Supplementary Information for

Modulating bioactivities of primary ammonium-tagged antimicrobial aliphatic polycarbonates by varying length, sequence and hydrophobic side chain structure

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Figure S1. SEM images of E. coli treated with R4 (128 μg/mL) (a) and no polymer (b)

(Protocol) The E. coli after incubation with cationic polycarbonate R4 at 128 μg/mL that is the double of the MIC for 24 h were harvested by centrifugation at 4,000 r.p.m. for 5 min. They were washed by PBS three times, and then fixed with a 1% glutaraldehyde solution overnight. The cells were further washed with DI water, followed by dehydration using a series of ethanol solutions with different volume contents (25, 50, 75, and 90%). The sample was placed on a carbon tape, which was further coated with platinum. The morphologies of the bacteria were observed using a field emission scanning electron microscope (Hitachi SU8000) operated at an accelerating voltage of 5.0 kV.
Figure S2. Bacterial growth of *E. coli* treated with different concentrations of polymers as a function of time. MICs (μg/mL): 64 (H1-a), 64 (H1-b), 64 (B1), 32 (R1), 250 (R2), 16 (R3), 64 (R4), 250 (PEI).
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S2. Polymer synthesis with detailed procedure

S2.1. Synthesis of MTC-BAE

![Chemical Structure of MTC-BAE]

In a three-necked 300 ml flask, 2,2-bis(hydroxymethyl)propionic acid (bis-MPA; 5.00 g, 36.5 mmol), bis(pentafluorophenyl)carbonate (PFC; 36.1 g, 91.5 mmol), and cesium fluoride (CsF; 1.11 g, 7.3 mmol) were dispersed in dry THF (115 mL), and the mixture was stirred at room temperature for 20 hours under nitrogen atmosphere. White precipitates were formed with reaction time and removed by filtration. The filtrate was then evaporated and the residue was dissolved in methylene chloride. The solution was cooled overnight at 4 °C to form precipitates as a byproduct pentafluorophenol. After the precipitates were filtered out, the filtrate was washed with saturated NaHCO₃ aq. and brine. The organic layer was then dried over MgSO₄ and evaporated and dried in vacuum. The residue was then recrystallized from toluene to form white crystals as a product (7.11 g, yield 58%).

**¹H-NMR (500 MHz, CDCl₃):** δ 4.86 (d, J = 12 Hz, 2H, CH₃H₃), 4.37 d, J = 12 Hz, 2H, CH₃H₃), 1.57 (s, 3H, CH₃).

S2.1.2. Synthesis of tert-butyl N-(2-hydroxyethyl)carbamate (Boc-AE)

To a solution (70 ml) of 2-aminoethanol (6.00 ml, 100 mmol) in dry methylene chloride, a dry methylene chloride solution (50 ml) of di-tert-butyl dicarbonate (21.8 g, 100 mmol) was gradually added in the nitrogen atmosphere at room temperature, and the mixed solution was kept stirring for 18 h. Afterward, the reaction mixture was then evaporated and dried in vacuum until the weight reaches unchanged. The crude residue was distilled in vacuum (137 °C, 11 torr) to obtain transparent oil as a product (15.0 g, yield 73%). **¹H-NMR (500 MHz, acetone-d₆):** δ 5.90 (br, 1H, NH), 3.555 (q, J = 7.5 Hz, 2H, OCH₂), 3.165 (q, J = 7.5 Hz, 2H, CH₂NH), 1.40 (s, 9H, C(CH₃)₃).

S2.1.3. Synthesis of 2-(tert-butoxycarbonylamino)ethyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate (MTC-BAE)
In a 50 ml flask, MTC-PF (2.0 g, 6.13 mmol), CsF (0.31 g, 2.02 mmol), and Boc-AE (1.01 g, 6.25 mmol) were dissolved in dry THF. The reaction mixture was stirred for 24 h in a nitrogen atmosphere. After precipitates were filtered out, the filtrate was evaporated in a reduced pressure. The residue was then dissolved in ethyl acetate (70 ml) and washed with saturated aqueous solution of NaHCO$_3$ twice, followed by brine twice. The organic layer was then dried over MgSO$_4$ and evaporated and dried in vacuum. The dried residue was recrystallized from a mixed solvent of diethyl ether and hexane (1: 2) to obtain white crystals as a product (1.17 g, yield 63%).

$^1$H-NMR (400 MHz, CDCl$_3$): δ 4.76 (br, 1H, NH), 4.70 (d, 2H, CH$_a$CH$_b$), 4.22 (d, 2H, CH$_a$CH$_b$), 4.28 (t, 2H, OCH$_2$CH$_2$), 3.43 (q, 2H, CH$_2$CH$_2$NH), 1.45 (s, 9H, C(CH$_3$)$_3$), 1.36 (s, 3H, CCH$_3$).

Figure S6. $^1$H NMR spectrum of MTC-BAE (CDCl$_3$, 400 MHz).
S2.2. Ring-opening polymerization (ROP)

S2.2.1. Typical ROP of MTC-BAE: Synthesis of Boc-H

In a nitrogen filled glove box, MTC-BAE (0.606 g, 2.0 mmol), 1-pyrenebutanol (5.5 mg, 0.02 mmol), TU (74.2 mg, 0.21 mmol) and SP (23.9 mg, 0.1 mmol) were stirred in dry CH$_2$Cl$_2$ (1.0 ml) at room temperature for 18 hours. After consumption of more than 90% of monomer was confirmed by $^1$H-NMR, a few drops of acetic anhydride was added and the mixture was stirred overnight. The solution was then reprecipitated into a mixed solvent of hexane and toluene (4: 1), and the precipitates were collected by centrifuge and dried in vacuum at room temperature until the weight became unchanged to give glassy solid as a precursor of H1-a (Boc-H1-a; 0.394 g, yield 65%). $^1$H-NMR (400 MHz,CDCl$_3$): $\delta$ 8.38-7.66 (m, Ar-$H$), 5.19-4.99 (br, NH), 4.23 (m, $CH_2OCOO$), 4.01 (m, OCH$_2$CH$_2$), 3.16 (m, CH$_2$H$_2$NH), 1.99 (s, OCOC$H_3$ end group), 1.36 (s, C(CH$_3$)$_3$), 1.16 (s, CH$_3$). SEC (DMF+0.1M LiBr): $M_n$ 20,000 g/mol, $D_M$ 1.21.

For a precursor of H1-b (Boc-H1-b), MTC-BAE (0.609 g, 2.0 mmol), TU (74.0 mg, 0.20 mmol) and SP (47.8 mg, 0.2 mmol) were used and stirred in dry CH$_2$Cl$_2$ (1.0 ml). No initiator was used (0.493 g, yield 81%). SEC (DMF+0.1M LiBr): $M_n$ 31,000 g/mol, $D_M$ 1.39.

For a precursor of H2 (Boc-H2), MTC-BAE (0.607 g, 2.0 mmol), 1-pyrenebutanol (5.6 mg, 0.02 mmol), TU (74.2 mg, 0.20 mmol) and SP (23.4 mg, 0.1 mmol) were used and stirred in dry CH$_2$Cl$_2$ (2.0 ml). The monomer was pre-dried with 5 wt% (relative to monomer) of CaH$_2$. The pre-drying was carried out in a glove box just before polymerization. MTC-BAE was dissolved in dry CH$_2$Cl$_2$ and CaH$_2$ was added. The mixed solution was stirred for at least 30 min and the CaH$_2$ was removed through a PTFE syringe filter (0.2 µm). The filtrate was directly used for polymerization adding initiator and catalysts (0.291 g, yield 48%). SEC (DMF+0.1M LiBr): $M_n$ 18,000 g/mol, $D_M$ 1.22.

![Figure S7. $^1$H NMR spectrum of Boc-H1-a (CDCl$_3$, 400 MHz).](image-url)
S2.2.2. Typical copolymerization of MTC-BAE and MTC-ME: Synthesis of Boc-R1 and Boc-R2

The copolymerization was carried out in a similar way as above mentioned. Instead, MTC-BAE (0.204 g, 0.68 mmol), MTC-ME (0.147 g, 0.68 mmol), benzyl alcohol (3.0 mg, 0.027 mmol), TU (24.9 mg, 0.068 mmol) and SP (15.8 mg, 0.068 mmol) were used and stirred in dry CH₂Cl₂ (1.35 ml) for 18 hours at which 97% of monomers were consumed (0.320 g, yield 91%). ¹H-NMR (500 MHz, CDCl₃): δ 7.41-7.36 (m, Ar-H), 5.16 (d, Ph-CH₂), 5.11-4.98 (br, NH), 4.46-4.25 (m, CH₂OCOO and COOCH₂CH₂O), 4.24-4.15 (m, OCH₂CH₂N), 3.64-3.53 (m, CH₂OCH₃), 3.41-3.33 (m, OCH₂CH₂N and OC₂H₃), 2.06 (s, OCOCH₃ end group), 1.45 (s, C (CH₃)₃), 1.28 (s, CH₂). SEC (THF): $M_n$ 7,100 g/mol, $D_M$ 1.30.

For a precursor of R2 (Boc-R2), MTC-BAE (0.152 g, 0.5 mmol), MTC-ME (0.327 g, 1.5 mmol), 1-pyrenebutanol (5.5 mg, 0.02 mmol), TU (74.0 mg, 0.20 mmol) and SP (23.4 mg, 0.1 mmol) were used and stirred in dry CH₂Cl₂ (2.0 ml) for 20 hours at which 95% of monomers were consumed (0.307 g, yield 64%). SEC (DMF+0.1M LiBr): $M_n$ 8,100 g/mol, $D_M$ 1.32.

Figure S8. ¹H NMR spectrum of Boc-R1 (CDCl₃, 500 MHz).
S2.2.3. Typical copolymerization of MTC-BAE and MTC-Et: Synthesis of Boc-R3

The copolymerization was carried out in a similar way as above mentioned. Instead, MTC-BAE (0.204 g, 0.68 mmol), MTC-Et (0.127 g, 0.68 mmol), benzyl alcohol (3.0 mg, 0.027 mmol), TU (24.9 mg, 0.068 mmol) and SP (15.8 mg, 0.068 mmol) were used and stirred in dry CH$_2$Cl$_2$ (1.35 ml) for 24 hours at which 91% of monomers were consumed (0.227 g, yield 69%). $^1$H-NMR (500 MHz,CDCl$_3$) : δ 7.41-7.36 (m, Ar-H), 5.16 (d, Ph-CH$_2$), 5.09-4.97 (br, NH), 4.40-4.24 (m, CH$_2$OCOO and COOC$_2$H$_5$), 4.24-4.14 (m, OCH$_2$CH$_2$N and OCH$_2$CH$_3$), 3.44-3.32 (m, OCH$_3$CH$_2$N), 2.06 (s, COCH$_2$ end group), 1.45 (s, C (CH$_3$)$_3$), 1.30-1.24 (m, CH$_2$ and CH$_2$CH$_3$). SEC (THF): $M_n$ 8,200 g/mol, $D_M$ 1.26.

Figure S9. $^1$H NMR spectrum of Boc-R3 (CDCl$_3$, 500 MHz).
S2.2.4. Typical copolymerization of MTC-BAE and MTC-Bn: Synthesis of Boc-R4

The copolymerization was carried out in a similar way as above mentioned. Instead, MTC-BAE (0.242 g, 0.80 mmol), MTC-Bn (0.200 g, 0.80 mmol), benzyl alcohol (3.4 mg, 0.032 mmol), TU (17.6 mg, 0.048 mmol) and SP (11.2 mg, 0.048 mmol) were used and stirred in dry CH$_2$Cl$_2$ (1.6 ml) for 24 hours at which 98% of monomers were consumed (0.318 g, yield 72%). $^1$H-NMR (500 MHz,CDCl$_3$) : $\delta$ 7.41-7.28 (br, Ar-$H$), 5.15 (s, Ph-$CH_2$), 5.07-4.92 (br, NH), 4.40-4.23 (m, $CH_2$OCOO), 4.23-4.12 (m, OCH$_2$), 3.41-3.27 (m, OCH$_2$CH$_2$N), 2.06 (s, OCOC$_3$H$_3$ end group), 1.44 (s, C (C$_3$H$_7$)$_3$), 1.26 (s, CH$_3$). SEC (THF): $M_n$ 9,500 g/mol, $\bar{D}_M$ 1.19.

Figure S10. $^1$H NMR spectrum of Boc-R4 (CDCl$_3$, 500 MHz).
S2.2.5. Sequential ROP of MTC-BAE and MTC-ME: Synthesis of Boc-B1

The sequential ROP to form a block copolymer was carried out in a similar way as above mentioned. First, MTC-ME (0.218 g, 1.0 mmol) was polymerized using benzyl alcohol (4.3 mg, 0.04 mmol), TU (3.7 mg, 0.01 mmol) and SP (2.4 mg, 0.01 mmol) in dry CH₂Cl₂ (1.0 ml) for 2 hours. The reaction was quenched by benzoic acid, and the polymer poly(MTC-ME) was isolated by reprecipitation in a mixture of hexane and toluene (4: 1) as a sticky material (0.15 g, yield 68%).

The poly(MTC-ME) (0.1 g) was then dissolved in dry CH₂Cl₂ (0.66 ml) and used as a macroinitiator to polymerize MTC-BAE (0.133 g, 0.44 mmol) in the presence of TU (1.6 mg, 0.004 mmol) and SP (1.1 mg, 0.004 mmol), at which 98% of monomers were consumed (0.318 g, yield 72%). The reaction was quenched by acetic anhydride for concomitant end capping, and the polymer was isolated as described above (0.120 g, yield 52%). ¹H-NMR (500 MHz, CDCl₃): δ 7.41-7.34 (m, Ar-H), 5.16 (s, Ph-CH₂), 5.13-4.98 (br, NH), 4.42-4.25 (m, CH₂OCOO and COOCH₂CH₂O), 4.25-4.14 (m, OCH₂CH₂N), 3.63-3.54 (m, CH₂OCH₃), 3.47-3.30 (m, OCH₂CH₂N and OCH₃), 2.06 (s, OCOCH₂ end group), 1.45 (s, C(CH₃)₃), 1.28 (s, CH₃). SEC (THF): $M_n$ 7,300 g/mol, $D_M$ 1.30.

Figure S11. ¹H NMR spectrum of Boc-B1 (CDCl₃, 500 MHz).
S2.3. Formation of ammonium side chains by deprotection of Boc group

S1.3.1. Synthesis of H1 and H2

To an acetonitrile solution (2.5 ml) of Boc-H1-a (192 mg, [Boc] = 0.63 mmol), trifluoroacetic acid (0.72 ml, 9.45 mmol) was slowly added at −5°C, and the mixture was kept at −5°C for 10 minutes, followed by room temperature for 6 hours at which more than 95% of Boc groups disappeared on 1H-NMR. Afterwards, the reaction mixture was precipitated in a mixed solvent of diethyl ether and hexane (1: 1), and the precipitates were isolated by decantation and centrifugation. The isolated residue was then dried in vacuum until the weight unchanged (199 mg, yield 99%). 1H-NMR (500 MHz, DMSO-d6): δ 8.23-7.95 (br, NH3+), 4.40-4.09 (m, CH2OCOO and COOC2H), 3.10 (s, CH2NH3+), 2.00 (s, OCOC2H end group), 1.20 (s, CH3). SEC (H2O/MeCN (1: 1) + 0.1M NaNO3): Mn 10,000 g/mol, Dn 1.25.

For the synthesis of H1-b, the same procedure was performed using Boc-H1-b (492 mg, [Boc] = 1.62 mmol), trifluoroacetic acid (1.86 ml, 24.3 mmol), and acetonitrile (6.5 ml). 422 mg, yield 82%. SEC (H2O/MeCN (1: 1) + 0.1M NaNO3): Mn 28,000 g/mol, Dn 1.22.

For the synthesis of H2, the same procedure was performed using Boc-H2 (84 mg, [Boc] = 0.28 mmol), 10N HCl aq. (0.28 ml, 2.8 mmol), and acetonitrile (1.1 ml). 65 mg, yield 99%. SEC (H2O/MeCN (1: 1) + 0.1M NaNO3): Mn 8,100 g/mol, Dn 1.24.

Figure S12. 1H NMR spectrum of H1-a (DMSO-d6, 500 MHz).
S2.3.2. Synthesis of R1 and R2

For the synthesis of R1, the same procedure was performed using Boc-R1 (320 mg, [Boc] = 0.61 mmol), trifluoroacetic acid (0.70 ml, 9.21 mmol), and acetonitrile (2.45 ml). 166 mg, yield 51%. 

\(^1\)H-NMR (500 MHz, DMSO-d\(_6\)): \(\delta 8.15-7.83\) (br, NH\(_3^+\)), \(7.38\) (s, Ar-H), \(5.13\) (Ph-CH\(_2\)), \(4.37-4.11\) (m, CH\(_2\)OCOO and COOC\(_2\)H\(_5\)), \(3.49\) (s, CH\(_2\)CH\(_2\)OCH\(_3\)), \(3.23\) (s, OCH\(_3\)), \(3.10\) (s, CH\(_2\)NH\(_3^+\)), \(2.00\) (s, OCOCH\(_2\)H\(_5\) end group), \(1.26-1.13\) (m, CH\(_2\)). \(M_n\) 10,100 g/mol (based on NMR).

For the synthesis of R2, the same procedure was performed using Boc-R2 (307 mg, [Boc] = 0.25 mmol), trifluoroacetic acid (0.38 ml, 5.0 mmol), and acetonitrile (5.0 ml). 147 mg, yield 47%. \(M_n\) 10,700 g/mol (based on NMR).

S2.3.3. Synthesis of R3

For the synthesis of R3, the same procedure was performed using Boc-R3 (227 mg, [Boc] = 0.46 mmol), trifluoroacetic acid (0.53 ml, 6.9 mmol), and acetonitrile (1.24 ml). 162 mg, yield 70%.

\(^1\)H-NMR (400 MHz, DMSO-d\(_6\)): \(\delta 8.17-7.85\) (br, NH\(_3^+\)), \(7.38\) (s, Ar-H), \(5.14\) (s, Ph-CH\(_2\)), \(4.38-4.15\) (m, CH\(_2\)OCOO and COOC\(_2\)H\(_5\)), \(4.10\) (q, \(J = 8.0\) Hz, COOC\(_2\)H\(_5\)CH\(_3\)), \(3.12\) (s, CH\(_2\)NH\(_3^+\)), \(2.00\) (s, OCOCH\(_2\)H\(_5\) end group), \(1.27-1.07\) (m, CC\(_2\)H\(_5\)CH\(_2\)H\(_2\)). \(M_n\) 8,400 g/mol (based on NMR).

![Figure S13. \(^1\)H NMR spectrum of R3 (DMSO-\(d_6\), 400 MHz).](image-url)
S2.3.4. Synthesis of R4

For the synthesis of R4, the same procedure was performed using Boc-R4 (200 mg, [Boc] = 0.36 mmol), trifluoroacetic acid (0.41 ml, 5.4 mmol), and acetonitrile (1.44 ml). 162 mg, yield 79%.

\(^1\)H-NMR (400 MHz, DMSO-d\(_6\)): \(\delta\) 8.16-7.83 (br, NH\(^+\)), 7.31 (s, Ar-H), 5.11 (s, Ph-CH\(_2\)), 4.37-4.12 (m, CH\(_2\)OCOO and COOC\(_2\)H), 3.10 (s, CH\(_2\)NH\(^+\)), 2.00 (s, OCOC\(_3\)H\(_2\) end group), 1.30-1.08 (m, CH\(_3\)). \(M_n\) 10,400 g/mol (based on NMR).

Figure S14. \(^1\)H NMR spectrum of R4 (DMSO-d\(_6\), 400 MHz).
S2.3.5. Synthesis of B1

For the synthesis of B1, the same procedure was performed using Boc-B1 (120 mg, \([\text{Boc}] = 0.23 \text{ mmol}\)), trifluoroacetic acid (0.30 ml, 3.5 mmol), and acetonitrile (0.92 ml). 98 mg, yield 82\%.

\(^1\text{H}-\text{NMR (500 MHz, DMSO-}d_6\text{)}: \delta 8.19-7.85 (\text{br, } \text{NH}_3^+), 7.38 (\text{s, } \text{Ar-H}), 5.13 (\text{s, } \text{Ph-CH}_2), 4.39-4.11 (\text{m, } \text{CH}_2\text{OCOO and COOC}_2\text{H}_5), 3.52-3.46 (\text{m, } \text{CH}_3\text{OCH}_3), 3.23 (\text{s, } \text{OCH}_3), 3.10 (\text{s, } \text{CH}_2\text{NH}_3^+), 2.01 (\text{s, } \text{OCOC}_2\text{H}_5 \text{end group}), 1.27-1.12 (\text{m, } \text{CH}_2). M_n 10,000 \text{ g/mol (based on NMR)}.

Figure S15. \(^1\text{H} \text{NMR spectrum of B1} \text{(DMSO-d}_6\text{, 500 MHz).}