Analytical Methods

Electronic Supplementary Information

Synthesis of azonia cyanine derivatives as NIR fluorescent probes for nucleic acid detection and cell imaging

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Table S1 Optical properties of dyes **1d–f** in different solvents.

Dyes	Solvent	$\lambda_{Abs,max}$ [a]	$\lambda_{Em,max}$ [a]	ε [b]	Φ [c]
1d	DCM	756	787	8.79	7.6
1d	TOL	728	777	2.74	6.9
1d	THF	735	783	3.86	7.1
1d	DMSO	729	795	3.3	18.2
1d	MeOH	757	786	5.78	2.8
1e	DCM	758	789	9.67	5.56
1e	TOL	730	778	3.26	7.58
1e	THF	737	780	5.00	10.2
1e	DMSO	733	793	3.75	9.19
1e	MeOH	732	780	5.40	4.79
1f	DCM	768	797	10.28	1.0
1f	TOL	751	784	3.82	3.1
1f	THF	752	792	5.54	1.7
1f	DMSO	749	797	3.88	1.3
1f	MeOH	769	796	8.19	0.4

[a] reported in nm; [b] ε reported in 10^4 $M^{-1}cm^{-1}$; [c] reported in %, ***HEDITCP*** (Φ = 0.159 in ethanol) was used as the reference compound.

Table S2 Optical properties of dyes **1d–f** toward DNA and RNA.

Compounds	$\lambda_{Abs,max}$ [a]	$\lambda_{Em,max}$ [a]	ε [b]	Φ [c]
1d	743	775	3.48	0.37
1d+DNA	673	785	3.04	0.35
1d+RNA	680	786	1.92	0.47
1e	668	783	2.46	0.21
1e+DNA	672	788	2.26	0.18
1e+RNA	681	778	1.19	0.19
1f	754	789	3.33	< 0.01
1f+DNA	688	794	2.14	< 0.01
1f+RNA	697	801	1.16	< 0.01

[a] reported in nm; [b] εreported in 10^4 $M^{-1}cm^{-1}$; [c] reported in %. ***HEDITCP*** (Φ = 0.159 in ethanol) was used as the reference compound.

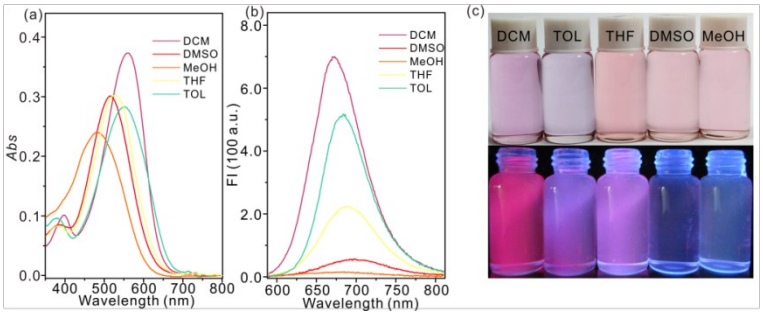


Fig. S1 Absorption spectra (a) and emission spectra (b, excited at 580nm, slit widths: 3 nm/1.5 nm) of dye **1b** (10 μM) in different solvents; (c) the photos of **1b** in different solvents under sunlight (top) and UV lamp (365 nm, down).

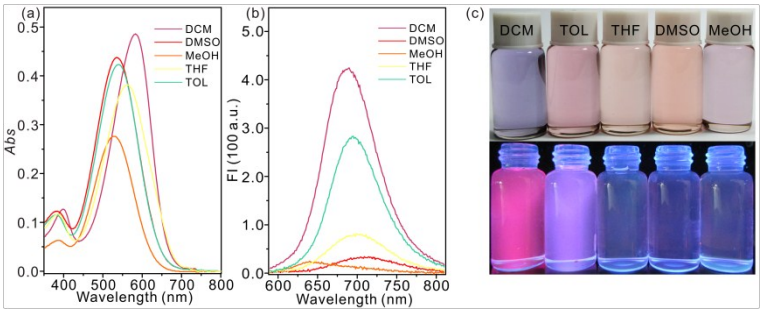
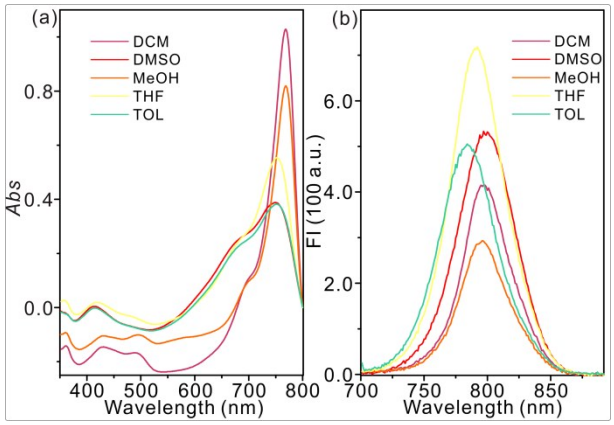


Fig. S2 Absorption spectra (a) and emission spectra (b, excited at 580 nm, slit widths: 3 nm/1.5 nm) of dye **1c** (10 μM) in different solvents; (c) the photos of **1c** in different solvents under sunlight (top) and UV lamp (365 nm, down).



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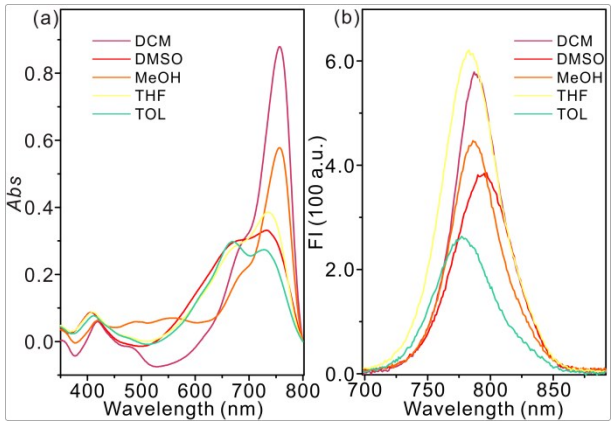

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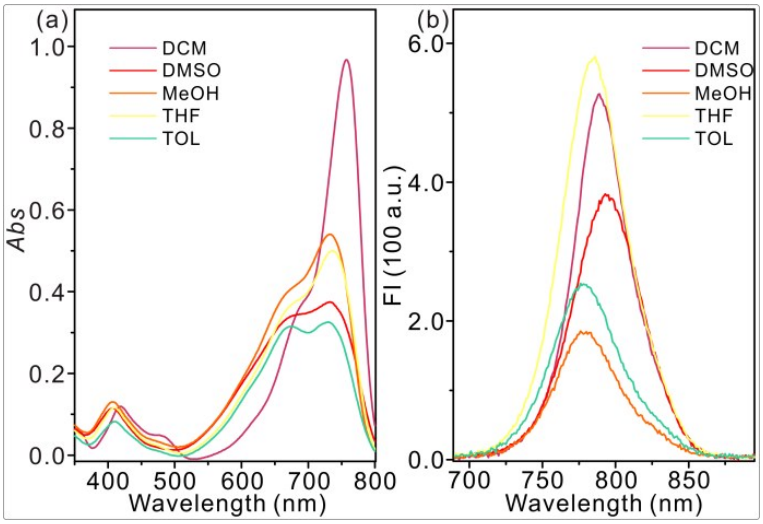

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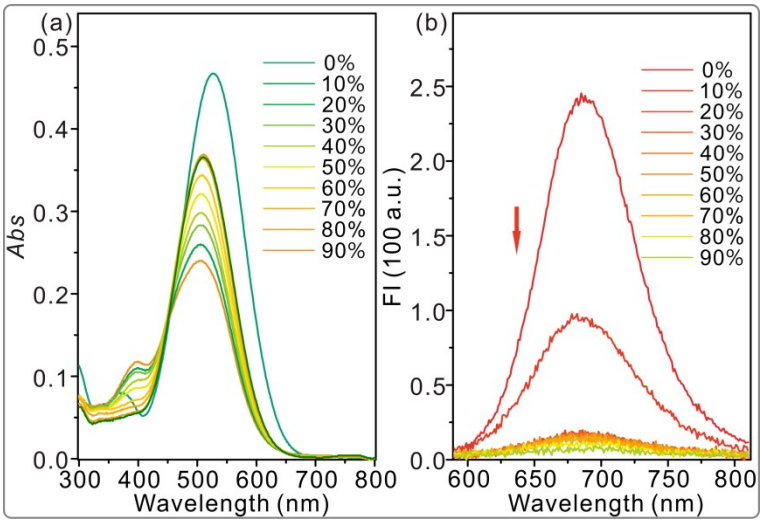
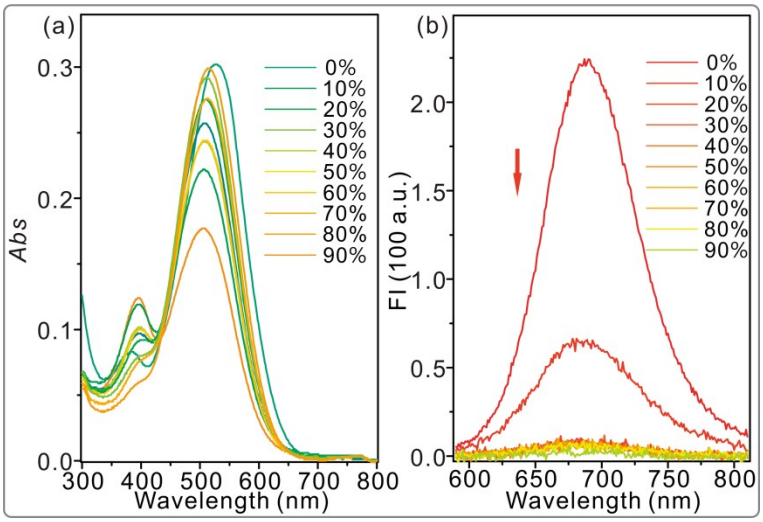


Fig. S6 Optical responses of dye **1a** (10 μM) in THF with different fraction of water (f_w). (a) Absorption spectra; (b) emission spectra (λ_{ex} = 580 nm, slit widths: 3 nm/1.5 nm).



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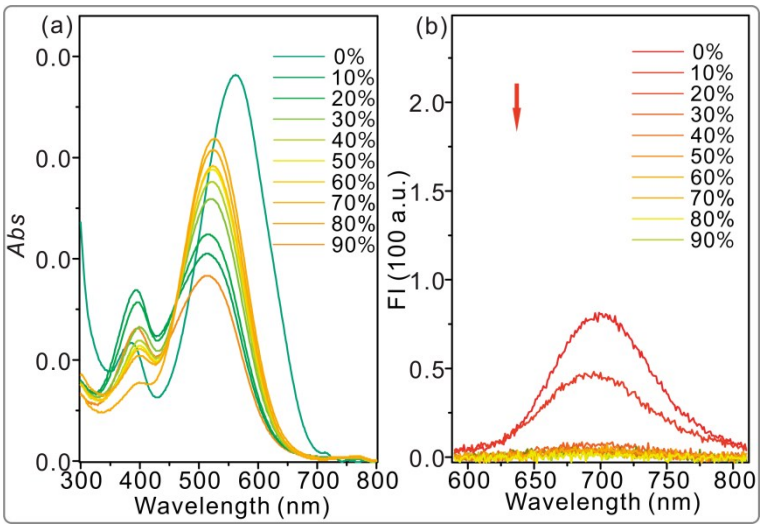

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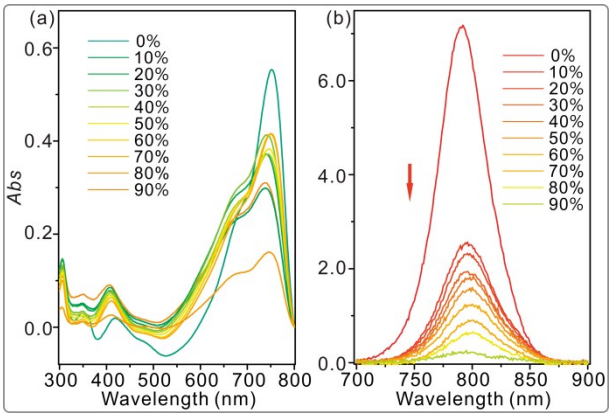

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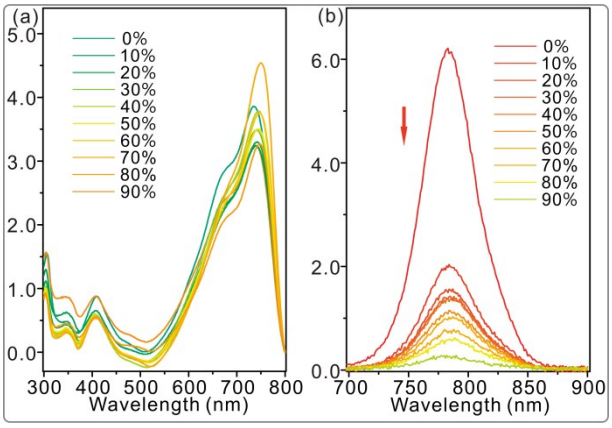

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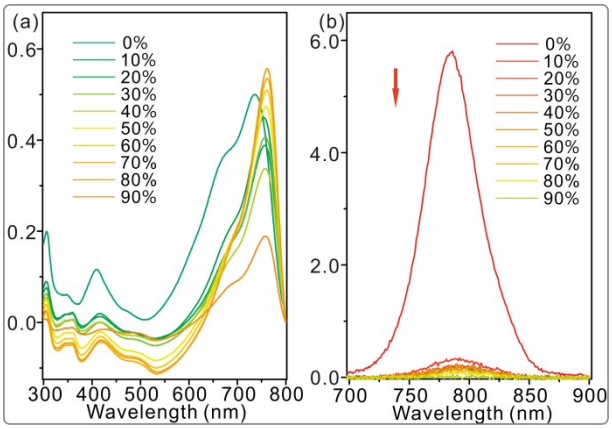

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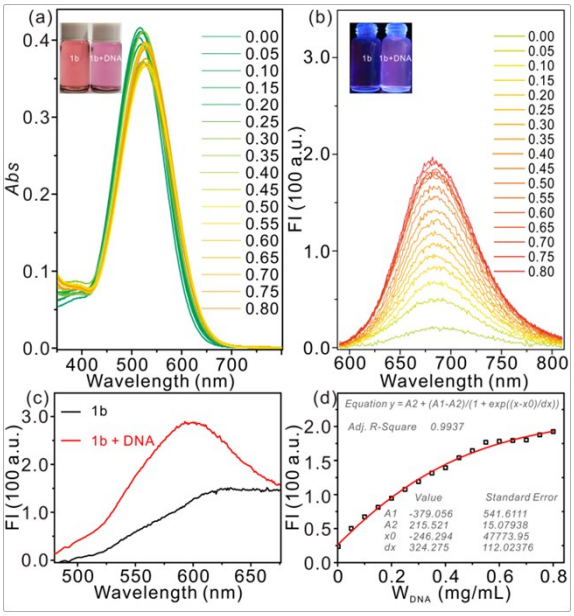

Fig. S12 Optical responses of dye **1b** (10 μM) toward DNA (0–800 μg·mL^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra, inset shows photograph of the samples before and after addition DNA; (b) emission spectra (λ_{ex} = 580 nm, slit widths: 3 nm/3 nm, inset shows photographs of the samples under 365 nm); (c) excitation spectra (λ_{em} = 681 nm); (d) fluorescence intensity toward different concentrations of DNA at 681 nm.

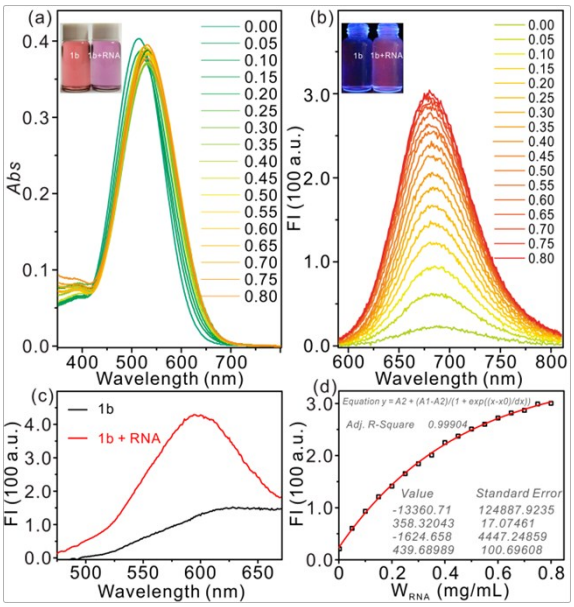

Fig. S13 Optical responses of dye **1b** (10 μM) toward RNA (0–800 μg·mL^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra, inset shows photograph of the samples before and after addition RNA; (b) emission spectra (λ_{ex} = 580 nm, slit widths: 3 nm/3 nm, inset shows photographs of the samples under 365 nm); (c) excitation spectra (λ_{em} = 678 nm); (d) fluorescence intensity toward different concentrations of RNA at 678 nm.

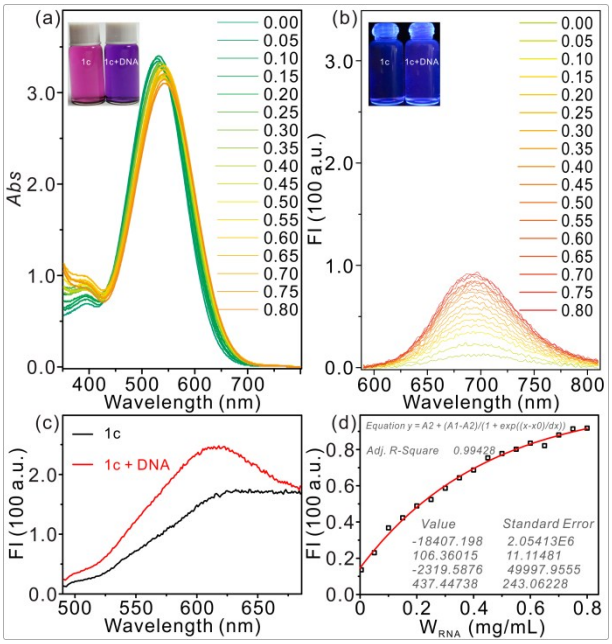

Fig. S14 Optical responses of dye **1c** (10 μM) toward DNA (0–800 μg·mL^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra, inset shows photograph of the samples before and after addition DNA; (b) emission spectra (λ_{ex} = 580 nm, slit widths: 3 nm/3 nm, inset shows photographs of the samples under 365 nm); (c) excitation spectra (λ_{em} = 695 nm); (d) fluorescence intensity toward different concentrations of DNA at 695 nm.

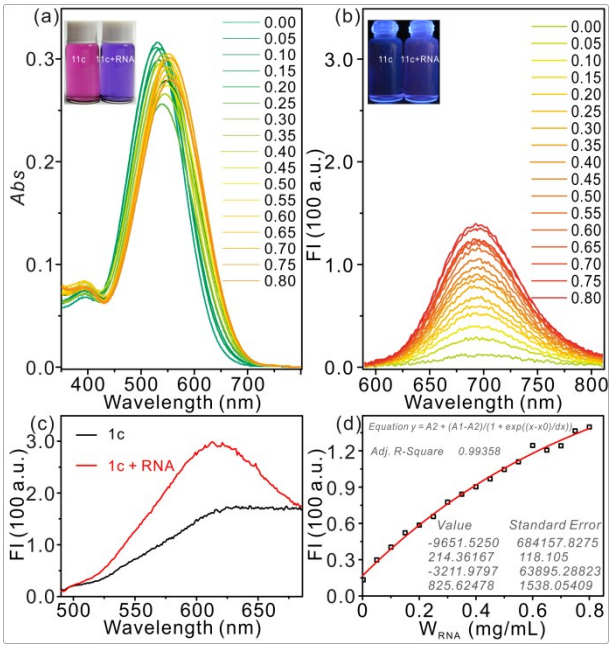

Fig. S15 Optical responses of dye **1c** (10 μM) toward RNA (0–800 μg·mL^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra, inset shows photograph of the samples before and after addition RNA; (b) emission spectra (λ_{ex} = 580 nm, slit widths: 3 nm/3 nm, inset shows photographs of the samples under 365 nm); (c) excitation spectra (λ_{em} = 697 nm); (d) fluorescence intensity toward different concentrations of RNA at 697 nm.

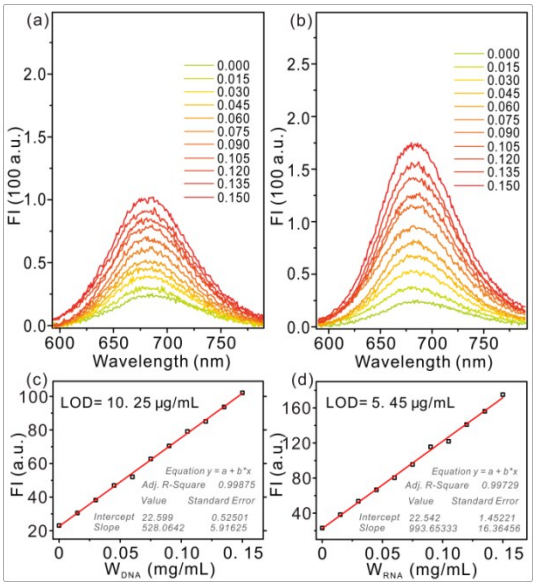

Fig. S16 Fluorescence emission spectrum of dye **1a** (10 μM) toward DNA (0–150 μg·L^{-1}) and RNA (0–150 μg·L^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO. (a) Emission spectra of dye **1a** in the presence of different concentrations of DNA (λex = 580 nm, slit widths: 3 nm/3 nm); (b) emission spectra of dye **1a** in the presence of different concentrations of RNA (λex = 580 nm, slit widths: 3 nm/3 nm); (c) fluorescene intensities toward different concentrations of DNA at 680 nm; (d) fluorescene intensities toward different concentrations of RNA at 680 nm.

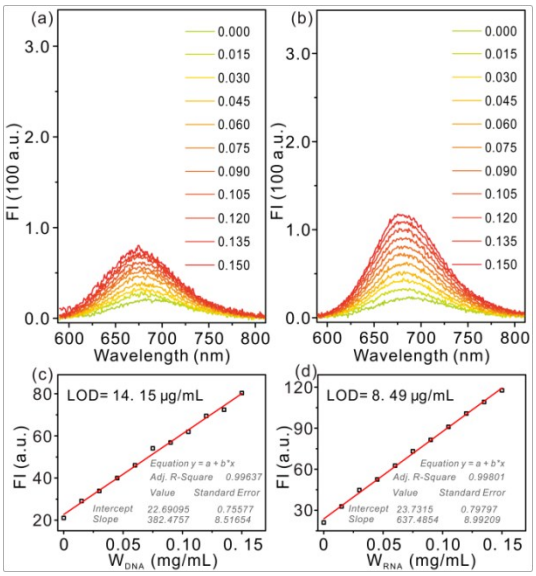

Fig. S17 Fluorescence emission spectrum of dye **1b** (10 μM) toward DNA (0–150 μg·L^{-1}) and RNA (0–150 μg·L^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO. (a) Emission spectra of dye **1b** in the presence of different concentrations of DNA (λex = 580 nm, slit widths: 3 nm/3 nm); (b) emission spectra of dye **1b** in the presence of different concentrations of RNA (λex = 580 nm, slit widths: 3 nm/3 nm); (c) fluorescene intensities toward different concentrations of DNA at 681 nm; (d) fluorescene intensities toward different concentrations of RNA at 678 nm.

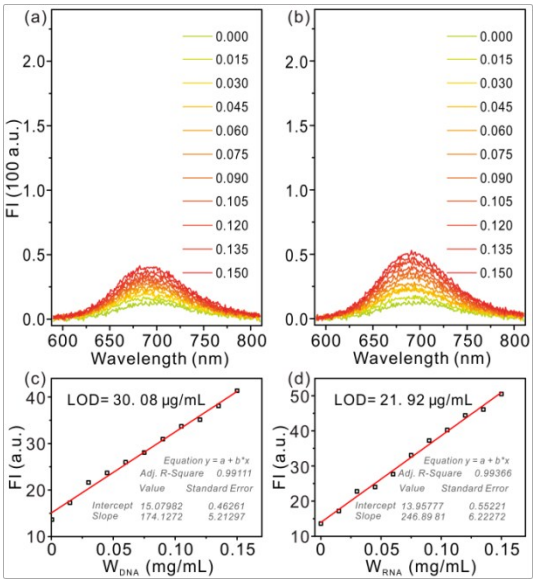

Fig. S18 Fluorescence emission spectrum of dye **1c** (10 μM) toward DNA (0–150 μg·L^{-1}) and RNA (0–150 μg·L^{-1}) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO. (a) Emission spectra of dye **1c** in the presence of different concentrations of DNA (λex = 580 nm, slit widths: 3 nm/3 nm); (b) emission spectra of dye **1c** in the presence of different concentrations of RNA (λex = 580 nm, slit widths: 3 nm/3 nm); (c) fluorescene intensities toward different concentrations of DNA at 695 nm; (d) fluorescene intensities toward different concentrations of RNA at 697 nm.

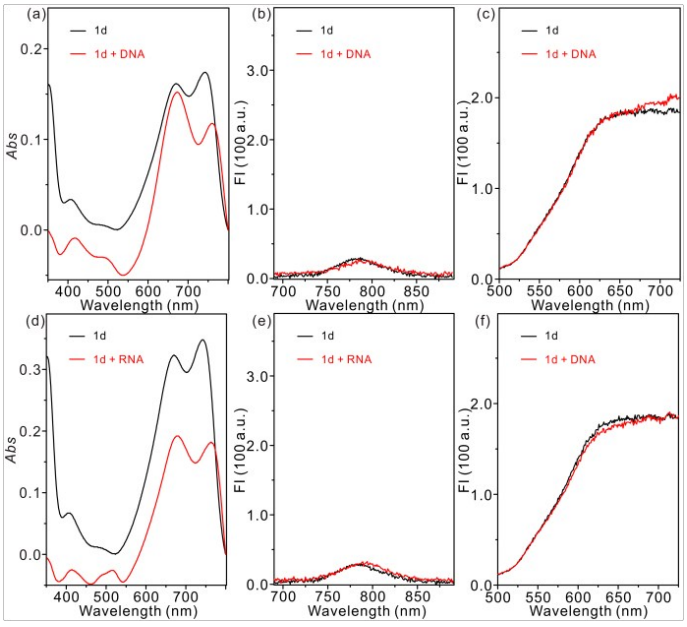

Fig. S19 Optical responses of dye **1d** (10 μM) in the presence or abpresence of DNA (800 μg·mL^{-1}, a, b, c) or RNA (650 μg·mL^{-1}, d, e, f) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra; (b) emission spectra (λ_{ex} = 680 nm, slit widths: 3 nm/3 nm); (c) excitation spectra (λ_{em} = 785 nm); (d) absorption spectra; (e) emission spectra (λex = 680 nm, slit widths: 3 nm/3 nm); (f) excitation spectra (λem = 786 nm).

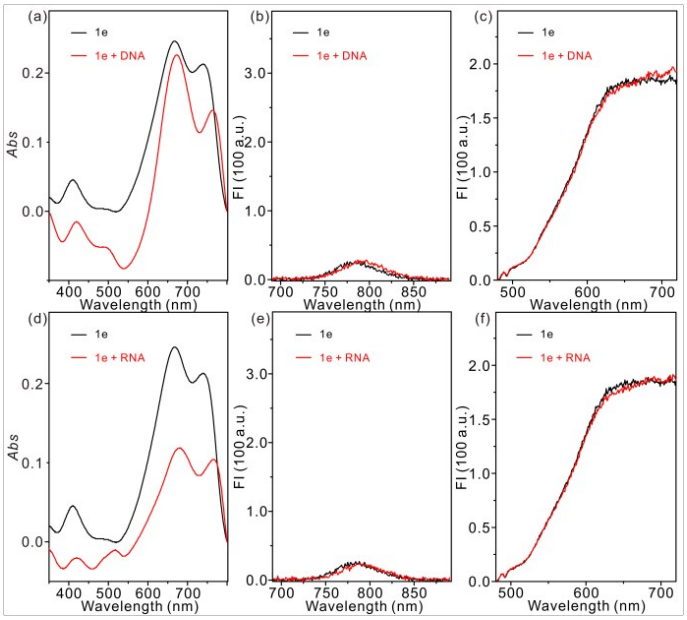

Fig. S20 Optical responses of dye **1e** (10 μM) in the presence or abpresence of DNA (800 μg·mL^{-1}, a, b, c) or RNA (650 μg·mL^{-1}, d, e, f) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra; (b) emission spectra (λ_{ex} = 680 nm, slit widths: 3 nm/3 nm); (c) excitation spectra (λ_{em} = 788 nm); (d) absorption spectra; (e) emission spectra (λex = 680 nm, slit widths: 3 nm/3 nm); (f) excitation spectra (λem = 778 nm).

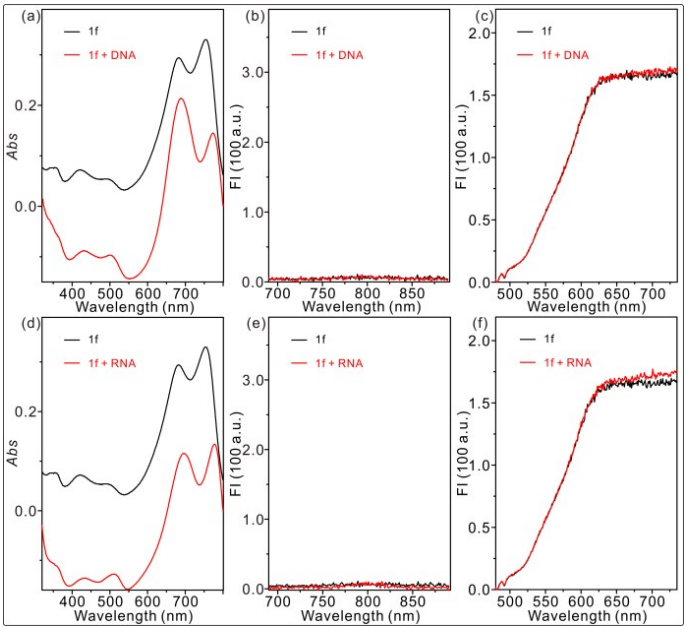

Fig. S21 Optical responses of dye **1f** (10 μM) in the presence or abpresence of DNA (800 μg·mL^{-1}, a, b, c) or RNA (650 μg·mL^{-1}, d, e, f) in Tris-HCl buffer (10 mM, pH=7.4) containing 10% DMSO (v / v). (a) Absorption spectra; (b) emission spectra (λ_{ex} = 680 nm, slit widths: 3 nm/3 nm); (c) excitation spectra (λ_{em} = 794 nm); (d) absorption spectra; (e) emission spectra (λex = 680 nm, slit widths: 3 nm/3 nm); (f) excitation spectra (λem = 801 nm).

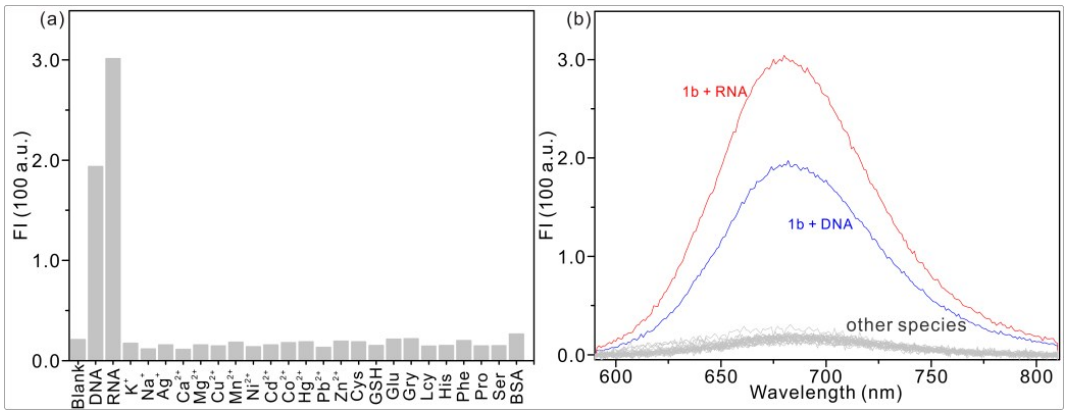

Fig. S22 The fluorescence intensity (a) and fluorescence spectra (b) of dye **1b** toward different analytes in Tris-HCl buffer solutions contained 10% (v / v) DMSO (10 mM, pH = 7.4). Analytes: 800 μg·mL^{-1} for DNA, RNA; 100 mM for Na^{+}, K^{+}, Ag^{+}; 5 mM for Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+},Hg^{2+}, Pb^{2+}, Zn^{2+}; 1 mM for Cys, GSH, Glu, Gry, Lcy, His, Phe, Pro, Ser and 5 mg mL^{-1} for BSA.

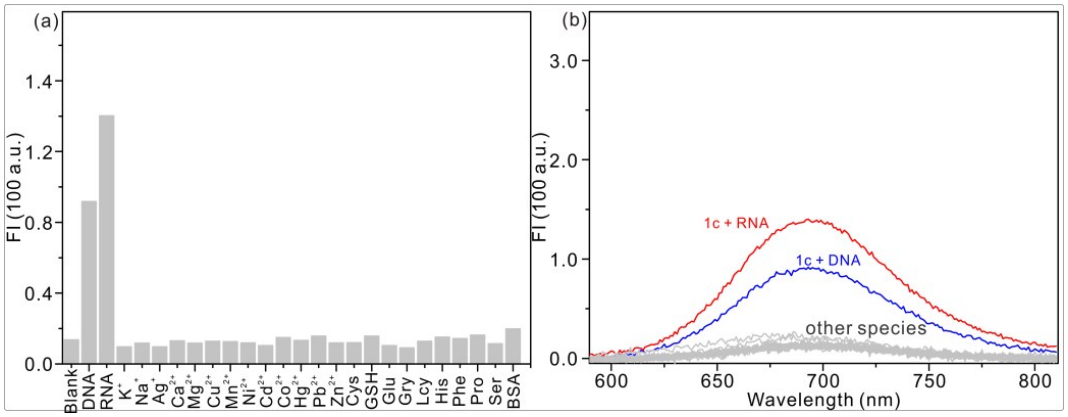

Fig. S23 The fluorescence intensity (a) and fluorescence spectra (b) of dye **1c** toward different analytes in Tris-HCl buffer solutions contained 10% (v / v) DMSO (10 mM, pH = 7.4). Analytes: 800 μg·mL^{-1} for DNA, RNA; 100 mM for Na^{+}, K^{+}, Ag^{+}; 5 mM for Ca^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+},Hg^{2+}, Pb^{2+}, Zn^{2+}; 1 mM for Cys, GSH, Glu, Gry, Lcy, His, Phe, Pro, Ser and 5 mg mL^{-1} for BSA.

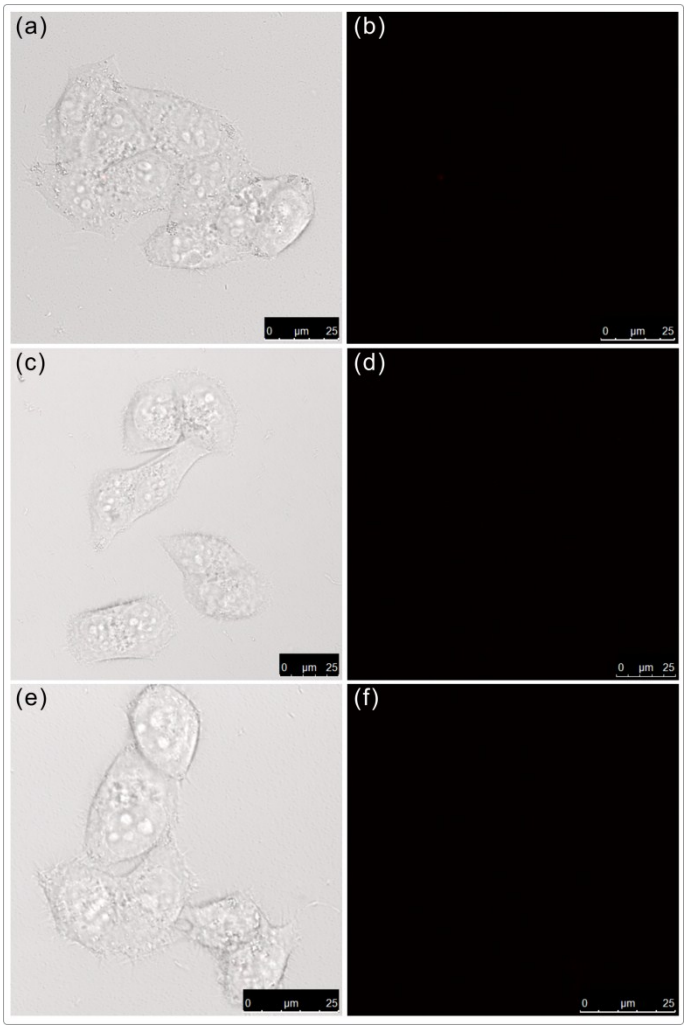

Fig. S24 Fluorescence confocal images of living HeLa cells with dyes **1a**–**c**. (a, b): Bright-field image and confocal image (red channel) of cells with **1a** (10 μM); (c, d): Bright-field image and confocal image (red channel) of cells with **1b** (10 μM); (e, f): Bright-field image and confocal image (red channel) of cells with **1c** (10 μM); (Red channel emission was collected in 575–750 nm upon excitation at 561 nm.

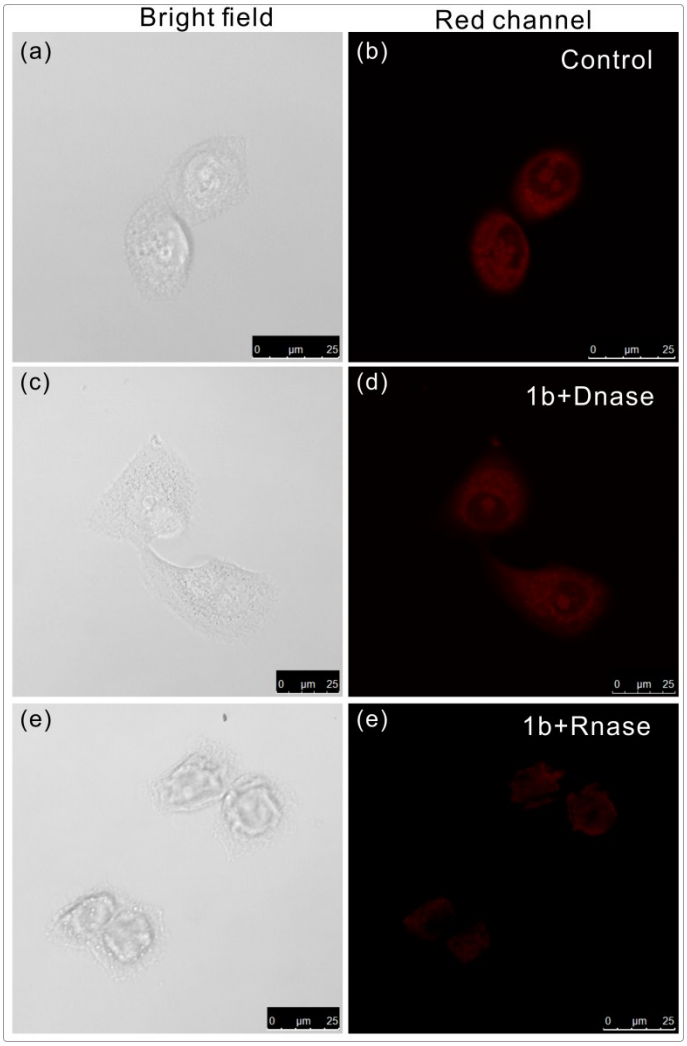

Fig. S25 Fluorescence confocal images of the digest experiment for dye **1b** (10 μM) with fixed HeLa cells. (a,b) Cells were incubated with **1b** in control experiments; (c,b) cells were incubated with **1a** and DNase (1 mg/mL); (e,f) cells were incubated with **1b** and RNase (10 mg/mL). Red channel emission was collected in 575–750 nm upon excitation at 561 nm.

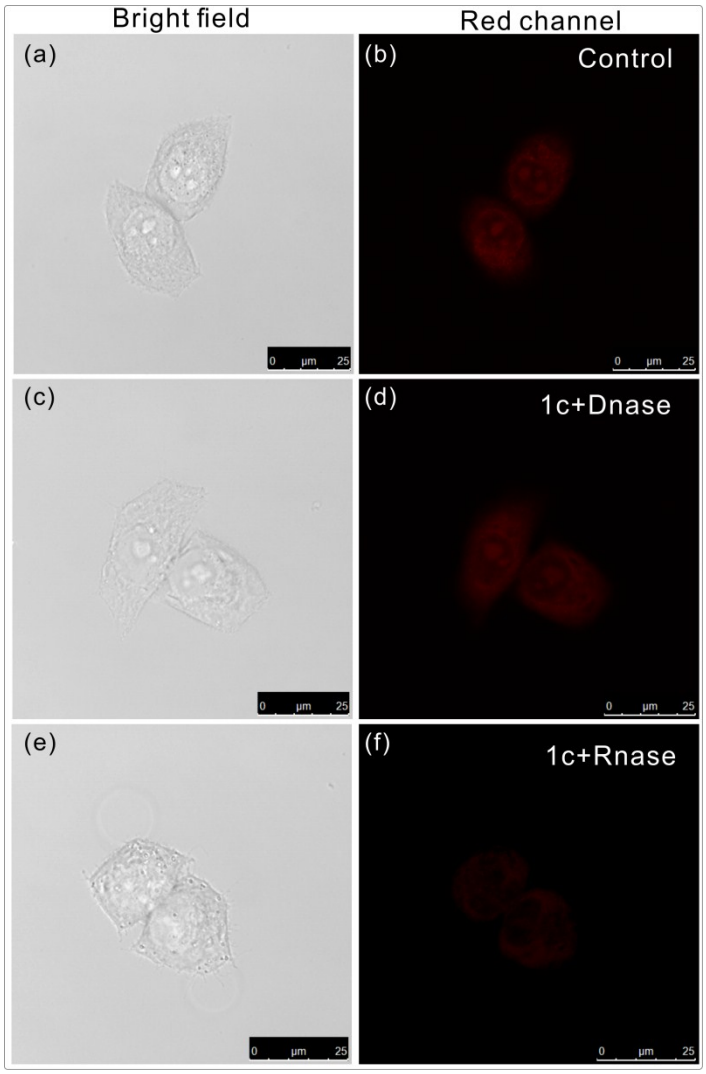

Fig. S26 Fluorescence confocal images of the digest experiment for dye **1c** (10 μM) with fixed HeLa cells. (a,b) Cells were incubated with **1c** in control experiments; (c,b) cells were incubated with **1a** and DNase (1 mg/mL); (e,f) cells were incubated with **1c** and RNase (10 mg/mL). Red channel emission was collected in 575−750 nm upon excitation at 561 nm.

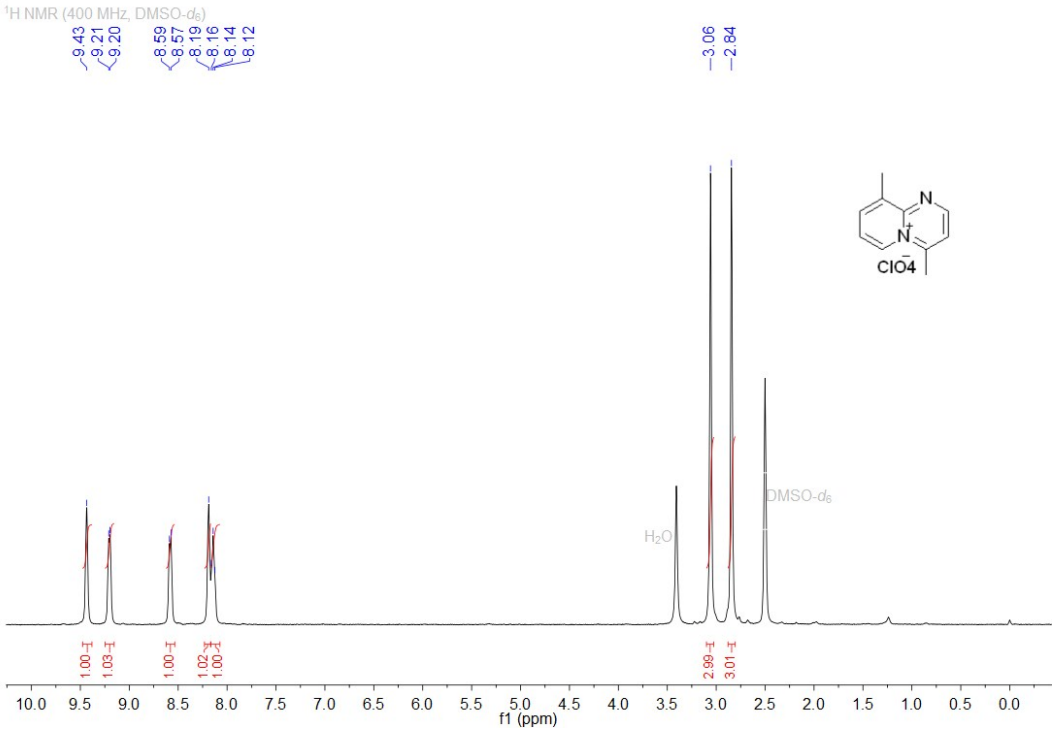

Fig. S27 ^{1}H NMR spectra of dye **3a**.

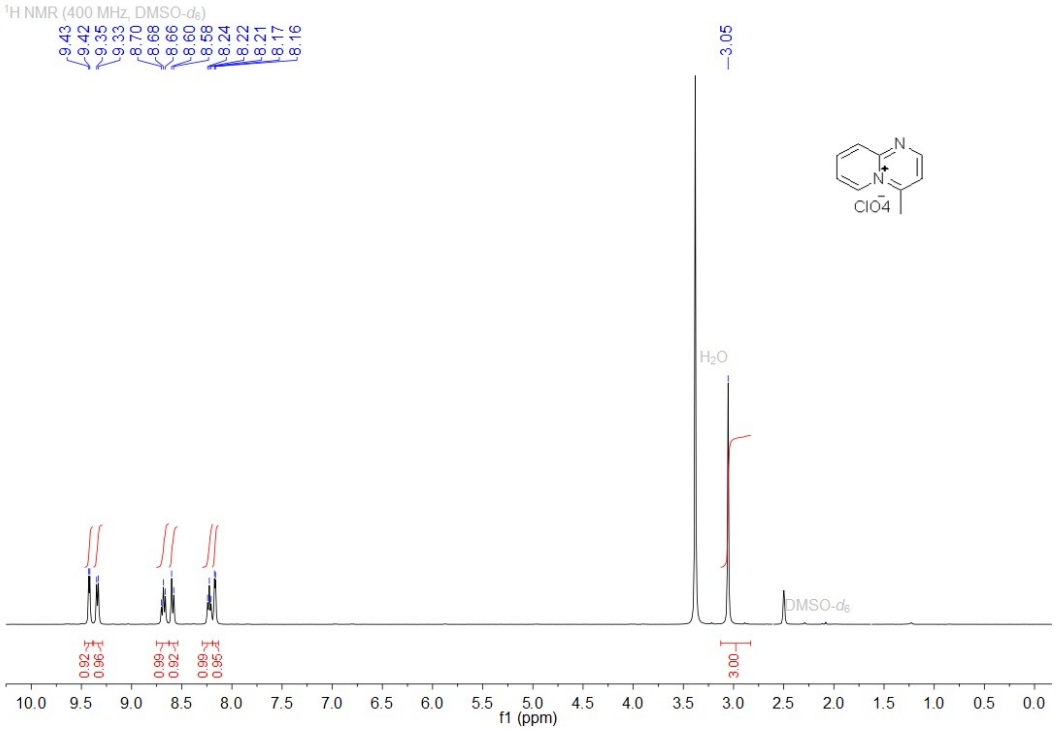

Fig. S28 ^{1}H NMR spectra of dye **3b**.

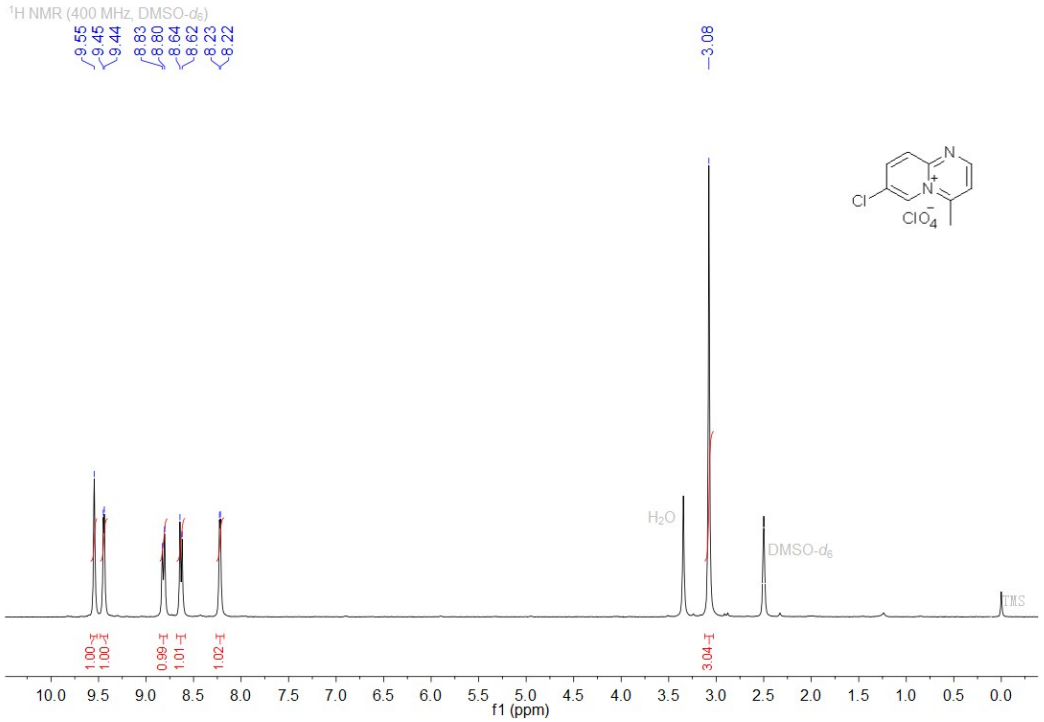

Fig. S29 ^{1}H NMR spectra of dye **3c**.

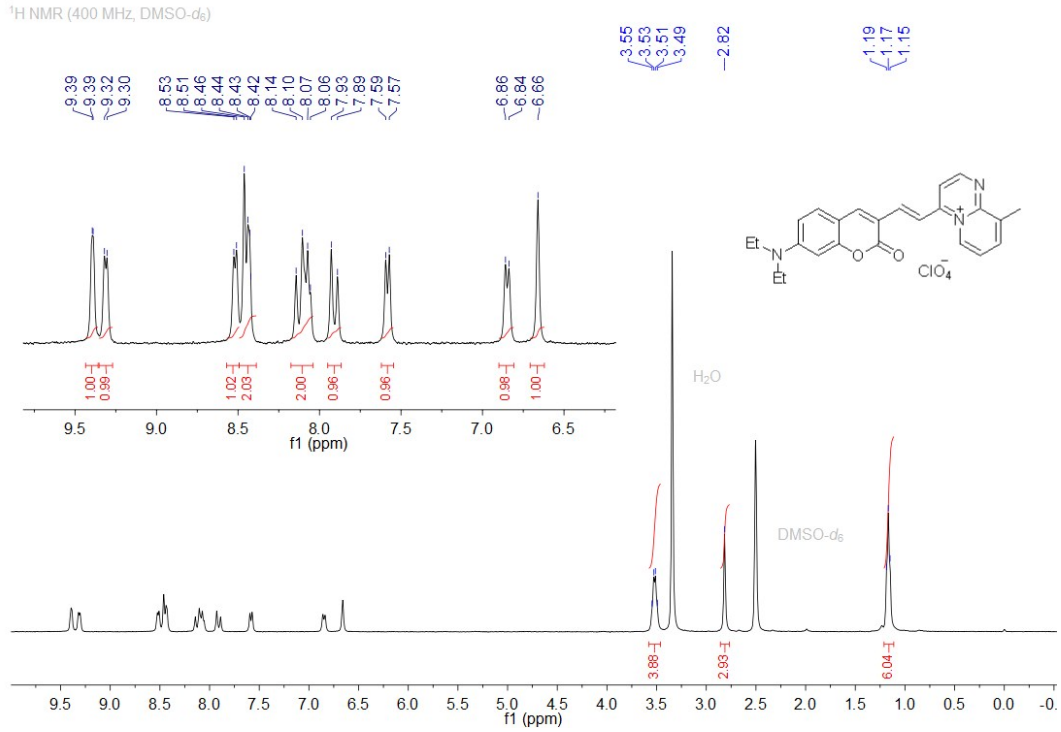

Fig. S30 ^{1}H NMR spectra of dye **1a**.

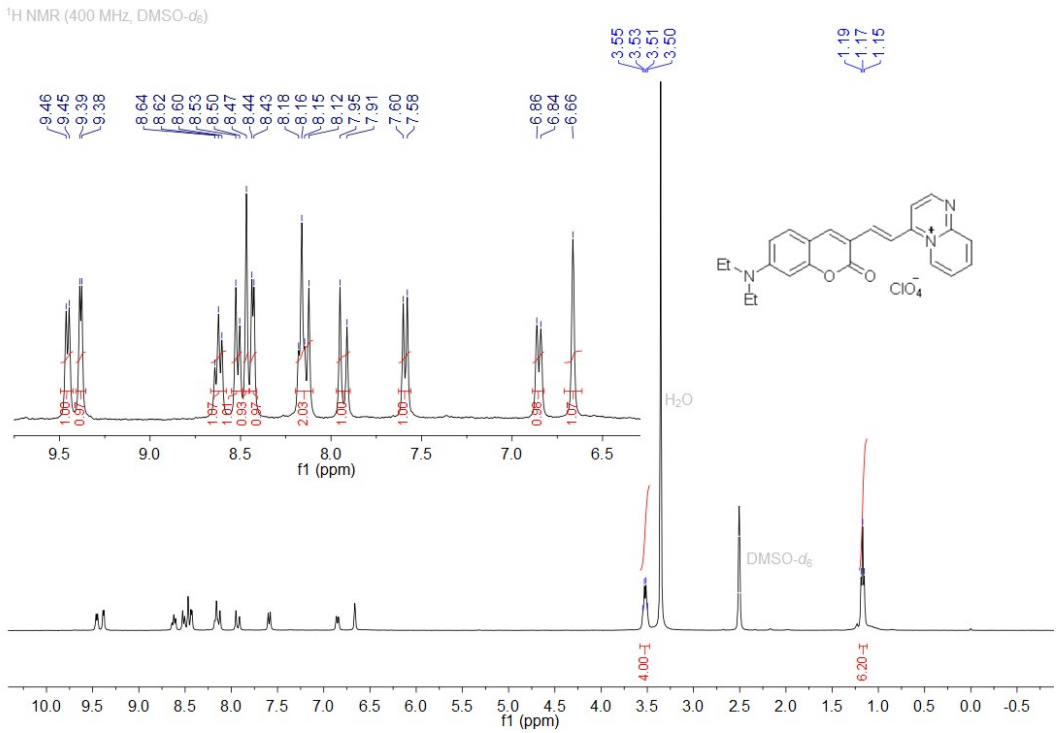

Fig. S31 ^{1}H NMR spectra of dye **1b**.

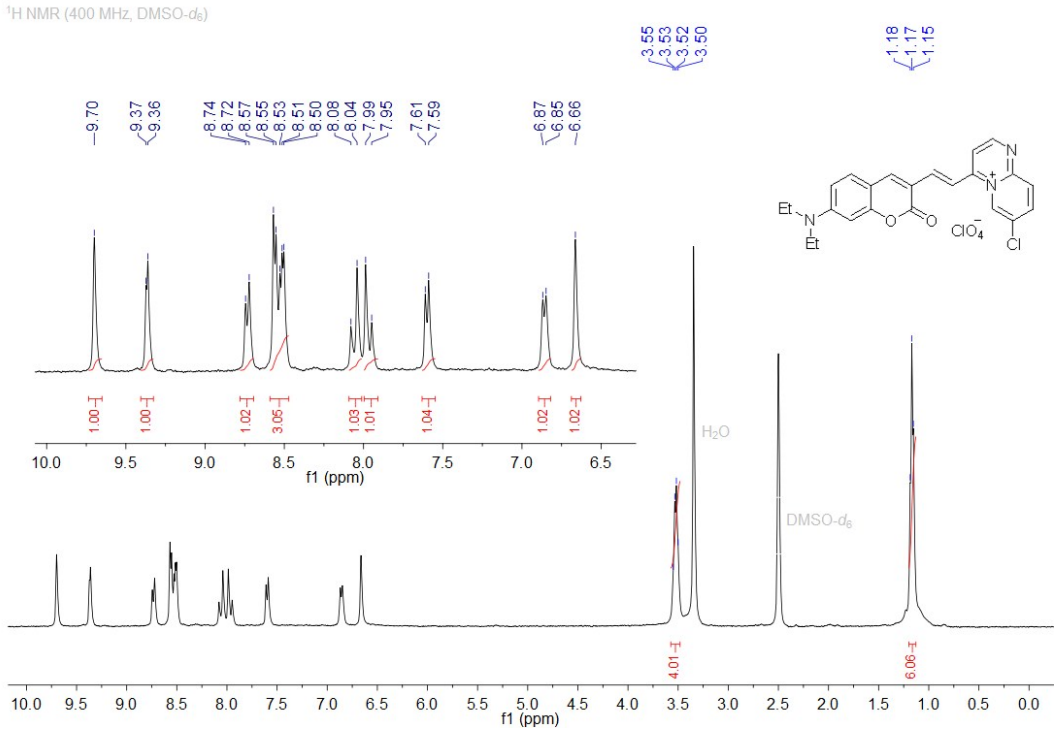

Fig. S32^{1}H NMR spectra of dye **1c**.

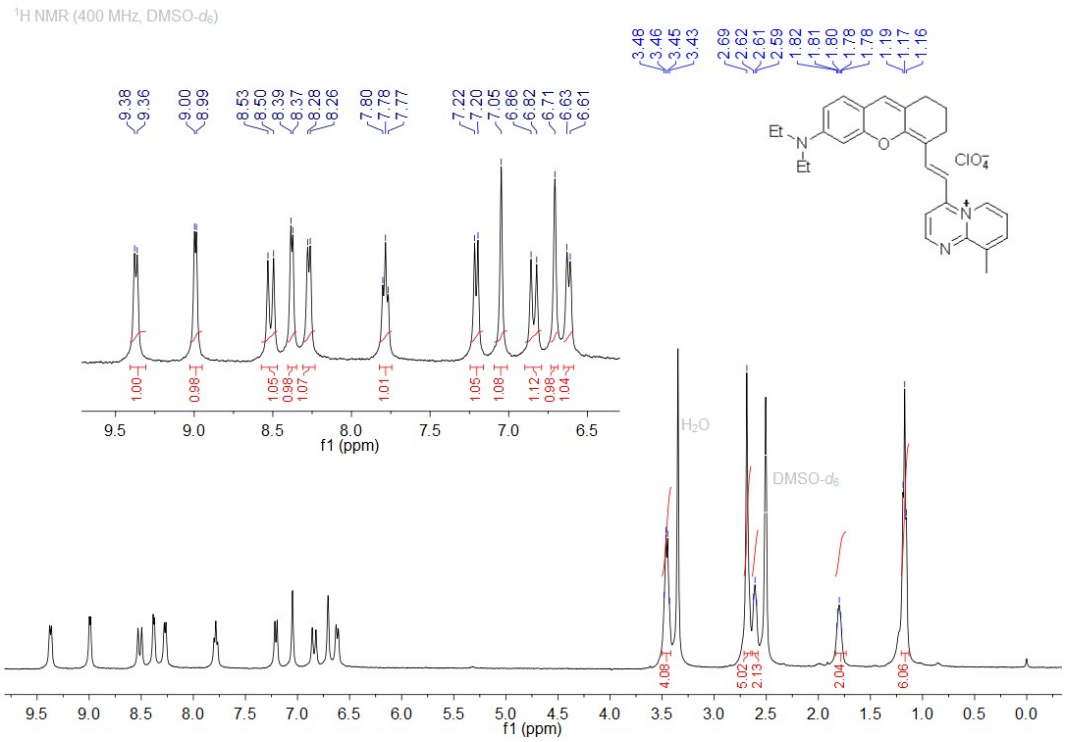

Fig. S33 ^{1}H NMR spectra of dye **1d**.

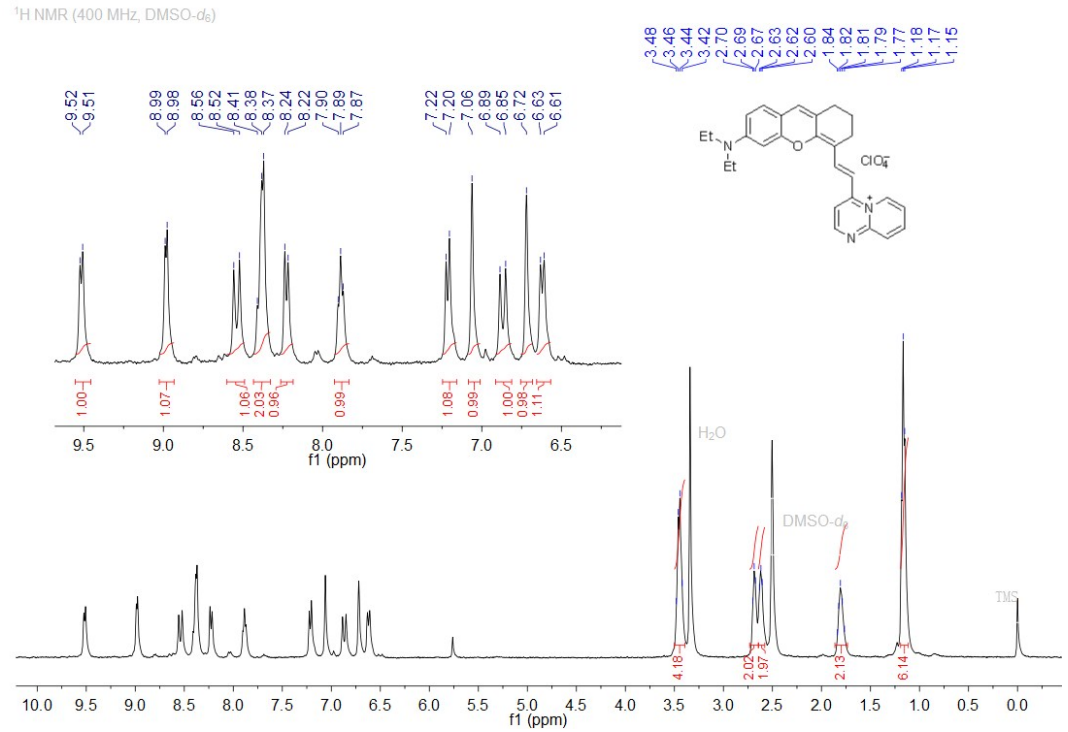

Fig. S34 ^{1}H NMR spectra of dye **1e**.

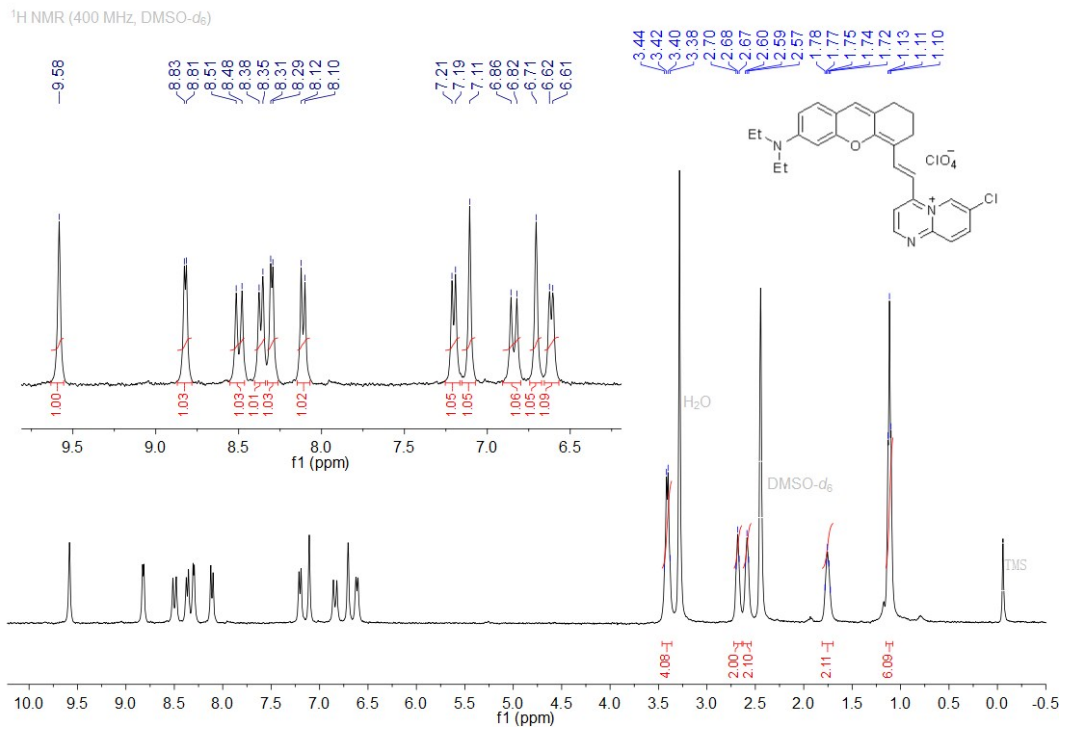

Fig. S35 ^{1}H NMR spectra of dye **1f**.

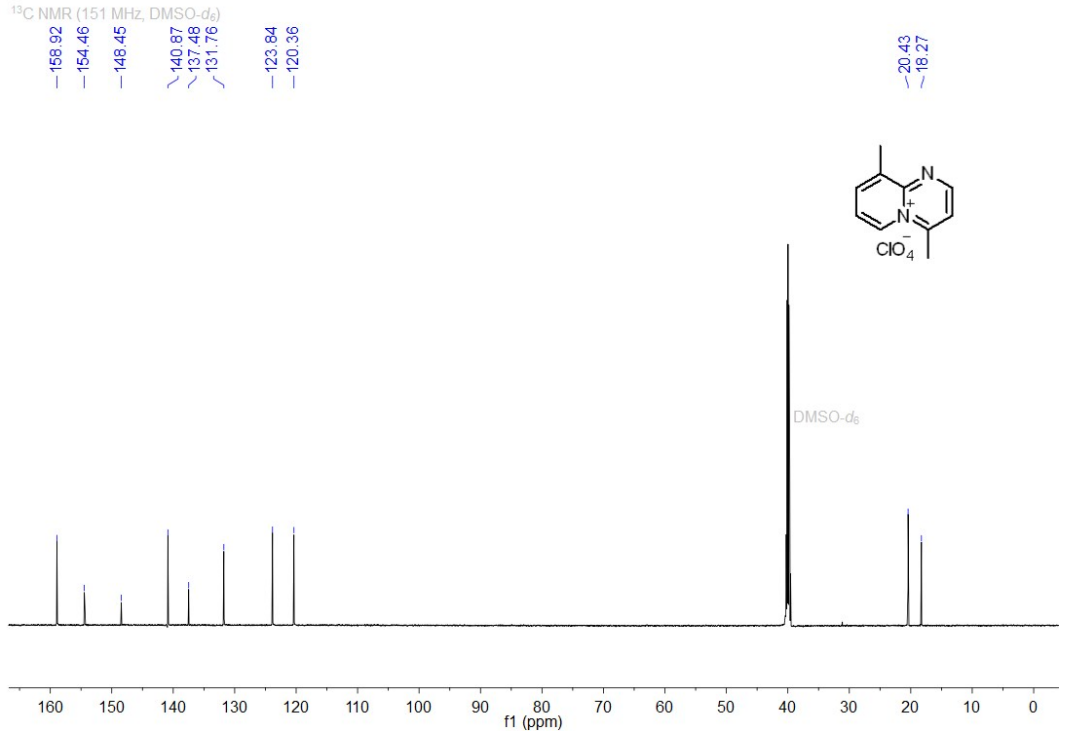

Fig. S36 ^{13}C NMR spectra of dye **3a**.

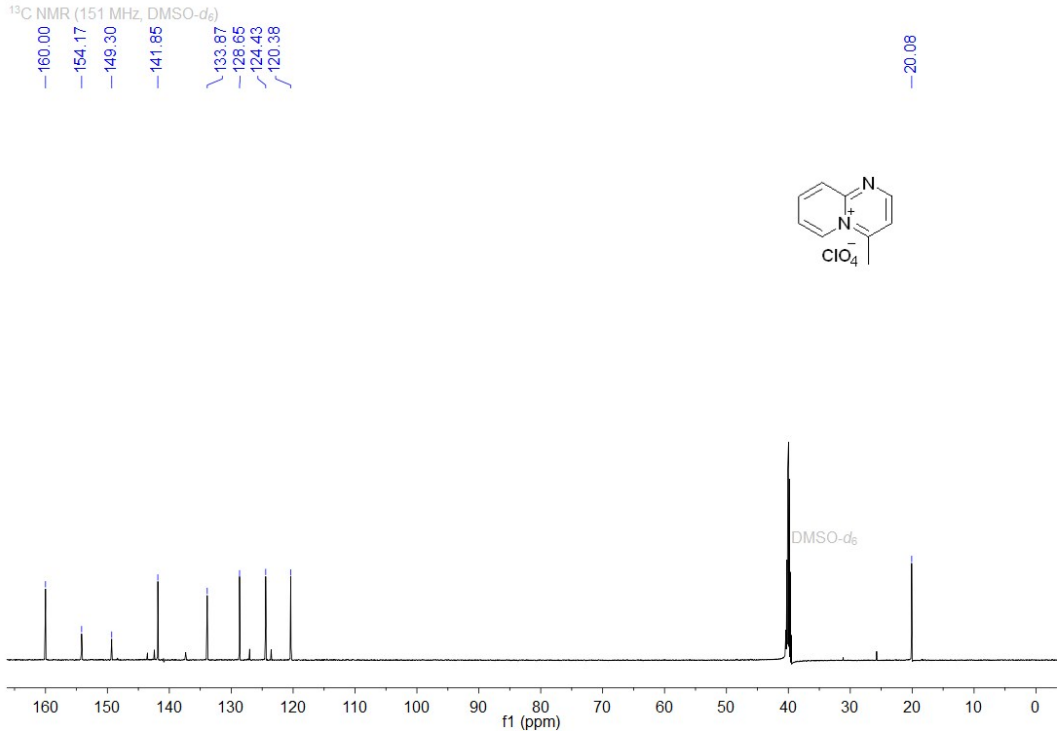

Fig. S37 ^{13}C NMR spectra of dye **3b**.

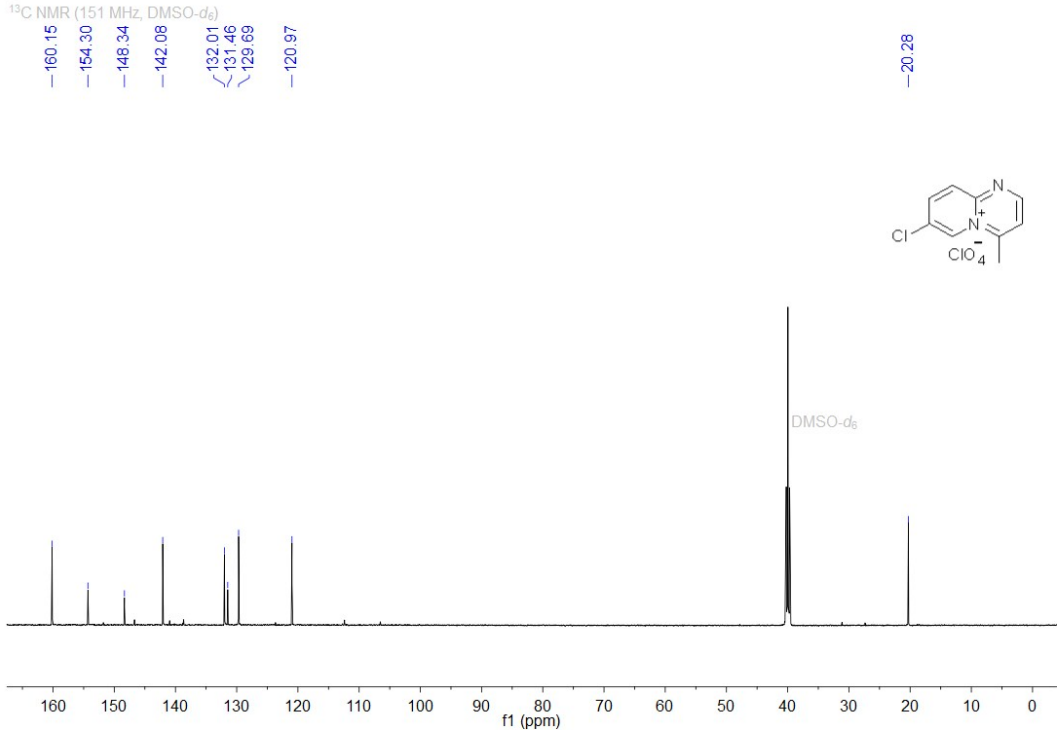

Fig. S38 ^{13}C NMR spectra of dye **3c**.

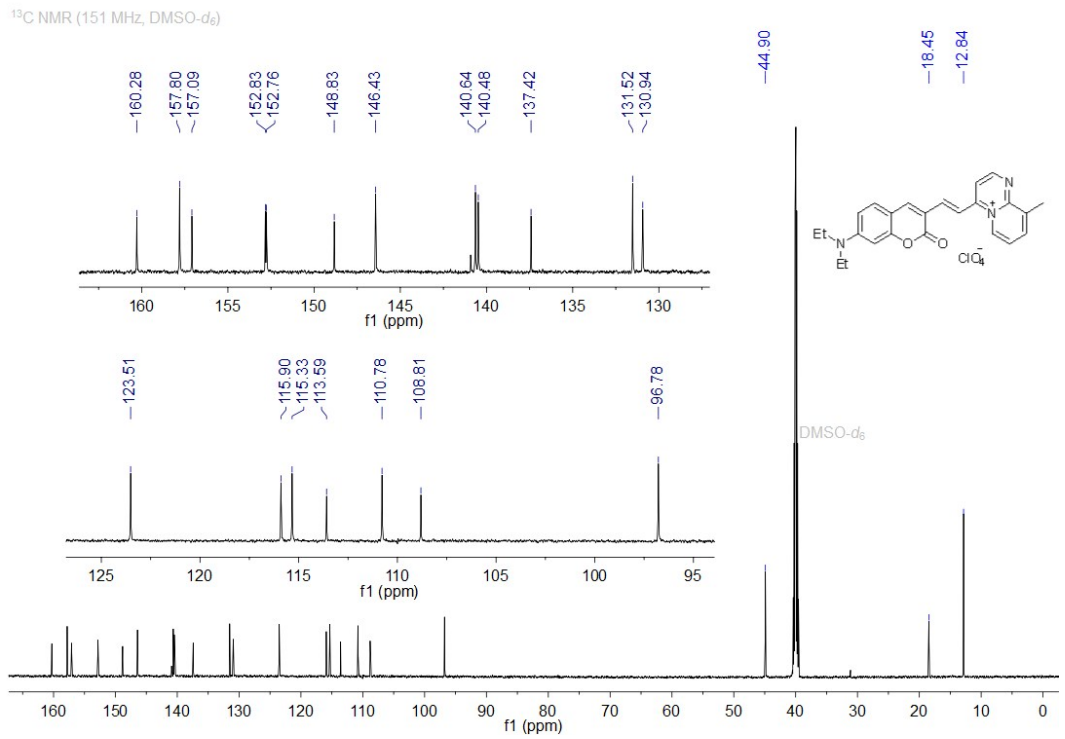

Fig. S39 ^{13}C NMR spectra of dye **1a**.

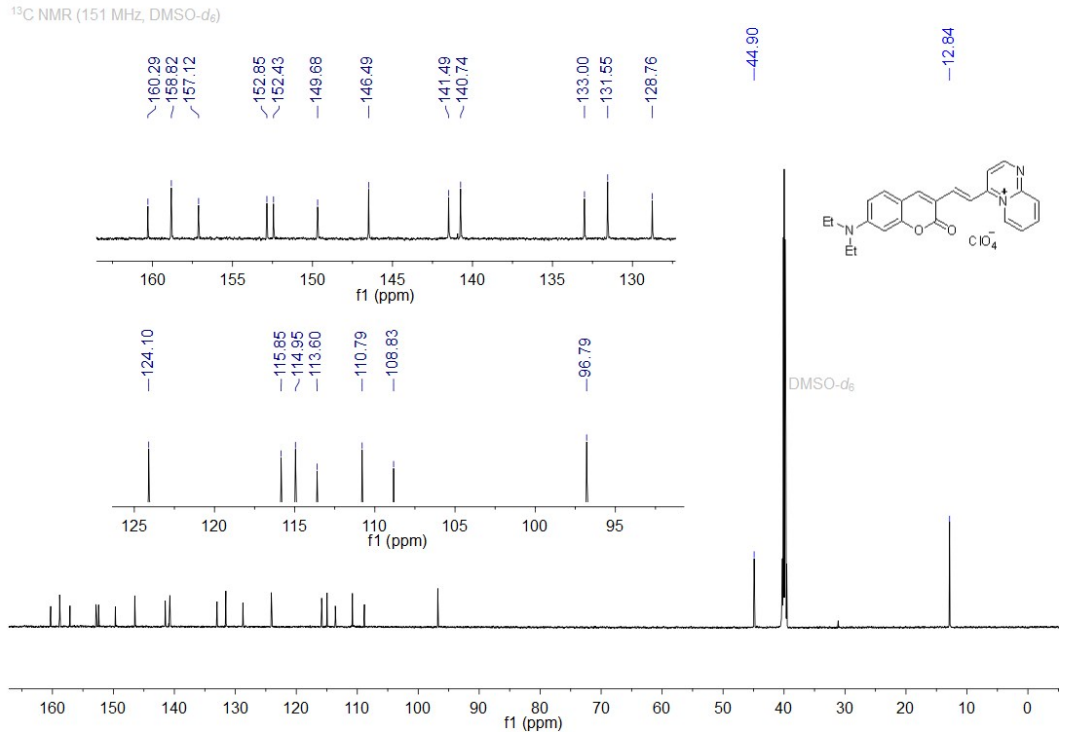

Fig. S40 ^{13}C NMR spectra of dye **1b**.

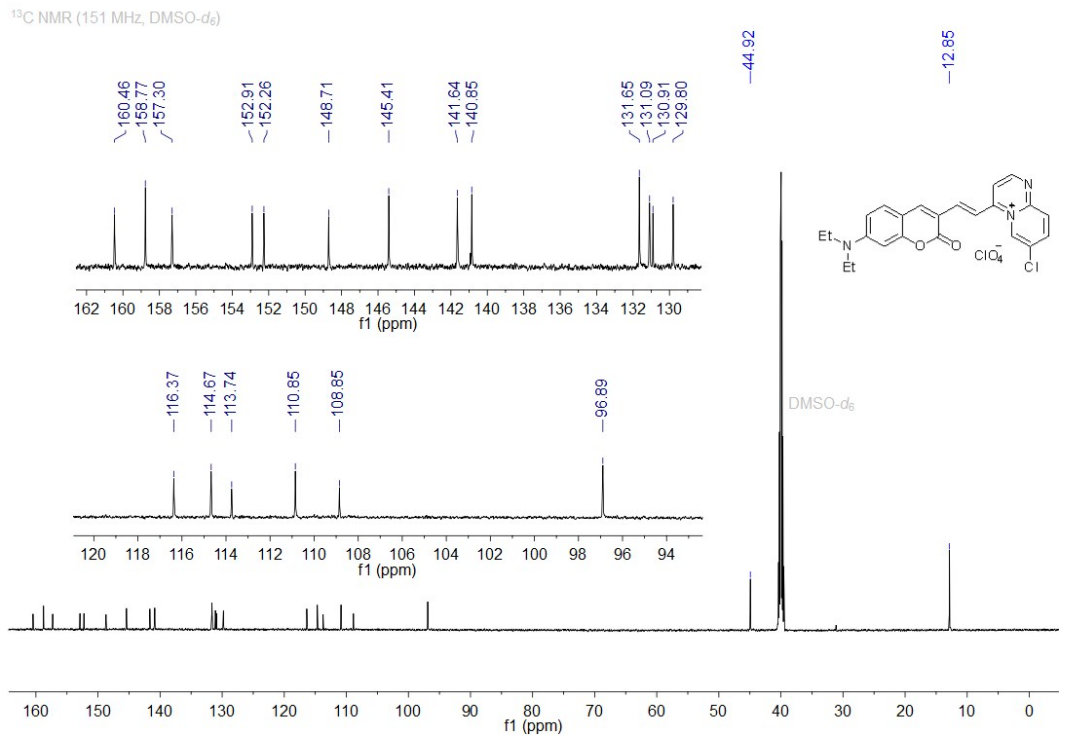

Fig. S41 ^{13}C NMR spectra of dye **1c**.

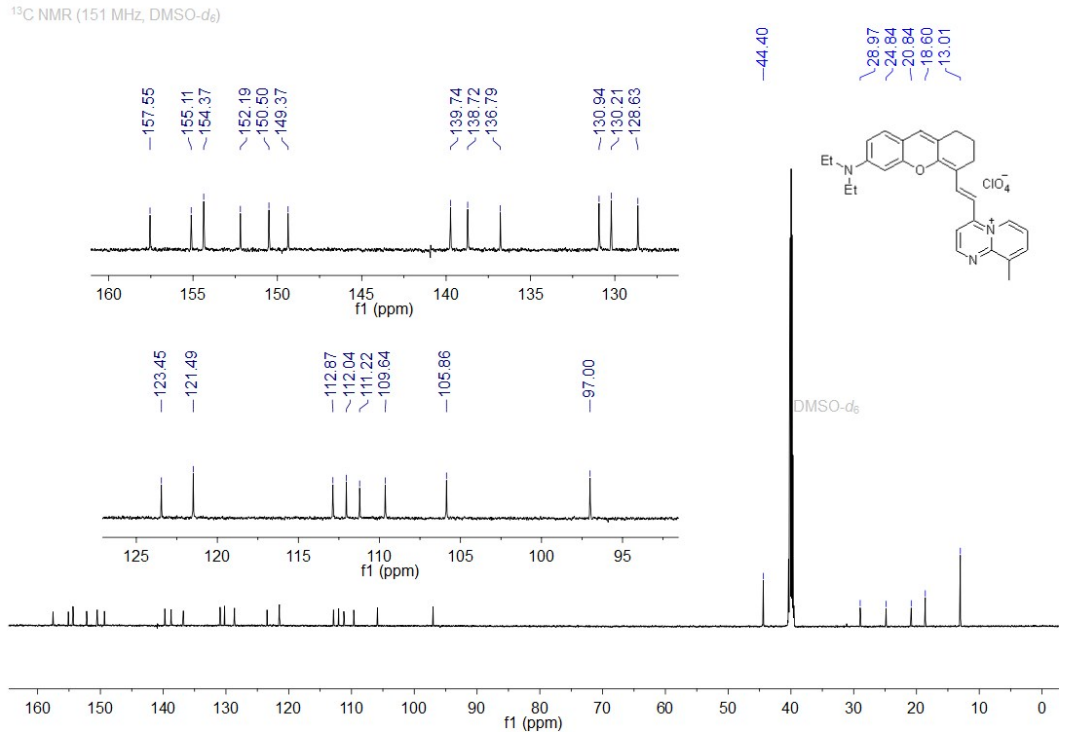

Fig. S42 ^{13}C NMR spectra of dye **1d**.

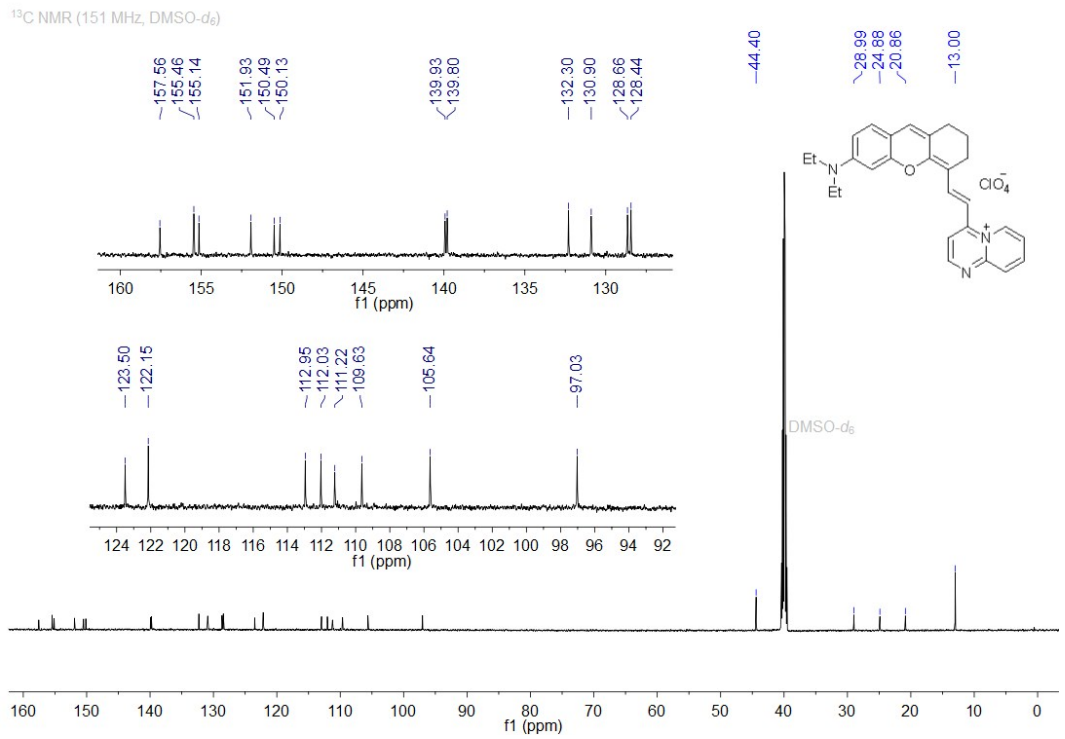

Fig. S43 ^{13}C NMR spectra of dye **1e**.

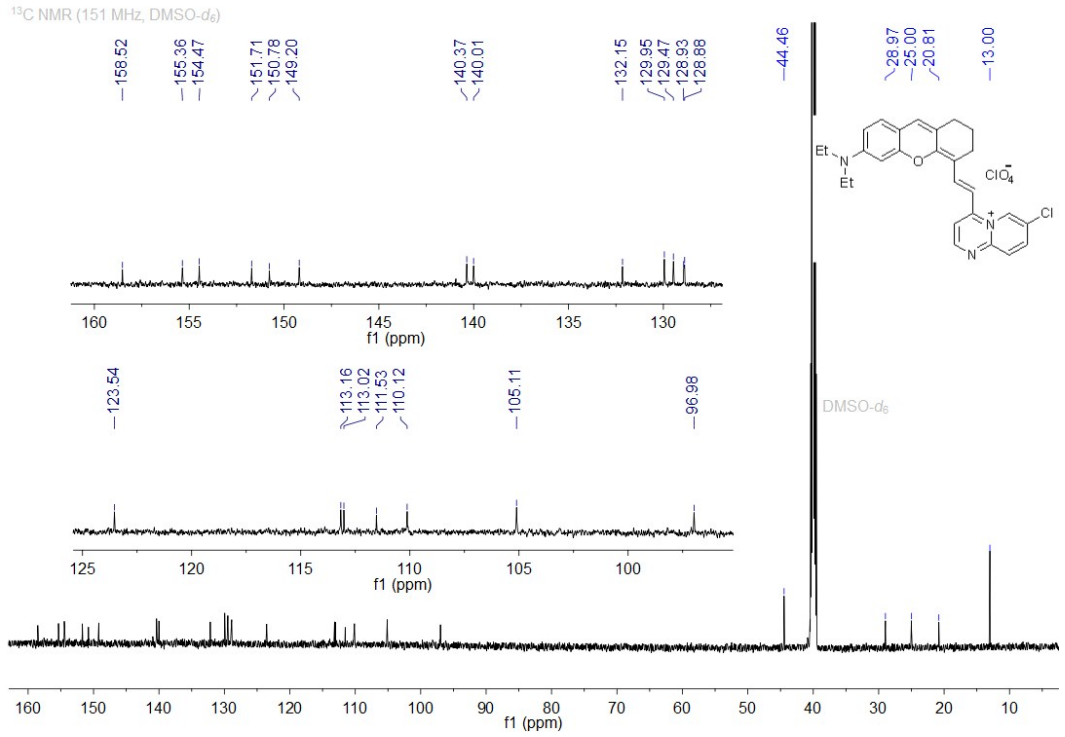

Fig. S44 ^{13}C NMR spectra of dye **1f**.

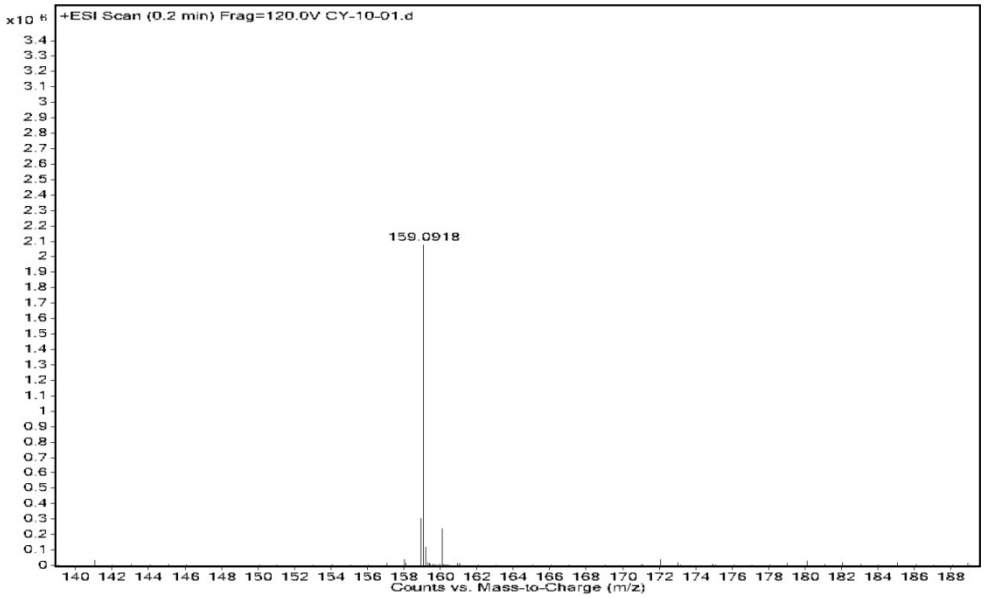

Fig. S45 HRMS(ESI⁺) spectra of dye **4a**.

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	120 ℃
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Waste

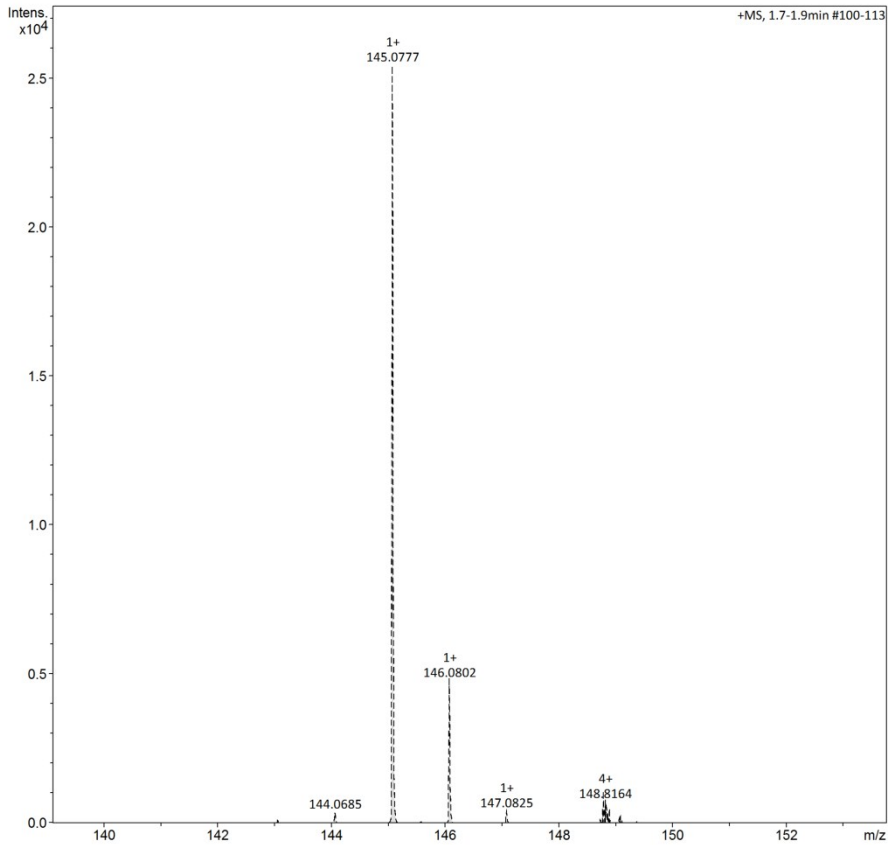

Fig. S46 HRMS(ESI⁺) spectra of dye **4b**.

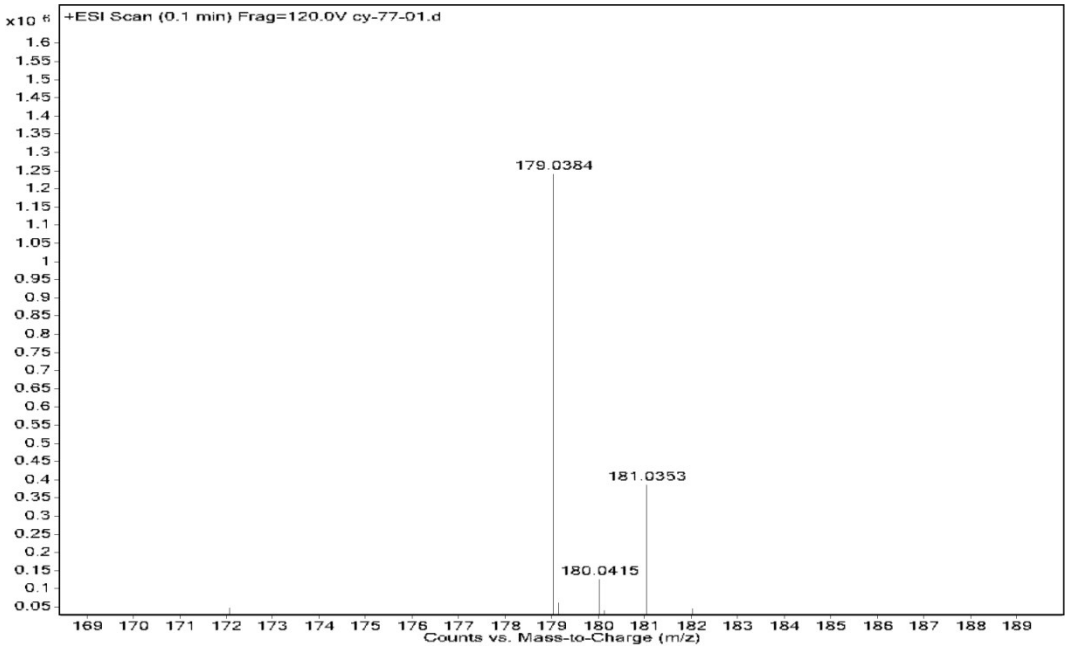

Fig. S47 HRMS(ESI$^+$) spectra of dye **4c**.

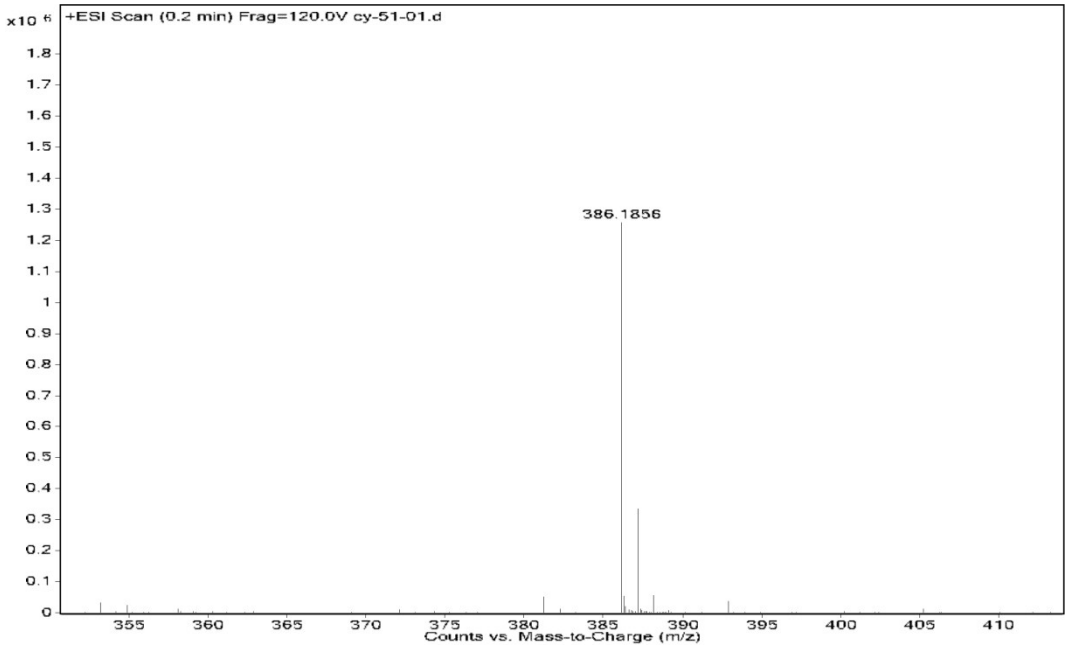

Fig. S48 HRMS(ESI$^+$) spectra of dye **1a**.

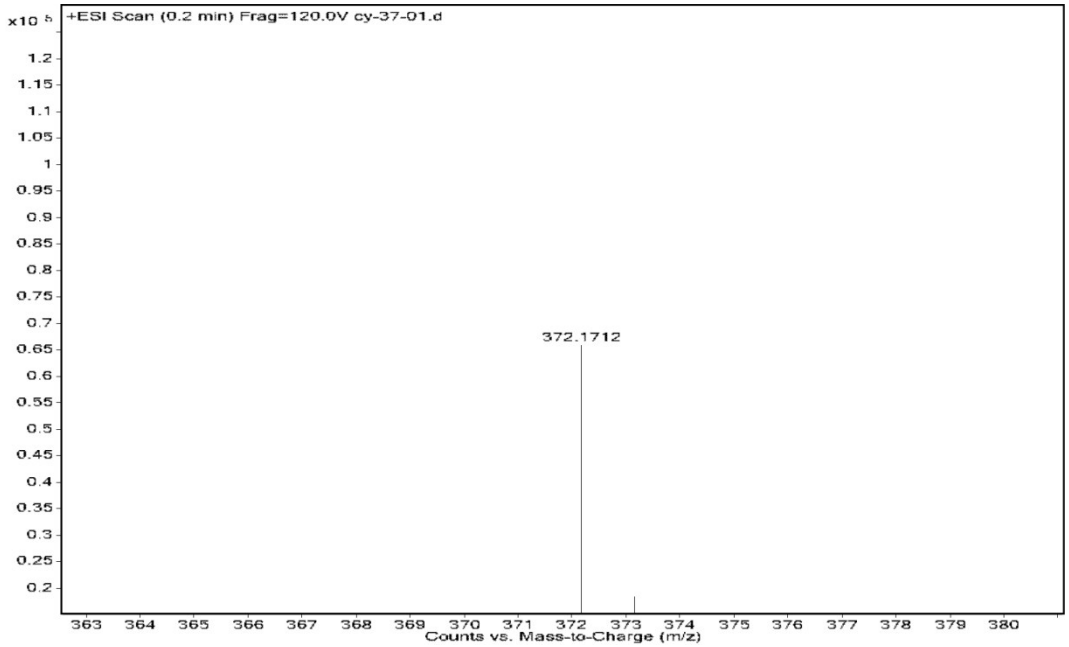

Fig. S49 HRMS(ESI$^+$) spectra of dye **1b**.

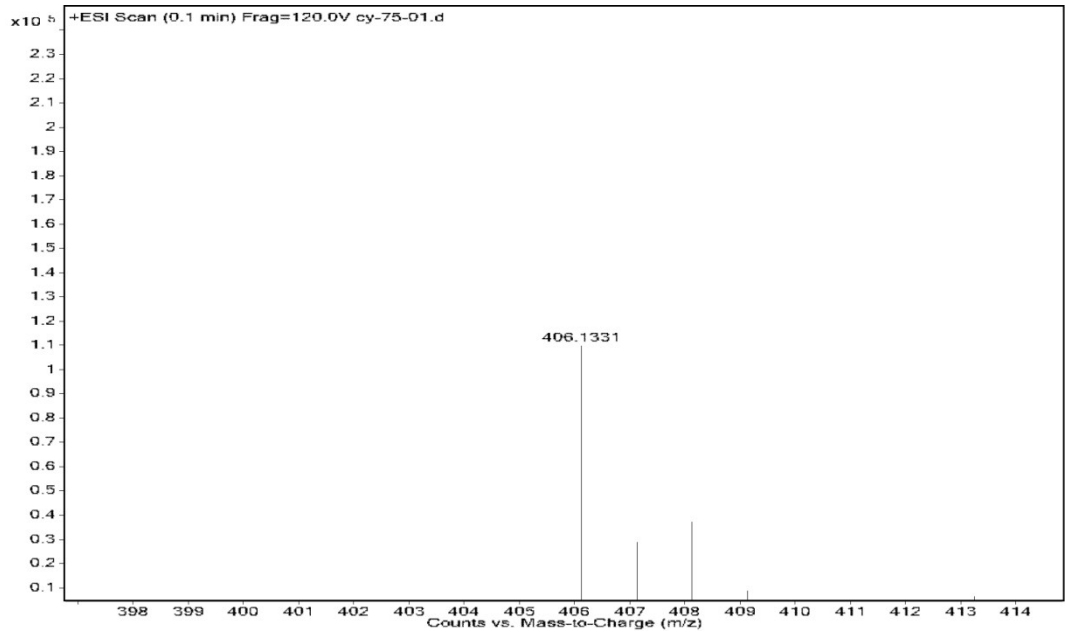

Fig. S50 HRMS(ESI$^+$) spectra of dye **1c**.

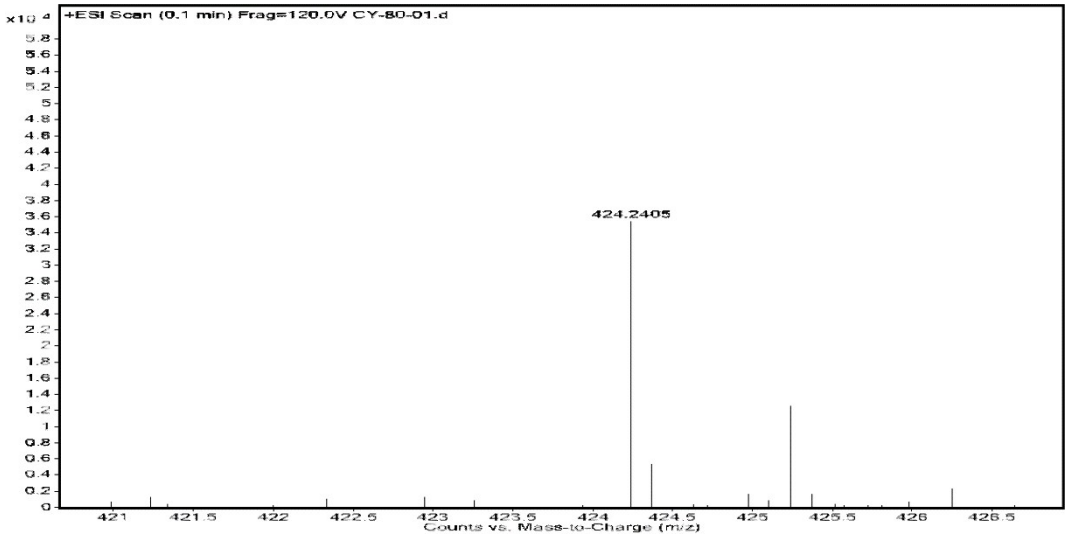

Fig. S51 HRMS(ESI$^+$) spectra of dye **1d**.

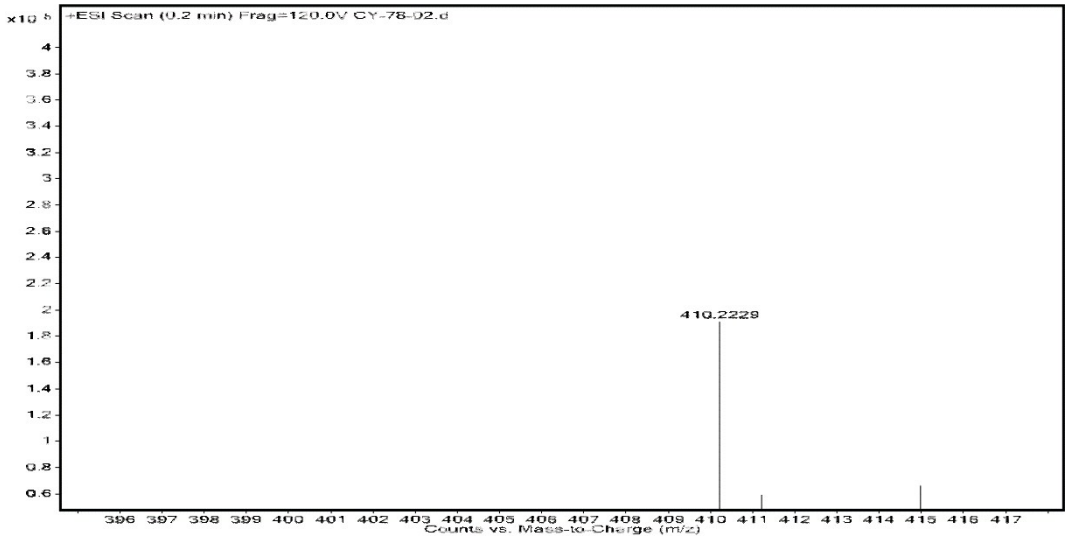

Fig. S52 HRMS(ESI$^+$) spectra of dye **1e**.

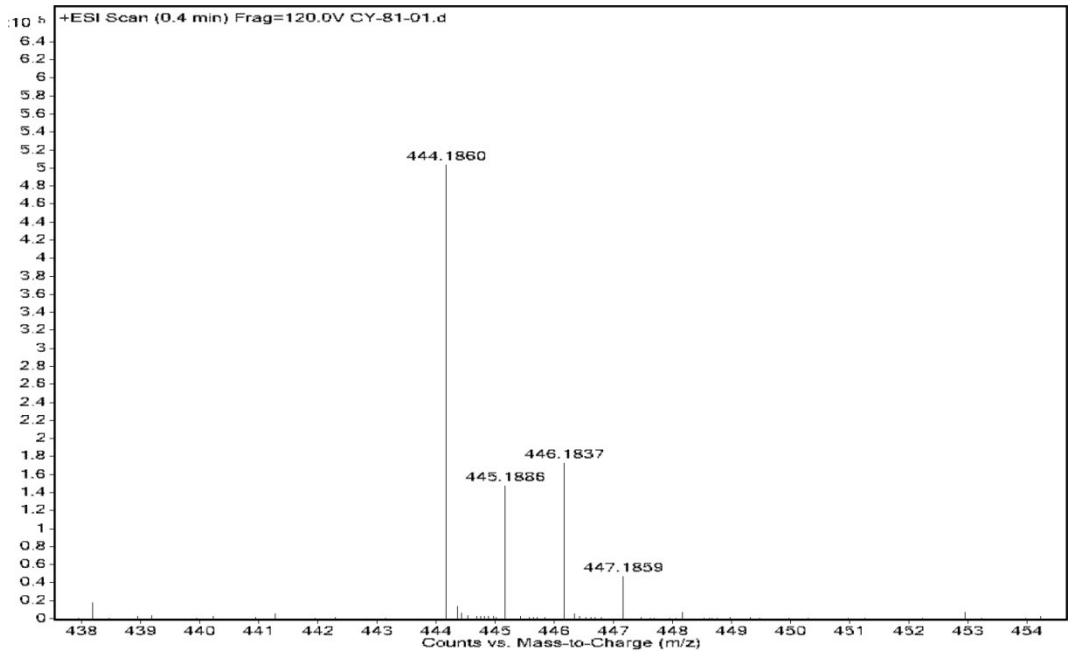

Fig. S53 HRMS(ESI$^+$) spectra of dye **1f**.

