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Supporting Information for:

Self-Immolative Polymers with Potent and Selective Antibacterial Activity by Hydrophilic Side Chain Grafting

Cansu Ergene and Edmund F. Palermo*

Department of Materials Science and Engineering, Rensselaer Polytechnic Institute, 110 8th St., Troy, New York 12180, United States

Table of Contents

General Experimental

Reagents were purchased commercially and used as received without further purification unless noted. 4,4'-methylenebis(2,6-dimethylphenol) was purchased from Tokyo Chemical Industry (USA). Potassium carbonate (K_2CO_3), allyl bromide (allyl Br), ammonium chloride (NH_4Cl), sodium chloride (NaCl), anhydrous sodium sulfates (Na_2SO_4), silver oxide (Ag_2O), tert-butyldimethylsilyl chloride (TBDMS-Cl), imidazole, 2-aminoethanethiol hydrochloride (cysteamine), poly(ethylene glycol) methyl ether thiol (M_n = 800g/mole and M_n= 2000 g/mole), 2,2- dimethoxy-2-phenylacetophenone (DMPA), cesium fluoride (CsF), Triton X-100, sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate were purchased from Sigma-Aldrich (USA). 1-tert-Butyl-2,2,4,4,4-pentakis(dimethylamino)-2λ^5,4λ^5-catenadi(phosphazene) (P_2-*t*-Bu base) (2.0 M solution in THF) was also purchased from Sigma-Aldrich and stored in a glove box under N_2 atmosphere. BacLight^{TM} Bacterial Viability Kit L-7007 and NHS-Rhodamine (5/6-carboxy-tetramethyl-rhodamine succinimidyl ester) were purchased from Thermo Fisher Scientific (USA). 10% (v/v) red blood cells (RBCs) was obtained from MP Biomedicals (USA). Organic solvents: diethyl ether (Et_2O), N,N-dimethylformamide (DMF), ethyl acetate, hexane, methanol (MeOH), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (USA). Isopropanol (iPrOH) was distilled before use. Anhydrous tetrahydrofuran (THF) was obtained from solvent purification system. Deionized water was purified using EDM Millipore purification system. Sephadex LH-20 was obtained from Sigma-Aldrich. Flash-column chromatography was employed using silica gel (60 Å pore size, 40-63 μm technical grade, Sigma-Aldrich). Thin-layer chromatography was performed on IB2-F J.T. Baker silica gel TLC (Germany).

Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using 500 MHz Agilent NMR spectrometer at 25 °C. NMR chemical shifts were reported in parts per million (ppm, δ) and referenced to tetramethylsilane ($(CH_3)_4Si$, 0.00 ppm) or to residual solvent signals ($CDCl_3$ (δ 7.27), $(CD_3)_2OS$ (δ 2.50), or CD_3OD (δ 3.31 and 4.78). Data are expressed as chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad) and integration. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded using 500 MHz Agilent NMR spectrometer at 25 °C. NMR chemical shifts were

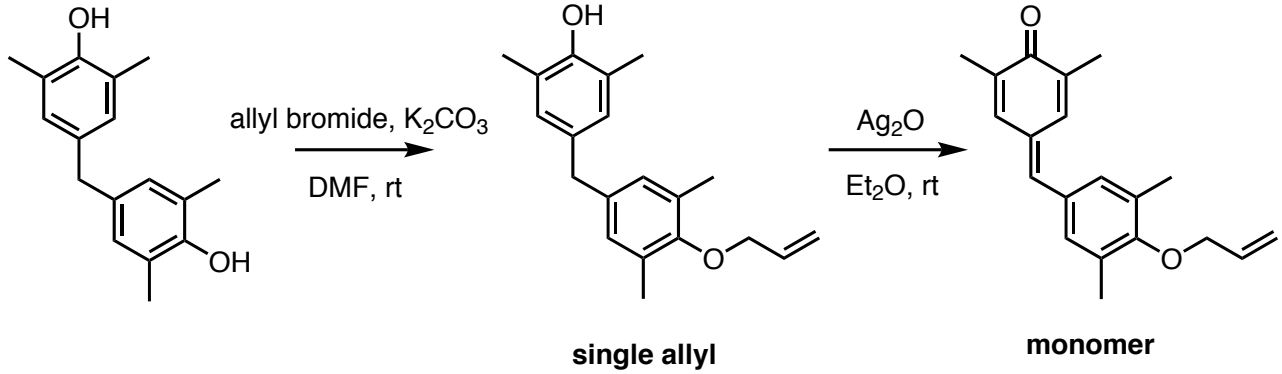
reported in parts per million (ppm, δ) and referenced to residual solvent signals ($CDCl_3$ (δ 77.0), or CD_3OD (δ 49.0).

Size exclusion chromatography (SEC) was performed on Agilent Technologies 1260 Infinity GPC system equipped with a refractive index detector and PLGel columns using THF and DMF as the mobile phase (flow rate: 1 mL/min, 25 °C for THF, flow rate: 1 mL/min, 45 °C for DMF). Molecular weight was calibrated using monodisperse polystyrene standards.

Laser scanning confocal microscopy (Zeiss LSM 510 Meta) was employed using Argon (458-488-514 nm) and HeNe1 (543 nm) lasers. 512 x 512 pixel images were recorded from single scan.

Mass spectra were measured on Thermo LTQ XL Orbitrap mass spectrometer (Thermo, Bremen, Germany) with electrospray ionization ion source. Samples were injected using an Agilent 1200 nano-HPLC system (Agilent, Palo Alto, CA) using an Agilent 1200 autosampler. The flow rate of the solvent was 50 μL/min. The injection volume was 1-2 μL. The data were collected in m/z range of 100-900 at the resolution of 30,000. The accuracy of mass measurements was ~3 ppm.

Synthesis of Monomer



Scheme S1 Synthesis of allyl-protected monomer, $\mathbf{M_0}$

Allyl Br (1.0 equiv) was added dropwise to a stirred mixture of 4,4'-methylenebis(2,6-dimethylphenol) (1.0 equiv) and K_2CO_3 (1.1 equiv) in DMF (0.4 M). After 24 h reaction at room temperature, the mixture was extracted with ethyl acetate and deionized H_2O. The organic layer was washed with saturated NH_4Cl solution and then brine. It was dried over anhydrous Na_2SO_4,

filtered to separate salts and concentrated via rotary evaporation. On TLC plate, there were three spots observed, assigned to compounds with double allyl and single allyl in the side chains, and unreacted starting material. The viscous oil was purified by silica gel column chromatography with gradient elution of solvents (10 – 33% ethyl acetate in hexanes) to afford compound with **single allyl** as a yellow oil (28%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.81 (s, 2H), 6.80 (s, 2H), 6.11 (m, 1H), 5.42 (d, 1H), 5.24 (d, 1H), 4.49 (s, 1H), 4.28 (d, 2H), 3.72 (s, 2H), 2.23 (s, 6H), 2.21 (s, 6H). ^{13}C NMR (500 MHz, $CDCl_3$): δ 154.20, 150.52, 137.14, 134.36, 133.11, 130.84, 129.08, 129.04, 123.04, 117.08, 73.19, 40.59, 16.49, 16.03. HRMS (ESI): calculated for $C_{20}H_{23}O_2$ 295.1704 g/mole, found 295.1683 g/mol.

Ag_2O (2.0 equiv) was added into the solution of **single allyl** compound (1.0 equiv) and Et_2O (0.1 M). The reaction was stirred for 16 h at room temperature. The mixture was filtered to remove silver oxide particles, concentrated via rotary evaporator and recrystallized in hot cyclohexane to afford yellow crystals. The **monomer** crystals were ground and dried in vacuum for 72 h. Dry monomer (67%) was store in inert atmosphere of glovebox for polymerization. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.55 (s, 1H), 7.15 (s, 2H), 7.07 (s, 1H), 7.03 (s, 1H), 6.13 (m, 1H), 5.46 (d, 1H), 5.30 (d, 1H), 4.37 (d, 2H), 2.34 (s, 6H), 2.08 (s, 3H), 2.06 (s, 3H)[1]. ^{13}C NMR (500 MHz, $CDCl_3$): δ 187.25, 157.32, 142.93, 139.07, 137.27, 135.30, 133.65, 131.70, 131.42, 131.30, 131.26, 117.57, 73.26, 16.94, 16.55, 16.21. HRMS (ESI): calculated for $C_{20}H_{23}O_2$ 295.1704 g/mole, found 295.1684 g/mol.

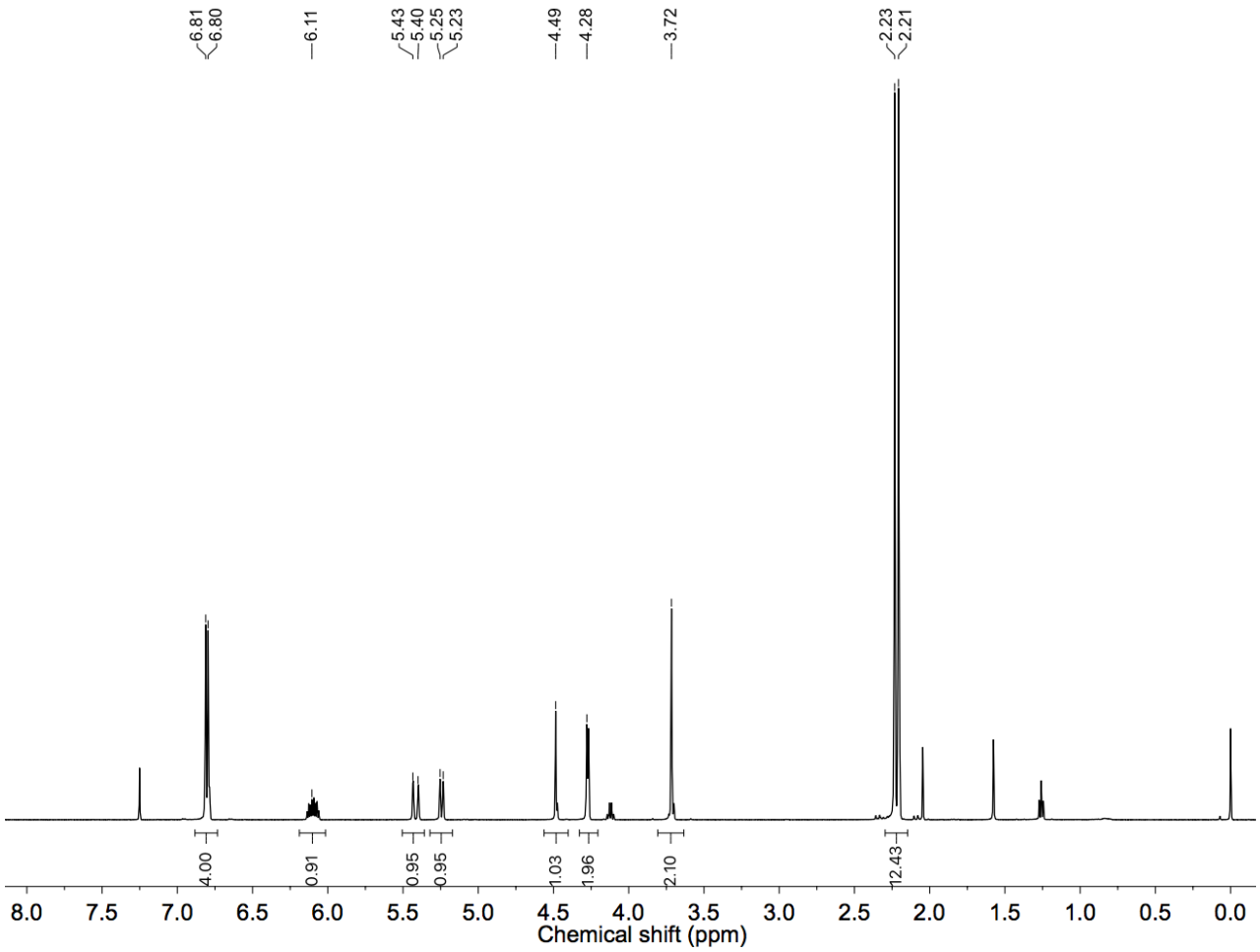


Figure S1 ^{1}H NMR spectrum of **single allyl** compound ($CDCl_3$, 500 MHz)

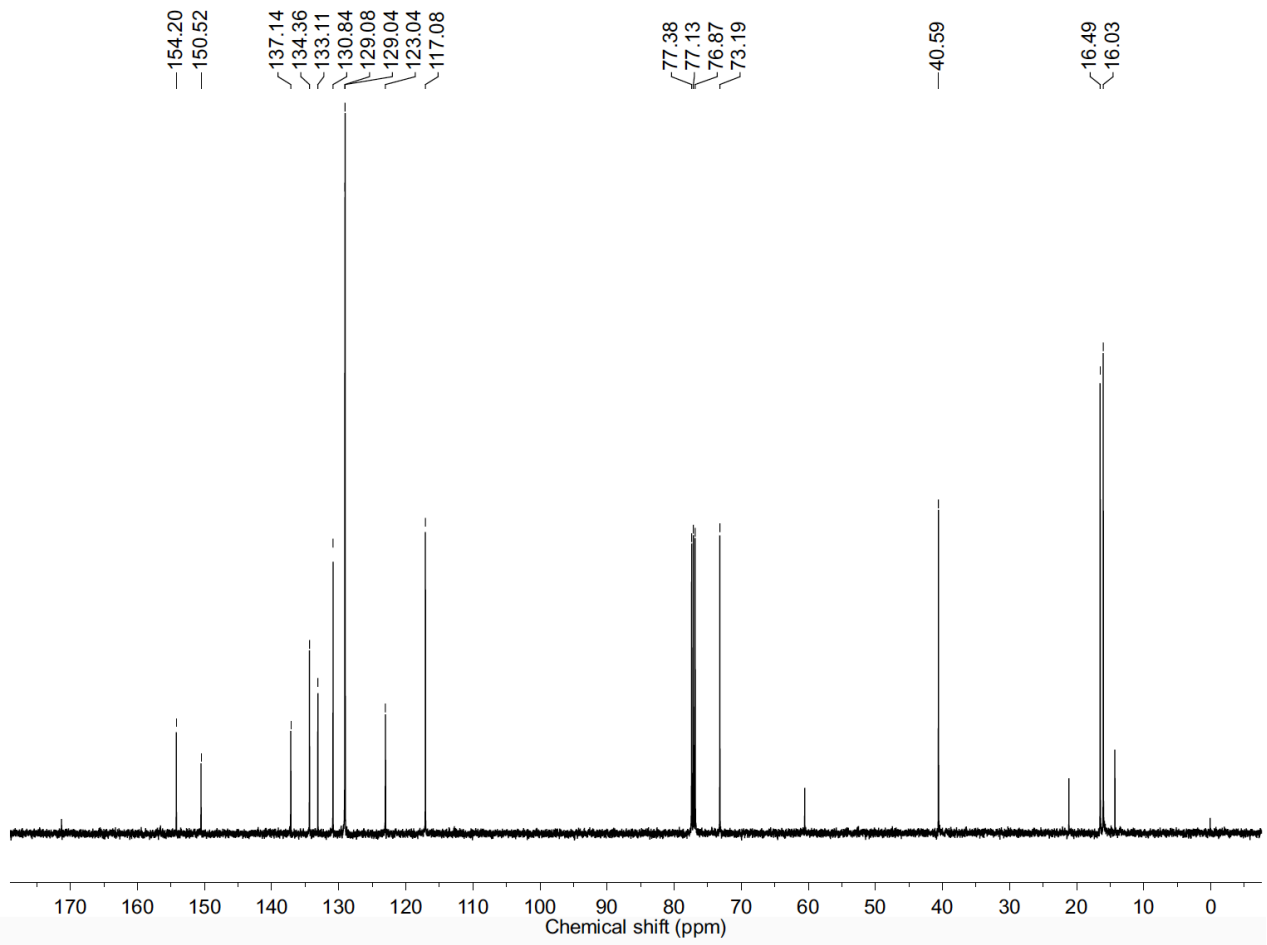


Figure S2 ^{13}C NMR spectrum of **single allyl** compound ($CDCl_3$, 500 MHz)

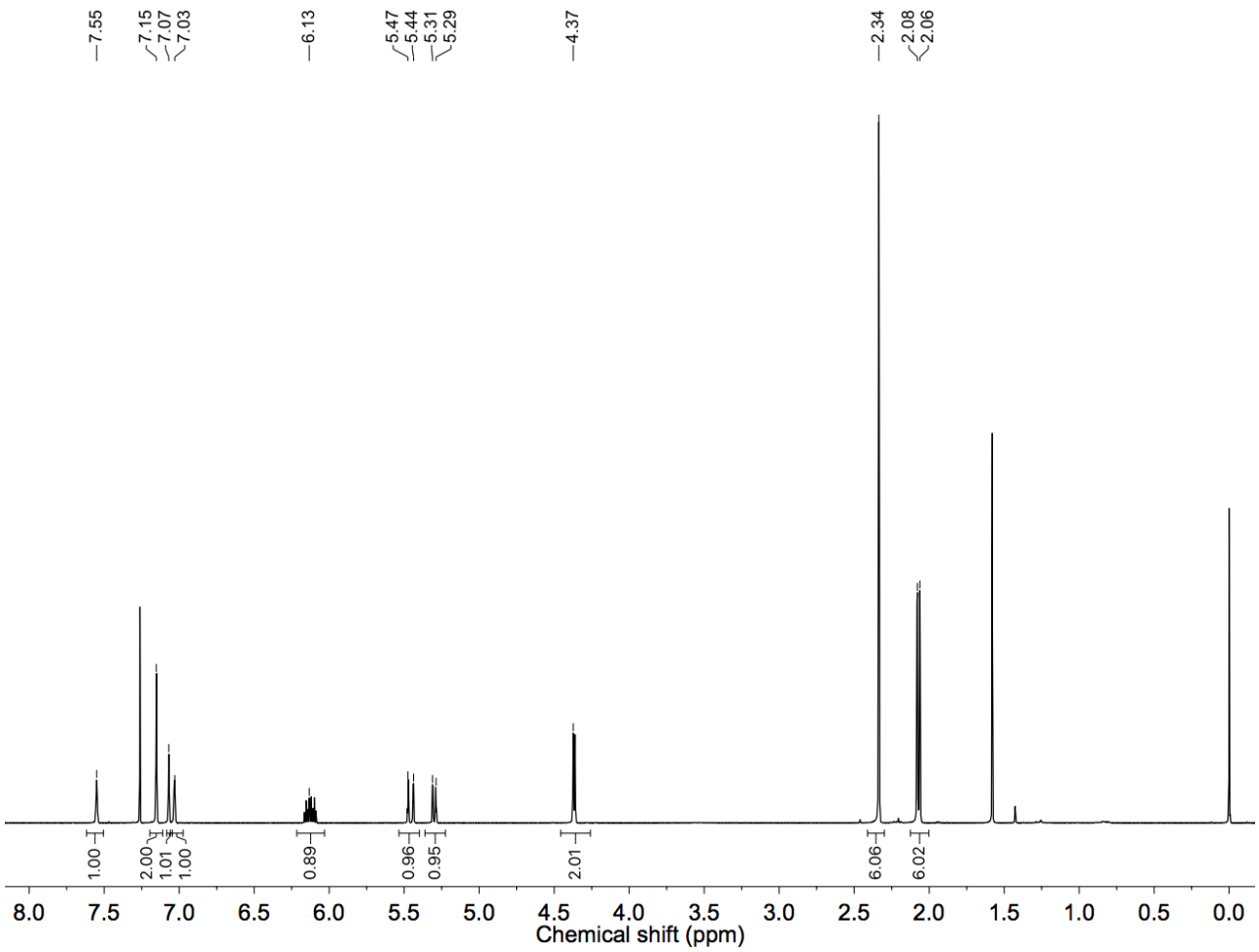


Figure S3 ^{1}H NMR spectrum of **monomer, M_0** ($CDCl_3$, 500 MHz)

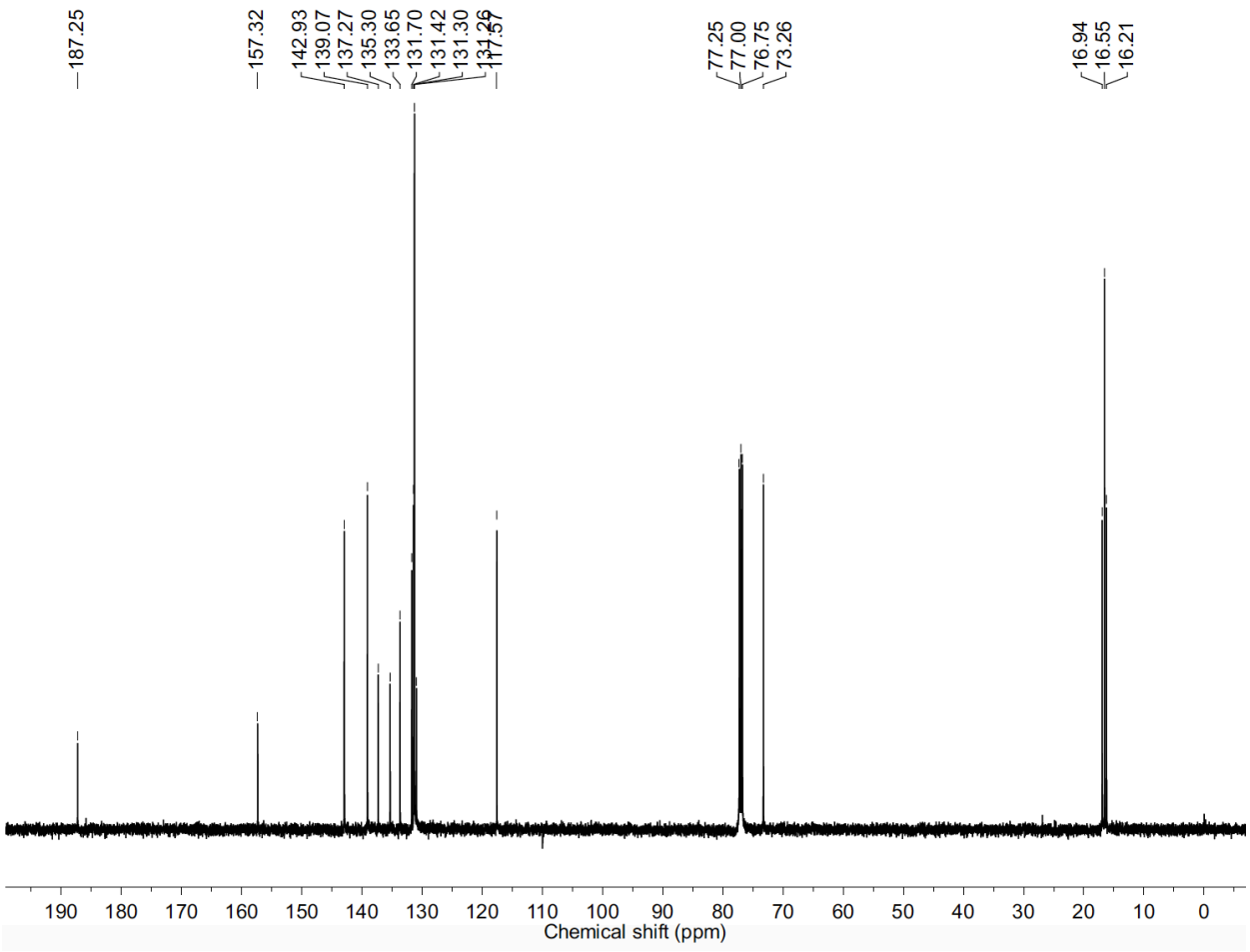
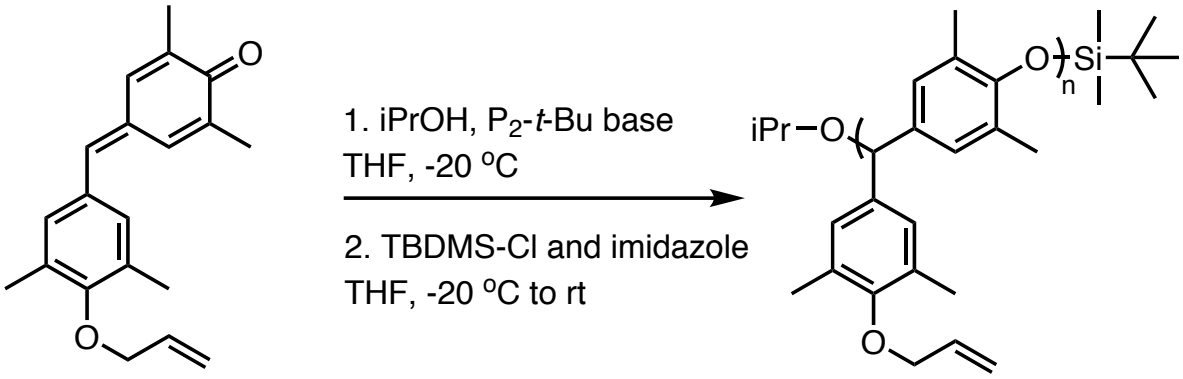


Figure S4 ^{13}C NMR spectrum of **monomer, M_0** ($CDCl_3$, 500 MHz)

Synthesis of Polymers

Monomer (1.0 equiv) was dissolved in anhydrous THF. A stock solution of distilled iPrOH (0.1 equiv) and 2.0 M P_2-*t*-Bu base solution (0.1 equiv) in THF was prepared in anhydrous THF (1:1 ratio). The chain length of polymer was tuned by altering the number of equivalents of initiator relative to monomer. Based on that, certain amount of initiator-base stock solution was added into monomer solution to pre-initiate and stirred for 1 h at room temperature. Final concentration of reaction was adjusted to 0.8 M. The reaction became dark red from bright yellow color after the addition of base. Following the initiation, polymerization was conducted in -20 °C for 4 h with stirring. All steps were carried out in glovebox under inert N_2 environment.

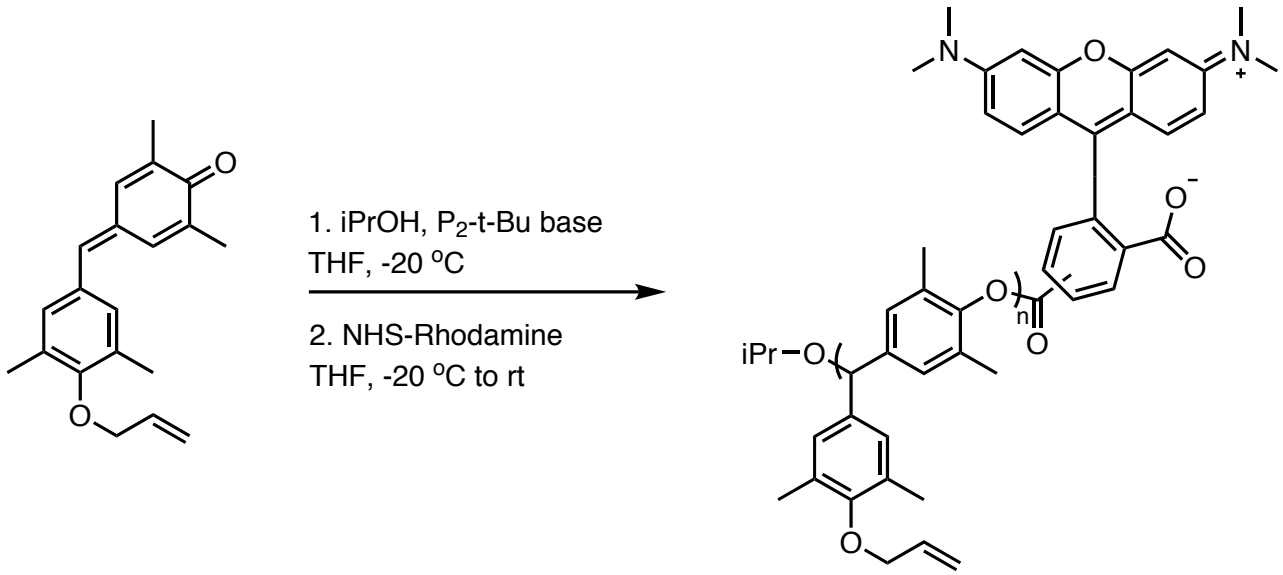
End-capping with TBDMS-Cl



Scheme S2 Polymerization and TBDMS end-capping

TBDMS-Cl (1.0 equiv) and imidazole (1.0 equiv) were dissolved in anhydrous THF and then injected into polymer reaction at -20 °C which immediately turned into orange-yellow color from dark red. Reaction was stirred for 24 h at -20 °C. It was allowed to warm to room temperature and continued to stir at this temperature for few hours. The polymer was precipitated in MeOH and collected via centrifuge. Excess MeOH was decanted. The polymer was redissolved in THF and precipitated in MeOH and centrifuged again, whole process was repeated three times. Polymers were dried in vacuum for 24 h. (All equivalents were relative to initiator). ^{1}H NMR (CD_2Cl_2, 500 MHz): δ 6.92 (bs, 4H), 6.08 (m, 1H), 5.52 (bs, 1H), 5.42 (d, 1H), 5.25 (d, 1H), 4.29 (bs, 2H), 2.21 (bs, 6H), 1.85 (s, 3H), 1.84 (s, 3H). *(1.01 (s, 9H) and 0.17 (s, 6H) ppm correspond to TBDMS end-capping group, integration in NMR spectrum was expressed based on single unit of the polymer)*

End-capping with NHS-Rhodamine



Scheme S3 Polymerization and NHS-Rhodamine end-capping

NHS-Rhodamine (5/6-carboxy-tetramethyl-rhodamine succinimidyl ester**)** (0.5 equiv) was dissolved in anhydrous THF and then injected into polymer reaction at -20 °C which immediately. Reaction was stirred for 24 h at -20 °C. It was allowed to warm to room temperature and continued to stir at this temperature for few hours. The polymer was precipitated in MeOH and collected via centrifuge. Excess MeOH was decanted. The polymer was redissolved in THF and precipitated in MeOH and centrifuged again, whole process was repeated three times. Polymers were dried in vacuum for 24 h. (All equivalents were relative to initiator). ^{1}H NMR (CD_2Cl_2, 500 MHz): δ 6.92 (bs, 4H), 6.08 (m, 1H), 5.52 (bs, 1H), 5.42 (d, 1H), 5.25 (d, 1H), 4.29 (bs, 2H), 2.21 (bs, 6H), 1.85 (s, 3H), 1.84 (s, 3H). *(integration in NMR spectrum was expressed based on single unit of the polymer)*

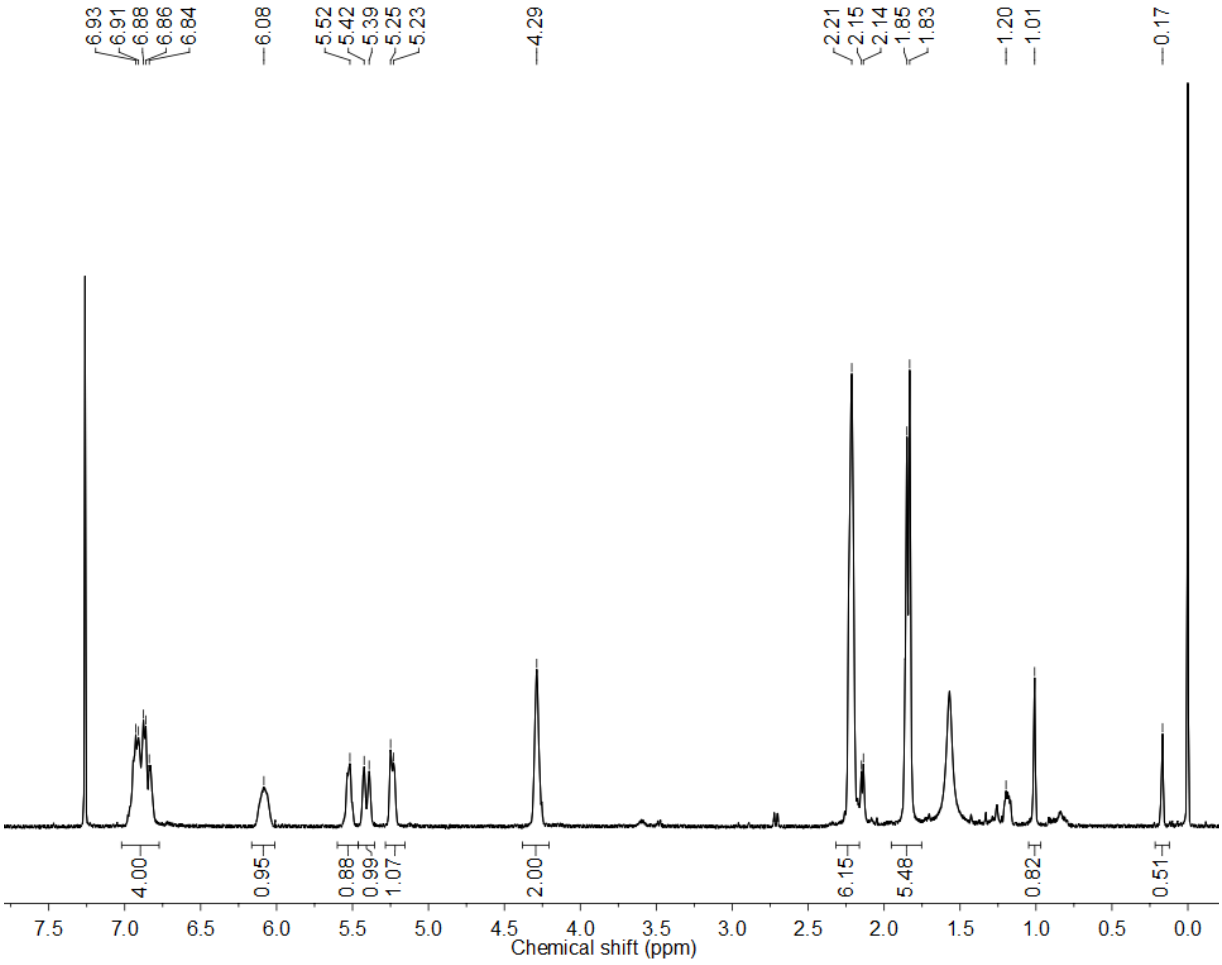


Figure S5 ^{1}H NMR spectrum of **P_0-1** ($CDCl_3$, 500 MHz)

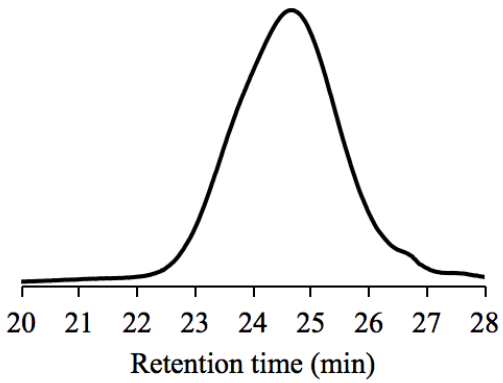


Figure S6 SEC trace of PBEs with allyl side groups **(P_o-1)**

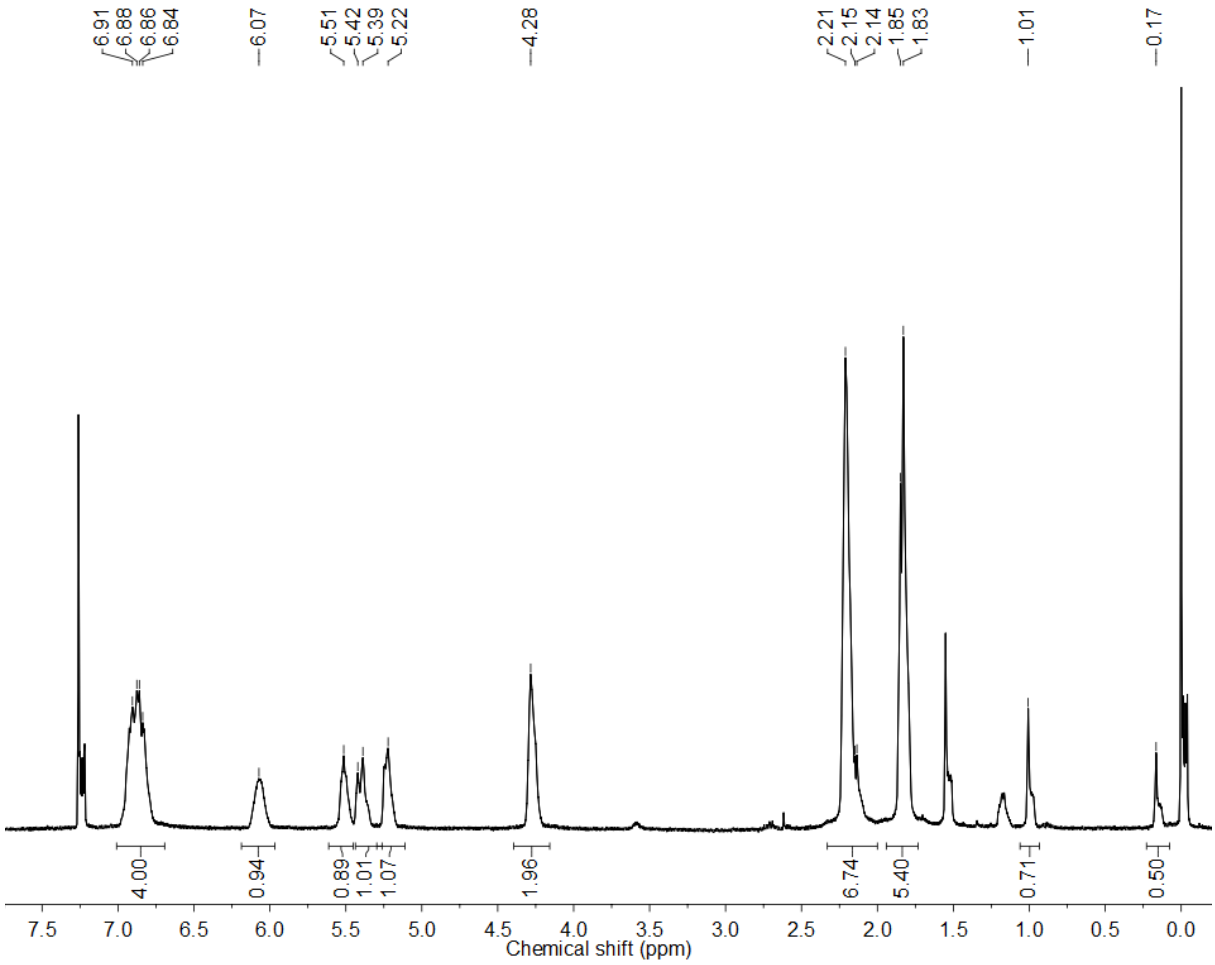


Figure S7 ^{1}H NMR spectrum of **P_0-2** ($CDCl_3$, 500 MHz)

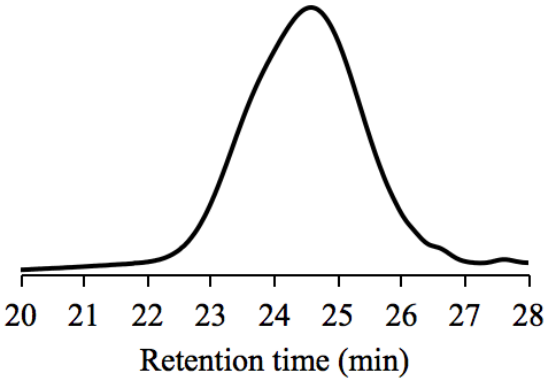


Figure S8 SEC trace of PBEs with allyl side groups (**Po-2**)

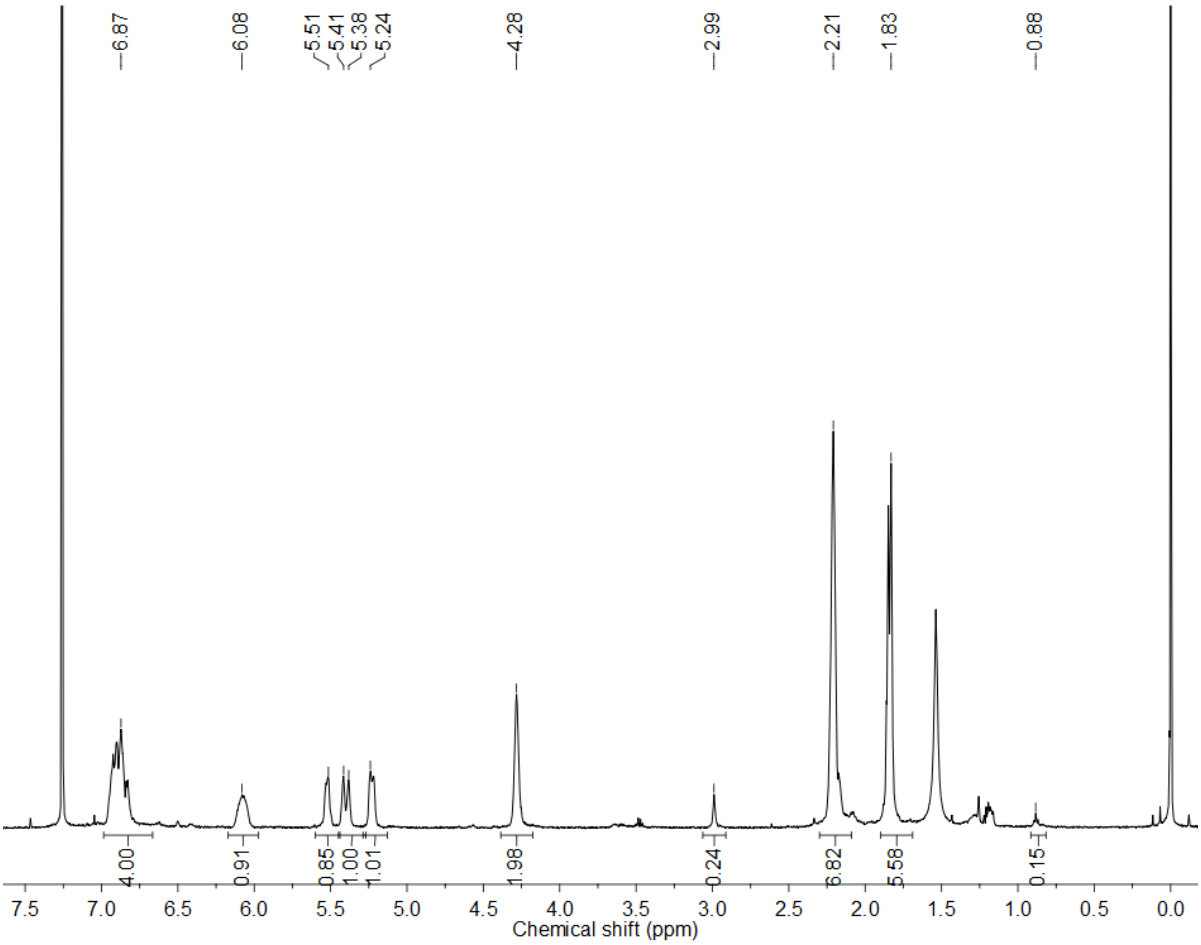


Figure S9 ^{1}H NMR spectrum of **RhB-P$_0$-1** ($CDCl_3$, 500 MHz)

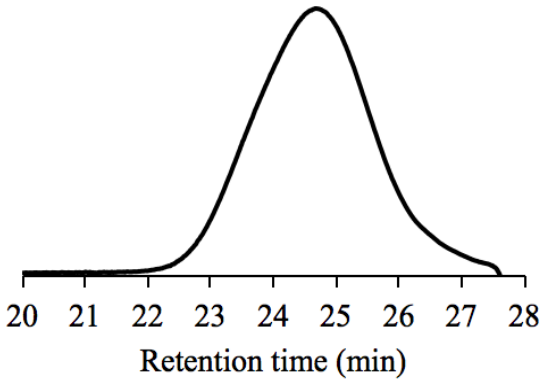


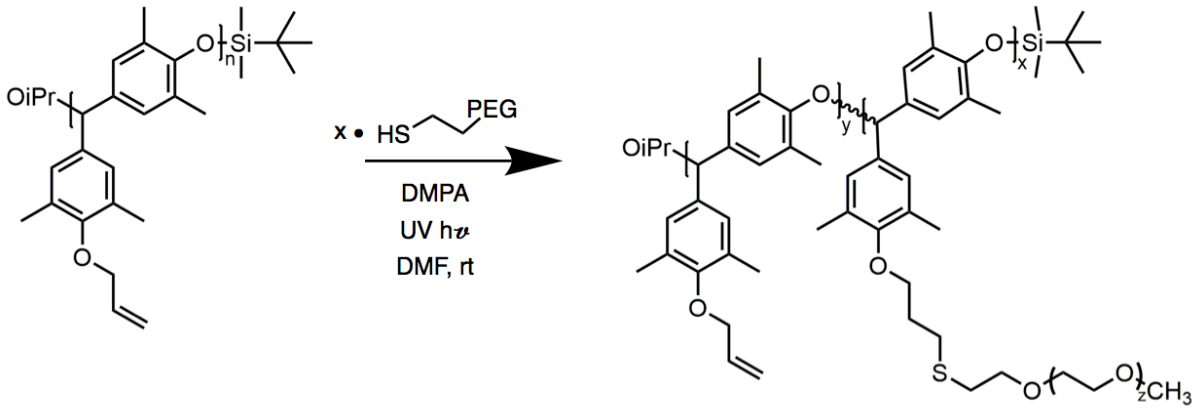
Figure S10 SEC trace of PBEs with allyl side groups (**RhB-P$_0$-1**)

Table S1
Summary of molecular weight and yielding of polymers.

Polymer	M_n (SEC) (Da)	M_w (SEC) (Da)	PDI	M_n (NMR) (Da)	Yield (%)
P_0-1	3558	5048	1.42	3406	71
P_0-2	3367	5270	1.57	3174	73
RhB-P_0-1	2955	4684	1.585	-	30

Side chain functionalization of polymers

PEGylation of allyl side chains



Scheme S4 PEGylation of polymers with pendant allyl side chains

Polymers (1.0 equiv) and poly (ethylene glycol) methyl ether thiol (0.x equiv relative to number of total allyl units) were dissolved in DMF (0.85 M). In an amber vial, DMPA (photo-initiator) (0.0x equiv) was prepared in DMF and added into reaction. Reaction was sealed and bubbled with N_2 for 5 mins to remove gases in solution. Sealed reaction was stirred in N_2 flow for 30 min at room temperature under irradiation of UV light (UV source: 100W, λ = 365 nm).

Rhodamine-tagged polymers were also PEGylated in same way.

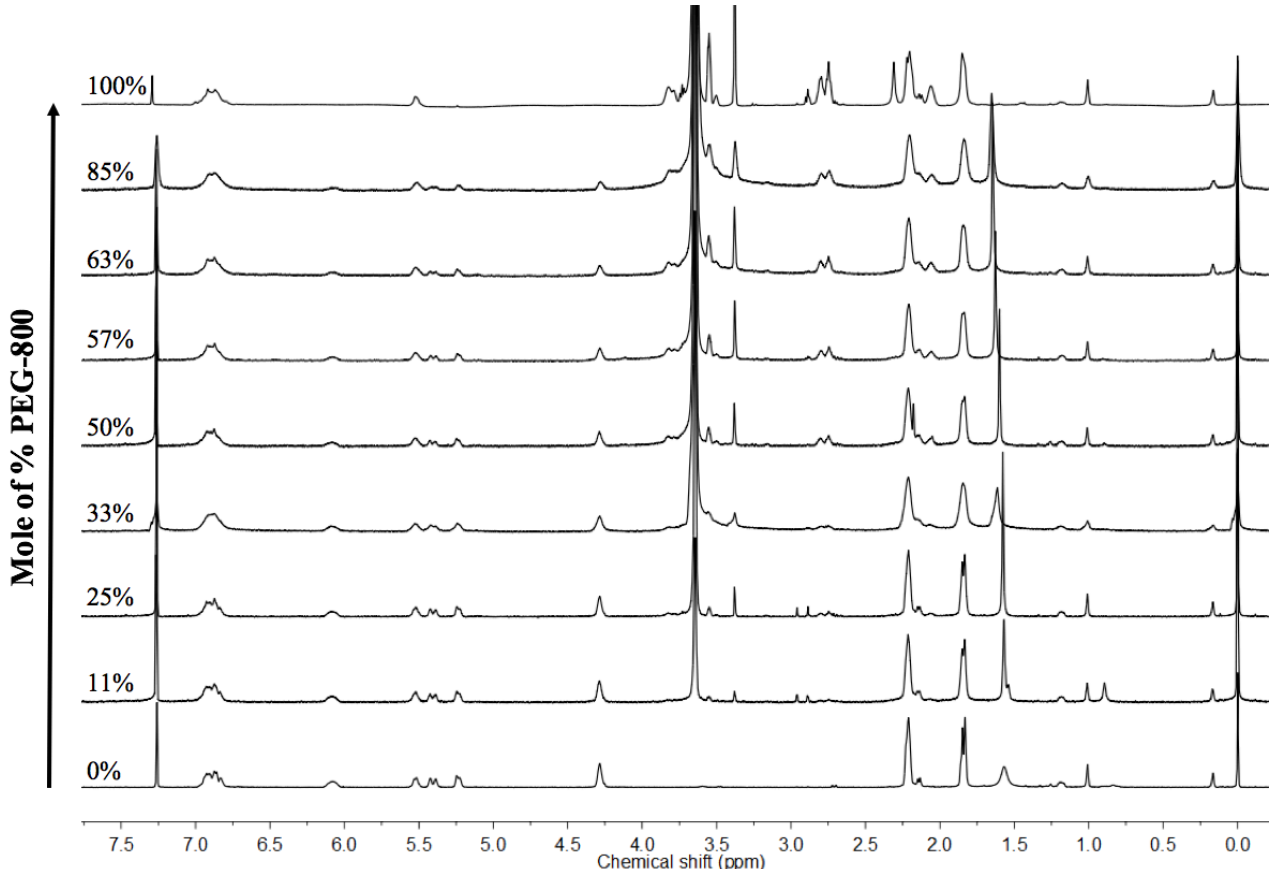


Figure S11 ^{1}H NMR spectra in $CDCl_3$ of PEGylated PBEs with increasing mole % of PEG-800 in the side chains.

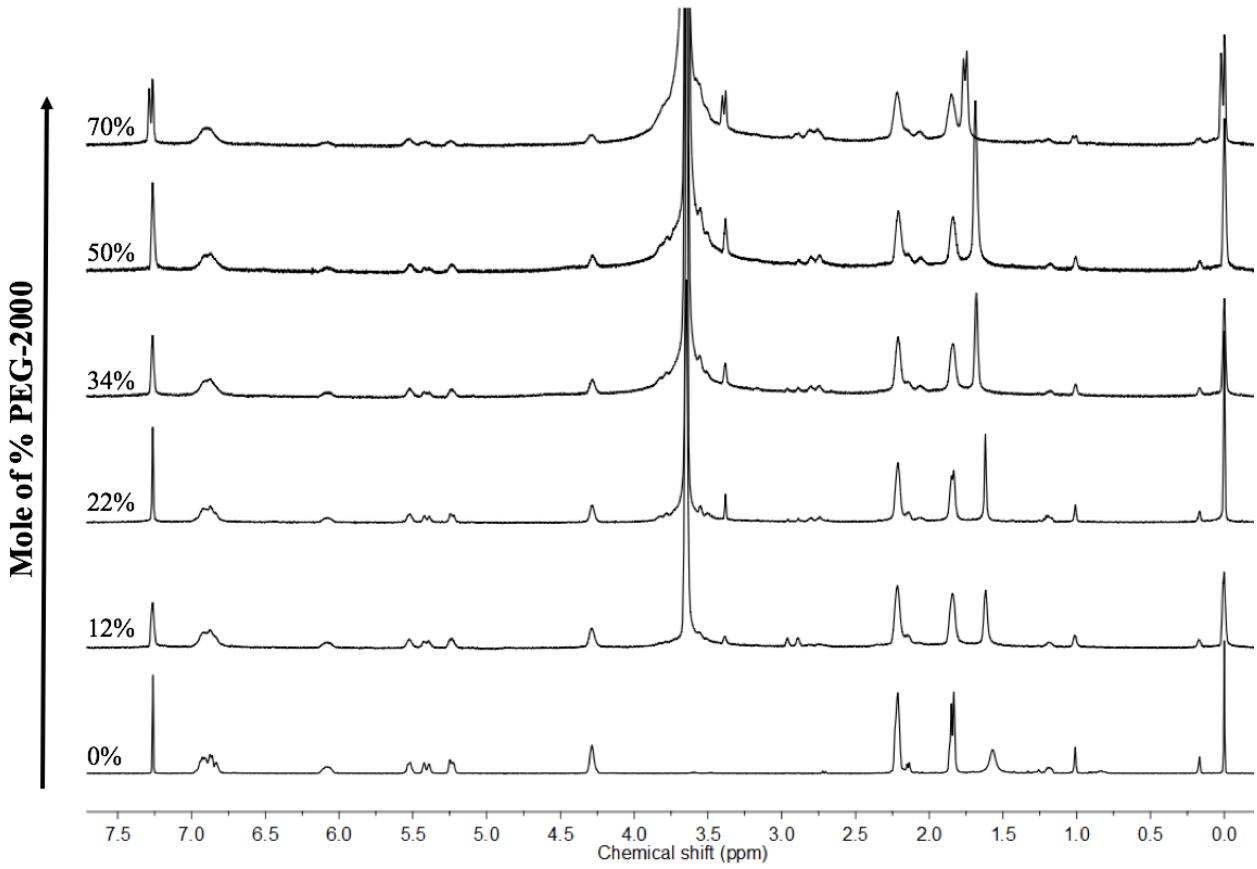
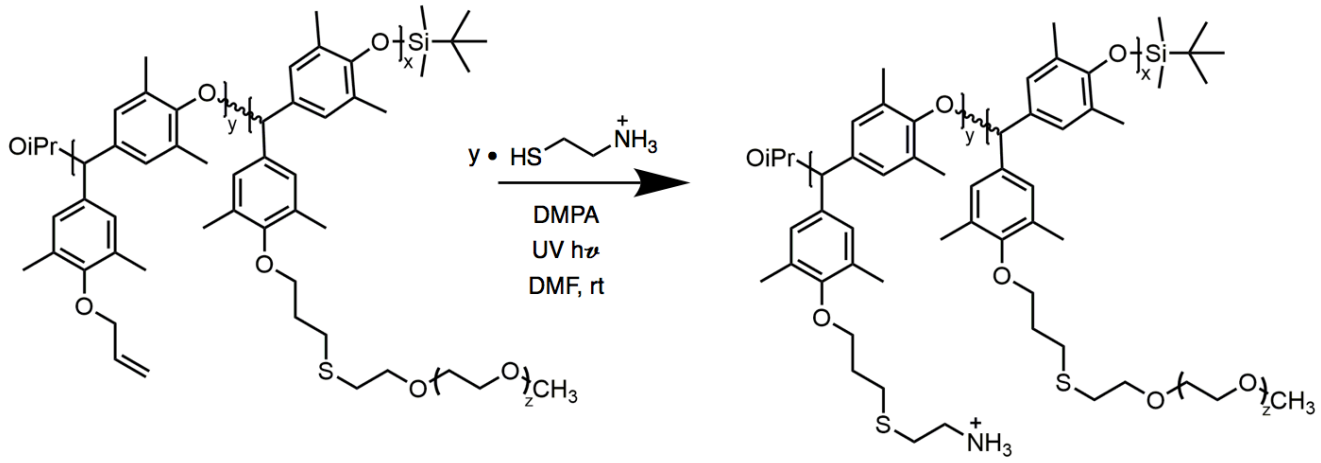


Figure S12 ^{1}H NMR spectra in $CDCl_3$ of PEGylated PBEs with increasing mole % of PEG-2k in the side chains.

Cysteamine functionalization of rest of allyl side chains



Scheme S5 Cysteamine side-chain functionalization of polymers with allyl groups

Into same reaction vial in which PEGylation was carried out, cysteamine (0.yy equiv relative to number of total allyl units) was added. In an amber vial, DMPA (photo-initiator) (0.00y equiv) was prepared in DMF and added into reaction. Reaction was sealed and bubbled with N_2 for 5 mins to remove gases in solution. Sealed reaction was stirred in N_2 flow for 30 min at room temperature under irradiation of UV light (UV source: 100W, λ = 365 nm). Once reaction was complete, DMF was evaporated and then polymers were purified from excess cysteamine and photoinitiator via running them in Sephadex LH-20 prepared in MeOH. ^{1}H NMR ($(CD_3)_2OS$, 500 MHz): δ 6.89 (bs), 5.50 (bs,), 3.85 (bs), 3.61 (bs), 3.51 (bs), 3.17 (bs), 2.85 (bs), 2.71 (bs) 2.68 (bs, 2H), 2.18 (bs), 1.80 (bs), 0.99 (bs), 0.15 (bs).

Rhodamine-tagged polymers were also functionalized in same way. Repeated precipitations and purification on LH-20 ensured that no unreacted dye remains in the polymer samples.

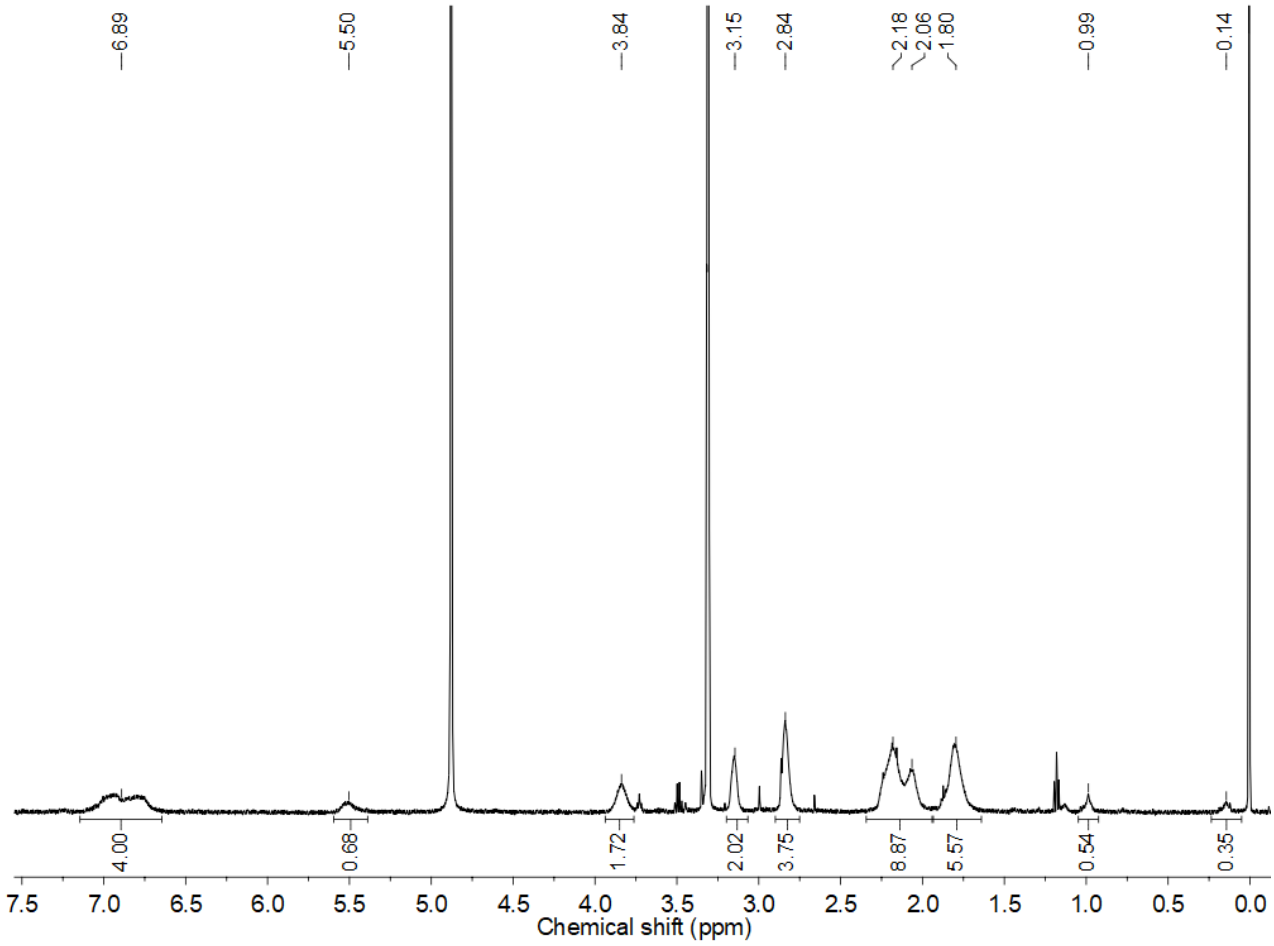


Figure S13 ^{1}H NMR spectrum of **P_1-0** (CD_3OD, 500 MHz)

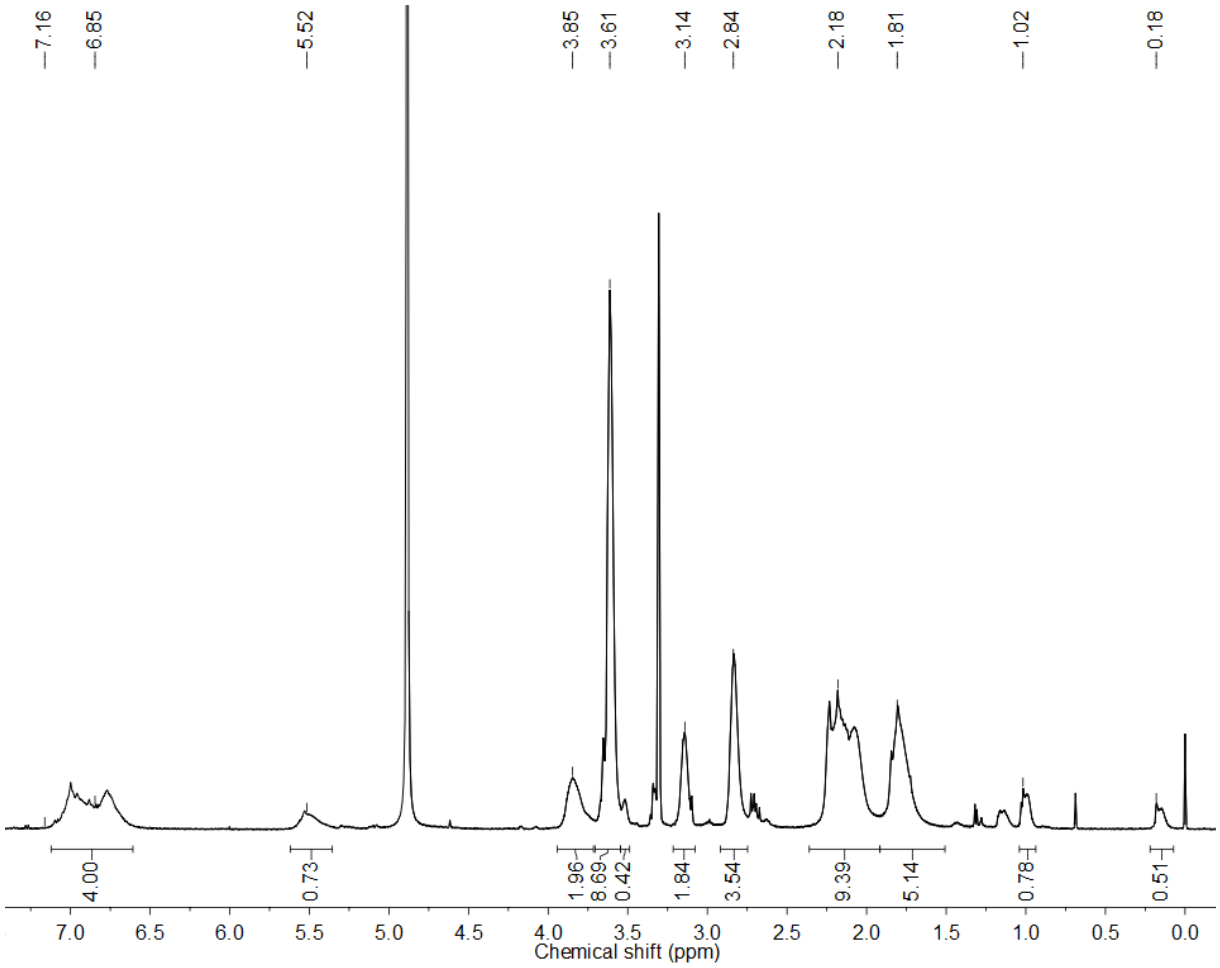


Figure S14 ^{1}H NMR spectrum of **P_1-11-PEG$_{800}$** (CD_3OD, 500 MHz)

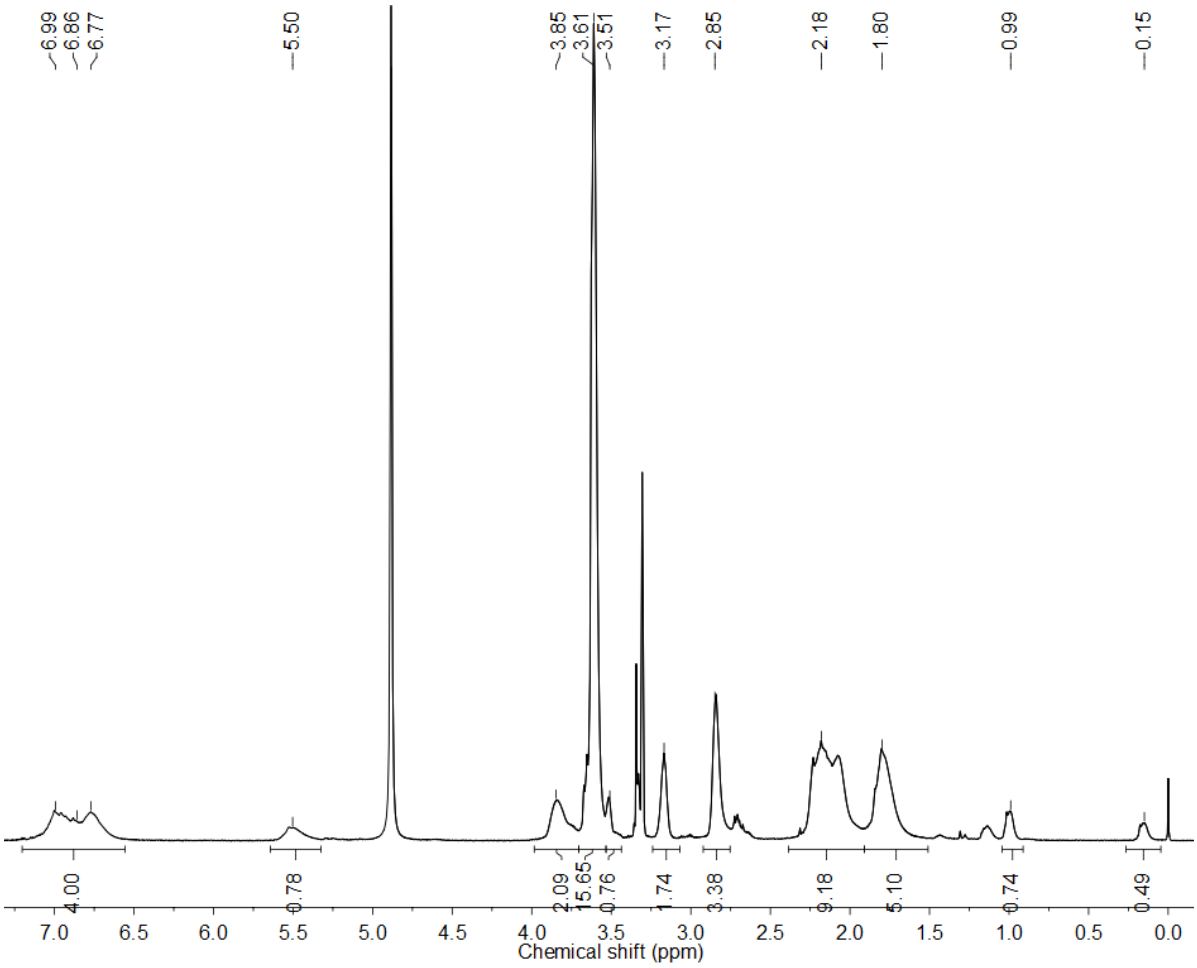


Figure S15 ^{1}H NMR spectrum of **P_1-25-PEG$_{800}$** (CD_3OD, 500 MHz)

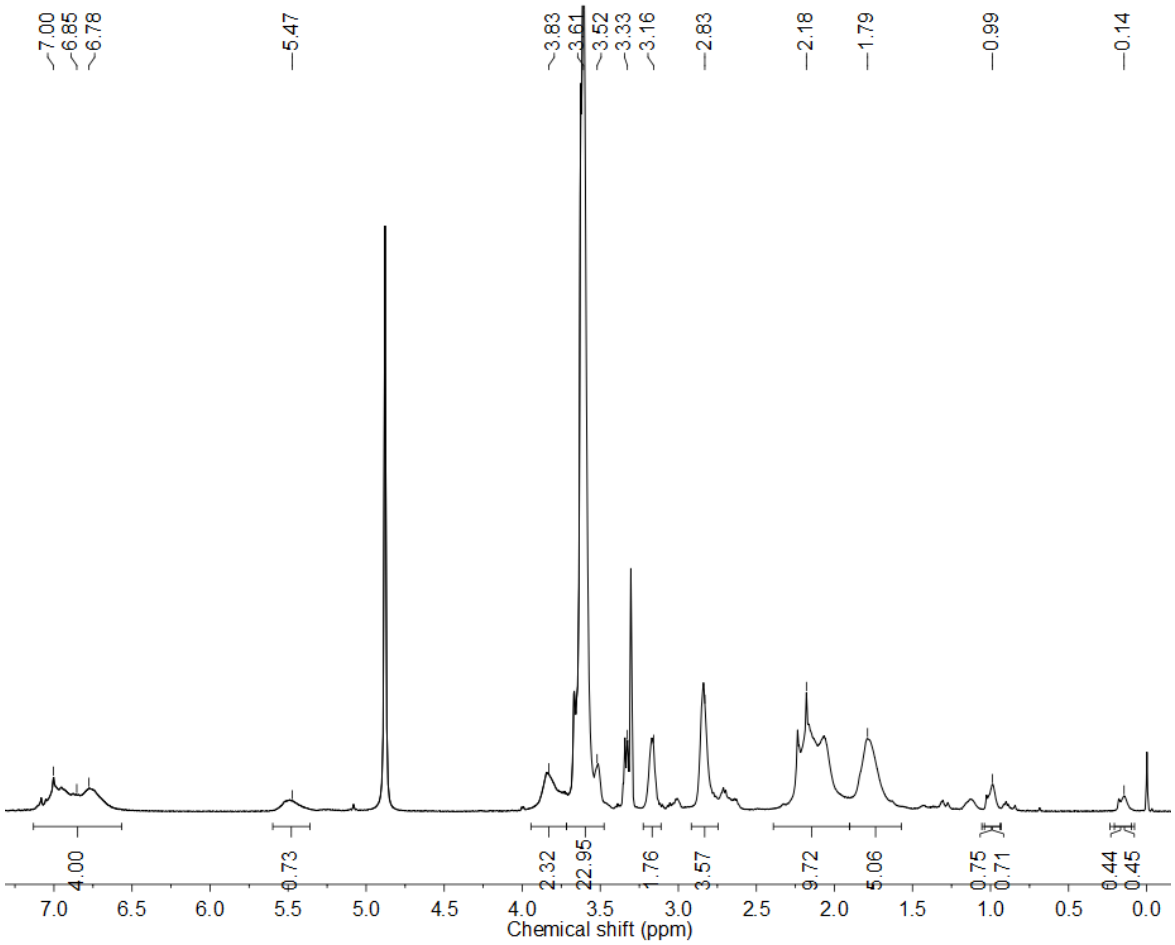


Figure S16 ^{1}H NMR spectrum of **P_1-33-PEG_{800}** (CD_3OD, 500 MHz)

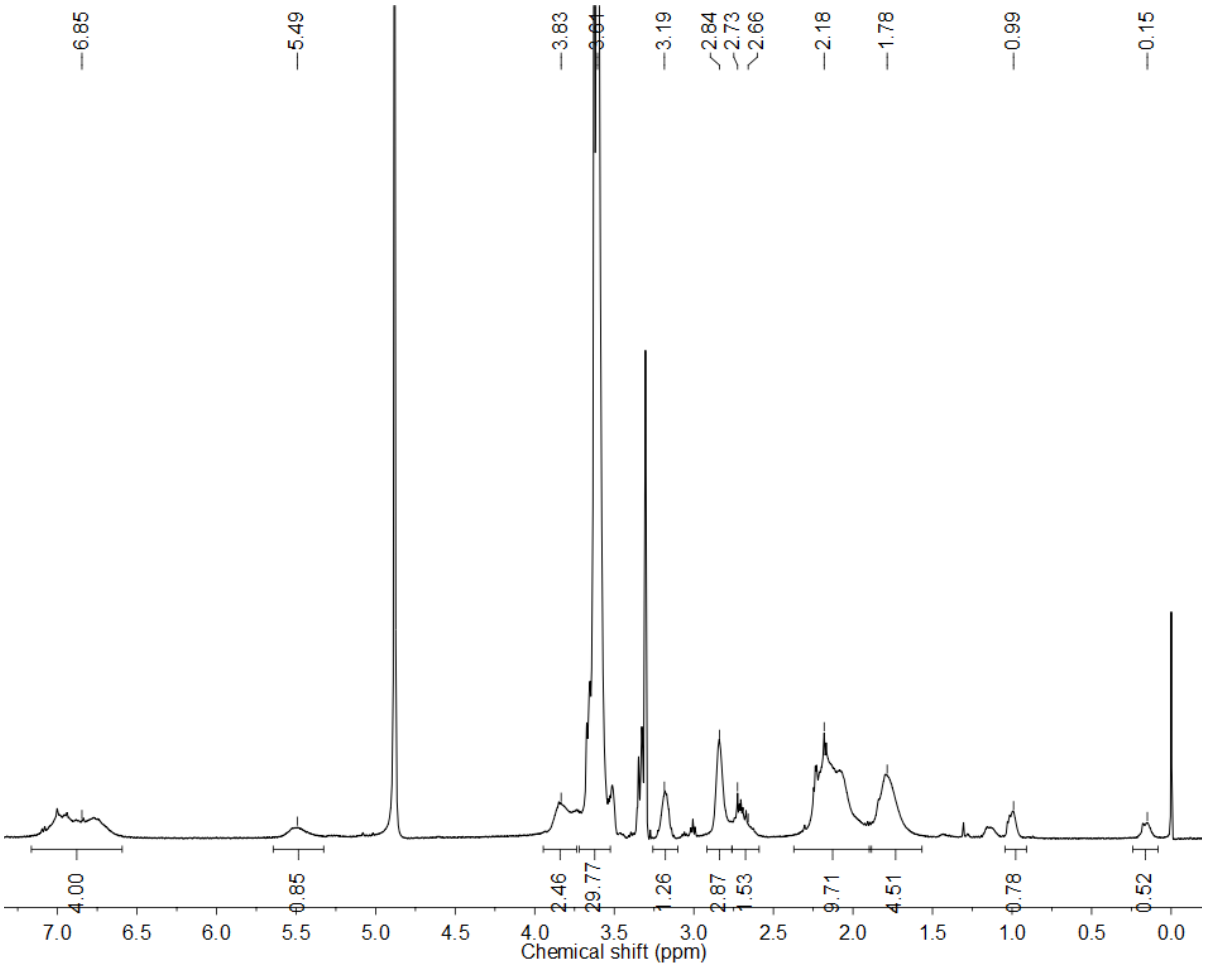


Figure S17 ^{1}H NMR spectrum of **P_1-50-PEG_{800}** (CD_3OD, 500 MHz)

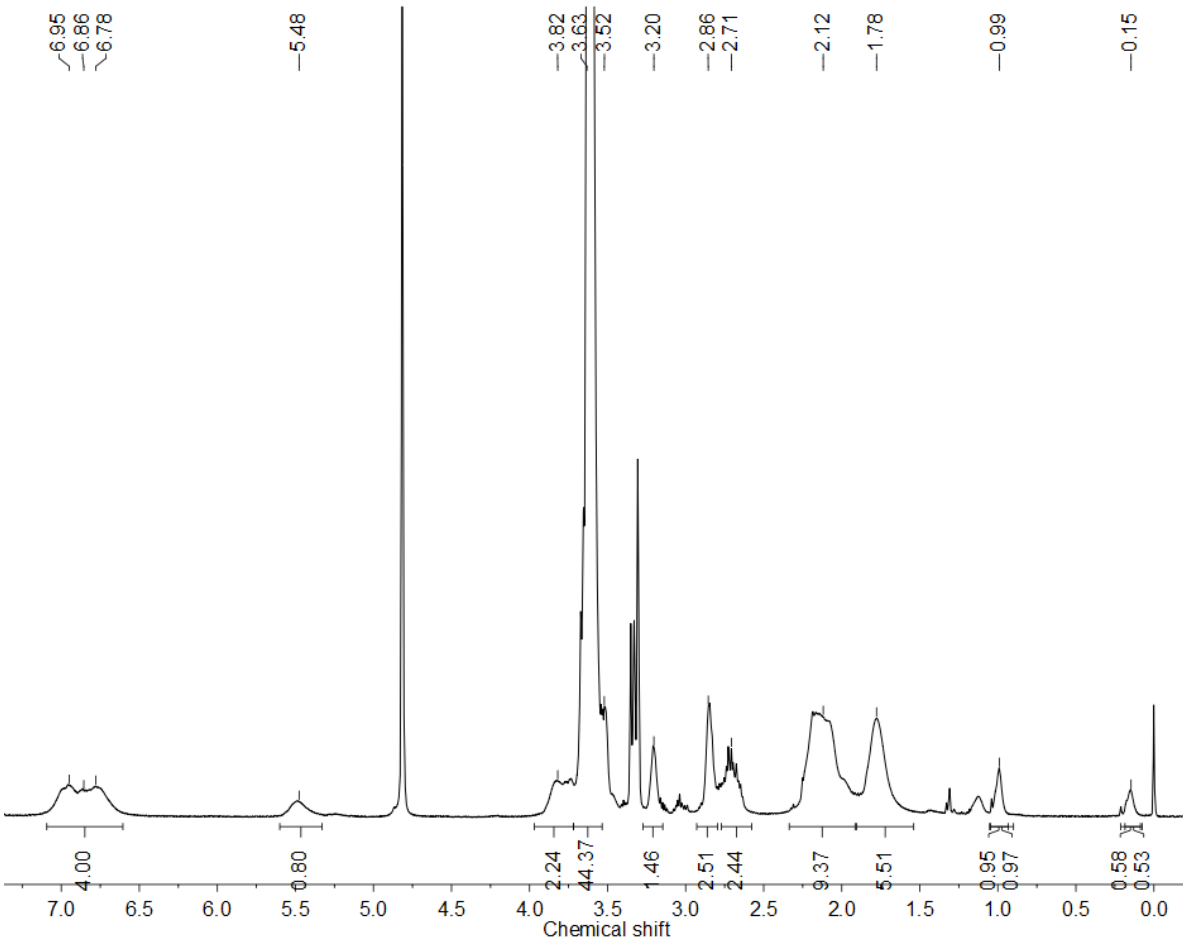


Figure S18 ^{1}H NMR spectrum of **P_1-57-PEG_{800}** (CD_3OD, 500 MHz)

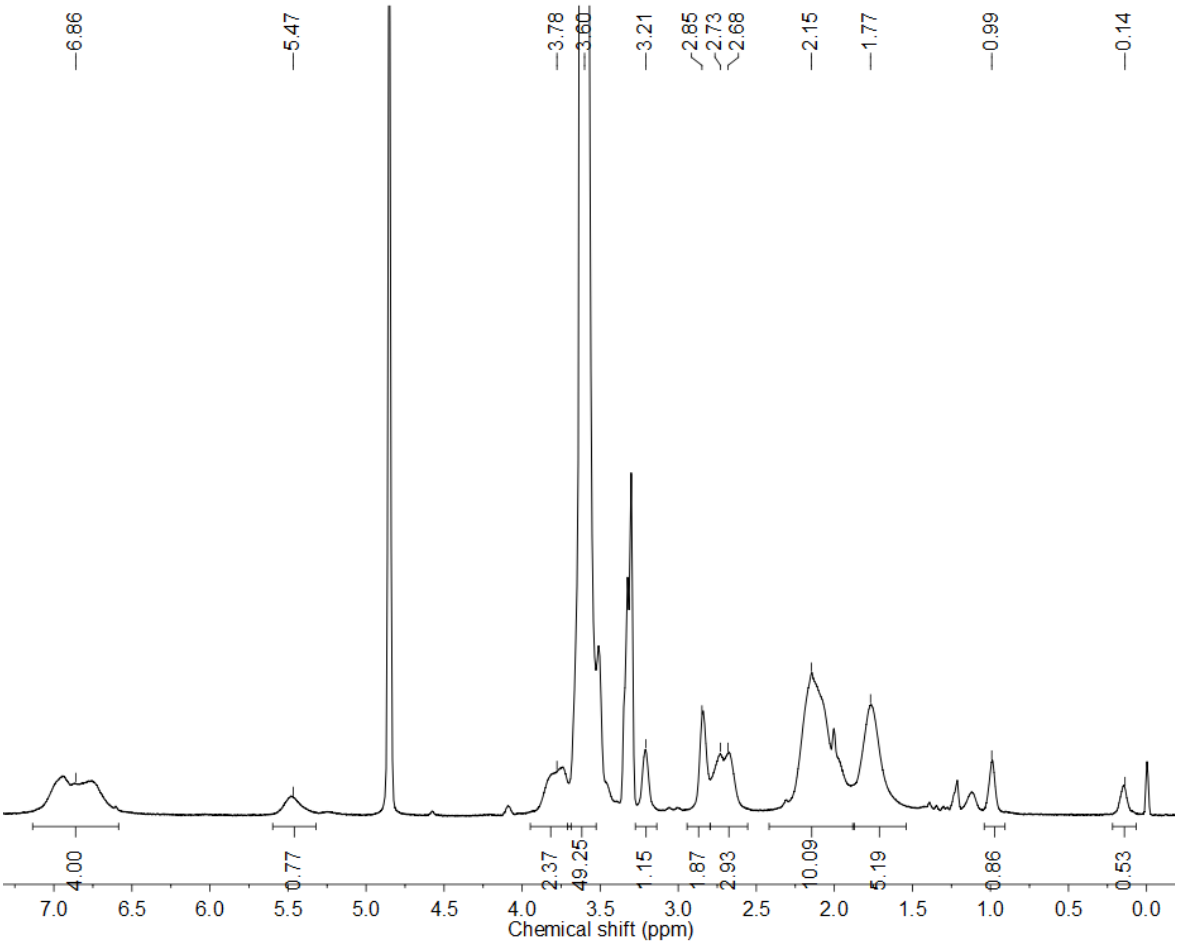


Figure S19 ^{1}H NMR spectrum of **P_1-63-PEG_{800}** (CD_3OD, 500 MHz)

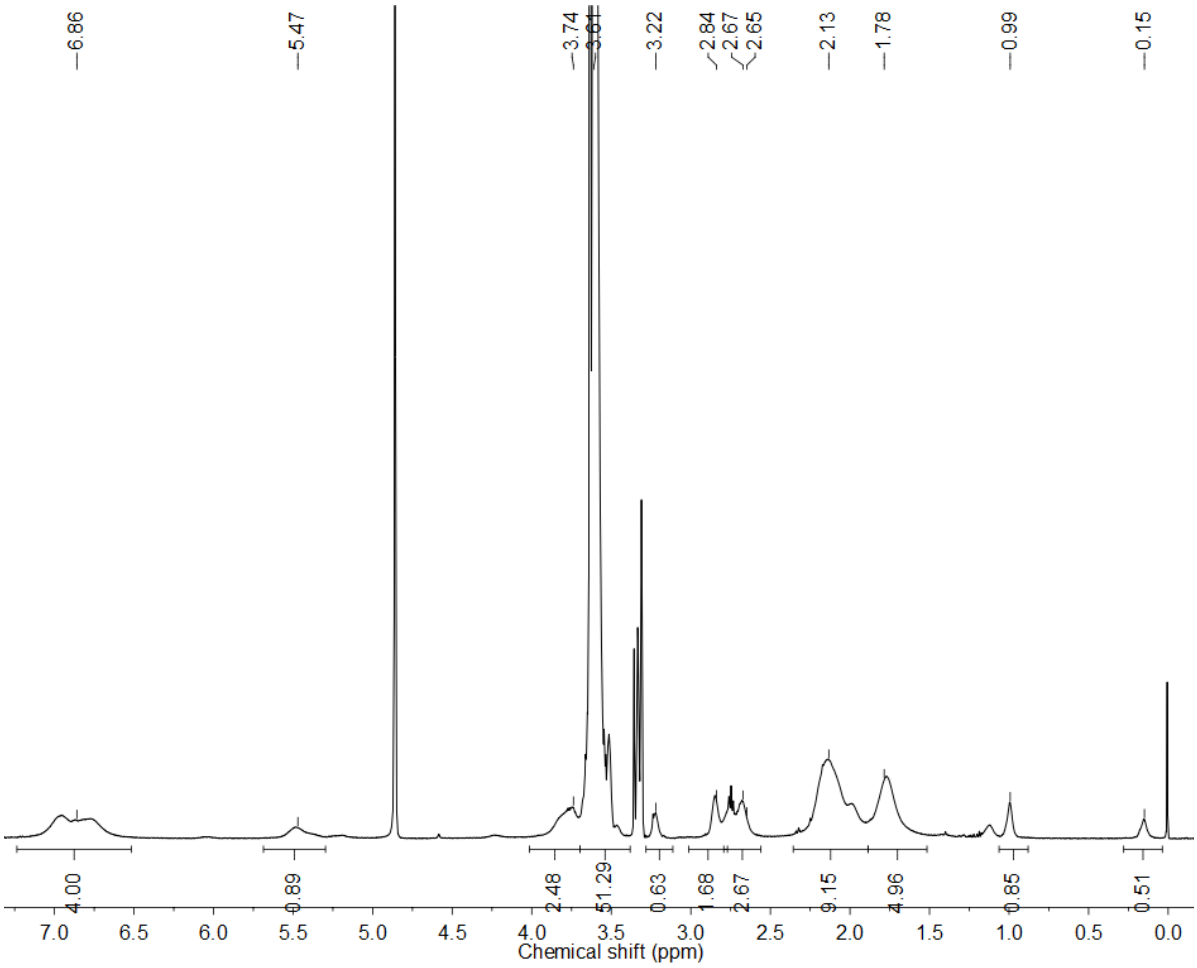


Figure S20 ^{1}H NMR spectrum of **P_1-85-PEG$_{800}$** (CD_3OD, 500 MHz)

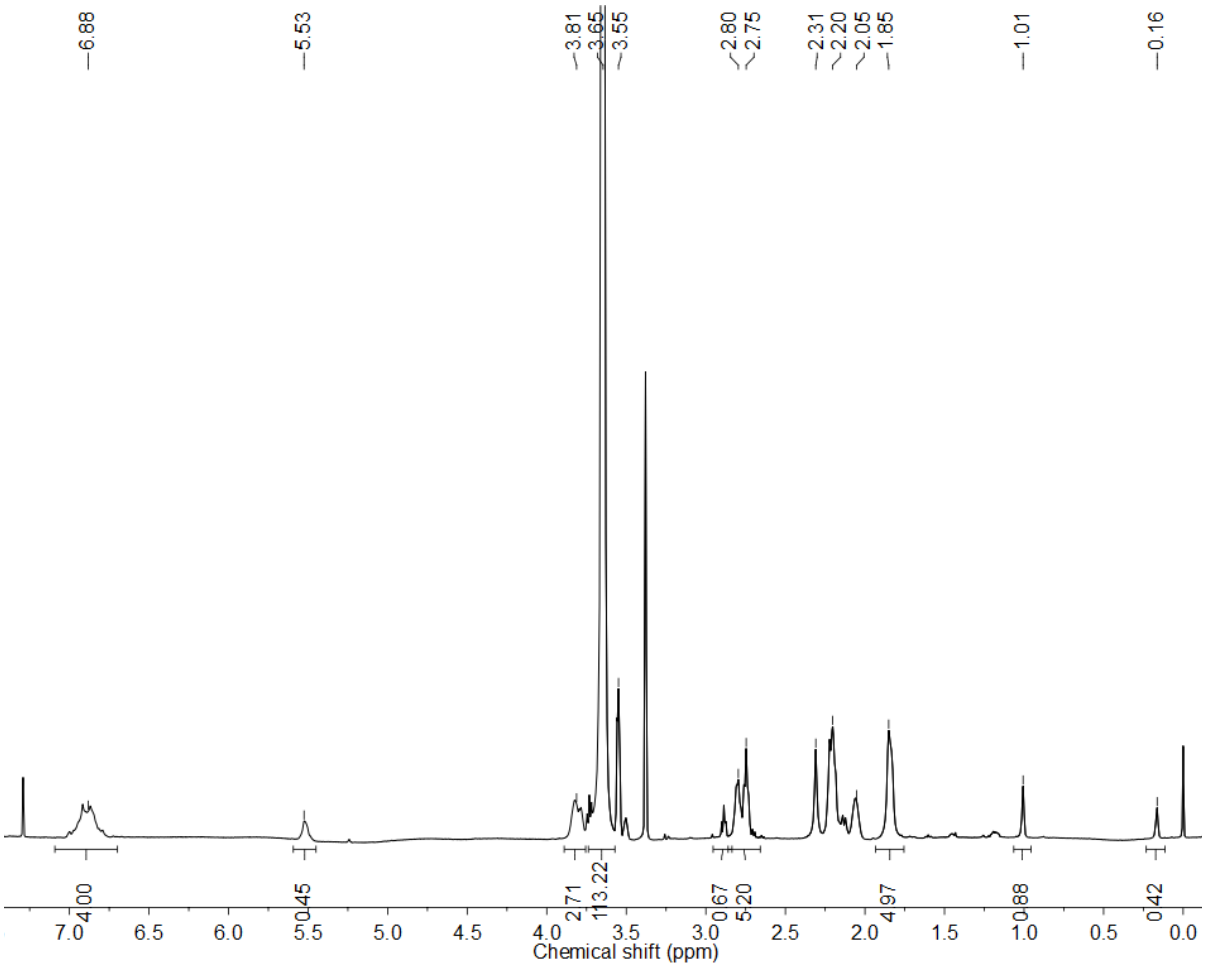


Figure S21 ^{1}H NMR spectrum of **P_1-100-PEG$_{800}$** ($CDCl_3$, 500 MHz)

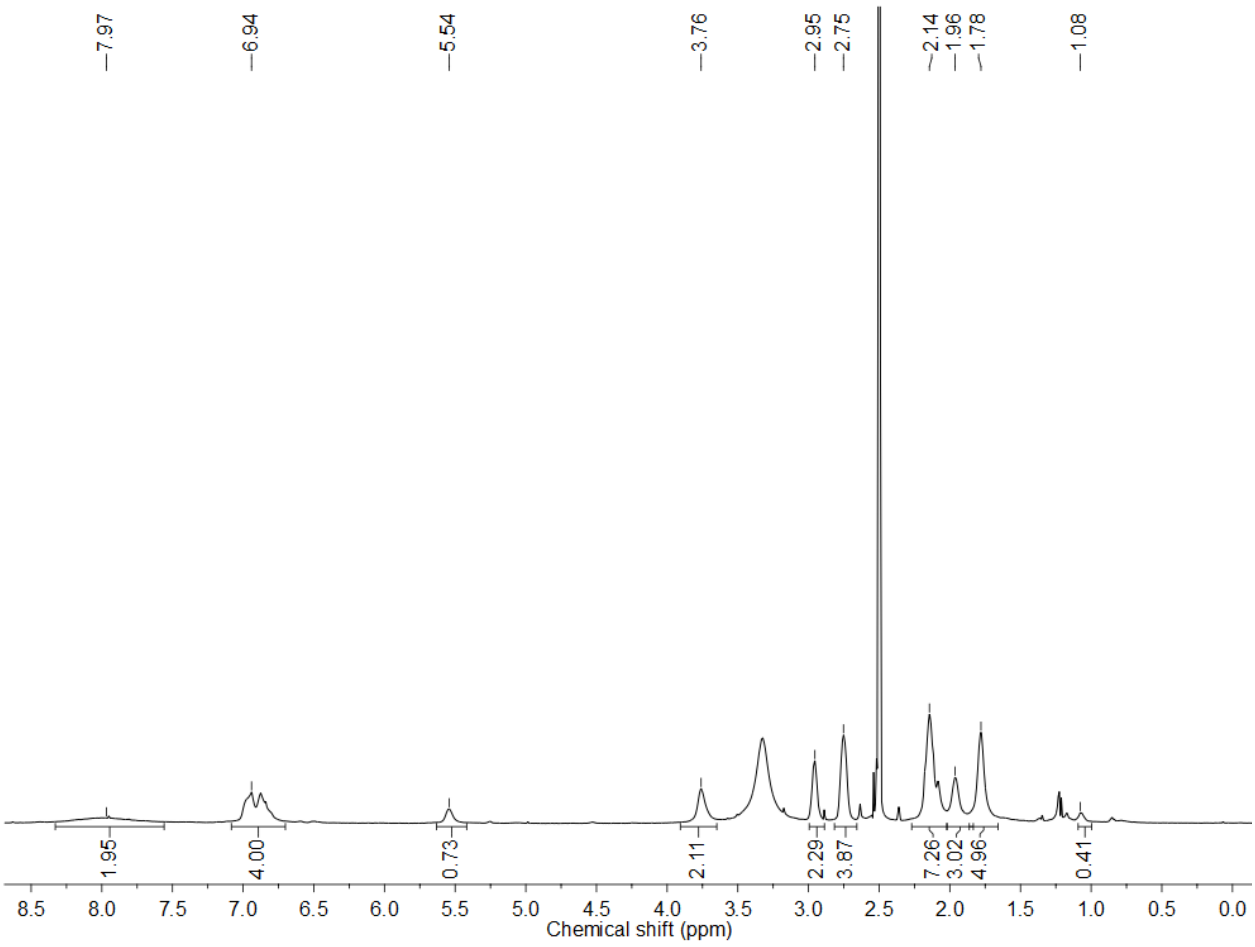


Figure S22 ^{1}H NMR spectrum of **RhB-P$_1$-0-PEG800** ($(CD_3)_2OS$, 500 MHz)

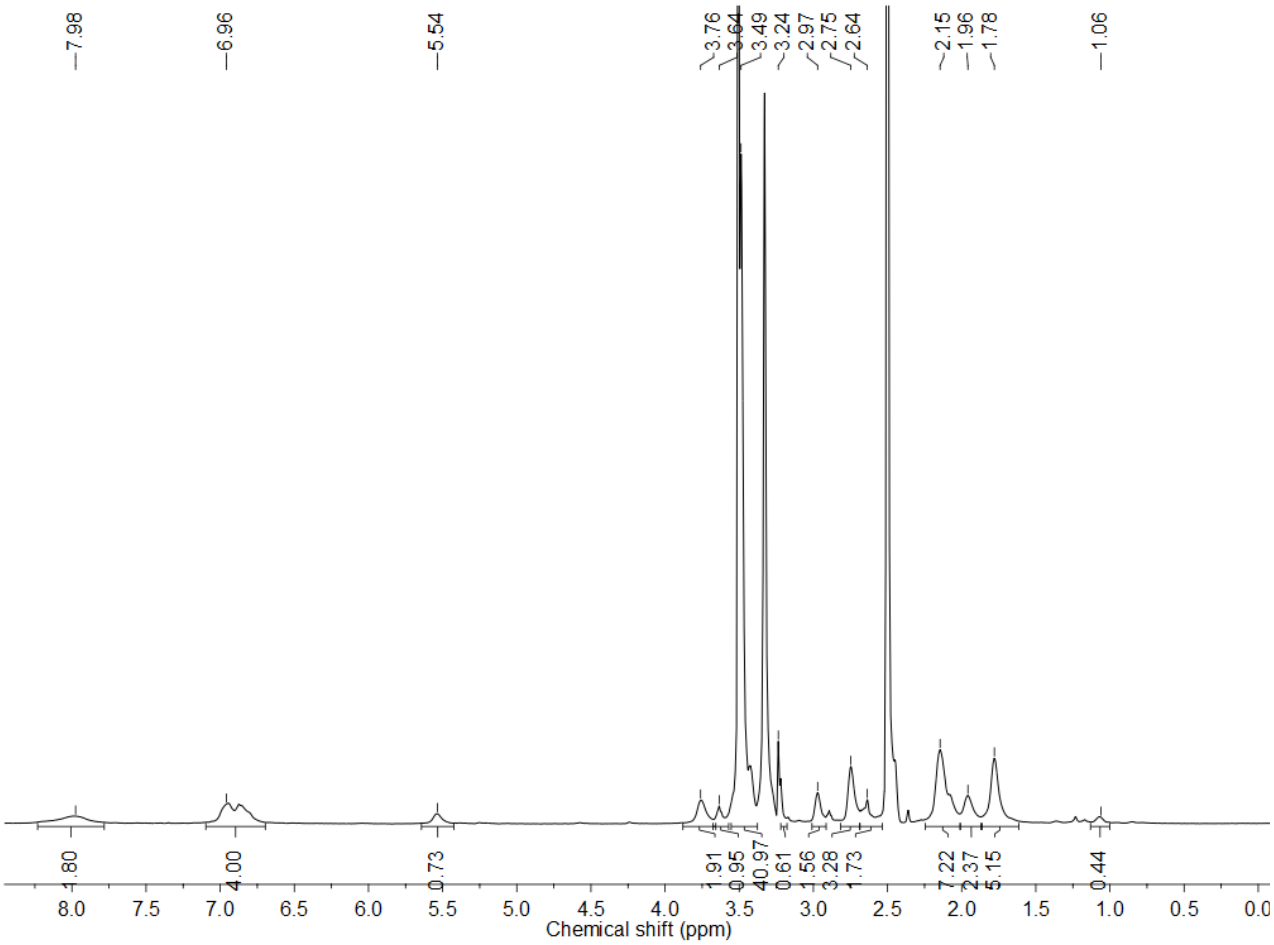


Figure S23 ^{1}H NMR spectrum of **RhB-P$_1$-40-PEG800** ($(CD_3)_2OS$, 500 MHz)

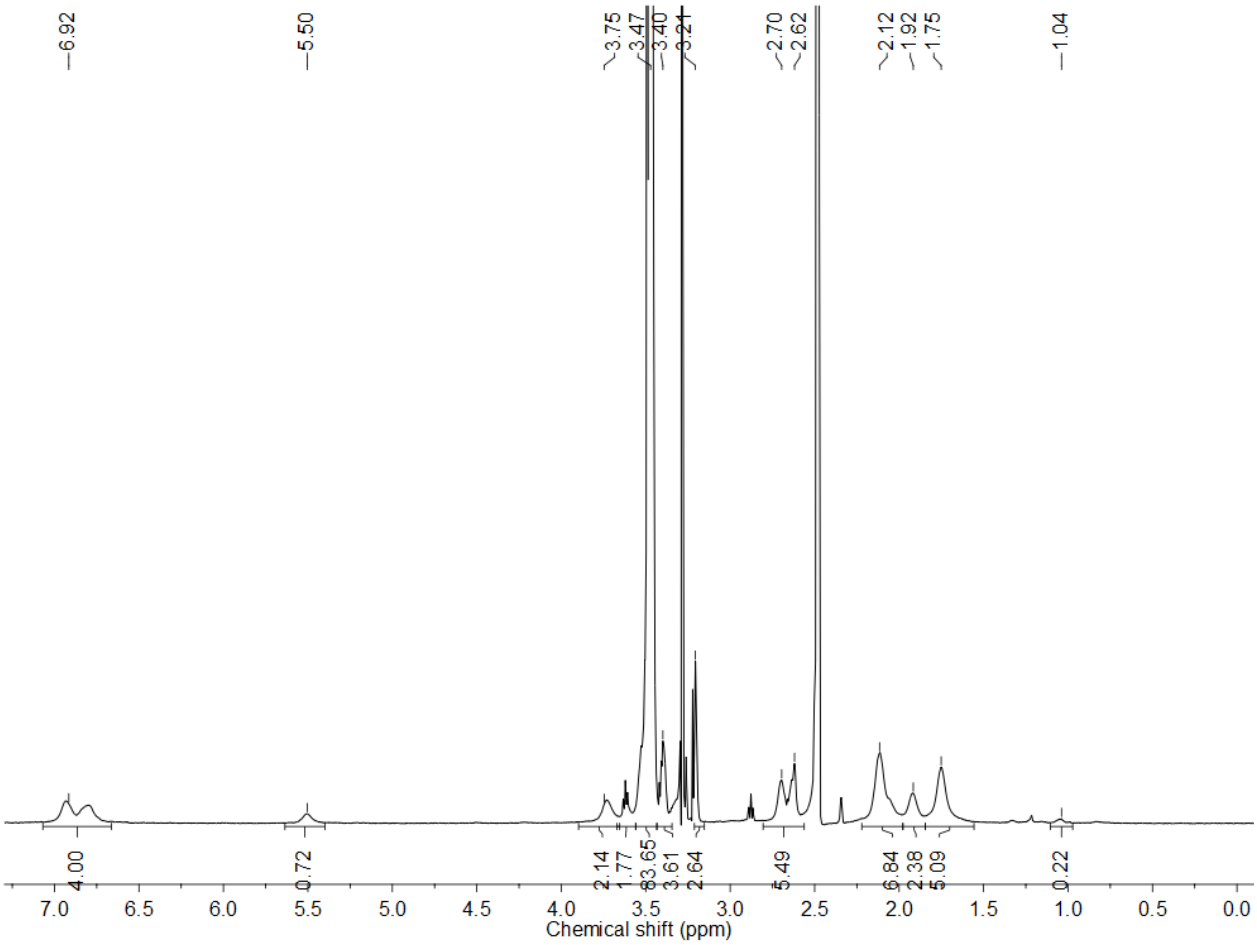


Figure S24 ^{1}H NMR spectrum of **RhB-P$_1$-100-PEG800** ($(CD_3)_2OS$, 500 MHz)

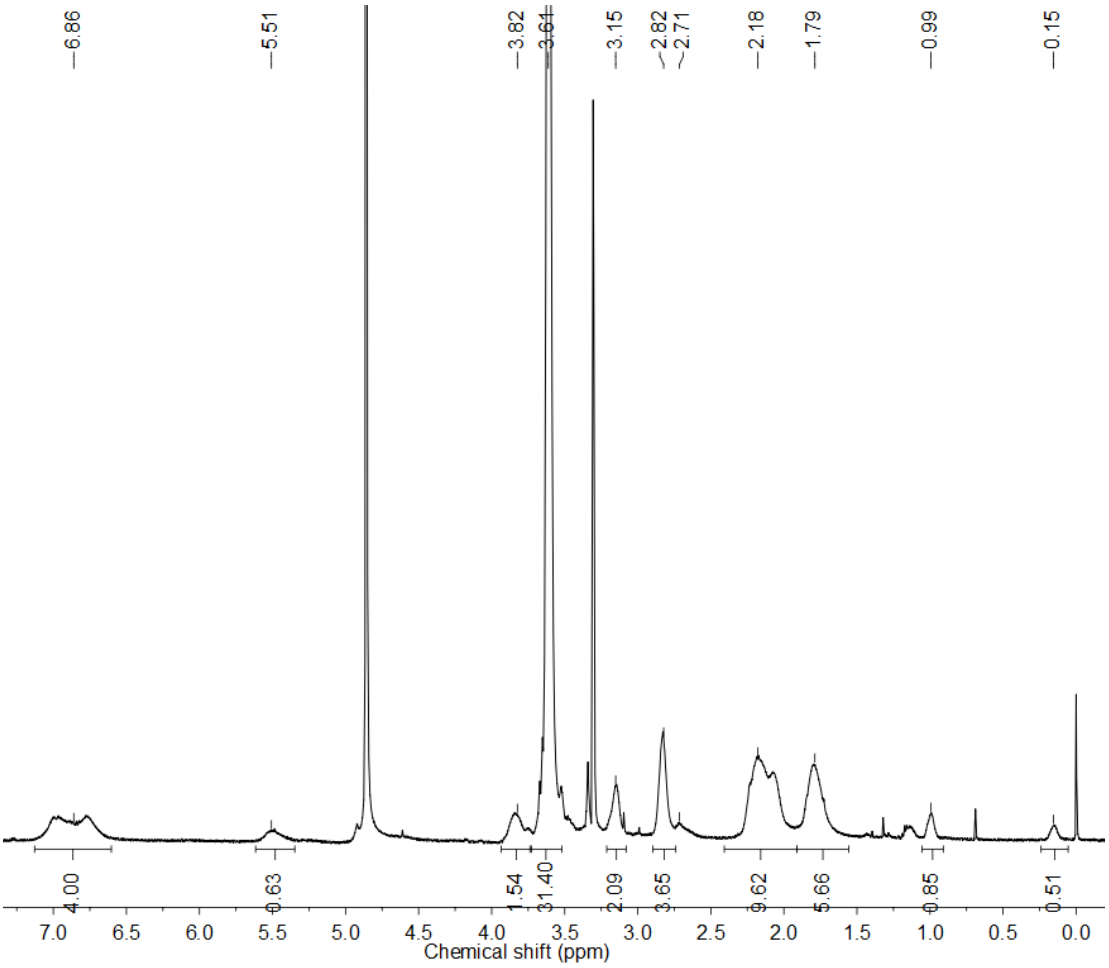


Figure S25 ^{1}H NMR spectrum of **P_1-12-PEG$_{2k}$** (CD_3OD, 500 MHz)

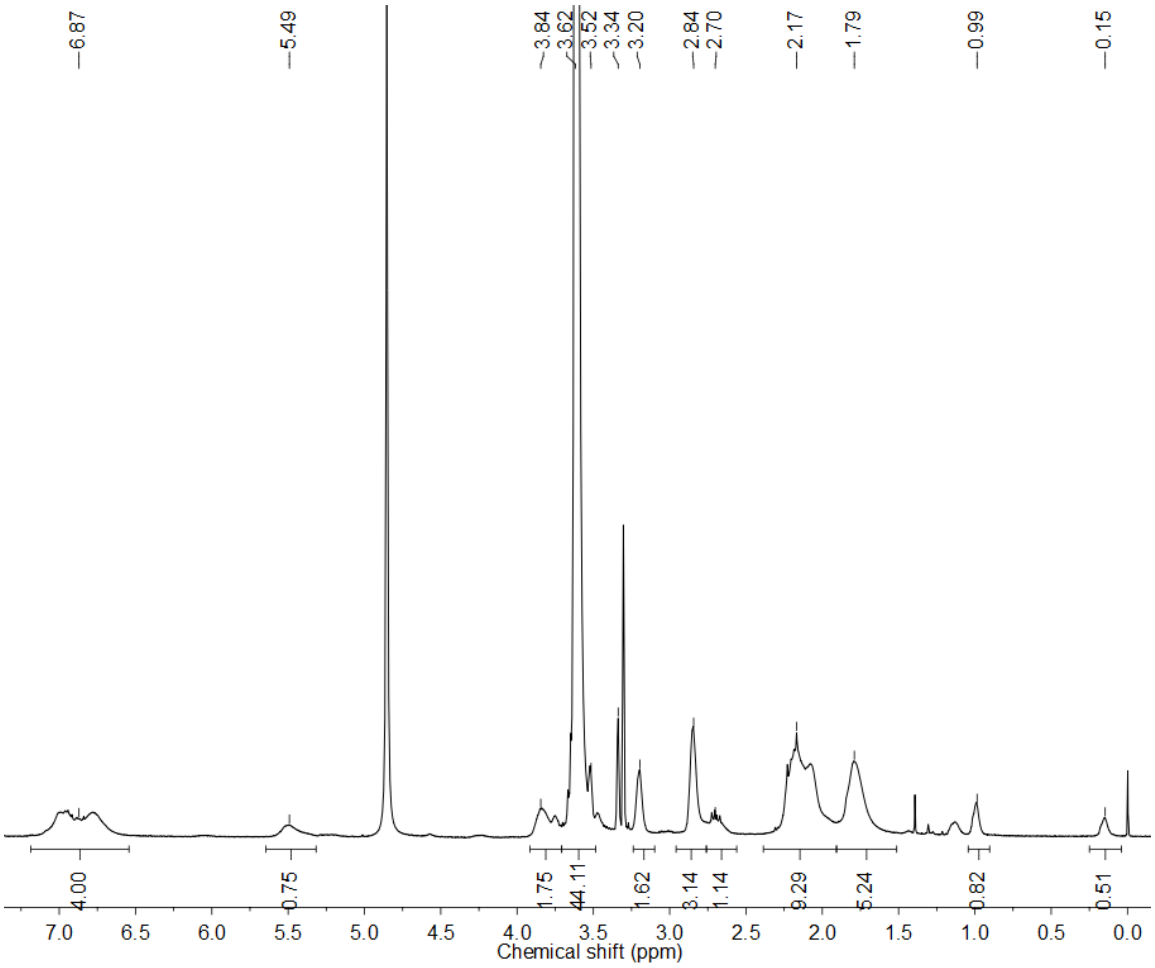


Figure S26 ^{1}H NMR spectrum of **P_1-26-PEG_{2k}** (CD_3OD, 500 MHz)

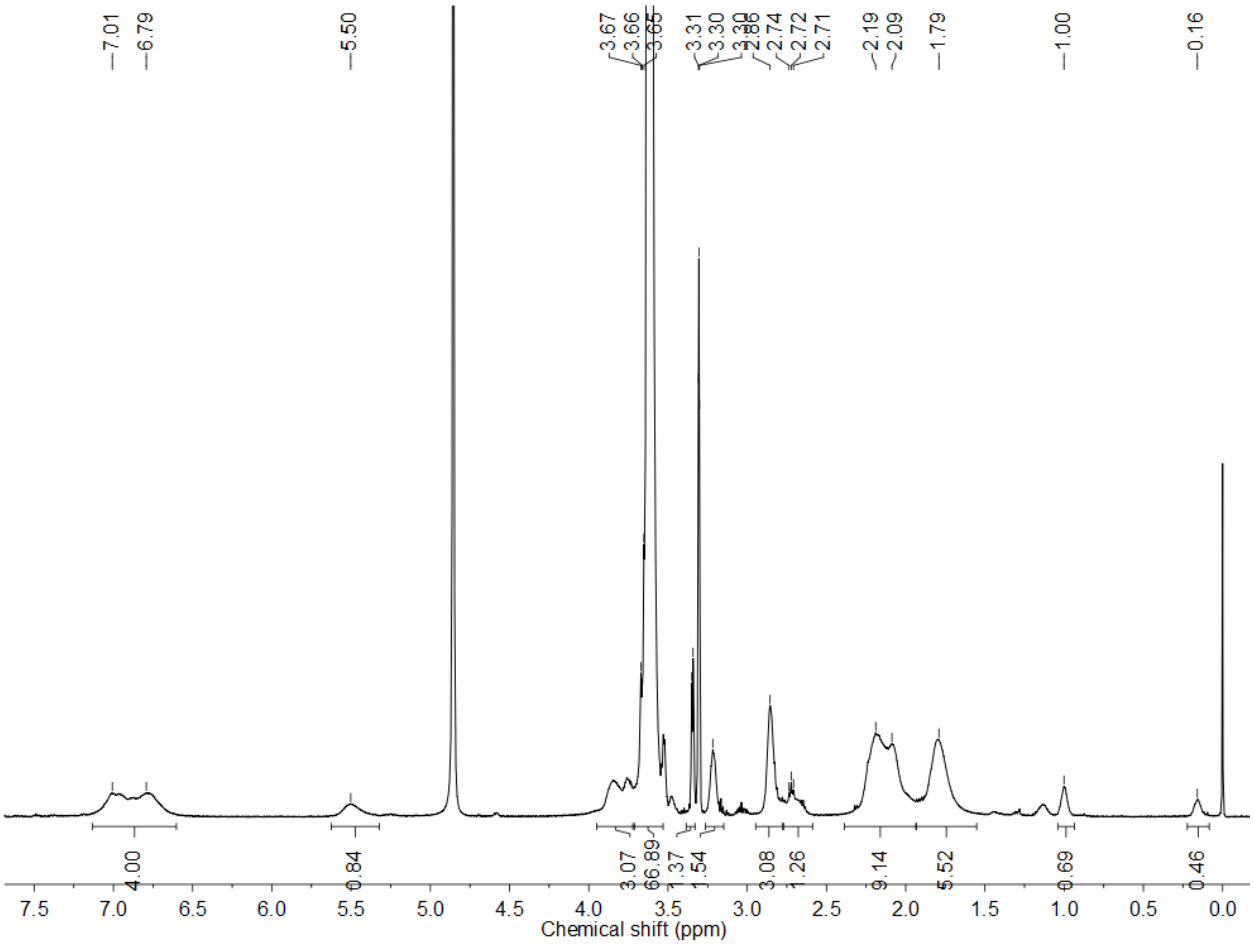


Figure S27 ^{1}H NMR spectrum of **P_1-34-PEG$_{2k}$** (CD_3OD, 500 MHz)

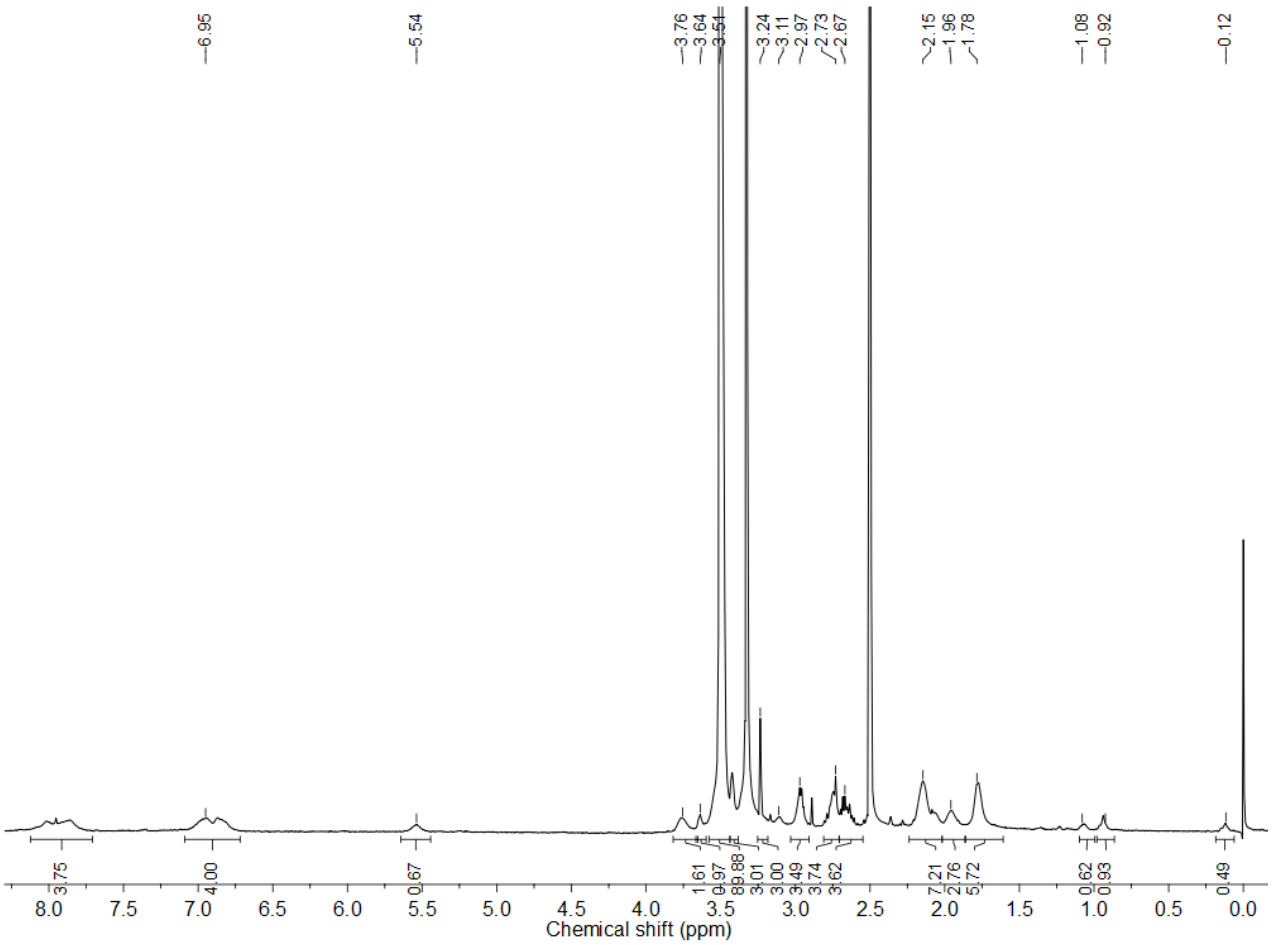


Figure S28 ^{1}H NMR spectrum of **P_1-52-PEG_{2k}** ($(CD_3)_2OS$, 500 MHz)

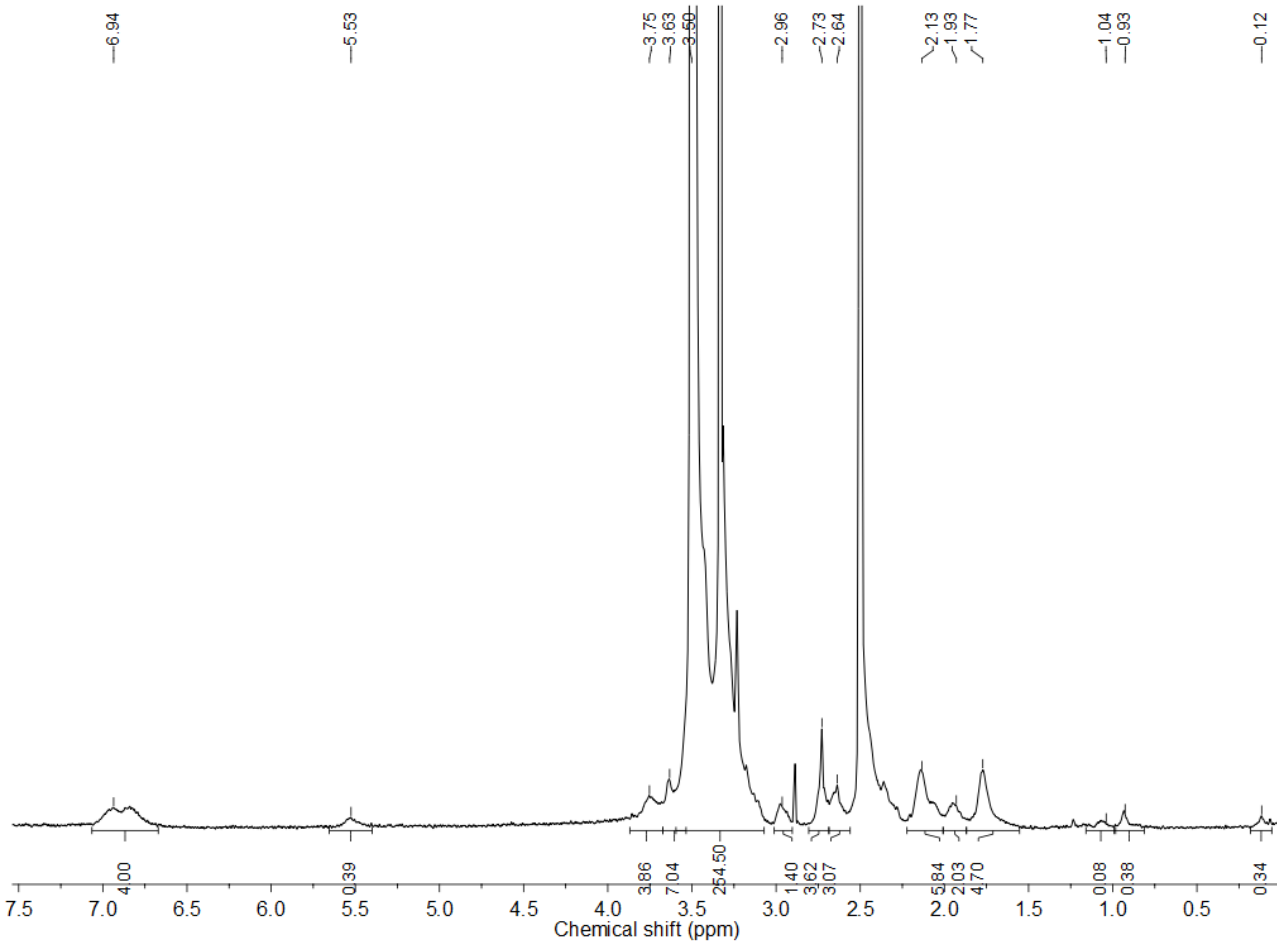
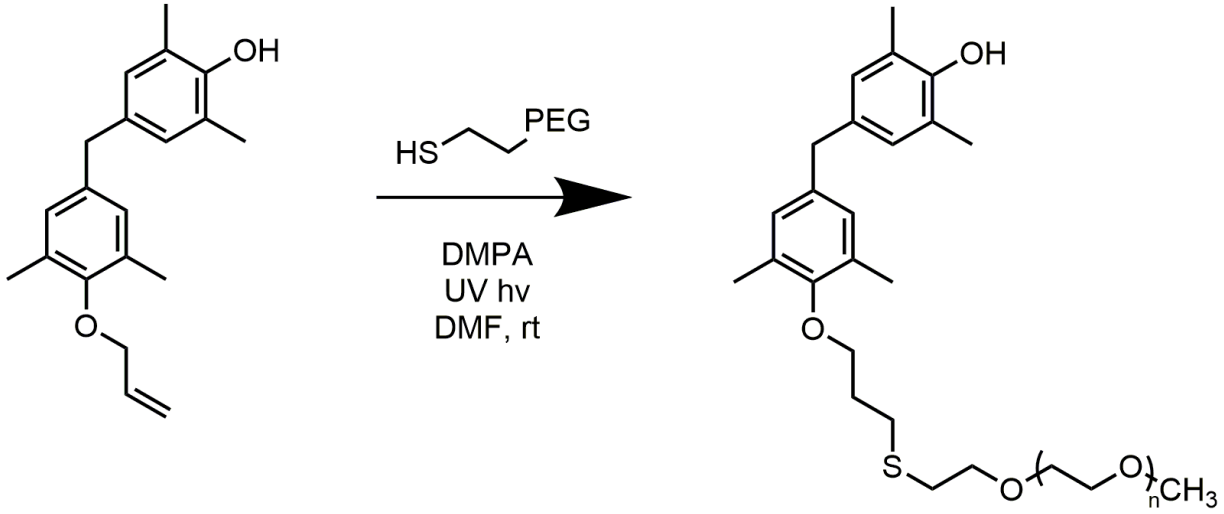


Figure S29 ^{1}H NMR spectrum of **P_1-72-PEG_{2k}** ($(CD_3)_2OS$, 500 MHz)

Side chain functionalization of monomers

PEGylation of single allyl compound



Scheme S4 PEGylation of **single allyl** compound, $\mathbf{M_2}$

Single allyl compound (1.0 equiv) and cysteamine (1.5 equiv) were dissolved in DMF (0.85 M). In an amber vial, DMPA (photo-initiator) (0.01 equiv) was prepared in DMF and added into reaction. Reaction was sealed and bubbled with N_2 for 5 min to remove gases in solution. Sealed reaction was stirred in N_2 flow for 2 h at room temperature under irradiation of irradiation of UV light (UV source: 100W, λ = 365 nm). Once the reaction was complete, it was concentrated via rotovap and dried under vacuum for 24 h. We couldn't separate excess PEG from the compound and used in tests as this. ^{1}H NMR ((CD_3OD, 500 MHz): δ 6.84 (s, 2H), 6.78 (s, 2H), 3.81 (t, 2H), 3.64 (bs), 3.55 (t, 2H), 2.80 (t, 2H), 2.74 (t, 2H), 2.22 (s, 6H), 2.20 (s, 6H), 2.08 (t, 2H). ^{13}C NMR (500 MHz, CD_3OD): δ 138.92, 131.49, 130.15, 129.76, 125.50, 72.97, 72.29, 71.56, 71.36, 59.10, 41.52, 32.33, 31.59, 30.51, 29.83, 16.72.

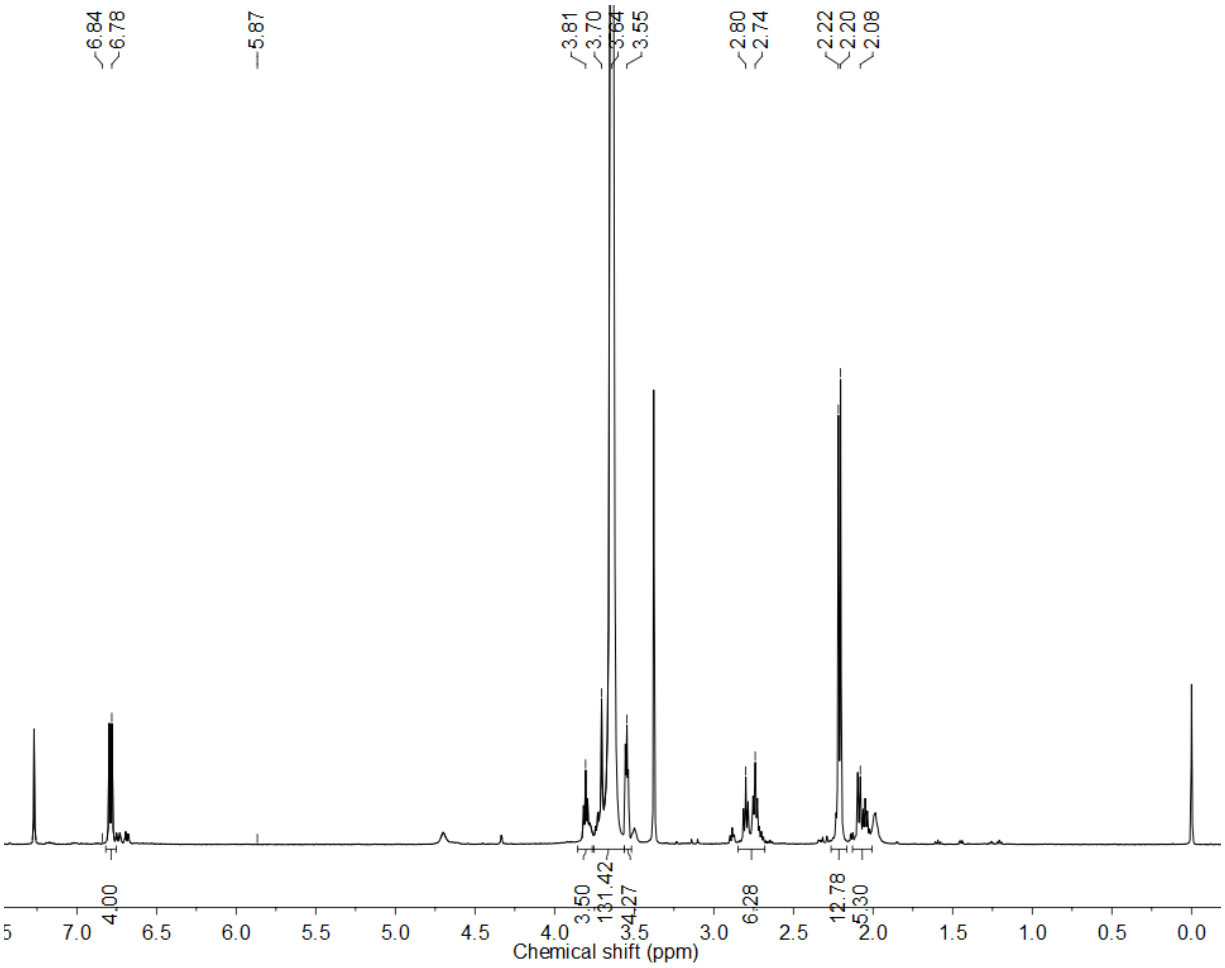


Figure S30 ^{1}H NMR spectrum of **primary amine-functionalized single allyl compound, M_2-PEG$_{800}$** ($CDCl_3$, 500 MHz)

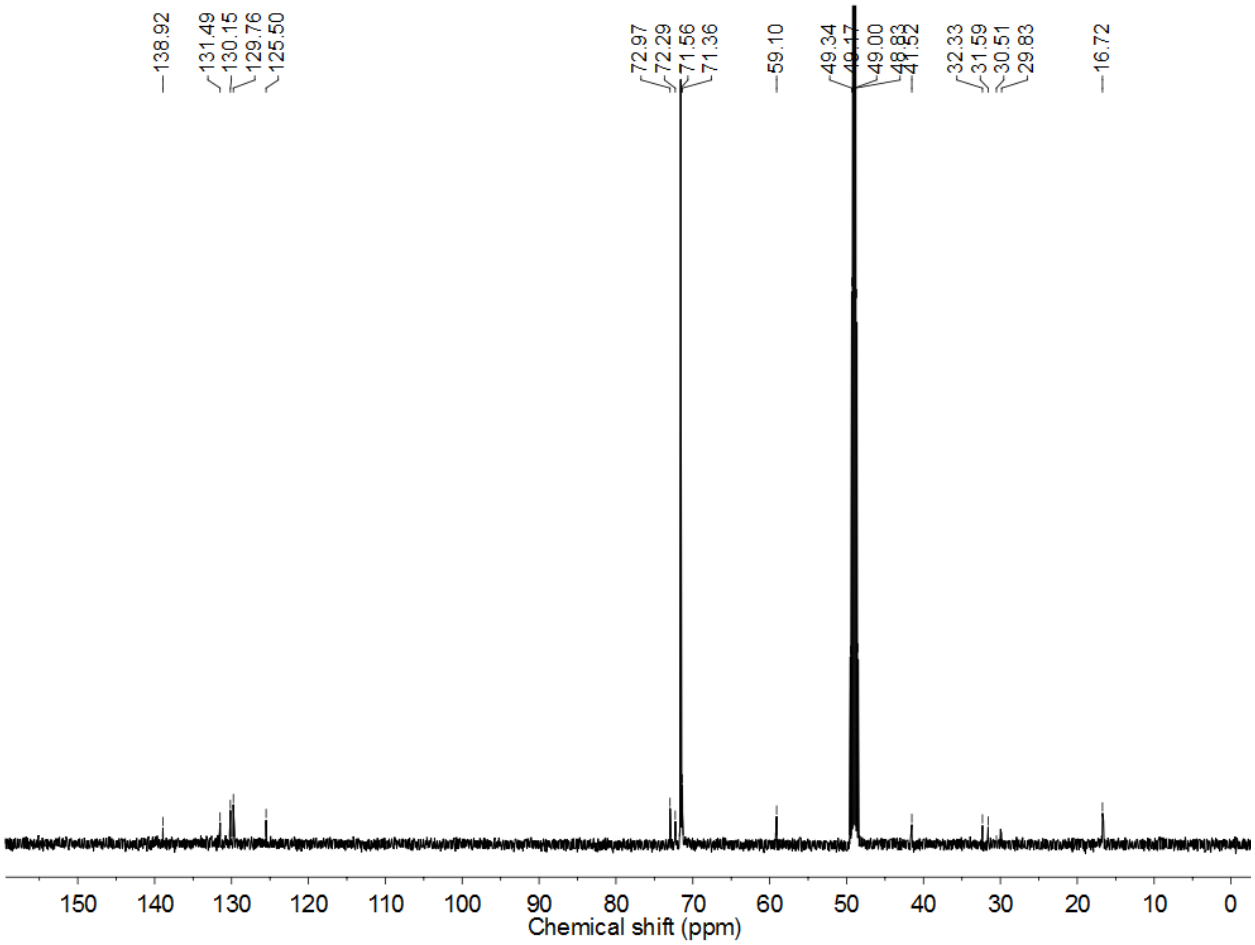
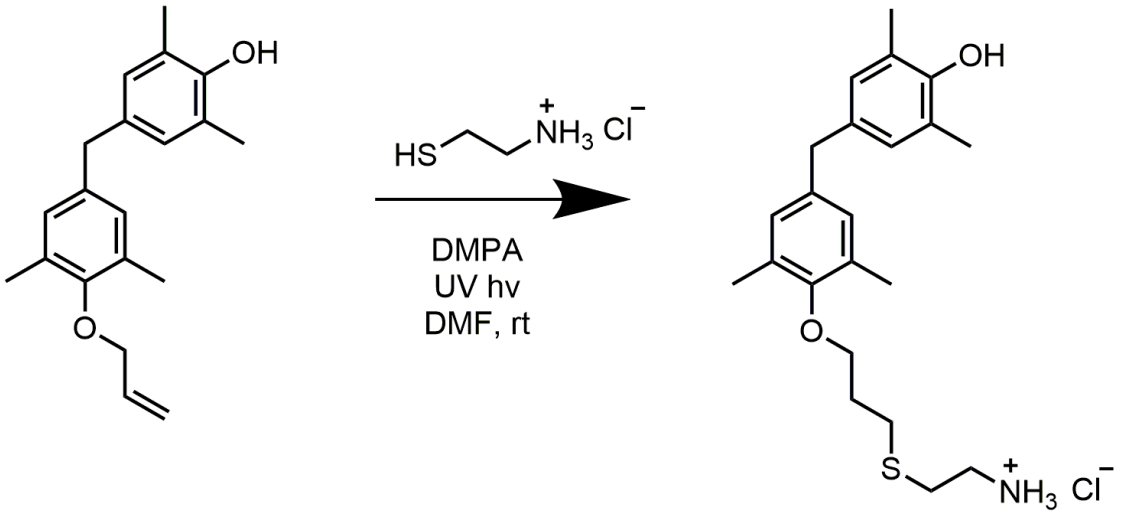


Figure S31 ^{13}C NMR spectrum of **primary amine-functionalized single allyl compound, M_2-PEG_{800}** (CD_3OD, 500 MHz)

Cysteamine side chain functionalization of single allyl compound



Scheme S6 Side chain modification of **single allyl** compound with cysteamine, $\mathbf{M_1}$

Single allyl compound (1.0 equiv) and cysteamine (1.1 equiv) were dissolved in DMF (34 mM). In an amber vial, DMPA (photo-initiator) (0.01 equiv) was prepared in DMF and added into reaction. Reaction was sealed and bubbled with N_2 for 5 min to remove gases in solution. Sealed reaction was stirred in N_2 flow for 2 h at room temperature under irradiation of irradiation of UV light (UV source: 100W, λ = 365 nm). Once the reaction, it was concentrated. It was purified by silica gel chromatography (10% MeOH in DCM). Oil product (yield: 8.3%) was concentrated via rotovap and dried under vacuum for 24 h. ^{1}H NMR ((CD_3OD, 500 MHz): δ 6.78 (s, 2H), 6.70 (s, 2H), 3.83 (t, 2H), 3.65 (s, 2H), 3.08 (t, 2H), 2.81 (td, 4H), 2.20 (s, 6H), 2.15 (s, 6H), 2.05 (p, 2H). ^{13}C NMR (500 MHz, CD_3OD): δ 154.96, 152.32, 139.01, 133.96, 131.41, 130.14, 129.74, 129.46, 129.20, 128.49, 125.49, 71.19, 41.46, 36.94, 31.23, 29.01, 16.70, 16.50. HRMS (ESI): calculated for $C_{22}H_{32}NO_2S$ 374.2148 g/mole; found 374.2137 g/mol.

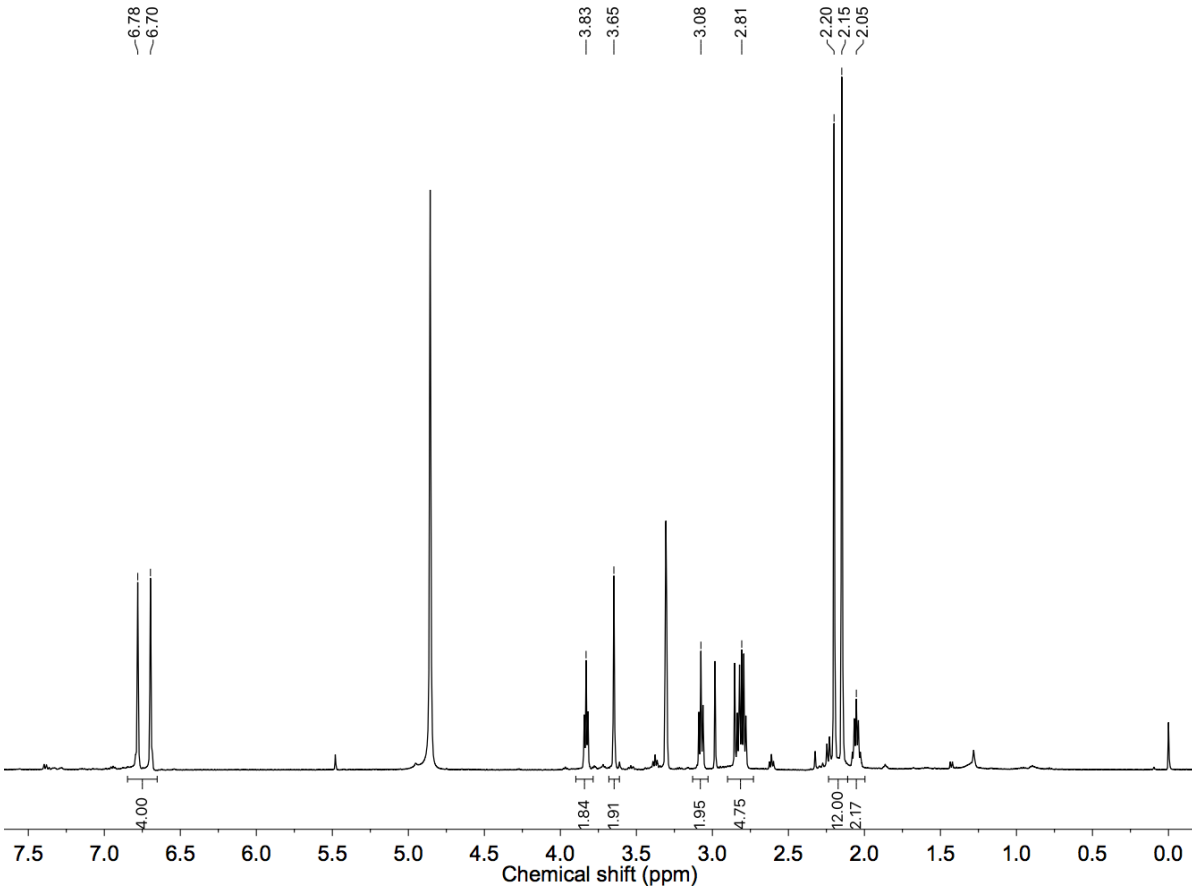


Figure S32 ^{1}H NMR spectrum of **primary amine-functionalized single allyl compound, M_1** (CD_3OD, 500 MHz)

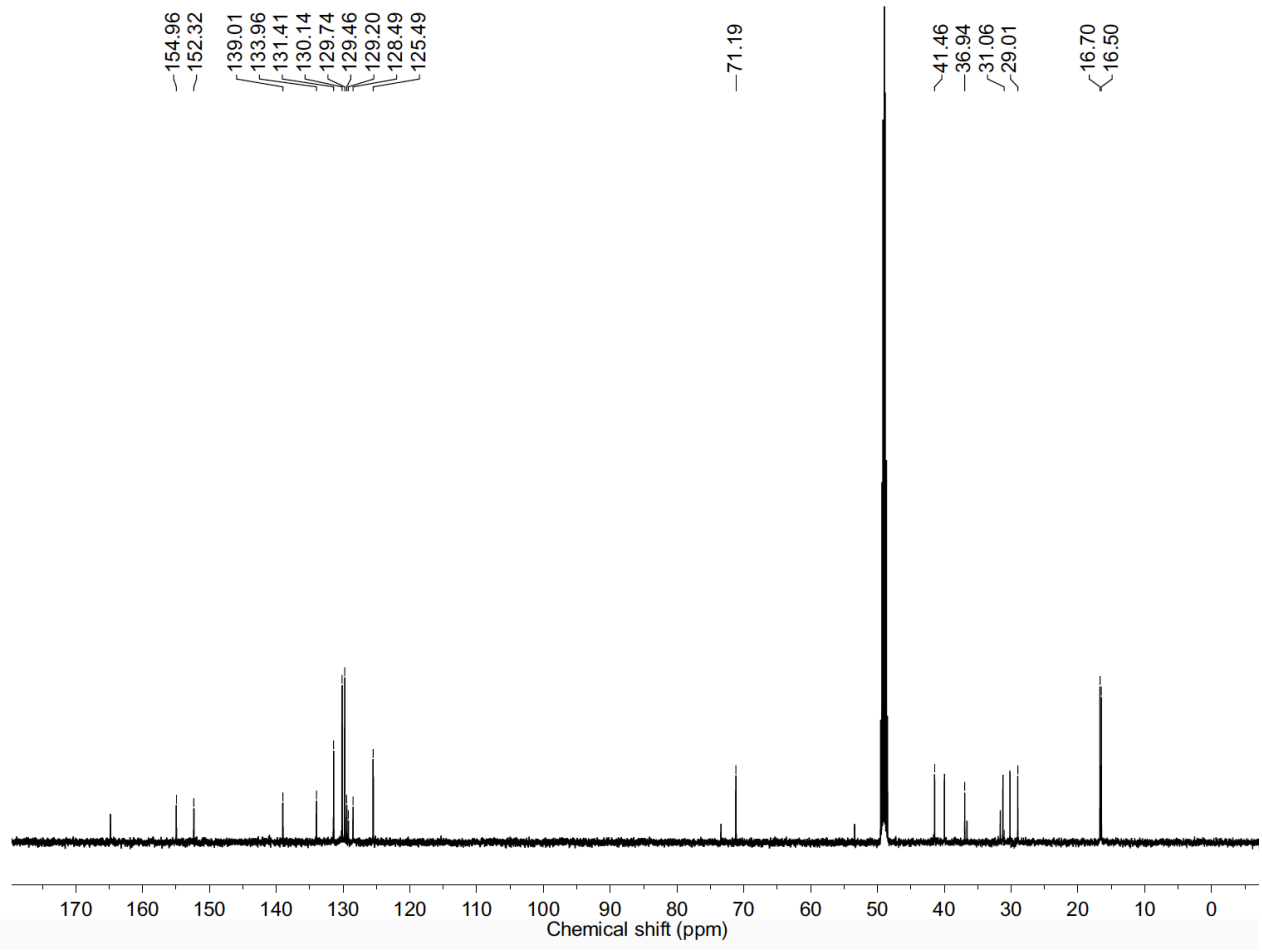


Figure S33 ^{13}C NMR spectrum of **primary amine-functionalized single allyl compound, M_1** (CD_3OD, 500 MHz)

Fluoride-triggered depolymerization

CsF (3.0 equiv) was added to **P_1-50-PEG_{800}** (1.0 equiv) in MeOH. Reaction was stirred for 16 h at room temperature. Reaction was concentrated by rotovap and monitored by ^{1}H NMR (in CD_3OD). The percent depolymerization was quantified using the ratio of integrated peak areas at ~ 5.5 ppm and the aromatic protons at 6.6-7.2 ppm.

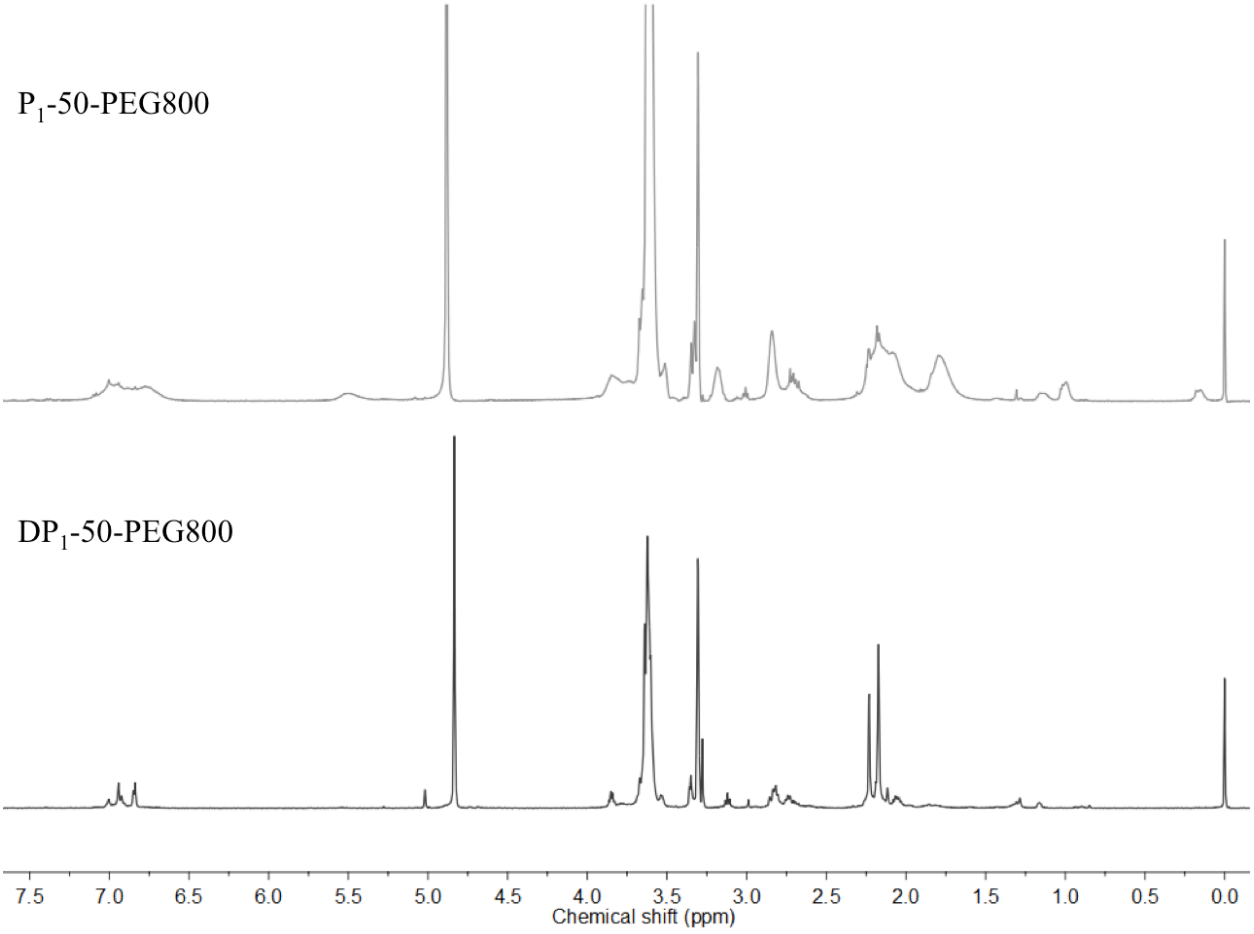


Figure S35 ^{1}H NMR spectra before and after depolymerization for **P_1-50-PEG_{800}**.

Control experiment *(no thiol present)*

Polymer $\mathbf{P_0}$ (1.0 equiv) was dissolved in DMF (0.85 M). In an amber vial, DMPA (photo-initiator) (0.1 equiv) was prepared in DMF and added into reaction. Reaction was sealed and bubbled with N_2 for 5 mins to remove gases in solution. Sealed reaction was stirred in N_2 flow for 60 min at room temperature under irradiation of UV light (UV source: 100W, λ = 365 nm).

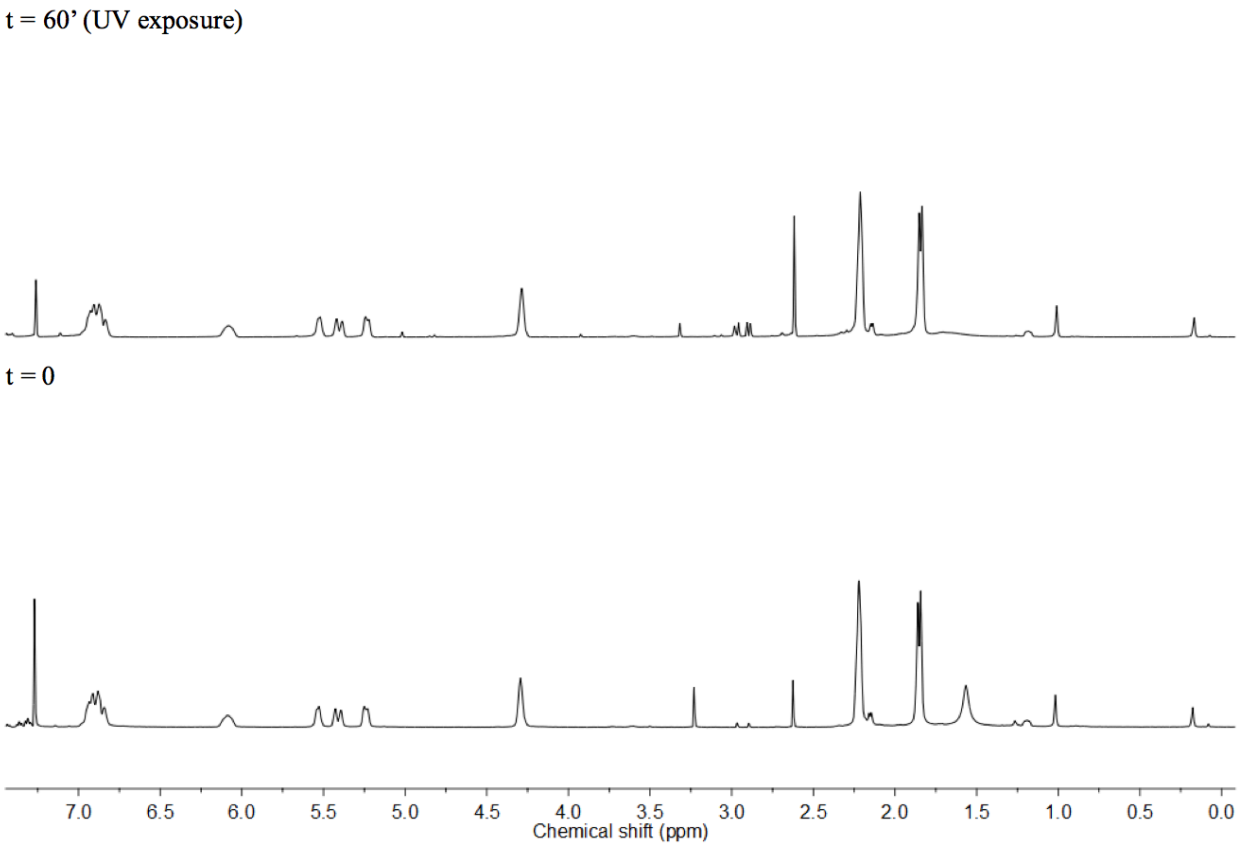


Figure S35 ^{1}H NMR spectra of control thiol-ene click reaction at t=0 and after 1 h UV (λ=365 nm) exposure (t=60')

Thiol-ene click chemistry: conversion of allyl side chains

Observed % of conversion of allyl side chains was determined by ^{1}H NMR peak integration.

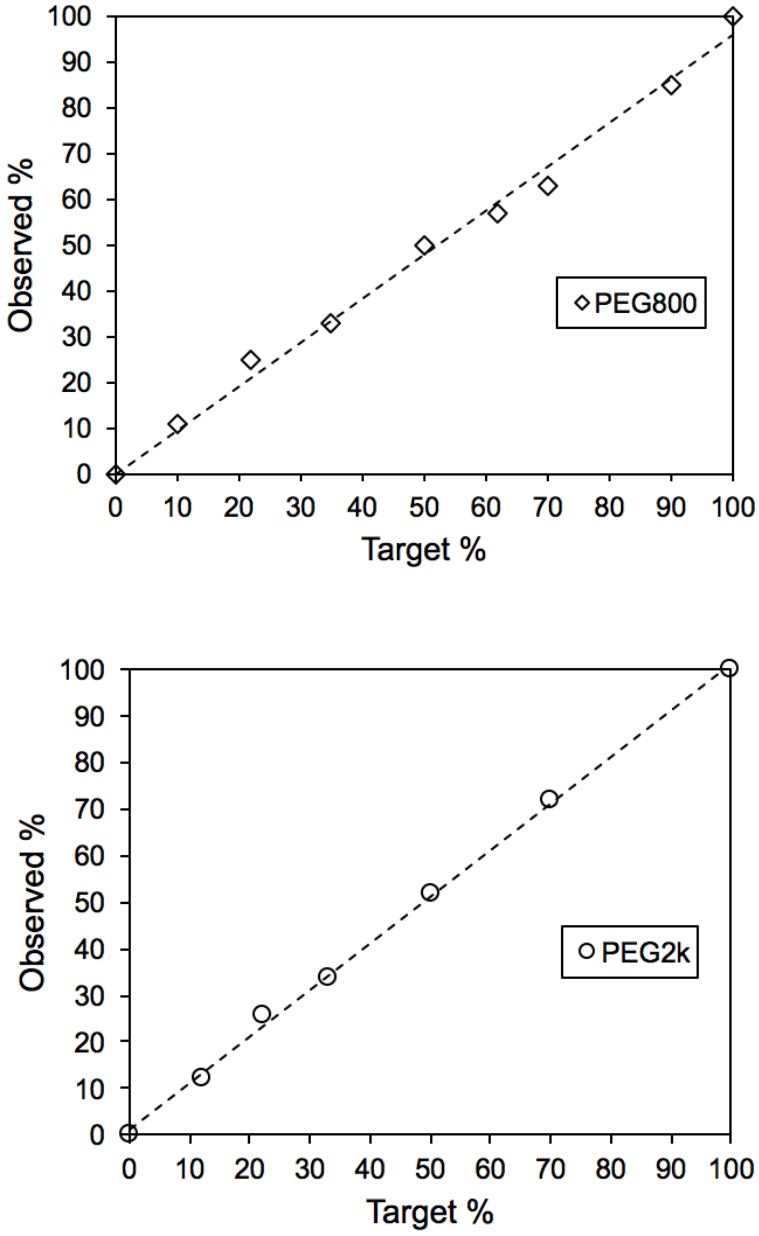
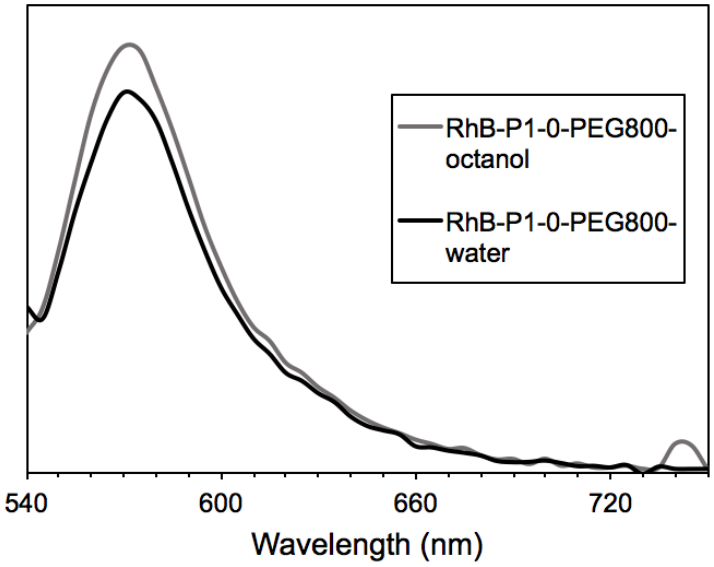
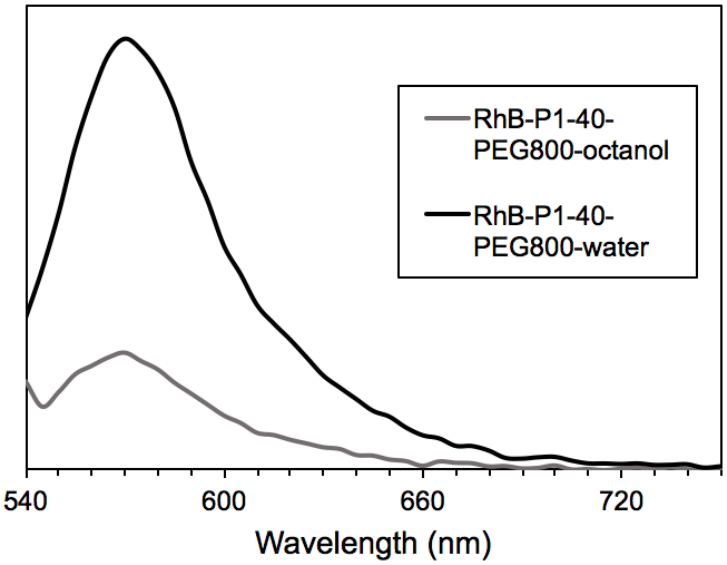


Figure S36 Target % of PEG side functionalization vs. Observed % PEG side functionalization

Partition coefficient (logP)

2 μl from 20 mg/ml DMSO stock solution of rhodamine-tagged polymers were added in 2 ml water, 2 ml octanol mixture in 15 ml centrifuge tubes (final concentration of polymer = 34 μM). Tubes are vortexed for 1 min and placed in orbital shaker for 16h at RT in dark. Next day, tubes were centrifuged to separate liquid phases. 100 μl from each phase was added in 1 ml MeOH. Emission spectra were recorded from 540 nm to 750 nm. (E_x = 525 nm, E_m = 575 nm for Rhodamine)





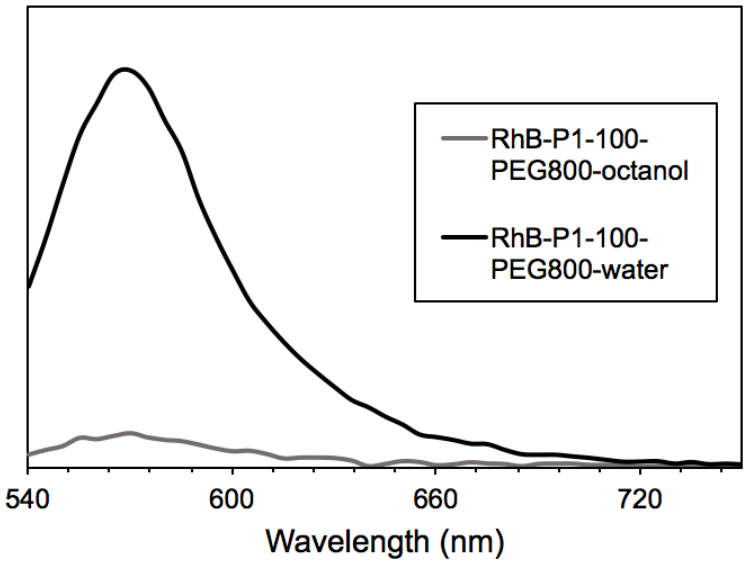


Figure S37 Fluoresence emission of rhodamine polymers

Bacterial strains and growth conditions

Table S2 Bacterium type and growth conditions. All broths and agars were obtained from Becton Dickinson (BD).

Bacterium	**Growth media**	**Growth agar**	**Growth Temperature (°C)**
Escherichia coli ATCC 25922	Mueller-Hinton broth	Mueller-Hinton	37
Pseudomonas aeruginosa ATCC 27853	Trypticase soy broth	Trypticase soy agar	37
Acinetobacter baumannii ATCC 17978	Mueller-Hinton broth	Mueller-Hinton	37
Staphylococcus aureus ATCC 25923	Mueller-Hinton broth	Mueller-Hinton	37
Staphylococcus aureus ATCC 33591	Mueller-Hinton broth	Mueller-Hinton	37
Enterococcus faecalis ATCC 29212	Brain Heart Infusion broth	Brain Heart Infusion agar	37

Antibacterial assays

The in vitro minimum bactericidal concentration (MBC) of polymers was assessed by the colony counting method. Polymer stock solutions were prepared in 50% DMSO-deionized H_2O with 2-fold serial dilutions of the stock, starting from 10 mg/ml to 5 μg/ml. A single colony of Escherichia coli ATCC 25922 was inoculated in Muller-Hinton (MH) broth at 37 °C in shaking incubator overnight. The turbid dilution was diluted to OD_{600} = 0.1 (measured by Molecular Devices SpectraMax M2), regrown for 90 min to midlogarithmic phase (OD_{600} = 0.5 – 0.6) in MH broth. Resulting suspension was centrifuged at 2000 rpm for 3 min and the supernatant was carefully removed by pipetting. Collected bacteria in centrifuge tube was resuspended in PBS (10mM phosphate, 137 mM sodium chloride) of pH 6.0. It was diluted to OD_{600} = 0.001 in PBS, corresponding to ~5 x 10^5 cfu/ml based upon colony counting on MH agar plates. The bacterial suspension (90 μl) was mixed with each polymer concentration (10 μl) in a sterile 96-well round-bottom polypropylene microplate (Chemglass #229590) and wrapped with parafilm. The microplate was incubated for 60 min at 37 °C in orbital shaker (180 rpm). After 60 min incubation, 10 μl of bacteria-polymer mixture was diluted in fresh PBS in same microplate to produce 10-fold and 10^2-fold dilutions. 10 μl of each dilution (0-10-10^2-fold) was pipetted onto MH agar plates, streaked and incubated 37 °C overnight. E. coli colony forming units (cfu) were counted. MBC was defined as the lowest concentration of polymer which induces at least 3 $\log_{10}$ reduction in the number of viable cells after incubation (mostly refers to ≥99.9% killing). All set of polymers were tested twice, each in duplicate, in different days. Final MBC values was determined by the average MBC of multiple tests. As a negative control, stock solution of DMSO was prepared in microplates with 2-fold serial dilutions, starting from 5% (v/v) and tested as polymers.

In addition to *E. coli*, polymers were performed against a broad range of bacteria. As gram-negative strains, *Acinetobacter baumannii* ATCC 17978 and *Pseudomonas aeruginosa* ATCC 27853 were used and incubated with polymers for 60 min. Gram-positive strains, including *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* ATCC 33591 and *Enterococcus faecalis* ATCC 29212 were incubated with polymers for 4 h. In each bacterial case, appropriate broths, agars and temperatures were used to grow bacteria as shown in **Table S2**.

Table S3 MBC of Rhodamine-tagged polymers against *E. coli*.

Polymer	MBC (μg/ml)
RhB-P_1-0-PEG800	31
RhB-P_1-40-PEG800	31
RhB-P_1-100-PEG800	>1000

Hemolysis assays

Hemolytic activity of polymers was determined by hemoglobin release assay using the same polymer stock solutions for MBC assays. 1 ml of 10% (v/v) sheep red blood cells was centrifuged at 1000 rpm for 5 min and washed with PBS of pH 6.0. The supernatant was carefully removed by pipetting. The RBCs were washed with PBS two more times. The resulting stock was diluted 10-fold in PBS to provide 1% (v/v) RBC assay stock. In a sterile 96-well round-bottom polypropylene microplate, 90 μl of 1% (v/v) RBC assay stock was mixed with 10 μl of each of the polymer dilution. As a negative control PBS, as a positive control 0.1% (v/v) Triton X-100 were used. Microplate was wrapped with parafilm, secured in orbital shaker at 37 °C and incubated at 180 rpm for 60 min. The microplate was centrifuged at 1000 rpm for 10 min. In another sterile microplate, 10 μl of supernatant was diluted in 90 μl PBS. The absorbance at 415 nm was recorded using a microplate reader. Hemolysis was plotted as a function of polymer concentration and the HC_{50} that is described as the polymer concentration causing 50% hemolysis relative to the positive control. This value was estimated by the fitting the experimental data to the function $H([P]) = 1/\{1+(K/[P])^n\}$ where H is the hemolysis fraction ($H = [OD_{415}(polymer)-OD_{415}(buffer)]/[OD_{415}(TritonX)-OD_{415}(buffer)]$), P is the polymer concentration, n and K are variable parameters. All experiments were repeated twice, each in duplicate, on different days. The absorbance values from each trial were averaged and then the HC_{50} was calculated.

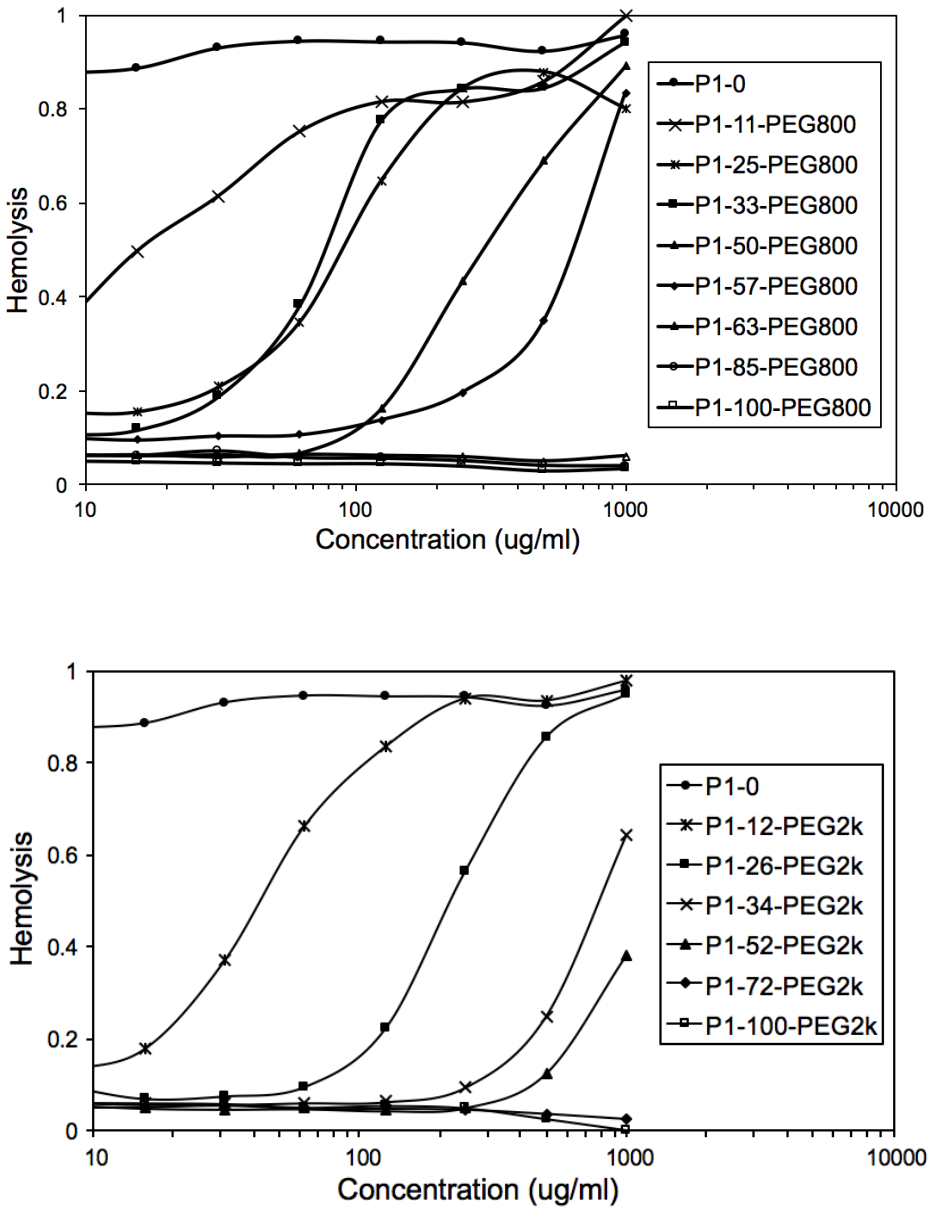


Figure S38 Hemolysis fraction vs. polymer concentration curves

Time-killing assay of *E. coli*

A single colony of *Escherichia coli* ATCC 25922 was inoculated in Muller-Hinton broth at 37 °C in shaking incubator overnight. The turbid dilution was diluted to OD_{600} = 0.1 (measured by Molecular Devices SpectraMax M2), regrown for 90 min to midlogarithmic phase (OD_{600} = 0.5 – 0.6) in MH broth. Resulting suspension was centrifuged at 2000 rpm for 3 min and the

supernatant was carefully removed by pipetting. Collected bacteria in centrifuge tube was resuspended in PBS of pH 6.0. It was diluted to OD_{600} = 0.001 in PBS. 1 ml of polymer stock solution (160 μg/ml in 8% (v/v) DMSO-PBS) was added in 4 ml of bacterial suspension to provide the final polymer concentration of 2 x MBC (32 μg/ml). Centrifuge tube was placed in orbital shaker at 37 °C. Every 5 to 10 min, 10 μl aliquots from the tube was pipetted onto MH agar plates and streaked as well as serial dilutions in fresh PBS to produce 10-fold dilutions (10^1, 10^2, and 10^3) (10 μl of each dilution was pipetted onto MH agar plates and streaked as well). Plates were incubated overnight at 37 °C, followed by colony counting. As a positive growth control, 1 ml of DMSO stock solution (8% (v/v) DMSO-PBS) was added in 4 ml of bacterial suspension to provide the final DMSO concentration in polymers (2%) and tested as polymers.

Confocal microscopy

A single colony of Escherichia coli ATCC 25922 was inoculated in Muller-Hinton broth at 37 °C in shaking incubator overnight. The turbid suspension was diluted to OD_{600} = 0.1, regrown for 90 min to midlogarithmic phase (OD_{600} = 0.5 – 0.6) in MH broth. Resulting suspension was centrifuged at 2000 rpm for 3 min and the supernatant was carefully removed by pipetting. Collected bacteria in centrifuge tube was resuspended in PBS of pH 6.0. Bacterial suspension was diluted to OD_{600} = 0.1 (~$5x10^7$ cells/ml). Polymer solution (310 μg/ml in 3.1% (v/v) DMSO-PBS) was added into 4 ml of bacterial suspension to provide the final concentration of polymer of 4 x MBC (62 μg/ml). The mixture was incubated with cells for 150 min. Once incubation was complete, mixture was centrifuged at 2000 rpm for 5 min and resuspended in 1 ml of imaging solution (10 mM HEPES, pH 7.4, containing 140 mM NaCl, 295 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM D-glucose). Cells were stained with 3 μl of SYTO 9 and propidium iodine (PI) solution (equal volumes of Component A and Component B) and incubated at room temperature for 15 min in dark. 5 μl of dyed bacterial suspension was placed on Nunc™ glass bottom dish (Thermo Fisher Scientific) and covered with glass cover slip. Cells were visualized under laser scanning confocal microscopy using Argon and HeNe1 lasers. SYTO9 (green dye) stain both live and dead cells, but PI (red dye) stains just dead cells. Same procedure above was applied for depolymerized byproducts of **P_1-50-PEG_{800}** after CsF treatment and negative control (0.62% DMSO-PBS).

Laser scanning confocal microscopy (Zeiss LSM 510 Meta) was employed using Argon (458-488-514 nm) and HeNe1 (543 nm) lasers. 512 x 512 pixel images were recorded from single scan or multiple z-stacks.

For rhodamine-tagged polymers, no live/dead assay kit was used. Polymers (**RhB-P_1-0-PEG800**, **RhB-P_1-40-PEG800** and **RhB-P_1-100-PEG800**) (no bacteria) were shaked at room temperature in PBS pH 6.0 for 2 hours and then imaged. Only HeNe1 (543 nm) laser was employed.

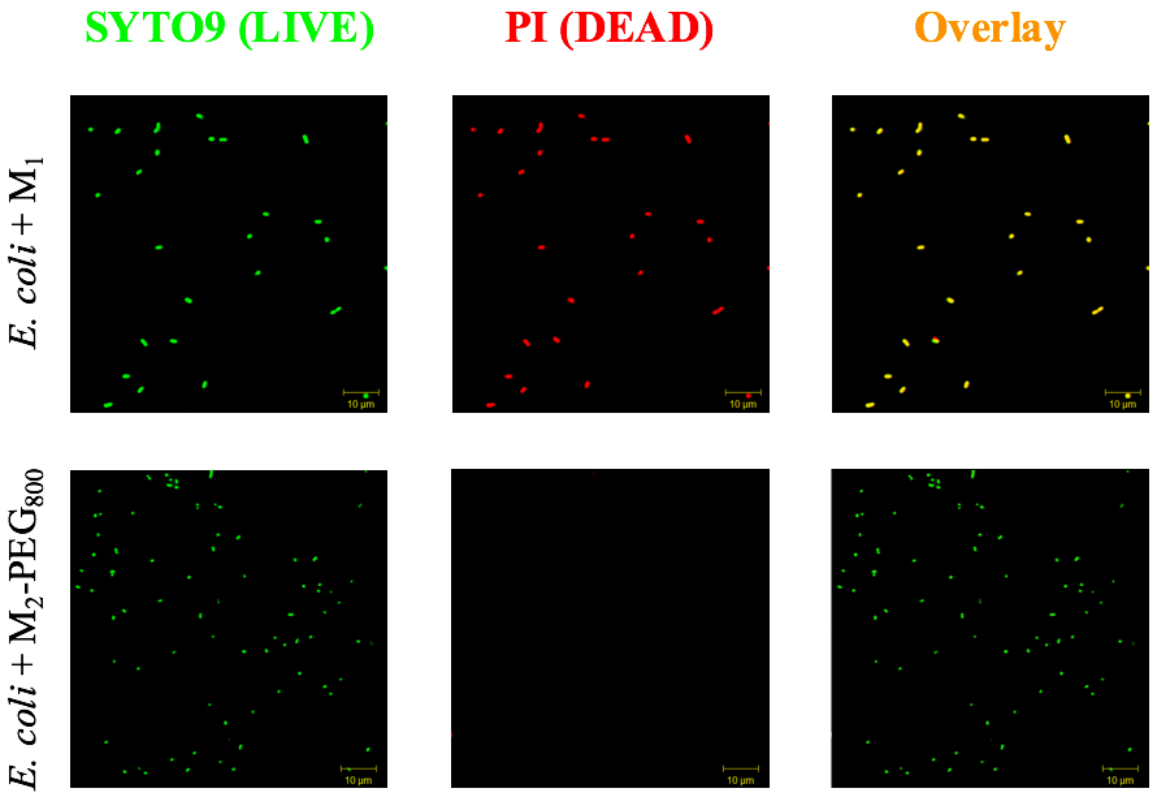


Figure S39 Confocal images of *E. coli* cells (first row) after exposure to **M_1**, (second row) after exposure to **M_2-PEG_{800}**. Scale bar is 10 µm in all images.