Synthesis and Antibacterial Activity of Immobilized Quaternary Ammonium Salts

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SYNOPSIS

Quaternary ammonium salts were immobilized on hydrophilic gels based on sucrose methacrylates (SM) and tested for their antibacterial properties. The cross-linked polymers were prepared by copolymerization of monomer–SM mixtures with 4-vinylpyridine and subsequent quaternization with 1-bromoctane and 1-bromoctadecan and by esterification of SM gels with 3-pyridine carboxylic acid chloride and quaternization. In addition, immobilized quaternary salts bonded by hydrophobic as well as by hydrophilic spacers were synthesized by esterification of SM gels with 11-bromundecanoic acid chloride and the tetraethylene glycol-based acid chloride **13c**, respectively, and subsequent reaction of the halogen-substituted gels with tertiary amines. Suspension tests for antibacterial properties of the immobilized bactericides against *Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus* demonstrated high activity of the quaternary salts bonded by the hydrophobic spacer. Advantageously, these insoluble bactericides can be applicated without contamination of the substrate; they can be removed easily and used repeatedly. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Polymeric disinfectants have received considerable attention in recent years with respect to important applications, e.g., as drugs with prolonged activity and less toxicity[1] and in antifouling coatings,[2-4] fiber finishing,[5,6] and water and air disinfection.[7-10] Soluble low molecular as well as polymer biocides[11,12] frequently give rise to problems like toxicity and a residual agent, especially when they are applied in water treatment, foodstuffs, and packaging materials. The problem of residues can be solved if the bactericide is insolubilized by covalent bonding to polymeric carriers or onto the surface of other insoluble materials. These carrier-bound disinfectants enfold their antimicrobial effect in the bound state and must be therefore distinguished from immobilized bactericides effecting after having been split off from the matrix. In the former case, the active agent is bonded by hydrolytically stable linkages, thus enabling prolonged antimicrobial action without significant release of toxic materials, whereas the desired release of the latter is achieved by linkages that may be split under mild conditions, resulting in contamination and low durability of the antibacterial action.[13-15]

Besides the prevention of toxic contamination of the substrate, insoluble disinfectants offer some additional advantages: They can be used in excess and they can be removed easily from solutions by filtration and applied repeatedly. Moreover, it might be possible to sterilize liquids continuously and to use agents that are prohibited for use in food applications because of their strong toxicities.

So far, insoluble carriers such as cellulose, starch, agarose, porous glass, and cross-linked styrene/divinylbenzene have been used as carriers for the immobilization of antibiotics,[16,17] metallorganic compounds,[7,8] and quaternary ammonium salts.[9,18-21] In addition, the antimicrobial activity of cross-linked 4-vinyl alkylpyridinium salts has been reported,[22] but later investigations demonstrated that this effect was due to adsorption of cells to the surface.[23]

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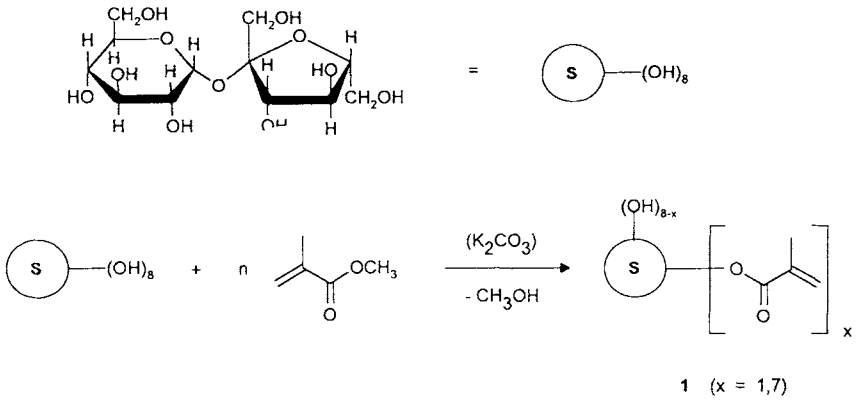
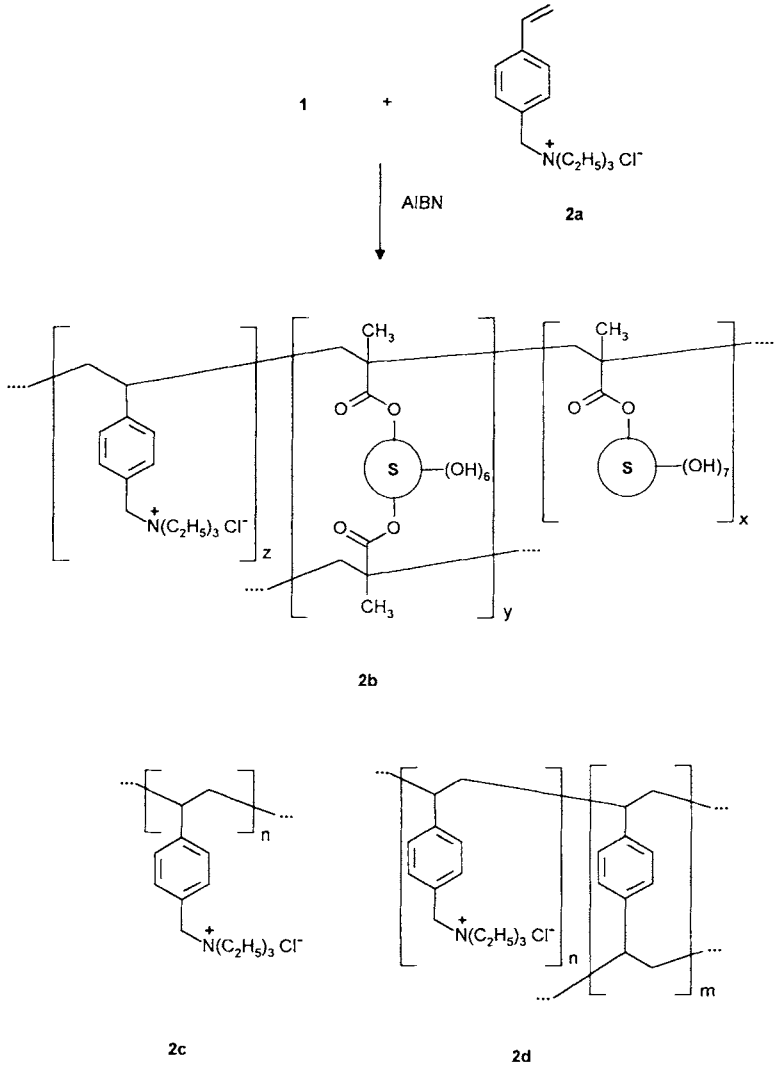


1 (x = 1,7)

Scheme 1



2b

2c

2d

Scheme 2

Recently, insoluble polymer beads based on cross-linked polystyrene–polyethyleneimine polymers,[24,25] on poly(glycidyl methacrylate)–tetraethylenepentamine,[26] and on activated alumina-supported poly(styrene-*co*-chloromethylstyrene)[27] were investigated for the removal of bacteria from water. A decrease of viable cell numbers was observed, due to the adsorption of cells to the surface of these polymers. The adsorbed cells were found to be alive and could be cultured again after rinsing.

Generally, few data concerning the influence of the nature of the carrier and the bactericidal group on the antibacterial activity, the hydrolytical stability, and durability of immobilized bactericides are available. The biocidal activity of linear polymers was suggested to be influenced strongly by both hydrophilic and hydrophobic interactions of the polymer with the cell surfaces.[28,29,31] Considering cross-linked polymers as carriers, the accessibility of the bactericidal groups as well as the swellability of the carrier in aqueous media is of importance, which can be influenced by spacer groups and the type and cross-linking density of the polymer.

Presuming that the appropriated combination of bactericide, spacer, and cross-linked carrier is essential for high bactericidal activity, we synthesized some immobilized quarternary ammonium salts, 2-nitrofurane derivatives, and biguanides based on cross-linked sucrose methacrylates and investigated their antimicrobial activity.

RESULTS AND DISCUSSION

Sucrose methacrylates (**1**, Scheme 1) were prepared by the transesterification of sucrose with methyl methacrylate.[30] The monomer mixture **1** was copolymerized with triethyl(4-vinylbenzyl)ammonium chloride (**2a**)[11] to yield the cross-linked bactericide **2b** (Scheme 2). For comparative tests, the homopolymer **2c** and the divinylbenzene–cross-linked polymer **2d** were prepared.

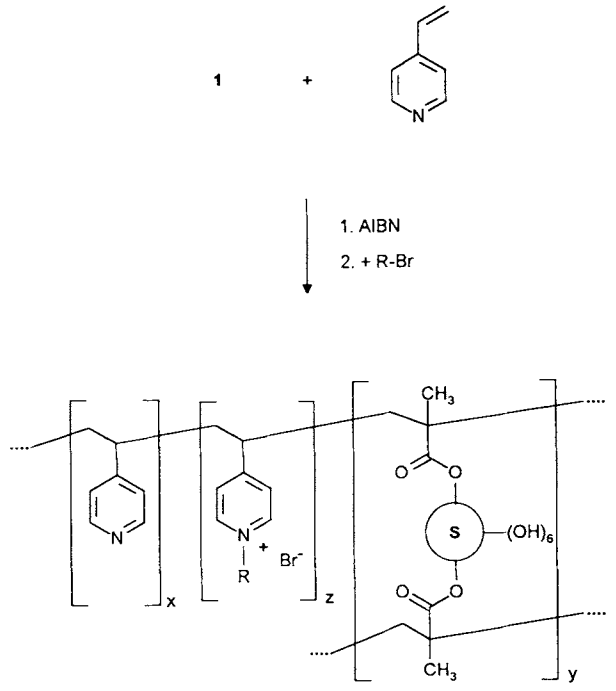
Copolymerization of **1** with 4-vinylpyridine and subsequent quaternization with 1-bromodecan, 1-bromooctadecane, and 5-nitro-2-(2-bromovinyl)furan afforded the immobilized ammonium salts **3a–c** (Scheme 3).

By radical polymerization of monomer mixtures **1**, cross-linked sucrose methacrylate (SM) gels (**4**) were prepared (Scheme 4). These SM gels have already been shown to be useful hydrophilic carriers for the immobilization of chelating groups, reagents, and catalytic active groups for applications in aqueous media.[32–36]

Bactericidal groups were bonded to **4** directly as well as by hydrophobic and hydrophilic spacers. Thus, the immobilized quaternary salts **6a** and **b** were prepared by esterification of **4** with 3-pyridine carboxylic acid chloride and subsequent reaction of the pyridine-substituted gels **5a** and **b** with 1-bromoctane (Scheme 5). Addition of lauric acid chloride in the esterification step yielded the hydrophobic gel **7b** containing *n*-octylpyridinium and dodecyl residues (Scheme 6).

Another carrier-bound salt **9b** containing no spacer between the carrier and the active group was obtained by the reaction of **4** with bromoacetic anhydride (**8a**) in DMF and subsequent stirring of the bromo-substituted gel **9a** with *N,N*-dimethyldodecylamine (Scheme 7).

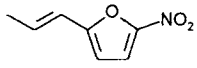
Quaternary salts immobilized by a hydrophobic spacer were prepared by esterification of **4** with 11-



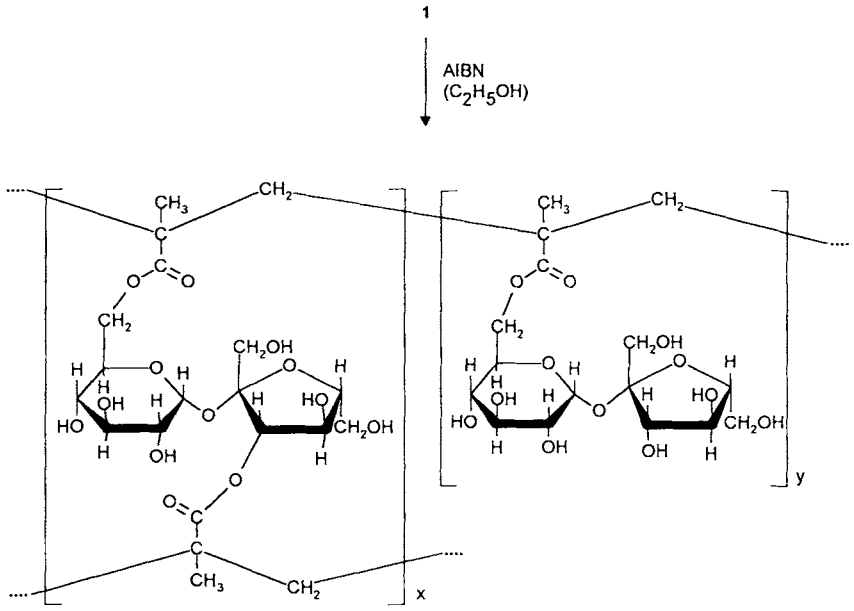
3a-c

3a: R = $-C_{10}H_{21}$, x = 0.7 z = 0.8

3b: R = $-C_{18}H_{37}$ x = 1.2 z = 0.3

3c: R =  x = 1.2 z = 0.3

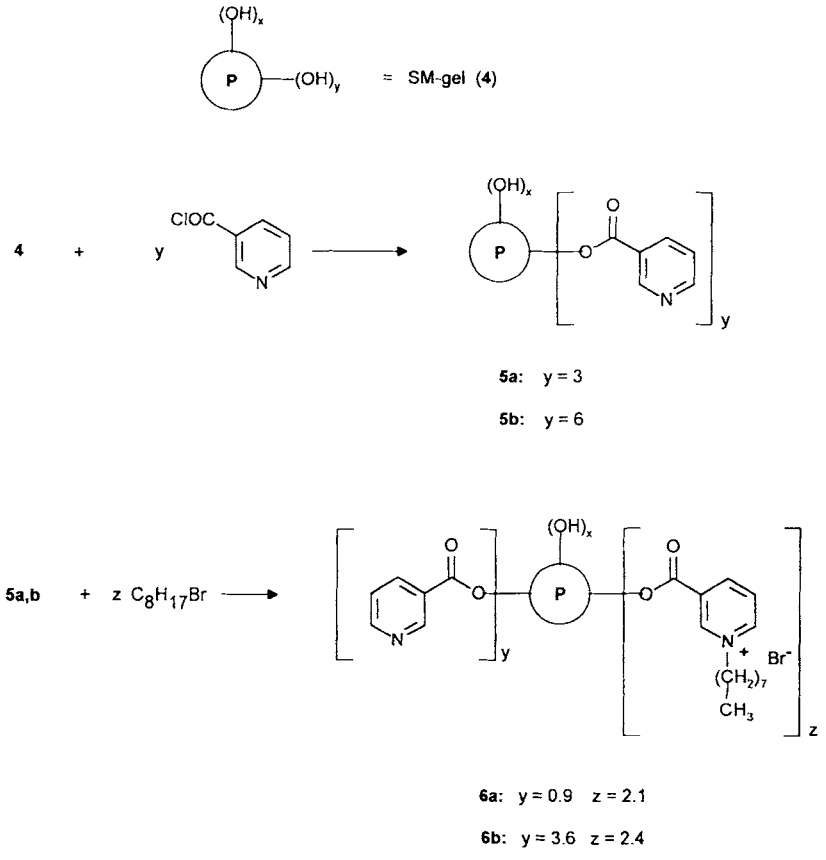
Scheme 3



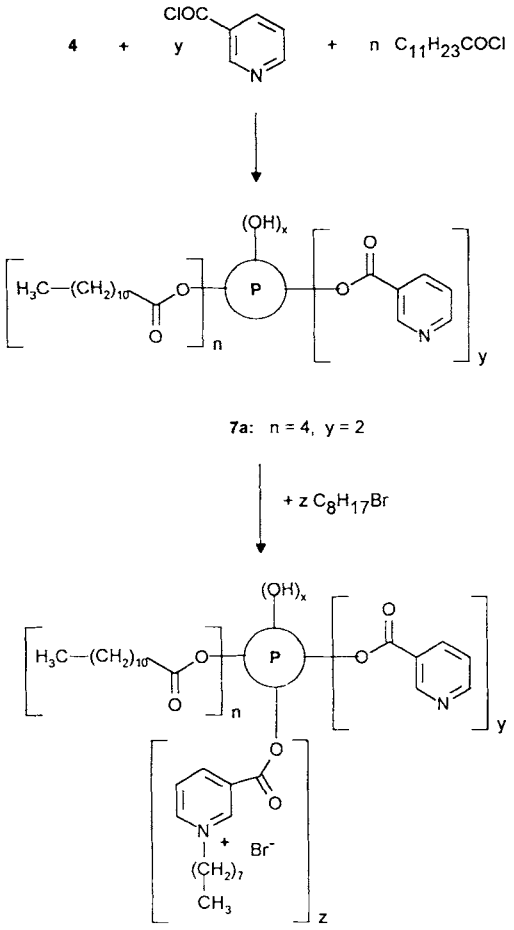
4

sucrose methacrylate gel (SM-gel)

Scheme 4



Scheme 5



7a: n = 4, y = 2

7b: n = 2, y = 3, z = 1

Scheme 6

bromundecanoyl chloride (**8b**) in the presence of tertiary amines and DMF as the swelling agent (Scheme 8). Thus, the immobilized bactericides **12a–h** were obtained using triethylamine (**10a–c**), dimethylbenzylamine (**10d**), 4-chlorobenzyl dimethylamine (**10e**), hydroxyethyl dimethylamine (**10f**), *N,N*-dimethyldodecylamine (**10g**), and pyridine (**10h**). Some gels were prepared also by stirring of the bromo-substituted carrier **11** with tertiary amines. In addition, a hydrophobic carrier-bound pyridinium salt **12i** was obtained by esterification of **4** with **8b** and lauric acid chloride (**8c**) in pyridine (Scheme 9).

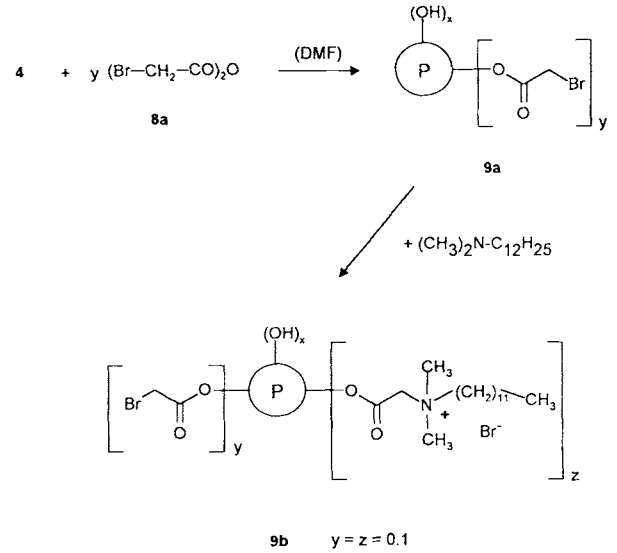
For the preparation of the hydrophilic spacer, one hydroxyl group of tetraethylene glycol (**13a**) was protected with dihydropyrane and subsequently reacted with bromoacetic acid. After splitting of the protecting acetal group, the acid **13c** was converted to the ω-chloro acid chloride **13d.** Crude **13c** was obtained also by reacting of the mono sodium salt of **13a** with 2-bromoacetic acid (Scheme 10). The salts **14a** and **14b** immobilized by a hydrophilic spacer were obtained by esterification of **4** with **13d** in the presence of pyridine and dimethylbenzylamine (Scheme 11).

Nitrofuryl residues as bactericidal groups[37] were immobilized by reaction of 2-nitrofuranealdehyde (**15**) with the amino-substituted SM carrier **4a** that was prepared by esterification of **4** with 4-nitrobenzoyl chloride and subsequent reduction of the nitro groups with sodium dithionite (**4a,** Scheme 12). Another carrier containing amino groups (**4b**) that was obtained by tosylation of **4** and substitution with the potassium salt of 2-aminoethanthiol failed to react with **15.**

Reaction of the hydrochloride of **4a** with the cyanoguanidin **17** yielded the immobilized bactericide **18** containing about 0.3 biguanide groups based on a sucrose unit (Scheme 13).

Antibacterial Activity of the Insoluble Bactericides

The antibacterial activity of the insoluble bactericides was tested by a suspension test[8] using *Escherichia coli, Staphylococcus aureus,* and *Micrococcus luteus* as test organisms and water samples contaminated by *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter* species. The solid material to be investigated was suspended in water containing a bacteria culture of known bacterial counts. The suspension was shaken and samples were taken after different

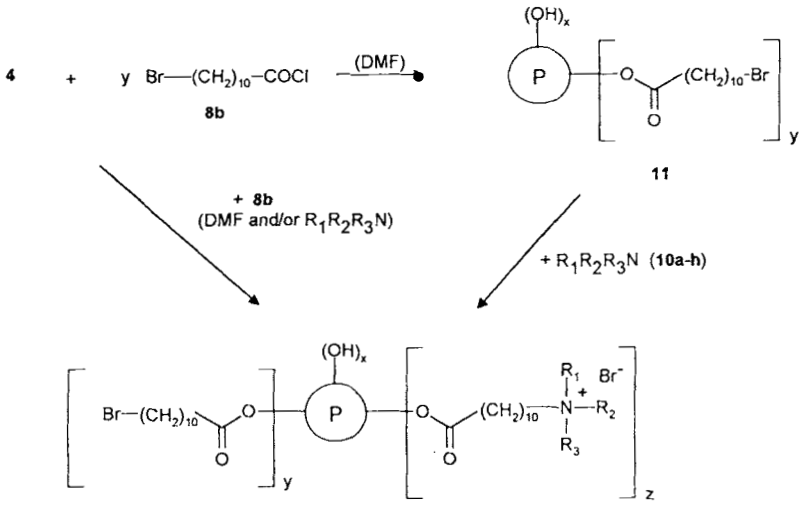


8a

9a

9b y = z = 0.1

Scheme 7



12a-h

10a,12a: $R_1 = R_2 = R_3 = CH_2CH_3$ y = 1.0 z = 0.4

10b,12b: $R_1 = R_2 = R_3 = CH_2CH_3$ y = 0.8 z = 0.9

10c,12c: $R_1 = R_2 = R_3 = CH_2CH_3$ y = 0 z = 0.5

10d,12d: $R_1 = R_2 = CH_3$, $R_3 =$ —CH_2—C$_6$H$_5$ y = 0.2 z = 0.4

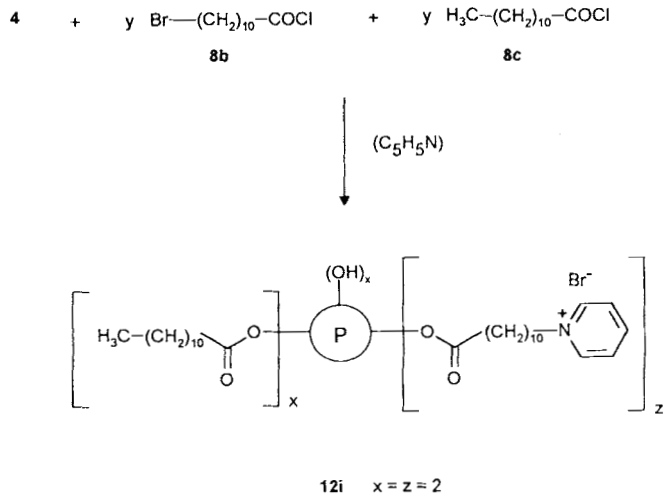
10e,12e: $R_1 = R_2 = CH_3$, $R_3 =$ —CH_2—C$_6$H$_4$—Cl y = 0 z = 0.1

10f,12f: $R_1 = R_2 = CH_3$, $R_3 =$ —CH_2—CH_2—OH y = 0 z = 0.2

10g,12g: $R_1 = R_2 = CH_3$, $R_3 = C_{12}H_{25}$, y = z = 0.1

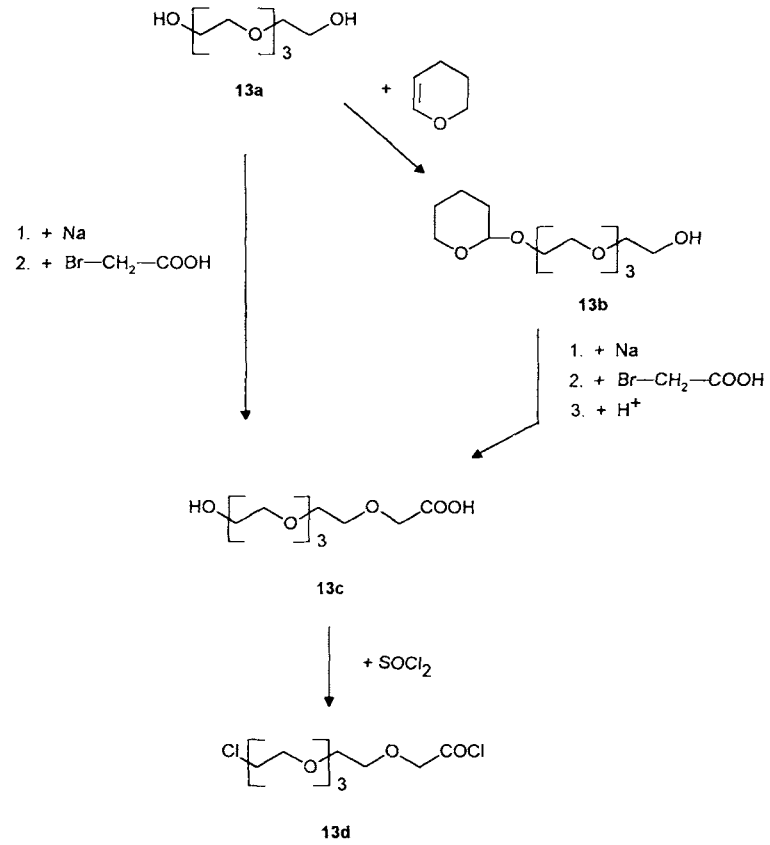
10h,12h: $R_1R_2R_3N =$ C$_5$H$_5$N y = 0.7 z = 2.8

Scheme 8



12i x = z = 2

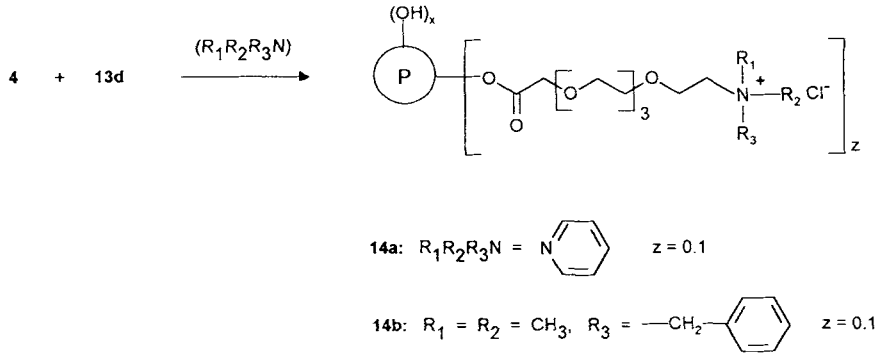
Scheme 9



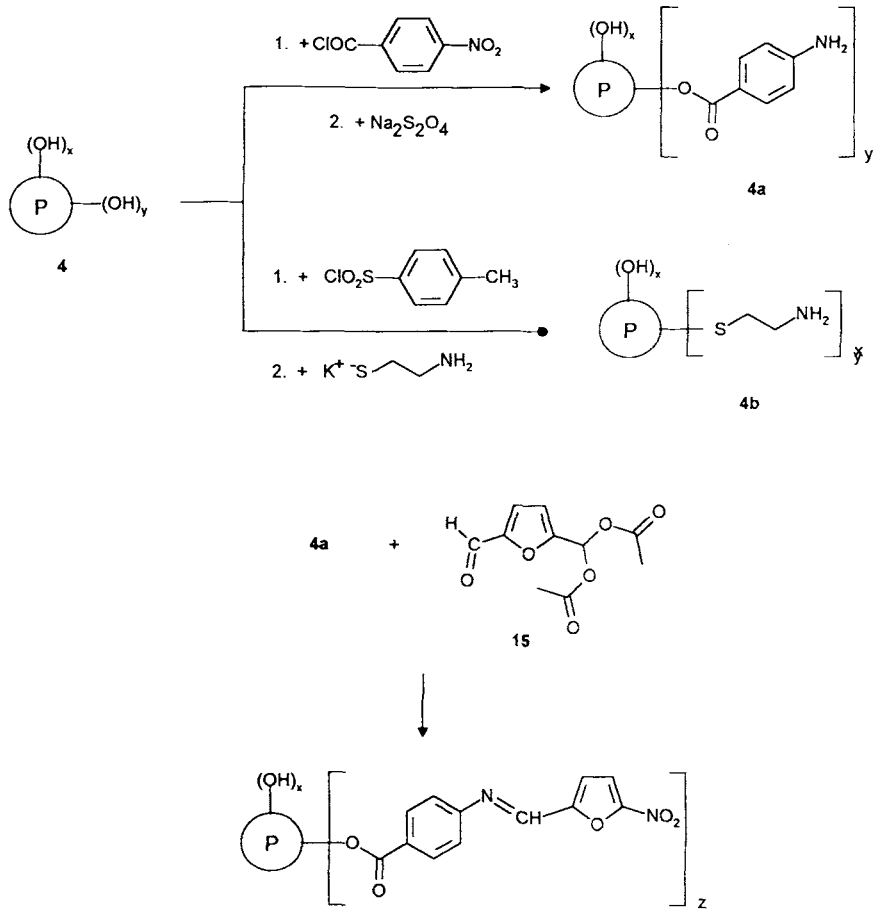
Scheme 10

times for count determination. According to the literature,[38] a substance is considered to be a bactericide if 99.9% of the inoculation is destroyed within 18 h. To determine germ losses in the water due to adsorption or inactivation, reference tests were performed with the carriers **4** and **11** containing no bactericidal groups. This test allows the differentiation between biostatic and biocidal effects and the investigation of the rate of germ destruction.

The results of the tests with *E. coli* (Table I) demonstrated that the immobilized bactericides containing the hydrophobic spacer (**12a–d, 12f, 12h, 12i**) exhibit excellent antibacterial activity if the content of active quaternary groups exceeds 0.1 mmol/g. The carrier-bound quaternary salts **12e** and **12g** as well as **14a** and **14b** with hydrophilic spacers were less effective, presumably due to the low concentration of active groups.



Scheme 11



Scheme 12

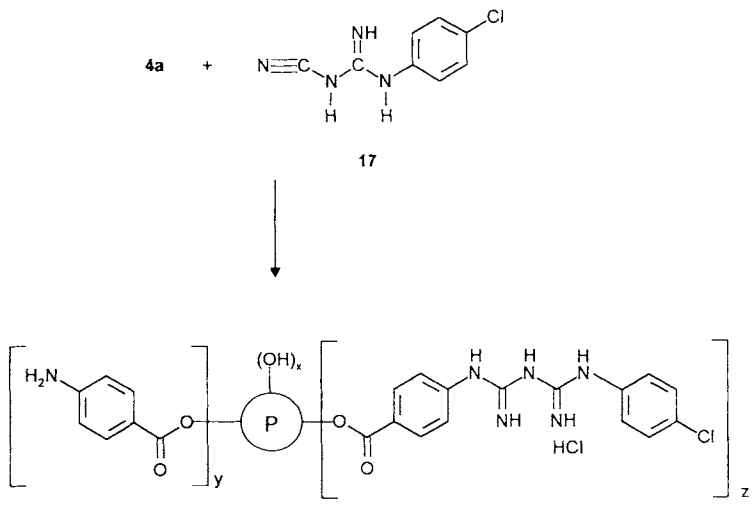
No activity was observed with the SM copolymers **3a–c,** which is attributed to the limited accessibility of the bactericidal groups. Interestingly, the gels **6a** and **b** and **7** containing bactericidal groups similar to **3a** and **3b** but which were prepared by polymer analogous reaction of the SM gel with 3-pyridinecarboxylic acid chloride and subsequent quaternization were found to be effective bactericides.

The soluble styrene monomer **2a** as well as the homopolymer **2c** showed high activity. Cross-linking of **2a** with divinylbenzene diminished the effectivness, whereas the activity of the analogous SM-cross-linked **2b** was excellent. Thus, the influence of the hydrophilic carrier is demonstrated.

The immobilized nitrofurane **16** exhibited no antibacterial properties, although low molecular 2-nitrofuranes are known to be effective bactericides.[37] The immobilized biguanide **18** was found to have limited antimicrobial efficiency. Presumably, its activity can be improved when the biguanide is bonded by a spacer to the carrier. Antibacterial tests of **12b, d,** and **h** and **14a** and **b** with the Gram-positive bacteria *S. aureus* and *M. luteus* demonstrated similar results (Table II).

Treatment of water samples contaminated by *Escherichia, Enterobacter, Klebsiella,* and *Citrobacter* species with **12d** or **12h** resulted in destruction of more than 98% of the inoculation, whereas complete disinfection was achieved by application of mixtures of **12d** and **12h.** Thus, the antimicrobial activity can be increased by application combinations of insoluble bactericides. No bactericidal effects were found with the unsubstituted SM Gel **4** and with gels containing 11-bromo-undecanoyl residues only (**11,** 2.1 mmol Br/g).

To investigate the hydrolytical stability of the carrier-bound ammonium salts, samples of **12d** and **12h** were stirred with water for 48 h, and after fil-



Scheme 13

tration of the insoluble material, the solution was added to bacteria suspensions. No decrease of the germ counts in these suspensions was observed. In addition, no organic substances could be detected by gas chromatographic analysis, thus demonstrating that no release of the immobilized disinfectants occurred and that the active groups are bonded by hydrolytically stable bonds.

In contrast to other insoluble quaternized polymers,[24] the decrease of cells is attributed predominantly to bactercidal effects and not to the reversible adsorption of viable cells. Samples of **12a** and **12d** were stirred in *E. coli* suspensions and subsequently rinsed with sterilized water. After spreading on agar plates, no cell proliferation was observed, thus indicating no adsorption of viable cells. This result was confirmed by identical elemental analysis of the samples before and after bacterial treatment. The durability of the insoluble bactericides was tested by repeated treatment of bacteria suspensions with the same sample. No decrease of the efficiency could be detected after six cycles. These results indicate no or negligible adsorption effects.

Conclusion

The results demonstrated that the antibacterial agents effecting against the cytoplasmic membrane of microbes such as quaternary salts or biguanides are still effective when bonded to suitable insoluble hydrophilic carriers such as SM gels. The bactericidal activity of the insoluble bactericides depends on the

- kind and concentration of the quaternary salt groups,
- accessibility of the active centers,
- nature of the spacer, and
- nature of the carrier.

Advantageously, the insoluble bactericides can be applied without contamination of the substrate and they can be removed easily and used repeatedly.

EXPERIMENTAL

Materials and Syntheses

Sucrose methacrylates (**1**) (degree of substitution 1.7) were prepared by transesterification of sucrose with methyl methacrylate (molar ratio 1 : 5) in the presence of K_2CO_3 and polymerized in ethanol to yield **4**.[30] 4-Chlorobenzyl-*N,N*-dimethylamine,[39] *N,N*-dimethyldodecylamine,[40] and **2a**[8] were prepared as described in the literature. The solvents were dried and purified by common methods.

Synthesis of 2c

2a (5.4 g, 0.02 mol) was polymerized under a nitrogen atmosphere in a mixture of DMF/water (100 mL, 1 : 1 vol.) with AIBN for 8 h. The polymer was precipitated into acetone, dissolved in methanol, and precipitated in acetone. This procedure was repeated three times. Yield: 4.8 g; molecular weight, 9240 (vapor pressure osmometry); and solubility in water, ca. 20%.

Table I Antibacterial Activity of the Polymers Against *E. coli*

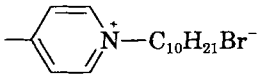
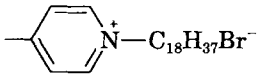
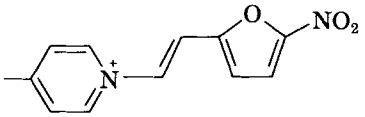
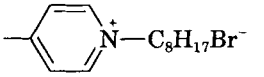
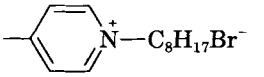
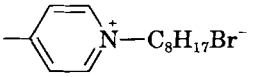
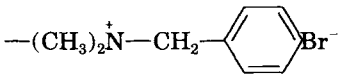
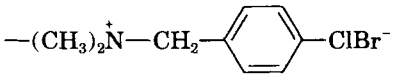
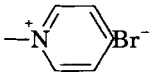
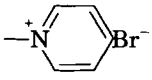
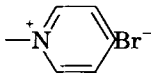
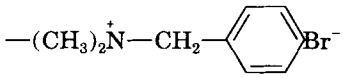
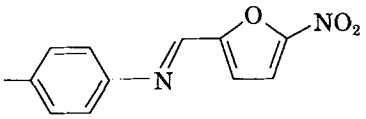
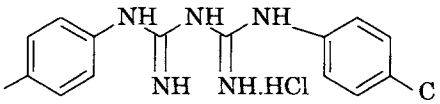
No.	Carrier	Spacer	Bactericidal Group	Active Groups (mmol/g)	Surviving Cells (%)
4	SM[a]	—	—	—[b]	100
11	SM	$-(CH_2)_{11}-$	—	—[b]	100
2a	Styrene (monomer)	—	$-\overset{+}{N}(C_2H_5)_3Cl^-$	3.9[b]	0
2c	Styrene (homopolymer)	—	$-\overset{+}{N}(C_2H_5)_3Cl^-$	3.9[b]	0
2d	Styrene/ divinylbenzene	—	$-\overset{+}{N}(C_2H_5)_3Cl^-$	2.6[b]	38
2b	SM/styrene	—	$-\overset{+}{N}(C_2H_5)_3Cl^-$	1.7[b]	0.9
3a	SM/ vinylpyridine	—		1.0[c]	100
3b	SM/ vinylpyridine	—		0.4[c]	100
3c	SM/ vinylpyridine	—		0.5[c]	100
6a	SM	—		1.8[b]	0.1
6b	SM	—		1.5[b]	0.1
7b	Hydrophobic SM	—		0.7[b]	23
9b	SM	—	$(CH_3)_2\overset{+}{N}-(CH_2)_{11}-CH_3Br^-$	0.2[c]	100
12a	SM	$-(CH_2)_{10}-$	$-\overset{+}{N}(C_2H_5)_3Br^-$	0.5[b]	0.3
12b	SM	$-(CH_2)_{10}-$	$-\overset{+}{N}(C_2H_5)_3Br^-$	0.9[d]	0.3
12c	SM	$-(CH_2)_{10}-$	$-\overset{+}{N}(C_2H_5)_3Br^-$	0.8[d]	0.3
12d	SM	$-(CH_2)_{10}-$		0.6[d]	0
12e	SM	$-(CH_2)_{10}-$		0.1[c]	30
12f	SM	$-(CH_2)_{10}-$	$(CH_3)_2\overset{+}{N}-CH_2-CH_2-OHBr^-$	0.4[d]	0.3
12g	SM	$-(CH_2)_{10}-$	$(CH_3)_2\overset{+}{N}-(CH_2)_{11}-CH_3Br^-$	0.2[b]	10
12h	SM	$-(CH_2)_{10}-$		1.8[d]	0
12i	Hydrophobic SM	$-(CH_2)_{10}-$		1.4[b]	0.5

Table I *(Continued)*

No.	Carrier	Spacer	Bactericidal Group	Active Groups (mmol/g)	Surviving Cells (%)
14a	SM	$-(CH_2CH_2O)_4-$		0.1[c]	25
14b	SM	$-(CH_2CH_2O)_4-$		0.1[c]	50
16	SM	—		1.3[c]	100
18	SM	—		0.5[c]	50

[a] SM: sucrose methacrylate gel.
[b] Amount of poymer 1.0 g; stirring time 1 h.
[c] Amount of poymer 1.0 g; stirring time 4 h.
[d] Amount of poymer 0.5 g; stirring time 0.5 h.

Synthesis of 2d

2a (6 g, 0.024 mol) was copolymerized with divinylbenzene (6.2 g, 0.0.24 mol) under a nitrogen atmosphere in water (150 mL) with AIBN for 10 h. The copolymer was filtered off, washed repeatedly with water and methanol, and dried under reduced pressure at 80°C. Yield: 7 g.

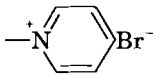
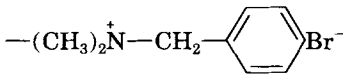
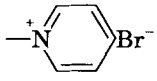
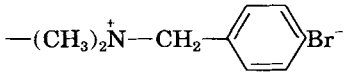
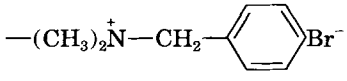
ANAL: N, 3.31%; Cl, 9.35%.

Synthesis of 2b

2a (6 g, 0.024 mol) was copolymerized with **1** (5 g) under a nitrogen atmosphere in ethanol (50 mL) with AIBN at 60°C for 12 h. The copolymer was filtered off, washed repeatedly with water and methanol, and dried under reduced pressure at 80°C. Yield: 6.3 g.

ANAL: N, 2.8%; Cl, 6.6%.

Table II Antibacterial Activity of the Polymers Against *S. aureus*[+)] and *M. luteus*[++)]

No.	Carrier	Spacer	Bactericidal Group	Active Groups (mmol/g)	Surviving Cells (%)
12h[+)]	SM	$-(CH_2)_{10}-$		1.8[d]	0
12d[+)]	SM	$-(CH_2)_{10}$		0.6[d]	0
12b[+)]	SM		$-\overset{+}{N}(C_2H_5)_3Br^-$	0.9[d]	2[d]
14a[+)]	SM	$(CH_2CH_2O)_4-$		0.1[c]	25
12d[++)]	SM	$-(CH_2)_{10}$		0.6[b]	0
14b[++)]	SM	$(CH_2CH_2O)_4-$		0.1[c]	14

[b–d] See footnotes [b–d] to Table I.

Synthesis of 3a–c

1 (26 g) and 4-vinylpyridine (6.3 g, 0.06 mol) were copolymerized in ethanol (250 mL) under a nitrogen atmosphere with AIBN at 70°C for 8 h. The product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 18 g.

ANAL: N, 3.6%.

The copolymer (5 g) was stirred with DMF (10 mL) at 50°C, then 1-bromdecan (10 g, 0.045 mol) was added and the mixture stirred at the same temperature for 12 h. The product (**3a**) was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 4.8 g **3a.**

ANAL: N, 2.91%; Br, 8.3%.

3b and **3c** were prepared by the same procedure using 1-bromoctadecane (12 g, 0.040 mol) and 5-nitro-2-(2-bromvinyl)furan (2 g, 0.009 mol), respectively. **3b,** yield: 4.9 g.

ANAL: N, 3.41%; Br, 2.84%.

3c, yield: 5 g.

ANAL: N, 3.14%: Br, 3.66%.

Synthesis of 6a

4 (4.3 g) was stirred in 50 mL pyridine at 20°C for 1 h and then 3-pyridine carboxylic acid chloride (3.82 g, 0.027 mol) was added. After stirring at 100°C for 10 h, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 5.8 g.

ANAL: N, 5.88%.

This product (4 g) was refluxed with *n*-octylbromide (10 g) in ethanol (30 mL) for 10 h. The solid was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 4.8 g.

ANAL: N, 4.40%; Br, 14.2%.

6b was prepared by the same method using **4** (9.6 g), 3-pyridine carboxylic acid chloride (17 g, 0.12 mol), and pyridine (100 mL), yielding a pyridine-substituted gel (21.5 g).

ANAL: N, 7.72%.

Four grams of this product was quaternized with *n*-octylbromide (2 g) to afford **6b;** yield: 4.7 g.

ANAL: N, 6.08%; Br, 12.3%.

Synthesis of 7b

4 (10 g) was stirred in 70 mL pyridine at 20°C for 1 h and then 3-pyridine carboxylic acid chloride (13 g, 0.075 mol) and lauric acid chloride (14 g, 0.06 mol) was added. After stirring at 100°C for 10 h, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 20 g.

ANAL: N, 4.25%.

This product (4 g) was refluxed with *n*-octylbromide (2.5 g) in ethanol (30 mL) for 10 h. The solid was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 4.1 g.

ANAL: N, 3.98%; Br, 5.3%.

Synthesis of 9b

4 (4.6 g) was stirred in 50 mL DMF at 80°C for 2 h and then bromacetic acid anhydride (11.4 g, 0.045 mol) and $NaHCO_3$ (1 g) were added. After stirring for 12 h at 20°C, the product was filtered and washed with water and ethanol. *N,N*-Dimethyldodecylamine (20 mL) was added and the mixture was stirred at 20°C for 12 h. The product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 4.2 g.

ANAL: N, 0.7%; Br, 1.4%.

Synthesis of Tetraethylenglykol-2-tetrahydropyranyl Ether (13b)

Tetraethylene glycol (**13a,** 48.5 g, 0.25 mol) was stirred with 3,4-dihydro-2H-pyran (21 g, 0.25 mol) and 2 drops of concentrated HCl at 20°C for 3 h. The mixture was fractionated to afford **13b** (40.8 g, 60%; bp 147–152°C/0.7 Pa).

Synthesis of 14-Chloro-3,6,9,12-tetraoxatetradecanoic Acid (13d)

Sodium (5.7 g, 0.25 mol) was dissolved in tetraethylene glycol-2-tetrahydropyranyl ether (81.8 g, 0.3 mol) by stirring for 48 h. The solution was heated to 100°C under a nitrogen atmosphere and 2-bromoacetic acid (20 g, 0.15 mol) was added. After stirring for 12 h at 100°C, the mixture was acidified with diluted HCl, the water was removed, and the residue was treated with isopropanol to precipitate the salt. The mixture was filtered and the solvent evaporated to yield 40 g crude product (**13c**), which contained some tetraethylene glycol.

13c was synthesized also by adding 2-bromoacetic (20 g, 0.15 mol) acid to a solution of sodium (7 g, 0.3 mol) in **13a** (200 g, 1 mol) at 100°C under a nitrogen atmosphere. After stirring for 2 h at the same temperature, crude **13c** (50 g) was isolated by the same procedure.

The acid **13c** (40 g) was refluxed in thionylchloride (71.6 g, 0.6 mol) with some drops of pyridine for 3 h. The excess thionylchloride was distilled off and the reaction mixture was fractionated to yield **13d** (18 g, 20%; bp. 65–67°C/1 Pa). IR (cm^{-1}): 2865, 1765, 750.

ANAL: Calcd for $C_{10}H_{18}Cl_2O_5$: C, 41.54%; H, 6.27%; Cl, 24.52%.
Found: C, 40.9%; H, 6.5%; Cl, 24.35%.

Synthesis of 11

4 (2.3 g) was stirred in 20 mL DMF at 80°C for 2 h and then 11-bromoundecanoyl chloride (8.5 g, 0.03 mol) was added. After stirring for 10 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 4.0 g.

ANAL: Br, 14.8%.

Synthesis of 12a

11 (2.1 g) was stirred in triethylamine (30 mL) at 50°C for 12 h. The mixture was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 2.1 g.

ANAL: N, 0.7%; Br, 13.6%.

General procedure for the synthesis of 12b and 12e–g

4 (2.3 g) was stirred in 50 mL DMF at 80°C for 2 h and then 11-bromoundecanoyl chloride (8.5 g, 0.03 mol) and the amine were added. After stirring for 12 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C.

12b: Triethylamine (9 mL); yield: 2.0 g.

ANAL: N, 1.4%; Br, 14.0%.

12e: 4-Chlorobenzyl-*N,N*-dimethylamine (6.9 g, 0.045 mol); yield: 2.0 g.

ANAL: N, 0.3%; Cl, 0.4%; Br, 0.9%.

12f: Dimethylethanolamine (10 mL); yield: 1.9 g.

ANAL: N, 0.6%; Br, 2.9%.

12g: *N,N*-Dimethyldodecylamine (10 mL); yield: 2.1 g.

ANAL: N, 0.4%; Br, 3.1%.

Synthesis of 12c

A mixture of **4** (2.3 g) and triethylamine (70 mL) was heated to reflux for 2 h. Then, 11-bromoundecanoyl chloride (14.2 g, 0.05 mol) was added and the reaction mixture was stirred at 80°C for 12 h. The gel was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 2 g.

ANAL: N, 1.3%; Br, 6.6%.

Synthesis of 12d

11 (1.7 g) was stirred in 20 mL of *N,N*-dimethylbenzylamine at 50°C for 12 h. The product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 1.7 g.

ANAL: N, 0.9%; Br, 7.8%.

Synthesis of 12h

4 (2.3 g) was stirred in 50 mL pyridine at 80°C for 2 h and then 11-bromoundecanoyl chloride (8.5 g, 0.03 mol) was added. After stirring for 12 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 2.1 g.

ANAL: N, 2.4%; Br, 18.2%.

Synthesis of 12i

4 (5 g) was stirred in 50 mL pyridine at 80°C for 2 h and then 11-bromoundecanoyl chloride (10 g, 0.035 mol) and lauric acid chloride (6 g, 0.033 mol) were added. After stirring for 12 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 11.8 g.

ANAL: N, 2.09%; Br, 10.71%.

Synthesis of 14a

4 (2.3 g) was stirred in 50 mL pyridine at 80°C for 2 h and then 14-chloro-3,6,9,12-tetraoxatetradecanoic acid chloride (11.6 g, 0.04 mol) was added. After stirring for 12 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 2.3 g.

ANAL: N, 0.4%; Cl, 0.6%.

Synthesis of 14b

4 (2.3 g) was stirred in 50 mL DMF at 80°C for 2 h and then 14-chloro-3,6,9,12-tetraoxatetradecanoic acid chloride (5.4 g, 0.02 mol) and *N,N*-dimethylbenzylamine (9 mL) was added. After stirring for 12 h at the same temperature, the product was filtered, washed repeatedly with water, ethanol, ace-

tone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 2.2 g.

ANAL: N, 0.3%; Cl, 0.4%.

Synthesis of 4a

4 (6.3 g) and 4-nitrobenzoylchloride (12.8 g, 0.07 mol) were stirred in 50 mL pyridine at 80°C for 6 h. The product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 9.2 g. IR: 1540 cm^{-1} (—NO_2). The nitro-substituted product was stirred at reflux temperature for 4 h in a mixture of pyridine (100 mL) and aqueous solution of sodium dithionite (35 g, 0.2 mol). After filtration, **4a** was washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 8.5 g.

ANAL: N, 2.6%.

Synthesis of 16

4a (3 g) was stirred in 50 mL water for 2 h. 5-Nitro-2-furaldehydediacetate (2.7 g, 0.075 mol) was refluxed in a mixture of methanol (12 mL), water (6 mL), and concentrated sulfuric acid (6 mL) for 5 min and added to the suspension of **4a.** The reaction mixture was stirred for 12 h at 20°C and the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 3 g.

ANAL: N, 4.3%.

Synthesis of 4b

4 (5 g) was stirred in 50 mL pyridine for 1 h. The mixture was cooled to 0°C and tosyl chloride (6.5 g, 0.034 mol) was added in several portions. After stirring at 20°C for 1 h and at reflux temperature for 20 h, the product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C.

Yield: 8 g; IR 1190 cm^{-1} (—SO_3H). A solution of potassium-2-aminoethylthiolate was prepared by adding cysteamin hydrochloride (10 g, 0.09 mol) to a solution of potassium (7 g, 0.17 mol) in *tert*-butanol (80 mL) and refluxing the mixture in benzene (160 mL) for 2 h. Then, the tosyl-substituted gel (5.5 g) was added and the reaction mixture was heated to reflux temperature for 20 h. The product was filtered off, washed with aqueous KOH (30%), and then repeatedly with water and acetone and was dried under reduced pressure at 80°C. Yield: 5.2 g.

ANAL: N, 1.1%; S, 2.3%.

Synthesis of 17

4-Chloroaniline hydrochloride (25.5 g, 0.15 mol, prepared by treating a solution of 4-chloroanilin in dioxane with HCl gas) and sodium dicyandiamide (15 g, 0.17 mol) were dissolved in water (100 mL) and stirred at 90°C for 3 h. The precipitate was filtered off and dissolved in 2*N* NaOH (500 mL). The solution was heated to 70°C and filtered from impurities and the product was precipitated with hydrochloric acid. Yield: 27.5 g (94%); Fp. 197–201°C.

Synthesis of 18

4a (5 g) was stirred in water (100 mL) and treated with HCl gas for 1 h. After washing with water **17** (5 g) was added and the mixture was stirred at 80°C for 16 h. The product was filtered, washed repeatedly with water, ethanol, acetone, and diethyl ether, and dried under reduced pressure at 80°C. Yield: 5.4 g.

ANAL: N, 4.5%; Cl, 4.8%.

Test of Antibacterial Properties

The bacteria used were *E. coli* DSM 613, *S. aureus* ATCC 6538, and *M. luteus* DSM 200 30. In addition, water samples containing about 10^3 cells/mL *Escherichia, Enterobacter, Klebsiella, and Citrobacter* species were used.

Each polymer (0.5–1 g) was wetted in 50 mL of sterilized water for 1 h. Then, 1 mL of a cell suspension (10^6–10^7 cells/mL) was added and stirred at 25°C for 0.5–4 h. Before sampling, the stirring of the suspension was stopped, enabling settling of the polymer. One milliliter of the contact suspension was spread on a standard-1 agar plate and kept at 37°C for 15–20 h. The number of viable cells in the suspension was calculated from that of the colonies formed on the plate.

For comparison measurements, the carriers **4** and **11** as well as pure cell suspensions were tested under the same conditions using serially diluted cell suspensions.

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