Synthesis and characterizations of a highly sensitive and selective fluorescent probe for hydrogen sulfide

Yan Huang, Changyu Zhang, Zhen Xi, Long Yi

PII: S0040-4039(16)30127-7
DOI: http://dx.doi.org/10.1016/j.tetlet.2016.02.017
Reference: TETL 47290

To appear in: *Tetrahedron Letters*

Received Date: 22 November 2015
Revised Date: 30 January 2016
Accepted Date: 3 February 2016

Please cite this article as: Huang, Y., Zhang, C., Xi, Z., Yi, L., Synthesis and characterizations of a highly sensitive and selective fluorescent probe for hydrogen sulfide, *Tetrahedron Letters* (2016), doi: http://dx.doi.org/10.1016/j.tetlet.2016.02.017

ELSEVIER

Tetrahedron Letters

journal homepage: www.elsevier.com

Synthesis and characterizations of a highly sensitive and selective fluorescent probe for hydrogen sulfide

Yan Huang[a,†], Changyu Zhang[a,†], Zhen Xi[b,*], Long Yi[a,*]

[a]*State Key Laboratory of Organic-Inorganic Composites, Beijing University of Chemical Technology, Beijing 100029, China. E-mail: yilong@mail.buct.edu.cn*

[b]*State Key Laboratory of Elemento-Organic Chemistry and Department of Chemical Biology, National Pesticide Engineering Research Center (Tianjin), Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin, 300071, China. E-mail: zhenxi@nankai.edu.cn*

[†] *The authors pay equal contributions to this work.*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Bioimaging
Fluorescence probe
Hydrogen sulfide
NBD amine
Thiolysis

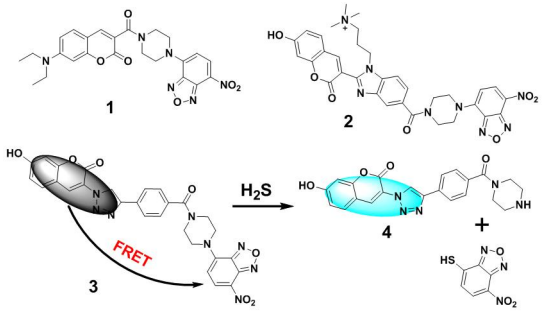
ABSTRACT

Hydrogen sulfide (H_2S) is an important endogenous signaling molecule with a variety of biological functions. To detect H_2S in living biological systems, herein we developed a new fluorescent probe for highly sensitive and selective sensing of H_2S in cells. The probe is based on coumarin-triazole as the fluorophore and thiolysis of the NBD (7-nitro-1,2,3-benzoxadiazole) amine as the receptor. Bioimaging experiments indicated that this probe could be used to monitor H_2O_2-induced H_2S biosynthesis in yeast cells. Our results show that such thiolysis of the NBD amine can be used for development of fluorescent H_2S probes.

Introduction

Hydrogen sulfide (H_2S) is an important endogenous signalling molecule with significantly biological functions.[1-3] The production of endogenous H_2S in different organs and tissues has been majorly attributed to three distinctive enzymatical pathways including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfur transferase (3-MPST) coupling with cysteine aminotransferase (CAT).[2] It has been proved that abnormal endogenous level of H_2S relates to numerous human diseases, including symptoms of Alzheimer's disease, Down syndrome, diabetes and liver cirrhosis.[3] Moreover, H_2S is proposed to play important roles in mediating a wide range of physiological processes, such as neurotransmission, vasodilation, inflammation, oxygen sensing, etc.[4] Although those studies indicated that numerous physiological and pathological processes were linked to levels of H_2S, the molecular mechanisms dictating how H_2S influences cellar signaling and interrelated biological events were insufficient understood. Therefore, it presents significant research value to develop efficient methods for detection of H_2S in biological systems.

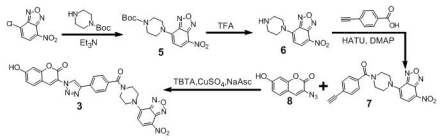
Traditionally, the main methods for H_2S detection are colorimetry, electrochemical assay, gas chromatography and sulfide precipitation.[5] However, recent research indicated that fluorescent methods with excellent sensitivity and selectivity were highly desirable for *in situ* and real-time visualization of H_2S in living biological systems.[6-11] These H_2S probes are mostly based on specific H_2S-induced reactions, including reduction-based probes,[6-8] metal sulfide precipitation-based probes[9] and nucleophile-based probes.[10] We have been interested in the biodetection of H_2S[11] and biothiols[12] for some time. In our previous work, the thiolysis of the NBD (7-nitro-1,2,3-benzoxadiazole) amine was explored for development of a FRET-based H_2S probe **1** (Scheme 1),[11a] which displayed good selectivity for H_2S over biothiols or SO_3^{2-}. However, Roubinet[13] et al. recently reported another NBD-amine-based probe **2** (Scheme 1) which 1) possessed no selectivity for S^{2-} and SO_3^{2-} and 2) could only react with Na_2S, but not NaHS in their tests.[13] To further investigate such thiolysis of the NBD amine for development of fluorescent H_2S probes, herein we reported the synthesis and characterizations of a new NBD-based probe **3**, which could be used to detect H_2S selectively and to monitor the H_2O_2-induced H_2S biosynthesis in yeast cells.



Scheme 1. Chemical structures of NBD-based fluorescent probes **1**-**3** and the reaction of **3** and H_2S to produce **4**.

Herein, we developed a new NBD-based fluorescent probe **3** based on click reaction of alkyne-containing NBD **7** and

azidocoumarin **8** (Scheme 2).[14] The resulted coumarin-triazole fluorophore was water-soluble and bright,[14b] whose fluorescence was quenched by the NBD moiety through the FRET effect, while the NBD fluorophore gave low fluorescence in aqueous solution.[11b] As result, the probe **3** exhibited weak fluorescence in buffer. Fluorescence of the coumarin-triazole could be released after thiolysis of the NBD amine by H_2S.



Scheme 2. The synthesis route for probe **3**.

The absorption spectrum of **3** displayed peaks at 490 nm and 394 nm (Fig. S1), which could be assigned as NBD and coumarin-triazole absorption, respectively. After reacting with H_2S (using Na_2S as an equivalent), the absorption at 490 nm decreased and a new peak at 535 nm appeared.[15] The solubility tests based on absorption spectra indicated that **3** had good water-solubility in PBS buffer (Fig. S2).

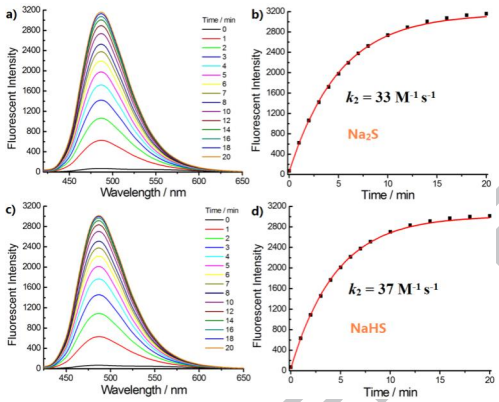


Figure 1. a) Time-dependent fluorescent spectra of 1 μM **1** with 100 μM Na_2S; b) Dynamic simulation (the red solid line) of fluorescence intensity at 486 nm versus time from (a); c) Time-dependent fluorescent spectra of 1 μM **1** with 100 μM NaHS; d) Dynamic simulation (the red solid line) of fluorescence intensity at 486 nm versus time from (c). All experiments were carried out in PBS (pH 7.4, 50 mM, containing 10% DMSO).

We then carried out the time-dependent fluorescent tests in PBS buffer at room temperature (Figure 1). **3** showed very weak background fluorescence at 486 nm, which could enhance about 45-fold upon treatment with 100 μM H_2S (using Na_2S as an equivalent). To obtain the reaction kinetics, the fluorescence signal at 486 nm was plotted as a function of time for data analysis (Figure 1b). The pseudo-first-order rate, k_{obs}, was found to be 3.3×10^{-3} s^{-1} by fitting the fluorescence intensity data with single exponential function. The reaction rate, k_2 ($k_2 = k_{obs}/[H_2S]$), was calculated as 33 M^{-1}s^{-1}. We also used NaHS as an equivalent of H_2S to examine the fluorescent response of **3** toward H_2S (Figure 1c and 1d). Results indicated that fluorescence at 486 nm enhanced about 44-fold, and the reaction rate (k_2) was about 37 M^{-1}s^{-1}. These studies clearly demonstrated that both S^{2-} and HS^- can trigger thiolysis of the NBD amine in physiological buffer. In fact, either Na_2S or NaHS in PBS buffer (pH 7.4) should majorly exist as HS^- anions and H_2S.[7] Therefore, both NaHS and Na_2S could be used as an equivalent H_2S for tests.

Encouraged by the above results, we further checked the fluorescence signal change of probe **3** with various concentrations (0-100 μM) of H_2S (Figure 2). As expected, a strong emission could be detected when the reaction mixture was excited at 394 nm. The fluorescence intensity at 486 nm was linearly related to the concentration of H_2S from 0 to 30 μM (Figure 2b). The detection limit of **3** was determined to be as low as 56 nM based on the 3σ/slope method.[11d] The results demonstrated that probe **3** could react with H_2S highly sensitively.

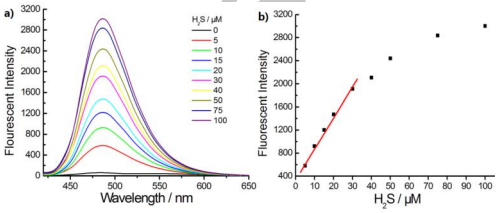


Figure 2. a) Fluorescence response of **3** upon increasing concentrations of H_2S (0-100 μM); b) linear relationship (R = 0.996) between fluorescence intensity at 486 nm of **3** and H_2S concentration.

A major challenge for H_2S detection in biological systems is to develop a selective probe that exhibits notably distinctive response to H_2S over other cellular molecules, especially for millimolar biothiols. To explore the selectivity of **3** for detection of H_2S, it was incubated with various biologically relevant species including reactive sulfur species (glutathione, cysteine, homocysteine, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$), reactive oxygen species (H_2O_2, NaOCl), $NaNO_2$, and cations (Zn^{2+}, Fe^{3+}). The fluorescence increase of millimolar biothiols, however, is far below that of H_2S. Among all the tested species, only SO_3^{2-} showed limited fluorescence response (Figure 3a), which was consistent with our previous observation.[11a] We then examined the competitive selectivity of **3** toward H_2S in the presence of SO_3^{2-}/HSO_3^- (Figure 3b). The results indicated that the fluorescence response of probe **3** toward H_2S over SO_3^{2-}/HSO_3^- was about 5-fold, implying that sulfite anions showed limited influence on H_2S detection. Therefore, probe **3** can be used to selectively detect H_2S in physiological buffer.

We also investigated the turn-on fluorescence response of **3** to H_2S under different pH values (Figure S3). The probe could functionalize over pH from 7.0 to 8.5. Under weak acid conditions, the fluorescence turn-on fold of **3** upon H_2S activation became smaller, because the thiolysis of the NBD amine became slower at weak acid buffers.[11g] To explore the plausible reaction mechanism, the stoichiometry of a reaction between **3** and H_2S was tested. Data from Job's plot implied the 1:1 stoichiometry for the reaction between **3** and H_2S (Figure S4).

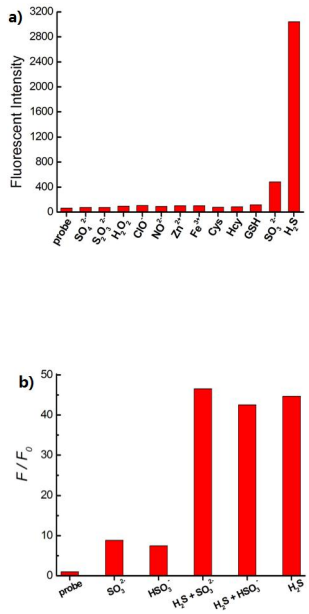


Figure 3. a) Fluorescence response (486 nm) of **3** (1 μM) upon incubation with various analytes (H_2S, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, NO_2^-, H_2O_2, ClO^-, Zn^{2+} and Fe^{3+} were 100 μM; Cys and Hcy were 1 mM; GSH was 5 mM) for 20 min; b) Competitive selectivity of **3** (1 μM) toward 100 μM SO_3^{2-}/HSO_3^- in the absence or presence of 100 μM H_2S after 20 min incubation.

To study the biological application of probe **3**, we preliminarily tested whether **3** could be used for sensing H_2S in yeast cells. The cells were treated with 5 μM **3** for 30 min and then washed by PBS. The resulted **3**-treated cells were then incubated with 200 μM H_2S for 20 min and subsequently imaged by a fluorescence microscopy. An obvious cyan fluorescence could be observed for the H_2S-treated cells (Figure S5), implying that probe **3** is cell-permeable and can image intracellular H_2S. We further examined whether **3** could be used to detect the H_2O_2-induced H_2S in yeast cells (Figure 4). In our previous work, the first H_2O_2-H_2S dual-response probe was successfully used for visualization of the H_2O_2-induced H_2S biogenesis in living HEK293 cells.[11f] Herein, yeast cells were stimulated with 100 or 200 μM H_2O_2 for 30 min and then incubated with 5 μM **3** for 20 min. As shown in Figure 4, significantly fluorescent enhancement can be observed in **3**-loaded cells upon H_2O_2 treatment (Figure 4e,4f), implying that H_2O_2 could induce endogenous H_2S production in yeast cells.[16] These preliminary tests suggested that probe **3** could be used to visualize endogenous H_2S in cells.

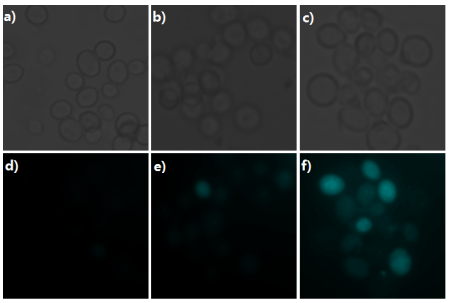


Figure 4. Fluorescent microscopy images of the H_2O_2-induced endogenous H_2S in yeast cells using **3**. Cells were incubated with (a,d) **3** (5 μM) for 20 min; (b,e) H_2O_2 (100 μM) for 30 min and then **3** (5 μM) for 20 min; (c,f) H_2O_2 (200 μM) for 30 min and then **3** (5 μM) for 20 min. (a-c) show the bright field images and (d-f) show the fluorescent images.

In summary, click synthesis of NBD amine and coumarin led to a new fluorescent probe **3** for selective H_2S detection in cells. Spectra studies indicated that **3** could rapidly react with H_2S using either Na_2S or NaHS as an equivalent in buffer. The selectivity study demonstrated that probe **3** could selectively detect H_2S even in the presence of SO_3^{2-}/HSO_3^-. Moreover, bioimaging experiments indicated that this probe could be used to monitor the H_2O_2-induced endogenous H_2S in yeast cells, which was the first *in-situ* observation of such phenomenon. Therefore, probe **3** could be a promising tool for potential applications in H_2S biology. This work further highlights that thiolysis of the NBD amine is a useful reaction for development of fluorescent H_2S probes.

Acknowledgments

This work was supported by the MOST (2010CB126102), NSFC (21332004, 21402007), 111 project (B14004).

References and notes

1. (a) Szabó, C. *Nat. Rev. Drug Discovery*, **2007**, *6*, 917; (b) Li, L.; Rose, P.; Moore, P. K. *Annu. Rev. Pharmacol. Toxicol.*, **2011**, *51*, 169.
2. (a) Predmore, B.; Lefer, D.; Gojon, G. *Antioxid. Redox Signaling*, **2012**, *17*, 119; (b) Whiteman, M.; Moore, P. K. *J. Cell. Mol. Med*., **2009**, *13*, 488; (c) Kimura, H. *Exp. Physiol.*, **2011**, *96*, 833.
3. (a) Kamoun, P.; Belardinelli, M.-C.; Chabli, A.; Lallouchi, K.; Chadefaux-Vekemans, B. *Am. J. Med. Genet. Part A*, **2003**, *116*, 310; (b) Yang, W.; Yang, G.; Jia, X.; Wu, L.; Wang, R. *J. Physiol*., **2005**, *569*, 519; (c) Fiorucci, S.; Antonelli, E.; Mencarelli, A.; Orlandi, S.; Renga, B.; Rizzo, G.; Distrutti, E.; Shah,V.; Morelli, A. *Hepatology*, **2005**, *42*, 539.
4. (a) Yang, G.; Wu, L.; Jiang, B.; Yang, B.; Qi, J.; Cao, K.; Meng, Q.; Mustafa, A. K.; Mu, W.; Zhang, S.; Snyder, H.; Wang, R. *Science*, **2008**, *322*, 587; (b) Li, L.; Bhatia, M.; Zhu, Y. Z.; Zhu, Y. C.; Ramnath, R. D.; Wang, Z. J.; Anuar, F. B.;Whiteman, M.; Salto-Tellez, M. P. K. *FASEB J.*, **2005**, *19*, 1196; (c) Peng, Y. J.; Nanduri, J.; Raghuraman, G.; Souvannakitti, D.; Gadalla, M. M.; Kumar G. K. *Proc. Natl. Acad. Sci. U.S.A.*, **2010**, *107*, 10719.
5. (a) Jime′nez, D.; Martinez-Manez, R.; Sancenon, F.; Ros-Lis, J. V.; Benito, A.; Soto, J. *J. Am. Chem. Soc*., **2003**, *125*, 9000; (b) Searcy, D. G.; Peterson, M. A. *Anal. Biochem.*, **2004**, *324*, 269; (c) Lawrence, N. S.; Davis, J.; Jiang, L.; Jones, T. G. J.; Davies, S. N.; Compton, R. G. *Electroanalysis*, **2000**, *12*, 1453; (d) Radford-Knoery, J.; Cutter, G. A., *Anal. Chem.*, **1993**, *65*, 976.

6. (a) Lippert, A. R.; New, E. J.; Chang, C. J. *J. Am. Chem. Soc.*, **2011**, *133*, 10078; (b) Peng, H.; Cheng, Y.; Dai, C.; King, A. L.; Predmore, B. L.; Lefer, D. J.; Wang, B. *Angew. Chem. Int. Ed.*, **2011**, *50*, 9672; (c) Montoya, L. A.; Pluth, M. D. *Chem. Commun.*, **2012**, *48*, 4767; (d) Chen, S.; Chen, Z.; Ren, W.; Ai, H. *J. Am. Chem. Soc.*, **2012**, *134*, 9589; (e) Das, S. K.; Lim, C. S.; Yang, S. Y.; Han, J. H.; Cho, B. R. *Chem. Commun.*, **2012**, *48*, 8395; (f) Wan, Q.; Song, Y.; Li, Z.; Gao, X.; Ma, H. *Chem. Commun.*, **2013**, *49*, 502; (g) Yu, C.; Li, X.; Zeng, F.; Zheng, F.; Wu, S. *Chem. Commun.*, **2013**, *49*, 403; (h) Chen, B.; Li, W.; Lv, C.; Zhao, M.; Jin, H.; Jin, H.; Du, J.;Zhang, L.; Tang, X. *Analyst*, **2013**, *138*, 946; (i) Wang, R.; Yu, F.; Chen, L.; Chen, H.; Wang, L.; Zhang, W. *Chem. Commun.*, **2012**, *48*, 11757; (j) Wu, M.-Y.; Li, K.; Hou, J.-T.; Huang, Z.; Yu, X.-Q. *Org. Biomol. Chem.*, **2012**, *10*, 8342; k) Lin, V. S.; Lippert, A. R.; Chang, C. J. *Proc. Natl. Acad. Sci. USA*, **2013**, *110*, 7131; l) Zhou, G. D.; Wang, H. L.; Ma, H. L.; Chen, X. Q. *Tetrahedron*, **2013**, *69*, 867.
7. For recent reviews: (a) Lin, V. S.; Chen, W.; Xian, M.; Chang, C. J. *Chem. Soc. Rev.*, **2015**, *44*, 4596. (b) Yu,F.; Han, X.; Chen, L. *Chem. Commun.*, **2014**, *50*, 12234.
8. (a) Yu, F.; Li, P.; Song, P.; Wang, B.; Zhao, J.; Han, K. *Chem. Commun.*, **2012**,*48*, 2852; (b) Wan, Q.; Song, Y.; Li, Z.; Gao, X.; Ma, H. *Chem. Commun.*, **2013**, *49*, 502; (c) Bae, S. K.; Heo, C. H.; Choi, D. J.; Sen, D.; Joe, E. H.; Cho, B. R.; Kim, H. M. *J. Am. Chem. Soc.*, **2013**, *135*, 9915; (d) Zhang, L.; Li, S.; Hong, M.; Xu, Y.; Wang, S.; Liu, Y.; Qian, Y.; Zhao, J. *Org. Biomol. Chem.*, **2014**, *12*, 5115; (e) Chen, B.; Wang, P.; Jin, Q. Tang, X. *Org. Biomol. Chem.*, **2014**, *12*, 5629.
9. (a) Sasakura, K.; Hanaoka, K.; Shibuya, N.; Mikami, Y.; Kimura, Y.; Komatsu, T.; Ueno, T.; Takuya, T.; Kimura, H.; Nagano, T. *J. Am. Chem. Soc.*, **2011**, *133*, 18003; (b) Wang, J.; Long, L.; Xie, D.; Zhan, Y. *J. Luminescence*, **2013**, *139*, 40.
10. (a) Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M. *Angew. Chem. Int. Ed.*, **2011**, *50*, 10327; (b) Liu, J.; Sun, Y.-Q.; Zhang, J.; Yang, T.; Cao,J.; Zhang, L.; Guo, W. *Chem. Eur. J.*, **2013**, *19*, 4717; (c) Cao, X.; Lin, W.; Zheng, K.; He, L. *Chem. Commun.*, **2012**, *48*, 10529; (d) Chen, Y.; Zhu, C.; Yang, Z.; Chen, J.; He, Y.; Jiao, Y.; He, W.; Qiu, L.; Cen, J.; Guo, Z. *Angew. Chem. Int. Ed.*, **2013**, *52*, 1688; (e) Xu, Z.; Xu, L.; Zhou, J.; Xu, Y.; Zhu, W.; Qian, X. *Chem. Commun.*, **2012**, *48*, 10871; (f) Qian, Y.; Karpus, J.; Kabil, O.; Zhang, S. Y.; Zhu, H. L.; Banerjee, R.; Zhao, J.; He, C. *Nat. Commun.*, **2011**, *2*, 495; (g) Hu, C.; Sun, W.; Cao, J.; Gao, P.; Wang, J.; Fan, J.; Song, F.; Sun, S.; Peng, X. *Org. Lett.*, **2013**, *15*, 2310; (h) Wang, X.; Sun, J.; Zhang, W.; Ma, X.; Lv, J.; Tang, B. *Chem. Sci.*, **2013**, *4*, 2551; (i) Qian, Y.; Zhang, L.; Ding, S.; Deng, X.; He, C.; Zheng, X.; Zhu, H.; Zhao, J. *Chem. Sci.*, **2012**, *3*, 2920; (j) Yuan, L.; Zuo, Q.-P. *Sensor Actuat. B-Chem.*, **2014**, *196*, 151; (k) Liu, T.; Xu, Z.; Spring, D. R.; Cui, J. *Org. Lett.*, **2013**, *15*, 2310; (l) Yuan, L.; Zuo, Q.-P. *Chem. Asian J.*, **2014**, *9*, 1544; (m) Huang, Z.; Ding, S.; Yu, D.; Huang, F.; Feng, G. *Chem. Commun.*, **2014**, *50*, 9185; (n) Yuan, L.; Jin, F.; Zeng, Z.; Liu, C.; Luo, S.; Wu, J. *Chem. Sci.*, **2015**, *6*, 2360.
11. (a) Wei, C.; Wei, L.; Xi, Z,; Yi, L. *Tetrahedron Lett.*, **2013**, *54*, 6937; (b) Wei, C.; Zhu, Q.; Liu, W.; Chen, W.; Xi, Z.; Yi, L. *Org. Biomol. Chem.*, **2014**, *12*, 479; (c) Wei, C.; Wang, R.; Wei, L.; Cheng, L.; Li, Z.; Xi, Z.; Yi, L. *Chem. Asian J.*, **2014**, *9*, 3586; (d) Wei, L.; Yi, L.; Song, F.; Wei, C.; Wang, B.; Xi, Z. *Sci. Rep.*, **2014**, *4*, 4521; (e) Wei, L.; Zhu, Z.; Li, Y.; Yi, L.; Xi, Z. *Chem. Commun.*, **2015**, *51*, 10463; (f) Yi, L.; Wei, L.; Wang, R.; Zhang, C.; Zhang, J.; Tan, T.; Xi, Z. *Chem. Eur. J.*, **2015**, *21*, 15167; (g) Zhang, J.; Wang, R.; Zhu, Z.; Yi, L.; Xi, Z. *Tetrahedron*, **2015**, *71*, 8572; (h) Zhang, C.; Wei, L.; Wei, C.; Zhang, J.; Wang, R.; Xi, Z.; Yi, L. *Chem. Commun.*, **2015**, *51*, 7505; (i) Zhu, Z.; Li, Y.; Wei, C.; Wen, X.; Xi, Z.; Yi, L. *Chem. Asian J.*, **2015**, DOI: 10.1002/asia.201500940.
12. (a) Yi, L.; Li, H.; Sun, L.; Liu, L.; Zhang C.; Xi, Z. *Angew. Chem. Int. Ed.*, **2009**, *48*, 4034; (b) Zhang, H.; Zhang, C.; Liu, R.; Yi L.; Sun, H. *Chem. Commun.*, **2015**, *51*, 2029; (c) Zhu, Z.; Wei, L.; Cheng, L.; Li, Z.; Xi Z.; Yi, L. *Tetrahedron Lett.*, **2015**, *56*, 3909; (d) Men, Y.; Li, Z.; Zhang, J.; Tong, Z.; Xi, Z.; Qiu, X.; Yi, L. *Tetrahedron Lett.*, **2015**, *56*, 5781.
13. Roubinet, B.; Bailly, L.; Petit, E.; Renard, P.-Y.; Romieu, A. *Tetrahedron Lett.*, **2015**, *56*, 1015.
14. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.*, **2001**, *40*, 2004; (b) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.*, **2004**, *6*, 4603.
15. Montoya,L. A.; Pearce, T. F.; Hansen, R. J.; Zakharov, L. N.; Pluth, M. D. *J. Org. Chem.*, **2013**, *78*, 6550.
16. Saiki, R.; Ogiyama, Y.; Kainou, T.; Nishi, T.; Matsuda, H.; Kawamukai, M. *BioFactors*, **2003**, *18*, 229.

Supplementary Material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.xx.xxx.

Graphical Abstract

Thiolysis of the NBD amine was employed for development of a highly sensitive and selective fluorescent H_2S probe, which could be used to detect H_2S in buffer and in cells.

Synthesis and characterizations of a highly sensitive and selective fluorescent probe for hydrogen sulfide

Yan Huang[a,†], Changyu Zhang[a,†], Zhen Xi[b,*], Long Yi[a,*]