ORGANIC LETTERS

2013
Vol. 15, No. 21
5412–5415

A Chemodosimeter for the Ratiometric Detection of Hydrazine Based on Return of ESIPT and Its Application in Live-Cell Imaging

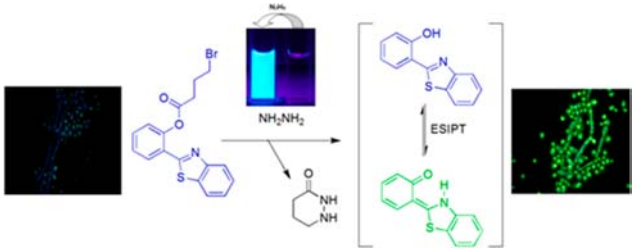
Shyamaprosad Goswami,*,† Sangita Das,† Krishnendu Aich,† Bholanath Pakhira,† Sukanya Panja,‡ Subhra Kanti Mukherjee,‡ and Sabyasachi Sarkar†

Department of Chemistry, Bengal Engineering and Science University, Shibpur, Howrah 711103, West Bengal, India and Department of Microbiology, The University of Burdwan, Burdwan 713101, West Bengal, India

spgoswamical@yahoo.com

Received August 14, 2013

ABSTRACT



A probe based on 2-(2′-hydroxyphenyl) benzothiazole (HBT) has been synthesized and used for the ratiometric detection of hydrazine. The probe is designed in such a way that the excited state intramolecular proton transfer (ESIPT) of the HBT moiety gets blocked. The chemodosimetric approach of hydrazine to the probe results in the recovery of the ESIPT by removal of a free HBT moiety through subsequent substitution, cyclization, and elimination processes. The probe is successfully demonstrated to enable the detection of hydrazine in live cells.

Hydrazine is well-known because of its flammable nature, and it finds wide use in rocket fuels.[1] It has high reactivity as a base and it can be used as a good reducing agent.[2] It plays essential roles in the chemical, pharmaceutical and agricultural industries as catalyst, corrosion inhibitor, textile dye, and pharmaceutical intermediate etc.[3]

By several yeasts and anammox organisms (anaerobic ammonia oxidizers) like *Brocadia anammoxidans*, hydrazine is formed in nature. Hydrazine is highly toxic and may affect the lungs, liver, spleen, and thyroid of humans and animals upon inhalation. It has been reported that hydrazine has mutagenic and carcinigenic effects.[4] According to the U.S. Environmental Protection Agency (EPA), "Increased incidences of lung, nasal cavity, and liver tumors have been observed in rodents exposed to hydrazine".[5] In fact, it has been classified as a potential human carcinogen by the EPA and the World Health Organization and suggested a low threshold limit value (TLV) of 10 ppb.[6] Therefore, the selective and sensitive detection of a trace amount of hydrazine has gained increasing attention. In recent years, the fluorometric method has been

† Bengal Engineering and Science University
‡ The University of Burdwan

(1) Zelnick, S. D.; Mattie, D. R.; Stepaniak, P. C. *Aviat., Space Environ. Med.* **2003**, *74*, 1285–1291.

(2) (a) Hydrazine and its derivatives. In *Kirk-Othmer Encyclopedia of Chemical Technology*, 5th ed.; Kroschwitz, J. I., Seidel, A., Eds.; Wiley: NewYork, 2005; Vol. 13, pp 562−607. (b) Ragnarsson, U. *Chem. Soc. Rev.* **2001**, *30*, 205–213.

(3) (a) Narayanan, S. S.; Scholz, F. *Electroanalysis* **1999**, *11*, 465–469. (b) Yamada, K.; Yasuda, K.; Fujiwara, N.; Siroma, Z.; Tanaka, H.; Miyazaki, Y.; Kobayashi, T. *Electrochem. Commun.* **2003**, *5*, 892–896.

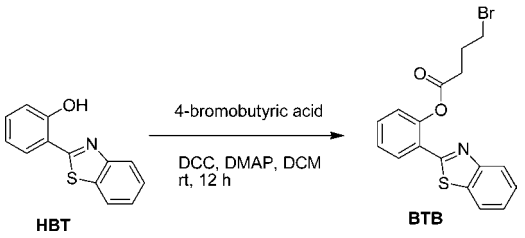
(4) International Agency for Research on Cancer. Re-evaluation of some organic chemicals, hydrazine, and hydrogen peroxide. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*; IARC: Lyon, 1999; Vol. 71, pp 991−1013; http://monographs.iarc.fr/ENG/Monographs/vol71/mono71-43.pdf.

(5) U.S. Environmental Protection Agency. *Integrated Risk Information System (IRIS) on Hydrazine/Hydrazine Sulfate*; National Center for Environmental Assessment, Office of Research and Development: Washington, D.C., 1999.

(6) (a) Choudhary, G.; Hansen, H. *Chemosphere* **1998**, *37*, 801–843. (b) Umar, A.; Rahman, M. M.; Kim, S. H.; Hahn, Y.-B. *Chem. Commun.* **2008**, 166–168.

10.1021/ol4026759
Published on Web 10/18/2013

Scheme 1. Synthetic Procedure of the Probe (BTB)



extensively used to detect diverse analytes because of its selectivity, sensitivity, easy operation, economy, and real-time detection.[7] In addition, ratiometric sensing is highly recommended because this property makes it feasible to evaluate the analytes more accurately with minimization of the background signal. Despite the carcinogenic and environmental pollutant effect of hydrazine, however, small molecule fluorescent probes for hydrazine detection are still very limited.[8] There are many interesting sensors reported for the chemodosimetric detection of various analytes involving intelligent chemical transformations, mainly the deprotection of a specific protecting group.[9] 2-(2′-Hydroxyphenyl) benzothiazole (HBT) is very familiar because of its intramolecularly hydrogen-bonded property, which exhibits excited state intramolecular proton transfer (ESIPT)[10] through tautomerization. The proton transfer, i.e, tautomerization, affords a huge bathochromic shift in the emission signal. There are a number of reactive probes reported based on the HBT moiety for the selective detection of different analytes via "protection−deprotection" sequence.[11] Inspired by all of these works, we report here a new bromo-ester derivative of 2-(2′-hydroxyphenyl) benzothiazole compound that selectively gets deprotected by hydrazine through substantial substitution−cyclization−elimination sequence to yield a deprotected HBT moiety. After the hydrazinolysis, the original phenolic form of the hidden probe becomes free again with the recovery of its excited state intramolecular proton transfer (ESIPT) property. To the best of our knowledge, this is the first example of a chemodosimetric detection of hydrazine using ESIPT mechanism with subsequent substitution−cyclization−elimination sequence. As shown in Scheme 1, the probe (BTB) was synthesized from the coupling reaction of 2-(2′-hydroxyphenyl) benzothiazole with 4-bromobutyric acid using DCC as coupling agent. This product (BTB) was well characterized by ^{1}H NMR, ^{13}C NMR, and HRMS (Figures S14−S17, Supporting Information).

The photophysical properties of BTB were investigated by monitoring the absorption and emission behavior upon addition of several anions, cations, and neutral bases, viz. Br^-, Cl^-, F^-, CN^-, HSO_3^-, I^-, NO_2^-, NO_3^-, N_3^-, HSO_4^-, SO_4^{2-}, SO_3^{2-}, PPi, PO_4^{3-}, S^{2-} (as their sodium salts), NH_2OH, NH_3, N_2H_4, ethylene diamine, Mg^{2+}, Cu^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+} and Mn^{2+} (as their chloride salts). Only hydrazine has been successful to perturb the absorption and emission signal of BTB, but other analytes cannot affect the absorption and emission profiles (Figure S5, Supporting Information). They are almost nonresponsive. The chromogenic signaling behavior of BTB (10 μM) was investigated in CH_3CN:H_2O (2:3, v/v, pH = 7.4, 1 mM HEPES buffer, 25 °C) solution, where it revealed a strong maximum at 285 nm. Upon incremental addition of hydrazine (0−2 equiv), the peak at 285 nm evidently decreased, and a new peak at 343 nm appeared with an isosbestic point at 329 nm. A moderate absorption band at 343 nm, characteristic of HBT, was detected upon addition of hydrazine (Figure 1). The change in the absorbance at 285 and 343 nm by hydrazine-assisted demasking process was identified by ratiometric analysis for the conversion of BTB to HBT moiety. Additionally, the absorbance ratio (A_{343}/A_{285}) at the two characteristic wavelengths of

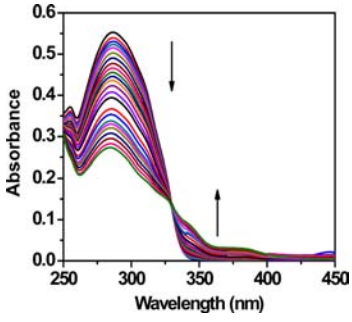


Figure 1. Change of UV−vis spectra of BTB (10 μM) with incremental addition of hydrazine (0−2 equiv). Spectra were recorded after 60 s of each mixing.

285 and 343 nm was measured after addition of 2 equiv of hydrazine, and a linear relationship was observed between (A_{343}/A_{285}) with [N_2H_4] in the range of 0−9 μM (Figure S4, Supporting Information). We elaborated the fluorogenic signaling behavior of BTB (10 μM) in CH_3CN:H_2O (2:3, v/v, and pH = 7.4, 1 mM HEPES buffer, 25 °C) solution toward the detection of hydrazine. In absence of hydrazine, the fluorescence profile of BTB shows only a

(7) (a) Zhang, J. F.; Zhou, Y.; Yoon, J.; Kim, J. S. *Chem. Soc. Rev.* **2011**, *40*, 3416–3429. (b) Chen, X.; Tian, X.; Shin, I.; Yoon, J. *Chem. Soc. Rev.* **2011**, *40*, 4783–4804. (c) Chen, X.; Pradhan, T.; Wang, F.; Kim, J. S.; Yoon, J. *Chem. Rev.* **2012**, *112*, 1910–1956.

(8) (a) Choi, M. G.; Hwang, J.; Moon, J. O.; Sung, J.; Chang, S.-K. *Org. Lett.* **2011**, *13*, 5260–5263. (b) Choi, M. G.; Moon, J. O.; Bae, J.; Lee, J. W.; Chang, S.-K. *Org. Biomol. Chem.* **2013**, *11*, 2961–2965. (c) Fan, J.; Sun, W.; Hu, M.; Cao, J.; Cheng, G.; Dong, H.; Song, K.; Liu, Y.; Sun, S.; Peng, X. *Chem. Commun.* **2012**, *48*, 8117–8119. (d) Chen, X.; Xiang, Y.; Li, Z.; Tong, A. *Anal. Chim. Acta* **2008**, *625*, 41–46. (e) Zhu, S.; Lin, W.; Yuan, L. *Anal. Methods* **2013**, *5*, 3450–3453. (f) Li, K.; Xu, H.-R.; Yu, K.-K.; Hou, J.-T.; Yu, X.-Q. *Anal. Methods* **2013**, *5*, 2653–2656. (g) Hu, C.; Sun, W.; Cao, J.; Gao, P.; Wang, J.; Fan, J.; Song, F.; Sun, S.; Peng, X. *Org. Lett.* **2013**, *15*, 4022–4025.

(9) (a) Quang, D. T.; Kim, J. S. *Chem. Rev.* **2010**, *110*, 6280–6301. (b) Yang, Y.; Zhao, Q.; Feng, W.; Li, F. *Chem. Rev.* **2013**, *113*, 192–270.

(10) Sytnik, A.; Kasha, M. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 8627–8630.

(11) (a) Hu, R.; Feng, J. A.; Hu, D. H.; Wang, S. Q.; Li, S. Y.; Li, Y.; Yang, G. Q. *Angew. Chem., Int. Ed.* **2010**, *49*, 4915–4918. (b) Kim, T. I.; Kang, H. J.; Han, G.; Chung, S. J.; Kim, Y. *Chem. Commun.* **2009**, 5895–5897. (c) Xu, Z.; Xu, L.; Zhou, J.; Xu, Y.; Zhu, W.; Qian, X. *Chem. Commun.* **2012**, *48*, 10871–10873. (d) Santra, M.; Roy, B.; Ahn, K. H. *Org. Lett.* **2011**, *13*, 3422–3426. (e) Maity, D.; Kumar, V.; Govindaraju, T. *Org. Lett.* **2012**, *14*, 6008–6011.

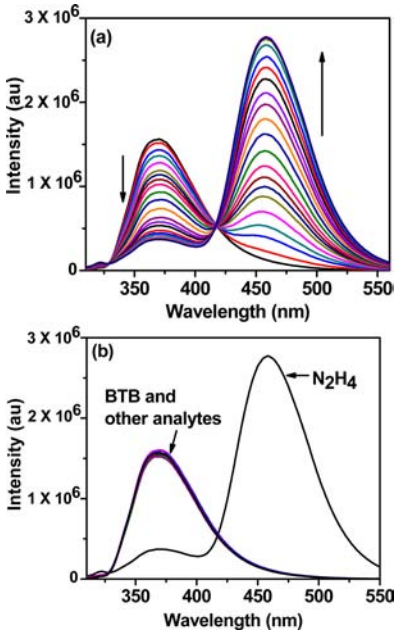
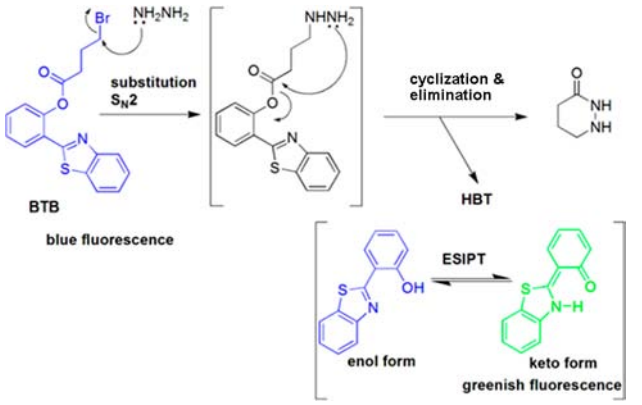


Figure 2. (a) Change of emission spectra of BTB (10 μM) with incremental addition of hydrazine (0−2 equiv). Spectra were recorded after 60 s of each mixing. (b) Fluorescence spectra of BTB (10 μM) in the presence of hydrazine and other representative cations, anions, and organic bases (2 equiv each). λ_{ex} = 300 nm.

strong signal at 368 nm (blue emission) upon excitation at 300 nm, which corresponds to the "enol-form" emission of HBT. But upon interaction with hydrazine, a prominent new emission band at 458 nm (greenish emission, correponds to "keto-form") developed, and upon progressive addition of hydrazine, the peak at 458 nm rapidly increased, and a good ratomertic spectrum was obtained with a well-defined isoemission point at 417 nm (Figure 2). These changes in the fluorescence spectra stopped when the amount of added hydrazine reached 1 equiv. At this moment the ratio of the emission intensities at 368 and 458 nm (I_{458}/I_{368}) became as high as 88 times as in the absence of hydrazine. The color of the fluorescence clearly changed from blue to bluish-green, which was observed by the naked eye after illumination under UV light. A linear relationship was observed between the ratio of fluorescence intensities (I_{458}/I_{368}) and concentration of hydrazine from 1−9.5 μM (Figure S3, Supporting Information). From the concentration-dependent fluorescence emission changes, the detection limit of BTB for the determination of hydrazine was estimated to be 6.6×10^{-8} M (2.2 ppb), which was much lower than that of TLV (10 ppb) recommended by the EPA and WHO.

The plausible mechanism behind the return of the ESIPT through the chemical transformation of BTB to HBT, induced by hydrazine, is shown in Scheme 2. The reaction of BTB with N_2H_4 was illustrated by involving two steps, at first the nucleophilic substitution to the bromo group and then the second nucleophilic addition

Scheme 2. Possible Mechanism of the Response of BTB to Hydrazine



to the ester carbonyl with subsequent intramolecular cyclization to release the HBT moiety. Thus was generated the phenolic HBT, which rapidly transformed to the keto-form upon excitation (ESIPT), and this is responsible for the green emission at a longer wavelength (458 nm, Stokes shift = 90 nm). The charge surface diagram supports the reaction sequence (Figure S11, Supporting Information). As shown in this diagram, the C-atom attached with bromo group is slightly positively charged (may be because of the −I effect of −Br group). Thus the first possible nucleophilic attack of hydrazine occurs at that center. To ensure the hydrazine induced chemical transformation of BTB to HBT, we have compared the emission spectra of free HBT with BTB after addition of hydrazine, and the result agrees well with the proposed hydrazine induced deprotection of BTB (Figure S7, Supporting Information). The ^{1}H NMR titration spectra of BTB with hydrazine also support the fact (Figure S19, Supporting Information). ESI TOF mass spectra further confirm the sensing mechanism (Figure S18, Supporting Information).

Reaction time is an important factor for chemodosimetric approach; therefore, the time required for the reaction of BTB with hydrazine was investigated in the same experimental condition after addition of 2 equiv of hydrazine. As shown in Figure S1 (Supporting Information), the fluorescence intensity increases with reaction time and then levels off at reaction time less than about 15 min (900 s). From this result, it is concluded that the probe is suitable for the rapid detection of hydrazine. A good linear relationship was observed between fluorescence intensity ratio (I_{458}/I_{368}) and reaction time from 0 to 480 s (Figure S2, Supporting Information). The time vs emission ratio (I_{458}/I_{368}) plot was obtained by using first order rate equation. We get the rate constant = k = (slope × 2.303) = 8.1×10^{-3} s^{-1}.

To further understand the relationship between the structural changes of BTB and after reaction with hydrazine and the optical response of BTB to hydrazine, we carried out a density functional theory (DFT) and time-dependent density functional theory (TD-DFT)

calculations with the B3LYP/6-311+G (d,p) method basis set using the Gaussian 03 program.[12] The HOMO and LUMO of the optimized BTB molecule (Figure 3) showed the expected extended conjugation in its HOMO and electron drift from benzene to ester group. Its electronic spectrum obtained from TD-DFT showed electronic transition (HOMO−LUMO) at 298.27 nm (Figure S10, Supporting Information, gas phase) close to the experimental value in mixed aqueous solution (285.7 nm) (Figure 1).

The TD-DFT calculation on the electronic transition of HBT showed transition from the enol form at 276.6 nm and of the keto form at 312.45 nm; the average of both

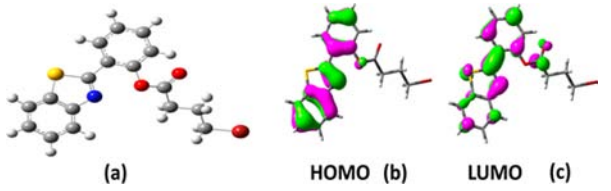


Figure 3. Optimized structure (a), HOMO (b), and LUMO (c) orbitals of BTB calculated at the DFT level using a B3LYP/6-311+G(d,p) basis set.

these transitions is close to the experimental transition. The enol form of HBT is more stabilized (19.38 kcal/mol) compared with its keto form. The HOMO−LUMO orbitals of enol and keto forms of HBT are shown in Figure S9 (Supporting Information).

We performed a biological study to test the ability of BTB to image hydrazine in live cells. As shown in Figure 4, the Candida cells incubated with the probe (10 μM) for 30 min showed a weak blue color emission from the intracellular area, but no green emission was observed. However, the cells treated with the probe were further incubated with hydrazine (20 μM), and the blue fluorescence changed to green. From these cell experiments, it is concluded that BTB could provide ratiometric detection for intracellular hydrazine.

(12) For references, see the Supporting Information.

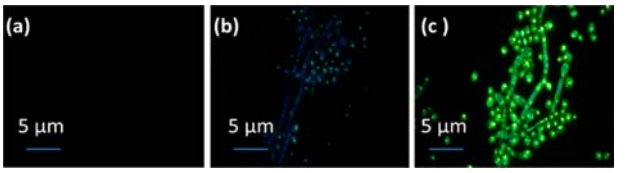


Figure 4. Fluorescence microscope images of (a) *Candida albicans* cells, (b) cells treated with BTB (10 μM), (c) cells treated with BTB (10 μM), further incubated with hydrazine (20 μM). Scale bar: 5 μm.

In order to investigate the practical application of this sensor we performed a "dip-stick" experiment. We prepared TLC plates immersed into acetonitrile solution of BTB, evaporated the solvent to dryness, and then immersed the plate to an N_2H_4 solution, when we observed the color of the plates changing from blue to green under UV light (Figure S8, Supporting Information).

Thus, in this letter, we have designed and synthesized a reactive probe for hydrazine based on the HBT moiety. The probe (BTB) exhibits sharp hydrazine promoting changes in the intensity ratios of both absorption and emission due to return of the ESIPT. DFT and TD-DFT calculations support the phenomenon. Moreover, live-cell imaging experiments establish the utility of this probe for tracking hydrazine in live cells. Thus, this ligand can be used to monitor hydrazine producing strains from natural samples. It can also be used to accurately determine concentration of hydrazine in any sample.

Acknowledgment. Authors thank DST and CSIR, Govt. of India, for financial support. S.D., K.A., and B. P. acknowledge CSIR for providing them with fellowships.

Supporting Information Available. The experimental details, synthetic procedures, characterization data, computational data, UV−vis and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.