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An ESIPT-based highly selective and sensitive probe for the detection of hydrogen sulfide

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ABSTRACT

A new excited state intramolecular proton transfer dye with red keto emission (620 nm) and remarkably large Stokes shift (240 nm) was discovered. Based on its unique red emission and large Stokes shift, it was developed to a novel fluorescent probe which can highly selectively and sensitively detect hydrogen sulfide.

Hydrogen sulfide (H_2S), well-known for its unpleasant rotten egg smell and traditionally considered as a toxic gas,[1] can be endogenously produced by enzymes such as cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopyruvate sulfurtransferase in mammalian systems.[1,2] Nevertheless, recent studies have demonstrated that H_2S can work as gaseous signaling compound (gasotransmitter) with importance on a par with that of the other two known endogenous gasotransmitters, nitricoxide (NO) and carbon monoxide (CO).[3] Moreover, H_2S has been recognized for mediating a wide range of physiological effects, such as vasodilation,[4] anti-oxidation,[5] anti-apoptosis,[6] and antiinflammation.[7] Any imbalance in H_2S has also been recognized to connect to various diseases such as Alzheimer's and Down's syndrome.[8] Therefore, accurate and reliable measurement of H_2S concentrations is needed and can provide useful information to study the function of H_2S in depth.

A number of techniques including polarography, gas chromatography, and colorimetry are available for the detection of H_2S.[9] However, these techniques require complicated procedures which do not allow for temporal monitoring and are destructive in nature. In recent years, fluorescent sensing has received great attention due to its simple operation and high sensitivity.[10] Thus, a fluorophore with a high quantum yield and large Stokes shift draws our interests. Recently, 2-(2-hydroxyphenyl) benzothiazole (HBT) and 2-(2′-hydroxyphenyl) benzoxazole (HBO) have received considerable attention because of their separated dual fluorescence in a four-level cycle.[11] In the ground state, HBT adopts the enol (E) form stabilized by intramolecular hydrogen bonding Upon photoexcitation at 320 nm, the excited enol (E^*) is quickly converted into the excited keto (K^*) tautomer by ESIPT on a subpicosecond time scale, which gives rise to an emission band (~500 nm) with a large Stokes shift (~180 nm). Nevertheless, the emission color of HBT/HBO is limited in the blue and green region, which may restrain the potential of their biological applications due to poor penetration and high photodamage.[12] Therefore, it is of great value to modify the structure of HBT/HBO and make the emission wavelength into the red region. On the other hand, many reported HBT/HBO derivatives still suffer from various shortcomings involving short emission wavelength, obvious hypochromatic shift, or the blockage of ESIPT process resulting in small Stokes' shift in strong polar solvents such as acetonitrile, methanol, and water.[13] Thus, it is challenging to tune the emission of HBT/HBO derivatives to longer wavelength, and still retain the ESIPT process for large Stokes' shift. To the best of our knowledge, among all the probes for H_2S detection based on ESIPT mechanism, rare of them have been found to have an emission exceed 600 nm, and most of them are at below 500 nm which may restrict their potential applications.[14] In this work, we present the development of HBTPP-S as a novel red fluorescent H_2S sensor based on 'turn-on' ESIPT mechanism (Scheme 1).

In a typical ESIPT process, upon photoexcitation, HBT can undergo fast proton transfer reaction in a sub picosecond time scale and imino nitrogen is converted into an amine group which

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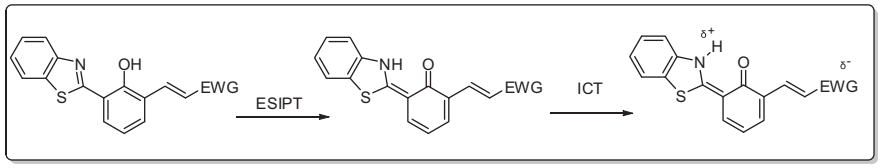
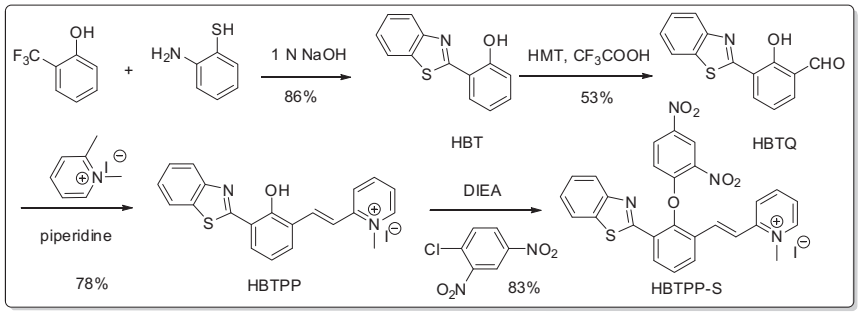


Scheme 1. Our design strategy.



Scheme 2. Synthetic scheme of HBTPP-S.

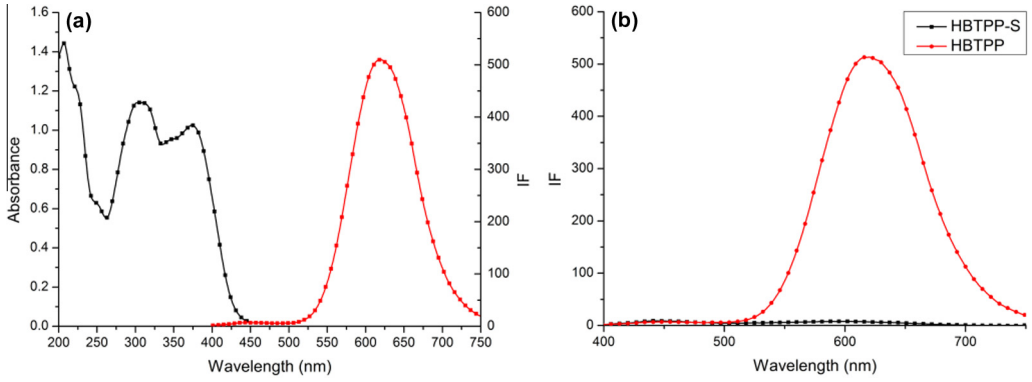


Figure 1. (a) Absorption and fluorescence spectra of HBTPP. (b) Fluorescence spectra of HBTPP and HBTPP-S. All absorption and fluorescence were recorded in a mixed solution of CH_3CN/PBS (50:50, v/v, pH = 7.4, 10 mM).

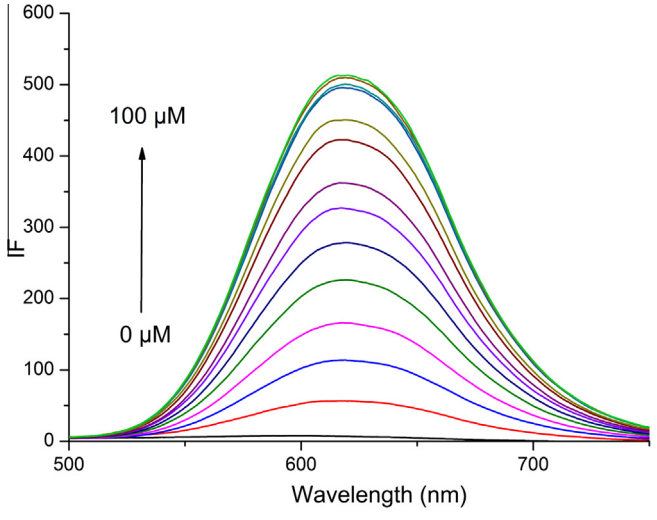


Figure 2. Emission spectra of probe HBTPP-S (5.0 μM) in a mixed solution of CH_3CN/PBS (50:50, v/v, pH = 7.4, 10 mM) upon addition of NaHS with λ_{ex} = 380 nm.

can serve as an electron donor group.[15] As we all know, when a dye possesses an electron rich group conjugated to an electron deficient group as a push–pull structure, intramolecular charge transfer (ICT) from the donor to the acceptor will proceed upon excitation.[16] Thus, if we appended an electron acceptor group in the *ortho*-position of phenol through double bond, it is logical to expect that the amine group formed by ESIPT process is capable of effective charge transfer into the electron acceptor unit and triggers the strong push–pull ICT interaction. This ESIPT coupled ICT process may lead to dramatic changes in fluorescent properties involving red shift keto emission and large Stokes shift. Furthermore, the ESIPT process can be hampered by a specific protecting group. Such a protected phenol can be used as a 'turn-on' molecular probe for detection or imaging of an analyte that can react with the probe to remove the protecting group (Scheme 2).

To test the above-mentioned hypothesis, we judiciously constructed HBTPP by the condensation reaction between HBTQ and 1,2-dimethylpyridin-1-ium iodide. HBTQ was prepared by the modified Duff reaction starting from HBT, which was synthesized by the established literature procedure.[17] With HBTPP in hand, we developed a fluorescent probe by connecting with 4-chloro-1,2-dinitrobenzene known as the protecting group for tyrosine in peptide synthesis.[18] These compounds synthesized were fully characterized by the standard NMR and mass spectrometry.

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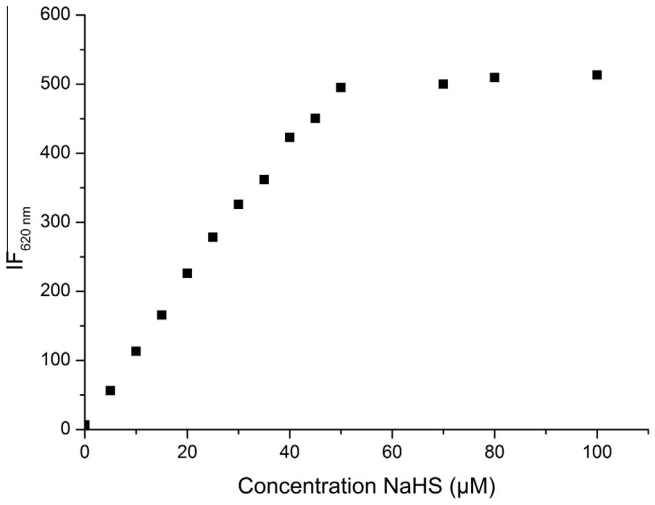

Figure 3. Fluorescence intensity changes at 620 nm of the probe HBTPP-S (5.0 μM) with the amount of NaHS in a mixed solution of CH_3CN/PBS (50:50, v/v, pH = 7.4, 10 mM).

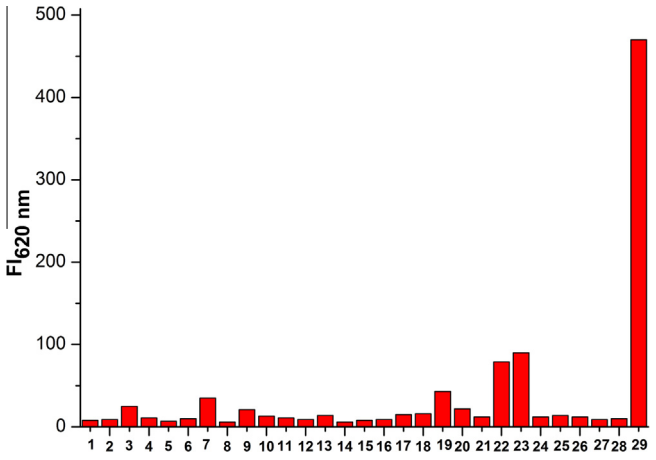

Figure 4. Fluorescence intensities of probe HBTPP-S upon addition of various biologically relevant species in a mixed solution of CH_3CN/PBS (50:50, v/v, pH = 7.4, 10 mM). Red bars representative anions, metal ions, reactive oxygen species, reactive nitrogen species, reducing agents, small-molecule thiols, and NaHS. F^-, Br^-, I^-, NO_3^-, AcO^-, CO_3^{2-}, and $H_2PO_4^-$, and CN^- (0.25 mM for each); K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} (1 mM for each); H_2O_2, ClO^-, ·OH, $^1O_2$, and NO (50 μM for each); SO_3^{2-}, $S_2O_3^{2-}$ (100 μM for each); 1 mM for ascorbic acid, cysteine, N-acetyl-cysteine(NAC), $C_6H_5NH_2$, $C_6H_5CH_2NH_2$, $H_2NCH_2CH_2NH_2$, $HOCH_2CH_2NH_2$; 10 mM for GSH, and 50 μM for NaHS. 1, probe HBTPP-S alone; 2, F^-; 3, Br^-; 4, I^-; 5, NO_3^-; 6, ACO^-; 7, CO_3^{2-}; 8, CN^-; 9, $H_2PO_4^-$; 10, K^+; 11, Ca^{2+}; 12, Mg^{2+}; 13, Zn^{2+}; 14, H_2O_2; 15, ClO^-; 16, ·OH; 17, $^1O_2$; 18, NO; 19, ascorbic acid; 20, SO_3^{2-}; 21, $S_2O_3^{2-}$; 22, cysteine; 23, GSH; 24, *N*-acetyl-cysteine(NAC); 25, $C_6H_5NH_2$; 26, $C_6H_5CH_2NH_2$; 27, $H_2NCH_2CH_2NH_2$; 28, $HOCH_2CH_2NH_2$; 29, NaSH.

The UV–vis spectrum of HBTPP exhibits major absorption peaks with maximum at 380 nm (Fig. 1). As expected, the fluorescence spectrum indeed shows emission peak in the red region at a wavelength of 620 nm, which is preferable for in vivo bioimaging due to the deep penetration ability and low background autofluorescence.[19] The obtained large Stokes shift between the excitation and the emission wavelengths of about 240 nm is a desirable feature for fluorescence probes, which assists in increasing the signal-to-noise ratio. Next, we measured the fluorescence spectrum of HBTPP-S in comparison with that of HBTPP. Since the phenolic hydroxyl group was masked by dinitrophenyl group resulting in the lost of ESIPT, as predicted, non-fluorescence was observed for HBTPP-S.

Subsequently, we performed the fluorescence titration studies of HBTPP-S toward NaSH (commercially available H_2S donor) in a mixed solution of CH_3CN/PBS (50:50, v/v, pH = 7.4, 10 mM). The absorption and fluorescence spectra of the solution of HBTPP-S treated with a series of NaHS (0–100 μM) were recorded. As shown in Figure 2, upon addition of NaHS, the initial absorption peak centered at 320 nm decreased gradually, along with a simultaneous emergence of the red-shifted new absorption peak at 380 nm. Moreover, the isosbestic point at 348 nm was observed. The free HBTPP-S displayed quite weak fluorescence. Importantly, with the addition of NaHS, as shown in Figure 3, the fluorescence intensity of HBTPP-S increased significantly at 620 nm due to the thiolysis of the dinitrophenyl ether by H_2S. In addition, the increase in fluorescence intensity is in a concentration dependent manner, reaching a maximum when the concentration of NaHS is at 50 μM.

To evaluate the specific nature of HBTPP-S for H_2S, we then examined the fluorescence enhancement of HBTPP-S incubated with various species (Fig. 4), most of which are biologically related. As expected, upon addition of the representative anions (F^-, Br^-, I^-, NO_3^-, AcO^-, CO_3^{2-}, and $H_2PO_4^-$) at 0.25 mM, metal ions (K^+, Ca^{2+}, Mg^{2+}, and Zn^{2+}) at 1 mM, reactive oxygen and nitrogen species (H_2O_2, ClO^-, ·OH, $^1O_2$, and NO), and reducing agents (ascorbic acid, SO_3^{2-}, $S_2O_3^{2-}$) at the biologically relevant concentrations, probe HBTPP-S shows no marked fluorescence enhancement. It should be noted that small-molecule thiols such as glutathione (GSH) at 10 mM and cysteine at 1 mM triggered only a small fluorescence enhancement and have nearly no interference to H_2S detection. By contrast, upon treatment of NaHS (50 μM) with the probe, a large fluorescence signal was observed. Therefore, the probe HBTPP-S shows high selectivity for the detection of H_2S (Fig. 4).

In conclusion, we have designed and synthesized HBTPP-S as a new ESIPT fluorescent probe for detection of H_2S based on thiolysis of dinitrophenyl ether. Due to the rapid conversion to the fluorescent compound HBTPP by H_2S, the solution of HBTPP-S shows remarkably red keto emission (620 nm) and large Stokes shift (240 nm), which is preferable for in vivo bioimaging. It is expected that HBTPP will be useful as a new platform for the development of various fluorescent probes and provided insight into the development of novel fluorescent chromophores based on ESIPT process.

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