Supplementary Material (ESI) for Dalton Transactions

Electronic Supporting Information

for

A Rhodamine-based Chemosensor for Cu^{2+} and Its Application in Bioimaging

Liang Huang[a], Xiao Wang[b], GuoQiang Xie[a], PinXian Xi[a], ZhengPeng Li[a], Min Xu[a], YongJie Wu[b], DeCheng Bai[b], ZhengZhi Zeng*[a]

[a]*State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, 730000 Lanzhou, P. R. China*
[b]*Pharmacology Lab of GanSu Province Key Laboratory of Preclinical Study for New Traditional Chinese Medicine, Lanzhou University, 730000 Lanzhou, P. R. China.*
Email: zengzhzh@yahoo.com.cn

Table of contents

1. Instruments, reagents and experimental procedures

^{1}H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with TMS as an internal standard and $CDCl_3$ as solvent. Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. HRMS were determined on a Bruker Daltonics APEXII 47e FT-ICR spectrometer.

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Methanol for spectra detection was HPLC reagent without fluorescent impurity.

Procedures of metal ion sensing

Stock solutions of the metal ions (2.5 mM) were prepared in deionized water. A stock solution of **L1**(1 mM) was prepared in DMF: CH_3CN (1:1 v/v). The solution of **L1** was then diluted to 20 μM with water/ CH_3CN (1:1 v/v). In titration experiments, each time a 2 mL solution of **1** (20μM) was filled in a quartz optical cell of 1 cm optical path length, and the Cu^{2+} stock solution was added into the quartz optical cell gradually by using a micro-pippet. Spectral data were recorded at 2 min after the addition. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2 mL solution of **L1** (20μM). For fluorescence measurements, excitation was provided at 495nm, and emission was collected from 508 to 650 nm.

Cell Culture

The EJ cell line was provided by Institute of Biochemistry and Cell Biology (China). Cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10 % FBS (Fetal Bovine Serum) in an atmosphere of 5 % CO_2, 95 % air at 37°C. Cells (5×10^8/L) were plated on 18 mm glass coverslips and allowed to adhere for 24 hours. Experiments to asses Cu^{2+} uptake were performed in the same media supplemented with 40 μM $CuCl_2$ for 0.5h.

Fluorescence Imaging

Fluorescent pictures were taken on Zeiss Leica inverted epifluorescence /reflectance laser scanning confocal microscope. Excitation of 1-loaded cells at 515 nm was carried out with a HeNe laser. Emission was collected using a 560 nm long-pass filter. Emission was collected from 570 to 625 nm. Before the experiments, cells were washed with PBS buffer and then incubated with 20 μM **L1** in DMF-PBS (1:49, v/v) for 5h at 37 °C. Cell imaging was then carried out after washing cells with PBS.

Binding Constant

The binding constant was calculated from the emission intensity - titration curves according to the equation

$I_F^0/ (I_F-I_F^0) = (1/f) [(1/K_S[M])+1]$,

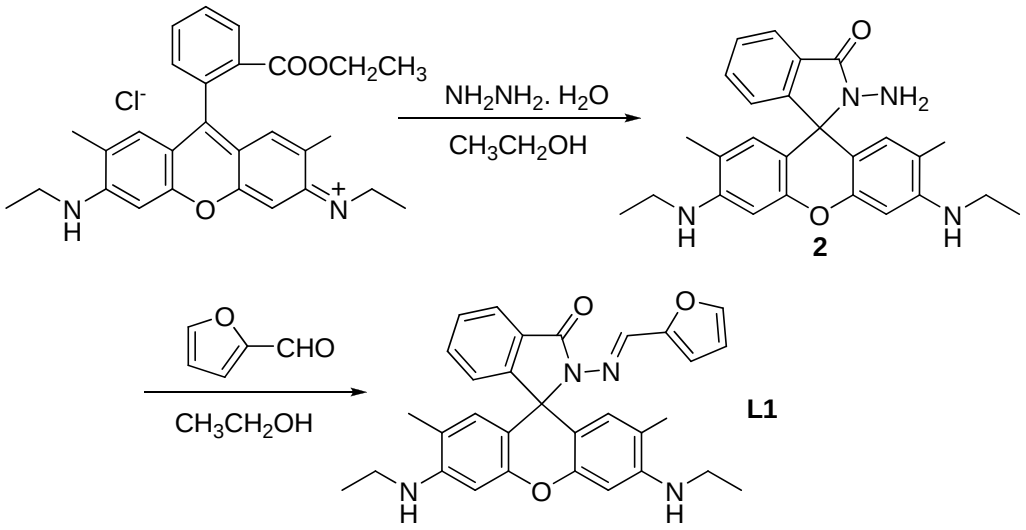
where I_F^0 is the emission intensity of **L1** at 552 nm, I_F is the emission intensity of **L1** at 552 nm upon the addition of different amount of Cu^{2+}, f is the fraction of the initial fluorescence which is accessible to the sensor, [M] is the concentration of Cu^{2+}. The association constant values K_S is given by the ratio intercept / slope.

The binding constant was also calculated from the absorption intensity - titration curves according to the equation

$A = A_0 + ((A_{lim} - A_0)/2C_0)(C_0 + [M] + 1/K - ((C_0 + [M] + 1/K)^2 - 4C0[M])^{0.5})$,

where A and A_0 are the absorbance for **L1** (at 529 nm) in the presence and absence of Cu^{2+}; C_0 is half of the concentration of **L1**; [M] is the concentration of the Cu^{2+}; and A_{lim} is the limiting value of the absorbance in the presence of excess Cu^{2+}.

2. Synthesis routes and characteristic data



Rhodamine 6G hydrazone **2** is prepared according to the literature method.[1]

Rhodamine 6G hydrozide (1.0 mmol, 0.428 g) and furan-2-carbaldehyde (1.0 mmol, 0.096 g) were mixed in boiling ethanol with 3 drops of acetic acid. After 4 h of stirring, brown precipitates obtained were filtered off, washed with ethanol/ether (1:1)

and dried over P_2O_5 under vacuum. Yield: 55 %. ^{1}H NMR (CDCl3, 400 MHz) δ (ppm): 8.06-8.027 (m, 2H), 7.434-7.455 (m, 2H), 7.362-7.365 (d, 1H *J* = 1.2 Hz), 7.261 (s, 2H), 6.366-6.393 (ss, 4H), 3.491 (b, 3H), 3.181-3.235 (q, 4H, *J*=7.2), 1.873 (s, 6H), 1.299-1.334 (t, 6H, *J* = 6.8 Hz). ^{13}C NMR (CDCl3, 100 MHz) δ (ppm):165.14, 150.98, 150.47, 147.60, 143.77, 135.31, 133.49, 128.16, 127.49, 123.50, 123.45, 118.51, 112.04, 111.52, 105.84, 96.90, 65.47, 38.35, 16.66, 14.73. ESI-MS m/z = 507.2 $[M + H]^+$, calc. for C27H29N5O2S =506.23.

3. Supplementary spectra data

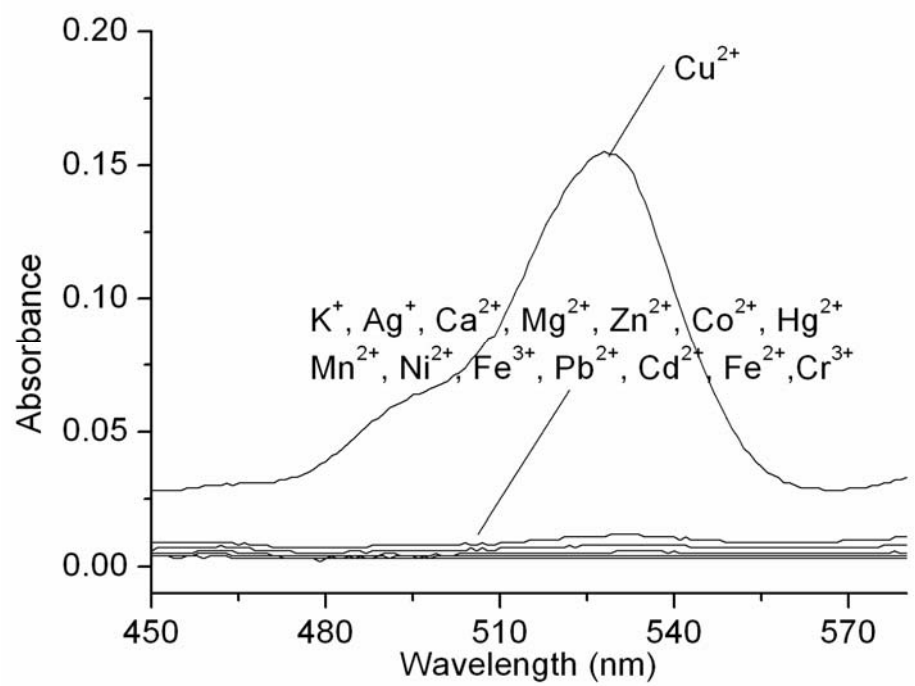
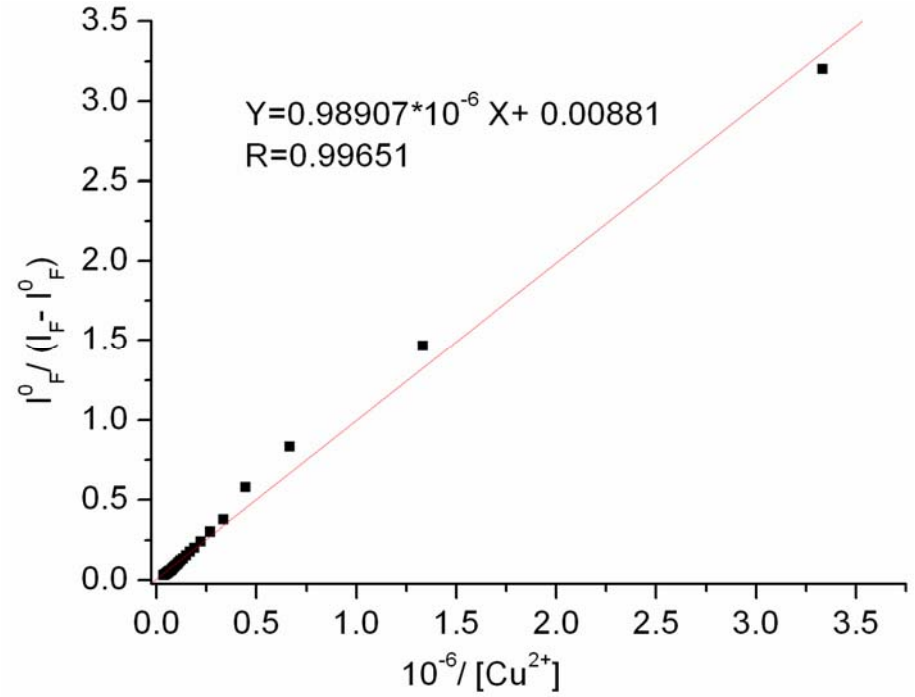


Fig. S1 Changes in the absorption spectra of **L1** (20 μM) in the presence of different metal ions (40 μM) in water/ CH_3CN (1:1 v/ v).

Fig.S2 (a) Photos of color changes of **L1** (20 μM) upon addition of 40 μM different metal ions in water/ CH_3CN (1:1 v/v) solutions.



Figu. S3 Fitting of Fluorescence titration curve of **L1** in water/ CH_3CN (1:1 v/v) solution.

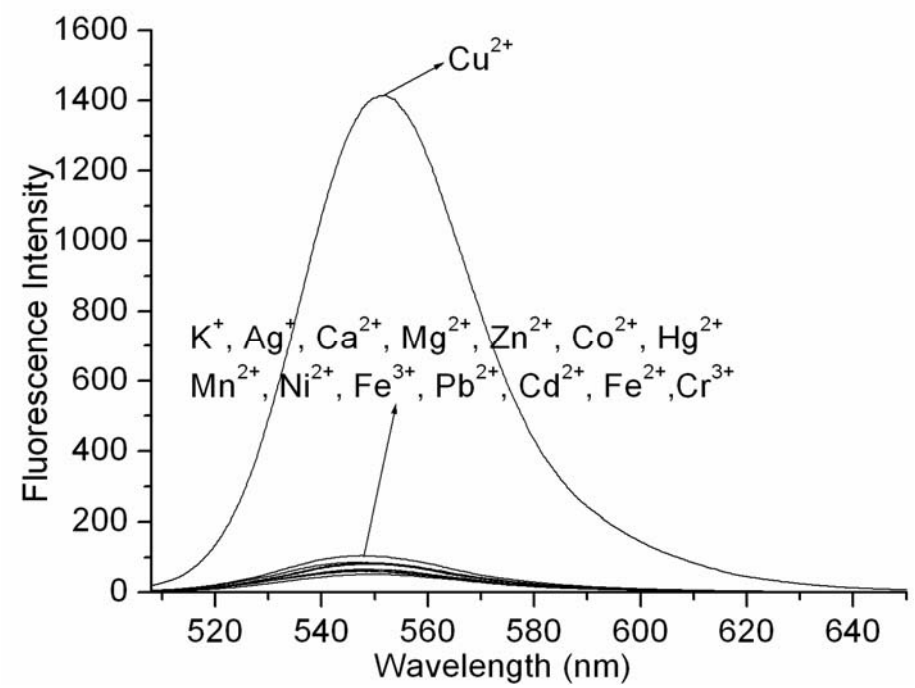


Fig. S4 Fluorescence spectra of **L1** (20μM) upon the addition of various metal ions (20μm for Cu^{2+}; 40μm for all other cations) in water/ CH_3CN (1:1 v/v) solution.

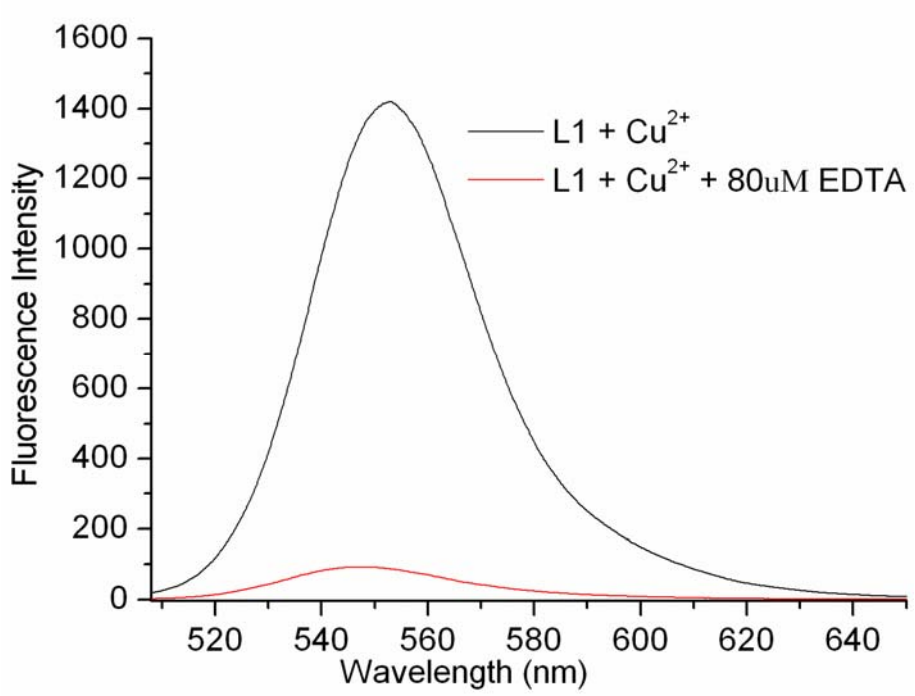
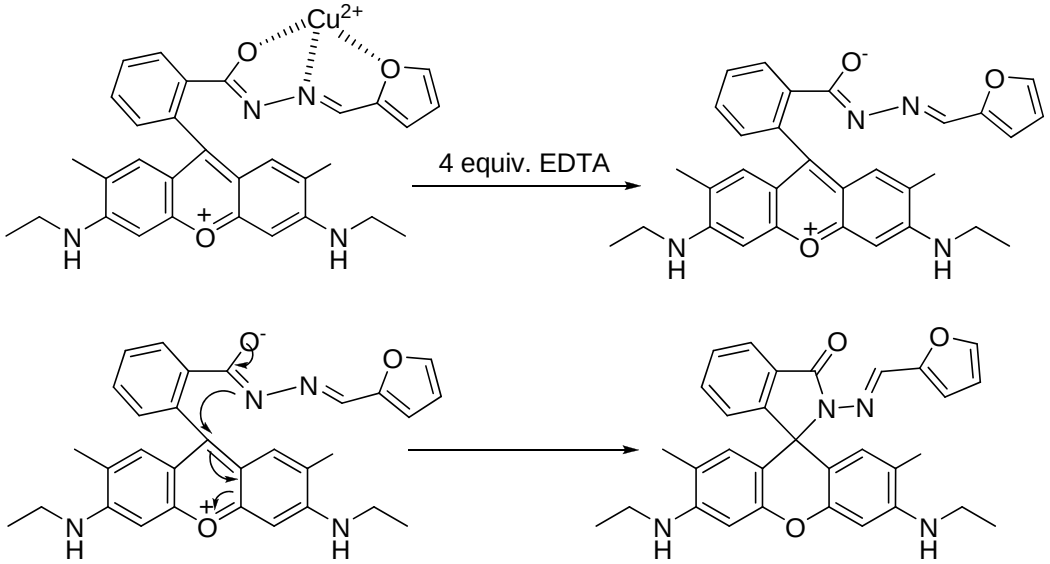


Fig. S5. Fluorescence spectra of 20 μM **L1** and 30 μM Cu^{2+} upon the addition of 80 μM EDTA in water/ CH_3CN (1:1 v/v) solution (λ_{ex}= 495 nm).

Reversible binding nature of Cu^{2+} with **L1**:



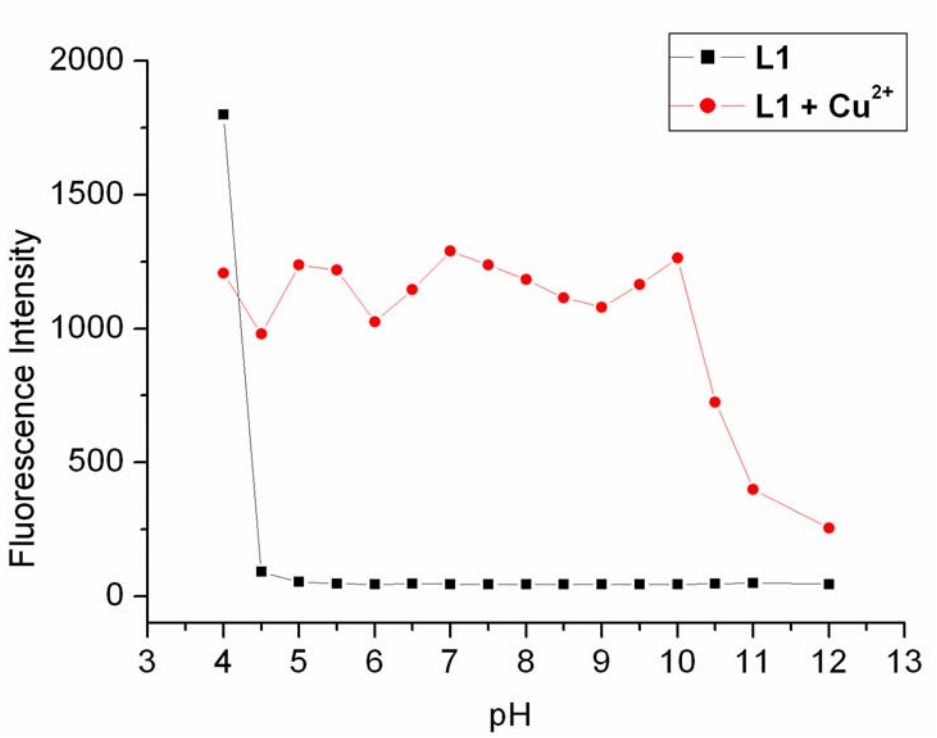


Fig. S6 Fluorescence spectra of **1** (20 μM) with the addition of Cu^{2+} (30 μM) in water/ CH_3CN (1:1 v/v) at various pH values (Ex. 495 nm; Em. 552 nm).

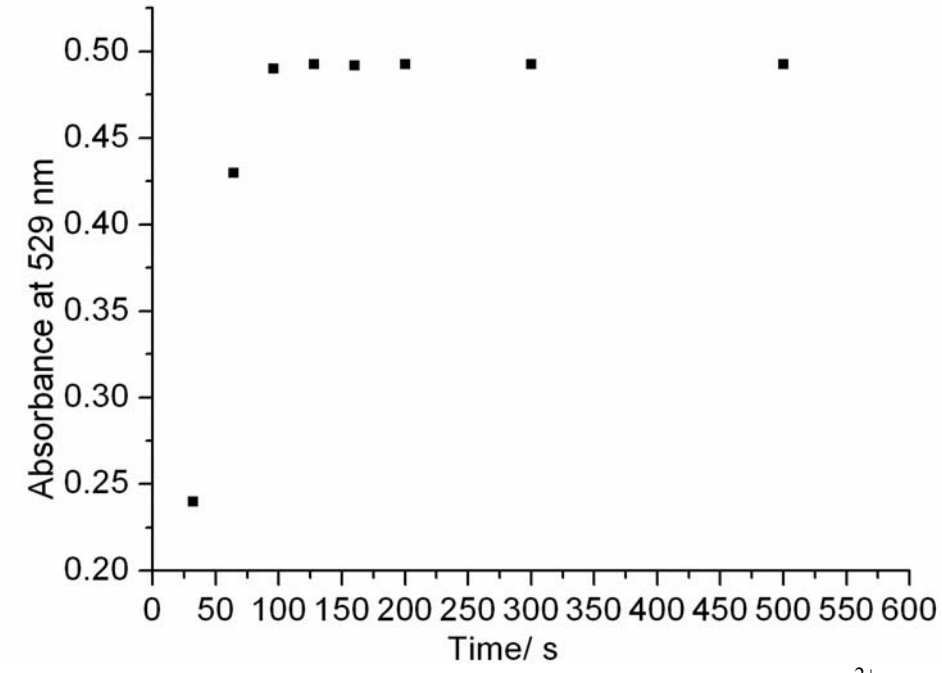


Fig. S7 Time course of the response of **L1** (20 μM) to 5 equiv Cu^{2+} in water/ CH_3CN (1:1 v/v) solution.

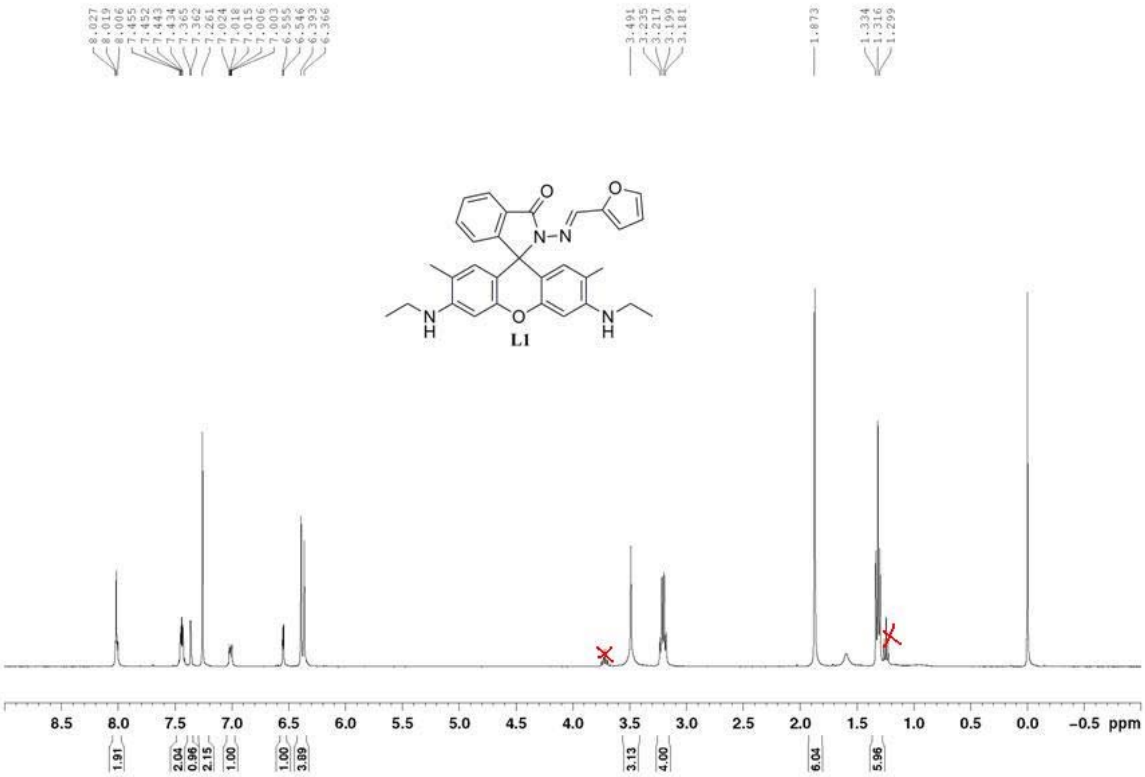


Fig.S8 ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of **L1**.

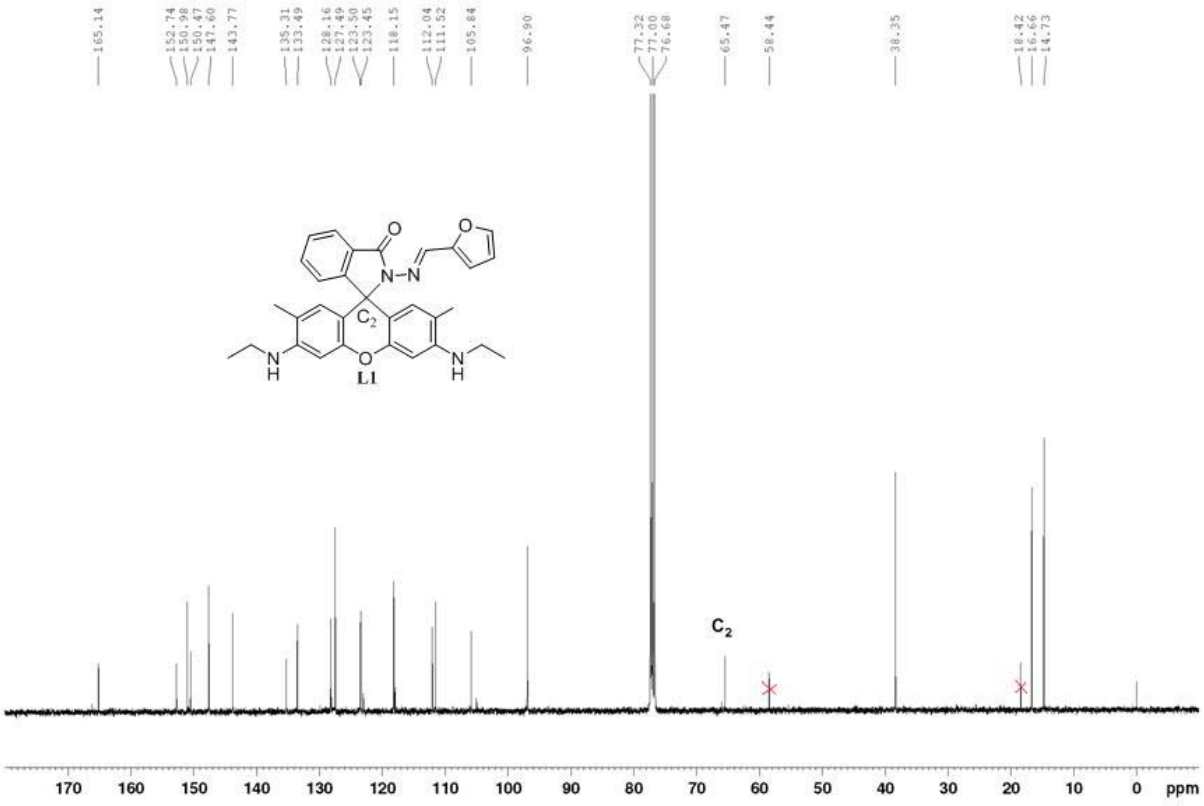


Fig. S9 ^{13}C NMR ($CDCl_3$, 100MHz) spectrum of **L1**.

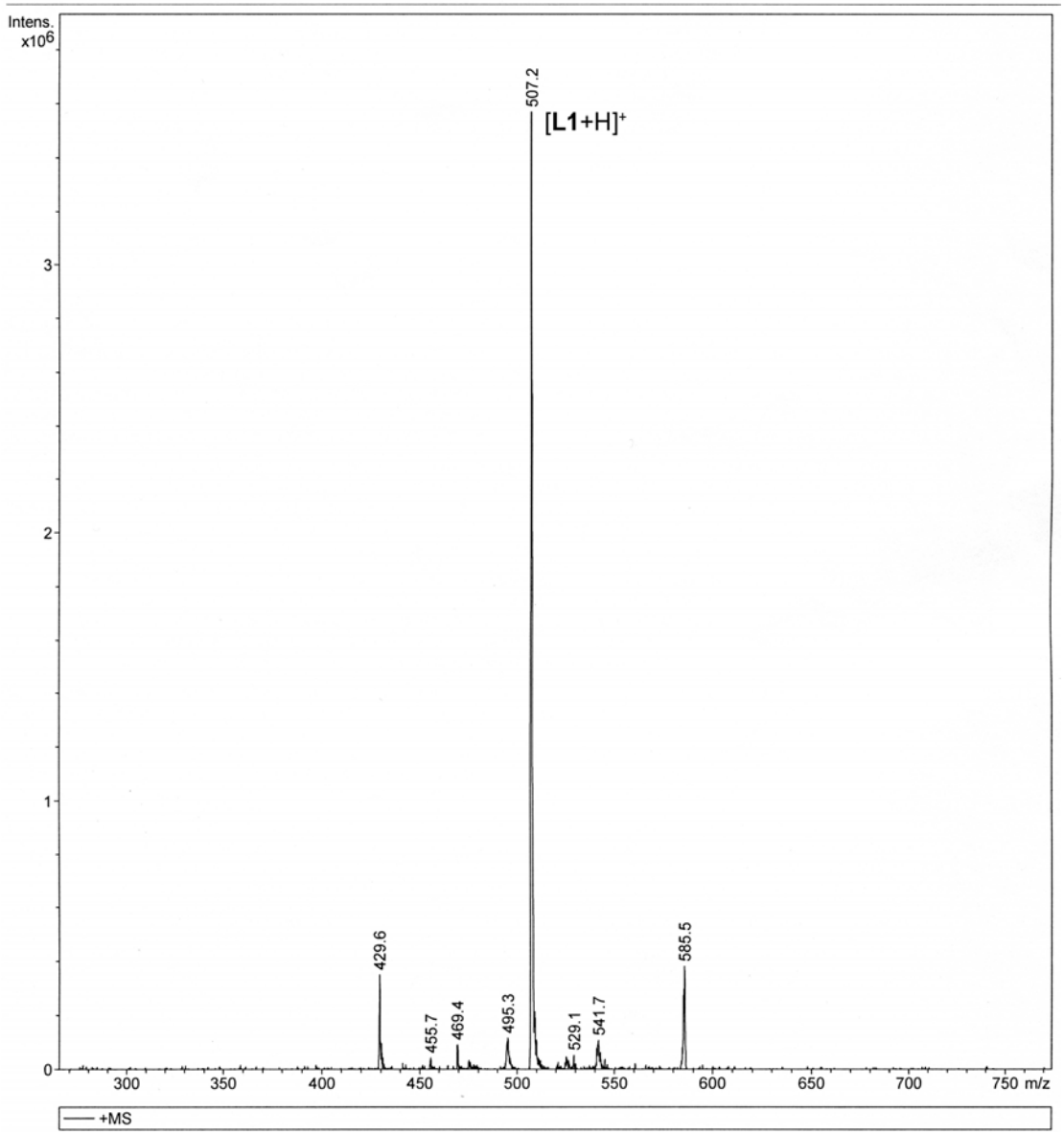


Fig.S10 ESI mass spectrum of **L1.**

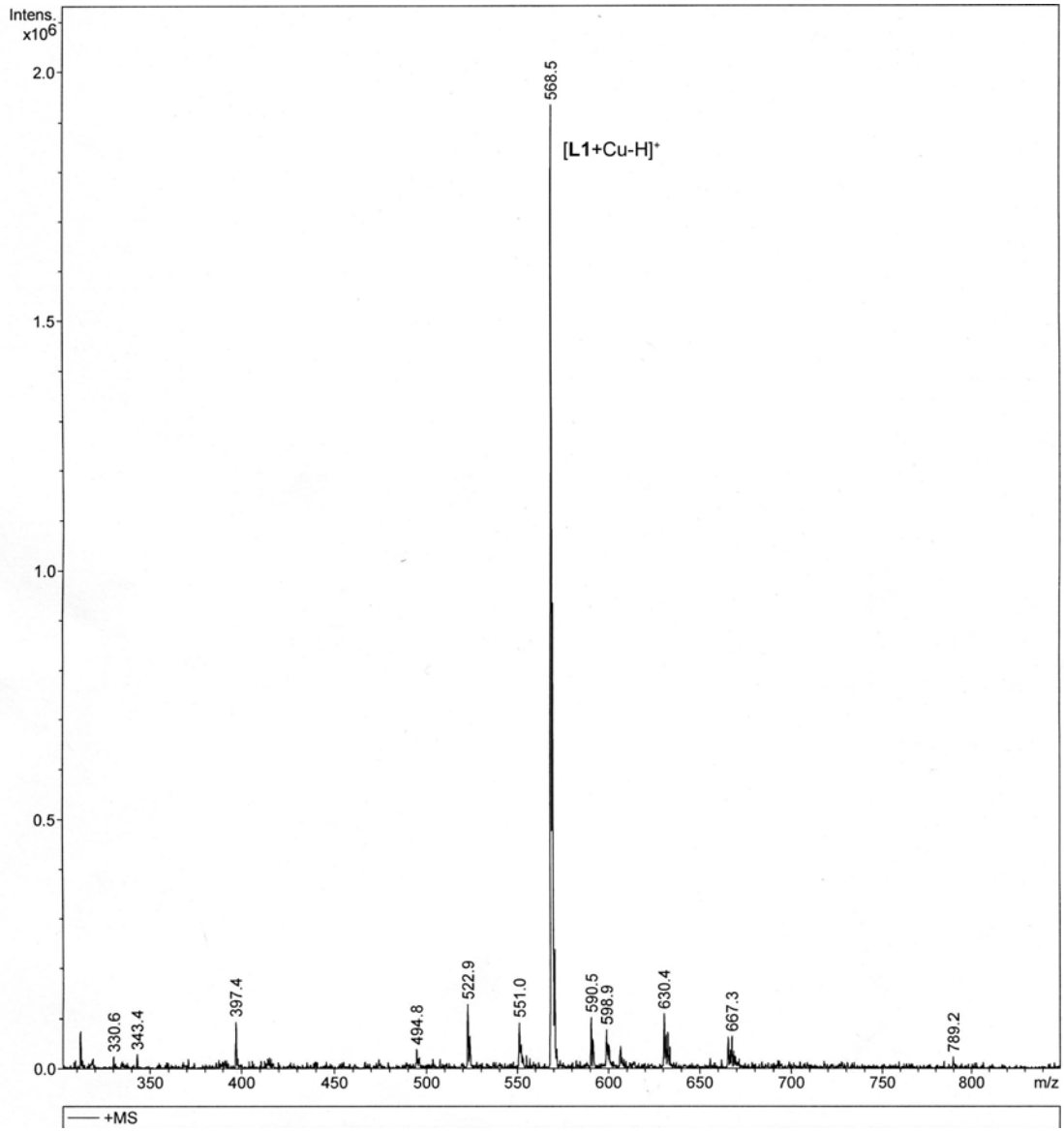


Fig.S11 ESI mass spectrum of 20 μM **L1** in the presence of 1.2 equiv of Cu^{2+} in water/ CH_3CN (1:1 v/v) solution, indicating the formation of [**L1** +Cu^{2+}-H].