Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

For

Synthesis of Water Soluble and Multi-Responsive Selenopolypeptides via Ring-Opening Polymerization of *N*-Carboxyanhydrides

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Materials

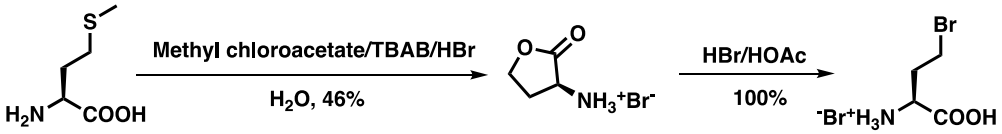
All chemicals were purchased from commercial sources and used as received unless otherwise specified. Hexamethyldisilazane (HMDS) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Superdry *N,N*-dimethylformamide (DMF) was purchased from J&K Scientific Ltd. Tetraethyleneglycol monomethyl ether and $_{L}$-methionine were purchased from D&B Ltd (Shanghai, China). Ethyl chloroacetate and tetrabutylammonium bromide (TBAB) were purchased from Sinopharm Chemical Reagent Co., Ltd. Hydrobromic acid and triethyleneglycol monomethyl ether were purchased from Aladdin. Selenium powder was purchased from Shanghai Macklin Biochemical Co. Ltd. The glutathione peroxidase assay kit was purchased from Beyotime Biotechnology. CellTiter 96 kit was purchased from Promega Inc (Beijing).

Instruments

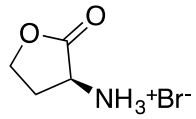
^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker ARX400 FT-NMR spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker ALPHA II FT-IR spectrometer using a KBr cell with a fixed path length of 0.2 mm. High-resolution mass spectrometry (HR-MS) analyses were performed on Q-Exactive Plus (Thermo Scientific). Selenium-containing amino acids were separated by a Cheetah Flash system (Agela technology) with a C18 column using mixed deionized water and methanol as the mobile phase. Size exclusion chromatography (SEC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 9-angle laser light scattering detector (MALLS, Wyatt Technology, Santa Barbara, CA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of MALLS was 658 nm and the temperature of both the refractive index and the MALLS detectors was 25 °C. Separations were performed using serially connected size exclusion columns (500, 10^3,10^4, and 10^5 Å Phenogel columns, 5 μm, 7.8 × 300 mm, Phenomenex, Torrance, CA) at a flow rate of 1.0 mL/min and 50 °C using DMF containing 0.1 M LiBr as the mobile phase. The *d*n/*d*c values were calculated offline by using the internal calibration system (by the ASTRA V software version 5.1.7.3 provided by Wyatt Technology). Circular dichroism (CD) spectra were recorded on Bio-Logic MOS-500 (France). Temperature-dependent CD spectra was recorded on Jasco J-810 spectropolarimeter. Temperature-dependent transmittance of the polypeptide solution was recorded on a Shimazu UV3600Plus with cell positioner CPS-100.

Experimental Procedures

Scheme S1 Synthesis of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide

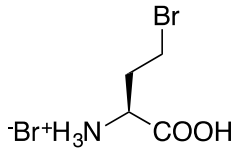


Synthesis of (*S*)-2-oxotetrahydrofuran-3-aminium bromide



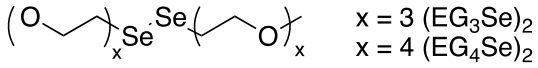
L-Methionine (30.0 g, 201 mmol), methyl chloroacetate (23.0 mL, 261 mmol), and tetrabutyl ammonium bromide (TBAB, 3.2 g, 10 mmol) were mixed in deionized water (150 mL) in a 500 mL single-neck flask. The mixture was heated at 90 °C in an oil bath and stirred for 9 h. After cooling to room temperature, the reaction was added HBr solution (40%, 41.0 mL) and further stirred overnight. The mixture was washed with dichloromethane (DCM, 200mL) twice and evaporated under vacuum to remove water. Crude product was precipitated out after cooling at 4 °C for 2 h. Pure product was obtained as a white crystal after washing with ethanol and drying in vacuum overnight (23.2 g, yield: 48%). HR-ESI-MS $[M + H]^+$ Calcd. for $C_4H_8NO_2$, 102.0550, found 102.0554. ^{1}H NMR (400 MHz, D_2O): δ 4.59 – 4.50 (m, 1H), 4.47 – 4.30 (m, 2H), 2.80 – 2.67 (m, 1H), 2.44 – 2.30 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 174.49, 67.37, 48.55, 26.76.

Synthesis of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide



To a thick-wall Schleck tube, L-homoserine lactone hydrobromide (23.2 g, 128 mmol) and hydrobromic acid in acetic acid (33%, 90 mL) were mixed. The system was sealed and stirred at 90 °C for 9 h (**high pressure warning**). After cooling to room temperature, the precipitate was filtrated and washed with ether to remove the residual acid. The final product was obtained as a white crystal following overnight vacuum drying (33.2 g, yield: 100%). HR-ESI-MS $[M + H]^+$ Calcd. for $C_4H_9BrNO_2$, 181.9811, found 181.9811. ^{1}H NMR (400 MHz, D_2O) δ 4.12 (t, J = 6.7 Hz, 1H), 3.52 (qdd, J = 10.8, 7.2, 6.1 Hz, 2H), 2.46 (dq, J = 15.3, 6.6 Hz, 1H), 2.29 (dq, J = 15.1, 6.7 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 171.30, 171.29, 51.41, 32.69, 28.02.

Synthesis of 2,5,8,11,18,21,24,27-octaoxa-14,15-diselenaoctacosane and 2,5,8,15,18,21-hexaoxa-11,12-diselenadocosane ($EG_xSe)_2$

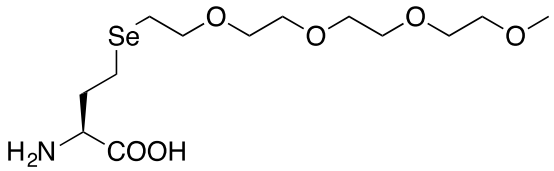


For $(EG_4Se)_2$ synthesis, tetraethyleneglycol monomethyl ether (18.5 g, 89 mmol) and NaOH (7.1 g, 178 mmol) was dissolved in deionized water (15 mL) in ice bathe, to which was added tetrahydrofuran (THF, 20 mL). the system was added 4-toluene sulfonyl chloride (Ts-Cl, 22.4 g, 117 mmol) in (THF 150 mL) in portion within 1 h, followed by stirring on ice for 1 h and

then at room temperature for 4 h. The solvent was removed by evaporation and the residue was dissolved in ethyl acetate (EA). The organic phase was washed with deionized water twice and saturated brine once (200 mL each). After desiccating with anhydrous Na_2SO_4 and vacuum evaporation of the solvent, 32.7 g clear oil (indicated as EG_4-OTs) was obtained and used without further purification.
Selenium powder (7.0 g, 89 mmol) was suspended in 5 M NaOH solution (100 mL), to which was added 85% hydrazine hydrate (5 mL, 85 mmol), and the system was stirred at room temperature overnight. Next day, EG_4-OTs (32.5 g, ~89 mmol) in THF (50 mL) was added dropwise to the Na_2Se_2 solution on ice bath. The system was stirred for 2 h before the removal of the ice bath, followed by another 24 h stirring at room temperature. Phase separation was expectable after the completion of the reaction. Deionized water (200 mL) was added and the resulting system was washed with EA (300 mL × 3). The organic phase was dried with Na_2SO_4 and the solvent was removed by rotary evaporation. The residue was purified by flash silica chromatography with petroleum ether (PE) and EA (to remove the *p*-toluenesulfonate). 19.0 g product was obtained as a yellow oil. (*Note*: there might be poly-selenide or other impurity remaining in the product. However, based on our experience, these impurities did not influence the following reactions. Thus, the product was used for the next step without further purification.)

$(EG_3Se)_2$ was synthesized by following a similar protocol starting from triethyleneglycol monomethyl ether.

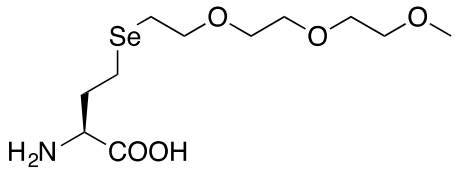
Synthesis of (*S*)-17-amino-2,5,8,11-tetraoxa-14-selenaoctadecan-18-oic acid (EG_4-SeHC)



(*S*)-3-bromo-1-carboxypropan-1-aminium bromide (3.3 g, 12.7 mmol) was dissolved in 30 mL methanol and cool to 0°C. To the system was added thionyl chloride (1.9 mL, 26.2 mmol) dropwise and stirred overnight. After the solvent removal, methyl ester of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide (compound **1**) was obtained.
In another flask, $(EG_4Se)_2$ (7.0 g) was dissolved in methanol (50 mL) and cooled to 0°C. Under the protection of nitrogen, sodium borohydride (1.05 g, 28.4 mmol) was added in four portions. After 1 h stirring, the ice bath was removed and the reaction was continued for another two hours. Then, compound **1** from the last step was dissolved in degassed methanol (25 mL) and poured to the system. The reaction was stirred at 50°C under the protection of nitrogen for 48 h. The solvent was removed by evaporation and the residue was dissolved in 20 mL deionized water for subsequent deprotection. Lithium hydroxide monohydrate (0.80 g, 19 mmol) was added and the reaction was stirred at room temperature for 1 h. The completion of deprotection was confirmed by thin layer chromatography (TLC) visualized by ninhydrin. The pH of the reaction was then adjusted to 5.0 using 1 M hydrogen chloride solution. The product was purified by C18 reverse phase chromatography using methanol/water (methanol gradient from 5% to 35%). The pure product was recovered as a white powder after lyophilization (4.1 g, yield: 85%). HR-ESI-MS $[M + H]^+$ Calcd. for $C_{13}H_{28}NO_6Se$, 374.1077, found 374.1069. ^1H NMR (400 MHz, D_2O) δ 3.77 – 3.64 (m, 3H), 3.62 – 3.56 (m, 10H), 3.56 – 3.48 (m, 2H), 3.28 (s, 3H), 2.73 (t, J = 6.7 Hz, 2H), 2.59 (t, J =

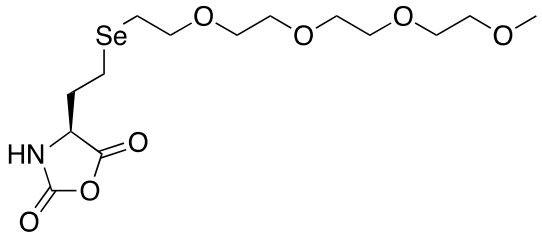
7.9 Hz, 2H), 2.21 – 2.02 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 173.94, 70.96, 70.22, 69.50, 69.37, 69.18, 58.01, 54.64, 31.47, 22.21, 18.18.

Synthesis of (*S*)-14-amino-2,5,8-trioxa-11-selenapentadecan-15-oic acid (EG_3-SeHC)



(*S*)-3-bromo-1-carboxypropan-1-aminium bromide (2.8 g, 11 mmol) was dissolved in 30mL methanol and cool to 0°C. Thionyl chloride (1.6mL, 22 mmol) was added dropwise and the system was stirred overnight. After the solvent removal, methyl ester of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide (compound **1**) was obtained.
In another flask, $(EG_3Se)_2$ (7.0 g) was dissolved in methanol (50 mL) and cooled to 0°C. Under the protection of nitrogen, sodium borohydride (0.9 g, 24.3 mmol) was added in four portions. After 1 h stirring, the ice bath was removed and the reaction was continued for another two hours. Then compound **1** from the last step was dissolved in degassed methanol (25 mL) and poured to the system. The reaction was stirred at 50 °C under the protection of nitrogen for 48 h. The solvent was removed by evaporation and the residue was dissolved in 20 mL deionized water for subsequent deprotection. Lithium hydroxide monohydrate (0.7 g, 16 mmol) was added and the reaction was stirred at room temperature for 1 h. The completion of deprotection was confirmed by TLC visualized by ninhydrin. The pH was then adjusted to 5.0 using 1 M hydrogen chloride solution. The pure product was recovered as a white powder after lyophilization (2.8 g, yield: 77%).HR-ESI-MS $[M + H]^+$ Calcd. for $C_{11}H_{24}NO_5Se$, 330.0808, found 330.0814. ^{1}H NMR (400 MHz, D_2O) δ 3.78 – 3.66 (m, 3H), 3.64 – 3.56 (m, 6H), 3.55 – 3.50 (m, 2H), 3.28 (s, 3H), 2.73 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 7.8 Hz, 2H), 2.22 – 2.01 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 173.94, 70.97, 70.25, 69.55, 69.49, 69.41, 69.19, 58.04, 54.68, 31.47, 22.24, 18.19.

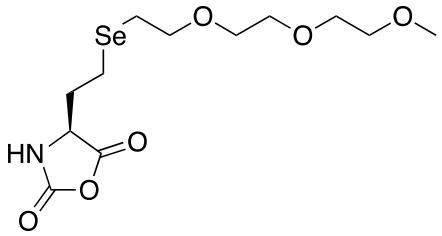
Synthesis of (*S*)-4-(2,5,8,11-tetraoxa-14-selenahexadecan-16-yl)oxazolidine-2,5-dione (EG_4-SeHC NCA)



Under the protection of nitrogen, EG_4-SeHC (1.0 g, 2.6 mmol), α-piene (0.6 mL, 3.8 mmol), and triphosgene (0.32, 1.08 mmol) were suspended in anhydrous THF (30 mL). The system was heated up to 50 °C for 4 h. The solvent was removed and the product was purified by silica gel chromatography in mixed EA/PE (EA gradient from 0% to 80%). Pure EG_4-SeHC NCA was recovered as a light yellow oil (0.55 g, yield: 52%). HR-ESI-MS $[M + Na]^+$ Calcd. for $C_{14}H_{25}NO_7SeNa$, 422.0688, found 422.0686. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 4.60 – 4.51 (m, 1H), 3.89 – 3.79 (m, 2H), 3.77 – 3.58 (m, 10H), 3.58 – 3.51 (m, 2H), 3.36 (s, 3H), 2.86 – 2.80 (m, 2H), 2.78 – 2.72 (m, 2H), 2.36 – 2.09 (m, 2H). ^{13}C NMR (101 MHz,

$CDCl_3$) δ 170.60, 152.09, 73.60, 71.85, 70.52, 70.39, 70.16, 69.84, 58.88, 57.46, 53.44, 32.66, 22.50, 18.89. FT-IR (4000-400 cm^{-1}): 1852 and 1785 (ν anhydride).

Synthesis of (*S*)-4-(2,5,8-trioxa-11-selenatridecan-13-yl)oxazolidine-2,5-dione (EG_3-SeHC NCA)



Under the protection of nitrogen, EG_3-SeHC (1.0 g, 2.8 mmol), α-piene (0.6 mL, 3.8 mmol), and triphosgene (0.32, 1.08 mmol) were suspended in anhydrous THF (30 mL). The system was heated up to 50 °C for 4 h. The solvent was removed and the product was purified by silica gel chromatography in mixed EA/PE (EA gradient from 0% to 80%). Pure EG_4-SeHC NCA was recovered as a light yellow oil (0.51g, yield: 48%).HR-ESI-MS $[M + Na]^+$ Calcd. for $C_{12}H_{21}NO_6SeNa$, 378.0426, found 378.0426. ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 4.56 – 4.47 (m, 1H), 3.94 – 3.80 (m, 2H), 3.80 – 3.52 (m, 8H), 3.38 (s, 3H), 2.95 – 2.78 (m, 2H), 2.78 – 2.73 (m, 2H), 2.37 – 2.11 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.41, 152.03, 73.42, 71.77, 70.42, 70.03, 69.88, 58.84, 57.36, 32.58, 22.80, 19.02. FT-IR (4000-400 cm^{-1}): 1854 and 1788 (ν anhydride).

General procedure for ROP of NCA

All polymerizations were carried out in a glovebox filled with ultrapure nitrogen. To a solution of EG_x-SeHC NCA in dry DMF (50 mg/mL), a certain amount of HMDS in dry DMF (0.5 M) was quickly added. The polymerizations were stirred at room temperature for at least 24 h and monitored by FT-IR. The M_n of the polymer was characterized by size exclusion chromatography. After the completion of the reaction, the polypeptides were precipitated in diethyl ether and collected by centrifugation. The products were then dissolved in water and passed through a flash size exclusion column (PD-10) to remove possible small molecular impurities. After lyophilization, the polymers were obtained as sticky gums (yield: 90-99%).

$P(EG_4\text{-SeHC})_n$
^1H NMR (400 MHz, D_2O) δ 4.50 – 4.06 (m, 1H), 3.75 – 3.50 (m, 14H), 3.31 (s, 3H), 2.90 – 2.53 (m, 4H), 2.40 – 1.98 (m, 2H).

$P(EG_3\text{-SeHC})_n$
^1H NMR (400 MHz, $CDCl_3$) δ 4.06 (s, 1H), 3.76 – 3.52 (m, 10H), 3.38 (s, 3H), 2.95 – 2.51 (m, 4H), 2.25 (s, 2H).

Measurement of GP_x activity of the selenopolypeptides

The GP_x activity of the selenopolypeptides was measured by following the instruction of the kit with slight modification. Briefly, in the presence of glutathione reductase, oxidized GSH is reduced by NADPH, which is expected to result in the decrease of absorption at 340 nm (ΔA340). Thus, ΔA340 for a given time can be used to indicate whether a substance could catalyze the oxidation of GSH by H_2O_2. In our experiments, the final concentration of the selenopolypeptides in the system was kept at 0.05 mg/mL with three parallel tests.

Measurement of the temperature-dependent transmittance

The polypeptides were dissolved in deionized water or PBS (pH 7.4) all at a final concentration of 1.0 mg/mL. Transmittance at 500 nm was recorded in a quartz cuvette with 1.0 cm path length. For each measurement, a certain temperature was set and the system was equilibrated for 2 minutes before the transmittance recording.

Oxidation and reduction of $P(EG_4\text{-SeHC})_{80}$

To 1.0 mg/mL $P(EG_4\text{-SeHC})_{80}$ in PBS was added. CD spectra of the reaction mixture were recorded, which indicated complete reaction after two hours. Then, to 2.5 mL of the mixture, DTT (75 μL × 1 M) was added to make a final concentration of 30 mM. The system was stirred at room temperature for 5 hours before passing through a size exclusion column PD-10 for desalting. The reduced polymer was lyophilization and re-dissolved in PBS in a concentration of 1.0 mg/mL for CD study.

Circular Dichroism

Circular dichroism spectra (190–250 nm) were recorded in a quartz cuvette of 0.1 cm path length. All spectra were recorded as an average of 3 scans. The spectra were reported in units of molar ellipticity [θ] ($deg \cdot cm^2 \cdot dmol^{-1}$). For oxidation kinetic study, the CD signal at 222 nm was recorded for 2 h immediately after the addition of H_2O_2. For temperature dependence study, the spectra were recorded in a quartz cuvette of 1.0 cm path length.

Cytotoxicity of the polymer

Raw 264.7, HUVEC, or HeLa cells were seeded in 96-well plate ($2x10^4$/well) and incubated for 24 h before the experiments. $P(EG_4\text{-SeHC})_{80}$ was dissolved in culture medium and incubated with the cells at varied concentrations for 24 h. The supernatant was aspirated and the cells were washed with PBS once. The relative viability of the cells was measured using Celltiter 96 following the manufacturer's instruction.

Supplementary Figures

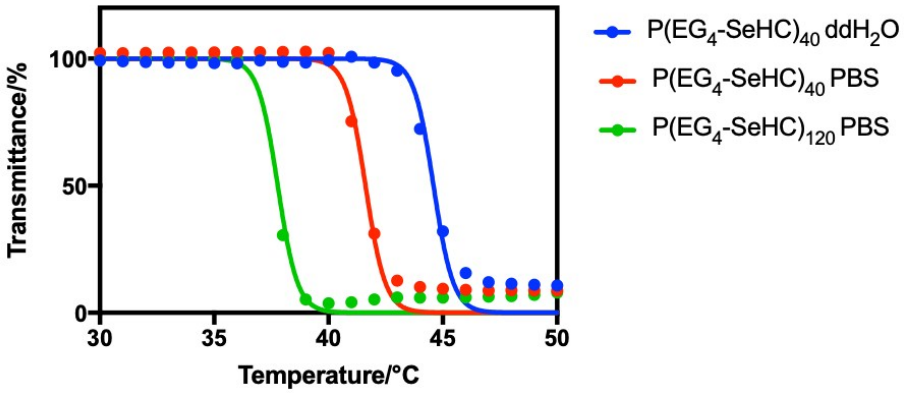


Figure S1 Temperature-dependent transmittance of $P(EG_4\text{-}SeHC)_{40}$ (1.0 mg/mL) in PBS and water, and $P(EG_4\text{-}SeHC)_{120}$ (1.0 mg/mL) in PBS.

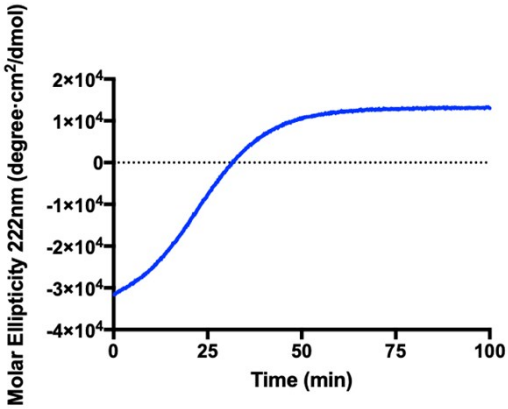


Figure S2 Oxidation kinetic monitored by CD signal at 222 nm. $P(EG_4\text{-}SeHC)_{40}$ (1.0 mg/mL) was dissolved in ddH_2O and treated with 20 mM H_2O_2. The ellipticity at 222 nm was monitored immediately after the addition of H_2O_2.

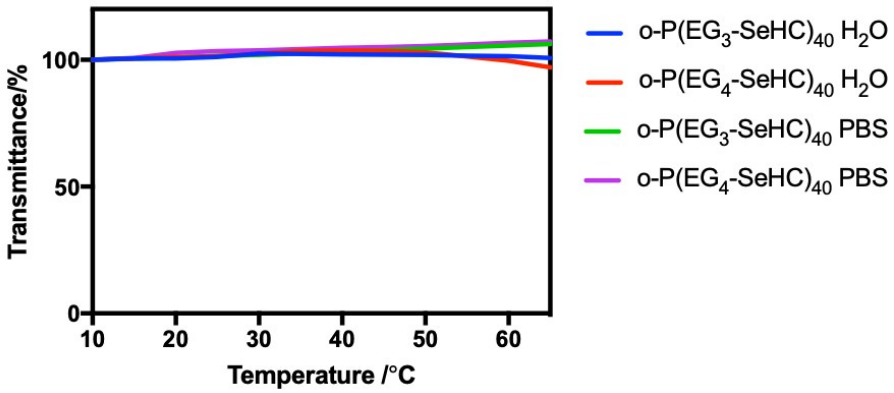


Figure S3 Transmittance of the oxidized selenopolypeptides $o\text{-}P(EG_3\text{-}SeHC)_{40}$ and $o\text{-}P(EG_4\text{-}SeHC)_{40}$ at different temperatures.

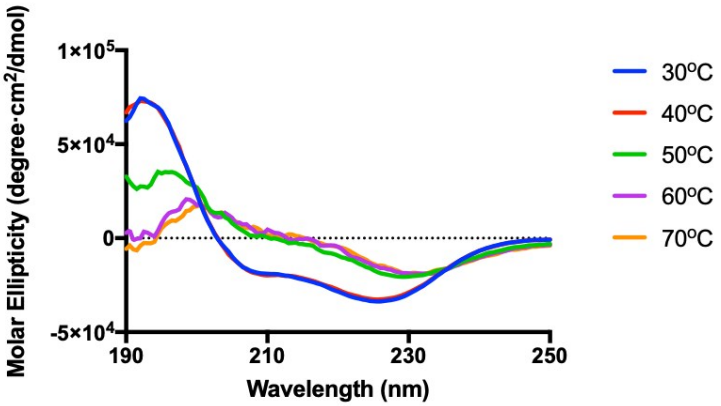


Figure S4 Temperature-dependent CD spectroscopy of $P(EG_4\text{-SeHC})_{80}$. $P(EG_4\text{-SeHC})_{40}$ (0.1 mg/mL) was dissolved in ddH_2O and the spectra was recorded at indicated temperature in a quartz cuvette of 1.0 cm path length.

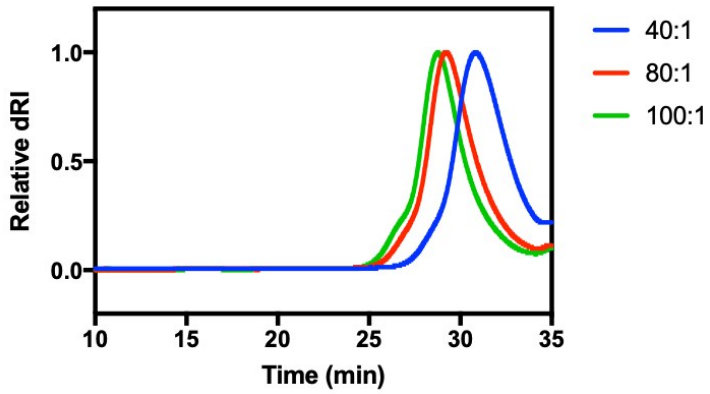


Figure S5 Size exclusion chromatography of $P(EG_4\text{-SeHC})$ prepared at different feeding M/I ratios.

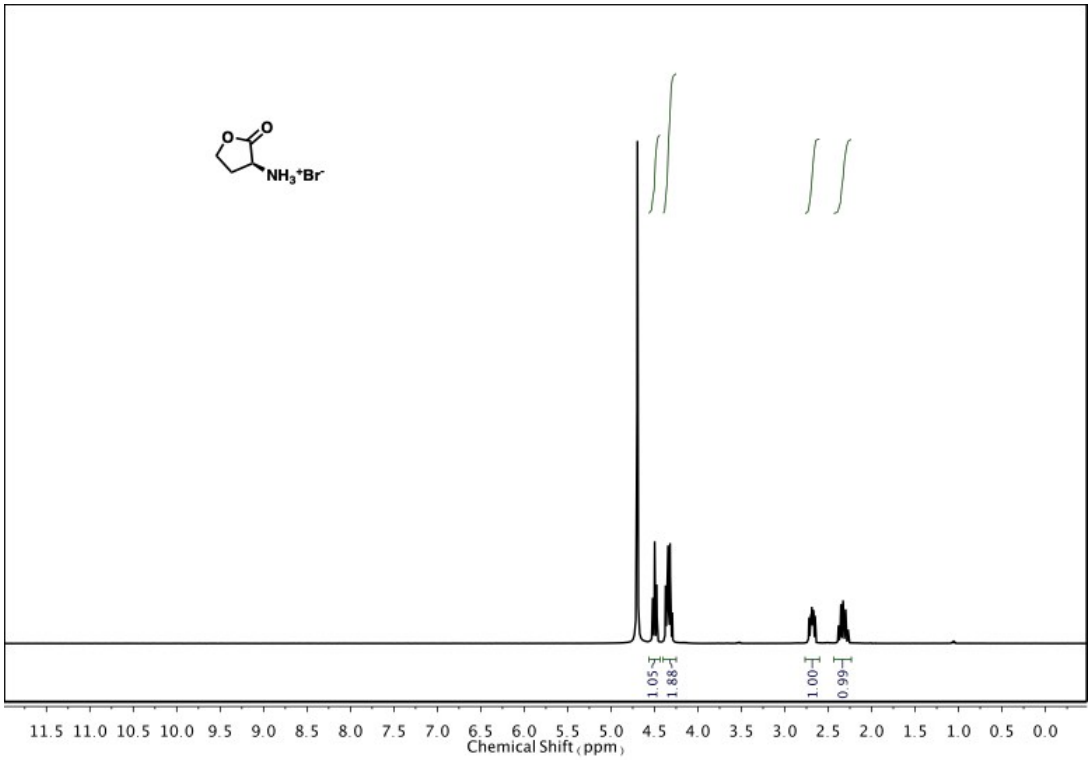


Figure S6 ^{1}H NMR of (*S*)-2-oxotetrahydrofuran-3-aminium bromide (solvent: D_2O)

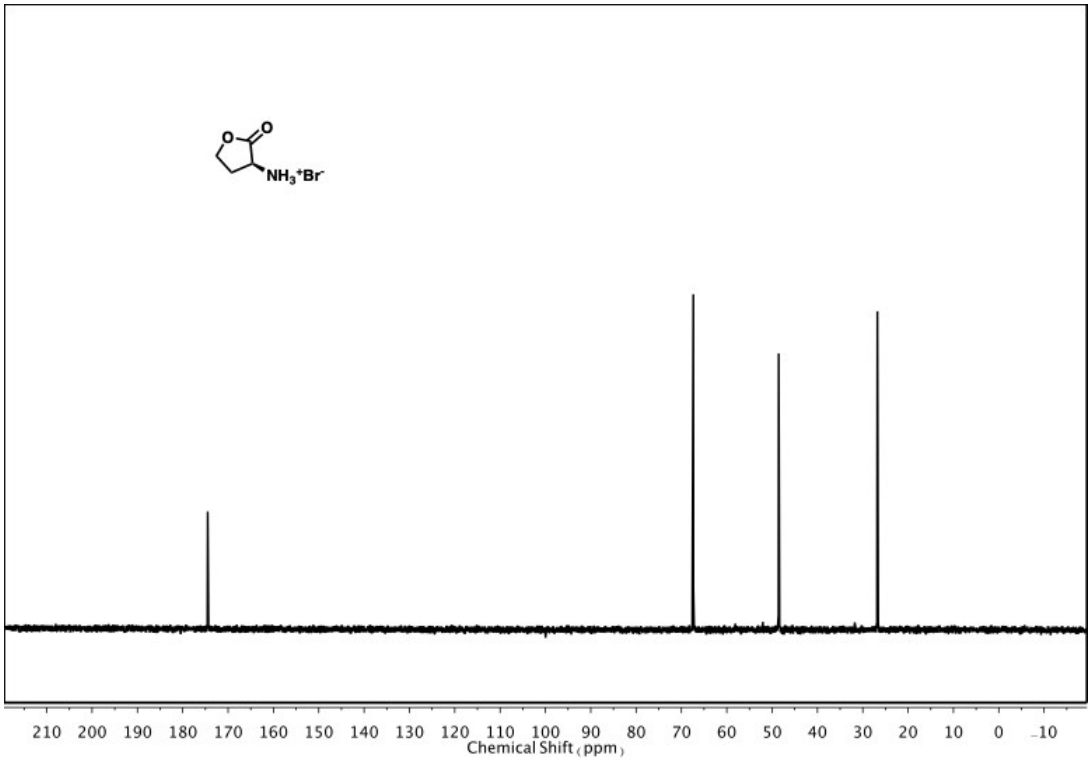


Figure S7 ^{13}C NMR of (*S*)-2-oxotetrahydrofuran-3-aminium bromide (solvent: D_2O)

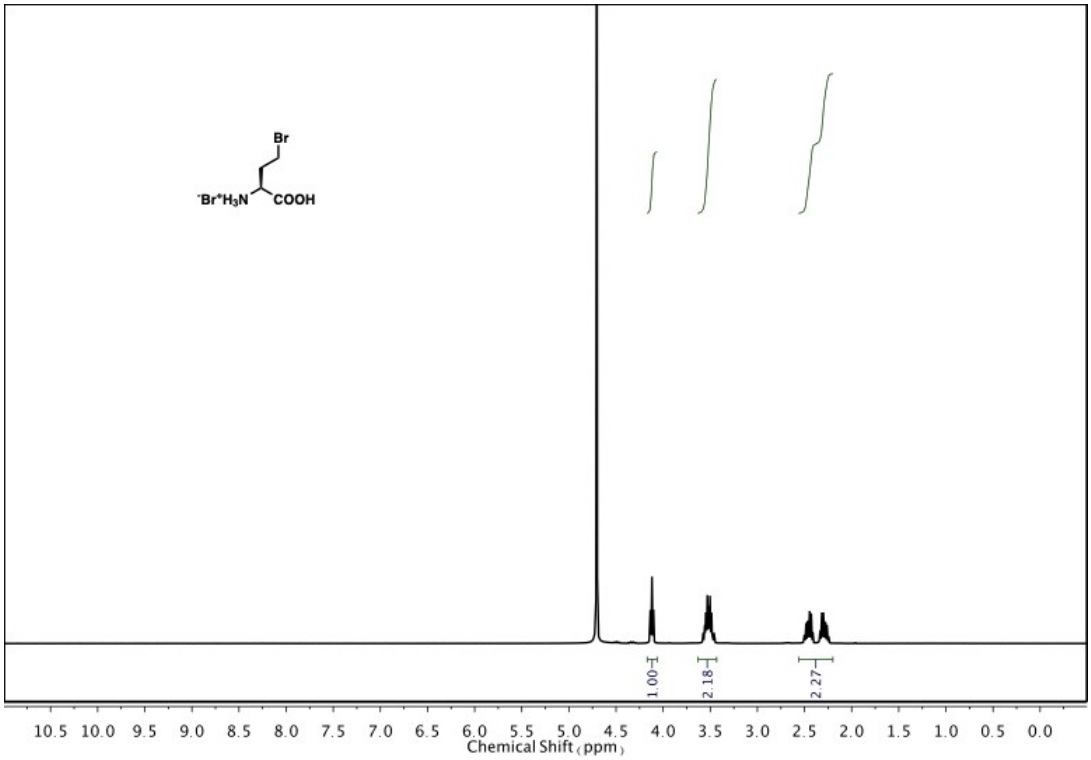


Figure S8 ^{1}H NMR of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide (solvent: D_2O).

Figure S9 ^{13}C NMR of (*S*)-3-bromo-1-carboxypropan-1-aminium bromide (solvent: D_2O).

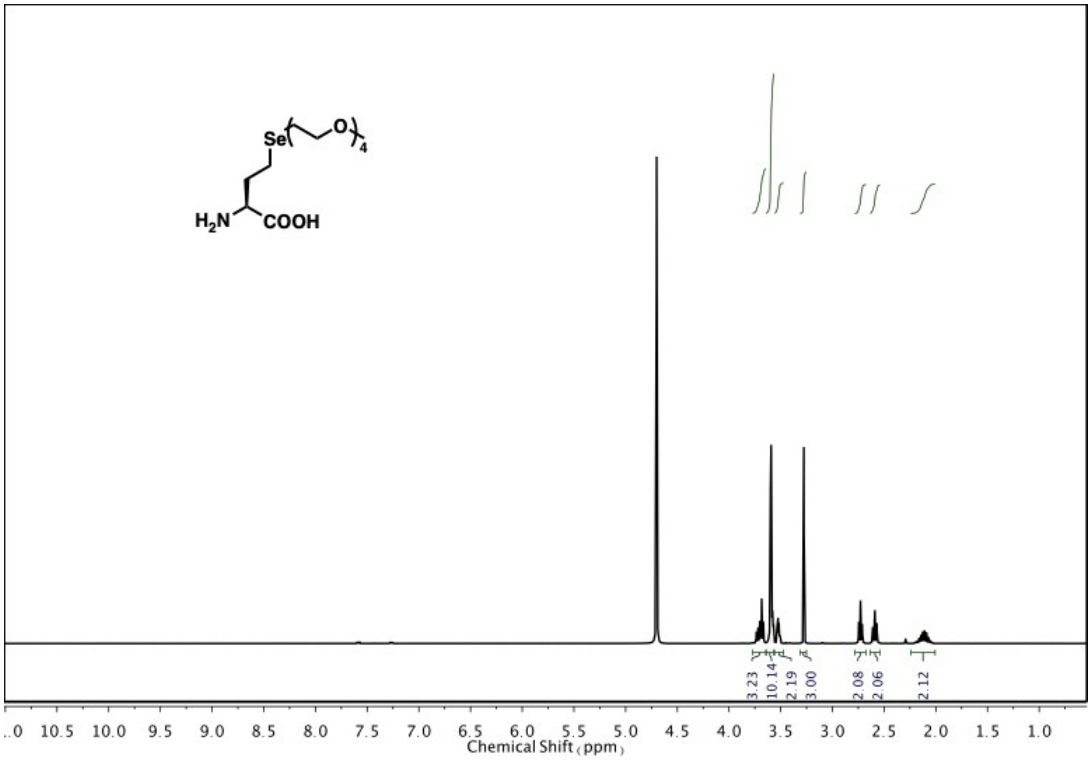


Figure S10 ^{1}H NMR of EG_4-SeHC (solvent: D_2O).

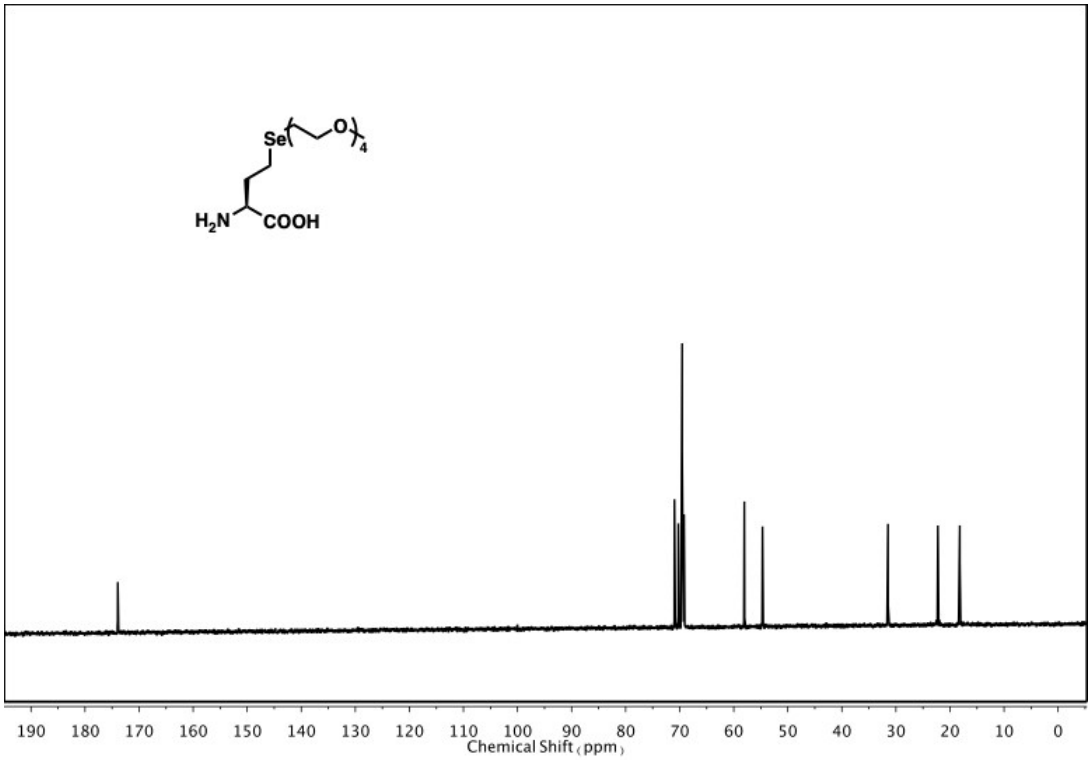


Figure S11 ^{13}C NMR of EG_4-SeHC (solvent: D_2O).

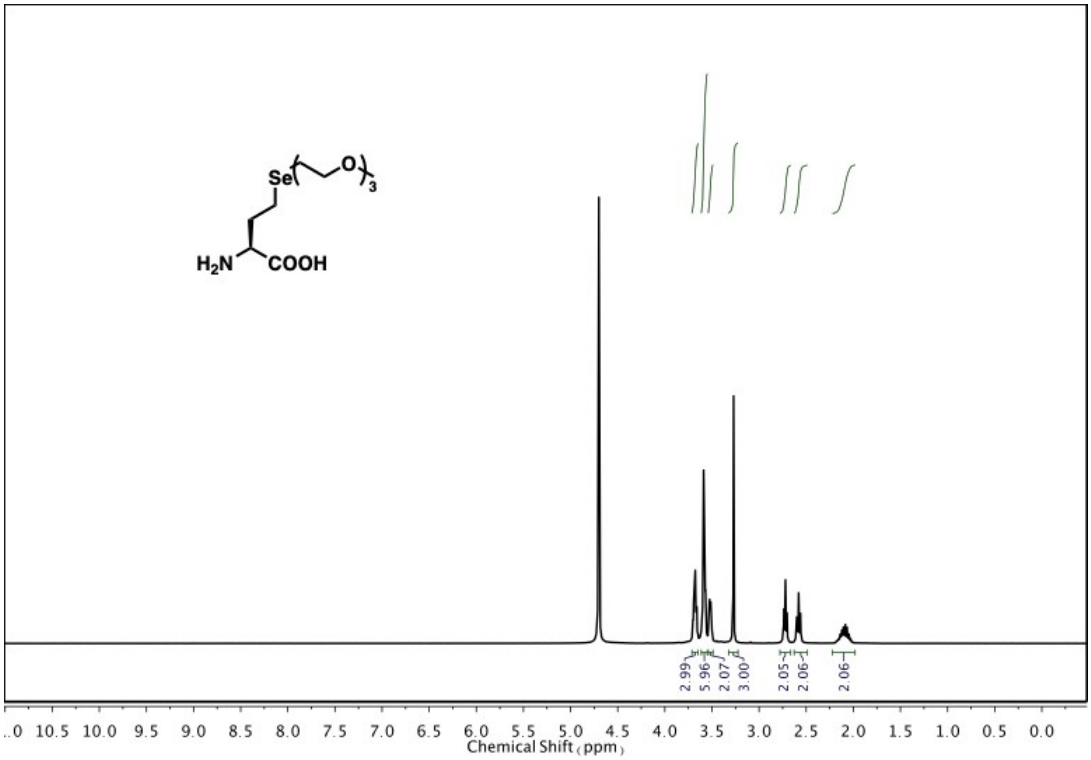


Figure S12 ^{1}H NMR of EG_3-SeHC (solvent: D_2O).

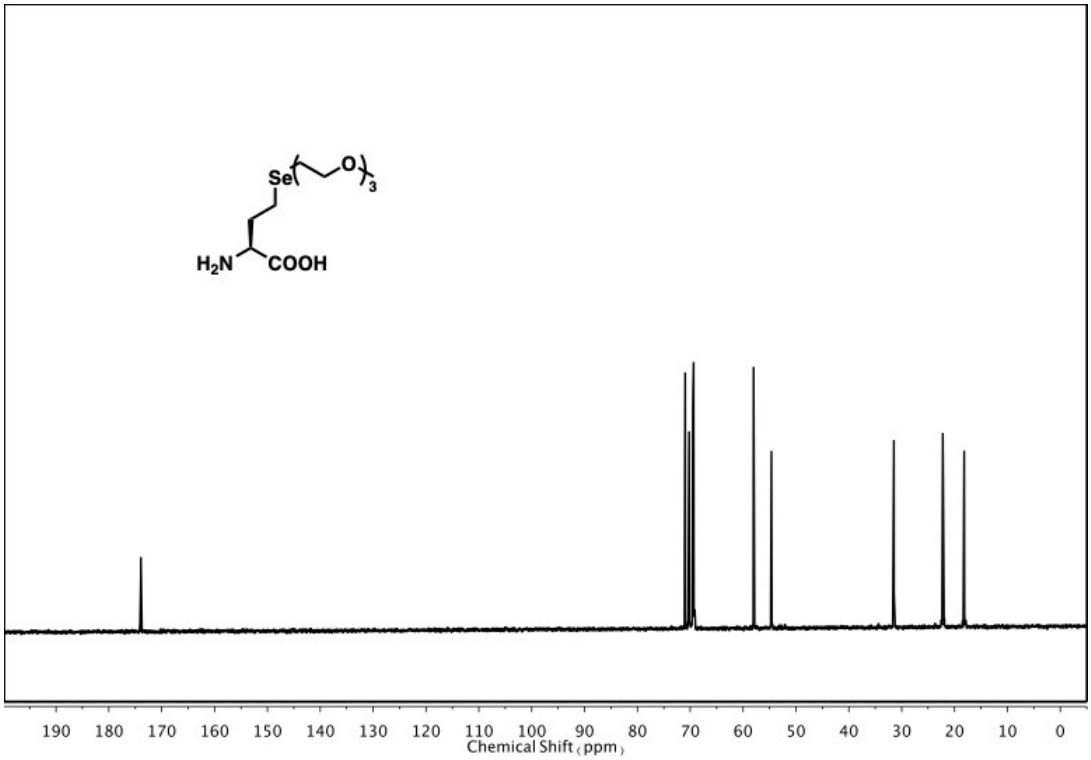


Figure S13 ^{13}C NMR of EG_3-SeHC (solvent: D_2O).

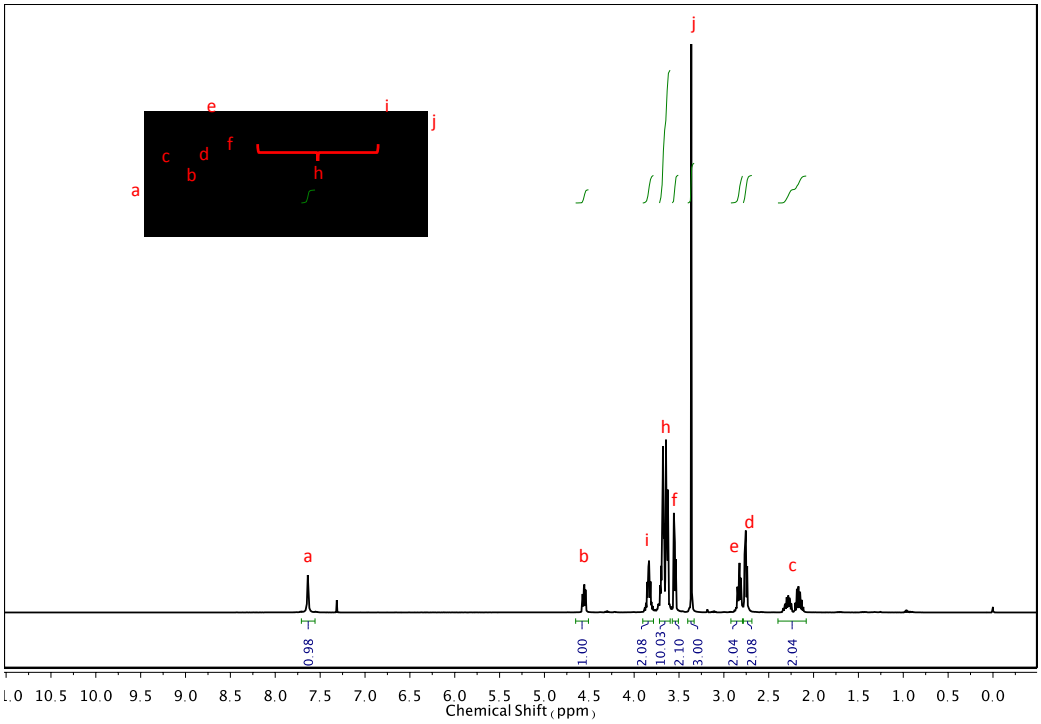


Figure S14 ^{1}H NMR of EG_4-SeHC NCA (solvent: $CDCl_3$).

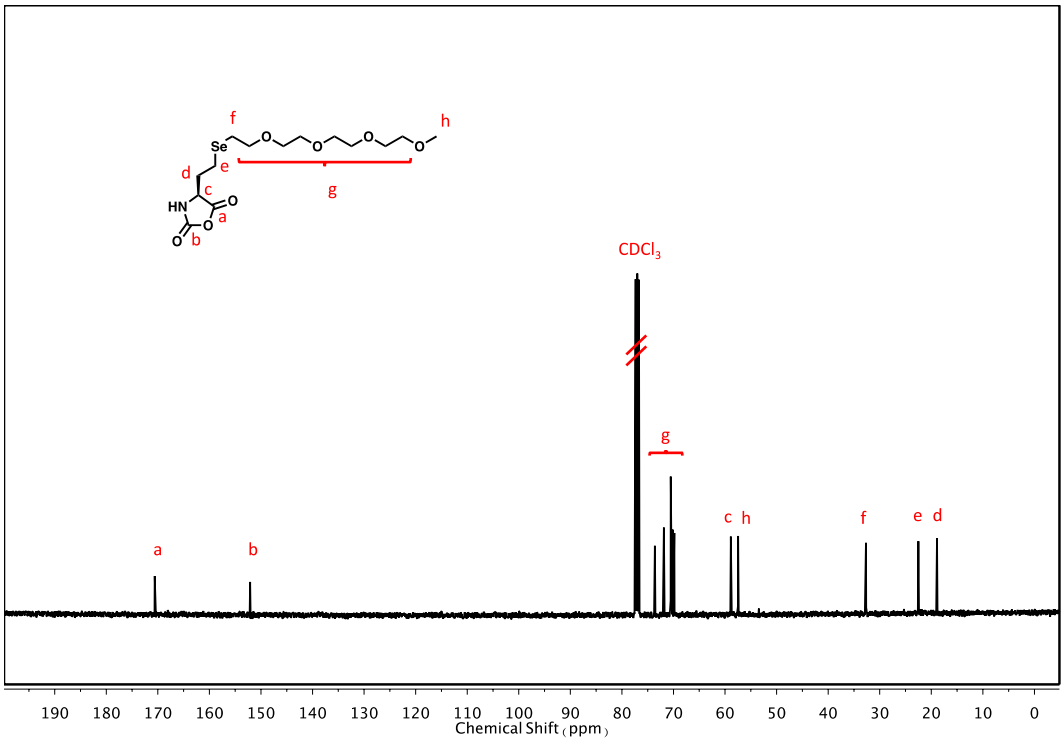


Figure S15 ^{13}C NMR of EG_4-SeHC NCA (solvent: $CDCl_3$).

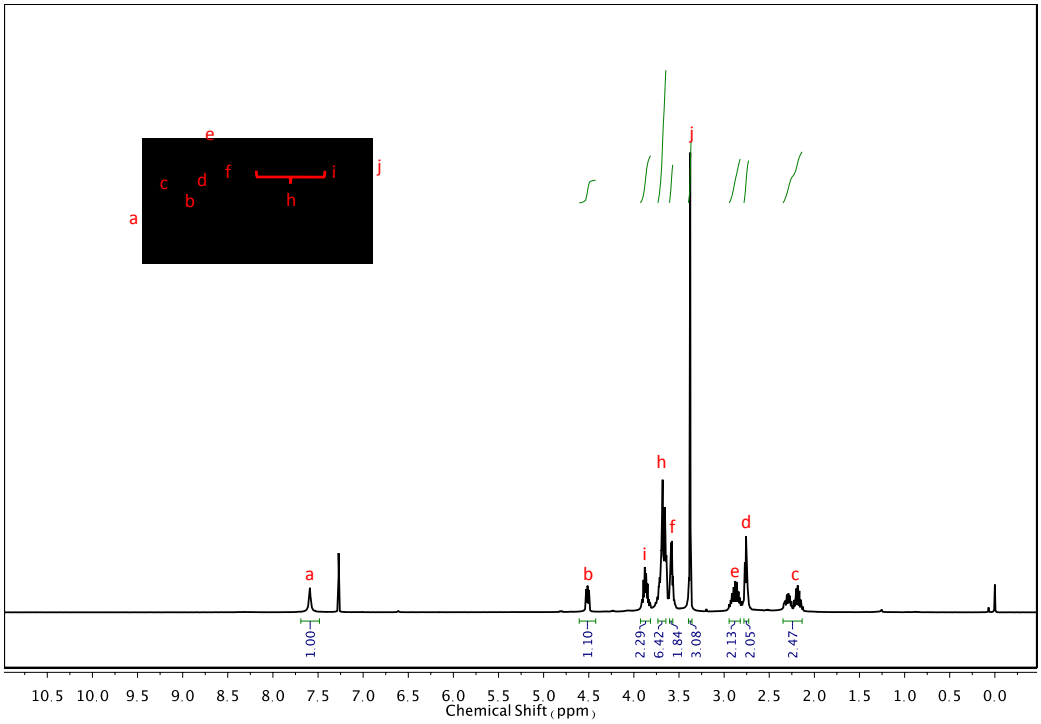


Figure S16 ^{1}H NMR of EG_3-SeHC NCA (solvent: $CDCl_3$).

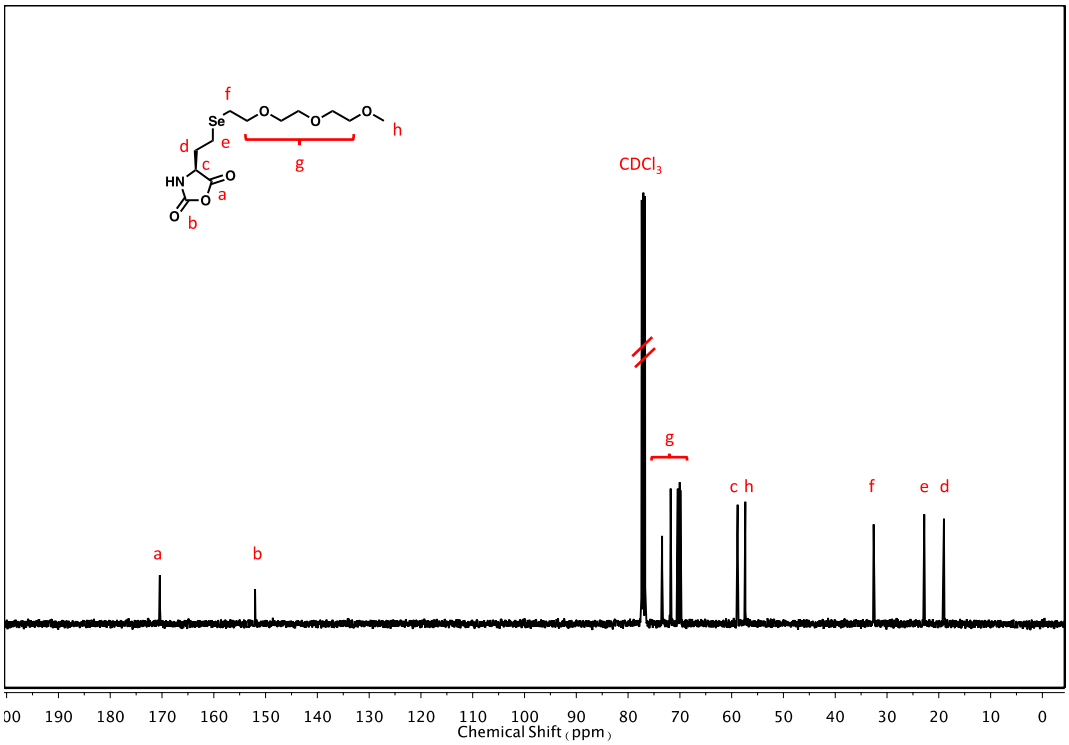


Figure S17 ^{13}C NMR of EG_3-SeHC NCA (solvent: $CDCl_3$).

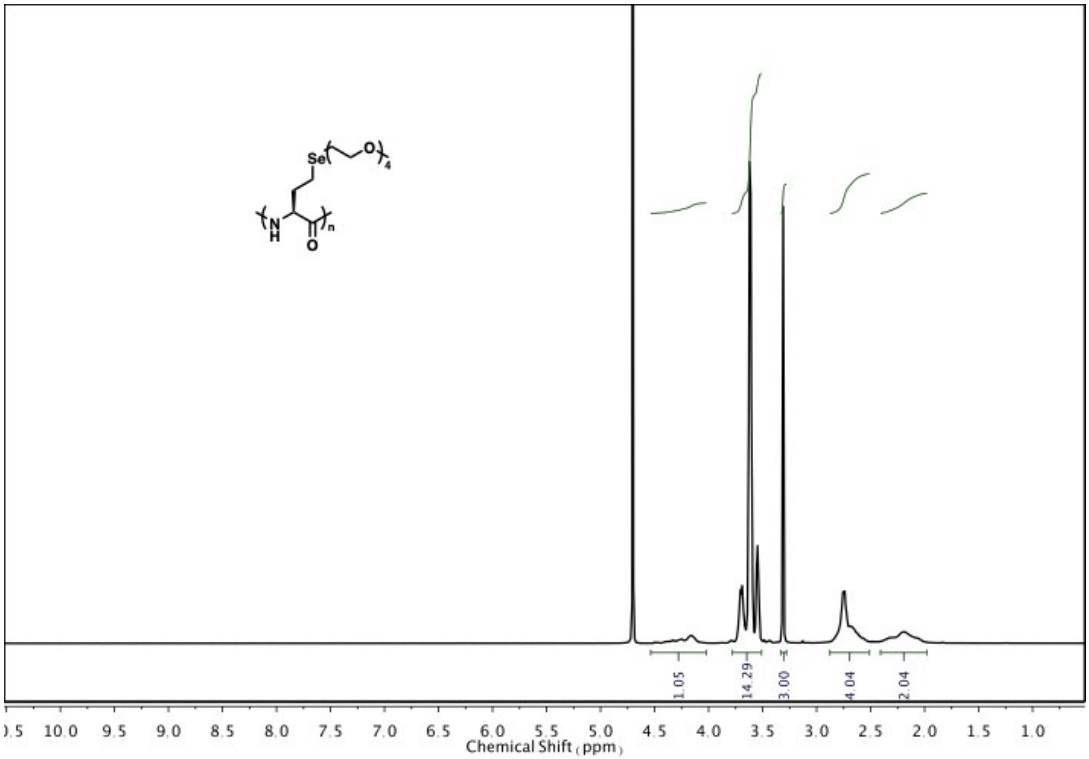


Figure S18 ^{1}H NMR of P(EG_4-SeHC) (solvent: D_2O).

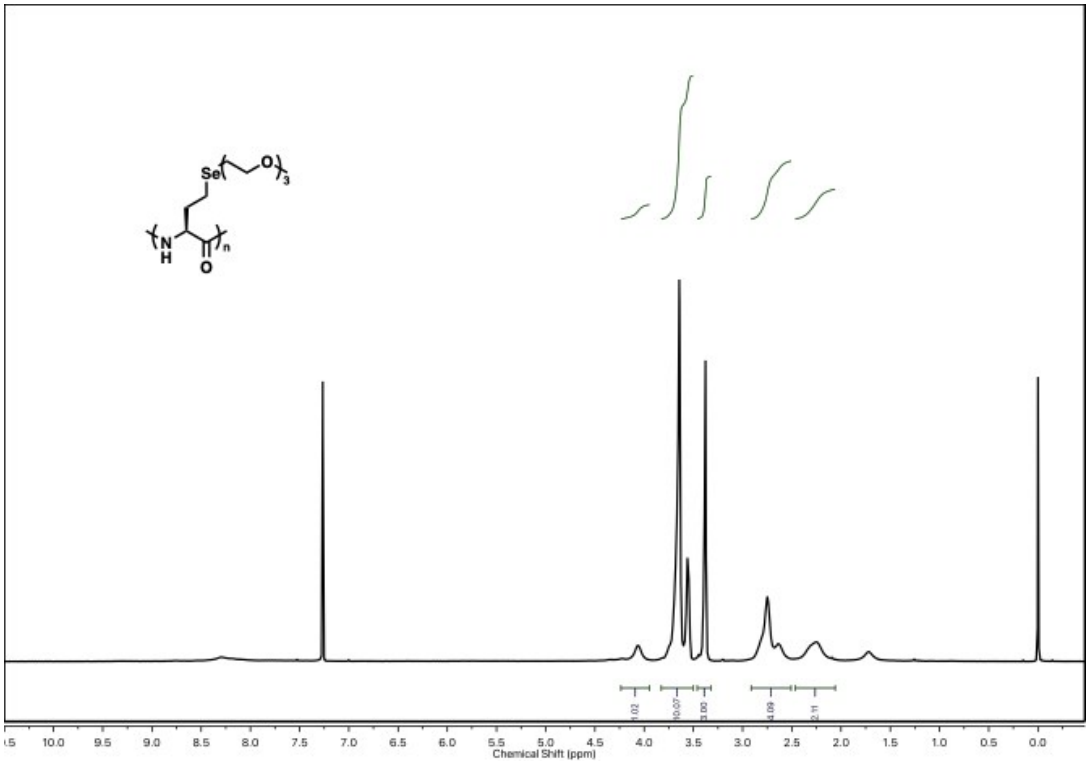


Figure S19 ^{1}H NMR of P(EG_3-SeHC) (solvent: $CDCl_3$).