Supporting information for

A Near-infrared Fluorescent Turn-on Probe for Fluorescence Imaging of Hydrogen Sulfide in Living Cells based on Thiolysis of Dinitrophenyl Ether

Xiaowei Cao, Weiying Lin,* Kaibo Zheng, Longwei He

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, P. R. China

E-mail: weiyinglin@hnu.edu.cn

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 spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an BRUKER 500 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope. 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.

Theoretical Methods. The calculations on compound **3** and 1,3-dinitrobenzene were carried out with the Gaussian 09 program package [1] by using density functional theory (DFT) and time-dependent DFT (TD-DFT): Becke's three-parameter functional combined with Lee, Yang, and Parr's correlation functional (B3LYP), [2] along with the 6-31G(d) basis set, were used. All the geometries and electronic properties were calculated by assuming the compound **3** and 1, 3-dinitrobenzene to be the isolated molecules.

Preparation of the test solution. The stock solution of the probe **NIR-H_2S** was prepared at 0.15 mM in ethanol. The solutions of various testing species were prepared from $CaCl_2$, $MgCl_2$, KI, NaCl, KBr, NaN_3, $Na_2S_2O_3{\cdot}5H_2O$, Na_2SO_3, $NaNO_2$, CH_3COONa, Na_2CO_3, $ZnCl_2$, ascorbic acid, GSH, cysteine, H_2O_2, NaClO in the twice-distilled water, and tetrabutylammonium cyanide in acetonitrile. Hydroxyl radicals were generated by reaction of Fe^{2+} with H_2O_2. Nitric oxide (NO) was generated from DEA/NONOate (stock solution 1 mM in 0.01M NaOH). Singlet oxygen ($^1O_2$) was generated from ClO^- and H_2O_2. The test solution of the probe **NIR-H_2S** (5.0 μM) in 3 mL 50 mM PBS buffer (pH 7.0) with 3 mM CTAB and 10% ethanol was prepared by placing 0.1 mL of the probe **NIR-H_2S** stock solution and 0.2 mL ethanol in 2.7 mL of the aqueous buffer. The resulting solution was shaken well and incubated with appropriate testing species for 10 min at 37

°C before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 650 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

Cytotoxicity assays. MCF-7 cells were grown in the modified Eagle's medium (MEM) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 ºC. Immediately before the experiments, the cells were placed in a 96-well plate, followed by addition of increasing concentrations of probe **NIR-H_2S** (99% MEM and 1% DMSO) or released nitro product (99% MEM and 1% DMSO). The final concentrations of the probe were kept from 0 to 20 μM (n = 4). The cells were then incubated at 37 ºC in an atmosphere of 5% CO_2 and 95% air at 37 ºC for 24 h, followed by MTT assays. Untreated assay with MEM (n = 3) was also conducted under the same conditions.

Cell culture and fluorescence imaging. MCF-7 cells were seeded in a 12-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C for 24 h. Immediately before the experiments, the cells were washed with PBS buffer. MCF-7 cells were then incubated with the probe **NIR-H_2S** (5 μM) and Hoechst 33342 (5 μM) for 20 min at 37 °C. After washing with PBS three times to remove the remaining dyes, the cells were then incubated with NaHS (10 μM, 100 μM) for another 10 min. The fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera. For a control experiment, MCF-7 cells were incubated with the probe **NIR-H_2S** (5 μM) and Hoechst 33342 (5 μM) in the culture medium for 30 min at 37 °C, and the fluorescence imaging was carried out after washing the cells with PBS buffer three times. For the co-localization experiments, MCF-7 cells were incubated with the probe **NIR-H_2S** (5 μM), Mito Tracker Green (0.5 μM), and Hoechst 33342 (5 μM) for 20 min at 37 °C. After washing with PBS three times to remove the remaining dyes, the cells were further incubated with NaHS (0 μM, 100 μM) for another 10 min. The fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera.

Synthesis of compound 3. 4,4-difluoro-8-(4-hydroxyphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene **1** (0.5 mmol, 170 mg), Fisher aldehyde **2** (0.65 mmol, 130 mg), piperidine (0.6 mL), p-TsOH (0.2 mg), and toluene (15 mL) were added to a 50 mL

round-bottomed flask equipped with a Dean-Stark trap and a reflux condenser. The reaction mixture was stirred at reflux under an N_2 atmosphere for 4 hours. The solvent was then removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (petroleum /CH_2Cl_2: 4/1) to yield the desired compound **3** as a blue solid (49 mg, 0.09 mmol, 19 %). ^{1}H NMR (500 MHz, $(CD_3)_2CO$)) δ 7.77 (t, *J* = 14 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.23 (t, *J* = 7.5 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.03 (d, *J* = 8.5 Hz, 2H), 6.93 (t, *J* = 7.0 Hz, 2H), 6.83 (s, 1H), 6.79 (s, 1H), 5.97 (s, 1H), 5.82 (d, *J* = 12.0 Hz, 1H), 3.36 (s, 3H), 2.49 (s, 3H), 1.65 (s, 6H), 1.52 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$)) δ 163.0, 159.0, 157.2, 149.6, 145.5, 144.1, 140.2, 138.4, 138.0, 137.4, 130.7, 128.8, 127.1, 122.5, 121.6, 119.9, 119.2, 116.6, 114.6, 108.1, 98.8, 55.0, 47.1, 28.8, 15.1, 14.4. MS(EI) m/z 523.3 $[M]^+$. HRMS(EI) m/z calcd for $C_{32}H_{32}BF_2N_3O([M]^+)$: 523.2606; Found 523.2624.

Synthesis of compound NIR-H_2S. Compound **3** (31 mg, 0.06 mmol), 1-fluoro-2,4-dinitrobenzene (22 mg, 0.12 mmol), and K_2CO_3 (16 mg, 0.12 mmol) were dissolved in anhydrous DMF (3 mL), and the reaction mixture was heated at 75 ˚C for 2 hours under N_2 atmosphere. Then the mixture was poured to petroleum (40 mL). The precipitate was collected by filtration and purified by flash column chromatography (petroleum /CH_2Cl_2 = 5/1) as eluent to obtain the compound **NIR-H_2S** as a blue solid (52 mg, 0.059 mmol, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.40 (dd, *J* = 9.0 Hz and 2.0 Hz, 1H), 7.62 (t, *J* = 13 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 2H), 7.24 (s, 2H), 7.22-7.18 (q, 2H), 7.08 (d, *J* = 9.0 Hz, 1H), 6.93 (s, 1H), 6.86 (s, 1H), 6.73 (d, *J* = 7.0 Hz, 1H), 6.54 (s, 1H), 5.95 (s, 1H), 5.70 (s, 1H), 3.22 (s, 3H), 2.56 (s, 3H), 1.58 (s, 3H), 1.52 (s, 3H), 1.45 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.0, 155.5, 154.1, 144.5, 142.4, 142.0, 140.0, 139.2, 137.4, 136.8, 134.3, 134.1, 131.5, 129.0, 128.0, 122.2, 121.7, 120.9, 120.8, 119.7, 118.8, 118.4, 113.8, 107.0, 98.5, 46.4, 36.1, 29.1, 28.8, 15.1, 14.3, 11.4. MS(EI) m/z 689.2 $[M]^+$. HRMS(EI) m/z calcd for $C_{38}H_{34}BF_2N_5O_5([M]^+)$: 689.2621; Found 689.2668.

Conversion of compound NIR-H_2S to compound 3. NaHS (6.0 mg, 0.11 mmol) was added into a solution of the compound **NIR-H_2S** (25 mg, 0.036 mmol) in ethanol (2 mL) with stirring at room temperature. After reaction for 10 min, the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (petroleum /CH_2Cl_2: 4/1) to give a blue

solid (16 mg, 0. 031 mmol, 86 %), which was characterized as compound **3** by the standard NMR and mass spectrometry. See the NMR and mass spectrometry data listed above for the compound **3**.

References

(1) The Gaussian 09 package refer to Gaussian 09, Revision B.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, **2010**.

(2) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98,* 5648-5652. (b) Dirac, P. A. M. *Proc. Royal Soc. (London) A* **1929**, *123,* 714-733. (c) Vosko, S. H.; Wilk, L.; Nusair, M.; *Can. J. Phys.* **1980,** *58,* 1200-1211. (d) Becke, A. D. *Phys. Rev. A* **1988**, *38,* 3098-3100. (e) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988,** *37,* 785-789.

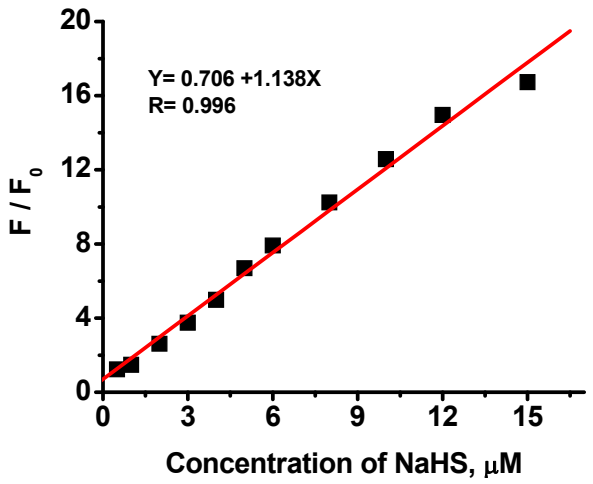

Figure S1. Plot of the fluorescent intensity ratio (F/F_0) as a function of the NaHS concentration. The concentration of the probe **NIR-H_2S** was 5 μM.

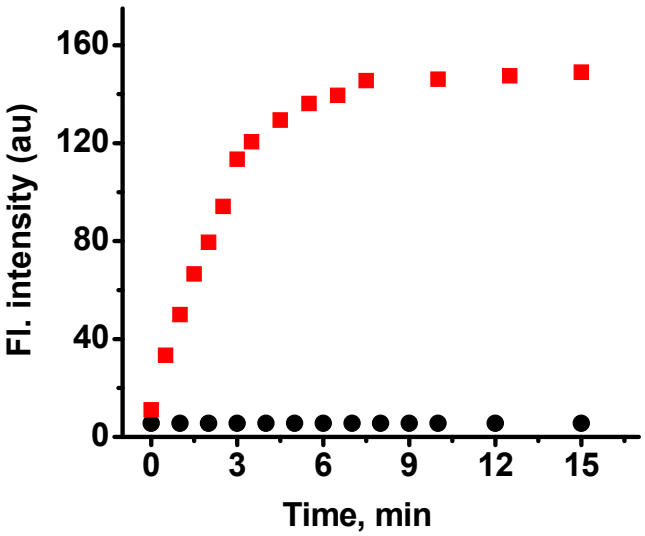

Figure S2. Time-dependent fluorescence intensity changes at 708 nm of the probe **NIR-H_2S** (5.0 μM) in the absence (●) or presence (■) of NaHS (25 μM) in the aqueous buffer at 37°C.

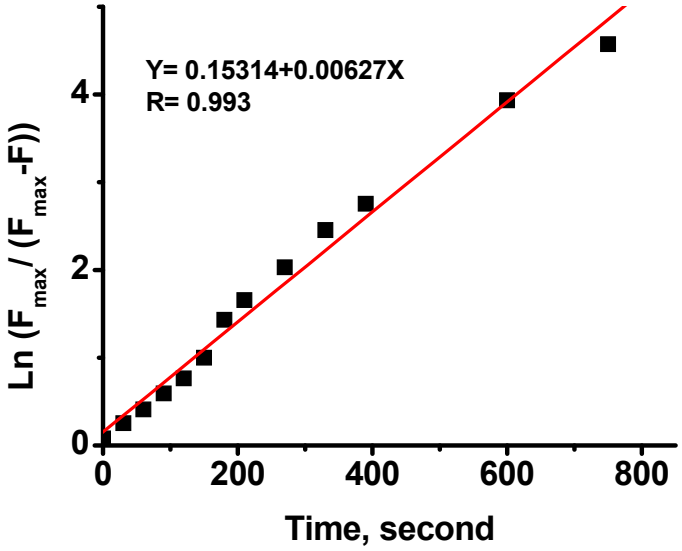

Figure S3. The *pseudo*-first-order kinetic plot of the reaction of the probe **NIR-H_2S** (5.0 μM) with NaSH (100 μM) in the aqueous buffer at 37℃.

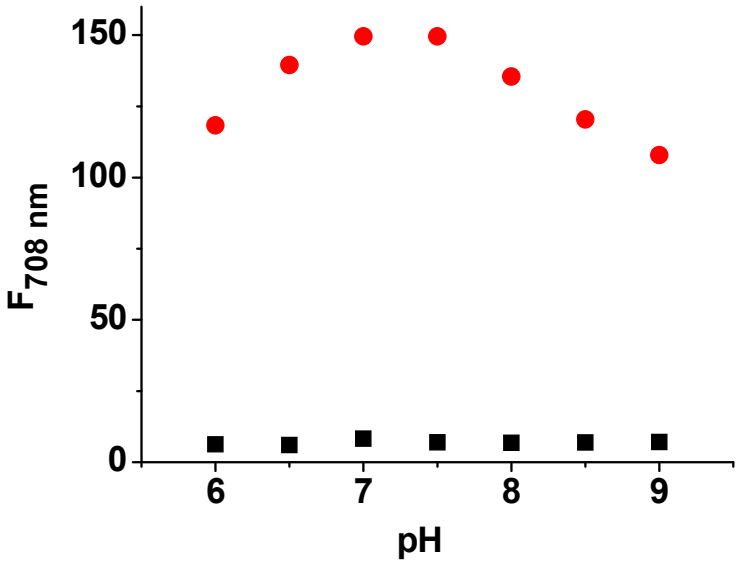

Figure S4. Fluorescence intensity changes of the probe **NIR-H_2S** (5.0 μM) at different pH values in the absence (■) or presence (●) of NaHS (25 μM).

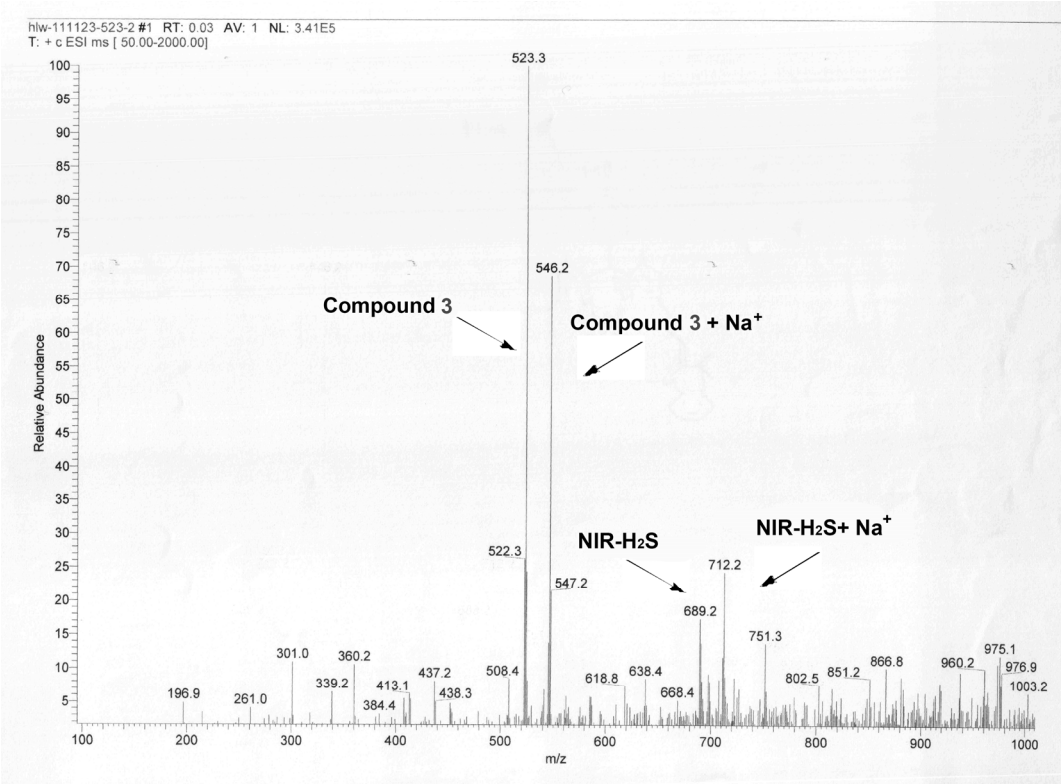

Figure S5. Mass spectrum of the probe **NIR-H_2S** (20 μM) with NaHS (40 μM).

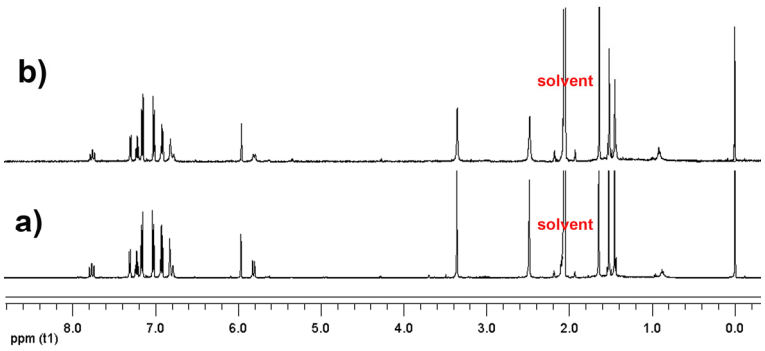

Figure S6. (a) ^{1}H NMR spectrum of the compound **3**. (b) ^{1}H NMR spectrum of the isolated product of the probe **NIR-H_2S** reacted with NaHS.

Table S1. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of the low-lying excited states of compound **3**. The data were calculated by TDDFT//B3LYP/6-31G(d) based on the optimized ground state geometries.

	Electronic Transition	TDDFT //B3LYP/6-31G(d)			
		Excitation Energy[a]	f[b]	Composition[c]	CI[d]
compound **3**	$S_0 \rightarrow S_1$	2.12 eV(582 nm)	0.8823	H→ L	0.7095
	$S_0 \rightarrow S_9$	3.76 eV (329 nm)	0.3261	H-5→ L	0.2318
				H-4→ L	0.1238
				H→ L+2	0.2725
				H→ L+3	0.3577
	$S_0 \rightarrow S_{10}$	3.94 eV (313 nm)	0.4619	H-5→ L	0.4965
				H-4→ L	0.3383
				H→ L+1	0.1956
				H→ L+7	0.1765

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength (only the f > 0.2 was considered). [c] H stands for HOMO and L stands for LUMO. [d]The CI coefficients are in absolute values.

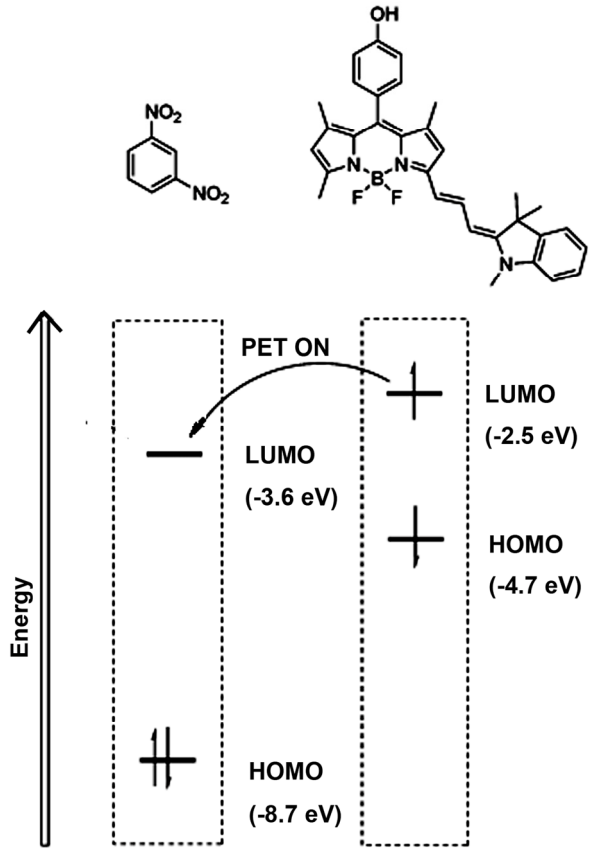

Figure S7. Frontier orbital diagram of compound **3** (the dye scaffold) and dinitrobenzene (the PET switch) for the probes **NIR-H_2S**. Orbital energies were calculated using Gaussian 09 B3LYP/ 6-31G(d) level.

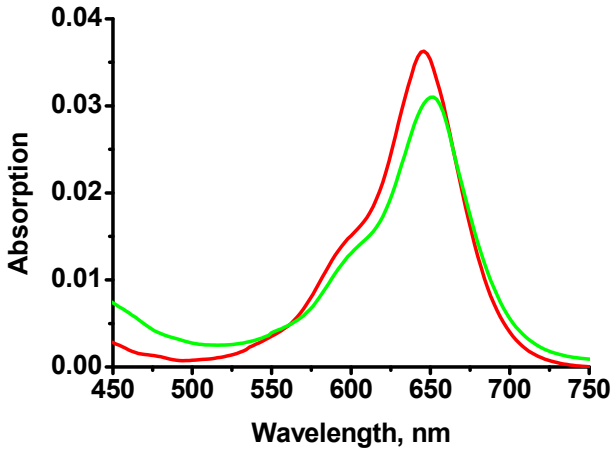

Figure S8. Absorption spectra of the probe **NIR-H_2S** (5.0 μM) in the aqueous buffer in the absence (red line) or presence (green line) of NaHS (25 μM).

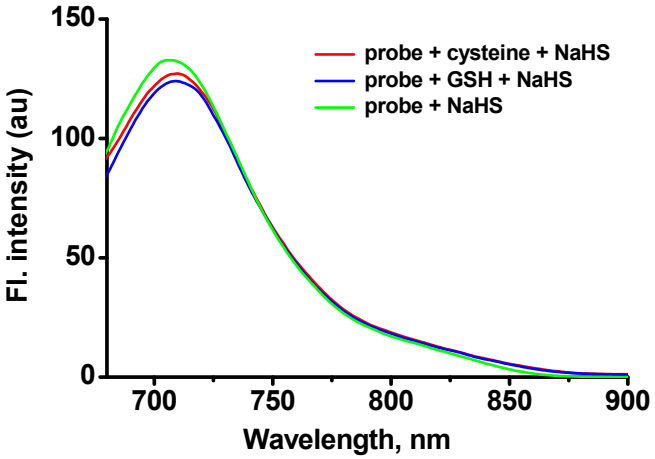

Figure S9. Fluorescence spectra of the probe **NIR-H_2S** (5.0 μM) in the presenec of only 25 μM NaHS (green line), in the presence of 25 μM NaHS + 1 mM cysteine (red line) or 25 μM NaHS + 10 mM GSH (blue line).

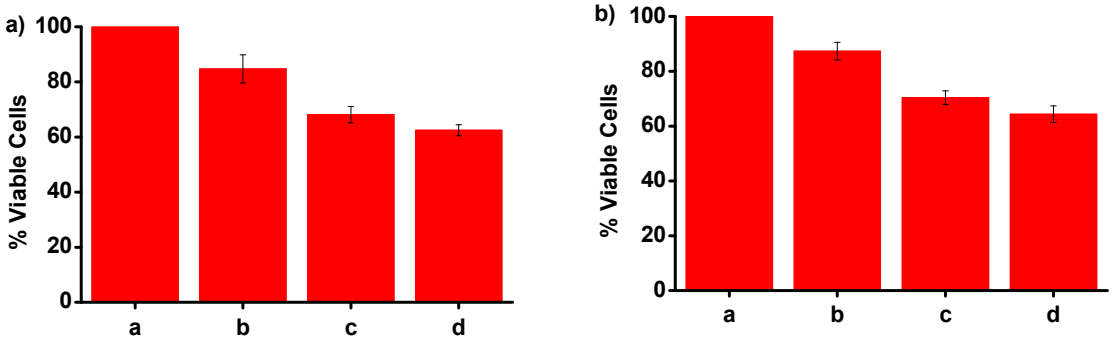

Figure S10. Cytotoxicity studies of (a) the probe **NIR-H_2S** and (b) the released nitro product for MCF-7 cells after 24 hours (a: 0; b: 5 μM; c: 10 μM; d: 20 μM).

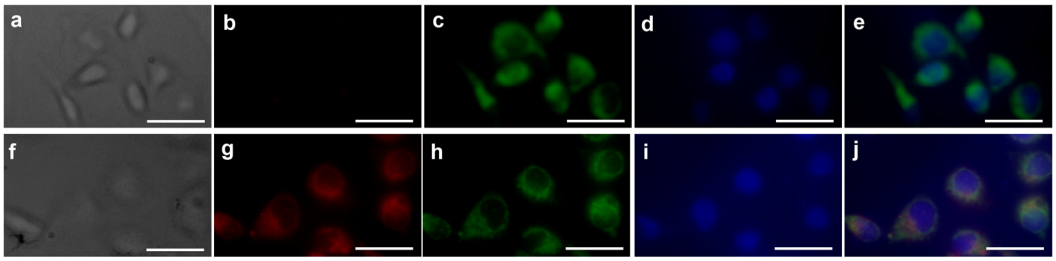

Figure S11. Fluorescence images of the probe **NIR-H_2S** with Mito Tracker Green and Hoechst 33342 in the living MCF-7 cells. (a)-(e) The cells were incubated with the probe **NIR-H_2S** (5 μM), Mito Tracker Green (0.5 μM), and Hoechst 33342 (5 μM) for 30 min. (a) Bright filed image; (b) Fluorescence image from the red channel; (c) Fluorescence image from the green channel; (d) Fluorescence image from the blue channel; (e) overlay of (b), (c), and (d); (f)-(j) The cells were pre-treated with the probe **NIR-H_2S** (5 μM), Mito Tracker Green (0. 5 μM), and Hoechst 33342 (5 μM) for 20 min, and then incubated with NaHS (100 μM) for another 10 min; (f) Bright filed image; (g) Fluorescence image from the red channel; (h) Fluorescence image from the green channel; (i) Fluorescence image from the blue channel; (j) overlay of (g), (h), and (i). Scale bar = 20 μm.

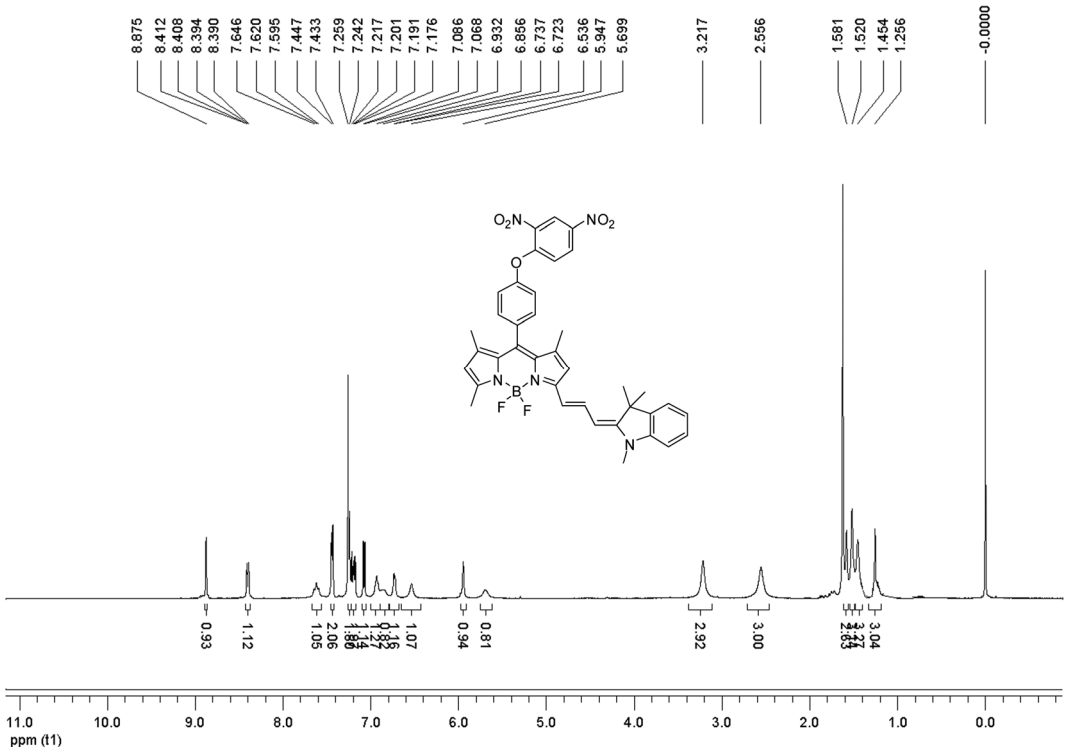

Figure S12. ^{1}H NMR spectrum of **NIR-H_2S**.

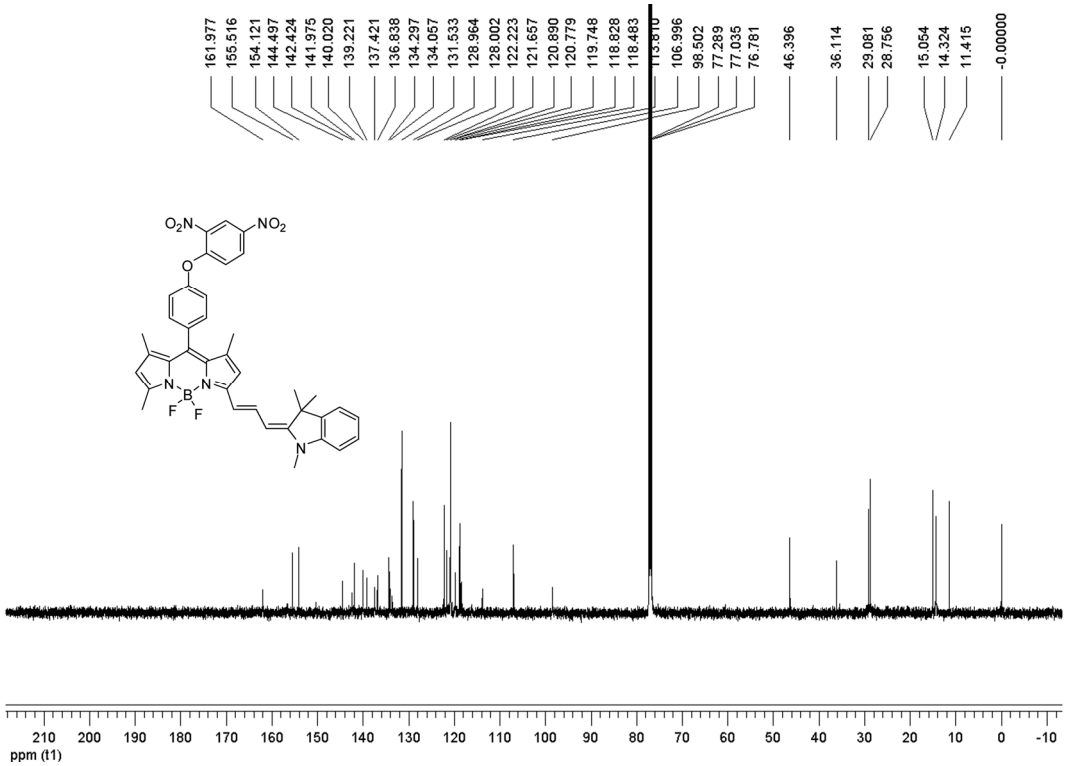

Figure S13. ^{13}C NMR spectrum of **NIR-H_2S**.