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A Specific Nucleophilic Ring-Opening Reaction of Aziridines as a Unique Platform for the Construction of Hydrogen Polysulfides Sensors

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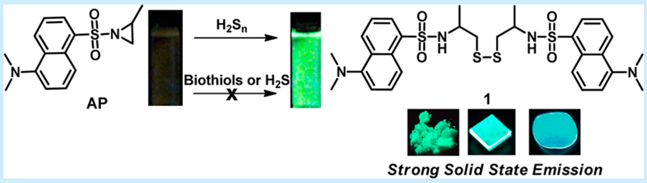
Supporting Information

ABSTRACT: A hydrogen polysulfide mediated aziridine ring-opening reaction was discovered. Based on this reaction, a novel H_2S_n-specific chemosensor (**AP**) was developed. **AP** showed high sensitivity and selectivity for H_2S_n. Notably, the fluorescent turn-on product (**1**) exhibited excellent two-photon photophysical properties, a large Stokes shift, and high solid state luminescent efficiency.



Reactive sulfur species (RSS), including biothiols, hydrogen sulfide (H_2S), sulfane sulfurs, and *S*-modified cysteine adducts (i.e., *S*-nitrosothiols, sulfenic acids, etc.), play important roles in redox biology.[1−4] Among these species, hydrogen polysulfides (H_2S_n, $n > 1$) have recently received particular attention as they are believed to be involved in H_2S-mediated signaling transduction.[5−10] Although H_2S has been extensively studied as a new signaling molecule in the past decade, the fundamental chemistry and physiological function of H_2S_n are still poorly understood. H_2S_n belong to the sulfane sulfur family and have very unique chemistry. H_2S_n can be derived from endogenous H_2S by the action of reactive oxygen species,[4,7,11−13] as redox partners of H_2S. In this regard, H_2S_n very likely coexist with H_2S *in vivo* and they work together to regulate sulfur redox balance. Recent studies suggested that H_2S_n might act as the real regulators in cellular signaling transduction.[5−10] Some biological activities that were originally ascribed to H_2S may actually be mediated by H_2S_n. One such case is S-sulfhydration (i.e., conversion of protein cysteine residues (−SH) to persulfides (−S−SH)).[4,14−20] This reaction was originally ascribed to H_2S. However, H_2S_n are recently found to be more effective than H_2S in S-sulfhydration.[6,7,12,13,21]

The field of H_2S_n is now rapidly growing, and more exciting biological activity exhibited by H_2S_n are to be discovered. In order to better understand the roles of H_2S_n, it is critical to develop efficient methods that can distinguish H_2S_n from other reactive sulfur species, especially H_2S and biothiols. This is still an underdeveloped field. So far the most commonly used method for H_2S_n detection is to measure UV absorption at 290−300 and 370 nm.[7] This low-sensitivity method is unsuitable for biological samples. Fluorescence-based methods could be ideal due to their rapid, sensitive fluorescent responses and spatiotemporal resolution capability.[22,23] Our laboratory recently discovered a H_2S_n-mediated aromatic substitution−cyclization and reported the first H_2S_n-specific fluorescent sensors based on this reaction.[24] The sensors utilize 2-fluoro-5-nitro-benzoic ester to trap H_2S_n and release the fluorophores. Although the sensors proved to be highly selective for H_2S_n, the competing reaction of the 2-fluoro-5-nitro-benzoic ester template with biothiols could cause the consumption of the sensors and higher sensor loading may be required. To solve this problem, further improvements of the sensor template or the discovery of novel H_2S_n specific reaction templates would be needed. Herein we report a unique reaction between aziridines and H_2S_n. Based on this reaction, a novel H_2S_n-specific sensor (**AP**) was prepared and evaluated. The physical properties of the actual fluorescent species generated from the reaction of H_2S_n were also studied.

In order to develop reaction-based fluorescent sensors for H_2S_n detection, the key is to identify specific reactions that only react with H_2S_n, but not react with biothiols such as glutathione (GSH) and cysteine (Cys) (which are ubiquitous in biological systems and concentrations can reach mM levels). The

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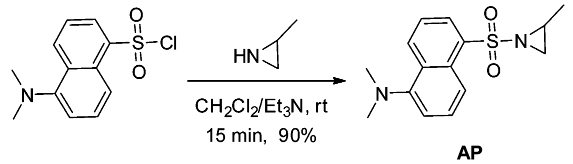
presence of H_2S (at μM levels) should also be taken into consideration. Due to α-effects, H_2S_n are expected to be weak acids. The estimated pK_a values of H_2S_n are in the range of 3 to 5.[25,26] For comparison, the pK_a values of H_2S and biothiols are in the range of 7 to 9.2 (H_2S, 7.0; Cys, 8.30; GSH, 9.2). Under physiological pH, H_2S_n should be stronger and more reactive nucleophiles than biothiols and H_2S. As such, our focus was on possible electrophiles which are reactive enough for H_2S_n, but not reactive toward H_2S and thiols.

Aziridines are popular electrophilic synthons for making amine-containing molecules. Ring-opening reactions of aziridines by many nucleophiles, including thiol-derivatives, have been reported.[27−30] In most cases the reactions were for synthetic purposes. High concentrations of reactants, organic solvents/bases, long reaction times, and elevated temperatures are often employed. It should be noted that although those studies revealed good reactivity of aziridines toward certain nucleophiles such as thiols, whether or not such reactions could be used for the detection of thiols or related biological molecules are still unclear. The low concentrations of analytes in biological systems and the mild, neutral, and aqueous reaction environments may make the reactions slow and nonproductive so the reaction signals cannot be visualized. So far the use of aziridine-based sensors for the detection and distinction of reactive sulfur species has not been well studied. We wondered if the strong nucleophilicity of H_2S_n under physiological conditions could be recognized by activated aziridine-based chemosensors. A *N*-sulfonylaziridine chemosensor was selected for this study.

In the design of the proposed chemosensor, we expected the aziridine moiety and strongly fluorescent dansyl group to be the recognition unit and signaling unit, respectively. The fluorescence of dansyl group should be effectively quenched or weakened via the twisted intramolecular charge transfer (TICT) effect. If the aziridine ring of the sensor could be opened upon reacting with H_2S_n, the resultant dansylamide derivative should possess strong fluorescence. With this idea in mind, an off−on fluorescent chemosensor, **AP**, was prepared from a simple coupling between dansyl chloride and 2-methylaziridine (Scheme 1).

Scheme 1. Preparation of AP



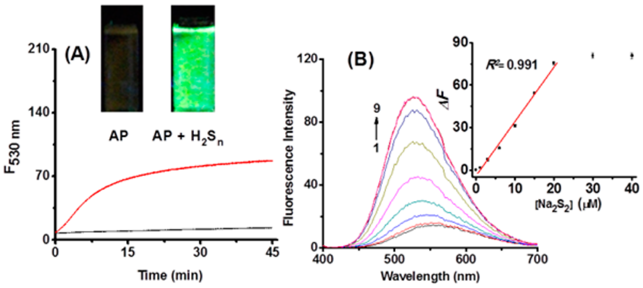


Figure 1. (A) Time-dependent fluorescence intensity changes of **AP** (10 μM) in the absence (black line) or presence (red line) of Na_2S_2 (25 μM). The inset depicts the fluorescence color change of the detection system after **AP** reacts with Na_2S_2. (B) Fluorescence emission spectra of **AP** (10 μM) with varied concentrations of Na_2S_2 (0, 1, 3, 6, 10, 15, 20, 30, 40 μM for curves 1−9, respectively). The inset depicts the plot of fluorescence increase value (ΔF) of the reaction system at $\lambda_{ex/em}$ = 350/530 nm against the corresponding reagent blank (without Na_2S_2).

With the sensor in hand, we first tested the time-dependent fluorescence changes of **AP** (10 μM) in the absence and presence of H_2S_n (25 μM). Freshly prepared Na_2S_2 was used in buffer solutions as the equivalent of H_2S_n. As shown in Figure 1A, **AP** showed very weak fluorescence with a low quantum yield (Φ = 0.01) in PBS buffer (pH 7.4), which was due to the TICT effect. The sensor appeared to be quite stable, as no fluorescence change was noted in 45 min. Upon the treatment of Na_2S_2, the fluorescence intensity at 530 nm increased dramatically, presumably due to a ring-opening reaction and the formation of the dansylamide derivative. This was also accompanied by a distinct fluorescence color change from weak yellow to bright green (see the inset of Figure 1A), which might be useful for the simple detection of H_2S_n by the naked eye. The maximum intensity could reach a plateau in 15 min, suggesting this was a fast detection. To ensure reproducibility, a reaction time of 30 min was used in all the experiments in this study. It should be noted that 50 μM CTAB was used in our experiments. Without CTAB, the reaction between **AP** and H_2S_n was found to be slow and nonproductive. This might be due to the poor stability of H_2S_n in water. The use of CTAB significantly increased reaction rates. The effects of CTAB could be attributed to the fact that (1) CTAB can increase the solubility of the sensor in aqueous buffers and (2) CTAB is a cationic surfactant, which may absorb a polysulfide anion (HS_n^-) and facilitate the reaction. We next studied the fluorescence responses of **AP** to Na_2S_2 at varied concentrations. Upon gradual introduction of Na_2S_2 (1 to 40 μM), increases in fluorescence emission at 530 nm were observed. This spectroscopic property displayed a large Stokes shift (180 nm), which should prevent serious self-quenching and fluorescence detection error due to excitation backscattering effects. We also noted that the fluorescence intensity increased linearly with the concentrations of Na_2S_2 in the range of 0−20 μM. The detection limit[31,32] was calculated to be 0.3 μM, suggesting the sensor was highly sensitive to H_2S_n. In addition, we studied the pH effects on this reaction and found that **AP** was a stable sensor in aqueous buffers and worked effectively in a pH range from 6 to 10 (Figure S1 in the Supporting Information).

Having proven the sensitivity of **AP** toward H_2S_n, we then turned our attention to examine the specificity of **AP**. In this study **AP** (10 μM) was treated with a series of biologically relevant sulfur species including GSH, Cys, Hcy, GSSG, H_2S, SO_3^{2-}, $S_2O_3^{2-}$, CH_3SSSCH_3, and S_8. The concentrations of these species were selected based on their biological relevance. As shown in Figure 2, none of these molecules gave a significant fluorescence response (columns 1−9). We also tested the responses of **AP** to common reactive oxygen species and some representative amino acids, including hydrogen peroxide (H_2O_2), hypochlorite (ClO^-), superoxide (O_2^-), the hydroxyl radical (·OH), singlet oxygen ($^1O_2$), alanine, proline, serine, lysine, tryptophan, and histidine. Again these species did not exhibit any significant fluorescence response (columns 10−20). H_2S_n are highly reactive species and may react with thiols to form persulfides. We then wondered if **AP** could effectively

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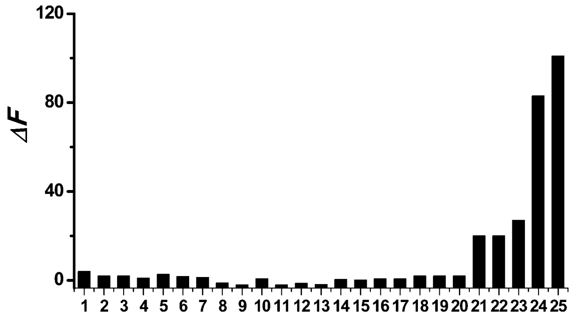
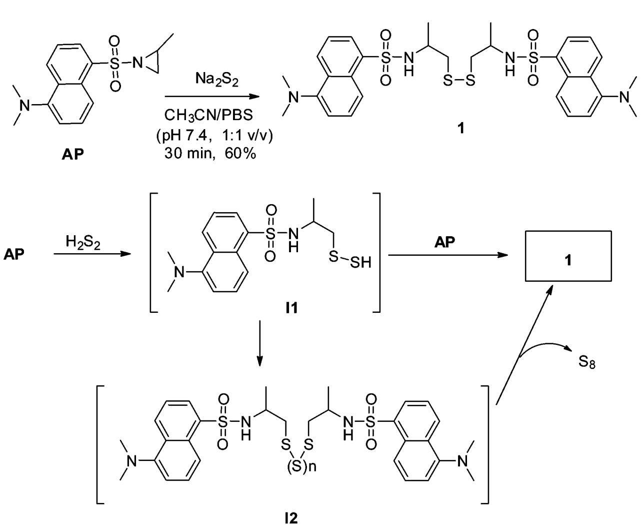


Figure 2. Fluorescence intensity increases (Δ*F*) of **AP** (10 μM) in the presence of various RSS, ROS, amino acids. (1) 10 mM GSH; (2) 500 μM Cys; (3) 100 μM Hcy; (4) 100 μM GSSG; (5) 100 μM Na_2S; (6) 100 μM $Na_2S_2O_3$; (7) 100 μM Na_2SO_3; (8) 100 μM CH_3SSSCH_3; (9) 100 μM S_8; (10) 250 μM H_2O_2; (11) 25 μM ClO^-; (12) 25 μM O_2^-; (13) 25 μM ·OH; (14) 25 μM $^1O_2$; (15) 100 μM alanine; (16) 100 μM proline; (17) 100 μM serine; (18) 100 μM lysine; (19) 100 μM tryptophan; (20) 100 μM histidine; (21) 100 μM GSH + 50 μM Na_2S_2; (22) 100 μM Cys + 50 μM Na_2S_2; (23) 100 μM Hcy + 50 μM Na_2S_2; (24) 25 μM Na_2S_2; (25) 25 μM ClO^- + 100 μM Na_2S.

identify H_2S_n in the presence of thiols. To this end, **AP** was applied in the mixtures of Na_2S_2 and thiols (columns 21–23). We still observed satisfactory fluorescence increases, albeit the values (Δ*F*) were lower as compared to that of Na_2S_2 only. This could be attributed to the loss of H_2S_n in the reactions with thiols.[6,7,13,33] Moreover, we tested the detection of in situ H_2S_n formation by **AP**. Nagy et al. reported that hypochlorite (ClO^-) could rapidly react with H_2S to form H_2S_n.[11] When Na_2S (100 μM) and ClO^- (25 μM) coexisted, **AP** gave a very strong fluorescence enhancement (column 25) and the value was even higher than that of Na_2S_2. Taken together, these results demonstrated the specificity and sensitivity of **AP** for H_2S_n.

To understand the fluorescence turn-on mechanism, the reaction between **AP** and Na_2S_2 was studied at the semi-synthetic scale (60 mM **AP**; 68 mM Na_2S_2) (Scheme 2). The reaction was found to be fast which completed within 30 min at room temperature. The major isolated product was disulfide **1** (60% yield). This result indicated that H_2S_2 could effectively open the aziridine ring of **AP** to form an intermediate **I1**. It was possible that **I1** reacted with another molecule of **AP** to form the final product **1**. Another possibility was that **I1** decomposed to form polysulfide **I2**, which was eventually converted to a stable disulfide product.[7] Given the good yield obtained in this reaction, we expected it could be used for the synthesis of disulfide derivatives.

Scheme 2. Reaction between AP and H_2S_n



The isolation of the disulfide product **1** allowed us to carefully study its fluorescence properties as a new fluorophore. As shown in Figure 3, this molecule displayed a strong green fluorescence with a moderate quantum yield (Φ = 0.25) in PBS buffer (pH 7.4). In addition, compound **1** exhibited interesting two-photon photophysical properties. The shape of the two-photon emission spectrum (λ_{ex} = 740 nm) closely resembles that obtained by single-photon excitation (λ_{ex} = 350 nm) (Figure 3A and Figure S2 in the Supporting Information). This characteristic suggested that **AP** might be a useful two-photon fluorescent sensor. Of particular interest is that compound **1** is a highly emissive fluorophore in solid states, such as in powder form and spin-coated thin films, and even in poly(methyl methacrylate) (PMMA) films. Both powder form and a spin-coated thin film of **1** were found to have bright green fluorescence (λ_{em} = 502 nm) under UV light (λ_{ex} = 365 nm). A PMMA film dispersing 0.8% compound **1** also emitted a strong blue-green fluorescence (λ_{em} = 466 nm), bearing the excellent efficiency of the solid-state emission (Φ = 1.00, calculated by using an integrating sphere[34,35]). The development of organic molecules bearing high solid state luminescent efficiency with a high absolute quantum yield remains a difficult task in the field of optoelectronic devices.[36] Currently available molecules with such properties are still quite scarce. Compared to most known organic solid-state luminescence molecules, the unique properties of fluorophore **1**, such as good solubility, easy and low-cost synthesis, and high absolute quantum yield, may endow it as a potential candidate for organic emitters and for application to solid-state lighting devices.

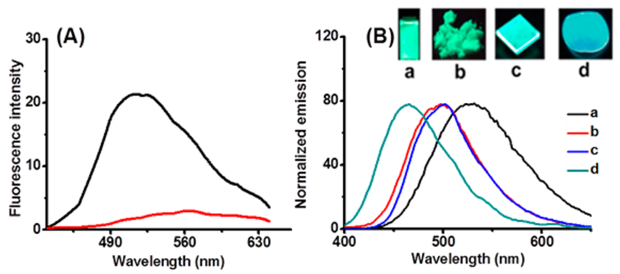


Figure 3. (A) Two-photon fluorescence emission spectra of **AP** (red line) and **1** (black line) (λ_{ex} = 740 nm). (B) Fluorescence emission spectra of **1** in different forms and corresponding fluorescence photos under UV light (λ_{ex} = 365 nm): (a) PBS buffer (pH 7.4); (b) solid powder; (c) spin-coated thin films; (d) PMMA solid films (film thickness is 50 μm) doped with 0.8% compound **1**.

To further evaluate the application of **AP** in biological samples, the detection of H_2S_n in diluted deproteinized bovine plasma[37] was performed (Figure S3 in the Supporting Information). Upon gradual introduction of Na_2S_2, steady fluorescence enhancements appearing at 530 nm were observed. The fluorescence response signals were lower than those obtained in PBS buffers. This may be due to the consumption of H_2S_2 by biothiols. Nevertheless a good linear correlation between the H_2S_2 concentration and the fluorescence intensity at 530 nm was obtained.

In summary, we have reported a unique ring-opening reaction of *N*-sulfonylaziridine by Na_2S_2 under mild conditions. This reaction was used to develop a specific fluorescent sensor **AP** for the detection of H_2S_n. The sensor was found to be selective and sensitive for H_2S_n while other reactive sulfur/oxygen species and amino acids could not turn on the fluorescence. Moreover, the fluorophore, i.e. compound **1**, exhibited excellent two-photon photophysical properties and a large Stokes shift. Given its high solid state luminescent efficiency, this molecule may be a potential candidate for organic emitters and for application to solid-state lighting devices.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic procedures, characteristic data, and experimental procedures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01194.

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Notes

The authors declare no competing financial interest.

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