Electronic Supplementary Information (ESI)

A selenium-contained aggregation-induced "turn-on" fluorescent probe for hydrogen peroxide

Ye-Xin Liao, Kun Li*, Ming-Yu Wu, Tong Wu and Xiao-Qi Yu*

Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, Chengdu, 610064, P.R. China.

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1. General experimental section

1.1 Materials:

Unless otherwise indicated, all reagents, and solvents were obtained from commercial suppliers, and were used without further purification. Column chromatography was performed on silica gel (Qingdao haiyang) 300-400 mesh. All solvents used in test systems was chromatographically pure. Ultrapure water was used throughout.

Stock solutions of probes **D-HMSe** and **HMSe** was prepared by dissolving the corresponding compound in chromatographically pure DMSO. H_2O_2 stock solution was prepared by diluting 30% H_2O_2 solution, and the concentration was determined from absorption at λ=240 nm (ε=43.6 $M^{-1}\cdot cm^{-1}$). ^tBuOOH was prepared by diluting 70% ^tBuOOH solution. ·OH was generated by Fenton reaction between $FeSO_4$ and H_2O_2, and concentration of ·OH was determined by H_2O_2. $^tBuOO\cdot$ was generated by reaction between $FeSO_4$ and ^tBuOOH, and concentration of $^tBuOO\cdot$ was determined by ^tBuOOH. $^1O_2$ was generated by the reaction of H_2O_2 with NaClO[1]. $O_2^{-}\cdot$ was generated from KO_2 solid diluted in DMSO. $ONOO^-$was generated by the reaction of H_2O_2 and $NaNO_2$[2] and stocked at -20 °C, the concentration was determined from absorption at λ=302 nm (ε=1670 $M^{-1}\cdot cm^{-1}$) in 0.1 M NaOH solution[3].ClO^- was prepared by diluting 5% NaClO aqueous solution, and the concentration was determined from absorption at λ=292 nm (ε=350 $M^{-1}\cdot cm^{-1}$)[3]. The fluorescence quantum yields in solution were calculated using a quinine bisulfate as a standard material ($5x10^{-6}$ M, 0.05 M H_2SO_4, Φ_s=0.55).

1.2 Instruments

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 with chemical shifts expressed in parts per million (in deuteriochloroform or DMSO-d6, Me_4Si as internal standard). Fluorescence spectra and fluorescence quantum yields in solid state were determined using a FluoroMax-4 Spectrofluoro photometer (HORIBA Jobin Yvon). UV/Vis absorption spectra were determined by a Hitachi PharmaSpec UV-1900 UV-Vis spectrophotometer. Mass spectral data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer . High performance liquid chromatography (HPLC) were perform on a Waters e2695 Separatins Module using Waters 2998 PDA detector equipped with an Symmetry C18 column(4.6 X 150mm, 5μm), CH_3CN (0.3%TFA) were used as eluents with a flow rate of 1ml/min. 280nm was used as wavelength. The measurements of dynamic light scattering (DLS) were carried out at 25 °C using a Zetasizer Nano-ZS 3690 system from Malvern Instruments equipped with a 633 nm He−Ne laser using backscattering detection with a fixed detector angle of 90°. TLC analyses were performed on silica gel GF 254. pH values were determined by a Leici pHS-25 (digital display) pH meter.

2. Synthetic procedures

Compound 1a[4], 3[5] and 4[6] was prepared according to the early literature. And compound 4 was used without further purification to next step after prepared.

Compound 1b

A mixture of compound 1a (1.50 g, 6.27 mmol), n-bromododecane (1.72 g, 6.89 mmol) and 33% (w/w) aqueous NaOH (1 g) in DMF (10 mL) was stirred at 60 °C for 3.5 h. Then solvent was removed in vacuo, and the residue was diluted with water and extracted with ethyl acetate (3 times). The organic layer was collected and washed water, then dried over anhydrous Na_2SO_4, and

concentrated in vacuo. The residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and giving a yellow liquid as product (1.58 g, yield 61.8%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, 1 H, *J* = 8.40 Hz), 7.79 (t, 2 H, *J* = 7.40 Hz), 7.73 (t, 1 H, *J* = 7.72 Hz), 7.65 (d, 1 H, *J* =7.40 Hz), 7.44 (d, 1 H, *J* = 7.76 Hz), 7.36-7.29 (m, 2 H), 3.99 (t, 2 H, *J* = 7.52 Hz), 1.73 (t, 2 H, *J* = 6.82 Hz), 1.29-1.17 (m, 18 H), 0.88 (t, 3 H, *J* = 13.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.3, 148.8, 143.2, 135.0, 133.3, 132.8, 131.0, 126.5, 124.9, 123.1, 122.4, 120.2, 110.1, 44.7, 31.9, 29.6, 29.5, 29.4, 29.30, 29.0, 26.7, 22.7, 14.1. HRMS (ESI) calcd for $C_{25}H_{34}N_3O_2^+$ $[M+H]^+$, m/z 408.2646 ; found, m/z 408.2650.

General synthetic process of compound 2

A mixture of compound 1 (4.9 mmol) and 20% (w/w) Pd/C (Pd, 10 wt% on carbon powder) in 30 mL CH_3OH was stirred vigorously under a hydrogen atmosphere at room temperature over night. After the reaction completed, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo to afford crude product. 2a was used to next step without further purification. 2b was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and giving a white solid as product (988 mg, yield 96%).^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68-7.65 (m, 1 H), 7.61 (d, 1 H, *J* = 7.40 Hz), 7.29-7.18 (m, 4 H), 6.85 (d, 1 H, *J* = 8.00 Hz), 6.70-6.66 (m, 1 H), 5.61 (s, 2 H), 4.20 (t, 2 H, *J* = 7.24 Hz), 1.63-160 (m, 2 H), 1.29-1.07 (m, 18 H), 0.87-0.84 (t, 3 H, *J* = 6.86 Hz).^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 152.6, 148.1, 143.1, 135.4, 130.8, 130.4, 122.5, 122.1, 119.2, 116.1, 116.0, 113.7, 111.2, 44.2, 31.6, 29.4, 29.3, 29.2, 29.1, 28.7, 22.6, 14.4. HRMS (ESI) calcd for $C_{25}H_{36}N_3^+$ $[M+H]^+$, m/z 378.2904 ; found, m/z 378.2907.

General synthetic process of compound 5

A mixture of compound 2 (1.33 mmol), triethylamine (2.00 mmol) and dry DCM (10 mL) in a 50 mL round-bottom flask was stirred in an ice/NaCl bath for 5 min, and a solution of compound 4 (2.00 mmol) prepared before in 10 mL dry DCM was added in 30 min. Then the reaction was kept in -15 °C bath over night. When the reaction completed, solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent, and gave the product.

Compound 5a:yellow solid (84.5 mg, yield 16.2%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 12.48 (s, 1H), 7.97-7.95 (d, 1 H, *J*= 8,00 Hz), 7.89-7.87 (d, 1 H, *J* = 7.52 Hz), 7.82-7.80 (dd, 1 H, *J* = 7.72 Hz, *J*= 0.82 Hz), 7.69-7.58 (m, 3 H), 7.56-7.52 (m, 1 H), 7.46-7.41 (m, 3 H), 7.16-7.08 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 166.8, 149.9, 144.2, 140.8, 138.1, 135.2, 132.4, 131.4, 130.7, 130.1, 129.5, 128.4, 128.1, 128.0, 126.3, 126.2, 122.8, 121.8, 119.4, 112.0. HRMS (ESI) calcd for $C_{20}H_{14}N_3OSe^+$ $[M+H]^+$, m/z 392.0297 ; found, m/z 392.0300.

Compound 5b: white solid (513.3 mg, yield 69.1%).^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.89-7.87 (dd, 1 H, *J* = 0.84 Hz, *J* = 7.76 Hz), 7.84 (d, 1H, *J*= 8.00 Hz), 7.70-7.63 (m, 4 H), 7.57-7.52 (m, 3 H), 7.43-7.39 (m, 1 H), 7.24-7.18 (m, 2 H), 3.91 (t, 2 H, *J* = 7.26 Hz), 1.45-1.43 (m, 2 H), 1.29-0.97 (m, 18 H), 0.85 (t, 3 H, *J* = 6.88 Hz).^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ 166.7, 151.1, 140.8, 138.9, 135.4, 132.5, 131.1, 129.5, 128.5, 127.5, 126.4, 122.8, 119.9, 111.5, 44.0, 31.8, 29.4, 29.3, 29.2, 28.7, 22.6, 14.4. HRMS (ESI) calcd for $C_{32}H_{38}N_3OSe^+$ $[M+H]^+$, m/z 560.2175; found, m/z 560.2180.

Compound D-HMSeO

20 mL HEPES buffer (20 mM, pH 7.40) was added to a methanol (5 mL) solution of compound D-HMSe (300 mg, 0.54 mmol), white solid appeared. 400 μL 30% H_2O_2 aqueous solution was then added. The reaction was stirred at ambient temperature for 2 h. Then reaction was filtered, and residue was collected and further purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluent. A white solid was obtained (233 mg, yield: 75%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (t, 2 H, J = 9.10 Hz), 7.87 (d, 1 H, J = 6.64 Hz), 7.81-7.73 (m, 3 H), 7.63-7.59 (m, 2 H), 7.50-7.43 (m, 4 H), 4.27 (m, 2 H), 1.73-1.71 (m, 2 H), 1.27-1.04 (m, 18 H), 0.85 (t, 3 H, J = 6.76 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3, 150.3, 148.2, 142.6, 136.6, 135.2, 134.9, 132.8, 131.5, 131.4, 131.3, 130.5, 129.3, 129.0, 128.4, 127.8, 123.0, 122.4, 119.3, 111.5, 44.5, 31.8, 29.4, 29.3, 29.2, 28.8, 28.6, 26.5, 22.6, 14.4. HRMS (ESI) calcd for $C_{32}H_{38}N_3O_2Se^+$ $[M+H]^+$, m/z 576.2124; found, m/z 576.2126.

3. UV-visible Absorbance Measurements

UV spectra of **D-HMSe** and **HMSe** were recorded at the concentration of 20 μM in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40.

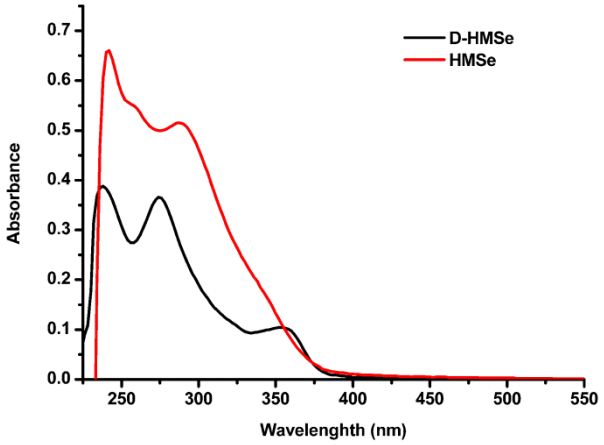

Figure S1 UV/Vis absorption spectra of **D-HMSe** (20 μM) and **HMSe** (20 μM) in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40.

4. Effect of different solvents

Different solvents including water, THF, CH_3CN, CH_3OH, DMF, and DMSO were used as the test solvents and fluorescence emission spectra were recorded. It exhibited significant fluorescence emission in water and shown very weak fluorescence emission in other solvents.

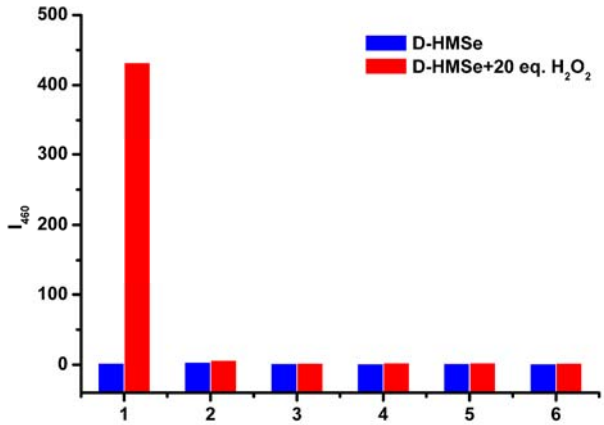

Figure S2 Effect of different solvents to relative fluorescence intensity of **D-HMSe** (10 μM) before (blue bar) and

after treated with H_2O_2 (200 μM) at ambient temperature for 1 h at 460 nm. The testing solvents include: 1. H_2O, 2. DMSO, 3.CH_3OH, 4. CH_3CN, 5. THF, 6. DMF. All of the solvents include 1% DMSO.

5. Fluorescence spectra of D-HMSe and D-HMSeO in solid state

Fluorescence emission spectra of **D-HMSe** and **D-HMSe** and **D-HMSeO** in solid state was recorded. **D-HMSeO** exhibits a strong fluorescence emission at 494 nm and **D-HMSe** shows nearly no fluorescence.

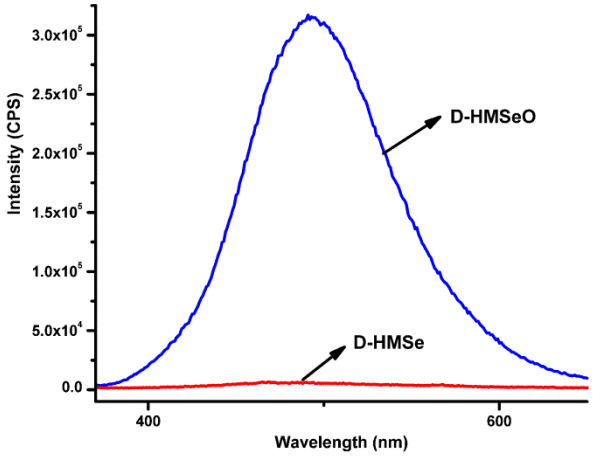

Figure S3 Fluorescence spectra of **D-HMSe** and **D-HMSeO** in solid state. λ_{ex}=347 nm, slit: 5/5 nm.

6. Effect of different ratio of DMSO and H_2O

Different ratios of HEPES buffer and DMSO (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) were prepared as the test solvents. **D-HMSe** (10 μM) and H_2O_2 (200 μM) were added and kept at ambient temperature for 1 h. Then emission spectra were recorded (Figure S4).

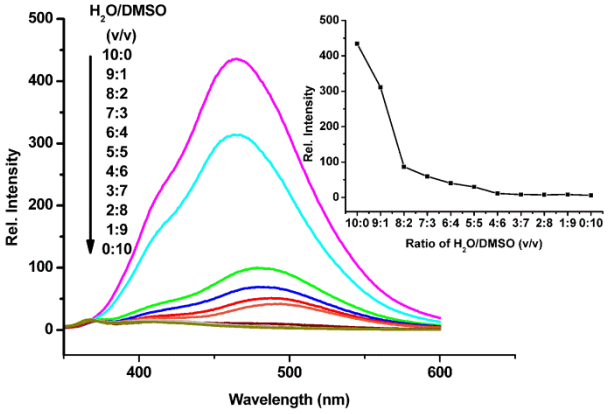

Figure S4 Fluorescence responds of the reaction between **D-HMSe** (10 μM) and H_2O_2 (200 μM) in different ratios of DMSO and H_2O as solvent at ambient temperature for 1 h.

7. HRMS of the reaction product between D-HMSe and H_2O_2

Oxidative product **D-HMSeO** was prepared from the reaction between **D-HMSe** and H_2O_2 at ambient temperature over night and further purified by column chromatography on silica gel. ESI-MS result shown that a major peak at m/z=576.2126 was found, which calcd for $C_{32}H_{38}N_3O_2Se^+$ $[(M+H)^+]$ m/z=576.2124.

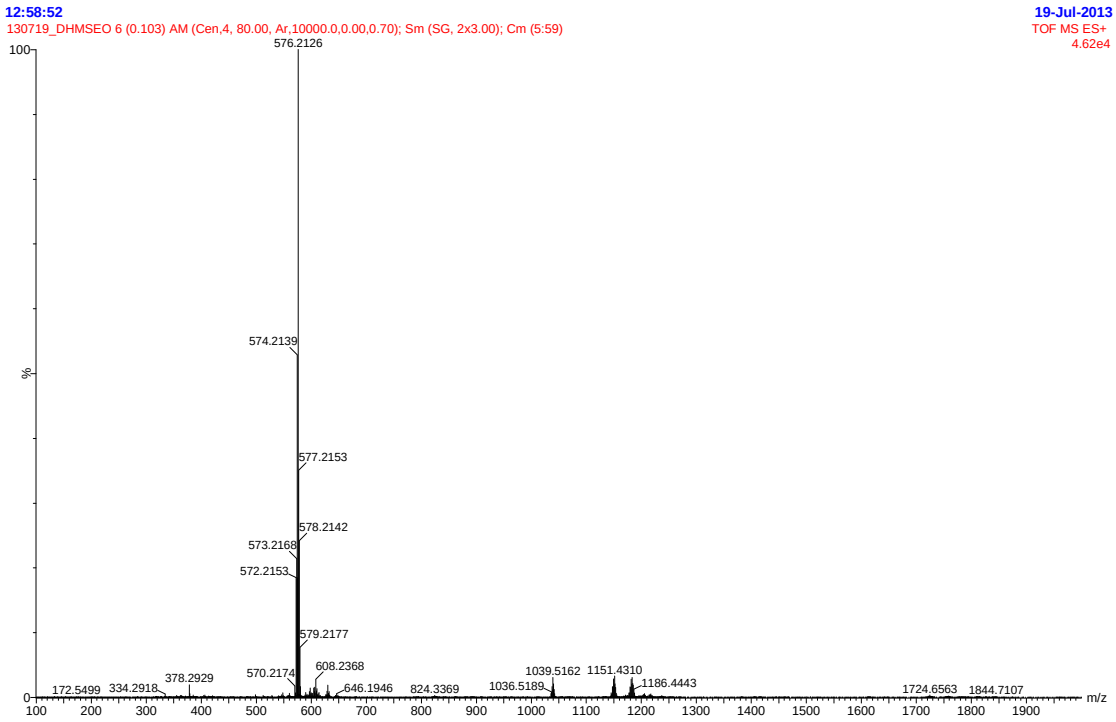

Figure S5 HRMS of the reaction product between **D-HMSe** and H_2O_2.

8. Dynamic light scattering (DLS) result

DLS was performed in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 at ambient temperature. After the addition of H_2O_2 to **D-HMSe**, the solution was kept at ambient temperature for 1 h.

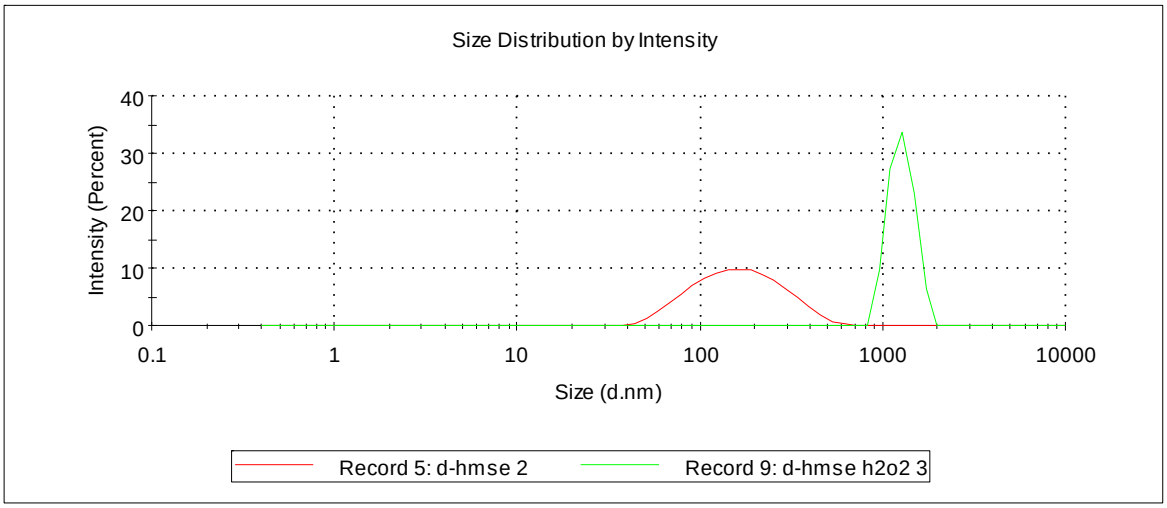

Figure S6 Dynamic light scattering result of **D-HMSe** (10 μM) before and after treated with H_2O_2 (200 μM) in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 at ambient temperature for 1 h.

9. Effect of pH value

pH solutions were prepared by using 50% NaOH solution to adjust the pH values of HEPES buffer (20 mM). The solutions of **D-HMSeO**, **D-HMSe** before and after treated with H_2O_2 in different pH solutions were prepared and kept at ambient temperature for 1 h, and spectra were

recorded then.

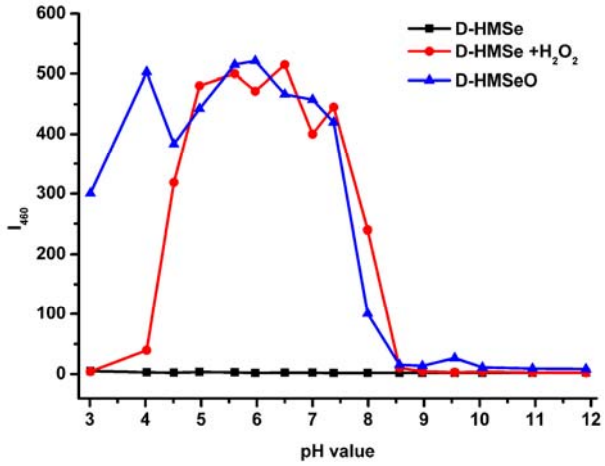

Figure S7 Effect of pH on the relative fluorescence intensity for 10 μM **D-HMSe** before (●), after (■) treated with 200 μM H_2O_2 and 10 μM **D-HMSeO** (▲) in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 for 1 h at ambient temperature. (The investigated pH values include 3.01, 4.02, 4.51, 4.97, 5.60, 5.97, 6.50, 7.00, 7.38, 7.99, 8.56, 8.97, 9.55, 10.05, 10.95 and 11.91).

10. Time-dependent UV spectra of D-HMSe reacted with H_2O_2

UV spectra of the process of **D-HMSe** reacting with H_2O_2 were recorded. The result is shown in Figure S8. With time going on, the three maximum absorption peaks changed gradually.

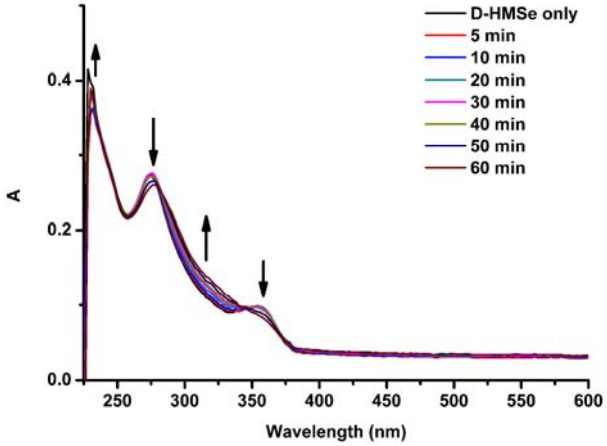

Figure S8 Time-dependent UV spectra of **D-HMSe** (20 μM) reacted with H_2O_2 (400 μM) in 60 min at ambient temperature in water (1% DMSO)

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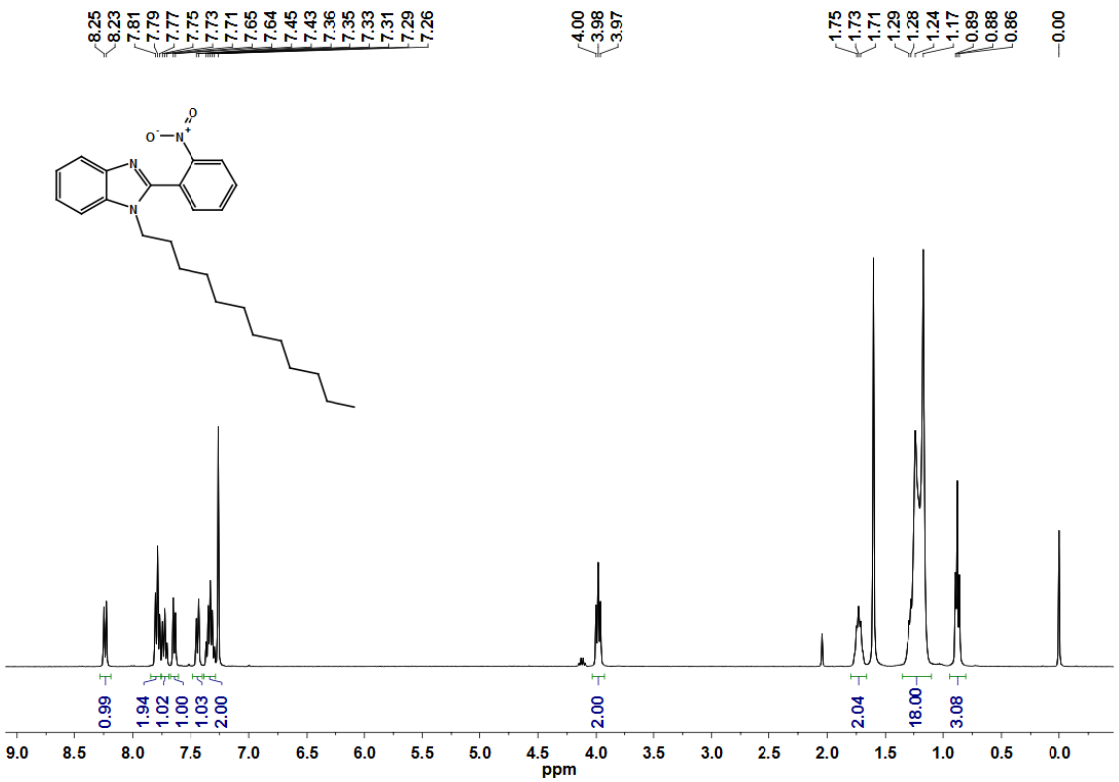

Figure S9 ^{1}H NMR spectra of compound 1 in $CDCl_3$.

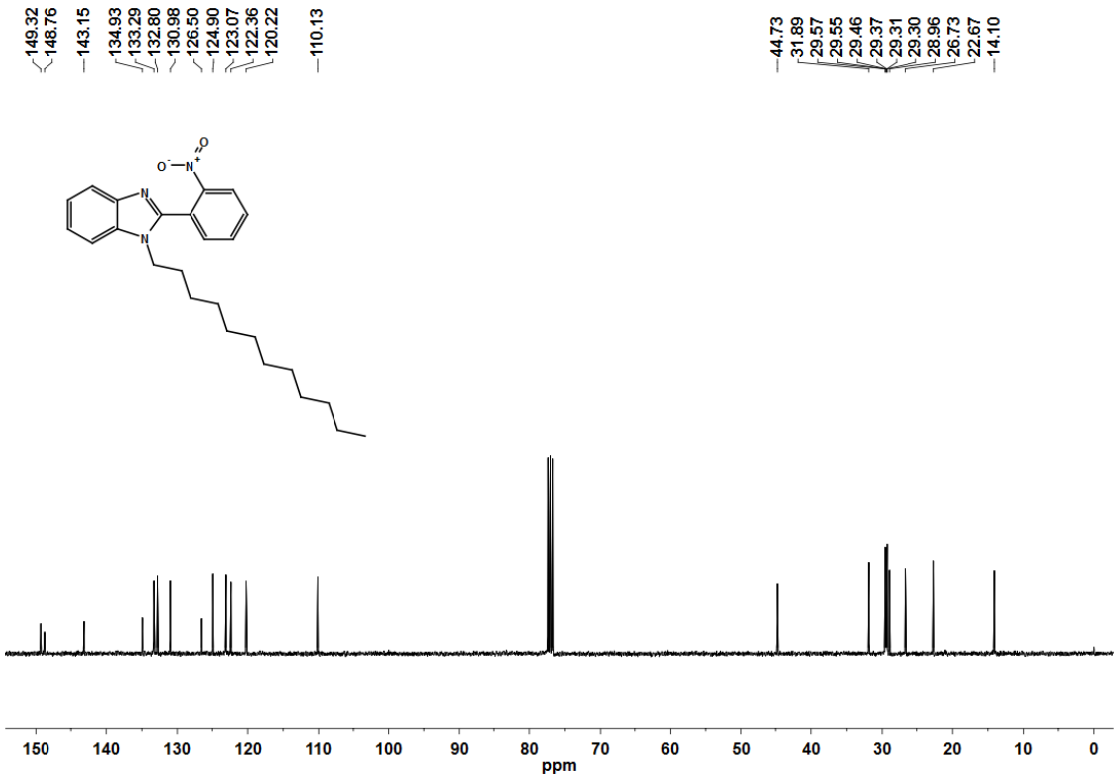

Figure S10 ^{13}CNMR spectra of compound 1 in $CDCl_3$.

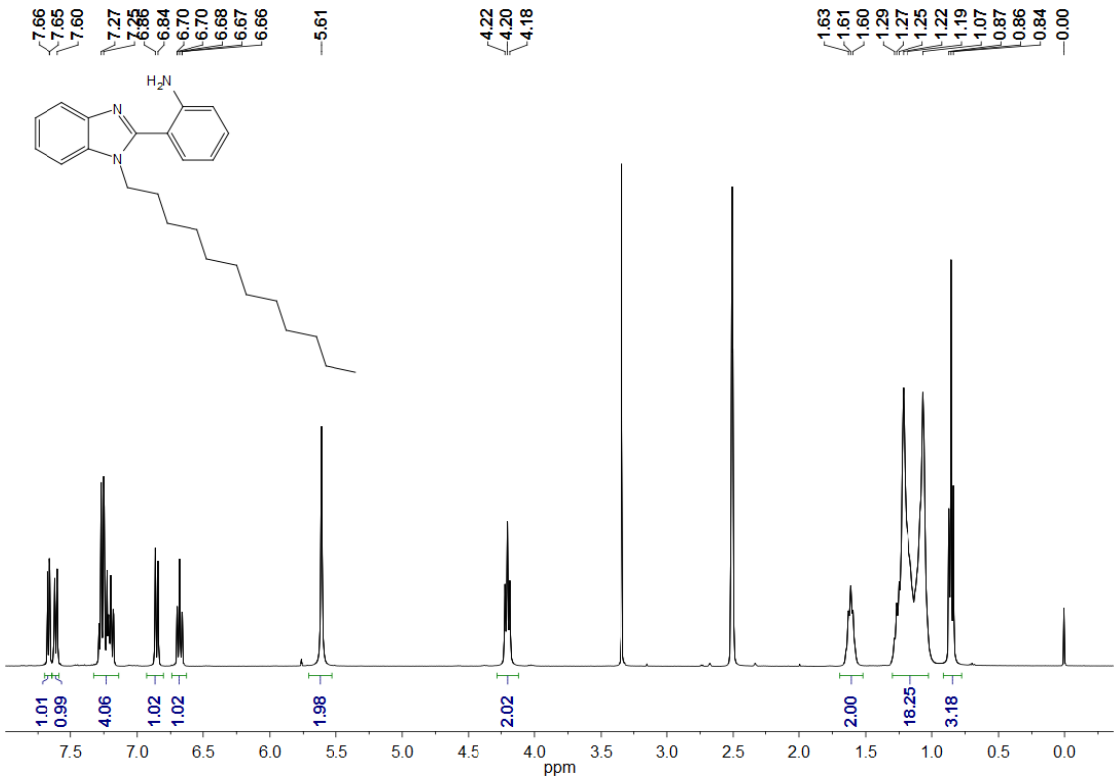

Figure S11 ^{1}H NMR spectra of compound 2b in DMSO-d_6.

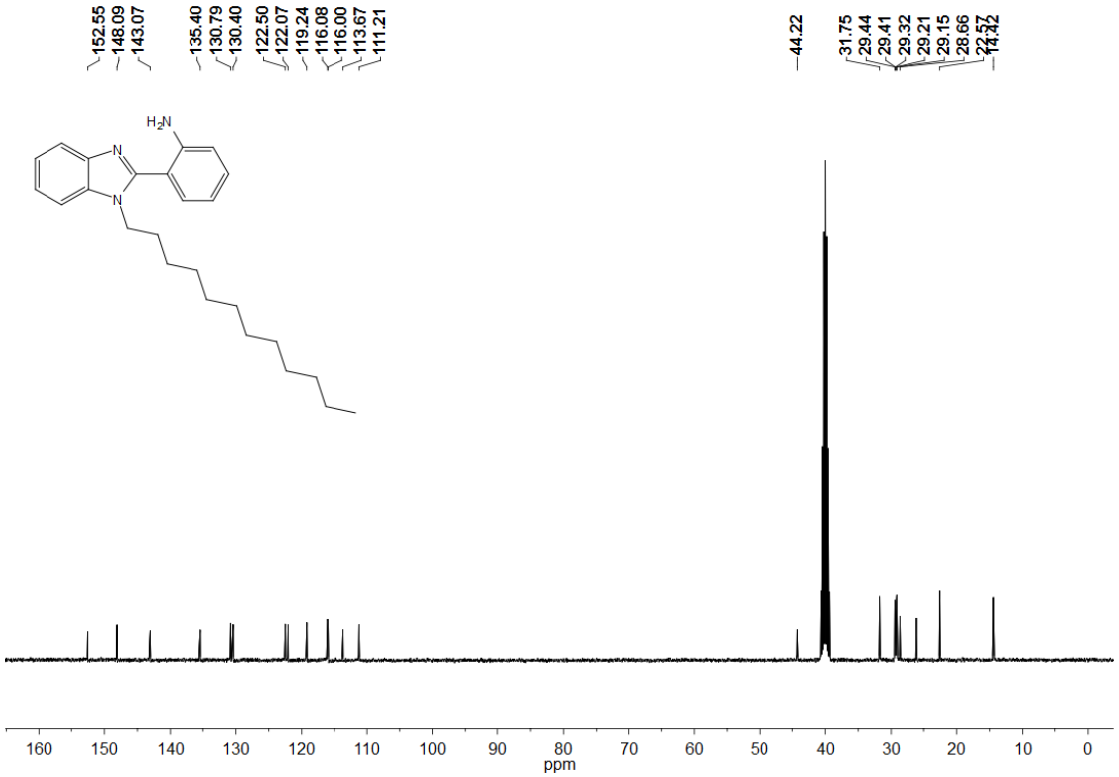

Figure S12 ^{13}C NMR spectra of compound 2b in DMSO-d_6.

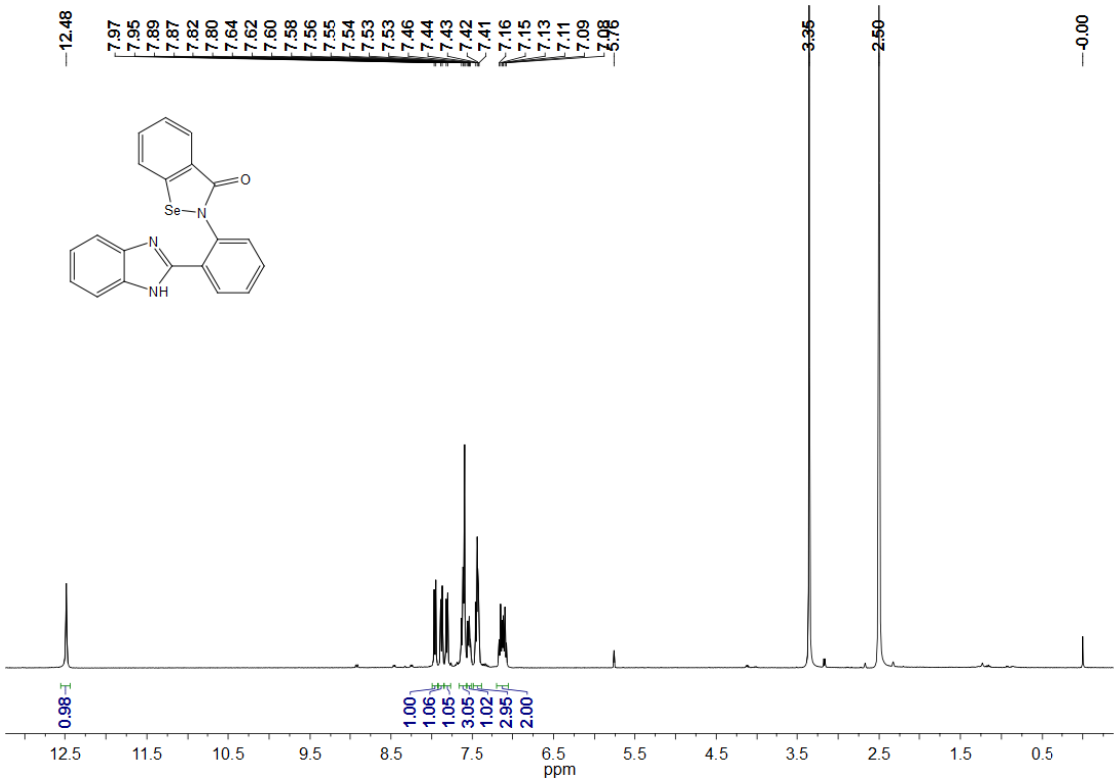

Figure S13 ^{1}H NMR spectra of compound **HMSe** in DMSO-d_6.

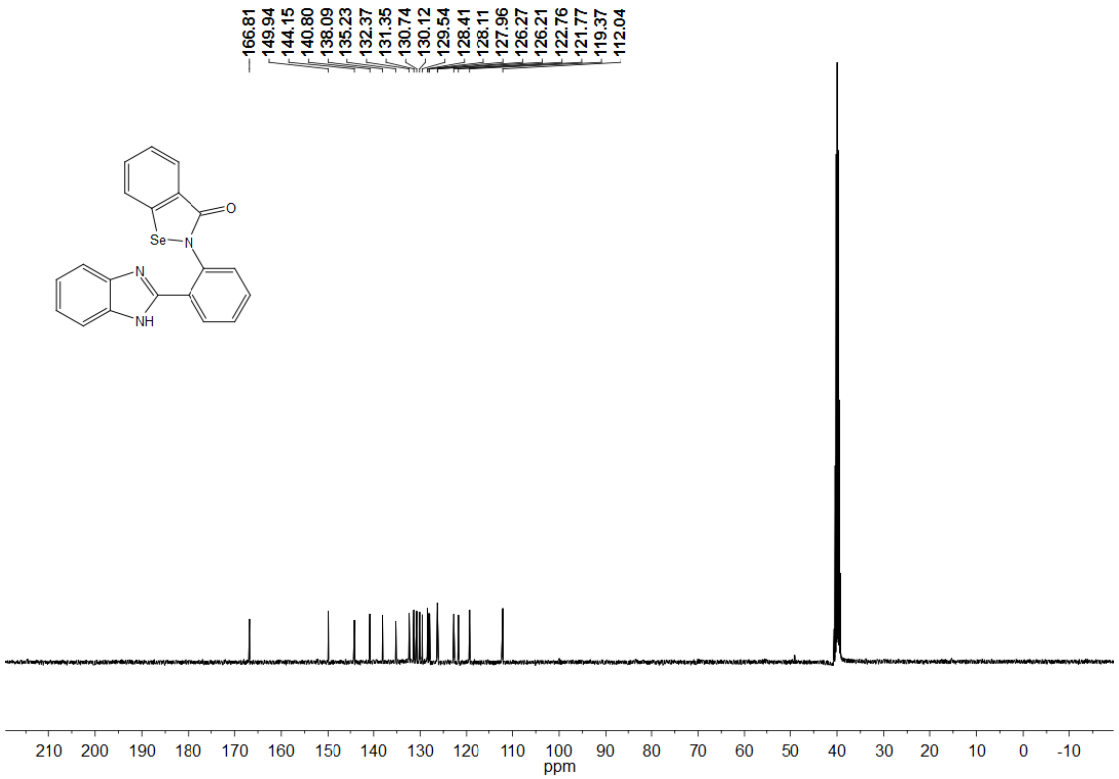

Figure S14 ^{1}H NMR spectra of compound **HMSe** in DMSO-d_6.

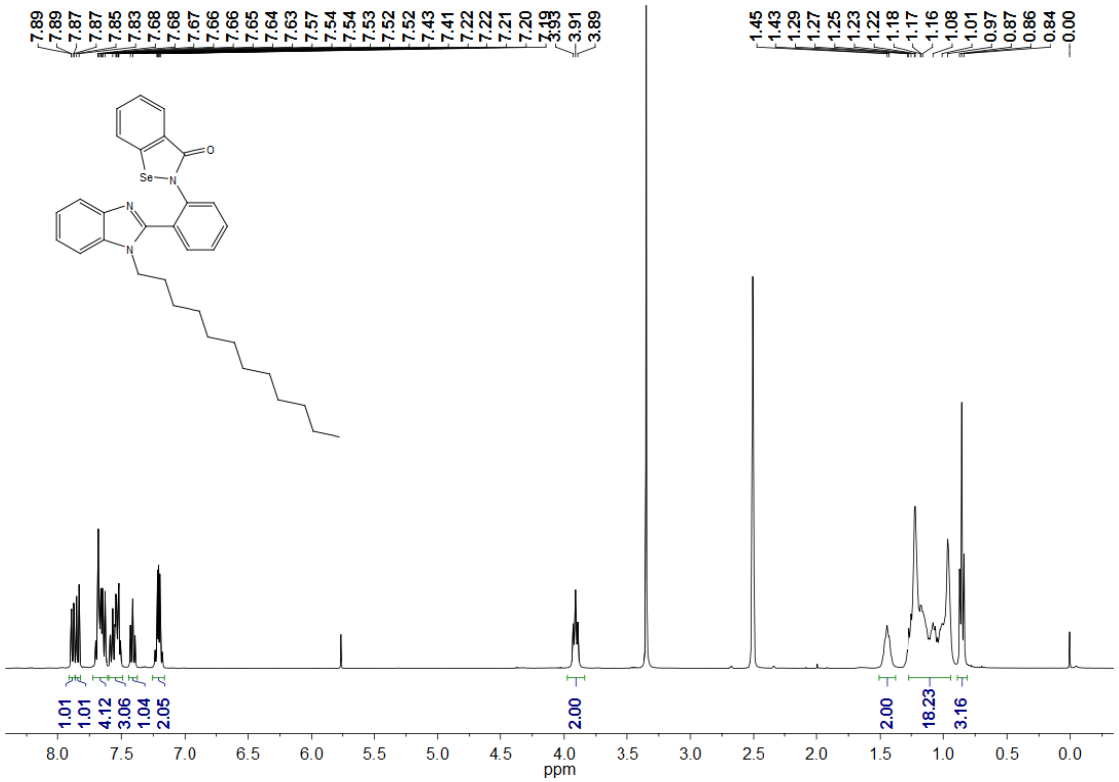

Figure S15 ^{1}H NMR spectra of compound **D-HMSe** in DMSO-d_6.

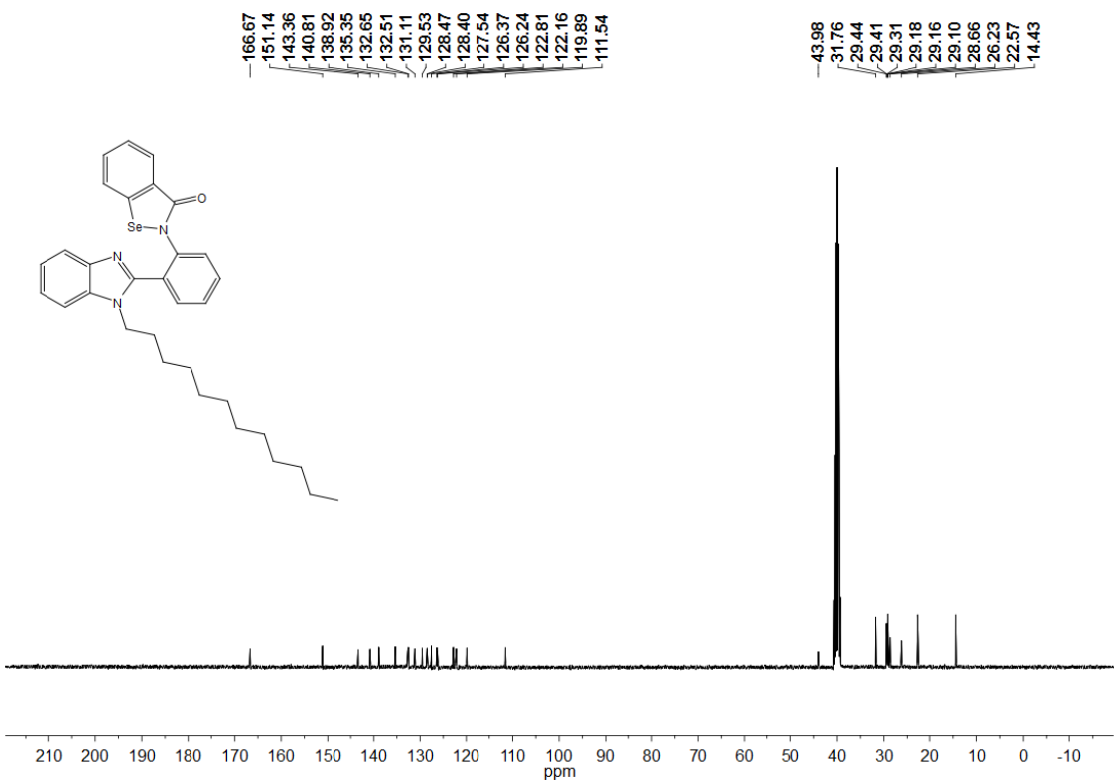

Figure S16 ^{13}C NMR spectra of compound **D-HMSe** in DMSO-d_6.

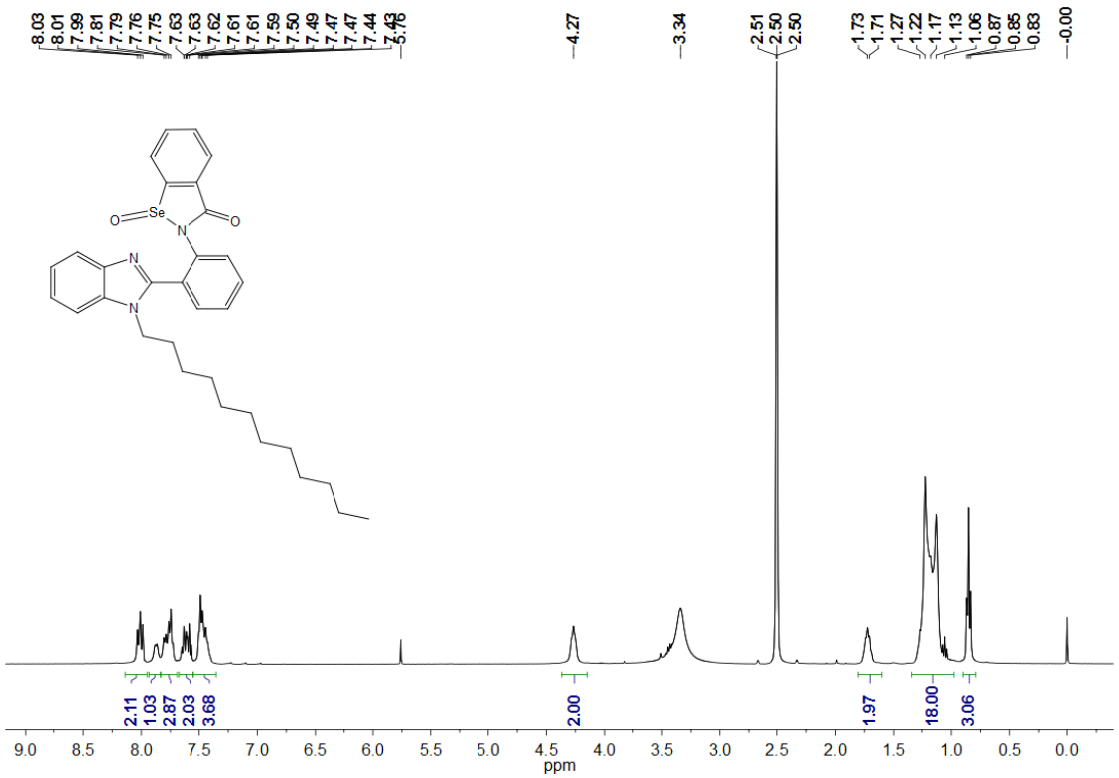

Figure S17 ^{1}H NMR spectra of compound **D-HMSeO** in DMSO-d_6.

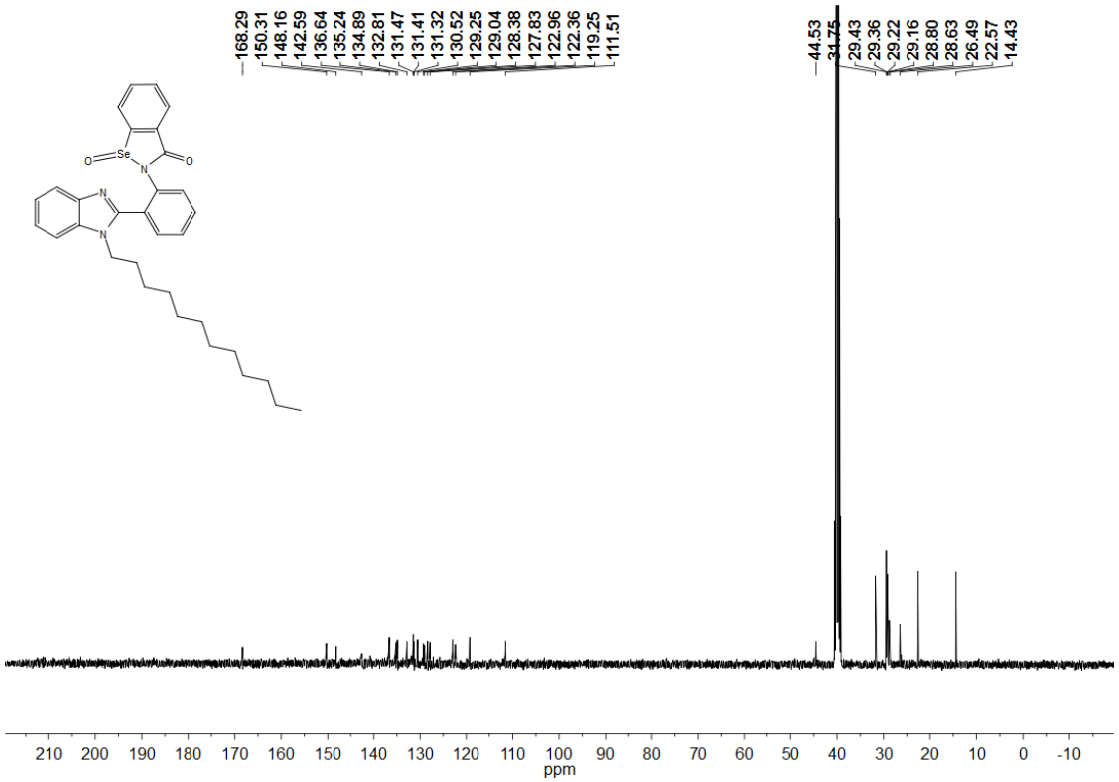

Figure S18 ^{13}C NMR spectra of compound **D-HMSeO** in DMSO-d_6.

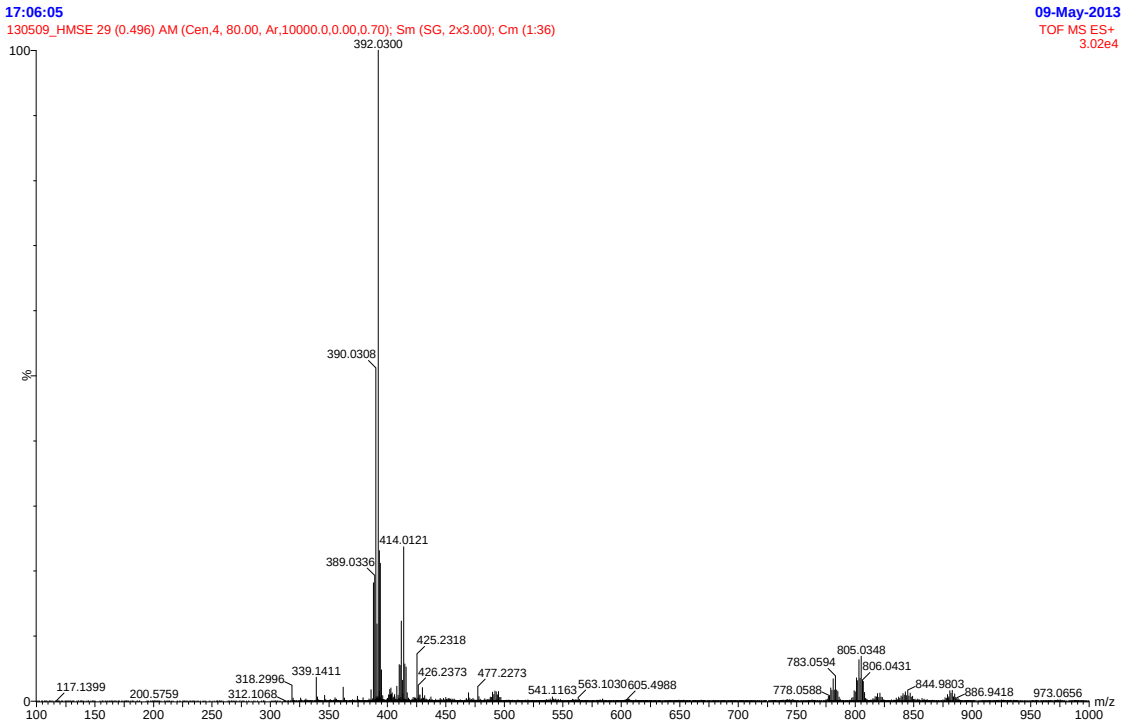

Figure S19 HRMS of compound **HMSe**.

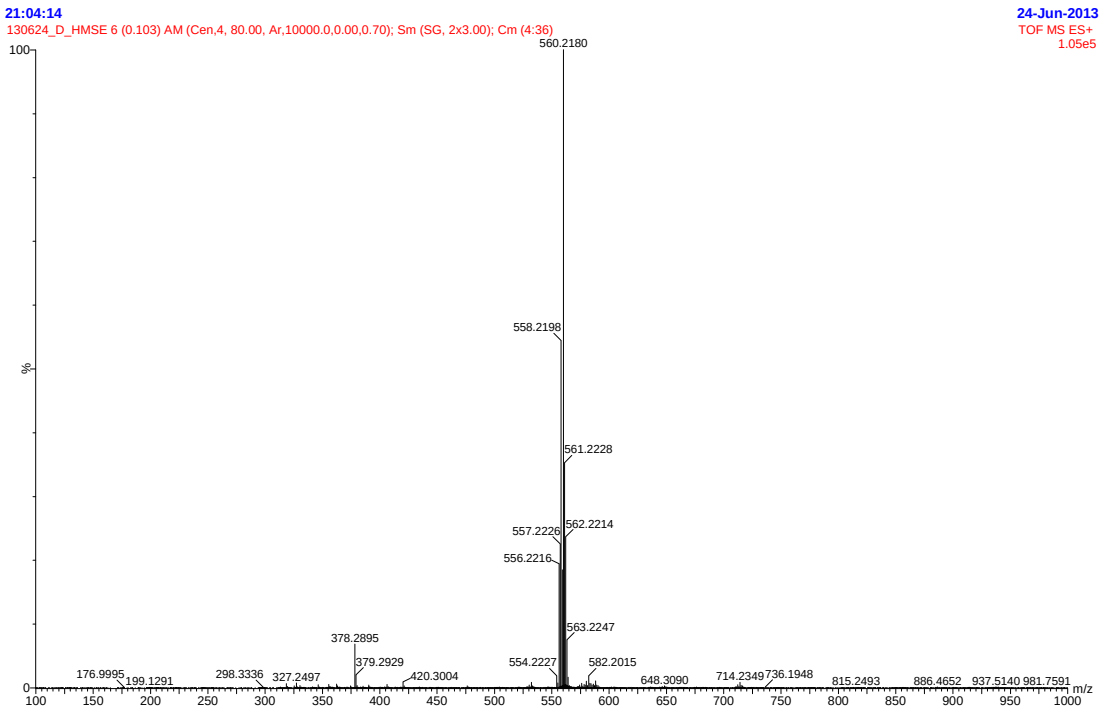

Figure S20 HRMS of compound **D-HMSe**.