Supporting Information for

Recognition of Copper and Hydrogen Sulfide in vitro Utilizing a Fluorescein Derivatives Indicator

Fengping Hou [a], Ju Cheng [b], Pinxian Xi [a], Fengjuan Chen [a], Liang Huang [a], Gouqiang Xie [a], Yanjun Shi [a], Hongyan Liu [a], Decheng Bai [b,*] and Zhengzhi Zeng [a,*]

[a] Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry and College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, PR China

[b] School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, PR China.

1. Abbreviations

ROS, Reactive oxygen species; RNS, Reactive nitrogen species; FA, 2-(5-formyl-6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid; SA, L(+)-Ascorbic acid; Cys, L-Cysteine ; SAS, Sulfoacetic acid disodium salt; MAP, Mercaptoacetic acid; Met, L-Methionine.

2. Instruments, reagents and experimental procedures

Instruments and reagents. All the materials for synthesis were purchased from commercial suppliers and used without further purification. Acetonitrile for spectra detection was HPLC reagent without fluorescent impurity. $CuCl_2$ and NaHS were analytical pure. ^1H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with TMS as an internal standard and DMSO 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. ESI-MS spectra were determined on a Bruker Daltonics Esquire 6000 spectrometer.

UV - Vis and Fluorometric Analysis. Stock solutions of various cations (1.0×10^{-3} mol · L^{-1}) were prepared in acetonitrile, and onions were prepared in deionized water. A stock solution of **L1** (1×10^{-3} mol · L^{-1}) was prepared in DMF. The solution of **L1** was then diluted to 1×10^{-5} mol · L^{-1} with HEPES - CH_3CN (6: 4, v/v, pH = 7.0). In titration experiments, each time a 2×10^{-3} L solution of **L1** (1×10^{-5} mol · L^{-1}) was filled in a quartz optical cell of 1 cm optical path length, and the ions stock solution were added into the quartz optical cell gradually by using a micro-pippet. Spectral data were recorded at 1 min after addition of the ions. In selectivity experiments, the test samples were prepared by placing appropriate amounts of ions stock into 2 mL solution of **L1** (2×10^{-5} mol · L^{-1}). For fluorescence measurements, excitation was provided at 494 nm, and emission was collected from 500 to 750 nm, slit was 5, 5nm.

Cell Culture. The Hep G2 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 for 24 hours.

Fluorescence Microscopy Imaging. After Hep G2 cells were grown on Petri dishes at 37 °C and in 5% CO_2 atmosphere for 24 h, then treated with **L1** (10 μM) and incubated for 0.5 h, Subsequently, the cells were treated with $CuCl_2$ (15 μM). Cells were incubated for another 0.5 h, different concentration of Na_2S was added. After incubating for 1 h, cells were washed with PBS for three times to remove free compound and ions before analysis. Then fluorescence microscope images were acquired. Excitation of loaded cells at 494 nm was carried out with a He Ne laser. Confocal microscopy optical setup was in multichannel mode. All confocal images were collected with a Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

Assessment of biocompatibility. The biocompatibility was determined in L929 mouse fibroblast cells line. The cell viability was evaluated using the modified MTT assay.[1] Basically, cells were plated at a density of 1×10^5 in 96-well plates 24 h prior to the exposure to the sensor. Different concentrations of **L1** and **L1**Cu (via interactions with DMEM (< 0.25 % DMSO) before used) were added to the wells and the cells were further incubated for for 24 h. After treatment, 10 μL of 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 5 mg · mL^{-1} in PBS) was added into each well. After 4 h of incubation, culture supernatants were aspirated, and purple insoluble MTT product was redissolved in 150 μL of dimethyl sulfoxide (DMSO) in 10 min. The concentration of the reduced MTT in each well was determined spectrophotometrically by subtraction of the absorbance reading at 630 nm from that measured at 570 nm using a microplate reader. All MTT experiments were performed in a set of five identical specimens, the maximum and minimum were deleted. The results were expressed as the mean ± standard deviation.

3. Related spectra and photographs

Table 1. Crystal data and structure refinement for compound L1 (Containing a CH_3OH formula)

Empirical formula	$C_{28}H_{18}N_2O_6$, CH_4O
Formula weight	510.49
Temperature(K)	296(2)
Wavelength(Å)	0.71073
Crystal system	Triclinic
space group	P-1
crystal habit	block
crystal color	yellow
Unit cell dimensions	
a (Å)	7.837(4)
b (Å)	12.342(6)
c (Å)	13.048(7)
α (°)	108.194(5)
β (°)	90.845(6)
γ (°)	90.795(6)
Volume (Å)	1198.7(11)
Z	2
Calculated density(Mg/m^3)	1.414
Absorption coefficient(mm^{-1})	0.102
F(000)	532
Crystal size(mm)	0.21 × 0.19 × 0.16
θ range for data collection(°)	2.60-25.50
Reflections collected	8647/4391
R (int)	0.0686
Data/restraints/parameters	4391/0/351
Final R indices [I>2σ(I)]	R1=0.0897, wR2=0.1529
R indices (all data)	R1=0.2081, wR2=0.1928
Absorption coefficient, *mu*/mm	0.102
Radiation type	multi-scan
radiation (A˚)	0.71073
Goodness of fit on F^2	0.997
Refinement method	Full-matrix least-squares on F^2
CCDC number	854854

Binding Constant

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

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

where I^0_F is the emission intensity of **L1** at 523 nm, I_F is the emission intensity of **L1** at 523 nm upon the addition of different amount of Cu (II) , f is the fraction of the initial fluorescence which is accessible to the sensor, [M] is the concentration of Cu (II) . The association constant values K_S is given by the ratio intercept / slope.

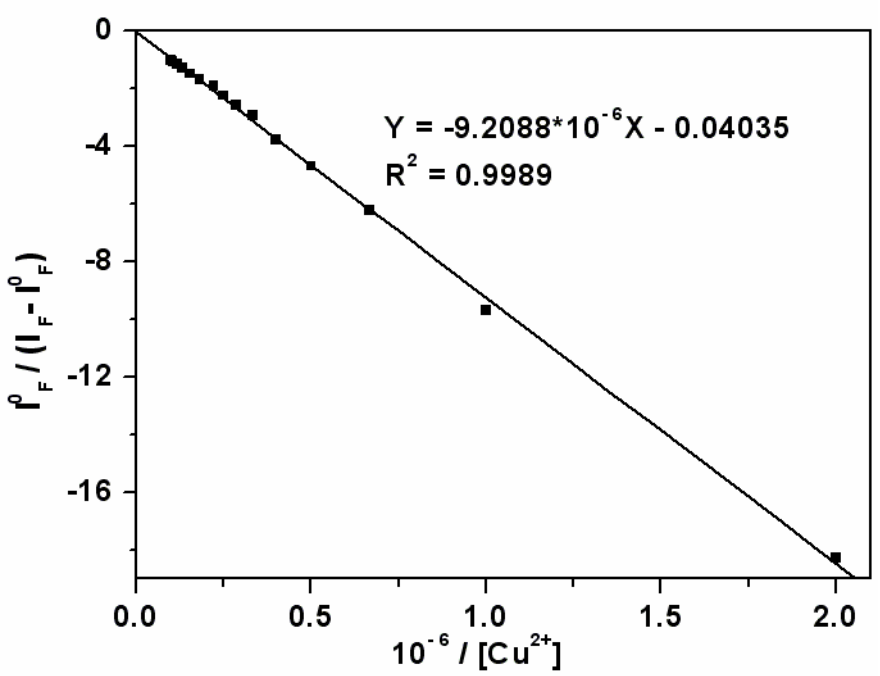

Figure S1. Fitting of Fluorescence titration curve of **L1** in HEPES/ CH_3CN (6: 4, v/v). The binding constant is $K_s = 4.38 \times 10^3\ M^{-1}$.

Calculation of detection limit[3, 4]

The detection limit was determined from the fluorescence titration data based on a reported method.[2, 3] According to the result of titrating experiment, the fluorescence intensity data at 523 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to these normalized fluorescence intensity data, and the point at which this line crossed the ordinate axis was considered as the detection limit.

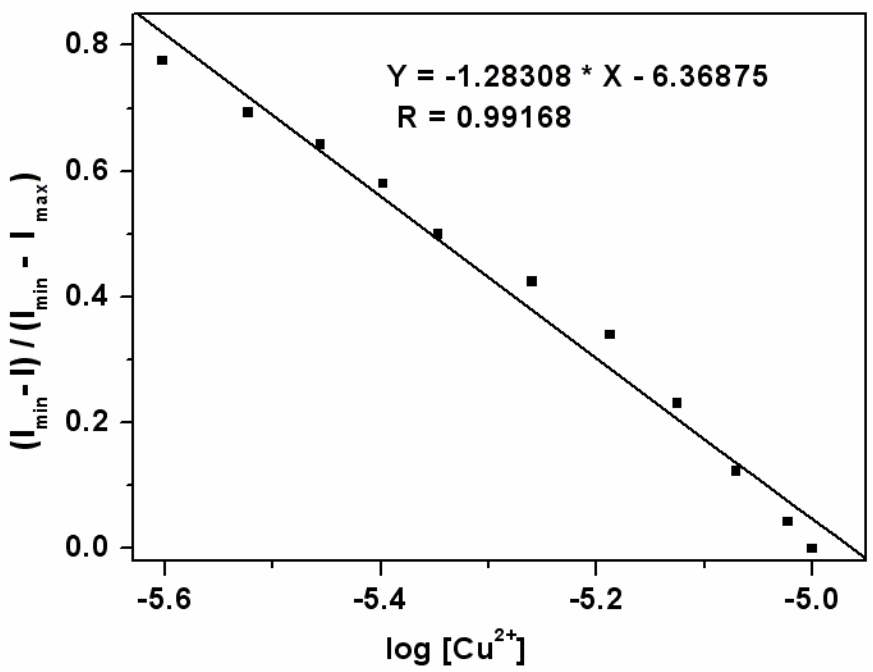

Figure S2. Normalized response of the fluorescence signal to changing Cu (II) concentrations. The detection limit of Cu (II) is 1.08×10^{-5}.

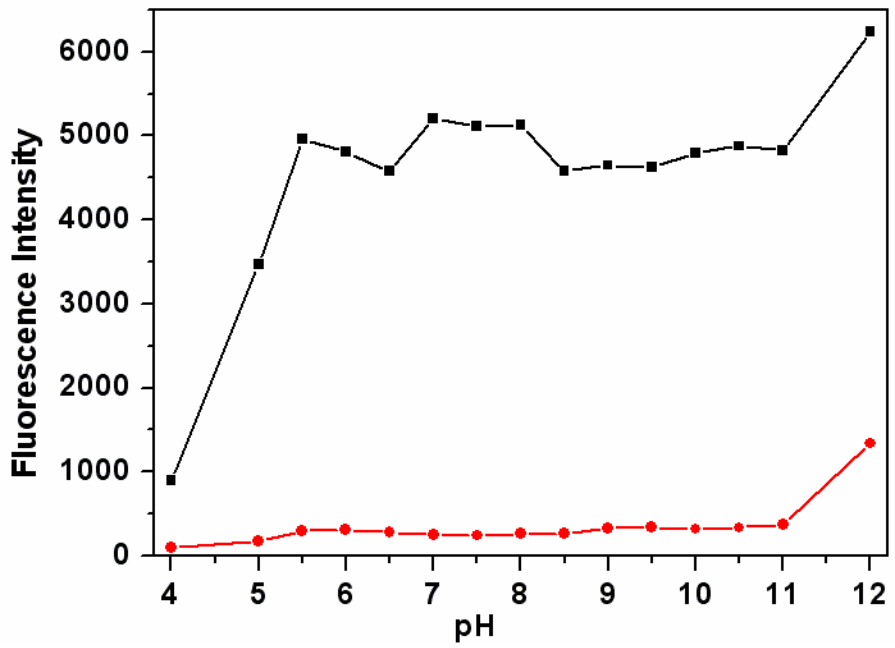

Figure S3. Fluorescence intensities at 523 nm of **L1** in the absence (black line) and presence (red line) of Cu (II) at various pH values at room temperature ([**L1**] =10 μM, [Cu (II)] =15 μM).

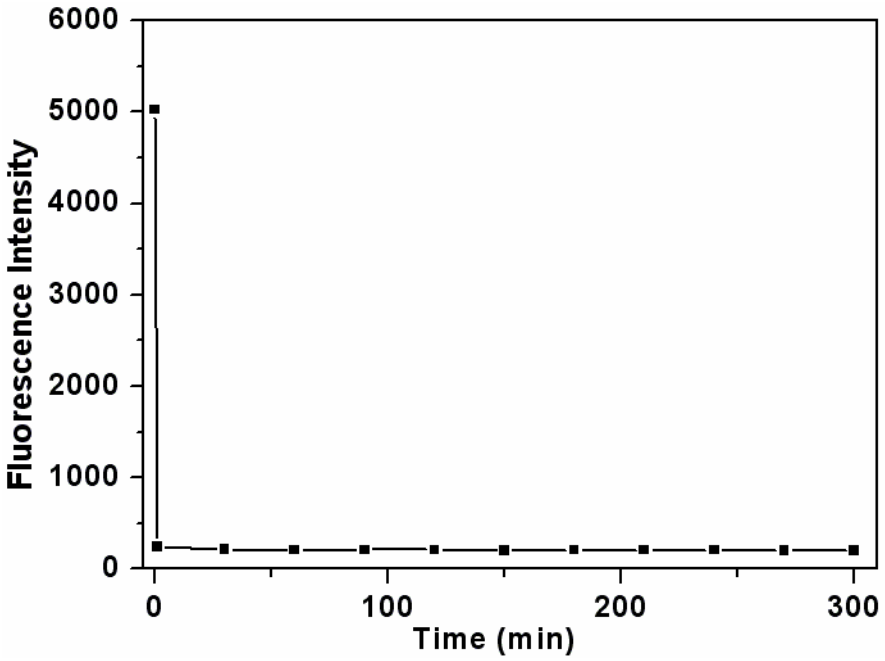

Figure S4. The time responses at 523 nm of 10 μM **L1** to 10 μM $CuCl_2$. Data were acquired at room tempreture in HEPES/ CH_3CN (6: 4, v/v, pH = 7.0) with excitation at λ_{ex} = 494nm.

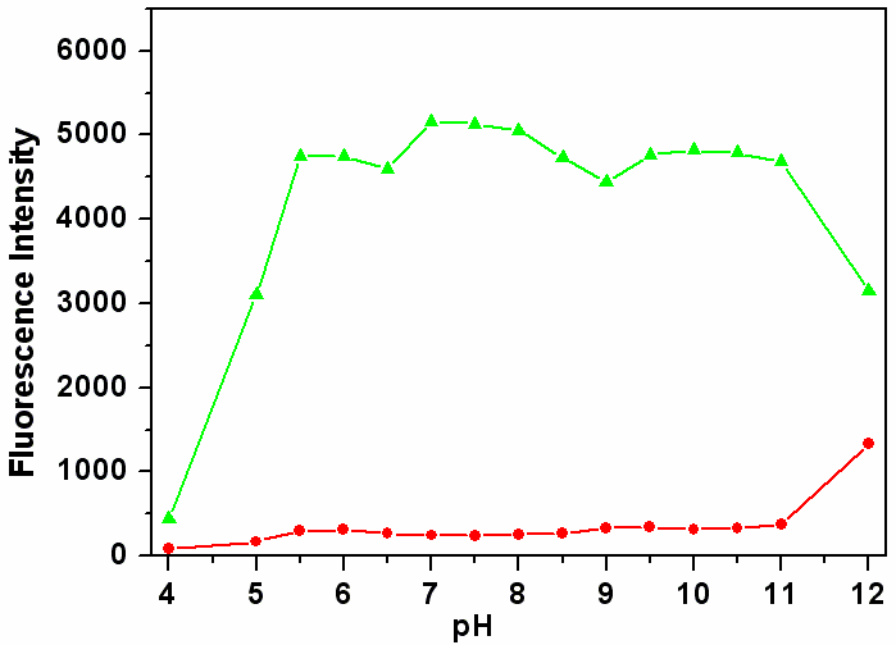

Figure S5. Fluorescence intensities at 523 nm of **L1**Cu in the absence (red line) and presence (green line) of H_2S at various pH values at room temperature ([**L1**Cu] =10 μM, H_2S =20 μM).

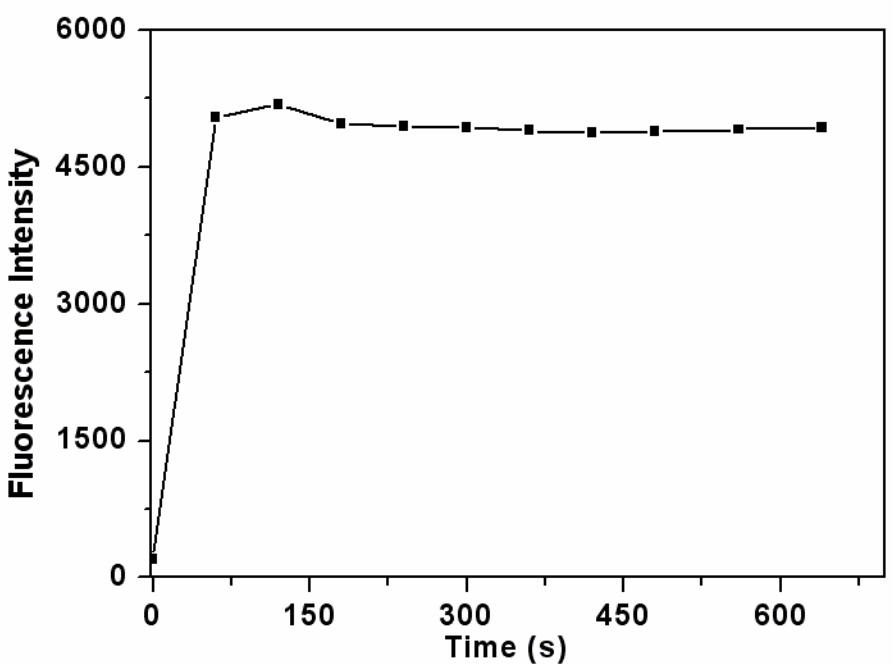

Figure S6. The time responses at 523 nm of 10 μM **L1** to 20 μM H_2S. Data were acquired at room tempreture in HEPES/ CH_3CN (6: 4, v/v, pH = 7.0) with excitation at λ_{ex} = 494nm.

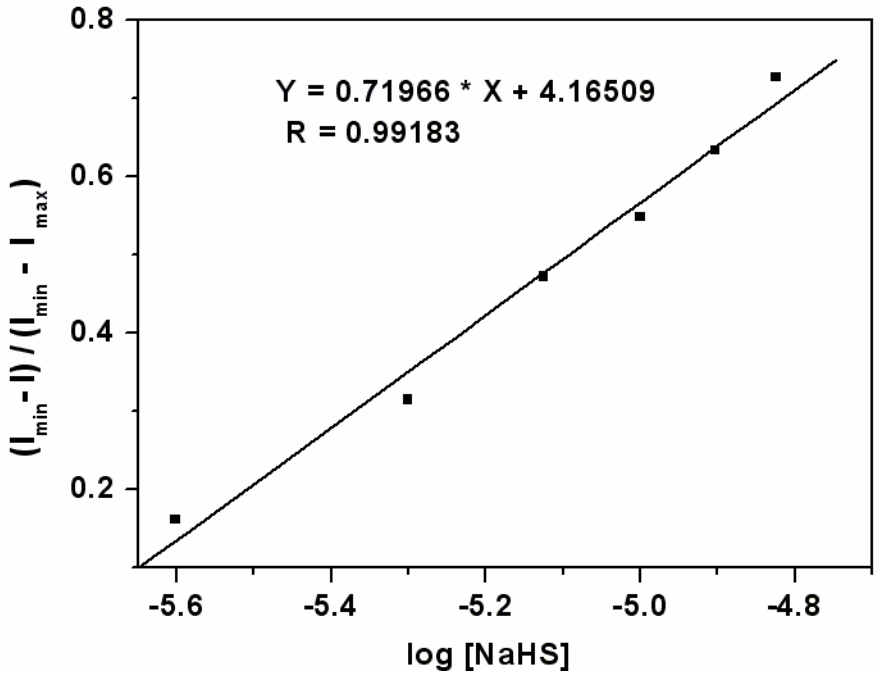

Figure S7. Normalized response of the fluorescence signal at 523 nm to changing H_2S concentrations. The detection limit of H_2S is 1.66 x 10^{-6}.

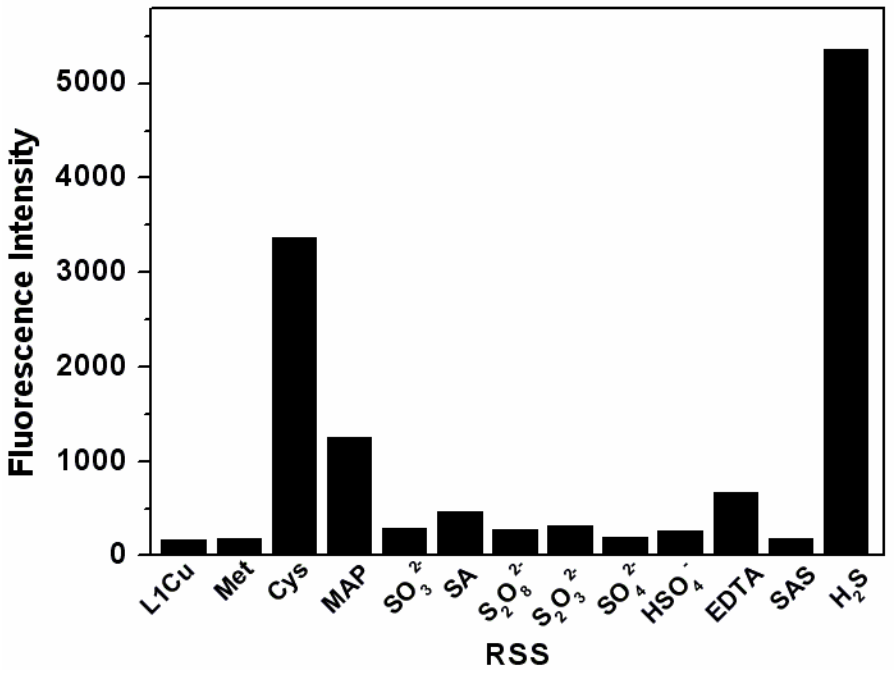

Figure S8. Fluorescence intensities at 523 nm of **L1** (10 μM) in the presence of various forms of sulfate, reducing substance in HEPES/ CH_3CN (6: 4, v/v, pH = 7.0). [RSS] = 200 μM, except for [Met] = [Cys] = [MAP] = 100 μM and [EDTA] = [H_2S] = 20 μM. (SA, L (+)-Ascorbic acid; Cys, L-Cysteine ; SAS, Sulfoacetic acid disodium salt; MAP, Mercaptoacetic acid)

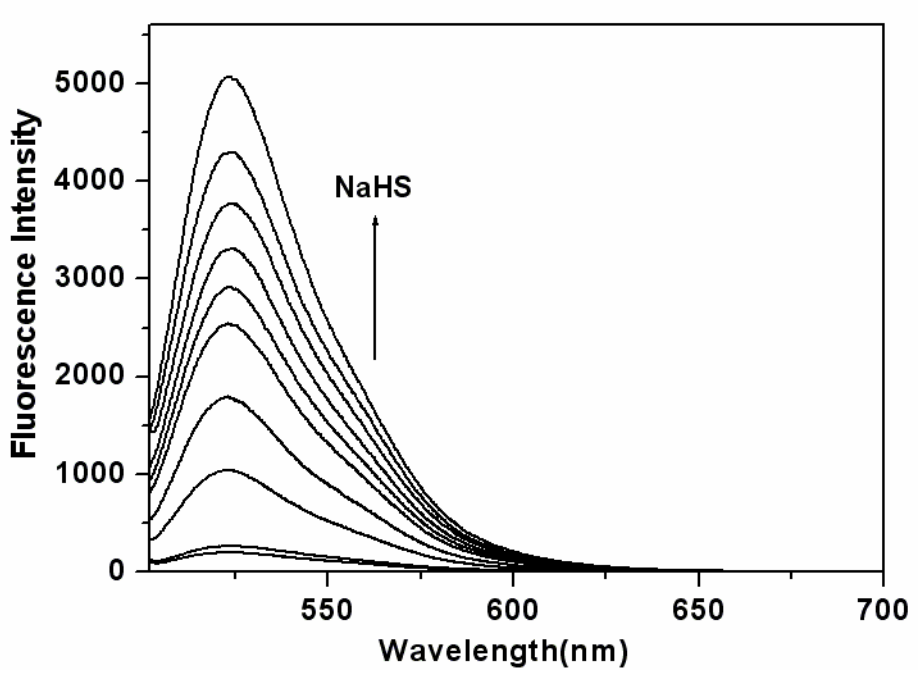

Figure S9. Fluorescence spectra of **L1**Cu (10 μM) in HEPES/ CH_3CN (6: 4, v/v, pH = 7.0) in the presence of different concentrations of H_2S (0- 2 equiv).

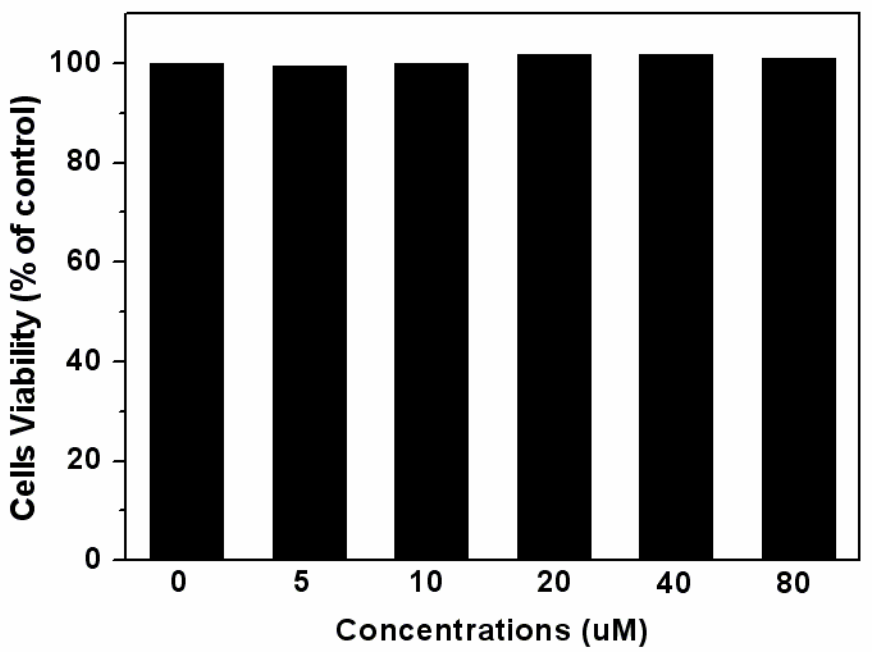

Figure S10. MTT assay of L929 cells cultured for 24 h in media containing various concentration of **L1**Cu.

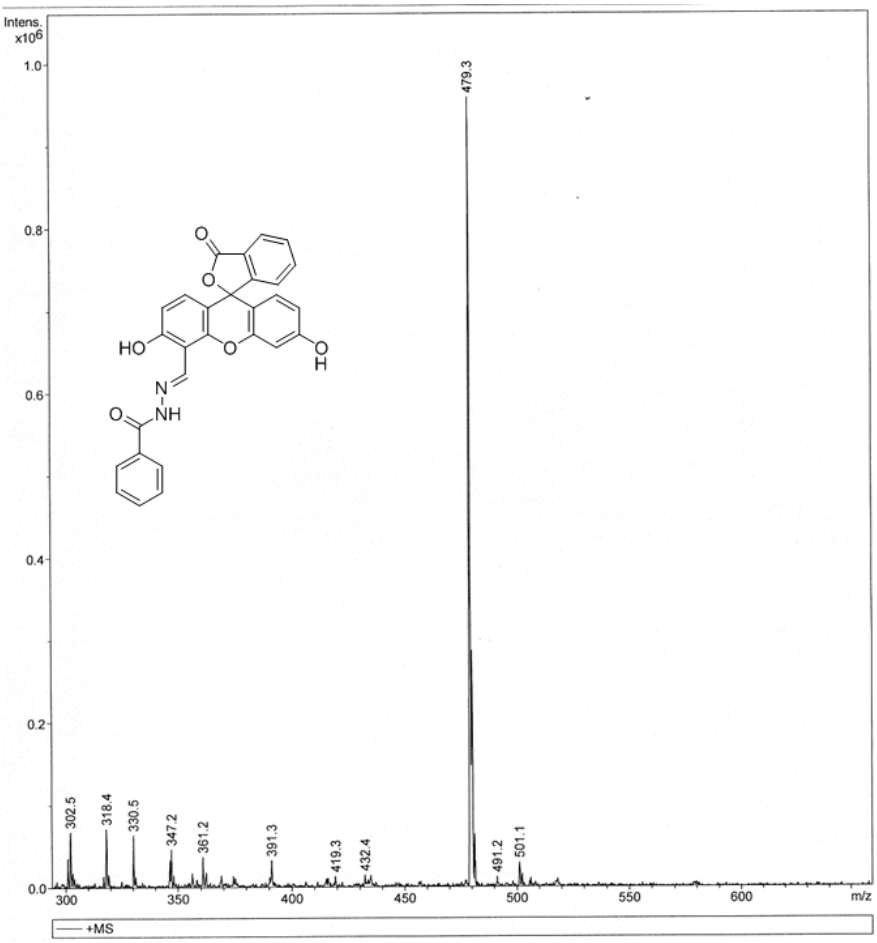

Figure S11. ESI mass spectrum of **L1**.

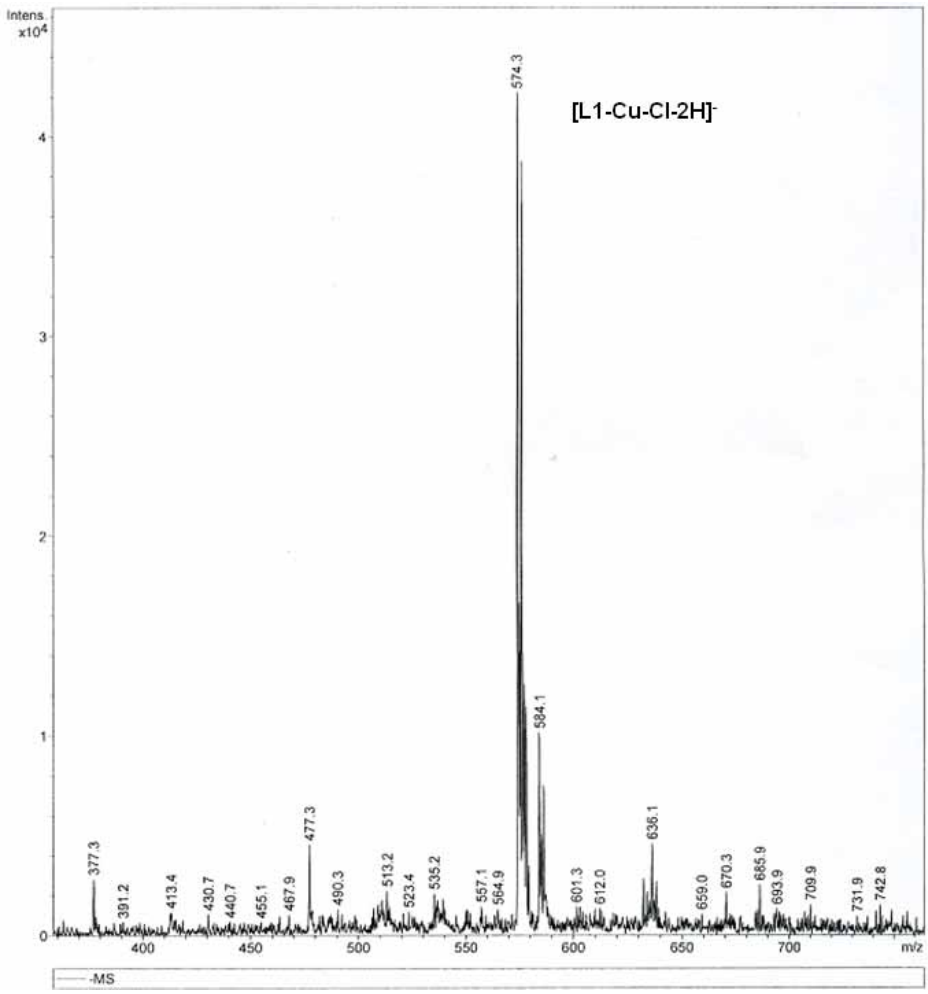

Figure S12. ESI mass spectrum of 20 μM **1** in the presence of 0.5 equiv of Cu (II) in ethanol.

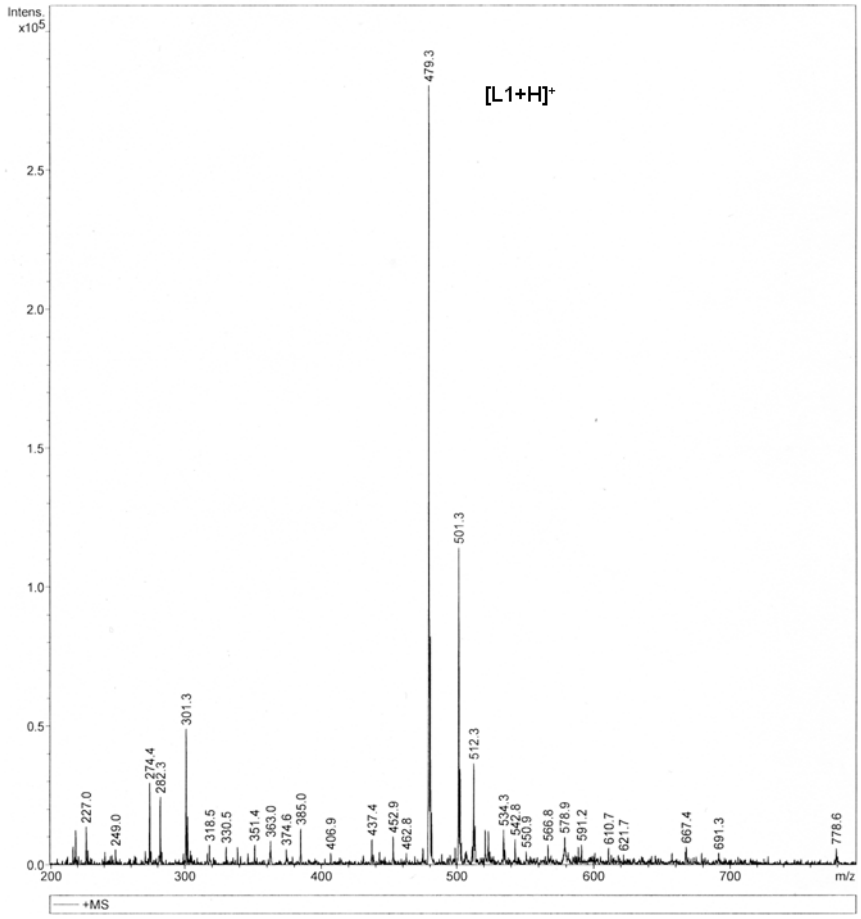

Figure S13. ESI mass spectrum of **L1**Cu in the presence of 2 equiv. of NaHS in methanol.

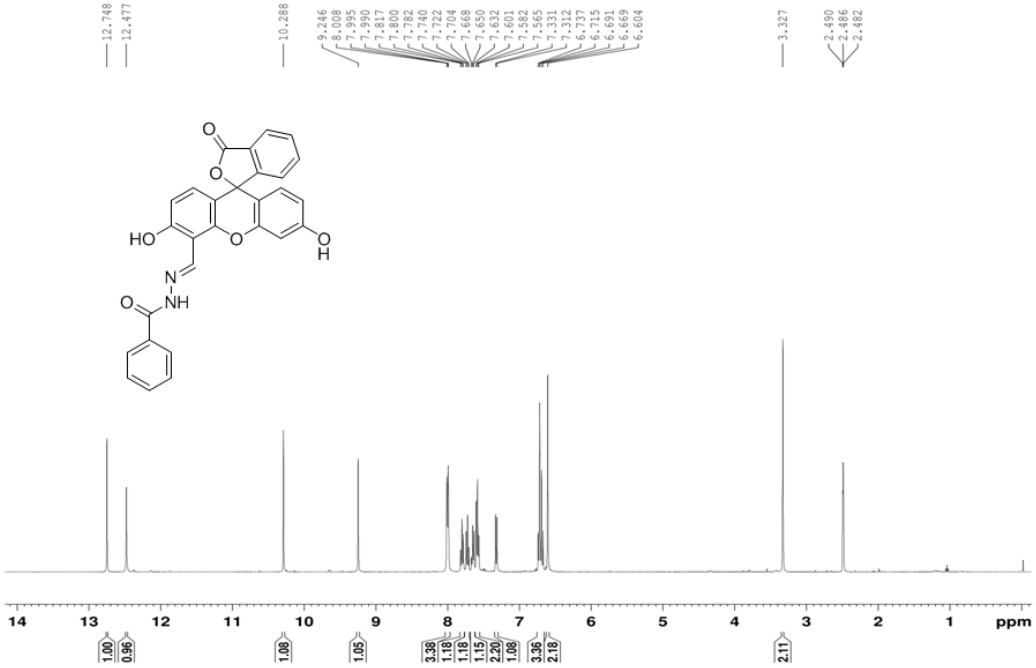

Figure S14. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of **L1**.

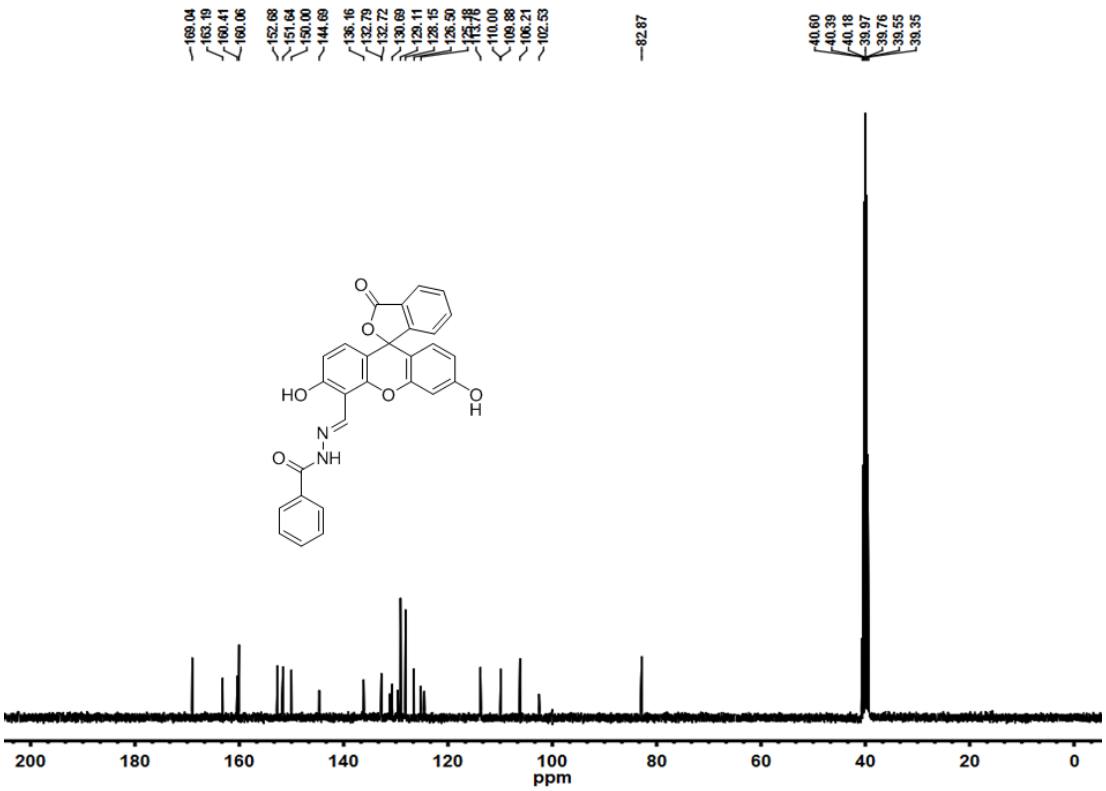

Figure S15. ^{13}C NMR ($CDCl_3$, 100MHz) spectrum of **L1**.

Reference

1. M. Mahmoudia, A. Simchi, M. Imani, M. A. Shokrgozar, A. S. Milani, U. O. Häfeli, P. Stroeve , *Colloids Surf B Biointerfaces*, 2010, **75**, 300.
2. W. H. Wang, O. Rusin, X. Y. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C.M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner, R. M. Strongin. *J. Am. Chem. Soc.* 2005, **127**, 15949.
3. M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland. *Anal. Chem.* 1996, **68**, 1414.
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