

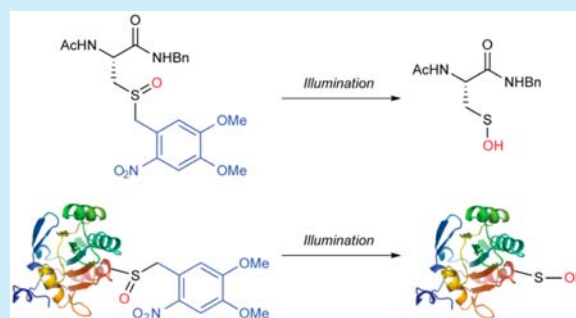
Light-Mediated Sulfenic Acid Generation from Photocaged Cysteine Sulfoxide

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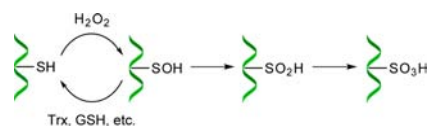
Supporting Information

ABSTRACT: S-Sulfenylation is a post-translational modification with a crucial role in regulating protein function. However, its analysis has remained challenging due to the lack of facile sulfenic acid models. We report the first photocaged cysteine sulfenic acid with efficient photodeprotection and demonstrate its utility by generating sulfenic acid in a thiol peroxidase after illumination in vitro. These caged sulfoxides should be promising for site-specific incorporation of Cys sulfenic acid in living cells via genetic code expansion.



The amino acid cysteine plays an essential role in redox signal transduction owing to its susceptibility to oxidation by various reactive oxygen species (ROS).¹ Among possible oxidative post-translational modifications of cysteine (Cys), S-sulfenylation has become the subject of growing attention in recent years.² During redox signaling or under conditions of oxidative stress, a reactive Cys thiol (-SH) can be oxidized to sulfenic acid (-SOH) by ROS, namely hydrogen peroxide (H₂O₂), and this process can be reversed by biological reductants, such as the enzyme, thioredoxin³ (Trx), or the tripeptide glutathione⁴ (GSH) (Scheme 1). To date, regulatory

Scheme 1. Biologically Relevant Cysteine Oxoforms



Cys sulfenic acid modifications have been identified in many signaling proteins such as tyrosine phosphatases,⁵ kinases,⁶ transcription factors,⁷ proteases,^a deubiquitinases,⁹ and ion channels.¹⁰ Aberrant protein sulfenylation has also been correlated with human pathologies, including cancer¹¹ and cardiovascular disease.¹⁰

While protein sulfenylation is an important post-translational modification, the analysis of this Cys oxoform has remained challenging due to the lack of facile sulfenic acid model systems. The challenge is significant given that sulfenic acids are often unstable, transient species that can rapidly react to form thiosulfinate and disulfide species.^{2b} Some progress has been made in defining sulfenic acid stabilization and properties in small-molecule models such as 1¹² and 2¹³ (Figure 1); however, such compounds still suffer from complex syntheses, poor aqueous solubility, do not adequately recapitulate the character-

Figure 1. Examples of small-molecule sulfenic acids stabilized via an intramolecular hydrogen bond (1) or steric effects (2).

istics or reactivity of Cys sulfenic acid, and cannot be stored for an extended period of time. In proteins, the stability of Cys sulfenic acid is determined by the surrounding microenvironment, and the absence of vicinal thiols¹⁴ and presence of basic residues¹⁵ are often cited as key features. Protein sulfenic acid formation in vitro and in cells is most often achieved by incubation with exogenous oxidants like H₂O₂, organo hydroperoxides,^{2b} or elevating endogenous ROS production via treatment with growth factor or insulin.^{1b} Nevertheless, uncontrolled oxidation of reactive Cys residue(s) stemming from such methods often makes it difficult to study sulfenylation of specific proteins at defined sites within redox signaling pathways.

Caged compounds are precursors of biologically active molecules that have been rendered inactive by installation of a photolabile protecting group (PPG) onto the essential functionality.¹⁶ After illumination, the PG is cleaved and the caged biomolecule is released irreversibly, thus revealing the active species. Photocaged Cys has been site-specifically incorporated to study thiol function and targeted covalent labeling in small molecules,¹⁷ peptides,¹⁸ and proteins.¹⁹ Photocaged selenocysteine has also been reported.²⁰ Despite

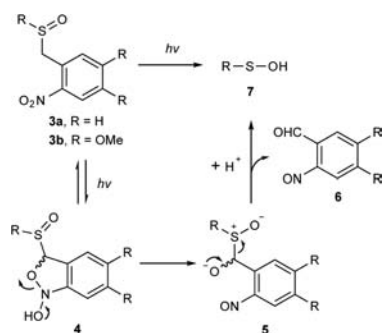
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these encouraging advances and the benefits of photocontrol, methods to incorporate defined Cys “oxoforms”, such as sulfenic acid, have not yet been described. Herein, we report the first photocaged Cys sulfenic acid analogues and establish conditions for efficient photodeprotection. We demonstrate the utility of this approach by generating Cys sulfenic acid in a thiol peroxidase, following illumination *in vitro*. Overall, these photocaged cysteine sulfenic acid analogues should have considerable utility for the site-specific incorporation of Cys sulfenic acid within small molecules, proteins, and eventually, living cells via genetic code expansion.

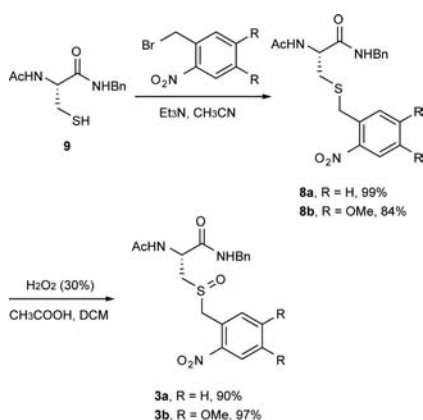
In the current investigation, we have explored a novel strategy based on a photolabile (*o*-nitrobenzyl, ONB or 4,5-dimethoxy-2-nitrobenzyl, DMNB) group to protect Cys sulfoxide (CysO) analogs (Scheme 2). In this approach, we reasoned that PG

Scheme 2. Proposed Mechanism for Formation of Sulfenic Acid from Caged Sulfoxide Species via Illumination



cleavage would proceed via benzoisoxazole 4, which would rearrange to give α -hydroxyl sulfoxide 5, and conclude with elimination of 6 to give sulfenic acid 7. We first focused our attention on the chemical synthesis of *o*-nitrobenzyl (ONB) and 4,5-dimethoxy-2-nitrobenzyl (DMNB) CysO and Cys analogues, such that photocleavage and reactivity of CysO and its reduced counterpart could be effectively compared (Scheme 3). In short,

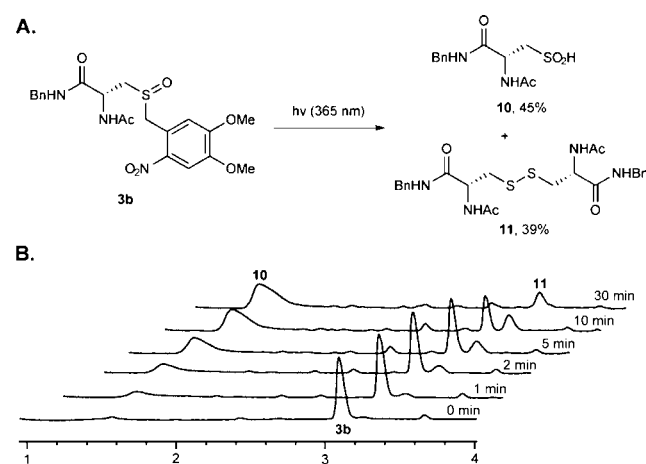
Scheme 3. Synthesis of Caged Sulfoxides 3a and 3b



photocaged Cys compounds (**8a**, **8b**) were generated from *N*-acetylcysteine benzylamide **9** following literature procedures.¹⁷ Subsequently, oxidation of photocaged Cys analogues to the corresponding sulfoxides (**3a**, **3b**) was achieved using H₂O₂ in the presence of acetic acid in excellent yields ($\geq 90\%$). In the absence of illumination, all compounds were stable in aqueous media at neutral pH values, as expected (Figure S1).

Next, we tested whether we could observe the formation of sulfenic acid from model caged sulfoxide **3b**. Illumination of **3b** ($\lambda_{\text{max}} = 350 \text{ nm}$, $\epsilon_{350 \text{ nm}} = 18590 \text{ M}^{-1} \text{ cm}^{-1}$, $\epsilon_{365 \text{ nm}} = 16500 \text{ M}^{-1} \text{ cm}^{-1}$, $\Phi_{\text{chem}} = 0.13$) with UV light at 365 nm (0.35 W/cm^2) led to complete consumption of **3b** and formation of sulfenic acid **10** (45% yield) and disulfide **11** (39% yield) as the major products (Scheme 4A). Formation of these products can be explained due

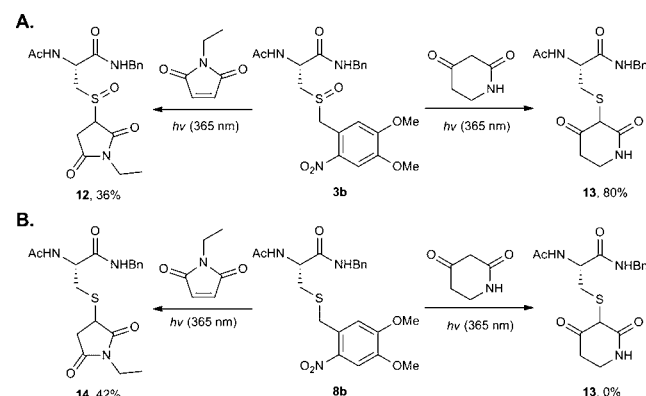
Scheme 4. (A) Uncaging Reaction of Caged Sulfoxide 3b under UV. (B) LC Traces of the Uncaging Reaction of 3b in 4 h



to the disproportionation reaction of sulfenic acid to sulfinic acid **10** and a thiol, which then reacts with sulfenic acid to give disulfide **11**.²¹ By measuring the consumption of **3b**, the pseudo-first-order rate constant (k_{obs}) for photocleavage was determined to be 0.17 min^{-1} (Scheme 4B and Figures S2 and S3).

To verify formation of the expected sulfenic acid product, we exploited the dual electrophilic and nucleophilic reactivity of this sulfur oxoform.^{1f,2b} When *N*-ethylmaleimide (NEM) was added to the reaction, the anticipated sulfoxide–NEM adduct **12** was formed (36% yield, Scheme 5A).²² Correspondingly, when

Scheme 5. Uncaging and Chemical Trapping of (A) Caged Sulfoxide 3b and (B) Reduced analogue 8b

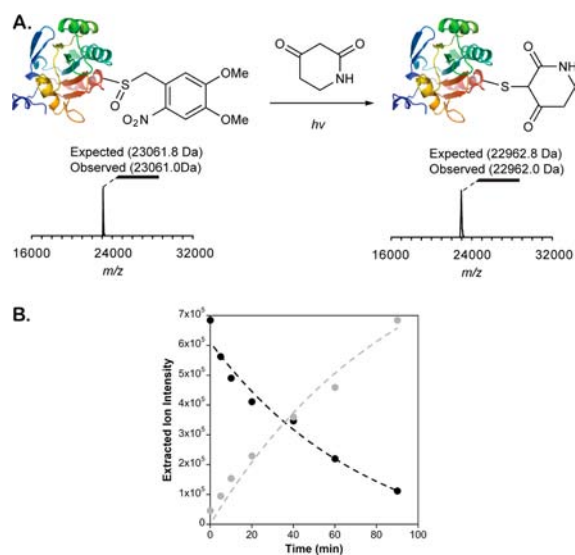


carbon-based nucleophiles like piperidine-2,4-dione (PD)²³ were included to trap the nascent sulfenic acid, the expected sulfide adduct **13** was observed (80% yield, Scheme 5A). Control experiments, performed under the same reaction conditions, clearly demonstrate that caged Cys analogue **8b** formed the appropriate sulfide adduct **14** with NEM (42% yield) and did not react with the PD nucleophile (Scheme 5B).²⁴ These data

establish formation of sulfenic acid from the caged sulfoxide starting material, as anticipated by our strategy.

To determine whether our approach could be applied to proteins, we next examined photolytic generation of Cys sulfenic acid from a DMNB-protected sulfoxide precursor in the thiol peroxidase, Gpx3 (Cys64Ser Cys82Ser Gpx3 was used to avoid multiple alkylation events). Caged Gpx3 Cys32 sulfoxide, termed Cys32O, was prepared by alkylation of the catalytic Cys32 of Gpx3 with dimethoxy-*o*-nitrobenzyl bromide (DMNB-Br), followed by oxidation with H₂O₂. During the oxidation step, we observed that methionines (Met) in Gpx3 were converted to Met sulfoxide (MetO) (expanded intact ESI-MS spectra presented in Figure S4).²⁵ As shown in Scheme 6A, illumination

Scheme 6. Photochemical Generation of Sulfenic Acid in a Protein. (A) ESI-MS Spectrum of Intact Gpx3. (B) Extracted Ion Intensities of Gpx3 Cys32O Consumption (Black Dots) and Gpx3-PD Formation (Gray Dots)



of Gpx3 Cys32O–DMNB at 365 nm followed by intact ESI-MS demonstrates the full conversion of the DMNB-caged sulfoxide to Gpx3 Cys32 sulfenic acid with nearly quantitative trapping of this intermediate by the PD nucleophile. Control photo-uncaging experiments, in which Gpx3 Cys32 was alkylated with DMNB-Br but not oxidized with H₂O₂, did not lead to the formation of PD-tagged Gpx3, as expected (Figure S3). A time-course study was also carried out to show controllable release of Gpx3 sulfenic acid via consumption of caged Gpx3 Cys32O ($k_{\text{obs}} = 0.013 \text{ min}^{-1}$) and robust formation of the anticipated Gpx3-PD adduct ($k_{\text{obs}} = 0.011 \text{ min}^{-1}$, Scheme 6B). Although removal of additional Cys residues and Met oxidation may limit the utility of our approach in this context, these experiments serve as an important proof-of-principle that CysO-DMNB can be efficiently uncaged in a protein model to afford Cys sulfenic acid.

Many applications of this approach can be envisioned, and we are particularly interested in the ability to genetically encode the site-specific incorporation^{26,27} of a photocaged Cys sulfenic acid residue into a target protein within cells. Evolution of the requisite orthogonal synthetase/tRNA pair to incorporate the aforementioned unnatural amino acid (UAA) will be reported in due course. At this stage, however, given the structural similarity with MetO, we deemed it essential to test whether caged CysO 15 (analogue of 3a with free amino and carboxylate groups,

Figure 2A) would be a substrate for the MetO repair enzyme (MsrA).²⁸ As shown in Figure 2B, MetO was efficiently reduced

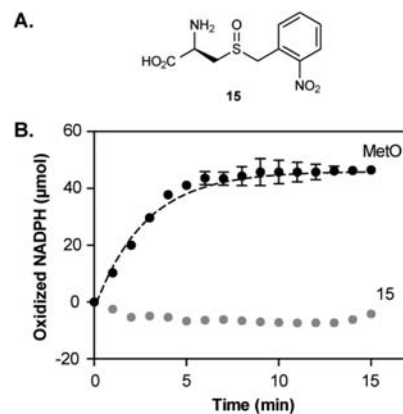


Figure 2. (A) Unprotected analogue of 3a. (B) MsrA activity assay with sulfoxide 15 (gray dots) and MetO (black dots) as putative substrates.

by MsrA, while 15 remained intact. These data demonstrate that photocaged CysO is not a substrate for MsrA, which bodes well for the intracellular stability and application of caged Cys sulfoxide toward genetic encoding of Cys sulfenic acid in future work.

In summary, we have designed, synthesized, and evaluated first-in-class photocaged cysteine sulfenic acid analogues. From practical and conceptual standpoints, this work represents an important advance for the thiol-based redox regulation and chemical biology communities due to the potential for site-specific incorporation of an oxidative cysteine post-translational modification. From a chemistry perspective, caged sulfenic acids should find broad application owing to their facile preparation and storage within a large range of scaffolds, including peptides and proteins. Evolution of the requisite orthogonal synthetase/tRNA pair for site-specific incorporation of Cys sulfenic acid within living cells via genetic code expansion is currently underway and will be reported in due course.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02981.

Experimental procedures and spectroscopic data (PDF)

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Notes

The authors declare no competing financial interest.

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