Nonionic Amphiphilic Compounds from Lysine as Molecular Mimics of Lecithins

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ABSTRACT: New monodisperse nonionic surfactant molecules, based on lysine with two fatty acid chains in the hydrophobic part and one or two polyoxyethylene methoxy-capped chains (EO_n-Me) in the hydrophilic headgroup, are synthesized as mimics of natural lecithins. Their surface-activity properties indicate that these compounds have surfactant behavior whose global hydrophobic contribution is comparable to that of one fatty chain.
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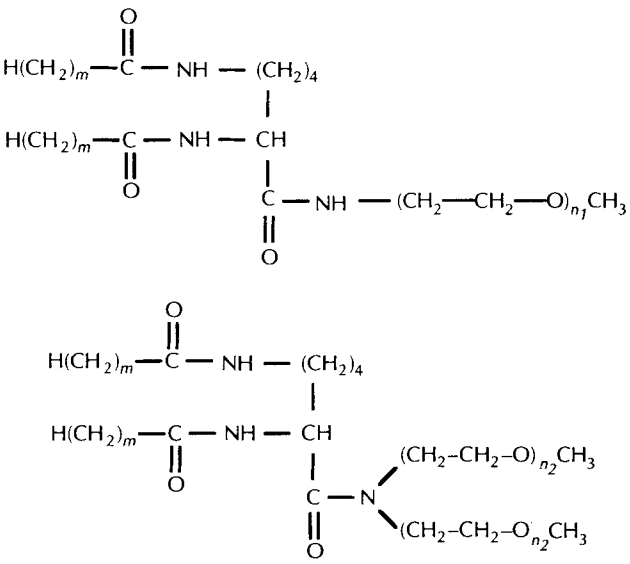
KEY WORDS: Amphiphilic compounds, critical micelle concentration, mimics of lecithins, surface tension.

In recent years, biocompatible surfactants have become increasingly important in biochemistry (1) and in biological (2) and chemical research applications (3). Preliminary work in our laboratory showed that synthetic nonionic molecular mimics of short-chain lecithins, based on trifunctional amino acids such as lysine (Lys), glutamic acid (Glu), and aspartic acid (Asp), were interesting nonhemolytic amphiphilic compounds, which could be applied as surfactants in biological systems (4,5). Synthesis of a large number of Lys, Asp, and Glu homologues with different hydrophobic/hydrophilic characteristics was carried out to determine the effects of several structural parameters (hydrophobic chainlength, polyoxyethylene chainlength, and number of polyoxyethylene chains) on the physicochemical properties and biological performances of these natural mimics.

This paper deals with a more detailed and systematic description of the synthesis and physicochemical properties in water of the nonionic short- and long-chain diacyl amphiphilic compounds from lysine with a structural resemblance to natural lecithins. Their toxic effects have been studied, and they are less hemolytic and less irritating than are conventional nonionic surfactants (6). Chemical structures of these amphiphiles are indicated in Scheme 1:



SCHEME 1

where m = 5, 7, 9, or 11 and n_1 = 2, 4, or 6 and n_2 =2 or 3. The code used is *mm*K*On*$_1$ and *mm*K*n*$_2$*n*$_2$. The letter "*K*" indicates lysine amino acid. Structures of these nonionic amphiphilic compounds are based on short- and long-chain N^{α}, N^{ε}–diacyl lysine and contain no active hydrogen in the POE hydrophilic head (they possess a methoxy-capped EO_n chain); hence they are more chemically inert. As with the lecithins, these compounds have two hydrophobic tails of different lengths and one hydrophilic head. In the latter compounds, however, the polar head is of the nonionic type (one or two chains of monomethylether-polyoxyethylene glycol with different EO units), whereas in the lecithins it is of the zwitterionic type. The central pivot in the structure of lecithins (glycerol) is imitated by the natural trifunctional amino acid lysine. The fatty acids and monomethylether-polyoxyethylene amine residues are introduce onto the α-, ω-amino or α-carboxylic functions of the lysine through amide bonds in place of the ester bonds in the lecithins. These amide bonds provide more resistance to hydrolysis than do the ester bonds in the lecithins. Synthesis and physicochemical properties of the Glu and Asp homologues will be discussed in subsequent publications.

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It is reasonable to suppose that most of these materials are acceptable from both toxicological and ecological points of view, given that in these synthetic compounds the biocompatible characteristics of both long-chain amino acids and nonionic POE surfactants converge on a structure mimic of lecithin.

EXPERIMENTAL PROCEDURES

All solvents were reagent-grade and were used without further purification except for tetrahydrofuran (THF), which was distilled from sodium benzophenone ketyl. BOP reagent (benzotriazole-1-yl-oxy-*tris*(dimethylamino)-phosphoniumhexafluorophosphate) was supplied by Propeptide (Paris). The progress of reaction and purity of the products were monitored on silica-gel TLC plates (Kieselgel 60 F_{254}; Merck, Darmstadt, Germany) with eluent ethyl acetate (AcOEt) or chloroform/methanol 7:3 (C/M). Purification was carried out by flash silica-gel column chromatography with the same eluents.

Retention times (R_f) were determined by HPLC model Merck–Hitachi D-2500 (Tokyo, Japan) with a ultraviolet-visible (UV-VIS) detector L-4250 (Tokyo, Japan) and a Lichrocart 125-4 Lichrospher 100 RP-18 column (Darmstadt, Germany). The chromatographic conditions were as follows: The mobile phase was a solvent gradient 0–100% of acetonitrile in water for 60 min; temperature of the column was between 293–303 K; detection at 210 nm, 0.4 aufs. Infrared (IR) spectra were obtained with a Nicolet 510 FTIR spectrometer (Warwick, United Kingdom). Melting points were determined with an electronic apparatus (electrothermal) and were not corrected. ^{1}H Nuclear magnetic resonance (NMR) and ^{13}C NMR were recorded with a Varian 200 MHz spectrometer (Palo Alto, CA). Surface-tension measurements were made with a Kruss tensiometer (Hamburg, Germany) by the Wilhelmy method. The principal characteristics of final compounds are indicated in Tables 1 and 2.

General procedure for preparing long-chain N^{α}, N^{ε}*-diacyl lysine derivatives (*mm*K00).* A solution of 0.121 mole (2.2 eq.) hexanoyl acid chloride were added dropwise to a well-stirred aqueous acetone (66:34) solution that contained 0.055 mole (1 eq.) sodium salt of lysine in a beaker equipped with a thermometer and two pH electrodes. The solution was maintained in a pH range of 9–12.5 by simultaneous addition of a 10% aqueous sodium hydroxide solution, and the temperature was kept below 10°C. After addition was completed, the solution was acidified with 10% HCl to a pH below 2. A precipitate formed, which was filtered and washed with water until pH 7 and finally recrystallized from hexane or ethyl ether. The results were as follows: N^{α}, N^{ε}–dihexanoyl lysine: 55K00: M.W.: 342.5; weight: 10.7 g; yield: 57%; m.p.: 72–73°C; HPLC: 19.3 min; TLC (SiO_2; $CHCl_3$:MeOH 7:3) R_f:0.65; $[\alpha]_D^{22}$: −16.4° (*c* = 2, EtOH/HCl 5N); anal. for.: $C_{18}H_{34}N_2O_4$: % calc. (found): C: 63.13 (62.98); H: 10.01 (10.03); N: 8.18 (8.14); N^{α},N^{ε}-dioctanoyl lysine: 77K00: M.W.: 398.6; weight: 11.6 g; yield: 53%; m.p.: 89–90°C; HPLC: 23.6 min; TLC (SiO_2; $CHCl_3$:MeOH 7:3) Rf: 0.65; $[\alpha]_D^{22}$: −10.4° (*c* = 2, EtOH/HCl 5 N); anal. for.: $C_{22}H_{42}N_2O_4$: % calc. (found): C: 66.29 (66.00); H: 10.62 (10.54); N: 7.03 (6.46); N^{α}, N^{ε}-didecanoyl lysine: 99K00: M.W.: 454.7; weight: 14.7 g; yield: 59%; m.p.: 115–116°C; HPLC: 39.3 min; TLC (SiO_2; $CHCl_3$:MeOH 7:3) Rf: 0.65; $[\alpha]_D^{22}$: −12.4° (*c* = 2, EtOH/HCl 5N); anal. for.: $C_{18}H_{34}N_2O_4$: % calc. (found): C: 68.68 (68.12); H: 11.08 (11.01); N: 6.16 (5.76); N^{α}, N^{ε}-didodecanoyl lysine 1111K00: M.W.: 510.8; weight: 17.1 g; yield: 61%; m.p.: 119–120°C; HPLC: 41.2 min; TLC (SiO_2; $CHCl_3$:MeOH 7:3) Rf: 0.65 $[\alpha]_D^{22}$: −12.8° (*c* = 2, EtOH/HCL 5N); anal. for.: $C_{30}H_{58}N_2O_4$: % calc. (found): C: 70.54 (70.55); H: 11.44 (11.42); N: 5.48 (5.40).

Spectral characteristics for diacyl lysines are as follows: IR (KBr): 3,300 cm^{-1} (NH); 2,950 cm^{-1} (*CH_2*); 1,715 cm^{-1} (*CO*-OH); 1,650 cm^{-1} (*CO*-N, amide I), 1,550 cm^{-1} (N-C=O, amide II); ^{1}H NMR (200 MHz δ, $CDCl_3$): 0.9 ppm (*t*, 6H, $2CH_3$); 1.2–1.8 ppm [*m*, 18H, 9 × CH_2]; 2.20 ppm (*m*, 4H, $2CH_2$-CO-NH); 3.2 ppm (*m*, 2H, *CH_2*-NH-CO); 4.4 ppm (*m*, 1H, CH); 6.2 ppm (*m*, 1H,-NH); 6.8 ppm (*d*, 1H, -NH); ^{13}C NMR (50 MHz, δ, $CDCl_3$): 13.9 ppm (CH_3-); 14.1 ppm (CH_3-); 22–39 ppm (CH_2); 52.1 ppm (CH-); 174.3 ppm (*CO*-NH); 174.4 ppm (*CO*-NH); 174.6 ppm (*CO*-OH).

*General procedure for preparing linear methyloxypolyethyleneglycol amines (0*n_1*).* A solution of 0.085 mole (10 mL)

TABLE 1
Principal Characteristics of Final Products of Code *mm*K0*n_1* (Series 3)

Compound	Molecular weight (g/mol)	m.p. (°C)	TLC[a] (R_f)	Yield (%)	HPLC[b] retention time (min)
55K02	443.6	109–111	0.70	59	19.7
77K02	499.7	110–112	0.70	68	27.8
99K02	555.8	117–118	0.75	51	36.1
1111K02	611.9	124–126	0.77	56	41.2
55K04	531.7	76–78	0.70	54	19.6
77K04	587.8	82–84	0.70	43	26.8
99K04	643.9	88–92	0.70	41	36.1
1111K04	770.1	91–93	0.75	47	42.1
55K06	651.8	72–74	0.70	49	19.7
77K06	707.9	76–78	0.70	43	27.8
99K06	764.1	80–83	0.70	31	36.1
1111K06	820.2	85–88	0.70	35	42.0

[a]Thin-layer chromatography (SiO_2; $CHCl_3$; MeOH 7:3).
[b]High-performance liquid chromatography.

TABLE 2
Principal Characteristics of Final Products of Code *mmKn$_2$n$_2$* (Series 5)

Compound	Molecular weight (g/mol)	m.p. (°C)	TLC[a] (R_f)	Yield (%)	HPLC[b] retention time (min)
55K22	545.8	Oil	0.70	37	19.7
77K22	601.9	Oil	0.70	43	27.8
99K22	658.0	39–41	0.70	38	36.1
1111K22	714.1	45–47	0.70	33	41.2
77K33	690.0	Oil	0.70	31	27.8
99K33	746.1	Oil	0.70	35	36.1

[a]Thin-layer chromatography (SiO_2; $CHCl_3$; MeOH 7:3).
[b]High-performance liquid chromatography.

methyloxyethyleneglycol in 30 mL methylene chloride was introduced to a round-bottom flask fitted with a dropping funnel. Then 0.17 mole (23.7 mL) triethylamine was added dropwise. After cooling the mixture to −10°C under constant agitation, 0.13 mole (24.3 g) tosyl chloride in 50 mL of methylene chloride was added. The reaction mixture was left to attain room temperature and diluted with 50 mL water. The organic phase was subsequently washed with 1N HCl solution and saturated $NaHCO_3$ and NaCl solutions. The organic phase was finally dried over $MgSO_4$ and evaporated under reduced pressure to obtain a crude oil that corresponded to the tosylmethyloxyethyleneglycol.

To obtain the corresponding azide, 0.0078 mole (21.5 g) the crude was dissolved in dimethylformamide (50 mL), and 0.162 mole (10.5 g) sodium azide and 0.008 mole (3 g) tetrabutylammonium hydrogen sulfate were added as catalysts. The mixture was stirred for 5 h at 40°C and for 15 h at room temperature. The mixture was diluted with water and extracted with ethyl ether (3 × 100 mL). The organic phase was washed with a saturated solution of NaCl and dried over $MgSO_4$.

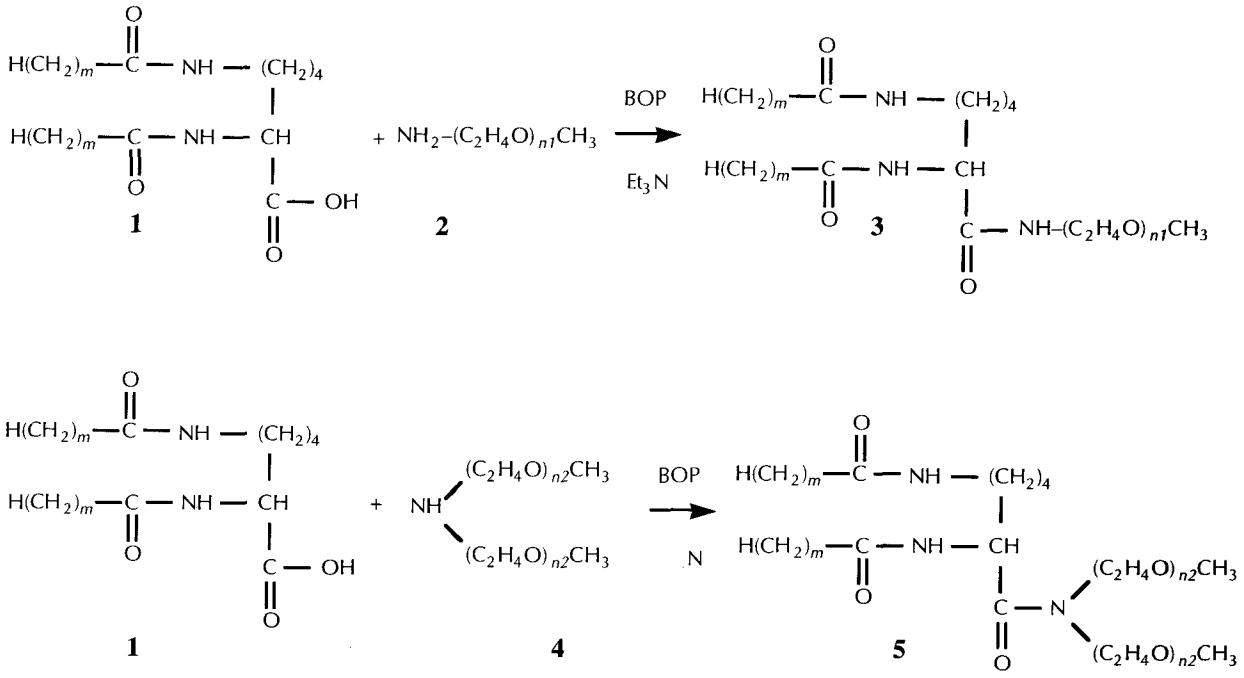
The methyloxyethyleneglycol amine was obtained by hydrogenation (under hydrogen pressure of 7 bars) of 0.047 mole (7 g) of the corresponding azide in 30 mL methanol with 0.7 g palladium on coal, and constant stirring was carried out for 4 h. At the end of the reaction, the solvent was evaporated under pressure, and a pure, colorless oil was obtained.

The results were as follows: Methyletherdiethyleneglycol amine: 02: M.W.: 119.2; weight: 5.1 g; yield: 89%; n_D^{22} = 1.435; Methylethertetraethyleneglycol amine: 04: M.W.: 207.3; weight: 7.2 g; yield: 81%; methyletherhexaethyleneglycol amine: 06: M.W.: weight: 7.3 g; yield: 61%.

General procedure for coupling N^{α}, N^{ε}*-diacyl lysine with methyloxyethyleneglycol amines by the BOP method (*mm*K0n$_1$).* A solution of 0.0029 mole (1 eq) dihexanoyl lysine in 20 mL $CHCl_3$ was added at room temperature to a well-stirred solution of 0.0044 mole (1.5 eq) methyl diethyleneglycol amine and 0.073 mole (2.5 eq) triethylamine in 20 mL $CHCl_3$, contained in a round-bottom flask. Then 0.003 mole (1 eq) of BOP reagent was added, and the mixture was stirred for 12 h. Afterward, the reaction mixture was extracted three times with 20 mL aqueous acid solution. The organic phase was washed again with 20 mL saturated $NaHCO_3$ (aq), dried over Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The product was purified by recrystallization from ethyl ether. The results are as follows: N^{α}, N^{ε}-dihexanoyl lysine methyl diethyleneglycol amide: 55K02: weight: 0.76 g; anal. for.: $C_{18}H_{34}N_2O_4$: % calc. (found): C: 62.27 (62.20); H: 10.22 (10.31); N: 9.47 (9.55); N^{α}, N^{ε}-dioctanoyl lysine methyl diethyleneglycol amide: 77K02: weight: 0.85 g; anal for.: $C_{27}H_{53}N_3O_5$: % calc. (found): C: 64.89 (64.88); H: 10.69 (10.65); N: 8.41 (8.49); N^{α}, N^{ε}-didecanoyl lysine methyl diethyleneglycol amide: 99K02: weight: 0.79 g; anal. for.: $C_{31}H_{61}N_3O_5$: % calc. (found): C: 66.99 (66.76); H: 11.06 (11.25); N: 7.56 (7.29); N^{α}, N^{ε}-didodecanoyl lysine methyl diethyleneglycol amide: 1111K02: weight: 0.67 g; anal. for.: $C_{35}H_{69}N_3O_5$: % calc. (found): C: 68.70 (68.49); H: 11.36 (11.50); N: 6.87 (6.62); N^{α}, N^{ε}-dihexanoyl lysine methyl tetraethyleneglycol amide: 55K04: weight: 0.95 g; anal. for.: $C_{27}H_{53}N_3O_7$: % calc. (found): C: 60.99 (59.19); H: 10.05 (9.89); N: 7.90 (7.71). MS-FAB$^+$-glycerol/MeOH (*m/e*): 555 (M^+ + 23, 100%), 533 (M^+ + 1, 38%), 435 (20%), 325 (30%), 242 (67%); N^{α}, N^{ε}-dioctanoyl lysine methyl tetraethyleneglycol amide: 77K04: weight: 0.63 g; anal. for.: $C_{31}H_{61}N_3O_7$: % calc. (found): C: 63.34 (63.39); H: 10.46 (10.61); N: 7.15 (7.16). MS-FAB$^+$-glycerol/MeOH (*m/e*): 589 (M^+ + 1, 55%), 463 (26%), 448 (10%), 381 (26%), 317 (7%), 277 (15%), 208 (100%); N^{α}, N^{ε}-didecanoyl lysine methyl tetraethyleneglycol amide: 99K04: weight: 0.58 g; anal. for.: $C_{35}H_{69}N_3O_7$: % calc. (found): C: 65.28 (65.40); H: 10.80 (11.01); N: 6.53 (6.53); N^{α}, N^{ε}-didodecanoyl lysine methyl tetraethyleneglycol amide: 1111K04: weight: 0.51 g; anal. for.: $C_{39}H_{77}N_3O_7$: % calc. (found): C: 66.91 (66.98); H: 11.09 (11.14); N: 6.00 (6.01). MS-FAB$^+$-glycerol/MeOH (*m/e*): 701 (M^+ + 1, 5%), 461 (7%), 369 (19%), 277 (66%), 242 (100%); N^{α}, N^{ε}-dihexanoyl lysine methyl hexaethyleneglycol amide: 55K06: weight: 0.89 g; anal. for.: $C_{31}H_{61}N_3O_9$: % calc. (found): C: 60.07 (60.10); H: 9.92 (9.99); N: 6.78 (7.03); N^{α}, N^{ε}-dioctanoyl lysine methyl hexaethyleneglycol amide: 77K06: weight: 0.70 g; anal. for.: $C_{35}H_{69}N_3O_9$: % calc. (found): C: 63.19 (61.26); H: 10.29 (9.87); N: 6.22 (5.91); N^{α}, N^{ε}-didecanoyl lysine methyl hexaethyleneglycol amide: 99K06: weight: 0.60 g; anal. for.: $C_{39}H_{77}N_3O_9$: % calc. (found): C: 63.99 (63.47); H: 10.60 (10.91); N: 5.74 (5.34); N^{α}, N^{ε}-didodecanoyl lysine methyl hexaethyleneglycol amide: 1111K06: weight: 0.51 g; anal. for.: $C_{43}H_{85}N_3O_9$: % calc. (found): C:

65.53 (65.43); H: 10.87 (10.74); N: 5.33 (5.69). Special characteristics for diacyl lysines methyl oxyethyleneglycol amides (*mm*K0n_1) are as follows: IR (KBr): 3,291 cm^{-1} (NH); 2,931 cm^{-1} (CH_2); 1,637 cm^{-1} (C=O, amide I); 1,560 cm^{-1} (N-C=O, amide II); 1,118 cm^{-1} (C-O-C); ^1H NMR (200 MHz, δ, $CDCl_3$): 0.95 ppm (*t*, 6H, $2CH_3$); 1.3–1.7 ppm (*m*, 18H, CH_2); 2.2 ppm (*m*, 4H, CH_2-CO); 3.25 ppm (*m*, 2H, -CH_2-CH_2-*CH_2*-NH-CO); 3.38 ppm (*s*, 3H, -OCH_3); 3.40–3.70 ppm [*m*, 8H, 2 × (CH_2-CH_2-O)]; 4.37 ppm (*m*, 1H, CH); 5.80 ppm (*t*, 1H, *NH*-CH_2-CH_2-CH_2); 6.42 ppm (*d*, 1H, *NH*-CH); 6.71 ppm (*t*, 1H, CO-*NH*-CH_2-CH_2-O); ^{13}C NMR (50 MHz, δ, $CDCl_3$): 13.92 ppm ($2CH_3$-); 22–40 ppm (12 × CH_2); 52.63 ppm (CH); 58.99 ppm (OCH_3); 69–72 ppm (2 × CH_2-CH_2-O); 171.75 ppm (1 HN-*C=O*, amide); 173.39 ppm (1 HN-*C=O*, amide): 173.49 ppm (1 HN-*C=O*, amide).

General procedure for coupling N^{α}, N^{ε}*-diacyl lysine with* N,N-bis*methyloxyethyleneglycol amines by BOP method* (mm*K*n_2n_2). A solution of 0.0029 mole (1 eq) dihexanoyl lysine in 20 mL $CHCl_3$ was added at room temperature to a well-stirred solution of 0.0044 mole (1.5 eq) N,N-*bis*(diethylene-monomethylether) amine and 0.073 mole (2.5 eq) triethylamine in 20 mL $CHCl_3$, contained in a round-bottom flask. BOP reagent [0.003 mole (1 eq)] was added, and the mixture was stirred for 24 h. The reaction mixture was extracted three times with 20 mL aqueous acid solution. The organic phase was washed again with 20 mL of an aqueous saturated solution of $NaHCO_3$, dried over Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The product was purified by column chromatography (silica gel, eluent $CHCl_3$/MeOH 98:2). The results are as follows: N^{α}, N^{ε}-dihexanoyl lysine N,N-*bis*methyl diethyleneglycol amide: 55K22: weight: 0.59 g; anal. for.: $C_{18}H_{34}N_2O_4$: % calc. (found): C: 61.62 (61.47); H: 10.16 (10.31); N: 7.70 (7.64); N^{α}, N^{ε}-dioctanoyl lysine N,N-*bis*methyl diethyleneglycol amide: 77K22: weight: 0.65 g; anal. for.: $C_{32}H_{63}N_3O_7$: % calc. (found): C: 63.86 (63.82); H: 10.55 (10.52); N: 6.98 (6.91). MS-FAB$^+$-glycerol/MeOH (*m/e*): 603 (M^+ + 1, 6%), 526 (17%), 381 (56%), 236 (8%), 222 (100%), 210 (57%); N^{α}, N^{ε}-didecanoyl lysine N,N-*bis*-methyl diethyleneglycol amide: 99K22: M.W.: 658.0; weight: 0.55 g; anal. for.: $C_{36}H_{71}N_3O_7$: % calc. (found): C: 65.72 (66.04); H: 10.88 (11.07); N: 6.39 (6.27); N^{α}, N^{ε}-didodecanoyl lysine N,N-*bis*methyl diethyleneglycol amide: 1111K22: weight: 0.46 g; anal. for.: $C_{40}H_{79}N_3O_7$: % calc. (found): C: 67.28 (66.98); H: 11.15 (10.95); N: 5.88 (5.77). N^{α},N^{ε}-dioctanoyl lysine N,N-*bis*methyl diethyleneglycol amide: 77K33: weight: 0.54 g; anal. for.: $C_{36}H_{71}N_3O_9$: % calc. (found): C: 62.67 (63.04); H: 10.37 (10.61); N: 6.09 (6.42). MS-FAB$^+$-glycerol/MeOH (*m/e*): 647 (6%), 588 (36%), 570 (11%), 468 (11%), 381 (65%), 310 (19%), 208 (100%); N^{α},N^{ε}-didecanoyl lysine N,N-*bis*methyl triethyleneglycol amide: 99K33: weight: 0.57 g; anal. for.: $C_{40}H_{79}N_3O_9$: % calc. (found): C: 64.40 (63.83); H: 10.67 (10.83); N: 5.63 (5.70); N^{α},N^{ε}-didodecanoyl lysine N,N-*bis*-methyl triethyleneglycol amide: 1111K33: weight: 0.52 g; anal. for.: $C_{44}H_{87}N_3O_9$: % calc. (found): C: 65.88 (65.47); H: 10.93 (10.83); N: 5.24 (5.11). Spectral characteristics for diacyl lysines N,N-*bis*methyloxyethyleneglycol amides (*mm*Kn_2n_2) are as follows: IR (film): 3,298 cm^{-1} (NH); 2,954 cm^{-1} (CH_2); 1,651 cm^{-1} (C=O, amide I); 1,547 cm-1 (N-C=O, amide II); 1,113 cm-1 (C-O-C); ^1H NMR (200 MHz, δ, $CDCl_3$): 0.9 ppm (*t*, 6H, $2CH_3$); 1.3–1.7 ppm (*m*, 18H, CH_2); 2.2 ppm (*m*, 4H, 2 × CH_2-CO); 3.25 ppm (*m*, 2H, -CH_2-*CH_2*-



SCHEME 2

NH-CO); 3.37 ppm (*s*, 6H, -OCH_3); 3.4–3.7 ppm [*m*, 16H, 2(CH_2-CH_2-O)$_2$]; 4.95 ppm (*m*, 1H, CH); 5.98 ppm (*t*, 1H, *NH*-); 6.45 ppm (*d*, 1H, *NH*-CH); ^{13}C NMR (50 MHz, δ, $CDCl_3$): 13.46 ppm (2 × *CH_3*); 21–49 ppm (16 × CH_2); 49.54 ppm (CH); 58.41–58.38 ppm (2 OCH_3); 68–72 ppm (4 × CH_2-CH_2-O); 172.31 ppm (1 HN-*C*=*O*, amide); 172.63 ppm (1 HN-*C*=*O*, amide); 173.11 ppm (1 N-*C*=*O*, amide).

RESULTS AND DISCUSSION

Synthesis. To accurately establish the relationship between the chemical structures of the new compounds and their properties, it was necessary to synthesize highly purified compounds in which the lengths of alkyl and polyoxyethylene chains were modified systematically, so that the hydrophobic part contained a definite number of methylene units and the hydrophilic part a definite number of EO units.

The strategy of synthesis described in Scheme 2 consisted of three main parts: (i) condensation of short- (hexanoyl, octanoyl) and long-chain (decanoyl, dodecanoyl) fatty acids to the lysine to prepare the N^{α}, N^{ε}-diacyl lysine homologues (hydrophobic part); (ii) preparation of hydrophilic methylpolyoxyethylene amine residues that contain a definite number of OE residues (hydrophilic part); (iii) condensation of the hydrophobic modified lysine with the hydrophilic moiety to obtain the final nonionic amphiphilic compounds from lysine (coupling part). Synthetic experimental procedures and results of compound characteristics were described earlier in this paper.

Pure and optically active short- and long-chain N^{α}, N^{ε}-diacyl lysine derivatives (**1**) were prepared in yields of about 60% by following the modified Schotten–Bauman procedure, which was developed by Takehara, *et al.* (7) to prepare long-chain Glu derivatives. The N^{α}, N^{ε}-diacyl lysine derivatives (**1**) were characterized by thin-layer chromatography (TLC) and ^{1}H NMR (nuclear magnetic resonance), ^{13}C NMR, and Fourier transform infrared (FTIR) spectroscopies. The purity of these compounds was checked by high-performance liquid chromatography (HPLC) and elemental analysis. In the ^{1}H NMR spectra, the two amide protons (NH) were observed between 6–6.7 ppm. The two carbonyl groups of the amide at 171–173.4 ppm and one carbonyl group of carboxylic acid at 174 ppm were observed in the ^{13}C NMR spectra. All compounds obtained were hygroscopic white solids with well-defined melting points. As expected, the melting point increased when the hydrocarbon chainlength increased.

The hydrophilic characteristics of the compounds were modified by introduction of monodisperse POE amine chains. The terminal hydroxyls of the POE amine chains were protected by O-methylation to prevent a progressive degradation of the final compounds due to the slow reaction of the terminal hydroxyls in the amide functions (8). The linear monodisperse polyoxyethylenemonomethylether amines (**2**) (n_1 = 2, 4, or 6) were prepared for the first time by reaction of tosyloxyethylene-monomethyl ether with sodium azide in a 1:1 molar ratio and subsequent hydrogenation of the azide intermediate. The overall yield of this reaction was approximately 65%. The N,N-*bis*(diethylene-monomethylether) amine (**4**) (n_2 = 2) was obtained from benzylamine as the starting material, to which tosyldiethylene-monomethylether was condensed in the presence of Na_2CO_3 and acetonitrile. Debenzylation by catalytic hydrogenation produced the desired amine. Overall yield of this reaction was about 60% (8,9). The ^{1}H NMR and ^{13}C NMR spectra confirmed the structures of **2** and **4**.

Given that the yield of the N,N-*bis*(triethylene-monomethylether) amine (**4**) (n_2 = 3) with the above-described benzylamine method was low (30%), compound **4** was prepared by the diethanol method, as reported by Guttmann (10). In this method, the trytildiethanolamine was condensed with tosyldiethylene-monomethylether, and subsequent deprotection of the trytil group was carried out. The overall yield of the reaction was about 60%. The reaction was monitored by TLC.

Silica-gel TLC showed that these amines were chromatographically pure compounds. Each amine showed a single spot at different R_f values depending on the number of the OE units. R_f values decreased regularly as the number of the OE units increased. The condensation of the hydrophobic modified lysine with the hydrophilic moiety (coupling part) was carried out by the well-known ester active of hydroxylbenzotriazole (HOBT) [formed *in situ* by reaction with BOP reagent benzotriazole-1-yl-oxy-*tris*(dimethylamino)-phosphoniumhexafluorophosphate] (11). The coupling reaction was fast and was easily monitored by TLC. Tables 1 and 2 summarize the main characteristics of the series of **3** and **5** compounds, respectively. The structures of the final **3** and **5** compounds were characterized by ^{1}H NMR, ^{13}C NMR, and FTIR, elemental analysis, and fast-atom bombardment /mass spectrometry (FAB/MS).

The purification of compound **3** was carried out by recrystallization from hexane or petroleum ether, and, in some cases, the residue was previously chromatographed with chloroform/methanol (C/M) as eluent. The purity of these compounds was checked by elemental analysis and HPLC. Only one peak was observed for all products by HPLC. The compounds of this series were white solids with a crystalline aspect with defined melting points between 72 and 126°C.

^{1}H NMR spectra showed the three amide protons (NH). The amide proton joined to the polyoxyethylene chain appeared in low fields as a triplet with a coupling constant of approximately 3 Hz. In the ^{13}C NMR spectra, only three amide carbonyl could be observed between 171–175 ppm. The absence of the carbonyl group corresponding to carboxylic acid (FTIR, ν = 1,740 cm^{-1}) indicated that the starting material, N^{α}, N^{ε}-diacyl lysine was not present. Compounds **5** were obtained in an oily form by column chromatography with C/M as eluent. Yields of the reactions were lower than those of series **3** compounds, probably because of the minor reactivity of the secondary amine due to steric hindrance vs. the primary amine. Purity of these compounds was checked by elemental analysis and HPLC.

After several recrystallizations, the elemental analysis of a

few compounds from series **3** and **5** were off by more than 0.5% for the carbon atom. They gave one peak, however, at the HPLC and spectral analysis consistent with the expected structures. In these cases, the solids probably crystallized with a small amount of water. Retention time of HPLC (see Tables 1 and 2) increased when the length of the hydrocarbon chain increased. No differences were observed, however, when the OE units were changed.

Physicochemical properties. All compounds of series **3** were not soluble in water, except the shorter homologues 55K0n_1 and 77K06. By contrast, the compounds of series **5** were all soluble in water, except the longest homologue 1111K22, whose solubility in water was approximately 10^{-5} molar at 25°C (see Table 3).

It is well known that there is a relationship between the crystallinity of a compound and its solubility in water (12). The more stable the crystallinity, the lower the water solubility. Solubility differences between the two series may be due to structural effects of the hydrophilic moiety in which the POE chains and the amides C(O)-N or C(O)-NH are involved. The geminal structure of the N,N-*bis*(POE-monomethylether) amine and the hydrophilicity of the C(O)-N amide group could lessen the tendency to crystallization of series **5**, giving rise to oily compounds soluble in water. The hydrophilicity of C(O)-N is equivalent to more than 5 EO units (9).

A systematic study to determine the cloud point of these compounds was not carried out in our study, although the water solubility behavior of their 1% (wt/vol) aqueous solutions was determined at the temperature range of 5–80°C. Clear solutions were obtained at up to 60°C for all compounds except for series *mm*K33, whose turbidity was observed at 10°C. According to the data reported in the literature, we expected that the cloud point of series **5**, which contains two short POE chains, would be lower than those of conventional nonionic surfactants. The hydration of these hydrophilic chains could probably be hindered by the effect of the configurational characteristics of the geminal structure of the POE chain capped with a methoxyl group (13,14). The study of the effect of the temperature on the surfactant's solubility behavior and on the cloud point as a function of surfactant concentration is currently in progress.

TABLE 3
Limits of Solubilities of *mm*K0n_1 and *mm*Kn_2n_2 in Water at 25°C

Code	Limit of solubility in water (mol L^{-1})
55K02	≥0.023
55K04	≥0.018
55K06	≥0.016
77K02	$<2 \times 10^{-6}$
77K04	$<1.7 \times 10^{-5}$
77K06	≥0.0015
99K02	$<1.8 \times 10^{-6}$
99K04	$<1.5 \times 10^{-6}$
99K06	$<1.4 \times 10^{-6}$
1111K02	$<1.6 \times 10^{-6}$
1111K04	$<1.4 \times 10^{-6}$
1111K06	$<1.3 \times 10^{-6}$
55K22	≥0.018
77K22	≥0.017
99K22	≥0.015
1111K22	$<1.4 \times 10^{-5}$
77K33	≥0.014
99K33	≥0.013
1111K33	$<1.3 \times 10^{-4}$

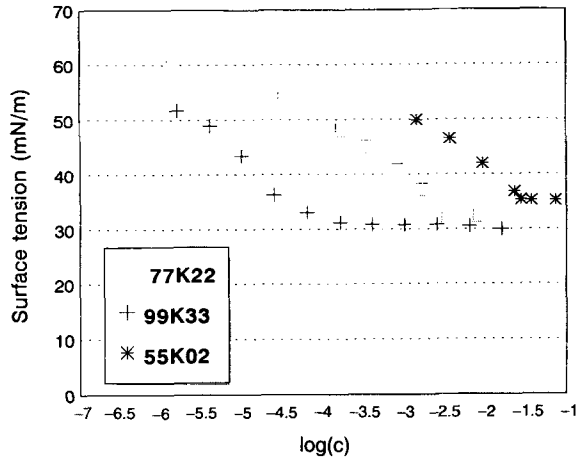


FIG. 1. Surface tension/log(*c*) curve for 55K02, 77K22, and 99K33 in water at 25°C.

To ascertain whether our amphiphilic compounds tended to adsorb at interfaces and act as surface-active materials, measurements were made at air/water interfaces of surface-active properties, such as surface tension (γ) and critical micellar concentration (CMC), of their aqueous solutions. The study was limited to the water-soluble compounds in which the surface-tension reduction of the aqueous solutions could be determined as a function of the log(*c*) at room temperature (see Fig. 1).

Maximum surface excess concentrations (Γ_{max} in mols/cm^2) and minimum areas per surfactant molecule (A_{min} in $nm^2 \times 10^2$) at the aqueous solution/air interface were obtained from the maximum slopes of surface tension (γ) vs. logarithm of the compound concentration [log(*c*)] plots with the classical Gibbs equation (15).

The interfacial properties of the water-soluble compounds of series **3** and **5** are listed in Tables 4 and 5, respectively. For comparison, the same data for some short-chain lecithin homologues are also shown in Table 5 (16).

These data suggest that the compounds of this study, even those of the shorter chains, are surface-active materials with an ability to form micelles (or aggregates) at concentrations in the millimolar range.

TABLE 4
Physicochemical Properties of Water-Soluble Compounds of Series 3 at 25°C

Compound	CMC (mM)	γ_{sat} (mN/m)	A_{min} ($nm^2 \times 10^2$)	Γ_{max} (mols/cm$^2 \times 10^{10}$)
55K02	34	35.3	87	1.9
55K04	44	35.4	93	1.8
55K06	35–55	35.1	—	—
77K06	1.6	35.6	102	1.6

TABLE 5
Physicochemical Properties of Water-Soluble Compounds of Series 5

Compound	CMC (mM)	γ_{sat} (mN/m)	A_{min} ($nm^2 \times 10^2$)	Γ_{max} (mols/$cm^2 \times 10^{10}$)
55K22[a]	57.0	35.5	103	1.6
77K22[a]	5.8	32.3	96	1.7
99K22[b]	0.88	32.9	79	2.1
77K33[b]	7.2	34.8	103	1.6
99K33[b]	0.97	30.8	85	1.9
55lecithin[c]	9.8	30.1	—	—
77lecithin[c]	0.17	24.1	—	—

[a]Measured at 25°C.
[b]Measured at 6°C.
[c]Reported by Wells; measured at 45°C (15). 55lecithin corresponds to dihexanoyl lecithin; 77lecithin corresponds to dioctanoyl lecithin.

For the same hydrophobic chainlength, the hydrophilic moiety does not significantly affect CMC values. This is reflected by CMC data for 55K04 and 55K22 homologue pairs or 77K06 and 77K33 homologue pairs. The surface-tension values (γ) vs. log(c) curve for the 55K06 compound showed a minimum, which is probably due to an unanalyzed surface-active impurity, which we were not able to remove. In this case, we assumed the CMC to be within a concentration range that was about the minimum. Consequently, the values for A_{min} and Γ_{max} were not determined.

CMC data of series **5** show that, when there is an increase of two methylene groups per hydrophobic chain, CMC is diminished by a factor of approximately 10. A similar magnitude was reported for monodisperse nonionic polyoxyethylene surfactants with one single chain of 8–14 carbon atoms when there was an increase of 2 methylene groups (17). These findings could indicate that the aggregate formed by our compounds depends essentially on the length of each chain and not on the total number of methylene units. It is conceivable that the two hydrophobic chains in one molecule of surfactant could interact in such a way that their global hydrophobic effect could be comparable to that of one chain.

For comparison, lower surface-tension values and CMC data in aqueous solutions at 25°C are shown in Table 5 for short-chain lecithins (16). For instance, aqueous solutions of the 77 lecithin homologue reduced the surface tension of water to 24.1 mN/m at a CMC of 0.17 mM, whereas their equivalent compounds in series **3** and **5** (77K06 and 77K22 or 77K33) reduced the surface-tension values to about 32–35 mN/m at a CMC range of 1.6–7.2 mM.

For lecithins, the contribution of the hydrophobic groups to the CMC parameter differs from those of our compounds. An increase in 2 methylene groups per chain in the lecithins decreased its previous value by 50 (16).

These results presumably indicate that, in the lecithins, the two short chains contribute to the whole molecular hydrophobic character, whereas in our compounds (at least for the homologues described in this study), only one short chain was responsible for the whole hydrophobicity in the molecule.

These differences could be attributed to the different structure of the central pivot in the trifunctional lysine amino acid and to the higher hydrophilicity of the amide groups when compared with the structure of natural lecithins.

A_m values (obtained from the surface excess, Γ_{max}) of the compounds of series **3** and **5** at the air/water interface were higher than those of the classical nonmethylated POE alcohols with a number of oxyethylene units of the same order of magnitude and the same hydrophobic chainlength. As in a normal series of conventional POE surfactants, the A_m values of the compounds in this study rise when EO units increase or when the alkyl chain decreases in both series of compounds **3** and **5**. According to Kuwamura and Takahashi (13,14), A_m data of these new surfactants should be discussed in terms of three structural factors: (i) hydrophobic chainlength, (ii) connecting trifunctional lysine group, and (iii) effect of the number of methyoxyl POE chains and OE units. Because the molecular area of surfactants is determined by the structural characteristics of the hydrophilic head, we suppose that (i) and (ii) are the main factors than influence A_m values of these compounds.

A theory of micellar structure, based on the geometry of various micellar shapes and the space occupied by the hydrophilic and hydrophobic groups of the surfactant molecules, has been developed by Israelachvili (18). We can thus determine the shape of the micelle. Thermodynamic parameters concerning the contribution per CH_2 groups to the standard free energy of adsorption and micellization, micellar shapes, and micellar weights of these compounds and some peculiarities of the phase separation in the water system of the compounds will be discussed and compared with natural lecithins in subsequent publications.

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