Supporting Information

A Molecular Engineering Platform for Enhanced Stokes Shift NIR-II Fluorophores Enabling High-Fidelity 1400 nm *In Vivo* Imaging.

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Table of Content

1. Experimental Section

Materials: Reagents and solvents were used as received from commercial suppliers unless otherwise specified. All reactions sensitive to air and moisture were conducted in oven-dried glassware under an argon atmosphere to maintain an inert environment. The reaction progress was monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel plates with UV indicator. Silica-gel flash column chromatography was carried out using silica gel with a mesh size of 300-400. Certain special chemicals were sourced from different reagent suppliers, including 2,7-dihydroxynaphthalene (98.2%) from Le Yan, 2-bromo-1,3-difluorobenzene from Le Yan, 1-bromo-3-chloropropane from Adamas, nBuLi from Adamas, and o-tolylmagnesium bromide from Adamas.

General Measurements: ^{1}H and ^{13}C spectra were recorded at room temperature on a Bruker AVANCE NEO 400 spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as a reference. Mass spectra (MS) were measured with Waters 2695 HPLC. The UV-Vis-NIR absorption spectra measurement was performed using a Shimadzu UV-2600i spectrophotometer. Fluorescence spectra were recorded by using a FLS980 fluorescence spectrometer (Edinburgh Instruments, UK). The *in vivo* fluorescence imaging was carried out with MARS (Artemis Intelligent Imaging) equipped with an InGaAs camera (NIRvana, Teledyne Princeton Instruments).

Quantum Yield (QY) Test: To determine the quantum yields (QYs) of three fluorophores in CH_2Cl_2, ESi5b, which has a known absolute fluorescence quantum yield, was used as the reference. The absolute quantum yield of ESi5b in CH_2Cl_2 is 10.7%.[1] Absorption spectra were recorded using a Shimadzu UV-2600i scanning spectrophotometer, and photoluminescence (PL) measurements were conducted using a FLS980 fluorescence spectrometer (Edinburgh Instruments, UK). The integrated emission intensities of EGe5 and ESi5b (reference) were recorded under identical excitation conditions. The intensities were plotted as a function of optical density (OD) for each sample, and the slopes were determined through linear fitting. The quantum yield (QY) of the sample was then calculated as follows:[2] QY_{ref}: Reference quantum yield value. N: Refractive index of the different solvents.

$$QY = QY_{ref} \cdot \frac{Slope}{Slope_{ref}} \cdot \frac{n^2}{{n_{ref}}^2}$$

Description of Single Crystal Testing Conditions and Refinement Method: Single crystals of $C_{38}H_{38}N_2O$, identified as NIR-842. All data were integrated with SAINT V8.40B and a multi-scan absorption correction using SADABS 2016/2 was applied.[3] The structure was solved by dual methods with SHELXT 2018/2 and refined by full-matrix least-squares methods against F2 using SHELXL 2018/3.[4, 5] All non-hydrogen atoms were refined with anisotropic displacement parameters. All C-bound hydrogen atoms were refined isotropic on calculated positions using a riding model with their Uiso values constrained to 1.5 times the Ueq of their pivot atoms for terminal sp^3 carbon atoms and 1.2 times for all other carbon atoms. Disordered moieties were refined using bond lengths restraints and displacement parameter restraints.

Spectral Measurements: A stock solution (5 mM) of fluorophores in DMSO was prepared. During the measurement, a sample solution was prepared by mixing an appropriate amount of the stock solution of fluorophores with the proper amounts of analytes (H_2O_2, NaClO, GSH, Cys), respectively, and finally diluted by MeCN: H_2O (v:v=1:1) to obtain the desired concentration. The mixtures were incubated for 1 min, and then fluorescence intensities of the probes were measured with a cuvette.

Animal Preparation: Animal Preparation：All experiments using female ICR mice (6-7 weeks old) were approved by the Zhejiang University IACUC (ZJU20220283). Mice were obtained from the Laboratory Animal Center of Sir Run Shaw Hospital and maintained under standard conditions: 12 h light/dark cycle; 22-24 °C; 40-60% humidity; ad libitum access to food and water.

Preparation of NIR-842 NPs: NIR-842 (3 mg) and DSPE-mPEG2000 (24 mg) were first dissolved in 1 mL of THF sonicated for 1 minute. After mixing, the solution was further sonicated for 5 minutes, followed by dropwise addition into vigorously stirred distilled water (3 mL). The self-assembled nanoparticles were obtained after 12 hours. The solution was filtered with 0.22 ummicroporous membrane for 3 times.

In vivo imaging: For in vivo whole-body NIR-II imaging experiment, The mice were depilated using a commercial hair removal cream, followed by intravenous administration of 200 μL of NIR-842 nanoparticles at a concentration of 3 mg/mL. All mice were anaesthetized by mask inhalation of isoflurane at various time points post injection and then imaged with the NIR-II fluorescence imaging system. The excitation was provided by an 880 nm laser, and the NIR emission was collected through different long pass filters (1100 nm, 1200 nm, 1350 nm, 1450 nm). All long-pass (LP) filters used in the experiment were sourced from Thorlabs.

Optical Setup for NIR-II Fluorescence Whole-Body Imaging: In vivo macroscopic fluorescence imaging was performed using a small animal imaging system (MARS, Artemis Intelligent Imaging) equipped with a NIRvana InGaAs camera. A fiber-coupled 880 nm laser provided shadow-free excitation illumination. The NIR emission was collimated by a 50 mm focal length SWIR lens (MARS-FAST, Artemis Intelligent Imaging, Shanghai, China).

2. Synthesis of fluorophores

2.1 Synthesis of NIR-842

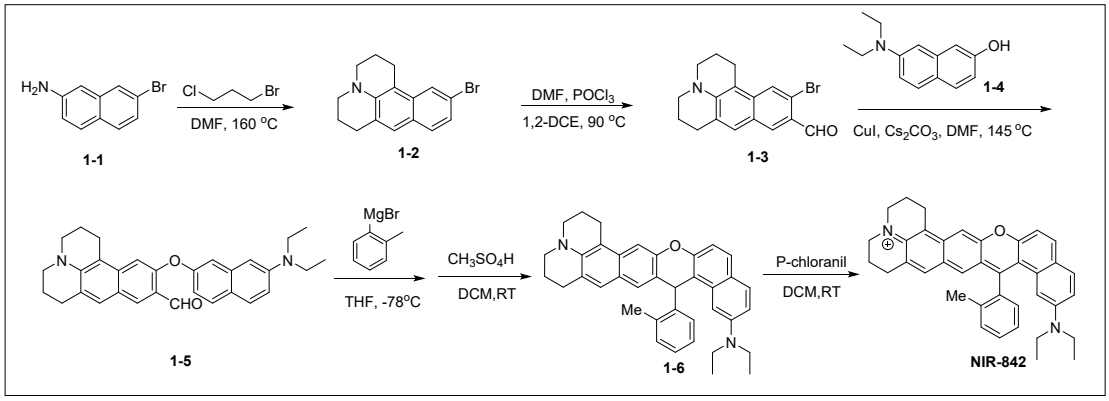

Fig. S1 Synthetic scheme of NIR-842.

bromo-2,3,6,7-tetrahydro-1H,5H-benzo[f]pyrido[3,2,1-ij] quinoline (1-2). Compound **1-1** (10 g, 45 mmol) was placed in a 250 mL round-bottom flask, followed by the addition of 10 ml of *N*, *N*-dimethylformamide (DMF) and 1-bromo-3-chloropropane (6.7 mL, 68 mmol). The mixture was heated to reflux at 160 °C and reacted for 24 hours. The reaction system was extracted with dichloromethane and concentrated in vacuo. The residue was purified by chromatography column to give **1-2** (8 g, 59%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 1.9 Hz, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.23 (s, 1H), 7.18 (dd, J = 8.5, 1.9 Hz, 1H), 3.22 (q, J = 5.6 Hz, 5H), 2.97 (t, J = 6.7 Hz, 2H), 2.92 - 2.88 (m, 2H), 2.14 - 2.06 (m, 2H), 2.05 - 1.97 (m, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 142.09, 133.53, 129.13, 125.71, 125.39, 124.93, 124.18, 123.46, 119.71, 110.15, 50.50, 49.86, 28.69, 23.40, 22.00, 21.67. HRMS (ESI) m/z calcd for $C_{16}H_{16}BrN$ $[M+H]^+$, 302.0544; Found 302.0539

11-bromo-2,3,6,7-tetrahydro-1H,5H-benzo[f]pyrido[3,2,1-ij] quinoline-10-carbaldehyde (1-3). Add N, N-dimethylformamide (0.51 mL, 6.57 mmol) to a 50 mL dried Schlenk flask. After cooling to 0°C, phosphorus oxychloride (1.16 mL, 6.57 mmol) was added dropwise, and the mixture was stirred for 30 minutes. Then, the resulting mixture was added to a solution of compound **1-2** (1 g, 3.23 mmol) in anhydrous 1,2-Dichloroethane (10 mL) at 90 °C for 3 hours, quenched by adding saturated Na_2CO_3 solution. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by chromatography column to give **1-3** (0.4 g, 36%) as a yellow soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (s, 1H), 8.13 (s, 1H), 7.78 (s, 1H), 7.32 (s, 1H), 3.35 - 3.27 (m, 4H), 2.94 (t, J = 6.6 Hz, 2H), 2.91 - 2.85 (m, 2H), 2.13 - 2.04 (m, 2H), 2.04 - 1.95 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.78, 144.58, 136.32, 131.51, 127.77, 126.55, 125.50, 125.02, 124.09, 121.28, 109.15, 50.50, 49.72, 28.68, 23.17, 21.45, 21.11. HRMS (ESI) m/z calcd for $C_{17}H_{16}BrNO$ $[M+Na]^+$, 352.0313; Found 352.0309.

7-(diethylamino) naphthalen-2-ol (1-4). A mixture of commercially available 2,7-dihydroxynaphthalene (10 g, 62.43 mmol), diethylamine (14.85 mL, 143.60 mmol), sodium metabisulfite (11.87 g, 62.43 mmol), and water (50 mL) was placed in a pressure vessel. The reaction was carried out at 130 °C for 2 h, then heated to 150 °C and maintained for 48 h. After cooling to room temperature, the mixture was filtered under vacuum using a Büchner funnel. The product was extracted with dichloromethane, and the combined organic layers were concentrated under reduced pressure. Purification by column chromatography on silica gel (eluent: ethyl

acetate/petroleum ether, 1:30 v/v) afforded the desired compound as a solid (7 g, yield 70%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 (d, *J* = 9.0 Hz, 1H), 7.52 (d, *J* = 8.6 Hz, 1H), 6.92 (dd, *J* = 9.2, 2.3 Hz, 1H), 6.87 (s, 1H), 6.76 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.69 (s, 1H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.33, 155.27, 149.18, 145.48, 144.17, 138.93, 136.61, 132.32, 130.96, 129.50, 128.87, 127.75, 124.40, 123.51, 122.48, 119.05, 118.87, 114.43, 111.34, 109.62, 104.55, 50.57, 49.78, 31.54, 30.16, 28.65, 23.39, 21.73, 21.22, 19.22, 13.77. HRMS (ESI) m/z calcd for $C_{14}H_{17}NO$ $[M+H]^+$, 216.1388; Found 216.1384.

((7-(diethylamino)naphthalen-2-yl)oxy)-2,3,6,7-tetrahydro-1H,5H-benzo[f]pyrido[3,2,1-ij]quinoline-10-carbaldehyde (**1-5**). Compound **1-3** (1 g, 3.03 mmol), cesium carbonate (1.68 g, 5.15 mmol), copper(I) iodide (0.12 g, 0.61 mmol), and 7-diethylamino-2-naphthol (0.72 g, 3.33 mmol) were placed in a Schlenk flask. Under nitrogen protection, anhydrous DMF (30 mL) was added, and the reaction was carried out at 145 °C for 48 hours. After cooling to room temperature, the mixture was filtered through a Büchner funnel (pre-coated with diatomite as a filter aid). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by chromatography column to give **1-5** (0.5 g, 35%) as a yellow soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.25 (s, 1H), 7.75 - 7.68 (m, 1H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.56 - 7.50 (m, 1H), 7.40 (s, 1H), 7.14 (s, 1H), 7.05 - 6.95 (m, 3H), 6.65 (s, 1H), 3.41 (q, *J* = 7.0 Hz, 2H), 3.30 (t, *J* = 5.8 Hz, 2H), 3.25 (t, *J* = 5.8 Hz, 2H), 2.91 (t, *J* = 6.4 Hz, 2H), 2.77 (t, *J* = 6.6 Hz, 2H), 2.05 - 1.96 (m, 3H), 1.72 (p, *J* = 6.8 Hz, 2H), 1.18 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.33, 155.27, 149.18, 145.48, 144.17, 138.93, 136.61, 132.32, 130.96, 129.50, 128.87, 127.75, 124.40, 123.51, 122.48, 119.05, 118.87, 114.43, 111.34, 109.62, 104.55, 50.57, 49.78, 31.54, 30.16, 28.65, 23.39, 21.73, 21.22, 19.22, 13.77. HRMS (ESI) m/z calcd for $C_{31}H_{32}N_2O_2$ $[M+H]^+$, 465.2542; Found 465.2531.

(R)**-N,N-diethyl-10-(o-tolyl)-2,3,6,7-tetrahydro-1H,5H,10H-benzo[7,8]xantheno[3,2-f]pyrido[3,2,1-ij]quinolin-12-amine (1-6)**. Compound **1-5** (200 mg, 0.43 mmol) was placed in an oven-dried Schlenk reactor. Under nitrogen atmosphere, 5 ml of THF was added as solvent. The reactor was cooled to -78 °C in a low-temperature reaction bath for 0.5 hours, followed by addition of o-tolylmagnesium bromide solution (3 eq). The mixture was stirred at room temperature for 5 hours. After completion of the reaction, the organic layer was extracted with ethyl acetate and concentrated. Dichloromethane was then added as solvent, and methanesulfonic acid (0.2 eq) was added dropwise with stirring for 6 hours. Reaction progress was monitored by TLC until complete consumption of the starting material. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by chromatography column to give **1-6** (167 mg, 72%) as a yellow oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 (d, 2H), 7.45 (s, 1H), 7.33 (s, 1H), 7.23 (d, *J* = 5.4 Hz, 1H), 7.11 (s, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.02 (dd, *J* = 6.9, 2.0 Hz, 1H), 6.97 - 6.89 (m, 2H), 6.85 (d, *J* = 8.9 Hz, 1H), 6.70 (s, 1H), 5.96 (s, 1H), 3.43 - 3.22 (m, 4H), 3.18 - 3.06 (m, 4H), 2.95 (t, *J* = 6.7 Hz, 2H), 2.81 (q, *J* = 6.0 Hz, 2H), 2.61 (s, 3H), 2.12 - 2.01 (m, 2H), 2.00 - 1.87 (m, 2H), 1.07 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.32, 148.72, 146.14, 145.67, 141.56, 133.88, 132.17, 131.18, 129.70, 129.22, 128.59, 127.67, 126.72, 126.07, 125.04, 124.04, 123.00, 121.33, 113.71, 113.21, 113.02, 110.07, 105.59, 101.65, 50.64, 50.05, 44.67, 39.03, 28.61, 23.62, 22.35, 21.99, 20.29, 12.69. (ESI) m/z calcd for $C_{38}H_{38}N_2O$ $[M+H]^+$, 539.3062 ; Found 539.3040.

(diethylamino)-10-(o-tolyl)-1,2,3,5,6,7-hexahydrobenzo[7,8]xantheno[3,2-f]pyrido[3,2,1-ij]quinolin-4-ium (NIR-842). Compound **1-6** (100 mg) was placed in a 50 ml round-bottom flask,

followed by the addition of 5 ml dichloromethane and 5 mg oxidant tetrachloro-1,4-benzoquinone. The reaction was carried out at room temperature for 24 hours, concentrated in vacuo. The residue was purified by chromatography column to give **1-7** (90 mg, 90%) as a green solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (d, *J* = 8.6 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.70 - 7.56 (m, 4H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.26 (s, 1H), 7.00 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.84 (d, *J* = 2.4 Hz, 1H), 3.63 (q, *J* = 6.0 Hz, 4H), 3.10 - 3.01 (m, 6H), 2.90 (d, *J* = 6.4 Hz, 2H), 2.24 - 2.14 (m, 4H), 2.07 (s, 3H), 1.01 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.01, 159.59, 150.22, 149.90, 149.21, 143.93, 138.56, 136.57, 135.13, 133.53, 133.47, 131.92, 131.00, 130.31, 129.76, 128.79, 128.09, 127.67, 127.55, 122.60, 120.24, 116.38, 113.86, 111.15, 109.81, 106.43, 102.84, 51.81, 50.95, 44.58, 29.68, 28.34, 22.97, 20.86, 20.35, 19.85, 12.80. HRMS (ESI) m/z calcd for $C_{38}H_{37}N_2O^+$ $[M]^+$, 537.2901; Found 537.2900.

2.2 Synthesis of NIR-864

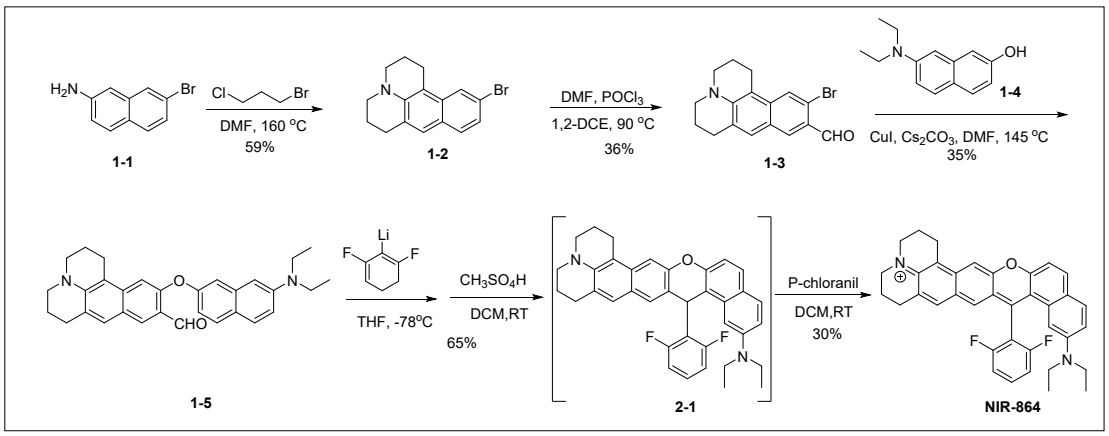

Fig. S2 Synthetic scheme of NIR-864.

Compounds **1-1** to **1-5** can be prepared according to the procedure described in Scheme 1.

12-**(diethylamino)-10-(2,6-difluorophenyl)-1,2,3,5,6,7-hexahydrobenzo[7,8]xantheno[3,2-f]pyrido[3,2,1-ij]quinolin-4-ium (NIR-864)**. Compound **1-5** (200 mg, 0.43 mmol) was placed in an oven-dried Schlenk reactor. Under nitrogen atmosphere, 5 ml of THF was added as solvent. The reactor was cooled to -78 °C in a low-temperature reaction bath for 0.5 hours, followed by addition of (2,6-difluorophenyl) lithium solution (3 eq). The mixture was stirred at room temperature for 5 hours. After completion of the reaction, the organic layer was extracted with ethyl acetate and concentrated. Dichloromethane was then added as solvent, and methanesulfonic acid (0.2 eq) was added dropwise with stirring for 6 hours. Reaction progress was monitored by TLC until complete consumption of the starting material. Quenched by adding saturated Na_2CO_3 solution. The aqueous layer was extracted with CH_2Cl_2, filtered and concentrated in vacuo. Followed by the addition of 5 ml dichloromethane and 5 mg oxidant tetrachloro-1,4-benzoquinone. The reaction was carried out at room temperature for 24 hours, concentrated in vacuo. The residue was purified by chromatography column to give **2-1** (47 mg, 30%) as a green solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (d, *J* = 8.4 Hz, 1H), 7.91 (s, 2H), 7.79 (d, *J* = 9.1 Hz, 2H), 7.60 (s, 1H), 7.44 (d, *J* = 15.9 Hz, 4H), 7.03 (d, *J* = 7.9 Hz, 1H), 6.97 (s, 1H), 3.71 - 3.63 (m, 4H), 3.14 (q, *J* = 6.9, 6.3 Hz, 4H), 3.06 - 3.00 (m, 2H), 2.97 - 2.92 (m, 2H), 2.21 (s, 2H), 2.09 (s, 2H), 1.08 (t, *J* = 6.8 Hz, 6H). Owing to the poor solubility of compound NIR-864, the signals in its ^{13}C NMR spectrum were weak. ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.77, 130.89, 128.81, 77.24, 77.03, 76.81, 68.18, 53.42, 44.80, 38.75,

30.38, 29.78, 29.70, 29.61, 29.32, 28.94, 27.22, 23.76, 22.99, 14.05, 12.73, 10.96. HRMS (ESI) m/z calcd for $C_{37}H_{33}F_2N_2O^+$ $[M]^+$, 559.2556; Found 559.2557.

2.3 Synthesis of NIR-820

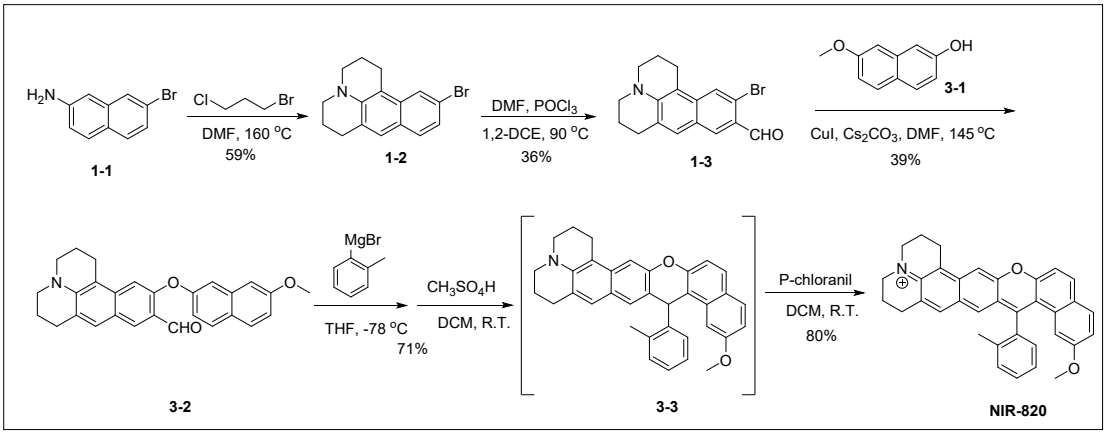

Fig. S3 Synthetic scheme of NIR-820.

Compounds **1-1** to **1-3** can be prepared according to the procedure described in Scheme 1. Compound **3-1** was obtained commercially.

((7-methoxynaphthalen-2-yl)oxy)-2,3,6,7-tetrahydro-1H,5H-benzo[f]pyrido[3,2,1-ij]quinoline-10-carbaldehyde (**3-2**). Compound **1-3** (1 g, 3.03 mmol), cesium carbonate (1.68 g, 5.15 mmol), copper(I) iodide (0.12 g, 0.61 mmol), and 7-methoxynaphthalen-2-ol (0.72 g, 3.33 mmol) were placed in a Schlenk flask. Under nitrogen protection, anhydrous DMF (30 mL) was added, and the reaction was carried out at 145 °C for 48 hours. After cooling to room temperature, the mixture was filtered through a Büchner funnel (pre-coated with diatomite as a filter aid). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by chromatography column to give **3-2** (0.5 g, 39%) as a yellow soild. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (s, 1H), 8.27 (s, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.9 Hz, 1H), 7.40 (s, 1H), 7.22 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.16 (s, 1H), 7.15 (d, *J* = 2.4 Hz, 1H), 7.06 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.34 - 3.28 (m, 2H), 3.27 - 3.22 (m, 2H), 2.92 (t, *J* = 6.3 Hz, 2H), 2.78 (t, *J* = 6.5 Hz, 2H), 2.01 (p, *J* = 6.4 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.11, 184.56, 173.02, 158.30, 157.25, 154.82, 144.29, 136.62, 135.77, 130.95, 129.76, 129.25, 127.79, 125.43, 122.70, 122.38, 117.44, 116.70, 112.17, 110.22, 109.59, 105.29, 77.45, 77.13, 76.81, 55.27, 50.57, 49.77, 28.67, 23.37, 21.70, 21.21. HRMS (ESI) m/z calcd for $C_{28}H_{25}NO_3$ $[M+Na]^+$, 446.1732; Found 446.1720.

12-methoxy-10-(o-tolyl)-1,2,3,5,6,7-hexahydrobenzo[7,8]xantheno[3,2-f]pyrido[3,2,1-ij]quinolin-4-ium (NIR-820). Compound **3-2** (400 mg, 0.43 mmol) was placed in an oven-dried Schlenk reactor. Under nitrogen atmosphere, 5 ml of THF was added as solvent. The reactor was cooled to -78 °C in a low-temperature reaction bath for 0.5 hours, followed by addition of o-tolylmagnesium bromide solution (3 eq). The mixture was stirred at room temperature for 5 hours. After completion of the reaction, the organic layer was extracted with ethyl acetate and concentrated. Dichloromethane was then added as solvent, and methanesulfonic acid (0.2 eq) was added dropwise with stirring for 6 hours. Reaction progress was monitored by TLC until complete consumption of the starting material. Quenched by adding saturated Na_2CO_3 solution. The aqueous layer was extracted with CH_2Cl_2, filtered and concentrated in vacuo. Followed by the addition of 5 ml

dichloromethane and 5 mg oxidant tetrachloro-1,4-benzoquinone. The reaction was carried out at room temperature for 24 hours, concentrated in vacuo. The residue was purified by chromatography column to give **3-3** (267 mg, 57%) as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, *J* = 8.9 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.72 (d, *J* = 8.9 Hz, 1H), 7.62 (s, 1H), 7.51 (dt, *J* = 17.8, 7.7 Hz, 2H), 7.44 (s, 1H), 7.37 (s, 1H), 7.34 (d, *J* = 5.1 Hz, 1H), 7.17 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.99 (d, *J* = 1.9 Hz, 1H), 3.87 - 3.79 (m, 2H), 3.71 (q, *J* = 6.4 Hz, 4H), 3.28 (s, 3H), 3.06 - 2.97 (m, 4H), 2.92 - 2.86 (m, 2H), 1.99 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.19, 151.09, 150.45, 142.01, 139.00, 132.33, 131.91, 131.42, 130.99, 130.80, 130.72, 129.60, 128.87, 127.96, 118.90, 115.13, 111.56, 106.00, 102.57, 77.46, 77.14, 76.82, 65.62, 54.99, 52.52, 51.63, 30.60, 28.18, 22.96, 20.76, 20.23, 19.88, 19.23, 13.79. HRMS (ESI) m/z calcd for $C_{35}H_{30}NO_2^+$ $[M+H]^+$, 496.2272; Found 496.2274.

2. Supporting Figures

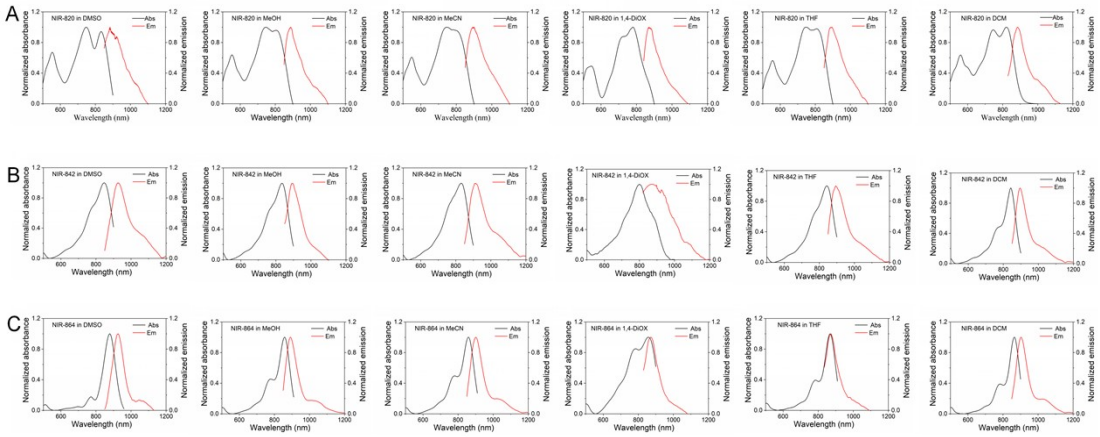

Fig. S4 Absorption and emission spectra of (A) NIR-820, (B) NIR-842, and (C) NIR-864 measured in different solvents: DMSO, MeOH, MeCN, 1,4-dioxane, THF, and DCM.

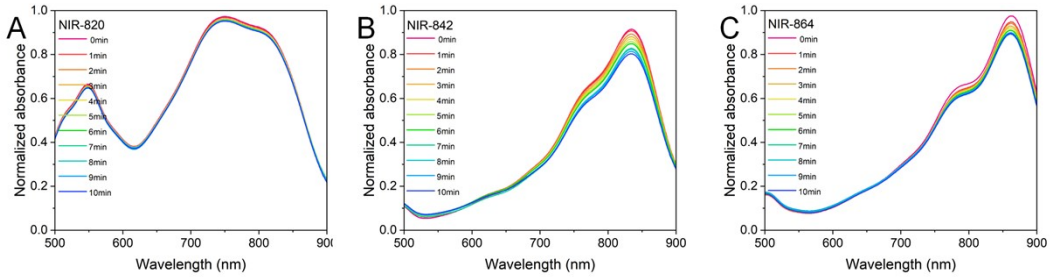

Fig. S5 Absorption spectra of (A) NIR-820, (B) NIR-842, and (C) NIR-864 measured under excitation with the power density of 0.5 W/cm^2 with varying irradiation durations.

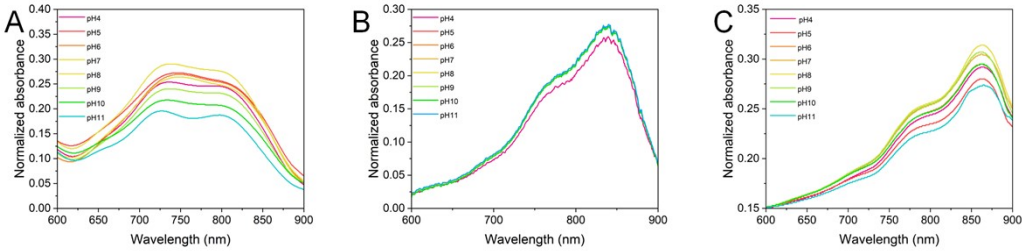

Fig. S6 Absorption spectra of (A) NIR-820, (B) NIR-842, and (C) NIR-864 measured in different pH conditions.

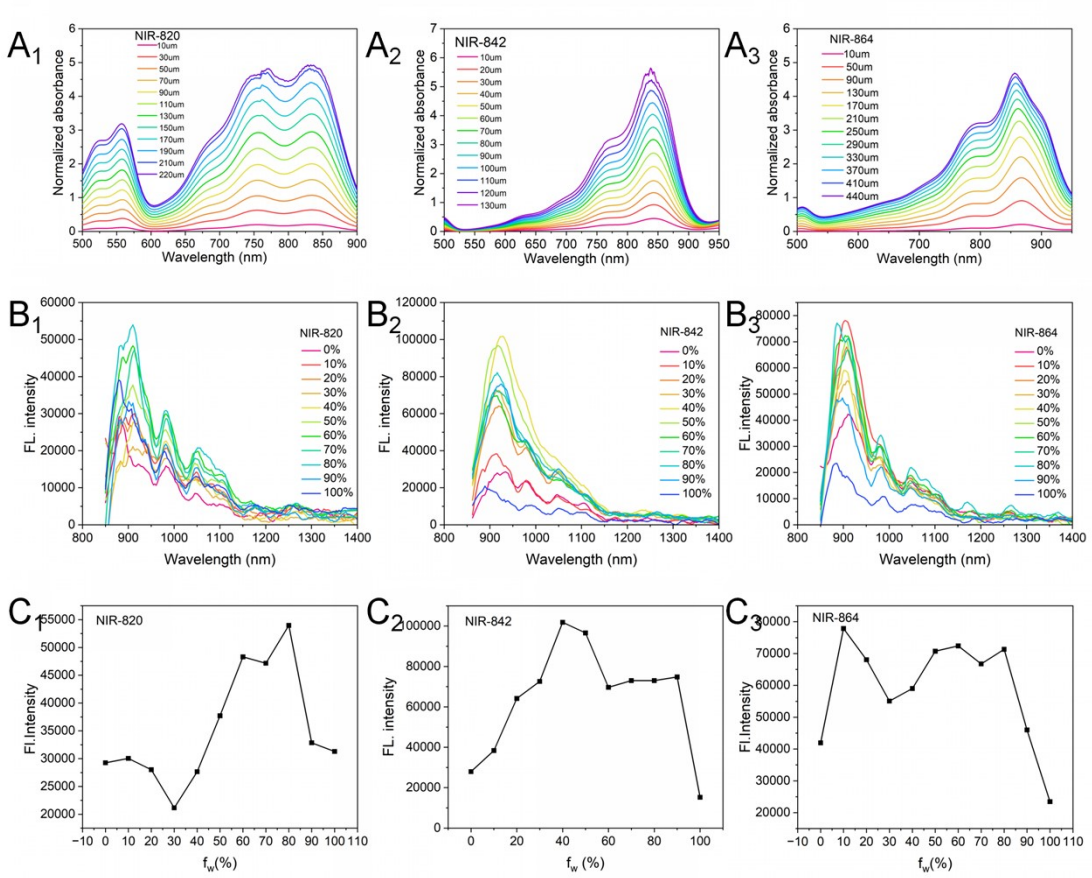

Fig. S7 (A_1-A_3) Absorption spectra of the three dyes in DCM; (B_1-B_3) Fluorescence emission spectra in THF upon incremental addition of water; (C_1-C_3) Normalized fluorescence intensity as a function of water fraction (f_w).

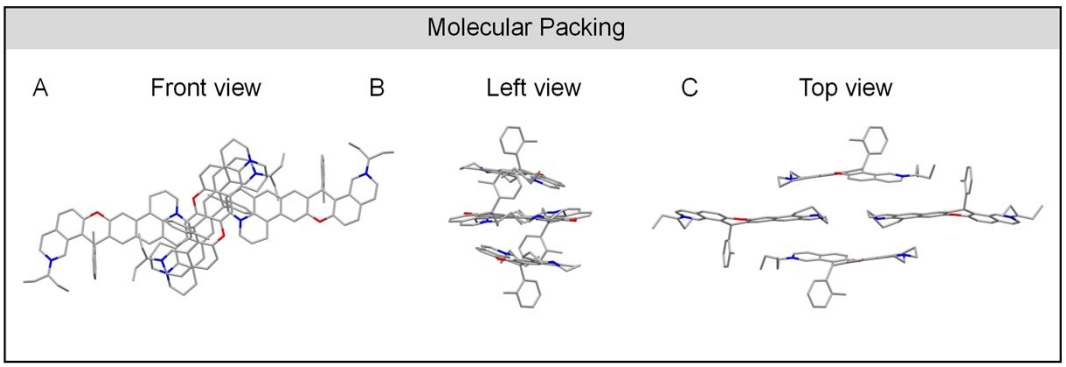

Fig. S8 The detailed stacking diagram of the compound 6 structure, i.e. (A) Front view, (B) Left view, and (C) Top view.

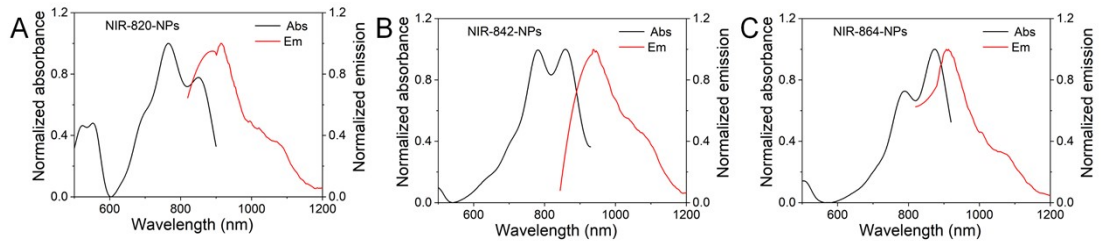

Fig. S9 Absorption and emission spectra of (A) NIR-820 NPs, (B) NIR-842 NPs, and (C) NIR-864 NPs measured in an aqueous solution.

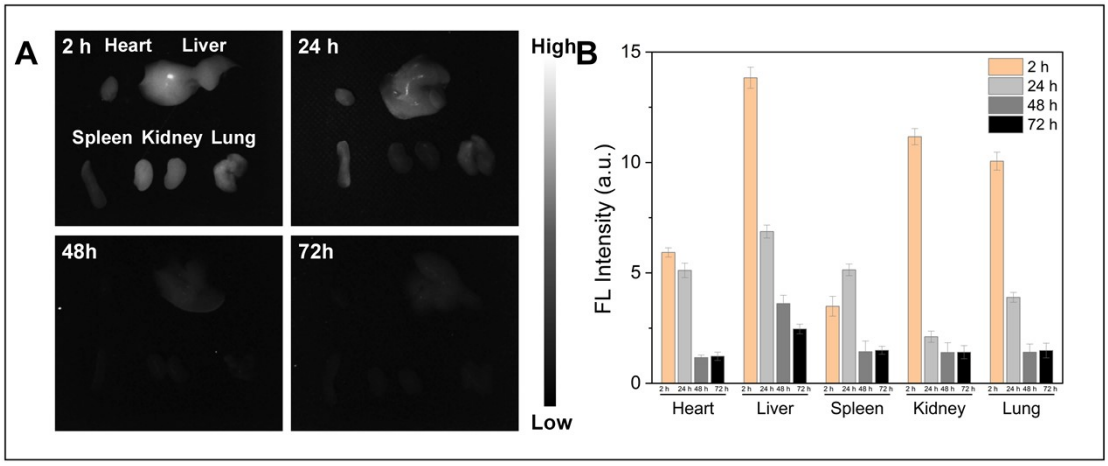

Fig. S10 Biodistribution of NIR-842 NPs. (A) Ex vivo NIR-II fluorescence images of major organs harvested from the mice at different time points and (B) The normalized fluorescence intensity.

4. Supporting Tables

Table S1. Photophysical parameters of three dyes.

Dye	non-polar	solvent	λabs	λem	Stokes shift	Φ[a] (%)	ε (L $M^{-1}cm^{-1}$)	Brightness ($cm^{-1}M^{-1}$)
NIR-820	moderately polar	N-hexane[b]	/	/	/	/	/	/
	moderately polar polar aprotic	DCM	820	874	54	0.556	22194	123.4
		THF	780	871	91	0.25	15275.0	38.2
		1,4-Diox	808	887	79	0.22	8572.2	18.9
	polar aprotic polar protic	DMSO	808	887	79	0.25	14605.6	36.5
		MeCN	812	895	83	0.317	16755.6	53.1
	polar protic	MeOH	800	900	100	0.329	18722.2	61.6
NIR-842	non-polar	N-hexane	/	/	/	/	/	/
	moderately polar	DCM	842	896	54	2.0	72361	1447.2
		THF	843	894	51	1.77	48041.7	850.3
		1,4-Diox	810	871	61	1.07	27755.6	297.0
	polar aprotic	DMSO	850	926	76	1.54	45841.7	706.0
		MeCN	832	914	82	1.79	46766.7	837.1
	polar protic	MeOH	836	894	58	1.81	45841.7	829.7

	non-polar	N-hexane	/	/	/	/	/	/
NIR-864	moderately polar	DCM	864	904	40	1.3	23944	311.3
		THF	868	873	5	1.05	19127.8	200.8
		1,4-Diox	862	873	11	0.759	8905.6	67.6
	polar aprotic	DMSO	880	924	44	0.83	16272.2	135.1
		MeCN	860	902	42	1.01	18361.1	185.4
	polar protic	MeOH	858	890	32	1.09	17927.8	195.4

[a] Absolute fluorescence quantum yield with ESi5b as standard (Φ = 10%) in DMSO.

[b] Due to poor solubility in non-polar solvents (n-hexane), no relevant photophysical properties of the dye could be detected.

Table S2. Crystal structure determination of compound 6.

CCDC number	2498509
Empirical formula	$C_{38}H_{38}N_2O$
Formula weight	538.70
Temperature [K]	170.00
Crystal system	monoclinic
Space group (number)	$P2_1/c$ (14)
a [Å]	17.138(4)
b [Å]	8.884(2)
c [Å]	18.772(4)
α [°]	90
β [°]	90.980(9)
γ [°]	90
Volume [Å^3]	2857.7(11)
Z	4
ρ_{calc} [gcm^{-3}]	1.252
μ [mm^{-1}]	0.365
F(000)	1152
Crystal size [mm^3]	0.02×0.05×0.06
Crystal colour	yellow
Crystal shape	plate
Radiation	$GaK\alpha$ (λ=1.34139 Å)
2θ range [°]	4.49 to 118.77 (0.78 Å)
Index ranges	−18 ≤ h ≤ 21 −11 ≤ k ≤ 11 −19 ≤ l ≤ 24
Reflections collected	23959
Independent reflections	6285 R_{int} = 0.0861 R_{sigma} = 0.0968
Completeness to θ = 53.594°	99.9 %
Data / Restraints / Parameters	6285 / 416 / 496
Absorption correction T_{min}/T_{max} (method)	0.6536 / 0.7516 (multi-scan)
Goodness-of-fit on F^2	0.979
Final *R* indexes [$I \geq 2\sigma(I)$]	R_1 = 0.0778 wR_2 = 0.1939
Final *R* indexes [all data]	R_1 = 0.1706 wR_2 = 0.2508
Largest peak/hole [eÅ^{-3}]	0.22/−0.30

Table S3. Photophysical parameters of three nanoparticles.

Dye	λabs[a]	λem	Stokes shift	Φ[b] (%)	ε (L $M^{-1}cm^{-1}$)	Brightness ($cm^{-1}M^{-1}$)
NIR-820 NPs	767	916	149	0.140	1665.6	2.3
NIR-842 NPs	861	937	76	0.298	3925.8	11.7
NIR-864 NPs	876	907	31	0.160	2239.3	3.6

[a] Maximum absorption wavelength in H_2O

[b] Absolute fluorescence quantum yield with ESi5b as standard (Φ = 10%) in DMSO.

^{1}H NMR, ^{13}C NMR and HRMS for 1-2

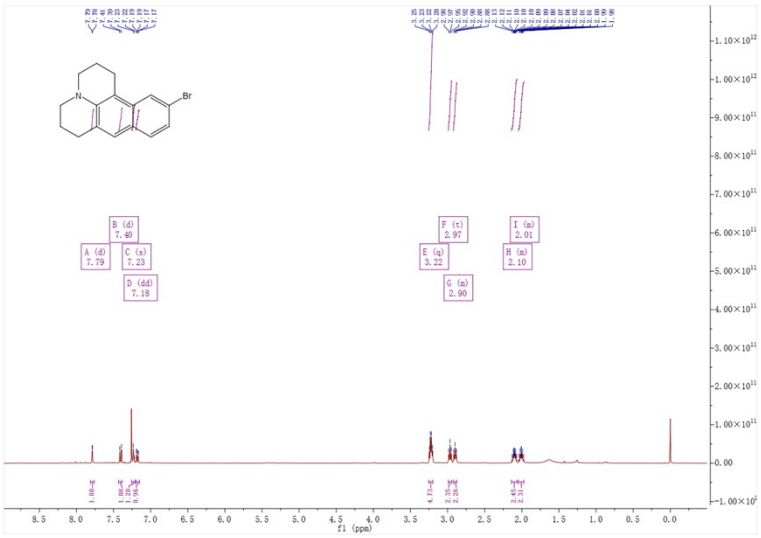

Fig. S6 ^{1}H NMR spectrum of 1-2.

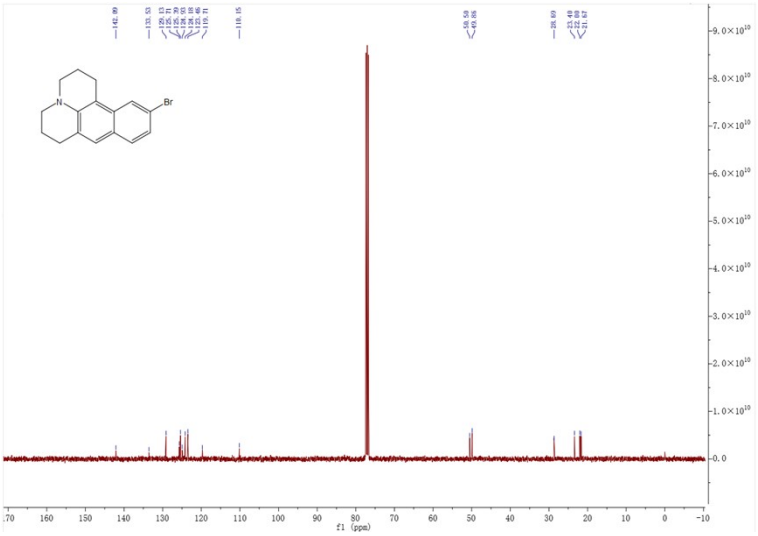

Fig. S7 ^{13}C NMR spectrum of 1-2.

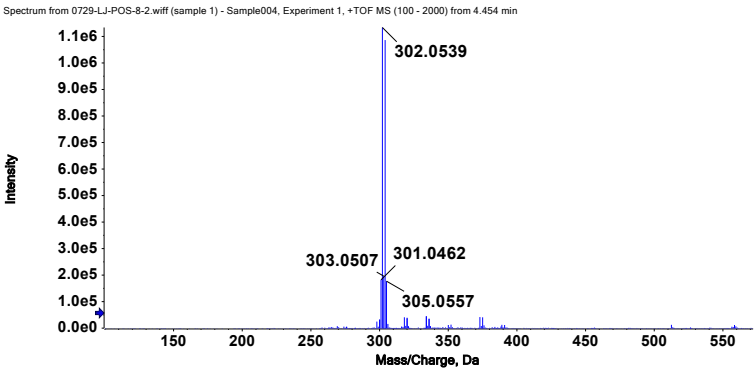

Fig. S8 The HRMS (ESI) of 1-2.

^{1}H NMR, ^{13}C NMR and HRMS for 1-3

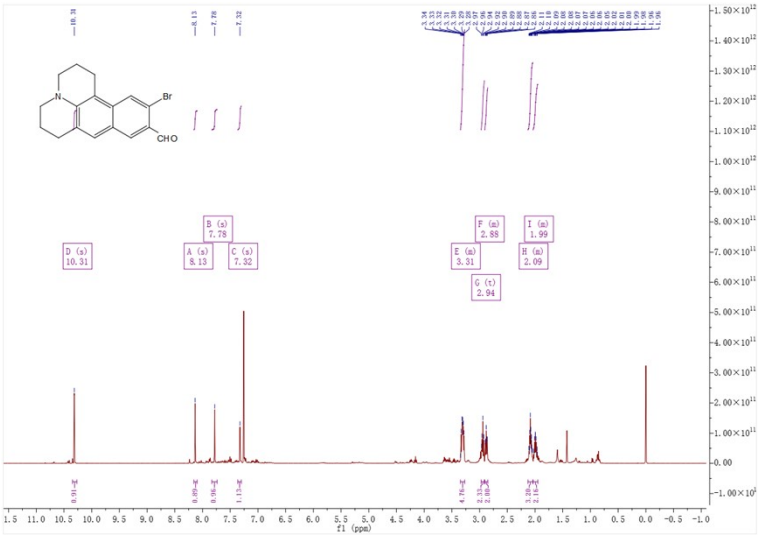

Fig. S9 ^{1}H NMR spectrum of 1-3.

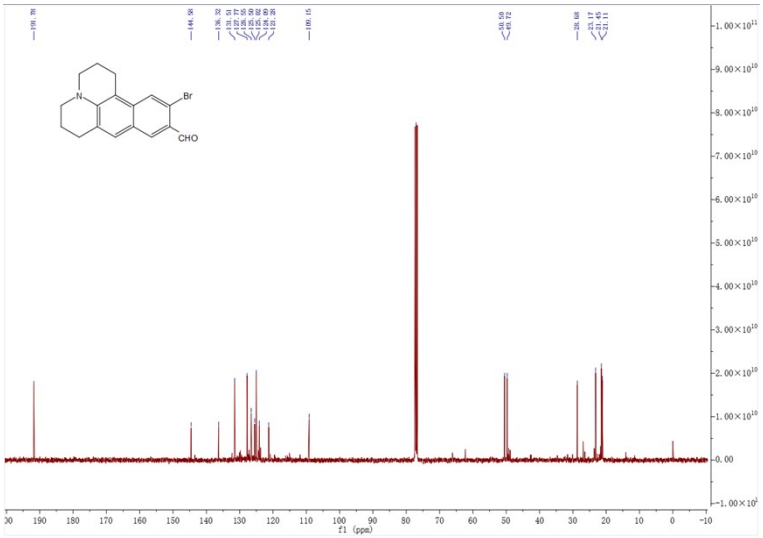

Fig. S10 ^{13}C NMR spectrum of 1-3.

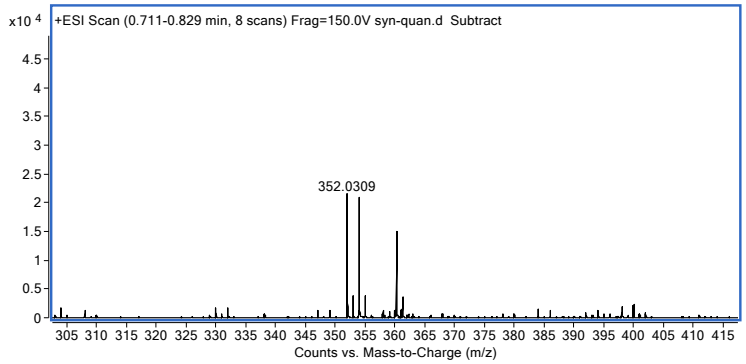

Fig. S11 The HRMS (ESI) of 1-3.

^{1}H NMR, ^{13}C NMR and HRMS for 1-4

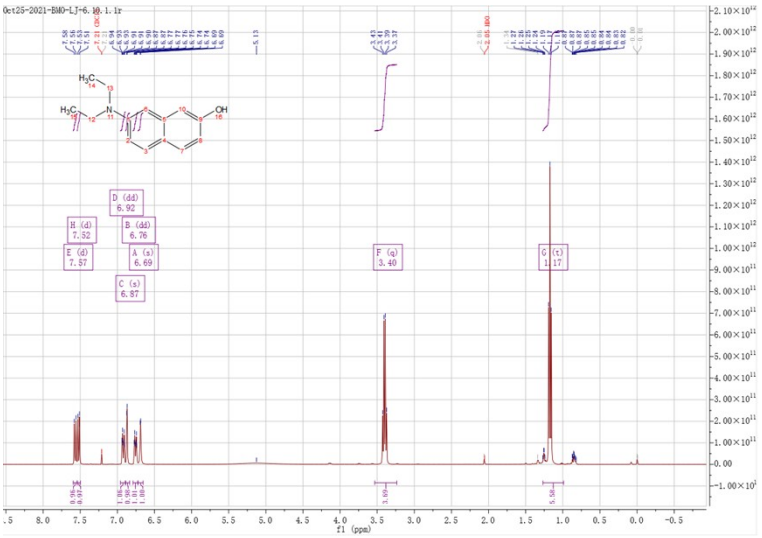

Fig. S12 ^{1}H NMR spectrum of 1-4.

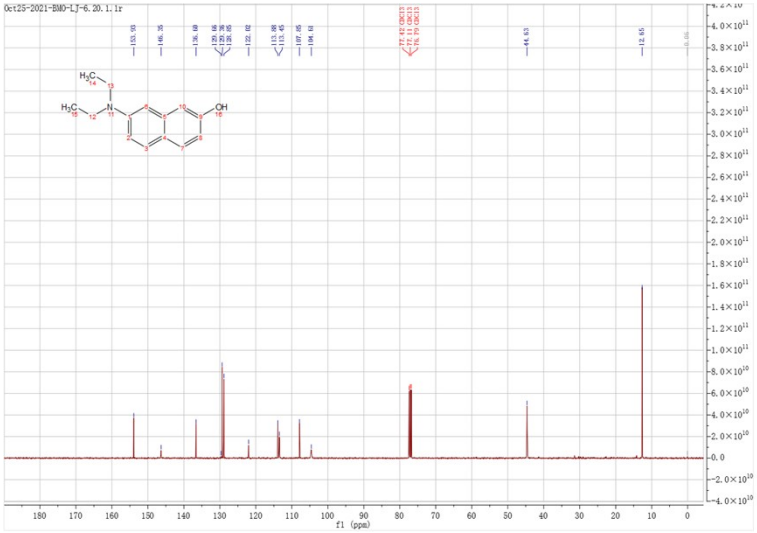

Fig. S13 ^{13}C NMR spectrum of 1-4.

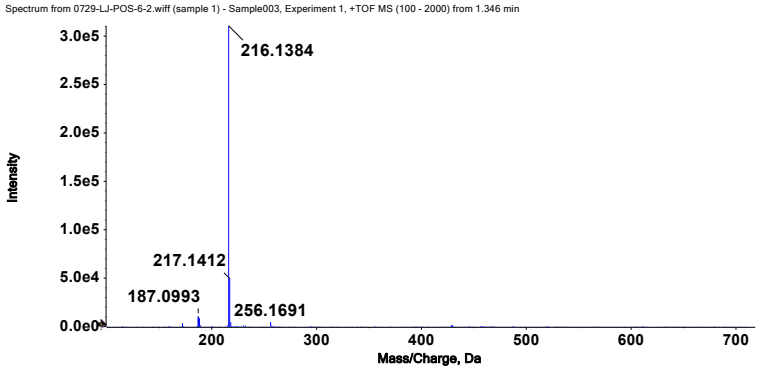

Fig. S14 The HRMS (ESI) of 1-4.

^{1}H NMR, ^{13}C NMR and HRMS for 1-5

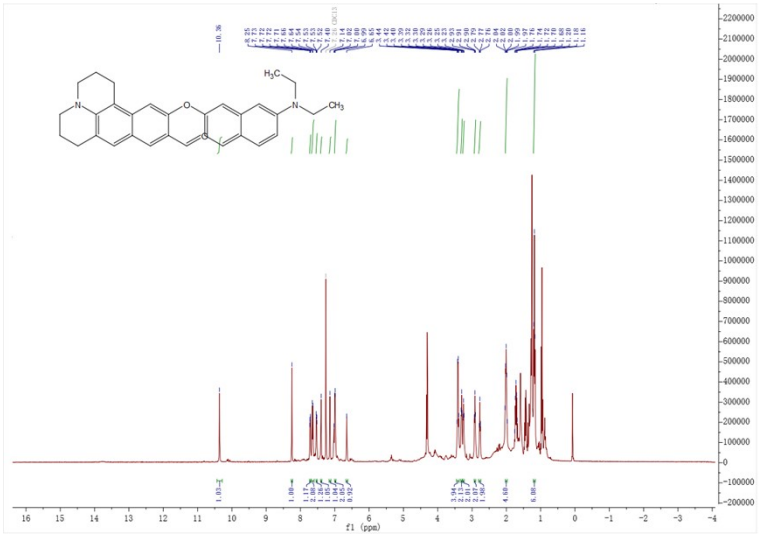

Fig. S15 ^{1}H NMR spectrum of 1-5.

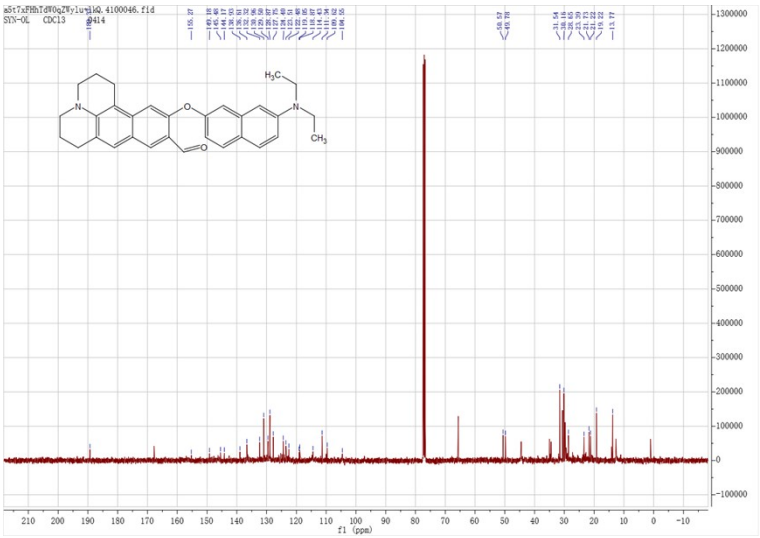

Fig. S16 ^{13}C NMR spectrum of 1-5.

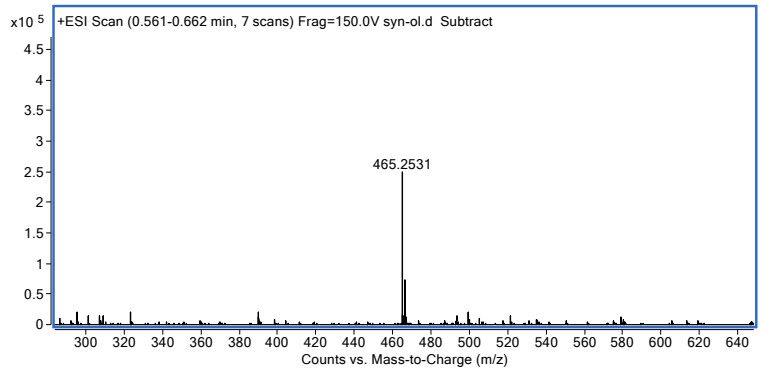

Fig. S17 The HRMS (ESI) of 1-5.

^{1}H NMR, ^{13}C NMR and HRMS for 1-6

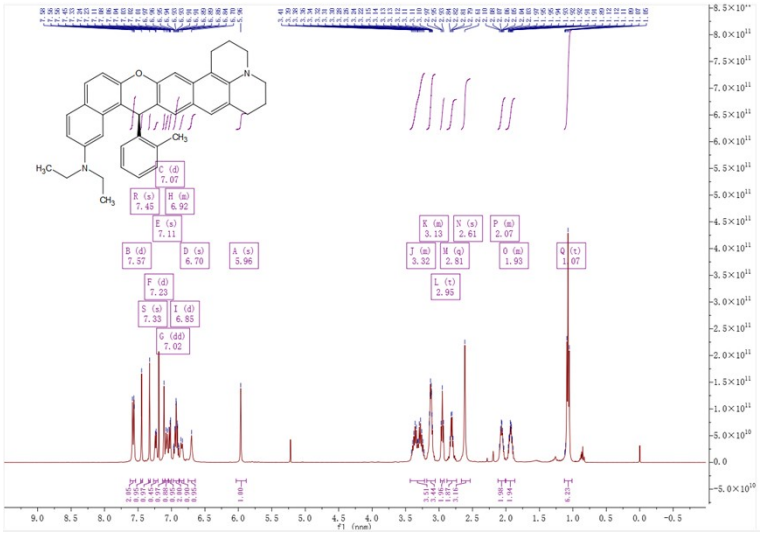

Fig. S18 ^{1}H NMR spectrum of 1-6.

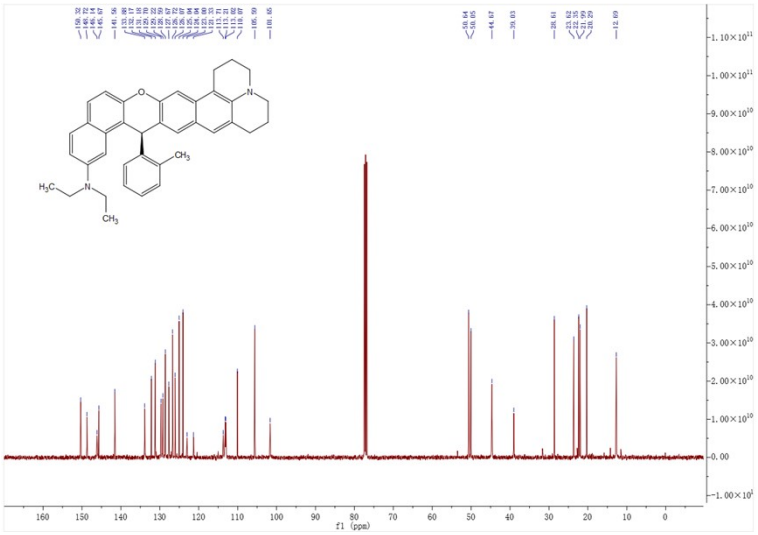

Fig. S19 ^{13}C NMR spectrum of 1-6.

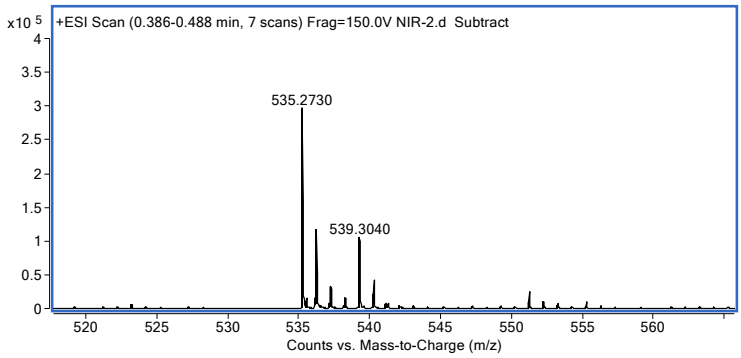

Fig. S20 The HRMS (ESI) of 1-6.

^{1}H NMR, ^{13}C NMR and HRMS for NIR-842

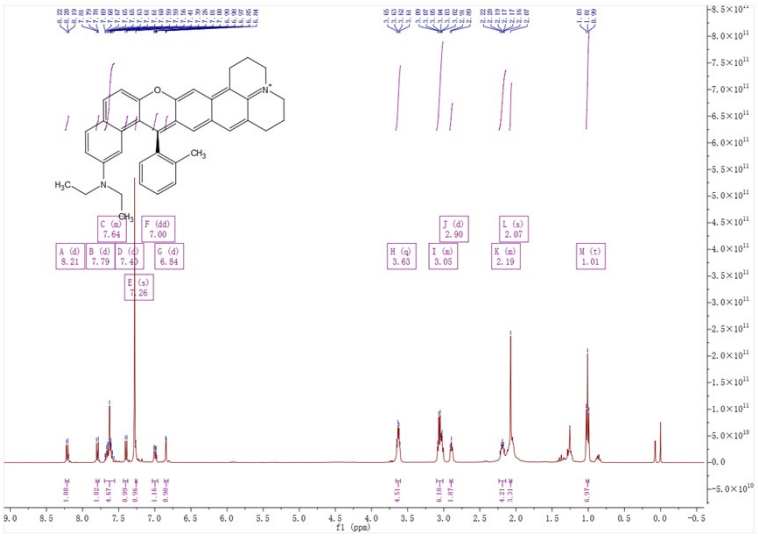

Fig. S21 ^{1}H NMR spectrum of NIR-842.

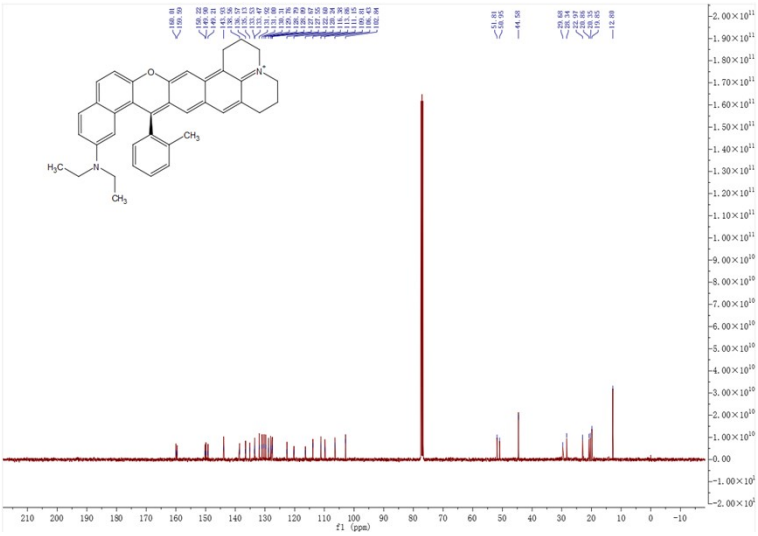

Fig. S22 ^{13}C NMR spectrum of NIR-842.

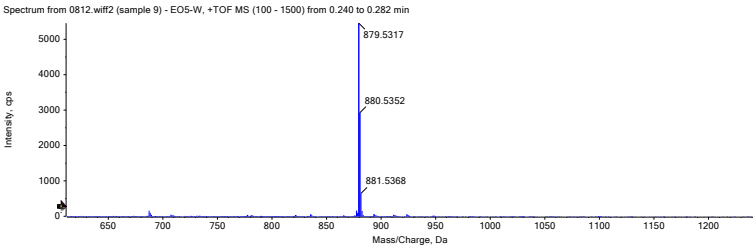

Fig. S23 The HRMS (ESI) of NIR-842.

^{1}H NMR, ^{13}C NMR and HRMS for NIR-864

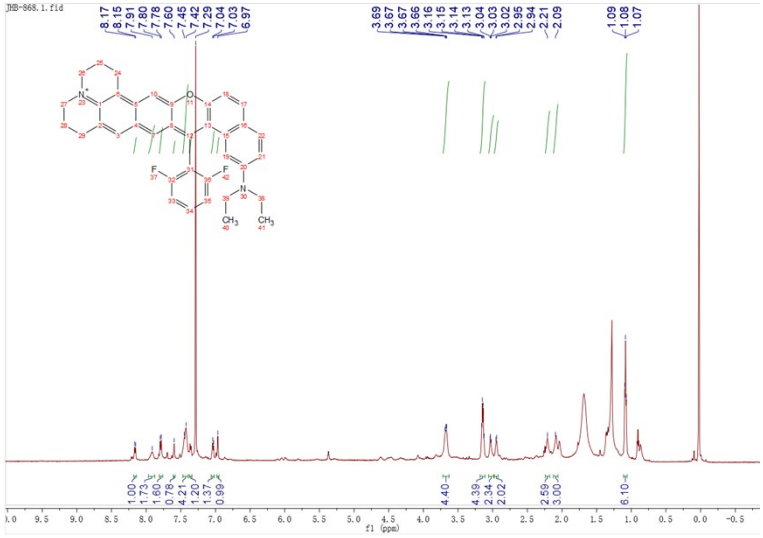

Fig. S24 ^{1}H NMR spectrum of NIR-864.

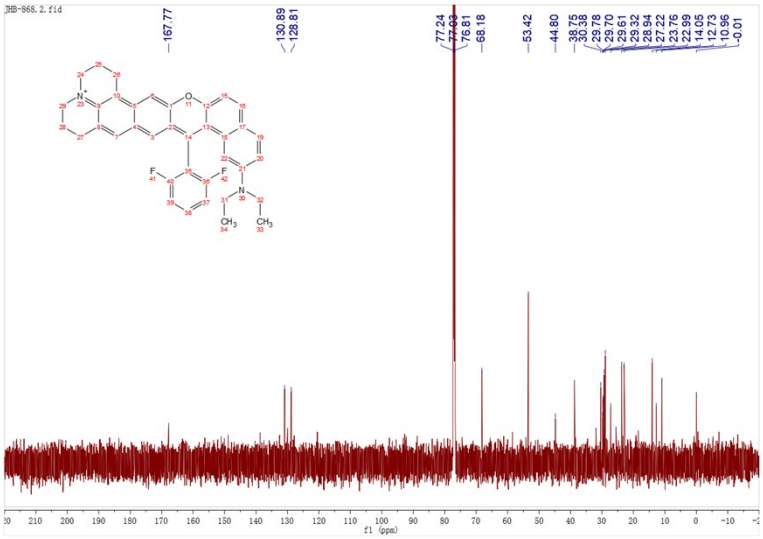

Fig. S25 ^{13}C NMR spectrum of NIR-864.(Owing to the poor solubility of compound NIR-864, the signals in its ^{13}C NMR spectrum were weak.)

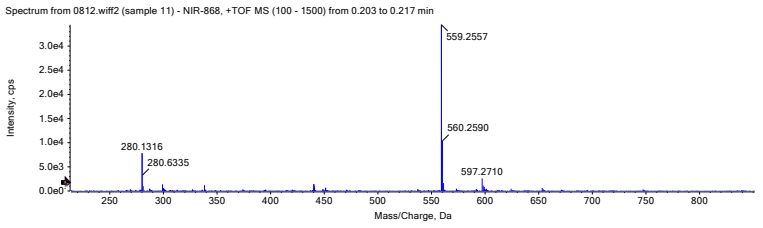

Fig. S26 The HRMS (ESI) of NIR-864.

^{1}H NMR, ^{13}C NMR and HRMS for 3-2

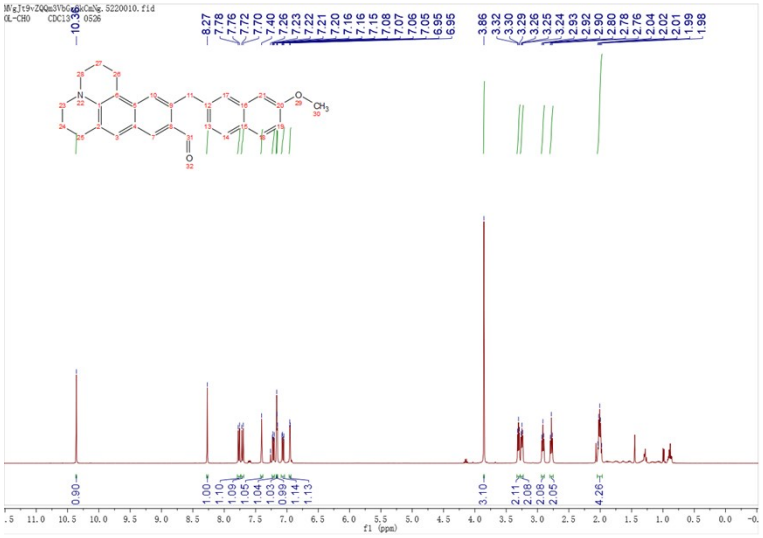

Fig. S27 ^{1}H NMR spectrum of 3-2.

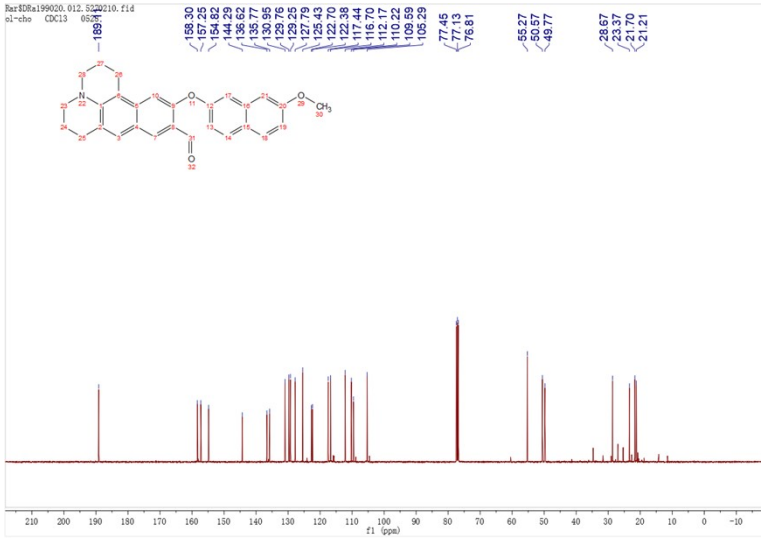

Fig. S28 ^{13}C NMR spectrum of 3-2.

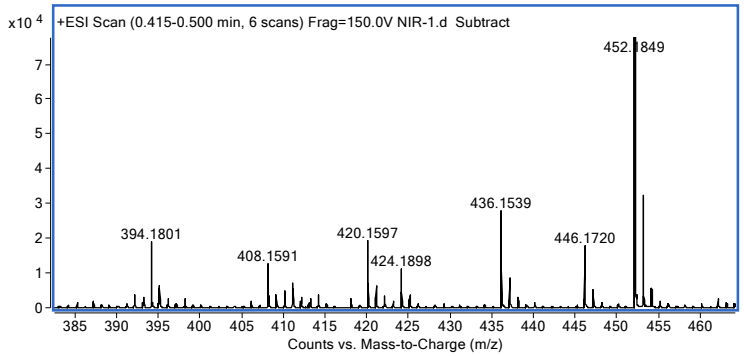

Fig. S29 The HRMS (ESI) of 3-2.

^{1}H NMR, ^{13}C NMR and HRMS for NIR-820

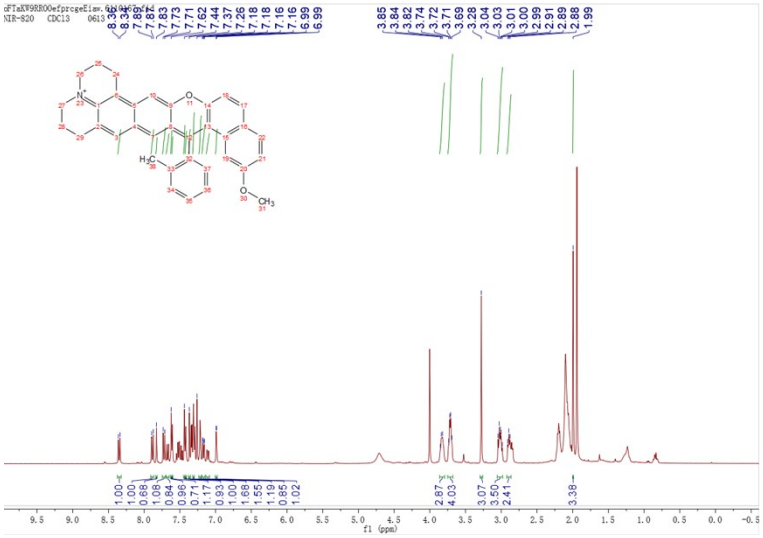

Fig. S30 ^{1}H NMR spectrum of NIR-820.

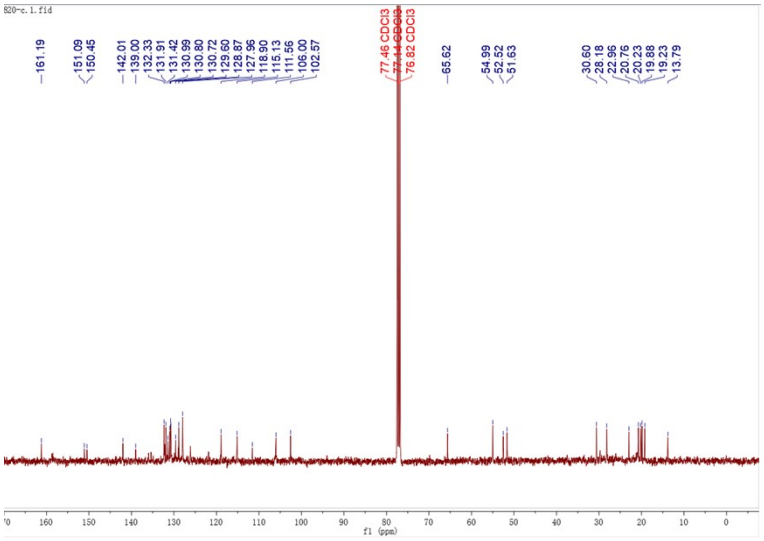

Fig. S31 ^{13}C NMR spectrum of NIR-820.

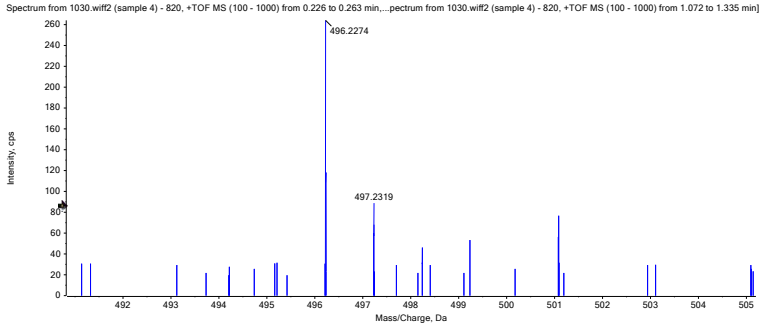

Fig. S32 The HRMS (ESI) of NIR-820.

References

1 Z. Lei, X. Li, X. Luo, H. He, J. Zheng, X. Qian, Y. Yang, *Angew. Chem. Int. Ed.* 2017, **56**, 2979-2983.

2 C. Würth, M. Grabolle, J. Pauli, M. Spieles, U. Resch-Genger, *Nat. Protoc.* 2013, **8**, 1535-1550.

3 L. Krause, R. Herbst-Irmer, G. M. Sheldrick, D. Stalke, *J. Appl. Crystallogr.* 2015, **48**, 3-10.

4 G. Sheldrick, *Acta Crystallogr., Sect. A* 2015, **71**, 3-8.

5 G. Sheldrick, *Acta Crystallogr., Sect. C* 2015, **71**, 3-8.