Acyl Selenyl Sulfides as the Precursors for Reactive Sulfur Species (Hydrogen Sulfide, Polysulfide, and Selenyl Sulfide)

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Abstract

Persulfides are receiving increased attention due to their links to hydrogen sulfide (H_2S) and hydrogen polysulfide (H_2S_n). Their close analogues selenyl sulfides (RSeSHs), however, have limited literature precedent, and their reactivity and possible role in biology are largely unknown. Here, we devised an acyl selenyl sulfide template to study RSeSH chemistry. Their stability and reactivity toward amines/thiols were studied. These compounds can produce H_2S or H_2S_2 under different conditions, suggesting that RSeSHs are possible intermediates.

Graphical Abstract
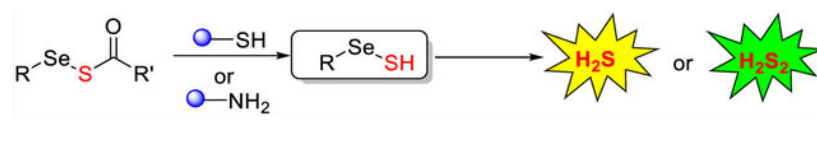
Reactive sulfur species (RSS) are a series of sulfur- containing molecules that play regulatory roles in biological systems. Recent studies have revealed that hydrogen sulfide (H_2S), persulfides (RSSHs), and hydrogen polysulfides (H_2S_n, n ≥ 2) are perhaps the most interesting RSS, and they exhibit functions such as inducing S-perthiolation in many proteins.[1,2] These molecules, in particular RSSH and H_2S_n, are highly reactive and unstable, which makes their research quite challenging. As such, much research effort has been put into developing chemical tools for delivering and detecting such RSS.[3] Although significant progress in understanding RSSH has been made, their close analogues, selenyl sulfide (RSeSHs), are still largely unexplored. Selenocysteine (Sec) is the 21st proteinogenic amino acid, and many selenoproteins are redox enzymes. Cys and Sec have many similar properties but only minor differences in terms of electronegativity, ionic radius, and available oxidation

states.[4] It is worth noting that the pK_a of the selenol group (~5.3) in Sec is much lower than the thiol in Cys (~8.3) and Sec has a lower redox potential than Cys (−381 vs −180 mV). These facts indicate that Sec is mostly deprotonated at physiological pH and very sensitive to redox regulation. The involvement of Sec in sulfur-related redox signaling is an interesting research topic. It is expected that Sec can readily react with certain reactive sulfur species to form selenyl sulfide (RSeSH), analogous to RSSH formation, though this has not been validated. A number of other questions about RSeSH remain unanswered, such as what their intracellular targets are and to what extent such reactions can impact signaling. A better understanding of RSeSH chemistry is needed to address these questions. It should be noted that the chemistry and properties of RSeSH are largely unknown in literature, except for a few theoretical studies.[5] In our previous works, we used acyl disulfides (1) and 9-fluorenylmethyl (Fm) disulfides (2) as the precursors for RSSH (Scheme 1), which could further produce H_2S in the presence of thiols like cysteine.[6] We also found that cyclic acyl selenyl sulfide (3) could serve as a precursor for RSeSH, which could also produce H_2S in the presence of cysteine.[7] Analogously, we envisioned that acyclic acyl selenyl sulfides (4) could serve as the precursors of RSeSH, and this would be a general method for the access of RSeSH and be used to explore their chemistry. Herein, we report the preparation of acyclic acyl selenyl sulfides and their reactions with nucleophiles (amine/thiol). Their production of H_2S and H_2S_2 under different treatments are also reported.

In the 1980s, acyclic acyl selenyl sulfides were studied as reagents to introduce seleno functionality into organic molecules.[8] These compounds were synthesized by reacting selenyl thioates with *m*-CPBA or reacting selenyl phthalimides with thioacids.[8] However, these methods rely on multistep operations and often yield many byproducts that make isolation and purification difficult. Here, we developed a simple method for synthesizing acyl selenyl sulfides under mild, one-pot conditions (Scheme 2). Briefly, selenyl chlorides 6 were generated upon the reaction between diselenide and SO_2Cl_2 and subsequently treated with thioacids to produce the desired acyl selenyl sulfides 7 in moderate to good yields. Overall, five substrates (7a–7e) were prepared and studied.

With these compounds in hand, we first tested their stability. Acyl selenyl sulfides were found to be reasonably stable in organic solvents like CH_2Cl_2 (dry, degassed, in dark). When their solutions were exposed to air and light, decomposition was noticed with the formation of diphenyl diselenide 5 and acyl disulfide 8 (Scheme 3a). This was likely due to a radical process.[9] When these compounds were exposed to aqueous buffers, slow but immediate decomposition was observed. We also noticed that the process was pH dependent as they decomposed faster under basic conditions than under acidic conditions (Figure S1). Diphenyl diselenide and elemental sulfur S_8 were identified to be the products. As such, a hydrolysis mechanism was proposed (Scheme 3b), with the generation of selenyl sulfide 9 as the intermediate and eventually producing 5 and H_2S_2 (which further degraded to S_8). It should be noted that in these decomposition processes we did not observe the formation of H_2S.

Given the structural similarity of acyl selenyl sulfides and acyl disulfides, we hypothesized that compounds 7 could serve as H_2S-releasing agents in the presence of cysteine. Therefore, we tested their H_2S-releasing ability in PBS buffers under the treatment of

cysteine. The produced H_2S was measured using a H_2S gas trapping experiment.[10] The results are shown in Figure 1. We expected that the change of acyl substitutions could affect the rates of thioester exchange with cysteine and regulate H_2S generation. Indeed, 7a showed the highest H_2S production (79%), while more hindered substrates 7b and 7c showed much decreased H_2S production (~40%). The alkyl selenide based substrate 7e also showed good H_2S production. These results demonstrated that H_2S release from these compounds could be regulated via structural modifications and the relative amounts of H_2S released could give access to researching the effects of different H_2S concentrations as they pertain to Se–S redox regulations.

To understand the mechanism of H_2S release from these compounds, we analyzed the reaction products of 7a and cysteine (5 equiv). Cystine, N-acetylcysteine, and diselenide 5 were found to be the products (Scheme 4a). In addition, the reaction was completed within 15 min. This indicated that the slow decomposition of 7a will not compete with its ability to release H_2S in the presence of cysteine. Although we were able to identify the products in this reaction, the nucleophilic residues of cysteine (–SH and –NH_2) could theoretically react with two possible electrophilic sites of 7a and both could lead to the formation of these products (vide infra). To better probe the mechanism, we decided to use simplified models to understand the reaction.

Benzylmercaptan (BnSH) and benzylamine ($BnNH_2$) were used to test the difference in reactivity between the two electrophilic sites on 7a. As shown in Scheme 4b, BnSH preferentially reacted with the selenium on 7a (to produce 10 as the major product), while $BnNH_2$ reacted only with the carbonyl to give 12 in almost quantitative yield. This type of reactivity is not surprising because thiols preferentially reacting with selenides over sulfides are known.[11] We also carried out a competition reaction with equal amounts of BnSH and $BnNH_2$ (Scheme 4c)and observed the formation of amide 12, diphenyl diselenide 5, benzyl disulfide 13, and selenyl sulfide 10. Based on these results, we proposed the mechanism of the reaction between 7a and cysteine as follows (Scheme 5): the reaction is initiated by a nucleophilic attack on the carbonyl by –NH_2 of cysteine resulting in the selenyl sulfide intermediate (PhSeSH). We have previously reported the presence of such an intermediate in cyclic acyl selenyl sulfides through similar reaction conditions.[7] From this intermediate two pathways can contribute to H_2S release: (a) a nucleophilic attack on the selenide by the SH of cysteine to liberate H_2S and selenyl sulfide 14, which further reacts with cysteine to produce cystine, and (b) a sulfide transfer with cysteine to form cysteine persulfide 15 and PhSeH. Persulfide 15 further reacts with cysteine to form cystine and release H_2S. PhSeH generated in both pathways can be oxidized to form PhSeSePh.

Apart from H_2S, hydrogen persulfide (H_2S_2) is believed to be another important signaling molecule, and its regulatory roles have been recently recognized.[12] The unique structure of the selenyl sulfide (RSeSH) intermediate made us suspect compounds 7 could also serve as H_2S_2 donors. We hypothesized that the RSeSH intermediate generated by the reaction of 7 and amines could degrade into diphenyl diselenide and H_2S_2. To test this hypothesis, a H_2S_2-specific fluorescent probe DSP-3[13] was used to monitor the reaction between 7a and butylamine. As shown in Figure 2, time- and concentration-dependent fluorescence increases were ob- served. We also used nitrofluorobenzoylate to trap in situ generated H_2S_2

(Scheme 6), and the desired product 16 was obtained in 22% yield. These results demonstrated the formation of H_2S_2 from the degradation of the RSeSH intermediate. Again, we did not observe H_2S formation in the reaction of 7a with butylamine.

Since donors like 7a have demonstrated their ability to release both H_2S and H_2S_2 we wondered if they could release these sulfur species when interacting with cells. Cell-imaging experiments were then conducted to address this question. As shown in Figure 3, H9c2 cells were first loaded with a selective fluorescent probe for H_2S (WSP5[14]) or H_2S_n (DSP-3) and then treated with 7a. Donor-treated cells showed much enhanced fluorescent signals with the H_2S probe. However, we did not observe much fluorescence enhancement with the H_2S_2 probe (data not shown). These results suggest that H_2S_2 production from the donors was favored, presumably due to the presence of cellular thiols like cysteine. While the donors could also produce H_2S_2 in theory, such a pathway might be minor, and the instability of H_2S_2 might also contribute to its lack of detection.

In summary, this work demonstrated that acyl selenyl sulfides could serve as the precursors for H_2S and H_2S_2, depending on the reaction conditions. Hydrolysis of acyl selenyl sulfides and their reactions with amine-based nucleophiles mainly lead to the formation of H_2S_2, while the reaction with thiols (such as cysteine) lead to H_2S generation. Selenyl sulfides are likely the key intermediates. RSeSHs are expected to be important regulating molecules involved in Sec- related redox signaling. Acyl selenyl sulfides should be useful tools for better understanding the chemical biology of RSeSH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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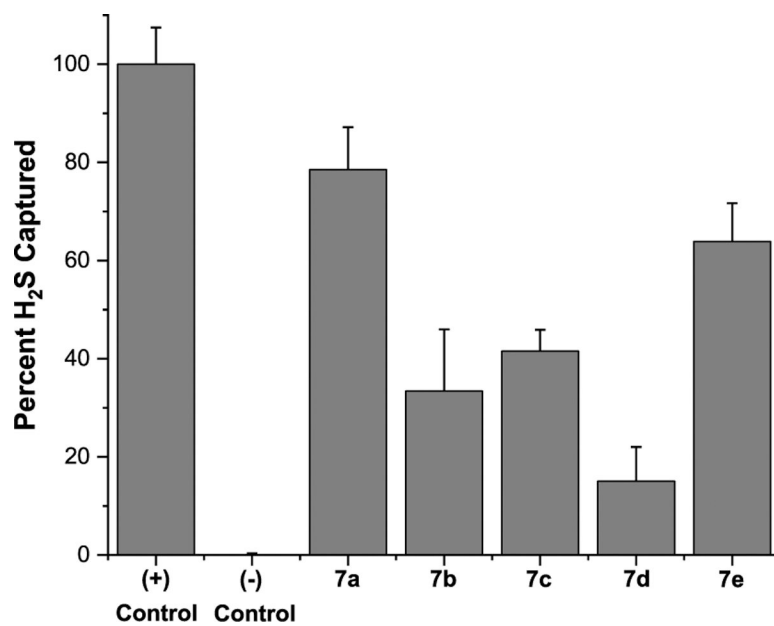
Figure 1.
Normalized H_2S production from 7a–e measured by H_2S gas trapping assay. (+) Control: Na_2S (0.5 mM). (−) Control: donor only (0.5 mM). 7a–7e: donor (0.5 mM) with Cys (2.5 mM).
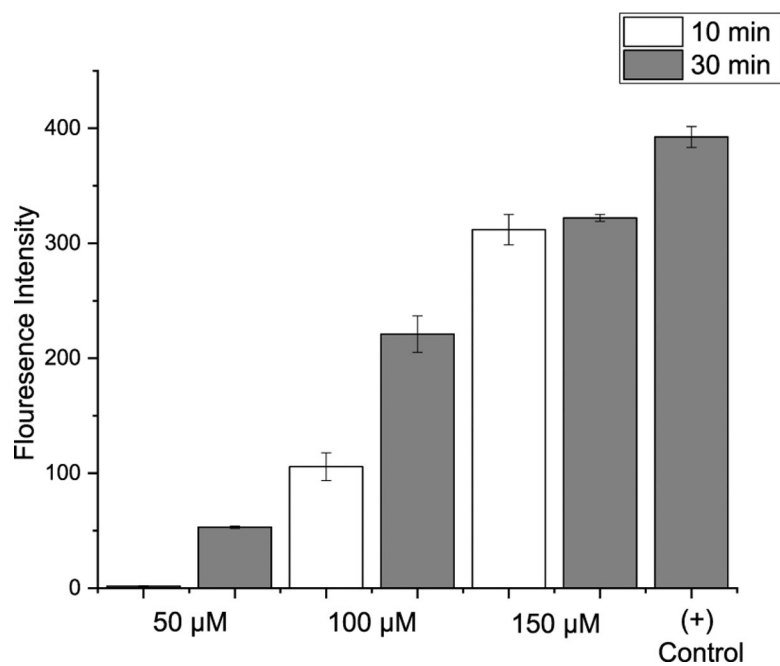
Figure 2.
Relative fluorescence intensities of DSP-3 toward the reaction between 7a and $BuNH_2$.
Testing solution contained DSP-3 (10 μM), 7a (50, 100, and 150 μM). (+)-Control: DSP-3
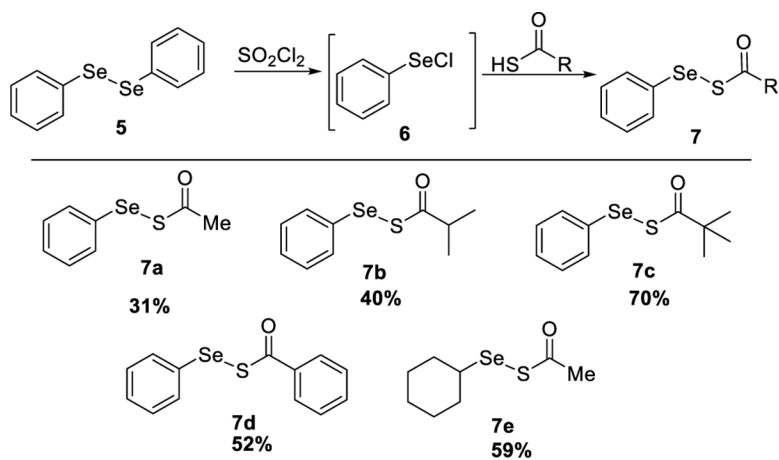(10 μM) and Na_2S_2 (50 μM).
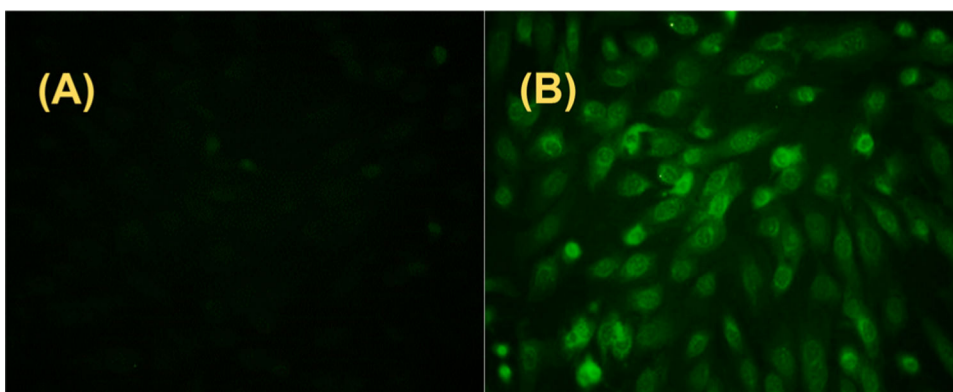
Figure 3.
Cell images of H_2S production from 7a in H9c2 cells. Cells were incubated with WSP5 (10 μM) for 30 min and then treated with vehicle (A) and 50 μM 7a (B) for 60 min.
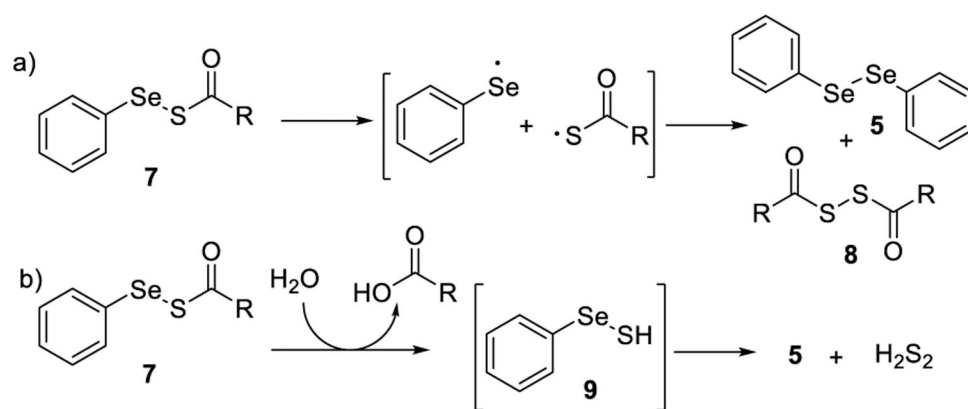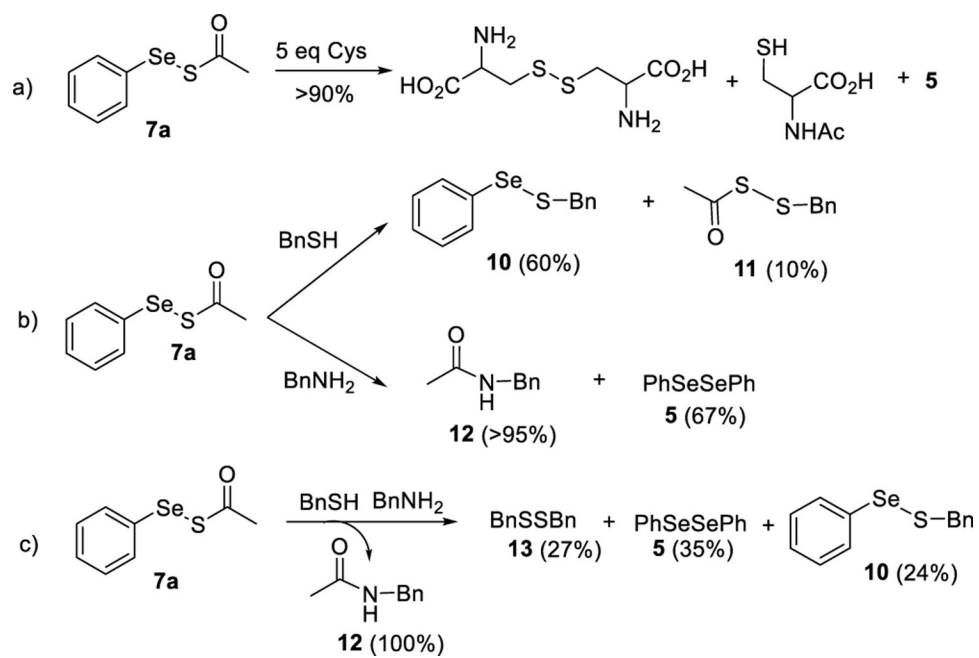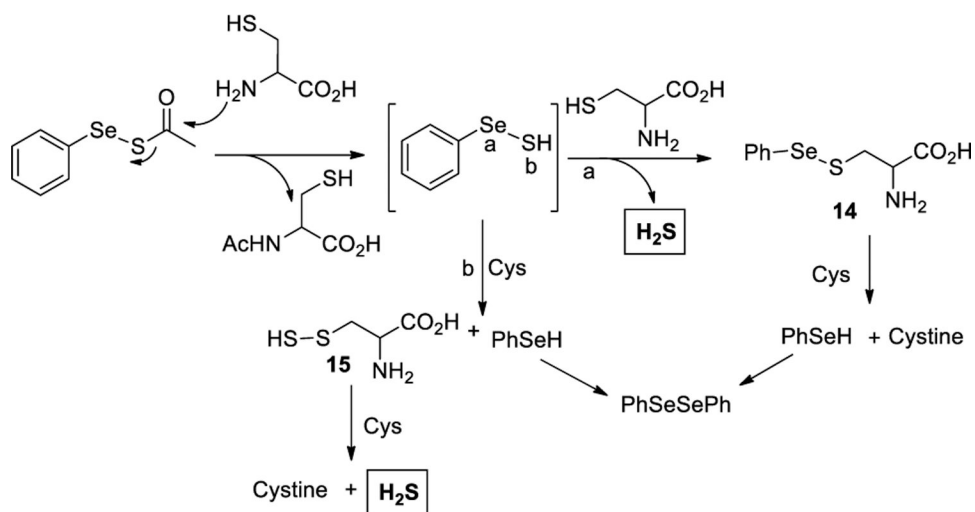
Previous Work

This Work

Nu: amines or thiols
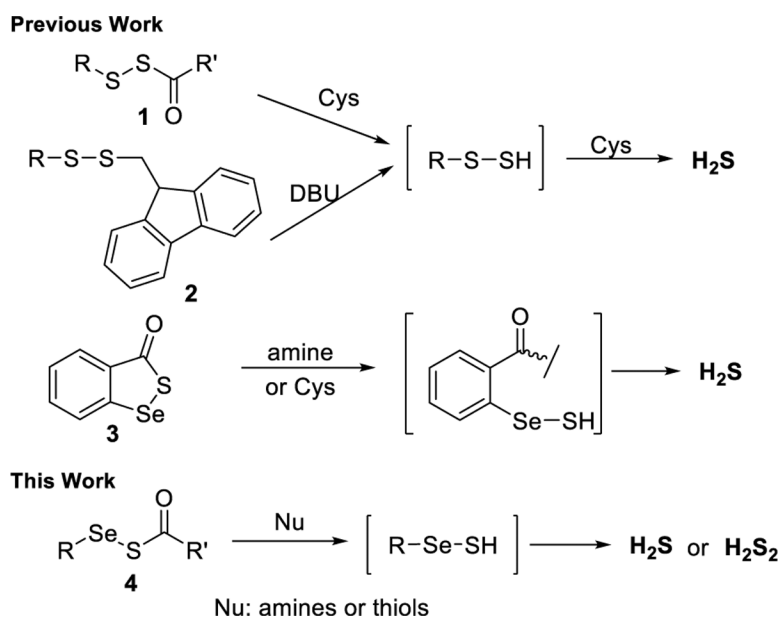
Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.