Supporting Information

A Hydrogen Sulfide (H_2S) Mediated Tandem Reaction of Selenenyl-Sulfides and its Application in Fluorescent Probe Development

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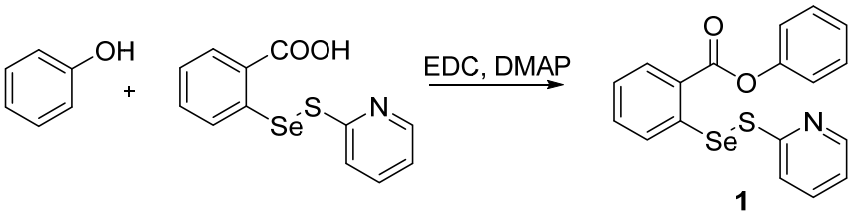
Materials and Instruments: ^{1}H NMR spectra and ^{13}C NMR were recorded at 600 MHz (Varian, VX 600) and are reported in parts per million (ppm) on the δ scale relative to residual $CDCl_3$ (δ 7.25), DMSO-d_6 (δ 2.50), $CHCl_3$ (δ 77.0), and DMSO (δ 39.5) respectively. NMR experiments were performed at room temperature. All reported melting points for solid materials were measured by Fisher-Johns melting point apparatus and not corrected. Absorption spectra were recorded on a Thermo 300 UV/VIS spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer.

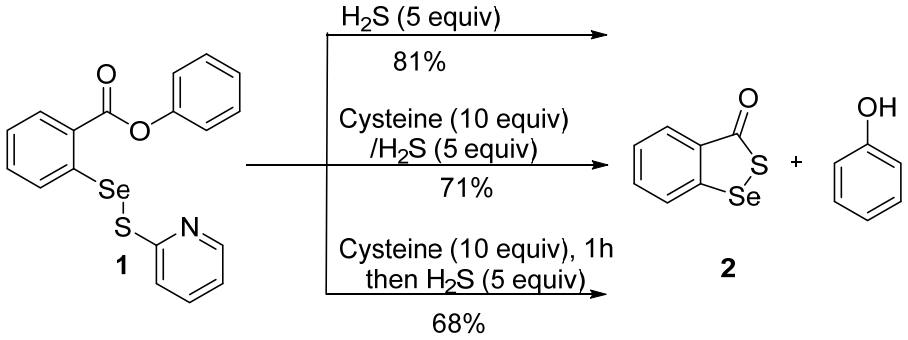
Solvents and Reagents: Reagents and solvents employed were of the highest grade available. Reagent grade solvents were used for either chromatography or extraction without further purification before use. Thiols (RSH), were purchased from Sigma-Aldrich and used as received. cyclic seleninate ester was synthesized using reported procedures.[1]

Chromatography: The progress of the reactions was monitored by analytical thin layer chromatography (VWR, TLC 60 F_{254} plates). Plates were visualized first with UV (254 nm). Flash column chromatography was performed using silica gel (230-400 mesh). The solvent compositions for all separations are on a volume/volume (v/v) basis.

Chemical synthesis



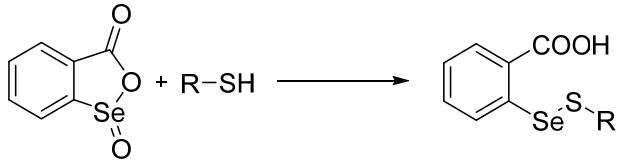
To a mixture of phenol (30 mg, 0.32 mmol), 2-((pyridine-2-ylthio)selanyl)benzoic acid (68 mg, 0.22 mmol), EDC (60 mg, 0.32 mmol) and a catalytic amount of DMAP was added CH_2Cl_2 (10 mL) at room temperature. The mixture was stirred for 3 hours. Then the solvent was removed under reduced pressure and the resulting residue was subjected to flash column chromatography (0 to 50% ethyl acetate/hexane gradient) for purification. The compound phenyl 2-((pyridine-2-ylthio)selenyl)benzoate **1**[2] was obtained as a white solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 4.9 Hz, 1H), 8.34 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.60 – 7.51 (m, 3H), 7.50 – 7.44 (m, 2H), 7.39 (t, J = 7.5 Hz, 1H), 7.34 – 7.29 (m, 1H), 7.24 – 7.21 (m, 1H), 7.05 (ddd, J = 7.2, 4.9, 1.3 Hz, 1H); 6.84 (d, J = 8.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ166.3, 158.5, 155.6, 150.5, 149.5, 138.7, 137.2, 134.1, 131.9, 129.6, 127.8, 126.3, 121.7, 121.6, 120.6, 115.3.



Scheme S1

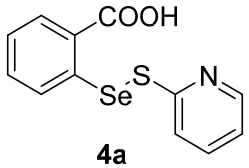
To a solution of 2-((pyridine-2-ylthio)selenyl)benzoate (40 mg, 0.1 mmol) in THF (2 mL) was slowly added a solution of Na_2S (126 mg, 0.52 mmol) in 10 mM PBS buffer (2 mL). The yellow solution was then stirred at room temperature for 3 hours. Then the reaction mixture was extracted with CH_2Cl_2. The combined organic extracts were concentrated *in vacuo*. Purification by flash column chromatography with 10% ethyl acetate/hexane afforded 2,1-benzothiaselenol-3-one **2**[2] and phenol as the isolated products with good yield (81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 7.7 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.64 (t, J = 6.9 Hz, 1H), 7.43 (t, J = 7.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ194.88, 143.79, 133.63, 130.51, 129.46, 127.07, 126.20.

In another two reactions cysteine was added as indicated in **Scheme S1**. The same protocol was applied and the corresponding yields were shown in **Scheme S1**.



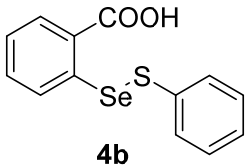
3 **4**

General procedure: To a solution of cyclic seleninate ester **3**[1] (2 mmol) in methanol (20 mL) was added RSH (6 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then concentrated and purified by flash column chromatography (methanol/dichloromethane 1/100).



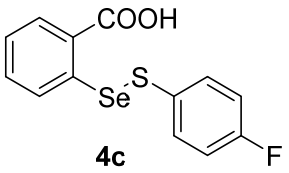
4a

Compound **4a**[2] was prepared by reacting 2-mercaptopyridine (667 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above, 465 mg, 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (ddd, J = 4.9,1.9,0.9 Hz, 1H), 8.10 (ddd, J = 7.7, 1.6, 0.4 Hz, 1H), 7.64 (ddd, J = 8.2, 7.3, 1.8 Hz, 1H), 7.58 (dt, J = 8.1, 1.1 Hz, 2H), 7.50 (ddd, J = 8.2, 7.3, 1.5 Hz, 1H), 7.34 (ddd, J = 7.7, 7.2, 1.1 Hz, 1H), 7.14 (ddd, J = 7.3, 4.9, 1.2 Hz, 1H).



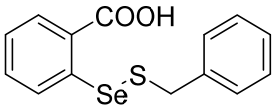
4b

Compound **4b** was prepared by reacting thiophenol (661 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4b** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 606 mg, 98% yield, mp 177.9-179.1 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (d, J = 8.2 Hz, 1H), 8.18 (dd, J = 7.8, 1.5 Hz, 1H), 7.59 – 7.53 (m, 1H), 7.49 (d, J = 7.5 Hz, 2H), 7.34 (t, J = 7.0 Hz, 1H), 7.27 – 7.20 (m, 2H), 7.16 (t, J = 7.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 175.13, 139.23, 136.04, 134.27, 132.44, 128.98, 128.92, 127.64, 126.77, 126.16, 126.03; HRMS m/z 308.9492 $[M-H]^-$; calcd for $C_{13}H_9O_2SSe^-$: 308.9488.



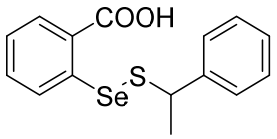
4c

Compound **4c** was prepared by reacting 4-fluorothiophenol (769 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4c** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 458 mg, 70% yield, mp 174.9-175.6 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (d, J = 7.7 Hz, 1H), 8.17 (dd, J = 7.8, 1.5 Hz, 1H), 7.62 – 7.54 (m, 1H), 7.47 (dd, J = 8.8, 5.1 Hz, 2H), 7.39 – 7.31 (m, 1H), 6.93 (t, J = 8.7 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.32, 139.03, 134.28, 132.53, 131.40, 131.35, 127.43, 126.25, 126.01, 116.15, 116.00; mass spectrum HRMS m/z 326.9406 $[M-H]^-$; calcd for $C_{13}H_8FO_2SSe^-$: 326.9394.



4d

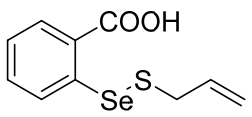
Compound **4d**[1] was prepared by reacting benzyl mercaptan (745 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above, 452 mg, 70% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (t, J = 8.9 Hz, 2H), 7.48 (t, J = 7.7 Hz, 1H), 7.29 (t, J = 7.5 Hz, 3H), 7.27-7.25 (m, 2H), 7.25 – 7.18 (m, 1H), 4.02 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.30, 138.86, 138.00, 133.75, 132.36, 128.94, 128.50, 127.40, 127.38, 126.04, 125.72; mass spectrum HRMS m/z 322.9634 $[M-H]^-$; calcd for $C_{14}H_{11}O_2SSe^-$: 322.9650.



4e

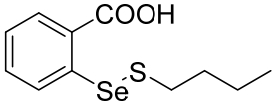
Compound **4e** was prepared by reacting 4-fluorothiophenol (829 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4e** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 405 mg, 60% yield, mp 149.3-150.5 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (d, J = 7.7 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H), 7.45 (t, J = 7.7 Hz, 1H), 7.33 (d, J = 6.9 Hz, 2H), 7.28 – 7.23 (m, 3H), 7.19 (t, J = 7.0 Hz, 1H), 4.08 (q, J = 7.0 Hz, 1H), 1.73 (d, J = 6.9 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.16, 142.68,

139.27, 133.67, 132.38, 128.47, 127.75, 127.51, 127.22, 126.18, 125.68, 49.09, 22.12; HRMS m/z 336.9807 [M-H]$^-$; calcd for $C_{15}H_{13}O_2SSe^-$: 336.9801.



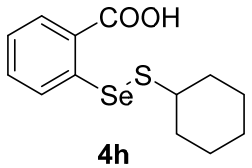
4f

Compound **4f** was prepared by reacting allyl mercaptan (445 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4f** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 465 mg, 85% yield, mp 94.1-96.2 ^oC; ^1H NMR (600 MHz, $CDCl_3$) δ 8.29 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 8.3 Hz, 1H), 7.60 (d, J = 7.0 Hz, 1H), 7.33 (d, J = 7.7 Hz, 1H), 5.85 (d, J = 7.2 Hz, 1H), 5.12 (d, J = 17.6 Hz, 1H), 5.06 (d, J = 9.9 Hz, 1H), 3.44 (d, J = 7.2 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.30, 138.86, 138.00, 133.75, 132.36, 128.94, 128.50, 127.38, 125.72, 42.16; HRMS m/z 272.9493 [M-H]$^-$; calcd for $C_{10}H_9O_2SSe^-$: 272.9488.



4g

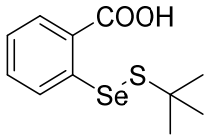
Compound **4g** was prepared by reacting butanethiol (541 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4g** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 433.5 mg, 75% yield, mp 82.8-83.5 ^oC; ^1H NMR (600 MHz, $CDCl_3$) δ 8.32 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 7.6 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 2.79 (t, J = 7.5 Hz, 2H), 1.64 (p, J = 7.5 Hz, 2H), 1.42 (h, J = 7.3 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.12, 139.37, 133.90, 132.55, 127.53, 126.25, 125.74, 37.28, 32.59, 21.72, 13.62; HRMS m/z 288.9809 [M-H]$^-$; calcd for $C_{11}H_{13}O_2SSe^-$: 288.9801.



4h

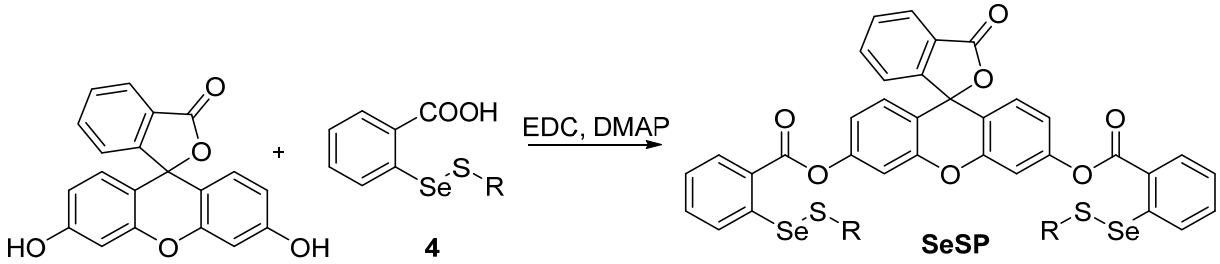
Compound **4h** was prepared by reacting cyclohexanethiol (696 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4h** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 548.6 mg, 87% yield, mp

160.6-161.9 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, J = 7.3 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.62 – 7.54 (m, 1H), 7.31 (t, J = 7.5 Hz, 1H), 2.74 (t, J = 11.0 Hz, 1H), 2.04 (d, J = 9.9 Hz, 2H), 1.75 (d, J = 12.2 Hz, 2H), 1.56 (d, J = 9.8 Hz, 1H), 1.39 (q, J = 12.0 Hz, 2H), 1.25 (p, J = 12.9, 12.3 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.12, 139.37, 133.90, 132.55, 127.53, 126.25, 125.74, 37.28, 32.59, 21.72, 13.62; HRMS m/z 314.9960 $[M-H]^-$; calcd for $C_{13}H_{15}O_2SSe^-$: 314.9958.

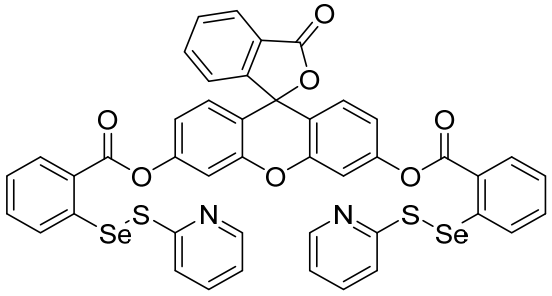


4i

Compound **4i** was prepared by reacting *tert*-butyl thiol (541 mg, 6 mmol) with **3** (430 mg, 2 mmol) using the general synthetic procedure described above. **4i** was isolated as white solid by flash column chromatography (methanol/dichloromethane = 1/100). 492 mg, 85% yield, mp 184.7-185.3 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (dd, J = 8.2, 1.1 Hz, 1H), 8.17 (dd, J = 7.8, 1.5 Hz, 1H), 7.61 – 7.52 (m, 1H), 7.35 – 7.28 (m, 1H), 1.38 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.93, 140.06, 133.57, 132.38, 128.19, 126.29, 125.71, 47.15, 30.86; HRMS m/z 288.9805 $[M-H]^-$; calcd for $C_{11}H_{13}O_2SSe^-$: 288.9801.

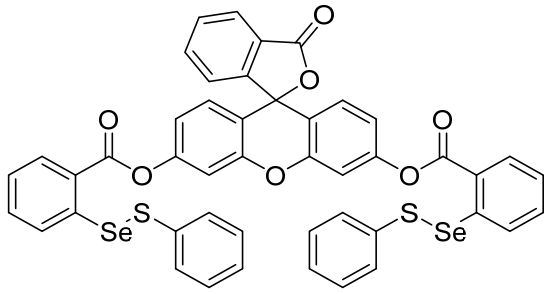


General procedure: To a mixture of fluorescein (1 mmol), compound **4** (2 mmol), EDC (2 mmol) and DMAP (0.1 mmol) was added CH_2Cl_2 (150 mL) at room temperature. The mixture was stirred overnight. The organic layer was washed with brine. After dried by anhydrous Na_2SO_4, the solvent was removed under reduced pressure and the resulting residue was subjected to flash column chromatography for purification.



SeSP1

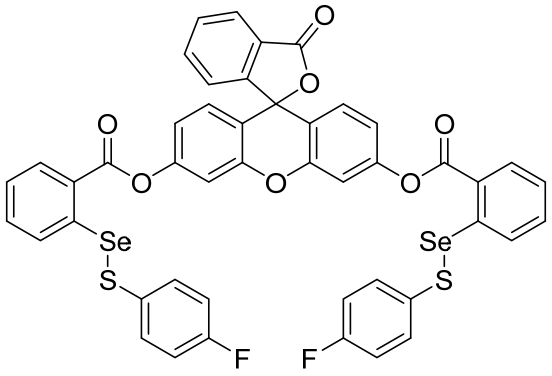
SeSP1 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4a** (620.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP1** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 596 mg, 65% yield, mp 142.6-143.7 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.43 (d, J = 3.9 Hz, 2H), 8.32 (d, J = 7.7 Hz, 2H), 8.12 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 7.7 Hz, 1H), 7.72 (t, J = 7.1 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.58 – 7.48 (m, 6H), 7.39 (t, J = 7.5 Hz, 2H), 7.28 (d, J = 2.3 Hz, 2H), 7.24 (d, J = 10.2 Hz, 1H), 7.03 (t, J = 5.6 Hz, 2H), 7.00 (dd, J = 8.7, 2.1 Hz, 2H), 6.94 (d, J = 8.6 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.07, 165.71, 158.28, 152.89, 151.86, 151.61, 149.57, 139.12, 137.17, 135.39, 134.41, 132.03, 130.16, 129.21, 129.00, 128.19, 127.96, 126.37, 126.11, 125.30, 124.08, 121.75, 120.70, 117.85, 117.03, 110.59; HRMS m/z 918.9586 $[M+H]^+$; calcd for $C_{44}H_{27}N_2O_7S_2Se_2^+$: 918.9590.



SeSP2

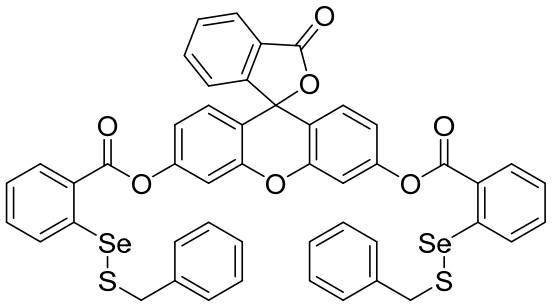
SeSP2 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4b** (618.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP2** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 549 mg, 60% yield, mp 114.2-115.2 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (dd, J = 7.8, 1.5 Hz, 2H), 8.24 (d, J = 8.1 Hz, 2H), 8.05 (d, J = 7.7 Hz, 1H), 7.71 (td, J = 7.5, 1.2 Hz, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.49 (d, J = 7.4 Hz, 4H), 7.41 – 7.36 (m, 2H), 7.26 (d, J = 2.3 Hz, 2H), 7.25 – 7.21 (m, 5H), 7.16 (t, J = 7.3 Hz, 2H), 6.98 (dd, J = 8.7, 2.3 Hz, 2H), 6.95 – 6.89 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ

169.10, 165.58, 152.95, 151.89, 151.61, 139.83, 135.92, 135.38, 134.31, 132.07, 130.14, 129.19, 128.99, 128.97, 127.84, 126.82, 126.23, 125.98, 125.29, 124.09, 117.87, 116.99, 110.60, 81.45; HRMS m/z 916.9681 $[M+H]^+$; calcd for $C_{46}H_{29}O_7S_2Se_2^+$: 916.9685.



SeSP3

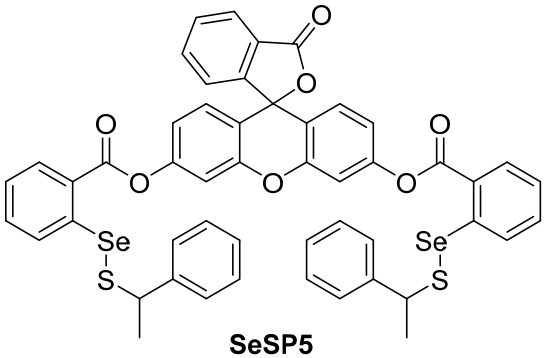
SeSP3 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4c** (654.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP3** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 666 mg, 70% yield, mp 111.6-112.7 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (d, J = 7.8 Hz, 2H), 8.28 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 7.5 Hz, 1H), 7.72 (t, J = 7.4 Hz, 1H), 7.67 (d, J = 7.4 Hz, 1H), 7.63 (t, J = 7.5 Hz, 2H), 7.48 (dd, J = 8.5, 5.1 Hz, 4H), 7.41 (t, J = 7.5 Hz, 2H), 7.26 (s, 2H), 7.23 (d, J = 7.6 Hz, 1H), 6.95 (q, J = 9.6, 8.5 Hz, 8H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.06, 165.54, 162.94, 161.31, 152.90, 151.84, 151.58, 139.62, 135.37, 134.30, 132.14, 131.46, 131.41, 130.14, 129.18, 127.62, 126.31, 125.96, 125.93, 125.30, 124.05, 117.83, 116.14, 110.57, 81.41; HRMS m/z 952.9491 $[M+H]^+$; calcd for $C_{46}H_{27}F_2O_7S_2Se_2^+$: 952.9497.



SeSP4

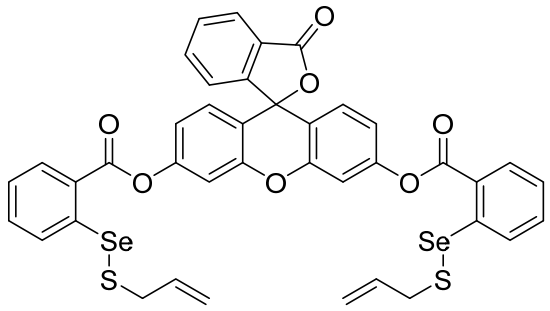
SeSP4 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4d** (646.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP4** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 641 mg, 68% yield, mp 121.2-122.1 ºC;

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.27 (d, J = 8.3 Hz, 2H), 8.16 (d, J = 8.1 Hz, 2H), 8.06 (d, J = 7.6 Hz, 1H), 7.72 (t, J = 7.5 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.51 (t, J = 6.9 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.30 (d, J = 7.3 Hz, 4H), 7.28 – 7.24 (m, 5H), 7.25 – 7.18 (m, 4H), 6.98 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 2H), 4.03 (s, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.11, 165.30, 152.98, 151.92, 151.58, 139.52, 137.94, 135.35, 133.80, 131.99, 130.10, 129.12, 128.93, 128.51, 127.63, 127.42, 125.95, 125.93, 125.81, 125.26, 124.07, 117.89, 116.87, 110.60, 81.48, 42.22; HRMS m/z 944.9994 $[M+H]^+$; calcd for $C_{48}H_{33}O_7S_2Se_2^+$: 944.9998.



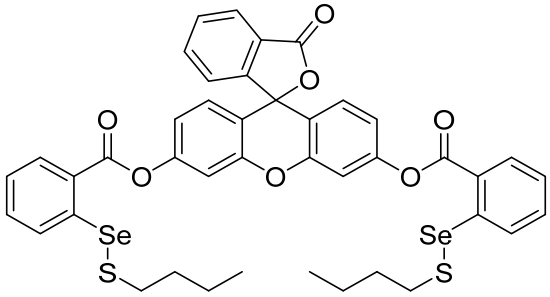
SeSP5

SeSP5 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4e** (675 mg, 2 mmol) using the general synthetic procedure described above. **SeSP5** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 728 mg, 75% yield, mp 130.6-131.3 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.25 (dd, J = 7.8, 1.5 Hz, 2H), 8.12 (d, J = 8.1 Hz, 2H), 8.05 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 7.4 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 7.3 Hz, 2H), 7.32 (t, J = 8.7 Hz, 6H), 7.26 (m, 6H), 7.21 (d, J = 7.7 Hz, 1H), 7.18 (t, J = 7.3 Hz, 2H), 6.97 (dd, J = 8.6, 2.3 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 4.08 (q, J = 6.9 Hz, 2H), 1.71 (d, J = 7.0 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.13, 165.26, 153.01, 151.94, 151.58, 142.60, 139.92, 135.34, 133.65, 131.92, 130.10, 129.11, 128.45, 127.96, 127.51, 127.20, 125.94, 125.73, 125.26, 124.06, 117.91, 116.85, 110.62, 81.49, 49.12, 22.08; HRMS m/z 973.0305 $[M+H]^+$; calcd for $C_{50}H_{37}O_7S_2Se_2^+$: 973.0311.



SeSP6

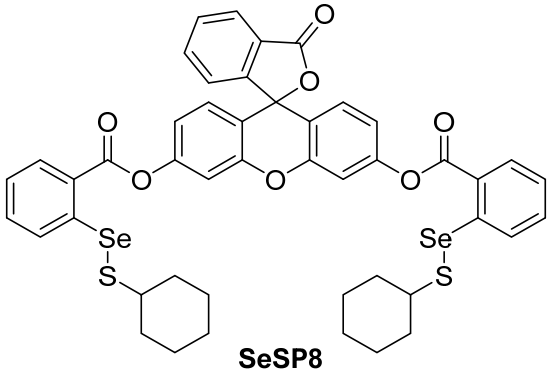
SeSP6 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4f** (546.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP6** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 421 mg, 50% yield, mp 99.7-101.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, J = 8.1 Hz, 2H), 8.29 (d, J = 7.9 Hz, 2H), 8.05 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.64 (q, J = 8.3 Hz, 3H), 7.38 (t, J = 7.6 Hz, 2H), 7.21 (d, J = 7.7 Hz, 1H), 6.96 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 5.84 (h, J = 7.9 Hz, 2H), 5.11 (d, J = 16.9 Hz, 2H), 5.05 (d, J = 9.9 Hz, 2H), 3.44 (d, J = 7.3 Hz, 4H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.11, 165.31, 151.91, 151.57, 139.79, 135.34, 133.88, 133.71, 132.07, 130.09, 129.12, 127.77, 126.05, 125.94, 125.88, 125.26, 124.06, 118.24, 117.89, 116.87, 110.61, 81.47, 40.27; HRMS m/z 844.9680 $[M+H]^+$; calcd for $C_{40}H_{29}O_7S_2Se_2^+$: 844.9685.



SeSP7

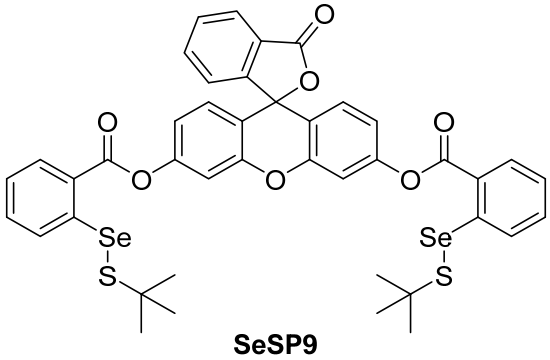
SeSP7 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4g** (578.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP7** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 682 mg, 78% yield, mp 97.1-98.6 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.35 (d, J = 8.2 Hz, 2H), 8.29 (d, J = 7.9 Hz, 2H), 8.05 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.64 (q, J = 8.3 Hz, 3H), 7.38 (t, J = 7.6 Hz, 2H), 7.21 (d, J = 7.7 Hz, 1H), 6.96 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 2.79 (t, J = 7.4 Hz, 4H), 1.63 (tt, J = 9.1 Hz, 4H), 1.41 (q, J = 7.4 Hz, 4H), 0.88 (t, J = 7.4 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.12, 165.29, 153.00, 151.93, 151.57, 140.03, 135.34, 133.90, 132.12, 130.09, 129.11, 127.72, 126.04,

125.94, 125.80, 125.25, 124.07, 117.90, 116.85, 110.61, 81.49, 37.29, 32.57, 21.70, 13.61; HRMS m/z 877.0309 $[M+H]^+$; calcd for $C_{42}H_{37}O_7S_2Se_2^+$: 877.0311.



SeSP8

SeSP8 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4h** (631 mg, 2 mmol) using the general synthetic procedure described above. **SeSP8** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 779 mg, 84% yield, mp 126.3-127.5 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, J = 8.2 Hz, 2H), 8.30 (dd, J = 7.9, 1.5 Hz, 2H), 8.06 (d, J = 7.2 Hz, 1H), 7.72 (td, J = 7.5, 1.3 Hz, 1H), 7.68 – 7.59 (m, 3H), 7.41 – 7.34 (m, 2H), 7.27 (s, 1H), 7.25 (s, 1H), 7.22 (d, J = 7.6 Hz, 1H), 6.98 (dd, J = 8.7, 2.3 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 2.75 (tt, J = 11.0, 3.7 Hz, 2H), 2.11 – 1.98 (m, 4H), 1.82 – 1.69 (m, 4H), 1.61 – 1.55 (m, 2H), 1.47 – 1.35 (m, 4H), 1.32 – 1.18 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.11, 165.26, 153.02, 151.96, 151.58, 140.70, 135.34, 133.74, 132.00, 130.09, 129.08, 128.09, 126.01, 125.94, 125.72, 125.25, 124.06, 117.92, 116.84, 110.63, 81.50, 48.76, 34.11, 26.26, 25.47; HRMS m/z 929.0618 $[M+H]^+$; calcd for $C_{46}H_{41}O_7S_2Se_2^+$: 929.0624.



SeSP9

SeSP9 was prepared by reacting fluorescein (332 mg, 1 mmol), compound **4i** (578.5 mg, 2 mmol) using the general synthetic procedure described above. **SeSP9** was isolated as white solid by flash column chromatography (ethyl acetate/hexane = 20/80). 717 mg, 82% yield, mp 123.4-124.0 ºC; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (d, J = 8.2 Hz, 2H), 8.27 (d, J = 7.8 Hz, 2H), 8.05 (d, J = 7.6

Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.58 (t, J = 7.6 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.26 (d, J = 13.9 Hz, 1H), 7.25 (s, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.98 (dd, J = 8.8, 2.3 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 1.36 (s, 18H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.13, 165.24, 153.05, 151.97, 151.58, 140.69, 135.33, 133.54, 131.93, 130.08, 129.08, 128.34, 126.06, 125.93, 125.75, 125.25, 124.05, 117.94, 116.83, 110.65, 81.50, 47.23, 30.82; HRMS m/z 877.0305 $[M+H]^+$; calcd for $C_{42}H_{37}O_7S_2Se_2^+$: 877.0311.

Preparation of the solutions and fluorescence measurements

The stock solutions of **SeSP1** (1 mM), **SeSP2** (1 mM) and **SeSP3** (1 mM) were prepared in DMSO, respectively. The solutions of various testing species were prepared from Cysteine (Cys), Homocysteine (Hcy), Alanine (Ala), Arginine (Arg), Lysine (Lys), Proline (Pro), Serine (Ser), GSH, $Na_2S·9H_2O$, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, in 10 mM PBS buffer. The stock solution of cetrimonium bromide (CTAB, 10 mM) was prepared in EtOH. All the test solutions need to be freshly prepared.

The concentration of H_2O_2 was determined using the absorption at 240 nm (ε = 43.6 $M^{-1}cm^{-1}$). Stock solution of HOCl was prepared by dilution of the commercial sodium hypochlorite solution and the concentration of ClO^- was determined using the absorption at 292 nm (ε = 360 $M^{-1}cm^{-1}$). Superoxide solution (O^{2-}) was prepared by adding KO_2 (1mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. Peroxynitrite solution ($ONOO^-$) was obtained according to the literature methods.[4] Stock solution of nitroxyl (HNO) was prepared from Anglie's salt in NaOH (0.01M).[5] Nitric oxide (NO) was obtained by using sodium 1-(Pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (V-PYRRO/NO) as the donor.[6]

Unless otherwise noted, all the measurements were carried out for 30 min at room temperature in PBS buffer (50 mM, pH 7.4) with 100 μM CTAB according to the following procedure. In a test tube, 3.0 mL of 50 mM PBS buffer (pH 7.4) and 40.0 μL of CTAB stock solution were mixed, followed by the addition of a required volume of testing species sample solution. The final volume of the reaction solution was adjusted to 3.96 mL with 50 mM PBS buffer (pH 7.4). And then 40 μL of **SeSP** stock slolution was added (10 μM). After mixing and then standing for 30 min at room temperature, the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence with λex = 498 nm. PMT detector voltage = 400V. In the meantime, a blank solution containing no testing species sample was prepared and measured under the same conditions for comparison.

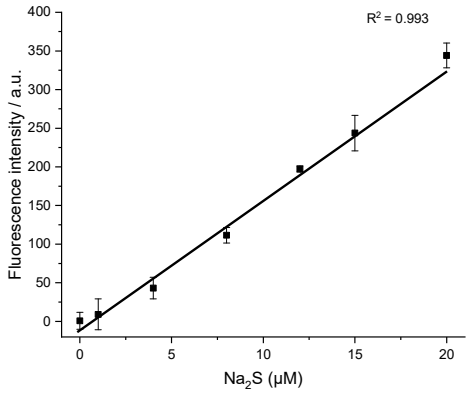


Figure S1. The linear fitting curve between the fluorescence intensity of SeSP1 (10 μM) and the concentration of Na_2S from 0 to 20 μM.

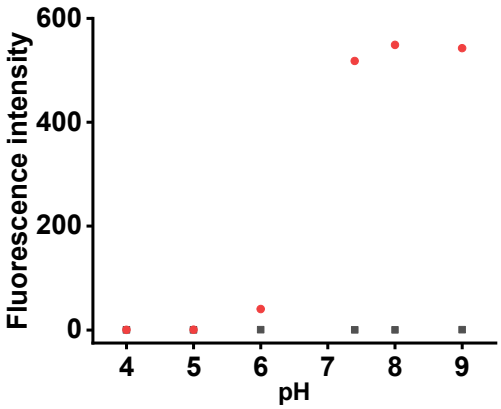


Figure S2. Fluorescence intensity changes of SeSP1 (10 μM) at different pH values in the absence (■) or presence (●) of Na_2S (50 μM). The reactions were carried out for 30 min at room temperature in PBS buffer (50 mM, pH 7.4) with 100 μM CTAB.

Cell culture and fluorescence imaging

HeLa cells were grown on glass-bottom culture dishes (Corning Inc.) in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 ºC under a humidified atmosphere containing 5% CO2 and 95% air. Cells were seeded into a 24 well-plate (1 x 10^5 cells/well) and medium was replaced every 2 days. Cells were used after getting 60%-80% confluent. Before use, the adherent cells were washed one time with PBS buffer solution.

For intracellular H_2S imaging, the cells were incubated with 30 μM SeSP1 in FBS-free DMEM at 37 ºC for 45 min. After removal of excess probe and washed with PBS (pH 7.4), the cells were

incubated with 50 μM or 100 μM H_2S donor (NSHD-1)[7] for 60 min in DMEM. Cell imaging was carried out after washing the cells one time with PBS (pH 7.4).

Measurement of endogenous H_2S changes

For L-cysteine experiment, cells were incubated with **SeSP1** (30 μM, with 5% DMSO) in DMEM at 37 ºC for 45 min, washed twice with PBS buffer (pH 7.4), and incubated with L-cysteine (1 mM), in DMEM at 37 ºC for 1 h. Fluorescent intensity was measured by a plate-reader (SpectraMax M5, Molecular Devices) after washing cells twice with PBS buffer (pH 7.4).

For a CSE inhibitor 2-amino-4-pentynoic acid (PAG) experiment, cells were incubated with PAG (1 mM) in DMEM for 1 h, washed with PBS buffer (pH 7.4), and incubated **SeSP1**(30 μM, with 5% DMSO) in DMEM at 37 ºC for 45 min. Then washed twice with PBS buffer (pH 7.4) and incubated with FBS free DMEM for 1 h. Fluorescent intensity was measured by a plate-reader (SpectraMax M5, Molecular Devices) after washing cells twice with PBS buffer (pH 7.4).

For a negative control experiment, cells were incubated with PAG (1 mM) in DMEM for 1 h, washed with PBS buffer (pH 7.4), and loaded **SeSP1** (30 μM, with 5% DMSO) in DMEM at 37 ºC for 45 min. Washed cells twice with PBS buffer (pH 7.4) and incubated with L-cysteine (1 mM), in DMEM at 37 ºC for 1 h. Fluorescent intensity was measured by a plate-reader (SpectraMax M5, Molecular Devices) after washing cells twice with PBS buffer (pH 7.4).

Cell viability assay

The cells were cultured in DMEM high glucose medium supplemented with 10% fetal bovine serum (FBS) at 37 ºC under an atmosphere of 5% CO_2 and 95% air. The cells were then inoculated in 96-well plates and cultured overnight. **SeSP1** in FBS-free medium were administered and cultured for 3 h. The cell viability was measured by cell counter kit (CCK)-8 (Dojindo Molecular Technologies, Inc. USA). The absorbance at 450 nm was measured with a microplate reader (Tecan US, Inc. USA). Optical density (OD) of the 9 wells in the indicated groups was used to calculate percentage of cell viability according to the formula below:

$$\text{Percentage of cell viability} = \text{OD treatment group} / \text{OD control group} \times 100\%$$

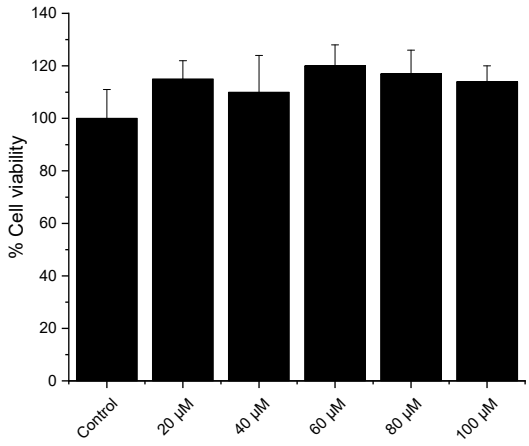


Figure S3. Cytotoxicity studies of SeSP-1 (20 μM; 40 μM; 60 μM; 80 μM; 100 μM) for HeLa cells.

References

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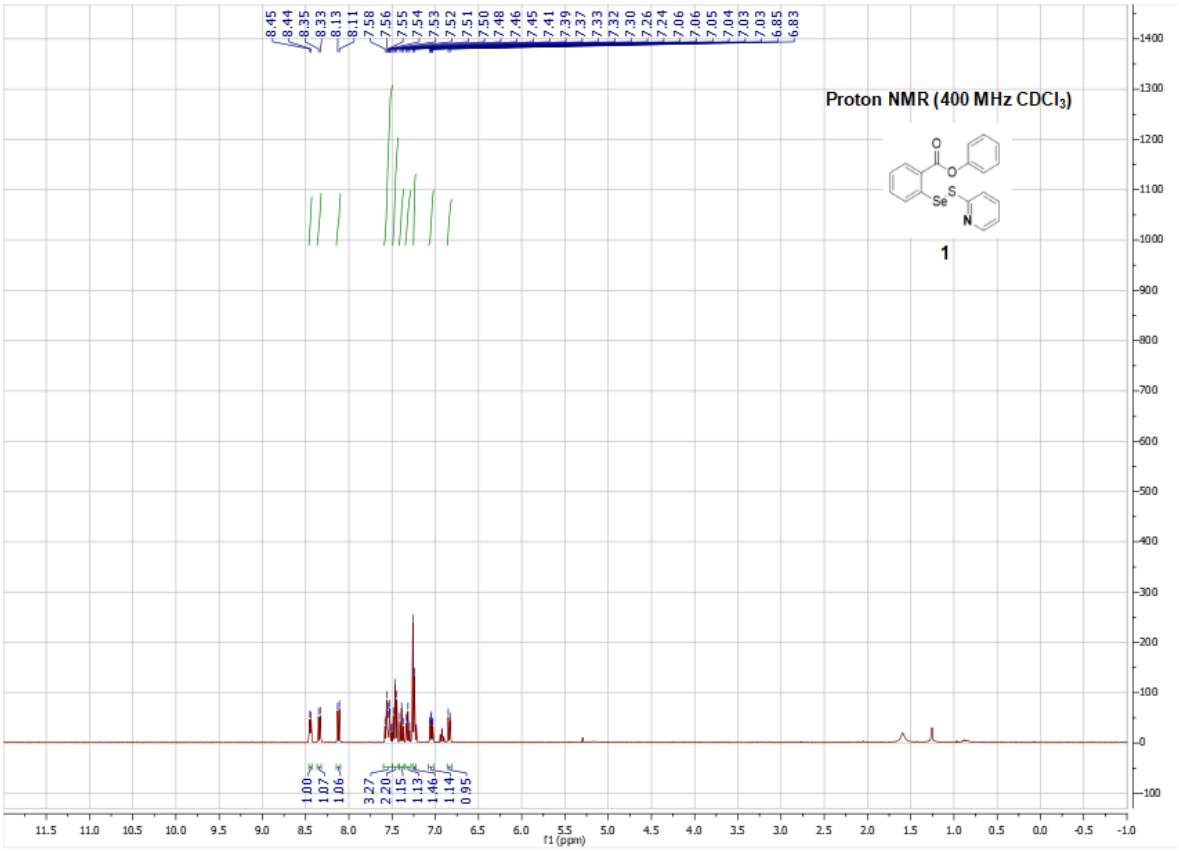
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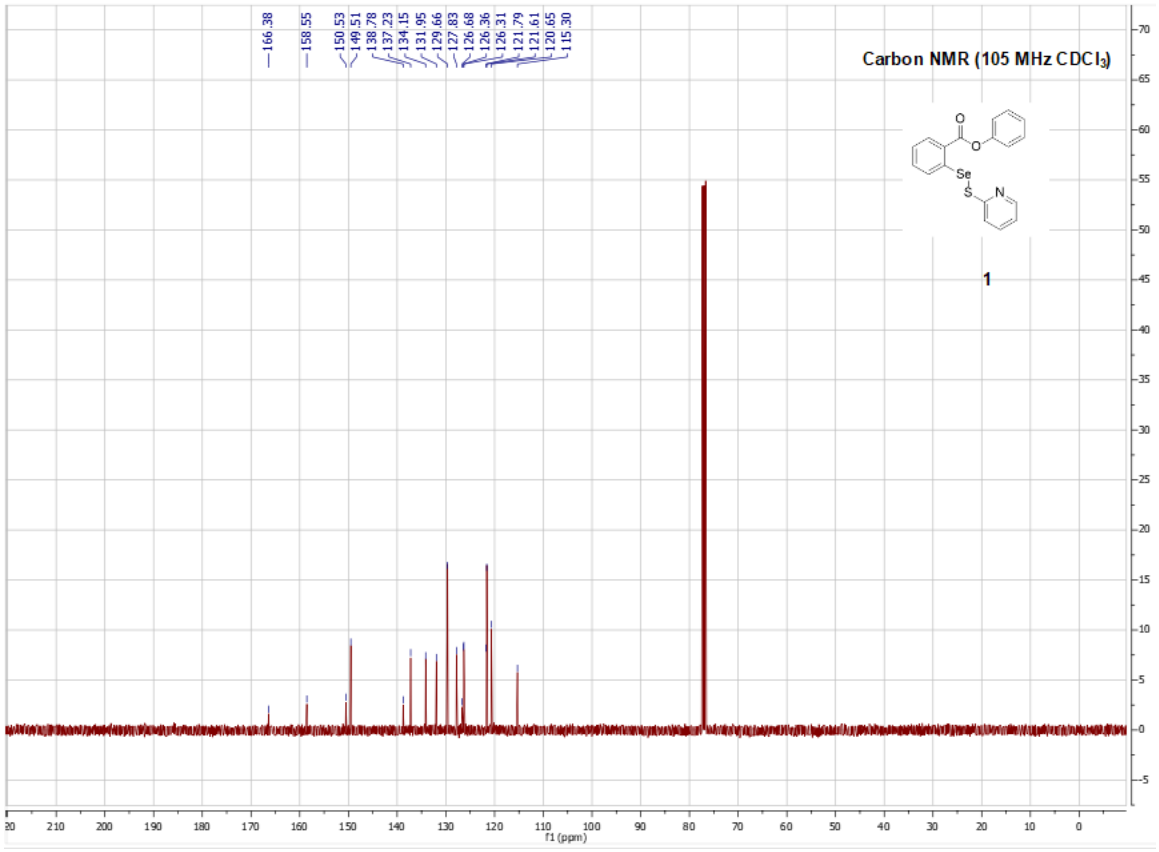
1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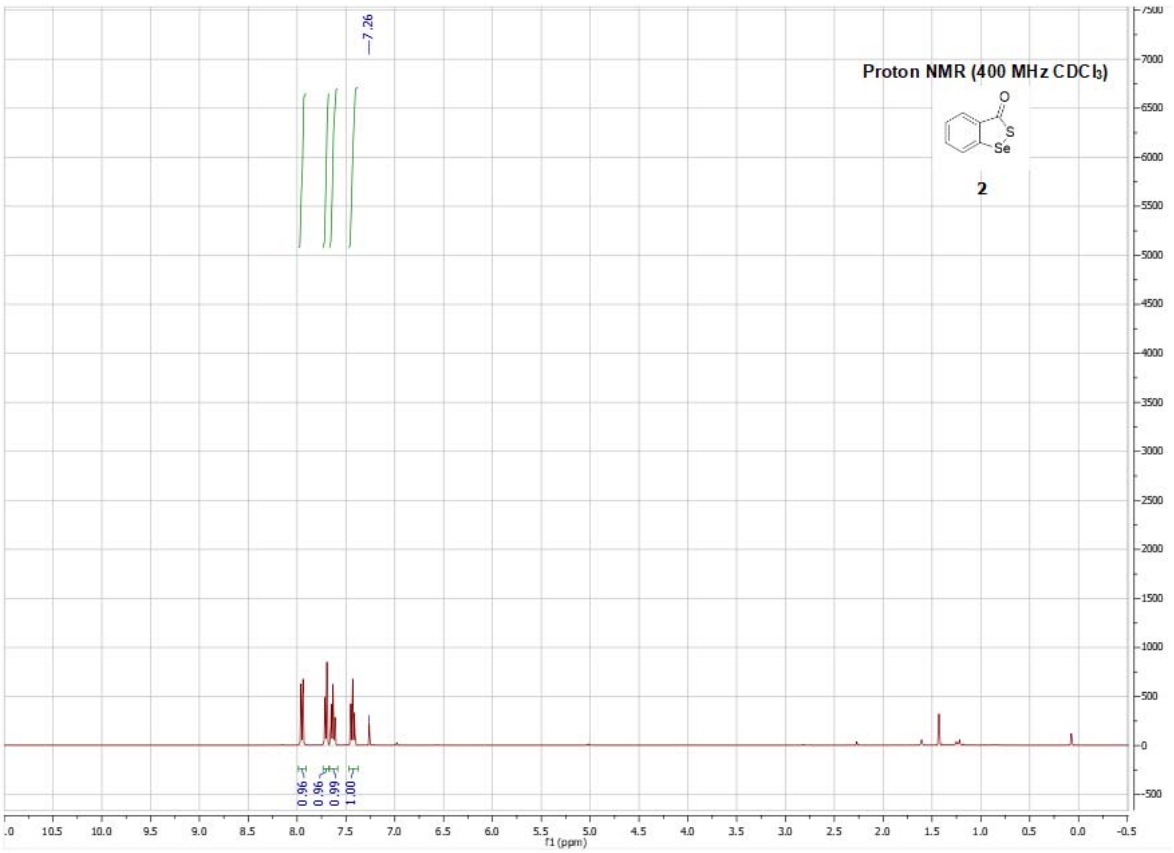
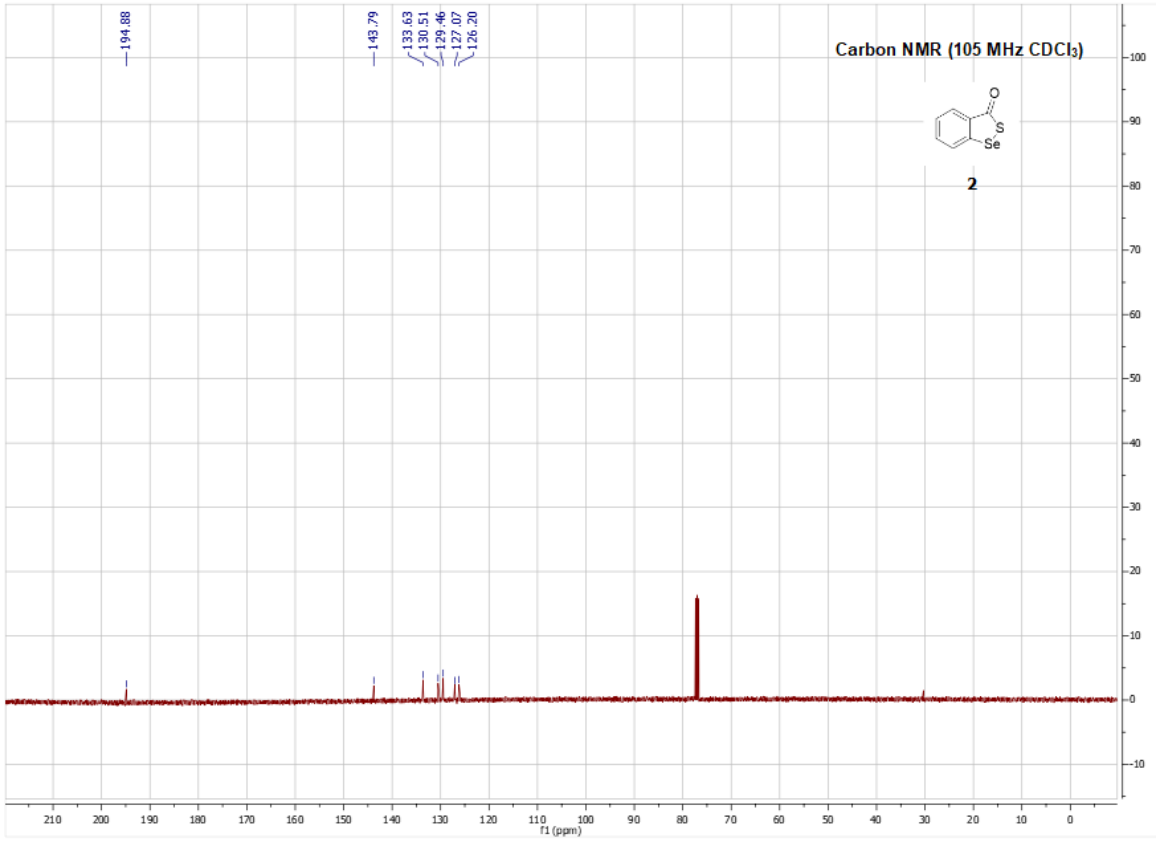
1


Proton NMR (400 MHz CDCl3)
2
7.26
0.96
0.96
0.99
1.00
f1 (ppm)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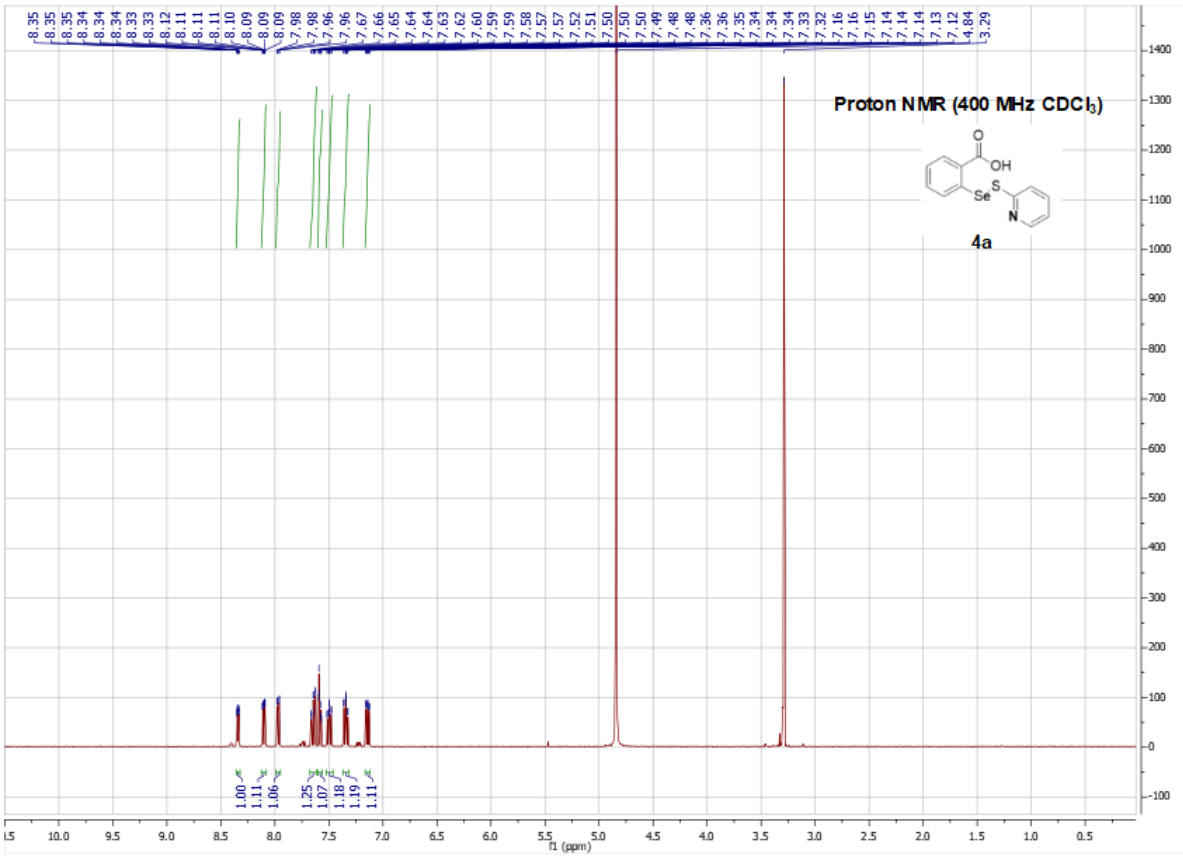
2
194.88
143.79
133.63
130.51
129.46
127.07
126.20
f1 (ppm)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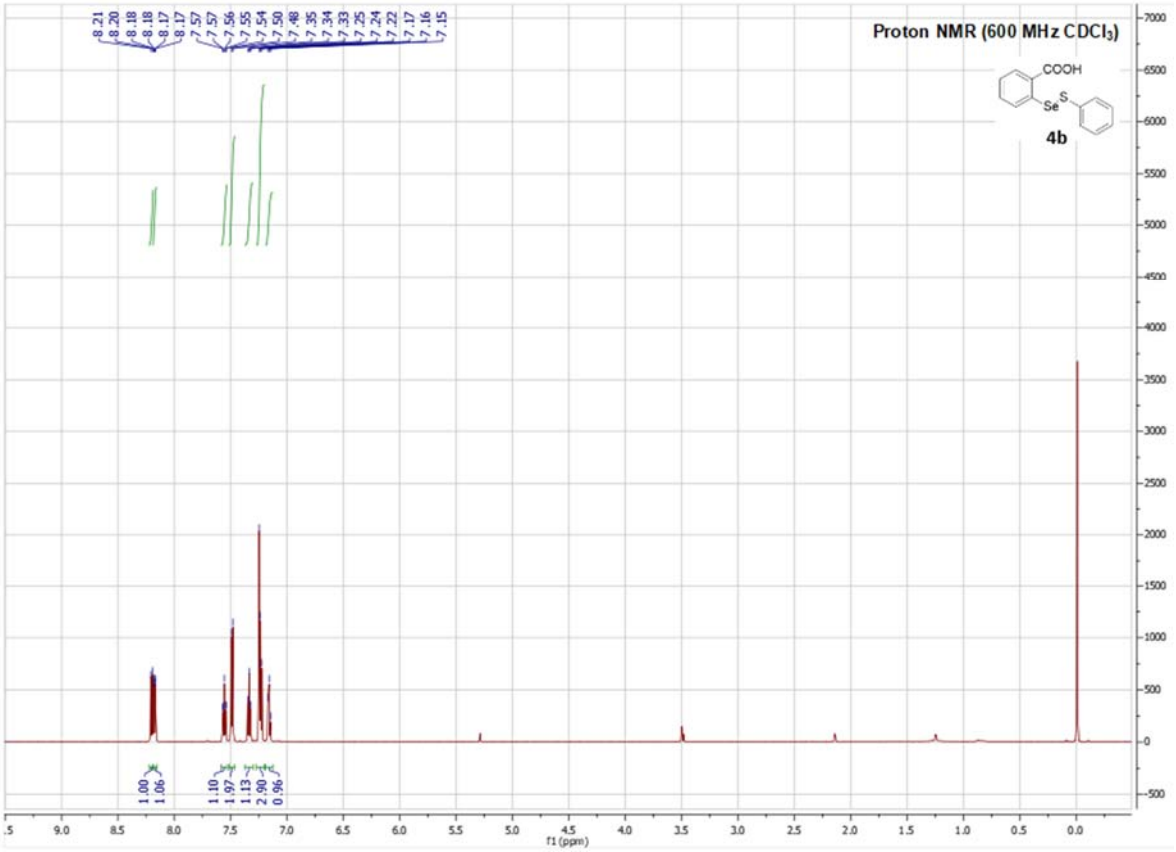
O
OH
S
Se
N
4a
f1 (ppm)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COOH
Se
S
4b
8.21
8.20
8.18
8.18
8.17
8.17
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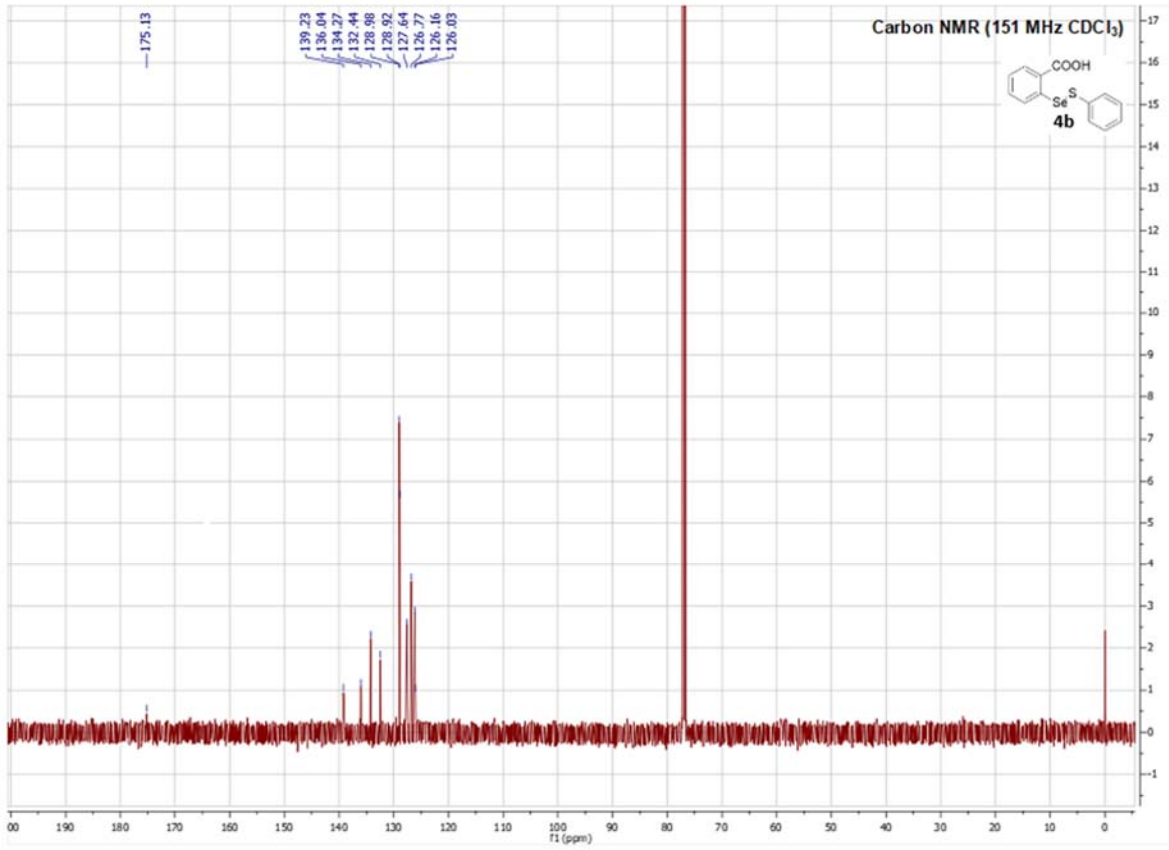
7.56
7.55
7.54
7.50
7.48
7.35
7.34
7.33
7.25
7.24
7.22
7.17
7.16
7.15
1.00
1.06
1.10
1.97
1.13
2.90
0.96
f1 (ppm)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COOH
Se
S
4b
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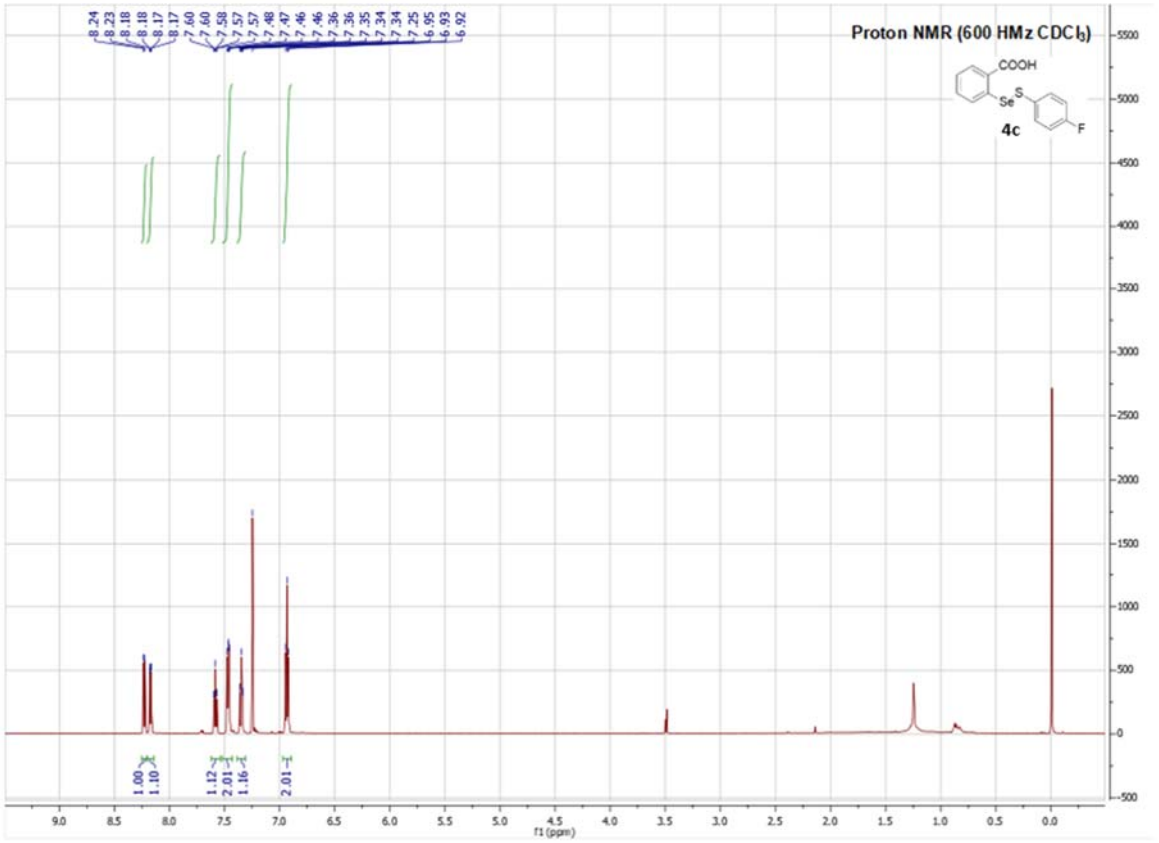
127.64
126.77
126.16
126.03
f1 (ppm)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COOH
Se
S
F
4c
8.24
8.23
8.18
8.18
8.17
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7.60
7.58
7.57
7.57
7.48
7.47
7.46
7.46
7.36
7.36
7.35
7.34
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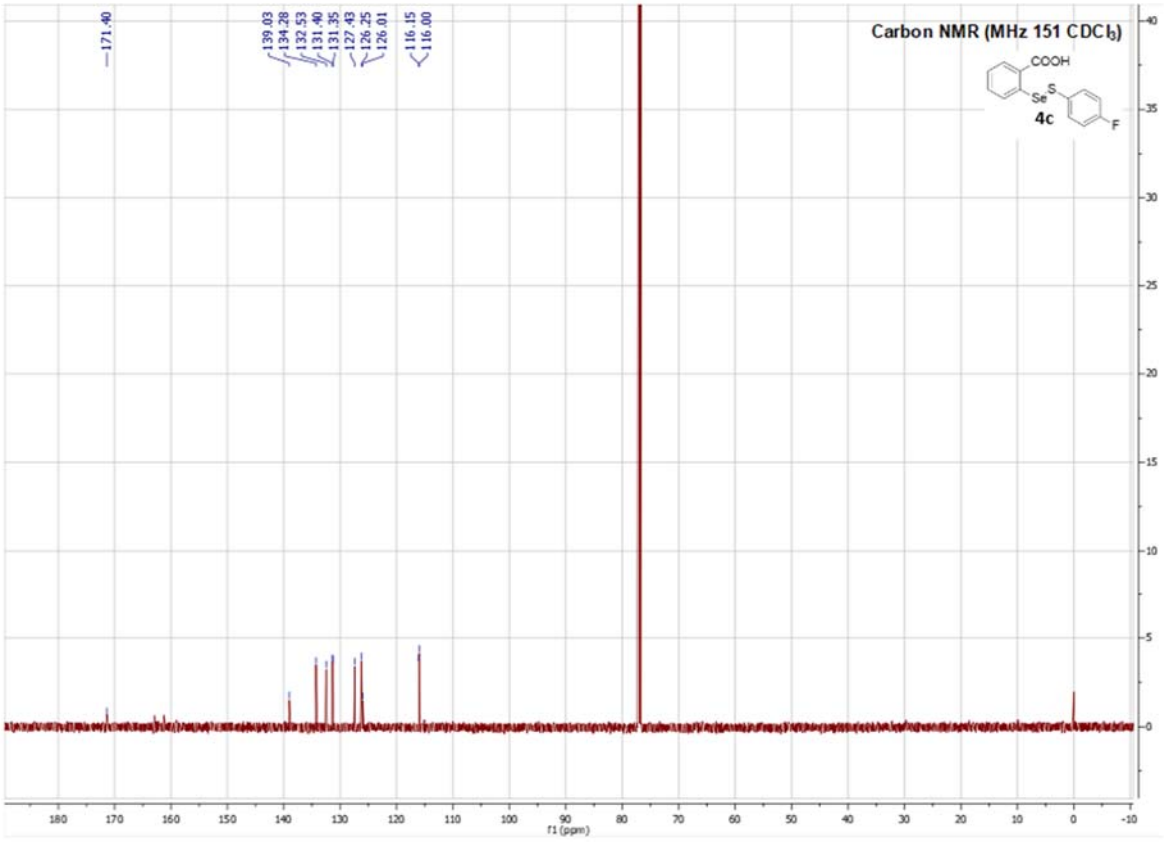
1.12
2.01
1.16
2.01
f1 (ppm)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COOH
Se
S
F
4c
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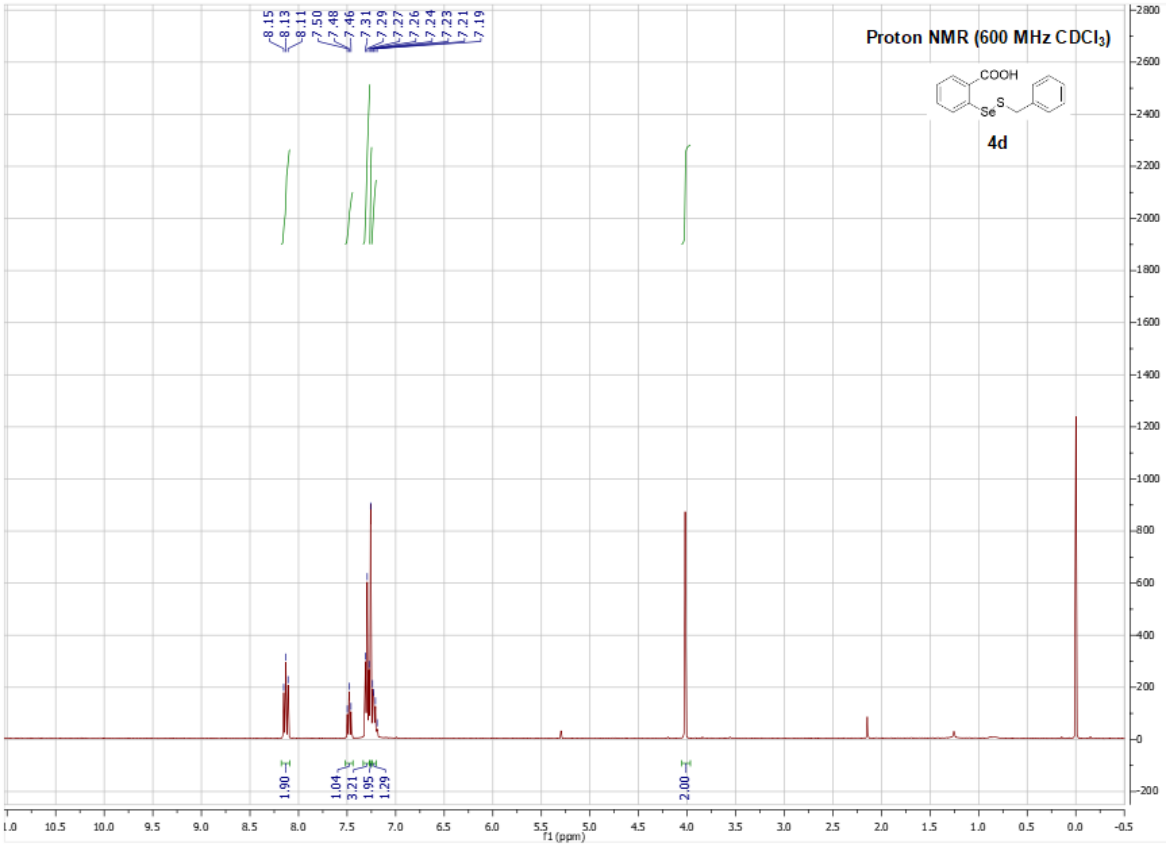
126.01
116.15
116.00
f1 (ppm)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COOH
Se
S
4d
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8.13
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7.48
7.46
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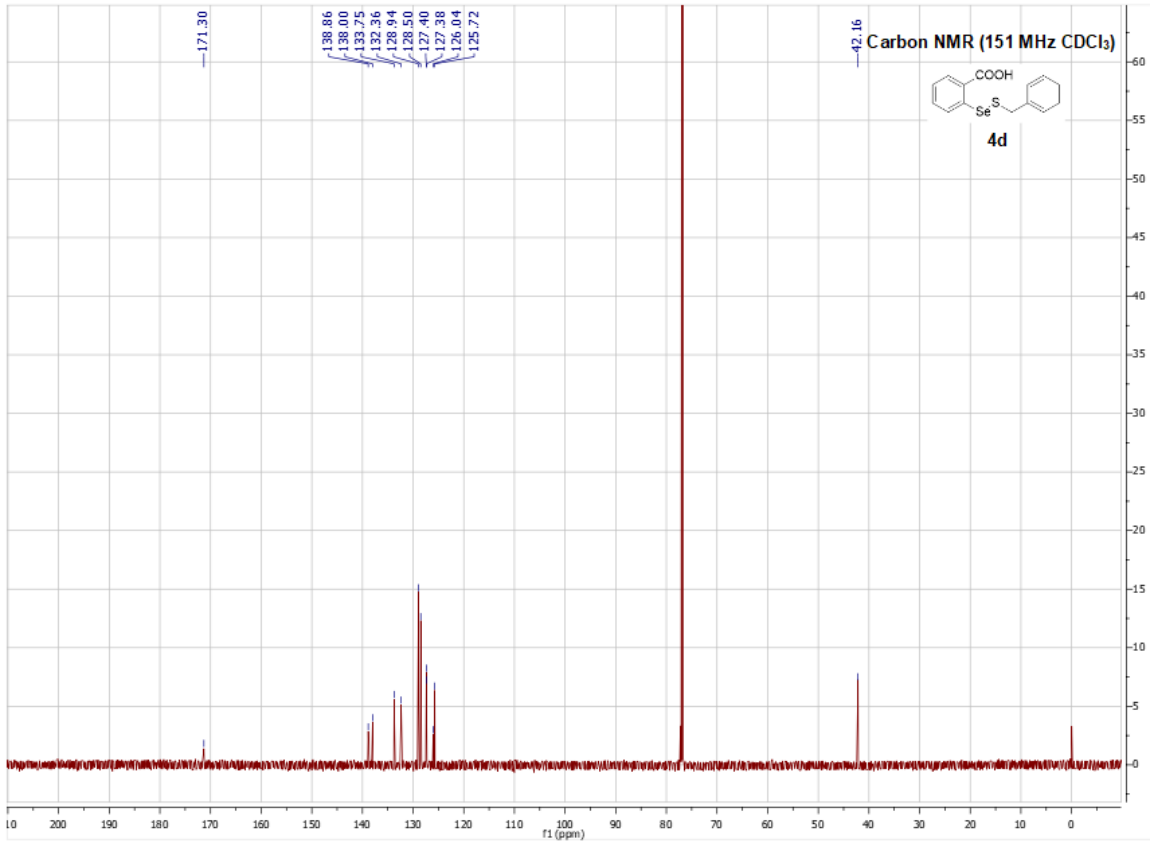
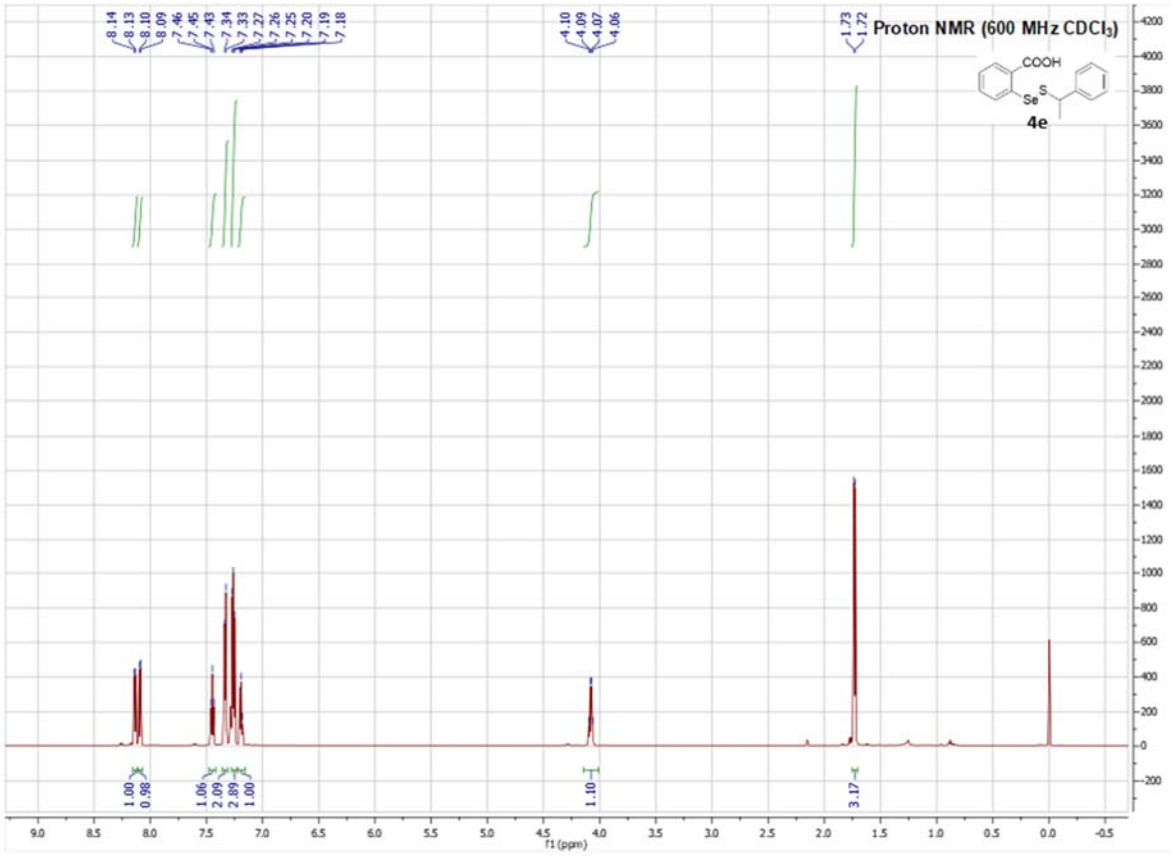
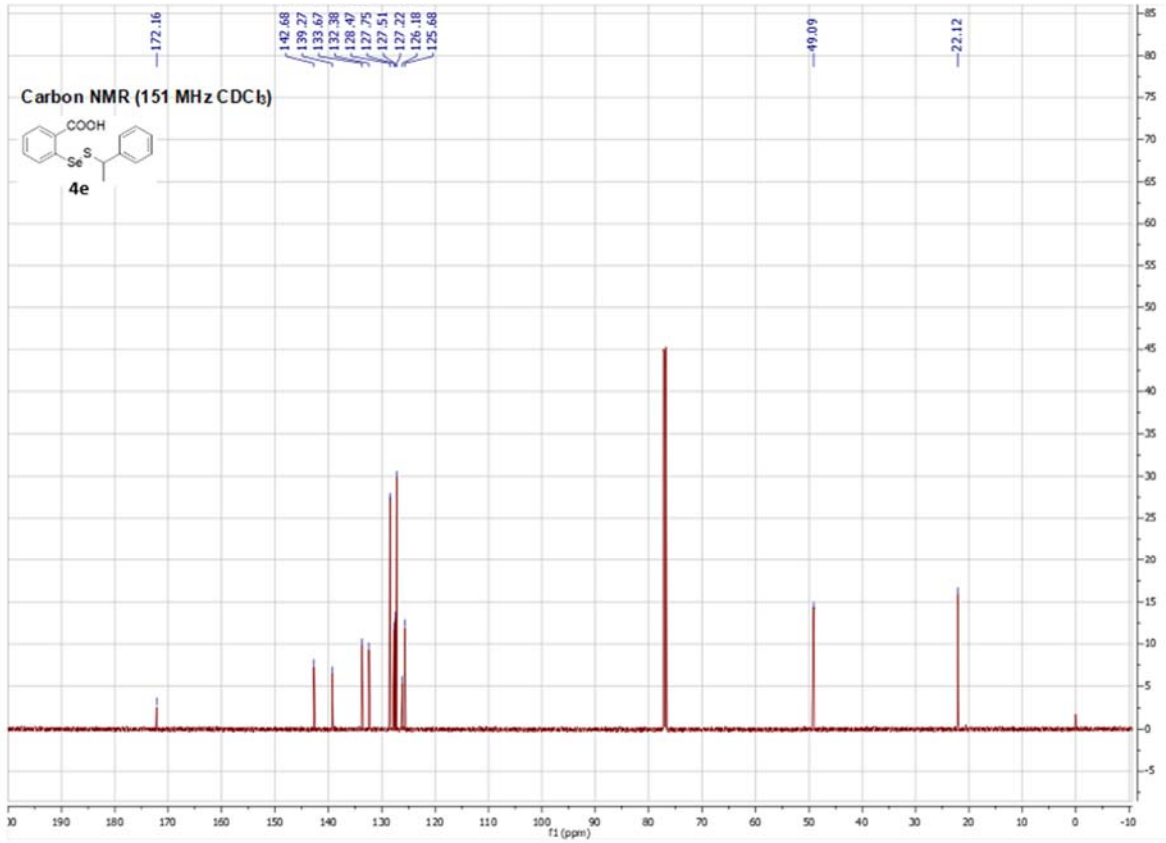
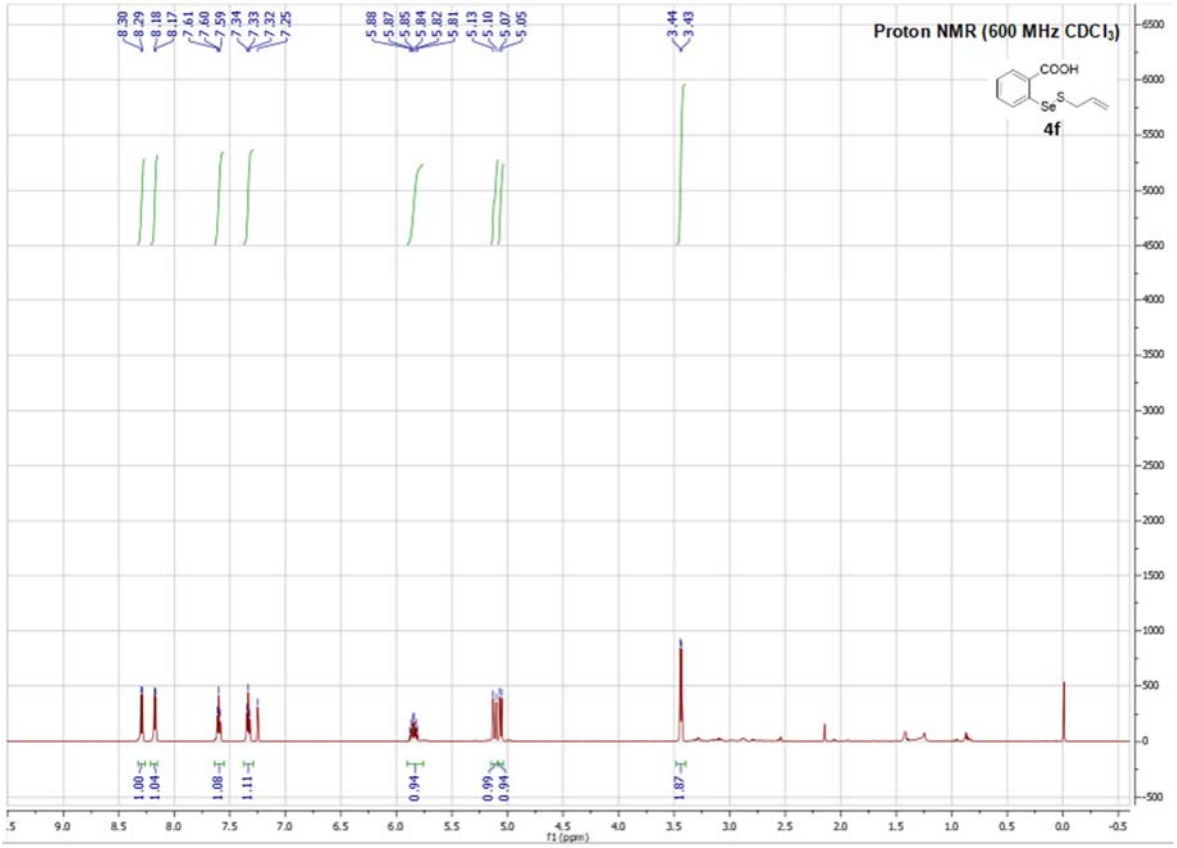
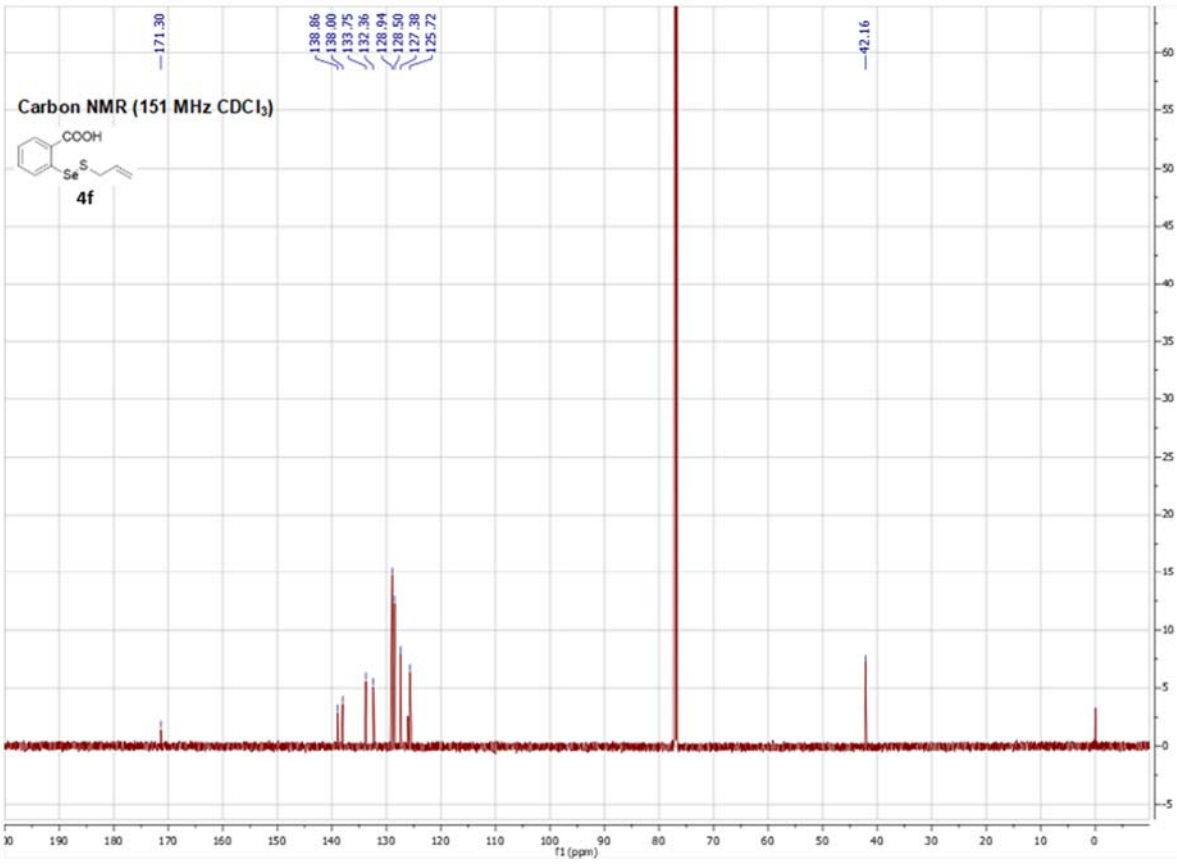
7.27
7.26
7.24
7.23
7.21
7.19
1.90
1.04
3.21
1.95
1.29
2.00
f1 (ppm)

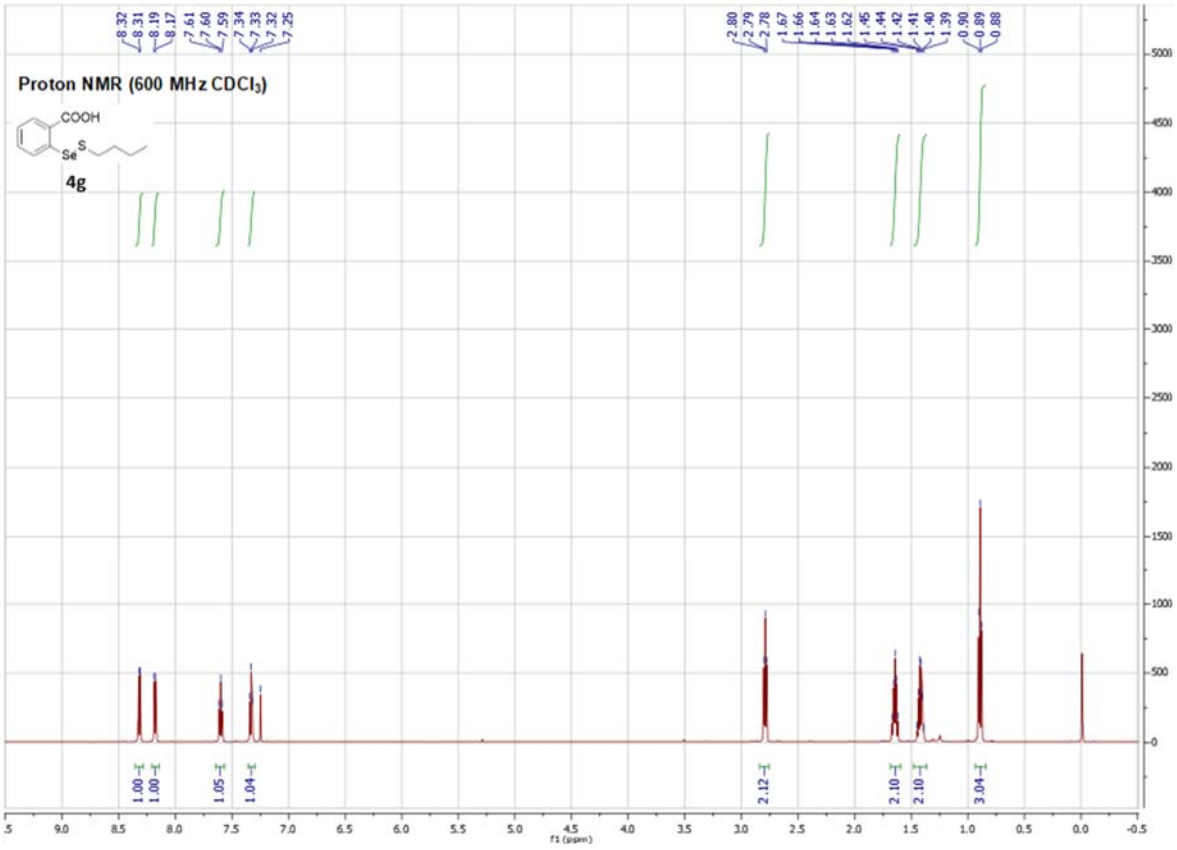

Carbon NMR (151 MHz CDCl3)
COOH
Se
S
4d
171.30
138.86
138.00
133.75
132.36
128.94
128.50
127.40
127.38
126.04
125.72
42.16
f1 (ppm)

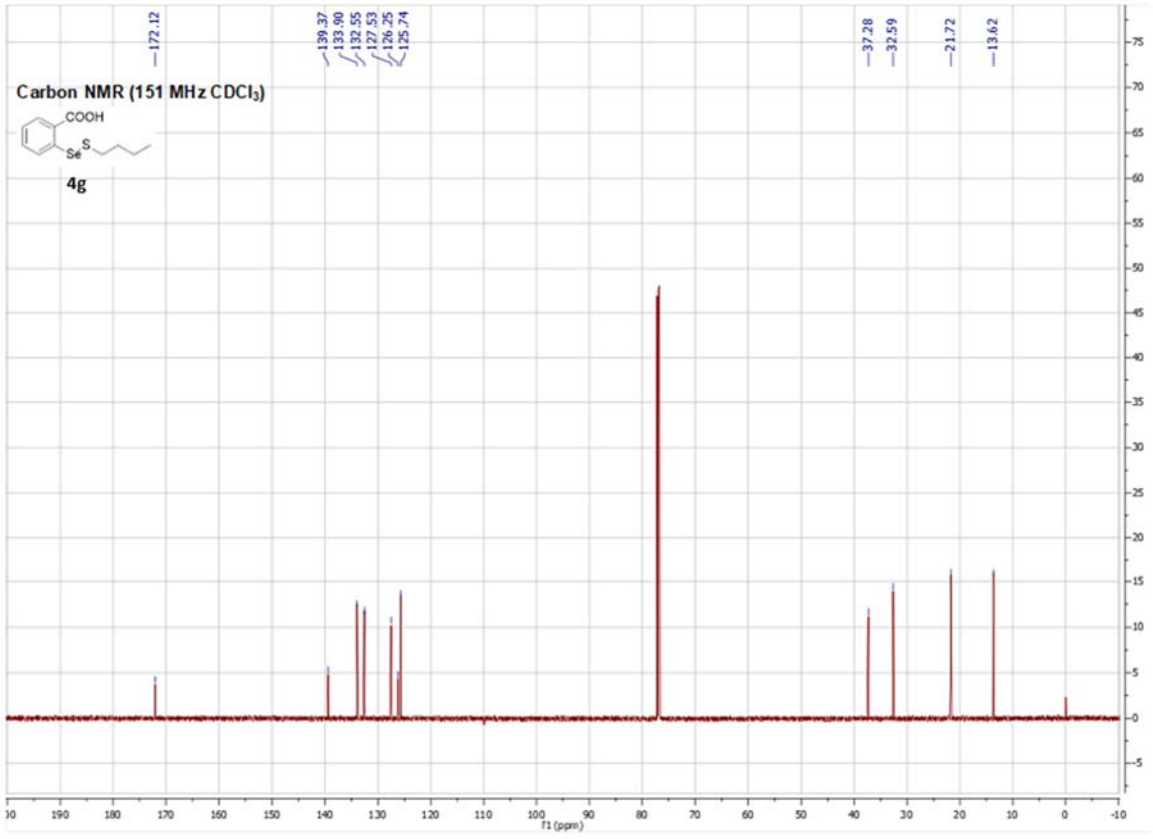

Proton NMR (600 MHz CDCl3)
4e

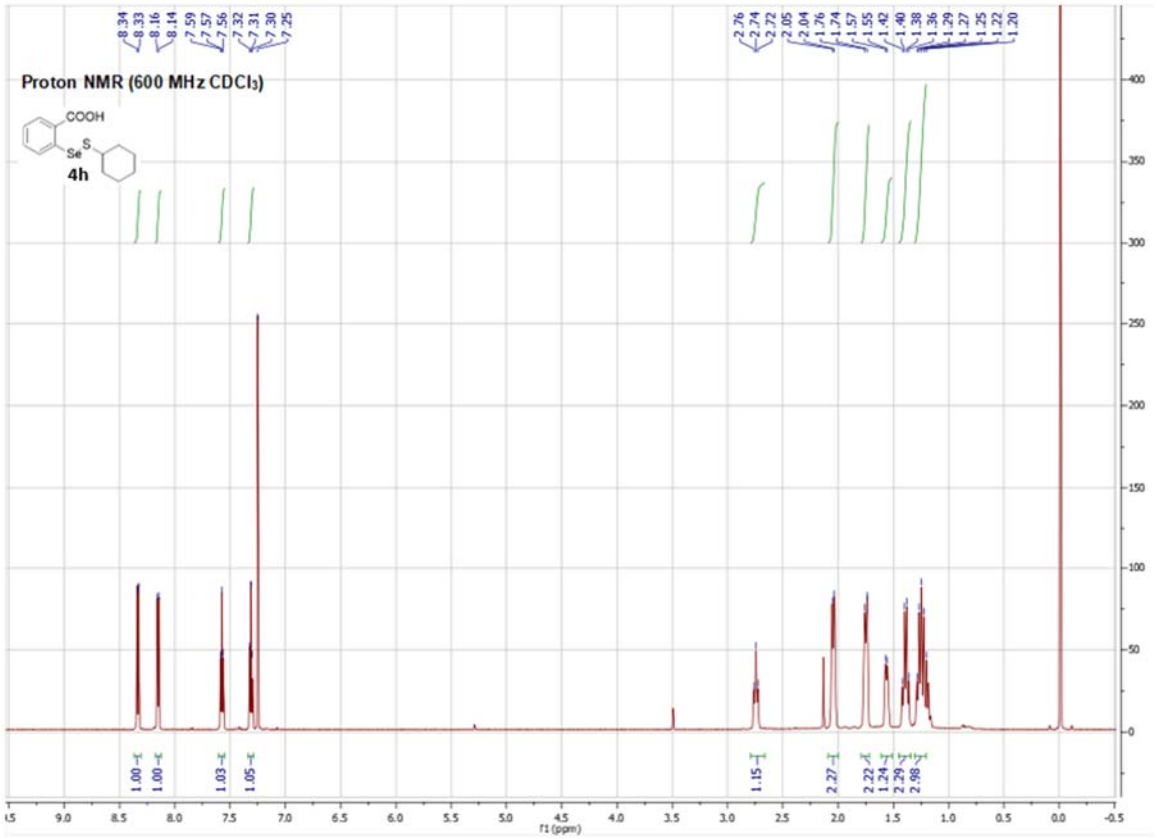

Carbon NMR (151 MHz CDCl3)
4e

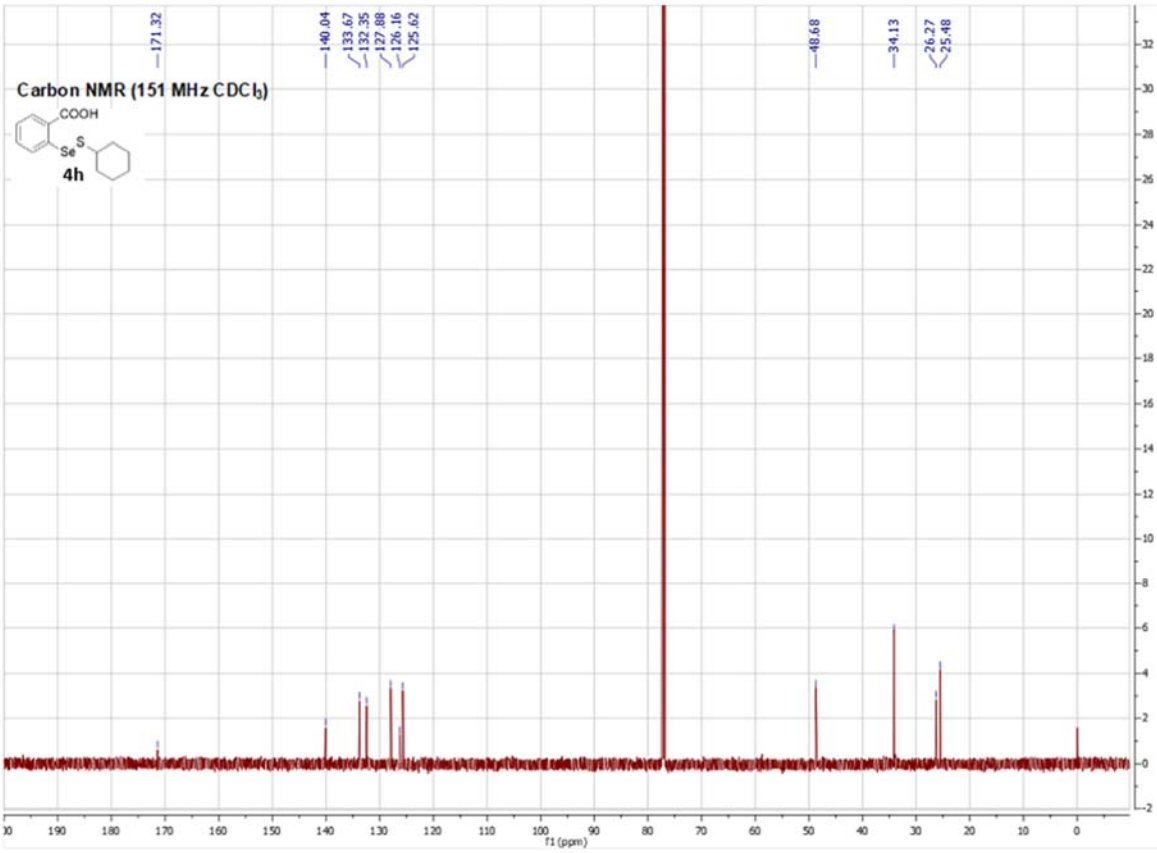

Proton NMR (600 MHz CDCl3)
COOH
Se
S
4f
8.30
8.29
8.18
8.17
7.61
7.60
7.59
7.34
7.33
7.32
7.25
5.88
5.87
5.85
5.84
5.82
5.81
5.13
5.10
5.07
5.05
3.44
3.43
1.00
1.04
1.08
1.11
0.94
0.99
0.94
1.87
f1 (ppm)

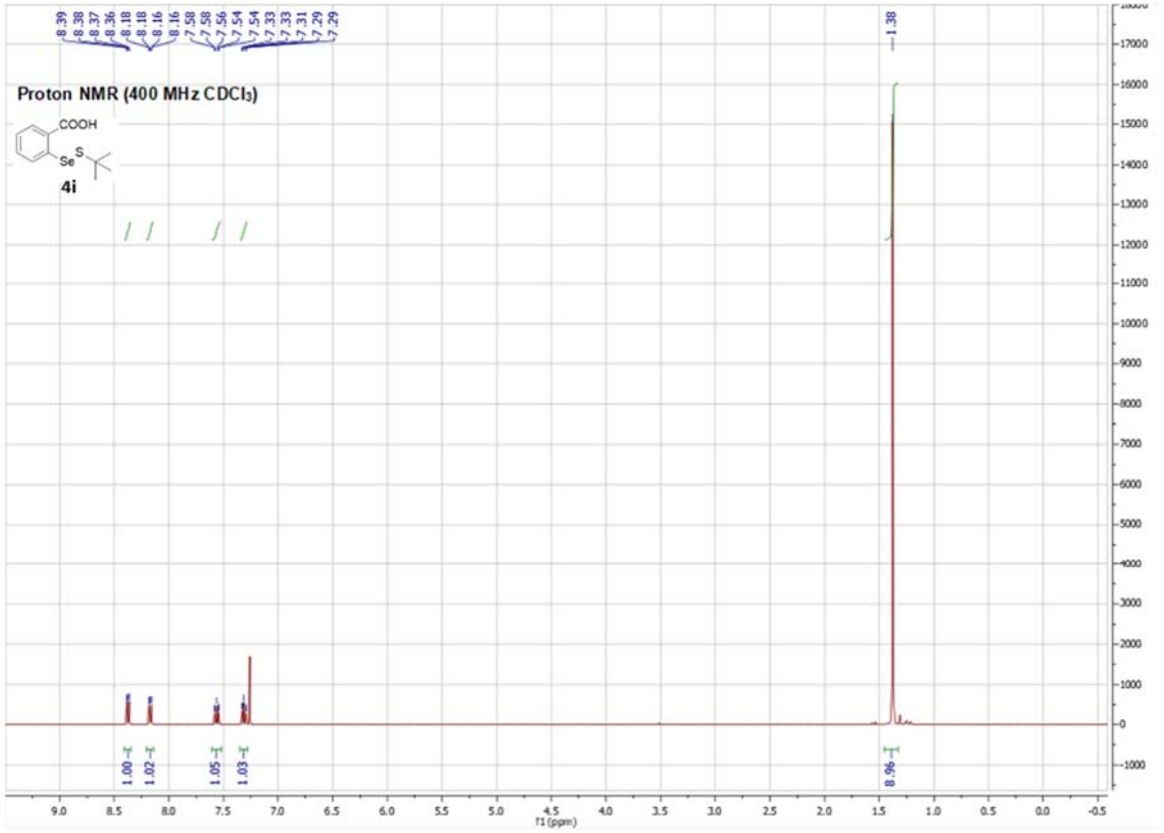

Carbon NMR (151 MHz CDCl3)
COOH
Se
S
4f
171.30
138.86
138.00
133.75
132.36
128.94
128.50
127.38
125.72
42.16
f1 (ppm)

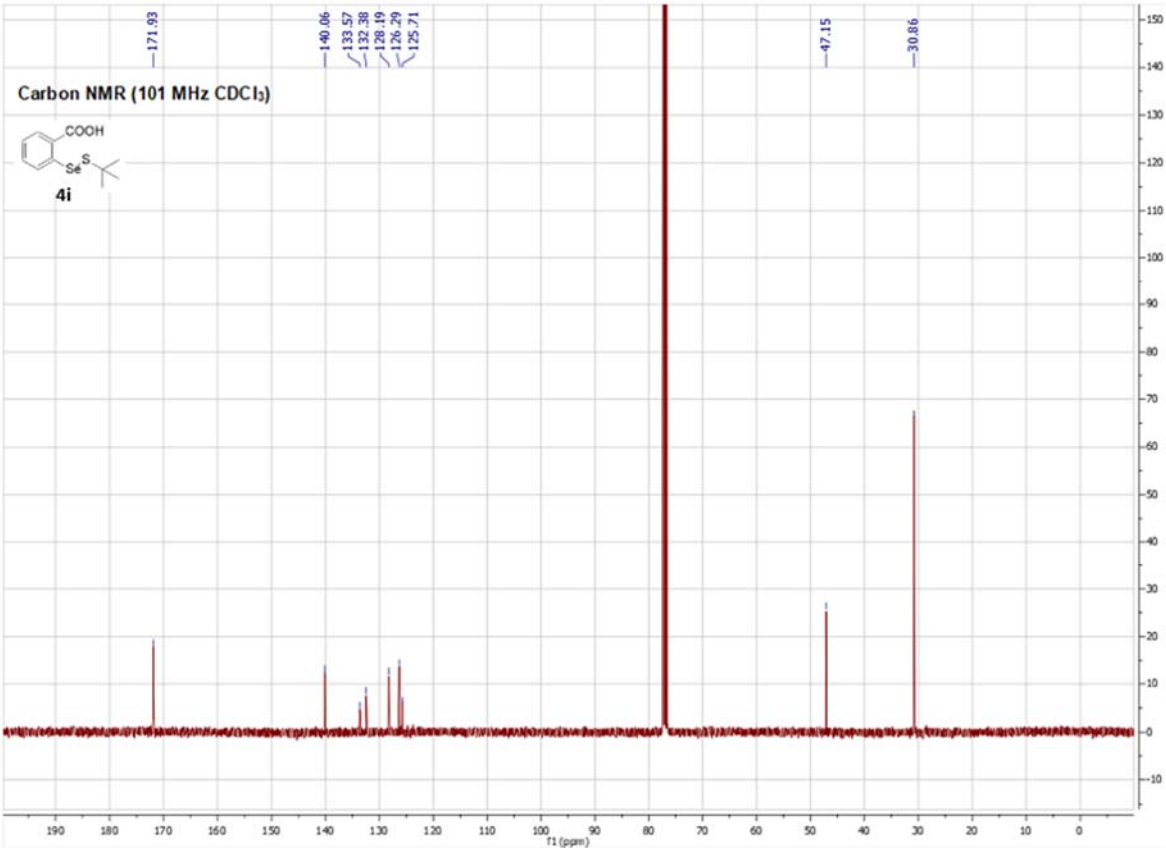

Proton NMR (600 MHz CDCl3)
COOH
Se
S
4g
8.32
8.31
8.19
8.17
7.61
7.60
7.59
7.34
7.33
7.32
7.25
2.80
2.79
2.78
1.67
1.66
1.64
1.63
1.62
1.45
1.44
1.42
1.41
1.40
1.39
0.90
0.89
0.88
1.00
1.00
1.05
1.04
2.12
2.10
2.10
3.04
f1 (ppm)

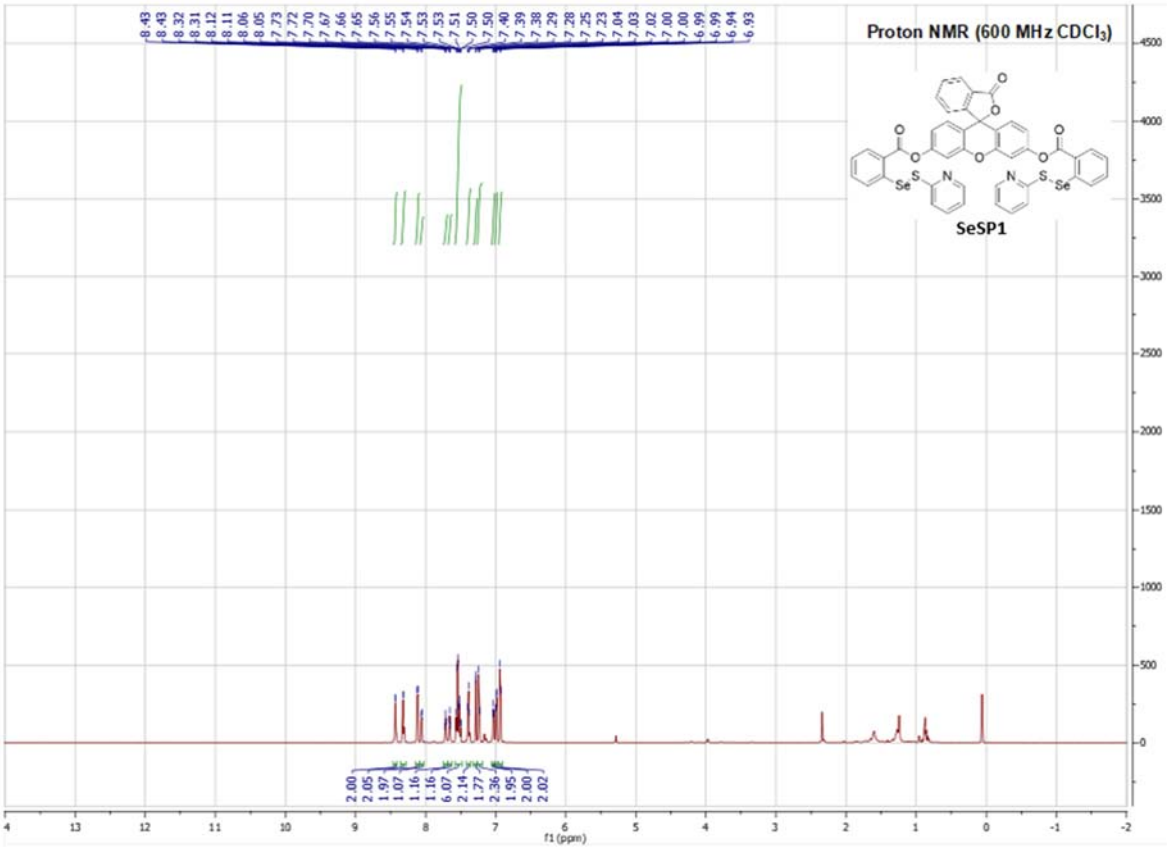

Carbon NMR (151 MHz CDCl3)
COOH
Se
S
4g
172.12
139.37
133.90
132.55
127.53
126.25
125.74
37.28
32.59
21.72
13.62
f1 (ppm)

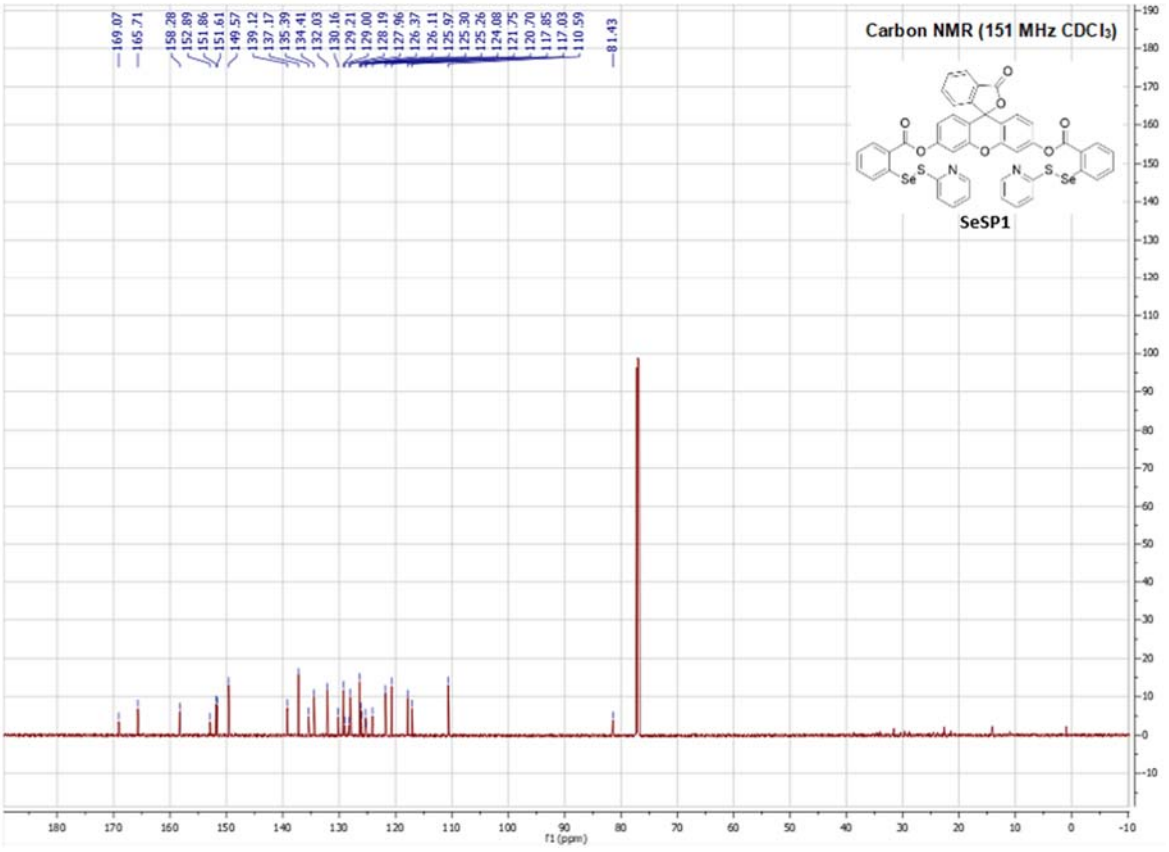

Proton NMR (600 MHz CDCl3)
COOH
Se
S
4h
8.34
8.33
8.16
8.14
7.59
7.57
7.56
7.32
7.31
7.30
7.25
2.76
2.74
2.72
2.05
2.04
1.76
1.74
1.57
1.55
1.42
1.40
1.38
1.36
1.29
1.27
1.25
1.22
1.20
1.00
1.00
1.03
1.05
1.15
2.27
2.22
1.24
2.29
2.98
f1 (ppm)

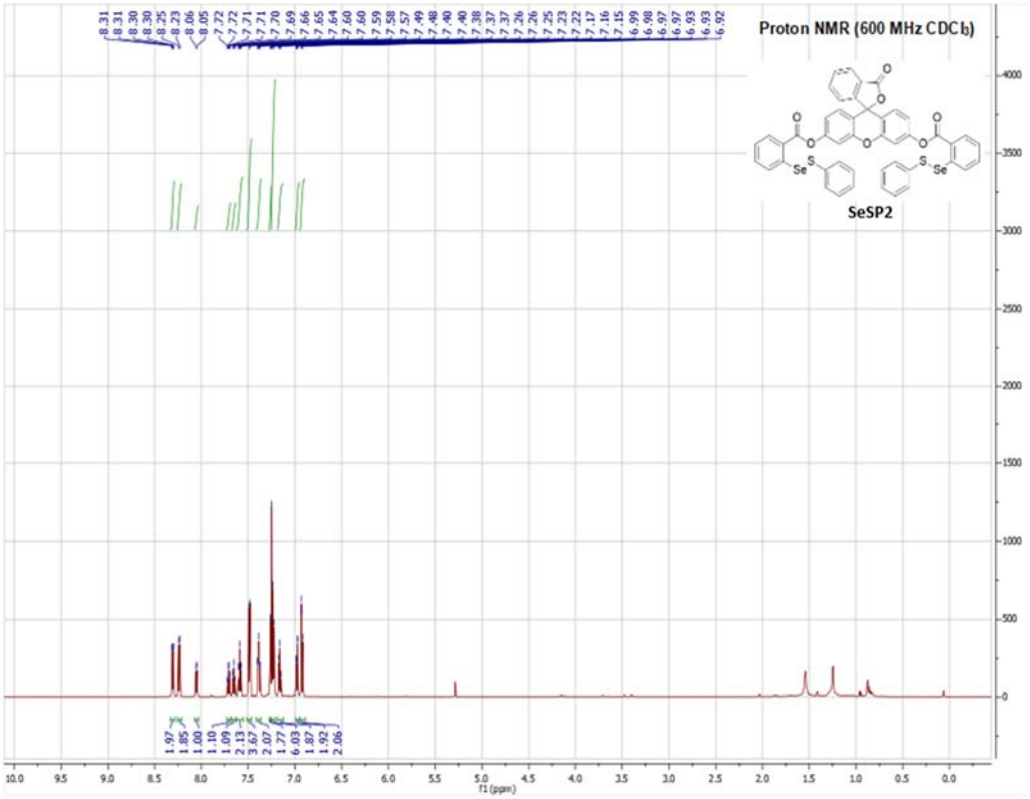

Carbon NMR (151 MHz CDCl3)
COOH
Se
S
4h
171.32
140.04
133.67
132.35
127.88
126.16
125.62
48.68
34.13
26.27
25.48
f1 (ppm)

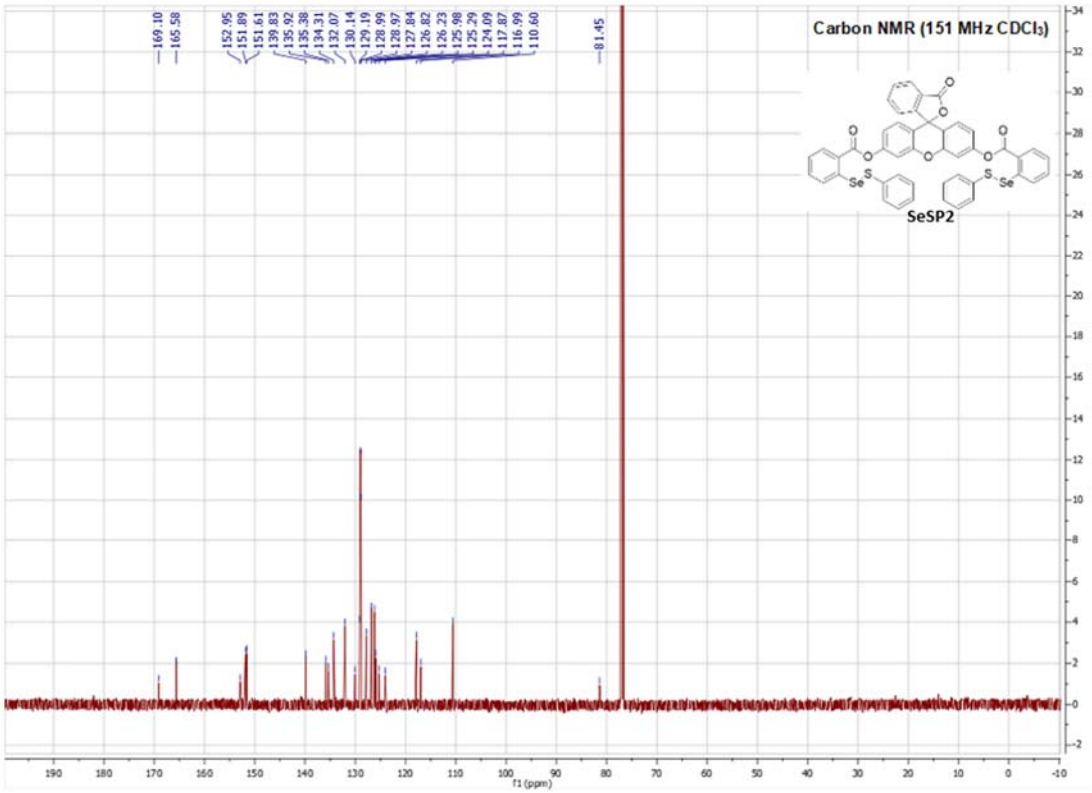

Proton NMR (400 MHz CDCl3)
4i
8.39
8.38
8.37
8.36
8.18
8.18
8.16
8.16
7.58
7.58
7.56
7.54
7.54
7.33
7.31
7.29
7.29
1.38
1.00
1.02
1.05
1.03
8.96
f1 (ppm)

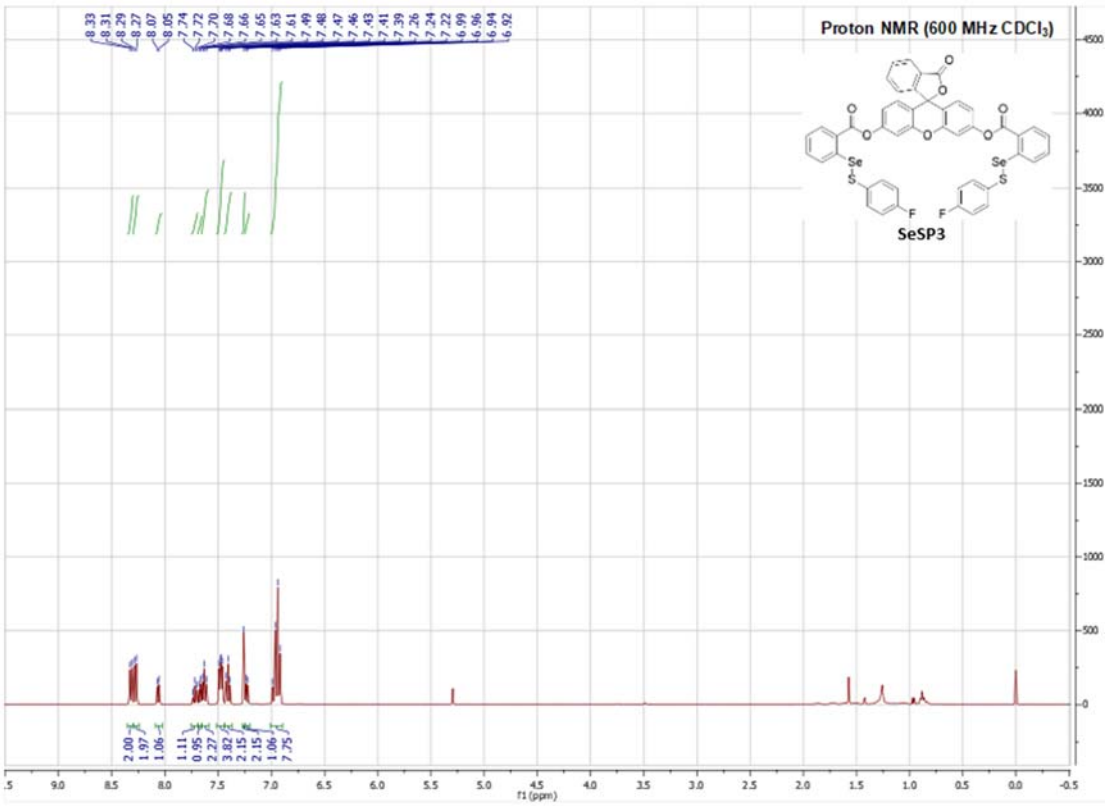

Carbon NMR (101 MHz CDCl3)
4i
171.93
140.06
133.57
132.38
128.19
126.29
125.71
47.15
30.86
f1 (ppm)

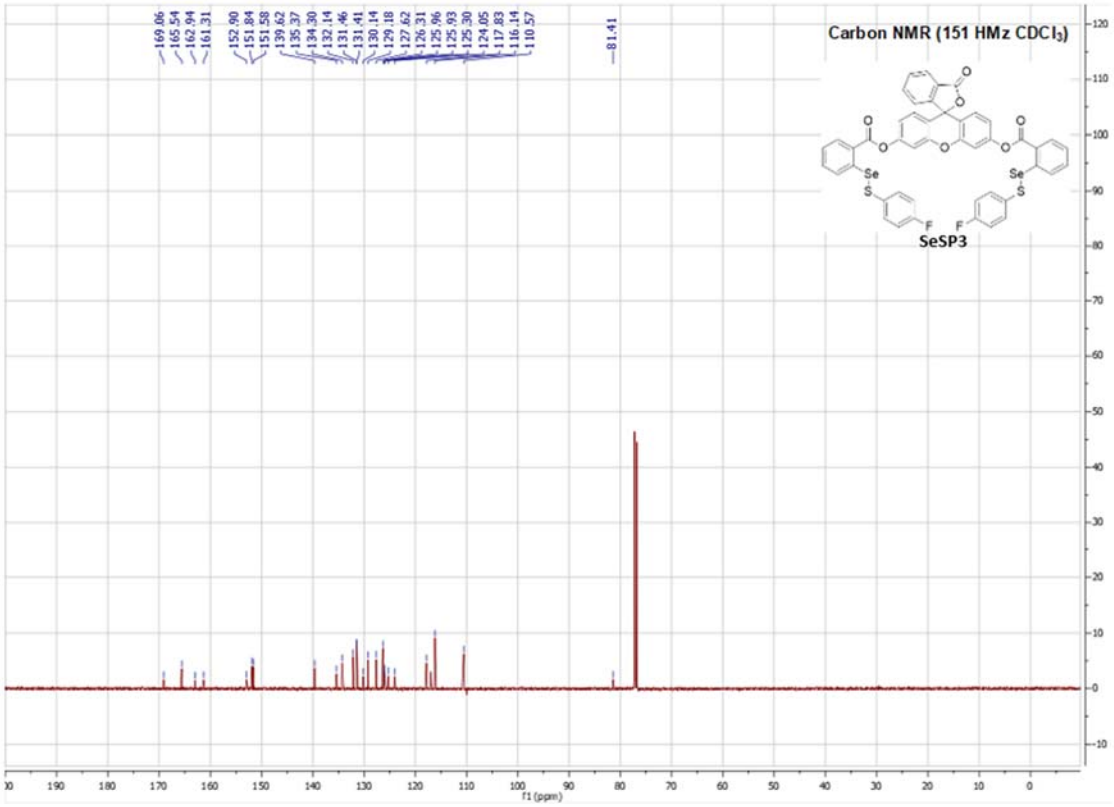

Proton NMR (600 MHz CDCl3)
SeSP1

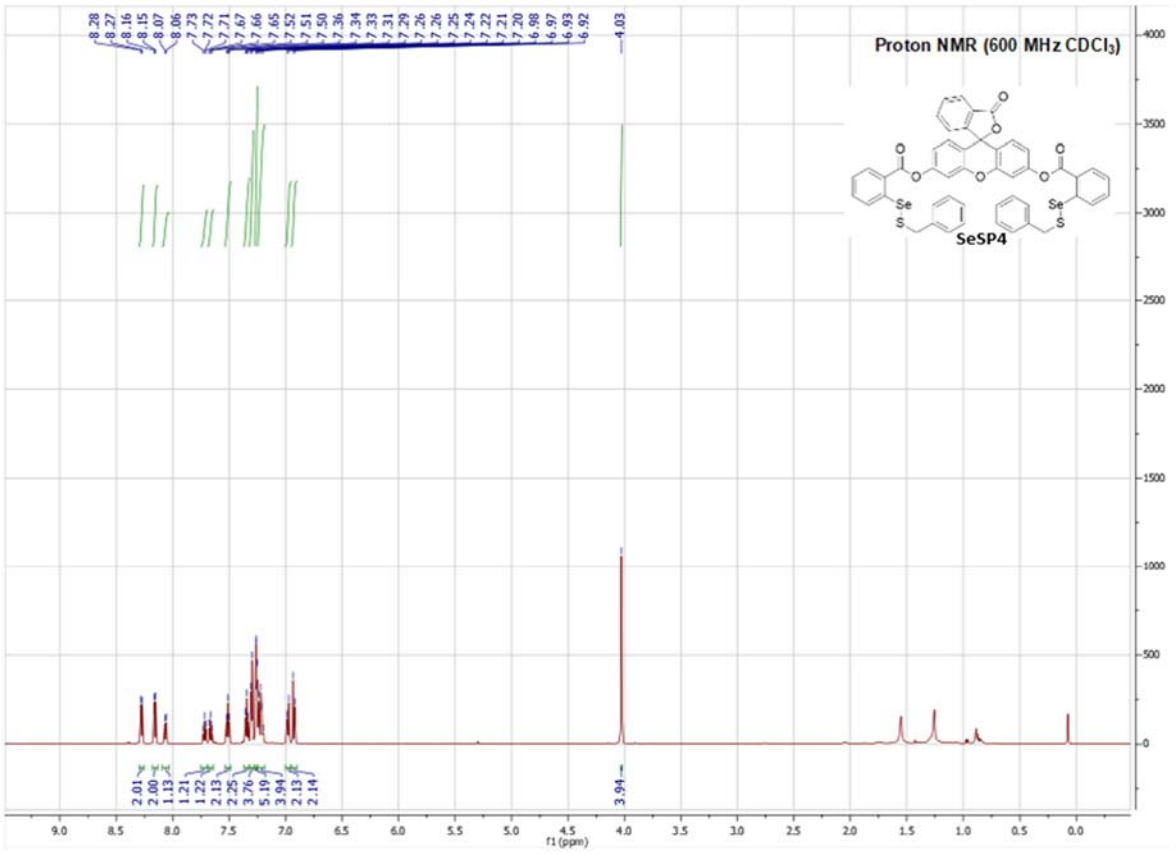

Carbon NMR (151 MHz CDCl3)
SeSP1

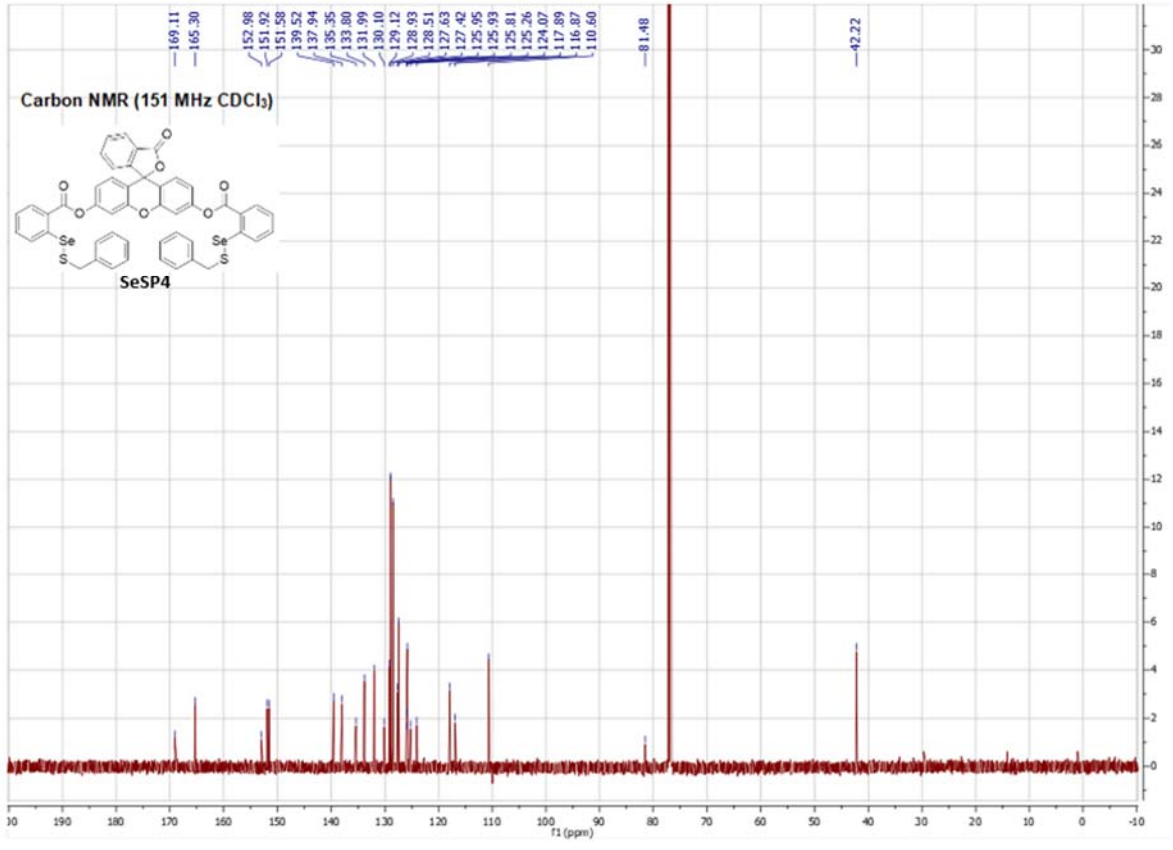

Proton NMR (600 MHz CDCl3)
SeSP2

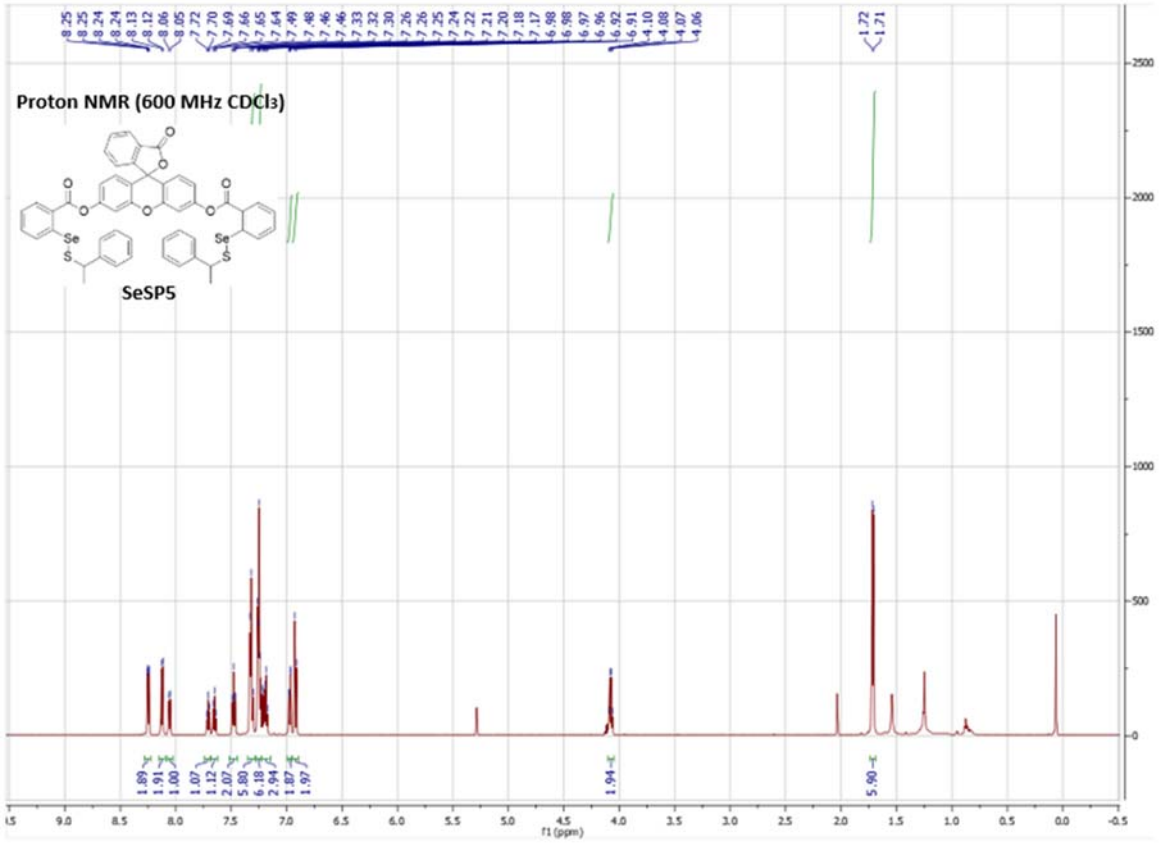

Carbon NMR (151 MHz CDCl3)
SeSP2

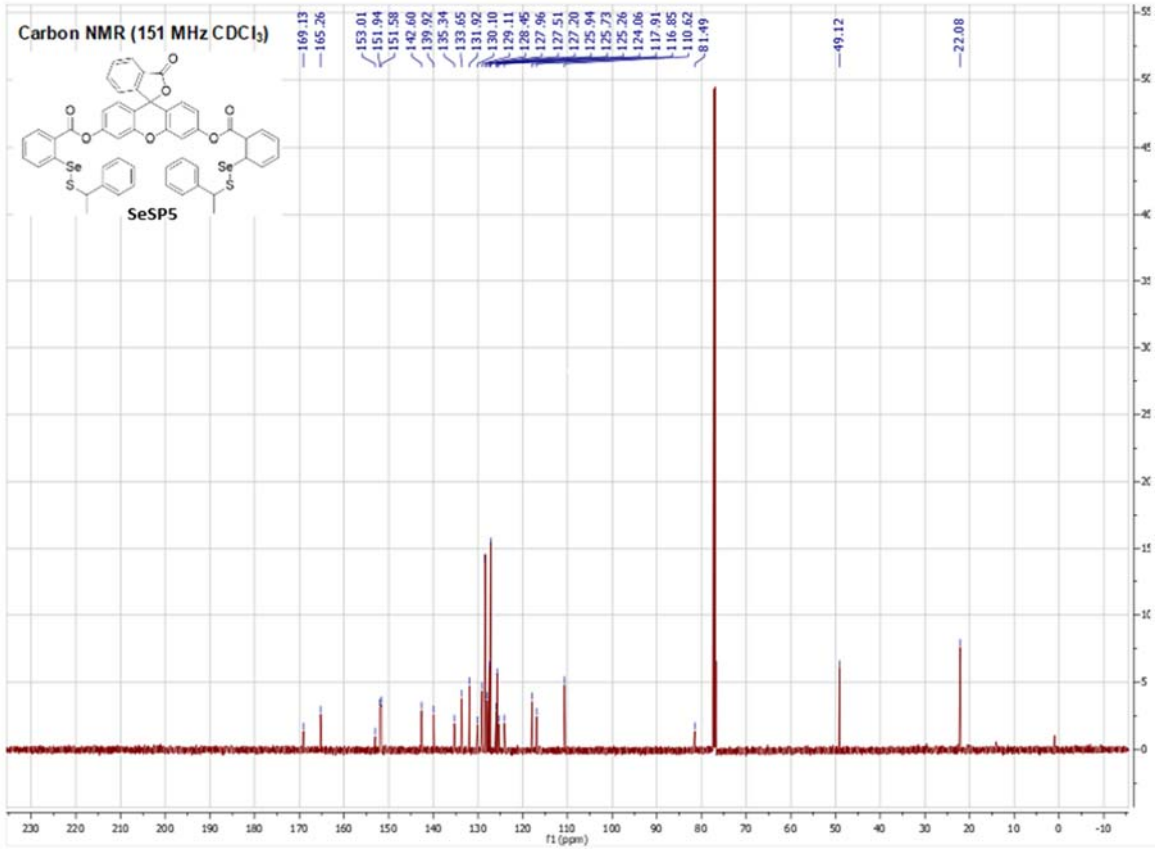

Proton NMR (600 MHz CDCl3)
SeSP3

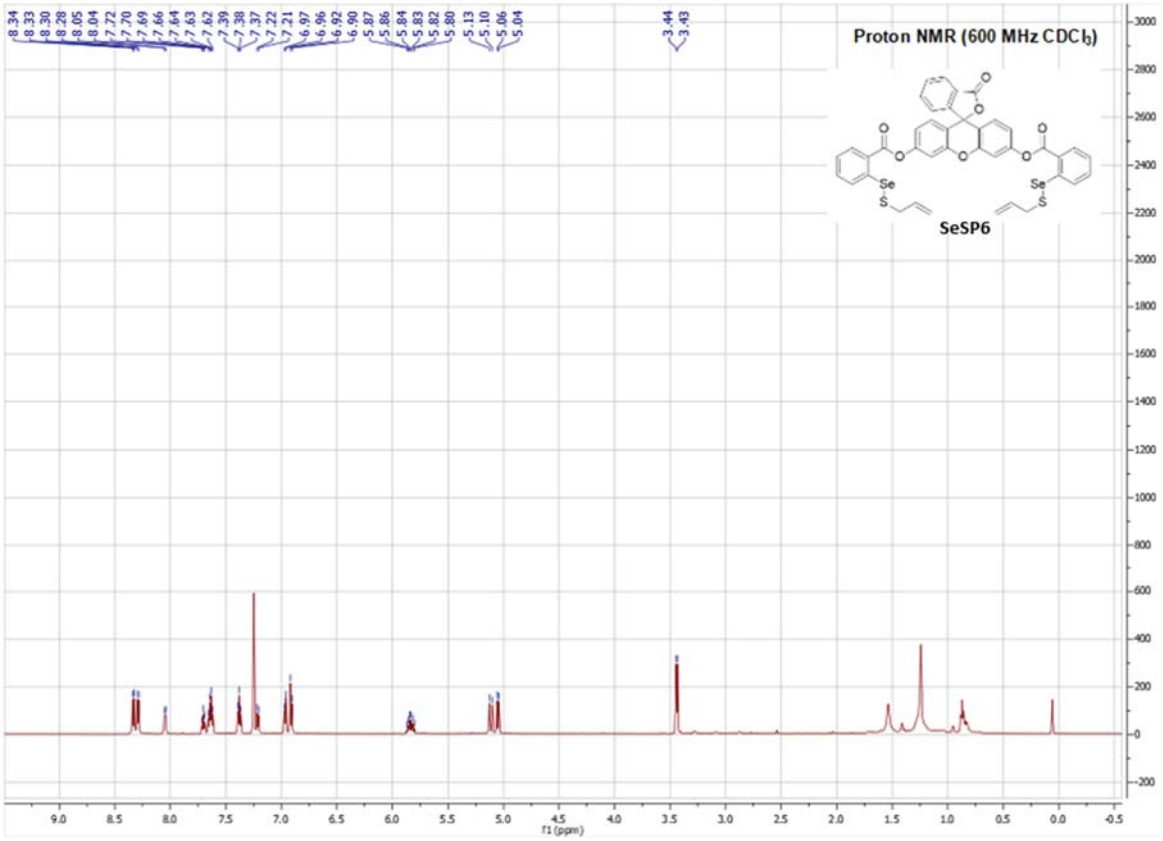

Carbon NMR (151 HMz CDCl3)
SeSP3

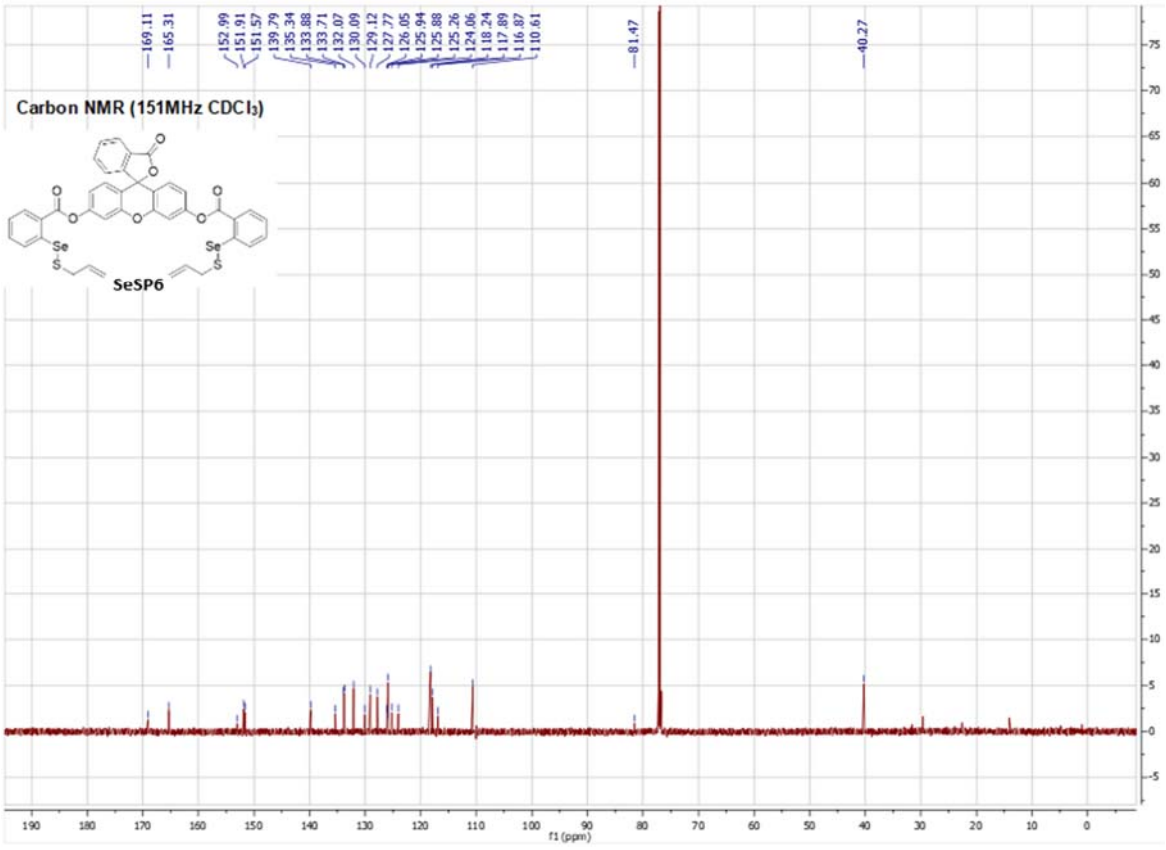

Proton NMR (600 MHz CDCl3)
SeSP4

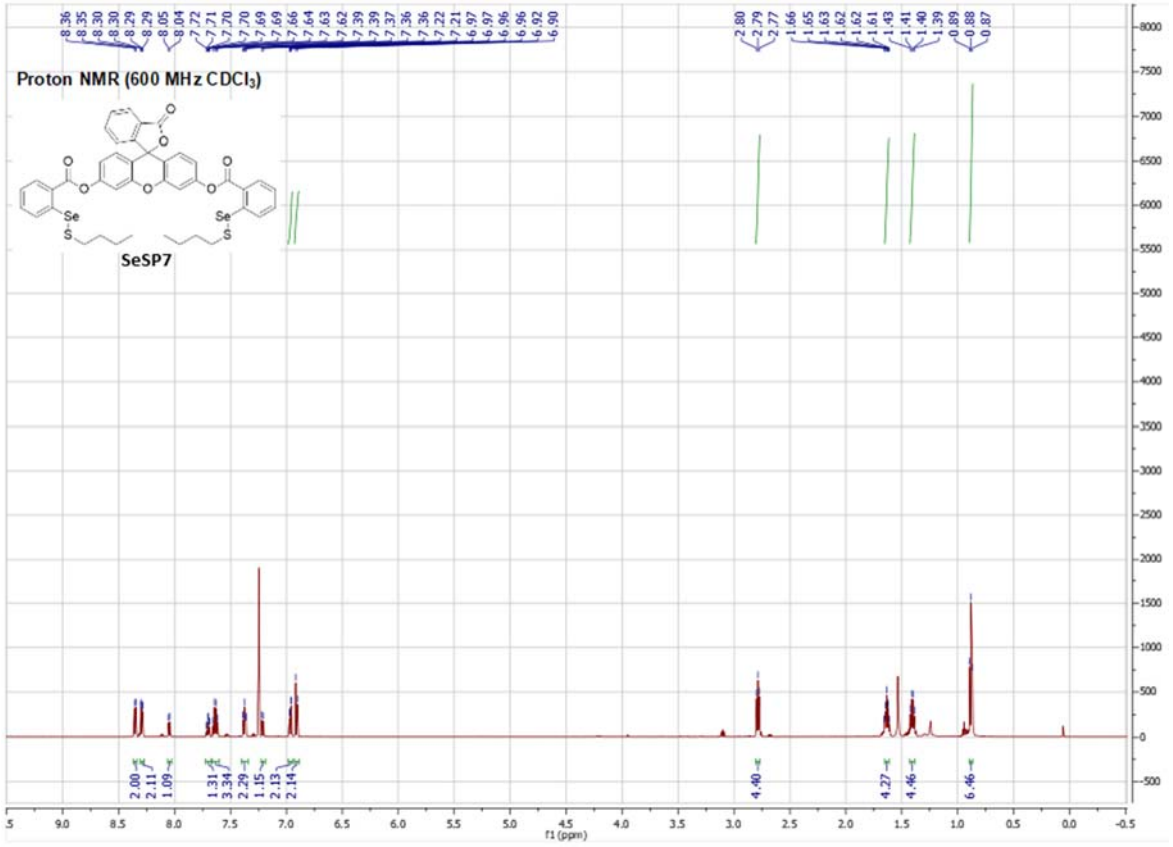

Carbon NMR (151 MHz CDCl3)
SeSP4

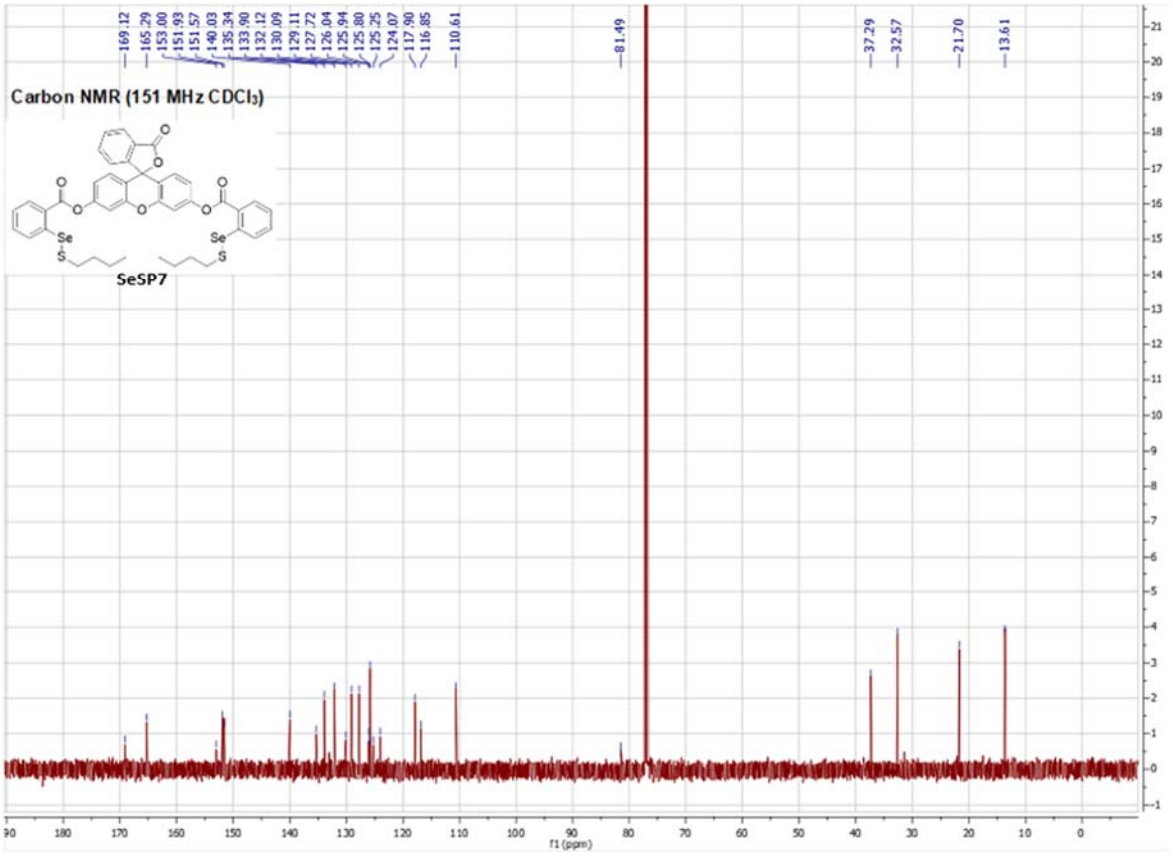

Proton NMR (600 MHz CDCl3)
SeSP5

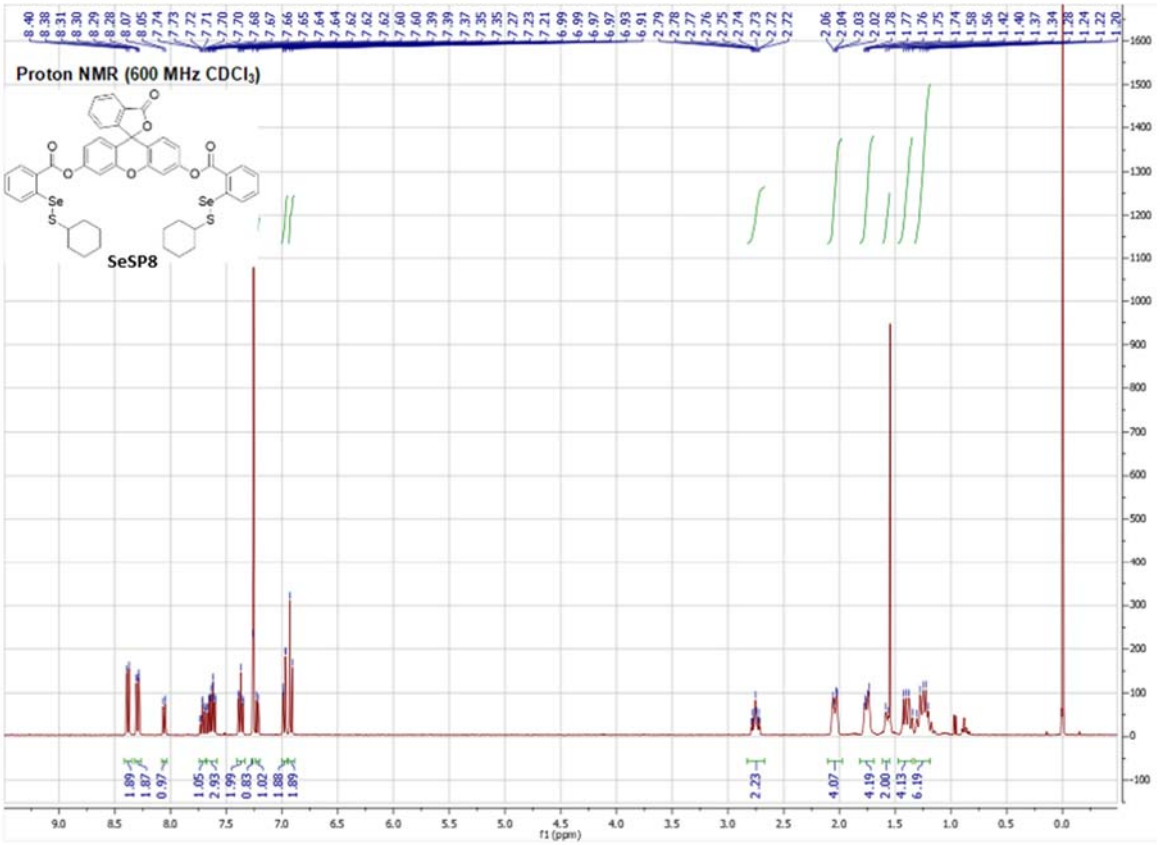

Carbon NMR (151 MHz CDCl3)
SeSP5

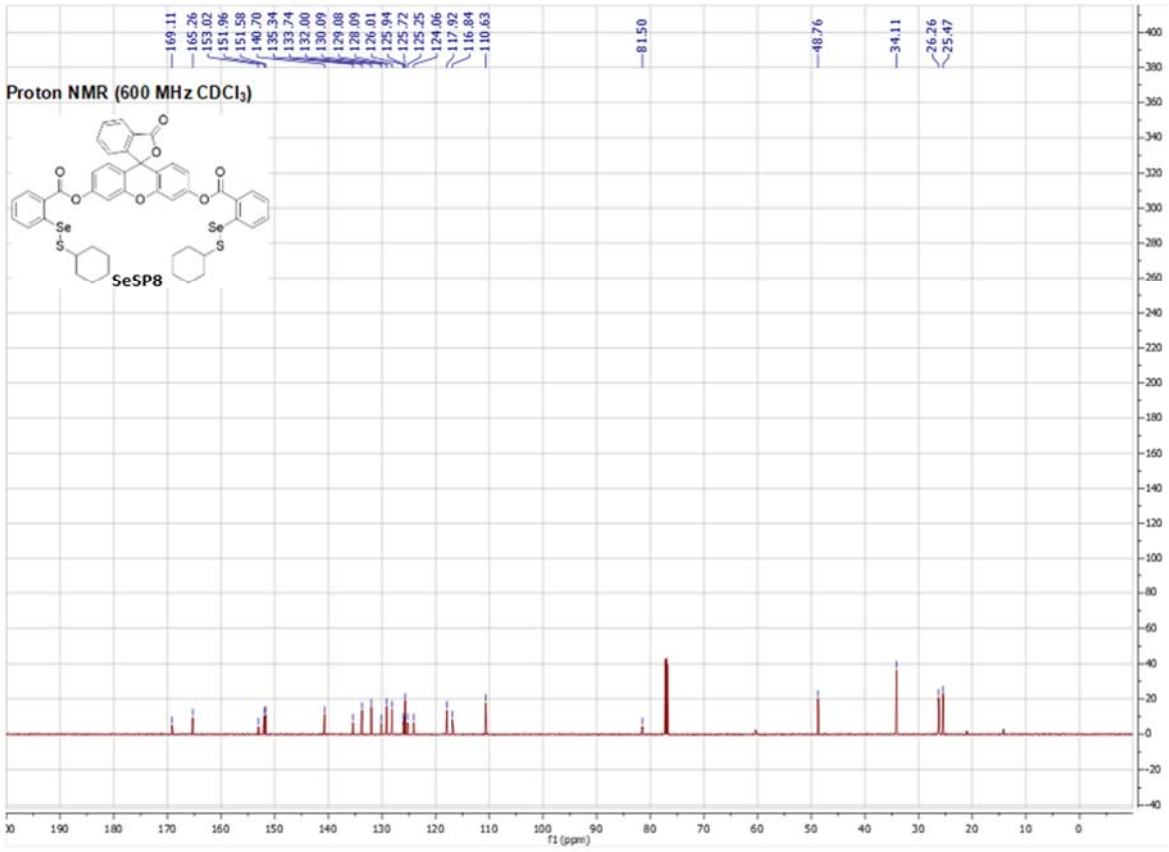

Proton NMR (600 MHz CDCl3)
SeSP6

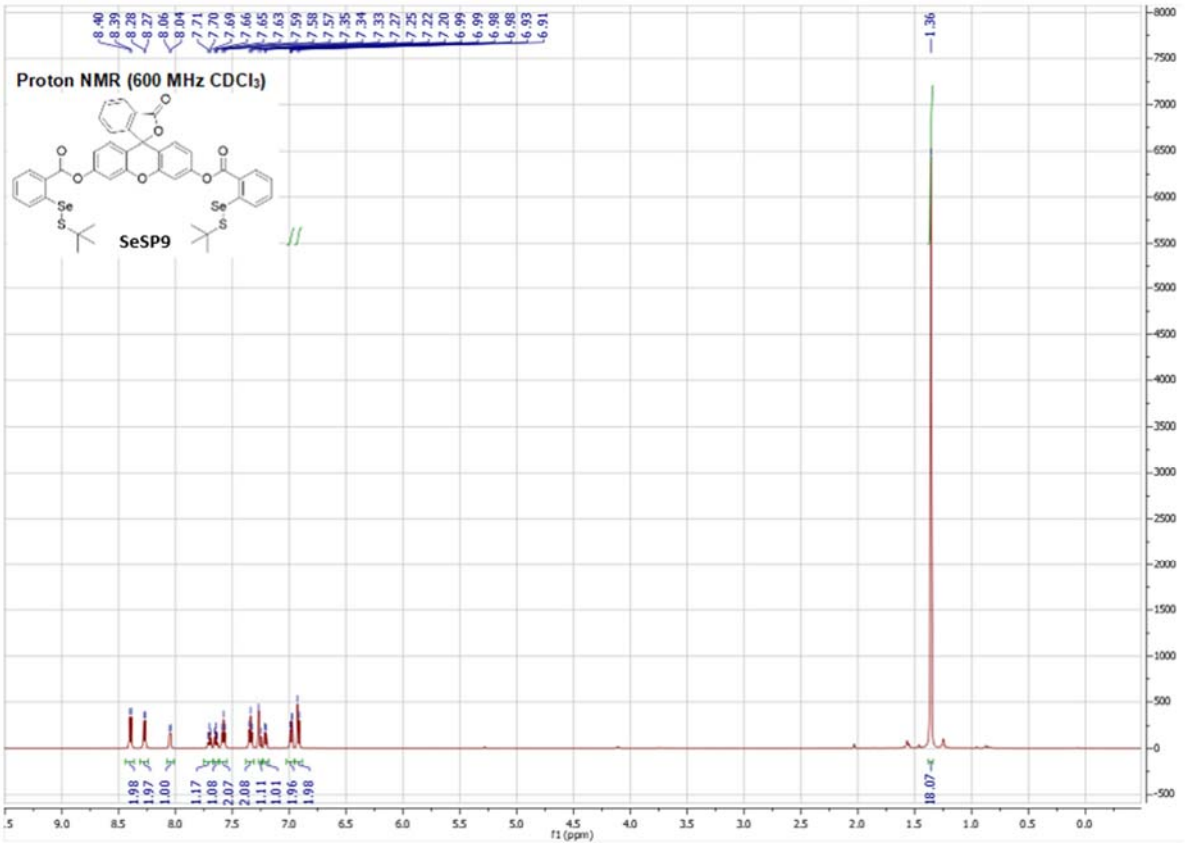

Carbon NMR (151MHz CDCl3)
SeSP6


Proton NMR (600 MHz CDCl3)
SeSP7
f1 (ppm)


Carbon NMR (151 MHz CDCl3)
SeSP7
f1 (ppm)


Proton NMR (600 MHz CDCl3)
SeSP8


Proton NMR (600 MHz CDCl3)
SeSP8


Proton NMR (600 MHz CDCl3)
SeSP9
8.40
8.39
8.28
8.27
8.06
8.04
7.71
7.70
7.69
7.66
7.65
7.63
7.59
7.58
7.57
7.35
7.34
7.33
7.27
7.25
7.22
7.20
6.99
6.98
6.93
6.91
1.36
1.98
1.97
1.00
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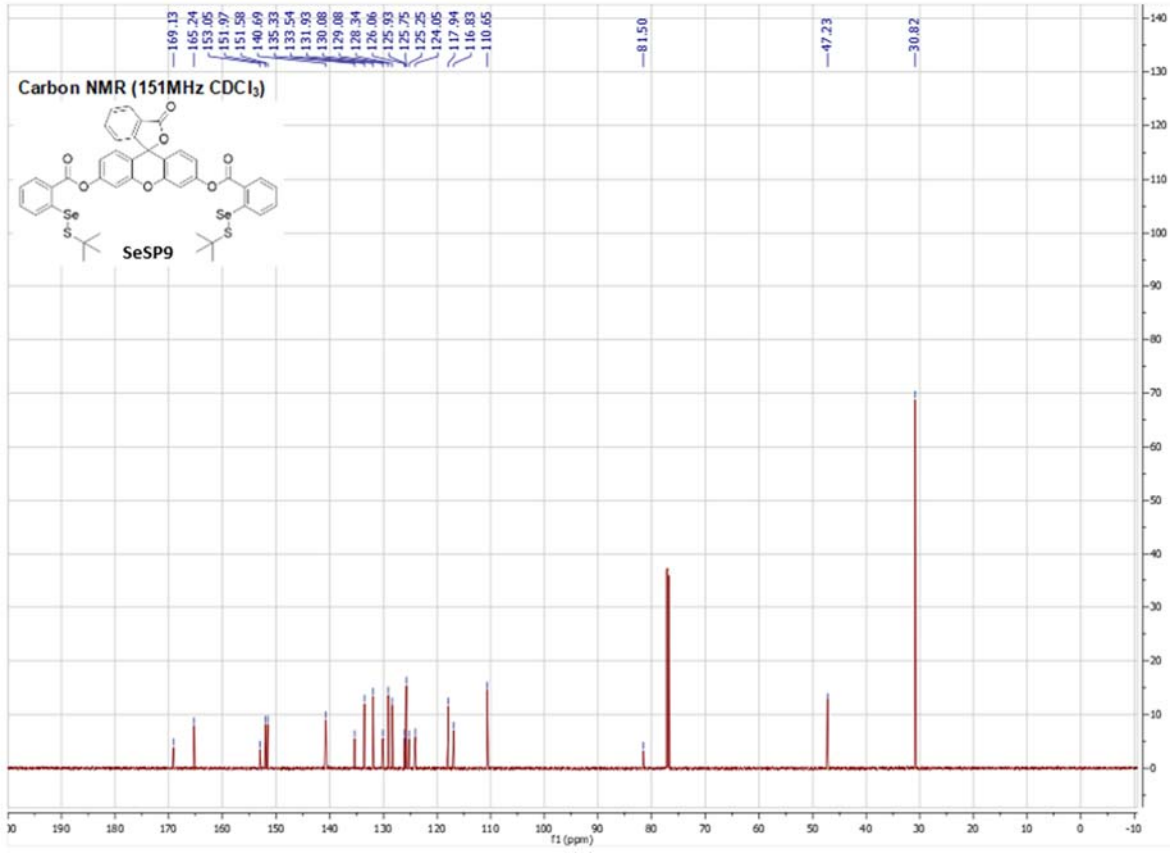
1.08
2.07
2.08
1.11
1.01
1.96
1.98
18.07
f1 (ppm)


Carbon NMR (151MHz CDCl3)
SeSP9
169.13
165.24
153.05
151.97
151.58
140.69
135.33
133.54
131.93
130.08
129.08
128.34
126.06
125.93
125.75
125.25
124.05
117.94
116.83
110.65
81.50
47.23
30.82
f1 (ppm)