Supplementary Information for

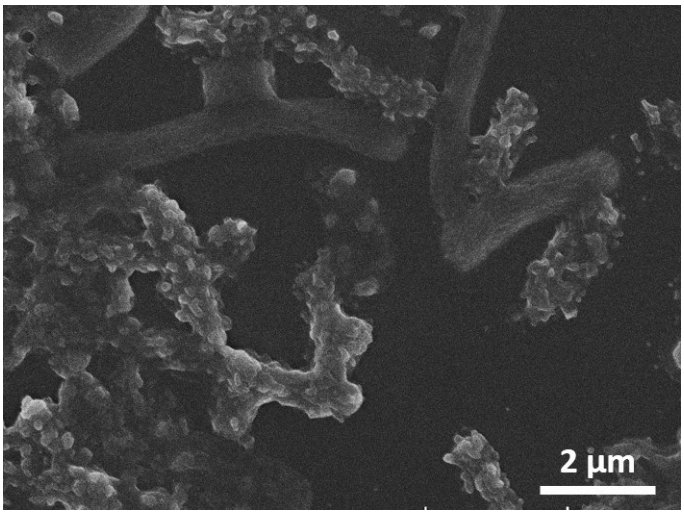
Modulating bioactivities of primary ammonium-tagged antimicrobial aliphatic polycarbonates by varying length, sequence and hydrophobic side chain structure

By Kazuki Fukushima,* Kohei Kishi, Keita Saito, Kazuki Takakuwa, Shunta Hakozaki, Shigekazu Yano

Contents:

S1. Supplemental figures

(a)



(b)

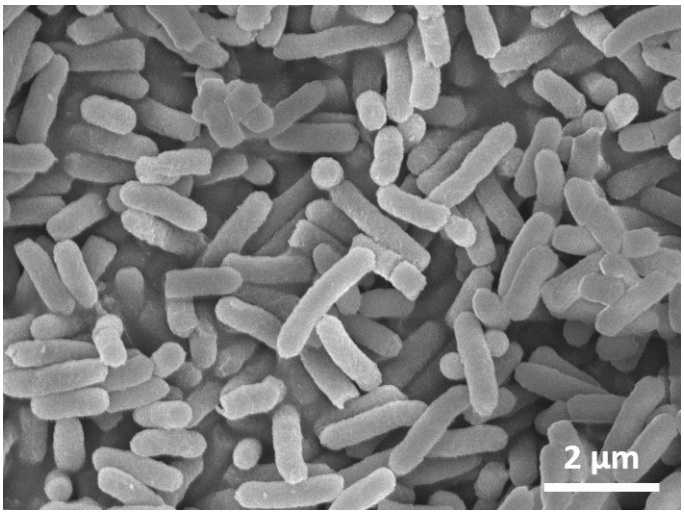


Figure S1. SEM images of *E. coli* treated with **R4** (128 µg/mL) (a) and no polymer (b)

(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.

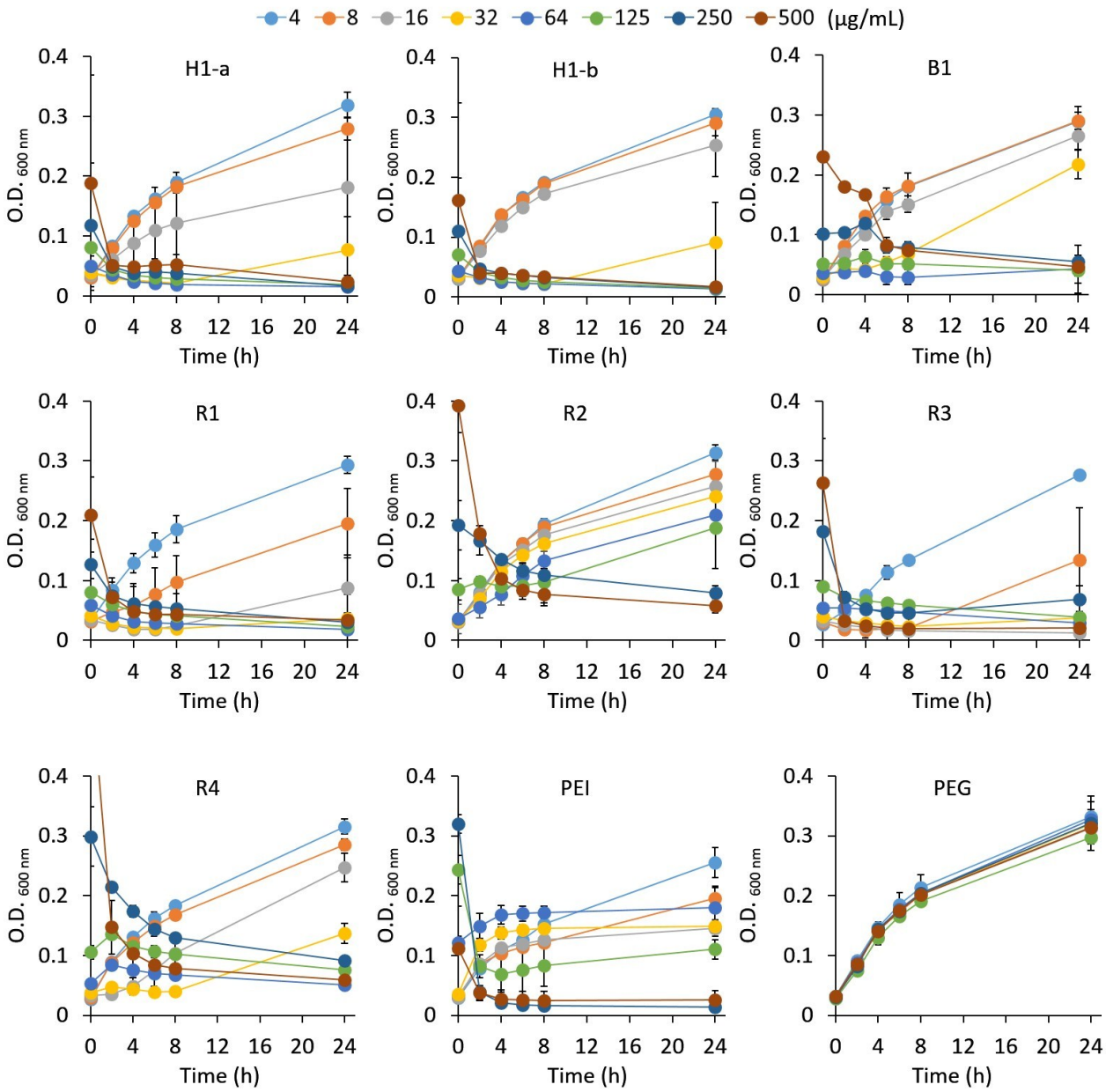


Figure S2. Bacterial growth of *E. coli* treated with different concentrations of polymers as a function of time. MICs (μg/mL): 64 (**H1-a**), 64 (**H1-b**), 64 (**B1**), 32 (**R1**), 250 (**R2**), 16 (**R3**), 64 (**R4**), 250 (PEI).

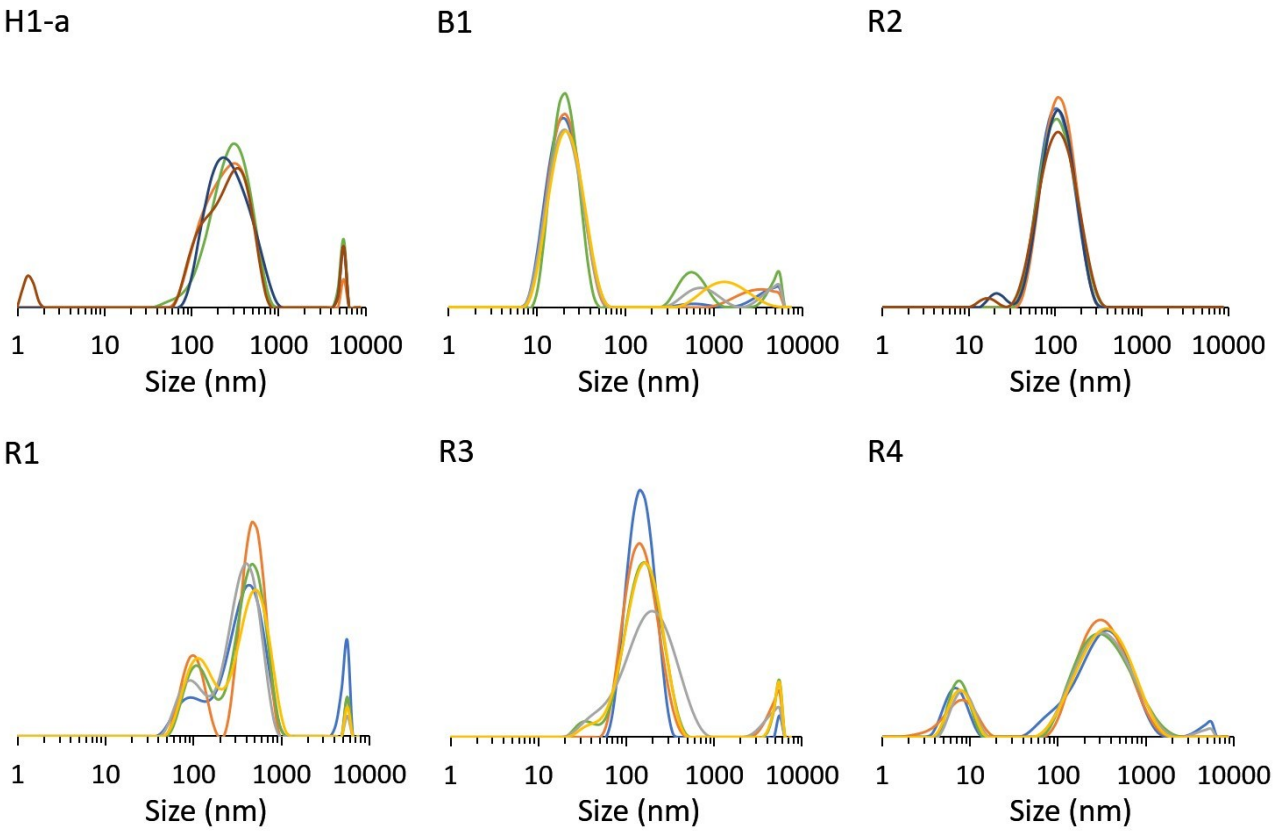


Figure S3. Size distribution of polymers in water measured by DLS.

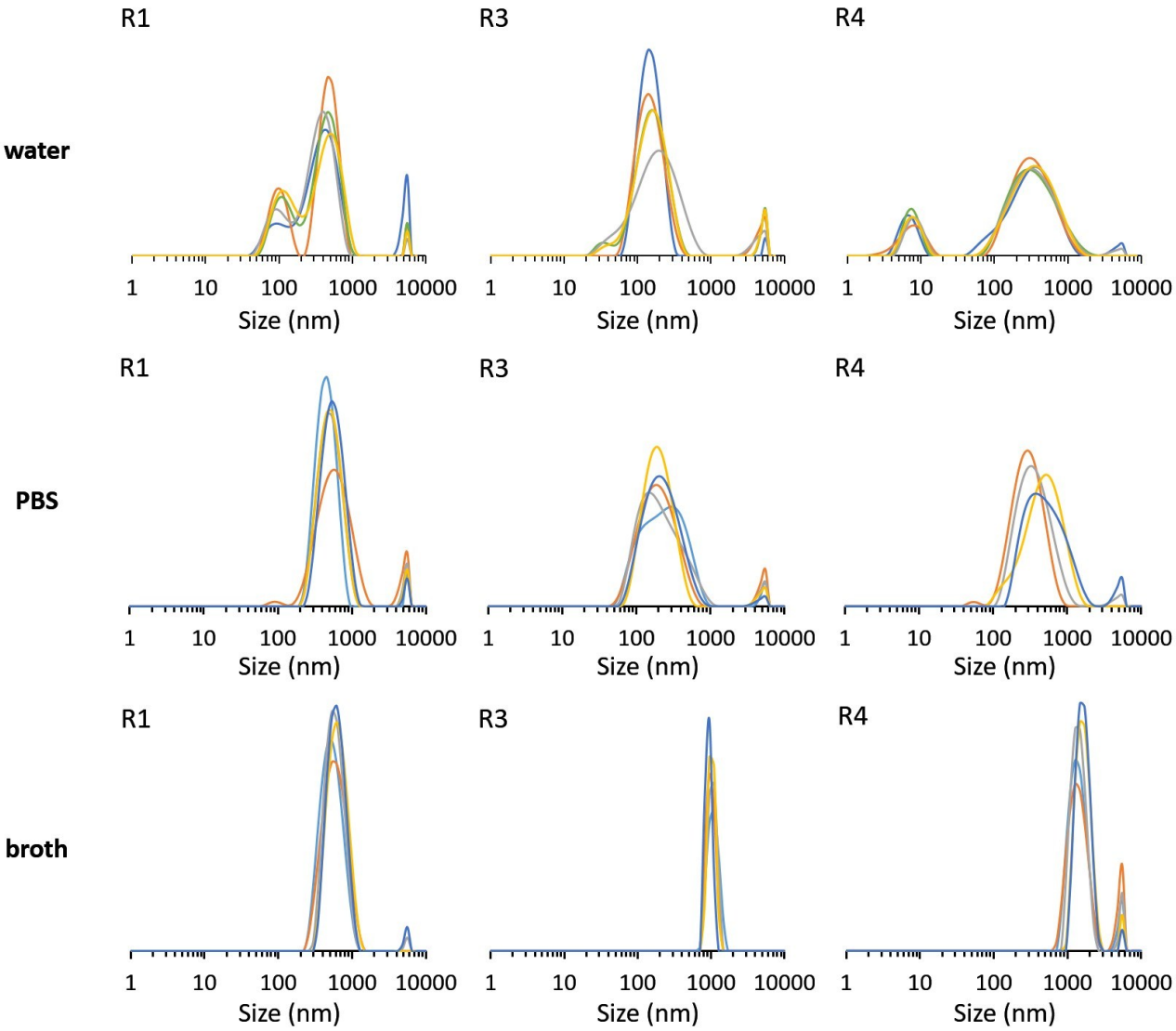


Figure S4. Size distribution of polymers in different media measured by DLS.

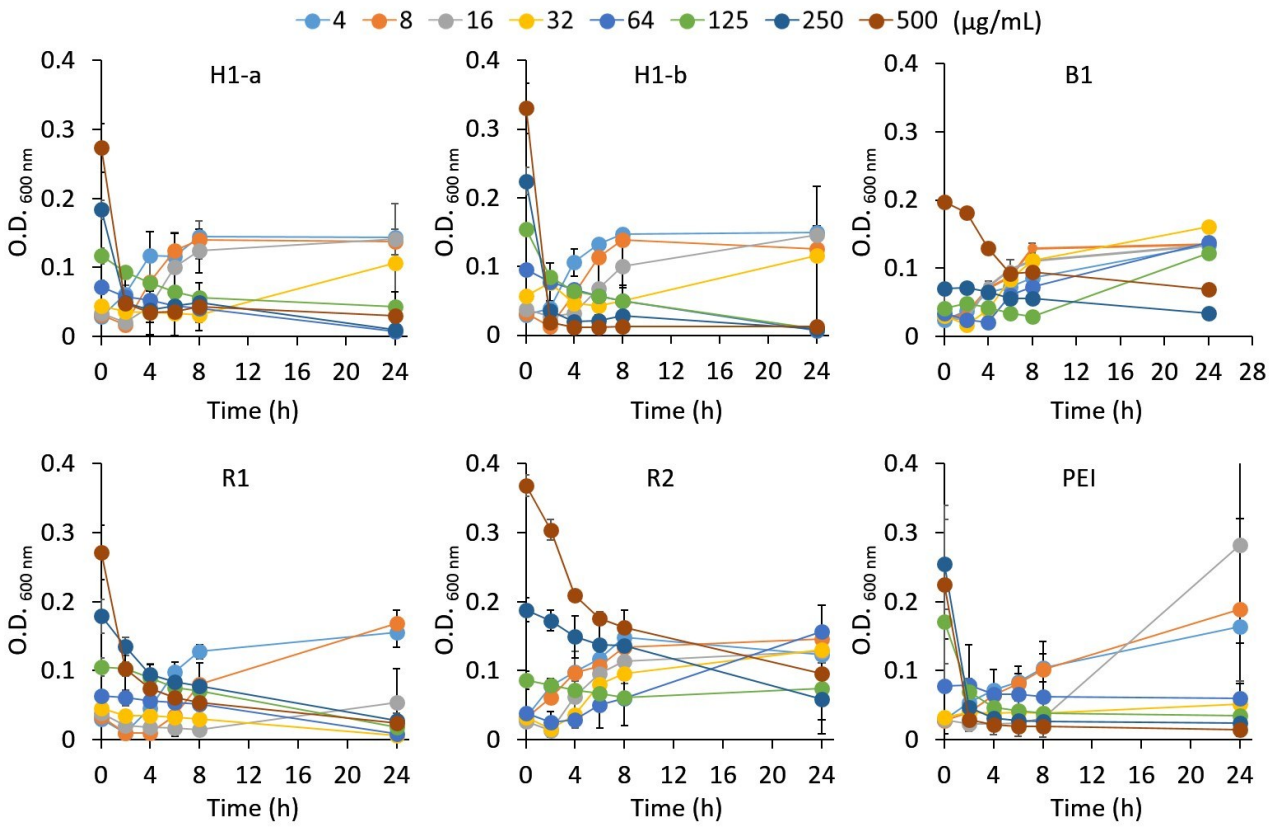
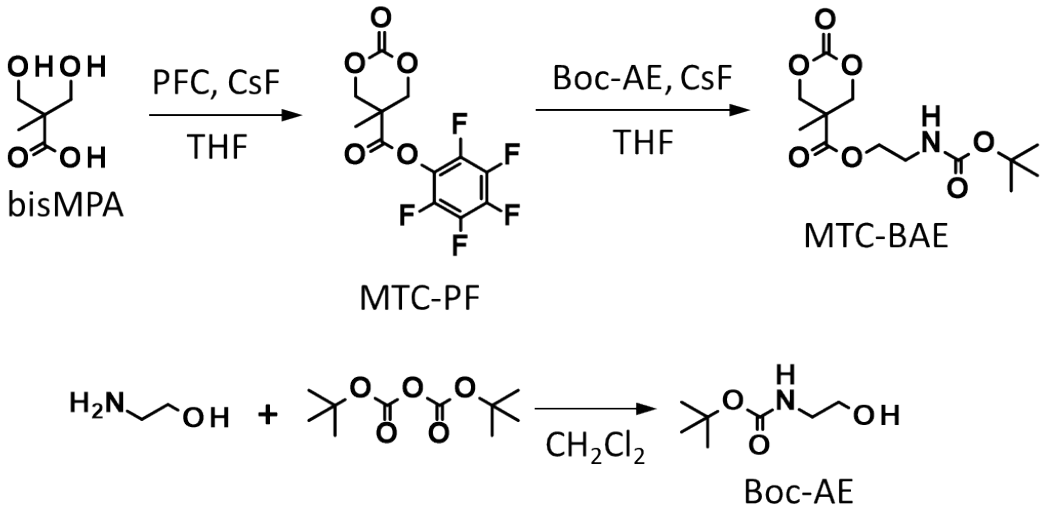


Figure S5. Bacterial growth of *B. subtilis* treated with different concentrations of polymers as a function of time. MICs (µg/mL): 64 (**H1-a**), 64 (**H1-b**), 250 (**B1**), 32 (**R1**), 125 (**R2**), 32 (PEI).

S2. Polymer synthesis with detailed procedure

S2.1. Synthesis of MTC-BAE



S2.1.1. Synthesis of 2,3,4,5,6-pentafluorophenyl 5-methyl-2-oxo-1,3-dioxane-5-carboxylate (MTC-PF)

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 $NaHCO_3$ aq. and brine. The organic layer was then dried over $MgSO_4$ 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%). ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 4.86 (d, J = 12 Hz, 2H, C$\underline{H_aH_b}$), 4.37 d, J = 12 Hz, 2H, C$\underline{H_aH_b}$), 1.57 (s, 3H, C$\underline{H_3}$).

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%). ^{1}H-NMR (500 MHz, acetone-d_6): δ 5.90 (br, 1H, N$\underline{H}$), 3.555 (q, J = 7.5 Hz, 2H, OC$\underline{H_2}$), 3.165 (q, J = 7.5 Hz, 2H, C$\underline{H_2}$NH), 1.40 (s, 9H, C (C$\underline{H_3}$)$_3$).

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, N*H*), 4.70 (d, 2H, C*H*$_a$C*H*$_b$), 4.22 (d, 2H, C*H*$_a$C*H*$_b$), 4.28 (t, 2H, OC*H*$_2$CH$_2$), 3.43 (q, 2H, CH$_2$C*H*$_2$NH), 1.45 (s, 9H, C (C*H*$_3$)$_3$), 1.36 (s, 3H, CC*H*$_3$).

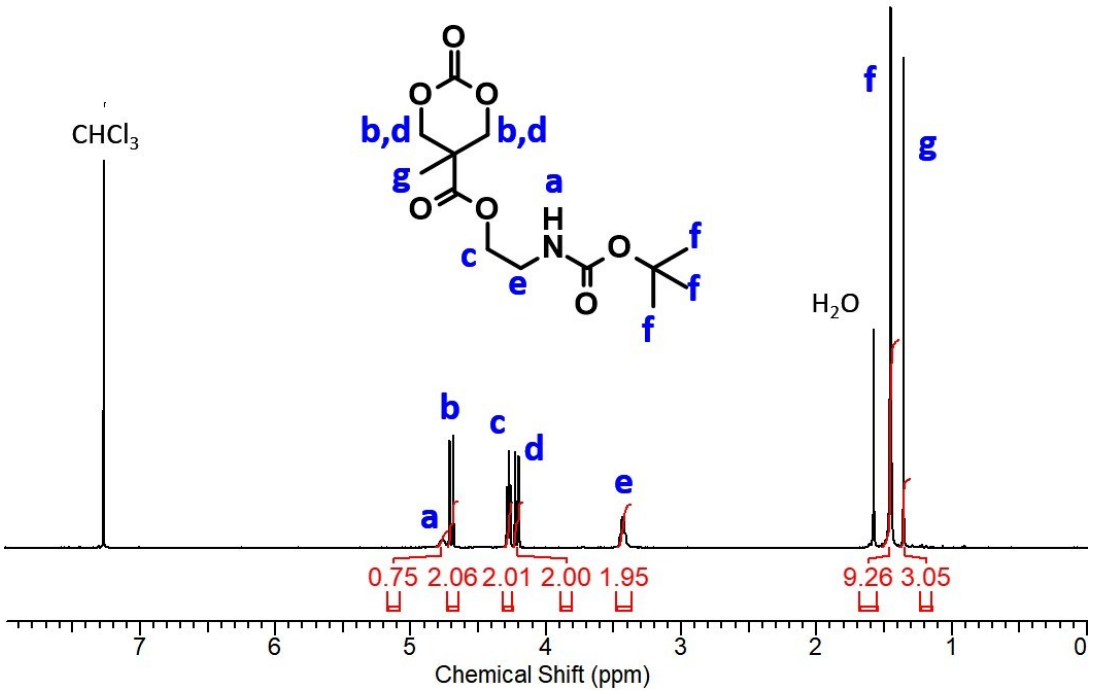


Figure S6. ^{1}H NMR spectrum of MTC-BAE ($CDCl_3$, 400 MHz).

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_2Cl_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,$CDCl_3$): δ 8.38-7.66 (m, Ar-*H*), 5.19-4.99 (br, NH), 4.23 (m, C*H*$_2$OCOO), 4.01 (m, OC*H*$_2$CH$_2$), 3.16 (m, CH$_2$C*H*$_2$NH), 1.99 (s, OCOC*H*$_3$ end group), 1.36 (s, C(C*H*$_3$)$_3$), 1.16 (s, C*H*$_3$). SEC (DMF+0.1M LiBr): M_n 20,000 g/mol, $Đ_M$ 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_2Cl_2 (1.0 ml). No initiator was used (0.493 g, yield 81%). SEC (DMF+0.1M LiBr): M_n 31,000 g/mol, $Đ_M$ 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_2Cl_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_2Cl_2 and CaH_2 was added. The mixed solution was stirred 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, $Đ_M$ 1.22.

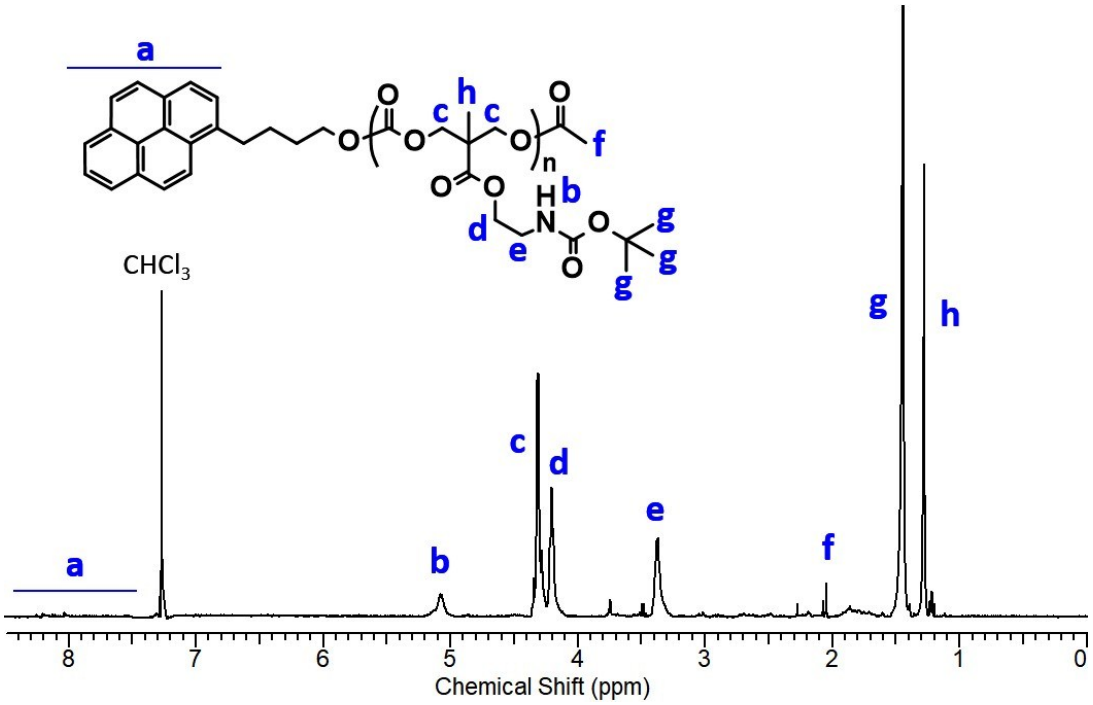


Figure S7. ^{1}H NMR spectrum of **Boc-H1-a** ($CDCl_3$, 400 MHz).

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 CH_2Cl_2 (1.35 ml) for 18 hours at which 97% of monomers were consumed (0.320 g, yield 91%). ^{1}H-NMR (500 MHz,$CDCl_3$) : δ 7.41-7.36 (m, Ar-*H*), 5.16 (d, Ph-C*H*$_2$), 5.11-4.98 (br, N*H*), 4.46-4.25 (m, C*H*$_2$OCOO and COOC*H*$_2$CH$_2$O), 4.24-4.15 (m, OC*H*$_2$CH$_2$N), 3.64-3.53 (m, C*H*$_2$OCH$_3$), 3.41-3.33 (m, OCH$_2$C*H*$_2$N and OC*H*$_3$), 2.06 (s, OCOC*H*$_3$ end group), 1.45 (s, C (C*H*$_3$)$_3$), 1.28 (s, C*H*$_3$). SEC (THF): M_n 7,100 g/mol, $Đ_M$ 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 CH_2Cl_2 (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, $Đ_M$ 1.32.

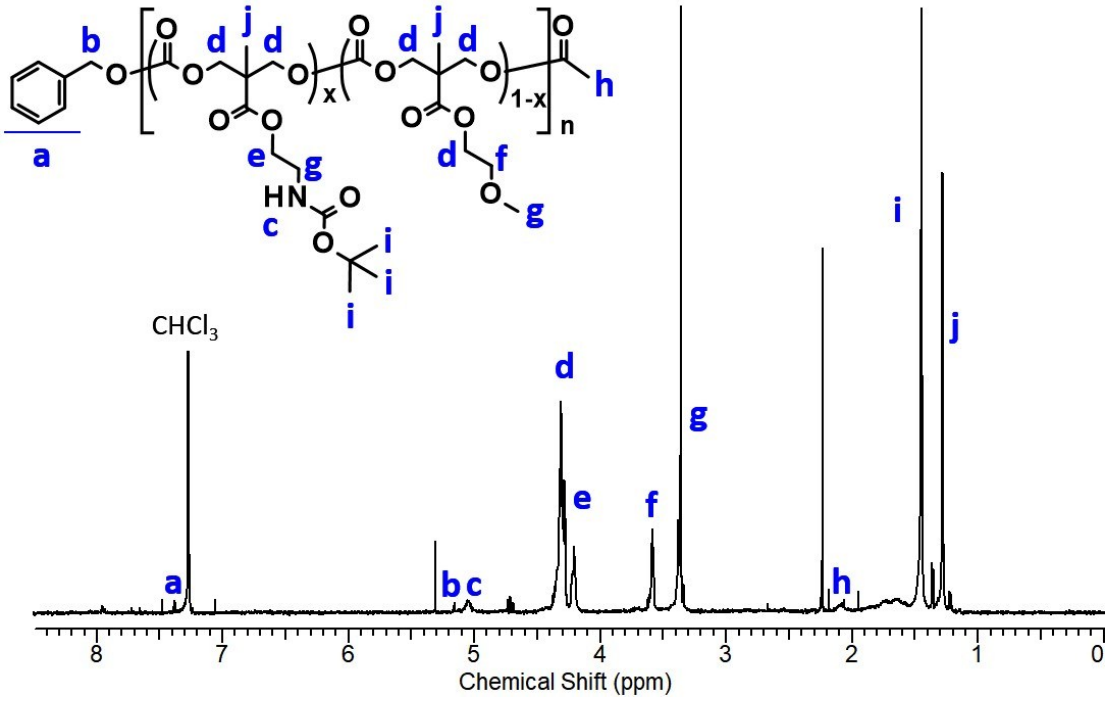


Figure S8. ^{1}H NMR spectrum of **Boc-R1** ($CDCl_3$, 500 MHz).

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_2Cl_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-C*H*$_2$), 5.09-4.97 (br, N*H*), 4.40-4.24 (m, C*H*$_2$OCOO and COOC*H*$_2$CH$_2$O), 4.24-4.14 (m, OC*H*$_2$CH$_2$N and OC*H*$_2$CH$_3$), 3.44-3.32 (m, OCH$_2$C*H*$_2$N), 2.06 (s, OCOC*H*$_3$ end group), 1.45 (s, C (C*H*$_3$)$_3$), 1.30-1.24 (m, C*H*$_3$ and CH$_2$C*H*$_3$). SEC (THF): M_n 8,200 g/mol, $Đ_M$ 1.26.

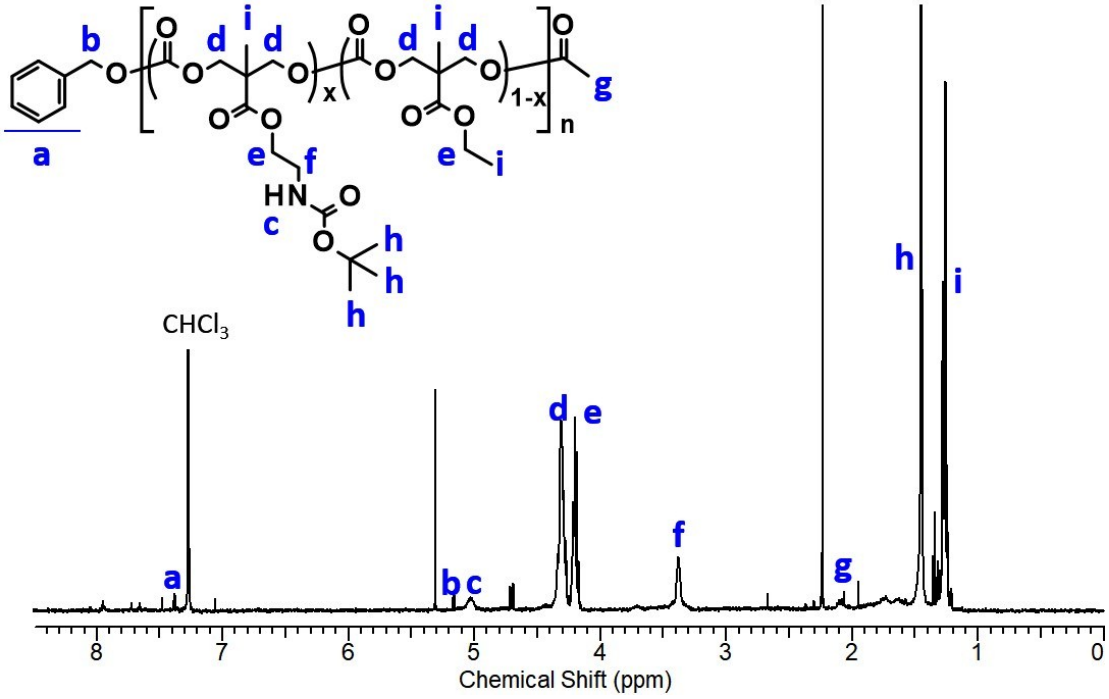


Figure S9. ^{1}H NMR spectrum of **Boc-R3** ($CDCl_3$, 500 MHz).

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_2Cl_2 (1.6 ml) for 24 hours at which 98% of monomers were consumed (0.318 g, yield 72%). ^{1}H-NMR (500 MHz,$CDCl_3$) : δ 7.41-7.28 (br, Ar-*H*), 5.15 (s, Ph-C*H*$_2$), 5.07-4.92 (br, N*H*), 4.40-4.23 (m, C*H*$_2$OCOO), 4.23-4.12 (m, OC*H*$_2$), 3.41-3.27 (m, OCH_2CH_2N), 2.06 (s, OCOC*H*$_3$ end group), 1.44 (s, C (C*H*$_3$)$_3$), 1.26 (s, C*H*$_3$). SEC (THF): M_n 9,500 g/mol, $Đ_M$ 1.19.

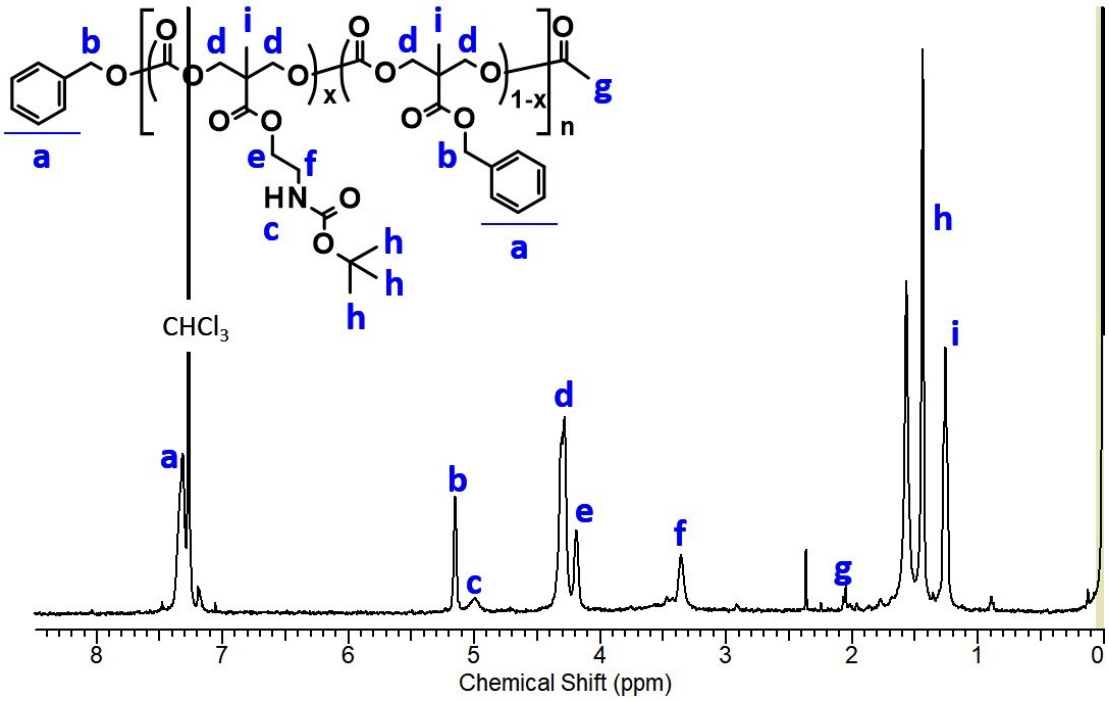


Figure S10. ^{1}H NMR spectrum of **Boc-R4** ($CDCl_3$, 500 MHz).

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 CH_2Cl_2 (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 CH_2Cl_2 (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%). ^{1}H-NMR (500 MHz,$CDCl_3$) : δ 7.41-7.34 (m, Ar-*H*), 5.16 (s, Ph-C*H*$_2$), 5.13-4.98 (br, N*H*), 4.42-4.25 (m, C*H*$_2$OCOO and COOC*H*$_2$CH$_2$O), 4.25-4.14 (m, OC*H*$_2$CH$_2$N), 3.63-3.54 (m, C*H*$_2$OCH$_3$), 3.47-3.30 (m, OCH$_2$C*H*$_2$N and OC*H*$_3$), 2.06 (s, OCOC*H*$_3$ end group), 1.45 (s, C (C*H*$_3$)$_3$), 1.28 (s, C*H*$_3$). SEC (THF): M_n 7,300 g/mol, $Đ_M$ 1.30.

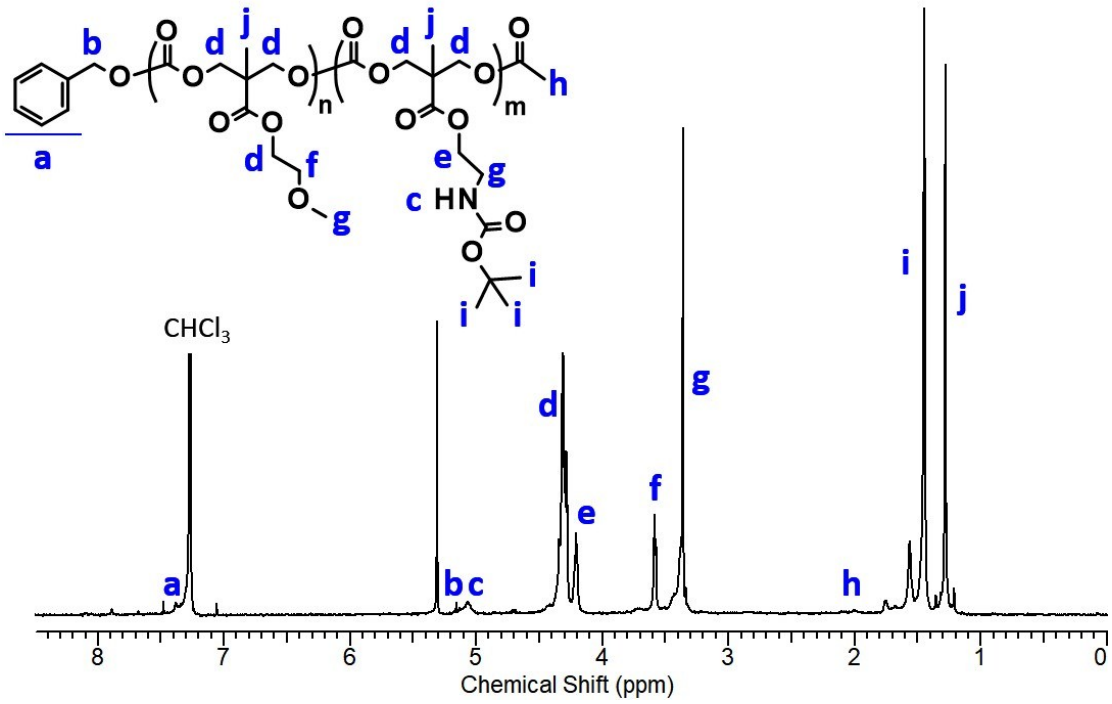


Figure S11. ^{1}H NMR spectrum of **Boc-B1** ($CDCl_3$, 500 MHz).

S2.3. Formation of ammonium side chains by deprotection of Boc gorup

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 ^{1}H-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%). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.23-7.95 (br, N$\underline{H_3}^+$), 4.40-4.09 (m, C$\underline{H_2}$OCOO and COOC$\underline{H_2}$), 3.10 (s, C$\underline{H_2}$NH$_3^+$), 2.00 (s, OCOC$\underline{H_3}$ end group), 1.20 (s, C$\underline{H_3}$). SEC (H_2O/MeCN (1: 1) + 0.1M $NaNO_3$): M_n 10,000 g/mol, $Đ_M$ 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 (H_2O/MeCN (1: 1) + 0.1M $NaNO_3$): M_n 28,000 g/mol, $Đ_M$ 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 (H_2O/MeCN (1: 1) + 0.1M $NaNO_3$): M_n 8,100 g/mol, $Đ_M$ 1.24.

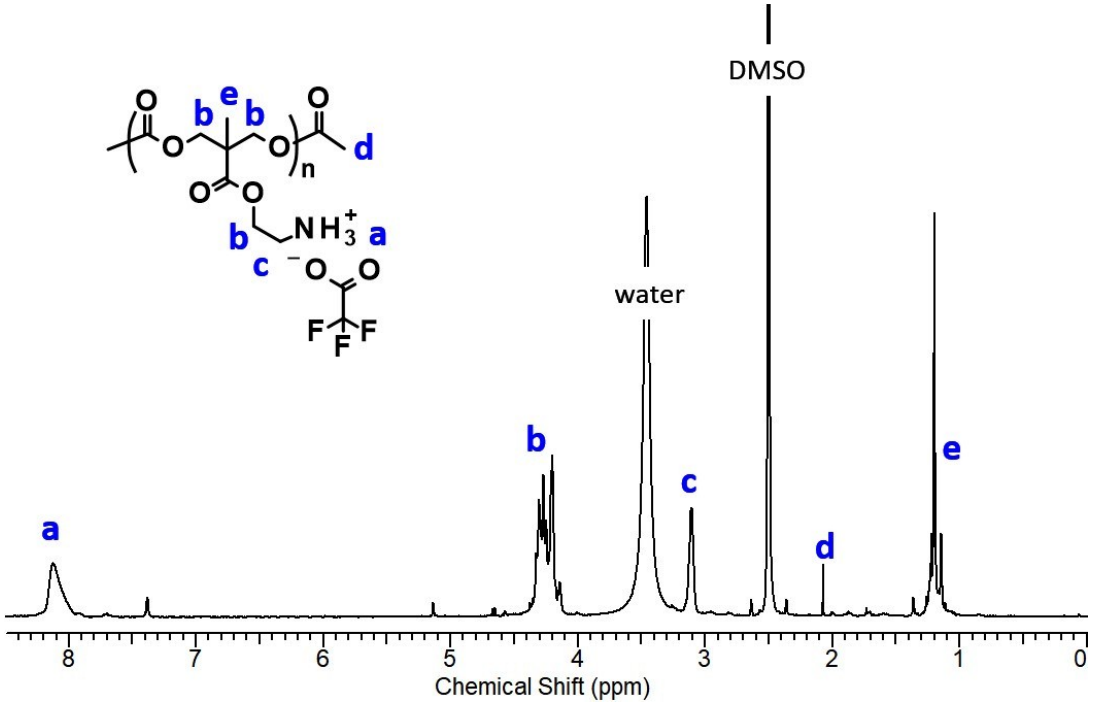


Figure S12. ^{1}H NMR spectrum of **H1-a** (DMSO-d_6, 500 MHz).

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$_6$): δ 8.15-7.83 (br, N$\underline{H_3}^+$), 7.38 (s, Ar-$\underline{H}$), 5.13 (Ph-C$\underline{H_2}$), 4.37-4.11 (m, C$\underline{H_2}$OCOO and COOC$\underline{H_2}$), 3.49 (s, CH$_2$C$\underline{H_2}$OCH$_3$), 3.23 (s, OC$\underline{H_3}$), 3.10 (s, C$\underline{H_2}$NH$_3^+$), 2.00 (s, OCOC$\underline{H_3}$ end group), 1.26-1.13 (m, C$\underline{H_3}$). 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$_6$): δ 8.17-7.85 (br, N$\underline{H_3}^+$), 7.38 (s, Ar-$\underline{H}$), 5.14 (s, Ph-C$\underline{H_2}$), 4.38-4.15 (m, C$\underline{H_2}$OCOO and COOC$\underline{H_2}$CH$_2$), 4.10 (q, J = 8.0 Hz, COOC$\underline{H_2}$CH$_3$), 3.12 (s, C$\underline{H_2}$NH$_3^+$), 2.00 (s, OCOC$\underline{H_3}$ end group), 1.27-1.07 (m, CC$\underline{H_3}$, CH$_2$C$\underline{H_3}$). M_n 8,400 g/mol (based on NMR).

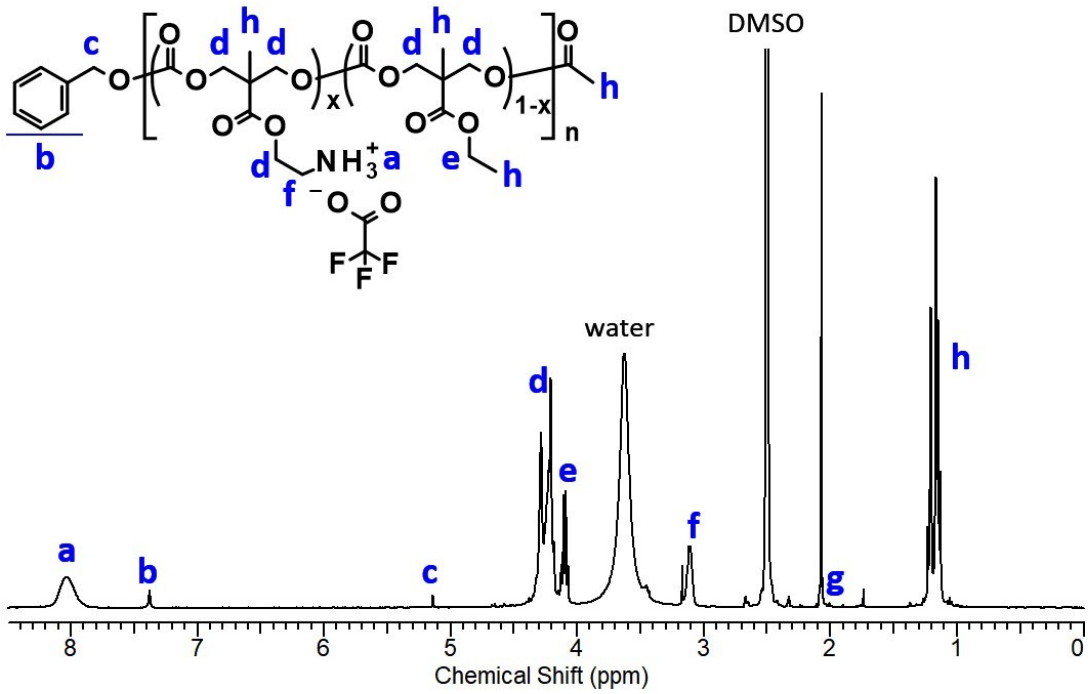


Figure S13. ^{1}H NMR spectrum of **R3** (DMSO-d_6, 400 MHz).

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$_6$): δ 8.16-7.83 (br, N$\underline{H_3}^+$), 7.31 (s, Ar-$\underline{H}$), 5.11 (s, Ph-C$\underline{H_2}$), 4.37-4.12 (m, C$\underline{H_2}$OCOO and COOC$\underline{H_2}$), 3.10 (s, C$\underline{H_2}$NH$_3^+$), 2.00 (s, OCOC$\underline{H_3}$ end group), 1.30-1.08 (m, C$\underline{H_3}$). M_n 10,400 g/mol (based on NMR).

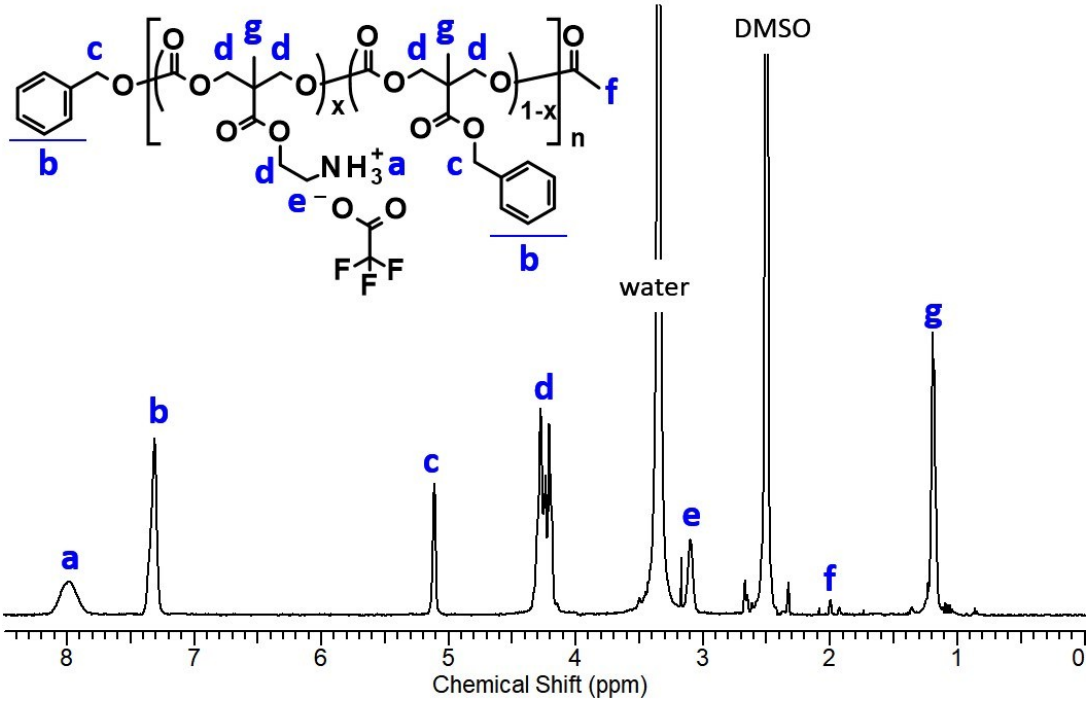


Figure S14. ^{1}H NMR spectrum of **R4** (DMSO-d_6, 400 MHz).

S2.3.5. Synthesis of **B1**

For the synthesis of **B1**, the same procedure was performed using **Boc-B1** (120 mg, [Boc] = 0.23 mmol), trifluoroacetic acid (0.30 ml, 3.5 mmol), and acetonitrile (0.92 ml). 98 mg, yield 82%. ^{1}H-NMR (500 MHz, DMSO-d$_6$): δ 8.19-7.85 (br, N$\underline{H_3}^+$), 7.38 (s, Ar-$\underline{H}$), 5.13 (s, Ph-C$\underline{H_2}$), 4.39-4.11 (m, C$\underline{H_2}$OCOO and COOC$\underline{H_2}$), 3.52-3.46 (m, C$\underline{H_2}$OCH$_3$), 3.23 (s, OC$\underline{H_3}$), 3.10 (s, C$\underline{H_2}$NH$_3^+$), 2.01 (s, OCOC$\underline{H_3}$ end group), 1.27-1.12 (m, C$\underline{H_3}$). M_n 10,000 g/mol (based on NMR).

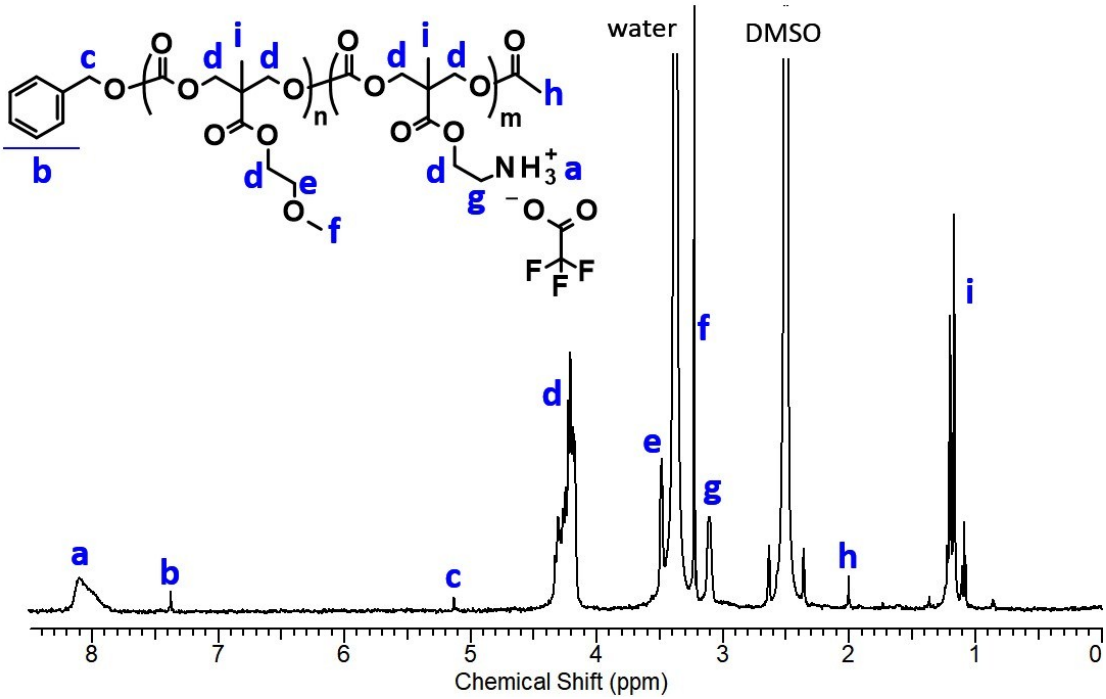


Figure S15. ^{1}H NMR spectrum of **B1** (DMSO-d_6, 500 MHz).