Supporting Information

A Near-Infrared Multifunctional Fluorescent Probe with Inherent Tomor-targeting Property for Bioimaging

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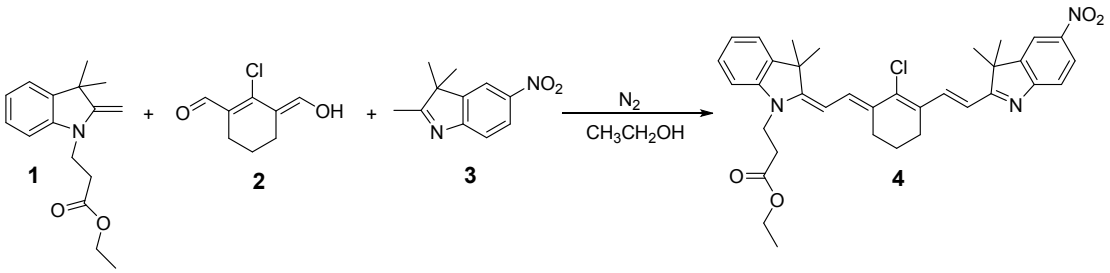
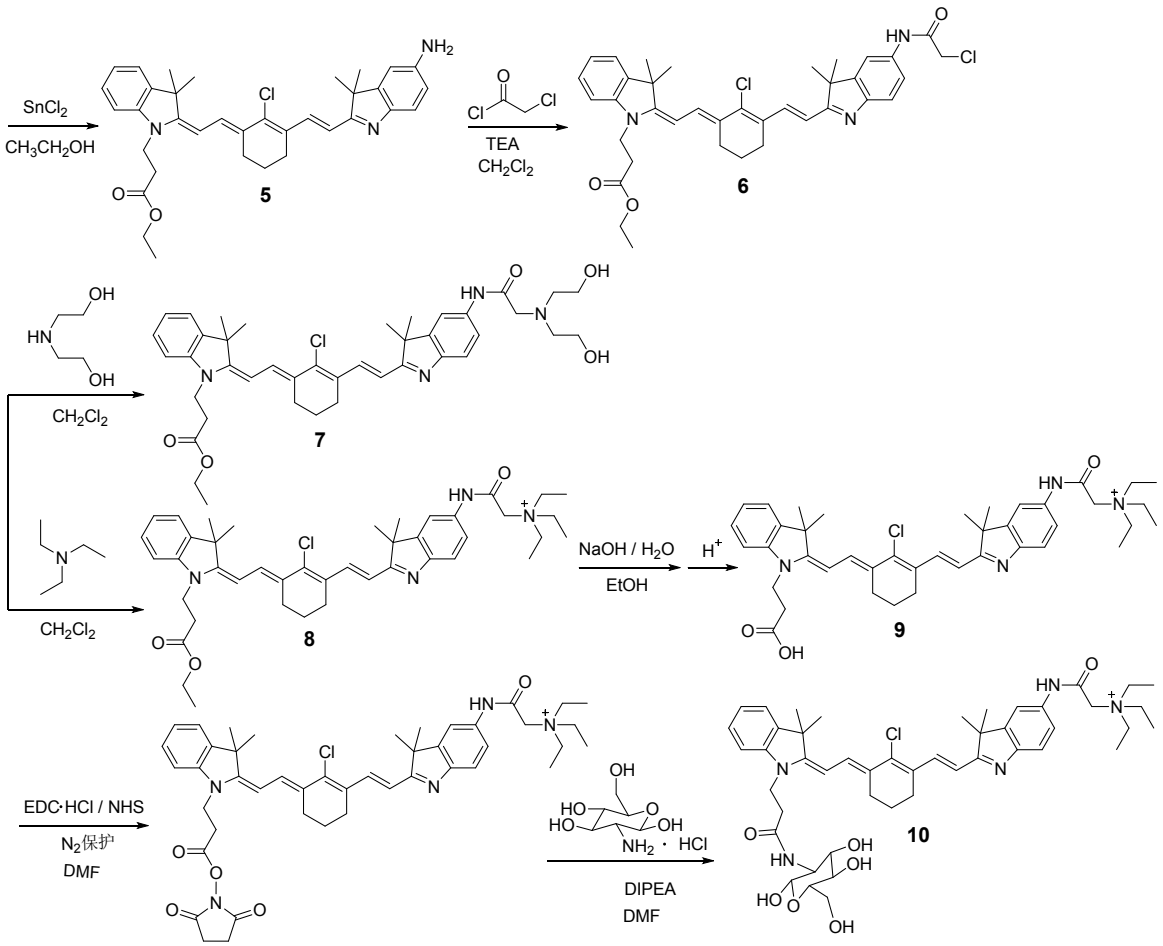
Materials and equipments

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), 2,3,3-trimethyl-5-nitro-3*H*-indole and ethyl 3-bromo propionate were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Rhodamine123 and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Poole, Dorset, England). Fetal bovine serum and cell culture media were purchased from Invitrogen (Carlsbad, CA, USA). Diethanolamine was obtained from Yantai Peiyang Fine Chemical Co. Ltd. (Shandong, China). Other reagents and solvents such as $POCl_3$ and TEA were purchased from Tianjin chemicals Co. (Tianjin, China) and used without further purification unless otherwise noted. DMF was purified by refluxing with calcium hydride for 4 h and then distilled. Deionized water was used throughout this work.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer and Bruker Avance 400 spectrometer, using TMS as an internal standard. High-resolution mass spectra were measured with a Bruker Daltonics micrOTOF-Q II instrument (ESI). All pH measurements were measured with a Sartorius basic pH-meter PB-10. UV-Vis spectra were obtained on an Evolution 300 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Jobin Yvon-Spex Fluorolog3 spectrofluorometer and Hitachi F-4500 spectrofluorometer. The photophysical properties of compound 10, including fluorescence quantum yield and lifetime were performed on a PTI QM/TM/NIR system (Photon Technology International, Birmingham, NJ, USA). The decayed curves of compound **10** emission at 810 nm excited by the high resolution laser (N_2 laser at 337nm tuned by laser dye PLD665) at 665 nm. Confocal fluorescence images of live cells were taken on a Leica TCS SP8. The *in vivo* images of mice were performed with a Bethold NightOWL LB 983 *in vivo* Imaging System equipped with a CCD camera.

Synthesis and characterization of compounds





Scheme S1 Synthetic routes of probes

3-(3,3-dimethyl-2-methyeneiodolinl-yl)-ethyl propionate (**1**) and 2-chloro-1-formyl-3-(hydroxymethylene)-cyclohex-1-ene (**2**) were prepared according to our previously reported method[1].

Synthesis of compound **4**: To a mixture of compound **1** (7.75 g, 29.88 mmol) and compound **2**（5.14 g, 29.78 mmol）in ethanol was added compound **3** (6.13 g, 30.01 mmol), then the resulting solution was stirred at 50 ℃ for 5 h under N_2 atmosphere. After cooling to room temperature, the reaction mixture was poured into brine (100 mL) and extracted with dichloromethane (3×30 mL). The organic phases were combined, dried over $MgSO_4$, filtered and concentrated to afford a red solid. The crude product was purified by flash chromatography with gradient elution (petroleum ether / ethyl acetate of 10/1 to 2/1, V/V) to obtain compound 4 as a red solid (7.10 g, 39.78 %).

^{1}H NMR (600MHz, $CDCl_3$) δ(ppm): 8.30(1H, d, *J* =16.2 Hz, -CH=CH-), 8.26(1H, d, *J* =9.0 Hz, ArH), 8.17(1H, d, *J* =2.4 Hz, ArH), 7.63(1H, d, *J* = 8.4 Hz,

ArH), 7.60(1H, d, *J* =12.6 Hz, -CH=CH-), 7.21-7.18(2H, m, ArH), 6.92(1H, t, *J* = 7.2 Hz, ArH), 6.74(1H, d, *J* = 7.8 Hz, ArH), 6.59(1H, d, *J* = 16.2 Hz, -CH=CH-), 5.55(1H, d, *J* = 12.6 Hz, -CH=CH-), 4.13(2H, q, *J* = 7.2 Hz, CH_2), 4.02(2H, t, *J* = 7.2 Hz, CH_2), 2.69(2H, t, *J* = 7.2 Hz, CH_2), 2.62-2.59(4H, m, CH_2), 1.93-1.89(2H, m, CH_2), 1.66(6H, s, CH_3), 1.53(6H, s, CH_3), 1.21(3H, t, *J* = 7.2 Hz, CH_3).

^{13}C NMR (150MHz, $CDCl_3$) δ(ppm): 189.15, 189.10, 171.45, 159.93, 159.05, 147.73 145.08, 143.65, 139.67, 139.05, 128.44, 127.86, 125.24, 124.78, 122.49, 121.85, 120.67, 120.06, 117.16, 116.91, 106.66, 93.64, 61.02, 53.00, 46.18, 38.29, 31.23, 29.69, 28.23, 26.88, 26.32, 24.31, 14.10.

HRMS (ESI Positive): calc. for $C_{35}H_{38}ClN_3O_4$, $[M+H]^+$ 600.2629, found 600.2623.

Synthesis of compound **5**: To an ethanol (50 mL) solution of compound **4**(3.60 g, 6.00 mmol), tin(II) chloride dihydrate (4.06 g, 15.00 mmol) was added, and then the resulting mixture was stirred at room temperature for 12 h. After this, the solvent was removed, then the residue was dispersed in ice and water mixture (150 mL) and alkalized to pH=8 with 2 mol/L NaOH solution. The suspension was extracted with dichloromethane (4×30 mL) and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to obtain the crude product as a red solid. Finally, the crude product was purified by flash chromatography with gradient elution (petroleum ether / ethyl acetate of 5/1 to 1/1, V/V) to offer the title compound as a red solid (2.67 g, 78.17%).

^{1}H NMR (400MHz, $CDCl_3$) δ(ppm): 7.97(1H, d, *J* =16.4 Hz, -CH=CH-), 7.50(1H, d, *J* =12.4 Hz, -CH=CH-), 7.41(1H, d, *J* =7.6 Hz, ArH), 7.19(1H, t, *J* =8.4 Hz, ArH, 1H, d, *J* =16.8 Hz, -CH=CH-), 6.90(1H, t, *J* =7.6 Hz, ArH), 6.71(1H, d, *J* =7.6 Hz, ArH), 6.66(1H, s, ArH), 6.63(1H, d, *J* =8.8 Hz, ArH), 5.53(1H, d, *J* =12.4 Hz, -CH=CH-), 4.14(2H, q, *J* = 7.2 Hz, CH_2), 4.01(2H, t, *J* = 7.2 Hz, CH_2), 3.77(12H, s, NH_2), 2.69(2H, t, *J* = 7.2 Hz, CH_2), 2.61-2.58(4H, m, CH_2), 1.91-1.88(2H, m, CH_2), 1.67(6H, s, CH_3), 1.48(6H, s, CH_3), 1.23(3H, t, *J* = 7.2 Hz, CH_3).

^{13}C NMR (150MHz, $CDCl_3$) δ(ppm): 180.48, 171.59, 157.41, 149.33, 146.66, 144,93, 143.90, 139.00, 136.33, 134.90, 126.98, 127.76, 125.34, 125.57, 121.80, 121.15, 120.98, 120.20, 114.26, 108.25, 106.33, 93.41, 60.97, 52.21, 45.90, 38.21, 31.21, 28.17, 26.87, 26.43, 25.00, 21.53, 14.10

HRMS (ESI Positive): calc. for $C_{35}H_{40}ClN_3O_2$, $[M+H]^+$ 570.2887, found

570.2886.

Synthesis of compound **6**: To a chilled dichloromethane solution (25 mL) of compound **5** (2.63 g, 4.61 mmol) and TEA (1.8 mL, 13.0 mmol) was dropwise added the solution of chloroacethyl chloride (450 μL, 5.98 mmol) in dichloromethane (10 mL), and the resulting mixture was stirred under an ice bath for 5 h. Then the mixture was poured into water (100 mL) and extracted with dichloromethane (3×30 mL). The organic phases were combined and dried over $MgSO_4$, concentrated to give the title compound without further purification (2.68 g, 89.93%).

^{1}H NMR (600MHz, $CDCl_3$) δ(ppm): 8.25(1H, s, -NHCO-), 8.02(1H, d, *J* =16.2 Hz, -CH=CH-), 7.66(1H, d, *J* =1.8 Hz, ArH), 7.50(1H, d, *J* =8.4 Hz, ArH), 7.45(1H, d, *J* =13.2 Hz, -CH=CH-), 7.25(1H, dd, J_1= 1.8 Hz, J_2= 7.8 Hz, ArH), 7.12-7.09(2H, m, ArH), 6.82(1H, t, *J* =7.2 Hz, ArH), 6.64(1H, d, *J* =7.8 Hz, ArH), 6.55(1H, d, *J* =16.2 Hz, -CH=CH-), 5.45(1H, d, *J* =12.6 Hz, -CH=CH-), 4.15(2H, s, CH_2), 4.05(2H, q, *J* =7.2 Hz, CH_2), 3.93(2H, t, *J* = 7.2 Hz, CH_2), 2.60(2H, t, *J* = 7.2 Hz, CH_2), 2.53-2.51(4H, m, CH_2), 1.84-1.79(2H, m, CH_2), 1.58(6H, s, CH_3), 1.43(6H, s, CH_3), 1.14(3H, t, *J* = 7.2 Hz, CH_3).

HRMS (ESI Positive): calc. for $C_{37}H_{41}Cl_2N_3O_3$, $[M+H]^+$ 646.2603, found 646.2605.

Synthesis of compound **8**: To a solution of compound **6** (1.04 g, 1.61 mmol) in dichloromethane (25 mL) was added TEA (1.0 mL, 7.2 mmol）) at room temperature under N_2 atmosphere. After stirring for 12 h, the mixture was poured into water (100 mL) and then extracted with dichloromethane (30 mL×3). The organic phases were combined and dried over $MgSO_4$, concentrated to give a purple red solid. The crude product was purified by flash chromatography (dichloromethane / methanol of 5/1, V/V) to obtain 1.01 g compound 8 as a purple red solid (88.60 % yield).

^{1}H NMR (400MHz, $CDCl_3$) δ(ppm): 11.60(1H, s, NH-C=O), 8.11(1H, d, *J* = 16.4 Hz, -CH=CH-), 7.82(2H, d, *J* =6.4 Hz, ArH), 7.57-7.53(2H, m, ArH, -CH=CH-), 7.21(2H, t, *J* =8.0 Hz, ArH), 6.92(1H, t, *J* =7.2 Hz, ArH), 6.74(1H, d, *J* =8.0 Hz, ArH), 6.64(1H, d, *J* = 16.4 Hz, -CH=CH-), 5.55(1H, d, *J* = 12.4 Hz, -CH=CH-), 4.78(2H, s, CH_2), 4.16(2H, q, *J* = 7.2 Hz, CH_2), 4.03(2H, t, *J* = 7.2 Hz, CH_2), 3.70(6H, q, *J* = 7.2 Hz, CH_2), 2.71(2H, t, *J* = 6.8 Hz, CH_2), 2.63-2.61(4H, m, CH_2), 1.95-1.90(2H, m, CH_2), 1.68(6H, s, CH_3), 1.54-1.51(15H, s, CH_3), 1.25(3H, t, *J* = 6.8 Hz, CH_3).

^{13}C NMR (150MHz, $CDCl_3$) δ(ppm): 183.71, 171.49, 160.93, 158.14, 150.77, 147.71, 143.78, 139.03, 137.87, 137.01, 135.33, 128.67, 127.78, 127.01, 125.47, 121.83, 120.42, 120.23, 120.12, 119.54, 113.60, 106.48, 93.51, 60.98, 57.65, 55.21, 52.73, 46.15, 46.04, 31.22, 28.19, 26.86, 26.37, 24.70, 21.45, 14.09, 8.43.

HRMS (ESI Positive): calc. for $C_{43}H_{56}ClN_4O_3^+$, $[M]^+$ (m/z) 711.4041, $[M+H]^{2+}$ (m/z) 356.2060, found 711.4037, 356.2058.

Compound **7** was obtained by the similar procedure in 79.86 % yield.

^{1}H NMR (400MHz, $CDCl_3$) δ(ppm): 9.80(1H, s, -NHCO-), 8.10(1H, d, *J* = 16.4 Hz, -CH=CH-),7.97(1H, s, ArH), 7.56-7.51(2H, m, ArH, -CH=CH-), 7.35(1H, d, *J* =8.0 Hz, ArH), 7.21(2H, t, *J* =8.0 Hz, ArH), 6.92(1H, t, *J* =7.2 Hz, ArH), 7.43(1H, d, *J* =7.6 Hz, ArH), 6.64(1H, d, *J* =16.4 Hz, -CH=CH-), 5.54(1H, d, *J* =12.4 Hz, -CH=CH-), 4.15(2H, q, *J* = 7.2 Hz, CH_2), 4.02(2H, t, *J* = 7.2 Hz, CH_2), 3.79(4H, t, *J* = 4.8 Hz, CH_2), 3.44(2H, s, CH_2), 2.89(4H, t, *J* = 4.8 Hz, CH_2), 2.70(2H, t, *J* = 7.2 Hz, CH_2), 2.62-2.60(4H, m, CH_2), 1.93-1.87(2H, m, CH_2), 1.68(6H, s, CH_3), 1.51(6H, s, CH_3), 1.24(3H, t, *J* = 7.2 Hz, CH_3).

^{13}C NMR (150MHz, $CDCl_3$) δ(ppm): 183.40, 171.55, 169.68, 157.98, 149.98, 147.97, 143.83, 139.05, 136.67, 136.18, 136.13, 128.67, 127.78, 126.71, 125.47, 121.83, 120.36, 120.28, 119.70, 119.13, 112.91, 106.42, 93.46, 60.99, 59.86, 58.46, 56.89, 52.72, 46.01, 31.22, 29.70, 28.20, 26.88, 26.39, 24.68, 18.44, 14.10.

HRMS (ESI Positive): calc. for $C_{41}H_{51}ClN_4O_5$, $[M+H]^+$ 715.3626, found 715.3925.

Synthesis of compound **9**: To an ethanol solution (25 mL) of intermediate **8** (1.82 g, 2.55 mmol), 2 mol/L NaOH solution (17.5 mL) was added, and then the resulting mixture was stirred at 50 ℃ for 3 h under N_2 atmosphere. After cooling to room temperature, the solvent was removed, then the residue was dissolved in water (50 mL) and acidified to pH=6 with 1 mol/L HCl solution. The precipitation was collected by filtration and washed with water (30 mL×3), then purified by flash chromatography with gradient elution (petroleum ether/ethyl acetate of 3:1 to methanol) to afford compound 9 as a blue solid (1.46 g, 83.91 %).

^{1}H NMR (400MHz, MeOD) δ(ppm): 8.14(1H, d, *J* = 16.0 Hz, -CH=CH-), 7.78(1H, s, ArH), 7.56(1H, d, *J* = 12.4 Hz, -CH=CH-), 7.45(2H, s, ArH), 7.20-7.15(2H, m, ArH), 6.90-6.85(2H, m, ArH), 6.55(1H, d, *J* = 16.0 Hz, -CH=CH-),

5.67(1H, d, J = 14.8 Hz, -CH=CH-), 4.94(2H, s, CH_2), 4.00(2H, t, J = 7.2 Hz, CH_2), 3.63(6H, q, J = 7.2 Hz, CH_2), 2.55-2.51(6H, m, CH_2), 1.82(2H, t, J = 7.2 Hz, CH_2), 1.62(6H, s, CH_3), 1.45(6H, s, CH_3), 1.38(9H, t, J = 7.2 Hz, CH_3).

HRMS (ESI Positive): calc. for $C_{41}H_{52}ClN_4O_3^+$, $[M]^+$ (m/z) 683.3728, $[M+H]^{2+}$ (m/z) 342.1903, found 683.3780, 342.1918 .

Synthesis of compound **10** (TM): The mixture of EDC·HCl (0.29 g, 1.62 mmol) and NHS (0.21 g, 1.82 mmol) was added to a solution of compound **9** (1.03 g, 1.51 mmol) in anhydrous DMF (15 mL) on an ice bath under N_2 atmosphere. After stirring for 1 h, the mixture was allowed to warm up to room temperature and continually stirred for 12 h in the dark. After this, *N,N*-diisopropylethylamine (DIPEA) (820 μL, 4.70 mmol) and D-glucosamine hydrochloride (0.39 g, 1.81 mmol) were added. The resulting mixture was stirred at room temperature for an additional 5 h under N_2 atmosphere, and then poured into acetone (50 mL). The precipitation was collected by filtration and purified by flash chromatography (CH_2Cl_2/Methanol of 10:1) to obtain the title compound as a red solid (0.73 g, 57.03 %).

^{1}H NMR (600MHz, MeOD) δ(ppm)：8.14(1H, d, J =15.6 Hz, -CH=CH-), 7.77(1H, d, J =28.8 Hz, -CH=CH-), 7.60-7.55(2H, m, ArH), 7.45(1H, dd, J_1= 2.4 Hz, J_2= 8.4 Hz, ArH), 7.19-7.14(2H, m, ArH), 6.87(1H, t, J =7.2 Hz, ArH), 6.77(1H, d, J =8.4 Hz, ArH), 6.56(1H, d, J =15.0 Hz, -CH=CH-), 5.59(1H, d, J =10.8 Hz, -CH=CH-), 5.32(1H, d, J =3.0 Hz, -NH-), 4.05(6H, q, J = 7.2 Hz, CH_2), 3.84-3.80(2H, m, CH_2), 3.79-3.76(2H, m, CH_2), 3.54(1H, d, J = 16.8 Hz, CH), 3.44(1H, t, J = 9.6 Hz, CH), 3.33-3.32(1H, m, CH), 2.66(2H, t, J = 6.6 Hz, CH_2), 2.62-2.58(4H, m, CH_2), 1.85(2H, t, J = 5.4 Hz, CH_2), 1.60(6H, s, CH_3), 1.45(6H, s, CH_3), 1.15(9H, t, J = 7.2 Hz, CH_3).

HRMS (ESI Positive): calc. for $C_{47}H_{63}ClN_5O_7^+$, $[M+H]^+$ 845.4494, found 845.4487.

Measurement of absorbance and fluorescence spectroscopy

Absorption spectra were recorded in a quartz cuvette (1 cm×1 cm) under ambient atmosphere at room temperature. Before the measurement, a stock solution of probe (1×10^{-3} M) was prepared by dissolving the required amount of **10** (0.0085 g) in ethanol and diluting it to the mark in a 10 mL volumetric flask. Further dilution yielded the working solution (1×10^{-5} M) by diluting 2.5 mL of stock solution to 250

mL with ethanol-water (2:8, V/V). For investigating the optical response of this probe towards pH, the accurate pH of the working solution was adjusted by 0.1 M NaOH and 0.1 M HCl (from 2.53 to 11.70), and then the absorption spectra at different pH value were recorded in the wavelength range of 300~1000 nm. Fluorescence spectra were collected in a quartz cuvette (1 cm×1 cm) at a 90-degree angle relative to the excitation light path. Excitation and emission slits were both 8 nm and the excitation wavelength was 760 nm. The Absorption spectra and fluorescence spectra of control molecule (compounds **7** and **8**, 1×10^{-5} M) upon pH were evaluated in the similar method.

The biologically relevant analytes, including metal ions (Na^+, K^+, Mg^{2+}, Zn^{2+}, Ca^{2+}) made from their chloride salts, thiols (GSH, Cys, Hcy) and H_2O_2 were dissolved in deionized water to obtain the stock solutions of the interferents (1×10^{-3} M). In selectivity experiments, working solution (9 mL) was added to a 10 mL volumetric flask, to which stock solutions of interferents (100 μL) were respectively added using a micropipettor, and then it was diluted to the mark with deionized water.

Measurement of absolute quantum yield

The absolute fluorescence quantum yield (ϕ_f) of solution of compound **10** (excitation at 760 nm) at room temperature was determined on a PTI QM/TM/NIR system (Photon Technology International, Birmingham, NJ, USA) with an integration sphere attachment (Labsphere, North Sutton, NH, USA). A circular quartz cuvette for the working solution of sample is set in the integrating sphere. The absolute photoluminescence QY is given by:

$$\phi_f = \frac{PN(em)}{PN(abs)} = \frac{\int L_{sample}}{\int E_{reference} - \int E_{sample}}$$

Where $PN_{(abs)}$and $PN_{(em)}$ are the number of photons absorbed by the sample and photons emitted from the sample, respectively. L_{sample} is the fluorescence intensity of the working solution of sample, $E_{\text{reference}}$ and E_{sample} are the excitation light intensities of the reference and the sample, respectively. Ethanol-water (2/8, *V/V*) solution was employed as reference.

***In vitro* cytotoxicity experiments**[2]

HeLa cells were used for *in vitro* cytotoxicity evaluation of the probe (**10**) based on the MTT assay. HeLa cells were seeded in a 96-well plate at a density of 5×10^4 cells/well in 100 μL DMEM with 10% FBS at 37 ℃ under 5% CO_2 humidified

atmosphere. After 24 h incubation, the original medium was replaced with fresh culture medium containing different concentrations of **10** (0.5, 1, 5 and 10 equiv. of testing concentration (1×10^{-5}M)) and then incubation for another 24 h (each concentration was done in triplicate). Afterwards, the medium in each well was then removed and washed with PBS buffer three times. Then solution of MTT (30 μL, 5 mg/mL in PBS buffer) and PBS buffer (1 mL) were added to each well followed by incubation for additional 4 h under the same condition. Subsequently, the medium in each well was removed and washed twice with PBS buffer, then 2 mL DMSO was added to dissolve the internalized purple formazan crystals. The plate was subjected to gently agitation for 30 min in order to dissolve all the crystals. The absorbance at wavelength of 570 nm was recorded on a microplate reader (Thermo Fisher). The average absorbance of the blank wells without cells was subtracted from the readings of the other wells. And then the cell viability was determined by the following equation: viability (%) = $A_x/A_{control}\times100$, (A_x is the average absorbance of the date with a certain concentration of **10**, $A_{control}$ is the average absorbance of the control wells in which the probe was absent.).

pH-dependent cell imaging and subcellular localization experiments[3]

HeLa cells were seeded in a 6-well plate at a density of 5×10^5 cells/well and incubated for 24 h for cell attachment. Afterwards, the original medium was removed and the cells were further incubated with 1×10^{-5} M of **10** in culture media for additional 2 h at 37 ℃ under 5% CO_2 humidified atmosphere. Then the culture medium was removed and washed twice with PBS buffer. For the pH-dependent cell imaging study, the above cells were treated with PBS buffer at various pH values from 4.0 to 7.0, and then confocal fluorescence imaging was acquired on Leica TCS SP8 confocal laser-scanning microscope with a 40×oil-immersion object lens. Fluorescence was excited at 633 nm and emission was collected by a 700~800 nm band pass filter.

Next, HeLa cells were incubated on another 6-well plate for subcellular localization study. After incubation for 24 h, **10** (1×10^{-5} M) was added to one of the well, and compounds **7** and **8** (1×10^{-5} M) were respectively added into another well as control. The culture medium was removed and washed twice with PBS buffer after incubated for 2 h, and then fresh culture medium containing rhodamine123 (1×10^{-6} M) was added to these wells and incubated for another 1 h. Afterwards, the medium was

removed and washed 3 times, and then the cells were treated with PBS buffer at pH 5 in order to activate the fluorescence of **10**, **8** and **7**. Subsequently, fluorescence images of probe (**10**, **8** and **7**) and rhodamine123 were measured.

***In vivo* imaging experiments**[4]

Male nude mice were purchased from Tianjin Medical University Cancer Institute and Hospital at 4 weeks of ages. All animal experiments were conducted according to protocols approved by the Animal Ethics Committee of the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College. For *in vivo* imaging, HeLa cells (5×10^6 cells suspended in 100 μL PBS) were inoculated into the right hind limb of the nude mice after anesthetization by injection 4% chloral hydrate (150 μL). After inoculation for two weeks, the mice were chosen for in vivo imaging experiments with the tumor diameters reached approximately 0.5 cm. With the HeLa tumor-bearing nude mice on the hand, **10** (1×10^{-5} M in PBS containing 1% DMSO, 200 μL) was injected in suit into the tumor-bearing nude mice and subcutaneously injected into the homologous normal tissus (left hind limb) with the same amount as a control.The fluorescence images of the tumor-bearing nude mice were performed at the special time points (before injection, 0.5 h, 1 h, 4 h, 8 h, 12 h and 24 h after injection) with a Bethold NightOWL LB 983 *in vivo* Imaging System equipped with the CCD camera. The excitation filter and emission filter were set as 630 nm and 800 nm respectively, the constant time was 0.1 s.

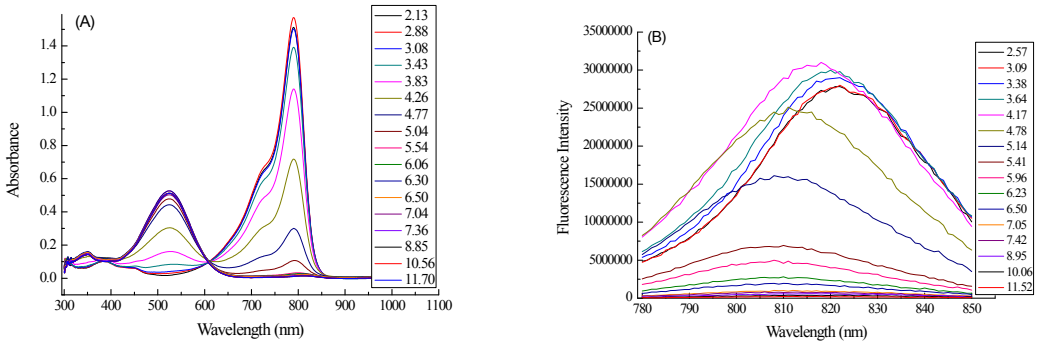


Fig.S1 UV absorption spectra (A) and fluorescence emission spectra (B) of compound **7** (1×10^{-5} M) upon pH in ethanol-water (2/8, V/V) solution

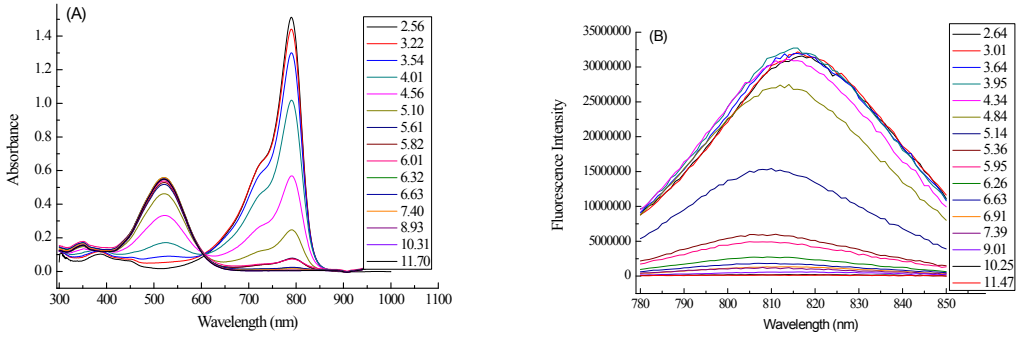


Fig.S2 UV absorption spectra (A) and fluorescence emission spectra (B) of compound **8** (1×10^{-5} M) upon pH in ethanol-water (2/8, V/V) solution

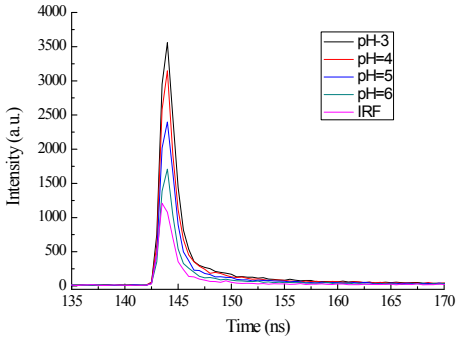


Fig.S3 The decayed curves of compound **10** at different pH values

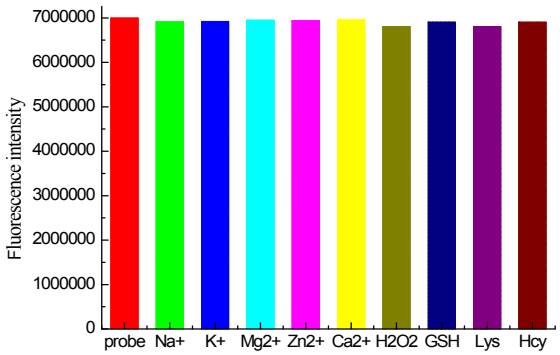


Fig.S4 The fluorescent intensity at 814 nm of **10** at pH = 4.01 in ethanol-water (2/8, V/V) solution upon addition of different interferents

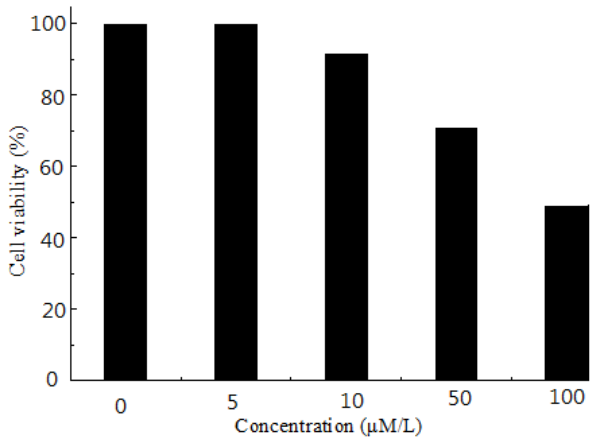


Fig.S5 Effect of probe **10** on the viability of HeLa cells

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^{1}H NMR and ^{13}C NMR spectra

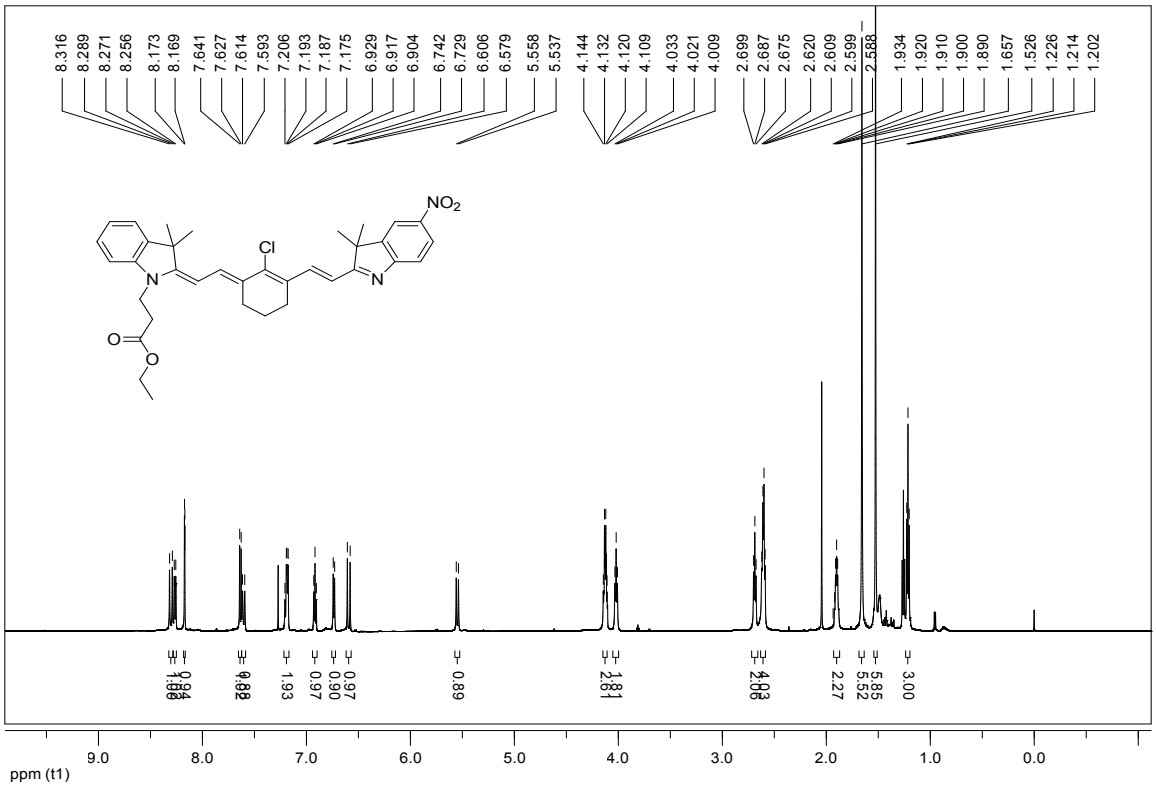

Fig. S6 ^{1}H NMR spectrum of compound **4**

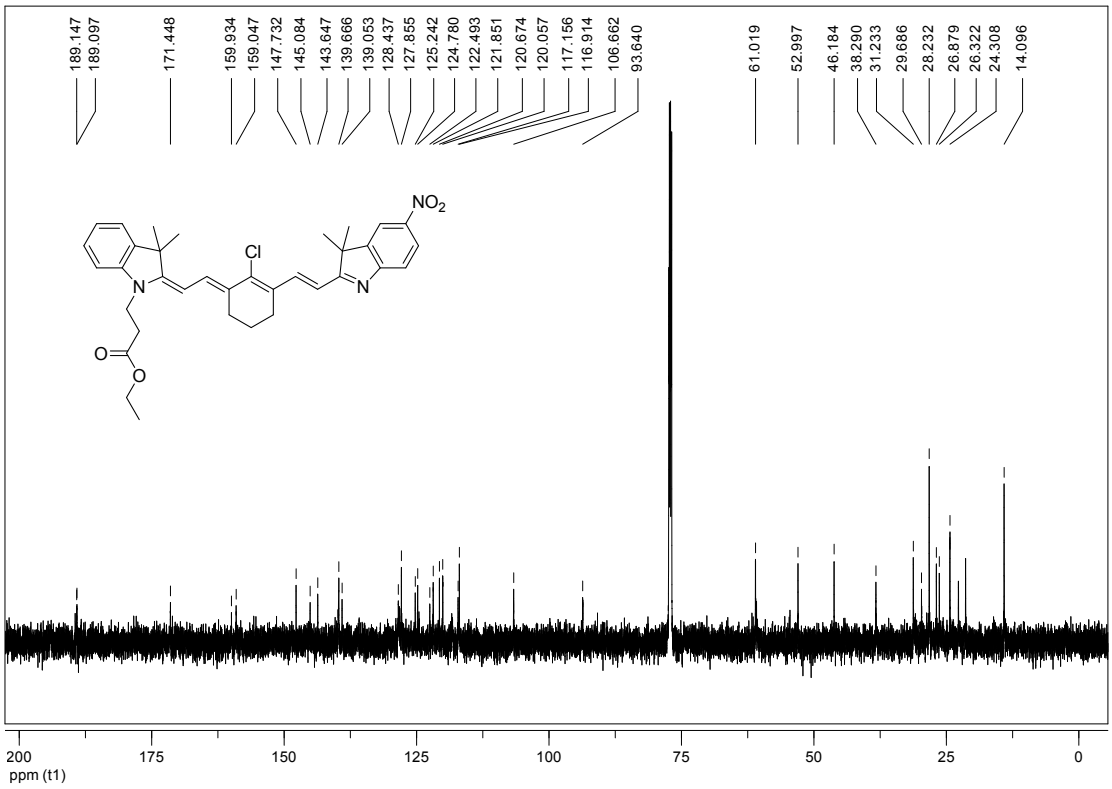

Fig. S7 ^{13}C NMR spectrum of compound **4**

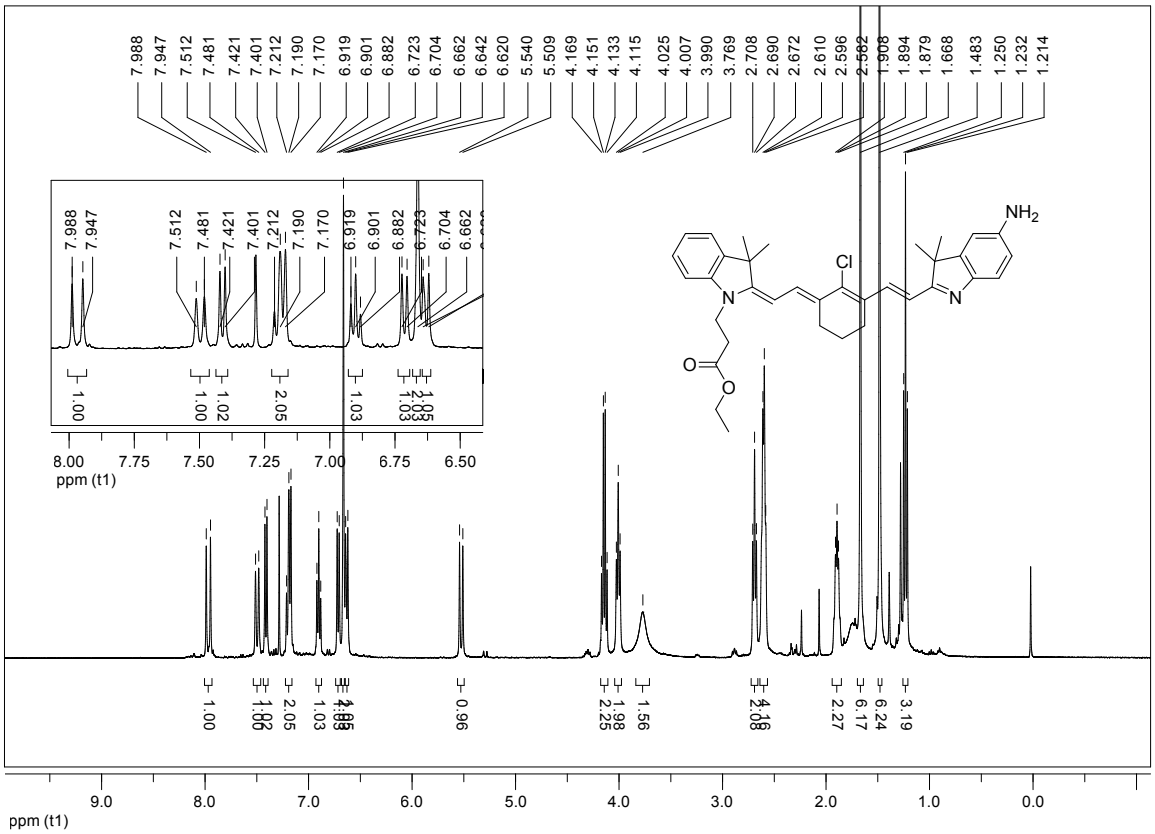

Fig. S8 ^{1}H NMR spectrum of compound **5**

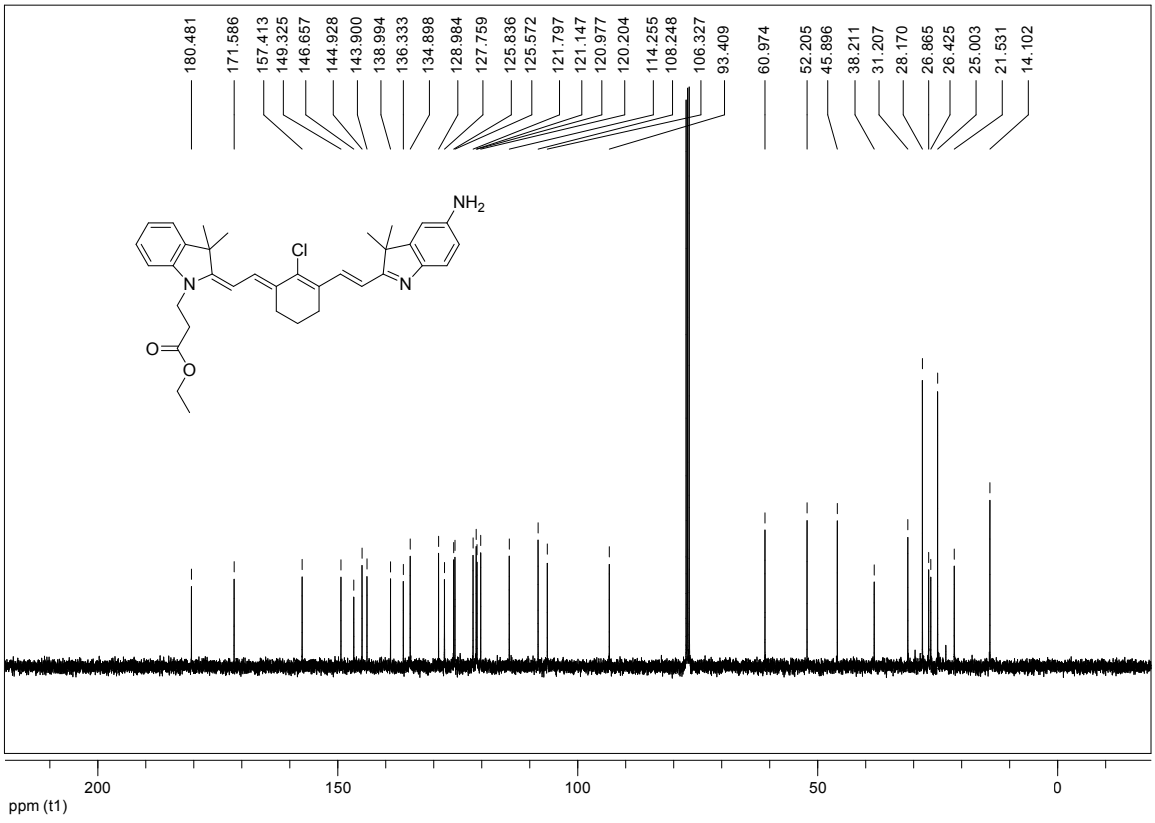

Fig. S9 ^{13}C NMR spectrum of compound **5**

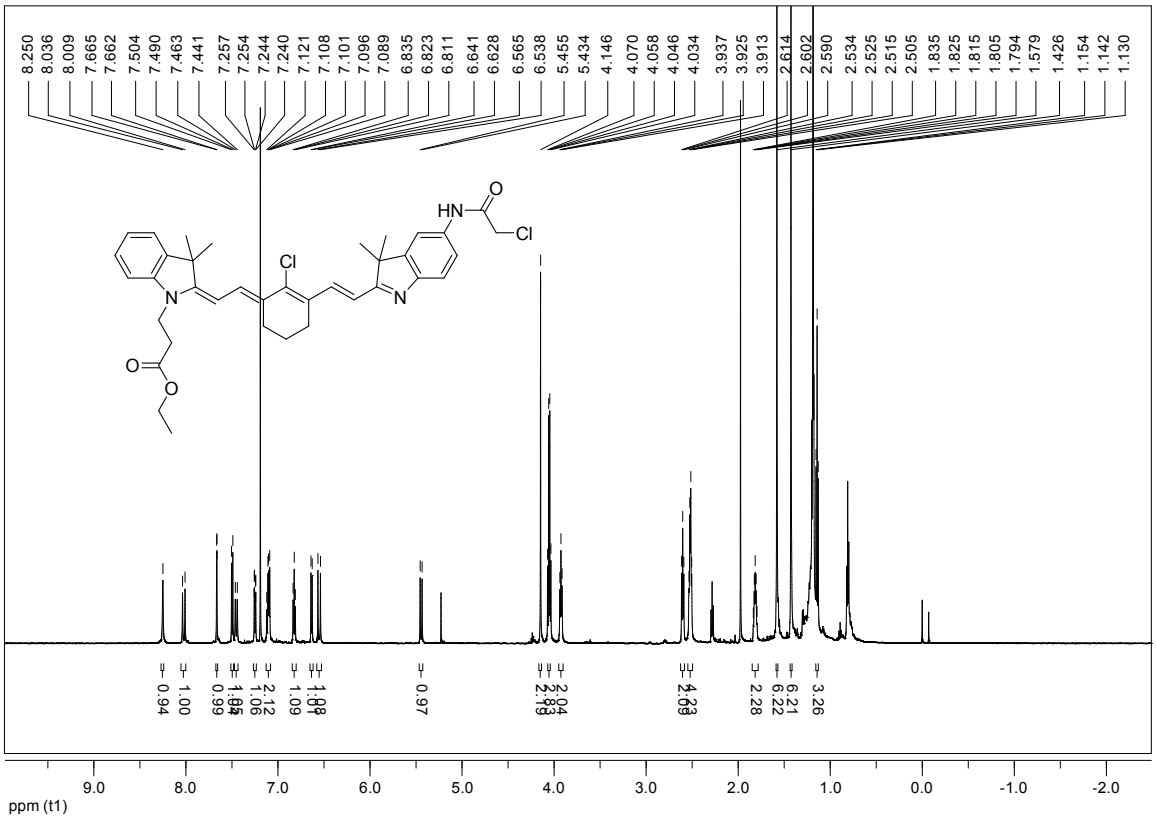

Fig. S10 [1]H NMR spectrum of compound **6**

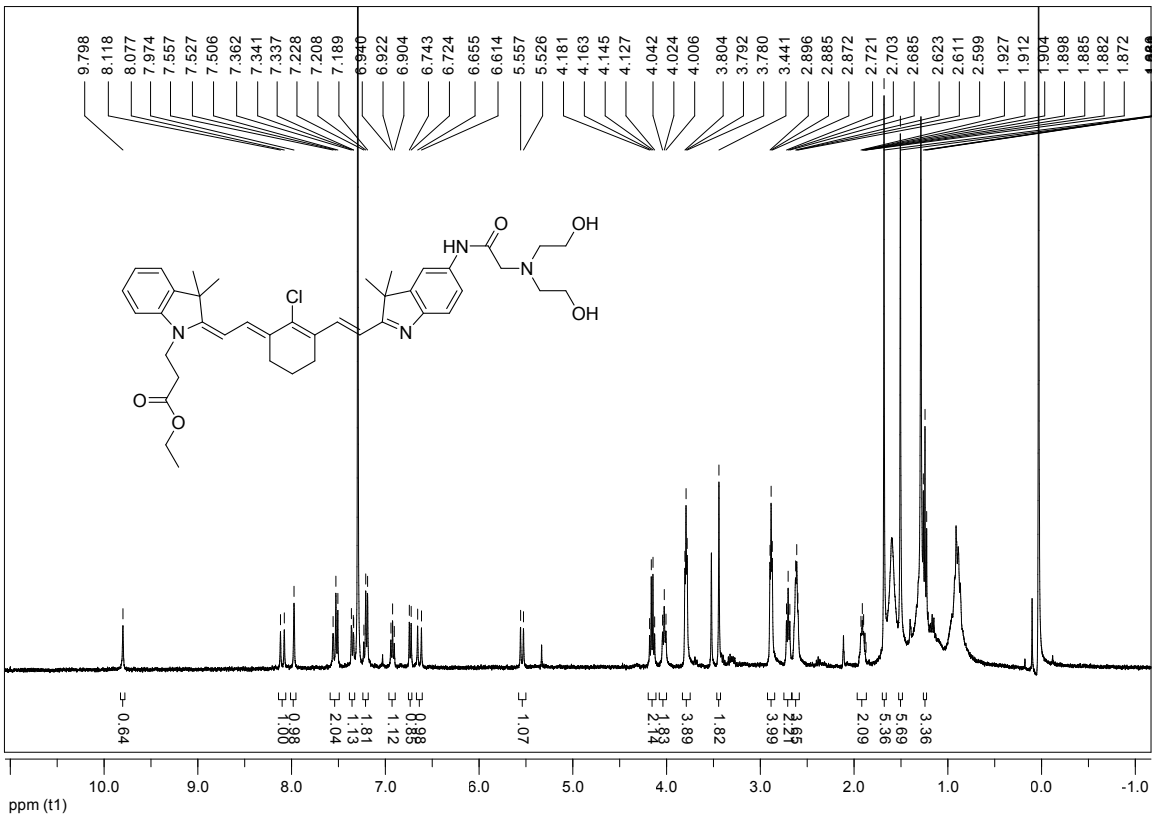

Fig. S11 [1]H NMR spectrum of compound **7**

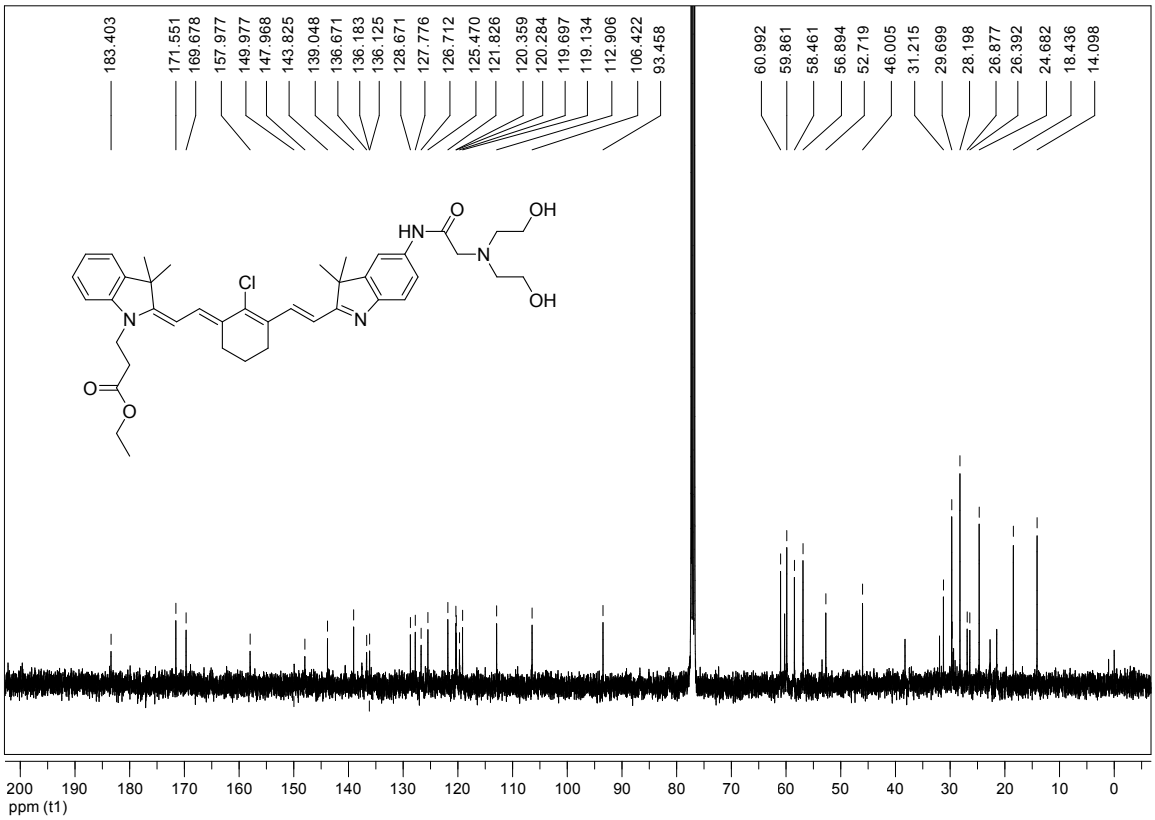

Fig. S12 ^{13}C NMR spectrum of compound **7**

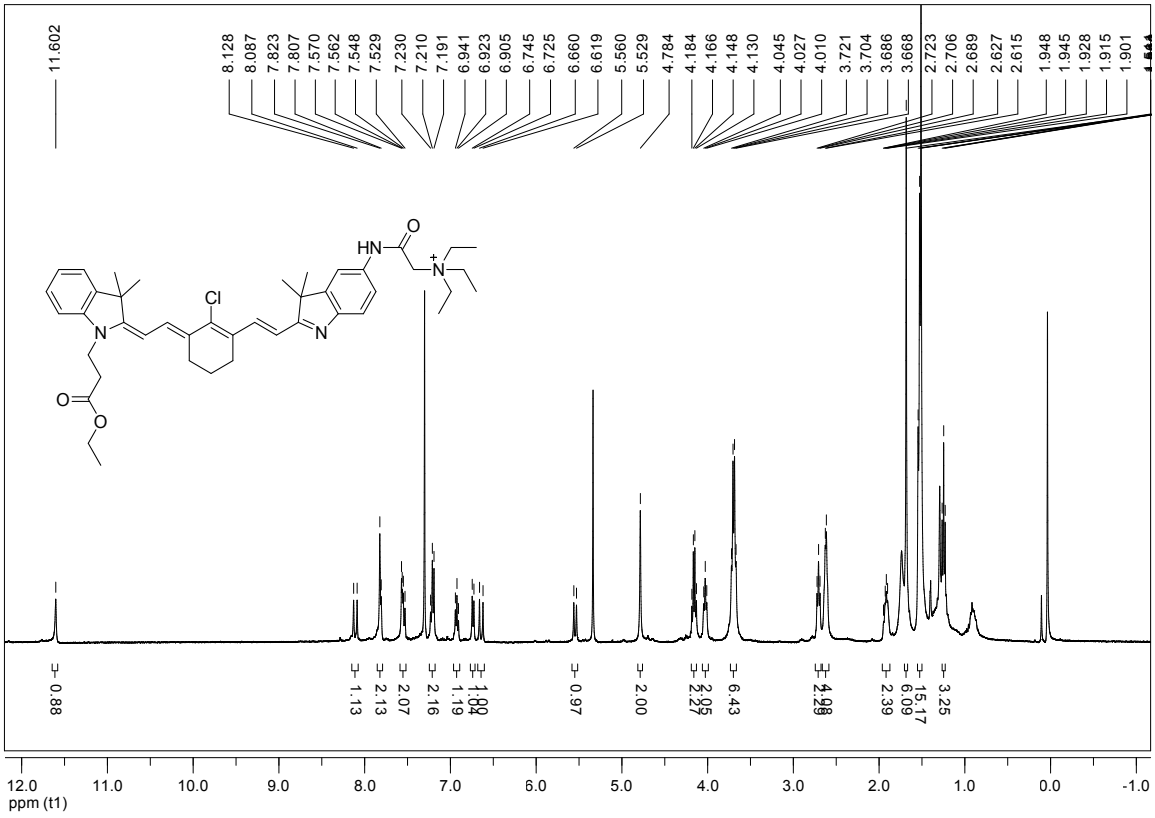

Fig. S13 ^{1}H NMR spectrum of compound **8**

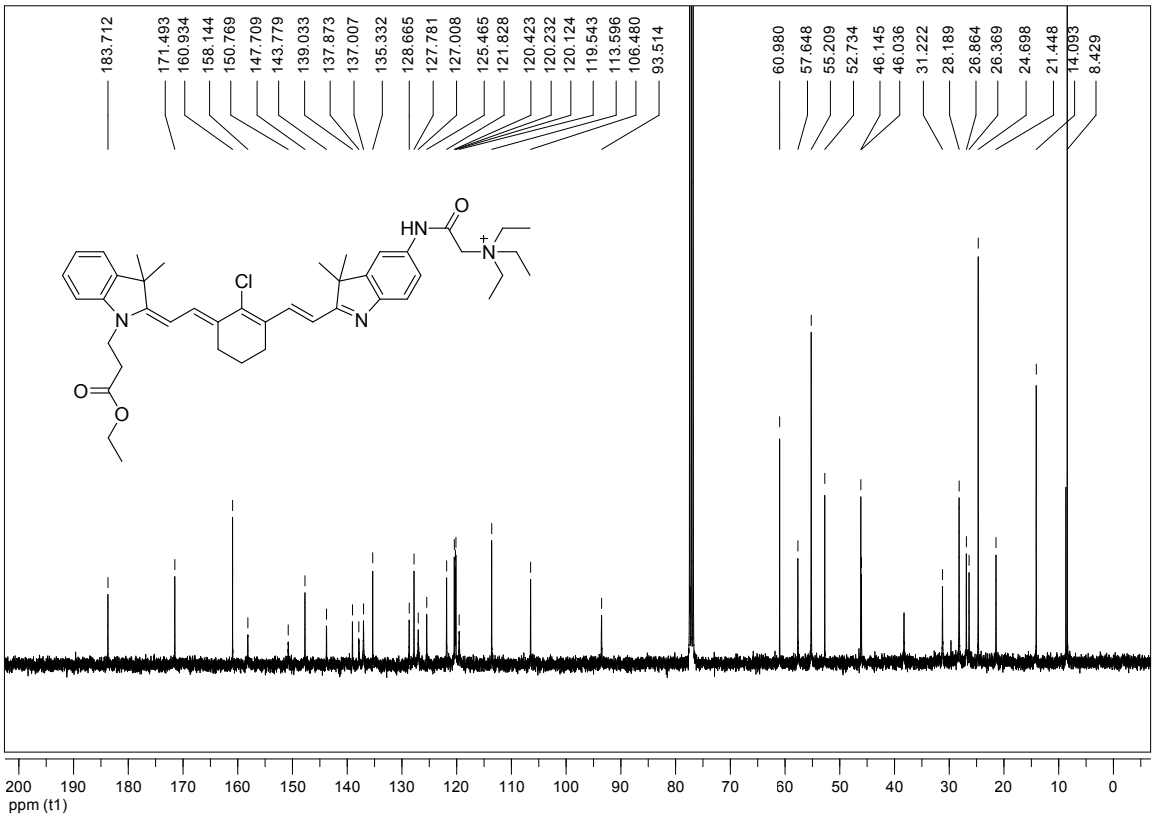

Fig. S14 ^{13}C NMR spectrum of compound **8**

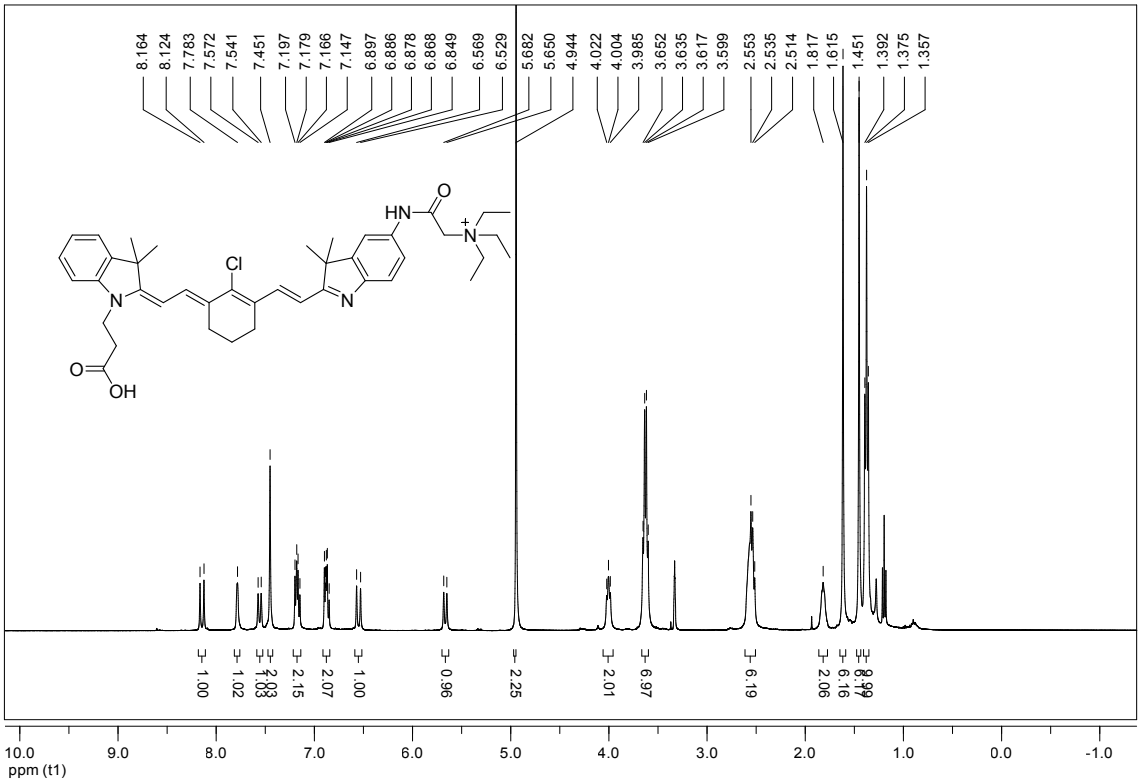

Fig. S15 ^{1}H NMR spectrum of compound **9**

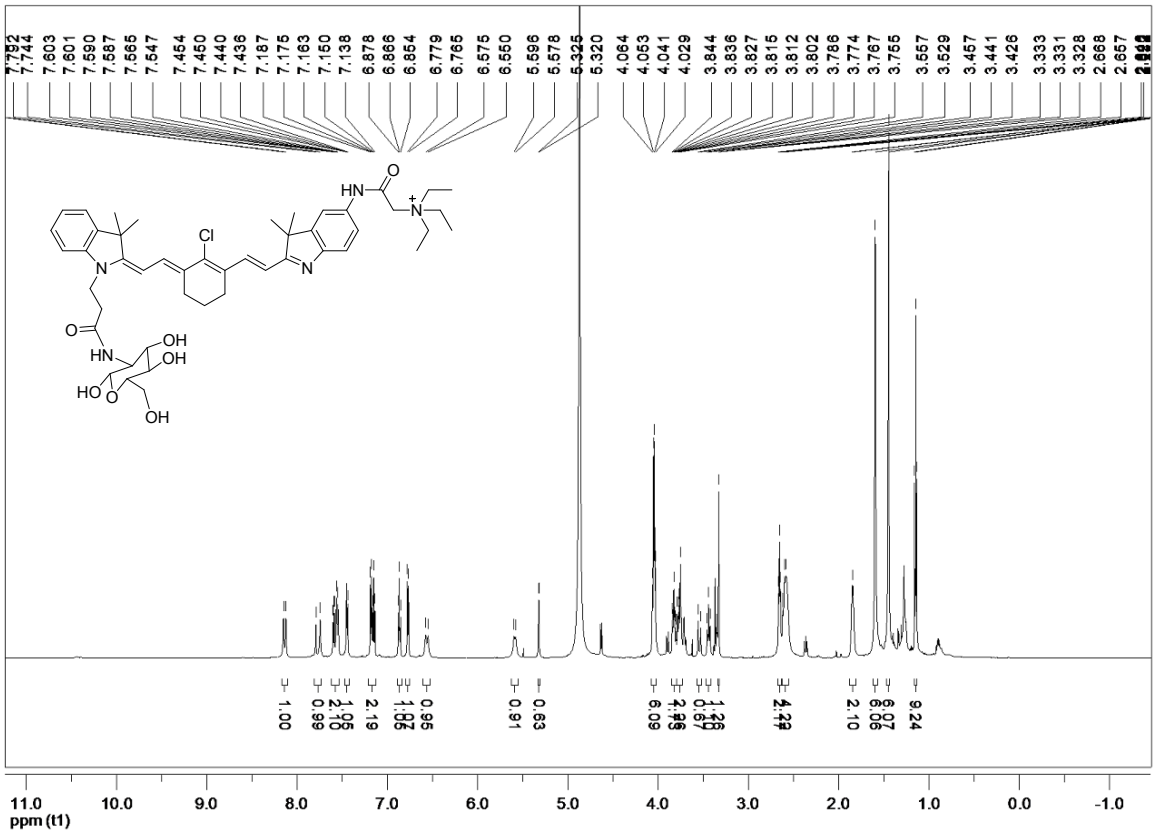

Fig. S16 ^{1}H NMR spectrum of compound **10**