Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information

ROS-triggered degradation of selenide-containing polymers based on selenoxide elimination

Lu Wang,[a] Kuixin Zhu,[a] Wei Cao,*[b] Chenxing Sun,[a] Chenjie Lu[a] and Huaping Xu*[a]

[a] Key Lab of Organic Optoelectronics and Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing 100084, China.
Email address: xuhuaping@mail.tsinghua.edu.cn

[b] Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, IL, 60208, USA.
Email address: wei.cao@northwestern.edu

1. *Experimental Procedures*

1.1 Materials

1,6-Hexanediol, 3-bromoproplonyl chloride, sodium borohydride, selenium powder, tellurium powder, hydrogen peroxide solution (30%) were products of Aladdin Chemical Company. 6-Brommohexanoyl chloride, 2, 4-toluenediisocyanate (TDI), diphenyl diselenide, poly(ethylene glycol) (PEG) monomethylether (M_w = 2000) were purchased from TCI. Dibutyltin dilaurate was obtained from J&K Scientific Ltd. Other organic chemicals and solvents employed in this work were analytical grade products purchased from Beijing Chemical Reagent Company. PEG was dehydrated in vacuum oven of 90 °C for 6 hours before use.

1.2 Instruments and Methods

The ^{1}H NMR and ^{13}C NMR spectra were measured on a BRUKER AVANCE III HD 400 (400 MHz) spectrometer at 25 °C. ^{77}Se NMR and ^{125}Te NMR spectra were recorded on a JEOL JNM-ECA 600 (600 MHz) spectrometer.

ESI-mass was carried out on a LTQ LC/MS apparatus.

Gel permeation chromatography (GPC) was performed using a breeze system from Waters Corporation (Styragel® Columns), which employed polystyrene as a standard and dimethyl formamide (DMF) as an eluent. Polystyrene standards were utilized for the GPC calibration.

The dynamic light scattering (DLS) measurement was analyzed at 25 °C on a Malvern 3000HS Zetasizer employing a monochromatic coherent He–Ne laser (633 nm) as the light source and the scattered light was detected at an angle of 90°.

Transmission electron microscopy (TEM) images were obtained using a JEM-2010 microscope with an accelerating voltage of 80 kV. All TEM samples were prepared by dropping a diluted solution of polymer micelles on a carbon-coated copper grid followed by staining using 1.5% uranyl acetate after complete drying.

1.3 Synthetic procedures of C6-C3Br and C6-C6Br

6-Hydroxyhexyl 3-bromopropanoate (C6-C3Br) or 6-hydroxyhexyl 6-bromohexanoate (C6-C6Br) was prepared under the reaction of 1,6-hexanediol and 3-bromoproplonyl chloride. A solution of 1,6-hexanediol (2.364 g, 20 mmol) in dry tetrahydrofuran (THF) was stirring at 0 °C in ice-water bath. A solution of 3-bromoproplonyl chloride (3.428 g, 20 mmol) or 6-brommohexanoyl chloride (4.270 g, 20 mmol) was then added in dropwise. The solution was stirred at room temperature for 7 h. The product was purified by a column chromatography method, using petroleum ether (PE)/ethyl acetate (EA) = 3:1 to 2:1 as eluate. 2.071 g of colorless liquid C6-C3Br was obtained with a yield of 41%. 2.3955 g of colorless liquid C6-C6Br was obtained with a yield of 40%.

C6-C3Br: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.12 (2H, t, $BrCH_2CH_2COO\mathit{CH_2}$), 3.63 (2H, t, $HO\mathit{CH_2}$), 3.57 (2H, t, $Br\mathit{CH_2}$), 2.90 (2H, t, $BrCH_2\mathit{CH_2}$), 1.74 (1H, s, $\mathit{H}OCH_2$), 1.65 (2H, m, $BrCH_2CH_2COOCH_2\mathit{CH_2}$), 1.57 (2H, m, $HOCH_2\mathit{CH_2}$), 1.39 (4H, m, $BrCH_2CH_2COOCH_2CH_2(\mathit{CH_2})_3CH_2OH$). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 170.72, 65.11, 62.88, 37.91, 32.67, 28.65, 26.09, 25.79, 25.47. ESI-Mass: m/z (M^+), calcd. 252.04; Found: 253.04 [$M+H^+$].

C6-C6Br: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.06 (2H, t, $BrCH_2(CH_2)_3CH_2COO\mathit{CH_2}$), 3.63 (2H, t, $HO\mathit{CH_2}$), 3.39 (2H, t, $Br\mathit{CH_2}$), 2.30 (2H, t, $BrCH_2(CH_2)_3\mathit{CH_2}COOCH_2$), 1.86 (2H, m, $BrCH_2(CH_2)_3CH_2COOCH_2\mathit{CH_2}$), 1.65 (1H, s, $\mathit{H}OCH_2$), 1.70-1.30 (12H, m, $BrCH_2(\mathit{CH_2})_3CH_2COO$ $CH_2CH_2(\mathit{CH_2})_3CH_2OH$). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm):173.68, 64.47, 62.89, 34.21, 33.58, 32.71, 32.51, 28.74, 27.77, 25.86, 25.51, 24.23. ESI-Mass: m/z (M^+), calcd. 294.08; Found: 295.09 [$M+H^+$].

1.4 Synthetic procedures of C6-C3Se and C6-C6Se

Bis(6-hydroxyhexyl) 3,3'-selenodipropanoate (C6-C3Se) or bis(6-hydroxyhexyl) 6,6'-selenodihexanoate) (C6-C6Se) was acquired through the reaction of disodium selenide and C6-C3Br or C6-C6Br. Disodium selenide was synthesized under reaction between Se powder (0.3158 g, 4 mmol) and excess amount of sodium borohydride (0.3632 g, 9.6 mmol) at 50 °C in water under a N_2 atmosphere. C6-C3Se (or C6-C6Se) was acquired through the reaction of disodium selenide and C6-C3Br (2.025 g, 8 mmol) or C6-C6Br (2.362 g, 8 mmol) in tetrahydrofuran (THF) for 12 h under a N_2 atmosphere. The product was purified by a column chromatography method, using PE/EA = 1:1 to 1:2 as eluate. 0.9310 g of yellow liquid C6-C3Se was obtained with a yield of 55%. 1.057 g of white solid C6-C6Se was obtained with a yield of 52%.

C6-C3Se: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.10 (4H, t, $\mathrm{SeCH_2CH_2COO}\mathit{CH_2}$), 3.64 (4H, t, $\mathrm{HO}\mathit{CH_2}$), 2.80 (4H, t, $\mathrm{SeCH_2}\mathit{CH_2}$), 2.72 (4H, t, $\mathrm{Se}\mathit{CH_2}$), 1.65 (4H, m, $\mathrm{SeCH_2CH_2COOCH_2}\mathit{CH_2}$), 1.59 (2H, s, $\mathit{H}\mathrm{OCH_2}$), 1.57 (4H, m, $\mathrm{HOCH_2}\mathit{CH_2}$), 1.39 (8H, m, $\mathrm{SeCH_2CH_2COOCH_2CH_2(}\mathit{CH_2}\mathrm{)_2CH_2OH}$). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 172.35, 64.82, 62.72, 35.82, 32.61, 28.63, 25.79, 25.44, 18.04. ^{77}Se NMR 600 MHz, $CDCl_3$, 298K) δ (ppm): 197.20 (1Se, s, *Se*). ESI-Mass: m/z (M^+), calcd. 426.15; Found: 449.14 [$M+Na^+$].

C6-C6Se: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.05 (4H, t, $\mathrm{SeCH_2(CH_2)_3CH_2COO}\mathit{CH_2}$), 3.62 (4H, t, $\mathrm{HO}\mathit{CH_2}$), 2.53 (4H, t, $\mathrm{Se}\mathit{CH_2}$), 2.29 (4H, t, $\mathrm{SeCH_2(CH_2)_3}\mathit{CH_2}\mathrm{COOCH_2}$), 1.70-1.30 (28H, m, $\mathrm{SeCH_2}\mathit{(CH_2)_3}\mathrm{CH_2COO}$ $\mathrm{CH_2CH_2(}\mathit{CH_2}\mathrm{)_3CH_2OH}$). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 173.86, 64.41, 62.88, 34.33, 32.72, 30.39, 29.54, 28.75, 25.87, 25.51, 24.63, 23.81. ^{77}Se NMR 600 MHz, $CDCl_3$, 298K) δ (ppm): 155.79 (1Se, s, *Se*). ESI-Mass: m/z (M^+), calcd. 510.25; Found: 533.24 [$M+Na^+$].

1.5 Synthetic procedures of C6-C3Se-Ph

6-Hydroxyhexyl 3-(phenylselanyl)propanoate (C6-C3Se-Ph) was prepared under the reaction of C6-C3Br, sodium borohydride and diphenyl diselenide. A solution of diphenyl diselenide in THF was stirred at room temperature. Then a sodium borohydride aqueous solution was added dropwise in it. Then after the orange solution change to colorless, a solution of C6-C3Br in THF was injected into the flask. The solution was stirred for 12 h at 50 °C under a N_2 atmosphere. The product was purified by a column chromatography method, using PE/EA = 2:1 as eluate. Colorless liquid was finally obtained with a yield of ~70%.

C6-C3Se-Ph: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 7.48, 7.27 (5H, m, *Ph*Se), 4.00 (2H, t, $\mathrm{PhSeCH_2CH_2COO}\mathit{CH_2}$), 3.45 (2H, t, $\mathrm{HO}\mathit{CH_2}$), 3.08 (2H, t, $\mathrm{PhSeCH_2}\mathit{CH_2}$), 2.68 (2H, t, $\mathrm{PhSe}\mathit{CH_2}$), 1.56 (2H, s, $\mathrm{PhSeCH_2CH_2COOCH_2}\mathit{CH_2}$), 1.45 (2H, m, $\mathrm{PhSeCH_2CH_2COOCH_2\ CH_2\ (CH_2)_2}\ \mathit{CH_2}\mathrm{CH_2}$), 1.30 (4H, m,

$PhSeCH_2CH_2COOCH_2$ CH_2 (CH_2)$_2$ CH_2CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 172.88, 133.47, 130.21, 128.04, 65.35, 62.49, 35.95, 33.44, 29.36, 26.46, 26.24, 22.46. ^{77}Se NMR 600 MHz, $CDCl_3$, 298K) δ (ppm): 300.08(1Se, s, *Se*). ESI-Mass: m/z (M^+), calcd. 330.07; Found: 353.06 [$M+Na^+$].

1.6 Synthetic procedures of C6-C3Te

Bis(6-hydroxyhexyl) 3,3'-tellurodipropanoate (C6-C3Te) was acquired through the reaction of disodium telluride and C6-C3Br. Disodium telluride was synthesized under reaction between Te powder (0.5104 g, 4 mmol) and excess amount of sodium borohydride (0.3632 g, 9.6 mmol) at 41 °C in water under a N_2 atmosphere. C6-C3Te was acquired through the reaction of disodium telluride and C6-C3Br (2.025 g, 8 mmol) in tetrahydrofuran (THF) for 12 h under a N_2 atmosphere. The product was purified by a column chromatography method, using PE/EA = 1:1 to 1:2 as eluate. 0.7242 g of rufous liquid C6-C3Se was obtained with a yield of 38%.

C6-C3Te: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.00 (4H, t, $TeCH_2CH_2COOCH_2$), 3.65 (4H, t, $HOCH_2$), 2.90 (4H, t, $TeCH_2$), 2.82 (4H, t, $TeCH_2CH_2$), 1.65 (4H, m, $SeCH_2CH_2COOCH_2CH_2$), 1.58 (2H, s, $HOCH_2$), 1.58 (4H, m, $HOCH_2CH_2$), 1.39 (8H, m, $TeCH_2CH_2COOCH_2CH_2(CH_2)_2CH_2OH$). ^{13}C NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 173.24, 64.81, 62.67, 37.36, 32.59, 28.62, 25.78, 25.43, -4.50. ^{125}Te NMR 600 MHz, d6-DMSO, 298K) δ (ppm): 327.10 (1Te, s, *Te*). ESI-Mass: m/z (M^+), calcd. 476.14; Found: 499.13 [$M+Na^+$].

1.7 Synthetic procedures of C6-C3Se-PEG2000 and C6-C6Se-PEG2000 block copolymer

The selenium-containing block copolymer C6-C3Se-PEG2000 and C6-C6Se-PEG2000 were synthesized as follows. Initially, C6-C3Se (2.0 mmol, 0.8508 g) or C6-C6Se (2.0 mmol, 1.019 g) was polymerized with an excess of TDI (2.2 mmol, 314.0 μL) using dibutyltin dilaurate (50.0 μL) as a catalyst at 50 °C in 20.0 mL of anhydrous THF with stirring for 5 h under a N_2 atmosphere. Then, PEG monomethyl ether (800.0 mg, M_w = 2000) was dissolved in 10 mL of anhydrous THF and injected into the flask. After reacting for another 24 h, the solvent was removed and the residue was washed with deionized water and ethanol three times, respectively. Both C6-C3Se-PEG2000 and C6-C6Se-PEG2000 were yellow, sticky solid.

C6-C3Se-PEG2000: ^{1}H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.20-4.05 (m,

$SeCH_2CH_2COOCH_2(CH_2)_4CH_2OCONH$), 3.64 (t, OCH_2CH_2 of PEG), 2.80 (t, $SeCH_2CH_2$), 2.71 (t, $SeCH_2$), 1.75-1.30 (m, $SeCH_2CH_2COOCH_2(CH_2)_4CH_2OCONH$). The molecular weight of the C6-C3Se-PEG2000 block copolymer was calculated according to the integral ratio of the peaks at 2.71 ppm, and 3.64 ppm and the known molecular weight of PEG. The selenium-containing blocks contained 47 repeating units. The M_n was calculated as approximately 3.22×10^4 Da. The M_n of C6-C3Se-PEG2000 determined by GPC in a DMF solution was 1.63×10^4 Da. The PDI was $M_w/M_n = 1.32$.

C6-C6Se-PEG2000: ^1H NMR (400 MHz, $CDCl_3$, 298K) δ (ppm): 4.21-4.01 (m, $SeCH_2(CH_2)_3CH_2COOCH_2(CH_2)_4CH_2OCONH$), 3.64 (t, OCH_2CH_2 of PEG), 2.53 (t, $SeCH_2$), 2.30 (t, $SeCH_2(CH_2)_3CH_2COOCH_2$), 1.80-1.31 (m, $SeCH_2CH_2COOCH_2(CH_2)_4CH_2OCONH$). Similar with C6-C3Se-PEG2000, the molecular weight of the C6-C6Se-PEG2000 block copolymer was calculated according to the integral ratio of the peaks at 2.53 ppm, and 3.64 ppm and the known molecular weight of PEG. The selenium-containing blocks contained 187 repeating units. The M_n was calculated as approximately 1.32×10^5 Da. The M_n of C6-C6Se-PEG2000 determined by GPC in a DMF solution was 3.27×10^4 Da. The PDI was $M_w/M_n = 1.46$.

1.8 Oxidation of C6-C3Se

6.38 mg C6-C3Se was dissolved in 0.6 mL $CDCN_3$ with a concentration of 25 mM. Different concentrations of H_2O_2 was also dissolved in the same solution with aconcentrations of 25 mM (1.0 eq.), 37.5 mM (1.5 eq.), 50 mM (2.0 eq.) and 62.5 mM (2.5 eq.), respectively. The whole oxidation process was analyzed by ^1H NMR spectra.

1.9 Fabrication and characterization of C6-C3Se-PEG2000 and C6-C6Se-PEG2000 micelles

C6-C3Se-PEG2000 or C6-C6Se-PEG2000 (15.0 mg) was dissolved in DMF (2.0 mL) and then added to deionized water (10.0 mL) under sonication dropwise. After that, the suspension was dialyzed in a dialysis bag (MWCO 3500) against deionized water for more than 24 h to remove DMF. After the dialysis process, the suspension was added to deionized water until the volume increased to 15 mL and finally obtained a micelle suspension of 1.0 mg mL^{-1} for further tests.

1.10 Measurement of the CAC

The C6-C3Se-PEG2000 or C6-C6Se-PEG2000 micelle solution (1.0 mg mL^{-1}) was diluted to different concentration, from 0.5 mg mL^{-1}, 0.2 mg mL^{-1}, to 2.0×10^{-5}, 1.0×10^{-5} and so on. Then we utilized DLS to measure the count rate of the solutions. The inflection point in the plot between count rate and concentration indicated the CAC of the polymer.

1.11 Oxidation of C6-C3Se-PEG2000 and C6-C6Se-PEG2000 micelles

H_2O_2 solution (1 mM) was prepared before oxidation. The C6-C3Se-PEG2000 or C6-C6Se-PEG2000 micelles suspension was sealed in a dialysis bag (MWCO 7000) and following by immersing in H_2O_2 solution. The vial was then moved to a shaking table at 37 °C. After oxidation, the micelle suspensions were measured by DLS and were dissolved in $CDCl_3$ for ^1H NMR analysis or DMF for GPC tests after freeze dried.

1.12 Oxidation of C6-C3Te

Firstly, 4.74 mg C6-C3Te was dissolved in 10 mL THF. Then the solution was added dropwise in 400 mL H_2O_2 aqueous solutions (50 μM or 100 μM). Then the reaction was stirred under room temperature overnight for around 12 h. Finally the oxidation product was purified and monitored by ^1H NMR analysis.

2. Results and Discussion

2.1 NMR spectra of C6-C3Br

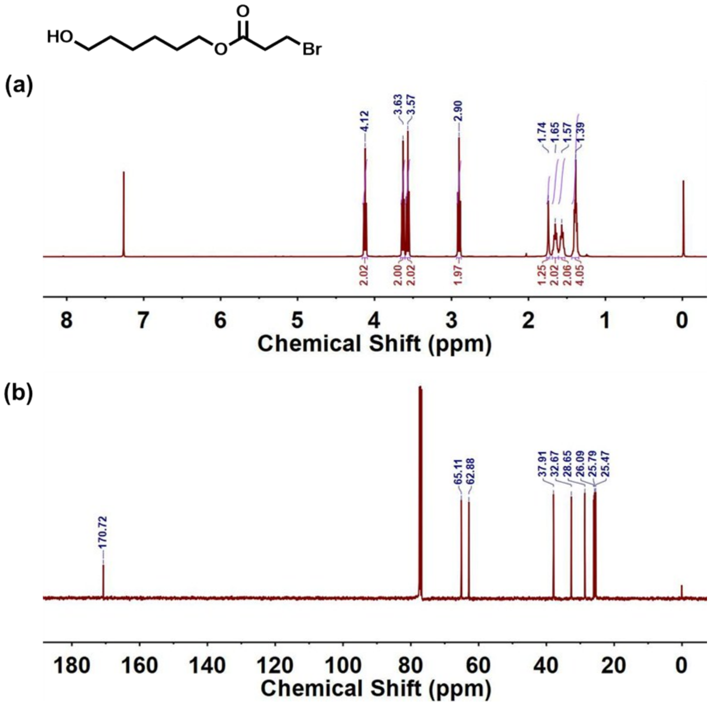


Figure S1. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of C6-C3Br (400 MHz, $CDCl_3$, 25 °C).

2.2 NMR spectra of C6-C6Br

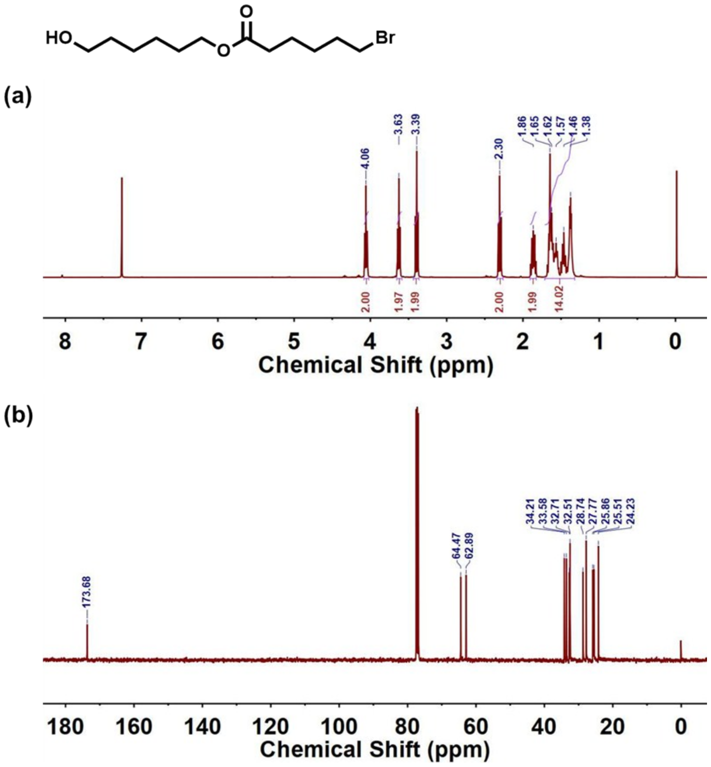


Figure S2. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of C6-C6Br (400 MHz, $CDCl_3$, 25 °C).

2.3 NMR spectra of C6-C3Se

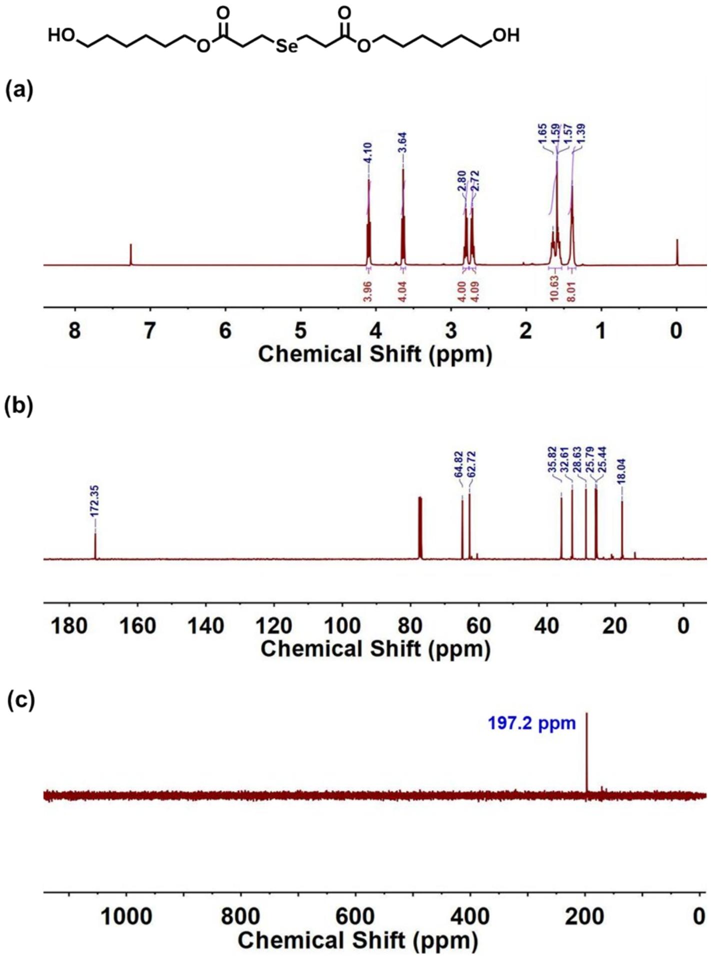


Figure S3. (a) ^{1}H NMR, (b) ^{13}C NMR and (c) ^{77}Se NMR spectra of C6-C3Se (400 MHz, CDCl$_3$, 25 °C).

2.4 NMR spectra of C6-C6Se

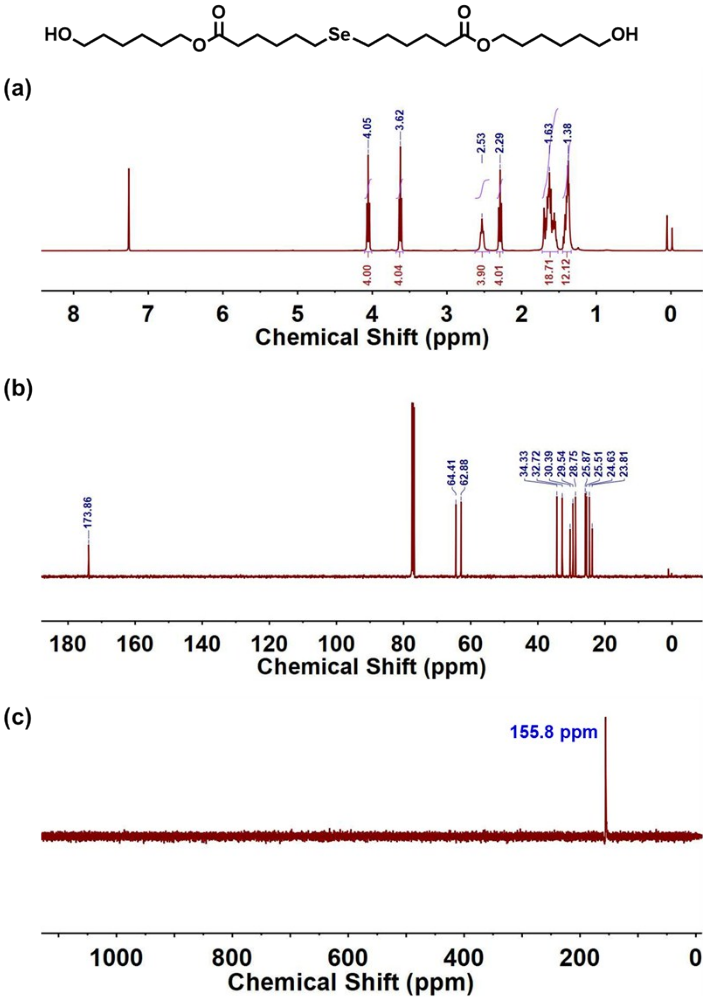


Figure S4. (a) ^{1}H NMR, (b) ^{13}C NMR and (c) ^{77}Se NMR spectra of C6-C6Se (400 MHz, $CDCl_3$, 25 °C).

2.5 NMR spectra of C6-C3Se-Ph

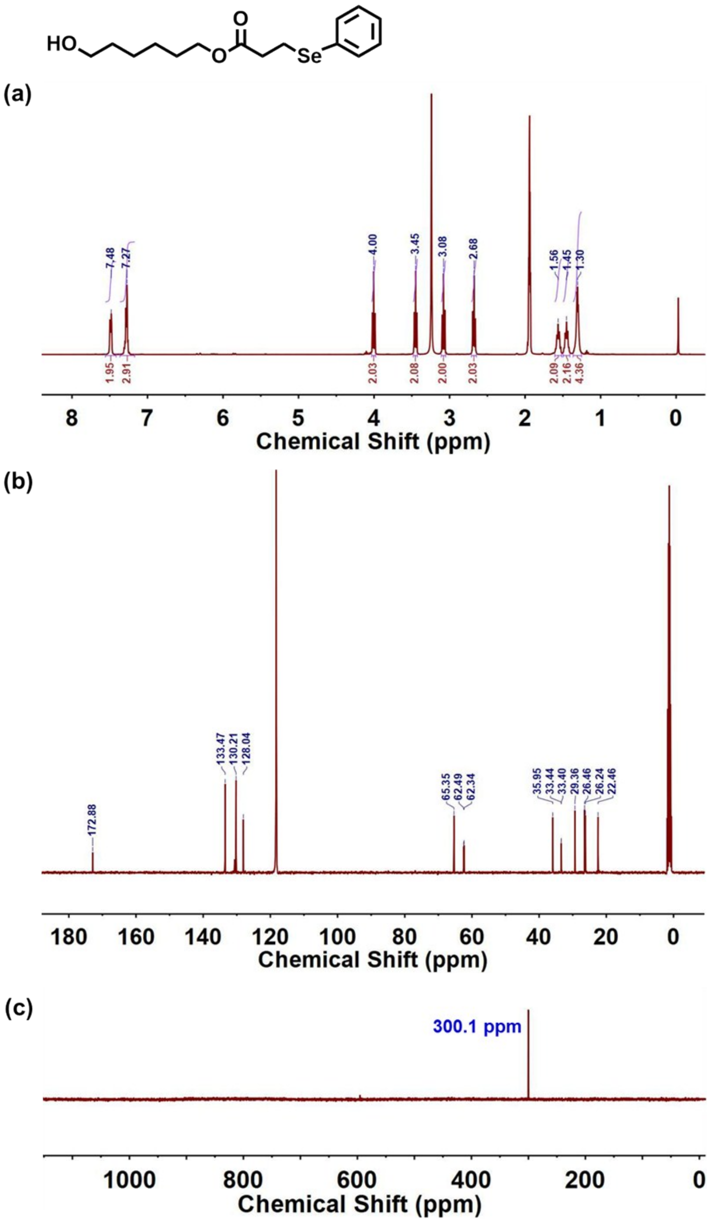


Figure S5. (a) ^{1}H NMR, (b) ^{13}C NMR and (c) ^{77}Se NMR spectra of C6-C3Se-Ph (400 MHz, $CDCl_3$, 25 °C).

2.6 NMR spectra of C6-ole

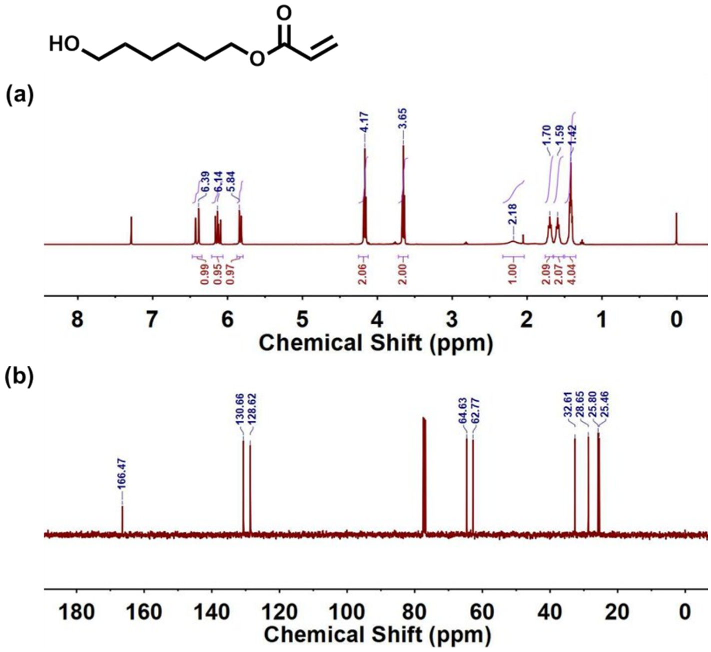
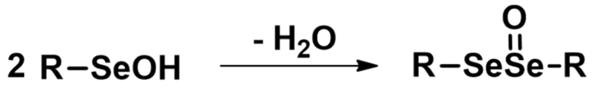


Figure S6. (a) ^{1}H NMR and (b) ^{13}C NMR spectra of C6-ole (400 MHz, $CDCl_3$, 25 °C).

2.7 Characterization of selenoseleninate as a byproduct of the oxidation-elimination reaction



$$2\ \mathrm{R{-}SeOH} \xrightarrow{-\mathrm{H_2O}} \mathrm{R{-}Se}\overset{\mathrm{O}}{\overset{\|}{\mathrm{Se}}}\mathrm{{-}R}$$

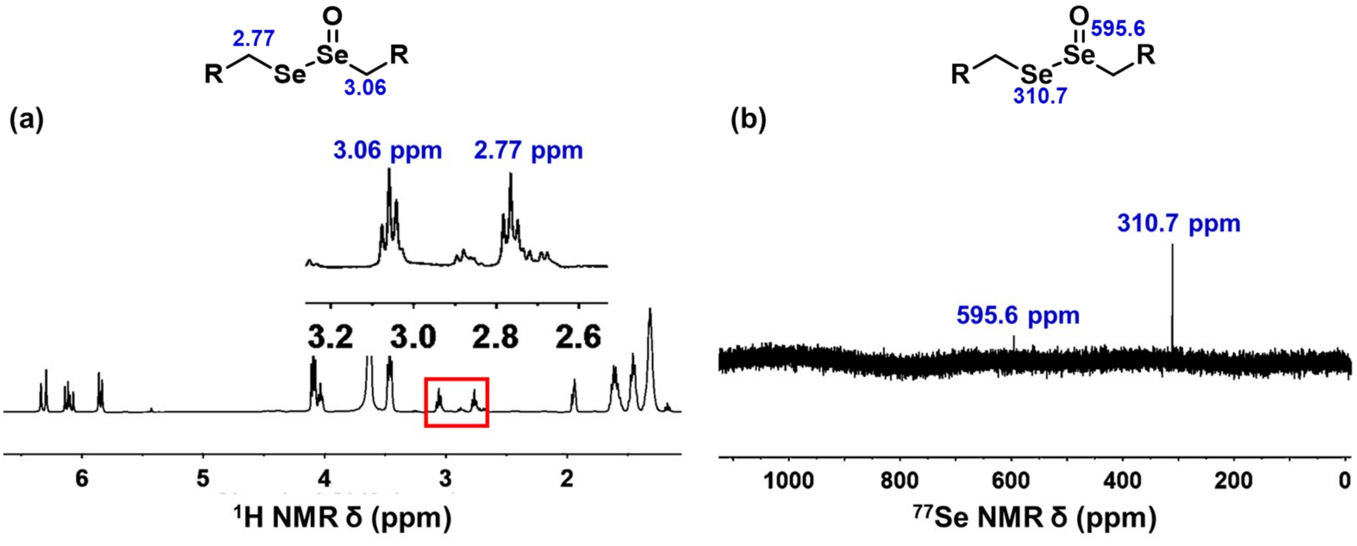


Figure S7. Characterization of the self-condensation of selenenic acid. (a) ^{1}H NMR and (b) ^{77}Se NMR spectra of concentrated C6-C3Se after oxidation by 2.0 eq H_2O_2.

2.8 ^{1}H NMR and GPC characterization of the selenium-containing polymer C6-C3Se-PEG2000

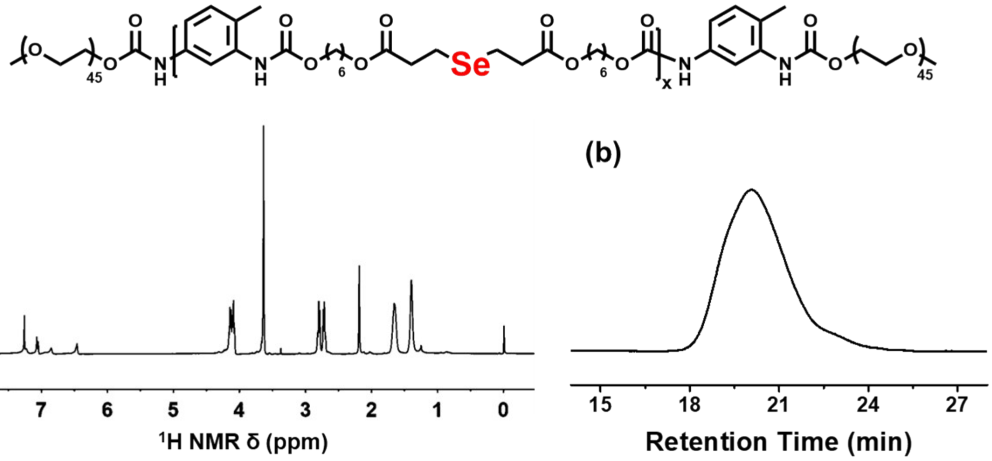


Figure S8. (a) ^{1}H NMR spectra (400 MHz, $CDCl_3$) (b) GPC plot of the selenium-containing polymer C6-C3Se-PEG2000.

C6-C3Se-PEG2000 was fabricated with a molecular weight of 3.22×10^4 Da (indicated by integrals of peaks in ^{1}H NMR spectrum). The M_n of C6-C3Se-PEG2000 determined by GPC in a DMF solution was 1.63×10^4 Da, which is smaller than the NMR calculated result. The PDI was $M_w/M_n = 1.32$.

In my view, the NMR result is more credible. The GPC determination based on calculating the relative molecular weight against the hydrodynamic volume of polystyrene standard samples. Since the conformation of this polymer is different from polystyrene, the molecular weight determined by GPC may be greatly underestimated.

2.9 DLS plots of C6-C3Se-PEG2000 after 24 h' oxidation in 1 mM H_2O_2.

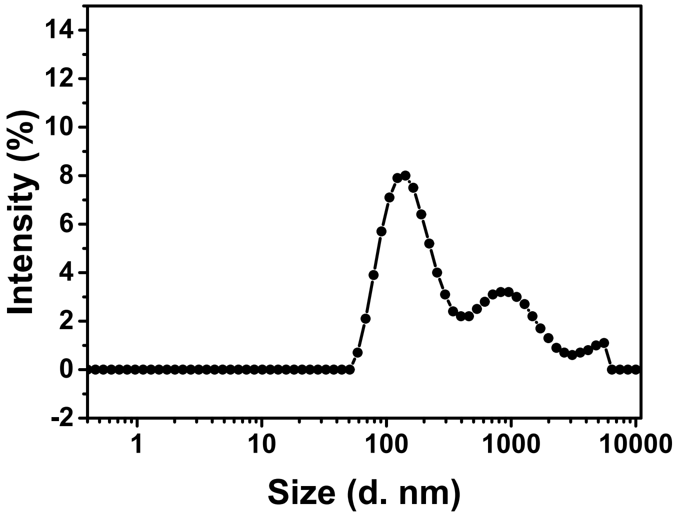


Figure S9. DLS plots of C6-C3Se-PEG2000 after 24 h' oxidation in 1 mM H_2O_2.

2.10 Oxidation of C6-C3Se-PEG2000 in the presence of H_2O_2 for different concentrations in 4 days

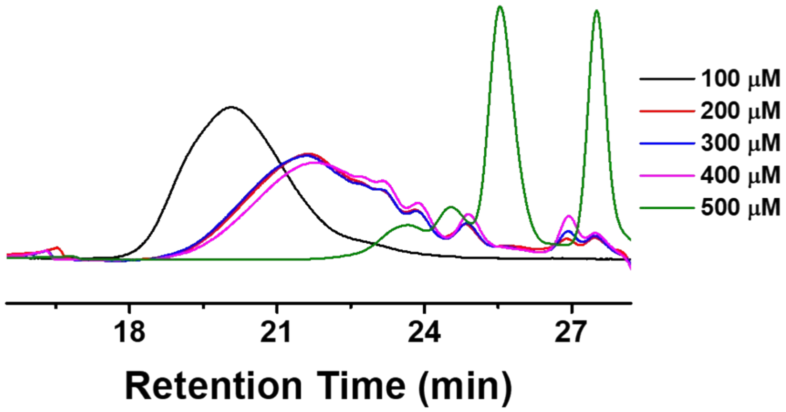


Figure S10. GPC plots for oxidation of C6-C3Se-PEG2000 in the presence of H_2O_2 for different concentrations in 4 days.

2.11 Oxidation of C6-C3Se-PEG2000 in the presence of 100 µM H_2O_2

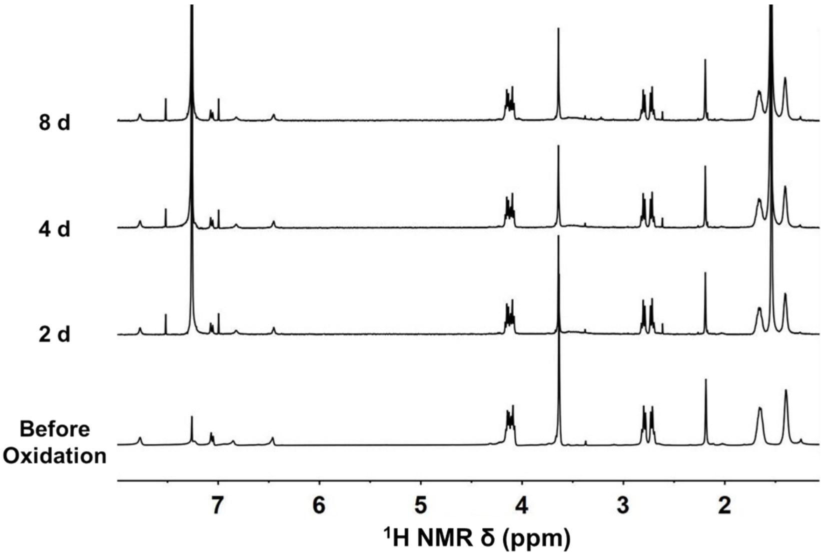


Figure S11. ^1H NMR spectra for oxidation of C6-C3Se-PEG2000 in the presence of 100 µM H_2O_2 (400 MHz, $CDCl_3$).

2.12 ^1H NMR and GPC characterization of the selenium-containing polymer C6-C6Se-PEG2000

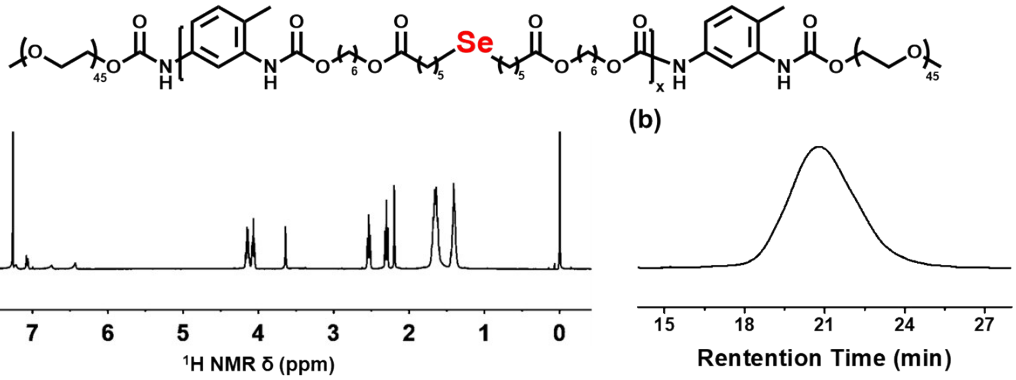


Figure S12. (a) ^{1}H NMR spectra (400 MHz, $CDCl_3$) (b) GPC plot of the selenium-containing polymer C6-C6Se-PEG2000.

The molecular weight calculated by integrals of peaks in ^{1}H NMR spectrum was approximately 1.32×10^5 Da. The M_n of C6-C3Se-PEG2000 determined by GPC in a DMF solution was 3.27×10^4 Da, which is smaller than the NMR calculated result. The PDI was $M_w/M_n = 1.46$.

2.13 TEM image of C6-C6Se-PEG2000 fully oxidized by 10 mM H_2O_2

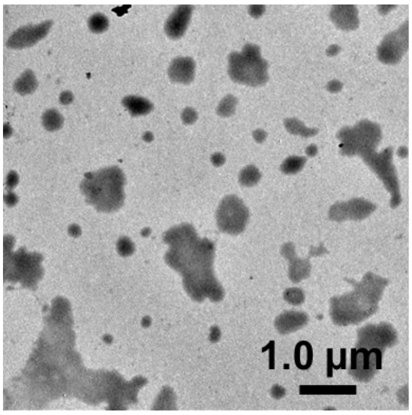


Figure S13. TEM image of C6-C6Se-PEG2000 fully oxidized by 10 mM H_2O_2.

2.14 NMR characterization of C6-C3Te

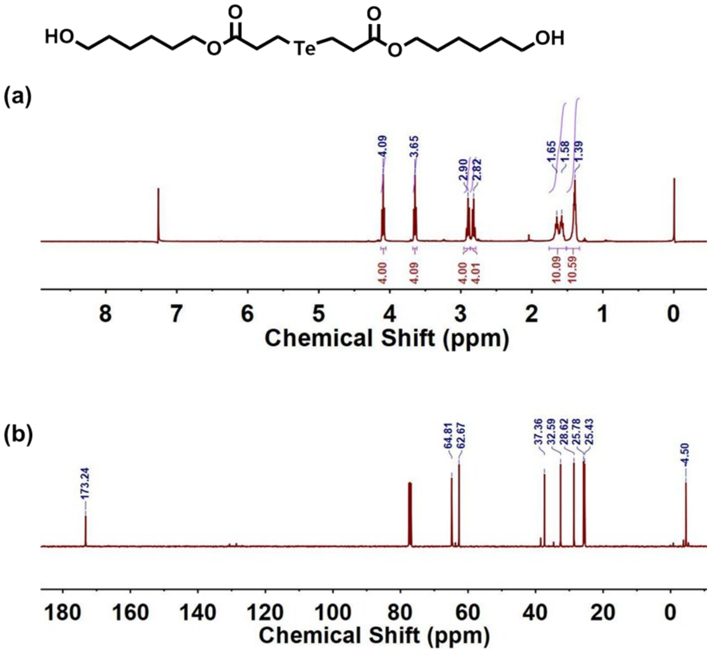


Figure S14 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of C6-C3Te (400 MHz, $CDCl_3$, 25 °C).