



Fast responding and selective near-IR Bodipy dye for hydrogen sulfide sensing†

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A Bodipy based, highly selective probe for hydrogen sulfide has been designed, synthesized and demonstrated to detect H₂S in living cells. In this design, the reduction of two arylazido groups change the charge transfer characteristics of the 3,5-distyryl substituents on the Bodipy core, producing a 20 nm bathochromic spectral shift in the absorption band, and quenching of the emission by 85% compared to the original intensity, through photoinduced electron transfer.

Hydrogen sulfide (H₂S) has a characteristic repulsive odor of rotten eggs, and plays crucial roles in biological processes; as a result, many groups worldwide are interested in potential agents that will allow its real-time monitoring.¹ Like the other two gaseous signaling molecules, carbon monoxide (CO)² and nitric oxide (NO),³ hydrogen sulfide is a biosynthetic gasotransmitter. These small gaseous molecules are different from the other messenger molecules regarding their production and function. Furthermore, because of their small size and charge neutrality, they can easily pass through the cellular membranes without affecting any cell signaling response.⁴ The important roles of H₂S in many metabolic processes, such as cardiovascular protection, neuroprotective effect, arrangement of cell growth, calcium homeostasis and regulation of neurotransmission are well established in the literature.⁵

H₂S is an example of reactive sulfur species, such as thiols, S-nitrosothiols, sulfenic acids and sulfite, produced enzymatically from cysteine in a series of reactions, mainly catalyzed by two pyridoxal 5'-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) and independently from pyridoxal 5'-phosphate by another enzyme, 3-mercaptopyruvate sulfur transferase (3MST).⁶ Also, elemental sulfur is another endogenous H₂S source.⁷

Biological imaging probes working in the near-IR region of the spectrum have attracted considerable attention in recent years, since the light used for excitation causes much less photodamage to cells compared to ultraviolet or visible light used in excitation of other probes. In addition, with near IR probes, cell autofluorescence is not an issue. On the other hand, Bodipy dyes, which are difluoroboron-chelated dipyrromethene derivatives, seem to be very good choices for designing novel H₂S probes due to their desirable properties, such as high quantum yields, chemical and photochemical stability, high molar absorption coefficients, and the fact that they allow straightforward access to near-IR emitting derivatives (Scheme 1).⁸

In this work, the Knoevenagel type chemistry has been used to obtain a near-IR emissive Bodipy derivative **1** with extended conjugation. Initially, we synthesized Bodipy **5** having three triethyleneglycol groups. To that end, compounds **3** and **4** were prepared according to the reports in the literature. Then Bodipy **5** was obtained by the reaction of aldehyde **4** and 3-ethyl-2,4-dimethylpyrrole. Finally, following recently established protocols, the condensation reaction of *p*-azidobenzaldehyde **3** yielded the probe **1** ($\Phi_f = 0.38$, cresyl violet in ethanol as a reference dye) in the analytically pure state following chromatographic purification. This reaction not only allowed the formation of the target probe in good yields, but also shifted emission and absorption wavelengths of the probe to the near-IR region.

H₂S probes functioning through the reduction of azido groups to amines are available in the literature.⁹ However, most of them do not respond fast enough. On the other hand, the probe proposed in this study (compound **1**) responds to H₂S with no time delay, practically immediately as the reagents are mixed.

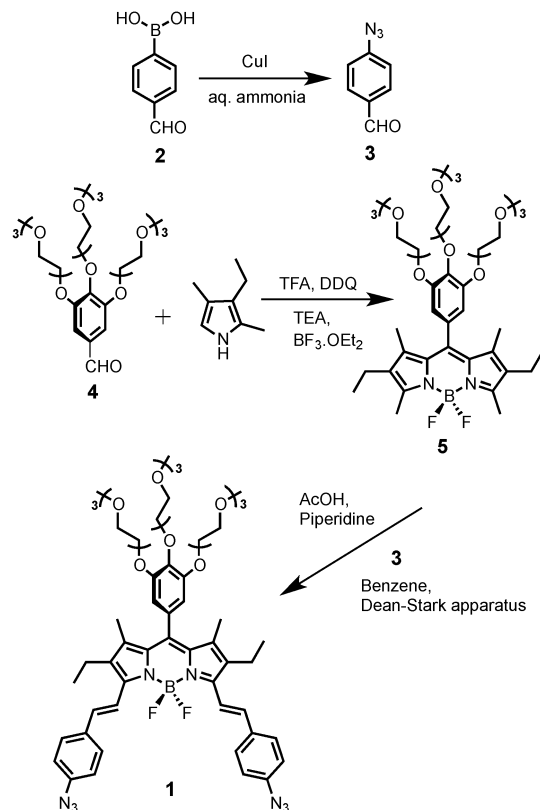
Upon titration of probe **1** with various concentrations of Na₂S at room temperature in the acetonitrile-buffer mixture (20 mM HEPES/CH₃CN, 40 : 60, v/v, pH = 7.20, 25 °C), a red shift of about 20 nm was observed in the electronic absorption spectrum (Fig. 1a); the color change was easily noticeable with

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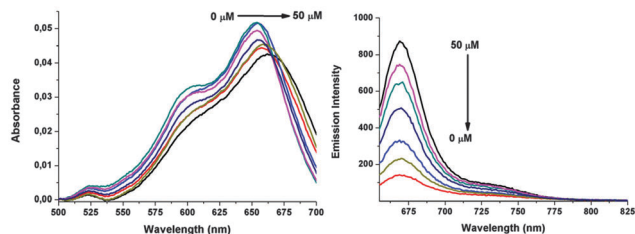
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† Electronic supplementary information (ESI) available: Experimental procedures, structural proofs. See DOI: 10.1039/c4cc00762j



Scheme 1 Synthesis of target probe 1.

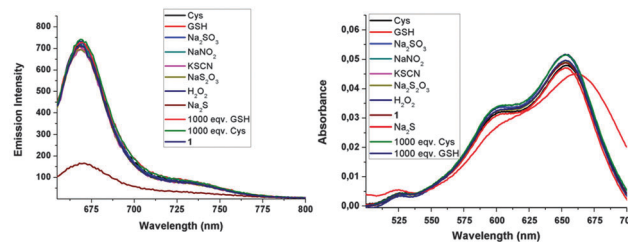
Fig. 1 Absorption and emission spectra of **1** (2.0 μM) in 20 mM HEPES: CH_3CN (40 : 60, v/v, pH = 7.20, 25 $^\circ\text{C}$) in increasing Na_2S concentrations. Excitation wavelength is 650 nm. Experiments were done in triplicate.

the naked eye. The actual ratio of $\text{S}^{2-}/\text{HS}^-/\text{H}_2\text{S}$ concentrations (speciation) is dictated by the pH of the buffer.

As shown in the fluorescence spectra (Fig. 1b), the emission of probe **1** was quenched upon increasing Na_2S concentrations at room temperature in 20 mM HEPES/ CH_3CN solutions (40 : 60, v/v, pH = 7.20, 25 $^\circ\text{C}$). The detection limit was determined (ESI^\dagger) to be 0.34 μM .

The reduction of the azido group to the amine group (mechanism presented in the ESI^\dagger) provides an alternative excited state process (photoinduced electron transfer, PET), which is responsible for quenching of the fluorescence emission.

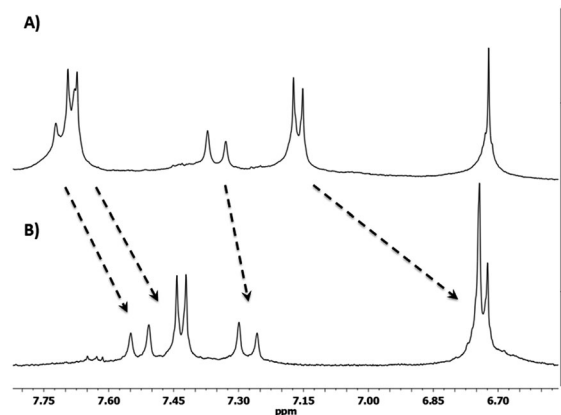
The selectivity of probe **1** for the sulfide species was also investigated. To that end, fluorescence and electronic absorption spectral data were collected for other reactive sulfur species (RSS), reactive oxygen species (ROS) and reactive nitrogen species (RNS). Emission data provide clear evidence that

Fig. 2 Absorption and emission spectra of probe **1** (2.0 μM) in 20 mM HEPES: CH_3CN (40 : 60, v/v, pH = 7.20, 25 $^\circ\text{C}$) in absence and presence of various anions. Added Na_2S concentration is 50 μM and anion concentrations were 100 μM . Excitation wavelength is 650 nm. Experiments were done in triplicate.

probe **1** offers very good selectivity for sulfide ions (Fig. 2a). No other species was able to reduce the two azide groups to amine groups; as a result no changes in the fluorescence emission spectra were observed. Competition experiments in the presence of a number of competing anions were also conducted (ESI^\dagger), again corroborating the selectivity of the probe. The electronic absorption spectra were unchanged as well. We also note that 1000 equivalents of glutathione or cysteine do not cause any changes in the emission intensity or absorbance spectrum of probe **1** (Fig. 2).

An NMR titration experiment was performed in order to investigate the changes in the chemical shifts of the aromatic protons during the reduction. Fig. 3 shows the partial ^1H NMR spectra of the probe before (Fig. 3a) and after (Fig. 3b) Na_2S addition in CD_3CN . The NMR spectrum clearly shows that all the aromatic protons of probe **1** were shifted upfield due to the formation of the electron donor amine group as expected. In addition, mass spectral (HRMS, ESI^\dagger) data following sulfide treatment of probe **1**, supports our structure assignment for the reduction product.

We also wanted to demonstrate the utility of probe **1** in living cells (Fig. 4). Human breast adenocarcinoma cells (MCF-7) were grown to confluence at 37 $^\circ\text{C}$ under 5% CO_2 in Dulbecco's Modified Eagle Serum (DMEM) containing 1% penicillin/streptomycin, 10% fetal bovine serum (FBS) and

Fig. 3 Stacked partial ^1H -NMR spectra of probe **1** (A) and the same spectrum after the addition of Na_2S (B) in acetonitrile- D_3 at 25 $^\circ\text{C}$.

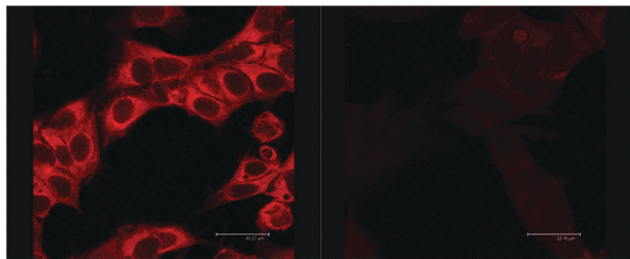


Fig. 4 Confocal microscopy images of showing the H₂S response of probe **1** in MCF-7 cells. (left) MCF-7 cells incubated with probe **1** (4 μM) for 30 min at 37 °C. (right) MCF-7 cells incubated with probe **1** (4 μM) for 2 h, after which 100 equiv. Na₂S was added. The cells were imaged after additional incubation for 30 min at 37 °C.

2 mM L-glutamine. MCF-7 cells were incubated with 4.0 μM probe **1** for 30 min at 37 °C and then washed with physiological saline to remove any excess amount of the probe. Under these conditions, confocal microscope imaging shows intense intracellular red fluorescence emission upon excitation at 633 nm. The cells, which were treated with probe **1**, were then incubated with 400 μM Na₂S in HEPES buffer for 2 hours at 37 °C. The quenching of intracellular fluorescence intensity of probe **1** was clearly visible under a microscope for the sulfide treated cells. Again, as noted for the spectroscopic experiments, the quenching seems to be only limited by the duration of incubation; the reaction seems to be very fast. Confocal microscope imaging was performed on a Leica TCS SP2 laser scanning microscope with an oil-immersion 40× objective lens.

In conclusion, we have developed a sensitive H₂S detection probe which operates much faster than the existing H₂S probes. The azide based probe **1** responds to the H₂S through reduction of two azido groups, resulting in instant quenching of the emission and a noticeable red shift in the absorbance spectrum. The highly selective and sensitive nature of probe **1** for H₂S over other reactive species demonstrates the potential utility of probe **1**. Moreover, due to the presence of hydrophilic moieties in its structure, the probe is water soluble and thus appropriate for biological applications. Thus, the added H₂S was successfully imaged inside the cells, suggesting the possibility of imaging endogenously produced H₂S in real-time and in the near IR region of the spectrum.

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