Supporting Information for

An ultra-fast illuminating fluorescent probe for monitoring formaldehyde in living cells, shiitake mushrooms, and indoors

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD or Agilent LC 1200/MS Q-TOF6520 spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescent images of solution and filter paper strip were excited by a 365 nm lighting of ZF-1 UV analyzer; The visual pictures were captured by LG (G4) mobile phone built-in camera; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; Ultrasonic extraction were carry out on KUDOS ultrasonic cleaner (SK2210HP); Centrifugalization was carried out on a ZONKIA high speed centrifuge (HC-2518); The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield. Fluorescence quantum yield (Φ_f) was determined by using rhodamine 6G (Φ_f = 0.95, in water, excitation at 488 nm) as the fluorescence standard. The quantum yield was calculated using the following equation.

$$\Phi_{F(X)} = \Phi_{F(S)} \, (A_S F_X / A_X F_S) \, (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvent used. Subscripts $_S$ and $_X$ refer to the standard and to the unknown, respectively.

HeLa cells culture. HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Imaging of FA in living cells. HeLa cells were incubated with 5.0 μM **R6-FA** for 30 minutes in an atmosphere of 5% CO_2 and 95% air, and then treated with 10 μM FA for 20 min. Subsequently, the cells were imaged using Nikon A1MP confocal microscope with excitation of 514 nm and emission collection of red channel of 550-580 nm.

Preparation of formaldehyde source from dried shiitake mushroom. The purchased dried shiitake mushrooms were removed stems and cut into small pieces. 2 g of sample was weighed and stored in a centrifuge tube, following 25 mL distilled water was added into the tube. The sealed tube was placed into an ultrasonic bath for extraction at 40 ^{o}C for 1 hour. After that, the sample was centrifuged at 6000 rpm for 10 min. The centrifuged sample was filtered and the filtrate was collected and stored up as the formaldehyde source.

Detection of formaldehyde from dried shiitake mushroom. 30 μL of probe **R6-FA** stock solution (1 mM) was diluted in PBS buffer (25 mM, pH 7.4) and DMF, and then the stored water extract of dried shiitake mushroom (100 μL) was added into the mixture as the final test solution (3 mL), which contained 10 μM **R6-FA** in PBS buffer solution with 50% DMF. The fluorescence spectra were recorded using fluorescence spectrophotometer with an excitation of 530 nm.

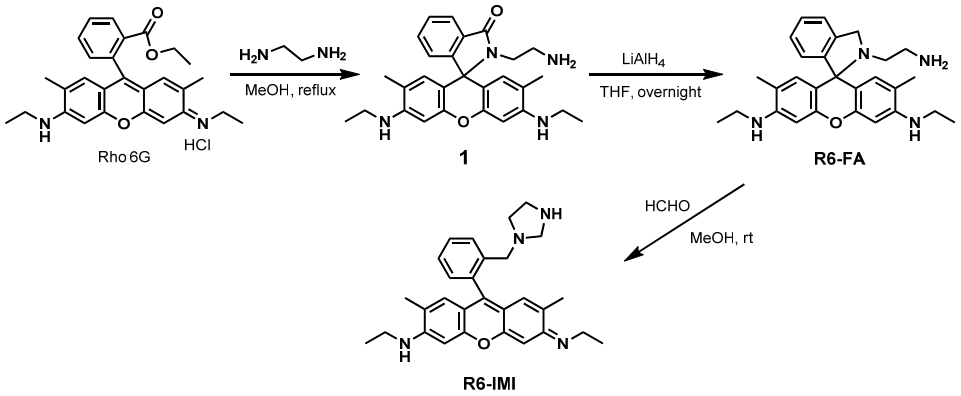
MBTH method for detecting formaldehyde from dried shiitake mushroom. 3-methyl-2-benzothiazolinone hydrazone (MBTH) (0.05 g) was dissolved in 50.0 mL water as the stock solution (1.0 mg/mL), and stored in refrigerator. Ammonium ferric sulfate (1.0 g) was dissolved in 0.1 M HCl and diluted to 100 mL with water as the stock solution (10.0 mg/mL). The various dosage of FA (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.7, 0.8, 0.9, 1.0 μg/mL) was added to the MBTH (20 μg/mL) in 5.0 mL aqueous solution, and incubated for 30 min. Then ammonium ferric sulfate (1.0 mg/mL) was added to the mixture, followed the absorption spectra of the mixture was recorded using an UV spectrometer and established the standard curve. To measure the FA level in mushroom, the absorption spectra of MBTH were recorded in the presence of 100 μL extract of mushroom using the above method. The FA level can be measured according to the linear equation.

Trace of formaldehyde in drawer. The filter paper was cut into strips as the test paper. The test papers were dipped into the **R6-FA** DMF solution (1 mM) for about 3 seconds and then flicked redundant liquid away. One test paper was put in ventilated indoor place for 5 minutes as control, and another was put in a drawer of plywood table used five months for the same time. Subsequently, the pictures of visual color and fluorescence color under excitation of 365 nm were captured with a camera.

MBTH method for detecting formaldehyde in drawer. 5 mL MBTH solution (20 μg/mL) was put in 250 mL flask, and then evacuate it using a vacuum pump. The evacuated flask was placed in the drawer and opened the valve to let air in. After shaking for 30 min, the ammonium ferric sulfate (1.0 mg/mL) was added to the mixture and the absorption spectra of the mixture was recorded using an UV spectrometer. The FA concentration can be measured according to the linear equation and the level of FA in drawer can be calculated via followed equation.

$$C = 5C_0/V_0$$

Where C and C_0 are FA concentrations in drawer and test solution, respectively; 5 is the volume of test solution; V_0 is the sampling air volume.



Synthesis of compound R6-FA. Compound **1** was prepared by reported method.[1] The mixture of compound **1** (50 mg, 0.1 mmol) and $LiAlH_4$ (50 mg, 1.3 mmol) was dissolved in 3 mL of dry THF. Under the protection of nitrogen, the mixture was stirred at room temperature overnight. The reaction was quenched by slowly adding ethanol. After gas evolution was complete, about 100 mL water was added and the reaction mixture was extract with dichloromethane (100 mL× 3). The organic phase was collected and dried using $MgSO_4$. The solvent was removed under reduced pressure affording the crude product, which was purified by flash chromatography column using ethanol/dichloromethane (v/v 1:4) to afford pale yellow solid as compound **R6-FA** (20 mg, yield 46 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 1.25-1.30 (t, *J* = 7.2 Hz, 6H), 1.95 (s, 6H), 2.45-2.48 (t, *J* = 6.0 Hz, 2H), 2.75-2.78 (t, *J* = 6.0 Hz, 2H), 3.17-3.22 (q, *J* = 7.1 Hz, 4H), 4.19 (s, 2H), 6.30 (s, 2H), 6.45 (s, 2H), 6.76-6.78 (d, *J* = 7.6 Hz, 1H), 7.19-7.23 (t, *J* = 7.0 Hz, 1H), 7.39-7.40 (1H); ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 14.79, 17.22, 39.12, 39.27, 47.41, 57.01, 97.32, 112.07, 119.31, 123.10, 125.63, 128.33, 128.89, 131.07, 139.88, 148.54, 150.08, 152.76. HRMS (EI) *m/z* calcd for $C_{28}H_{34}N_4O$ (M^+): 442.2718. Found 442.2727.

Synthesis of compound R6-IMI. The compound **R6-FA** (22 mg, 0.05 mmol) was dissolved in methanol (3 mL), and then 40% FA aqueous solution (38 mg, 0.5 mmol) was added. The mixture was immediately turned red and stirred at room temperature for 30 min. After the reaction was completed, about 50 mL water was added into the flask and the reaction mixture was extracted with dichloromethane/methanol (v/v 95/5; 100 mL × 3). The organic phase was collected and dried using $MgSO_4$. The solvent was removed under reduced pressure affording the crude product, which was purified by flash chromatography column using ethanol/dichloromethane (v/v 1:5) to afford pink solid as compound **R6-IMI** (9 mg, yield 39.6 %). ^{1}H NMR (400 MHz, $CDCl_3$), δ

(ppm): 1.41-1.45 (t, J = 6.6 Hz, 6H), 2.28 (s, 6H), 2.44 (2H), 2.59 (2H), 3.27 (s, 2H), 3.57-3.58 (4H), 3.68 (s, 2H), 6.73 (s, 2H), 6.80 (s, 2H), 7.21-7.24 (1H), 7.51-7.53 (2H), 7.57-7.61 (1H); ^{13}C NMR (100 MHz, CD_3OD), δ (ppm): 14.18, 17.71, 39.61, 44.64, 50.17, 61.44, 64.36, 95.11, 114.66, 127.07, 129.37, 130.46, 131.03, 131.38, 131.72, 134.00, 137.75, 157.87, 159.00. HRMS (EI) *m/z* calcd for $C_{29}H_{34}N_4O$ (M^+): 454.2724. Found 454.2731. Note: the compound was also synthesized in an aqueous solution, but the yield was lower than that in the methanol system, thus it was prepared in the methanol solution.

Reference

1 B. Wang, J. Hai, Z. Liu, Q. Wang, Z. Yang and S. Sun, *Angew. Chem. Int. Ed.*, 2010, **49**, 4576 –4579.

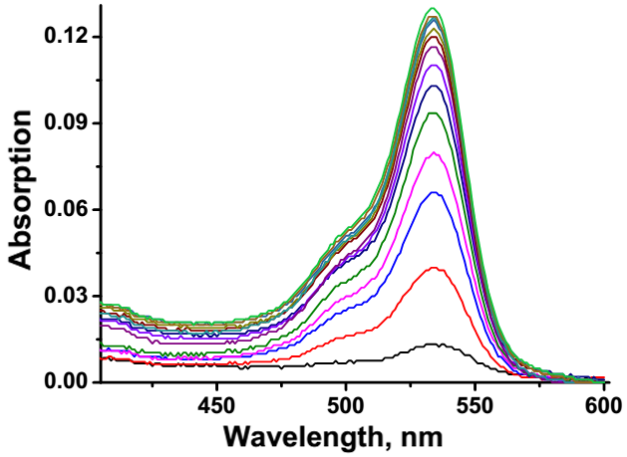


Fig. S1 Absorption spectra of **R6-FA** (10 μM) in the presence FA (0-20 μM) in aqueous buffer.

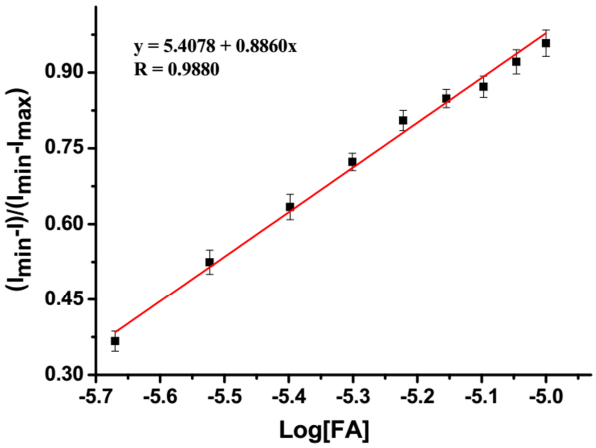


Fig. S2 The linear relationship between emission intensity (at 560 nm) and concentrations of FA (2.0-10.0 μM).

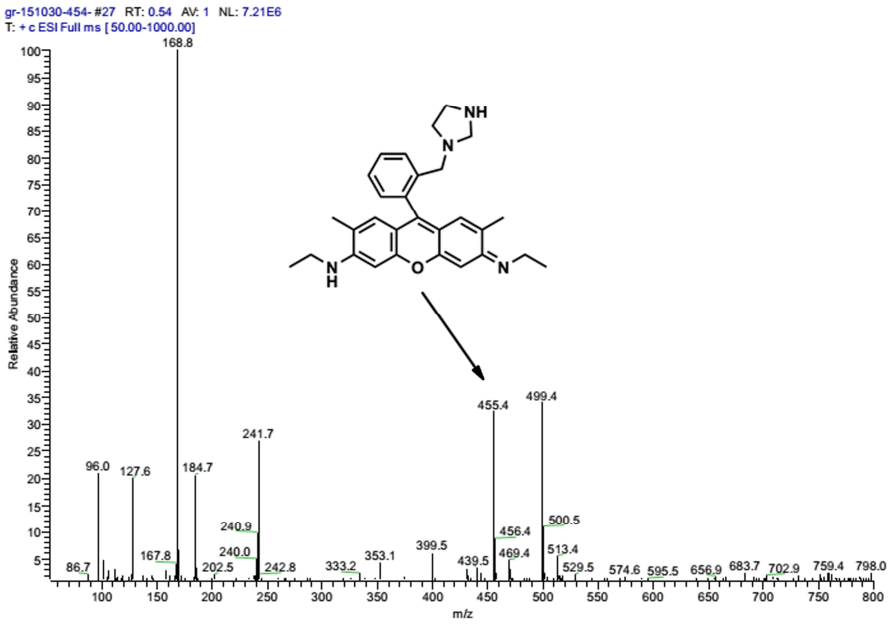


Fig. S3 ESI-MS spectrum of compound **R6-FA** in the presence of FA in pH 7.4 PBS buffer. The peak of 499.4 m/z may be due to the complicated reaction under the electrospray ionization conditions.

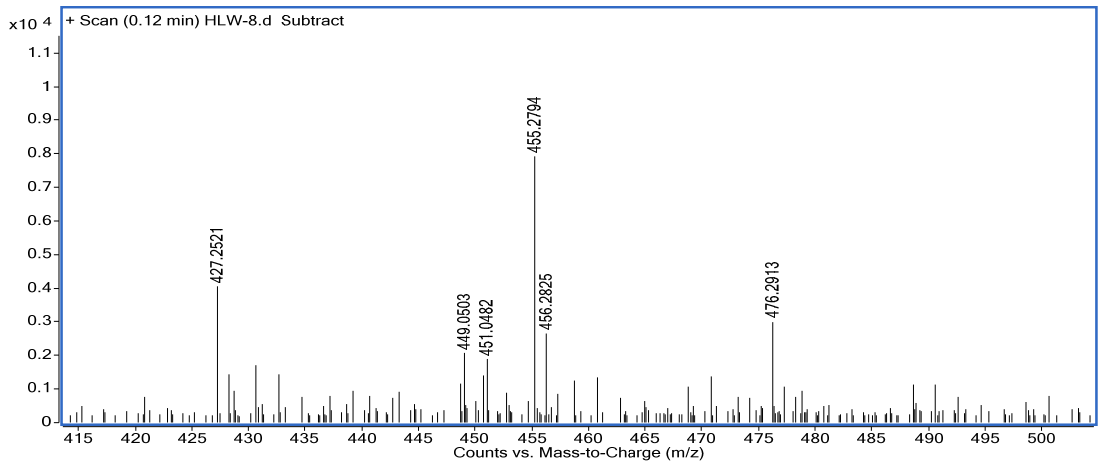


Fig. S4 ESI-HRMS spectrum of compound **R6-FA** in the presence of FA in pH 7.4 PBS buffer. HRMS (ESI) *m/z* calcd for **R6-IMI** ($C_{29}H_{35}N_4O$ ($[M+H]^+$): 455.2805.

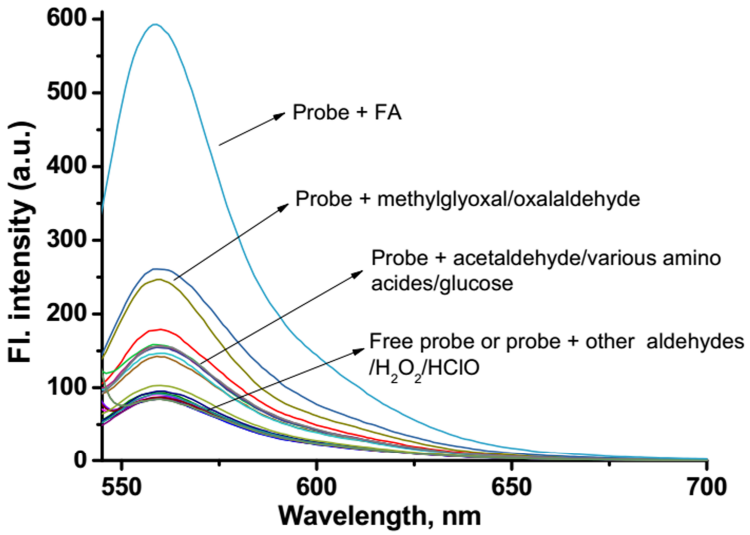


Fig. S5 Fluorescence responses of **R6-IMI** (10 μM) in the presence of various relevant analytes in PBS buffer (pH 7.4, containing 50 % DMF).

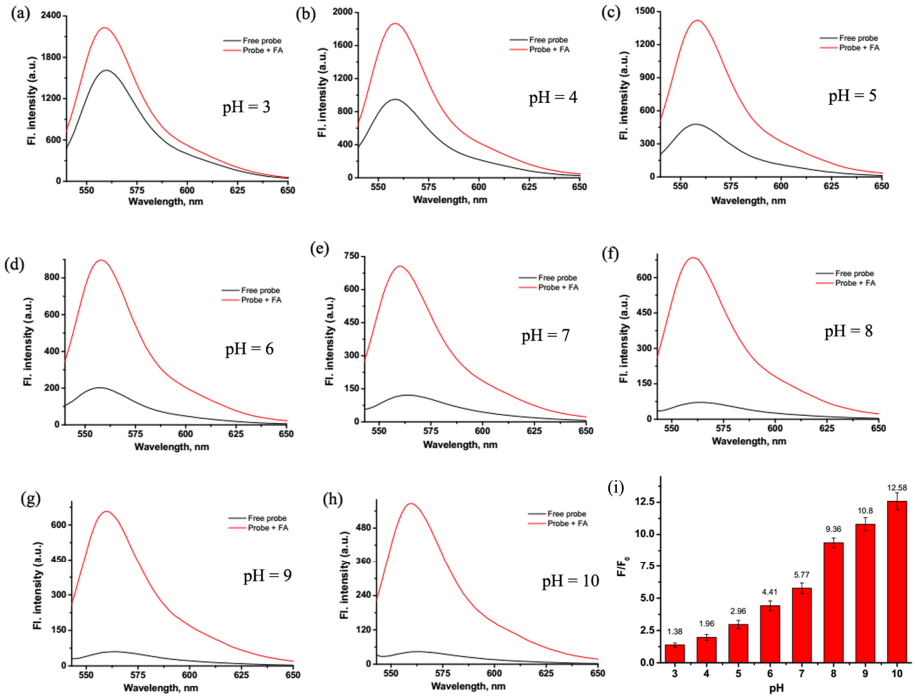


Fig. S6 (a-h) Fluorescence spectra of **R6-IMI** (10 μM) and (i) the enhancement ratio of fluorescence intensity (at 560 nm) in the presence of FA (20 μM) in various pH (3.0-10.0) PBS buffer (containing 50 % DMF). The numerical values showed in figure (i) are the enhancement ratios.

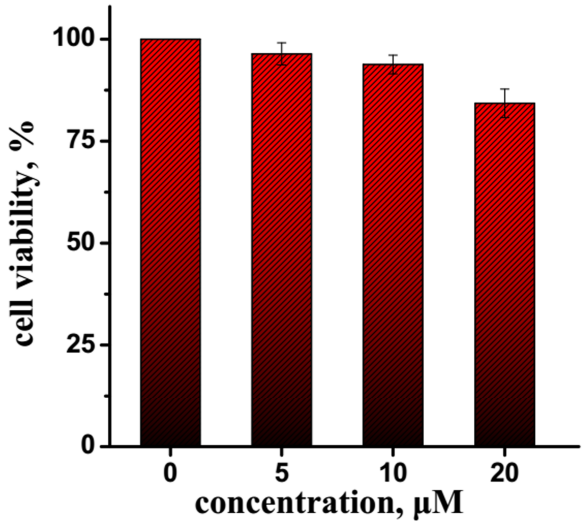


Fig. S7 Cytotoxicity of **R6-FA** evaluated on HeLa cells by MTT assays. The cells were incubated with the probe for 24 h.

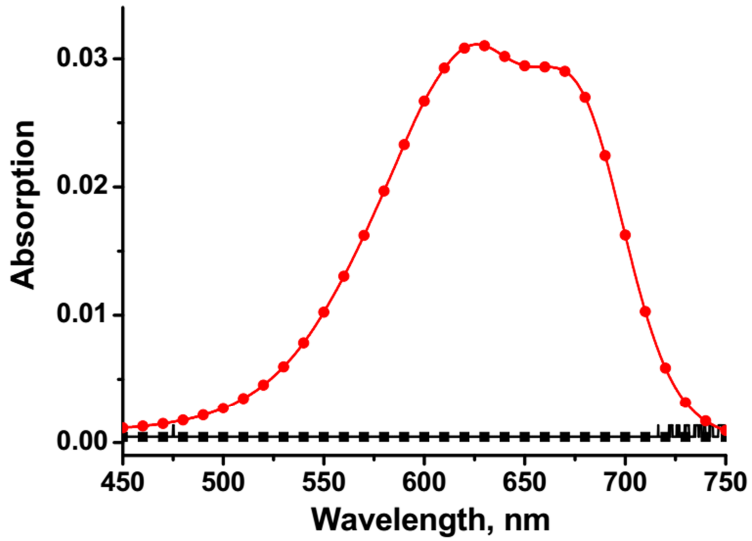


Fig. S8 The absorption spectra of **R6-FA** in the presence of indoor FA.

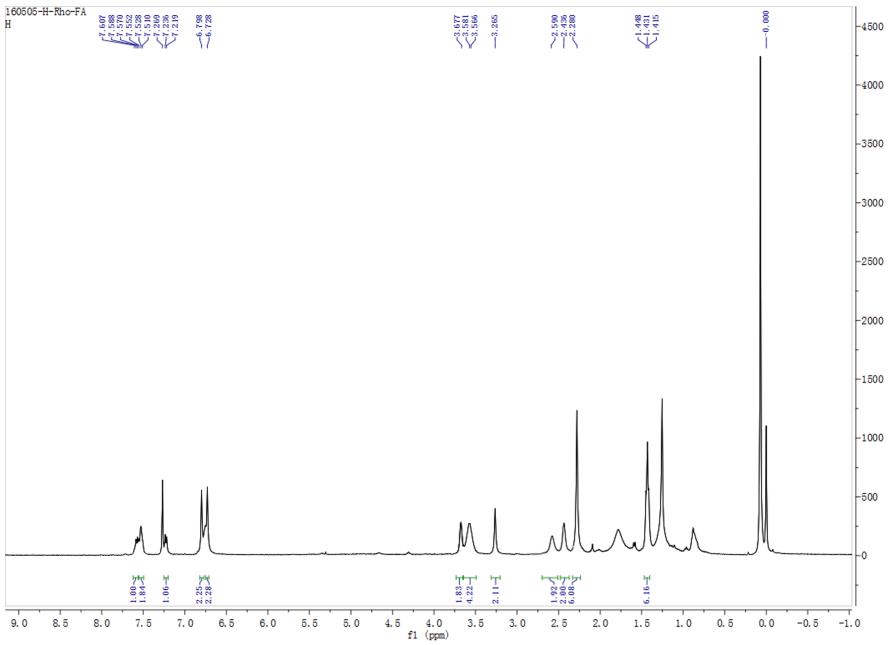


Fig. S9 ^{1}H NMR spectrum of the product **R6-IMI** isolated from the reaction between **R6-FA** and FA.

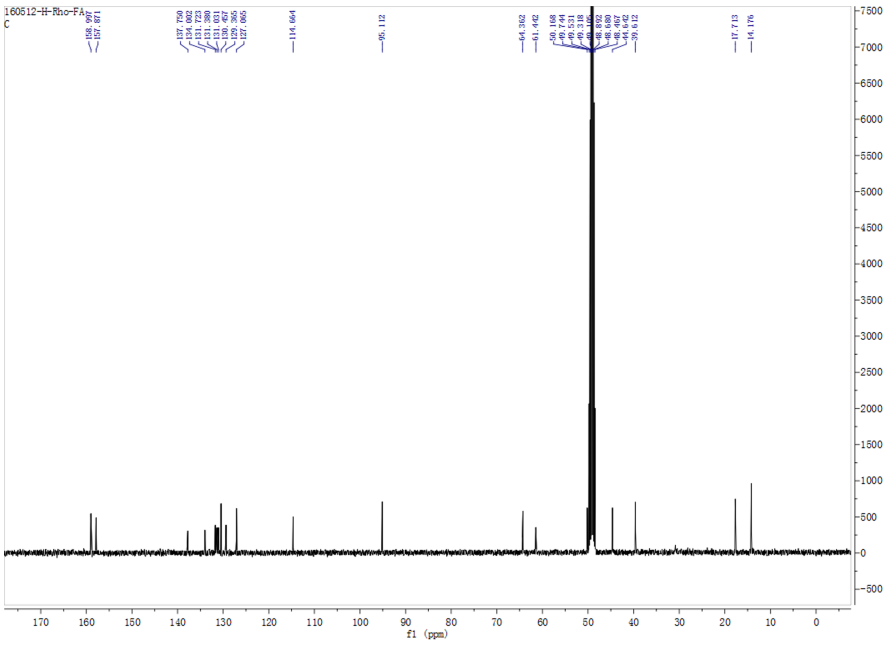


Fig. S10 ^{13}C NMR spectrum of the product **R6-IMI** isolated from the reaction between **R6-FA** and FA.

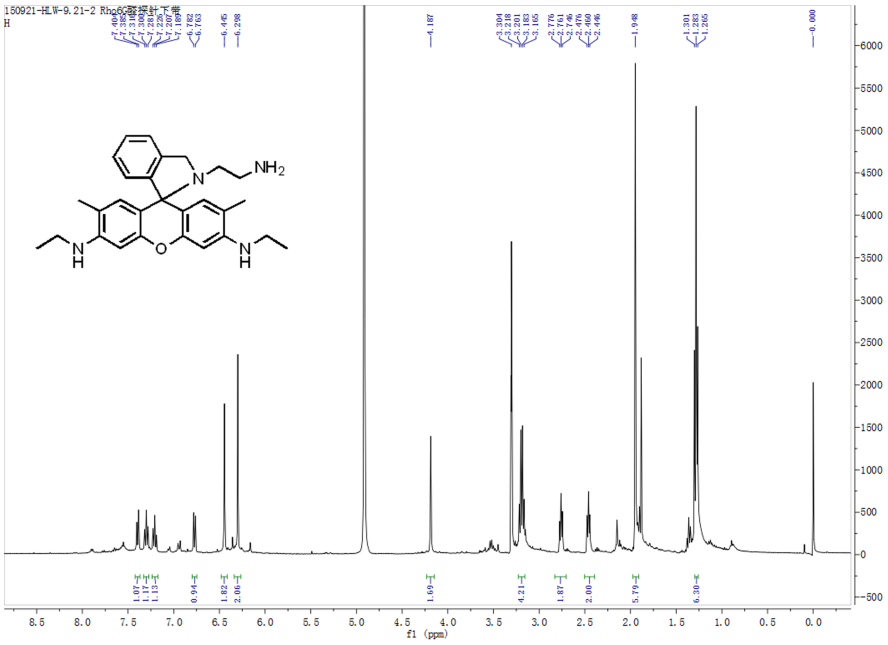


Fig. S11 ^{1}H NMR spectrum of compound **R6-FA** in CD_3OD.

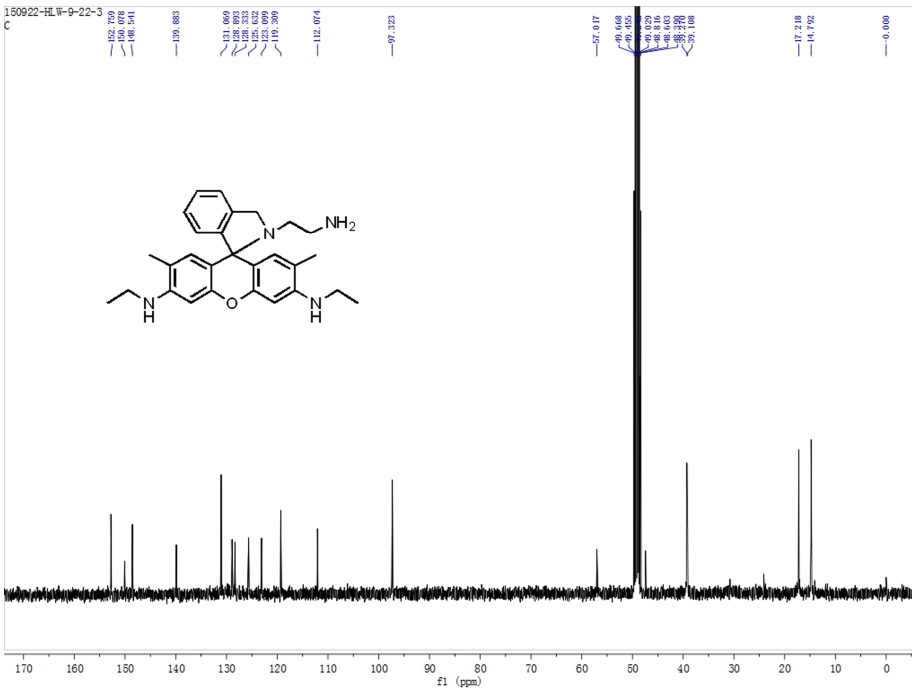


Fig. S12 ^{13}C NMR spectrum of compound **R6-FA** in CD_3OD.