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Light-induced Hydrogen Sulfide Release from “Caged” *gem*-Dithiols

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Abstract

“Caged” *gem*-dithiol derivatives that release H_2S upon light stimulation have been developed. This new class of H_2S -donors was proven, by various spectroscopic methods, to generate H_2S in an aqueous/organic medium as well as in cell culture.

Hydrogen sulfide (H_2S) is a notorious toxic gas known for many years to be detrimental to humans. Recently this molecule has been identified as a cell-signaling mediator and constitutes a member of the gasotransmitter family, together with its congeners nitric oxide (NO) and carbon monoxide (CO).¹ The endogenous generation of H_2S has been predominantly attributed to the enzymatic actions of cystathionine- β -synthase (CBS), cystathionine- γ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST or 3-MPST).² In specific tissues, these enzymes utilized cysteine (Cys), homocysteine (Hcy) or other cysteine derivatives to produce H_2S in a controllable manner. Once H_2S is produced, in addition to carrying out its biological functions, H_2S can be rapidly metabolized into two other forms as acid-labile sulfur (Fe-S cluster) and bound-sulfane sulfur. Both could in turn serve as *in vivo* H_2S sources.³

In the past decade a number of studies have revealed significant roles of H_2S in physiology and pathology.^{1,2} Among many attributes given to endogenous production of H_2S and/or exogenous administration of H_2S , critical functions are especially exerted in the cardiovascular and nervous systems, and regulating inflammation.⁴ However, mechanisms underlying these biological responses are still unclear. These physiological and pathological activities may be derived from biological chemistry occurring at the molecular level. For example, H_2S is highly reactive toward reactive oxygen and nitrogen species including hydrogen peroxide (H_2O_2),⁵ superoxide ($\text{O}_2^{\cdot-}$)⁶ and peroxynitrite (OONO^-),⁷ establishing its role as an antioxidant. H_2S can also react with RSNO to produce thionitrous acid, HSNO (the smallest *S*-nitrosothiol), which serves as a cell permeable nitrosylating agent.⁸ In addition, H_2S can modify protein cysteine residues to give sulfhydrated proteins (protein-S-SH), which are believed to be a critical pathway in regulating protein functions.⁹

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Supporting Information Available (Synthetic procedures, spectroscopic data, and experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>).

The rapid and constant growth of the H₂S-biomedical research has led a concomitant need of research tools. Recent advances on H₂S-fluorescent sensors and H₂S donors are perfect examples.¹⁰ In particular, H₂S-donors are very attractive as many studies have highlighted the therapeutic potentials of exogenous administration of H₂S.¹¹ Among commonly used donors most researchers are using inorganic sulfide salts such as NaSH and Na₂S. However, H₂S generation from these salts occurs rapidly. It is difficult to control the timing of release, which therefore cannot mimic the endogenous production of H₂S.¹² In some cases, the biological effects displayed by sulfide salts may not represent physiological events induced by the actions of H₂S. Instead, it may be a systematic response to excess amounts of H₂S.¹³

In contrast to inorganic sulfide salts, organic H₂S donors can exert continuous and controllable H₂S release at concentrations relative to endogenous levels. Currently, several types of organic H₂S-donors have been developed and their mechanisms of H₂S production are diverse (Scheme 1).^{11,14} Our group has recently disclosed two types of controllable donors: *N*-(benzoylthio)-benzamide based donors and persulfide-based donors.¹⁵ Both types are utilizing biological thiols, such as cysteine and glutathione, as the triggers to promote H₂S generation. In addition to the thiol-activation mechanism, we expect that a platform capable of generating H₂S upon external stimulus should be of great interest. Such donors would enable steady and localized concentrations of H₂S at desired timing and cellular locations. In this context, photocaged H₂S donors are potential candidates. Herein we report the design, synthesis, and evaluation of a series of photo-activated H₂S donors.

The idea of caged-H₂S donors was based on the structure of geminal-dithiols (*gem*-dithiols). It is known that *gem*-dithiols are unstable species, particularly in aqueous environments, and H₂S can be formed as a decomposition by product.¹⁶ Therefore we envisioned *gem*-dithiols were useful templates for H₂S donor design. Introduction of protecting groups on free-SH of *gem*-dithiols should lead to stable derivatives as H₂S donors. In addition, we should be able to manipulate the deprotection strategy to achieve controllable H₂S release. As the first step to develop *gem*-dithiol based donors, we decided to test photo-activation strategy. As shown in Scheme 2, our target was compounds **1**, in which the SH groups were protected with a photo-sensitive 2-nitrobenzyl group. Upon light irradiation, the *gem*-dithiol intermediate **1A** should be produced and subsequent hydrolysis of **1A** would liberate H₂S.

The synthesis of this type of donor is illustrated in Scheme 3. Briefly, commercially available 2-nitrobenzyl bromide **2** was treated with thiourea in THF to produce the thiuronium bromide salt **3**. Hydrolysis of **3** in the presence of sodium metabisulfite (Na₂S₂O₅) provided 2-nitro benzenemethanethiol **4** in high yield. Finally compound **4** was coupled with acetone in the presence of catalytic amount of TiCl₄ to give a model donor **1a**.

With the model donor in hand, we examined its H₂S generation capability. The standard methylene blue method was used to monitor H₂S generation (the mechanistic scheme of this method is shown in supporting information). In this study, a 200 μM solution of **1a** in pH 7.4 phosphate buffer/acetonitrile (1:1) was prepared. The compound appeared to be stable and no H₂S release was detected. However, when the solution was subjected to UV irradiation at 365 nm, we observed a time-dependent H₂S production. The concentrations of H₂S reached a maximum of ~36 μM in about 7 min and dropped afterwards, presumably due to volatilization of H₂S gas (Figure 1).^{12a}

To confirm the signals shown in Figure 1 were indeed from H₂S, we recorded the UV-Vis spectra of methylene blue generated from the photolysis of **1a** and compared with the spectra obtained using Na₂S, a standard H₂S precursor. As shown in Figure 2, these absorbance spectra showed identical patterns and the levels increased when irradiation was prolonged.

Given the potential applications of photo-activated donors for site-specific delivery of H₂S and the diverse cellular pH values, we also studied the effects of pH on H₂S releasing activity of **1a**. In mild acidic medium (pH=5.5), H₂S level was found to be much higher than the level generated at neutral pH (Figure 3). This result may indicate the intermediacy of *gem*-dithiol, in which the hydrolysis should be an acid-facilitated process. In contrast, H₂S concentration dropped slightly under mild basic pH (8.2). It should be noted that the donor did not produce any H₂S under these pH values if the irradiation of UV light was absent.

Having demonstrated UV light-induced H₂S generation of **1a**, we turned our attention to other derivatives **1b–1f**, which were prepared using the same protocol shown in Scheme 3. We wondered if structural modifications could modulate H₂S release capability. As shown in Figure 4, alkyl-substituted donors **1b–1d** exhibited similar H₂S release capability as the model compound **1a**. However, aryl-substituted compounds **1e** and **1f** showed very low activity.

Although the methylene blue method has been widely used to evaluate H₂S donors, this method requires strong acidic conditions and is destructive to biological samples like cells. We expected the photo-activated donors would be used in cell-based studies therefore non-destructive methods for continuously testing H₂S generation in such samples are needed. The fluorescent probes are appropriate for this purpose. To this end, WSP-1, a H₂S-specific fluorescent probe developed by our group,¹⁷ was used to monitor the photolysis of **1a** in buffers (the structure and reaction mechanism of WSP-1 is shown in the supporting information). In this study, a 200 μ M solution of **1a** in pH 7.4 phosphate buffer/acetonitrile (1:1) was prepared. The solution was subjected to UV irradiation at 365 nm and aliquots were withdrawn from the solution at a given time and then detected by WSP-1. We observed a time-dependent H₂S production, similar to that observed when using the methylene blue method. As shown in Figure 5, fluorescence signal increased dramatically when **1a** was under photolysis (aliquot taken at 9 min). The intensity was approximately 66 folds higher than the solution without light irradiation. The results proved that fluorescence method is appropriate for the evaluation of photo-activated H₂S donors.

Finally we wondered if these donors could be used to selectively deliver H₂S to cells and conducted a cell-based assay to address this question. In this study, HeLa cells were first incubated with **1d** (200 μ M) for 30 min and the mixture was then exposed to UV-light (365 nm) for 15 min. After that, cells were washed and re-suspended in new media. WSP-1 (50 μ M) was applied into the system to monitor H₂S in cells. As expected, cells treated with **1d** under irradiation showed much stronger fluorescent signals than cells treated with **1d** but no irradiation (Figure 5).

In summary, a series of photo-activated H₂S donors based on the structure of *gem*-dithiol were prepared and evaluated. Our evidence demonstrates the capabilities of these compounds as specific H₂S donors. These “caged” donors could allow spatial and temporal release of H₂S, and study real time H₂S activities. Screening other photo-labile groups and examining different activation mechanisms to unmask *gem*-dithiols are ongoing in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Time-dependent H₂S release of **1a** in pH 7.4 phosphate buffer/acetonitrile (1:1).

Figure 2. Spectra of methylene blue assay. Blue line: Na₂S (50 μ M). Other lines: H₂S release from **4a** upon irradiation at different times.

Figure 3.
 H_2S release of **1a** ($200 \mu\text{M}$) under different pH

Figure 4.
 H_2S release of **1a–1f**. Donor concentration: 200 μM . Under continuous irradiation.

Figure 5.

H₂S-release of **1a** detected by fluorescence: a) WSP-1 only (100 μ M); b) WSP-1 (100 μ M) and **1a** (200 μ M), in the absence light; c) WSP-1 (100 μ M) and **1a** (200 μ M), in the presence of light.

Figure 5. H₂S-release of **1d** in HeLa cells: a) **1d** (200 μ M) and WSP-1 (50 μ M), no UV-irradiation; b) **1d** (200 μ M) and WSP-1 (50 μ M), under UV-irradiation.

Scheme 1.
Representative organic H₂S donors

Scheme 2.
The design of photo-activated H₂S donors

Scheme 3.
Synthesis of photo-activated H_2S donors