A Highly Sensitive and Water Soluble Fluorescent Probe for Rapid Detection of Hydrogen Sulfide in Living Cells

Xin Li,*[, a] Yanling Gong,[a] Kai Wu,[b] Steven H. Liang,[c] Ji Cao,[a] Bo Yang,[a] Yongzhou Hu,[a] and Yifeng Han*[, b]

[a] College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

[b] Department of Chemistry, Zhejiang Sci-Tech University, Hangzhou, 310018, China

[c] Division of Nuclear Medicine and Molecular Imaging, Massachusetts General Hospital and Department of Radiology, Harvard Medical School, 55 Fruit St., Boston, MA 02114, USA

SUPPORTING INFORMATION

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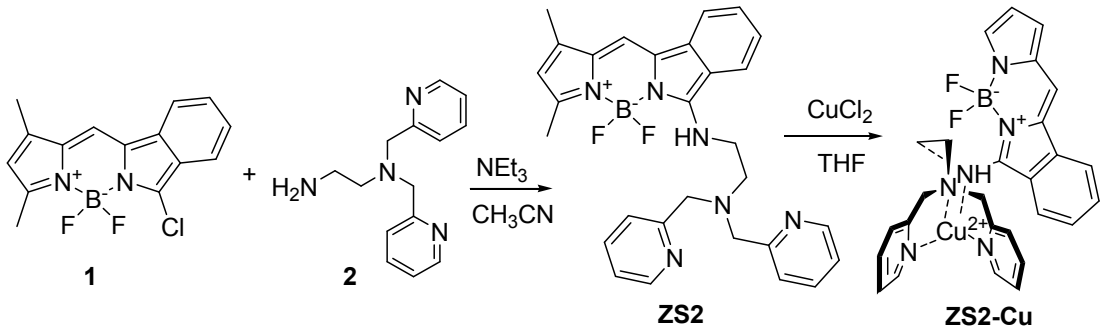
General Experimental

α-Chloro-BODIPY (**1**) and *N*, *N*-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (**2**) were prepared referring to literature procedures.[1]

Isolated yields refer to 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 agen. 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 (400 MHz) at 25 °C. ^{13}C-NMR spectra were recorded on a Bruker Fourier transform spectrometer (100 Hz) spectrometer and were calibrated using residual undeuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16). All chemical shifts were given in ppm and coupling constants (*J*) in Hz. The following abbreviations or combinations thereof were used to explain the multiplicities: s = singlet, d = doublet, m = multiplet. 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). Absorption spectra were acquired using a Hitachi U-3010 spectrophotometer. Fluorescence measurements were carried out on a PE LS45 fluorescence spectrometer.

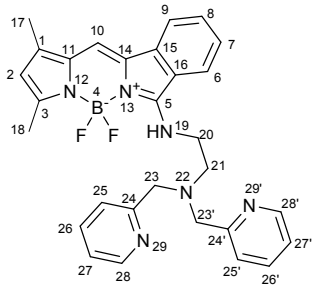
Scheme S1. Synthesis of ZS2 and ZS2-Cu



ZS2: To a stirred solution of α-Chloro-BODIPY (**1**, 100 mg, 0.330 mmom) and *N*, *N*-bis(pyridin-2-ylmethyl)ethane-1,2-diamine (**2**, 400 mg, 1.65 mmol) in acetonitrile (15.0 mL) was added

[1] L. Jiao, C. Yu, M. Liu, Y. Wu, K. Cong, T. Meng, Y. Wang, E Hao, *J. Org. Chem.*, 2010, **75**, 6035–8; K. Kiyose, H. Kojima, Y. Urano, T. Nagano, *J. Am. Chem. Soc.*, 2009, **131**, 10077-82.

slowly triethylamine (500 µL, 3.58 mmol). The system was then heated to reflux. After 12 hours, the reaction was cooled down to ambient temperature and ethyl acetate (50 mL) was added to dilute the system. The mixture was then washed subsequently with H_2O (3 x 15 mL) and brine (1 x 15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (SiO_2: EtOAc / petroleum ether, 1:1 to give the product as a red solid (150 mg, 90% yield).



Rf = 0.43 (40:1 CH_2Cl_2:CH_3OH)

δ_H (400 MHz, $CDCl_3$): 8.48 (2 H, d, *J* 4.5, H-28 and H-28'), 7.79 (2 H, m, H-26 and H-26'), 7.67 (4 H, m, H-25, H-25', H-6 and H-9), 7.43 (1 H, m, H-8), 7.18 (1 H, m, H-7), 7.05 (2 H, m, H-27), 6.83 (1 H, s, H-10), 5.90 (1 H, s, H-2), 3.91 (4 H, s, H-23 and H-23'), 3.86 (2 H, m, H-20), 2.96 (2 H, m, H-21), 2.56 (3 H, s, H-18), 2.20 (3 H, s, H-17).

δ_C (100 MHz, $CDCl_3$): 158.43, 156.58, 148.87, 140.16, 137.72, 137.04, 131.21, 128.42, 127.59, 126.03, 124.56, 123.33, 122.42, 119.82, 113.52, 106.02, 60.42, 51.87, 41.57, 13.90, 11.02.

IR (ν_{max}, cm^{-1}): 1620 (C=N), 1601 (C=N), 1445 (C=C), 1430 (C=C), 1263 (B-F), 1140 (C-N), 1042 (C-N).

ESI-HRMS (m/z): $[M+H]^+$ calc'd. for $C_{29}H_{28}BCuF_2N_6$: 511.2593; found 511.2601.

ZS2-Cu: To a solution of **ZS2** (50 mg, 0.10 mmol) in THF (50 mL) was added $CuCl_2 \cdot 2H_2O$ (17 mg, 0.10 mmol, 1.0 eq). The mixture was stirred at ambient temperature in dark for 2 days. Evaporation of the volatile solvent under reduced pressure gave the crude product which was rinsed with THF (10 mL) to yield **ZS2-Cu** as a black red powder quantitatively.

ESI-HRMS (m/z): $[M+Cu^{2+}-H]^+$ calc'd. for $C_{29}H_{28}BCuF_2N_6$: 572.1733; found 572.1735.

Fluorometric analysis

All fluorescence measurements were carried out at room temperature in PBS (10 mmol, pH 7.4)

which was prepared with DI water and purged with nitrogen for 5 minutes before use. The probes were dissolved in CH_3CN to make a 1.0 mM stock solution, which was diluted to the required concentrations with PBS for measurements. NaHS was dissolved in the above mentioned deoxygenated PBS to make a stock solution of 1.0 mM and only fresh solutions were used. All fluorometric experiments were repeated at least three times.

Fluorescence quantum yield measurements

Fluorescence quantum yields were obtained by comparison with fluorescein (Φ 0.95, in 0.1 M NaOH) with the following equation where Σ[F] is the integrated fluorescence intensity, Abs is absorbance at λex 496 nm, and n represents the refractive index. For PBS and 0.1 M NaOH, we used refractive indices of 1.334 and 1.335 respectively.

$$\Phi_{sample} = \Phi_{standard} \times (Abs_{standard}/Abs_{sample}) \times (\Sigma[F_{sample}]/\Sigma[F_{standard}]) \times (n_{sample}^2/n_{standard}^2)$$

Detection limit

The detection limit of **ZS2-Cu** was determined as the concentration of NaHS that resulted in a threefold increase in the fluorescence intensity at 546 nm compared to **ZS2-Cu** (0.5 μM) in PBS (10 mM, pH 7.4, 25°C). The slit width was 5 nm for both excitation and emission.

Cell lines

HeLa cell line was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells 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 captions respectively, the images were collected on a fluorescence microscope (LEICA DMI 4000B) upon excitation at 488 nm.

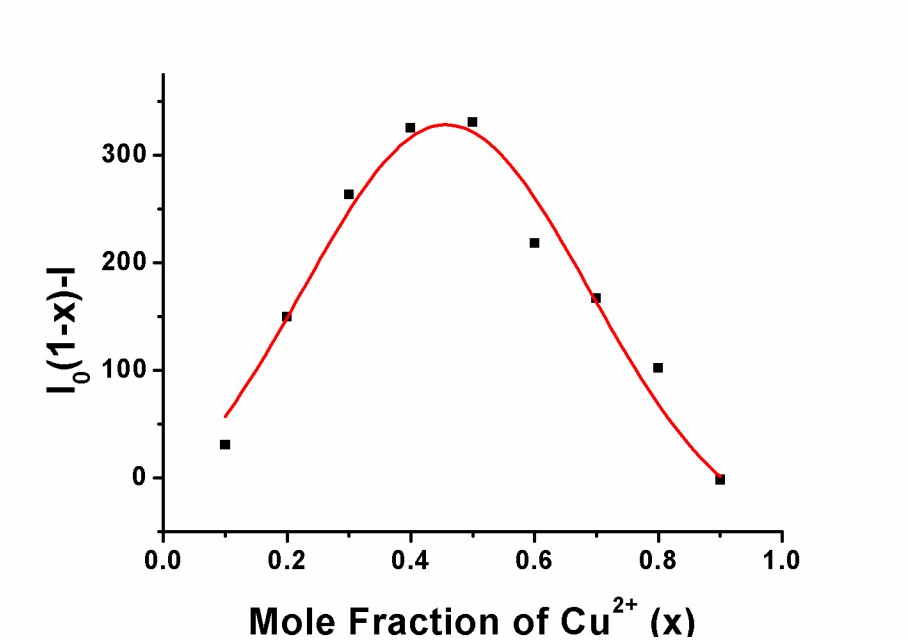


Fig. S1 Job's plot for determining the stoichiometry of **ZS2** and Cu^{2+} in PBS (10 mM, pH 7.4, 25°C). The total concentration of **ZS2** and Cu^{2+} was 20 μM and the excitation wavelength was 480 nm.

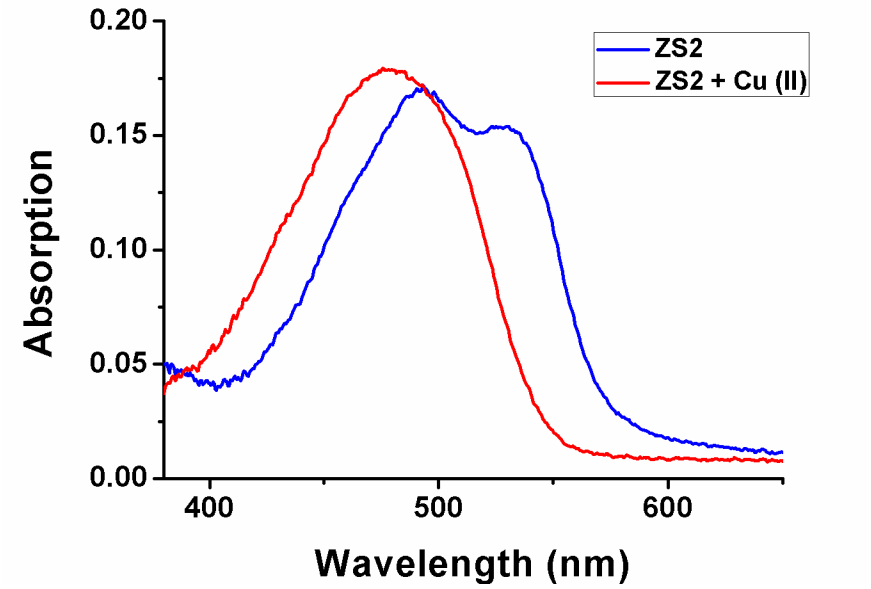


Fig. S2 UV-vis spectrum of **ZS2** (10 μM) in PBS (10 mM, pH 7.4, 25°C) and that of the system after the addition of 1.0 eq of $CuCl_2$.

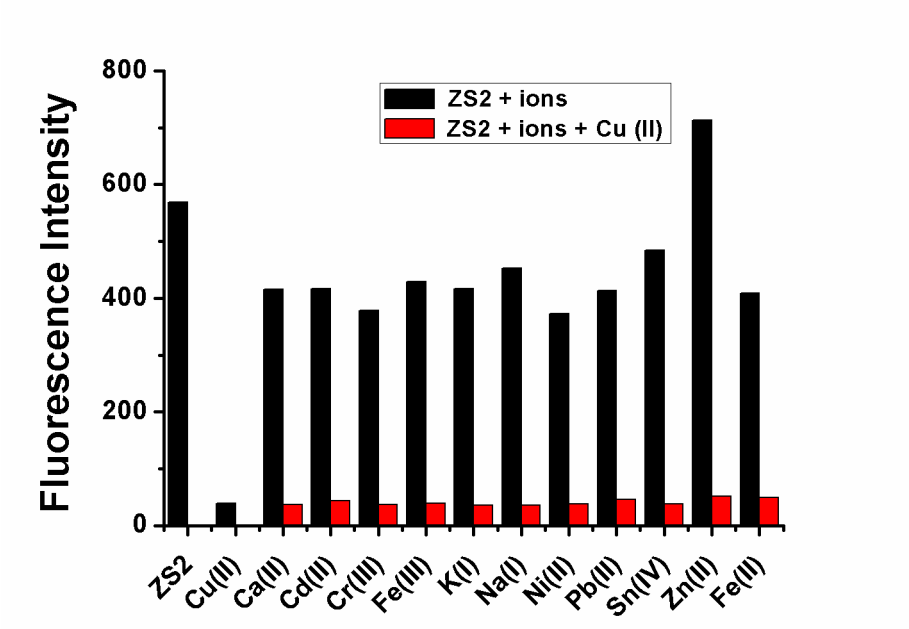


Fig. S3 Fluorescence responses of **ZS2** towards various cations with or without the presence of Cu^{2+}. Data were obtained as the fluorescence intensity at 546 nm (λex 480 nm) in PBS buffer (10 mM, pH 7.4, 25°C) right after treating **ZS2** with the cation indicated. The slit width was 3 nm for both excitation and emission. The concentrations of **ZS2** and the cations were both 10 μM. For the coexistence experiments, both Cu^{2+} and the tested substrates were kept at a final concentration of 10 μM.

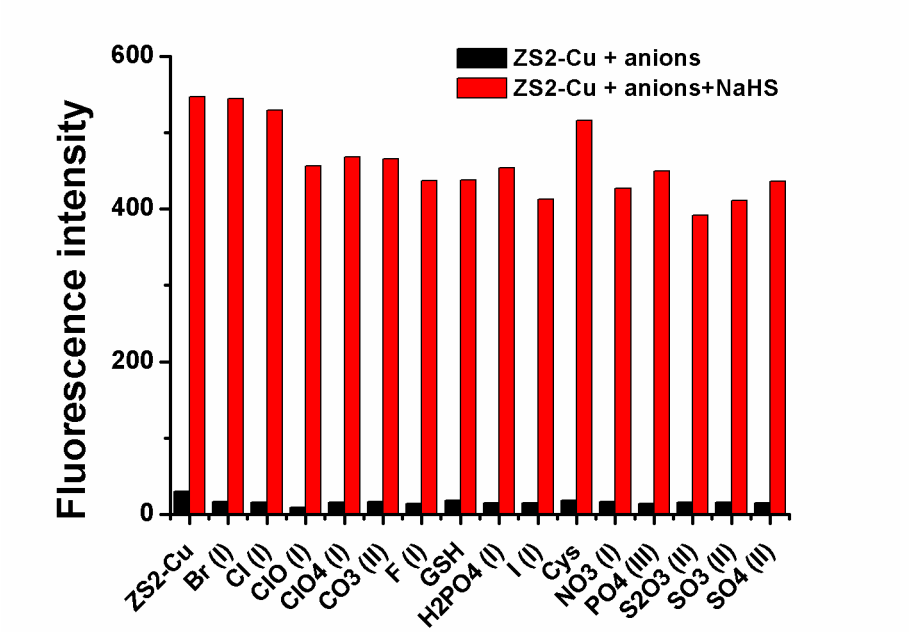


Fig. S4 Fluorescence responses of **ZS2-Cu** towards NaHS in the presence of other anions or

biothiols. Data were obtained as the fluorescence intensity at 546 nm (λex 480 nm) in PBS buffer (10 mM, pH 7.4, 25°C) immediately after treating **ZS2-Cu** with the anion indicated alone (black) or both the anion and NaHS (red). The slit width was 3 nm for both excitation and emission. The concentrations of **ZS2-Cu** and the anions were 10 μM and 20μM, respectively. For the coexistence experiments, both NaHS and the tested substrates were kept at a final concentration of 20 μM.

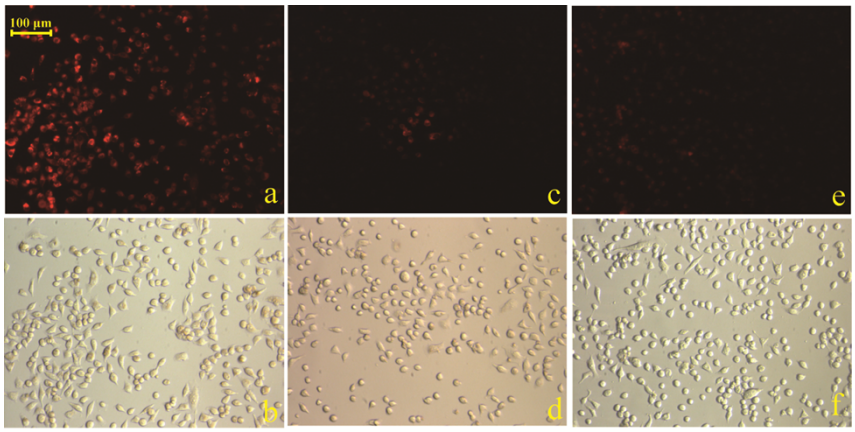
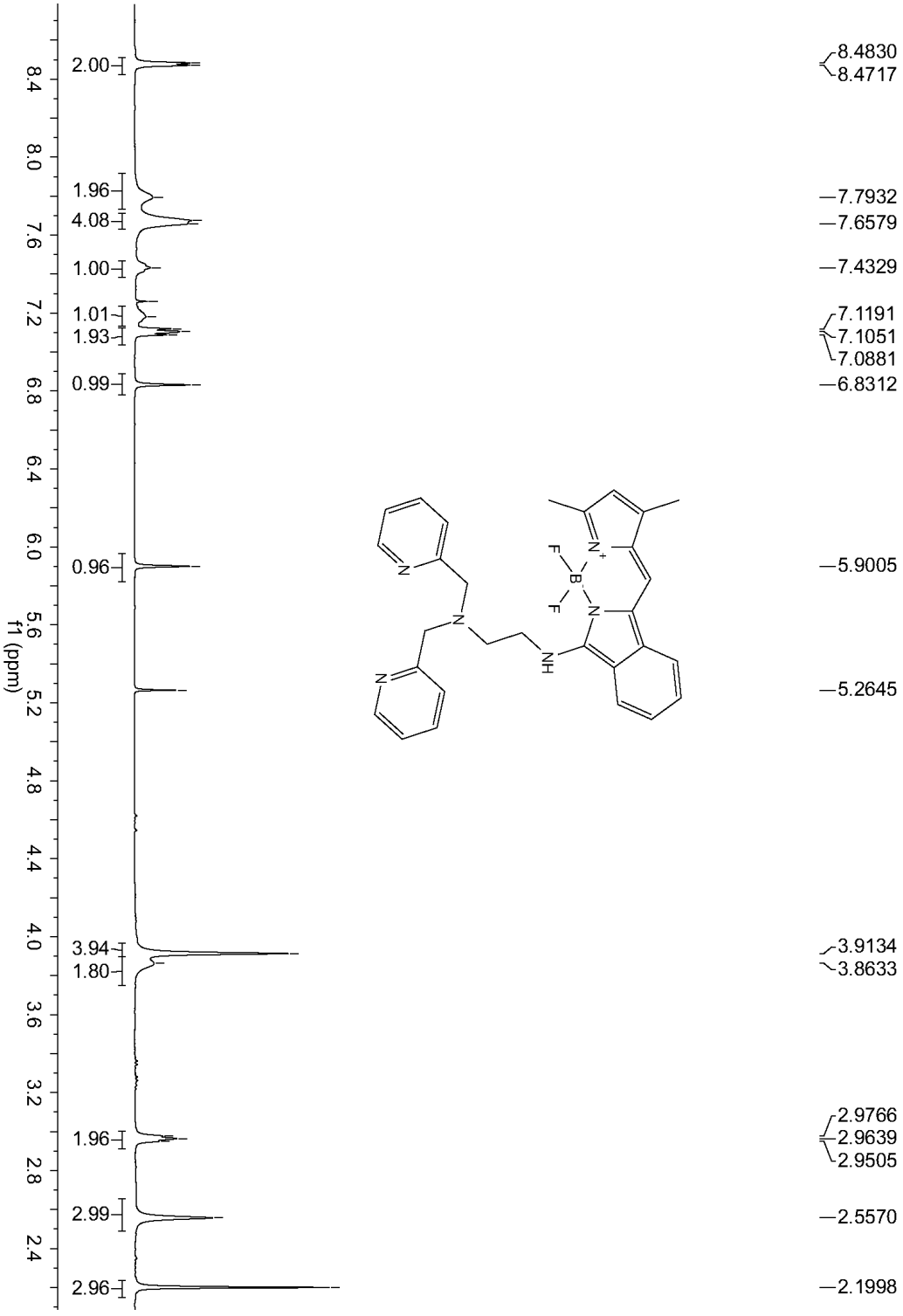


Fig. S5 ZS2 acts as a switch-off probe for Cu^{2+} and a switch-on probe for H_2S in live cells. (a, b) Cells were incubated with **ZS2** (5 μM) for 15 min to result in strong intracellular fluorescence. (c, d) Incubating **ZS2**-prereated cells (15 min) with $CuCl_2$ (10 μM) incurred a great decrease in the fluorescence. (e, f) Treatment of cells contacted with **ZS2** and Cu^{2+} subsequently with NaHS (100 μM) rescued the intracellular fluorescence (15 min). Images were collected on a fluorescence microscope (LEICA DMI 4000B) upon excitation at 488 nm (a, c, e) or under bright field (b, d, e).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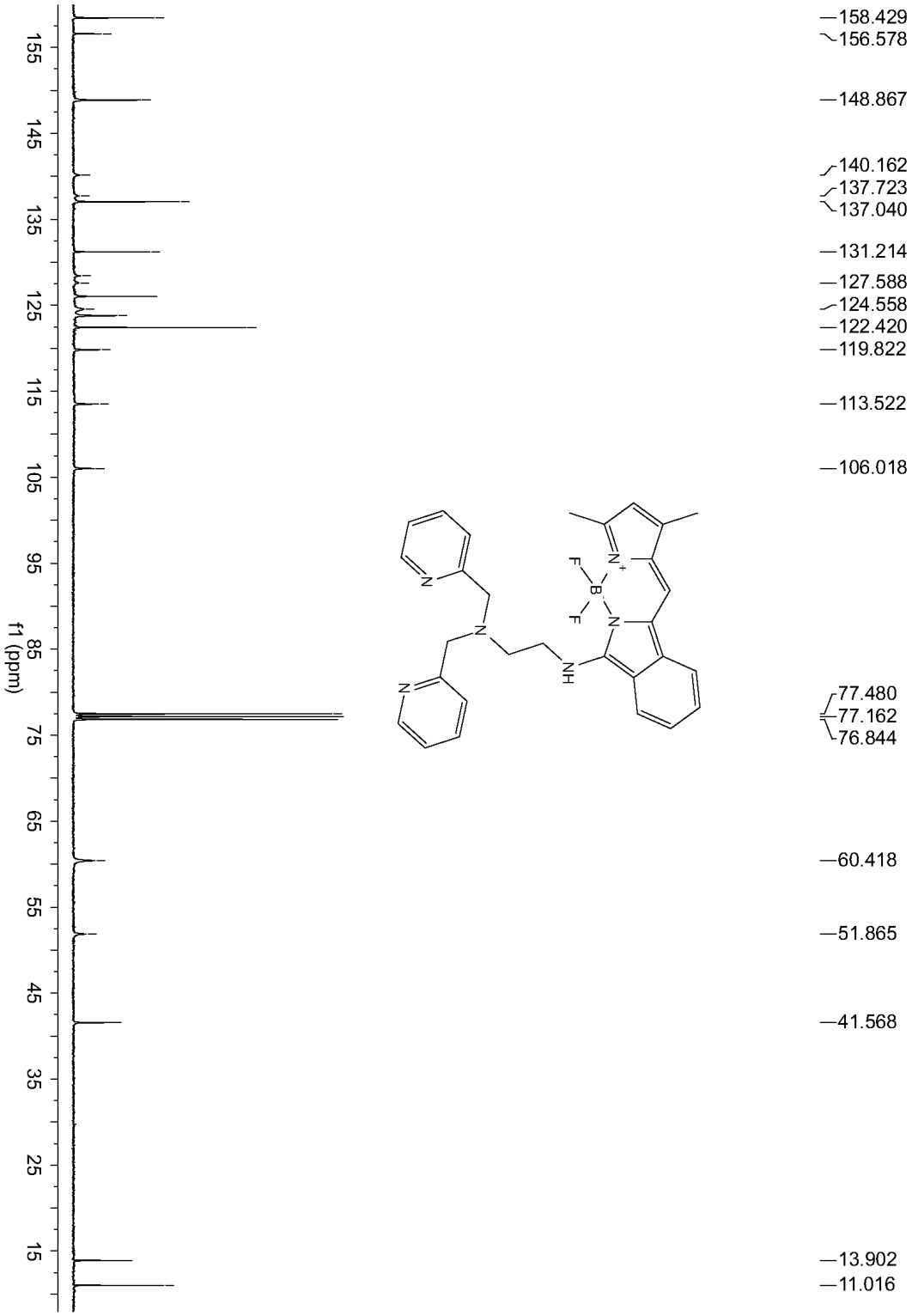
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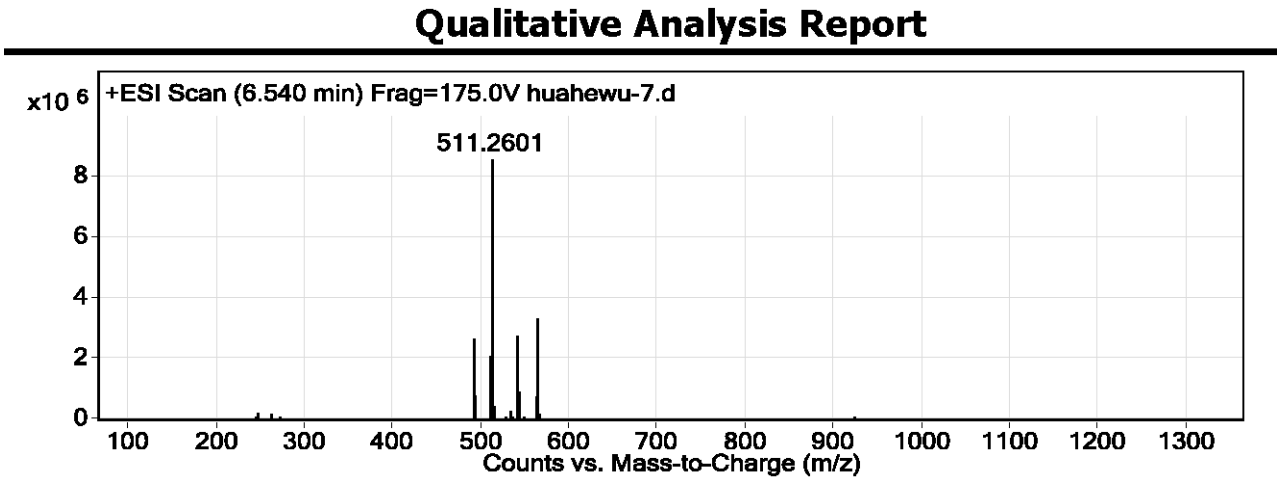
HRMS spectra of compound ZS2



HRMS spectra of compound ZS2-Cu