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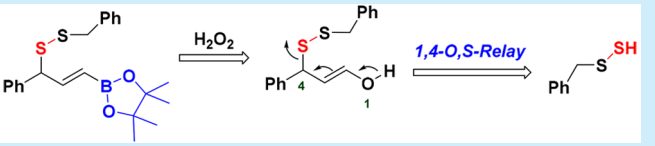
A Vinyl-Boronate Ester-Based Persulfide Donor Controllable by Hydrogen Peroxide, a Reactive Oxygen Species (ROS)

Prerona Bora, Preeti Chauhan, Suman Manna, and Harinath Chakrapani*

Department of Chemistry, Indian Institute of Science Education and Research Pune, Pune 411 008, Maharashtra, India

Supporting Information

ABSTRACT: A vinyl boronate ester-based persulfidating agent that is selectively activated by hydrogen peroxide, which is a reactive oxygen species (ROS), and efficiently generated a persulfide by a hitherto unexplored 1,4-O,S-relay mechanism is reported. This donor was found to protect cells from cytotoxicity induced by oxidants, and the major byproduct is cinnamaldehyde, which is widely used in the food industry as an additive.



Hydrogen sulfide (H_2S) has emerged as an important mediator of redox cellular processes, especially in the context of cellular responses associated with oxidative stress.[1−3] During several disease-like conditions, cells are exposed to increased reactive oxygen species (ROS), which contribute to neurodegenerative disorders, inflammation, diabetes, tumor progression, and aging.[4−8] A mechanism by which H_2S exerts its effects is protein persulfidation (or *S*-sulfhydration),[3] which is an oxidative post-translational modification where a cysteine (Cys-SH) residue is modified to Cys-SSH group.[1] Ambient protein persulfidation in cells is symptomatic of normal functioning in certain cells;[4] a corollary to this observation is that diminished persulfidation is associated with stressed or diseased states. For example, diminished persulfidation of parkin, an E3 ubiquitin ligase that contains a reactive cysteine residue, is correlated with decreased rescue of damaged neurons. Increasing parkin persulfidation appears to protect neurons by removing damaged proteins. This finding has tremendous implications in the treatment of neurodegenerative diseases such as Parkinson's disease.[9] However, this correlation does not hold for other proteins, underscoring the importance of developing new tools to interrogate the chemical biology of persulfidation under disease-relevant conditions.[1] Furthermore, since persulfides (RSS^-) are superior reductants, compared with RS^-, these species have gained traction as important intermediates in countering oxidative stress.[10,11] However, since persulfides are unstable in biological milieu, it is challenging to generate these reactive sulfur species in a controllable manner,[10,11] hence the growing interest in small-molecule persulfidating agents as tools to interrogate redox chemical biology of this reactive sulfur species.

An ideal donor would respond to elevated ROS to produce a protein persulfidating agent. The general strategy to generate a persulfide in situ involves stimuli responsive deprotection, followed by electronic rearrangement or a relay mechanism to release a persulfide.[12,13] Subsequent exchange of the sulfhydryl group between the persulfidating agent and a protein occurs to induce protein persulfidation (Figure 1a). To closely mimic inflammatory conditions, the donor would ideally need to be triggered by hydrogen peroxide, which is a stable ROS.[14] The first major class of persulfidating agents are based on a 1,2-O,S-relay mechanism, where the oxygen and sulfur are placed adjacent to each other (Figure 1b).[15] These donors respond to

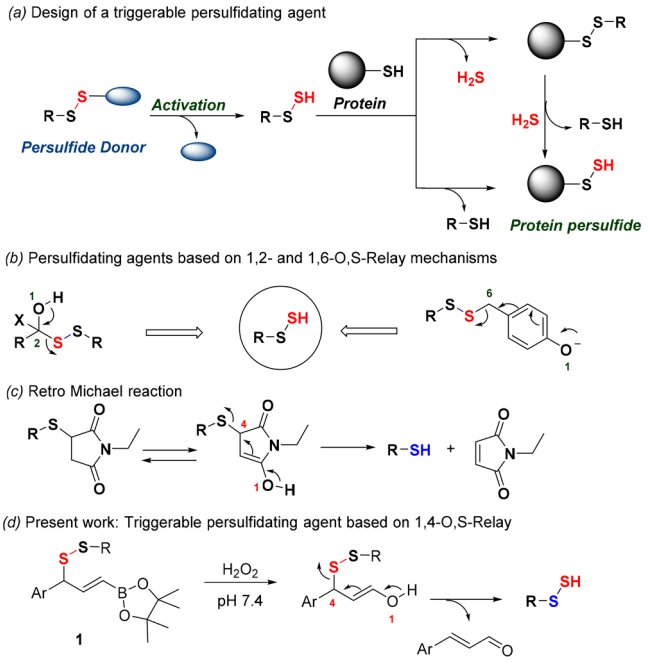


Figure 1. (a) Design of a triggerable protein *S*-sulfhydrating agent. (b) Key intermediates of some reported triggerable persulfide donors that operate via a 1,2- or a 1,6-O,S-relay mechanism. (c) An example of a retro Michael reaction: the first step is presumably the generation of an enol(ate), which generates a thiol by a 1,4-O,S-relay mechanism. (d) Design of a ROS-triggered persulfide donor that is expected to operate by a 1,4-O,S-relay mechanism.

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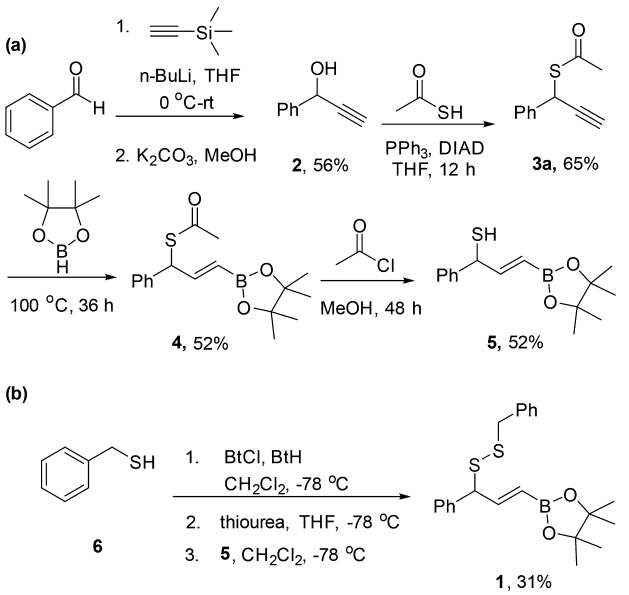
a nucleophile,[15,16] enzyme,[17−19] or fluoride.[20] These donors, while useful, have limited selectivity toward oxidative stress conditions. The next class of persulfidating agents involves a 1,6-O,S-relay mechanism, where the oxygen is masked in the form of a boronate ester, which is a substrate for oxidation by ROS such as hydrogen peroxide.[21] As a testament to its therapeutic utility, this donor was shown to protect cells from oxidative stress. The formation of a quinone-methide[22] during persulfide delivery is possibly a limitation of this method.

To address these major gaps, we considered an alternate design for a ROS-triggerable persulfide donor, which was based on a hitherto unexplored 1,4-O,S-relay mechanism.[12] Formally, this type of rearrangement is a retro Michael reaction involving a thiol as a leaving group (Figure 1c).[23,24] Here, the enol undergoes a 1,4-O,S-relay mechanism to generate a thiol and an α,β-unsaturated carbonyl compound. Since the pK_a of R-SSH (6.2) is somewhat lower than a thiol (7−9), it was envisaged that this group might depart under these conditions.[11] The carbonyl was masked as a vinyl boronate ester **1**, which is known to undergo oxidation in the presence of hydrogen peroxide to generate an aldehyde (Figure 1d).[25,26] The byproduct of decomposition of **1** is cinnamaldehyde, which is a constituent of cinnamon oil, and has been classified as Generally Recognized As Safe (GRAS). Together, **1** should respond to oxidative stress to generate a persulfide and relatively innocuous byproducts.

To synthesize **1** (Scheme 1a), benzaldehyde and TMS-acetylene were reacted in the presence of *n*-butyllithium to

Scheme 1. (a) Synthesis of Thiol 5; (b) Reaction of Benzylthiol (6) with 5 Affords the Desired Compound 1[a]



[a]BtCl = 1-chlorobenzotriazole and BtH = benzotriazole.

produce the secondary alcohol **2**.[27] Under Mitsunobu reaction conditions, **2** was converted to the thioacetate **3a** in 65% yield.[28] Hydroboration of **3a** gave **4** as the *trans* isomer (vicinal olefinic, J = 17.2 Hz). Deprotection of the thioacetate in the presence of acetyl chloride afforded the thiol **5** in 52% crude yield. Next, using a reported protocol, the reaction of benzyl thiol **6** with **5** afforded the desired product **1** in 31% yield.[29]

First, to ascertain the reactivity of **1** toward ROS, **1** was incubated with H_2O_2 (10 equiv) in pH 7.4 buffer at 37 °C. HPLC analysis of the reaction mixture revealed the complete disappearance of this compound in 90 min (Figure S1a in the Supporting Information). A time course of this decomposition was obtained, and curve fitting to a first-order equation gave a rate constant k_1 of 5.3 × 10^{-2} min^{-1} (Figure 2a). The

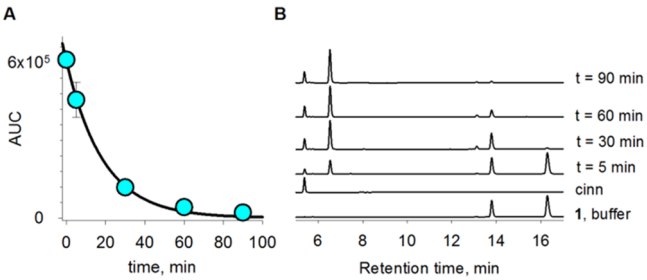


Figure 2. (a) Decomposition of **1**, as monitored by HPLC analysis. Curve fitting to first-order decomposition gave a rate constant (k_1) of 5.3 × 10^{-2} min^{-1}. (b) HPLC analysis of the reaction of **1** with hydrogen peroxide in the presence of monobromobimane (mBBr) at 37 °C in pH 7.4 buffer (containing 20% CH_3CN).

estimated half-life of **1** under these conditions is 13 min. This value is comparable with arylboronate ester decomposition (0.09 min^{-1}), suggesting no significant difference in the mechanism of hydrogen peroxide-mediated oxidation. To study the potential for **1** to generate a persulfide, we used a method developed by Binghe Wang and co-workers, where they used 1-fluoro-2,4-dinitrobenzene (**7**, FDNB) to trap the persulfide.[17] The resulting compound **8** (Figure 3) can be detected by HPLC analysis.

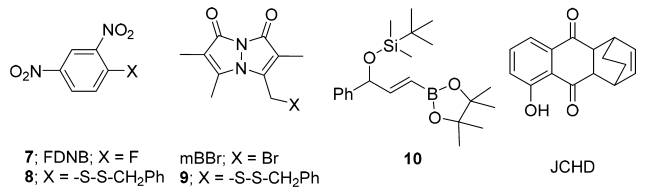


Figure 3. Structures of key tools and compounds used in this study.

Compound **8** was synthesized using a reported procedure and HPLC analysis showed a distinct peak at 11.3 min (Figure S3a in the Supporting Information). When **1** (retention time (RT) = 16.3 min) was coincubated with FDNB in the presence of H_2O_2, we found nearly complete decomposition in 90 min with a concomitant formation of **8** (Figure S3b in the Supporting Information). Because of the susceptibility of persulfides to decompose, this experiment was performed at room temperature. In the absence of hydrogen peroxide, we did not observe the formation of **8** (Figure S3d in the Supporting Information). These data support the generation of a persulfide when **1** was reacted with H_2O_2. The rate constant for the formation of **8** (k_3) was 0.15 min^{-1} and is a proxy to the persulfide formation rate (Scheme 2).

An independent assay based on monobromobimane (mBBr) was next used for detecting persulfides (Figure 2b). Reaction of mBBr with a persulfide is expected to produce the disulfide **9** (Figure 3). When **1** was reacted with hydrogen peroxide at 37 °C in the presence of mBBr, we find a distinct peak that is attributable to the formation of **9** (m/z = 369.07; observed,

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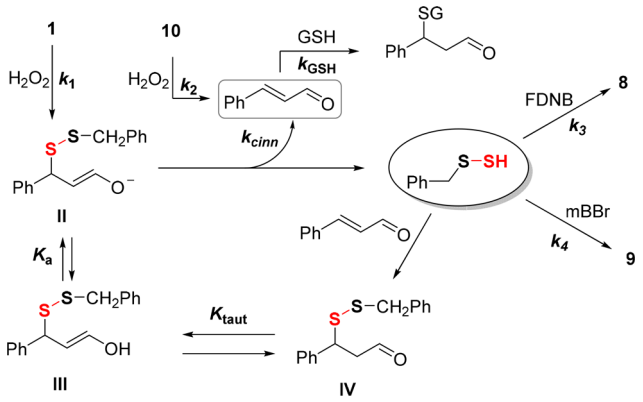
Scheme 2. Mechanism of Persulfide Formation during the Oxidation of 1[a]



[a]The rate constant for decomposition of **1** was $k_1 = 5.3 \times 10^{-2}$ min^{-1}, whereas that for the decomposition of **10** was $k_2 = 4.8 \times 10^{-2}$ min^{-1}. The rate constant for the formation of cinnamaldehyde during the decomposition of **1** in the presence of FDNB was $k_{cinn} = 12.8 \times 10^{-2}$ min^{-1}. The rate constants for the formation of **8** ($k_3 = 0.15$ min^{-1}) and **9** ($k_4 = 0.12$ min^{-1}) were comparable.

369.25; see Figure S4 in the Supporting Information). The time course for formation of this compound was monitored, and a rate constant of k_4 of 0.12 min^{-1} was obtained. This rate constant is comparable with k_1. Furthermore, the value of k_3 was determined at 25 °C, and considering rate effects on temperature, we suggest that the values of k_1, k_3, and k_4 are similar.

Next, the silylated derivative **10** was synthesized in two steps from the propargyl alcohol **2** (Scheme S1 in the Supporting Information). Compound **10** should undergo decomposition in the presence of hydrogen peroxide but does not generate a persulfide. When **10** was incubated in the presence of H_2O_2, indeed, we find evidence for the formation of cinnamaldehyde (Figure S2 in the Supporting Information).

Curve fitting yielded a rate constant (k_2) of 4.8×10^{-2} min^{-1} that was comparable in value with the rate of decomposition of **1** (Scheme 2). The yield of cinnamaldehyde under these conditions was 73%, which is comparable with the yield of **8** during incubation of **1** with hydrogen peroxide and FDNB (Figure S3b in the Supporting Information). Phillips and co-workers have previously demonstrated the use of vinyl boronate esters in the deprotection of alcohols with pK_a values of >11.[26] Since the estimated pK_a value of *tert*-butyldimethylsilanol is 15, this result supports the use of such vinylboronate esters for the release of poorly acidic alcohols as well.

When **1** was incubated in the presence of hydrogen peroxide, we similarly observed the formation of cinnamaldehyde, but with diminished yield (Figure S1a in the Supporting Information). This diminished yield could be due to the collateral consumption of cinnamaldehyde by the persulfide. To test this possibility, we incubated **1** in the presence of H_2O_2 and mBBr, and we found that the yield of cinnamaldehyde was significantly better (~70%; Figure 2b). The rate constant for the formation of cinnamaldehyde (k_{cinn}) was 12.8×10^{-2} min^{-1} (Scheme 2). Persulfides, being good one-electron reductants, have a high propensity to undergo oxidation to form tetrasulfides (RSSSSR) and polysulfides (RS_nR).[30] Although all assays were performed in the presence of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent, to prevent the decomposition of H_2O_2 and the subsequent radical-based oxidation of persulfides, the possibility of polysulfide formation cannot be ruled out. Furthermore, in cells, we would expect cinnamaldehyde to react with other thiols such as glutathione, which typically occurs in millimolar concentrations. When cinnamaldehyde was reacted with glutathione, the pseudo-first-order rate constant for this reaction was found as 2.3×10^{-2} min^{-1}, which translates to a half-life of ~30 min (Figure S5 in the Supporting Information). Previously, cinnamaldehyde was found to react with thiols as well as bovine serum albumin;[31] the half-life of the latter reaction was in the range of 3−8 min. These data suggested the possibility of cellular thiols competitively reacting with cinnamaldehyde and possibly sparing the persulfide to conduct protein persulfidation.

We next estimated the selectivity of **1** toward activation by H_2O_2. We tested **1** against a variety of oxidants in the presence of FDNB and tested if the persulfide adduct **8** was produced. We find no evidence for decomposition of **1** or the formation of **8** under these conditions (Figure S6a in the Supporting Information). In a separate assay with **10**, we found no evidence for the formation of cinnamaldehyde, except when **10** was treated with H_2O_2 (Figure S6c in the Supporting Information). Together, these data support the excellent selectivity of the vinyl boronate ester functional group toward oxidation by hydrogen peroxide.

The proposed mechanism for the reaction of **1** with hydrogen peroxide involves the oxidation of the vinyl boronate ester (likely the boronic acid in pH 7.4 buffer) to produce an enolate intermediate, which decomposes to produce the persulfide and cinnamaldehyde. Since the value of k_{cinn} was comparable in magnitude with that of k_3 and k_4, it is likely that the formation of the persulfide and cinnamaldehyde is concerted. Previously, retro Michael reactions involving thioethers of *N*-ethylmaleimide have been reported.[23,24] These reactions are extremely slow (half-lives ranging from ~1−7 days). Hence, the present method appears to be distinct from the previous reports due to the direct generation of an enolate **II** that rapidly rearranges to produce a persulfide. The equilibrium constant for tautomerism (K_{taut}), which is defined as [keto]/[enol] for aliphatic aldehydes, is in the range of 10^{-3}−10^{-4}.[32]

These data suggest that, once formed, **III** would likely equilibrate to the aldehyde **IV** (Scheme 2). If the aldehyde (keto form) is indeed produced, it is likely that the generation of the persulfide might be extremely slow. Our attempts to detect this aldehyde **IV** were unsuccessful. Since the yields of products are in excess of 70%, we reasoned that tautomerism may not be a major competitive process. Thus, it is likely that the enolate **II**, once formed, would rapidly rearrange to generate the persulfide. The estimated pK_a for the enol is 9−10, likely to be deprotonated in pH 7.4.[33] Previously, in basic solution, the enolate was found to be the dominant form for certain aldehydes.[33] Although the operating pH is 7.4, it appears to stabilize the enolate sufficiently to promote the 1,4-O,S-relay.

Lastly, since persulfides are reported to mitigate oxidative stress, we tested the ability of **1** to protect colon carcinoma DLD-1 cells from cytotoxicity induced by elevated ROS. Colon cells are constantly exposed to xenobiotics and stress induced by pathogens. It has been reported that hydrogen sulfide is an important mediator of stress response in the gut.[34−36] Using DLD-1 colon carcinoma cells, a cell viability assay was first

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conducted with increasing doses of **1**, and no significant inhibition up to 100 μM was observed (Figure S7a in the Supporting Information). Next, using menadione, which is a known redox cycling agent and inducer of oxidative stress, we evaluated the protective effects of **1**.[37,38] DLD-1 cells were treated with menadione and viable cells were measured (Figure S7b in the Supporting Information). We found significant cell killing at 50 μM (30% viable cells); this concentration was chosen to study possible cytoprotective effects of **1**. When **1** was cotreated with menadione, a dose-dependent cytoprotection was observed (Figure 4a). A cell viability assay conducted

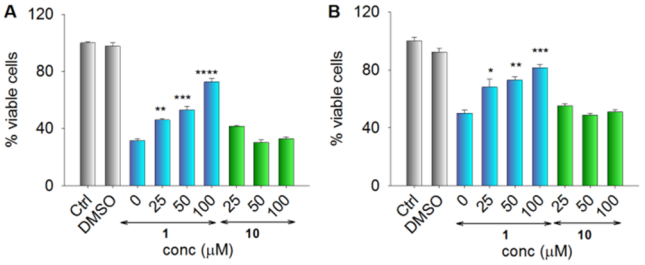

Figure 4. (a) Cytoprotective effects of compound **1** against menadione (50 μM). Results are expressed as mean ± SEM (n = 3). [Legend: (**) $p < 0.01$, (***) $p < 0.001$, (****) $p < 0.0001$ vs menadione.] A similar assay was conducted with **10**. No significant effect on the percentage of viable cells during incubation of **10** with menadione was observed. (b) Cytoprotective effects of compound **1** against JCHD (50 μM). Results are expressed as mean ± SEM (n = 3). [Legend: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, vs JCHD.] A similar assay was conducted with **10**, and no significant effect was observed.

with **10** revealed that this compound was not significantly cytotoxic (Figure S7a). However, **10** was unable to protect cells from menadione-induced cytotoxicity, supporting the importance of the persulfide in cytoprotective effects (Figure 4a). Next, JCHD, which is a derivative of juglone, was used to simulate increased ROS within cells. This compound has been previously characterized to generate ROS in pH 7.4 buffer under ambient aerobic conditions and increase ROS levels in cells (Figure S8 in the Supporting Information). Again, we find significant cytotoxicity induced at 50 μM by JCHD (Figure S7b).[39−41] The persulfide donor **1** protected cells from JCHD-induced toxicity, while **10** showed no effect (Figure 4b). Thus, the results of these cell studies demonstrate the potential for this new donor to protect cells from xenobiotics and oxidative stress.

In summary, we report a new class of 1,4-O,S-relay mechanism-based persulfide donors with a unique retro Michael reaction as the key step. Generation of persulfide from this donor was independently validated by two assays, and the mechanism is consistent with experimental data. The major byproducts that are produced appear well tolerated by cells and this observation is encouraging for further development of this donor. We found that **1** was able to protect cells from oxidative stress induced by exogenous ROS generators. Taken together, this compound is a valuable addition to the growing redox toolbox to understand the chemical biology of reactive sulfur species better while progressing toward new classes of sulfur-based therapeutic agents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03471.

Synthesis and characterization data, analytical data, and protocols (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: harinath@iiserpune.ac.in.

ORCID

Harinath Chakrapani: 0000-0002-7267-0906

Notes

The authors declare no competing financial interest.

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