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Aldehyde group assisted thiolysis of dinitrophenyl ether: a new promising approach for efficient hydrogen sulfide probes†

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We report herein a new approach, which combines fast nucleophilic addition of H_2S to an aldehyde group and the subsequent intramolecular thiolysis of dinitrophenyl ether, and can be used to develop efficient and effective H_2S probes.

Much attention has been focussed in the last few years on the development of small molecular probes for hydrogen sulfide (H_2S) since the recognition of the biological significance of H_2S recently.[1] Among them, the fluorescent detection method is highly attractive due to its simplicity, high sensitivity, low cost and ability of real-time detection. Accordingly, highly selective and sensitive fluorescent probes with rapid response for H_2S under mild conditions have become high in demand, as they have huge potential benefits for a better and deeper understanding of the biological functions of H_2S.

Since 2011, a number of fluorescent probes for H_2S have been developed based on several significant chemical properties of H_2S, such as good reducing property,[2,3] high binding affinity towards copper ions,[4] specific nucleophilic addition to unsaturated double bonds,[5] dual nucleophilicity[6] as well as efficient thiolysis of dinitrophenyl ether.[7] Among them, using the dual nucleophilicity of H_2S is especially attractive for H_2S probes because a high selectivity for H_2S can be well guaranteed by this strategy.[6] Although the advantage of this strategy has been utilized in several innovative H_2S probes,[6] most of them showed a delayed response time (typically >20 min) to detect H_2S. Actually, this is a common problem for most reaction-based H_2S probes reported to date, which is a disadvantage for efficient capturing of the transient H_2S.[1] Besides, some other drawbacks of these reported probes can also be found such as either they need a relatively high probe loading due to the probe consumption by other thiols,[6a] or need complicated synthetic procedures,[6b–d] or need the use of surfactants to promote the reactions.[6f,h,i] Therefore, new methods are highly expected to overcome these shortcomings.

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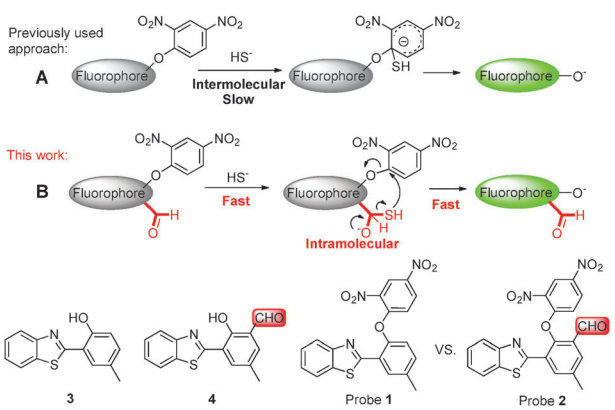
We are interested in the thiolysis of dinitrophenyl ether for H_2S probes because the dinitrophenyl ether is very easy to be introduced to a fluorophore, and such probes have very low background fluorescence due to the strong quenching effect of the nitro group. However, the reported probes for H_2S based on this approach by several groups[7a–d] including us[7e] used intermolecular thiolysis of dinitrophenyl ether (A in Scheme 1), and they generally suffered from low reaction rates and thus long response times are also needed. Although addition of a surfactant such as cetyltrimethylammonium bromide (CTAB)[7a,e] or Tween-20[7c] can enhance the reaction rates significantly, the use of surfactant limits the applications of these probes. Since an intramolecular reaction can be performed more efficiently than an intermolecular one, we thought that the thiolysis reaction could proceed much faster if it was switched into an intramolecular fashion. Using the unique dual nucleophilic characters of H_2S, we envisaged that combining the fast nucleophilic addition of H_2S to an aldehyde group and the subsequent spontaneous intramolecular instead of intermolecular thiolysis of dinitrophenyl ether could achieve this purpose (B in Scheme 1). Motivated by this concept, we herein report that this new approach can be very promising to develop efficient H_2S probes.

To investigate the feasibility of our design concept, we chose a known excited state intramolecular proton transfer (ESIPT)



Scheme 1 Sensing of hydrogen sulfide by thiolysis of dinitrophenyl ether, the ESIPT dyes and probes in this work.

dye, compound **3** in Scheme 1, as the fluorophore, because this type of dye has been widely used to construct fluorescent probes due to their large Stokes shift and good photostability.[6f,8] In addition, it can be easily prepared, and an aldehyde group can also be easily introduced to the *ortho* position of the hydroxyl group in this dye to afford an aldehyde containing fluorophore **4**. Thus, two new probes (probe **1** and **2**) for the purpose of this study can be readily obtained by protection of the hydroxyl group in **3** and **4** with a dinitrophenyl group, respectively. The synthesis of these two probes is illustrated in Scheme S1 (ESI†), and their structures are confirmed by NMR, IR and HR-MS analysis. Detailed synthetic procedures and structure characterization are given in the ESI.†

Since **3** and **4** are the expected thiolysis products of probe **1** and **2** with H_2S, respectively, we first checked the differences in their optical spectra. As shown in Fig. S1 (ESI†), **3** absorbs light in the UV range (λ_{max} 335 nm), however, λ_{max} of **4** red-shifted to the visible light region (λ_{max} 445 nm), which indicates that **4** is visible light excitable. Fluorescent spectra showed that both probe **1** and **2** are non-fluorescent, while **3** and **4** show much stronger fluorescence. Besides, **4** shows not only longer emission wavelength, but also higher fluorescence quantum yield than **3** (**4**: λ_{em} 545 nm, Φ 0.18 *vs.* **3**: λ_{em} 480 nm, Φ 0.04), indicating that **4** is a better fluorophore (Fig. S1b, ESI†). Anyway, if the thiolysis reactions of both probes with H_2S are fast, then both of them should give rapid and significant optical signal changes.

The sensing ability of probe **1** and **2** for H_2S was then investigated in 20 mM PBS buffer (pH 7.4) with 25% CH_3CN (v/v) at 25 °C. Under these conditions, we observed that addition of 10 equiv. of NaHS (a standard source for H_2S) to the probe **1** and **2** (20 μM of each) solutions resulted in significant differences. As shown in Fig. 1, the probe **1** solution showed very small changes upon addition of NaHS and even the reaction time was prolonged to 18 h. In contrast, the probe **2** solution showed fast and significant optical changes within a few minutes upon addition of NaHS, and meanwhile, the colorless solution of **2** changed into yellow, and gave out strong green fluorescence as compound **4**. Kinetic studies showed that the reaction (20 μM probe **2** + 100 μM NaHS) was completed at about 2 min (Fig. S2, ESI†). The observed pseudo-first-order rate constant k_{obs} at 25 °C was determined to be about 2.077 min^{-1} ($t_{1/2} \approx 0.334$ min) and the emission intensity was found to increase about 120-fold at 545 nm. Since the reaction between probe **1** and NaHS cannot complete even after 18 hours under the same conditions (Fig. S2, ESI†), probe **2** responds at least more than hundreds of times faster than probe **1** for H_2S, indicating that our design concept works very well. In fact, probe **2** is able to detect H_2S more rapidly than most of the reported reaction-based probes for H_2S.[1–3,5,6] In addition, it was also found that probe **2** can work with less amount of CH_3CN and can work over a wide range of pH with the optimal pH around 7.0 (Fig. S3, ESI†). All these results indicate that this new combined approach is effective and attractive.

To confirm the thiolysis of dinitrophenyl ether and release of **4**, the reaction product of probe **2** with NaHS was isolated and subjected to thin layer chromatography (TLC) and ^1H NMR analysis, and the results proved that the product is **4** (Fig. S4 and S5, ESI†). Considering that the aldehyde group in probe **2** provides a larger steric hindrance to the dinitrophenyl ether moiety for a direct thiolysis by H_2S and the low reactivity of probe **1** for H_2S, the sensing process of probe **2** for HS^- most likely happened through route B as shown in Scheme 1.

The above results show that probe **2** can be used as a rapid and sensitive dual colorimetric and fluorescent turn-on sensor for HS^- in aqueous solution. To shed further light on the sensitivity of probe **2** for H_2S, the changes in its fluorescence were investigated by addition of various concentrations of NaHS. As shown in Fig. S6 (ESI†), probe **2** is reasonably stable under the test conditions, and upon addition of 0.6–10 equiv. HS^-, the fluorescence intensity of the probe **2** solutions showed significant enhancement within 5 min. When the reaction was monitored after 5 min of HS^- addition, a saturation of fluorescence could be observed after the addition of more than 2 equiv. HS^- (Fig. S7, ESI†). In addition, we also observed a good linearity between the fluorescent intensity at 545 nm and the concentration of HS^- in the range of 0–12 μM (Fig. 2), thus, the detection limit (S/N = 3) of probe **2** for HS^- is calculated to be about 48 nM under the test conditions. This indicates that probe **2** has high sensitivity for H_2S.

We then investigated the selectivity of probe **2** for HS^- over various competitors such as F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, SCN^-, CO_3^{2-}, HCO_3^-, $H_2PO_4^-$, CN^-, SO_4^{2-}, HSO_4^-, SO_3^{2-}, HSO_3^-, $S_2O_3^{2-}$, $S_2O_7^{2-}$, cysteine (Cys), homocysteine (Hcy), glutathione (GSH), *N*-acetyl-cysteine (NAC), $C_6H_5NH_2$, $C_6H_5CH_2NH_2$, $H_2NCH_2CH_2NH_2$ and $HOCH_2CH_2NH_2$, and the results showed that probe **2** is highly

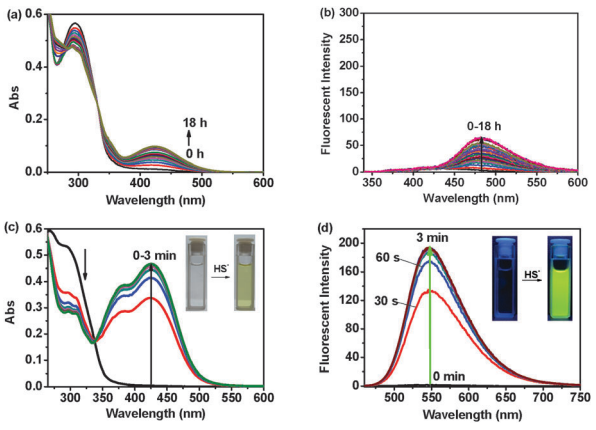


Fig. 1 UV-vis and fluorescent spectral changes of probe **1** (a and b, respectively) and probe **2** (c and d, respectively) against time in the presence of HS^- (10 equiv.). All spectra were measured in PBS buffer (20 mM, pH 7.4) with 25% CH_3CN (v/v) at 25 °C with [probe] = 20 μM.

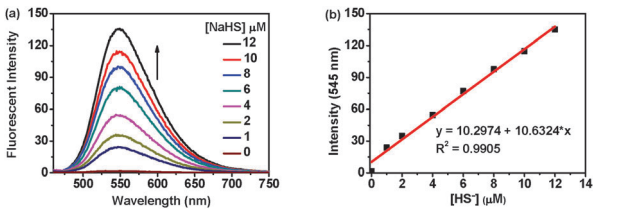


Fig. 2 (a) Fluorescence spectral changes of probe **2** (20 μM) upon addition of different concentrations of HS^- in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v). The final concentrations of HS^- are 0, 1, 2, 4, 6, 8, 10, 12 μM, respectively. (b) A linear calibration curve between the fluorescent intensity changes of probe **2** at 545 nm and the concentration of HS^- in the range of 0 to 12 μM. Each spectrum was collected at 5 min after HS^- addition. λ_{ex} = 440 nm, slit width: d_{ex} = d_{em} = 5 nm.

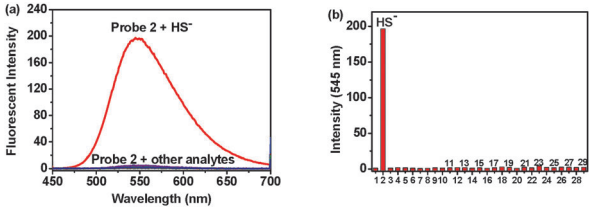


Fig. 3 (a) Fluorescent spectral changes of probe **2** (20 μM) in the presence of various analytes (10 eq.). (b) Fluorescent intensity changes of probe **2** (20 μM) at 545 nm for various analytes (200 μM). λ_{ex} = 440 nm, d_{em} = d_{ex} = 5 nm. The numbers (1–29) represent probe **2** with analytes: (1) none, (2) HS^-, (3) F^-, (4) Cl^-, (5) Br^-, (6) I^-, (7) SCN^-, (8) CO_3^{2-}, (9) AcO^-, (10) NO_3^-, (11) NO_2^-, (12) PO_4^{3-}, (13) HPO_4^{2-}, (14) $H_2PO_4^-$, (15) $S_2O_3^{2-}$, (16) $S_2O_7^{2-}$, (17) SO_4^{2-}, (18) SO_3^{2-}, (19) HSO_3^-, (20) HSO_4^-, (21) CN^-, (22) GSH, (23) Hcy, (24) Cys, (25) NAC, (26) $C_6H_5NH_2$, (27) $C_6H_5CH_2NH_2$, (28) $H_2NCH_2CH_2NH_2$, (29) $HOCH_2CH_2NH_2$ respectively.

selective for HS^-. As shown in Fig. 3 and Fig. S8 and S9 (ESI†), only addition of HS^- resulted in significant optical changes to the probe **2** solution. In contrast, addition of other analytes showed almost no effect. Notably, detection of HS^- using probe **2** in the presence of these analytes is still effective (Fig. S10, ESI†), even biothiols Cys, Hcy, GSH and NAC are present in a millimolar range (Fig. S11, ESI†). Despite the fact that aryl-aldehydes have been reported to react with biothiols such as Cys to form stable thiazolidines,[9] our experiments showed that compound **4**, the product of probe **2** with HS^-, is not fluorescent responsive to Cys even upon incubation for more than 10 hours (Fig. S12, ESI†). Therefore, all these results indicate that probe **2** is highly selective for HS^-.

The ability of probe **2** to visualize H_2S in living cells was also tested. As shown in Fig. 4, no fluorescence was observed when HeLa cells were incubated with probe **2** only. In contrast, addition of H_2S to the cells pre-incubated with probe **2** produced strong green fluorescence. This result indicates that probe **1** can be used to visualize H_2S in living cells.

In summary, we reported herein a new approach, which uses an aldehyde group to facilitate the thiolysis of dinitrophenyl ether, and can be used to develop more efficient probes for H_2S. Using this approach, we showed that a new dual colorimetric and fluorescent probe (probe **2**) was able to sense H_2S with rapid response (<5 min), high selectivity and sensitivity, and was also able to detect H_2S in living cells, indicating that this approach is applicable and very promising. We believe that this approach could aid the design of future H_2S probes for efficient and effective detection of H_2S.

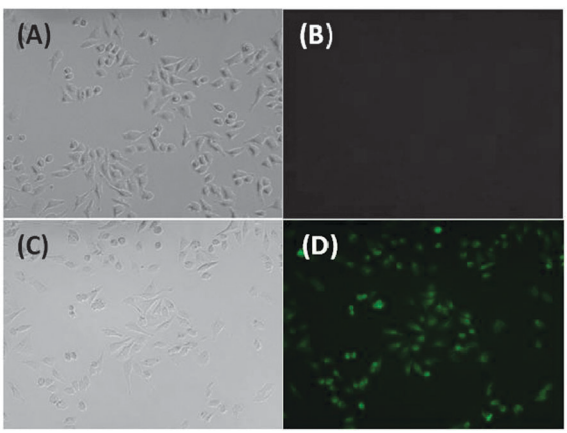


Fig. 4 Fluorescence imaging of H_2S in HeLa cells incubated with 20 μM probe **2**. (A) and (B) show that cells were incubated with **2** only for 20 min. (C) and (D) show that cells were pre-incubated with **2** for 20 min, and then incubated with 200 μM H_2S for 30 min. (A) and (C) are bright field images, (B) and (D) are fluorescence images of (A) and (C), respectively.

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