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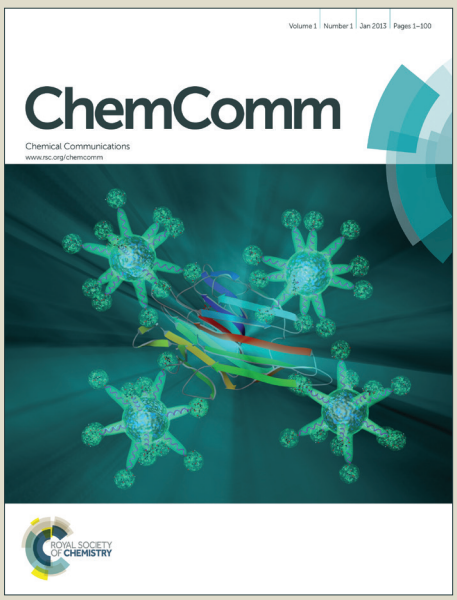

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Fast Responding and Selective Near-IR Bodipy Dye for Hydrogen Sulfide Sensing

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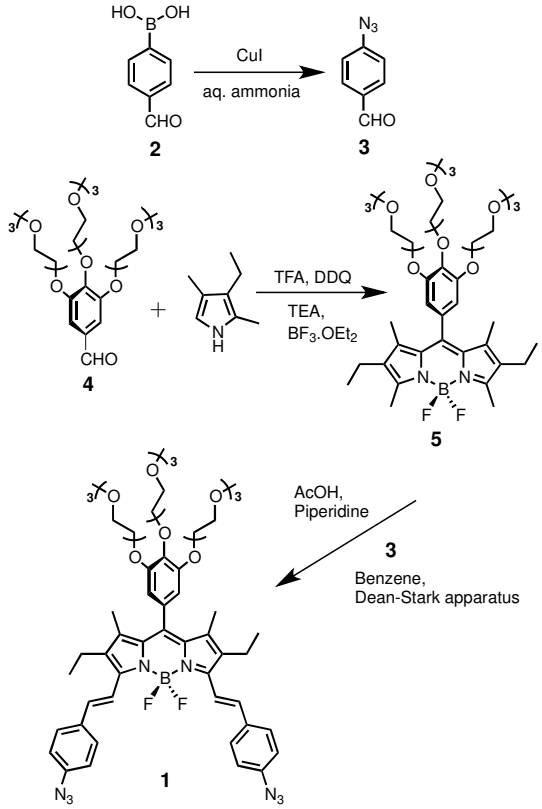
Tugba Ozdemir,[a] Fazli Sozmen,[a] Sevcan Mamur,[c] Turgay Tekinay,[c] and Engin U. Akkaya*[,a,b]

A Bodipy based, highly selective probe for hydrogen sulfide has been designed, synthesized and demonstrated to detect H_2S 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 (H_2S) 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 H_2S 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]

H_2S is an example of reactive sulfur species such as, thiols, *S*-nitrosothiols, sulfenic acids and sulfite, produced enzymatically from cysteine with 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 H_2S 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 exciting 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 H_2S probes due to their desirable properties such as; high quantum yields, chemical and photochemical stability, high molar absorption coefficients, and the fact that



Scheme 1. Synthesis of target probe **1**.

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they allow straightforward access to near-IR emitting derivatives.[8]

In this work, 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, compound **3** and **4** were prepared according to literature reports. Then, Bodipy **5** was obtained by the reaction of the aldehyde **4** and 3-ethyl-2,4-dimethylpyrrole. Finally, following recently established protocols, condensation reaction of *p*-azidobenzaldehyde **3** yielded the probe **1** (Φ_f= 0.38, cresyl violet in ethanol as a reference dye) in analytically pure state following chromatographic purifications. This reaction allowed not only the formation of target probe in good yields, but also emission and absorption wavelengths of the probe were shifted to near-IR.

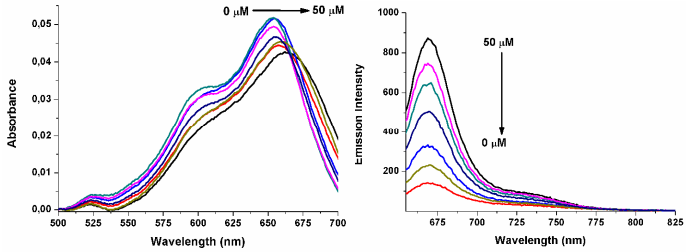

Figure 1. Absorption and emission spectra of **1** (2.0 μM) in 20 mM HEPES:CH_3CN (40:60, v/v, pH=7.20, 25 °C) in increasing Na_2S concentrations. Excitation wavelength is 650 nm. Experiments were done in triplicate.

H_2S probes functioning through the reduction of azido group 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 H_2S with no time delay, practically immediately as the reagents are mixed.

Upon titration of probe **1** with various concentrations of Na_2S at room temperature in acetonitrile-buffer mixture (20 mM HEPES/CH_3CN, 40:60, v/v, pH=7.20, 25 °C), the red shift about 20 nm was observed in the electronic absorption spectrum (Figure 1a), the color change was easily noticeable with naked eye. The actual ratio of $S^{2-}/HS^-/H_2S$ concentrations (speciation) is dictated by the pH of the buffer.

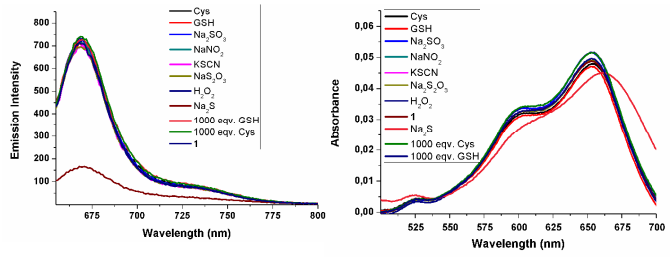

Figure 2. Absorption and emission spectra of probe **1** (2.0 μM) in 20 mM HEPES:CH_3CN (40:60, v/v, pH=7.20, 25 °C) in absence and presence of various anions. Added Na_2S concentration is 50 μM and anion concentrations were 100 μM. Excitation wavelength is 650 nm. Experiments were done in triplicate.

As shown in the fluorescence spectra (Figure 1b), the emission of probe **1** was quenched with increased Na_2S concentrations at room temperature in 20 mM HEPES/CH_3CN solutions (40:60, v/v, pH=7.20, 25 °C). The detection limit was determined (ESI) to be 0.34 μM.

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

Selectivity of the probe **1** for sulfide species was also investigated. To that end, fluorescence and electronic absorption spectral data were collected with other reactive sulfur species (RSS), reactive oxygen species (ROS) and reactive nitrogen species (RNS). Emission data provides clear evidence that the probe **1** offers very good selectivity for sulfide ions (Figure 2a). No other species were 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 done (ESI), 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 the probe **1** (Figure 2).

An NMR titration experiment was performed in order to investigate the changes in the chemical shifts of the aromatic protons during the reduction. Figure 3 shows the partial ^1H NMR spectra of the probe before (Figure 3a) and after (Figure 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 electron donor amine group as expected. In addition, mass spectral (HRMS, ESI) data following sulfide treatment of the probe **1**, supports our structure assignment for the reduction product).

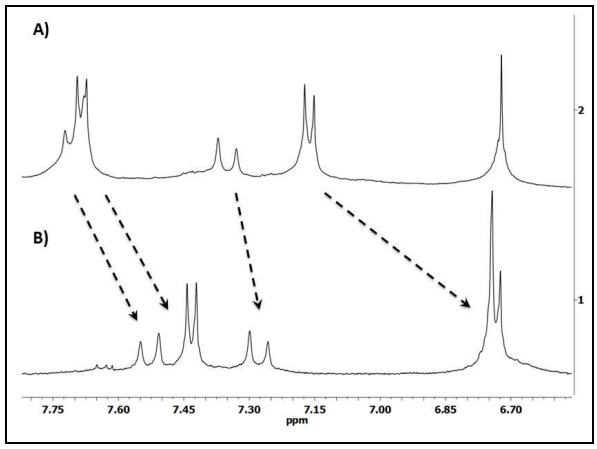

Figure 3. Stacked partial ^1H-NMR spectra probe **1** (A) and the same spectrum after the addition of Na_2S (B) in acetonitrile-D_3 at 25 °C.

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We also wanted to demonstrate the utility of the probe **1** in living cells (Figure 4). Human breast adenocarcinoma cells (MCF-7) were grown to confluence at 37°C under 5% CO_2 in Dulbecco's Modified Eagle Serum (DMEM) containing 1% penicillin/streptomycin, 10% fetal bovine serum (FBS) and 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 image 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 Na_2S in HEPES buffer for 2 hours at 37 °C. Under microscope, the quenching of intracellular fluorescence intensity of the probe **1** was clearly visible 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 with a Leica TCS SP2 laser scanning microscope with an oil-immersion 40x objective lens.

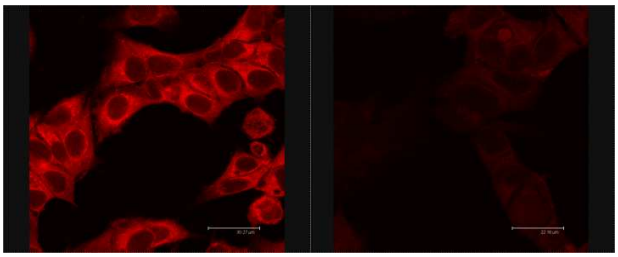

Figure 4. Confocal microscopy images of showing the H_2S response of probe **1** in MCF-7 cells. (Left) MCF-7 cells incubated with probe **1** (4 μM) for 30 min. at 37 °C (Right) MCF-7 cells incubated with probe **1** (4 μM) for 2 h, after which 100 eqv. Na_2S was added. The cells were imaged after additional incubation for 30 min. at 37 °C.

In conclusion, we developed a sensitive H_2S detection probe which is operating much faster compared to existing H_2S probes. Azide based probe **1** gives response the H_2S through reduction of two azido groups, resulting instant quenching of the emission and noticeable red shift in absorbance. The highly selective and sensitive nature of the probe **1** for H_2S over other reactive species demonstrates the potential utility of the 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, added H_2S was successfully imaged inside the cells, suggesting a possibility of imaging endogenously produced H_2S in real-time and in the near IR region of the spectrum.

Notes and references

[a] UNAM-National Nanotechnology Research Center, Bilkent University, 06800 Ankara, Turkey.

[b] Department of Chemistry, Bilkent University, 06800 Ankara, Turkey.

[c] Life Sciences Practice and Research Center, Gazi University, 06500 Ankara, Turkey.

† Electronic Supplementary Information (ESI) available: : Experimental procedures, structural proofs. See DOI: 10.1039/c000000x/

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