# Supporting Information

## Quaternary Ammonium Salt based NIR-II Probes for *in vivo* Imaging

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### 1. Materials and General Experimental Methods

All air and moisture sensitive reactions were carried out in flame-dried glassware under an inert atmosphere. Reactive liquid compounds were measured and transferred by gas-tight syringes and were added in the reaction flask through rubber septa. Tetrahydrofuran (THF) was freshly distilled from sodium/benzophenone. Dichloromethane, toluene, and DMF were distilled from $CaH_2$. $PEG_{2000}$-$NH_2$ was purchased from Thermo Scientific (Rockford, IL). All other standard synthesis reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) or Fisher Scientific, and used without further purification. Fetal bovine serum was purchased from GIBCO® (Lot:422234) and human albumin serum was purchased from Calbiochem® (Lot: 2765871). The cell line was obtained from the American Type Tissue Culture Collection (Manassas, VA). Female athymic nude mice (nu/nu) were purchased from Charles River Laboratories (Boston, MA).

Analytical thin layer chromatography was performed on glass-backed silica gel plates with F254 indicator. Compounds were visualized under UV lamp or by developing in iodine, phosphomolybdic acid solution or with a potassium permanganate solution followed by heating on a hot plate to approximately 350 °C. Flash chromatography was performed on 200-400 mesh silica gel with technical grade solvents which were distilled prior to use.$^1$H NMR spectra were recorded on a Bruker AV400 at 400 MHz as $CDCl_3$ solutions with tetramethylsilane (δ = 0 ppm) as the internal standard. $^{13}$C spectra were obtained on the same instruments at 100 MHz with $CDCl_3$ (δ = 77 ppm) as the internal reference. Chemical shifts are reported in parts per million (ppm). Multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), etc. Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) by Stanford Protein and Nucleic Acid Biotechnology Facility, Stanford University (AB SCIEX 5800 TOF/TOF MALDI) Mass spectrometer System, CHCA as matrix, reflector mode was chosen. High-resolution MS was carried out with a Thermo LTQ XL Orbitrap instrument. Analytical or preparative high-performance liquid chromatography (HPLC) was performed on a DIONEX ultimate 3000 instrument with PDA detection (column: PrincetonSPHER-300 C4, 5μm, 250 mm ×4.6 mm or 10.0 mm; mobile phase: water/acetonitrile with 0.1 % TFA)。

### 1.1.Characterization of optical probes

UV–vis-NIR absorption spectroscopy of the probes was recorded on an Agilent 8453 UV spectrophotometer. Fluorescence imaging was recorded on a NIR-II Uninano Imaging System and NIRvana 640 x 512 InGaAs camera (Princeton Instruments).

### 1.2. Photostability Assay

The absorption values of ICG and **Q8PNap** in 10%FBS DMEM solution (pH= 7.4) were tested with Agilent 8453 UV spectrophotometer and adjusted to 0.1 (OD value). The above solutions were placed in a 96-well plate, and fluorescence intensities were recorded at different time

points for a total period of 2 h under 808 nm laser (140 mM/cm$^2$), 200 ms exposure time and 1000 long-pass filter. Fluorescence intensities were represented as means (n= 5), and fitted to a non-lineear regression one-phase exponential decay.

### 1.3. Cytotoxicity Assay

Cytotoxicity of the compounds was evaluated by using MTT (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, St. Louis, MO) assay. Murine fibroblast NIH-3T3 cells were plated into a 96 well plate at a concentration of 4 x $10^3$ cells/well in Dulbecco's modified Eagle medium (DMEM) with 10% FBS at 37 ℃ and 5% $CO_2$, and 24 h later, the cells were incubated with **Q8PNap**/FBS with different concentrations (0.1 mM, 50 µM, 25 µM, 12.5 µM, 6.25 µm, 0 µM) for 48 h. Old medium was taken out and new medium was added in 48 h later. Then 20 µL of the 5mg/mL MTT stock solution was added and incubated for 4 hours. After removing medium, 100 µL of dimethylsulfoxide was added to dissolve the formazan crystals precipitates. After shaking the cell plate for 5 min, the absorbance (A) at a wavelength of 570 nm was measured with a Tecan microplate reader. The normalized cell viability was calculated by (A4T/Ablank), (Y values). The concentrations of Q8PNap/FBS were calculated in log scales (X values). Mean value and standard deviation were calculated using Graph Pad Prism version 6.0 (Graph Pad Software). Dose-response data table was chosen, with nonlinear regression the curve was obtained. Error bars represent standard deviation. All samples were done in triplicate and the experiment was replicated five times.

### 1.4. Quantum yield

In order to measure the quantum yield of compounds, a reference fluorophore emitting in the NIR-II was chosen. While there is some debate over the true quantum yield of IR-26, 0.05% is the accepted value. The quantum yield was calculated in the following manner:

$$QY = QY_{ref} \times \frac{n^2}{n_{ref}^2} \left( \frac{A_{ref}}{I_{ref}} \right) \frac{I_{sample}}{A_{sample}}$$

Instead of comparing the integrated fluorescence intensity at a single concentration to that of the reference, 5 difference concentrations at or below OD 0.1 (roughly OD 0.1, 0.08, 0.06, 0.04, and 0.02) were measured and the integrated fluorescence was plotted against absorbance for both IR-26 andcompounds. Comparison of the slopes led to the determination of the quantum yields.

### 1.5. Cells and Animal Models

U87 MG (human glioma) and NIH 3T3 (mouse embryonic fibroblast cell) were cultured in DMEM containing high glucose (Gibco), all of which were supplemented with 10% FBS and 1% penicillin–streptomycin. The cells were expanded in tissue culture dishes and kept in a humidified atmosphere of 5% $CO_2$ at 37 °C. The medium was changed every other day. A confluent monolayer was detached with 0.5% trypsin and dissociated into a single-cell suspension for inoculation.

Nude mice (Charles River Laboratories, USA) were maintained in pathogen free conditions until imaging. All animal experiments were performed under the approval of Stanford University's Administrative Panel on Laboratory Animal Care. For glioblastoma inoculation, 5 to 7-week-old nude mice were inoculated with ten million U87MG cells in 150 µL of serum-free medium on the left/right front shoulder. Tumors were allowed to grow for approximately 25 days before imaging.

### 1.6. *In Vivo* NIR-II Imaging

All NIR-II images including pre- and post- injection of the probe were collected on a 640 × 512 pixel two-dimensional InGaAs array (Princeton Instruments). The excitation laser was an 808 nm laser diode at a power density of 140 mW $cm^{-2}$. Emission was typically collected with a 1,000 nm LP (long-pass) filter. A lens set was used to obtain tunable magnifications ranging from 1× (whole body) to 2.5× (high magnification) by changing the relative position of two NIR achromats (75 mm and 200 mm, Thorlabs). A binning of one and a variable exposure time were used for Uninano Imaging System and InGaAs camera (640 × 512 pixel) to capture images in the NIR-II window.

### 1.7. *Ex Vivo* Biodistribution

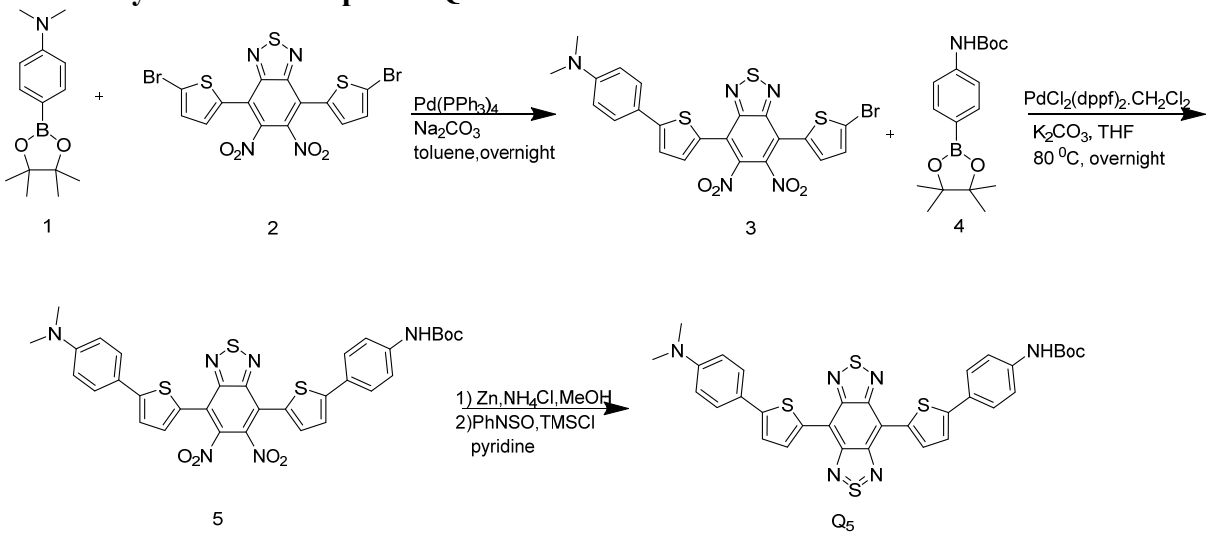
Ex vivo biodistribution studies were further performed at 48 h post-injection of **Q8PNap**/FBS (200 nmol) through tail vein to evaluate its distribution in vital organs including heart, liver, spleen, lung, kidney, brain, stomach, intestine. The organs were harvested and imaged under NIR-II imaging at 1,000 nm LP filter with 50 ms exposure time.

### 1.8. Statistical Analysis

The fluorescence measurement was performed to quantitate NIR fluorescence signal intensity through the Image J 1.45× software (National Institutes of Health, Bethesda, MD). The line graphs were analysis by graphpad prism 6.0. Guass fitted of lymph node using origin 2018. Data are given as mean ± standard deviation.

## 2. Experiment procedures of chemical synthesis:

### 2.1. The synthesis of compound Q5




**Scheme S1**. The synthetic route of compound **Q5**

***4-(5-(7-(5-bromothiophen-2-yl)-5,6-dinitrobenzo[c][1,2,5]thiadiazol-4-yl)thiophen-2-yl)-N,N-dimethylaniline 3***

To added *N*,*N*-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **1** (200 mg, 0.8 mmol) , 4,7-bis(5-bromothiophen-2-yl)-5,6-dinitrobenzo[*c*][1,2,5]thiadiazole **2** (487.97 mg, 0.89 mmol) in 30 mL fresh distilled toluene were added to sodium carbonate (169 mg, 1.6 mmol) solution in 10 mL distilled water under nitrogen atmosphere. Tetrakis(triphenylphosphine)palladium (92 mg, 0.08 mmol), and triphenylphosphine (42 mg, 0.016 mmol) were added to the reaction mixture. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (60 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (30 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$,

concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether: ethyl acetate, 2:1 ) to yield the product **3** as a purple solid (470 mg, 72% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, *J* = 15.8, 6.4 Hz, 4H), 7.19 (dd, *J* = 22.8, 4.0 Hz, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 3.02 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 132.7，130.8，127.2，121.9，112.2，40.2.

***Tert-butyl (4-(5-(7-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-5,6-dinitrobenzo[c][1,2,5]thiadiazol-4-yl)thiophen-2-yl)phenyl)carbamate 5***

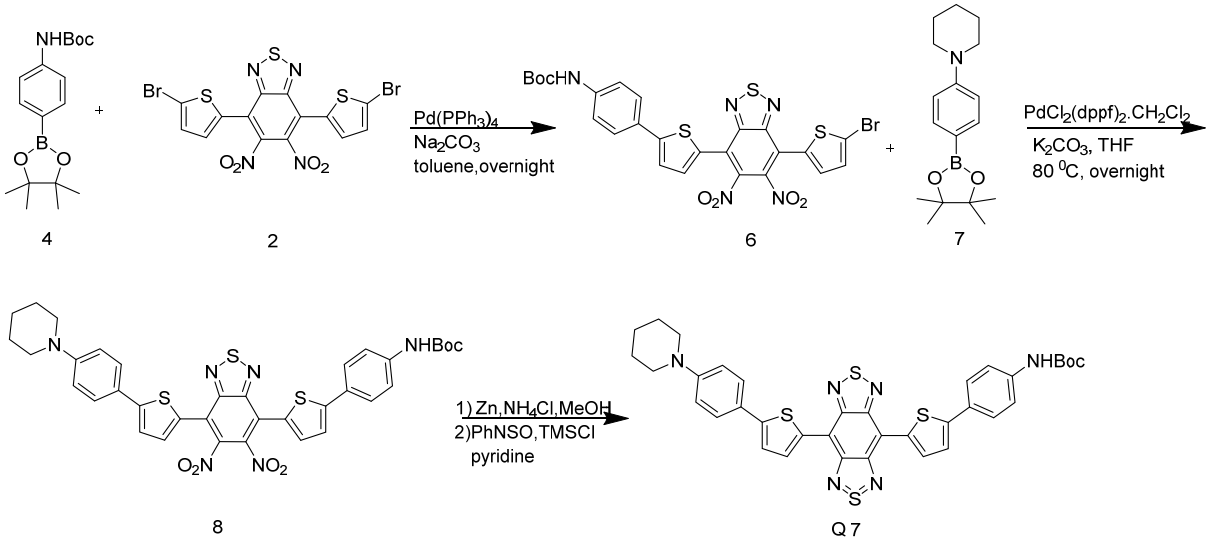
To added **3** (30mg, 0.027mmol) and *tert*-butyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)carbamate **4** (8.8mg, 0.027mmol)in 10 mL fresh distilled THF were added to potassium carbonate (7.5 mg, 0.054 mmol) solution in 3 mL distilled water under nitrogen atmosphere. Dichloro[1,1'-bis(diphenylphosphino)ferrocene]-palladium dichloromethane adduct (2.2 mg, 0.0027 mmol) were added to the reaction mixture. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (30 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (25 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (petroleum ether: dichloromethane, 2:1) to yield the product **5** as a purple solid (15.3 mg, 80% yield).$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, *J* = 17.0, 8.7 Hz, 4H), 7.50 (d, *J* = 4.0 Hz, 1H), 7.46 (d, *J* = 4.0 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 2H), 7.32 (d, *J* = 4.0 Hz, 1H), 7.25 (d, *J* = 4.0 Hz, 1H), 6.72 (d, *J* = 8.9 Hz, 2H), 6.60 (s, 1H), 3.02 (s, 6H), 1.54 (s, 9H). HRMS (ESI) calcd for $C_{33}H_{29}N_6O_6S_3$ $[M + H]^+$ : 701.12, found 701.1260.

**Compound Q5:**

Zinc dust (311 mg, 4.8 mmol) and $NH_4Cl$ (76 mg, 1.43 mmol) were added to a stirred solution of compound **5** (28 mg, 0.04 mmol) in dichloromethane (2 mL) and 90% methanol (3 mL) under nitrogen atmosphere. After being stirred at room temperature for 4 h, the solution was filtered through Celite pad, diluted with dichloromethane (20 mL), and washed with water (8 mL x 3), saturated aqueous $NaHCO_3$, and saturated aqueous brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to afford a yellow solid which was utilized for the next step without further purification.

To a yellow solution in anhydrous pyridine (2 mL) was added *N*-thionylaniline (0.17 mL, 1.43 mmol) and chlorotrimethylsilane (0.25 mL, 2.87 mmol). The mixture was heated in an oil bath at 80°C for overnight. The reaction mixture was allowed to cool down to room temperature, poured into iced water, extracted with dichloromethane. The combined organic layer was washed with water, saturated aqueous brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (dichloromethane: methanol, 5:1) to yield the product **Q5** as a dark solid (9 mg, two step 34% yield). MALDI-TOF/TOF Calcd for: $C_{33}H_{28}N_6O_2S_4$ [M]: 668.12, found: 668.25.

**2.2 The synthesis of compound Q6**




**Scheme S2**. The synthetic route of compound **Q6**

***Tert-butyl(4-(5-(7-(5-bromothiophen-2-yl)-5,6-dinitrobenzo[c][1,2,5]thiadiazol-4-yl) thiophen-2-yl) phenyl)carbamate 6***

To added *tert*-butyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)carbamate (4) (11.6mg, 0.036 mmol) , 4,7-bis(5-bromothiophen-2-yl)-5,6-dinitrobenzo[*c*][1,2,5]thiadiazole (2) (20 mg, 0.036 mmol)in 10 mL fresh distilled toluene were added to sodium carbonate (12.2 mg, 0.087mmol) solution in 2 mL distilled water under nitrogen atmosphere. Tetrakis(triphenylphosphine)palladium (4.2 mg, 0.0036 mmol), and triphenylphosphine (2 mg, 0.0072 mmol) were added to the reaction mixture. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (20 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (10 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether: dichloromethane, 1:1) to yield the product **6** as a purple solid (12 mg, 51% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, $J$ = 8.7 Hz, 2H), 7.51 (d, $J$ = 4.0 Hz, 1H), 7.44 (d, $J$ = 8.5 Hz, 2H), 7.34 (d, $J$ = 4.0 Hz, 1H), 7.25 (d, $J$ = 4.0 Hz, 1H), 7.19 (d, $J$ = 4.0 Hz, 1H), 6.59 (s, 1H), 1.54 (s, 9H). HRMS (ESI) calcd for $C_{25}H_{19}N_5O_6S_3$ $[M + H]^+$ : 659.96, found 659.9610.

***Tert-butyl(4-(5-(5,6-dinitro-7-(5-(4-(piperidin-1-yl)phenyl)thiophen-2-yl)benzo[c][1,2,5] thiadiazol-4-yl) thiophen-2-yl)phenyl)carbamate 8***

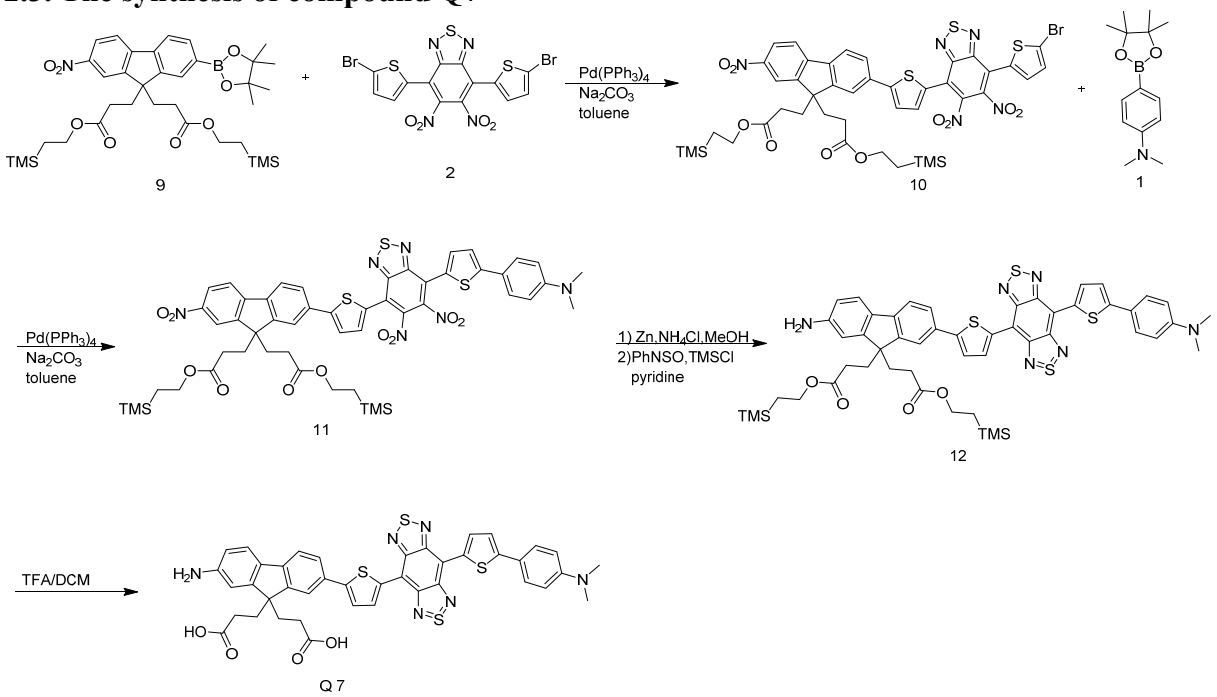
To added **6** (20 mg, 0.03 mmol) and 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine (**7)** (13 mg, 0.045 mmol) in 10 mL fresh distilled THF were added to potassium carbonate (6.2 mg, 0.045 mmol) solution in 3 mL distilled water under nitrogen atmosphere. Dichloro[1,1'-bis(diphenylphosphino)ferrocene]-palladium dichloromethane adduct (2.5 mg, 0.003 mmol) were added to the reaction mixture. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (23 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (10 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether : dichloromethane, 5:1 ) to yield the product 8 as a blue solid (12.6 mg, 58% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, $J$ = 17.7, 8.7 Hz, 4H), 7.52 – 7.41 (m, 4H), 7.33 (d, $J$ = 4.0 Hz, 1H), 7.28 (d, $J$ = 4.0 Hz, 1H), 6.94 (d, $J$ = 8.9 Hz, 2H), 6.61 (s, 1H), 3.32 – 3.21 (m, 4H), 1.71 (d, $J$ = 3.6 Hz, 4H), 1.63 (d, $J$ = 4.9 Hz, 2H), 1.54 (s, 9H). HRMS (ESI) calcd for $C_{36}H_{33}N_6O_6S_3$ $[M + H]^+$ :741.15, found 741.1536.

**Compound Q6:**

Zinc dust (388 mg, 6 mmol) and $NH_4Cl$ (85 mg, 1.75 mmol) were added to a stirred solution of compound **8** (37 mg, 0.05 mmol) in dichloromethane (2 mL) and 90% methanol (3 mL) under nitrogen atmosphere. After being stirred at room temperature for 4 h, the solution was filtered through Celite pad, diluted with dichloromethane (20 mL), and washed with water (8 mL x 3), saturated aqueous $NaHCO_3$, and saturated aqueous brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to afford a yellow solid which was utilized for the next step without further purification.

To a yellow solution in anhydrous pyridine (3.5 mL) was added *N*-thionylaniline (0.22 mL, 1.75 mmol) and chlorotrimethylsilane (0.31 mL, 3.6 mmol). The mixture was heated in an oil bath at 80°C for overnight. The reaction mixture was allowed to cool down to room temperature, poured into iced water, extracted with dichloromethane. The combined organic layer was washed with water, saturated aqueous brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (dichloromethane: methanol, 1:1) to yield the product **Q6** as a dark solid (16 mg, two step 45% yield). MALDI-TOF/TOF Calcd for: $C_{36}H_{32}N_6O_2S_4$ [M]: 708.15, found [M]: 708.23.

### 2.3. The synthesis of compound Q7




**Scheme S3**. The synthesis route of compound **Q7**

**Synthesis of Compound 10**

To a solution of bis(2-(trimethylsilyl)ethyl) 3,3'-(2-nitro-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9H-fluorene-9,9-diyl)dipropionate (**9**) (156 mg, 0.229 mmol) and 4,7-bis(5-bromothiophen-2-yl)-5,6-dinitrobenzo[c][1,2,5]thiadiazole (**2**) (137 mg, 0.25 mmol) in 15 mL fresh distilled toluene were added to sodium carbonate (47.7 mg, 0.45 mmol) solution in 3 mL distilled water under nitrogen atmosphere. Tetrakis(triphenylphosphine)palladium (26 mg, 0.02 mmol), and triphenylphosphine (10.5 mg, 0.016 mmol) were added to the reaction mixture. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (30 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (25 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate, 5:1 ) to yield the product

**10** as a blue dark solid (140 mg, 60% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.41 – 8.25 (m, 2H), 7.89 (d, *J* = 7.6 Hz, 2H), 7.80 (d, *J* = 7.3 Hz, 1H), 7.73 (s, 1H), 7.57 (s, 2H), 7.30 (d, *J* = 4.1 Hz, 1H), 7.22 (d, *J* = 3.4 Hz, 1H), 3.97 (dd, *J* = 9.7, 7.4 Hz, 4H), 2.57 (t, *J* = 7.4 Hz, 4H), 1.61 (d, *J* = 4.8 Hz, 4H), 0.88 – 0.77 (m, 4H), -0.03 (s, 18H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.7, 151.8, 150.3, 149.3, 147.5, 146.7, 141.7, 139.4, 134.5, 132.3, 131.3，130.8, 129.4, 126.6, 125.0, 124.3, 122.3, 121.0, 120.6, 119.7, 118.6, 62.8, 54.5, 34.4, 29.1, 17.2, -1.6.

**Synthesis of Compound 11**

Compound **10** (56 mg, 0.229 mmol) and *N,N*-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**1**) (212 mg, 0.208 mmol) were dissolved in 10 mL dry toluene under nitrogen atmosphere. Sodium carbonate (43 mg, 0.416 mmol) dissolved in 3mL distilled water, tetrakis(triphenylphosphine)palladium (24 mg, 0.0208 mmol), and triphenylphosphine (11 mg, 0.041 mmol) were added to the mixture solution. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (23 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (15 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate, 5:1 ) to yield the product **11** as a blue dark solid (132 mg, 60% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.32 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.25 (d, *J* = 2.0 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.77 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.70 (s, 1H), 7.59 – 7.47 (m, 5H), 7.27 (s, 1H), 6.72 (d, *J* = 8.9 Hz, 2H), 3.99 – 3.87 (m, 4H), 3.03 (s, 6H), 2.54 (t, *J* = 8.3 Hz, 4H), 1.58 (dd, *J* = 15.9, 8.4 Hz, 4H), 0.84 – 0.78 (m, 4H), -0.05 (s, 18H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.3, 154.6, 152.4, 151.9, 150.9, 149.1, 140.8, 136.3, 134.3, 133.4, 128.8, 126.4, 125.9, 123.7, 123.5，123.3, 122.4, 120.6, 120.2, 113.8, 64.4, 56.1, 41.9, 35.9, 30.7, 18.8, -0.0. MALDI-TOF/TOF Calcd for: $C_{51}H_{54}N_6O_{10}S_3Si_2$ [M]:1062.26, found:1062.23.

**Synthesis of Compound 12**

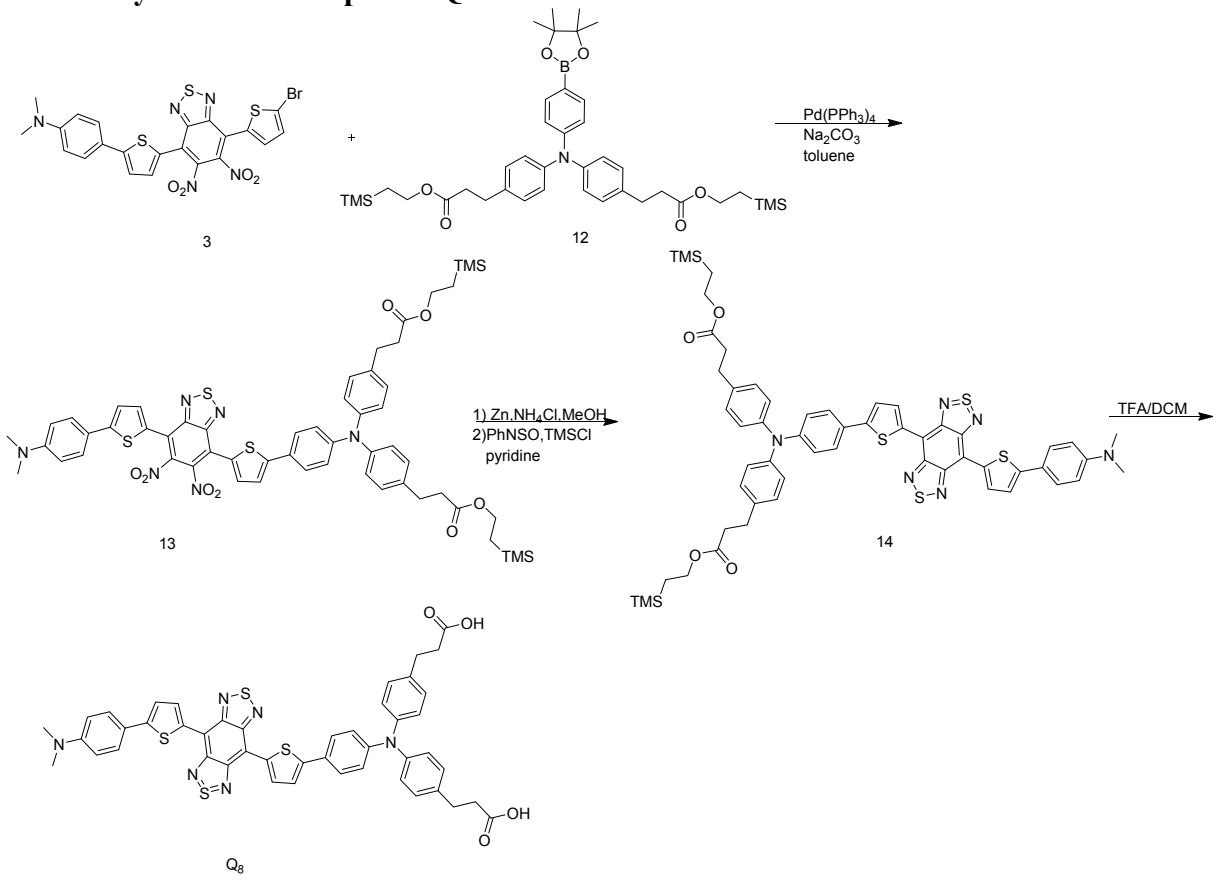
Zinc dust (366 mg, 5.64 mmol) and $NH_4Cl$ (89 mg, 1.69 mmol) were added to a stirred solution of compound 11 (50 mg, 0.047 mmol) in dichloromethane (3 mL) and 90% methanol (4 mL) under nitrogen atmosphere. After being stirred at room temperature for 4 h, the solution was filtered through Celite pad, diluted with dichloromethane (28 mL), and washed with water (10 mL x 3), saturated aqueous $NaHCO_3$, and saturated aqueous brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to afford a yellow solid which was utilized for the next step without further purification.

To a dark yellow solution in anhydrous pyridine (2 mL) was added *N*-thionylaniline (0.2 mL, 1.69 mmol) and chlorotrimethylsilane (0.3 mL, 3.38 mmol). The mixture was heated in an oil bath at 80°C for overnight. The reaction mixture was allowed to cool down to room temperature, poured into iced water, extracted with dichloromethane. The combined organic layer was washed with water, saturated aqueous brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (dichloromethane: methanol, 100:1) to yield the product 12 as a dark solid (24 mg, two step 50% yield). MALDI-TOF/TOF Calcd for: $C_{51}H_{56}N_6O_4S_4Si_2$ [M]: 1000.28, found: 1000.29.

**Synthesis of Compound Q7**

To a solution of compound 12 (20 mg, 0.019 mmol) in DCM (3 mL) was cooled at 0 °C. Trifluoroacetic acid (3 mL) was added and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was slowly warmed to ambient temperature. The solvent was removed in vacuo and the crude product was washed by dichloromethane to yield the desired compound Q7 (12 mg, 80%) as a dark solid which was used for the next step without further purification. MALDI-TOF/TOF Calcd for: $C_{41}H_{32}N_6O_4S_4$ [M]: 800.14, found: 800.15.

## 2.4. The synthesis of compound Q8




**Scheme S4**. The synthesis route of compound **Q8**

**Synthesis of Compound 13**

Bis(2-(trimethylsilyl)ethyl)3,3'-(((4-(5-(7-(5-(4-(dimethylamino)phenyl)thiophen-2-yl)-5,6 dinitrobenzo[c][1,2,5]thiadiazol-4-yl)thiophen-2-yl)phenyl)azanediyl)bis(4,1-phenylene)) dipropionate (**3**) (150 mg, 0.25 mmol) and bis(2-(trimethylsilyl)ethyl) 3,3'-(((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)azanediyl)bis(4,1-phenylene))dipropionate (**12**) (196 mg，0.275 mmol) were dissolved in 15 mL dry toluene under nitrogen atmosphere. Sodium carbonate (52 mg, 0.5 mmol) dissolved in 5 mL distilled water, tetrakis(triphenylphosphine)palladium (28mg, 0.025 mmol), and triphenylphosphine (13 mg, 0.05 mmol) were added to the mixture solution. The reaction mixture was stirred at 80 ℃ for overnight. After cooling to room temperature, the solvent was removed in vacuo. Water (20 mL) was added to the residue, and the resulting solution was washed with ethyl acetate (17 mL x 3), then washed with saturated aqueous brine. After dried over anhydrous $MgSO_4$, concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (petroleum ether: ethyl acetate, 5:1 ) to yield the product **13** as a blue dark solid (128 mg, 47% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.39 (m, 6H), 7.23 (d, $J$ = 4.0 Hz, 1H), 7.19 (d, $J$ = 4.0 Hz, 1H), 7.12 (d, $J$ = 7.5 Hz, 1H), 7.07 (d, $J$ = 8.3 Hz, 4H), 7.01 – 6.96 (m, 5H), 6.66 (d, $J$ = 8.7 Hz, 2H), 4.20 – 4.08 (m, 4H), 2.97 (s, 6H), 2.87 (t, $J$ = 7.8 Hz, 4H), 2.57 (t, $J$ = 7.8 Hz, 4H), 0.94 (dd, $J$ = 10.7, 6.2 Hz, 4H), -0.00 (s, 18H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.5，154.0，152.4，150.0，146.8，137.4，130.7，128.6，126.4，123.7，113.6，64.1，41.7，37.5，31.8，18.7，0.0. MALDI-TOF/TOF Calcd for: $C_{56}H_{60}N_6O_8S_3Si_2$ [M]:1096.32, found:1096.35

**Synthesis of Compound 14**

Zinc dust (351 mg, 5.4 mmol) and $NH_4Cl$ (140 mg, 1.62 mmol) were added to a stirred solution of compound **13** (50 mg, 0.045 mmol) in dichloromethane (7 mL) and 90% methanol (11 mL) under nitrogen atmosphere. After being stirred at room temperature for 4 h, the solution was filtered through Celite pad, diluted with dichloromethane (20 mL), and washed with water (10 mL x 3), saturated aqueous $NaHCO_3$, and saturated aqueous brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under vacuum to afford a yellow solid which was utilized for the next step without further purification. To a dark yellow solution in anhydrous pyridine (2 mL) was added *N*-thionylaniline (0.18 mL, 1.62 mmol) and chlorotrimethylsilane (0.28 mL, 3.24 mmol). The mixture was heated in an oil bath at 80°C for overnight. The reaction mixture was allowed to cool down to room temperature, poured into iced water, extracted with dichloromethane. The combined organic layer was washed with water, saturated aqueous brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (dichloromethane : methanol, 100:1) to yield the product **14** as a dark solid (21 mg, two step 45% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.94 (d, *J* = 3.1 Hz, 2H), 7.66 (dd, *J* = 20.9, 8.7 Hz, 4H), 7.40 (dd, *J* = 17.2, 4.1 Hz, 2H), 7.19 – 7.03 (m, 10H), 6.75 (d, *J* = 8.8 Hz, 2H), 4.24 – 4.13 (m, 4H), 3.03 (s, 6H), 2.93 (t, *J* = 7.8 Hz, 4H), 2.62 (t, *J* = 7.8 Hz, 4H), 1.02 – 0.96 (m, 4H), 0.05 (s, 18H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.5, 146.9, 137.0, 130.6, 128.0, 126.2, 124.2, 113.6, 69.4, 64.1, 41.7, 37.5, 31.8, 18.7, -0.0. MALDI-TOF/TOF Calcd for: $C_{56}H_{60}N_6O_4S_4Si_2$ [M]:1064.31, found: 1064.30

**Synthesis of Compound Q8**

To a solution of compound **15** (20 mg, 0.018 mmol) in DCM (1 mL) was cooled at 0 °C. Trifluoroacetic acid (1 mL) was added and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was slowly warmed to ambient temperature. The solvent was removed in vacuo and the crude product was washed by dichloromethane to yield the desired compound **Q8** (10mg, 67%) as a dark solid which was used for the next step without further purification. MALDI-TOF/TOF Calcd for: $C_{46}H_{36}N_6O_4S_4$ [M]: 864.17, found: 864.4.

**2.5. The synthesis of compound Q8P**

To a solution of **Q8** (2 mg, 0.00231mmol) in 200 μL DMSO was added $NH_2$-$PEG_{2000}$ (4.6mg, 0.00231 mmol), DIPEA 20 μL, EDC (0.44mg, 0.00231 mmol) and 4-DMAP (0.05mg, 0.231 μmol). The reaction was stirred 4h at room temperature then purified by HPLC (Thermo scientific hypersil GOLD C4 column). The final product **Q8P** was confirmed using MALDI-TOF-MS. Expected M.W. 2860, measured M.W. 2740.8.

**2.6. The synthesis of compound Q8PNap**

To a solution of Q8P (2 mg, 0.71 μmol) in 1mL $CH_3CN$ was added 2-(bromomethyl) naphthalene (1.56 mg, 7.1 μmol). The reaction was stirred 2h at 50 °C then purify by HPLC (Thermo scientific hypersil GOLD C4 column). The final product **Q8PNa**p was confirmed using MALDI-TOF-MS. Expected M.W. 2881, measured M.W. 2873.0.

**3. Results**

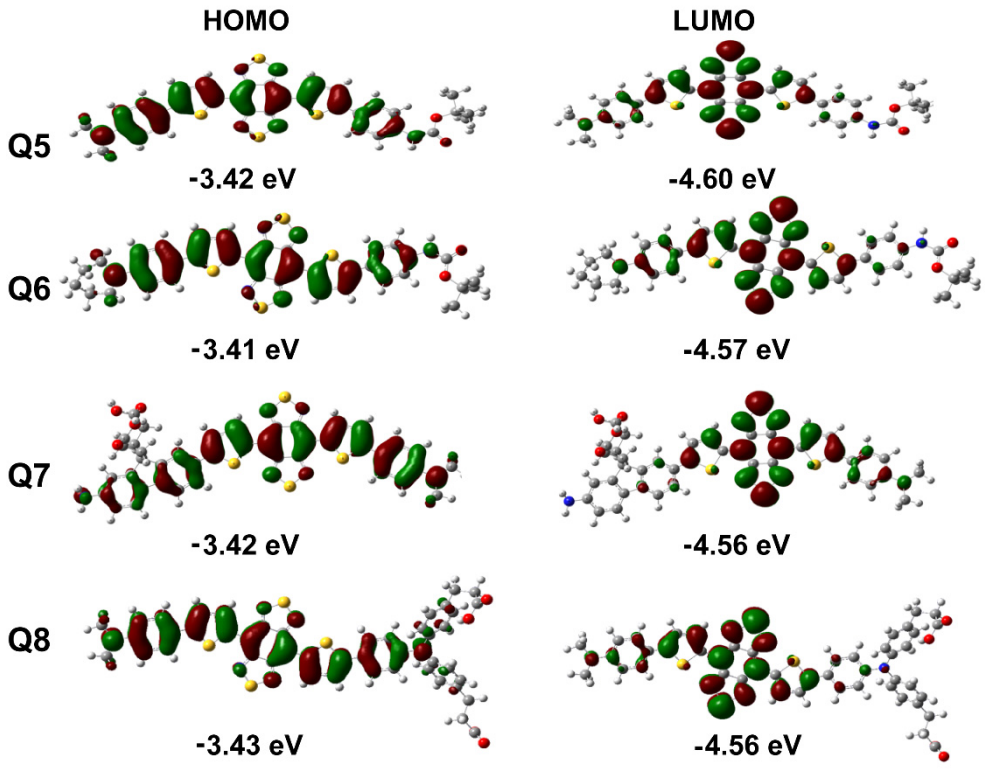
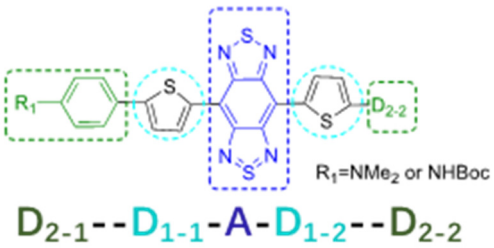



**Figure S1.** Calculated HOMOs and LUMOs of **Q5-Q8** at the optimally B3LYP/6-31G(d) scrf method with Gaussian 09 software.

**Table S1**. LUMO composition analysis results of the molecular fluorophores **Q5, Q6, Q7** and **Q8** based on Hirshfeld method by Multiwfn.




| Probes | $D_{2-1}$ | $D_{1-1}$ | Acceptor | $D_{1-2}$ | $D_{2-2}$ |
|---|---|---|---|---|---|
| **Q5** | 6.147% | 11.652% | 70.828% | 8.489% | 2.88% |
| **Q6** | 2.818% | 8.323% | 70.441% | 11.824% | 6.592% |
| **Q7** | 6.056% | 11.575% | 70.455% | 8.508% | 3.718% |
| **Q8** | 6.238% | 11.636% | 70.243% | 8.452% | 3.429% |

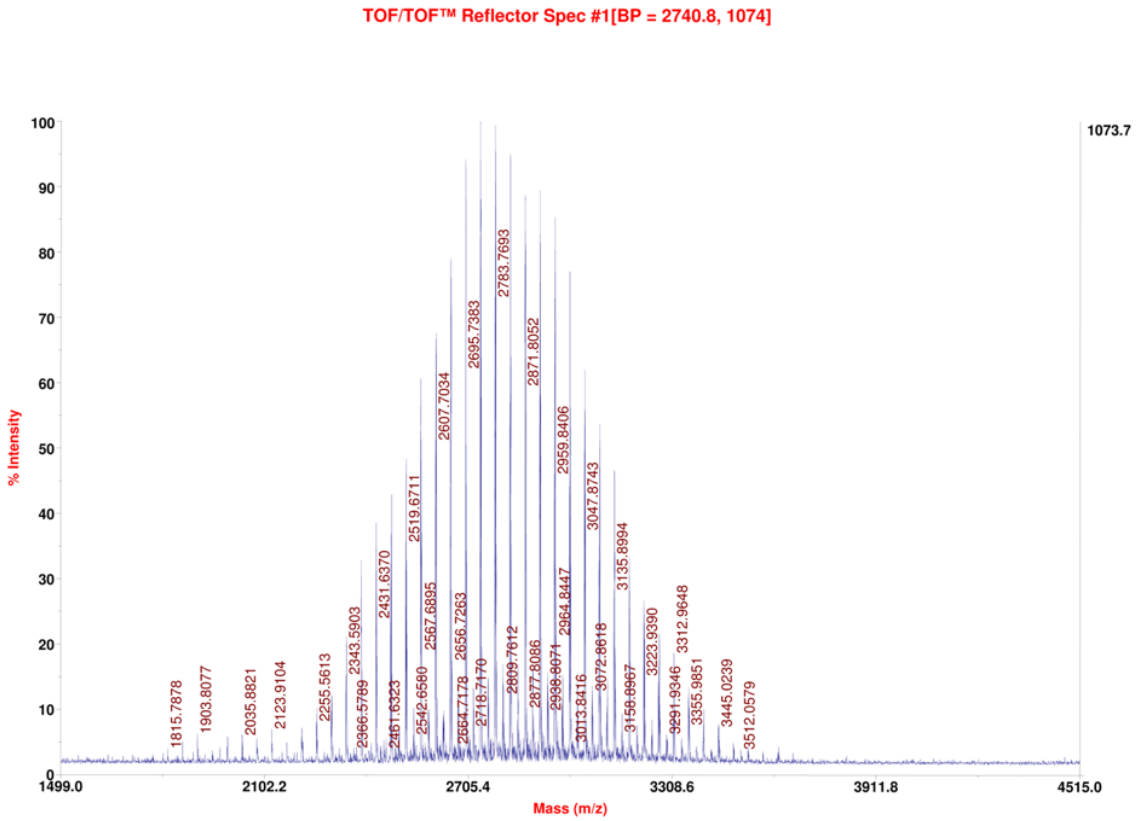



**Figure S2.** MALDI-TOF-MS of **Q8P**. AB SCIEX 5800 TOF/TOF MALDI Mass spectrometer System, CHCA as matrix, reflector mode was chosen.

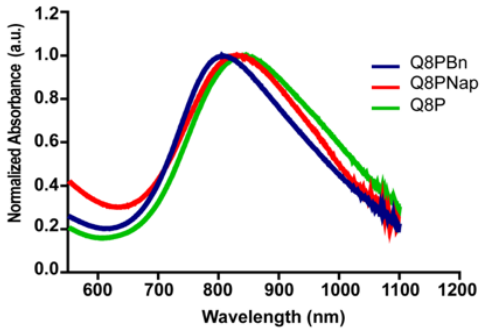



**Figure S3.** The absorption characteristic of **Q8P, Q8PNap** and **Q8PBn** in water.

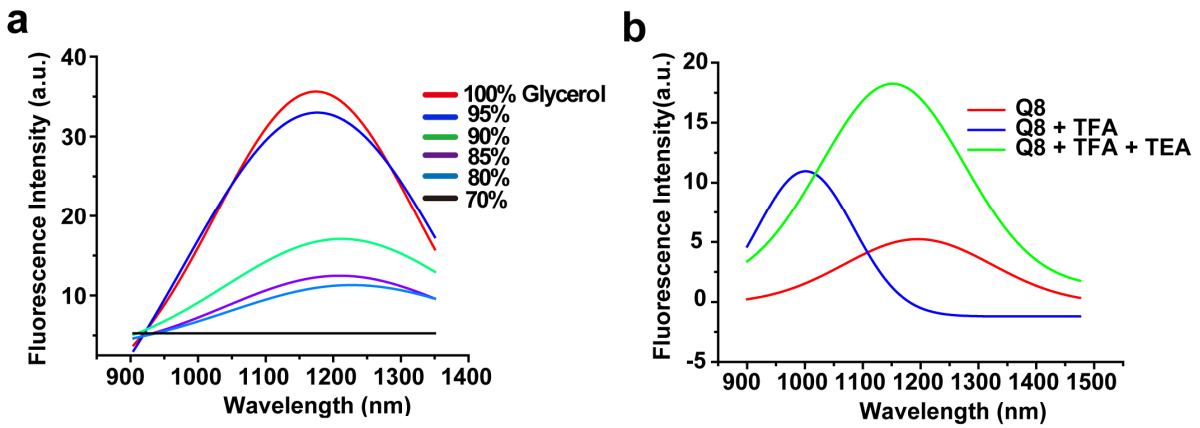



**Figure S4.** (a) The Fluorescence intensity of **Q8P** in different glycerol / water ratio. (b) The emission peaks of **Q8** when TFA/TEA were added.

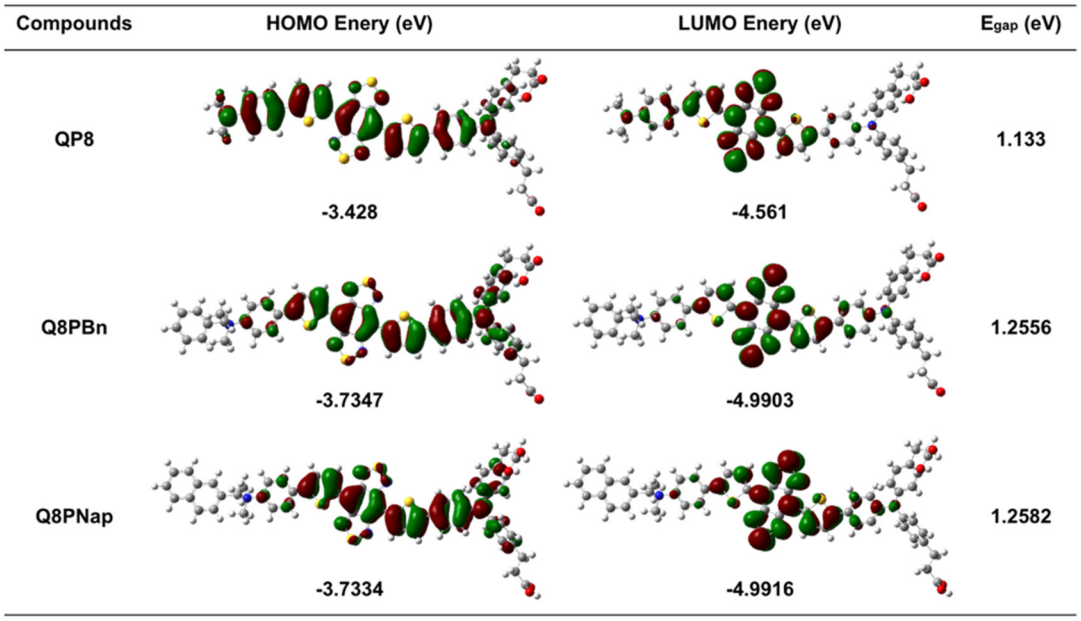



**Figure S5.** Comparison of HOMO and LUMO orbital surfaces of **Q8P, Q8PBn** and **Q8PNap** using DFT B3LYP/6-31G(d) scrf = (cpcm, solvent=dichloromethane) method. $E_{gap} = E_{LUMO} - E_{HOMO}$.

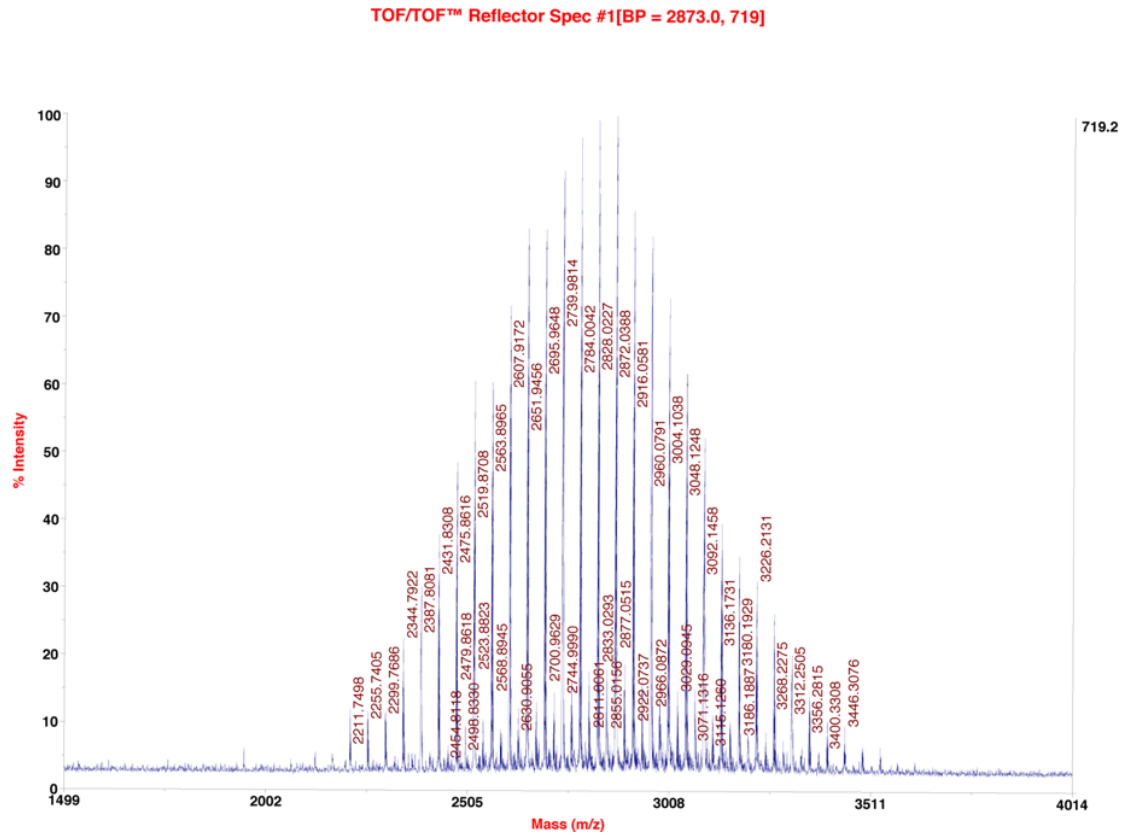



**Figure S6.** MALDI-TOF-MS of **Q8PNap**. AB SCIEX 5800 TOF/TOF MALDI Mass spectrometer System, CHCA as matrix, reflector mode was chosen.

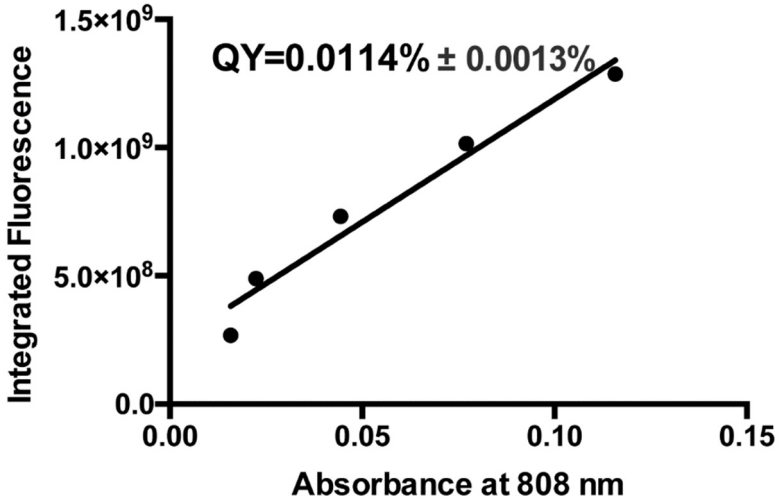



**Figure S7.** Quantum yield of **Q8PNap**/FBS. Linear fit was used to calculate quantum yield by comparing the slopes to reference IR26 in DCE solution (QY=0.05%).

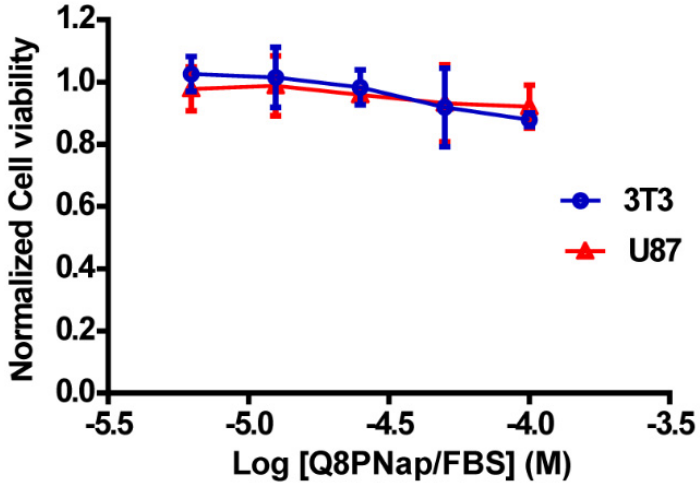



**Figure S8.** MTT assay of **Q8PNap**/FBS ( incubating time :48 h) which indicated that there was no observed toxicity to U87MG and NIH3T3 cells with different concentrations of compounds tested.

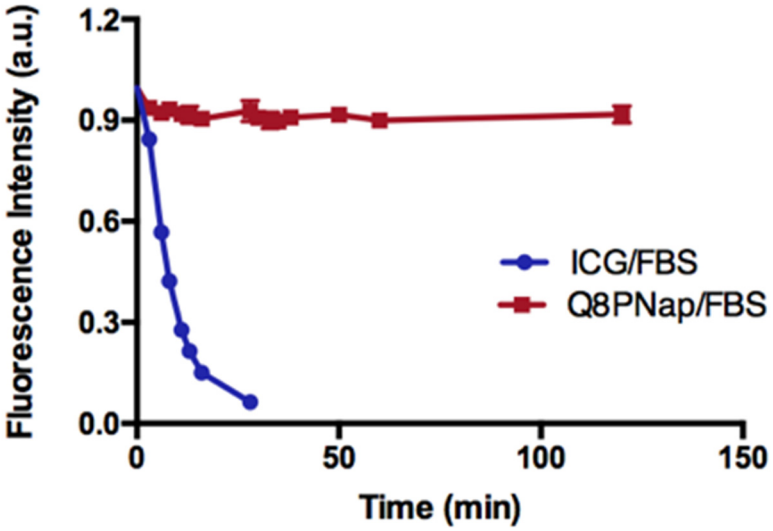



**Figure S9**. The photostability characteristics of ICG and Q8PNap/FBS under 808 nm laser.

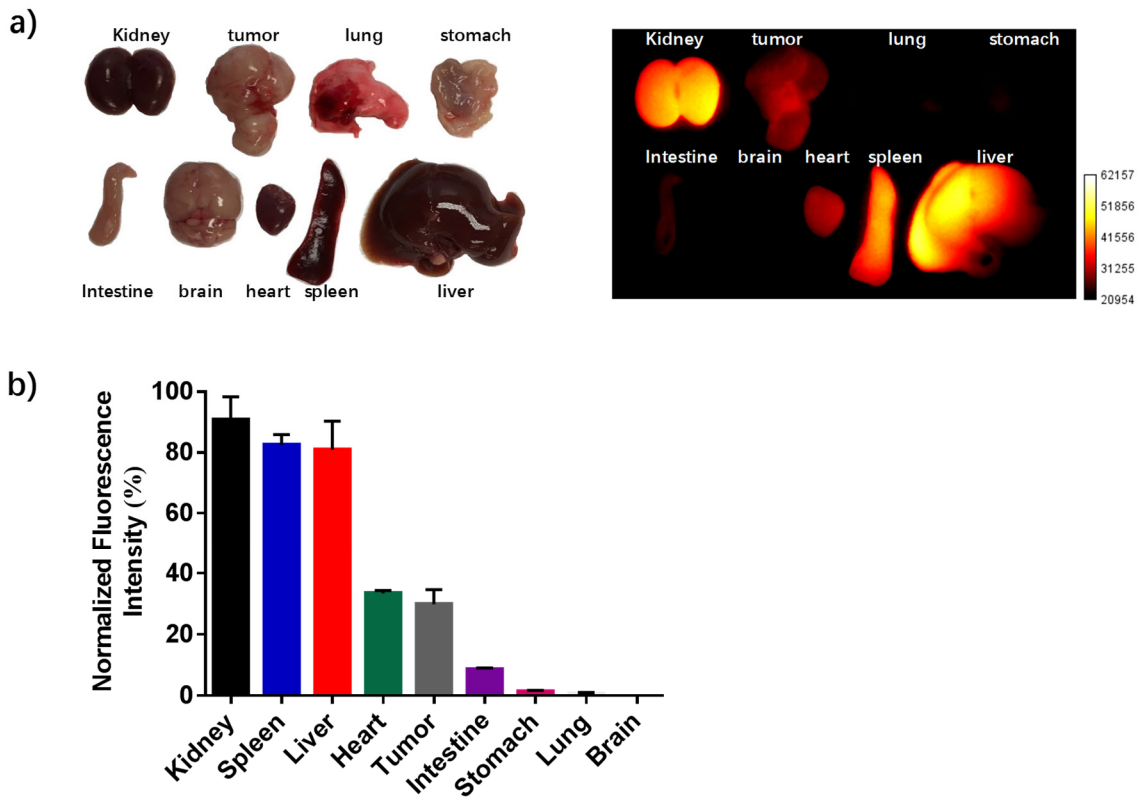



**Figure S10.** (a) *Ex vivo* photograph and NIR-II imaging of the vital organs at 48 h after **Q8PNap**/FBS injection under 808 nm excitation (1000 nm LP filter and 50 ms exposure time. (b) The fluorescence intensity percentage of the vital organs at 48 h after **Q8PNap**/FBS injection under 808 nm excitation (1000 nm LP filter and 50 ms exposure time).

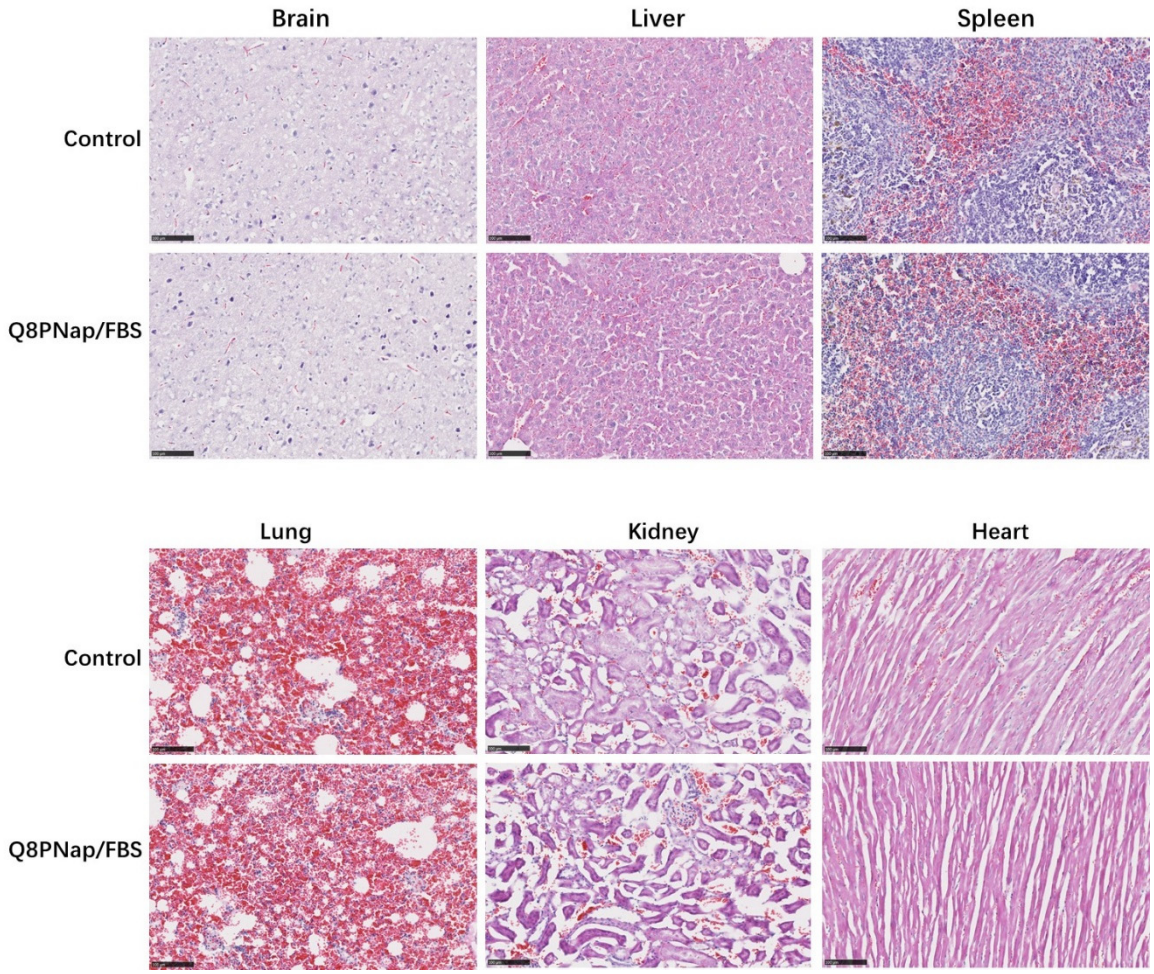



**Figure S11.** The representative H&E stained images of major organs including brain, liver, spleen, lung, kidney and heart collected from the untreated mice and **Q8PNap**/FBS injected mice at 48 h post-injection. No obvious organ damage or lesion was observed for **Q8PNap**/FBS treated mice. scale bar:100 μm.