Conjugation of Ciprofloxacin with Poly(2-oxazoline)s and Polyethylene Glycol via End Groups

Martin Schmidt,[†] Simon Harmuth,[†] Eva Rebecca Barth,[†] Elena Wurm,[†] Rita Fobbe,[‡] Albert Sickmann,[‡] Christian Krumm,[†] and Joerg C. Tiller*[,†]

[†]Biomaterials and Polymer Science, Department of Bio- and Chemical Engineering, TU Dortmund, Emil-Figge-Straße 66, 44227 Dortmund, Germany

[‡]Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., Otto Hahn-Straße 6b, 44227 Dortmund, Germany

S *Supporting Information*

ABSTRACT: The antibiotic ciprofloxacin (CIP) was covalently attached to the chain end of poly(2-methyloxazoline) (PMOx), poly(2-ethyloxazoline) (PEtOx), and polyethylene glycol (PEG), and the antimicrobial activity of these conjugates was tested for *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Kleisella pneumoniae*. Chemical structures of the conjugates were proven by ^{1}H NMR and electron spray ionization mass spectrometry. The direct coupling of PMOx and CIP resulted in low antimicrobial activity. The coupling via a spacer afforded molecular weight dependent activity with a molar minimal inhibitory concentration that is even higher than that of the pristine CIP. The antimicrobial activity of the conjugates increases in the order of PMOx < PEtOx < PEG. Conjugation of CIP and a quaternary ammonium compound via PMOx did not result in higher activity, indicating no satellite group or synergistic effect of the different biocidal end groups.
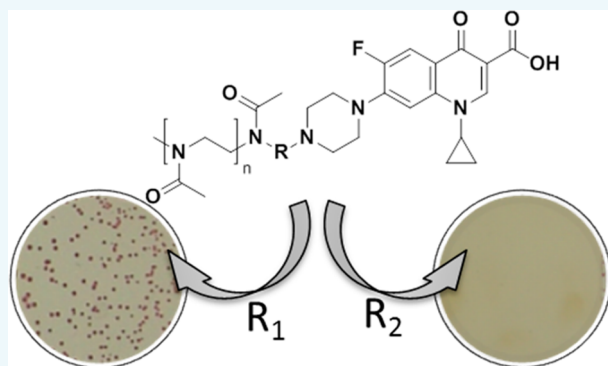
INTRODUCTION

In times of rapid spread of bacterial infections, there is a growing demand for new antibiotics because the abusive use of antibiotics afforded the development of numerous resistant bacterial strains. The latter are becoming a great problem of modern medicine. Although, there are many developments in finding new antibiotics, the number of such drugs that reach the market is rather limited with approximately one new antibiotic per year.[1,2] This is partially due to exploding costs for drug development and approval. Formulation and derivatization of existing antibiotics is a promising alternative to shorten the time of development for new antimicrobial agents. Most often, the functional groups of such drugs are modified to change charge density, solubility, degradability, selectivity, and efficiency.[3,4]

Moreover, formulations based on macromolecules such as nanoparticles, liposomes, or molecular micelles are mostly optimized to change bioavailability.[5−7] Another very successful way to prepare efficient derivatives of antibiotics is their combination with polymers. Macromolecules as carriers for therapeutics become increasingly important alternatives because such polymer antibiotic conjugates (PACs) afford lower toxicity, increase solubility, and prolonged activity.[8,9] In 2013, two of the top 10 selling drugs in the United States were polymer therapeutics.[10] In most cases, polymer PACs are used as temporary antibiotic-carriers, for instance, by using degradable polymer matrices[11−13] or hydrolyzable binding to nondegradable polymer matrices.[14−20] Preparing polymer antibiotic conjugates

which are designed without the intention of releasing antibiotics is another successful concept in drug design but less often used. Overall, PACs are mostly used for detoxification of anticancer therapeutics such as doxorubicin,[21−23] but surprisingly, there are only few examples of permanently bound polymer antibiotic conjugates, which focus on the treatment of bacterial infections.[24] Polyethylene glycol (PEG) is commonly used for conjugation of drug conjugates. Nathan et al. coupled penicillin V and hydrophilic cephradine to PEG-lysine polyurethane via two different strategies. Penicillin V was introduced by a hydrolyzable ester group to the polymer, and cephradine was bound by a hydrolytically stable amid bond. Whereas the first example is a nonpermanent PAC, which showed activity after the release of penicillin V, the second permanently bound PAC showed no antimicrobial activity at all.[19] PEG was also used for the conjugation of hydrophilic tobramycin by end group coupling via amid bonds. Du et al. also reported that the prepared PACs show a lower antimicrobial activity in solution against *Pseudomonas aeruginosa* (*P.a.*) than free tobramycin. The more remarkable result was achieved when the PACs were tested against *P.a.* biofilms. In this case, the PAC shows a 3.2 fold higher activity against the resilient biofilms than tobramycin, which indicates the potential of such permanently bound PACs.[25] Lawson et al.

A

prepared telechelic nondegradable conjugates of PEG and vancomycin. These conjugates possess an acrylate functionality as the second terminal end for further surface modification. In solution, the PEG–PACs revealed 6–75 fold decrease in biocidal activity against *Staphylococcus epidermidis*.[26] After surface modification with these PACs, the surfaces revealed a 7–8 log reduction in bacterial colony forming units, which is exceptional for an antimicrobial surface.[27,28] Turos et al. published a study in which the covalent conjugation of *N*-thiolated *β*-lactam antibiotic with polyacrylates toward nanoparticles was performed. In all cases, the nanoparticles show better activity than the antibiotic containing monomers even with no difference to methicillin-resistant *Staphylococcus aureus* (MRSA).[29] An innovative concept for the application of PACs was investigated by the group of Rimmer. Polymyxin B (PMX) was covalently attached to a highly branched poly(*N*-isopropylacrylamid) (PNIPAM). Therefore, the biocidally active functional group of PMX was removed to introduce a potential binding site for PNIPAM. The resulting conjugate is able to precipitate *P.a.* by binding of the cyclic peptide of PMX to the lipopolysaccharide in the membrane of *P.a.* The caused aggregation decreased the lower critical solution temperature of PNIPAM below 37 °C, which leads to precipitation of this aggregates in water solutions. This represents a very smart method for the detection of Gram-negative bacteria in aqueous solution.[30] In general, permanent PACs are a promising way to improve antibiotic performance, but more examples are needed to truly judge this.

Poly(2-oxazoline)s (POx) are nontoxic polymers with adjustable hydrophilicity and readily functionalizable end groups.[31–33] They are considered as an alternative to PEG.[34,35] So far, POx have not been investigated as PAC. However, end group conjugation with quaternary ammonium compounds is a well-established way of preparing biocidal polymers; sometimes, such polymeric biocides even surpass the molar activity of their low molecular weight pendants.[36] This is achieved by the introduction of a second nonbiocidal end group, which is able to enhance or to weaken the antimicrobial efficiency.[37,38] This second end group is called satellite group, and in our latest investigations, we could show that this satellite group can be chemically or enzymatically altered for switching the antimicrobial activity of the macromolecules over several orders of magnitude.[39] Therefore, the conjugation of poly(2-oxazoline)s with antibiotics as functional end groups might be a suitable concept for preparing new biocidally active poly(2-oxazoline)s PACs. In the present studies, ciprofloxacin (CIP)-PMOx PACS are synthesized and explored regarding their antimicrobial activity and hemotoxicity.

RESULTS

CIP contains two suitable functional groups, the carboxylic acid and the piperazine group, that might be used in the termination-step of the cationic ring-opening polymerization of 2-R-2-oxazolines or other coupling reactions (see Figure 1).

First, CIP was reacted with the living cationic polymer chains of poly(2-methyloxazoline) (PMOx). It was found by ^{1}H NMR spectroscopy and electron ionization mass spectrometry (ESI-MS) that 80% of the polymer chains carry a CIP (Figure S1, Supporting Information). ^{1}H NMR spectroscopy reveals that 13% of the polymer chains are conjugated via the carboxyl group of CIP. The other 87% of the PMOx chains are coupled to CIP via the piperazine ring (Figure S2).

In order to obtain CIP-PACs with more uniform structures, *tert*-butyloxycarbonyl (Boc) was chosen to protect the piperazine
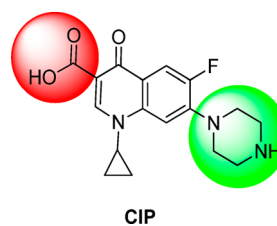
Figure 1. Ciprofloxacin. The carboxylic acid group is in red and the piperazine group in green.

group of CIP (compare Scheme 1 to the ^{1}H NMR spectrum in Figure S3).

The deprotonated carboxylic acid group was used for reaction with the living end of PMOx. After cleaving the protecting group with TFA, Me-PMOx-O-CIP was obtained. The structure of the polymer was characterized by ^{1}H NMR spectroscopy (Figures S4–S5) and ESI-MS before and after deprotection. The found *m/z* values correlate with the expected masses of the polymer Me-PMOx-O-CIP-Boc and Me-PMOx-O-CIP (Figure 2).

Additionally, a PAC was prepared by modification of the piperazine group with PMOx. For this purpose, the carboxylic acid group of CIP was protected by an ethyl ester group, introduced by esterification with ethanol (Figure S6). This derivative reacted with the living end of PMOx. It was proven by ^{1}H NMR spectroscopy that 70% of the polymer chains are functionalized with CIP (Figure S7). The protecting group was cleaved by alkaline hydrolysis to get Me-PMOx-N-CIP (Figure S8).

In order to introduce a spacer between PMOx and CIP, a polymer-analogue implementation was developed. The strategy for the modification of PMOx is pictured in Scheme 2. At first the active polymer chains were functionalized with ethylene diamine (Figure S9).[40] In a second step, an amino-reactive derivate of CIP was synthesized after a protocol of Kerns.[41,42] To this end, CIP was coupled with *α,α′*-dichloro-*p*-xylene and obtained with 49% yield and high purity (>98%) confirmed by ^{1}H NMR spectroscopy (Figure S10).

As seen in Figure 3, all signals in the ^{1}H NMR spectrum can be assigned to the expected polymer structure, indicating that polymer chains were successfully functionalized with the CIP-derivative. The analysis of the obtained ^{1}H NMR spectrum results in a degree of polymerization of 32 suggesting quantitative conversion.

The ESI-MS of the derivative is shown in Figure 4. The found degree of polymerization of 30 matches that determined in the ^{1}H NMR. Eighty-six percent of all signals in the ESI-MS could be assigned to macromolecules with the methyl starter and the CIP end group, respectively. Nine percent of all polymer molecules were attributed to proton initiated polymers. This is due to chain transfer reactions that occur during the polymerization caused by *β*-H-cleavage.[37,43]

Previous works have shown that poly(2-oxazoline)s equipped with antimicrobial end groups,[36,39] which were derived from low molecular biocides such as DTAC show a great potential with regard to their antimicrobial activity and selectivity. By modification of these biocidal functionalized polymers with an antibiotic end group, both antibiotics and biocides would be combined in a single macromolecule.

The previously described polymer DDA-X-PMOX$_{30}$-EDA[37] (^{1}H NMR, Figure S11) was used as precursor for coupling with CIP as depicted in Scheme 3.

Scheme 1. Synthesis of Me-PMOx−CIP with the Direct Termination Strategy
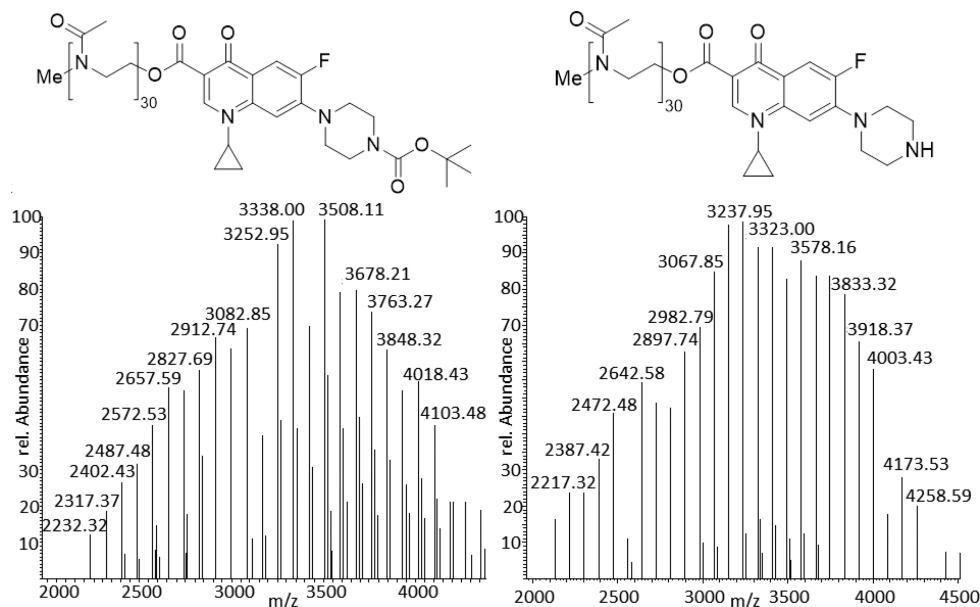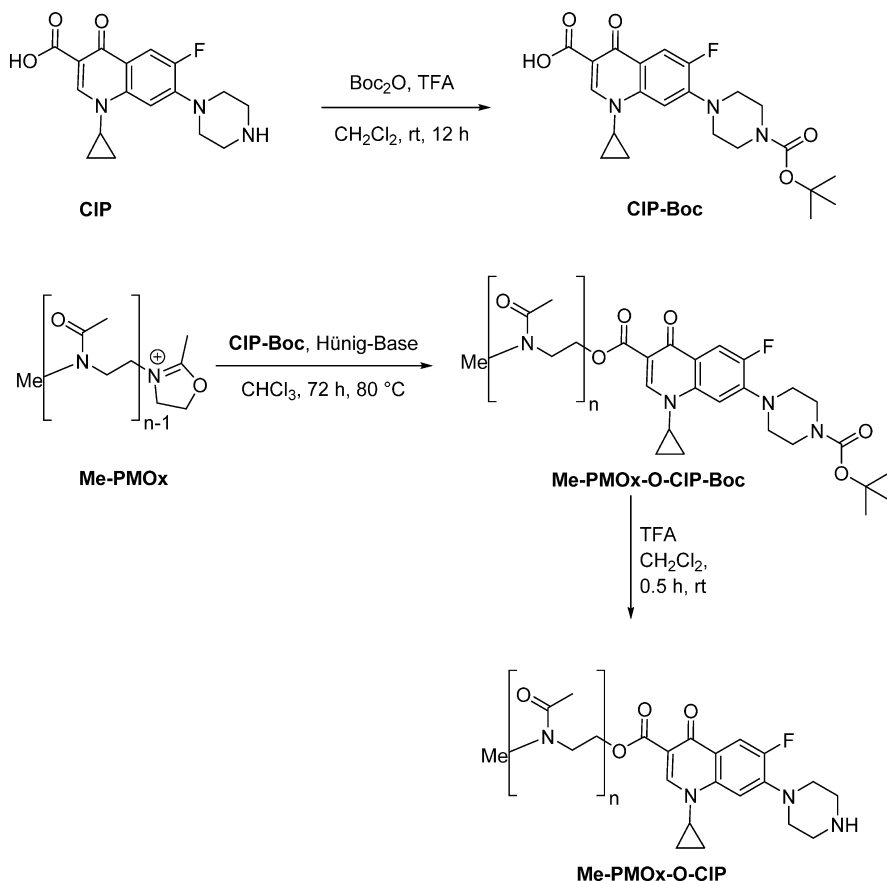

Figure 2. ESI-MS of polymer Me-PMOx-O-CIP-Boc and Me-PMOx-O-CIP.

DDA-X-PMOX$_{30}$-EDA-CIP was characterized by ^{1}H NMR spectroscopy and ESI-MS. The initiating DDA-X and terminating CIP-functionalities can be clearly assigned to the signals in the obtained ^{1}H NMR spectrum and the degree of functionality could be determined to 99% (see Figure 5).

According to this high degree of modification, more than 99% of the masses obtained by ESI-MS measurements represent species with the CIP-end group (Figure 6). More than 85% of these polymers contain a DDA-X at the other end. Fourteen percent of the polymers chains were initiated by protons. This is similar to the above-described results found for Me-PMOx-EDA-CIP. The analytical data of all synthesized polymers including the varied molecular weight species are given in Table 1 and Table 2 (^{1}H NMR spectrum, Figure S12−S16).

C

Scheme 2. Synthesis of Me-PMOx-EDA, the CIP-Spacer, and the Polymer Analogue Implementation of the CIP-Spacer to Me-PMOx-EDA-CIP
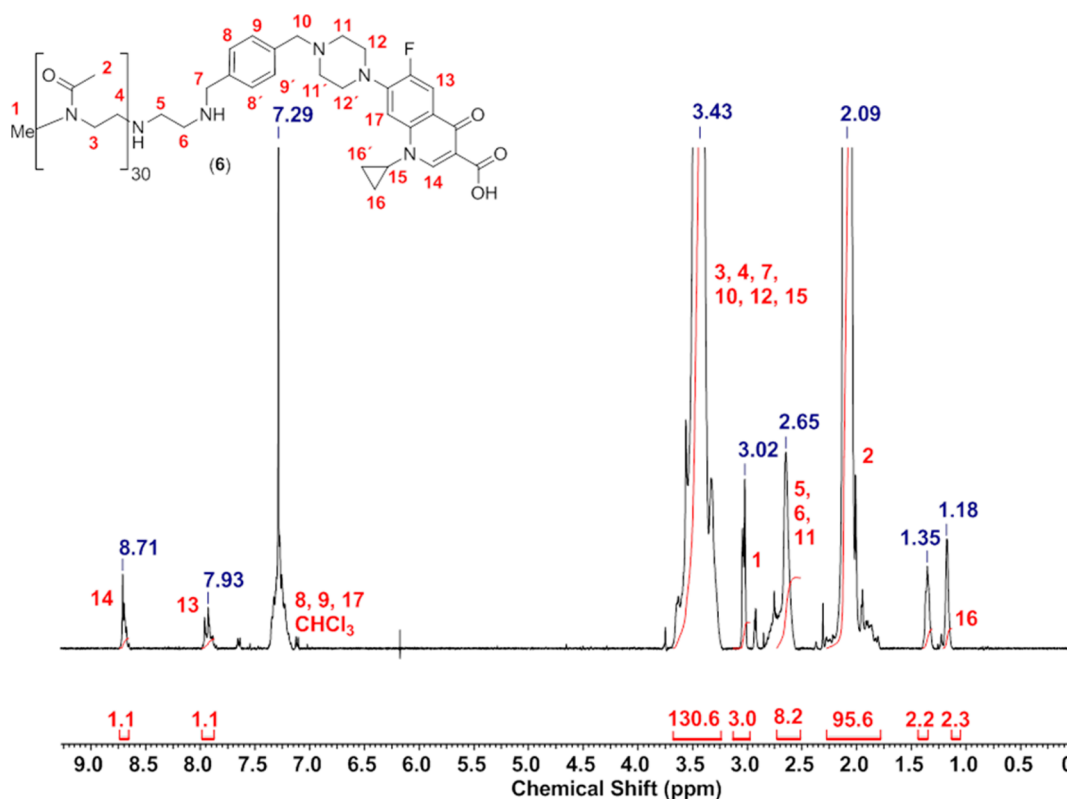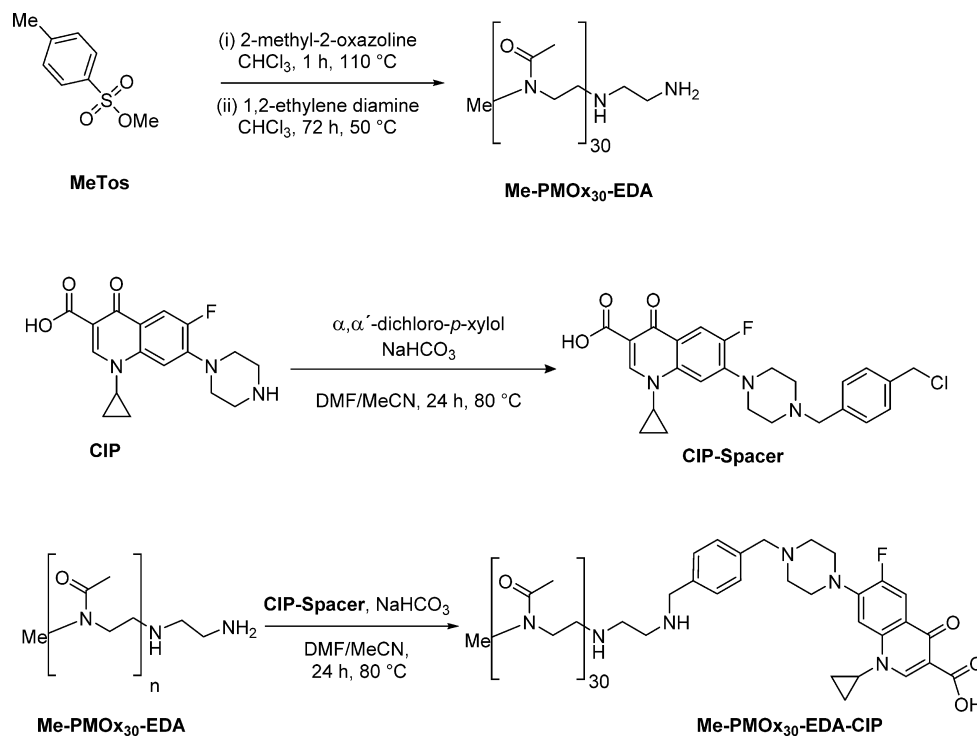

Figure 3. ^{1}H NMR of Me-PMOx-EDA-CIP in CDCl$_3$.

Additionally, 2-ethyl-2-oxazoline was used for further variation of the polymer backbone. For this purpose, methyl p-toluenesulfonate (MeTos) was used as initiating agent, and the living polymer chains were functionalized with EDA (Figure S17) and reacted with the CIP-spacer as described above. The

analytical data and the ^{1}H NMR spectrum of this poly(2-ethyloxazoline) (Me-PEtOx-EDA-CIP) is given in Figure S18. The ^{1}H NMR spectrum suggests a degree of modification of 93%, which is similar to the respective PMOx-CIP conjugate. Further, polyethylene glycol (PEG) was conjugated with CIP.
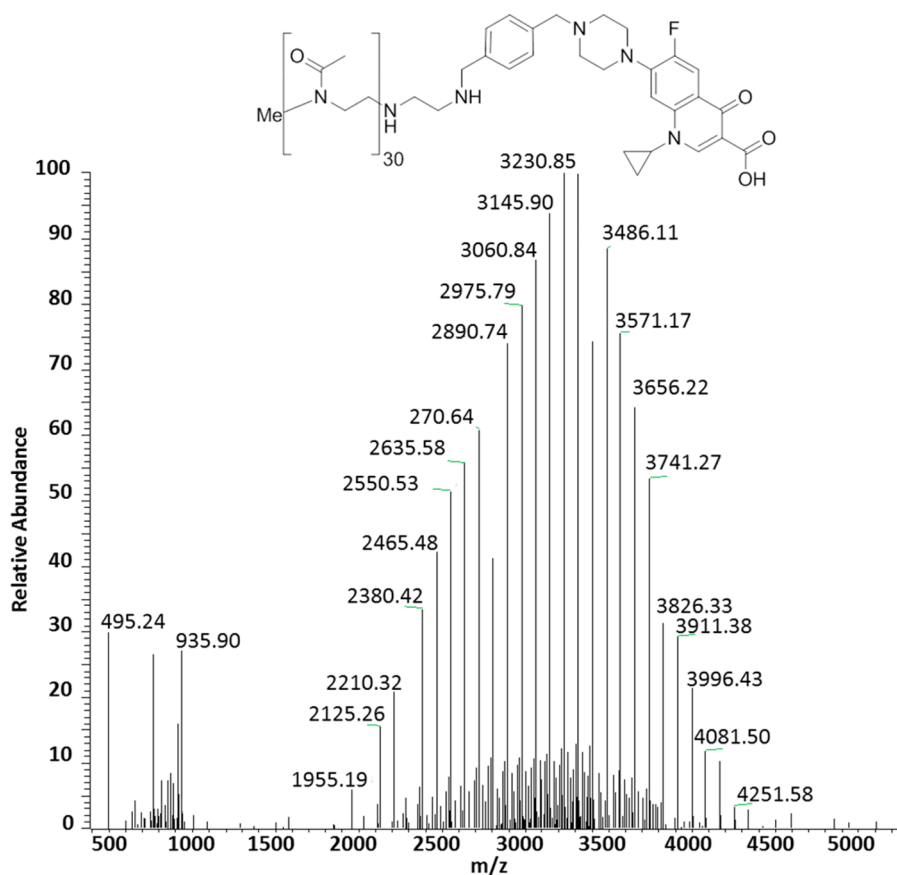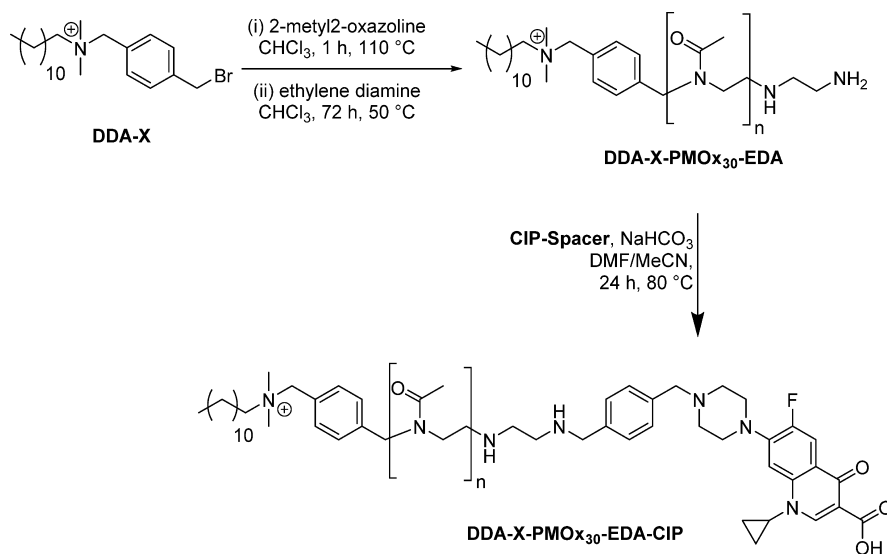
D

Figure 4. ESI-MS of Me-PMOx-EDA-CIP.

Scheme 3. Synthesis of DDA-X-PMOx-EDA-CIP

Therefore, PEG was equipped with an EDA end group. This was achieved by mesylation of the OH end group of PEG and a subsequent nucleophilic substitution reaction with EDA. This terminal amino group offers the possibility to use the same coupling route already applied for the POx derivatives (Scheme 4; ^{1}H NMR spectrum, Figure S19–S21). The ^{1}H NMR spectrum reveals 90% conversion with respect to CIP.

The minimal inhibitory concentrations (MIC) of the synthesized PACs were investigated. This concentration displays

the concentration of an antimicrobial active compound at which 99% of bacteria are inhibited in growth. Direct termination of CIP with PMOx results in a $MIC_{S.aureus}$ value of 1250 $\mu g \cdot mL^{-1}$ (390 $\mu mol \cdot L^{-1}$). This low activity seems to indicate that conjugation with PMOx drastically lowers the activity of the antibiotic CIP ($MIC_{S.aureus}$ = 0.5 $\mu g \cdot mL^{-1}$ (1.29 $\mu mol \cdot L^{-1}$)). As shown by ^{1}H NMR spectroscopy, the termination occurs at the carboxylic as well as at the secondary amine group of CIP. It is known from literature that low molecular derivatives of CIP
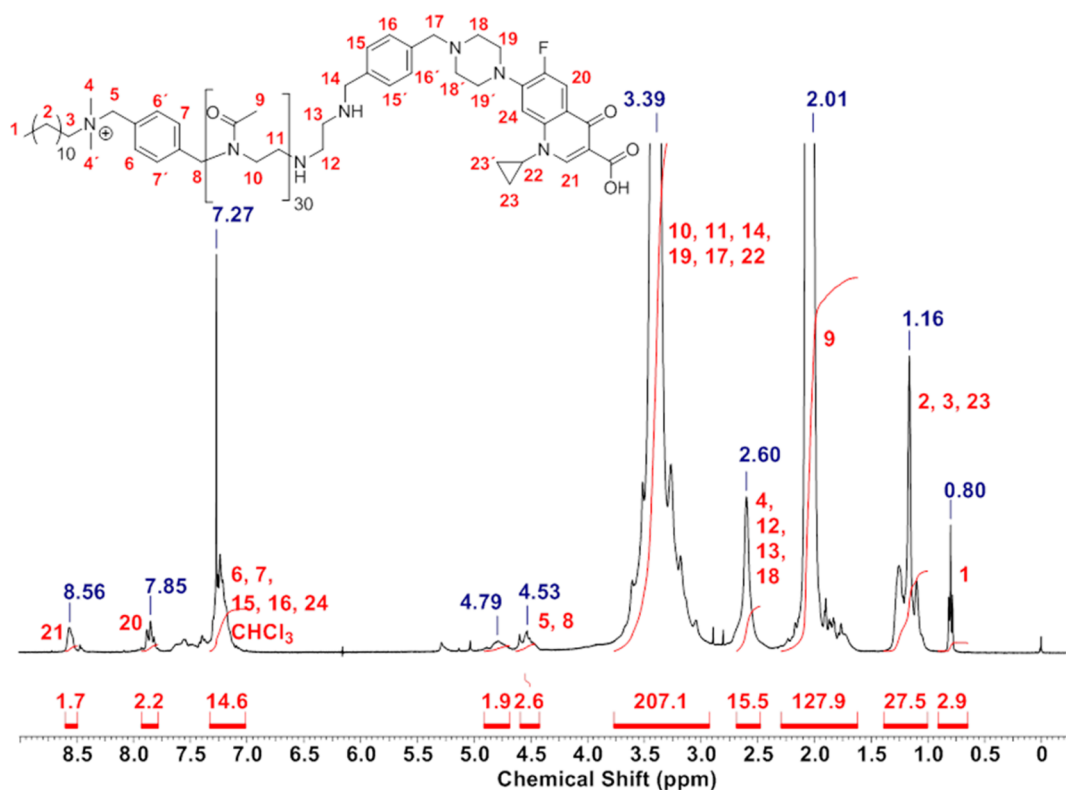
Figure 5. ^{1}H NMR messurement of DDA-X-PMOx-EDA-CIP in CDCl$_3$.

exhibit reduced biological activity when modified at the carboxylic group,[44,45] while modification of the piperazine ring results in an antibacterial activity on the same level as the unmodified fluoroquinone.[4]

The derivative PMOxylated at the carboxylic group (Me-PMOx-O-CIP) exhibits a MIC$_{S.aureus}$ value of 625 μg·mL^{-1} (189 μmol·L^{-1}), while the respective conjugate modified at the amine group (Me-PMOx-N-CIP) shows MIC$_{S.aureus}$ 1250 μg·mL^{-1} (260 μmol·L^{-1}). These results show that direct conjugation of CIP with PMOx is in contrast to the literature for low molecular weight groups not dependent on the modified functional group. Nevertheless, conjugation drastically lowers the antimicrobial activity of the antibiotic CIP. The biological activity of CIP is the inhibition of the intracellular enzyme gyrase.[46] The reduced activity of the PMOx-CIP conjugates could be caused by a reduced affinity to the enzyme or lowered diffusion ability into the bacterial cell. Thus, alternative PMOx-CIP conjugates that have a spacer between the antibiotic and the polymer were synthesized (Me-PMOx-EDA-CIP, see Scheme 2). Several conjugates with different PMOx lengths were prepared and tested against *S. aureus*. As seen in Figure 7, the Me-PMOx-EDA-CIP conjugates exhibit a strong antimicrobial activity against this microorganism. The molar activity is linearly increasing with shorter PMOx chain lengths. The molar MIC$_{S.aureus}$ value of Me-PMOx$_{10}$-EDA-CIP is even 5 times lower than that of CIP. This shows that conjugation of CIP with PMOx via a spacer leads to highly active PACs.

The PACs with PMOx$_{10}$ and PMOx$_{30}$ were tested against 4 further clinically relevant ubiquitous bacterial strains, being the Gram positive, caries causing strain, *Streptococcus mutans*, and the three Gram negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

The comparison of the two PACs Me-PMOx$_{30}$-EDA-CIP (Figure 8a) and Me-PMOx$_{10}$-EDA-CIP (Figure 8b) reveals the same trend for the *S. mutans* and for *S. aureus*. The antimicrobial molar activity Me-PMOx$_{10}$-EDA-CIP is 3 times higher than that of the pristine antibiotic (MIC$_{S.mutans}$ = 0.3 μmol·L^{-1}). A different picture is found for the Gram negative bacterial strains. In all cases, the conjugates show a drastically lower activity than CIP against the respective bacterial strain. While the conjugates show a very good antimicrobial activity against *E. coli* after an evaluation standard of Fortuniak et al.,[47] the conjugates are only moderately active against *P. aeruginosa* and *K. pneumoniae*.

In order to judge the influence of the nature of the polymer backbone on the activity of the CIP-PACs, conjugates with poly(2-ethyloxazoline) and poly(ethylene glycol) were prepared accordingly. The MIC values of these conjugates shown in Figure 8c−d are generally lower than those of the respective Me-PMOx$_{30}$-EDA-CIP but show a similar trend for the different bacterial strains. Thus, the conjugates exhibit excellent activity against *S. aureus, S. mutans*, and *E. coli*. The very good activity of Me-PEG$_{45}$-EDA-CIP against *K. pneumoniae* is rather surprising. Altogether, the data clearly show that the nature of the polymer backbone strongly influences the antimicrobial activity of the CIP conjugates. Generally, the activity decreases in the order PEG > PEtOx > PMOx. The greatest influence was found for *K. pneumoniae*. The MIC$_{K.pneumoniae}$ is 143 μmol·L for Me-PMOx$_{30}$-EDA-CIP, 37 μmol·L for Me-PEtOx$_{30}$-EDA-CIP, and 4.47 μmol·L for Me-PEG$_{45}$-EDA-CIP.

We hypothesize that the reason for the higher molar antimicrobial activity of the CIP conjugates compared to pristine CIP is a higher binding constant for bacterial gyrase. Therefore, the attached polymer chain might additionally hinder ATP binding to the enzyme.
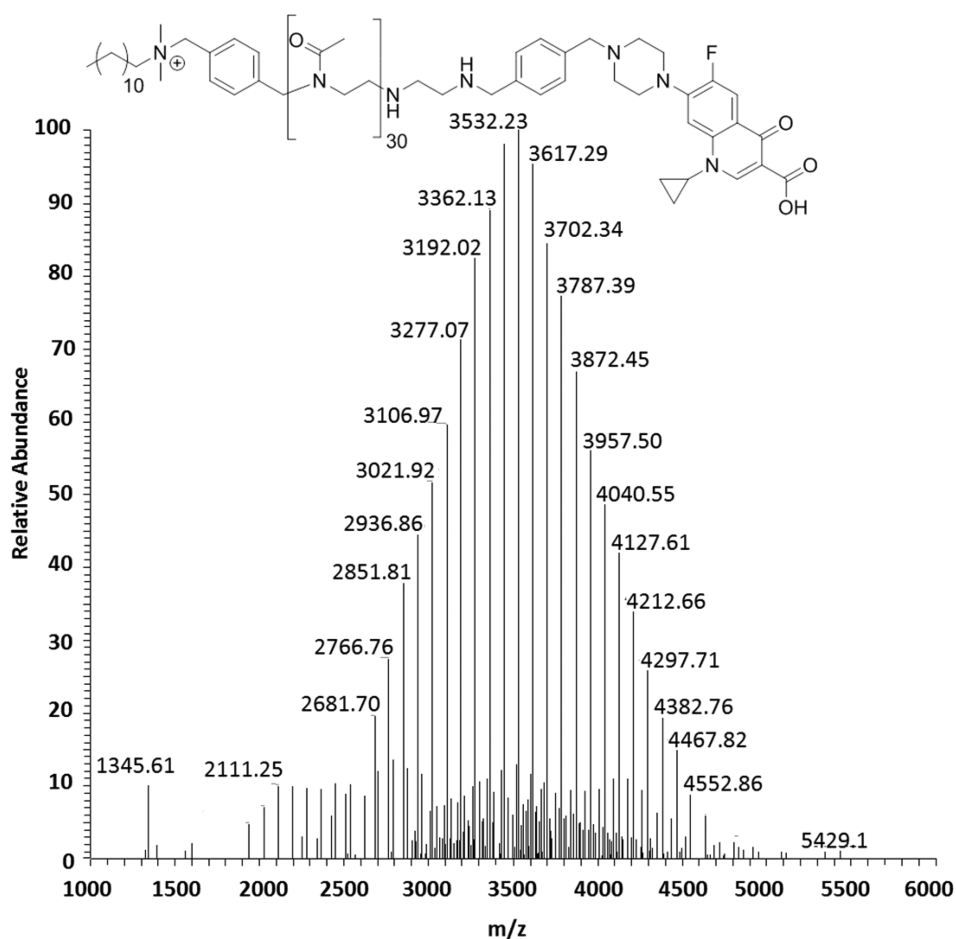
Figure 6. ESI-MS of DDA-X-PMOx-EDA-CIP.

Table 1. Analytic Data of the PACs, Characterized by ^{1}H NMR Spectroscopy and ESI-MS

polymer	DP_{set}[a]	DP_{NMR}[b]	$M_{n,NMR}$ [g·mol^{-1}]	F^d_{NMR}[c]	DP_{esi}	$M_{n,ESI}$ [g·mol^{-1}]	F^d_{ESI}[d]	PDI_{ESI}[e]
Me-PMOx$_{30}$-EDA-CIP	30	32	3200	> 99%	31	3100	80%	1.03
DDA-X-PMOx$_{30}$-EDA-CIP	30	43	4300	> 99%	31	3400	85%	1.03
Me-PMOx$_{30}$-CIP	30	36	3400	86%	35	3200	78%	1.03
Me-PMOx$_{30}$-CIP-Boc	30	25	2600	54%	35	3400	75%	1.02
Me-PMOx$_{30}$-O-CIP	30	46	4341	90%	34	3300	54%	1.06

[a]Degree of polymerization set by the ratio of monomer to initiator according to [initiator] = [monomer]·DP_{set}^{-1}. [b]Degree of polymerization determined by ^{1}H NMR spectroscopy via comparison of the respective signals caused by the initiating group and the signals caused by the protons of the polymer backbone. [c]Degree of functionalization determined by ^{1}H NMR spectroscopy via comparison of the respective signals caused by the initiating and the terminal CIP groups. [d]Degree of functionalization determined by ESI-MS. The targeted molecular weights were rated by their intensity with respect to the intensity sum of all obtained molecular weight peaks. [e]Calculated from the data of the ESI-MS by rating all obtained peaks with their corresponding intensity.

As shown previously, the group distal to the biocidal end group, the satellite group, of a polymer biocide conjugate is dramatically influencing the activity of the whole macromolecule.[39] In order to investigate if this is also the case for CIP PACs, the biocidal group DDA-X (compare Scheme 3) was introduced as satellite group for the PMOx-CIP conjugate. The MIC values of the DDA-X-PMOx-EDA-CIP diagrammed in Figure 8e and f show that the DDA-X group does not strongly influence the antimicrobial activity of the conjugates for most bacterial strains. This might be due to the fact that CIP is an enzyme inhibitor and that satellite groups are not influencing those as previously shown in the example of polymeric collagenase inhibitors.[48] Further, there might not be a synergistic effect of quaternary ammonium compounds and CIP because of

the distant sites of action in the bacterial cell. The only significant influence was found for *E. coli*, where the DDA-X group increased the activity of the conjugates by a factor of 3 to 16.

Additionally, the hemocompatibility of the prepared PACs was explored. Therefore, the hemolytic concentration at which 50% of the porcine red blood cells (HC_{50}) are lysed was determined. All synthesized PACs were found to have HC_{50} values above 5000 μg·mL^{-1} indicating low hemocytotoxicity (Table S.1).

■ CONCLUSIONS

The goal of this work was to investigate permanently bound polymer antibiotic conjugates of CIP and different hydrophilic polymers. It could be shown that direct conjugation of CIP by using the latter as termination reagent of the cationic ring
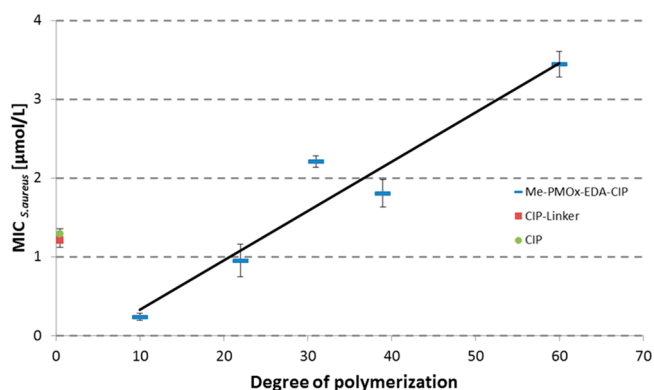
Table 2. Analytical Data of PACs, Which Were Characterized by ^{1}H NMR Spectroscopy

polymer	DP_{set}[a]	DP_{NMR}[b]	$M_{n,NMR}$ [g·mol^{-1}]	F^d_{NMR}[c]
Me-PMOx$_{30}$-CIP-OEt	30	53	4900	70%
Me-PMOx$_{30}$-N-CIP	30	52	4800	54%
Me-PMOx$_{10}$-EDA-CIP	10	12	1300	>99%
DDA-X-PMOx$_{10}$-EDA-CIP	10	10	1600	87%
Me-PMOx$_{20}$-EDA-CIP	20	22	2400	>99%
Me-PMOx$_{40}$-EDA-CIP	40	39	3800	>99%
Me-PMOx$_{60}$-EDA-CIP	60	58	5400	87%
Me-PEtOx$_{30}$-EDA-CIP	30	34	3800	93%
Me-PEG$_{45}$-EDA-CIP	45	46	2600	90%

[a]Degree of polymerization was set by the ratio of monomer to initiator according to [initiator] = [monomer]·DP_{set}^{-1}. [b]Degree of polymerization determined by ^{1}H NMR spectroscopy via comparison of the respective signals caused by the initiating group and the signals caused by the protons of the polymer backbone. [c]Degree of functionalization determined by ^{1}H NMR spectroscopy via comparison of the respective signals caused by the initiating and the terminal CIP groups.

Figure 7. Degree of polymerization against $MIC_{S.aureus}$ value [μmol·L^{-1}] for Me-PMOx-EDA-CIP.

opening polymerization of 2-methy-2-oxazoline is successful but leads to lowly antimicrobial conjugates. Functional group selective termination of PMOx resulted in poorly active conjugates as well, indicating that the conjugation of CIP with hydrophilic polymers might not be a promising approach. Conjugates of CIP and PMOx prepared via a spacer showed high antimicrobial activity, which are dependent on the molecular weight of the polymer, even exceeding the activity of pristine CIP. Additionally prepared conjugates with poly(2-ethyloxazoline) (PEtOx) as well as PEG revealed a strong dependence of the CIP conjugate activity increasing in the order PMOx < PEtOx < PEG. The introduction of the biocidal satellite group DDA-X did not strongly improve activity for most bacterial strains.

It could be shown that permanent conjugation of hydrophilic polymers and antibiotics via the polymer end group is a promising approach toward antibiotics with different activity patterns for various bacterial strains. Therefore, the coupling strategy, including the use of spacers, as well as the nature and the

length of the macromolecules play a crucial role. So far, a satellite group effect could not be found when using CIP. Future work will be dedicated to further optimize this system regarding a more suited spacer and exploring the properties of the conjugates with respect to biodegradability, blood plasma half life, and building up of bacterial resistances. Additional antibiotics will be conjugated as well.

■ EXPERIMENTAL PROCEDURES

Materials. All reactions, purifications, and polymerizations were carried out under an inert atmosphere. Chloroform (AppliChem) was distilled under reduced pressure from aluminum oxide (Merck) and stored in a flask with 4 Å molecular sieves. N,N-dimethylformamide (VWR) and acetonitrile (Merck) were distilled from diphosphorus pentoxide (VWR), then from potassium carbonate (VWR) and stored over 3 Å molecular sieves. The water content was determined by Karl Fischer titration (<0.5 ppm). The monomers 2-methyl-2-oxazoline and 2-ethyl-2-oxazoline (MOx, EtOx, Sigma-Aldrich) were distilled from CaH$_2$ (ABCR). N,N-dimethyldodecylamine (DDA, Sigma-Aldrich) and ethylene diamine (EDA, ABCR) were distilled under reduced pressure. α,α'-Dibromo-p-xylene (ACROS, TCI) was recrystallized in chloroform. Ciprofloxacin (Alfa aesar, Sigma-Aldrich, TCI), α,α'-dichloro-p-xylene (TCI

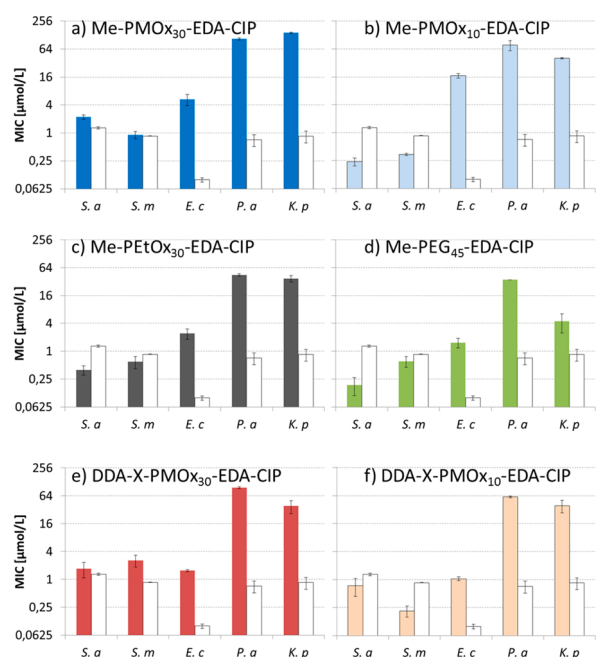## Scheme 4. Synthesis Route Used for the Preparation of Me-PEG-EDA-CIP
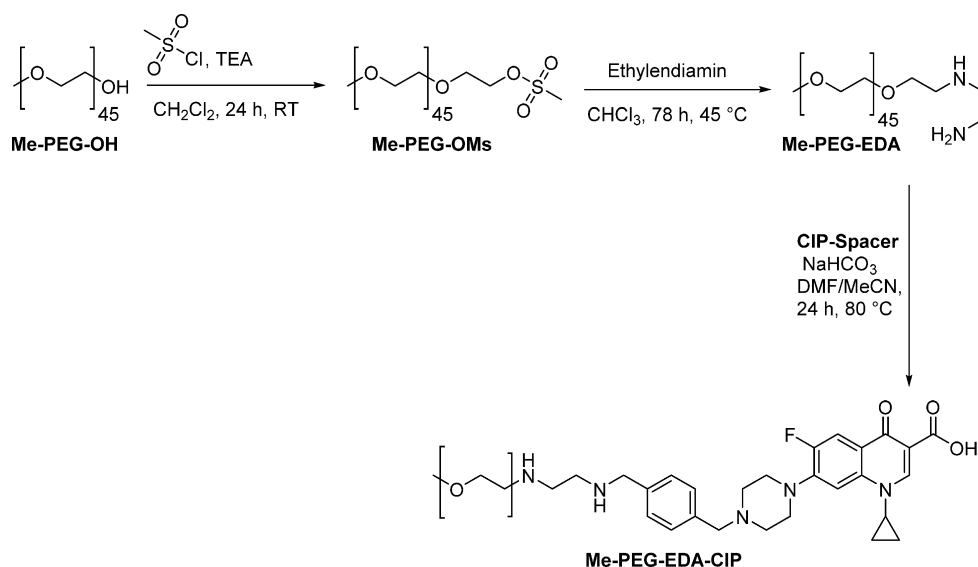
Figure 8. Molar MIC values for the different PACs (a−f), against *S. aureus* (*S.a.*), *S. mutans* (*S.m.*), *E. coli* (*E.c.*), *P. aeruginosa* (*P.a.*), and *K. pneumoniae* (*K.p.*) in comparison to the molar MIC value of CIP against each strain (white columns). All measurements were performed in triplicate, and the error bars are the standard deviation. (Compare to Table 4 in Experimental Procedures).

Europa), 2,3,5-triphenyltetrazolium chloride (AppliChem), polyethylene glycol 2000 monomethyl ether (Fluka), mesyl chloride (Sigma-Aldrich), sodium hydrogen carbonate (Merck), sodium chloride (Fischer), sodium dihydrogen phosphate dihydrate (Merck), sodium hydroxide (Merck), sodium citrate (Sigma-Aldrich), citric acid monohydrate (Sigma-Aldrich), glucose monohydrate (Sigma-Aldrich), hydrochloric acid (VWR), acetic acid (VWR), formic acid (Merck), and nutrient broth (ISO, APHA, VWR) were used without further purifications. The germs *Escherichia coli* (Gram-negative, ATCC 25922), *Klebsiella pneumoniae* (Gram-negative, ATCC 13883), *Pseudomonas aeruginosa* (Gram-negative, ATCC 17423), *Staphylococcus aureus* (Gram-positive, ATCC 25323), and *Streptococcus mutans* (Gram-positive, ATCC 25175) were provided by the German Resource Center for Biological Material (DSMZ). Fresh porcine blood was provided by a local butcher shop and immediately implemented to erythrocyte concentrates.

Measurements. ^{1}H NMR spectra were recorded in deuterated solvents (CDCl$_3$, DMSO-d_6) using FT-appliances of Bruker, types DPX-300 (300 MHz), DRX-400 (400 MHz), DRX-500 (500 MHz), or FT-appliances of Varian type Inova 500 (500 MHz). The residual protons of the not fully deuterated solvents served as an internal standard.

The mass spectrometry measurements were performed on a Thermo LTQ-FT-ICR-Ultra mass spectrometer (linear iron trap−Fourier transform ion cyclotron resonance mass spectrometer). The polymer samples were dissolved in distilled water with 30 vol % acetonitrile and 0.1 vol % formic acid. The polymer samples were measured in a concentration of 10 pmol·μL^{-1}. For direct infusion, a 5 μL sample was injected using a Chip-A-384 emitter, (with 5 μm i.d.), resulting in a flow rate of ∼200 nL min^{-1}. Nominal nitrogen back pressure and ESI voltage were adapted to the respective analyte, varying between 0.4 and 1.4 psi and 1.8 kV, respectively. The profile mass spectra were obtained

as full scan data in positive mode, by accumulation of 2 μscans in the mass range m/z 225−2000 with a resolution of 100.000 (at m/z 400), AGC target value 1×10^6. In the range of m/z 600−1400, the obtained mass spectra contained charges up to 12. Signals were deconvoluted using Xtract RAW. Polymer distribution was determined based on the monoisotopic peaks.

The calculation of the theoretical molecular masses was performed by using the following isotopes: ^{12}C = 12.000 g·mol^{-1}, ^{16}O = 15.995 g·mol^{-1}, ^{14}N = 14.003 g·mol^{-1}, ^{1}H = 1.008 g·mol^{-1}, ^{79}Br = 78.918 g·mol^{-1}, ^{1}H$^+$ = 1.0072766 g·mol^{-1}, and ^{19}F = 18.998 g·mol^{-1}. The UV/vis measurements were recorded on a double-beam spectrophotometer Specord 210 of the Analytik Jena company by 25 °C.

Syntheses. *4-(Bromomethyl)-N-dodecyl-N,N-dimethyl-benzene Ammonium Bromide (DDA-X).* A suspension of α,α'-dibromo-*p*-xylene (5.00 g, 18.94 mmol, 1 equiv) in chloroform (15 mL) was stirred, and a solution of *N,N*-dimethyldodecylamine (DDA; 4.04 g, 18.94 mmol, 1 equiv) in chloroform (7.5 mL) was added dropwise at 42 °C for 4 h. The mixture was stirred for another 30 min at this temperature. The solvent was concentrated to one-third of its initial volume. The residue was stored at 0 °C for 6 h, and the unreacted α,α'-dibromo-*p*-xylene was crystallized and filtered off. Chloroform was removed in vacuum, and tetrahydrofuran (150 mL) was added. The byproduct was precipitated and was removed by filtration. The solution was concentrated in vacuum to give the highly viscous product (5.48 g, 13.78 mmol, 77%). ^{1}H NMR (300 MHz, chloroform-*d*) δ = 0.69−0.95 (t, *J* = 6.8 Hz 3 H), 1.06−1.46 (m, 18 H), 1.58−1.90 (m, 2 H), 3.28 (s, 6 H), 3.41−3.57 (m, 2 H), 4.57 (s, 2 H), 5.15 (s, 2 H), 7.43 (d, *J* = 8.42 Hz, 2 H), 7.68 (d, *J* = 8.42 Hz, 2 H) ppm.

7-(4-(4-(Chloromethyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquino-line-3-carboxylic Acid (CIP-Spacer). α,α'-Dichloro-*p*-xylene (875 mg, 5.00 mmol, 5 equiv) and NaHCO$_3$ (168 mg, 2 mmol, 2 equiv) were dissolved in a mixture of *N,N*-dimethylformamide and acetonitrile (1:1, 4 mL). Ciprofloxacin (331 mg, 1.00 mmol, 1 equiv) was added to the solution at room temperature. The reaction mixture was stirred at 80 °C for 24 h. The product was precipitated in Et$_2$O, the supernatant was decanted, and the solid residue was purified by column chromatography (SiO2; CH$_2$Cl$_2$/MeOH = 20:1) to get the product as slightly yellow solid (230 mg, 0.489 mmol, 49%). R_f = 0.48.

^{1}H NMR (300 MHz, chloroform-*d*) δ = 1.15−1.25 (m, 2 H), 1.32−1.44 (m, 2 H), 2.69 (t, *J* = 4.80 Hz, 4 H), 3.36 (t, *J* = 4.80 Hz, 4 H), 3.52 (td, *J* = 6.77, 2.93 Hz, 1 H), 3.61 (s, 2 H), 4.61 (s, 2 H), 7.31−7.42 (m, 5 H), 8.02 (d, *J* = 13.17 Hz, 1 H) 8.77 (s, 1 H) ppm.

7-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid (CIP-Boc). To a suspension of ciprofloxacin (4.00 g, 12.08 mmol, 1 equiv) in 140 mL of dry dichloromethane, triethylamine (5.02 mL, 36.24 mmol, 3.00 equiv) and di-*tert*-butyl dicarbonate (3.10 mL, 14.50 mmol, 1.20 equiv) were added at room temperature and stirred for 12 h. After cooling to −5 °C, the suspension was filtered. The collected precipitate was dissolved in 200 mL of dichloromethane and washed with an aqueous solution of acetic acid (0.3 M, 100 mL). The solution was dried with sodium sulfate, filtered, and concentrated in vacuum to get the crude product. The product was purified by recrystallization in a mixture of dichloromethane and hexane (300:100 mL) to get CIP-Boc as a yellow solid (1.031 g, 2.39 mmol, 20%).

^{1}H NMR (400 MHz, chloroform-d) δ = 1.19−1.22 (m, 2H), 1.38−1.42 (m, 2H), 1.50 (s, 9H), 3.29−3.31 (m, 4H), 3.55 (tt, 1H, J = 7.1 Hz, J = 3.3 Hz), 3.66−3.67 (m, 4H), 7.35 (d, 1H, J = 7.1 Hz), 7.95 (d, 1H, J = 12.9 Hz), 8.70 (s, 1H).

Ethyl 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (CIP-OEt). To an ice-cooled suspension of ciprofloxacin (2.00 g, 6.04 mmol, 1 equiv) and 50 mL of ethanol, thionyl chloride (8.76 mL, 120.80 mmol, 20 equiv) was added dropwise. After 15 h under reflux, the solution was concentrated under reduced pressure. The resulting solid was dissolved in an aqueous solution of potassium carbonate and extracted with dichloromethane three times. The collected organic phases were dried with sodium sulfate, and the solvent was removed under reduced pressure. The crude product was recrystallized in acetonitrile to get CIP-OEt as a white solid (1.41 g, 3.92 mmol, 65%).

^{1}H NMR (400 MHz, chloroform-d) δ = 1.12−1.16 (m, 2H), 1.32 (q, 2H, J = 6.4 Hz), 1.41 (t, 3H, J = 7.0 Hz), 3.09−3.11 (m, 4H), 3.23−3.26 (m, 4H), 3.43 (dt, 1H, J = 7.0, J = 3.3 Hz), 4.39 (dt, 2H, J = 7.2 Hz), 7.26−7.28 (m, 1H), 8.04 (d, 1H, J = 13.3 Hz), 8.54 (s, 1H) ppm.

Methoxy-poly(ethylene glycol)-mesylate (Me-PEG-OMs). Polyethylene glycol 2000 monomethyl ether (4.00 g, 2.00 mmol, 1.00 equiv) was dried under reduced pressure at 100 °C for 2 h. The dry polymer was dissolved in 100 mL of dichloromethane, and triethylamine (2.77 mL, 20.00 mmol, 10.0 equiv) was added. The reaction mixture was cooled to 0 °C, and mesyl chloride (1.48 mL, 20.00 mmol, 10.0 equiv) was slowly added. After stirring for 24 h at room temperature, the solvent was removed. The residue was dissolved in water and extracted with dichloromethane (3 × 50 mL). The collected organic phases were precipitated in diethyl ether. The precipitating step was performed three times to get Me-PEG-OMs as a white solid (2.39 g, 1.15 mmol, 58%).

^{1}H NMR (500 MHz, chloroform-d) δ = 3.04 (s, 3H) 3.33 (s, 3H) 3.42−3.78 (m, 168 H) 4.30−4.36 (m, 2 H) ppm.

Methoxy-poly(ethylene glycol)-ethylene Diamine (Me-PEG-EDA). Me-PEG-OMs (208 mg, 0.10 mmol, 1.00 equiv) was dissolved in 5 mL of chloroform. Ethylene diamine (66.66 μL, 1.00 mmol, 10.0 equiv) was added to the stirred solution, and the reaction mixture was stirred for 48 h at 78 °C. The polymeric raw product was precipitated in diethyl ether and dialyzed against distilled water for 24 h using benzoylated cellulose membranes (1000 MWCO). The water was removed by lyophilization, and purified Me-PEG-EDA was obtained in a yield of 85% (170 mg, 0.083 mmol, 83%).

^{1}H NMR (500 MHz, chloroform-d) δ = 2.43−2.98 (m, 6 H) 3.36 (s, 3 H) 3.62 (s, 182 H) ppm.

General Procedure for the Polymerization of Polymers with 30 Repeating Units. The required concentration of the initiator (DDA-X or methyl tosylate) was calculated with the start-concentration of monomer and the desired degree of polymerization DP_{set} according to [initiator] = [monomer]·DP_{set}^{-1}. The initiator (DDA-X; 0.149 g or methyl tosylate; 0.059 g, 0.375 mmol, 1 equiv) was dissolved in chloroform (5 mL) at room temperature, and the monomer was added (2-methyl-2-oxazoline; 1.00 g or 2-ethyl-2-oxazoline; 1.11 g, 11.25 mmol, 30 equiv) to the solution. The polymerization was conducted in a CEM Discover synthesis microwave. The reaction temperature was monitored with a vertically focused IR temperature sensor. The vessels were heated (110 °C for DDA-X and 90 °C for methyl tosylate) using the maximum available power (300 W) under magnetic stirring. After reaching the desired temperature, the

power was reduced to a basis level maintaining the target temperature, and the vessels were cooled by compressed air shocks within the next 2 to 4 min to maintain at the adjusted temperature. The corresponding reaction times varied with the DP_{set} between 1 and 5.5 h.

Termination with Ethylene Diamine. A 10 fold molar excess (based on the initiator molarity) of ethylene diamine was added to the living polymer chains and heated to 45 °C for 72 h.

Termination with CIP or CIP-Derivatives. For functionalization with CIP, a 10 fold molar excess (based on the initiator amount) was used, and the mixture was heated to 70 °C for 48 h.

Purification. The raw polymeric product was precipitated in diethyl ether and dialyzed against distilled water for 24 h using benzoylated cellulose membranes (1000 MWCO). The water was removed by lyophilization, and purified polymers were obtained in yields of 69%−93%.

Purifications of Direct CIP Terminated Polymers. After precipitation of the polymers in diethyl ether, they were suspended in water and stirred for 12 h. Then, the suspension was filtered with a 0.2 μm syringe filter, and the filtrate was lyophilized to get the purified polymer in yields of 33%−97%.

DDA-X-PMOx$_{30}$-EDA: ^{1}H NMR (500 MHz, DMSO-d_6) δ = 0.72−0.93 (m, 3 H), 1.25 (br. s, 18 H), 1.67−2.16 (m, 117 H,) 2.51−2.72 (m, 6 H), 2.95 (br. s, 6 H), 3.08−3.71 (m, 165 H), 4.37−4.74 (m, 4 H), 7.04−7.84 (m, 4 H) ppm.

Me-PMOx$_{30}$-EDA: ^{1}H NMR (500 MHz, chloroform-d) δ = 1.74−2.20 (m, 81 H), 2.66−2.89 (m, 4 H), 2.91−3.02 (m, 3H), 3.10−3.72 (m, 112 H) ppm.

Me-PMOx$_{30}$-CIP: ^{1}H NMR (400 MHz, chloroform-d) δ = 1.37 (d, J = 6.53 Hz, 3 H) 1.90−2.34 (m, 110 H) 2.96−3.07 (m, 3 H) 3.20−3.62 (m, 141 H) 7.11 (d, J = 7.78 Hz, 1 H) 7.68 (d, J = 8.03 Hz, 1 H) 8.71 (s, 1 H) ppm.

Me-PMOx$_{30}$-CIP-Boc: ^{1}H NMR (400 MHz, chloroform-d) δ = 1.11 (br. s, 2 H) 1.44 (s, 4 H) 1.82−2.36 (m, 75 H) 2.67 (br. s,

Table 3. Used Bacteria Strains and Their Incubation Conditions

bacteria strain	DSM	ATCC	Gram straining	incubation conditions
Escherichia coli	1103	25922	negative	nutrient broth, pH 6.8, 37 °C
Klebsiella pneumoniae	30104	13883	negative	nutrient broth, pH 7.0, 37 °C
Pseudomonas aeruginosa	50078	17423	negative	nutrient broth, pH 7.0, 30 °C
Staphylococcus aureus	1104	25923	positive	nutrient broth, pH 7.3, 37 °C
Streptococcus mutans	20523	24178	positive	nutrient broth, pH 7.3, 37 °C

2 H) 2.97−3.10 (m, 3 H) 3.14−3.78 (m, 102 H) 4.31−4.44 (m, 1 H) 7.11 (d, J = 8.03 Hz, 1 H) 7.67 (d, J = 8.03 Hz, 1 H) 8.43−8.57 (m, 1 H) ppm.

Me-PMOx$_{30}$-CIP-OEt: ^{1}H NMR (400 MHz, chloroform-d) δ = 1.04−1.31 (m, 3 H) 1.34 (t, J = 7.03 Hz, 2 H) 1.81−2.29 (m, 162 H) 2.95−3.04 (m, 3 H) 3.13−3.61 (m, 200 H) 4.25−4.36 (m, 1 H) 7.06−7.13 (m, 1 H) 7.56−7.68 (m, 1 H) 8.48 (s, 1 H) ppm.

Me-PEtOx$_{30}$-EDA: ^{1}H NMR (300 MHz, chloroform-d) δ = 1.11 (br. s, 94 H) 2.14−2.49 (m, 61 H) 2.59 (br. s, 6 H) 3.02 (s, 3 H) 3.28−3.78 (m, 120 H) ppm.

General Procedure of CIP-Linking. Ciprofloxacin-derivate (93.8 mg, 2.00 mmol, 2 equiv) and NaHCO$_3$ (16.8 mg, 2.00 mmol, 2 equiv) were suspended in a mixture of *N,N*-

Table 4. MIC Tests in μmol·L^{-1} against Different Germs, Two Gram-Positive Strains (*S. aureus* and *S. mutans*) and Three Gram-Negative Strains (*E. coli*, *P. aeruginosa*, and *K. pneumonia*)[a]

	MIC [μmol·L^{-1}]				
samples	*S.a.*	*S.m.*	*E.c.*	*P.a.*	*K.p.*
Me-PMOx$_{10}$-EDA-CIP	0.2 ± 0.1	0.3 ± 0.02	17 ± 2	78 ± 19	41 ± 1.5
Me-PMOx$_{30}$-EDA-CIP	2.2 ± 0.2	0.9 ± 0.2	5.3 ± 1.5	105 ± 5	143 ± 6.1
DDA-X-PMOx$_{10}$-EDA-CIP	0.7 ± 0.3	0.2 ± 0.05	1.0 ± 0.1	60 ± 2.5	39 ± 11.7
DDA-X-PMOx$_{30}$-EDA-CIP	1.7 ± 0.6	2.6 ± 0.7	1.5 ± 0.1	96 ± 4.1	38 ±. 11.9
Me-PEtOx-EDA-CIP	0.4 ± 0.1	0.6 ± 0.2	2.4 ± 0.6	44 ± 2.5	37 ± 5.9
Me-PEG-EDA-CIP	0.2 ± 0.1	0.6 ± 0.2	1.5 ± 0.4	35.0 ± 0.01	4.5 ± 2
CIP	1.3 ± 0.1	0.9 ± 0.01	0.1 ± 0.01	0.7 ± 0.2	0.6 ± 0.2
CIP-spacer	1.2 ± 0.1	4.3 ± 0.9	5.1 ± 2.4	10 ± 0.2	12 ± 5

[a]Additional MIC values for all other synthesized polymers in μmol·L^{-1} and in μg·mL^{-1} are given in Tables S2−S3.

dimethylformamide and acetonitrile (1:1, 4 mL). The polymer (DDA-X-PMOx$_{30}$-EDA; 300 mg, Me-PMOx$_{30}$-EDA; 219 mg, Me-PEtOx$_{30}$x-PMOx$_{30}$-EDA; 305 mg, Me-PEG$_{45}$-EDA; 205 mg, 0.10 mmol, 1 equiv) was added to the suspension at room temperature. The reaction mixture was stirred at 80 °C for 24 h. The product was precipitated in Et$_2$O, and the supernatant was decanted. The residue was dissolved in water, and polymers were dialyzed in membranes of Roth (ZelluTrans) with a molecular cutoff of 2000 g·mmol^{-1}. Polymers with a lower molecular weight were dialyzed two times in membranes with a cutoff of 1000 g·mmol^{-1}. The water was removed by lyophilization, and the purified polymers were obtained in yields of 45%−85%.

DDA-X-PMOx$_{30}$-EDA-CIP: ^{1}H NMR (400 MHz, chloroform-*d*) δ = 0.80 (t, *J* = 6.78 Hz, 3 H), 0.99−1.25 (m, 22 H), 1.65−2.28 (m, 128 H), 2.60 (br. s., 17 H), 3.00−3.76 (m, 206 H), 4.39−5.39 (m, 6 H), 7.27 (s, 8 H), 7.56 (m, 1H), 7.85 (m, 1 H) 8.56 (m, 1 H) ppm.

Me-PMOx$_{30}$-EDA-CIP: ^{1}H NMR (400 MHz, chloroform-*d*) δ = 1.18 (br. s., 2 H), 1.35 (br. s., 2 H), 1.70−2.30 (m, 79 H), 2.54−2.86 (m, 8 H), 3.01−3.05 (m, 2 H), 3.24−3.72 (m, 107 H), 7.23−7.33 (m, 6 H), 7.89−7.99 (m, 1 H) 8.65−8.74 (m, 1 H) ppm.

Me-PEtOx$_{30}$-EDA-CIP: ^{1}H NMR (500 MHz, chloroform-*d*) δ = 0.90−1.29 (m, 98 H) 1.36 (d, *J* = 6.50 Hz, 2 H) 2.07−2.47 (m, 70 H) 2.65 (br. s., 8 H) 2.96−3.09 (m, 3 H) 3.22−3.79 (m, 135 H) 7.19−7.41 (m, 6 H) 7.98 (d, *J* = 13.00 Hz, 1 H) 8.73 (s, 1 H) ppm.

Me-PEG$_{45}$-EDA-CIP: ^{1}H NMR (500 MHz, chloroform-*d*) δ = 1.10−1.20 (m, 2 H) 1.32−1.41 (m, 2 H) 2.55−2.72 (m, 8 H) 3.28−3.36 (m, 4 H) 3.37 (s, 3 H) 3.46−3.80 (m, 173 H) 7.29 (s, 7 H) 7.84−8.01 (m, 1 H) 8.65−8.75 (m, 1 H) ppm.

Deprotection of the Boc-Group. The protecting group was cleaved of from Me-PMox$_{30}$-CIP-Boc (260 mg, 0.076 mmol, 1 equiv) with a mixture of dichloromethane and trifluoroacetic acid (TFA) (1:1 vol %, 4 mL). The raw polymeric product was purified as described above. The raw polymeric product was precipitated in diethyl ether and dialyzed against distilled water for 24 h using benzoylated cellulose membranes (1000 MWCO). The water was removed by lyophilization.

^{1}H NMR (400 MHz, chloroform-*d*) δ = 1.02−1.14 (m, 2 H) 1.30 (br. s., 2 H) 1.75−2.27 (m, 135 H) 2.95−3.08 (m, 3 H) 3.41 (m, 168 H) 4.28−4.46 (m, 1 H) 7.11 (d, *J* = 7.78 Hz, 1 H) 7.64 (d, *J* = 7.78 Hz, 1 H) 8.43−8.60 (m, 1 H) ppm.

Hydrolysis of Ethyl Ester. Two hundred milligrams (0.041 mmol, 1 equiv) of the respective polymer was dissolved in 10 mL of 0.015 M aqueous sodium hydroxide solution and stirred for 12 h at 50 °C. After cooling to room temperature, the reaction mixtures were neutralized with 1 M HCl and lyophilized. The

dried polymers were suspended in CHCl$_3$ and filtered by a 0.45 μm PTFE filter and precipitated in Et$_2$O. The polymers were resolved in CHCl$_3$, and the solvent was evaporated.

^{1}H NMR (400 MHz, chloroform-*d*) δ = 1.13−1.39 (m, 2 H) 2.01 (m, 163 H) 2.98 (m, 3 H) 3.39 (m, 204 H) 7.08 (m, 1 H) 7.59 (m, 1 H) 8.69 (s, 1 H) ppm.

Bacterial Susceptibility: Minimal Inhibitory Concentration (MIC). Stock solutions of the respective bacterial strains were prepared from a stock pellet from DSMZ. The stock pellet of the respective bacterial strain was suspended in 50 mL of nutrient both (standard 1, 25 g in 1 L distilled water) and incubated under the conditions given in Table 3 for 24 h. The confluent medium was centrifuged (3000 rpm, 10 min), and the supernatant was decanted. The residue was suspended in 50 mL of sterile PBS buffer (8.77 g NaCl, 1.56 g NaH$_2$PO$_4$ 2H$_2$O, adjusted to pH 7.0 with 0.1 M NaOH) and then centrifuged (3000 rpm, 10 min) again. This step was repeated three times. In the last step, the bacterial residue was suspended in 10 mL of sterile PBS buffer and mixed with 10 mL of a sterile-filtered 50% glycerin-solution. The stock solution was stored at −20 °C.

Fifty microliters of the respective stock solution was added to 25 mL of nutrient broth (standard 1). The solution was incubated for 24 h (For conditions, see Table 3). The bacterial concentration of the suspension was controlled by UV/vis-spectroscopy at 541 nm/25 °C and diluted with nutrient broth to ~10^7 cells mL^{-1}.

A sample of the polymer (5.00 mg) was dissolved in pure nutrient broth (4.00 mL), and a dilution series was prepared with halved concentrations in every following sample. Every probe was incubated with 20 μL of the diluted bacterial stock solution at 37 or 30 °C (see Table 3) for 24 h. Positive and negative controls were incubated with the polymer samples. The MIC was visually observed, and then 100 μL of a 2,3,5-triphenyltetrazolium chloride (TCC)-solution in water (1 mg mL^{-1}) was added. After a further 3 h of incubation, the samples with a bacterial cell concentration >1% showed a red coloration. The MIC was the sample with the lowest polymer concentration without red coloration. The effective molar concentration of the CIP conjugate was calculated from the average molecular weight determined by ^{1}H NMR. The concentration of unmodified CIP was less than 0.05 mol % in all cases according to the respective ESI MS.

Hemocompatibility (HC$_{50}$). Sodium citrate (26.30 g, Sigma Aldrich), citric acid monohydrate (3.27 g, Sigma-Aldrich), sodium dihydrogen phosphate dihydrate (2.51 g, Merck), and glucose monohydrate were dissolved in 1.00 L of distilled water and sterile filtered (0.2 μm). The pH was adjusted to 7.38 with a sterile sodium hydroxide solution (1 M) to give the CPD buffer.

Fresh, nonsalted porcine blood (10 mL) was centrifuged (4000 rpm) for 15 min. The supernatant plasma was then rejected, and the obtained crude erythrocyte sediment was washed (six times) with a sterile, isotonic sodium solution (6.00 g·L^{-1}). The erythrocyte sediment was suspended in a CPD buffer solution (10 mL) after the final washing step. The thus-prepared erythrocyte suspensions were stored at 4 °C for a maximum of 5 days. The polymer samples (40−80 mg) were dissolved in CPD buffer solution (800 µL), and a dilution series was prepared. A small amount of the erythrocyte concentrate was slowly brought to room temperature and added (200 µL) to every sample. The final mixtures have a concentration of approximately 5 × 10^8 red blood cells per mL based on counting with a Neubauer-Improved counting chamber; the test series was incubated for 1 h at 37 °C and then centrifuged (13500 rpm, 5 min). The supernatant liquid was further diluted with CPD buffer (1:20), and the absorbance of the released hemoglobin was measured in a UV/vis spectrometer at 541 nm and 25 °C. The concentration at which 50% of the erythrocytes were destroyed (HC$_{50}$) was determined against a positive control that was treated with 2 µL of Triton X and a negative buffer control containing no active substance.

■ ASSOCIATED CONTENT

ⓈSupporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00393.

> ESI-MS and NMR spectra as well as tables with the antimicrobial and hemotoxical evaluation of different PACS (PDF)

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: joerg.tiller@udo.edu.

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B., and Bartlett, J. (2009) Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis. 48*, 1−12.

(2) Spellberg, B., Powers, J. H., Brass, E. P., Miller, L. G., and Edwards, J. E. (2004) Trends in Antimicrobial Drug Development: Implications for the Future. *Clin. Infect. Dis. 38*, 1279−1286.

(3) Craig, W. A. (2004) Overview of newer antimicrobial formulations for overcoming pneumococcal resistance. *American Journal of Medicine Supplements 117*, 16−22.

(4) Vila, J., Sánchez-Céspedes, J., Sierra, J. M., Piqueras, M., Nicolás, E., Freixas, J., and Giralt, E. (2006) Antibacterial evaluation of a collection of norfloxacin and ciprofloxacin derivatives against multiresistant bacteria. *Int. J. Antimicrob. Agents 28*, 19−24.

(5) Cheng, Y., Qu, H., Ma, M., Xu, Z., Xu, P., Fang, Y., and Xu, T. (2007) Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: An in vitro study. *Eur. J. Med. Chem. 42*, 1032−1038.

(6) López-Cebral, R., Romero-Caamaño, V., Seijo, B., Alvarez-Lorenzo, C., Martín-Pastor, M., Concheiro, Á., Landin, M., and Sanchez, A. (2014) Spermidine Cross-Linked Hydrogels as a Controlled Release Biomimetic Approach for Cloxacillin. *Mol. Pharmaceutics 11*, 2358−2371.

(7) Ghosh, S., Chakraborty, P., Saha, P., Acharya, S., and Ray, M. (2014) Polymer based nanoformulation of methylglyoxal as an antimicrobial agent: efficacy against resistant bacteria. *RSC Adv. 4*, 23251−23261.

(8) Hoste, K., De Winne, K., and Schacht, E. (2004) Polymeric prodrugs. *Int. J. Pharm. 277*, 119−131.

(9) Duncan, R., Gac-Breton, S., Keane, R., Musila, R., Sat, Y. N., Satchi, R., and Searle, F. (2001) Polymer−drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to clinic. *J. Controlled Release 74*, 135−146.

(10) Duncan, R. (2014) Polymer therapeutics: Top 10 selling pharmaceuticals — What next? *J. Controlled Release 190*, 371−380.

(11) Woo, G. L. Y., Mittelman, M. W., and Santerre, J. P. (2000) Synthesis and characterization of a novel biodegradable antimicrobial polymer. *Biomaterials 21*, 1235−1246.

(12) Woo, G. L. Y., Yang, M. L., Yin, H. Q., Jaffer, F., Mittelman, M. W., and Santerre, J. P. (2002) Biological characterization of a novel biodegradable antimicrobial polymer synthesized with fluoroquinolones. *J. Biomed. Mater. Res. 59*, 35−45.

(13) GAC, S., Coudane, J., Boustta, M., Domurado, M., and Vert, M. (1999) Synthesis, Characterisation and In Vivo Behaviour of a Norfloxacin-Poly(L-Lysine Citramide Imide) Conjugate Bearing Mannosyl Residues. *J. Drug Targeting 7*, 393−406.

(14) Sobczak, M., Nałęcz-Jawecki, G., Kołodziejski, W. L., Goś, P., and Żółtowska, K. (2010) Synthesis and study of controlled release of ofloxacin from polyester conjugates. *Int. J. Pharm. 402*, 37−43.

(15) Parwe, S. P., Chaudhari, P. N., Mohite, K. K., Selukar, B. S., Nande, S. S., and Garnaik, B. (2014) Synthesis of ciprofloxacin-conjugated poly (L-lactic acid) polymer for nanofiber fabrication and antibacterial evaluation. *Int. J. Nanomed. 9*, 1463.

(16) Namazi, H., and Kanani, A. (2009) Investigation diffusion mechanism of β-lactam conjugated telechelic polymers of PEG and β-cyclodextrin as the new nanosized drug carrier devices. *Carbohydr. Polym. 76*, 46−50.

(17) Kugel, A., Chisholm, B., Ebert, S., Jepperson, M., Jarabek, L., and Stafslien, S. (2010) Antimicrobial polysiloxane polymers and coatings containing pendant levofloxacin. *Polym. Chem. 1*, 442−452.

(18) Greenwald, R. B., Zhao, H., Xia, J., and Martinez, A. (2003) Poly(ethylene glycol) Transport Forms of Vancomycin: A Long-Lived Continuous Release Delivery System. *J. Med. Chem. 46*, 5021−5030.

(19) Nathan, A., Zalipsky, S., Ertel, S. I., Agathos, S. N., Yarmush, M. L., and Kohn, J. (1993) Copolymers of lysine and polyethylene glycol: a new family of functionalized drug carriers. *Bioconjugate Chem. 4*, 54−62.

(20) Pichavant, L., Bourget, C., Durrieu, M.-C., and Héroguez, V. (2011) Synthesis of pH-Sensitive Particles for Local Delivery of an Antibiotic via Dispersion ROMP. *Macromolecules 44*, 7879−7887.

(21) Řihová, B., Jelínková, M., Strohalm, J., Šubr, V., Plocová, D., Hovorka, O., Novák, M., Plundrová, D., Germano, Y., and Ulbrich, K. (2000) Polymeric drugs based on conjugates of synthetic and natural macromolecules.: II. Anti-cancer activity of antibody or (Fab′)2-targeted conjugates and combined therapy with immunomodulators. *J. Controlled Release 64*, 241−261.

(22) Haag, R., and Kratz, F. (2006) Polymer Therapeutics: Concepts and Applications. *Angew. Chem., Int. Ed. 45*, 1198−1215.

(23) Zhao, Y.-J., Wei, W., Su, Z.-G., and Ma, G.-H. (2009) Poly(ethylene glycol) prodrug for anthracyclines via N-Mannich base linker: Design, synthesis and biological evaluation. *Int. J. Pharm. 379*, 90−99.

(24) Ferguson, E. L., Azzopardi, E., Roberts, J. L., Walsh, T. R., and Thomas, D. W. (2014) Dextrin−Colistin Conjugates as a Model Bioresponsive Treatment for Multidrug Resistant Bacterial Infections. *Mol. Pharmaceutics 11*, 4437−4447.

(25) Du, J., Bandara, H. M. H. N., Du, P., Huang, H., Hoang, K., Nguyen, D., Mogarala, S. V., and Smyth, H. D. C. (2015) Improved Biofilm Antimicrobial Activity of Polyethylene Glycol Conjugated Tobramycin Compared to Tobramycin in Pseudomonas aeruginosa Biofilms. *Mol. Pharmaceutics 12*, 1544−1553.

(26) Lawson, M. C., Shoemaker, R., Hoth, K. B., Bowman, C. N., and Anseth, K. S. (2009) Polymerizable Vancomycin Derivatives for Bactericidal Biomaterial Surface Modification: Structure−Function Evaluation. *Biomacromolecules 10*, 2221−2234.

(27) Bieser, A. M., and Tiller, J. C. (2011) Mechanistic Considerations on Contact-Active Antimicrobial Surfaces with Controlled Functional Group Densities. *Macromol. Biosci. 11*, 526−534.

(28) Siedenbiedel, F., Fuchs, A., Moll, T., Weide, M., Breves, R., and Tiller, J. C. (2013) Star-Shaped Poly(styrene)-block-Poly(4-vinyl-N-methylpyridiniumiodide) for Semipermanent Antimicrobial Coatings. *Macromol. Biosci. 13*, 1447−1455.

(29) Turos, E., Shim, J.-Y., Wang, Y., Greenhalgh, K., Reddy, G. S. K., Dickey, S., and Lim, D. V. (2007) Antibiotic-conjugated polyacrylate nanoparticles: New opportunities for development of anti-MRSA agents. *Bioorg. Med. Chem. Lett. 17*, 53−56.

(30) Sarker, P., Shepherd, J., Swindells, K., Douglas, I., MacNeil, S., Swanson, L., and Rimmer, S. (2011) Highly Branched Polymers with Polymyxin End Groups Responsive to Pseudomonas aeruginosa. *Biomacromolecules 12*, 1−5.

(31) Tauhardt, L., Frant, M., Pretzel, D., Hartlieb, M., Bucher, C., Hildebrand, G., Schroter, B., Weber, C., Kempe, K., Gottschaldt, M., et al. (2014) Amine end-functionalized poly(2-ethyl-2-oxazoline) as promising coating material for antifouling applications. *J. Mater. Chem. B 2*, 4883−4893.

(32) Weber, C., Becer, C. R., Baumgaertel, A., Hoogenboom, R., and Schubert, U. S. (2009) Preparation of Methacrylate End-Functionalized Poly(2-ethyl-2-oxazoline) Macromonomers. *Des. Monomers Polym. 12*, 149−165.

(33) Krumm, C., Konieczny, S., Dropalla, G. J., Milbradt, M., and Tiller, J. C. (2013) Amphiphilic Polymer Conetworks Based on End Group Cross-Linked Poly(2-oxazoline) Homo- and Triblock Copolymers. *Macromolecules 46*, 3234−3245.

(34) Farrugia, B. L., Kempe, K., Schubert, U. S., Hoogenboom, R., and Dargaville, T. R. (2013) Poly(2-oxazoline) Hydrogels for Controlled Fibroblast Attachment. *Biomacromolecules 14*, 2724−2732.

(35) Adams, N., and Schubert, U. S. (2007) Poly(2-oxazolines) in biological and biomedical application contexts. *Adv. Drug Delivery Rev. 59*, 1504−1520.

(36) Waschinski, C. J., Herdes, V., Schueler, F., and Tiller, J. C. (2005) Influence of Satellite Groups on Telechelic Antimicrobial Functions of Polyoxazolines. *Macromol. Biosci. 5*, 149−156.

(37) Fik, C. P., Krumm, C., Muennig, C., Baur, T. I., Salz, U., Bock, T., and Tiller, J. C. (2012) Impact of Functional Satellite Groups on the Antimicrobial Activity and Hemocompatibility of Telechelic Poly(2-methyloxazoline)s. *Biomacromolecules 13*, 165−172.

(38) Waschinski, C. J., Barnert, S., Theobald, A., Schubert, R., Kleinschmidt, F., Hoffmann, A., Saalwächter, K., and Tiller, J. C. (2008) Insights in the Antibacterial Action of Poly(methyloxazoline)s with a Biocidal End Group and Varying Satellite Groups. *Biomacromolecules 9*, 1764−1771.

(39) Krumm, C., Harmuth, S., Hijazi, M., Neugebauer, B., Kampmann, A.-L., Geltenpoth, H., Sickmann, A., and Tiller, J. C. (2014) Antimicrobial Poly(2-methyloxazoline)s with Bioswitchable Activity through Satellite Group Modification. *Angew. Chem., Int. Ed. 53*, 3830−3834.

(40) Konieczny, S., Leurs, M., and Tiller, J. C. (2015) Polymer Enzyme Conjugates as Chiral Ligands for Sharpless Dihydroxylation of Alkenes in Organic Solvents. *ChemBioChem 16*, 83−90.

(41) Kerns, R. J., Rybak, M. J., Kaatz, G. W., Vaka, F., Cha, R., Grucz, R. G., and Diwadkar, V. U. (2003) Structural features of piperazinyl-linked ciprofloxacin dimers required for activity against drug-resistant strains of Staphylococcus aureus. *Bioorg. Med. Chem. Lett. 13*, 2109−2112.

(42) Kerns, R. J., Rybak, M. J., Kaatz, G. W., Vaka, F., Cha, R., Grucz, R. G., Diwadkar, V. U., and Ward, T. D. (2003) Piperazinyl-linked fluoroquinolone dimers possessing potent antibacterial activity against drug-resistant strains of Staphylococcus aureus. *Bioorg. Med. Chem. Lett. 13*, 1745−1749.

(43) Litt, M., Levy, A., and Herz, J. (1975) Polymerization of Cyclic Imino Ethers. X. Kinetics, Chain Transfer, and Repolymerization. *J. Macromol. Sci., Chem. 9*, 703−727.

(44) Chu, D. T., and Fernandes, P. B. (1989) Structure-activity relationships of the fluoroquinolones. *Antimicrob. Agents Chemother. 33*, 131−135.

(45) Tillotson, G. S. (1996) Quinolones: structure-activity relationships and future predictions. *J. Med. Microbiol. 44*, 320−324.

(46) LeBel, M. (1988) Ciprofloxacin: Chemistry, Mechanism of Action, Resistance, Antimicrobial Spectrum, Pharmacokinetics, Clinical Trials, and Adverse Reactions. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 8*, 3−30.

(47) Fortuniak, W., Mizerska, U., Chojnowski, J., Basinska, T., Slomkowski, S., Chehimi, M., Konopacka, A., Turecka, K., and Werel, W. (2011) Polysiloxanes With Quaternary Ammonium Salt Biocidal Functions and Their Behavior When Incorporated Into a Silicone Elastomer Network. *J. Inorg. Organomet. Polym. Mater. 21*, 576−589.

(48) Fik, C. P., Konieczny, S., Pashley, D. H., Waschinski, C. J., Ladisch, R. S., Salz, U., Bock, T., and Tiller, J. C. (2014) Telechelic Poly(2-oxazoline)s with a Biocidal and a Polymerizable Terminal as Collagenase Inhibiting Additive for Long-Term Active Antimicrobial Dental Materials. *Macromol. Biosci. 14*, 1569−1579.