Soft Antimicrobial Agents: Synthesis and Activity of Labile Environmentally Friendly Long Chain Quaternary Ammonium Compounds

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A series of soft quaternary ammonium antimicrobial agents, which are analogues to currently used quaternary ammonium preservatives such as cetyl pyridinium chloride and benzalkonium chloride, were synthesized. These soft analogues consist of long alkyl chain connected to a polar headgroup via chemically labile spacer group. They are characterized by facile nonenzymatic and enzymatic degradation to form their original nontoxic building blocks. However, their chemical stability has to be adequate in order for them to have antimicrobial effects. Stability studies and antibacterial and antiviral activity measurements revealed relationship between activity, lipophilicity, and stability. Their minimum inhibitory concentration (MIC) was as low as 1 μg/mL, and their viral reduction was in some cases greater than 6.7 log . The structure−activity studies demonstrate that the bioactive compounds (i.e., MIC for Gram-positive bacteria of <10 μg/mL) have an alkyl chain length between 12 and 18 carbon atoms, with a polar headgroup preferably of a small quaternary ammonium group, and their acquired inactivation half-life must be greater than 3 h at 60 °C.

Introduction

The shortage of new antibacterial drugs and increasing resistance of bacteria to antimicrobial agents[1] has been of some concern. Long chain quaternary ammonium compounds exert antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as against some pathogenic species of fungi and protozoa.[2] These quaternary ammonium compounds, in general, have toxic effects toward mammalian cells. In humans and animals they are considered too toxic for systemic applications, but acceptable for topical applications. The sustained toxicity and environmental impact of these antibacterial compounds are related to their chemical stability. To overcome these limitations, a series of chemically labile derivatives of long chain quaternary ammonium compounds, so-called soft analogues, have been synthesized and tested both in vitro and in vivo.[3,4] Soft compounds can be defined as biologically active compounds that are readily degraded into nontoxic and biologically inactive products in vivo, as well as in the environment. The concept of soft antibacterial agents was proposed by Bodor over 20 years ago,[5] whereby the environmental impact and toxicity of cetylpyridinium chloride was reduced by a soft drug approach. Although these analogues did not possess sufficient chemical stability to cause acceptable antibacterial effect, the concept was shown to be effective. Similar compounds such as labile L-carnitine esters[6] have also been shown to possess antibacterial activity.[7]

This report discloses the synthesis and evaluation of new soft analogues of cetylpyridinium chloride and benzalkonium chloride possessing greater efficiency, enhanced bacterial activity, and chemical stability.[8] The selection of building blocks was partly based on compounds found in marine lipids, with polar quaternary ammonium as a headgroup and a chemically labile spacer. It was expected that this kind of chemically labile compounds would be more environmental friendly than currently used hard compounds.[9] In short, the main object of this study was to synthesize antimicrobial compounds that are relatively labile ("soft") and environmentally friendly and hopefully less likely to induce bacterial resistance toward these agents.

Chemistry

Quaternary ammonium compounds with various forms of quaternary ammonium headgroups, spacers, and lipophilic alkyl chains were synthesized. The majority of these compounds are made from natural building blocks such as fatty acids, alcohols, or amides. Seven of these compounds were commercially available (used as a intermediates or for reference) and few have previously been described.[5,10] The headgroup was either pyridinium or small trialkyl moiety (trimethyl, triethyl, or tributyl), the spacer was either ester or amide group, consisting of one to three carbons, and the lipophilic alkyl chain consisted in most cases of a C12 to C18 chain, which has been shown to afford the best bioactivity.[2,6] The spacer group was designed to be labile in order for the compound to be converted to original nontoxic building blocks, both chemically and enzymatically. The relationship between the nature of the labile spacer group and the antibacterial activity was inves-

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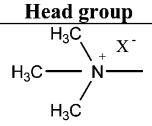
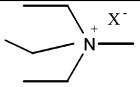
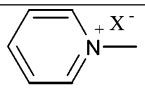
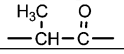
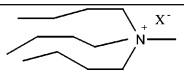
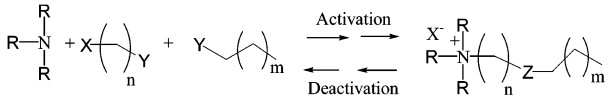
Table 1. Compounds Synthesized and Evaluated in This Study

No	Head group	Spacer	Alkyl chain
1 a		-CH_2CH_2-	-OH
1 b	-	-CH_2CH_2-	-$OOC(CH_2)_{10}CH_3$
2 a		-CH_2CH_2-	-OH
2 b	-	-CH_2CH_2-	-$OOC(CH_2)_{10}CH_3$
2 c	-	-CH_2CH_2-	- $OOC(CH_2)_{14}CH_3$
2 d	-	-CH_2CH_2-	-$HNOC(CH_2)_{14}CH_3$
2 e	-	-CH_2CH_2-	Cod liver fatty acids
3 a		-CH_2CH_2-	-$CH_2(CH_2)_8CH_3$
3 b	-	-CH_2-	-$OOCCH_3$
3 c	-	-CH_2-	- $OOC(CH_2)_{10}CH_3$
3 d	-	-CH_2CH_2-	-OH
3 e	-	-CH_2CH_2-	- $OOC(CH_2)_{10}CH_3$
3 f	-	-CH_2CH_2-	- $OOC(CH_2)_6CH_3$
3 g	-	-CH_2CH_2-	- $OOC(CH_2)_{14}CH_3$
3 h	-	-CH_2CH_2-	- $OOC(CH_2)_{16}CH_3$
3 i	-	-CH_2CH_2-	- $OOC(CH_2)_7CH{=}CH(CH_2)_7CH_3$
3 k	-	-CH_2CH_2-	-$HNOC(CH_2)_{14}CH_3$
3 l	-	-CH_2CH_2-	Cod liver fatty acids
3 m	-	-CH_2CH_2-	Saturated Cod liver fatty acids
3 n	-	-$CH_2CH_2CH_2$-	-OH
3 o	-	-$CH_2CH_2CH_2$-	-$OOC(CH_2)_{10}CH_3$
3 p	-	-$CH_2CH_2CH_2$-	-$OOC(CH_2)_{14}CH_3$
3 q	-	-CH_2CO-	-OH
3 r	-	-CH_2CO-	-OCH_2CH_3
3 s	-	-CH_2CO-	-$OCH_2(CH_2)_4CH_3$
3 t	-	-CH_2CO-	-$OCH_2(CH_2)_{14}CH_3$
3 u	-	-CH_2CO-	-$OCH_2(CH_2)_{16}CH_3$
3 v	-	-CH_2CO-	-$HN(CH_2)_{11}CH_3$
3 w	-	-CH_2CO-	-$HN(CH_2)_{17}CH_3$
3 x	-	-CH_2CH_2CO-	-OH
3 y	-	-CH_2CH_2CO-	-$OCH_2(CH_2)_{14}CH_3$
3 z	-		-OCH_2CH_3
4 a		-CH_2CH_2-	-OH
4 b	-	-	-$OOC(CH_2)_{10}CH_3$
4 c	-	-	-$OOC(CH_2)_{14}CH_3$
5	Benzalkonium Chloride		

Scheme 1. Example of the Synthetic Route and Route of Deactivation for the Compounds



R = CH_3 **Serie 1**
R = CH_2CH_3 **2**
R = Pyridine **3**
R = $(CH_2)_3CH_3$ **4**

X = Br or Cl, n = 1-3, m = 0-16, Y = NH, OH or COOH
Z = COO or CONH

tigated. The general synthetic and degradation route of the compounds is shown in Scheme 1. The NMR and the elemental analysis of several compounds (**2b**, **2c**, **3o**, **3p**, **3v**, **3w**, and **4b**) showed the presence of free fatty acids impurities (<5%), which lacked antibacterial activity. Some analogues were synthesized from mixtures of fatty acids obtained from cod liver oil. Since cod liver fatty acids are high in unsaturated fatty acids, one aspect of this study was to investigate the effect of degree of unsaturation on the antibacterial effect.

Results and Discussion

The antibacterial properties of the compounds were determined with strains of *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*. The bacterial strains represent important Gram-positive and Gram-negative species. Their antibacterial activity was assessed by measuring minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) (Table 2). Some of the soft antibacterial agents possessed antibacterial activity comparable to that of their parent hard compounds, i.e., cetylpyridinium chloride (**3a**) and benzalkonium chloride (**5**). A mixture of several different antibacterial agents was also tested, denoted as **6a**−**f** in Table 2, and their composition is given in Table 3. However, the antibacterial activity of the mixtures appeared to be comparable to that of the individual compounds.

Quaternary ammonium compounds **1a**, **2a**, **3d**, **3n**, **3q**, **3x**, and **4a**, intermediates with no alkyl chain, lacked antibacterial activity. Compounds **3b**, **3r**, and **3z** were also inactive. We tested all of the free fatty acids, the possible degradation products, and solvents used in

Table 2. Antibacterial Activity for the Compounds in μg/mL

	Enterococcus faecalis ATCC 29212		*Staphylococcus aureus* ATCC 25923		*Escherichia col* ATCC 25922		*Pseudomonas aeruginosa* ATCC 27853	
no.	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
1a	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
1b	16	64	16	16	64	64	250	250
2a	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
2b	32	32	16	16	16	125	250	250
2c	8	16	2	4	64	64	250	250
2d	not determined							
2e	15	-	7.5	-	250	-	500	-
3a[a]	<0.5	8	<0.5	4	16	-	500	1000
3b	320	3200	3200	3200	3200	3200	3200	3200
3c	10	200	50	50	100	400	3200	3200
3d	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
3e	2	100	<6.25	50	25	100	200	200
3f	>2000	>2000	500	500	1000	1000	>2000	>2000
3g	8	8	1	1	32	32	250	1000
3h	8	8	1	2	500	500	250	250
3i	4	62	8	16	62	125	250	250
3k	7.5	-	2.0	-	60	-	250	-
3l	7.5	-	15	-	250	-	>2000	-
3m	16	64	8	16	500	500	≥2000	≥2000
3n	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
3o	4	16	4	4	125	125	250	250
3p	1	4	<0.25	1	250	250	500	500
3q	12800	12800	6400	12800	12800	12800	12800	12800
3r	>12800	>12800	>12800	>12800	>12800	>12800	>12800	>12800
3s	>12800	>12800	>12800	>12800	>12800	>12800	>12800	>12800
3t	>12800	>12800	12800	12800	6400	12800	6400	6400
3u	>12800	>12800	>12800	>12800	>12800	>12800	>12800	>12800
3v	4	8	4	4	125	125	125	125
3w	<0.25	8	<0.25	4	1000	>2000	1000	>2000
3x	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
3y	250	500	250	250	>1000	>1000	>1000	>1000
3z	>12800	>12800	>12800	>12800	>12800	>12800	>12800	>12800
4a	not determined							
4b	4	16	2	8	125	125	500	1000
4c	4	8	2	2	125	250	250	1000
5[a]	1	4	1	8	16	64	64	64
6a	8	16	8	32	125	125	500	500
6b	32	125	8	16	500	500	≥2000	≥2000
6c	16	16	4	32	250	500	≥	≥2000
6d	32	32	8	16	125	250	≥2000	≥2000
6e	32	32	16	250	125	250	500	1000
6f	64	64	32	125	125	250	1000	1000

[a] **3a** = Cetylpyridinium chloride and **5** = benzalkonium chloride.

Table 3. Composition in Percentage for the Mixture Compounds **6a**−**f**

compound	**3e**	**3g**	**3h**	**4b**	**4c**	**2b**	**2c**	**3w**	**3v**
6a	-	-	-	-	-	-	-	43.5	56.5
6b	-	-	-	-	-	58.1	41.9	-	-
6c	-	-	-	59.9	40.1	-	-	-	-
6d	24.6	50.8	24.6	-	-	-	-	-	-
6e	26.1	-	-	23.5	-	23.4	-	-	27.0
6f	60.4	-	-	-	-	39.6	-	-	-

the synthesis, and they had negligible antibacterial activity (MIC > 250 μg/mL); therefore, the activity was due to the antibacterial properties of the compounds and not to that of impurities or degradation products. The long chain compounds **1b, 2,b, 2e**, **3i**, **3l**, and **3m** were able to inhibit bacterial growth and even kill some bacteria at as low concentration as 10 μg/mL. However, compounds **2c**, **3e**, **3g**, **3h**, **3p**, **3k, 3o**, **3v, 3w**, **4b**, and **4c** were inhibitory at concentrations <2 μg/mL, which is comparable to the parent hard compounds cetylpyridinium chloride (**3a**) and benzalkonium chloride (**5**). The betaine ester analogues **3s, 3t**, and **3u**, with the ester linkage reversed vs the novel compounds herein (i.e., the alkyl chain is a fatty alcohol residue, essential in comparison with the fatty acids derivatives due to their lack of chemical stability) have been tested previously.[10−12] The novel compounds killed the bacteria at relatively fast rate (Figure 1), and the relatively small difference in values for MIC and MLC confirms that these compounds are bactericidal. Cod liver derivatives (**2e**, **3l**, and **3m**) were also highly active, but not as active as the compounds with single defined alkyl chains, probably due to the fact that these are mixtures of very active long alkyl chain compounds and relatively inactive short alkyl chain compounds. There was no difference in antibacterial activity between compounds containing saturated alkyl chains and corresponding unsaturated chains.

Selected compounds were tested for anti-HSV-1 activity at concentrations up to 100 μg/mL (Table 4). Compound **4b** showed the greatest antiviral activity with ≥6.7 log (about 5 million-fold) reduction in virus titer compared to the control. At a 50 μg/mL concentration, three compounds **2c**, **3e**, and **4b** had lost most of their activity, while the others still caused significant reduction in a virus titer. Compound **3a** (the parent hard compound) was the most active at low concentrations and could be diluted to 12.5 μg/mL without losing its

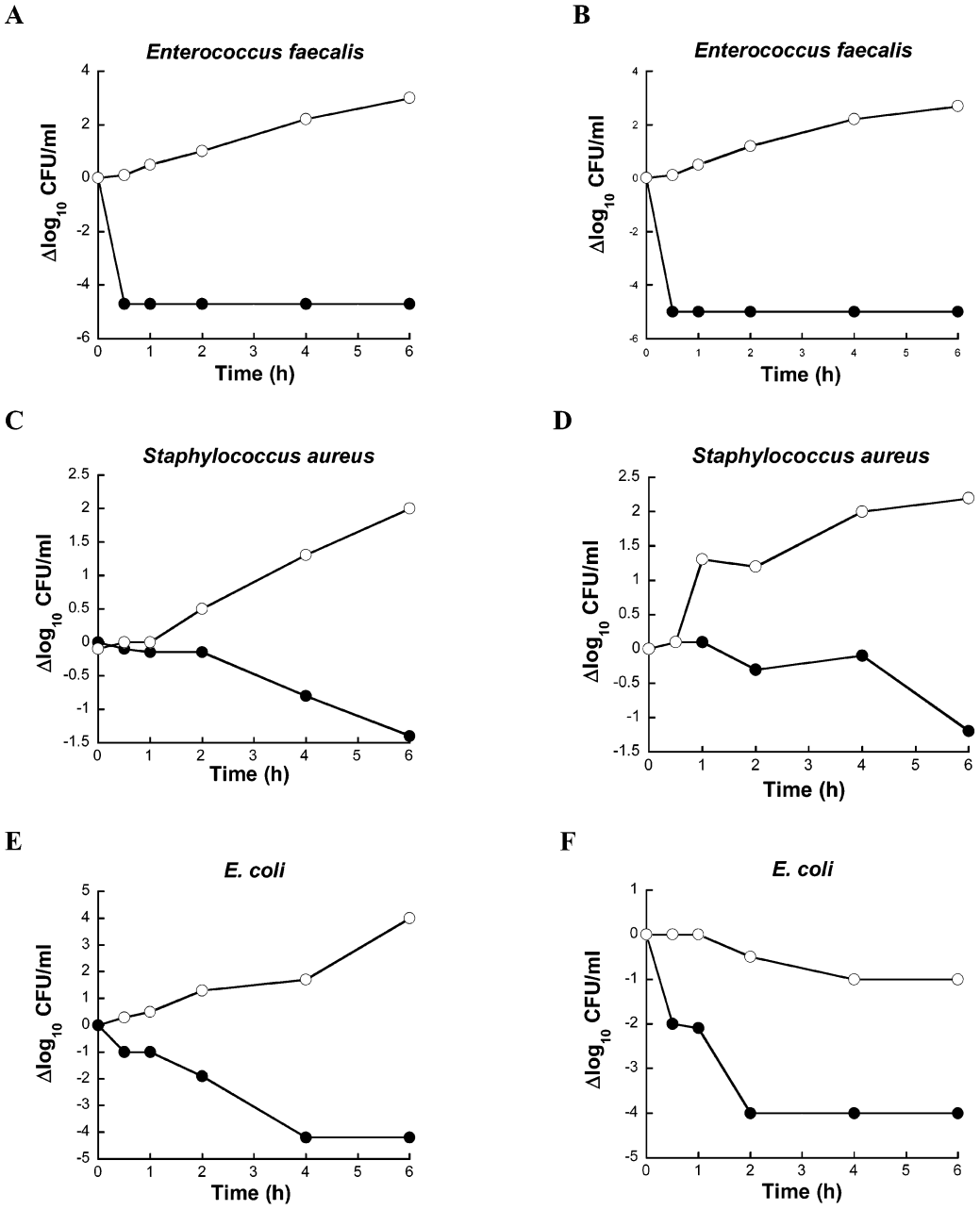


Figure 1. Effect on same type of bacteria against time (●) compared with control (○) for compounds **3g** (A, C, E) and **3h** (B, D, F). Amount used was 4 times the MIC (4 μg/mL for *Staph. aureus*).

Table 4. Antiviral Activity of Compounds against HSV-1 after 2 h Treatment at 37 °C. Virus Titer (Log_{10} $TCID_{50}$/0.1 mL)[a] after Treatment with the Following Concentrations of Compounds (μg/mL)[b]

compound	100	50	25	12.5	6.25	control
2c	0.80 ± 0.17	5.73 ± 0.25	6.33 ± 0.29	7.00 ± 0.30	ND[c]	7.00 ± 0.30
3a	2.5_T[d]	2.5_T	1.5_T	1.5_T	6.43 ± 0.12	7.10 ± 0.30
3e	1.63 ± 0.12	6.73 ± 0.25	6.73 ± 0.25	6.80 ± 0.36	7.03 ± 0.29	7.30 ± 0.00
3h	1.30 ± 0.36	1.63 ± 0.58	2.90 ± 1.22	6.10 ± 0.69	6.90 ± 0.35	7.10 ± 0.35
3k	0.80 ± 0.17	0.90 ± 0.17	2.90 ± 0.17	6.07 ± 0.40	6.47 ± 0.25	6.57 ± 0.12
3v	1.5_T	1.5_T	5.40 ± 0.17	6.57 ± 0.40	ND	7.00 ± 0.30
4b	0.5 ± NA[e]	6.00 ± 0.30	6.63 ± 0.12	7.00 ± 0.30	7.00 ± 0.30	7.20 ± 0.17

[a] Mean (±standard deviation) of three measurements for each compound. [b] Final concentrations of compounds. [c] ND, not determined. [d] $_T$, not possible to measure due to toxic effects on Vero cells. [e] NA, not available.

activity; however, it was also the most toxic toward Vero cells. Compounds **2d** and **3h** showed high antiviral activity at 25 μg/mL (reduced the virus titer about 3 to 4 log), without showing any toxicity toward the Vero cells, even at the highest concentration tested, i.e., 100 μg/mL.

Accelerated degradation studies were performed in aqueous buffer solutions at pH 6.0 and 60 °C. The observed pseudo-first-order constants (k_{obs}) shown in Table 5 indicate that both the structure of the spacer group as well as the lipophilicity affect great variations between the compounds depending on how labile spacer

Table 5. Observed Degradation Rate (k_{obs}) in Aqueous Buffer Solutions, at pH 6.0 at 60 °C, ± Standard Deviation, $n = 3$. Other Compounds Were Intermediates or Not Structurally Similar and Not Active

compound	k_{obs} (h^{-1})	compound	k_{obs} (h^{-1})
1b	0.2052 ± 0.0026	**3k**	ND[a]
2b	0.2070 ± 0.0043	**3p**	ND[a]
2c	0.1805 ± 0.0121	**3o**	0.0107 ± 0.0018
2d	ND[a]	**3s**	0.4911 ± 0.0272
3a	ND[a]	**3t**	5.2817 ± 0.3801
3c	0.3333 ± 0.0157	**3u**	8.4202 ± 1.7302
3d	0.0402 ± 0.0019	**3v**	0.0933 ± 0.0142
3e	0.2034 ± 0.0111	**3w**	0.1071 ± 0.0099
3f	0.6213 ± 0.0044	**3y**	0.6111 ± 0.0202
3g	0.0546 ± 0.0057	**4b**	0.3690 ± 0.0136
3h	0.0864 ± 0.0065	**4c**	0.5299 ± 0.0447
3i	0.0473 ± 0.0037	**5**	ND[a]

[a] No degradation was observed during 72 h.

Table 6. Observed and Predicted Antibacterial Activity, and Data Used to Derive SAR

	Log(1/MIC)				
	observed	predicted	Δ	CLogP	Log $t_{1/2}$
2b	−1.20	−0.80	−0.41	4.90	0.52
3c	−1.51	−2.90	1.40	2.03	0.31
3e	−0.90	−2.08	1.17	1.97	0.33
3f	−2.69	−2.38	−0.31	0.01	0.62
3g	0.00	−0.37	0.37	3.94	1.10
3h	0.00	−0.29	0.29	4.92	0.90
3i	−0.90	−0.02	−0.88	4.70	1.17
3o	−0.60	0.04	−0.64	2.46	1.81
3p	0.60	1.46	−0.86	4.43	2.36
3s	−4.10	−3.40	−0.70	−1.13	0.15
3t	−4.10	−3.06	−1.04	3.78	−0.90
3u	−4.10	−2.98	−1.12	4.76	−1.10
3y	0.42	−0.89	1.31	4.27	0.62
3v	−0.60	−0.85	0.24	3.27	0.92
3w	0.60	−0.43	1.03	4.86	0.81
4b	−0.30	−0.61	0.31	6.37	0.27
4c	−0.30	−0.13	−0.17	8.34	0.12

is connecting the alkyl chain with the trialkylammonium group and also a small variation between the alkyl chain lengths. Stability studies in both types of agar revealed no differences between the two agars, with regard to the observed degradation rate and relative rate compared to the half-lives obtained at 60 °C and pH 6.0. Half-life ($t_{1/2}$), of the most unstable active compound (**3c**), was 7 h in both types of agar and up to 36 h for compound **3v** at 37 °C. The stability relationship was done using the MIC values of *Staphylococcus aureus*, and there appear to be a relationship between activity and half-life of the compounds ($R^2 = 0.50$) according to our calculations, but it is not highly significant ($p = 0.111$), and relationship between activity and the ClogP values ($R^2 = 0.25$, $p = 0.133$) is less significant. However, when all those factors were combined in structure−activity relationship (SAR), there is a strong relationship between all these parameters. The relationship follows the following equation:

$$\text{Log (1/MIC)} = 1.316 \text{ Log } t_{1/2} + 0.350 \text{ CLog P} - 3.202 \text{ (stdev} = 0.91)$$

$$R^2 = 0.73,\ p = 0.004$$

Comparison of measured values and calculated values is shown in Table 6. The correlation shows that even though a given compound has adequate lipophilicity, its antibacterial activity will only be adequate if its chemical stability is sufficient to allow the compound to express its activity for sufficient duration of time. Increasing the lipophilicity further, beyond what is observed in the present data set, is not expected to increase activity of the compounds. Other investigators have shown that activity will decrease when the alkyl chains are longer than C18.[15−18] In addition, a further increase in stability is not expected to increase antibacterial activity, as the most stable compounds in the present data set are just as active as the hard compounds, benzalkonium chloride (**5**) and cetylpyridinum chloride (**3a**).

Conclusion

Compounds were synthesized, possessing various alkyl chain lengths, various polar quarter ammonium headgroups, and different spacers. The bactericidal activities of some of the compounds were very good, and the most active compounds (MIC for Gram-positive bacteria <10 μg/mL) have alkyl chain length between 12 and 18 carbon atoms, a polar headgroup (preferably small quaternary ammonium group), and a half-life in aqueous solutions of at least 3 h at 60 °C. These compounds could replace currently used compounds resulting in less residue impact on the environment, with multipurpose usage against bacterial and viral infections.

Experimental Section

Melting points were determined on a Gallenkamp melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were measured on a 250 MHz Bruker AC 250P NMR spectrometer with tetramethylsilane (Me_4Si) as an internal reference and $CDCl_3$ or D_2O as a solvent. Both ^{1}H NMR and ^{13}C NMR spectral data are reported in parts per million (δ) relative to Me_4Si. Elemental analysis was performed at the Microanalytical Laboratorium Institut for Physical Chemistry at University of Vienna, Austria. Compounds **1a**, **1b**, **3a**, and **5** are commercially available and not synthesized in this study, compounds **2a**, **3n**, **3q**, **3x**, and **4a** are commercial available intermediates that were synthesized for this study. All chemicals were of synthetic, analytical or HPLC grade. All chemicals used are commercial available and were from Merck, Sigma-Aldrich, ICN or Rathburn. The refined cod liver fatty acids were donated from Lysi ehf, Reykjavik.

Triethyl-(2-hydroxyethyl)ammonium Bromide (2a). Equimolar amounts of triethylamine (2.8 g, 28 mmol) and bromoethanol (3.5 g, 28 mmol) were mixed at 0 °C in a cooling water bath and then heated slowly until the solution turned to solid. The mixture was cooled to room temperature, anhydrous ether was added, and the solid was washed thoroughly with ether. Recrystallization from ethyl acetate:methanol (50:50) afforded white crystals (5.4 g, yield 85%). Mp 210−212 °C. ^{1}H NMR (D_2O) δ 3.98 (t, 2H, $J = 6$ Hz), 3.36 (q, 6H, $J = 7.5$ Hz), 3.22 (t, 2H, $J = 6$ Hz), 1.28 (t, 9H, $J = 7.5$ Hz). ^{13}C NMR ($CDCl_3$) δ 59.9, 55.9, 49.1, 9.2. Anal. ($C_8H_{20}BrNO$) C, H, N.

General Procedure for Compounds 2b,c. Equimolar amounts of corresponding fatty acid chloride (6 mmol) and compound **2a** were mixed, sonicated, and heated at reflux in CH_2Cl_2 overnight. The mixture was cooled to room temperature and evaporated, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. The crude compound was recrystallized in a mixture of ethyl acetate:EtOH (10:1), affording white crystals. Yields 89−93%.

(2-Dodecanoyloxyethyl)triethylammonium Bromide (2b). ^{1}H NMR ($CDCl_3$) δ 4.53 (t, 2H, $J = 7.0$ Hz), 3.85 (t, 2H, $J = 7.0$ Hz), 3.58 (q, 6H, $J = 7.2$ Hz), 2.30 (t, 2H, $J = 7.8$ Hz), 1.56 (m, 2H), 1.40 (t, 9H, $J = 7.2$ Hz), 1.22 (m, 16H), 0.84 (t, 3H, $J = 7.0$ Hz). ^{13}C NMR ($CDCl_3$) δ 172.7, 57.1, 55.8, 54.3,

46.0, 33.7, 31.8, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 24.5, 22.6, 14.0, 8.1. Mp 143−147 °C. Anal. ($C_{20}H_{42}BrNO_2$) H, N; C: calcd, 58.81; found, 57.18.

(2-Hexadecanoyloxyethyl)triethylammonium Bromide (2c). ^{1}H NMR ($CDCl_3$) δ 4.52 (t, 2H, J = 7.0 Hz), 3.84 (t, 2H, J = 7.0 Hz), 3.59 (q, 6H, J = 7.2 Hz), 2.29 (t, 2H, J = 7.8 Hz), 1.56 (m, 2H), 1.39 (t, 9H, J = 7.2 Hz), 1.21 (m, 24H), 0.83 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 172.7, 57.1, 55.8, 54.3, 46.0, 33.7, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 24.5, 22.6, 14.0, 8.1. Mp 157−162 °C. Anal. ($C_{24}H_{50}BrNO_2$) H, N; C: calcd, 62.05; found, 60.42.

General Procedure for Compounds 3b, 3r, and 3z. Equimolar amounts (30 mmol) of the corresponding acyloxyalkyl halide and pyridine were mixed and heated to 70−90 °C for 3−6 h in water bath. The mixture was cooled to room temperature, anhydrous ether was added, and the mixture was triturated in anhydrous ether overnight. The solid was collected by filtration, washed thoroughly with ether, and purified by silica gel chromatography. Yields 72−86%.

Acetyloxymethylpyridinium Chloride (3b). ^{1}H NMR ($CDCl_3$) δ 9.43 (d, 2H, J = 7.5 Hz), 8.55 (q, 1H, J = 7.5 Hz), 8.20 (q, 2H, J = 7.5 Hz), 6.80 (s, 2H), 1.21 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.0, 148.2, 144.9, 129.1, 79.7, 33.3, 17.0.

1-Ethyloxycarbonylmethylpyridinium Chloride (3r). ^{1}H NMR ($CDCl_3$) δ 9.48 (d, 2H, J = 6.5 Hz), 8.49 (q, 1H, J = 7.5 Hz), 8.10 (q, 2H, J = 7.0 Hz), 6.55 (s, 2H), 1.60 (m, 2H), 0.85 (t, 3H, J = 7.0 Hz).

1-Ethyloxycarbonylethylpyridinium Chloride (3z). ^{1}H NMR ($CDCl_3$) δ 9.45 (d, 2H, J = 6.0 Hz), 8.45 (q, 1H, J = 7.5 Hz), 8.05 (q, 2H, J = 7.1 Hz), 6.38 (m, 1H), 4.17 (d, 3H, J = 7.0 Hz), 3.62 (m, 2H), 0.80 (t, 3H, J = 7.5 Hz).

1-Dodecanoyloxymethylpyridinium Chloride (3c). Adopted from Bodor et al.[5] Equimolar amounts (32 mmol) of the corresponding acyloxyalkyl chloride and paraformaldehyde were mixed and heated between 90 and 100 °C for 3−6 h. The resulting crude chloromethyl ester was purified by silica gel chromatography. A mixture of 1-dodecanoyloxymethyl chloride and pyridine was heated at 90 °C for 3h. The mixture was cooled to room temperature, anhydrous ether was added, and the mixture was stirred in anhydrous ether overnight. The solid was isolated by filtration and washed thoroughly with ether. Mp 71−75 °C and yield 68%. ^{1}H NMR ($CDCl_3$) δ 9.44 (d, 2H, J = 7.5 Hz), 8.57 (q, 1H, J = 7.5 Hz), 8.18 (q, 2H, J = 7.5 Hz), 6.82 (s, 2H), 2.33 (m, 2H), 1.54 (m, 2H), 1.18 (bs, 16H), 0.81 (t, 3H, J = 6.7 Hz). ^{13}C NMR ($CDCl_3$) δ 172.6, 147.7, 145.6, 128.2, 79.7, 33.4, 31.8, 29.5, 29.4, 29.2, 29.1, 29.0 28.9, 24.2, 22.5, 14.0.

1-(2-Hydroxyethyl)pyridinium Bromide (3d). Equimolar amounts of pyridine (2.5 g, 32 mmol) and bromoethanol (3.9 g, 32 mmol) were mixed and heated slowly until the solution turned to a solid. The mixture was cooled to room temperature, anhydrous ether was added, and the solid was washed thoroughly with ether. Recrystallization in ethanol afforded white crystals (6.1 g, 95% yield). Mp 99−103 °C. ^{1}H NMR (D_2O) δ 8.83 (dd, 2H, J = 7 Hz), 8.58 (q, 1H, J = 7.5 Hz), 8.08 (q, 2H, J = 7.5 Hz), 4.72 (t, 2H, J = 5.5 Hz), 4.07 (t, 2H, J = 5.5 Hz). Anal. ($C_7H_{10}BrNO$) C, H, N.

General Procedure for Compounds 3e−h. Equimolar amounts of corresponding fatty acid chloride (10 mmol) and compound **3d** were mixed and heated at 70 °C for 3−6 h. The mixture was cooled to room temperature, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. The crude product was crystallized from dichloromethane:diethyl ether (1:1). Yields 86−91%.

1-(2-Dodecanoyloxyethyl)pyridinium Bromide (3e). ^{1}H NMR ($CDCl_3$) δ 9.56 (d, 2H, J = 7.5 Hz), 8.53 (q, 1H, J = 7.5 Hz), 8.12 (q, 2H, J = 7.5 Hz), 5.39 (t, 2H, J = 5 Hz), 4.61 (t, 2H, J = 5 Hz), 2.22 (m, 2H), 1.44 (m, 2H), 1.18 (bs, 16H), 0.81 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 172.8, 145.7, 145.6, 128.1, 62.6, 60.4, 33.2, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0 28.9, 24.2, 22.5, 14.0. Mp 78−83 °C. Anal. ($C_{19}H_{32}BrNO_2$)N; C: calcd 59.06; found 59.64, H: calcd, 8.35; found 8.87.

1-(2-Octanoyloxyethyl)pyridinium Bromide (3f). ^{1}H NMR ($CDCl_3$) δ 9.42 (d, 2H, J = 7.5 Hz), 8.55 (q, 1H, J = 7.5 Hz), 8.12 (q, 2H, J = 7.5 Hz), 5.30 (t, 2H, J = 5 Hz), 4.58 (t, 2H, J = 5 Hz), 2.22 (m, 2H), 1.43 (m, 2H), 1.16 (bs, 8H), 0,80 (t, 3H, J = 6.7 Hz). ^{13}C NMR ($CDCl_3$) δ 172.9, 145.5, 128.2, 62.6, 60.4, 33.6, 31.4, 28.8, 24.7, 22.4, 20.9, 13.9. Oil at rt.

1-(2-Hexadecanoyloxyethyl)pyridinium Bromide (3g). ^{1}H NMR ($CDCl_3$) δ 9.53 (d, 2H, J = 7.5 Hz), 8.55 (q, 1H, J = 7.5 Hz), 8.12 (q, 2H, J = 7.5 Hz), 5.38 (t, 2H, J = 5 Hz), 4.60 (t, 2H, J = 5 Hz), 2.22 (m, 2H), 1.44 (m, 2H), 1.19 (bs, 24H), 0.82 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ 172.8, 145.8, 128.1, 62.6, 60.4, 33.2, 31.7, 29.7, 29.6, 29.5 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.2, 22.5, 14.1. Mp 78−82 °C. Anal. ($C_{23}H_{40}BrNO_2$) H, N; C: calcd 62.43; found 63.18. **1-(2-Eicosanoyloxyethyl)-pyridinium Bromide (3h).** ^{1}H NMR ($CDCl_3$) δ 9.53 (d, 2H, J = 7.5 Hz), 8.51 (q, 1H, J = 7.5 Hz), 8.12 (q, 2H, J = 7.5 Hz), 5.41 (t, 2H, J = 5 Hz), 4.61 (t, 2H, J = 5 Hz), 2.24 (m, 2H), 1.46 (m, 2H), 1.21 (bs, 28H), 0.84 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 172.9, 145.7, 128.1, 62.6, 60.4, 33.7, 31.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 24.5, 22.6, 14.0. Mp 79−83 °C. Anal. ($C_{25}H_{44}BrNO_2$)C, H, N.

1-(2-(*cis*-9)-Octadecenoyloxyethyl)pyridinium Bromide (3i). Equimolar amounts of oleic acid (1.0 g, 3.5 mmol) and compound **3d** (0.7 g, 3.5 mmol) were mixed with an excess (35%) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) (0.9 g) in CH_2Cl_2 and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP) (0.01 g, 0.1 mmol) and heated at reflux overnight. The mixture was cooled to room temperature and concentrated under reduced pressure. Cold anhydrous ether was added, and the mixture was stirred for 2 h. An impure solid was isolated by filtration and washed thoroughly with cold ether. This was purified by silica gel chromatography to afford a yellowish oil (1.3 g, 81% yield). ^{1}H NMR ($CDCl_3$) δ 9.40 (d, 2H, J = 7.5 Hz), 8.47 (q, 1H, J = 7.5 Hz), 8.03 (q, 2H, J = 7.5 Hz), 6.09 (m, 1H), 5.23 (m, 2H, J = 5 Hz), 5.16 (m, 1H), 4.50 (t, 2H, J = 5 Hz), 2.11 (m, 2H), 1.35 (m, 2H), 1.09 (bs, 20H), 0.70 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 172.6, 145.6, 145.3, 129.2, 62.3, 60.1, 55.1, 33.7, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 24.5, 22.6, 14.0.

General Procedure for Compounds 2d and 3k. Equimolar amounts of palmitoyl chloride (2.0 g, 7.3 mmol) and 2-chloroethylammonium chloride (0.9 g, 7.3 mmol) were mixed and heated at 70 °C for 24 h. CH_2Cl_2 was added and heated at reflux for 24 h. The mixture was cooled to a room temperature and purified by silica gel chromatography, affording a clear oil (1.8 g, 79% yield). ^{1}H NMR ($CDCl_3$) δ 5.90 (bs, 1H), 3.61 (m, 4H), 2.20 (m, 2H), 1.20 (m, 12H), 0.87 (t, 3H, J = 6.5 Hz).^{13}C NMR ($CDCl_3$) δ 140.1, 44.2, 41.1, 36.7, 31.0, 29.7, 29.3, 29.1, 29.0, 25.7, 22.7, 14.1. Equimolar amounts of triethylamine or pyridine were mixed with the product and refluxed overnight. The mixture was cooled to a room temperature, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. Purification by chromatography afforded white crystals. Yields 73−81%.

Triethyl-(2-hexadecanoylaminoethyl)ammonium Chloride (2d). IR (KBr) 1651 cm^{-1} (carbamyl),^{1}H NMR ($CDCl_3$) δ 4.43 (t, 2H, J = 7.5 Hz), 3.93 (t, 2H, J = 6.5 Hz), 3.14 (q, 6H, J = 7.0 Hz), 2.56 (m, 2H), 1.40 (t, 9H, J = 7.5 Hz), 1.18 (m, 24H), 0.83 (t, 3H, J = 6.5 Hz). Mp 109−113 °C.

1-(2-Hexadecanoylaminoethyl)pyridinium Chloride (3k). IR (KBr) 1651 cm^{-1} (carbamyl), ^{1}H NMR ($CDCl_3$) δ 9.47 (d, 2H, J = 7.5 Hz), 8.59 (t, 1H, J = 7.0 Hz), 8.41 (t, 1H, J = 7.5 Hz), 8.00 (t, 2H, J = 7.5 Hz), 6.25 (t, 2H, J = 6.3 Hz), 3.90 (q, 2H, J = 5.0 Hz), 2.12 (t, 2H, J = 7.5 Hz), 1.39 (m, 2H), 1.25 (m, 24H), 0.84 (t, 3H, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$) δ 174.6, 145.7, 144.9, 127.8, 60.6, 39.7, 36.0, 31.8, 29.8, 29.6, 29.3, 29.1, 29.0, 25.6, 22.6, 14.1. Mp 123−127 °C. Anal. ($C_{23}H_{41}(Br_{0.7}$-$OH_{0.3})NO_2$) C, H, N. Compounds **2e** and **3l**: equimolar amounts of corresponding quaternary ammonium compound (**3q** or **2a**) (4 mmol) and refined cod liver fatty acids were mixed with excess (35%) of EDAC in CH_2Cl_2 and a catalytic amount of DMAP and heated at reflux overnight. The mixture was cooled to room temperature and evaporated, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. The crude compound was purified by

silica gel chromatography, affording a clear or yellowish oil. Yields 56−68%.

(2-Cod liver oil ethyl)triethylammonium Bromide (2e). ^{1}H NMR ($CDCl_3$) δ 5.42 (m, 3H), 4.75 (t, 2H, J = 7.0 Hz), 3.85 (t, 2H, J = 7.0 Hz), 3.53 (q, 6H, J = 7.5 Hz), 2.70 (m, 1H), 2.00 (m, 2H), 1.58 (m, 2H), 1.40 (t, 9H, J = 7.5 Hz), 1.22 (m, 20H), 0.85 (t, 3H, J = 7.0 Hz).

1-(2-Cod liver oil ethyl)pyridinium Bromide (3l). ^{1}H NMR ($CDCl_3$) δ 9.50 (d, 2H, J = 7.5 Hz), 8.52 (t, 1H, J = 7.0 Hz), 8.21 (t, 1H, J = 7.5 Hz), 5.42 (m, 4H), 4.65 (m, 2H), 3.45 (m, 4H), 2.00 (m, 2H), 1.58 (m, 2H), 1.22 (m, 22H), 0.85 (t, 3H, J = 7.0 Hz).

1-(2-Cod liver oil sat. ethyl)pyridinium Bromide (3m). Same as for **3l** except the refined cod liver fatty acids had been perhydrogenated with H_2 and palladium on carbon catalysis. Yield (54%). ^{1}H NMR ($CDCl_3$) δ 9.55 (d, 2H, J = 7.5 Hz), 8.55 (t, 1H, J = 7.0 Hz), 8.12 (t, 1H, J = 7.5 Hz), 5.41 (m, 1H), 4.62 (m, 2H), 3.46 (m, 1H), 1.97 (m, 2H), 1.49 (m, 2H), 1.21 (m, 24H), 0.84 (t, 3H, J = 7.0 Hz).

1-(3-Hydroxypropyl)pyridinium Chloride (3n). Equimolar amounts of pyridine (2.5 g, 32 mmol) and 3-chloro-1-propanol (3.0 g, 32 mmol) were mixed and slowly heated to 70 °C and stirred for 3 h. The mixture was cooled to room temperature, anhydrous ether was added, and the solid was washed thoroughly with ether. Recrystallization in ethyl acetate:MeOH (14:1) afforded white crystals (4.6 g, 83% yield).

General Procedure for Compounds 3o−p. Equimolar amounts of corresponding fatty acid chloride (5 mmol) and compound **3n** were mixed and heated at 70 °C for 3−6 h. The mixture was cooled to room temperature, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. Purified by silica gel chromatography and recrystallized in mixture of ethyl acetate:MeOH (10:1) affording white crystals. Yields 82−89%.

1-(3-Dodecanoyloxypropyl)pyridinium Chloride (3o). ^{1}H NMR ($CDCl_3$) δ 9.61 (d, 2H, J = 7.5 Hz), 8.47 (t, 1H, J = 7.5 Hz), 8.10 (t, 2H, J = 7.5 Hz), 5.08 (t, 2H, J = 7.5 Hz), 4.13 (t, 2H, J = 6.3 Hz), 2.39 (t, 2H, J = 6.3 Hz), 2.12 (t, 2H, J = 7.5 Hz), 1.43 (m, 2H), 1.18 (m, 16H), 0.78 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4, 145.5, 145.1, 128.4, 60.5, 59.0, 33.9, 31.7, 30.7, 29.4−28.9, 24.6, 22.5, 14.0. Mp 149−152 °C. Anal. ($C_{20}H_{34}ClNO_2$) H, N; C: calcd, 67,49; found 66. 71. **1-(3-Hexadecanoyloxypropyl)pyridinium Chloride (3p).** ^{1}H NMR ($CDCl_3$) δ 9.54 (d, 2H, J = 5.0 Hz), 8.51 (t, 1H, J = 7.5 Hz), 8.13 (t, 2H, J = 7.5 Hz), 5.09 (t, 2H, J = 6.3 Hz), 4.16 (t, 2H, J = 6.3 Hz), 2.42 (t, 2H, J = 6.3 Hz), 2.16 (t, 2H, J = 7.5 Hz), 1.48 (m, 2H), 1.22 (m, 24H), 0.83 (t, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4, 145.5, 145.2, 128.4, 60.5, 59.0, 33.9, 31.7, 30.8, 29.4, 29.3, 29.1, 29.0, 24.6, 22.5, 14.0. Mp 129−133 °C. Anal. ($C_{24}H_{42}ClNO_2$) N; C: calcd 69.96; found 66.71, H: calcd. 10.27; found 10.77.

1-Carboxymethylpyridinium Chloride (3q). A mixture of chloroacetic acid (2.5 g, 26 mmol) in equimolar amounts of pyridine (2.1 g, 26 mmol) was heated to 70−80 °C until all the mixture had turned solid. Washed thoroughly with ether and recrystallization from ethanol which afford white crystals (4.6 g, 92% yield). Mp 118−122 °C. ^{1}H NMR (D_2O): δ 8.84 (q, 2H, J = 7 Hz), 8.62 (q, 1H, J = 8 Hz), 8.12 (q, 2H, J = 8 Hz), 5.43 (s, 2H). ^{13}C NMR (D_2O): δ 176.4, 149.0, 147.6, 130.8, 59.5.

General Procedure for Compounds 3s−u. Chloroacetyl chloride in excess amount was mixed with corresponding fatty alcohol (10 mmol) and heated between 60 and 70 °C for 4 h. The resultant chloro ester was purified by silica gel chromatography and crystallized in MeOH (except the hexyl chloro ester was liquid). Structure verified by NMR. Cetyl chloro ester: ^{1}H NMR ($CDCl_3$) δ 4.18 (t, 2H, J = 7.0 Hz), 4.05 (s, 2H), 1.65 (m, 2H), 1.25 (m, 24H), 0.83 (t, 3H, J = 7.0 Hz). Steryl chloro ester: ^{1}H NMR ($CDCl_3$) δ 4.20 (t, 2H, J = 7.0 Hz), 4.07 (s, 2H), 1.66 (m, 2H), 1.24 (m, 28H), 0.83 (t, 3H, J = 7.0 Hz).

A mixture of chloroester and pyridine was mixed and heated at 60 °C for 4 h. The mixture was cooled to a room temperature, anhydrous ether was added, and the mixture was triturated in anhydrous ether. The solid was isolated by filtration and washed thoroughly with ether, which afforded a white solid (except hexanoyl derivative was yellowish oil). Yields 76−82%.

1-Hexyloxycarbonylmethylpyridinium Chloride (3s). ^{1}H NMR ($CDCl_3$) δ 9.50 (d, 2H, J = 6.0 Hz), 8.48 (q, 1H, J = 7.65 Hz), 8.05 (q, 2H, J = 7.15 Hz), 6.38 (s, 2H), 4.17 (t, 2H, J = 7.0 Hz), 1.63 (m, 2H), 1.24 (m, 4H), 0.85 (t, 3H, J = 7.0 Hz). Mp oil at rt.

1-Hexadecyloxycarbonylmethylpyridinium Chloride (3t). ^{1}H NMR ($CDCl_3$) δ 9.50 (d, 2H, J = 6.7 Hz), 8.47 (q, 1H, J = 7.75 Hz), 8.04 (q, 2H, J = 7.75 Hz), 6.43 (s, 2H), 4.17 (t, 2H, J = 7.0 Hz), 1.64 (m, 2H), 1.24 (m, 24H), 0.87 (t, 3H, J = 7.0 Hz). Mp 113−114 °C.

1-Octadecyloxycarbonylmethylpyridinium Chloride (3u). ^{1}H NMR ($CDCl_3$) δ 9.52 (d, 2H, J = 6.7 Hz), 8.46 (q, 1H, J = 7.75 Hz), 8.04 (q, 2H, J = 7.75 Hz), 6.43 (s, 2H), 4.17 (t, 2H, J = 7.0 Hz), 1.64 (m, 2H), 1.24 (m, 28H), 0.86 (t, 3H, J = 7.0 Hz). Mp 38−39 °C.

Compounds 3v−w. Chloroacetyl chloride was mixed in excess amount with corresponding fatty amine (6 mmol) and heated between 60 and 70 °C for 4 h. The excess of chloroacetyl chloride was removed in a vacuum. The obtained chloro amide and pyridine was mixed and heated at 60 °C for 4 h. The mixture was cooled to room temperature, anhydrous ether was added, and the mixture was triturated in anhydrous ether. The white solid was isolated by filtration and washed thoroughly with ether. Purified by silica gel chromatography, yields (70−81%).

1-Dodecylcarbamoylmethylpyridinium Chloride (3v). IR (KBr) 1671 cm^{-1} (carbamyl). ^{1}H NMR ($CDCl_3$) δ 9.34 (bs, 3H), 8.43 (t, 1H, J = 7.5 Hz), 8.03 (t, 2H, J = 7.5 Hz), 5.95 (s, 2H), 3.19 (q, 2H, J = 7.5 Hz), 1.54 (m, 2H), 1.22 (m, 18H), 0.85 (t, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$) δ 163.5, 146.0, 145.0, 127.6, 62.5, 40.3, 31.9, 29.5−29.0, 27.0, 22.6, 14.1. Mp 83−87 °C. Anal. ($C_{19}H_{33}ClN_2O$) H, N; C: calcd 69.94; found 65.05

1-Octadecylcarbamoylmethylpyridinium Chloride (3w). IR (KBr) 1651 cm^{-1} (carbamyl). ^{1}H NMR ($CDCl_3$) δ 9.35 (bs, 3H), 8.43 (t, 1H, J = 7.5 Hz), 8.03 (t, 2H, J = 7.5 Hz), 5.94 (s, 2H), 3.20 (q, 2H, J = 7.5 Hz), 1.56 (m, 2H), 1.23 (m, 30H), 0.86 (t, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3$) δ 163.4, 146.0, 145.0, 127.6, 62.5, 40.3, 31.9, 29.6−29.0, 27.0, 22.6, 14.1. Mp 93−97 °C. Anal. ($C_{25}H_{45}ClN_2O$) H, N; C: calcd. 70.64; found 68.86.

1-(2-Carboxyethyl)pyridinium Chloride (3x). A mixture of chloropropionic acid (3.5 g, 32 mmol) in equimolar amounts of pyridine (2.5 g, 32 mmol) was heated to 70−80 °C until the mixture had turned solid. Washed thoroughly with ether and recrystallization in 50/50 mixture of ethyl acetate and ethanol afforded white crystals (5.4 g, 90% yields). Mp 157−160 °C. ^{1}H NMR (D_2O) δ 8.90 (q, 2H, J = 5.5 Hz), 8.54 (q, 1H, J = 7.5 Hz), 8.05 (q, 2H, J = 7.5 Hz), 4.87 (t, 2H, J = 6 Hz), 3.17 (t, 2H, J = 6 Hz). ^{13}C NMR (D_2O) δ 176.0, 148.5, 147.3, 130.6, 59.3, 36.9. Anal. ($C_8H_{10}ClNO_2$) C, H, N.

1-(2-Hexadecyloxycarbonylethyl)pyridinium Chloride (3y). Equimolar amounts of hexadecanoic acid (1.0 g, 3.9 mmol) and compound **3x** (0.7 g, 3.9 mmol) were mixed with an excess (35%) of EDAC (1.0 g) in CH_2Cl_2 and a catalytic amount of DMAP (0.01 g, 0.1 mmol), heated to reflux overnight. The mixture was cooled to room temperature and concentrated under reduced pressure. Cold anhydrous ether was added, and the mixture was stirred for 2 h. The solid was isolated by filtration and washed thoroughly with cold ether. Purified by silica gel chromatography afforded white solid (1.1 g, 67% yield).^{1}H NMR ($CDCl_3$) δ 9.48 (d, 2H, J = 7.0 Hz), 8.45 (q, 1H, J = 7.7 Hz), 8.03 (q, 2H, J = 7.7 Hz), 5.43 (t, 2H, 5.0 Hz), 4.27 (t, 2H, J = 5.0 Hz), 1.62 (m, 2H), 1.22 (m, 24H), 0.88 (t, 3H, J = 7.0 Hz). Mp 97−101 °C. Anal. ($C_{24}H_{42}ClNO_2 \cdot H_2O$): C, N; H: calcd. 10.31; found 10.77.

Tributyl-(2-hydroxyethyl)ammonium Bromide (4a). A mixture of bromoethanol (3.5 g, 28 mmol) in equimolar amounts of tributylamine (5.2 g, 28 mmol) was heated to 70−80 °C and stirred overnight. Washed thoroughly with ether, afford white solid (6.7 g, yields 78%). ^{1}H NMR ($CDCl_3$) δ 4.62 (t, 2H, J = 5.5 Hz), 4.07 (t, 2H, J = 5.5 Hz), 3.42 (t, 6H, J =

7.5 Hz), 1.72 (m, 6H), 1.43 (m, 6H), 0.97 (t, 9H, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 59.9, 58.3, 49.4, 29.3, 21.1, 13.5. Mp 63−67 °C.

General Procedure Compounds 4b,c. Equimolar amounts of corresponding fatty acid chloride (6 mmol) and compound **4a** were mixed, sonicated, and refluxed in CH_2Cl_2 overnight. The mixture was cooled to room temperature and evaporated, anhydrous ether was added, and the solid was isolated by filtration and washed thoroughly with ether. The obtained compound was recrystallized in mixture of ethyl acetate:MeOH (10:2), affording white crystals. Yields 64−76%.

Tributyl-(2-dodecanoyloxyethyl)ammonium Bromide (4b). ^{1}H NMR ($CDCl_3$) δ 4.52 (bs, 2H), 3.91 (bs, 2H), 3.41 (t, 6H, J = 7.7 Hz), 2.26 (t, 2H, J = 7.4 Hz), 1.69 (m, 6H), 1.55-(m, 2H), 1.40 (m, 6H), 1.20 (m, 24H), 0.97 (t, 9H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 172.7, 59.5, 57.5, 34.0, 31.6, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 24.7, 24.5, 22.9, 20.0, 13.6, 13.5. Mp 60−64 °C. Anal. ($C_{26}H_{54}BrNO_2$) H, N; C: calcd. 63.39; found 62.16.

Tributyl-(2-dodecanoyloxyethyl)ammonium Bromide (4c). ^{1}H NMR ($CDCl_3$) δ 4.53 (bs, 2H), 3.92 (bs, 2H), 3.42 (t, 6H, J = 7.9 Hz), 2.28 (t, 2H, J = 7.6 Hz), 1.70 (m, 6H), 1.57(m, 2H), 1.41 (m, 6H), 1.22 (m, 16H), 0.98 (t, 9H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ 172.7, 59.5, 57.6, 34.0, 31.7, 29.4, 29.3, 29.2, 29.1, 24.5, 22.5, 20.0, 13.6, 13.5. Mp 79−83 °C. Anal. ($C_{30}H_{62}BrNO_2$) C, H, N.

HPLC Measurements. The HPLC system consisted of Merck Hitachi L-7100 gradient solvent delivery system with a L-7400 UV detector, using (a) 150 mm, 4.6 mm i.d., 5 μm bead, C18 reverse-phase column; (b) cyanocolumn 150 mm, 4.6 mm i.d., 5 μm; (c) 75 mm, 4.6 mm i.d., 3 μm bead, C18 reverse-phase column; (d) LiChrosorb NH_2, 5 μm. The detection was performed at 230 or 254 nm wavelength, and the flow rate was 1.5 mL/min. The mobile phases and retention times for each compound were as follows: (**3c**) (a) gradient system of acetonitrile, 0.015% octasulfonic acid in H_2O (40−100% acetonitrile in 15 min), 4.0 min, (**3e**) acetonitrile, acetic acid, H_2O (90:0.5:9.5), 4.1 min; (b) (**3s**) MeOH, 0.05 M phosphoric acid (92:8), 2.1 min. (**3t**) 2.6 min. (**3u**) 3.6 min. (**3q**) 3.0 min. (c) MeOH, 1% acetic acid in H_2O (55:45), (**3g**) 2.8 min. (**3h**) 3.6 min. (**3i**) 3.3 min. (**3w**) (81:19), 2.9 min. (**3v**) (68:32), 3.4 min. (**3o**) (70:30), 2.1 min. (**3p**) (75:25), 3.8 min. (d) 0.015% octasulfonic acid in H_2O, MeOH (80:20) (**3d**) 2.5 min.

Degradation Rate Studies. Stock solution (0.1 mg/mL) was prepared in ethanol, and 30 μL of this solution was added to 1.5 mL an aqueous buffer solution. The degradation was measured by periodic HPLC analysis of buffered solutions heated on the sample rack. The degradation rate constants (k_{obs}) were obtained by linear regression of the peak integration. The degradation rates of the compounds were determined at pH 6 in 10 mM phosphate buffer at 60 °C (±0.2 °C). Stability of selected compounds (**3c**, **3e**, and **3v**) was also determined in diluted (1:4) agar at 37 °C.

Compounds that did not have a good chromophore were measured at Encode Inc. in a LC-MS/MS Quattro Ultima from Micromass with Hewlett-Packard 1100 gradient pump and HCT PAL autosampler from CTC Analytics.

Lipophilicity Calculation. Octanol−water partition coefficient (ClogP) was calculated online (www.syrres.com) according to structure. Two compounds had sufficient aqueous solubility for the octanol−water partition coefficients determination and were used to compare to the calculation. These compounds were measured according to the shake flask method.[13] Literature values for the known compounds were also used to compare to the calculated values. The calculations were less than 10% from the experimental values.

Antibacterial Test. The antibacterial tests were performed at the Department of Clinical Microbiology, Landspitali University Hospital. The method used was a microtiter dilution, performed according to the NCCLS standard.[14] It was adapted to the specific needs related to the solubility of the compounds. In the case of poor water solubility, as small a concentration of dimethyl sulfoxide (Sigma) as possible was used as a solvent, and in one of the test series it was not possible to obtain the value 1 μg/mL for the doubling dilutions. The media used for MIC measurements was Mueller Hinton broth (Oxoid) and blood agar (heart infusion agar (Oxoid) with 5% defibrinated horse blood) for measurements of MLC. Measurements are expressed in μg/mL. The broth dilutions and the agar plates were incubated for 24h, at 37 °C, in ambient air. The bacterial strains, *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), selected were type strains from the ATCC (American Type Culture Collection), representing clinically important species recommended by the NCCLS as quality control strains for susceptibility testing.

Antiviral Test. Vero cells (African green monkey kidney cell line) were grown in RPMI-1640 media with 2.0 mM L-glutamine, 0.05 mg/mL gentamicin, 0.375% (w/v) sodium bicarbonate, and 10% heat-inactivated fetal bovine serum (FBS). The maintenance medium (MM) for Vero cell monolayers was RPMI-1640 with 2% FBS. All media was obtained from GIBCO, Paisley, Scotland. Herpes simplex virus type 1 (HSV-1) strain MacIntyre (American Type Culture Collection, Rockville, MD) was grown in Vero cells. Virus stocks with an infectivity titer of $10^{6.5}$−$10^{7.5}$ $TCID_{50}$ (50% Tissue Culture Infective Dose) per 100 μL were used in the experiments. The virus was titrated by inoculation of 10-fold dilutions in MM into monolayers of Vero cells in 96-well microtiter tissue culture plates (Nunc, Roskilde, Denmark). One hundred microliters of each virus dilution was inoculated into quadruplicate wells. The plates were incubated at 37 °C in a humidified incubator with 5% CO_2 in the air and examined for cytophatic effect daily for 5 days. Virus titers were calculated by the method of Reed and Muench (1938). Stock solutions of the compounds were made in Hanks balanced salt solution (HBSS) at a concentration of 1 mg/mL. Two-fold dilutions of each stock solution were made in MM, and each dilution was mixed with an equal volume (100μL) of HSV-1 in 12 × 75 mm polystyrene round-bottom tubes (Falcon). The mixtures were incubated at 37 °C in a water bath for 2 h. The action of the compounds was then stopped by diluting the mixtures 10-fold in MM. Virus mixed with MM served as a control. The virus titers of the mixtures were determined by an inoculation of 10-fold dilutions into cell culture as previously described. The difference in the titer (log) of the compound−virus mixture and the titer (log) of the control mixture i.e., the reduction in viral infectivity, was used as a measure of the antiviral activity of the compounds.

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