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O→S Relay Deprotection, A General Approach for the Design of Controllable Donors of Reactive Sulfur Species

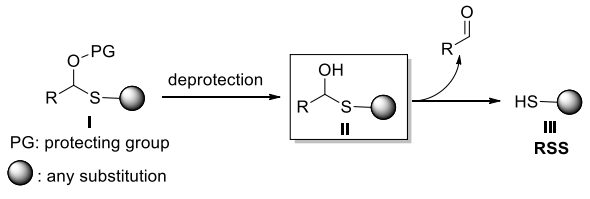
Jianming Kang,[†] Shi Xu,[†] Miles N. Radford, Wenjia Zhang, Shane S. Kelly, Jacob J. Day, and Ming Xian*

Dedicated to Prof. Jin-Pei Cheng on the occasion of his 70th birthday

Abstract: Reactive sulfur species (RSS) are biologically important molecules. Among them, hydrogen sulfide (H_2S), hydrogen polysulfides (H_2S_n, n>1), persulfides (RSSH), and *S*-nitroso hydrogen sulfide (HSNO) are believed to play regulatory roles in sulfur-related redox biology. However, these molecules are unstable and difficult to handle. Their instability is the major roadblock hindering their biological understanding. Having the access to their reliable and controllable precursors (or donors) is the prerequisite for the study of these sulfur species. In this work, we report the preparation and evaluation of a series of *O*-silyl mercaptan based sulfur containing molecules. These compounds can undergo pH- or F^- mediated desilylation to release the corresponding H_2S, H_2S_n, RSSH, and HSNO in a controlled fashion. This O→S relay deprotection serves as a general strategy for the design of pH- or F^- triggered RSS donors. Moreover, we have demonstrated that the O-silyl groups in the donors could be changed to other protecting groups like esters. This should allow the development of RSS donors with other activation mechanisms (such as esterase-activated donors).

Reactive sulfur species (RSS) are a group of sulfur containing molecules existing in biological systems and play regulatory roles. Representative RSS include thiols (like cysteine and glutathione), hydrogen sulfide (H_2S), persulfides (RSSH), polysulfides (RSS_nSR, n>0), hydrogen polysulfides (H_2S_n, n>1), as well as *S*-modified cysteine adducts such as nitrosothiols (RSNO) and sulfenic acids (RSOH).[1] Since the discovery of H_2S as a nitric oxide (NO)-like signaling molecule, understanding chemical biology of H_2S and H_2S-derived RSS, especially RSSH, H_2S_n, and HSNO, has become a fast growing research field.[1] On the other hand, many of these RSS are unstable and highly reactive molecules, causing significant difficulty in their studies. To solve this problem, considerable efforts have been put into the development of unique releasing systems (e.g. donors) or *in situ* generation systems for RSS.[2] Meanwhile, the development of specific and sensitive detection methods for RSS has also attracted much attention.[3] Progress in chemical tool development, especially those targeting H_2S, has dramatically advanced the RSS field. However, reliable and controllable releasing systems for other RSS including RSSH, H_2S_n, HSNO, etc., are still lacking. Current methods for generating these species rely on the use of commercially available inorganic salts (e.g. Na_2S_n for H_2S_n), or 'in-situ' generation of RSSH or HSNO based on the reactions of H_2S (with RSSR to form RSSH; with RSNO to form HSNO). The unavoidable sulfide impurity in the salts and the presence of H_2S in the reaction mixtures make these methods less useful as it is impossible to clearly differentiate the properties of the desired RSS from that of H_2S. The 'ideal' method for specific RSS generation should produce the desired RSS molecules in a clean, controllable, non-H_2S or other RSS involved, and free of redox conditions (because RSS are sensitive to redox conditions). With these considerations in mind, we herein report a general strategy for the design of RSS releasing systems that meet the aforementioned criteria. Most importantly, this modular design can be easily adopted to release a number of RSS including H_2S, H_2S_n, RSSH, HSNO, etc.

In the design of RSS releasing systems, specific sulfur (*S*-) protecting groups are often needed to cage the sulfur containing molecules and also serve as the triggers for the release. However, sulfur-based protecting groups that can be deprotected under bio-compatible conditions are rather limited. In contrast, oxygen (*O*-) based protecting groups are much more developed. A large number of *O*-protection groups are known and have been employed in *O*-based prodrug or sensor developments. We wondered if *O*-protection/deprotection could be used in *S*-cage/decage. That would require O→S relay protection or deprotection. We envisioned a geminal thiol-hydroxyl moiety could be useful for this design. As shown in Scheme 1, the removal of the *O*-protection group on substrate **I** should generate a geminal thiol-hydroxyl derivative **II**, which should be unstable and readily degrade to form the SH-containing molecule **III**. This achieves the O→S relay deprotection. We also envisioned many different *S*-derivatives of **I** could be prepared. Therefore, a variety of RSS can be released by this general method.



Scheme 1. The design of a general approach for RSS releasing

Based on this hypothesis, we believed the *O*-protected geminal hydroxyl-mercaptans should be the common precursors for all RSS releasing compounds. As the first proof-of-concept, we decided to prepare a series of *O*-silyl mercaptans (**1** and **2**, Scheme 2). These compounds were prepared from the corresponding aldehyde reacting with silyl chloride and H_2S gas.[4] These compounds were found to be quite stable. They could be

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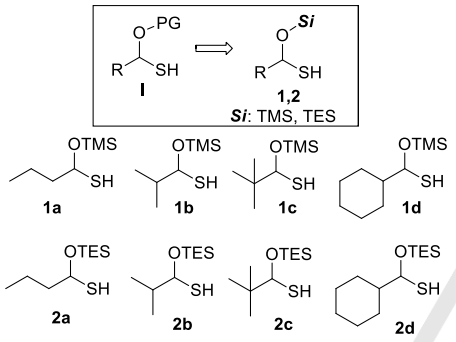
purified by column chromatography and fully characterized (see the supporting information (SI)). Due to the sensitivity of silyl groups toward acidic conditions, **1** and **2** were expected to be pH-sensitive H_2S donors. As such, H_2S release profiles of **1**/**2** were measured in buffers using a H_2S-selective electrode. Indeed, TMS-protected donors (**1a-d**) exhibited pH- and structure-dependent H_2S releasing ability. In general, more and faster H_2S release was observed under more acidic pH. This is shown in Figure 1 using **1d** as the example. Other donors' profiles are shown in Fig. S1 in the SI. Their structures also played a role in controlling H_2S release as more hindered donors tended to release slower and less H_2S. TES-protected donors (**2a-d**) showed enhanced stability. These donors released H_2S in a much slower fashion. In particular, **2c** and **2d** released barely detectable H_2S under these conditions. To further confirm their H_2S production in buffers we also carried out H_2S gas trapping experiments. The representative results were shown in Fig. S2, which matched well with the H_2S-electrode results. These results indicate that *O*-silyl mercaptans could serve as effective H_2S donors and their H_2S release profiles could be controlled by structural modifications.



Scheme 2. The structures of *O*-silyl mercaptans

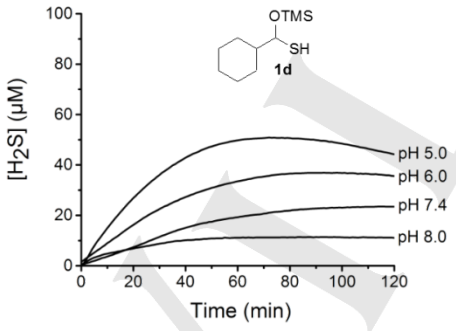
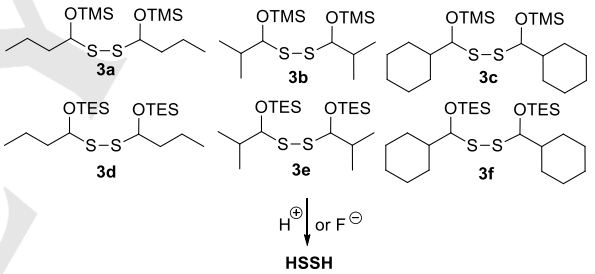


Figure 1. Time-dependent H_2S release curves of **1d** (100 μM) in different pH buffers.

H_2S_n are another group of sulfur-based signaling molecules that have received increasing attention recently.[1,5] Due to their instability in aqueous environments, slow and controllable H_2S_n donors are expected to be useful research tools. However, this has not been well studied. Only a recent work by Wang *et al.* reported acyl disulfides as H_2S_n donors.[6] It is known that acyl disulfides are highly reactive and can easily react with nucleophiles like amines.[7] As such, off-target effects of acyl disulfide based donors could be a concern. Seeking more stable and controllable H_2S_n donors has become one of our research goals. We realized that *O*-silyl mercaptans could be readily converted to the corresponding disulfides and thus prepared a series of such compounds (**3a-f**, Scheme 3). It should be noted that in theory these compounds should just release H_2S_2 upon desilylation, given their structural identity. However, regardless of what the starting H_2S_n species is, in solutions mixed polysulfides (including H_2S_2, H_2S_3, H_2S_4, etc.) will be formed rapidly. Therefore, these compounds were expected to be H_2S_n donors. **3a-f** were then tested for their pH-dependent degradation to release H_2S_n. As shown in Table 1, the time needed to reach complete degradation of these donors varies based on their structures. Again, more hindered donors showed slower degradation and more acidic conditions could promote faster degradation. It should be noted that H^+-promoted H_2S_n production from these donors could be detected by DSP-3,[8] a specific H_2S_n fluorescent senor (Figure S3 in SI). We also used DSP-3 to measure fluoride-triggered H_2S_n release from the donors. Figure 2 showed the time-dependent release profiles of these donors. Structural effects were obvious. With these results we believe *O*-silyl disulfides are effective H_2S_n donors.



Scheme 3. Structures of H_2S_n donors (**3a-f**)

Table 1. Time needed to release H_2S_n from the donors[a]

Donors	pH=5.0	pH=6.0	pH=7.4
3a	2.5 h	6 h	10 h
3b	4 h	12 h	14 h
3c	4.8 h	>24 h	>24 h
3d	7 h	14 h	20 h
3e	12 h	16 h	23 h
3f	13 h	>24 h	>24 h

[a]donor concentration: 20 mM, in DMF/PBS buffer (4/1)

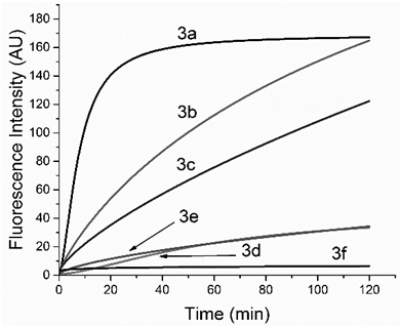


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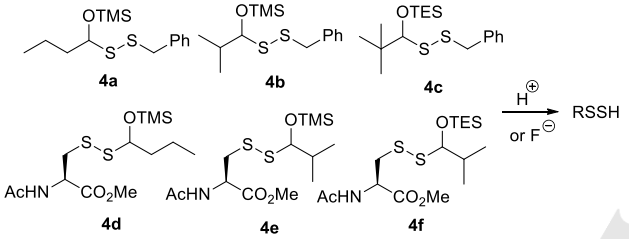
Figure 2. Time-dependent H_2S_n release from **3a-3f** (indicated by fluorescence changes). Donors (0.5 mM) were treated with TBAF (2.0 mM) in ethanol and then sensed by DSP-3.

Persulfides (RSSH) are another group of biologically important RSS.[1,9] The formation of persulfides on proteins has been recognized as a critical posttranslational modification. It provides a possible mechanism by which RSS alter the functions of a wide range of cellular proteins and enzymes. Methods that allow easy and reliable access to persulfides are the prerequisite for the study of persulfides. Previously, acyl disulfides (RSSAc)[10] and 9-fluorenylmethyl disulfides (RSSFm)[11] have been developed as acid- or base-triggered persulfide donors, respectively. We envisioned that *O*-silyl mercaptan derived unsymmetric disulfides could serve as a new category of persulfide donors and would benefit the study of persulfides. With this idea in mind, a series of such disulfides (**4a-f**, Scheme 4) were prepared (see SI for syntheses and characterization).



Scheme 4. Structures of persulfide donors (**4a-f**)

With **4a-f** in hand, we measured their pH- and fluoride-dependent decomposition to release persulfides. As shown in Table 2, the times needed to complete the decomposition were used to assess their release profiles. Clearly, TMS-based substrates (**4a**, **4b**, **4d**, **4e**) could be triggered to release persulfides in pH 5.0 and 6.0 solutions. They could also release persulfides in pH 7.4, but more slowly. The corresponding products were found to be a mixture of disulfide/trisulfides, which are the typical end products of persulfides. TES-based donors (**4c**, **4f**), in contrast, appeared to be quite stable and no decomposition in those buffer systems was observed. When these donors were treated with KF, they all underwent decomposition via varing rates. The effects of structure on the rates were obvious.

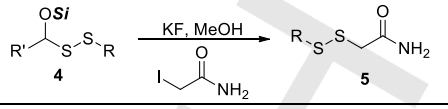
Table 2. Time needed to release persulfides from the donors

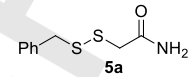
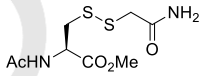
Donors	pH=5.0[a]	pH=6.0[a]	pH=7.4[a]	w/ KF[b]
4a	3.5 h	4 h	>12 h	10 min
4b	4 h	4.3 h	>12 h	15 min
4c	n.a.[c]	n.a.	n.a.	>12 h
4d	3.8 h	4 h	>12 h	5 min
4e	4 h	4.5 h	>12 h	5 min
4f	n.a.	n.a.	n.a.	8 h

[a]donor concentration: 20 mM, in DMF/PBS buffer (4/1); [b]donor concentration: 20 mM, in MeOH. [c]no reaction detected.

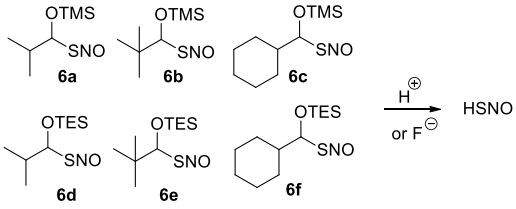
To further prove persulfides were generated from these donors, we used iodoacetamide to capture the persulfides. As shown in Table 3, in all the cases the desired trapping products (**5a** or **5b**) were obtained in moderate to high yields. These results indicate compounds **4a-f** are efficient persulfide donors.

Table 3. Trapping persulfides with iodoacetamide



substrate	yield of **5**	product
4a	94%	
4b	92%	
4c	90%	
4d	38%	
4e	36%	
4f	64%	

HSNO is the simplest *S*-nitrosothiols and has been proposed to be the crucial intermediate in the 'crosstalk' between NO and H_2S.[12] However, the biochemistry (formation, transport, decomposition) of HSNO remains poorly understood. Recent results show that HSNO could be generated from the treatment of RSNO with H2S,[12] or from NO and H_2S gases in high concentration.[13] These methods are not ideal for HSNO studies as other reactive species (like NO, H_2S) could also present. Therefore, it is impossible to attribute the observed activities only to HSNO. Methods that allow 'clean' generation of HSNO (without the interference of other RSS) are critical for better understanding HSNO chemical biology. To this end, we found *O*-silyl mercaptans (**1**, **2**) could be readily converted to the corresponding SNO adducts (as shown in Scheme 5). These compounds showed the characteristic red color of *S*-nitrosothiols (due to $n\rightarrow\pi^*$ transition of the SNO motif).[14] Their representative UV-vis spectra are shown in Figure S4 in SI. Their absorbance peaks around 565 nm were observed (with ε=11~15 L/mol/cm). Like typical alkyl S-nitrosothiols, **6a-f** were found to be unstable. But their identity could be confirmed by NMR and MS analysis (see SI).



Scheme 5. Structures of HSNO donors (**6a-f**)

We next studied H^+ and F^- triggered HSNO release from these compounds. Both conditions led to the formation of the corresponding aldehydes, indicating the release of HSNO. Most importantly, the formation of HSNO could be observed by direct MS analysis (Figure 3), showing the desired HSNO peak. The ultimate degradation product from HSNO was found to be elemental sulfur and we did not observe the formation of H_2S. Overall, these results indicate *O*-silyl SNO compounds like **6a-f** are clean and H_2S-independent HSNO donors.

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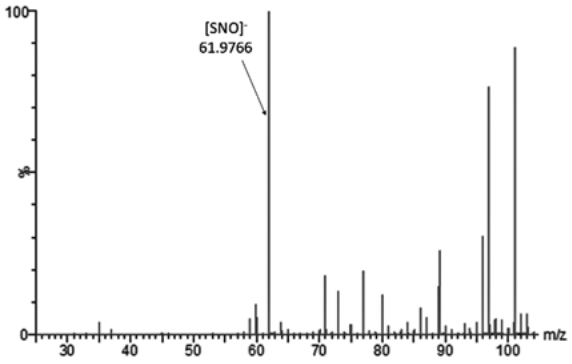
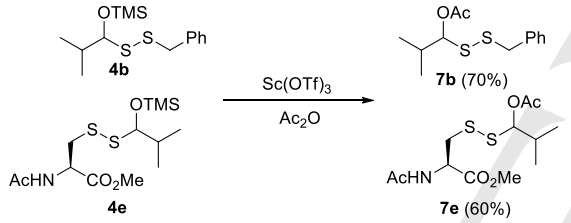


Figure 3. ESI-TOF MS spectrum of HSNO (negative mode)

Finally we wondered if the *O*-silyl groups could be changed to other biocompatible *O*-protection groups. If so, it would expand the scope of this strategy and lead to other activation mechanisms for RSS release. As the first proof-of-concept, we tested the direct protection group switch from *O*-silyl to *O*-acetyl. As shown in Scheme 6, the treatment of **4b** or **4e** with Ac_2O in the presence of $Sc(OTf)_3$ as the catalyst led to the formation of the desired *O*-acetyl persulfide donors **7b** and **7e** in good yields. Compounds like **7b**/**7e** could serve as esterase-triggered persulfide donors.[15] Enzyme-triggered release of small biologically active molecules is a useful strategy in exploring their biological functions.[16]



Scheme 6. *O*-Silyl to *O*-Acetyl protecting group switch

In summary, we report herein the preparation and evaluation of a series of *O*-silyl mercaptan based sulfur containing derivatives. These molecules can undergo pH and F^- mediated desilylation to release the corresponding reactive sulfur species (RSS) such as H_2S, H_2S_n, RSSH, and HSNO. This O→S relay deprotection serves as a general strategy for the design of controllable RSS donors. It should be noted that some of the donors (such as **3a-f**, **4d-f**) were obtained as the mixtures of diastereomers, due to two chiral centers in their structures. However, the desired RSS were produced as single compounds upon deprotection. While we only validated donors for H_2S, H_2S_n, RSSH, and HSNO in this study, we expect donors for other RSS like RSOH, RSO_2H, RSeSH, SO_2, etc., could also be developed using this concept. Given the significance of RSS in redox biology and the lack of appropriate generation methods for many of them, our strategy should be very useful in further exploration of these sulfur species. Moreover, we have demonstrated that *O*-silyl groups of the donors could be changed to other protecting groups like esters. This should allow the development of RSS donors with other activation mechanisms (such as esterase-activated donors). More studies using this strategy for the design of RSS based donors, pro-drugs, and specific imaging sensors are currently being developed in our laboratory and will be reported in due courses.

Acknowledgements

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Keywords: relay deprotection • sulfide • persulfide • polysulfide • nitrosothiol• prodrug

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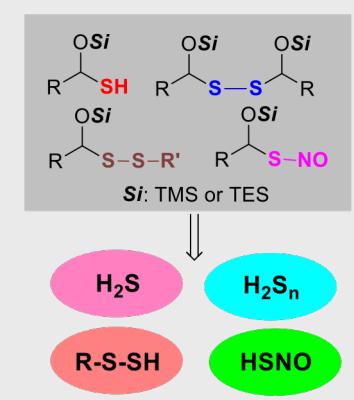
A General Structural Template for Sulfur Precursors/Prodrugs: *O*-Silyl mercaptan derived compounds can be developed as controllable donors for reactive sulfur species such as H_2S, H_2S_n, RSSH, and HSNO. This O→S relay deprotection can serve as a general strategy for the design of sulfur-based chemical tools such as prodrugs and imaging sensors.



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O→S Relay Deprotection, A General Approach for the Design of Controllable Donors of Reactive Sulfur Species