Supporting Information

Photocontrollable Hydrogen Sulfide Donor using Ketoprofenate Photocages

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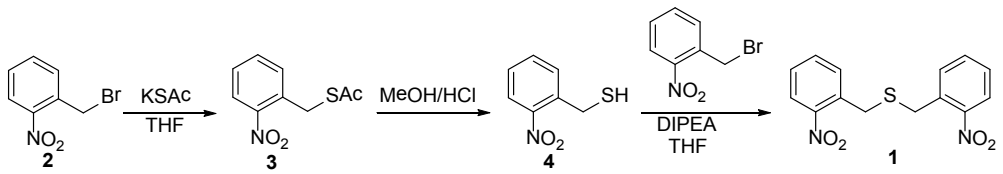
General methods and materials. Melting point was determined using a Yanagimoto micromelting point apparatus. Proton nuclear magnetic resonance spectra (^{1}H-NMR) and carbon nuclear magnetic resonance spectra (^{13}C-NMR) were recorded on a JEOL JNM-LA500 or JEOL JNM-A500 spectrometer in the indicated solvent. Chemical shifts (δ) are reported in parts per million relative to the internal standard, tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-SX102A mass spectrometer. Analytical HPLC was performed with a Shimadzu instrument equipped with an ODS-3 column (20 × 250 mm, GL Science). Ultraviolet–visible-light spectra were recorded on an Agilent 8453 spectrophotometer. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, Nacalai Tesque, and Kanto Kagaku, and used without purification. Flash column chromatography was performed using silica gel 60 (particle size 0.046-0.063 mm) supplied by Taiko Shoji. Photoirradiation was performed by using the light source of an Asahi Spectra MAX-302 irradiation apparatus.

Scheme S1. Preparation of **1**.



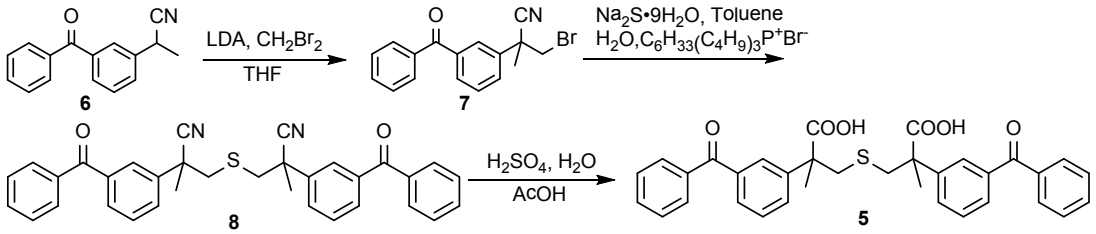
1-{[(2-nitrophenyl)methyl]sulfanyl}ethan-1-one (3). 1-(Bromomethyl)-2-nitrobenzene (5.22 g, 24.1 mmol) and potassium acetylsulfanide (14.6 g, 128 mmol) were dissolved in THF under an N_2 atmosphere. The solution was stirred overnight at room temperature. The residue was suspended in water and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, and evaporated to give 5.17 g of **3** (quantitative yield): ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.13 (1H, d, J = 8.1 Hz), 7.63 (1H, d, J = 7.1 Hz), 7.57 (1H, t, J = 7.5 Hz), 7.43 (1H, t, J = 7.8 Hz), 4.43 (2H, s), 2.33 (3H, s).

(2-nitrophenyl)methanethiol (4). Thioacetate **3** (1.49 g, 7.1 mmol) was dissolved in MeOH under an N_2 atmosphere, then 3.5 mL of HCl in MeOH was added dropwise, and the mixture was stirred overnight at 60 C°. The reaction mixture was acidified with 2 N HCl on an ice bath and evaporated. The reaction mixture was purified by

silica gel column chromatography ($CHCl_3$:*n*-hexane = 1:9 to 7:3) to give 1.10 g (92%) of **4** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 8.01 (1H, d, *J* = 4.1 Hz), 7.59 (1H, t, *J* = 7.5 Hz), 7.48 (1H, d, *J* = 1.2 Hz), 7.42 (1H, t, *J* = 8.6 Hz), 4.03 (2H, d, *J* = 8.5 Hz), 2.15 (1H, t, *J* = 8.5 Hz) ^{13}C NMR ($CDCl_3$, 125 MHz) 137.00, 133.74, 133.73, 131.57, 128.25, 125.36, 26.46; MS (EI) m/z: 169 $[M]^+$.

1-nitro-3-{[(3-nitrophenyl)methane]sulfanylmethyl}benzene (1). To a solution of **4** (0.30 g, 1.4 mmol) and 1-(bromomethyl)-2-nitrobenzene (0.19 g, 1.1 mmol) in THF (freshly distilled) was added dropwise DIPEA (0.18 g, 1.4 mmol). The mixture was stirred overnight at room temperature, then evaporated, and the residue was purified by silica gel flash column chromatography ($CHCl_3$ / *n*-hexane = 1:9 to $CHCl_3$ only) to give 0.25 g (72%) of **1** as a white powder: mp 121.5-123.9 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, δ; ppm) 7.95 (2H, dd, *J* = 1.5, 8.5 Hz), 7.62 (2H, m), 7.52–7.49 (4H, m), 4.02 (4H, s) ^{13}C NMR (DMSO-d_6, 125 MHz, δ; ppm) 138.64, 138.50, 137.22, 133.22, 133.88, 130.36, 30.34; MS (FAB) m/z: 305 $[M+H]^+$; Anal. Calcd. for $C_{14}H_{12}N_2O_4S$:C, 55.25; H, 3.97; N, 9.21. Found: C, 54.94; H, 4.04; N, 9.17.

Scheme S2. Preparation of **5**.

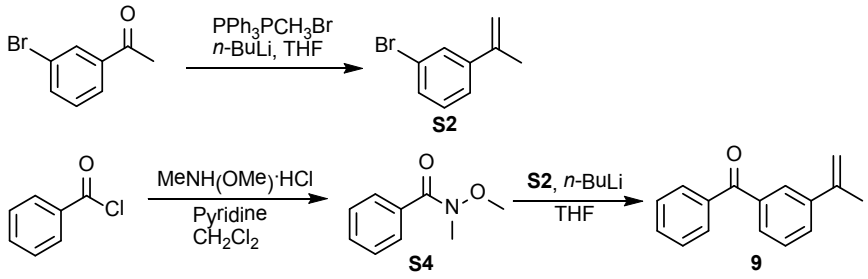


2-(3-Benzoyl-phenyl)-3-bromo-2-methyl-propionitrile (7). To a solution of 2-(3-benzoylphenyl)propionitrile (1.03 g, 4.42 mmol) in anhydrous THF (8 mL) was added LDA (2 M solution, 3 mL, 6 mmol). The mixture was stirred at -78 °C in a MeOH bath. The solution turned deep blue in color, indicating the presence of the anion of the nitrile. To this mixture was added dibromomethane (2.48 g, 14.3 mmol), after which the reaction mixture was removed from the MeOH bath and allowed to warm to room temperature. When the deep blue color of the nitrile anion had faded to pale yellow (5 h), the reaction mixture was suspended in 100 mL of H_2O. The whole was extracted with 50 mL of CH_2Cl_2, and the extract was dried over Na_2SO_4, and filtered. The solvent was removed under reduced pressure to give 1.50 g of **7** as an organic solid (quantitative yield). White crystals were obtained after recrystallization from hot EtOH: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.89 (1H, s), 7.77–7.81 (4H, m), 7.63 (1H, t, *J* = 7.4 Hz), 7.57 (1H, dd, *J* = 8.0, 7.7 Hz) 7.51 (2H, dd, *J* = 7.9, 7.5 Hz) 3.73 (1H, d, *J* = 10.7 Hz) 3.69 (1H, d, *J* = 10.7 Hz) 1.94 (3H, s).

2-(3-benzoylphenyl)-3-{[2-(3-benzoylphenyl)-2-cyano-2-methylethyl]sulfanyl}-2-methylpropanenitrile (8). To a mixture of **7** (3.46 g, 10.54 mmol), sodium sulfide nonahydrate (2.53 g, 10.54 mmol) and tributylhexadecylphosphonium bromide (1.61 g, 3.16 mmol) was added toluene (20 mL) and H_2O (20 mL) under an N_2 atmosphere. The solution was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*–hexane = 1/2) gave 2.27 g (81%) of **8** as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.83 (2H, d, *J* = 6.2 Hz), 7.80–7.69 (8H, m), 7.63–7.59 (2H, m), 7.55–7.47 (6H, m) 3.08 (4H, quintet, *J* = 14 Hz) 1.82 (6H, d, *J* = 9.5 Hz); MS (EI) m/z: 528 (M^+).

2-(3-benzoylphenyl)-3-{[2-(3-benzoylphenyl)-2-carboxy-2-methylethyl]sulfanyl}-2-methylpropanoic acid (5). A mixture of **8** (1.75 g, 1.1 mmol), H_2O (50 mL), H_2SO_4 (50 mL) and acetic acid (50 mL) was refluxed overnight, and then rapidly cooled in an ice-water bath. The reaction mixture was diluted with H_2O, and extracted with CH_2Cl_2. The combined organic solution was extracted with 1 N NaOH, and the aqueous solution was washed with AcOEt. The basic aqueous layer was then acidified with 10 % HCl, and extracted with AcOEt. Filtration, concentration of the filtrate in vacuo, and purification of the residue by silica gel flash column chromatography ($CHCl_3$/MeOH = 10/1) gave 1.39 g of **5** (73%) as a crude solid, which was recrystallized from MeCN to give a white solid: mp 126–130 °C; ^{1}H NMR (CD_3OD, 500 MHz, δ; ppm) 7.73 (6H, m), 7.61 (6H, m), 7.51–7.45 (6H, m) 2.95 (4H, q, *J* = 13, 37 Hz) 1.62 (6H, s). ^{13}C NMR ($CDCl_3$, 125 MHz, δ; ppm) 198.43, 178.23, 144.62, 138.77, 133.91, 132.06, 131.14, 129.80, 129.59, 129.56, 129.27, 52.92, 45.50, 22.86; MS (FAB) m/z: 567 (M^++1); Anal. Calcd. for $C_{34}H_{30}O_6S$·10/11H_2O: C, 70.04; H, 5.50. Found: C, 70.01; H, 5.44.

Scheme S3. Preparation of **9**.



1-bromo-3-(prop-1-en-2-yl)benzene (S2). Methyltriphenylphosphonium bromide (7.0 g, 19.6 mmol) was suspended in tetrahydrofuran (15 mL) and the suspension was cooled to 0 °C. *n*-Butyllithium (1.6 M in hexane, 12.4 mL, 19.6 mmol) is added slowly to it. The resulting solution was stirred for 1 h at 0 °C. A solution of 3-bromoacetophenone (3.0 g, 15.1 mmol) in tetrahydrofuran (10 mL) was then added slowly via an addition funnel. The resulting mixture was warmed to room temperature, stirred overnight, and then cooled to 0°C. The reaction was quenched with saturated aqueous ammonium chloride solution. The aqueous phase was collected in a separatory funnel and extracted with hexane. The combined organic solution was washed with brine and dried over Na_2SO_4. Filtration, evaporation, and silica gel flash column chromatography (*n*-hexane) gave 2.5 g of 1-bromo-3-(prop-1-en-2-yl)benzene (83%) as a colorless liquid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.59 (1H, t, J = 2.0 Hz) 7.40–7.37 (2H, m) 7.19 (1H, t, J = 7.5 Hz) 5.37 (1H, s) 5.12 (1H, s) 2.13 (3H, s).

***N*-methoxy-*N*-methylbenzamide (S4).** Pyridine (12.1 mL, 149.4 mmol) was slowly added to a mixture of benzoyl chloride (10 g, 71.1 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (7.3 g, 74.7 mmol) in CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. After the addition of 1 N HCl, the phases were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and filtered. The solvent was removed *in vacuo* to afford 11.6 g of *N*-methoxy-*N*-methylbenzamide as a colorless oil (99%): ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.67 (2H, m) 8.75 (1H, d, J = 7.5 Hz) 7.47–7.44 (1H, m) 7.42–7.39 (2H, m) 3.59 (3H, s) 3.36 (1H, s)

(Phenyl) {3-(prop-1-en-2-yl)phenyl}methanone (9). To a solution of 1-bromo-3-(prop-1-en-2-yl)benzene (424 mg, 2.15 mmol) in THF (2.5 mL) was added *n*-butyllithium (1.6 M in hexane, 1.34 mL, 2.15 mmol), keeping the temperature below -78 °C. The resulting reaction mixture was stirred at -78 °C for 30 minutes followed by addition of *N*-methoxy-*N*-methylbenzamide (319 mg, 1.93 mmol). When the reaction was completed, the solution was quenched with saturated NaH_4Cl and allowed to warm to room temperature. It was then poured into water and extracted with AcOEt. The organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*–hexane = 1/20) gave 179 mg (37%) of **9** as a white solid: mp 60–62 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.90 (1H, m) 7.83–7.81 (2H, m) 7.70–7.66 (2H, m) 7.61–7.58 (1H, m) 7.49 (2H, t, J = 7.5 Hz) 7.44 (1H, t, J = 7.5 Hz) 5.42 (1H, s) 5.16 (1H, m) 2.18 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz, δ; ppm) 196.83, 142.48, 141.50, 137.65, 137.62, 132.47, 130.09, 130.08, 129.42, 129.11, 129.10, 128.30, 128.30, 128.29, 128.13, 126.98, 113.63, 21.79; MS (EI) m/z: 222 (M^+); Anal. Calcd. for $C_{16}H_{14}O \cdot 1/5H_2O$: C, 85.07; H, 6.43. Found: C, 85.04; H, 6.43.

Irradiation of sample solutions.
For photoirradiation experiments, a quartz flat cuvette was placed at a distance of 9 cm from a lens attached to a MAX-302 light source (Asahi Spectra, Tokyo) with a xenon lamp (300 W), and irradiated with UV-A light (300 – 350 nm) through a ND filter at room temperature for the indicated duration.

Fluorescence measurement with HSip-1.
Sample solutions (800 μL) containing compound **1** or **5** (100 μM) and HSip-1 (1 μM) in 30 mM HEPES buffer (pH 7.4, containing 1% DMSO) were irradiated or incubated in the dark. Emission spectra (λex = 491 nm, λem = 516 nm) were determined 30 minutes thereafter using a RF-5300 PC spectrofluorophotometer (Shimadzu, Kyoto, Japan).

Assay of H_2S release with methyleneblue.
Methyleneblue assay was carried out as described previously with some modifications.[1]

Calibration curve. A 5, 10, 15, 20 mM solution of Na_2S in MiliQ were prepared and used as the stock solution. Aliquots of 1 μL of the Na_2S stock solution were dissolved in a 30 mM HEPES buffer (1%DMSO, pH 7.4) to obtain

the standard solutions in 5, 10, 15, 20 μM, respectively. To 500 μL aliquot of the respective solution was added zinc acetate (1% w/v, 100 μL). Subsequently, *N*,*N*-dimethyl-p-phenylenediamine sulfate (20 mM, 150 μL) in 7.2 M HCl was added followed by $FeCl_3$ (30 mM, 150 μL) in 1.2 M HCl. The reaction mixture was incubated for 20 minutes. After centrifugation (6,200 rpm) for 5 minutes, absorbance (670 nm) of aliquots of the resulting solution (800 μL) was determined with a UV-Vis spectrometer. (Each reaction was performed in triplicate).

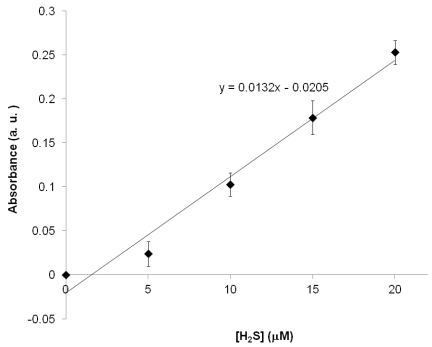


Fig S1. Calibration curve for H_2S in 30 mM HEPES buffer containing 1% DMSO (pH 7.4).

To 500 μL of a 30 mM HEPES buffer solution (pH 7.4) containing 100 μM **5** (500 μL) with or without photoirradiation was added zinc acetate (1% w/v, 100 μL). Next, *N*,*N*-dimethyl-p-phenylenediamine sulfate (20 mM, 150 μL) in 7.2 M HCl was added, followed by $FeCl_3$ (30 mM, 150 μL) in 1.2 M HCl. The reaction mixture was incubated for 20 minutes. After centrifugation (6,200 rpm) for 5 minutes, the absorbance (670 nm) of aliquots of the resulting solution (800 μL) was determined with a UV-Vis spectrometer. The H_2S concentration of each sample was calculated from a calibration curve obtained using sodium sulfide nonahydrate.

HPLC analysis for detection of photoproduct.

Sample solutions of **5** (100 μM) in 30 mM HEPES buffer (pH 7.4, containing 1% DMSO) were irradiated for 400 seconds. An aliquot (100 μL) of each solution was mixed with DMSO (100 μL) and the mixture was loaded onto a Inertsil ODS-3 column (5 mm; 150 × 4.6 mm) fitted on a Shimadzu HPLC system. The eluates were monitored with a photodiode array detector. Milli-Q water containing 0.1% FA (A) and MeOH containing 0.1% FA (B) were used as developing solvents. Gradient conditions were as follows: 0 min, A 50% and B 50% → 15 min, A 15% and B 85% → 25 min, A 15% and B 85% → 30 min, A 50% and B 50% → 35 min, A 50% and B 50%.

H_2S detection in serum.

Calibration curve. A 10, 20, 30, 40 mM solution of sodium sulfide nonahydrate in MiliQ were prepared and used as the stock solution. Aliquots of 1 μL of the Na_2S stock solution were dissolved in a fetal bovine serum (purchased from Bio West) to obtain the standard solutions in 10, 20, 30, 40 μM, respectively. Reaction aliquots (500 μL) were collected into 1.5 mL vials containing zinc acetate (1% w/v, 50 μL) and trichloroacetic acid (10%, 300 μL) in every 5 minutes. The resulting solution was centrifuged for 5 minutes (12,000 rpm). The clear solution (600 μL) was transferred to another vial. Subsequently, *N,N*-dimethyl-*p*-phenylenediamine sulfate (20 mM, 75 μL) in 7.2 M HCl was added followed by $FeCl_3$ (30 mM, 75 μL) in 1.2 M HCl. The reaction mixture was incubated for 20 minutes. After centrifugation (12,000 rpm) for 5 minutes, absorbance (670 nm) of aliquots of the resulting solution (600 μL) was determined with a UV-Vis spectrometer. (Each reaction was performed in triplicate).

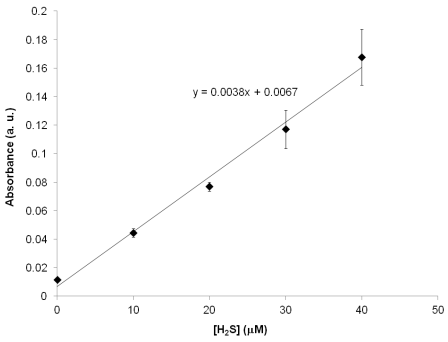


Fig S2. Calibration curve for H_2S in serum.

To 500 μL of a fetal bovine serum containing **5** with or without photoirradiation were added zinc acetate (1% w/v, 50 μL) and trichloroacetic acid (10%, 300 μL) every 5 minutes. The resulting solution was centrifuged for 5 minutes (12,000 rpm). The clear solution (600 μL) was transferred to another vial. Subsequently, *N,N*-dimethyl-*p*-phenylenediamine sulfate (20 mM, 75 μL) in 7.2 M HCl was added, followed by $FeCl_3$ (30 mM, 75 μL) in 1.2 M HCl. The reaction mixture was incubated for 20 minutes. After centrifugation (12,000 rpm) for 5 minutes, the absorbance (670 nm) of an aliquot of the resulting solution (600 μL) was determined with a UV-Vis spectrometer. The H_2S concentration of each sample was calculated from a calibration curve obtained with sodium sulfide nonahydrate.

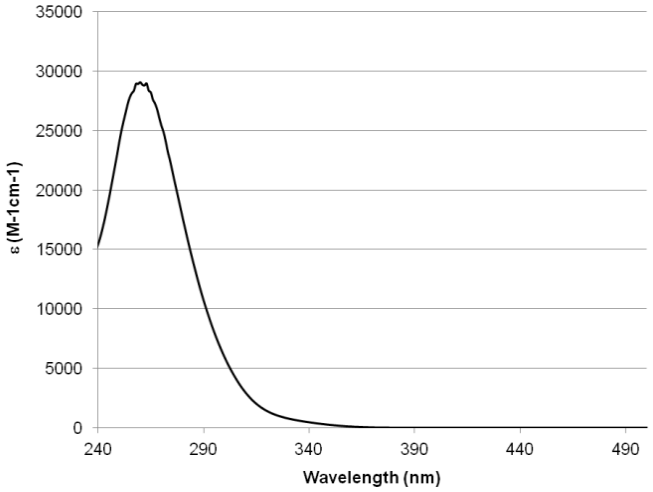


Figure S3. UV-visible absorbance spectrum of a solution of **5** (100 μM) in pH 7.4 HEPES buffer (1% DMSO as a co-solvent).

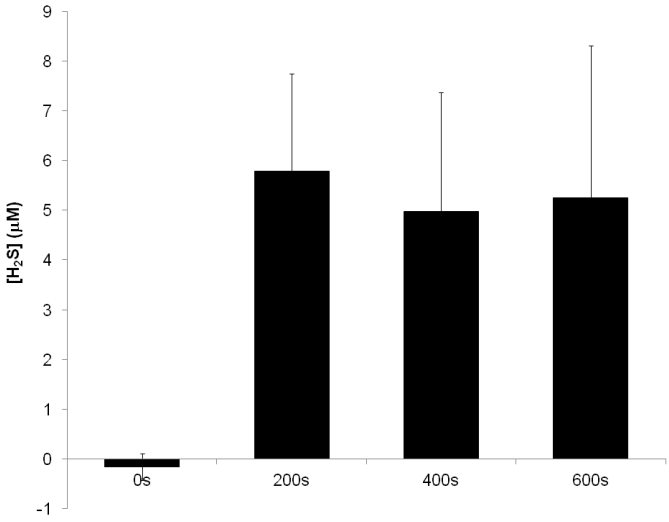
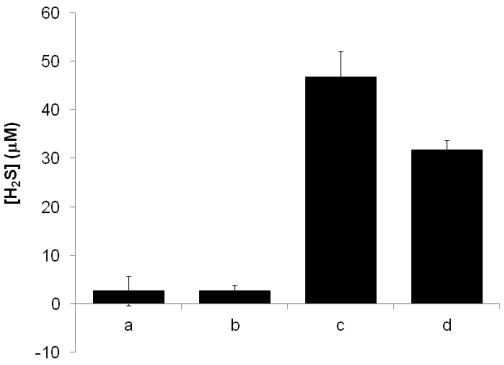


Figure S4. Release of H_2S from **5** (100 μM) in bovine serum as detected by the methyleneblue method. Absorbance of formed methyleneblue after irradiation of **5** was determined at 670 nm. Solution containing **5** irradiated for 200, 400 and 600 seconds. The data represents the average of three experiments with standard deviations.

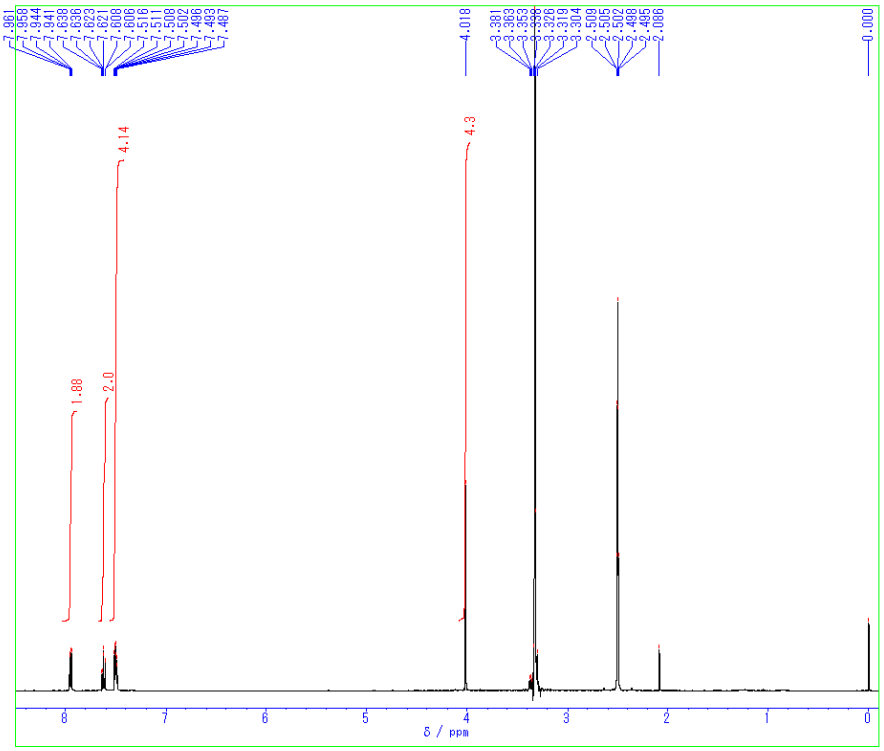


500 µM **5**	-	+	+	+
hν	-	-	+	+
60 min incubation	+	+	+	-

Figure S5. Evaluation of the stability of compound **5** in serum. Release of H_2S from **5** (100 µM) in serum detected by the methyleneblue method after 60 min incubation at 37 °C. Formation of methyleneblue after irradiation of **5** for various time periods with different light intensities was determined from the absorbance at 670 nm. The data represents the average of three independent experiments with standard deviations.

(1) Y. Zhao, H. Wang and M. Xian, *J. Am. Chem. Soc.*, 2011, **133**, 15.

NMR charts of 1, 5 and 9



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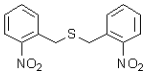

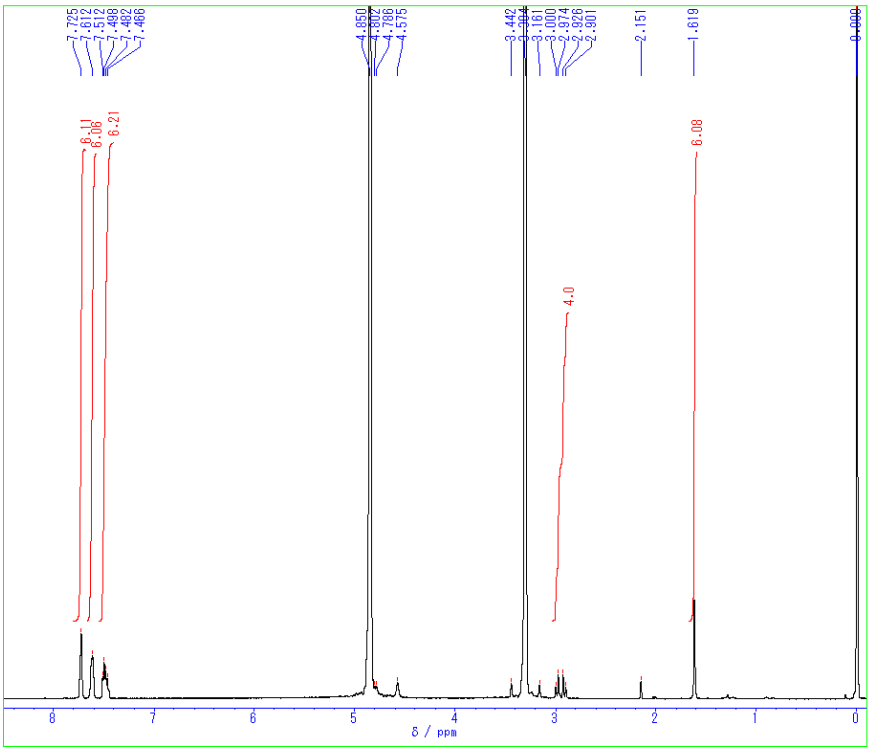

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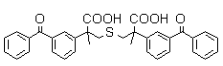

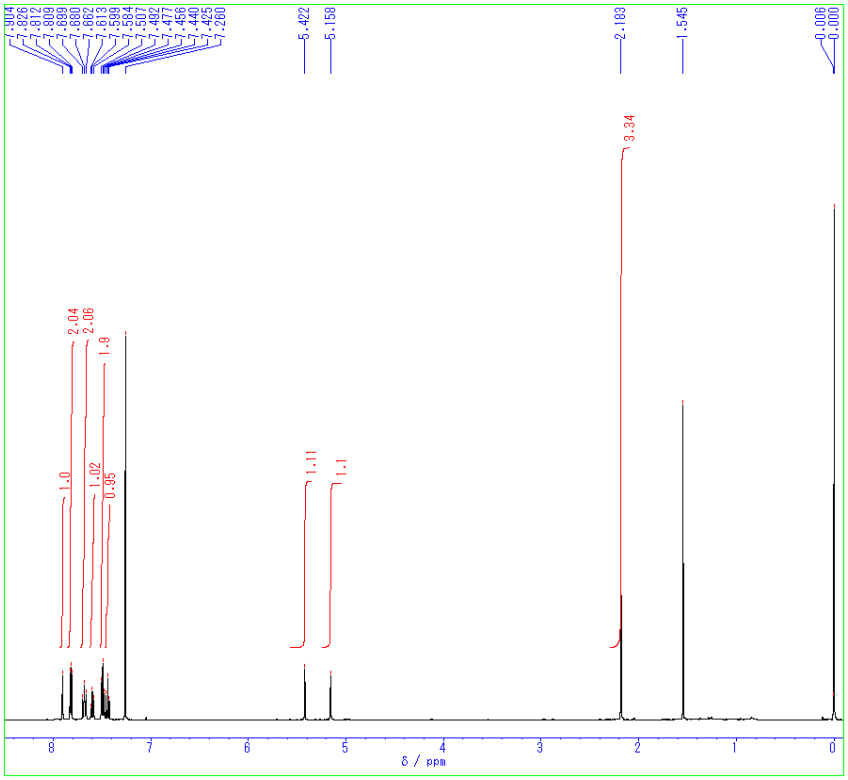

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PI3 0.0 ms
Loop1 0
Point 131072(ZeroFill: ×4)
Scan 16
DummyScan 0
Frequecy(Span) 8012.82 Hz
AcqTime 4.0894 s
PD 1.5 s
RGain 32
Broad.Factor 0.25 Hz
ExMode NON
IrrNuc ^{13}C
IrrFreq 0.0 MHz
IrrSet 0.0 kHz
IrrFine 0.0 Hz
IrrPulse 0 µs
IrrAttn 0
Spinning 16.0 Hz
Temperature 26.0 ℃
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