Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Rodamine-based lysosome-targeted fluorescence probes: high pH sensitivity and their imaging application in living cells

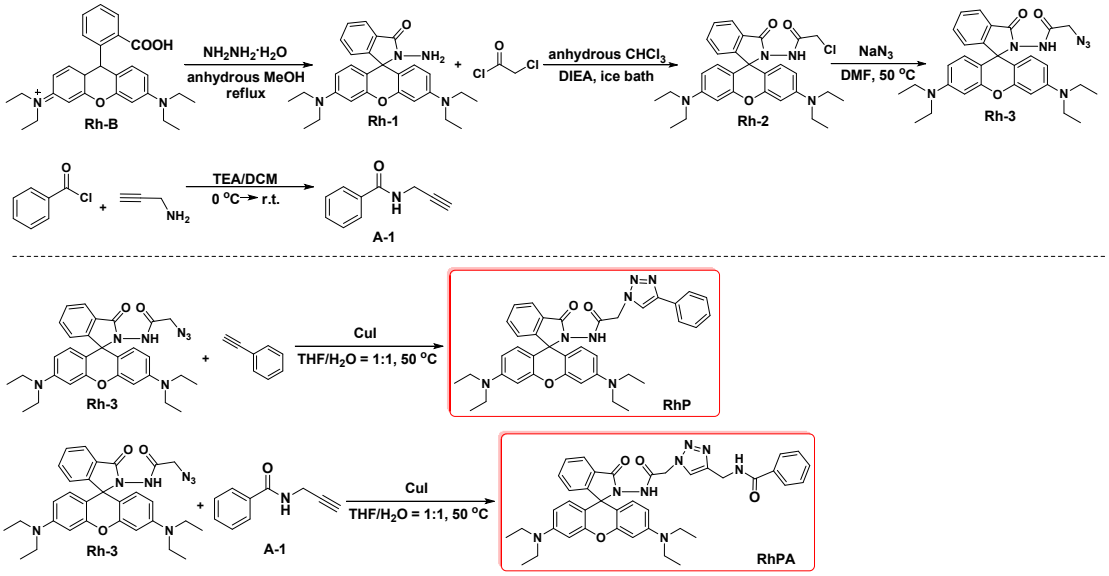
Contents

1. General remarks for experimental

^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-MS and HRMS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics Bio TOF mass spectrometer, respectively. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Fluorescence emission spectra were obtained using FluoroMax-4 Spectrofluorophotometer (HORIBA Jobin Yvon) at 298 K. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies. NucBlue® Live Cell Stain，LysoTracker green DND-26 and MitoTracker Green was purchased from Invitrogen.

Birtton-Robison (B-R) buffer solutions consist of 40 mM boric acid, 40 mM phodphoric acid, 40 mM acetic acid and 20 mM sodium hydroxide were used for tuning pH values.[1] All samples for fluorescence experiments were performed in different pH B-R buffer solution for 30 min before measurement.

2. Synthesis of various compounds



Scheme S1 Synthesis of **RhP** and **RhPA**

Rh-1, **Rh-2** and **A-1** was synthesized according to the literature [1, 2].

Synthesis of Rh-3: Under nitrogen, Rh-2 (650 mg, 1.2 mmol) was dissolved in DMF (15 ml), then sodium azide (234 mg, 3.6 mmol) was added to the solution very carefully. The mixture was stirred at 50℃ for 12 h. After the reaction, the solution extracted with CH_2Cl_2 (3×20 mL) and the combined organic layer was washed with brine (3×20 mL). After the solution was dried over

anhydrous sodium sulfate, the solvent was removed under the reduced pressure and the residue was further purified by column chromatography to afford **Rh-3** (620 mg, 95.6%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 9.91 (s, 1H), 7.84 (d, *J* = 6.9 Hz, 1H), 7.63 – 7.48 (m, 2H), 7.03 (d, *J* = 7.2 Hz, 1H), 6.51 (d, *J* = 9.3 Hz, 2H), 6.34 (d, *J* = 6.6 Hz, 4H), 3.99 (s, 2H), 3.34 (s, 8H), 1.09 (t, *J* = 6.8 Hz, 13H). **Rh-3**: m/z $[M+H]^+$ 540.2719 found, 540.2723 calcd.

Synthesis of RhP: **Rh-3** (270 mg, 0.5 mmol), phenylacetylene (109 μl, 1 mmol) and CuI (190mg, 1 mmol) were added in 20 ml THF-water (v:v, 1:1). The mixture was stirred at 50℃ for 24 h, and monitored by TLC. Then the solution was cooled down to room temperature and the solvent was removed under reduced pressure. After that, the solution was poured into distilled water and extracted with CH_2Cl_2 (3×30 mL). The combined extracts were dried over anhydrous sodium sulfate. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography to afford **RhP** (289.8 mg, 90.38%) as a light pink solid. ^{1}H NMR (400 MHz, DMSO) δ 10.20 (s, 1H), 8.27 (s, 1H), 7.83 (t, *J* = 6.9 Hz, 3H), 7.59 – 7.51 (m, 2H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.03 (d, *J* = 6.9 Hz, 1H), 6.52 (d, *J* = 9.0 Hz, 2H), 6.34 (d, *J* = 8.0 Hz, 4H), 5.13 (s, 2H), 3.34 – 3.26 (m, 8H), 1.07 (t, *J* = 7.0 Hz, 12H). **RhP**: m/z $[M+H]^+$ 642.5195 found, 642.3193 calcd; m/z $[M+Na]^+$ 664.2944 found, 664.3012 calcd.

Synthesis of RhPA: **Rh-3** (345 mg, 0.64 mmol), **A-1** (153 mg, 0.96 mmol) and CuI (243 mg, 1.28 mmol) were added in 20 ml THF-water (v:v, 1:1). The mixture was stirred at 50℃ for 24 h, and monitored by TLC. Then the solution was cooled down to room temperature and the solvent was removed under reduced pressure. After that, the solution was poured into distilled water and extracted with CH_2Cl_2 (3×30 mL). The combined extracts were dried with sodium sulfate anhydrous. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography to afford **RhPA** (350.6 mg, 78.4%) as a light purper solid. ^{1}H NMR (400 MHz, DMSO) δ 10.10 (s, 1H), 9.04 (s, 1H), 7.88 (d, *J* = 7.3 Hz, 2H), 7.82 (d, *J* = 7.0 Hz, 1H), 7.74 (s, 1H), 7.58 – 7.49 (m, 3H), 7.46 (t, *J* = 7.3 Hz, 2H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.52 (d, *J* = 8.5 Hz, 2H), 6.33 (d, *J* = 9.1 Hz, 4H), 5.06 (s, 2H), 4.49 (d, *J* = 5.1 Hz, 2H), 3.36 – 3.29 (m, 8H), 1.07 (t, *J* = 6.8 Hz, 12H). **RhPA**: m/z $[M+H]^+$ 699.3410 found, 699.3407 calcd; m/z $[M+Na]^+$ 721.3154 found, 721.3227 calcd.

3. Imaging of living cell

Hela cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37^{o}C in a 5% CO2/95% air incubator. For fluorescence imaging, cells (4×103/well) were passed on confocal dishes and incubated for 24h. Immediately before the staining experiment, cells were washed twice with PBS (10 mM): dish **1** incubated with 1μM LysoTracker Green, one drop NucBlue and 5μM **RhP** for 30 min at 37℃; dish **2** incubated with 1μM MitoTracker Green, one drop NucBlue and 5μM **RhP** for 30 min at 37℃; dish **3** incubated with 1μM LysoTracker Green, one drop NucBlue and 5μM **RhPA** for 30 min at 37℃; dish **4** incubated with 1μM MitoTracker Green, one drop NucBlue and 5μM **RhPA** for 30 min at 37℃. Then wash each dish with PBS (10 mM) for 3 times, and analyzed with a confocal fluorescence microscope. NucBlue(the blue emission) in 420-470 nm was collected using an excitation wavelength of 405 nm, LysoTracker Green and MitoTracker Green (the green

emission) in 500-540 nm was collected using an excitation wavelength of 488 nm, **RhP** and **RhPA** (the red emission) in 565-620 nm was collected using an excitation wavelength of 552 nm.

4. Spectral data

Henderson-Hasselbach-type mass action equation: $pK_a = pH - \log [(I_{max}-I)/(I-I_{min})]$

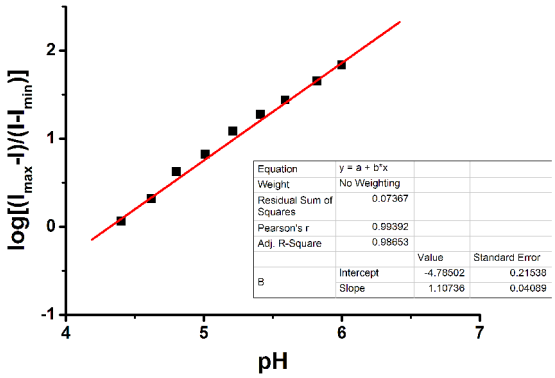


Figure S1 The calculation for the pK_a value of **RhP**

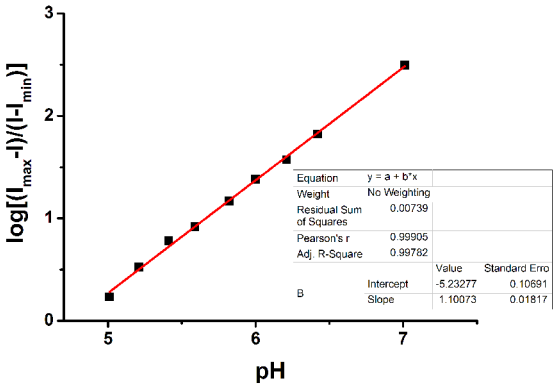


Figure S2 The calculation for the pK_a value of **RhPA**

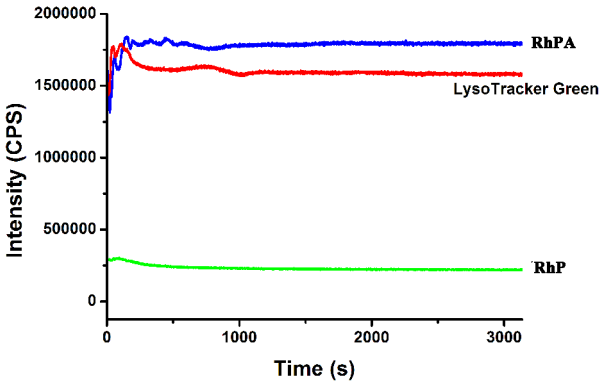


Figure. S3 Fluorescence spectra of **RhP** (5 μM) and **RhPA** (5 μM) in B-R buffer (pH 4.40), Fluorescence spectra of LysoTracker Green (1μM) in PBS buffer solution (10 mM, pH 7.40)

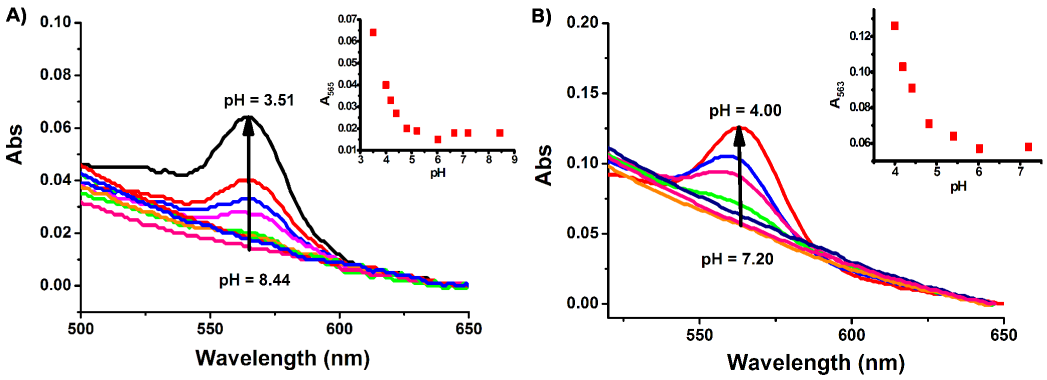
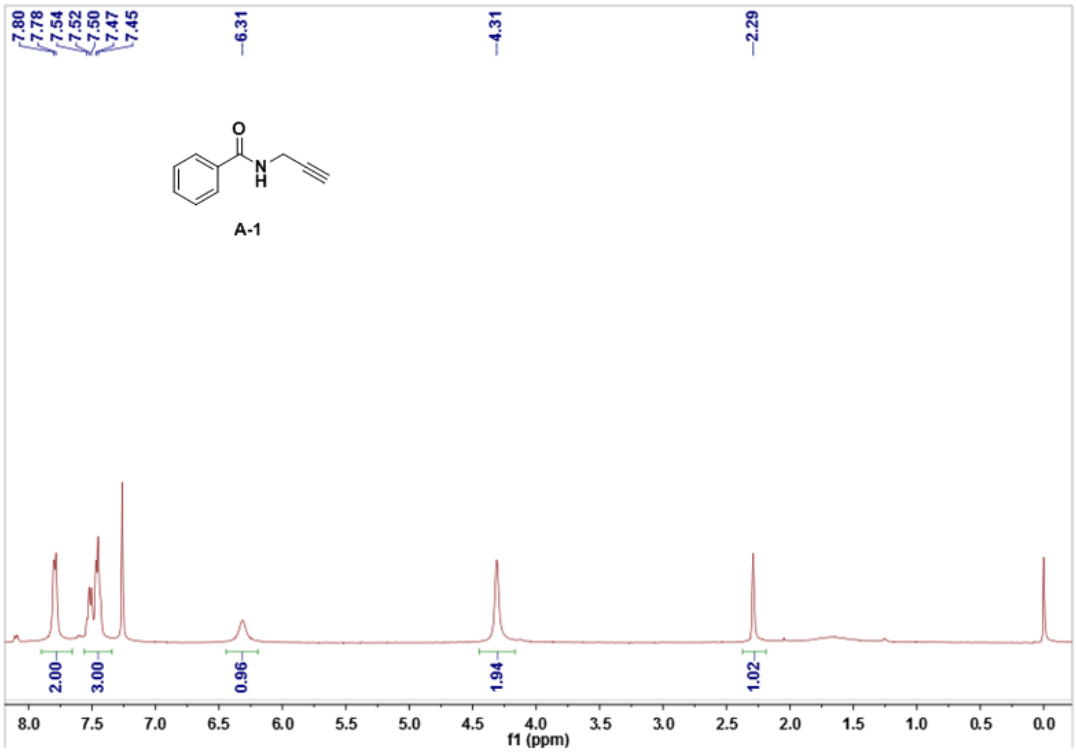


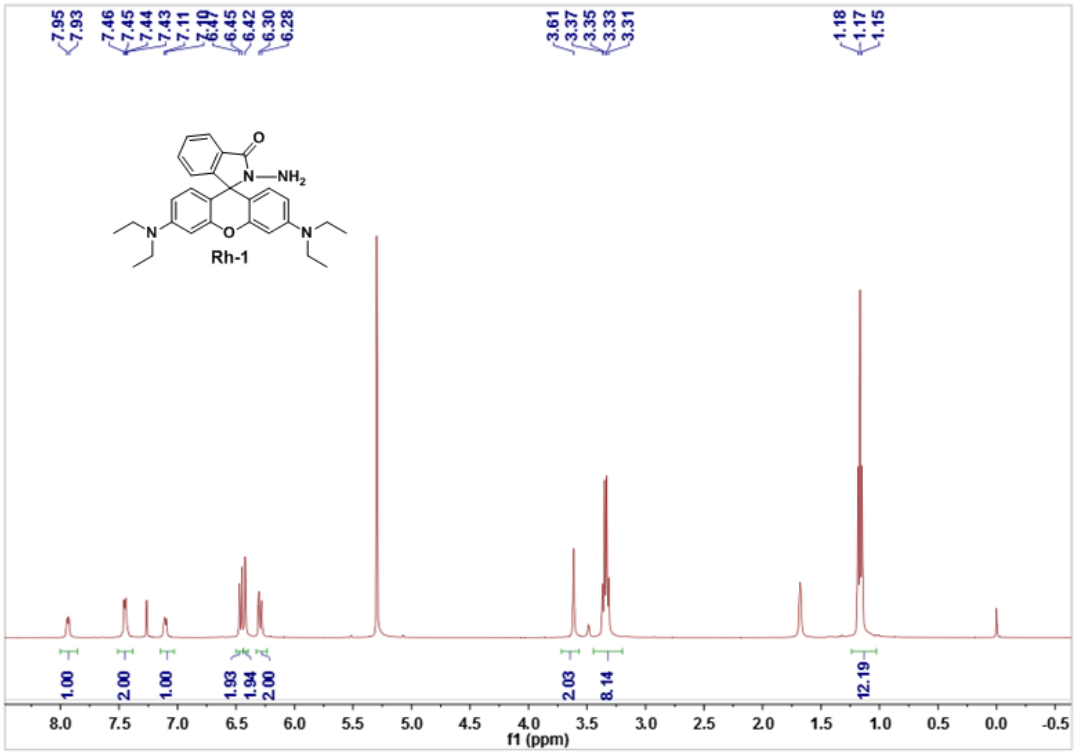
Fig. S4 A) Absorption spectral changes of **RhP** (10 μM) in B-R buffer solution at different pH values. Inset: Plot of the absorption intensity. pH 3.51, 4.00, 4.19, 4.41, 4.82, 5.21, 6.02, 6. 66, 7.20, 8.44. B) A) Absorption spectral changes of **RhPA** (10 μM) in B-R buffer solution at different pH values. Inset: Plot of the absorption intensity. pH 4.00, 4.19, 4.41, 4.82, 5.40, 5.81, 6.02, 7.20.

5. Product Analysis

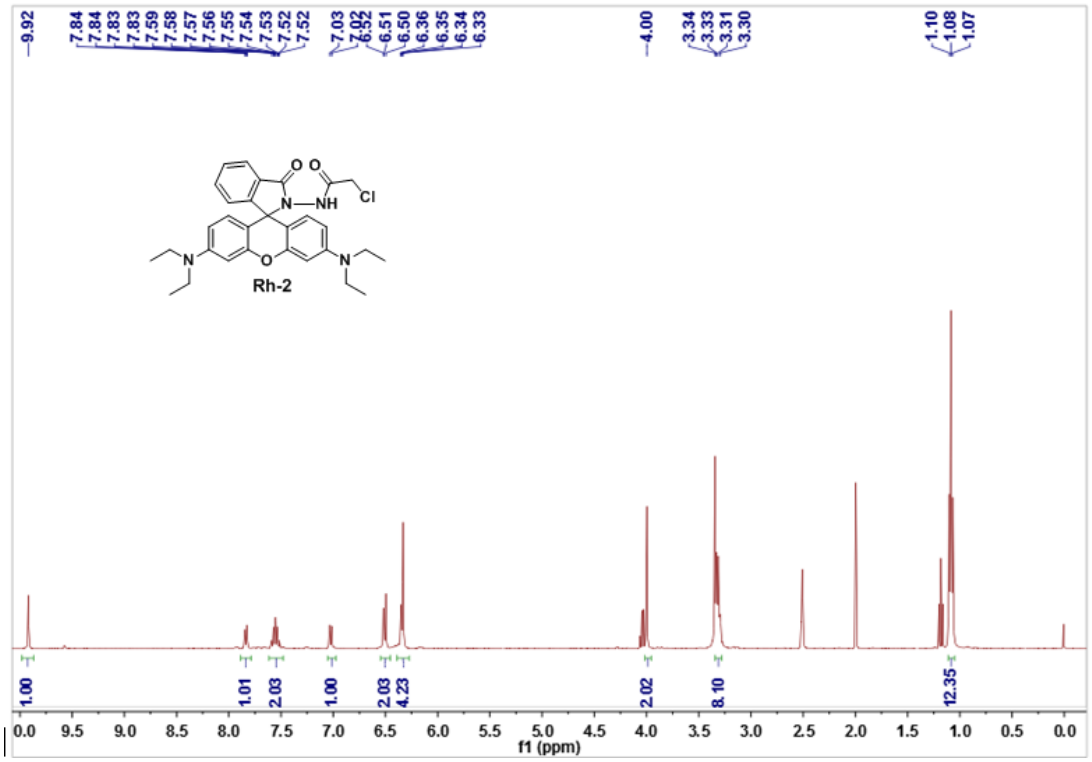
^{1}H NMR spectra of **A-1** in $CDCl_3$



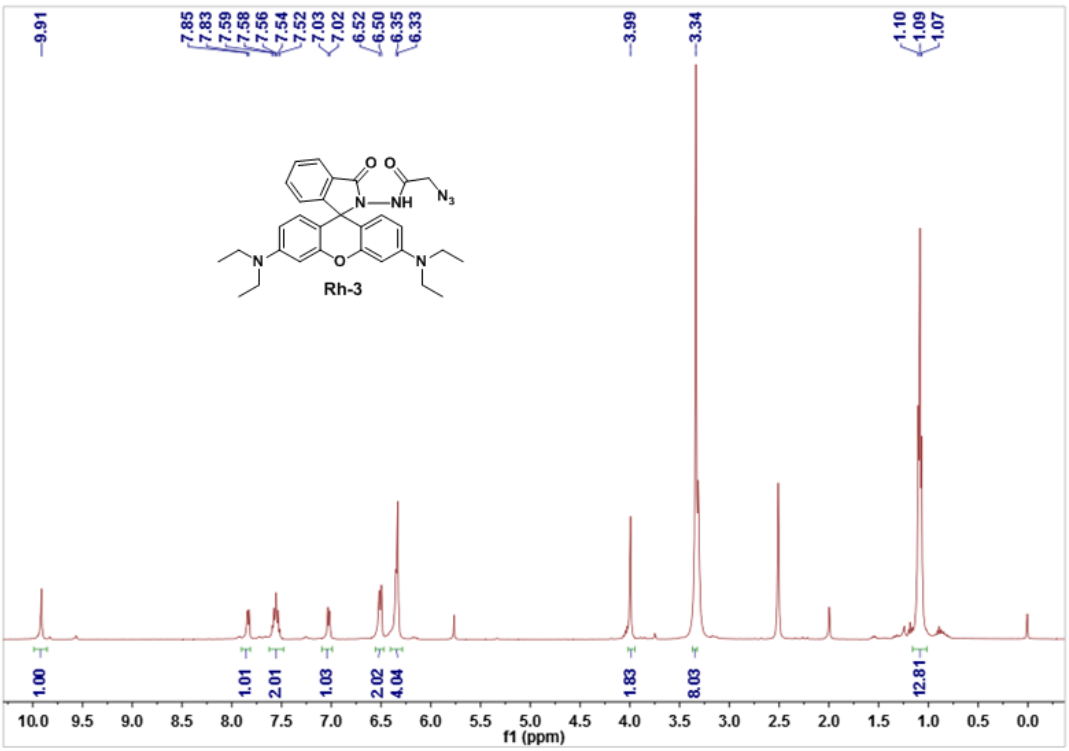
^{1}H NMR spectra of **Rh-1** in $CDCl_3$



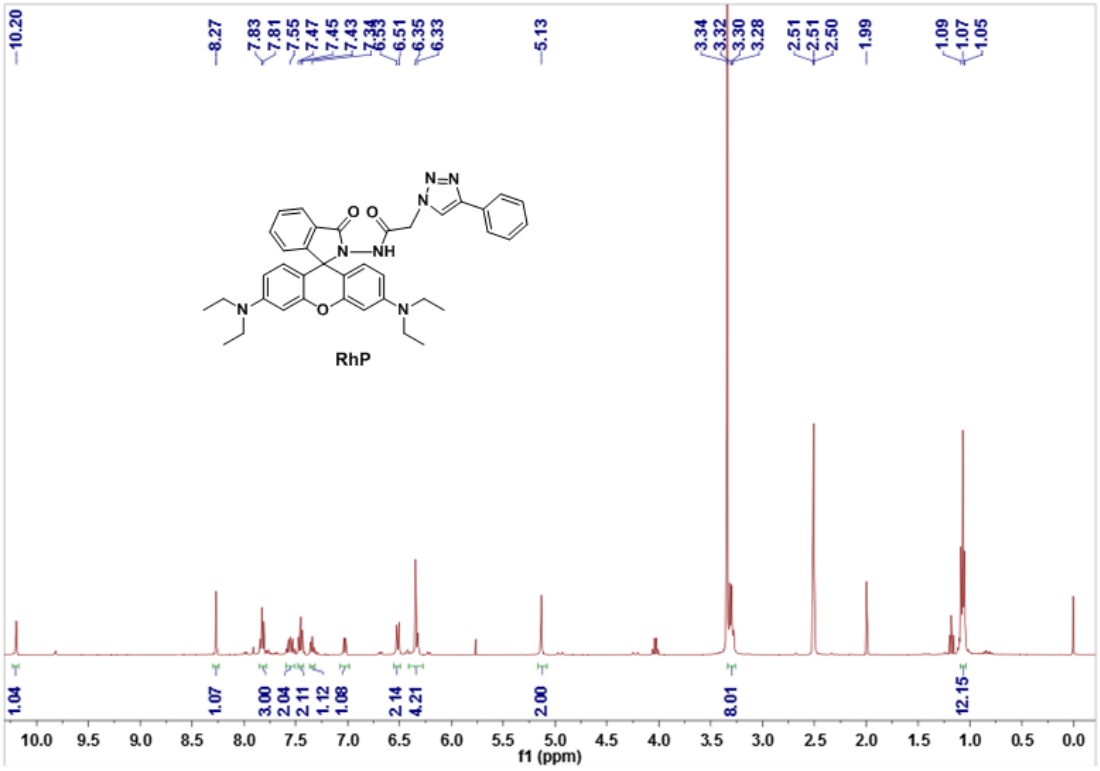
^{1}H NMR spectra of **Rh-2** in DMSO-d_6



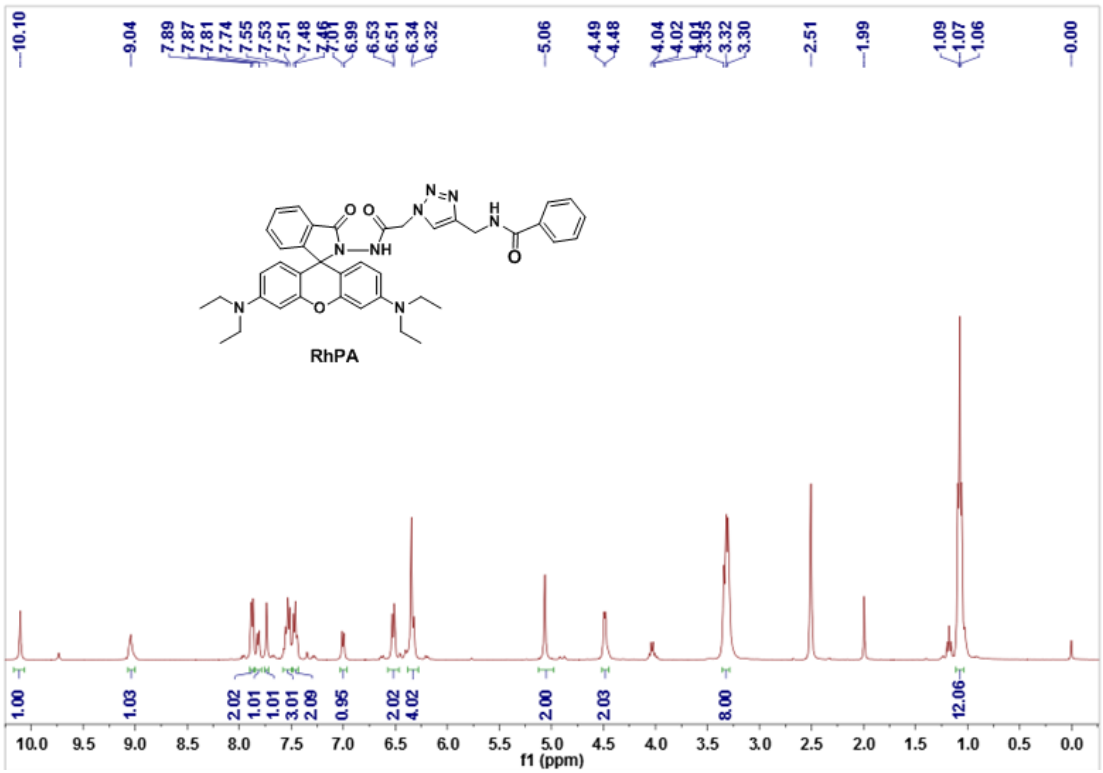
^{1}H NMR spectra of **Rh-3** in DMSO-d_6



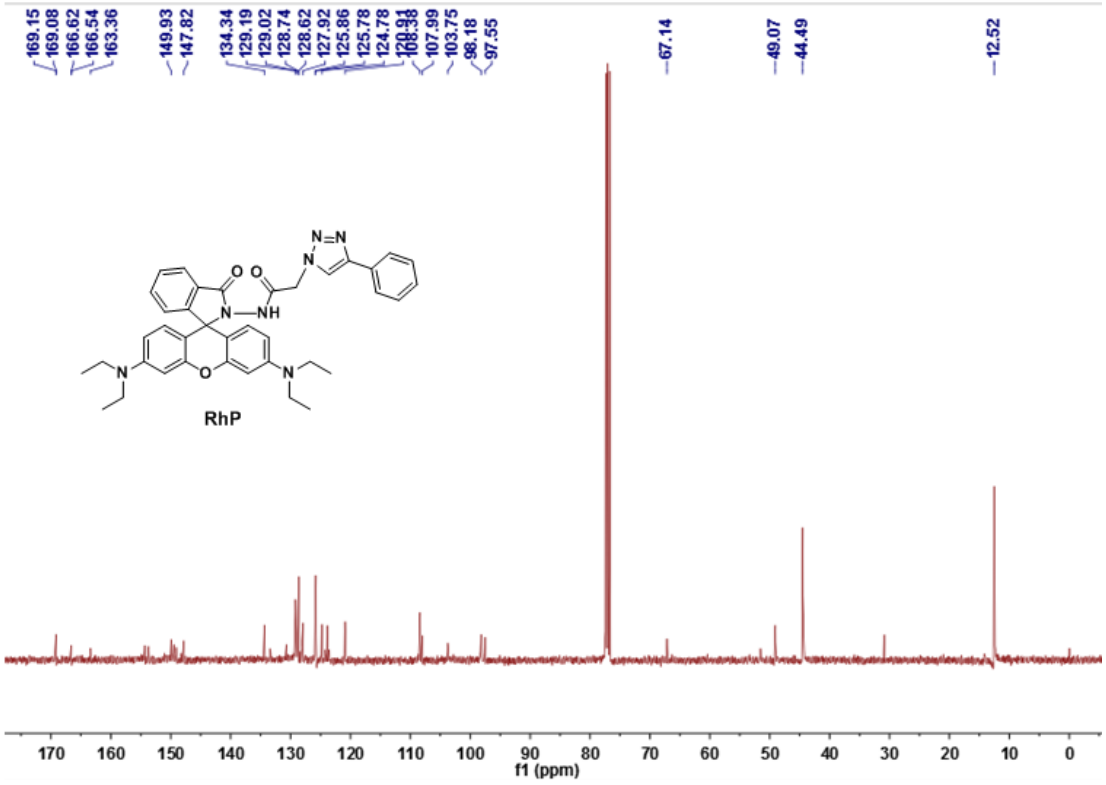
^{1}H NMR spectra of **RhP** in DMSO-d_6



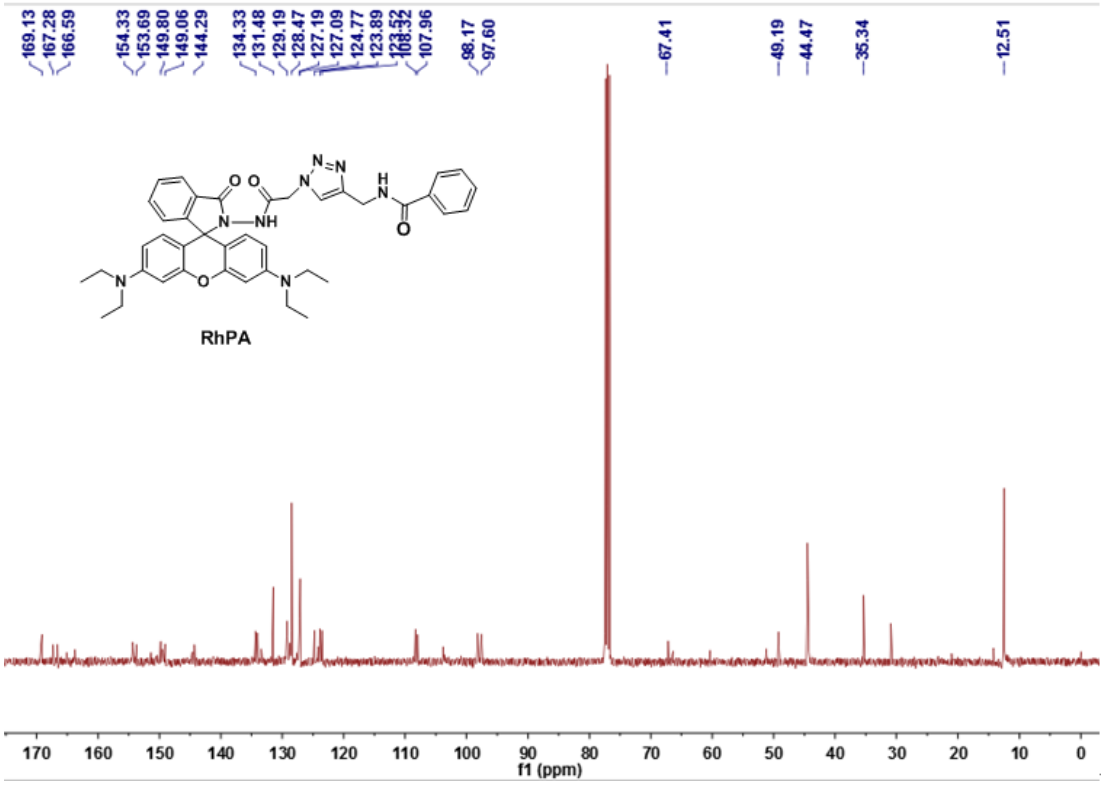
^{1}H NMR spectra of **RhPA** in DMSO-d_6



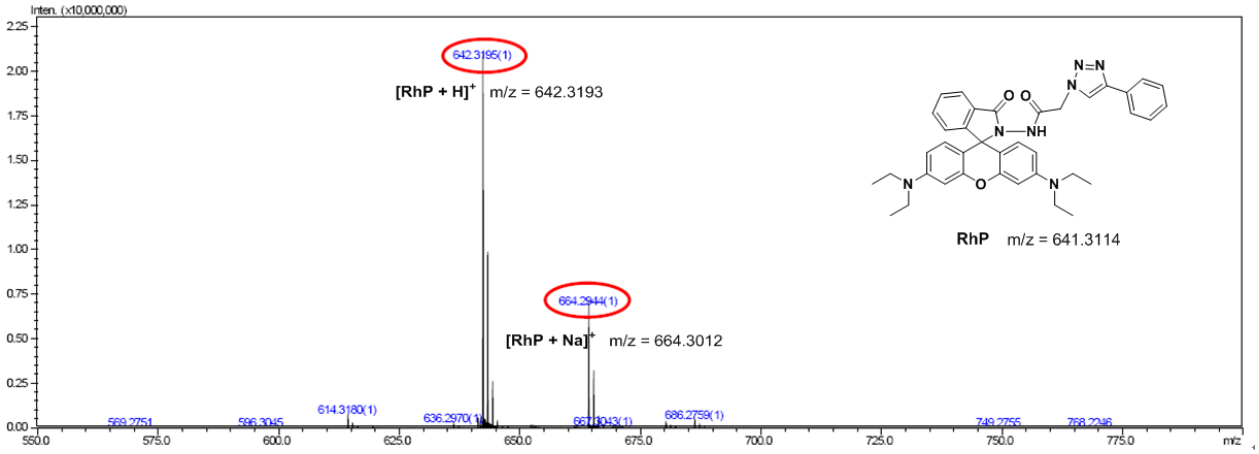
^{13}C NMR spectra of **RhP** in $CDCl_3$



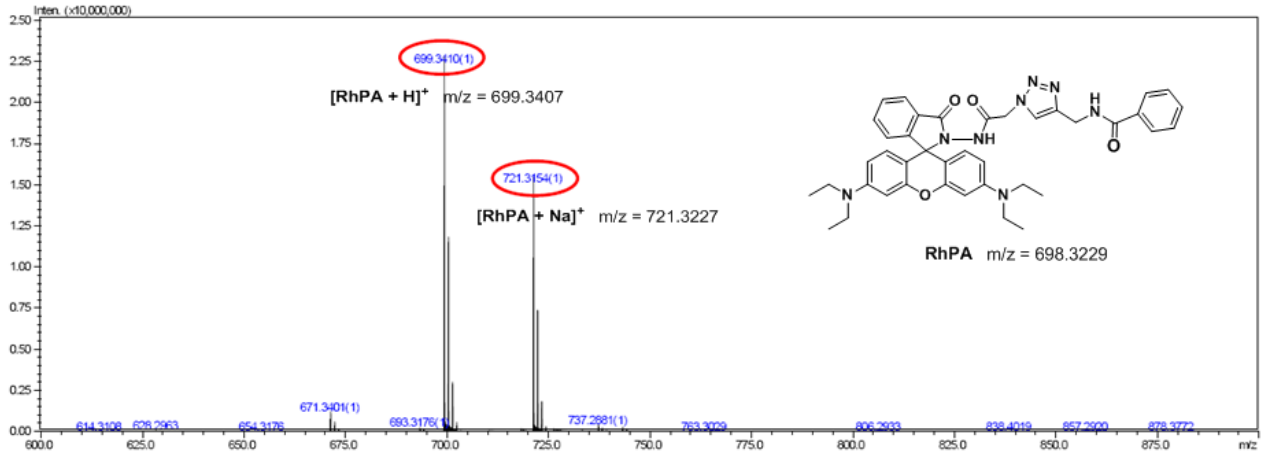
^{13}C NMR spectra of **RhPA** in $CDCl_3$



ESI-MS of **RhP**



ESI-MS of **RhPA**



6. Reference

[1] J. T. Hou, K. Li, K. K. Yu, M. Z. Ao, X. Wang, X. Q. Yu, Analyst 138 (2013) 6632-6638.

[2] S. Ji, X. Meng, W. Ye, Y. Feng, H. Sheng, Y. Cai, J. Liu, X. Zhu, Q. Guo, Dalton Trans. 43 (2014) 1583-1588.