Dry NMe_3 gas was passed into dry acetone (2.0 mL) in a screw-top pressure tube at 0 °C till the volume of the resulting solution was 10 mL. Then the corresponding bromide **10** (0.28 g, 0.42 mmol) was dissolved in dry acetone (2.0 mL) and added to the pressure tube at 0 °C. The reaction mixture was allowed to stir at room temperature for 12 h. During the reaction, a gummy solid was formed which stacks to the walls of the pressure tube. $CHCl_3$ was added to the reaction mixture, and the resulting mixture was transferred into a round-bottom flask. First, NMe_3 was removed by heating with hot water, and then the solvent was evaporated. Final compound **T3** (65%) was purified by repetitive precipitations from a $CHCl_3$/EtOAc/*n*-hexane mixture. The compound was found to be an extremely hygroscopic solid. FTIR (KBr): 1751 cm^{-1} (C═O str). ^1H NMR (300 MH_Z, $CDCl_3$): δ 0.88 (t, terminal $-CH_3$, −3H), 1.26 (br s, $(-CH_2-)_{13}$, 26H), 3.36 (s, $-^+N(CH_3)_3 \times 3$, 27H), 4.25 (s, $-CH_2O- \times 3$, 6H), 5.57 (*s*, $-CH_2N^{+-} \times 3$, 6H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ 14.085, 22.67, 22.92, 29.35, 29.53, 29.72, 30.35, 31.92, 32.29, 39.93, 44.82, 54.335, 62.55, 69.12, 165.26. ESI-MS: *m/z* M^{3+} $[C_{33}H_{68}N_3O_6]^{3+}$ $2Br^-$ (78.91), calcd 760.3, found 760.3; M^{3+} Br^- (78.91) Br^- (80.91), calcd 762.3, found 762.4; M^{3+} $2Br^-$ (80.91), calcd 764.3, found 764.3, (M^{3+} Br^- (78.91))/2, calcd 340.7; found 340.7; (M^{3+} Br^- (80.91))/2, calcd 341.7, found 341.6; M^{3+}/3, calcd 200.8, found 200.9. Anal. Calcd for $C_{33}H_{68}N_3O_6Br_3,H_2O$: C, 46.05; H, 8.2; N, 4.88. Found: C, 45.67; H, 8.05; N, 4.43.

2,2-Bis(methyl *N*-ethanoate pyridium bromide)hexadecyl *N*-Ethanoate Pyridinium Bromide (BEPB, P3). 10 (0.07 g, 0.105 mmol) was dissolved in dry acetone (4 mL), and the resulting mixture added to a screw-top pressure tube. Dry pyridine (1 mL) was added, and the mixture was heated at 50 °C for 48 h. A brown solid was formed on the wall of the pressure tube. The reaction mixture was cooled at room temperature, and solvent was decanted carefully from the pressure tube. The solid residue was dissolved in $CHCl_3$ and precipitated upon addition of *n*-hexane several times. During the process of precipitation the compound initially came out of the solution as a dense yellow liquid. The solvent could be separated from this by careful decantation to afford a sticky solid material, **P3** (62%). The compound was found to be an extremely hygroscopic solid. FT-IR (KBr): 1750 cm^{-1} (C═O str). ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, terminal $-CH_3$, 3H), 1.26 (br m, $(-CH_2-)_{13}$, 26H), 4.26 (*s*, $-CH_2O- \times 3$, 6H), 6.51 (s, $-COCH_2- \times 3$, 6H), 8.09 (t, *m-H*, 6H), 8.56 (t, *p-H*, 3H), 9.75 (d, $J = 5.4$ Hz, *o-H*, 6H). ^{13}C NMR (133.3 MHz, $CDCl_3$): δ 14.09, 22.71, 27.16, 27.32, 27.91, 28.09, 29.36, 29.72, 29.96, 31.94, 39.84, 42.26, 61.57, 61.79, 65.87, 67.46, 128.04, 146.37, 146.79, 165.99. ESI-MS: *m/z* M^{3+} $[C_{39}H_{56}N_3O_6]^{3+}$ $2Br^-$ (78.91), calcd 820.7, found 820.6; M^{3+} Br^- (80.91) Br^- (78.91), calcd 822.6, found 822.3; M^{3+} $2Br^-$ (80.91), calcd 824.7, found 824.2; (M^{3+} Br^- (78.91))/2, calcd 370.8; found 370.8; (M^{3+} Br^- (80.91))/2, calcd 371.8, found 371.6; M^{3+}/3, calcd 220.9, found 220.9. Anal. Calcd for $C_{39}H_{56}N_3O_6Br_3 \cdot 3H_2O$: C, 48.9; H, 6.5; N, 4.4. Found: C, 48.7; H, 6.4; N, 4.5.

Microorganisms and Culture Conditions. The microbicidal activity of these new multiheaded amphiphiles was tested against both Gram-negative and Gram-positive bacteria. The Gram-negative bacteria examined in the present study were *E. coli* (DH5α) and *S. sonnei* (AK1). The Gram-positive bacteria were *S. aureus* (clinical isolate) and *E. faecalis* (clinical isolate). The LB medium (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl in 1000 mL of sterile distilled water (pH ≈ 7)) was used as a liquid medium. The LB agar (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, and 15 g of agar in 1000 mL of sterile distilled water) was used as a solid medium for all the experiments. The stock solution of the amphiphiles was made in autoclaved sterile water.

Freeze-dried ampules of *E. coli* were opened, and a loopful of culture was spread to give single colonies on LB agar and incubated at 37 °C for 24 h. A single colony was picked up with a sterile micropipet tip, placed in 10 mL of liquid LB medium, and inoculated with constant shaking at 37 °C for 10 h to give a working concentration of 10^8-10^9 cells/mL before every experiment.

A single colony of *S. sonnei* was picked up with a small tip, placed in 10 mL of liquid LB medium, and inoculated with constant shaking at 37 °C for 12 h to give a working concentration of 10^7-10^8 cells/mL before every experiment.

S. aureus and *E. faecalis* grown on one blood agar plate were used to isolate a single colony with a small tip, placed in 10 mL of liquid LB, and inoculated with constant shaking at 37 °C for 10 h for *S. aureus* to give a working concentration of 10^9-10^{10} cells/mL and 12 h for *E. faecalis* to give a working concentration of 10^7-10^8 cells/mL before every experiment.

MBC. A 100 μL sample of the overnight culture was added to 12−15 glass test tubes (autoclaved) with different amounts of liquid LB medium, and then different amounts of stock amphiphile solution were added such that the overall volume of the samples was 2 mL and the same amount of bacteria was present in every test tube. For control experiments, sterile water was added to one of the test tubes instead of amphiphile solution. Then all the tubes were inoculated at 37 °C for 5 h with constant shaking. After that each tube was diluted depending upon the requirement, and 100 μL was spread on an LB agar plate inside the laminar flow. The plates were incubated at 37 °C for 24 h before the colonies were counted. The counting was done three times every time. The MBC was taken as the lowest amphiphile concentration at which there was no colony present in the plate after 24 h of incubation at 37 °C.

Bacterial Killing Assays. Overnight cultures were diluted with liquid LB medium to give a working concentration of

10^8−10^{10} cells/mL. The amphiphile stock solution was added at higher than MBC values, and this suspension was incubated at room temperature inside the laminar flow. The concentration of **T1** and **P1** used was 50 μM, whereas 20 μM was used for all other amphiphiles. At regular intervals after amphiphile addition, 10 μL samples were removed every time, and kept at 4 °C. After the experiments, all the samples were diluted accordingly, and 100 μL was spread onto LB agar plates. Then the plates were incubated at 37 °C for 24 h, and the viable cells were counted. The control experiment was also performed where amphiphile was not added.

Acknowledgment. This work was supported by grants from the Department of Biotechnology and Chemical Biology Unit, JNCASR.

References

(1) Domagk, G. A new class of disinfectants. *Dtsch. Med. Wochenschr*. **1935**, *61*, 829−832.
(2) Hugo, W. B.; Russel, A. D. In *Types of antimicrobial agents, Principles and practice of disinfection, preservation*, 2nd ed.; Russel, A. D., Hugo, W. B., Ayliffe, G. A. J., Eds.; Blackwell Scientific Publications: Oxford, 1992; pp 7−68.
(3) Merianos, J. J. Quaternary ammonium antimicrobial compounds. In *Disinfection, sterilization, and preservation*, 4th ed.; Block, S. S., Ed.; Lea and Febiger: Philadelphia, 1991; pp 225−255.
(4) Ahlstrom, B.; Chelminska-Bertilsson, M.; Thompson, R. A.; Edebo. L. Submicellar complexes may initiate the fungaicidal effects of cationic amphiphilic compounds on Candida albicans. *Antimicrob. Agents Chemother.* **1997**, *41*, 544−550.
(5) Allenmark, S.; Lindstedt, M.; Edebo, L. Article and a prophylactic composition containing a microbicide. U.S. Pat. 5080902, 1992.
(6) Wong, Y.-L.; Hubieki, M. P.; Curfaman, C. L.; Doncel, G. F.; Dudding, T. C.; Savle, P. S.; Gandour, R. D. A structure-activity study of spermicidal and anti-HIV properties of hydroxylated cationic surfactants. *Bioorg. Med. Chem.* **2002**, *10*, 3599−3608.
(7) Bodor, N., Kaminski, J. K.; Selk, S. Soft drugs. 1. Labile quaternary ammonium salts as soft antimicrobials. *J. Med. Chem.* **1980**, *23*, 469−474.
(8) Linstedt, M.; Allenmark, S.; Thompson, R. A.; Edebo, L. Antimicrobial activity of betaine esters, quaternary ammonium amphiphiles which spontaneously hydrolyze into nontoxic components. *Antimicrob. Agents Chemother*. **1990**, *34*, 1949−1954.
(9) Ahlstrom, B.; Chelminska-Bertilsson, M.; Thompson, R. A.; Edebo, L. Long-chain alkanoylcholines, a new category of soft antimicrobial agents that are enzymatically degradable. *Antimicrob. Agents Chemother.* **1995**, *39*, 50−55.
(10) Thompson, R. A.; Allenmark, S. Effects of molecular association on the rates of hydrolysis of long-chain alkyl betaines (alkoxycarbonyl-N,N,N-trialkylmethanaminium halides. *Acta Chem. Scand.* **1989**, *43*, 690−693.
(11) Thompson, R. A.; Allenmark, S. Factors influencing the micellar catalyzed hydrolysis of long-chain betainates. *J. Colloid Interface Sci.* **1992**, *148*, 241−246.
(12) Allenmark, S.; Lindstedt, M.; Edebo, L. Prophylactic agent for controlling venereal diseases. Eur. Pat. 0298065A2, 1989.
(13) Haldar, J., V. K. Aswal, P. S. Goyal; S. Bhattacharya. Molecular modulation of surfactant aggregation in water: effect of the incorporation of multiple head groups on micellar properties. *Angew. Chem., Int. Ed.* **2001**, *40*, 1228−1232.
(14) Ikeda, T.; Yamaguchi, H.; Tazuke, S. New polymer biocides: synthesis and antibacterial activities of polycations with pendant biguanide groups. *Antimicrob. Agents Chemother.* **1984**, *26*, 139−144.
(15) Franklin, T. J., Snow, G. A., Eds. Antiseptics, antibiotics and the cell membrane. *Biochemistry of Antimicrobial Action*; Chapman and Hall, Ltd.: London, 1981; pp 58−78.
(16) Willemen, H. M.; de Smet, L. C. P. M.; Koudijs, A. Stuart, M. C. A.; Heikamp-de Jong, I. G. A. M.; Marcelis, A. T. M.; Sudhölter,E. J. R. Micelle formation and antimicrobial activity of cholic acid derivatives with three permanent ionic head groups. *Angew. Chem., Int. Ed.* **2002**, *41*, 4275−4277.
(17) Bernard, E.; Faucon, J. F.; Dufourcq, J. Phase separations induced by melittin in negatively-charged phospholipid bilayers as detected by florescence polarization and differential scanning calorimetry. *Biochim. Biophys. Acta* **1982**, *688*, 152−162.
(18) El Mashak, E. M.; Tocanna, J. F. Polymyxin B-phosphatidylglycerol interactions. A monolayer (π, ΔV) study. *Biochim. Biophys. Acta* **1980**, *596*, 165−179.
(19) Costerton, J. W.; Cheng, K. J. The role of the bacterial cell envelope in antibiotic resistance. *J. Antimicrob. Chemother*. **1975**, *1*, 363−377.
(20) Haldar, J., V. K. Aswal, P. S. Goyal; S. Bhattacharya. Aggregation Properties of Novel Cationic Surfactants with Multiple Pyridinium Head Groups. Small-Angle Neutron Scattering and Conductivity Studies. *J. Phys. Chem. B* **2004**, *108*, 11406−11411.
(21) Corey, E. J.; Suggs, J. W. Pyridinium chloro chromate. Efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, *31*, 2647−2650.
(22) Bhattacharya, S.; Haldar, S. Synthesis of novel cationic lipids with fully or partially non-scissile linkages between the hydrocarbon chains and pseudoglycerol backbone. *Proc.—Indian Acad. Sci., Chem. Sci.* **2002**, *114*, 197−201.
(23) Newkome, G. R.; Yao, Z.; Baker, G. R.; Gupta, V. K. Micelles. Part 1. Cascade Molecules: A new approach to micelles. A[27]-Arborol. *J. Org. Chem.* **1985**, *50*, 2003−2004.

JM049106L