J Surfact Deterg (2008) 11:223–230
DOI 10.1007/s11743-008-1076-4

ORIGINAL ARTICLE

Synthesis and Properties of Novel Antifungal Gemini Compounds Derived from *N*-Acetyl Diethanolamines

Marcelo C. Murguía · Laura M. Machuca · María C. Lurá · María I. Cabrera · Ricardo J. Grau

Received: 9 January 2008 / Accepted: 30 April 2008 / Published online: 11 July 2008

Abstract A series of new N-acetylated non-ionic and cationic gemini surfactants (**3a–f**) having dimeric structures derived from primary and tertiary amines with variably long tails (C_8–C_{12}–C_{18}) were synthesized. In addition, N-acetylated monomeric analogues **6a** and **6b** were prepared and their antifungal potency and surface properties were also determined. Critical micelle concentration (CMC), effectiveness of surface tension reduction (γ_{CMC}), surface excess concentration (Γ), and area per molecule at the interface (A) were also determined and the resulting values indicate that the cationic series is characterized by good surface-active and self-aggregation properties. For the first time, all surfactants were tested to evaluate their antifungal properties using the method for the broth macrodilution test (M27-A2, NCCLS). Four microbial strains were used to perform the study: *Candida parapsilosis* (ATCC 22019), *C. albicans* (ATCC 64548), and a wild-type strain of *C. parasilosis* and *Saccharomyces cerevisiae* (ATCC 9763). The antimicrobial activity was measured by yeast growth inhibition expressed as minimum inhibitory concentration (MIC) values. Results were compared to those obtained for their monomeric analogues and for a commercially available reference compound (Fluconazole). Gemini **3b**, **3e** and **3f** were found to be the most potent compounds. The results show *S. cerevisiae* as the most sensitive strain. In contrast, the wild strain of *C. parapsilosis* was resistant.

Keywords Non-ionic dimeric compounds · Cationic dimeric compounds · Antifungal activity · Surface-active properties · Gemini surfactants

M. C. Murguía · L. M. Machuca · M. I. Cabrera · R. J. Grau (✉)
Laboratorio de Química Fina. Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Universidad Nacional del Litoral (U.N.L.) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Parque Tecnológico del Litoral Centro, Ruta Nacional 168, Paraje El Pozo 3000 Santa Fe, Argentina
e-mail: cqfina@santafe-conicet.gov.ar

M. C. Murguía
e-mail: mmurguia@santafe-conicet.gov.ar

M. C. Lurá
Cátedra de Microbiología General. Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral (U.N.L.). Ciudad Universitaria, Ruta Nacional 168, Paraje El Pozo 3000 Santa Fe, Argentina

Introduction

The general denomination of antifungals includes numerous substances with different chemical structures and action modes. However, the progressive incidence of the infections caused by fungi as well as the detection of more resistant strains stress the need to go deeper into the development of novel antifungals with appreciable advantages over those already known.

In the field of applied organic chemistry, the production of amphiphile molecules with new and interesting properties has increased in the last decade [1–11]. Surfactants are included among some of the most versatile chemical substances, and are found in fine chemicals and specialties such as drugs, shampoos, and cosmetics [3]. Surfactants have an amphipathic structure with hydrophobic moieties connected to polar or hydrophilic groups. The nature of polar groups has allowed their classification into anionic, cationic, zwitterionic, and non-ionic compounds. According to the number of hydrophobic tails and their geometric arrangement, there are different types of surfactants [1].

A conventional surfactant has a single hydrocarbon tail connected to an ionic or polar head group. Distinctively, gemini compounds have a symmetrically arranged geometric structure possessing, in sequence, a long hydrocarbon chain, an ionic group, a rigid or flexible spacer, a second ionic group, and another hydrocarbon tail [2]. These compounds may cause disruption of the membrane of microorganisms or inhibition of enzymes essential to their growth [12–27]. Moreover, these compounds have an excellent biodegradability and low toxicity [5–7].

According to these properties, we have synthesized new N-acetylated gemini compounds having dimeric structures derived from primary and tertiary amines with variable long tails (C_8–C_{12}–C_{18}). The synthesized compounds (**3a–f**) have a molecular structure able to interact with the fungi membrane and to promote their free entrance to the cell wall. Non-ionic compounds (**3a–c**) have a β-hydroxy amino group, which can inhibit enzymes important for the life of microorganisms such as glycosyl transferase [25, 27]. Cationic compounds (**3d–f**) have two positive charges derived from bis-quaternary ammonium salts favouring their adsorption at the cell surface and the interaction with the opposite charges of the cell membrane [25–28]. For example, two synthesized gemini compounds are shown in Fig. 1. In this contribution, the synthesis procedures and chemical characterization of the synthesized N-acetylated gemini surfactants are presented. Their tensioactive properties are determined and their antifungal activity is evaluated using broth dilution tests in comparison to a commercially available reference (Fluconazole) and N-acetylated monomeric analogues.

Experimental Procedures

Chemicals

All chemicals for the synthesis of compounds were reagent grade commercial materials and used without further purification. *N*-acetyl diethanolamine **1** and *N*-acetyl monoethanolamine **4** were synthesized by the reaction of di- and monoethanolamine with acetic anhydride (130 °C, 1.5 h) following a procedure previously reported [29]. Ethanol was freshly distilled over magnesium turnings to give absolute ethanol. Fluconazole (Merck) was used as a commercially available reference compound. The purity and chemical structure of the synthesized compounds were checked by TLC, HRMS, and NMR spectra.

Synthesis of *N*,*N*-Bis[2-(oxiran-2-yl) methoxyethyl]acetamide (**2**)

A 1:20:6:0.05 molar mixture of **1** (0.60 g, 4.0 mmol), (±)-epichlorohydrin (7.40 g, 80.0 mmol), NaOH (0.60 g, 24.0 mmol), and tetrabutylammonium hydrogen sulfate (TBAB) (Strem) (0.068 g, 0.20 mmol) was heated at 30 °C under vigorous stirring (700 rpm) for 5 h. The solid material was filtered, washed with CH_2Cl_2 (150 mL) and the washing solvent evaporated to dryness. The residue was partitioned between Et_2O (2 × 150 mL) and brine (80 mL). The organic phases obtained were combined, dried over $MgSO_4$, filtered, and the organic solvent evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with hexane/ethyl acetate (3:1) as eluants. Compound **2** was obtained in good yield (80%). Physical data of **2**: Oil pale yellow. IR (KBr): ν = 761.8, 804.3, 933.5, 1105.1, 1251.7, 1336.6, 1458.1, 1637.5, 2862.2, 2999.1 cm^{-1}. ^1H NMR [200 MHz, ($CDCl_3$)]: δ = 2.05 (s, 3H), 2.44–2.61 (m, 4H), 2.98–3.03 (m, 2H), 3.18–3.22 (dd, 2H J = 4.1, 3.9), 3.24–3.27 (m, 4H), 3.38–3.42 (dd, 2H J = 4.3, 2.7), 3.53–3.57 (t, 4H J = 6.5). ^{13}C NMR [50 MHz, ($CDCl_3$)]: δ = 20.71, 41.64, 44.64, 44.67, 53.96, 67.07, 71.90, 169.10. MS: spectra m/z (% rel. int.) = 260 (M^+, 2), 230 (1), 202 (8), 172 (4), 144 (3), 130 (60), 112 (5), 86 (15), 70 (10), 55 (90), 43 (100).

General Procedure for the Synthesis of Bis-alkylamine Compounds (**3a–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** (0.397 g, 1.53 mmol) and tetrabutyl ammonium bromide (TBABr) (Strem) (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 18 h at 30 °C. Then, the solvent was removed under reduced pressure, and purification was achieved via silica gel column chromatography using dichloromethane/ethanol (1:1) as eluants. The above-mentioned general procedure gave **3a–c** as

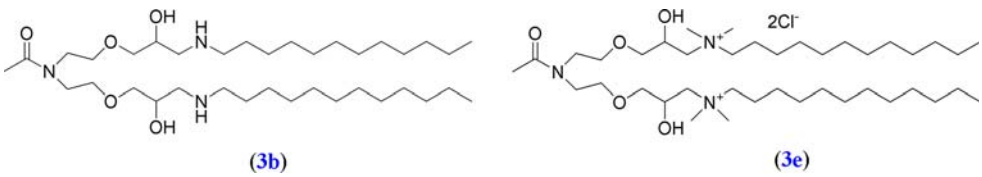


Fig. 1 Chemical structures of the dimeric non-ionic (**3b**) and cationic (**3e**) compounds under study

isolated pure compounds, in 40, 33, and 30%, respectively. The physical data of the synthesized products are as follows:

N,N-Bis[2-(3-octylamino-2-hydroxypropoxy)ethyl]acetamide (**3a**): Oil pale yellow. IR (KBr): $\nu = 760.0$, 930.2, 1120.0, 1460.3, 1620.0, 2850.1, 2930.4, 3450.1 cm^{-1}. ^1H NMR [200 MHz, ($CDCl_3$)]: $\delta = 0.90$ (t, 6H $J = 2.3$), 1.22–1.38 (m, 24H), 2.05 (s, 3H), 2.59–2.72 (m, 8H), 2.76–2.80 (t, 4H $J = 12$), 3.14–3.17 (m, 4H), 3.26 (t, 4H $J = 6$), 3.52 (t, 4H $J = 5$), 4.01–4.06 (m, 2H). ^{13}C NMR [50 MHz]: δ= 14.20, 20.70, 23.10, 27.41, 29.30, 29.80, 30.35, 32.35, 41.64, 44.64, 48.84, 52.45, 66.80, 71.21, 72.90, 169.10. FAB-HRMS: Calcd. for $C_{28}H_{59}N_3O_5$: 517.7963. Found: 517.7955.

N,N-Bis[2-(3-dodecylamino-2-hydroxypropoxy)ethyl] acetamide (**3b**): White waxy product. IR (KBr): $\nu = 760.4$, 930.1, 1120.1, 1460.3, 1620.0, 2850.7, 2930.0, 3450.1 cm^{-1}. ^1H NMR [200 MHz, ($CDCl_3$)]: $\delta = 0.94$ (t, 6H J = 2.7), 1.21–1.40 (m, 40H), 2.07 (s, 3H), 2.57–2.73 (m, 8H), 2.78 (t, 4H $J = 10$), 3.14–3.18 (m, 4H), 3.25 (t, 4H $J = 6$), 3.54 (t, 4H $J = 5$), 3.98–4.06 (m, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.18$, 20.70, 22.69, 27.18, 29.39, 29.62, 29.63, 29.65, 29.73, 29.76, 31.94, 41.64, 44.64, 48.84, 52.45, 66.80, 71.21, 72.90, 169.0. FAB-HRMS: Calcd. for $C_{36}H_{75}N_3O_5$: 630.0122. Found: 630.0118.

N,N-Bis[2-(3-octadecylamino–2-hydroxypropoxy)ethyl] acetamide (**3c**): White waxy product. IR (KBr): $\nu = 760.3$, 930.2, 1120.6, 1460.1, 1620.3, 2850.1, 2930.4, 3450.0 cm^{-1}. ^1H NMR [200 MHz, ($CDCl_3$)]: $\delta = 0.88$ (t, 6H $J = 3.1$), 1.20–1.41 (m, 64H), 2.05 (s, 3H), 2.59–2.72 (m, 8H), 2.80 (t, 4H $J = 7.8$), 3.13–3.17 (m, 4H), 3.24 (t, 4H $J = 7.1$), 3.43–3.46 (m, 4H), 3.52 (t, 4H $J = 6.5$), 4.01–4.06 (m, 2H). ^{13}C NMR [50 MHz]: $\delta = 14.01$, 19.77, 20.70, 27.18, 29.29, 29.36, 29.38, 29.40, 29.62, 29.66, 29.68, 29.71, 29.76, 32.01, 41.64, 44.64, 48.84, 52.45, 66.80, 71.21, 72.90, 169.10. FAB-HRMS: Calcd. for $C_{48}H_{99}N_3O_5$: 798.3361. Found: 798.3359.

General Procedure for the Synthesis of Bis-alkyldimethylammonium salts (**3d–f**)

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** (0.397 g, 1.53 mmol) and TBABr (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 18 h at 30 °C. Then, the solvent was evaporated under reduced pressure at room temperature, and the residue was purified by column chromatography. The unreacted tertiary amine was removed from the residue using ethyl acetate/ethanol/water/ammonium hydroxide 28–30 wt % (4:1:0.25:0.5) solvent system as eluant. The eluates containing the desired products were evaporated under reduced pressure. Then, the addition of the HCl solution provided the corresponding counter ions. Finally, compounds **3d–f** were lyophilized. The above-mentioned general procedure yielded **3d–f** as isolated pure compounds, in 92, 61, and 39%, respectively. The physical data of the synthesized products are as follows:

N,N-Bis[2-(3-octyldimethylammonio-2-hydroxypropoxy) ethyl]acetamide dichloride (**3d**): Oil pale yellow. IR (KBr): $\nu = 760.0$, 930.2, 1120.1, 1460.4, 1620.0, 2850.1, 2930.3, 3450.0 cm^{-1}. ^1H NMR [200 MHz, (D_2O)]: $\delta = 0.81$–0.93 (m, 10H), 1.25–1.45 (m, 16H), 1.94–1.97 (m, 4H), 2.05 (s, 3H), 3.12–3.14 (m, 6H), 3.23–3.27 (m, 4H), 3.32 (s, 12H), 3.35–3.45 (m, 4H), 3.53–3.56 (m, 4H), 3.80–4.02 (m, 6H). ^{13}C NMR [50 MHz]: $\delta = 14.07$, 20.70, 22.68, 24.13, 26.31, 27.19, 28.46, 31.73, 41.64, 44.64, 59.43, 63.50, 64.84, 66.93, 70.11, 70.25, 169.10. FAB-HRMS $(M - Cl)^+$: Calcd. for $C_{32}H_{69}Cl_2N_3O_5$: 611.3727. Found: 611.3716.

N,N-Bis[2-(3-dodecyldimethylammonio-2-hydroxypropoxy)ethyl]acetamide dichloride (**3e**): White waxy product. IR (KBr): $\nu = 760.1$, 930.4, 1120.0, 1460.0, 1620.5, 2850.0, 2930.1, 3450.2 cm^{-1}. ^1H NMR [200 MHz, (D_2O)]: $\delta = 0.84$–0.91 (m, 10H), 1.22–1.40 (m, 32H), 1.94–2.01 (m, 4H), 2.06 (s, 3H), 3.12–3.16 (m, 6H), 3.25–3.27 (m, 4H), 3.32 (s, 12H), 3.36–3.45 (m, 4H), 3.53–3.57 (m, 4H), 3.79–4.04 (m, 6H). ^{13}C NMR [50 MHz]: $\delta = 14.05$, 20.70, 22.62, 23.79, 26.08, 27.88, 29.37, 29.45, 29.61, 29.62, 31.68, 41.64, 44.64, 44.64, 59.43, 63.50, 64.84, 66.93, 70.11, 70.25, 169.10. FAB-HRMS $(M–Cl)^+$: Calcd. for $C_{40}H_{85}Cl_2N_3O_5$: 723.5886. Found: 723.5875.

N,N-Bis[2-(3-octadecyldimethylammonio-2-hydroxypropoxy)ethyl]acetamide dichloride (**3f**): White waxy product. IR (KBr): $\nu = 760.0$, 930.1, 1120.0, 1460.0, 1620.2, 2850.1, 2930.0, 3450.0 cm^{-1}. ^1H NMR [200 MHz, (D_2O)]: $\delta = 0.83$–0.90 (m, 10H), 1.21–1.40 (m, 56H), 1.94–1.98 (m, 4H), 2.05 (s, 3H), 3.12–3.16 (m, 6H), 3.25–3.27 (m, 4H), 3.32 (s, 12H), 3.38–3.45 (m, 4H), 3.53–3.56 (m, 4H), 3.79–4.04 (m, 6H). ^{13}C NMR [50 MHz]: $\delta = 13.10$, 19.77, 20.70, 23.79, 26.08, 26.87, 27.88, 29.22, 29.29, 29.36, 29.38, 29.40, 29.62, 29.66, 29.68, 29.70, 32.01, 41.64, 44.64, 59.43, 63.50, 64.84, 66.93, 70.11, 70.25, 169.10. FAB-HRMS $(M–Cl)^+$: Calcd. for $C_{52}H_{109}Cl_2N_3O_5$: 891.9125. Found: 891.9113.

Synthesis of *N*-[2-(oxiran-2-yl)methoxyethyl] acetamide (**5**)

According to the general procedure for compound **2**, a 1:20:6:0.05 molar mixture of *N*-(2-hydroxyethyl)acetamide **4** (0.65 g, 6.3 mmol), (±)-epichlorohydrin (11.64 g, 126.0 mmol), NaOH (1.51 g, 37.8 mmol), and TBAB (0.107 g, 0.315 mmol), was heated at 30 °C under

vigorous stirring (700 rpm) for 5 h. After workup, 0.781 g of **5**, 78% yield and >98% purity (by ^{1}H NMR) were isolated as a pure compound. Physical data of **5**: Oil pale yellow. IR (KBr): ν = 761.6, 804.0, 932.7, 1105.1, 1252.0, 1336.3, 1458.4, 1637.5, 2862.1, 2998.8 cm^{-1}. ^{1}H NMR [200 MHz, ($CDCl_3$)]: δ = 2.02 (s, 3H), 2.45–2.61 (m, 2H), 3.03 (m, 1H), 3.18–3.48 (m, 6H), 6.35 (s, 1H). ^{13}C NMR [50 MHz, ($CDCl_3$)]: δ = 22.27, 35.44, 44.47, 53.96, 71.90, 73.12, 174.04. MS: spectra *m/z* (% rel. int.) = 159 (M^+, 2), 144 (3), 130 (60), 112 (5), 86 (15), 70 (10), 55 (90), 43 (100).

Synthesis of *N*-[2-(3-dodecylamino-2-hydroxypropoxy) ethyl]acetamide (**6a**)

According to the general procedure for compounds **3a–c**, a 1.25:1:0.1 molar mixture of dodecylamine (0.354 g, 1.912 mmol), monoglycidyl ether **5** (0.243 g, 1.53 mmol) and tetrabutyl ammonium bromide (TBABr) (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 18 h at 30 °C. After workup, the general procedure gave **6a** in 86% yield, as isolated pure product. Physical data of (**6a**): Pale yellow, waxy material. IR (KBr): ν = 760.4, 930.1, 1120.1, 1460.3, 1620.0, 2850.7, 2930.0, 3450.1 cm^{-1}. ^{1}H NMR [200 MHz, ($CDCl_3$)]: δ = 0.88–0.92 (m, 3H), 1.20–1.41 (m, 20H), 1.91 (s, 3H), 2.57–2.79 (m, 4H), 3.14–3.46 (m, 6H), 4.03–4.05 (m, 1H). ^{13}C NMR [50 MHz]: δ = 14.10, 22.27, 27.18, 29.39, 29.76, 31.94, 35.44, 48.84, 52.45, 71.21, 72.90, 174.04. FAB-HRMS: Calcd. for $C_{19}H_{40}N_2O_3$: 344.5401. Found: 344.5399.

Synthesis of *N*-[2-(3-dodecyldimethylammonio-2-hydroxypropoxy)ethyl]acetamide chloride (**6b**)

According to the general procedure for compounds **3d–f**, a 1.25:1:0.1 molar mixture of *N,N*-dimethyldodecylamine (0.407 g, 1.912 mmol), monoglycidyl ether **5** (0.243 g, 1.53 mmol) and TBABr (0.049 g, 0.153 mmol) was added to absolute ethanol (12 mL), and the solution was stirred for 18 h at 30 °C. After workup, the general procedure yielded **6b** as pure product, in 80% yield. Physical data of (**6b**): White waxy product. IR (KBr): ν = 760.1, 930.4, 1120.0, 1460.0, 1620.5, 2850.0, 2930.1, 3450.2 cm^{-1}. ^{1}H NMR [200 MHz, (D_2O)]: δ = 0.84–0.88 (m, 3H), 1.24–1.39 (m, 18H), 1.91 (s, 3H), 1.93–1.97 (m, 2H), 3.14–3.16 (m, 2H), 3.32 (s, 6H), 3.38–3.51 (m, 4H), 3.86–3.51 (m, 4H), 3.86–4.01 (m, 2H), 4.71–4.74 (m, 1H). ^{13}C NMR [50 MHz]: δ = 14.04, 22.27, 23.79, 26.87, 29.37, 29.45, 29.61, 31.68, 35.44, 59.43, 63.50, 66.93, 70.11, 70.25, 70.89, 174.02. FAB-HRMS (M–Cl)$^+$: Calcd. for $C_{21}H_{45}ClN_2O_3$: 373.6018. Found: 373.6013.

Analytical Methods

Structures of the prepared compounds were confirmed by their spectral data. Infrared (IR) spectra were recorded on a Shimadzu 8201 PC spectrophotometer; ^{1}H- and ^{13}C-NMR spectra on a Bruker FT-200 spectrometer, using D_2O 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. High-resolution mass spectrometric measurements were conducted at the Mass Spectrometry Facility of the University of California at Riverside, United States. Gas-liquid chromatographic (GLC) analyses were performed on a Shimadzu GC-17AATF chromatograph equipped with a methyl silicone capillary column (30 m × 0.32 mm, 0.25 μm 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 standards to perform quantitative GC analyses.

The surface tension of aqueous solutions (pH = 7) 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 [30]. The surface tension was then measured three times for each sample with a 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 versus 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 there from. The optimal cross-section surface area A occupied by the surfactant head group at the air/water interface was estimated from the surface excess concentration, Γ. The latter one was calculated by applying the Gibbs adsorption isotherm equation: $\Gamma = -(1/n\ 2.303\ RT)\ (d\gamma/d\ \log\ C)$, where γ is the surface tension and C is the surfactant concentration and n equals 2. The area per molecule at the interface was calculated from $A = 1/N\Gamma$ where N is Avogadro's number [31]. Results are summarized in Table 1.

Antimicrobial Activity

Antifungal activities of the dimeric and monomeric compounds were evaluated using the method for the broth

Table 1 Surface activity and aggregation data of synthesized cationic compounds[a]

Compound	CMC (mM)	C_{20} (mM)	γ_{CMC} (mN m^{-1})	pC_{20}	CMC/C_{20}	$\Gamma \times 10^6$ (mol/m^2)	$A \times 10^{20}$ (m^2)
3d[b]	34.787	5.475	31.84	2.26	6.35	2.10	78.88
3e[b]	2.455	0.410	34.58	3.39	5.99	1.85	90.14
3f[b]	0.219	0.049	39.24	4.31	4.47	1.58	105.18
6b[c]	4.974	0.854	33.94	3.06	5.82	1.95	84.94

[a] Experimental conditions: temperature 20 ± 0.5 °C. Aqueous solution at pH 7. Experimental uncertainties are estimated to be ±0.03 mM on CMC and ±1 mN m^{-1} on γ_{CMC} values

[b] Dimeric cationic compounds

[c] Monomeric cationic compound

macro dilution test (M27-A2, National Committee for Clinical Laboratory Standards-NCCLS) [32]. The culture media used to determine the antifungal activities was Sabouraud dextrose (Britania S.A). Fluconazole was used as the reference drug for positive control. Although the Fluconazole is an azole derivative, which does not have bis-amino alcohols or bis-quaternary ammonium groups, it was chosen as standard because it is a potent antifungal agent. Minimum inhibitory concentrations (MIC) were expressed in μg mL^{-1}. The MIC means the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test. Four microorganisms were used: *Candida parapsilosis* (ATCC 22019), *C. albicans* (ATCC 64548), a wild-type strain of *C. parapsilosis*, and *Saccharomyces cerevisiae* (ATCC 9763). Cationic compound concentrations (**3d–f** and **6b**) (water-soluble agents) were in the range of 64–0.03125 μg mL^{-1}. Non-ionic compound stock solutions were prepared in an appropriate solvent (methyl sulfoxide, DMSO), reducing the final solvent concentration to 1%. The concentration range for these water-insoluble agents (**3a–c** and **6a**) was the same as that used for the cationic compounds. All the tests included drug-free and yeast-free controls. Tests with non-ionic compounds also included 1% DMSO as a dilution control.

Results and Discussion

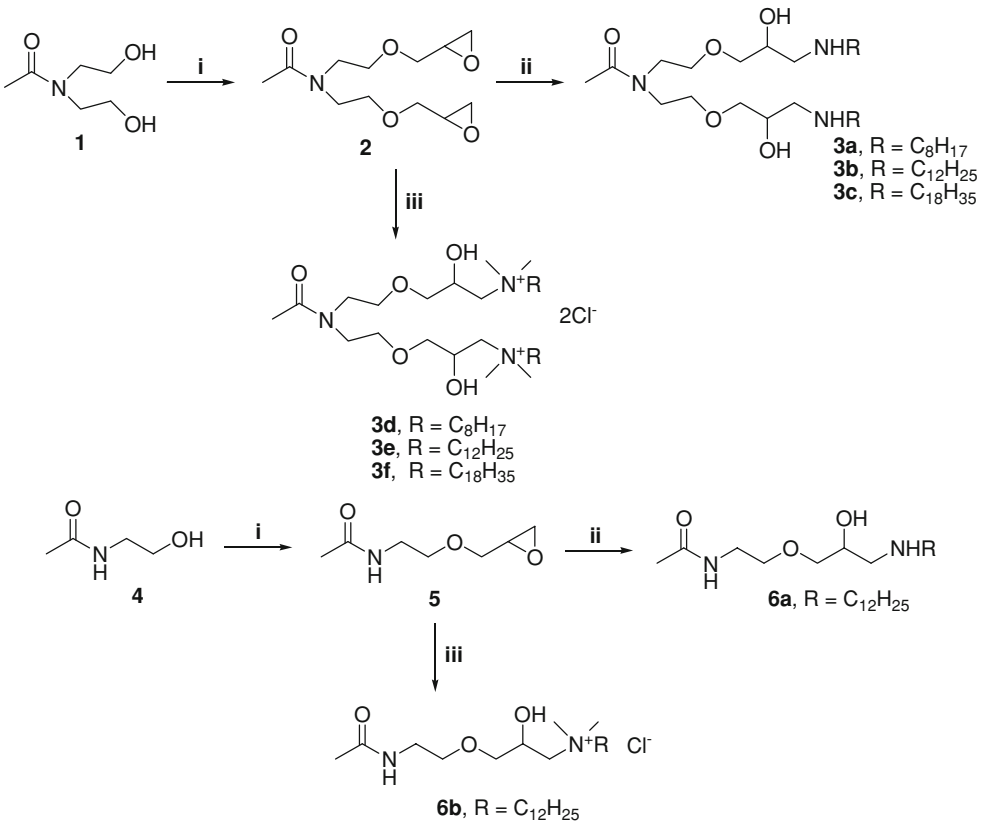
Synthesis

N-acetylated dimeric (**3a–f**) and monomeric (**6a–b**) compounds were prepared following the well-known two-step procedure described by Tewari et al. [27] (see Scheme 1). Key intermediates **2** and **5** were obtained by Williamson's etherification of **1** and **4** with (±)-epichlorohydrin under phase-transfer catalysis (PTC) conditions in 84 and 78% yields, respectively. N-acetylated non-ionic gemini surfactants (**3a–c**) were synthesized via oxirane ring opening with primary amines (C_8–C_{12}–C_{18}) under PTC conditions, obtaining a 40, 33, and 30% yield, respectively. The N-acetylated monomeric analogues (**6a**) and (**6b**) were obtained in 86 and 80% yields, respectively. Likewise, the reaction of the N-acetylated intermediate **2** with tertiary amines yielded **3d–f**, in 92, 61, and 39% yield, respectively. The nucleophilic substitution reactions involving amines were assisted by tetrabutyl ammonium bromide (TBABr) as phase transfer catalyst, and no attempt was made to optimize yields. The structures of all compounds were confirmed by spectral (IR, ^1H and ^{13}C NMR) and HRMS methods. All analytical methods indicated high levels of purity of the N-acetylated dimeric and monomeric surfactants.

Surface Properties

The solubility in water of the cationic compounds largely exceeded that of the non-ionic homologues. Regarding tensioactive and self-aggregation properties, non-ionic compounds **3a–c** and **6a** are able to remain dispersed in a stable state but their poor solubility prevented a reliable determination of their tensioactive properties and, therefore, these were not measured. However, cationic compounds **3d–f** and **6b** exhibited clear solutions and displayed a sharp break in the surface tension versus concentration (on log scale) curves indicating a well-defined CMC and surface tension at the CMC (γ_{CMC}), as shown in Fig. 2. All N-acetylated cationic compounds were active at lower concentrations than CMC, so its antifungal activity was not due to the formation of aggregates. 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. The critical micelle concentration (CMC), the ability to lower surface tension above the CMC (γ_{CMC}), the efficiency of adsorption at the water/air interface [pC_{20}, C_{20}: the concentration (mM) of surfactant in the bulk phase required to produce a 20 mN m^{-1} reduction in the surface of water, $pC_{20} = -\log C_{20}$], the ratio of cationic surfactants (CMC/C_{20}), the amount of surfactant

Scheme 1 Reagents and conditions: (**i**) (±)-epichlorohydrin, aq. NaOH, tetrabutylammonium hydrogen sulfate, 30 °C, 5 h; (**ii**) **2**, primary amines, tetrabutylammonium bromide (TBABr), ethanol, 30 °C, 18 h; (**iii**) **2**, *N,N*-dimethylamines, TBABr, ethanol, 30 °C, 18 h



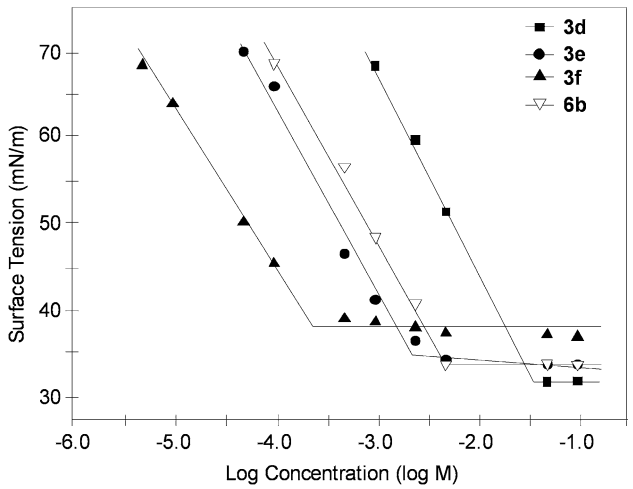


Fig. 2 Surface tension vs. logarithm of the aqueous molar concentration (log C) of synthesized dimeric (**3d–f**) and monomeric (**6b**) cationic compounds at pH 7 and 20 °C

adsorbed per unit area of the saturated interface (Γ) and the interfacial area occupied by the surfactant molecule (*A*) are summarized in Table 1. These values are in agreement with those characterizing the proper quantitative behavior of moderate to good surfactants. A very good linear relationship between the CMC and the number of carbon atoms (*N*) in the alkyl chain of homologue compounds was found for the cationic series: $\log \mathrm{CMC} = -1.655 - 0.097\,N$ ($R^2 = 0.999$). The pC_{20} values indicate that the three dimeric cationic compounds are good surfactants, and the CMC/C_{20} values show a slightly greater capability of compound **3f** to be adsorbed at the interfaces. The surface excess Γ and the area per molecule *A* vary with the molecular structure. Indeed, the area per molecule at air/water interface of the headgroup in the surfactants of the cationic series was found to be within a range of 78.88–105.18 Å^2, 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 closely 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 N-acetylated cationic dimeric compounds (**3d–f**) and monomeric salt (**6b**) satisfy the primary requirement of being moderate to good surfactants.

Antimicrobial Activity

The MIC values of these six new gemini surfactants were obtained in comparison to Fluconazole and to N-acetylated monomeric analogues (Table 2). The maximum inhibitory activity was found for **3f** (0.25 μg mL^{-1})

Table 2 Antifungal activity of dimeric (**3a–f**) and monomeric (**6a–b**) compounds synthesized

Compound	R	MIC (μg mL^{-1})			
		C. parapsilosis ATCC 22019	*C.albicans* ATCC 64548	*C. parapsilosis* wild- type	*S.cerevisiae* ATCC 9763
3a	$-C_8H_{17}$	64	>64	64	16
3b	$-C_{12}H_{25}$	8	8	16	4
3c	$-C_{18}H_{35}$	64	>64	>64	8
3d	$-C_8H_{17}$	>64	>64	>64	>64
3e	$-C_{12}H_{25}$	16	8	16	8
3f	$-C_{18}H_{35}$	0.25	32	16	2
6a	$-C_{12}H_{25}$	64	64	64	16
6b	$-C_{12}H_{25}$	64	64	>64	16
Fluconazole		16	<5	8	16

against *C. parapsilosis* (ATCC 22019). Compounds **3b**, **3e** and **3f** were found to be the most potent compounds. In general, the less active molecules were **3a** and **3d**. Compounds **3b** and **3e** showed a similar activity for all strains. Monomeric analogues **6a** and **6b** were active only for *S. cerevisiae* (ATCC 9763). The results showed that *S. cerevisiae* was the most sensitive strain. In contrast, the wild strain of *C. parapsilosis* was resistant. *C. parapsilosis* (ATCC 22019), the strain recommended by the NCCLS, was inhibited. The value obtained for this strain, when **3f** is used, would be important for medical treatment. Results obtained for gemini compounds were comparable to the values attained for the reference highly active antifungal (Fluconazole). Further studies will be carried out using filamentous fungi and the fungicide or fungistatic effects will be evaluated from death curves of the microorganisms.

Acknowledgments The authors wish to express their gratitude to the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and to the Universidad Nacional del Litoral (UNL) of Argentina, for the financial support granted to this contribution.

References

1. Menger FM, Keiper JS (2000) Gemini surfactants. Angew Chem Int Ed Engl 39:1906–1920
2. Zana R (2002) Dimeric (gemini) surfactants: effect of the spacer group on the association behavior in aqueous solution. J Colloid Interface Sci 248:203–251
3. Zana R, Xia J (2004) Gemini surfactants, surfactant science series. Marcel Dekker, New York
4. Menger FM, Migulin VA (1999) Synthesis and properties of multiarmed geminis. J Org Chem 64:8916–8921
5. Menger FM, Mbadugha BNA (2001) Gemini surfactants with a disaccharide spacer. J Am Chem Soc 123:875–885
6. Zhu Y, Masuyama A, Kirito Y, Okahara M, Rosen MJ (1992) Preparation and properties of glycerol-based double- or triple-chain surfactants with two hydrophilic ionic groups. J Am Oil Chem Soc 69:626–632
7. Devínsky F, Lacko L, Mlynarcik D, Racansky V, Krasnec L (1985) Relationship between critical micelle concentration and minimum inhibitory concentrations for some none-aromatic quaternary ammonium salts and amine oxides. Tenside Deterg 22:10–15
8. Murguía MC, Grau RJ (2001) Synthesis of new pentaerythritol-based gemini surfactants. Synlett 8:1229–1232
9. Murguía MC, Vaillard SE, Grau RJ (2001) A facile, selective preparation of monoketals from pentaerythritol and ketones. Synthesis 7:1093–1097
10. Murguía MC, Cabrera MI, Grau RJ (2002) Selective preparation of key intermediates for the synthesis of gemini surfactants by phase-transfer catalysis. React Kinet Catal Lett 75:205–211
11. Murguía MC, Cabrera MI, Guastavino JF, Grau RJ (2005) New oligomeric surfactants with multiple-ring spacers: synthesis and tensioactive properties. Colloids Surf A Physicochem Eng Asp 262:1–7
12. Kopecky F (1996) Micellization and other associations of amphiphilic antimicrobial quaternary ammonium salts in aqueous solutions. Pharmazie 51:135–144
13. Dubnicková M, Pisárcik M, Lacko I, Devínsky F, Mlynarcik D, Balgavý P (1997) Gemini amphiphiles: antimicrobial activity, micellization and interaction with phospholipid bilayers. XIIIth school on biophysics of membrane transport. Cell Mol Biol Lett 2(Suppl. 1):215–216
14. Dubnicková M, Yaradaikin S, Lacko I, Devínsky F, Gordeliy VI, Balgavý P (2004) Effects of gemini surfactants on egg phosphatidylcholine bilayers in the fluid lamellar phase. Colloids Surf B Biointerfaces 34:161–164
15. Horniak L, Ebringer L, Balgavý P (1990) Interaction of bactericidal bis-ammonium salts with model and bacterial membranes. IUMS congress: bacteriology and mycology, Osaka, Japan, 16–22 September, Book of abstracts, 171
16. Iwamoto S, Otsuki M, Sasaki Y, Ikeda A, Kikuchi J (2004) Gemini peptide lipids with ditopic ion-recognition site. Preparation and functions as an inducer for assembling of liposomal membranes. Tetrahedron 60:9841–9847
17. Bell PC, Bergsma M, Dolbnya IP, Bras W, Stuart MCA, Rowan AE, Feiters MC, Engberts JBFN (2003) Transfection mediated by gemini surfactants: engineered escape from the endosomal compartment. J Am Chem Soc 125:1551–1558
18. David S, Pérez L, Infante MR (2002) Sequestration of bacterial lipopolysaccharide by bis(args) gemini compounds. Bioorg Med Chem Lett 12:357–360

19. Diz M, Manresa A, Pinazo A, Erra P, Infante MR (1994) Synthesis, surface active properties and antimicrobial activity of new bis-quaternary ammonium compounds. J Chem Soc (Perkin Trans. 2), 1871–1876.
20. Okazaki K, Maeda T, Nagamune H, Manabe Y, Kourai H (1997) Synthesis and antimicrobial characteristics of 4, 4′-(α, ω-polymethylenedithio) bis(1-alkylpyridinium iodide)s. Chem Pharm Bull (Tokyo) 45:1970–1974
21. Maeda T, Manabe Y, Yamamoto M, Yoshida M, Okazaki K, Nagamune H (1999) Synthesis and antimicrobial characteristics of novel biocides, 4, 4′-(1, 6-Hexamethylenedioxy dicarbonyl) bis(1-alkylpyridinium iodide)s. Chem Pharm Bull (Tokyo) 47:1020–1023
22. Iyer S, Rele SM, Baskaran S, Chaikof EL (2003) Design and synthesis of hyaluronan-mimetic gemini disaccharides. Tetrahedron 59:631–638
23. Shirai A, Maeda T, Nagamune H, Matsuki H, Kaneshina S, Kourai H (2005) Biological and physicochemical properties of gemini quaternary ammonium compounds in which the positions of a cross-linking sulfur in the spacer differ. Eur J Med Chem 40:113–123
24. Chaudier Y, Zito F, Barthélémy P, Stroebel D, Améduri B, Popot J-L, Pucci B (2002) Synthesis and preliminary biochemical assessment of ethyl-terminated perfluoroalkylamine oxide surfactants. Bioorg Med Chem Lett 12:1587–1590
25. Massi L, Guittard F, Géribaldi S, Levy R, Duccini Y (2003) Antimicrobial properties of highly fluorinated bis-ammonium salts. Int J Antimicrob Agents 21:20–26
26. Massi L, Guittard F, Géribaldi S (2000) Application en Tant que Biocides des Composés QUATERFLUO®, MC Patent Number 2452
27. Tewari N, Tiwari VK, Tripathi RP, Chaturvedi V, Srivastava A, Srivastava R, Shukla PK, Chaturvedi AK, Gaikwad A, Sinha S, Srivastava BS (2004) Synthesis of galactopyranosyl amino alcohols as new class of antitubercular and antifungal agents. Bioorg Med Chem Lett 14:329–332
28. Botana López L, Landoni MF, Martin-Jiménez T (eds) (2002) Farmacología Terapéutica Veterinaria. Mc Graw-Hill Int, Madrid
29. Vogel AI (1989) Vogel′s textbook of practical organic chemistry, 5th edn. Wiley, New York
30. Pisárcik M, Rosen MJ, Polakovicová M, Devínsky F, Lacko I (2005) Area per surfactant molecule values of gemini surfactants at the liquid-hydrophobic solid interface. J Colloid Interface Sci 2:560–565
31. Chlebicki J, Wegrzynska J, Maliszewska I, Oswiecimska M (2005) Preparation, surface-active properties, and antimicrobial activities of bis-quaternary ammonium salts from amines and epichlorhidrin. J Surfact Deterg 8:227–232
32. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts. M27-A2. NCCLS, Wayne, Pa, USA (2002)

Author Biographies

Marcelo C. Murguía is an MS in Chemistry at U.N.C, Professor and Dr in Biology at U.N.L., and Member of CONICET's Research Staff.

Laura M. Machuca is a graduate in Biotechnology from UNL and Research Assistant from CONICET.

María C. Lurá is a Professor and Dr. in Biology at U.N.L. and Member of UNL's Research Staff.

María I. Cabrera is a Dr in Chemical Engineering at U.N.L. and member of CONICET's Research Staff.

Ricardo J. Grau is a Professor and Dr in Chemical Engineering at U.N.L. and Member of CONICET's Research Staff.