JOC The Journal of Organic Chemistry
Cite This: J. Org. Chem. 2019, 84, 14469−14475
Article
pubs.acs.org/joc

Development of Acid-Mediated H_2S/COS Donors That Respond to a Specific pH Window

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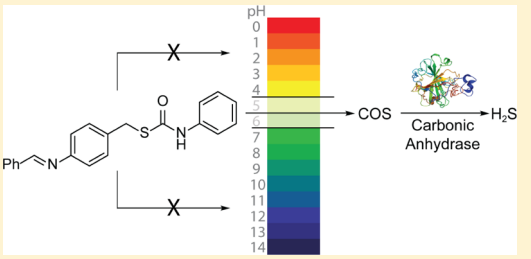
Supporting Information

ABSTRACT: Hydrogen sulfide (H_2S) is a biologically relevant molecule, and recent efforts have focused on developing small molecular donors that deliver H_2S on demand. Acid-activated donors have garnered significant interest due to the potential application of such systems in myocardial ischemia injury or for suppressing tumor growth. In this work, we report a new strategy for tuning H_2S delivery to a specific pH window. Specifically, we utilize self-immolative thiocarbamates with an imine-derived triggering group. After imine hydrolysis, the self-immolative decomposition releases carbonyl sulfide (COS), which is quickly hydrolyzed to H_2S by carbonic anhydrase. Although acid-mediated hydrolysis results in imine cleavage, environments that are too acidic result in protonation of the aniline intermediate and results in inhibition of COS/H_2S release. Taken together, this mechanism enables access to donor motifs that are only activated within specific pH windows. Here, we demonstrate the design, preparation, and pH evaluation of a series of imine-based COS/H_2S donor motifs, which we anticipate that will have utility in investigating H_2S in acidic microenvironments.



INTRODUCTION

Hydrogen sulfide (H_2S) is an important biological signaling molecule and the most recent addition to the gasotransmitter family alongside nitric oxide and carbon monoxide.[1] Endogenous H_2S production is primarily attributed to four main enzymes including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), 3-mercaptopyruvate sulfur transferase, and cysteine aminotransferase through cysteine and homocysteine catabolism. H_2S can also be generated through nonenzymatic pathways such as the thiol-mediated release of H_2S from allium- and garlic-derived polysulfides.[2] Once generated, H_2S is involved in different physiological processes, including K_{ATP} channel activation[3] as well as antioxidant and antiapoptotic signaling.[4] In efforts to modulate H_2S levels in different systems, most studies have used inorganic sulfide salts, such as sodium sulfide (Na_2S) and sodium hydrosulfide (NaSH), as exogenous H_2S sources. Although these sulfide sources increase endogenous H_2S levels, the large and immediate dose can result in unwanted toxicity and sulfide oxidation.[5] To better understand and leverage H_2S levels in various biological systems, a variety of chemical tools have been developed in the last decade and include CSE and CBS inhibitors, H_2S donors, and activity-based H_2S probes for H_2S detection.[6−8]

Of the wide array of available H_2S donors, one common strategy to engineer H_2S release is to leverage water- or acid-mediated hydrolysis. For example, GYY4137 is one of the most widely used synthetic H_2S donors and releases H_2S slowly upon hydrolysis in the water at physiological pH (Figure 1a).[9] Similarly, deprotection of certain thioacetals[10,11] generates an unstable *gem*-dithiol intermediate that is hydrolyzed through an acid-mediated mechanism to release H_2S. In more recent examples, JK donors, which are based on the phosphorothioate core of GYY4137, were developed and designed to undergo an intramolecular cyclization upon protonation of the P−S moiety.[12] Highlighting potential applications of pH-activated donors, the JK family of donors showed cytoprotective effects in cell models of oxidative damage and cardioprotective effects in an in vivo mouse model of myocardial ischemia-reperfusion injury. More broadly, mildly acidic pH environments are found during ischemia injury, within the extracellular environment of cancerous cells, and in certain subcellular compartments, such as the lysosome. These acidic environments when taken in combination with the prior work showing the beneficial effects

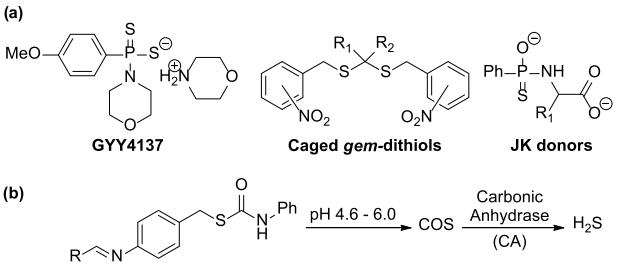


Figure 1. (a) Representative examples of current acid-labile H_2S donors. (b) Design of pH-dependent carbonyl sulfide (COS)/H_2S release from caged thiocarbamate scaffolds.

Received: July 10, 2019
Published: September 3, 2019

DOI: 10.1021/acs.joc.9b01873
J. Org. Chem. 2019, 84, 14469−14475

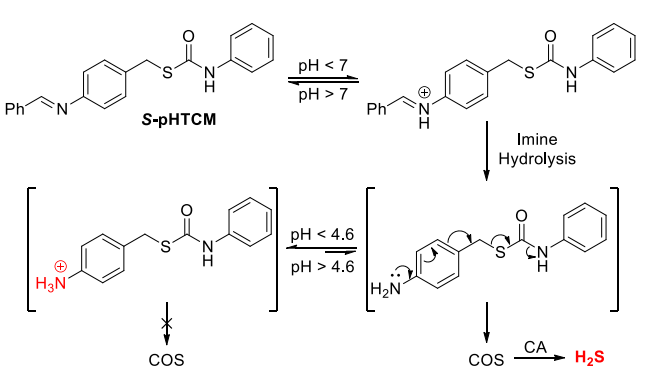
of H_2S in myocardial ischemia-reperfusion injury[13] and the ability to induce cell cycle arrest to suppress tumor growth[14] suggest the potential application for acid-activated H_2S donors. Aligned with these potential opportunities, a key need remains developing chemistry that enables access to donors that can be activated in specific pH ranges rather than just at increasing rates at more acidic pH. Such a strategy could be useful in developing design strategies for oral administration of hydrolysis-based H_2S donor motifs.

To address this challenge, we viewed that triggerable caged thiocarbamates could be modified to develop donor motifs activated within a specific pH range. Caged thiocarbamates have recently emerged as a highly tunable class of donors that undergo a triggered, self-immolative elimination to release carbonyl sulfide (COS), which is rapidly hydrolyzed to H_2S by the ubiquitous enzyme carbonic anhydrase (CA).[15−17] Examples of triggers employed within this scaffold include reactive oxygen species,[18−20] esterases,[21−23] light,[24−26] and cysteine.[27] We envisioned that using a pH-sensitive group, such as an imine, as the trigger could be used to develop acid-triggered COS/H_2S donors (Figure 1b). Here, we demonstrate that the use of an imine trigger, when coupled to the mechanism of 1,6-elimination required in the self-immolative thiocarbamates, provides donors that respond within a specific pH window. This new class of donors improves on the pH activation specificity of current acid-labile donors by providing H_2S delivery within a specific pH range. We expect this pH activation specificity will be useful in different applications requiring compound stability in strongly acidic or basic environments prior to H_2S release.

RESULTS AND DISCUSSION

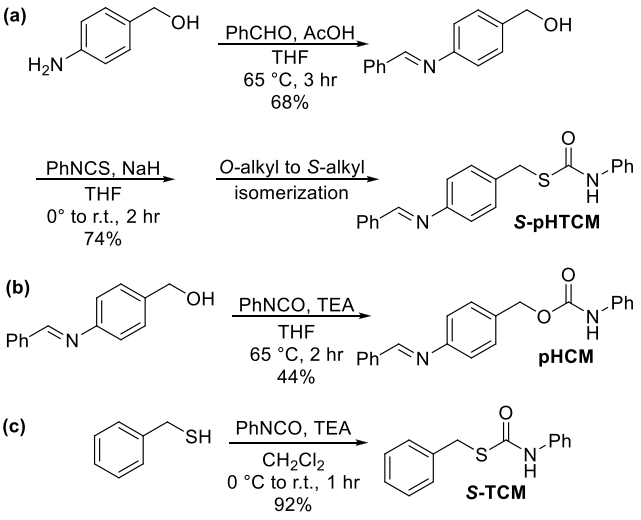
Donor Design. To develop donor motifs that are activated within a specific pH range, we chose to use an imine as the acid-sensitive trigger because imines are readily hydrolyzed under acidic conditions and have been used previously to initiate the 1,6-benzyl elimination of carbamate-containing prodrugs.[28] In our designed system, imine cleavage would generate a *p*-aminobenzylcarbamothioate intermediate, which would undergo a subsequent 1,6-elimination to release COS (Scheme 1). Although imine hydrolysis is acid-mediated, if the solution is too acidic, then the resultant aniline intermediate will be protonated, which will inhibit the 1,6-elimination and should decrease the rate of COS release. Similarly, the imine should be stable under basic conditions and not release COS. Combining these parameters, we expected that the efficiency of the imine-based donor would peak at pH values between the pK_a of the iminium ($pK_a \sim 5-7$) and anilinium ion ($pK_a \sim 4.6$). In contrast to other available acid- or hydrolysis-based donors, this would result in a pH window for activation rather than a direct dependence of the release rate with pH.

Scheme 1. Proposed pH-Dependent COS/H_2S-Release Pathway: Basic Conditions Decrease the Rate of Imine Hydrolysis; Strongly Acidic Conditions Protonate the Aniline Intermediate and Prevent COS Release



Synthesis. To test our hypothesis that the acid-triggered thiocarbamate donors undergo a pH-dependent release, we prepared a pH-sensitive imine-containing thiocarbamate donor. Treatment of 4-aminobenzyl alcohol with benzaldehyde in the presence of acetic acid formed 4-(benzylideneamino)-benzyl alcohol, which was coupled with phenyl isothiocyanate in the presence of sodium hydride to obtain the *O*-alkyl thiocarbamate isomer (Scheme 2a). Although analogous *O*-alkyl thiocarbamate donors have been prepared and are stable under ambient conditions, we found that the *O*-alkyl thiocarbamate with the imine trigger isomerized to the *S*-alkyl pH-sensitive thiocarbamate (**S-pHTCM**) isomer both in the solution and also in the solid state (Figure S1). Thione−thiol isomerization is well known to occur in thiocarbamates via the Newman−Kwart rearrangement[29,30] but typically requires high temperatures or catalysts.[31,32] Similarly, benzylic Newman−Kwart rearrangements have recently been reported to occur at elevated temperatures.[33] We hypothesize that the electron-donating imine substituent aids in the stabilization of the benzylic carbocation intermediate formed in the thiocarbamate rearrangement and, thus, may facilitate this isomerization under more mild conditions.

Scheme 2. (a) Synthetic Scheme for Acid-Triggered Thiocarbamate COS/H_2S Donor (S-pHTCM), (b) Carbamate Control Compound (pHCM), and (c) Triggerless Control Compound (S-TCM)



In addition to **S-pHTCM**, the corresponding pH-sensitive carbamate (**pHCM**) and triggerless *S*-alkyl thiocarbamate (**S-TCM**) were prepared as control compounds to confirm that the COS/H_2S release is triggered by imine hydrolysis of the thiocarbamate. In contrast to the model thiocarbamate, **pHCM** should undergo the same imine hydrolysis to release CO_2 instead of COS while generating the same byproducts as **S-pHTCM**. In the absence of the imine trigger, **S-TCM** is not expected to decompose and release COS.

DOI: 10.1021/acs.joc.9b01873
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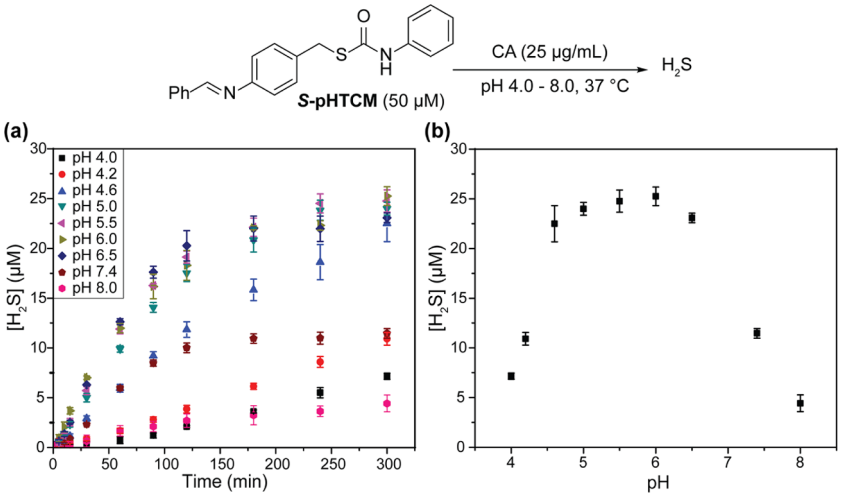


Figure 2. (a) COS/H_2S release from **S-pHTCM** (50 μM) at pH 4.0−8.0 containing CA (25 μg/mL). (b) pH curve of H_2S concentration at 5 h. Experiments were performed in quadruplicate with results expressed as mean ± standard deviation (SD) (n = 4).

Measurement of H_2S-Release. To evaluate COS/H_2S release from this series of compounds, we used the colorimetric methylene blue (MB) assay.[34] We measured COS/H_2S release from **S-pHTCM** (50 μM) across a range of pH values (4.0−8.0) in the presence of CA (25 μg/mL) at 37 °C. We confirmed CA activity at pH 5.5 using a p-nitrophenyl acetate assay (see Figure S2). To span this range of pH, we used sodium citrate-buffered and phosphate-buffered saline (PBS) solutions. We observed no buffer dependence when evaluating H_2S release from **S-pHTCM** in citrate and PBS buffer at pH 6.0, as shown in Figure S3. Consistent with our expectations, we observed more efficient COS/H_2S release in weakly acidic conditions from pH 4.6 to 6.5 than from pH values outside of this range (Figure 2). The rates of H_2S release have a similar pH dependence, but because the rate-limiting step of the reaction may change as a function of pH and not all of the H_2S release curves peaked at the same levels, we chose to measure H_2S concentration at 5 h for these investigations (Figure S4). Further supporting our hypothesis, we found that the inflection points of the pH response curve (4.3 and 7.3) matched the expected pK_a values of the iminium and the anilinium ions (Figure S5). Taken together, these data support the mechanism of COS/H_2S release outlined in Scheme 1.

To confirm that H_2S release from **S-pHTCM** requires both imine hydrolysis and the thiocarbamate moiety, we measured the H_2S release from the **pHCM** and **S-TCM** control compounds at pH 5.5. Under identical conditions, as those for **S-pHTCM**, we failed to observe H_2S generation from either **pHCM** or **S-TCM** (Figure 3). In the absence of CA, **S-pHTCM** showed a significantly reduced rate of H_2S release (Figure S6). To further support the proposed release mechanism, we monitored the reaction by high-performance liquid chromatography (HPLC) analysis (Figures S7 and S8). Consistent with the proposed 1,6-elimination mechanism, we observed benzaldehyde, aniline, and 4-aminobenzyl alcohol during the course of the reaction (Scheme 3).

We next investigated the tolerance of the acid-mediated cleavage and self-immolation to the presence of different biological nucleophiles by measuring H_2S release from **S-pHTCM** at pH 5.5 in the presence of different analytes. We did not observe a significant change in the donor efficiency in

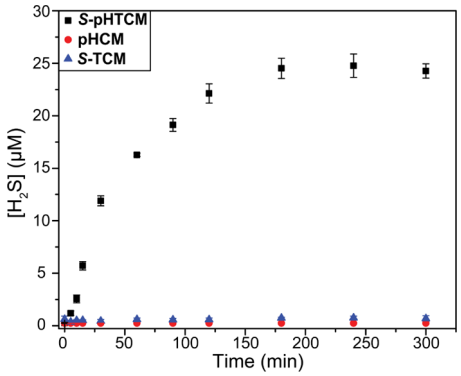
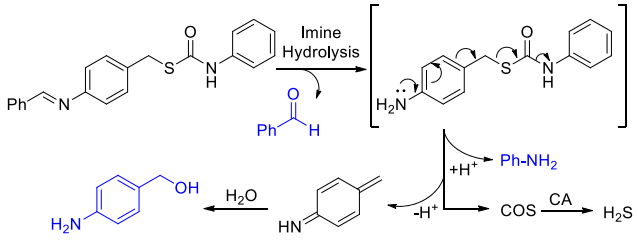


Figure 3. H_2S release from **S-pHTCM**, triggerless (**S-TCM**), and carbamate (**pHCM**) control compounds (50 μM) in citrate buffer (10 mM, pH 5.5) containing CA (25 μg/mL) at 37 °C. Experiments were performed in quadruplicate with results expressed as mean ± SD (n = 4).

Scheme 3. Proposed Mechanism of Acid-Triggered COS/H_2S Release from Caged Thiocarbamate Donors with Byproducts of Self-immolation



the presence of 250 μM of glutathione (GSH), Cys, Lys, Ser, Hcy, Gly, or GSSG, which demonstrates the compatibility of our approach with common nucleophiles and reactive species (Figure 4). In the presence of higher levels of GSH (5 mM), however, we did observe modest inhibition. We do not view this as a significant problem, however, because about 90% of GSH is localized in the cytosol, about 10% is localized in the mitochondria and the endoplasmic reticulum, leaving negligible GSH levels in acidic cellular compartments.[35] To further investigate whether this observed inhibition was observed at physiological pH values, we repeated the measurement at pH

DOI: 10.1021/acs.joc.9b01873
J. Org. Chem. 2019, 84, 14469−14475

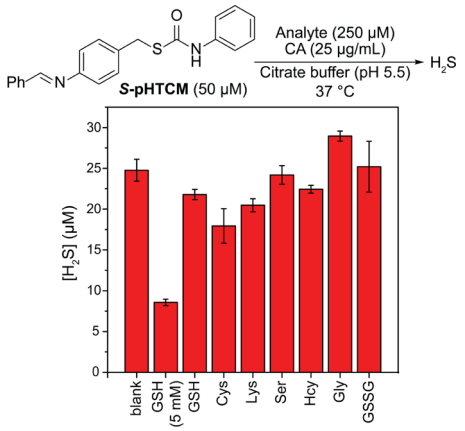


Figure 4. COS/H_2S release at 5 h from **S-pHTCM** (50 μM) in citrate (pH 5.5) in the presence of CA (25 μg/mL) and various analytes (250 μM unless otherwise noted): no analyte, GSH (5 mM), GSH, Cys, Lys, Ser, Hcy, Gly, and GSSG. Experiments were performed in triplicate with results expressed as mean ± SD (n = 3).

7.4 in the presence of 5 mM GSH and did not observe a significant difference in efficiency in the absence or presence of GSH (Figure S9). These data suggest that high concentrations of GSH should not interfere with the application of this triggering motif in normal cellular environments.

Although our primary goal was to develop chemistry that enabled donor response within a specific pH range, we also wanted to demonstrate the feasibility of appending the developed motif to commonly used subcellular targeting groups that direct compounds to acidic subcellular compartments. To demonstrate this compatibility, we prepared a thiocarbamate donor (**Lyso-pHTCM**), as well as the associated control compounds (**Lyso-pHCM** and **Lyso-TCM**), with an aminoethyl−morpholine group, which has been used previously to direct compounds to the lysosome. We measured the H_2S release efficiency from the compounds and found that **Lyso-pHTCM** showed a 33% H_2S release efficiency over 5 h and that the control compounds did not release H_2S (Figure 5). Although beyond the scope of the present investigations, we anticipate that these compounds may be of use in investigating the role of lysosomal H_2S delivery in various contexts.

CONCLUSIONS

Based on the broad utility of hydrolysis- and acid-sensitive H_2S donors, we developed a new strategy that enables for the efficiency of H_2S release to be tuned to specific pH ranges. This response profile is in contrast to currently available acid-mediated H_2S donor motifs. By including an acid-sensitive imine group on a caged thiocarbamate, we demonstrated that the acid-mediated imine hydrolysis in combination with inhibitory protonation of aniline product after hydrolysis resulted in an operative pH response range of 4.6−6.5. The imine-based donor also shows good tolerance to a wide array of biological nucleophiles at physiologically relevant concentrations. We also demonstrated the modularity of our approach by attaching a common lysosomal targeting group to the donor motif and revealed that H_2S release is still efficient in this targeted construct. Overall, we anticipate that this approach will provide new opportunities to tune donor motifs to respond to specific pH windows associated with subcellular organelles and that the developed chemistry will enable specific pH windows to be targeted to other acidic microenvironments.

EXPERIMENTAL SECTION

Materials and Methods. Reagents were purchased from Sigma-Aldrich, Tokyo Chemical Industry (TCI), Fisher Scientific, and VWR. Column chromatography was performed with 230−400 mesh silica gel. Deuterated solvents were purchased from Cambridge Isotope Laboratories. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker 500 MHz or Varian Inova 500 MHz instrument. Chemical shifts are reported relative to residual protic solvent resonances. Air-free experimental procedures were performed in an Innovative Atmospheres N_2-filled glovebox or using Schlenk technique. UV−vis spectra were acquired on an Agilent Cary 60 UV−vis spectrometer. Mass spectrometric data was acquired by the University of Illinois, Urbana Champaign MS Facility or on a Xevo Waters ESI LC/MS instrument.

General Procedure for H_2S Detection. In a N_2-filled glovebox, scintillation vials with septa caps were filled with 20 mL of degassed buffer (citrate/PBS, pH 4.0−8.0, 10 mM). All stock solutions were

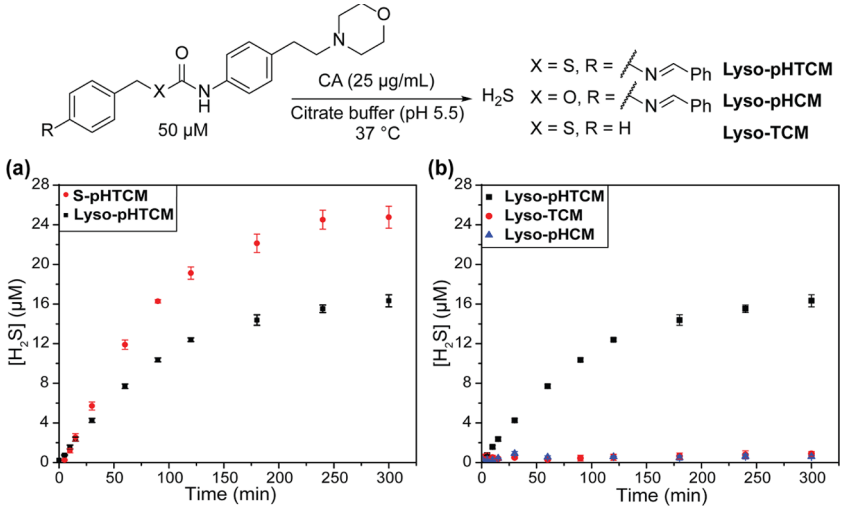


Figure 5. (a) H_2S release comparison of **Lyso-pHTCM** and **S-pHTCM**. (b) H_2S release from **Lyso-pHTCM** and related control compounds. All experiments performed in quadruplicate in citrate buffer (pH 5.5, 10 mM) containing CA (25 μg/mL) at 37 °C. Results expressed as a mean ± SD (n = 4).

DOI: 10.1021/acs.joc.9b01873
J. Org. Chem. 2019, 84, 14469−14475

prepared in the glovebox with degassed solvents. The 10 mM donor stock solution was prepared in dimethyl sulfoxide (DMSO), and a 10 mg/mL carbonic anhydrase (CA) stock solution was prepared in Millipore H_2O. Each scintillation was charged with 50 μL of the CA stock solution to achieve a final concentration of 25 μg/mL. The solutions were thermally equilibrated to 37 °C while stirring for 20 min. During this time, methylene blue cocktail solutions (0.3 mL) were prepared in 1.5 mL disposable cuvettes. The methylene blue cocktail solutions contained 60 μL of 1% (w/v) $Zn(OAc)_2$, 120 μL of 30 mM $FeCl_3$ in 1.2 M HCl, and 120 μL of 20 mM *N,N*-dimethyl-*p*-phenylene diamine in 7.2 M HCl. To each solution, 100 μL of the 10 mM donor stock solution was added to reach a final concentration of 50 μM. Immediately after donor addition at t = 0 min, a 0.3 mL reaction aliquot was removed and added to a methylene blue cocktail solution. This process was repeated at 5, 10, 15, 30, 60, 90, 120, 180, 240, and 300 min time points. The reaction aliquots added to the methylene blue cocktail solutions were mixed thoroughly and incubated for 1 h at room temperature in the dark, after which the absorbance values at 670 nm were measured.

MB Assay Calibration Curve. The methylene blue cocktail solution (0.5 mL) and PBS pH 7.4 buffer (0.5 mL) were added to 1.5 mL disposable cuvettes. A NaSH stock solution (100 mM) was prepared in degassed Millipore H_2O under an inert atmosphere and diluted to 1 mM. Immediately after dilution, aliquots of the NaSH stock solution were added to the methylene blue solutions to reach final concentrations of 10, 20, 30, 40, and 50 μM. The solutions were mixed and incubated at room temperature for 1 h, after which the absorbance values at 670 nm were measured.

Syntheses. *4-(Benzylideneamino)benzyl Alcohol.* (Modified from the previous report.)[28] 4-Aminobenzyl alcohol (0.50 g, 4.1 mmol) and benzaldehyde (0.82 mL, 8.2 mmol) were added to anhydrous tetrahydrofuran (THF) (15 mL) in the presence of $MgSO_4$ (~1 g). Glacial AcOH was then added dropwise (~4 drops), and the resultant reaction mixture was refluxed for 2 h. The reaction was then quenched with a solution of saturated $NaHCO_3$ (30 mL) and extracted with EtOAc (3 × 15 mL). The organic layers were combined, washed with deionized H_2O (30 mL) and brine (30 mL), and dried over $MgSO_4$. The drying agent was removed by filtration, and the organic solvent was removed under reduced pressure. The product was isolated and purified by column chromatography using EtOAc/hexanes (10−100% gradient; 5% NEt_3) to yield a yellow solid (343 mg, 68% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 8.63 (s, 1 H), 8.02−7.87 (m, 2 H), 7.59−7.48 (m, 3 H), 7.36 (d, J = 8.2 Hz, 2 H), 7.24 (d, J = 8.2 Hz, 2 H), 5.18 (t, J = 5.7 Hz, 1 H), 4.51 (d, J = 5.7 Hz, 2 H).^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 160.1, 149.9, 140.5, 136.1, 131.4, 128.8, 128.6, 127.3, 120.8, 62.6. HRMS (ASAP TOF) (m/z): [M + H]$^+$ cacld for $C_{14}H_{14}NO$, 212.1075; found, 212.1090.

S-4-(Benzylideneamino)benzyl Phenylcarbamothioate (S-pHTCM). 4-(Benzylideneamino)-benzyl alcohol (60.0 mg, 0.284 mmol) and phenyl isothiocyanate (41 μL, 0.34 mmol) were combined in anhydrous THF (15 mL). The reaction mixture was cooled to 0 °C before adding NaH (18 mg, 0.75 mmol). The reaction mixture was then stirred at room temperature for 2 h. The solvent was removed under reduced pressure, and the crude mixture was purified by column chromatography with EtOAc/hexane (4−34% gradient; 5% NEt_3). The product was isolated as a yellow solid, which isomerizes from the *O*-alkyl to *S*-alkyl isomer over 3 days in the solid state (73 mg, 74% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.35 (s, 1 H), 8.62 (s, 1 H), 8.02−7.82 (m, 2 H), 7.59−7.44 (m, 5 H), 7.41 (d, J = 8.2 Hz, 2 H), 7.31 (dd, J = 8.4, 7.2 Hz, 2 H), 7.23 (d, J = 8.2 Hz, 2 H), 7.05 (t, J = 7.4 Hz, 1 H), 4.19 (s, 2 H). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 164.3, 160.6, 150.3, 138.9, 136.6, 136.0, 131.5, 129.6, 128.9, 128.8, 128.6, 123.4, 121.1, 119.0, 32.6. HRMS (ES + TOF) (m/z): [M + H]$^+$ cacld for $C_{21}H_{19}N_2OS$, 347.1218; found, 347.1212.

*4-(Benzylideneamino)benzyl Phenylcarbamate (**pHCM**).* 4-(Benzylideneamino)benzyl alcohol (61 mg, 0.28 mmol) and phenyl isocyanate (31 μL, 0.28 mmol) were added to anhydrous THF (15 mL). The reaction mixture was cooled to 0 °C before the addition of NEt_3 (0.50 mL, 3.6 mmol), after which the reaction mixture was refluxed for 2 h. The reaction was quenched with brine (30 mL) and extracted with EtOAc (3 × 15 mL). The organic layers were combined, washed with deionized H_2O (30 mL) and brine (30 mL), and dried over $MgSO_4$. The drying agent was removed by filtration, and the solvent was removed under reduced pressure. The crude mixture was purified by column chromatography with EtOAc/hexane (4−34% gradient; 5% NEt_3) to afford the product as a white solid (41 mg, 44%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.76 (s, 1 H), 8.63 (s, 1 H), 7.94 (dd, J = 7.3, 2.2 Hz, 2 H), 7.63−7.37 (m, 7 H), 7.37−7.20 (m, 4 H), 6.99 (t, J = 7.4 Hz, 1 H), 5.17 (s, 2 H). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 161.0, 153.4, 151.3, 139.1, 136.0, 134.3, 131.6, 129.3, 128.8, 128.8, 128.7, 122.4, 121.1, 118.2, 65.5. HRMS (ES + TOF) (m/z): [M + H]$^+$ cacld for $C_{21}H_{19}N_2O_2$, 331.1447; found, 331.1435.

*S-Benzyl Phenylcarbamothioate (**S-TCM**).* Benzyl mercaptan (61 μL, 0.52 mmol) and phenyl isocyanate (58 μL, 0.51 mmol) were combined in anhydrous THF (15 mL). The reaction mixture was cooled to 0 °C before adding NEt_3 (0.1 mL), after which the reaction was stirred at room temperature for 1 h. The reaction was then quenched with brine (30 mL) and extracted with EtOAc (3 × 15 mL). The organic layers were combined and dried over $MgSO_4$. The organic layer was filtered, and the solvent was removed under reduced pressure. The product was isolated by column chromatography using EtOAc/hexane (4−34% gradient; 5% NEt_3) to afford the product as a white solid (120 mg, 92% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.33 (s, 1 H), 7.55−7.46 (m, 2 H), 7.39−7.20 (m, 7 H), 7.05 (t, J = 7.4 Hz, 1 H), 4.15 (s, 2 H). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 164.3, 138.9, 138.7, 128.9, 128.7, 128.4, 127.0, 123.4, 119.0, 32.9. HRMS (ASAP TOF) (m/z): [M + H]$^+$ cacld for $C_{14}H_{14}NOS$, 244.0796; found, 244.0791.

4-(2-Aminoethyl)morpholine Phenyl Isothiocyanate. 4-(2-Morpholinoethyl) aniline (61 mg, 0.30 mmol) was added to anhydrous CH_2Cl_2 (20 mL). The reaction mixture was cooled to 0 °C, then a solution of 1,1′-thiocarbonyldiimidazole (53 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise. The reaction was stirred at room temperature for 2 h, after which the solvent was removed under reduced pressure. The crude mixture was purified by column chromatography with MeOH/CH_2Cl_2 (2−20% gradient) and isolated as a yellow oil (180 mg, 86% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22−7.11 (m, 4 H), 3.73 (t, J = 4.6 Hz, 4 H), 2.79 (dd, J = 9.7, 6.3 Hz, 2 H), 2.57 (dd, J = 9.6, 6.4 Hz, 2 H), 2.50 (t, J = 4.6 Hz, 4 H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 140.0, 135.0, 130.0, 129.3, 125.9, 67.1, 60.5, 53.8, 33.1. HRMS (ASAP TOF) (m/z): [M + H]$^+$ cacld for $C_{13}H_{17}N_2OS$, 249.1062; found, 249.1078.

S-4-(Benzylideneamino)benzyl(4-(2-morpholinoethyl)phenyl) Carbamothioate (Lyso-pHTCM). 4-(Benzylideneamino)benzyl alcohol (100 mg, 0.473 mmol) and morpholine isothiocyanate (118 mg, 0.473 mmol) were added to anhydrous THF (20 mL). The reaction mixture was cooled to 0 °C before the addition of NaH (18 mg, 0.75 mmol) and then stirred for 10 h at room temperature. The solvent was removed under reduced pressure. The crude mixture was purified by column chromatography with EtOAc/hexane (8−66% gradient; 5% NEt_3) to afford the product as a white solid that isomerizes from the *O*-alkyl to *S*-alkyl isomer over 4 days (77 mg, 36%).^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.26 (s, 1 H), 8.61 (s, 1 H), 7.95−7.89 (m, 2 H), 7.56−7.48 (m, 3 H), 7.43−7.37 (m, 4 H), 7.22 (d, 2 H), 7.15 (d, 2 H), 4.17 (s, 2 H), 3.56 (d, J = 5.0 Hz, 4 H), 2.67 (t, J = 7.7 Hz, 2 H), 2.46 (t, 1 H), 2.40 (s, 4 H). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 164.6, 161.0, 150.7, 137.3, 137.1, 136.5, 136.0, 132.0, 130.1, 129.5, 129.3, 129.1, 121.6, 119.6, 66.7, 60.5, 53.7, 33.1, 32.3. HRMS (ES + TOF) (m/z): [M + H]$^+$ cacld for $C_{27}H_{30}N_3O_2S$, 460.2059; found, 460.2076.

*S-4-(Benzylideneamino)benzyl(4-(2-morpholinoethyl)phenyl) Carbamate (**Lyso-pHCM**).* 4-(2-Morpholinoethyl) aniline (61 mg, 0.30 mmol) and triphosgene (41 mg, 0.36 mmol) were combined in anhydrous CH_2Cl_2 (15 mL). The reaction was cooled to 0 °C and NEt_3 (0.2 mL, 5 equiv) was added. After stirring at 0 °C for 2 h, the reaction mixture was purged with N_2 before adding a solution of phenyl imine benzyl alcohol (74 mg, 0.35 mmol) in anhydrous

DOI: 10.1021/acs.joc.9b01873
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CH_2Cl_2 (5 mL) dropwise. The reaction was stirred at room temperature for 2 h, after which the solvent was removed under reduced pressure. The crude mixture was purified by column chromatography with EtOAc/hexane (12−100% gradient; 5% NEt_3) to yield the product as a white solid (70.1 mg, 54% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.66 (s, 1 H), 8.63 (s, 1 H), 7.98−7.90 (m, 2 H), 7.57−7.50 (m, 3 H), 7.50−7.44 (m, 2 H), 7.37 (d, J = 8.1 Hz, 2 H), 7.33−7.26 (m, 2 H), 7.17−7.09 (m, 2 H), 5.15 (s, 2 H), 3.56 (t, J = 4.7 Hz, 4 H), 2.66 (dd, J = 9.3, 6.4 Hz, 2 H), 2.46 (dd, J = 9.2, 6.5 Hz, 2 H), 2.40 (s, 4 H). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ: 160.9, 153.4, 151.2, 136.9, 135.9, 134.4, 134.3, 131.6, 129.2, 128.9, 128.8, 128.7, 121.0, 118.2, 66.2, 65.4, 60.2, 53.3, 31.8. HRMS (ASAP_TOF) (m/z): [M + H]$^+$ cacld for $C_{27}H_{30}N_3O_3$, 444.2287; found, 444.2282.

*S-Benzyl(4-(2-morpholinoethyl)phenyl) Carbamothioate (**Lyso-TCM**).* 4-(2-Morpholinoethyl) aniline (61 mg, 0.29 mmol) and triphosgene (41 mg, 0.35 mmol) were combined in anhydrous CH_2Cl_2 (15 mL). The reaction mixture was cooled to 0 °C and NEt_3 (0.20 mL, 1.5 mmol) was added. The reaction mixture was warmed from 0 °C to room temperature and stirred overnight, after which it was purged with N_2 before adding benzyl mercaptan (41 μL, 0.35 mmol) dropwise. The reaction mixture was stirred at room temperature for 10 h. The solvent was removed under reduced pressure, and the product was purified by column chromatography with MeOH/CH_2Cl_2 2−20% gradient to afford the product as a yellow solid (41 mg, 39% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1 H), 7.43−7.28 (m, 6 H), 7.27−7.21 (m, 1 H), 7.18−7.12 (m, 2 H), 4.14 (s, 2 H), 3.56 (t, J = 4.7 Hz, 4 H), 2.67 (t, 2 H), 2.47 (t, J = 8.0 Hz, 1 H), 2.40 (s, 4 H). ^{13}C: NMR (126 MHz, DMSO-d_6) δ: 164.6, 139.3, 137.3, 129.5, 129.2, 128.9, 127.4, 119.5, 66.7, 60.5, 55.4, 53.7, 40.5, 33.4, 32.3. HRMS (ES+ TOF) (m/z): [M + H]$^+$ cacld for $C_{20}H_{25}N_2O_2S$, 357.1637; found, 357.1641.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.9b01873.

H_2S release data, HPLC data, and NMR spectra (PDF)

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the NIH (MDP; R01GM113030), NSF/GRFP (AKG; DGE-1842486), and the Dreyfus Foundation. The NMR and MS facilities in CAMCOR are supported through NSF awards CHE-0923589 and CHE-1625529. We thank Nathanael Lau for assistance with the cover art associated with this article.

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