Supporting Information

Mitochondrial specific H_2S_n fluorogenic probe for live cell imaging by rational utilization of a dual-functional-photocage group

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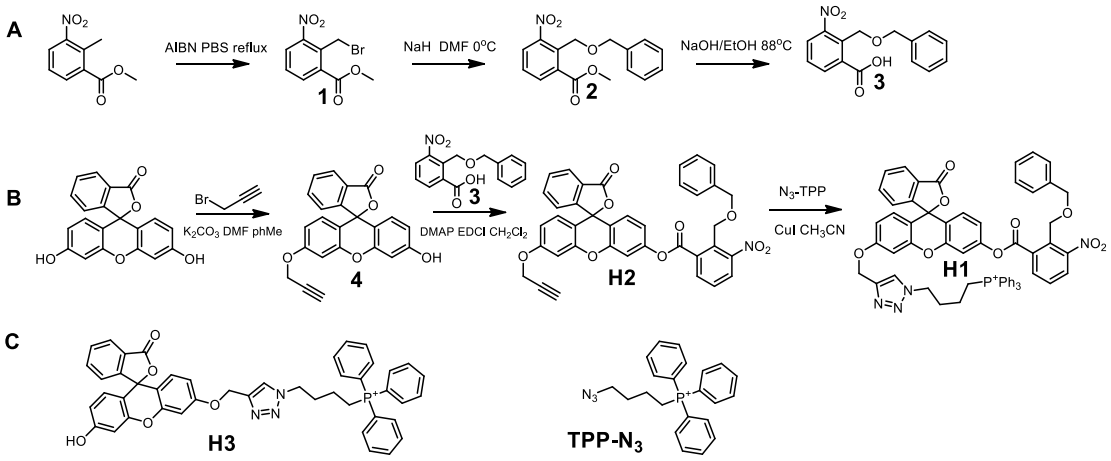
1. General procedures:

All reactions were carried out under dry nitrogen protection. Silica 60 (200-300 mesh, Silicycle) for column chromatography. All reagents and solvents are purchased from commercial suppliers and are used without further purification unless otherwise noted. *N*, *N*-dimethylformamide (DMF) and dichloromethane (CH_2Cl_2, DCM) were distilled by CaH_2. Petroleum ether (PE, 60~90°C), DCM, ethyl acetate (EA) and methanol (MeOH) were used as eluents for flash column chromatography with Merck silica gel (0.040-0.063). Reaction progress was monitored by TLC on pre-coated silica plates (250 μm thickness) and spots were visualized by ceric ammonium molybdate, basic $KMnO_4$, UV light or iodine. The ^1H and ^{13}C NMR spectra were collected in $CDCl_3$ or DMSO-d_6 at 25°C using an Avance AV-300 spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.50 ppm, MeOD = 3.31 ppm). ^1H NMR coupling constants (*J*) are reported in Hertz (Hz) and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), br d (broad doublet), dd (doublet of doublet), dt (doublet of triplet), dq (doublet of quartet), tq (triplet of quartet). Mass spectra were recorded on a Finnigan LCQ mass spectrometer, a Shimadzu LC-IT-TOF spectrometer. Analytical HPLC was carried out on Shimadzu LC-IT-TOF or LC-ESI systems equipped with an autosampler, using reverse-phase Phenomenex Luna 5 μm C_{18} 100 Å 50 × 3.0 mm columns, and the flow rate was 0.6 mL/min. Absorption spectra were recorded using Synergy HTX microplate reader or a Shimadzu UV-3600 UV-Vis-NIR spectrophotometer. Photoluminescent spectra were

recorded using a HITACHI F4600 fluorescence spectrophotometer with excitation slit widths of 5 nm and emission slit widths of 10 nm. All photochemical reactions were conducted in an ultraviolet analyzer ZF-20D. All the measurements were performed at room temperature. All images were acquired on Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System equipped with objective LD C-Apochromat 63x/1.15 W Corr M27, cell incubator with temperature control resolution ± 0.1 ºC, 405 nm Diode laser, Argon ion laser (458, 488 and 514 nm), HeNe laser (543 and 594 nm), and a 633 nm laser, with 8 channels AOTF for simultaneous control of 8 laser lines. A PMT detector ranging from 420 nm to 700 nm for steady-state fluorescence were used. Internal photomultiplier tubes were used to collect the signals in 8-bit unsigned 1024×1024 pixels at a scan speed of 200 Hz. Images were processed with Zeiss User PC Advanced for LSM system (BLUE).

2. Synthesis and characterization:



Scheme S1 Synthetic route of probe **H1**, **H2** and the structure of **H3** and **TPP-N_3**.

methyl 2-(bromomethyl)-3-nitrobenzoate (1)

Compounds are synthesized according to literature procedures[1]. methyl-2-methyl-3-nitrobenzoate (1.95 g, 0.01 mol) was brominated with a brominating agent selected from *N*-Bromobutanimide (1.78 g, 0.01 mol) in carbon tetrachloride (15 ml) followed by addition of azobisisobutyronitrile (0.274 g, 0.001 mol). The reaction mixture was heated to 60ºC for about 1.5 hours, then up to 85ºC for another 1.5 hours. The progress of the reaction was monitored by TLC upon completion of the reaction. Solvent was removed under vacuum to obtain crude compounds. The mixture was poured into saturated ammonium chloride aqueous solution, and then extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The resulting crude material was purified by flash column chromatography to afford methyl 2-(bromomethyl)-3-nitrobenzoate as white solid (2.33 g, 85% yield) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (d, J = 7.83 Hz, 1 H), 7.97 (d, J =8.13 Hz, 1 H), 7.53 (t, J = 8.1 Hz, 1 H), 5.16 (s, 2 H), 4.00 (s, 3 H).

methyl 2-((benzyloxy)methyl)-3-nitrobenzoate (2)

Compounds are synthesized according to literature procedures [2]. Benzyl alcohol (0.4 g, 3.7 mmol) was added into 10 mL of dry DMF and cooled down under an ice-water bath, followed by adding 50% oil dispersion NaH (178 mg, 3.7 mmol)). After stirring in an ice bath for 30 min and no gas from the round bottom flask, a solution of **1** (1 g, 3.65 mmol) dissolved in 1 mL of DMF was added. After stirring for 1 hour at room temperature, 10 mL water was added to quench the reaction followed by extraction with ethyl acetate (20 mL×3). The combined ethyl acetate layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resultant crude material was purified by flash column chromatography to afford **2** as white solid (2.33 g, 45% yield). ^{1}H NMR(300MHz, $CDCl_3$): δ 7.86 (t, *J* = 7.86 Hz, 2 H), 7.47 (t, *J* = 7.98 Hz, 1 H), 7.25 - 7.31 (m, 5 H), 5.01 (s, 2 H), 4.47 (s, 2 H), 3.83 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.01, 156.70, 150.91, 134.75, 134.71, 132.81, 132.32, 129.49, 129.26, 128.91, 128.74, 128.00, 127.83, 68.23, 53.26, 36.80.

2-((benzyloxy)methyl)-3-nitrobenzoic acid (3)

Compounds are synthesized according to literature procedures[3]. A solution of **2** (3.01 g, 0.01 mol) and sodium hydroxide (0.8 g, 0.02 mol) in ethanol-water (8:2, 12.5 mL) was heated to reflux for 3.5 hours. The solvent was evaporated to dryness, and the residue was dissolved in water. The residue in the flask was neutralized with hydrochloric acid and extracted by ethyl acetate. Then organic layer was washed with brine, dehydrated with anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash column chromatography. to afford **3** as white solid (2.583 g, 90% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (t, *J* = 7.77 Hz, 1 H), 7.90 (t, *J* = 8.19 Hz, 1 H), 7.55 (t, *J* = 7.92 Hz, 1 H), 7.25 - 7.31(m, 5 H), 5.06 (s, 2 H), 4.53 (s, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.30, 151.23, 133.75, 133.46, 132.71, 128.64, 128.41, 127.92, 127.87, 127.24, 77.44, 77.02, 76.59, 73.72, 65.19.

3'-hydroxy-6'-(prop-2-yn-1-yloxy)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (4)

Compounds are synthesized according to literature procedures[4]. A mixture of fluorescein (200 mg, 0.601 mmol), 3-bromoprop-1-yne (71.52 mg, 0.601 mmol) and K_2CO_3 (828 mg, 6 mmol) in dry DMF (5 mL) was stirred for 10 hours at room temperature. The solvent was then removed under reduced pressure. The obtained residue was redissolved in ethyl acetate and washed with 1 M $NaHCO_3$ (5 mL), and water (50 ml) saturated NaCl solution (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield the crude product. Purification with column chromatography (silica gel, EA/PE=1:1) was afforded **4** as a yellow solid (66.6 mg, 30% yield).^{1}H NMR(300 MHz, $CDCl_3$): δ 10.13 (s, 1 H), 8.01 (d, *J* = 7.59 Hz, 1 H), 7.82 (t, *J* = 7.44 Hz, 1 H), 7.75 (t, *J* = 7.32 Hz, 1 H), 7.29 (d, *J* = 7.38 Hz, 1 H), 7.00 (s, 1 H), 6.66 - 6.76 (m, 3 H) 6.58 (s, 2 H), 4.88 (s, 2 H), 3.60 (s, 1 H), ^{13}C NMR (75 MHz, $CDCl_3$): 164.73, 159.34, 152.08, 151.72, 135.14, 129.14, 128.45, 128.01, 126.46, 123,97, 117.19, 112.43, 110.27, 102.25, 94.50, 82.13, 76.21, 73.70, 65.39, 56.09, 54.15, 29.67.

3-oxo-3'-(prop-2-yn-1-yloxy)-3H-spiro[isobenzofuran-1,9'-xanthen]-6'-yl-2-((ben zy loxy)methyl)-3-nitrobenzoate (H2)

Compounds are synthesized according to literature procedures[5].To a mixture of **4** (370 mg, 1.0 mmol), Compound **3** (287 mg, 1.0 mmol), EDC (250 mg, 1.3 mmol) and DMAP (12.2 mg, 0.1 mmol) was added into dry CH_2Cl_2 (25.0 mL). The mixture was stirred for 24 hours at room temperature. Then the solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography to afford **H1** as pale yellow solid (102.4 mg, 40% yield).^{1}H NMR(300 MHz, $CDCl_3$): δ 8.07 (t, *J* = 7.74 Hz, 2 H), 7.96 (d, *J* = 4.5 Hz, 1 H), 7.54 - 7.71 (m, 3 H), 7.25 - 7.34 (m, 5 H), 7.19 (d, J = 7.14 Hz, 1H), 7.10 (s, 1 H), 6.89 (s, 1 H), 6.80(s, 2 H), 6.76 (s, 2H), 5.07 (s, 2 H), 4.74 (s, 2 H), 4.84 (s, 2 H), 3.65(s, 1 H). IT-TOF-MS: m/z $[M]^+$ caled: 639.6, Found: 640.5.

(4-(4-(((3'-((2-((benzyloxy)methyl)-3-nitrobenzoyl)oxy)-3-oxo-3H-spiro[isobenzof uran-1,9'-xanthen]-6'-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)butyl)triphenylphosp honium (H1)

Compounds are synthesized according to improved literature procedures[6]. Compound **H2** (0.64 g, 1.0 mmol) was stirred in CH_3CN (5 mL) at room temperature for 30 min under nitrogen. Then (4-azidobutyl) triphenylphosphonium (0.36 g, 1.0 mmol), CuI (0.76 g, 4 mmol) were added, and the mixture was stirred at room temperature. This progression of the reaction was monitored by TLC. After the reaction was finished, the suspension was filtered and the product as the solid part was washed successively with MeOH. Then the crude product was purified by flash column chromatography to afford **H1** as white solid (500 mg, 50% yield).^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, *J* = 4.6 Hz, 1 H), 8.23 (s, 1H), 8.16 (d, *J* = 4.7Hz, 1 H), 8.07 (d, *J* = 4.6Hz, 1 H), 7.89 - 7.93 (m, 3 H), 7.75 - 7.82 (m, 14 H), 7.33 - 7.36 (m, 2 H), 7.23-8.01 (m, 6 H), 7.14 (s, 1 H), 7.07 (dd, J_1 = 2.6 Hz, J_2 = 6.5 Hz, 1 H), 6.91 (d, *J* = 5.2 Hz, 1 H), 6.84 (dd, J_1 = 2.7 Hz, J_2 = 6.8 Hz, 1 H), 6.76 (d, *J* = 5.1 Hz, 1 H), 5.24 (s, 2 H), 4.99 (s, 2 H), 4.45 (s, 4 H), 1.98 - 2.04 (m, 2 H), 1.51 - 1.65 (m, 4 H). IT-TOF-MS: m/z $[M]^+$ called: 1000.0, Found: 1001.3.

3. Spectroscopic Materials and Methods

All spectra were measured in 25 mM PBS buffer, pH 7.35, 37°C. The limit of detection was calculated according to the literatures [7, 8] and Figure 2C in maintext. The calibration curve was obtained from the plot of fluorescence intensity at 525 nm as a function of the analyte concentration (Na_2S_2). The regression curve equation was then obtained for the lower concentration part.

$$LOD = 3 \times \sigma / k$$

where k is the slope of the curve equation, and σ represents the standard deviation for the probe's fluorescence intensity in the absence of Na_2S_2.

Detection limit (DL) = 3 ×2.19 / 43.4 = 151.38 nM.

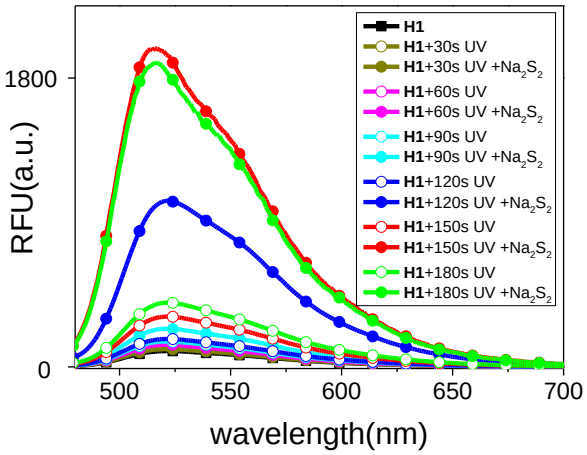


Figure S1. Fluorescence spectra of **H1** responded with Na_2S_2 after different UV irradiation times. Experiments were performed in 25 mM PBS buffer, pH 7.35, 37°C at a probe concentration of 10 μM (1% DMSO final). The solution was first treated with UV irradiation (365 nm, 4 w) for different time periods. Then Na_2S_2 (100 μM) was added into the solution. Finally, the fluorescent spectra were obtained using a Synergy HTX microplate reader. The excitation wavelength was 458 nm. As is shown in figure, the fluorescence of **H1** would not switch on if there is no addition of Na_2S_2 even after long time UV irradiation. 150 s UV irradiation was chosen to conduct further experiments.

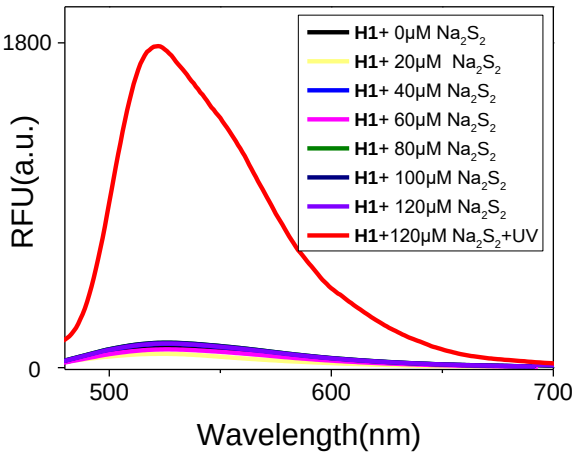


Figure S2. Fluorescence spectra of **H1** in the presence of different amounts of Na_2S_2. Experiments were performed in 25 mM PBS buffer, pH 7.35, 37°C at a concentration of 10 μM (1% DMSO final). The solution was first treated with or without UV irradiation (365 nm, 4 W) for 150 s. Then Na_2S_2 (0, 20, 40, 60, 80, 100, 120 μM) was added into the solution. Finally, the fluorescent spectra were obtained using a Synergy HTX microplate reader. The excitation wavelength was 458 nm. As is shown in figure, without UV irradiation, **H1** would not be sensitive to Na_2S_2. While, with addition of Na_2S_2, a huge fluorescent enhancement was observed.

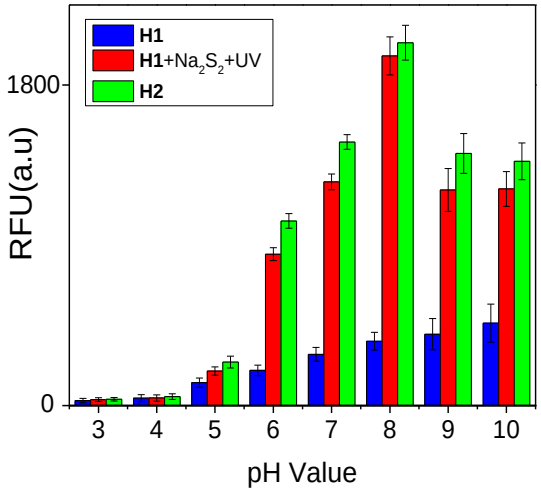


Figure S3. The fluorescence intensities at 525 nm of **H1**(10 μM), **H1**(10 μM)+Na_2S_2(100 μM)+UV(150 s) and **H3** (10 μM) at various pH values (3-10) at room temperature. The excitation wavelength was 458 nm. The result indicated that **H1** could perform well in a biologically relevant pH range (6~8).

4. Selectivity

Fluorescence detection of all the analytes *in vitro* was performed in PBS buffer containing 10 μM **H1** with 150 s UV irradiation. Some analytes were fresh prepared as below.[9, 10] The final concentrations of analytes were adjusted to 100 μM in PBS buffer. Fluorescence intensity at 520 nm was measured after 1 hour mixing.

CysSS$_n$H: Cys (100 μM) was reacted with NaHS (1 mM) in the presence of sodium nitroprusside (SNP, NO donor, 500 μM) in 20 mM Tris-HCl buffer (pH 7.4) at room temperature for 30 min.

GSS$_n$H: GSH (100 μM) was reacted with NaHS (1 mM) in the presence of SNP (500 μM) in 20 mM Tris-HCl buffer (pH 7.4) at room temperature for 30 min.

CysSS$_n$Cys: Cys (20 mM) was reacted with 20 mM NaHS in the presence of I_2 (20 mM) in 20 mM Tris-HCl buffer (pH 7.4) at room temperature for 15 min.

GSS$_n$G: GSH (20 mM) was reacted with 20 mM NaHS in the presence of I_2 (20 mM) in 20 mM Tris-HCl buffer (pH 7.4) at room temperature for 15 min.

GSS$_n$H: GSS$_n$G (5 mM) was incubated with glutathione reductase (GSR, 10 U/mg, Sigma) and NADPH (10 mM) in 40 mM sodium phosphate buffer (pH 7.4) at room temperature for 10 min.

GSSH: 2 mM glutathione (oxidized form) was reacted with 2 mM freshly Sodium sulfide (Na_2S) in 20 mM Tris-HCl buffer (pH 7.4) at 37 ^oC for 15 min.

CysSSH was synthesized by L-Cystine (oxidized form) in the same way as GSSH.

The hydropolysulfides and hydropersulfides of cysteine and glutathione solution was used immediately.

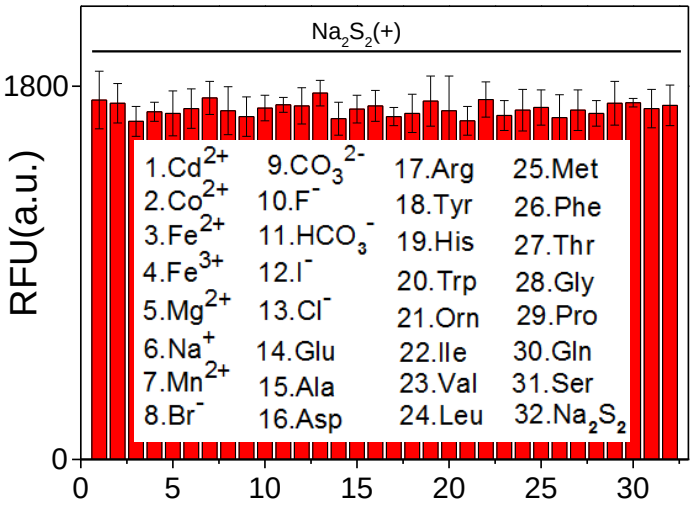


Figure S4. Fluorescent intensity changes of **H1** (10 μM, 150 s UV irradiation) after addition of Na_2S_2 and other species together. 100 μM cation and ion ((1) $Cd(NO_3)_2$, (2) $Co(NO_3)_2$, (3) $FeCl_2$, (4) $Fe(NO_3)_3$, (5) $Mg(NO_3)_2$, (6) $NaNO_3$, (7) $Mn(NO_3)_2$, (8) NaBr, (9) Na_2CO_3, (10) NaF, (11) $NaHCO_3$, (12) NaI, (13) NaCl); 500 μM amino acids ((14) Glu, (15) Ala, (16) Asp, (17) Arg, (18) Tyr, (19) His, (20) Trp, (21) Orn, (22) Ile, (23) Val, (24) Leu , (25) Met, (26) Phe, (27) Thr, (28) Gly, (29) Pro, (30) Gln, (31) Ser), (32) Na_2S_2. All samples were contained 100 μM Na_2S_2.

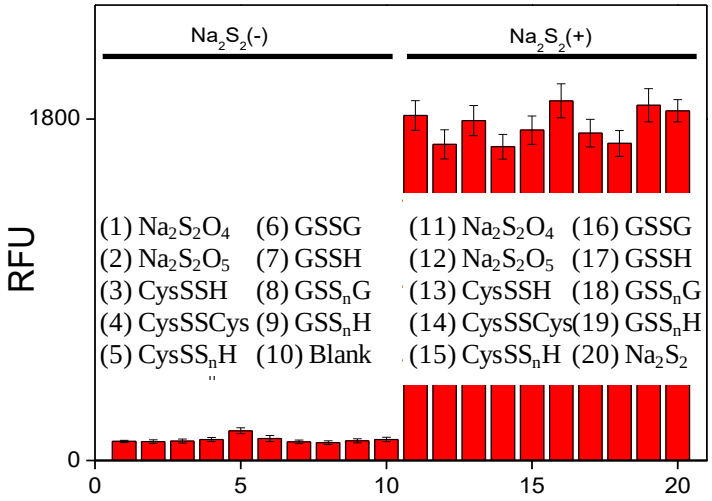


Figure S5 Fluorescence response of **H1** (10 μM) to various biological related hydropersulfides and hydropolysulfides (100 μM) in the absence ((1)~(10)) or presence ((11)~(20)) of Na_2S_2 after 150 s UV irradiation. (1) $Na_2S_2O_4$, (2) $Na_2S_2O_5$, (3) CysSSH, (4) CysSSCys, (5) $CysSS_nH$, (6) GSSG, (7) GSSH, (8) GSS_nG and (9) GSS_nH. (3)~(20) is fresh prepared.

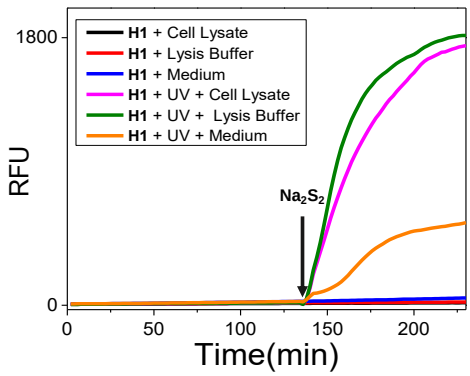


Figure S6 Time-dependent fluorescent intensity changes of **H1** (10 μM) with cell lysate (0.2 mg/ml), lysis buffer (PBS with 0.1% Triton X-100) or medium (DMEM with 10% FBS) with or without 150 s UV irradiation. 100 μM Na_2S_2 was added after 140 min incubation. For cell lysate, cells were lysed by sonication in lysis buffer (PBS with 0.1% Triton, pH = 7.4). After centrifugation (13,000×g, 13 min, 4°C), the supernatant was immediately incubated with **H1** or **H1** with 150 s UV irradiation. The medium was chosen Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS). For a 100 μL reaction, 5 μL **H1** probe (final 10 μM) was added into 95 μL cell lysate (0.2 mg/ml final), medium or lysis buffer, respectively. After 5 min incubation, the solutions were treated with 150 s UV irradiation. The fluorescent intensity was measured using microplate reader (λ_{ex} = 458 nm; λ_{em} = 525 nm). After 140 min, Na_2S_2 (final 100 μM) was added into the reactions. The intensity was recorded for another 120 min. According to the result, after around 1.5 hours incubation, there is still no significant fluorescent enhancement, suggesting the ester bond of the probe is stable in cell lysate and medium under the detection time scale.

5. Cell culture and Cell proliferation assay

HepG2 cells were cultured in DMEM containing supplemented with 10% fetal bovine serum (FBS), in 100.0 mg/L streptomycin and 100 IU/mL penicillin.

The cytotoxicity of UV was determined by using MTT assay (Sigma-Aldrich). Briefly, HepG2 cells were grown to 20%~30% confluency (~4000 cells) in 96-wll plates under normal cell culture condition. After overnight incubations, cells were irradiated with UV for 0, 2, 4, 6 min using a 365 nm hand-handle 4 W UV lamp. Subsequently, cells were further incubated for 72 hours. Finally, the assay was conducted using MTT formazan according to the manufacturer protocol. Results are summarized in Figure S7. Results were shown that short UV irradiation time cause negligible cell death.

The cytotoxicity of **H1** and **H2** was determined by using the XTT colorimetric cell proliferation kit (Roche) following manufacturer's guidelines. Briefly, cells were grown to 20~30% confluency (since they will reach 80~90% confluency within 48 to 72 hours in the absence of compounds) in 96-well plates under the conditions described above. The medium was aspirated, and then washed with PBS, and then treated, in duplicate, with 0.1 mL of the medium containing different concentrations of **H1** and **H2** (1~100 μM). Probes was applied from DMSO stocks whereby DMSO never

exceeded 1% in the final solution. The same volume of DMSO was used as a negative control. After a total treatment time of 24 hours, proliferation was assayed using the XTT colorimetric cell proliferation kit (Roche) following manufacturer reference. DMSO was used as a negative control. Results are summarized in Figure S8.

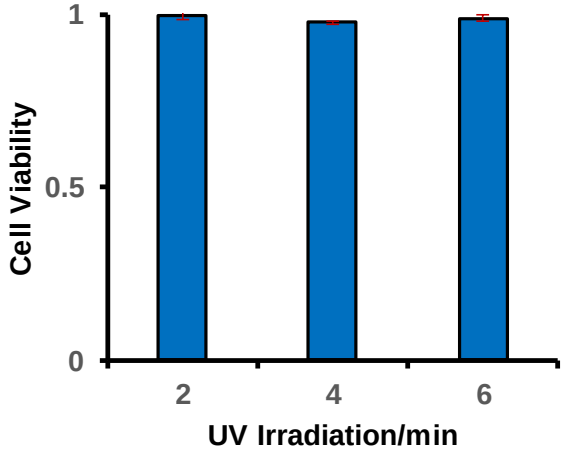


Figure S7 Cytotoxicity of UV irradiation towards HepG2 cells from an MTT assay.

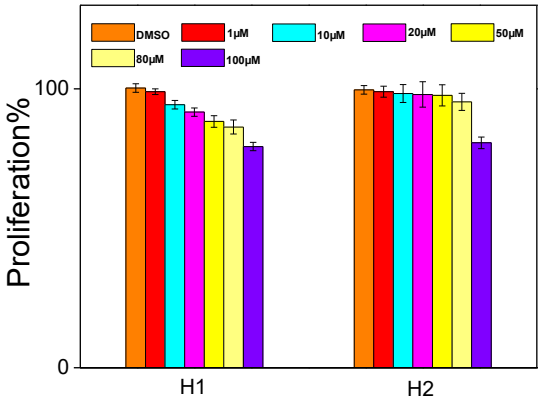


Figure S8 The toxicity of probe **H1** and **H2** against HepG2.

6. Confocal microscopy for bioimaging

HepG2 cells were seeded on glass chassis (Mattek) and grown to confluency 70~80%. **H1** and **H2** (10 μM prepared in fresh medium) were added to each well, respectively. Cells were further incubated for 60 min, washed three times with PBS buffer solution. Na_2S_2 (100 μM) was added into each well for further 30 min incubation. Cells were then irradiated with UV for 150 s and the sample was incubated at 37 ºC for 60 min. During half of this incubation period, 50 nM Mito-tracker Red CMXRos dye was added. Finally, cells were washed three times with PBS and imaged with the Zeiss LSM880 NLO (2+1 with BIG) Confocal Microscope System. Mito-tracker Red CMXRos dye (200 nM, Ex = 543 nm and PMT range: 570-650 nm) from Invitrogen was used here. Meanwhile, as the negative control, another identical set of samples was similarly treated as described above without UV-irradiation (Control 1) or without the addition of Na_2S_2 (Control 2).

Background signals of all images were verified to be nearly zero by imaging the same cells treated with Control 1.

7. ^{1}H and ^{13}C NMR spectra.

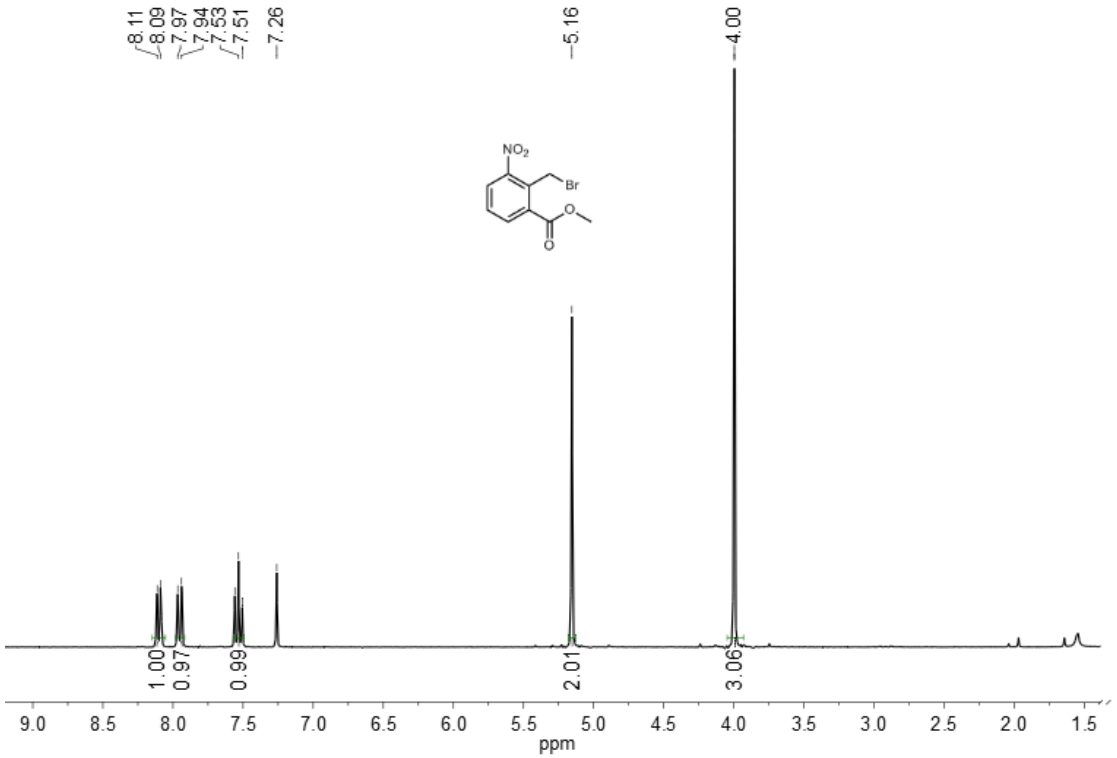


Figure S9 ^{1}H NMR spectrum of Compound 1.

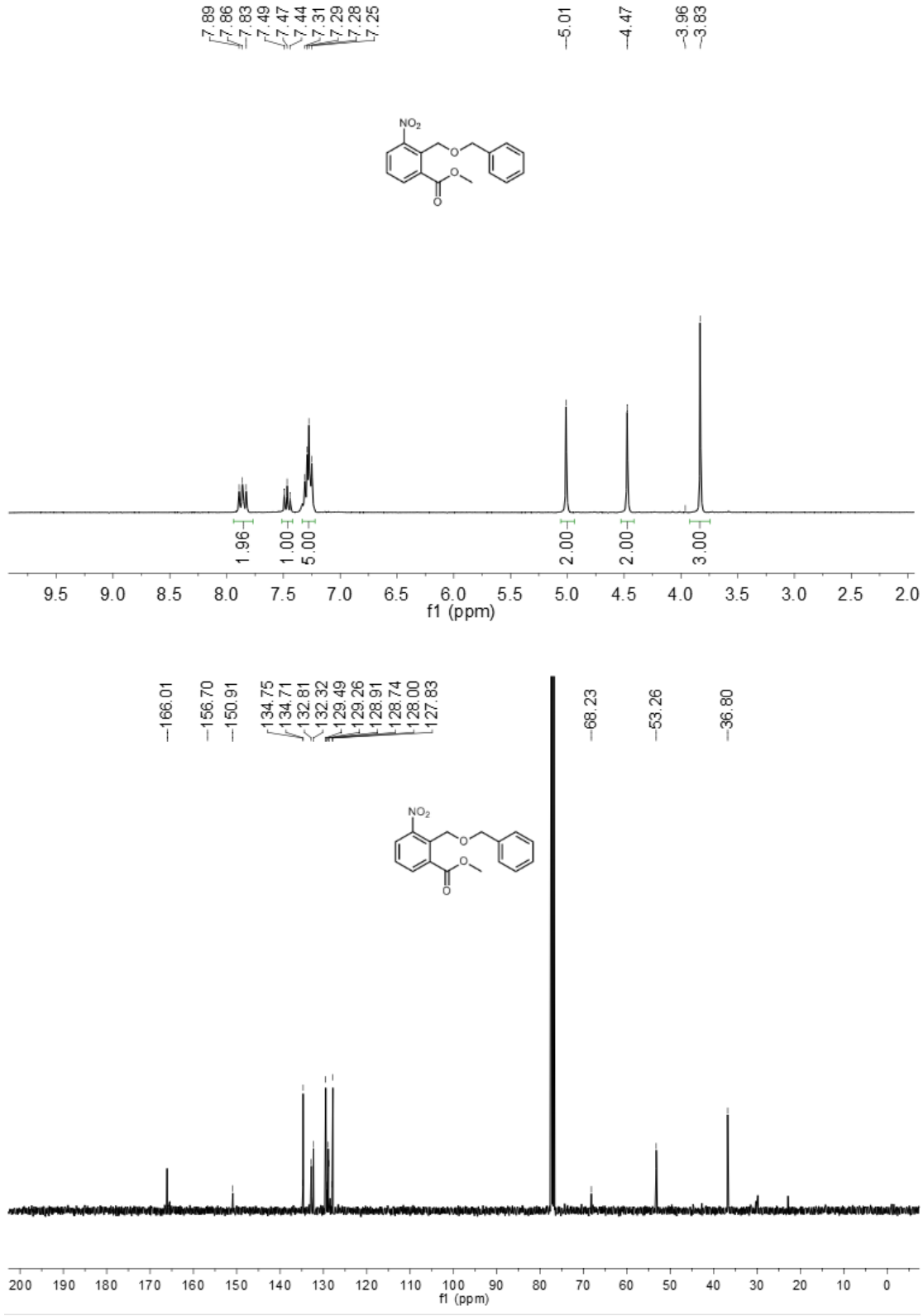


Figure S10 ^{1}H NMR and ^{13}C NMR spectrum of Compound 2.

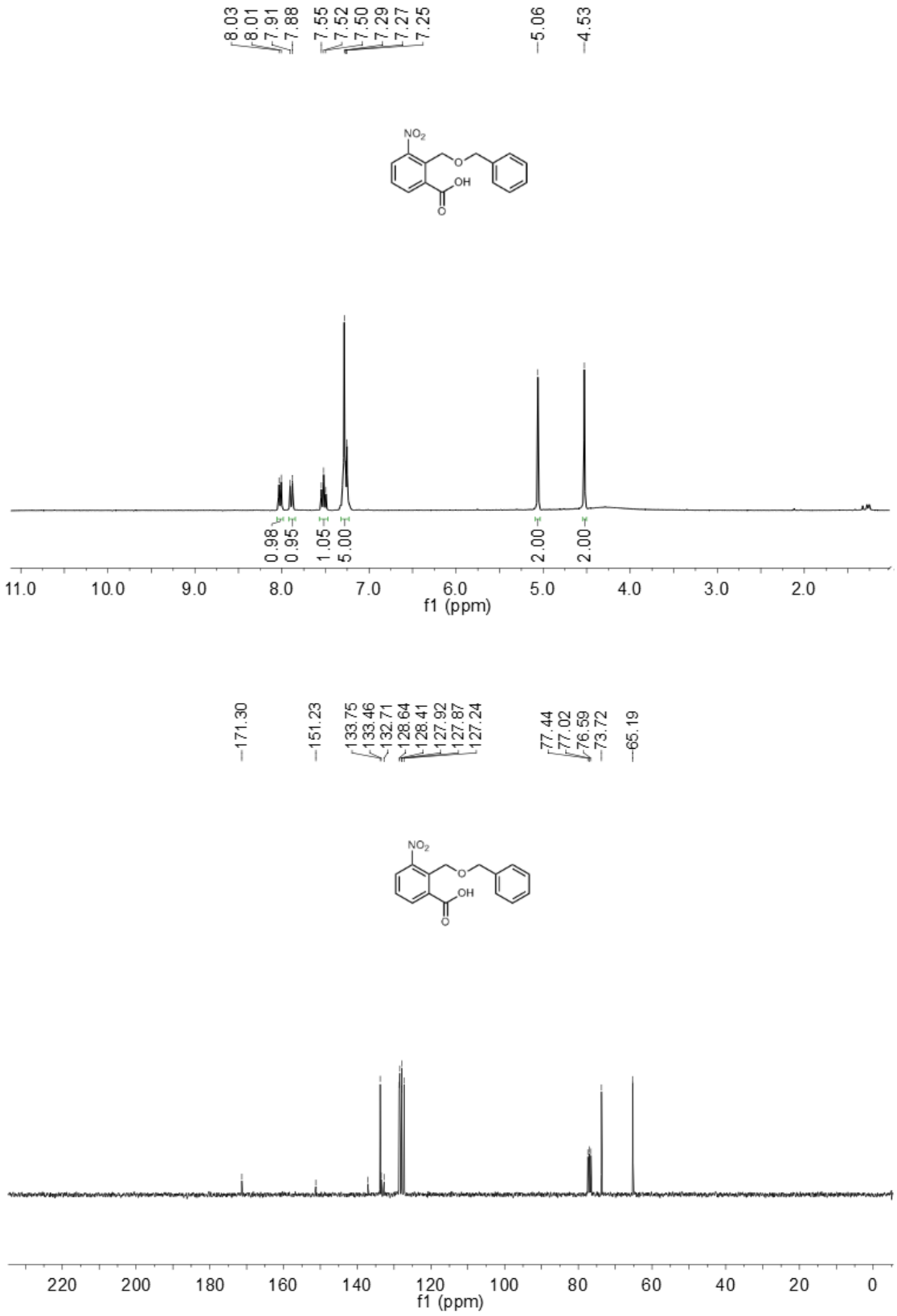


Figure S11 ^{1}H NMR and ^{13}C NMR spectrum of Compound 3.

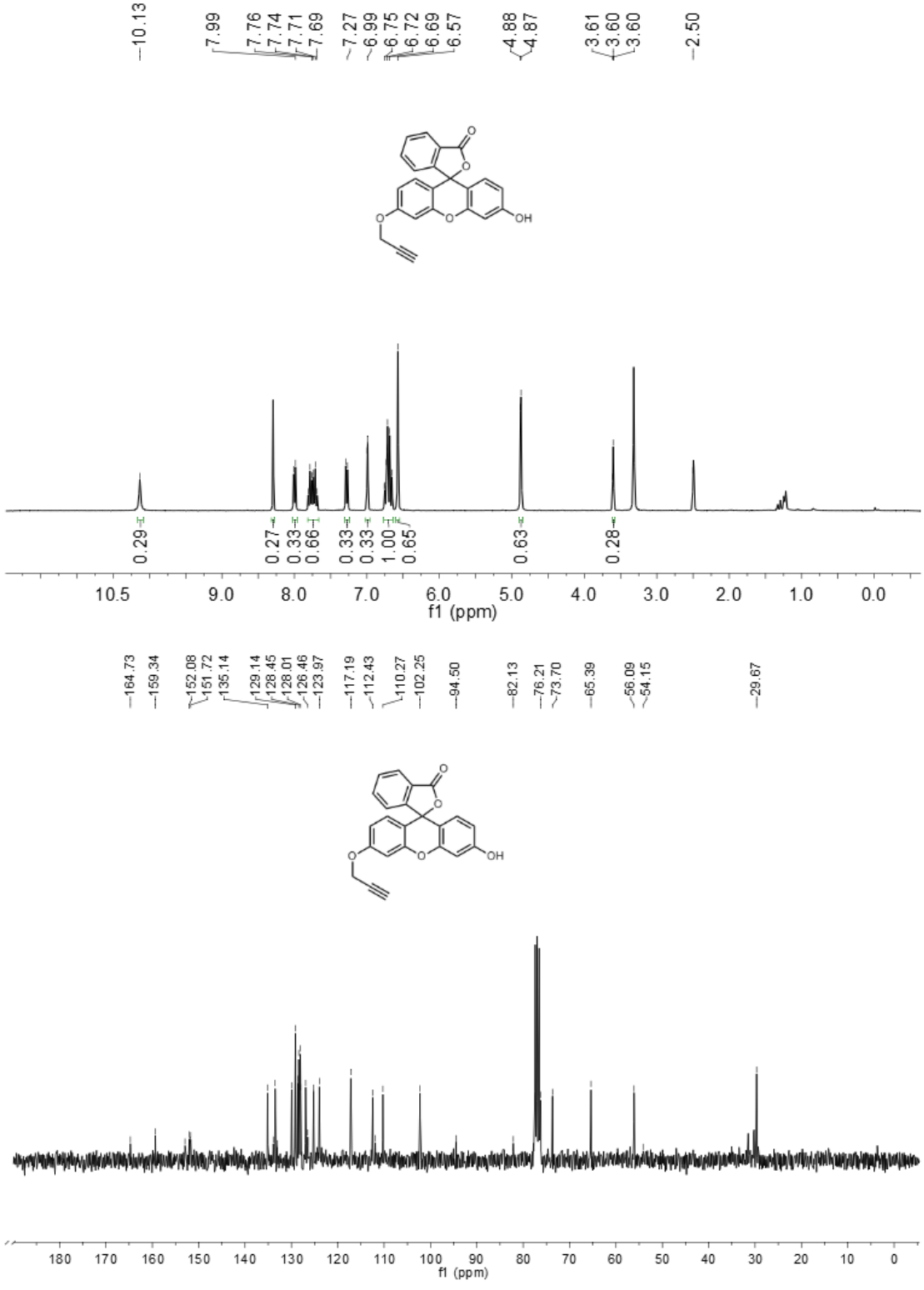


Figure S12 ^{1}H NMR and ^{13}C NMR spectrum of Compound 4.

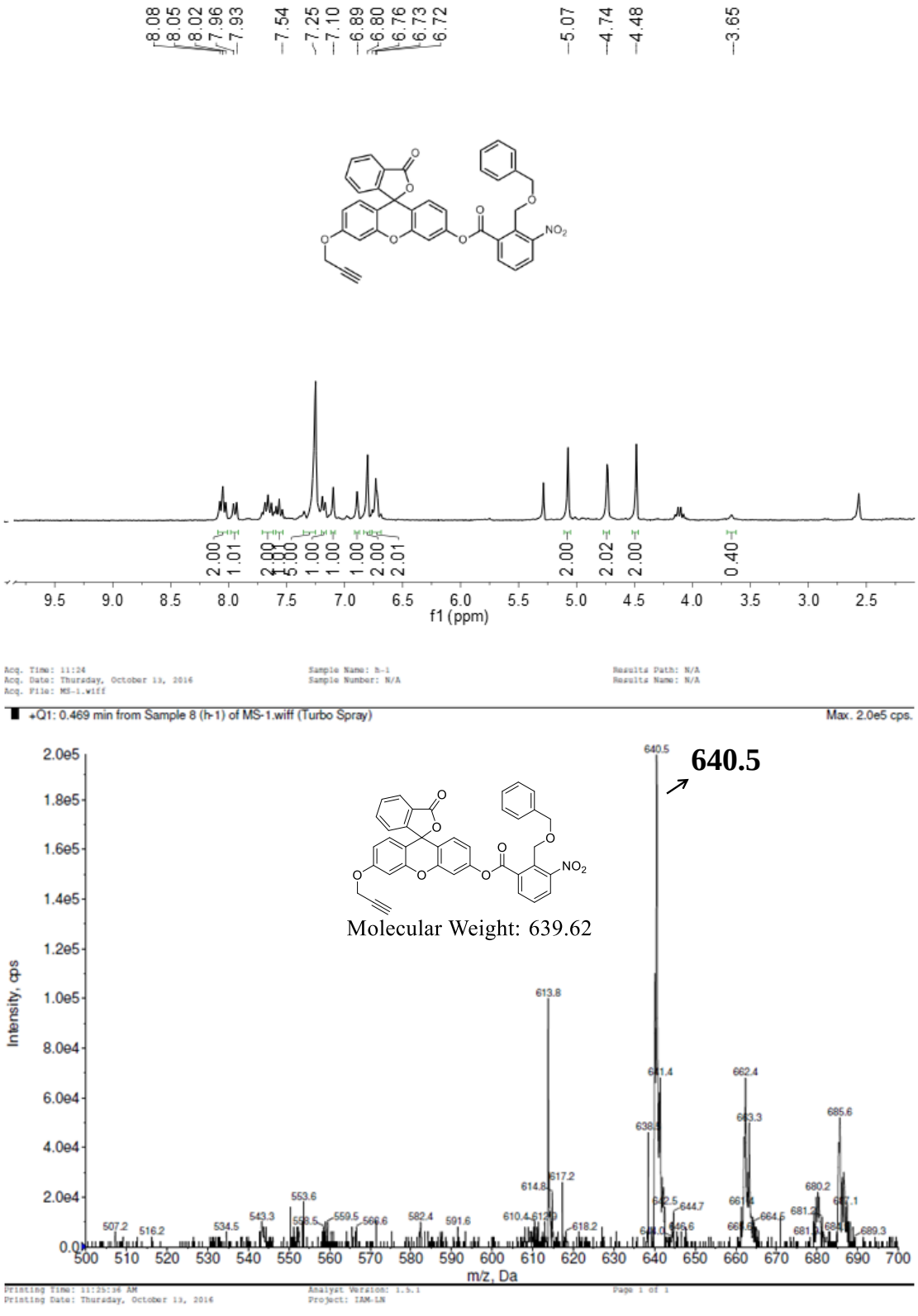
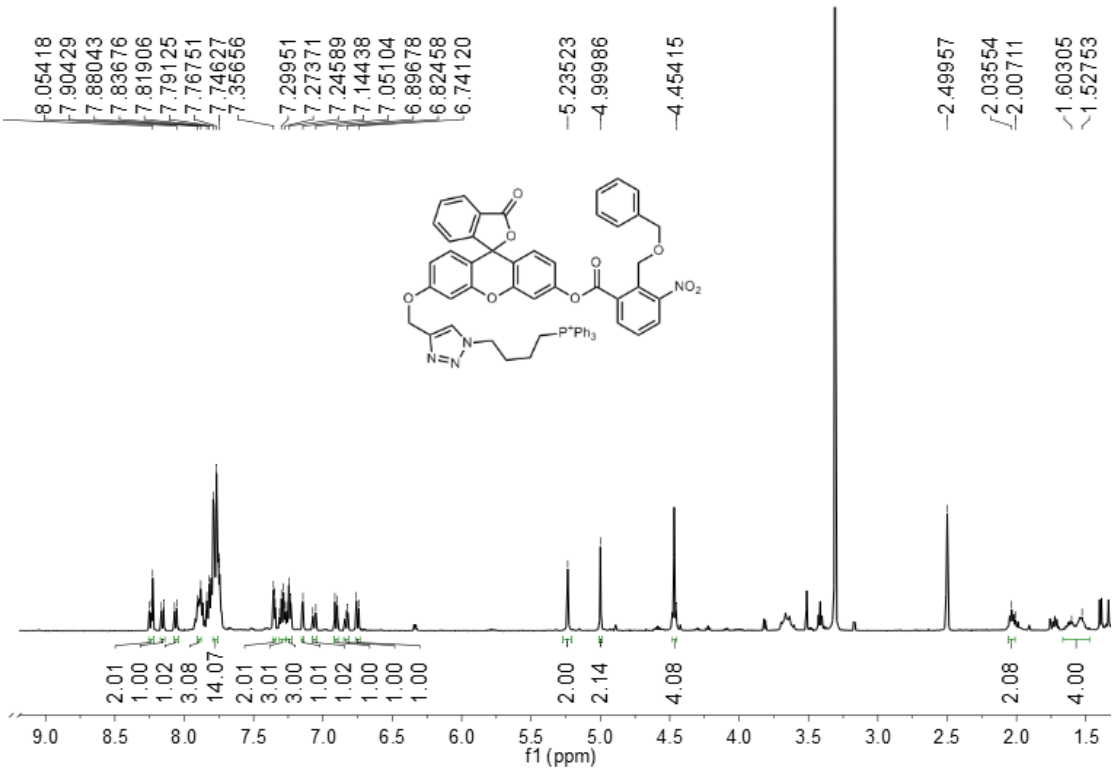


Figure S13 ^{1}H NMR and MS spectrum of Compound **H2**.



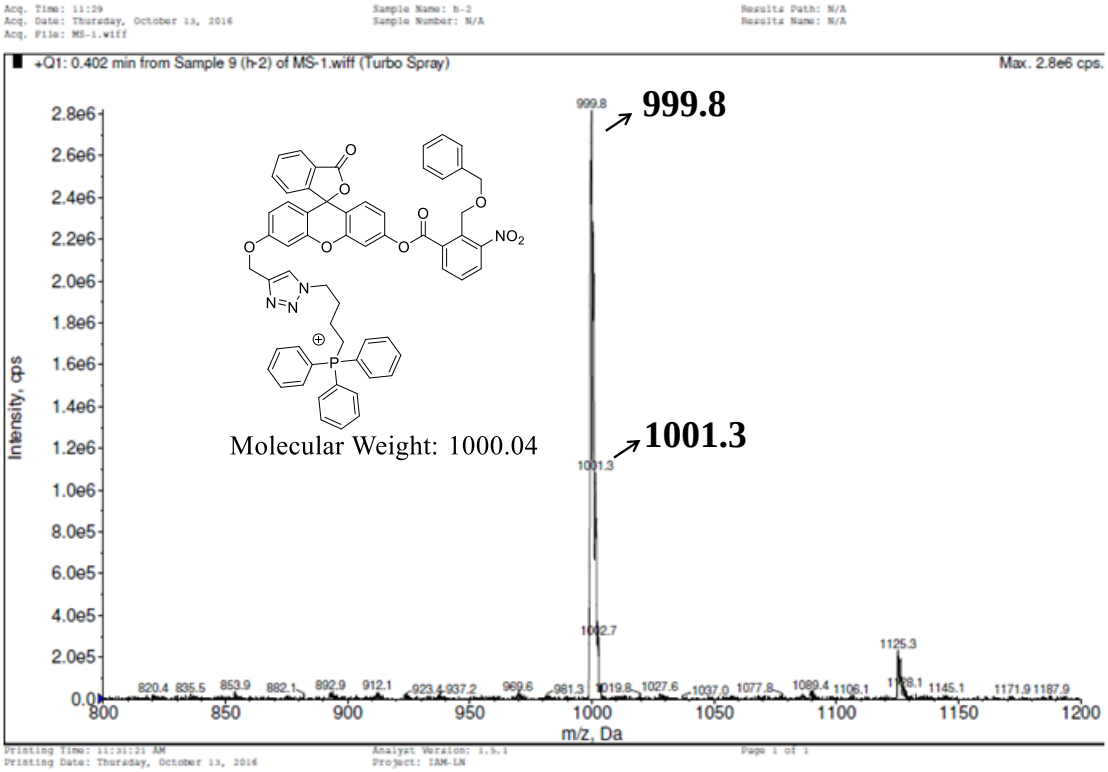


Figure S14 ^{1}H NMR and MS spectrum of **H1**.

8. References

[1] B. Peng, A. Thorsell, T. Karlberg, H. Schüler, S. Q. Yao, Small molecule microarray based discovery of PARP14 inhibitors. *Angew. Chem. Int. Ed.*, 2017, **56**, 248-253.

[2] Y. Kikuchi, J. Nakanishi, T. Shimizu, H. Nakayama, S. Inoue, K. Yamaguchi, H. Iwai, Y. Yoshida, Y. Horiike, T. Takarada, M. Maeda, Arraying heterotypic single cells on photoactivatable cell-culturing substrates. *Langmuir*, 2008, **24**, 13084-13095.

[3] K. Arimitsu, H. Kimura, T. Kajimoto, M. Ono, Y. Ohmomo, M. Yamashita, M. Node, H. Saji, Novel design and synthesis of a radioiodinated glycolipid analog as an acceptor substrate for N-acetylglucosaminyltransferase V. *J. Labelled Compd. R.*, 2013, **56**, 562-566.

[4] J. F. McGoura, H. B. Kramer, M. M. Mackeen, K. dI Gleria, M. Altun, B. M. Kessler, Fluorescence-based active site probes for profiling deubiquitinating enzymes. *Org. Biomol. Chem.*, 2012, **10**, 3379-3383.

[5] A. Kamal, P. S. Srikanth, M. V. P. S. Vishnuvardhan, G. B. Kumar, K. S. Babu, S. M. Ali Hussaini, J. S. Kapure, A. Alarifi, Combretastatin linked 1,3,4-oxadiazole conjugate's as a potent tubulin polymerization inhibitors. *Bioorg. Chem.*, 2016, **65**, 126-136.

[6] G. Colombano, C. Travelli, U. Galli, A. Caldarelli, M. G. Chini, P. L. Canonico, G. Sorba, G. Bifulco, G. C. Tron, A. A. Genazzani, A novel potent nicotinamide phosphoribosyltransferase inhibitor synthesized via click chemistry. *J. Med. Chem.*, 2010, **53**, 616-623.

[7] H. Li, Q. Yao, J. Fan, J. Du, J. Wang, X. Peng, A two-photon NIR-to-NIR fluorescent probe for imaging hydrogen peroxide in living cells. *Biosens. Bioelectron.*, 2017, **94**, 536-543.

[8] Y. Wang, Q. Meng, R. Zhang, H. Jia, C. Wang, Z. Zhang, A mitochondria-targeted ratiometric probe for the fluorescent and colorimetric detection of SO_2 derivatives in live cells. *J Lumin.*, 2017, **192**, 297-302.

[9] T. Ida, T. Sawa, H. Ihara, Y. Tsuchiya, Y. Watanabe, Y. Kumagai, M. Suematsu, H. Motohashi, S. Fujii, T. Matsunaga, M. Yamamoto, K. Ono, N. O. Devarie-Baez, M. Xian, J. M. Fukuto, T. Akaike, Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 7606-7611.

[10] X. Han, F. Yu, X. Song, L. Chen, Quantification of cysteine hydropersulfide with a ratiometric near-infrared fluorescent probe based on selenium-sulfur exchange reaction. *Chem. Sci.*, 2016, **7**, 5098-5107.