Electronic Supplementary Material (ESI) for ChemComm.


# Polyselenonium salts: Synthesis through sequential selenium-epoxy 'click' chemistry and Se-alkylation

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## General Methods and Materials

Glycidyl methacrylate, ethyl α-bromoisobutyrate (EBiB) 4,4'-dinonyl-2,2'-dipyridyl (dNbpy), Cu(I)Cl, dimethyl diselenide, diphenyl diselenide, acetic anhydride, hexanoic anhydride, triethylamine (TEA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 4-dimethylaminopyridine (DMAP), sodium borohydride ($NaBH_4$), iodomethane, iodopentane, silver tetrafluoroborate ($AgBF_4$), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC6538), red blood cells (sterile defibrinated sheep's blood, RBC), were purchased from commercial sources. NMR spectra were recorded on a Varian NMR system 500 MHz spectrometer, using DMSO-$d_6$ and $CDCl_3$ as the solvents. Gel permeation chromatography (GPC) (against polystyrene standards) was carried out in 1% LiBr in DMF using a Waters system (Waters 1515 pump, Water 2414 refractive 5 index detector) instrument with four styragel HR 0.5, HR 5E, HR 4, HR5 columns. Gel permeation chromatography (GPC) (against polystyrene standards) was carried out in THF as well using a Waters system (Waters 1515 pump, Water 2414 refractive 5 index detector) instrument with three styragel HR 0.5, HR 2, HR 4 columns. LC-MS/MS was carried out on a HP 1000 coupled with an Agilent 6130 single quadruple mass detector from Agilent technologies. Antibacterial tests and hemolysis assays were performed using a microplate reader (Spectra Max 340, Molecular Devices, Sunnyvale, CA USA).

## Polymer synthesis

Synthesis of PGMA (**1**): glycidyl methacrylate (3 g, 35.17 mmol), 4,4'-dinonyl-2,2'-dipyridyl (dNbpy) (56 mg, 0.14 mmol), ethyl α-bromoisobutyrate (EBiB) (13 mg, 0.07 mmol), and THF (2 mL) was added to a schlenk tube and purged by bubbling Ar for 30 min. Cu(I)Cl (7 mg, 0.07 mmol) was added and Ar purging was continued for another 5 min. The reaction mixture was then stirred under inert atmosphere at room temperature for 5 h. After this time, the reaction mixture was cooled to room temperature and precipitated into hexane, filtered, and passed through a small plug of silica gel using DCM as an eluent. The organic solvent was reduced under low pressure and then precipitated into hexane. The obtained white powder was then dried under high vacuum conditions. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 4.30

(br s, COO**CH$_2$**), 3.74 (br s, COO**CH$_2$**), 3.21 (br s, CH$_2$**CH**CH$_2$O), 2.80 (br s, COOCH$_2$CH**CH$_2$**O), 2.66 (br s, COOCH$_2$CH**CH$_2$**O), 2.17 – 0.57 (br m, backbone). GPC(DMF): $M_n$ = 18900 g/mol, $M_w$ = 22500 g/mol, $Đ$ = 1.21.

Synthesis of polymers **2** and **3** through selenium-epoxy reaction: To a solution of **1** (1 g, 7.04 mmol of epoxy units) and diselenide (3.52 mmol (0.5 eq. per epoxide)) in THF (9 mL) at 0 °C, a solution of sodium borohydride (7.74 mmol (2.2 eq. per diselenide)) in water (1 mL) was added dropwise. The reaction was exothermic and the evolution of hydrogen gas could be observed. The reaction mixture was then stirred at room temperature for 1 hour. After this time, the reaction mixture was precipitated into methanol twice and hexane twice then dried under high vacuum conditions.

**2**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.45 (br s, -Se**Ph**), 7.21 (br m, -Se**Ph**), 5.34 (br s, CH(**OH**)CH$_2$Se), 3.88 (br m, COO**CH$_2$CH**(OH)), 3.02 (br d, CH(OH)**CH$_2$**Se), 2.17 – 0.57 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 267.8. GPC(DMF): $M_n$ = 24400 g/mol, $M_w$ = 29300 g/mol, $Đ$ = 1.20.

**3**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 5.14 (br s, CH(**OH**)CH$_2$Se), 3.86 (br m, COO**CH$_2$CH**(OH)), 2.63 (br d, CH(OH)**CH$_2$**Se), 2.01 (br s, -Se**CH$_3$**), 1.96 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 53.9. GPC(DMF): $M_n$ = 22300 g/mol, $M_w$ = 27400 g/mol, $Đ$ = 1.23.

Synthesis of polymers **4** and **5** through esterification reaction: To a solution of **2** or **3** (1.26 mmol of hydroxyl groups), TEA (5.06 mmol) and DMAP (0.38 mmol) in 5 mL DCM was added hexanoic anhydride (3.79 mmol) at 0 °C. The cooling was removed, and the reaction mixture was stirred at room temperature for overnight. After this time, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, reduced under low pressure, precipitated into hexane thrice and then dried under high vacuum conditions.

**4**: $^1$H NMR (500 MHz, $CDCl_3$) δ 7.49 (br s, -Se**Ph**), 7.24 (br s, -Se**Ph**), 5.15 (br s, OCH$_2$**CH**CH$_2$Se), 4.15 (br d, O**CH$_2$**CHCH$_2$Se), 3.10 (br s, OCH$_2$CH**CH$_2$**Se), 2.16 (br s, CHOCO**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$), 1.52 (br s, CHOCOCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 1.25 (br s, CHOCOCH$_2$CH$_2$**CH$_2$CH$_2$**CH$_3$), 0.86 (br s, CHOCOCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**), 1.99 – 0.63 (br m, backbone). $^{77}$Se NMR (95 MHz, $CDCl_3$) δ 261.3. GPC(DMF): $M_n$ = 41600 g/mol, $M_w$ = 48700 g/mol, $Đ$ = 1.17.

**5**: $^1$H NMR (500 MHz, $CDCl_3$) δ 5.18 (br s, OCH$_2$**CH**CH$_2$Se), 4.14 (br d, O**CH$_2$**CHCH$_2$Se), 2.73 (br s, OCH$_2$CH**CH$_2$**Se), 2.34 (CHOCO**CH$_2$**CH$_2$CH$_2$CH$_2$CH$_3$), 2.08 (br s, -Se**CH$_3$**), 1.64

(br s, CHOCOCH$_2$**CH$_2$**CH$_2$CH$_2$CH$_3$), 1.34 (br s, CHOCOCH$_2$CH$_2$**CH$_2$CH$_2$**CH$_3$), 0.91 (br s, CHOCOCH$_2$CH$_2$CH$_2$CH$_2$**CH$_3$**), 1.99 – 0.63 (br m, backbone). $^{77}$Se NMR (95 MHz, $CDCl_3$) δ 53.9. GPC(DMF): $M_n$ = 36000 g/mol, $M_w$ = 42900 g/mol, $Đ$ = 1.19.

Synthesis of polymer **2**-AC: To a solution of **2** (50 mg, 0.168 mmol of hydroxyl group), TEA (70 mg, 0.67 mmol) and DMAP (6 mg, 0.05 mmol) in 2 mL THF was added acetic anhydride (51 mg, 0.50 mmol) at 0 °C. The cooling was removed, and the reaction mixture was stirred at room temperature for overnight. After this time, the reaction mixture was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, reduced under low pressure, precipitated into hexane thrice and then dried under high vacuum conditions. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.48 (br s, -Se**Ph**), 7.24 (br s, -Se**Ph**), 5.10 (br s, OCH$_2$**CH**CH$_2$Se), 4.09 (br d, O**CH$_2$**CHCH$_2$Se), 3.13 (OCH$_2$CH**CH$_2$**Se), 1.85 (br s, CHOCO**CH$_3$** + backbone), 1.84 – 0.56 (br m, backbone). GPC(THF): $M_n$ = 58600 g/mol, $M_w$ = 69200 g/mol, $Đ$ = 1.18.

Synthesis of 3-(phenylselanyl)propane-1,2-diol: To a solution of 3-bromopropane-1,2-diol (1.0 g, 6.45 mmol) and benzeneselenol (2.03 g, 12.9 mmol) in 6 mL MeCN was added TEA (5.22 g, 51.6 mmol) and the reaction mixture was stirred at 75 °C for 6 h. After this time, the reaction mixture was cooled to room temperature, diluted with DCM and washed with water. The organic solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent = DCM/MeOH 95:5) which gave about 1.31 g of 3-(phenylselanyl)propane-1,2-diol as a white powder (yield = 88%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.50 – 7.44 (m, 2H), 7.35 – 7.15 (m, 3H), 4.98 (d, $J$ = 5.1 Hz, 1H), 4.65 (t, $J$ = 5.5 Hz, 1H), 3.74 – 3.56 (m, 1H), 3.44 – 3.33 (m, 2H), 3.17 – 2.85 (m, 2H). $^{13}$C NMR (126 MHz, DMSO-$D_6$) δ 131.2, 130.9, 129.1, 126.1, 70.8, 65.0, 31.5. $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 262.5. ESI (observed: 255.0 $[M+Na]^+$, calc. 255.0 for $C_9H_{12}O_2NaSe$).

**2**-Mimic: A suspension of 3-(phenylselanyl)propane-1,2-diol (500 mg, 2.16 mmol), acetic acid (649 mg, 10.8 mmol), EDCI (2.07 g, 10.8 mmol), and DMAP (132 mg, 1.08 mmol) was stirred at 0 °C in 10 mL DMF for 1 hr. Then the reaction mixture was stirred at room temperature for overnight. After this time, the reaction was diluted with DCM and washed with water, 0.1 M HCl solution, followed by saturated $NaHCO_3$. The organic solvent was removed under reduced pressure which gave about 552 mg of the product as a colorless oil (yield = 81%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.61 – 7.42 (m, 2H), 7.40 – 7.19 (m, 3H), 5.10 (dtd, $J$ = 7.0, 6.1, 3.3 Hz, 1H), 4.30 – 4.05 (m, 2H), 3.24 – 3.12 (m, 2H), 1.98 (s, 3H), 1.90 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$D_6$) δ 170.2, 169.8, 131.9, 129.5, 129.4, 127.1,

70.7, 64.0, 26.6, 20.6, 20.6. $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 272.8. ESI (observed: 339.0 $[M+Na]^+$, calc. 339.0 for $C_{13}H_{16}O_4NaSe$).

Synthesis of polymers **6-11** through selenium-alkylation: To a solution of polymer (50 mg/mL) and $AgBF_4$ (1.25 eq per seleno-ether units) in MeCN was added alkyl iodide (4 eq per seleno-ether unit) and the reaction mixture was stirred under an inert atmosphere in the dark at 50 °C for 24 hr. After this time, AgI was removed by syringe filter and the filtrate was precipitated into diethyl ether. Polymer was dissolved in MeCN, transferred to a dialysis tube (cutoff 1k Da), and then dialyzed against DI water for 1 day. Polymer solution was lyophilized to dryness to give the product.

**6**: $^1$H NMR (500 MHz, DMSO-$D_6$) δ 7.93 (br s, -Se$^+$**Ph**), 7.68 (br m, -Se$^+$**Ph**), 5.66 – 4.84 (br m, $OCH_2\mathbf{CH}CH_2Se^+$), 4.59 – 3.43 (br m, $O\mathbf{CH_2}CH\mathbf{CH_2}Se^+$), 3.05 (br s, -Se$^+$(**$CH_3$**)), 2.33 – 1.77 (br m, $CHOCO\mathbf{CH_2}CH_2CH_2CH_2CH_3$), 1.35 (br s, $CHOCOCH_2\mathbf{CH_2}CH_2CH_2CH_3$), 1.21 (br s, $CHOCOCH_2CH_2\mathbf{CH_2CH_2}CH_3$), 0.83 (br s, $CHOCOCH_2CH_2CH_2CH_2\mathbf{CH_3}$). 2.19 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 379.5.

**7**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 5.33 (br s, $OCH_2\mathbf{CH}CH_2Se^+$), 4.04 (br d, $O\mathbf{CH_2}CHCH_2Se^+$), 3.49 (br s, $OCH_2CH\mathbf{CH_2}Se^+$), 2.76 (br s, -Se$^+$(**$CH_3$**)$_2$), 2.37 ($CHOCO\mathbf{CH_2}CH_2CH_2CH_2CH_3$), 1.57 (br s, $CHOCOCH_2\mathbf{CH_2}CH_2CH_2CH_3$), 1.30 (br s, $CHOCOCH_2CH_2\mathbf{CH_2CH_2}CH_3$), 0.89 (br s, $CHOCOCH_2CH_2CH_2CH_2\mathbf{CH_3}$), 2.19 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 311.8.

**8**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.93 (br s, -Se$^+$**Ph**), 7.69 (br m, -Se$^+$**Ph**), 6.07 (br s, $CH(\mathbf{OH})CH_2Se^+$), 4.41 – 3.46 (br m, $\mathbf{CH_2CH}(OH)\mathbf{CH_2}Se^+\mathbf{CH_2}CH_2CH_2CH_2CH_3$), 1.59 (br s, $Se^+(CH_2\mathbf{CH_2}CH_2CH_2CH_3)$), 1.25 (br s, $Se^+(CH_2CH_2\mathbf{CH_2CH_2}CH_3)$), 0.77 (br s, $Se^+(CH_2CH_2CH_2CH_2\mathbf{CH_3})$), 2.19 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 412.6, 411.5.

**9**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 5.99 (br s, $CH(\mathbf{OH})CH_2Se^+$), 4.18 (br s, $COOCH_2\mathbf{CH}(OH)$), 3.85 (br d, $COO\mathbf{CH_2CH}(OH)$), 3.33 (br m, $CH(OH)\mathbf{CH_2}Se^+(\mathbf{CH_2}CH_2CH_2CH_2CH_3)$), 2.74 (br s, -Se$^+$(**$CH_3$**)), 1.78 (br s, $Se^+(CH_2\mathbf{CH_2}CH_2CH_2CH_3)$), 1.36 (br s, $Se^+(CH_2CH_2\mathbf{CH_2CH_2}CH_3)$), 0.91 (br s, $Se^+(CH_2CH_2CH_2CH_2\mathbf{CH_3})$), 2.19 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 330.5, 329.2.

**10**: $^1$H NMR (500 MHz, DMSO-$d_6$) δ 7.90 (br s, -Se$^+$**Ph**), 7.65 (br s, -Se$^+$**Ph**), 6.07 (br s, $CH(\mathbf{OH})CH_2Se$), 4.39 – 3.44 (br m, $COO\mathbf{CH_2CH}(OH)\mathbf{CH_2}Se$), 3.03 (br s, - Se$^+$(**$CH_3$**)), 2.17 – 0.57 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 382.7, 387.8.

**11**: $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 6.01 (br s, CH(**OH**)$CH_2Se^+$), 4.17 (br s, $COOCH_2$**CH**(OH)), 3.86 (br d, COO**$CH_2$**CH(OH)), 3.34 (br m, CH(OH)**$CH_2$**$Se^+$), 2.74 (br s, -$Se^+$(**$CH_3$**)**$_2$**), 2.19 – 0.56 (br m, backbone). $^{77}$Se NMR (95 MHz, DMSO-$D_6$) δ 302.7.

**Antibacterial assay**

Bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* were grown in Luria-Bertani (LB) medium at 37 °C overnight. *E. coli* cell suspension was diluted with fresh LB medium to an optical density of 0.01 at 600 nm ($OD_{600}$) and suspension of *S. aureus* was diluted with $OD_{600}$ ~ 0.05. Polymers were first dissolved in DMSO at 50 mg/mL and diluted with PBS buffer (pH 7.4) to different concentrations and sterilized by irradiation in a UV light for 15 min before mixing 1:1 with bacterial cell suspensions in a 96 well plate. Samples in all experiments were tested in quadruplicate. All samples and controls were incubated at 37 °C for overnight. The absorbance at 600 nm was measured with a microplate reader. Inhibitory percentages were calculated by the following equation.

$I = \{(A_{sample}-A_{negative})/(A_{positive}-A_{negative})\}$x100 %

($A_i$ = the absorbance of bacteria with polymer samples, $A_{negative}$ = the absorbance of LB medium without bacteria, $A_{positive}$ = the absorbance of bacteria without polymer samples)

**Hemolysis assay**

Hemolytic activity of the polymers was determined using sheep's red blood cells (RBCs). RBCs were pelletized by centrifuging 1 mL of the blood and then washing the RBCs at 4 times with PBS buffer (pH 7.4) solution. The crosslinked micelles were re-dispersed in PBS buffer. Then, 1mL of samples and 25 μL of the RBCs were mixed and incubated at room temperature for 2h. After incubation, the mixtures were re-pelletized by centrifuging and 200 μL of the supernatant of each sample were put into wells in a 96-well plate. The absorbance at 540 nm was measured with a microplate reader. The percent hemolysis was calculated by following equation.

$I = \{(A_i-A_{negative})/(A_{positive}-A_{negative})\}$x100 %

($A_i$ = the absorbance of samples solution, $A_{negative}$ = the absorbance of PBS solution, $A_{positive}$ = the absorbance of DI)

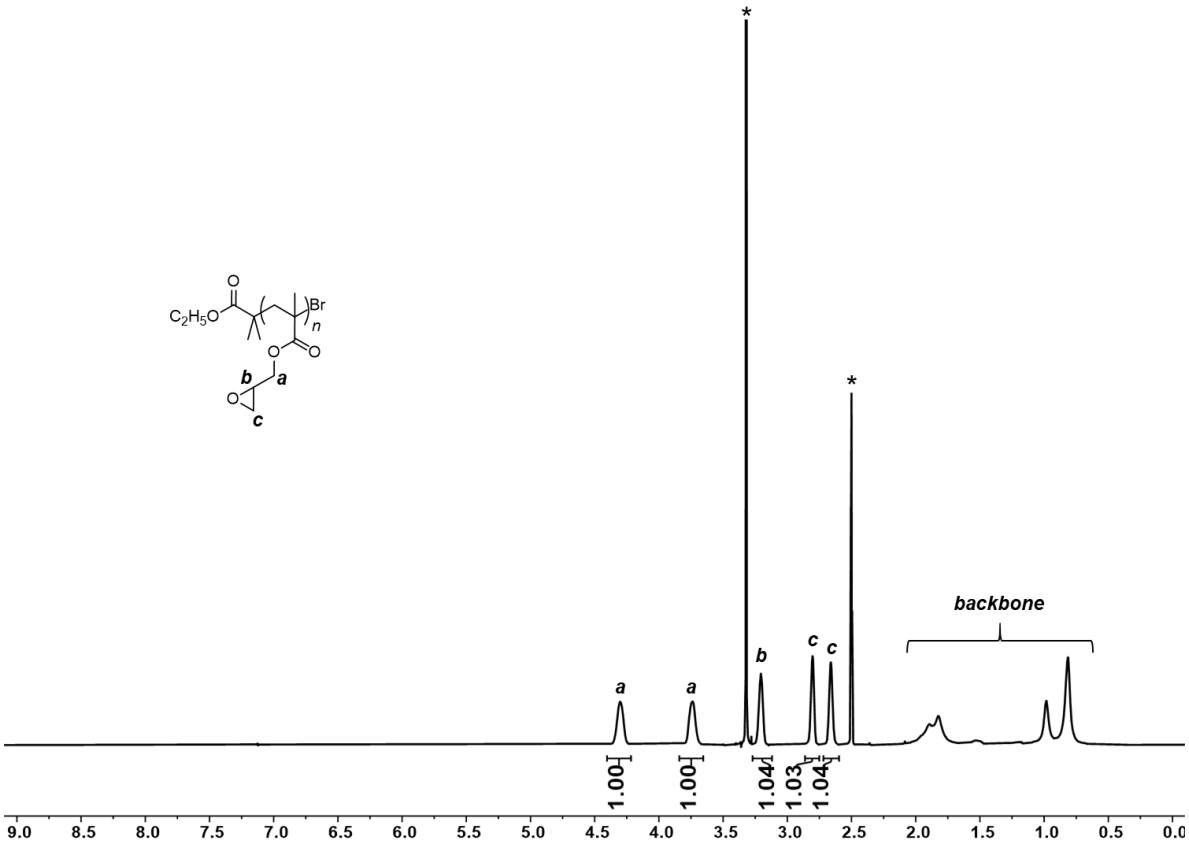


Figure S1. Full range $^{1}$H-NMR spectrum of **1** (DMSO-$d_6$).

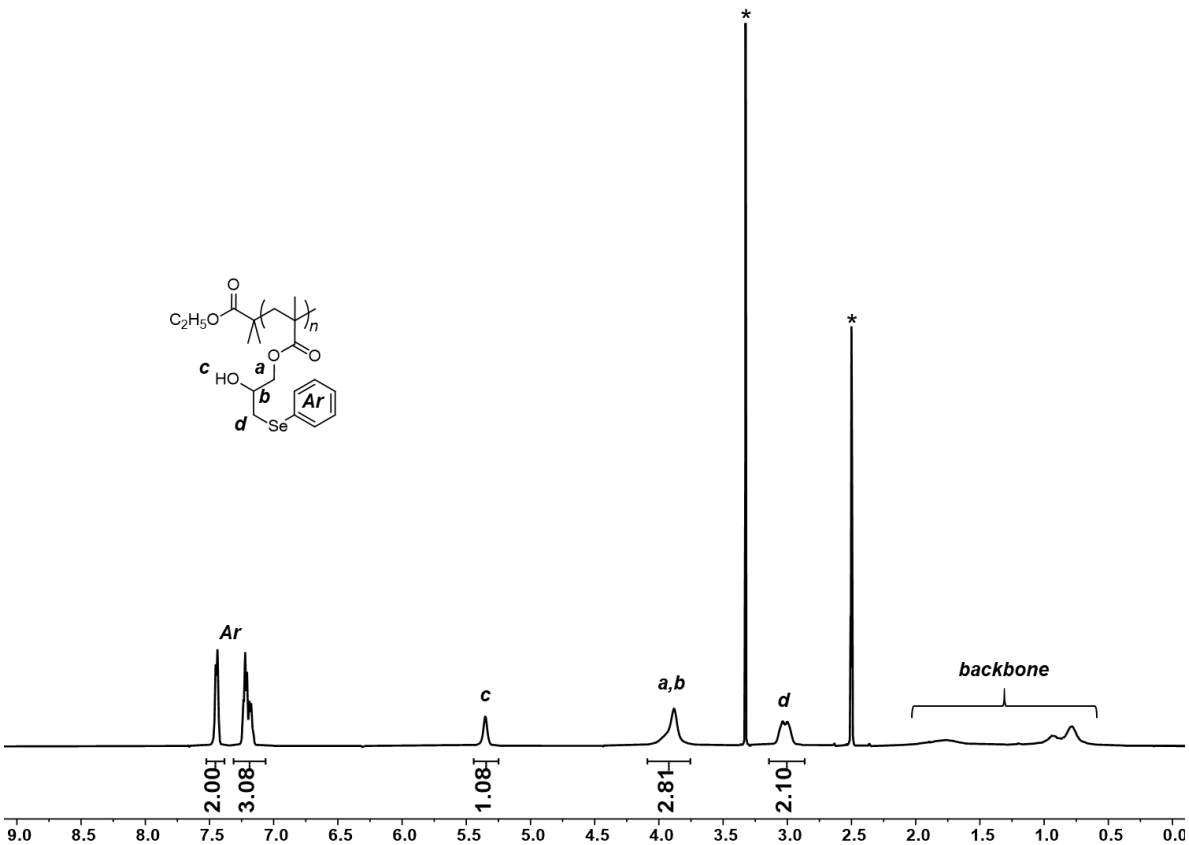


Figure S2. Full range $^{1}$H-NMR spectrum of **2** (DMSO-$d_6$).

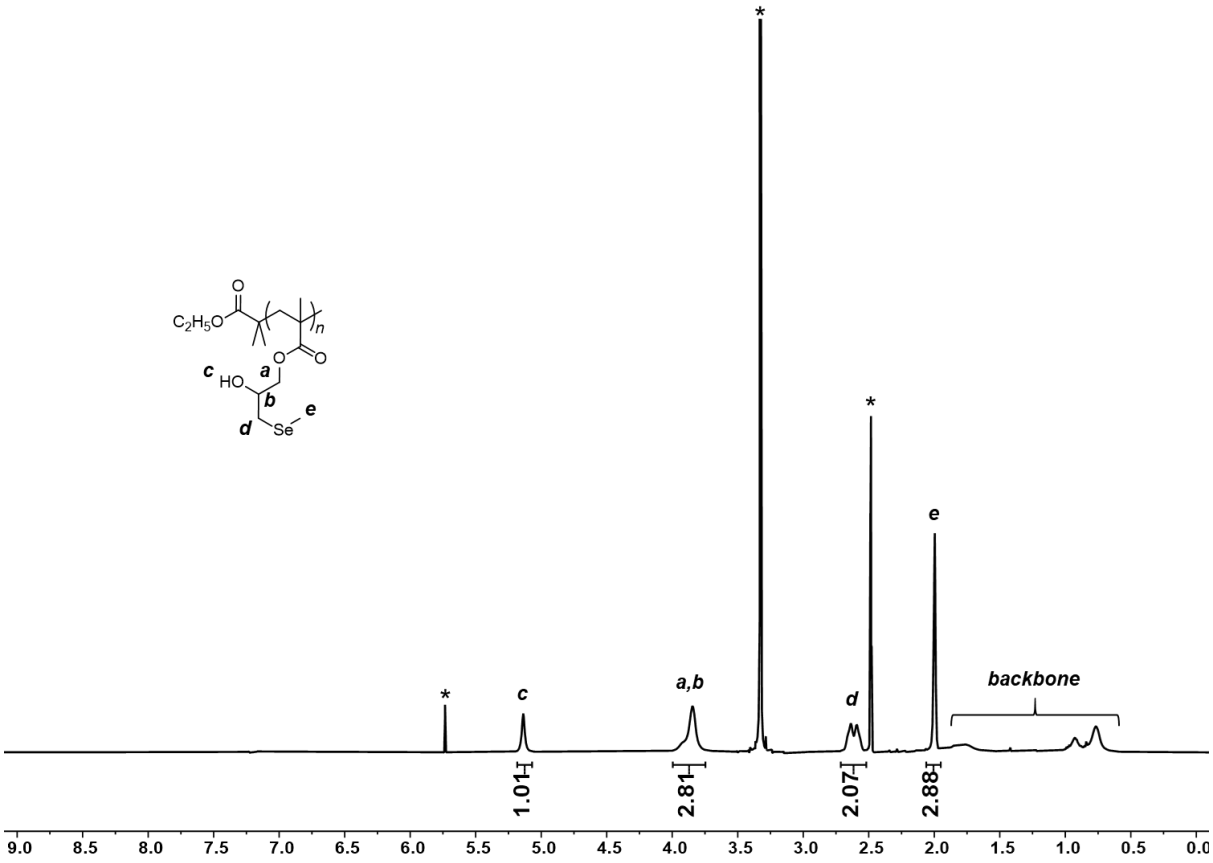


Figure S3. Full range $^{1}$H-NMR spectrum of **3** (DMSO-$d_6$).

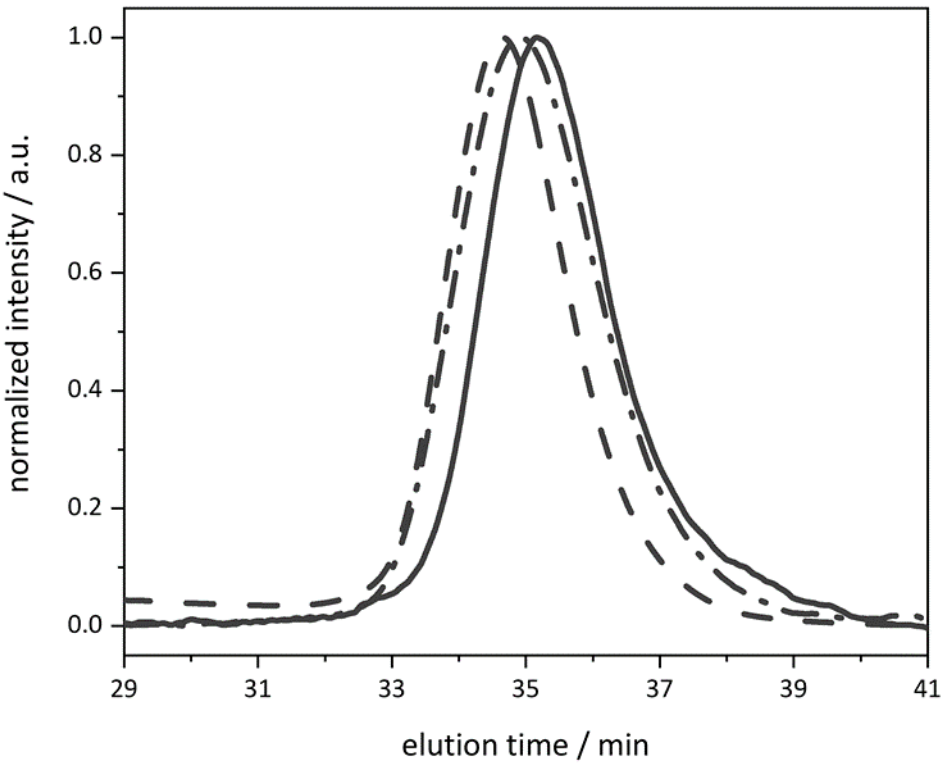


Figure S4. GPC traces for **1** (line), **2** (dash), and **3** (dash dot) (DMF).

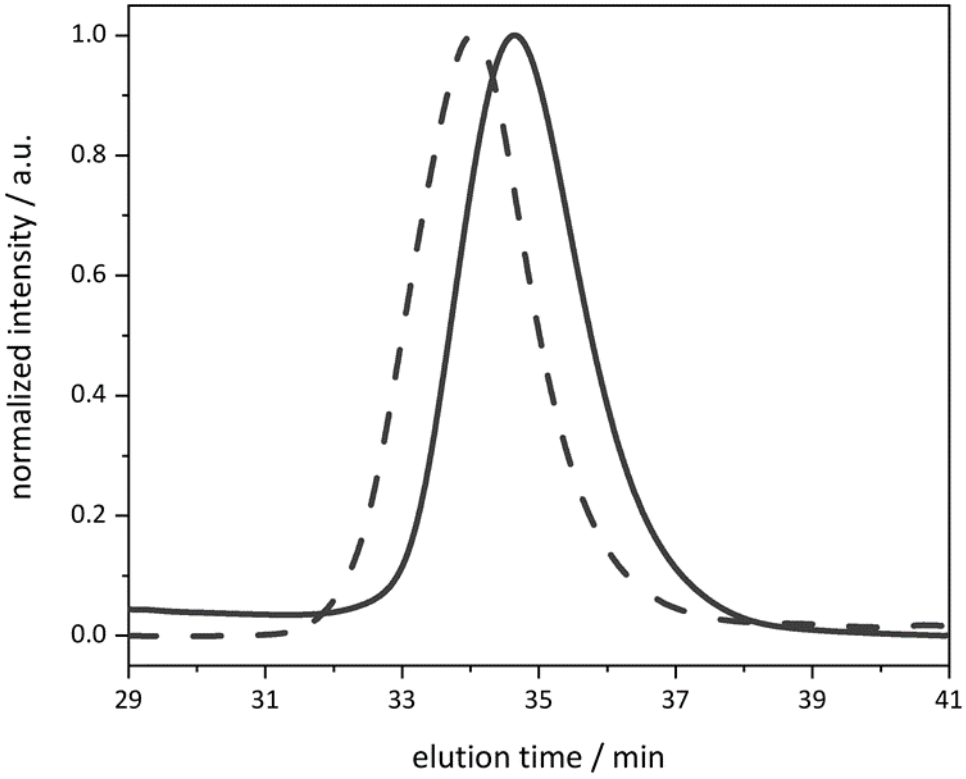


Figure S5. GPC traces for **2** (line) and **4** (dash) (DMF).

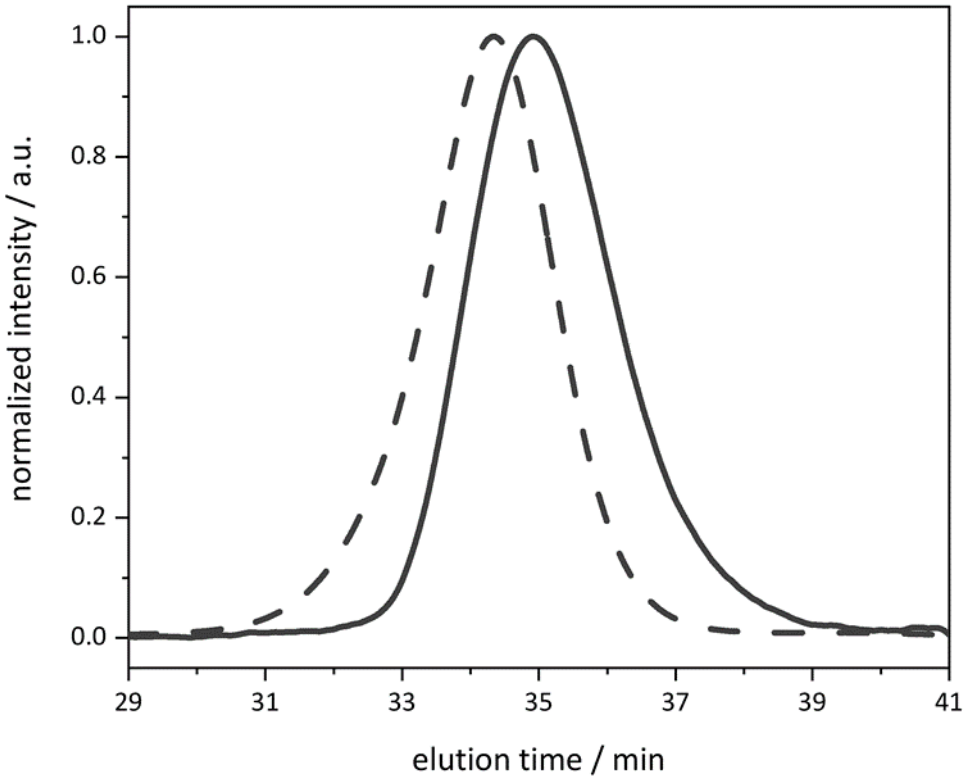


Figure S6. GPC traces for **3** (line) and **5** (dash) (DMF).

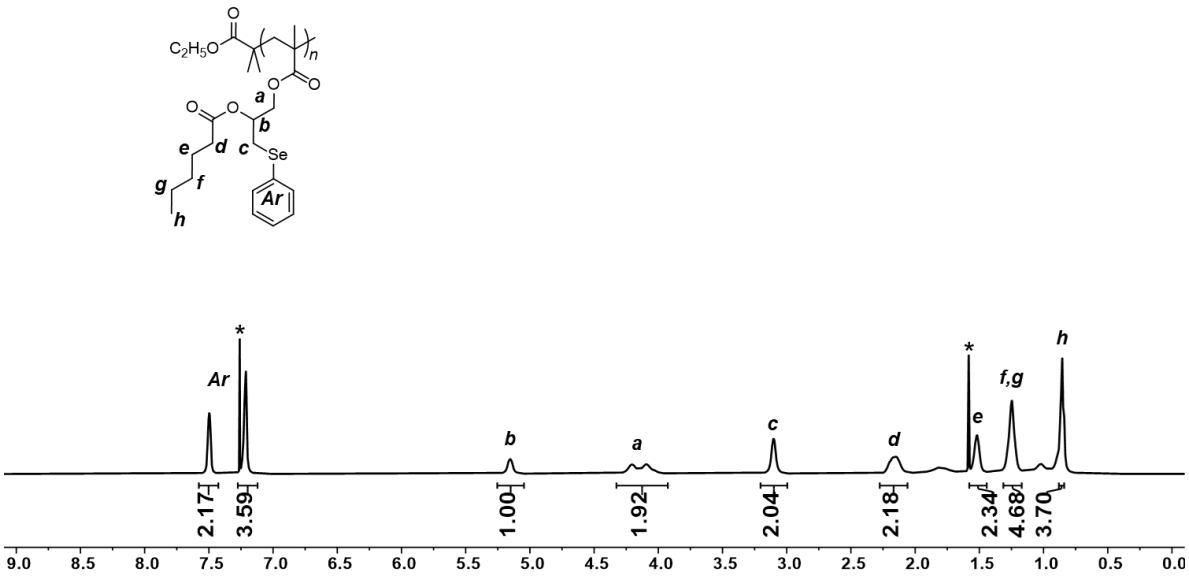


Figure S7. Full range $^{1}$H-NMR spectrum of **4** ($CDCl_3$)

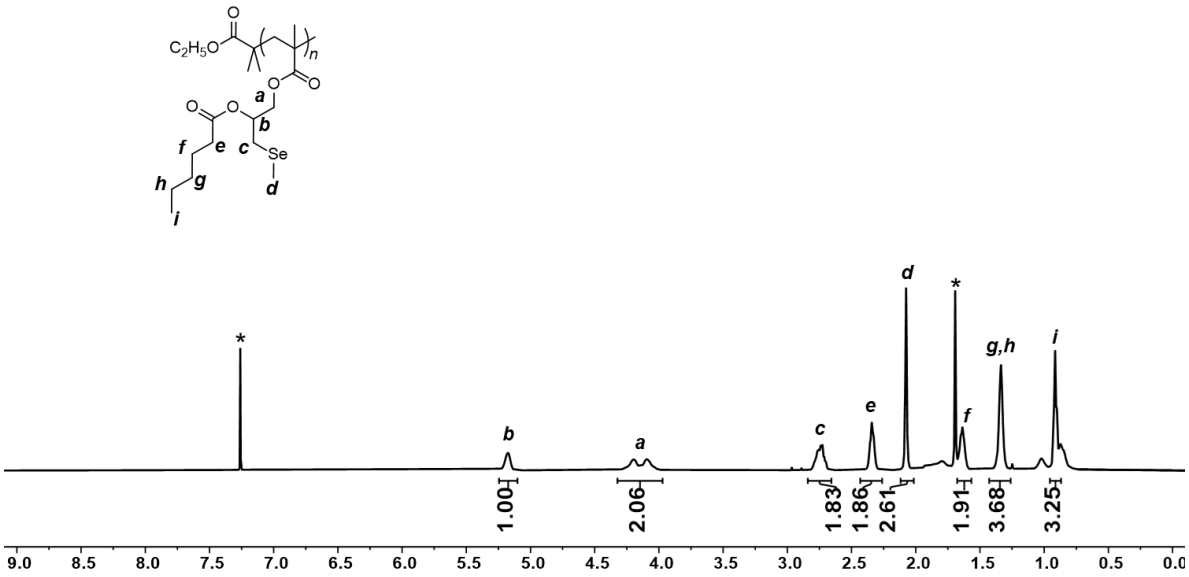


Figure S8. Full range $^{1}$H-NMR spectrum of **5** ($CDCl_3$)

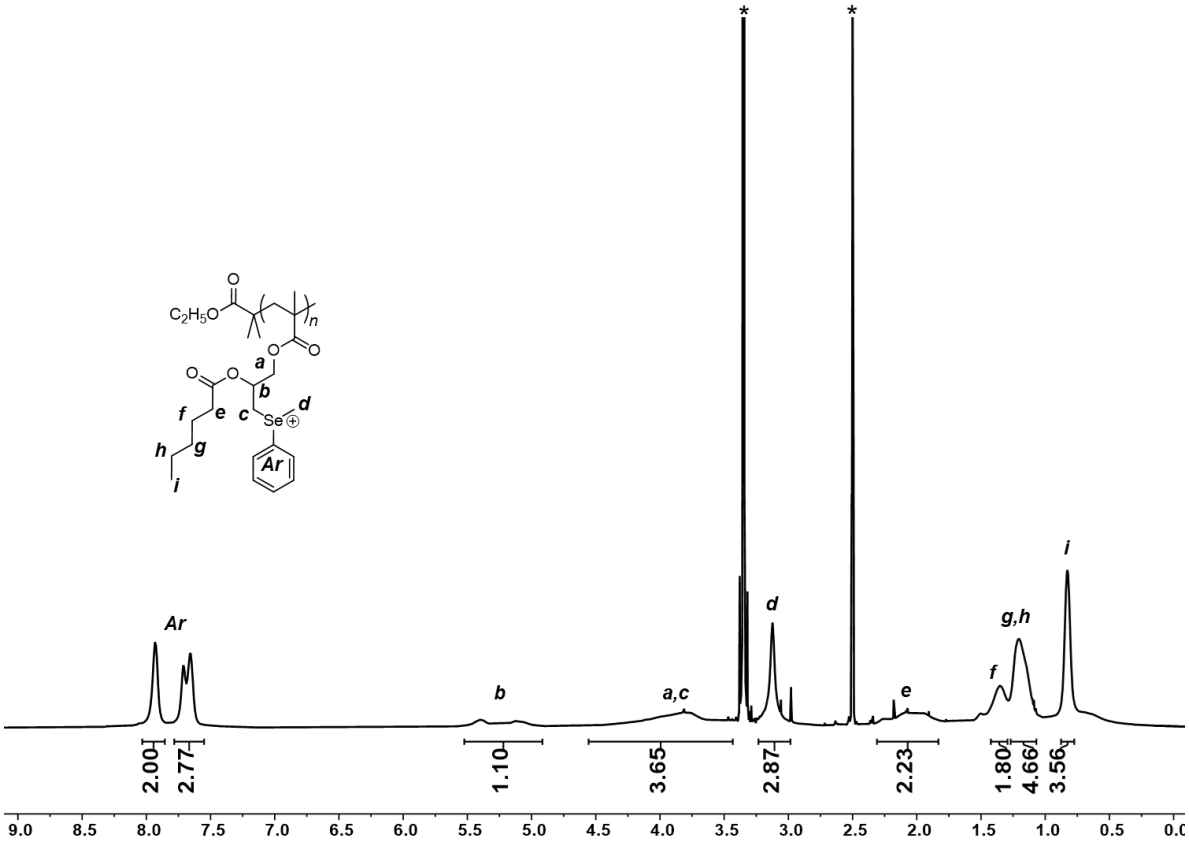


Figure S9. Full range $^{1}$H-NMR spectrum of **6** (DMSO-$d_6$).

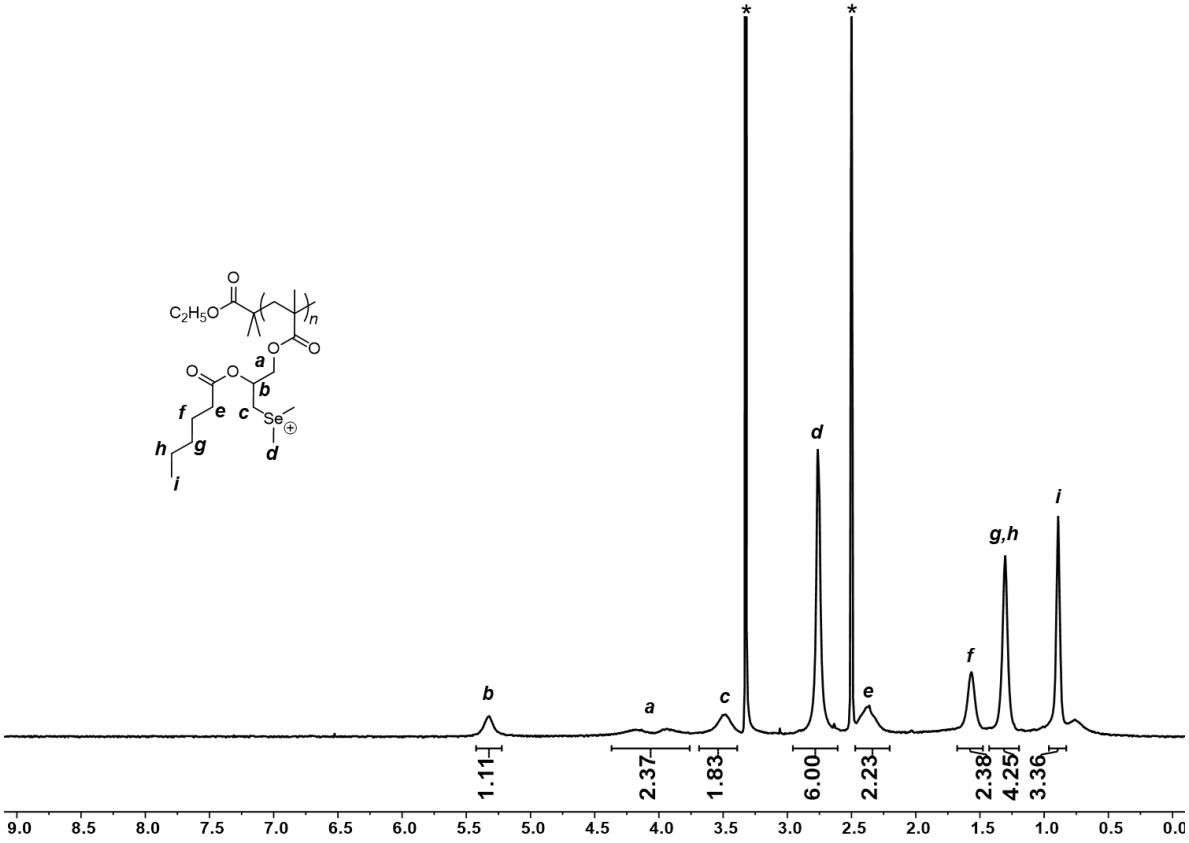


Figure S10. Full range $^{1}$H-NMR spectrum of **7** ((DMSO-$d_6$)

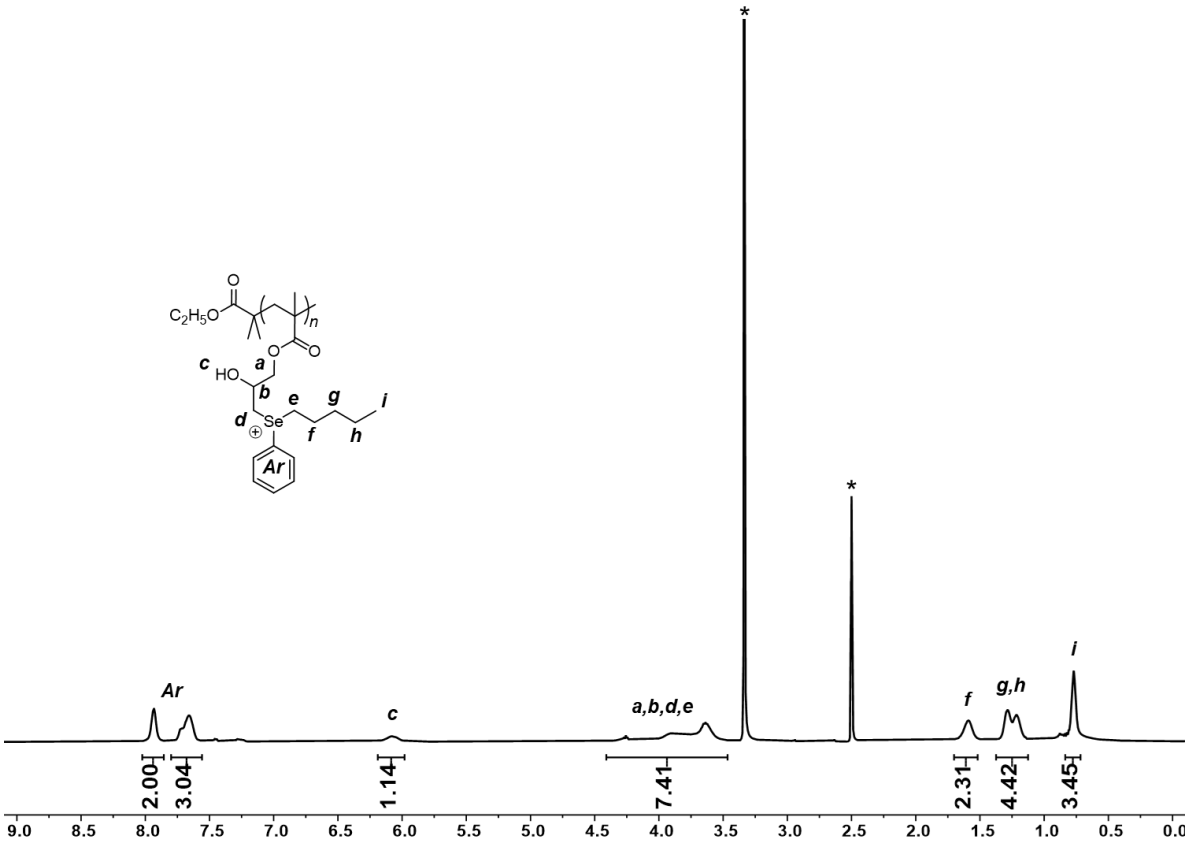


Figure S11. Full range $^{1}$H-NMR spectrum of **8** ((DMSO-$d_6$)

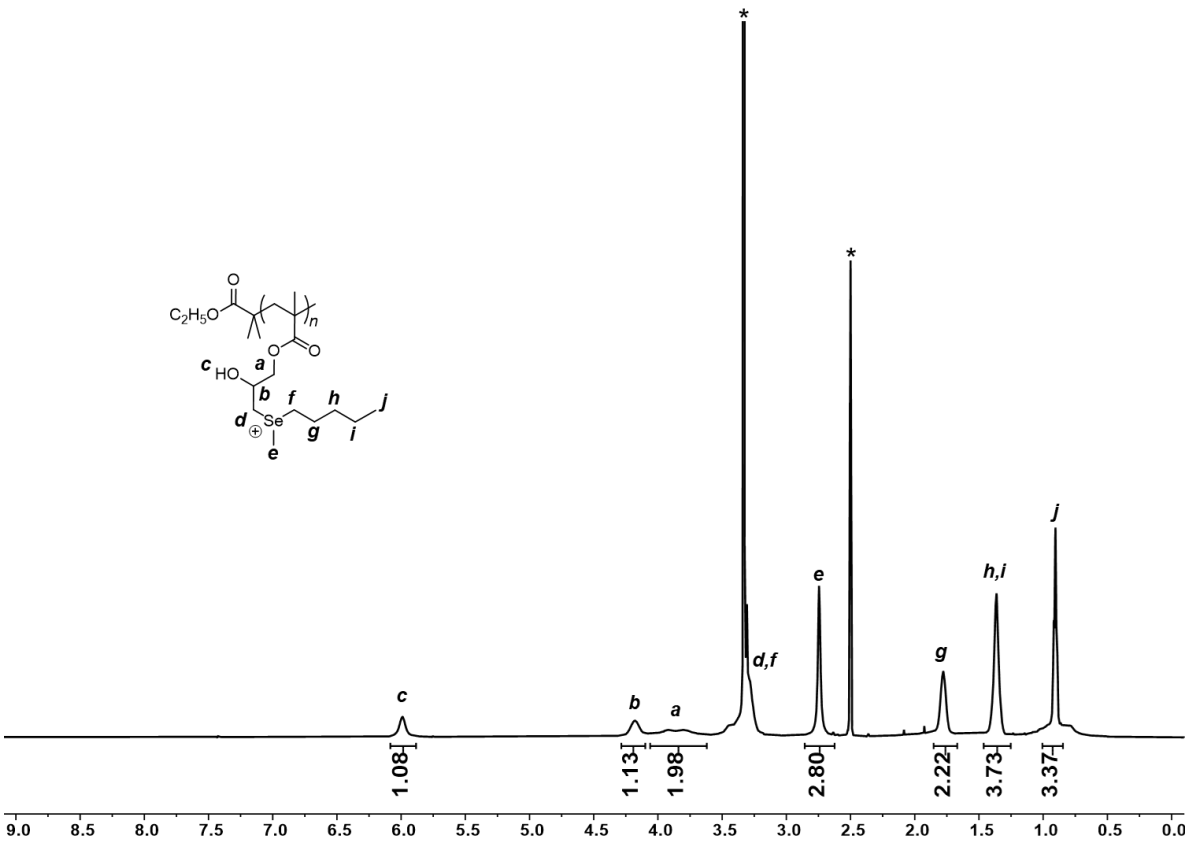


Figure S12. Full range $^{1}$H-NMR spectrum of **9** ((DMSO-$d_6$).

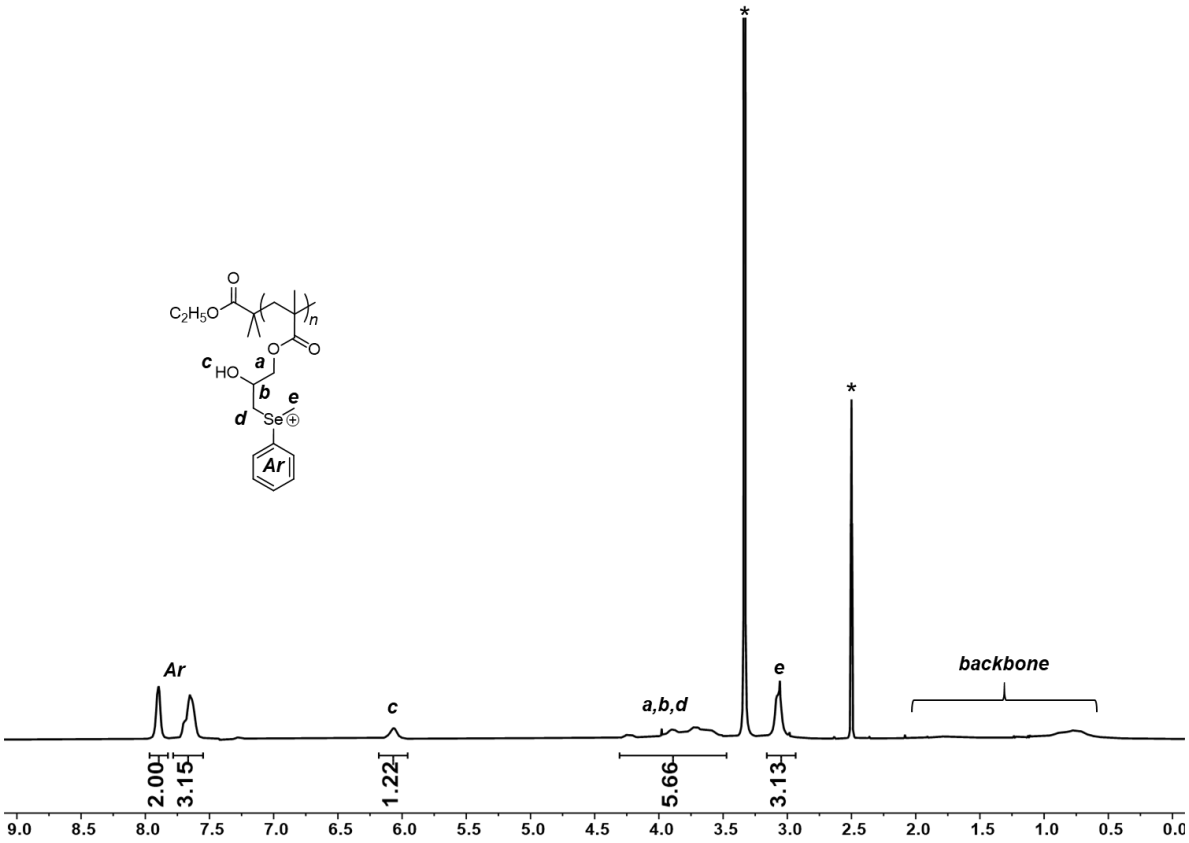


Figure S13. Full range $^{1}$H-NMR spectrum of **10** ((DMSO-$d_6$)

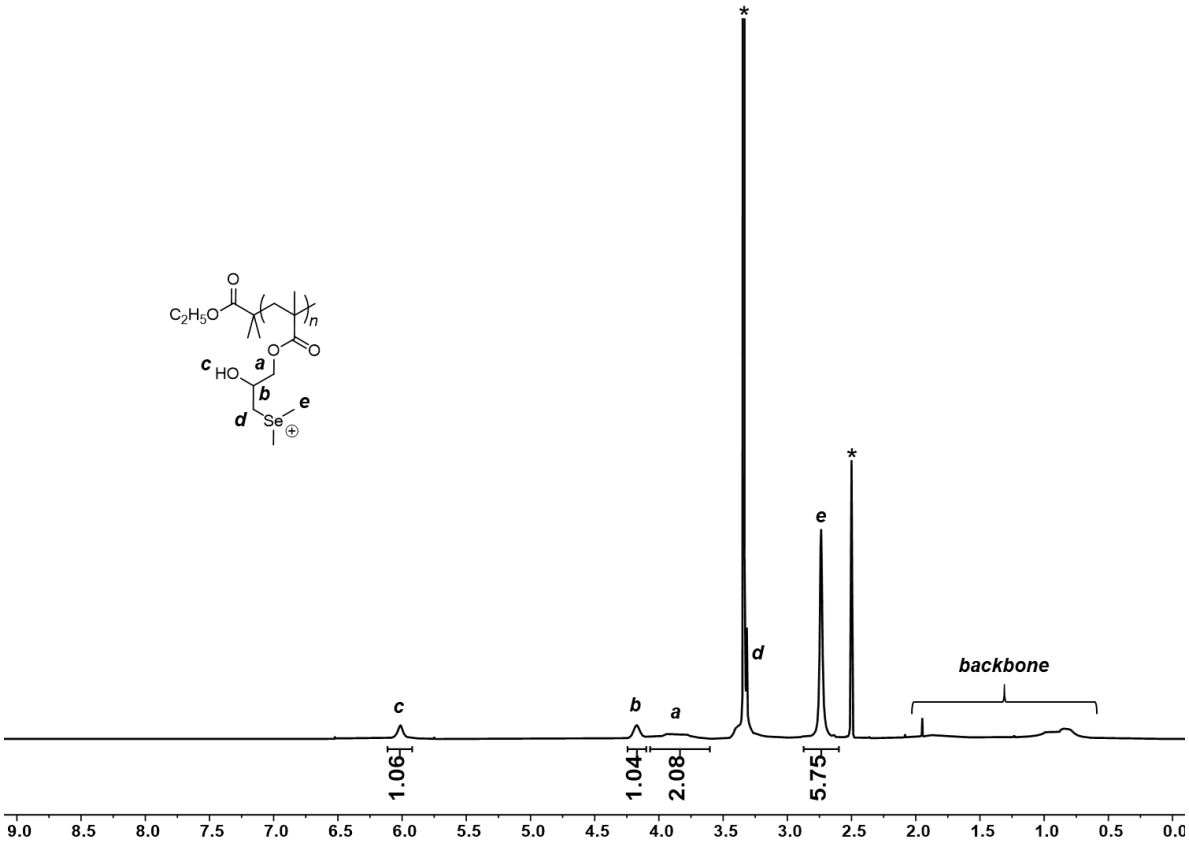


Figure S14. Full range $^{1}$H-NMR spectrum of **11** ((DMSO-$d_6$)

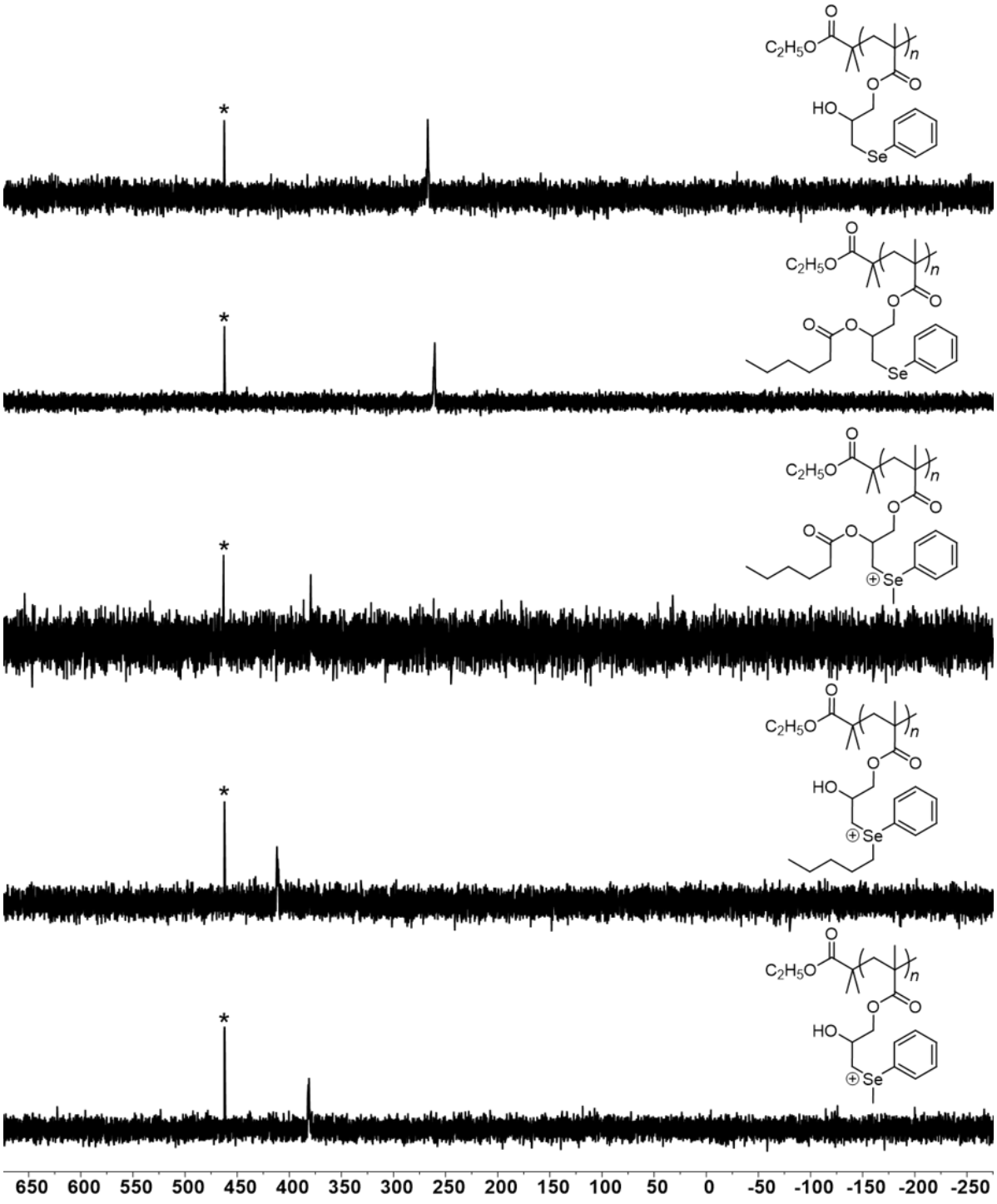


Figure S15. $^{77}$Se-NMR spectra of **2**, **4**, **6**, **8** and **10**. The reference PhSeSePh (463 ppm) is shown with an asterisk.

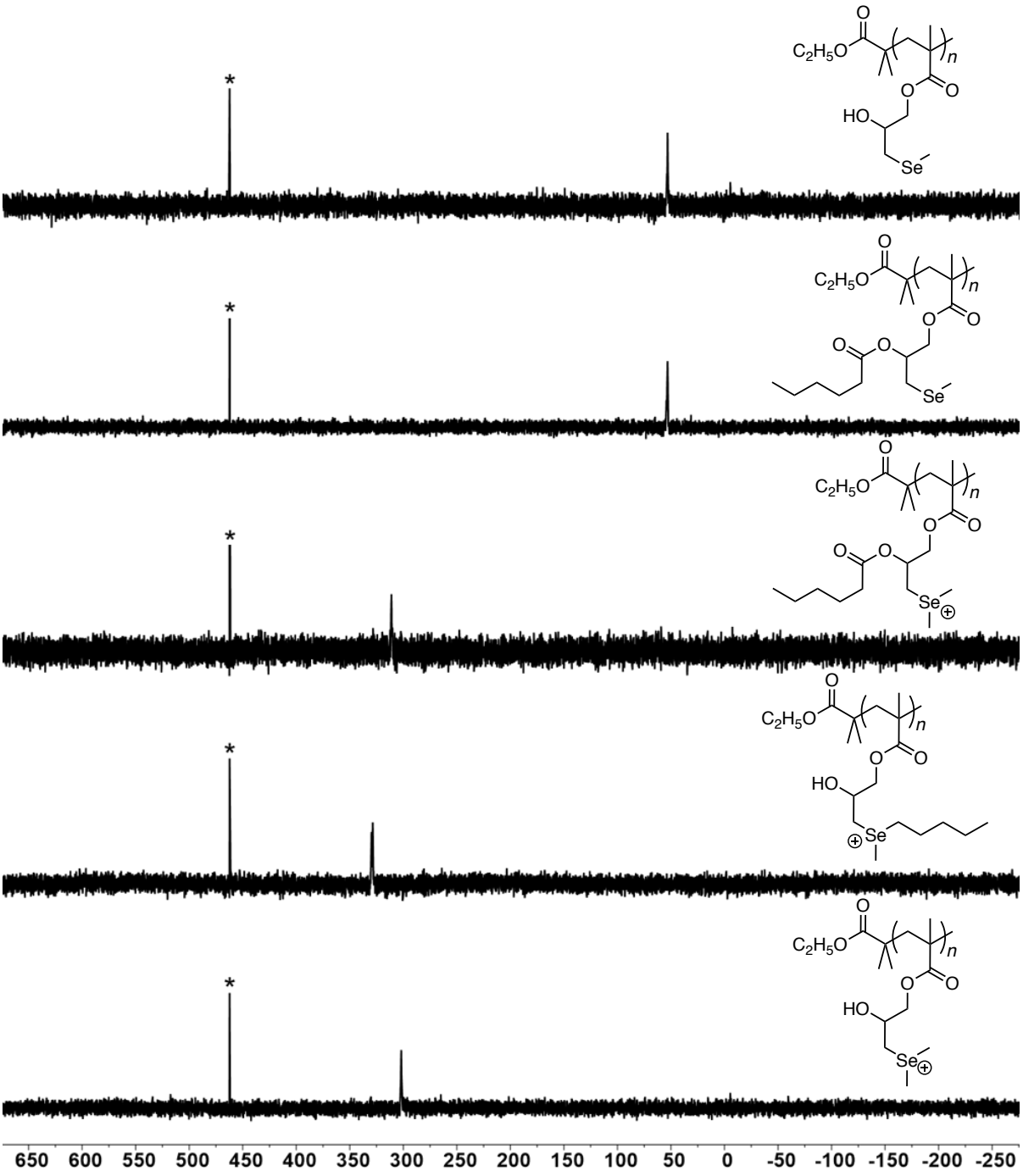


Figure S16. $^{77}$Se-NMR spectra of **3**, **5**, **7**, **9** and **11**. The reference PhSeSePh (463 ppm) is shown with an asterisk.