Supporting Information for

Development of a Vinyl Ether-functionalized Polyphosphoester as a Template for Multiple Post-polymerization Conjugation Chemistries and Study of Core Degradable Polymeric Nanoparticles

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EXPERIMETNAL SECTION

Materials. Ethylene glycol vinyl ether (EVE), 2-(2-methoxyethoxy)ethanethiol, 2,2-dimethoxy-2-phenylacetophenone (DMPA), diethyl ether, triethylamine (TEA), methanol (MeOH) and benzoic acid were used as received from Sigma-Aldrich Company or TCI America. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was used as received from Thermo Fisher Scientific Inc. Benzyl alcohol (BnOH), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 4-methylbenzyl alcohol, 4-methylbenzyl mercaptan were purchased from Sigma-Aldrich Company, and they were distilled from calcium hydride prior to use. *p*-toluenesulfonic acid monohydrate (PTSA) was purchased from Sigma-Aldrich Company, and it was dried by azeotropic distillation in toluene three times prior to use. α-Methoxy-ω-hydroxy poly(ethylene glycol) 2,000 Da ($mPEG_{44}$-OH) was purchased from Rapp Polymere, and it was dried by azeotropic distillation in toluene three times prior to use. The dried benzyl alcohol, DBU, 4-methylbenzyl alcohol, 4-methylbenzyl mercaptan, PTSA and $mPEG_{44}$-OH were stored in an argon-filled glove box until use. Dichloromethane (DCM) and *N*,*N*-dimethylformamide (DMF) were dried through columns (J. C. Meyer Solvent Systems, Inc.). Spectra/Pro® membranes (MWCO 6–8 kDa, Spectrum Medical Industries, Inc., Laguna Hills, CA) were used for dialysis. RAW 264.7 and OVCAR-3 cell lines, as well as RPMI and DMEM media were purchased from the American Type Culture Collection. Media additives (fetal bovine serum, penicillin/streptomycin) were purchased from Sigma Aldrich Company. The cell culture 96-well flat bottom plates were purchased from Corning Costar Co. The CellTiter 96 non-radioactive cell proliferation assay was obtained from Promega Co.

Instrumentation. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Inova 300 MHz spectrometers interfaced to a UNIX computer using VnmrJ software. Chemical shifts were referenced to solvent resonance signals. For ^{31}P NMR spectroscopy, phosphoric acid (85 wt. % in H_2O) at 0 ppm was used as an external standard. IR spectra were recorded on an IR Prestige 21 system (Shimadzu Corp.) and analyzed by using the IRsolution software.

The polymer molecular weight and molecular weight distribution were determined by Gel Permeation Chromatography (GPC). The GPC was conducted on a Waters 1515 HPLC (Waters Chromatography, Inc.) equipped with a Waters 2414 differential refractometer, a PD2020 dual-angle (15° and 90°) light scattering detector (Precision Detectors, Inc.), and a three-column series (PL gel 5 μm Mixed C, 500 Å, and 104 Å, 300 × 7.5 mm columns; Polymer Laboratories, Inc.). The system was equilibrated at 40 °C in THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL/min. Polymer solutions were prepared at a known concentration (3–5 mg/mL) and an injection volume of 200 μL was used. Data collection and analysis were performed with Precision Acquire software and Discovery 32 software (Precision Detectors, Inc.), respectively.

Glass transition temperatures (T_g) were measured by differential scanning calorimetry (DSC) on a Mettler-Toledo DSC822® (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 10 °C/min. Measurements were analyzed by using Mettler-Toledo Star^e v. 7.01 software. The T_g was taken as the midpoint of the inflection tangent, upon the second heating scan. Thermogravimetric analysis (TGA) was performed under N_2 atmosphere using a Mettler-Toledo model TGA/SDTA851e, with a heating rate of 5 °C/min and cooling rate of 5 °C/min. Measurements were analyzed by using Mettler-Toledo Star^e v. 7.01 software.

Dynamic light scattering (DLS) measurements were conducted using Delsa Nano C (Beckman Coulter, Inc., Fullerton, CA) equipped with a laser diode operating at 658 nm. Size measurements were made in nanopure water (n = 1.3329, η = 0.890 cP at 25 ± 1 °C). Scattered light was detected at 165° angle and analyzed using a log correlator over 70 accumulations for a 3.0 mL sample in a glass sizing cell (4.0 mL capacity). The samples in the glass sizing cell were equilibrated for 30 minutes before measurements were made. The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of *ca.* 10 kcps. Calculation of the particle size distribution and distribution averages was performed using CONTIN particle size distribution analysis routines. The peak averages of histograms from number distributions out of 70 accumulations were reported as the average diameters of the particles.

The zeta potential values of the nanoparticles were determined by Delsa Nano C particle analyzer (Beckman Coulter, Fullerton, CA) equipped with a 30 mW dual laser diode (658 nm). The zeta potential of the particles in suspension was obtained by measuring the electrophoretic movement of charged particles under an applied electric field. Scattered light was detected at a 30° angle at 25 °C. The zeta potential was measured at five regions in the flow cell and a weighted mean was calculated. These five measurements were used to correct for electroosmotic flow that was induced in the cell due to the surface charge of the cell wall. All determinations were repeated three times.

Transmission electron microscopy (TEM) images were collected on a JEOL 1200EX operating at 100 kV and micrographs were recorded at calibrated magnifications using a SIA-15C CCD camera. The samples as aqueous solutions (5 μL) were deposited onto carbon-coated copper grids. Excess sample was wicked off using filter paper and the grids were allowed to dry

in the air for 1 min. Following this, the grids were stained with 5 μL of a 2% uranyl acetate aqueous solution. Excess stain was wicked off by using filter paper after 20 seconds. The sample grids were dried under vacuum overnight before analysis.

Atomic force microscopy (AFM) imaging was performed by using a MFP-3D system (Asylum Research) using a standard silicon probe (VISTAprobes, resonance constant: 62 kHz, tip radius: 15 nm, spring constant: 3 N/m). For AFM sample preparation, the sample was dissolved in nanopure water at 0.25 mg/mL and 20 μL of the sample was spin coated onto a glass coverslip. All AFM samples were stored at room temperature under vacuum prior to use.

Electrospray ionization (ESI) in negative ion mode was carried on a quadruple ion trap mass spectrometer (LCQ-DECA, ThermoFinnigan, San Jose, CA). The sample was directly infused at a flow rate of 6 μL/min. The spray voltage was set at -4.5 kV. Sheath gas and auxiliary gas flow rates were 50 and 10 arbitrary units, respectively. Transfer capillary temperature was held at 250 °C. MS/MS experiments were performed on the same instrument at a relative collision energy of 30−32%. Xcalibur 2.0 software package (ThermoFinnigan) was used for data acquisition and processing.

Gas chromatography-mass spectrometry (GC-MS) was performed on Ultra GC/DSQ (ThermoElectron, Waltham, MA). Chromatography was carried out using an Rxi-5ms column (60 m × 0.25 mm with 0.25 μm film thickness) (Restek; Bellefonte, PA). Helium was used as a carrier gas at constant flow of 1.5 mL/min. GC inlet was held at 225 °C while transfer line and ion source were held at 250 °C. An aliquot of 1 μL of sample was injected in splitless mode. The oven temperature was maintained at 50 °C for 5 min and raised to 320 °C at 20 °C/min.

Electron impact ionization at 70eV was used for ionization and mass spectra were acquired in full scan mode in the range of 30-500 m/z.

Matrix assisted laser desorption ionization (MALDI) experiments were performed on a Voyager DE-STR mass spectrometer (Applied Biosystems, Foster City, CA) under optimized conditions in positive linear mode. Ions were generated by a pulsed nitrogen laser at 337 nm and accelerated through 25 kV. 100 laser shots were used per spectrum. Trans-2-[3-(4-*t*-butyl-phenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) and potassium trifluoroacetate (KTFA) were used as a matrix and cationization reagent, respectively. The sample, KTFA, and matrix were prepared at concentration of 1, 10 and 20 mg/mL respectively. The sample solution was mixed with the matrix and KTFA at a volume ratio of 1:5:1. About 0.5 μL of this mixture was deposited on a stainless steel sample holder. After air-dried, the sample was analyzed using MALDI TOF MS.

Synthesis of Monomer, 2-Ethylene glycol vinyl ether-1,3,2-dioxaphospholane 2-oxide (EVEP). A solution of COP (5.0026 g, 35.11 mmol) in 10 mL of anhydrous DCM was added dropwise to a stirred solution of EVE (3.0908 g, 35.08 mmol) and TEA (4.89 mL, 3.55 g, 35.1 mmol) in 50 mL of anhydrous DCM at 4 °C. The reaction mixture was allowed to stir for 12 h at 4 °C and then for 30 min at rt. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure. Diethyl ether (200 mL) was then added to precipitate the remaining triethylammonium chloride. After the removal of the precipitates by filtration and the solvent *in vacuo*, the clear viscous liquid EVEP was collected (5.13 g, 76% yield). IR: 3045–2800, 1622, 1500–1432, 1368, 1285, 1198, 1070, 1024, 993, 926, 835, 764 cm^{-1}. ^{1}H NMR ($CDCl_3$, ppm): δ 6.44 (dd, J = 14.4 Hz, J = 6.9 Hz, 1H, CH_2OCHCH_2), 4.46–4.26 (m, 6H, $POCH_2CH_2OP$ and $POCH_2CH_2OCHCH_2$), 4.16 (dd, J = 14.4 Hz, J = 2.4 Hz, 1H, $CH_2OCHCHH$), 4.01 (dd, J = 6.9

Hz, J = 2.4 Hz, 1H, $CH_2OCHCHH$), 3.86 (m, 2H, $POCH_2CH_2OCHCH_2$). ^{13}C NMR ($CDCl_3$, ppm): δ 151.3, 87.3, 66.8 (d, J = 23.4 Hz), 66.6 (d, J = 24.3 Hz), 66.1 (d, J = 10.2 Hz). ^{31}P NMR ($CDCl_3$, ppm): δ 17.91. +ESI MS: calculated $[M+H]^+$ for $C_6H_{11}O_5P$: 195.0422, found: 195.0419.

Kinetic Study of the Homopolymerization of EVEP *via* ROP by Using an Organocatalyst, DBU. In a glovebox, a solution of EVEP (1.0050 g, 5.18 mmol) and benzyl alcohol (5.34 μL, 5.58 mg, 0.052 mmol) in anhydrous DCM (1.0 mL) was placed in a flamed-dried 5 mL shell vial with a magnetic stir bar. DBU (23.1 μL, 23.5 mg, 0.15 mmol) was added into the mixture solution. 70 μL of samples were extracted at 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, and 18 min and quenched immediately by adding a solution of benzoic acid (excess). Conversion of monomer to polymer was calculated by ^{31}P NMR spectroscopy. A portion of the collected samples was purified by precipitation in diethyl ether twice and dried *in vacuo* before injection into the THF GPC to obtain the number average molecular weight, M_n, and polydispersity index, PDI.

Synthesis of Homopolymer, Poly(ethylene glycol vinyl ether phosphotriester)$_{50}$ (PEVEP$_{50}$). In a glovebox, a solution of EVEP (1.9991 g, 10.30 mmol) and benzyl alcohol (10.7 μL, 11.2 mg, 0.10 mmol) in anhydrous DCM (2.0 mL) was placed in a flamed-dried 5 mL shell vial with a magnetic stir bar. DBU (46.2 μL, 47.0 mg, 0.31 mmol) was added into the mixture solution. After 9 min of stirring, the reaction was quenched immediately by adding a solution of benzoic acid (excess). The product was dialyzed (MWCO 6–8 kDa) in MeOH followed by DCM overnight. A viscous liquid was collected after removal of the solvent *in vacuo* (0.5588 g, 56% yield). GPC (THF): M_n = 4,040 g/mol, PDI = 1.05. IR: 3700–3300, 3025–2825, 1622, 1454, 1425–1350, 1323, 1269, 1198, 1022, 964, 812 cm^{-1}. ^1H NMR (CD_2Cl_2, ppm): δ 7.43–7.34 (m, 5H, aromatic ring), δ 6.49 (dd, J = 14.4 Hz, J = 6.9 Hz, 50H, CH_2OCHCH_2), 5.08 (d, J = 8.1 Hz, 2H, $POCH_2Ar$), 4.34–4.15 (broad, 350H, $POCH_2CH_2OP$, $POCH_2CH_2OCHCH_2$ and

$CH_2OCHCHH$), 4.06 (broad dd, J = 6.9 Hz, J = 2.1 Hz, 50H, $CH_2OCHCHH$), 3.94–3.86 (broad t, 100H, $POCH_2CH_2OCHCH_2$). ^{13}C NMR (CD_2Cl_2, ppm): δ 151.9, 129.1, 128.6, 87.7, 67.4 (d, J = 28.2 Hz), 67.2–66.6 (m). ^{31}P NMR (CD_2Cl_2, ppm): δ -0.68. DSC: (T_g) = -39 °C. TGA in N_2: 225 °C, 10% mass loss; 245 °C, 10% mass loss; 285 °C, 22% mass loss; 48% mass remaining above 285 °C.

Thiol-ene "Click" Reaction of Vinyl Ether Side Chain Moieties of $PEVEP_{50}$ with 2-(2-Methoxyethoxy)ethanethiol. A solution of $PEVEP_{50}$ (0.3003 g, 0.035 mmol), 2-(2-methoxyethoxy)ethanethiol (0.6 mL, 0.6 g, 4 mmol), and DMPA (0.2302 g, 0.898 mmol) in 10 mL of MeOH was irradiated under UV irradiation (365 nm, 6 W) for 1 h while being stirred. The reaction mixture was purified by precipitated in diethyl ether twice and dialysis (MWCO 6–8 kDa) in MeOH followed by DCM overnight. A viscous liquid was collected after the removal of solvent *in vacuo* (0.3651 g, 68% yield). GPC (THF): M_n = 6,020 g/mol, PDI = 1.07. IR: 3675–3175, 3050–2700, 1675–1600, 1456, 1356, 1273, 1197, 1094, 1020, 970, 812 cm^{-1}. ^1H NMR (CD_2Cl_2, ppm): δ 7.45–7.30 (m, 5H, aromatic ring), 5.09 (d, J = 8.1 Hz, 2H, $POCH_2Ar$), 4.40–4.05 (broad, 300H, $POCH_2CH_2OCH_2CH_2S$ and $POCH_2CH_2OP$), 3.72–3.44 (broad, 500H, $POCH_2CH_2OCH_2CH_2SCH_2CH_2OCH_2CH_2OCH_3$), 3.33 (s, 150H, $OCH_2CH_2OCH_3$), 2.78–2.69 (broad q, 200H, $OCH_2CH_2SCH_2CH_2O$). ^{13}C NMR (CD_2Cl_2, ppm): δ 129.1, 128.5, 72.4, 71.5 (d, J = 18.9 Hz), 70.7, 70.1 (d, J = 27.9 Hz), 67.6, 67.0, 59.2, 32.4 (d, J = 25.5 Hz). ^{31}P NMR (CD_2Cl_2, ppm): δ -0.68. DSC: (T_g) = -64 °C. TGA in N_2: 280 °C, 70% mass loss; 30% mass remaining above 280 °C.

Acetalization of the Vinyl Ether Side Chain Moieties of $PEVEP_{50}$ with 4-Methylbenzyl Alcohol. In a glovebox, a solution of $PEVEP_{50}$ (0.1004 g, 0.010 mmol) and dried 4-methylbenzyl alcohol (128 mg, 1.05 mmol) in 0.5 mL of DMF were placed in a flame-dried 5

mL shell vial, and a solution of PTSA (20 mg, 0.12 mmol) in 0.5 mL of DMF was added into the solution. After 5 min of stirring, the reaction was quenched by adding TEA (excess). The reaction mixture was purified by dialysis (MWCO 6–8 kDa) in DMF followed by DCM overnight. A viscous liquid was collected after the removal of solvent *in vacuo* (74.1 mg, 67% yield). IR: 3700–3200, 3075–2775, 1622, 1454, 1430–1357, 1323, 1267, 1200, 1020, 966, 806, 750 cm^{-1}. ^{1}H NMR (CD_2Cl_2, ppm): δ 7.44–7.32 (m, 5H, aromatic ring), 7.22 (d, J = 7.5 Hz, 18H, $OCH_2CCHCH(CHCH)CCH_3$), 7.14 (d, J = 7.8 Hz, 18H, $OCH_2CCHCH(CHCH)CCH_3$), 6.49 (dd, J = 14.4 Hz, J = 6.9 Hz, 25H, CH_2OCHCH_2), 5.08 (d, J = 8.1 Hz, 2H, $POCH_2Ar$), 4.87–4.75 (broad q, 9H, $OCH(CH_3)O$), 4.59 and 4.45 (d, J = 11.4 Hz, 18H, OCH_2ArCH_3), 4.38–4.10 (broad, 325H, $POCH_2CH_2OP$, $OPOCH_2CH_2O$ and $CH_2OCHCHH$), 4.05 (broad dd, 25H, $CH_2OCHCHH$), 3.94–3.84 (broad, 68H, $POCH_2CH_2OCHCH_2$ and $POCH_2CH_2OCH(CH_3)O$), 3.83–3.61 (b, 48H, $OPOCH_2CH_2OH$), 2.32 (s, 27H, OCH_2ArCH_3), 1.33 (d, J = 5.1 Hz, 27H, $OCH(O)CH_3$). ^{13}C NMR (CD_2Cl_2, ppm): δ 151.90, 137.85, 135.83, 129.52, 128.30, 99.87, 87.66, 70.58, 68.1–67.6 (broad), 67.3 (d, J = 28.2 Hz), 67.2–66.4 (broad), 64.02 (d, J = 28.8 Hz), 21.40, 20.05. ^{31}P NMR (CD_2Cl_2, ppm): δ -0.68. DSC: (T_g) = -27 °C. TGA in N_2: 225 °C, 26% mass loss; 295 °C, 28% mass loss; 46% mass remaining above 295 °C.

Thio-acetalization of Vinyl Ether Side Chain Moieties of $PEVEP_{50}$ with 4-Methylbenzyl Mercaptan. In a glovebox, a solution of $PEVEP_{50}$ (0.1005 g, 0.010 mmol) and dried 4-methylbenzyl mercaptan (136 μL, 0.141 g, 1.02 mmol) in 0.5 mL of DMF were placed in a flame-dried vial, and a solution of PTSA (21 mg, 0.12 mmol) in 0.5 mL of DMF was added into the solution. After 5 min of stirring, the reaction was quenched by adding TEA (excess). The reaction mixture was purified by dialysis (MWCO 6–8 kDa) in DMF followed by DCM overnight. A viscous liquid was collected after the removal of solvent *in vacuo* (73.6 mg, 73%

yield). IR: 3700–3200, 3045–2835, 1622, 1454, 1425–1350, 1323, 1265, 1200, 1020, 966, 812, 739 cm^{-1}. ^1H NMR (CD_2Cl_2, ppm): δ 7.44–7.32 (m, 5H, aromatic ring), 7.20 (d, J = 8.1 Hz, 8H, $SCH_2CCHCH(CHCH)CCH_3$), 7.11 (d, J = 7.8 Hz, 8H, $SCH_2CCHCH(CHCH)CCH_3$), 6.50 (dd, J = 14.4 Hz, J = 6.9 Hz, 28H, CH_2OCHCH_2), 5.09 (d, J = 8.1 Hz, 2H, $POCH_2Ar$), 4.71 (q, J = 6.3 Hz, 4H, $SCH(CH_3)O$), 4.38–4.09 (broad, 328H, $POCH_2CH_2OP$, $OPOCH_2CH_2O$ and $CH_2OCHCHH$), 4.06 (broad dd, 28H, $CH_2OCHCHCH$), 3.94–3.83 (broad, 64H, $POCH_2CH_2OCHCH_2$ and $POCH_2CH_2OCH(CH_3)S$), 3.83–3.58 (broad, 62H, $OPOCH_2CH_2OH$ and SCH_2Ar), 2.31 (s, 12H, OCH_2ArCH_3), 1.51 (d, J = 6.3 Hz, 12H, $OCH(O)CH_3$). ^{13}C NMR (CD_2Cl_2, ppm): δ 151.91, 129.66, 129.31, 105.54, 100.33, 87.68, 70.67, 67.4 (d, J = 28.8 Hz), 67.2–66.6 (broad), 64.42, 61.75, 32.83, 22.34, 21.34, 19.89. ^{31}P NMR (CD_2Cl_2, ppm): δ -0.67. DSC: (T_g) = -31 °C. TGA in N_2: 240 °C, 22% mass loss; 290 °C, 22% mass loss; 56% mass remaining above 290 °C.

Synthesis of Diblock Copolymer, α-Methoxy Poly(ethylene glycol)$_{44}$-*block*-poly(ethylene glycol vinyl ether phosphotriester)$_{33}$, mPEG$_{44}$-*b*-PEVEP$_{33}$. In a glove box, a solution of EVEP (1.2950 g, 6.67 mmol) and $mPEG_{44}$-OH (134 mg, 0.067 mmol) in anhydrous DCM (1.3 mL) was placed in a flame-dried 5 mL shell vial with a magnetic stir bar. DBU (30 µL, 31 mg, 0.20 mmol) was injected into the mixture solution. After 6 min of stirring, the reaction was quenched by adding a solution of benzoic acid (excess). The reaction mixture was purified by dialysis in MeOH followed by DCM overnight. A viscous solid was collected after the removal of solvent *in vacuo* (0.6776 mg, 79% yield). GPC (THF): M_n = 6,550 g/mol, PDI = 1.09. IR: 3700–3325, 3020–2780, 1620, 1454, 1323, 1269, 1200, 1022, 972, 814, 748 cm^{-1}. ^1H NMR (CD_2Cl_2, ppm): δ 6.49 (dd, J = 14.4 Hz, J = 6.9 Hz, 33H, CH_2OCHCH_2), 4.34–4.12 (broad, 231H, $POCH_2CH_2OP$, $POCH_2CH_2OCHCH_2$ and $CH_2OCHCHH$), 4.06 (broad dd, J = 6.9 Hz, J =

2.1 Hz, 33H, $CH_2OCHCH\mathit{H}$), 3.94–3.86 (broad t, 66H, $POCH_2C\mathit{H}_2OCHCH_2$), 3.61–3.58 (broad, 176H, $CH_3OC\mathit{H}_2C\mathit{H}_2O$), 3.33 (s, 3H, $C\mathit{H}_3OCH_2CH_2OCH_2$). ^{13}C NMR (CD_2Cl_2, ppm): δ 151.9, 87.7, 71.0, 67.4 (d, J = 28.2 Hz), 67.2–66.6 (broad). ^{31}P NMR (CD_2Cl_2, ppm): δ -0.71. DSC: (T_g) = -38 °C. TGA in N_2: 250 °C, 16% mass loss; 265 °C, 10% mass loss; 300 °C, 27% mass loss; 47% mass remaining above 300 °C.

General Procedure for the Self-assembly of $mPEG_{44}$-*b*-$PEVEP_{33}$. The amphiphilic diblock copolymer, $mPEG_{44}$-*b*-$PEVEP_{50}$, (1.0 mg) was suspended into nanopure water (for TEM and AFM analyses), 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (10 mM, pH 7.4) or ammonium acetate buffer (10 mM, pH 5.0) (for DLS and zeta potential measurements) in a vial and stirred at room temperature for 30 min.

Investigation of the Micelle Stability in Aqueous Solution by Monitoring the Changes in Hydrodynamic Diameters and Intensity, Scattered by Micelles, as Measured by DLS. Amphiphilic diblock copolymers, $mPEG_{44}$-*b*-$PEVEP_{33}$, were dissolved (1 mg/mL) in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (10 mM, pH 7.4) or ammonium acetate buffer (10 mM, pH 5.0) in a vial. The prepared solutions were stirred at room temperature or incubated in a shaker at 37 °C and, hydrodynamic diameter and intensity, scattered by the micelles were monitored by using DLS over a period of time.

Investigation of the Degradability of the PEVEP Backbone Segment in Aqueous Solution by Using ^{31}P NMR Spectroscopy. A solution of amphiphilic diblock copolymers, $mPEG_{44}$-*b*-$PEVEP_{33}$, (10 mg/mL) in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (10 mM in D_2O, pH 7.4) or sodium acetate buffer (10 mM in D_2O, pH 5.0) was incubated in a shaker at 37

°C and, and the backbone degradability of PEVEP was monitored by ^{31}P NMR spectroscopy over a period of time.

Investigation of the Stability of the Vinyl Ether Side Chain Moieties in Aqueous Solution by Using ^{1}H NMR Spectroscopy. A solution of amphiphilic diblock copolymers, mPEG$_{44}$-*b*-PEVEP$_{33}$, (10 mg/mL) in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (10 mM in D_2O, pH 7.4) or sodium acetate buffer (10 mM in D_2O, pH 5.0) was incubated in a shaker at 37 °C and, the disappearance of the vinyl group and the appearance of acetaldehyde ^{1}H resonance peaks were monitored by using ^{1}H NMR spectroscopy over a period of time.

Preparation and Identification of the Degradation Products of the Micelles. A solution of amphiphilic diblock copolymers, mPEG$_{44}$-*b*-PEVEP$_{33}$, (6 mg/mL) in ammonium acetate buffer (D_2O, 10 mM, pH 5.0) was incubated in a shaker at 37 °C until the micelle detection was not possible by DLS and the complete disappearance of ^{31}P resonance signal from the intact PEVEP backbone was confirmed by ^{31}P NMR spectroscopy. The solution was lyophilized into a powder, and then analyzed by ESI, GC and MALDI-TOF MS.

Preparation of Degradation Products for Cytotoxicity Tests. A solution of amphiphilic diblock copolymers, mPEG$_{44}$-*b*-PEVEP$_{33}$, (9 mg/mL) in phosphate buffered saline (PBS) (10 mM, pH 7.4) was incubated in a shaker at 37 °C until the micelles were not detectable by DLS. The solution was lyophilized into a powder and was used for the cytotoxicity tests.

Cytotoxicity Assays. Human ovarian adenocarcinoma cells (OVCAR-3) (5×10^3 cells/well) and RAW 264.7 mouse macrophages (2×10^4 cells/well) were plated in 96-well plates in RPMI-1640 medium and Dulbecco's Modified Eagle's Medium (DMEM) (20% and 10% fetal bovine serum, for the OVCAR-3 and RAW 264.7, respectively, and 1% penicillin/streptomycin). Cells

were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 atmosphere. The medium was replaced with a fresh medium 24 h after seeding, and 1 h prior to the addition of the various formulations at concentrations ranged from 18.0×10^{-3} to 1.76 μg/mL of polymer dissolved in phosphate buffered saline (PBS). For each well, 20 μL of each formulation was added to 100 μL of the medium. Negative controls were created by addition of 20 μL of PBS to wells containing 100 μL of the medium. The cells were incubated for 24 h, and after this period, the media was replaced with 100 μL of the complete medium. Then, 20 μL of the MTS combined reagent was added to each well (Cell Titer 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega Co.). The cells were incubated with the reagent for 2 h for RAW 264.7 cells, and 3 h for OVCAR-3 cells, at 37 ˚C in a humidified atmosphere containing 5% CO_2 protected from light. Absorbance was measured at 490 nm by using SpectraMax M5 (Molecular Devices Co.). The cell viability was calculated based on the relative absorbance to the control untreated cells.

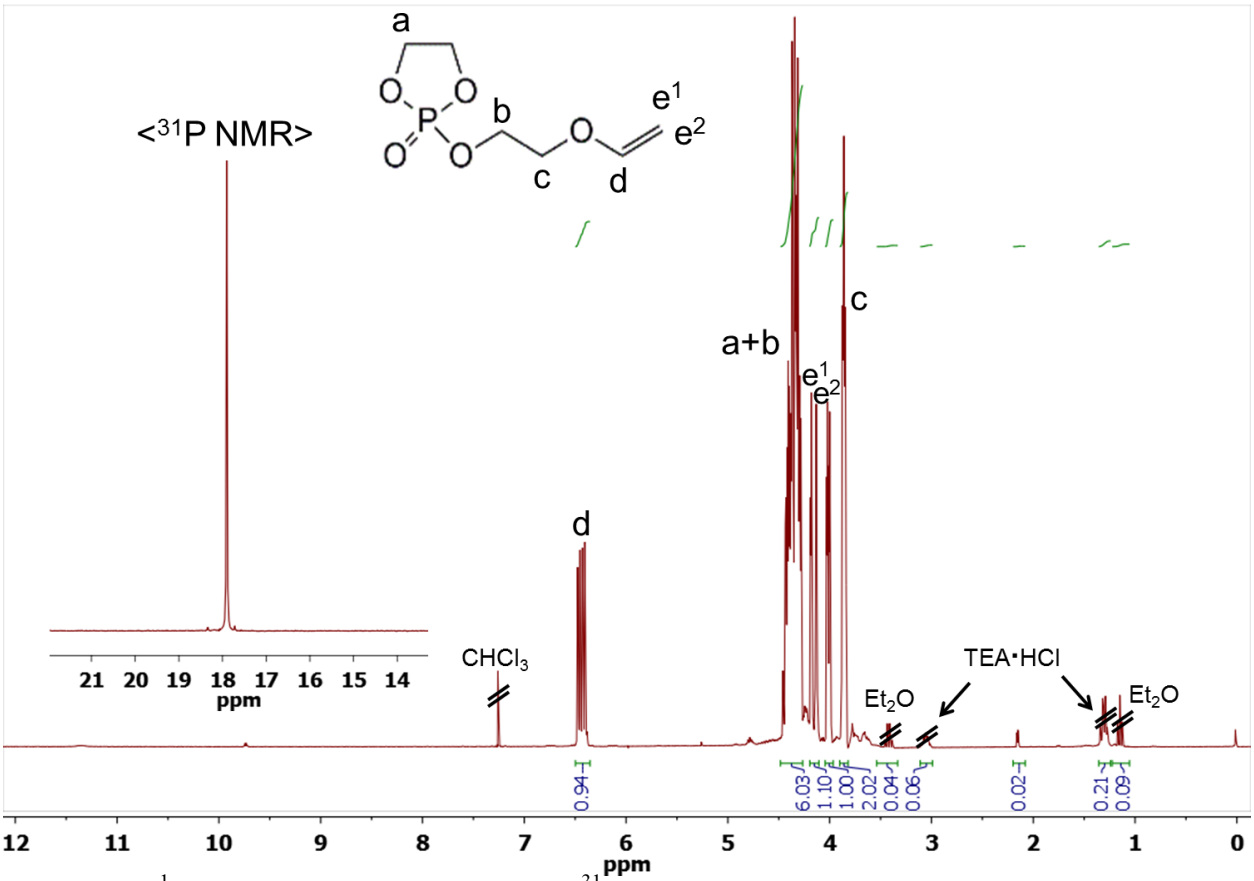


Figure S1. ^{1}H (300 MHz, $CDCl_3$, ppm) and ^{31}P (121 MHz, $CDCl_3$, ppm, inset) NMR spectra of **1**.

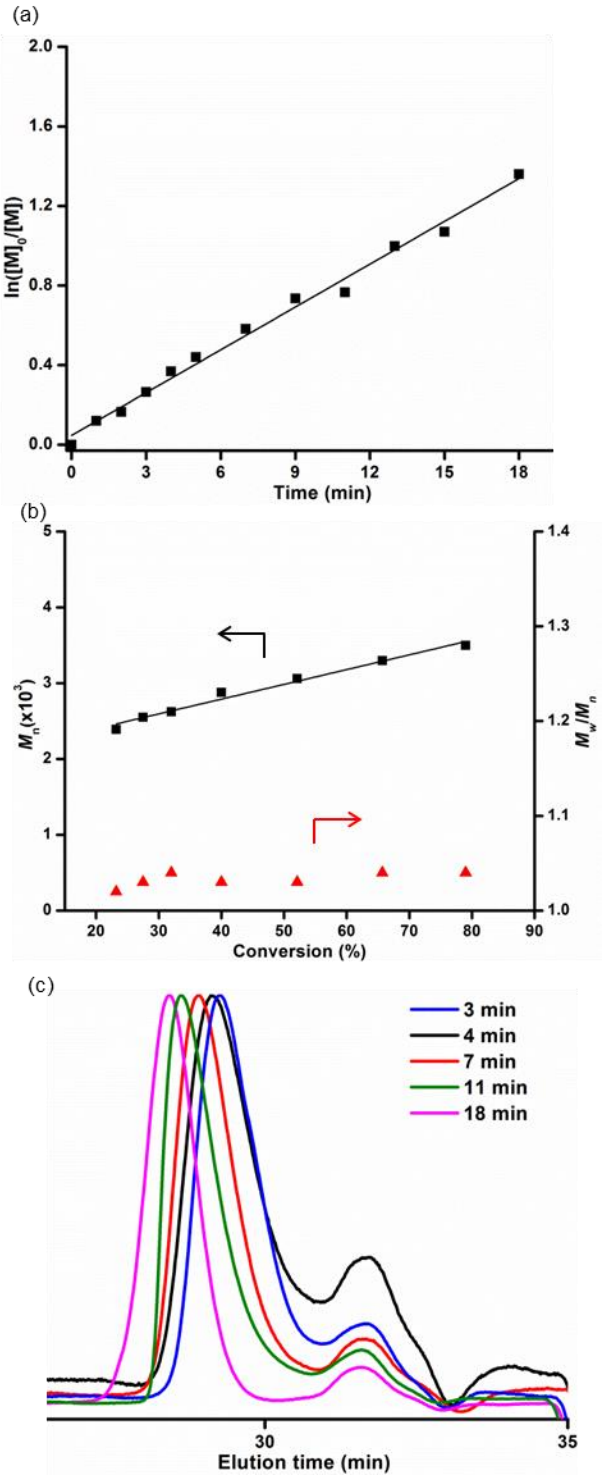


Figure S2. (a) Kinetic plots of $\ln([M]_0/[M])$ *vs.* polymerization time (min), obtained from ^{31}P NMR spectroscopy data. (b) Kinetic plots of M_n and M_w/M_n *vs.* monomer conversion in ROP of

1, obtained from GPC analysis. (c) GPC traces as a function of elution time (min), after work-up of aliquots of the polymerization mixture by quenching with a solution of excess of benzoic acid in DCM and precipitation into diethyl ether. Conditions: [EVEP] = 5.2 M in DCM, [EVEP]:[BnOH]:[DBU] = 100:1:3.

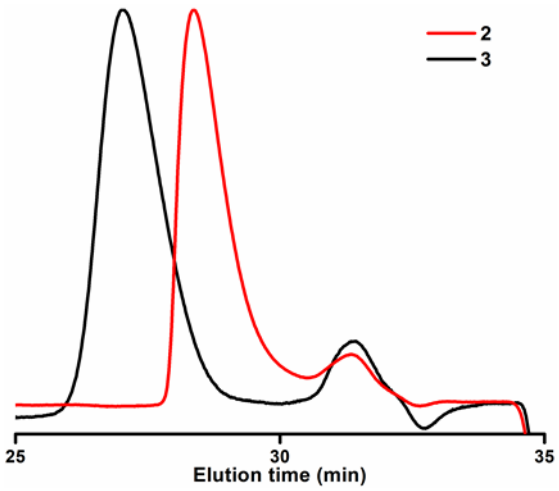


Figure S3. GPC traces of **2** and **3**, before and after thiol-ene "click" reaction, respectively, as a function of elution time (min).

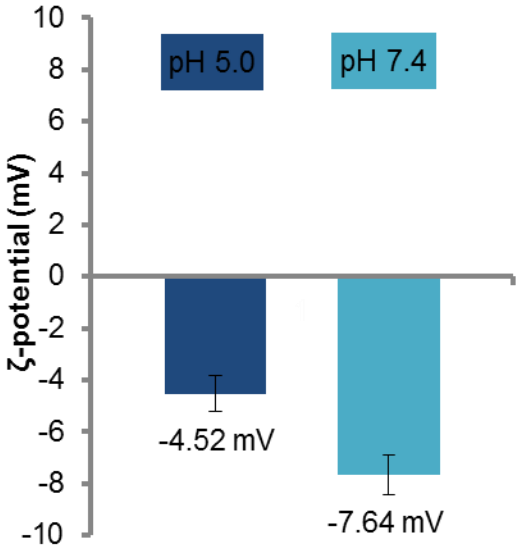


Figure S4. ζ-Potential values of **7** in acetate buffer solutions at pH 5.0 and in MOPS buffer solutions at pH 7.4. The average values and their standard deviations, from three measurements, are shown.

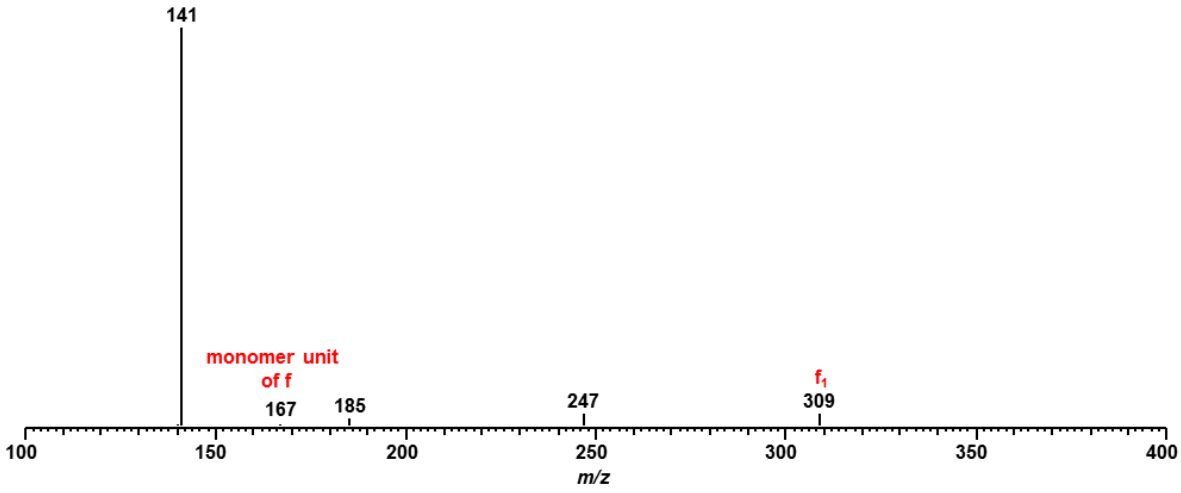


Figure S5. MS/MS spectrum of *m/z* 309.

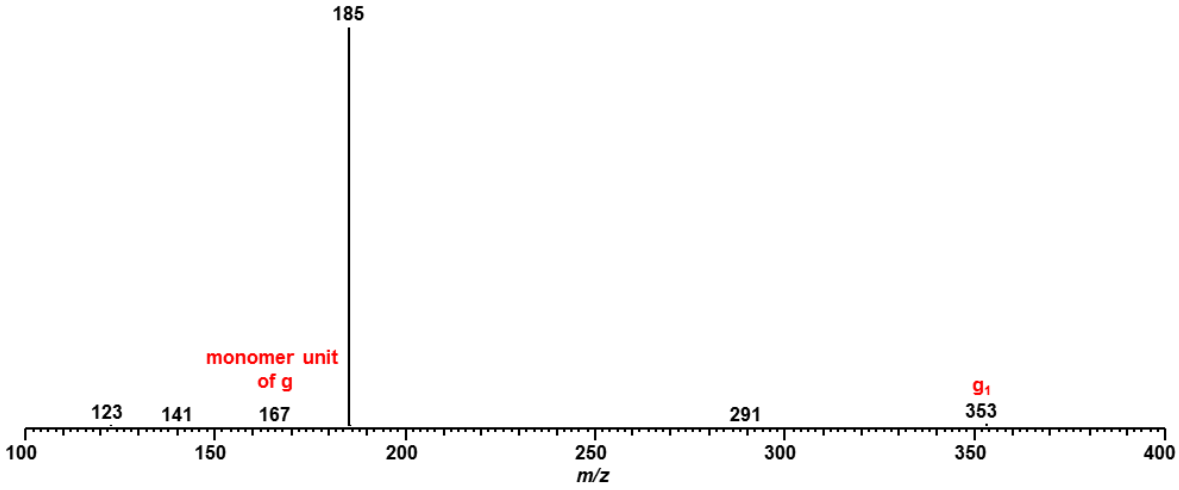
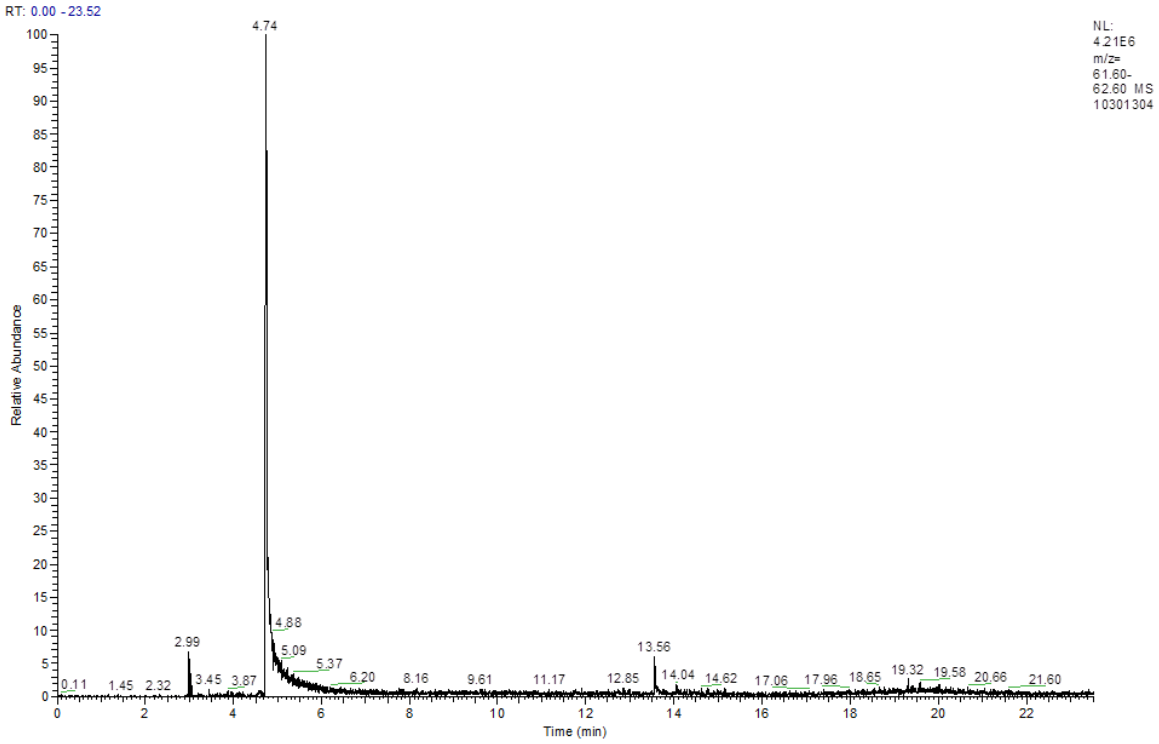


Figure S6. MS/MS spectrum of *m/z* 353.

(a)


RT: 0.00 - 23.52
NL:
4.21E6
m/z=
61.60-
62.60 MS
10301304
Relative Abundance
Time (min)
4.74
2.99
4.88
5.09
5.37
13.56
0.11
1.45
2.32
3.45
3.87
6.20
8.16
9.61
11.17
12.85
14.04
14.62
17.06
17.96
18.65
19.32
19.58
20.66
21.60

(b)

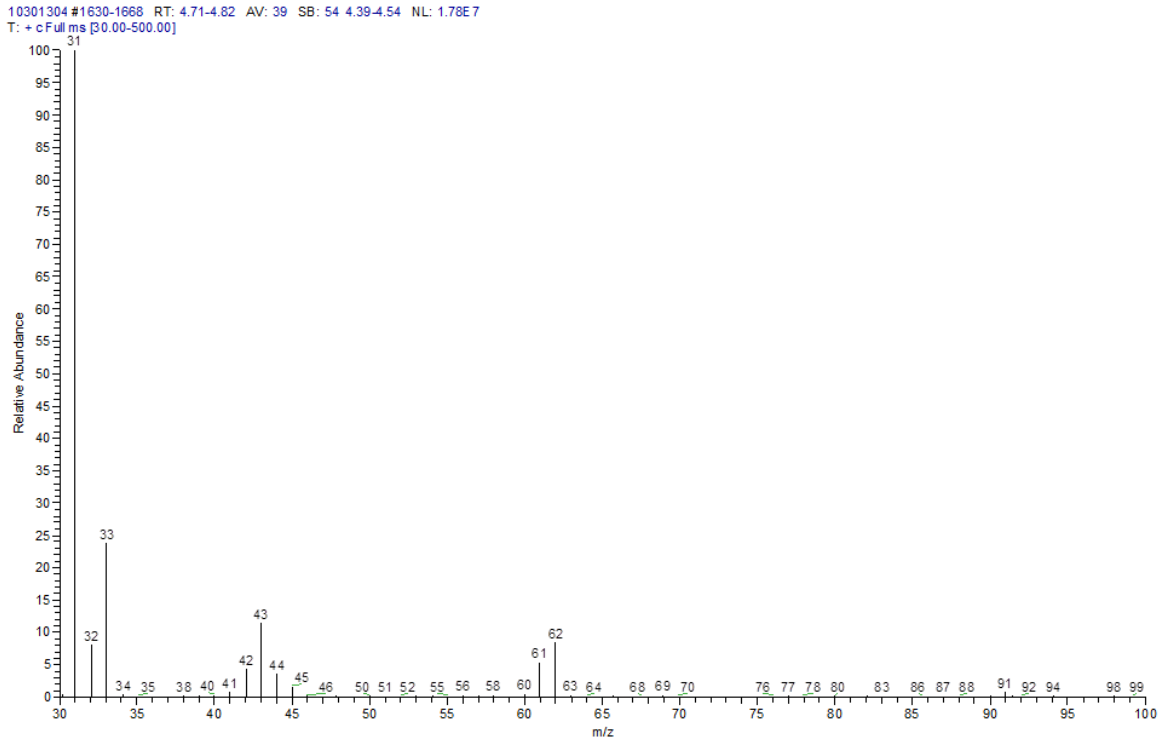

10301304 #1630-1668 RT: 4.71-4.82 AV: 39 SB: 54 4.39-4.54 NL: 1.78E7
T: + c Full ms [30.00-500.00]
Relative Abundance
m/z
31
33
32
43
42
44
45
62
61

Figure S7. GC MS analysis of the degradation products of **8**. (a) Extracted ion chromatogram at *m/z* 62. (b) EI MS at retention time 4.74 min.

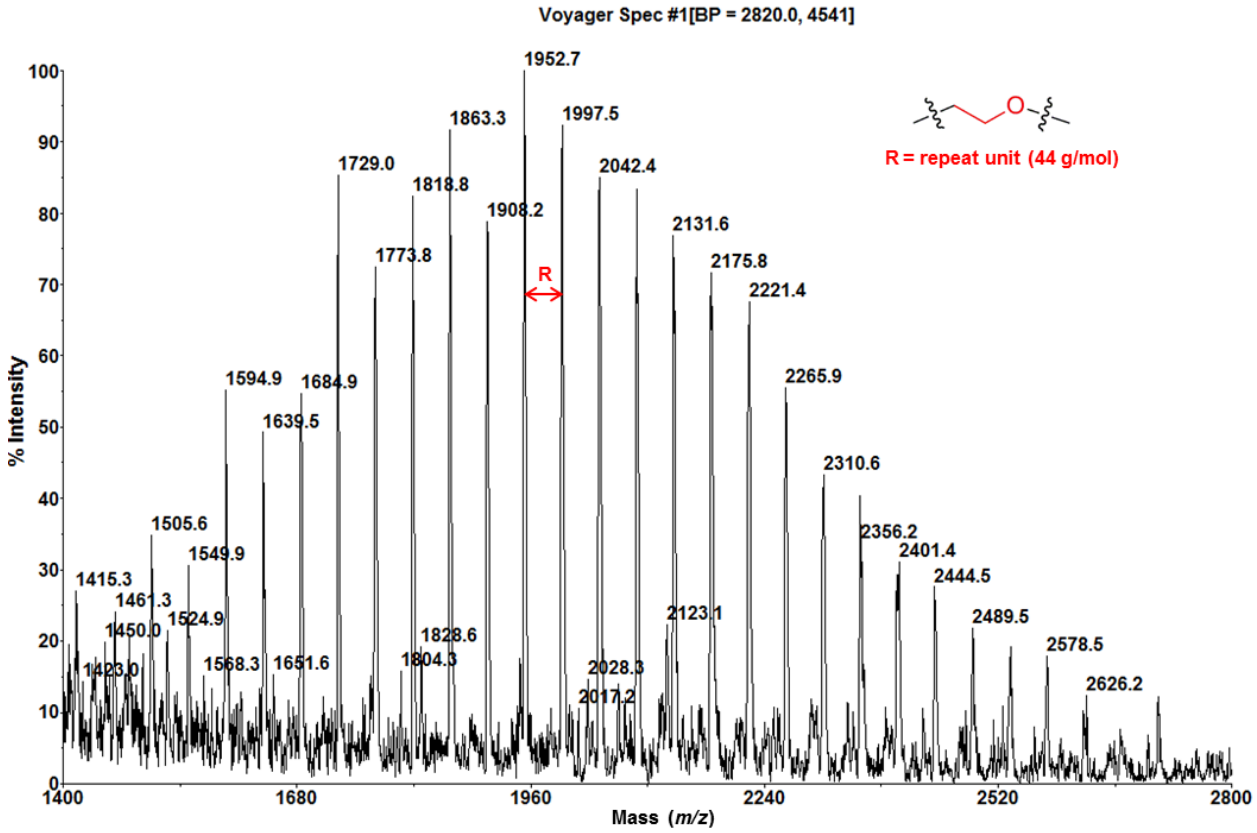


Figure S8. MALDI-TOF MS spectrum of the degradation products of **8**.

Additional Thermal Studies Regarding the Suppression of the Crystallinity of PEG in $mPEG_{44}$-*b*-$PEVEP_{33}$. Since the ability of the PEVEP block segment to suppress the crystallization of PEG was unexpected, additional experiments were conducted to confirm the finding by contrasting the $mPEG_{44}$-*b*-$PEVEP_{33}$ diblock behavior to two analogous homopolymers, $mPEG_{44}$-OH and $PEVEP_{50}$, and an equimolar physical blend of $mPEG_{44}$-OH and $PEVEP_{50}$. Furthermore, we present these studies in contrast to liquid nitrogen crash-cooled (CC) samples of $mPEG_{44}$-OH, $mPEG_{44}$-*b*-$PEVEP_{33}$ and the $mPEG_{44}$-OH/$PEVEP_{50}$ blend (Figure S9), in order to kinetically capture PEG domains in an amorphous state and enhance the ability to observe PEG T_g values. Looking at the T_m region for PEG in the $mPEG_{44}$-*b*-$PEVEP_{33}$ and $mPEG_{44}$-OH/$PEVEP_{50}$ blend samples, for both crash-cooled and ambient-cooled samples, it was clear that (regardless of the cooling rate) the PEVEP block completely suppresses the crystallization of PEG for the diblock copolymer system and that the blend still expresses a high degree of PEG crystallinity. This result made a strong case for the diblock structure being critical to the observed suppression of PEG crystallization, not merely an effect of the presence of PEVEP. When crash-cooled, the $mPEG_{44}$-OH sample presented T_g at *ca.* -17 °C, the crash-cooled $mPEG_{44}$-OH/$PEVEP_{50}$ blend displayed a minor transition in the same range, however, the $mPEG_{44}$-*b*-$PEVEP_{33}$ diblock showed an uncertain fluctuation of the baseline over the broad range from -20 to +30 °C, which complicated the analysis and prevented determination of whether there may be amorphous PEG-rich domains in the diblock sample that were capable of exhibiting a glass transition. Comparison of the $PEVEP_{50}$, $mPEG_{44}$-*b*-$PEVEP_{33}$ and blend samples over the -40 to -25 °C range indicated a virtually identical heat curve for T_g of PEVEP at *ca.* -35 °C.

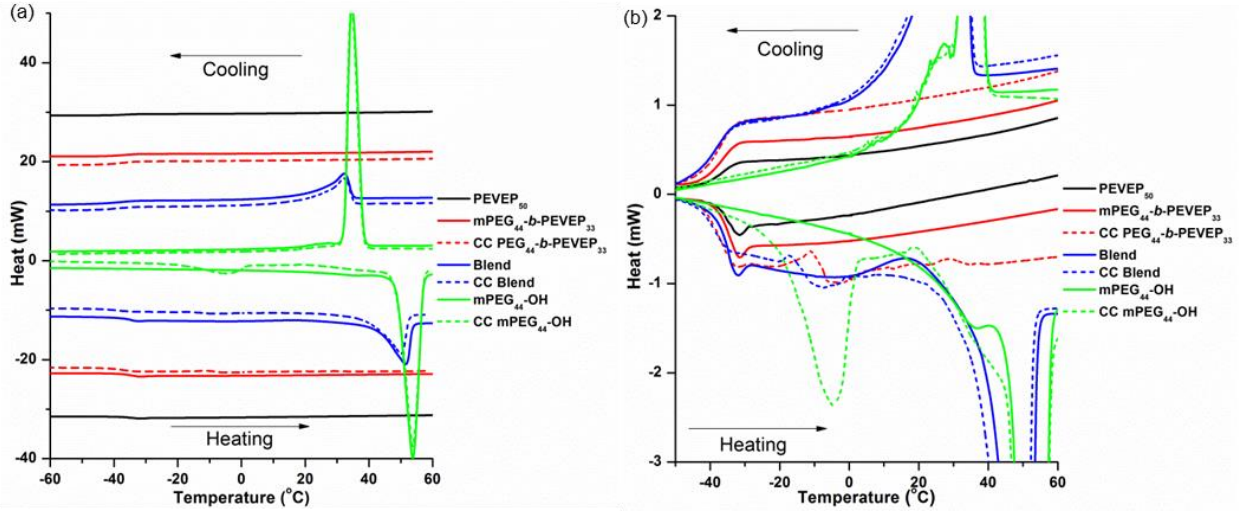


Figure S9. DSC traces for $mPEG_{44}$-OH, $PEVEP_{50}$, $mPEG_{44}$-*b*-$PEVEP_{33}$ and a physical mixture of $mPEG_{44}$-OH and $PEVEP_{50}$. The crash-cooling (CC) samples are those which were crash-cooled from 100 °C into liquid nitrogen before heating. (a) Trace over the region of interest (-60–60 °C) for the system, offset to increase clarity. (b) Rescaled trace (-50–60 °C) to contrast the glass transition regions for all samples.