Electronic Supplementary Information for

A Fluorescent Probe for the Rapid Detection of Hydrogen Sulfide in Blood Plasma and Brain Tissues in Mice

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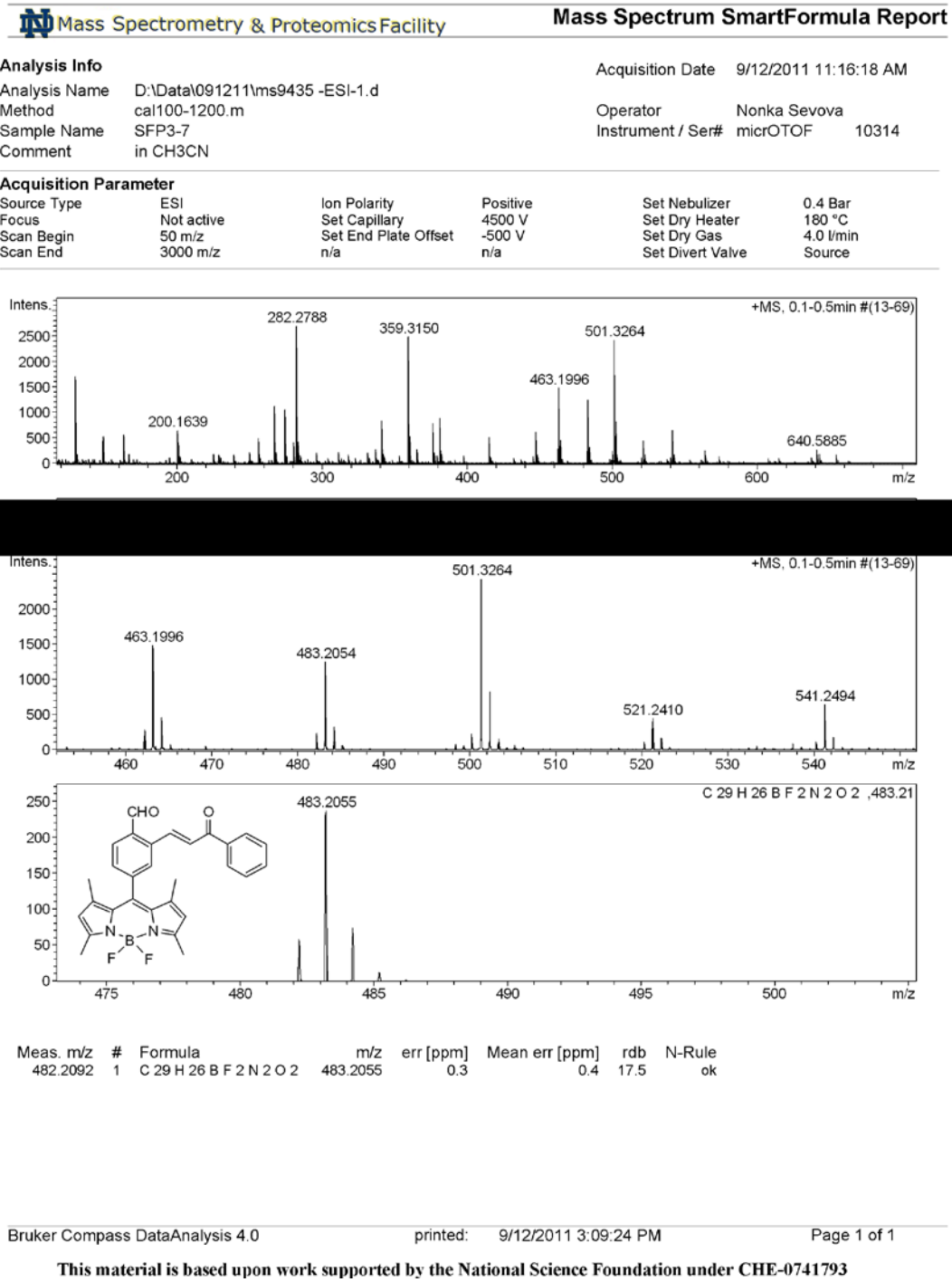
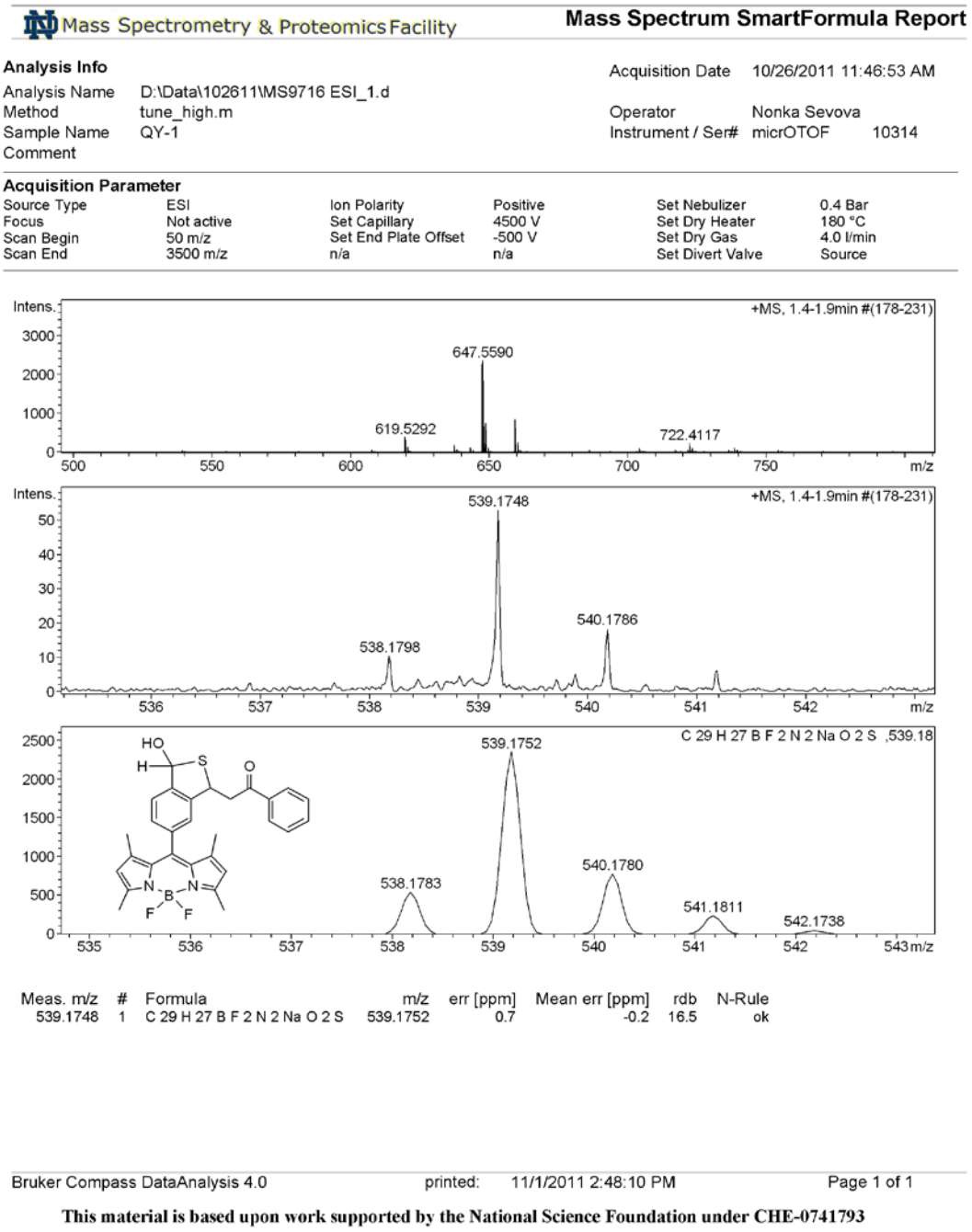
General Information: All reagents were purchased from Sigma-Aldrich. All solvents were distilled before use unless otherwise noted. Eight-week old male C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and Yangzhou University. Phosphate-Buffered Saline PBS, Cat# BM-220 was purchased from Boston Bioproducts. Chromatographic purification of products was accomplished by using forced-flow chromatography on EM Science Geduran silica gel 60 (35-75 μm). Thin layer chromatography was performed on EM Science silica gel 60 F254 plates (250 μm). Visualization of the developed chromatogram was accomplished by UV lamp. Nuclear magnetic resonance (NMR) spectra were acquired on Bruker DRX-500/400 operating at 125/100 MHz for ^{1}H NMR and ^{13}C NMR, respectively; residual protio solvent signals were used as internal standards for calibration purposes. Data for ^{1}H NMR are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), integration, coupling constant (Hz). High-Resolution Mass was performed by Mass Spectrometry Facility at University of Norte Dame. All fluorescence measurements were recorded on a Varian Cary Eclipse Fluorescence Spectrophotometer and Hitachi F4600 Fluorescence Spectrophotometer.



This material is based upon work supported by the National Science Foundation under CHE-0741793

Figure S1a. HR-MS identification of SFP-3 probe (**8**) (calculated for $C_{29}H_{26}BF_2N_2O_2$ $(M+H)^+$ 483.2055; found 483.2054) prior to the addition of sulfide.



This material is based upon work supported by the National Science Foundation under CHE-0741793

Figure S1b. HRMS identification for the sulfide addition product of SFP-3 probe **8a** (yield 40%) (calculated for $C_{29}H_{27}BF_2N_2NaO_3S$ $(M+Na)^+$ 539.1752; found 539.1748).

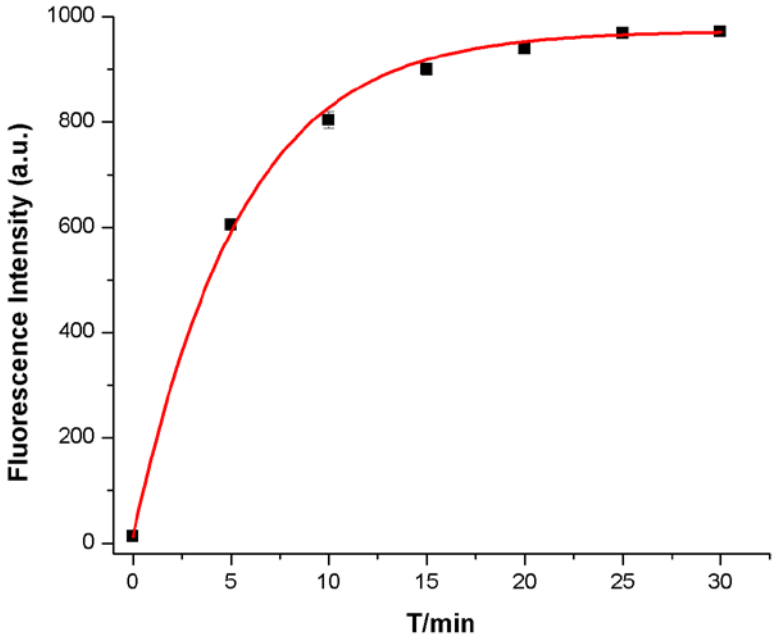


Figure S2. SFP-3 probe (10 μM) incubated with 100 μM Na_2S in PBS buffer (20 mM, pH 7.0, 40% CH_3CN) at 25 ^oC after 0-5-10-15-20-25-30-min. The data represent the average of three independent experiments. Excitation: 500nm, slit, 5nm; emission: 505-600 nm, slit, 5nm, 450V.

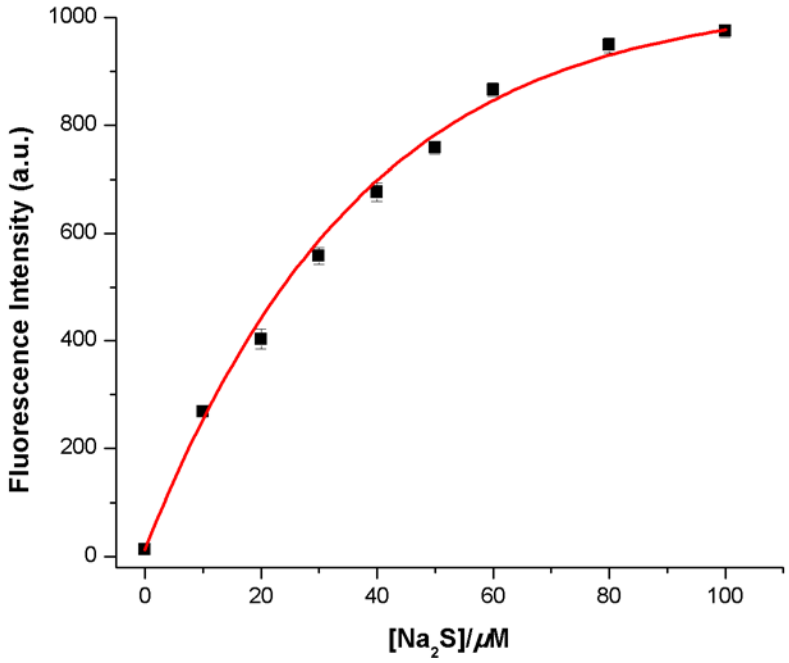


Figure S3. SFP-3 probe (10 μM) incubated with different concentrations of Na_2S (10, 20, 30, 40, 50, 60, 80 and 100 μM) at 25 ^oC for 20 min (20 mM PBS, PH 7.0, 40% CH_3CN), excitation: 500 nm, slit, 5 nm; emission: 505-600 nm, slit, 5 nm, 450V.

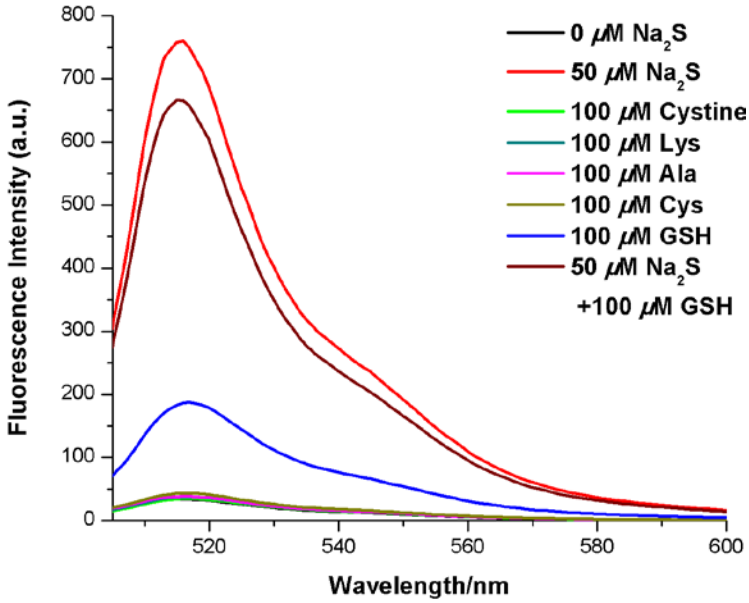


Figure S4. SFP-3 probe (10 μM) incubated with Na_2S and various thiols or amino acid in 20 mM PBS (PH 7.0, 40% CH_3CN) at 25 ^oC for 20 min. Excitation:500 nm, slit, 5 nm; emission: 505-600 nm, slit, 5 nm, 450V.

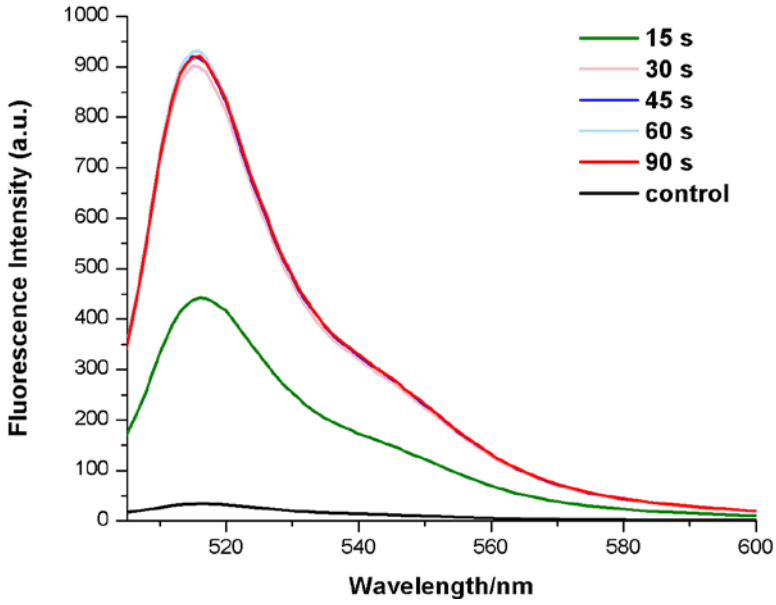


Figure S5. Fluorescence spectra of 10 μM SFP-3 measured in the presence of 1 μl H_2S buffers that H_2S gas had been bubbled through for varying lengths of time. SFP-3 probe was allowed to incubate in the buffers for 30 min at 25 ^oC. Measurements performed in 20 mM PBS buffer (pH 7.0, 40% CH_3CN). Excitation: 500 nm, emission: 505–600 nm. All H_2S buffers were prepared by adding 10 ml DI H_2O to a 25 ml Schlenk tube and subsequently bubbling nitrogen through for 30 min. H_2S was then bubbled through for various lengths of time using a 0.8 mm needle. Ventilation rate was maintained at 1 bubble per second.

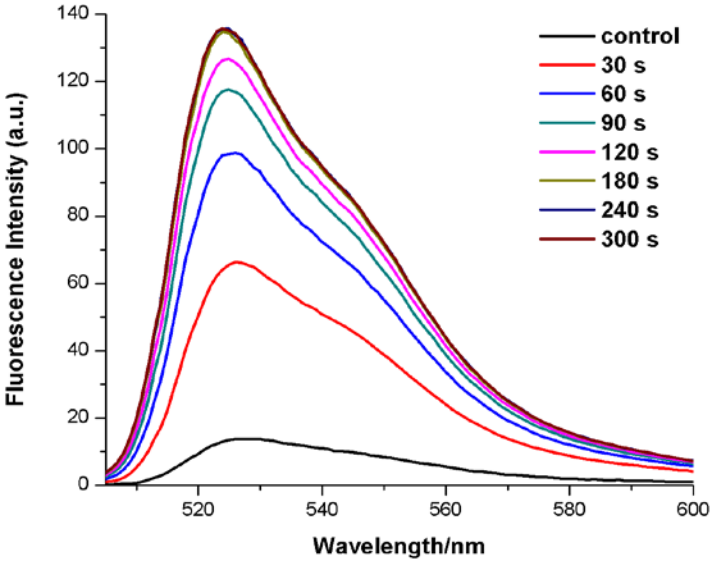


Figure S6. Fluorescence spectra of the SFP-3 probe (100 μM) incubated with 100 μM Na_2S after 30 s, 60 s, 90 s, 120 s, 180 s, 240 s, 300 s in bovine serum (40% CH_3CN) at 25 ^oC. Excitation: 500 nm, emission: 505–600 nm. The data represent the average of four independent experiments.

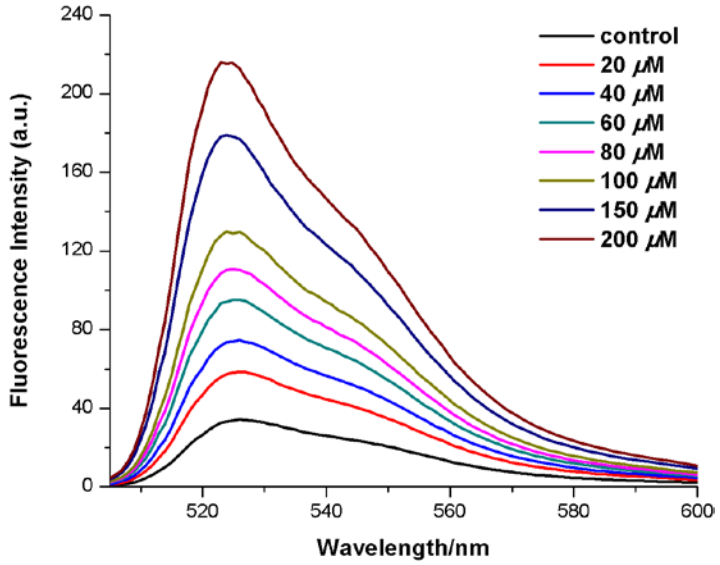


Figure S7. Fluorescence spectra of the SFP-3 probe (100 μM) incubated with 0, 20, 40, 60, 80, 100, 150, 200 μM Na_2S after 3 min in bovine serum (40% CH_3CN) at 25 ^oC. Excitation: 500 nm, emission: 505–600 nm. The data represent the average of four independent experiments.

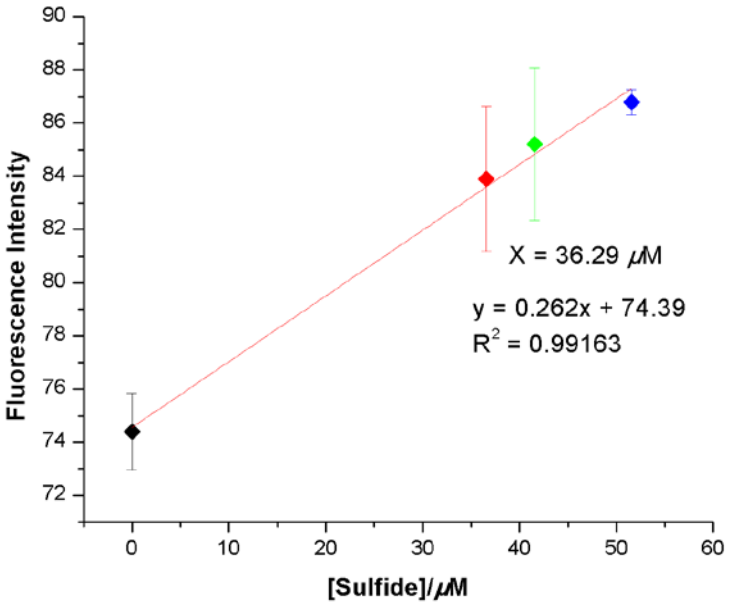


Figure S8. Determination of sulfide concentration in Spiked mouse blood plasma using Na_2S as internal standard (final concentrations 0, X, X+5, X+15 μM). Four eppendorf tubes containing 100 μL blood were centrifuged at 6000 rpm for 2 min. 1 μL $ZnCl_2$ (76 mM, final concentration 1 mM. as 0 point), DI H_2O (as X point), Na_2S (375 μM, final concentration 5 μM, as X+5 μM point), Na_2S (1.125 mM, final concentration 15 μM, as X+15 μM point) were spiked into 45 μL blood plasma respectively, then 30 μL CH_3CN was added respectively to precipitate the protein in plasma[1]. The samples were centrifuged at 6000 rpm for 1min, 70 μL supernatant liquid was added into 140 μL CH_3CN/H_2O buffer (40% CH_3CN) to precipitate proteins again, then centrifuged at 6000 rpm for 5 min, get supernatant liquid samples which will be used to detect in PBS buffer. 50 μL sample was added into 30 μL 40 mM PBS buffer (final concentration 12 mM, pH 7.0), after that, 20 μL 100 μM SFP-3 probe (final concentration 20 μM, 40% CH_3CN) was added. Emission spectra (λex = 500 nm, emission at 517 nm) were collected after the mixture were incubated at 37 ^oC for 30 min. The data represent the average of three independent experiments.

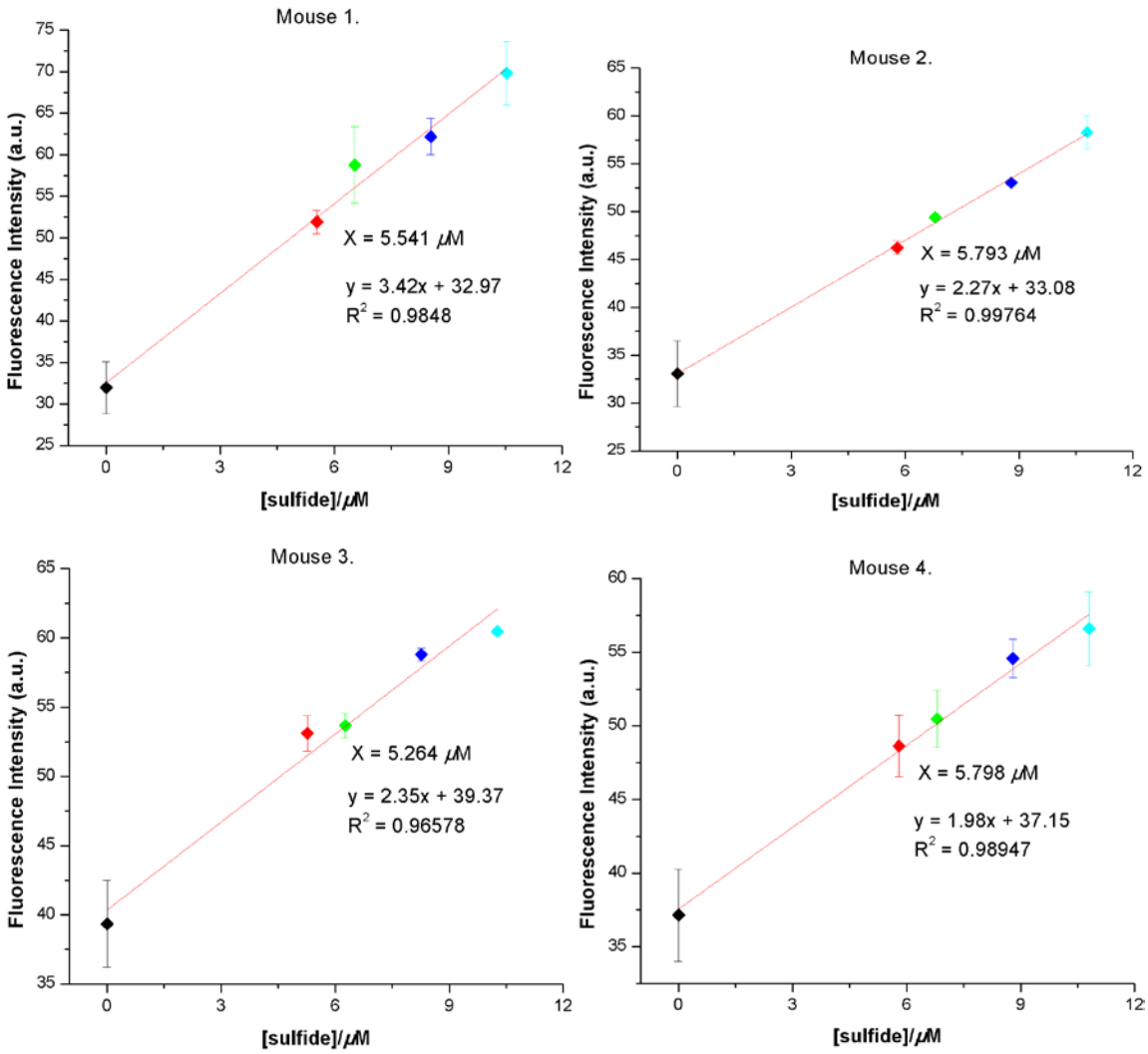


Figure S9. Determination of sulfide concentration in the fresh mouse blood plasma with SFP-3 probe (see the following H_2S detection protocols). Sulfide concentrations in mouse blood plasma 1, 2, 3, 4 were 55.41 μM, 57.93 μM, 52.64 μM, 57.98 μM, respectively. The average sulfide concentration is 55.99 ± 2.54 μM.

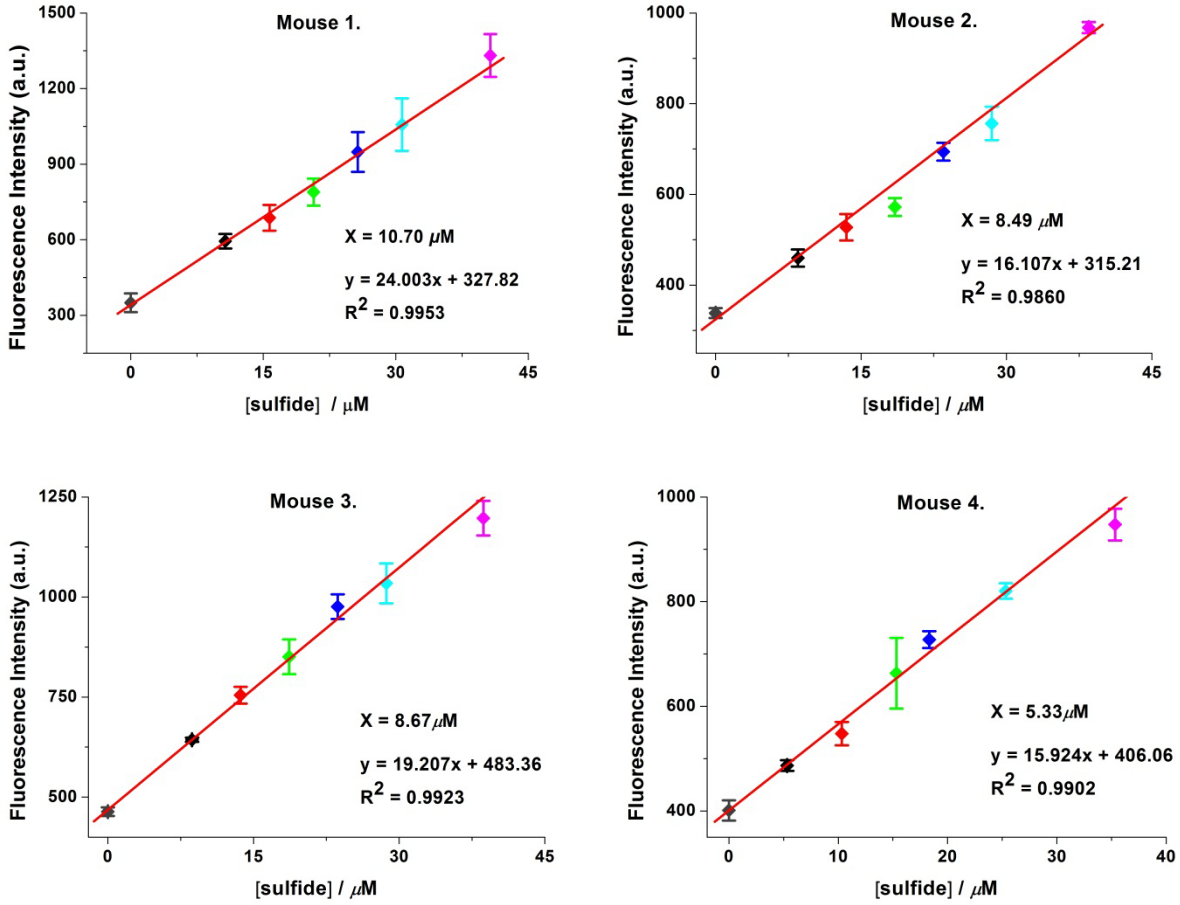


Figure S10. Determination of sulfide concentrations in mice brain tissues with SFP-3 probe (see the following H_2S detection protocols). Sulfide concentrations in mice brain homogenates (2 %, w/v) were 10.70 μM, 8.49 μM, 8.67 μM, 5.33 μM, respectively. Total protein concentrations in mice brain homogenates (1 %, w/v) were 0.80 g/L, 0.50 g/L, 0.56 g/L, 0.50 g/L, respectively. The H_2S concentrations in mice brain tissues were expressed as μmol/g protein. The H_2S concentrations in mice brain were 6.69 μmol/g protein, 8.49 μmol/g protein, 7.74 μmol/g protein, 5.33 μmol/g protein, respectively. The average H_2S concentration was 7.06 ± 1.37 μmol/g protein. Excitation: 400 nm, slit, 5 nm; emission: 505-600 nm, slit, 5 nm, 500V.

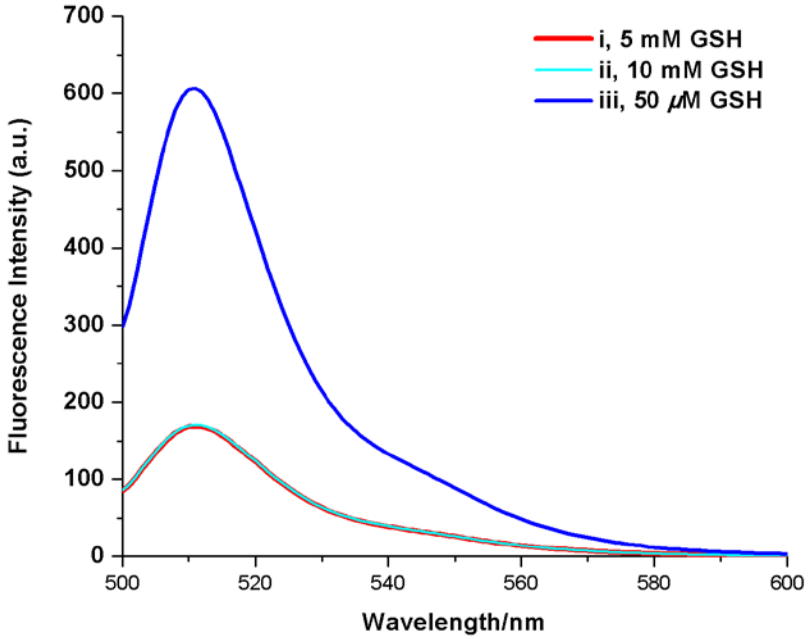
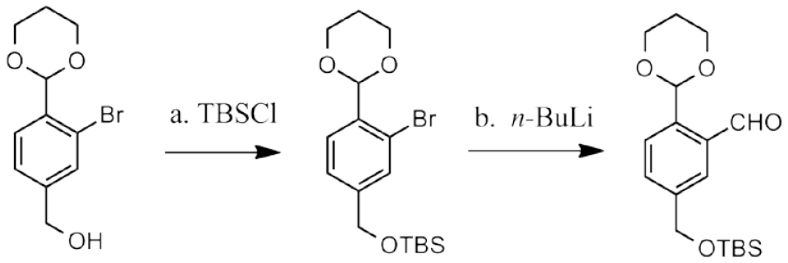


Figure S11. SFP-3 probe (10 μM) incubated with 50 μM Na_2S and GSH (5, 10 mM) in 20 mM PBS (PH 7.4, 40% CH_3CN) at 25 ^oC for 60 min. Excitation:496 nm, slit, 5 nm; emission: 500-600 nm, slit, 5 nm, 300V, at 240 nm/min. Fluorescence measurements were carried out at room temperature on a Hitachi Fluorescence Spectrophotometer F-7000. The data represent the average of three independent experiments.

Supplementary Methods

Synthetic protocols of compounds 1-3



1 2 3

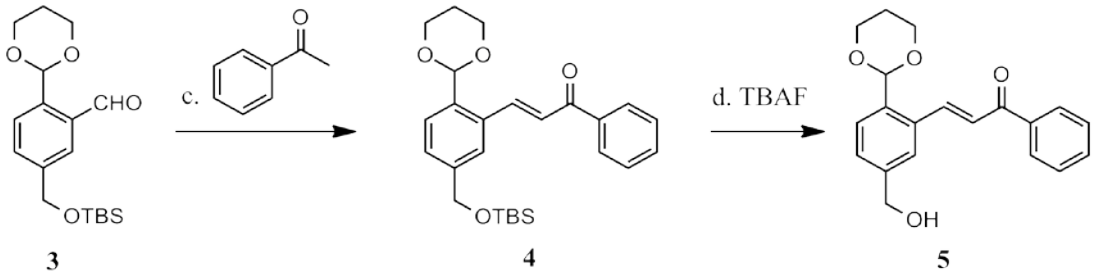
((3-bromo-4-(1,3-dioxan-2-yl)benzyl)oxy)(tert-butyl)dimethylsilane (2)

A solution of TBSCl (331 mg, 2.21 mmol) in dry DMF (2 mL) was added by using a syringe to a solution of compound **1** (500 mg, 1.84 mmol) and imidazole (461 mg, 3.68 mmol) in dry DMF (2 mL) in a 25 mL Schlenk tube. The reaction mixture was stirred at room temperature for 6 h. The reaction was quenched with deionized water (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with water and brine and then dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography on SiO_2 to give the purified product, colorless oil. Yield 82 %. TLC (silica, hexane:EtOAc, 4:1 v/v): R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.63 (d, *J* = 8.0 Hz, 1 H), 7.50 (s, 1 H), 7.26 (d, *J* = 8.0 Hz, 1 H), 5.74 (s, 1 H), 4.96 (s, 2 H), 4.25 (dd, *J* = 5.0, 11.0 Hz, 2 H), 3.99–4.04 (m, 2 H), 2.01–2.25 (m, 1 H), 1.3 (dd, *J* = 1.0, 13.5 Hz, 1 H), 0.92 (s, 9 H), 0.08 (s, 6 H) ; ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.2, 136.1, 130.1, 127.9, 125.1, 122.3, 101.0, 67.7, 64.1, 26.0, 25.8, 18.5, -5.2; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{17}H_{28}BrO_3Si$, 387.0991; found, 387.0968 .

5-(((tert-butyldimethylsilyl)oxy)methyl)-2-(1,3-dioxan-2-yl)benzaldehyde (3)

To a solution of compound **2** (500 mg, 1.30 mmol) in THF (4 mL) cooled to -78^{o}C under nitrogen was added *n*-BuLi (240 µL, 2.55 mmol) in hexane dropwise with the temperature kept below -70^{o}C. After 1 h, dry DMF (812 µL, 5.2 mmol) was added dropwise. The resulting solution was allowed to gradually warm to room temperature and stirring was continued for 2h. The reaction was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with water and brine and then dried with Na_2SO_4. The solvent was evaporated and the crude product was purified by column chromatography on SiO_2 to give the purified product, white solid. Yield 67 %. TLC (silica, hexane:EtOAc, 4:1 v/v): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.54 (s, 1 H), 7.88 (s, 1 H), 7.60–7.68 (m, 2 H), 6.02 (s, 1 H), 4.80 (s, 2 H), 4.27–4.29 (m, 2 H), 4.02–4.07 (m, 2 H), 2.25–2.28 (m, 1 H), 1.48–1.50 (m, 1 H), 0.95 (s, 9 H),

0.11 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 192.4, 142.9, 138.4, 133.8, 131.0, 127.4, 127.2, 100.2, 67.7, 64.2, 26.0, 25.7, 18.4, -5.2; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{18}H_{28}O_4Si$, 337.1835; found, 337.1819.



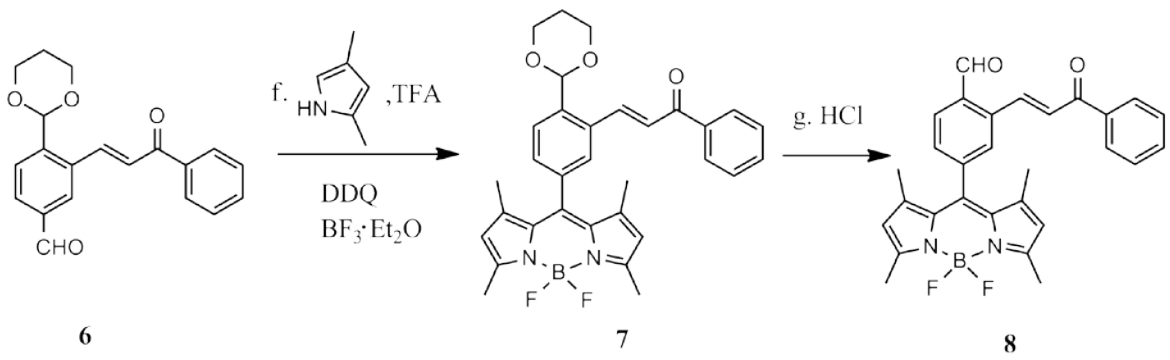
3 4 5

(E)-3-(5-(((tert-butyldimethylsilyl)oxy)methyl)-2-(1,3-dioxan-2-yl)phenyl)-1-phenylprop-2-en-1-one (4)

5 N NaOH (2.1 mL) was added dropwise into a stirred solution of aldehyde **3** (359 mg, 1.07 mmol) and acetophenone (125 µL, 1.07 mmol) in 5 mL MeOH. The reaction mixture was continuously stirred at room temperature for 2 h. After, the mixture was filtered to collect the solid, which was then washed with water to obtain the crude product. The crude product was then purified by column chromatography on SiO_2 to give the purified product as a yellow solid. Yield 74 %. TLC (silica, hexane:EtOAc, 4:1 v/v): R_f = 0.45; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (d, *J* = 15.6 Hz, 1 H), 8.05 (d, *J* = 7.2 Hz, 2 H), 7.76 (s, 1 H), 7.58–7.64 (m, 2 H), 7.51–7.54 (m, 2 H), 7.45 (d, *J* = 16.0 Hz, 1 H), 7.38 (d, *J* = 8.0Hz, 1 H), 5.75 (s, 1 H), 4.80 (s, 2 H), 4.27–4.30 (m, 2 H), 3.99–4.05 (m, 2 H), 2.22–2.26 (m, 1 H), 1.46 (d, *J* = 13.2Hz, 1 H), 0.99 (s, 9 H), 0.13 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.8, 142.6, 142.4, 138.2, 136.4, 133.1, 132.6, 128.6, 128.5, 127.5, 127.2, 124.0, 123.8, 100.3, 67.5, 64.3, 25.9, 25.6, 18.3, -5.3; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{26}H_{35}O_4Si$, 439.2304; found, 439.2301.

(E)-3-(2-(1,3-dioxan-2-yl)-5-(hydroxymethyl)phenyl)-1-phenylprop-2-en-1-one (5)

A solution of **4** (394 mg, 0.9 mmol) and TBAF (1.0M in THF, 856 μL, 0.9 mmol) in THF (8 mL) was stirred at RT for 30 min, then it was quenched with aq. sat. $NaHCO_3$ (6 mL) and extracted with EtOAc (3 x 100 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography on SiO_2 to give the purified product, a yellow solid. Yield 82 %. TLC (silica, hexane:EtOAc, 1:2 v/v): R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (d, *J* = 15.5 Hz, 1 H), 7.96–8.02 (m, 2 H), 7.71 (s, 1 H), 7.55–7.60 (m, 2 H), 7.47–7.50 (m, 2 H), 7.35–7.43 (m, 2 H), 5.71 (s, 1 H), 4.70 (s, 2 H), 4.24–4.27 (m, 2 H), 3.97–4.02 (m, 2 H), 2.20–2.25 (m, 1 H), 1.44(d, *J* = 13.5 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.2, 142.5, 142.1, 138.2, 136.9, 133.4, 132.9, 128.8, 128.7, 128.4, 127.4, 124.9, 124.0, 100.3, 67.6, 64.6, 25.7; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{20}H_{21}O_4$, 325.1440; found, 325.1431.



(E)-4-(1,3-dioxan-2-yl)-3-(3-oxo-3-phenylprop-1-en-1-yl)benzaldehyde (6)

A mixture of compound **5** (330 mg, 1.02 mmol), PCC (332 mg, 1.53 mmol) and Celite (300 mg) in CH_2Cl_2 (10 ml) was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite and a silica gel pad and then evaporated to obtain the crude product. This was purified by column chromatography on SiO_2 to give a white solid. Yield 93 %. TLC (silica, hexane:EtOAc, 1:2 v/v): R_f = 0.8; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.04 (s, 1 H), 8.25 (d, *J* = 15.5 Hz, 1 H), 8.20 (s, 1 H), 8.03 (d, *J* = 7.0 Hz, 2 H), 7.87–7.95 (m, 2 H), 7.56–7.58 (m, 1 H), 7.43–7.53 (m, 3 H), 5.75 (s, 1 H), 4.26–4.28 (m, 2 H), 3.98–4.03 (m, 2 H), 2.21–2.24 (m, 1 H), 1.47 (d, *J* = 13.0 Hz, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 191.6, 190.3, 143.0, 140.8, 137.8, 136.7, 134.5, 133.1, 130.9, 128.7, 128.6, 128.0, 127.6, 125.2, 99.4, 67.6, 25.6; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{20}H_{19}O_4$, 323.1283; found, 323.1281.

(E)-10-(4-(1,3-dioxan-2-yl)-3-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (7)

Compound **6** (85mg, 0.264 mmol) and 2,4-dimethylpyrrole (54.4 μL, 0.53 mmol) were dissolved in 100 mL of dry CH_2Cl_2 under N_2. One drop of TFA was added, after the solution was stirred at room temperature for 12 h, DDQ (66 mg, 0.29 mmol) was added, and stirring was continued for 1.5 h. Then 184 μL of Et_3N and 163 μL of $BF_3{\cdot}Et_2O$ were added, and the solution was stirred at room temperature for 2 h. The reaction mixture was washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by column chromatography on SiO_2 to give the purified product, a red foam. Yield 22 %. TLC (silica, CH_2Cl_2): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (d, *J* = 16.0 Hz, 1 H), 8.02 (d, *J* = 7.5 Hz, 2 H), 7.82 (d, *J* = 7.5 Hz, 1 H), 7.73 (s, 1 H), 7.57–7.60 (m, 1 H), 7.48–7.52 (m, 1 H), 7.46 (d, *J* = 15.5 Hz, 1 H), 7.38 (dd, *J* = 1.5, 8.0 Hz, 1 H), 6.00 (s, 2 H), 5.83 (s, 1 H), 4.33 (dd, *J* = 4.5, 11.0 Hz, 2 H), 4.05–4.10 (m, 2 H), 2.56 (s, 6 H), 2.26–2.34 (m, 1 H), 1.51 (d, *J* = 13.5 Hz, 1 H), 1.42 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.2, 156.0, 143.1, 141.0, 140.4, 138.7, 137.9, 136.0, 134.5, 133.1, 131.4, 129.5, 128.8, 128.7, 128.2, 126.3, 124.4, 121.6, 99.9, 67.8, 25.7, 15.0, 14.7; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{32}H_{32}BF_2N_2O_3$, 541.2474; found, 541.2506.

(E)-5,5-difluoro-10-(4-formyl-3-(3-oxo-3-phenylprop-1-en-1-yl)phenyl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (8)

10 N HCl (708 μL) was added to a solution of compound **7** (85 mg, 0.16 mmol) in acetone (4 mL) and stirred at room temperature for 2 h. The reaction was quenched with water (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with water, a saturated $NaHCO_3$ solution, and brine, and then dried with Na_2SO_4. The crude product was purified by column chromatography on SiO_2 to give the purified product, a red foam. Yield 81 %. TLC (silica, CH_2Cl_2): R_f = 0.7; ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.44 (s, 1 H), 8.62 (d, *J* = 16.0 Hz, 1 H), 8.05 (d, *J* = 8.0 Hz, 1 H), 8.01 (d, *J* = 7.5 Hz, 2 H), 7.78 (s, 1 H), 7.54–7.60 (m, 2 H), 7.48–7.51 (m, 2 H), 7.45 (d, *J* = 16.0 Hz, 1 H), 6.02 (s, 2 H), 2.56 (s, 6 H), 1.41 (s, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 190.9, 189.8, 156.7, 142.7, 141.1, 139.5, 138.9, 138.2, 137.5, 134.5, 133.4, 132.8, 130.9, 130.0, 128.9, 128.8, 127.9, 127.6, 121.9, 15.0, 14.8; HRMS (ESI^+): $(M+H)^+$ calcd. for $C_{29}H_{26}BF_2N_2O_2$, 483.2055; found, 483.2054.

Quantum Yields

Quantum yields were determined using fluorescein as a standard according to a published method[2]. For SFP-3 and fluorescein, the absorbance spectra were measured within an absorbance range of 0.01 to 0.1. The quantum yield was calculated according to the equation: $\Phi_{sample} = \Phi_{standard} (Grad_{sample}/Grad_{standard})(\eta^2_{sample}/\eta^2_{standard})$; where Φ is the quantum yield, $\Phi_{fluorescein}$ = 0.79 in 0.1 M NaOH, Grad is the slope of the plot of absorbance versus integrated emission intensity, and η is the refractive index of the solvent.

SFP-3, $\varepsilon = 1.12 * 10^5\ M^{-1}\ cm^{-1}$, Φ= 0.10.

Protocols for H_2S Measurement

Fluorometric analysis.

All fluorescence measurements were carried out at room temperature on a Varian Cary Eclipse Fluorescence Spectrophotometer. The samples were excited at 500 nm with the excitation and emission slit widths set at 5 nm. The emission spectrum was scanned from 505 nm to 600 nm at 120 nm/min. The photomultiplier voltage was set at 450 V. The probe was dissolved in CH_3CN to make a 1.0 mM stock solution, which was diluted to the required concentration for measurement.

SFP-3 stock solution preparation

SFP-3 (1.3 mg, 0.002697 mmol) was dissolved into CH3CN (1.08 mL) to get 2.5 mM stock solution, which was then diluted to 1.0 mM stock solution for general use.

Na_2S stock solution (20 mM) preparation[3]

5 mg EDTA was dissolved in 10 mL DI H_2O in a 25 mL Schlenk tube. The solution was purged vigorously with nitrogen for 15 min. Then 48 mg sodium sulfide ($Na_2S{\cdot}9H_2O$) was dissolved in the solution under nitrogen. The resulting solution was 20 mM Na_2S, which was then diluted to 1.0 – 2.0 mM stock solution for general use.

SFP-3 Fluorescence responses to H_2S

Fig 2a. 1 µL of SFP-3 stock solution (1.0 mM) (for a final concentration: 10 μM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 39 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL Na_2S stock solution (1.0 mM) was added (for a final concentration of 100 μM). The mixture was then incubated at room temperature. Emission spectra (λ_{ex} = 500 nm) were collected at 0, 5, 10, 15, 20, 25, and 30 min.

Fig 2b. 1 µL of SFP-3 stock solution (1.0 mM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM), 39 µL CH_3CN and DI H_2O (9, 8, 7, 6, 5, 4, 2, and 0 µL respectively) in a 1.5 mL eppendorf tube. Na_2S stock solution (1, 2, 3, 4, 5, 6, 8, and 10 µL, 1.0 mM, respectively) was added (for a final concentration of 10, 20, 30, 40, 50, 60, 80, and 100 μM, respectively). Emission spectra (λ_{ex} = 500 nm) were collected after the mixture was incubated at room temperature for 20 min.

SFP-3 selectivity tests to other thiols and amino acids

Fig 2c. Selectivities for SFP-3 probe (10 μM) were measured by fluorescence responses at 20 min in PBS buffer (20 mM, pH 7.0, 40% CH_3CN) at 25 ^{o}C. Excitation: 500 nm, emission: 505–600 nm. The data represent the average of three independent experiments.

Na_2S (0 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. Then 10 µL DI H_2O was added.

Na_2S (50 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 5 µL DI H_2O and 5 µL Na_2S stock solution (1.0 mM) was then added.

Cystine (100 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL cystine (1.0 mM) was then added.

Lys (100 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL Lys (1.0 mM) was then added.

Ala (100 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL Ala (1.0 mM) was then added.

Cys (100 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL Cys (1.0 mM) was then added.

GSH (100 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 10 µL GSH (1.0 mM) was then added.

GSH (100 μM) + **Na_2S** (50 μM): 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 μM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM) and 30 µL CH_3CN in a 1.5 mL eppendorf tube. 4 µL H_2O, 1 µL GSH (10 mM), and 5 µL Na_2S stock solution (1.0 mM) were then added.

SFP-3 Fluorescence responses to H_2S gas buffer

Fig 2d. 10 µL of SFP-3 stock solution (100 μM) (for a final concentration: 10 µM) was dissolved into a mixed solution of 50 µL PBS buffer (pH 7.0, 40 mM), 9 µL DI H_2O, and 30 µL CH_3CN in a 1.5 mL eppendorf tube. Then the mixture was incubated with 1 μl H_2S buffer (bubbling H_2S 10 min–saturated solution) at 25 ^oC. Emission spectra (λ_{ex} = 500 nm) were collected from 5 s–300 s.

Fluorescence response of SFP-3 probe in Bovine plasma

Figure 3a: Fluorescence spectra of the SFP-3 probe (100 μM) incubated with 100 μM Na_2S in bovine serum (40% CH_3CN) at 25 ^oC. Excitation: 500 nm, emission: 505–600 nm. The data represent the average of four independent experiments.

20 µL Na_2S stock solution (1.0 mM) (for a final concentration: 100 µM) was spiked into a 1.5 mL eppendorf tube containing 100 µL Bovine plasma. Then, 80 µL of SFP-3 probe stock solution (250 μM) (for a final concentration 100 μM) was added, the mixture were incubated at 25 ^oC. The emission spectra were collected at 30, 60, 90, 120, 180, 240 and 300 s, respectively.

Figure 3b: 1.0 mM Na_2S stock solution (0, 4, 8, 12, 16, and 20 µL, respectively) were spiked into a 1.5 mL eppendorf tube containing 100 µL Bovine plasma and DI H_2O (20, 16, 12, 8, 4, and 0 µL, respectively) (for Na_2S final concentration 0, 20, 40, 60, 80, 100 μM, respectively). Then, 80 µL of SFP-3 probe stock solution (250 μM) (for a final concentration 100 μM) was added, Emission spectra (λ_{ex} = 500 nm) were collected after the mixture were incubated at 25 ^oC for 3min.

2.0 mM Na_2S stock solution (15 and 20 µL, respectively) were spiked into a 1.5 mL eppendorf tube containing 100 µL Bovine plasma and DI H_2O (5 and 0 µL, respectively) (for a final concentration 150 and 200 μM, respectively). Then, 80 µL of SFP-3 probe stock solution (250 μM) (for a final concentration 100 μM) was added, Emission spectra (λ_{ex} = 500 nm) were collected after the mixture were incubated at 25 ^oC for 3min.

Detection of H_2S in mouse blood plasma

Table 1 and Figure S9: The animal protocol is approved by the Institutional Animal Care and Use Committee at the University of Chicago. Eight-week old male C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were anesthetized with ketamine/xylazine and blood was drawn with a heparinized (~50 USP/ml) syringe from the inferior vena cava of male C57BL6/J mice. Mouse blood (500 µL) was centrifuged in a eppendorf tube at 6000 rpm for 5min. 10 µL blood plasma was added into 50 µL bovine plasma, then 0, 1, 3, 5 µL Na_2S stock solution (100 μM) were spiked into the sample as internal standard (a final H_2S concentration X, X+1, X+3, X+5 μM, respectively), after that, 40 µL 50 μM SFP-3 probe (final concentration 20 μM. 40% CH_3CN) was added. Emission spectra (λex = 500 nm, emission at 516 nm) were collected after the mixture were incubated at 37 ^oC for 3 min. Zero point was obtained by adding 1 µL, 100 mM (final concentration 1 mM) $ZnCl_2$ to trap H_2S in the sample. The data represent the average of three independent experiments.

Detection of H_2S in mouse brain tissues

Table 1 and Figure S10: All animal procedures are approved by the Institutional Animal Care and Use Committee at Nanjing University. Eight-week old male C57BL/6 mice were purchased from Yangzhou University. For the measurement of H_2S, mice were sacrificed and brains were immediately removed, homogenized with a 9 volumes (w/v) of ice-cold 100 mM PBS buffer (pH 7.4) and centrifuged at 10,000 g for 10 min at 4 ^oC. **All the above operations were performed in ice bath, and the homogenate supernatants were immediately used for the following determination.** All fluorescence measurements were recorded on a Hitachi F4600 Fluorescence Spectrophotometer. Total protein concentrations in mice brain were determined using the Coomassie Blue method.

Determination of sulfide concentration in spiked brain homogenates using Na_2S as internal standard (X, X+5, X+10, X+15, X+20, X+30 μM). 20 μL of 10% homogenates supernatant (final concentration 2%, w/v) was added into Eppendorf tubes with 30 μL PBS buffer (100 mM, pH 7.4), 30 μL CH_3CN and DI H_2O (10, 9.5, 9, 8.5, 8, and 7 μL respectively). Then 0, 0.5, 1, 1.5, 2, 3 μL Na_2S stock solution (1.0 mM) were spiked into the sample as internal standard, after that, 10 μL 1.0 mM SFP-3 probe (final concentration 100 μM) was added. Emission spectra (λ_{ex} = 400 nm, emission at 520 nm) were collected after the mixture were incubated at 37 ^oC for 20 min. Zero point was obtained by adding 1 μL, 100 mM (final concentration 1 mM) $ZnCl_2$ to trap H_2S in the sample. The H_2S concentration of each sample was calculated by a calibration curve of Na_2S and results were expressed as μmol/g protein. The data represent the average of three independent experiments.

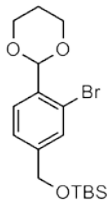
References

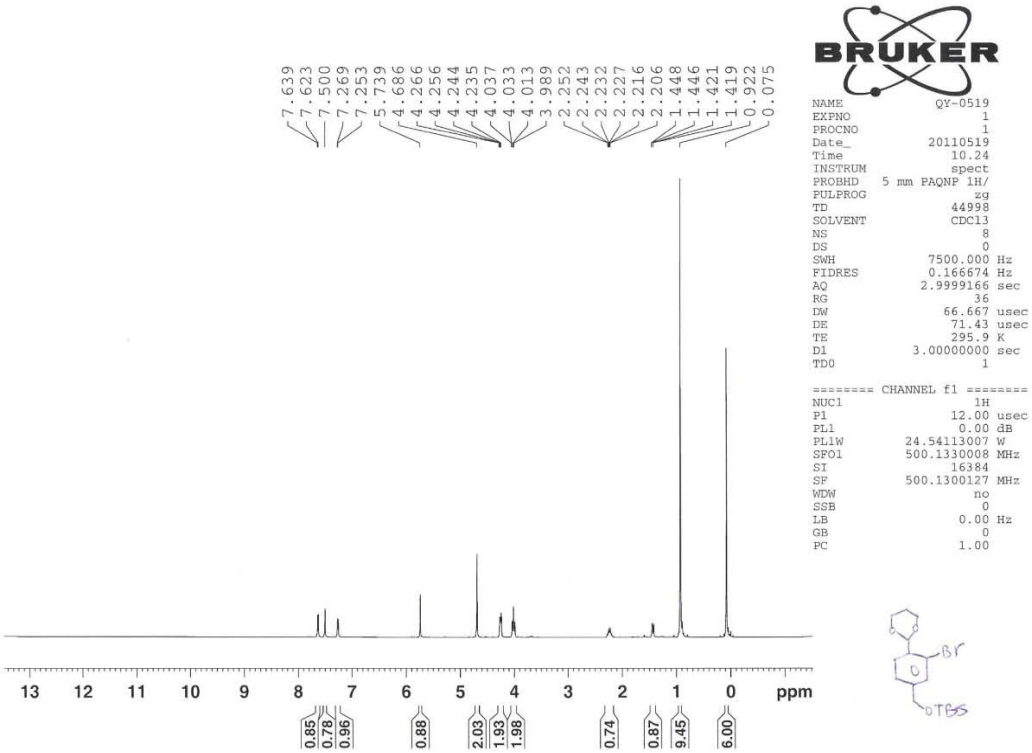
1. S. Sreejith, K. P. Divya, A. Ajayaghosh. *Angew. Chem. Int. Ed*. **2008**, *120*, 8001-8005.

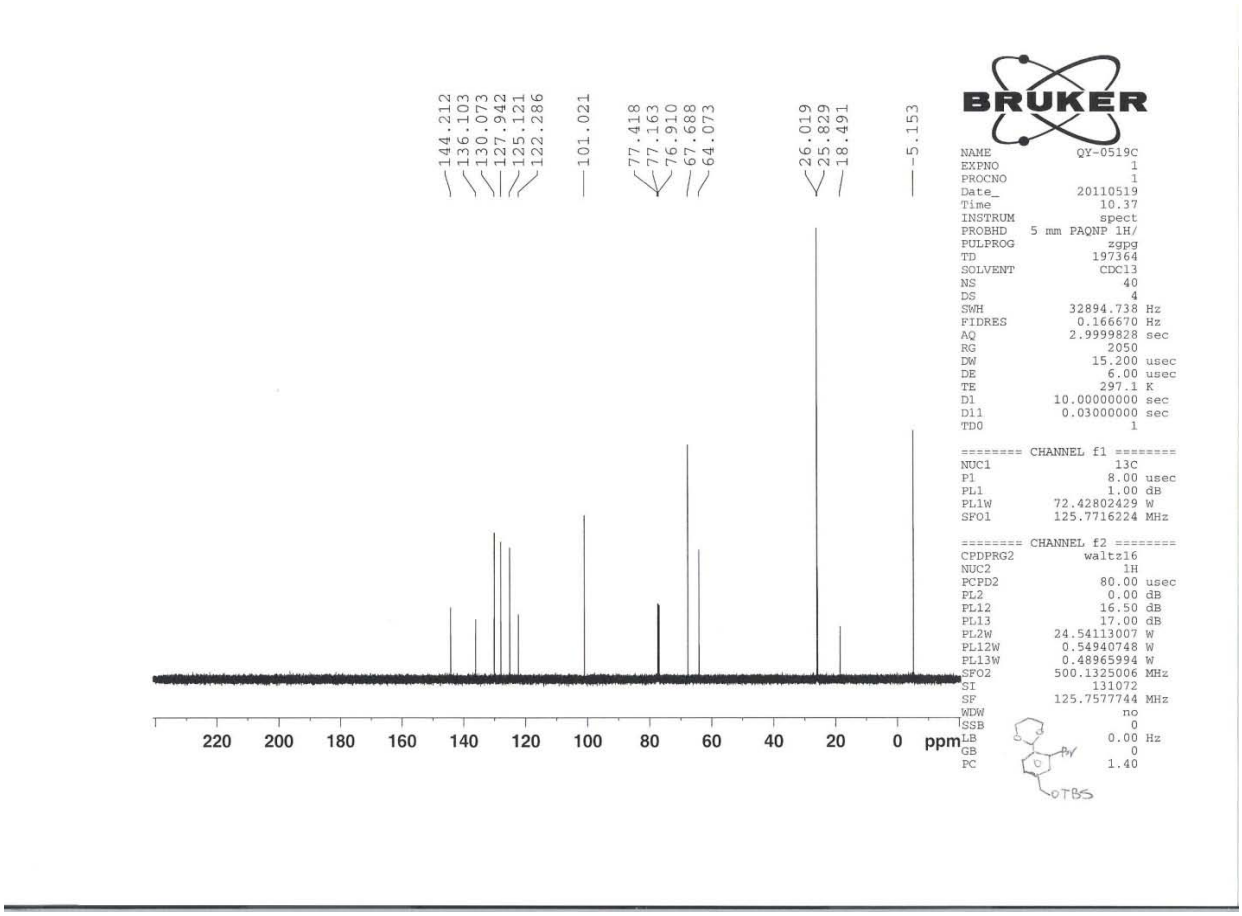
2. A. T. R. Williams, S. A. Winfield, J. N. Miller. *Analyst*. **1983**, *108*, 1067–1071.

3. Y. Zhao, H. Wang, M. Xian. *J. Am. Chem. Soc.*, **2011**, *133*, 15–17.

NMR spectra of chemical compounds



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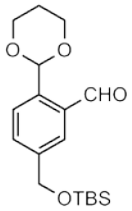
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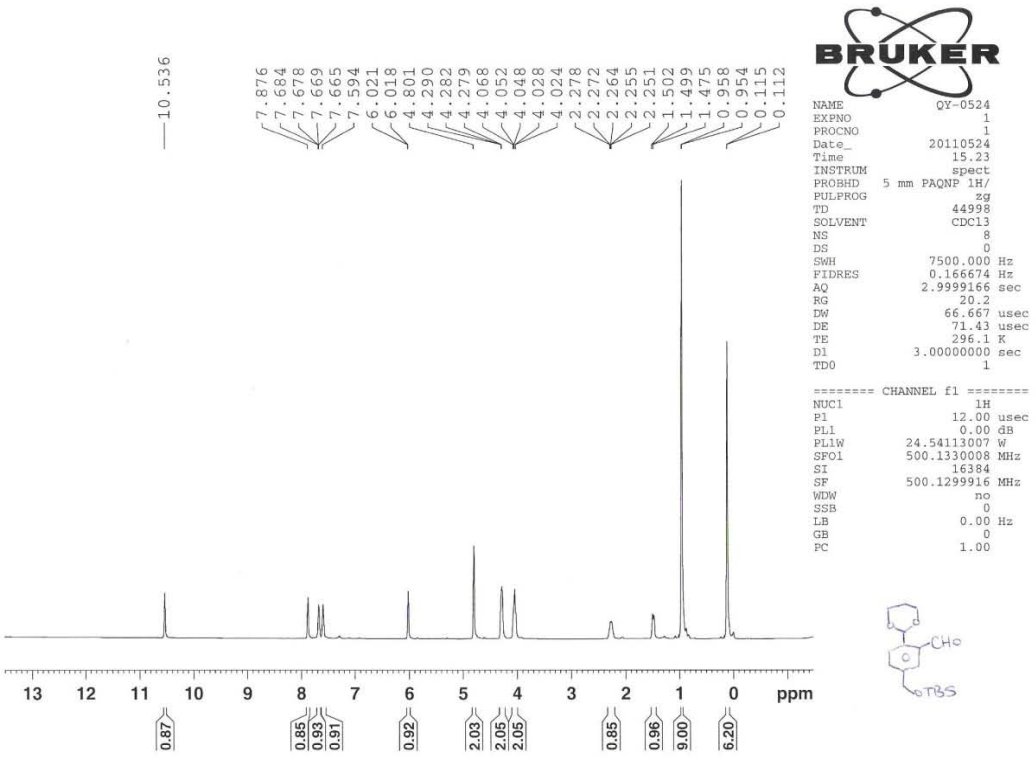
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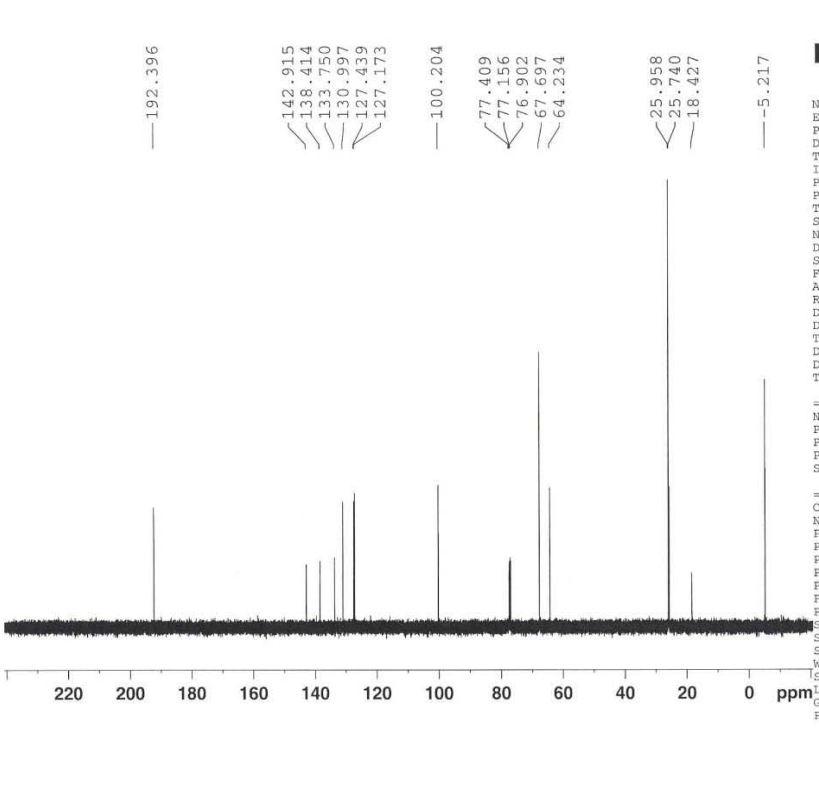
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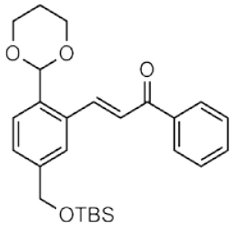
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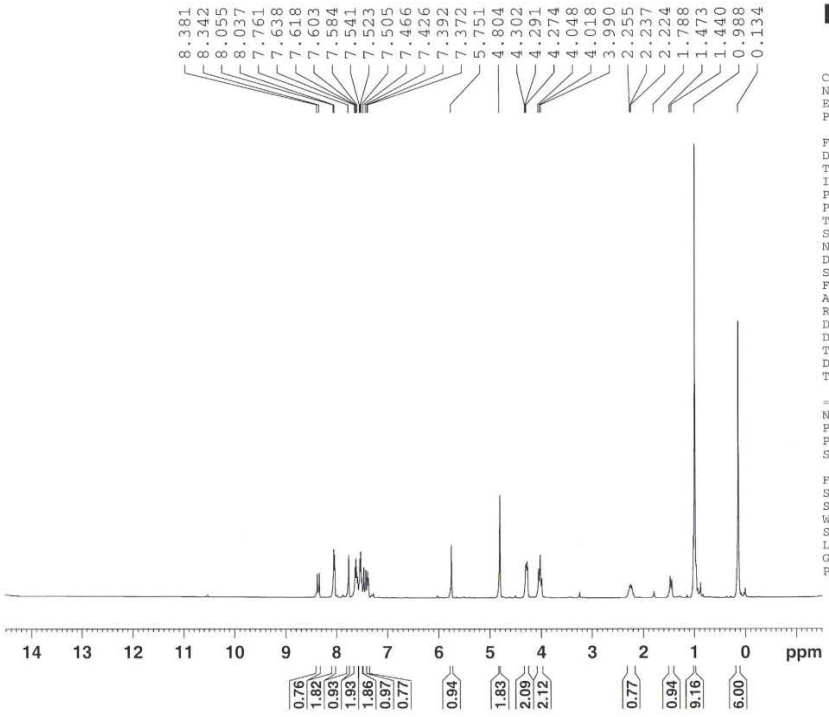
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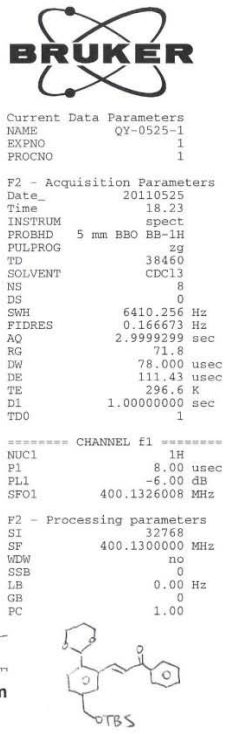
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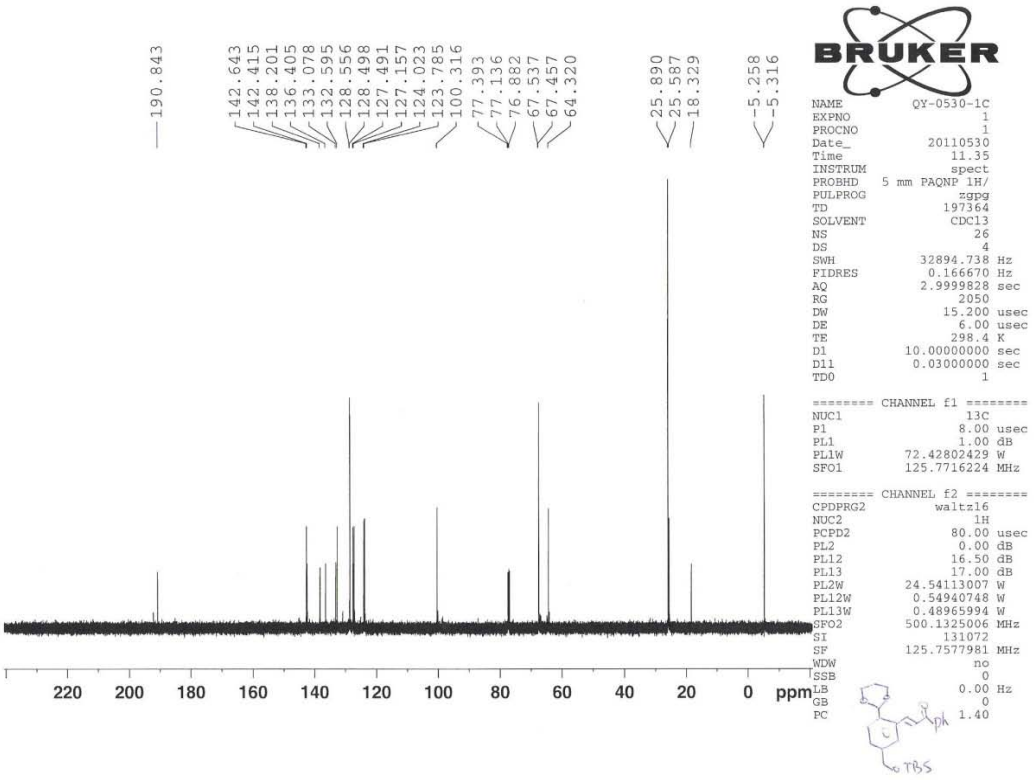
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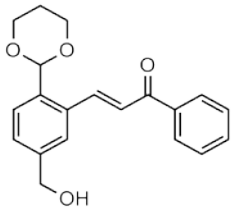

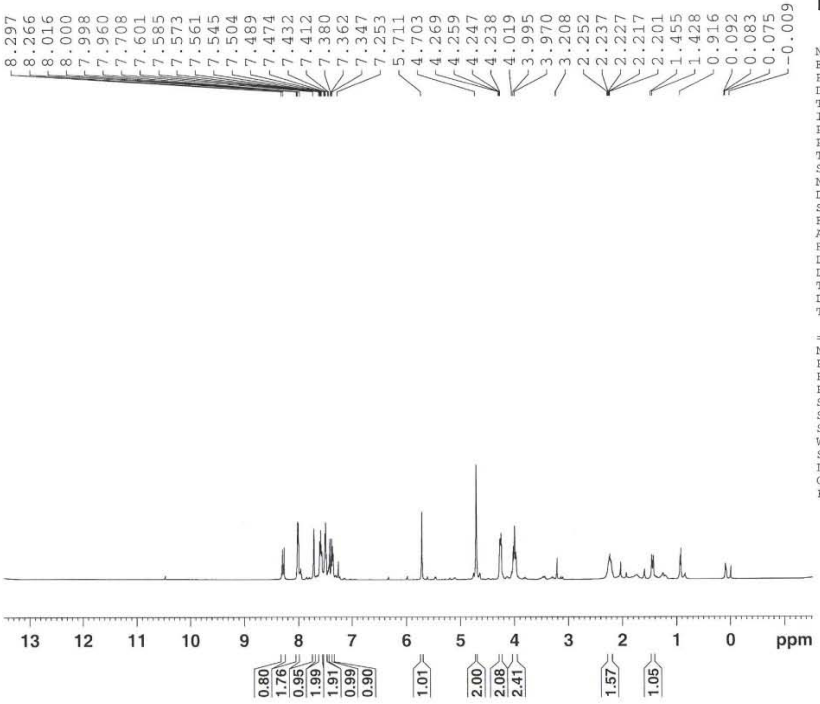

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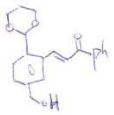

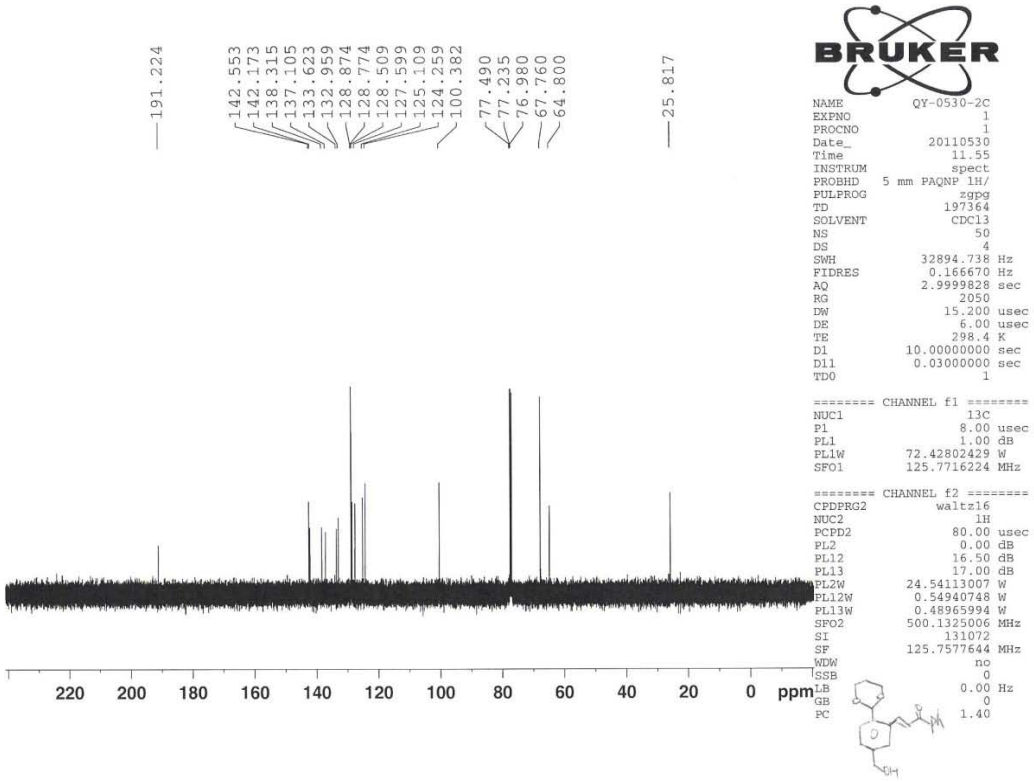
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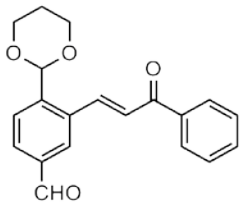
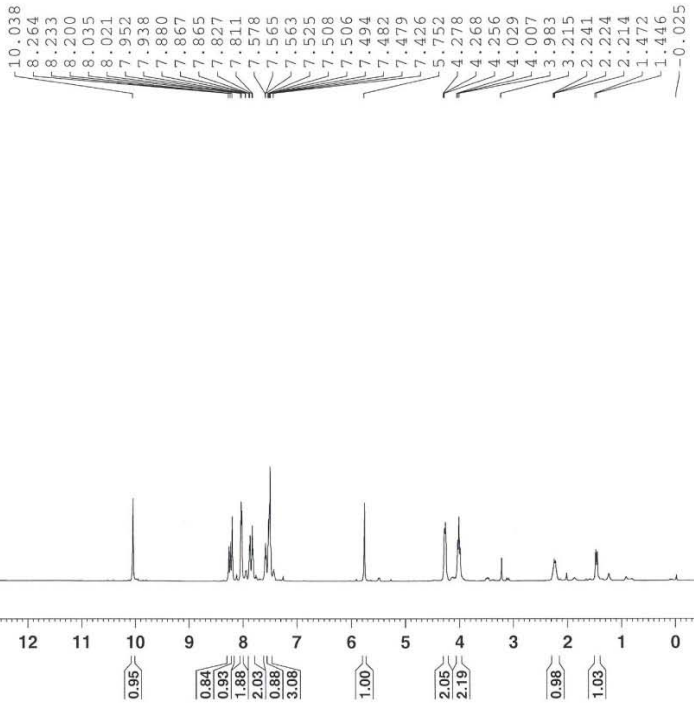
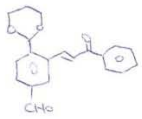
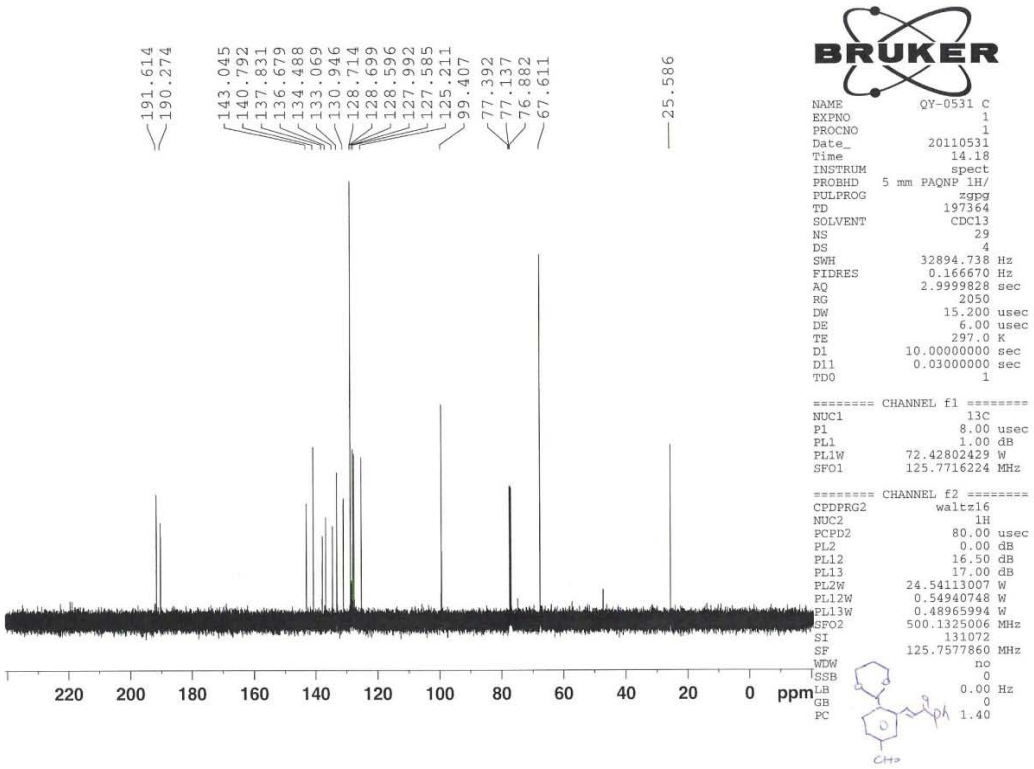


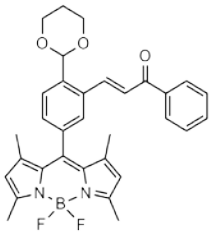


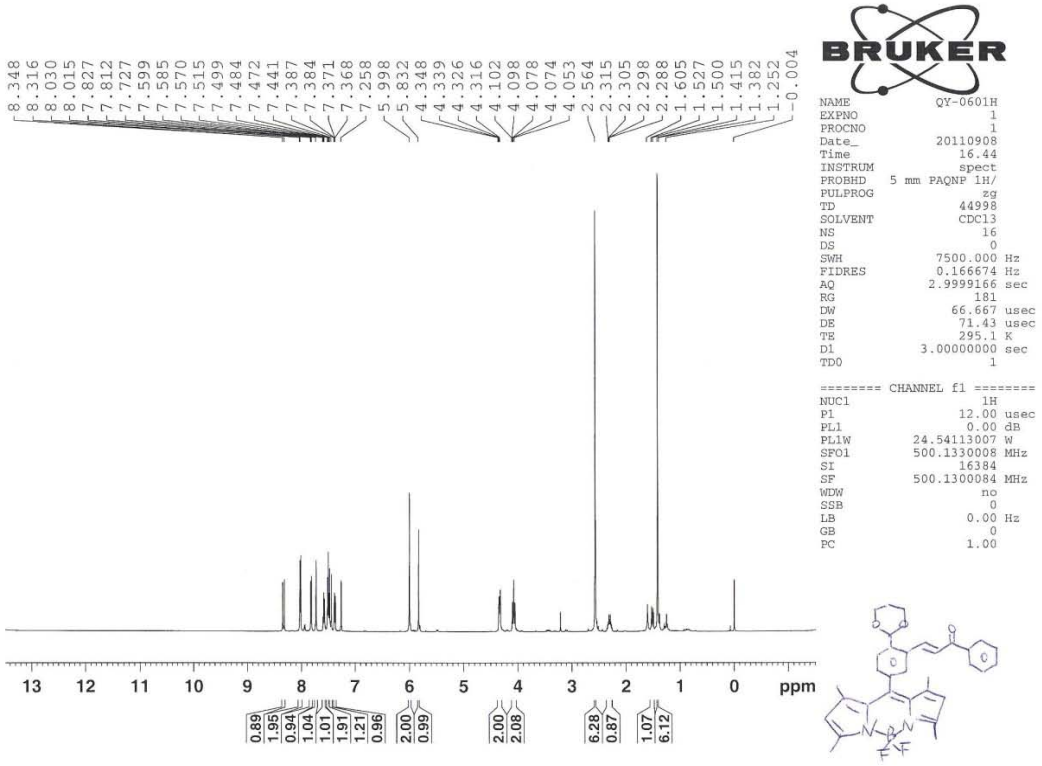
BRUKER

NAME	QY-0531
EXPNO	1
PROCNO	1
Date_	20110531
Time	14.08
INSTRUM	spect
PROBHD	5 mm PAQNP 1H/
PULPROG	zg
TD	44998
SOLVENT	CDCl3
NS	8
DS	0
SWH	7500.000 Hz
FIDRES	0.166674 Hz
AQ	2.9999166 sec
RG	40.3
DW	66.667 usec
DE	71.43 usec
TE	295.9 K
D1	3.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.00 usec
PL1	0.00 dB
PL1W	24.54113007 W
SFO1	500.1330008 MHz
SI	16384
SF	500.1300102 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00



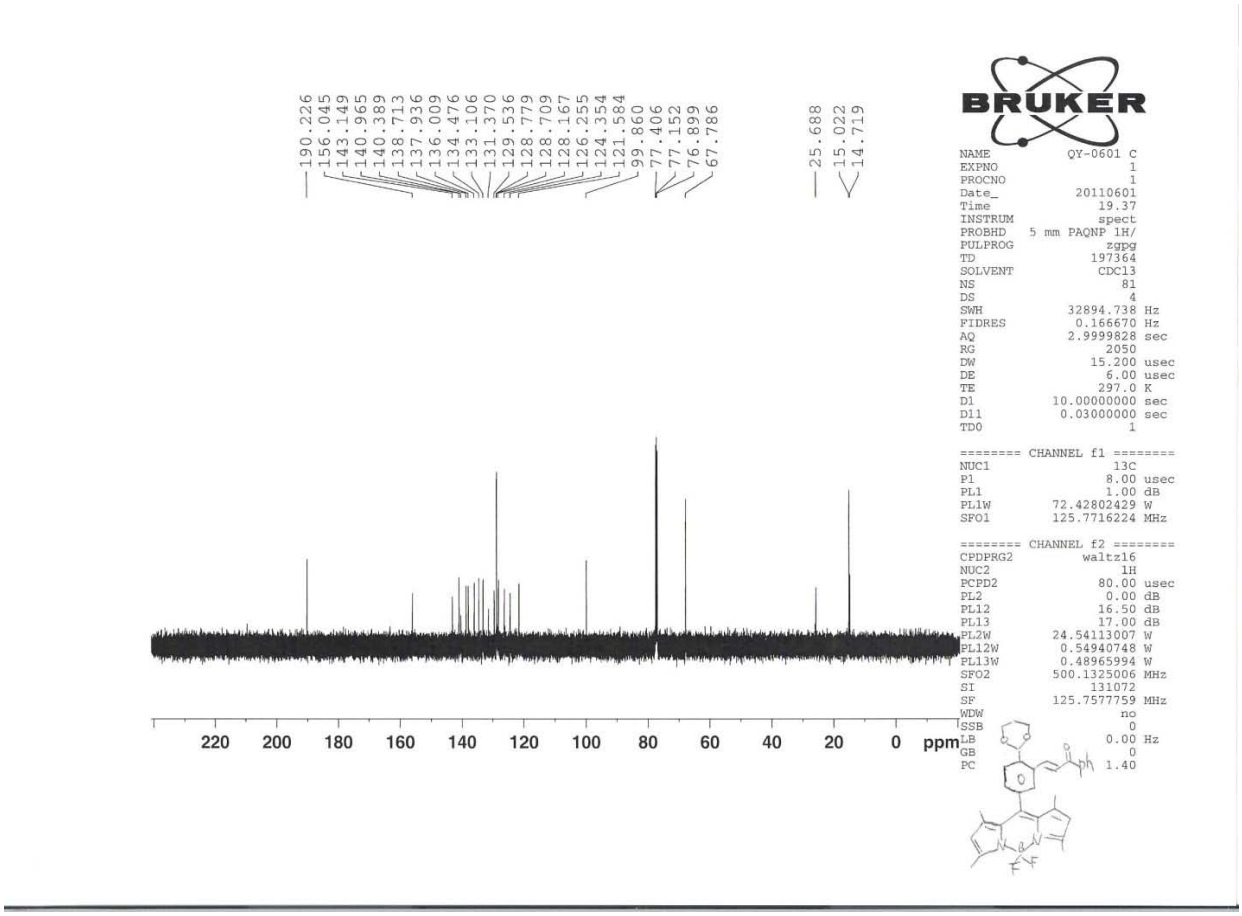

191.614
190.274
143.045
140.792
137.831
136.679
134.488
133.069
130.946
128.714
128.699
128.596
127.992
127.585
125.211
99.407
77.392
77.137
76.882
67.611
25.586
220
200
180
160
140
120
100
80
60
40
20
0
ppm
BRUKER
NAME QY-0531 C
EXPNO 1
PROCNO 1
Date_ 20110531
Time 14.18
INSTRUM spect
PROBHD 5 mm PAQNP 1H/
PULPROG zgpg
TD 197364
SOLVENT CDCl3
NS 29
DS 4
SWH 32894.738 Hz
FIDRES 0.166670 Hz
AQ 2.9999828 sec
RG 2050
DW 15.200 usec
DE 6.00 usec
TE 297.0 K
D1 10.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 1.00 dB
PL1W 72.42802429 W
SFO1 125.7716224 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 16.50 dB
PL13 17.00 dB
PL2W 24.54113007 W
PL12W 0.54940748 W
PL13W 0.48965994 W
SFO2 500.1325006 MHz
SI 131072
SF 125.7577860 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
Ph
CH3

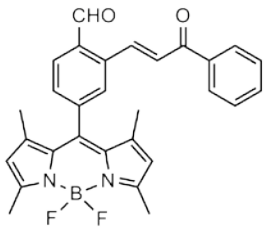


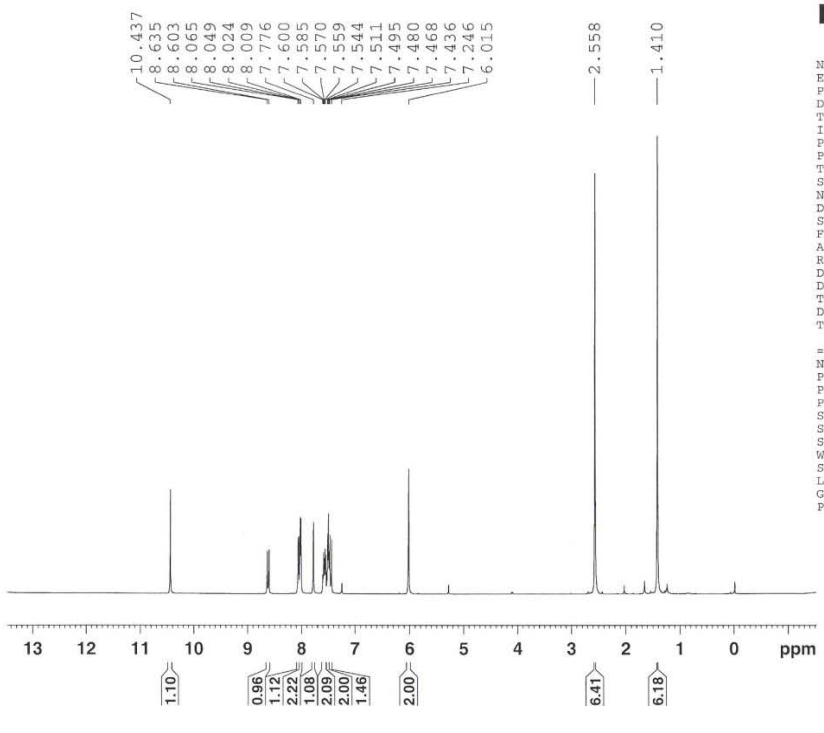


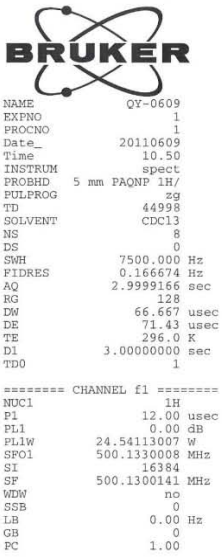
BRUKER

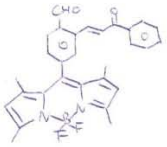
NAME	QY-0601H
EXPNO	1
PROCNO	1
Date_	20110908
Time	16.44
INSTRUM	spect
PROBHD	5 mm PAQNP 1H/
PULPROG	zg
TD	44998
SOLVENT	CDCl3
NS	16
DS	0
SWH	7500.000 Hz
FIDRES	0.166674 Hz
AQ	2.9999166 sec
RG	181
DW	66.667 usec
DE	71.43 usec
TE	295.1 K
D1	3.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	12.00 usec
PL1	0.00 dB
PL1W	24.54113007 W
SFO1	500.1330008 MHz
SI	16384
SF	500.1300084 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

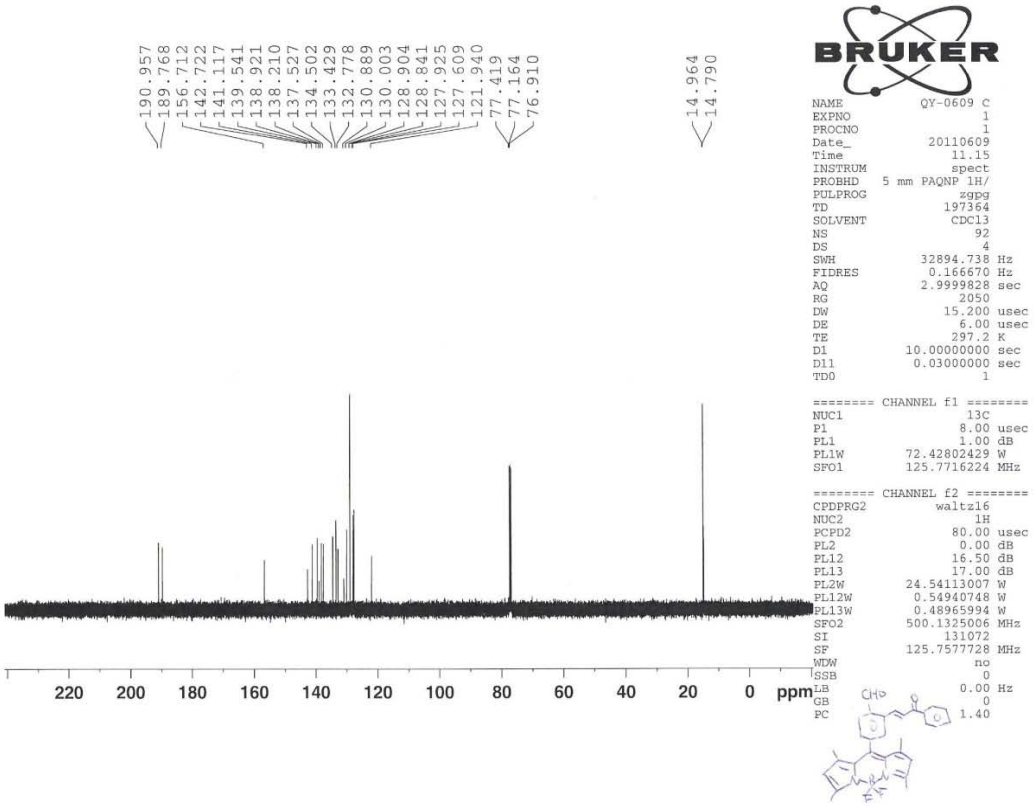

190.226
156.045
143.149
140.965
140.389
138.713
137.936
136.009
134.476
133.106
131.370
129.536
128.779
128.709
128.167
126.255
124.354
121.584
99.860
77.406
77.152
76.899
67.786
25.688
15.022
14.719
220
200
180
160
140
120
100
80
60
40
20
0
ppm
BRUKER
NAME QY-0601 C
EXPNO 1
PROCNO 1
Date_ 20110601
Time 19.37
INSTRUM spect
PROBHD 5 mm PAQNP 1H/
PULPROG zgpg
TD 197364
SOLVENT CDCl3
NS 81
DS 4
SWH 32894.738 Hz
FIDRES 0.166670 Hz
AQ 2.9999828 sec
RG 2050
DW 15.200 usec
DE 6.00 usec
TE 297.0 K
D1 10.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 1.00 dB
PL1W 72.42802429 W
SFO1 125.7716224 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 16.50 dB
PL13 17.00 dB
PL2W 24.54113007 W
PL12W 0.54940748 W
PL13W 0.48965994 W
SFO2 500.1325006 MHz
SI 131072
SF 125.7577759 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40


CHO
O
N
B
N
F
F


10.437
8.635
8.603
8.065
8.049
8.024
8.009
7.776
7.600
7.585
7.570
7.559
7.544
7.511
7.495
7.480
7.468
7.436
7.246
6.015
2.558
1.410
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.10
0.96
1.12
2.22
1.08
2.09
2.00
1.46
2.00
6.41
6.18


BRUKER
NAME QY-0609
EXPNO 1
PROCNO 1
Date_ 20110609
Time 10.50
INSTRUM spect
PROBHD 5 mm PAQNP 1H/
PULPROG zg
TD 44998
SOLVENT CDCl3
NS 8
DS 0
SWH 7500.000 Hz
FIDRES 0.166674 Hz
AQ 2.9999166 sec
RG 128
DW 66.667 usec
DE 71.43 usec
TE 296.0 K
D1 3.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 12.00 usec
PL1 0.00 dB
PL1W 24.54113007 W
SFO1 500.1330008 MHz
SI 16384
SF 500.1300141 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00




190.957
189.768
156.712
142.722
141.117
139.541
138.921
138.210
137.527
134.502
133.429
132.778
130.889
130.003
128.904
128.841
127.925
127.609
121.940
77.419
77.164
76.910
14.964
14.790
220
200
180
160
140
120
100
80
60
40
20
0
ppm
BRUKER
NAME QY-0609 C
EXPNO 1
PROCNO 1
Date_ 20110609
Time 11.15
INSTRUM spect
PROBHD 5 mm PAQNP 1H/
PULPROG zgpg
TD 197364
SOLVENT CDCl3
NS 92
DS 4
SWH 32894.738 Hz
FIDRES 0.166670 Hz
AQ 2.9999828 sec
RG 2050
DW 15.200 usec
DE 6.00 usec
TE 297.2 K
D1 10.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 8.00 usec
PL1 1.00 dB
PL1W 72.42802429 W
SFO1 125.7716224 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 16.50 dB
PL13 17.00 dB
PL2W 24.54113007 W
PL12W 0.54940748 W
PL13W 0.48965994 W
SFO2 500.1325006 MHz
SI 131072
SF 125.7577728 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40
CHO