Selective and Sensitive Detection of Hydrogen Sulfide in Live Cells

Juan Cheng, Baihao Shao, Shuai Zhang, Yongzhou Hu and Xin Li*

College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

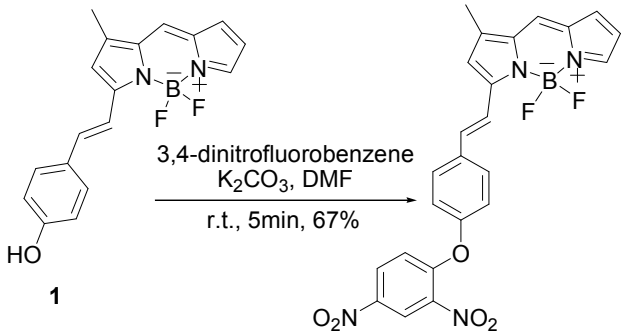
* Corresponding author. Tel/fax.: +86-571-8898-1051; e-mail: lixin81@zju.edu.cn

Supplementary data

1. General experimental methods

All reagents were purchased from commercially available suppliers, and used without further purification unless otherwise stated. BODIPY fluorophore **1** was synthesized according to literature procedures. Reactions were monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation using UV light as the visualizing agen. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. ^{1}H NMR spectrum was recorded on a Bruker Fourier transform spectrometer (500 MHz) at 25^{o}C. ^{13}C NMR spectrum was recorded on a Bruker Fourier transform spectrometer (125 MHz) spectrometer and were calibrated using residual undeuterated $CDCl_3$ as an internal reference (^{1}H NMR = 7.26, ^{13}C NMR = 77.16). All chemical shifts were given in ppm and coupling constants (*J*) in Hz. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, m = multiplet. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). Fluorescence measurements were carried out on a SHIMADZU RF-5301pc spectrofluorimeter.

2. Synthesis and characterization of PS1



BODIPY **1**

BODIPY **1** was synthesized according to literature procedures in 42% yield as a red powder. [1]

m. p. ＞250^{o}C

^{1}H NMR (500 MHz, DMSO) δ 10.18 (s, 1H), 7.75 (d, *J* = 16.2 Hz, 1H), 7.71 (s, 1H), 7.64 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 2H), 7.26 (d, *J* = 16.2 Hz, 1H), 7.13 (s, 1H), 7.04 (d, *J* = 3.7 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.48 (dd, *J* = 3.7, 2.1 Hz, 1H), 2.33 (s, 3H).

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1 J. S. Lee, N. Y. Kang, Y. K. Kim, A. Samanta, S. Feng, H. K. Kim, M. Vendrell, J. H. Park, Y. T. Chang, *J. Am. Chem. Soc.* 2009, **131**, 10077.

Probe **PS1**

To a stirred solution of BODIPY **1** (50 mg, 0.15 mmol) in DMF (2.0 mL) was added K_2CO_3 (21 mg, 0.15 mmol) and 2, 4-dinitrofluorobenzene (34 mg, 0.18 mmol). After being stirred at room temperature for 5 min, the reaction was quenched by the addition of H_2O (5 mL). The mixture was then extracted with EtOAc (10 mL x 3) and the combined organic phases was washed sequentially with H_2O (5 mL x 3) and brine (5 mL x 3). After being dried over anhydrous Na_2SO_4, the mixture was concentrated under reduced pressure and the resulting crude residue was purified by column chromatography on silica gel (petroleum:EtOAc = 6:1) to yield the product as a dark solid (50 mg, 67% yield).

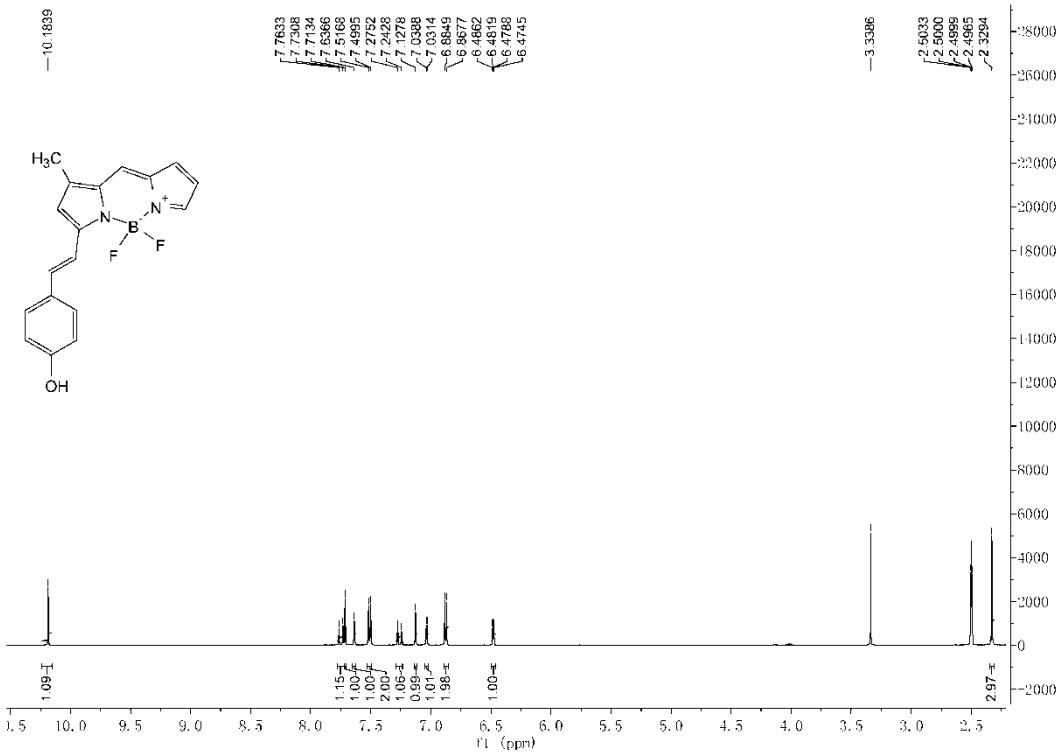
m. p. ＞250 °C

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (d, *J* = 2.7 Hz, 1H), 8.35 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.70 (m, 3H), 7.61 (d, *J* = 16.3 Hz, 1H), 7.35 (d, *J* = 16.3 Hz, 1H), 7.22 (s, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 9.2 Hz, 1H), 6.98 (d, *J* = 3.7 Hz, 1H), 6.76 (s, 1H), 6.49 (m, 1H), 2.34 (s, 3H).

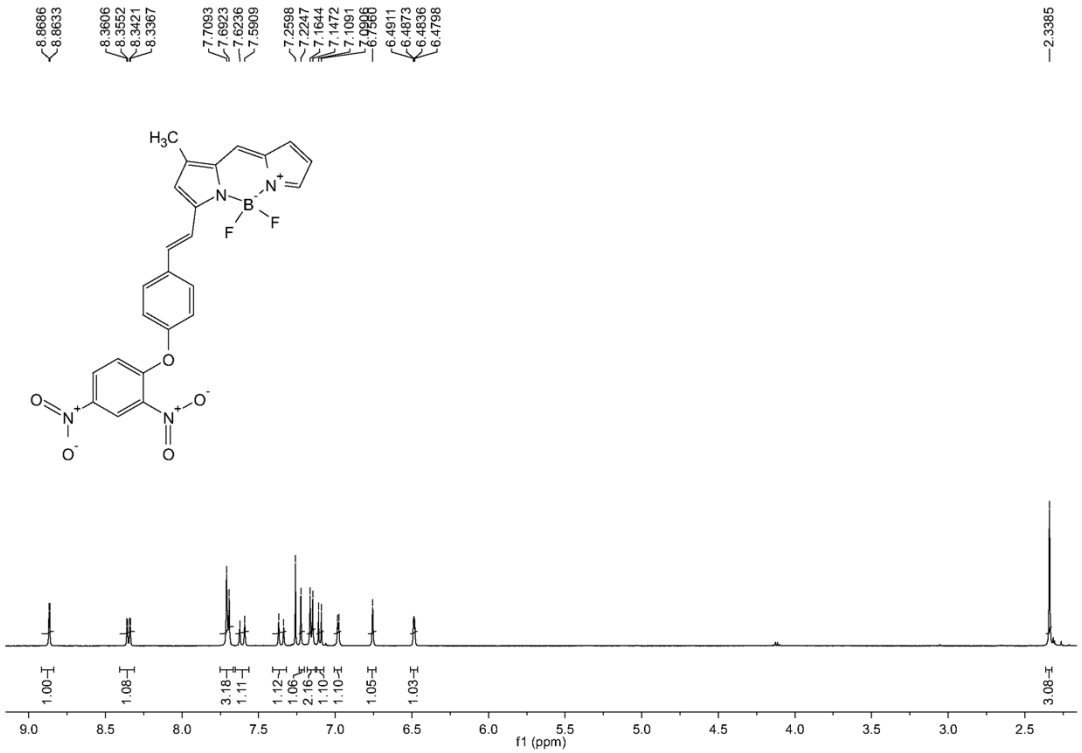
^{13}C NMR (126 MHz, $CDCl_3$) δ 157.80, 155.71, 154.69, 144.70, 141.97, 140.09, 139.95, 137.81, 137.49, 134.48, 133.59, 130.13, 129.01, 127.07, 124.00, 122.31, 120.94, 119.73, 119.13, 117.13, 117.08, 11.66.

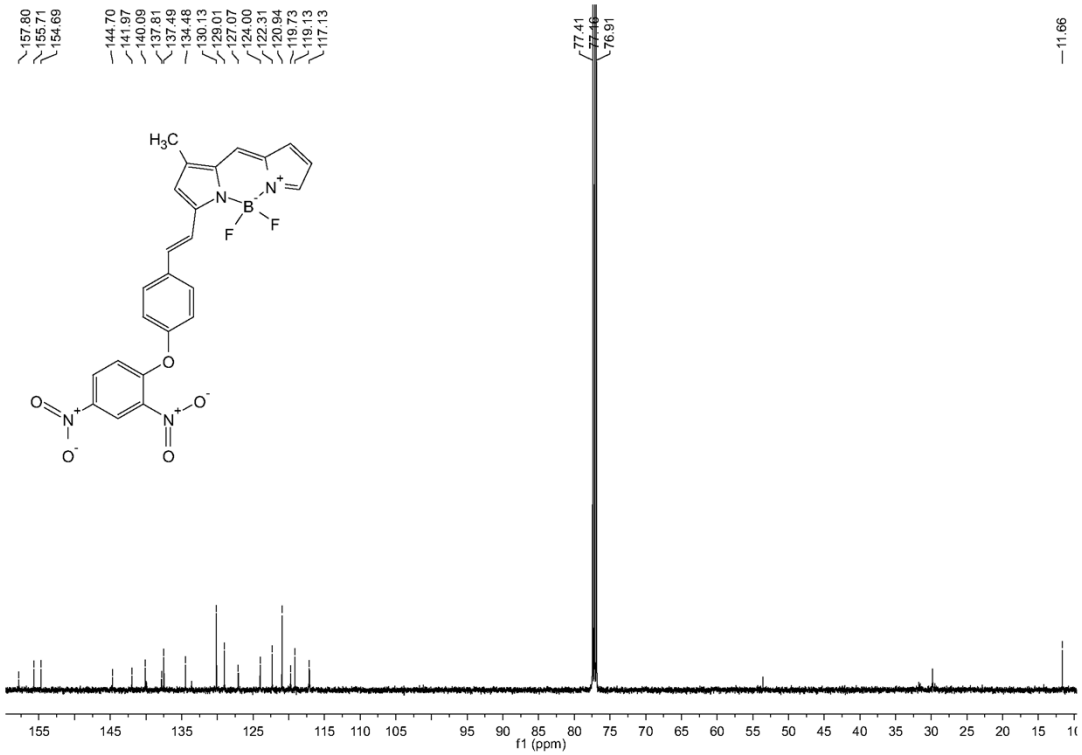
Mass data: ESI-HRMS (m/z): $[M+Na]^+$ calc'd. for $C_{24}H_{17}BF_2N_4NaO_5$ = 513.1158; found 513.1153

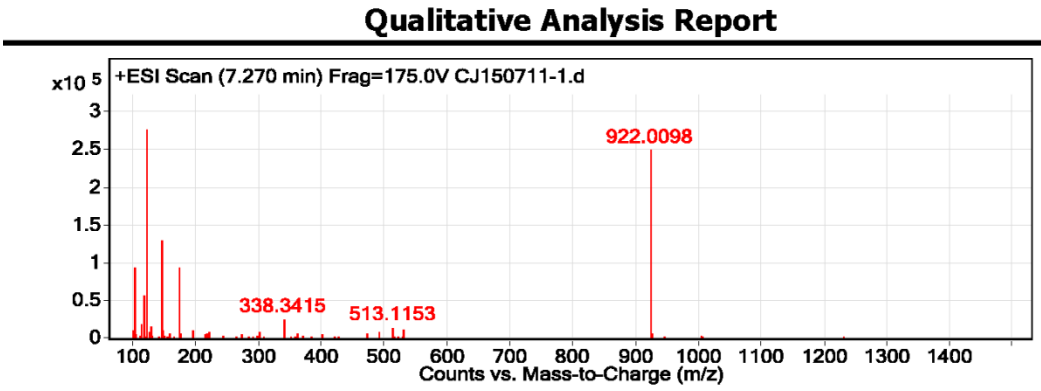
3. ^{1}H NMR spectra of BODIPY **1**



4. ^{1}H NMR ^{13}C NMR and HRMS spectra of **PS1**







5. Supplementary figures

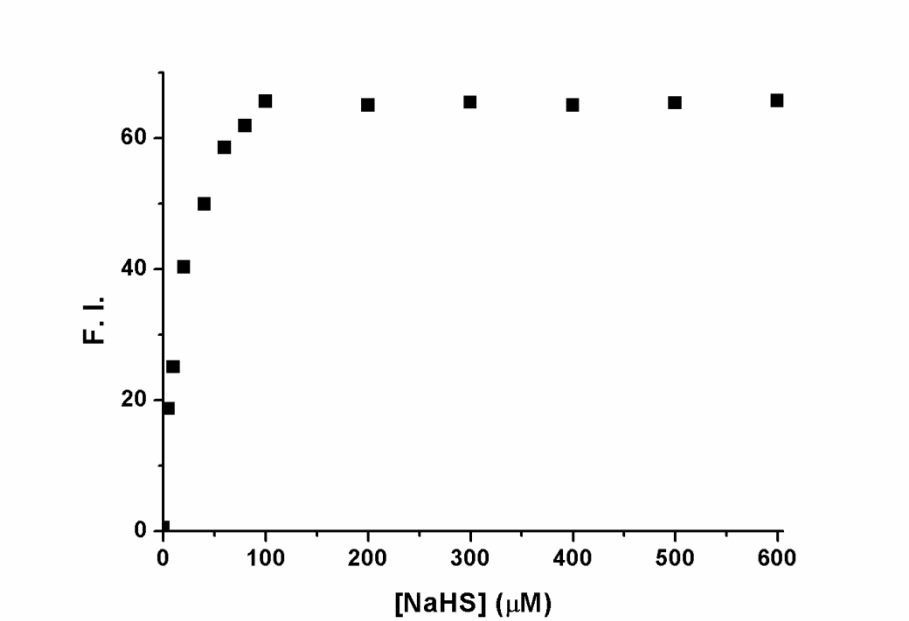


Fig. S1 Plot of the fluorescent intensity of **PS1** (5 μM) at 570 nm after treated with various concentrations of NaHS for 20 min *versus* NaHS concentration. Data were collected in PBS (10 mM, PH=7.4, 5% ethanol, 37°C) with CTAB (100 μM) as a cationic surfactant. λ_{ex}=535 nm. Slit widths: 10 nm for excitation and 5 nm for emission.

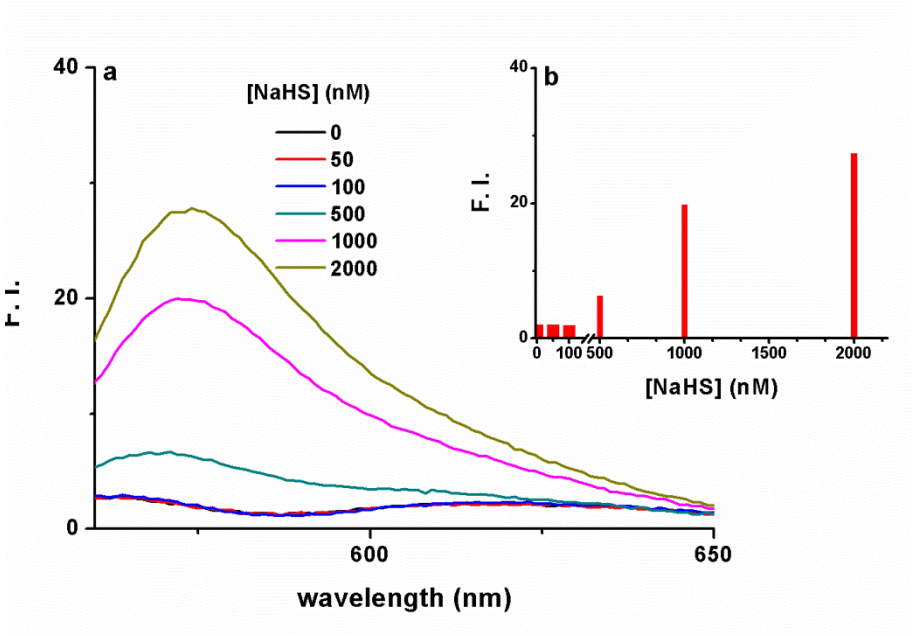


Fig. S2 Detection limit determination. The detection limit of **PS1** was determined to be the concentration of NaHS that induced three-fold fluorescence intensity enhancement. a) Fluorescent spectra of **PS1** (5 μM) in the presence of various concentrations of NaHS. b) Fluorescent intensity of **PS1** (5 μM) at 570 nm after treated with various concentrations of NaHS for 20 min. Measurements were conducted in PBS (10 mM, PH=7.4, 5% ethanol, 37°C) with CTAB (100 μM). λ_{ex}=535 nm. Slit widths: 10 nm for excitation and 10 nm for emission.