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Reactive Oxygen Species-Triggered Tunable Hydrogen Sulfide Release

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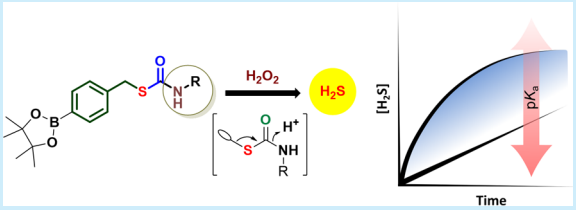
Supporting Information

ABSTRACT: A series of carbamothioates with tunable release of H_2S after activation by reactive oxygen species are reported. The half-lives of H_2S release could be tuned from 24 to 203 min by varying the basicity of the amine.



Hydrogen sulfide (H_2S) is an important gaseous cellular antioxidant and signaling agent that mediates numerous processes.[1−3] Diminished H_2S has been associated with various diseases like neurological disorders, cardiovascular diseases, gastrointestinal diseases, inflammation, etc.[1−3] Most of these conditions are associated with an increased level of reactive oxygen species (ROS).[4,5] Hydrogen sulfide (H_2S) is known to stimulate the cellular antioxidant machinery as a cytoprotective measure.[4] Treatment with H_2S induces translocation of nuclear factor NRF2 to enhance its binding with anti-inflammatory response element (ARE) and in turn increase the production of glutathione, a free-radical scavenger. Thus, delivery of hydrogen sulfide to areas associated with inflammation is of therapeutic interest.[6−8] Left untreated, inflammation can accentuate tissue damage and accelerate further degeneration. The major problem with delivery of hydrogen sulfide is that the antioxidant properties of hydrogen sulfide are largely dependent on the location and the rate of release. The rate of release can be modulated by using different classes of donors. For example, NaSH, an inorganic source of H_2S, can be used for fast release, while GYY4137 is commonly used for prolonged release of hydrogen sulfide. They are found to have contrasting effects on inflammation. In a study by Moore and co-workers, NaSH was found to exacerbate the condition by increasing the levels of pro-inflammatory cytokines like TNF-α or IL-6,[9,10]while GYY4137 attenuated the levels of these pro-inflammatory cytokines.[11,12] In yet another study by Wang and co-workers, the superior cardioprotective effects of diallyl trisulfide-based nanoparticle over NaSH was demonstrated in a myocardial ischemia reperfusion model.[13] These observations underscore the importance of modulating release of exogenous hydrogen sulfide. While a number of nanoparticle-based methods for slowing down release of hydrogen sulfide are known, it is desirable to have a class of small molecules where tunable release is possible.[14,15] Next, the other problem is to localize delivery of hydrogen sulfide. Here, a number of classes of H_2S donors which localize H_2S are known and some utilize physiologically relevant stimuli to release H_2S.[16] However, none to our knowledge display stimuli-responsiveness as well as tunability by using a physiologically relevant trigger.[17] Here, we report arylboronate ester-based carbamothioates that are triggerable by ROS and after activation, the release of H_2S can be varied by modifying the basicity of the amine.

Recently, ROS-activated carbonyl sulfide (COS) donors were reported by Pluth and co-workers.[18,19] This donor is triggered by hydrogen peroxide, a relatively stable ROS to generate COS, which undergoes hydrolysis to produce hydrogen sulfide. Hydrolysis of COS is accelerated by catalytic carbonic anhydrase (CA), a widely prevalent enzyme.[20] This donor has the distinct advantage of being triggered by a metabolite (H_2O_2) that is frequently associated with inflammation.[1−3] The mechanism of COS release involves the generation of a phenolate **I**, which undergoes self-immolation to produce the anion **II**, which fragments to produce COS and an amine (Figure 1a).The penultimate step in this process, wherein the loss of COS and RNH_2 occurred, was found to be the step with the highest barrier (Figure 1a).[19] We hypothesized that a similar intermediate was possible by starting from the carbamothioate (Figure 1b). The thiocarbamate anion **V**, which is generated by self-immolation of **IV**, is expected to undergo fragmentation to produce COS. This process involves the protonation of **V**, which we proposed would depend on the basicity of the amine. Our laboratory had previously reported esterase-sensitive COS/H_2S donors where we found some evidence for the dependence of rate of hydrogen sulfide generation on the basicity of the amine. For example, the rate of hydrogen sulfide generation from the benzylamine derivative (0.014 min^{-1}; pK_a of amine, 9.34) was significantly slower when compared with the aniline derivative (0.032 min^{-1}, pK_a 5.34).[21] We therefore envisaged that the

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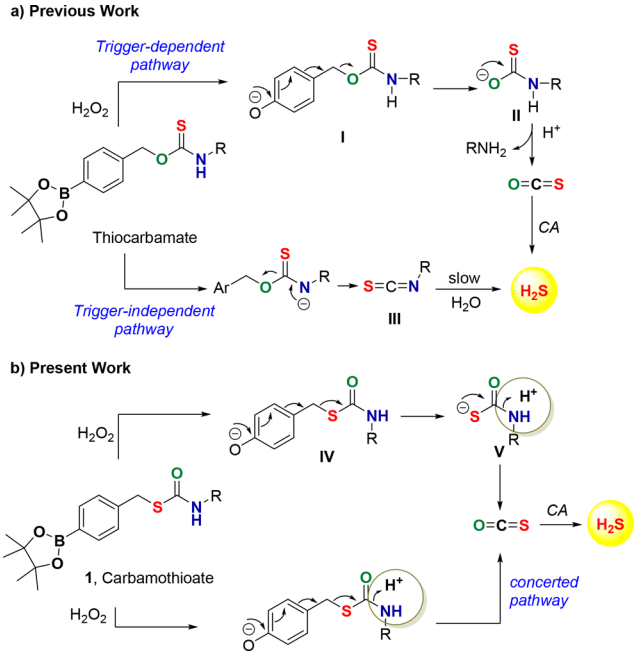
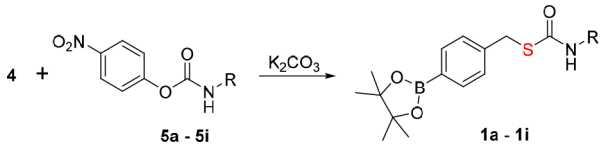


Figure 1. Arylboronate esters of thiocarbamates are activated by hydrogen peroxide to produce COS: (a) reported donors that generate hydrogen sulfide; (b) design of tunable COS/H_2S donors.

basicity of the amine would have a significant impact on the release rate after activation. Although thiocarbamates have been similarly evaluated, the range of pK_a's was small (1−5), and correspondingly, the range of rates of release was also somewhat limited.[19] Furthermore, these thiocarbamates also undergo decomposition by pathways that did not depend on ROS: thiocarbamates can undergo deprotonation and subsequent rearrangement to produce an isothiocyanate, which is known to undergo hydrolysis in buffer to produce hydrogen sulfide (Figure 1a).

In order to test our hypothesis, we chose amines with a range of pK_a values of roughly 7 units (see Table 1).[22] The compounds were synthesized in four steps starting from (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol **3** to give the thiol **4**, with 82% overall yield (Scheme 1). Next,

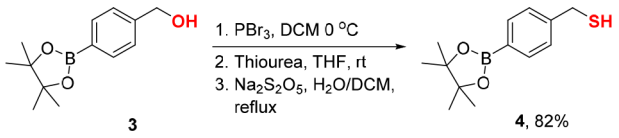
Table 1. Synthesis of Carbamothioates



entry	R	pK_a RNH_2[a]	carbamate	prod	% yield
1	(3-COOMe)Ph	4.75	**5a**	**1a**	28
2	4-OCH_2CH_2OHPh	5.03[b]	**5b**	**1b**	32
3	(4-OMe)Ph	5.34	**5c**	**1c**	27
4	(4-NO_2)$PhCH_2$	8.36[b]	**5d**	**1d**	23
5	4-(*O*-propargyl)-$PhCH_2$	9.18[b]	**5e**	**1e**	41
6	$PhCH_2$	9.34	**5f**	**1f**	21
7	$CH_3CH_2CH_2$	10.53	**5g**	**1g**	27
8	*tert*-butyl	10.45	**5h**	**1h**	
9	pyrrolidine	11.27	**5i**	**1i**	
10	mesalamine methyl ester	4.13[b]	**5j**	**1j**	52

[a] pK_a values are from ref 22. [b] SciFinder was used.

Scheme 1. Synthesis of Thiol 4



p-nitrophenylcarbamate **5a** was synthesized using a reported protocol (Table 1, entry 1). This carbamate was reacted with thiol **4** to give carbamothioate **1a** (Table 1, entry 1).Compound **2** (Figure 2) was synthesized as a negative

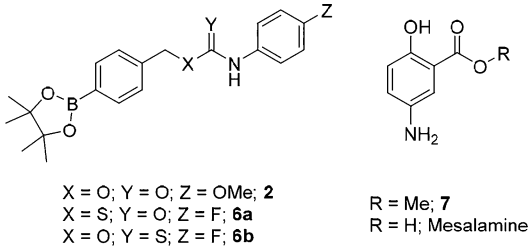


Figure 2. Structures of compounds **2**, **6**, **7**, and mesalamine.

control from the reaction of **3** with **5c** (see the Supporting Information). Compound **2** should cleave in the presence of H_2O_2 but will not produce H_2S (Figure 2).

The hydroxyethyl derivative **5b** was next synthesized in four steps using a reported protocol.[23]This amine was chosen due to its improved aqueous solubility. Similarly, carbamates **5c−g** were converted to the corresponding carbamothioate using a similar methodology (Table 1, entries 2−7). Under these reaction conditions, the *tert*-butylamine carbamate **5h** decomposed, and we were unable to synthesize the corresponding carmabothioate. In an effort to synthesize secondary amine-based derivatives, the carbamate of pyrrolidine **5i** was synthesized. This compound, however, did not react with **4** possibly due to the low reactivity of carbamates derived from secondary amines.

We first tested the ability of compounds to produce H_2S by using a methylene blue formation assay, which is frequently used for the detection of H_2S. Briefly, the donors pretreated with CA were independently incubated with 10 equiv of H_2O_2 in phosphate-buffered saline (PBS, pH = 7.4, 50 mM) at 37 °C, the resulting mixture was exposed to the rest of the methylene blue reagents, and absorbance was recorded (see the Supporting Information).

The formation of methylene blue complex was confirmed by measuring absorbance at 676 nm, and the donors showed varying levels of H_2S release (30 min, Figure 3a; 2 h, see Figure S1b). The absorbance profile of **1g** is shown and was found to be similar to that of authentic Na_2S (see Figure S1a). As expected, no H_2S formation from compound **2** was observed as it lacks the ability to form COS (Figure 3a). Having established the H_2S release from the donors, we next studied the effect of the basicity of the amine (pK_a) on H_2S release. Using the methylene blue protocol for monitoring hydrogen sulfide release, we studied the time course of H_2S release (see Figure S2). Pseudo-first-order rate constants were obtained, and half-lives were calculated (Table 2). Compound **1c** with ansidine as the leaving group was found to have the fastest rate of H_2S release with half-life of 23.9 min^{-1} (Table 2).

On the other hand, compound **1g** with propylamine as the leaving group, turned out to be the slowest H_2S donor with the

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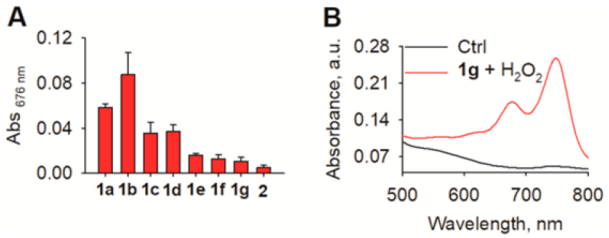

Figure 3. (a) Measurement of H_2S release in the presence of H_2O_2 using methylene blue after 30 min incubation. (b) Representative absorbance trace of the methylene blue assay conducted with **1g** in the presence of hydrogen peroxide after 4 h. Ctrl represents **1g** alone.

Table 2. Kinetics of H_2S Release

compd	pK_a	k^a	$t_{1/2}$ (min)	relative rate
1a	4.75	0.026	26.7	0.90
1b	5.03	0.017	40.8	0.59
1c	5.34	0.029	23.9	1.00
1d	8.36	0.004	173.3	0.14
1e	9.18	0.0045	154.0	0.16
1f	9.34	0.0049	141.4	0.17
1g	10.53	0.0034	203.8	0.12
1j	4.13	0.0145	54.8	0.50
6a	4.66	0.10[b]	43.3[c]	0.55
6b	4.66	1.16[b]	n.d.	n.d.

[a]All values are in min^{-1} unless otherwise mentioned. [b]Values are in M^{-1} s^{-1}. [c]Calculated from a pseudo-first-order rate constant of 0.016 min^{-1}.[19]

half-life of 203.8 min^{-1}. Consistent with our hypothesis, we observed a fast rate of H_2S release from aniline-based derivatives with pK_a values in the range 4.75 to 5.34 compared to derivatives with pK_a values in the range of 8.36−10.53 (Table 2). Relative rates were determined, and an 8-fold difference in the rate of H_2S release from our donors was recorded.

Linear regression analysis of relative rates and pK_a of the corresponding amine showed a good correlation, suggesting that it is possible to modulate pK_a to change hydrogen sulfide release rate (Figure 4). While this paper was in preparation, 4-fluoroaniline derivative **6** was synthesized and found to generate H_2S when exposed to hydrogen peroxide.[19] The pK_a of 4-fluoroaniline was 4.66, and the rate constant was 0.01 M^{-1} s^{-1}. The pseudo-first-order rate constant reported by them was 0.016 min^{-1} (data is from Figure S3, Supporting Information, from ref 19). When this data point was included

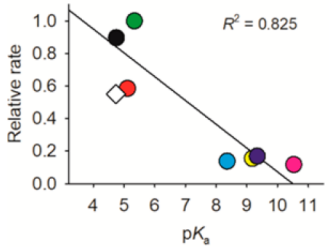

Figure 4. Linear regression analysis of relative rates of hydrogen sulfide release upon treatment of the compound with hydrogen peroxide with the pK_a of the amine. The data for this plot are available in Table 2. The white square indicates the relative rate data for the 4-fluoroaniline derivative **6a**.[19]

in the plot, we found that the position of **6a** (white square, Figure 4) was nearly identical to the derivative of the amines of comparable basicity (**1b**, Table 1). Again, this was a testament to the predictability of H_2S release from carbamothioates.

For a better understanding of the mechanism of decomposition, we monitored the decomposition of compound **1c** with concomitant formation of *p*-anisidine by HPLC analysis. Compound **1c** when incubated in PBS readily converted to boronic acid. Upon treatment of **1c** with H_2O_2 we observed complete disappearance of the compound in 30 min, which is in accordance with the previous reports suggesting fast reactivity of boronic acids with H_2O_2.[24] Formation of a new peak after 30 min suggested the formation of an intermediate which decomposed over a period of 2 h to generate *p*-anisidine (see Figure S4). The rate of *p*-anisidine formation (0.036 min^{-1}) was found to be in accordance with the rate of H_2S release (0.029 min^{-1}). A similar pattern was observed in the case of **1g**. Here, **1g** was converted to boronic acid in buffer which upon reaction with H_2O_2 led to the formation of an intermediate within 30 min (see Figure S5). The intermediate formed in the case of **1g** gradually decomposed over a period of 6 h (see Figure S6). Despite repeated attempts, we were unable to characterize this intermediate. However, on the basis of its absorbance in the UV region, it is unlikely to be the **V** (Figure 1b) as aliphatic amines have poor absorbance at this wavelength. It is therefore possible that the intermediate is **IV**, whose self-immolation rate is also dependent on the basicity of the amine, and the observed data would be consistent with this step being the rate-determining step (see Scheme S1). We are unable to provide experimental support for this hypothesis at this time. In order to test the stability of compounds in buffer, **1g** was incubated in phosphate buffer (pH 7.4) at 37 °C for 5 h. We found nearly quantitative recovery of the compound, suggesting that the compounds are stable under reported conditions (see Figures S5 and S6).

It is intriguing that the compounds synthesized and evaluated by Pluth and co-workers, i.e., **6a** and its thiocarbamate isomer **6b**, have distinct rates with nearly 12-fold difference in their second-order rate constants for hydrogen sulfide release (Table 2).[19] This observation is not consistent with the decomposition of **II**, which is proposed as the common intermediate, being the rate-determining step since they should produce hydrogen sulfide at the same rate.[19] Further work clearly needs to be done to characterize these differences better. Lastly, the trigger-independent pathways that may contribute to producing hydrogen sulfide are not relevant when carbamothioates are used (see Scheme S1). Thus, due to their predictable mechanism, the use of carbamothioates (**1**) reported herein may offer significant advantages over other hydrogen sulfide donors.

Lastly, hydrogen sulfide has been used for the treatment of gastrointestinal disorders associated with inflammation.[25,26] In a study by Wallace and co-workers, H_2S contributed to gastric mucosal defense by resisting injury induced by exogenous substances such as NSAIDs (corrodes the stomach lining), stress, or ischemia reperfusion.[29,30] The underlying cytoprotective activity of H_2S was attributed to its ability to inhibit leukocyte adherence to the vascular endothelium, markedly caused due to the pathogenesis of NSAIDs. Wallace and co-workers reported H_2S−NSAID hybrids (mesalamine, Figure 2, or naproxen)[27] which retained the anti-inflammatory properties of the NSAID alone[28] and also significantly reduced the

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gastric mucosal damage caused by them. Several H_2S-releasing NSAIDs have been previously synthesized and evaluated.[29,30] However, using ROS as a trigger, to our knowledge, a hybrid compound has not been reported. We synthesized **1j**, which is a derivative of mesalamine, a clinically used NSAID for the treatment of colitis (Table 1, entry 10).[28] This compound was found to release H_2S (Figure 5a), and its release profile

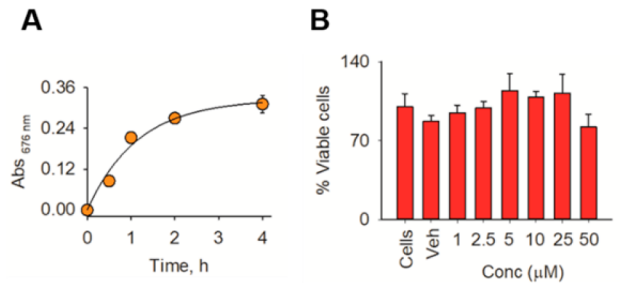

Figure 5. (a) Representative kinetic plot of H_2S release from **1j** in the presence of H_2O_2 as determined by a methylene blue assay. Curve fitting yielded a pseudo-first-order rate constant of 0.0145 min^{-1}. (b) Cell viability assay conducted with MCF-7 breast cancer cell line with **1j** during 24 h. Veh is DMSO.

(pseudo-first-order rate constant, 0.0145 min^{-1}) was comparable with that of amine **1b** (pK_a 5.03). The H_2S response obtained was selective toward H_2O_2 (see Figure S7). Decomposition studies showed the formation of the methyl ester of mesalamine **7** (see Figure S8). The compound formed could in turn be hydrolyzed to the active NSAID in the presence of esterase (widely prevalent in cells; see Figure S9). The hybrid compound was well tolerated by cells as studied by a standard cell viability assay (Figure 5b), and further studies are presently underway to characterize the anti-inflammatory properties. Thus, taken together, we report ROS-activated COS/H_2S[31−37] donors whose rates of hydrogen sulfide release could be modulated by modifying the basicity of the amine. We also report a novel H_2S−NSAID hybrid that decomposes when exposed to ROS to produce H_2S.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01356.

Synthesis, characterization data, and protocols for assays (PDF)

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Notes

The authors declare no competing financial interest.

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