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Hydrogen Sulfide Mediated Tandem Reaction of Selenenyl Sulfides and Its Application in Fluorescent Probe Development

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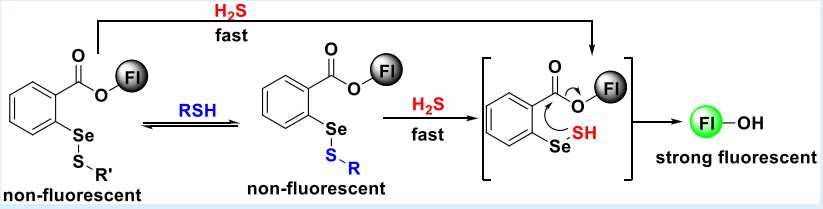
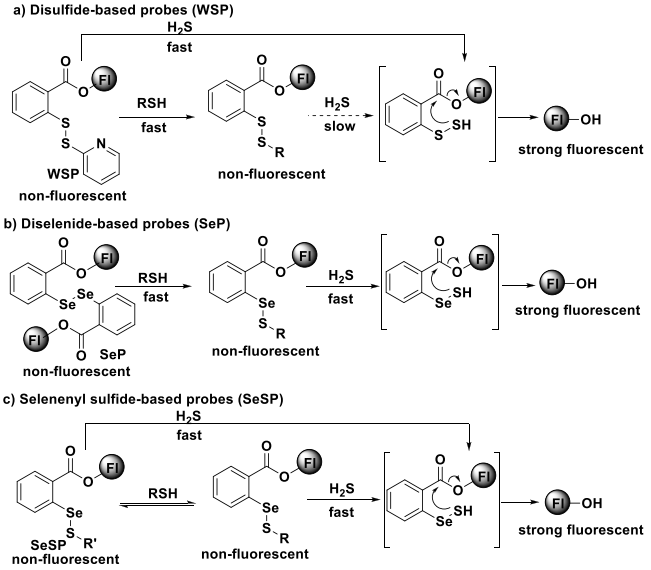
Supporting Information



ABSTRACT: A unique reaction between H_2S and a selenenyl sulfide containing benzoate ester template was discovered. This reaction could be specifically triggered by H_2S and lead to ester bond cleavage. The reaction was not affected by the presence of thiols such as glutathione and cysteine. With this reaction, a series of fluorescent probes were synthesized and evaluated. The probes exhibited high sensitivity/selectivity for H_2S in both buffers and cells.

Hydrogen sulfide (H_2S) is a critical gasotransmitter in biological systems.[1,2] Current studies have proven that H_2S exists in different tissues and regulates cellular redox status and various physiological functions involved in human disease and health.[3−7] However, the exact mechanisms of its action remain elusive mainly due to the lack of reliable and noninvasive methods for H_2S detection in biological samples. Traditional detection methods, such as colorimetric measurements, electrochemical assays, and gas chromatography methods, often require complicated post-mortem processing, which could lead to the destruction of samples.[8−11] Thus, they cannot be applied in real-time and noninvasive detection. Alternatively, fluorescent probes have obvious advantages due to their great temporal and spatial sampling capability. This topic has been well studied in recent years, and a large number of such probes have been invented.[12−15] In general, these probes are reaction-based sensors, meaning their optical changes are based on specific H_2S reactions. As such, exploring novel and more specific reactions of H_2S is important for the development of the next generation of H_2S probes. Our group reported in 2011 the first nucleophilic reaction-based design strategy for H_2S probes (**WSP**).[16,17] These probes utilize a unique tandem reaction: H_2S reacts with pyridyl disulfide to form a persulfide intermediate, which then undergoes an intramolecular cyclization to release the fluorophore. This is specific for H_2S, not for other biothiols (Scheme 1a). While the selectivity of **WSP** for H_2S is excellent, the probes can be consumed by thiols (RSH), which could lead to decreased sensitivity when high concentrations of RSH are presented. To address this problem, in 2015, we developed diselenide-based probes **SeP**s (Scheme 1b).[18] While a **SeP** can react with RSH, the resulting Se−S intermediate still retains high reactivity toward H_2S, which eventually leads to H_2S-mediated cyclization and fluorophore release. This design overcomes the problem of **WSP**. However, it is not flexible enough to tune their reactivity toward H_2S as two identical fluorophore moieties are needed in **SeP**. Moreover, this diselenide template often shows poor solubility in aqueous systems. To further optimize these nucleophilic reaction-based probes, we envisioned a design using the selenenyl sulfide (Se−S) template,[19] inspired by the intermediate structure shown in

Scheme 1. Design Concept of SeSP Probes



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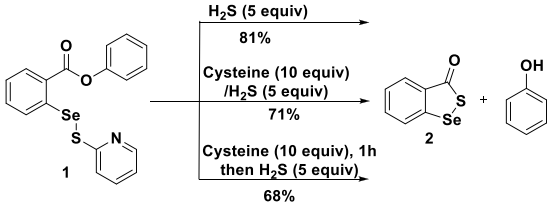
Scheme 1b. We reasoned that this template (shown as **SeSP** in Scheme 1c) should have reactivity similar to that of **SeP** toward H_2S, but it would allow easier modification and tuning of the structures of the probes. Herein, we report the study of the H_2S-mediated tandem reaction with the selenenyl sulfide template. We also prepared and evaluated a series of fluorescent probes based on this reaction.

As shown in Scheme 1c, the probe template is based on the intermediate of **SeP** probes' reaction with RSH. Some studies have revealed that nucleophilic attack by RSH at the Se atom is thermodynamically favorable and kinetically faster.[20] Moreover, in this template there should be a strong Se---O interaction with the C═O carbonyl group which would facilitate the attack of −SH at the Se atom rather than at the S atom.[21] Therefore, the selenenyl sulfide linkage of the probe is expected to be highly reactive to both H_2S and cellular thiols (Cys and GSH). With H_2S, the −Se−S− linkage should be converted to −Se−SH rapidly. The following cyclization should produce benzothiaselenol-3-one and release the fluorophore to turn on fluorescence. With thiols (RSH), the original −Se−S− linkage will be converted into another −Se−S− linkage in an equilibrium process, and this should not turn on fluorescence. Nor would it consume the probe. Only when H_2S is present will the equilibrium be broken to release the fluorophore. Overall, the SeP probes are expected to be specific for H_2S and will not be affected by the presence of biothiols.

With this idea in mind, we first prepared a model substrate **1** and tested its reaction with H_2S and cysteine. The reaction between **1** and H_2S (Scheme 2) was fast. The expected

Scheme 2. Model Reactions of the −Se−S− Template

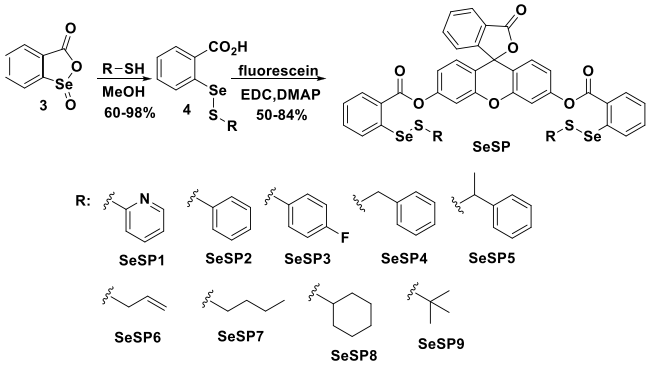


products **2** and phenol were produced in very good yields. The reaction was equally effective when H_2S and cysteine coexisted. Even when **1** was pretreated with cysteine for 1 h and then treated with H_2S, the same products were obtained. These results demonstrated that the selenenyl sulfide template indeed could effectively capture H_2S and the presence of biothiols will not cause any problems.

To validate the application of this reaction template in the design of H_2S fluorescent probes, we next synthesized a series of possible probes (Scheme 3). These compounds employed fluorescein as the common fluorophore due to its excellent fluorescence property. It is also known that the fluorescence of fluorescein can be quenched upon acylation on hydroxy groups. Different R groups were used in the probe structures to explore their effects on the reactivity/selectivity of the −Se−S− linkage to H_2S. Their synthesis was straightforward. Briefly, a known starting material **3**[22] was treated with various thiols to yield −Se−S−-containing benzoic acids **4a**−**4i**. Subsequent esterification with fluorescein gave the desired probes **SeSP1**−**9**. Their characterization data can be found in the Supporting Information.

With these compounds in hand, we then studied their fluorescence responses to H_2S in different conditions. All of

Scheme 3. Synthetic Route and Structures of SeSP1−9



these molecules showed nonfluorescence in buffers due to esterification of two −OH groups of fluorescein. However, upon treatment with 50 μM H_2S (using Na_2S as the equivalent) for 30 min, **SeSP1**−**3** gave significant fluorescence increases while **SeSP4**−**9** showed much smaller fluorescence enhancement (Figure 1). These results indicated that S-aryl

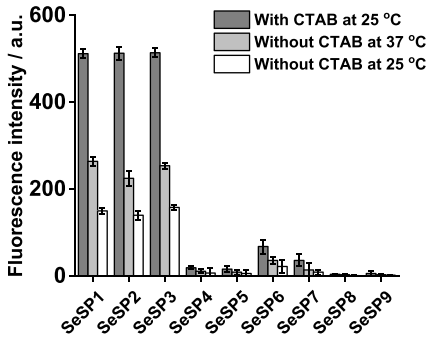


Figure 1. Fluorescence intensity of SeSP (10 μM) with 50 μM Na_2S. Reaction time: 30 min, in PBS (50 mM, pH 7.4).

substituents made the −Se−S− linkage more reactive to H_2S, while substrates with S-alkyl substituents (**SeSP4**−**9**) seem to react with H_2S slowly.

It should be noted that we tested the effects of cetrimonium bromide (CTAB) in the responses of the probes in the studies shown in Figure 1. As a cationic surfactant, CTAB is normally considered not biologically friendly as it could disrupt the cell membrane. In our previous works, we found that the use of a small amount of CTAB could dramatically increase the probes' reactivity (e.g., turn-on rates) and sensitivity (e.g., enhanced fluorescence intensity) in aqueous systems.[17] The effects of CTAB can be attributed to two reasons: (1) with CTAB the solubility of the probe in aqueous buffers is increased, and (2) as a cationic surfactant CTAB may absorb sulfide anion. This would facilitate the reaction between sulfide anion and the probe.

As **SeSP1**−**3** showed better performance than other probes, these three probes were evaluated. As shown in Figure 2, time-dependent responses demonstrated they were fast turn-on probes for H_2S because the maximum signals could be reached in 5−15 min. They were also found to be very sensitive as their fluorescence turn-on folds were at 500−700 for **SeSP2** and **SeSP3** or even 1800 for **SeSP1**.

We next tested the selectivity of **SeSP1**−**3** for H_2S. These probes were treated separately with some representative sulfur species such as GSH, Cys, homocysteine (Hcy), SO_3^{2-}, SO_4^{2-},

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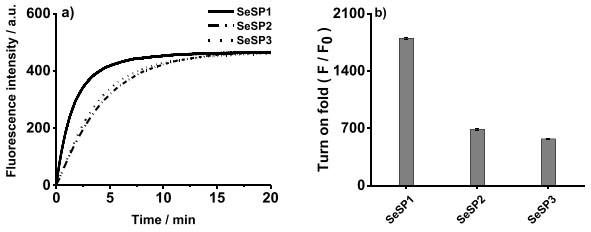


Figure 2. (a) Time-dependent fluorescence responses of SeSP (10 μM) to Na_2S (50 μM) with 100 μM CTAB at 25 °C. (b) Turn-on fold of SeSP (10 μM) to Na_2S (50 μM) with 100 μM CTAB at 25 °C.

and $S_2O_3^{2-}$. We also tested the responses of **SeSP1−3** to several reactive oxygen species, reactive nitrogen species, and selected amino acids: alanine, lysine, arginine, proline, serine, hypochlorite (ClO^-), superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), nitroxyl (HNO), and nitric oxide (NO). As shown in Figure 3, these molecules did not

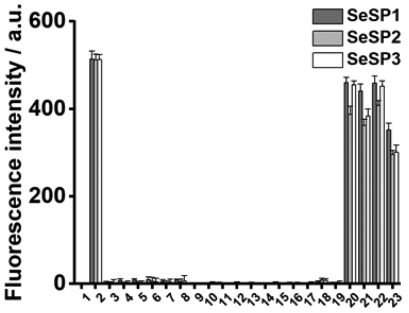
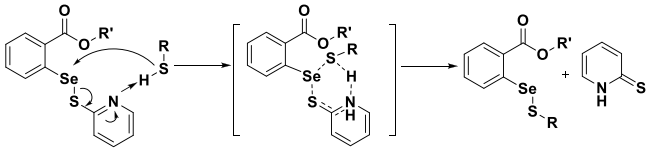


Figure 3. Fluorescence intensity of **SeSP1−3** (10 μM) to different sulfur molecules: (1) probes alone; (2) 50 μM Na_2S; (3) 0.2 mM Na_2SO_3; (4) 0.2 mM $Na_2S_2O_3$; (5) 0.2 mM Na_2SO_4; (6) 0.2 mM Cys; (7) 1 mM GSH; (8) 0.1 mM Hcy; (9) 0.1 mM Ala; (10) 0.1 mM Arg; (11) 0.1 mM Lys; (12) 0.1 mM Pro; (13) 0.1 mM Ser; (14) 25 μM ClO^-; (15) 25 μM O_2^-; (16) 0.25 mM H_2O_2; (17) 0.1 mM $ONOO^-$; (18) 0.1 mM Anglie's salt (HNO donor); (19) 0.1 mM V-PYRRO/NO (NO donor); (20) 0.1 mM Hcy + 50 μM Na_2S; (21) 1 mM GSH + 50 μM Na_2S; (22) 0.2 mM Cys + 50 μM Na_2S; (23) 1 mM GSH + 0.2 mM Cys + 0.1 mM Hcy +50 μM Na_2S. Reaction time: 30 min, rt, in PBS (50 mM, pH 7.4, 100 μM CTAB).

cause significant fluorescence increase. When H_2S coexisted with high concentrations of biothiols (Cys, GSH, Hcy), **SeSP1−3** still elicited strong fluorescence. These results demonstrated that **SeSP** were not only highly selective for H_2S but also very resistant to probe consumption by biothiols.

While **SeSP1−3** showed excellent properties, the results shown in Figures 2 and 3 indicated that **SeSP1** was better than the other two. This superiority may be attributed to the nitrogen atom in pyridine deprotonates thiol to form a six-membered ring intermediate and accelerate the reaction.[23] (Scheme 4).

Scheme 4. Proposed Mechanism and Intermediates for SeSP1



As **SeSP1** was identified as the best in this series, we then tested its detection limit for H_2S. It was tested with different concentrations of H_2S (0−60 μM) (Figure 4). The

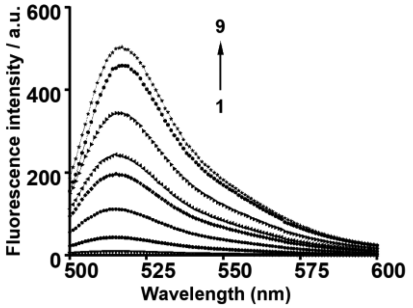


Figure 4. Fluorescence emission spectra of **SeSP1** (10 μM) with different concentrations of Na_2S (0, 1, 4, 8, 12, 15, 20, 40, 60 μM for curves 1−9. Reaction time: 30 min, room temperature in PBS (50 mM, pH 7.4, 100 μM CTAB).

fluorescence intensities were linearly related to the concentrations in the range of 1−20 μM (Figure S1). The detection limit of **SeSP1** was found to be 6.7 nM, indicating **SeSP1** was suitable for detecting H_2S in biological systems. We also tested pH effects on the sensor and **SeSP1** worked well in normal biological pH (7−9) (Figure S2).

To demonstrate the application of **SeSP1**, it was used in H_2S imaging in HeLa cells. Briefly, freshly cultured cells were incubated with **SeSP1** (10 μM) for 45 min. Cells were then washed with PBS to remove excess SeSP1. No significant fluorescence in cells was observed (Figure 5). However, after

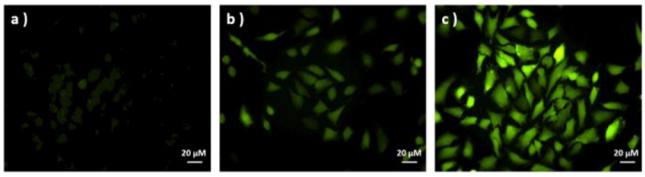


Figure 5. Fluorescence images of H_2S in HeLa cells. Cells were treated with SeSP1 (45 min), washed, and subjected to different treatments: (a) control (no H_2S donor was added); (b) 50 μM H_2S donor (60 min); (c) 100 μM H_2S donor (60 min).

treatment with a H_2S donor (**NSHD-1**[24]) for 60 min, we observed obvious fluorescence and the intensity was correlated to the concentration of donor applied. Moreover, cell viability assay showed **SeSP1** was almost noncytotoxic (Figure S3). These data suggest **SeSP1** is cell permeable and can be used for visualizing H_2S fluctuation in cells. Of note, CTAB was not needed for cell imaging.

Finally, we wondered if **SeSP1** could measure endogenous H_2S concentration changes. We treated HeLa cells with either L-Cys (an H_2S biosynthetic substrate) or 2-amino-4-pentynoic acid (PAG, a CSE inhibitor). The fluorescence intensity changes in cells loaded **SeSP1** were measured by a plate reader and referred to control (detailed protocols were presented in the Supporting Information). As shown in Figure 6, L-Cys treated cells showed enhanced fluorescence, while PAG-pretreated cells showed decreased fluorescence. These experiment results suggested **SeSP1** is suitable for monitoring endogenous H_2S changes.

In summary, we reported here a unique reaction between H_2S and the selenenyl sulfide template. This reaction was

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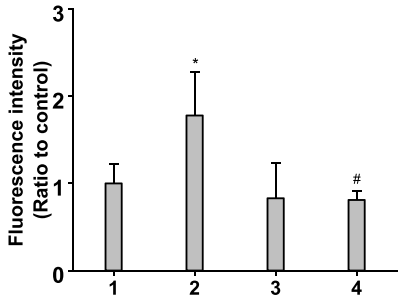

Figure 6. Measurement of endogenous H_2S in HeLa cells: (1) SeSP1 only; (2) 1 mM L-Cys; (3) 1 mM PAG; (4) 1 mM PAG + 1 mM L-Cys. Data is shown as the mean ± SD $n = 5$, $^*P < 0.01$ vs control group, $^{\#}P < 0.01$ vs L-Cys group.

found to be specific for H_2S, with no interference by thiols. A series of fluorescent probes based on this reaction were synthesized and evaluated. Among them, **SeSP1** showed high sensitivity and selectivity to H_2S. It solved the major problem of previously reported **WSP** and **SeP** probes. **SeSP1** should be a useful research tool for understanding H_2S functions in biology. The selenenyl sulfide template is also expected to be a suitable scaffold in the development of novel H_2S probes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02844.

Experimental procedures and characterization of each compound (PDF)

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Notes

The authors declare no competing financial interest.

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