Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2016

Supporting Information for

A TICT based fluorescent probe for rapid and specific detection of hydrogen sulfide and its bio-imaging applications

Mingguang Ren, Beibei Deng, Xiuqi Kong, Kai Zhou, Keyin Liu, Gaoping Xu and Weiying Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Biological Science and Technology, University of Jinan, Jinan, Shandong 250022, P.R.

Email: weiyinglin2013@163.com

*Correspondence to: Weiying Lin, Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Biological Science and Technology, University of Jinan, Jinan, Shandong 250022, P.R. China. Email: weiyinglin2013@163.com.

Table of contents

Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

HeLa cells culture

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C and 5% CO_2 atmosphere for overnight and then incubated for 24 h in the presence of **BH-HS** at different concentrations (0, 5, 10, 20, 30, 50 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 500 μL sodium dodecyl

sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 558 nm using a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **BH-HS**.

Imaging of H_2S in living cells

1) Imaging of exogenous H_2S in HeLa cells and colocation experiment

Before the experiments, the HeLa cells were plated on 6-well plates and allowed to adhere for 24 h. the cells were washed with PBS (pH=7.4) buffer three times. Subsequently, incubating with probe **BH-HS** (5 μM) (containing 0.1 % DMSO as a cosolvent) for another 20 min at 37 ˚C, the HeLa cells were rinsed with PBS three times, and the cells were incubated with H_2S for 30 min at 37 ˚C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

2) Imaging of endogenous H_2S in HeLa cells

Before the experiments, the HeLa cells were plated on 6-well plates and allowed to adhere for 24 h and then Cysteine (100 μM) treated for 6 h. For the control experiments, the cells without treated with Cysteine were cultivation for 6 hours under the same conditions. The cells were washed with PBS (pH=7.4) buffer. Subsequently, incubating with chemodosimeter **BH-HS** (5 μM) (containing 0.1 % DMSO as a cosolvent) for another 30 min at 37 ˚C, and then washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

Theoretical Calculations

The frontier molecular orbitals (HOMO, LUMO and LUMO+1) of **BH-HS** were determined by the density functional theory (DFT) calculations using the Gaussian 09 software. The exchange-correlation functional of B3LYP with Becke's three parameter form was adopted and the basis set of 6-31G(d) was used in the calculations.

Synthesis

Synthesis of compound HB-HS

Compound **1** (200 mg, 0.51 mmol, 1eq) and compound **2** (160 mg, 0.51 mmol, 1eq) were dissolved in anhydrous ethanol (20 mL). The reaction mixture was refluxed for 12 h with an inert atmosphere of nitrogen, and then the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (CH_2Cl_2 to CH_2Cl_2 /methanol = 100: 4, v/v) to afford the compound **BH-HS** as a reddish powder (118 mg, yield: 60.5%). UV/Vis (PBS:DMF=1:1) λ_{max} 384 nm (ε = 1.08 $\times 10^4$ L·mol^{-1}·cm^{-1}) and 501 nm (ε = 2.61 $\times 10^4$ L·mol^{-1}·cm^{-1}); ^{1}H-NMR (400 MHz, DMSO-d_6) δ .95 – 7.81 (m, 2H), 7.67 – 7.50 (m, 2H), 7.20 (d, J = 8.7 Hz, 2H), 7.02 – 6.84 (m, 2H), 4.53 (dd, J = 13.5, 6.7 Hz, 2H), 3.02 (s, 4H), 2.82 (s, 2H), 2.57 (s, 2H), 1.76 (d, J = 7.0 Hz, 6H), 1.55 (d, J = 12.2 Hz, 3H), 1.40 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 179.75 , 163.24 , 155.77 , 149.13 , 146.07 , 144.41 , 142.32 , 140.55 , 139.69 , 132.59 , 129.70 , 128.97 , 128.86 , 124.93 , 122.59 , 114.41 , 107.81 , 51.50 , 43.62 , 40.36 , 27.82 , 15.67 , 15.29 , 14.52 , 13.94 , 13.21 . HRMS (ESI) m/z calcd for $C_{35}H_{40}BF_2N_4^+$ $[M]^+$: 565.3393; found 565.3308.

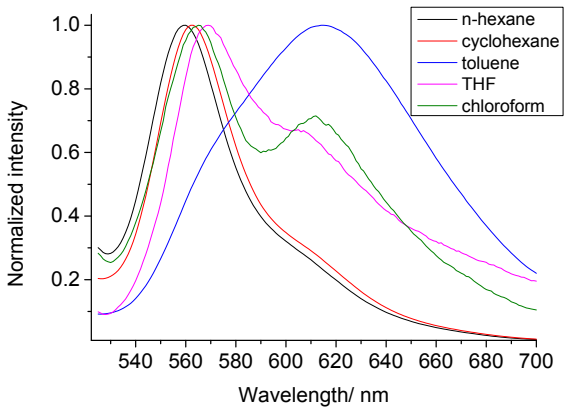

Figure S1 Fluorescence spectra of **BH-HS** in different polarity of solvents n-hexane, cyclohexane, toluene, THF, chloroform. (λ_{ex}= 515 nm)

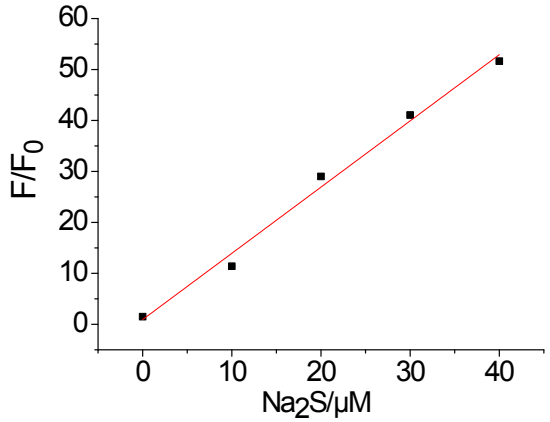

Figure S2 Fluorescence intensity ratio (F/F_0) changes at 535 nm of **BH-HS** (10mM) with the amount of Na_2S. The spectra were recorded after incubation of the probe with Na_2S for 3 min.

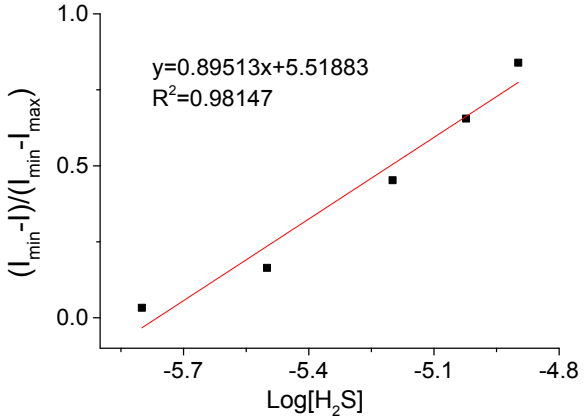

Figure S3 Normalized response of the fluorescence signal to changing H_2S concentrations.

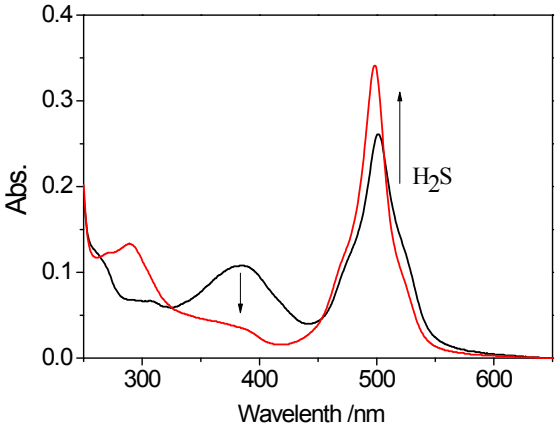

Figure S4 Absorption spectra of the probe **BH-HS** (10 μM, black line) and **BH-HS+Na_2S** (red line) in PBS buffer (pH 7.4, 50 % DMF).

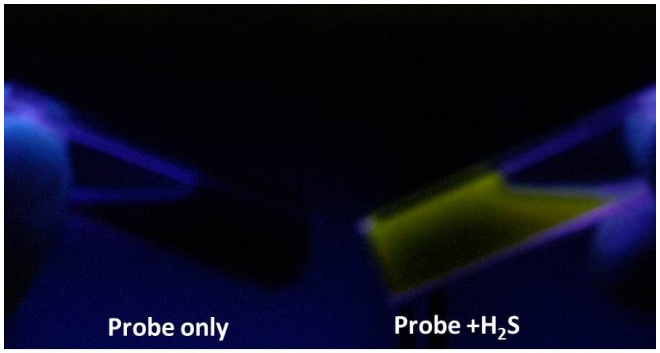

Figure S5 The fluorescence color changes of the probe **BH-HS** solution (0.5 mM) in PBS buffer solution (pH 7.4, containing 50 % DMF as a co-solvent) with 365 nm ultraviolet light. a) probe **BH-HS** solution (0.5 mM) only; b) probe **BH-HS** solution and Na_2S (10 eq).

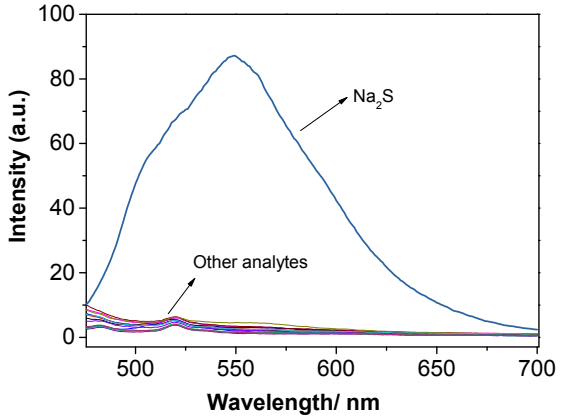

Figure S6 The fluorescence spectra changes of probe **BH-HS** (10 μM) in the presence of various analytes (100 μM) in PBS buffer (pH 4.7, containing 50% DMF as a cosolvent).

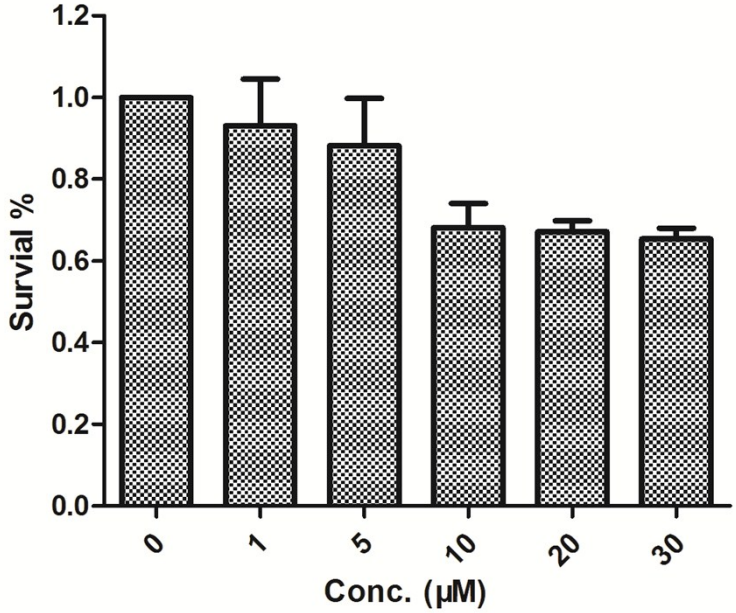

Figure S7 Cytotoxicity assays of **BH-HS** at different concentrations for HeLa cells.

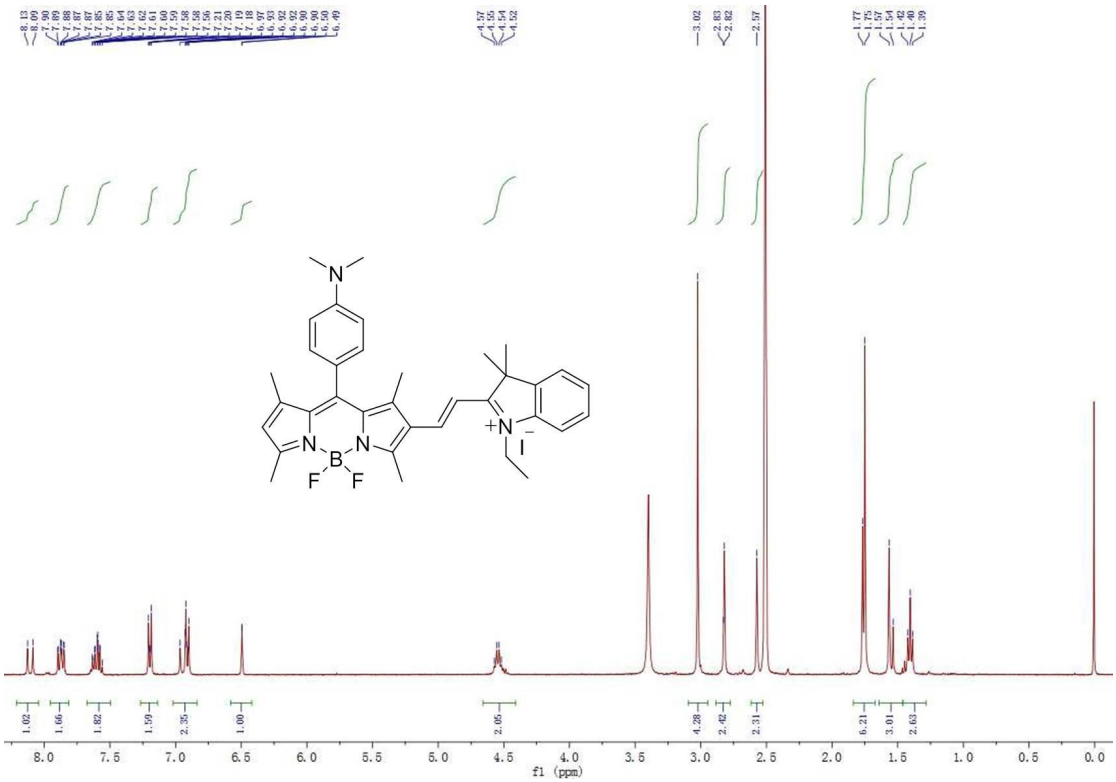

Figure S8 ^{1}H-NMR (DMSO-d_6) spectrum of **BH-HS**.

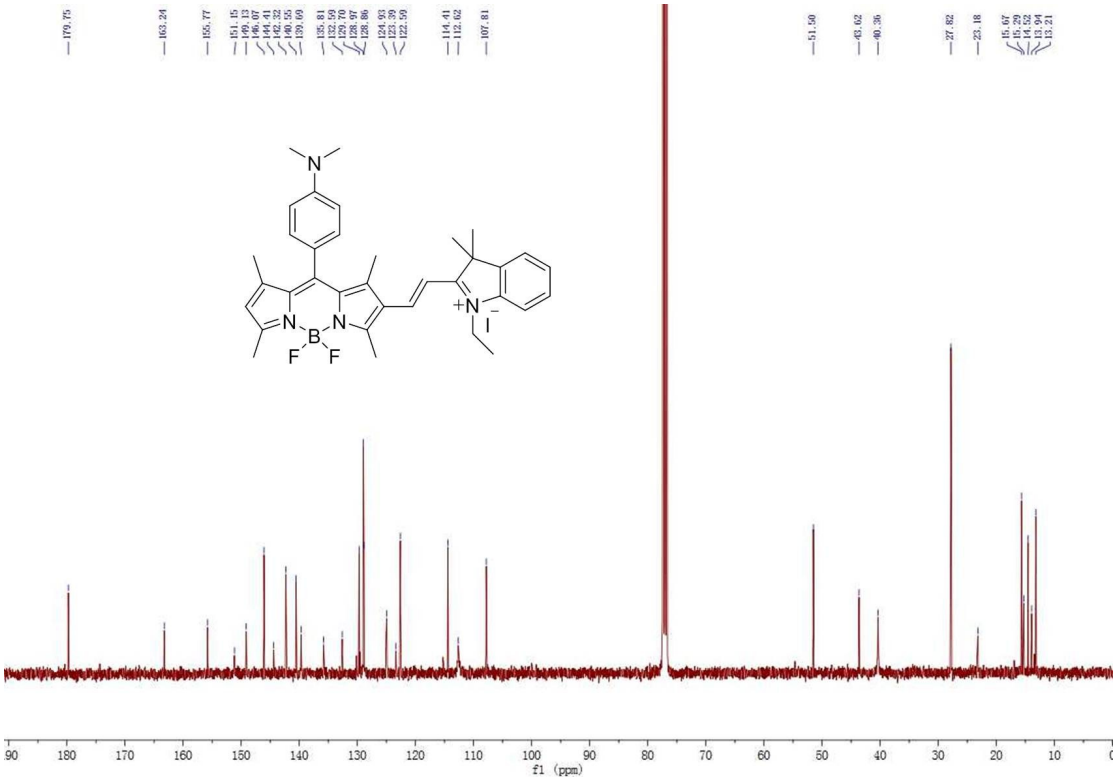

Figure S9 ^{1}C-NMR (DMSO-d_6) spectrum of **BH-HS**.

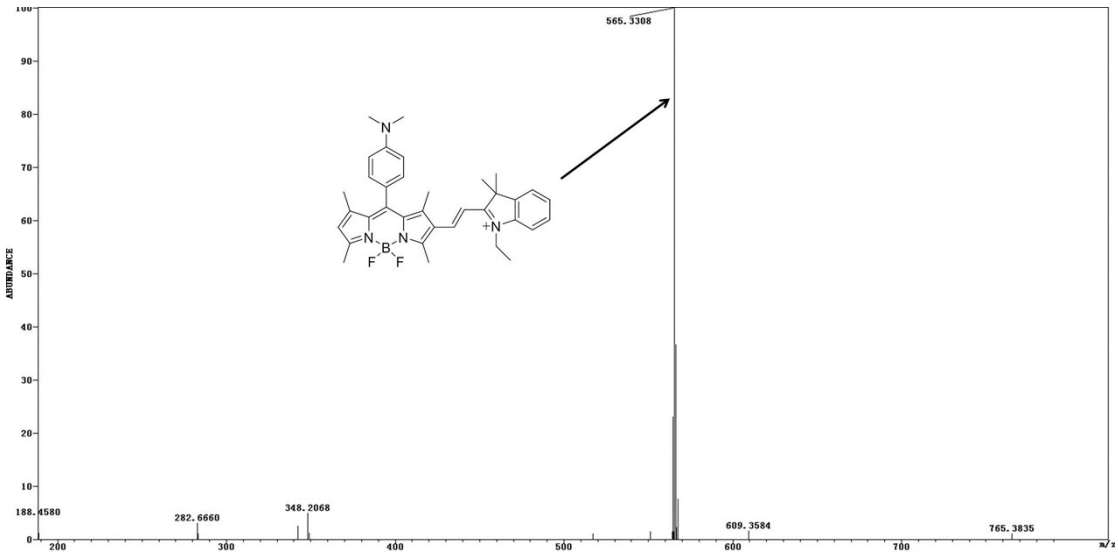

Figure S10 HRMS (ESI) spectrum of **BH-HS**.