Synthesis, Surface-Active Properties, and Antimicrobial Activities of New Double-Chain Gemini Surfactants

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Abstract: A novel series of neutral and cationic dimeric surfactants were prepared involving ketalization reaction, Williamson etherification, and regioselective oxirane ring opening with primary and tertiary alkyl amines. The critical micelle concentration (CMC), effectiveness of surface tension reduction (γ_{CMC}), surface excess concentration (Γ), and area per molecule at the interface (A) were determined and values indicate that the cationic series is characterized by good surface-active and self-aggregation properties. For the first time, we reported the antimicrobial activities against representative bacteria and fungi for dimeric compounds. The antimicrobial activity was found to be dependent on the target microorganism (Gram-positive bacteria > fungi > Gram-negative bacteria), as well as both the neutral or ionic nature (cationic > neutral) and alkyl chain length (di-C₁₂ > di-C₁₈ > di-C₈) of the compounds. The cationic di-C₁₂ derivative was found to have equipotent activity to that of benzalkonium chloride (BAC) used as standard.

Key words: neutral dimeric compounds, cationic dimeric compounds, antimicrobial activity, surface-active properties, gemini surfactants

1 INTRODUCTION

Over the last two decades, the growing demand for high-performance surface-active agents has multiplied the interest of the academic and industrial communities for the development of new gemini surfactants¹⁻⁵. Their identical amphiphilic moieties connected by a spacer group impart them unique surface-active properties and self-assembly patterns, and also play a significant role in their antimicrobial performances^{6,7}. A brief review of the specific literature reveals that the studies enlarge upon the physicochemical properties, but the survey of the antimicrobial activities emerging thereof is less common. As a rule, gemini surfactants are more efficient in lowering surface tension and have much lower critical micelle concentration (CMC) than those of their monomeric counterparts.

A similar rule is usually invoked for their antimicrobial potencies. This singular combination of detergent-like structures with antimicrobial activities is among the key features that researchers look for disinfectant and sanitizer agents to be used in industrial and human health fields⁸⁻¹⁰. Thus, diverse articles focused on searching novel geminis with antibacterial and antifungal properties have lately been reported in the literature¹¹⁻¹⁴. Some contributions in this connection involve gemini compounds having new active moieties, such as heteroatoms^{15,16}, aromatic and non-aromatic cyclic substituents^{17,18}, or perfluorinated chains¹⁹⁻²³, among others. Nevertheless, it still remains an ample research area for synthesizing improved geminis efficiently and with low cost.

In this paper, we present in detail the preparation

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method, surface-active properties, and the antimicrobial activity of neutral and cationic series of new gemini compounds having a flexible, hydrophilic spacer and two alkyl chains of varying length. The molecular design and synthetic strategy were outlined to link the two hydrophilic head groups through a spacer having multiple oxygen atoms (1,3-dioxane ring, ether, and hydroxyl groups) conferring wettability properties. The structures of the synthesized compounds are illustrated in Scheme 1.

Scheme 1 Chemical Structure of the Studied Dimeric Neutral (a) and Cationic (b) Surfactants.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

All the chemicals used in synthetic procedures were of reagent grade quality and used as received.

2.2 Synthesis of 1,5-dioxaspiro[5.5]undecane-3,3-dimethanol 2

The reaction was carried out in a 100 mL three-neck round-bottomed flask equipped with a magnetic stirring bar and a Dean-Stark trap topped with a condenser^{24,25}. A catalytic amount of *p*-toluenesulfonic acid monohydrate (5% w/w relative to the cyclohexanone) was added to a pentaerythritol (2.995 g, 22 mmol) and benzene-dimethylformamide (40 : 60) mixture (50 mL) at room temperature. The well-stirred dispersion was warmed to 80°C until complete dissolution, and cyclohexanone (1.571 g, 15 mmol) was then added dropwise while the reaction mixture was heated to 115°C for 48 hours. The water formed during the reaction was removed by distillation and collected in the Dean-Stark trap. The reaction was stopped when there was no further increase in the collected water. After cooling, the reaction mixture was poured into water (30 mL), neutralized with potassium carbonate and three-fold extracted with dichloromethane (30 mL). The organic layer was dried with MgSO₄ and evaporated in vacuo. After Kugelrohr distillation and column chromatography on silica gel by eluting with ether-light petroleum (80 : 20) mixture, the compound 2 was obtained as a white solid in good

yield (90 %) and good selectivity (92 %). Physical data of 2: White solid. Melting point (m.p.) 123–124°C. IR (KBr): ν = 920.0, 1039.6, 1062.7, 1107.1, 1369.4, 2856.4, 2922.0, 3273.0 cm⁻¹. ¹H NMR [200 MHz, (CD₃)₂SO]: δ = 1.31–1.57 (m, 6H), 1.61–1.78 (m, 4H), 3.27–3.48 (m, 4H), 3.61 (s, 4H), 4.47 (t, 2H *J* = 6.5). ¹³C NMR [50 MHz, (CD₃)₂SO]: δ = 22.11 (CH₂-4'), 25.19 (CH₂-3' – CH₂-5'), 32.25 (CH₂-2' – CH₂-6'), 40.36 (C), 60.63, 60.79, 61.19 (CH₂O – CH₂OH), 96.88 (C-1'). MS: spectra *m/z* (% rel. int.): 216 (M⁺, 6), 187 (13), 173 (96), 160 (4), 125 (5), 101 (6), 99 (14), 83 (34), 71 (40), 55 (100), 41 (72). Anal. calcd. for C₁₁H₂₀O₄ (Mol. W. 216.278): C 61.08, H 9.32, O 29.59. Found: C 61.01, H 9.61, O 29.38.

2.3 Synthesis of 1,5-dioxaspiro[5.5]undecane-3,3-dimethyldiglycidyl ether 3

The Williamson etherification was carried out in solid-liquid reaction system with 2 and (±)-epichlorohydrin using tetrabutylammonium hydrogen sulfate (TBAB) as phase-transfer catalyst yielding the diglycidyl ether 3, as key intermediate^{26,27}. A 1 : 10 : 6 : 0.1 molar mixture of 2 (0.843 g, 3.90 mmol), (±)-epichlorohydrin (3.608 g, 39.0 mmol), NaOH (0.936 g, 23.40 mmol), and TBAB (0.132 g, 0.39 mmol), was heated at 30°C under vigorous stirring (700 rpm) during 1.5 hours. The solid material was filtered, washed with CH₂Cl₂ (150 mL) and the washing solvent evaporated to dryness. The residue was partitioned between Et₂O (2 × 150 mL) and brine (80 mL). The two organic layers were combined, dried over MgSO₄, filtered and the organic solvent evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with acetone/hexane (3 : 7) as eluants. The compound 3 was obtained as an oil pale yellow, in good yield (92 %) and excellent selectivity (98.9 %). Physical data of 3: Oil pale yellow. IR (KBr): ν = 1100, 1460.0, 2980.0 cm⁻¹. ¹H NMR [200 MHz, (CDCl₃)]: δ = 1.44–1.51 (m, 10H), 2.45–2.60 (m, 4H), 2.96 (m, 2H), 3.46–3.64 (m, 4H), 3.80 (s, 4H), 4.13 (m, 2H), 4.23 (m, 2H). ¹³C NMR [50 MHz, (CDCl₃)]: δ = 22.00 (CH₂-4'), 24.26 (CH₂-3' – CH₂-5'), 33.22 (CH₂-2' – CH₂-6'), 41.58 (C), 44.73 (CHCH₂O), 49.91 (CH), 63.19 (CCH₂O), 86.08 (CHCH₂O), 99.28 (C-1'). MS: spectra *m/z* (% rel. int.) = 328 (M⁺, 3), 299 (4), 285 (12), 113 (10), 83 (42), 55 (95), 31 (100). Anal. calcd. for C₁₇H₂₈O₆ (Mol. W. 328.406): C, 62.17; H, 8.59; O, 29.24. Found: C, 62.28; H, 8.51; O, 29.21.

2.4. Synthesis of Bis-alkylamine compounds (4a-c)

A 2.5 : 1 : 0.1 molar mixture of alkylamine (octylamine 0.494 g; dodecylamine 0.708 g; octadecylamine 1.022 g; i.e. 3.825 mmol), diglycidyl ether 2 (0.331 g, 1.530 mmol) and TBAB (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 20 hours at 30°C. Then, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel with CH₂Cl₂/EtOH (1 : 1) as eluants. The above-mentioned general procedure gave 4a-c in 55, 65,

and 51% yields, respectively. Physical data of synthesized products are as follows.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-octylamino-propan-2-ol) 4a: White waxy product. IR (KBr): $\nu = 1100.0, 2950.0, 3640.0 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.88\text{--}0.92$ (t, 6H $J = 6.6$), 1.27–1.35 (m, 24H), 1.37–1.61 (m, 10H), 2.57–2.72 (m, 8H), 2.76–2.80 (m, 4H), 3.09–3.41 (m, 4H), 3.76–3.85 (m, 4H), 3.87–3.91 (m, 2H), 4.10 (s, 2H), 4.20 (s, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.20, 22.00, 23.10, 24.26, 27.41, 29.30, 29.80, 30.35, 32.35, 34.22, 41.11, 48.84, 52.45, 62.88, 63.07, 69.41, 74.04, 98.28$. Anal. calcd. for $\text{C}_{33}\text{H}_{66}\text{N}_2\text{O}_6$ (Mol. W. 586.887): C, 67.51; H, 11.36; N, 4.74; O, 16.39. Found: C, 67.53; H, 11.33; N, 4.77; O, 16.37.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-dodecylamino-propan-2-ol) 4b: White waxy product. IR (KBr): $\nu = 1110.0, 2955.0, 3640.1 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.80\text{--}0.92$ (t, 6H $J = 6.4$), 1.22–1.33 (m, 40H), 1.37–1.60 (m, 10H), 2.57–2.72 (m, 8H), 2.76–2.80 (t, 4H $J = 7.05$), 3.08–3.41 (m, 4H), 3.77–3.84 (m, 4H), 3.87–3.90 (m, 2H), 4.10 (s, 2H), 4.20 (s, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.10, 22.00, 22.69, 24.26, 27.18, 29.39, 29.59, 29.62, 29.63, 29.65, 29.73, 29.73, 29.76, 31.24, 34.22, 41.11, 48.84, 52.45, 62.88, 63.07, 69.41, 74.04, 98.28$. Anal. calcd. for $\text{C}_{41}\text{H}_{82}\text{N}_2\text{O}_6$ (Mol. W. 699.100): C, 70.41; H, 11.83; N, 3.98; O, 13.78. Found: C, 70.44; H, 11.82; N, 4.00; O, 13.74.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-octadecylamino-propan-2-ol) 4c: Oil pale yellow. IR (KBr): $\nu = 1090.3, 2956.0, 3644.1 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.86\text{--}0.94$ (t, 6H $J = 6.8$), 1.19–1.41 (m, 52H), 1.42–1.59 (m, 10H), 1.94–2.01 (m, 8H), 2.57–2.71 (m, 8H), 2.76–2.80 (m, 4H), 3.09–3.41 (m, 4H), 3.77–3.84 (m, 4H), 3.87–3.91 (m, 2H), 4.15 (s, 2H), 4.20 (s, 2H), 5.22–5.28 (m, 4H). ^{13}C NMR [50 MHz]: $\delta = 13.95, 22.57, 24.26, 27.18, 29.41, 29.73, 29.76, 31.60, 31.99, 34.22, 41.11, 48.84, 52.45, 63.07, 69.41, 74.04, 98.28, 130.70$. Anal. calcd. for $\text{C}_{53}\text{H}_{102}\text{N}_2\text{O}_6$ (Mol. W. 863.387): C, 73.71; H, 11.91; N, 3.22; O, 11.16. Found: C, 73.73; H, 11.90; N, 3.24; O, 11.13.

2.5 Synthesis of Bis-alkyldimethylammonium salts (5a-c)

A 2.5 : 1 : 0.1 molar mixture of *N,N*-dimethylalkylamine (*N,N*-dimethyloctylamine 0.601 g, *N,N*-dimethyldodecylamine 0.815 g, *N,N*-dimethyloctadecylamine 1.129 g; *i.e.*, 3.825 mmol), diglycidyl ether **2** (0.331 g, 1.530 mmol) and TBAB (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 20 hours at 30°C. Then, the solvent was evaporated under reduced pressure at room temperature, and the residue was purified by column chromatography using a two-step procedure to afford the desired product **5a**. The unreacted tertiary amine was removed from the residue using ethyl acetate/acetone/ NH_4OH (4 : 1 : 0.5) solvent system as eluant. This procedure

was repeated using $\text{CH}_2\text{Cl}_2/\text{EtOH}$ (1 : 1) as eluants, and the eluates containing the desired products were evaporated under reduced pressure. Further addition of the HCl solution provides the corresponding counterions. The above-mentioned general procedure gave **5a-c** in 95, 97, and 61% yields, respectively. Physical data of synthesized products are as follows.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-octyldimethylammonio-propan-2-ol) dichloride 5a: White waxy product. IR (KBr): $\nu = 1091.0, 2950.0, 3640.0 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.83\text{--}0.93$ (m, 10H), 1.27–1.37 (m, 16H), 1.39–1.54 (m, 10H), 1.92–1.99 (m, 4H), 3.12–3.16 (m, 6H), 3.32 (s, 12H), 3.34–3.41 (m, 4H), 3.82–4.05 (m, 6H), 4.13 (s, 2H), 4.23 (s, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.07, 22.00, 22.68, 24.13, 26.31, 27.19, 28.46, 31.73, 34.22, 41.11, 59.43, 60.92, 63.07, 66.93, 68.57, 71.20, 98.28$. Anal. calcd. for $\text{C}_{37}\text{H}_{76}\text{Cl}_2\text{N}_2\text{O}_6$ (Mol. W. 715.915): C, 62.05; H, 10.73; Cl, 9.89; N, 3.89; O, 13.44. Found: C, 62.07; H, 10.70; Cl, 9.90; N, 3.91; O, 13.42.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-dodecyldimethylammonio-propan-2-ol) dichloride 5b: White waxy product. IR (KBr): $\nu = 1110.0, 2950.0, 3640.0 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.83\text{--}0.91$ (m, 10H), 1.21–1.40 (m, 32H), 1.37–1.60 (m, 10H), 1.95–2.01 (m, 4H), 3.12–3.16 (m, 6H), 3.32 (s, 12H), 3.33–3.41 (m, 4H), 3.77–4.05 (m, 8H), 4.13 (s, 2H), 4.23 (s, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.04, 22.00, 22.62, 23.79, 24.26, 26.08, 26.87, 29.37, 29.45, 29.61, 29.62, 31.68, 34.22, 41.11, 59.43, 60.92, 63.50, 66.93, 68.57, 71.20, 98.27$. Anal. calcd. for $\text{C}_{45}\text{H}_{92}\text{Cl}_2\text{N}_2\text{O}_6$ (Mol. W. 828.128): C, 65.27; H, 11.18; Cl, 8.55; N, 3.36; O, 11.64. Found: C, 65.26; H, 11.19; Cl, 8.56; N, 3.38; O, 11.61.

1,5-dioxaspiro[5.5]undecane-3,3-Bis(1-methoxy-3-octadecyldimethylammonio-propan-2-ol) dichloride 5c: White waxy product. IR (KBr): $\nu = 1085.9, 2940.0, 3640.0 \text{ cm}^{-1}$. ^1H NMR [200 MHz, (CDCl_3): $\delta = 0.85\text{--}0.94$ (m, 10H), 1.25–1.32 (m, 40H), 1.39–1.60 (m, 10H), 1.94–1.98 (m, 4H), 3.12–3.16 (m, 6H), 3.32 (s, 12H), 3.34–3.40 (m, 4H), 3.79–4.05 (m, 6H), 4.13 (s, 2H), 4.23 (s, 2H), 5.29–5.32 (m, 4H). ^{13}C NMR [50 MHz]: $\delta = 13.95, 22.00, 22.57, 23.79, 26.08, 27.88, 29.42, 29.69, 29.76, 31.60, 31.99, 34.22, 41.11, 59.43, 60.92, 63.07, 66.93, 68.57, 71.20, 98.28, 130.70$. Anal. calcd. for $\text{C}_{57}\text{H}_{112}\text{Cl}_2\text{N}_2\text{O}_6$ (Mol. W. 992.415): C, 68.95; H, 11.39; Cl, 7.17; N, 2.80; O, 9.69. Found: C, 68.98; H, 11.37; Cl, 7.14; N, 2.82; O, 9.69.

2.6 Analytical methods

Melting points (m.p.) were determined on a Büchi 510 micro melting point apparatus and were not corrected. Infrared (IR) spectra were recorded on a Shimadzu 8201 PC spectrophotometer; ^1H and ^{13}C NMR spectra on a Bruker FT-200 spectrometer, using $(\text{CD}_3)_2\text{SO}$ and CDCl_3 as solvent.

Chemical shifts (δ) were reported in ppm related to internal tetramethylsilane. Mass spectra (MS) were performed on a Shimadzu GCMS-QP 5000 spectrometer. Elemental analyses were performed at the Galbraith Laboratories, Inc., Knoxville, United States. Gas-liquid chromatographic (GLC) analyses were performed on a Shimadzu GC-17AATF chromatograph equipped with a methyl silicone capillary column (30 m $\times$ 0.32 mm, 0.25 μ m film thickness) and flame ionization detector. Column chromatography was performed on silica gel (70-230 mesh ASTM). Isolated and authenticated compounds were used as internal standard to perform quantitative GC analyses.

The surface tension of aqueous solutions (pH7) was measured at 20°C using a semiautomatic tension-meter apparatus (Cole-Parmer Surface Tensiomat 21) by the Du Nouy ring method. Calibration was performed against a range of standard liquids obtaining an excellent agreement with the reference values. A time-dependent surface tension behavior was observed by an increase of the experimental values over successive measurements at each concentration. This behavior has been related to the difficulties of gemini in organizing at the air/water interface. The surface tension was then measured three times for each sample within 40 min interval between each reading to ensure equilibrium data. The critical micelle concentration (CMC) values were determined using a series of aqueous solutions at various concentrations, and estimated from the break point of each surface tension vs. concentration (on log scale) curves. The ability of these compounds to lower surface tension at the CMC (γ_{CMC}) and reduced by 20 mN/m (C_{20} or pC_{20}) were calculated therefrom. The optimal cross-section surface area A occupied by the surfactant headgroup at the air/water interface was estimated from the surface excess concentration Γ . Results are summarized in Table 1.

2.7 Antimicrobial activity

The antimicrobial activity of the new compounds was tested against reference strains of Gram-positive (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 6538, and *Listeria monocy-*

togenes ATCC 15313) and Gram-negative (*Escherichia coli* ATCC 11105, *Pseudomonas aeruginosa* ATCC 9027) bacteria, and fungi (*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404). Antibacterial and antifungal activities were evaluated using a protocol adapted from the European²⁸⁾ and ASTM norms²⁹⁾. The antimicrobial activity was assessed by using the filter paper disk diffusion method, and by measuring the minimum inhibitory concentration (MIC) in two antibiotic media. Initial screening for antibacterial and antifungal activity was performed by impregnating 6 mm sterile filter paper disks with 100 μ g of each substance diluted in ethanol and dried at 37°C. Benzalkonium chloride (BAC) (Fluka) was used as reference drug for positive control and ethanol was used as negative control. Although the BAC is a benzyl ammonium compound, which does not have bis-amino alcohols or bis-quaternary ammonium groups, it was chosen as standard because it is a common antiseptic agent. Disks were distributed on the surface of dried agar plate inoculated with the microorganisms at approximately 10⁶ cfu/mL, using different antibiotic media: AM1 (pH 6.6) (Difco), AM11 (pH 7.9) (Difco), and Sabouraud dextrose agar (only for fungal) (Difco). Plates were incubated at 37°C for 24 hs. The presence of an absolute inhibition zone around the disk was used as a criterion for the definition of active compound. The test was performed twice. Results, expressed as the diameter (in mm) of inhibition zones, are summarized in Table 2. All prepared compounds were also assayed on a quantitative basis determining the MICs for each microorganism, and BAC was used as positive control. Each compound was dissolved and serially diluted with distilled water. Dilutions were incorporated in melted antibiotic medium 1 (pH 6.6) and 11 (pH 7.9) (Difco), homogenized and poured into 90 mm Petri dishes. When dry, each plate was inoculated in duplicate with approximately 10⁴ cfu/spot by means of a Steers multi-inoculator. The MIC was defined as the lowest concentration of the tested sample at which the microorganism colonies were not visible to the naked eye after 24 h of incubation at 37°C. MICs values (μ mol/L) are summarized in Table 3.

Table 1 Surface Activity and Aggregation Data of Synthesized Dimeric Cationic Compounds (#).

Compound	CMC (mM)	C_{20} (mM)	γ_{CMC} (mN/m)	pC_{20}	CMC/ C_{20}	$\Gamma \times 10^6$ (mol/m ²)	$A \times 10^{20}$ (m ²)
5a	3.61	0.568	38.98	3.24	6.35	2.62	63.27
5b	1.54	0.146	35.93	3.84	10.55	2.59	64.17
5c	0.38	0.038	34.61	4.42	10.0	2.87	57.82

(#) Experimental conditions: Temperature 20 $\pm$ 0.5°C. Aqueous solution at pH 7. Experimental uncertainties are estimated to be $\pm$ 0.03 mM on CMC and $\pm$ 1 mN/m on γ_{CMC} values.

Table 2 Antibacterial and Antifungal *in vitro* Activity of Six Synthesized Surfactants Using the Disc-diffusion Method.^a

Microorganism	Compound ^b													
	Medium AM1							Medium AM11						
	4a	4b	4c	5a	5b	5c	BAC ^c	4a	4b	4c	5a	5b	5c	BAC ^c
<i>E. coli</i>	-	-	-	-	++	-	++	-	-	-	-	+	-	+
<i>P. aeruginosa</i>	-	-	-	-	+	-	+	-	-	-	-	+	-	+
<i>M. luteus</i>	-	+	+	-	+++	++	++	-	-	++	+	+++	++	++
<i>S. Aureus</i>	-	+	-	-	++	+	++	-	-	-	+	++	+	++
<i>B. subtilis</i>	-	+	-	-	+++	+	+++	-	-	+	+	+++	+	+++
<i>L. monocytogenes</i>	-	-	-	-	++	+	++	-	-	-	-	+++	+	++
<i>C. albicans</i>	-	+	-	-	++	+	++	-	-	-	-	++	+	++
<i>A. niger</i>	-	-	+	-	++	+	++	-	-	+	-	+++	+	+++

^a Zones of growth inhibition in: - : 0-6 mm; + : 7-12 mm; ++: 13-20 mm; +++ > 21 mm.^b For structures of compounds, see **Scheme 2**.^c Benzalkonium chloride (BAC).**Table 3** Antibacterial and Antifungal *in vitro* Activity Expressed as MIC of the Dimeric-C₁₂ and C₁₈ Cationic Compounds in Two Antibiotic Medium.^a

Microorganism	MIC of compounds ($\mu\text{mol/L}$) ^b					
	Medium AM1			Medium AM11		
	5b	5c	BAC ^c	5b	5c	BAC ^c
<i>E. coli</i>	38	1032	28	19	1032	28
<i>P. aeruginosa</i>	154	1032	14	154	1032	28
<i>M. luteus</i>	4	16	14	4	64	4
<i>S. Aureus</i>	14	128	14	14	64	4
<i>B. subtilis</i>	38	64	14	38	64	28
<i>L. monocytogenes</i>	38	64	14	38	64	28
<i>C. albicans</i>	38	128	28	38	64	14
<i>A. niger</i>	19	128	28	19	128	4

^a Minimum Inhibitory Concentrations (MIC) were expressed in $\mu\text{mol/L}$.^b The MIC was defined as the lowest concentration of the tested sample at which the microorganism colonies were not visible to the naked eye after 24 h of incubation at 37°C.^c Benzalkonium chloride (BAC).

3 RESULTS AND DISCUSSION

3.1 Synthesis

The new series of the neutral and cationic dimeric surfactants were easily prepared following the three-step procedure outlined in **Scheme 2**. The intermediate compound **2** was obtained by ketalization of the pentaerythritol **1** with cyclohexanone, with a benzene-DMF mixture as solvent and in the presence of *p*-toluenesulfonic acid monohydrate as homogeneous catalyst (pathway a). The diglycidyl ether

3 was obtained by etherification of **2** with ($\pm$)-epichlorohydrin via solid-liquid phase-transfer catalysis using tetrabutylammonium bromide (TBAB) as catalyst (pathway b). The β -hydroxy amino or ammonium alkyl functionalization was performed by regioselective oxirane ring opening with either primary amines to obtain the neutral compounds **4a-c** (pathway c) or tertiary amines to prepare the cationic compounds **5a-c** (pathway d). The nucleophilic substitutions involving tertiary amines were assisted by TBAB as

Scheme 2 Synthetic Pathways to Neutral and Cationic Dimeric Compounds.

(a) Pentaerythritol (1), cyclohexanone, *p*-toluenesulfonic acid monohydrate, benzene-DMF (40:60), 115°C, 48 h; (b) Diol (2), ($\pm$)-epichlorohydrine, NaOH, TBAB, 30°C, 1.5 h; (c) Diglycidyl ether (3), primary alkylamine, TBAB, ethanol, 30°C, 20 h; (d) 3, *N,N*-dimethylalkylamine, TBAB, ethanol, 30°C, 20 h.

phase transfer catalyst. Experimental details and spectral data are given in the following section.

3.2. Surface properties

The solubility in water of the cationic compounds largely exceeded that of the neutral homologues. For instance, the solubility of compound **5b** in water at 20°C was found to be nearly six hundred times that of neutral compound **4b**. Regarding tensioactive and self-aggregation properties, neutral compounds **4a-c** are able to remain dispersed in a stable state but its poor solubility prevented a reliable determination of their tensioactive properties and, therefore, these were not measured. However, cationic compounds **5a-c** exhibited clear solutions and displayed a sharp break of the surface tension vs concentration (on log scale) curves and a final plateau indicating a well-defined CMC and surface tension at the CMC (γ_{CMC}), as shown in Fig. 1. The surface excess, Γ , at the air-water interface was calculated by applying the Gibbs adsorption isotherm equation. The area per molecule at the interface was estimated from the corresponding value of Γ . Table 1 summarizes the CMC, C_{20} , γ_{CMC} , pC_{20} , CMC/ C_{20} , Γ , and A values, which are in agreement with those characterizing the proper quantitative behavior of good surfactants. A very good linear relationship between the CMC and the number of carbon atoms (N) in the alkyl chain of homologues compounds was found for the cationic series: $\log \text{CMC} = -1.655 - 0.097 N$ ($R^2=0.999$). The pC_{20} values indicate that the three-cationic compounds are good surfactants, and the CMC/ C_{20} values show a slightly greater capability of compound **5b** to be adsorbed at the interfaces. The surface excess Γ and the area per molecule A practically do not

Fig. 1 Surface Tension vs. Logarithm of the Aqueous Molar Concentration (log C) of Synthesized Dimeric Cationic Compounds at pH 7 and 20°C.

vary for the compounds of the cationic series. Indeed, the area per molecule at air/water interface of headgroup in the surfactants of the cationic series was found to be within a very close range of 57.82–64.17 Å², showing a somewhat smaller area per molecule with increasing tail length. This would be attributable to the flexibility of the spacing group and stronger intermolecular van der Waals forces at increasing chain lengths. These relatively lower head-group surface areas can lead to a more close-packed arrangement yielding a more effective self-assembling. The described behavior is consistent with most of the results reported in the literature for oligomeric surfactants and

indicates that all three cationic dimeric compounds satisfy the primary requirement of being good surfactants.

3.3 Antimicrobial activity

The antimicrobial activity of dimeric compounds **4a-c** and **5a-c** on various prokaryotic and eukaryotic microorganisms was evaluated by the disk-diffusion assay (Table 2) and determination of the minimum inhibitory concentration (MIC, in units of $\mu\text{mol/L}$) (Table 3). For comparison, the MICs of a commercially available germicide, benzalkonium chloride (BAC), were also determined under the same experimental conditions.

Table 2 summarizes the results of the disk-diffusion assay. Unexpectedly, the neutral **4a**, **4b**, and **4c**, and cationic **5a** derivatives did not exhibit appreciable *in vitro* antimicrobial activity over all tested microorganisms. However, the cationic **5b** and **5c** derivatives were found to have activity against Gram-positive bacteria – *M. luteus*, *S. aureus*, *B. subtilis*, and *L. monocytogenes* – and fungi – *C. albicans* and *A. niger* –, but a moderate activity against Gram-negative bacteria – *E. coli* and *P. aeruginosa* –. As expected, the Gram-negative bacteria were less sensitive than the Gram-positive bacteria because their outer membranes are less permeable to amphiphilic compounds. The biological activity of the neutral compounds was somewhat lower than their cationic homologues, and it may be attributable to the high density of negative charges that teichonic acids confer to Gram-positive bacterial membranes. Differences between the inhibition zones in AM1 and AM11 media are also observable for compound **5b** against fungi *A. niger*, which is known to be less adapted for growing in alkaline media. We attribute the good performance of di- C_{12} and di- C_{18} cationic compounds to its relative higher preference towards adsorption at the interfaces (see CMC/C_{20} values in Table 1) yielding a higher concentration at the bacterial cell wall, and an optimum balance between the hydrophilic and lipophilic regions to disrupt cell membranes of Gram-positive bacteria and fungi.

Table 3 shows the MICs values ($\mu\text{mol/L}$) for the compounds **5b** and **5c** exhibiting the best performance in the preliminary screening. MICs values corroborated an antimicrobial potency dependent on the target microorganism (Gram-positive bacteria > fungi > Gram-negative bacteria), as well as both the neutral or ionic nature (cationic > neutral). Gram-negative bacteria were inhibited at the MIC levels greater than $154 \mu\text{mol/L}$ (AM1 and AM11). The compound **5b** exhibited a good antibacterial potency against *M. luteus* with MIC values of $4 \mu\text{mol/L}$ (AM1 and AM11), and against *S. aureus* with MIC values of $14 \mu\text{mol/L}$ (AM1 and AM11). This compound was also active against *A. niger* with MIC values of $19 \mu\text{mol/L}$ (AM1 and AM11).

4 CONCLUSIONS

Neutral and cationic series of new dimeric β -hydroxy amino or ammonium surfactants were synthesized using a facile method, which affords good-to-excellent yields. The synthesized compounds were obtained in high purity by a simple purification procedure on column chromatography. CMC , γ_{CMC} , C_{20} , pC_{20} , CMC/C_{20} , Γ , and A values indicate that the cationic series is characterized by good surface-active and self-aggregation properties. For the first time, we reported the antimicrobial activities against representative bacteria and fungi for dimeric compounds. The antimicrobial potency was found to be dependent on the target microorganism (Gram-positive bacteria > fungi > Gram-negative bacteria), as well as both the neutral or ionic nature (cationic > neutral) and alkyl chain length (di- C_{12} > di- C_{18} > di- C_8) of the compounds. All neutral compounds and di- C_8 cationic compounds were found to be almost inactive. The cationic di- C_{12} compounds demonstrated to be the most potent antimicrobial surface-active agents from the synthesized series, and was found to be comparable to BAC against Gram-positive bacteria and fungi *in vitro*. The promising results shown by the di- C_{12} cationic compound have prompted further research. These studies will be reported in due course.

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