N-Pyridineium-2-yl Darrow Red Analogue: Unique Near-Infrared Lysosome-Biomarker for the Detection of Cancer Cells

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1. Figures

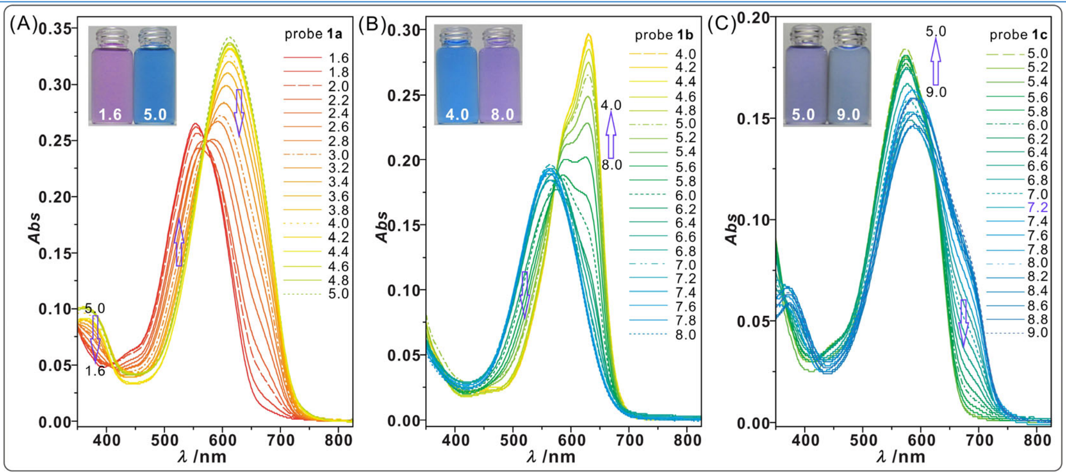

Figure S1. Absorption properties of probes **1a**–**1c** (10 μM) in various disodium hydrogen phosphate-citric acid buffers with different pH containing 10% DMSO: (A) probe **1a**; (B) probe **1b**; (C) probe **1c**. The insets are pH-dependent photographs of the samples.

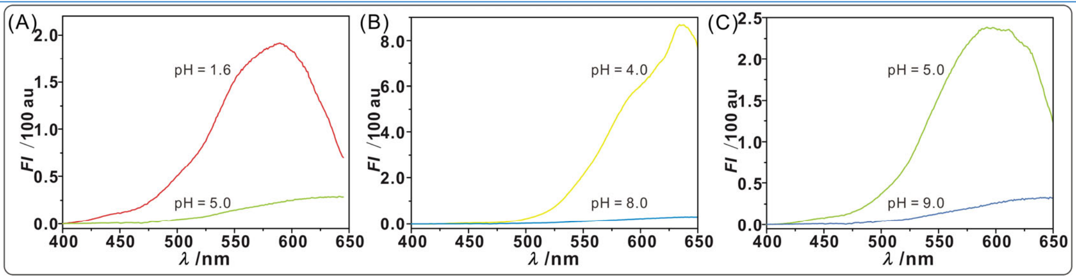

Figure S2. Excitation spectra of probes **1a**–**1c** (10 μM) under acidic and basic conditions. (A) probe **1a**, λ_{em} = 657 nm; (B) probe **1b**, λ_{em} = 668 nm; (C) probe **1c**, λ_{em} = 663 nm.

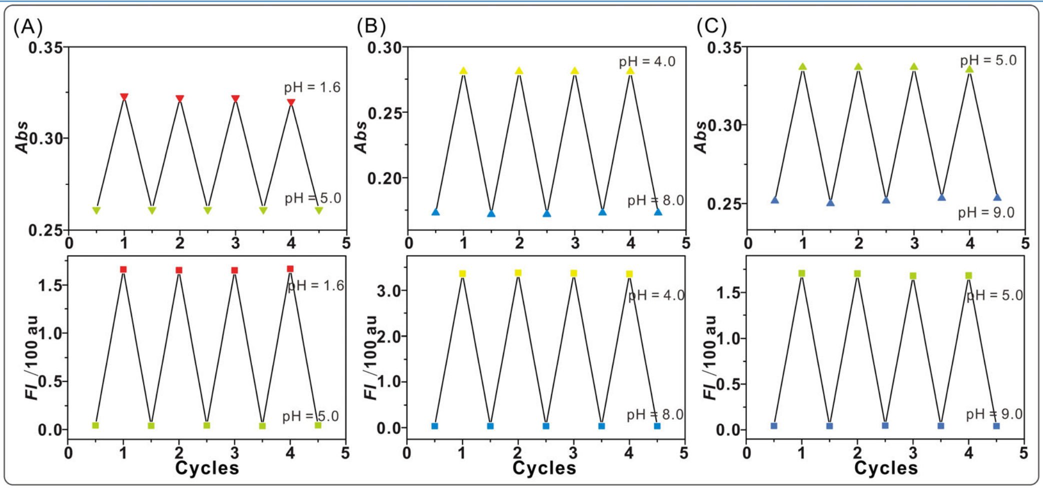

Figure S3. The reversible absorption (top) and emission (bottom) responses of probes **1a**–**1c** with changes between basic and acidic conditions. (A) probe **1a**; (B) probe **1b**; (C) probe **1c**.

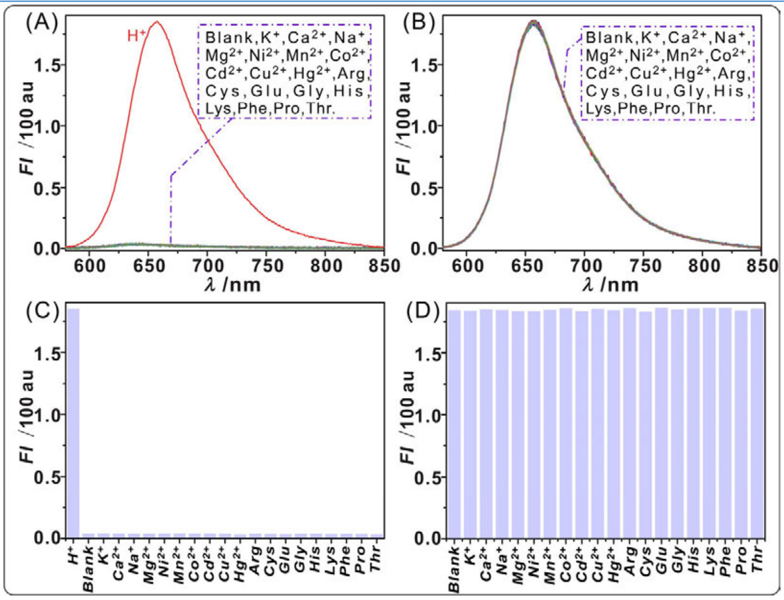

Figure S4. Fluorescence responses of **1a** (10 μM) towards different analytes in buffer solutions containing 10% DMSO; K^+ (100 mM), Ca^{2+} (0.5 mM), Na^+ (100 mM), Mg^{2+} (0.5 mM), Ni^{2+} (0.3 mM), Mn^{2+} (0.3 mM), Co^{2+} (0.3 mM), Cd^{2+} (0.3 mM), Cu^{2+} (0.3 mM), Hg^{2+} (0.3 mM), Arg (0.1 mM), Cys (0.1 mM), Glu (0.1 mM), Gly (0.1 mM), His (0.1 mM), Lys (0.1 mM), Phe (0.1 mM), Pro (0.1 mM) and Thr (0.1 mM) were included. (A, C) Tested in disodium hydrogen phosphate-citric acid buffer (pH 9.0); (B, D) Tested in citric acid buffer (pH 1.6).

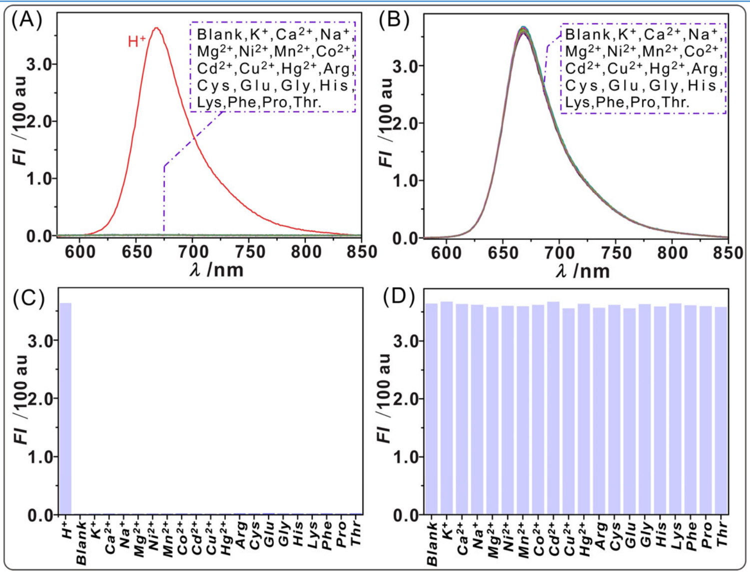

Figure S5. Fluorescence responses of **1b** (10 μM) towards different analytes in buffer solutions containing 10% DMSO; K^+ (100 mM), Ca^{2+} (0.5 mM), Na^+ (100 mM), Mg^{2+} (0.5 mM), Ni^{2+} (0.3 mM), Mn^{2+} (0.3 mM), Co^{2+} (0.3 mM), Cd^{2+} (0.3 mM), Cu^{2+} (0.3 mM), Hg^{2+} (0.3 mM), Arg (0.1 mM), Cys (0.1 mM), Glu (0.1 mM), Gly (0.1 mM), His (0.1 mM), Lys (0.1 mM), Phe (0.1 mM), Pro (0.1 mM) and Thr (0.1 mM) were included. (A, C) Tested in disodium hydrogen phosphate-citric acid buffer (pH 9.0); (B, D) Tested in citric acid buffer (pH 1.6).

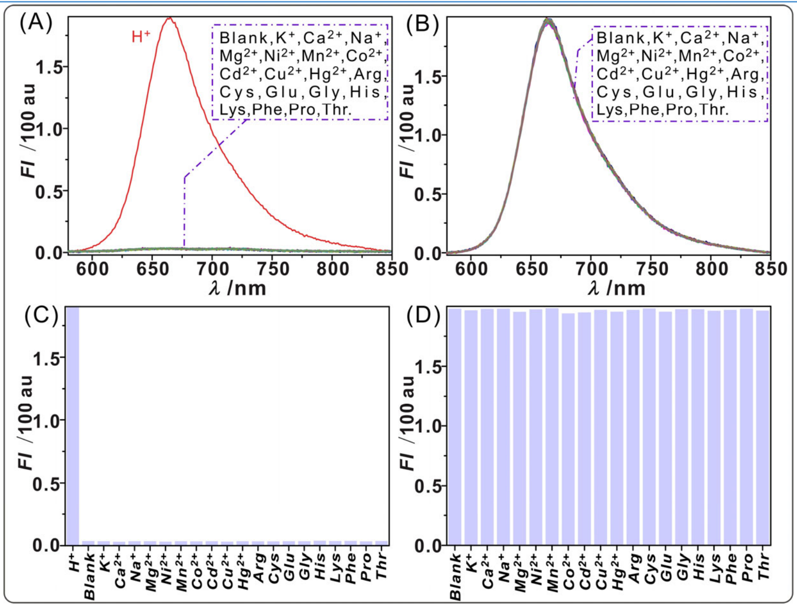

Figure S6. Fluorescence responses of **1c** (10 μM) towards different analytes in buffer solutions containing 10% DMSO; K^+ (100 mM), Ca^{2+} (0.5 mM), Na^+ (100 mM), Mg^{2+} (0.5 mM), Ni^{2+} (0.3 mM), Mn^{2+} (0.3 mM), Co^{2+} (0.3 mM), Cd^{2+} (0.3 mM), Cu^{2+} (0.3 mM), Hg^{2+} (0.3 mM), Arg (0.1 mM), Cys (0.1 mM), Glu (0.1 mM), Gly (0.1 mM), His (0.1 mM), Lys (0.1 mM), Phe (0.1 mM), Pro (0.1 mM) and Thr (0.1 mM) were included. (A, C) Tested in disodium hydrogen phosphate-citric acid buffer (pH 9.0); (B, D) Tested in citric acid buffer (pH 1.6).

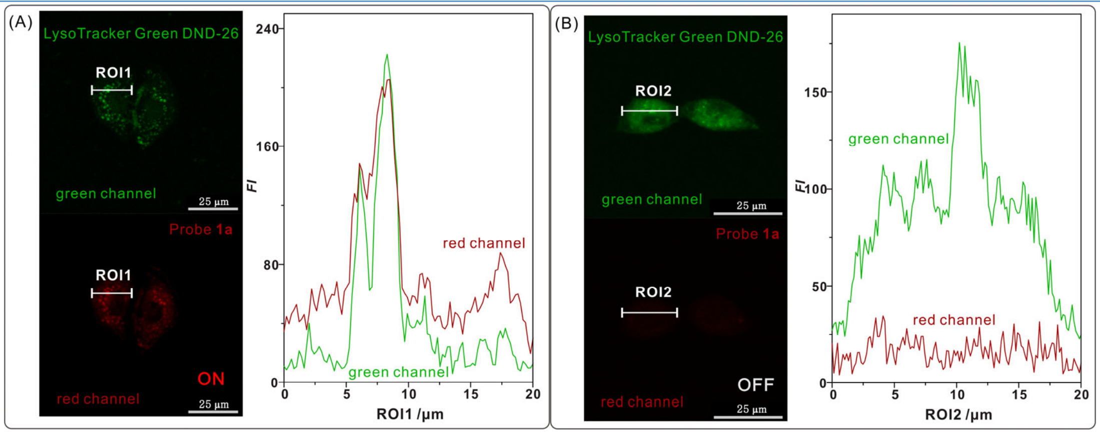

Figure S7. Fluorescence intensities in regions of interest (ROI1 and ROI2) of KB cancer cells (A) and V79 normal cells (B) with LysoTracker Green DND-26 and probe **1a** in green and red channels.

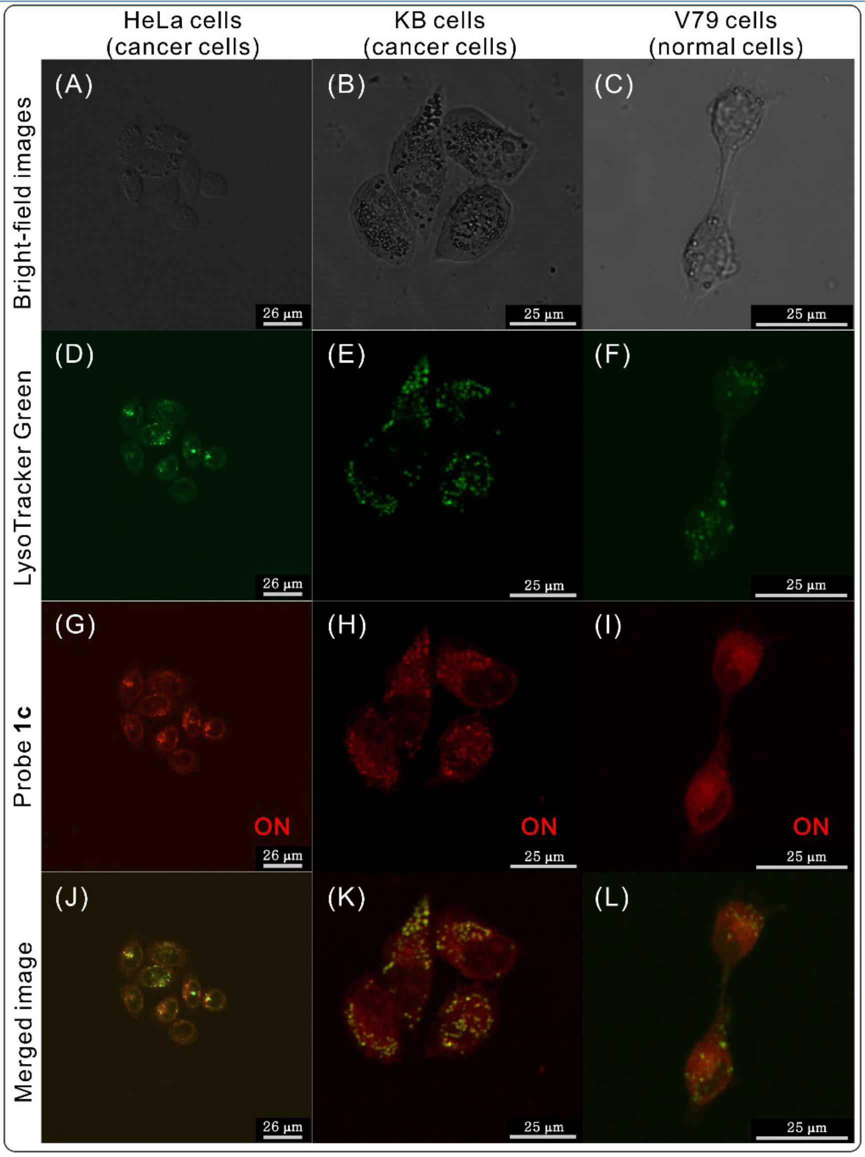

Figure S8. Fluorescence confocal images of HeLa (A, D, G, H), KB (B, E, H, K) and V79 (C, F, I, L) cells with probe **1c** and standard LysoTracker. (A–C) bright-field transmission images; (D–F) confocal images (green channel) of cells with LysoTracker Green DND-26 (50 nM); (G–I) confocal images (red channel) of cells with probe **1c** (10 μM); (J–L) merged images of green and red channels. Green channel emission was collected in 505–550 nm upon excitation at 488 nm, and red channel emission was collected in 590–795 nm upon excitation at 561 nm.

2. Experimental section

2.1. Materials and apparatus

All reagents and solvents (synthetic or analytical grade) were purchased from TCI Development Co., Ltd. (Tokyo, Japan), Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Energy Chemical Co., Ltd. (Shanghai, China). *N*-Ethyl-2-methyl-4-nitrosoaniline hydrochloride was prepared by the reported method.[1] The anion-exchange resin

(717 anion exchange resin) was washed to the chloride salt form before use. Disodium hydrogen phosphate-citric acid buffer (pH 1.6–9.0) were prepared with 0.2 M disodium hydrogen phosphate and 0.1 M citric acid by the reported way,[2] and theoretical pH values of the buffers were used for the buffer solution containing 10% (v/v) dimethyl sulfoxide (DMSO).

^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian 300–600 MHz spectrometers, and solvent peak or TMS were used as an internal standard, which were underlined in supporting spectra. High-resolution mass spectra were recorded on a Finnigan MAT95 mass spectrometer (ESI^{+}). Infrared (IR) spectra were recorded on a Nicolet 5200 IR spectrometer using solid samples dispersed in KBr pellets. Metling points were determined on an X-4 microscope electron thermal apparatus (Taike, China).

UV-vis spectra were obtained with a Shimadzu UV-1800 spectrophotometer. Fluorescence emission spectra were performed with fused quartz cuvette (10 mm × 10 mm) on Shimadzu RF-5301PC spectroscope with R-928 photomultiplier at room temperature; the excitation and emission slit widths are set at 1.5 nm and 3 nm respectively. The pH values were measured with a Lei-Ci (pH-3C) digital pH meter (Shanghai, China) using a combined glass-calomel electrode.

Cells were cultured in Roswell Park Memorial Instrument (RPMI-1640). Fluorescence confocal images were recorded with Leica TCS SP5 II and PerkinElmer UltraView VoX confocal laser scanning microscopes, the images were handled with their attached software.

2.2. Synthesis

2.2.1. 9-(Ethylamino)-10-methylbenzo[*a*]phenoxazin-7-ium Nitrate (4)

To a boiling solution of 2-naphthol (12.82 g, 88.9 mmol) and anhydrous zinc chloride (6.82 g, 50.1 mmol) in anhydrous ethanol (340 mL), *N*-ethyl-2-methyl-4-nitrosoaniline hydrochloride (17.84 g, 88.9 mmol) was added in small portions within one hour, and the mixture was heated under reflux for two more hours. The dark red precipitate was filtered from the hot reaction mixture, *N*-monoalkyl Meldola's Blue with zinc chloride was obtained as red powder, 20.80 g, yield 72.0%. The above product was dissolved in hot water (1.25 L), and filtrated to remove insoluble impurities, then concentrated nitric acid (52 mL) was added dropwise, the solution was stood at room temperature overnight. The nitrate product was collected by filtration and dried. Red amorphous solid, 17.12 g, yield 76.0%, overall yield 54.7%, mp 193.0–195.0 °C. IR ν (KBr, cm^{-1}): 3424, 3151, 2026, 1709, 1640, 1385, 1110. ^{1}H NMR (400 MHz, CD_3OD) δ 9.04 (d, *J* = 8.3 Hz, 1H), 8.48 (d, *J* = 9.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 7.95–7.81 (m, 4H), 7.34 (s, 1H), 3.86 (q, *J* = 7.4 Hz, 2H), 2.55 (s, 3H), 1.49 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 162.6, 151.9, 145.5, 145.0, 139.5, 138.3, 134.5, 133.3, 132.9, 131.5, 130.9, 130.1, 129.5, 123.9, 117.0, 96.0, 41.6, 17.9, 14.0. HRMS-ESI^{+}: m/z = 289.1345 (calcd for $[M\text{-}NO_3^{-}]^{+}$, 289.1341).

2.2.2. *N*-Ethyl-10-methyl-5-(pyridin-2-ylimino)-5H-benzo[*a*]phenoxazin-9-amine (5a)

A solution of compound **4** (3513.6 mg, 10.0 mmol), 2-aminopyridine (2823.3 mg, 30.0 mmol) and ethanol (420 mL) was reflux for 24 h. The solvent was removed, and the residue was purified by column chromatography on silica gel eluenting with methanol/chloroform (v/v = 1/70) to give compound **5a**. Dark red solid, 1032.8 mg, yield 27.1%, mp 239.0–241.0 °C. IR ν (KBr, cm^{-1}): 3441, 2920, 2344, 2027, 1600, 1577, 1385, 1306, 1130; ^{1}H NMR (400 MHz, *$CDCl_3$:CD_3OD = 8:1 (v/v)*) δ 8.63–8.45 (m, 2H), 8.46 (dd, *J* = 5.2, 1.7 Hz, 1H), 7.75 (td, *J* = 7.7, 1.9 Hz, 1H), 7.71–7.63 (m, 2H), 7.37 (s, 1H), 7.09–7.05 (m, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.30 (s, 1H), 6.25 (s, 1H), 3.21 (q, *J* = 7.2 Hz, 2H), 2.16 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, *$CDCl_3$:CD_3OD = 8:1 (v/v)*) δ 163.7, 158.4, 157.6, 149.2, 148.7, 145.3, 142.0, 138.3, 132.5, 131.6, 130.4, 130.2, 130.1, 125.2, 125.0, 123.8, 119.2, 118.9, 117.0, 98.7, 94.8, 38.2, 16.8, 14.5. HRMS-ESI$^+$: m/z = 381.1720 (calcd for $[M+H]^+$, 381.1715).

2.2.3. *N*-Ethyl-10-methyl-5-(pyridin-3-ylimino)-5*H*-benzo[*a*]phenoxazin-9-amine (5b)

Compound **5b** was synthesized by compound **4** (3513.6 mg, 10.0 mmol) and 3-aminopyridine (2823.3 mg, 30.0 mmol) using a similar procedure to compound **5a**. Dark red solid, 617.2 mg, yield 16.2%, mp 239.0-241.0 °C; IR ν (KBr, cm^{-1}): 3432, 3181, 2027, 1640, 1594, 1551, 1401, 1315, 1111; ^{1}H NMR (400 MHz, *$CDCl_3$:CD_3OD = 8:1 (v/v)*) δ 8.63 (dd, *J* = 7.1, 2.0 Hz, 1H), 8.55 (dd, *J* = 7.3, 2.0 Hz, 1H), 8.33 (dd, *J* = 4.5, 1.9 Hz, 1H), 8.23 (d, *J* = 2.1 Hz, 1H), 7.71–7.64 (m, 2H), 7.38–7.33 (m, 3H), 6.26 (s, 1H), 6.21 (s, 1H), 3.23 (q, *J* = 7.2 Hz, 2H), 2.17 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, *$CDCl_3$:CD_3OD=8:1(v/v)*) δ 158.4, 149.4, 149.3, 148.4, 145.3, 144.0, 142.2, 142.0, 132.5, 131.5, 130.4, 130.24, 130.17, 128.9, 125.05, 125.03, 124.1, 124.0, 119.2, 97.6, 94.9, 38.4, 38.3, 16.8, 14.4. HRMS-ESI$^+$: m/z = 381.1708 (calcd for $[M+H]^+$, 381.1715).

2.2.5. *N*-Ethyl-10-methyl-5-(pyridin-4-ylimino)-5*H*-benzo[*a*]phenoxazin-9-amine (5c)

Compound **5c** was synthesized by compound **4** (5270.4 mg, 15.0 mmol) and 4-aminopyridine (4234.9 mg, 45.0 mmol) using a similar procedure to compound **5a**. Dark red solid, 458.3 mg, yield: 8.0%, mp 247.0–248.0 °C; IR ν (KBr, cm^{-1}): 3420, 2926, 2364, 2029, 1601, 1572, 1560, 1488, 1385, 1189, 1130, 1015; ^{1}H NMR (400 MHz, *$CDCl_3$:CD_3OD = 8:1 (v/v)*) δ 8.64 (d, *J* = 7.7 Hz, 1H), 8.51–8.47 (m, 3H), 7.72–7.65 (m, 2H), 7.40 (s, 1H), 6.93 (d, *J* = 5.2 Hz, 2H), 6.30 (s, 1H), 6.13 (s, 1H), 3.25 (q, *J* = 7.2 Hz, 2H), 2.19 (s, 3H), 1.34 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, *$CDCl_3$:CD_3OD = 8:1 (v/v)*) δ 160.4, 157.3, 149.6, 149.3, 145.4, 141.6, 132.0, 131.6, 130.6, 130.5, 130.3, 130.2, 125.2, 124.0, 119.6, 116.8, 105.1, 97.5, 94.8, 38.3, 16.9, 14.4. HRMS-ESI$^+$: m/z = 381.1712 (calcd for $[M+H]^+$, 381.1715).

2.2.6. *N*-Ethyl-5-((1-ethylpyridine-1-ium-2-yl)imino)-5*H*-benzo[*a*]phenoxazin-9-amine Chloride (1a)

The solution of **5a** (380.5 mg, 1.0 mmol), iodoethane (0.80 mL, 10.0 mmol), chloroform (7 mL) and methanol (7mL) was heated under reflux for 18 h. After removal of solvents, the residue was purified by column chromatography on silica gel eluting with methanol/chloroform (v/v, from 1:15 to 1:5). The crude product was further purified by recrystallization with chloroform, then the iodide salt of **1a** was dissolved in methanol and passed through 717 anion-exchange resin (chloride form), chloride salt of **1a** was obtained after evaporation. Dark blue crystals, 290.1 mg, yield 65.2%; mp > 250.0 °C. IR ν (KBr, cm^{-1}): 3441, 2970, 2363, 2026, 1631, 1591, 1560, 1534, 1385, 1310, 1187, 1123, 1013. ^{1}H NMR (400 MHz, *CD_3OD*) δ 8.71 (d, *J* = 8.0 Hz, 1H), 8.57 (d, *J* = 6.5 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.20 (t, *J* = 8.0 Hz, 1H), 7.80 (t, *J* = 7.6 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.51 (s, 1H), 7.38 (t, *J* = 7.0 Hz, 1H), 6.73 (s, 1H), 6.55 (s, 1H), 4.56 (q, *J* = 7.2 Hz, 2H), 3.38 (q, *J* = 7.2 Hz, 2H), 2.24 (s, 3H), 1.52 (t, *J* = 7.2

Hz, 3H), 1.32 (t, *J* = 7.2 Hz, 4H). ^{13}C NMR (75 MHz, *CD_3OD*) δ 163.2, 158.7, 154.3, 153.1, 148.0, 145.3, 143.6, 138.0, 132.9, 132.3, 132.0, 131.7, 130.6, 129.2, 126.7, 125.5, 124.7, 120.4, 119.5, 97.3, 94.5, 51.7, 39.3, 17.6, 15.5, 14.3. HRMS-ESI$^+$: m/z = 409.2028 (calcd for [M – Cl$^-$]$^+$, 409.2028).

2.2.7. *N*-Ethyl-5-((1-ethylpyridine-1-ium-3-yl)imino)-5H-benzo[*a*]phenoxazin-9-amine Chloride (1b)

Compound **1b** was synthesized by compound **5b** (380.5 mg, 1.0 mmol) and iodoethane (0.80 mL, 10.0 mmol) using a similar procedure to compound **1a**. Dark red power, 285.2 mg, yield 64.1%, mp > 250.0 °C. IR ν (KBr, cm^{-1}): 3442, 2966, 2363, 2026, 1640, 1597, 1572, 1560, 1483, 1385, 1311, 1195, 1129, 1014. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ 8.87 (br, 2H), 8.55 (d, *J* = 7.9 Hz, 1H), 8.47 (d, *J* = 7.9 Hz, 1H), 8.16–8.10 (m, 2H), 7.81 (t, *J* = 7.5 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.40 (br, 1H), 6.45 (s, 1H), 6.35 (s, 1H), 6.31 (s, 1H), 4.65 (q, *J* = 7.2 Hz, 2H), 3.28–3.21 (m, 2H), 2.17 (s, 3H), 1.60 (t, *J* = 7.2 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, *DMSO-d_6*) δ 159.0, 151.4, 150.6, 149.6, 145.0, 138.6, 138.2, 136.9, 136.8, 131.0, 130.84, 130.79, 129.9, 129.8, 128.3, 124.8, 124.3, 123.3, 121.0, 96.3, 93.4, 56.2, 37.4, 17.1, 16.3, 13.7. HRMS-ESI$^+$: m/z = 409.2041 (calcd for [M – Cl$^-$]$^+$, 409.2028).

2.2.8. *N*-Ethyl-5-((1-ethylpyridine-1-ium-4-yl)imino)-5H-benzo[*a*]phenoxazin-9-amine Chloride (1c)

Compound **1c** was synthesized by compound **5c** (380.5 mg, 1.0 mmol) and iodoethane (0.80 mL, 10.0 mmol) using a similar procedure to compound **1a**. Dark red power, 276.4 mg, yield 62.1%, mp > 250.0 °C. IR ν (KBr, cm^{-1}): 3448, 2969, 2365, 2023, 1636, 1594, 1571, 1560, 1478, 1449, 1385, 1312, 1129, 1015. ^{1}H NMR (400 MHz, *CD_3OD*) δ 8.60 (d, *J* = 7.0 Hz, 3H), 8.36 (d, *J* = 8.0 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 6.6 Hz, 2H), 7.36 (s, 1H), 6.36 (s, 1H), 6.30 (s, 1H), 4.48 (q, *J* = 7.3 Hz, 2H), 2.17 (s, 3H), 1.63 (t, *J* = 7.3 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, *CD_3OD*) δ 166.7, 159.8, 153.3, 152.0, 147.4, 145.1, 139.2, 132.9, 132.0, 131.8, 131.7, 130.6, 127.8, 126.5, 124.7, 124.0, 120.2, 97.3, 94.7, 56.1, 39.2, 17.5, 16.7, 14.4. HRMS-ESI$^+$: m/z = 409.2020 (calcd for [M – Cl$^-$]$^+$, 409.2028).

2.3. Optical assays

Stock solutions of probes **1a**–**1c** (100.0 μM) were prepared in volumetric flasks (100 mL) with DMSO, each test solution (10 μM) was prepared in a volumetric flask (10 mL) with the corresponding stock solution and various buffer solutions to give a total volume of 10.0 mL in the pH responsible absorption and emission tests.

Stock solutions of various ions were prepared in volumetric flasks (10 mL) with concentrations of NaCl (1.0 M), KCl (1.0 M), $MgSO_4$ (5.0 mM), $CaCl_2$ (5.0 mM), $CoCl_2$ (3.0 mM), $CuCl_2$ (3.0 mM), $MnCl_2$ (3.0 mM), $NiCl_2$ (3.0 mM), $CdCl_2$ (3.0 mM), $HgCl_2$ (3.0 mM) in twice distilled water; Stock solutions of all kinds of amino acids were all prepared in volumetric flasks (100 mL) with concentrations of 1.0 mM in doubly distilled water. In selectivity and competition assays, the test solutions were prepared in volumetric flasks (10 mL) with 1 mL stock solution of probes and 1 mL stock solution of corresponding ions or amino acids' solutions, diluted with 0.2 M disodium hydrogen phosphate solution (pH=9.0) or 0.1 M citric acid solution (pH = 1.6).

Relative fluorescent quantum yields were obtained by the reported equation and Oxazine 1 (Φ = 0.14, ethanol) was used as the reference,[3] reported refractive index of water-DMSO solution was used for the buffer solution containing 10% DMSO.[4]

The reversible absorption and emission responses of probes **1a**–**1c** were obtained by the 50 mL test solutions, which were made by 5.0 mL of stock solutions diluted to 50.0 mL with water, the pH values were adjusted with hydrochloric acid (12 M) and sodium hydroxide (12 M) solutions.

2.4 Cell culture and confocal microscopy study

Cells were cultured with 10% calf serum, penicillin (100 U•mL^{-1}), streptomycin (100 μg•mL^{-1}) and 250 μM L-glutamine at 37°C in a 5:95 CO_2-air incubator. The cells were cultured for 2 d in a 35 mm diameter glass-bottomed dish with 10 mm microwell NO.0 cover glasses (0.085–0.13 mm thickness).

Cells were washed with phosphate buffered saline (PBS) solution for three times, followed by incubation with culture medium including probes (10 μM) and LysoTracker Green DND-26 (50 nM) for 10–15 min. Fluorescence confocal images of the stained cells were obtained. Green channel emission was collected in 505–550 nm upon excitation at 488 nm, and red channel emission was collected in 590–795 nm upon excitation at 561 nm.

3. Appendix

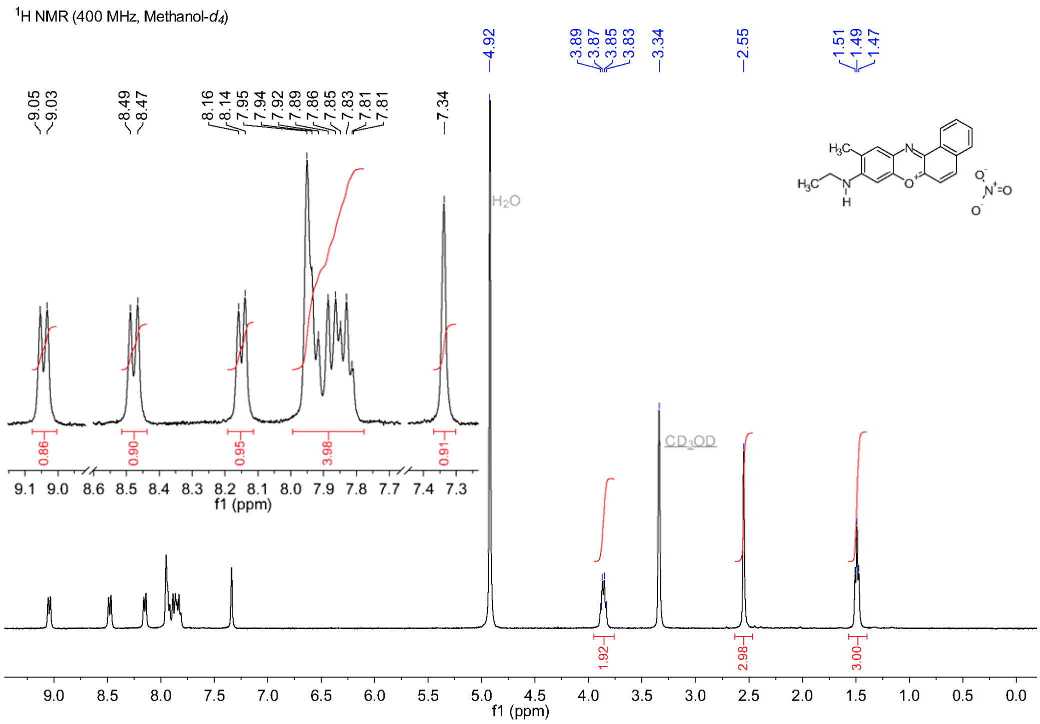

Figure S9. ^{1}H-NMR of compound **4**.

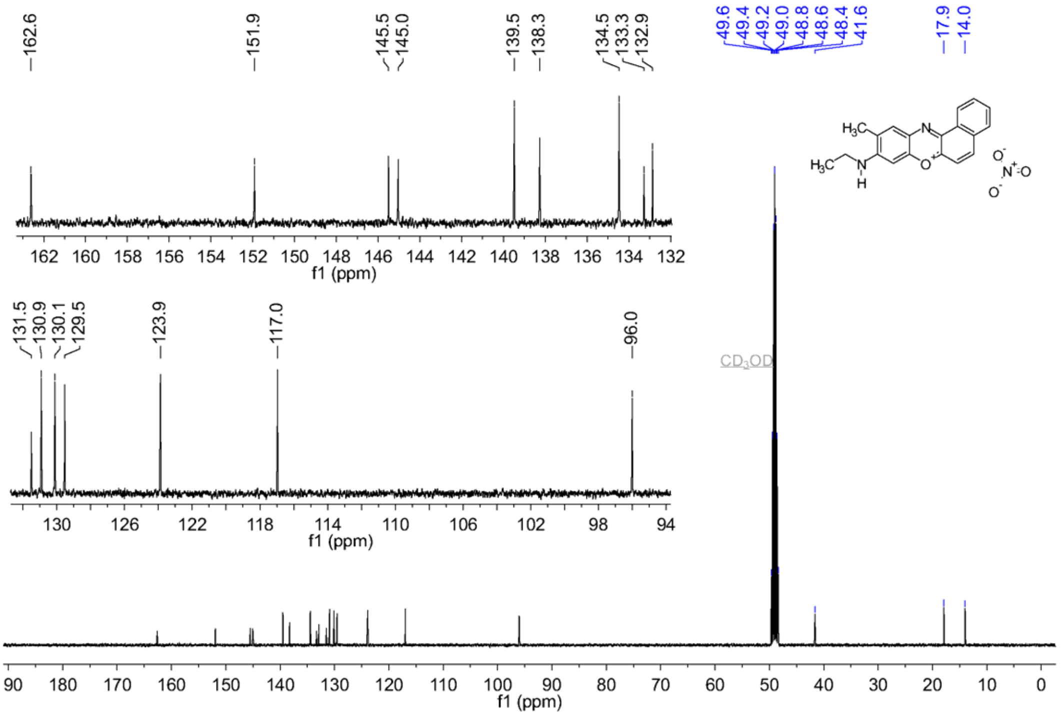

Figure S10. ^{13}C-NMR of compound **4**.

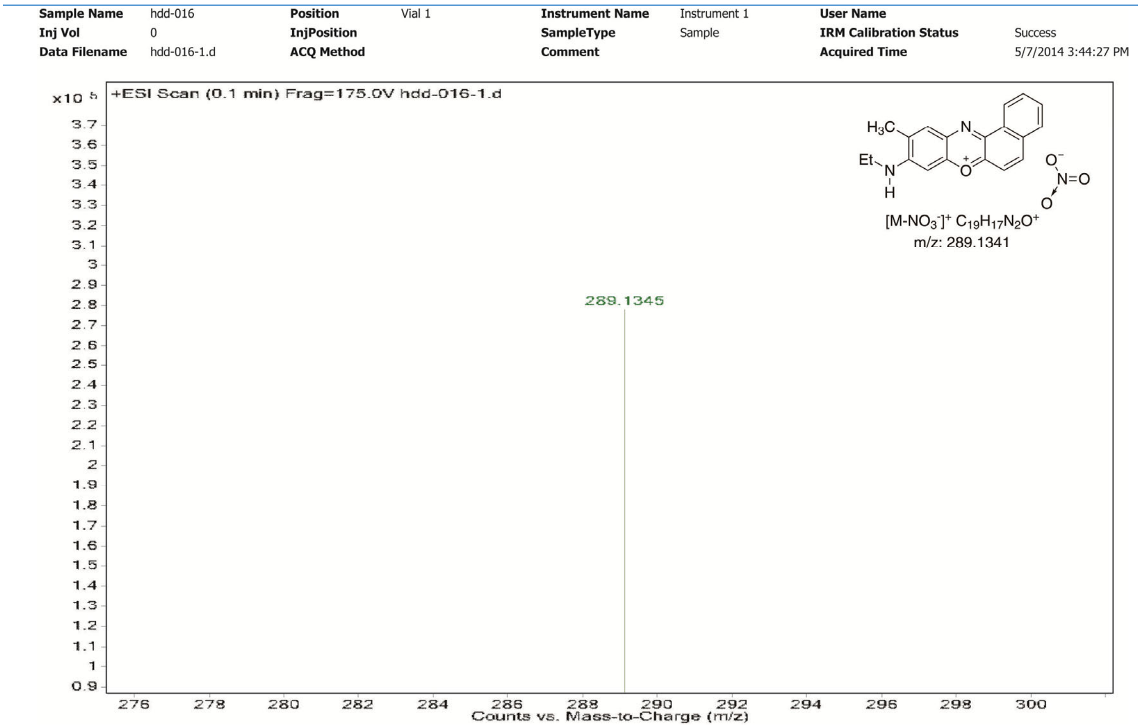

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Figure S11. HRMS(ESI$^+$) of compound **4**.

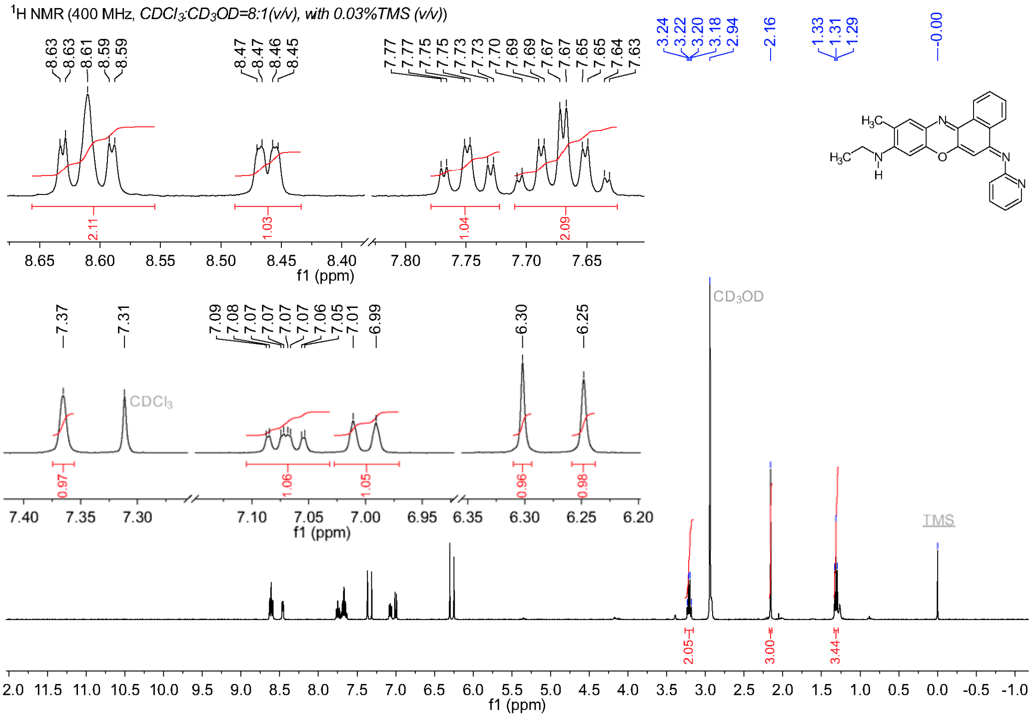


Figure S12. ^{1}H-NMR of compound **5a**.

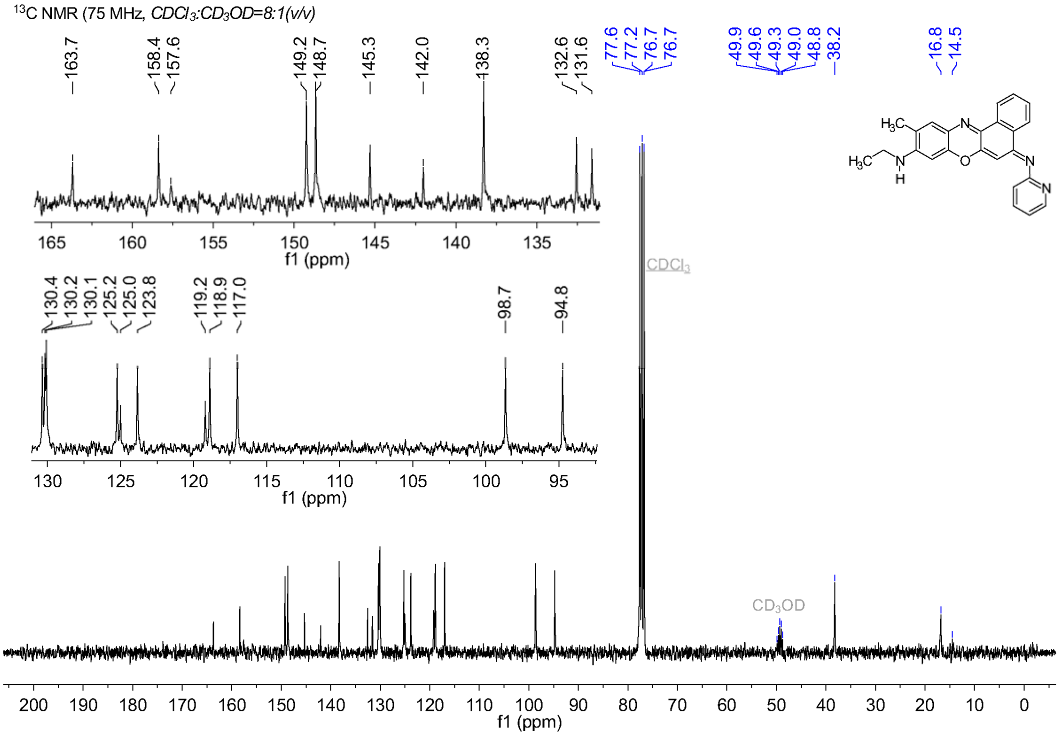
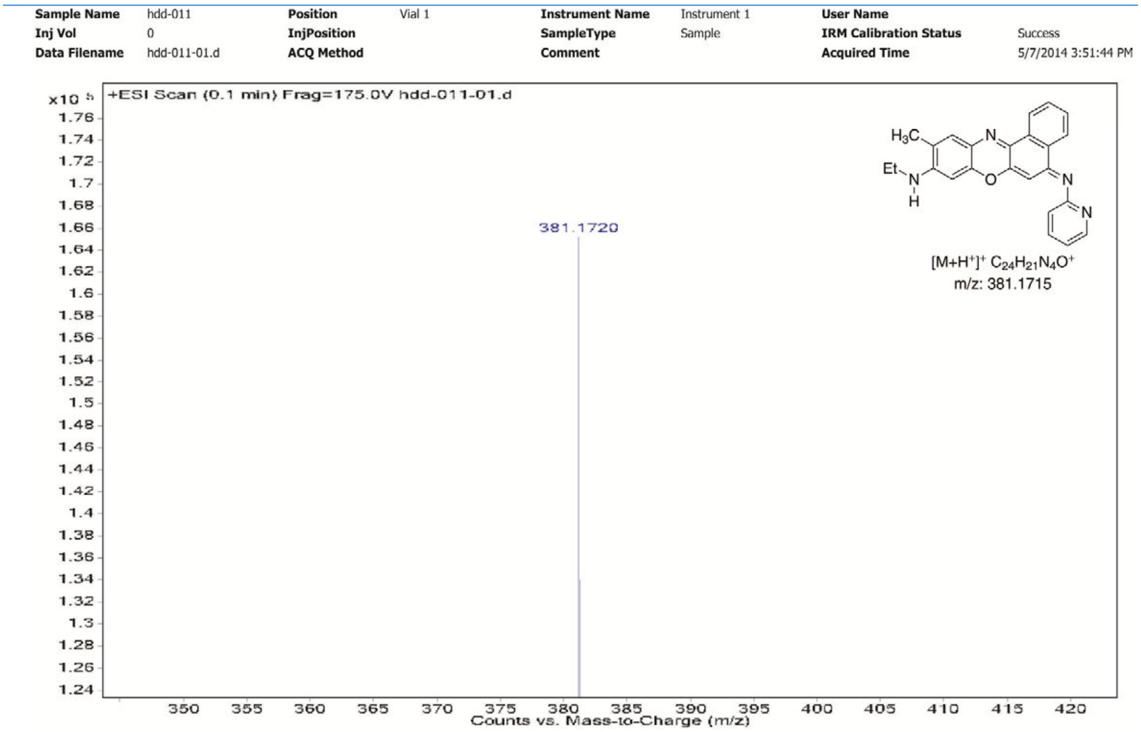


Figure S13. ^{13}C-NMR of compound **5a**.



Sample Name hdd-011 **Position** Vial 1 **Instrument Name** Instrument 1 **User Name**
Inj Vol 0 **InjPosition** **SampleType** Sample **IRM Calibration Status** Success
Data Filename hdd-011-01.d **ACQ Method** **Comment** **Acquired Time** 5/7/2014 3:51:44 PM

Figure S14. HRMS(ESI$^+$) of compound **5a**.

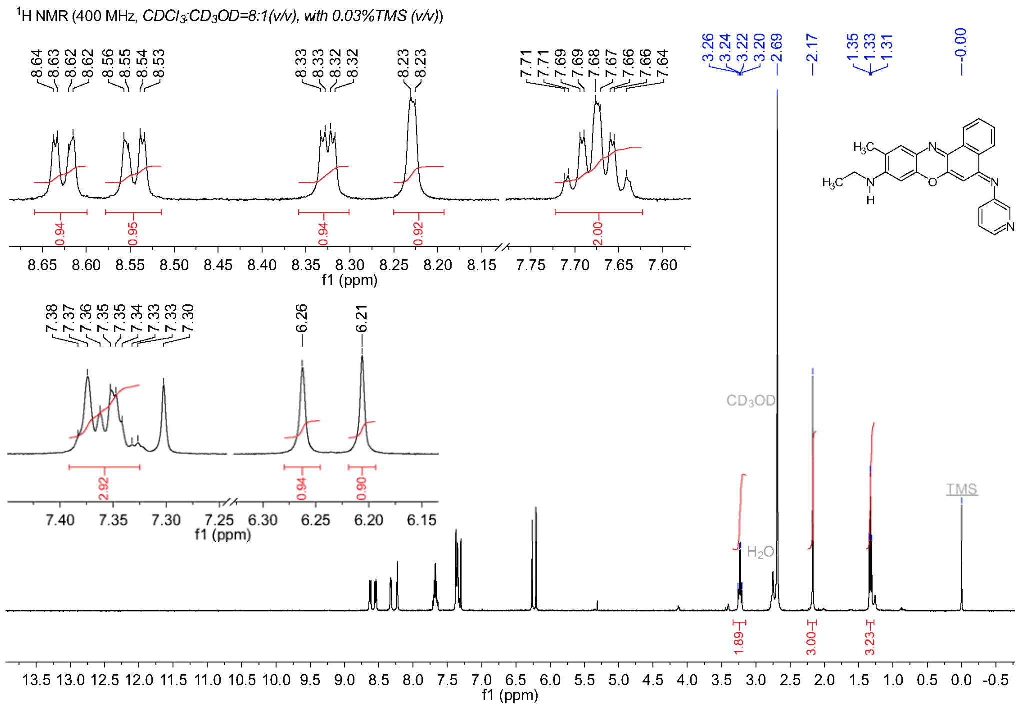


Figure S15. ^{1}H-NMR of compound **5b**.

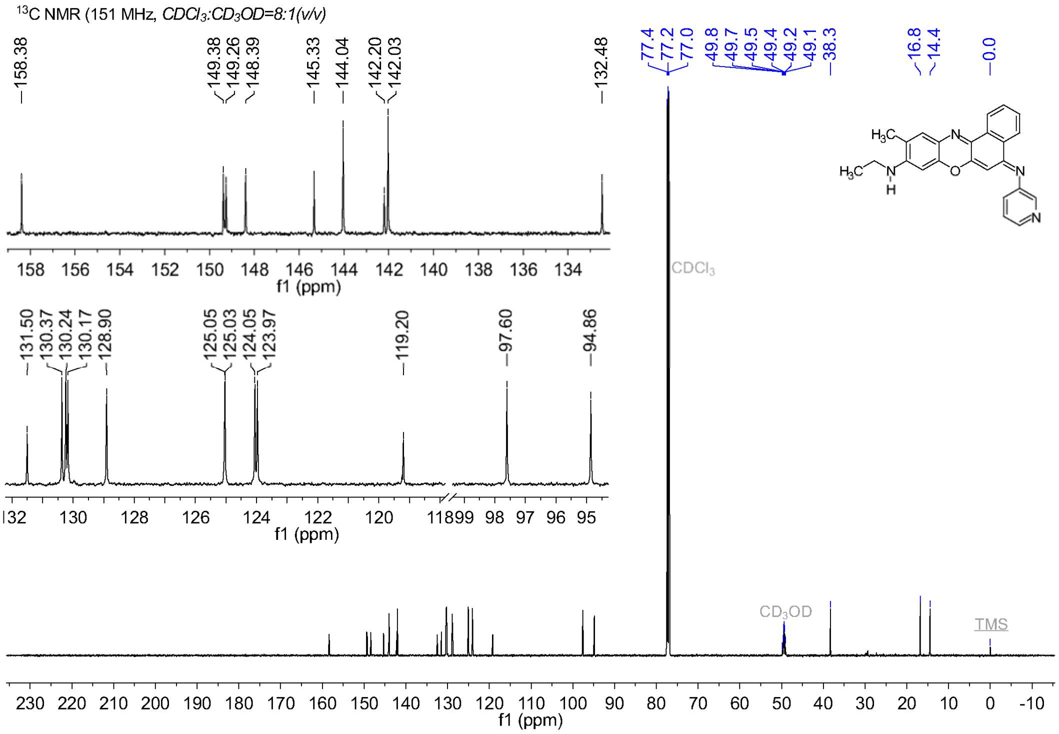
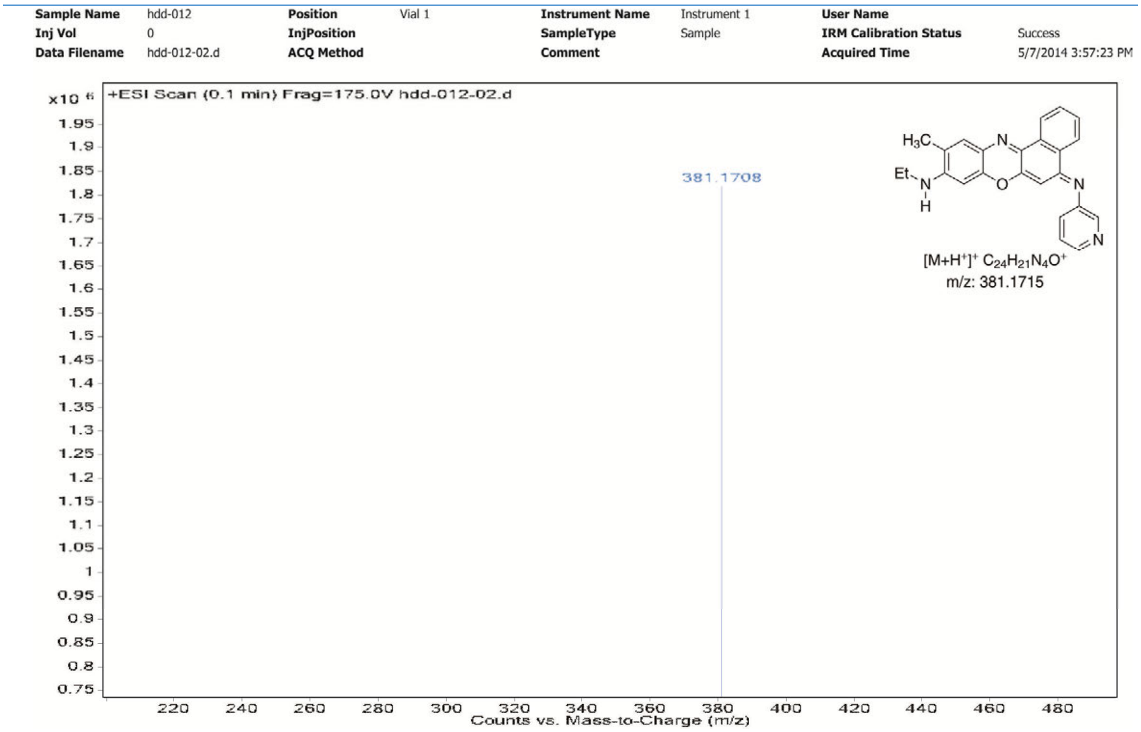


Figure S16. ^{13}C-NMR of compound **5b**.



Sample Name	hdd-012	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	0	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	hdd-012-02.d	**ACQ Method**		**Comment**		**Acquired Time**	5/7/2014 3:57:23 PM

Figure S17. HRMS(ESI$^+$) of compound **5b**.

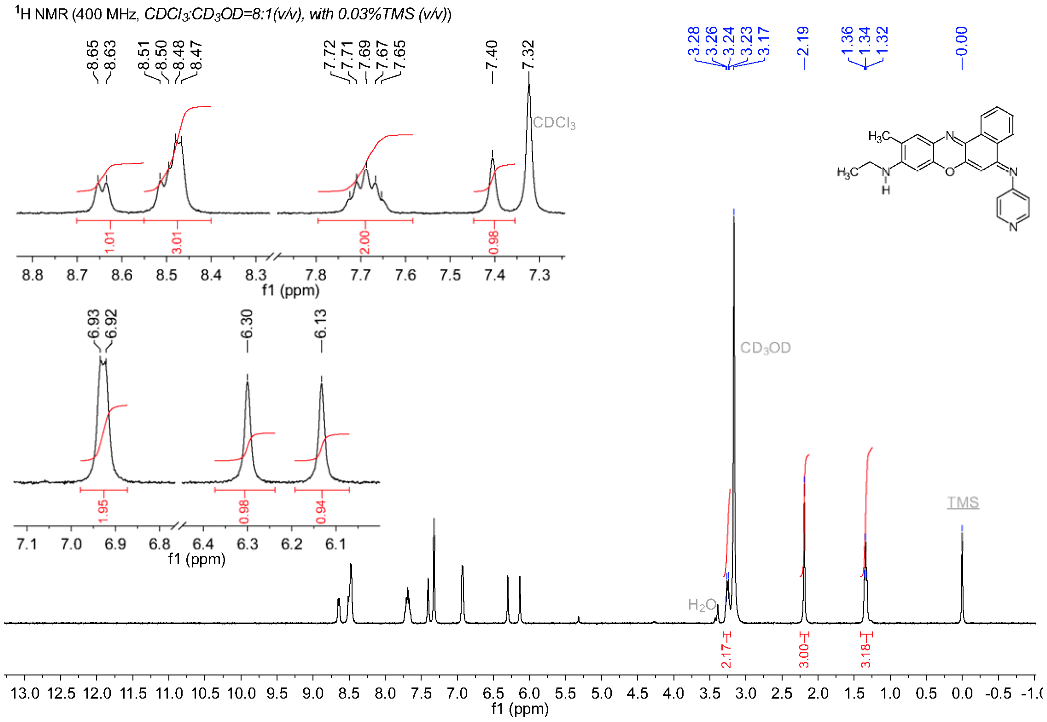


Figure S18. ^{1}H-NMR of compound **5c**.

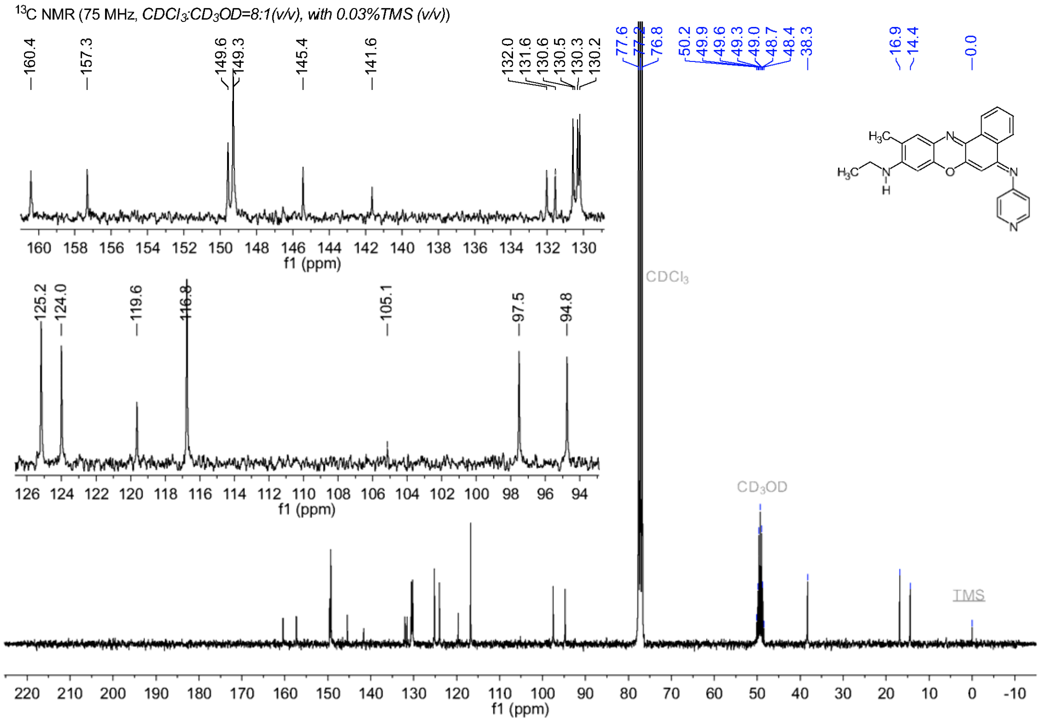
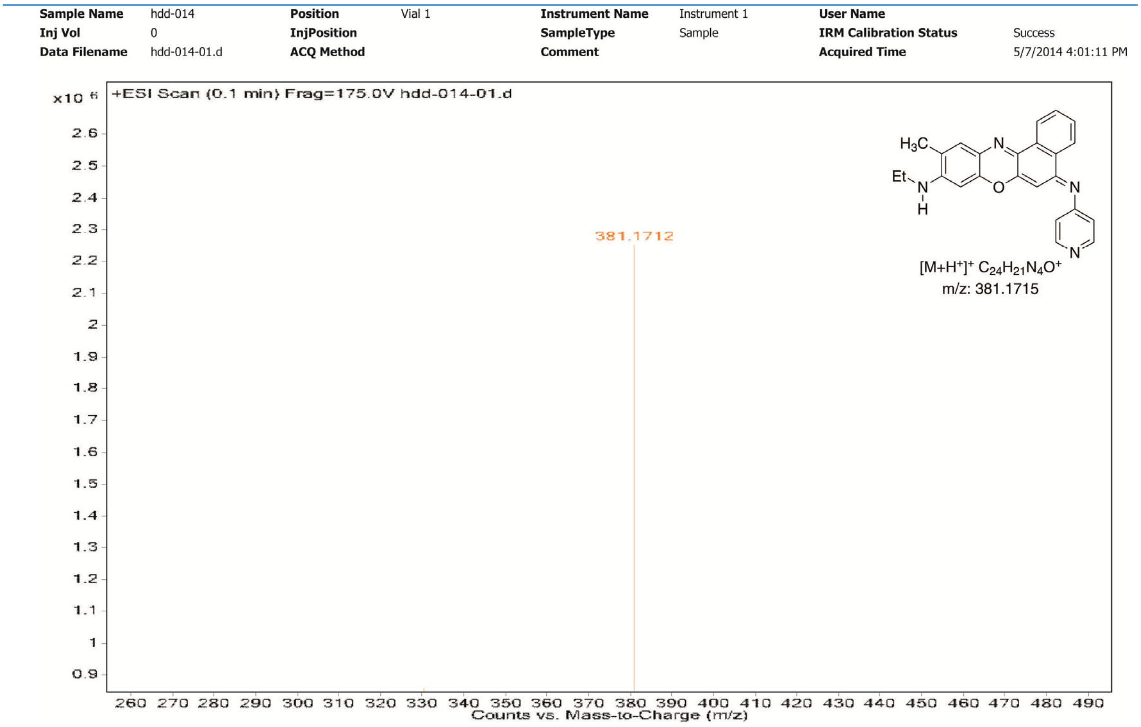


Figure S19. ^{13}C-NMR of compound **5c**.



Sample Name	hdd-014	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	0	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	hdd-014-01.d	**ACQ Method**		**Comment**		**Acquired Time**	5/7/2014 4:01:11 PM

Figure S20. HRMS(ESI$^+$) of compound **5c**.

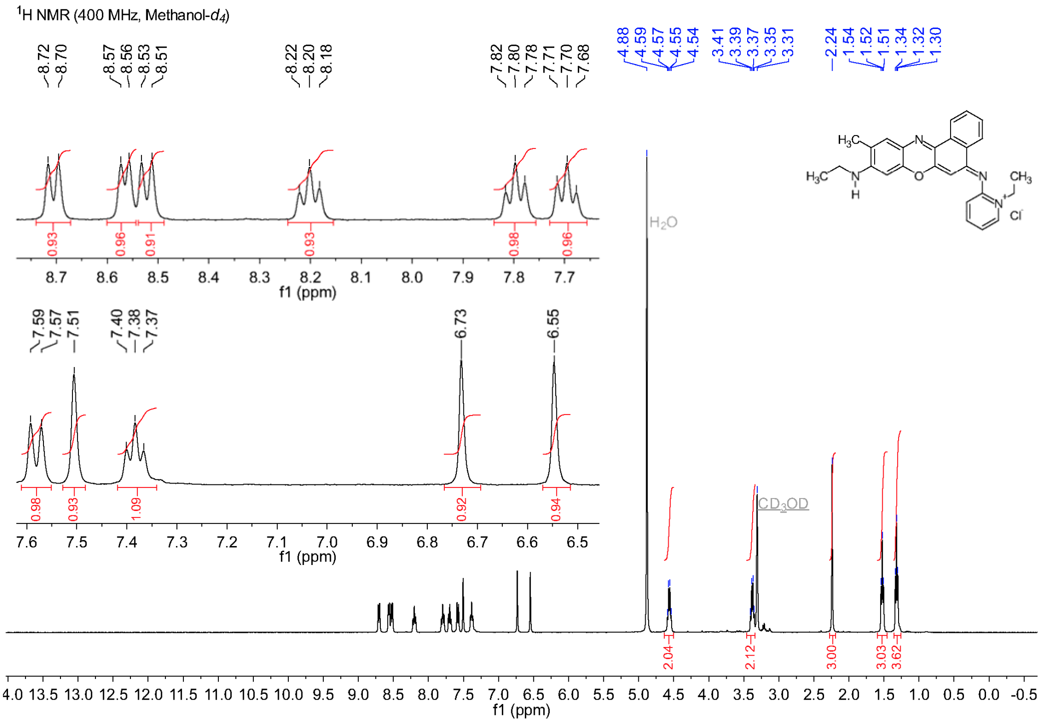


Figure S21. ^{1}H-NMR of compound **1a**.

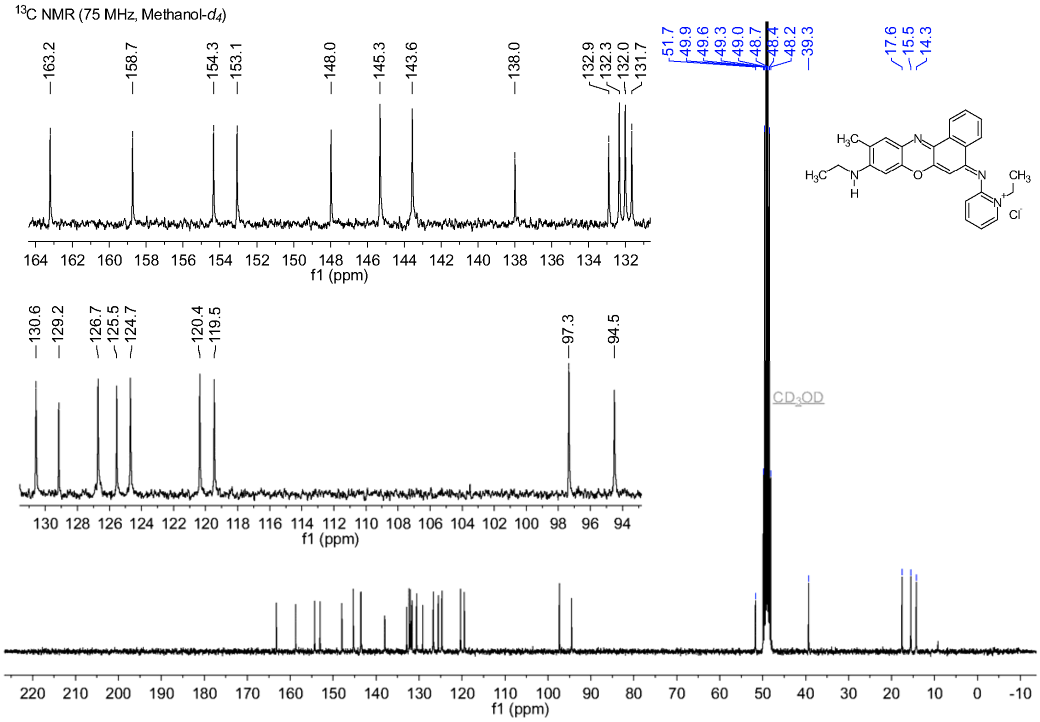
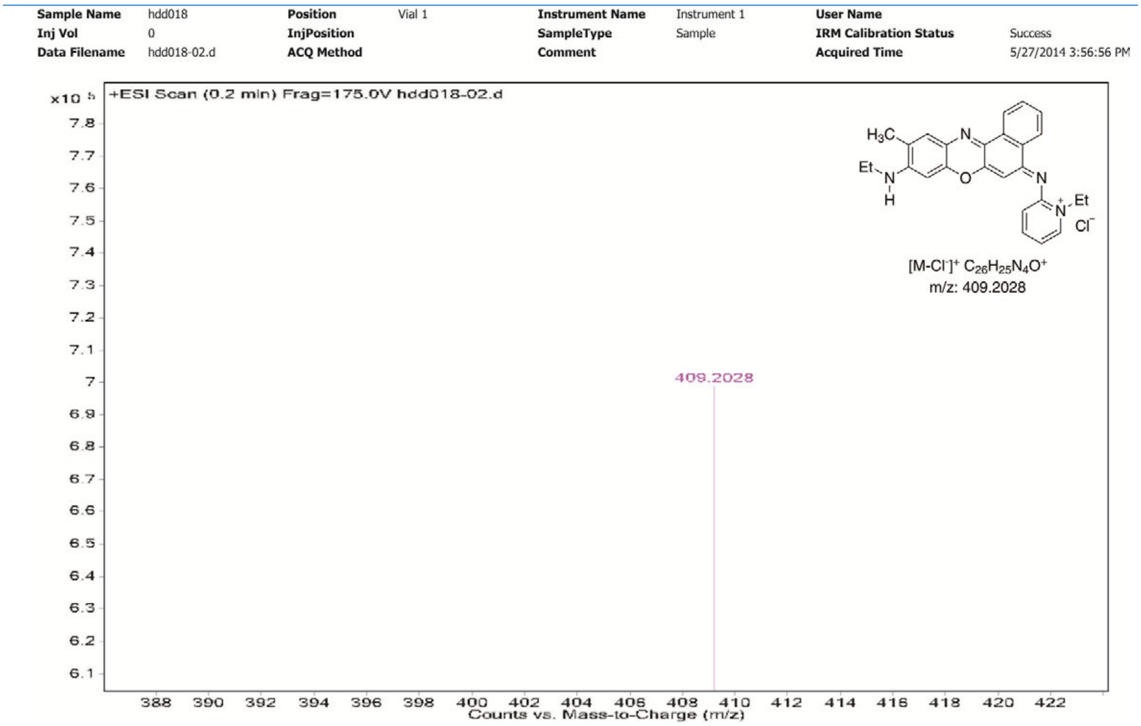


Figure S22. ^{13}C-NMR of compound **1a**.



Sample Name hdd018 **Position** Vial 1 **Instrument Name** Instrument 1 **User Name**

Inj Vol 0 **InjPosition** **SampleType** Sample **IRM Calibration Status** Success

Data Filename hdd018-02.d **ACQ Method** **Comment** **Acquired Time** 5/27/2014 3:56:56 PM

Figure S23. HRMS(ESI$^+$) of compound **1a**.

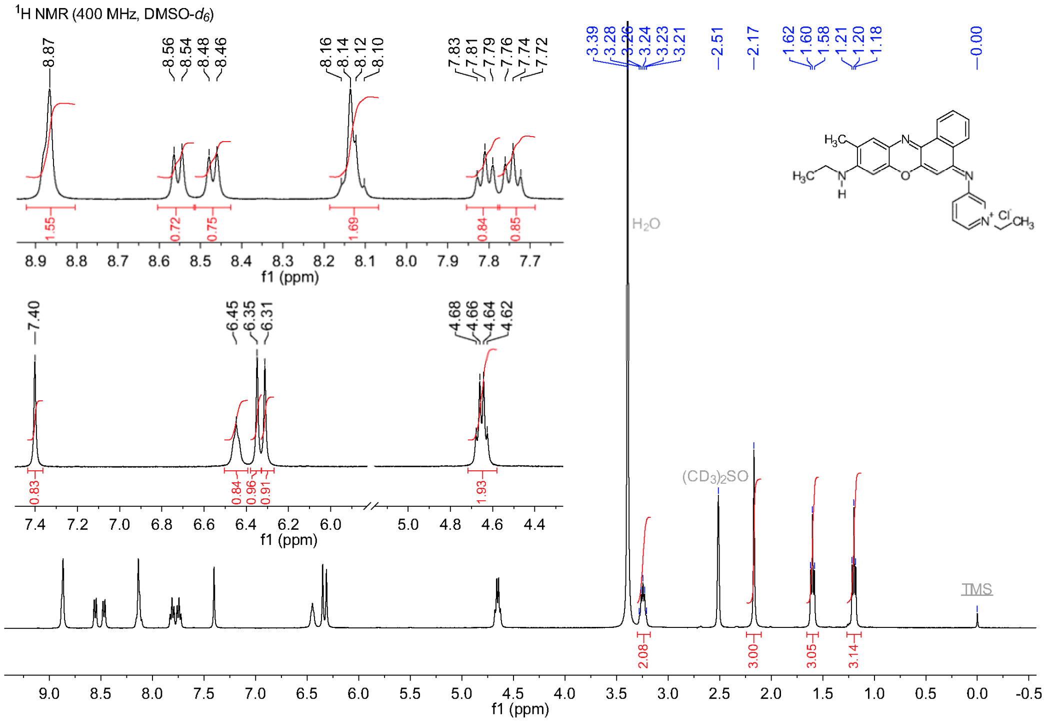


Figure S24. ^{1}H-NMR of compound **1b**.

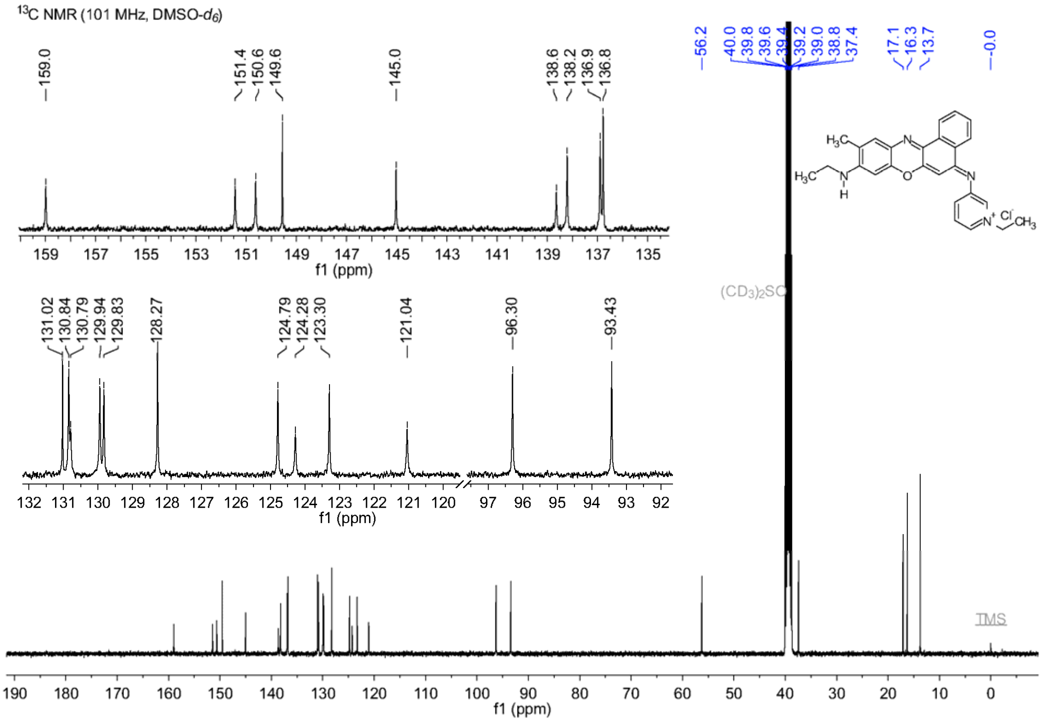
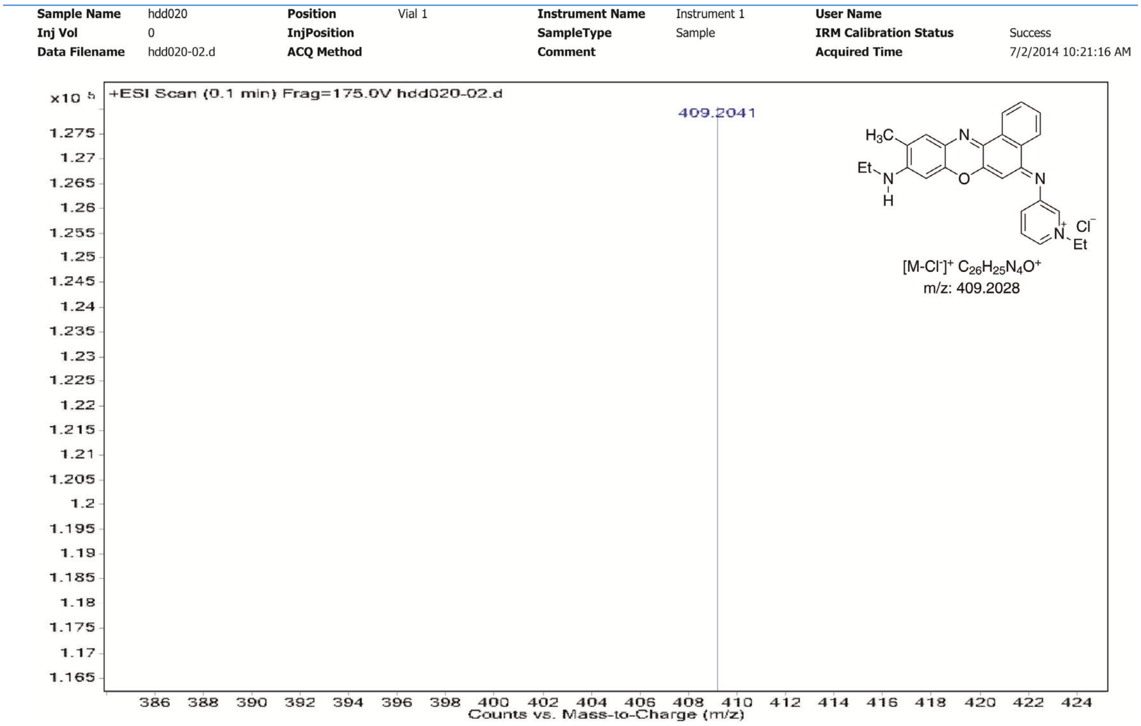


Figure S25. ^{13}C-NMR of compound **1b**.



Sample Name	hdd020	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	0	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	hdd020-02.d	**ACQ Method**		**Comment**		**Acquired Time**	7/2/2014 10:21:16 AM

Figure S26. HRMS(ESI$^+$) of compound **1b**.

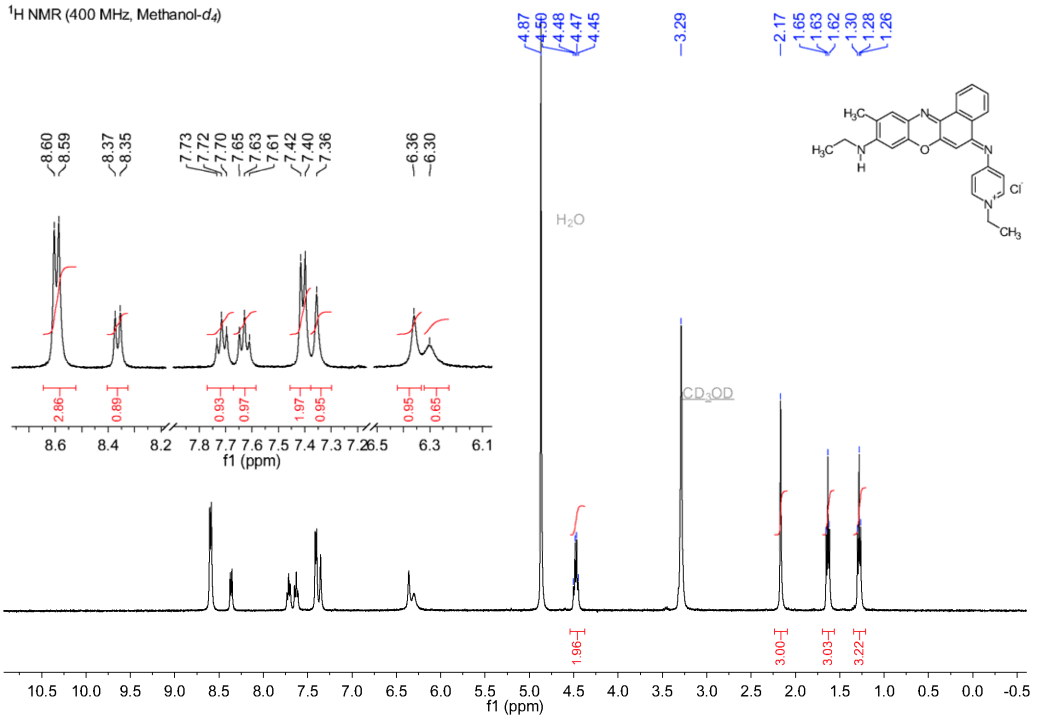


Figure S27. ^{1}H-NMR of compound **1c**.

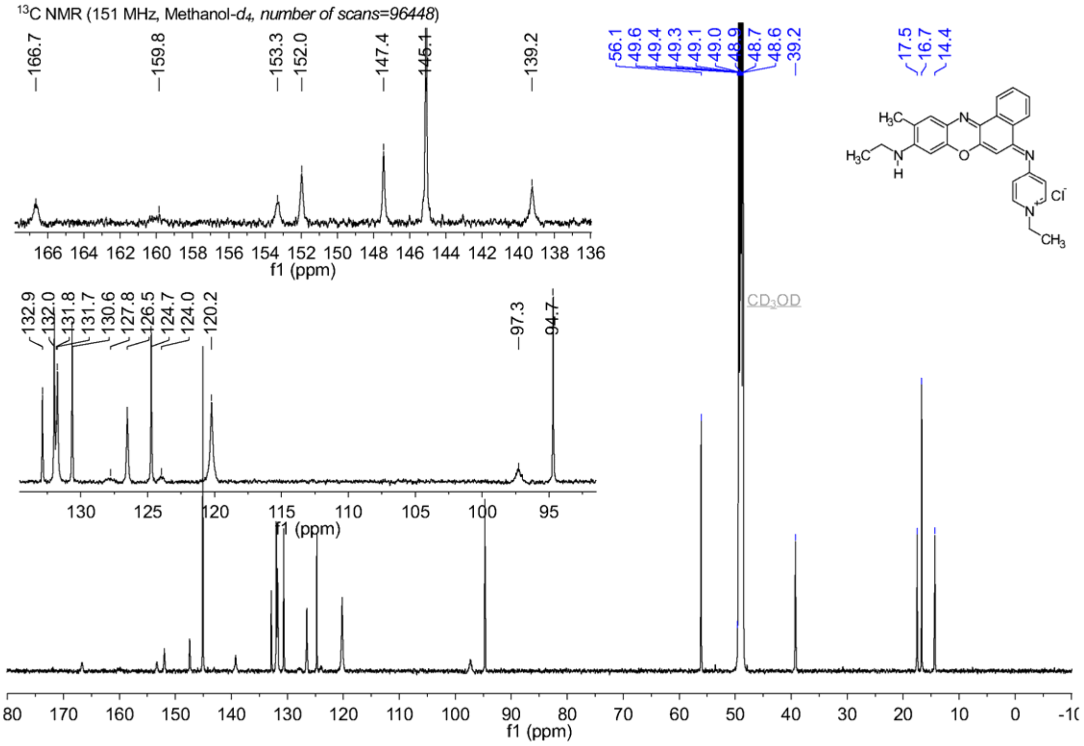
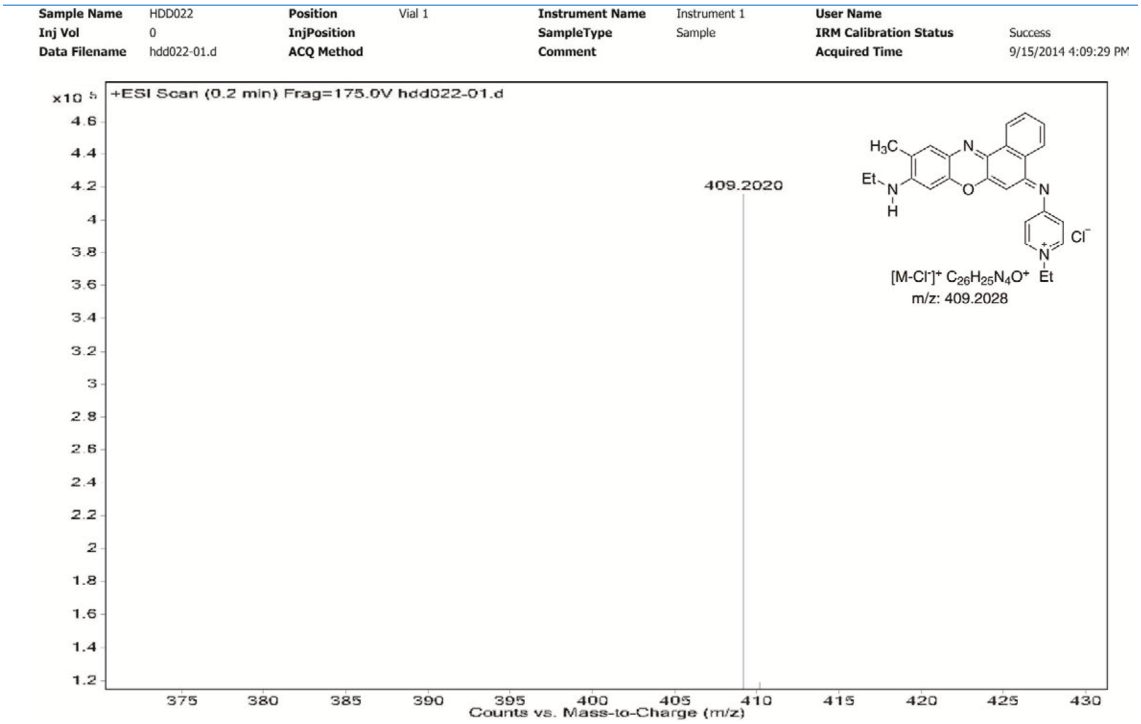


Figure S28. ^{13}C-NMR of compound **1c**.



Sample Name	HDD022	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	0	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	hdd022-01.d	**ACQ Method**		**Comment**		**Acquired Time**	9/15/2014 4:09:29 PM

Figure S29. HRMS(ESI$^+$) of compound **1c**.

4. References

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