Organic LETTERS

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Visible-Light-Triggered Uncaging of Carbonyl Sulfide for Hydrogen Sulfide (H_2S) Release

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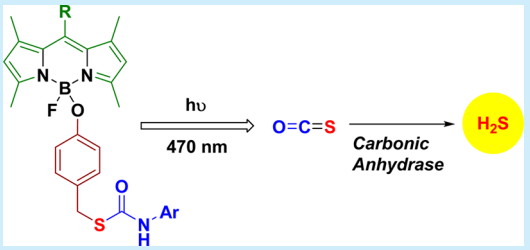
Supporting Information

ABSTRACT: Generation of hydrogen sulfide (H_2S) is challenging and few methods are capable of localized delivery of this gas. Here, a boron dipyrromethene-based carbamothioate (BDP-H_2S) that is uncaged by visible light of 470 nm to generate carbonyl sulfide (COS), which is rapidly hydrolyzed to H_2S in the presence of carbonic anhydrase, a widely prevalent enzyme, is reported.



Gaseous species derived from carbon, nitrogen, and sulfur are emerging as major mediators of cellular processes. Some have been termed as "gasotransmitters", which are small gaseous molecules that can act as signal transmitting agents within cells as well as across cells. For example, hydrogen sulfide (H_2S) is known to mediate a number of physiological processes.[1] Due to its highly diffusible nature and multiple targets within cells, cell signaling by H_2S is often complex and can depend on local concentrations of this species.[2,3] This underscores the need for spatiotemporally controlled generation of this gas under ambient conditions. A number of donors of H_2S with varying degrees of control over release of H_2S are known.[4,5] Among these, light-triggered donors have distinct advantages for localized delivery.[6−8] On account of the ability to direct the light source, generation of H_2S in a spatially controlled manner is possible. Although small molecule-based light-triggered H_2S generation methodologies are known, they all are based on ultraviolet light. For example, Xian and co-workers have reported a 2-nitrobenzyl-based donor that when exposed to 365 nm light produces a geminal dithiol, which undergoes hydrolysis to generate H_2S (Figure 1a). Nakagawa and co-workers have reported two scaffolds based on thioethers that are triggered by UV light to produce H_2S (Figure 1a).[7,8] More recently, Connal and co-workers have reported photogenerated thiobenzaldehydes as H_2S donors.[9] While these methods offer excellent spatiotemporal control, the phototoxicity associated with UV light is a major limitation.[10] Thus, a method for photouncaging of a H_2S source under ambient visible light conditions is yet unavailable.

Recently, 4-arylalkoxy-boron dipyrromethene (4-OAr-BODIPY)[11] has been used to deliver histamine, an important mediator of inflammation and allergy.[12] Our group[13] and others[14−17] have shown that carbonyl sulfide (COS)[18] is an excellent donor of H_2S. The gas COS is known to undergo rapid hydrolysis in the presence of the widely occurring enzyme carbonic anhydrase (CA),[19] to produce H_2S. Pluth and co-workers have recently reported a UV light activated COS/H_2S

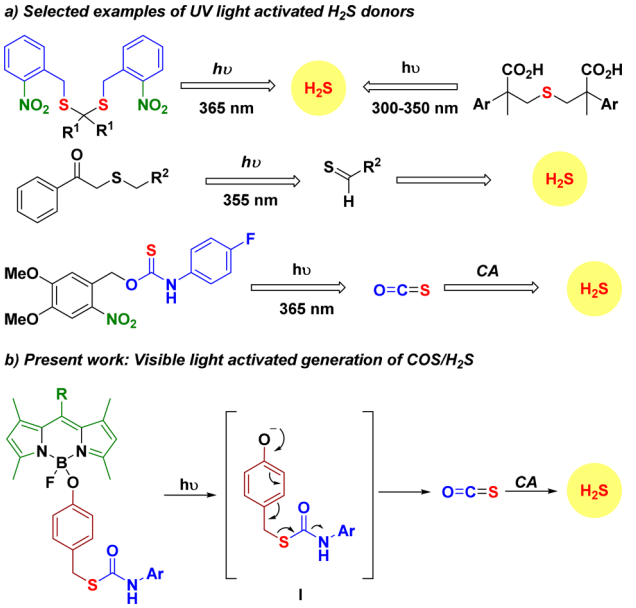
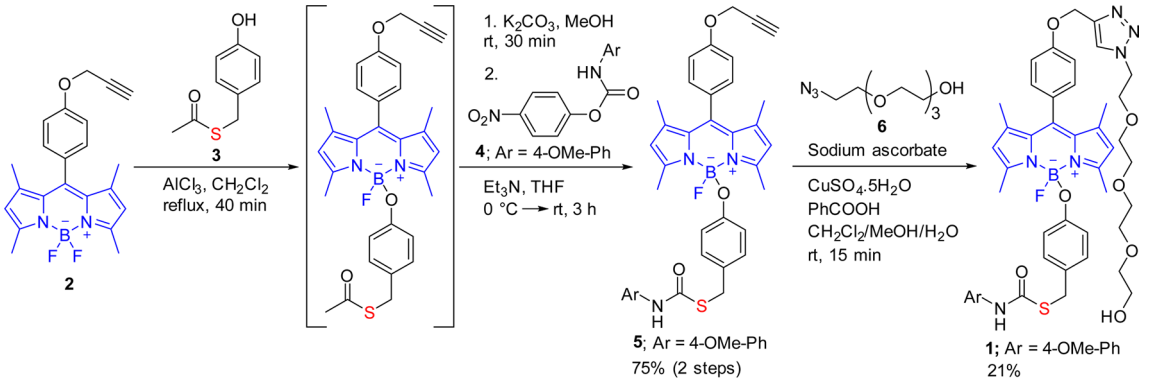


Figure 1. (a) Selected UV-light-activated methodologies for H_2S generation R^1 = various alkyl groups; Ar = PhCOPh; R^2 = aryl group. (b) Design of a visible light-triggered COS/H_2S donor. Visible-light-mediated cleavage of the B−O bond produces an intermediate **I** that undergoes self-immolation to generate COS that undergoes hydrolysis in the presence of CA to generate H_2S.

Received: July 23, 2017

DOI: 10.1021/acs.orglett.7b02259
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of H_2S Donor 1



donor (Figure 1a).[20] Again, the potential for phototoxicity due to prolonged UV exposure may be a limitation of this method. Therefore, masking COS in the form of a carbamothioate with attachment of this functional group to a BODIPY-based photolabile group was considered (Figure 1b). Upon exposure to visible light, this compound is expected to undergo cleavage to produce COS/H_2S.

Compound **2** (Scheme 1) was first synthesized using a reported methodology (see Supporting Information (SI)).[21] Next, 4-*S*-(hydroxybenzyl) ethanethioate **3** was prepared, and reaction of **3** with **2** in the presence of a Lewis acid $AlCl_3$ gave a thioacetate (crude) as an intermediate, which was subsequently hydrolyzed to the corresponding thiol (not isolated). Reaction of the crude thiol with the 4-nitrophenylcarbamate **4** gave **5** in 75% yield over two steps. Due to the increased hydrophobicity associated with BODIPY derivatives, incorporation of a short oligo-ethylene glycol functional group that should increase aqueous solubility was considered. Using a copper-mediated click reaction, the desired compound, BDP-H_2S **1**, was prepared in 21% yield.

Using a reported method, BDP-H_2S was incubated in MeOH and exposed to 470 nm light for 20 min. HPLC analysis (detection wavelength, 500 nm) revealed nearly complete decomposition of **1** (Figure 2a; also see Figure S1, SI). Urano and co-workers have reported that uncaging of the 4-aryloxy-BODIPY derivative[12] is accomplished by a photoinduced electron transfer (PeT)[22,23] process that results in the formation of a charge-separated intermediate, involving a cation radical of the aryloxy group and the anion radical of BODIPY. This results in the cleavage of the B−O bond by solvolysis, and the expected product in methanol is the B−OMe derivative. The time course of decomposition of **1** was studied, and a first-order rate constant of 0.16 min^{-1} was obtained (Figure 2b). During photolysis of **1**, HPLC analysis revealed the formation of a new product (Figure 2a), which was characterized by mass spectrometry as the methoxy-derivative **7** (Figure 2d, Figure S5, SI). The time course for formation of **7** gave a first order rate constant of 0.20 min^{-1} (Figure 2b). Together, these data suggest cleavage of **1** is accompanied by rapid solvolysis to produce **7**.

When photolysis of **1** was monitored by HPLC attached with a fluorescence detector, a distinct peak attributable to **7** was formed (see SI, Figure S2). Thus, photocleavage of **1**, a weakly fluorescent compound, in methanol produces **7**, which is highly fluorescent (excitation 470 nm, emission 540 nm).

This enhancement in fluorescence is attributable to the differences in the quenching ability of the O-aryl group in **1**

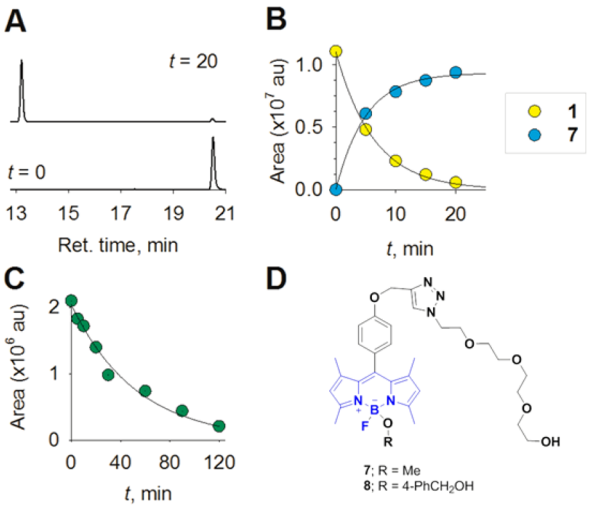


Figure 2. (a) HPLC traces for **1** before and after irradiation with 470 nm light (30 mW/cm^2) for 20 min in MeOH showed nearly complete disappearance of **1** with concomitant formation of an intermediate at retention time of 13.2 min, which was later identified by mass spectrometry analysis as BDP-OMe (**7**). (b) Time course of disappearance of **1** and formation of intermediate **7** during the same time period was determined by HPLC analysis. Curve fitting yielded first-order rate constants for disappearance of **1** as 0.16 min^{-1} and formation of **7** as 0.20 min^{-1}. (c) Time course of disappearance of intermediate **I** was followed by HPLC analysis. The rate constant for decomposition was found as 0.02 min^{-1}, (d) Proposed structure of the intermediate **7** produced during photolysis of **1** in methanol and the negative control **8**.

when compared with the OMe group in **7**. In a separate experiment, the fluorescence enhancement was monitored by a microwell plate reader and the fluorescence enhancement after irradiation was >20-fold (Figure 3a). In order to test whether this fluorescence enhancement also occurred within cells, human cervical cancer HeLa cells were incubated with **1** for 2 min followed by imaging using a fluorescence microscope. A small fluorescence signal at 540 nm (excitation 470 nm) was seen under these conditions (Figure 3b, dark). However, when HeLa cells pretreated with **1** were irradiated with a 470 nm light source for 2 min, and subsequent fluorescence enhancement was studied by microscopy, a significant enhancement in fluorescence signal was recorded (Figure 3b and 3c, light versus dark).

A cell viability assay was next conducted to assess the cytotoxicity during photocleavage of **1**. HeLa cells were independently incubated with **1** at various concentrations,

DOI: 10.1021/acs.orglett.7b02259
Org. Lett. XXXX, XXX, XXX−XXX

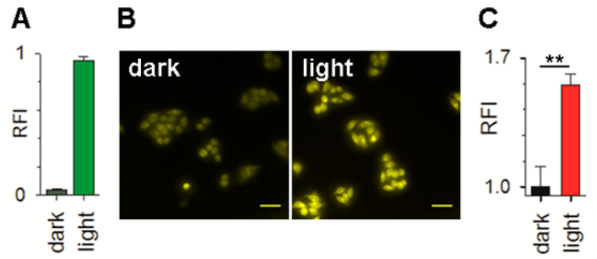


Figure 3. (a) Enhancement in fluorescence (excitation 470 nm, emission 540 nm) when **1** (50 μM) was irradiated with 470 nm light; (b) HeLa cells pretreated with **1** (10 μM) were irradiated for 2 min by 470 nm light and imaging showed increased fluorescence signal (YFP channel); Scale bar = 50 μm (c) Fluorescence enhancement data for the cellular experiment. **p-value = 0.002.

and these cells were exposed to 470 nm light for 5 min. We found no evidence for inhibition of proliferation of HeLa cells by **1** during photocleavage suggesting that this compound was not cytotoxic (Figure 4). The compound itself was not toxic in the dark (Figure S9a), and the absorbance at 570 nm from this compound was not significant (Figure S9b).

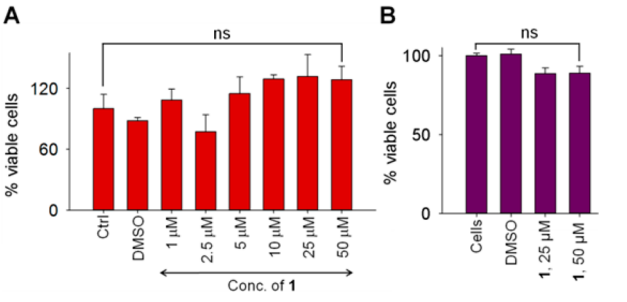


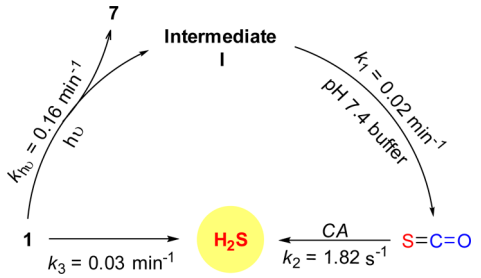
Figure 4. Effect of **1** on cell viability was determined by two independent assays: (a) HeLa cells with increasing doses of **1** followed by irradiation with 470 nm light for 5 min (24 h incubation, MTT assay). (b) A549 cells with increasing doses of **1** followed by irradiation with 470 nm light for 5 min (24 h incubation, crystal violet assay). The differences in viability are not significant (ns) as determined by Student's t-test.

A crystal violet assay was independently conducted using the A549 human lung cancer cell line to validate our results: the compound was not significantly cytotoxic (Figure 4b). Together, these results indicate that **1** is nontoxic, is cell permeable, and can be cleaved by a visible light source within cells to generate a fluorescence signal.

Cleavage of the B−O bond results in the formation of a phenolate-intermediate **I**, which has been previously reported to generate COS (Scheme 2).[13] The time course of decomposition of **I** was monitored in pH 7.4 buffer (see Figure S3, SI), and this intermediate disappeared within 2 h (Figure 2c). The rate constant for decomposition was found to be 0.02 min^{-1}. In the presence of carbonic anhydrase (CA), COS is known to generate H_2S (Scheme 2). In order to test this hypothesis, **1** was irradiated using visible light at 470 nm in methanol and the resulting mixture was incubated in pH 7.4 buffer in the presence of CA. The formation of H_2S was monitored by a methylene blue assay. If H_2S were produced, a characteristic UV−visible absorption spectrum with absorption maxima at 676 nm would be expected.

When this experiment was conducted, as predicted, an increase in absorption at 676 nm confirming the ability of **1** to

Scheme 2. Proposed Mechanism for H_2S Generation during Photocleavage of BDP-H_2S 1 in Methanol Followed by Incubation in pH 7.4 Buffer[a]



[a]For further details, see Scheme S2, SI.

produce H_2S was observed (Figure 5a). In the absence of CA or when incubated in the dark, **1** was incapable of generating H_2S

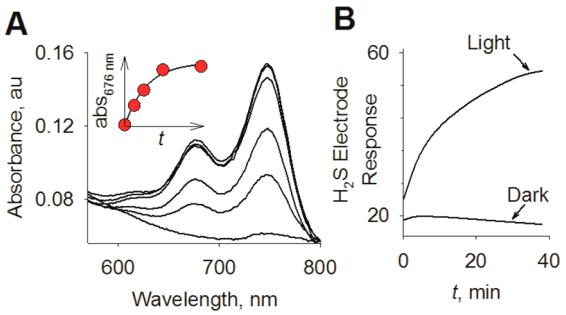


Figure 5. (a) A methylene blue formation assay was used to determine H_2S release from **1** when irradiated with 470 nm light for 25 min followed by exposure to carbonic anhydrase in pH 7.4 buffer. Inset, time course of increase in absorbance at 676 nm during 2 h. Curve fitting to a pseudo-first-order release gave a rate constant of 0.03 min^{-1} (see SI). (b) H_2S generation during incubation of **1** after irradiation with visible light followed by exposure to CA in pH 7.4 buffer was assessed by a H_2S-sensitive electrode. Dark: A similar experiment was conducted in the absence of light.

in 2 h (Figure S6, SI). The time course of H_2S release showed a gradual increase over 2 h (Figure 5a, inset). The rate constant for H_2S release was found to be 0.03 min^{-1}, which is comparable with the disappearance of intermediate **I** (Figure 2c). The yield of H_2S was found to be in the range 30%−40%; this efficiency is comparable with the previously reported photouncaging of histamine that produced a similar yield of the amine.[12]

Next, a H_2S-sensitive electrode was used to independently verify the generation of H_2S from **1**. Here, the irradiated sample of **1** in methanol was incubated in pH 7.4 buffer in the presence of CA and H_2S generation was monitored by the electrode. As expected, a distinct signal attributable to H_2S was recorded (Figure 5b). No detectable signal was observed when the donor was incubated under similar conditions in the absence of light. BDP-H_2S **1** was found to be stable in pH 7.4 buffer during 2.5 h incubation in the dark as determined by HPLC analysis (see SI, Figure S4). Together, these data confirm the ability of **1** to generate H_2S only when exposed to visible light of 470 nm.

Compound **8** was next synthesized (see Figure 2d, Scheme S1, SI) and was similarly irradiated with 470 nm light in methanol. The resultant reaction mixture was incubated in pH 7.4 buffer and was analyzed for H_2S production by a methylene

DOI: 10.1021/acs.orglett.7b02259
Org. Lett. XXXX, XXX, XXX−XXX

blue assay. As expected, no evidence for the formation of H_2S under these conditions was found (see SI, Figure S7).

Much like the other biological gases nitric oxide (NO),[24−30] carbon monoxide (CO),[31] and sulfur dioxide (SO_2),[32−37] both chemical and biological tools to generate and detect H_2S are necessary. The ability to effectively localize H_2S presents numerous opportunities to study the biology of this gas as well as progress toward site-directed delivery of H_2S for therapeutic purposes.[38−41] Again, BDP-H_2S that we report herein has distinct advantages over the existing class of UV-activated H_2S donors. Although blue light can cause moderate oxidative stress in cells,[10] the short irradiation times and low intensity of light required for H_2S release do not compromise cell viability (Figure 4). The formation of a quinone-methide intermediate may be a limitation for therapeutic use of this compound. However, to our knowledge, this is the first example of a visible light activated H_2S donor, and it is anticipated that this tool will lay the platform for delivery of this gas under ambient conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b02259.

Compound characterization data, spectra, and assay protocols (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the Department of Science and Technology (DST, Grant No. EMR/2015/000668) and Council for Scientific and Industrial Research (CSIR) for financial support.

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DOI: 10.1021/acs.orglett.7b02259
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