Synthesis, Surface Active Properties and Antimicrobial Activity of New Bis Quaternary Ammonium Compounds

M. Diz,[a] A. Manresa,[b] A. Pinazo,[a] P. Erra[a] and M[a]R. Infante*,[a]
[a] *Centro de Investigación y Desarrollo (CSIC), Barcelona, Spain*
[b] *Departamento de Microbiología, Facultad de Farmacia, Universidad de Barcelona, Spain*

New dimeric surfactants **1** and **2** containing two saturated hydrocarbon chains and two quaternary ammonium salts linked through an alkene spacer chain with amide and disulfide bonds have been prepared from a betaine type amphoteric surfactant. They show a high effectiveness of adsorption in comparison with their single chain homologues. Surfactants **1** and **2** are very water soluble compounds with extraordinary micelle-forming properties. Both are very active against a wide range of microorganisms including gram negative bacteria.

A surfactant molecule normally contains both one hydrophilic (water soluble) group and one hydrophobic (water insoluble) chain.[1,2] Surfactant properties have attracted growing attention for use in biochemistry, biological and chemical research applications.[3] Numerous structural modifications have been carried out to increase hydrophobicity in an effort to enhance the surface activity of surfactants. In many cases, however, there is a consequential loss of water solubility which is often a practical disadvantage (*e.g.* an increase in the length of the hydrophobic single-chain alkyl trimethylammonium surfactants increases their tendency to adsorb at an interface or to form micelles; solubility in water falls while oil solubility rises.[4]

Although reported previously,[5a] several papers have been published in the last decade addressing the synthesis and micellization properties of a new generation of bifunctional cationic surfactants, called bis(quaternary ammonium halide) surfactants or bis(Quats); these contain two hydrophobic chains, two quaternary ammonium groups and one alkene[5b-7] or heteroatomic[8,9] spacer chain (Y) per molecule. Their general chemical structure is shown in Fig. 1 where R = C_mH_{2m} + 1 (hydrophobic chain), Y = $(CH_2)_n$, NCH_3, or O (spacer group) and X = Br or Cl (counter ion).

The growing interest in these bifunctional surface active agents results from their unusual physical chemical properties (*i.e.* very low critical micelle concentration and high solubilizing capacity[10]) and their biological research applications (*i.e.* they can act as pore models for the diffusion of salts through biological membranes[11a] or as models for biological membranes).[11b] Also, the function of bis(Quats) as phase-transfer catalysts has been documented recently.[12]

This paper describes the synthesis, surface activity in water and antimicrobial properties of a new type of bis-quaternary surfactant **1** (DABK), Fig. 2. The molecule is based on two saturated hydrocarbon chains and a complex polar group consisting of two quaternary ammonium salts linked through an alkene spacer chain containing amide and disulfide bonds. This compound represents the first example of a series of homologues with hydrophobic chains in the range m = 10–14; further members of the series are currently being prepared with a view to extending the investigation.

Compound **1** and its reduced counterpart can be considered as a functionalized surfactant. They were designed as effective surfactants to offer convenient preparation by covalent coupling *via* a disulfide–thiol interchange reaction to thiol-containing substrates (*e.g.* SH-groups of cysteine side chains in enzymes and other proteins), so modifying their surface and biological properties.[13] Furthermore, these compounds could constitute an interesting micellar catalysis surfactant system for application in thiol–disulfide reactions.

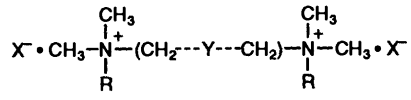


Fig. 1 Schematic representation of a bis(Quat) molecule

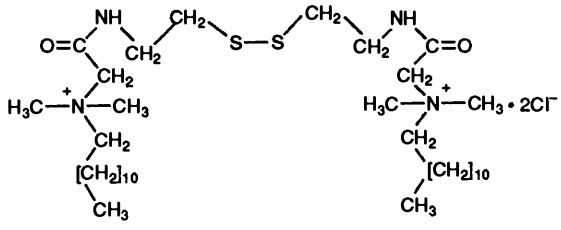


Fig. 2 Compound **1**: *N*,*N*′-bis(*N*-dodecyl-*N*,*N*-dimethylglycine)cystamine dihydrochloride (DABK)

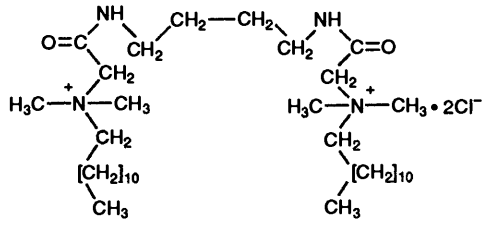


Fig. 3 Compound **2**: *N*,*N*′-bis(*N*-dodecyl-*N*,*N*-dimethylglycine)1,4-diaminobutane dihydrochloride (DABB)

This paper also describes the synthesis and properties of compound **2** (DABB), an analogue of compound **1** in which the spacer chain does not contain a disulfide bond, Fig. 3

To ascertain whether or not the presence of the disulfide bond in the spacer chain has an effect on the surface and antimicrobial properties, the characteristics of compound **1** are compared with those of **2**. Moreover, in order to determine the effect of the bifunctional structure on their intrinsic surfactant properties, these compounds are also compared with two commercial cationic surfactants, compounds **3** (DTAB) and **4** (HTAB). Possessing one quaternary ammonium salt as the hydrophilic head and one hydrocarbon chain of 12 and 16 methylene groups, respectively,[4] DTAB and HTAB can be considered as single-chain homologues of compounds **1** and **2**. Both DTAB and HTAB are regarded as conventional bactericidal cationic surfactants of potential practical interest, Fig. 4.

Compound **1** is a derivative of glycine and cystamine (dithioethyldiamine) and is potentially more biodegradable

than the classical alkanediyl-α-bis(dimethylalkylammonium halide) surfactants described in the literature.[4–6] The expected products of the hydrolysis of compound **1** are *N*-dodecyl-*N*,*N*-dimethylglycine (a well known 'soft' amphoteric surfactant) and cystamine, neither of which is dangerous from either toxicological or ecological standpoints.

Synthetic pathways for the preparation of compounds **1** and **2** are outlined in Scheme 1 pathways 1a, 1b and 2).

The preparation of compounds **1** (1a, 1b) and **2** (2) was carried out utilizing the readily available *N*-dodecyl-*N*,*N*-dimethylglycine as a source of both the hydrophobic and the quaternary ammonium moieties. In the case of compound **1**, cystamine (1a) or cysteamine (1b) (β-mercaptoethylamine) were used as sources of disulfide and nucleophilic amino functions. Thus, compound **1** was obtained by directly coupling *N*-dodecyl-*N*,*N*-dimethylglycine with cystamine (1a) or cysteamine (1b) in the absence of S-protection. The subsequent oxidation of the monomer thiol derivatives gave the required disulfide linkage.

A preliminary trial of the acidic function activation of the glycine derivative was carried out by formation of the acid chloride derivative with Cl_2SO.[14] This procedure was selected because of the high reactivity of the associated carbonyl towards any available nucleophilic group. Nevertheless yields in the coupling reaction were very poor (*ca.* 20%) owing to the low stability of this acid chloride even with the rigorous exclusion of moisture. Finally, activation of the acidic function of the glycine derivative was achieved by the classical formation of the mixed anhydride using isobutyl chloroformate (IBCF) as the reagent and *N*-methylmorpholine as tertiary base.[15]

The aminolysis of the mixed anhydride intermediate by the amino groups of cystamine (1a) preferentially yielded the monoacylated derivative of cystamine (yield: 50%), the addition of a second portion of intermediate to the crude reaction mixture being necessary to obtain compound **1** in a yield of 60%.

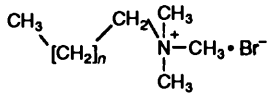
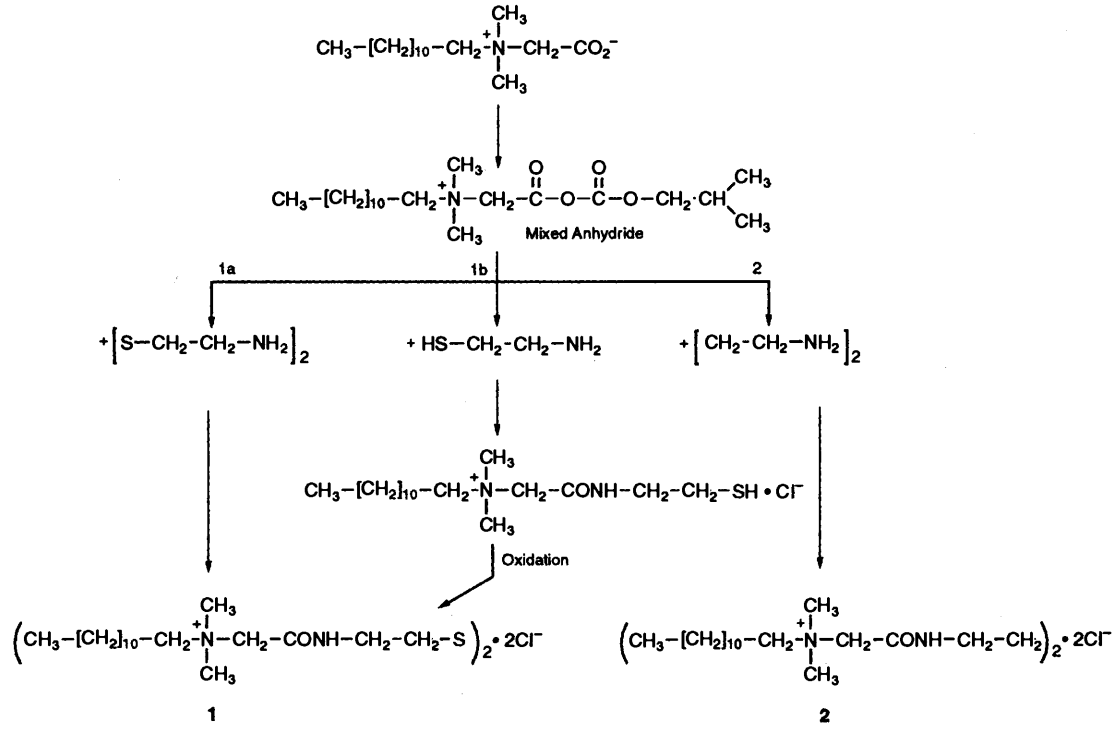


Fig. 4 Compound **3**: dodecyltrimethylammonium bromide (DTAB; n = 10); **4**: Hexadecyltrimethylammonium bromide (HTAB; n = 14)

The aminolysis of the mixed anhydride intermediate by the amino group of cysteamine (1b) yielded the monomeric thiol derivative in a yield of 80%. By subsequent oxidation (water at pH 7.0, O_2, ultrasound), **1** was obtained in a 90% yield. The procedure has already been described for the synthesis of *N*,*N*-dipalmitoylcystamine used to prepare functionalized liposomes for therapeutic applications.[16] It is noteworthy that this reaction succeeded without prior protection of the sulfydryl groups. Cysteamine was more easily and effectively accessible to the coupling reaction than cystamine and no formation of the thioester derivative was detected during the reaction. An intramolecular transfer reaction of the acyl group from the sulfur atom to the nitrogen atom (S→N) probably takes place. This has earlier been observed for some aminothiols (*e.g.* for the *S*-acetyl derivative of cysteamine)—this intramolecular transfer reaction proceeds rapidly at a pH value greater than 5, with the formation of the *N*-acetyl cysteamine derivative.[17] Little (less than 10%) urethane was formed[18] by either route, being readily eliminated by diethyl ether extractions. The final products from both routes were purified by column chromatography on silica gel and were identical with each other on the basis of their IR, ^{1}H NMR, elemental analyses and EM-FAB spectrometry; all were consistent with the expected structure of compound **1**. The purity of compound **1** was determined by two-phase mixed indicator titration method for the quantitative analysis of cationic surfactants.[19] Data were consistent with a compound containing two cationic groups per molecule at a purity of 98%.

Compound **2** was obtained in one step by the addition of 1,4-diaminobutane and methylmorpholine in dimethylformamide (DMF) to the mixed anhydride intermediate formed between *N*-dodecyl-*N*,*N*-dimethylglycine and IBCF. The addition of a second portion of the intermediate was not necessary. All analytical data were consistent with the structure of compound **2**.

In order to determine their potential applicability, solubilities in 100 g of water at 20 °C were determined in the absence or presence of sodium chloride (Table 1).



Scheme 1

Table 1 Solubility of synthetic compounds at 20 °C in the presence and the absence of 10% NaCl

Compound	Aqueous medium	Saline medium
DABK	≤25%	≤20%
DABB	≤20%	≤20%
DTAB	≤40%	≤20%
HTAB	≤0.3%	≤0.15%

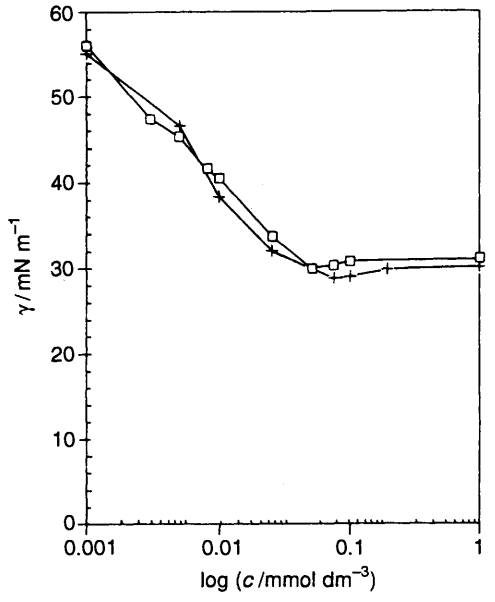


Fig. 5 Surface tension against log concentration at 25 °C

An isotropic liquid phase (probably with a cylindrical micellar structure[6]) was observed at concentrations ≤20–25%. The presence of a common soluble electrolyte such as NaCl did not induce at 20 °C precipitation of a notable amount of an amorphous solid phase of surfactant from aqueous solution as is normal in conventional cationic surfactants. These data show that the intrinsic hydrophilicities of compounds **1** and **2** are very large, comparable indeed to those reported for compound **3**. These findings could indicate that the high solubilities for compounds **1** and **2** depend essentially on the hydrophilic character of the complex polar portion: two quaternary trimethyl ammonium groups linked through a spacer chain which contain two amide bonds per molecule and that the disulfide bond has no significance.

Since these new compounds have been designed in the light of their potential use as surfactants, their fundamental surface active properties in water have been evaluated.

One of the main characteristics of surfactants is their tendency to adsorb at interfaces in an oriented fashion as a consequence of its amphipatic structure. For this adsorption it is important to determine the amount of surfactant absorbed per unit area of the saturated interface or saturation absorption, which is a measure of how much of the interface has been changed by the surfactant and depends on the structural groupings in the surfactant molecule and its orientation at the interfaces. The effectiveness of adsorption is related to the interfacial area occupied by the surfactant molecule; the smaller the effective cross-sectional area of the surfactant at the interface the greater its effectiveness of adsorption.[20] Micelle formation is another alternative mechanism to adsorption for removing hydrophobic groups from contact with water in which many interfacial phenomena such as solubilization, surface or interfacial tension reduction, *etc.* are involved. For surfactants

Table 2 Surface activity properties of DABK, DABB, DTAB and HTAB at 25 °C

Compound	γ/mN m^{-1}	CMC/10^{-5} mol dm^{-3}	A_m/Å^2 mol^{-2}
DABK	31	4.1	109
DABB	30	3.9	110
DTAB	37	1600	84
HTAB	33	100	84

in aqueous solution the adsorption and micellization processes are both related with the hydrophobic–hydrophilic balance of the molecule and increase with increase the hydrophobic character of surfactant, since it distorts the structure of the water and therefore increases the free energy of the system.[20]

Their critical micellar concentrations (CMC), determined from the break point of each surface tension/concentration curve in Fig. 5, ability to lower surface tension above the CMC (γ_{CMC}), and the area per molecule (A_m) of these surfactants are all summarized in Table 2 along with the reference data for compounds **3** and **4** measured under the same conditions.

Compared with the single-chain homologues (which apparently have the same hydrophobic–hydrophilic characteristic), **1** and **2** showed very small CMC values. The CMC data are one order of magnitude lower than those in the literature for other series of bis(Quat) type surfactants with the same chain length but different spacer chains. Thus, $CMC_{N,N'\text{-bis[(dodecyloxycarbonyl-methyl)dimethyl]-1,2-ethanediammonium}\cdot 2Br^-}$ is 2×10^{-4} mol dm^{-3},[9] while $CMC_{N,N\text{-bis[(dodecyl)dimethyl]-1,2-octanediammonium}\cdot 2Br^-}$ is 8.9×10^{-4} mol dm^{-3}.[9] Compared with the corresponding single chain surfactant with one cationic group, and even with other bis(Quat) analogues, the double chain surfactants **1** and **2** have superlative micelle-forming properties. The surface tension lowering properties above the CMC are however, of the same order as the conventional ones. These properties are similar to those of non-ionic single-chain surfactants which are much more efficient than conventional ionic surfactants in these respects. Following the work of Rosen, who has studied the surface activity of 'gemini surfactants' with two hydrophilic anionic heads and two or three hydrophobic tails,[11] these results suggest that, compared to corresponding single-chain surfactants, two alkyl chains in one molecule linked by a such spacer chain can make a positive contribution to the adsorption and micellar properties because inter- or intra-molecular hydrophobic interactions may be strengthened probably by an effect of the spacer chain nature.

As expected, they exhibited higher values of A_m than those of compounds **3** and **4** regardless the spacer chain. The areas per molecule of these compounds are of the same order of magnitude to that of bis(dodecyl quaternary ammonium bromide), a surfactant linked by a hydrocarbon spacer (C_sH_{2s}, $s = 4$); for that compound the area is $A_m = 110$ Å^2.[7] These values are, however, lower than twice the A_m of the single-chain homologues which rather suggests that the hydrophobic interactions of these dimeric surfactants with two chains of 12 carbon atoms are stronger than those of a single-chain surfactant homologue with 16 carbon atoms. This dimeric structure appears to cause a more close-packed arrangement in the water/air interface and in consequence a more effective adsorption. In line with the observations of Zana, high A_m values for compounds **1** and **2** indicate that the surface area occupied by these molecules is determined by the spacer chain which can affect strongly the distribution of distances between polar heads[7] and in consequence the hydrophobic interaction between the chains.

The antimicrobial activity of the compounds was determined

Table 3 MIC [(μg cm^{-3})$^{-1}$] of DABK (K), DABB (B) and HTAB

Microorganism	K	B	HTAB
Gram-negative			
Alcaligenes faecalis ATCC 8 750	8	8	16
Citrobacter freundii ATCC 11 606	8	16	> 16
Klebsiella pneumoniae ATCC 13 882	8	8	16
Pseudomonas aeruginosa ATCC 27 853	16	32	> 16
Bordetella bronchiseptica ATCC 4 617	8	8	8
Escherichia coli ATCC 23 231	8	16	16
Salmonella typhimurium ATCC 14 028	16	16	> 16
Serratia marcescens ATCC 13 880	16	16	> 16
Gram-positive			
Bacillus pumilus ATCC 7 061	8	16	> 16
Bacillus subtilis ATCC 6 633	8	16	16
Micrococus luteus ATCC 9 341	4	8	16
Staphylococcus epidermidis ATCC 14 990	16	16	> 16
Staphylococcus aureus ATCC 25 178	4	—[a]	—
Corynebacterium agropyri CM	0.125	0.5	0.5
Micrococcus aurianticus ATCC 11 731	2	2	4
Bacillus cereus ATCC 11 778	2	—	> 16
Streptococcus faecium ATCC 19 434	2	—	—
Enterococcus faecalis ATCC 19 433	16	16	> 16
Yeast			
Candida albicans ATCC 10 231	16	16	> 16

[a] Not determined.

on the basis of minimum inhibitory concentrations (MIC), measured as described in ref. 21. A limited but systematic investigation was carried out to determine the antimicrobial activities of **1** and **2** against 19 selected microorganisms; the results are given in the Table 3 together with those for compound **4**. Little information has been found in the literature on the antimicrobial activity of bis(Quats). Our work with a large number of selected microorganisms thus offers much more reliable information than heretofore on the antimicrobial activity of these new compounds.

Table 3 shows that, at relatively low concentrations, DABK and DABB are more effective than HTAB. The new compounds were active against both gram-positive and -negative organisms. As expected from the high lipid content of the cell membranes,[22] gram-negative bacteria were somewhat more resistant than gram-positives. No significant differences could be observed between DABK, which has a disulfide bridge (–S–S–) and its analogue counterpart, DABB; the MIC values were low for both products suggesting that the presence of the –S–S–group did not affect the antimicrobial activity. Once again the bifunctional structure of these bis(Quats) seems to be responsible for this high activity compared with their single-chain homologue. The results with our compounds are of the same order of magnitude as those of other bis(Quats) with the same hydrocarbon but different spacer chain.[23]

It has been established[24,25] that the antimicrobial action of the mono(Quat) salts is related to their physical rather than to their chemical properties. Ferguson[24] proposed that the mechanism of action of these compounds depends primarily on a physical relationship between the external surface of the microbial cell membrane and the surfactant phase, and is related to the solubility of the compound in the medium, its relative surface activity, and its ability to form micelles which is in turn closely related to its solubilizing properties. Following Ferguson's principle,[24] the high antimicrobial activity of these bis(Quats) in comparison with the single chain HTAB might be related to the greater physical chemical efficiency of these new compounds.

The successful prediction on a rational basis of the biological activity of compounds may be achieved by considering structural modifications of a known bioactive molecule (*e.g.* HTAB); such changes may be located either in the polar or hydrophobic moieties. In our case, the design of the bifunctional cationic surfactants DABK and DABB demonstrate good hydrophilicity as well as fundamental surface-active properties and very high antimicrobial activity. It may be difficult to achieve these effects solely by structural modification of general single-chain surfactants.

It is expected that these surfactants, in particular DABK are very efficient materials to change the physiochemical and biological properties of proteins which contain disulfide bonds in their structure.

Experimental

Materials.—Cystamine dihydrochloride, cysteamine hydrochloride and 1,4-diaminobutane were purchased from Fluka (Synthetic grade). *N*-Dodecyl-*N*,*N*-dimethylaminobetaine (DAB) was kindly prepared by Tenneco España S.A. Div. Marchon Surfac. It was purified by extraction with anhydrous ethanol and recrystallized from an HCl–ethanol–diethyl ether mixture. The purity of this material was checked by TLC, two-phase mixed indicator method for zwitterionic surfactants,[26] elemental analyses and ^{1}H NMR spectroscopy. Isobutyl chloroformate (IBCF) was purchased from Fluka A.G.

DMF was dried over 4 Å molecular sieves for 8 h. Prior to use it was bubbled for 3 h with a stream of nitrogen. TLC was carried out using Merck silica gel 60 plates and used throughout the synthetic procedures to monitor the course of the reaction and the homogeneity of the products. The solvent systems were A: butanol–pyridine–acetic acid–water (60:20:6:24); B: chloroform–methanol–acetic acid–water (60:25:2:4) and C: ethyl acetate–methanol (50:50). Primary amino groups were detected on the TLC with ninhydrin spray,[27] disulfide functions with nitroprusiate after reduction to sulfydryl groups[28] and quaternary ammonium groups with the Dragendorf spray.[29] The purity and structure of final products was checked by elemental analyses, ^{1}H NMR (Bruker WP 80 MHz frequency Spectrometer) and EM-FAB [MS9-VG VG11B50 Spectrometer] analyses, and the two-phase indicator method for cationic surfactants.[19] The formation of the amide linkage –CO–NH–was confirmed by FTIR [Nicolette 510 Spectrometer] and ^{13}C NMR (Varian Gemini 200 MHz frequency Spectrometer) spectroscopic analyses.

Preparation of DABK.—(a) *Starting from cystamine.* IBCF (4.7 g; 34 mmol) was added to a solution of DAB (9.02 g; 29.3 mmol) and *N*-methylmorpholine (3.5 g; 35 mmol) in DMF (50 cm^3) at −15 °C. After 5 min a chilled solution of cystamine dihydrochloride (2.2 g; 9.7 mmol) and *N*-methylmorpholine (2.3 g; 23 mmol) in DMF–H_2O (20 cm^3, 22:1 by vol.) was added to the reaction mixture. The solution was stirred for 1 h at 0 °C, left overnight at room temp. and evaporated under vacuum. The resulting crude product was dissolved again in 20 cm^3 of DMF, cooled to −15 °C and added to a solution containing IBCF (2.3 g; 17.2 mmol), DAB (5.2 g; 17.2 mmol) and *N*-methylmorpholine (1.7 g; 17.2 mmol) in DMF (50 cm^3). The new reaction mixture was stirred for 1 h at 0 °C, left overnight at room temp. and evaporated under vacuum.

In order to eliminate the urethane by-product the resulting crude product was washed with diethyl ether. It was then dissolved in $CHCl_3$ and washed successively with aqueous 10% citric acid, 10% NaCl and finally with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuum. The residue was subjected to preparative column chromatography (3.0 × 50 cm) on silica gel 60 (40–60 m), eluting with $CHCl_3$–MeOH–AcOH–H_2O (60:25:2:4 v/v). The fractions containing the pure product were evaporated and

the product was repeatedly lyophilized from H_2O, yielding a very hygroscopic white material. Yield 50%; R_f 0.43 (A) and 0.50 (B); ν(KBr)/cm^{-1} 3400 (NH), 2900 ([CH_2]$_n$), 1680 (amide I), 1560 (amide II) and 1270 (S–C); δ_H($CDCl_3$; TMS) 0.9 (3 H, t, CH_3–[CH_2]$_{10}$–), 1.2–1.8 (22 H, m, CH_3–[CH_2]$_{10}$$CH_2$–N–), 3.3 (6 H, s, –$CH_2$–N[$CH_3$]$_2$–$CH_2$), 3.1 (2 H, m, –CO–NH–$CH_2$–$CH_2$–S–), 3.4 (2 H, m, CO–NH–CH_2–CH_2–S–), 3.5 (2 H, m, N[CH_3]$_2$–CH_2–CO) and 6.2 (2 H, m, CO–NH); δ_C($CDCl_3$; TMS) 164.885 (CO–NH); m/z 663 (M^+, 2%) (C, 59.07; H, 10.46; N, 7.65; S, 4.37 Found: C, 59.0; H, 10.2; N, 6.9; S, 4.4%).

(*b*) *Starting from cysteamine.* IBCF (4.7 g; 34 mmol) was added to a solution of DAB (9.02 g; 29.3 mmol) and *N*-methylmorpholine (3.5 g; 34.5 mmol) in DMF (50 cm^3) at −15 °C. After 5 min, a chilled solution of cysteamine hydrochloride (3.3 g; 29.3 mmol) and *N*-methylmorpholine (3.5 g; 35 mmol) in DMF (25 cm^3) was added to the reaction mixture. The solution was stirred for 1 h at 0 °C, left overnight at room temp. and evaporated under vacuum. The resulting product was dissolved in $CHCl_3$ and washed successively with aqueous 10% citric acid, 10% NaCl and water.

The resulting oil was dissolved in water at pH 7.0 and treated in an ultrasonic bath with a stream of O_2 until the nitroprusiate thiol reaction was negative. In order to purify the crude product, column chromatography was carried out as for DABK. Yield 80%; R_f (A) 0.43; ν(KBr)/cm^{-1} 3400 (NH), 2900 ([CH_2]$_n$), 1680 (amide I), 1560 (amide II) and 1270 (S–C); δ_H($CDCl_3$; TMS) 0.9 (3 H, t, CH_3–[CH_2]$_{10}$–), 1.2–1.8 (22 H, m, CH_3–[CH_2]$_{10}$$CH_2$–N–), 3.3 (6 H, s, –$CH_2$–N[$CH_3$]$_2$–$CH_2$), 3.1 (2 H, m, –CO–NH–$CH_2$–$CH_2$–S–), 3.4 (2 H, m, CO–NH–CH_2–CH_2–S–), 3.5 [2 H, m, N(CH_3)$_2$—CH_2–CO] and 6.2 (2 H, m, CO–NH); δ_C($CDCl_3$, TMS) 164.885 (CO–NH); m/z 663 (M^+, 2%) (C, 59.07; H, 10.46; N, 7.65; S, 4.37 Found: C, 59.8; H, 10.6; N, 7.7; S, 4.5%).

Preparation of DABB.—IBCF (4.7 g; 34 mmol) was added to a solution of DAB (9.02 g; 29.3 mmol) and *N*-methylmorpholine (3.5 g; 34.5 mmol) in DMF (50 cm^3) at −15 °C. After 5 min, a chilled solution of 1,4-diaminobutane (0.86 g; 9.7 mmol) and *N*-methylmorpholine (19.4 mmol) in DMF (20 cm^3) was added to the reaction mixture. The solution was stirred for 1 h at 0 °C, left overnight at room temp. and evaporated under vacuum. The purification of the residue was carried out as for DABK. Yield 40%; R_f (A) 0.53; ν(KBr)/cm^{-1} 3400 (NH), 2900 ([CH_2]$_n$), 1680 (amide I) and 1560 (amide II); δ_H($CDCl_3$; TMS) 0.9 (3 H, t, CH_3–[CH_2]$_{10}$–), 1.2–1.4 (20 H, m, CH_3–[CH_2]$_{10}$ CH_2–N–), 1.6–1.8 (2 H, m, NH–CH_2–CH_2), 1.2–1.4 [6 H, s, –CH_2–N–(CH_3)$_2$–CH_2] 3.8 (2 H, m, –CO–NH–CH_2–CH_2), 3.5 (2 H, m, CO–NH–CH_2–CH_2–), 3.5 [2 H, m, N(CH_3)$_2$–CH_2–CO] and 6.2 (2 H, m, CO–NH); δ_C($CDCl_3$; TMS) 165.565 (CO–NH) (C, 64.74; H, 11.47; N, 8.39 Found: C, 64.7; H, 11.1; N, 8.3%).

Surface Tension Measurements (γ).—A Du Noüy tensiometer (Lauda) with a platinum ring was used. All solutions were prepared with double distilled water. Water/surfactant solutions of different concentrations were prepared and allowed to equilibrate in for 15–30 min at 25 °C in appropriate cells.

Methods for Critical Micellar Concentration (CMC), Saturation Absorption (Γ) *and Area per Molecule* (A_m).—CMC values for DABK and DABB were determined from the surface tension/concentration curves at 25 °C. The saturation adsorption values (Γ) at the air–water interface and the area per molecule (A_m) were calculated as in ref. 30 using the Gibbs adsorption equation: $\Gamma = 1\ (d)_T/4.30RT(d\ \log c)_T$ and $A_m = (N_A \times \Gamma)^{-1}$.

Determination of MICs.—Antimicrobial activities were determined on the basis of MIC values, defined as the lowest concentration of antibacterial agent inhibiting the development of visible growth after 24 h of incubation at 37 °C. These were determined in liquid medium using a two-fold serial antibiotic dilution technique.[21] A quick spense II microdilution system (Dynatech, Chantilly, VA) was used to prepare broth microdilution panels containing twofold dilutions of antibacterial agent in 0.15 cm^{-3} of Mueller–Hintom (MH) broth (Oxoid Ltd, Basingstoke, England). Panels were inoculated with each test organism to yield a final inoculum of 6×10^4 CFU cm^{-3} and incubated for 24 h at 37 °C.

A wide range of microorganisms used as a susceptibility test were used. Gram-negative bacteria included: *Alcaligenes faecalis* ATCC 8750, *Citrobacter freundii* ATCC 11606, *Klebsiella pneumoniae* ATCC 13882, *Pseudomonas aeruginosa* ATCC 27853, *Bordetella bronchiseptica* ATCC 4617, *Escherichia coli* ATCC 23231, *Salmonella typhimurium* ATCC 14028, *Serratia marcescens* ATCC 13880. Gram-positive bacteria were: *Bacillus pumilus* ATCC 7061, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus aureus* ATCC 25178, *Corynebacterium agropyri* CM, *Micrococcus aurianticus* ATCC 11731, *Bacillus cereus* ATCC 11778, *Streptococcus faecium* ATCC 19434 and *Enterococcus faecalis* ATCC 19433. One yeast strain was also employed: *Candida albicans* ATCC 10231.

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