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Cationic Surfactants Derived from Lysine: Effects of Their Structure and Charge Type on Antimicrobial and Hemolytic Activities

A. Colomer,[†] A. Pinazo,[†] M. A. Manresa,[§] M. P. Vinardell,[#] M. Mitjans,[#] M. R. Infante,[†] and L. Pérez*,[†]

[†]*Departamento de Tecnología Química y de Tensioactivos, IQAC, CSIC, C/Jordi Girona 18-26, 08034 Barcelona, Spain,* [§]*Departament de Microbiología, and* [#]*Departament de Fisiología, Facultat de Farmacia, Universitat de Barcelona, Avinguda Joan XIII s/n, 08028 Barcelona, Spain*

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Three different sets of cationic surfactants from lysine have been synthesized. The first group consists of three monocatenary surfactants with one lysine as the cationic polar head with one cationic charge. The second consists of three monocatenary surfactants with two amino acids as cationic polar head with two positive charges. Finally, four gemini surfactants were synthesized in which the spacer chain and the number and type of cationic charges have been regulated. The micellization process, antimicrobial activity, and hemolytic activity were evaluated. The critical micelle concentration was dependent only on the hydrophobic character of the molecules. Nevertheless, the antimicrobial and hemolytic activities were related to the structure of the compounds as well as the type of cationic charges. The most active surfactants against the bacteria were those with a cationic charge on the trimethylated amino group, whereas all of these surfactants showed low hemolytic character.

Introduction

Cationic surfactants have two important properties: they are easily absorbed at solid/liquid interphases and can interact with the cellular membranes of microorganisms. Consequently, compounds of this type behave as good antimicrobial agents, and for many years they have been used as disinfectants in hospitals and in the food industry.[1,2] Recently, it has been shown that cationic amphiphiles have great potential in new therapeutic biomedical applications as antimicrobial and antifungal agents in human infections. They can also be used in gene therapy (cationic vesicles made from cationic surfactants, gemini surfactants in particular, can encapsulate RNA or DNA for cellular transfer),[3−5] as vehicles for certain drugs[6,7], and as modifiers of the physicochemical and biological properties of biomaterials (prosthesis and synthetic veins).[8] These types of therapeutic applications require the use of stable surfactants under sterilization conditions that do not present hemolytic or cytotoxic activities. Moreover, the antimicrobial activity of amphiphilic compounds can be of great interest for some of these applications.

Classic cationic surfactants derived from quaternary ammonium show good antimicrobial activity and are capable of forming vesicles to transport drugs and genetic material.[9] However, these compounds present hemolytic activity;[10] therefore, they are not suitable for biomedical applications. Furthermore, their use is seriously questioned from an environmental point of view as they are not readily biodegradable and are toxic to aquatic organisms. The new generation of cationic surfactants derived from quaternary ammonium, the ester-QUATS type, improves the biodegradation, but these surfactants are still toxic to the aquatic environment and present problems of stability because they hydrolyze easily in both acidic and basic conditions as well as at high temperatures.[11]

Amino acid based surfactants constitute an important class of natural surface-active molecules of great interest to organic and physical chemists as well as biologists with an unpredictable number of basic and industrial applications.[12−14] Our research group has synthesized and studied several new antimicrobial cationic surfactants derived from amino acids with different structures: monocatenary or single chain (one head/one tail), gemini (two heads/two tails linked by a spacer chain), and glycerolipid (one head/one or two tails linked together through a glycerol skeleton).[15] The presence of the cationic guanidine group of arginine in these amphiphilic structures gives a wide variety of compounds with a rich phase behavior and strong antimicrobial activity. They present physicochemical properties similar to those of classic cationic surfactants but are more environmentally friendly and less toxic to the eyes and skin than the conventional compounds.[16] It has been demonstrated that the presence of monocatenary arginine surfactants improves the transfection efficiency when they are conjugated to cationic liposome systems.[17] Cationic gemini surfactants based on arginine are extraordinarily active at the surface.[18,19] They have critical micelle concentrations (CMC[a]) much lower than those of the monocatenary arginine-based surfactant homologues while maintaining their biological properties (antimicrobial activity, biodegradation,

*Author to whom correspondence may be addressed (phone: 34 93 4006164; fax: (+33) 34 93 2045904; e-mail: lourdes.perez@cid.csic.es).

[a] Abbreviations: CMC, critical micelle concentration; Cbz, carbobenzyloxy, MIC, minimum inhibitory concentration ; DTAB, dodecyltrimethylammonium bromide; NMR, nuclear magnetic resonance; MHB, Mueller−Hinton broth; BOP, benzotriazol-1-yl-oxytris-(dimethylamino)phosphonium hexafluorophosphate; DABCO, 1,4-diazabicycle(2.2.2)octane.

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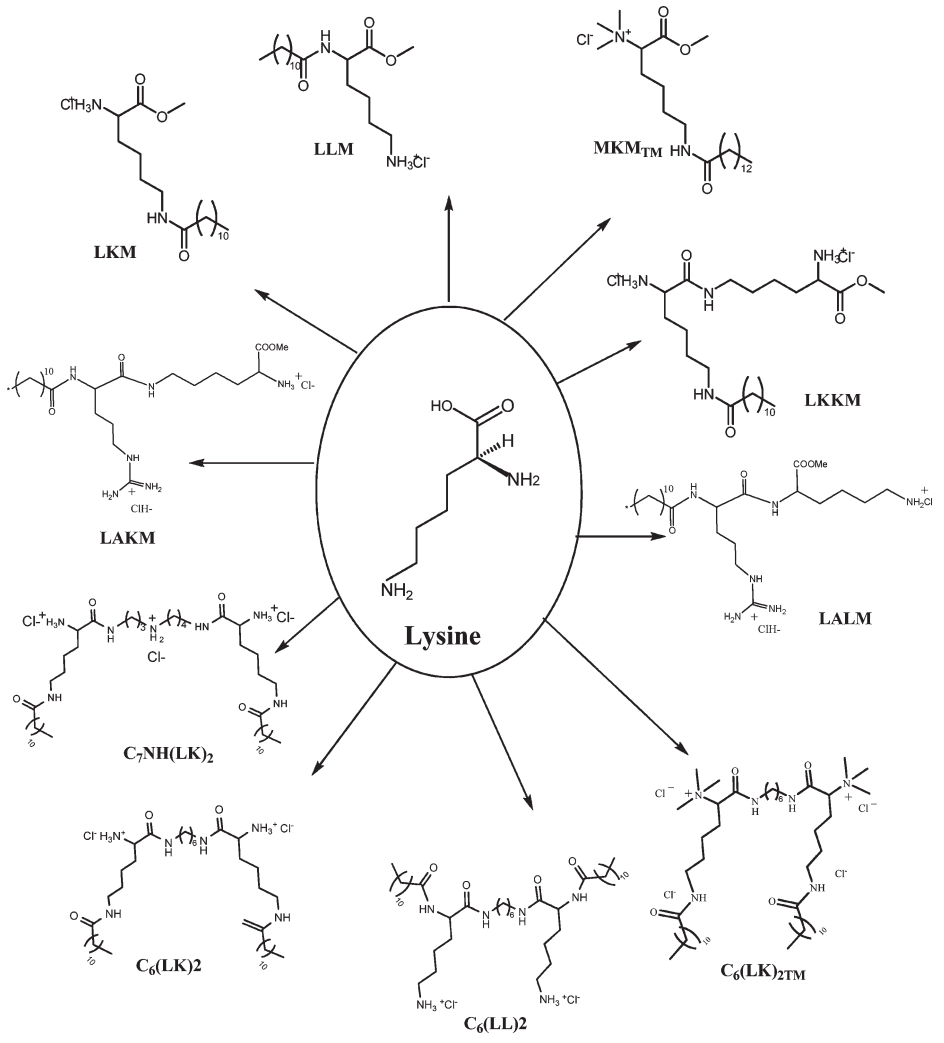


Figure 1. Chemical structures of new synthetic surfactants from lysine.

and toxicity) unchanged. However, these compounds present toxicity levels that make them unsuitable for applications in which the compound has to be in contact with biological systems. Therefore, it is interesting to extend this area of research to obtain surfactants with properties similar to the arginine derivatives but with lower toxicity against human cells.

In this work we report the synthesis and characterization of a new series of lysine-based surfactants designed for special biomedical applications. The amino acid lysine, with two amino groups of different pK_a/basicity values and one carboxylic acid function, offers the possibility to design a wide range of surfactant architectures, which is an excellent opportunity for tailoring the biological as well as surfactant aggregation behavior for multipurpose uses.

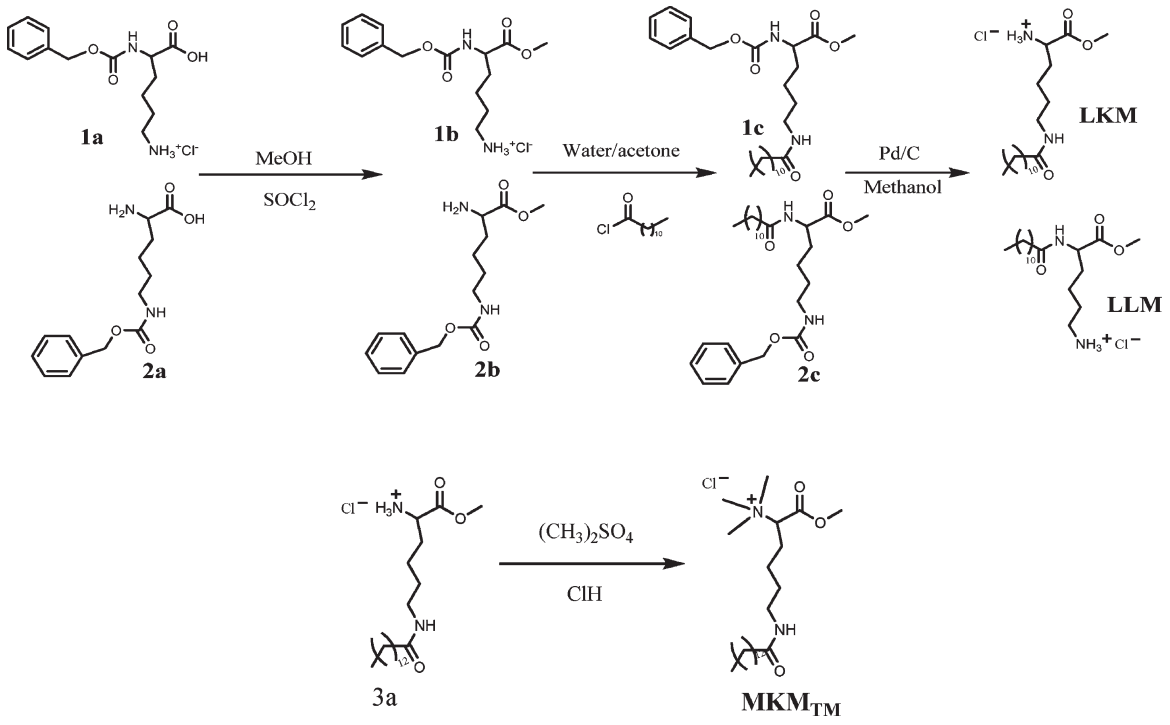
Figure 1 shows the chemical structures and acronyms of the synthesized surfactant molecules. We have prepared monocatenary and gemini lysine-based cationic surfactants in which the nature of the head polar group, the character of the spacer, and the type and quantity of the cationic charge in the headgroup region have been systematically varied. The alkyl chain has always been 12 carbon atoms except for one of the monocatenary surfactant that contains 14 carbon atoms. The following compounds have been prepared.

(a) Three monocatenary surfactants with one lysine as the cationic polar head (one cationic charge) and one alkyl chain: N^{α}-lauroyl-lysine methyl ester (LLM) with one alkyl chain of 12 carbon atoms and one positive charge on the ε-amino group of the lysine, N^{ε}-lauroyl-lysine methyl ester (LKM) with one alkyl chain of 12 carbon atoms and the positive charge on the α-amino group of the lysine, and N^{ε}-mirystoyl-N^{α} -trimethyl-lysine methyl ester (MKM) with one alkyl chain of 14 carbon atoms and the positive charge lying on the trimethylated ε-amino group.

(b) Three monocatenary surfactants with two amino acids as the cationic polar head, two positive charges, and one alkyl chain of 12 carbon atoms: N^{α}-lauroylarginine-N^{α}-lysine methyl ester (LALM) with two positive charges, one on the guanidine group of arginine and the other on the ε-amino group of lysine; N^{α}-lauroylarginine-N^{ε}-lysine methyl ester (LAKM) with one positive charge on the guanidine group of arginine and the other on the α-amino group of lysine; and N^{ε}-lauroyl-lysine-N^{ε}-lysine methyl ester (LKKM) in which the two positive charges lie on the α-amino group of the lysines.

(c) Four gemini surfactants in which the spacer chain and the number and type of cationic charges have been regulated: N^{ε},N^{ω}-bis(N^{ε}-lauroyl-lysine) α,ω-hexylendiamide ($C_6(LK)_2$), with two positive charges on the α-amino groups of the lysine and a methylene-based spacer chain; N^{α},N^{ω}-bis(N^{α}-lauroyl-lysine) α,ω-hexylendiamide ($C_6(LL)_2$), with two positive charges on the ε-amino groups of the lysine and a methylene-based spacer chain; N^{ε},N^{ω}-bis(N^{ε}-lauroyl-N^{α}-trimethyl-lysine) α,

Scheme 1. Synthetic Pathway for the Synthesis of Single-Chain Lysine Surfactants



ω-hexylendiamide ($C_6(LK)_{2\,TM}$), with two positive charges on the two trimethylated α-amino groups of the lysine and a methylene-based spacer chain; and finally N^{ε},N^{ω}-bis(N^{ε}-lauroyl-lysine) α,ω-spermidindiamide ($C_7NH(LK)_2$), with a spermidine-based spacer and two positive charges on the α-amino groups of the lysine but with a third positive charge on the spacer chain.

These compounds may represent a step forward from a scientific and application point of view, first, because of the growing need for new nontoxic and biodegradable systems and, second, because of the importance of exploring new systems with novel properties.

For all of these new surfactants, we have studied the micellization process and their antimicrobial and hemolytic activities to determine the influence of different structural parameters on their physicochemical and biological properties.

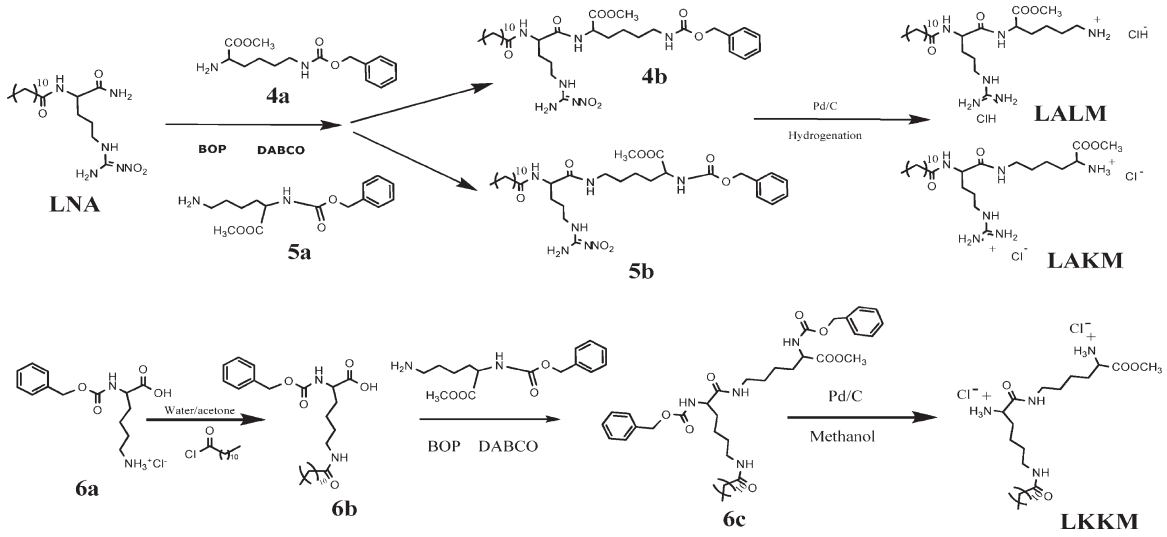
Results and Discussion

Synthesis. One of the most important variables in the structure of cationic surfactants is the length of the alkyl chain. There are numerous studies demonstrating that the aggregation and biological properties of a surfactant are not determined only by the hydrophobic tail but by the hydrophilic head, including the size and the cationic charge (type, number, and spatial positioning of the positive charge).

In this work, we have changed the position of the cationic charge. Lysine is a dibasic amino acid consisting of two amino groups with different basicities because the α-amino group has a $pK_a = 8.9$ and the ε-amino group a $pK_a = 10.5$. This feature can be used to obtain two different types of cationic surfactants (see Figure 1): compounds with the positive charge on the α-amino group and compounds with the positive charge on the ε-amino group. We have prepared monocatenary and gemini N^{α}- and N^{ε}-acyl lysine derivatives with a dodecyl or tetradecyl aliphatic chain as cationic surfactants. These compounds are made from natural fatty acid and amino acid organic building blocks. For monocatenary surfactants, the polar group consists of one or two positively charged amino acids, lysine and arginine, whereas the gemini group consists of two monocatenary N^{α}- or N^{ε}-lysine amphiphiles connected at the level of the headgroup by a spacer chain of different ionic character. In all cases, all building blocks are linked by amide bonds to design biodegradable molecules that are benign to the environment.

Monocatenary *N*-Lauroyl-lysine Surfactants with One Positive Charge on the Polar Head. Monocatenary *N*-lauroyl-lysine surfactants with one positive charge on the polar head, LLM and LKM, were prepared using commercially available N^{α}-carbobenzyloxy-lysine·HCl (N^{α}-Cbz-lysine·HCl) (**1a**) or N^{ε}-Cbz-lysine·HCl (**2a**), respectively, as starting material. Because lysine has two amino groups of similar reactivity, the use of N^{α}- or N^{ε}-protected lysine is an essential requirement for the synthesis of these compounds. A three-step procedure has been used to obtain these compounds (Scheme 1). The first step consisted of the preparation of the N^{α}-Cbz-lysine methyl ester (**1b**)/N^{ε}-Cbz-lysine methyl ester (**2b**) HCl salts (α-carboxylic group protection). Then, N^{ε}-lauroyl-N^{α}-Cbz-lysine methyl ester (**1c**)/N^{α}-lauroyl-N^{ε}-Cbz-lysine methyl ester (**2c**) was obtained by acylation of the ε/α-amino group with lauroyl chloride. The reaction progress was monitored by HPLC, and after 4 h, a 92% conversion of the starting reagent was obtained. The purification of compounds was carried out by silica gel chromatography. Satisfactory HPLC analyses were obtained for these materials, yielding fast atom bombardment mass spectroscopy (FABMS) and nuclear magnetic resonance (NMR) spectra, consistent with the target compounds. The third step involved the removal of the Cbz group by catalytic hydrogenation of the **1c** and **2c** using Pd over charcoal to give LLM and LKM, respectively. The reaction was carried out by controlling the pH to prevent the hydrolysis of the ester linkage present in these compounds. Pure compounds were obtained after several crystallizations in methanol/acetonitrile.

Scheme 2. Synthetic Pathway for the Synthesis of Single-Chain Diamino Acid Surfactants



The chemical structure of these compounds was checked by NMR. The proton ^{1}H and carbon ^{13}C NMR spectra were in concordance with the proposed structures. Elemental analyses for the derivatives were in good agreement with the calculated ones. MKM was obtained following the same procedure as described for LKM. The trimethylation of MKM (**3a**) was carried out using the classical reaction with dimethyl sulfate and potassium carbonate.

Monocatenary *N*-Lauroyl Surfactants with Two Positive Charges on the Polar Head. The second group of surfactants consisted of three cationic surfactants in which the polar head has two positive charges provided by two basic amino acids bonded by peptide linkages: arginine−lysine (LAKM), arginine−lysine (LALM), and lysine−lysine (LKKM). The general procedure for the preparation of these surfactants is shown in Scheme 2. To prepare LALM and LAKM, the temporary protected lauroylnitroargine (LNA) was used. LNA was synthesized following the procedure described in ref 18. The introduction of the strongly electronegative nitro function decreased the basic nature of the guanidine group, thus facilitating the incorporation of the lauroyl residue by the formation of an amide bond without the competition of the guanidine side chain. Compounds **4b** and **5b** (Scheme 2) were obtained by condensation of LNA with the free amino group of the N^{ε}- or N^{α}-Cbz-lysine methyl ester (**4a** and **5a**, respectively). In this reaction, the reagent benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) was used as condensation agent in the presence of 1,4-diazabicycle(2.2.2)octane (DABCO) base. The formation of LKKM was achieved using the same procedure as shown in Scheme 2. In this case the lauroyl N^{α}-Cbz-lysine methyl ester (**6b**) was condensed to N^{α}-Cbz-lysine methyl ester. These reactions occurred readily in CH_2Cl_2 solutions at room temperature and were essentially complete within 1 h. This approach was chosen because it allowed us to perform the synthesis in a short time and on a large scale. Finally, total deprotection of the guanidine group of the arginine and the amino groups of the lysine were carried out by removal of the nitro and Cbz groups by means of hydrogenolysis to return the corresponding target surfactants with yields on the order of 95%. As we had previously observed,[20] the elimination of the Cbz group was quite rapid, but the nitro group required more time (6 h) as well as some pressure (3 atm). The reaction was carried out by controlling the pH to prevent the hydrolysis of the ester linkage present in these compounds. Hydrochloric acid was added to obtain the surfactants in the form of hydrochloride salts. Typical overall yields of these products (LALM, LAKM, LKKM) were 65−75%. The proton ^{1}H and carbon ^{13}C NMR spectra of these compounds showed all peaks corresponding to the target surfactants. The MS spectra also agreed with the proposed structure. In addition, the results from the combustion analysis experiments confirmed the high purity of the compounds (on the order of 98−99%).

Gemini Surfactants with Spacer Chains of Different Cationic Character. Four gemini surfactants, $C_6(LK)_2$, $C_6(LL)_2$, $C_6(LL)_{2\ TM}$, and $C_7NH(LK)_2$, were prepared via the synthetic outline displayed in Scheme 3. Gemini $C_6(LK)_2$ and $C_6(LL)_2$ with two amino cationic charges per molecule were synthesized by condensation of the Cbz-protected $N^{\alpha}/N^{\varepsilon}$-lauroyl-lysine (LZL/LZK in Scheme 3) to both amino primary groups of the α,ε-hexylenediamine. This condensation was carried out using BOP as coupling agent and DABCO as base. This method was used by our group in the past to obtain gemini surfactants from arginine with very good yields.[18] Target compounds were easy to isolate by recrystallization from hot methanol (MeOH). Spermidine instead of hexylenediamine was used as spacer chain for the preparation of $C_7NH(LK)_2$, which contains three cationic charges in the overall molecule. Removal of the Cbz protecting group was achieved using the same procedure as described above. Gemini $C_6(LK)_{2\ TM}$ with two cationic charges of the quaternary ammonium type was obtained in good yield by trimethylation of both N^{α}-terminal amino groups of the $C_6(LK)_2$ molecule with excess of dimethyl sulfate using anhydrous potassium carbonate (K_2PO_3) as base. Finally, the sulfate anion was changed by the chlorhydrate using a cationic exchange column.

The symmetrical derivatives display simplified ^{1}H and ^{13}C NMR spectra that facilitate their interpretation. All of the symmetrical H and C have the same chemical shift, and the spectra are very similar to those of the corresponding single-chain compound. Only the presence of the signals corresponding to the spacer chain indicates the dimeric character of the surfactant. The structures of all these surfactants were also confirmed by UPLC-MS, and the purity was checked by combustion analysis.

Scheme 3. Synthetic Pathway for the Synthesis of Gemini Surfactants

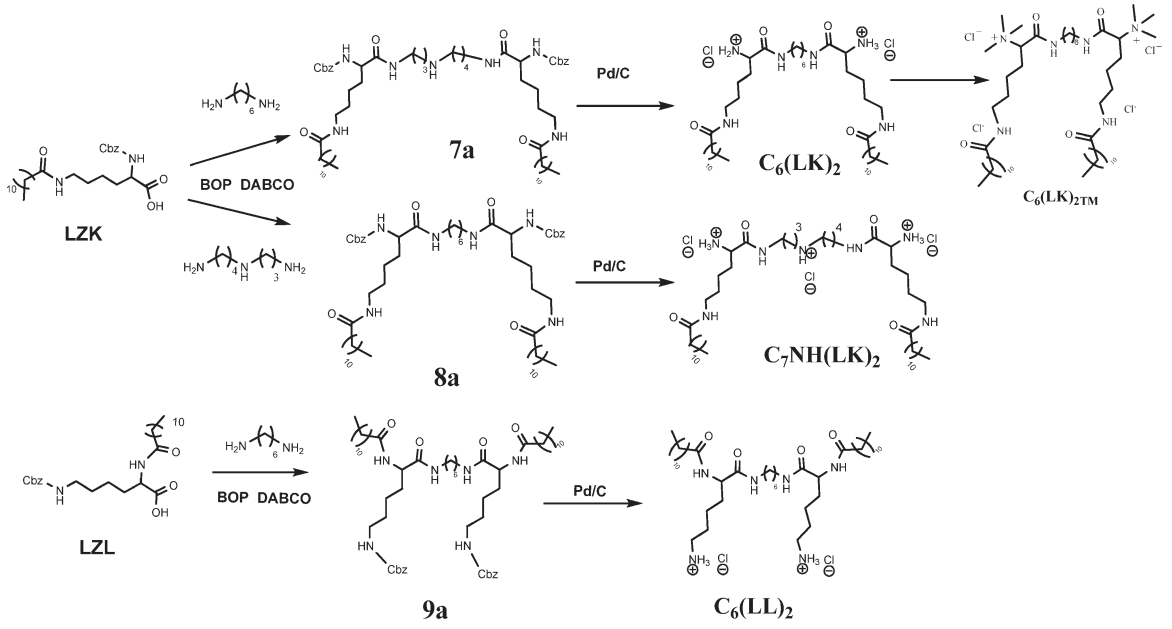


Table 1. HPLC Retention Times and CMCs of Lysine Surfactants

surfactant	HPLC retention time (min)	CMC (mM)
LAKM	11.4	26
LALM	11.4	25
LKKM	12.8	22
LKM	13.4	5.5
LLM	14.8	7.2
MKM_{TM}	17.7	2.2
$C_6(LL)_2$	17.6	0.74
$C_6(LK)_2$	17.8	0.50
$C_6NH(LK)_2$	18.7	1.9
$C_6(LK)_{2\ TM}$	20.5	0.75

Critical Micelle Concentration. To investigate the effect of the structure, number of cationic charges, and their position on the aggregation properties, the CMC was determined for each *N*-acyl-lysine-based surfactants. The conductivity of aqueous solutions at 25 °C was measured at the adequate concentration range. Conductivity of the aqueous solutions increased linearly with increasing concentration up to break points due to micellization and counterion binding that corresponds to the CMC of these surfactants (see Figure S1 in the Supporting Information).

Table 1 shows the CMCs obtained for each new surfactant. Micellization (or CMC value) of the surfactants with one lysine in the polar head, LKM, LLM, and MKM_{TM}, took place at 7.2, 5.5, and 2.2 mM, respectively. These values are similar to those reported for monocatenary arginine cationic surfactants with the same hydrocarbon chain length[21] and lower than the CMC corresponding to the commercial 12-carbon, straight-chain cationic surfactants such as dodecyltrimethylammonium bromide (DTAB),[22] which is approximately 10 mM. The position and type of the cationic charge do not affect significantly the concentration at which these surfactants form molecular aggregates. Roy et al.[23] prepared a series of cationic surfactants with various headgroup structures and a constant tail of 16 carbon atoms, in terms of flexibility/rigidity, size/area, and hydrophilicity, to investigate their effect on the CMC values obtained by surface tension and fluorescence measurements. Roughly, the structure differences in the polar headgroup did not have any notable effect on the CMC values.

As expected, diamino acid surfactants (LAKM, LALM, LKKM) showed higher CMC values (1 order of magnitude) than the monocatenary ones. Increasing the number of amino acids increased the hydrophilicity of surfactants in water. Such improvements in the solubility lowered the tendency of surfactants to form micelles in water and increased the CMC. In this case, neither the position of the cationic charge nor the type of amino acids on the polar head affected the CMC value. The micellization was mainly governed by the number of cationic charges on the polar head as well as the length of the alkyl chain.

It is well known that the gemini structure increases hydrophobic interactions, giving rise to compounds with very low CMC and high efficiency by reducing the surface tension of water.[24] In general, gemini surfactants show CMC values about 1 order of magnitude lower than their corresponding monocatenary surfactants. In the present work, the same behavior has been observed (Table 1). $C_6(LL)_2$, $C_6(LK)_2$, and $C_6(LK)_{2\ TM}$ have comparable CMC values 1 order of magnitude lower than those of LKM and LLM. $C_7NH(LK)_2$ showed a higher CMC value; the third positive charge in the spacer chain increased the hydrophilic character of the molecule, increasing the CMC. Gemini surfactants from arginine with the same alkyl chain and spacer lengths gave a CMC of 0.4 mM by conductivity measurements.[25] Table 1 also includes the retention time of all compounds obtained by HPLC. The retention time is mainly affected by the polarity of the compound. Except for $C_7NH(LK)_2$, the CMC correlates well with the retention times. The results indicate that the micellization process is governed by the hydrophobic/hydrophilic balance and not by the type of cationic charge.

Antimicrobial Activity. Due to the positively charged polar head, cationic surfactants can easily disturb bacterial membranes and in general have antimicrobial activity.[26] Bacterial cell surface is usually negatively charged, and hence, the adsorption of cationic amphiphiles onto the negatively charged cell

Table 2. MIC Values Obtained for the Monocatenary Derivatives with One or Two Amino Acids on the Polar Head at pH 7.4

	one amino acid			two amino acids		
	LKM μM (μg/mL)	LLM μM (μg/mL)	MKM_{TM} μM (μg/mL)	LALM μM (μg/mL)	LAKM μM (μg/mL)	LKKM μM (μg/mL)
Gram-positive						
Bacillus subtilus	331 (125)	331 (125)	35 (16)	28 (16)	218 (125)	115 (63)
Staphylococcus epidermidis	166 (63)	83 (31)	17 (8)	218 (125)	436 (250)	115 (63)
Micrococcus luteus	331 (125)	83 (31)	35 (16)	28 (16)	436 (250)	115 (63)
Staphylococcus aureus	331 (125)	166 (63)	35 (16)	54 (31)	436 (250)	229 (125)
Candida albicans	166 (63)	166 (63)	35 (16)	218 (125)	436 (250)	R[a]
Gram-negative						
Pseudomonas aeruginosa	R	331 (125)	R	218 (125)	R	459 (250)
Klebsiella pneumoniae	331 (125)	331 (125)	278 (128)	218 (125)	R	459 (250)
Escherichia coli	166 (63)	331 (125)	R	218 (125)	R	229 (125)

[a] R, resistant microorganism at the highest concentration tested (250 μg/mL).

Table 3. MIC Values Obtained for Gemini Surfactants at pH 7.4

	gemini surfactants			
	$C_6(LL)_2$ μM (μg/mL)	$C_6(LK)_2$ μM (μg/mL)	$C_7NH(LK)_2$ μM (μg/mL)	$C_6(LK)_{2\ TM}$ μM (μg/mL)
Gram-positive				
B. subtilus	308 (250)	308 (250)	142 (125)	16 (15)
S. epidermidis	77 (63)	38 (31)	142 (125)	16 (15)
M. luteus	154 (125)	R[a]	142 (125)	67 (60)
S. aureus	154 (125)	R	R	67 (60)
C. albicans	R	R	285 (250)	67 (60)
Gram-negative				
P. aeruginosa	R	R	142 (125)	R
K. pneumoniae	R	R	R	R
E. coli	308 (250)	R	R	R

[a] R, resistant microorganism at the highest concentration tested (250 μg/mL).

surface is facilitated by electrostatic interactions, along with hydrophobic interactions.[27] Development of cationic amphiphiles having antibacterial properties with considerable biocompatibility is of great interest in biomedical chemistry.

In this work we have studied the influence of the number and position of cationic charges as well as the number of alkyl chains on the bactericidal effect of cationic surfactants from lysine. The antimicrobial activity of the lysine derivatives was investigated by determining the minimal inhibitory concentration (MIC). Eight representative bacterial strains, four Gram-positive and three Gram-negative, and one yeast were chosen for studying antimicrobial activity in vitro. The results of the antimicrobial tests for all lysine-synthesized derivatives are reported in Tables 2 (monocatenary surfactants) and 3 (gemini surfactants).

The lysine derivative surfactants are moderate antimicrobial agents against Gram-positive bacteria, whereas they present low activity against the Gram-negative ones. Gram-positive bacteria have a rather simple cell wall composed of a peptidoglycan layer, which allows amphiphiles to penetrate it without difficulty.[28] The external layer of the outer membrane of the Gram-negative bacteria is almost entirely composed of lipopolysaccarides and proteins that restrict the entrance of biocides.

For the monocatenary lysine derivatives, the most active compound is by far MKM_{TM}. This compound presents high activity against Gram-positive microorganisms as well as against *Candida albicans*. The cationic charge is situated on the quaternized amino group, and the charge density of the molecules does not depend on the pH. LLM was found to be more active than LKM. The differences between these two surfactants are the position of the cationic charge. LLM has the charge on the ε-amino group, whereas LKM has the charge on the α-amino group.

Among the monocatenary surfactants with two amino acids on the polar head, LALM was the more active, having broad-spectrum activity against the Gram-positive and Gram-negative bacteria. LALM and LKKM were generally more active than the rest of the monocatenary compounds, with the exception of the quaternized compounds. The hydrophilic character of these molecules increased considerably due to the cationic charges and the higher size of the polar group; in fact, the CMC was higher and the retention time by HPLC smaller. As a consequence of these factors, these molecules showed the highest solubility in pure water as well as in the culture medium used for MIC determinations. Haldar et al.[26] found that the incorporation of multiple head groups in cationic surfactants led to impressive antibacterial activity. Lipo-α-peptides and their lipo-β-peptide counterparts with four amino acids on the polar head and one C_{16} lipid tail showed good antimicrobial activity.[29] Moreover, it has been also reported that polycationic neomycin−lipid conjugates with several protonated amino groups on the polar head displayed higher activity that the lysine derivatives presented in this work.[30] The adsorption of amphiphiles with multicationic head groups onto the negatively charged bacterial cell surface is expected to take place to a greater extent than monocationic amphiphiles through electrostatic interactions because of the higher charge density on multihead cationic surfactants.

It was also observed that the exchange of one of the amino acids can drastically modify antimicrobial properties (see LAKM and LKKM), and the exchange of the position of one of the two positive charges also affects antimicrobial activity (see LAKM and LALM). On the other hand, in reference to the alkyl chain length, there are numerous studies about the influence of this parameter on antimicrobial activity. For surfactants with the same headgroup, the optimum antimicrobial activity is obtained for a determined alkyl chain length, usually in the range of $C_{12}-C_{14}$. It is probable that the optimal chain length for these diamino acid surfactants is $C_{14}-C_{16}$ because the polar head volume is considerably larger. For monoacyl lysine derivatives the alkyl chain did not significantly affect antimicrobial activity,[31] but lipopeptides with two lysines as polar head and one alkyl chain of 16 carbons atoms showed very good antimicrobial activity against Gram-positive and Gram-negative bacteria.[32]

Antimicrobial activity of gemini lysine surfactants is shown in Table 3. We found large differences in the MIC values of these surfactants. Following the same trend as the monoacyl derivatives, the higher activity was also obtained for the compound with the positive charge on the quaternized amino group. In fact, $C_6(LK)_{2\ TM}$ presented notable activity compared with the other compounds. It can be also observed that the gemini with the positive charges on the α-amino group was ineffective against six of the eight bacteria tested. Moreover, this gemini surfactant did not show activity against Gram-negative bacteria even at concentrations as high as 308 μM.

It can be stated that for the same alkyl chain length, the gemini surfactants from lysine have lower antimicrobial activity than the corresponding monocatenary ones. These results contrast with those obtained for Bis(Quats) gemini surfactants, which usually exhibit higher antibacterial activity than conventional surfactants.[33,34] For alkyl chains of 12 carbon atoms, gemini surfactants from arginine had lower activity than the corresponding monocatenary, but greater activity was obtained with the alkyl chain of 10 carbon atoms.[18] This could indicate that for lysine gemini compounds a shorter alkyl chain could also lead to a higher antimicrobial activity. On the other hand, the introduction of spermidine as spacer chain with one additional positive charge did not enhance antimicrobial activity of these gemini surfactants.

In general, the antibacterial activity of these lysine derivatives is lower than that shown by cationic surfactants from arginine derivatives with similar chemical structures.[18] These variations could be attributed to differences between the pK_a values of the molecules. MIC values decreased with the increase of pK_a of the amino group in which the cationic charge is situated. The headgroup charge of these surfactants is modulated by the proportion of dissociation of the protonated amino group, and this dissociation depends on the specific architecture of the molecule. Surfactants of the N^{α}-acyl type have the ε-amino group protonated, and the pK_a of these molecules is around 10−12. However, surfactants of the N^{ε}-acyl type have the α-amino group protonated with a pK_a around 8.[35] At pH equal to the pK_a, the amino acid will be 50% protonated. As the pH is decreased by >2 units below the pK_a, it may be assumed that the amino acid is fully protonated. This would mean that the average charge is 1. Given that the pK_a of the protonated α-amino group of the N^{ε}-lysine derivatives is around 8, the average charge of the compound could be lower than 1 at the pH of the test medium (pH 7.4); hence, the compounds have more difficulty in disrupting the bacterial membrane. Previous studies also showed that neomycin B derived cationic lipids containing a guanidinylated polycationic headgroup exhibited greater antimicrobial potency than the neomycin B derived cationic lipid homologues containing an amine-based polycationic headgroup.[36]

As for the effect of the compound's net charge on its bactericidal activity, we have determined the antimicrobial activity at different pH values from 5.4 to 8 (Figure 2). At pH 5.4, it is expected that all of the amino groups, the α-amino as well as the ε-amino, were fully protonated with a net average charge of 1. The results obtained are summarized in Tables S1, S2, and S3 in the Supporting Information. It can be observed that for the compounds with the charge on the α-amino group (LKM, LAKM, and $C_6(LK)_2$) the activity increased as the pH decreased (Figure 2). These results confirm that the net average charge of the molecule is one of the factors that affect antimicrobial activity. Another factor that also affects this property is the type of cationic charge. The activity of the chlorhydrated surfactants increased considerably as the pH increased, but the activity obtained at the lower pH tested is still inferior to those shown by the quaternized ones.

In summary, these studies indicate that lysine cationic surfactants with very different structures show moderate antimicrobial activity against tested bacteria. The position of the positive charge, the type of cationic charge, and the number of alkyl chains determine the extent of antimicrobial activity.

Hemolytic Activity. Hemolysis by surfactants is a process of great importance for research and practical purposes. The human erythrocyte lacks internal organelles and is the most widely used cell membrane system to study surfactant−membrane interactions.[37] Surfactant intercalation into the cell membrane leads to changes in the membrane molecular organization and increases membrane permeability that concludes with cell lyses. In addition, the potential use of surfactants as drug delivery systems makes the evaluation of hemolysis of great importance, and it is used as a measure for cytotoxicity and a model for mammalian cells because erythrocytes are extremely fragile.[38] The polar head type could affect the capability of surfactants to disrupt the fragile cellular surface of the erythrocytes. However, hemolysis depends on the adsorption of the surfactant to components of the membrane surface, which is influenced by electrostatic attractions between the surfactant molecules and membrane components, among other factors.

Despite the potential use of cationic surfactants in a wide range of pharmaceutical and biotechnological applications, structure−membrane toxicity relationships are poorly developed. Alkyl chain length, type of alkyl chain (hydrocarbonated or fluorinated), structure type (monocatenary or gemini surfactants), position of the cationic charge, number of cationic charges, counterion type, and headgroup hydrophobicity can affect significantly the hemolytic activity of surfactants. Nowadays, it is still not well established how these molecular parameters determine the cytotoxicity of surfactants. Moreover, the influence of some of these parameters changes depending on surfactant type. There are some surfactant analogues in which the hemolysis increases when the alkyl chain increases,[39,40] whereas in other groups the hemolysis decreases as the alkyl chain increases.[41] On the other hand, in the case of the gemini amphiphiles the role of the spacer and its length remains unknown.

Evaluation of the concentration that induces the hemolysis of 50% of the erythrocytes (HC_{50}) was determined and

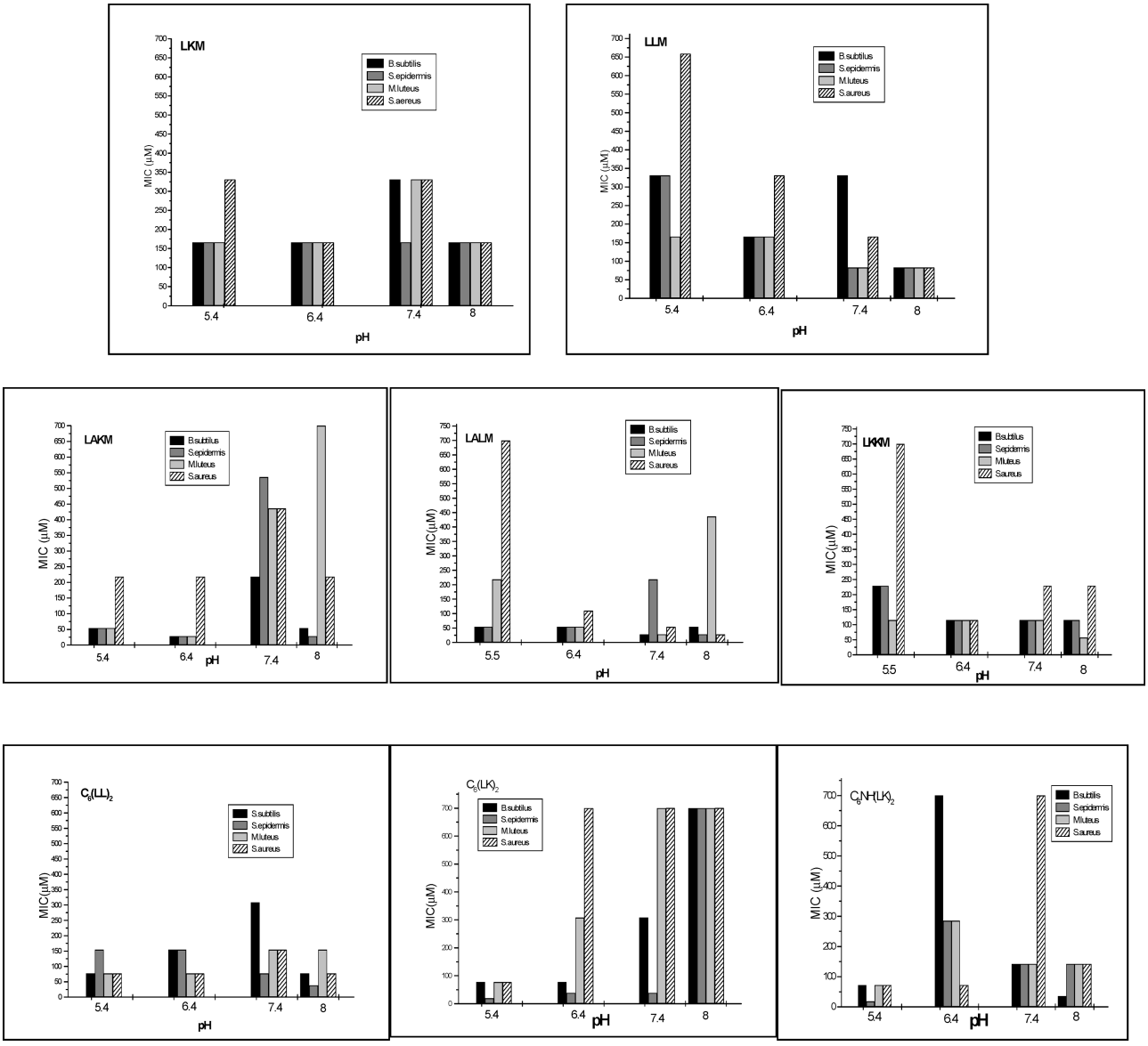


Figure 2. MIC values for Gram-positive microorganisms at different pH values.

quantified from plots of percentage hemolysis as a function of amphiphile concentration. Table 4 shows the HC_{50} values obtained for lysine derivatives. In general, these surfactants showed significantly lower hemolytic activity than cationic surfactants based on quaternary ammonium.[42−44] The activity of these new lysine surfactants on red blood cells was also lower than those reported for cationic fluorinated compounds in which hemolysis decreased drastically by increasing the degree of fluorination and length of the hydrophobic tail.[45] The analogue derivatives with the arginine amino acid also present higher hemolytic character.[18] It has been reported that for cationic amino acid surfactants, the cytotoxicity changed when one amino acid was replaced by another, and the presence of more hydrophobic groups surrounding the positive charge gave rise to lower cytotoxicity.[46]

Our results indicate that in general single-chain surfactants are less hemolytic than the corresponding gemini amphiphiles. This same trend has also been described for Bis(Quats) gemini surfactants and their corresponding monoQuats.[42,47] The molecular shape and the relationship between the hydrophilic and hydrophobic moieties constitute determining characteristics of the way in which monomers interact and probably also of the way in which they interact with lipids in the erythrocyte membrane. The molecular shape also determines intra- and intermolecular hydrophobic interactions. It is well-known that the gemini structure enhances hydrophobic interactions, giving rise to surfactants than can aggregate at very low concentrations. The increase of the hemolytic

Table 4. Hemolysis of Lysine-Based Surfactants

surfactant	HC_{50} (μM)
LAKM	791
LALM	1353
LKKM	290
LKM	391
LLM	199
MKM_{trim}	52
$C_6(LL)_2$	475
$C_6(LK)_2$	116
$C_7NH(LK)_2$	12
$C_6(LK)_{2trim}$	11

character of the gemini surfactants could be due to the increase of these interactions; in fact, it has been reported that this type of interaction controls hemolytic activity, whereas charge interactions have been suggested to be more important for antibacterial activity.[48] The hydrophobicity and the capacity of forming micelles seem to influence significantly the hemolysis of these compounds. It can be observed that the less active compounds are the diamino acid surfactants, whereas the most hydrophobic surfactants, the gemini, present the highest activity against erythrocytes. However, we have compounds with similar hydrophobicity and CMC values (the two diamino acid surfactants or the two monocatenary lysine derivatives) that exhibit different HC_{50} values. Concerning the gemini surfactants, $C_7NH(LK)_2$ was the most soluble in aqueous media and showed the highest CMC value, but it was by far the most active against erythrocytes. This suggests that the new cationic charge improves the interaction of the compound with the membrane.

From these results, it can be stated that the number of charges, the density of charge, and the position of the cationic charges play also an important role in the hemolytic activity of the compounds. LLM was nearly 2 times more potent than LKM in causing erythrocyte lysis, and it was also more potent in killing bacteria. Different behaviors can be observed for gemini and diamino acid surfactants; surprisingly, in these cases the less toxic compounds are those with the positive charge on the α-amino group, these compounds being the most active against bacteria. These results are in concordance with those obtained by Sambhy et al.,[49] who reported new pyridinium polymers in which the spatial relationship between the positive charge and the pendent alkyl chain changed drastically hemolytic activity.

In general, except for $C_7NH(LK)_2$ and $C_6(LK)_2$, the lysine derivatives described in this work possess antibacterial activity to Gram-positive bacteria at lower concentrations than those in which they show erythrocyte toxicity. The safe concentration range is remarkably wide in the case of diamino acid surfactants and $C_6(LL)_2$. It is worth noting that the observed HC_{50} of LALM was 1385 μM, a 50-fold higher concentration than the MICs to the Gram-positive bacteria and a 6-fold higher concentration than the MICs against the Gram-negative. The possible use of these compounds as antimicrobials in clinical applications can be analyzed from their therapeutic index, the HC_{50}/MIC ratio. A high therapeutic index means wide safe concentration range. The most suitable surfactants for these applications are the diamino acid derivatives with therapeutic index values ranging from 24 to 48. Gemini surfactants ($C_7NH(LK)_2$ and $C_6(LK)_2$) seem to be the less appropriate to use as antimicrobial agents in systemic applications, but some of them are still active against bacteria at concentrations at which they do not exhibit hemolytic activity. Nevertheless, it is noteworthy that we have obtained very active cationic gemini surfactants with good solubility in aqueous media and with low hemolytic character. They can be promising compounds for biotechnological applications in which active cationic compounds are required and reducing mammalian cell toxicity is essential. The use of very active compounds minimizes the amount of surfactant in human cells and increases the safety of these systems.

Experimental Procedures

Materials. All solvents were of reagent grade and were used without further purification. Lauroyl and myristoyl chloride acids were from Fluka. *N*-Benzyloxycarbonyl-L-lysine hydrochloride (*N*-Cbz-L-lysine·HCl) was obtained from Calbiochem-Novabiochem AG (Switzerland). Trifluoroacetic acid (TFA), thionyl chloride ($SOCl_2$), and palladium on activated charcoal (Pd/C, 10%) were supplied by Merck (Darmstadt, Germany). Deuterated solvents were purchased from Eurotop. Mueller−Hinton broth (MHB) was purchased from Difco Laboratories (USA). Water from a Milli-Q Millipore system was used to prepare aqueous solutions.

The progress of the reactions as well as the purity of the final compounds was monitored by HPLC, model Merck-Hitachi D-2500, using UV−vis detector L-4250 at 215 nm. A Lichrospher 100 CN (propylcyano) 5 μm, 250 × 4 mm, column was used. A gradient elution profile was employed from the initial solvent composition of A/B 75:25 (by volume), changing during 24 min to a final composition of 5:95; solvent A was 0.1% (v/v) TFA in H_2O, and solvent B was 0.085% of TFA in H_2O/CH_3CN 1:4. The flow rate through the column was 1.0 mL min^{-1}.

Methods. The structures of the pure compounds were checked by ^1H and ^{13}C NMR analyses, which were recorded on a Varian spectrometer at 499.803 (^1H) and 125.233 (^{13}C) MHz, respectively, using the deuterium signal of the solvent as the lock. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). All measurements were carried out on 0.6 mL samples in 5 mm tubes using a 5 mm indirect broadband probe. ^{13}C NMR spectra were recorded under composite decoupling to eliminate $^{13}C-^1H$ coupling. The distortionless enhancement by polarization transfer spectra (DEPT) were run in a standard way to separate the CH/CH_3 and CH_2 lines phased up and down, respectively.

Mass spectroscopy (MS) spectra with fast atom bombardment (FAB) or electrospray techniques were carried out with a VG-QUATTRO from Fisons Instruments. Elemental analysis of the final compounds was also achieved.

Conductivity. Conductivity was measured using an Orion Cond. Cell 011010A with platinized platinum electrodes in conjunction with a Thermo Orion 550A with a cell constant of 0.998 cm^{-1}. The cell constant was calibrated periodically with NaCl/KCl solutions of known conductivities and was used for calculating the conductivity of the surfactant solution. The conductivity of water was subtracted from the measured conductivity of each sample. Measurements were made at increasing concentration to minimize errors from possible contamination from the electrode. Samples for conductivity measurements were prepared by weight in Millipore ultrapure water.

Antimicrobial Activity. MIC Determinations. Antimicrobial activities were determined in vitro on the basis of the MIC values,[50] defined as the lowest concentration of antimicrobial agent that inhibits the development of visible growth after 24 h of incubation at 37 °C. The compounds tested were dissolved in MHB in the concentration range of 0.1−256 μg/mL, and no precipitate was observed at the highest concentration of the surfactant. The MHB was prepared according to the manufacturer's instructions. Then 10 μL of a nutrient broth starter culture of each bacterial strain was added to achieve final inoculums of ca. $5 \times 10^{-4}-5 \times 10^{-5}$ colony forming units mL^{-1}. The cultures were incubated overnight at 37 °C. Nutrient broth medium without the compound served as control. The growth of the microorganisms was determined visually after incubation for 24 h at 37 °C. The development of turbidity in an inoculated medium is a function of growth. A rise in turbidity reflects increases in both mass and cell number. Changes in turbidity were correlated with changes in cell numbers. The lowest concentration of antimicrobial agent at which no visible turbidity was observed was taken as the MIC.

Seven bacteria and one fungus were used for MIC determinations. Gram-negative bacteria included *Escherichia coli* ATCC 27325, *Klebsiella pneumonia* ATCC 9721, and *Pseudomonas aeroginosa* ATCC 9721. Gram-positive bacteria included *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25178, *Staphylococcus epidermidis* ATCC 155-1, and *Micrococcus*

luteus ATCC 9341. The fungus used was *Candida albicans* ATCC 10231.

Hemolytic Activity. Erythrocytes were washed three times in phosphate buffer isotonic saline (PBS), containing 22.2 mmol/L Na_2HPO_4, 5.6 mmol/L KH_2PO_4, and 123.3 mmol/L NaCl, in distilled water (pH 7.4). Erythrocytes were then suspended in PBS at a cell density of 8×10^9 cells/mL.

A series of different volumes of surfactant solution (1 mg/mL), ranging from 10 to 80 μL, were placed in polystyrene tubes, and an aliquot of 25 μL of erythrocyte suspension was added to each tube. The tubes were incubated at room temperature with shaking for 10 min. Following incubation, the tubes were centrifuged (5 min at 5000 rpm). The degree of hemolysis was determined by comparing the absorbance (540 nm) of the supernatant with that of the control samples totally hemolyzed with distilled water.

Preparation of the Single-Chain or Monocatenary Lysine Surfactants LKM, LLM, and MKM (Scheme 1). (a) General Procedure for the Synthesis of $N^{\alpha}/N^{\varepsilon}$-Cbz-lysine Methyl Ester·HCl (1b, 2b). MeOH (400 mL) was cooled to −80 °C using acetone/solid carbon dioxide, and then $N^{\alpha}/N^{\varepsilon}$-Cbz-LysOH·HCl (**1a**, **2a**) (25 g, 0.089 mol) was added. Thionyl chloride (19.5 mL, 0.027 mol) was added dropwise, and the mixture was stirred at room temperature for 20 h. The end of the reaction was checked by HPLC with the decrease in the peak area corresponding to the starting materials. Methanol, excess thionyl chloride, and HCl formed during the reaction were eliminated by successive vacuum evaporations. After lyophilization, a white solid corresponding to the title product was obtained.

Analytical Data and Spectral Assignments for 1b. Yield, 97%; HPLC, t_r = 5.8 min; MW, 330.5 g/mol; elem anal. found, C, 51.13; H, 6.89; N, 8.11; calcd for $C_{15}H_{23}O_4N_2Cl \cdot H_2O$, C, 51.65; H, 7.17; N, 8.03; ^{1}H NMR, δ_H (CD_3OD) 1.4−1.8 [m, 6H, 3 $-CH_2-$, lateral chain of lysine], 2.8 [t, 2H, $-CH_2-NH_3^+$], 3.7 [s, 3H $-COO-CH_3$], 4.2 [m, 1H, $-CH-$, lysine], 5.1 [s, 2H, $-CH_2-$, Cbz group], 7.2−7.3 [m, 5H, $-CH-$, aromatic ring]; ^{13}C NMR, δ_C (CD_3OD), 23.8, 27.9, 32.0 [$-CH_2-$, lateral chain of lysine], 40.4 [$-CH_2-NH_3^+$], 52.7 [$-COO-CH_3$], 55.1 [$-CH-$, lysine], 67.6 [$-CH_2-$, Cbz group], 128.8, 129.0, 129.4 [$-CH-$, aromatic ring], 138.1 [$-C-$, aromatic ring], 158.6 [$-COO-$, Cbz group], 174.3 [$-COO-CH_3$].

Analytical Data and Spectral Assignments for 2b. Yield, 97%; HPLC, t_r = 5.8 min; MW, 330.5 g/mol; elem anal. found, C, 51.27; H, 6.93; N, 8.07; calcd for $C_{15}H_{23}O_4N_2Cl \cdot H_2O$, C, 51.65; H, 7.17; N, 8.03; ^{1}H NMR, δ_H (CD_3OD), 1.5−1.9 [m, 6H, 3 $-CH_2-$, lateral chain lysine], 3.1 [t, 2H, $-CONH-CH_2-$], 3.8 [s, 3H, $-CO-OCH_3$], 4.0 [t, 1H, $-CH-$, lysine], 5.0 [s, 2H, $-CH_2-$, Cbz group], 7.3 [m, 5H, $-CH-$, aromatic ring]; ^{13}C NMR, δ_C(CD_3OD) 23.0, 27.9, 30.9 [$-CH_2-$, lateral chain of lysine], 41.1 [$-CONH-CH_2-$], 53.6 [$-COO-CH_3$], 53.8 [$-CH-$, lysine], 67.3 [$-CH_2-$, Cbz group], 128.7, 128.9, 129.4 [$-CH-$, aromatic ring], 138.3 [$-C-$, aromatic ring], 158.9 [$-COO-$, Cbz group], 170.9 [$-COO-CH_3$].

(b) General Procedure for the Synthesis of N^{α}-Cbz-N^{ε}-Lauroyl-lysine Methyl Ester (1c) and N^{α}-Lauroyl-N^{ε}-Cbz-lysine Methyl Ester (2c). A solution of acetone/water (30 mL) containing 0.015 mol (5 g) of **1b**/**2b** was placed in a round-bottom flask. To this solution was added dropwise 0.015 mol of lauroyl chloride, and stirring was continued at room temperature for 4 h. After completion of the reaction, the mixture was acidified with HCl and a white solid precipitated. The resulting solid was dissolved in MeOH (25 mL) and extracted three times with petroleum ether to remove the corresponding fatty acid excess. The solvent was evaporated, and the solid was dissolved in chloroform ($CHCl_3$) (25 mL). Thereafter, this solution was shaken with water to eliminate water-soluble impurities. Finally, the solid was purified by silica acid flash chromatography: 100 mL of silica (Chromagel 60A CC, 70−230) was packed into a flash chromatography column. The corresponding solid (3 g of **1c**/**2c**) was dissolved in $CHCl_3$ and loaded into the column and eluted from $CHCl_3$ to $CHCl_3$/MeOH. The fractions containing the pure target products were pooled. The identification of the products was carried out by HPLC, elemental analysis, ^{1}H NMR, and ^{13}C NMR.

Analytical Data and Spectral Assignments for 1c. Yield, 91%; HPLC, t_r = 19.5 min; MW, 476.2 g/mol; ESI-MS, *m*/*z* 477 corresponding to $(M + H)^+$; ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.6 [m, 24H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.0 [t, 2H, $-CH_2-CONH-$], 2.9 [m, 2H, $-CONH-CH_2-$], 3.6 [s, 3H, $-COO-CH_3$], 3.9 [m, 1H, $-CH-$, lysine], 5.0 [s, 2H, $-CH_2-$, Cbz group], 7.2−7.3 [m, 5H, $-CH-$, aromatic ring]; ^{13}C NMR, δ_H (DMSO-d_6) 13.7 [$-CH_3-$, alkyl chain], 21.9, 22.7, 25.1, 28.5, 28.6, 28.7, 28.8, 28.8, 30.2, 31.1 [$-CH_2-$, alkyl chain and lysine lateral chain], 35.3 [$-CH_2-CONH-$], 37.9 [$-CONH-CH_2-$], 51.6 [$-COO-CH_3$], 53.8 [$-CH-$, lysine], 67.5 [$-CH_2-$, Cbz group], 128.7, 128.9, 129.4 [$-CH-$, aromatic ring], 138.1 [$-C-$, aromatic ring], 158.6 [$-COO-$, Cbz group], 171.8 [$-CONH-$], 172.7 [$-COO-$].

Analytical Data and Spectral Assignments for 2c. Yield, 89%; HPLC, t_r = 19.4 min; MW, 476.2 g/mol; ESI-MS, *m*/*z* 477 corresponding to $(M + H)^+$; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.8 [m, 24H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.2 [t, 2H $-CH_2-CONH-$], 3.1 [m, 2H $-CONH-CH_2-$], 3.6 [s, 3H, $COO-CH_3$], 4.3 [m, 1H, $-CH-$, lysine], 5.0 [s, 2H, CH_2, Cbz group], 7.2−7.3 [m, 5H, $-CH-$, aromatic ring]; ^{13}C NMR, δ_H (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.7, 24.1, 26.9, 30.2, 30.4, 30.6, 30.7, 32.1, 33.0 [$-CH_2-$, alkyl chain and lysine lateral chain], 36.7 [$-CO-CH_2-(CH_2)_9-$], 41.4 [$-CH_2-NH-CO$], 52.6 [$-COO-CH_3$], 53.6 [$-CH-$, lysine], 67.3 [$-CH_2-$, Cbz group], 128.7, 128.9, 129.4 [$-CH-$, aromatic ring], 138.4 [$-C-$ aromatic ring], 158.9 [$-COO-$, Cbz group], 174.3 [$-CONH-$], 176.5 [$-COO-$].

(c) General Procedure for the Synthesis of N^{ε}-Lauroyl-lysine (LKM) and N^{α}-Lauroyl-lysine (LLM) Methyl Ester Hydrochloride Salts. LKM and LLM were obtained by hydrogenation of the corresponding pure **1c** and **2c** (0.002 mols) in 30 mL of MeOH/HCl (HCl/lysine derivative moles = 1.3) using Pd in activated charcoal (10% Pd) as catalyst. The reaction was carried out at atmospheric pressure. The mixture was stirred for 30 min. Given that HCl is present in the reaction medium, the final surfactants were obtained as HCl salts. At the end of the reaction, the catalyst was filtered off on Celite. The solvent was evaporated under reduced pressure. Pure compounds were obtained by several crystallizations from methanol/acetone.

Analytical Data and Spectral Assignments for LKM. Yield, 70%; HPLC, t_r = 14.8 min; MW, 378.4 g/mol; ESI-MS, *m*/*z* 343.2 (M^+ without Cl^-); elem anal. found, C, 57.02; H, 11.20; N, 7.27; calcd for $C_{19}H_{39}O_3N_2Cl \cdot 1.2H_2O$, C, 56.99; H, 10.35; N, 6.99; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.9 [m, 24H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.18 [t, 2H, $-CH_2-CONH-$], 3.1 [t, 2H, $-CONH-CH_2-$], 3.8 [s, 3H, $-COO-CH_3$], 4.0 [m, 1H, $-CH-$, lysine]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.2, 23.7, 27.0, 29.9, 30.3, 30.4, 30.6, 30.7, 31.1, 33.0 [$-CH_2-$, alkyl chain and lysine lateral chain], 37.1 [$-CH_2-CONH-$], 39.6 [$-CONH-CH_2-$] 53.6 [$-COO-CH_3$], 53.8 [$-CH-$, lysine], 170.9 [$-CONH-$], 176.4 [$-COO-$].

Analytical Data and Spectral Assignments for LLM. Yield, 72%; HPLC, t_r = 14.8 min; MW, 378.4 g/mol; ESI-MS, *m*/*z* 343.2 (M^+ without the Cl^-); elem anal. found, C, 59.30; H, 10.40; N, 7.25; calcd for $C_{19}H_{39}O_3N_2Cl$, C, 60.2; H, 10.37; N, 7.39; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.9 [m, 24H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.2 [t, $-CH_2-CONH-$], 2.9 [t, 2H, $-CONH-CH_2-$], 3.7 [s, 3H, $-COO-CH_3$], 4.3 [t, 1H, $-CH-$, lysine]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.74, 23.8, 26.9, 27.9, 30.2, 30.4, 30.6, 30.7, 31.8, 33.8 [$-CH_2-$, alkyl chain and lysine lateral chain], 36.6 [$-CH_2-CONH-$], 40.4 [$-CONH-CH_2-$], 52.7 [$-COO-CH_3$], 53.3 [$-CH-$, lysine], 173.9 [$-CONH-$], 176.5 [$-COO-$].

(d) General Procedure for the Synthesis of N^{ε}-Myristoyl-N^{ε}-trimethyl-lysine (MKM_{TM}). MKM was prepared using the procedure described for the preparation of LLM and LKM. Then, potassium carbonate was added to a solution of MKM (0.005 mol) in methanol until pH 10. After that, dimethyl sulfate (0.015 mol) was added dropwise, and the mixture was stirred at room temperature until the reactants were consumed. The solvent was evaporated and crystallized in methanol/acetone. The sulfate salts thus obtained were treated with HCl in methanol to obtain the corresponding chloride surfactants.

Analytical Data and Spectral Assignments for MKM_{TM}. Yield, 70%; HPLC, $t_r = 17.7$ min; MW, 449.1 g/mol; ESI-MS, m/z 413.6 (M^+ without the Cl^-); elem anal. found, C, 53.4; H, 10.0; N, 4.6; calcd for $C_{24}H_{49}O_3N_2Cl \cdot 5H_2O$, C, 53.4; H, 10.7; N, 5.1; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.9 [m, 24H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.2 [t, $-CH_2-CONH-$], 2.9 [t, 2H, $-CONH-CH_2-$], 3.7 [s, 3H, $-COO-CH_3$], 4.3 [t, 1H, −CH−, lysine]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.74, 23.8, 27.0, 27.2, 30.2, 30.4, 30.6, 30.7, 33.0, 37.1 [$-CH_2-$, alkyl chain and lysine lateral chain], 39.3 [$-CH_2-CONH-$], 52.7 [$-COO-CH_3$], 52.8 [−CH−, lysine], 64.1 [CH_2-N-], 75.7 [$-N-(CH_3)_3$], 173.9 [$-CONH-$], 176.5 [$-COO-$].

Preparation of the Single-Chain Diamino Acid Surfactants (Scheme 2). (a) General Procedure for the Synthesis of N^{α}-Lauroylnitroarginine-N^{α}-lysine(Cbz) Methyl Ester (4b) and N^{α}-Lauroylnitroarginine-N^{ε}-lysine(Cbz) Methyl Ester (5b). A N^{α}-lauroylnitroarginine (LNA) solution (20 mmol) in 50 mL of CH_2Cl_2 was treated with 40 mmol of DABCO. Then, 20 mmol of **4a** or **5a** was added under stirring until total solubilization of the compounds was reached. Finally, 20 mmol of BOP was added, and the mixture was stirred for 3 h. After complete conversion of the starting LNA, the mixture was cooled to 0 °C, and the crude precipitate was isolated by filtration. The solid was washed with hexane and water and dried over vacuum. Flash chromatography on silica gel 60, using $CHCl_3$/MeOH as mobile phase, afforded the pure compound, which was crystallized from MeOH/ACN to yield the target compounds **4b** and **5b**.

Analytical Data and Spectral Assignments for 4b. Yield, 70%; HPLC, $t_r = 16.5$ min; MW, 677.8 g/mol; ESI-MS, m/z 700 (M + Na); ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 3H $-CH_3$, alkyl chain], 1.2−1.7 [m, 28H, $-CH_2$, alkyl chain and lateral chain of arginine and lysine], 2.1 [t, 2H, $-CH_2-CONH-$], 2.9 [t, 2H, $-CONH-CH_2-$], 3.2 [t, 2H, $-NH-CH_2-$, guanidine], 3.6 [s, 3H, $-COO-CH_3$], 4.2 [m, 1H, −CH−, arginine], 4.3 [m, −CH−, lysine], 5.0 [s, 2H, $-CH_2-$, Cbz group], 7.2−7.3 [m, 5H, −CH−, aromatic ring]; ^{13}C NMR, δ_C(DMSO-d_6) 13.6 [$-CH_3$, alkyl chain], 21.8, 22.4, 25.0, 28.3, 28.4, 28.5, 28.7, 28.8, 29.1, 29.2, 30.3, 31.0 [$-CH_2-$, alkyl chain and lateral chain of arginine and lysine], 35.0 [$-CH_2-CONH-$], 38.0 [$-CONH-CH_2-$], 40.1 [$-NH-CH_2-$, guanidine], 51.5 [$-COO-CH_3$], 51.7 [−CH−, lysine], 51.9 [−CH−, arginine], 64.9 [$-CH_2-$, Cbz group], 127.4, 127.6, 128.0 [−CH−, aromatic ring], 137.1 [−C−, Cbz group], 159.2 [$-COO-$, Cbz group], 160.9 [−C−, guanidine], 171.6 [$-CONH-$, lysine], 172.0 [$-CONH-$, arginine], 172.1 [$-COO-$].

Analytical Data and Spectral Assignments for 5b. Yield, 70%; HPLC, $t_r = 15.6$ min; MW, 677.8 g/mol; ESI-MS, m/z 700 (M + Na); ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.7 [m, 28H, $-CH_2-$, alkyl chain and lateral chain of arginine and lysine], 2.2 [t, 2H, $-CH_2-CONH-$], 3.1 [m, 2H, $-CONH-CH_2-$], 3.2 [m, 2H, $-NH-CH_2-$, guanidine], 3.7 [s, 3H, $-COO-CH_3$], 4.1 [m, 1H, −CH−, arginine], 4.3 [m, 1H, −CH−, lysine], 5.0 [s, 2H, $-CH_2-$, Cbz group], 7.2−7.3 [m, 5H, −CH−, aromatic ring]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.7, 24.1, 26.9, 29.8, 30.3, 30.4, 30.6, 30.7, 32.1, 33.0 [$-CH_2$, alkyl chain and lateral chain of arginine and lysine], 36.8 [$-CH_2-CONH-$], 39.9 [$-CONH-CH_2-$], 41.6 [$-NH-CH_2-$, guanidine], 52.6 [$-COO-CH_3$], 54.2 [−CH−, lysine], 55.4 [−CH−, arginine], 67.6 [$-CH_2-$, Cbz group], 128.7, 128.9, 129.4 [−CH−, aromatic ring], 138.1 [−C−, Cbz group], 158.6 [−COO−, Cbz group], 160.9 [−C−, guanidine], 174.0 [−NH−CO−, lysine], 174.6 [−NH−CO, arginine], 176.3 [−COO−].

(b) General Procedure for the Synthesis of N^{ε}-Lauroyl-lysine-(carbobenzoxy)-N^{ε}-lysine(carbobenzoxy) (6c). A solution of N^{ε}-lauroyl-lysine (**6b**) (20 mmol) in 50 mL of CH_2Cl_2 was treated with 40 mmol of DABCO. Then, 20 mmol of N^{α}-carbobenzoxylysine methyl ester was added under stirring until the compounds were completely solubilized. Finally, 20 mmol of BOP was added, and the mixture was stirred for 3 h. After complete conversion of the starting material, the mixture was cooled to 0 °C and the precipitate was isolated by filtration. Flash chromatography on silica gel 60, using $CHCl_3$/MeOH as mobile phase, afforded the pure compound, which was crystallized from MeOH/ACN to yield the target compound **6c**.

Analytical Data and Spectral Assignments for 6c. Yield, 65%; HPLC, $t_r = 18.7$ min; MW, 738.9 g/mol; ESI-MS, m/z 738.6; ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.6 [m, 30H, $-CH_2-$, alkyl chain and lysine lateral chain], 2.0 [t, 2H, $-CH_2-CONH-$], 3.01 [m, 4H, 2 $-CH_2-NHCO-$], 3.6 [s, 3H, $-COO-CH_3$], 3.9 [m, 1H, −CH−, lysine], 4.0 [m, 1H, −CH−, lysine], 5.0 [m, 4H, 2 $-CH_2-$, Cbz group], 7.3−7.4 [m, 10H, −CH−, aromatic ring]; ^{13}C NMR, δ_C(DMSO-d_6) 13.7 [$-CH_3-$, alkyl chain], 21.9, 22.6, 22.7, 25.1, 28.4, 28.5, 28.6, 28.7, 28.8, 30.2, 31.1, 31.6 [$-CH_2-$, alkyl chain and lysine lateral chain], 35.3 [$-CH_2-CONH-$], 36.3 [$-CONH-CH_2-$], 38.0 [$-CONH-CH_2-$], 51.6 [$-COOCH_3$], 53.7, 54.6 [−CH−, lysine], 65.2, 65.3 [$-CH_2-$, Cbz group], 127.4, 127.5, 127.6, 128.1, 128.2 [−CH−, aromatic ring], 136.8, 136.9 [−C−, aromatic ring], 155.7, 156.0 [$-COO-$, Cbz group], 171.5, 171.7 [$-CONH-$], 172.7 [$-COO-$].

(c) General Procedure for the Synthesis of N^{α}-Lauroylarginine-N^{α}-lysine Methyl Ester (LALM), N^{α}-Lauroylarginine-N^{ε}-lysine Methyl Ester (LAKM), and N^{ε}-Lauroyl-lysine-N^{ε}-lysine Methyl Ester (LKKM). Compounds LALM, LAKM, and LKKM were obtained by hydrogenation of the corresponding pure **4b**, **5b**, and **6c** (0.002 mol) in 30 mL of MeOH/HCl (HCl/lysine derivative moles = 2.3) using Pd in activated charcoal (10% Pd) as catalyst. The reaction was carried out at 2 atm. Given that HCl is present in the reaction medium, the final surfactants were obtained as HCl salts. At the end of the reaction, the catalyst was filtered off on Celite. The solvent was evaporated under reduced pressure. Pure compounds were obtained by several crystallizations from MeOH/ACN.

Analytical Data and Spectral Assignments for LALM. Yield, 91%; HPLC, $t_r = 11.4$ min; MW, 571.6 g/mol; ESI-MS, m/z 499 (M^+ without the Cl^-); elem anal. found, C, 47.2; H, 9.28; N, 14.0; Cl, 11.7; calcd for $C_{25}H_{52}Cl_2N_6O_4 \cdot 3H_2O$, C, 48.0; H, 9.3; N, 13.5; Cl, 11.2; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.8 [m, 28H, $-CH_2-$, alkyl chain and lateral chain of arginine and lysine], 2.2 [t, 2H, $-CH_2-CONH-$], 2.9 [m, 2H, $-NH-CH_2-$, guanidine], 3.2 [m, 2H, $^+H_3N-CH_2-$], 3.7 [s, 3H, $-COO-CH_3$] 4.3 [m, 1H, −CH−, arginine], 4.4 [m, 1H, −CH−, lysine]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.7, 26.2, 26.9, 27.8, 30.0, 30.3, 30.5, 30.6, 30.7, 31.6, 33.0 [$-CH_2-$, alkyl chain and lateral chain of arginine and lysine], 36.7 [$-CH_2-CONH-$], 40.4 [$-CH_2-NH-$, guanidine], 41.9 [$^+H_3N-CH_2-$], 53.2 [$-COO-CH_3$], 53.3 [−CH−, lysine], 54.4 [−CH−, arginine], 158.6 [−C−, guanidine], 173.8 [$-CONH-$], 174.4 [$-CO-NH-$], 176.5 [$-COO-$].

Analytical Data and Spectral Assignments for LAKM. Yield, 92%; HPLC, $t_r = 11.4$ min; MW, 571.6 g/mol; ESI-MS, m/z 499 (M^+ without the Cl^-); elem anal. found, C, 49.0; H, 9.1; N, 13.9; Cl, 12.3; calcd for $C_{25}H_{52}Cl_2N_6O_4 \cdot 2H_2O$, C, 49.5; H, 9.2; N, 13.8; Cl, 11.7; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, $-CH_3$, alkyl chain], 1.2−1.9 [m, 28H, $-CH_2$, alkyl chain and lateral chain of arginine and lysine], 2.3 [t, 2H, $-CH_2-CONH-$], 3.2 [m, 4H, $-CH_2-NH-$, $-^+H_3N-CH_2$], 3.8 [s, 3H, $-COO-CH_3$], 4.0 [m, 1H, −CH−, arginine], 4.2 [−CH−, lysine]; ^{13}C NMR, δ_C(CD_3OD) 14.4 [$-CH_3$, alkyl chain], 23.0, 23.1, 23.7, 26.5, 26.9, 29.6, 29.7, 30.0, 30.3, 30.4, 30.5, 30.6, 30.7, 30.9, 31.0, 33.0 [$-CH_2-$, alkyl chain and lateral chain of arginine and lysine],

36.8 [−$\underline{C}H_2$−CONH−], 39.6 [^+H_3N−$\underline{C}H_2$−], 42.0 [−$\underline{C}H_2$−NH−], 53.6 [−COO−$\underline{C}H_3$], 53.8 [−$\underline{C}$H−, lysine], 54.7 [−$\underline{C}$H−, arginine], 158.6 [−$\underline{C}$−, arginine], 170.9 [−$\underline{C}$ONH−], 174.3 [−$\underline{C}$ONH−], 176.6 [−$\underline{C}$OO−].

Analytical Data and Spectral Assignments for LKKM. Yield, 91%; HPLC, t_r = 12.8 min; MW, 543.6 g/mol; ESI-MS, *m*/*z* 472.4; ^{1}H NMR, δ_H (CD_3OD) 0.9 [t, 3H −$\underline{C}H_3$, alkyl chain], 1.2−1.9 [m, 30H, $\underline{C}H_2$−, alkyl chain and lysine lateral chain], 2.2 [t, 2H, −$\underline{C}H_2$−CONH−], 3.2 [m, 4H, 2 −CONH−$\underline{C}H_2$], 3.84 [t, 3H, −COO−$\underline{C}H_3$], 3.9, 4.0 [m, 2H, −$\underline{C}$H−, lysine]; ^{13}C NMR, δ_C(CD_3OD) 14.4 [−$\underline{C}H_3$, alkyl chain], 23.2, 23.4, 23.7, 27.1, 29.7, 30.0, 30.4, 30.6, 30.7, 31.1, 32.2, 33.0 [−$\underline{C}H_2$−, alkyl chain and lysine lateral chain], 37.2 [−$\underline{C}H_2$−CONH−], 39.7 [−CO-NH−$\underline{C}H_2$], 40.0 [−CONH−$\underline{C}H_2$], 53.6 [−COO$\underline{C}H_3$], 53.8, 54.45 [−$\underline{C}$H−, lysine], 170.2 [−$\underline{C}$ONH−], 170.9 [−$\underline{C}$ONH−], 176.4 [$\underline{C}$OO−].

Preparation of the Gemini Surfactants (Scheme 3). (a) General Procedure for the Synthesis of N^{ε}-Lauroyl-N^{α}-carbobenzoxylysine (LZK) and N^{α}-Lauroyl-N^{ε}-carbobenzoxylysine (LZL). In a beaker equipped with a thermometer and a pH electrode, N^{α}-carbobenzoxy-lysine or N^{ε}-carbobenzoxylysine (0.050 mmol) was dissolved in 300 mL of water/acetone (38:62, v/v) solution. After stirring, NaOH was added until pH 10, and the mixture was cooled at 0 °C while lauroyl chloride (0.052 mmol) was added dropwise. The solution was held in the pH range of 9−10 by the simultaneous addition of a 10% aqueous NaOH solution, and the temperature was maintained below 10 °C. After completion of the addition, HCl (10%) was added until pH 2; the resulting precipitate was filtered and washed with water until pH 7 and dried over P_2O_5. Then, the solid was washed with diethyl ether and crystallized from methanol to yield the pure compounds LZK and LZL.

Analytical Data and Spectral Assignments for LZK. Yield, 90%; HPLC, t_r = 16.6 min; MW, 462.2 g/mol; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 3H, −$\underline{C}H_3$, alkyl chain], 1.2−1.8 [m, 24H, −$\underline{C}H_2$−, alkyl chain and lysine lateral chain], 2.1 [t, 2H, −$\underline{C}H_2$−CONH−], 3.1 [t, 2H, −CONH−$\underline{C}H_2$−], 4.1 [m, 1H, −$\underline{C}$H−, lysine], 5.0 [s, 2H, −$\underline{C}H_2$−, Cbz group], 7.3 [m, 5H, −$\underline{C}$H−, aromatic ring]; ^{13}C NMR, δ_C(CD_3OD) 14.4 [−$\underline{C}H_3$, alkyl chain], 23.7, 24.2, 27.0, 29.9, 30.3, 30.4, 30.6, 30.7, 32.3, 33.0 [−$\underline{C}H_2$−, alkyl chain and lysine lateral chain], 37.1 [−$\underline{C}H_2$−CONH−], 40.0 [−CONH−$\underline{C}H_2$−], 55.2 [−$\underline{C}$H−, lysine], 67.5 [−$\underline{C}H_2$−, Cbz group], 128.7, 128.9, 129.4 [−$\underline{C}$H−, aromatic ring], 138.2 [−$\underline{C}$−, aromatic ring], 158.6 [−$\underline{C}$O−, Cbz group], 175.9 [−$\underline{C}$ONH−], 176.2 [$\underline{C}$OOH].

Analytical Data and Spectral Assignments for LZL. Yield, 90%; HPLC, t_r = 16.6 min; MW, 462.6 g/mol; ^{1}H NMR, δ_H (CD_3OD) 0.9 [t, 3H, −$\underline{C}H_3$, alkyl chain], 1.2−1.8 [m, 24H, −$\underline{C}H_2$, alkyl chain and lysine lateral chain], 2.1 [t, 2H, −$\underline{C}H_2$−CONH−], 3.2 [t, 2H, −Cbz−NH−$\underline{C}H_2$−], 4.2 [m, 1H, −$\underline{C}$H−, lysine], 5.1 [s, 2H, −$\underline{C}H_2$−, Cbz group], 7.3 [m, 5H, −$\underline{C}$H−, aromatic ring]; ^{13}C NMR, δ_C(CD_3OD) 14.4 [−$\underline{C}H_3$, alkyl chain], 23.7, 24.1, 27.0, 26.9, 30.2, 30.4, 30.6, 30.7, 32.1, 33.0 [−$\underline{C}H_2$−, alkyl chain and lysine lateral chain], 36.7 [−$\underline{C}H_2$−CONH], 41.4 [−Cbz−NH−$\underline{C}H_2$−], 53.4 [−$\underline{C}$H−, lysine], 67.3 [−$\underline{C}H_2$−, Cbz group], 128.7, 128.9, 129.4 [−$\underline{C}$H−, aromatic ring], 138.4 [−$\underline{C}$−, aromatic ring], 158.9 [−$\underline{C}$O−, Cbz group], 175.5 [−$\underline{C}$ONH−], 176.4 [−$\underline{C}$OOH].

(b) General Procedure for the Synthesis of N^{α},N^{ω}-Bis(N^{α}-lauroyl-N^{ε}-carbobenzoxylysine) α,ω-Hexylendiamide (9a), N^{ε},N^{ω}-Bis(N^{ε}-lauroyl-N^{α}-carbobenzoxylysine) α,ω-Hexylendiamide (8a), and N^{ε},N^{ω}-Bis(N^{ε}-lauroyl-N^{α}-carbobenzoxylysine) α,ω-Spermidindiamide (7a). A solution of LZK or LZL (20 mmol) in 50 mL of CH_2Cl_2 was treated with 50 mmol of DABCO. Then, 20 mmol of hexanediamine or spermidine was added under stirring until the total solubilization of the compounds. Finally, 40 mmol of BOP was added, and the mixture was stirred for 3 h. After complete conversion of the starting materials, the mixture was cooled to 0 °C, and the crude precipitate was isolated by filtration. The solid was washed with hexane and water and dried over vacuum. Pure compound was obtained by several crystallizations from methanol.

Analytical Data and Spectral Assignments for 9a. Yield, 70%; HPLC, t_r = 21.7 min; MW, 1005.4 g/mol; ESI-MS, *m*/*z* 1028 (M + Na); ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 6H, −$\underline{C}H_3$, alkyl chain], 1.2−1.6 [m, 56H, −$\underline{C}H_2$−, alkyl chain and lysine lateral chain], 2.1 [t, 4H, −$\underline{C}H_2$−CONH−], 3.0 [t, 8H, 2 −NH−$\underline{C}H_2$−, −CO-NH−$\underline{C}H_2$−, −$\underline{C}H_2$−CONH−], 4.1 [m, 2H, −$\underline{C}$H−], 4.99 [s, 4H, 2 −$\underline{C}H_2$−, Cbz group], 7.0−7.3 [−$\underline{C}$H−, aromatic ring]; ^{13}C NMR, δ_C(DMSO-d_6) 13.6 [−$\underline{C}H_3$, alkyl chain], 21.8, 22.4, 25.0, 25.7, 28.4, 28.5, 28.7, 28.9, 31.0, 31.6 [−$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spacer chain], 35.0 [−$\underline{C}H_2$−CONH−], 38.1 [−NH−$\underline{C}H_2$−], 40.1 [−CONH−$\underline{C}H_2$−], 52.2 [−$\underline{C}$H−], 64.9 [−$\underline{C}H_2$−, Cbz group], 127.4, 128.0 [−$\underline{C}$H−, aromatic ring], 137.1 [−$\underline{C}$−, aromatic ring], 155.8 [−$\underline{C}$O−, Cbz group], 171.3 [−$\underline{C}$ONH−], 171.8 [−$\underline{C}$ONH−].

Analytical Data and Spectral Assignments for 7a. Yield, 63%; HPLC, t_r = 22.4 min; MW, 1034.4 g/mol; ESI-MS, *m*/*z* 1057 (M + Na); ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 6H, −$\underline{C}H_3$, alkyl chain], 1.2−1.8 [m, 54H, −$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spacer chain], 2.1 [t, 4H, −$\underline{C}H_2$−CONH−], 2.9 [m, 4H, −$\underline{C}H_2$−NH−$\underline{C}H_2$−, spermidine], 3.1 [t, 4H, 2 −$\underline{C}H_2$−NHCO−], 3.2 [m, 4H, CONH−$\underline{C}H_2$−(Spe)$_4$−$\underline{C}H_2$−NH$\underline{C}$O], 3.9 [m, 2H, 2 −$\underline{C}$H−], 5.0 [m, 4H, −$\underline{C}H_2$−, Cbz group], 7.3 [m, 10H, −$\underline{C}$H−, aromatic ring]; ^{13}C NMR, δ_C (DMSO-d_6) 14.4 [−$\underline{C}H_3$, alkyl chain], 23.7, 24.1, 24.3, 24.4, 27.1, 27.3, 27.5, 30.0, 30.2, 30.3, 30.4, 30.6, 30.7, 21.2, 32.4, 32.7, 33.0, 36.6 [−$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spermidine], 37.1 [−$\underline{C}H_2$−CONH−] 39.2 [−CONH−$\underline{C}H_2$−(Spe)$_4$−$\underline{C}H_2$−NHCO−], 39.8, 39.9 [−$\underline{C}H_2$−NHCO−], 43.2, 45.9 [−$\underline{C}H_2$−NH−$\underline{C}H_2$−, spermidine], 56.7, 56.9 [−$\underline{C}$H−], 65.2−67.6 [−$\underline{C}H_2$−, Cbz group], 126.9, 127.9, 128.7, 128.8, 129.0, 129.5 [−$\underline{C}$H−, aromatic ring], 138.1 [−$\underline{C}$−, aromatic ring], 158.4 [−$\underline{C}$O−, Cbz group], 175.2, 176.2 [−$\underline{C}$O-NH−], 176.3, 176.4 [−$\underline{C}$H−$\underline{C}$ONH−].

Analytical Data and Spectral Assignments for 8a. Yield, 70%; HPLC, t_r = 21.4 min; MW, 1005.4 g/mol; ESI-MS, *m*/*z* 1028 (M + Na); ^{1}H NMR, δ_H (DMSO-d_6) 0.8 [t, 6H, −$\underline{C}H_3$, alkyl chain], 1.3−1.8 [m, 56H, −$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spacer chain], 2.1 [t, 4H, 2 −$\underline{C}H_2$−CONH−], 3.1 [m, 8H, CO-NH−$\underline{C}H_2$−(CH$_2$)$_4$−$\underline{C}H_2$−NHCO, 2 −CONH−$\underline{C}H_2$], 3.9 [m, 2H, −$\underline{C}$H−], 5.0 [m, 4H, 2 −$\underline{C}H_2$−, Cbz group], 7.3 [m, 10H, −$\underline{C}$H−, aromatic ring]; ^{13}C NMR, δ_C (DMSO-d_6) 13.6 [−$\underline{C}H_3$, alkyl chain], 21.8, 22.6, 25.0, 25.7, 28.4, 28.7, 31.0, 31.6 [−$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spacer chain], 35.3 [−$\underline{C}H_2$−CONH−], 38.0 [−CONH−$\underline{C}H_2$], 38.2 [−CONH−$\underline{C}H_2$], 54.5 [−$\underline{C}$H−], 65.1 [−$\underline{C}H_2$−, Cbz group], 127.4, 128.0 [−$\underline{C}$H−, aromatic ring], 136.9 [−$\underline{C}$−, aromatic ring], 155.6 [−$\underline{C}$O−, Cbz group], 171.3 [−$\underline{C}$ONH−], 171.7 [−$\underline{C}$ONH−].

(c) General Procedure for the Synthesis of N^{α},N^{ω}-Bis(N^{α}-lauroyl-lysine) α,ω-Hexylendiamide ($C_6(LL)_2$), N^{ε},N^{ω}-Bis(N^{ε}-lauroyl-lysine) α,ω-Hexylendiamide ($C_6(LK)_2$), and N^{ε},N^{ω}-Bis(N^{ε}-lauroyl-lysine) α,ω-Spermidindiamide ($C_7NH(LK)_2$). Compounds $C_6(LL)_2$, $C_6(LK)_2$, and $C_7NH(LK)_2$ were obtained by hydrogenation of the corresponding pure **7a**, **8a**, and **9a** (0.003 mmol) in 30 mL of MeOH/HCl (HCl/lysine derivative mol = 2.3) using Pd in activated charcoal (10% Pd) as catalyst. The reaction was carried out at room temperature and atmospheric pressure. Given that HCl is present in the reaction medium, the final surfactant was obtained as HCl salt. At the end of the reaction, the catalyst was filtered off on Celite. The solvent was evaporated under reduced pressure. Pure compound was obtained by crystallizations from MeOH/ACN.

Analytical Data and Spectral Assignments for $C_6(LL)_2$. Yield, 96%; HPLC, t_r = 17.6 min; MW, 810.0 g/mol; ESI-MS, *m*/*z* 738 (M^+ without 2Cl$^-$); elem anal. found, C, 60.8; H, 10.6; N, 10.0; Cl, 8.3; calcd for $C_{42}H_{86}N_6O_4Cl_2 \cdot 1.5H_2O$, C, 60.2; H, 10.6; N, 10.1; Cl, 8.4; ^{1}H NMR, δ_H (CD_3OD) 0.9 [t, 6H, 2 −$\underline{C}H_3$, alkyl chain], 1.2−1.8 [m, 56H, −$\underline{C}H_2$−, alkyl chain, lysine lateral chain, and spacer chain], 2.2 [t, 4H, −$\underline{C}H_2$−CONH−], 2.9 [t, 4H, 2 −CONH−$\underline{C}H_2$−], 3.1 [m, 4H, −$\underline{C}H_2$−NH_3^+], 4.2 [m, 2H, 2 −$\underline{C}$H−]; ^{13}C NMR, δ_C(CD_3OD), 14.4 [−$\underline{C}H_3$, alkyl chain], 23.7, 23.9, 26.9, 27.3, 28.0, 30.2, 30.3, 30.4, 30.5, 30.6, 30.7, 32.5,

33.0 [−CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 36.8 [−CH_2−CONH−], 40.1 [CO−NH−CH_2−], 40.4 [−CH_2−NH_3^+], 54.5 [−CH−], 174.1 [−CONH−], 176.3 [−CONH−].

Analytical Data and Spectral Assignments for $C_6(LK)_2$. Yield, 96%; HPLC, t_r = 17.8 min; MW, 810.0 g/mol; ESI-MS, *m*/*z* 738 (M^+ without $2Cl^-$); elem anal. found, C, 56.6; H, 10.4; N, 9.6; Cl, 9.0; calcd for $C_{42}H_{86}N_6O_4Cl_2 \cdot 4H_2O$, C, 57.1; H, 10.6; N, 9.5; Cl, 8.0; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 6H, 2 −CH_3, alkyl chain], 1.3−1.8 [m, 56H, −CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 2.1 [t, 4H, 2 −CH_2−CONH−], 3.1 [t, 8H, −CO-NH−CH_2−$(CH_2)_4$−CH_2−NHCO−, 2 −CONH−CH_2−], 3.8 [m, 2H, 2 −CH−]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [−CH_3, alkyl chain], 23.2, 23.7, 27.1, 27.5, 30.0, 30.1, 30.3, 30.4, 30.6, 30.7, 32.3, 33.0 [−CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 37.8 [−CH_2−CONH−], 39.8 [−CONH−CH_2−], 40.4 [−CONH−CH_2−], 54.4 [−CH−], 169.9 [−CO−NH−], 176.3 [−CO−NH−].

Analytical Data and Spectral Assignments for $C_7NH(LK)_2$. Yield, 92%; HPLC, t_r = 18.7 min; MW, 875.5 g/mol; ESI-MS, *m*/*z* 676 (M^+ without $3Cl^-$); elem anal. found, C, 55.5; H, 10.3; N, 10.3; Cl, 12.0; calcd for $C_{42}H_{86}N_6O_4Cl_2 \cdot 2.5H_2O$, C, 56.0; H, 10.3; N, 10.6; Cl, 11.5; ^{1}H NMR, δ_H (CD_3OD) 0.8 [t, 6H, −CH_3, alkyl chain], 1.2−2.0 [m, 54H, −CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 2.2 [t, 4H, −CH_2−CO−NH−Lys], 3.1 [m, 4H, CONH−CH_2−(Spe)−CH_2−NHCO−], 3.2 [t, 4H, 2 −CONH−CH_2−], 3.3 [m, 4H, −CH_2−$NH^+{}_2$−CH_2−, 3.9 [m, 2H, 2 −CH−]; ^{13}C NMR, δ_C (CD_3OD) 14.4 [−CH_3], 23.3, 23.7, 24.5, 27.1, 27.3, 29.8, 30.3, 30.4, 30.6, 30.7, 32.1, 32.2, 33.0 [−CH_2, alkyl chain, lysine lateral chain, and spermidine], 36.9 [−CH_2−CONH−], 37.3 [−CONH−CH_2−], 39.6, 39.9 [CONH−CH_2−(Spe)−CH_2−NHCO−], 46.5, 48.3 [−CH_2−$NH^+{}_2$−CH_2], 54.4 [−CH−], 170.80 and 170.22 [−CO−NH−], 176.62 and 176.61 [−CO−NH−].

(d) General Procedure for the Synthesis of N^α,N^ω-Bis(N^α-lauroyl-N^ϵ-trimethyl-lysine) α,ω-Hexylendiamide ($C_6(LK)_{2\ TM}$). To a solution of gemini $C_6(LK)_2$ (0.003 mmol) in MeOH was added K_2CO_3 until pH 10. Then dimethyl sulfate (0.014 mmol) was added dropwise, and the mixture was stirred at room temperature until the reactants were consumed. The solvent was evaporated to give the ammonium salts and was crystallized in MeOH/ACN. The sulfate salts thus obtained were treated with HCl in MeOH to obtain the corresponding chloride surfactants.

Analytical Data and Spectral Assignments for ($C_6(LK)_{2\ TM}$). Yield, 70%; HPLC, t_r = 20.5 min; MW, 894.2 g/mol; ESI-MS, *m*/*z* 411.3; elem anal. found, C, 58.2; H, 10.9; N, 8.3; Cl, 7.2; calcd for $C_{48}H_{98}N_6O_4Cl_2 \cdot 5H_2O$, C, 58.5; H, 10.9; N, 8.5; Cl, 7.2; ^{1}H NMR, δ_H (CD_3OD) 0.9 [t, 6H −CH_3, alkyl chain], 1.2−1.8 [m, 56H, −CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 2.2 [t, 4H, −CH_2−CO−NH−], 3.1 [s, 18H, N−CH_3], 3.2 [m, 4H, CONH−CH_2−$(CH_2)_4$−CH_2−NHCO], 3.4 [t, 4H CH_2−N^+], 4,3 [m, 2H, −CO−CH−NHCO]; ^{13}C NMR, δ_C(CD_3OD) 14.4 [−CH_3, alkyl chain], 23.4, 23.7, 26.9, 27.3, 30.2, 30.4, 30.5, 30.6, 30.7, 32.6, 33.0 [−CH_2−, alkyl chain, lysine lateral chain, and spacer chain], 36.9 [−CH_2−CONH−] 40.2 [−CONH−CH_2−], 53.5 [$N(CH_3)_3$], 54.4 [−CH−], 67.4 [−CH_2−N^+−], 174.1 [−CO−NH−], 176.3 [−CO−NH−].

Conclusions

Cationic surfactants from lysine, monoalkyl derivatives with one lysine on the polar head, monoalkyl derivatives with two amino acids on the polar head, and gemini surfactants have been prepared using simple and fast chemical methodologies. The CMC of these surfactants mainly depended on the hydrophobicity of the molecule. Nevertheless, the antimicrobial activity as well as the hemolytic character depended also on the charge density, the type of amino acid present on the polar head, and the position of the cationic charge. Diamino acid surfactants have CMC values around 20 mM, and they can be suitable for applications that require antimicrobial compounds against Gram-positive and Gram-negative bacteria with very low cytotoxicity. Monolysine derivatives with CMC values around 3−7 mM are active against a wide range of bacteria, and they still have low hemolytic character. Finally, gemini surfactants will be adequate for applications that need nontoxic cationic active surfactants that aggregate at very low concentrations with soft antimicrobial properties.

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Supporting Information Available: Tables listing the MIC at different pH values as well as conductivity curves against concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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