Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Coumarin-DPA-Cu(II) as A Chemosensing Ensemble toward Histidine Determination in Urine and Serum

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Experimental Section

General remarks for experimental

^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA Jobin Yvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies.

Fluorescence analysis.

1. Cu^{2+} titration: Fluorescence emission spectra were obtained with a Xenon lamp and 1.0 cm quartz cells. The probe **HS-1** (15 μL, 1 mM, DMSO) was added to a quartz cell containing 3.0 mL HEPES (20 mM, pH 7.4). Then appropriate aliquots of $Cu(NO_3)_2$ (0.5 mM) were added to the mixture and the fluorescence was measured. The excitation and emission slits were set to 2.0 and 2.0 nm, respectively.

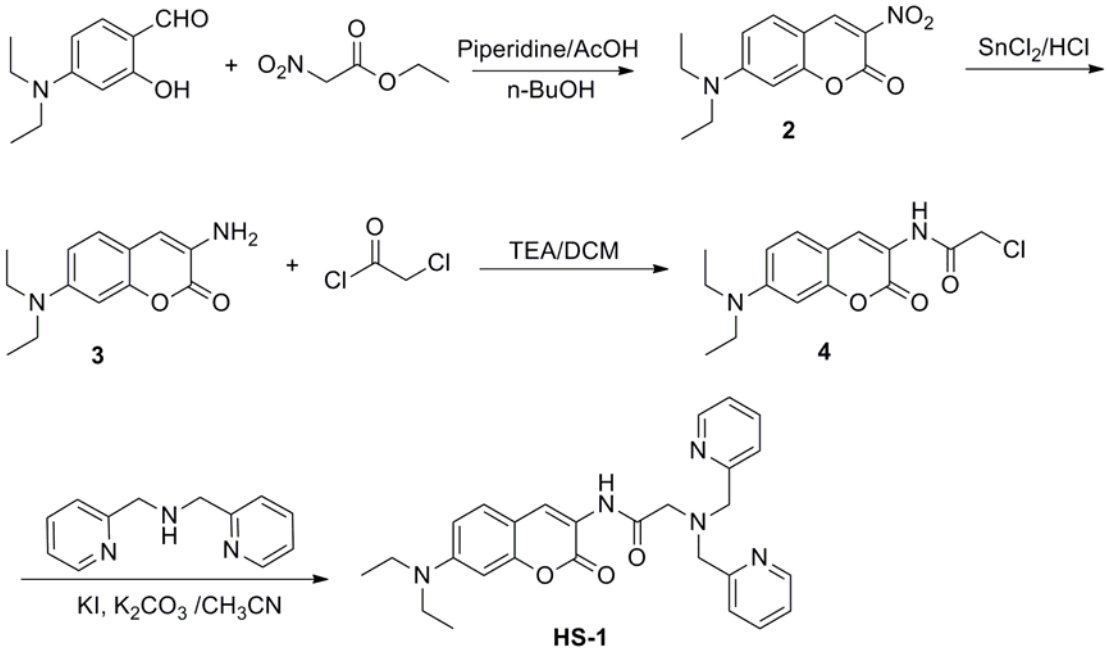
2. His titration: The probe **HS-1** (250 μL, 1 mM, MeCN) and the $Cu(NO_3)_2$ (500 μL, 0.5 mM) were added to a 50.0 mL volumetric flask before dilution with HEPES (20 mM, pH 7.4). The mixture was equilibrated for minutes and 3.0 mL of the mixture was transferred to a quartz cell. Then appropriate aliquots of histidine (6 mM) were added to the mixture and the fluorescence was measured. The excitation and emission slits were set to 2.0 and 2.0 nm, respectively.

3. Determination of histidine concentration in urine. Human urine samples were collected from healthy adult. 1 mL of the urine sample was transferred to 5.0 mL volumetric flasks, then His was added as internal standard (spiked His concentrations in volumetric flasks: 0, 120, 240 μM) following by diluting to scale with HEPES. Then 100 μL of the spiked urine was transferred into the quartz cell with 3.0 mL of HEPES containing **HS-1**-Cu^{2+} (5 μM) (final His concentrations tested: 0, X, X+4, X+8 μM), and the fluorescence was measured. X was measured to be 3.4 and 6.6 μM, respectively. Considering that the urine was diluted 150 times totally before measured, the His concentrations in these two urine samples were 510 and 990 μM, respectively.

4.Fetal calf serum titration. The serum (1 mL) was first deproteinized by mixing with 4 mL methanol in a 10 mL centrifugal tube, shaking for minutes, and centrifuging at 8000 rpm for 15 min at 4 ^{o}C. Then 100, 200, 300, 500, 1000 μL of deproteinized fetal calf serums were transferred into a 5.0 mL volumetric flask following by diluting with HEPES containing **HS-1**-Cu^{2+} (5 μM). The fluorescence was measured after incubating for minutes.

5. Determination of histidine concentration in fetal calf serum. 100 μL of the deproteinized fetal calf serums were transferred into clean tubes and 400 μL of HEPES were added for dilution. Then His was added as internal standard (spiked His concentrations in volumetric flasks: 0, 120, 240 μM). Then 100 μL of the spiked serum was transferred into the quartz cell with 3.0 mL of HEPES containing **HS-1**-Cu^{2+} (5 μM) (final His concentrations tested: 0, X, X+4, X+8 μM), and the fluorescence was measured. X was measured to be 3.3 μM, resulting in an original His concentration in fetal calf serum of 495 μM.

Synthesis of various compounds



Scheme S1 The preparation of **HS-1**

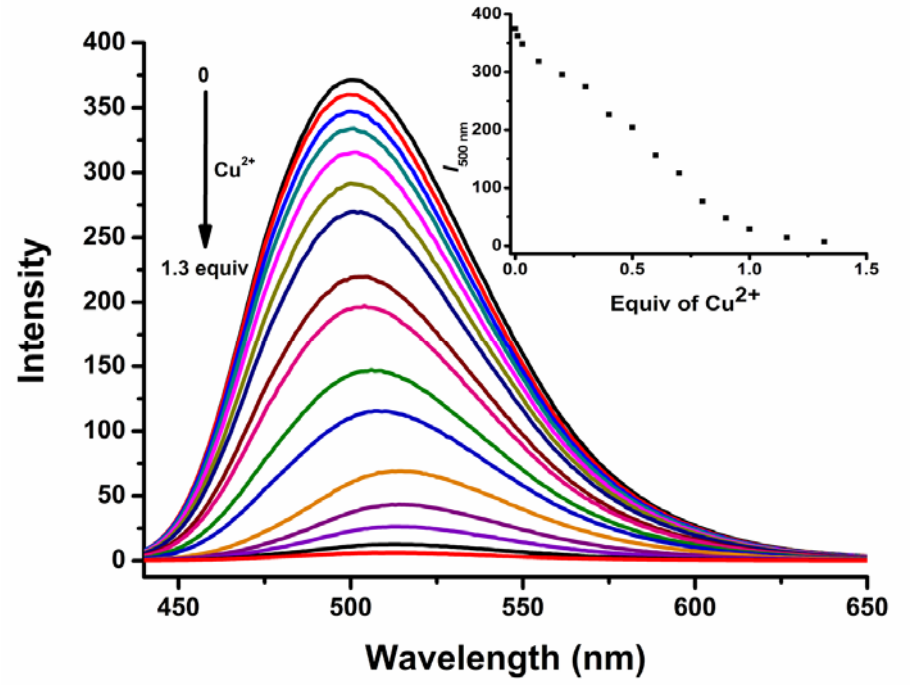


Figure S1 The titration profile of **HS-1** (5 μM) toward Cu^{2+} in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent) (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

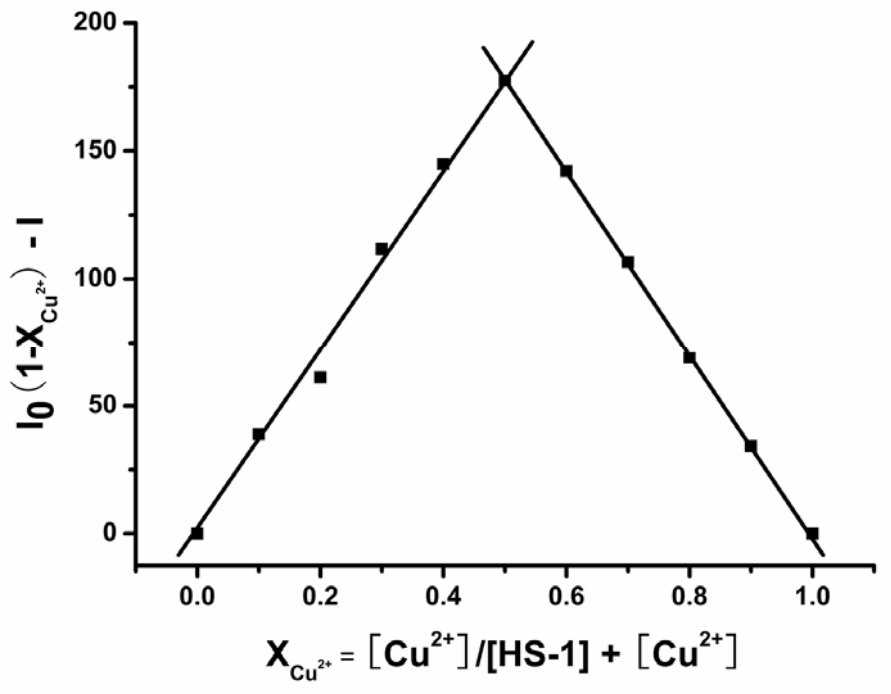


Figure S2: The job plot of a 1:1 complex of **HS-1** with Cu^{2+}.

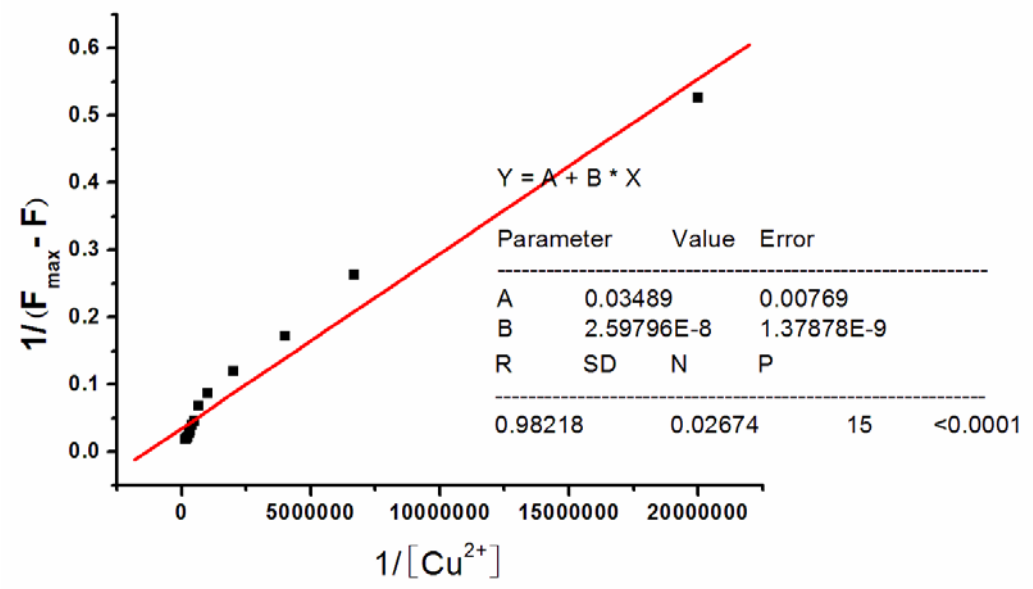


Figure S3. The binding constant of **HS-1**-Cu^{2+} complex was calculated using B-H expression.

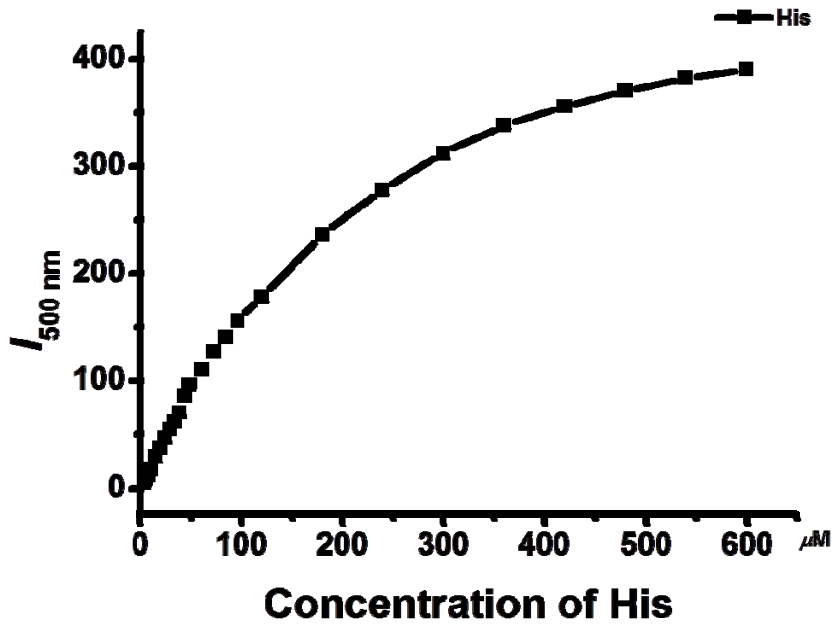


Figure S4. Fluorescence intensity changes at 500 nm of **HS-1**-Cu^{2+} upon addition of His in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent) (λ_{ex} =410 nm).

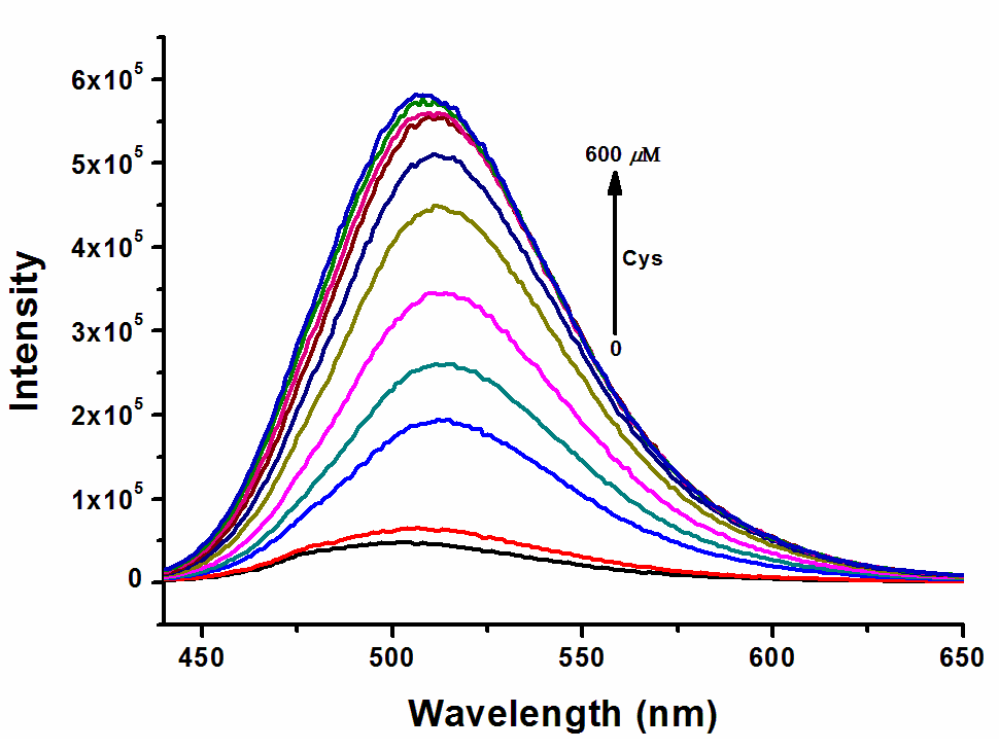


Figure S5. The titration profile of **HS-1**+Cu^{2+} (5 μM) toward Cys in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

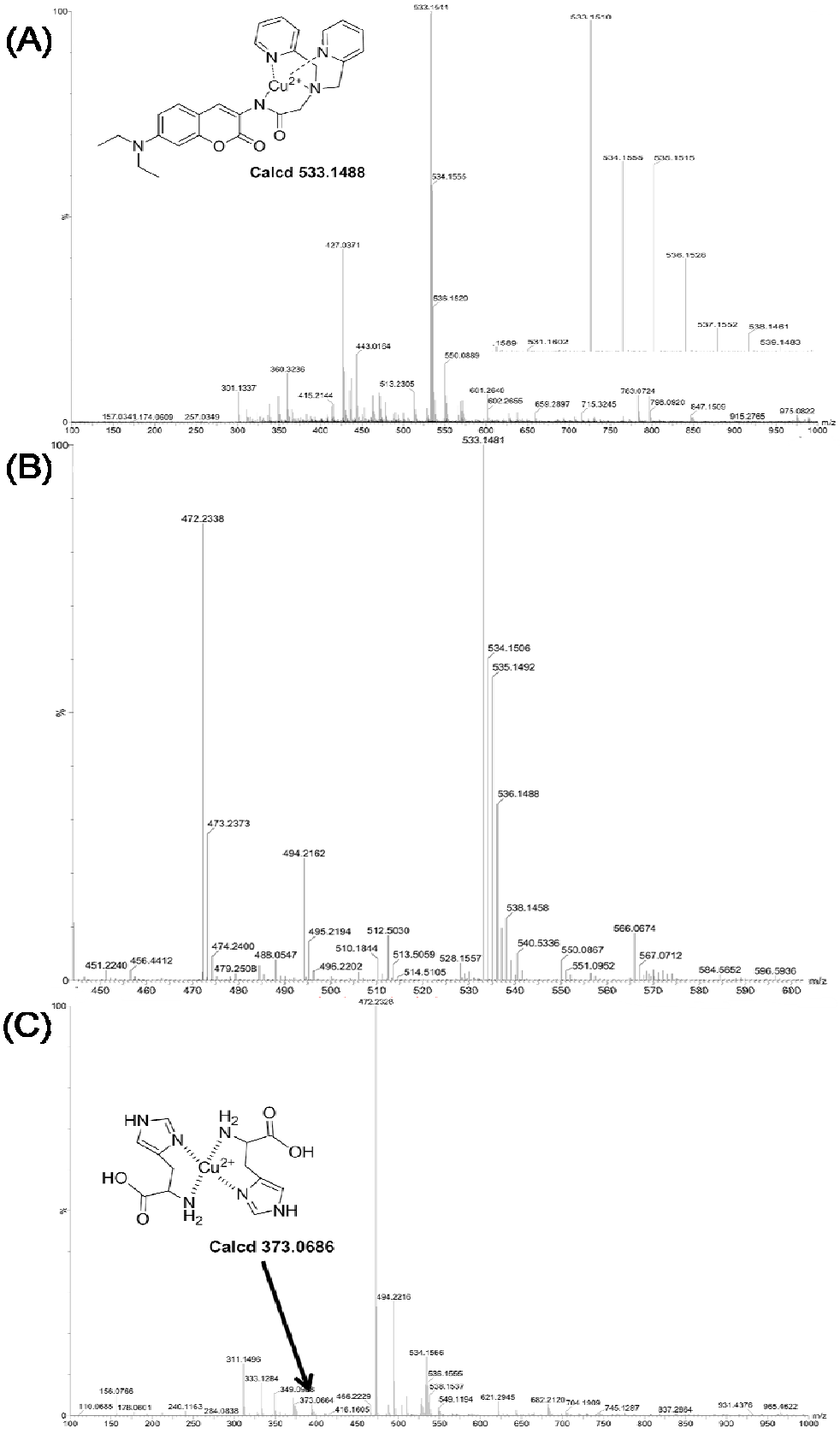


Figure S6. ESI spectra of (A) **HS-1**-Cu^{2+}, (B) upon addition of 4 equiv His and (C) upon addition of 40 equiv His.

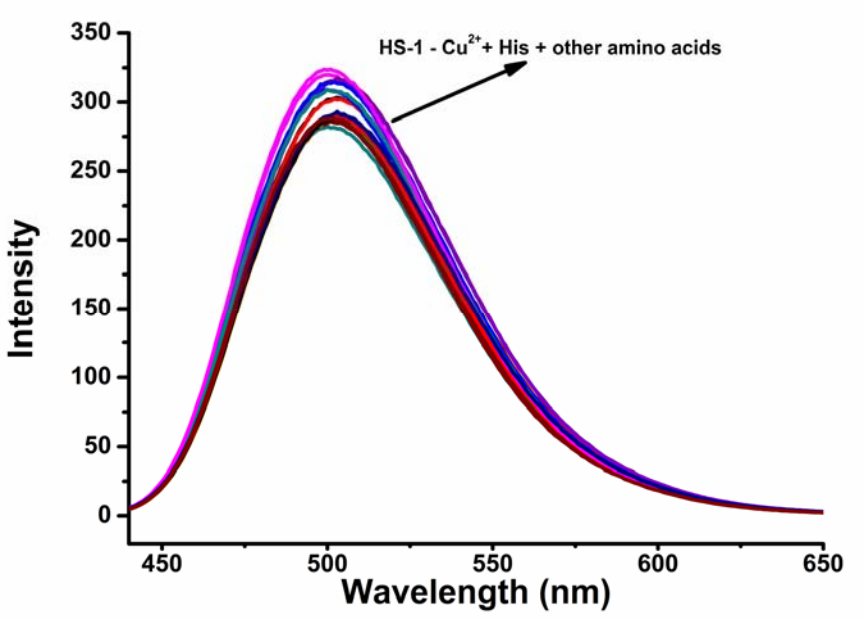


Figure S7. Fluorescence response of **HS-1**+Cu^{2+} (5 μM) toward amino acid (100 equiv) in the presence of His (50 equiv) in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent) (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

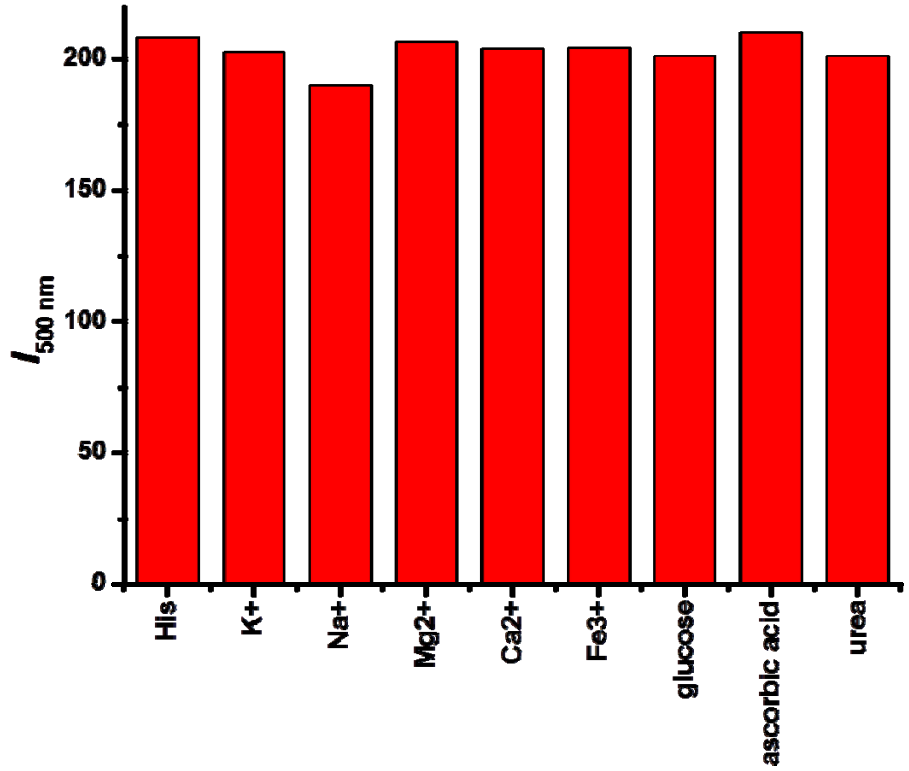


Figure S8 Fluorescence response of **HS-1**+Cu^{2+} (5 μM) toward common biological species in the presence of His (250 μM) in HEPES (20 mM, pH = 7.4, containing 0.5% DMSO as cosolvent). K^+ 4mM, Na^+ 4 mM, Mg^{2+} 400 μM, Ca^{2+} 400 μM, Fe^{3+} 20 μM, glucose 240 μM, ascorbic acid 500 μM, urea 500 μM. (λ_{ex} = 410 nm, slits: 2 nm/2 nm).

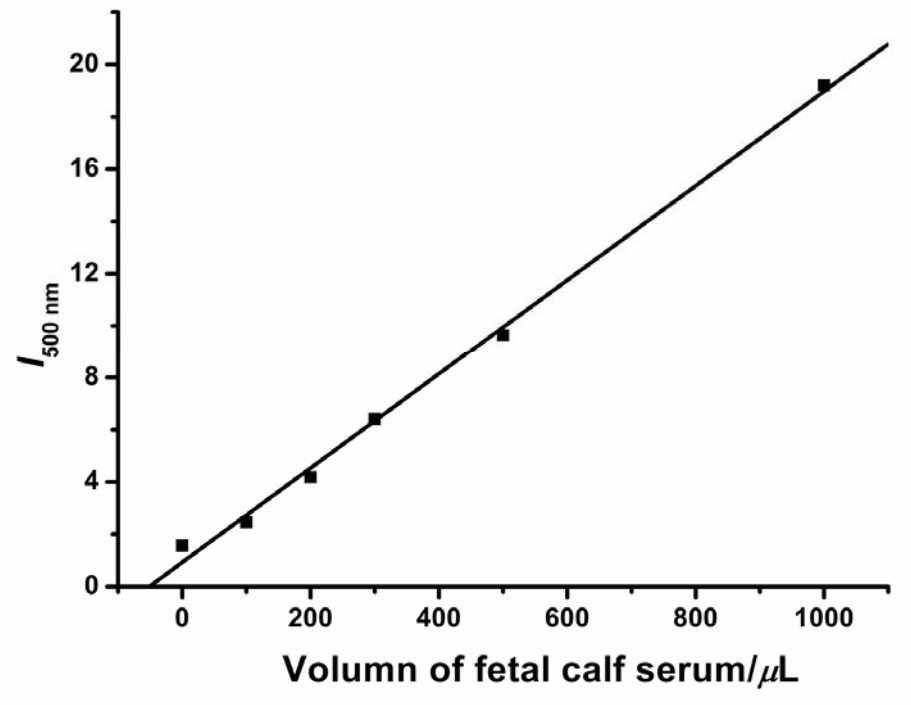
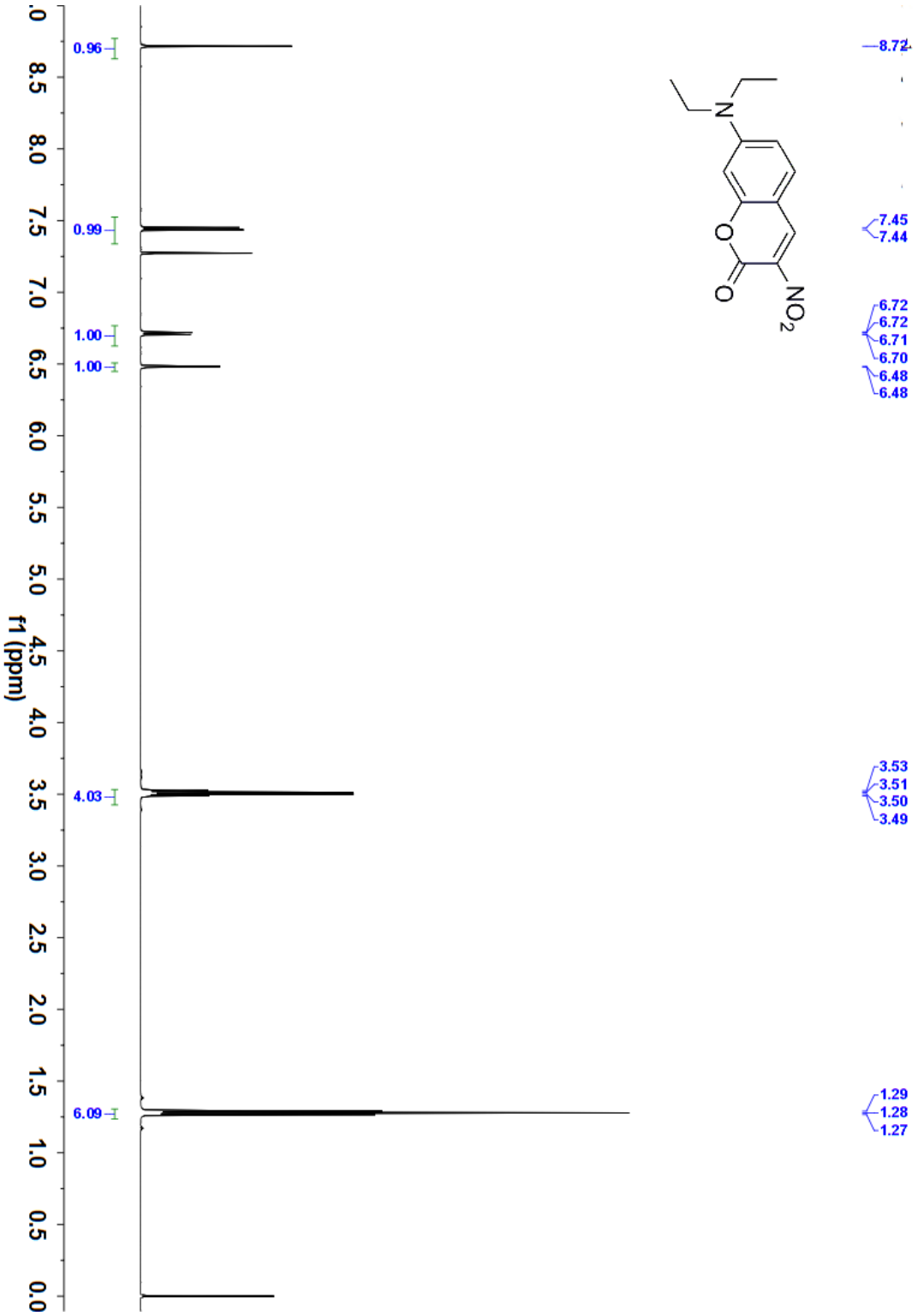
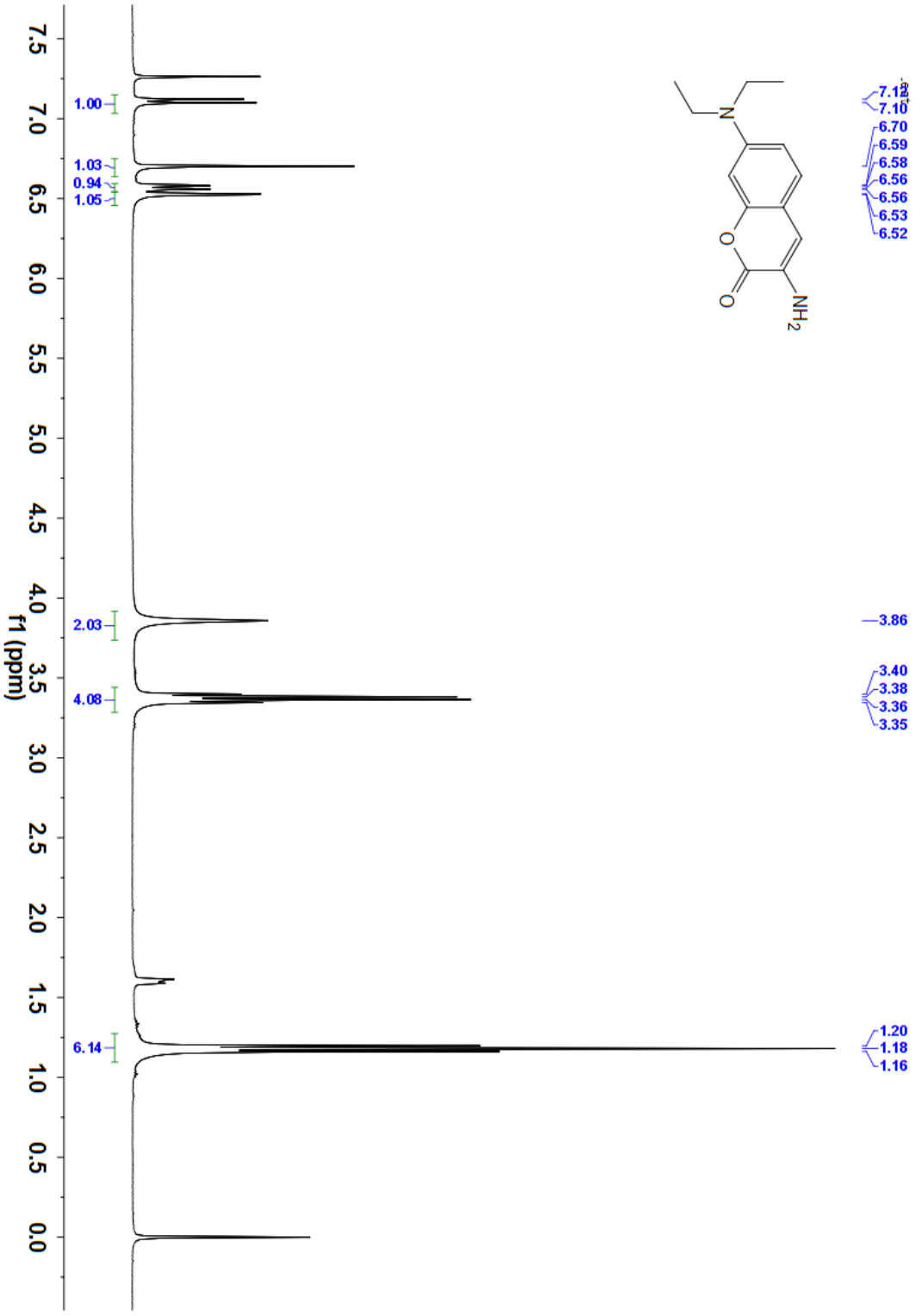


Figure S9 Fluorescence intensity of **HS-1**-Cu^{2+} (5 μM) at 500 nm upon addition of fetal calf serum (0, 100 μL, 200 μL, 300 μL, 500 μL, 1000 μL,).

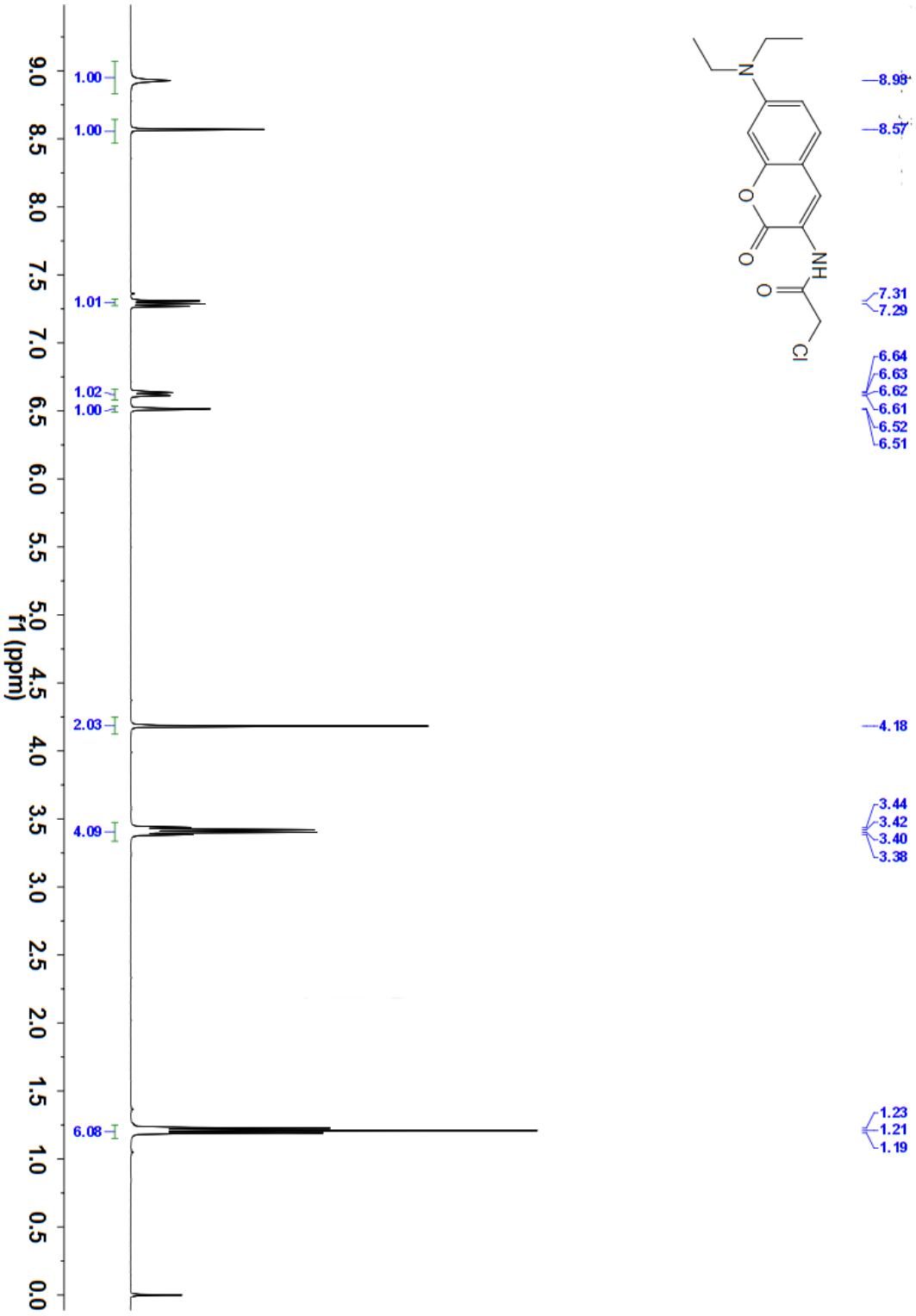
^{1}H-NMR Spectrum of **2** in $CDCl_3$ (600 MHz):



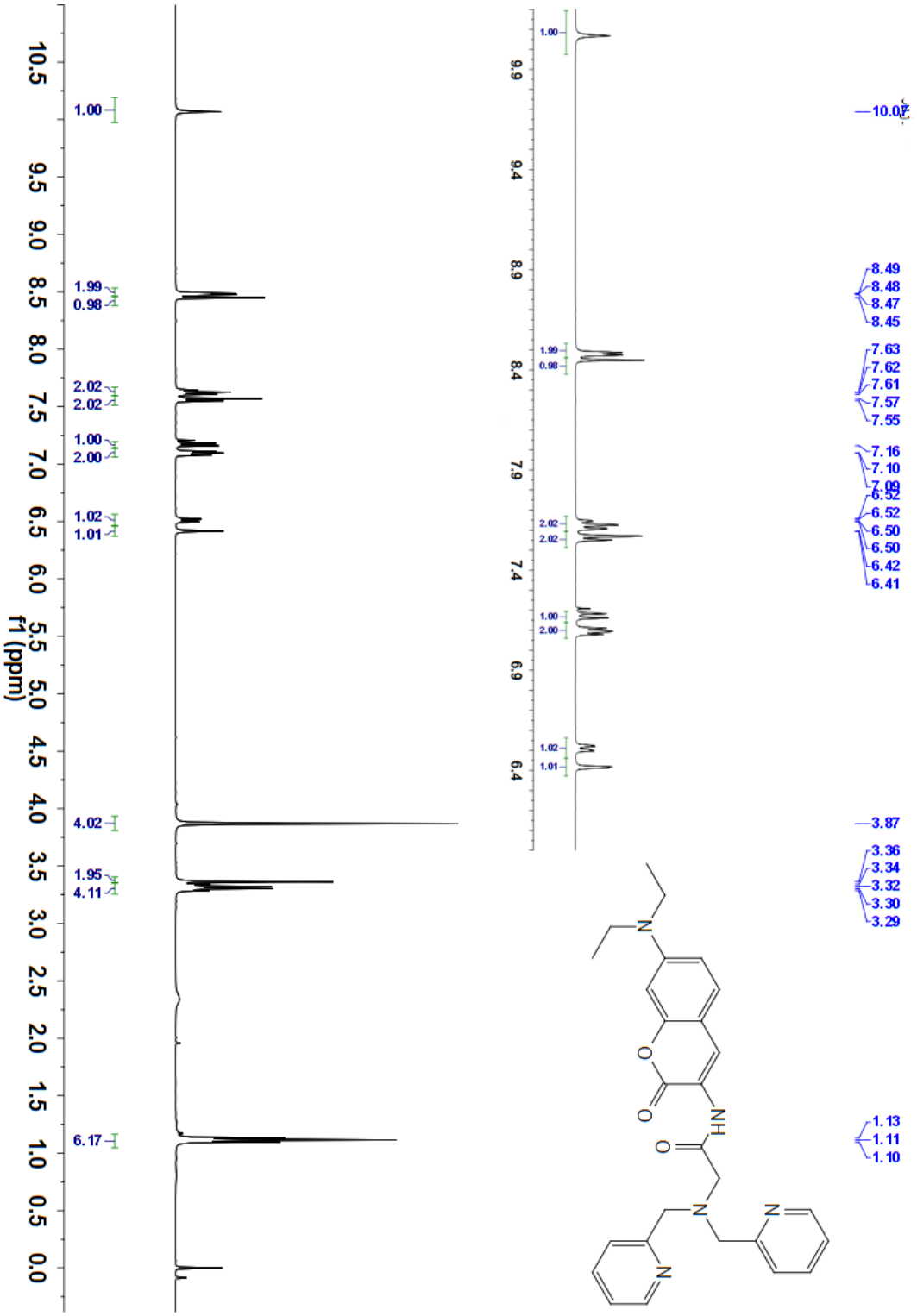
^{1}H-NMR Spectrum of **3** in $CDCl_3$ (400 MHz):



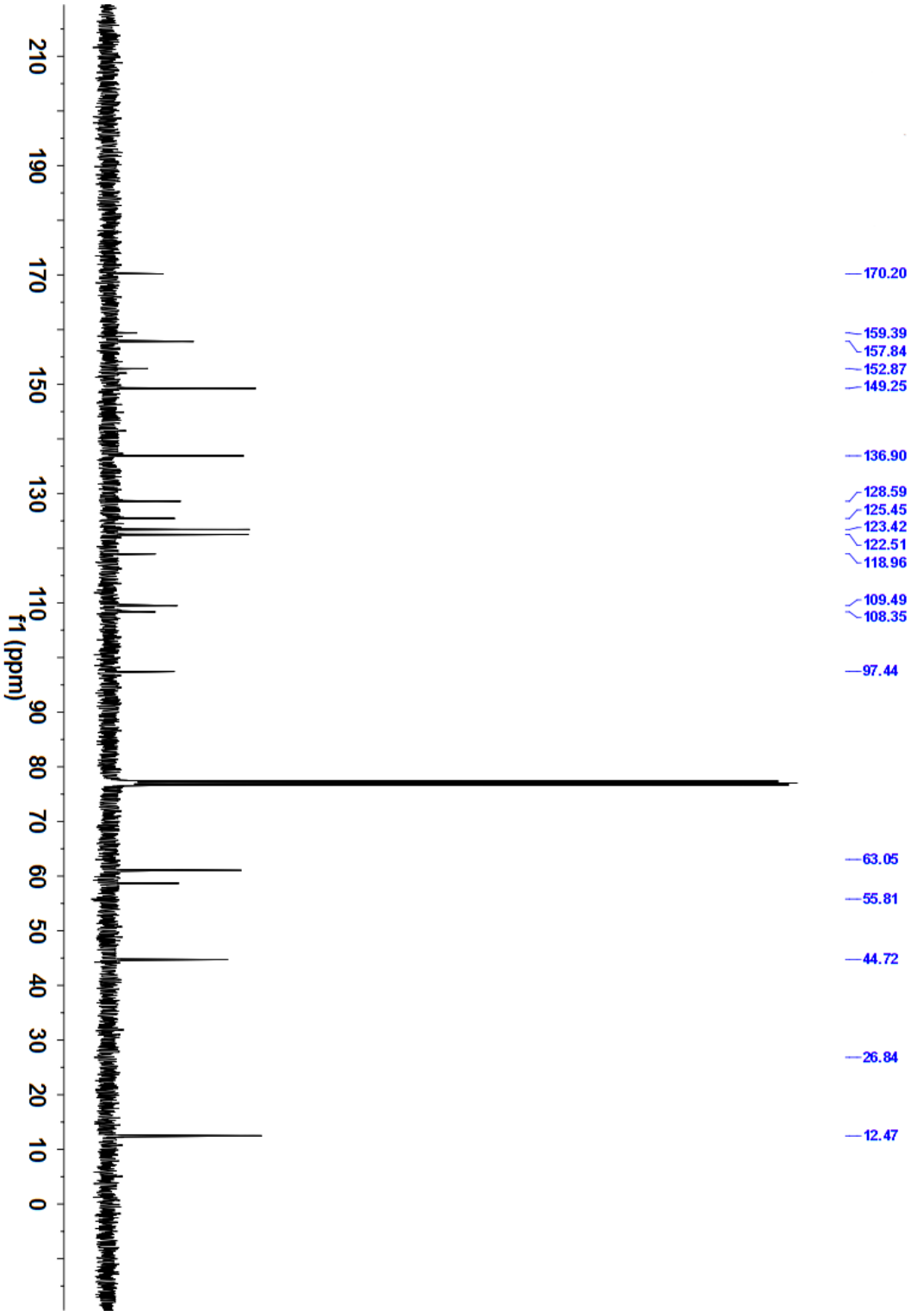
^{1}H-NMR Spectrum of **4** in $CDCl_3$ (400 MHz):



^{1}H-NMR Spectrum of **HS-1** in $CDCl_3$ (400 MHz):



^{13}C-NMR Spectrum of **HS-1** in $CDCl_3$ (100 MHz):



HRMS spectra of **HS-1**