Supporting Information

Thionoesters: A Native Chemical Ligation-Inspired Approach to Cysteine-Triggered H_2S Donors

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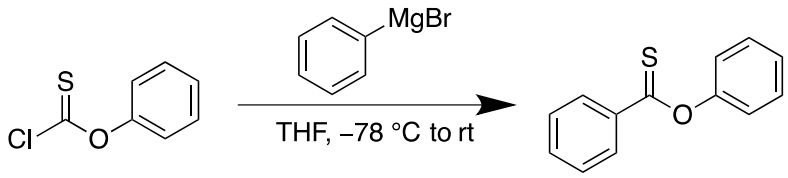
Materials and Methods.
Reagents were purchased from Sigma-Aldrich, Tokyo Chemical Industry (TCI) or VWR and used directly as received. *S*-Phenyl benzothioate was synthesized according to a previous report.[1] *N*-phenylthiobenzamide was synthesized according to a previous report.[2] Methyl 2-phenyl-4,5-dihydrothiazole-4-carboxylate (**CysDHT**) was synthesized according to a previous report.[3] Deuterated solvents were purchased from Cambridge Isotope Laboratories and used as received. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker 500 MHz instrument. Chemical shifts are reported relative to residual protic solvent resonances. Silica gel (SiliaFlash F60, Silicycle, 230-400 mesh) was used for column chromatography. All air-free manipulations were performed under an inert atmosphere using standard Schlenk technique or an Innovative Atmospheres N_2-filled glove box. UV-Vis spectra were acquired on an Agilent Cary 60 UV-Vis spectrophotometer equipped with a Quantum Northwest TC-1 temperature controller at 25 °C ± 0.05 °C.

Synthesis



O-Phenyl benzothioate (**DPTE**) was synthesized using a slightly-modified procedure.[1] Phenyl chlorothionoformate (14.8 mmol, 1.1 equiv.) was added to anhydrous THF (20 mL) at -78 °C under N_2. While stirring, phenylmagnesium bromide (12.8 mmol, 1.0 M in THF, 1.0 equiv.) was added dropwise, and the reaction solution was stirred for 1 h at -78 °C. After 1 h, the reaction solution was allowed to warm to room temperature and stirred for an additional 2 h. The reaction was then quenched by addition of deionized H_2O (30 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extractions were washed with brine (30 mL), and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure, and the desired product purified by column chromatography (10% CH_2Cl_2 in hexanes, R_f = 0.33). The resultant product was isolated as a bright orange-yellow liquid. 938 mg (34%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.37 (d, J = 7.3 Hz, 2H), 7.63 (t, J = 7.5 Hz, 1H), 7.48 (q, J = 7.2 Hz, 4H), 7.33 (t, J = 7.4 Hz, 1H), 7.14 (d, J = 7.5 Hz, 2H). $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) δ: 211.22, 155.03, 138.11, 133.46, 129.76, 129.44, 128.42, 126.53, 122.31. HRMS-EI$^+$ (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{10}OS$, 214.04524; found, 214.04478.

HPLC Analysis.
HPLC analysis was performed on an Agilent 1260 HPLC instrument with a Poroshell 120 EC-C18 4.6x100 mm column and monitored at 280 nm. Solvent A: 95% H_2O, 5% MeOH, Solvent B: 100% MeCN. Gradient: 35% Solvent A/65% Solvent B for 2 min. Change to 100% Solvent B over 4 min and hold for 6.5 min. Change to 35% Solvent A/65% Solvent B over 0.5 min and hold for 4.5 min. Flow Rate: 0.5 mL/min, 2 μL injection.

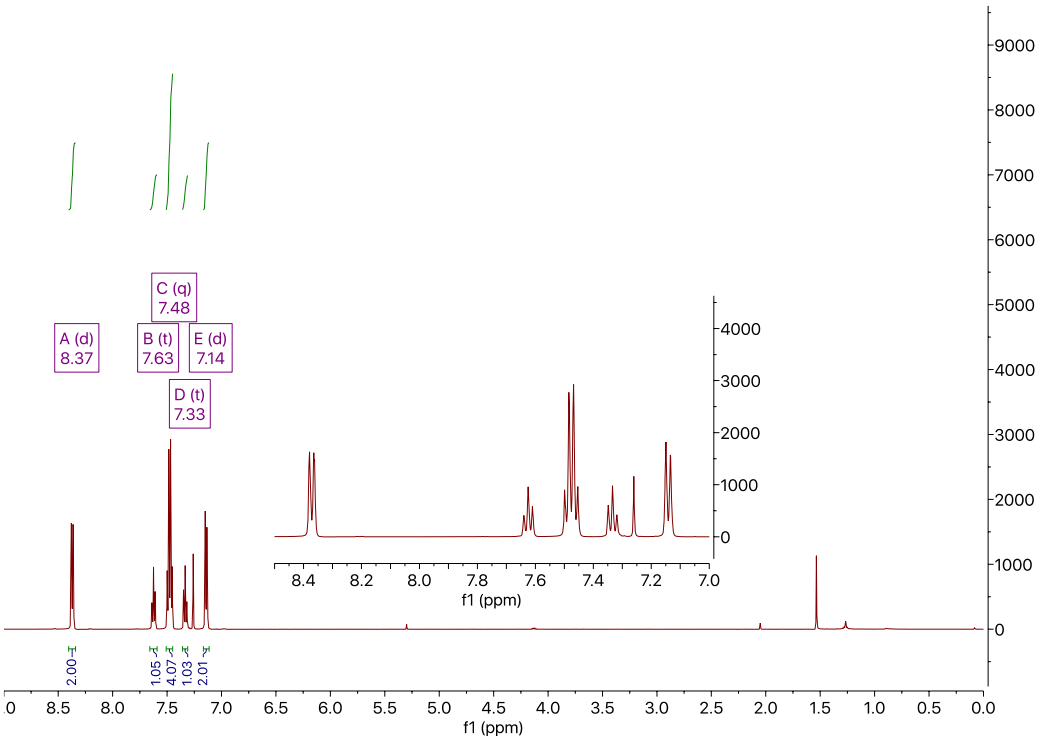


Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **DPTE**.

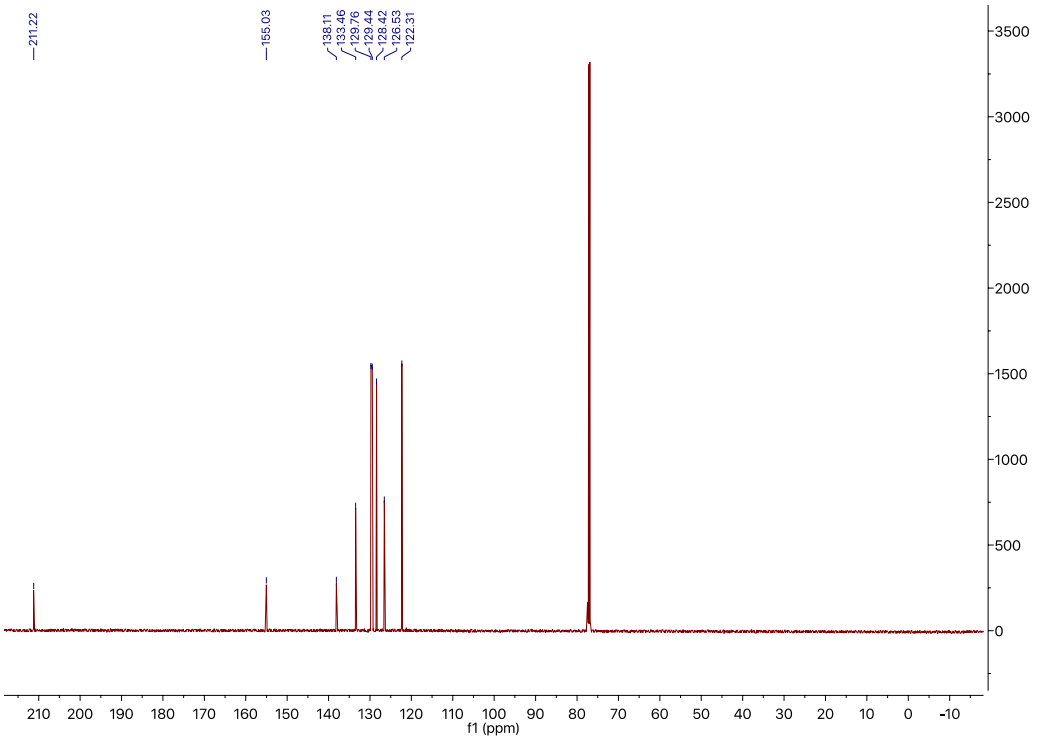


Figure S2. ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) spectrum of **DPTE**.

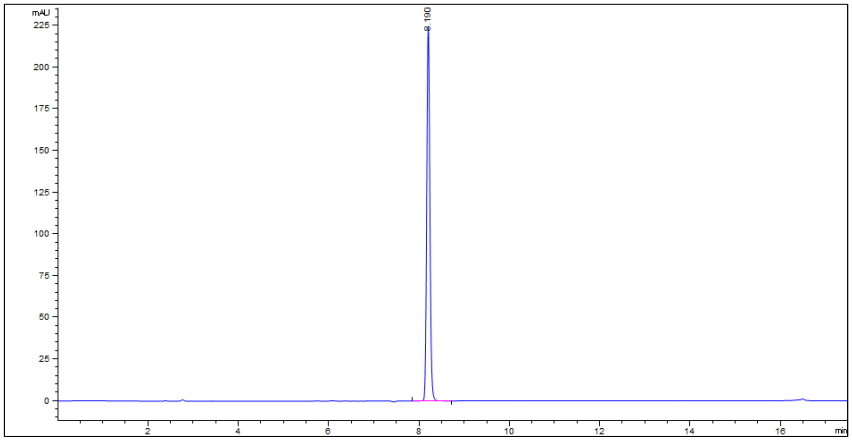


Figure S3. HPLC trace of **DPTE**.

H_2S Detection Materials and Methods. Phosphate buffered saline (PBS) tablets (1X, CalBioChem) were used to prepare buffered solutions (140 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) in deionized water. Buffer solutions were sparged with N_2 to remove dissolved oxygen and stored in an N_2-filled glovebox. Donor stock solutions (in DMSO) were prepared inside an N_2-filled glovebox and stored at -25 °C until immediately before use. Trigger stock solutions (in PBS) were freshly prepared in an N_2-filled glovebox immediately before use.

General Procedure for Measuring H_2S Release via Methylene Blue Assay (MBA). Scintillation vials containing 20 mL of PBS were prepared in an N_2-filled glovebox. To these solutions, 20 μL of 500 mM analyte stock solution (in PBS) was added for a final concentration of 500 μM. While stirring, the solutions were allowed to thermally equilibrate in heating block at the desired temperature for approximately 20-30 min. Immediately prior to donor addition, 0.5 mL solution of the methylene blue cocktail were prepared in disposable 1.5 mL cuvettes. The methylene blue cocktail solution contained: 200 μL of 30 mM $FeCl_3$ in 1.2 M HCl, 200 μL of 20 mM *N*,*N*-dimethyl-*p*-phenylene diamine in 7.2 M HCl, and 100 μL of 1% (w/v) $Zn(OAc)_2$. To begin an experiment, 20 μL of 25 mM donor stock solution (in DMSO) was added for a final concentration of 25 μM. At set time points after the addition of donor, 500 μL reaction aliquots were added to the methylene blue cocktail solutions and incubated for 1 h at room temperature shielded from light. Absorbance values at 670 nm were measured 1 h after addition of reaction aliquot. Each experiment was performed in quadruplicate unless stated otherwise.

MBA Calibration Curve. Solutions containing 0.5 mL of the methylene blue cocktail and 0.5 mL PBS containing 500 μM cysteine were freshly prepared in disposable cuvettes (1.5 mL). Under inert conditions, a 10 mM stock solution of NaSH (Strem Chemicals) in PBS was prepared and diluted to 1 mM. Immediately after dilution, 1 mM NaSH was added to 1.0 mL solutions for final concentrations of 10, 20, 30, 40, and 50 μM. Solutions were mixed thoroughly, incubated at room temperature for 1 h, and shielded from light. Absorbance values at 670 nm were measured after 1 h.

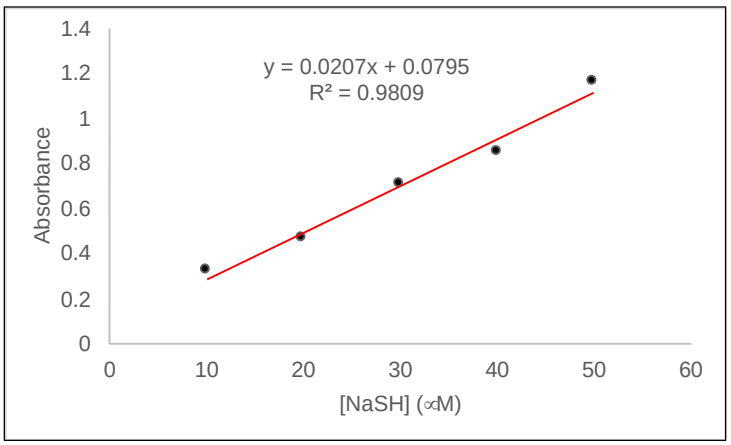
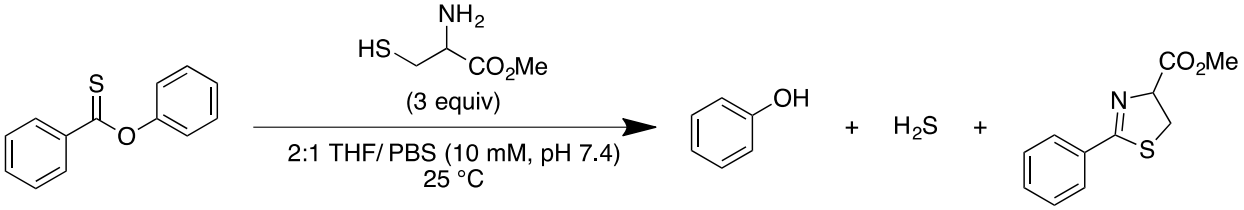


Figure S4. MBA calibration curve generated using known concentrations of NaSH.

Reaction Product Analysis



DPTE (0.39 mmol, 1.0 equiv.) was dissolved in 15 mL of 2:1 THF/PBS (10 mM, pH 7.4). L-cysteine methyl ester hydrochloride (1.17 mmol, 3.0 equiv.) was added in a single portion and stirred at room temperature for 2 h. The reaction mixture was diluted with deionized H_2O (~ 20 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure, and the purified by preparative thin layer chromatography (1:1 CH_2Cl_2 in hexanes). **DPTE** R_f = 0.75 (63.6 mg, 76%) and **CysDHT** R_f = 0.03 (11.0 mg, 13%) were isolated and characterized by ^{1}H and ^{13}C{^{1}H} NMR spectroscopy. The significant recovery of **DPTE** is likely due to the lack of buffering capacity in the preparative-scale reaction.

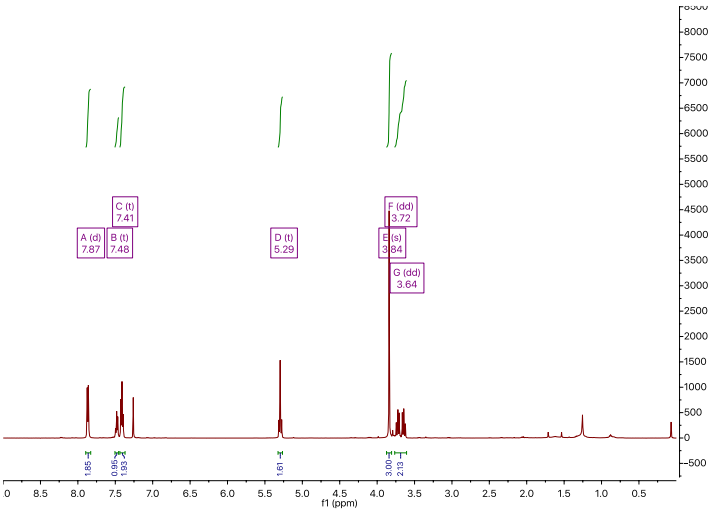


Figure S5. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of isolated **CysDHT**

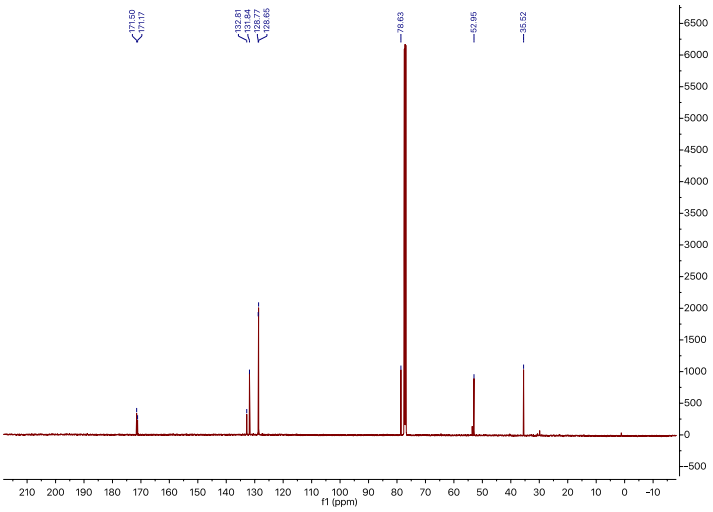
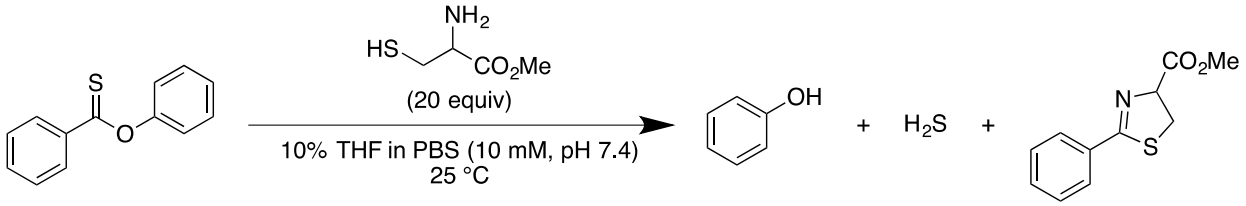


Figure S6. $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) spectrum of isolated **CysDHT**.

Reaction Product Analysis via HPLC



To a 20 mL solution of 10% THF in PBS (10 mM, pH 7.4) containing 2 mM L-cysteine methyl ester (20 equiv.), 20 µL of 100 mM **DPTE** in THF was added and stirred at room temperature. After 1 h, a 1 mL reaction aliquot was analyzed by HPLC. HPLC analysis was performed on an Agilent 1260 HPLC instrument with a Poroshell 120 EC-C18 4.6x100 mm column and monitored at 280 nm. Solvent A: 95% H_2O, 5% MeOH, Solvent B: 100% MeCN. Gradient: 35% Solvent A/65% Solvent B for 2 min. Change to 100% Solvent B over 4 min and hold for 6.5 min. Change to 35% Solvent A/65% Solvent B over 0.5 min and hold for 4.5 min. Flow Rate: 0.5 mL/min, 4 µL injection. The concentration of **CysDHT** and phenol present at the end of the reaction were determined by measurement against calibration curves for each compound.

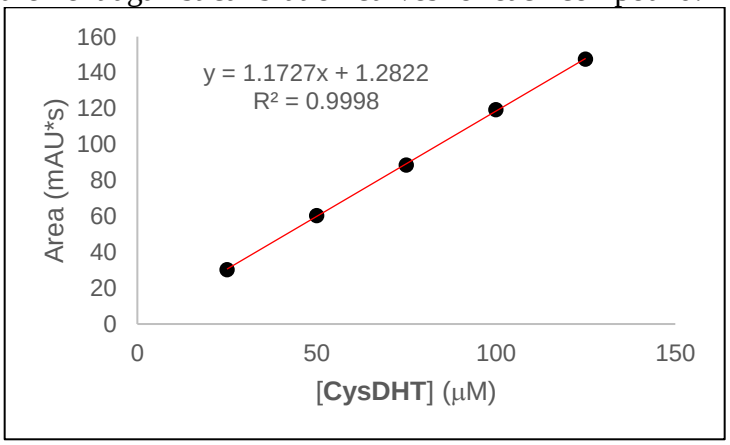


Figure S7. HPLC calibration curve of **CysDHT**

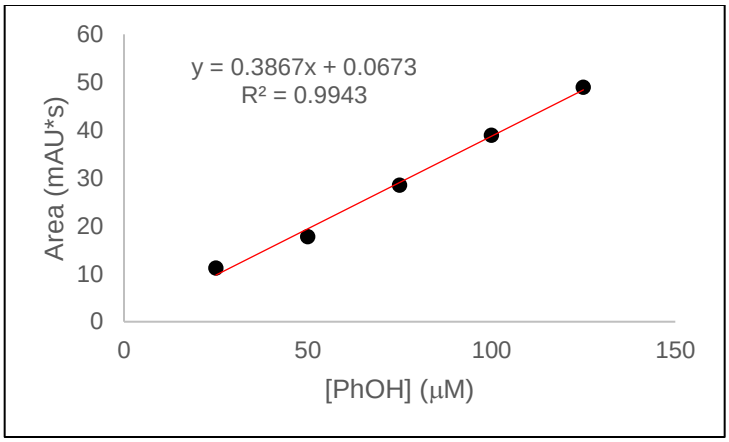


Figure S8. HPLC calibration curve of phenol

References:

1. Lim, Y.W.; Hewitt, R.J.; Burkett, B.A. *Eur. J. Org. Chem.* **2015**, *2015*, 4840-4842.
2. Zhang, G.; Liu, C.; Yi, H.; Meng, Q.; Bian, C.; Chen, H.; Jian, J.-X.; Wu, L.-Z.; Lei, A. *J. Am. Chem. Soc.* **2015**, *137*, 9273-9280.
3. Kim, T.S.; Lee, Y.J.; Jeong, B.S.; Park, H.G.; Jew, S.S. *J. Org. Chem.* **2006**, *71*, 8276-8278.