Supporting Information

Acyl Selenylsulfides as the Precursors for Reactive Sulfur Species (Hydrogen Sulfide, Polysulfide, and Selenylsulfide)

Akil Hamsath,[a] Yingying Wang,[a] Chun-tao Yang,[a,b] Shi Xu,[a] Danica Cañedo,[a] Wei Chen,[a] and Ming Xian[*,a]

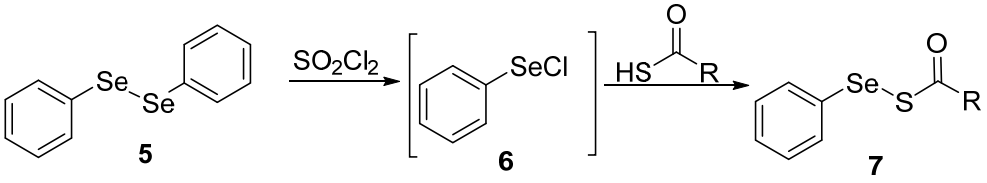
[a]Department of Chemistry, Washington State University, Pullman, Washington 99164, United States

[b]Affiliated Cancer Hospital & Institute of Guangzhou Medical University, Guangzhou 510095, China

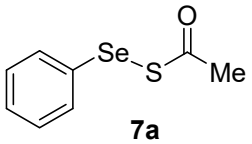
Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-NMR spectra were recorded on a 400 MHz spectrometer unless otherwise stated. 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 Thermo Scientific Evolution 300 UV-Vis Spectrophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer.

Experimental Procedures and Compound Characterization

Preparation of compounds 7a-7e

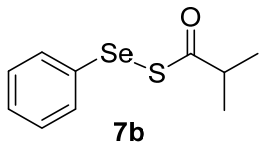


Scheme S1



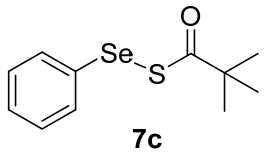
Compound **7a**: Diphenyl diselenide (310 mg, 1.0 mmol) was dissolved in 20 mL CH_2Cl_2. To this solution was added SO_2Cl_2 (190 µL, 2.4 mmol). The resulting dark red reaction was stirred at room temperature for 5 min. Thioacetic acid (340 µL, 4.8 mmol) was then added and the resulting pale yellow mixture was stirred for 1 hour. The mixture was quenched with saturated $NaHCO_3$ and extracted 3 times with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated in vaccuo. 140 mg (0.61 mmol) of **7a** was obtained as a yellow oil by flash chromatography (hexane : toluene =

2.5:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.59 (m, 2H), 7.32 – 7.25 (m, 3H), 2.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ192.0, 132.6, 131.3, 129.3, 128.7, 29.2; mass spectrum (HRMS) calcd for $C_8H_9OSSe^+$ $[M+H]^+$ 232.9539 found: 232.9541; yield: 31%.



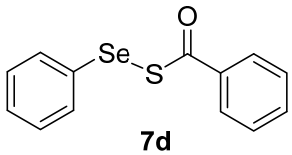
7b

Compound **7b**[1] was prepared from thioisobutyric acid using the same procedure as **7a**. 205 mg (0.79 mmol) of **7b** was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.57 (m, 2H), 7.30 – 7.27 (m, 3H), 3.01 (sep, *J* = 6.9 Hz, 1H), 1.24 (d, *J* = 6.9 Hz, 6H); yield: 40%



7c

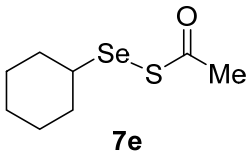
Compound **7c** was prepared from thiopivalic acid using the same procedure as **7a**. 380 mg (1.4 mmol) of **7c** was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.55 (m, 2H), 7.30 – 7.26 (m, 3H), 1.31 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0, 132.2, 131.5, 129.2, 128.4, 46.9, 27.7; mass spectrum (HRMS) calcd for $C_{11}H_{14}NaOSSe^+$ [M+Na] 296.9828 found: 296.9847; yield: 70%



7d

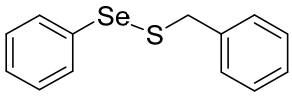
[1] Kato, S.; Yasui, E.; Terashima, K.; Ishihara, H.; Murai, T., *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3931 – 3942.

Compound **7d**[2] was prepared from thiobenzoic acid using the same procedure as **7a**. 305 mg (1.0 mmol) of **7d** was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 2H), 7.72 – 7.66 (m, 2H), 7.63 – 7.57 (m, 1H), 7.50 – 7.44 (m, 2H), 7.32 – 7.27 (m, 3H); yield: 52%



7e

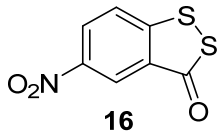
Compound **7e** was prepared from thioacetic acid and dicyclohexyl diselenide using the same procedure as **7a**. 280 mg (1.2 mmol) of **7e** was obtained as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.20 – 3.15 (m 1H), 2.52 (s, 3H), 2.05 – 2.00 (m, 2H), 1.79 – 1.71 (m, 2H), 1.62 – 1.57 (m, 1H), 1.53 – 1.43 (m, 2H), 1.36 – 1.20 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.3, 46.3, 33.4, 29.3, 26.7, 25.5; mass spectrum (HRMS) calcd for $C_8H_{14}NaOSSe^+$ [M+Na] 260.9828 found: 260.9821; yield: 59%.



10

Compound **10: 7a** (230 mg, 1.0 mmol) was dissolved in 3 mL CH_2Cl_2. To this solution was added benzyl mercaptan (120 μL, 1.0 mmol) in one portion. The resulting yellow solution was stirred overnight at room temperature. The mixture was concentrated in vacuo. 170 mg (0.60 mmol) of **10** was obtained as a yellow oil by flash chromatography (hexane : toluene = 10:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.49 (m, 2H), 7.29 – 7.27 (m, 5H), 7.25 – 7.23 (m, 3H), 4.05 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 131.9, 130.1, 129.4, 129.2, 129.1, 128.5, 127.4, 127.3, 42.4; mass spectrum (HRMS) calcd for $C_{13}H_{12}NaSSe^+$ [M+Na] 302.9723 found: 302.9731; yield: 60%

[2] Toru, T.; Nishigaki, M.; Seko, T.; Kanefusa, T.; Maekawa, E., *Synthesis.* **1985,** *9*, 878 – 879.



16

Compound **16**: **7a** (230 mg, 1.0 mmol) was dissolved in 10 mL CH_2Cl_2. To this solution was added n-butyl amine (99 µL, 1.0 mmol) in one portion. The resulting yellow solution was stirred for 5 min at room temperature. To this solution nitrofluorobenzoylate (130 mg, 0.50 mmol) was added in one portion and the mixture was stirred for one hour. The mixture was concentrated in vacuo. 23 mg (0.11 mmol) of **16** was obtained as a pale white solid by flash chromatography (hexane : ethyl acetate = 20:1). Yield: 22%. Compound **16** is a known compound.[3]

H_2S release from 7 in the presence of cysteine

L-cysteine stock solution (Solution A): L-cysteine was dissolved in 50 mM PBS buffer to afford a 120 mM stock solution. Compound **7** stock solution (Solution B): **7** was dissolved in THF to afford a 30 mM stock solution.

H_2S release measurement. To 3.9 mL phosphate buffer (pH 7.4) and 920 µL of THF solution in a 20 mL glass scintillation vial was added 100 µL of solution A for a total L-cysteine concentration of 2.4 mM (solution C). A 1.5 mL eppendorf vial containing 500 µL of 1% zinc acetate and a quarter of a 4.25 cm (in diameter) filter paper was gently placed in the scintillation vial containing solution C. Then 80 µL of solution B was added to solution C under argon and capped for a total **7** concentration of 0.5 mM. The resulting solution was incubated at room temperature for 4 hours. Afterwards, the contents of the Eppendorf vial was transferred to a 4 mL glass vial containing 500 µL of N,N-dimethyl-1,4-phenylenediamine sulfate (20 mM in 7.2 M HCl) and 500 µL ferric chloride (30 mM in 1.2 M HCl). The absorbance (at 670 nm) of the resulting solution was measured (after incubating for

[3] Liu, C.; Chen, W.; Shi, W.; Peng, B.; Zhao, Y.; Ma, H.; Xian, M., *J. Am. Chem. Soc.* **2014,** *136*, 7257–7260.

15 min). H_2S concentration was calculated based on a calibration curve of Na_2S. H_2S release data of all the donors were measured by this method.

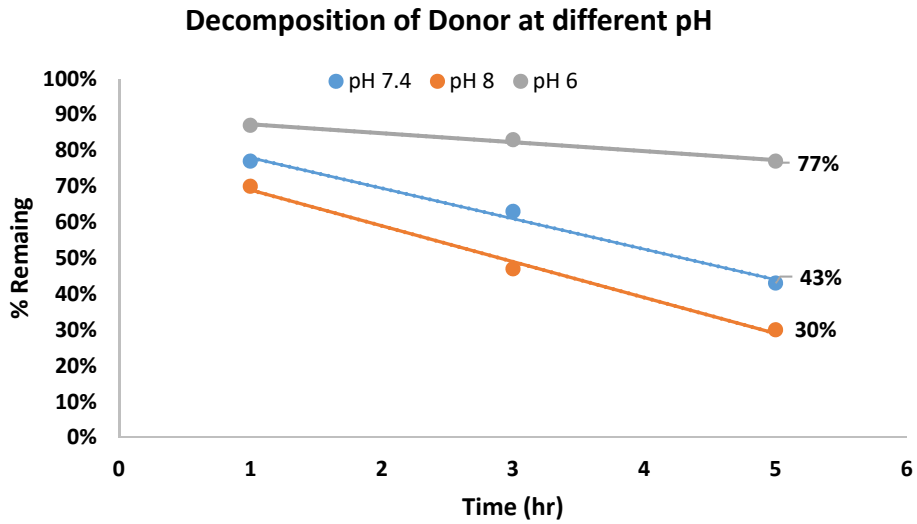


Figure S1. Decomposition of **7a** in a mixture of THF/phosphate buffer (1/5) at pH 6, 7.4 and 8.

7a stock solution (solution A): **7a** was dissolved in THF to afford a 30 mM stock solution.

Decomposition Test. To a 100 mL round bottom flask was added 11 mL THF and 48 mL of 50 mM phosphate buffer (pH 7.4). Then 1 mL of solution A was added to this flask and the resulting solution was incubated at room temperature. At 1 h, the solution was transferred to a 200 mL separatory funnel and extracted with CH_2Cl_2 (3x25 mL). The organic layers were combined, dried, filtered, and concentrated in vacuo. Acetophenone (12 µL, 0.10 mmol) was added to the residue as an internal standard for NMR yield analysis. The acyl protons of acetophenone (2.60 ppm in $CDCl_3$) were compared to the acyl protons of **7a** (2.55 ppm in $CDCl_3$) for NMR yield calculations. This procedure was also used to analyze the reaction at 2 and 3 hour reaction time with varied pH. Decomposition of **7a** is reported by the percent remaining (Figure S1).

Fluorescent measurement of H_2S_2 release from donors under $nBuNH_2$ treatment

DSP-3 stock solution (solution A): DSP-3 was dissolved in DMSO to afford a 0.5 mM stock solution.

CTAB stock solution (solution B): CTAB was dissolved in ethanol to afford a 5.0 mM stock solution.

7a stock solution (solution C): **7a** was dissolved in THF to afford a 40 mM stock solution.

N-Butylamine stock solution (solution D): n-Butylamine was dissolved in THF to afford a 40 mM stock solution.

H_2S_2 fluorescent measurement. H_2S_2 generation was initiated by adding 50 μL of solution C to a solution of 50 μL solution D in 3.9 mL THF, making both the donor's and $nBuNH_2$ concentrations 0.5 mM. The solution was incubated at room temperature. At different time points (10 min and 30 min) 400 μL of the solution aliquot was taken and transferred into a freshly prepared solution containing 3.5 mL of 25 mM PBS 7.4 buffer, 80 μL of solution A and 20 μL of solution B, making the donor concentration 50 μM and DSP-3 concentration 10 μM. After 5 min at room temperature, the fluorescence emission intensity at 515 nm was recorded (excitation at 490 nm). This same procedure was duplicated with varying concentrations of equal amounts donor and $nBuNH_2$ (1.0 mM, 3.0 mM and 5.0 mM). The data was plotted in Figure 2.

A methylene blue experiment were also carried out to test if H2S was generated in the reaction of the donor with $nBuNH_2$. However, we did not observe significant H_2S signal.

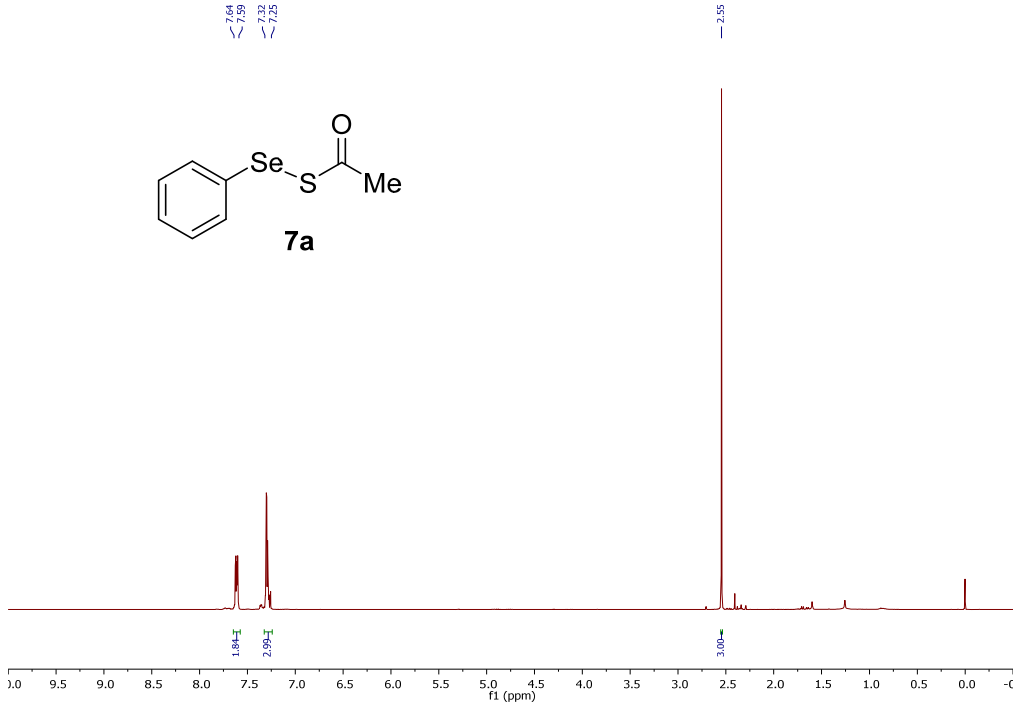
Cell Imaging Experiment.

H9c2 cells were bought from American Type Culture Collection (ATCC). They were maintained in DMEM supplemented with 10% (v/v) FBS at 37 °C under a humidified atmosphere containing 5% CO_2. Before use, the adherent cells were washed one time with PBS. For imaging studies, the cells were pre-incubated with 10 μM WSP5 or DSP-3 in PBS-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 50 μM **7a** for 60 min in

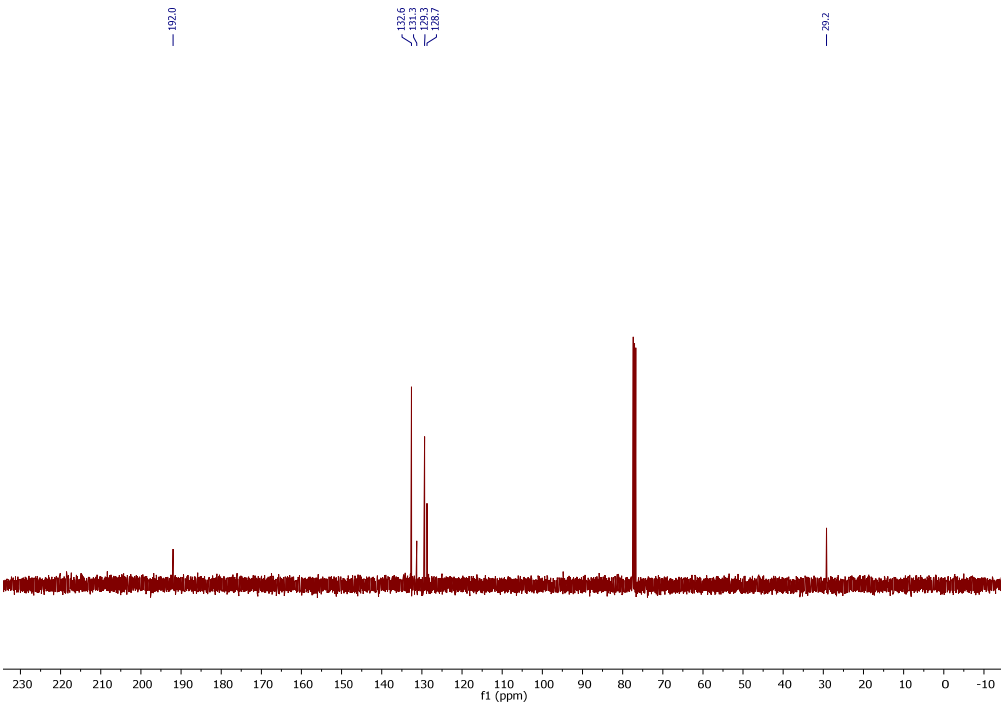
FBS free medium (pH 7.4, containing 50 μM CTAB). Cell imaging was carried out after washing the cells one time with PBS (pH 7.4). The intracellular H_2S-triggered fluorescence was visualized under a Nikon microscope.

Compound **7a**

^{1}H NMR (400 MHz, $CDCl_3$)

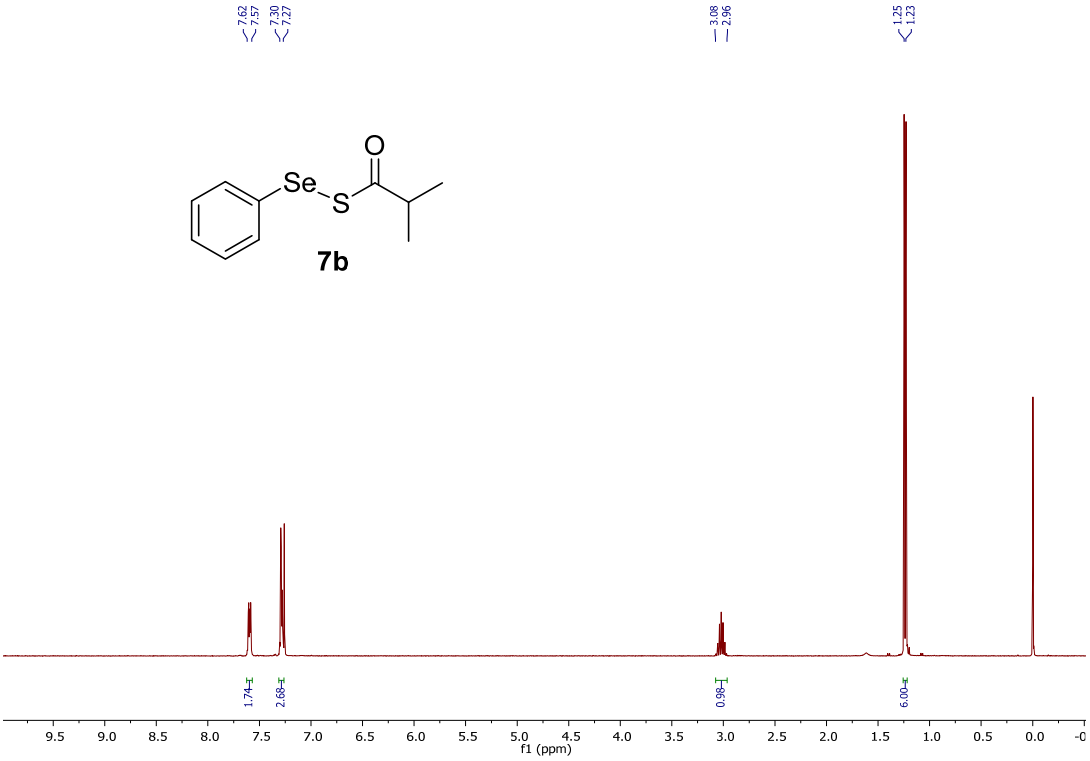


^{13}C NMR (100 MHz, $CDCl_3$)



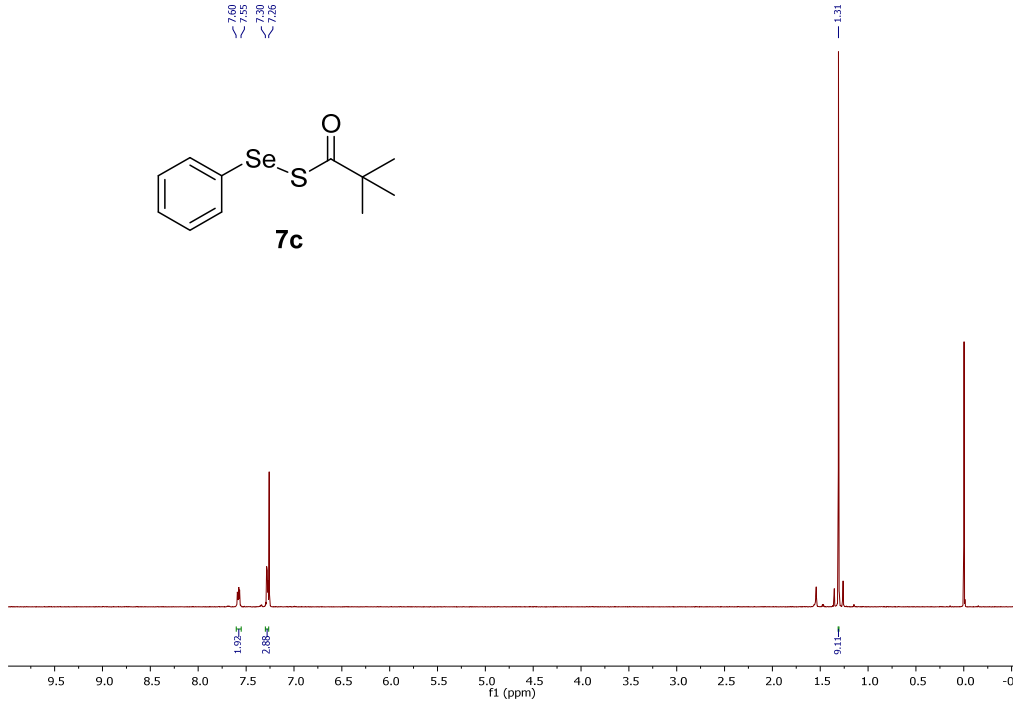
Compound **7b**

^{1}H NMR (400 MHz, $CDCl_3$)

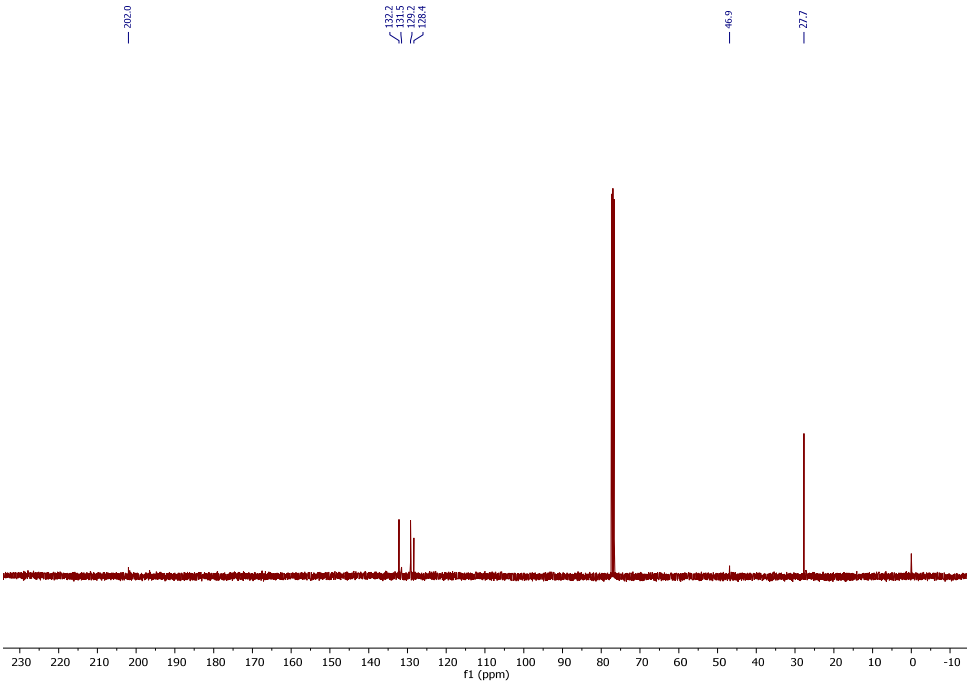


Compound **7c**

^{1}H NMR (400 MHz, $CDCl_3$)

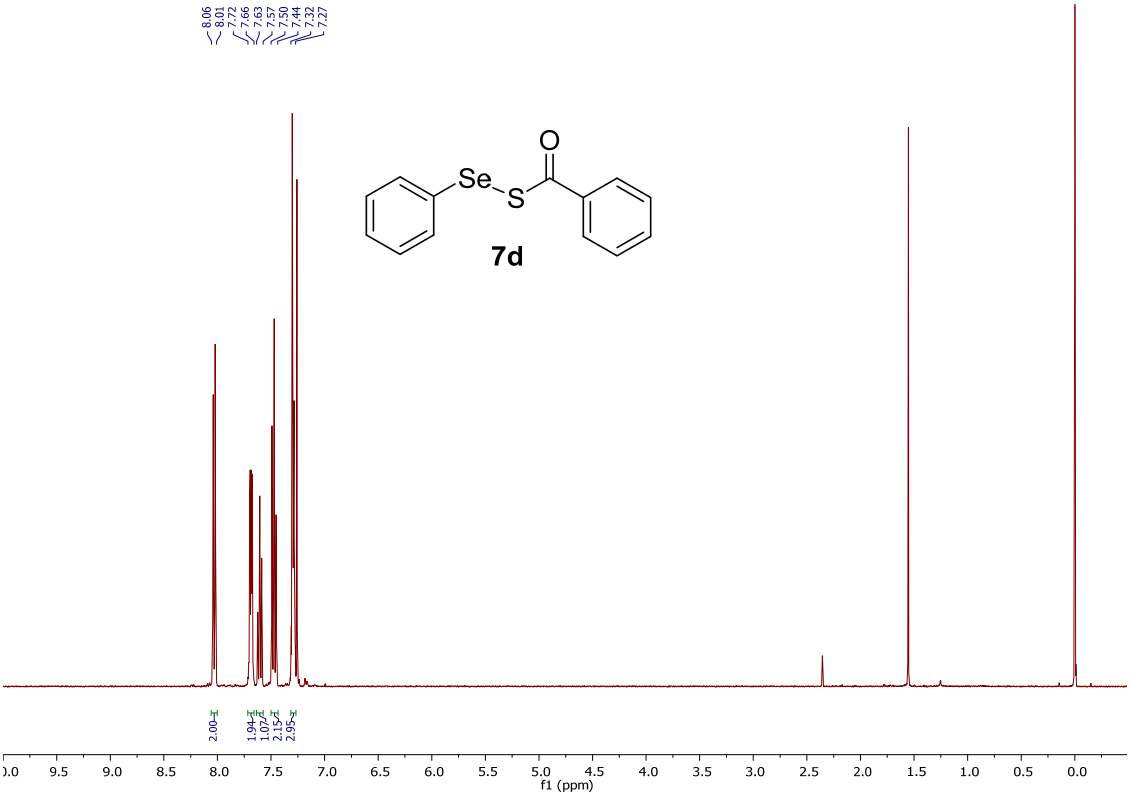


^{13}C NMR (100 MHz, $CDCl_3$)



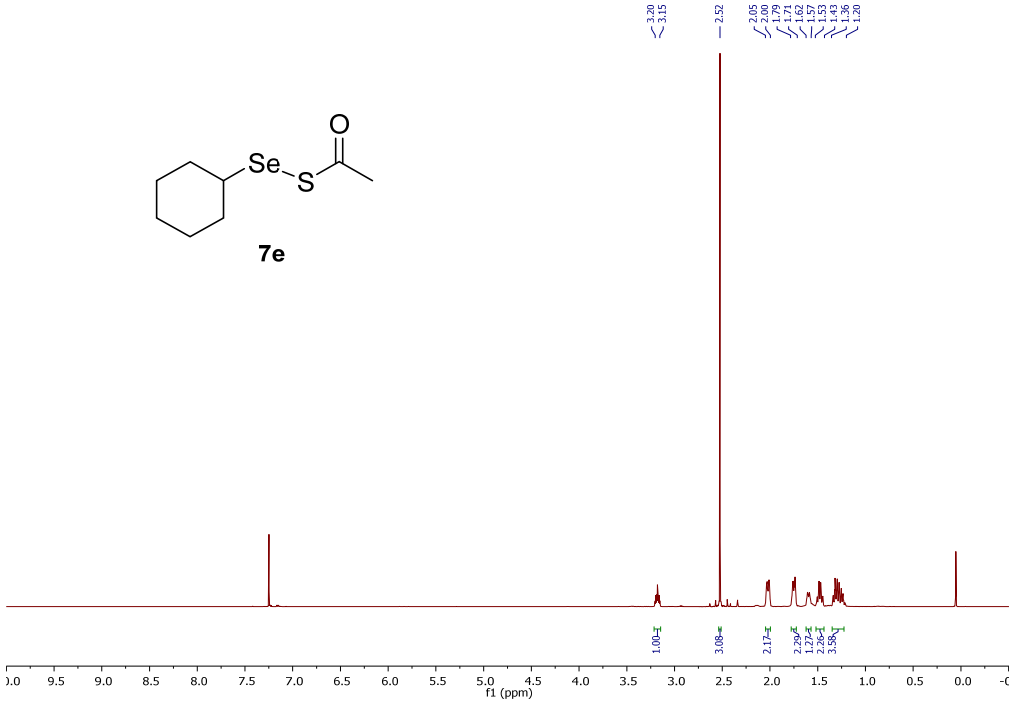
Compound **7d**

^{1}H NMR (400 MHz, $CDCl_3$)



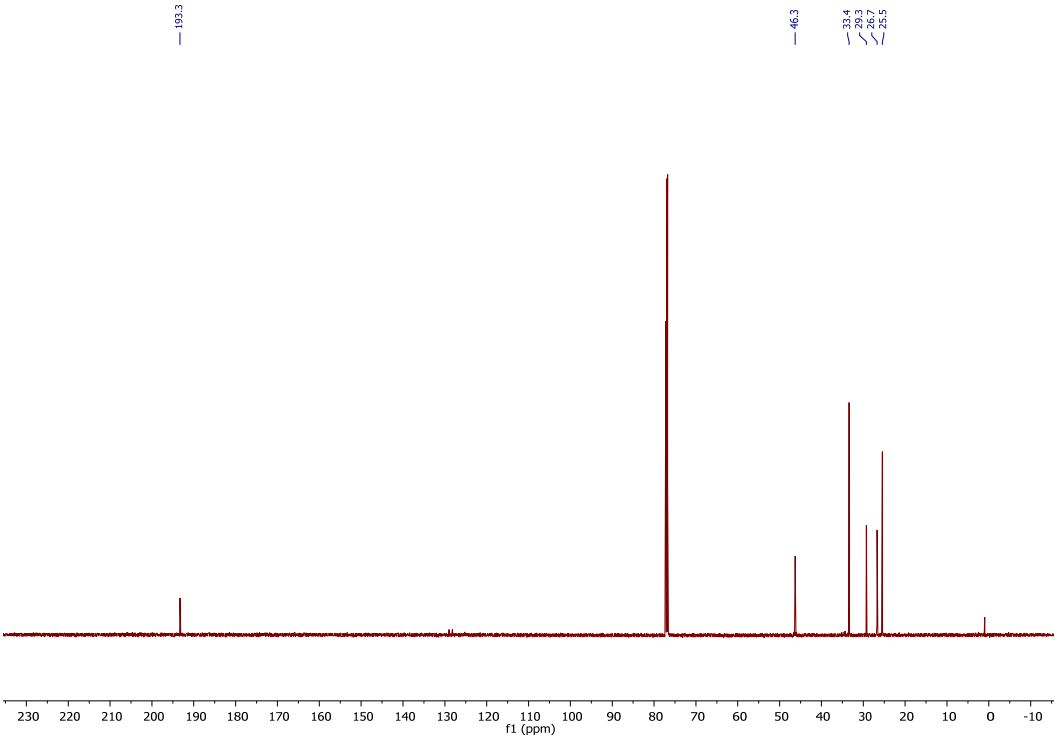
Compound **7e**

^{1}H NMR (600 MHz, $CDCl_3$)



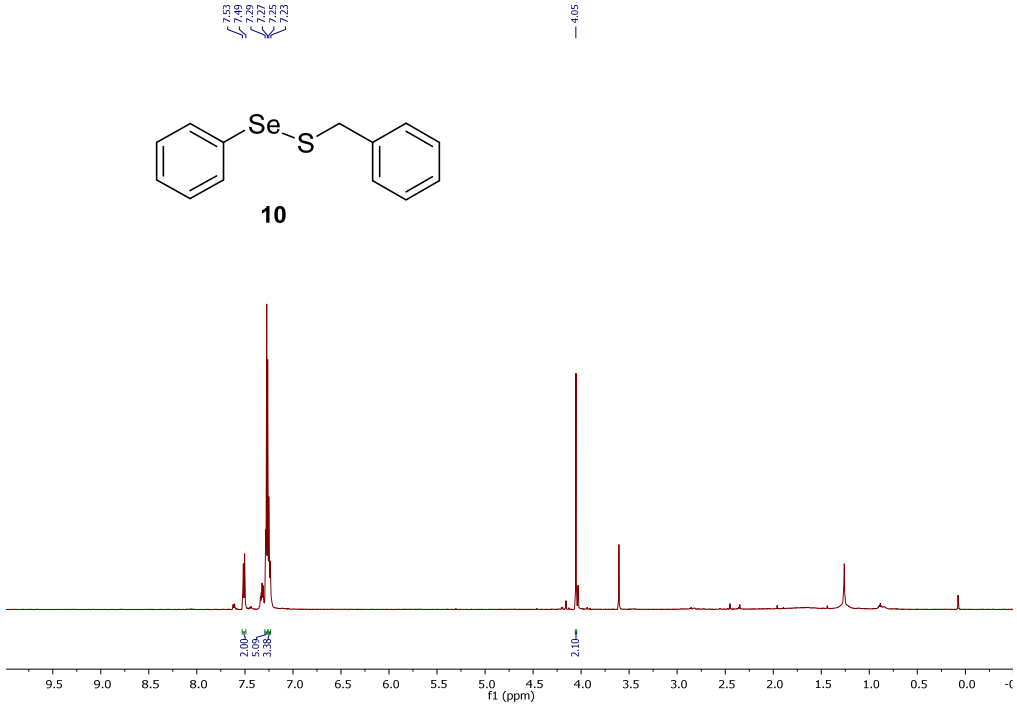
7e

^{13}C NMR (150 MHz, $CDCl_3$)



Compound **10**

^{1}H NMR (600 MHz, $CDCl_3$)



^{13}C NMR (150 MHz, $CDCl_3$)