An ICT-based Fluorescent Switch-on Probe for Hydrogen Sulfide in Living Cells

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SUPPORTING INFORMATION

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General Experimental

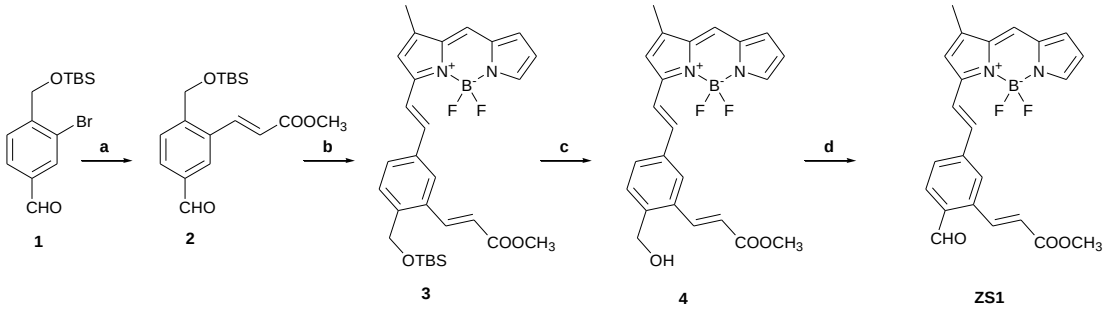
All reactions were carried out under an inert nitrogen atmosphere unless otherwise stated. All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated.

Acetonitrile (MeCN) and triethylamine (Et_3N) were purified by distillation over CaH_2. 3-Bromo-4-((tert-butyldimethylsilyloxy)methyl)benzaldehyde (**1**) and 4, 4-difluoro-1,3-dimethyl-4-bora-3a,4a-diaza-s-indacene were prepared referring to literature procedures with some modifications.[1]

Isolated yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. 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 agent and iodine as the developing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, P. R. ^{1}H-NMR spectra were recorded on a Bruker Fourier transform spectrometer (500 MHz or 400 MHz) at 25 °C. ^{13}C-NMR spectra were recorded on a Bruker Fourier transform spectrometer (125 MHz or 100 Hz) spectrometer and were calibrated using residual undeuterated solvent as an internal reference (for $CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16; for DMSO: ^{1}H NMR = 2.50, ^{13}C NMR = 39.52;). 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, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were recorded on a Bruker Vector 22 spectrophotometer as KBr pellets. High resolution mass spectra (HRMS) were recorded on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). Fluorescence measurements were carried out on a JASCO FP 6500 spectrofluorimeter with slit widths to be 1 and 3 for excitement and emission respectively. Agilent 1200 Series HPLC system was used with a Diamonsil C18 column (5μm, 200 x 4.6 mm) using a concentration gradient from 65:35 to 85:15 (acetonitrile:H_2O) as eluent at a flow rate of 1.0 mL/min and monitored at 569 nm for HPLC analysis.

[1] Y. Qian, J. Karpus, O. Kabil, S. Zhang, H. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, **2**, 495; J. S. Lee, N. Y. Kang, Y. K. Kim, A. Samanta, S. Feng, H. K. Kim, M. Vendrell, J. H. Park and Y. T. Chang, *J. Am. Chem. Soc.*, 2009, **131**, 10077-82.

Scheme S1. Synthesis of ZS1



(a) PPh_3, $Pd(OAc)_2$, methyl acrylate, Et_3N, dry CH_3CN, reflux, 18 hours, 41%; (b) 4, 4-difluoro-1,3-dimethyl-4-bora-3a,4a-diaza-s-indacene, piperidine, CH_3COOH, benzene, reflux, Dean-stark trapper, 4 hours, 39%; (c) HF aqueous solution, CH_3CN, ambient temperature, 1 hour, 81%; (d) PCC, Na_2SO_4, CH_2Cl_2, ambient temperature, 1 hour, 37%;

Synthesis of intermediate 2

To a dry flask loaded with **1** (185 mg, 0.587 mmol), PPh_3 (46 mg, 0.17 mmol) and $Pd(OAc)_2$ (13 mg, 0.058 mmol) was added dry CH_3CN (5.0 mL), dry Et_3N (0.25 mL, 1.8 mmol) and a solution of methyl acrylate (0.69 mL, 7.6 mmol) in CH_3CN (5.0 mL) sequentially. The mixture was stirred under a nitrogen atmosphere at ambient temperature for 10 minutes first then was heated to reflux. After 18 hours, the reaction mixture was allowed to cool down to ambient temperature. H_2O (10 mL) was added to quench the reaction. The insoluble part was removed by filtration and the filtrate was extracted with EtOAc (3×10 mL). The combined organic phases were washed with brine (1×10 mL), dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The remaining residue was purified by flash column chromatography (SiO_2, petroleum ether/EtOAc, 25:1) to give the product as a pale yellow oil (80 mg, 41% yield).

Rf = 0.35 (15:1, petroleum ether:EtOAc)

δ_H (500 MHz, $CDCl_3$): 10.02 (1 H, s), 8.04 (1 H, s), 7.91 (1 H, d, *J* 18.0), 7.88 (1 H, d, *J* 8.0, 1.6), 7.71 (1 H, d, *J* 8.0), 6.47 (1 H, dd, *J* 16.0), 4.88 (2 H, s), 3.82 (3 H, s), 0.94 (9 H, s), 0.13 (6 H, s)

δ_C (126 MHz, $CDCl_3$): 191.36, 166.73, 146.44, 140.05, 135.58, 132.99, 130.81, 127.80, 127.45, 121.08, 62.69, 51.74, 25.76, 18.22, -5.42

IR (cm^{-1}): 2951, 2857, 1707, 1260, 1186, 1078, 842

ESI-HRMS (m/z): $[M+Na]^+$ calc'd. for $C_{18}H_{26}O_4Si$: 357.1498; found 357.1483.

Synthesis of intermediate 3

AcOH (0.10 mL) and piperidine (0.10 mL) were added sequentially to a flask containing benzene (10 mL) with stirring, followed by the addition of compound **2** (177 mg, 0.53 mmol) and 4, 4-difluoro-1,3-dimethyl-4-bora-3a,4a-diaza-s-indacene (105 mg, 0.45 mmol). The solution was stirred under reflux for 4 hours with continuously separation of H_2O by a Dean-stark apparatus. After being cooled to ambient temperature, the mixture was diluted with EtOAc (10 mL), transferred to a

separation funnel, washed with H_2O (1×10 mL) and brine (1×10 mL), dried over anhydrous Na_2SO_4, and concentrated by rotarory evaporation under reduced pressure. The crude product was then purified by flash column chromatography (SiO_2, petroleum ether/EtOAc, 15:1) to give the product as a red solid (100 mg, 39% yield).

Rf = 0.40 (8:1, petroleum ether:EtOAc)

δ_H (500 MHz, $CDCl_3$): 7.95 (1 H, d, *J* 15.9), 7.70 (2 H, m), 7.64 (2 H, m), 7.52 (1 H, d, *J* 8.0), 7.35 (1 H, d, *J* 16.3), 7.18 (1 H, m), 6.95 (1 H, d, *J* 3.4), 6.75 (1 H, s), 6.47 (1 H, m), 6.43 (1 H, d, *J* 15.9), 4.84 (2 H, s), 3.83 (3 H, s), 2.31 (3 H, s), 0.94 (9 H, s), 0.13 (6 H, s)

δ_C (126 MHz, $CDCl_3$): 167.26, 158.40, 144.65, 141.90, 141.24, 139.46, 138.94, 137.88, 135.23, 133.38, 133.17, 128.83, 128.30, 126.49, 126.42, 123.55, 120.37, 119.00, 117.29, 116.75, 63.02, 51.89, 25.99, 18.44, 11.61, -5.17

IR (cm^{-1}): 1758, 1319, 1301, 1193, 1120, 949

ESI-HRMS (m/z): $[M+Na]^+$ calc'd. for $C_{29}H_{35}BF_2N_2O_3Si$: 559.2376; found 559.2372.

Synthesis of intermediate 4

To a solution of compound **3** (90 mg, 0.17 mmol) in CH_3CN (10 mL) was added an aqueous solution of HF (~70%, 0.20 mL). The reaction was stirred at ambient temperature for 1 hour then EtOAc (30 mL) was added to dilute the solution. The mixture was transferred to a separation funnel, washed with H_2O (3×10 mL) and brine (1×10 mL), dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure. The product was purified by flash column chromatography (SiO_2, petroleum ether/CH_2Cl_2/EtOAc, 5:20:1) as a red solid (58 mg, 81% yield).

Rf = 0.30 (3:1, petroleum ether:EtOAc)

δ_H (500 MHz, DMSO): 7.98 (1 H, d, *J* 15.9), 7.93 (1 H, s), 7.83 (1 H, s), 7.80 (1 H, d, *J* 16.4), 7.76 (1 H, s), 7.69 (1 H, dd, *J* 8.0, 1.3), 7.56 (1 H, d, *J* 8.0), 7.47 (1 H, d, *J* 16.4), 7.14 (1 H, d, *J* 3.7), 7.09 (1 H, s), 6.57 (1 H, d, *J* 15.9), 6.54 (1 H, m), 5.41 (1 H, t, *J* 5.3), 4.65 (2 H, d, *J* 5.3), 3.76 (3 H, s), 2.36 (3 H, s)

δ_C (101 MHz, DMSO): 166.38, 157.77, 145.45, 142.96, 141.12, 139.74, 139.17, 137.30, 134.82, 133.08, 133.05, 129.26, 128.04, 126.98, 126.52, 125.24, 119.83, 117.73, 117.61, 116.79, 60.84, 51.61, 11.21

IR (cm^{-1}): 3446, 2926, 1592, 1520, 1394, 1285, 1144, 1069

ESI-HRMS (m/z): $[M+Na]^+$ calc'd. for $C_{23}H_{21}BF_2N_2O_3$: 445.1511; found 445.1501.

Synthesis of ZS1

A mixture of compound **4** (57 mg, 0.14 mmol), PCC (58 mg, 0.27 mmol) and anhydrous Na_2SO_4 (500 mg) in CH_2Cl_2 (10 mL) was stirred at ambient temperature for 1 hour, then was filtered through celite. The filtrate was evaporated by rotarory evaporation under reduced pressure to give the crude product which was purified by flash column chromatography (SiO_2, petroleum ether/CH_2Cl_2/EtOAc, 30:40:1) as a red solid (21 mg, 37% yield).

Rf = 0.45 (9:1, petroleum ether:EtOAc)

δ_H (400 MHz, DMSO): 10.20 (1 H, s), 8.52 (1 H, d, *J* 16.0), 8.05 (1 H, d, *J* 8.0), 8.02 (1 H, s), 7.94 – 7.81 (4 H, m), 7.60 (1 H, d, *J* 16.2), 7.22 (1 H, d, *J* 3.5), 7.11 (1 H, s), 6.64 (1 H, d, *J* 16.0), 6.59 (1 H, s), 3.78 (3 H, s), 2.37 (3 H, s)

δ_C (101 MHz, DMSO): 192.73, 166.10, 156.28, 145.23, 141.15, 140.71, 140.57, 137.70, 137.19, 135.88, 133.97, 133.66, 133.59, 128.26, 127.84, 127.62, 126.33, 122.36, 120.77, 117.68, 117.51, 51.73, 11.21

IR (cm^{-1}): 2987, 2942, 2875, 1734, 1665, 1395, 1292, 1185, 1032

ESI-HRMS (m/z): $[M+Na]^+$ calc'd. for $C_{23}H_{19}BF_2N_2O_3$: 443.1354; found 443.1341.

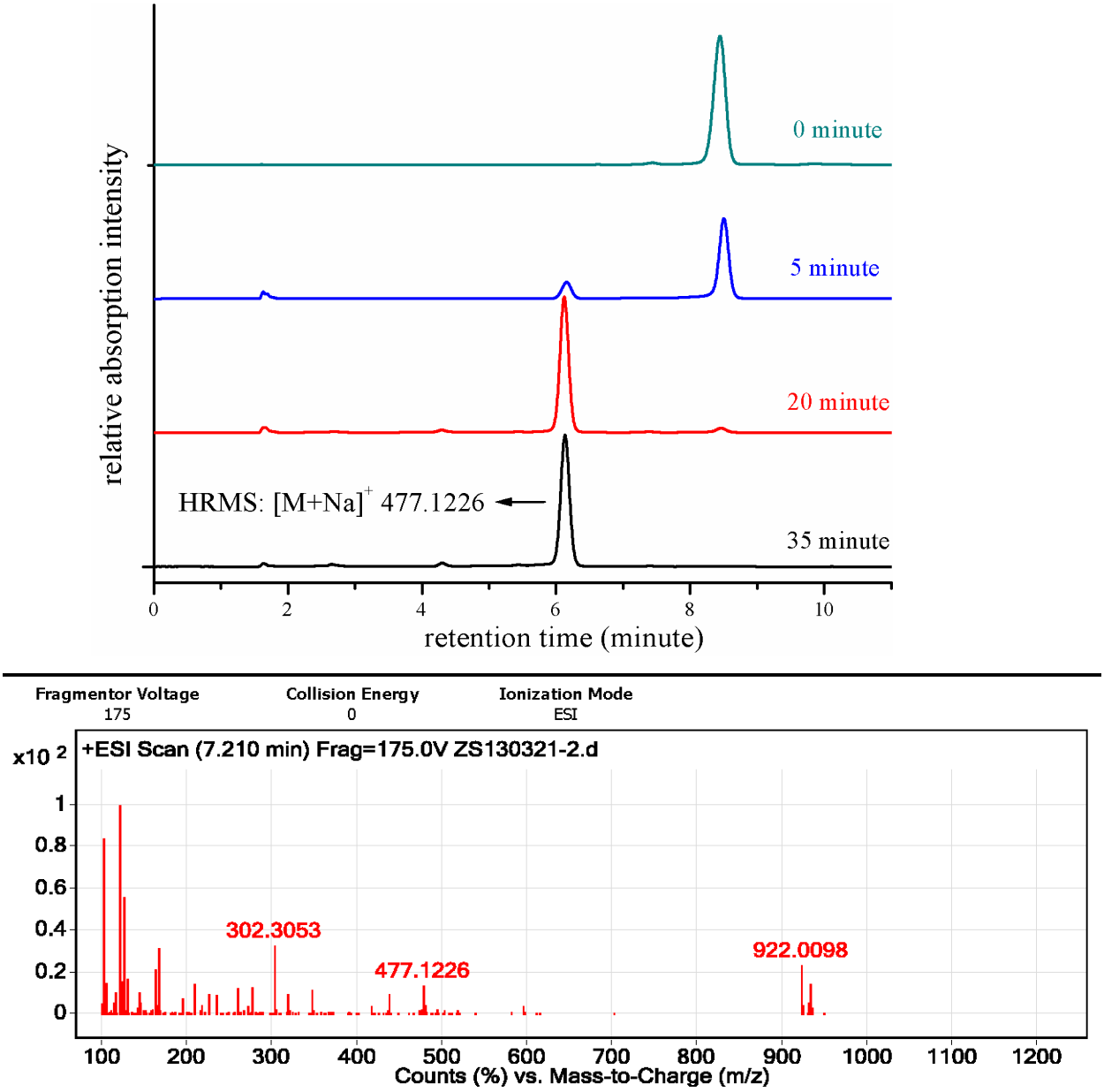
Cell lines

Raw 264.7 macrophage cell line was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and KHOS cell line was a gift from Dr. Lingtao Wu (Children's Hospital Los Angeles, CA). All the cell lines were cultured in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum in a humidified atmosphere of 5%CO_2 at 37°C.

Imaging experiments

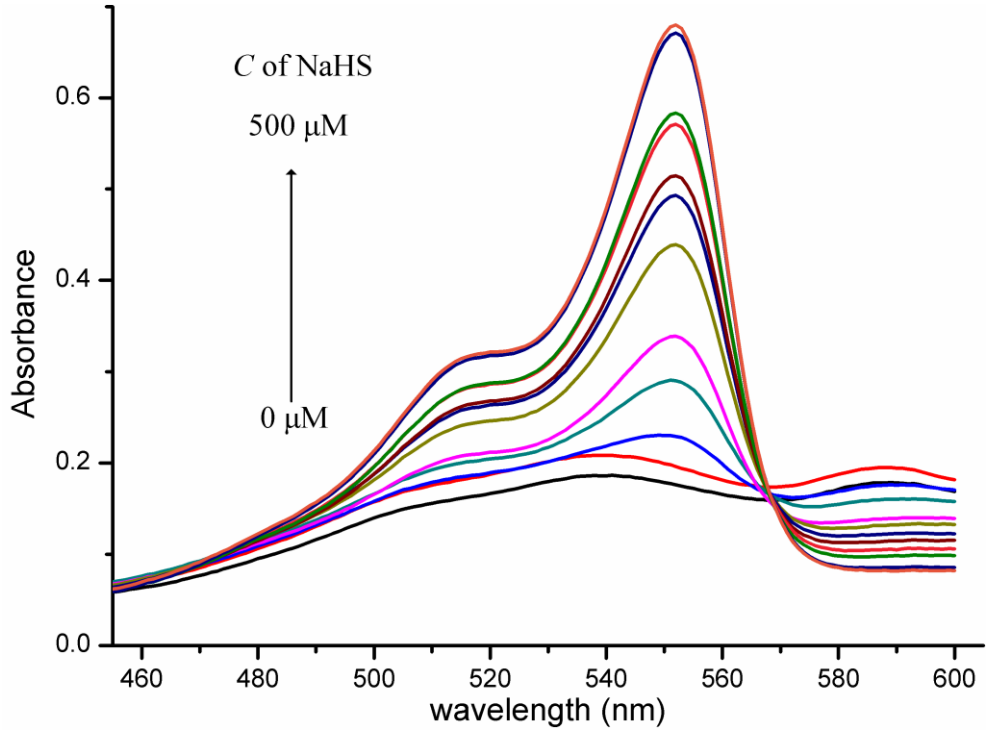
Exponentially growing cells (at a density of 20000-40000 cells per well, respectively) were seeded in 24-well plate. Cells were cultured at 37°C in a 5%CO_2 atmosphere for 24 h before they were exposed to reagents. After the staining steps as described in figure legends respectively, the images were collected at 620 nm on a fluorescence microscope (LEICA DMI 4000B) upon excitation at 550 nm, and the fluorescence intensity was analyzed by the software Image Pro Plus 6.0.

Fig. S1 Monitoring the response of ZS1 towards NaHS *via* HPLC-HRMS



The traces are of the reaction solution of **ZS1** (500 μM) and NaHS (5 mM) in PBS buffer (10 mM, pH 7.4, 37°C, 50% CH_3CN) as time elapsed. The HRMS spectra is that of the newly formed product.

Fig. S2 UV Responses of ZS1 towards Different Concentrations of NaHS



A gradual increase of UV absorbance intensity accompanied by a bathochromic-shift (absorbance maximum from 535 nm to 550 nm) was observed when **ZS1** was treated with gradual increased concentrations of NaHS. Spectra were taken in PBS buffer (10 mM, pH 7.4, 37°C, 20% CH_3CN) after 60 minutes' incubation with the concentration of **ZS1** to be 10 μM and those of NaHS to be 0, 10, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 μM.

Fig. S3 Quantified data of the three replicate imaging experiments on RAW 246.7 cells

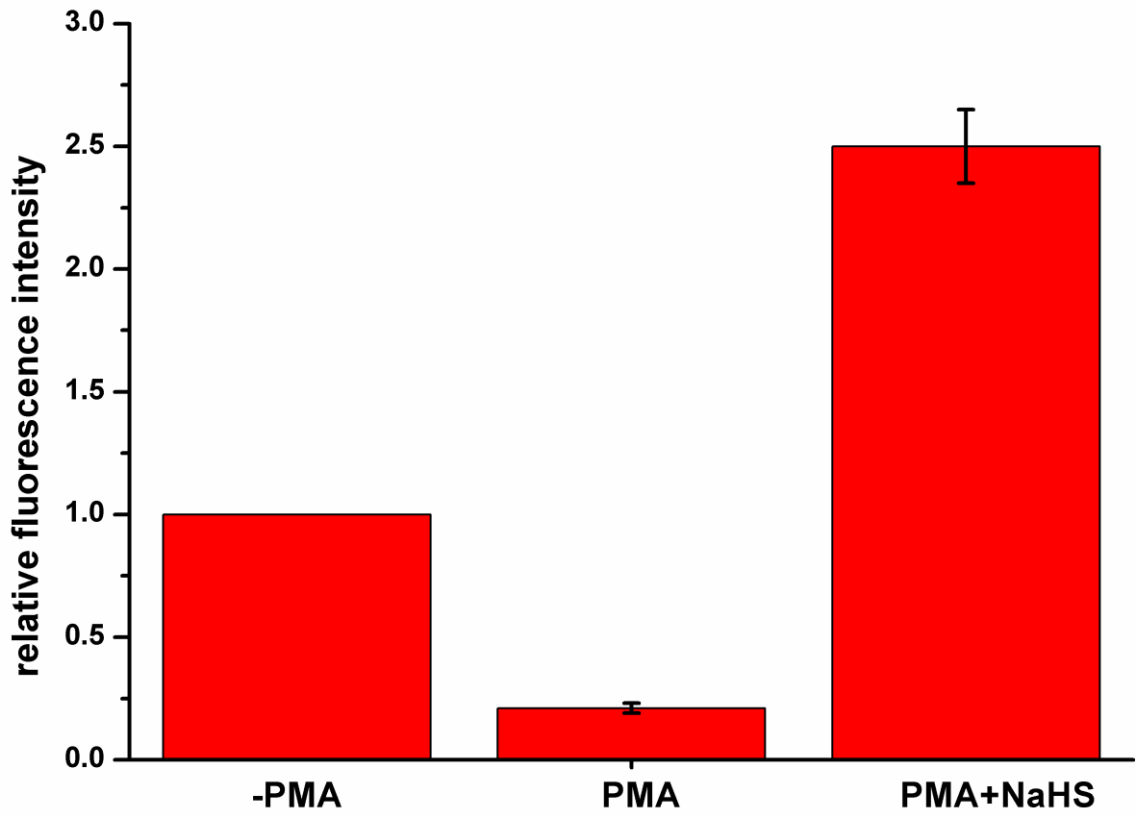
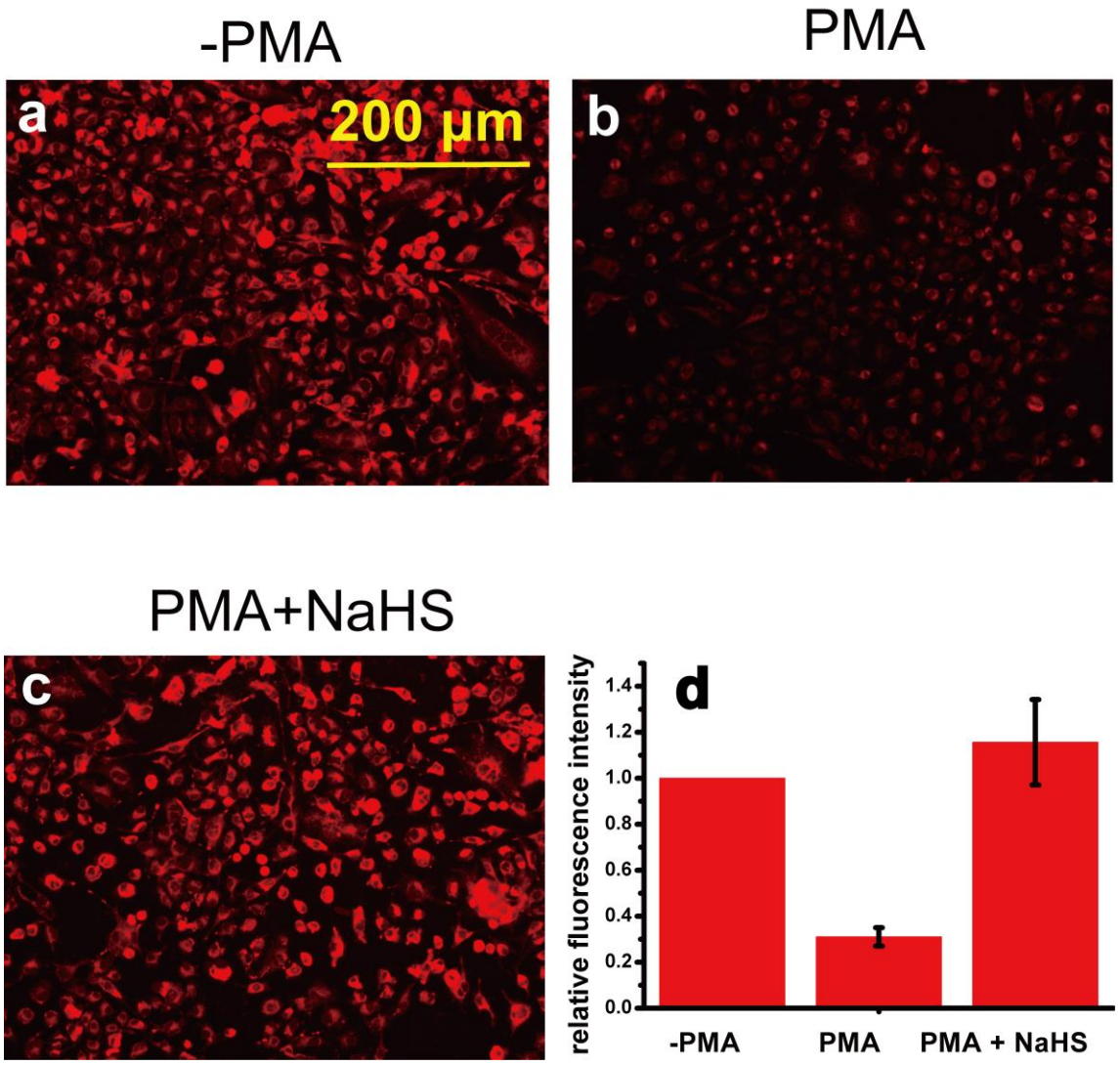
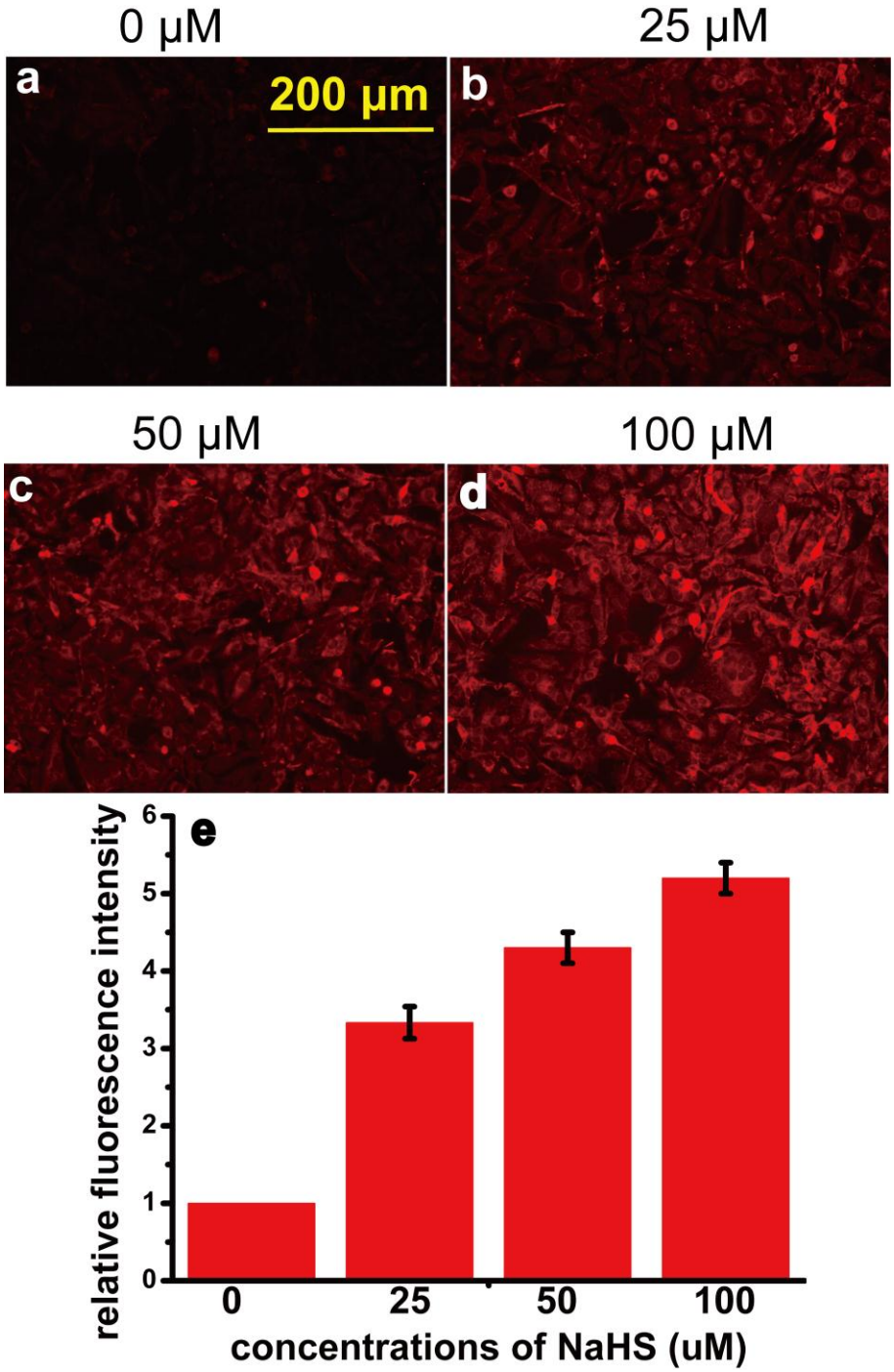


Fig. S4 ZS1 responds to endogeneous H_2S in KHOS cells



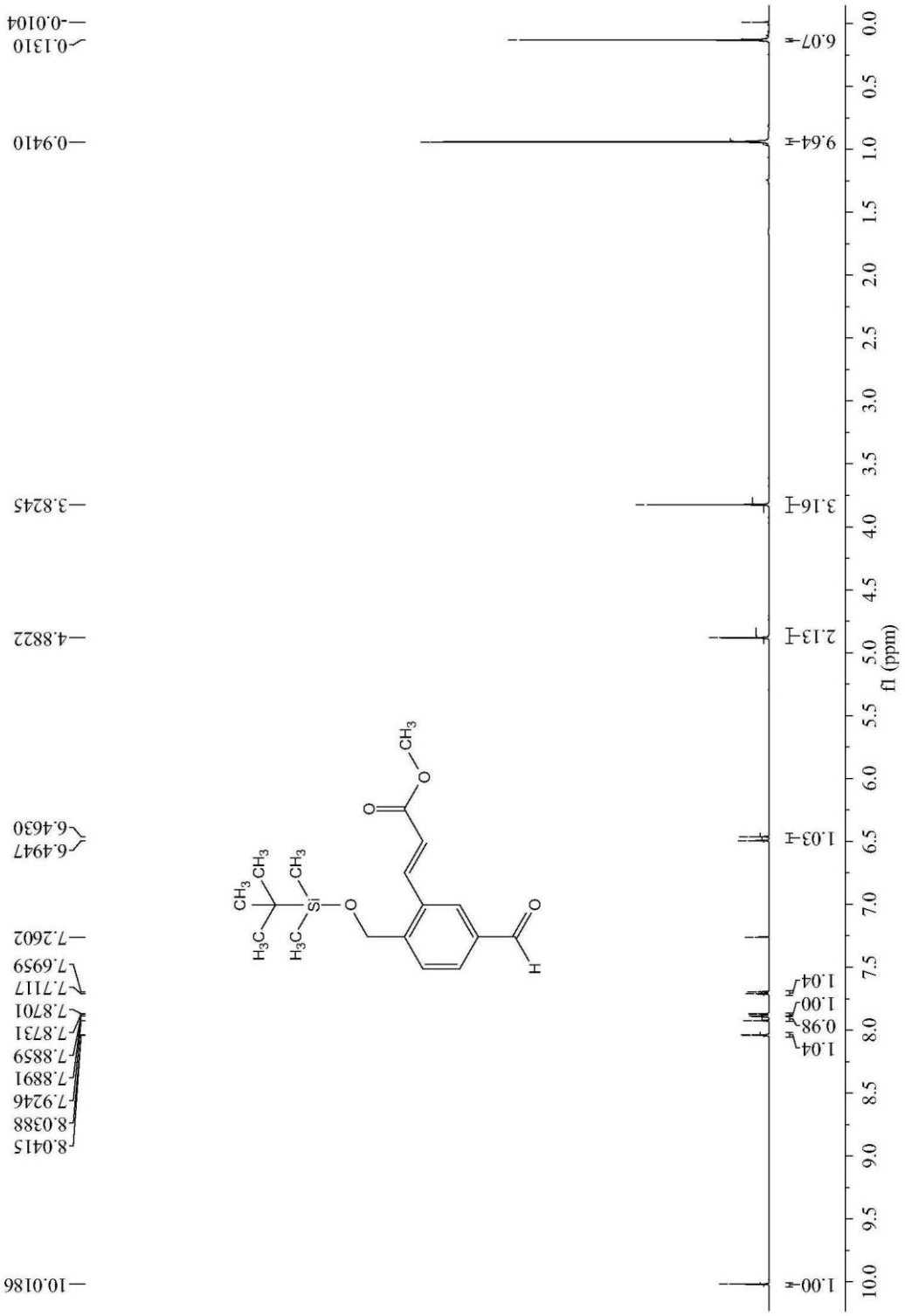
(a) Intact KHOS cells were treated with **ZS1** (5 μM) for 15 mins before imaging. (b) Cells were incubated with **PMA** (1 μg/ml) before being stained with **ZS1** (5 μM). (c) Cells pre-stimulted by PMA (2 μg/ml) were treated with NaHS (100 μM, 30 mins) after incubating with **ZS1** (5 μM, 15 mins). Images were collected at 620 nm on a fluorescence microscope (LEICA DMI 4000B) upon excitation at 550 nm. Images shown are representatives of replicate experiments (n =3) and the quantified data of the three replicate experiments are shown in image d.

Fig. S5 ZS1 responds to NaHS in a concentration-dependent manner in KHOS cells

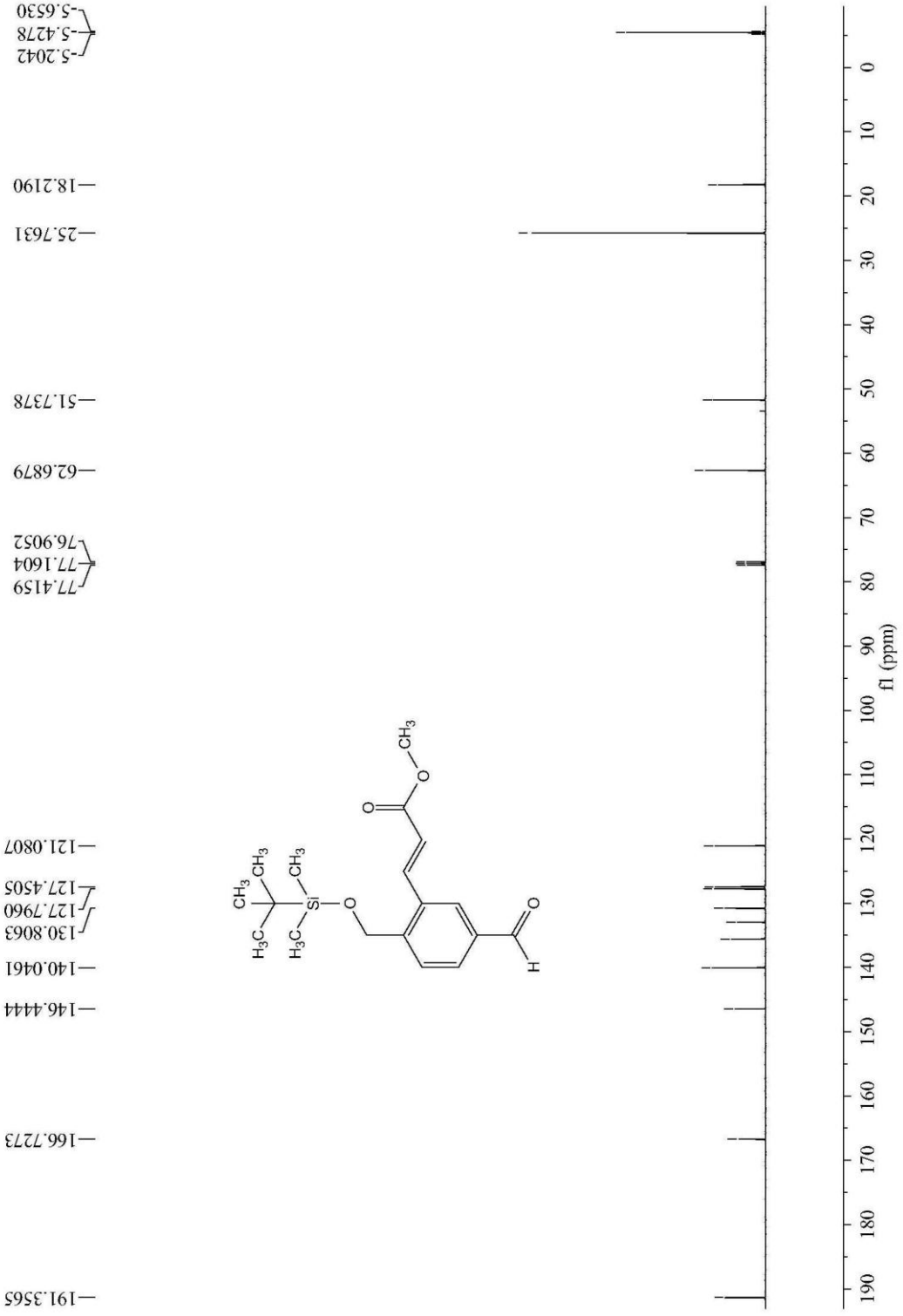


KHOS cells were pretreated with *N*-methylmaleimide (1 mM) for 15 mins. After twice of quick wash with PBS buffer, the cells were then incubated with **ZS1** (5 μM) for 15 mins. After that, the cells were once again washed with PBS twice quickly and then fresh culture medium containing varying concentrations of NaHS was added. Images were obtained using a fluorescence microscope (LEICA DMI 4000B) using a filter upon excitation at 550 nm. Images shown are representatives

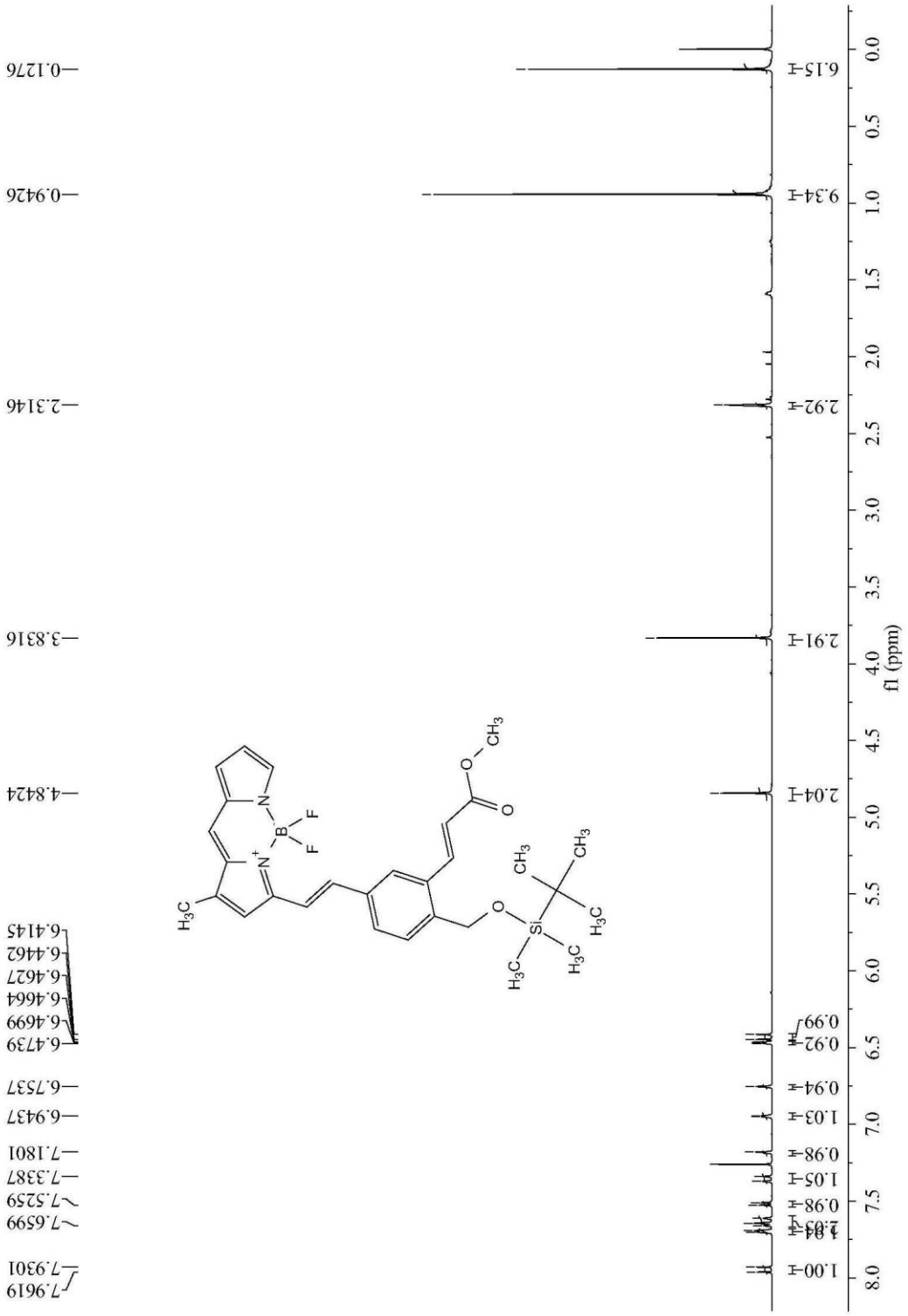
of replicate experiments (n =3). NaHS concentration: 0 μM (a), 25μM (b), 50μM (c), 100 μM (d) and image e represents the the quantified data of the three replicate experiments.



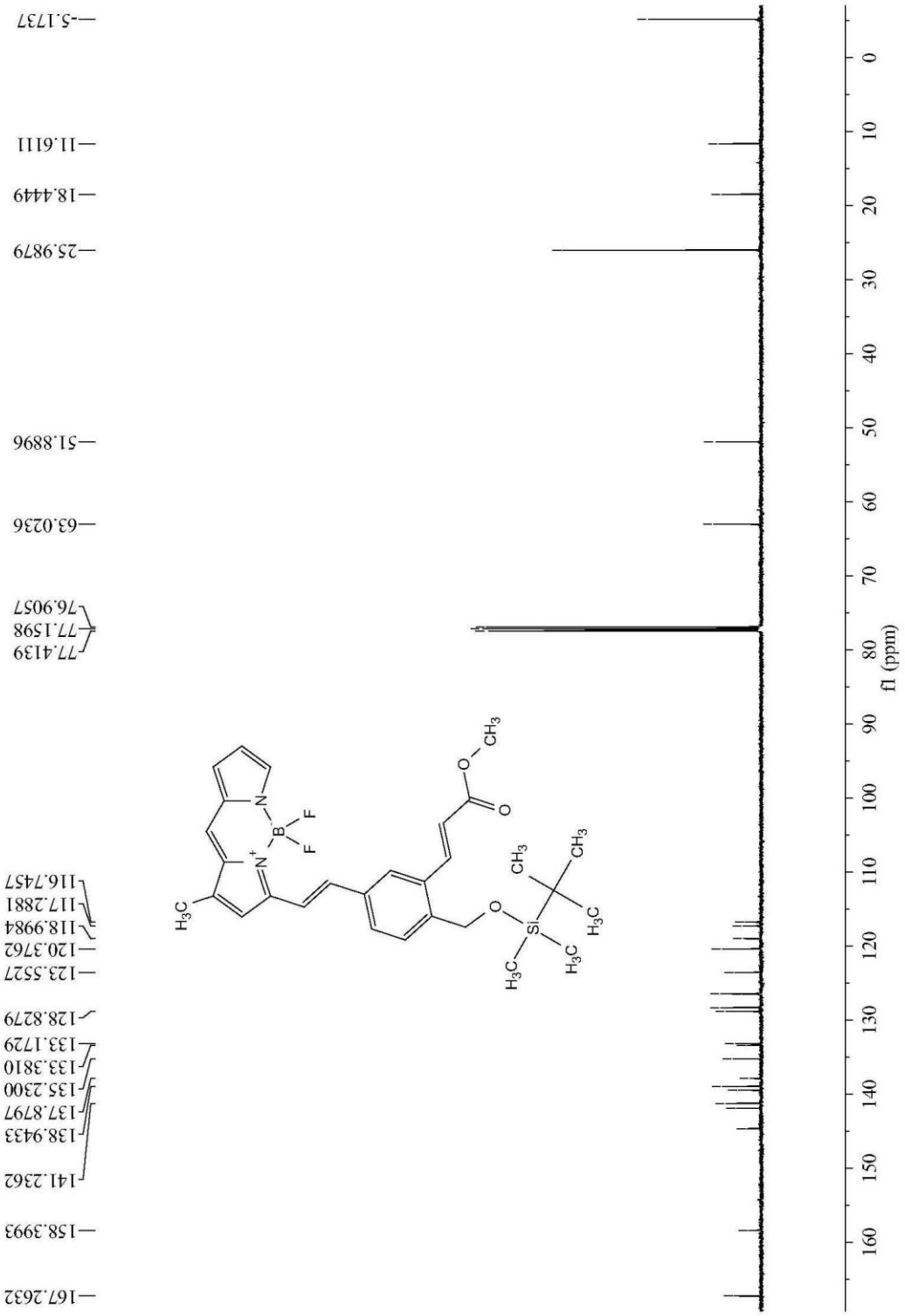
^{1}H NMR trace of intermediate **2**



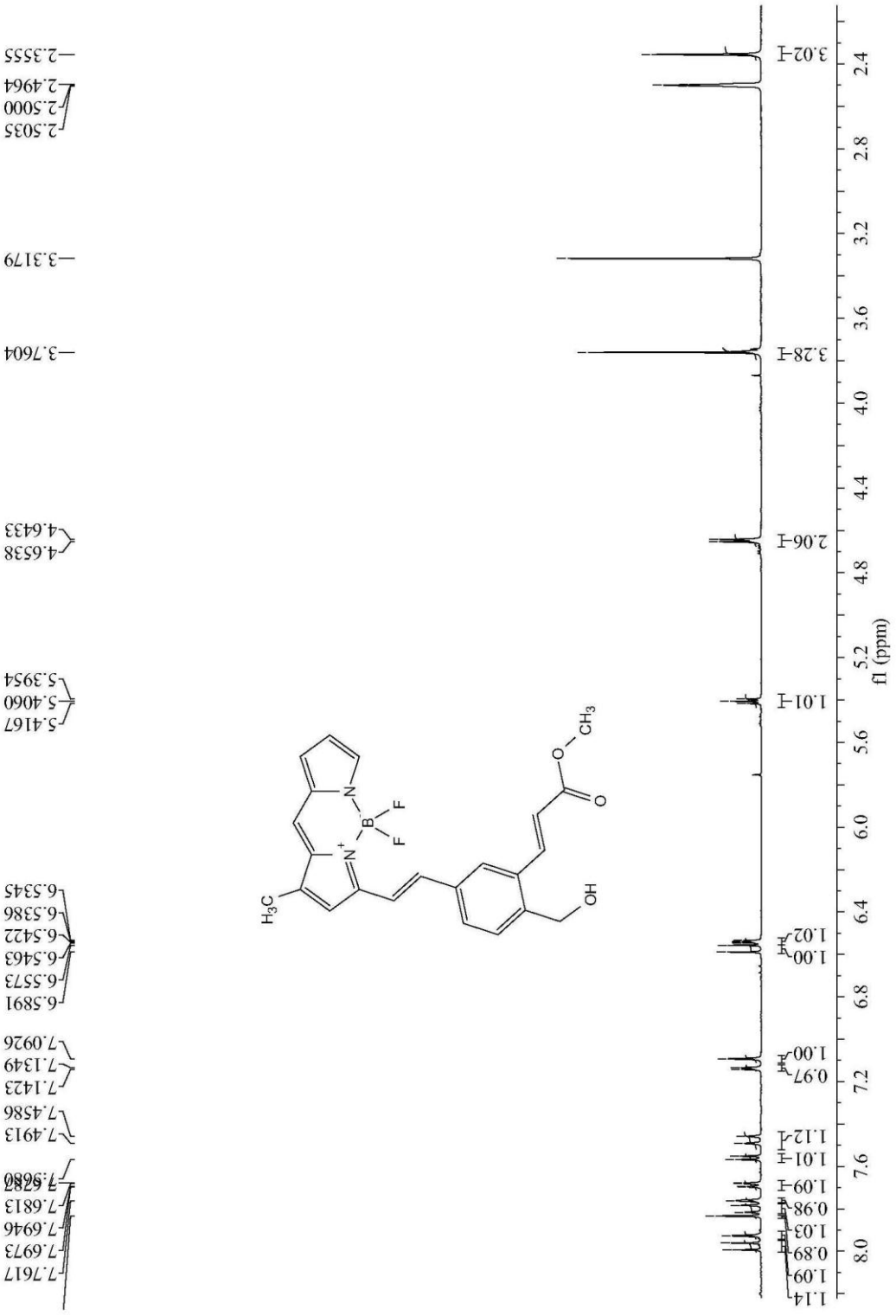
^{13}C NMR trace of intermediate **2**



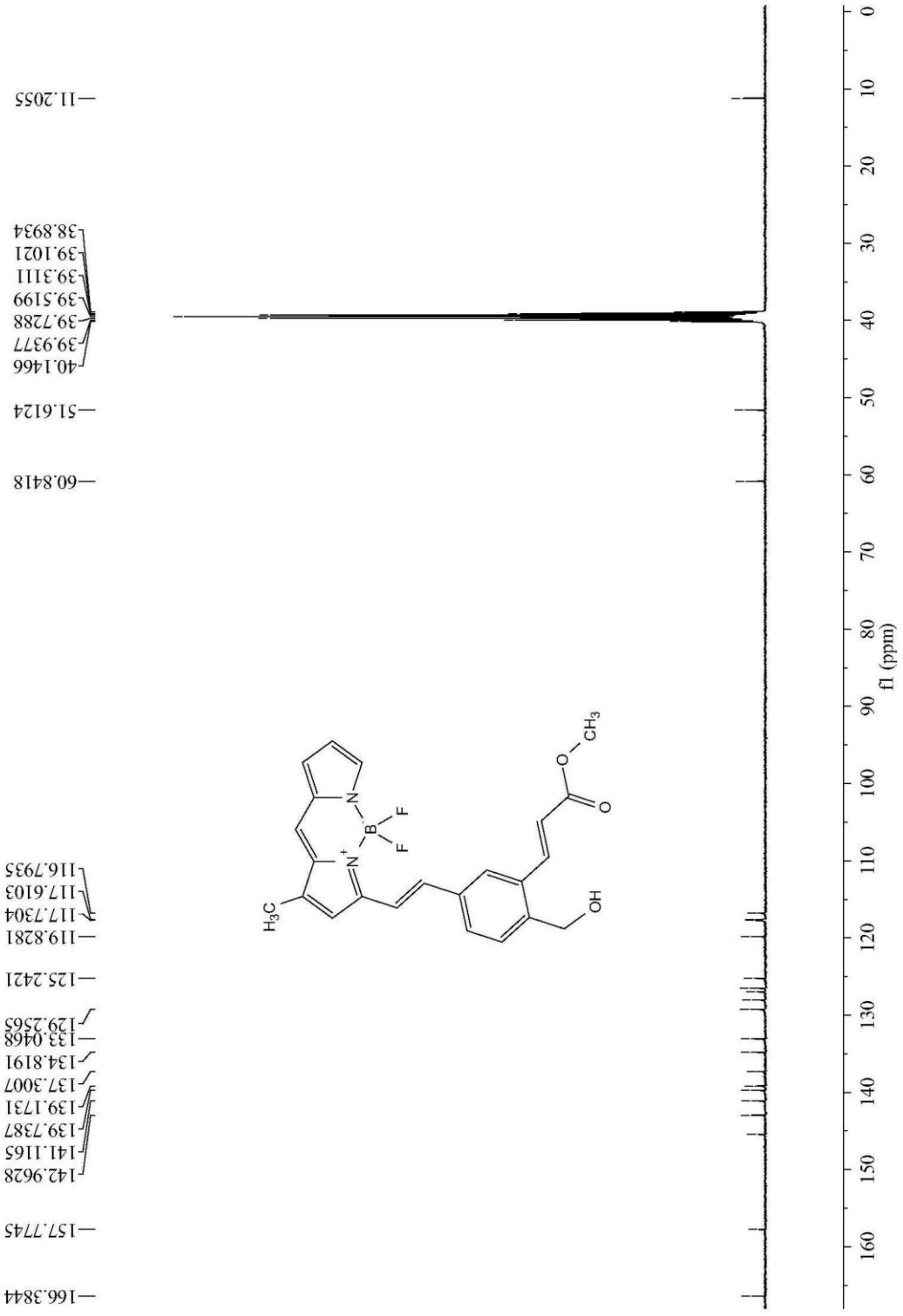
^{1}H NMR trace of intermediate **3**



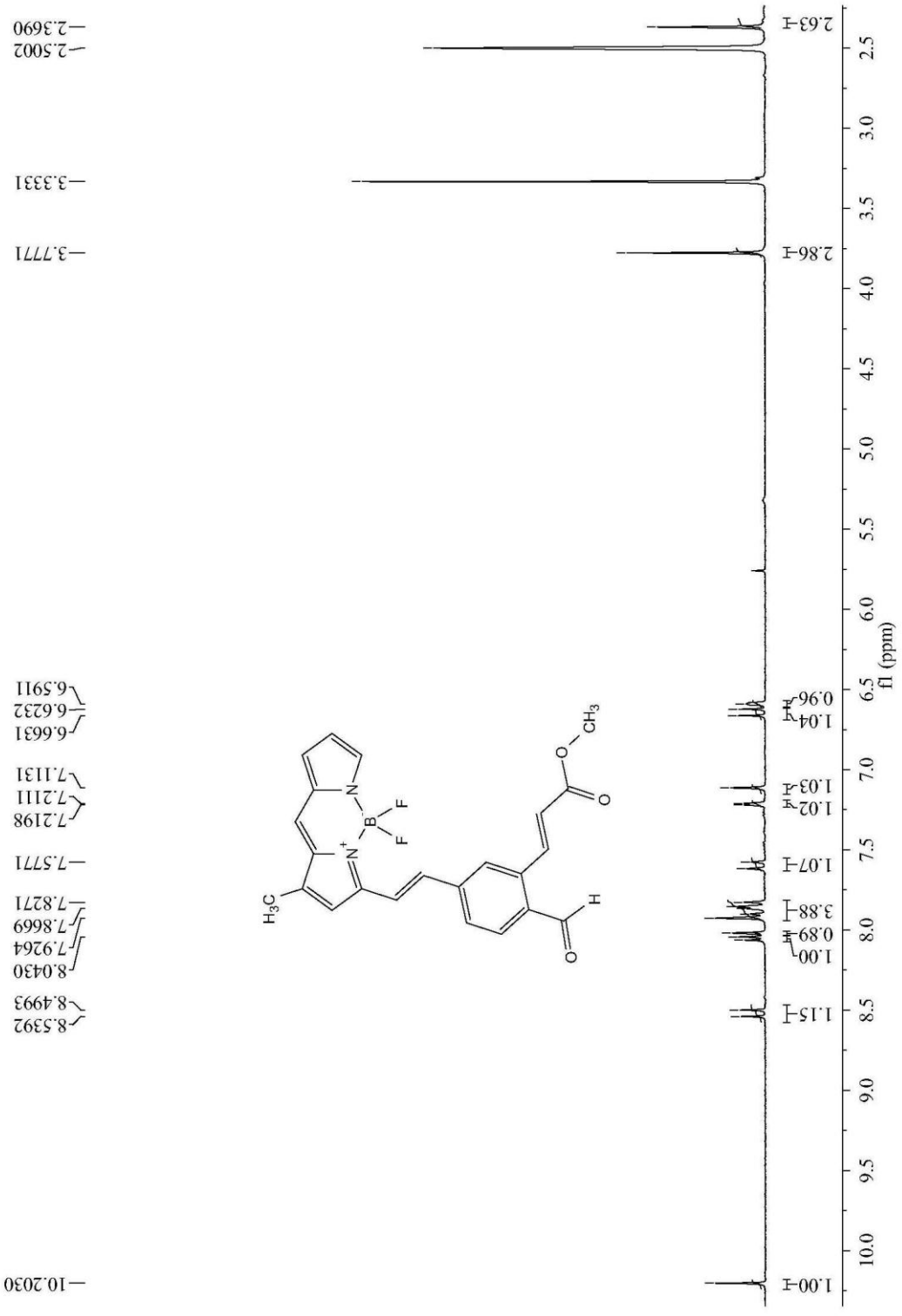
^{13}C NMR trace of intermediate **3**



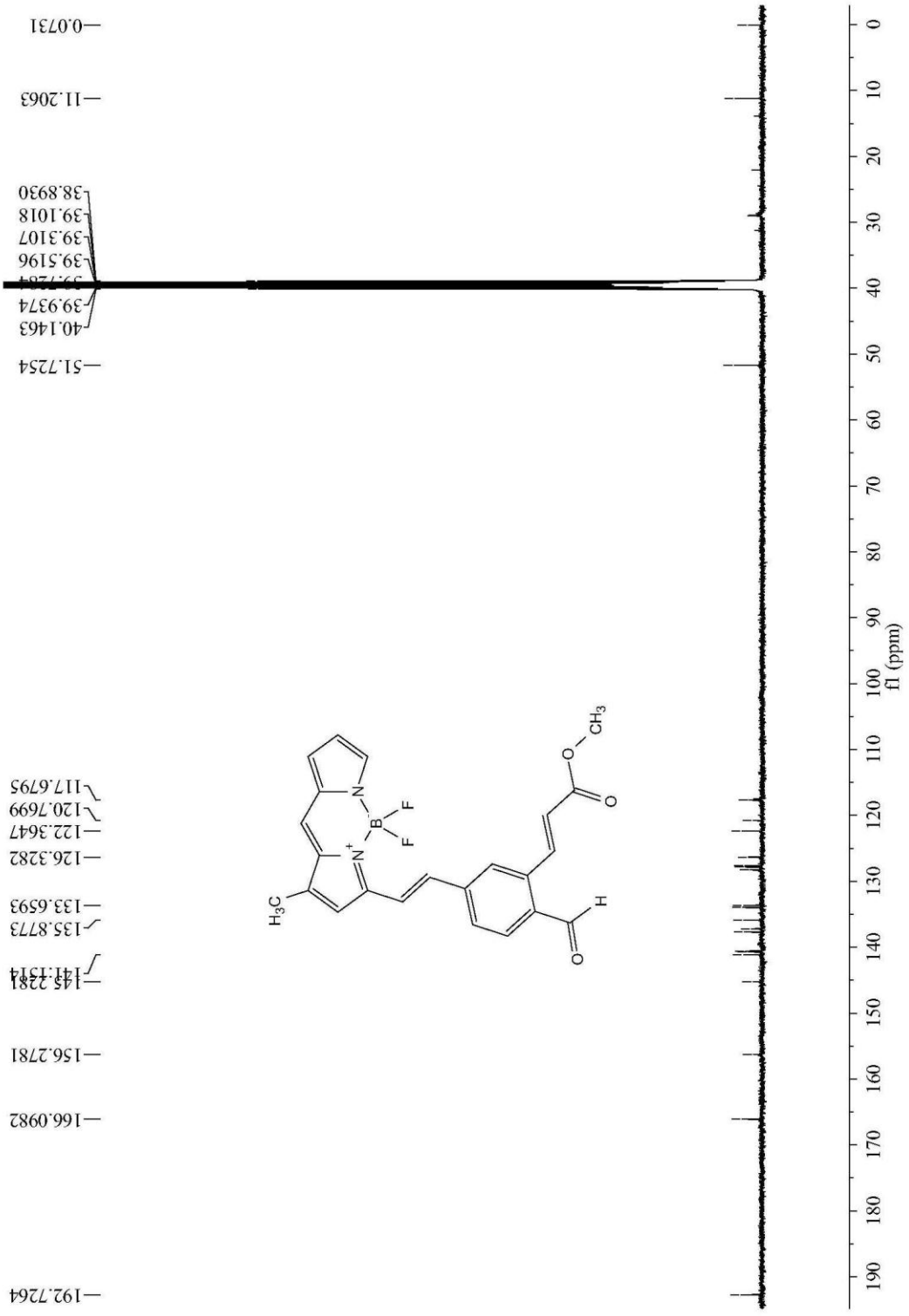
^{1}H NMR trace of intermediate **4**



^{13}C NMR trace of intermediate **4**



^{1}H NMR trace of probe **ZS1**



^{13}C NMR trace of probe **ZS1**