Trapping hydrogen sulfide (H_2S) with diselenides: the application in the design of fluorescent probes

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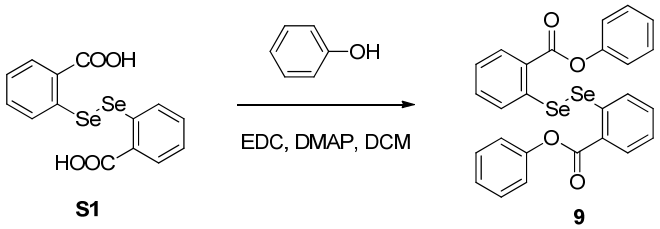
Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) for ^{13}C NMR. Absorption spectra were recorded on a Lambda 20 UV/VIS spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on a Cary Eclipse fluorescence spectrophotometer.

Chemical Synthesis

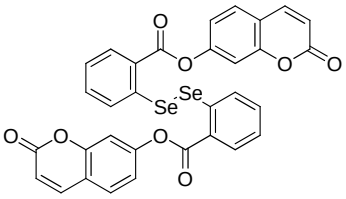


S1 **9**

Model compound **9**: to a solution of compound **S1**[1] (500 mg, 1.25 mmol) in CH_2Cl_2 (30 ml) was added EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (576 mg, 3 mmol), DMAP (4-dimethylaminopyridine) (60 mg, 0.5 mmol) and phenol (470 mg, 5 mmol). The mixture was allowed to stir at rt overnight. The mixture was then washed with saturated

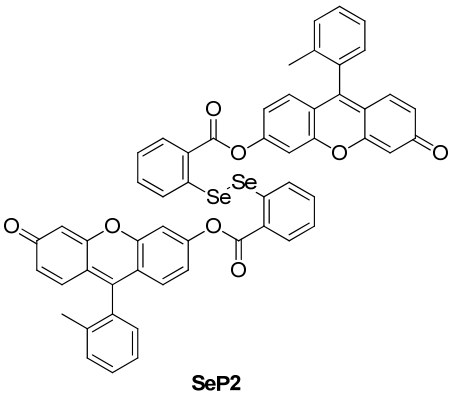
[1] He, J.; Li, D.; Xiong, K.; Ge, Y.; Jin, H.; Zhang, G.; Hong, M.; Tian, Y.; Yin, J.; Zeng, H. *Bioorg. Med. Chem.* **2012**, *20*, 3816-3827.

NH_4Cl solution and brine and was extracted with CH_2Cl_2 (20 ml × 2). The combined organic layers were dried (by $MgSO_4$) and concentrated under reduced pressure. The resulted crude material was purified by flash column chromatography. Compound **9** was obtained as a white solid (560 mg, 80 %). ^{1}H NMR (300 MHz, CD_3Cl) δ 7.18-7.30 (m, 5H), 7.32-7.37 (m, 3H), 7.42-7.46 (m, 6H), 7.90 (d, *J*=7.8 Hz, 2H), 8.31-8.34 (m, 2H); ^{13}C NMR (75 MHz, CD_3Cl) δ 166.06, 150.77, 136.10, 134.19, 132.18, 130.84, 129.77, 129.72, 127.49, 126.40, 121.86; MS (ESI^+) m/z 577.0 ($M+Na^+$); HRMS m/z 576.9444 $[M+Na]^+$; calcd for $C_{26}H_{18}NaO_4Se_2$ 576.9433. IR (cm^{-1}) 1700, 1485, 1268, 1183, 1023, 919, 744. m. p. 114-115 °C.



SeP1

Probe **SeP1** was prepared using the same method for compound **9**. ^{1}H NMR (300 MHz, CD_3Cl) δ 6.45 (d, *J*=9.6 Hz, 2H), 7.24-7.25 (m, 1H), 7.27-7.28 (m, 1H), 7.30-7.31 (m, 2H), 7.36-7.41 (m, 2H), 7.45-7.51 (m, 2H), 7.59 (d, *J*= 8.7 Hz, 2H), 7.75 (d, *J*= 9.3 Hz, 2H), 7.91 (d, *J*= 7.8 Hz, 2H), 8.34 (dd, *J*= 7.5, 1.5 Hz, 2H); MS (ESI^+) m/z 712.9 ($M+Na^+$); HRMS m/z 712.9220 $[M+Na]^+$; calcd for $C_{32}H_{18}NaO_8Se_2$ 712.9230. IR (cm^{-1}) 1702, 1618, 1454, 1242, 1119, 1020, 984, 741. m. p. 275-276 °C.

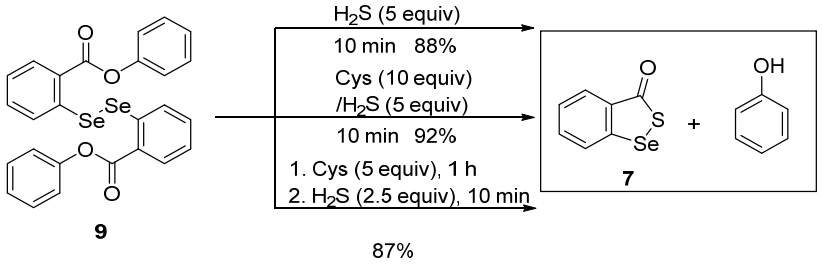


SeP2

Probe **SeP2** was prepared using the same method as for compound **9**. ^{1}H NMR (300 MHz, CD_3Cl) δ 2.12 (s, 6H), 6.46 (d, 2H), 6.60 (dd, 2H), 6.97-7.00 (m, 2H), 7.14 (m, 4H), 7.19-7.21 (m, 2H), 7.39-7.49 (m, 12H), 7.90 (dd, *J*=8.1, 0.9 Hz, 2H), 8.33 (dd, J=7.5, 1.5 Hz,

2H); ^{13}C NMR (75 MHz, CD_3Cl) δ 186.1, 165.2, 158.6, 154.2, 153.2, 148.1, 136.5, 136.4, 134.7, 132.4, 132.2, 131.2, 130.9, 130.8, 130.7, 129.9, 129.4, 129.3, 126.6, 126.4, 120.7, 118.9, 118.6, 110.6, 106.5, 19.8; MS (ESI^+) m/z 993.1 ($M+Na^+$); HRMS m/z 971.0657 $[M+H]^+$; calcd for $C_{54}H_{35}O_8Se_2$ 971.0662. IR (cm^{-1}) 1702, 1618, 1454, 1242, 1119, 1020, 984, 741.. m.p. 270-271 °C.

Model Reactions



Scheme S1

To the solution of **9** (55.4 mg, 0.1 mmol) in THF (4 mL) and PBS buffer (4 mL, 10 mM, pH 7.4) was added $Na_2S{\cdot}9H_2O$ (96 mg, 0.5 mmol). The mixture was stirred for 0.5 hour at rt and then diluted with CH_2Cl_2. The organic layer was separated and dried by $MgSO_4$, and concentrated. Purification by flash column chromatography afforded compound **7**[2] as yellow solid (39 mg, 88% yield). 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.

Stability of product 7

To the solution of **7** (21.6 mg, 0.1 mmol) in THF (2 mL) and PBS buffer (2 mL) was added alanine (44.5 mg, 0.5 mmol) or glycine (37.5 mg, 0.5 mmol) or lysine (73 mg, 0.5 mmol) seperately. The mixture was stirred for 1 h at rt and no reaction was observed (by TLC). The reaction was then diluted with CH_2Cl_2. The organic layer was separated, dried by $MgSO_4$, and concentrated. Compound **7** was recovered by flash column chromatography in 90-95% yields.

Quantum Yields

The quantum yield was calculated according to the equation:

$$\Phi_{sample} = \Phi_{standard} \times (I_{sample}/I_{standard}) \times (A_{standard}/A_{sample}) \times (n_{sample}/n_{standard})^2$$

[2] Mhizha, S.; Mlochowski, J *Synth. Commun.* **1997**, *27*, 283.

Φ denotes the quantum yield; *I* denotes the area under the fluorescence band; *A* denotes the absorbance at the excitation wavelength; n denotes the refractive index of the solvent. For quantum yield of **SeP1**, it was determined using 7-hydroxycoumarin as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of 7-hydroxycoumarin excited at 330 nm in sodium phosphate buffer (0.1 M; pH 7.4), which has a quantum efficiency of 0.76. For quantum yield of **SeP2**, Quantum yield was determined using 2-Methyl TokyoGreen as a standard by comparing the area under the corrected emission spectrum of the test sample with that of a solution of fluorescein excited at 491 nm in 0.1 N NaOH, which has a quantum efficiency of 0.85.

Preparation of the solutions and fluorescence measurements

The stock solutions of **SeP1** (0.7 mM) and **SeP2** (2 mM) were prepared in DMSO, respectively. The solutions of various testing species were prepared from Cysteine (Cys), GSH, $Na_2S·9H_2O$, $Na_2S_2O_3$, Na_2SO_3, Na_2SO_4, in 10 mM PBS buffer. All the test solutions need to be freshly prepared.

Unless otherwise noted, all the measurements were carried out for 5 min at room temperature in CH_3CN/PBS buffer (10 mM, pH 7.4, 1:1, v/v) according to the following procedure. In a test tube, 1.5 mL of PBS buffer (pH 7.4) and 1.5 mL of acetonitrile were mixed, and then 42 μL of the stock solution of **SeP1** or 15 μL of the stock solution of **SeP2** were added. The resulted solution was mixed well, followed by the addition of a requisite volume of testing species sample solution. After mixing and then standing for 5 min at room temperature, the reaction solution was transferred into a 1-cm quartz cell to measure fluorescence with λ_{ex} = 340 nm (for **SeP1**) or 498 nm (for **SeP2**). 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.

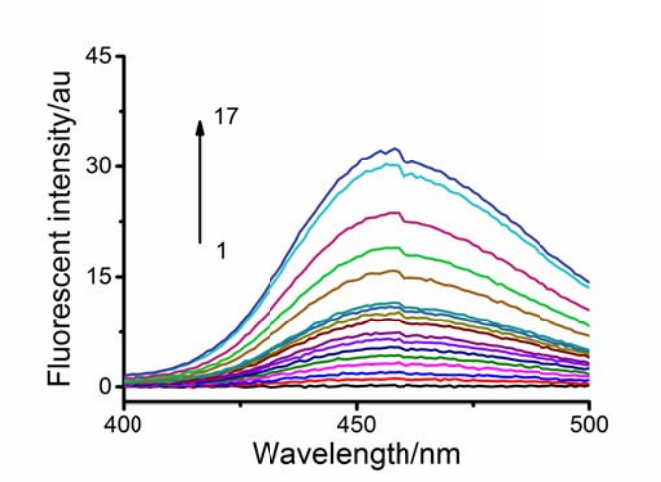


Figure S1. Fluorescence emission spectra of **SeP1** (10 μM) with varied concentrations of Na_2S (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 μM for curves 1-17, respectively). λ_{ex} = 498 nm. The reactions were carried out for 5 min at room temperature in CH_3CN/PBS buffer (10 mM, pH 7.4, 1:1, v/v).

Cell culture and fluorescence imaging

HeLa cells were grown on glass-bottom culture dishes (Corning Inc.) in DMEM supplemented with 10% (v/v) FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37 °C under a humidified atmosphere containing 5% CO_2. Before use, the adherent cells were washed one time with FBS-free DMEM. For intracellular H_2S imaging, the cells were incubated with 50 μM **SeP2** in FBS-free DMEM at 37 °C for 30 min. After removal of excess probe and washed with PBS (pH 7.4), the cells were incubated with 100 μM Na_2S for 30 min in PBS buffer (pH 7.4, containing 100 μM CTAB). Cell imaging was carried out after washing the cells three times with PBS (pH 7.4). All microscopy images were taken on an EVOS fl fluorescence microscope from Advanced Microscopy Group (AMG).

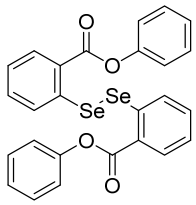
Measurement of endogenous H_2S changes

Human neuroblastoma cells (SH-SY5Y) were cultured in Eagle's medium/Ham's F-12 50/50 Mix (DMEM/F12, Cellgro) supplemented with 10 % fetal bovine serum (FBS, ATCC) and penicillin (100 Units/ml)/streptomycin (100 μg/ml) as described previously.[3] Cells were seeded into a 96 well-plate (2 x 10^4 cells/well) and medium was replaced every 2 days. Cells

[3] Marutani, E.; Kosugi, S.; Tokuda, K.; Khatri, A.; Nguyen, R.; Atochin, D. N.; Kida, K.; Van Leyen, K.; Arai, K.; Ichinose, F. *J. Biol. Chem*., **2012**, *287*, 32124-32135.

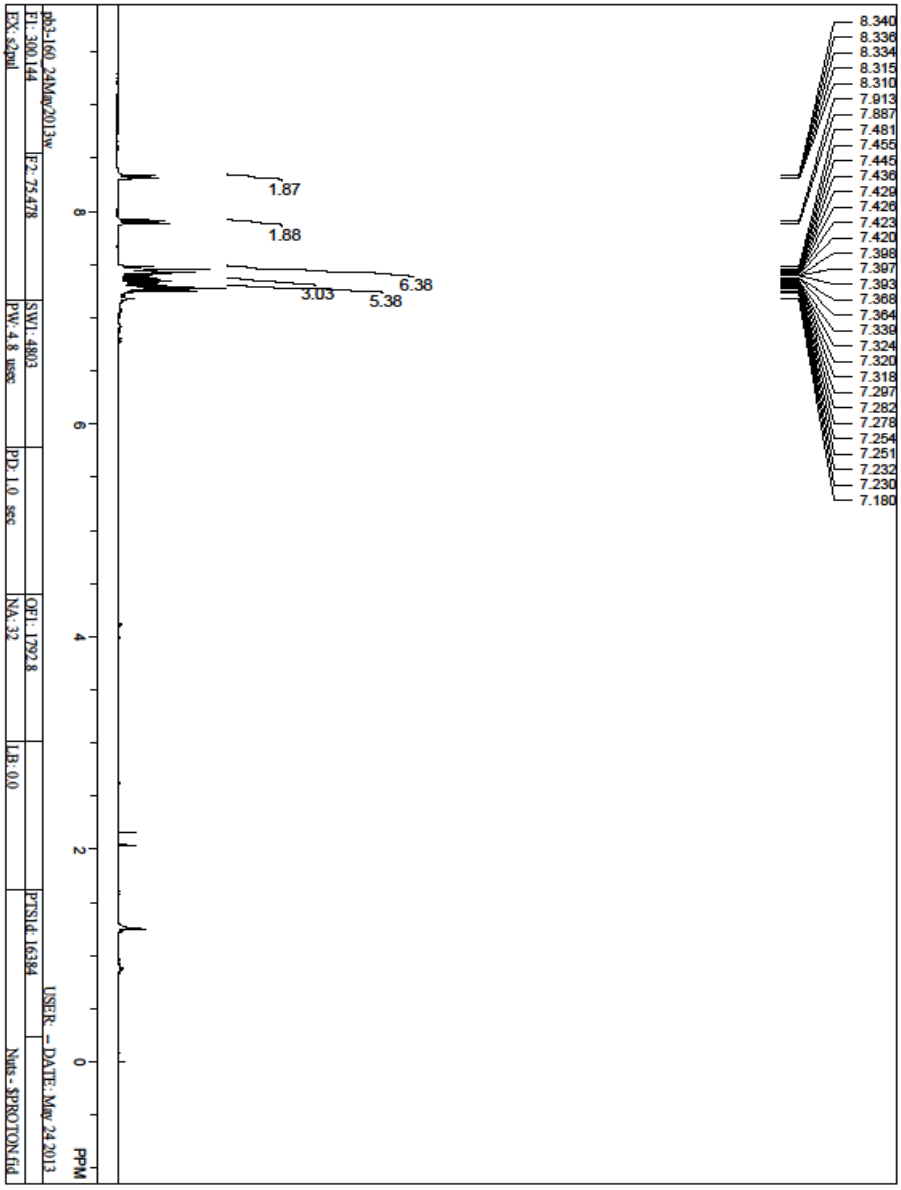
were used after getting 80-90 % confluent.

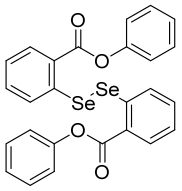
For L-cysteine or D-cysteine experiment, cells were incubated with **SeP2** (10 μM, with 0.1 % DMSO) in Hank's Balanced Salt Solution (HBSS) at 37°C for 30 min, washed twice with warmed HBSS, and incubated with L-cysteine (10 mM), D-cysteine (10 mM), or Na_2S (0.1 mM) in DMEM/F12 (0.1 % FBS) at 37°C for 2 h. Fluorescent intensity was measured by a plate-reader (SpectraMax M5, Molecular Devices) after washing cells twice with HBSS. For a CBS activator S-adenosylmethyonine (SAM, AK Scientific) or a CBS inhibitor aminooxyacetic acid (AOAA) experiment, cells were incubated with SAM (2 mM) or AOAA (2 mM) in DMEM/F12 (0.1 % FBS) for 24 h, washed with HBSS, and loaded **SeP2**. Fluorescent intensity was measured by a plate-reader (SpectraMax M5, Molecular Devices) after washing cells twice with HBSS.



9

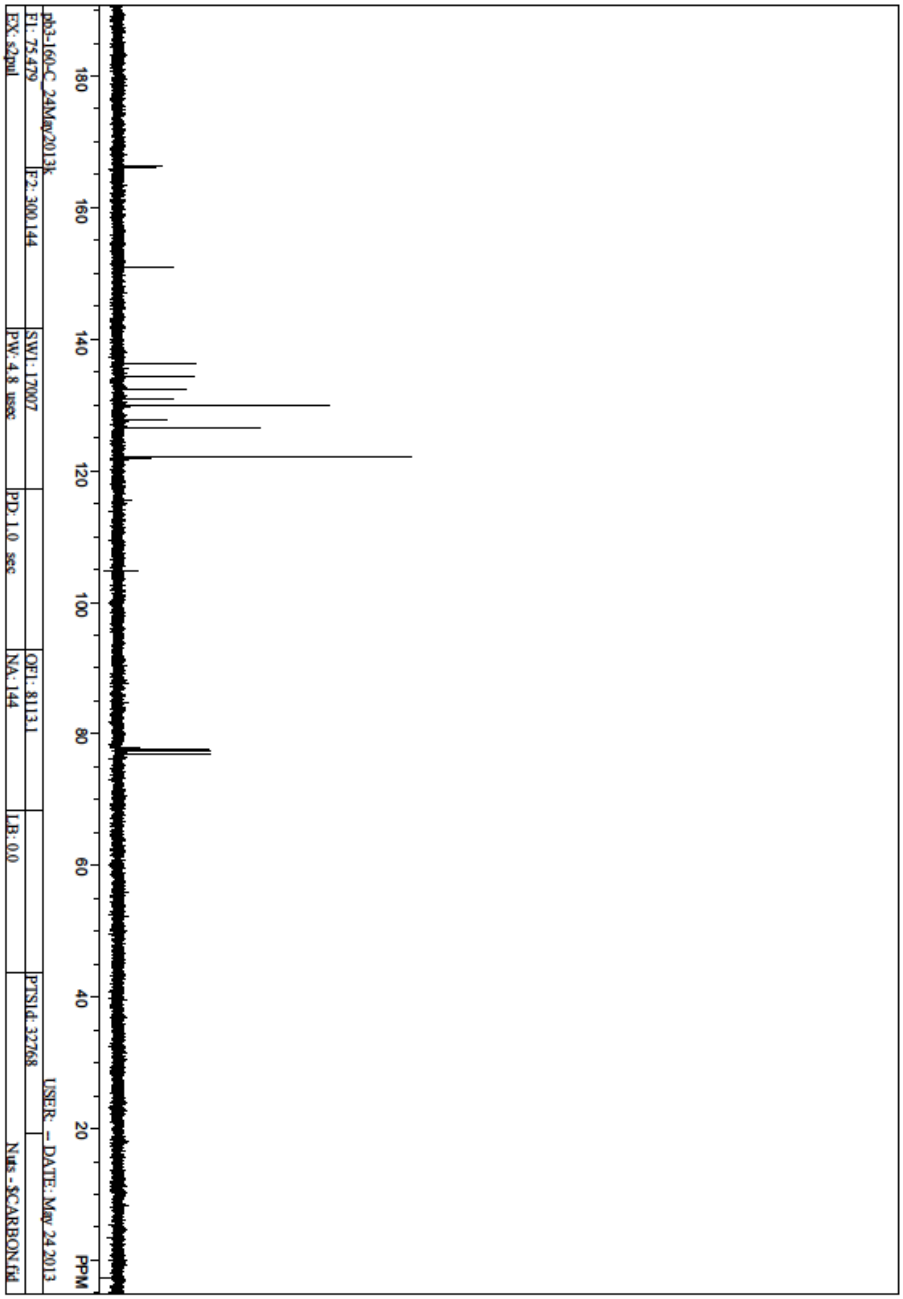
^{1}HNMR (300 MHz, $CDCl_3$)

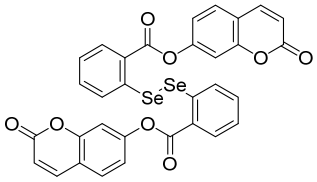




9

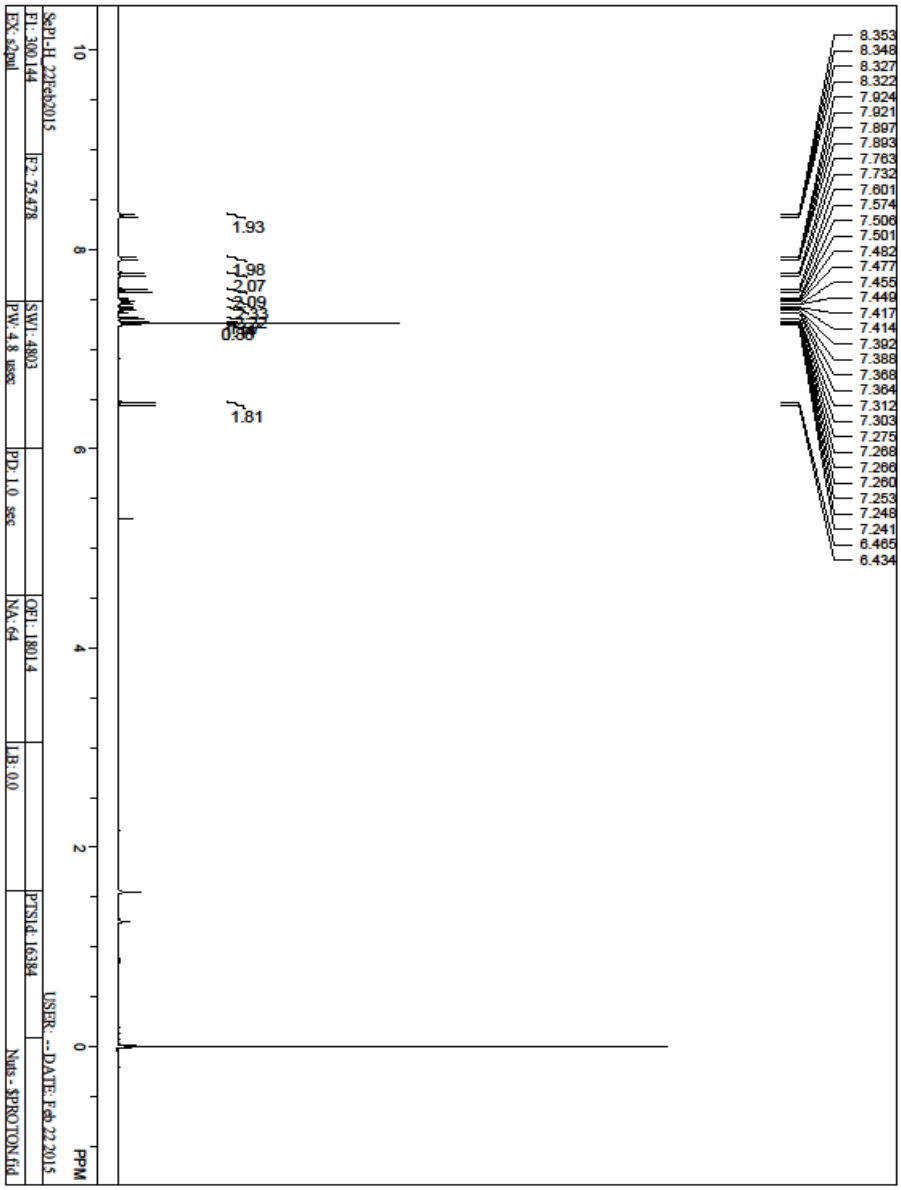
^{13}CNMR (75 MHz, $CDCl_3$)

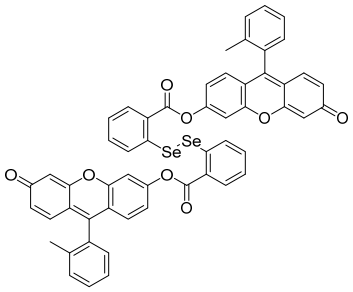




SeP1

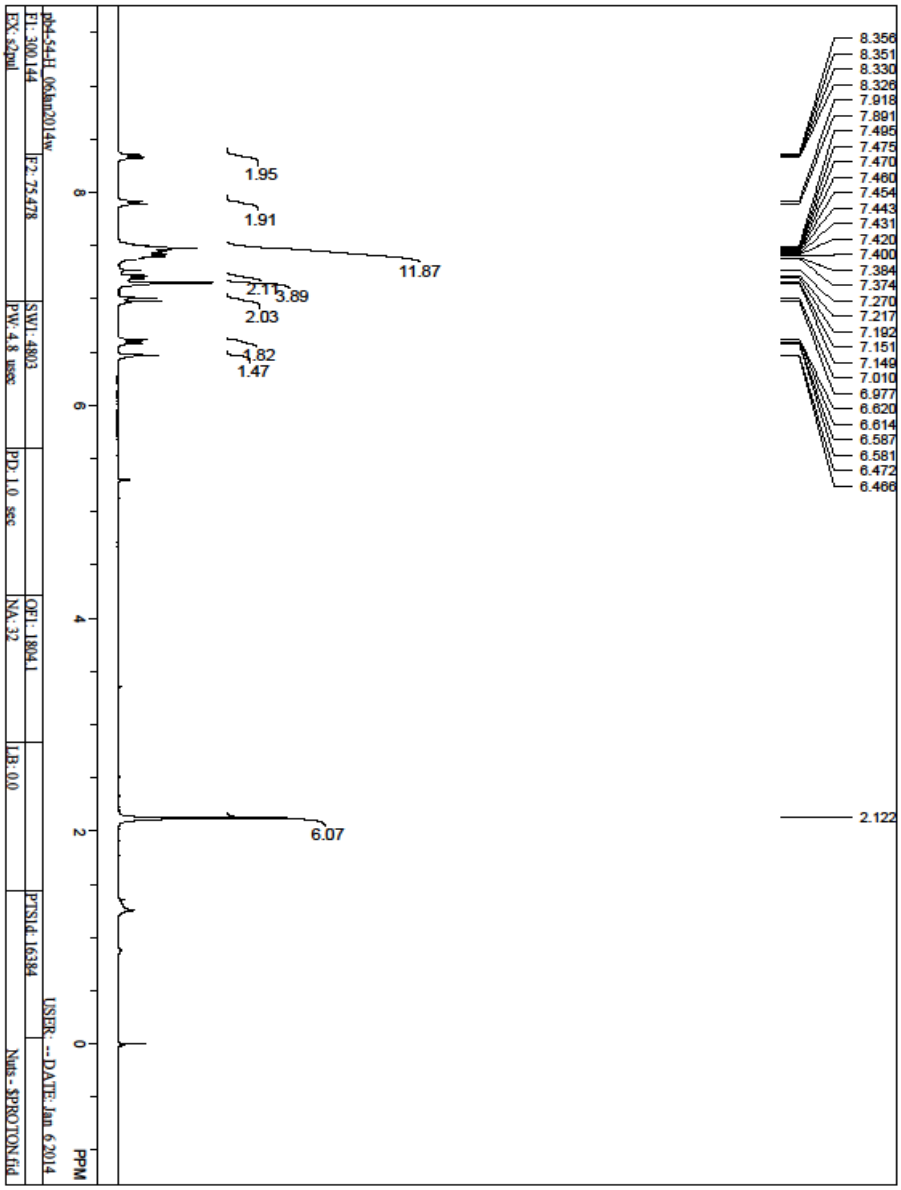
^{1}HNMR (300 MHz, $CDCl_3$)

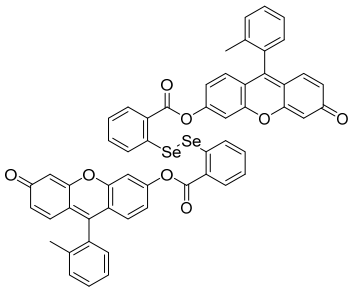




SeP2

^{1}HNMR (300 MHz, $CDCl_3$)





SeP2

^{13}CNMR (75 MHz, $CDCl_3$)