ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

Light-Induced Hydrogen Sulfide Release from "Caged" *gem*-Dithiols

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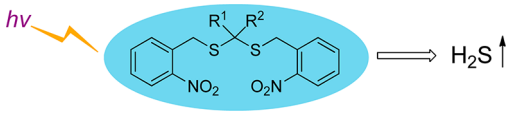
Received April 22, 2013

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 ($O_2^{\bullet-}$),[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

(1) (a) Li, L.; Rose, P.; Moore, P. K. *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 169. (b) Olson, K. R.; Donald, J. A.; Dombkoski, R. A.; Perry, S. F. *Repir. Physiol. Neurobiol.* **2012**, *184*, 117. (c) Wang, R. *Physiol. Rev.* **2012**, *92*, 791. (d) Fukuto, J. M.; Carrington, S. J.; Tantillo, D. J.; Harrison, J. G.; Ignarro, L. J.; Freeman, B. A.; Chen, A.; Wink, D. A. *Chem. Res. Toxicol.* **2012**, *25*, 769.

(2) (a) Kabil, O.; Banerjee, R. *J. Biol. Chem.* **2010**, *285*, 21903. (b) Kimura, H. *Amino Acids* **2011**, *41*, 113. (c) Jhee, K. H.; Kruger, W. D. *Antioxid. Redox Signal.* **2005**, *7*, 813. (d) Zhao, W.; Zhang, J.; Lu, Y.; Wang, R. *EMBO J.* **2001**, *20*, 6008.

(3) (a) Ubuka, T. *J. Chromatogr.* **2002**, *781*, 227. (b) Ishigami, M.; Hiraki, K.; Umemura, K.; Ogasawara, Y.; Ishii, K.; Kimura, H. *Antioxid. Redox Signal.* **2009**, *11*, 205. (c) Shen, X.; Peter, E. A.; Bir, S.; Wang, R.; Kevil, C. G. *Free Radical Biol. Med.* **2012**, *52*, 2276.

(4) (a) Whitffield, N. J.; Kreimier, E. L.; Verdial, F. C.; Skovgaard, N.; Olson, K. R. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2008**, *294*, R1930. (b) Li, J.; Whiteman, M.; Guan, Y. Y.; Neo, K. L.; Cheng, Y.; Lee, S. W.; Zhao, Y.; Baskar, R.; Tan, C. H.; Moore, P. K. *Circulation* **2008**, *117*, 2351. (c) Predmore, B. L.; Lefer, D. J.; Gojon, G. *Antioxid. Redox Signal.* **2012**, *17*, 119. (d) Abe, K.; Kimura, H. *J. Neurosci.* **1996**, *16*, 1066. (e) Qu, K.; Lee, S. W.; Bian, J. S.; Low, C. M.; Wong, P. T. H. *Neurochem. Int.* **2008**, *52*, 155. (f) Fiorucci, S.; Distrutti, E.; Cirino, G.; Wallace, J. L. *Gastroenterology* **2006**, *131*, 259. (g) Wallace, J. L.; Caliendo, G.; Santagada, V.; Cirino, G.; Fiorucci, S. *Gastroenterology* **2007**, *132*, 261.

(5) Geng, B.; Yang, J.; Qi, Y.; Zhao, J.; Pang, Y.; Du, J.; Tang, C. *Biochem. Biophy. Res. Commun.* **2004**, *313*, 362.

(6) Cheng, L.; Geng, B.; Yu, F.; Zhao, J.; Jiang, H.; Du, J.; Tang, C. *Amino Acids* **2008**, *34*, 573.

(7) Whiteman, M.; Armstrong, J. S.; Chu, S. H.; Jia-Ling, S.; Wong, B. S.; Cheung, N. S.; Halliwell, B.; Moore, P. L. *Neurochemistry* **2004**, *90*, 765.

(8) Filipovic, M. R.; Miljkovic, J. Lj.; Nauser, T.; Royzen, M.; Klos, K.; Shubina, T.; Joppenol, W. H.; Lippard, S. J.; Ivanvic-Burmazovic, I. *J. Am. Chem. Soc.* **2012**, *134*, 12016.

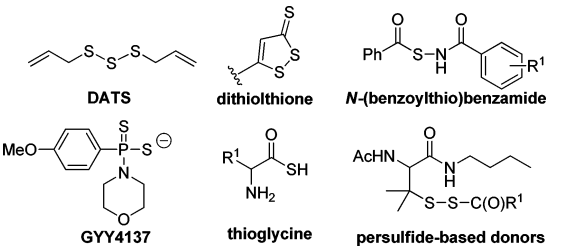
10.1021/ol401118k

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]

The rapid and constant growth of the H_2S-biomedical research has led to a concomitant need of research tools. Recent advances on H_2S-fluorescent sensors and H_2S donors are perfect examples.[10] In particular, H_2S donors are very attractive as many studies have highlighted the therapeutic potentials of exogenous administration of H_2S.[11] Among commonly used donors, most researchers are using inorganic sulfide salts such as NaHS and Na_2S. However, H_2S generation from these salts occurs rapidly. It is difficult to control the timing of release, which therefore cannot mimic the endogenous production of H_2S.[12] In some cases, the biological effects displayed by sulfide salts may not represent physiological events induced by the actions of H_2S. Instead, it may be a systematic response to excess amounts of H_2S.[13]

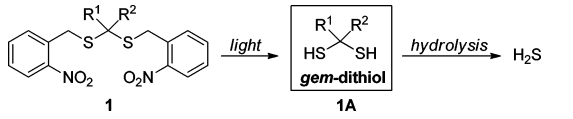
In contrast to inorganic sulfide salts, organic H_2S donors can exert continuous and controllable H_2S release at concentrations relative to endogenous levels. Currently, several types of organic H_2S donors have been developed and their mechanisms of H_2S production are diverse (Scheme 1).[11,14] Our 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 H_2S generation. In addition to the thiol-activation mechanism, we expect that a platform capable of generating H_2S upon external stimulus should be of great interest. Such donors would enable steady and localized concentrations of H_2S at desired timing and cellular locations. In this context, photocaged H_2S donors are potential candidates. Herein we report the design, synthesis, and evaluation of a series of photoactivated H_2S donors.

Scheme 1. Representative Organic H_2S Donors



The idea of caged-H_2S 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 H_2S can be formed as a decomposition byproduct.[16] Therefore, we envisioned *gem*-dithiols were useful templates for H_2S donor design. Introduction of protecting groups on free −SH of *gem*-dithiols should lead to stable derivatives as H_2S donors. In addition, we should be able to manipulate the deprotection strategy to achieve controllable H_2S release. As the first step to develop *gem*-dithiol based donors, we decided to test photoactivation strategy. As shown in Scheme 2, our target was compounds **1**, in which the SH groups were protected with a photosensitive 2-nitrobenzyl group. Upon light irradiation, the *gem*-dithiol intermediate **1A** should be produced and subsequent hydrolysis of **1A** would liberate H_2S.

Scheme 2. Design of Photoactivated H_2S Donors



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 thiouronium bromide salt **3**. Hydrolysis of **3** in the presence of sodium metabisulfite ($Na_2S_2O_5$) provided 2-nitrobenzenemethanethiol **4** in high yield. Finally, compound **4** was coupled with acetone in the presence of catalytic amount of $TiCl_4$ to give a model donor **1a**.

With the model donor in hand, we examined its H_2S generation capability. The standard methylene blue method was used to monitor H_2S generation (the mechanistic scheme of this method is shown in the 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 H_2S release was detected. However, when the solution was subjected to UV irradiation at 365 nm, we observed a time-dependent H_2S production. The concentrations of H_2S reached a maximum of ~36 μM in about 7 min and dropped afterward, presumably due to volatilization of H_2S gas (Figure 1).[12a]

(9) (a) Mustafa, A. K.; Gadalla, M. M.; Sen, N.; Kim, S.; Mu, W.; Gazi, S. K.; Barrow, R. K.; Yang, G.; Wang, R.; Snyder, S. H. *Sci. Signal.* **2009**, *2*, ra72. (b) Krishnan, N.; Fu, C.; Pappin, D. J.; Tonks, N. K. *Sci. Signal.* **2011**, *4*, ra86. (c) Paul, B. D.; Snyder, S. H. *Nat. Rev. Mol. Cell Bio.* **2012**, *13*, 499.

(10) For selected reviews, see: (a) Lin, V. S.; Chang, C. J. *Curr. Opin. Chem. Biol.* **2012**, *16*, 1. (b) Chan, J.; Dodani, S. C.; Chang, C. J. *Nat. Chem.* **2012**, *4*, 973.

(11) (a) Kashfi, K.; Olson, K. R. *Biochem. Pharmacol.* **2012**, *85*, 689. (b) Olson, K. R. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2011**, *301*, R297. (c) Whiteman, M.; Trionnaire, Le; Chopra, M.; Fox, B.; Whatmore, J. *Clin. Sci.* **2011**, *121*, 459. (d) Caliendo, G.; Cirino, G.; Santagada, V.; Wallace, J. L. *J. Med. Chem.* **2010**, *53*, 6275.

(12) (a) DeLeon, E. R.; Stoy, G. F.; Olson, K. R. *Anal. Biochem.* **2012**, *421*, 203. (b) Calvert, J. W.; Coetzee, W. A.; Lefer, D. J. *Antioxid. Redox Signaling* **2010**, *12*, 1203.

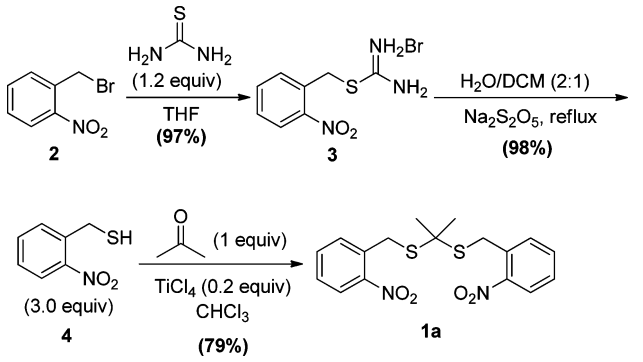
(13) Olson, K. R. *Front. Physiol.* **2013**, *4*, 1.

(14) Zhou, Z.; von Wantoch Rekowski, M.; Coletta, C.; Szabo, C.; Bucci, M.; Cirino, G.; Topouzis, S.; Papapetropoulos, A.; Gianns, A. *Bioorg. Med. Chem.* **2012**, *20*, 2675.

(15) (a) Zhao, Y.; Wang, H.; Xian, M. *J. Am. Chem. Soc.* **2011**, *133*, 15. (b) Zhao, Y.; Bhushan, S.; Yang, C.; Otsuka, H.; Stein, J. D.; Pacheco, A.; Peng, B.; Devarie-Baez, N. O.; Aguilar, H. C.; Lefer, D. J.; Xian, M. *ACS Chem. Biol.* **2013**dx.doi.org/10.1021/cb400090d.

(16) (a) Cairns, T. L.; Evans, G. L.; Larchar, A. W.; Mckusick, B. C. *J. Am. Chem. Soc.* **1952**, *74*, 3982. (b) Berchtold, G. A.; Edwards, B. E.; Campaigne, E.; Carmack, M. *J. Am. Chem. Soc.* **1959**, *81*, 3148. (c) Voronkov, M.; Shagun, L.; Ermolyuk, L.; Timokhina, L. *J. Sulfur Chem.* **2004**, *25*, 131.

Scheme 3. Synthesis of Photoactivated H_2S Donors



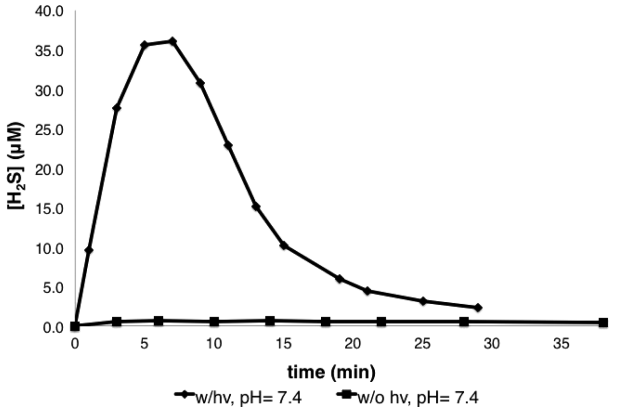


Figure 1. Time-dependent H_2S release of **1a** in pH 7.4 phosphate buffer/acetonitrile (1:1).

To confirm the signals shown in Figure 1 were indeed from H_2S, we recorded the UV−vis spectra of methylene blue generated from the photolysis of **1a** and compared with the spectra obtained using Na_2S, a standard H_2S 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 photoactivated donors for site-specific delivery of H_2S and the diverse cellular pH values, we also studied the effects of pH on H_2S releasing activity of **1a**. In mild acidic medium (pH = 5.5), H_2S 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, H_2S concentration dropped slightly under mild basic pH (8.2). It should be noted that the donor did not produce any H_2S under these pH values if the irradiation of UV light was absent.

Having demonstrated UV light-induced H_2S generation of **1a**, we turned our attention to other derivatives **1b**–**f**, which were prepared using the same protocol shown in Scheme 3. We wondered if structural modifications could

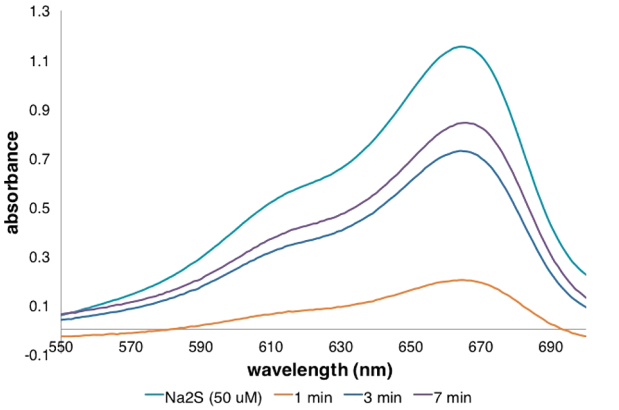


Figure 2. Spectra of methylene blue assay. Blue line: Na_2S (50 μM). Other lines: H_2S release from **1a** upon irradiation at different times.

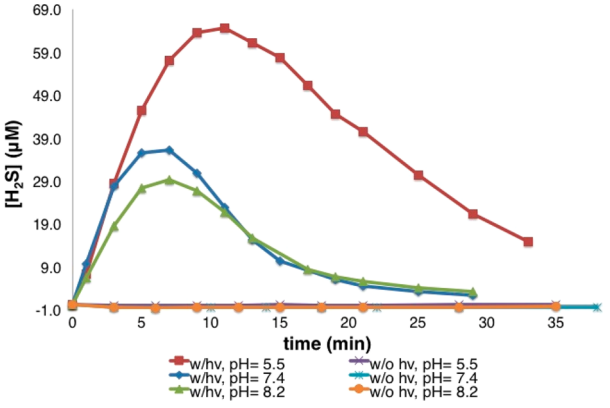


Figure 3. H_2S release of **1a** (200 μM) at different pH's.

modulate H_2S release capability. As shown in Figure 4, alkyl-substituted donors **1b**–**d** exhibited similar H_2S 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 H_2S donors, this method requires strong acidic conditions and is destructive to biological samples like cells. We expected the photoactivated donors would be used in cell-based studies; therefore nondestructive methods for continuously testing H_2S generation in such samples are needed. The fluorescent probes are appropriate for this purpose. To this end, WSP-1, a H_2S-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 μM solution of **1a** in pH 7.4 phosphate buffer/acetonitrile (1:1) was prepared.

(17) Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M. *Angew. Chem., Int. Ed.* **2011**, *123*, 10511.

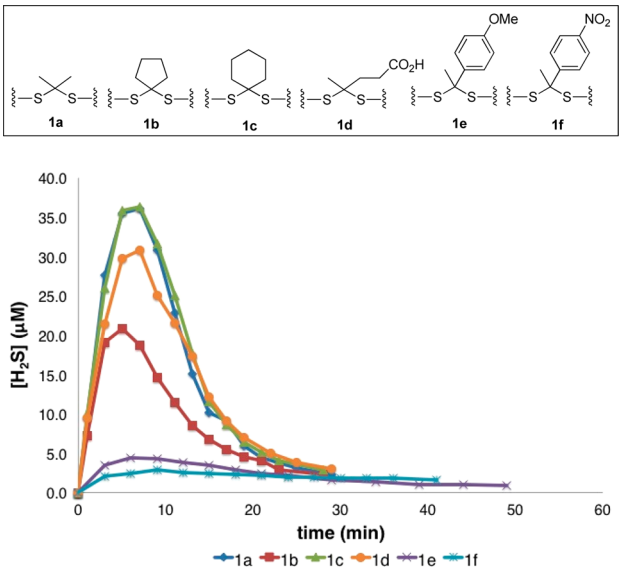


Figure 4. H_2S release of **1a**−**f**. Donor concentration: 200 μM. Under continuous irradiation.

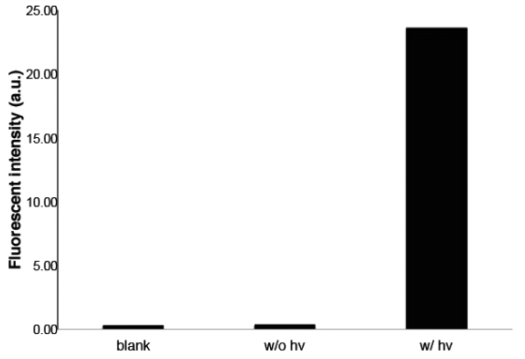


Figure 5. H_2S release of **1a** detected by fluorescence: (a) WSP-1 only (100 μM); (b) WSP-1 (100 μM) and **1a** (200 μM), in the absence of light; (c) WSP-1 (100 μM) and **1a** (200 μM), in the presence of light.

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 H_2S 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 photoactivated H_2S donors.

Finally, we wondered if these donors could be used to selectively deliver H_2S to cells and conducted a cell-based assay to address this question. In this study, HeLa cells were first incubated with **1d** (200 μM) for 30 min, and the mixture was then exposed to UV light (365 nm) for 15 min. After that, cells were washed and resuspended in new media. WSP-1 (50 μM) was applied into the system to monitor H_2S in cells. As expected, cells treated with **1d** under irradiation showed much stronger fluorescent signals than cells treated with **1d** but no irradiation (Figure 6).

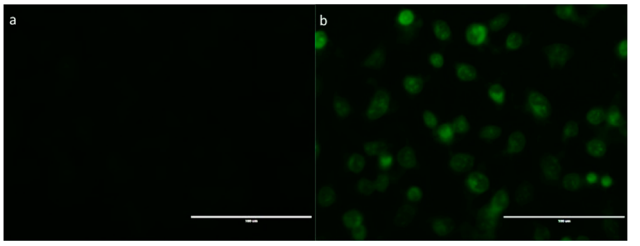


Figure 6. H_2S release of **1d** in HeLa cells: (a) **1d** (200 μM) and WSP-1 (50 μM), no UV irradiation; (b) **1d** (200 μM) and WSP-1 (50 μM), under UV irradiation.

In summary, a series of photoactivated H_2S donors based on the structure of *gem*-dithiol were prepared and evaluated. Our evidence demonstrates the capabilities of these compounds as specific H_2S donors. These "caged" donors could allow spatial and temporal release of H_2S, and study real time H_2S activities. Screening other photolabile groups and examining different activation mechanisms to unmask *gem*-dithiols are ongoing in our laboratory.

Acknowledgment. This work is supported by an American Chemical Society−Teva USA Scholar Grant and the NIH (R01GM088226).

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 authors declare no competing financial interest.