Organic Chemistry Frontiers

Electronic Supplementary Information

Synthesis and optical properties of cyanine dyes with aromatic azonia skeleton

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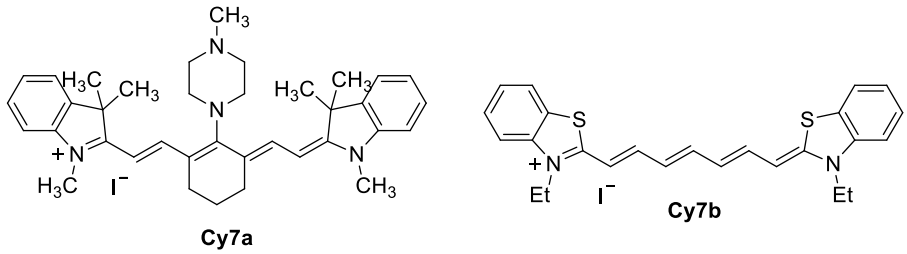
Index

1. Experimental section

1.1. Materials

Starting materials were purchased from Shanghai Mackin Biochemical Co., Ltd., 9 Ding Chemistry Co., Ltd. (Shanghai, China) and Chinasun Specialty Products Co., Ltd. (Shanghai, China), and solvents (synthetic or analytical grade) were purchased from commercial suppliers and were used as received. 2-(2-(2-Chloro-3-(2-(1,3,3-trimethylindolin-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1,3,3-trimethyl-3H-indol-1-ium iodide (**2**) was synthesized by reported method,[1] 3-ethyl-2-(-7-(3-ethylbenzo[d]thiazol-2(3H)-ylidene)hepta-1,3,5-trien-1-yl)benzo[d]thiazol-3-ium iodide (**Cy7b**, CAS number: 3071-70-3) were obtained from Sigma Chemical Co. (USA). 1,3,3-Trimethyl-2-(2-(2-(4-methylpiperazin-1-yl)-3-(2-(1,3,3-trimethylindolin-2-ylidene)eth-ylidene)cyclohex-1-en-1-yl)vinyl)-3H-indol-1-ium iodide (**Cy7a**) was synthesized according to reported method.[2] Flash chromatography was performed with silica gel (200-300 mesh). The citric acid-NaOH-HCl (pH = 1.4 to 6.6) and Tris-HCl (pH = 6.6 to 9.0) buffer were used in the titration experiments.

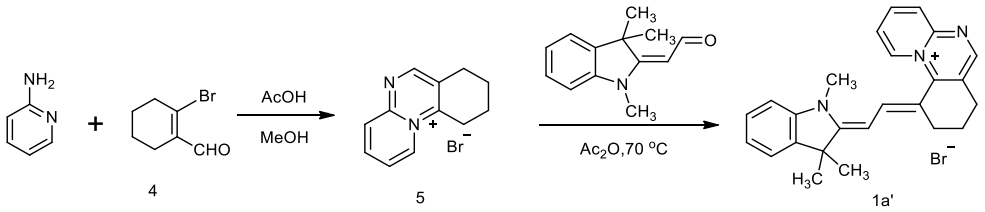


Scheme S1 The structures of **Cy7a** and **Cy7b** in photofading experiments.

1.2. Apparatus

Melting points were determined on an X-4 microscope electron thermal apparatus (Taike, China). Infrared (IR) spectra were recorded on a VERTEX 70 IR spectrometer using KBr plates. The thermogravimetric analysis (TGA) were recorded on PerkinElmer TGA 4000. NMR spectra were recorded on Varian or Bruker spectrometers (400−600 MHz), and tetramethylsilane (TMS) or the solvent peaks were used for internal standard. High resolution mass spectra were recorded on a Finnigan MAT95 mass spectrometer in ESI^+ mode. The pH values were measured with a Lei-Ci (pH-3C) digital pH-meter (Shanghai, China) using a combined glass-calomel electrode. All experiments were carried out at room temperature. Absorption spectra were obtained with a ShimadzuUV-1800 spectrophotometer. Fluorescence spectra were performed on Shimadzu RF-5301PC spectroscope with R-928 photomultiplier.

1.3. Synthesis of dye **1a'** in another way



Scheme S2 A direct route for synthesis of final product **1a'**

Compound **4** and **5** were synthesized as reported.[3,4] The compound **5** (60.0 mg, 0.23 mmol) and acetaldehyde (92.6 mg, 0.46 mmol) were placed in a round bottom flask containing acetic anhydride (4 mL), after heating for 3 h at 60 °C, the solution was added 20 ml of water slowly under stirred, after 1 hour later, the solvent was evaporated under

reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol = 50:1 to 18:1 v/v) to afford **1a'** as a dark brown solid (40.5 mg, 32 % yield). The ^{1}H NMR of **1a'** was shown in Fig. S13, and was the same as the ^{1}H NMR of **1a** (Fig. S7).

1.4. Absorption and Fluorescence titration

Stock solutions (100 μM) were prepared in a volumetric flask (100 mL) with DMSO (100.0 mL). Each test solution (5 μM) was prepared in a volumetric flask (10 mL) with 500 μL stock solution and corresponding buffer solution to give a total volume of 10.0 mL. Absorption and fluorescence spectra were obtained with 1.0-cm quartz cells. All the relative fluorescence quantum yields were determined and calculated with the following equation: $\Phi_x/\Phi_{st} = [A_{st}/A_x][n_x^2/n_{st}^2][D_x/D_{st}]$, where st: standard; x: sample. Φ: quantum yield; A: absorbance at the excitation wavelength; D: area under the fluorescence spectra on an energy scale; n: the refractive index of the solution, and the refractive index of mixed solution of DMSO and water can be calculated from the reported document. The **Oxazine 1** (Φ = 0.14 in ethanol) was used as standard.

1.5. Photofading experiment

Dyes were dissolved in acetonitrile with a concentration of 1.0×10^{-5} M. Solutions of the samples were irradiated with a 500 W Philips iodine–tungsten lamp at room temperature. The distance between the samples and the lamp was 25 cm. An 8 cm thick cold trap (60 g·L^{-1} $NaNO_2$) was set up between the 20 mL transparent glass bottle and the lamp to eliminate the heat and absorb short wavelength light. The photostability was in the terms of remaining absorption (%) calculated from the change of absorption intensity at the absorption maximum before and after irradiation.[5]

1.6. Thermogravimetric analysis experiment

The temperature is measured in the sample holder. Highpurity nitrogen was used for the tests at a flow rate of 150 mL/min. The nitrogen was purged for 20 min, before starting the heating program, to establish an inert environment. The sample mass was 5 mg. The experiments started with a drying session (a heating rate of 30 K/min up to 383 K with a holding time of 30 min). The subsequent thermal decomposition was carried out at a slow heating rate (10 K/min to a final temperature of 666 K) to keep possible heat/mass-transfer intrusions at a minimum.

1.7. Selectivity experiment

Stock solutions of probes (100 μM) were prepared in a volumetric flask (100 mL) with DMSO (100.0 mL). Stock solutions of various ions were prepared in volumetric flasks (10 mL) with concentrations of KCl (1.0 M), NaCl (1.0 M), $CaCl_2$ (5 mM), $MgSO_4$ (5 mM), $ZnCl_2$ (3 mM), $NiCl_2$ (3 mM), $MnCl_2$ (3 mM), $CoCl_2$ (3 mM), $CdCl_2$ (3 mM), $CuCl_2$ (3 mM), $HgCl_2$ (3 mM) in doubly distilled water. Stock solutions of all kinds of amino acids (GSH, Cys, Hcy, Gly, Pro, Trp, Glu, Lys, His, Arg, Phe, Leu and Ser) were all prepared in volumetric flasks (100 mL) with concentrations of 1 mM in doubly distilled water. Each test solution was prepared in a volumetric flask (10 mL) with 500 μL stock solution of probes and 1 mL stock solution of corresponding ions or amino acids solutions, diluted with Tris-HCl (pH = 9.0) or citric acid-NaOH-HCl (pH = 1.4 or 2.5) for competition assay to give a total volume of 10 mL.

1.8. Cell culture and fluorescent imaging of HeLa and Ges-1 cells

HeLa and Ges-1 cells were cultured in Roswell Park Memorial Institute culture medium (RPMI-1640) supplemented with 10% calf serum, penicillin (100 U·mL^{-1}), streptomycin (100 μg·mL^{-1}) and L-glutamine (2.5×10^{-4} M) at 37 ^{o}C in a 5:95 CO_2-air incubator. Before used, the cells were first loaded onto a glass-bottomed coverslip with a diameter of 35 mm and cultured for 48 h. The LysoTracker Green DND-26 (50 nM) and dyes **1a**, **1b**, **1c**, **1d** and **1f** (7.5 μM) were used in the

fluorescent imaging subsequently. Firstly, the PBS buffer (1 mL, pH = 7.4) was added, and cells were incubated for 5 min, then cells were incubated with commercial LysoTracker Green DND-26 (50 nM) and appropriate concentration of probes for 20 min. Fluorescence images of the stained cells were obtained with a Leica SP2 laser confocal scanning microscope equipped with a 561 nm laser head. Green channel emission was collected in 505−550 nm upon excitation at 488 nm, and red channel emission was collected in 580−790 nm upon excitation at 561 nm.

2. Figures

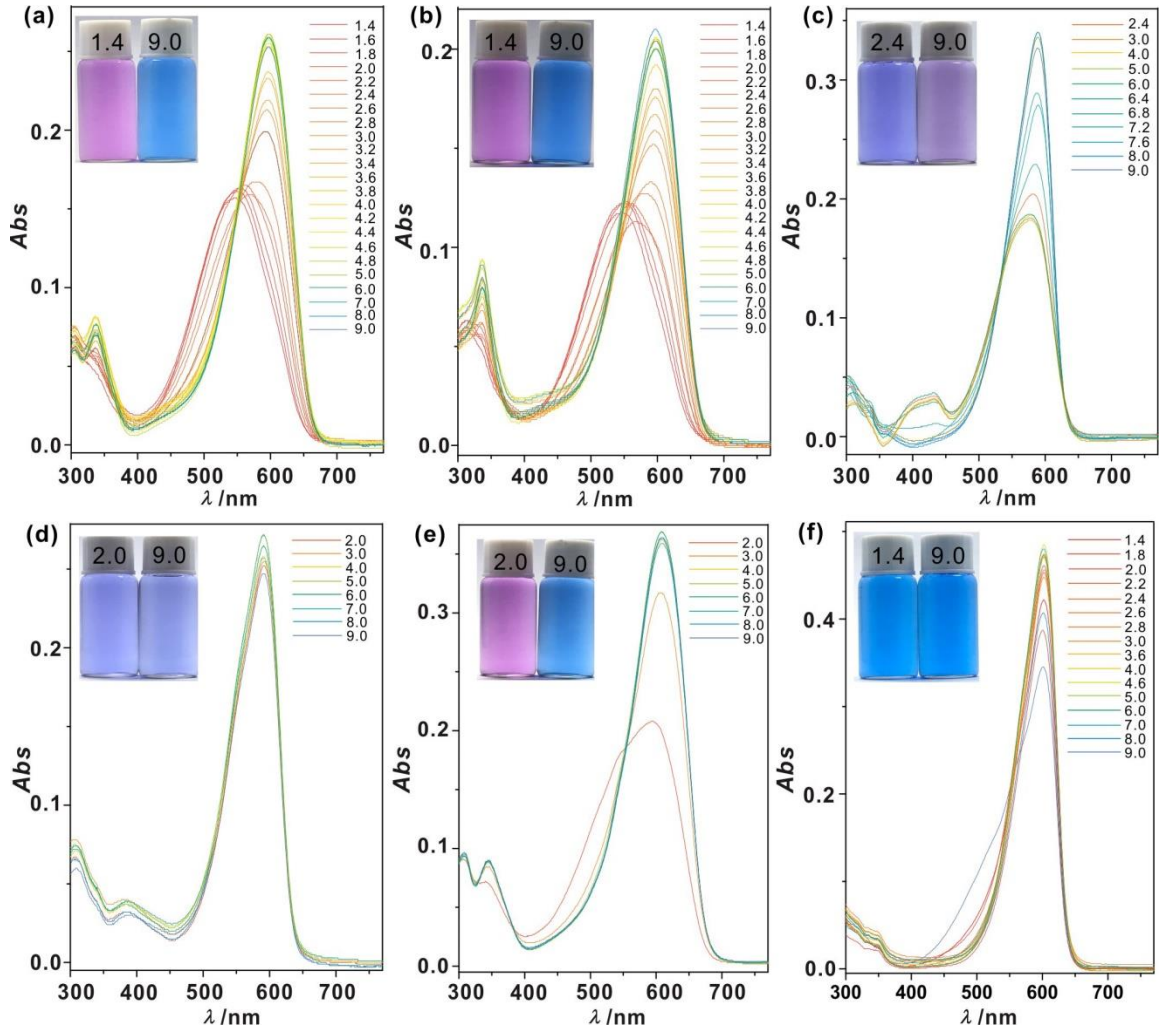


Fig. S1. Absorption properties of dyes **1a–f** (5 μM) in different pH buffer solutions containing 5% DMSO; (a) dye **1a**; (b) dye **1b**; (c) dye **1c**; (d) dye **1d**; (e) dye **1e**; (f) dye **1f**. The insets are pH-dependent photographs of the samples.

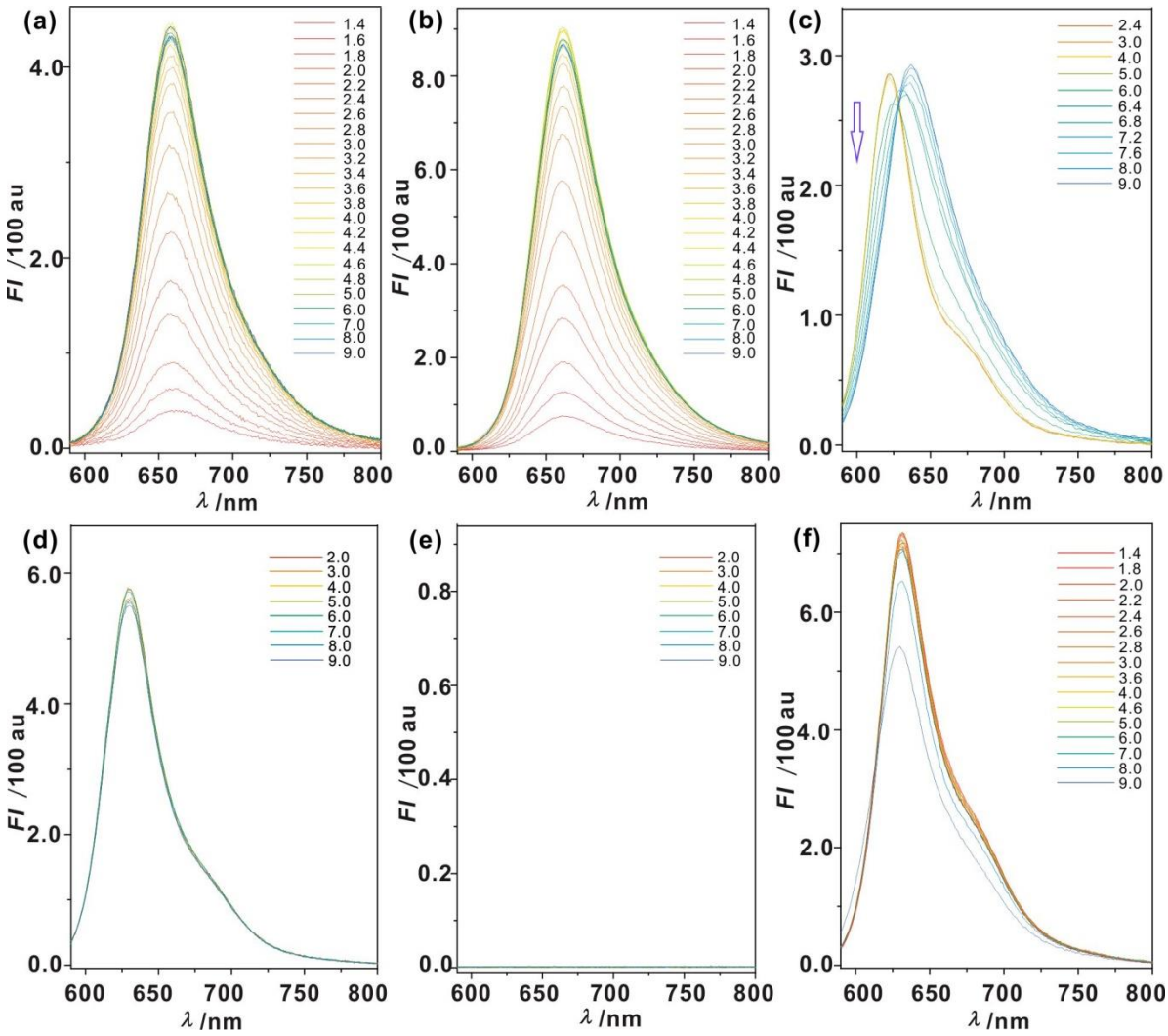


Fig. S2. Emission properties of dyes **1a–f** (5 μM) in different pH buffer solutions containing 5% DMSO: (a) dye **1a** (slit widths: 5 nm/5 nm); (b) dye **1b** (slit widths: 5 nm/5 nm); (c) dye **1c** (slit widths: 3 nm/3 nm); (d) dye **1d** (slit widths: 3 nm/3 nm); (e) dye **1e** (slit widths: 3 nm/5 nm); (f) dye **1f** (slit widths: 5 nm/5 nm). All emission spectrum were collected in 590–800 nm upon excitation at 580 nm.

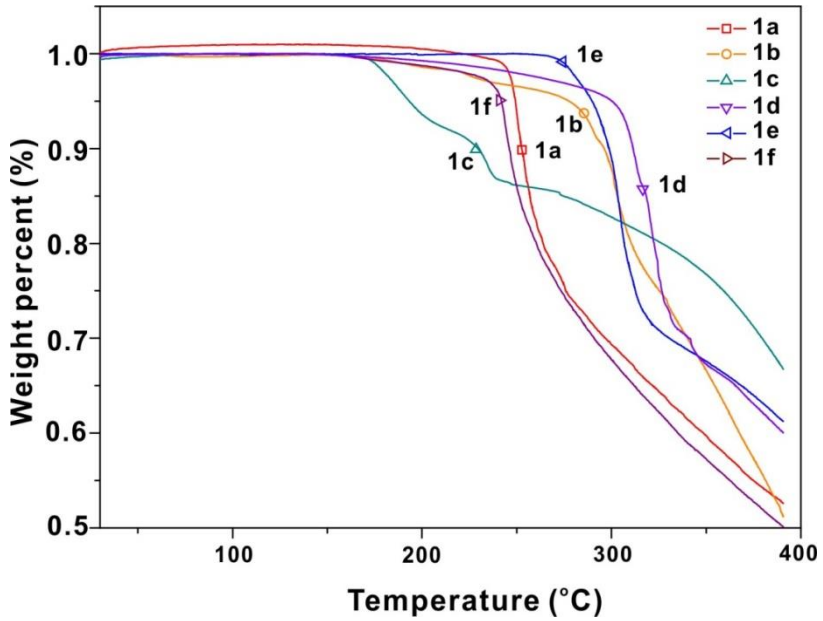


Fig. S3. Thermal stabilities of dyes **1a–f**.

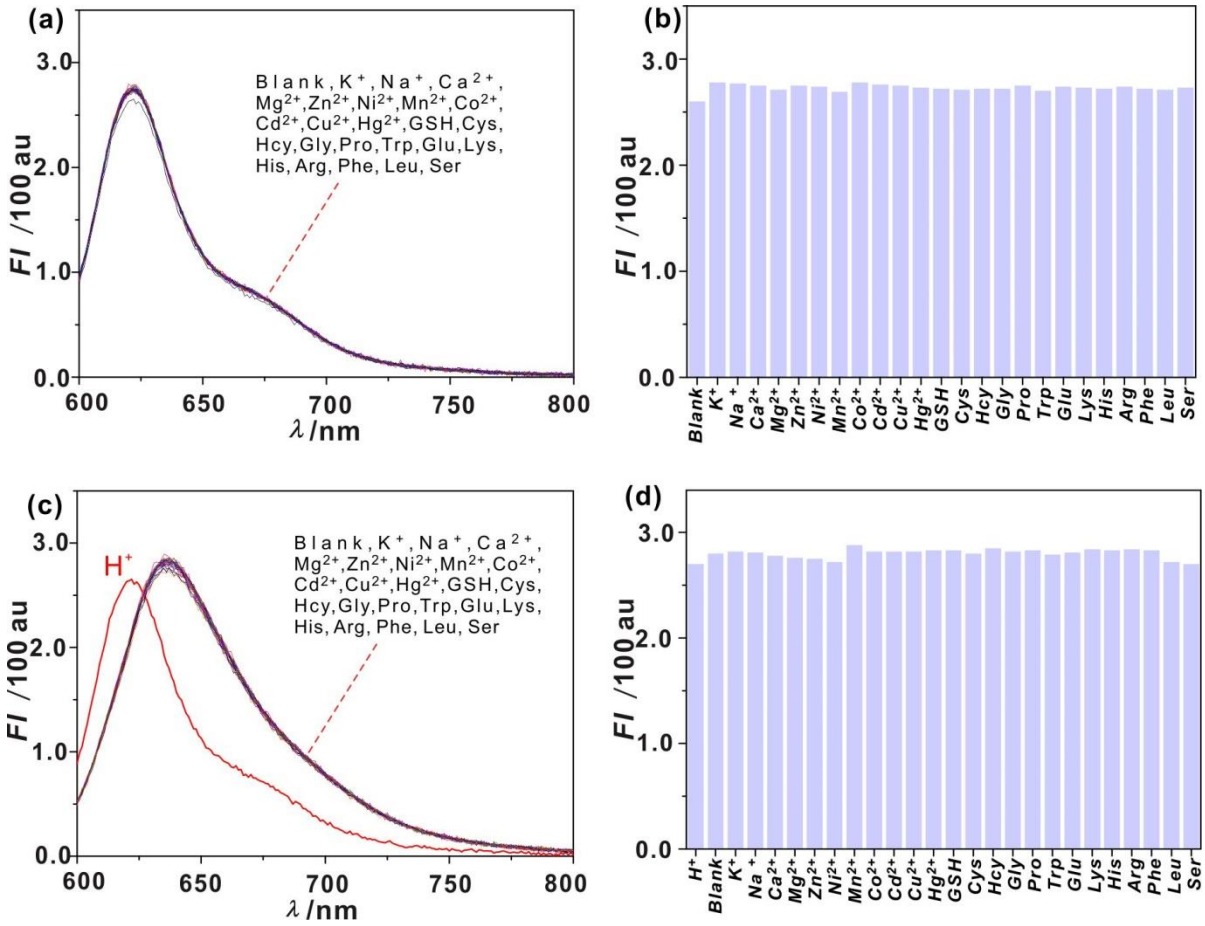


Fig. S4. Fluorescence responses of dye **1c** (5 μM) to competitive species, K^+ (100 mM), Na^+ (100 mM), Ca^{2+} (0.5 mM), Mg^{2+} (0.5 mM), Zn^{2+} (0.3 mM), Ni^{2+} (0.3 mM), Mn^{2+} (0.3 mM), Co^{2+} (0.3 mM), Cd^{2+} (0.3 mM), Cu^{2+} (0.3 mM), Hg^{2+} (0.3 mM), GSH (0.1 mM), Cys (0.1 mM), Hcy (0.1 mM), Gly (0.1 mM), Pro (0.1 mM), Trp (0.1 mM), Glu (0.1 mM), Lys (0.1mM), His (0.1 mM), Arg (0.1 mM), Phe (0.1 mM), Leu (0.1 mM) and Ser (0.1 mM) were included. (a, b) Tested under acidic conditions (pH = 1.4 or 2.4); (c, d) tested under alkaline conditions (pH = 9.0).

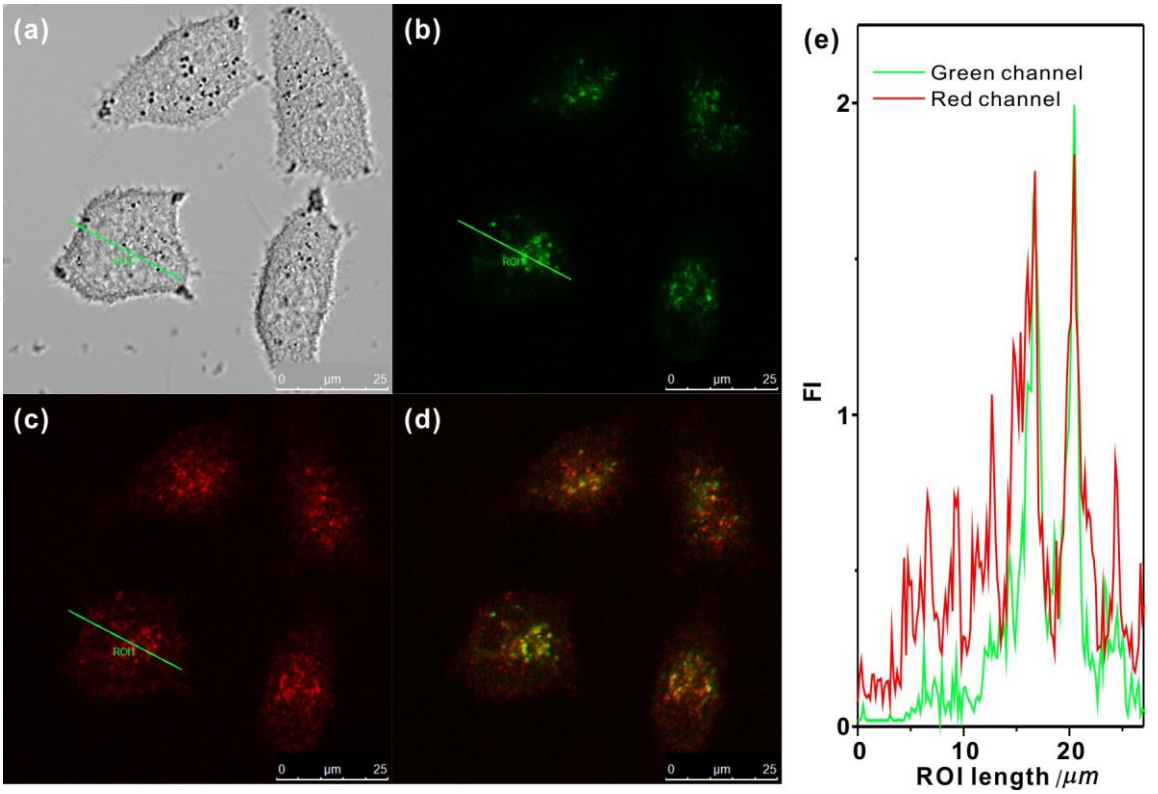


Fig. S5. Fluorescence confocal images of dye **1c** (7.5 μM) with Ges-1(a, b, c, d) cell and ROIs (e) analysis. (a) Bright-field images; (b) confocal images (green channel) of cells with LysoTracker Green DND-26 (50 nM); (c) confocal images (red channel) of cells with the dye **1c** (7.5 μM); (d) merged images of green and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells. Green channel emission was collected in 505-550 nm upon excitation at 488 nm, and red channel emission was collected in 580-790 nm upon excitation at 561 nm.

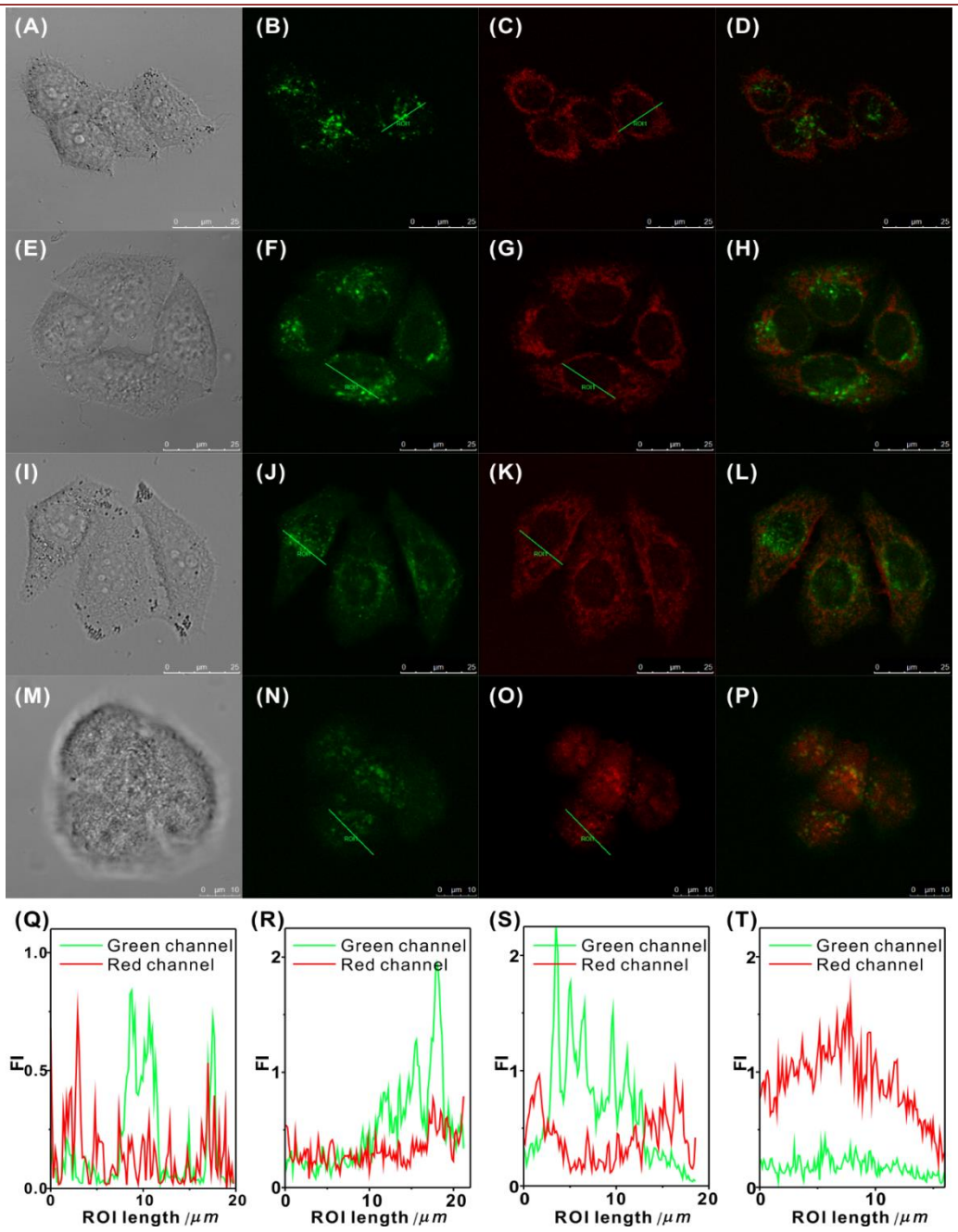


Fig. S6. Fluorescence confocal images of HeLa cells with dyes **1a**, **1b**, **1d** and **1f** (7.5 μM) and their ROIs analysis. (A, B, C, D, Q) dye **1a**; (E, F, G, H, R) dye **1b**; (I, J, K, L, S) dye **1d**; (M, N, O, P, T) dye **1f**; (A, E, I, M) Bright-field images; (B, F, J, N) confocal images (green channel) of cells with LysoTracker Green DND-26 (50 nM); (C, G, K, O) confocal images (red channel) of cells with the dyes (7.5 μM); (D, H, L, P) merged images of corresponding green and red channels; (Q, R, S, T) fluorescence intensities of the ROIs across the cells, (Q) ROIs of (B) and (C), (R) ROIs of (F) and (G), (S) ROIs of (J) and (K), (T) ROIs of (N) and (O). Green channel emission was collected in 505–550 nm upon excitation at 488 nm, and red channel emission was collected in 580-790 nm upon excitation at 561 nm.

3. Appendix

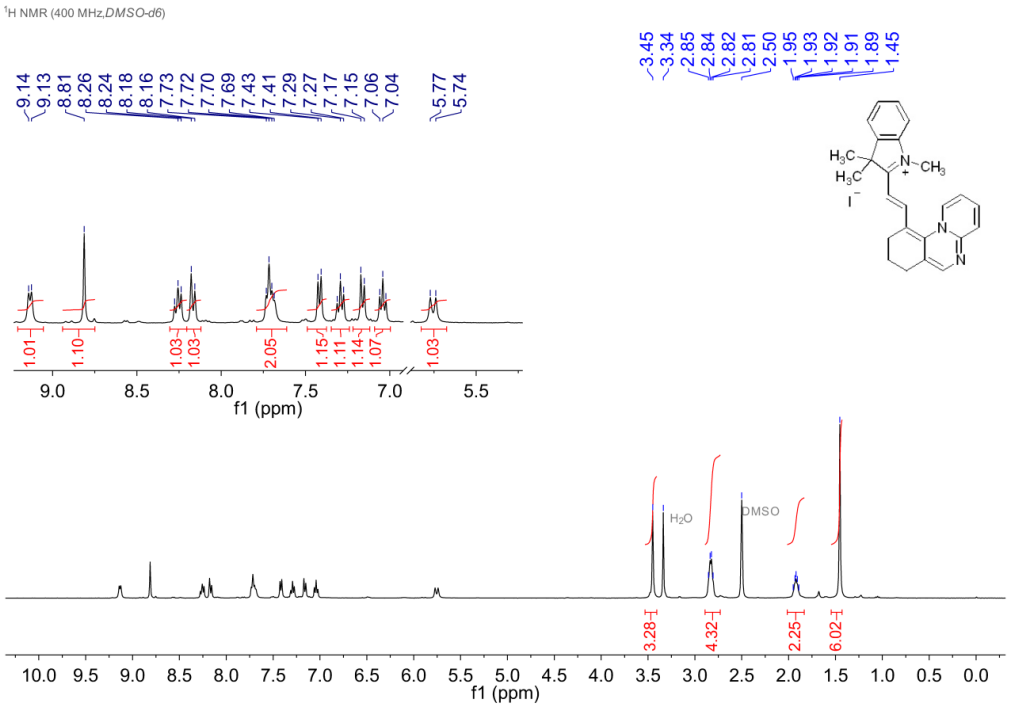


Fig. S7. ^{1}H NMR of dye **1a**.

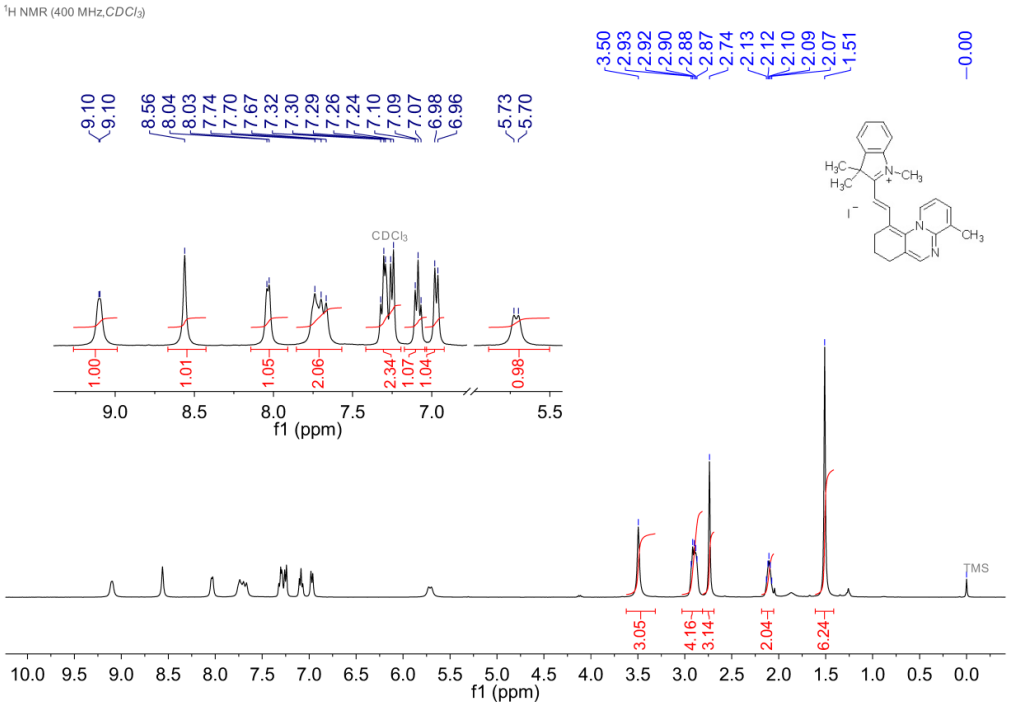


Fig. S8. ^{1}H NMR of dye **1b**.

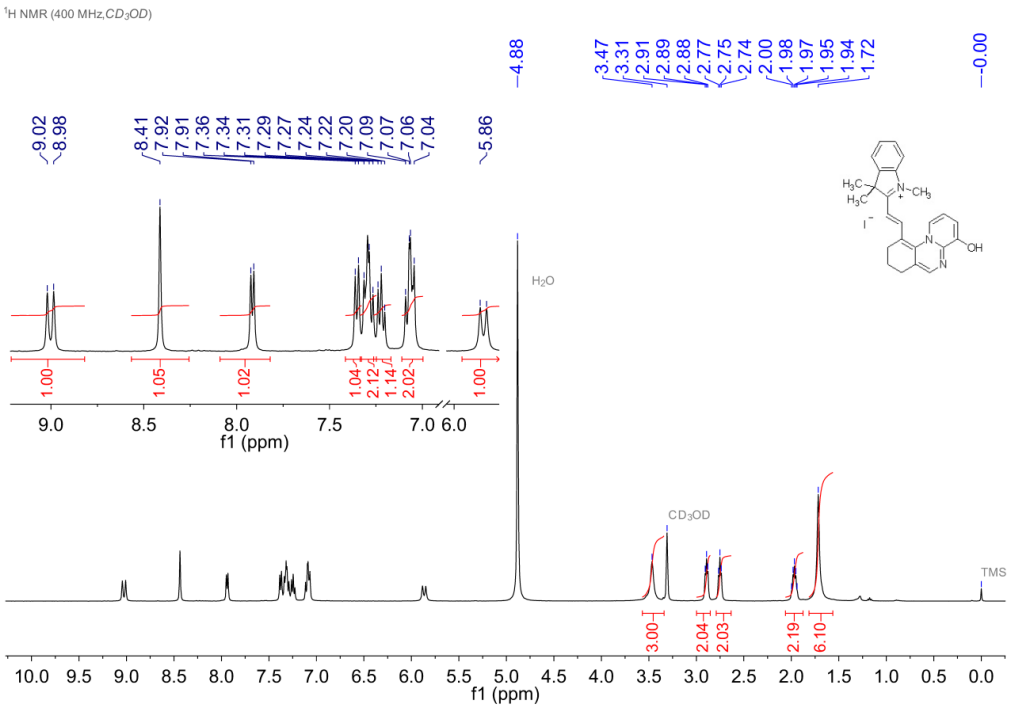


Fig. S9. ^{1}H NMR of dye **1c**.

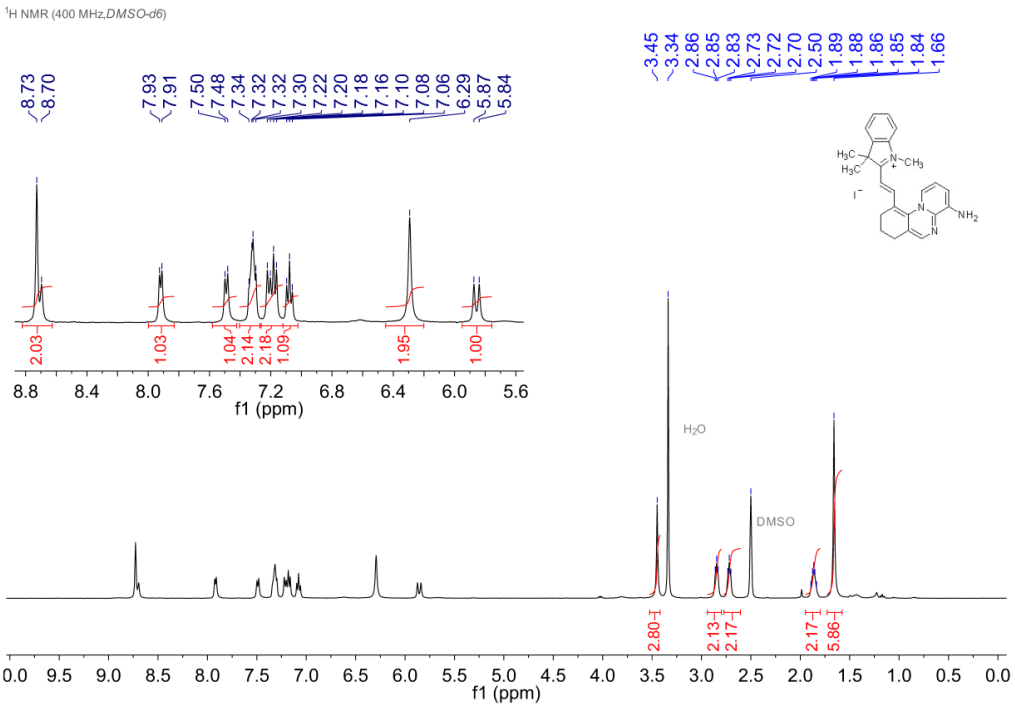


Fig. S10. ^{1}H NMR of dye **1d**.

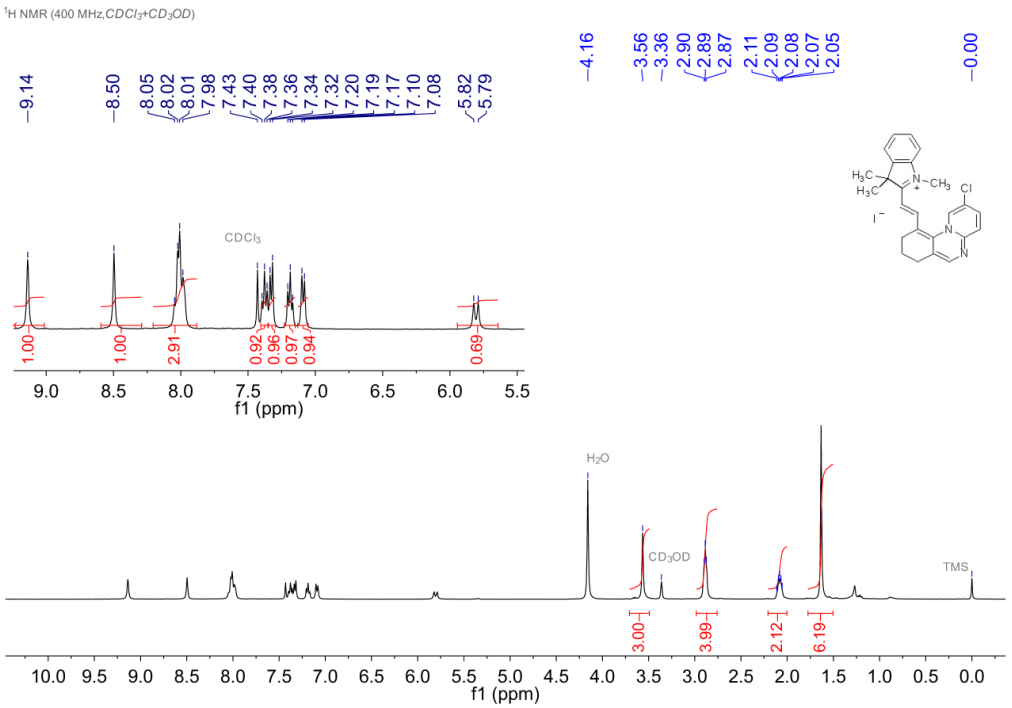


Fig. S11. ^{1}H NMR of dye **1e**.

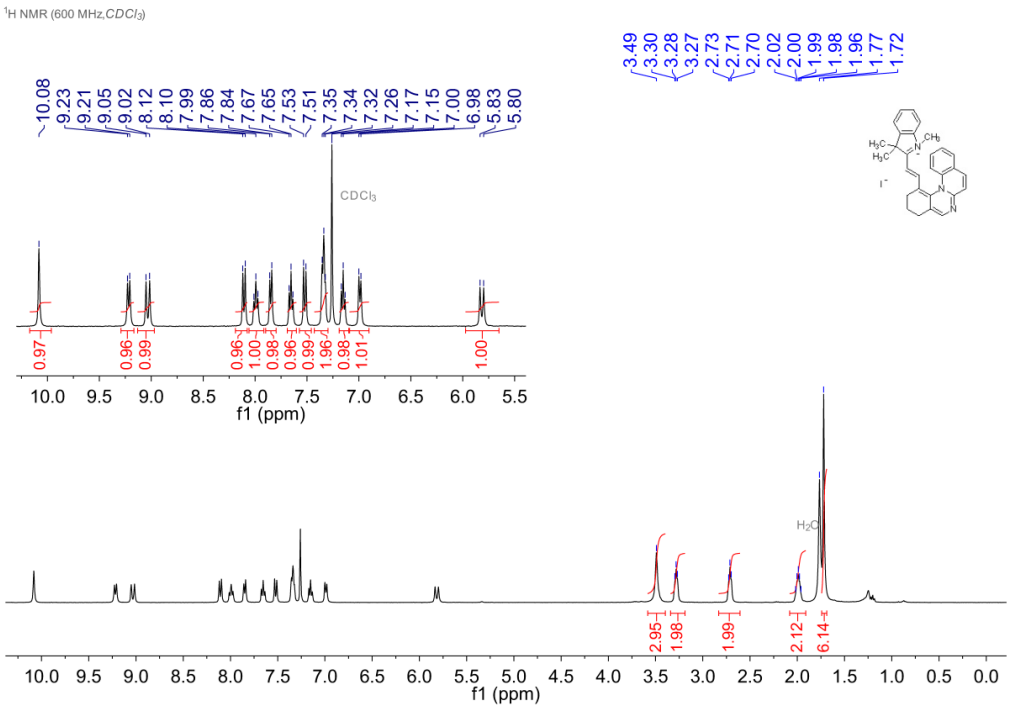


Fig. S12. ^{1}H NMR of dye **1f**.

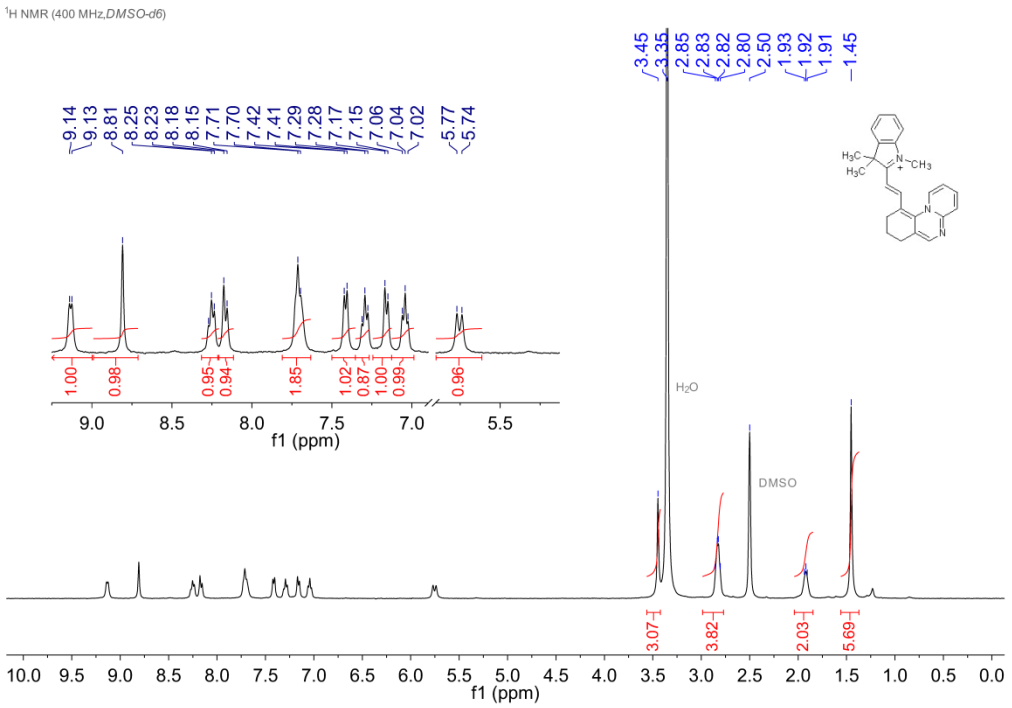


Fig. S13. ^{1}H NMR of dye **1a'**.

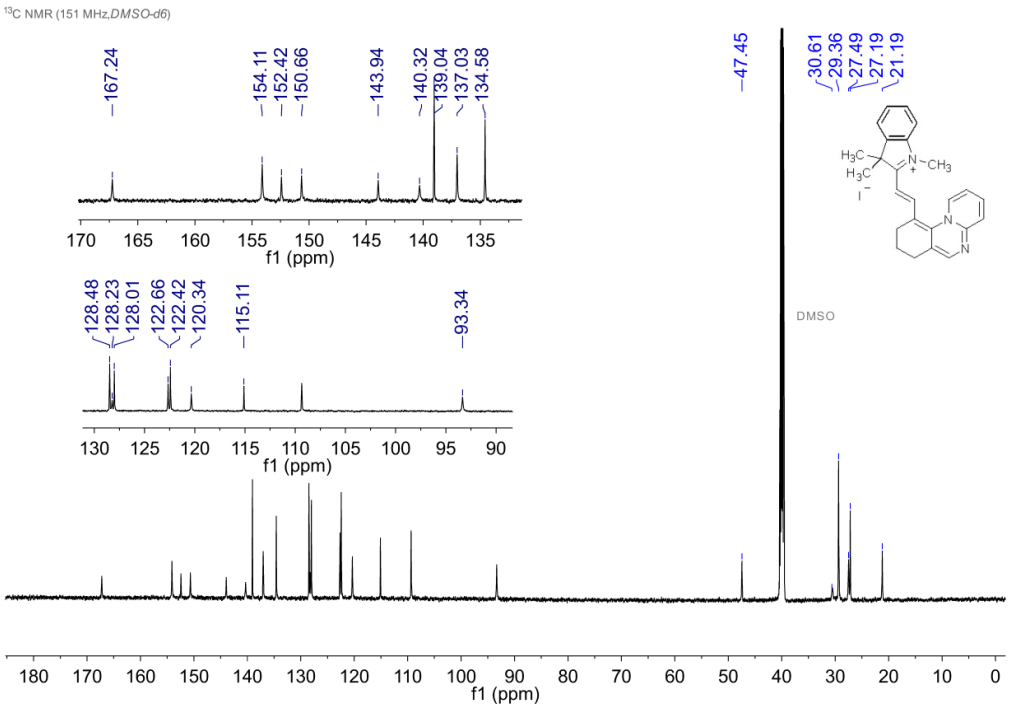


Fig. S14. ^{13}C NMR of dye **1a**.

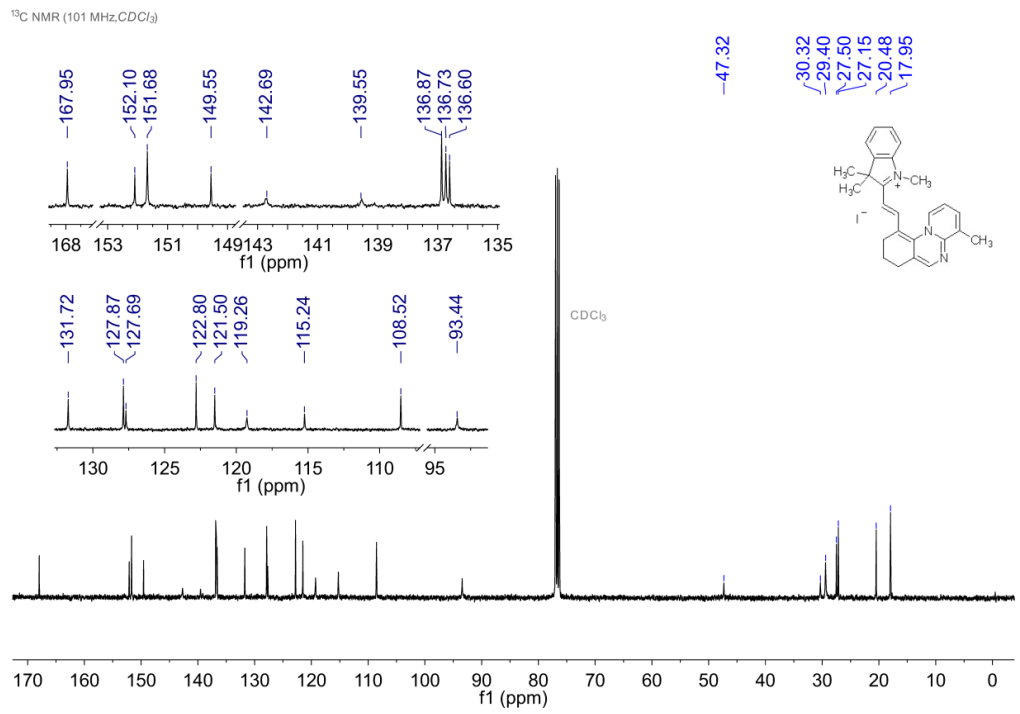


Fig. S15. ^{13}C NMR of dye **1b**.

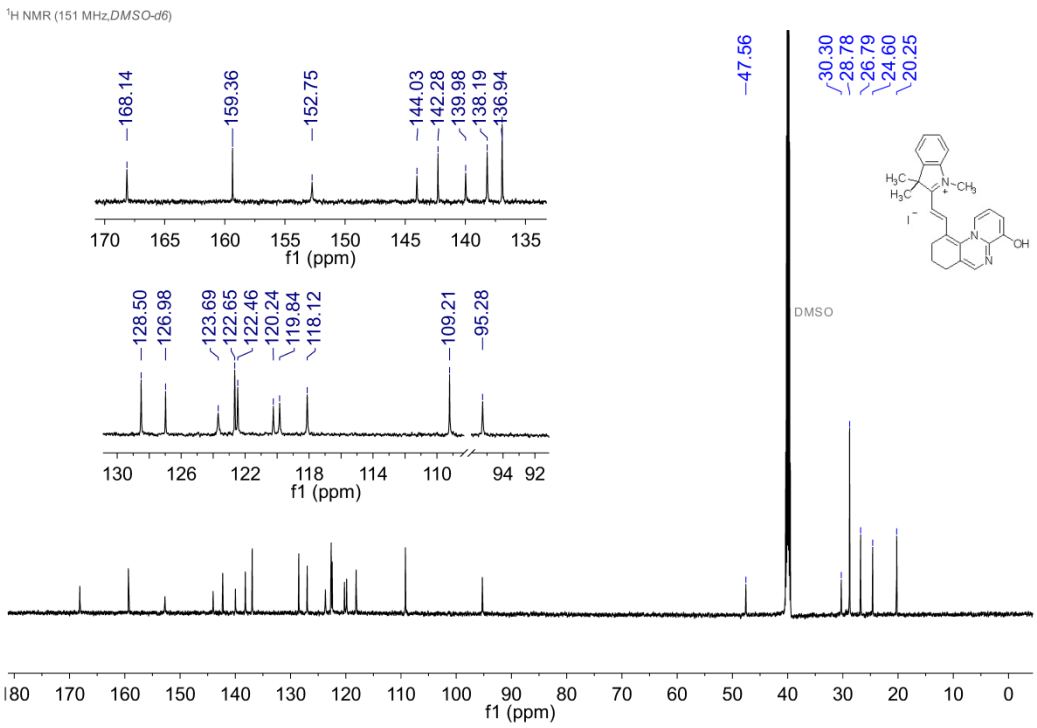


Fig. S16. ^{13}C NMR of dye **1c**.

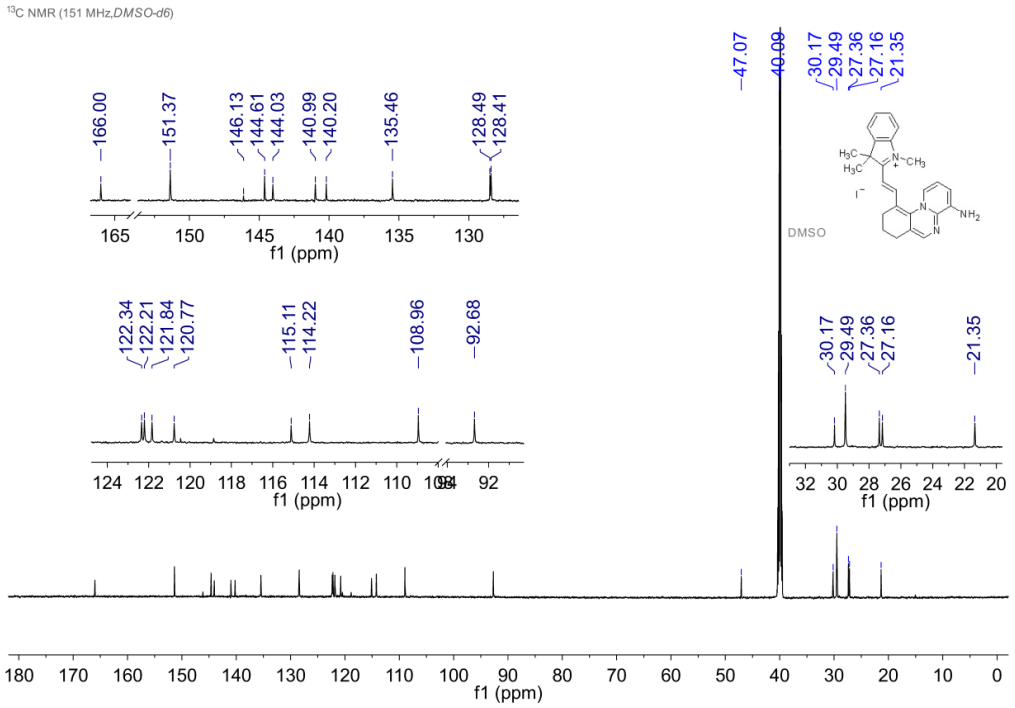


Fig. S17. ^{13}C NMR of dye **1d**.

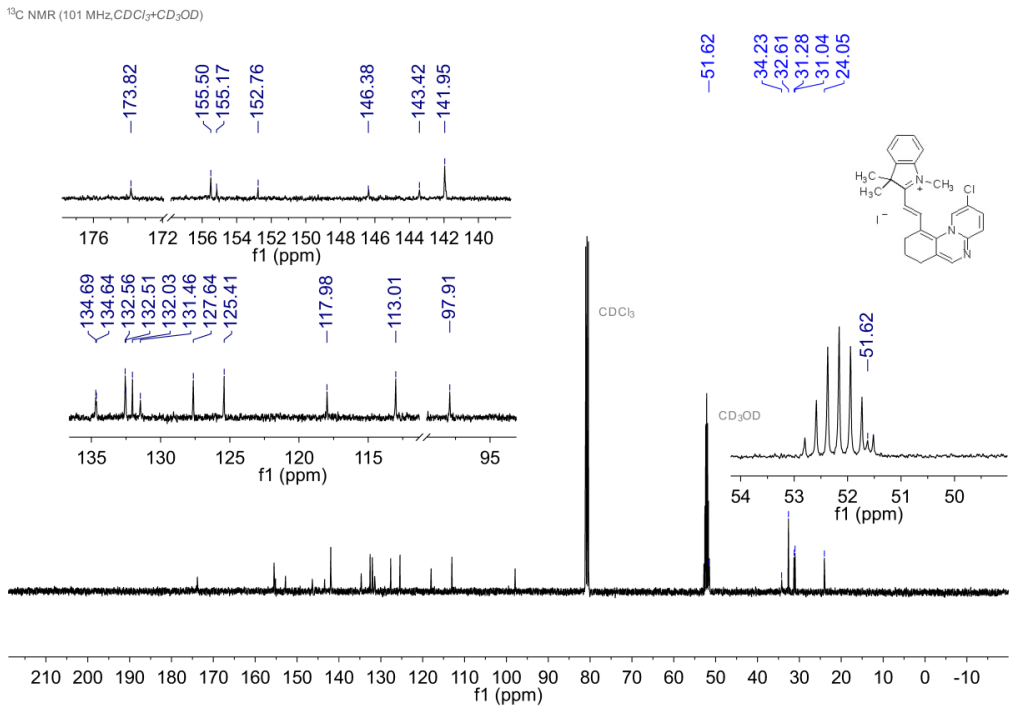


Fig. S18 ^{13}C NMR of dye **1e**.

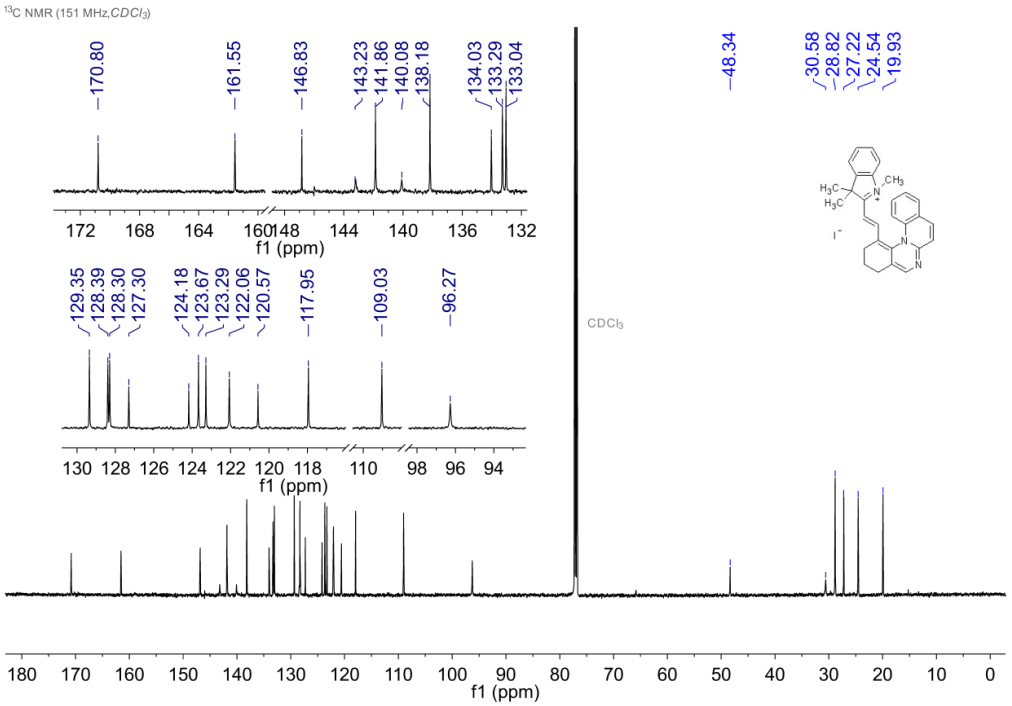


Fig. S19 ^{13}C NMR of dye **1f**.

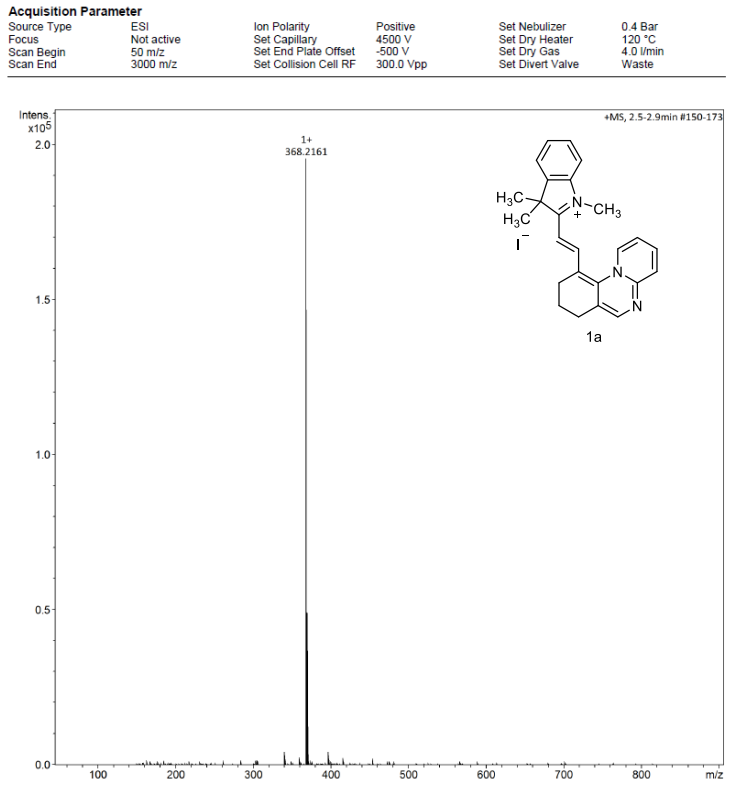


Fig. S20 HRMS of dye **1a**.

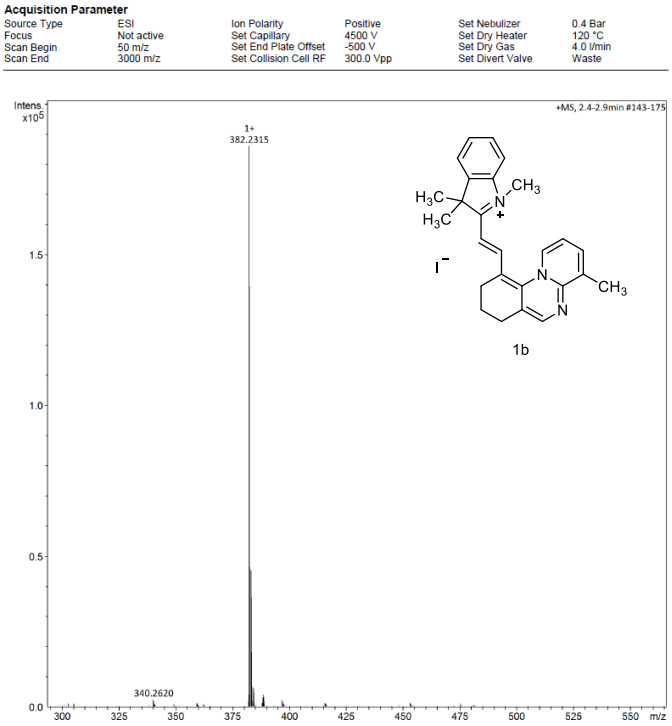


Fig. S21 HRMS of dye **1b**.

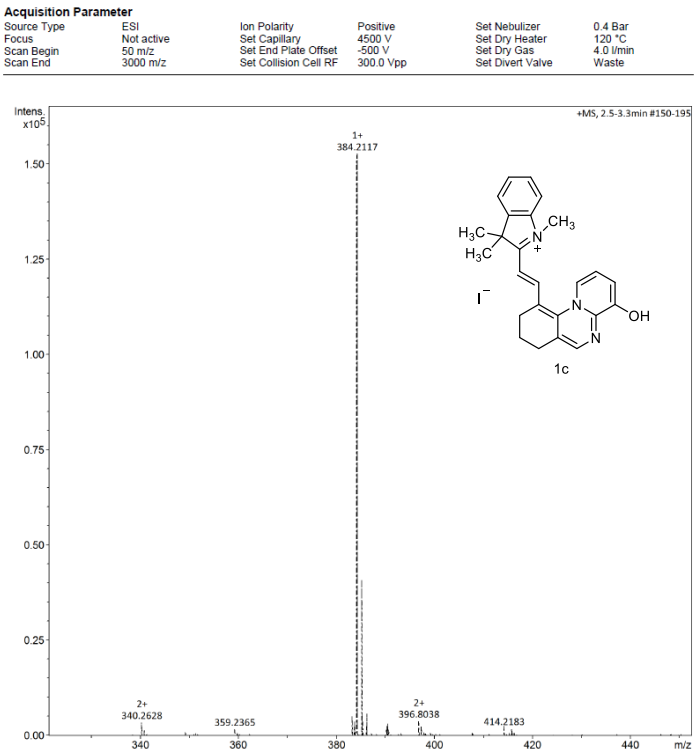


Fig. S22 HRMS of dye **1c**.

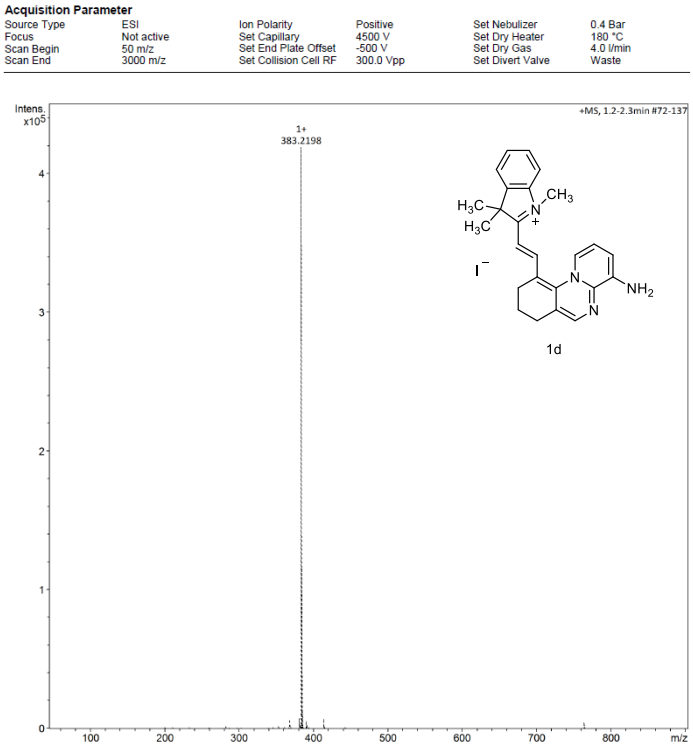


Fig. S23 HRMS of dye **1d**.

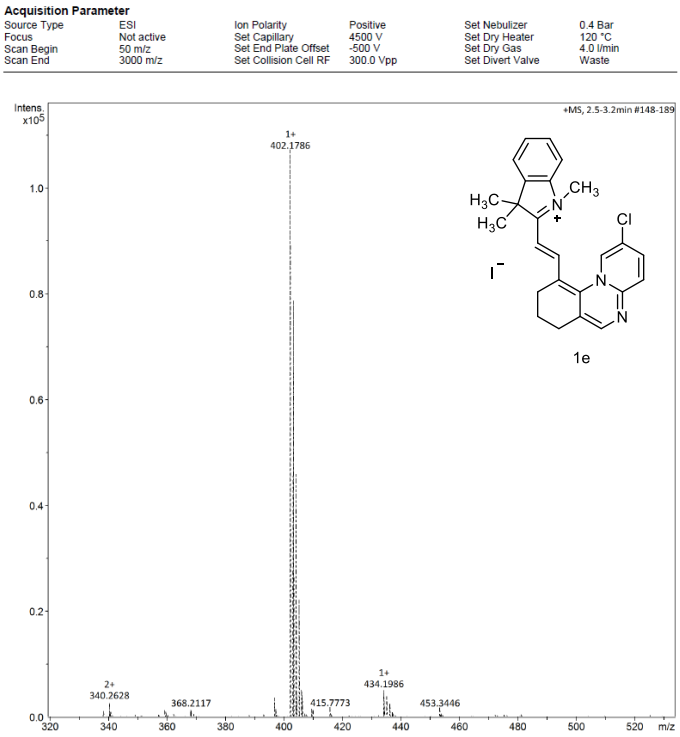


Fig. S24 HRMS of dye **1e**.

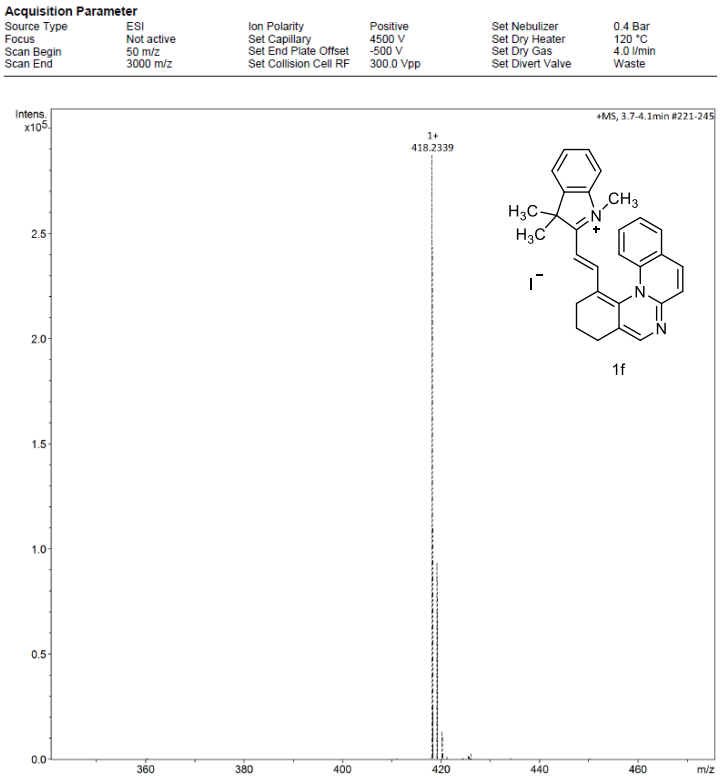


Fig. S25 HRMS of dye **1f**.

4. Reference

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