Electronic Supplementary Information for

Spirolactamized benzothiazole-substituted N,N-diethylrhodol: a new platform to construct ratiometric fluorescent probes

Hui Wen, Qian Huang, Xiao-Feng Yang* and Hua Li

Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry & Materials Science, Northwest University, Xi'an 710069, (P.R. China); Fax: (+86) 29-88303798; E-mail: xfyang@nwu.edu.cn

Table of contents

Materials and Instruments

3-Diethylaminophenol and 2-aminothiophenol were obtained from Aladdin reagent Co. (Shanghai, China); *o*-phthalic anhydride was obtained from Xi'an chemical reagent factory; 2, 4-dihydroxybenzaldehyde was obtained from Shanghai Darui fine chemical Co., Ltd. Column chromatography was conducted over silica gel (200 - 300 mesh) obtained from the Qingdao Ocean Chemicals. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried by standard methods prior to use. Double-distilled water was used throughout the experiments. Analytical grade of metal chlorate (Zn^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cr^{3+}), nitrate (Ag^{+}, Cu^{2+}, Pb^{2+}) or sulfate (Mn^{2+}, Fe^{3+}) were dissolved in double distilled water to prepare the stock solutions of metals ions. Cu^{+} stock solution was prepared by dissolving tetrakis(acetonitrile)copper(I) hexafluorophosphate in acetonitrile.

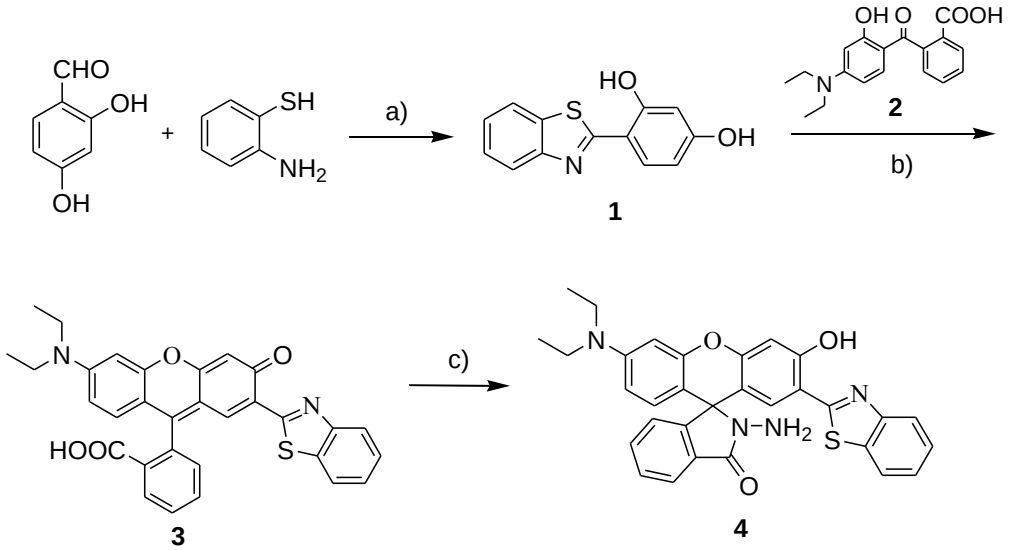
The fluorescence spectra and relative fluorescence intensity were measured with a Shimadzu RF-5301 spectrofluorimeter with a 10mm quartz cuvette. Absorption spectra were recorded using a Shimadzu UV-2550 spectrophometer. High-resolution mass spectra were collected using a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonics Corp.,USA) in electrospray ionization (ESI) mode. ^{1}H and ^{13}C NMR spectra were recorded on an INOVA-400 spectrometer (Varian Unity), using tetramethylsilane (TMS) as the internal standard. The pH measurements were carried out on a Sartorius PB-10 pH meter. All the measurements were operated at room temperature at about 298 K.

General procedure for the spectra measurement

A stock of solution of 4 (1.0 mM) was prepared in DMF. In a set of 10 mL volumetric tubes containing 1.0 mL of HEPES (10.0 mmol L^{-1}, pH=7.4), 3.0 mL of

CH_3CN and 50 μL of probe **4** (1.0 mmol L^{-1}), different concentrations of metal ions were added and the reaction mixture was diluted to 10.0 mL with water. The resulting solution was kept at room temperature (25 ^oC) for 20 min, and then the absorption or fluorescence spectra were recorded. The fluorescence ratio of I_{575}/I_{450} was measured with the excitation and emission wavelengths at 358 and 575/450 nm, respectively.

Synthesis



Scheme S1. Synthesis of **3** and **4**. Reagents and conditions: a) sodium metabisulfite, DMF, reflux 2 h; b) CH_3SO_3H, 90 °C, 24 h; c) $NH_2NH_2•H_2O$, C_2H_5OH, reflux 5 h.

Preparation of 2-(2',4'-dihydroxyphenyl) benzothiazole (**1**)

Compound **1** was synthesized according to the reported procedure.[1] Briefly, to a solution of 2-aminothiophenol (3.12 mmol, 0.33 mL) and 2,4-dihydroxybenzaldehyde (3.16 mmol, 0.446 g) in DMF (10 mL), sodium metabisulfite ($Na_2S_2O_5$, 0.610 g) was added and the reaction mixture was refluxed for 2 h. After cooling, the resulting solution was added dropwise into water (200 mL). The precipitate was filtered off, washed with water (20 mL × 3) and dried to afford the crude product, which was further purified by recrystallization from methanol to give **1** as a light yellow solid (0.38 g, yield 50%). ^1H NMR (400MHz, d_6-DMSO): δ11.68 (s, 1H), 10.19 (s, 1H), 8.07 (d, 1H, J =7.6 Hz), 7.97 – 7.90 (m, 2H), 7.49 (t, 1H, J =7.4 Hz), 7.38 (t, 1H, J =7.4 Hz), 6.46 (bs, 2H).

Preparation of 2-(4-Diethylamino-2-hydroxybenzoyl) benzoic acid (**2**)

Compound **2** was prepared from o-phthalic anhydride and 3-diethylaminophenol according to the literature procedures.[2] ^{1}H NMR (400MHz, d_6-DMSO): δ13.11 (s, 1H), 12.59 (s, 1H), 7.97 (d, 1H, J = 7.2 Hz), 7.68 (t, 1H, J = 7.2 Hz), 7.60 (t, 1H, J = 7.4 Hz), 7.37 (d, 1H, J = 7.2 Hz), 6.80-6.78 (m, 1H), 6.17 (d, 1H, J = 9.2 Hz), 6.08 (bs, 1H), 3.37 (q, 4H, J = 6.4 Hz), 1.08 (t, 6H, J = 6.2 Hz).

Preparation of compound **3**

A suspension of **1** (0.41 mmol, 0.1 g) and **2** (0.41 mmol, 0.13 g) in methanesulfonic acid (5 mL) was stirred at 90 °C for 24 h. The reaction mixture was cooled to room temperature and then poured in ice-cold water (30 mL). The precipitate was filtered, washed with brine (3×7 mL), and then dried under vacuum to give the crude product, which was further purified by preparative thin-layer chromatography ($CHCl_3$-C_2H_5OH, 20:1, v/v) to afford compound **3** as a purple solid (116 mg, yield 54.4%). ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.19 (d, 1H, J = 6.8 Hz), 8.03-7.99 (m, 2H) ,7.82 (d, 1H, J = 8.0 Hz), 7.69-7.62 (m, 2H), 7.37 (t, 1H, J = 7.4 Hz), 7.28 (q, 2H, J = 7.2 Hz), 6.71 (d, 3H, J = 5.6 Hz), 6.41 (s, 1H), 1.13 (t, 6H, J = 6.4 Hz). ^{13}C NMR(100 MHz, d_6-DMSO): δ169.16, 162.82, 162.70, 156.81, 154.55, 154.48, 151.78, 151.21, 135.91, 135.83, 131.55, 131.51, 131.43, 130.16, 129.39, 128.960, 127.49, 127.44, 125.73, 123.99, 121.79, 121.69, 110.36, 110.24, 103.86, 103.79, 103.743, 96.24, 44.38, 12.47. HRMS (ESI): *m/z* calcd for $C_{31}H_{25}N_2O_4S$ $[M + H]^+$ 521.1530, found 521.1536.

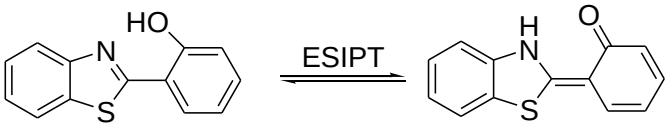
Preparation of compound **4**

Rhodol hydrazide **4** was prepared in high yield from rhodol **3** according the procedure we reported previously.[3] To a suspended solution of **3** (1 mmol, 0.52 g) in ethanol (20 mL) was added an excess of hydrazine monohydrate (3.0 mL, 80%,) and the solution was refluxed for 5 h with stirring. The resulting solution was evaporated in vacuo to give a brown oil, which was then recrystallized from ethanol–water to afford the desired compound **4** (358 mg, yield 67.1%). ^{1}H NMR (400MHz, d_6-DMSO): δ8.05 (d, 1H, J = 7.6 Hz), 7.93 (d, 1H, J = 8.0 Hz), 7.80 - 7.87 (m, 1H),7.58 (s, 1H), 7.53 (t, 2H, J = 3.4 Hz), 7.44 (t, 1H, J = 7.6 Hz), 7.35 (t, 1H, J = 7.4

Hz), 7.09 (d, 1H, *J* = 4.8 Hz), 6.92 (s, 1H), 6.44 (bs, 1H), 6.36 (q, 2H, *J* = 9.2 Hz), 4.5 (s, 2H), 3.31 (q, 4H, *J* = 6.4 Hz), 1.07 (t, 6H, *J* = 6.8 Hz). ^{13}C NMR(100 MHz, DMSO): δ165.65, 163.31, 154.62, 152.55, 151.68, 151.37, 148.39, 134.73, 132.92, 129.68, 128.73, 127.82, 126.26, 124.68, 123.72, 122.68, 122.03, 121.86, 116.12, 111.83, 109.41, 108.53, 105.03, 103.45, 97.59, 64.74, 43.81, 12.54. HRMS (ESI): *m/z* calcd for $C_{31}H_{25}N_4O_3S$ $[M - H]^-$ 533.1653, found 533.1662.

Reference

1. K. M. Khan, F. Rahim, S. A. Halim, M. Taha, M. Khan, S. Perveen, Zaheer-ul-Haq, M. A. Mesaik and M. I. Choudhary, *Bioorg. Med. Chem.*, **2011**, 19, 4286.
2. E. Nakata, Y. Yukimachi, H. Kariyazono, S. Im, C. Abe, Y. Uto, H. Maezawa, T. Hashimoto, Y. Okamoto and H. Hori, *Bioorg. Med. Chem.*, **2009**, 17, 6952.
3. X.-F. Yang, X.-Q. Guo and Y.-B. Zhao, *Talanta*, **2002**, 57, 883.



HBT

enol form — keto form

emission at short wavelenght — emission at long wavelenght

Figure S1. Structures of the enol and keto forms of HBT.

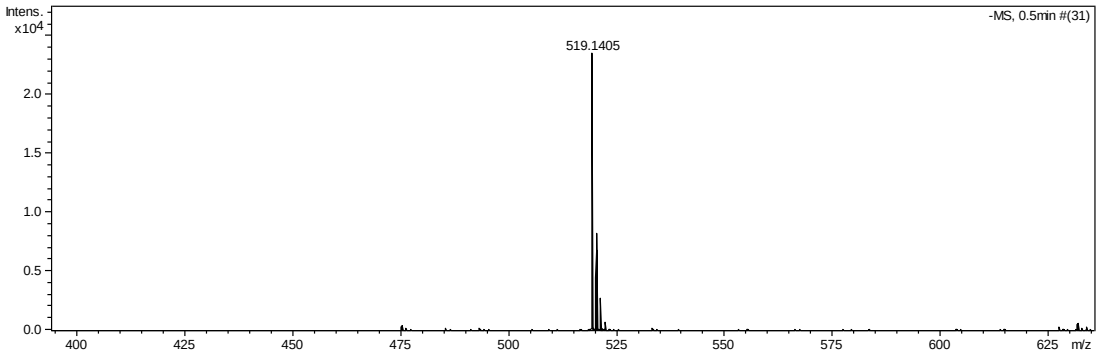


Figure S2. HRMS confirmed formation of compound **3** in the reaction of **4** with Cu^{2+}. $Cu(NO_3)_2 \cdot 3H_2O$ (26.7 mg) and probe **4** (36.2 mg) were mixed in 30 mL of CH_3CN, and then 10 mL of H_2O was added. The mixture was refluxed for 4 h, and then the desired product was separated by TLC with $CHCl_3$-CH_3OH (10:1, v/v), which was analyzed by HRMS after appropriate dilution.

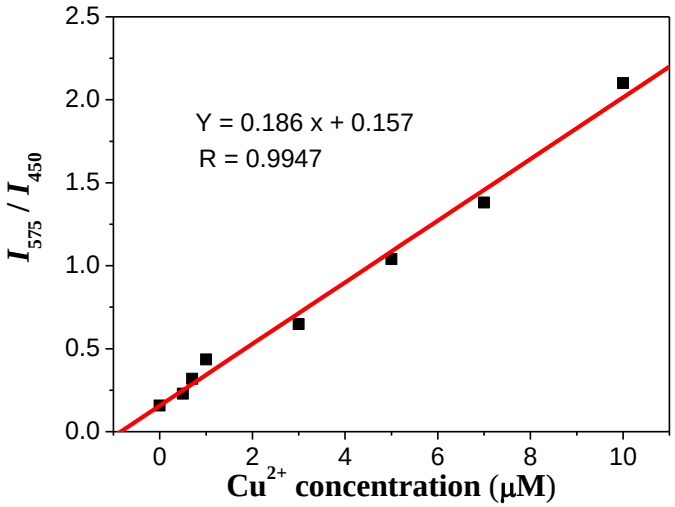


Figure S3. Plot of the fluorescence ratio (I_{575}/I_{450}) as a function of Cu^{2+} concentration.

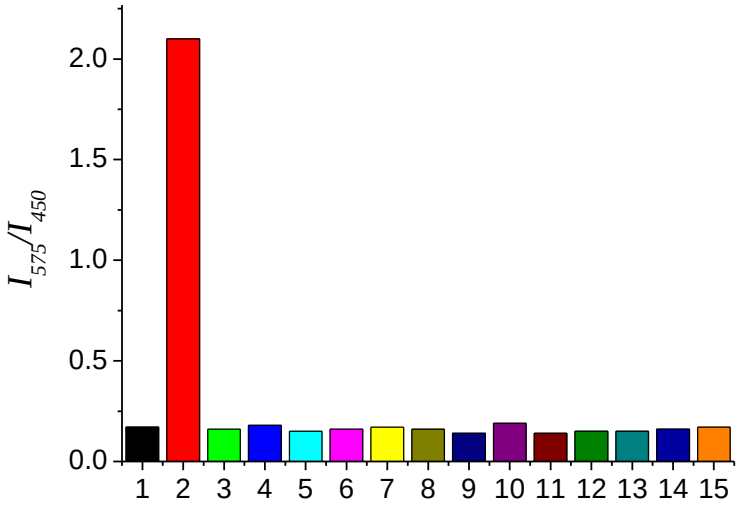


Figure S4. Ratiometric response (I_{575}/I_{450}) of **4** (5 μM) to various metal ions (2 equiv) in a mixed solution of CH_3CN:HEPES (30: 70, v/v, pH = 7.4, 10 mM). (1), **4** only; (2) Cu^{2+}; (3) Zn^{2+}; (4) Mg^{2+}; (5) Mn^{2+}; (6) Ag^{+}; (7) Hg^{2+}; (8) Pb^{2+}; (9) Cd^{2+}; (10) Co^{2+}; (11) Cr^{3+}; (12) Fe^{3+}; (13) Ni^{2+}; (14) Fe^{2+}; (15) Cu^{+}. λ_{ex} = 358 nm.

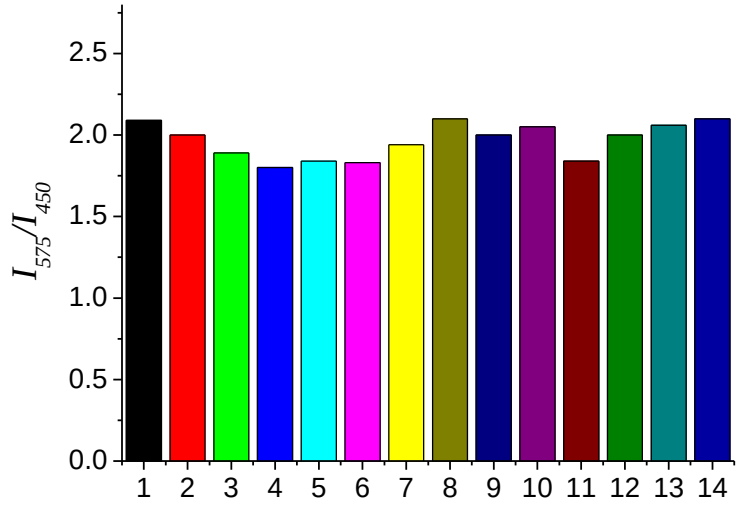


Figure S5. Fluorescence ratiometric response (λ_{ex} = 358 nm) of probe **4** (5 μM) to Cu^{2+} (2 equiv) in the presence of 5 equiv of different competing metal ions. (1) Cu^{2+}; (2) Zn^{2+}; (3) Mg^{2+}; (4) Mn^{2+}; (5) Ag^{+}; (6) Hg^{2+}; (7) Pb^{2+}; (8) Cd^{2+}; (9) Co^{2+}; (10) Cr^{3+}; (11) Fe^{3+}; (12) Ni^{2+}; (13) Fe^{2+}; (14) Cu^{+}.

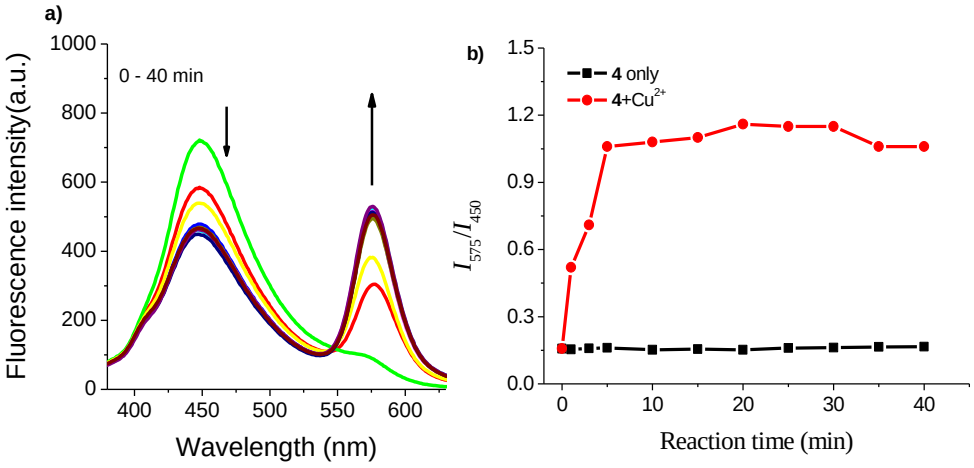


Figure S6. Time-depend fluorescence spectral (a) and emission ratio (I_{575}/I_{450}) changes (b) of probe **4** (5 μM) upon adding Cu^{2+} (1 equiv) in a mixed solution of CH_3CN:HEPES (30: 70, v/v, pH = 7.4, 10 mM). Data were acquired at 25 ^oC with excitation at 358 nm.

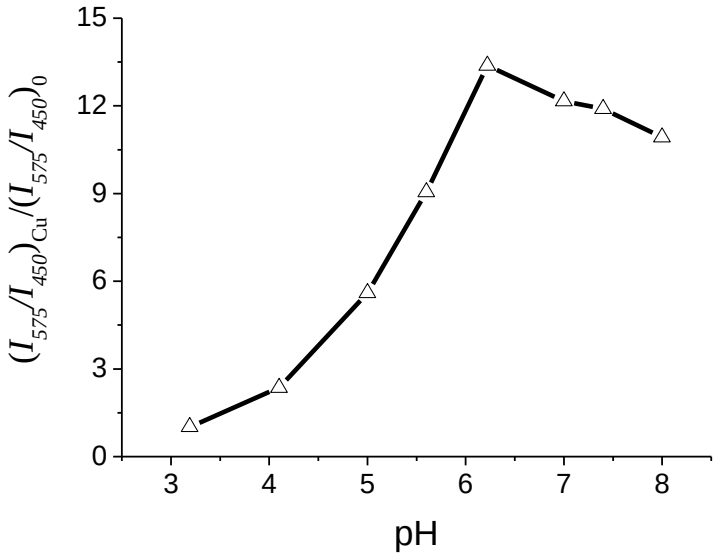


Figure S7. The enhancement factor of emission ratio (I_{575}/I_{450}) for probe **4** (5 μM) in the presence of Cu^{2+} (2 equiv) at various pH values. The $(I_{575}/I_{450})_0$ and $(I_{575}/I_{450})_{Cu}$ represent the emission ratio of probe **4** in the absence and presence of Cu^{2+}, respectively.

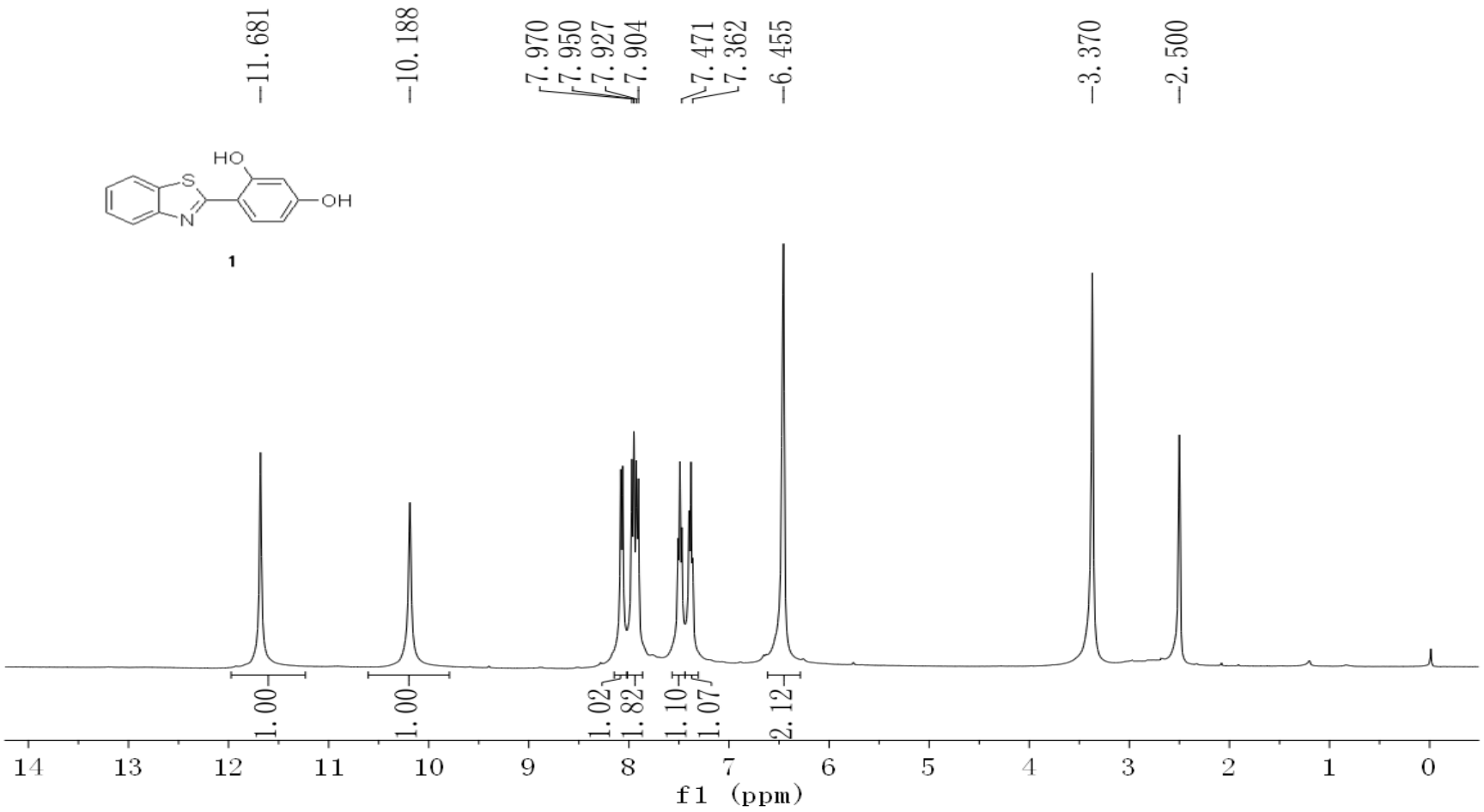


Figure S8. ^{1}H NMR spectrum of **1** in DMSO-d_6.

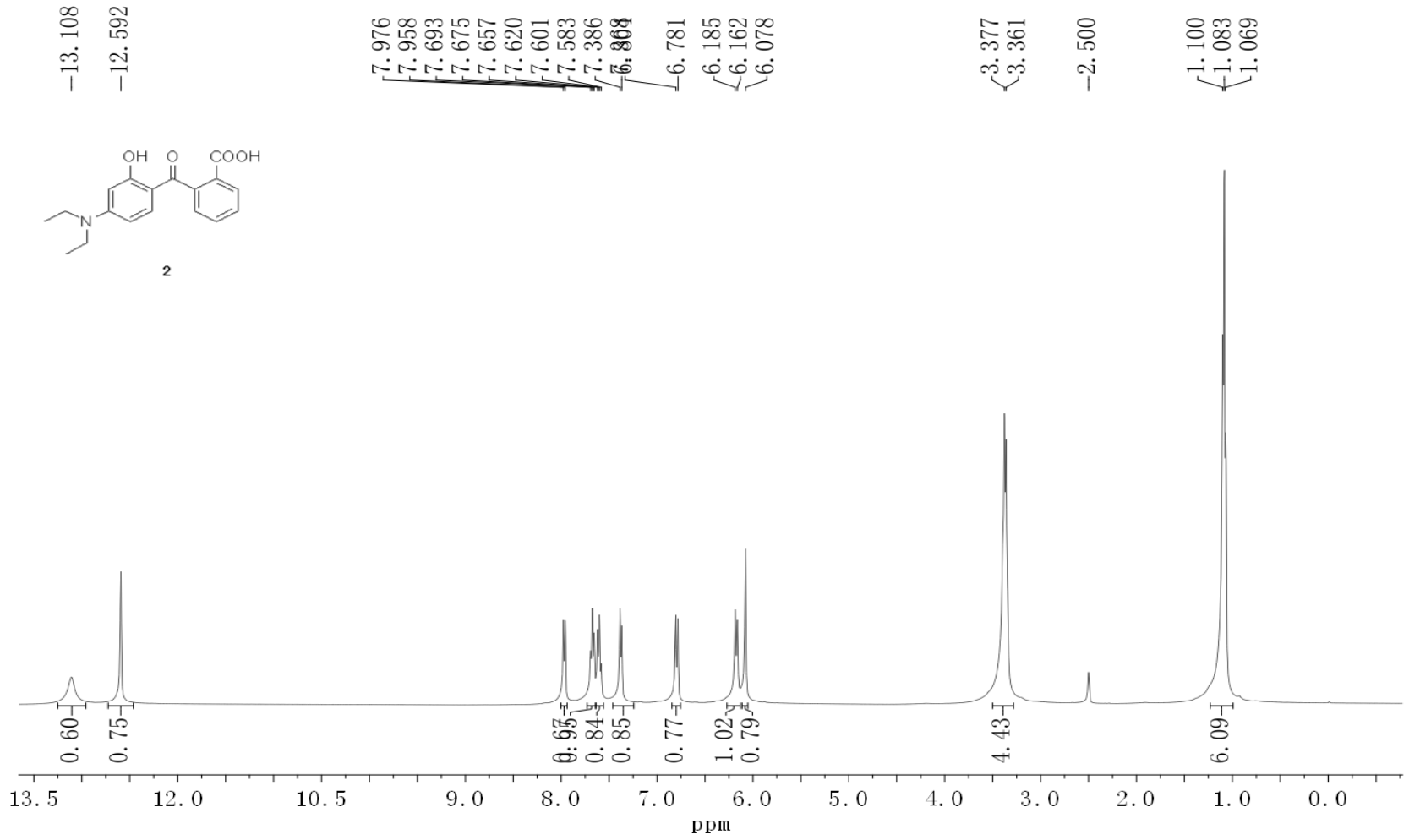


Figure S9. ^{1}H NMR spectrum of **2** in DMSO-d_6.

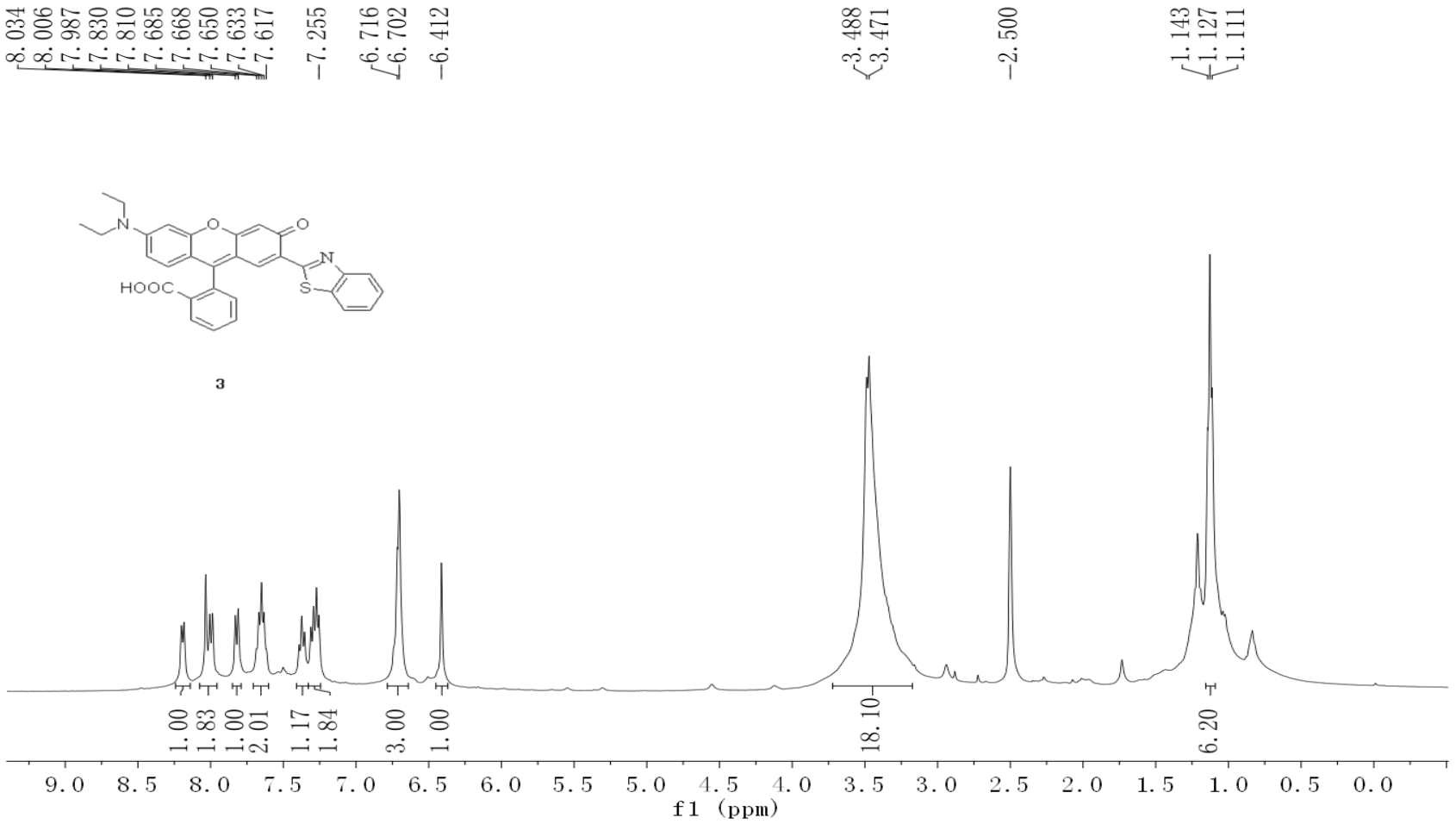


Figure S10. ^{1}H NMR spectrum of **3** in DMSO-d_6. The peak of methylene (-NCH_2CH_3) was overlapped with H_2O in solvent, so it can not be precisely integrated.

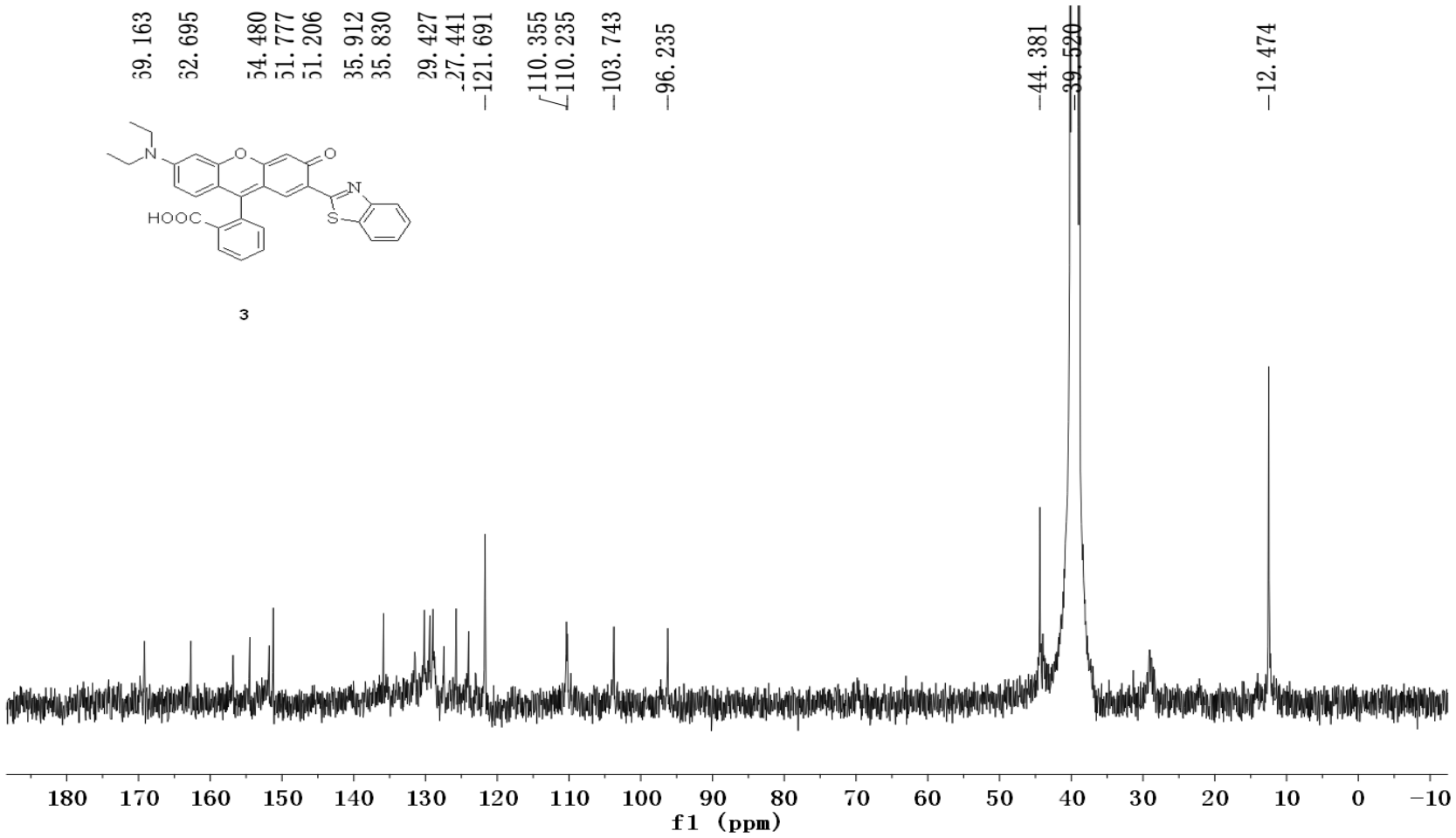


Figure S11. ^{13}C NMR spectrum of **3** in DMSO-d_6.

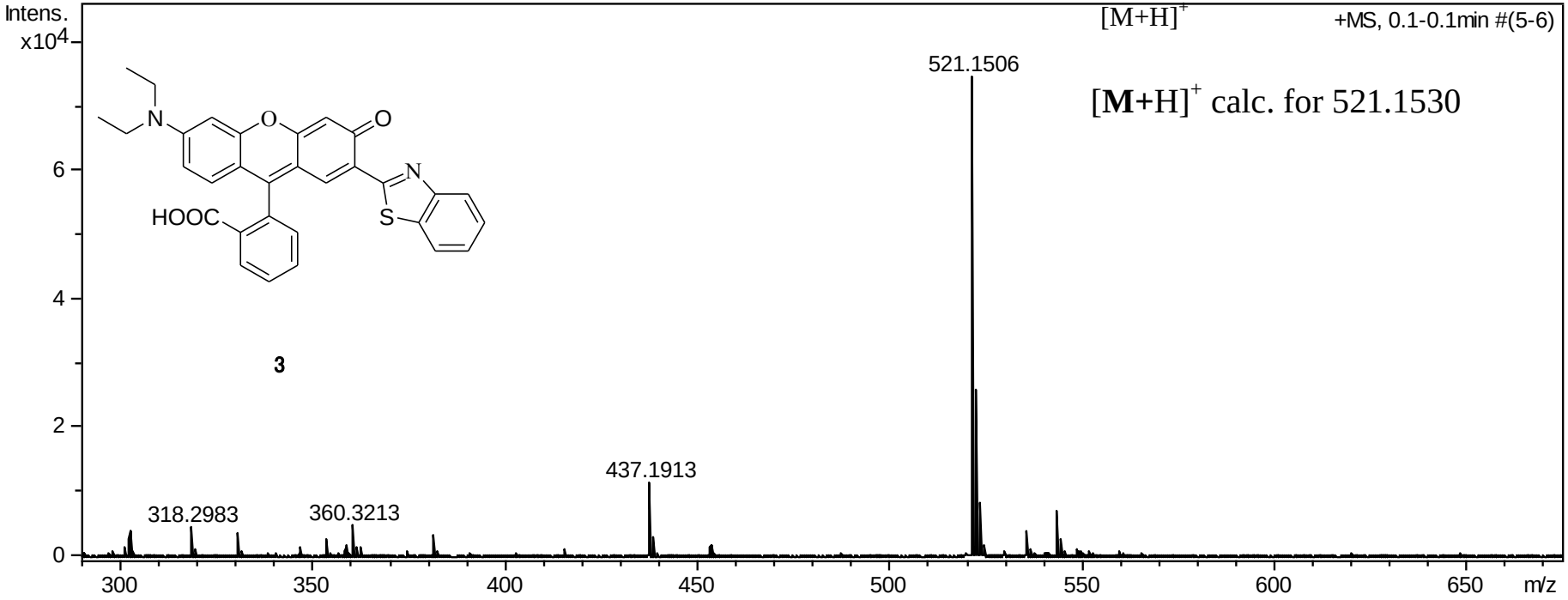


Figure S12. HRMS of **3**.

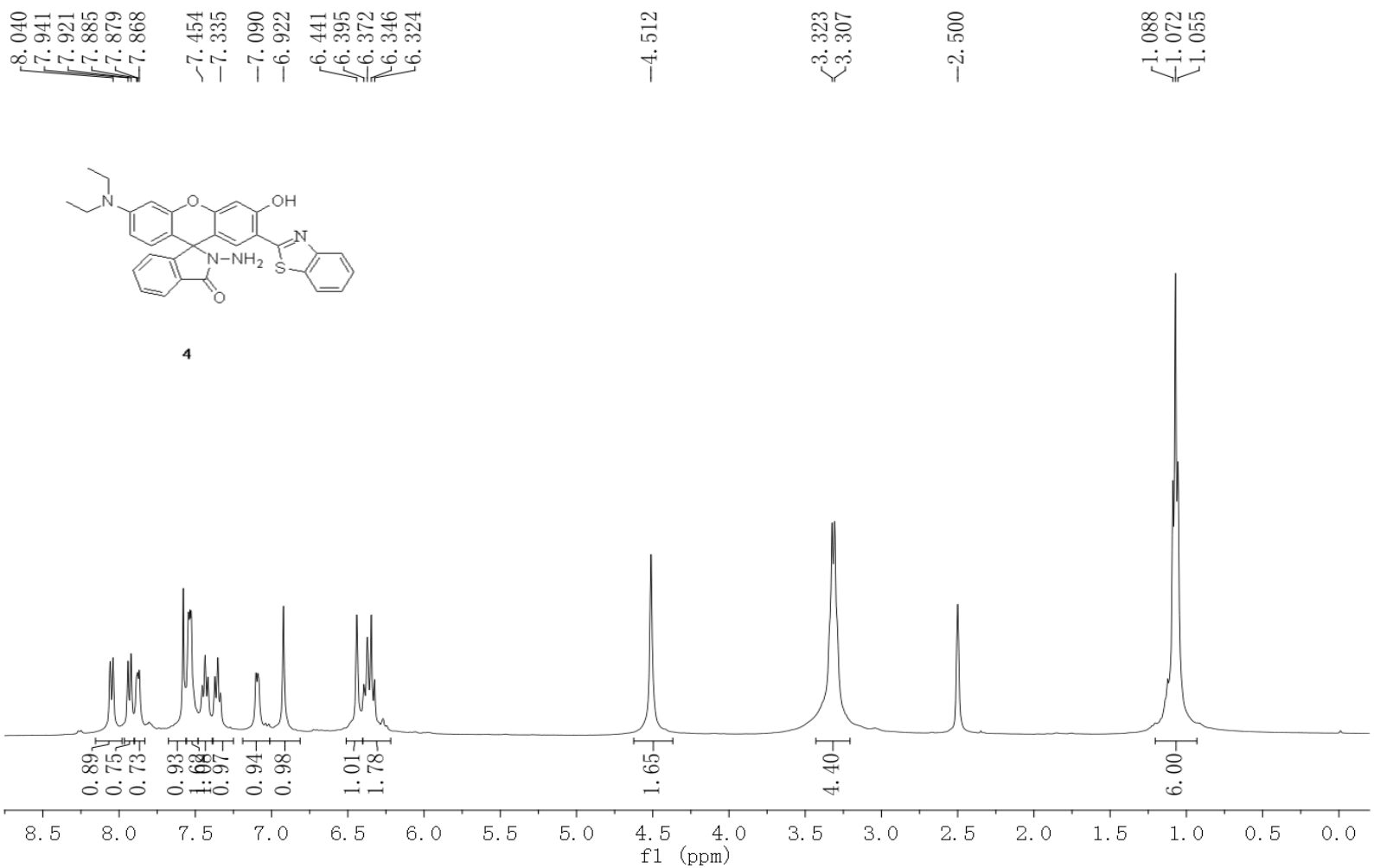


Figure S13. ^{1}H NMR spectrum of **4** in DMSO-d_6.

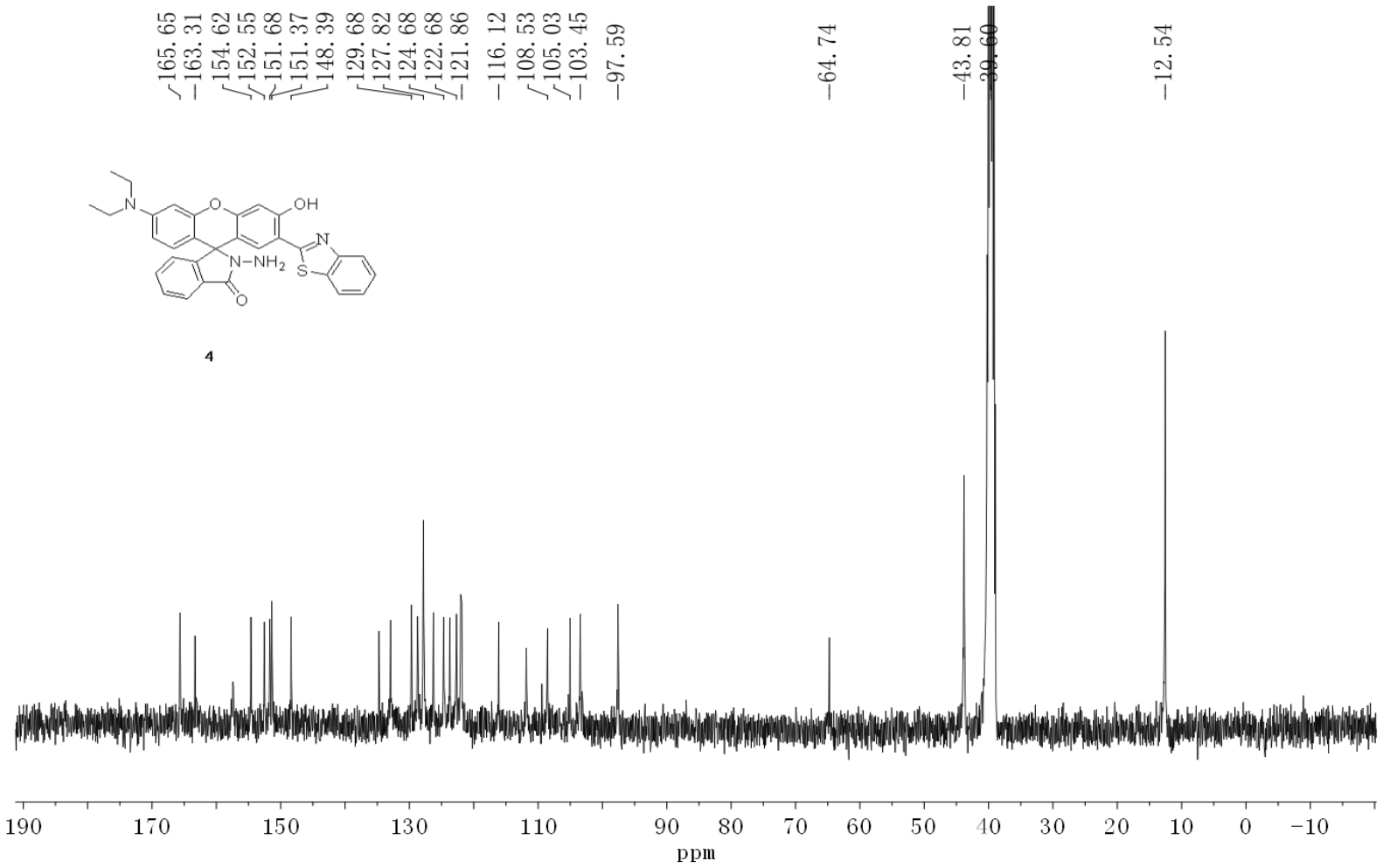


Figure S14. ^{13}C NMR spectrum of **4** in DMSO-d_6.

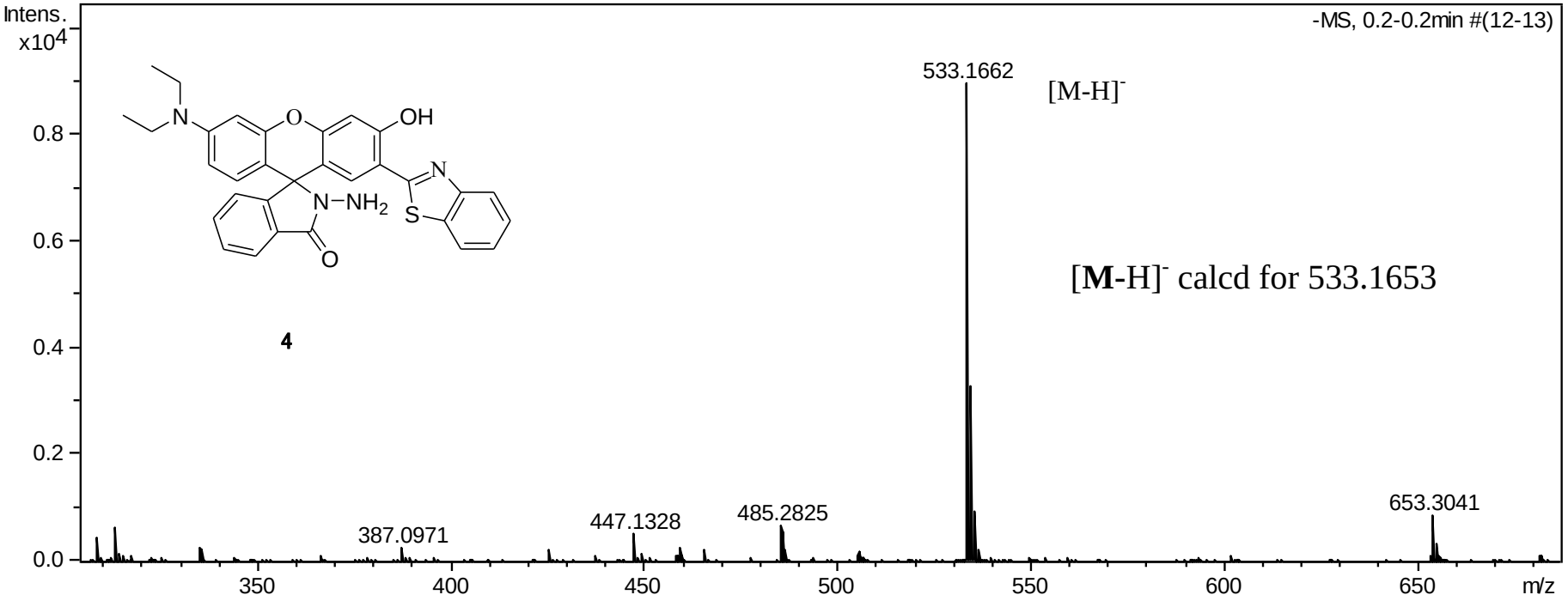


Figure S15. HRMS of **4**.