Activatable Water Soluble Probes Enhance Tumor Imaging by Responding to Dysregulated pH and Exhibiting High Tumor-to-liver Fluorescence Emission Contrast

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Supporting Information

Contents

1. General information

Unless stated otherwise, all reactions were carried out under an atmosphere of argon using standard Schlenk techniques. All organic solvents were purchased from Fisher Scientific or Sigma Aldrich and purified with a solvent purification system (Innovative Technology). Sulfo-Cyanine5 carboxylic acid was purchased from Lumiprobe Corporation. Indocyanine green was purchased from Fisher Scientific. Dulbecco's Modified Eagle Medium (DMEM), RPMI-1640 Medium, and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. LysoTracker Green and DAPI were purchased from Life Technologies. Sephadex LH-20 was purchased from GE Healthcare. ^{1}H NMR spectra were recorded on a Varian Mercury 500 MHz or Agilent Mercury 400 MHz spectrometer in chloroform-d or methanol-d. All signals were reported in ppm with chloroform signal at 7.26 ppm or methanol signals at 3.31, 4.87 ppm as a standard. Data for ^{1}H NMR were recorded as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartiplet, m = multiplet or unresolved, coupling constant(s) in Hz, integration). ^{13}C NMR spectra were recorded on an Agilent Mercury 400 MHz spectrometer in chloroform-d. All signals are reported in ppm with the internal chloroform signal at 77.0 ppm as a standard. Column chromatography was performed either manually or on a Teledyne-Isco CombiFlash. The fluorescence emission spectra were obtained on a Hitachi fluorometer (F-7500 model). The UV-Vis spectroscopy study was performed on a Shimadzu UV-Vis spectrophotometer (UV-1800 model). Confocal microscopy imaging was performed using a Zeiss LSM 700 Confocal and images were analyzed using ImageJ (NIH). The whole body and *ex vivo* organ fluorescence imaging was performed on an IVIS Lumina System (Caliper Life Sciences). Luminescence measurements were performed on a Tecan InfiniTe F/M200 Pro microplate reader.

2. Supplemental Tables and Figures

2.1 Modified reaction conditions of Knoevenagel condensation.[a]

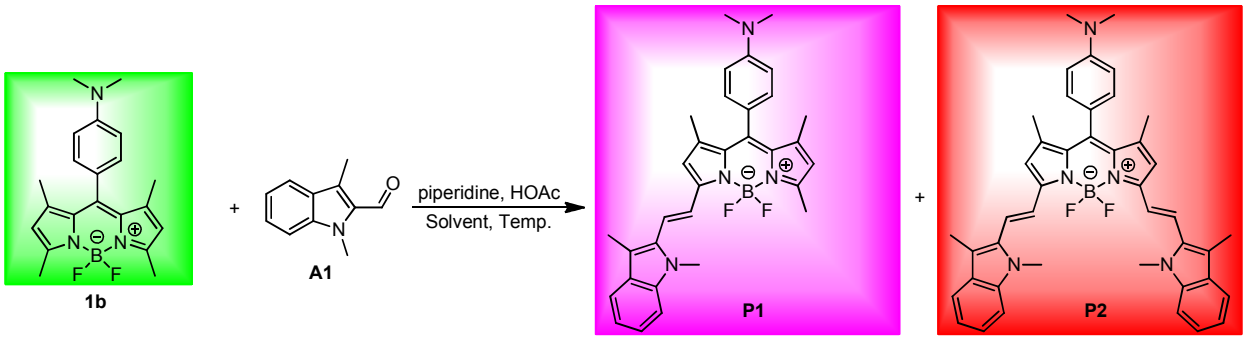


Table S1. Reaction optimization of Knoevenagel condensation reaction.

Entry	Solvent	Temp. (°C)	Time (h)	Product	Conversion
1	Toluene	120	24	P1, P2	—[c]
2[b]	Toluene	120	24	P1, P2	—[c]
3	EtOH	85	24	P1, P2	—[c]
4	CH_3CN	85	1.5	P1	> 80%
5	CH_3CN	85	8	P2	> 90%

[a]Reaction conditions: **1b** (0.03 mmol), **A1** (0.18 mmol), piperidine (60 μL), and HOAc (36 μL) in solvent (1 mL) with 4 Å molecular sieves. [b]Piperidine/TsOH as catalyst. [c]Not determined.

The condensation of a tetramethyl-BODIPY scaffold with an aromatic aldehyde was usually refluxed in dry toluene in the presence of piperidine and glacial acetic acid (classic Knoevenagel conditions).[1-4] Any water formed during the reaction was removed azeotropically by heating in a Dean-Stark apparatus. However, these reactions usually require high temperature and long reaction time. Moreover, the conversions for such reactions were low.[1-4] After screening, we found acetonitrile was the optimized solvent for such condensation reactions. **P1** was mainly formed after 1.5 h in refluxing CH_3CN (entry 4). However, **P2** could be mainly obtained after an extended reaction time (8 h, entry 5).

2.2 Photo-physical properties of NIR BODIPYs.

Table S2. Spectroscopic properties of **2b**, **3b**, and **5a-e**.

Probe	Solvent	$\lambda_{abs,\ max}$ (nm)[a]	$\lambda_{em,\ max}$ (nm)[b]	Φ_{FL}[c]	pK_a
2b	DMSO	705	746	—[d]	
3b	DMSO	660	730	—[d]	
5a	H_2O	645	660	0.09	
5b-1	H_2O	640	651	0.18[e]	2.9
5b-2	H_2O	648	668	0.23[e]	3.1
5c	H_2O	648	670	0.22[e]	4.5
5d	H_2O	648	667	0.23[f]	1.75
5e	H_2O	648	668	0.25[e]	4.0

[a]The UV-Vis spectroscopy study was performed on a Shimadzu UV-Vis spectrophotometer (UV-1800 model). [b]The fluorescence emission spectra were obtained on a Hitachi fluorometer (F-7500 model). [c]The reported quantum yield was calculated according to the following equation: $\Phi_{sample} = \Phi_{standard}\ (A_{standard} / A_{sample})\ (F_{sample} / F_{standard})\ (n_{sample} / n_{standard})^2$, where "Φ" is the quantum yield, "A" is the absorbance at the excitation frequency, "F" is the integrated area under the emission curve, and "n" is the refractive index of the solvent used. Sulfo-Cyanine5 carboxylic acid (sulfo-Cy5) (Φ_{fl} = 0.2) in water was used as the fluorescence standard. [d]Not determined. [e]In 0.01 M aqueous HCl. [f]In 0.1 M aqueous HCl.

2.3 Supplemental Figures.

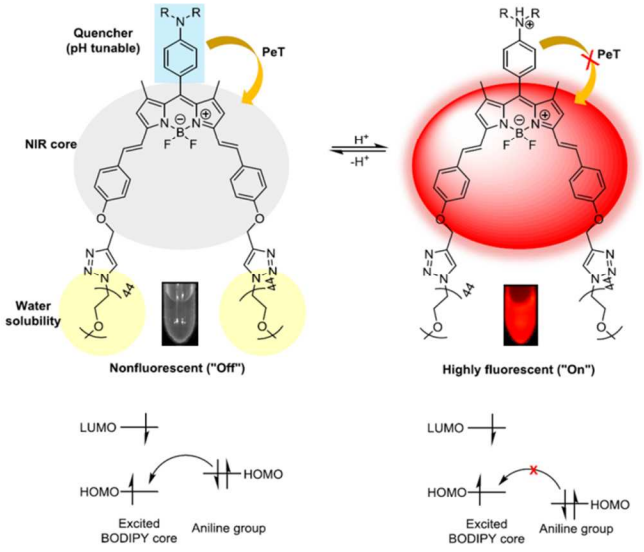


Figure S1. Water-soluble PEGylated NIR BODIPYs activate in response to a decrease in pH by photo-induced electron transfer (PeT) mechanism. Electron flow is involved in a PeT process from the aniline group to the excited BODIPY core as shown in the frontier molecular orbital diagram.

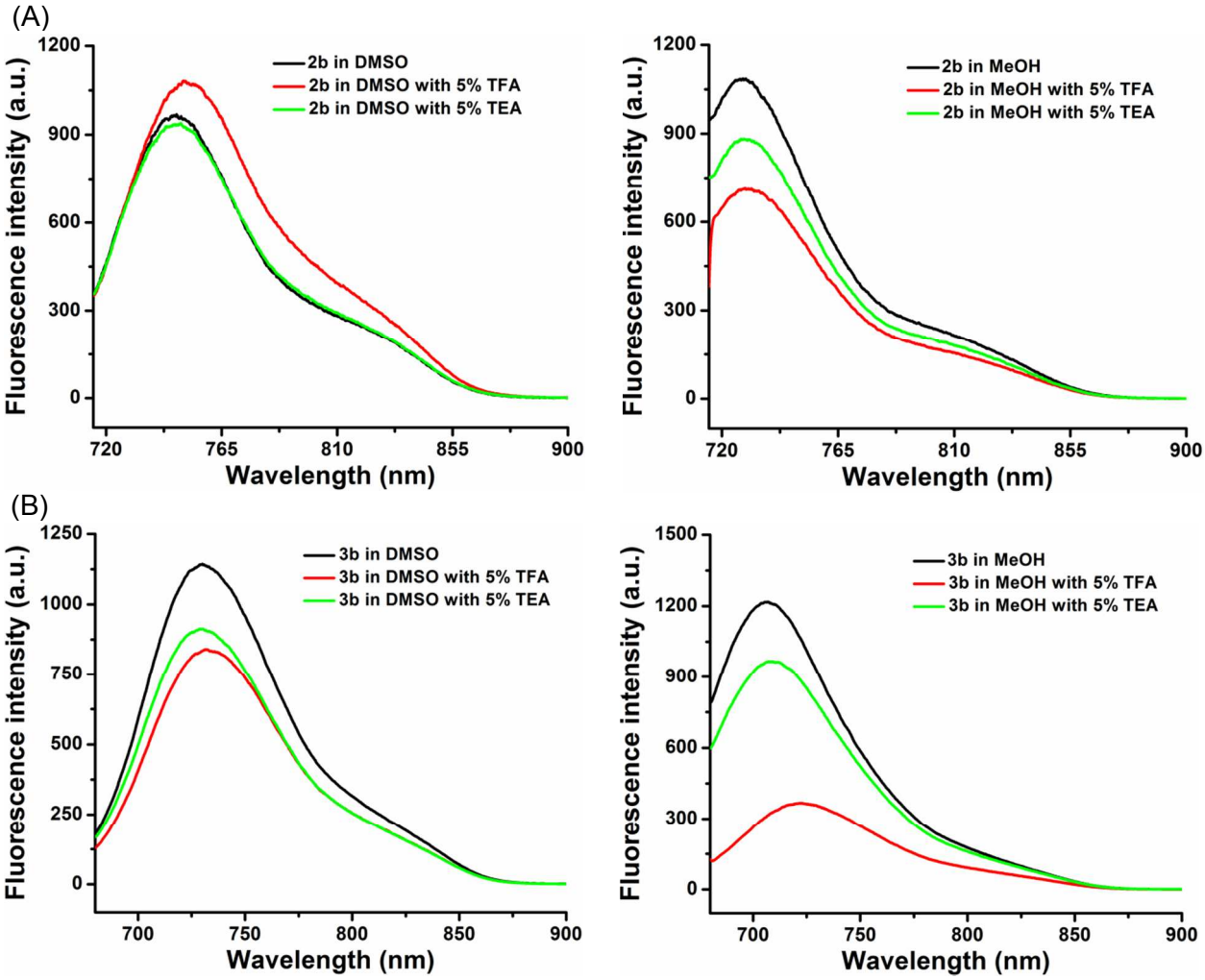


Figure S2. (A) Fluorescence emission spectra of **2b** in DMSO (10 μM) and MeOH (10 μM). Each sample was excited at 705 nm, and emission spectra were collected from 715 to 900 nm. (B) Fluorescence emission spectra of **3b** in DMSO (10 μM) and MeOH (10 μM). Each sample was excited at 660 nm, and emission spectra were collected from 680 to 900 nm.

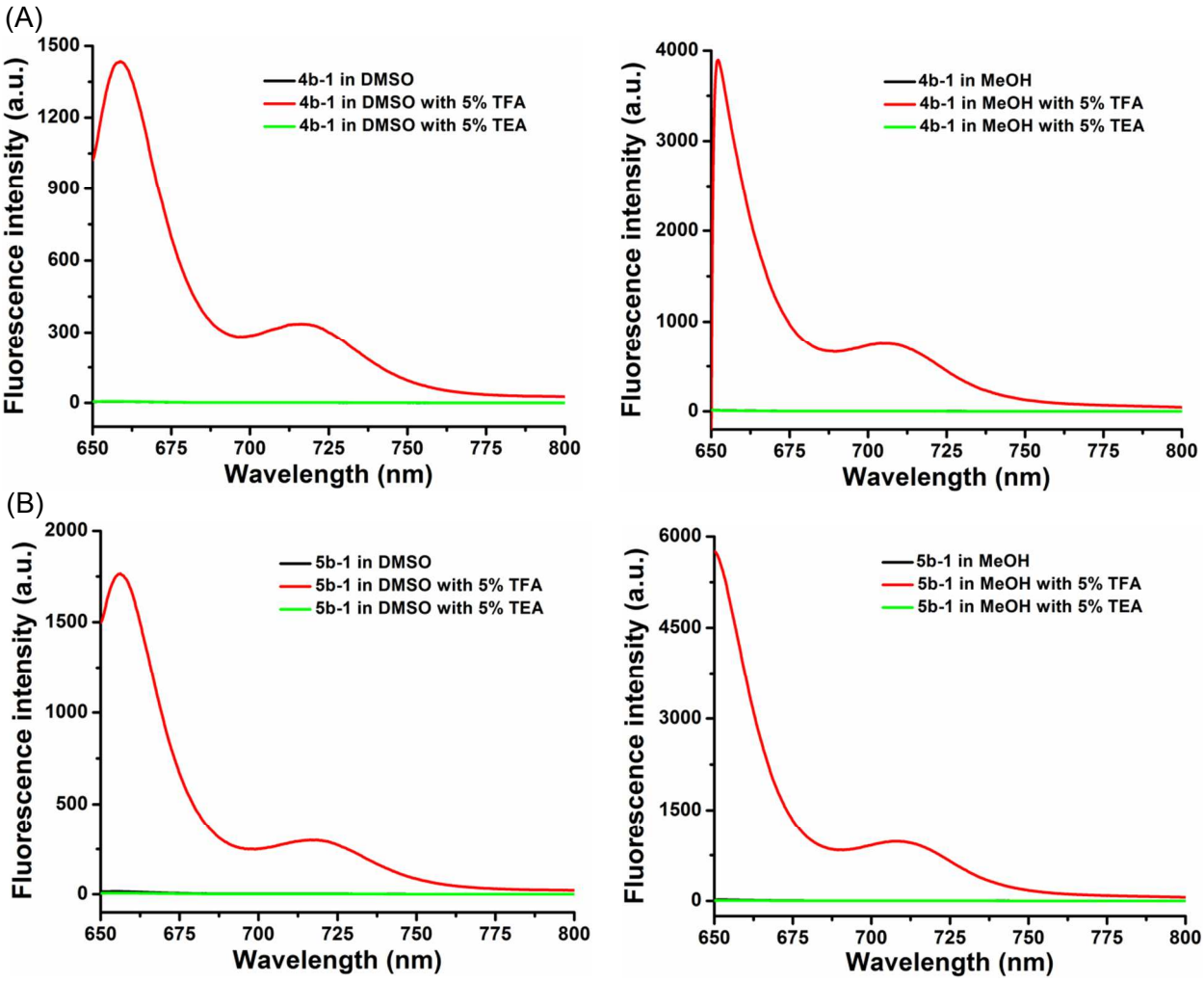


Figure S3. (A) Fluorescence emission spectra of **4b-1** in DMSO (10 μM) and MeOH (10 μM). **4b-1** can only turn on in acidic DMSO or MeOH. Each sample was excited at 640 nm, and emission spectra were collected from 650 to 800 nm. (B) Fluorescence emission spectra of **5b-1** in DMSO (10 μM) and MeOH (10 μM). **5b-1** can only turn on in acidic DMSO or MeOH. Each sample was excited at 640 nm, and emission spectra were collected from 650 to 800 nm.

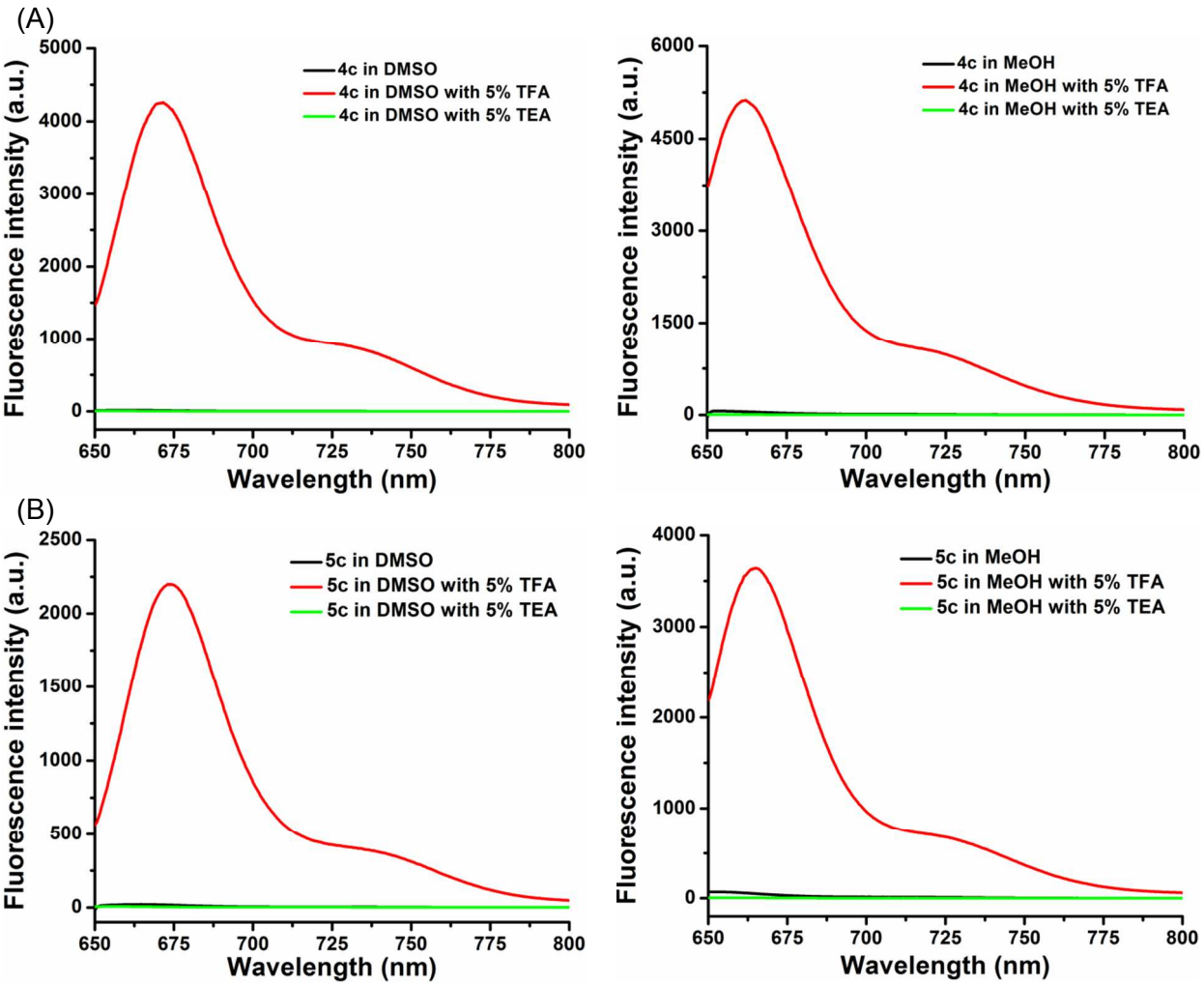


Figure S4. To help understand the origin of the increased T/L ratio, the tumor and liver were homogenized and **5c** was extracted into methanol to completely isolate **5c**. Probe **5c** is off in neutral methanol, but will immediately turn on in acidic methanol. Thus, we were able to calibrate and quantify the total uptake of **5c** in the tissues after adding a small amount of trifluoroacetic acid (TFA) to the methanol extracts. (**A**) Fluorescence emission spectra of **4c** in DMSO (10 μM) and MeOH (10 μM). **4c** can only turn on in acidic DMSO or MeOH. Each sample was excited at 640 nm, and emission spectra were collected from 650 to 800 nm. (**B**) Fluorescence emission spectra of **5c** in DMSO (10 μM) and MeOH (10 μM). **5c** can only turn on in acidic DMSO or MeOH. Each sample was excited at 640 nm, and emission spectra were collected from 650 to 800 nm.

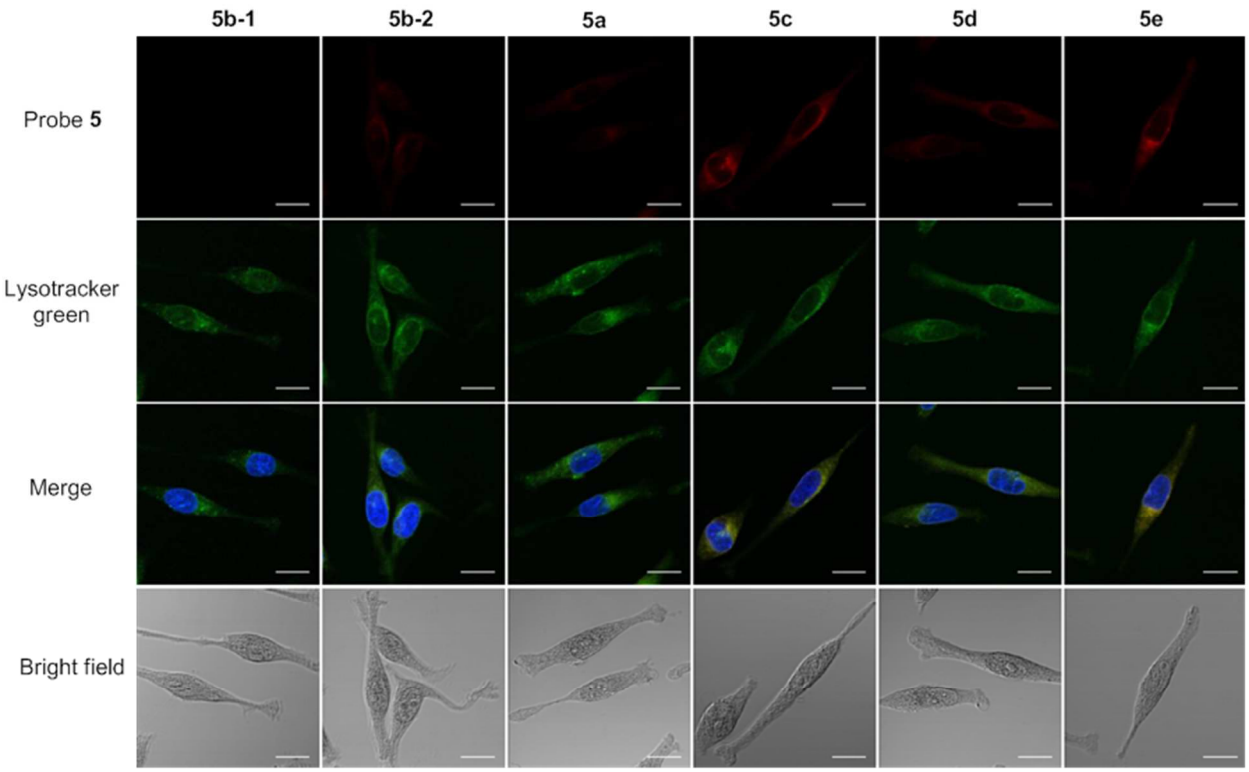


Figure S5. Confocal fluorescence microscope images (20X) of the **5** series of probes in Hela cells. Cells (10,000 cells/well) were incubated with **5** (5.0 μM) for 30 min at 37 °C, and the media was replaced with fresh media containing lysotracker green (0.5 μM) and incubated for 20 min. Then the cells were fixed with 4% paraformaldehyde. Cell nucleus was stained with DAPI (0.3 μM). The NIR probe is red; lysosomes are green; and the cell nuclei are blue. Co-localization is seen as yellow. **5a** did not exhibit bright fluorescence due to low quantum yield and uptake in short time. Scale bar = 20 μm.

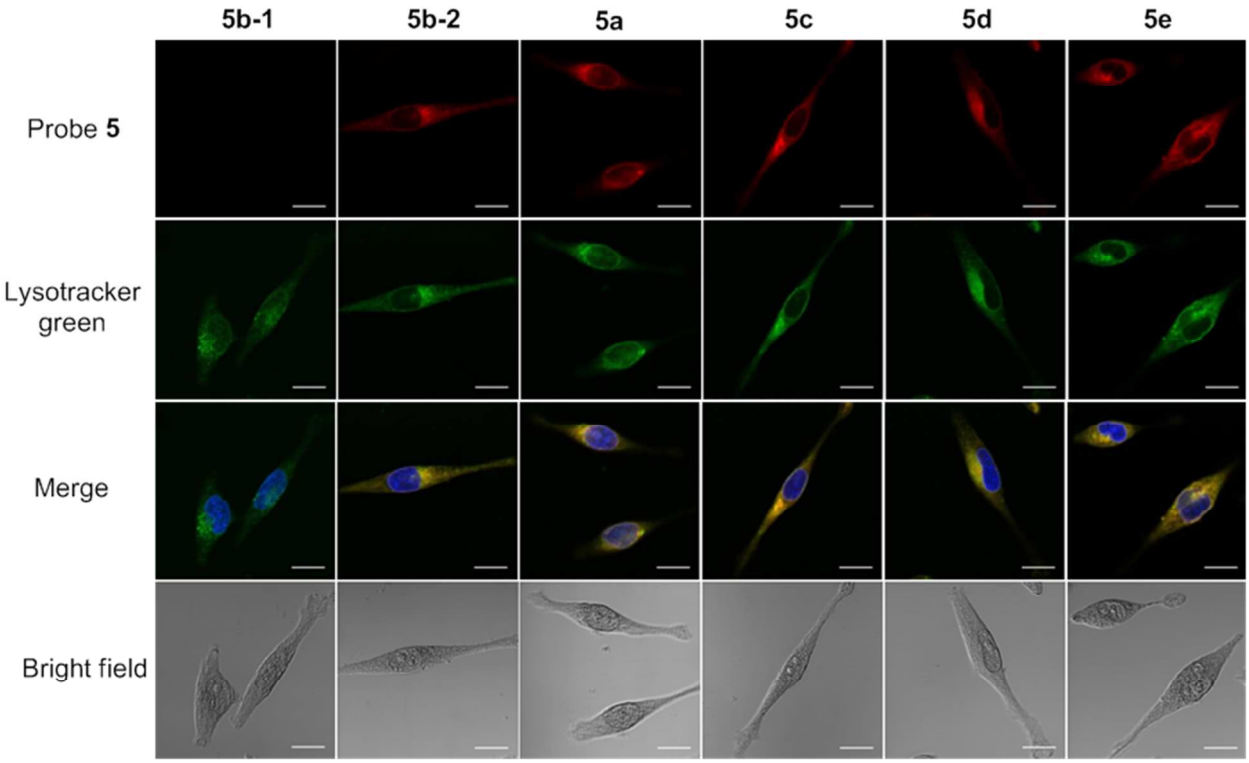


Figure S6. Confocal fluorescence microscope images (20X) of the **5** series of probes in Hela cells. Cells (10,000 cells/well) were incubated with **5** (5.0 μM) for 4 h at 37 °C, and the media was replaced with fresh media containing lysotracker green (0.5 μM) and incubated for 30 min. Then the cells were fixed with 4% paraformaldehyde. Cell nucleus was stained with DAPI (0.3 μM). The NIR probe is red; lysosomes are green; and the cell nuclei are blue. Co-localization is seen as yellow. Scale bar = 20 μm.

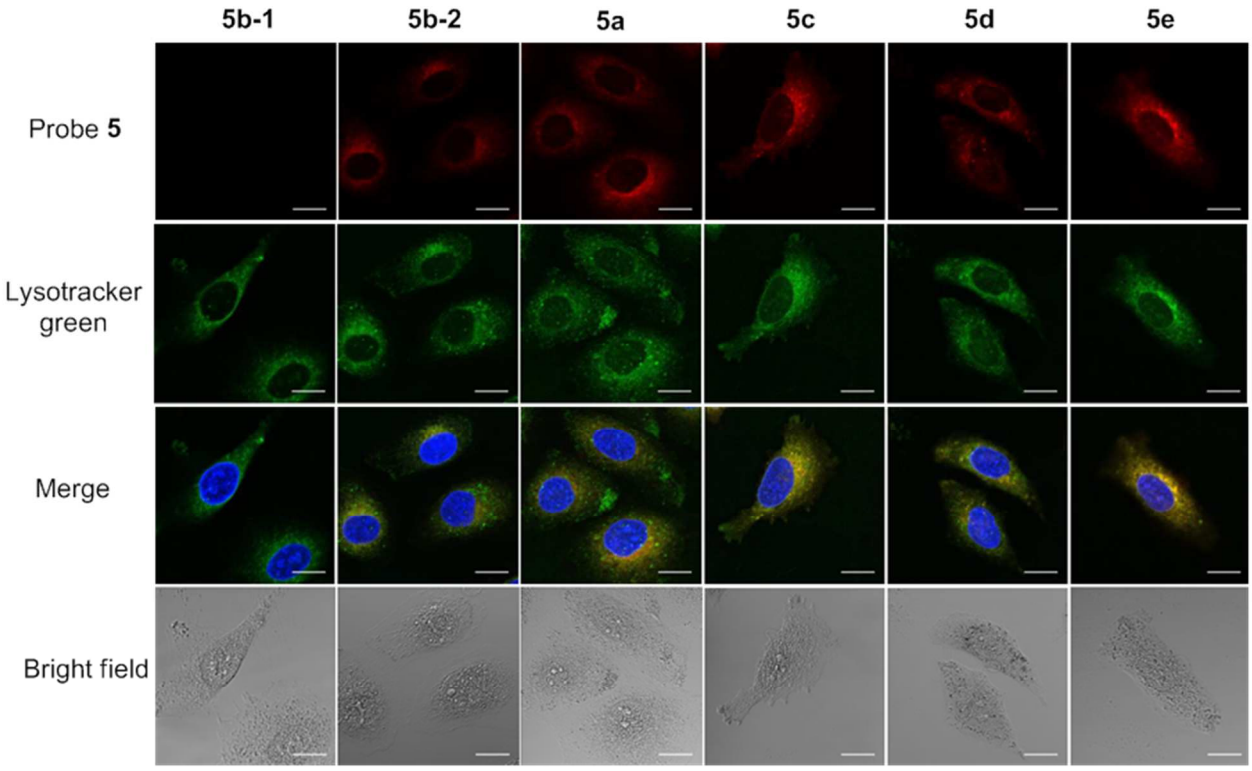


Figure S7. Confocal fluorescence microscope images (20X) of the **5** series of probes in Huh7 cells. Cells (10,000 cells/well) were incubated with **5** (5.0 μM) for 4 h at 37 °C, and the media was replaced with fresh media containing lysotracker green (0.5 μM) and incubated for 30 min. Then the cells were fixed with 4% paraformaldehyde. Cell nucleus was stained with DAPI (0.3 μM). The NIR probe is red; lysosomes are green; and the cell nuclei are blue. Co-localization is seen as yellow. Scale bar = 20 μm.

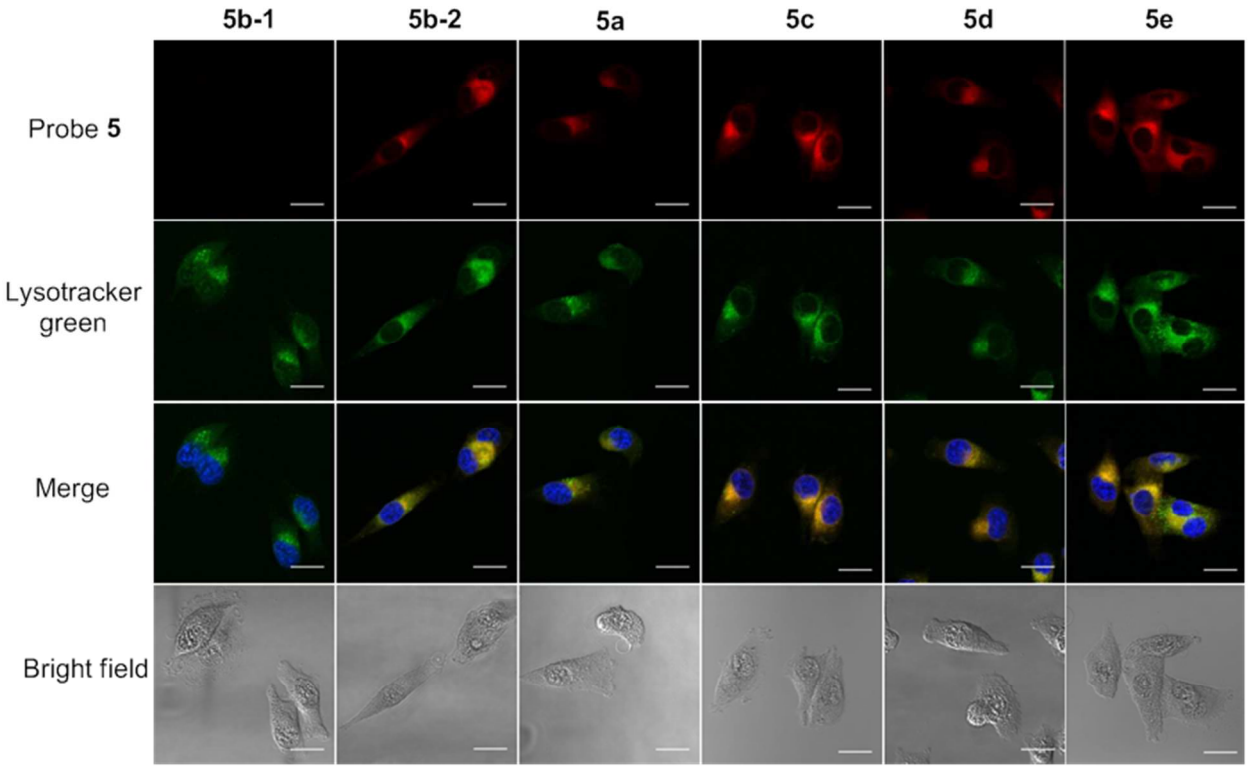


Figure S8. Confocal fluorescence microscope images (20X) of the **5** series of probes in HCC4017 cells. Cells (10,000 cells/well) were incubated with **5** (5.0 μM) for 4 h at 37 °C, and the media was replaced with fresh media containing lysotracker green (0.5 μM) and incubated for 30 min. Then the cells were fixed with 4% paraformaldehyde. Cell nucleus was stained with DAPI (0.3 μM). The NIR probe is red; lysosomes are green; and the cell nuclei are blue. Co-localization is seen as yellow. Scale bar = 20 μm.

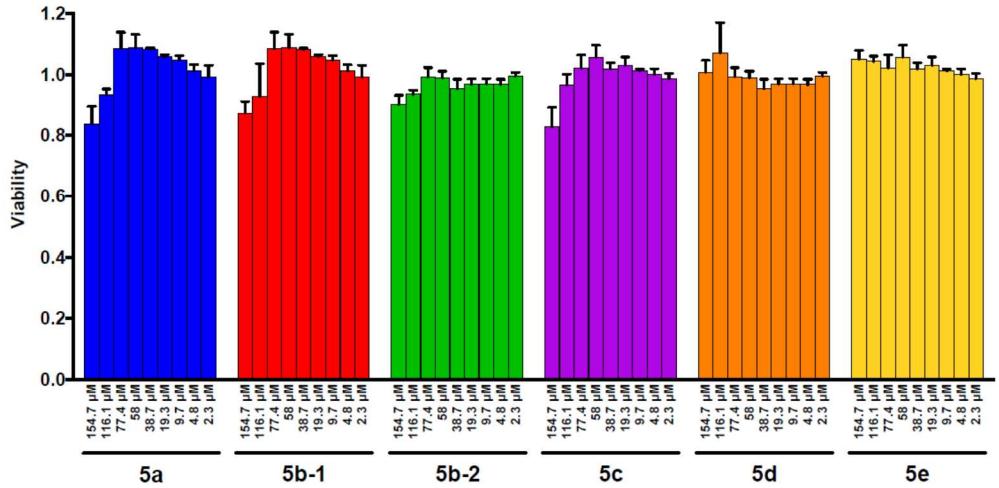


Figure S9. CellTiter-Glo luminescent cell viability assay in Hela cells. Cell viability was measured after addition of **5a-e** with different concentrations and 24 hours incubation compared to untreated cells (n = 3).

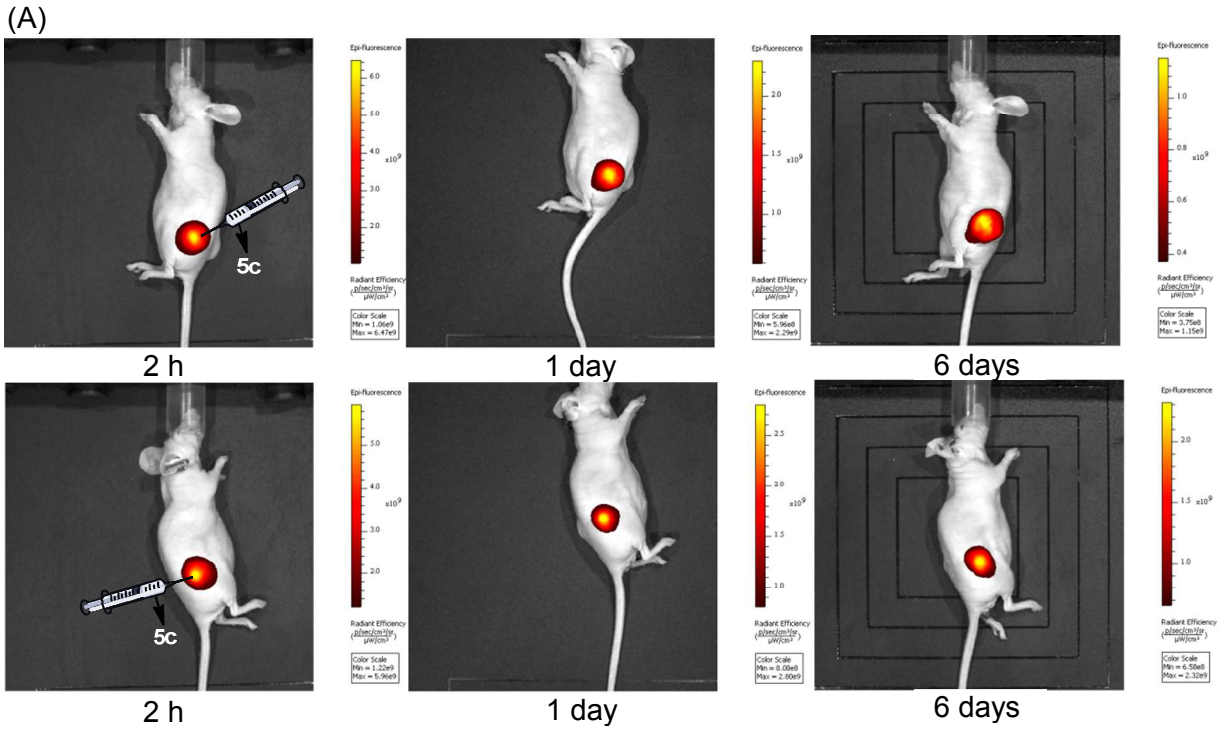


Figure S10. Probe **5c** for MDA-MB-231 tumor imaging *in vivo* and *ex vivo*. (A) Nude mice bearing two MDA-MB-231 tumors were injected with a dosage of 0.5 mg/kg **5c** (10 μM, 200 μL in PBS) via intratumoral injection. NIR fluorescence images at selected time-points were captured. (B) At 6 days post-injection, mice were sacrificed and organs were collected and imaged using an IVIS Lumina imaging system.

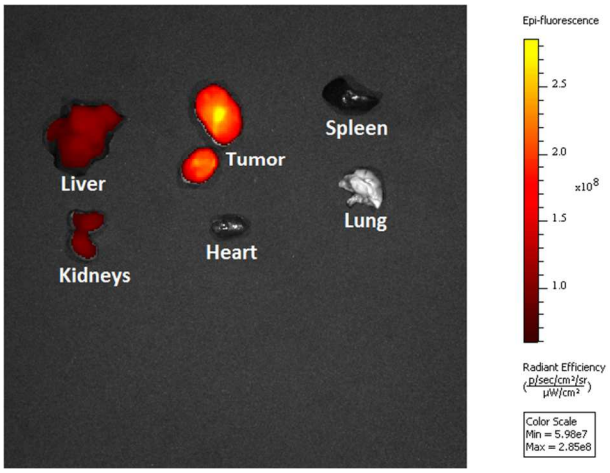


Figure S11. Representative *ex vivo* fluorescence images of harvested tumors and organs of MDA-MB-231 tumor-bearing mice sacrificed at 24 h post-injection. Nude mice bearing two MDA-MB-231 tumors were intravenously administered with probe **5c** (10 μM, 200 μL in PBS) at a dose of 0.5 mg/kg via the tail vein and, 24 h later, organs were collected for NIR imaging using an IVIS Lumina imaging system.

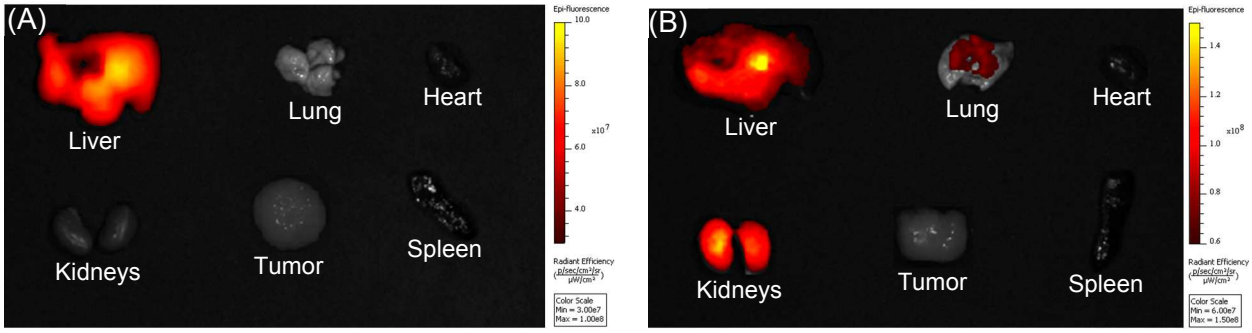


Figure S12. Representative *ex vivo* fluorescence images of collected tumors and organs of MDA-MB-231 tumor-bearing mice sacrificed at 15 min post-injection. (A) MDA-MB-231 tumor-bearing mice were intravenously injected with ICG (10 μM, 200 μL in PBS) via the tail vein and, 15 min later, organs were collected for NIR imaging. (B) MDA-MB-231 tumor-bearing mice were intravenously injected with sulfo-Cy5 (10 μM, 200 μL in PBS) via the tail vein and, 15 min later, organs were collected for NIR imaging.

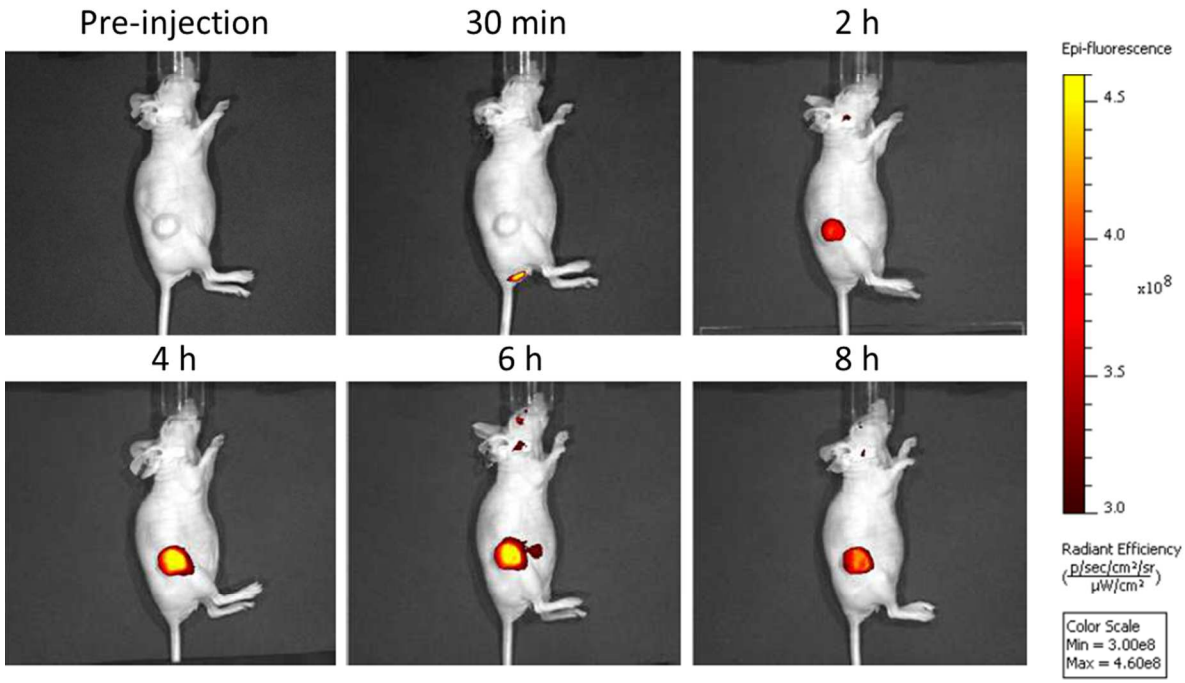


Figure S13. Time-dependent *in vivo* fluorescence images of subcutaneous HCC4017 tumor-bearing mice after intravenous injection of 0.5 mg/kg (10 μM, 200 μL) pH-activatable probe **5c** *via* the tail vein.

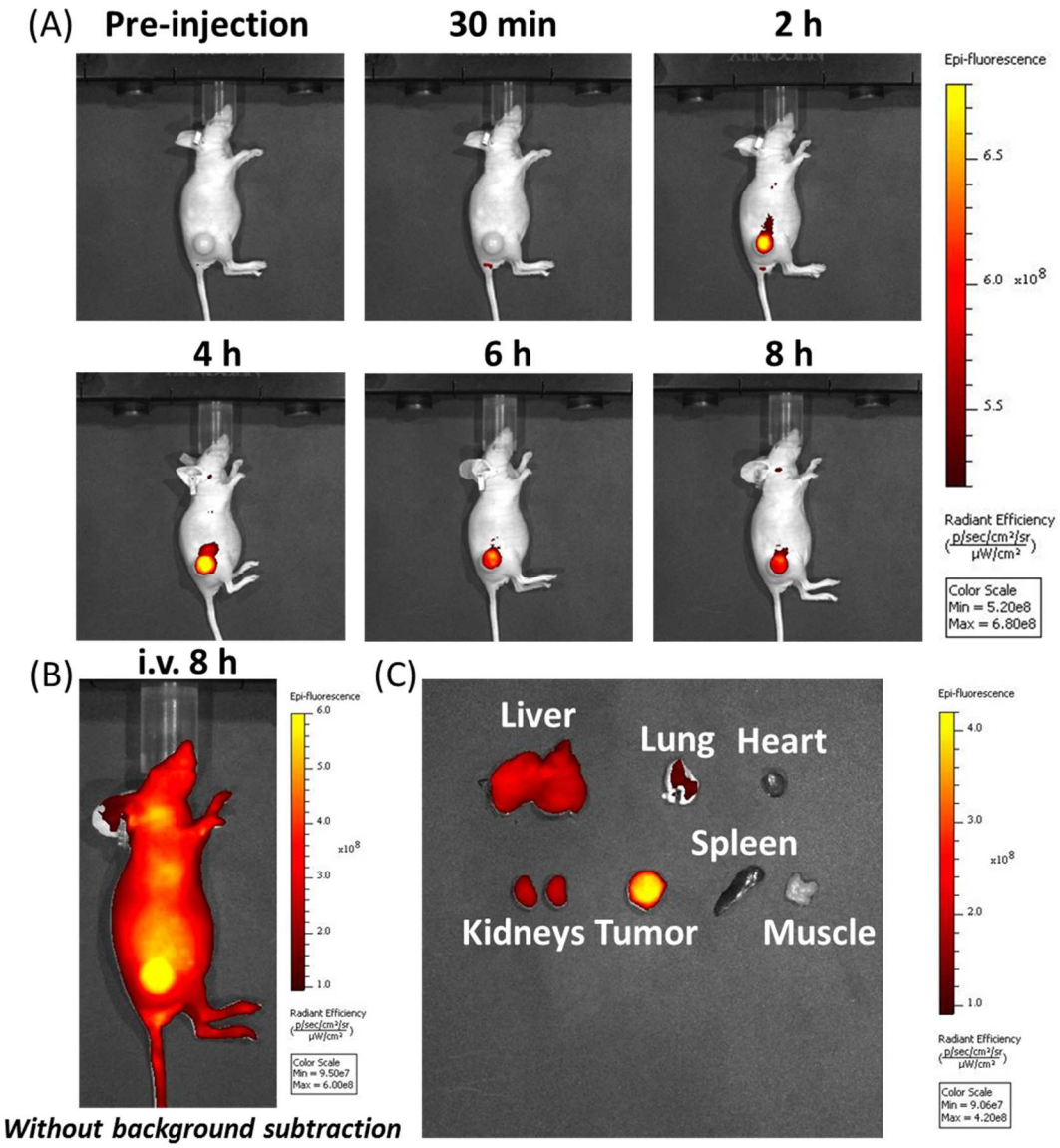


Figure S14. Probe **5c** for Hela tumor imaging *in vivo* and *ex vivo*. (A) Time-dependent *in vivo* fluorescence images of subcutaneous Hela tumor-bearing mice after intravenous injection of 0.5 mg/kg (10 µM, 200 µL) pH-activatable probe **5c** *via* the tail vein. (B) NIR fluorescence imaging 8 h post i.v. administration of **5c** without intensity scale adjusted. (C) At 8 h post-injection, mice were sacrificed and organs were collected and imaged using an IVIS Lumina imaging system.

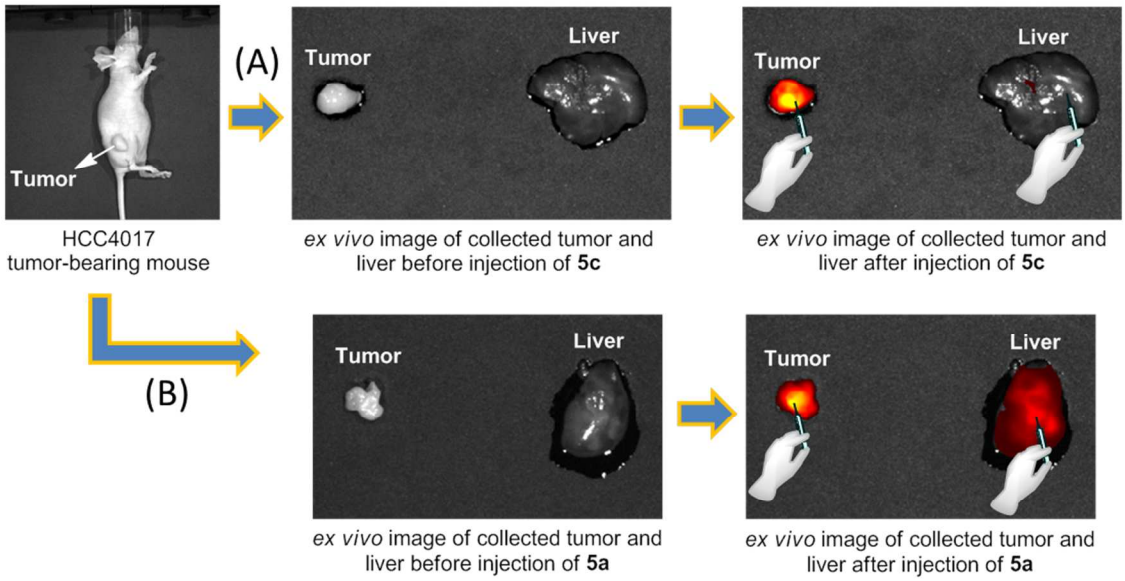
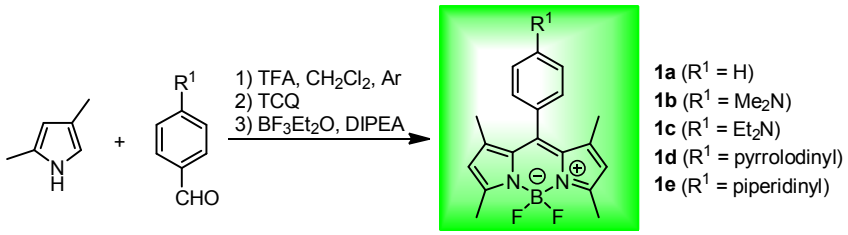


Figure S15. Illustration of microenvironment in tumor and liver. (A) The tumor and liver were harvested from a HCC4017 tumor-bearing mouse. *Ex vivo* imaging of collected tumor and liver was performed 10 min after direct injection of **5c** (1 μM, 100 μL). (B) *Ex vivo* image of collected tumor and liver was performed after injection of **5a** (1 μM, 100 μL) at 10 min post-injection.

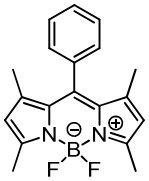
3. Synthesis of NIR probes

3.1 General procedure for the synthesis of BODIPYs **1a-e**.[5, 6]

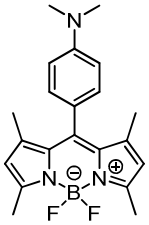


2,4-dimethylpyrrole (1.0 mL, 10.0 mmol) and the appropriate *N,N*-dialkylaminobenzaldehyde or benzaldehyde (5.0 mmol) were dissolved in 600 mL of anhydrous dichloromethane (DCM) containing a catalytic amount of trifluoroacetic acid (TFA). The resulting reddish solution was stirred overnight at ambient temperature under an Ar atmosphere. Tetrachloro-1,4-benzoquinone (*p*-chloranil) (1.23 g, 5.0 mmol) was added and the mixture was stirred for 1 h. Then the DCM was removed under reduced pressure. The residue thus obtained was dissolved in 300 mL of dry toluene containing *N,N*-diisopropylethylamine (DIPEA) (6 mL), and the resulting solution was stirred at ambient temperature. $BF_3 \cdot OEt_2$ (6 mL) was then slowly added, and stirring was continued for 2 h. The reaction mixture was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by flash column

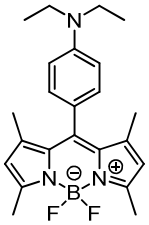
chromatography over silica gel using DCM/hexanes as the eluent. Then the collected product was recrystallized in CH_2Cl_2/hexanes (10/90), affording **1a-e** as brown-red solid.



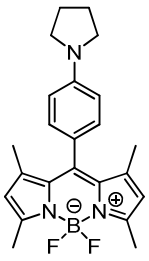
1a Yield = 16%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-7.48 (m, 3H), 7.29-7.26 (m, 2H), 5.98 (s, 2H), 2.56 (s, 6H), 1.37 (s, 6H).



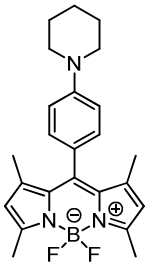
1b Yield = 45%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.05 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 2H), 5.97 (s, 2H), 3.02 (s, 6H), 2.55 (s, 6H), 1.48 (s, 6H).



1c Yield = 27%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 5.95 (s, 2H), 3.40 (q, *J* = 7.2 Hz, 4H), 2.54 (s, 6H), 1.51 (s, 6H), 1.19 (t, *J* = 7.2 Hz, 6H).



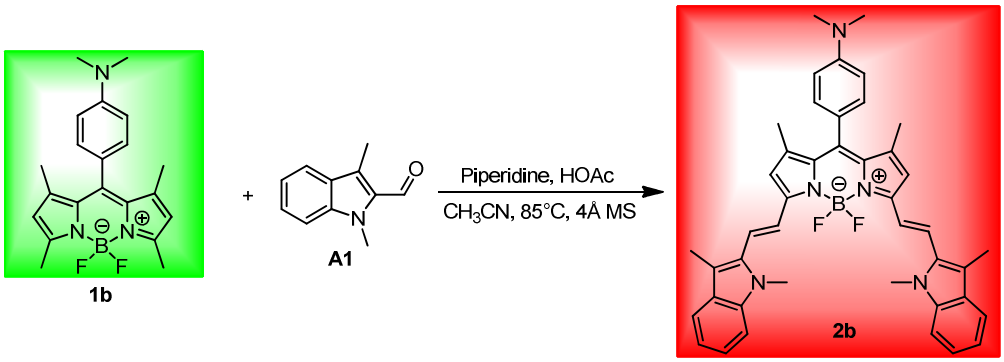
1d Yield = 32%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 5.96 (s, 2H), 3.33 (t, *J* = 6.4 Hz, 4H), 2.55 (s, 6H), 2.07-2.04 (m, 4H), 1.51 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.55, 148.10, 143.56, 143.25, 132.27, 128.77, 121.14, 120.74, 111.79, 47.53, 25.43, 14.79, 14.54. LRMS-ESI (*m/z*): Exact mass calcd for $C_{23}H_{27}BF_2N_3^+$ $[M+H]^+$: 394.2; Found: 394.2.



1e

Yield = 30%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (d, J = 8.8 Hz, 2H), 7.00 (d, J = 8.0 Hz, 2H), 5.96 (s, 2H), 3.23 (t, J = 5.3 Hz, 4H), 2.54 (s, 6H), 1.74-1.73 (m, 4H), 1.64-1.58 (m, 2H), 1.46 (s, 6H).

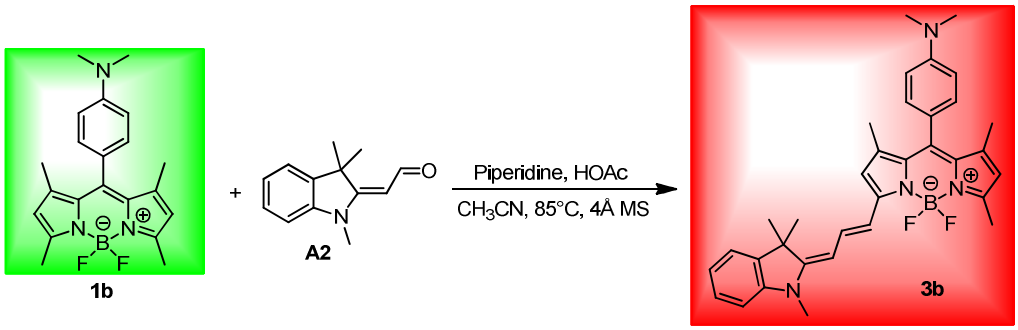
3.2 Synthesis of NIR BODIPY **2b**.



BODIPY **1b** (184 mg, 0.5 mmol) and 1,3-dimethyl-1*H*-indole-2-carbaldehyde (520 mg, 3.0 mmol) were refluxed in a mixture of dry acetonitrile (20 mL), piperidine (1.0 mL), glacial acetic acid (0.6 mL), and 4 Å molecular sieves (~50 pellets) under an Ar atmosphere. The reaction mixture was stirred at 85 °C for 8 h. Then the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography over silica gel using ethyl acetate/hexanes as the eluent. The collected product was then recrystallized in CH_2Cl_2/hexanes (10/90), affording **2b** as gold-brown solid.

Yield = 65%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J = 16.8 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 16.4 Hz, 2H), 7.30-7.24 (m, 4H), 7.14-7.10 (m, 4H), 6.81 (d, J = 8.4 Hz, 2H), 6.68 (s, 2H), 3.88 (s, 6H), 3.04 (s, 6H), 2.56 (s, 6H), 1.59 (s, 6H). ^{13}C NMR (100 MHz, CD_2Cl_2): δ 152.2, 151.4, 143.0, 140.3, 139.2, 134.9, 134.5, 129.8, 129.1, 124.0, 123.8, 122.5, 121.3, 119.8, 119.7, 117.3, 114.6, 112.8, 109.7, 40.7, 31.4, 15.3, 10.8. LRMS-ESI (*m/z*): Exact mass calcd for $C_{43}H_{43}BF_2N_5^+$ $[M+H]^+$: 678.4; Found: 678.2.

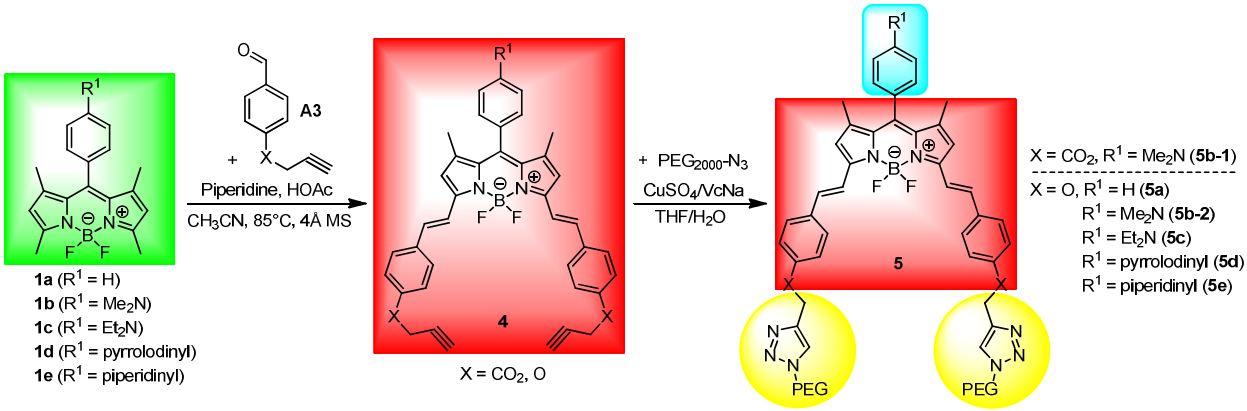
3.3 Synthesis of NIR BODIPY **3b**.



BODIPY **1b** (184 mg, 0.5 mmol) and the Fisher aldehyde (131 mg, 1.3 mmol) were refluxed in a mixture of dry acetonitrile (20 mL), piperidine (1.0 mL), glacial acetic acid (0.6 mL), and 4 Å molecular sieves (~50 pellets) under an Ar atmosphere. The reaction mixture was stirred at 85 °C for 2 h. Then the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography over silica gel using ethyl acetate/hexanes as the eluent. The collected product was then recrystallized in CH_2Cl_2/Hexanes (10/90), affording **3b** as brown-green solid.

Yield = 50%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55 (t, *J* = 13.5 Hz, 1H), 7.20 (t, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 7.0 Hz, 1H), 7.10-7.09 (m, 2H), 6.91-6.78 (m, 4H), 6.69 (d, *J* = 7.5 Hz, 1H), 6.49 (s, 1H), 5.91 (s, 1H), 5.66 (d, *J* = 11.5 Hz, 1H), 3.18 (s, 3H), 3.02 (s, 6H), 2.55 (s, 3H), 1.60 (s, 6H), 1.52 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6, 155.4, 150.4, 149.9, 144.6, 143.2, 139.1, 138.7, 138.1, 135.2, 134.2, 131.4, 129.3, 127.8, 123.0, 121.6, 120.4, 119.2, 117.4, 114.2, 112.2, 106.6, 98.4, 46.1, 40.4, 30.9, 29.2, 28.7, 15.0, 14.4. LRMS-ESI (*m/z*): Exact mass calcd for $C_{34}H_{38}BF_2N_4^+$ $[M+H]^+$: 551.3; Found: 551.2.

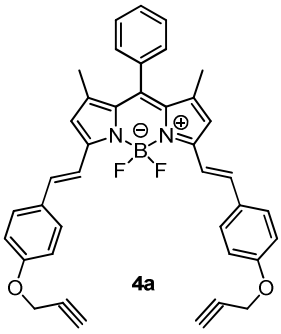
3.4 General experimental procedure for synthesis of **5a-e**.



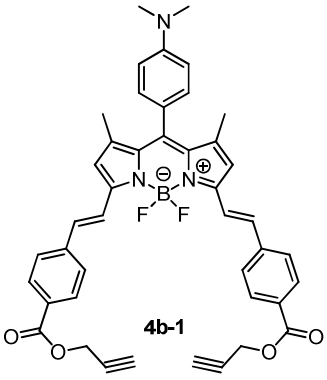
BODIPY **1** (0.5 mmol) and the appropriate propargyl benzaldehyde **A3** (3.0 mmol) were refluxed in a mixture of dry acetonitrile (20 mL), piperidine (1.0 mL), glacial acetic acid (0.6 mL), and 4 Å molecular sieves (~50 pellets) under an Ar atmosphere. The reaction mixture was

stirred at 85 °C for 8 h. Then the solvent was removed under reduced pressure. The crude product was first purified by flash column chromatography over silica gel using ethyl acetate/hexanes as the eluent to remove the excess **A3**. Subsequently, the eluent was changed to DCM/hexanes to obtain the desired product. The collected product was then recrystallized in CH_2Cl_2/hexanes (10/90), yielding **4** as gold-brown or dark-blue solid.

NIR BODIPY **4** (0.04 mmol), methoxy-poly(ethylene glycol)-azide (PEG-N_3, average M_n = 2000, 192 mg, 0.096 mmol), $CuSO_4$ (1.3 mg, 0.008 mmol), and sodium ascorbate (VcNa, 15.8 mg, 0.08 mmol) were added to a 10 mL schlenk flask. The vessel was evacuated and back-filled with Ar. Then the flask was cooled to 0 °C for 20 minutes and 4 mL THF (degassed) was added via a syringe, followed by 1 mL DI water (degassed). The reaction mixture was slowly warmed to room temperature and stirred overnight. After evaporation of THF, the residue was dialyzed in DI water for 6 h and lyophilized to obtain a blue powder. The collected product was then purified by column chromatography through a Sephadex LH-20 resin using MeOH as the eluent, affording **5a-e** as blue solid.

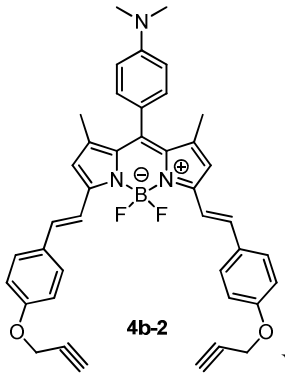


Yield = 49%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.59 (m, 6H), 7.51-7.49 (m, 3H), 7.34-7.32 (m, 2H), 7.21 (d, *J* = 16.0 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.62 (s, 2H), 4.74 (d, *J* = 2.4 Hz, 4H), 2.56 (t, *J* = 2.4 Hz, 2H), 1.44 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.2, 152.6, 141.9, 138.4, 135.5, 135.2, 133.2, 130.4, 129.03, 128.95, 128.9, 128.5, 117.7, 117.5, 115.2, 78.2, 75.8, 55.8, 14.6. LRMS-ESI (*m/z*): Exact mass calcd for $C_{39}H_{32}BF_2N_2O_2^+$ $[M+H]^+$: 609.3; Found: 609.4.

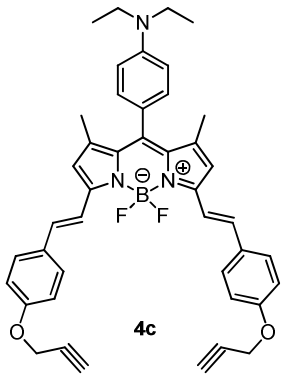


Yield = 18%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 8.4 Hz, 4H), 7.84 (d, *J* = 16.0 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 4H), 7.25 (d, *J* = 16.0 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.8 Hz, 2H), 6.68 (s, 2H), 4.95 (d, *J* = 2.4 Hz, 4H), 3.04 (s, 6H), 2.54 (t, *J* = 2.4

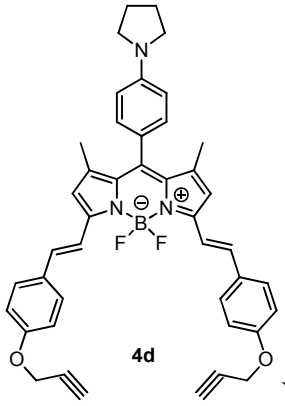
Hz, 2H), 1.58 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 151.6, 150.8, 143.0, 141.8, 141.4, 134.7, 134.3, 130.3, 129.0, 127.3, 121.82, 121.79, 118.0, 112.3, 77.7, 75.0, 52.5, 40.3, 15.0. LRMS-ESI (*m/z*): Exact mass calcd for $C_{43}H_{37}BF_2N_3O_4^+$ $[M+H]^+$: 708.3; Found: 708.2.



Yield = 61%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.58 (m, 6H), 7.19 (d, *J* = 16.0 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 4H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.61 (s, 2H), 4.74 (d, *J* = 2.0 Hz, 4H), 3.03 (s, 6H), 2.55 (t, *J* = 2.4 Hz, 2H), 1.55 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.1, 152.1, 150.6, 142.1, 140.0, 134.9, 134.0, 130.5, 129.2, 128.9, 122.4, 117.9, 117.2, 115.2, 112.2, 78.3, 75.8, 55.8, 40.3, 14.9. LRMS-ESI (*m/z*): Exact mass calcd for $C_{41}H_{37}BF_2N_3O_2^+$ $[M+H]^+$: 652.3; Found: 652.2.

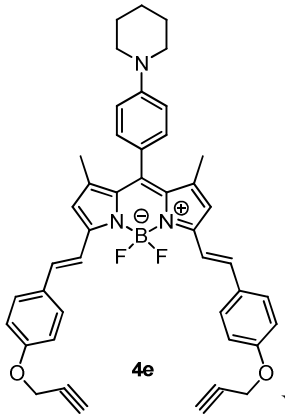


Yield = 60%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.58 (m, 6H), 7.19 (d, *J* = 16.4 Hz, 2H), 7.06 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 4H), 6.75 (d, *J* = 8.8 Hz, 2H), 6.61 (s, 2H), 4.74 (d, *J* = 2.4 Hz, 4H), 3.41 (q, *J* = 7.2 Hz, 4H), 2.55 (t, *J* = 2.4 Hz, 2H), 1.58 (s, 6H), 1.21 (t, *J* = 7.2Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.0, 152.0, 148.1, 142.1, 140.3, 134.8, 134.0, 130.5, 129.3, 128.8, 121.4, 117.9, 117.2, 115.1, 111.9, 78.3, 75.8, 55.8, 44.3, 15.0, 12.4. LRMS-ESI (*m/z*): Exact mass calcd for $C_{43}H_{41}BF_2N_3O_2^+$ $[M+H]^+$: 680.3; Found: 680.2.



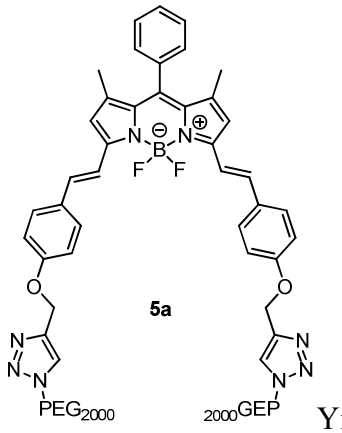
4d

Yield = 86%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64-7.58 (m, 6H), 7.19 (d, *J* = 16.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 4H), 6.73 (d, *J* = 5.6 Hz, 2H), 6.61 (s, 2H), 4.74 (d, *J* = 2.4 Hz, 4H), 3.38 (m, 4H), 2.55 (t, *J* = 2.0 Hz, 2H), 2.09 (m, 4H), 1.56 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.0, 152.0, 148.1, 142.2, 140.4, 134.8, 134.1, 130.5, 129.2, 128.8, 121.4, 118.0, 117.1, 115.1, 111.7, 78.3, 75.8, 55.8, 47.6, 25.4, 15.0. LRMS-ESI (*m/z*): Exact mass calcd for $C_{43}H_{39}BF_2N_3O_2^+$ $[M+H]^+$: 678.3; Found: 678.2.

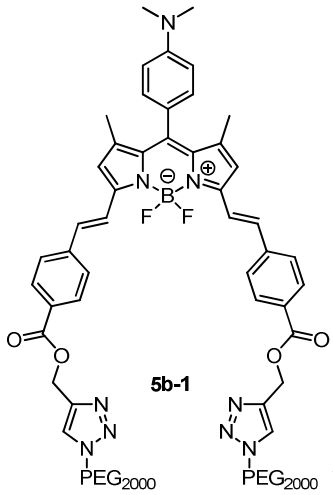


4e

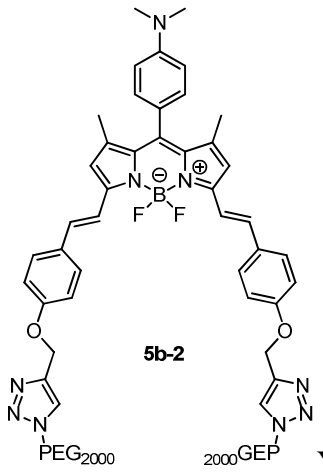
Yield = 50%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64-7.58 (m, 6H), 7.19 (d, *J* = 16.0 Hz, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.03-7.00 (m, 6H), 6.61 (s, 2H), 4.74 (d, *J* = 2.4 Hz, 4H), 3.24 (t, *J* = 5.6 Hz, 4H), 2.55 (t, *J* = 2.4 Hz, 2H), 1.78-1.72 (m, 4H), 1.65-1.61 (m, 2H), 1.53 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.1, 152.4, 152.2, 142.1, 139.4, 135.1, 133.8, 130.5, 129.1, 128.9, 125.0, 117.9, 117.3, 116.2, 115.2, 78.3, 75.8, 55.8, 50.1, 25.6, 24.2, 14.9. LRMS-ESI (*m/z*): Exact mass calcd for $C_{44}H_{41}BF_2N_3O_2^+$ $[M+H]^+$: 692.3; Found: 692.2.



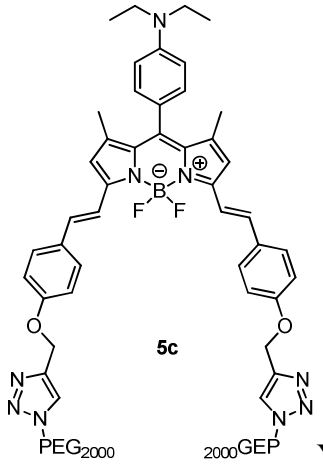
Yield = 92%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.19 (s, 2H), 7.64-7.55 (m, 9H), 7.43-7.39 (m, 4H), 7.12 (d, *J* = 8.4 Hz, 4H), 6.83 (s, 2H), 5.27 (s, 4H), 4.62 (t, *J* = 4.8 Hz, 4H), 3.90 (t, *J* = 4.8 Hz, 4H), 3.64-3.57 (m, 344H), 3.35 (s, 6H), 1.48 (s, 6H).



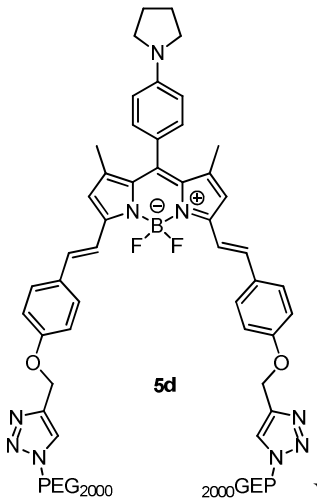
Yield = 93%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.22 (s, 2H), 8.09 (d, *J* = 8.4 Hz, 4H), 7.82-7.75 (m, 6H), 7.50 (d, *J* = 16.4 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.90 (s, 2H), 5.48 (s, 4H), 4.64 (t, *J* = 4.8 Hz, 4H), 3.92 (t, *J* = 4.8 Hz, 4H), 3.63-3.57 (m, 344H), 3.35 (s, 6H), 3.04 (s, 6H), 1.62 (s, 6H).



Yield = 84%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.19 (s, 2H), 7.63-7.56 (m, 6H), 7.38 (d, *J* = 16.4 Hz, 2H), 7.14-7.10 (m, 6H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.80 (s, 2H), 5.26 (s, 4H), 4.61 (t, *J* = 4.8 Hz, 4H), 3.90 (t, *J* = 4.8 Hz, 4H), 3.63-3.57 (m, 344H), 3.35 (s, 6H), 3.04 (s, 6H), 1.59 (s, 6H).

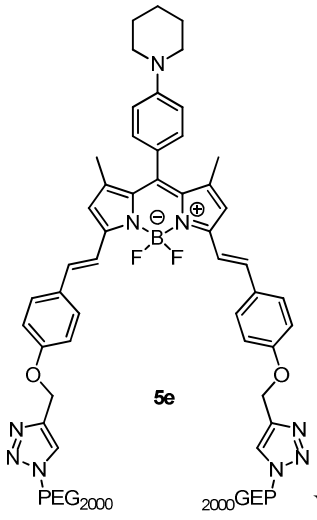


Yield = 91%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.19 (s, 2H), 7.62 (d, *J* = 8.4 Hz, 4H), 7.57 (d, *J* = 17.6 Hz, 2H), 7.38 (d, *J* = 16.0 Hz, 2H), 7.13-7.10 (m, 6H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.80 (s, 2H), 5.27 (s, 4H), 4.62 (t, *J* = 4.8 Hz, 4H), 3.90 (t, *J* = 4.8 Hz, 4H), 3.63-3.57 (m, 348H), 3.35 (s, 6H), 1.63 (s, 6H), 1.21 (t, *J* = 7.2 Hz, 6H).



5d

Yield = 86%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.18 (s, 2H), 7.61 (d, *J* = 8.8 Hz, 4H), 7.56 (d, *J* = 16.4 Hz, 2H), 7.38 (d, *J* = 16.0 Hz, 2H), 7.12-7.09 (m, 6H), 6.80 (s, 2H), 6.73 (d, *J* = 8.4 Hz, 2H), 5.26 (s, 4H), 4.61 (t, *J* = 4.8 Hz, 4H), 3.89 (t, *J* = 4.8 Hz, 4H), 3.64-3.56 (m, 344H), 3.35-3.33 (m, 10H), 2.07 (m, 4H), 1.60 (s, 6H).



5e

Yield = 85%; ^{1}H NMR (400 MHz, CD_3OD): δ 8.18 (s, 2H), 7.62 (d, *J* = 8.8 Hz, 4H), 7.56 (d, *J* = 16.0 Hz, 2H), 7.39 (d, *J* = 16.4 Hz, 2H), 7.17-7.10 (m, 8H), 6.81 (s, 2H), 5.27 (s, 4H), 4.61 (t, *J* = 4.8 Hz, 4H), 3.90 (t, *J* = 4.8 Hz, 4H), 3.63-3.56 (m, 344H), 3.35 (s, 6H), 3.30 (m, 4H), 1.75-1.73 (m, 4H), 1.65 (m, 2H), 1.58 (s, 6H).

4. Cell culture and confocal microscopy

All cell lines were cultured in high glucose Dulbecco's Modified Eagle's Medium or RPMI-1640 medium containing 5% fetal bovine serum (FBS). Cells were maintained at 37 °C under a humidified atmosphere with 5% CO_2. (DMEM for Hela, Huh7, and A549 cell lines. RPMI for MDA-MB-231, SKOV3, and HCC4017 cell lines.)

Prior to confocal imaging studies, cells were seeded at a density of 10,000 cells per well in 8-chambered cover glass slides (Nunc) in 300 μL of media and allowed to attach for 24 hours. On the following day, the media was removed and replaced with fresh DMEM or RPMI (300 μL). The probe of interest (**5a-e**) (5 μM) was added and the cells were incubated for 30 min or 4 h. Then the cells were washed twice with phosphate buffered saline (PBS) and incubated with the LysoTracker Green DND-26 (0.5 μM, 200 μL) for 30 min. The cells were fixed by 4% PFA (200 μL) and stained with DAPI (0.3 μM, 200 μL) for 10 min. After labeling, cells were washed twice with PBS and incubated with fresh PBS for fluorescence imaging. Confocal microscopy imaging was performed using a Zeiss LSM 700 Confocal. All imaging settings were held constant across all experiments imaging different probes. The use of identical settings allowed for proper comparison of different dyes. Images were analyzed using ImageJ (NIH).

5. Cell viability studies

HeLa cells were seeded in white opaque flat-bottom 96-well plates at a cell density of 10,000 cells per well in 100 μL growth medium. After 24 h to allow cell adhesion, the medium was exchanged for 180 μL fresh growth medium. Probes **5a-e** were serially diluted in PBS. Probe dilutions (100 μL) were added to the cells via multichannel pipette for final micelle concentrations of 2.3 to 154.7 μM. The cells were then incubated for 24 hours. After this time, cell viability was evaluated by the CellTiter-Glo assay and normalized to a PBS control, n = 3.

6. *In vivo* imaging and biodistribution of breast, lung, and cervical tumor-bearing mice

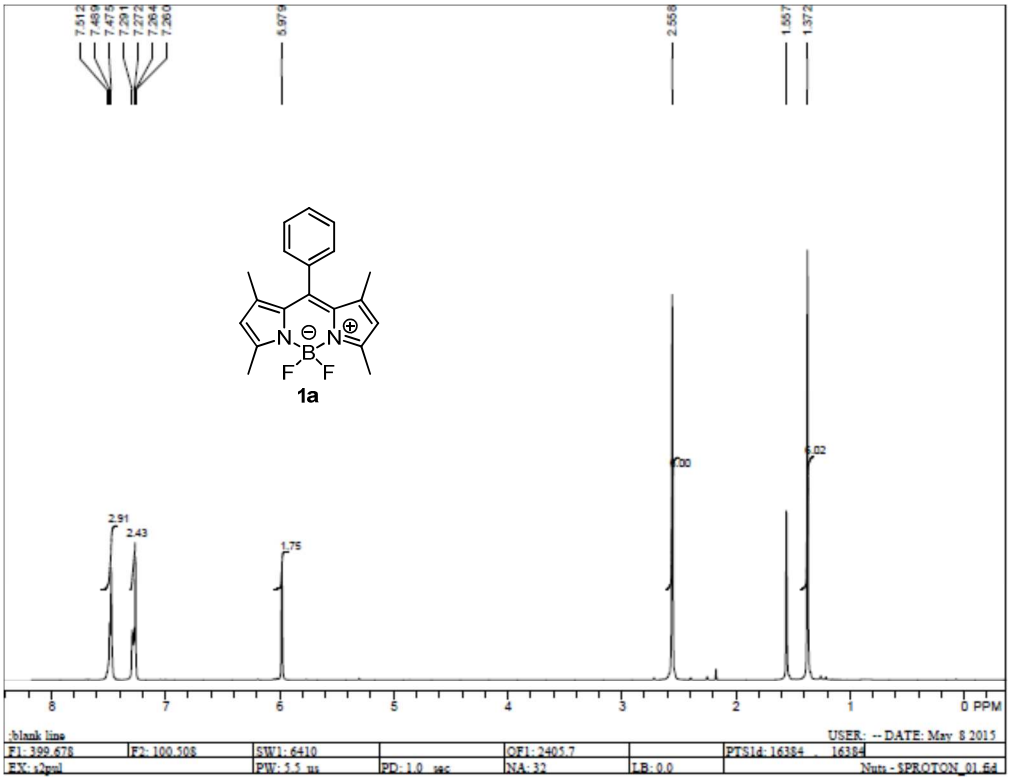
Female athymic nude Foxn1^{nu} mice were purchased from Harlan Laboratories. All experiments were approved by the Institutional Animal Care and Use Committees of The University of Texas Southwestern Medical Center and were consistent with local, state and federal regulations as applicable. MDA-MB-231, HCC4017, or Hela tumor cells (5×10^6) in 100 μL HBSS/Matrigel (v/v, 50/50) were injected subcutaneously into each flank of 6 weeks old nude mice. After three (for breast and cervical tumor) or five (for lung tumor) weeks, when the tumors reached adequate size, probe **5c** (10 μM) in 200 μL PBS was injected intravenously through the tail vein (0.5 mg/kg dose i.v.). In the control groups, the "always on" probe **5a** or sulfo-Cy5 or ICG (10 μM, 200 μL) was administrated i.v. For the intratumoral injection, 200 μL of **5c** in PBS (10 μM) was directly injected into the tumor. Mice were anesthetized with 2.5 % isofluorane in oxygen at selected time points and the whole body NIR fluorescence images (the Cy5.5 filter was used) were captured using an IVIS Lumina imaging system (Caliper Life Sciences). At the end point (6 h, 8 h, or 24 h), mice were euthanized and their livers, tumors, lungs, hearts, kidneys, spleens, and muscle were collected. *Ex vivo* fluorescence imaging of these

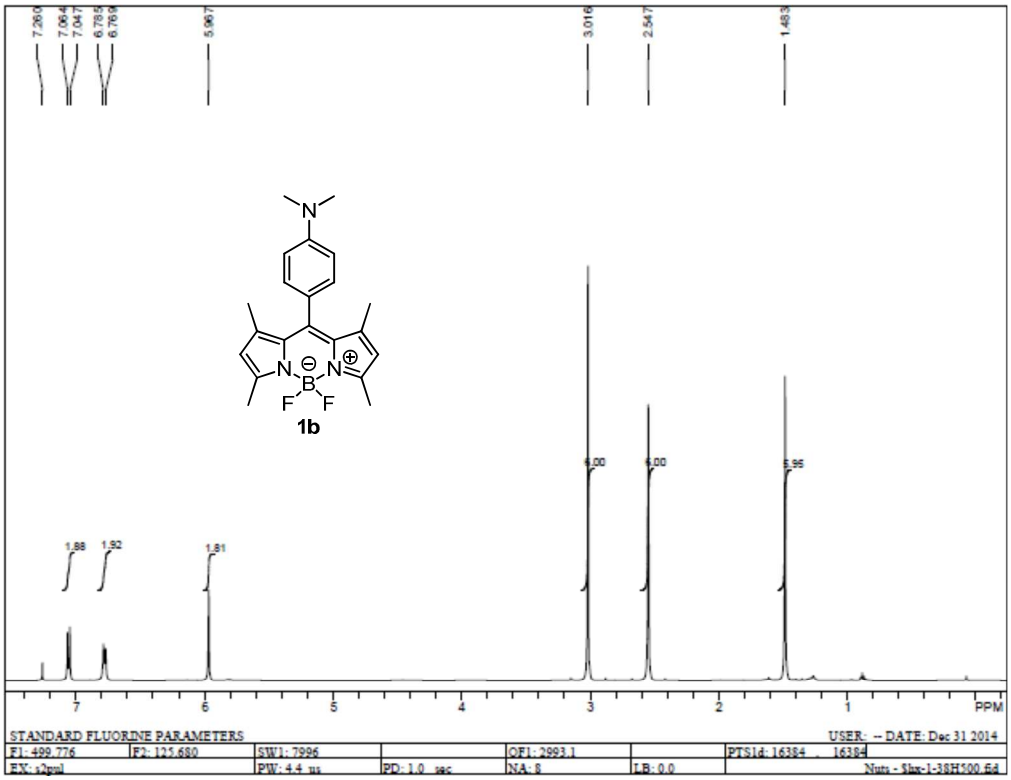
organs was immediately performed on the IVIS Lumina. The fluorescent signal of organs was normalized to the fluorescent signal in the muscle within a representative region of interest (ROI).

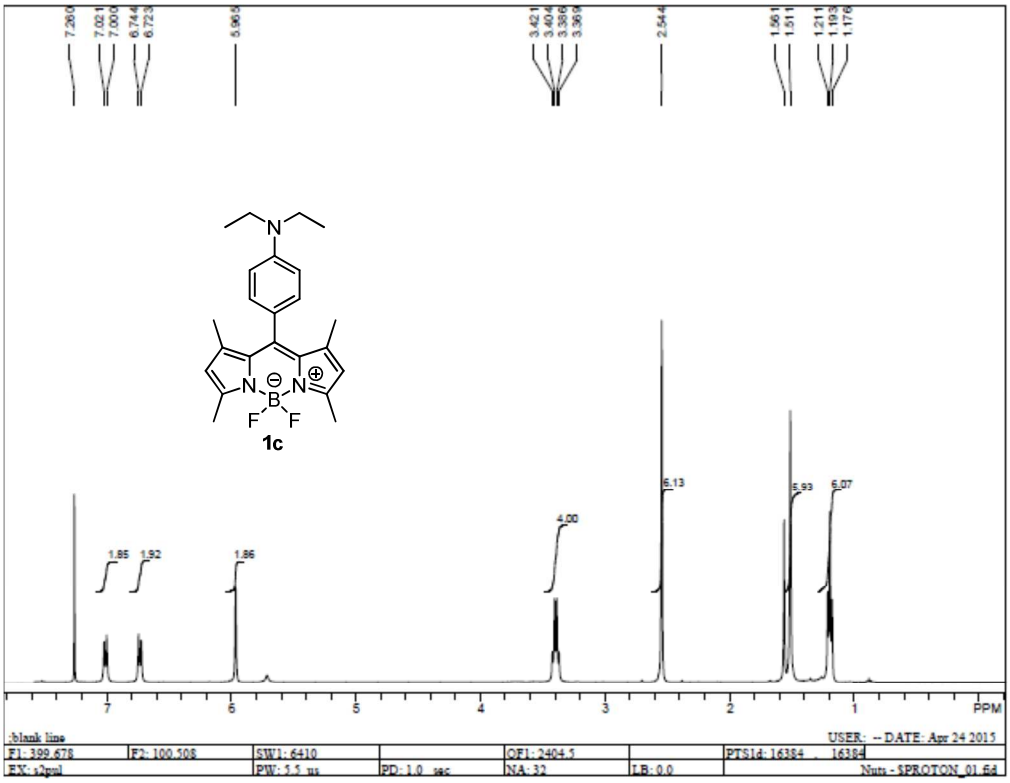
7. *Ex vivo* analysis of liver and tumor

Female athymic nude Foxn1^{nu} mice (n = 3) bearing subcutaneous MDA-MB-231 tumors were intravenously injected with pH-activatable probe **5c** at a dose of 0.5 mg/kg (10 μM, 200 μL in PBS) *via* the tail vein. The mice were sacrificed at 8 hours post-injection. Liver and tumor were immediately collected and frozen in OCT (Tissue-Tek, Sakura Finetek, Torrance, CA) at -20 °C for 20 min. Then the frozen tissues were cut into 10 μm sections using Leica CM3050 S cryostat. The sections were fixed in cold acetone (-20 °C) for 10 min, washed with PBS for 5 min (3×), and mounted with medium containing DAPI (Vector Laboratories Inc., CA, USA). Fluorescence images were captured on a Zeiss LSM 700 Confocal and analyzed using ImageJ (NIH).

8. ^{1}H NMR and ^{13}C NMR spectra of the probes



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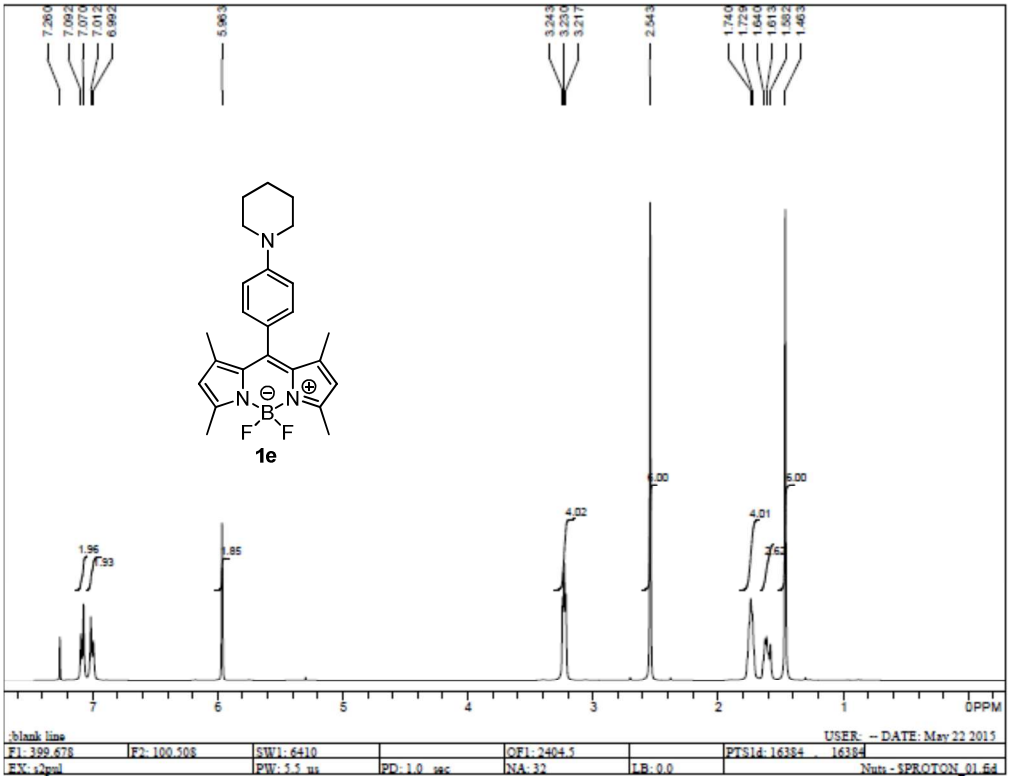
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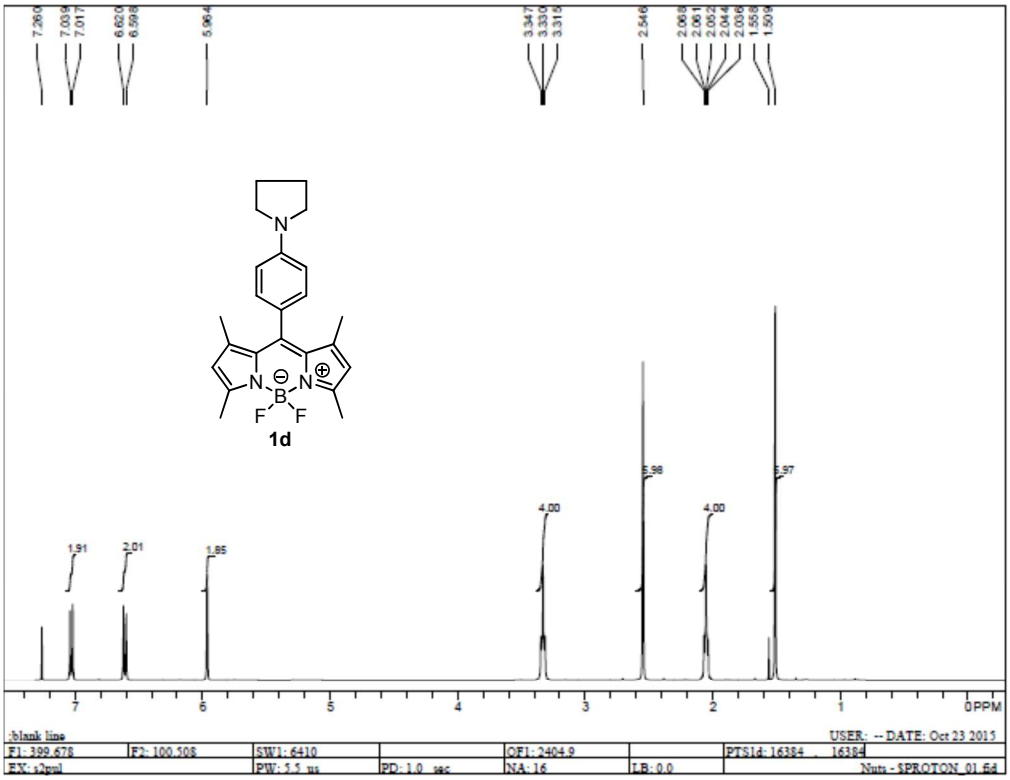
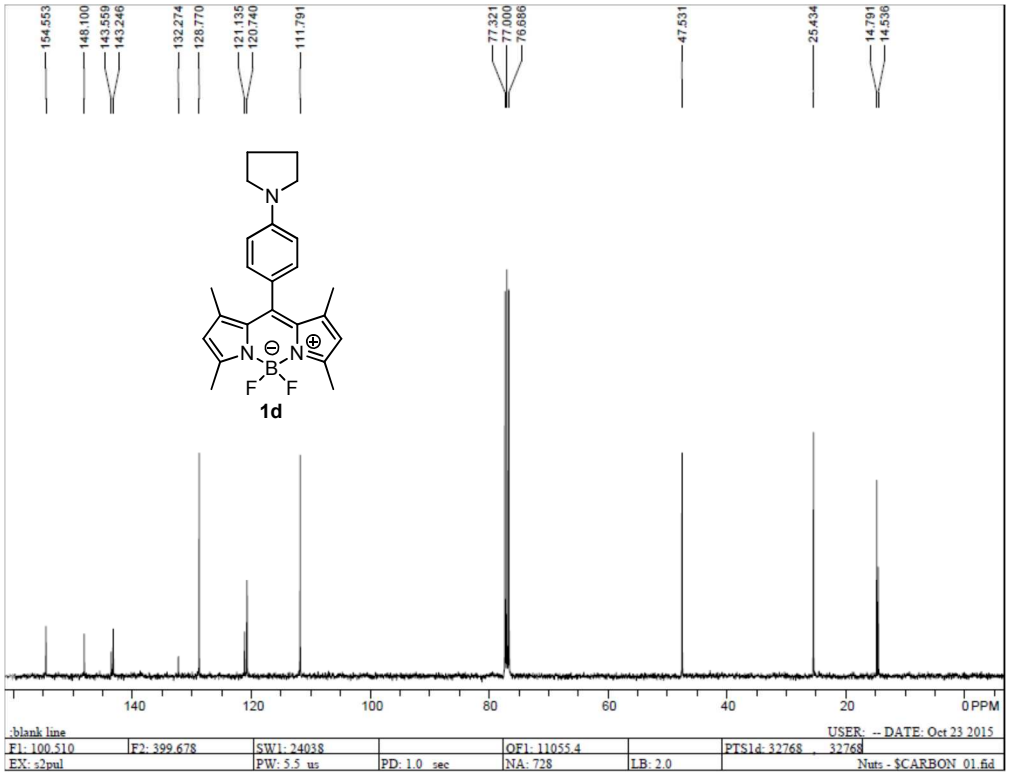
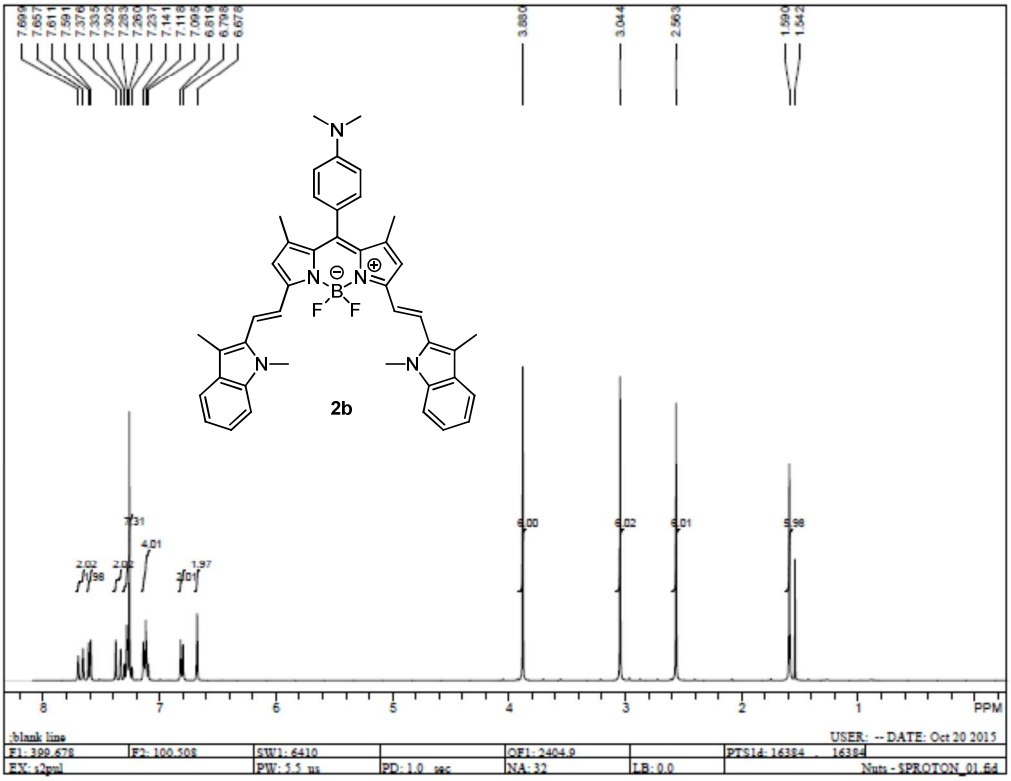
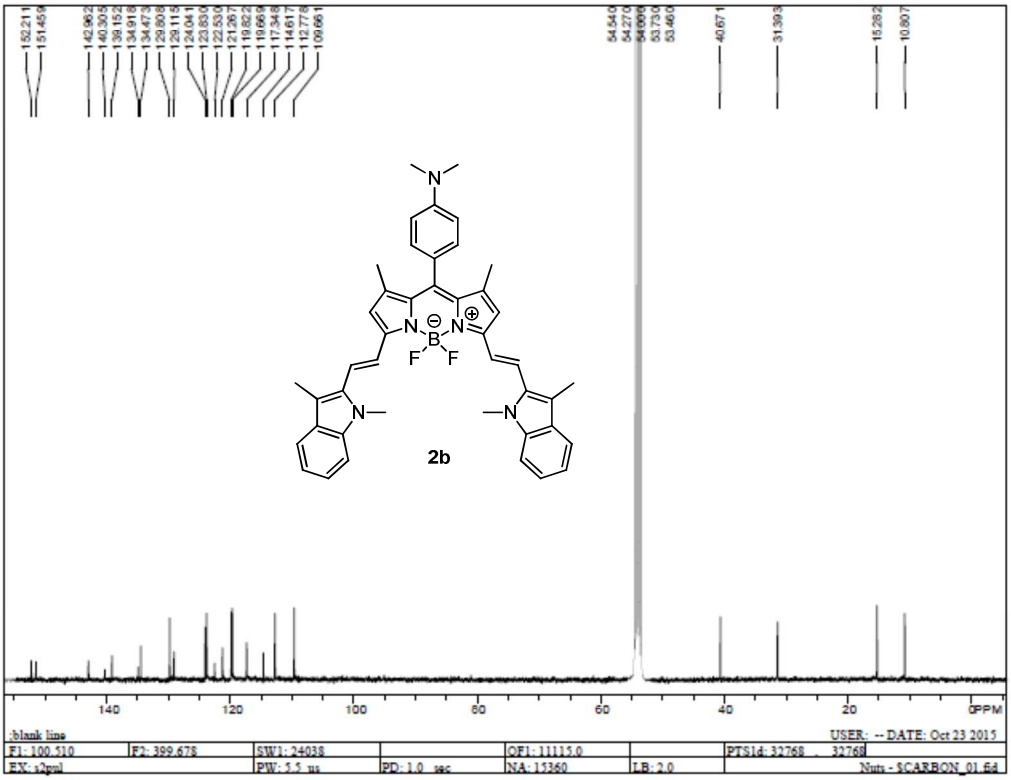
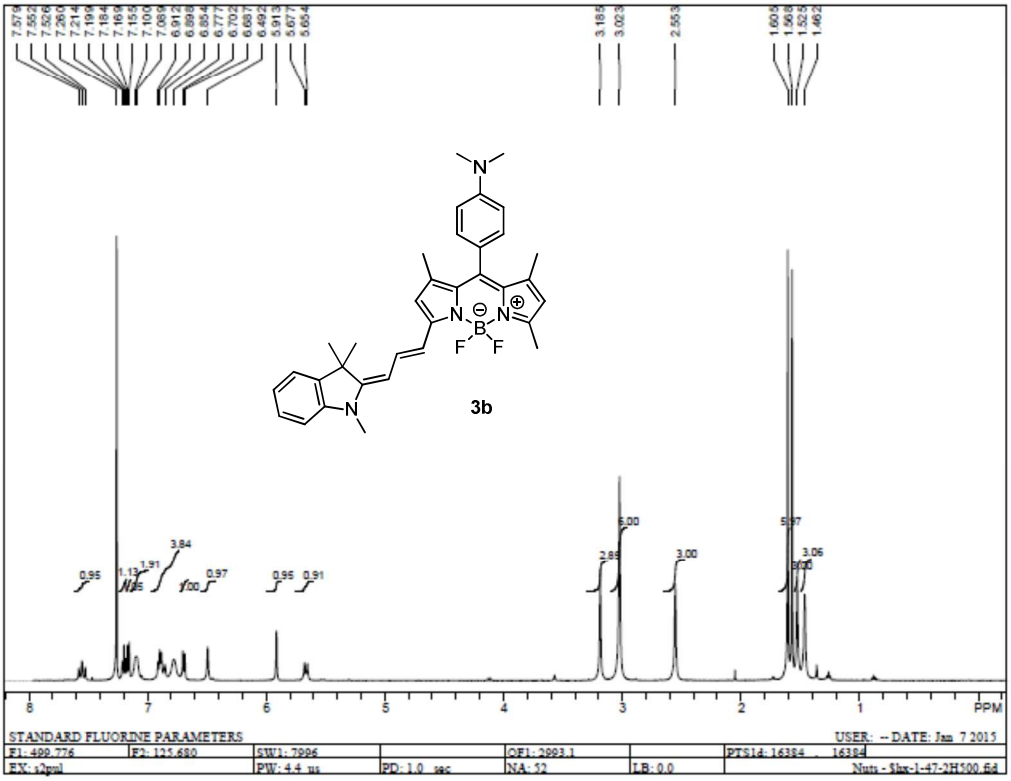
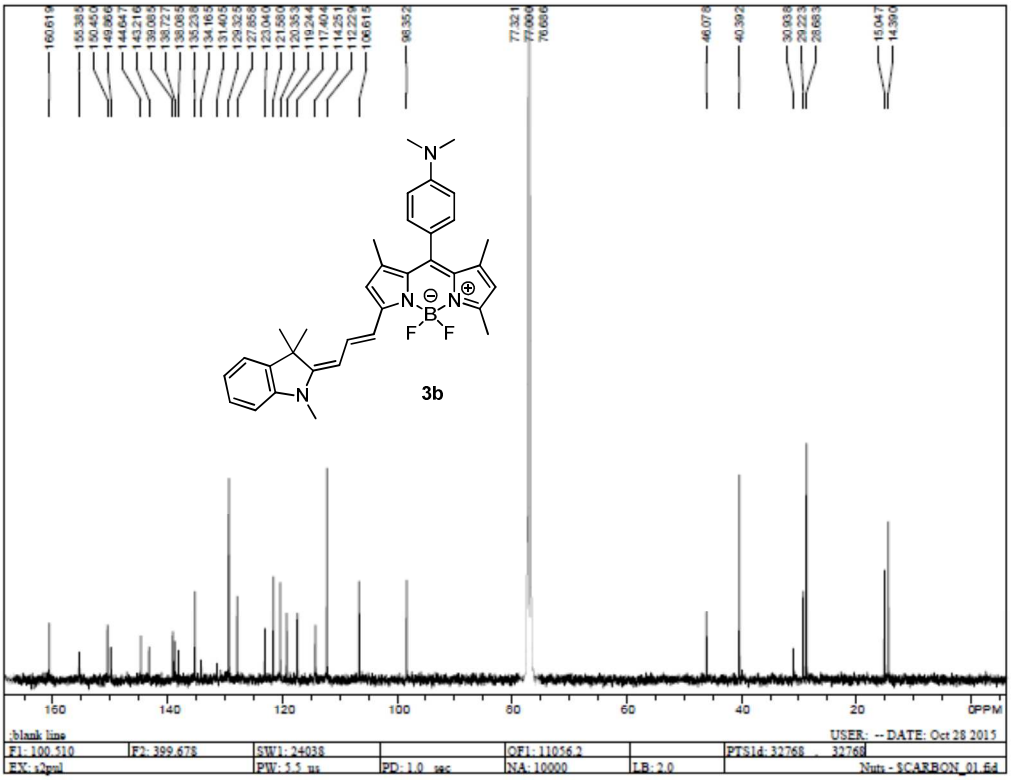

7.260
7.039
7.017
6.620
6.598
5.964
3.347
3.330
3.315
2.546
2.068
2.061
2.052
2.044
2.036
1.558
1.509
1d
1.91
2.01
1.85
4.00
5.98
4.00
5.97
7
6
5
4
3
2
1
0 PPM
;blank line
USER: -- DATE: Oct 23 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.9
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 16
LB: 0.0
Nuts - $PROTON_01.fid

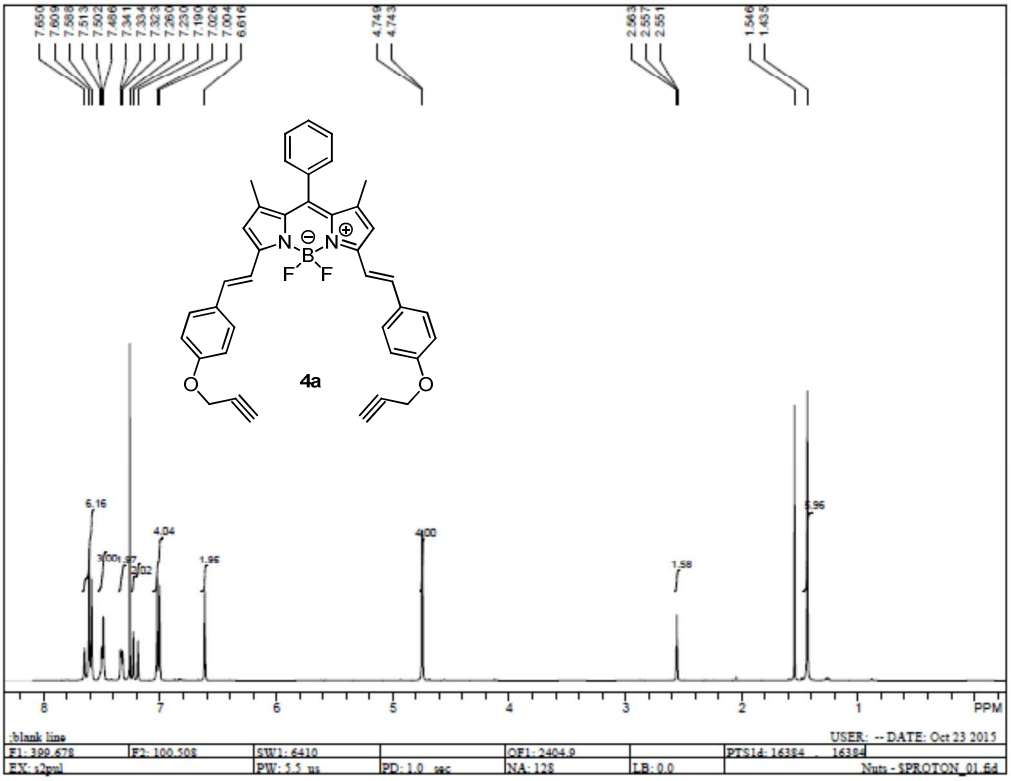

154.553
148.100
143.559
143.246
132.274
128.770
121.135
120.740
111.791
77.321
77.000
76.686
47.531
25.434
14.791
14.536
1d
140
120
100
80
60
40
20
0 PPM
;blank line
USER: -- DATE: Oct 23 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11055.4
PTS1d: 32768 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 728
LB: 2.0
Nuts - $CARBON_01.fid

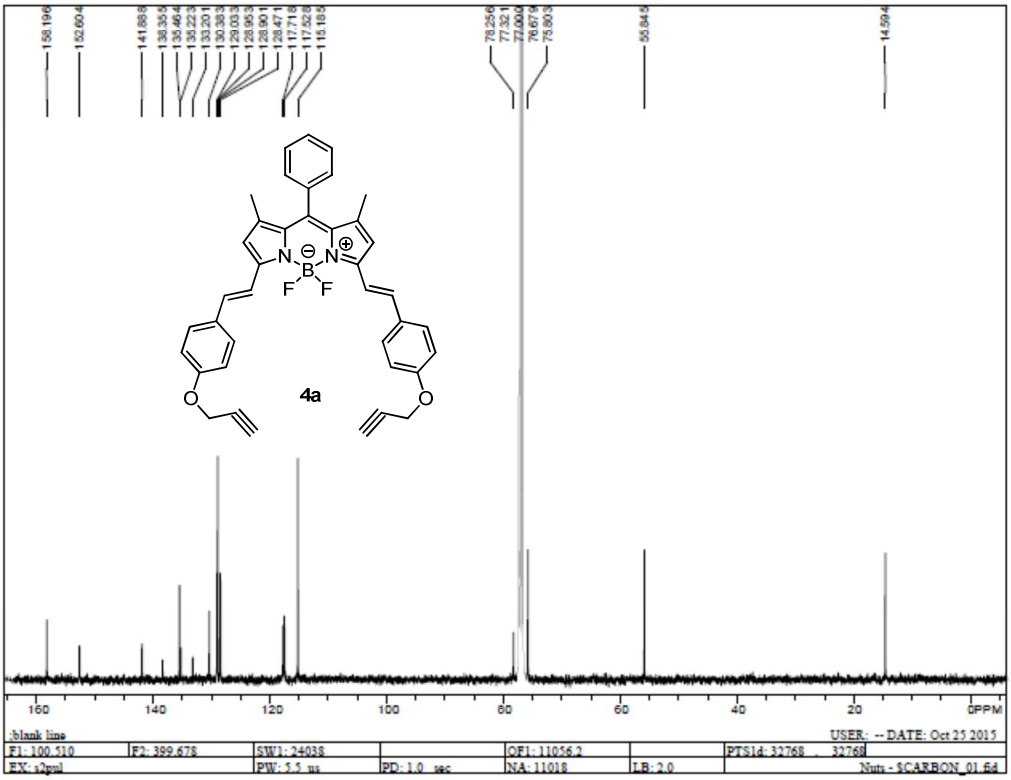

2b
PPM
;blank line
USER: -- DATE: Oct 20 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.9
PTS1d: 16384 . 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 32
LB: 0.0
Nuts - $PROTON_01.fid

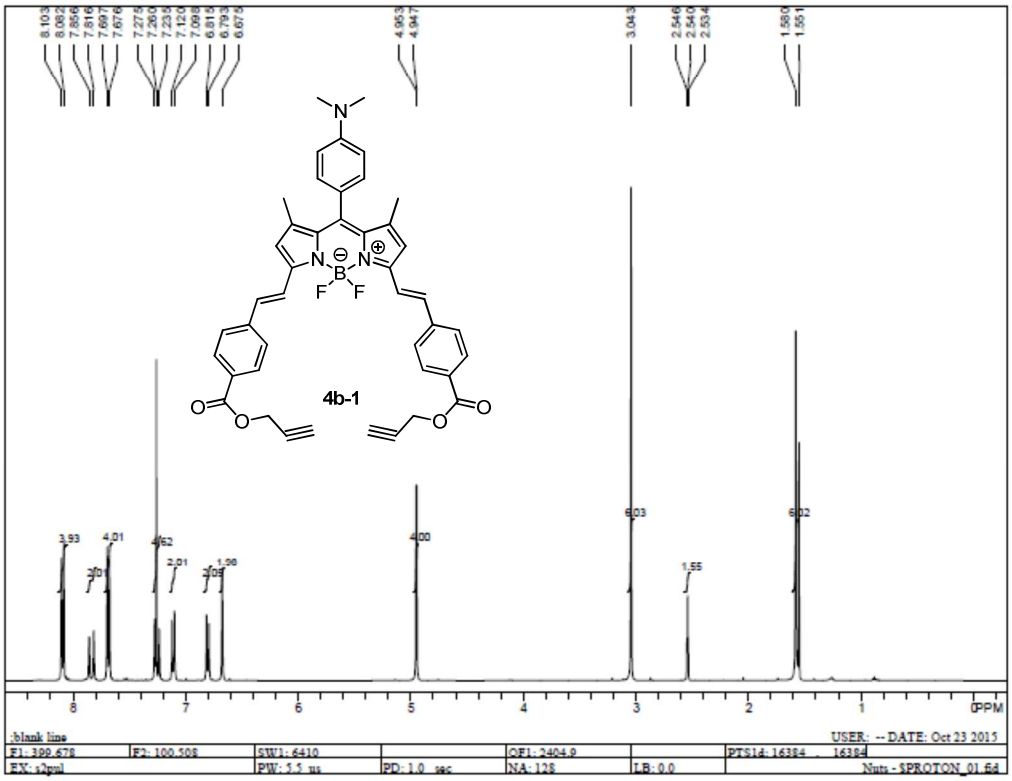

2b
PPM
;blank line
USER: -- DATE: Oct 23 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11115.0
PTS1d: 32768 . 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 11360
LB: 2.0
Nuts - $CARBON_01.fid

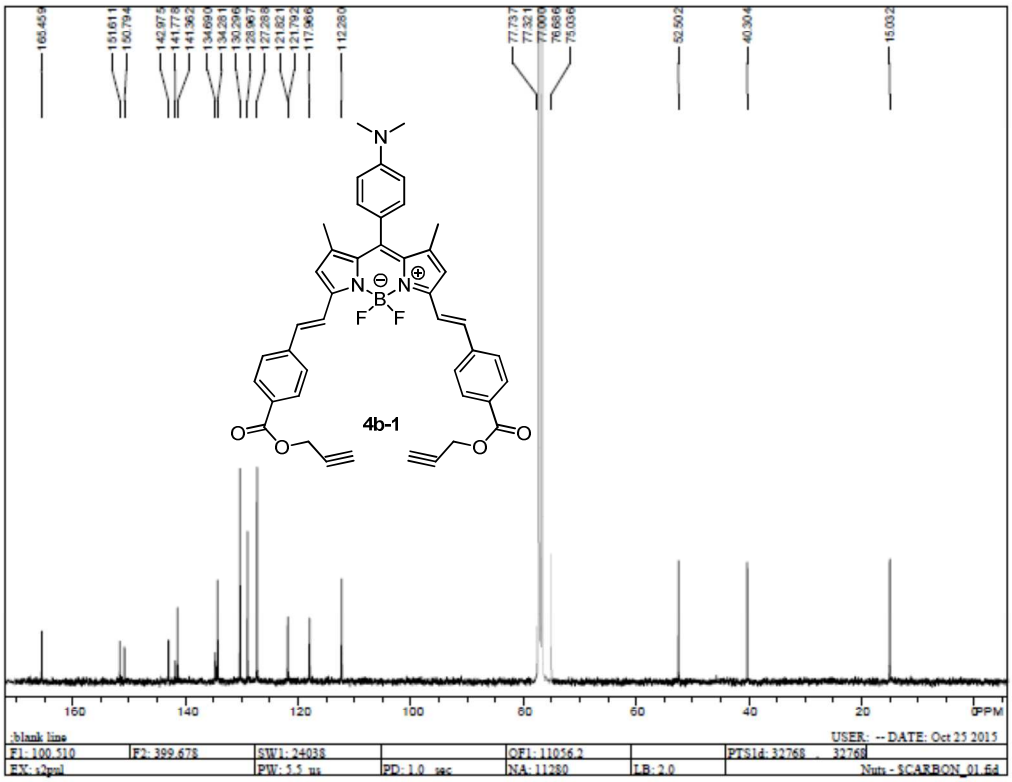

3b
STANDARD FLUORINE PARAMETERS
USER: -- DATE: Jan 7 2015
F1: 499.776 F2: 125.680 SW1: 7996 OF1: 2993.1 PTS1d: 16384 16384
EX: s2pul PW: 4.4 us PD: 1.0 sec NA: 52 LB: 0.0 Nuts - 5hx-1-47-2H500.fid
PPM

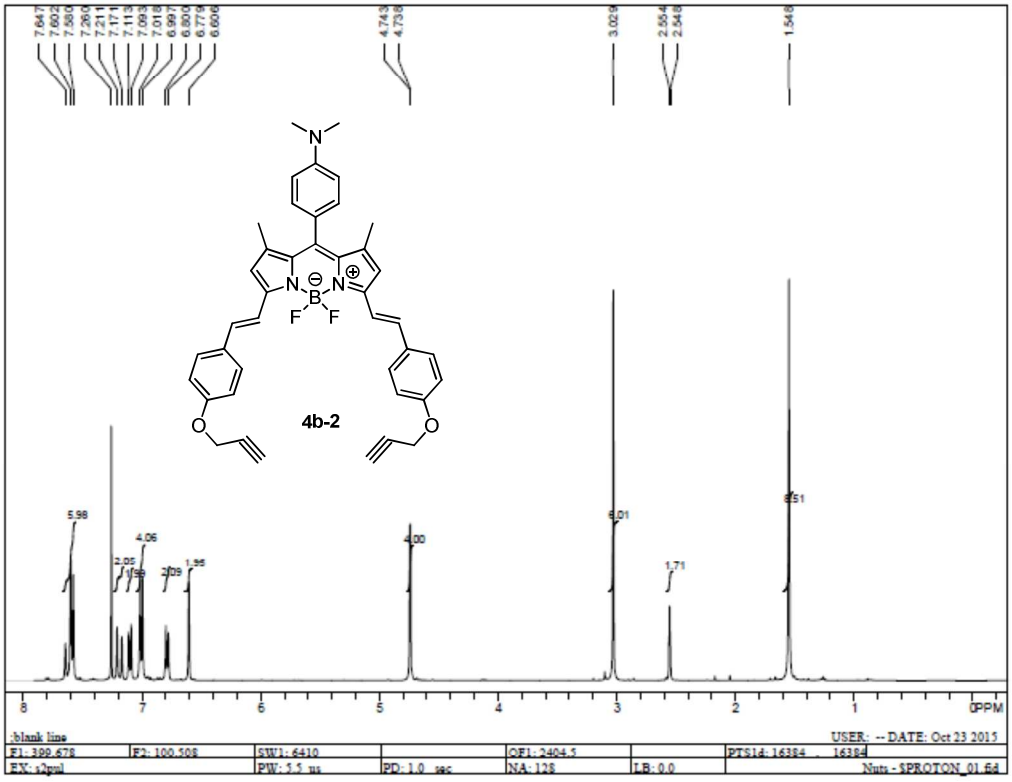

3b
blank line
USER: -- DATE: Oct 28 2015
F1: 100.510 F2: 399.678 SW1: 24038 OF1: 11056.2 PTS1d: 32768 32768
EX: s2pul PW: 5.5 us PD: 1.0 sec NA: 10000 LB: 2.0 Nuts - $CARBON_01.fid
PPM

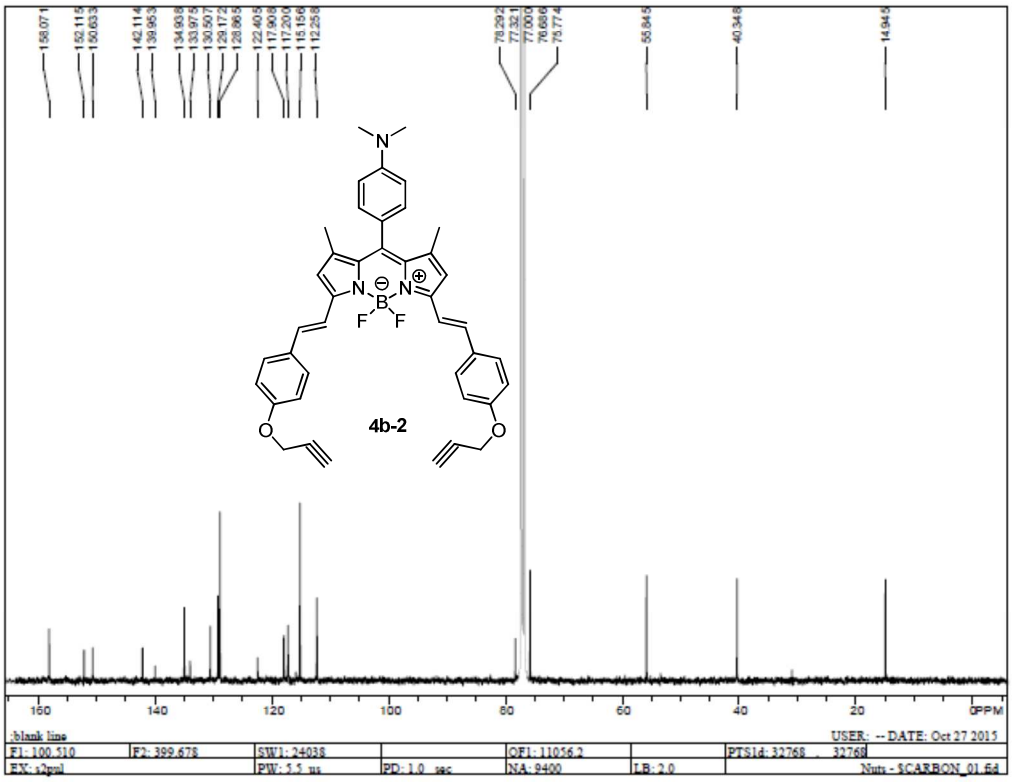

7.650
7.609
7.588
7.513
7.502
7.486
7.341
7.334
7.323
7.260
7.230
7.190
7.026
7.004
6.616
4.749
4.743
2.563
2.557
2.551
1.546
1.435
4a
6.16
4.04
1.96
4.00
1.58
1.96
PPM
;blank line
USER: -- DATE: Oct 23 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.9
PTS1d: 16384 . 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 128
LB: 0.0
Nuts - $PROTON_01.fid

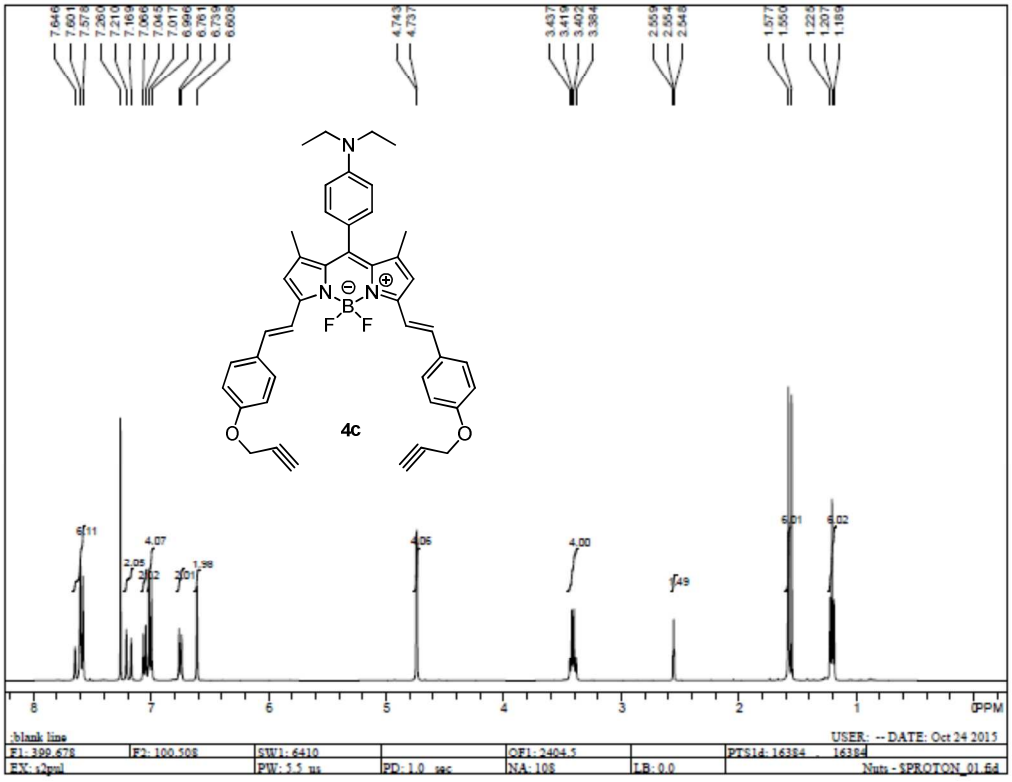

158.196
152.604
141.888
136.305
135.464
135.223
133.201
130.383
129.003
128.901
128.471
117.718
117.528
115.185
78.256
77.321
77.000
76.679
75.803
55.845
14.594
4a
160
140
120
100
60
60
40
20
0PPM
;blank line
USER: -- DATE: Oct 25 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11056.2
PTS1d: 32768 . 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 11018
LB: 2.0
Nuts - $CARBON_01.fid

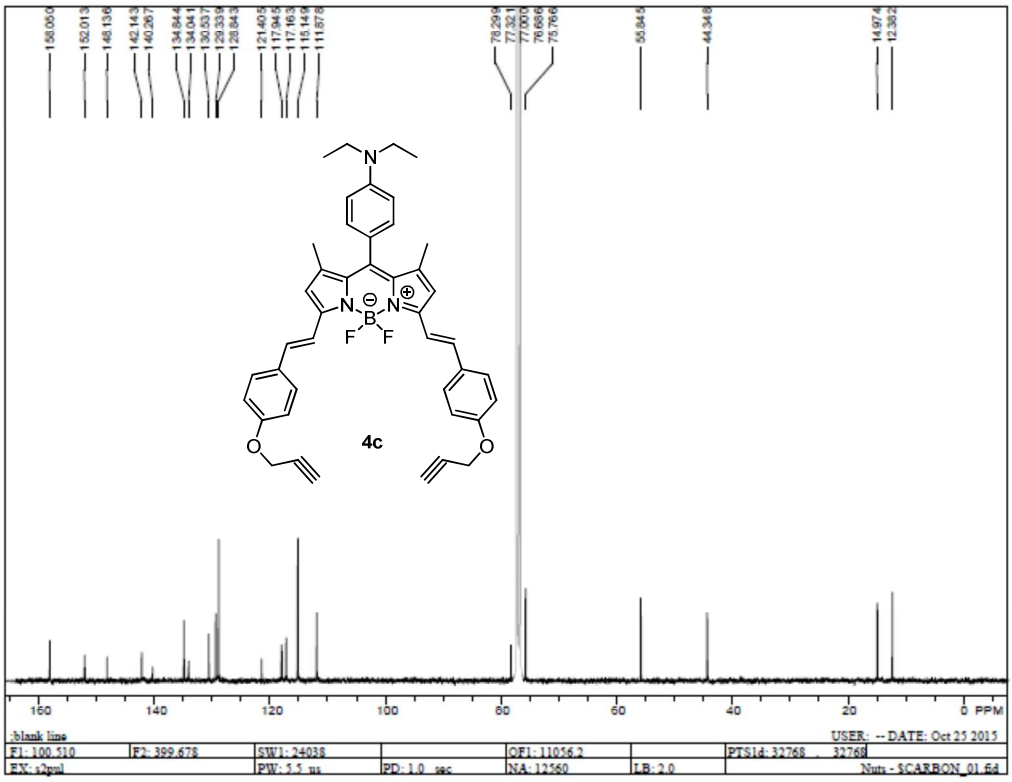

8.103
8.082
7.856
7.816
7.697
7.676
7.275
7.260
7.233
7.120
7.098
6.815
6.793
6.675
4.953
4.947
3.043
2.546
2.540
2.534
1.580
1.551
4b-1
3.93
4.01
4.62
2.01
2.05
1.96
4.00
6.03
1.55
6.02
8
7
6
5
4
3
2
1
0PPM
;blank line
USER: -- DATE: Oct 23 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.9
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 128
LB: 0.0
Nuts - $PROTON_01.fid

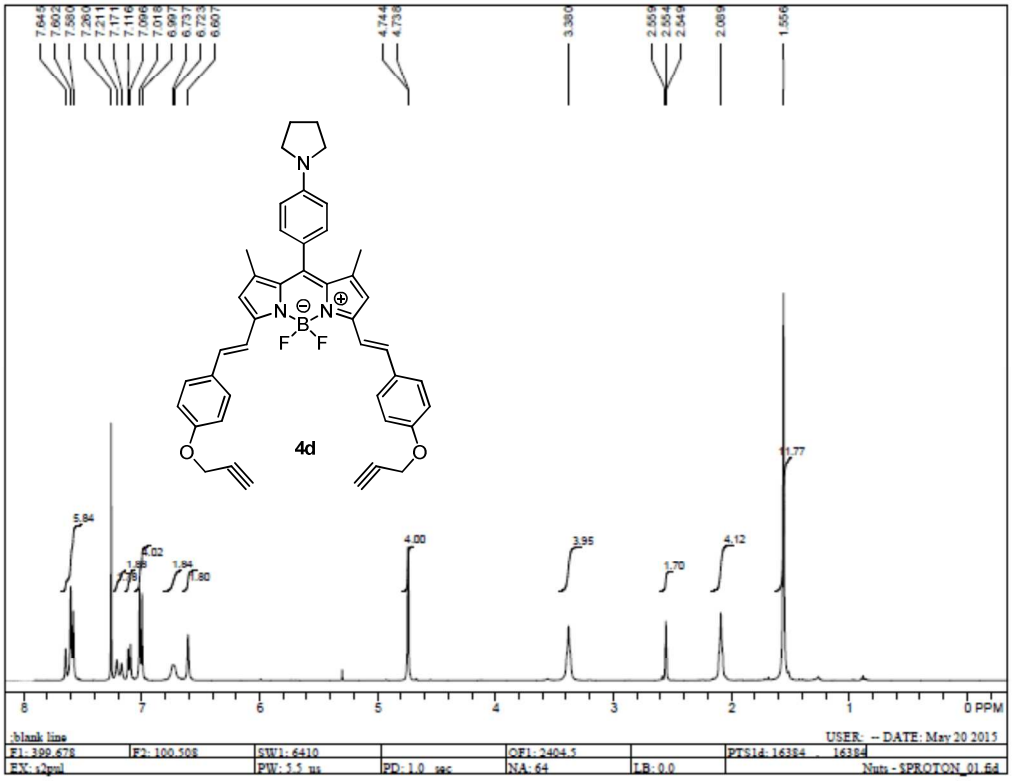

165.459
151.611
150.794
142.975
141.778
141.362
134.690
134.281
130.296
128.967
127.288
121.821
121.792
117.966
112.280
77.737
77.321
77.000
76.686
75.036
52.502
40.304
15.032
4b-1
160
140
120
100
80
60
40
20
0PPM
;blank line
USER: -- DATE: Oct 25 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11056.2
PTS1d: 32768 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 11280
LB: 2.0
Nuts - $CARBON_01.fid

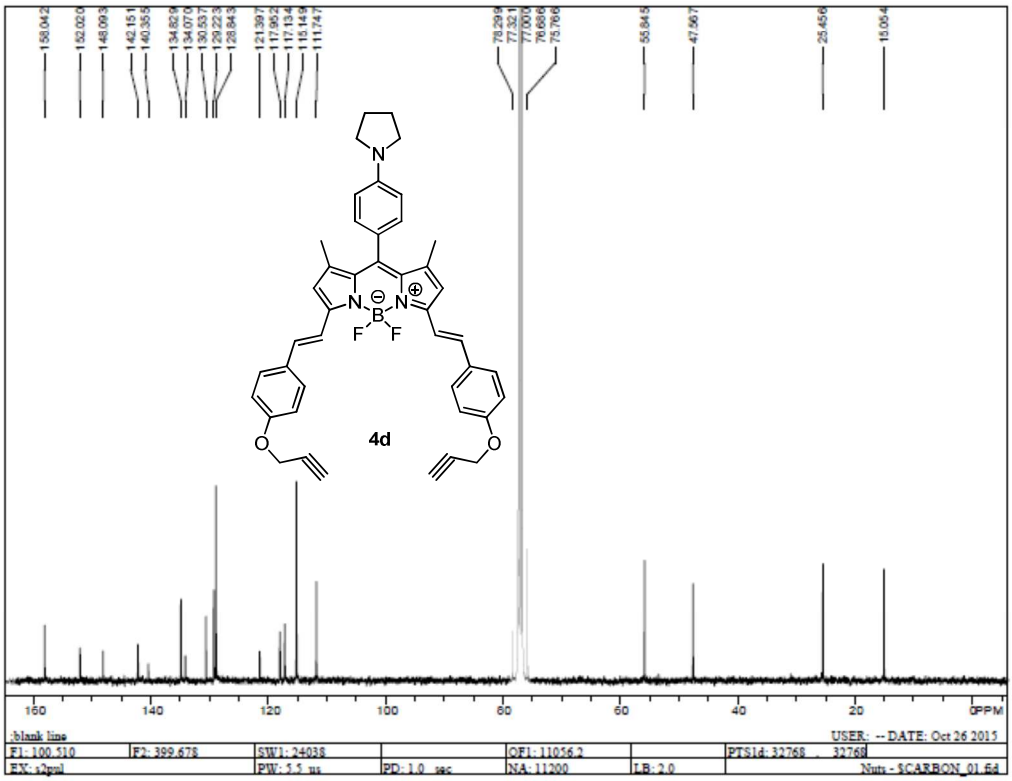

4b-2
;blank line
USER: -- DATE: Oct 23 2015
F1: 399.678 F2: 100.508 SW1: 6410 OF1: 2404.5 PTS1d: 16384 16384
EX: s2pul PW: 5.5 us PD: 1.0 sec NA: 128 LB: 0.0 Nuts - $PROTON_01.fid

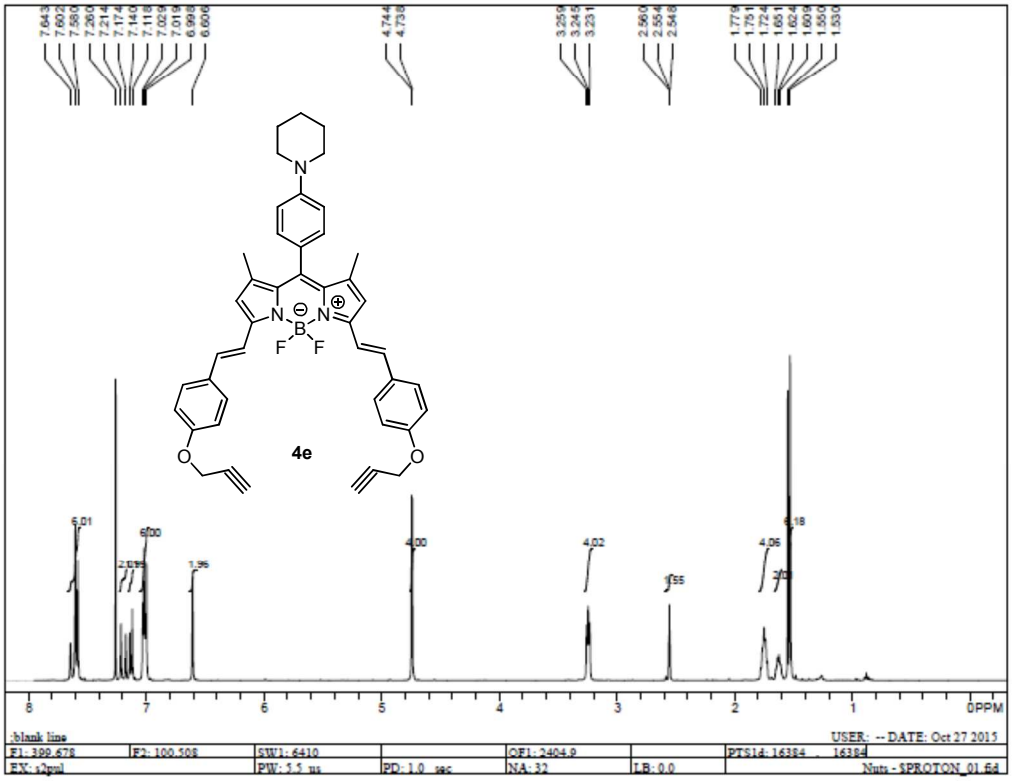

4b-2
;blank line
USER: -- DATE: Oct 27 2015
F1: 100.510 F2: 399.678 SW1: 24038 OF1: 11056.2 PTS1d: 32768 32768
EX: s2pul PW: 5.5 us PD: 1.0 sec NA: 9400 LB: 2.0 Nuts - $CARBON_01.fid

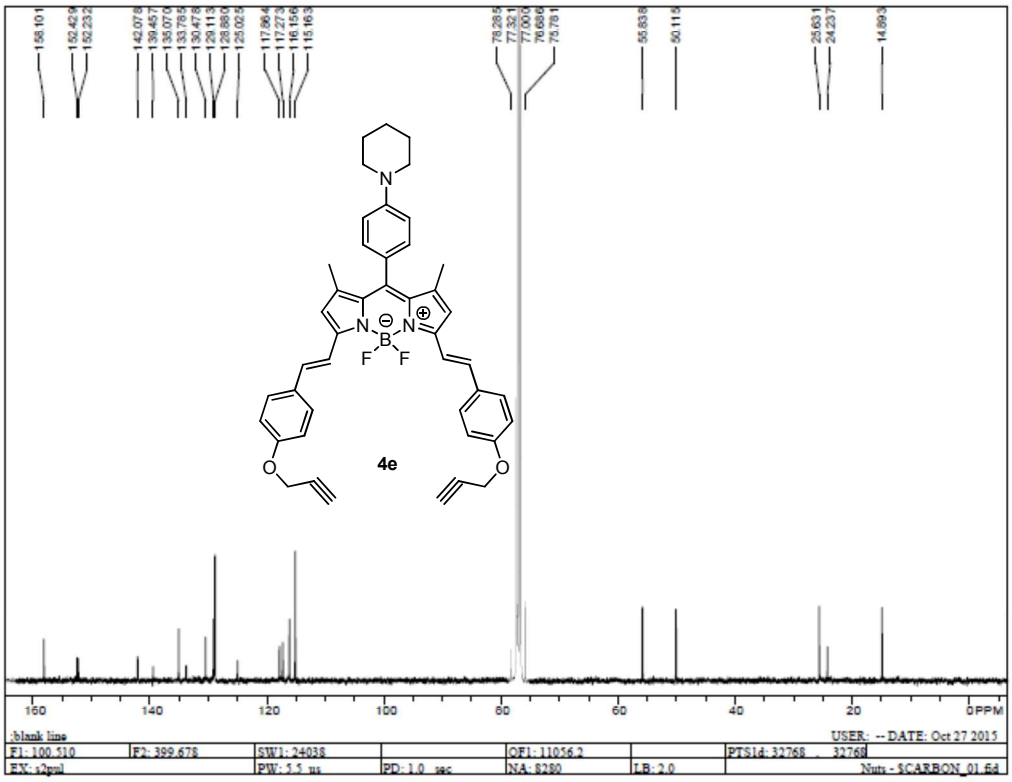

7.646
7.601
7.578
7.260
7.210
7.169
7.066
7.045
7.017
6.996
6.761
6.739
6.608
4.743
4.737
3.437
3.419
3.402
3.384
2.559
2.554
2.548
1.577
1.560
1.225
1.207
1.189
4c
6.11
2.05
2.02
4.07
2.01
1.99
4.06
4.00
1.49
6.01
6.02
8
7
6
5
4
3
2
1
0PPM
;blank line
USER: -- DATE: Oct 24 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.5
PTS1d: 16384 . 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 108
LB: 0.0
Nuts - $PROTON_01.fid

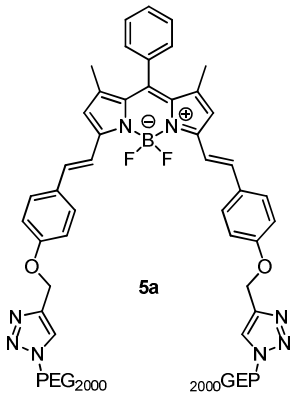

158.050
152.013
148.136
142.143
140.267
134.844
134.041
130.537
129.339
128.843
121.405
117.945
117.163
115.149
111.678
78.299
77.321
77.000
76.686
75.766
55.845
44.348
14.974
12.392
4c
160
140
120
100
60
60
40
20
0 PPM
;blank line
USER: -- DATE: Oct 25 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11056.2
PTS1d: 32768 . 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 12560
LB: 2.0
Nuts - $CARBON_01.fid


4d
5.84
4.00
3.95
1.70
4.12
11.77
8
7
6
5
4
3
2
1
0 PPM

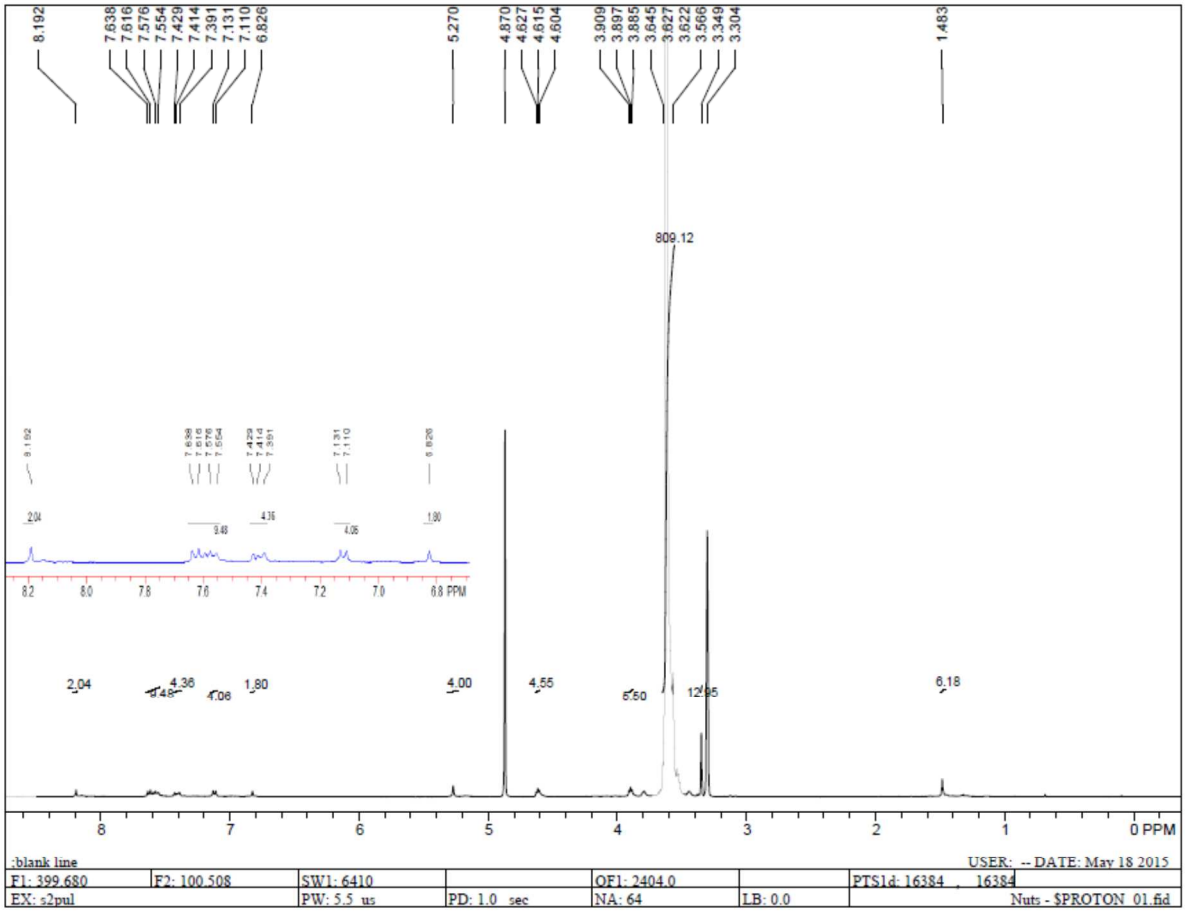

4d
160
140
120
100
80
60
40
20
0PPM

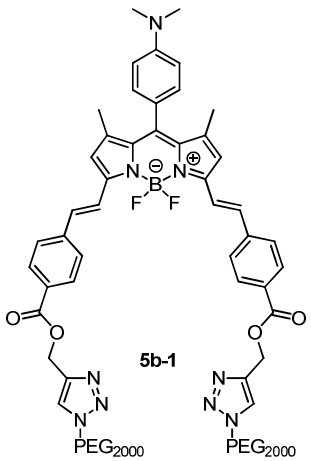

4e
6.01
6.00
1.95
4.00
4.02
3.55
4.06
6.18
8
7
6
5
4
3
2
1
0PPM
;blank line
USER: -- DATE: Oct 27 2015
F1: 399.678
F2: 100.508
SW1: 6410
OF1: 2404.9
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 32
LB: 0.0
Nuts - $PROTON_01.fid

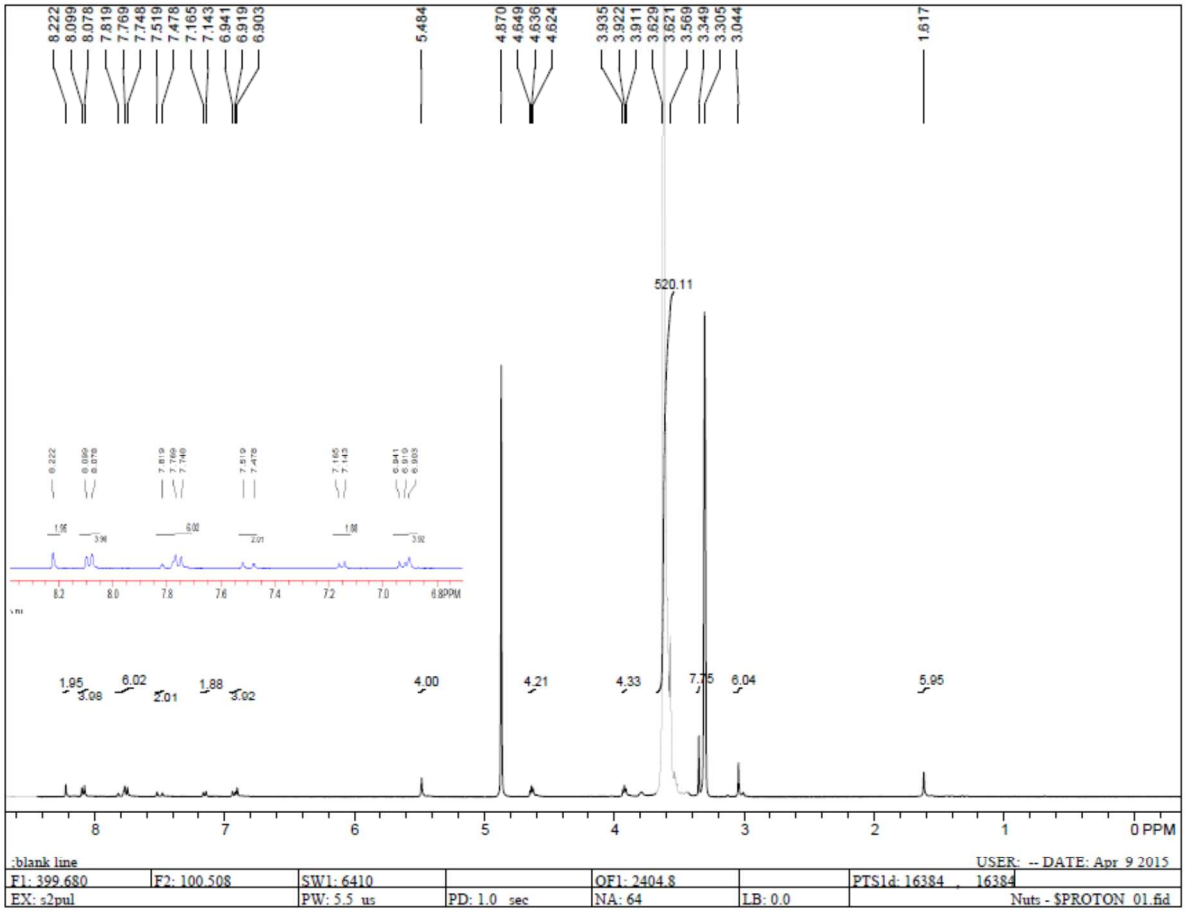

4e
158.101
152.429
152.232
142.078
139.457
135.070
133.785
130.478
129.113
128.880
125.025
117.864
117.273
116.156
115.163
78.285
77.321
77.000
76.686
75.781
55.838
50.115
25.631
24.237
14.693
160
140
120
100
80
60
40
20
0PPM
;blank line
USER: -- DATE: Oct 27 2015
F1: 100.510
F2: 399.678
SW1: 24038
OF1: 11056.2
PTS1d: 32768 32768
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 8280
LB: 2.0
Nuts - $CARBON_01.fid

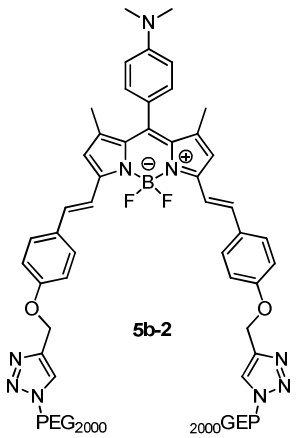

PEG2000
2000GEP

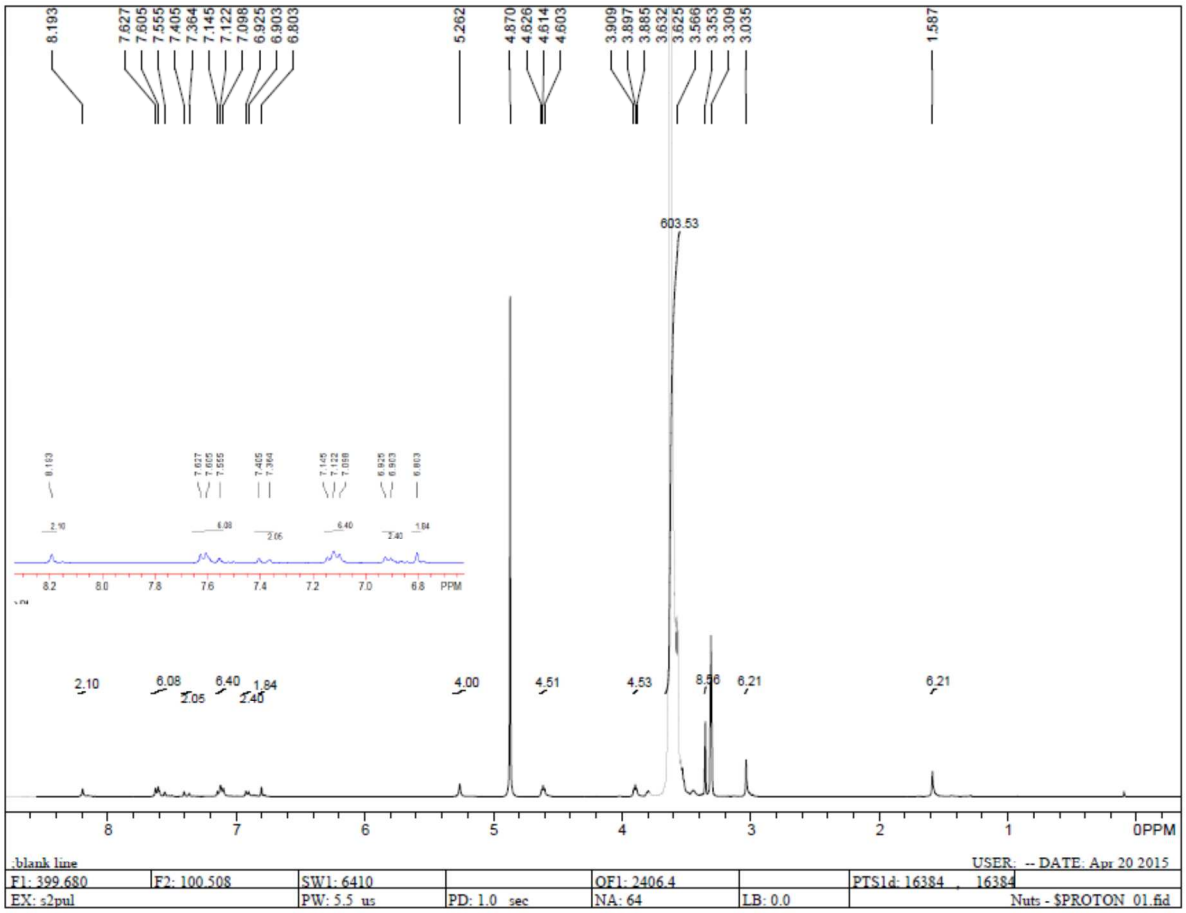
5a

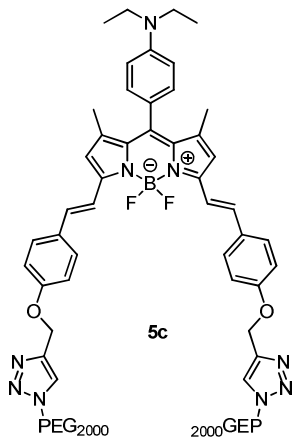

8.192
7.638
7.616
7.576
7.554
7.429
7.414
7.391
7.131
7.110
6.826
5.270
4.870
4.627
4.615
4.604
3.909
3.897
3.885
3.645
3.627
3.622
3.566
3.349
3.304
1.483
809.12
2.04
4.36
1.80
4.00
4.55
5.50
12.95
6.18
0 PPM
:blank line
USER: -- DATE: May 18 2015
F1: 399.680
F2: 100.508
SW1: 6410
OF1: 2404.0
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 64
LB: 0.0
Nuts - $PROTON_01.fid

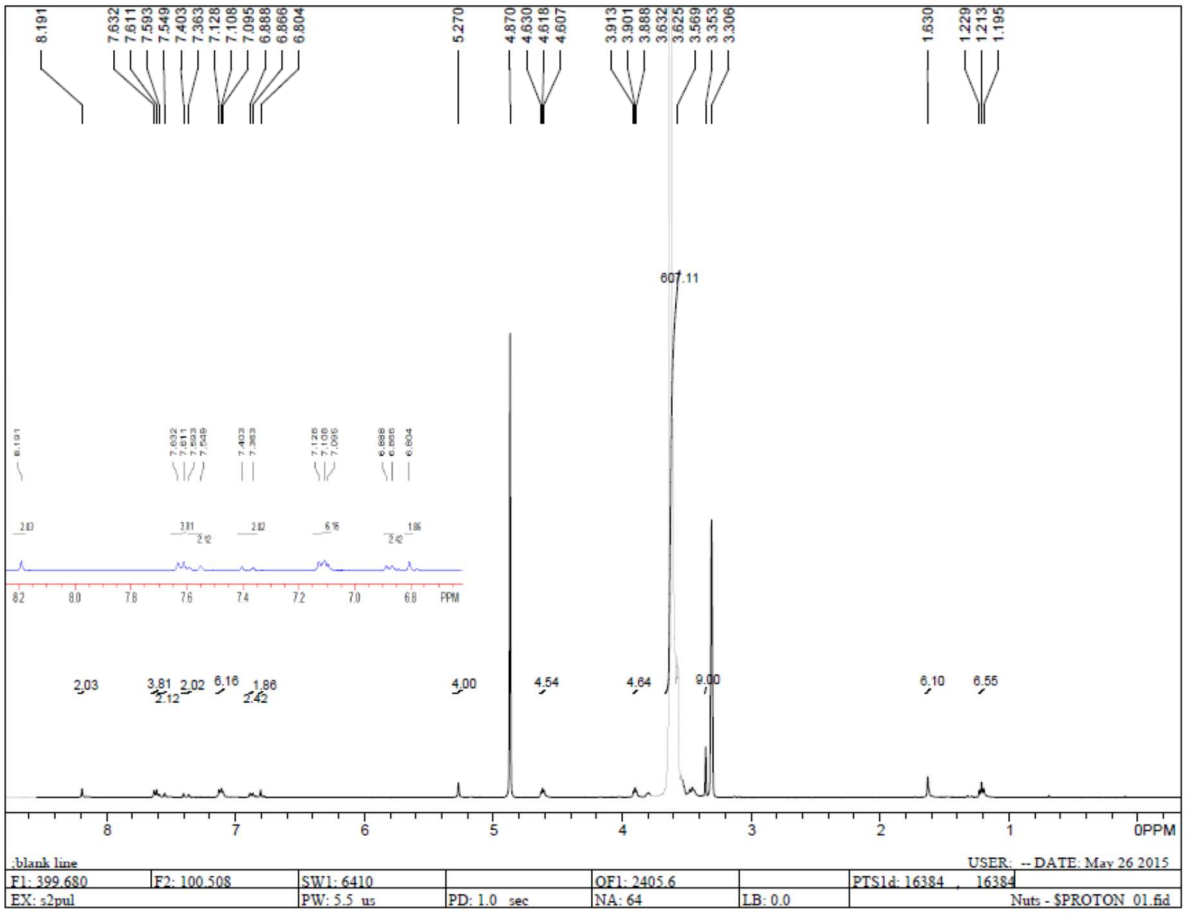

5b-1
PEG2000
PEG2000

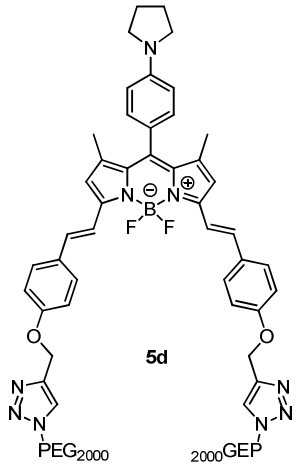

8.222
8.099
8.078
7.819
7.769
7.748
7.519
7.478
7.165
7.143
6.941
6.919
6.903
5.484
4.870
4.649
4.636
4.624
3.935
3.922
3.911
3.629
3.621
3.569
3.349
3.305
3.044
1.617
520.11
1.95
3.08
6.02
2.01
1.88
3.02
4.00
4.21
4.33
7.75
6.04
5.95
8
7
6
5
4
3
2
1
0 PPM
:blank line
USER: -- DATE: Apr 9 2015
F1: 399.680
F2: 100.508
SW1: 6410
OF1: 2404.8
PTS1d: 16384 , 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 64
LB: 0.0
Nuts - $PROTON_01.fid


5b-2
PEG2000
2000GEP


8.193
7.627
7.605
7.555
7.405
7.364
7.145
7.122
7.098
6.925
6.903
6.803
5.262
4.870
4.626
4.614
4.603
3.909
3.897
3.885
3.632
3.625
3.566
3.353
3.309
3.035
1.587
603.53
2.10
6.08
2.05
6.40
1.84
2.40
4.00
4.51
4.53
8.56
6.21
6.21
PPM
0PPM
.blank line
USER: -- DATE: Apr 20 2015
F1: 399.680
F2: 100.508
SW1: 6410
OF1: 2406.4
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 64
LB: 0.0
Nuts - $PROTON_01.fid


5c
PEG2000
2000GEP


8.191
7.632
7.611
7.593
7.549
7.403
7.363
7.128
7.108
7.095
6.888
6.866
6.804
5.270
4.870
4.630
4.618
4.607
3.913
3.901
3.888
3.632
3.625
3.569
3.353
3.306
1.630
1.229
1.213
1.195
607.11
2.03
3.81
2.12
2.02
6.16
1.86
1.42
4.00
4.54
4.64
9.00
6.10
6.55
PPM
0PPM
.blank line
USER: -- DATE: May 26 2015
F1: 399.680
F2: 100.508
SW1: 6410
OF1: 2405.6
PTS1d: 16384 16384
EX: s2pul
PW: 5.5 us
PD: 1.0 sec
NA: 64
LB: 0.0
Nuts - $PROTON_01.fid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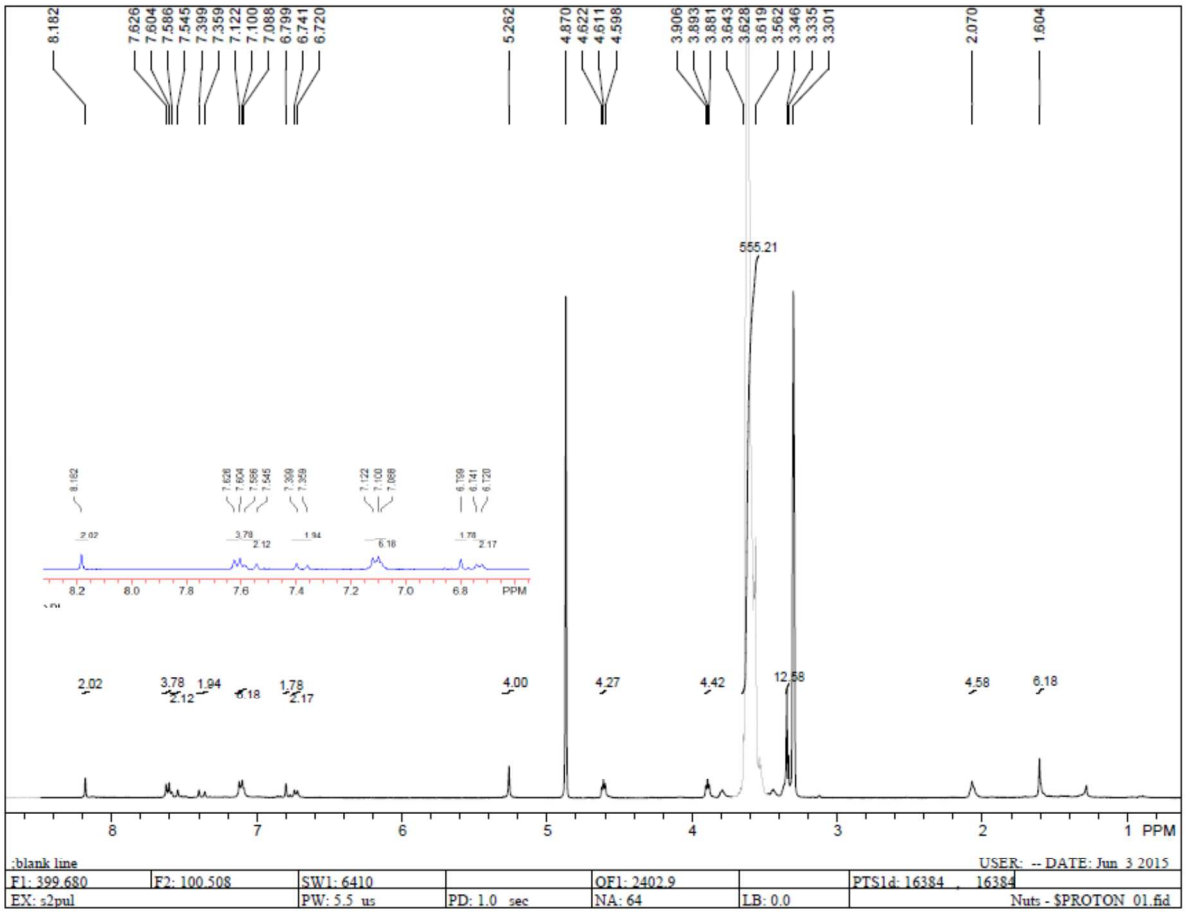
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4.42
12.58
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6.18
8
7
6
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4
3
2
1 PPM
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F2: 100.508
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OF1: 2402.9
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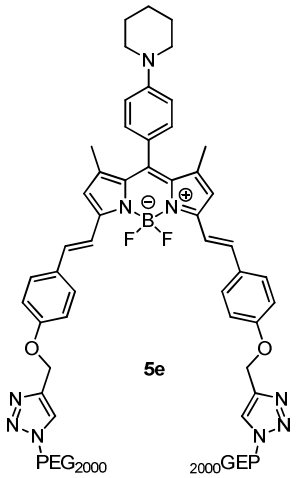
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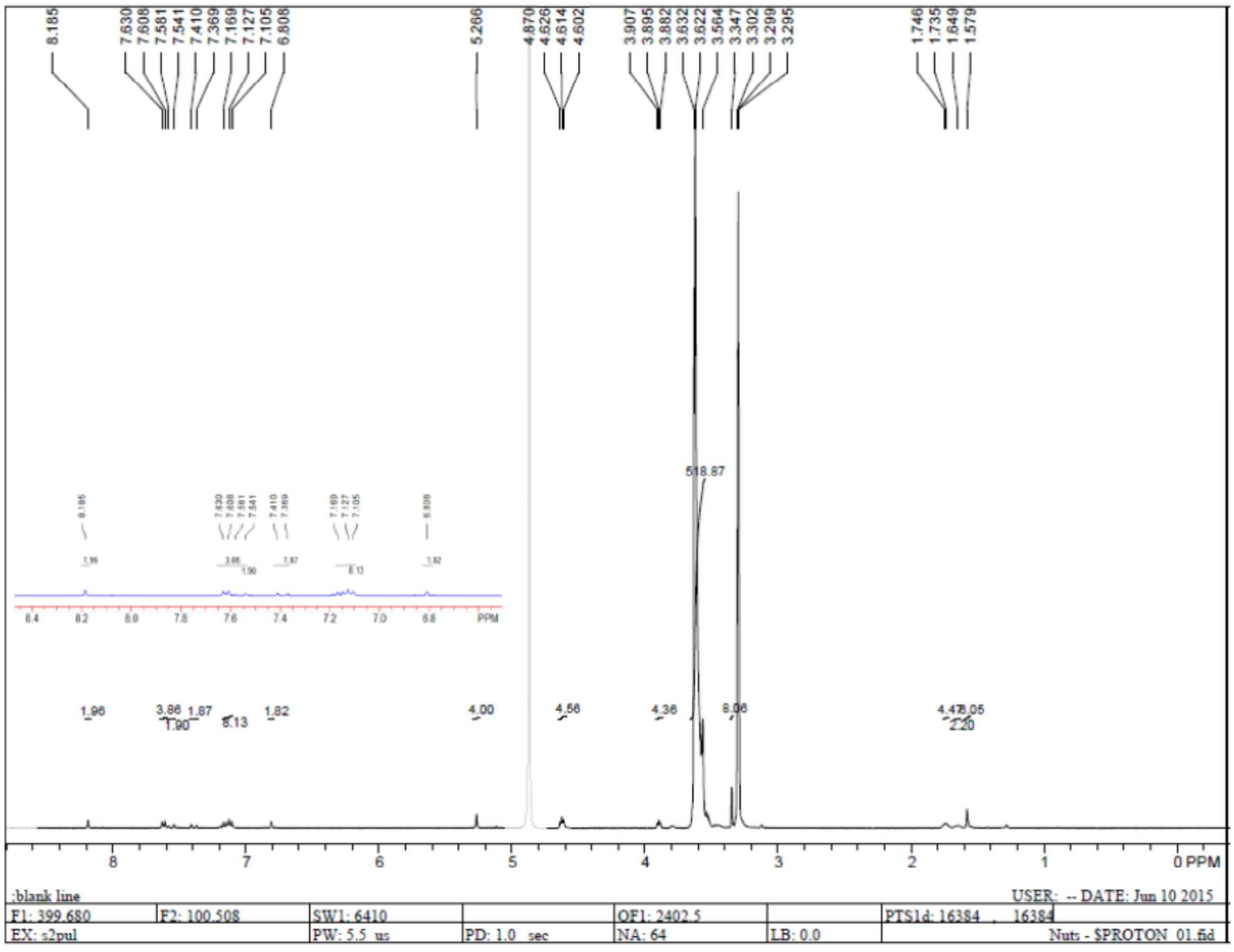
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9. References

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