Supporting Information

A selective and sensitive near-infrared fluorescent probe for real-time detection of Cu(I)

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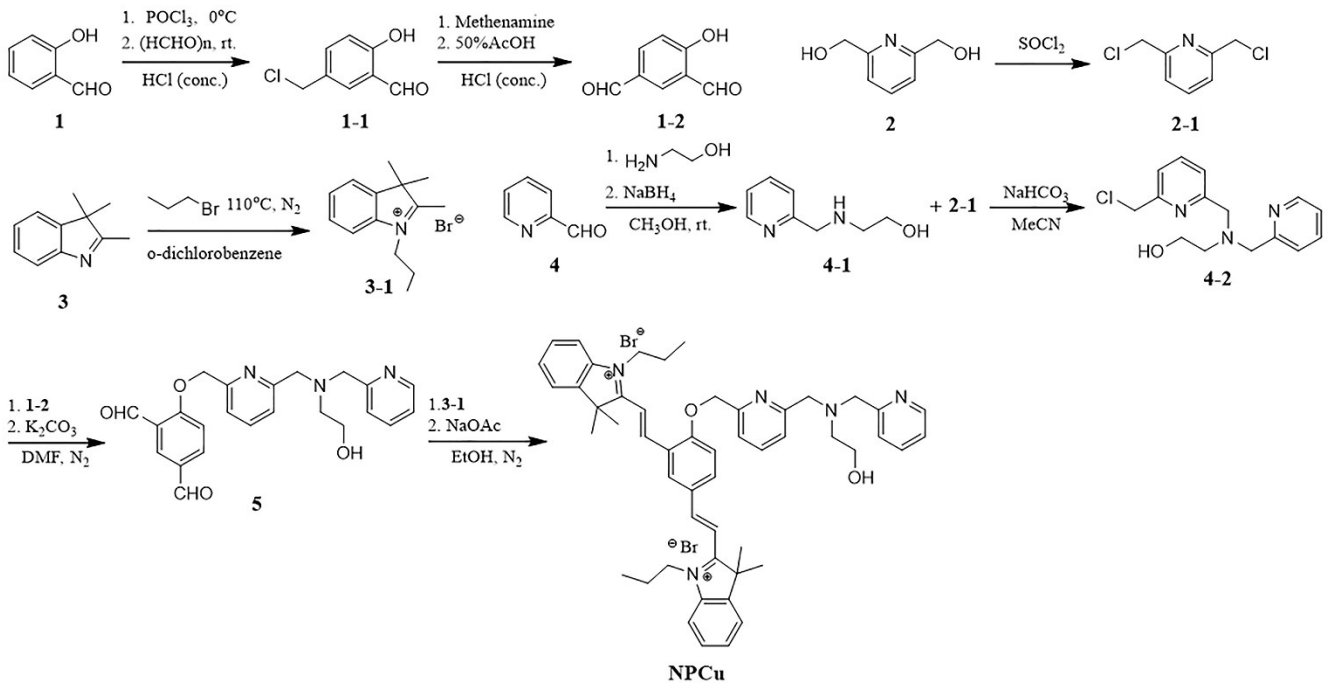
1. Synthesis



Scheme 1 Synthesis procedure of Cu^{+} probe **NPCu**

5-(chloromethyl)-2-hydroxybenzaldehyde(compound 1-1)

This compound was obtained by the following modification of the published procedure.[1] A solution of salicylaldehyde (10.01 g, 81.9 mmol) and excess polyoxymethylene (19.17 g) in concentrated hydrochloric acid (150 ml) was stirred at 5 °C for 30 min, and then treated dropwise with phosphorus oxychloride (5 ml). After addition, the mixture was stirred at room temperature for 28 h to perform white solid. After completion of the reaction (by TLC), the mixture was filtered and the filter cake was washed 4-5 times with distilled water, dried in vacuum and recrystallized with petroleum ether to afford a needle like white solid **1-1** (6.62 g, 47.4% yield), mp: 86-88 °C (lit.[2]mp: 84-86 °C).

4-hydroxyisophthalaldehyde (compound 1-2)

This compound was obtained as described in the literature[1]. A solution of compound **1-1** (6.42 g, 37.4 mmol) and methenamine (6.82 g, 48.6 mmol) in 50% acetic acid was stirred at 115 °C for 1 h. Then 20 ml of HCl (conc.) was added and the reaction was kept stirring at the same condition for several minutes (detected with TLC). The mixture was cooled down to 0 °C after completion and a yellowish precipitate was generated. After filtration, the filter cake was washed with ice water (25 ml*2) and dried in vacuum to give a yellowish solid **1-2** (2.0 g, 51.86%), mp:110-112 °C (lit.[2]mp: 106-108 °C).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.77 (s, 1H), 10.33 (s, 1H), 9.89 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 8.01 (dd, J = 8.8, 2.4 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.49, 190.46, 165.90, 136.10, 132.44, 128.80, 122.99, 118.65.

2,6-bis(chloromethyl)pyridine (compound 2-1)

This compound was obtained by the following modification of the published procedure.[3] To a stirred solution of pyridine-2,6-diyldimethanol (2 g, 14.4 mmol) in ether, 15 ml of thionyl chloride was added dropwise over 1 h at 0 °C. Then, the mixture was refluxed for 5 h. 20 ml of toluene was added dropwise after cooling down to room temperature and a white precipitate was generated. The filter cake was washed with toluene (5 ml*2) and then dissolved in 200 ml of distilled water, and the mixture was adjusted pH = 7 with K_2CO_3. The precipitate was filtered, washed with H_2O (5 ml*2) and dried in vacuum. The crude product was recrystallized with petroleum ether to afford a white solid **2-1** (1.28 g, 57.3% yield), mp: 72-74 °C (lit.[4] mp: 68-70 °C).
^{1}H NMR (400MHz, DMSO-d_6) δ: 7.89 (t, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 4.77 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.63, 139.02, 123.23, 46.94.

2,3,3-trimethyl-1-propyl-3H-indol-1-ium bromide (compound 3-1)

This compound was obtained by the following modification of the published procedure[5] which did not include any characterization of the product. A mixture of 2,3,3-trimethyl-3H-indole (20.00 g, 0.13 mol) and 1-bromopropane (23.16 g, 0.19 mol) was dissolved in 50 ml of *o*-dichlorobenzene with stirring at 25 °C under a nitrogen atmosphere. Then the reaction mixture was kept stirring at 110 °C for 12 h. The reaction was detected by TCL (cyclohexane: acetone = 3:1). When the reaction was completed and cooled down to room temperature naturally, 30 ml of isopropyl ether was poured to give the precipitates. Then the filter cake was washed with acetone (15 ml*3), dried at 45 °C in vacuum to afford a pink solid **3-1** (17 g, 47.96% yield).
^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.02 (dd, J=8.0, 4.0Hz, 1H), 7.86 (dd, J=8.0, 4.0Hz, 1H), 7.67–7.58 (m, 2H), 4.51–4.42 (m, 2H), 2.87 (s, 3H), 1.95–1.81 (m, 2H), 1.55 (s, 6H), 1.00 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.01, 142.26, 141.51, 129.78, 129.32, 123.92, 115.96, 54.56, 49.22, 22.48, 21.21, 14.54, 11.16.

2-((pyridin-2-ylmethyl)amino)ethan-1-ol (compound4-1)

This compound was obtained by the following modification of the published procedure[6] which did not include any characterization of the product. To an anhydrous methanol (15 ml) solution of ethanolamine (2.96 g, 48.1 mmol), 2-pyridinecarboxaldehyde (5.00 g, 46.7 mmol) in 10 ml of methanol was added dropwise at 0 °C and stirred for 5 h at room temperature. When most of the raw materials had been consumed (detected by TLC), $NaBH_4$ (1.80 g, 47.3 mmol) was added in small portion and the reaction mixture was kept stirring at rt. overnight. Then the reaction mixture was concentrated *in vacuo* and the crude product was passed through silica column chromatography using MeOH/DCM from 0 to 10% as eluent to afford a yellow oil compound **4-1** (6.30 g, 88.7% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.49 (ddd, J = 5.2, 2.0, 1.2 Hz, 1H), 7.74 (td, J = 8.0, 2.0 Hz, 1H), 7.41 (dt, J = 8.0, 1.2 Hz, 1H), 7.23 (ddd, J = 8.0, 5.2, 1.2 Hz, 1H), 3.80 (s, 2H), 3.48 (t, J = 5.6 Hz, 2H), 2.60 (t, J = 5.6 Hz, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 160.66, 149.19, 136.89, 122.31, 122.25, 60.81, 54.92, 51.69

2-(((6-(chloromethyl)pyridin-2-yl)methyl)(pyridin-2-ylmethyl)amino)ethan-1-ol (compound 4-2)

This compound was synthesized as described in the literature.[7] Compound **2-1** (374.2 mg, 2.1 mmol) and $NaHCO_3$ (84.8 mg, 1.0 mmol) were mixed together in 30 ml of MeCN and stirred at 70 °C for 40 min, then an acetonitrile solution (15 ml) of compound **4-1** (161.4 mg, 11 mmol) was added slowly. The mixture was stirred at 70 °C overnight and cooled to room temperature. Insoluble materials were removed by filtration. Then the filtrate was concentrated on a rotary evaporator and the crude product was purified by silica gel column chromatography (MeOH/EA from 0 to 8%, v/v) to afford a yellowish oil compound **4-2** (148 mg, 47.83% yield).
^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.47 (d, J = 5.2 Hz, 1H), 7.81 (t, J = 8.0 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.55 (dd, J = 8.0, 5.2 Hz, 2H), 7.40 (d, J = 8.0 Hz, 1H), 7.24 (dd, J = 8.0, 5.2 Hz, 1H), 4.74 (s, 2H), 4.53 (s, 1H), 3.80 (s, 4H), 3.52 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.12, 159.83, 155.79, 149.05, 138.04, 136.83, 123.01, 122.42, 122.33, 121.76, 60.52, 60.45, 59.46, 56.57, 47.25.

4-((6-(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)pyridin-2-yl)methoxy)isophthalaldehyde (compound 5)

This compound was obtained by the following modification of the published procedure[8] which did not include any characterization of the product. To a solution of compound **3-1** (492.4 mg, 3.3 mmol) dissolved in 2 ml of anhydrous DMF, K_2CO_3 powder (602.6 mg, 4.4 mmol) and compound **4-2** (318.0 mg, 1.1 mmol) in 3 ml of DMF was added slowly in succession. Then the reaction mixture was degassed for 10 min by purging nitrogen gas and stirred at 70 °C for 12 h. After completion (detected by TLC), the reaction mixture was cooled. The insoluble substance was removed, then the residue was extracted with EA (15 ml*3) and washed with H_2O (15 ml) three times. The organic layer was dried over anhydrous sodium sulfate, and the filtrate was concentrated on a rotary evaporator. Finally, the crude compound was purified by silica gel column chromatography (MeOH/DCM from 0 to 7%) to afford a yellowish oil compound **5** (0.70 g, 62.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.47 (s, 1H), 9.95 (s, 1H), 8.46 (d, J = 4.8 Hz, 1H), 8.26 (d, J = 2.4 Hz, 1H), 8.14 (dd, J = 8.0, 2.4 Hz, 1H), 7.83 (t, J = 8.0 Hz, 1H), 7.77-7.69 (m, 1H), 7.57-7.48 (m, 4H), 7.23 (t, J = 6.0 Hz, 1H), 5.46 (s, 2H), 4.53 (s, 1H), 3.80 (s, 4H), 3.50 (t, J = 6.4 Hz, 2H), 2.59 (t, J = 6.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.64, 189.12, 164.65, 162.66, 159.88, 159.84, 154.95, 149.04, 137.94, 136.80, 130.70, 129.87, 124.95, 122.95, 122.39, 122.33, 120.23, 115.14, 71.66, 60.53, 60.41, 59.49, 56.55.

2.Experiment procedure of fluorescence determination

The color changes of NPCu towards various Cu^+ concentrations

To 2 mL of NPCu solution (50 μM) was added the same volume of Cu^+ (20 eq.) in PBS buffer (25 mM, pH 7.2, 2 mM GSH) from 0 to 150 μM and incubated at 37 °C for 2 hours. The fluorescence intensity was measured at 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 180, and 240 min, respectively.

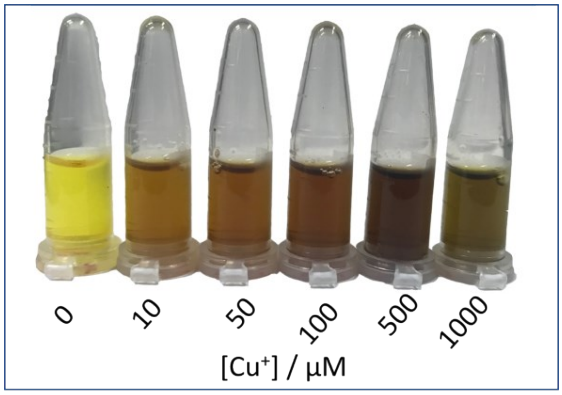


Fig. S1 Color changes of NPCu (250 μM) in 25 mM PBS buffer (pH = 7.2, 2 mM GSH) at 37 °C before and after the addition of various concentrations of Cu^+ (From left to right: 0, 10, 50, 100, 500 and 1000 μM).

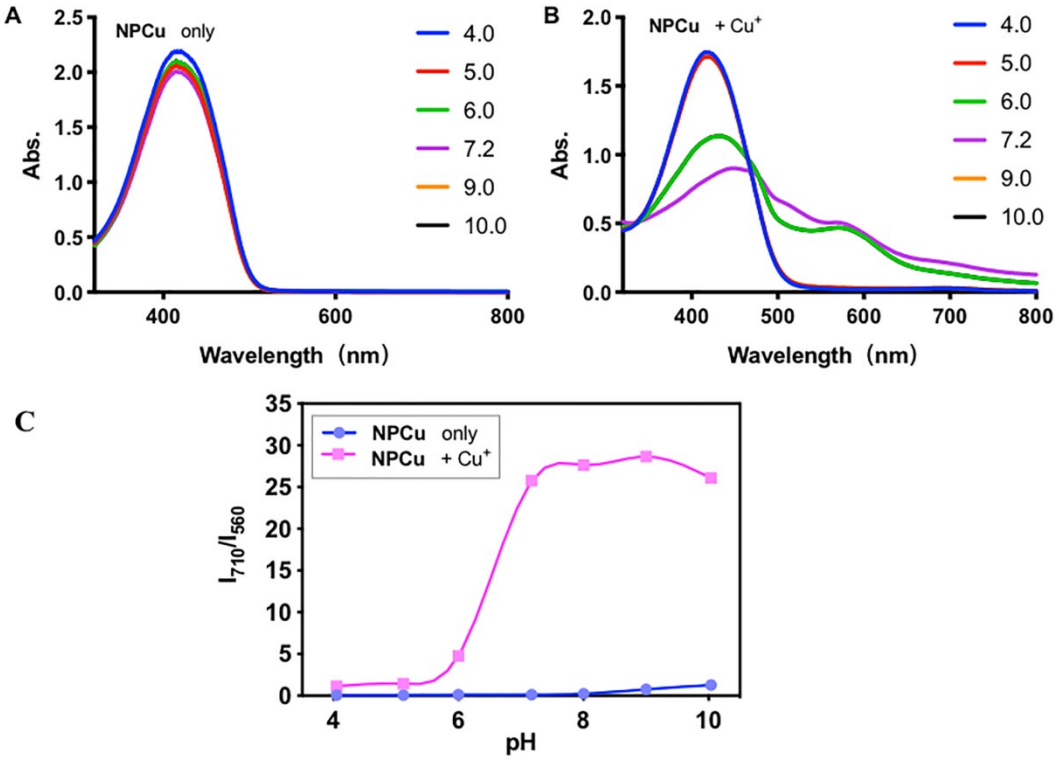


Fig. S2 UV-vis absorption (A, B) spectra and ratiometric fluorescence intensity [I_{710}/I_{560}] (C) in aqueous solution at different pH of NPCu (50 μM) before and after addition of Cu^+ (500 μM), generated by in situ reduction of $[Cu(CH_3CN)_4]PF_6$ with 2 mM glutathione.

3.Cytotoxicity Assay

Cytotoxicity studies were performed using the MTT assay. A549 cells were seeded in 96-well plates at a density of 5000 cells per well. After 24 h incubation, the culture medium was replaced by probe NPCu solutions at various concentrations (0-200 μM) respectively. The cells were further cultured at 37 °C for another 24 h. The solution was then discarded, and freshly prepared MTT solution (20 μL; 5 mg/mL in culture medium) was added to each well. This was followed by incubation at 37 °C for 4 h. After removal of the MTT medium solution, DMSO (150 μL) was added to each well, and the plate was shaken for 10 min to dissolve the formed precipitates. The absorbance values of the wells were read with a microplate reader at 490 nm.

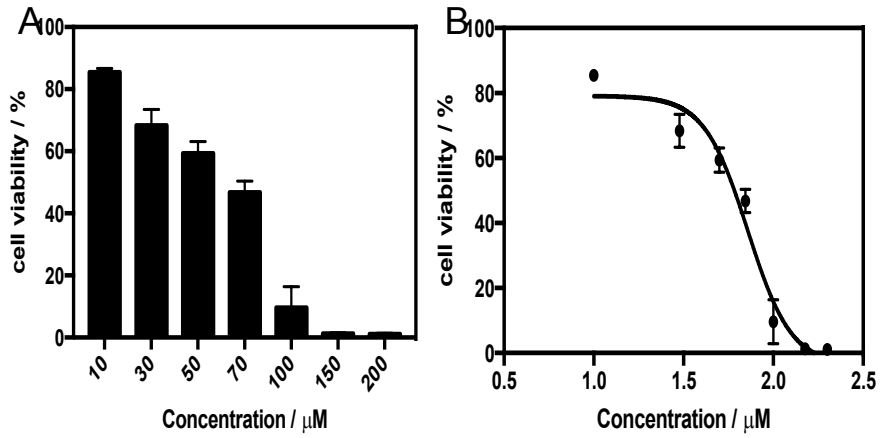
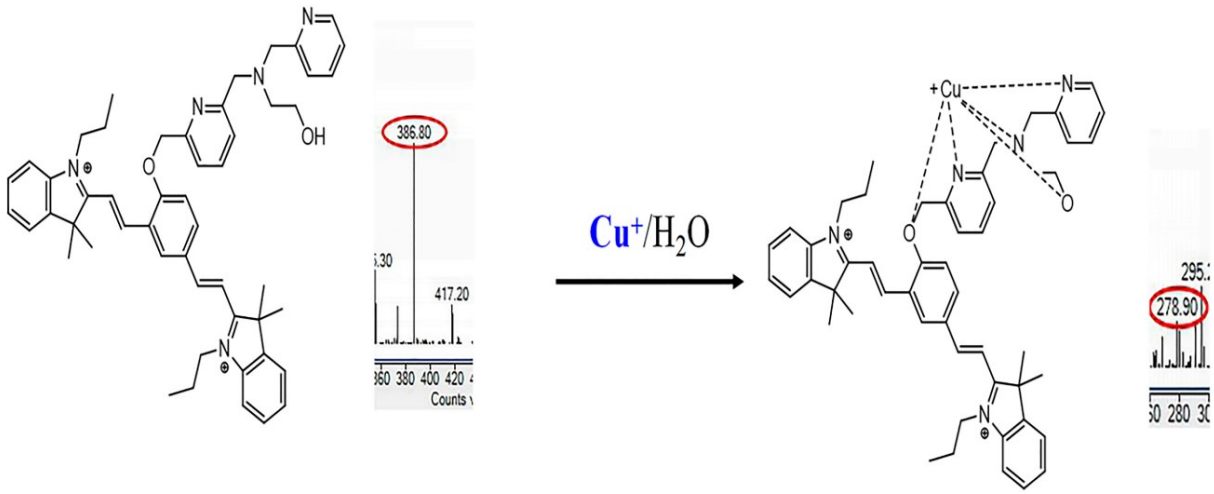


Fig. S3 The MTT assay of probe NPCu toward A549 cell

4.Liquid chromatography-mass spectrometry analysis

The mixing time of 60 minutes ensures complete reaction. In order to support the proposed mechanism, various ESI-MS spectra of the reaction between **NPCu** and Cu $^{+}$ solution were collected at different times. The peak at m/z was 386.80 monitored to detect **NPCu**. The peak at m/z 278.90 was the complex when **NPCu** reacted with Cu^{+}. The peak at m/z was 273.30 and 517.30 the product after the reaction of NPCU and Cu^{+}.

The mass spectrometer was operated in positive ion electrospray mode. The chromatographic separation was performed on an analytical mass spectrometer column (Capcell PAK C18, 50×2.00 mm, 5μm).The column temperature is set to 50. The analyte was eluted with a mobile phase containing 0.3% formic acid Acidic water and methanol. Analyzer software was used for data acquisition and analysis(version 1.4.2; Applied Biosystems, Fo ster City, CA, US



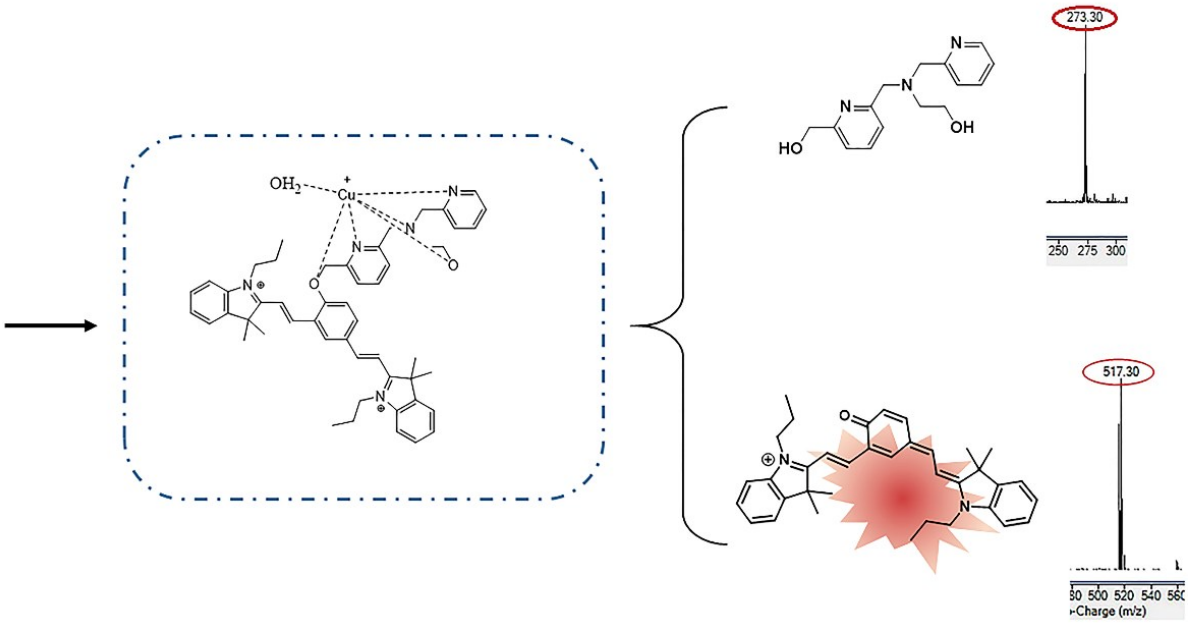


Fig. S4 The supposed mechanism of **NPCu** with Cu^{+}

5.NMR spectra

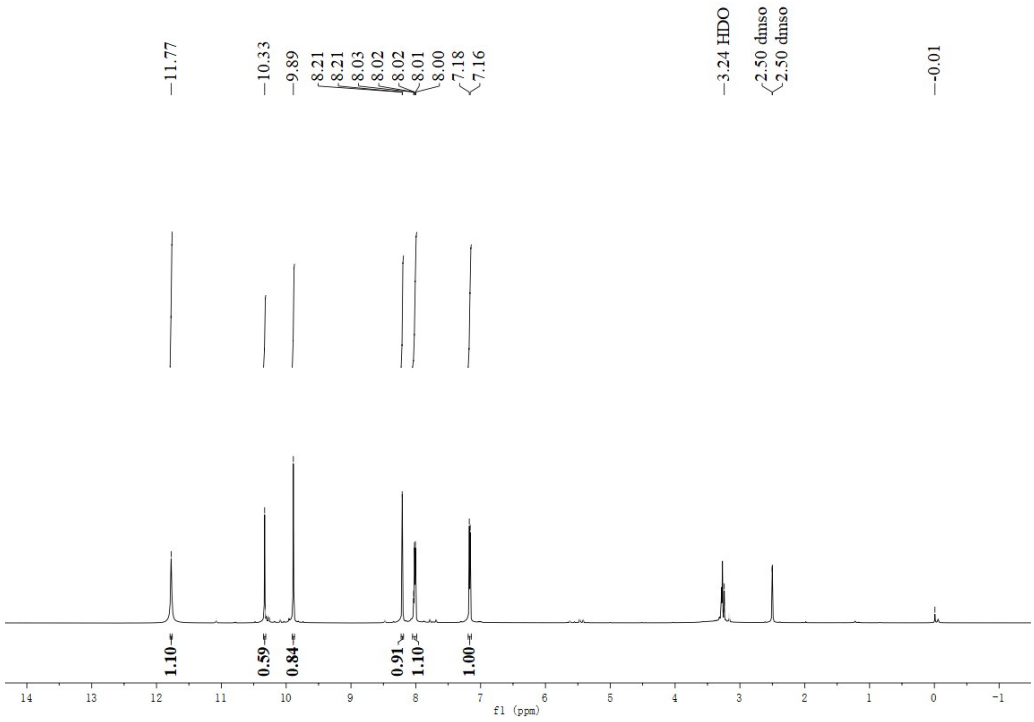


Fig. S5 [1]HNMR spectrum of the **compound 1-2**

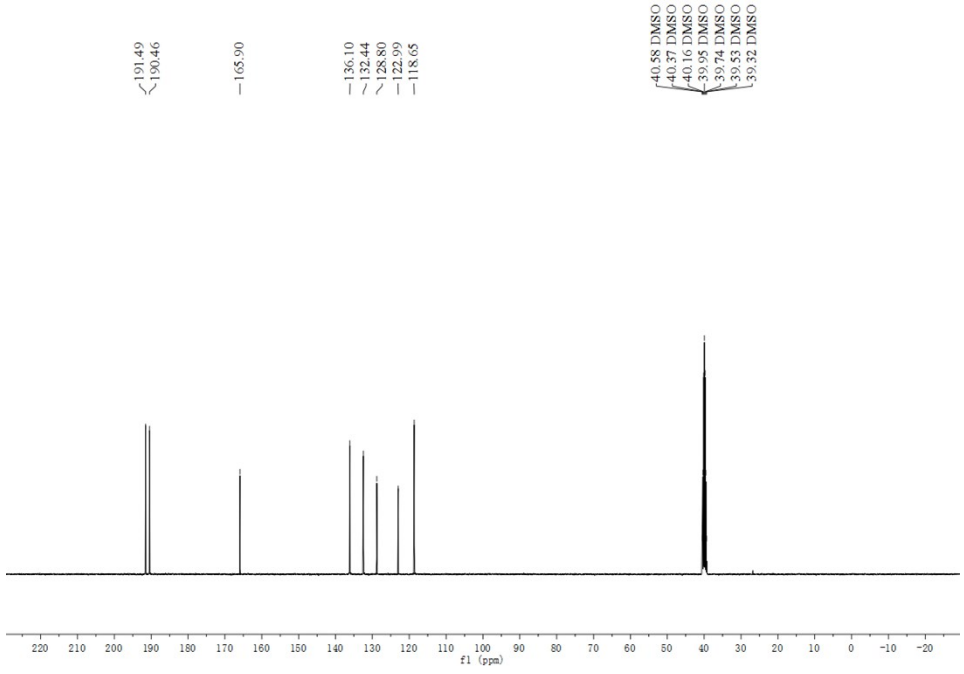


Fig. S6 [13]CNMR spectrum of the **compound 1-2**

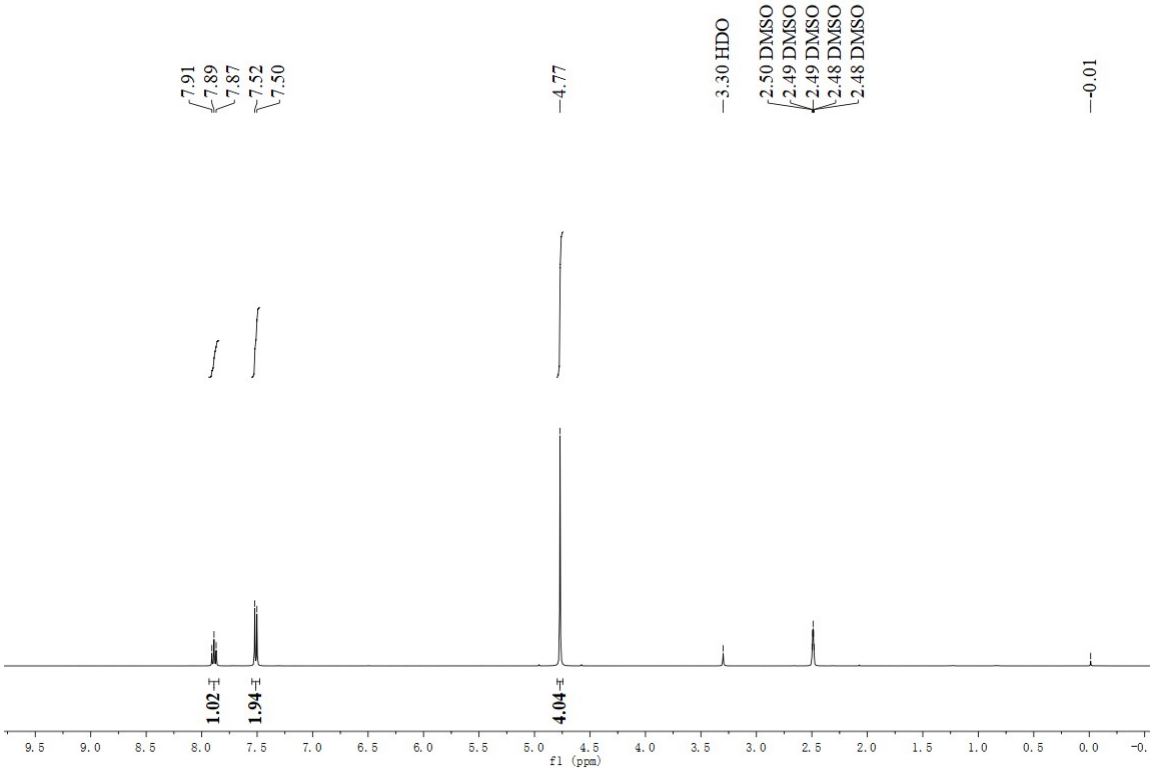


Fig. S7 [1]HNMR spectrum of the **compound 2-1**

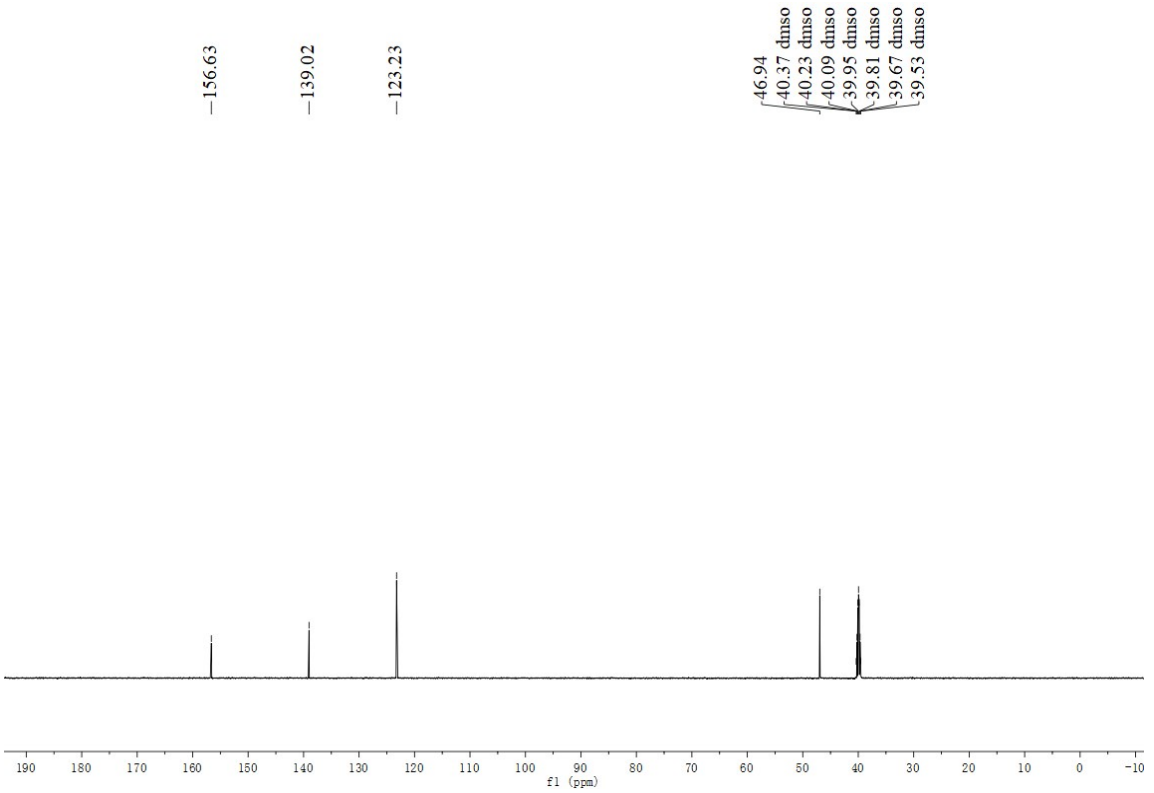


Fig. S8 [13]CNMR spectrum of the **compound 2-1**

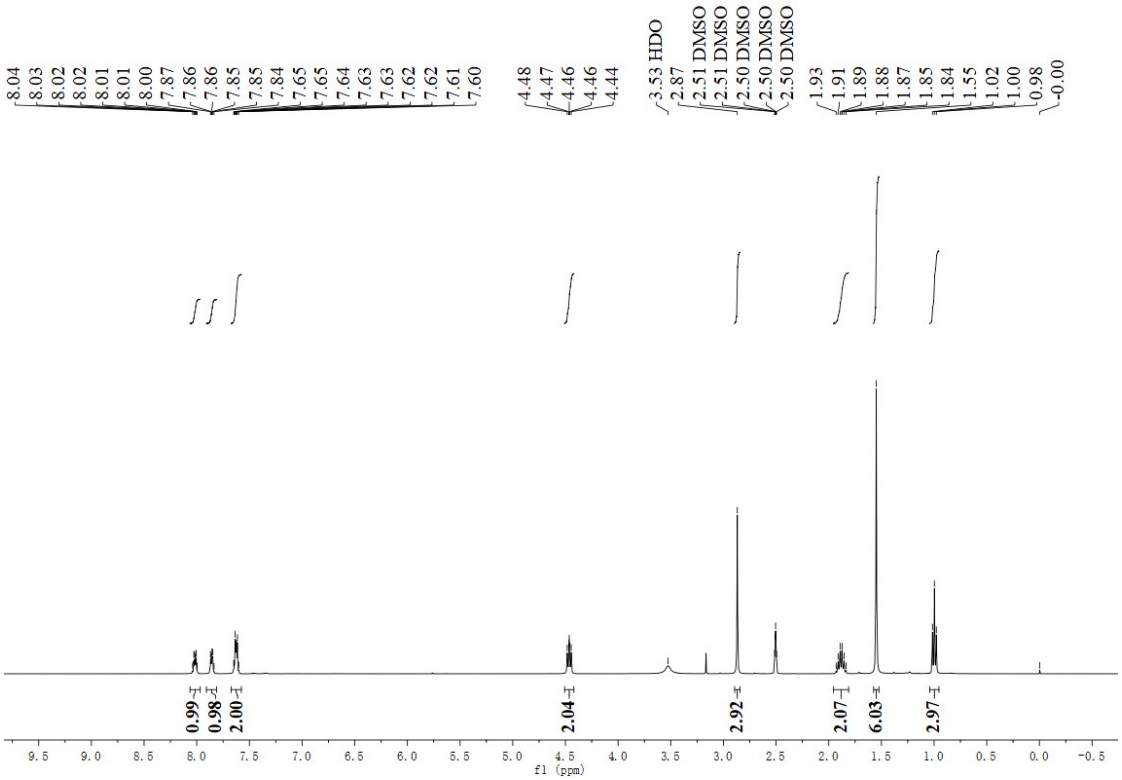


Fig. S9 [1]HNMR spectrum of the **compound 3-1**

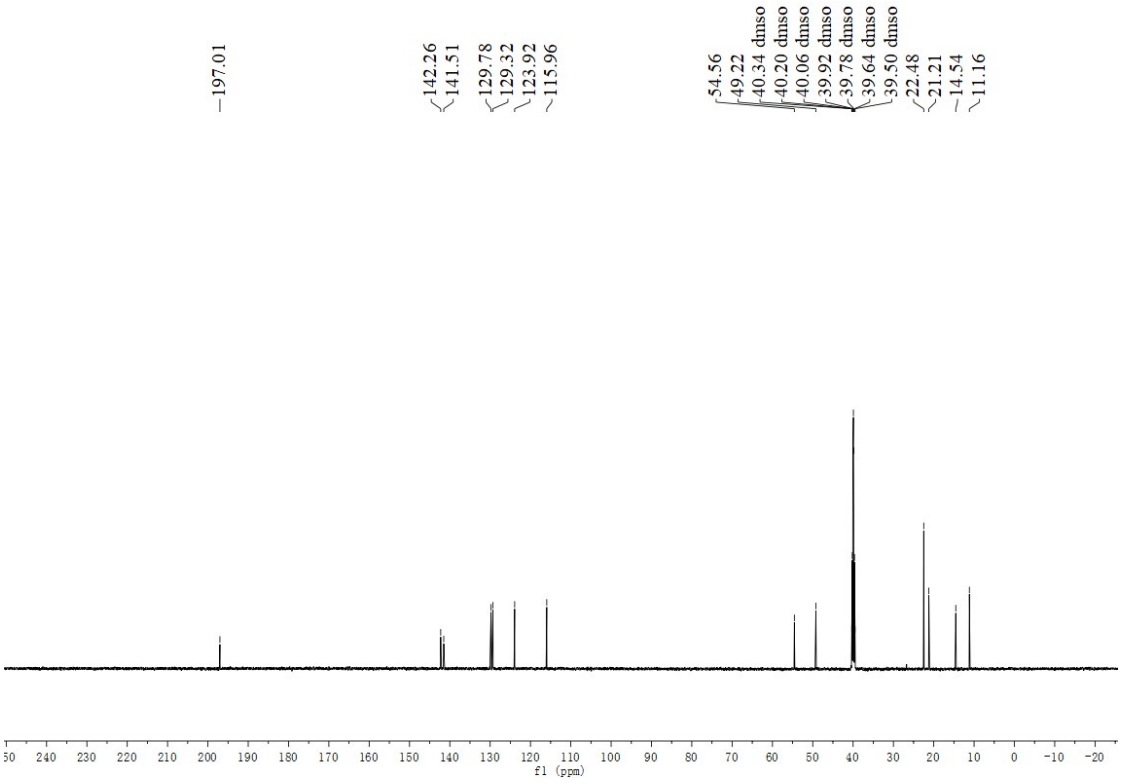


Fig. S10 [13]CNMR spectrum of the **compound 3-1**

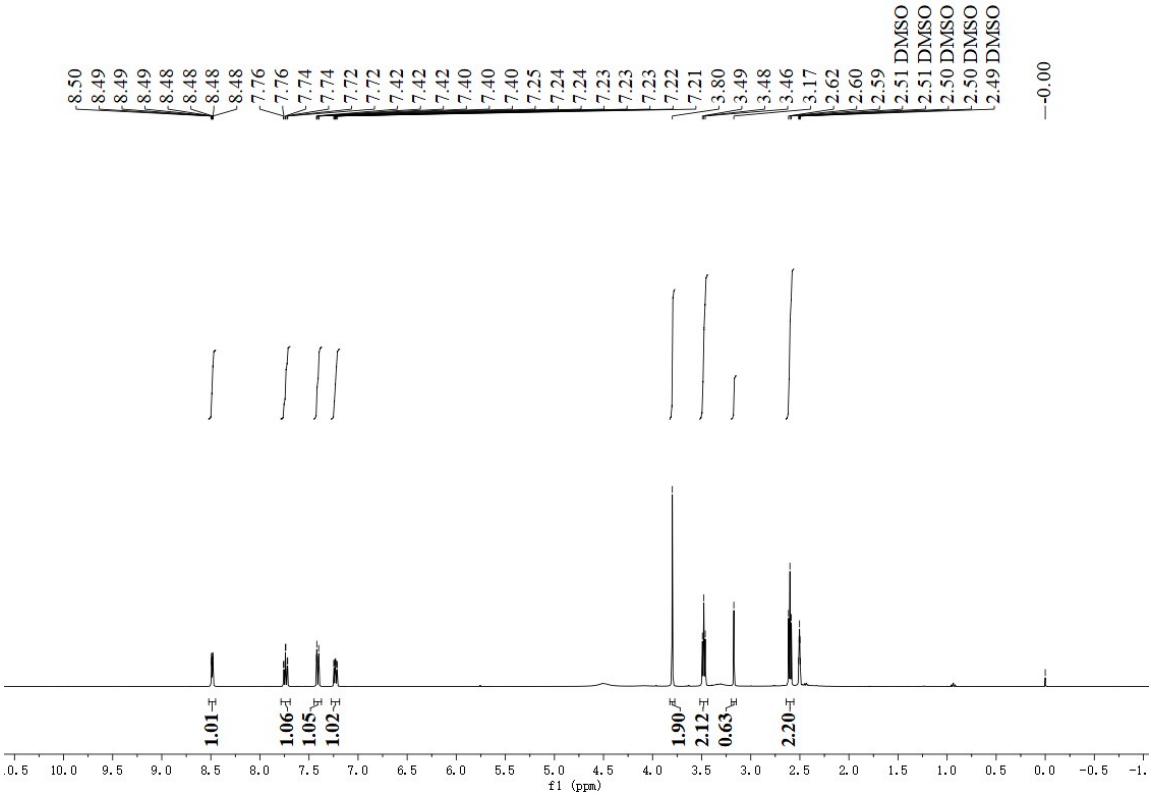


Fig. S11 [1]HNMR spectrum of the **compound 4-1**

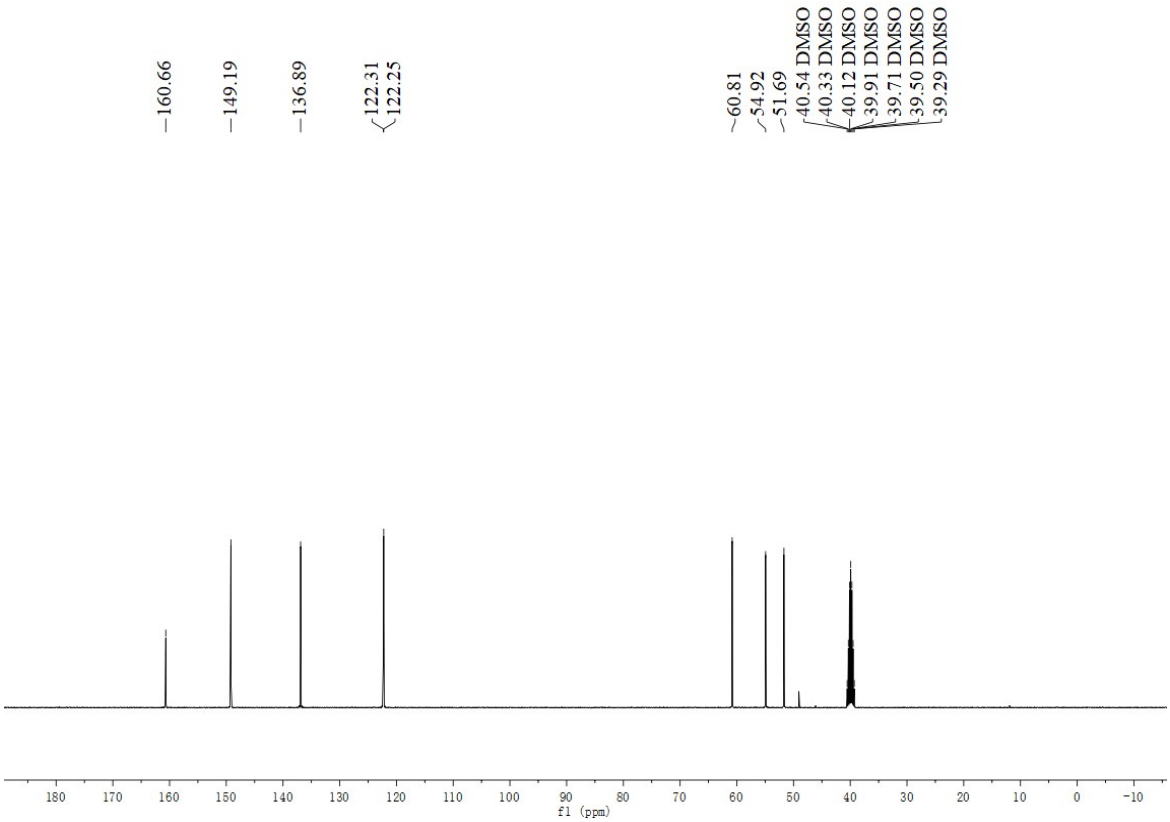


Fig. S12 [13]CNMR spectrum of the **compound 4-1**

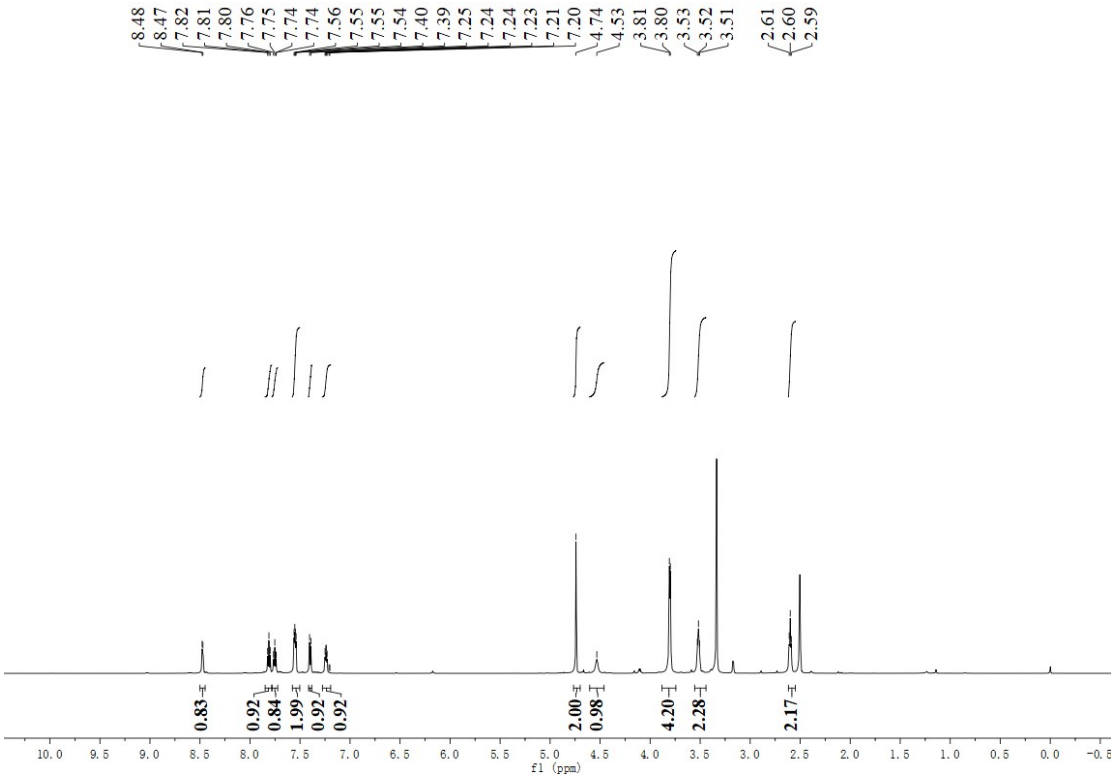


Fig. S13 [1]HNMR spectrum of the **compound 4-2**

Fig. S14 [13]CNMR spectrum of the **compound 4-2**

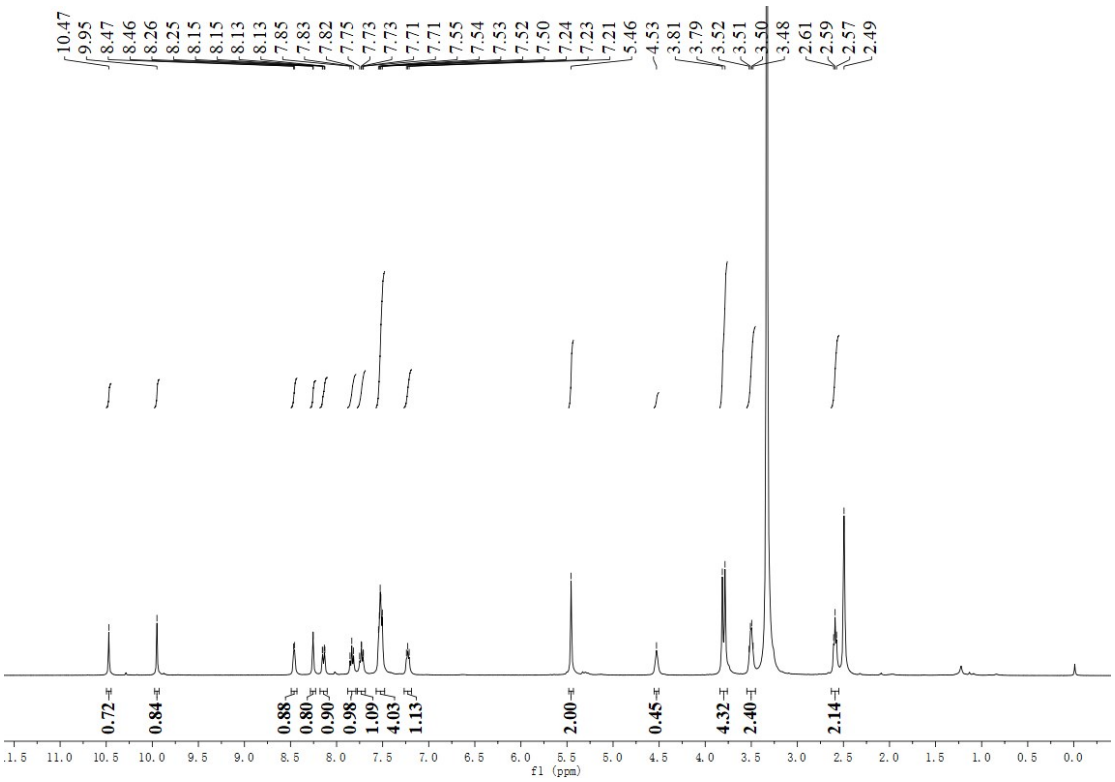


Fig. S15 [1]HNMR spectrum of the **compound 5**

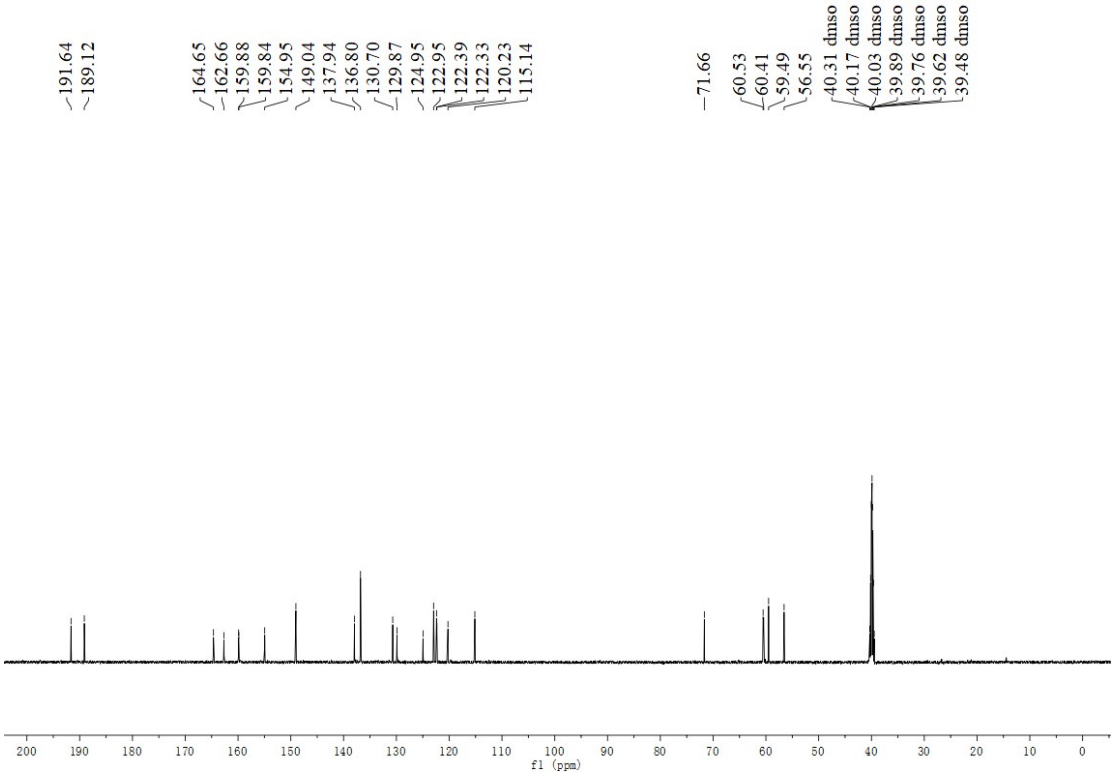


Fig. S16 [13]CNMR spectrum of the **compound 5**

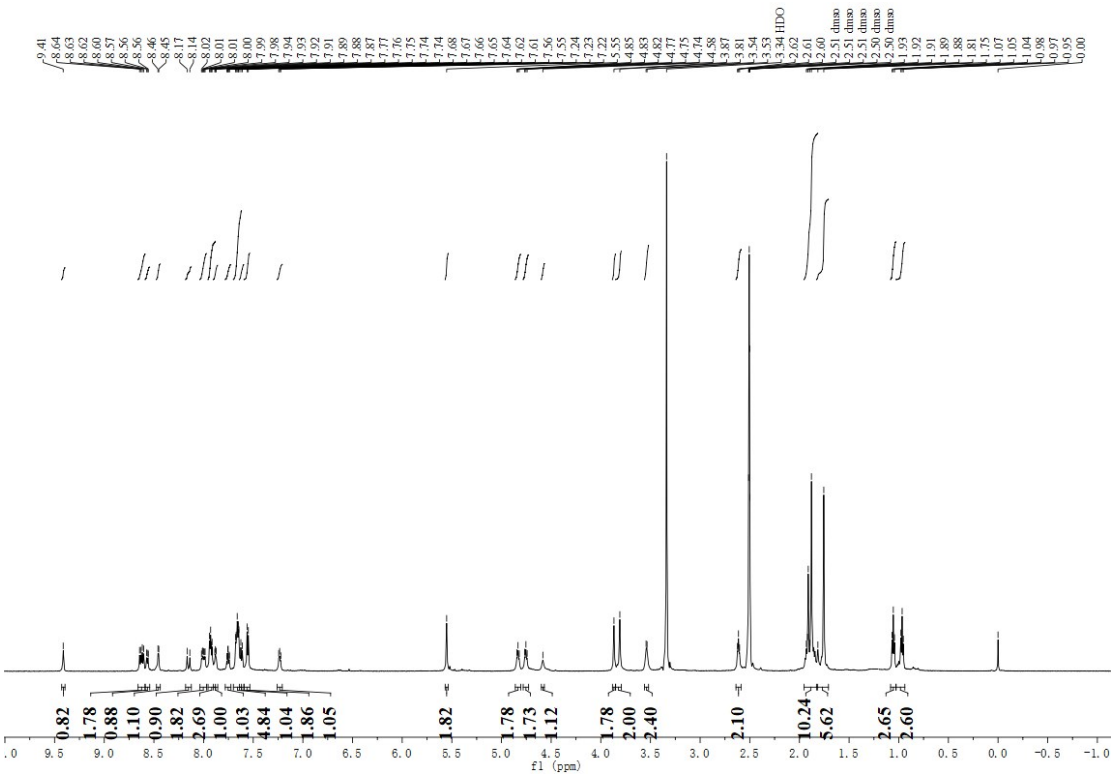


Fig. S17 ^{1}HNMR spectrum of the compound **NPCu**

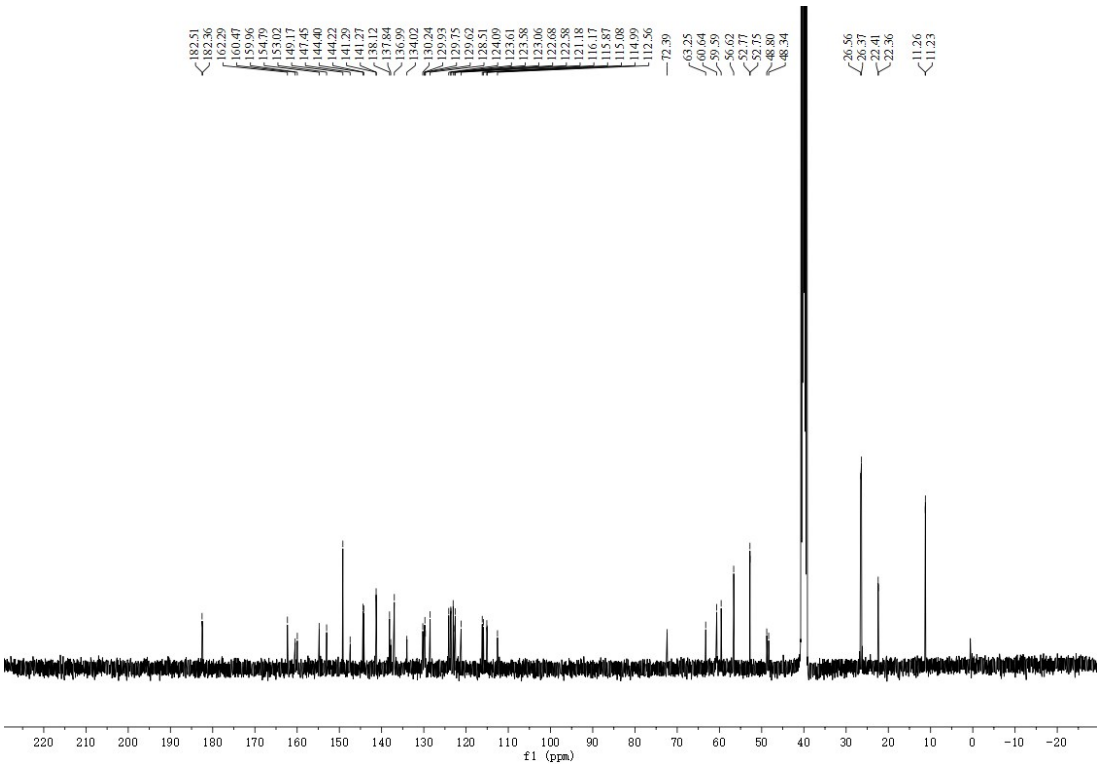
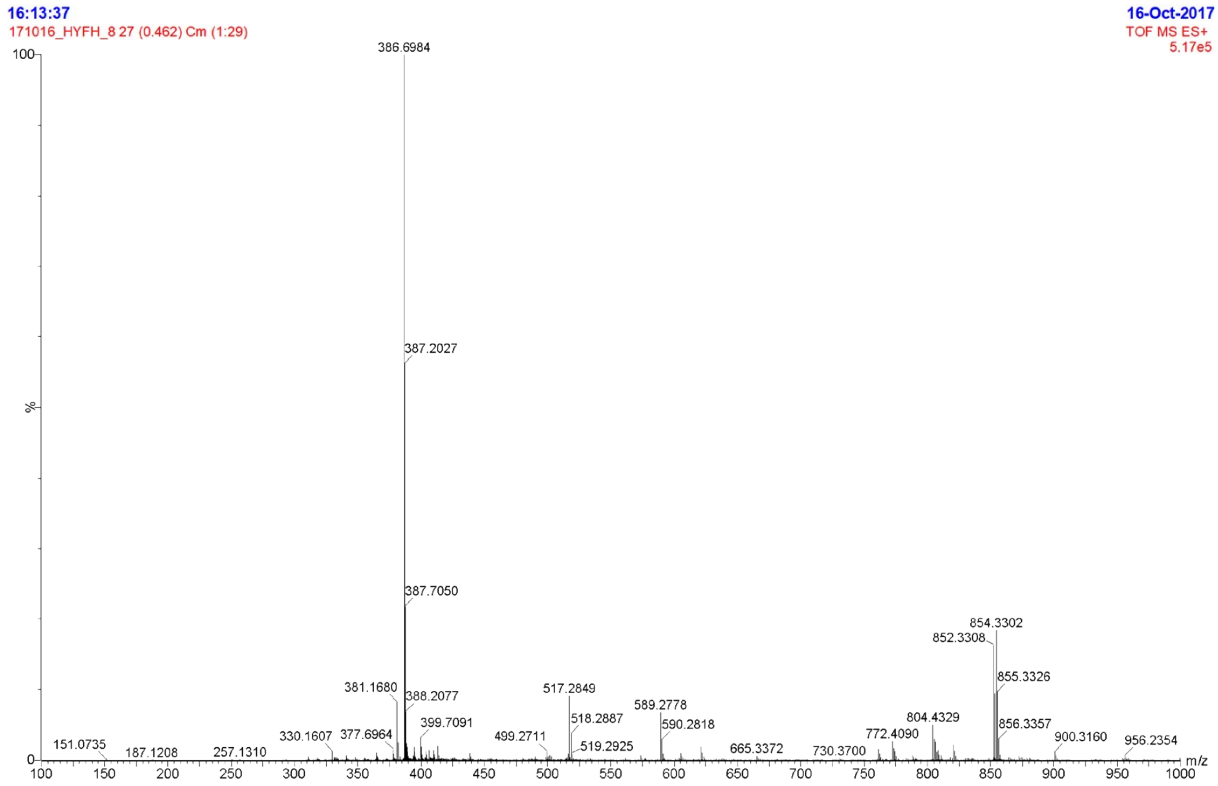


Fig. S18 ^{13}CNMR spectrum of the compound N**PCu**



HRMS spectrum of the compound **NPCu**

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