SUPPORTING INFORMATION

Table of Contents

1.Experimental Procedures

Materials and synthesis. Rhodamine B (98%, TCI), fluorescein (98%, TCI), ethylenediamine (99%, Sigma-Aldrich), bromotriphenylethylene (98%, Energy Chemical), 4-formylphenylboronic acid (98%, Energy Chemical), tetrabutyl ammonium bromide (TBAB, 99%, Energy Chemical), tetrakis(triphenylphosphine) palladium(0) (99%, Energy Chemical), sodium borohydride (NaH_4B, 98%, Energy Chemical), succinic anhydride (99%, J&K), 4-(dimethyl-amino)-pyridine (DMAP, 99%, Aldrich), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU, 98%, Energy Chemical), tert-Butyl *N*-(2-bromoethyl)carbamate (98%, Energy Chemical), trifluoroacetic Acid (TFA, 99%, Energy Chemical), lithium hydroxide ($LiOH·H_2O$, 98%, Energy Chemical). Dichloromethane (CH_2Cl_2) were purified by stirring over calcium hydride for 24 h followed by distillation. Other reagents were purchased from Beijing Chemical Works and used as received.

Synthesis of RB-NH_2. Ethane-1, 2-diamine (6.01 g, 100.0 mmol) was added to a solution of rhodamine B (4.79 g, 10.0 mmol) in ethanol (100 mL). The mixture was refluxed for 18 h and then evaporated to dryness under a vacuum. After that, the crude solid was dissolved in CH_2Cl_2 and then washed with brine and deionized water. The organic layer was dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with MeOH/CH_2Cl_2 (v/v = 3/97) as an eluent to give orange powder with 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 8.01 – 7.81 (m, 1H), 7.53 – 7.34 (m, 2H), 7.15 – 6.99 (m, 1H), 6.51 – 6.30 (m, 4H), 6.26 (dd, J = 11.8, 3.5 Hz, 2H), 3.32 (q, J = 9.4 Hz, 8H), 3.18 (t, J = 8.8 Hz, 2H), 2.40 (t, J = 8.8 Hz, 2H), 1.15 (t, J = 9.4 Hz, 12H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm = 168.8, 153.7, 153.5, 149.0, 132.6, 131.4, 128.9, 128.2, 124.0, 122.9, 108.3, 105.9, 97.9, 65.1, 44.5, 44.0, 41.0, 12.7.

Synthesis of TPE-CHO. Bromotriphenylethylene (2.01 g, 6.0 mmol) and 4-formylphenylboronic acid (1.35 g, 9.0 mmol) were dissolved in the mixture of toluene (40 mL), TBAB (0.19 g, 0.6 mmol) and 1.2 M potassium carbonate aqueous solution (10 mL). The mixture was stirred at room temperature for 0.5 h under Ar gas followed by adding $Pd(PPh_3)_4$ (60 mg, 5.3×10^{-3} mmol) and then heated to 90 °C for 24 h. After that the mixture was poured into water and extracted with ethyl acetate. Then the organic layer was dried over with anhydrous sodium sulfate. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with CH_2Cl_2/n-hexane (v/v = 1/2) as an eluent to give faint yellow powder with 95% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm = 9.90 (s, 1H), 7.61 (d, J = 7.9 Hz, 2H), 7.25–7.07 (m, 11H), 7.07–6.92 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ ppm = 191.9, 150.6, 143.1, 143.0, 142.9, 139.8, 134.3, 132.0, 131.3, 131.2, 129.2, 127.9, 127.8, 127.1, 126.9, 126.8.

Synthesis of TPE-RB. RB-NH_2 (4.13 g, 5.0 mmol) was slowly added to a solution of TPE-CHO (1.80 g, 5.0 mmol) and $MgSO_4$ (6.00 g, 50 mmol) in CH_2Cl_2/ absolute Methanol (v/v = 1:1, 50 mL) at ambient temperature. The reaction was stirred for 6 h and $MgSO_4$ filtered out with a Büchner. Then the solution was cooled in an ice bath to 0° C. Sodium borohydride powder (0.95 g, 2.5 mmol) was then added to the reaction solution and the mixture was stirred overnight at room temperature. After filtration, the solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with MeOH/CH_2Cl_2 (v/v = 5/95) as an eluent to give orange powder with 90% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.90 (dd, *J* = 6.0, 2.8 Hz, 1H), 7.46 – 7.37 (m, 2H), 7.13 – 6.93 (m, 16H), 6.89 (d, *J* = 1.3 Hz, 4H), 6.45 – 6.34 (m, 4H), 6.23 (dd, *J* = 8.9, 2.6 Hz, 2H), 3.45 (s, 2H), 3.30 (q, *J* = 6.9 Hz, 10H), 2.40 (t, *J* = 6.7 Hz, 2H), 1.14 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 168.5, 153.7, 153.3, 148.7, 143.8, 143.7, 143.7, 1421, 140.8, 140.7, 138.2, 132.3, 131.3, 131.3, 131.2, 131.2, 131.11, 128.7, 128.0, 127.6, 127.6, 127.5, 127.4, 126.3, 126.3, 126.3, 123.8, 122.7, 108.1, 105.6, 97.7, 64.9, 53.0, 47.6, 44.3, 40.1, 12.6.

Synthesis of TPE-RB-COOH. TPE-RB (4.14 g, 5.0 mmol), succinic anhydride (0.60 g, 6.0 mmol) and 4-dimethylaminopyridine (DMAP, 0.74 g, 6.0 mmol) were dissolved in absolute CH_2Cl_2 (50 ml). The mixture was stirred at ambient temperature for 4 hours, then the mixture was washed with 1N HCl, $NaHCO_3$ and deionized water. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on a silica gel column with MeOH/CH_2Cl_2 (v/v = 10/90) as an eluent to give orange powder with 98% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm = 7.91 (dd, *J* = 7.6, 4.1 Hz, 1H), 7.43 (td, *J* = 9.1, 8.4, 3.9 Hz, 2H), 7.18 – 6.68 (m, 20H), 6.40 (dd, *J* = 11.5, 3.2 Hz, 4H), 6.24 (dd, *J* = 11.9, 3.5 Hz, 2H), 4.23 (d, *J* = 16.9 Hz, 2H), 3.30 (q, *J* = 9.4 Hz, 8H), 3.10, 2.61 (dd, *J* = 10.5, 5.9 Hz, 4H), 2.53 (dd, *J* = 10.0, 5.7 Hz, 4H), 1.14 (t, *J* = 9.3 Hz, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ ppm = 176.0, 172.4, 168.1, 153.6, 153.3, 148.9, 143.9–143.4, 142.9, 140.9, 140.6, 135.6, 132.8, 131.8, 131.6–131.1, 128.9, 128.4, 127.8, 126.5, 125.8, 124.0, 122.9, 108.4, 108.0, 104.9, 98.0, 97.7, 65.1, 49.3, 45.1, 44.4, 38.3, 30.0, 27.4, 12.7.

Synthesis of FL-2Boc. Fluorescein (2.82 g, 8.5 mmol), K_2CO_3 (4.69 g, 34.0 mol) and tert-Butyl *N*-(2-bromoethyl)carbamate (7.60 g, 34.0 mmol) were dissolved in DMF (50 mL) and heated to 65 °C for 12 h. The mixture was concentrated under reduced pressure then diluted with brine (100 mL) and extracted with EtOAc. And the combined organic extracts further washed with brine. The organic layer was dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with EtOAc /n-hexane (v/v = 1/3) as an eluent to give FL-2Boc with 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm = δ 8.28 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.75 (td, *J* = 7.5, 1.4 Hz, 1H), 7.68 (td, *J* = 7.6, 1.4 Hz, 1H), 7.36–7.29 (m, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.88 (dd, *J* = 16.0, 9.3 Hz, 2H), 6.81–6.70 (m, 1H), 6.55 (dd, *J* = 9.7, 1.8 Hz, 1H), 6.46 (d, *J* = 1.8 Hz, 1H), 5.03 (s, 1H), 4.36 (s,

1H), 4.14 (t, *J* = 5.2 Hz, 2H), 4.06 (t, *J* = 5.5 Hz, 2H), 3.57 (q, *J* = 5.5 Hz, 2H), 3.27–2.98 (m, 2H), 1.45 (s, 18H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 185.5, 165.2, 163.1, 158.7, 155.8, 155.6, 154.1, 149.9, 134.3, 132.9, 131.5, 130.5, 130.2, 130.13, 129.8, 129.0, 117.8, 115.0, 113.5, 106.0, 101.2, 79.8, 79.7, 68.0, 64.8, 39.9, 39.4, 28.4, 28.3.

Synthesis of FL-Boc. FL-2Boc (1.17 g, 1.89 mmol) was dissolved in THF (20 mL). $LiOH{\cdot}H_2O$ (1.58 g, 37.70 mmol) in H_2O (20 mL) was added to THF solution. The mixture solvent was stirred for 3-5 hours. The process of hydrolytic reaction was checked by TLC. After the reaction completed, THF was removed under reduced pressure. 1 M HCl aqueous solution was added to the solution for adjusting pH value to 3-4. Then the product was extracted with DCM. The organic layer was combined and dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with EtOAc /n-hexane (v/v = 2/3) as an eluent to give FL-Boc (yellow powder) with 85% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm = δ 8.00 (d, *J* = 7.4 Hz, 1H), 7.62 (dt, *J* = 18.0, 7.3 Hz, 2H), 7.13 (d, *J* = 7.4 Hz, 1H), 6.75 (d, *J* = 1.6 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 6.57 (d, *J* = 4.6 Hz, 3H), 5.23 (d, *J* = 5.6 Hz, 1H), 4.00 (t, *J* = 5.2 Hz, 2H), 3.51 (q, *J* = 5.6 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm = δ 170.2, 160.3, 159.0, 158.9, 156.4, 153.1, 152.5, 152.5, 135.2, 129.8, 129.1, 129.1, 126.7, 125.0, 124.1, 112.7, 111.9, 111.4, 110.3, 103.1, 101.5, 80.1, 67.4, 40.0, 28.4.

Synthesis of FL-NH_2. FL-Boc (0.78 g, 1.64 mmol) was dissolved in DCM (20 mL). TFA (5 mL) was added and then stirred for 2-4 hours. The reaction progression was checked by TLC. After the reaction completed, the solution was concentrated under reduced pressure. Then co-evaporation with toluene afforded the crude product. Finally, it was purified by precipitating with diethyl ether to give FL-NH_2 with 95% yield. ^{1}H NMR ($(CD_3)_2CO$, 300 MHz) δ ppm = δ 7.98 (d, *J* = 7.5 Hz, 1H), 7.76 (dt, *J* = 21.0, 7.4 Hz, 2H), 7.25 (d, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 5.3 Hz, 1H), 6.80 (s, 1H), 6.73 (d, *J* = 4.3 Hz, 2H), 6.65 (s, 2H), 4.50 (t, *J* = 5.0 Hz, 2H), 4.26 (t, *J* = 5.0 Hz, 2H). ^{13}C NMR ($(CD_3)_2CO$, 75 MHz) δ ppm = δ 169.5, 160.7-160.4, 154.0, 153.3, 153.2, 136.1, 130.8, 130.0, 127.7, 125.4, 124.9, 120.0, 116.1, 113.5, 113.4, 113.0, 112.7, 111.2, 103.4, 102.8, 102.6, 102.5, 83.4, 66.0, 65.5, 64.7, 58.6, 47.5, 41.3, 40.0.

Synthesis of RTF. TPE-RB-COOH (0.46 g, 0.50 mmol), HATU (0.21 g, 0.55 mmol) and TEA (350 μL, 2.50 mmol) were dissolved by anhydrous DCM (10 mL) and DMF (10 mL), and stirred for 3 hours. Then, FL-NH_2 (0.29 mg, 0.60 mmol) was added to the solution. After continuous stirred for 12 h, the solution was concentrated under reduced pressure and decentralized with brine (15 mL). The product was extracted with EtOAc. Then, the combined organic layer further was washed with water and dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with DCM / MeOH (v/v = 100/5) as an eluent to give **RTF** with 80% yield. ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ ppm = δ 9.18 (s, 1H), 7.97 (dt, *J* = 5.1, 2.9 Hz, 1H), 7.82 (dd, *J* = 6.4, 2.3 Hz, 1H),

7.71 (ddt, *J* = 11.0, 7.7, 3.9 Hz, 2H), 7.51 (dtd, *J* = 7.4, 5.8, 5.3, 2.8 Hz, 2H), 7.40 (t, *J* = 5.6 Hz, 1H), 7.22–7.14 (m, 1H), 7.14–6.83 (m, 20H), 6.82–6.74 (m, 2H), 6.71–6.60 (m, 4H), 6.49–6.28 (m, 6H), 4.20 (d, *J* = 31.8 Hz, 2H), 4.05 (dt, *J* = 15.5, 5.7 Hz, 2H), 3.52 (dq, *J* = 13.3, 6.8, 6.3 Hz, 2H), 3.36 (q, *J* = 7.1 Hz, 8H), 3.23–3.03 (m, 4H), 2.44 (s, 4H), 1.13 (t, *J* = 6.9 Hz, 12H). ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ ppm = δ 172.9, 172.8, 172.4, 172.3, 169.4, 168.3, 167.8, 161.4, 160.3, 154.3, 153.9, 153.2, 149.8, 149.6, 144.5, 144.4, 143.2, 141.9, 141.7, 141.5, 141.4, 137.6, 136.8, 136.0, 133.3, 132.2, 132.1, 131.9, 131.8, 130.6, 130.0, 129.8, 129.5, 129.0, 128.9, 128.4, 128.3, 127.7, 127.2, 126.9, 125.3, 124.8, 124.6, 123.1, 113.3, 113.1, 112.9, 112.5, 111.4, 109.2, 109.0, 106.4, 106.0, 103.3, 102.2, 102.1, 98.4, 98.3, 83.4, 68.0, 67.8, 65.6, 65.4, 49.4, 45.9, 44.8, 44.8, 39.2, 39.1, 31.6, 31.5, 28.3, 12.9, 12.8.

Synthesis of FL-spirolactam. Ethane-1, 2-diamine (6.01 g, 100.0 mmol) was added to a solution of fluorescein (3.32 g, 10.0 mmol) in ethanol (100 mL). The mixture was refluxed for 4 d and then evaporated to dryness under a vacuum. After that, the crude solid was dissolved in CH_2Cl_2 and then washed with brine and deionized water. The organic layer was dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and recrystallized to give product FL-s. ^{1}H NMR (CD_3OD, 400MHz) δ ppm = δ 7.93 – 7.84 (m, 1H), 7.62 – 7.51 (m, 2H), 7.06 (dd, *J* = 5.9, 2.2 Hz, 1H), 6.61 (d, *J* = 2.2 Hz, 2H), 6.53 – 6.34 (m, 4H), 3.18 (t, *J* = 7.0 Hz, 2H), 2.32 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR (CD_3OD, 100MHz) δ ppm = δ 170.53, 160.77, 154.79, 154.30, 134.40, 131.90, 129.94, 129.88, 125.06, 123.78, 113.70, 110.59, 103.80, 66.67, 43.80, 40.97.

Synthesis of RTF-s. TPE-RB-COOH (0.46 g, 0.50 mmol), HATU (0.21 g, 0.55 mmol) and TEA (350 μL, 2.50 mmol) were dissolved by anhydrous DCM (10 mL) and DMF (10 mL), and stirred for 3 hours. Then, FL-s (0.22 mg, 0.60 mmol) was added to the solution. After continuous stirred for 12 hours, the solution was concentrated under reduced pressure and decentralized with brine (15 mL). The product was extracted with EtOAc. The combined organic layer further was washed with water and dried over anhydrous $MgSO_4$. Finally, the solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with DCM / MeOH (v/v = 100/3) as an eluent to give **RTF-s** with 90% yield. ^{1}H NMR ($CDCl_3$, 400MHz) δ ppm = δ 9.25 (d, *J* = 19.8 Hz, 2H), 7.85 (dd, *J* = 18.3, 7.5 Hz, 2H), 7.39 (ddt, *J* = 25.4, 15.8, 7.2 Hz, 4H), 7.15 – 6.74 (m, 21H), 6.70 (d, *J* = 7.9 Hz, 2H), 6.61 (d, *J* = 9.0 Hz, 1H), 6.51 (d, *J* = 9.6 Hz, 2H), 6.45 – 6.25 (m, 6H), 6.17 (d, *J* = 8.8 Hz, 2H), 4.08 (d, *J* = 19.6 Hz, 2H), 3.47 – 3.18 (m, 10H), 3.14 (s, 2H), 3.08 (s, 4H), 2.73 – 2.21 (m, 4H), 1.21 – 0.96 (m, 12H). ^{13}C NMR ($CDCl_3$, 100MHz) δ ppm = δ 172.5, 172.0, 169.5, 168.4, 158.7, 153.4, 153.3, 152.7, 152.7, 149.0, 143.8, 143.6, 143.5, 142.7, 141.2, 140.8, 140.6, 140.3, 135.9, 132.9, 131.6, 131.3, 131.2, 130.6, 128.7, 128.4, 127.9, 127.6, 127.6, 126.6, 126.4, 126.3, 126.0, 123.9, 122.9, 113.0, 112.8, 109.3, 108.3, 104.3, 103.3, 97.6, 77.3, 65.6, 65.2, 45.3, 44.3, 40.7, 39.6, 39.1, 38.4, 30.9, 27.7, 12.6.

Preparation of RTF EtOH-H_2O mixtures. With the aid of a pH meter (with an organic solvent resistant pH electrode), different pH values Britton-Robinson buffer solutions, from pH 2.0 to 8.0, were prepared by using H_3PO_4-HAc-H_3BO_3 and ultrapure water, and HCl aqueous solution (pH 1.0) was also prepared for adjusting the pH values of EtOH-H_2O mixture solvents. For precise control of the pH value at determined water fraction (f_w), pre-experiments were needed. If the f_w was less than or equal to 30%, the salt of buffer solutions precipitated out, especially in basic solution. And if the f_w was great than or equal to 80%, **RTF** easily aggregate precipitated. In both cases, the precipitation did bad influence in fluorescence measurement. Thus, the appropriate f_w for the research was 35-75%.

pKa. The *pKa* values for rhodamine and fluorescein units of **RTF** were estimated from changes in the fluorescence intensity with various pH values by using the relationship, $\log[(I_{max}-I)(I-I_{min})] = pH - pKa$, where I_{max}, I_{min}, and I were the maximum, minimum, and observed fluorescence intensity at a given pH, separately.

Preparation of RTF gels. In the process of preparing the multicolour emission gels, the **RTF** solutions under various f_w and pH were used to dissolve 4-arm-PEG-SH (20 K). The mass percent concentration of 4-arm-PEG-SH was 8%. For quickly preparing the gels, small amounts of the hydrogen peroxide solution were added to the precursor solution. Then the solution was poured into the model and sealed. The model was warmed to 40 °C for 1-12 h. After crosslinking, the gels were demoulded from the model. The gels were support structure for **RTF** molecule or NPs and the emission colour of **RTF** kept, because **RTF** was not bonded with the gels.

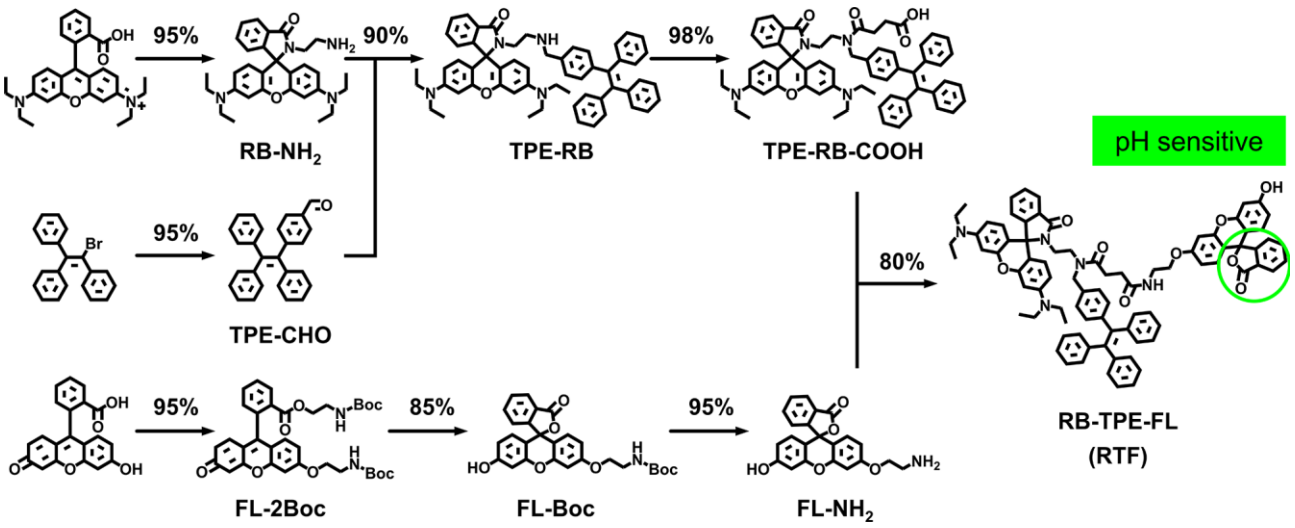
Cell culture and bioimaging. HeLa (human cervical cancer) cell lines were cultured in DMEM supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL). The cells were maintained in a humidified atmosphere of 5/95 (v/v) of CO_2/air at 37 °C. Two days before imaging, the cells were passed and plated on glass bottomed dishes. The cells were treated and incubated with **RTF** NPs at 37 °C under 5% CO_2 for 12 h. The cells were washed three times with phosphate buffered saline (PBS) and then cell images were obtained using a CLSM.

Characterizations. ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were measured on a Bruker Avance III 400 HD or Bruker Fourier 300 spectrometer. Mass spectra of the intermediate products and **RTF-s** were recorded on an Autoflex III Matrix-Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) or a QP2010 gas chromatgraphy mass spectrometrometry (GCMS) mass spectrometer. For the final product **RTF**, high-resolution Fourier Transform Ion Cyclotron Resonance Mass spectrum (FT-ICR-MS) was recorded on an 9.4T FT-ICR-MS Solarix. UV-vis spectra of the samples were measured on a Lambda950 spectrometer. Fluorescence spectra were carried out on a Cary Eclipse photoluminescence

spectrometer (upon excitation at 315 nm) with setting the excitation filter and emission filter for remove the frequency-doubled reflection. The 1931 Commission Internationale del'Éclairage (CIE) coordinates and chromaticity diagrams were obtained from the corresponding fluorescence emission spectra by a software (CIE1931xy, model V.1.6.0.2a) according to the computational formula of CIE standard observer. Fluorescence quantum yields were recorded on an Edinburgh Instruments FLS980 spectrometer with integrating spheres. Fluorescence lifetime was recorded by time-correlated single photon counting on an Edinburgh Instruments FLS980 spectrometer and instrument response function (IRF) was measured by scattering diluted concentration of Ludox. Dynamic light scattering (DLS) were obtained on Malvern Instrument NanoZS (ZEN3600) equipped with a He-Ne laser (632.8 nm, 4.0mW) by non-invasive backscattering (173°). NPs and cell imaging observations were performed on an OLYMPUS biological confocal laser scanning microscope (CLSM, model: FV1000-IX81).

2. Synthetic route and characterizations of RTF

2.1. Synthetic route of RTF



Scheme S1 Synthetic route of **RTF**.

2.2. ^{1}H and ^{13}C NMR spectra for preparing RTF

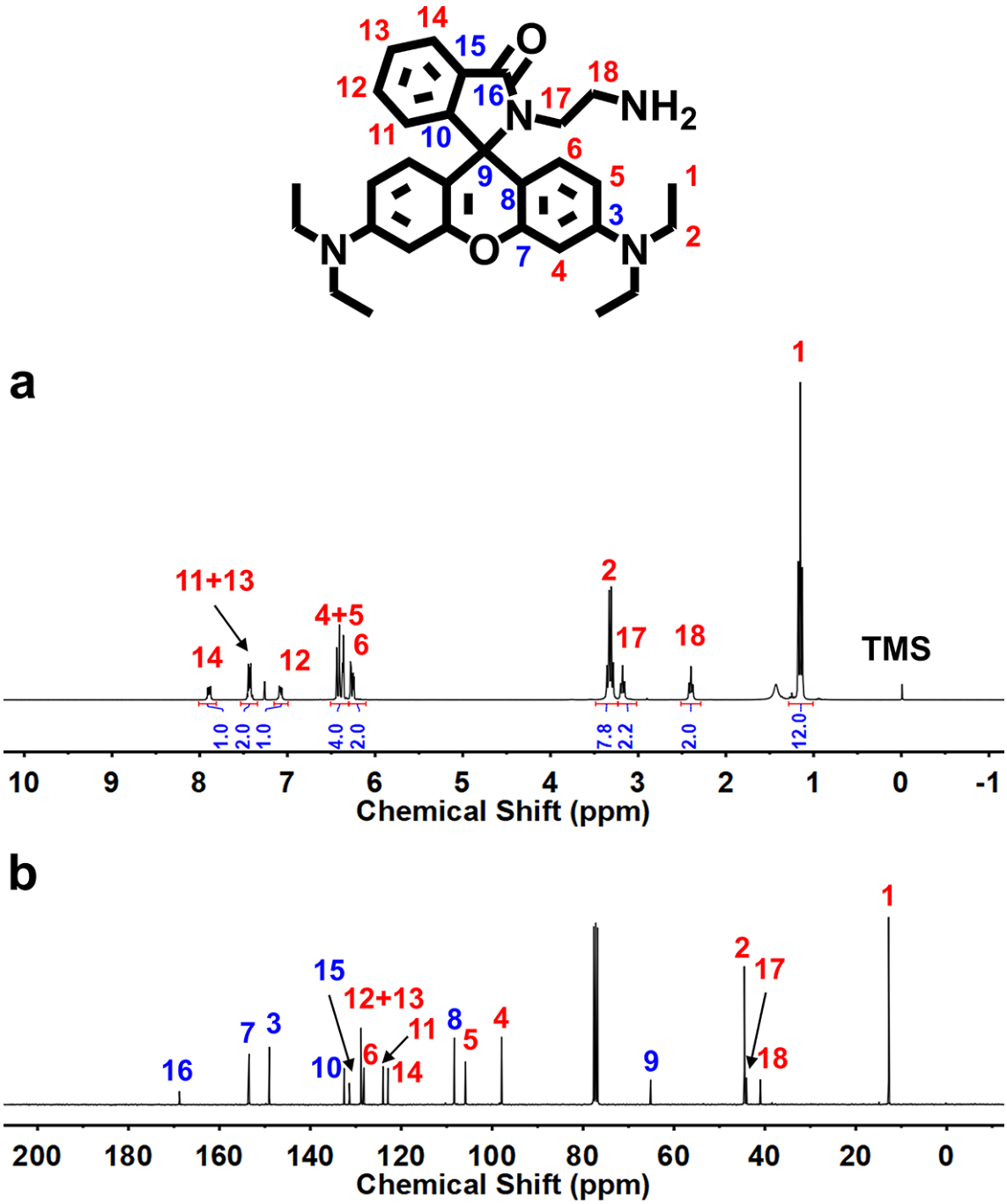


Fig. S1 ^{1}H (a) and ^{13}C (b) NMR spectra of RB-NH_2 in $CDCl_3$ solution.

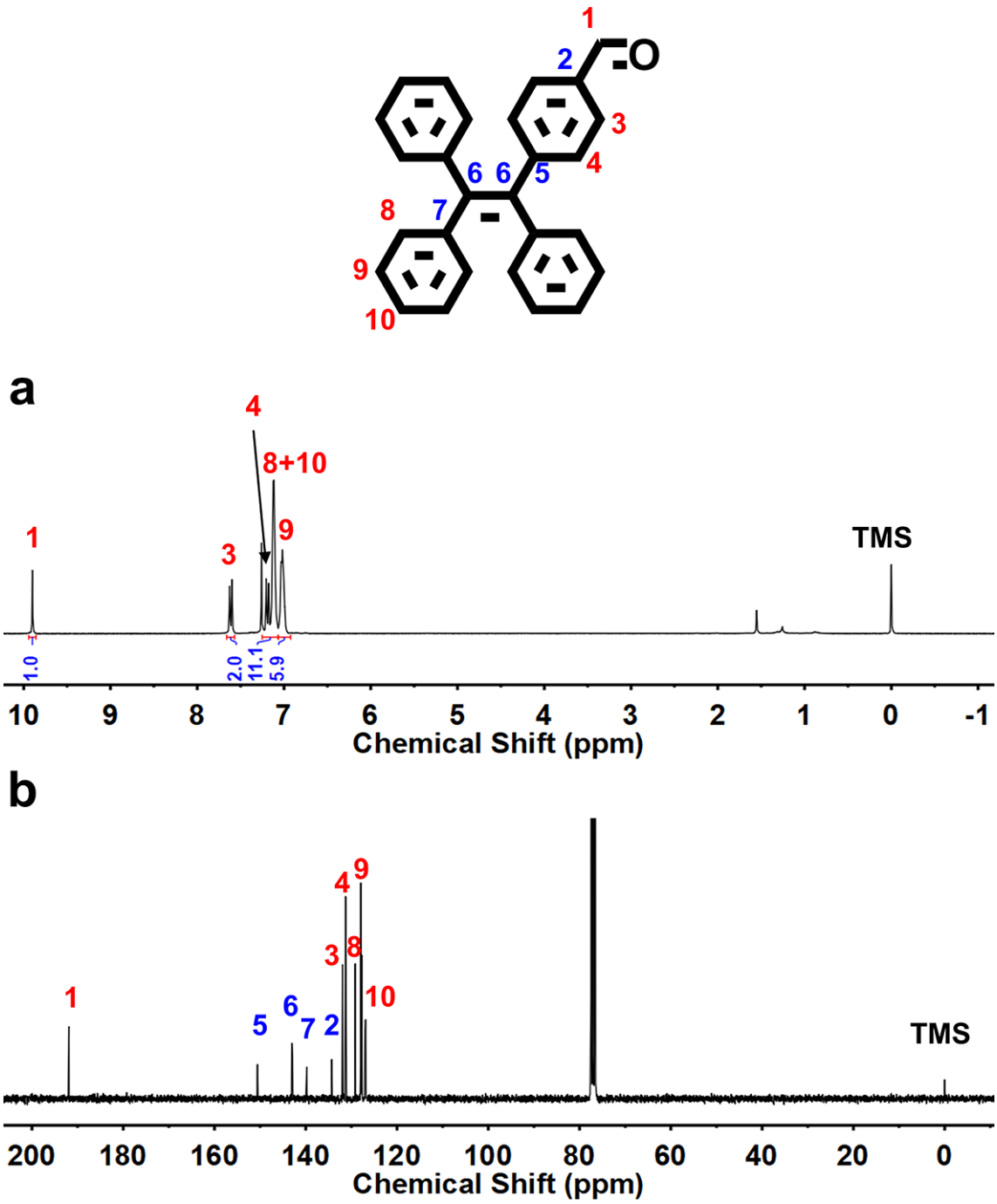


Fig. S2 ^{1}H (a) and ^{13}C (b) NMR spectra of TPE-CHO in $CDCl_3$ solution.

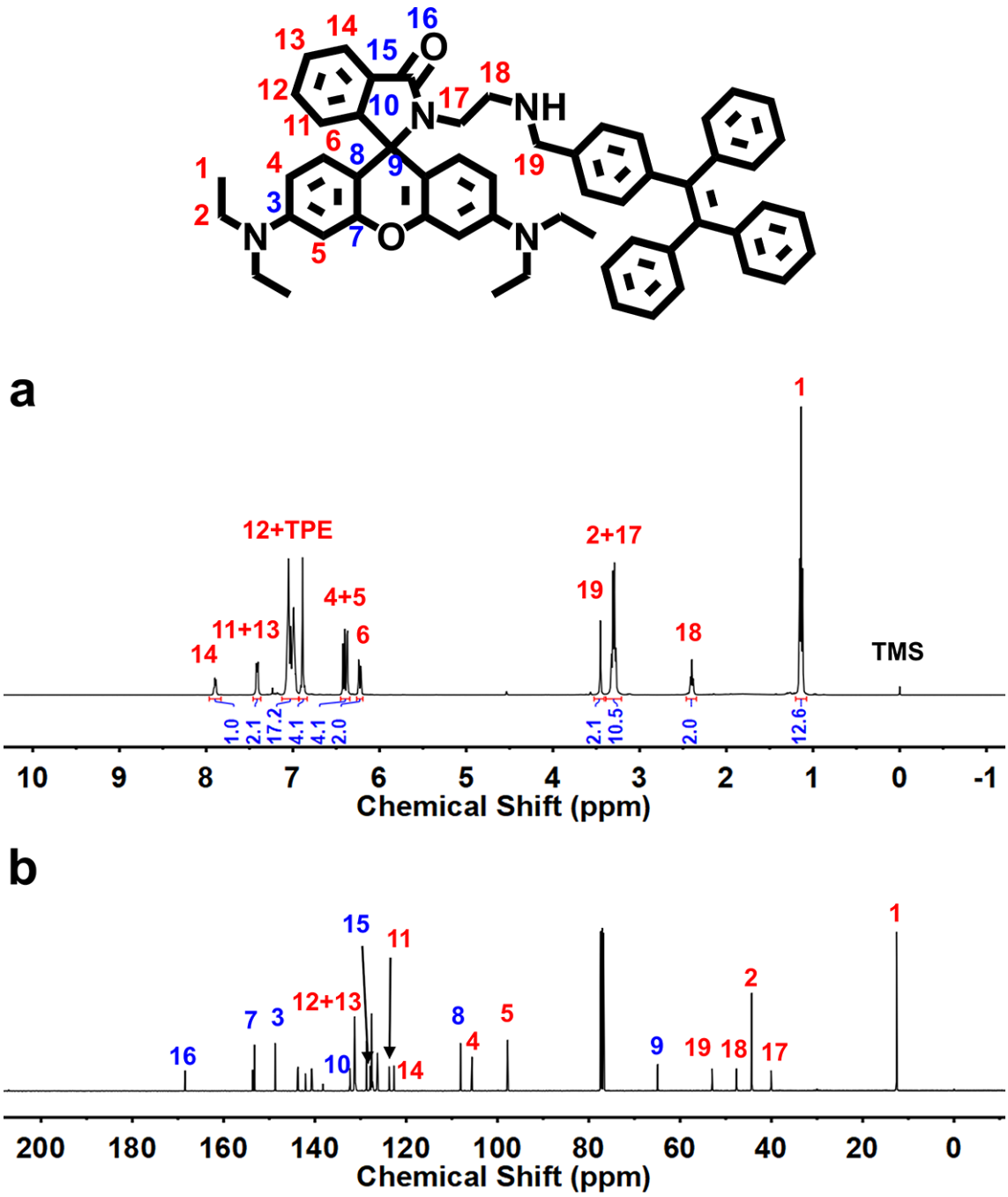


Fig. S3 ^{1}H (a) and ^{13}C (b) NMR spectra of TPE-RB in $CDCl_3$ solution.

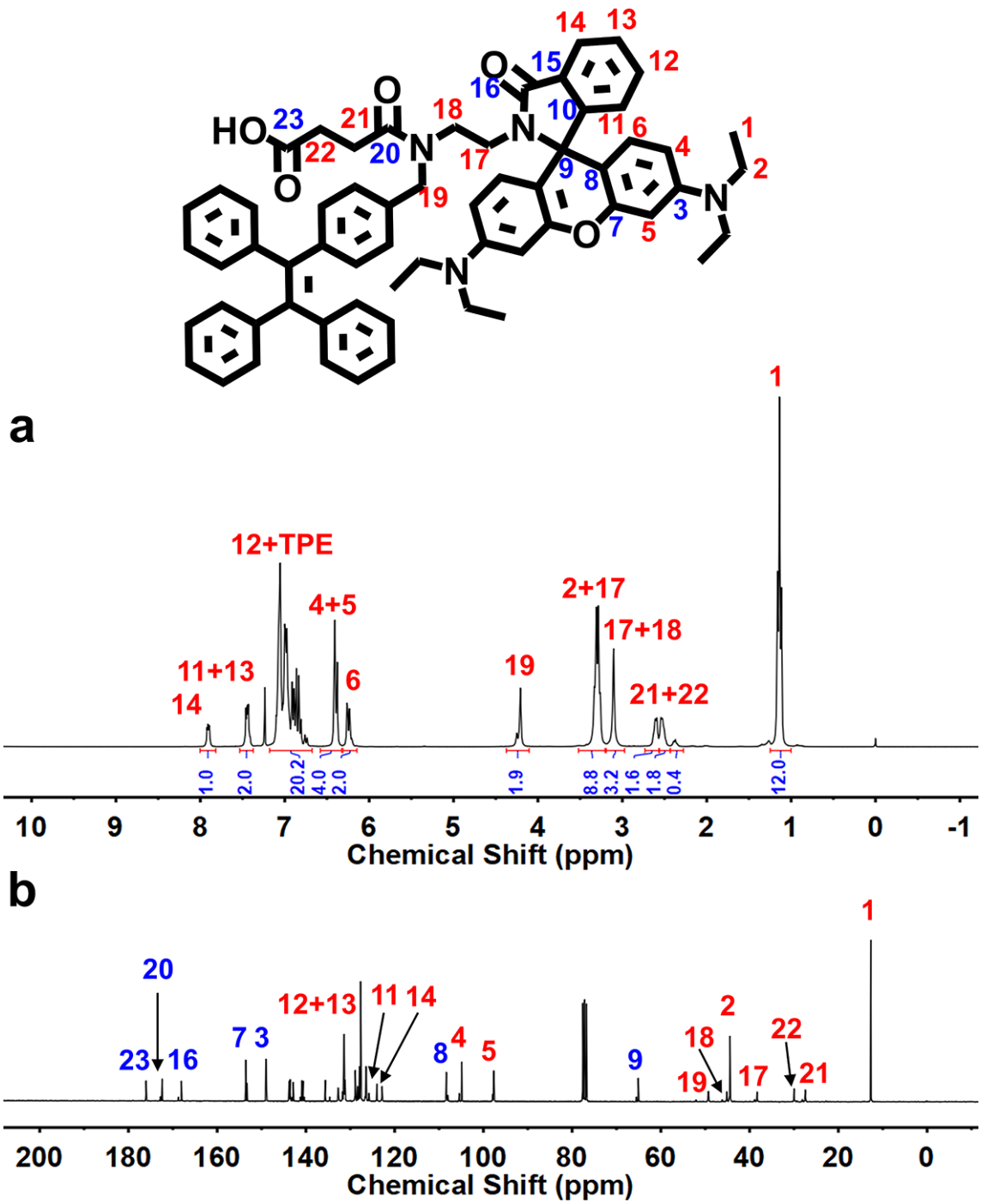


Fig. S4 ^{1}H (a) and ^{13}C (b) NMR spectra of TPE-RB-COOH in $CDCl_3$ solution.

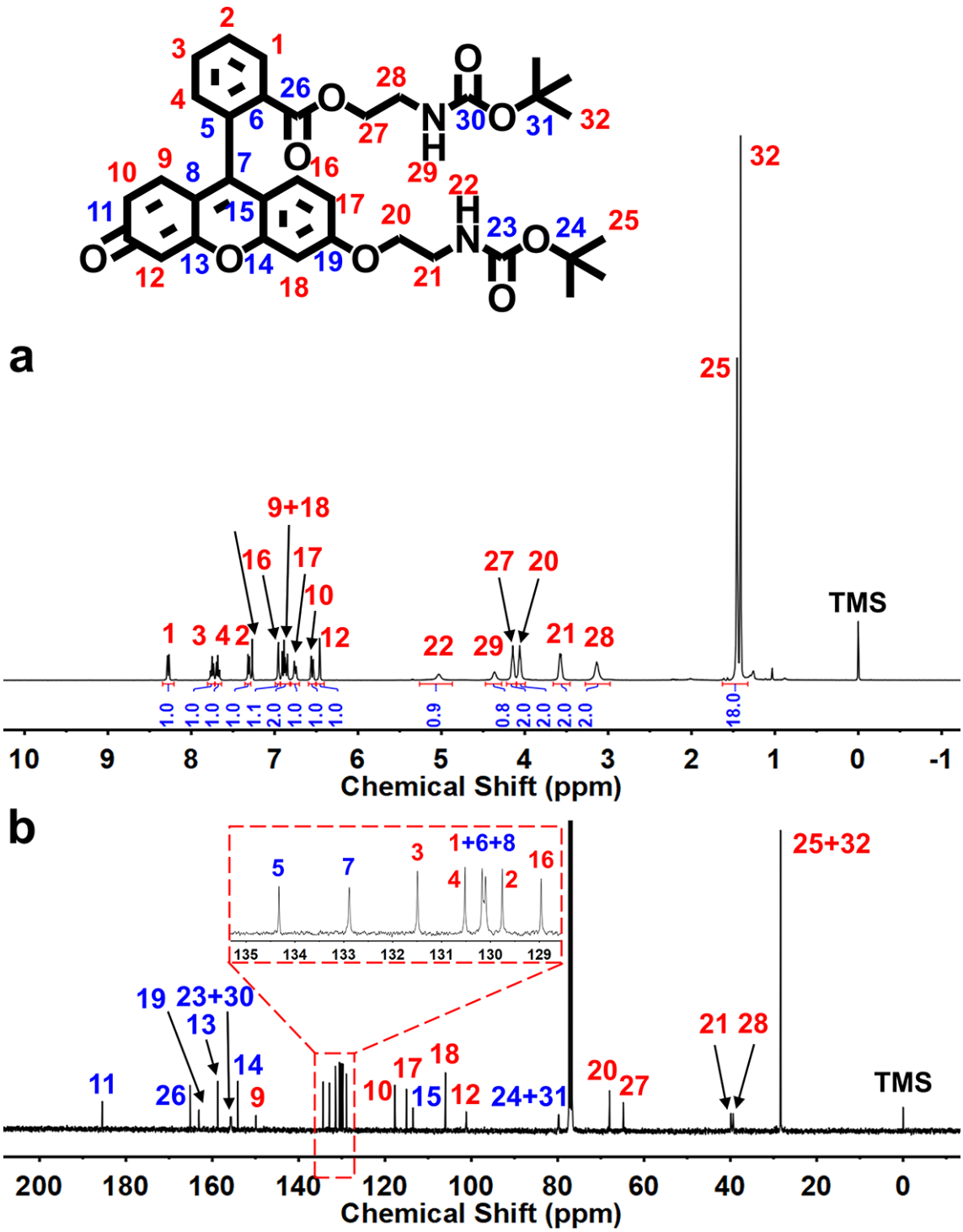


Fig. S5 ^{1}H (a) and ^{13}C (b) NMR spectra of FL-2Boc in $CDCl_3$ solution.

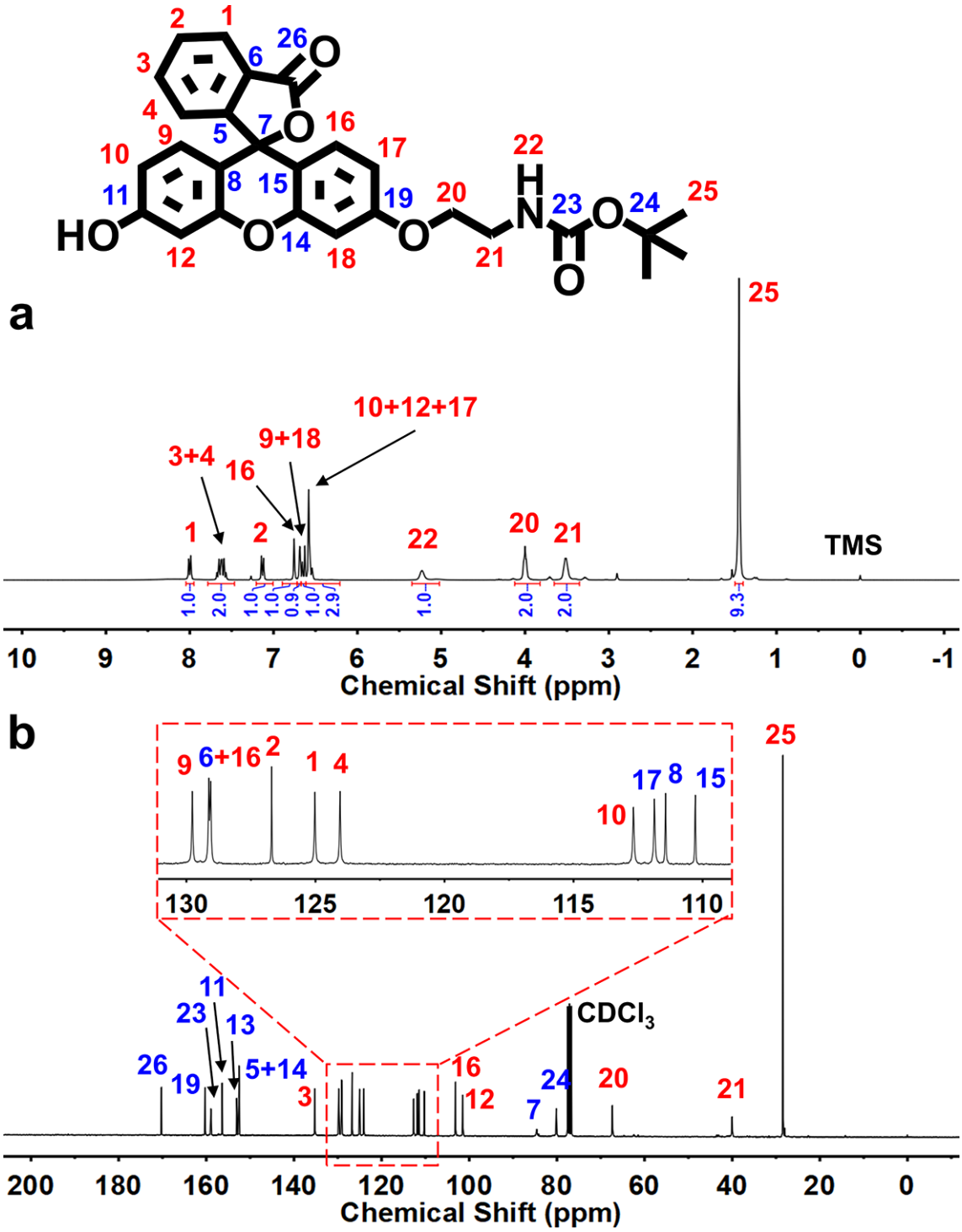


Fig. S6 ^{1}H (a) and ^{13}C (b) NMR spectra of FL-Boc in $CDCl_3$ solution.

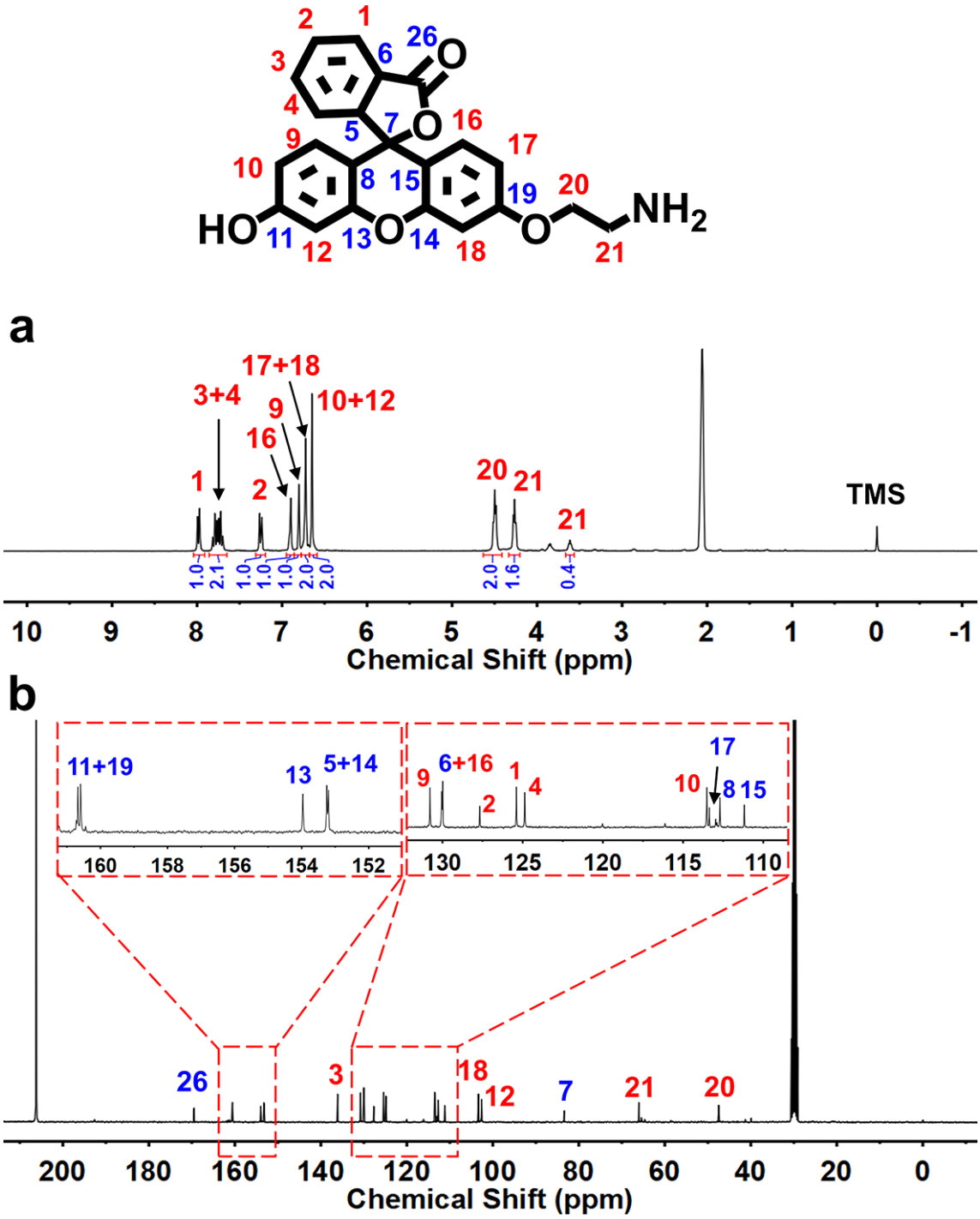


Fig. S7 ^{1}H (a) and ^{13}C (b) NMR spectra of FL-NH_2 in $(CD_3)_2CO$ solution.

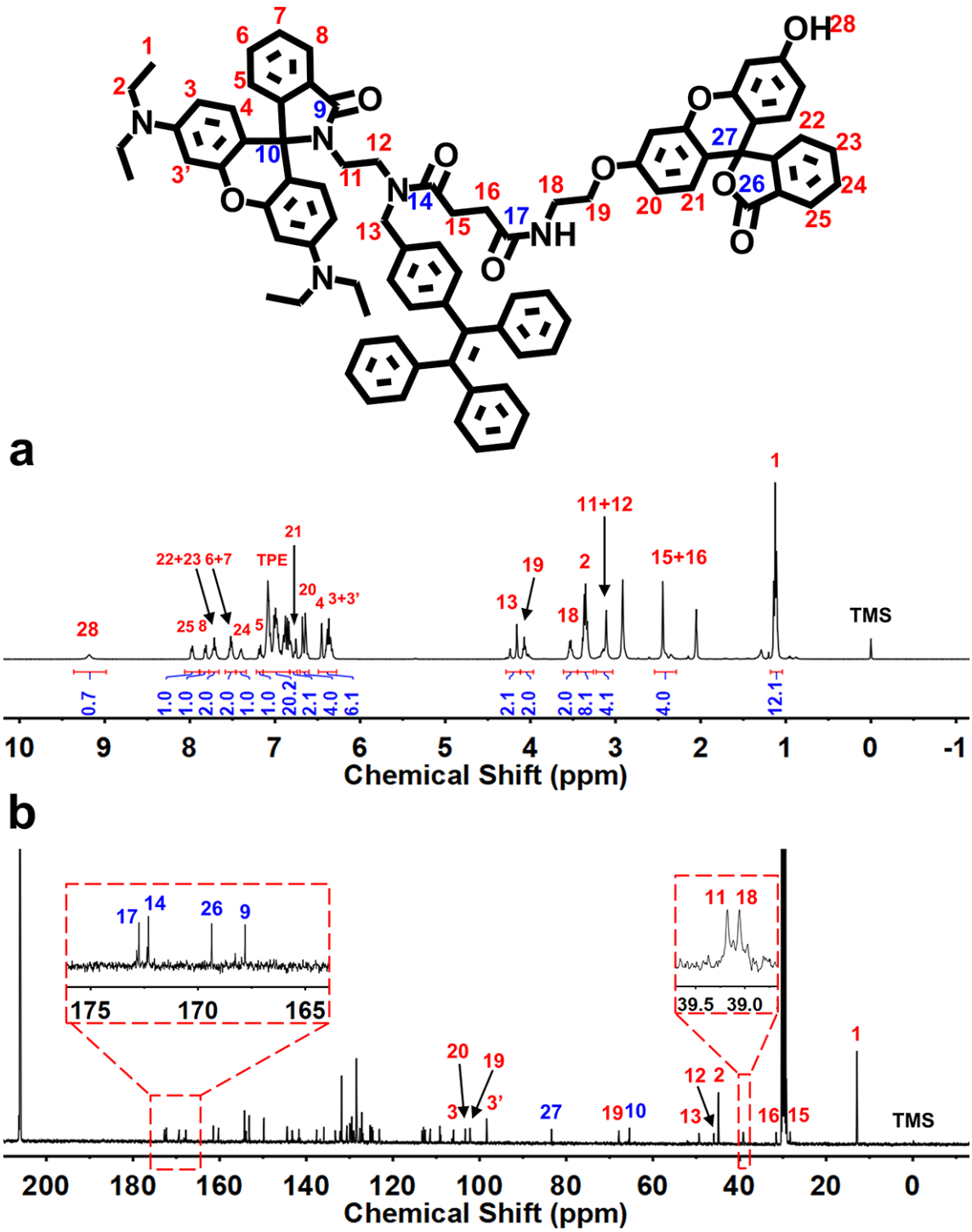


Fig. S8 ^{1}H (a) and ^{13}C (b) NMR spectra of RTF in $(CD_3)_2CO$ solution.

2.3. Mass spectra (MS)

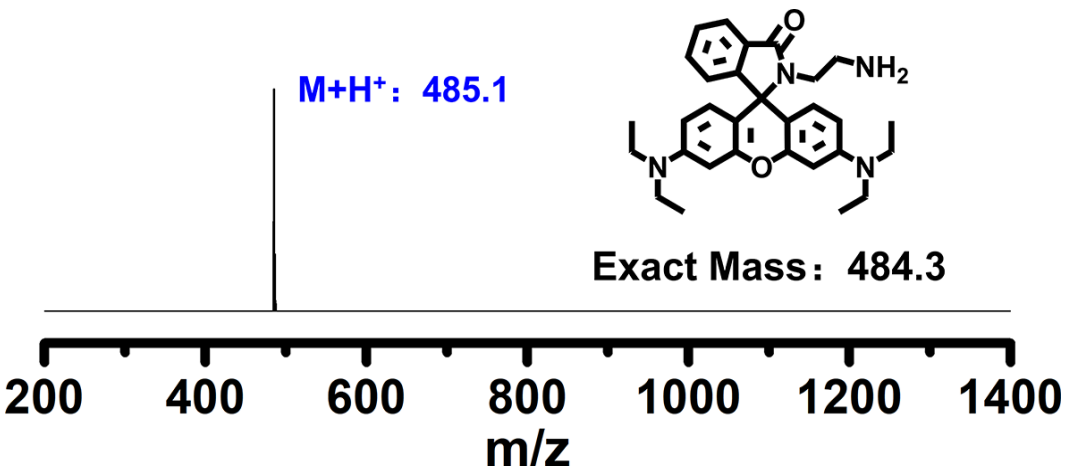


Fig. S9. MALDI-TOF mass spectrum of RB-NH_2.

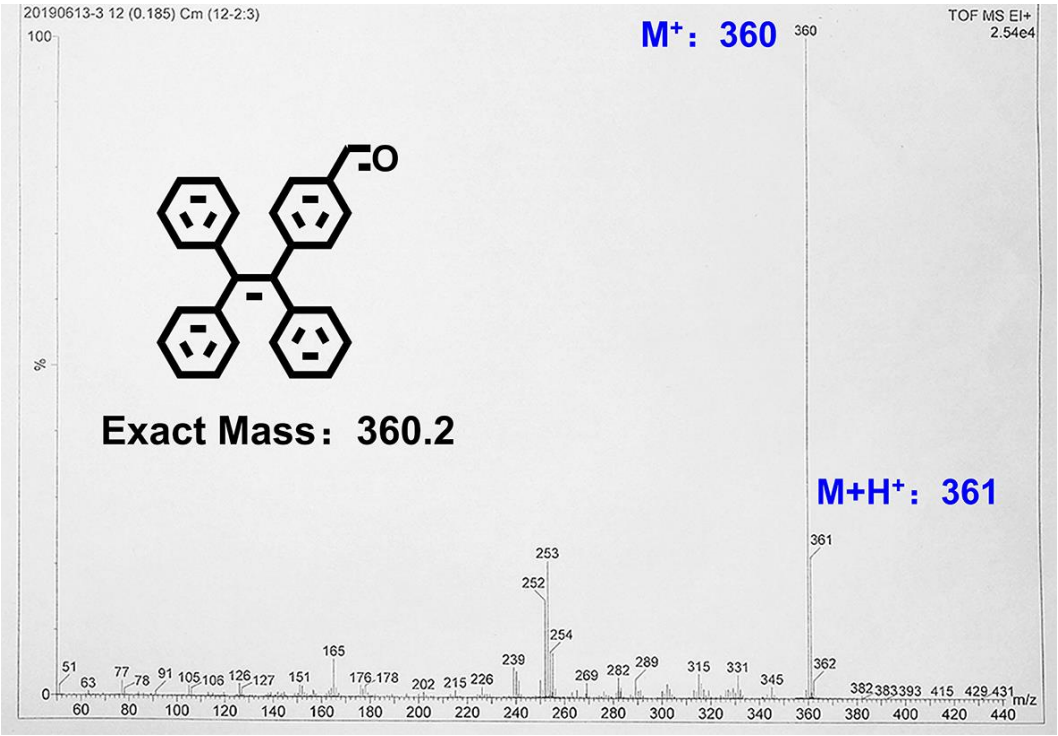


Fig. S10 Electron impact mass spectrum of TPE-CHO.

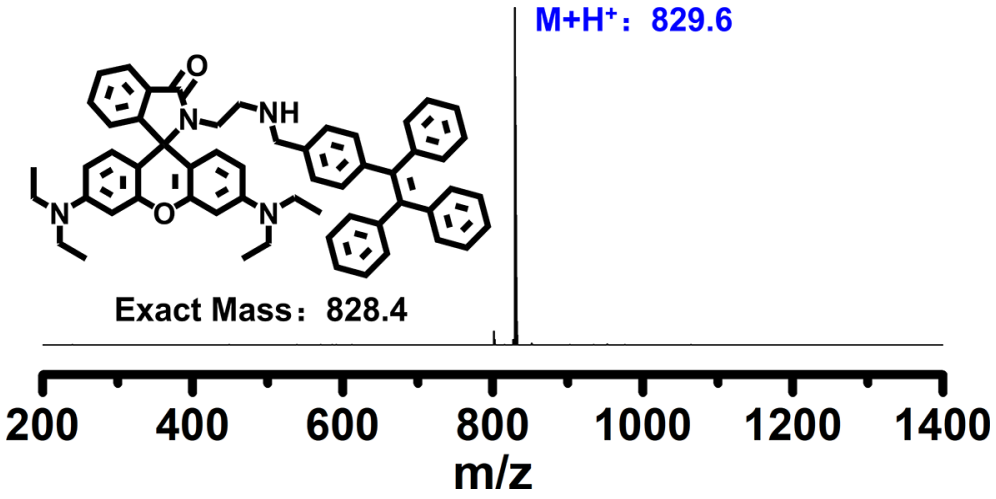


Fig. S11. MALDI-TOF mass spectrum of TPE-RB.

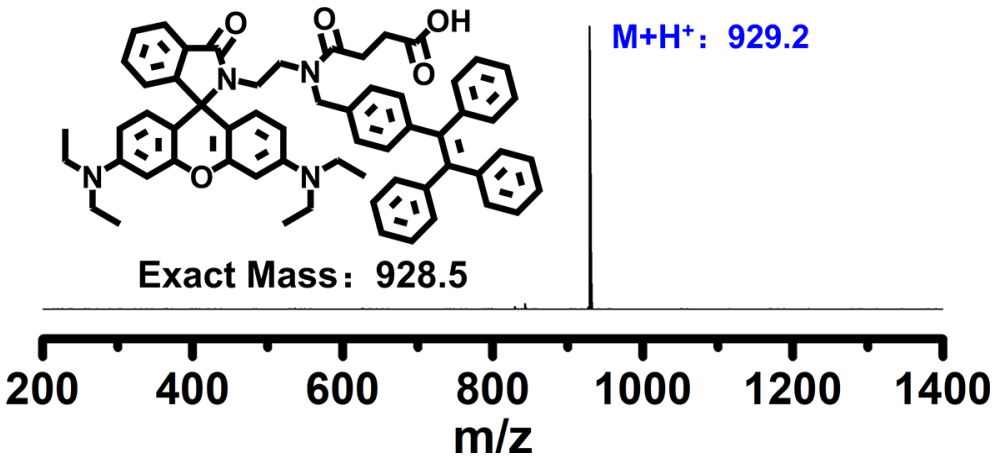


Fig. S12 MALDI-TOF mass spectrum of TPE-RB-COOH.

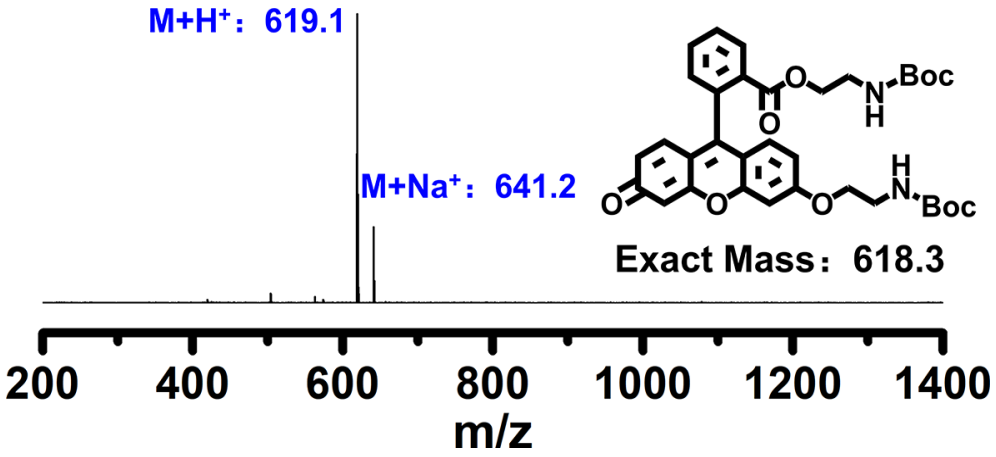


Fig. S13 MALDI-TOF mass spectrum of FL-2Boc.

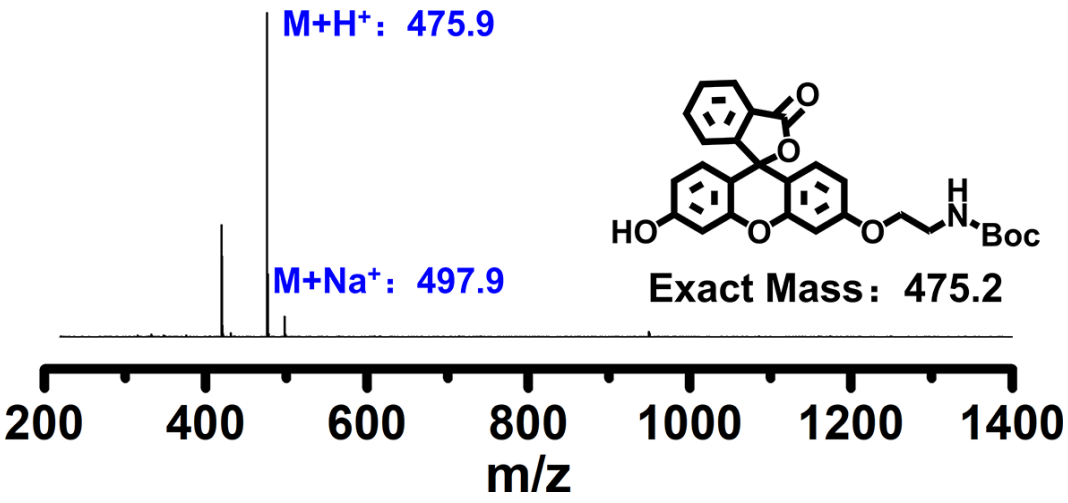


Fig. S14 MALDI-TOF mass spectrum of FL-Boc.

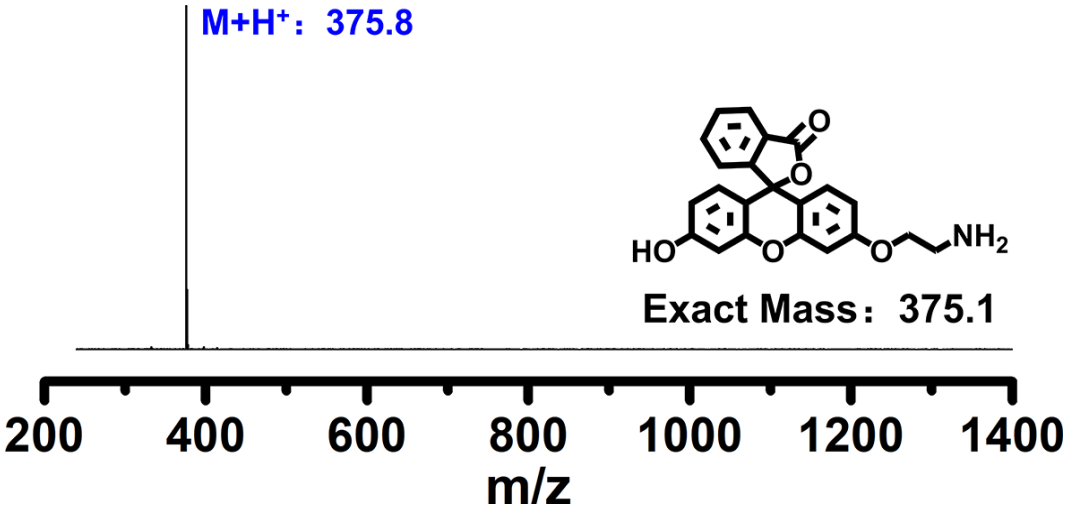


Fig. S15 MALDI-TOF mass spectrum of FL-NH_2.

3. pH-responsive behaviors of RTF

3.1. p*K*a of RB and FL units

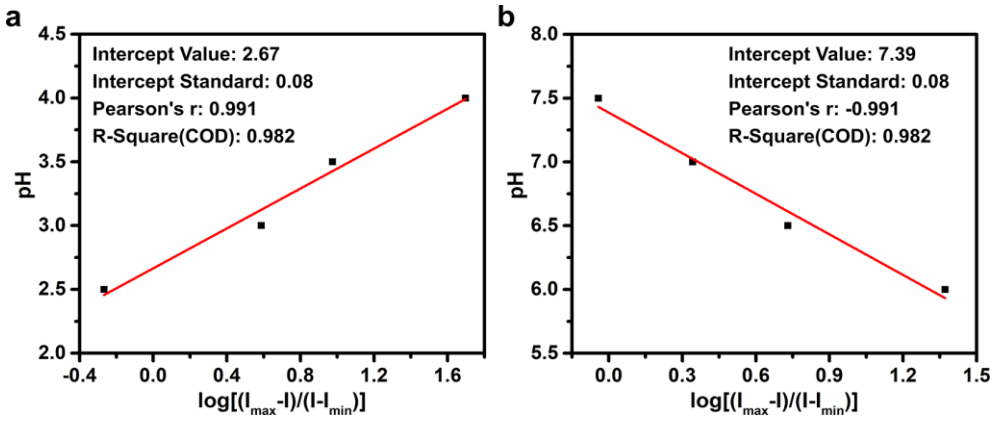


Fig. S16 (a) Plot of pH vs log[(I_{max}-I)(I-I_{min})], where I was the observed fluorescence intensity of **RTF** (*c* = 20 μM) at 582 nm. The intercept value was the p*K*a value (2.67 ± 0.08) of equilibrium between the ring-opened form and the lactam form of rhodamine unit. (b) Plot of pH vs log[(I_{max}-I)(I-I_{min})], where I was the observed fluorescence intensity of **RTF** (*c* = 20 μM) at 515 nm. The intercept value was the p*K*a value (7.39 ± 0.08) of equilibrium between the ring-opened form and the lactam form of fluorescein unit.

3.2. Reversible pH response of RTF

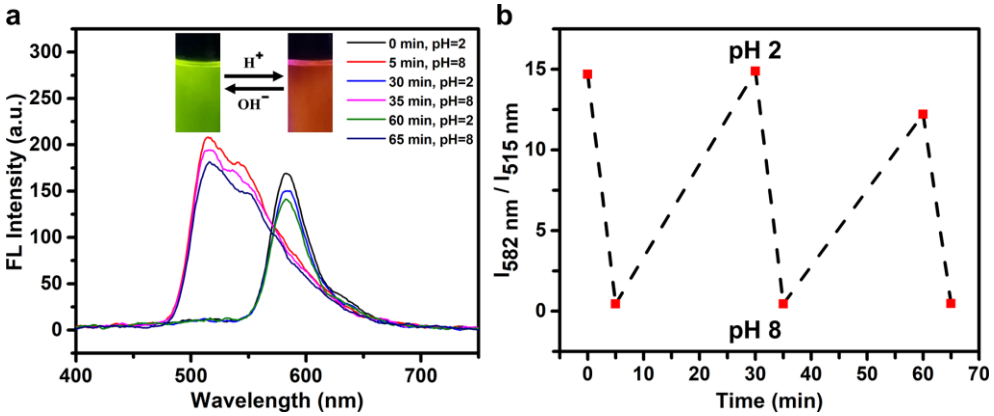


Fig. S17 (a) Reversible fluorescence changes of **RTF** (20 μM) upon excitation at 315 nm. (b) Reversible fluorescence ratio ($I_{582\ nm}$ and $I_{515\ nm}$ was the observed fluorescence intensity of **RTF** at 582 nm and 515 nm, respectively) changes between pH 2 and pH 8 EtOH-H_2O (3/2, v/v) mixture solution (pH 2) was prepare by Britton-Robinson buffer solutions and chromatographically pure EtOH with the aid of a pH meter. Then the pH value was adjusted by adding little amount of 3 M of NaOH with a micropipette for pH 8 and the fluorescent signal was monitored. After the fluorescence intensity tending to be stable, the pH was readjusted to 2 by adding little amount of 3 M of HCl with a micropipette. Repeating the operations made the pH values changed between 2 and 8.

4. Fluorescence of RTF at different pH values

4.1. Fluorescence spectra of RTF at different f_w and pH values

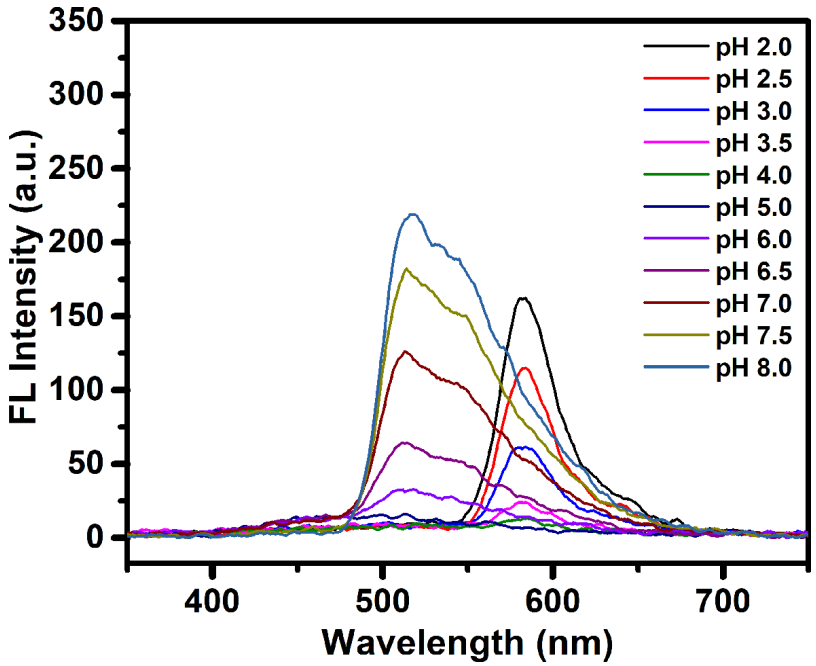


Fig. S18 Fluorescence spectra of **RTF** at f_w 45.0% with different pH values.

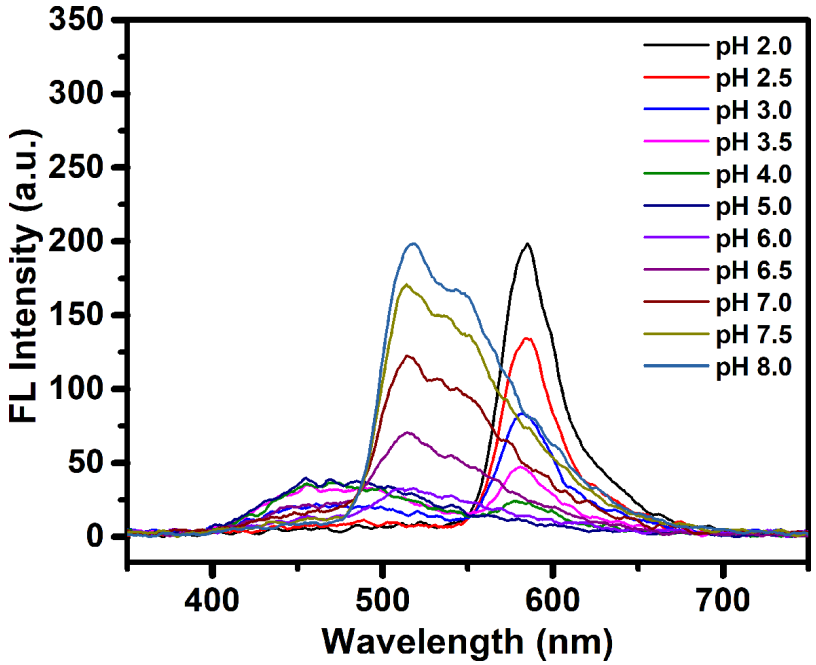


Fig. S19 Fluorescence spectra of **RTF** at f_w 50.0% with different pH values.

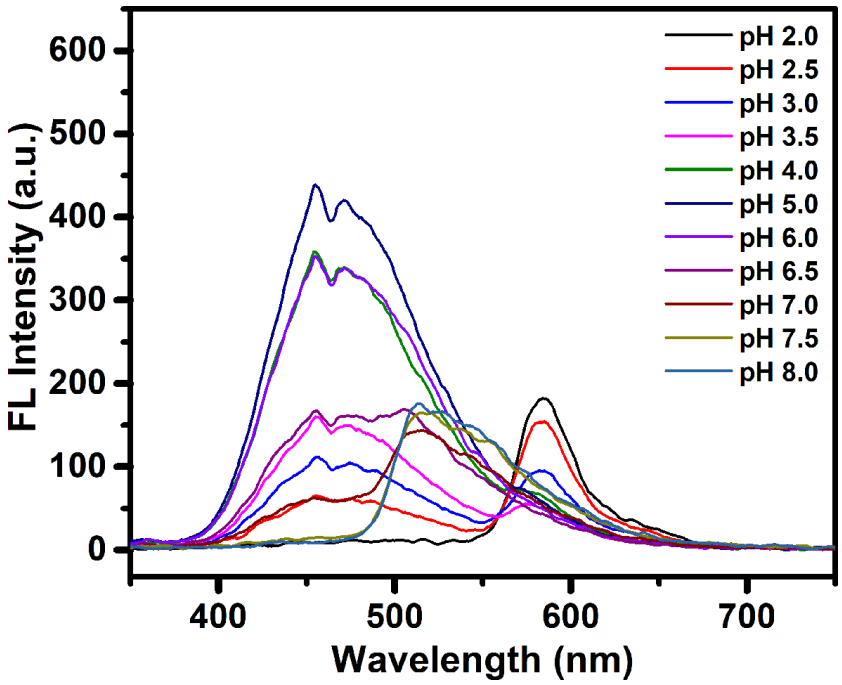


Fig. S20 Fluorescence spectra of **RTF** at f_w 52.5% with different pH values.

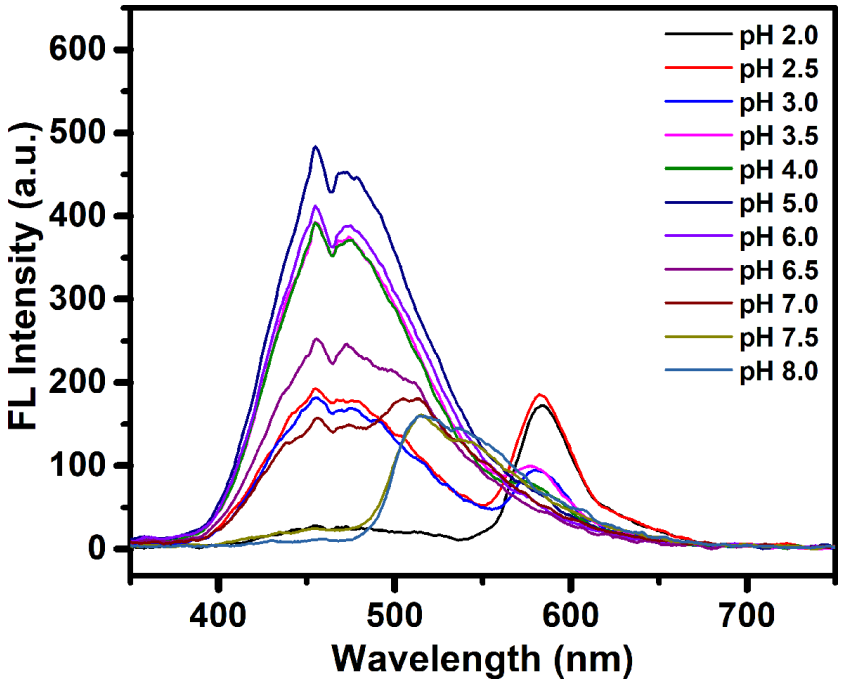


Fig. S21 Fluorescence spectra of **RTF** at f_w 55.0% with different pH values.

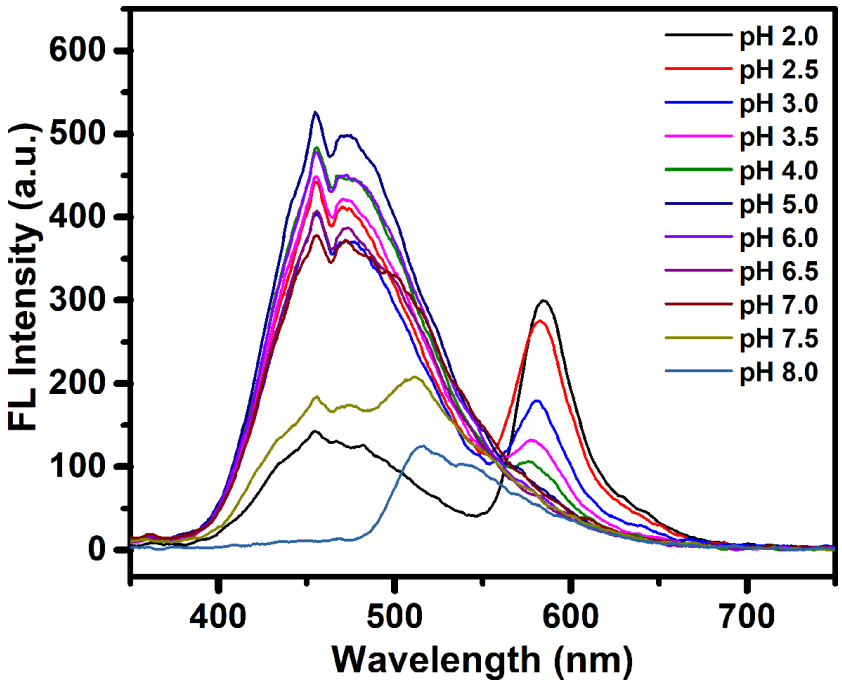


Fig. S22 Fluorescence spectra of **RTF** at f_w 60.0% with different pH values.

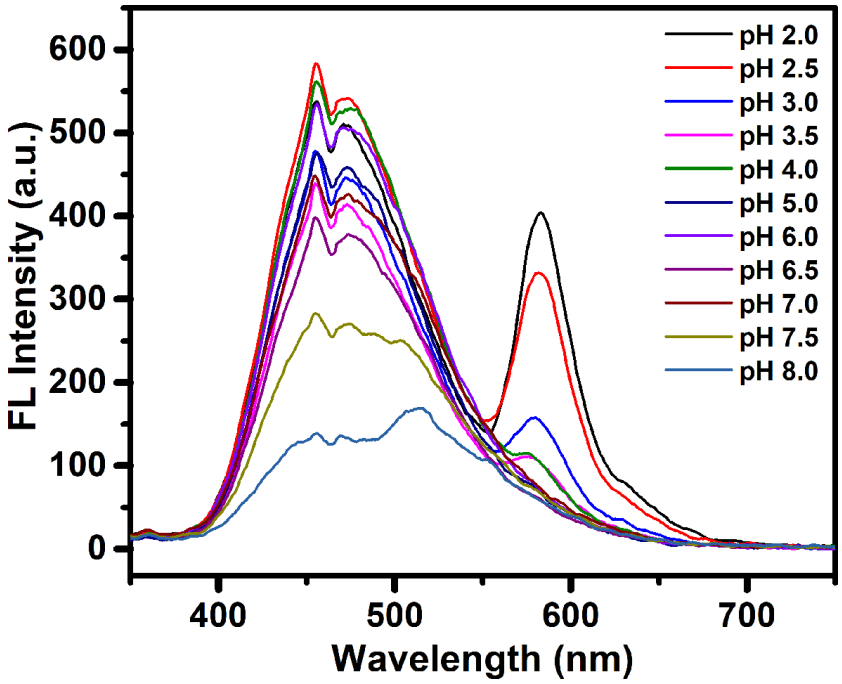


Fig. S23 Fluorescence spectra of **RTF** at f_w 65.0% with different pH values.

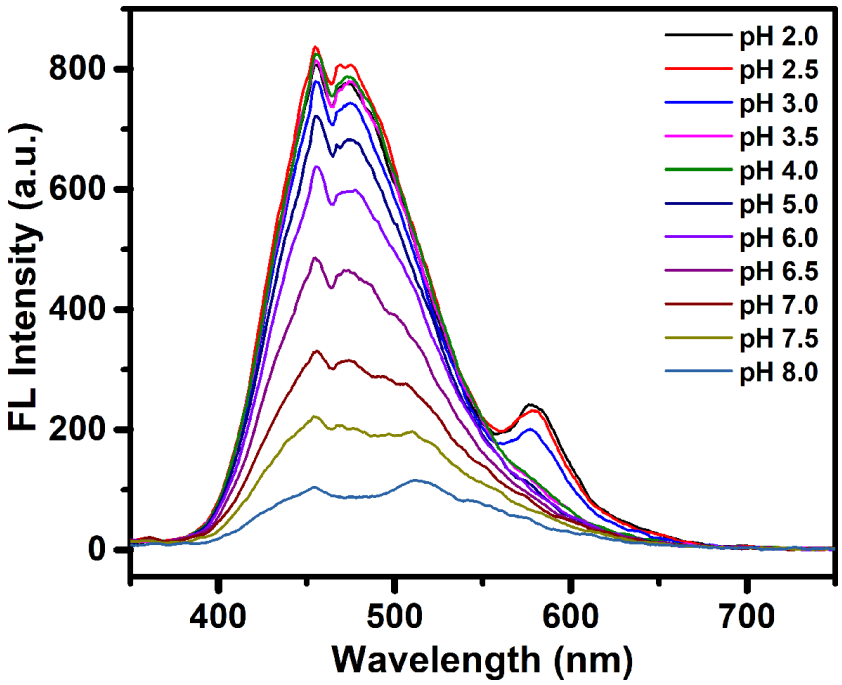


Fig. S24 Fluorescence spectra of **RTF** at f_w 75.0% with different pH values.

4.2. CIE of RTF at different f_w and pH values

Table S1 CIE coordinates of RTF at f_w 35.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.5226	0.4383	583
2.5	0.4983	0.4288	582
3.0	0.4469	0.4160	580
3.5	0.3857	0.3897	581
4.0	0.2659	0.3048	457
5.0	-	-	
6.0	-	-	
6.5	0.3170	0.5513	514
7.0	0.3261	0.5831	514
7.5	0.3316	0.5905	514
8.0	0.3317	0.6013	515

Table S2 CIE coordinates of RTF at f_w 45.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.5155	0.4354	581
2.5	0.5075	0.4300	584
3.0	0.4662	0.4158	584
3.5	0.3899	0.3876	582
4.0	0.3473	0.3862	585
5.0	-	-	
6.0	-	-	
6.5	0.2975	0.5058	512
7.0	0.3141	0.5566	513
7.5	0.3277	0.5922	514
8.0	0.3323	0.6002	516

Table S3 CIE coordinates of RTF at f_w 50.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.5299	0.4351	585
2.5	0.5084	0.4249	585
3.0	0.4185	0.3762	582
3.5	0.3181	0.3223	581
4.0	0.2603	0.2930	472
5.0	0.2094	0.2748	455
6.0	0.2221	0.3267	503
6.5	0.2754	0.4648	514
7.0	0.3057	0.5350	514
7.5	0.3227	0.5692	514
8.0	0.3268	0.5818	519

Table S4 CIE coordinates of RTF at f_w 52.5.% with different pH values.

pH	x	y	Peak/nm
2.0	0.5104	0.4272	584
2.5	0.3786	0.3428	585
3.0	0.2824	0.2850	456
3.5	0.2139	0.2449	455
4.0	0.1831	0.2246	455
5.0	0.1740	0.2202	455
6.0	0.1770	0.2326	455
6.5	0.2004	0.2913	505
7.0	0.2595	0.4296	515
7.5	0.3183	0.5594	514
8.0	0.3237	0.5743	513

Table S5 CIE coordinates of RTF at f_w 55.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.4648	0.3940	583
2.5	0.2877	0.2816	455
3.0	0.2356	0.2532	456
3.5	0.1929	0.2301	455
4.0	0.1846	0.2226	455
5.0	0.1749	0.2202	455
6.0	0.1739	0.2219	455
6.5	0.1860	0.2536	456
7.0	0.2153	0.3231	512
7.5	0.3038	0.5296	515
8.0	0.3223	0.5716	517

Table S6 CIE coordinates of RTF at f_w 60.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.3696	0.3241	584
2.5	0.2505	0.2617	456
3.0	0.2235	0.2483	456
3.5	0.1998	0.2368	455
4.0	0.1861	0.2286	456
5.0	0.1761	0.2248	455
6.0	0.1753	0.2278	455
6.5	0.1783	0.2353	456
7.0	0.1874	0.2576	456
7.5	0.2135	0.3189	511
8.0	0.3110	0.5577	516

Table S7 CIE coordinates of RTF at f_w 65.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.2650	0.2685	456
2.5	0.2435	0.2592	455
3.0	0.2077	0.2400	455
3.5	0.1936	0.2330	455
4.0	0.1827	0.2295	456
5.0	0.1757	0.2268	456
6.0	0.1747	0.2302	456
6.5	0.1782	0.2357	455
7.0	0.1829	0.2462	455
7.5	0.1967	0.2725	455
8.0	0.2252	0.3342	514

Table S8 CIE coordinates of RTF at f_w 75.0% with different pH values.

pH	x	y	Peak/nm
2.0	0.2007	0.2365	456
2.5	0.1961	0.2344	455
3.0	0.1922	0.2325	455
3.5	0.1725	0.2213	455
4.0	0.1728	0.2228	455
5.0	0.1730	0.2236	455
6.0	0.1771	0.2333	456
6.5	0.1826	0.2436	455
7.0	0.1948	0.2650	456
7.5	0.2063	0.2831	454
8.0	0.2308	0.3300	511

5.Fluorescence quantum yield of white fluorescence

Table S9 The CIE coordinates and quantum yields of white fluorescence of RTF with different concentration.

Color	C /μM	pH	f_w /%	CIE Coordinates /(x, y)	Quantum Yield /%
White	20	3.5	50	(0.3181, 0.3223)	0.7
	20	2.0	60	(0.3696, 0.3241)	6.7
	40	2.0	60	(0.3111, 0.2877)	11.5
	80	2.0	60	(0.3074, 0.2874)	12.2

6. Data of RTF-s

6.1. ^{1}H and ^{13}C NMR spectra for preparing RTF-s

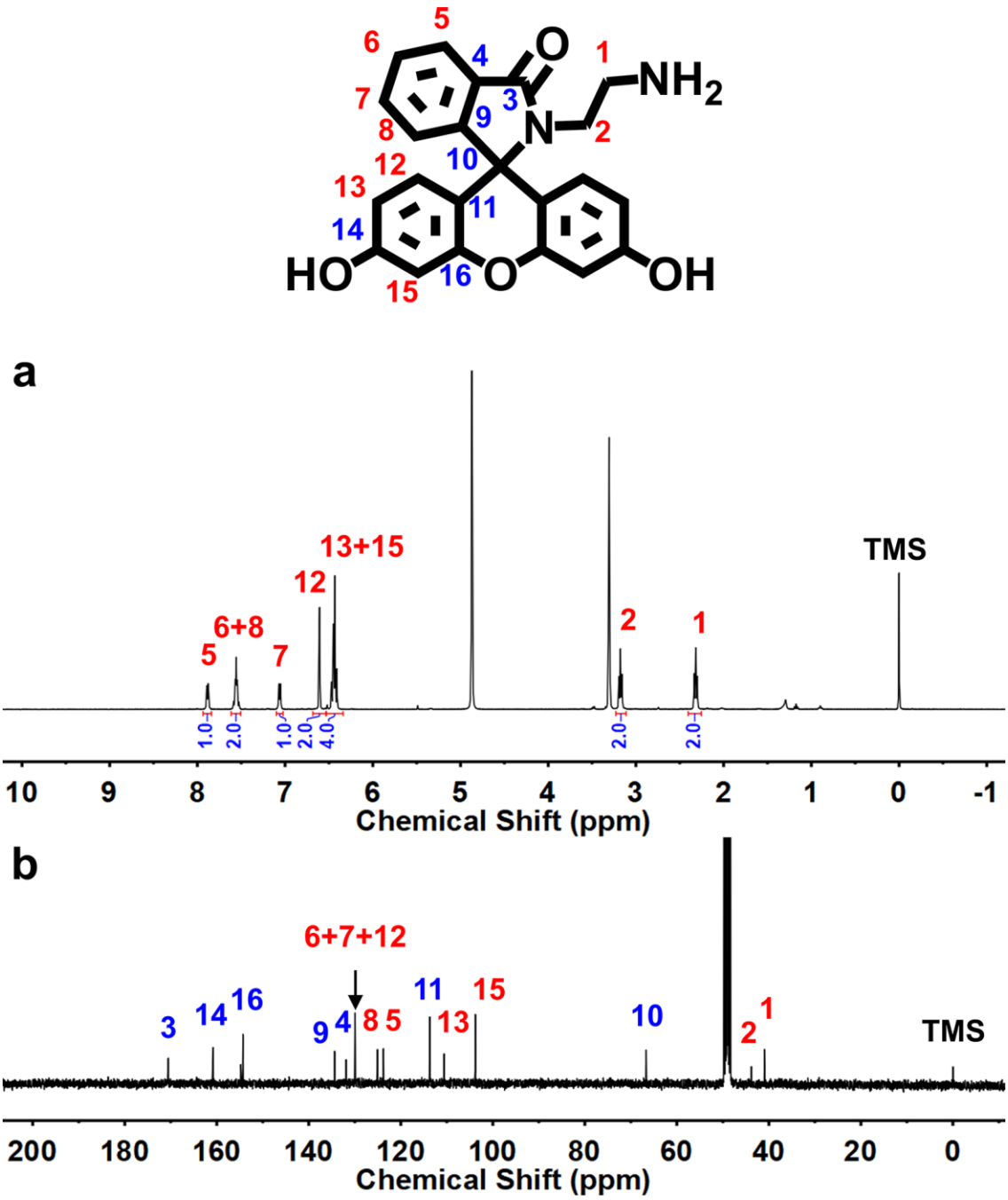


Fig. S25 ^{1}H (a) and ^{13}C (b) NMR spectra of product FL-s in CD_3OD solution.

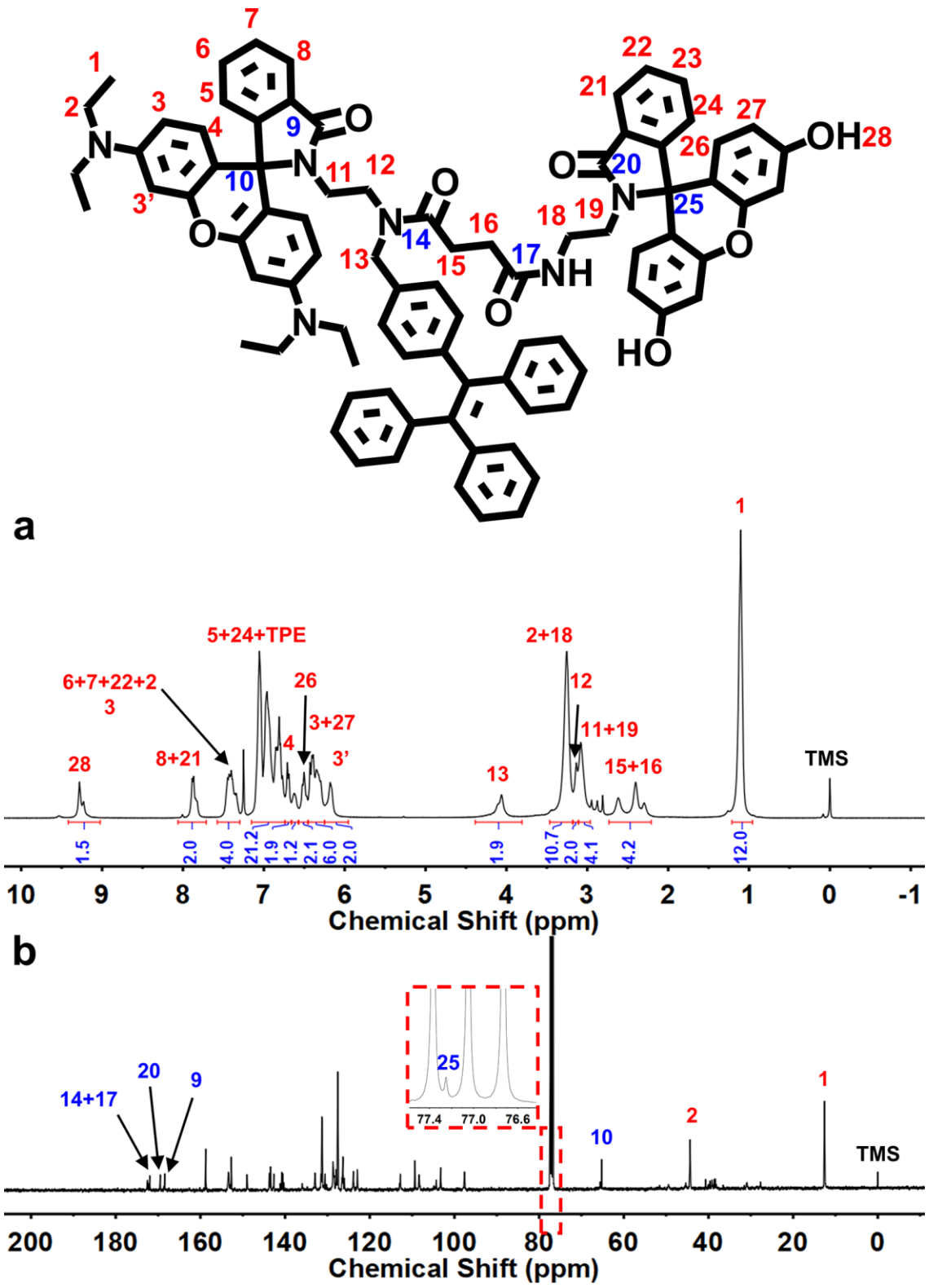


Fig. S26 ^{1}H (a) and ^{13}C (b) NMR spectra of RTF-s in $CDCl_3$ solution.

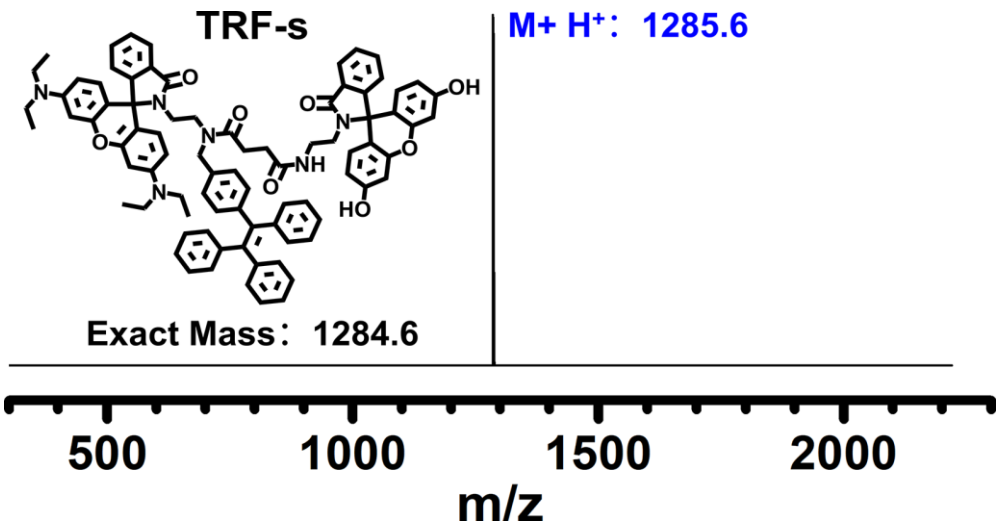


Fig. S27 MALDI-TOF mass spectrum of **RTF-s**.

6.2. Fluorescence of RTF-s

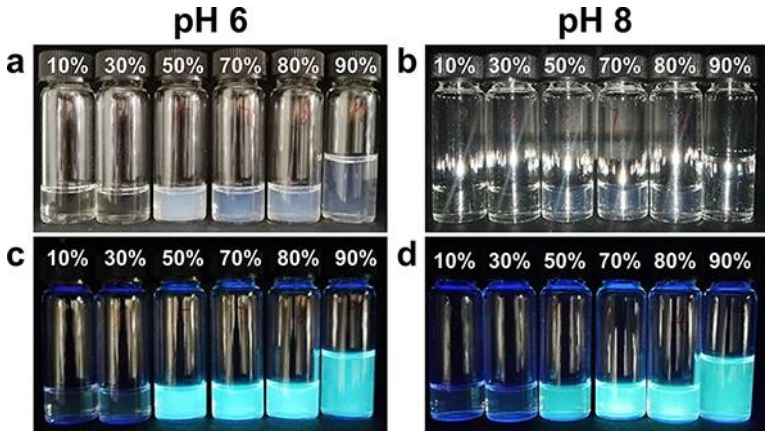


Fig. S28 At pH 6 (a and c) and pH 9 (b and d), the photographs were **RTF-s** with different water fraction. The photographs were taken under nature light (a and b) and 365 nm UV lamp (c and d). The spirolactam remained in the closed form in basic environment and the closed fluorescein unit did not produce green fluorescence.

7. Fluorescence of the mixed system of RB, FL and TPE dyes

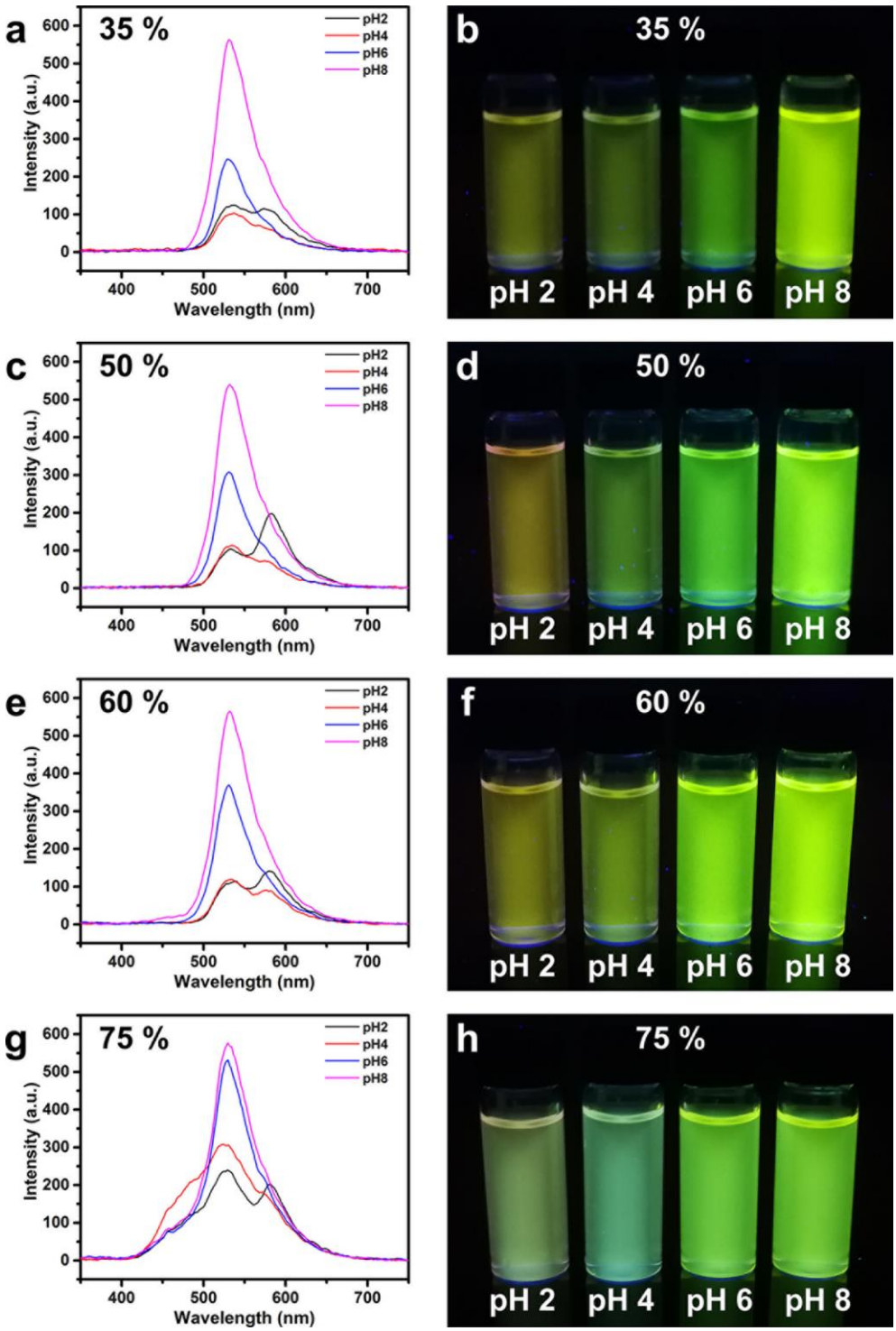


Fig. S29 (a, c, e, g) The fluorescence spectra of ethanol/water solutions containing RB, FL and TPE dyes with different water fraction and pH (λ_{em} = 315 nm, all the concentration of the components was 20 μM). (b, d, f, h) The fluorescence photographs of the mixed system under UV lamp.

8. The aggregation processes of RTF with increasing f_w

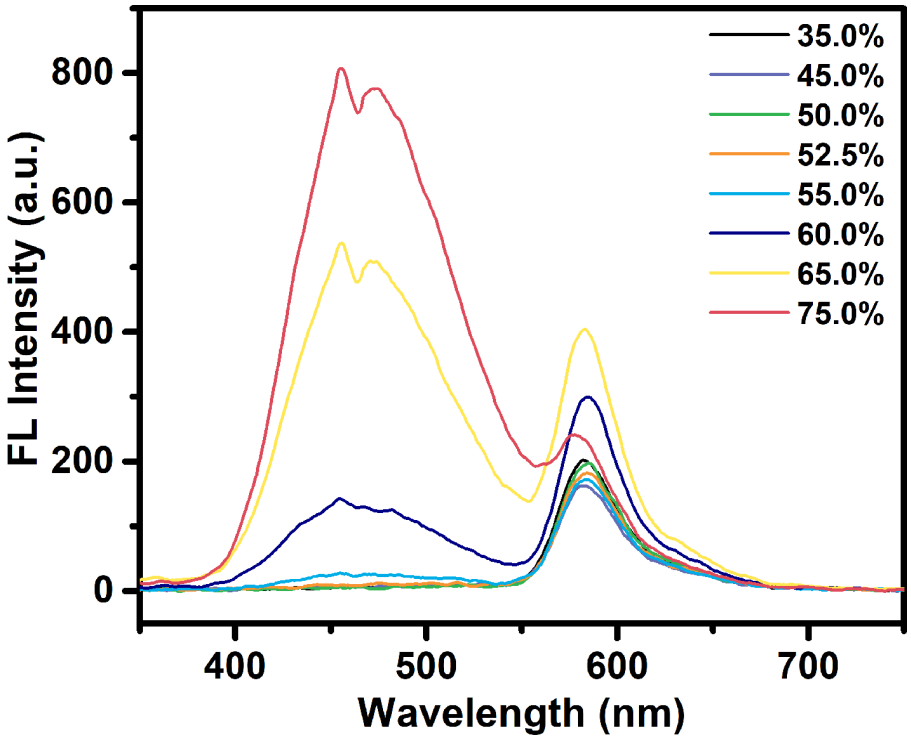


Fig. S30 Fluorescence spectra of **RTF** at pH 2.0 with different f_w.

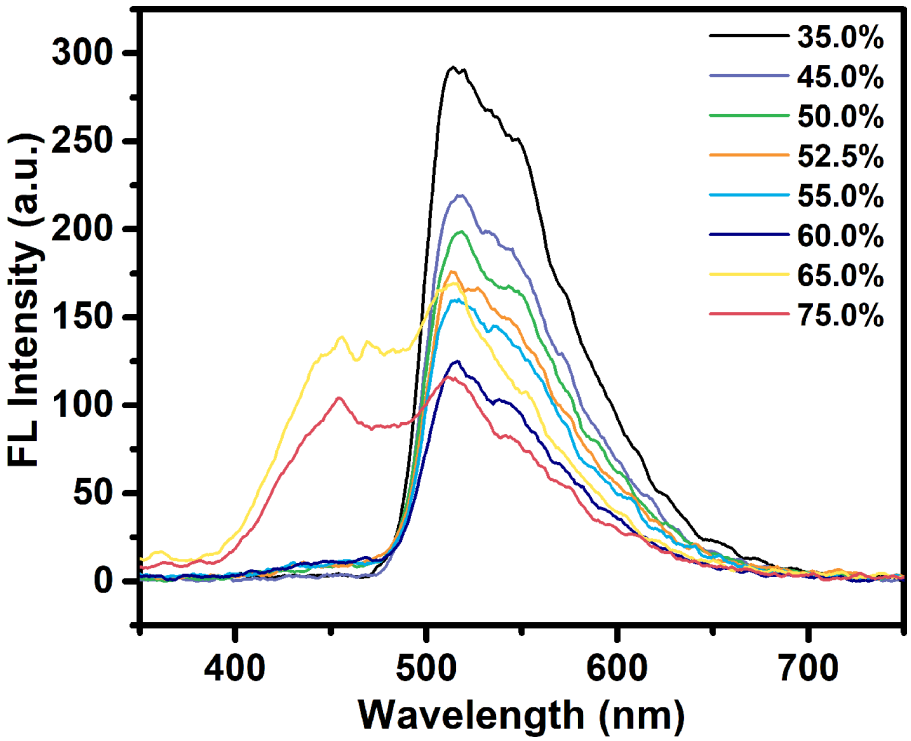
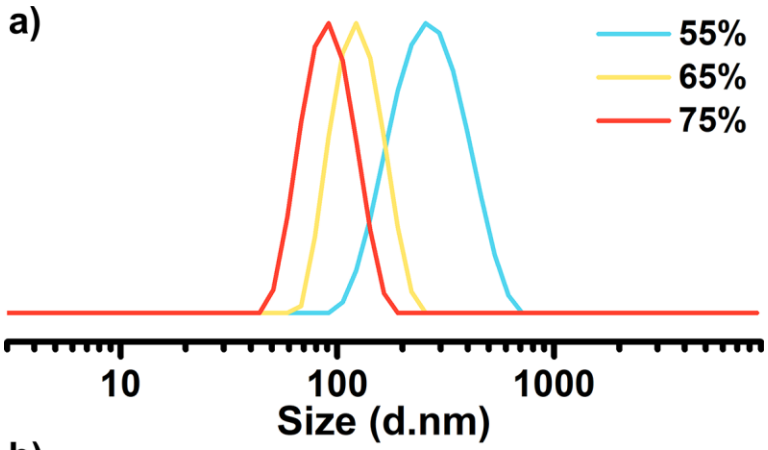


Fig. S31 Fluorescence spectra of **RTF** at pH 8.0 with different f_w.

a)

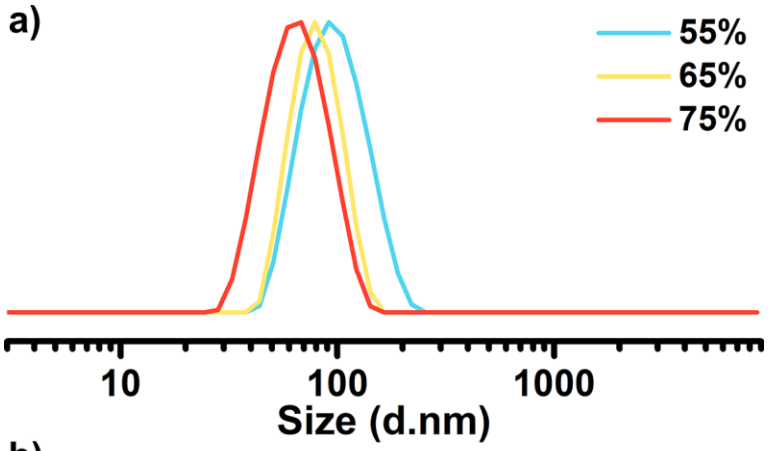


b)

f_w (%)	55	65	75
Z-Average (d.nm)	250	118	87
PDI	0.197	0.092	0.048

Fig. S32 Size distribution of **RTF** at f_w 55%, 65%, 75% with pH 2.0.

a)



b)

f_w (%)	55	65	75
Z-Average (d.nm)	89	77	65
PDI	0.134	0.047	0.214

Fig. S33 Size distribution of **RTF** at f_w 55%, 65%, 75% with pH 8.0.

9. FRET process

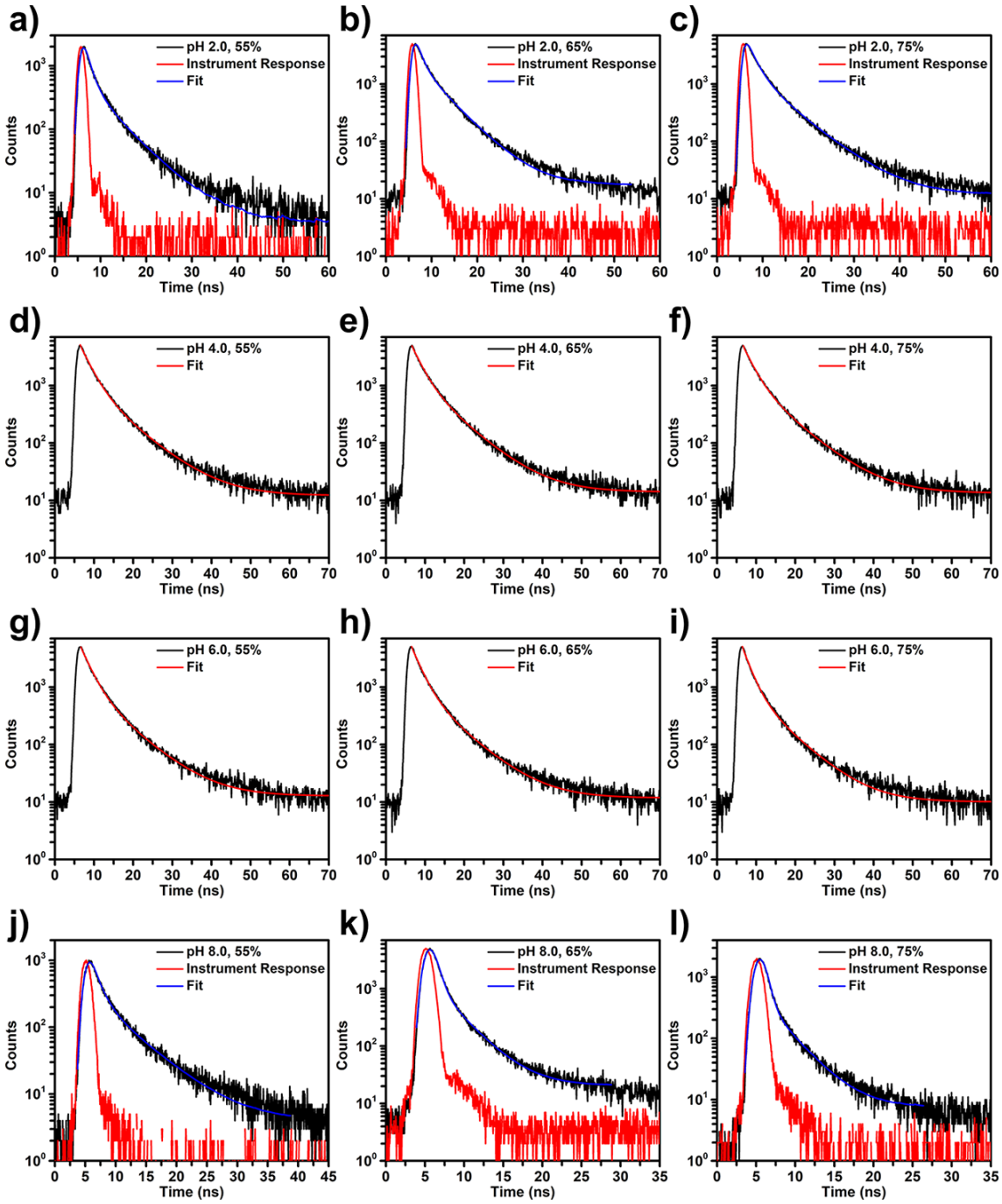


Fig. S34 Time-resolved fluorescence decay curves of **RTF** with different f_w and pH. (For fluorescence lifetime less than 4 ns, instrument response function were measured to reduce errors by the adopted deconvolution.)

10. The pH response of RB and FL units

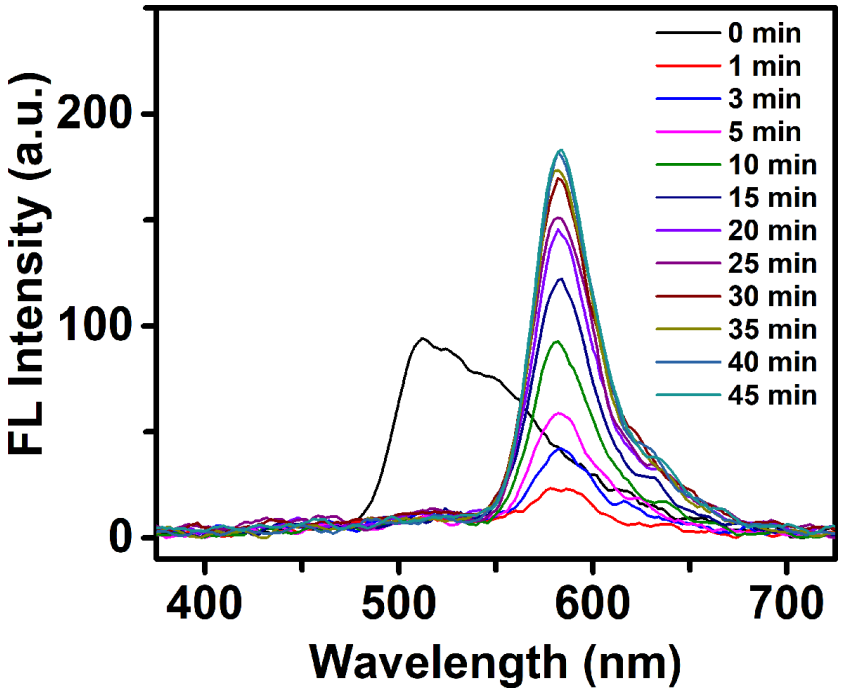


Fig. S35 At pH 2 and f_w = 35%, fluorescence spectra of **RTF** with time.

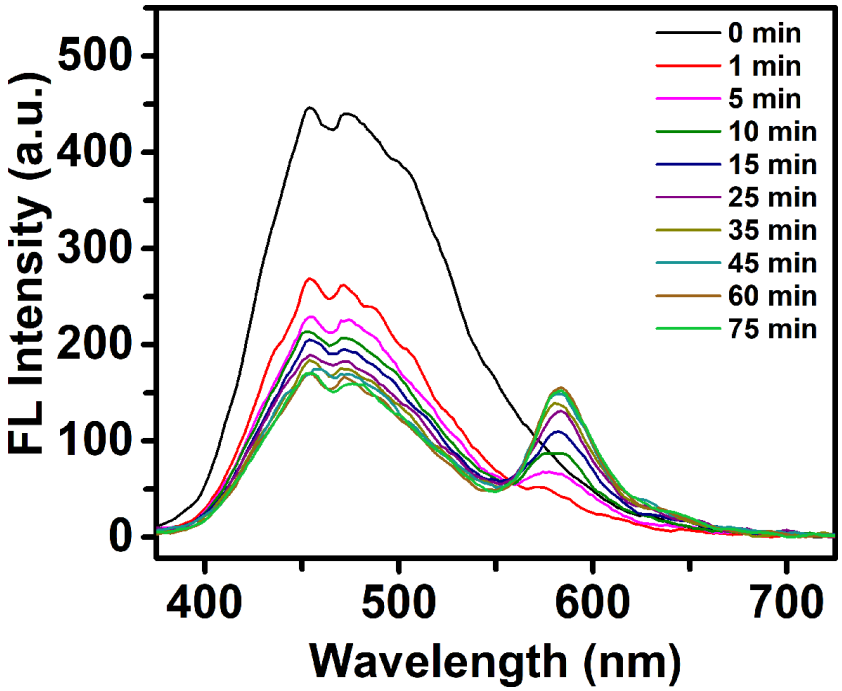


Fig. S36 At pH 2 and f_w = 55%, fluorescence spectra of **RTF** with time.

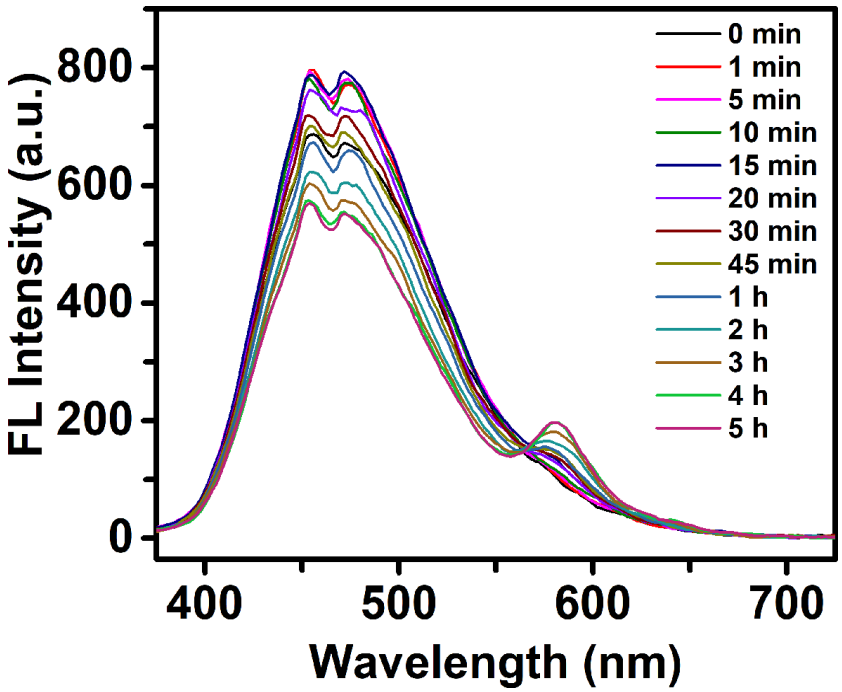


Fig. S37 At pH 2 and f_w = 75%, fluorescence spectra of **RTF** with time.

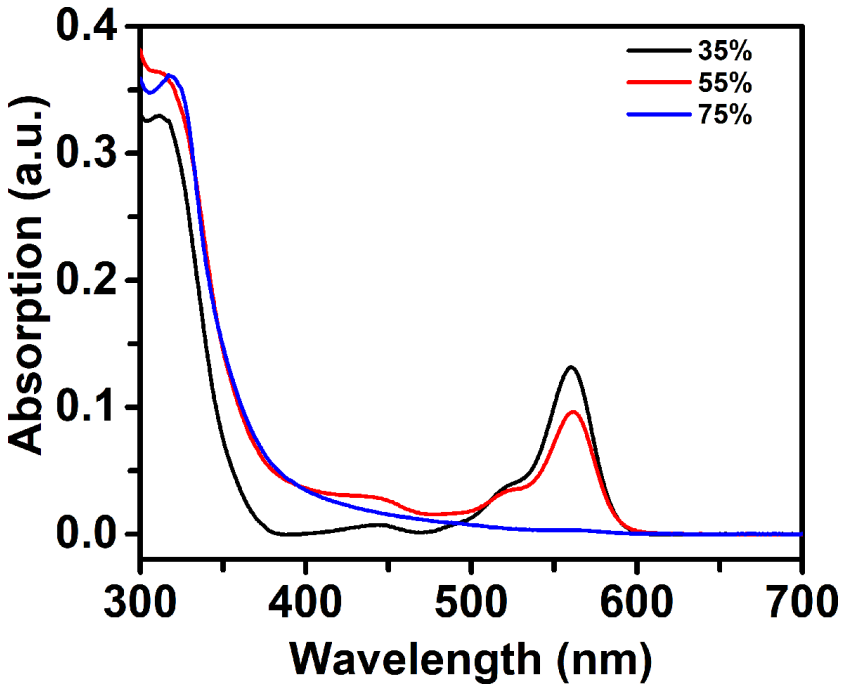


Fig. S38 At pH 2, UV/Vis absorption spectra of **RTF** (after stability) with different f_w.

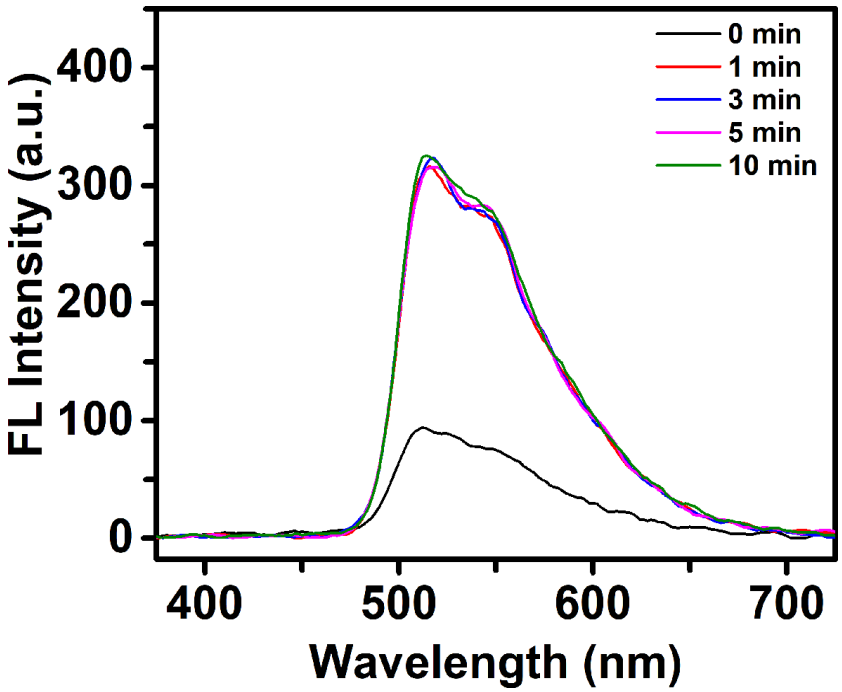


Fig. S39 At pH 8 and f_w = 35%, fluorescence spectra of **RTF** with time.

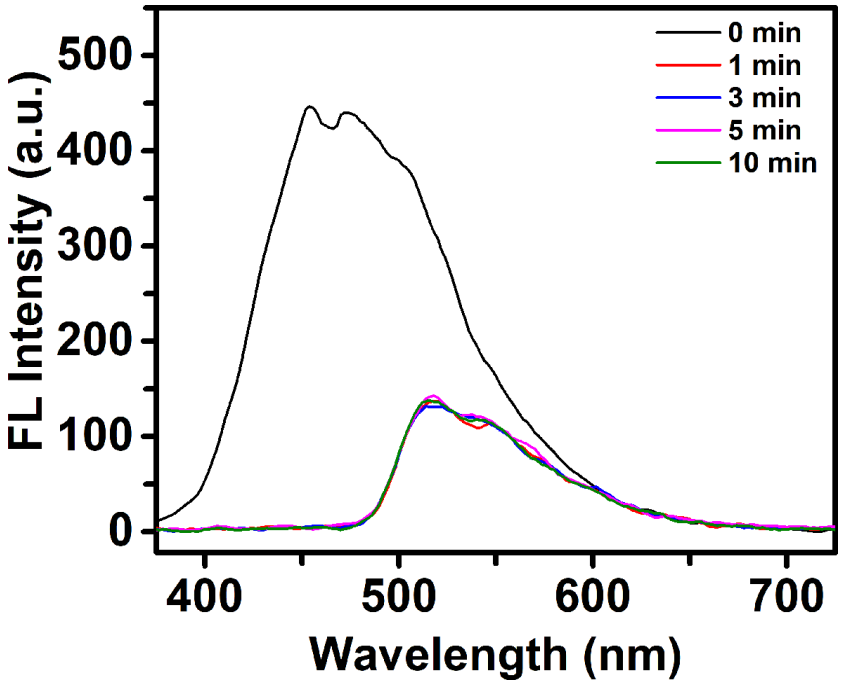


Fig. S40 At pH 8 and f_w = 55%, fluorescence spectra of **RTF** with time.

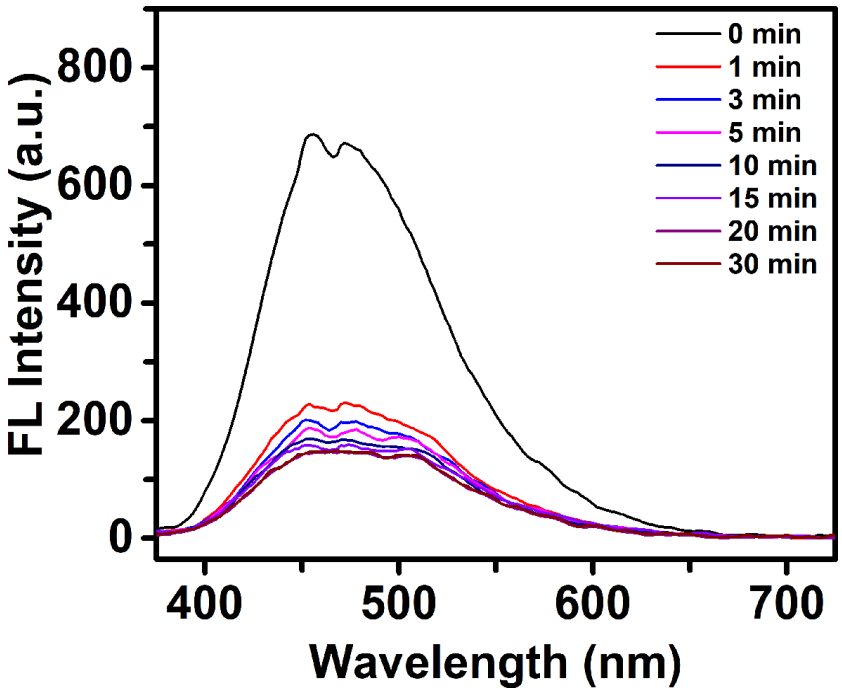


Fig. S41 At pH 8 and f_w = 75%, fluorescence spectra of **RTF** with time.

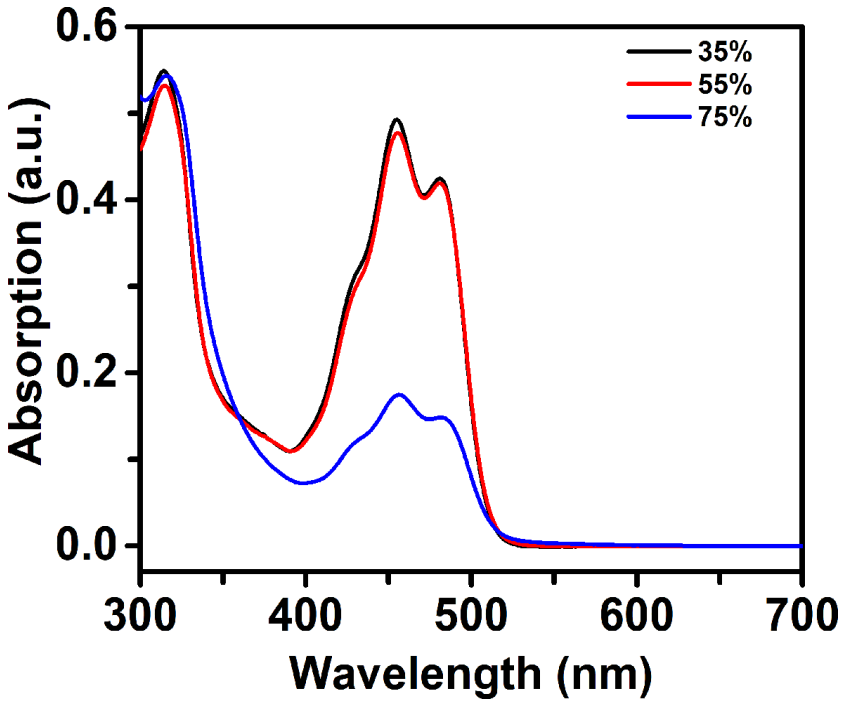


Fig. S42 At pH 8, UV/Vis absorption spectra of **RTF** (after stability) with different f_w.

11. Preparation and characterization of RTF NPs

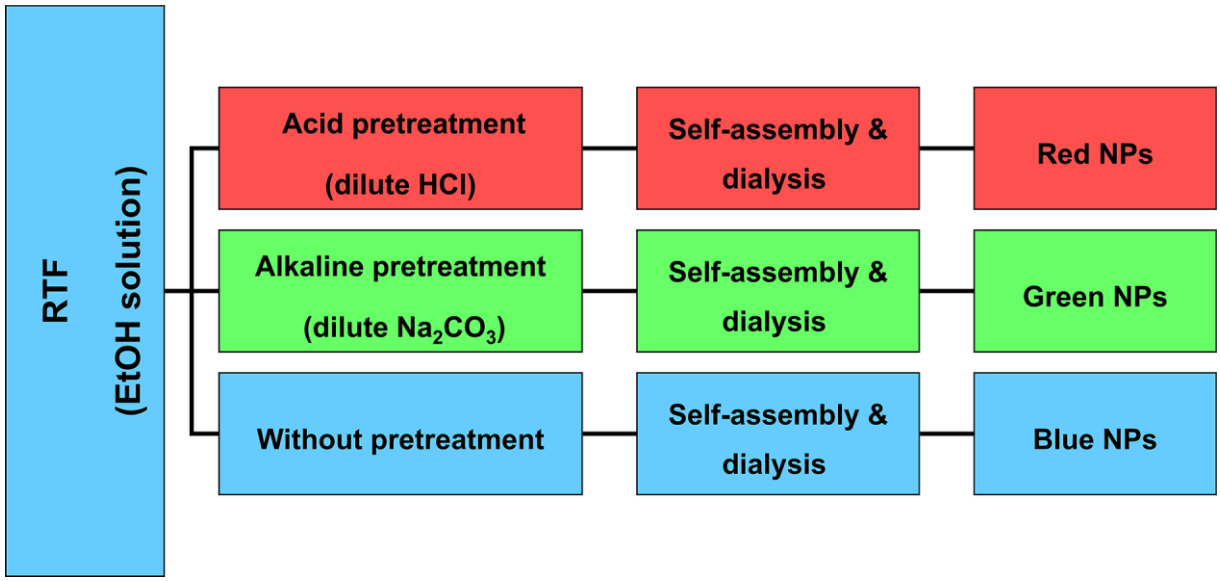


Fig. S43 The process of preparing red, green and blue NPs by self-assembly. A typical self-assembly aggregate solution was prepared as following: **RTF** (15 mg) was dissolved in EtOH (2 mL). Then deionized water (8 mL) was added dropwise into the solution at the rate of 0.05 mL/min via a syringe pump. The colloidal dispersion was further stirred for another 1 h. The organic solvent was removed by dialysis (MW cutoff, 100-500 Da) against deionized water for 24 hours with renewing distilled water every 6 hours.

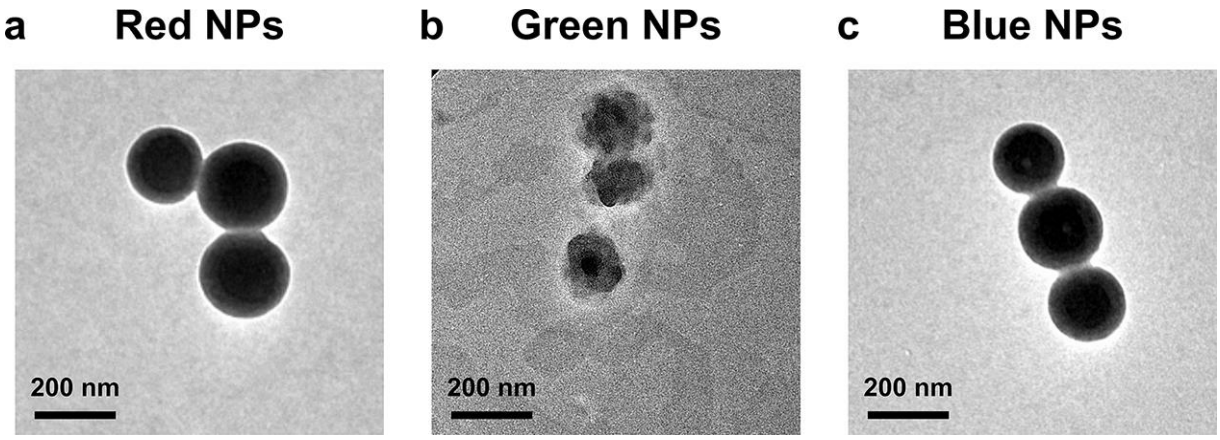


Fig. S44 TEM images of red, green and blue NPs.

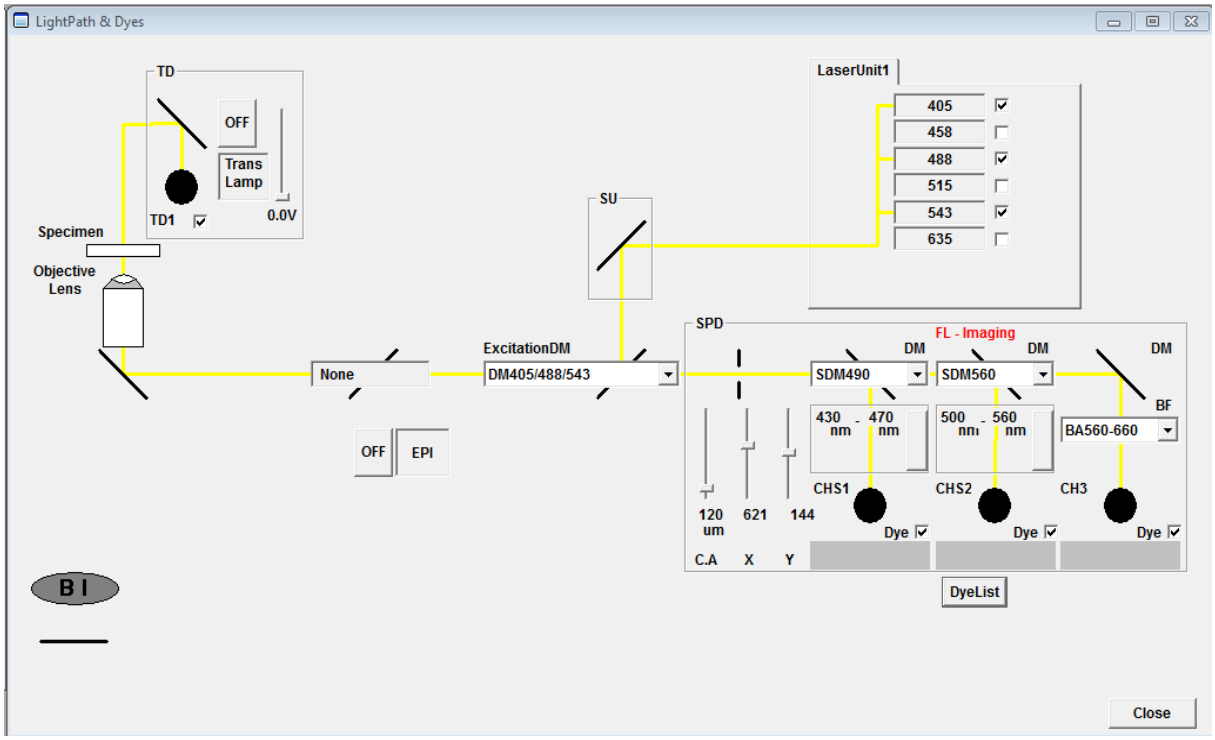


Fig. S45 The lightpath of the laser scanning confocal microscopy for multichannel imaging.

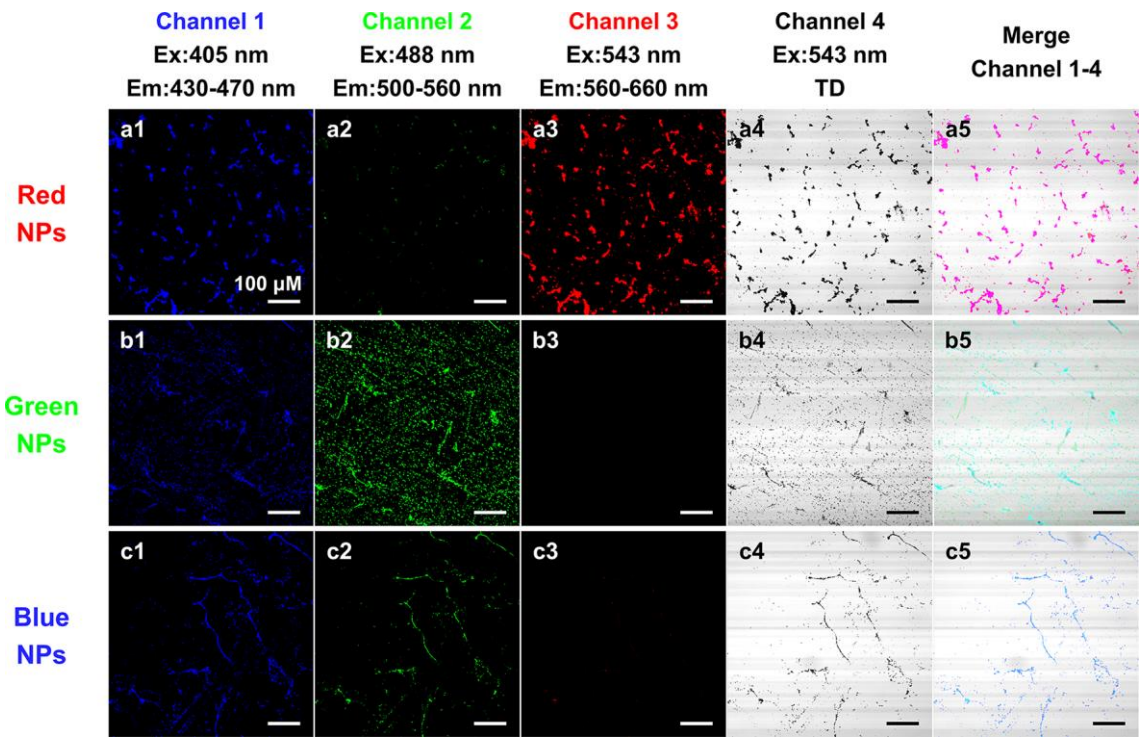


Fig. S46 Photographs of red, green and blue NPs used to realize multichannel imaging with different channels by a LSCM. Channel 1, 2 and 3 were set for TPE, FL and RB unit emission, respectively. Scale bars: 100 μm.

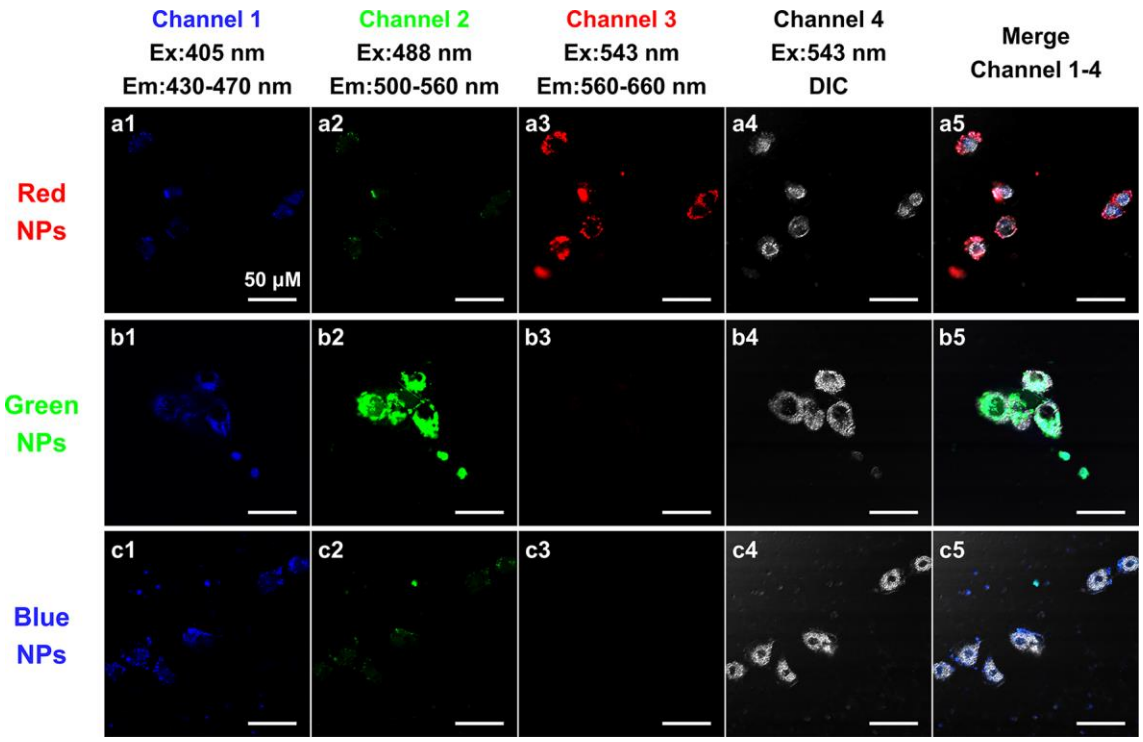


Fig. S47 Photographs of red, green and blue NPs used to realize living cell imaging with different channels by a LSCM. Channel 1, 2 and 3 were set for TPE, FL and RB unit emission, respectively. Scale bars: 50 μm.

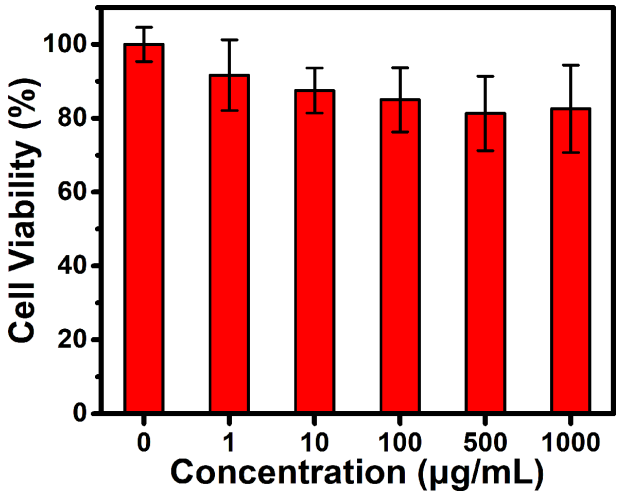


Fig. S48 The cell cytotoxicity of blue **RTF** NPs was investigated by CCK-8 assay using Hela cells. Cells were seeded onto a 96-well plate at a density of 1×10^4 cells per well in 200 μL medium. The cytotoxicity is dose-dependent after exposure to cells for 24 h. After removal of the culture media from cell culture plates, 100 mL of fresh culture media and 10 mL of CCK-8 kit solutions were immediately added and homogeneously mixed and then incubated for 2 h in incubator. Finally, 100 mL of reaction solutions were put into a new 96-well plate. The optical density of each well at 450 nm was read by a microplate reader. (Error bars represent the standard deviation)

12. References

(1). Mandal S., *et al. RSC. Adv*. **2015**, *5*, 103350-103357.

(2). Huang M. H., *et al. Acta Pharm. Sin. B* **2014**, *4*, 447-453.

(3). Adamczyk M., Grote J. *Synth. Commun.* **2001**, *31*, 2681-2690.

(4). Adamczyk M., Grote J. *Tetrahedron Lett.* **2000**, *41*, 807-809.