Supporting Information

A Fluorescent Ratiometric Chemodosimeter for Cu^{2+} Based on TBET and Its Application in Living Cells

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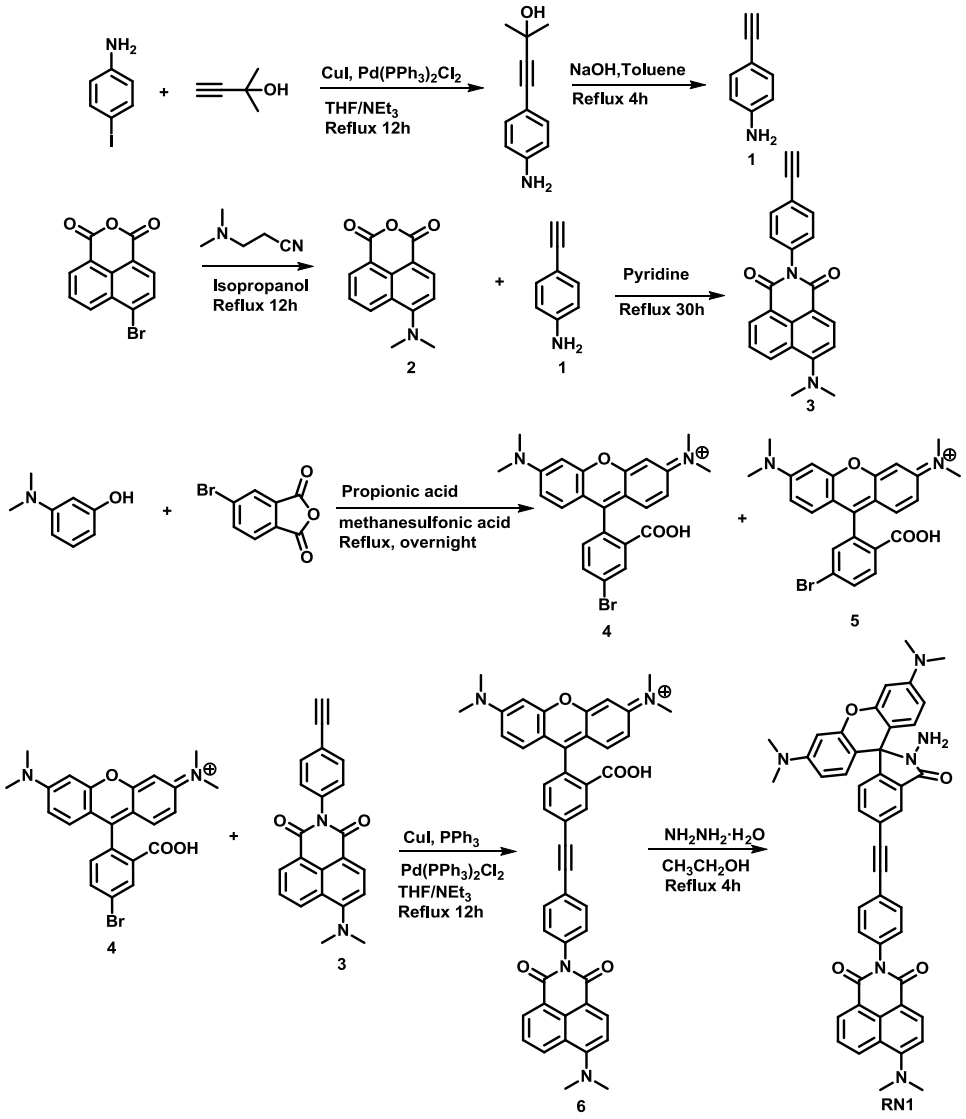
Instruments and experimental procedures

General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Analyte solutions of NaCl, KCl, $CaCl_2$, $MgCl_2·6H_2O$, $CoCl_2·6H_2O$, $CuCl_2·2H_2O$, $BaCl_2·2H_2O$, $NiCl_2·6H_2O$, $HgCl_2$, $CrCl_3$, $ZnCl_2$, $CdCl_2$, $FeCl_3·6H_2O$, $AgNO_3$, $NaNO_3$, NaBr, $NaH_2PO_4·2H_2O$, NaAc, $NaClO_4$, KI, K_2CO_3, $Na_2S·9H_2O$, Na_2HPO_4, and NaClO were prepared by dissolving the salts in distilled water to final concentrations of 5.0 mM for $CuCl_2$ and 25 mM for the other ions. **RN1** was dissolved in dimethyl sulphoxide (DMSO) to produce 5 mM stock solutions. Slight variations in the pH of the solutions were achieved by adding minimal volumes of NaOH or HCl. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer. Chemical shifts (δ) were reported as ppm (in $CDCl_3$ or DMSO, with TMS as the internal standard). Mass spectrometric (MS) data were obtained with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. Fluorescence measurements were performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer. All pH measurements were performed using a Model PHS-3C meter.



Scheme S1. The synthesis route for the compound **RN1**.

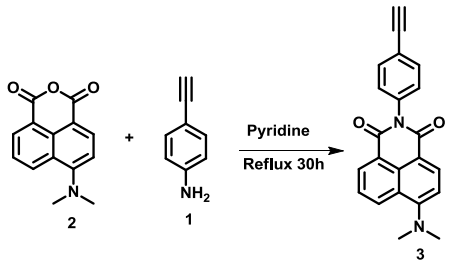
Synthesis

Synthesis of **1**

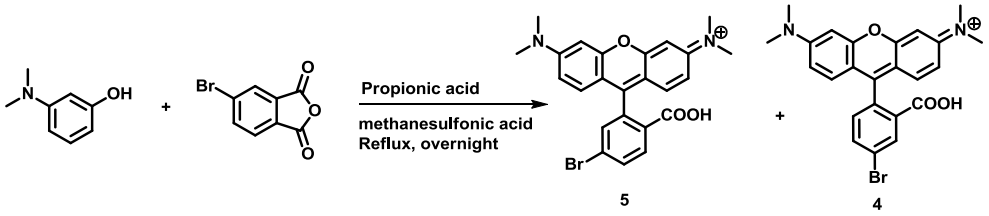
1 was synthesized from 4-iodoaniline by the procedure published in literature[1].

Synthesis of **2**

2 was synthesized as previously described[2].



Synthesis of 3: A solution of **1** (1.5 mmol, 176 mg), 4-*N*, *N*-dimethylamino-1,8-naphthalic anhydride (240 mg, 1 mmol) and $Zn(OAc)_2$ (180 mg, 1 mmol) in 5 mL pyridine was refluxed for 30 h. After completion of the reaction by TLC, the solvent was evaporated. The crude product was purified by column chromatography with CH_2Cl_2/hexane (4/1) and get the desired yellow solid product (300 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.61 (d, 1H, *J* = 8.0 Hz), 8.52 (d, 1H, *J* = 4.0 Hz), 8.49 (d, 1H, *J* = 4.0 Hz), 7.70 (t, 1H, *J* = 4.0 Hz), 7.65(d, 2H, *J* = 8.0 Hz), 7.28 (d, 2H, *J* = 8.0 Hz), 7.15 (d, 1H, *J* = 8.0 Hz), 3.15 (s, 6H), 3.12 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 44.79, 77.85, 83.17, 113.35, 114.71, 122.45, 123.07, 124.94, 125.36, 128.94, 130.74, 131.51, 131.71, 133.02, 133.14, 136.18, 157.35, 163.99, 164.60 ppm.

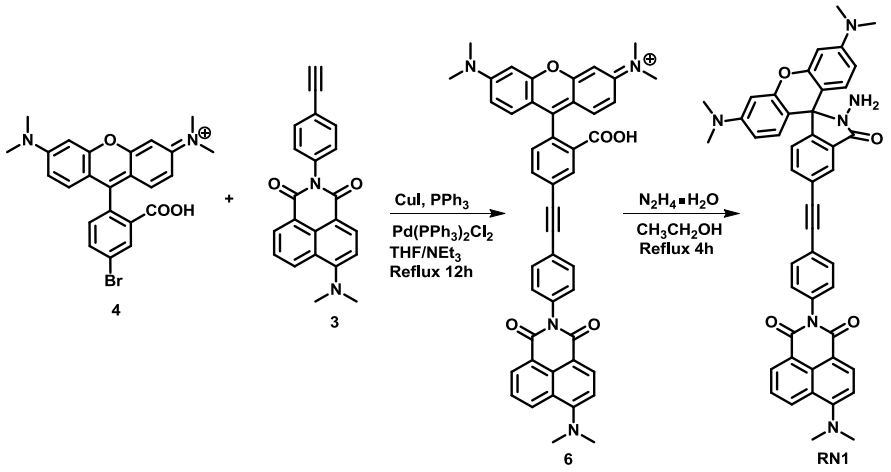


Synthesis of 4 and 5: A mixture of 3-dimethylaminophenol (7.2 g, 52.8 mmol), 4-bromophthalic anhydride (4.8 g, 21.1 mmol), propionic acid (90 mL) and methanesulfonic acid (0.5 mL) was heated at refluxing for 25 h. The resultant dark mass was dissolved in dichloromethane (1000 mL), washed with water, saturated sodium chloride over Na_2SO_4. The solution was concentrated to give a crude mixture of **4** and **5**. The isomers were sepateted by three repetitive silica gel

chromatography eluting with a gradient of methanol (0–15%) in dichloromethane. Concentration of the faster eluting product and slower eluting product afforded 2.12 g of compound **4** and 3.23 g of compound **5**.

Compound 4: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.90 (d, 2H, *J* = 4.0 Hz), 7.49 (s, 1H), 6.56 (d, 2H, *J* = 8.0 Hz), 6.51 (d, 2H, *J* = 8.0 Hz), 6.49 (s, 2H), 3.33 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.38, 45.85, 97.52, 108.63, 110.36, 123.59, 126.19, 127.29, 129.61, 129.77, 135.74, 153.80, 154.46, 168.32, 170.12 ppm.

Compound 5: ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.15 (d, 1H), 7.93 (d, 1H, *J* = 8.0 Hz), 7.19 (d, 1H, *J* = 8.0 Hz), 6.57 (d, 2H, *J* = 8.0 Hz), 6.50 (s, 2H), 6.46 (d, 2H, *J* = 8.0 Hz), 3.33 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.23, 45.90, 98.45, 105.99, 108.77, 126.17, 126.49, 127.43, 128.69, 129.72, 132.92, 152.19, 152.86, 154.83, 168.94 ppm.



Synthesis of RN1: A mixture of **4** (0.5 mmol, 233 mg), **3** (0.5 mmol, 170 mg), 35 mg (0.05 mmol) of $PdCl_2(PPh_3)_2$, and PPh_3 (26 mg, 0.1 mmol), 4.8 mg (0.025 mmol) of CuI, THF (20 mL), NEt_3 (5 mL) under nitrogen, upon the temperature reached 95 °C and refluxed 12 h after completion of the reaction by TLC, evaperated the solvent, the crude product was purified by column chromatography with CH_2Cl_2/CH_3OH/NEt_3 (500/6/6) and afforded the target product

6 as a purplish red (274 mg, 75%). A solution of **6** (0.01 mmol, 73 mg), excess 98% $N_2H_4 \cdot H_2O$ (1 mL) was resolved in 5 mL of ethanol and refluxed 6 h, evaperated the solvent the crude product was purified by column chromatography with CH_2Cl_2/EtOAc (10/3) get the desired product **RN1** (55 mg, 74%).

Compound 6: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.64 (d, 1H, *J* = 4.0 Hz), 8.55 (t, 2H, *J* = 8.0 Hz), 8.20 (s, 1H), 7.74 (d, 1H, *J* = 8.0 Hz), 7.74-7.69 (m, 3H), 7.35 (d, 2H, *J* = 8.0 Hz), 7.17 (t, 2H, *J* = 8.0 Hz), 6.69(d, 2H, *J* = 8.0 Hz), 6.52 (d, 2H, *J* = 8.0 Hz), 6.46 (d, 2H, *J* = 8.0 Hz) , 3.16(s, 6H), 3.03 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.28, 45.90, 63.29, 88.43, 90.74, 98.10, 107.22, 109.38, 113.21, 114.31, 122.75, 122.89, 124.82, 124.86, 124.95, 125.22, 128.62, 129.05, 129.14, 129.33, 130.72, 131.45, 131.88, 132.51, 133.13, 136.28, 137.06, 152.81, 153.54, 157.44, 163.97, 164.61, 168.91, 170.13 ppm.

Compound RN1: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (d, 1H, *J* = 4.0 Hz), 8.52 (t, 2H, *J* = 8.0 Hz), 8.13 (s, 1H), 7.71 (t, 1H, *J* = 8.0 Hz), 7.70 (d, 2H, *J* = 8.0 Hz), 7.65(d, 1H, *J* = 8.0 Hz), 7.32 (d, 2H, *J* = 8.0 Hz), 7.16 (d, 1H, *J* = 8.0 Hz), 7.07 (d, 1H, *J* = 8.0 Hz), 6.60(s, 2H), 6.56 (d, 2H, *J* = 8.0 Hz), 6.46 (d, 2H, *J* = 8.0 Hz) , 3.15(s, 6H), 3.14 (s, 2H), 3.00 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.39, 44.78, 65.82, 89.13, 89.97, 99.28, 106.22, 108.99, 113.36, 114.77, 123.13, 123.22, 123.56, 123.89, 124.94, 125.39, 126.33, 127.96, 129.02, 130.31, 130.75, 131.51, 131.68, 132.56, 133.14, 135.85, 135.92, 151.01, 151.44, 153.47, 157.37, 164.04, 164.64, 165.39 ppm.

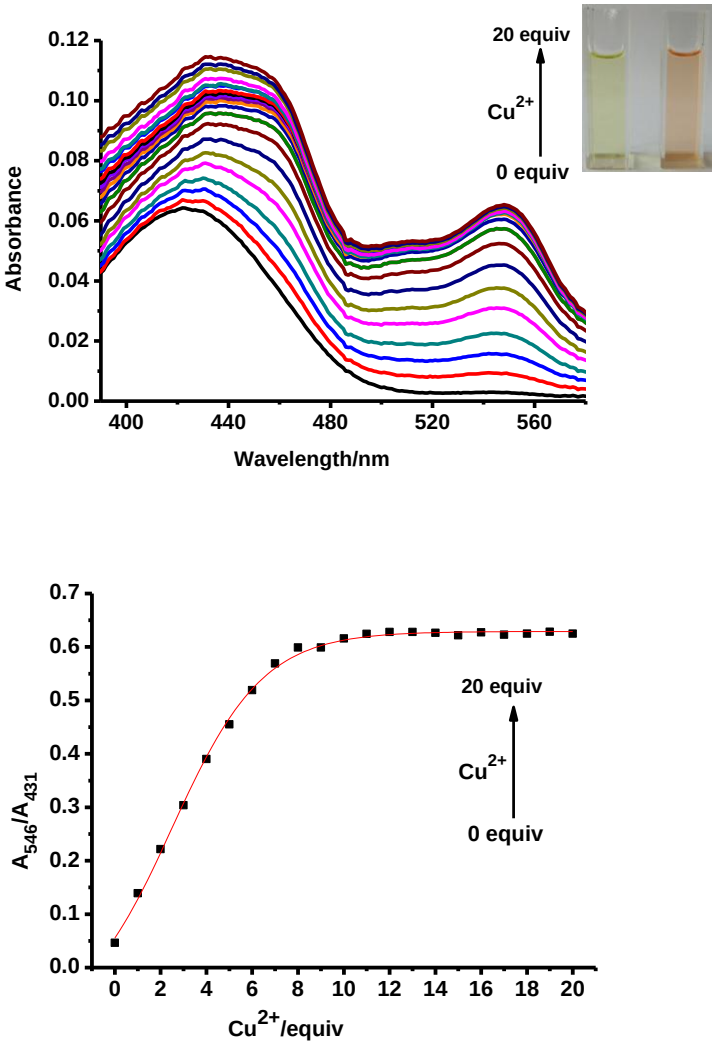


Figure S1. UV- vis spectra of **RN1** (5 μM) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM), in the presence of Cu^{2+} (0─20 equiv). Inset showing the change in color before and after the addition of Cu^{2+}; λ_{ex} = 420 nm.

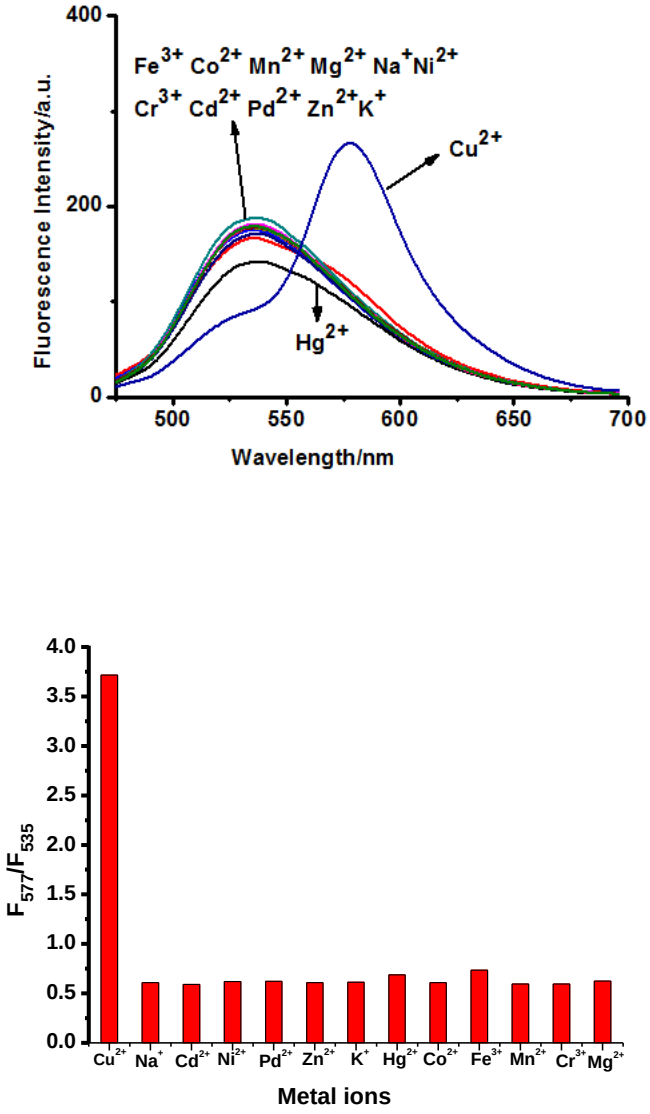


Figure S2. Fluorescence ratio (F_{577}/F_{535}) of **RN1** (5 μM) in the presence of various analytes (50 μM) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM); λ_{ex} = 420 nm.

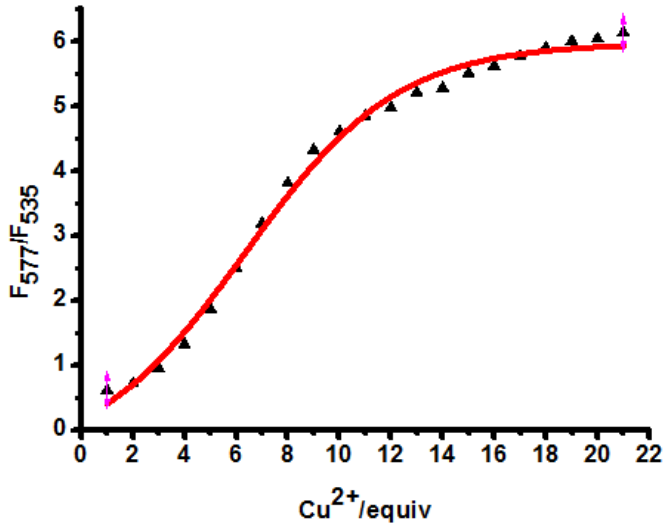


Figure S3. Fluorescence spectra of **RN1** (5 μM) in response to the presence of Cu^{2+} (0—20 equiv) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM); λ_{ex} = 420 nm.

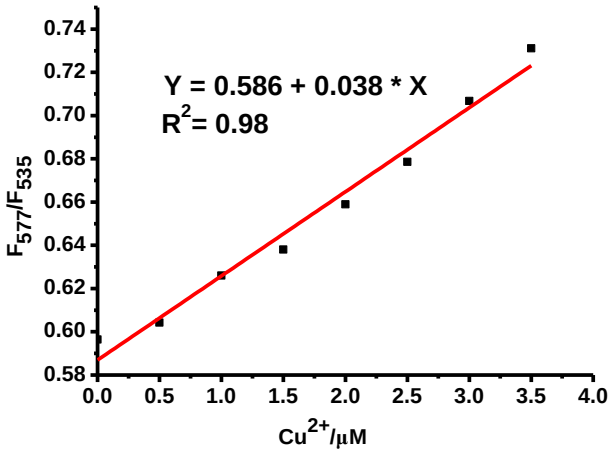


Figure S4. The ratiometric fluorescence responses (F_{577}/F_{535}) of **RN1** (5 μM) to various concentrations of Cu^{2+} (0—3.5 μM) in CH_3CN/H_2O (20/1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM); λ_{ex} = 420 nm.

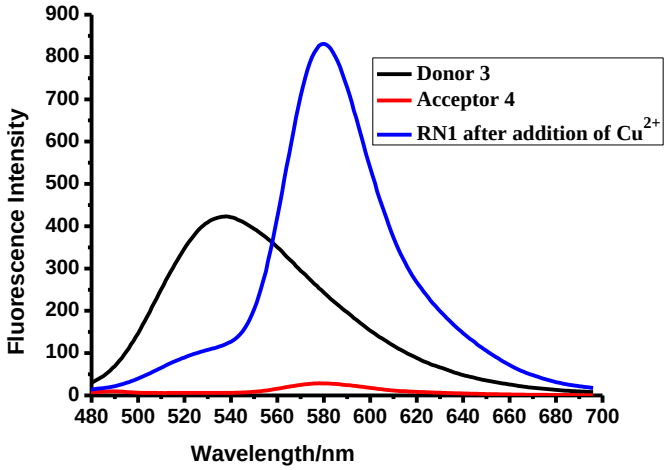


Figure S5. Fluorescence responses of Donor **3** (5 μM), Acceptor **4** (5 μM) and **RN1** (5 μM) after addition of Cu^{2+} in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM); λ_{ex} = 420 nm.

Energy transfer efficiency = [(fluorescence of donor) - (fluorescence of donor in cassette)/ (fluorescence of donor)] ×100

For **RN1,** energy transfer efficiency = [423.51-79.22]/423.51 ×100 = 81

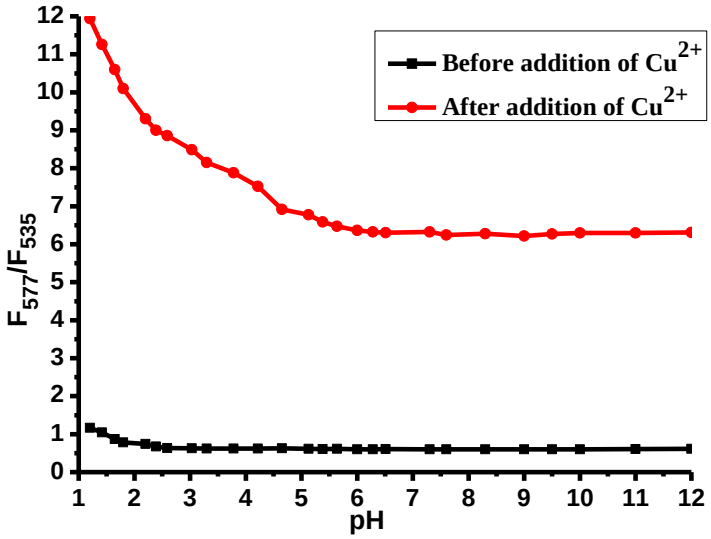


Figure S6. Before and after the addition of Cu^{2+} respectively, the effect of pH on the ratiometric fluorescence responses (F_{577}/F_{535}) of **RN1** (5 μM) in CH_3CN/H_2O (20:1, v/v) buffered with Tris-HCl (pH 7.4, 10 mM). The pH of solution was adjusted by aqueous solution of NaOH (aq, 1 M) or HCl (aq, 1 M); λ_{ex} = 420 nm.

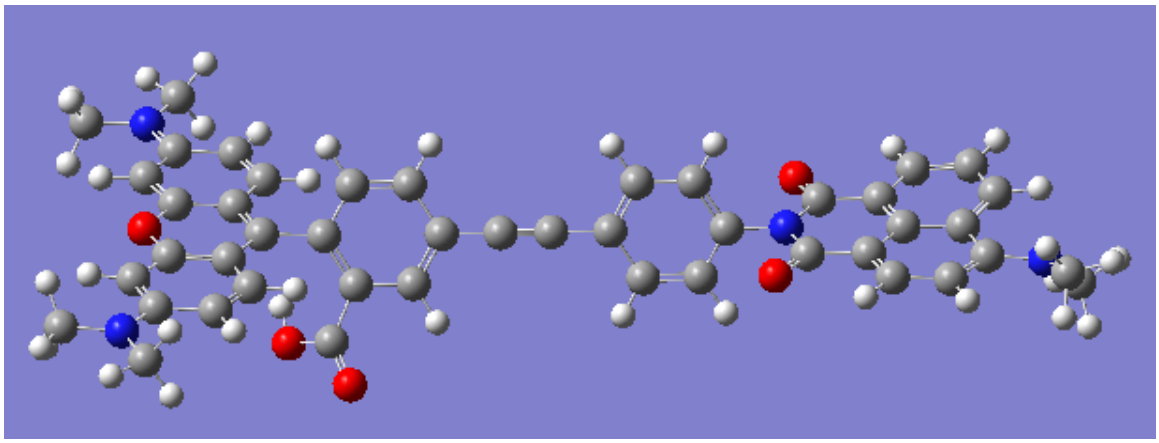


Figure S7. The spatial structure of compound **6**

Cell incubation

The mammalian cells MCF-7 were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 ºC under 5% CO_2. The probe solution of **RN1** was prepared in the solvent of DMSO in 5mL volumetric flask, and then added (5 μM) to the cells and incubation for another 30 min followed. The cells were washed three times with phosphate-buffered saline (PBS). Fluorescence imaging was performed using an OLYMPUS FV-1000 inverted fluorescence microscope with a 100×objective lens. Then 50 μM Cu^{2+} was added into the above cell solution, after culturing for another 30 min, and the white light and fluorescence pictures were obtained with same methods.

Cytotoxicity test

Measurement of cell viability was evaluated by the reduction of MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide) to formazan crystals by mitochondrial dehydrogenases (Mosmann,1983). MCF-7 cells were seeded in 96-well microplates (Nunc, Denmark) at a density of 1×10^5 cells/mL in 100 μL medium containing 10% FBS. After 24 h of cell attachment, cells were cultured in medium with 5 μM of **RN1** for 6 h and 12 h, respectively. Cells in culture medium without **RN1** were used as the control. Six replicate wells were used for each control and test concentration. Plates were then washed with 100 μL/well PBS before 10 μL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37ºC for another 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple products were lysed in 200 μL DMSO. The plate was shaken for 10 min and the absorbance was measured at 570 nm and 630 nm using a microplate reader (Thermo Fisher Scientific). Cell viability was expressed as a percent of the control culture value.

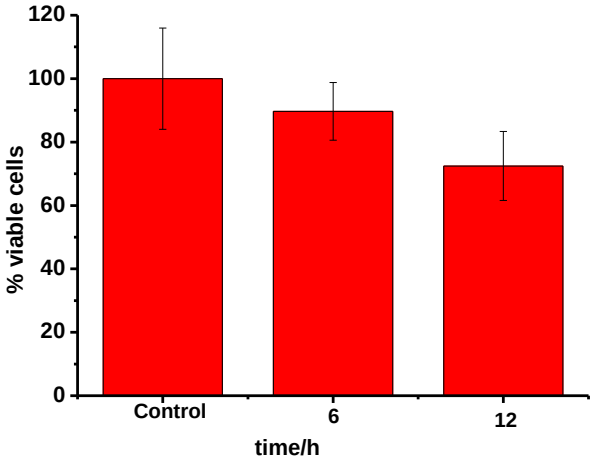


Figure S8. Cytotoxicity studies of **RN1.**

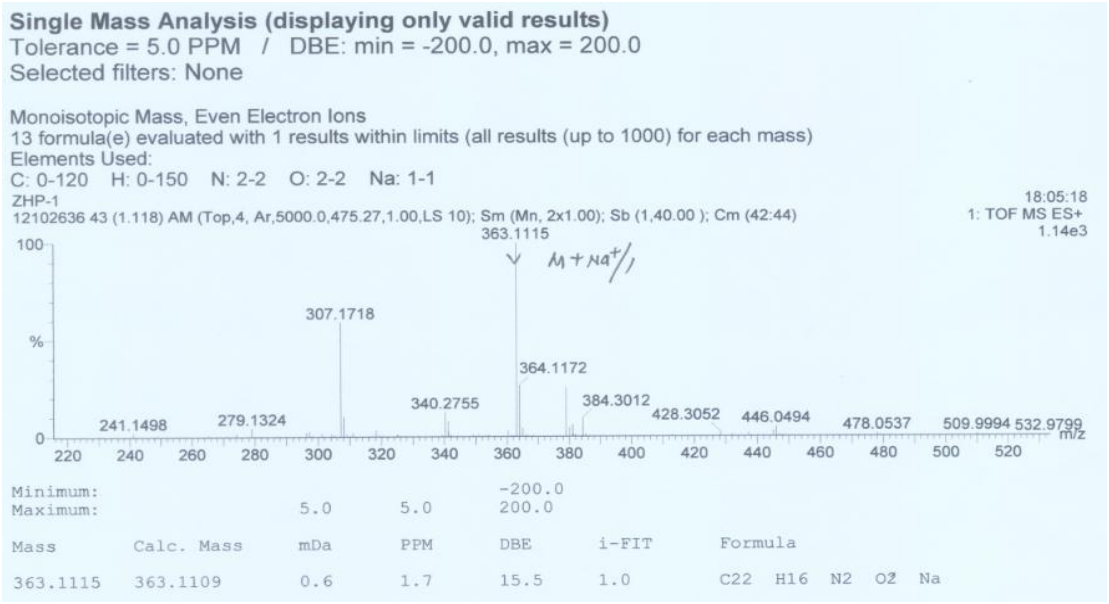


Figure S9. TOF mass of compound **3.**

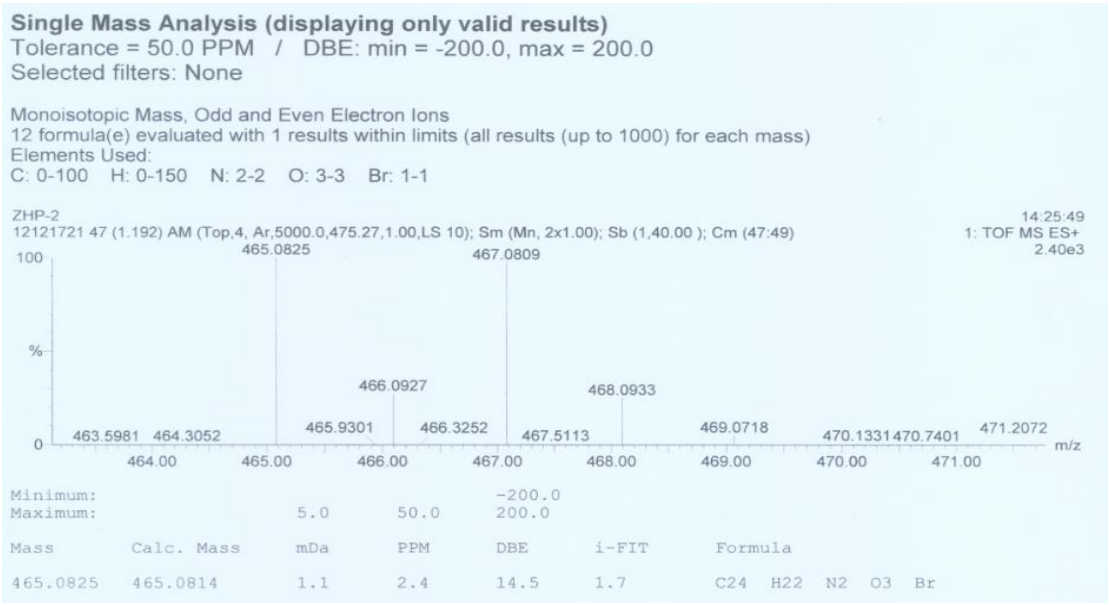


Figure S10. TOF mass of compound **4.**

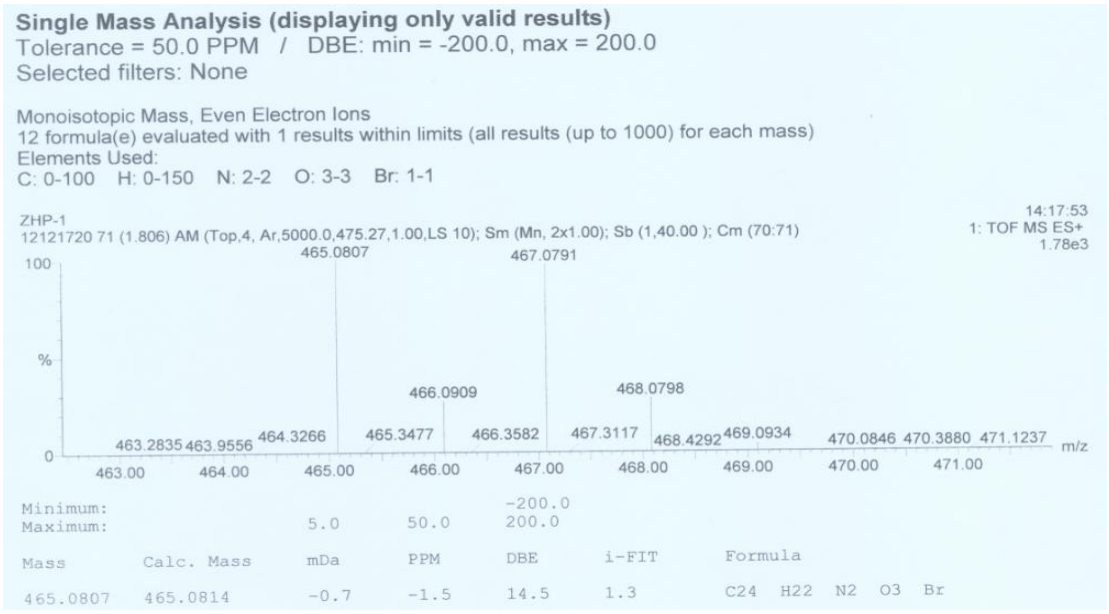


Figure S11. TOF mass of compound **5.**

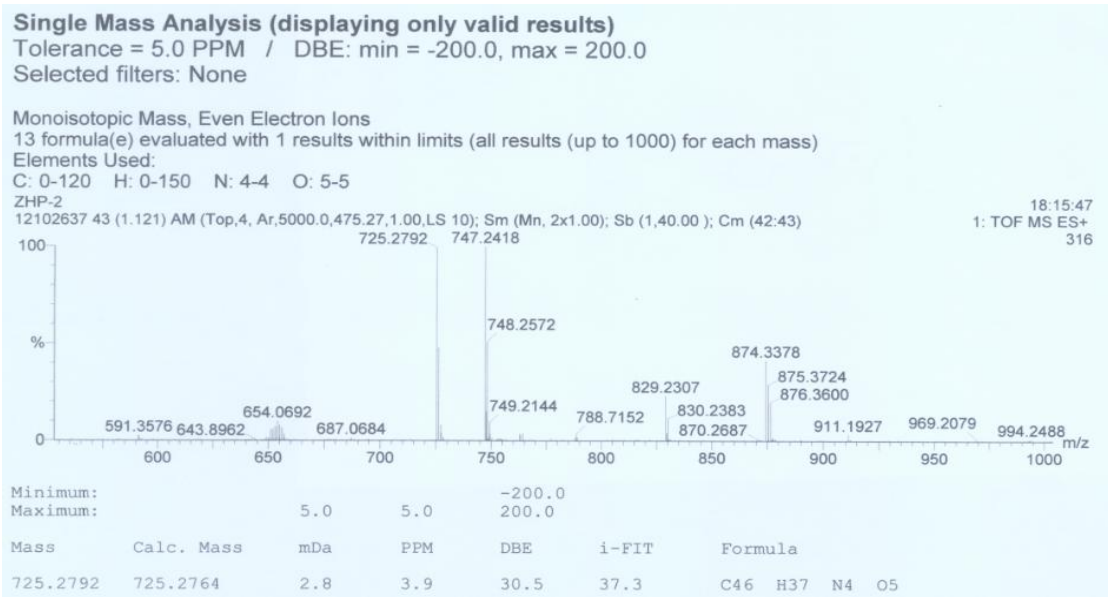


Figure S12. TOF mass of compound **6.**

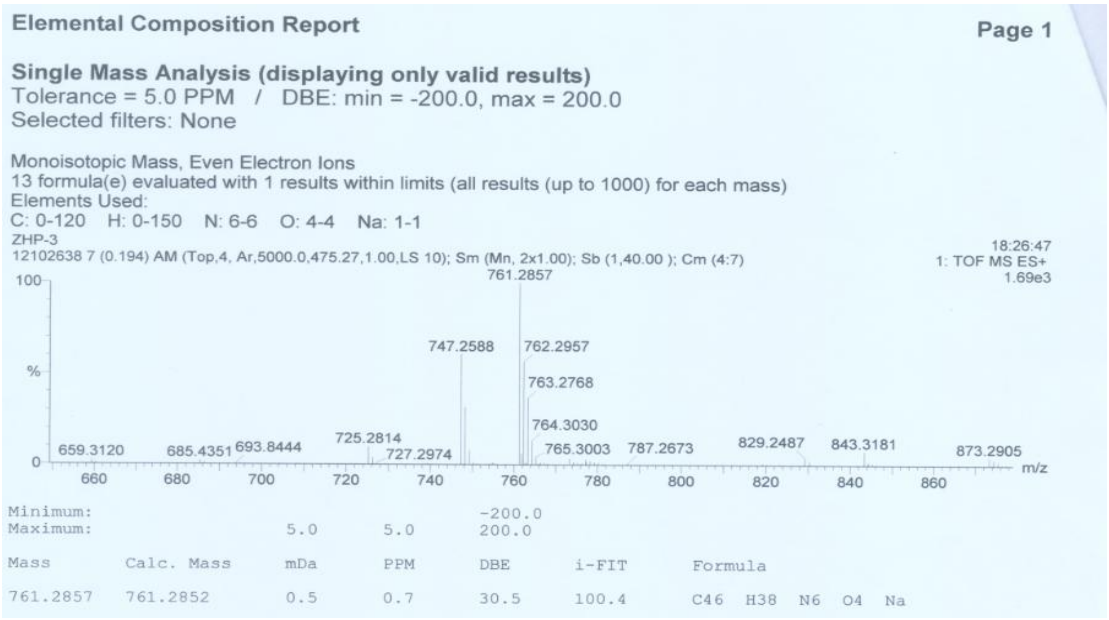


Figure S13. TOF mass of **RN1.**

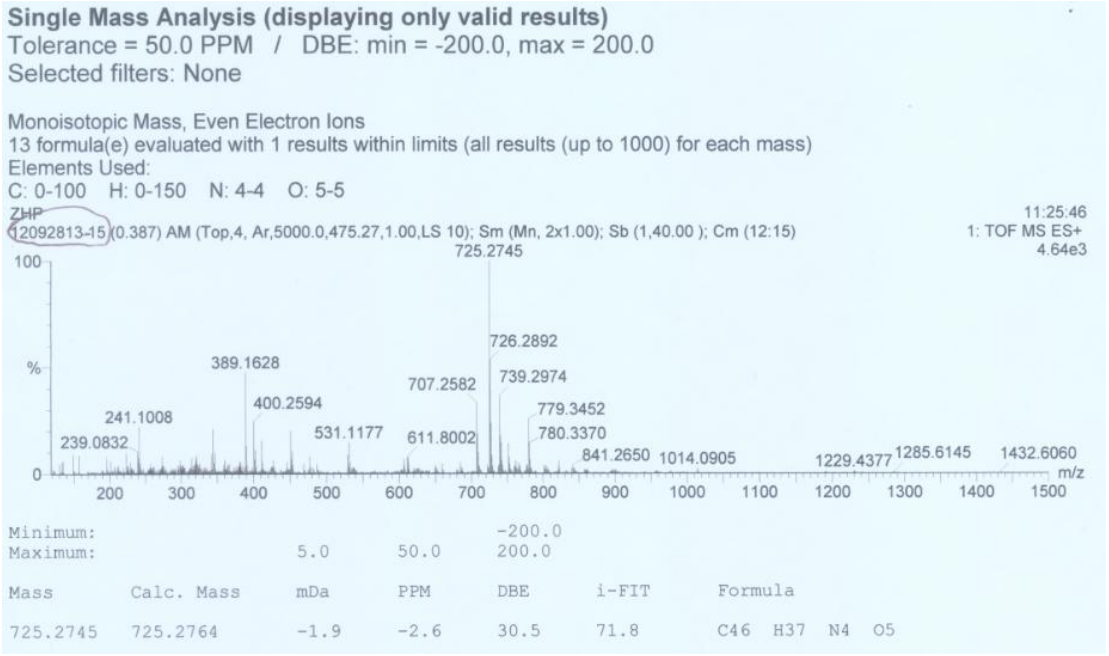


Figure S14. TOF mass of **RN1-Cu^{2+}** complex, a peak at m/z 725.2745 corresponding to compound **6** was observed after the addition of Cu^{2+} to **RN1** aqueous solution, which suggested that Cu^{2+}-induced the hydrolysis and opening of the spirolactam ring of rhodamine moiety.

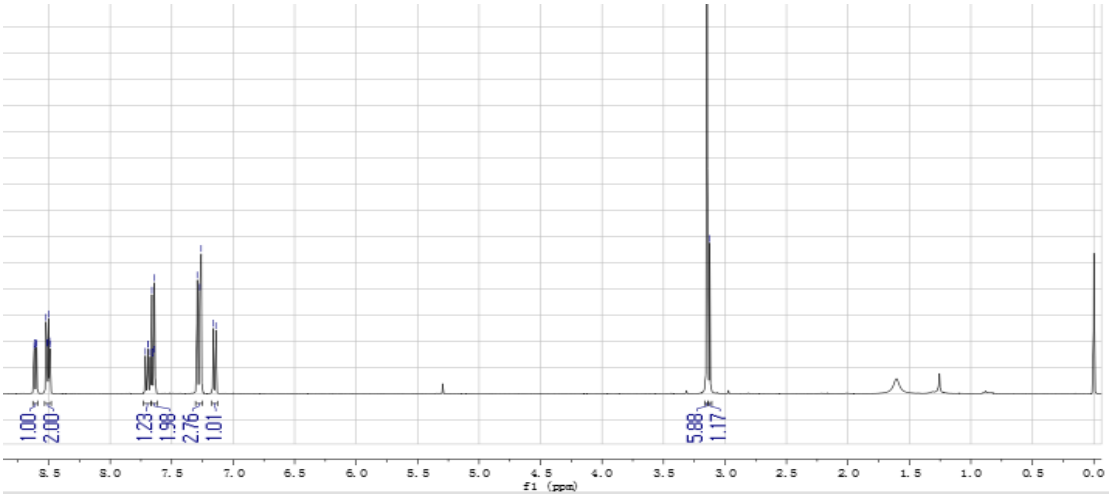


Figure S15. ^{1}H NMR spectrum of compound **3** recorded in $CDCl_3$.

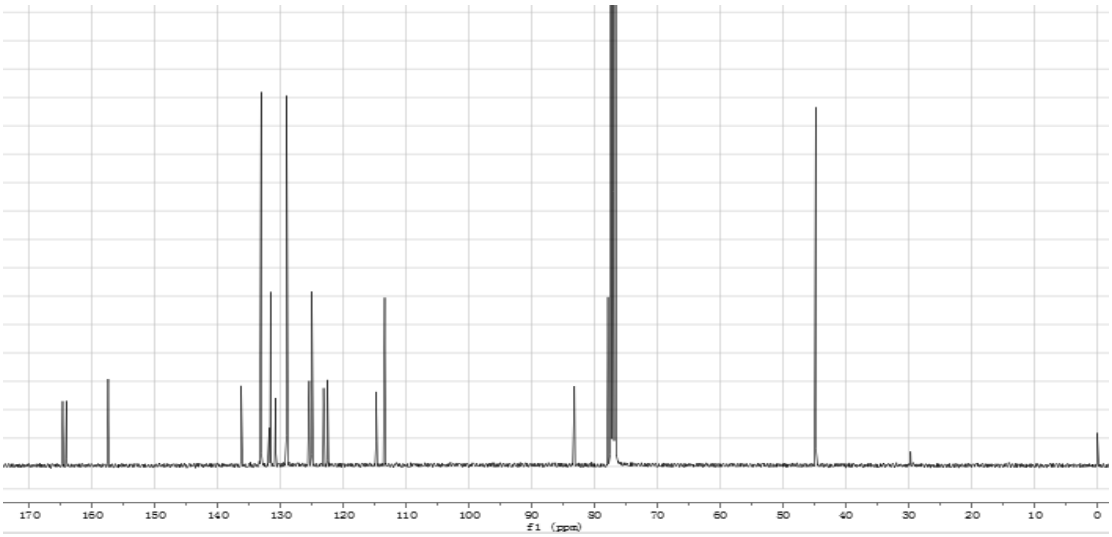


Figure S16. ^{13}C NMR spectrum of compound **3** recorded in $CDCl_3$.

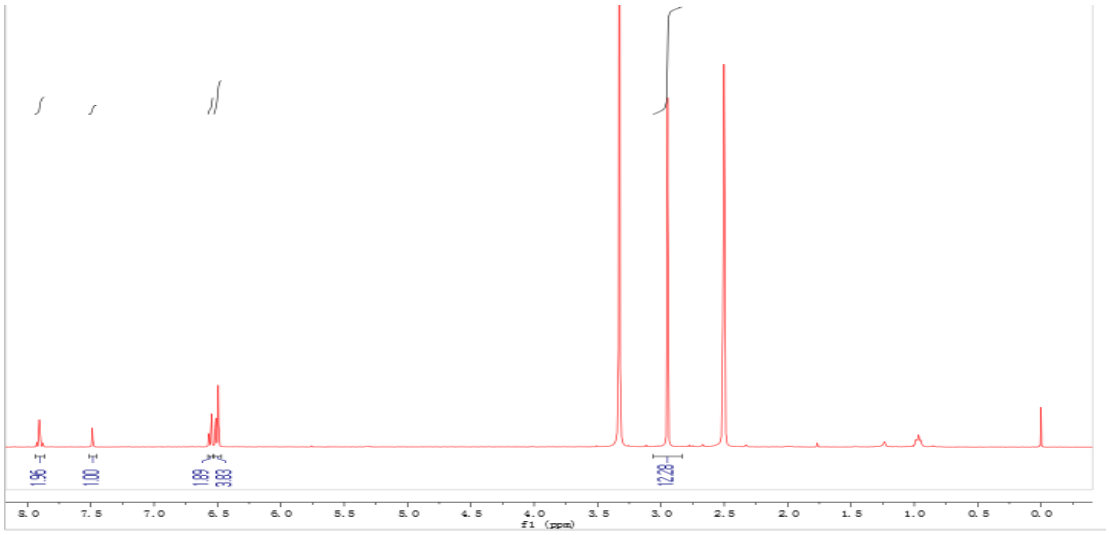


Figure S17. ^{1}H NMR spectrum of compound **5** recorded in DMSO-d_6.

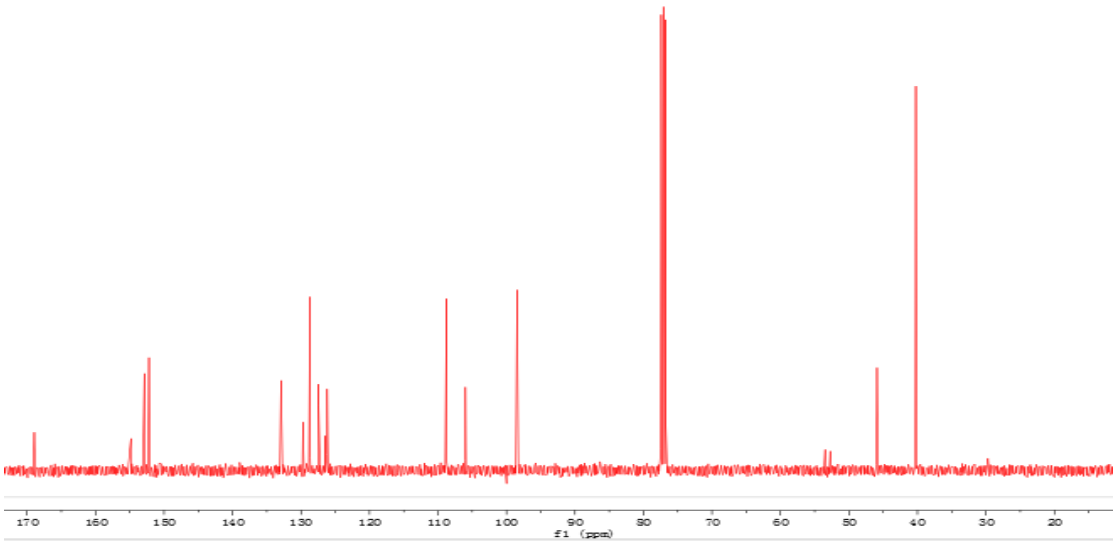


Figure S18. ^{13}C NMR spectrum of compound **5** recorded in $CDCl_3$.

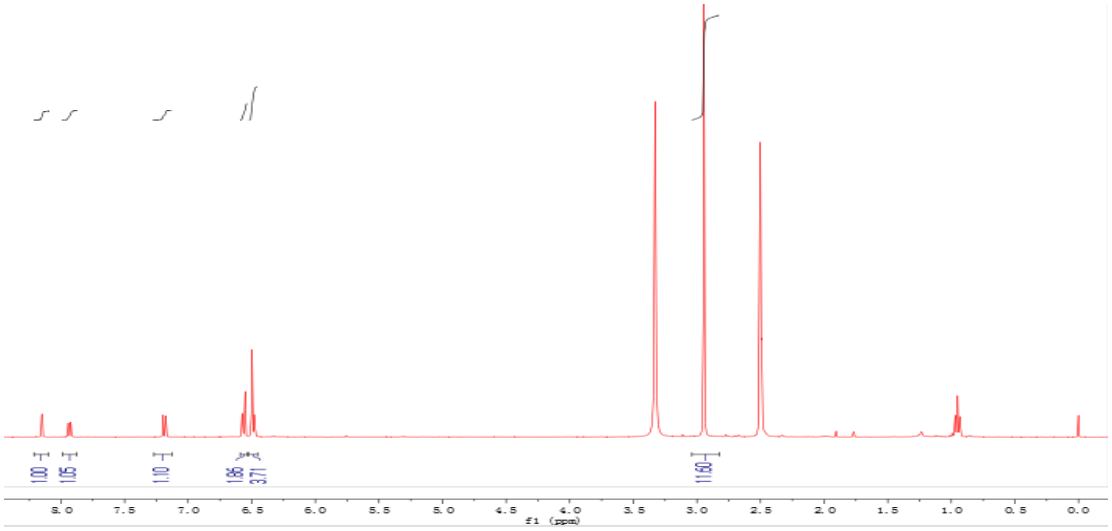


Figure S19. ^{1}H NMR spectrum of compound **4** recorded in DMSO-d_6.

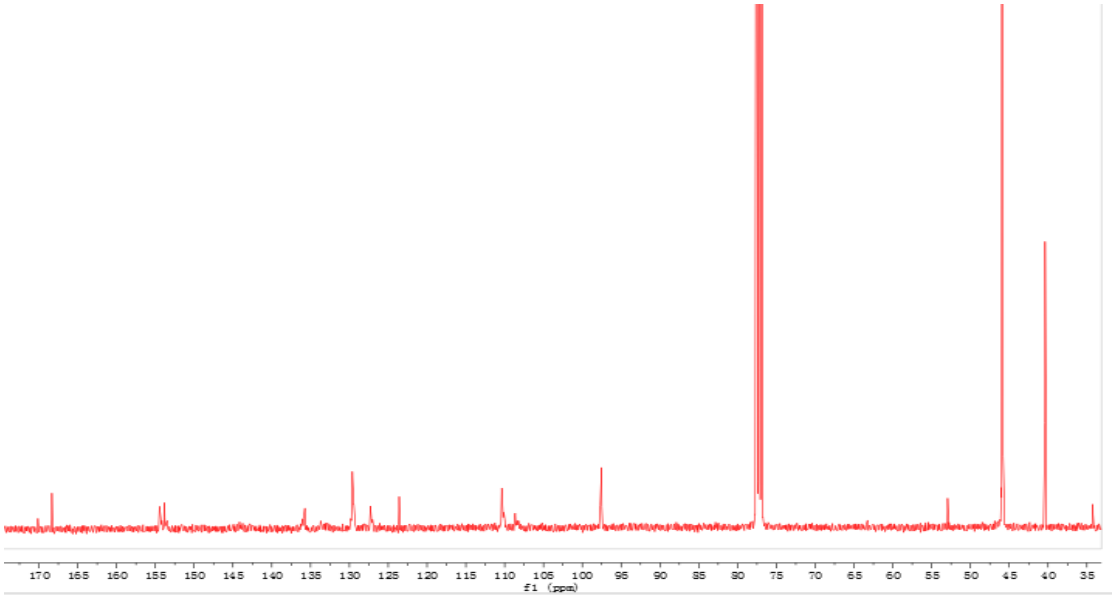


Figure S20. ^{13}C NMR spectrum of compound **4** recorded in $CDCl_3$.

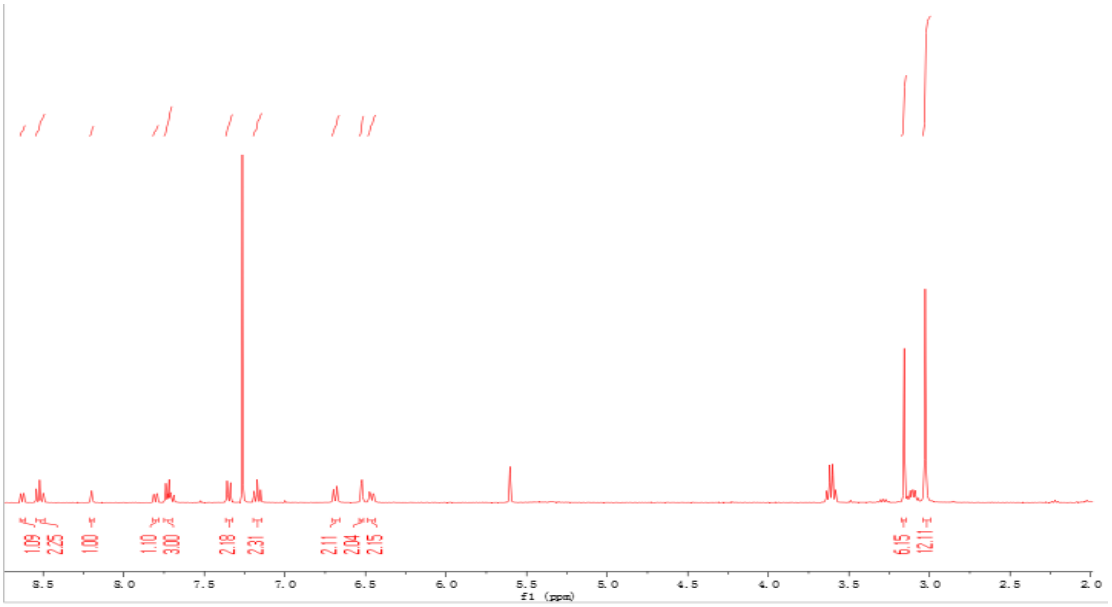


Figure S21. ^{1}H NMR spectrum of compound **6** recorded in $CDCl_3$.

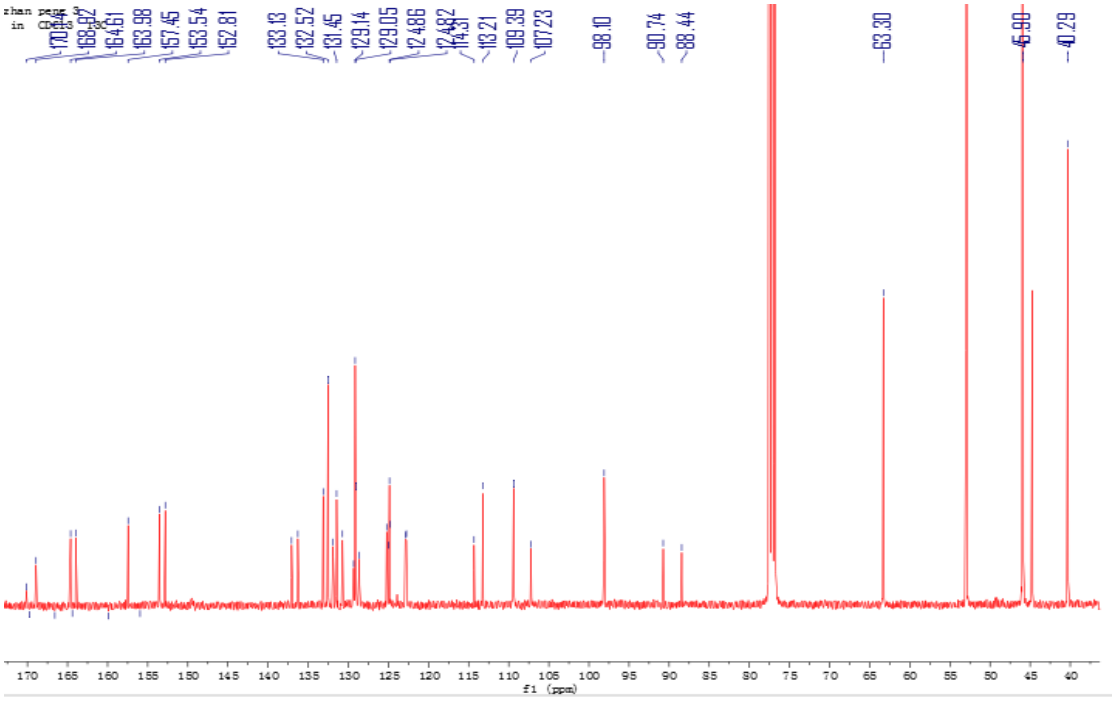


Figure S22. ^{13}C NMR spectrum of compound **6** recorded in $CDCl_3$.

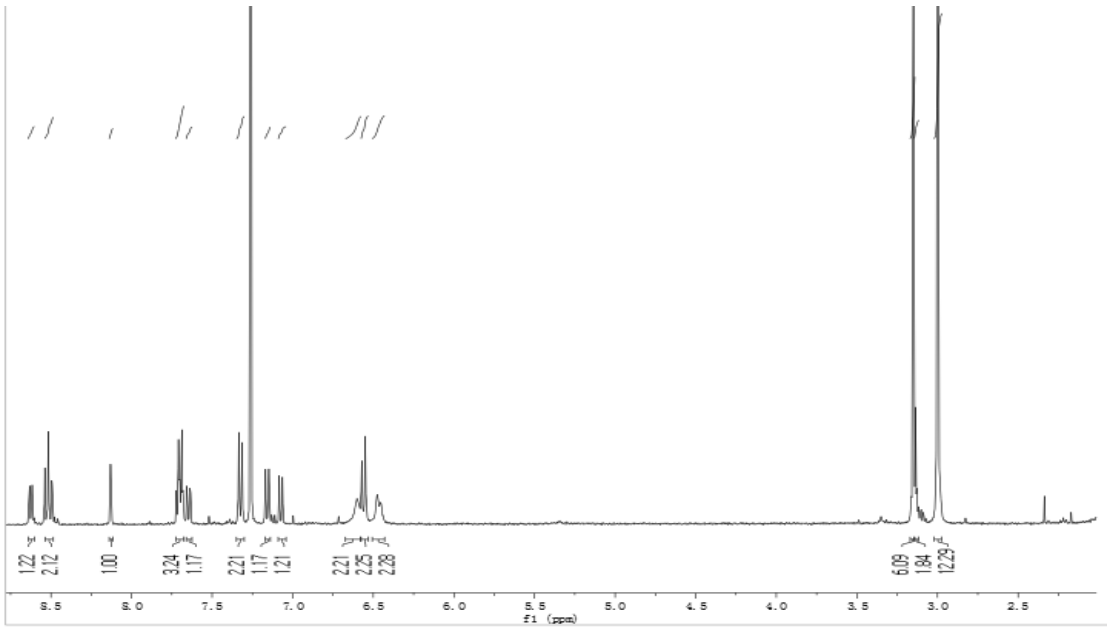


Figure S23. ^{1}H NMR spectrum of **RN1** recorded in $CDCl_3$.

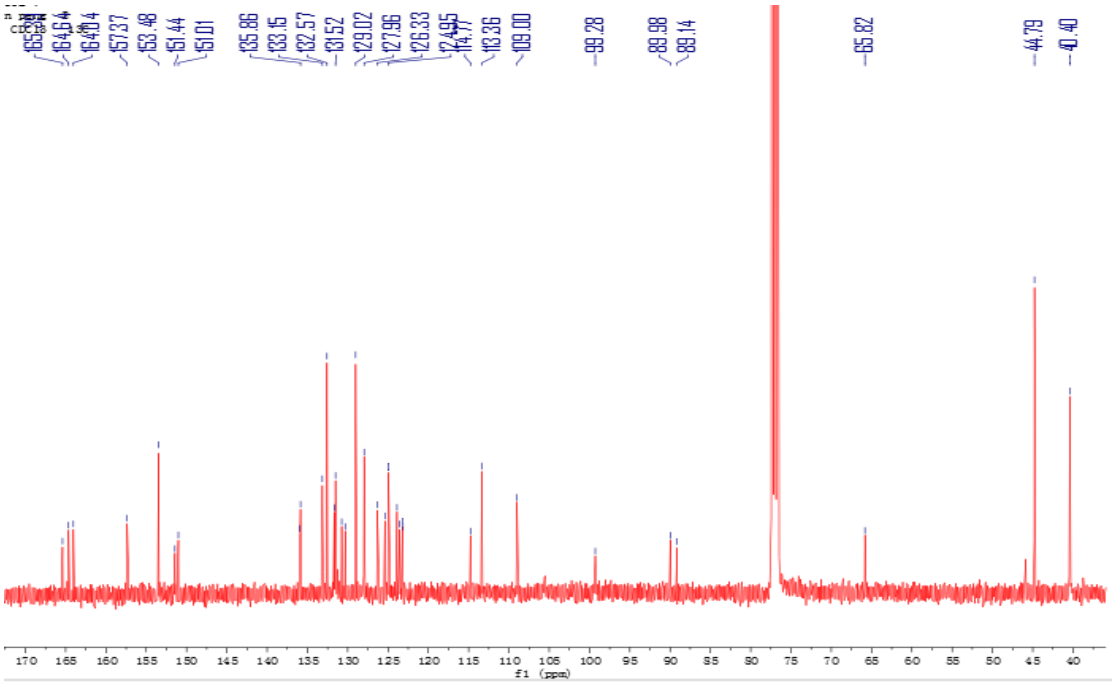


Figure S24. ^{13}C NMR spectrum of **RN1** recorded in $CDCl_3$.

Reference

1. A. K. Flatt, Y. Yao, F. Maya and J. M. Tour, *J. Org. Chem.* **2004**, *69*, 1752.

2. G. Loving and B. Imperiali, *J. Am. Chem. Soc.* **2008**, *130*, 13630.