Supporting information

Esterase-sensitive trithian-based hydrogen sulfide donors

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Materials and Methods

Instrumentations: ^{1}H and ^{13}C NMR were recorded at 400 MHz (JEOL, ECG400) and reported in parts per million (ppm) on the δ scale relative to residual DMSO-d_6 (δ 2.50 for ^{1}H, δ 39.5 for ^{13}C NMR). All reported melting points were measured by Fisher-Johns melting point apparatus and not corrected. Mass spectra were recorded using an electrospray ionization mass spectrometry (ESI, Thermo Finnigan). Mass data were reported in units of *m/z* for $[M+H]^+$. Fragments of the hydrolysis of donors were recorded by Gas chromatography coupled with a 5975C mass selective detector (GC-MS, Agilent 7890N). For the gas chromatography separation, an HP-5MS fused silica capillary column (5% diphenyl-95% dimethyl polysiloxane, 30 m × 0.25 mm i.d., J&W Scientific Agilent Tech, USA). The GC-MS parameters for the analysis were as follow: 50°C isothermal for 3 min, 15°C min^{-1} up 305°C and isothermal for 5 min. The carrier gas (Helium, purity 99.999%) was used in the constant flow mode at 1.5 mL/min. A microplate reader (Infinite M1000, TECAN Group Ltd.) was used to obtain the optical density (absorbance) from cells at 450 nm. Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies) was used to measure the concentration of H_2S released from donors.

Solvents and Reagents: Reagents and solvents were used reagent grade. Benzene was distilled under nitrogen atmosphere before use. Solvents were used for either chromatography or extraction without further purification. 1,3,5,-trithiane, cuprous chloride (CuCl), *tert*-butyl peroxybenzoate and *tert*-butyl peroxyacetate were purchased from Aldrich and used without further purification.

***Chromatography*:** The progress of the reactions was monitored by analytical thin layer chromatography (TLC). Plates were visualized with UV (254 nm) and then illuminated by CAM stain reagent (2.5 g of ammonium molybdate tetrahydrate and 1 g of cerium ammonium sulfate in a solution of 10% sulfuric acid in water). A flash column chromatography using ethyl acetate/hexanes was performed using silica gel (230-400 mesh). The solvent compositions for all separations are on a volume/volume (v/v) basis.

Concentration determination of H_2S released from donors by a fluorescent probe (WSP-5):[s1]

Preparation of stock solutions. In separate vials, donors were dissolved in DMSO to afford 20 mM of each stock solution. 6.0 mg Esterase (18 unit/mg esterase from porcine liver esterase, Aldrich) was dissolved in 1.0 mL PBS (pH 7.4) to provide a 100 unit/mL esterase stock solution. A fluorescent probe, WSP-5, was prepared following a literature method [S1] and the solid was dissolved in DMSO to prepare a 2.5 mM stock solution. Cetyl trimethyl ammonium bromide (CTAB) was dissolved in ethanol to afford a 100 mM stock solution.

Measurement of H_2S concentration by a fluorescent probe WSP-5. **3b** and **3c** (final concentration: 200 μM) were added to 10 mL of PBS buffer (pH 7.4) containing esterase (1 unit/mL) at 37°C. Based on the specified time-intervals, aliquots of 100 μL samples were taken out and added to 100 μL PBS containing 50 μM of WSP-5 and 100 μM of CTAB in a 1 mm cuvette. The final volume of the solution was adjusted to 4 mL with PBS buffer (pH 7.4). After mixing with a cuvette cap at room temperature, the fluorescence intensities at 535 nm were recorded. The concentrations of H_2S in each time were determined depending on the standard curve of hydrogen sulfide which obtained by a literature method.[S1]

S1. B. Peng, W. Chen, C. Liu, E. W. Rosser, A. Pacheco, Y. Zhao, H. C. Aguilar and M. Xian, *Chemistry*, 2014, **20**, 1010-1016.

Cell viability assay

H9c2 cells were purchased from the American Tissue Culture Collection and cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 IU/ml penicillin, and 100 ug/ml streptomycin at 37°C. H9c2 cells were transferred and cultured in 96-well plates with 4 duplicate wells in each group. When

80~90% confluence was reached, the cells were treated with H_2S donors (20 mM stock solutions were prepared in DMSO) at different concentrations in 1% DMSO in medium and incubated for 24 h. To test cell viability, the cell counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Inc.) solution (10 μL) at a 1:10 dilution with FBS-free DMEM/F12 (100 μL) was added to each well followed by a further 3 h incubation with 5% CO_2 at 37°C. Absorbance was automatically measured at 450 nm with a microplate reader (Infinite M1000, TECAN Group Ltd.). The mean optical density (OD, absorbance) of 4 wells in the indicated groups was used to calculate the percentage of cell viability as follows: percentage of cell viability = $(A_{treatment} - A_{blank}) / (A_{control} - A_{blank}) \times 100\%$ (where, A = absorbance).

GC-MS Analysis

In separate vials, compound **3b** and **3c** was dissolved in MeOH and cooled to 0°C. To each vial 5 equiv of lithium hydroxide was directly added and the reactions were monitored by TLC. The compounds were disappeared less than 5 min. Each aliquot was taken and diluted with MeOH and then filtered before GC-MS analysis.

(1) GC spectra of **3b** and **3c**

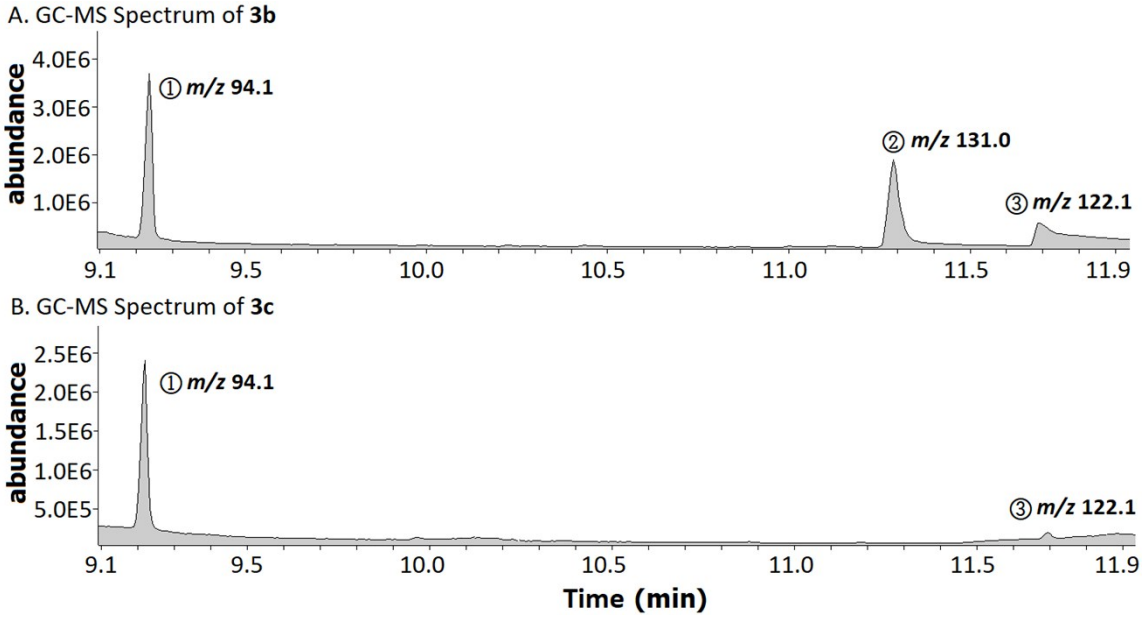


Fig. S1 GC-MS chromatograms of hydrolyzed products of **3b** (A) and **3c** (B).

MS analysis of each individual peak from GC-MS chromatogram

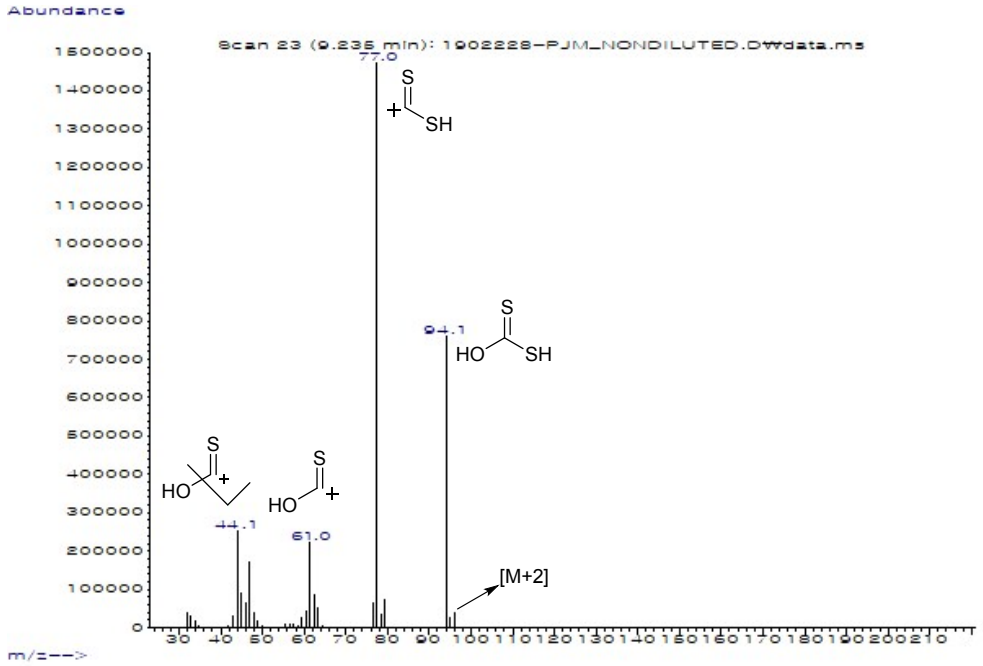


Fig. S2 MS spectrum of peak ① identified as compound **5** (*m/z* [M+] 94.1, t_R = 9.2 min)

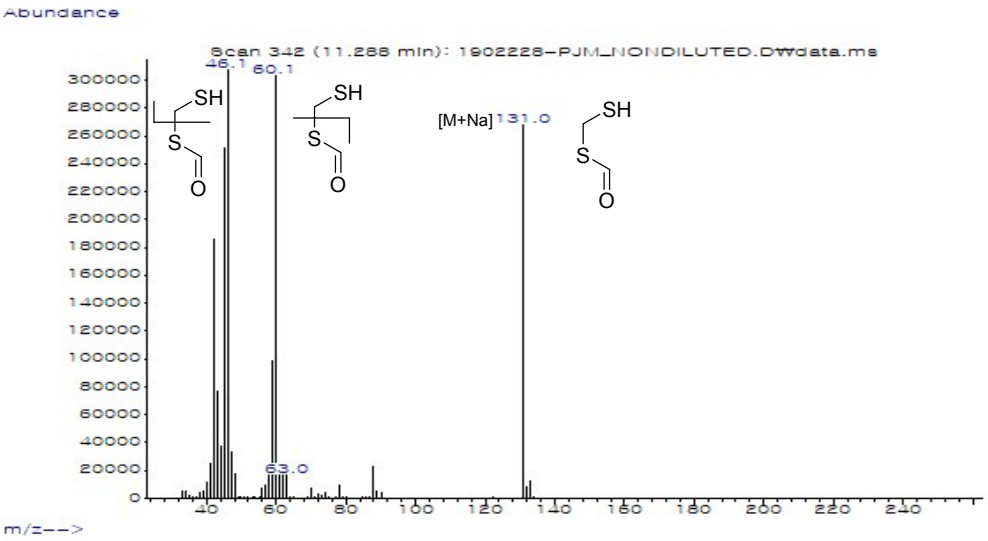


Fig. S3 MS spectrum of peak ② identified as compound **5** (*m/z* [M+Na] 131.0, t_R = 11.2 min)

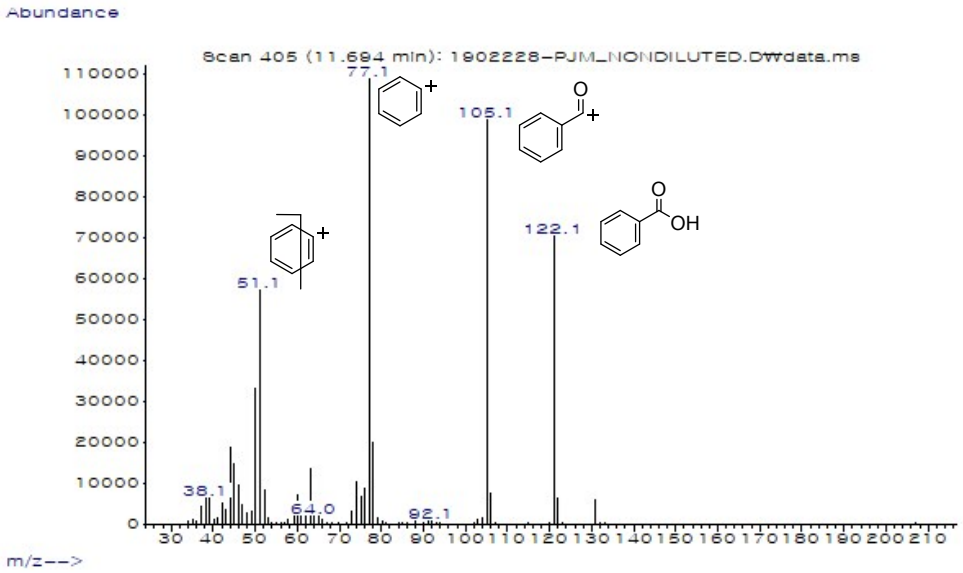
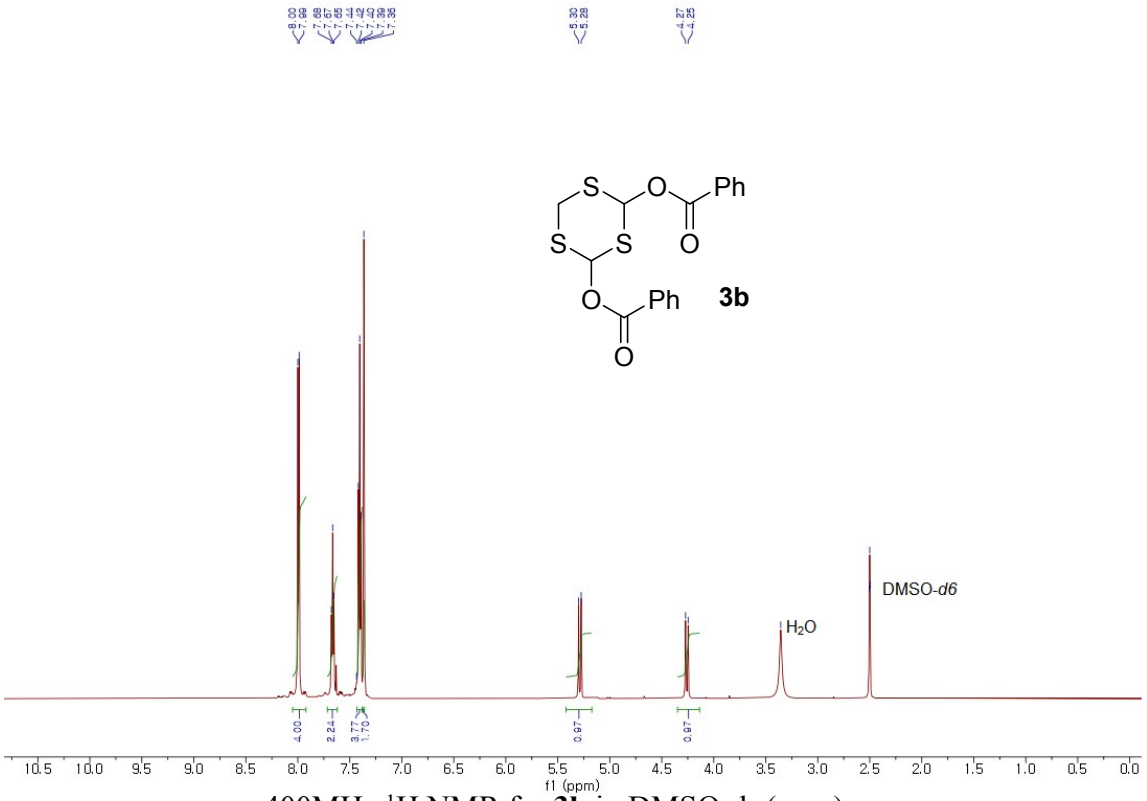


Fig. S4 MS spectrum of peak 3 identified as compound **5** (*m/z* [M+] 122.1, t_R = 11.7 min)

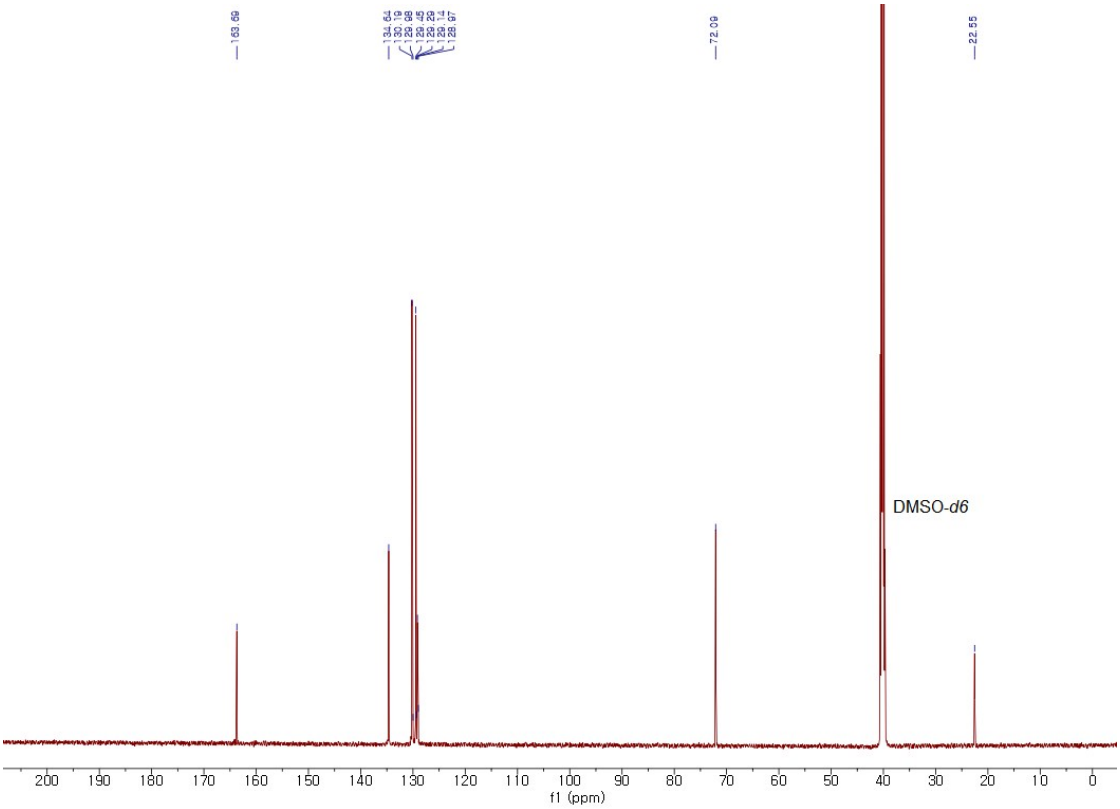
Procedure for the synthesis of 1,3,5-trithiane-based H_2S donors

1,3,5-trithiane-2,4-diyl dibenzoate **3b:** To a solution of 1,3,5-trithiane (554 mg, 3.50 mmol) in dry benzene (30 mL) was added a 50% solution of *tert*-butyl peroxybenzoate (2.28 mL, 12.3 mmol) followed by 12 mmol % of anhydrous CuCl (47 mg) under N_2 atmosphere. The solution was refluxed for 1 h at 81°C until the reaction was complete (TLC monitoring). The reaction mixture was quickly filtered through a Celite 545 pad to remove the cuprous salts with ethyl acetate. The filtrate was evaporated under reduced pressure at room temperature. The crude product was purified by the crystallization using benzene/hexanes systems. This crystallization was carried out by a 1:1 mixture of benzene and hexanes then the solid was recrystallized by a 7:3 mixture of the same solvents to afford a pure desired compound (320 mg, 31%) as a white solid. mp 152~156°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.99 (d, J = 7.6 Hz, 4H), 7.67 (t, J = 7.3 Hz, 2H), 7.40 (t, J = 7.7 Hz, 4H), 7.36 (s, 1H), 7.36 (s, 1H), 5.29 (d, J = 14.7 Hz, 1H), 4.26 (d, J = 14.7 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-D_6) δ 163.69, 134.64, 130.19, 129.98, 129.45, 129.29, 129.14, 128.97, 72.09, 22.55; HRMS *m/z* calcd for $C_{17}H_{15}O_4S_3$ $[M+H]^+$ 379.0132, found 379.0138.

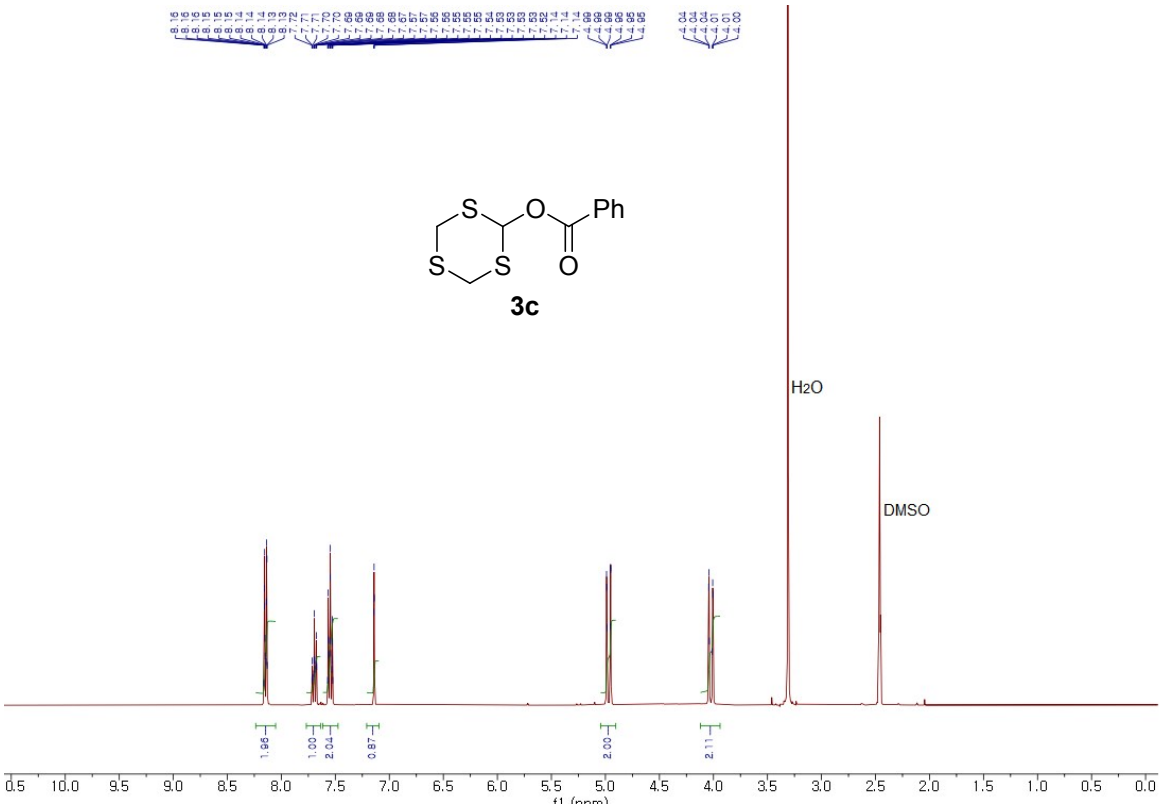
1,3,5-trithian-2-yl benzoate **3c:** To a solution of 1,3,5-trithiane (158 mg, 1.14 mmol) in dry benzene (15 mL) was added a 50% solution of *tert*-butyl peroxybenzoate (111 mg, 0.571 mmol) followed by 10 mmol % of anhydrous CuCl (14 mg) under N_2 atmosphere. The solution was refluxed for 1 h at 80°C until the reaction was complete (TLC monitoring). The reaction mixture was quickly filtered through a Celite 545 pad to remove the cuprous salts with ethyl acetate. The filtrate was evaporated under reduced pressure at room temperature. The crude product was purified by the crystallization using benzene/hexanes and then hexanes/DCM systems several times. This crystallization was carried out by a 1:1 mixture of benzene and hexanes then the solid was recrystallized by a 1:1 mixture of hexanes:DCM. A short flash column chromatography using 5% EtOAc in hexanes to remove polar impurities afforded the compound (94 mg, 32%) as a white solid. mp 134~137°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18-8.11 (m, 2H), 7.74-7.65 (m, 1H), 7.59-7.50 (m, 2H), 7.14 (s, 1H), 5.01-4.93 (m, 2H), 4.07-3.98 (m, 2H); ^{13}C NMR (100 MHz, DMSO-D_6) δ 163.54, 134.74, 130.45, 129.64, 129.37, 73.35, 40.57, 40.43, 40.29, 40.15, 40.01, 39.87, 39.74, 29.20; HRMS *m/z* calcd for $C_{10}H_{11}O_2S_3$ $[M+H]^+$ 258.9921, found 258.9928.



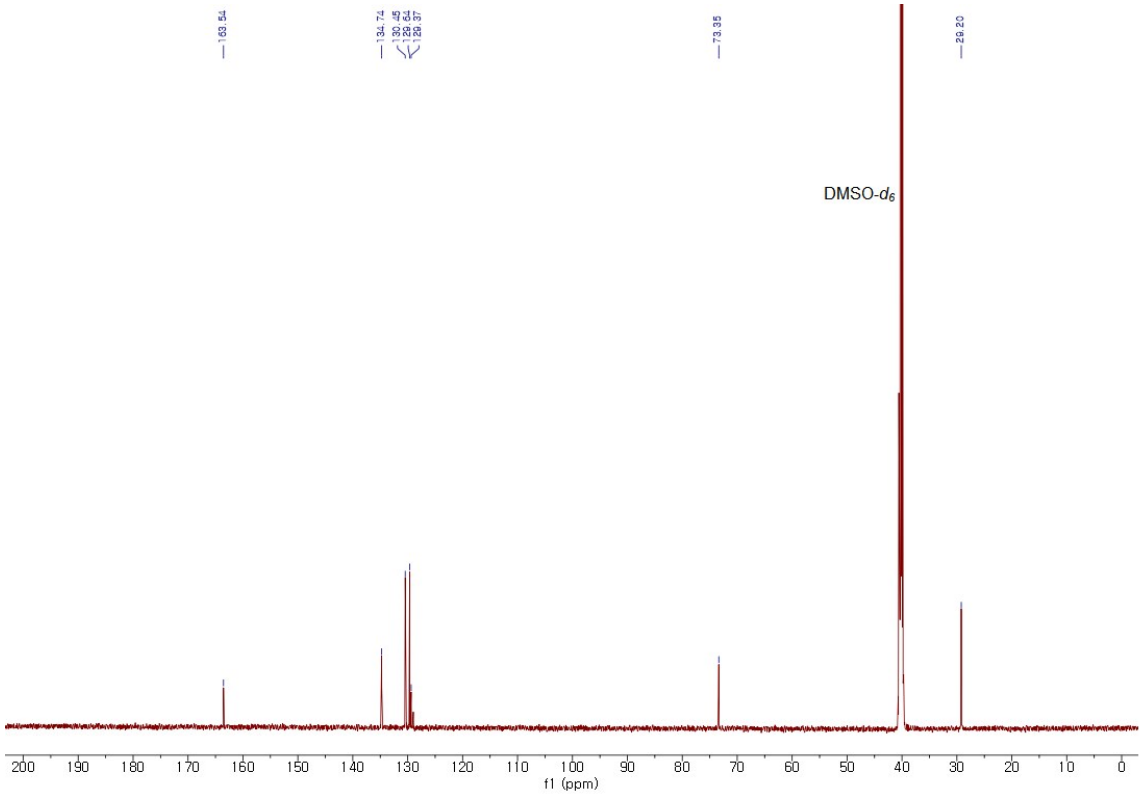
400MHz ^{1}H NMR for **3b** in DMSO-d_6 (ppm)



151MHz ^{13}C NMR for **3b** in DMSO-d_6 (ppm)



400MHz ^{1}H NMR for **3c** in DMSO-d_6 (ppm)



151MHz ^{13}C NMR for **3c** in DMSO-d_6 (ppm)