

Antibacterial Properties of Main-Chain Cationic Polymers Prepared through Amine-Epoxy 'Click' Polymerization

Junki Oh, Seung-Jin Kim, Min-Kyu Oh and Anzar Khan*

Email: anzar@korea.ac.kr

Experimental Details

Materials

Poly(ethylene glycol) diglycidyl ether ($M_n = 500$), 1,4 butanediol glycidyl ether, poly(propylene oxide) diglycidyl ether ($M_n = 380$), propylamine, butylamine, pentylamine, hexylamine, heptylamine, iodomethane, iodoethane, iodopropane, iodobutane, hydrochloric acid (37%), silver tetrafluoroborate, acetonitrile were purchased from Sigma Aldrich. Ethylene glycol diglycidyl ether was purchased from TCI (Tokyo Chemical Industry).

Escherichia coli (ATCC25922), *Staphylococcus aureus* (ATCC6538), *Mycobacterium smegmatis* (ATCC19420), PBS buffer (0.1 M PBS with 0.05 M NaCl, pH 7.4), Luria– Bertani (LB) medium (tryptone 10 g/L, yeast extract 5 g/L, and NaCl 10 g/L), tryptic soy broth (TSB) medium (tryptone 17 g/L, soytone 3 g/L, dextrose 2.5 g/L, NaCl 5 g/L, dipotassium phosphate 2.5 g/L) with tween 80 supplement and red blood cells (sterile defibrinated sheep's blood, RBC) were used as purchased.

Characterizations

The bacterial surfaces were characterized by a field emission-scanning electron microscope (FE-SEM, Hitach S-4800) operated at 10 kV.

GPC measurements were conducted with three styragel HR 0.5, HR 2, and HR 4 Columns. PS standards in THF were used for the relative molecular weight determinations.

NMR spectra were measured on a Varian NMR System 500 MHz spectrometer, using CDCl_3 , $\text{DMSO-}d_6$ and D_2O as the deuterated solvents at 500 MHz for $^1\text{H-NMR}$. $^1\text{H-NMR}$ spectra were measured on UNITY-INOVA 500 from VARIAN. INC., using D_2O at 500 MHz.

The bacteria were viewed under an Olympus BX53 microscope equipped with a 470 nm excitation filter and a 540 nm emission filter for green fluorescence and 530 nm excitation filter and a 590 nm emission filter for red fluorescence.

General procedure for polymerization

Alkane amine monomer (2 eq.) is added very slowly and drop-wise to a diglycidyl ether monomer (1 eq.) solution (50 wt%) in water. Both monomers are used as received from commercial suppliers without any further purification. After complete addition of the amine monomer, the reaction mixture is stirred at room temperature for 48 hours. After this time, the reaction mixture is freeze-dried and used for further studies without any purification.

PEG N(Bu) (1): $^1\text{H NMR}$ (500 MHz, Deuterium Oxide) δ 4.01 – 3.39 (broad multiplet, OCH), 2.75 – 2.45 (broad multiplet, NCH₂), 1.47 (broad signal, CH₂CH₂CH₃), 1.30 (broad signal, CH₂CH₃), 0.90 (t, $J = 7.4$ Hz, CH₃). GPC(THF): $M_n = 5700$, $M_w = 7300$, PDI (M_w/M_n) = 1.28.

PPO N(Bu) (4): $^1\text{H NMR}$ (500 MHz, Chloroform-*d*) δ 3.90 – 3.24 (broad multiplet, OCH), 2.50 (broad multiplet, NCH₂), 1.40 (broad signal, CH₂CH₂CH₃), 1.27 (broad signal, CH₂CH₃), 1.13 (broad signal, CHCH₃), 0.94 – 0.83 (broad signal, CH₂CH₃). GPC(THF): $M_n = 2200$, $M_w = 3800$, PDI (M_w/M_n) = 1.72.

EO N (Bu) (7): ^1H NMR (500 MHz, Deuterium Oxide) δ 4.01 – 3.43 (broad multiplet, OCH), 2.85 – 2.42 (broad multiplet, NCH₂), 1.48 (broad signal, CH₂CH₂CH₃), 1.31 (broad signal, CH₂CH₃), 0.91 – 0.84 (broad signal, CH₃). GPC(THF): $M_n = 900$, $M_w = 1300$, PDI (M_w/M_n) = 1.44.

BD N(Bu) (10): ^1H NMR (500 MHz, Chloroform-*d*) δ 3.80 (broad signal, HOCH), 3.52 – 3.29 (broad multiplet, OCH₂), 2.64 – 2.39 (broad multiplet, NCH₂), 1.68 – 1.57 (broad multiplet, OCH₂CH₂), 1.41 (broad signal, CH₂CH₂CH₃), 1.33 – 1.20 (broad signal, CH₂CH₃), 0.89 (t, $J = 7.3$ Hz, CH₃). GPC(THF): $M_n = 3800$, $M_w = 6200$, PDI (M_w/M_n) = 1.63.

BD N(Pr) (13): ^1H NMR (500 MHz, Chloroform-*d*) δ 3.81 (broad signal, HOCH), 3.53 – 3.28 (broad multiplet, OCH₂CH₂), 2.61 – 2.41 (broad multiplet, NCH₂), 1.62 (broad signal, OCH₂CH₂), 1.52 – 1.36 (broad signal, CH₂CH₃), 0.86 (t, CH₃). GPC(THF): $M_n = 3000$, $M_w = 4200$, PDI (M_w/M_n) = 1.40.

BD N(Pen) (14): ^1H NMR (500 MHz, Chloroform-*d*) δ 3.85 (broad signal, HOCH), 3.55 – 3.21 (broad multiplet, OCH₂), 2.59 (broad multiplet, NCH₂), 1.63 (broad signal, OCH₂CH₂), 1.46 (broad signal, NCH₂CH₂), 1.36 – 1.17 (broad multiplet, CH₂), 0.87 (t, $J = 9.5$ Hz, CH₃). GPC(THF): $M_n = 4500$, $M_w = 6100$, PDI (M_w/M_n) = 1.35.

BD N(Hex) (15): ^1H NMR (500 MHz, Chloroform-*d*) δ 3.86 (broad signal, HOCH), 3.53 – 3.31 (broad multiplet, OCH₂), 2.61 (broad multiplet, NCH₂), 1.63 (broad signal, OCH₂CH₂), 1.47 (broad signal, NCH₂CH₂), 1.27 (broad signal, CH₂), 0.87 (t, CH₃). GPC(THF): $M_n = 2800$, $M_w = 5200$, PDI (M_w/M_n) = 1.86.

BD N(Hep) (16): ^1H NMR (500 MHz, Chloroform-*d*) δ 3.86 (broad signal, HOCH), 3.43 (broad multiplet, OCH₂), 2.61 (broad multiplet, NCH₂), 1.63 (broad signal, OCH₂CH₂), 1.47 (broad signal, NCH₂CH₂), 1.26 (broad signal, CH₂), 0.87 (t, CH₃). GPC(THF): $M_n = 3700$, $M_w = 5900$, PDI (M_w/M_n) = 1.61.

General procedure for protonation of the polymers

The polymer to be protonated was dissolved in DI water. Hydrochloric acid was then added and the reaction mixture was stirred for a few minutes. After this time, the polymer was purified using a dialysis membrane for overnight. Finally, the aqueous solution is freeze-dried and used for further studies without any purification.

PEG N(H/Bu) (2): ^1H NMR (500 MHz, Deuterium Oxide) δ 4.33 – 4.17 (broad multiplet, HOCH), 3.90 – 3.22 (broad multiplet, OCH₂, NCH₂), 1.69 (broad signal, CH₂CH₂CH₃), 1.39 (broad signal, CH₂CH₃), 0.93 (broad signal, CH₃).

PPO N(H/Bu) (5): ^1H NMR (500 MHz, Chloroform-*d*) δ 4.26 (broad signal, HOCH), 3.94 – 3.27 (broad multiplet, OCH, OCH₂, NCH₂), 1.74 (broad signal, CH₂CH₂CH₃), 1.41 (broad signal, CH₂CH₃), 1.17 (broad signal, CHCH₃), 0.95 (t, $J = 7.4$ Hz, CH₂CH₃).

EO N (H/Bu) (8): ^1H NMR (500 MHz, Deuterium Oxide) δ 4.32 – 2.89 (broad multiplet, OCH, OCH₂, NCH₂), 1.64 (broad multiplet, CH₂CH₂CH₃), 1.41 – 1.25 (broad multiplet, CH₂CH₃), 0.89 (broad signal, CH₃).

BD N(H/Bu) (11): ^1H NMR (500 MHz, Deuterium Oxide) δ 4.27 – 4.14 (broad multiplet, HOCH), 3.62 – 3.48 (broad multiplet, OCH₂), 3.42 – 3.20 (broad multiplet, NCH₂), 1.69 (broad signal, CH₂CH₂CH₃), 1.60 (broad signal, OCH₂CH₂), 1.42 – 1.28 (broad signal, CH₂CH₃), 0.97 – 0.85 (broad signal, CH₃).

General procedure for alkylation of polymers

The polymer (0.2 g) and silver tetrafluoroborate (1.2 eq., per repeating unit) was dissolved in acetonitrile (2 mL). After that, excess alkyl iodide (4 eq., per repeating unit) is added very slowly and drop-wise to the reaction mixture. After complete addition, the reaction mixture is stirred at 65 °C for 48 hours. After this time, the acetonitrile solution is precipitated into cold diethyl ether. The precipitate was separated by centrifugation and the collected solid was purified using a dialysis membrane for overnight. Finally, the aqueous solution is freeze-dried and used for further studies without purification.

PEG N(Bu/Bu) (3): ¹H NMR (500 MHz, Deuterium Oxide) δ 4.39 – 4.22 (broad multiplet, HOCH), 3.95 – 3.15 (broad multiplet, OCH₂, NCH₂), 1.48 – 1.29 (broad multiplet, CH₂CH₃), 1.01 – 0.83 (broad multiplet, CH₃).

PPO N(Bu/Bu) (6): ¹H NMR (500 MHz, Deuterium Oxide) δ 4.26 (broad multiplet, HOCH), 3.99 – 3.25 (broad multiplet, OCH, OCH₂, NCH₂), 1.74 (broad multiplet, CH₂CH₂CH₃), 1.40 (broad multiplet, CH₂CH₃), 1.18 (broad multiplet, CHCH₃), 0.97 (t, *J* = 7.2 Hz, CH₃).

EO N(Bu/Bu) (9): ¹H NMR (500 MHz, Deuterium Oxide) δ 4.27 (broad multiplet, HOCH), 3.94 – 3.16 (broad multiplet, OCH, OCH₂, NCH₂), 1.71 (broad multiplet, CH₂CH₂CH₃), 1.39 (broad multiplet, CH₂CH₃), 0.96 (broad signal, CH₃).

BD N(Bu/Bu) (12): ¹H NMR (500 MHz, Deuterium Oxide) δ 4.25 (br m, HOCH), 3.65 – 3.24 (broad multiplet, OCH₂, NCH₂), 1.84 – 1.56 (broad multiplet, CH₂CH₂CH₃), 1.47 – 1.30 (broad multiplet, CH₂CH₃), 1.02-0.88 (broad multiplet, CH₃).

BD N(Bu/Bu) (12): ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.45 (broad multiplet, OH), 4.15 (broad multiplet, HOCH), 3.57 – 3.17 (broad multiplet, OCH₂, NCH₂), 1.58 (broad multiplet, CH₂CH₂CH₃), 1.28 (broad signal, CH₂CH₃), 0.91 (broad signal, CH₃).

BD N(Pr/Pen) (17): ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.46 (broad multiplet, OH), 4.15 (broad multiplet, HOCH), 3.57 – 3.16 (broad multiplet, OCH₂, NCH₂), 1.88 – 1.42 (broad multiplet, OCH₂CH₂, NCH₂CH₂), 1.27 (broad multiplet, CH₂), 0.88 (broad signal, CH₃).

BD N(Et/Hex) (18): ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.63 – 5.32 (broad multiplet, OH), 4.14 (broad multiplet, HOCH), 3.62- 3.17 (broad multiplet, OCH, NCH₂), 1.74 - 1.48 (broad multiplet, OCH₂CH₂, NCH₂CH₂), 1.37 – 1.05 (broad multiplet, NCH₂CH₃, CH₂), 0.87 (broad signal, CH₃).

BD N(Me/Hep) (19): ¹H NMR (500 MHz, DMSO-*d*₆) δ 5.45 (broad multiplet, OH), 4.18 (broad multiplet, HOCH), 3.53 – 3.23 (broad multiplet, OCH, NCH₂), 3.08 (broad multiplet, NCH₃), 1.70-1.47 (broad multiplet, OCH₂CH₂, NCH₂CH₂), 1.35-1.19 (broad multiplet, CH₂), 0.87 (broad signal, CH₃).

Minimum Inhibitory Concentration (MIC_{>90}) determination

Bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* were grown in Luria-Bertani (LB) medium and *Mycobacterium smegmatis* was grown in tryptic soy broth (TSB) medium at 37 °C overnight. *E. coli* cell suspension was diluted with fresh LB medium to an optical density of 0.01 (inoculum of 10⁷ CFU mL⁻¹) at 600 nm (OD₆₀₀) and suspension of *S. aureus* was diluted with OD₆₀₀ ~ 0.05 (inoculum of 10⁷ CFU mL⁻¹). *M. smegmatis* was diluted with fresh TSB medium to an OD₆₀₀ ~ 0.03 (inoculum of 10⁶ CFU mL⁻¹).

Polymers were first dissolved in DMSO at 50 mg/mL and diluted with PBS buffer (pH 7.4) to different concentrations and sterilized by irradiation in a UV light for 15 min before mixing 1:1 with bacterial cell suspensions in a 96 well plate.

LB medium without any polymer solution was inoculated and used as a positive control and LB medium without any bacteria was used as a negative control. Samples in all experiments were tested in quadruplicate. All samples and controls were incubated at 37 °C for overnight. Inhibitory percentages were calculated by the following equation.

$$\text{inhibition \%} = \frac{OD_{600}(\text{sample}) - OD_{600}(\text{negative control})}{OD_{600}(\text{positive control}) - OD_{600}(\text{negative control})} \times 100 \%$$

$OD_{600}(\text{sample})$ indicates the OD_{600} value for bacteria after overnight incubation with polymer sample.

$OD_{600}(\text{negative control})$ indicates the OD_{600} value for overnight incubation without any bacteria.

$OD_{600}(\text{positive control})$ indicates the OD_{600} value for bacteria after overnight incubation without any polymer.

MIC is defined as the lowest polymer concentration to inhibit >90% bacterial growth ($MIC_{>90}$).

Hemolysis assay

Hemolytic activity of the polymers was determined using sheep's blood. Red blood cells (RBCs) were pelletized by centrifuging 1 mL of the blood and washing the pellet at least four times with PBS buffer solution. A polymer solution of known concentration was diluted with PBS buffer (pH 7.4) to different concentrations and vigorously stirred. Appropriate amounts of polymers and 25 μL of the RBC suspension were added to PBS solution, achieving desired concentrations in a total volume of 1000 μL . The samples were incubated at room temperature for 2 h and afterward, the samples were centrifuged to re-pelletize the blood cells. 200 μL of the supernatant of each sample were placed into wells in a 96-well plate and the absorbance at 540 nm was measured. The percent hemolysis was calculated by following equation.

$$\text{Percent Hemolysis} = \frac{OD_{540}(\text{Sample}) - OD_{540}(\text{negative control})}{OD_{540}(\text{positive control}) - OD_{540}(\text{negative control})} \times 100 \%$$

$OD_{540}(\text{sample})$ indicates the OD_{540} value after 2 h incubation with polymer sample.

$OD_{540}(\text{negative control})$ indicates the OD_{540} value after 2 h incubation with PBS buffer.

$OD_{540}(\text{positive control})$ indicates the OD_{540} value after 2 h incubation with DI water.

Microorganism morphology

E. Coli and *S. aureus* with or without polymers were examined using Field Emission Scanning Electron Microscopy (FE-SEM, Hitach S-4800) operated at 10 kV. *E. Coli* and *S. aureus* were grown in LB medium at 37 °C overnight. Cell suspensions were diluted to $OD_{600} = 1.0$ (inoculum of $\sim 10^9$ CFU mL^{-1}). After making the suspensions, 40 μg of **BD N(Bu/Bu)** was added to a 1 mL cell stock solution of *E. coli* or *S. Aureus*. For the control, cell suspensions without any polymer were used. After incubation at 37 °C for 24 h, all samples were then fixed in glutaraldehyde solution (2.5%) for 2 h at room temperature. The bacteria cells were dehydrated using graded ethanol solutions (30, 50, 70, 90 and 100% v/v in water) and dried under vacuum chamber and observed by FE-SEM.

LIVE/DEAD bacterial viability assays

E. Coli were grown in LB medium at 37 °C overnight. Cell suspensions were diluted to OD₆₀₀ = 1.0 (inoculum of ~10⁹ CFU mL⁻¹). After making the suspensions, 80 µg of **BD N(Bu)** or 1000 µg of **EO N(Bu)** were added to 1 mL cell stock solution of *E. coli*. For control, cell suspensions without any polymer were used. After incubation at 37 °C for 4 h or 24 h, 1.0 mL of LIVE/DEAD BacLight (Kit L7012) commercial solution, which was prepared by adding 3 µL of SYTO (3.34 mM) and 3 µL of propidium iodide (20 mM) to 2 mL of PBS buffer, was added to the solutions. After incubation in the dark for 15 min, cells were viewed under an Olympus BX53 microscope equipped with a 470 nm excitation filter and a 540 nm emission filter for green fluorescence, and 530 nm excitation filter and a 590 nm emission filter for red fluorescence.

Kinetic assay

Cultures of *E. coli* and *S. aureus* (inoculum of 10⁵ CFU mL⁻¹) were incubated with 2 x MIC **12** in LB broth at 37 °C. At defined intervals (10, 30, 60, 120 min), 100 µL of each culture was plated onto LB agar containing no antibiotics and incubated at 37 °C 24 h. Cell viability was assessed by determining CFU·mL⁻¹ values. Each assay was performed in triplicate.

Streptomyces venezuelae (ATCC 15439) assay

Polymers were first dissolved in DMSO at 50 mg/mL and diluted with PBS buffer (pH 7.4) to different concentrations and sterilized by irradiation in a UV light for 15 min before mixing 1:1 with bacterial cell suspensions.

Polymers and Cultures of *Streptomyces venezuelae* (inoculum of 10⁶ CFU mL⁻¹) was grown in tryptic soy broth (TSB) medium at 30 °C overnight.

Antibiotic assay

Bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* were grown in Luria-Bertani (LB) medium and *Mycobacterium smegmatis* was grown in tryptic soy broth (TSB) medium at 37 °C overnight. *E. coli* cell suspension was diluted with fresh LB medium to an optical density of 0.01 at 600 nm (inoculum of 10⁷ CFU mL⁻¹) (OD₆₀₀) and suspension of *S. aureus* was diluted with OD₆₀₀ ~ 0.05 (inoculum of 10⁷ CFU mL⁻¹). *M. smegmatis* was diluted with fresh TSB medium to an OD₆₀₀ ~ 0.03 (inoculum of 10⁶ CFU mL⁻¹).

Antibiotics (kanamycin and carbenicillin) dissolved in 50% H₂O solution at 50 mg/mL were diluted with PBS buffer (pH 7.4) to different concentrations and sterilized by irradiation in a UV light for 15 min before mixing 1:1 with bacterial cell suspensions in a 96 well plate.

LB medium without any polymer solution was inoculated and used as a positive control and LB medium without any bacteria was used as a negative control. Samples in all experiments were tested in quadruplicate. All samples and controls were incubated at 37 °C for overnight. Inhibitory percentages were calculated as mentioned before.

Outer membrane permeabilization assay

The outer membrane permeabilization activity of polymer **12** was determined by an NPN (N-phenyl-naphthylamine) assay. *E. coli* cells (OD₆₀₀ = 0.05, ~10⁸ CFU mL⁻¹) were harvested (4000 rpm, 4 °C, 10 min), washed, and re-suspended in 5 mM glucose/5 mM HEPES buffer at pH 7.2. Then, 30 µg of polymer **12** in HEPES buffer (3 X MIC₉₀) was added to a cuvette containing 1 mL of cells and 10 µM NPN. The excitation and emission wavelengths used

were 350 nm and 420 nm, respectively (slit width was 10 nm in both cases). The uptake of NPN as a measure of outer membrane permeabilization was monitored by the increase in fluorescence of NPN for 10 min. The control experiment was carried out any polymer.

Inner membrane permeabilization assay

E. coli cells ($OD_{600} = 0.05$, $\sim 10^8$ CFU mL⁻¹) were harvested (4000 rpm, 4 °C, 10 min), washed, and re-suspended in PBS buffer at pH 7.2. Then, 30 µg of polymer **12** in PBS buffer (3 X MIC₉₀) was added to a cuvette containing 1 mL of cells and 15 µM propidium iodide (PI). The excitation wavelength was 535 nm (slit width: 10 nm), and the emission wavelength was 617 nm (slit width: 10 nm). The uptake of PI was measured by the increase in fluorescence of PI for 10 min. The control experiment was carried out any polymer.

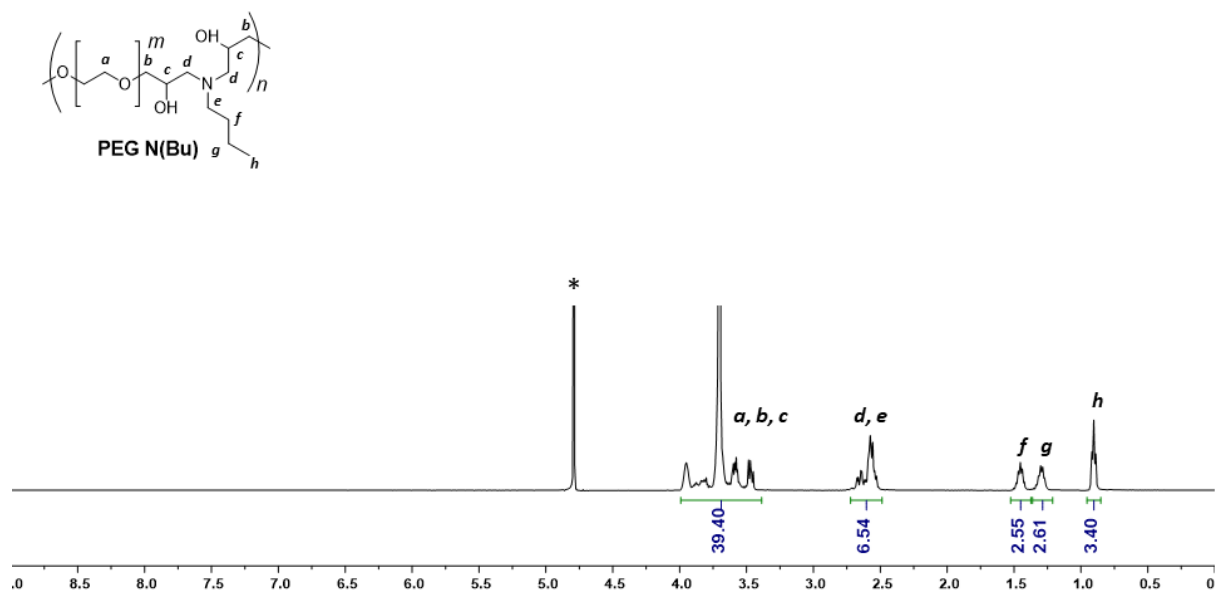


Figure S1. ¹H-NMR of polymer **PEG N(Bu) (1)** in D₂O.

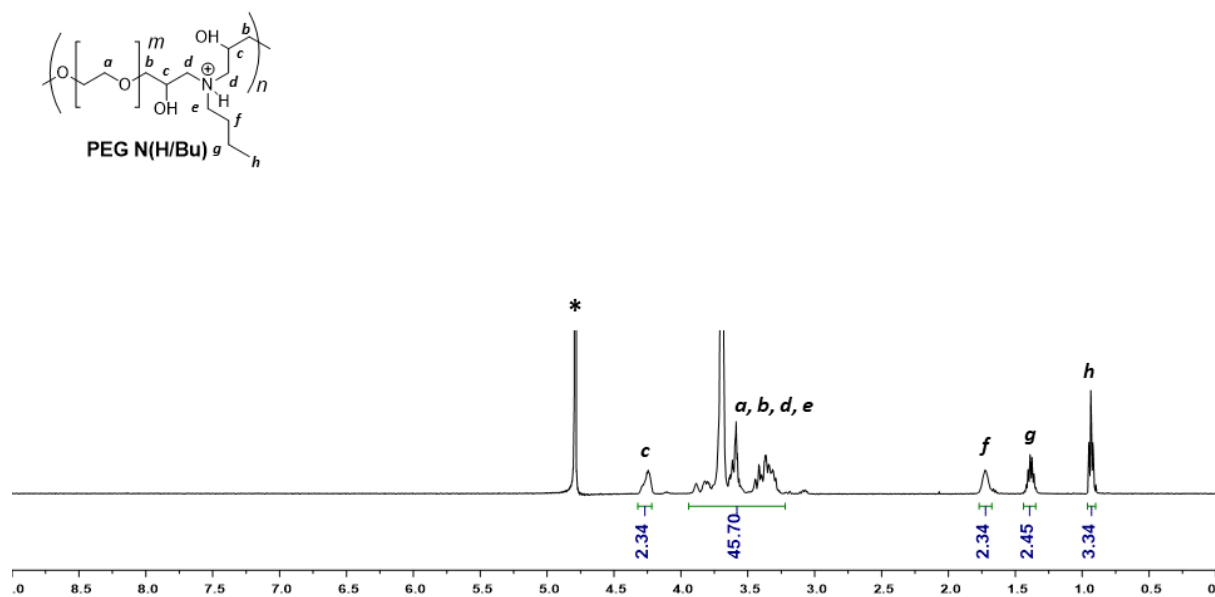


Figure S2. ¹H-NMR of polymer **PEG N(H/Bu) (2)** in D₂O

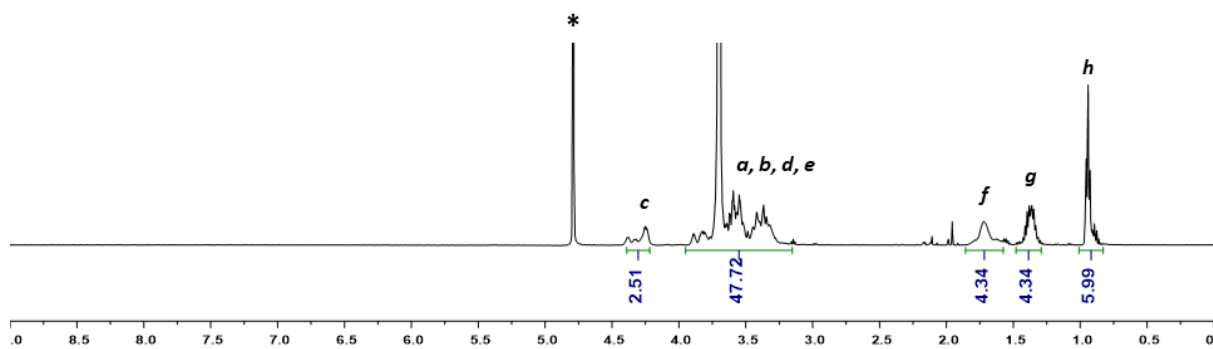
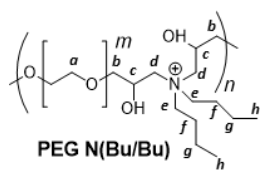


Figure S3. $^1\text{H-NMR}$ of polymer **PEG N(Bu/Bu) (3)** in D_2O

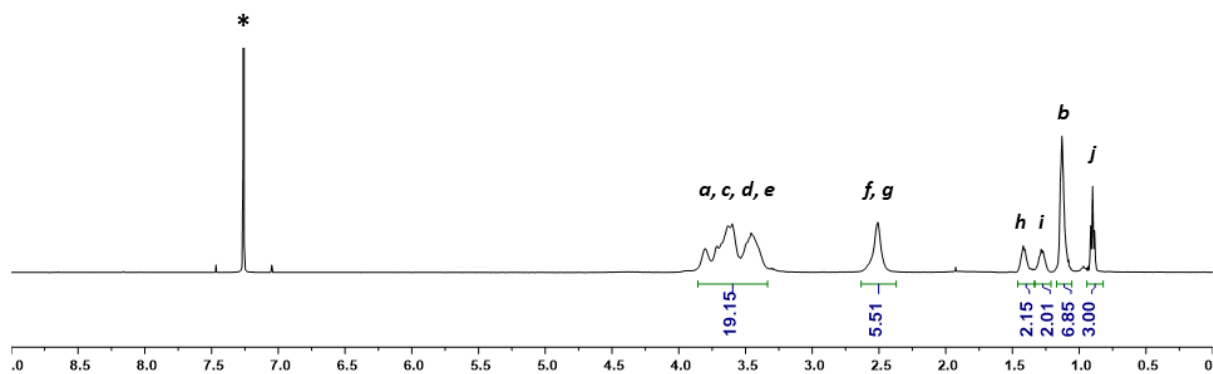
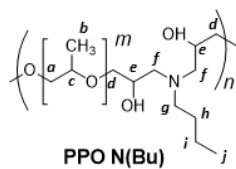


Figure S4. $^1\text{H-NMR}$ of polymer **PPO N(Bu) (4)** in CDCl_3 .

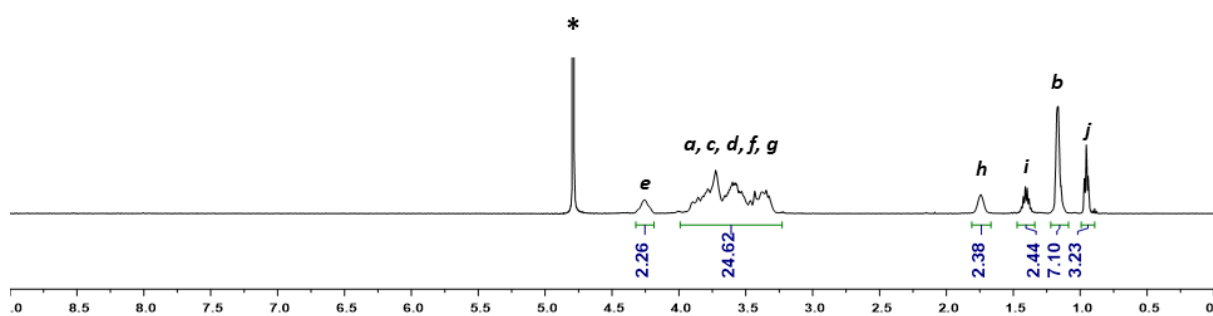
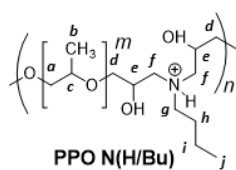


Figure S5. $^1\text{H-NMR}$ of polymer **PPO N(H/Bu) (5)** in D_2O

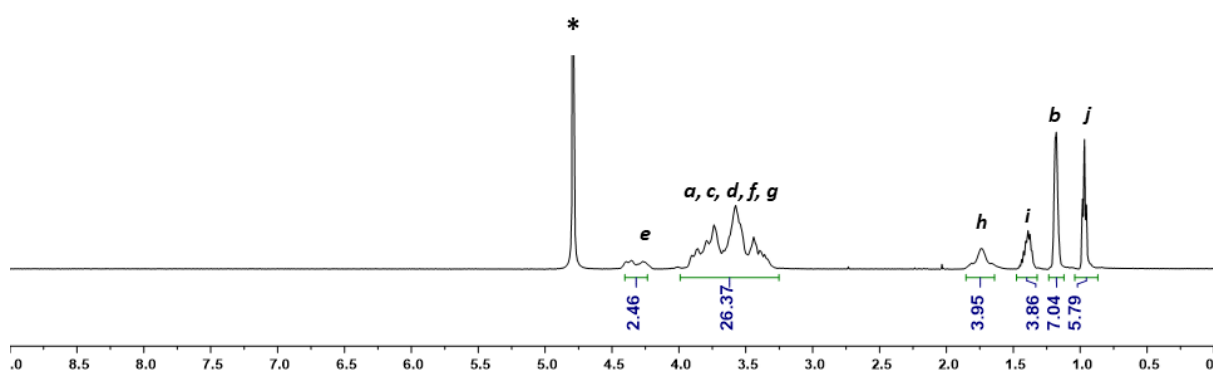
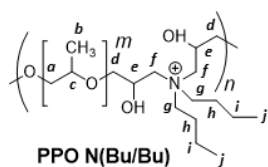


Figure S6. $^1\text{H-NMR}$ of polymer **PPO N(Bu/Bu) (6)** in D_2O

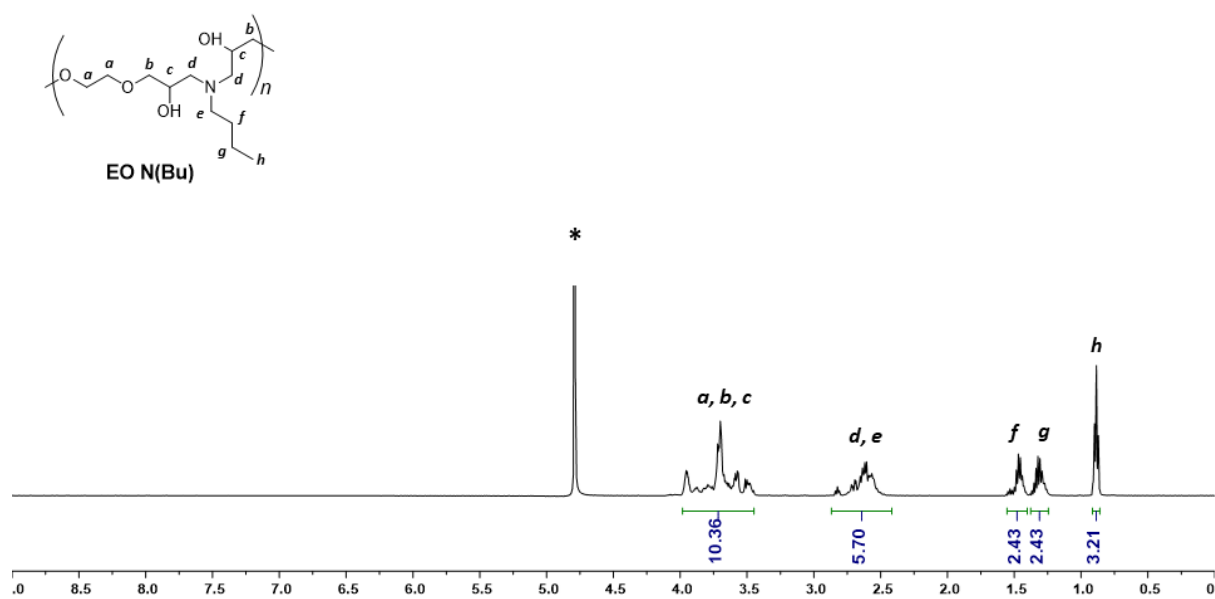


Figure S7. ¹H-NMR of polymer **EO N(Bu) (7)** in D₂O.

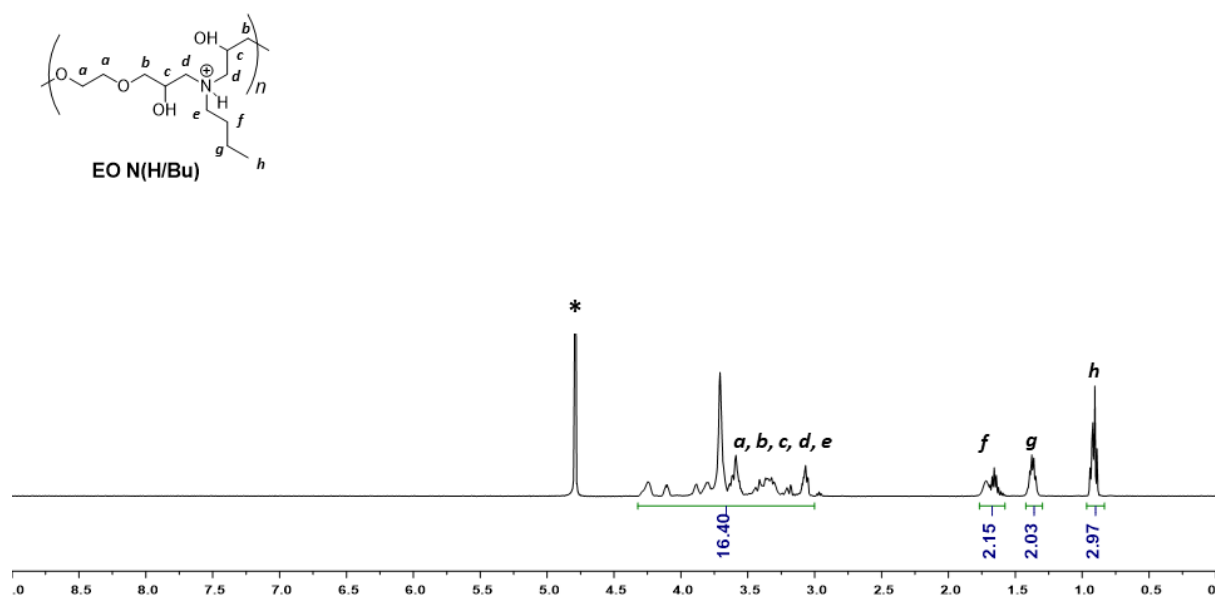


Figure S8. ¹H-NMR of polymer **EO N(H/Bu) (8)** in D₂O

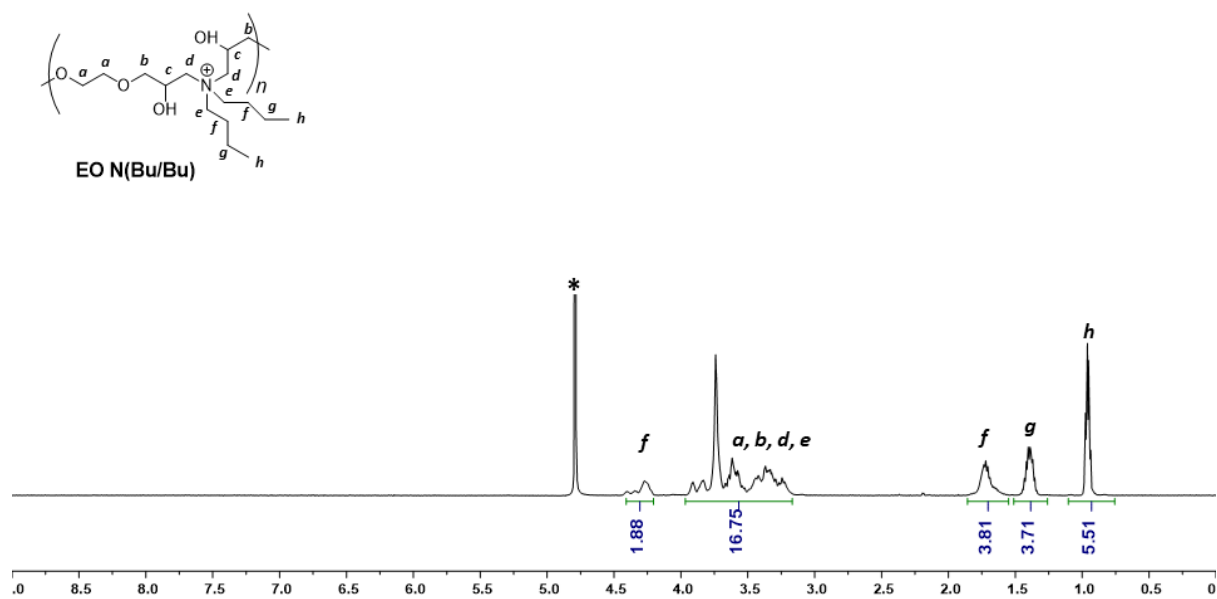


Figure S9. ¹H-NMR of polymer **EO N(Bu/Bu) (9)** in D₂O

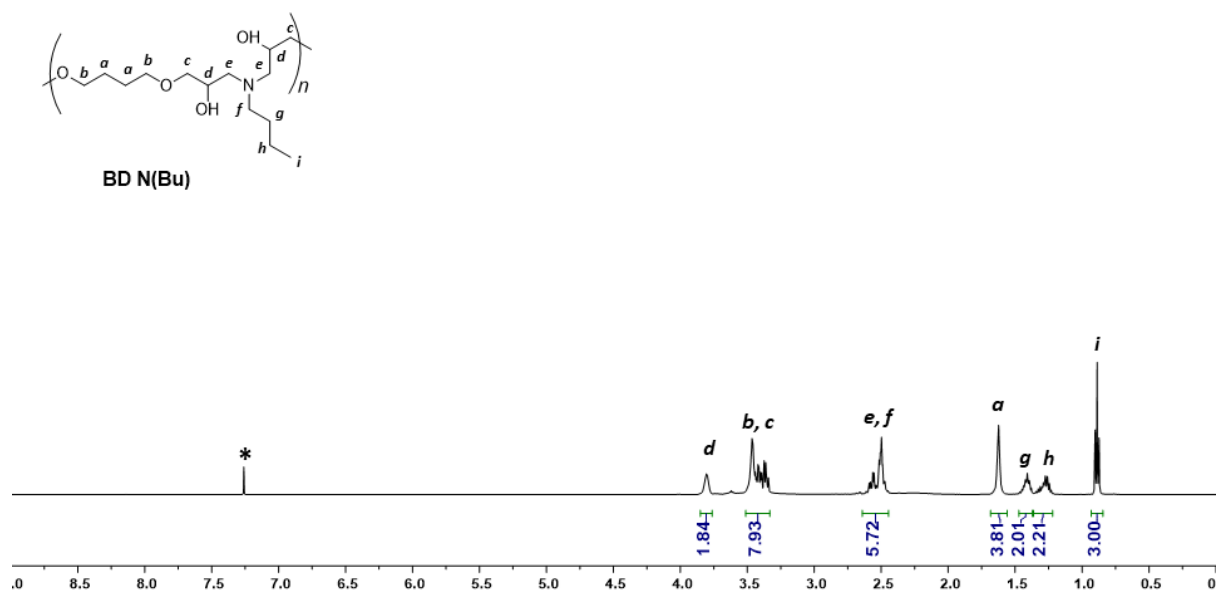


Figure S10. ¹H-NMR of polymer **BD N(Bu) (10)** in CDCl₃.

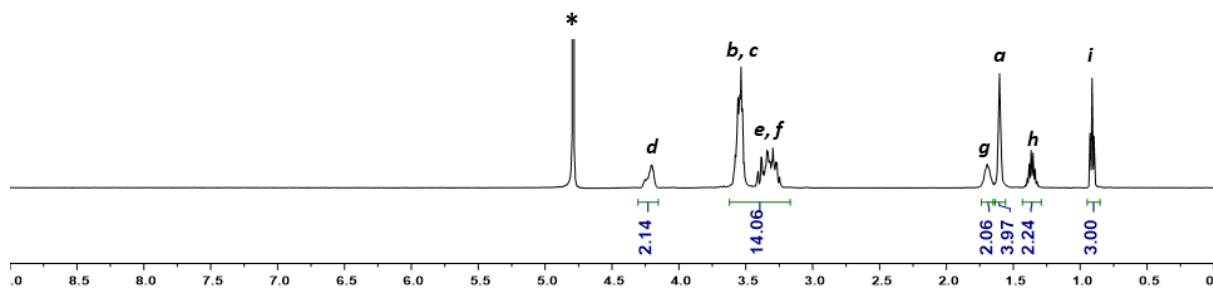
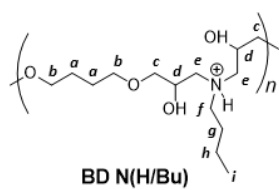


Figure S11. 1H -NMR of polymer **BD N(H/Bu) (11)** in D_2O

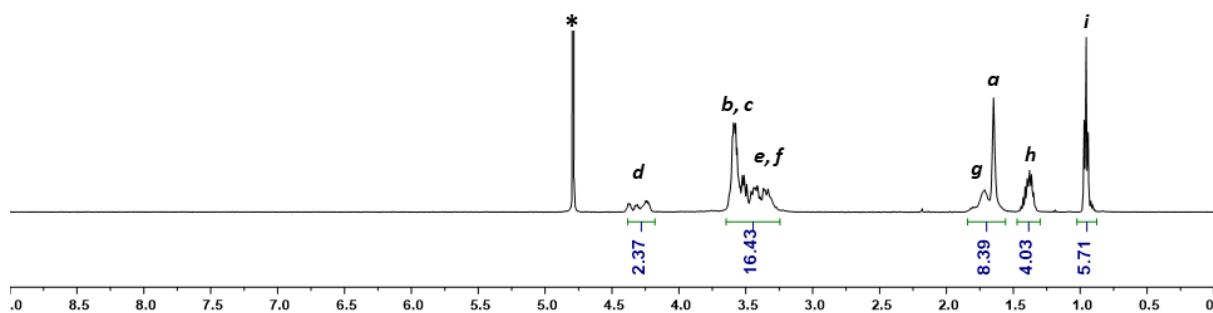
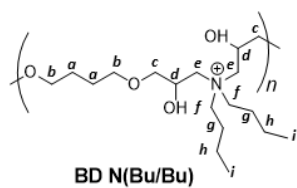


Figure S12a. 1H -NMR of polymer **BD N(Bu/Bu) (12)** in D_2O

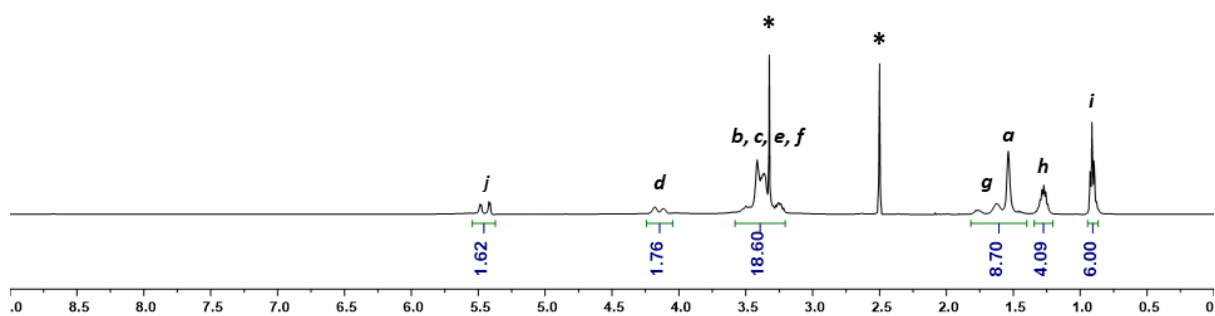
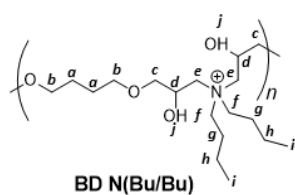


Figure S12b. $^1\text{H-NMR}$ of polymer **BD N(Bu/Bu) (12)** in $\text{DMSO-}d_6$.

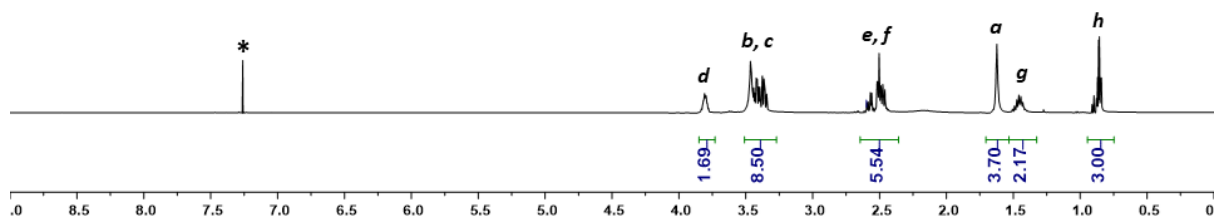
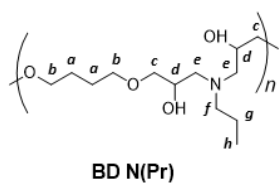


Figure S13. $^1\text{H-NMR}$ of polymer **BD N(Pr) (13)** in CDCl_3 .

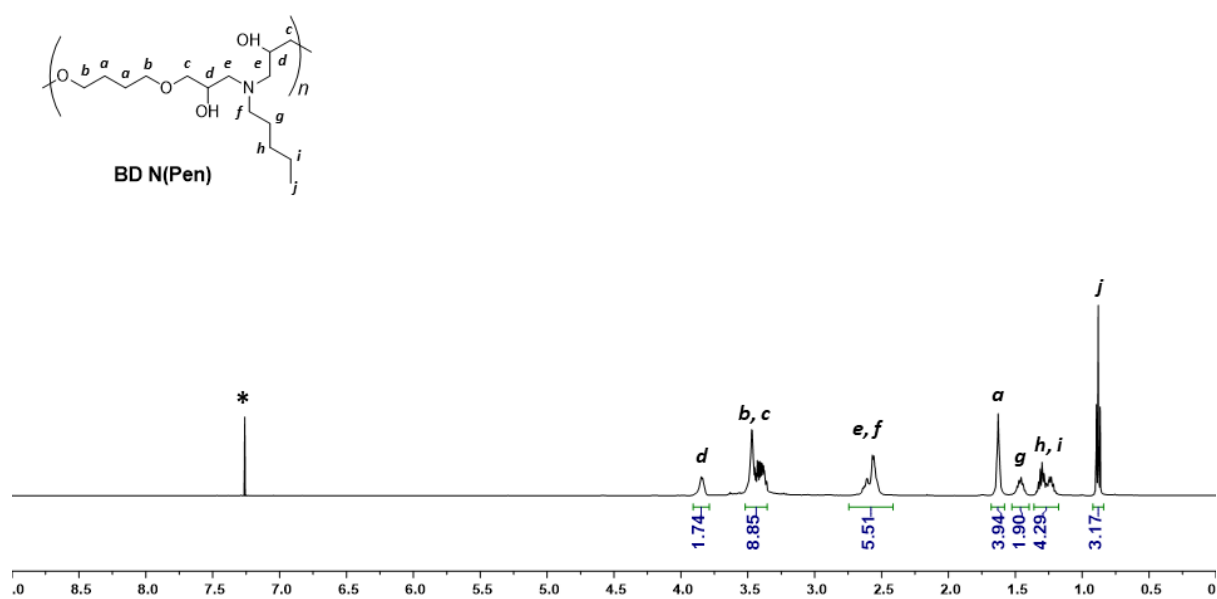


Figure S14. ¹H-NMR of polymer **BD N(Pen)** (14) in CDCl₃.

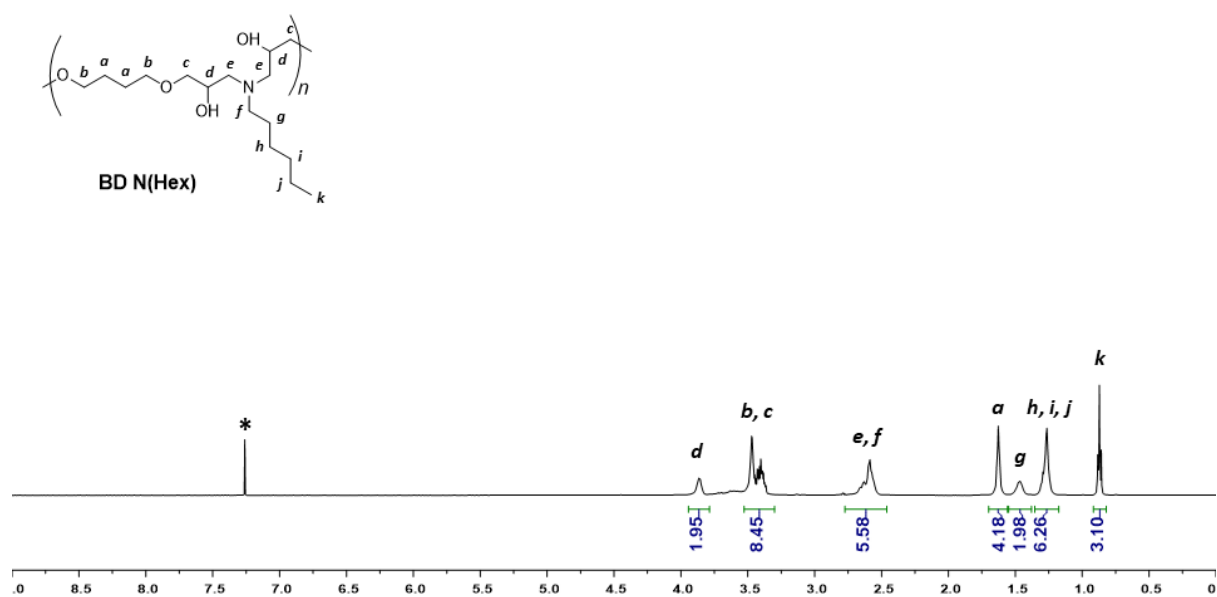


Figure S15. ¹H-NMR of polymer **BD N(Hex)** (15) in CDCl₃.

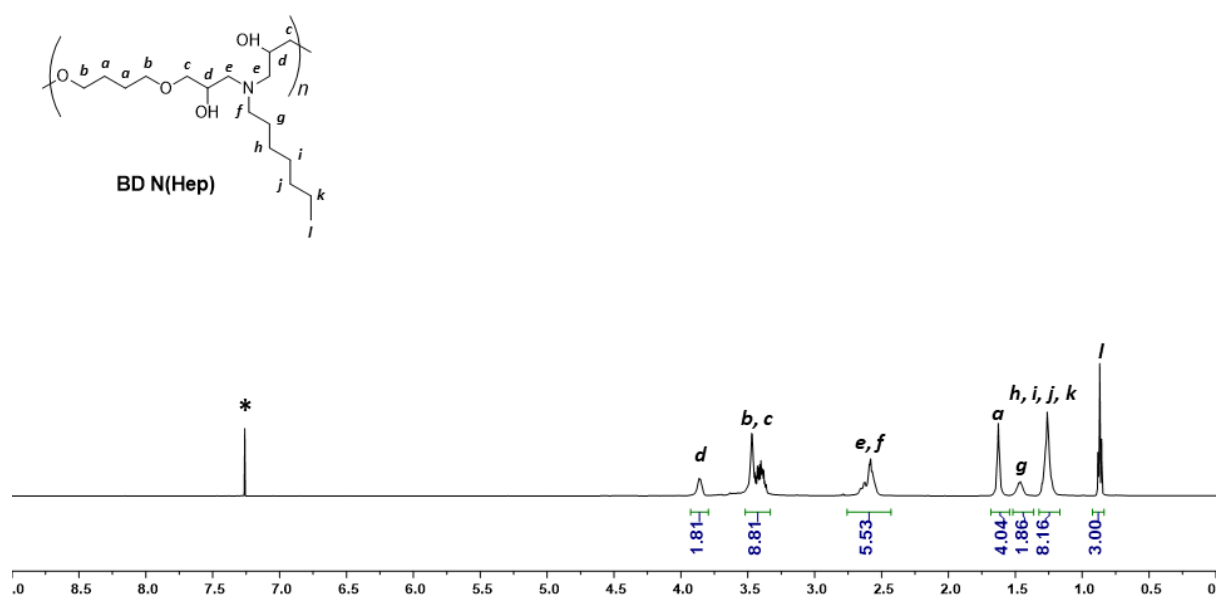


Figure S16. ¹H-NMR of polymer **BD N(Hep)** (16) in CDCl₃.

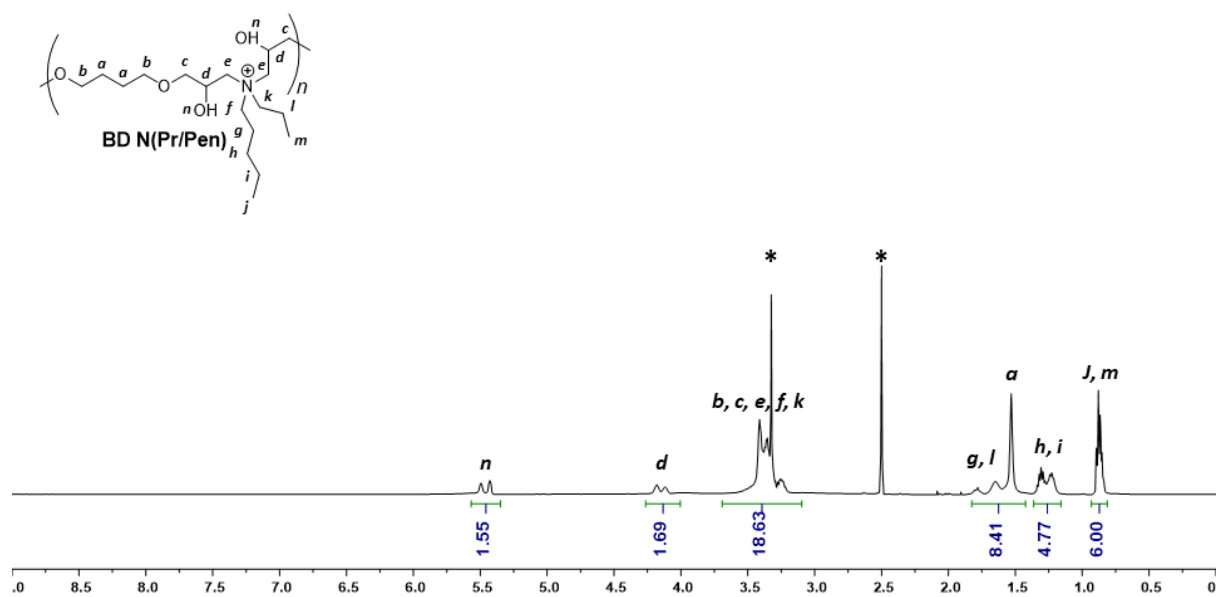


Figure S17. ¹H-NMR of polymer **BD N(Pr/Pen)** (17) in deuterated DMSO-*d*₆.

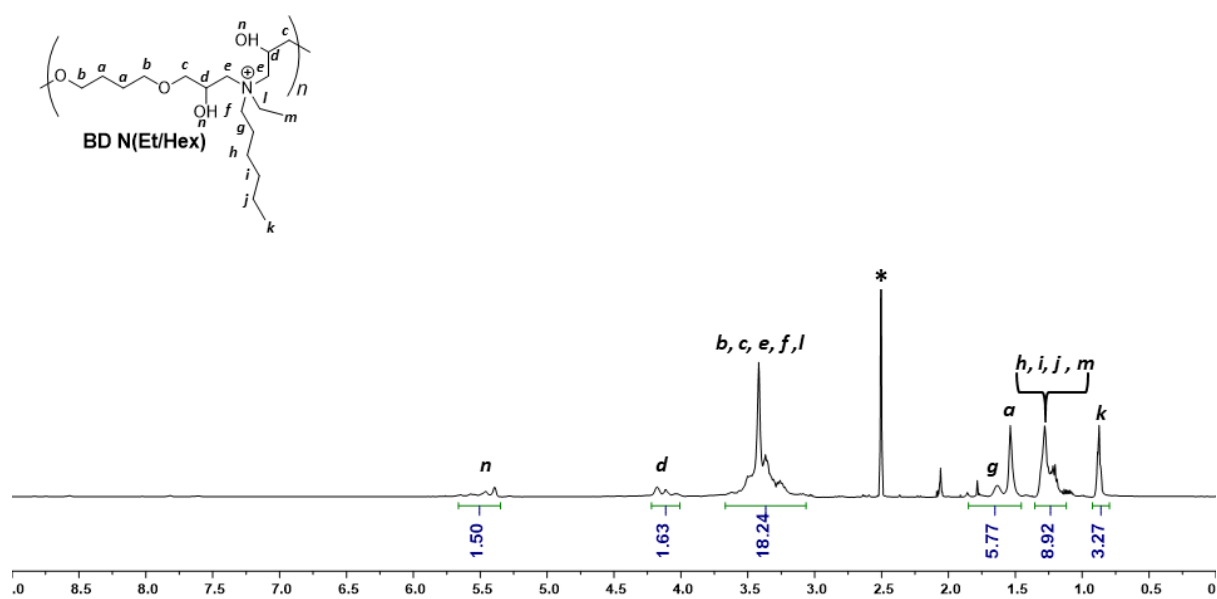


Figure S18. ¹H-NMR of polymer **BD N(Et/Hex) (18)** in deuterated DMSO-d₆.

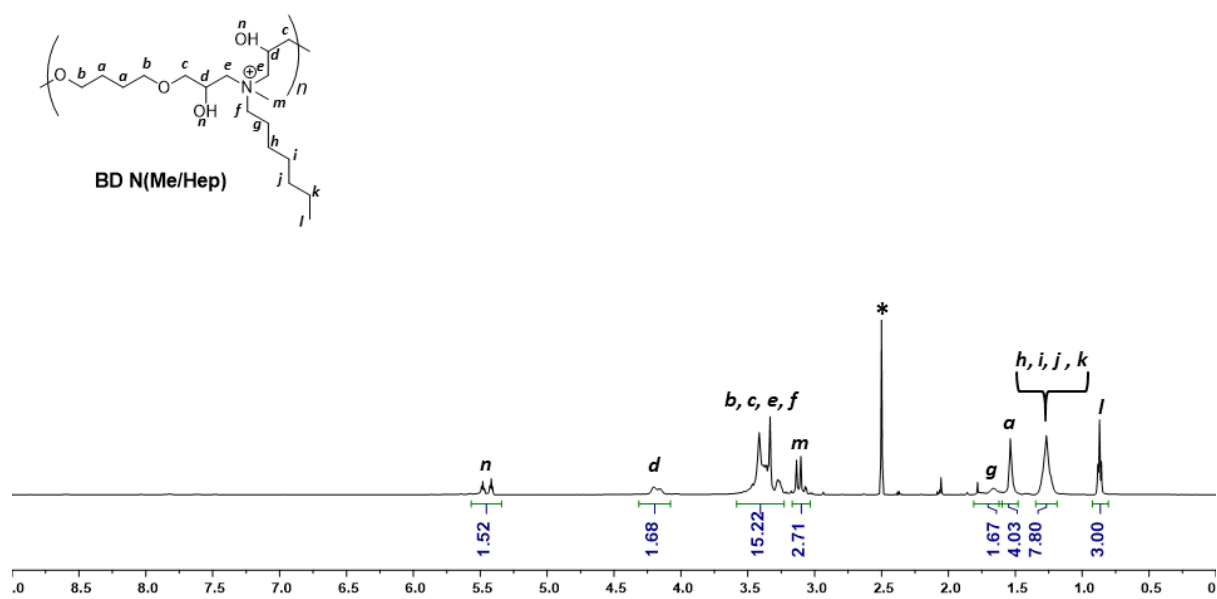


Figure S19. ¹H-NMR of polymer **BD N(Me/Hep) (19)** in deuterated DMSO-d₆.

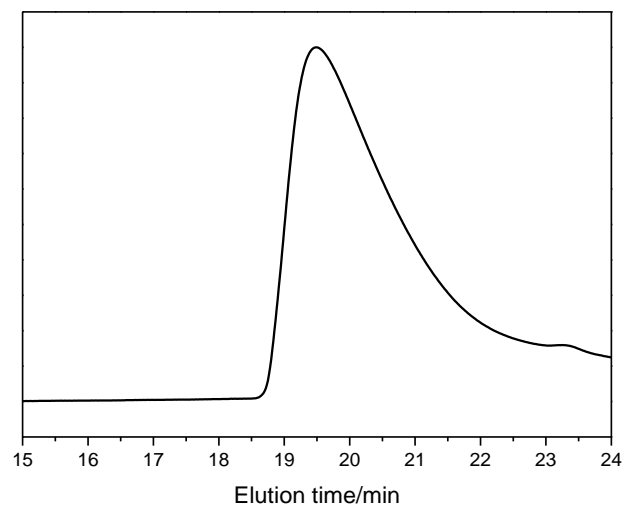


Figure S20. GPC trace (THF) of polymer **PEG N(Bu) (1)**.

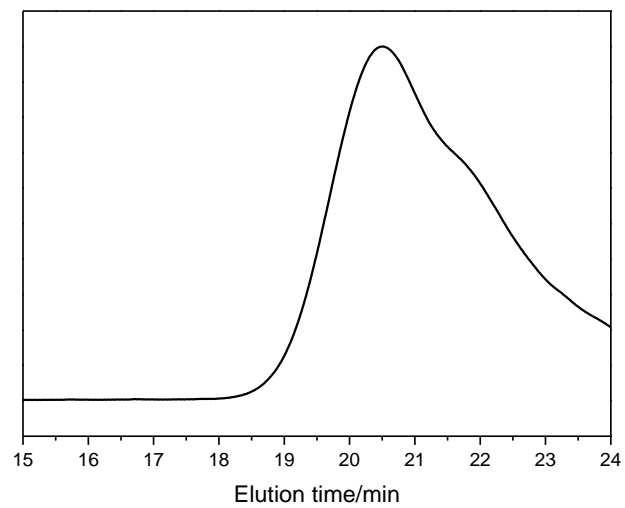


Figure S21. GPC trace (THF) of polymer **PPO N(Bu) (4)**.

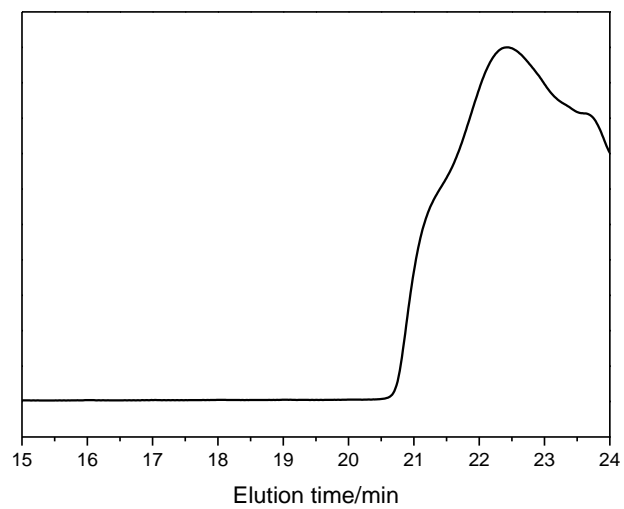


Figure S22. GPC trace (THF) of polymer **EO N(Bu) (7)**.

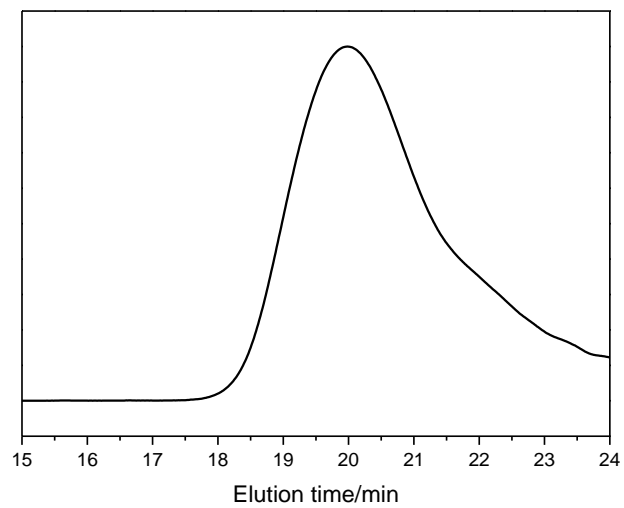


Figure S23. GPC trace (THF) of polymer **BD N(Bu) (10)**.

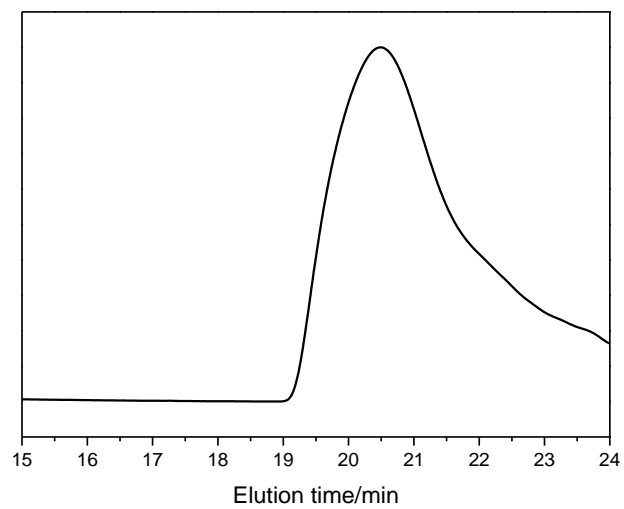


Figure S24. GPC trace (THF) of polymer **BD N(Pr) (13)**.

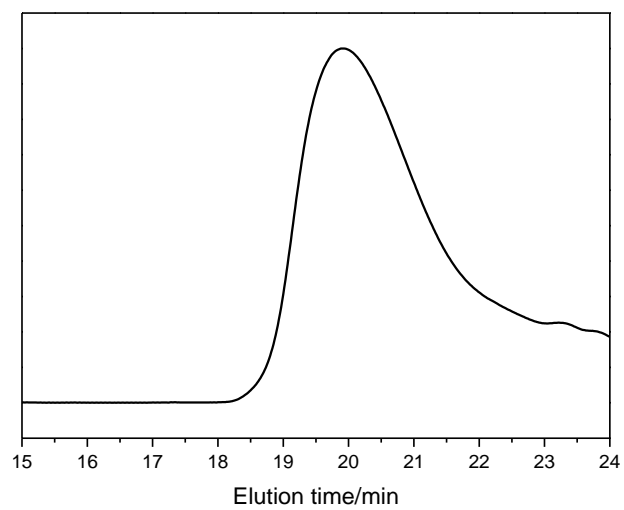


Figure S25. GPC trace (THF) of polymer **BD N(Pen) (14)**.

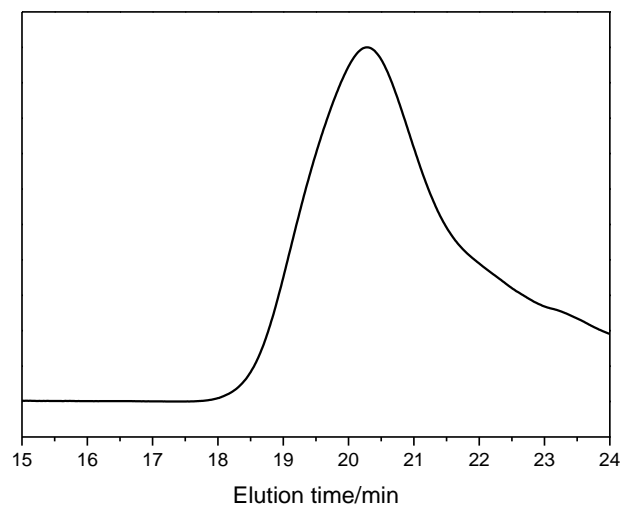


Figure S26. GPC trace (THF) of polymer **BD N(Hex) (15)**.

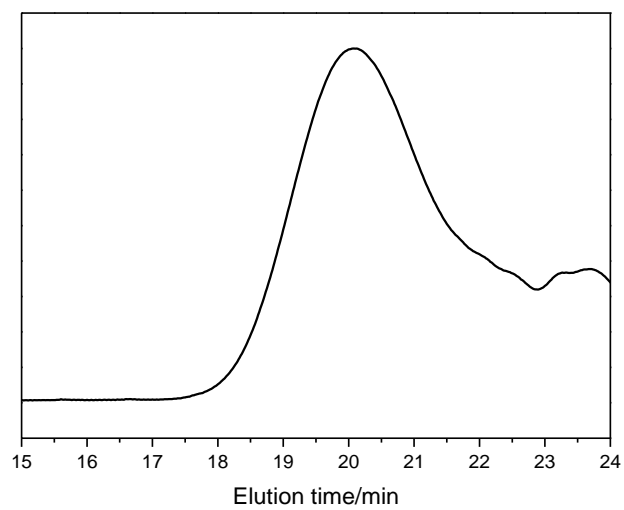


Figure S27. GPC trace (THF) of polymer **BD N(Hep) (16)**.

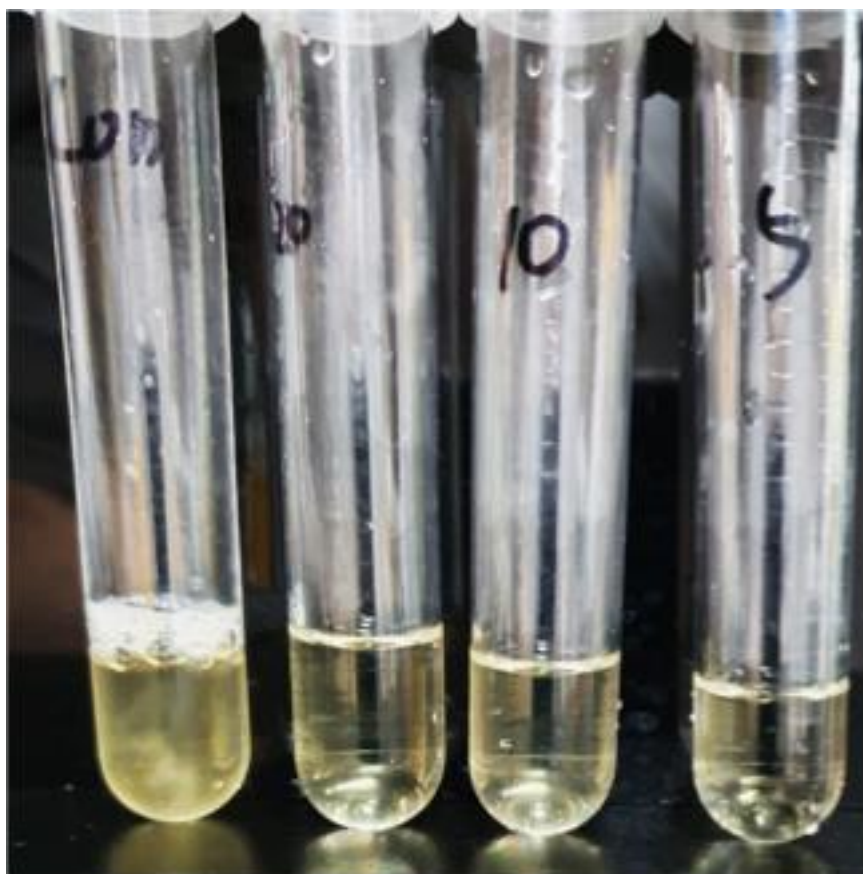
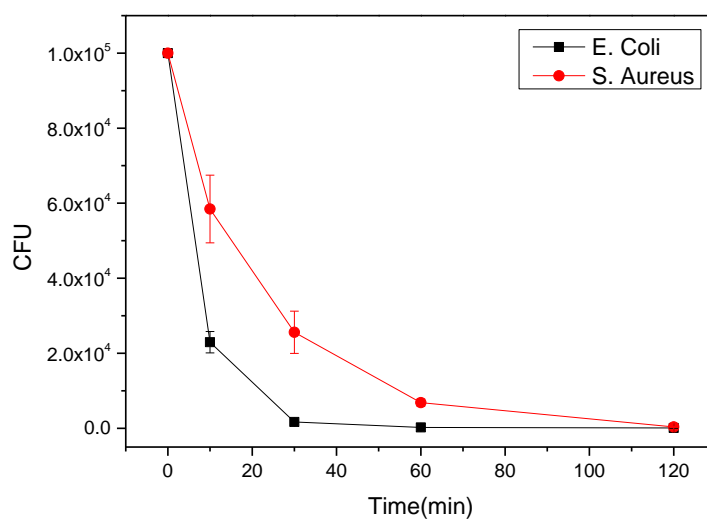
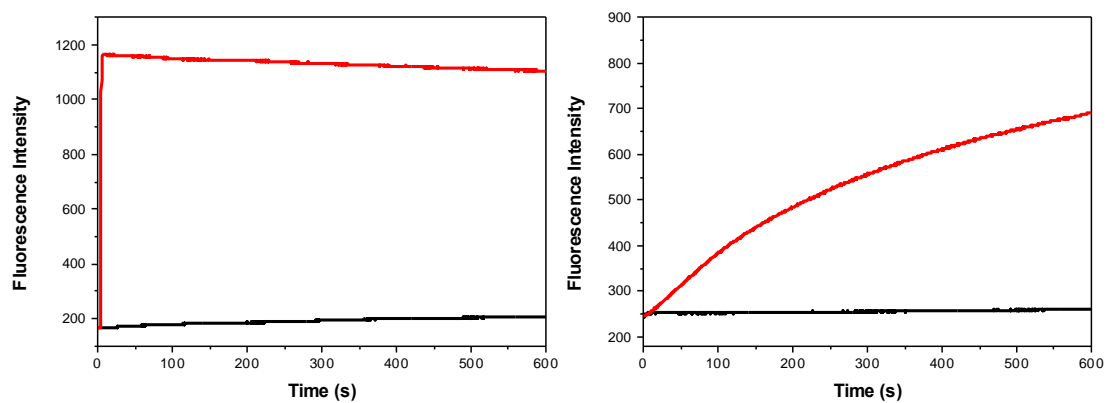


Figure S28. Clearing of the antibiotic producing *streptomyces venezuelae* bacterial suspension (left) upon treatment with 20 (second to left), 10 (second to right), and 5 (right) $\mu\text{g/mL}$ concentration of polymer **12**.



S29. Bacterial death as a function of time while using polymer **12** as an antimicrobial agent.



S30. Outer-membrane permeabilization of *E. coli* by cationic polymer **12** as measured by the increase in 1-*N*-phenyl-naphthylamine fluorescence (red line, left image). Inner-membrane permeabilization of *E. coli* by cationic polymer **12** as measured by the increase in propidium iodide fluorescence (red line, right). The black trace is from the control experiments carried out in absence of **12**.