From Multifunctionalized Poly(ethylene imine)s toward Antimicrobial Coatings

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Primary amine groups of branched poly(ethylene imine) (PEI) were functionalized with quaternary ammonium groups, alkyl chains of different length, allylic and benzylic groups in a one-step reaction, using a carbonate coupler. The structure of the obtained amphiphilic polymers was determined by means of ^{1}H and ^{13}C NMR spectroscopy. Depending on their hydrophilic/hydrophobic balance, the obtained polymers can be used as water-soluble disinfectants and for antimicrobial coating materials. The bactericidal properties of some of the amphiphilic polymers against Gram-negative and Gram-positive bacteria were investigated. Minimal inhibitory concentrations (log 4 reduction of bacterial growth) against *Escherichia coli* and *Bacillus subtilis* were determined in the range of 0.3−0.4 mg/mL and 0.03−0.04 mg/mL for water-soluble polymers. Glass slides coated with functionalized PEIs showed a reduction of colony forming units of at least 95%, at best 99.9%, against *E. coli* and *B. subtilis*.

Introduction

Functional and furthermore responsive properties of polymers present a topic of increasing importance in polymer science. Well-established examples are thermoresponsive hydrogels, self-organization of block copolymers in bulk and in thin films, dispersants and surface active polymers in coatings and inkjet technology. Yet regarding functionality synthetic polymers are by far inferior to natural macromolecules, e.g., enzymes, molecular motors, chaperons, pores, etc. On first glance a relatively simple functionality, like an amphiphilic behavior, i.e., latent amphiphilicity that is only fully developed in contact with an interface, has to our knowledge not been realized in a satisfactory way.

Here we report an attempt to discuss such water-soluble polymers with a strong affinity to lipid membranes. The rational of the concept is to substitute a water-soluble hyperbranched macromolecule, i.e., poly(ethylene imine), by alkyl chains and ammonium groups in such a way that the water solubility is preserved but that the polymers will at the same time gain the ability to adsorb at a lipid membrane. These amphipathic molecules are of interest for the preparation of new antimicrobial polymers and bactericidal or bacteria repellent surfaces, in order to provide solutions for one of the biggest problems of modern medicine. Over the past decade, worldwide networks reported an increasing resistance of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecium*, and *Escherichia coli* against currently available antibiotics.[1,2] Moreover, methicillin-resistant *S. aureus* (MRSA) is becoming a community-based as well as a hospital-based problem. Hospital- and community-acquired *E. coli* infection will pose an increasing challenge to health care systems in the years to come. Many developmental antibiotics are based on existing classes of antimicrobial agents and therefore raise concern that antibiotic resistance will develop quickly, notably through cross-resistance with existing agents.

The problem may be treated with complementary approaches. Beside the development of new therapeutic agents (new classes of antibiotic agents,[3] host-defense peptides, or synthetic analogues,[4−6] aminoglycosides,[7] etc.) and the improvement of hygiene compliance,[8] the spread of bacterial infections can be actively fought by rendering surfaces antimicrobial. There are two major categories of antimicrobial surfaces, those resulting from incorporation of the antimicrobial throughout the bulk and those resulting from a surface coating.

Objects with a biostatic in the bulk of the material rely on migration of the biostatic toward the surface to provide an antimicrobial effect. Migration can occur within crystal polymer interstices and in the amorphous matrix. Some surface-coating products claim a disinfection action by allowing biocides to leach out of the polymer film onto the surface. These low molecular weight release systems can, however, be toxic to higher organisms and promote the growth of resistant strains.

Significant release of biocide can be prevented or restricted to a minimum if the biocidal moiety is covalently linked within a thin polymer film. The demand for nonrelease systems is mostly focused to household objects (telephones, computer keyboards, door knobs, clothing, children's toys, table surfaces, kitchen utensils, and refrigerator wall) but also to medical applications such as containments, fabrics, certain medical devices, but also for implants and surgical instruments.

It is known that various amphiphilic polycations possess antibacterial properties.[9−16] Recently, a substantial amount of work has been done on immobilizing hydrophobic polycations onto surfaces of glass, plastics, or cellulose.[17−21] For example, high-density polyethylene slides were coated with a nanolayer of silica, followed by the covalent attachment of poly(vinyl-*N*-hexylpyridinium).[18] Hydroxyl groups on cellulose filter paper or free amine groups on amino glass slides were reacted with 2-bromoisobutyryl bromide to produce the active atom transfer radical polymerization (ATRP) initiator. Controlled radical polymerization of 2-(dimethylamino)ethyl methacrylate gave grafted polymer chains of controlled molecular weights and low dispersity. Subsequent quaternization of the amino groups with ethyl bromide provided the biocidal functionality.[20] Poly(ethylene imine) (PEI, 750 kDa) was consecutively *N*-alkylated with a poly(styrene-*co*-butyl methacrylate-*co*-allyl bromide), an

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alkyl bromide, and iodomethane to obtain a *N*-copolymer,*N*-alkyl,*N*-methyl-PEI. Glass and polyethylene slides were dip-coated with solutions of the *N*-copolymer,*N*-hexyl,*N*-methyl-PEI or *N*-dodecyl,*N*-methyl-PEI.[21]

Because the mechanisms of the antimicrobial action of polycations is still not fully understood, the properties required need to be tuned and optimized in each case, and it is particularly promising to test a large library of polymers to reach the requirements.

Previously we reported on a new approach for the preparation of amphiphilic antimicrobial polymers based on a one-step multifunctionalization of PEI with functionalized cyclic carbonates.[22] Here we report the synthesis of functionalized cyclic carbonates and their reactions with PEI. The rational behind our approach is to find a hydrophilic/hydrophobic balance that assures just water solubility but approaches amphipathic properties. Depending on the hydrophilic/hydrophobic balance and the presence of cross-linkable groups, the synthesized polymers can be applied as a coating or as a water-based formulation. Treated surfaces and water solutions were tested for their antibacterial activity against Gram-positive *Bacillus subtilis* and Gram-negative *E. coli*.

Experimental Section

Materials. Starting materials used for the synthesis were of high purity. Glycerol (Acros Organics), 1,4-diazabicyclo[2.2.2]octane (DABCO, Aldrich), dimethyl carbonate (Acros Organics), 1-hexylamine (BASF), 1-dodecylamine (Acros Organics), 1-octadecylamine (Aldrich), allylamine (Aldrich), pyridine (Aldrich), *N*,*N*-dimethylformamide (DMF, Acros Organics), phenylchloroformate (Fluka), methyl iodide (Acros Organics), 3-dimethylamino-1-propylamine (Aldrich), di(ethylene glycol) dimethacrylate (DEgDMA, Aldrich), 2,2′-azobis(2-methylpropionitrile) (AIBN, Merck), and PEI (M_w ~25 000 by light scattering (LS) M_n ~10 000 by gel permeation chromatography (GPC), water-free, Aldrich) were used as received.

Microorganisms. The strains employed in this work were the Gram-negative bacterium *E. coli* (DSMZ 498) and the Gram-positive bacterium *B. subtilis* (DSMZ 347).

Solutions. Nutrient solution pH 7 contained 5 g of peptone, 3 g of meat extract per liter of bidistilled water. Phosphate-buffered saline (PBS) contained 9.0 g of NaCl per liter of 0.1 M disodium hydrogen phosphate/sodium dihydrogen phosphate buffer solution adjusted to pH 6.5. Soft agar was prepared from 10.0 g of peptone, 3.0 g of meat extract, 6.0 g of NaCl, and 7.0 g of agar-agar per liter of bidistilled water. All solutions were autoclaved for 15 min at 120 °C prior to use.

Instruments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 FT-NMR spectrometer at 300 and 75 MHz, respectively. Chloroform-*d* ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6) were used as solvents, and tetramethylsilane (TMS) served as an internal standard. C, H, and N elemental analysis was performed on a Heraeus CHN-O-Rapid Elementar Vario EL instrument. A thermal shaker (Heidolph), climate chamber (Vötsch), microplate incubator/reader GENIOS PRO (Tecan), photometer Cary 100 (VARIAN), drying oven, and clean bench (Kendro) were used for the antimicrobial assessments.

Preparation of Coated Slides. Commercially available glass slides (18 mm × 18 mm, Menzel-Gläser) were ultrasonicated in 2-propanol for 4 min and dried at room temperature under N_2 flow. Thin films were prepared by casting from 150 μL of a 1 wt % solution of **PEI-8**, **PEI-9** in methanol or a solution of 100 mg of **PEI-10**, 10 mg of DEgDMA, and 1 mg of AIBN per gram of methanol, followed by air-drying overnight at room temperature or by drying for 2 h at room temperature and in oven at 60 °C for 16 h.

Antibacterial Assessments of Polymer Water Solutions. *(i) Growth Test.* Suspensions of strains with known colony forming units (CFU; *E. coli*, 6×10^8 CFU/mL; *B. subtilis*, 8×10^7 CFU/mL) were incubated at 37 °C in nutrient solutions with different concentrations of the test samples. The growth of the bacteria was followed during the incubation over 20 h by measuring the optical density at 612 nm every 30 min by using a microplate reader/incubator. The minimal inhibitory concentration (MIC) corresponds to the concentration of the test substance at which a log 4 reduction of the growth of the inoculated bacteria was observed by comparison with control samples without test substance. This test does not clarify whether the substance is bactericidal or bacteriostatic. Experiments were triplicated. The standard deviations obtained from the triplication are not significant in comparison with the growth curves. The growth curves obtained from samples with different polymer concentrations are significantly different.

(ii) Growth Test After Exposure. Microorganisms were washed twice with PBS pH 6.5 and were exposed in the first step for 24 h at 30 °C in PBS to different concentrations of the polymer; thereafter, an aliquot was transferred to nutrient solution to monitor the proliferation potency of the exposed microorganisms. Calibration curves were obtained by incubation of defined CFU/mL without test substance.

Antibacterial Assessments of Coated Slides. Glass slides were sterilized at 80 °C in a drying oven (Kendro) for 20 min, covered with 50 μL of a diluted suspension of strains in nutrient solution ($1-2 \times 10^4$ CFU/mL), incubated in a climate chamber at 30 °C and 90% humidity, kept at room temperature for 30 min, covered with 1.2 mL of soft agar (38 °C), shortly shaken, and incubated overnight at 37 °C in a drying oven. The growth inhibition (%) was determined by dividing the number of colonies by 500 (number of colonies in the control test, without coating) and multiplied by 100. Experiments were triplicated.

Synthesis: (Hydroxymethyl)-1,3-dioxolan-2-one. Glycerol (23.12 g, 251 mmol), dimethyl carbonate (DMC) (67.51 g, 754 mmol), and DABCO (281 mg, 2.51 mmol) were mixed and heated at 75 °C for 10 h. After distillation of MeOH and excess DMC, glycerol carbonate was used without further purification for the synthesis of the dicarbonate **1**. ^{1}H and ^{13}C NMR spectra correspond to the data reported in the literature.[22]

(2-Oxo-1,3-dioxolan-4-yl)methyl Phenyl Carbonate (1). Glyceryl carbonate (19.5 g, 165 mmol) was dissolved in dry CH_2Cl_2 (200 mL) and pyridine (14.5 g, 183 mmol). The solution was cooled to 0 °C, phenylchloroformate (25.9 g, 165 mmol) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 16 h at room temperature. Pyridine hydrochloride was removed by filtration, and the solution was washed with H_2O and brine. The organic phase was dried over Na_2SO_4, and the solvent was removed by distillation (~20 mbar, 40 °C). The dicarbonate **1** was further purified by recrystallization from toluene, and a fine colorless powder was obtained (yield: 82%). ^{1}H and ^{13}C NMR spectra correspond to the data reported in the literature.[22]

(2-Oxo-1,3-dioxolan-4-ylmethyl) Hexylcarbamate (A6). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (10.00 g, 42.0 mmol) was dissolved in dry THF (100 mL). After cooling the solution to 0 °C, a solution of hexylamine (4.29 g, 42.4 mmol) in dry THF (50 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 16 h at room temperature. Solvents were removed by distillation (~20 mbar, 40 °C). The cyclic carbonate **A6** was purified by recrystallization from warm Et_2O/THF (5:2, v:v, 330 mL) to yield light brown pellets (8.21 g, 80%). ^{1}H NMR (DMSO-d_6): δ = 0.85 (t, 3H, CH_3), 1.16−1.30 (s, 6H, CH_2^{alkane}), 1.30−1.44 (m, 2H, NHCH$_2$CH_2), 2.96 (dt, 2H, NHCH_2), 4.11−4.32 (m, 3H, OCOOCH^aH^b, NHCOOCH_2), 4.55 (dd, 1H, OCOOCH^{a}H^b), 4.94−5.03 (m, 1H, OCH), 7.33 (t, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ = 13.8 (CH_3), 22.0 (CH_2CH$_3$), 25.8, 29.2, 30.9 (3C, CH_2^{alkane}), 40.3 (NHCH_2), 63.0 (NHCOOCH_2), 65.8 (OCOOCH_2), 74.8 (OCH), 154.7 (OCOO), 155.6 (OCONH) ppm.

(2-Oxo-1,3-dioxolan-4-yl)methyl Dodecylcarbamate (A12). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (2.00 g, 8.40 mmol) was dissolved in dry THF (20 mL). After cooling the solution to 0 °C, a suspension of dodecylamine (1.56 g, 8.40 mmol) in dry THF (10 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 16 h at

room temperature. Solvents were removed by distillation (~20 mbar, 40 °C). The cyclic carbonate **A12** was purified by recrystallization from $CHCl_3/Et_2O$ (15 mL/30 mL) to yield a colorless powder (2.35 g, 85%). ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, CH_3), 1.19−1.36 (s, 18H, CH_2^{alkane}), 1.41−1.56 (m, 2H, NHCH$_2$CH_2), 3.16 (dt, 2H, NHCH_2), 4.22−4.38 (m, 3H, OCOOCH^aH^b, NHCOOCH_2), 4.55 (dd, 1H, OCOOCH^aH^b), 4.86−4.95 (m, 1H, OCH), 5.08−5.18 (b, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_3), 22.7 (CH_2CH$_3$), 26.7, 29.3−29.8 (8C, CH_2^{alkane}), 31.9 (NHCH$_2$CH_2), 41.3 (NHCH_2), 63.3 (NHCOOCH_2), 66.0 (OCOOCH_2), 74.5 (OCH), 154.8 (OCOO), 155.6 (OCONH) ppm. Anal. Calcd for $C_{17}H_{31}NO_5$: C, 61.98; H, 9.49; N, 4.25%. Found: C, 61.11; H, 9.75; N, 4.04%.

(2-Oxo-1,3-dioxolan-4-yl)methyl Octadecylcarbamate (A18). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (5.00 g, 21.0 mmol) was dissolved in dry THF (50 mL). After cooling the solution to 0 °C, a suspension of octadecylamine (5.65 g, 21.0 mmol) in dry THF (20 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 16 h at room temperature. The solvents were removed by distillation (~20 mbar, 40 °C). The cyclic carbonate **A18** was purified by recrystallization from $CHCl_3$ (100 mL) to yield a colorless powder (7.36 g, 85%). ^{1}H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, CH_3), 1.198−1.40 (s, 30H, CH_2^{alkane}), 1.42−1.58 (m, 2H, NHCH$_2$CH_2), 3.17 (dt, 2H, NHCH_2), 4.24−4.38 (m, 3H, OCOOCH^aH^b, NHCOOCH_2), 4.54 (dd, 1H, OCOOCH^aH^b), 4.86−5.03 (m, 2H, OCH, NH) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (CH_3), 22.7 (CH_2CH$_3$), 26.7, 29.3−29.8 (14C, CH_2^{alkane}), 31.9 (NHCH$_2$CH_2), 41.3 (NHCH_2), 63.3 (NHCOOCH_2), 65.9 (OCOOCH_2), 74.4 (OCH), 154.6 (OCOO), 155.5 (OCONH) ppm.

(2-Oxo-1,3-dioxolan-4-yl)methyl Benzylcarbamate (B). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (5.00 g, 21.0 mmol) was dissolved in dry THF (25 mL). After cooling the solution to 0 °C, a solution of benzylamine (2.25 g, 21.0 mmol) in dry THF (25 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 20 h at room temperature. Solvents were removed by distillation (~20 mbar, 40 °C). The solid raw product was treated with ether and was filtered and washed with ether to yield a brown solid (3.86 g, 73%). The cyclic carbonate **B** (2.98 g) was further purified by recrystallization from warm THF/water (30 mL/40 mL) to yield colorless crystals (2.36 g, 58%). ^{1}H NMR (DMSO-d_6): δ = 4.11−4.34 (m, 5H, OCOOCH^aH^b, NHCOOCH_2, NHCH_2), 4.56 (t, ^3J = 8.6 Hz, 1H, OCOOCH^aH^b), 4.94−5.08 (m, 1H, OCH), 7.20−7.38 (m, 5H, CH^{aryl}), 7.95 (t, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6): $\delta = 43.7$ (NHCH_2), 63.2 (NHCOOCH_2), 65.8 (OCOOCH_2), 74.7 (OCH), 126.7 (C$H^{p\text{-aryl}}$), 126.9 (2C, C$H^{o\text{-aryl}}$), 128.2 (2C, C$H^{m\text{-aryl}}$), 139.4 (C^{aryl}), 154.6 (OCOO), 155.8 (OCONH) ppm. Anal. Calcd for $C_{12}H_{13}NO_5$: C, 57.37; H, 5.22; N, 5.58%. Found: C, 57.90; H, 4.97; N, 5.60%.

(2-Oxo-1,3-dioxolan-4-yl)methyl Allylcarbamate (D). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (20.00 g, 83.96 mmol) was dissolved in dry THF (200 mL). After cooling the solution to 0 °C, a solution of allylamine (6.012 g, 105.2 mmol) in dry THF (40 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 16 h at room temperature. Solvents were removed by distillation (~20 mbar, 40 °C) to yield a yellow oil (22.82 g, 94%) containing phenol (ca. 27 wt %). This raw product could be used without further purification for the reaction with amines. A small amount (2.30 g) was purified by chromatography on silica gel with pentane/AcOEt (1:1) (v/v) as eluting solvent to yield a colorless solid (1.40 g, 85%). ^{1}H NMR ($CDCl_3$): δ = 3.79 (t, ^3J = 5.6 Hz, 2H, NHCH_2), 4.24−4.42 (m, 3H, OCOOCH^aH^b, NHCOOCH_2), 4.57 (t, ^3J = 8.6 Hz, 1H, OCOOCH^aH^b), 4.94−5.08 (m, 1H, OCH), 5.11−5.26 (m, 2H, CH=CH_2), 5.51 (br, 1H, NH), 5.76−5.92 (m, 1H, CH=CH$_2$) ppm. ^{13}C NMR ($CDCl_3$): $\delta = 43.3$ (NHCH_2), 63.3 (NHCOOCH_2), 65.8 (OCOOCH_2), 74.3 (OCH), 116.0 (CH=CH_2), 133.9 (CH=CH$_2$), 154.7 (OCOO), 155.4 (OCONH) ppm.

3-*N*,*N*,*N*-Trimethyl-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonylamino)propan-1-ammonium Iodide (QI). In a two-neck flask equipped with thermometer and dropping funnel, dicarbonate **1** (4.00 g, 16.79 mmol) was dissolved in dry THF (40 mL). After cooling the solution to 0 °C, a solution of 3-dimethylamino-1-propylamine (1.72 g, 16.89 mmol) in dry THF (10 mL) was slowly added, and the temperature was kept below 5 °C. The reaction was stirred for 20 h at room temperature. A solution of MeI (2.1 mL, 34 mmol) in THF (10 mL) was added at 80 °C and under stirring for 1 h. After stirring for an additional hour under reflux, the solid was filtered and dried at room temperature in vacuo (10^{-3} mbar) to obtain a slightly yellow powder in quantitative yield. ^{1}H NMR (DMSO-d_6): δ = 1.76−1.90 (m, 2H, NHCH$_2$CH_2), 2.97−3.18 (m, 11H, NHCH_2, N$^+$(CH_3)$_3$), 3.29−3.38 (m, 2H, N$^+$CH_2), 4.12−4.35 (m, 3H, OCOOCH^aH^b, NHCOOCH_2), 4.60 (t, ^3J = 8.7 Hz, 1H, OCOOCH^aH^b), 4,99−5.08 (m, 1H, OCH), 7.49 (t, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6): δ = 22.9 (NHCH$_2$CH_2), 35.3 (NHCH_2), 52.2 (3C, N$^+$(CH_3)$_3$), 63.2 (2C, NHCOOCH_2, N$^+$CH_2), 65.9 (OCOOCH_2), 74.7 (OCH), 154.6 (OCOO), 155.6 (OCONH) ppm.

Table 1. Reactants for the Preparation of the Polymers **PEI-1**−**10**, Method of Purification, and Yields

	reactants mass (mg)								purification	yield
polymer	**PEI**	**QsI**[a]	**QI**	**A6**	**A12**	**A18**	**B**	**D**	method	(%)
PEI-1	500	495			568		8.7		1	80
PEI-2	500		563		478		7.3		2	85
PEI-3	500	417		356			7.3		2	86
PEI-4	500	500			382		7.3		1	84
PEI-5	500	417			478		7.3		2	84
PEI-6	500	333			574		7.3		1	81
PEI-7	500	250			669		7.3		1	66
PEI-8	500	167			765		7.3		2	75
PEI-9	500	417				600	7.3		2	82
PEI-10	2000	833			956			1173	1	82

[a] Quaternary ammonium functionalized carbonate derived from 3-chloro-1,2-propanediol.

General Procedure for the Functionalization of PEI with Functional Cyclic Carbonates: PEI-1−**10.** To a solution of PEI in DMF (10 mL, 30 mL for **PEI-10**) at 60 °C a mixture of functional cyclic carbonates (Table 1) was added. The solution was stirred at 60 °C for 20 h to 3 days. The conversion was monitored by ^{1}H NMR spectroscopy. Purification method 1: after addition of Et_2O (100 mL) the two-phase system was stirred vigorously for 10 min. The upper layer (Et_2O with impurities) was removed by decantation. Another Et_2O portion was added, and the separation procedure was repeated until the bottom layer became quite viscous and no change was observed. After drying in vacuum at room temperature, a slightly yellow highly viscous material was obtained. Purification method 2: the polymer solution was precipitated into Et_2O/pentane (1:1, v:v, 100 mL), and the two-phase system was stirred vigorously for 10 min. The upper layer (Et_2O/pentane with impurities) was removed by decantation, and the residue was dissolved into MeOH (5 mL) and precipitated into another portion of Et_2O/pentane. The separation procedure was repeated until the bottom layer became quite viscous and no change was observed. After drying in vacuum at room temperature, a slightly yellow highly viscous material was obtained.

Results and Discussion

Hydrophobic polycations act as antimicrobials.[9,17,18] It was our goal to prepare polycationic PEIs decorated with hydrophobic groups and to determine their antimicrobial properties. The synthetic approach comprises two steps: (i) synthesis of functional ethylene carbonates and conversion of the primary amine groups of PEI with these molecules.[22] We introduced quaternary ammonium groups and alkyl chains onto PEI by reaction of the primary amine groups, i.e., the terminal groups, with specifically functionalized cyclic carbonates and formation of a stable urethane group (Figure 1).

Synthesis of Functional Ethylene Carbonates. Beside the quaternary ammonium functionalized carbonate **QsI** described previously,[22] we prepared functionalized carbonate couplers with

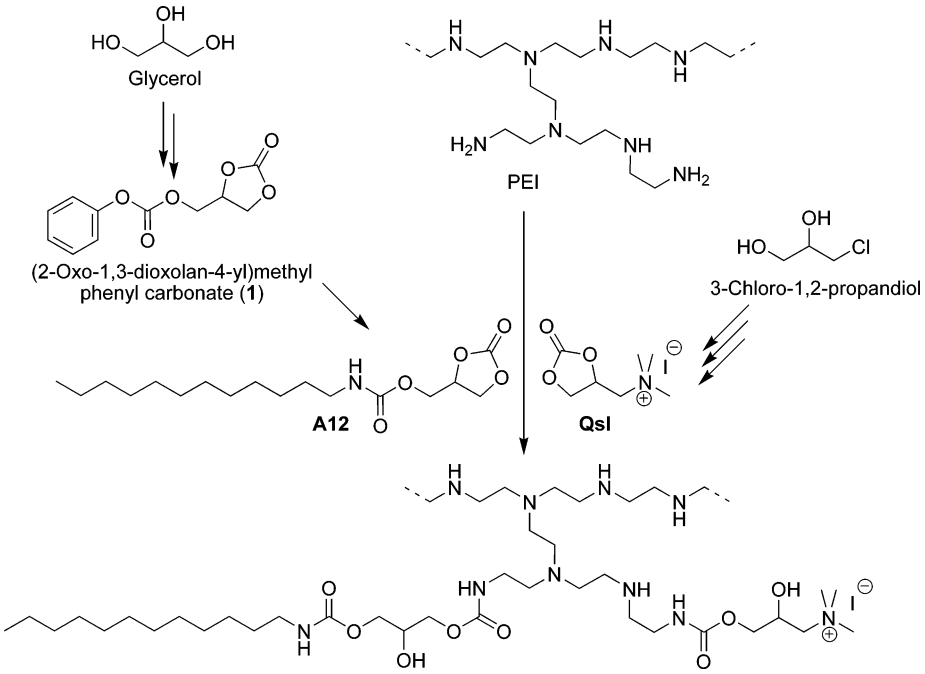

Figure 1. Synthesis strategy of hydrophobic polycations using functional ethylene carbonates and poly(ethylene imine) (PEI).

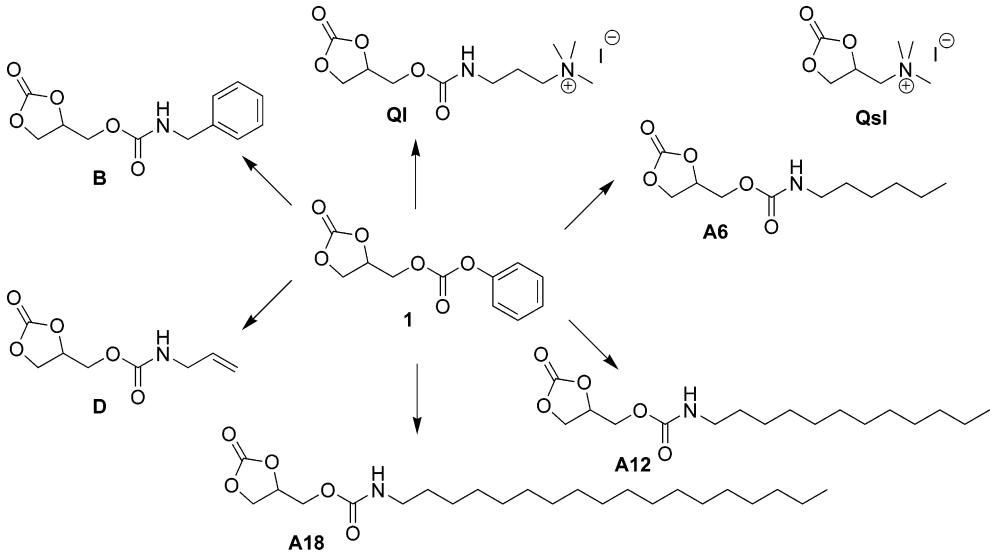

Figure 2. Structures of functionalized cyclic carbonates based on dicarbonate **1** and the alternative quarternary ammonium functionalized cyclic carbonate **QsI** (ref 22).

linear alkyl chains **An** ($n = 6$, 12, 18), ammonium groups **QI**, with allylic groups **D** suitable for photochemical cross-linking reactions, and with benzylic groups **B** for labeling the polymer. The synthesis of these functional carbonates starts with (2-oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate (**1**) and corresponding amines (Figure 2).

The dicarbonate **1** has two electrophilic sites with different reactivity: a highly reactive phenyl ester carbonate and a less reactive α-glycol carbonate. At low temperature (0−25 °C), the phenyl ester carbonate reacts with an amine and forms a urethane group; it should be noticed that the only observed leaving group is phenol. The cyclic carbonate reacts via ring-opening at slightly higher temperature (25−60 °C) but only after total consumption of the phenyl ester carbonate. According to this observation, the dicarbonate **1** was reacted at 0 °C with an equimolar amount of the desired amine. The quaternary ammonium salt **QI** was synthesized in a one-pot-two-step synthesis by addition of 3-dimethylamino-1-propylamine to a solution of dicarbonate **1** followed by addition of a solution of a methylation agent.

Synthesis of Functional Poly(ethylene imine)s. Each specifically functionalized carbonate coupler consists of a five-membered ring carbonate (1,3-dioxolan-2-one) on one side and a functional group on the other. The branched PEI used in our studies had a molecular weight (M_w) of 25 000. The ratio of primary, secondary, and tertiary amine groups is ca. 31:39:30 as determined by quantitative ^{13}C NMR spectroscopy.[23] These values are similar to data previously published for branched PEI and obtained by NMR spectroscopy[24] but slightly different from data obtained by titration.[25] In this study we report only the modification of the primary amine groups; this means that only 30% of the repeating units will be or can be converted. In other words, the degree of functionalization with respect to all repeating units can be chosen within a range between 0% and 30%. The primary amine groups of PEI react with the cyclic carbonate of a functionalized coupler via ring-opening with formation of a stable urethane and a hydroxyl group introducing the new functions into the substrate. The similar reactivity of the different functionalized carbonate couplers allows a one-

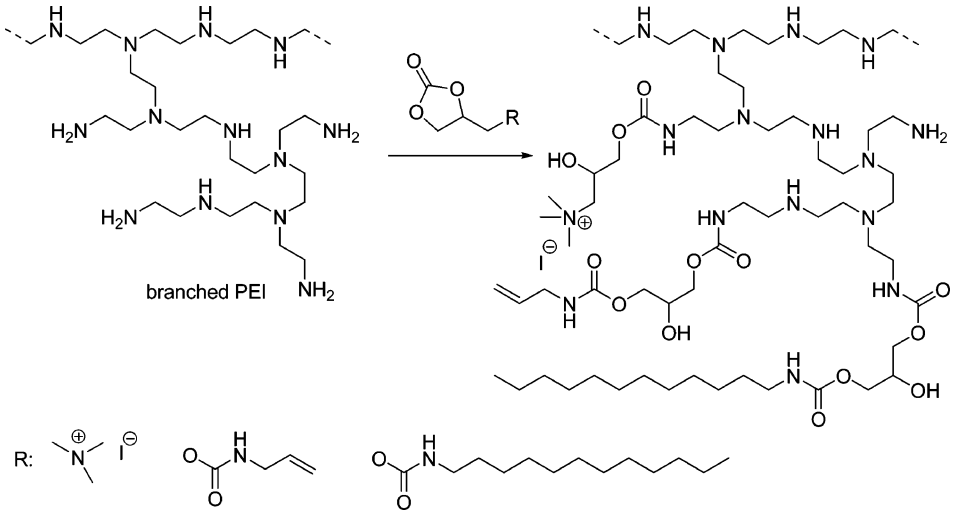

Figure 3. Synthesis of functional PEIs bearing cross-linkable allyl groups, quaternary ammonium groups, and dodecyl chains using functionalized carbonate couplers.

Table 2. Composition of the Functionalized Samples Obtained by Reaction of PEI with the Functional Carbonate Couplers

	functional carbonate in the feed[a] (functionalized EI repeating unit)[b]						
polymer	**QsI**	**QI**	**A6**	**A12**	**A18**	**B**	**D**
PEI-1	14.85 (12.3)			14.85 (11.2)		0.3 (0.13)	
PEI-2		12.5 (16.6)		12.5 (14.2)		0.25 (n.d.)[c]	
PEI-3	12.5 (12.7)		12.5 (13.5)			0.25 (0.17)	
PEI-4	15.0 (15.1)			10.0 (9.8)		0.25 (0.20)	
PEI-5	12.5 (12.0)			12.5 (12.2)		0.25 (0.23)	
PEI-6	10.0 (9.3)			15.0 (15.0)		0.25 (0.18)	
PEI-7	7.5 (6.6)			17.5 (18.4)		0.25 (0.21)	
PEI-8	5.0 (4.3)			20.0 (20.2)		0.25 (0.20)	
PEI-9	12.5 (14.3)				12.5 (14.6)	0.25 (0.28)	
PEI-10	6.25 (7.1)			6.25 (7.1)			12.5 (14.3)

[a] Ratio of functional cyclic carbonates to 100 ethylene imine (EI) units in the feed. [b] Percentage of primary amine groups, converted upon reaction with functional cyclic carbonates to the corresponding urethane groups as determined by NMR. [c] Not determined due to the level of the baseline noise.

step multifunctionalization of PEI; several functionalized carbonate couplers with different types of functional groups, e.g., quaternary ammonium groups, double bonds, and alkyl chains, were added simultaneously to a solution of PEI (Figure 3). Conditions for this reaction have been chosen with purpose: DMF was used as a solvent because of its ability to dissolve all the reactants and because it is chemically inert with respect to the cyclic carbonate. In order to achieve high conversion, the reaction mixture was stirred at 60 °C for at least 20 h. The multifunctionalized PEIs were recovered after precipitation. DMF was nearly quantitatively removed by multiple precipitation into ether/pentane (1:1, v:v) from highly concentrated polymer solution in MeOH. After drying under vacuum, polymers were obtained as slightly yellow solids (**PEI-10** as viscous oil). The relatively low yield of **PEI-7** (66%) is explained by the fact that, due to the low density of charges, the lower molecular weight fractions of the products are lost during purification (method 1). With improvement of the purification process (method 2), the yield was increased. **PEI-8**, which contains more alkyl groups and less cationic groups, was isolated in higher yield (75%) than **PEI-7** (cf., Experimental Section, Table 1).

NMR analysis of the reaction products demonstrated that a full conversion of all primary amine groups is difficult to reach. The reaction with a desired degree of functionalization of 30% (**PEI-1**) using 14.85 mol % **QsI**, 14.85 mol % **A12**, and 0.3 mol % **B** gave similar NMR results as polymers obtained with a desired degree of functionalization of 25% (**PEI-5**). Table 2 lists the different polymers that have been prepared. The samples **PEI-2**−**9** with a degree of functionalization of 25% were prepared from PEI using a coupler with a cationic group (**QsI** or **QI**) and a hydrophobic coupler (**An**) in different ratios. Additionally, benzyl groups were introduced in **PEI-2**−**9** in low concentration. **PEI-10** was prepared using PEI, 6.25 mol % **QsI**, 6.25 mol % **A12**, and 12.5 mol % **D**. All polymers were prepared according to the general procedure and by using the amount of material given in Table 1.

Characterization of Functional Poly(ethylene imine)s. The structure of the polymers was investigated by means of ^{1}H and ^{13}C NMR spectroscopy; the assignment was based on the results for model compounds obtained from functionalized carbonate couplers with amines.[22] The NMR spectra of **PEI-10** will be discussed exemplarily (Figures 4 and 5). In the ^{1}H NMR spectrum the coupling between PEI and the functional carbonates was proven by the appearance of a new signal for the urethane proton (signal 10) which, however, overlaps with the signals of the urethane protons 16A and 16D of the carbonates. Further, the CH_2−NH protons adjacent to the urethane group (signal 8′) are shifted to lower field compared to the CH_2−NH_2 protons of the starting material (signal 8). In addition the CH_3 and CH_2 protons of the alkane chain (signals 18A, 19A−27A, and 28A), the CH_3 protons of the ammonium moiety (15Q) and the terminal allyl protons (signals 18D and 19D) were clearly observed. In the ^{13}C NMR spectrum the characteristic signals for the building blocks—the propyl carbons (signal 12Q−14Q) and glycerol carbons (signal 12A−14A, 12′A−14′A,

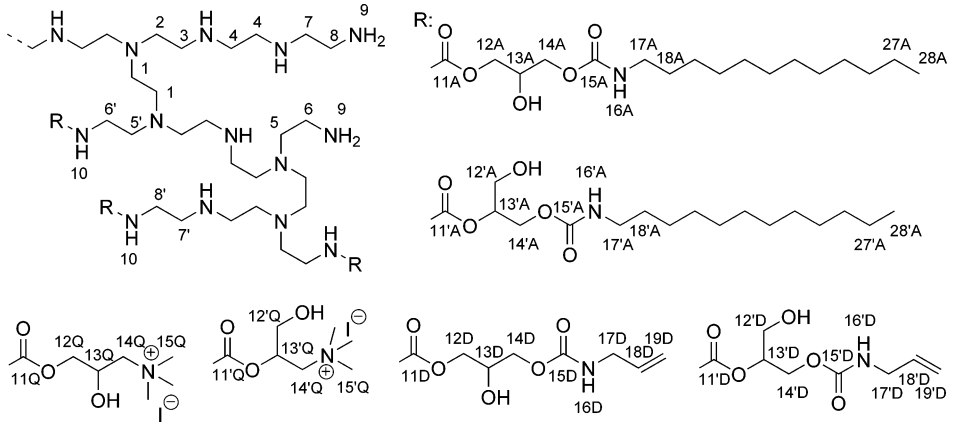

Figure 4. Structure of **PEI-10** with NMR assignment: repetitive ethylene imine units (signals 1−8), primary and secondary amines (signal 9), converted primary ethylene imine repeating units (signals 5′−8′), proton of primary amine converted into urethane groups (10), ammonium building blocks (signals 11Q−15Q and 11′Q−15′Q for the protons of two positional isomers), alkane building blocks (signals 11A−28A and 11′A−28′A for the protons of the two positional isomers), and double-bond building blocks (signals 11D−19D and 11′D−19′D for the protons of the two positional isomers).

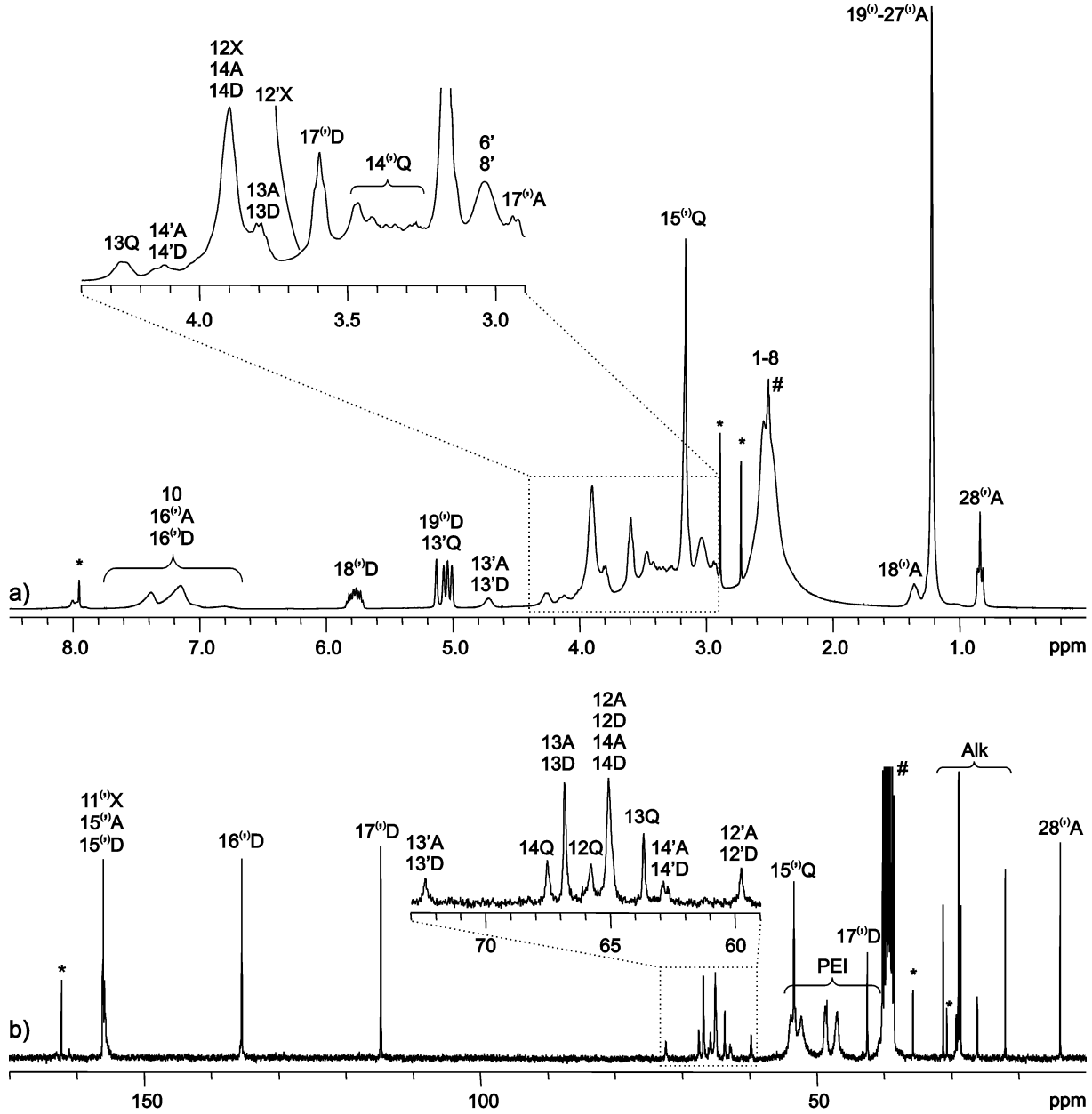

Figure 5. NMR spectra of **PEI-10** in DMSO-d_6: (a) ^{1}H and (b) ^{13}C (# = residual solvent peak, ∗ = DMF, PEI = polymer chain carbons, Alk = alkane carbons, X = A, D, and Q).

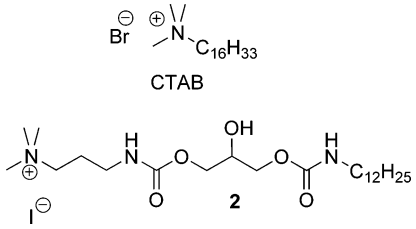

Figure 6. Structures of CTAB and **2**.

12D−14D, 12′D−14′D)—were observed. In addition, the NMR spectra showed no characteristic signals of the methylene group in the cyclic carbonates proving full conversion.

On the basis of the ^{1}H NMR results, the composition of the polymers was calculated. Table 2 summarizes the results; the values calculated from NMR spectra and the expected values from the feed reagents are in good agreement, proving high conversion of the primary amine groups.

Results of the Antibacterial Assessment. The different functionalized PEIs were tested for their MIC in solution regarding the effect of (i) the length of the alkyl chains, (ii) the hydrophilic/hydrophobic balance, and (iii) the kind of spacer between the cationic moiety and the polymer. The influence of the length of the alkyl chains was studied on **PEI-3**, **PEI-1**, or **PEI-9** containing ammonium groups and hexyl, dodecyl, and octadecyl groups, respectively, in a 1:1 ratio. For evaluating the influence of the hydrophilic/hydrophobic balance PEI was functionalized with ammonium and the dodecyl groups in different ratios: 6:4 in **PEI-4**, 5:5 in **PEI-1** and **PEI-5**, 4:6 in **PEI-6**, 3:7 in **PEI-7**, 2:8 in **PEI-8**. The influence of the spacer length was studied on **PEI** samples substituted with **A12** as hydrophobic group and **QsI** or **QI** as cationic groups in a ratio of 1:1: **PEI-1** substituted with **A12** and **QsI** and **PEI-2** substituted with **A12** and **QI**. The MIC of the polymers **PEI-1**−**4** and **PEI-6** was determined by means of a growth test. **PEI-5** has the same properties as **PEI-1**. The antimicrobial properties of **PEI-8** and **PEI-9** in solution were not evaluated due to the water insolubility of these polymers. Although **PEI-7** is soluble in water, it precipitated in PBS/nutrient solution, which did not allow a correct evaluation. The proliferation curves were monitored during incubation by measuring the optical density. We define the MIC of the polymer samples as the concentration at which the logarithm of the number of CFU decreases by 4 (which means a growth inhibition of 99.99%). For comparison purpose, the same investigation was done for cetyltrimethylammonium bromide (CTAB) and a low molecular weight molecule (**2**) prepared via coupling of **A12** with dimethylaminopropyl amine followed by quaternization.[22] Both molecules contain quaternary ammonium and alkyl groups (Figure 6).

The modified PEIs have an MIC between 0.3 and 0.4 mg/mL and between 0.03 and 0.04 mg/mL against *E. coli* and *B. subtilis*, respectively, except for **PEI-3**. As previously shown,[13,26] cationic amphiphilic polymers are less efficient against Gram-negative bacteria, due to the negative charges of the outer membrane of these organisms.[27] The MIC against *E. coli* of the polymer with hexyl groups (**PEI-3**) is higher than 0.5 mg/mL, and for this reason **PEI-3** is much less efficient than the one with dodecyl groups (**PEI-1**). The efficiency decreases also for the polymer with more alkyl groups (**PEI-6**) but is similar for the polymer with less alkyl groups (**PEI-4**). If the length of the spacer between the quaternary ammonium group and the polymer backbone is increased (**PEI-2**), the MIC is slightly lower against *E. coli* and similar against *B. subtilis*. This is reasonable since the active cationic groups are more effective when located farther apart from the backbone. **PEI-2** has an MIC against *E. coli* of 8 μmol/L, which is smaller by a factor of 3 than that of the reference molecule CTAB and smaller by more than a factor of 6 than that of the corresponding low molecular weight molecule **2** (Table 3).

Table 3. Minimal Inhibitory Concentration (MIC) of **PEI-1**−**4**, **PEI-6**, CTAB, and **2** in Aqueous Solution against *E. coli* and *B. subtilis*

	MIC			
	(mg/mL)		(μmol/L)[a]	
substance	*E. coli*	*B. subtilis*	*E. coli*	*B. subtilis*
PEI-1	0.3	0.03	11	1.1
PEI-2	0.3	0.04	8	1.1
PEI-3	>0.5	0.03	>19	1.1
PEI-4	0.3	0.03	11	1.1
PEI-6	0.4	0.04	14	1.4
CTAB	0.01	0.003	27	8.2
2	0.03	0.012	52	21

[a] Determined on the basis of M_n of PEI given by the producer plus the molecular weights of the functionalizing fragments.

Table 4. Bactericidal Activity against *E. coli* of **PEI-1**−**4** and **PEI-6** in Solution in the Two-Step Exposure/Growth Test (Exposure at a Polymer Concentration of 1 mg/mL; Dilution, 1:40 for Growth Test)

polymer	growth inhibition (%)
PEI-1	99.96
PEI-2	98.5
PEI-3	96.5
PEI-4	98.5
PEI-6	98.5

A two-step testing was applied in order to determine whether the substances are bactericidal or bacteriostatic. In the first step, the bacteria were exposed to the substance in different concentrations under nongrowth conditions in PBS. In the second step, an aliquot of the suspension was diluted (1:10, 1:20, 1:40, and 1:200) and transferred into nutrient solution. The growth was observed by measuring the optical density. During exposure (first step), the test substance interacted with the bacteria under nonproliferation conditions; however, upon dilution, the polymer concentration was reduced below the MIC enabling again proliferation. A lag phase was observed during monitoring the growth, and the proliferation rate was determined by comparing the results with the calibration curves. As an example, the results for *E. coli* with a polymer concentration of 1 mg/mL during the exposure and at a dilution of 1:40 during growth are shown in Table 4. When the effects of **PEI-1** and **PEI-3** are compared it seems likely that under nongrowth conditions the kind of the hydrophobic residues exhibit the greatest impact on the proliferation ability of *E. coli* (C12 is better than C6). Under these conditions a ratio of 1:1 of cationic to hydrophobic groups seems to have the highest impact on the proliferation ability of *E. coli*, the cationic group **QI** being less effective than **QsI**.

The good results obtained in solution encouraged us to prepare also coatings and to investigate their bactericidal efficiency against *E. coli* and *B. subtilis*. As a first possibility the hydrophobic **PEI-8** (functionalized with dodecyl and cationic groups with a molar ratio of 8:2) and **PEI-9** (functionalized with octadecyl and cationic groups in a ratio 1:1) were used because of their water insolubility. An alternative would be to use a soluble polymer and to cross-link it. With this purpose PEI was functionalized with quaternary ammonium groups, dodecyl chains, and cross-linkable double bonds with a molar ratio of 1:1:2 (**PEI-10**) and thermally cross-linking with

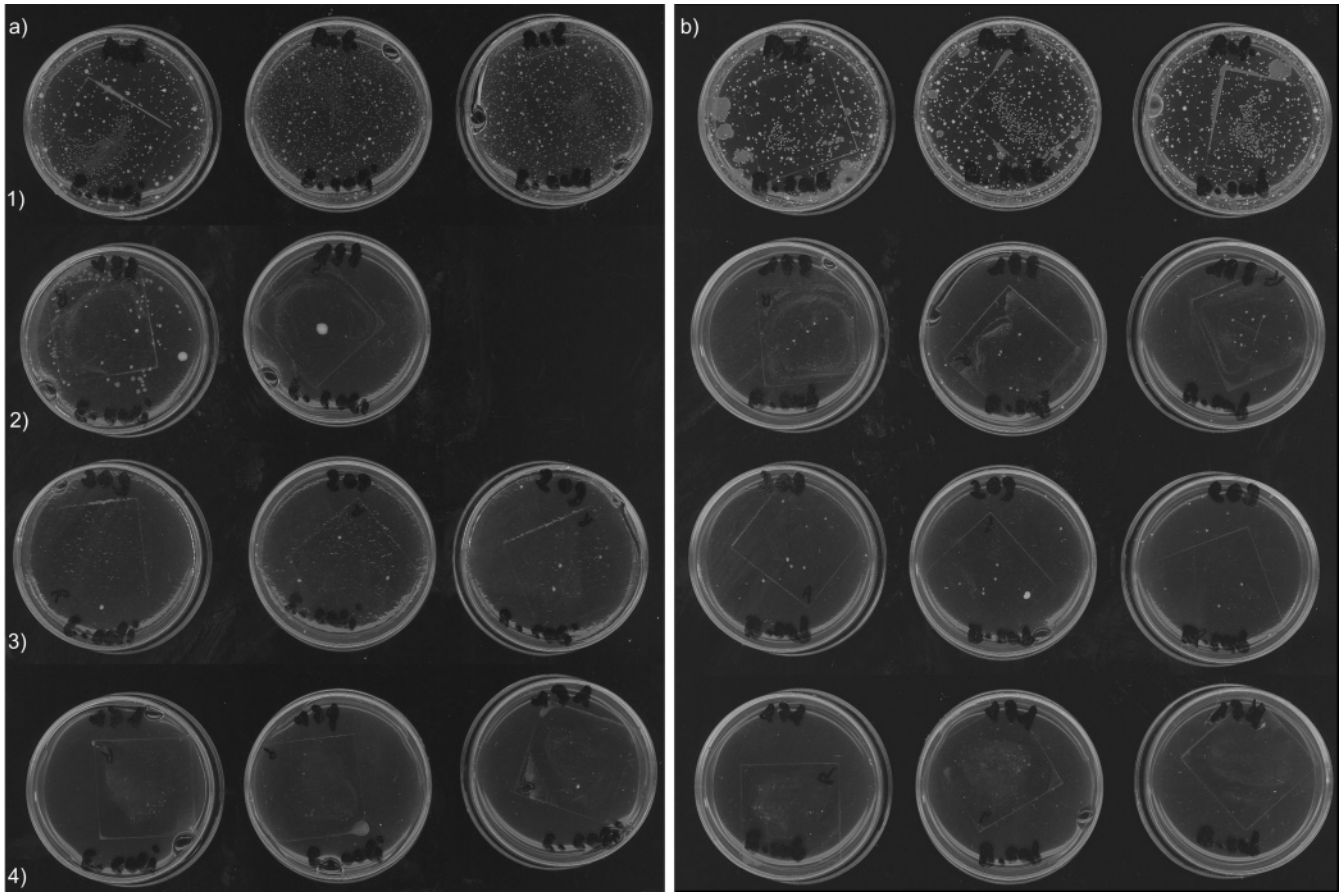

Figure 7. Pictures obtained by scanning the Petri dishes containing glass slides (1), glass slides coated with **PEI-8** (2), with **PEI-9** (3), and with cross-linked **PEI-10** (4) which were covered with 50 μL of suspension of *E. coli* (a) and *B. subtilis* (b) ($1-2 \times 10^4$ CFU/mL in nutrient solution) followed by incubation, covering with soft agar, and incubation overnight at 37 °C. Each white dot corresponds to a colony grown from a single surviving bacterial cell.

Table 5. Growth Inhibition of *E. coli* and *B. subtilis* on Glass Slides Coated with **PEI-8**, **PEI-9**, and Cross-Linked **PEI-10**

	growth inhibition	
	E. coli	*B. subtilis*
polymer	(%)	(%)
PEI-8	95.3	98.5
PEI-9	99.5	98.4
PEI-10[a]	99.9	99.9

[a] **PEI-10** was cross-linked using DEgDMA as cross-linking agent and AIBN as initiator.

DEgDMA as cross-linking agent and AIBN as an initiator (weight ratio polymer/DEgDMA/AIBN 100:10:1). It was found that after 18 h, the methanol-insoluble part reached 70 wt % of the initial mass. Coated glass slides were prepared by casting **PEI-8**−**10** (solution of **PEI-10** containing cross-linker and initiator in the ratio mentioned above) from methanol solutions. The obtained coatings were transparent and colorless. The growth inhibition effect against *E. coli* and *B. subtilis* was investigated by first exposing a suspension of bacteria in nutrient solution ($1-2 \times 10^4$ CFU/mL) to the surface. After incubation and addition of soft agar and further incubation under optimum growth conditions, the CFU were counted. The coatings were highly effective against both Gram-negative and Gram-positive bacteria (Figure 7). A growth inhibition higher than 95% was found for each sample (Table 5). The best results (99.9% for both) were obtained with the cross-linked polymer **PEI-10**, where none or only a single colony was observed. This is explained by the fact that the hydrophobicity of **PEI-10** is less prominent than that of both other polymers, since it exhibits a molar ratio cationic/dodecyl groups of 1:1, whereas **PEI-8** exhibits a molar ratio of 1:4 and **PEI-9** has longer alkyl chains. It was effectively demonstrated that, with other parameters being unchanged, increasing the hydrophobicity of the polycations strengthens the hydrophobic/hydrophobic attraction of the alkyl chains within the coating and hence reduces the bactericidal efficiency.[21]

To determine if part of the polymer is transferred from the coating into the exposure solution during the exposure time polymer-coated glass surfaces were covered with 50 μL of nutrient solution without bacteria and incubated as described. Thereafter, 40 μL of the exposure solution was transferred to a Petri dish and inoculated with *B. subtilis* (4×10^4 CFU/mL). This solution was exposed again for 2 h at 30 °C and 90% humidity, covered with soft agar, and incubated overnight. The solution exposed to the surface coated with **PEI-8** and with **PEI-10** showed a strong growth inhibition (~60% and 65%) and the solution exposed to the surface of **PEI-9** a light growth inhibition (~46%) of *B. subtilis*. Even so, these polymers are insoluble in water; they are able to swell in aqueous media. In the experiment described we used a nutrient solution containing meat extract and peptone in order to test whether some of the polymer is transferred to the solution. It must be admitted that the proteins (amphiphilic molecules) from the meat extract and peptone can act as surfactant and promote the dissolution of a small amount of the hydrophobic polymer. It should be also mentioned that no detachment of the coating was observed after water rinsing or after storage under air.

Conclusions

We have developed a novel method for a one-step multifunctionalization of polyamines utilizing functional cyclic carbonate couplers as modification agent for PEI. By applying this method, PEI was successfully functionalized with cationic quaternary ammonium salts, hydrophobic alkyl chains, and cross-linkable allyl groups. The obtained hydrophilic polymers act as antibacterial substances in solution. Coating glass with the most hydrophobic or cross-linkable ones also resulted in antimicrobial properties. Due to the observation that polymers leach out of the coating, the improvement of the cross-linking method or the development of a covalent bond between the polymer and the surface is ongoing. Additionally, PEI with new hydrophobic groups is synthesized in order to extend the available selection of antimicrobial substances not leading to microbial resistance. We believe that the strategy demonstrated in this article might lead to many applications in hygiene, food, and cosmetics industries.

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