**Supporting Information**

# Naphthylamine–rhodamine-based ratiometric fluorescent probe for the determination of $Pd^{2+}$ ions

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**Reagents and instruments**

All starting materials were purchased and used without further purication. All solvents were analytical grade. The stock solution of **RN** and $PtCl_2$ were prepared in DMSO. The stock solution of $PdCl_2$ was prepared in 3:1 (v/v) MeOH/brine. The stock solution of $RuCl_3$ and $RhCl_3$ were prepared in 1:1 (v/v) MeOH/$H_2O$. The stock solution of other metal salts used in the experiments were prepared in distilled water. $^1H$ NMR and $^{13}C$ NMR spectra were obtained by Bruker Avance II 400M spectrometer (in DMSO-d or $CDCl_3$, TMS as internal standard). Mass spectrometry data were obtained by HP1100 LC/MSD mass spectrometer or LTQ Orbitrap XL TM mass spectrometer. Fluorescence spectra were obtained by Agilent Cary Eclipse fluorescence spectrophotometer. Absorption spectra were obtained by Agilent 8453 UV-Visible spectrophotometer. The pH values were obtained by PHS-3C pH meter model.

The bioimaging experiments on living mice utilized the NightOWL II LB983 small animal *in vivo* imaging system equipped with a sensitive Charge Coupled Device (CCD) camera, with the excitation at 480 nm and the 600 ± 20 nm emission filter. Healthy mice (seven weeks old, 20–25 g) was used, and animals had free access to food and water. All the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Dalian Medical College, China.

**Synthesis of R2**: Rhodamine B (**RB**, 5 g, 10.4 mmol) and ethylenediamine (9 mL, 134.8 mmol) were dissolved in ethanol (50 mL) in a 250 mL flask, then the mixture was heated at reflux for 7 h. After ethanol was removed under vacuum, the residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH, 10:1) to give **R2** as a pale yellow powder (4.7 g, yield: 93%). $^1H$ NMR (400 MHz, $CDCl_3$, $\delta$ ppm): 7.94 – 7.86 (m, 1H), 7.50 – 7.38 (m, 2H), 7.14 – 7.03 (m, 1H), 6.43 (dd, $J$ = 8.8, 4.0 Hz, 2H), 6.37 (d, $J$ = 2.6 Hz, 2H), 6.27 (dd, $J$ = 8.9, 2.6 Hz, 2H), 3.42 – 3.24 (m, 8H), 3.19 (t, $J$ = 6.6 Hz, 2H), 2.43 (t, $J$ = 6.6 Hz, 2H), 1.16 (t, $J$ = 7.0 Hz, 12H). ES-API: $[M+H]^+$, calcd: $m/z$ = 485.29, found: $m/z$ = 485.3.

**Synthesis of RN**: 3-Amino-2-naphthoic acid (200 mg, 1.1 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 250 mg, 1.3 mmol) and catalytic amount of 4-dimethylaminopyridine (DMAP) were add to a 100 mL flask with dichloromethane (20 mL), shaked to mix, then added **R2** (500 mg, 1.0 mmol) to the flask and reflux for 12 h. After dichloromethane was removed under vacuum, the residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH, 100:1) to give **RN** as a yellow powder (232

mg, yield: 33%). $^{1}$H NMR (400 MHz, $CDCl_3$, $\delta$ ppm): 8.06 (s, 2H), 7.94 (dd, J = 5.8, 2.8 Hz, 2H), 7.81 (d, J = 8.1 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.48 – 7.43 (m, 2H), 7.41 – 7.34 (m, 2H), 7.25 – 7.19 (m, 1H), 7.13 – 7.06 (m, 1H), 6.94 (s, 1H), 6.47 (d, J = 8.9 Hz, 2H), 6.41 (s, 2H), 6.28 (dd, J = 8.9, 2.5 Hz, 2H), 3.55 – 3.41 (m, 2H), 3.30 (q, J = 7.1 Hz, 8H), 3.19 (dt, J = 22.8, 11.3 Hz, 2H), 1.15 (t, J = 7.1 Hz, 12H). $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$ ppm): 168.6, 167.8, 154.1, 152.8, 148.6, 146.0, 135.8, 132.9, 130.2, 128.9, 128.5, 127.6, 125.3, 124.8, 123.7, 122.5, 121.7, 120.7, 108.4, 105.0, 97.6, 64.5, 43.8, 37.9, 29.2, 12.6. HRMS: $[M+H]^+$, calcd: *m/z* = 654.3444, found: *m/z* = 654.3422.

**Fluorescence quantum yield ($\Phi$) measurements.**

The relative fluorescence quantum yields were determined with Rhodamine B ($\Phi_F$ = 0.97) in ethanol as a standard and calculated using the following equation.[1]

$\Phi_x = \Phi_s (F_x / F_s) (A_s / A_x) (\lambda_{exs}/\lambda_{exx}) (n_x/n_s)^2$

Where Φ represents quantum yield; F stands for integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; $\lambda_{ex}$ is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions ($10^{-7}$-$10^{-8}$ mol/L), the refractive indices of the solutions were replaced with those of the solvents); and the subscripts x and s refer to the unknown and the standard, respectively.

Therefore, the fluorescence quantum yield of **RN** and its $Pd^{2+}$ complex were 0.08 and 0.14, respectively.

**References**:

1. Velapoldi, R. A.; Tønnese, H. H. *J. Lumin.* **2004**, 14, 465-472.

**Dissociation constant measurements.**

The dissociation constant was determined by fluorescence titration method for **RN** with $Pd^{2+}$, the result was obtained by Origin software simulation.[2]

Therefore, the dissociation constant for **RN** with $Pd^{2+}$ was $7.75 \times 10^{-6}$ M.

**References**:

2. Goswami, S.; Sen, D.; Das, N. K.; Fun, H.; Quah, C. K. *Chem. Commun.* **2011**, 47, 9101.

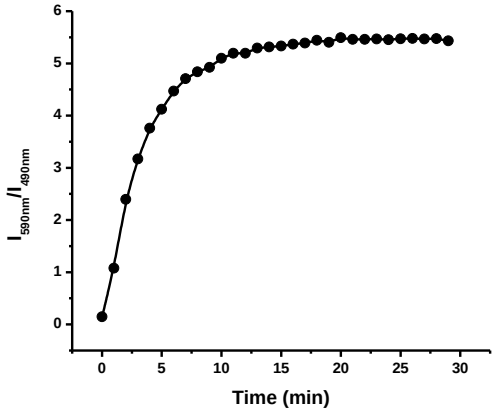


**Figure S1.** Time-dependent fluorescent intensities ratio ($I_{590\ nm}/I_{490\ nm}$) change of **RN** (10 μM) with $PdCl_2$ (10 μM) in EtOH/$H_2O$ (1:1, v/v) at room temperature, $\lambda_{ex}$ = 420 nm.

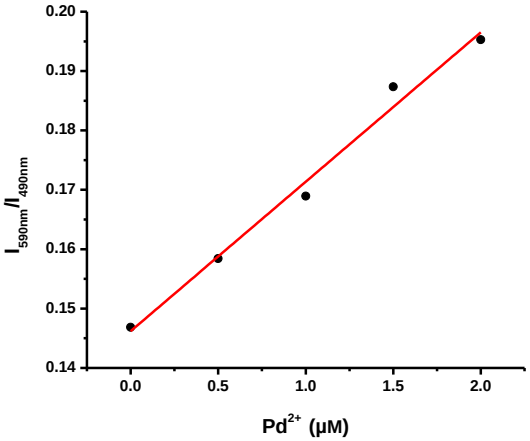


**Figure S2.** Fluorescent intensities ratio ($I_{590\ nm}/I_{490\ nm}$) change of **RN** (10 μM) in the presence of different concentrations of $Pd^{2+}$ (0 – 2 μM). $R^2$ = 0.9836.

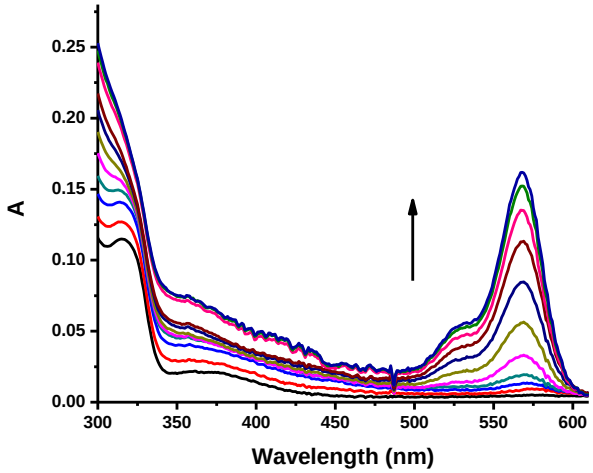


**Figure S3.** Absorption spectra of **RN** (10 μM) upon titration of $PdCl_2$ (0 – 10 μM) in EtOH/$H_2O$ (1:1, v/v).

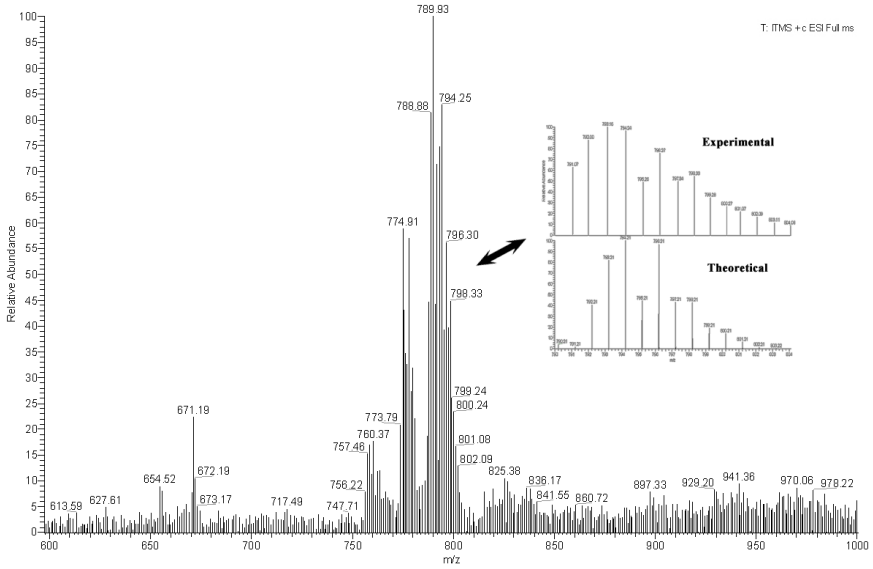


**Figure S4.** MS of [**RN**+$Pd^{2+}$+$Cl^{-}$]$^{+}$.

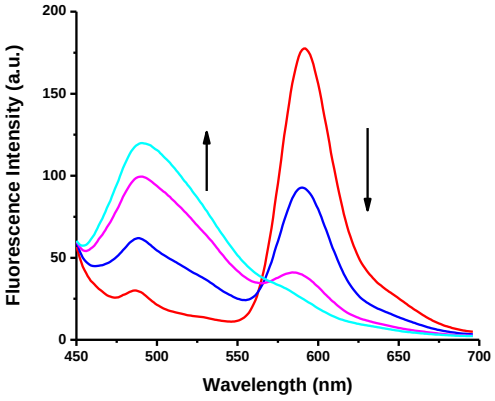


**Figure S5.** Fluorescence spectral changes of **RN** (10 μM)/$Pd^{2+}$ (10 μM) upon addition of EDTA-2Na (0 – 30 μM) in EtOH/$H_2O$ (1:1, v/v). $\lambda_{ex}$ = 420 nm.

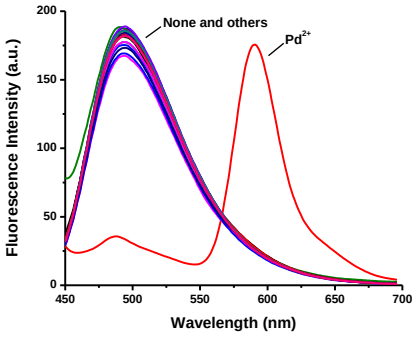


**Figure S6.** Fluorescence spectra of **RN** (10 μM) in the presence of different metal ions (10 μM for $Pd^{2+}$ and 20 μM for others) in EtOH/$H_2O$ (1:1, v/v). $\lambda_{ex}$ = 420 nm.

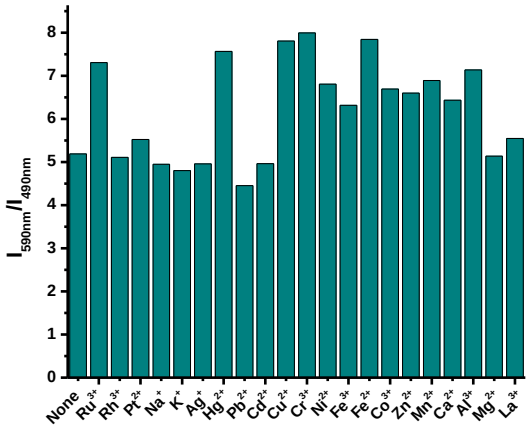


**Figure S7.** Fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) of **RN** (10 μM) after addition of $Pd^{2+}$ (10 μM) in the presence of other metal ions (20 μM) in EtOH/$H_2O$ (1:1, v/v). $\lambda_{ex}$ = 420 nm.

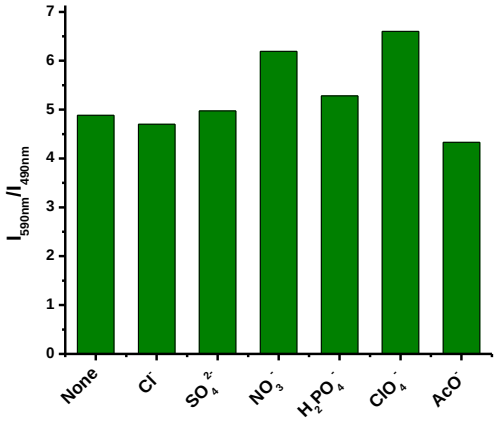


**Figure S8.** Fluorescence intensity ratio ($I_{590\ nm}/I_{490\ nm}$) of **RN** (10 μM) after addition of $Pd^{2+}$ (10 μM) in the presence of common anions (20 μM) in EtOH/$H_2O$ (1:1, v/v). $\lambda_{ex}$ = 420 nm.

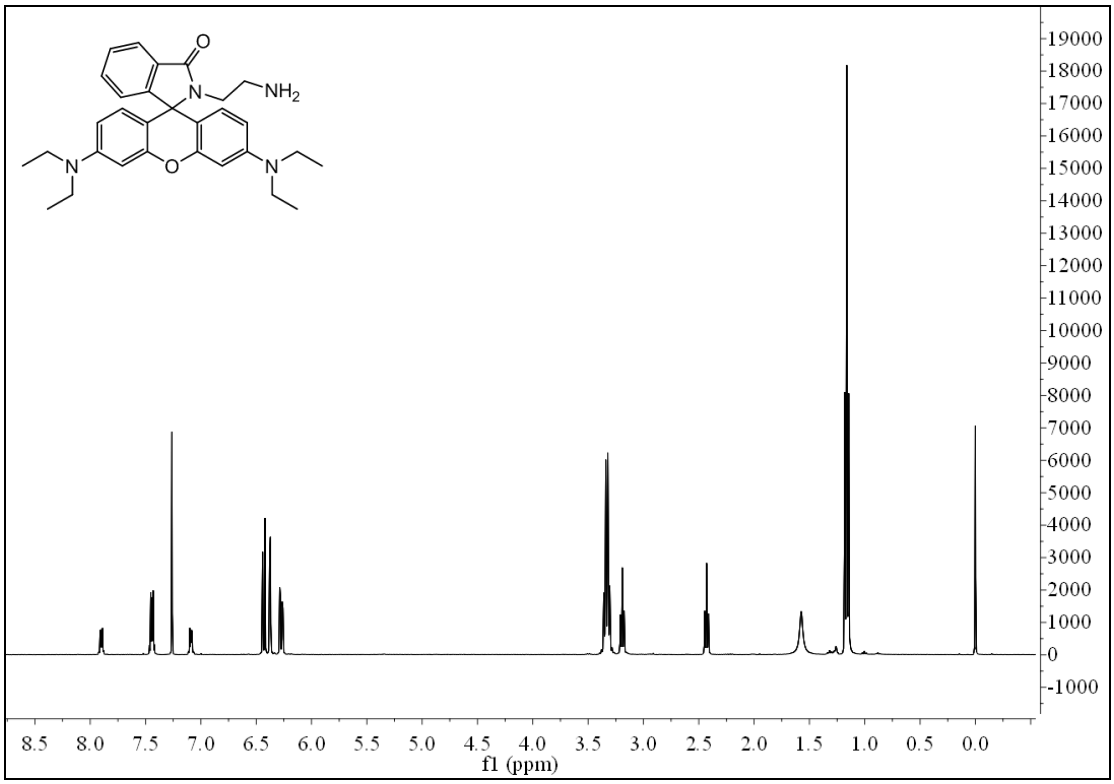


**Figure S9.** $^{1}$H NMR of **R2**.

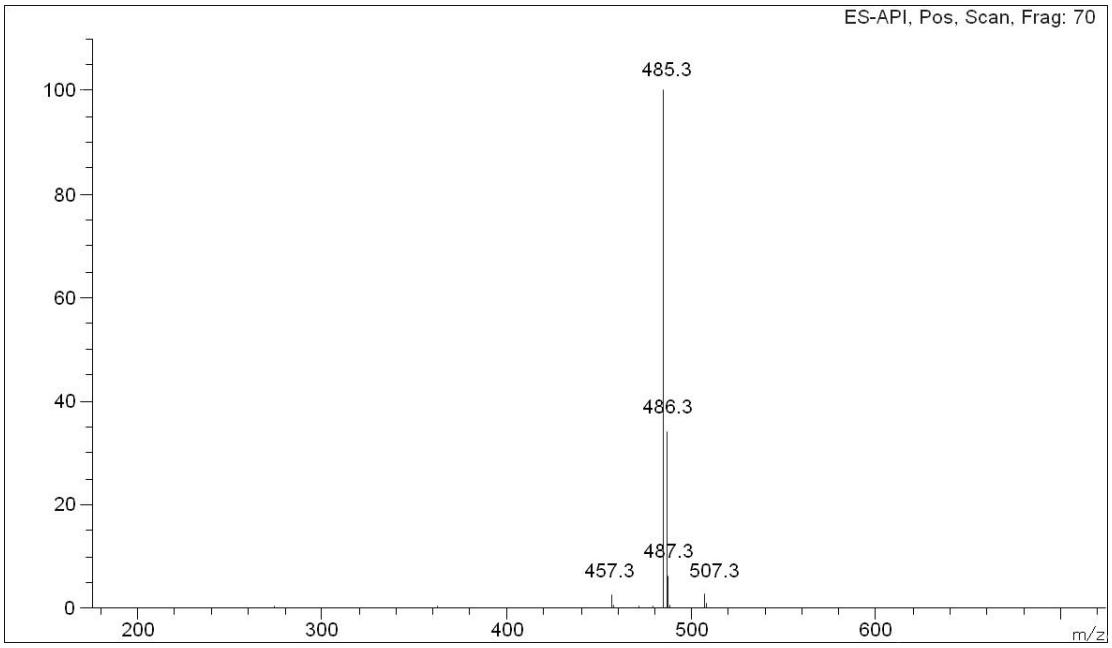


**Figure S10.** MS of **R2**.

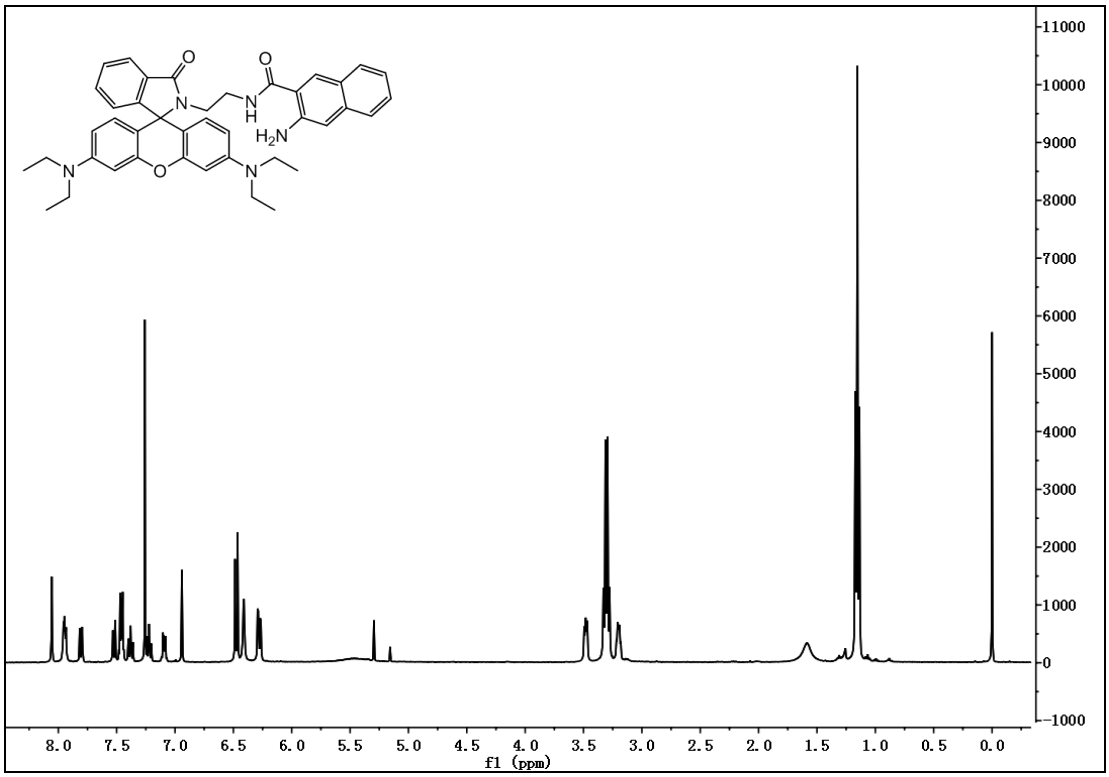


**Figure S11.** $^{1}$H NMR of **RN**.

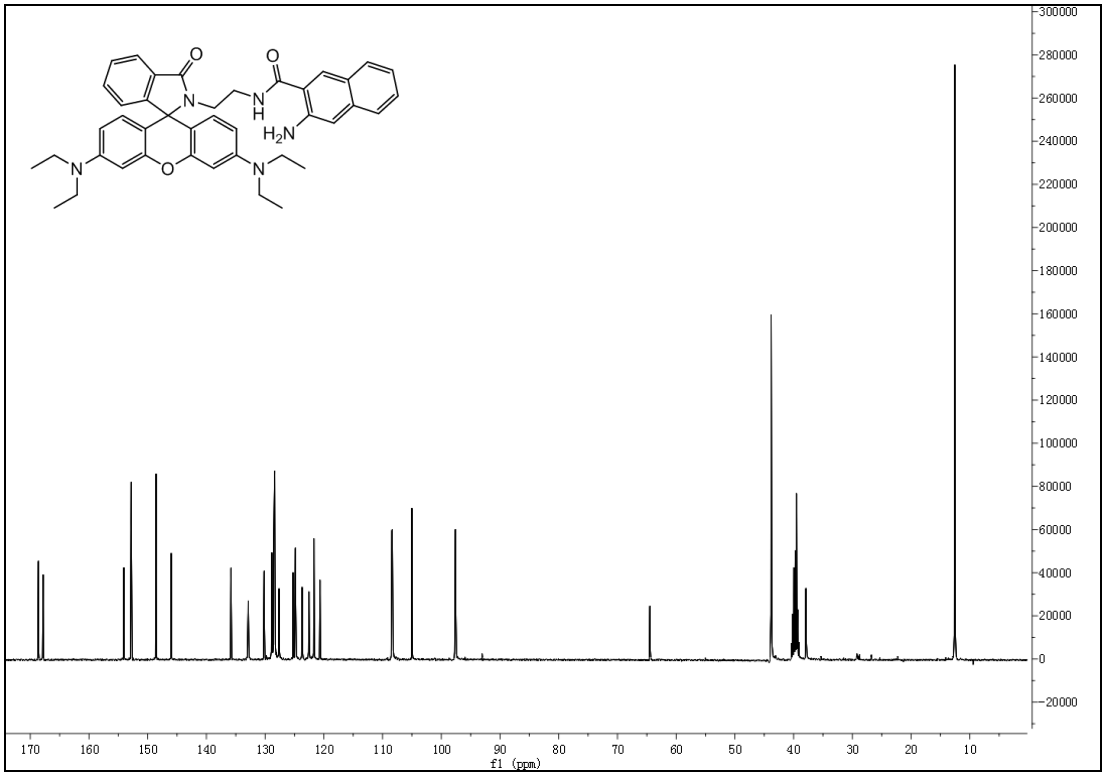


**Figure S12.** $^{13}$C NMR of **RN**.

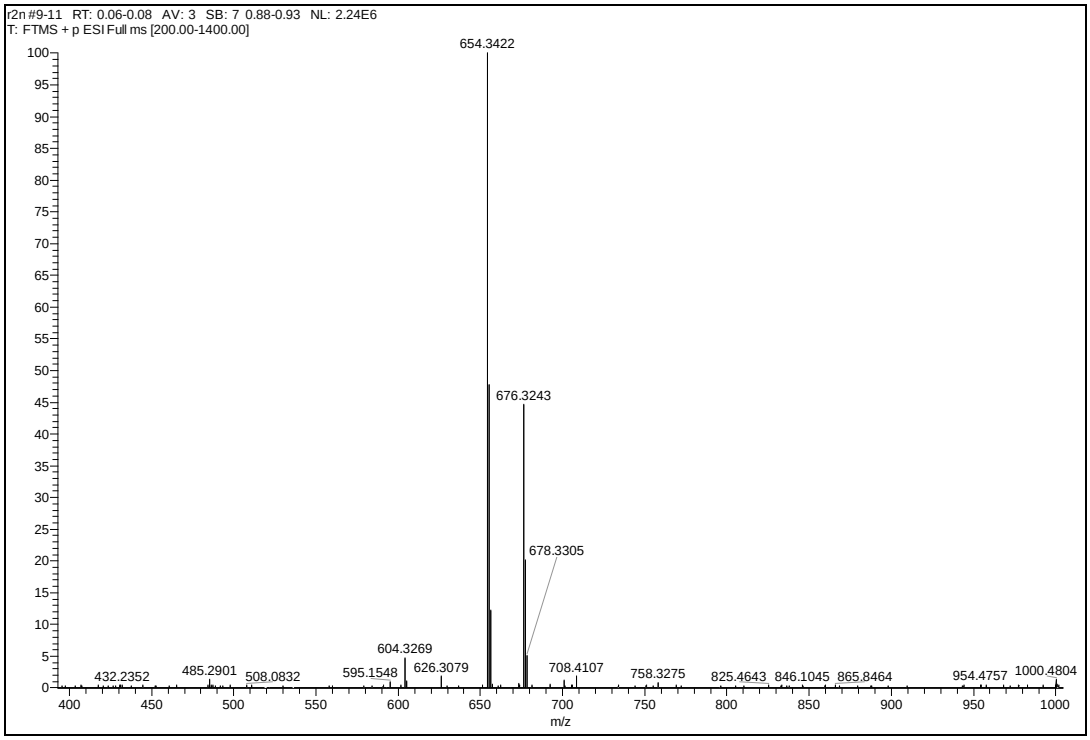


**Figure S13.** HRMS of **RN**.