The application of amide unit in the construction of neutral functional dyes for mitochondrial staining

Wei Ma, ‡[a] Bing Xu, ‡[b] Ru Sun, [a] Yu-Jie Xu,*[b] Jian-Feng Ge*[,a,c]

[a] College of Chemistry, Chemical Engineering and Material Science, Soochow University, Suzhou, Jiangsu 215123, China.
[b] State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection and Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou, Jiangsu 215123, China.
[c] Jiangsu Key Laboratory of Medical Optics, Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou, Jiangsu 215123, China

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1.Table

Table S1 A summary of optical changes of dyes **2a-e** in different solvents.

Dyes	Solvents	$\lambda_{Abs,\ max}$ [a]	$\lambda_{Em,\ max}$ [a]	Stokes shift [a]	ε [b]	Φ [c, d]
2a	H_2O	555	*ND*[e]	*ND*[e]	2.23	*ND*[e]
2a	DMSO	563	639	76	4.33	86
2a	MeOH	563	644	81	4.21	45
2a	$CHCl_3$	554	604	50	4.32	94
2a	THF	540	604	64	4.12	96
2a	TOL	538	591	53	4.02	89
2b	H_2O	585	*ND*[e]	*ND*[e]	2.37	*ND*[e]
2b	DMSO	564	630	66	6.07	75
2b	MeOH	563	635	72	5.70	63
2b	$CHCl_3$	552	597	45	5.98	90
2b	THF	540	592	52	5.74	95
2b	TOL	536	589	53	4.78	93
2c	H_2O	596	*ND*[e]	*ND*[e]	4.99	*ND*[e]
2c	DMSO	563	655	92	5.36	89
2c	MeOH	563	660	97	4.60	45
2c	$CHCl_3$	552	621	69	5.09	92
2c	THF	539	628	59	4.94	95
2c	TOL	535	618	83	2.96	93
2d	H_2O	573	*ND*[e]	*ND*[e]	2.18	*ND*[e]
2d	DMSO	561	640	79	3.15	83
2d	MeOH	560	657	97	3.10	27
2d	$CHCl_3$	551	607	56	3.31	87
2d	THF	538	601	63	3.24	96
2d	TOL	535	586	51	3.05	92
2e	H_2O	558	*ND*[e]	*ND*[e]	2.32	*ND*[e]
2e	DMSO	563	620	57	3.92	83
2e	MeOH	562	629	67	3.75	23
2e	$CHCl_3$	553	585	32	3.86	74
2e	THF	539	581	42	3.87	90
2e	TOL	535	583	48	3.72	81

[a] Reported in nm. [b] Cresyl violet (Φ = 0.578 in ethanol) was used as the reference dye. [c] reported in $10^4\ M^{-1}\ cm^{-1}$. [d] not detectable. The water solutions contained 1% DMSO.

Table S2 A summary of optical changes of dyes **3a-e** in different solvents.

Dyes	Solvents	$\lambda_{Abs,\ max}$ [a]	$\lambda_{Em,\ max}$ [a]	Stokes shift [a]	ε [b]	Φ [c, d]
3a	H_2O	398	489	91	2.04	38
3a	DMSO	386	482	96	3.16	80
3a	MeOH	388	471	83	3.28	89
3a	$CHCl_3$	388	457	69	3.22	65
3a	THF	379	469	90	3.34	68
3a	TOL	379	458	79	3.29	55
3b	H_2O	393	481	88	3.05	31
3b	DMSO	387	483	96	3.08	77
3b	MeOH	386	475	89	3.41	85
3b	$CHCl_3$	386	459	73	3.29	52
3b	THF	376	459	83	3.44	48
3b	TOL	379	460	81	3.36	65
3c	H_2O	391	479	88	2.94	50
3c	DMSO	385	472	87	3.47	78
3c	MeOH	386	469	83	3.61	93
3c	$CHCl_3$	385	455	70	3.57	97
3c	THF	376	462	86	3.76	57
3c	TOL	378	456	78	3.49	55
3d	H_2O	390	482	92	1.78	55
3d	DMSO	383	475	92	3.15	71
3d	MeOH	384	470	86	3.29	65
3d	$CHCl_3$	380	457	77	3.49	54
3d	THF	374	459	85	3.51	48
3d	TOL	374	457	83	3.38	52
3e	H_2O	395	483	88	2.55	67
3e	DMSO	385	470	85	3.31	90
3e	MeOH	386	468	82	3.48	92
3e	$CHCl_3$	385	458	73	3.39	67
3e	THF	376	455	79	3.69	45
3e	TOL	375	459	84	3.21	46

[a] Reported in nm. [b] Coumarin 153 (Φ = 0.546 in ethanol) was used as the reference dye. [c] reported in 10^4 M^{-1} cm^{-1}. [d] not detectable. The water solutions contained 1% DMSO.

2.Figures

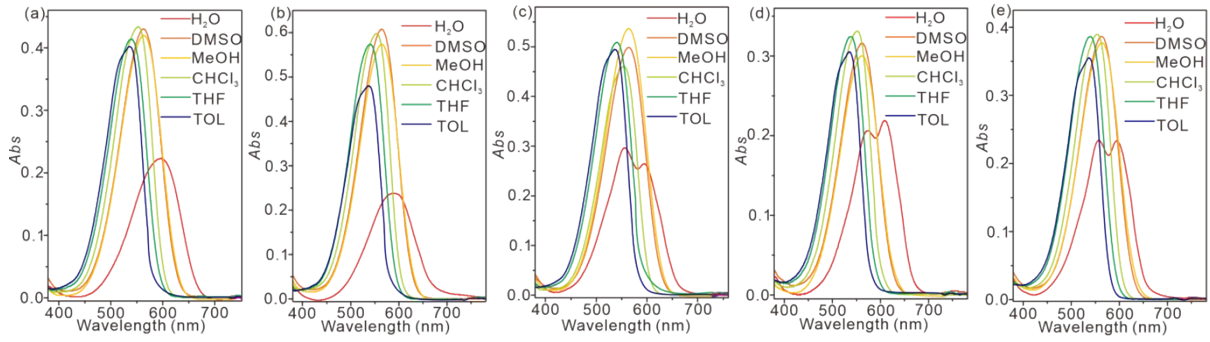

Fig. S1 Absorption spectra of **2a** (a), **2b** (b), **2c** (c) **2d** (d) and **2e** (e)with the concentration of 10 μM in different solvents.

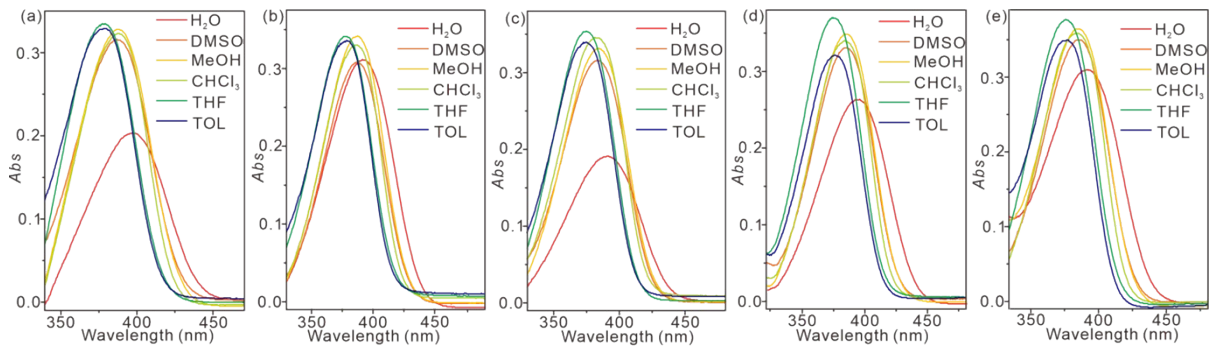

Fig. S2 Absorption spectra of **3a** (a), **3b** (b), **3c** (c) **3d** (d)and **3e** (e) with the concentration of 10 μM in different solvents.

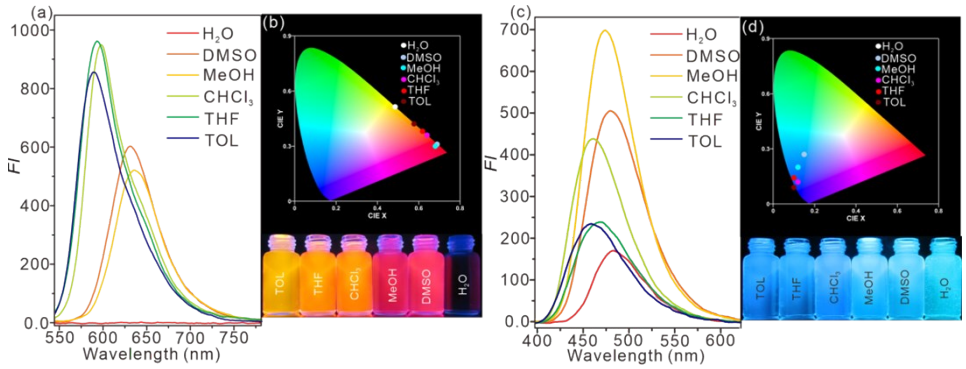

Fig. S3 Emission spectra of **2b** (a) and **3b** (c) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **2b** was excited at 540 nm, slit widths: 1.5 nm/3 nm;**3b** was excited at 390 nm, slit widths: 1.5 nm/1.5 nm.

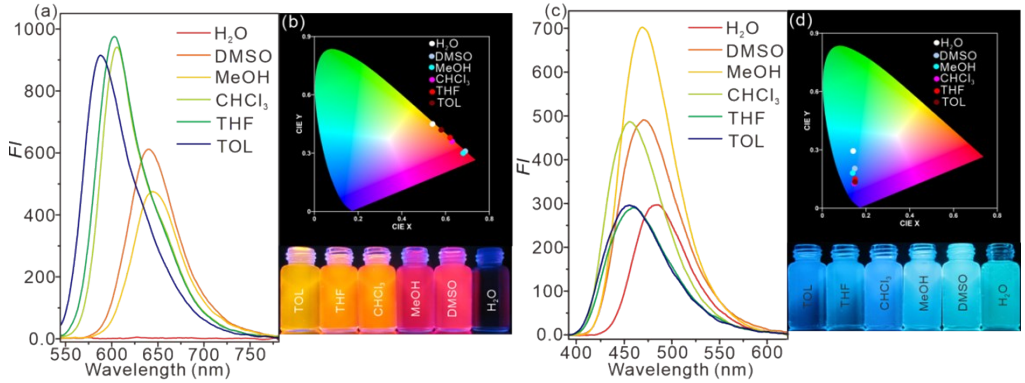

Fig. S4 Emission spectra of **2c** (a) and **3c** (c) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **2c** was excited at 540 nm, slit widths: 1.5 nm/3 nm; **3c** was excited at 390 nm, slit widths: 1.5 nm/1.5 nm.

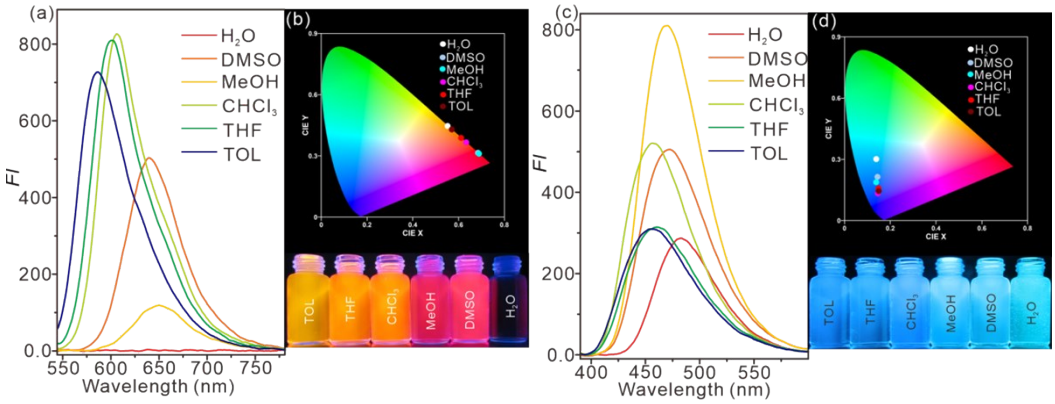

Fig. S5 Emission spectra of **2d** (a) and **3d** (c) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **2d** was excited at 540 nm, slit widths: 1.5 nm/3 nm; **3d** was excited at 390 nm, slit widths: 1.5 nm/1.5 nm.

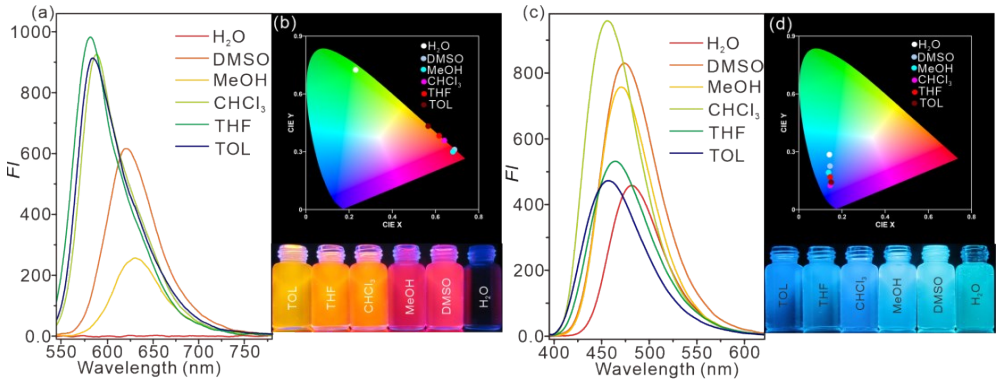

Fig. S6 Emission spectra of **2e** (a) and **3e** (c) in different solvents, inset showed photographs of them under 365 nm irradiation in dark condition and their CIE chromaticity diagram; **2e** was excited at 540 nm, slit widths: 1.5 nm/3 nm; **3e** was excited at 390 nm, slit widths: 1.5 nm/1.5 nm.

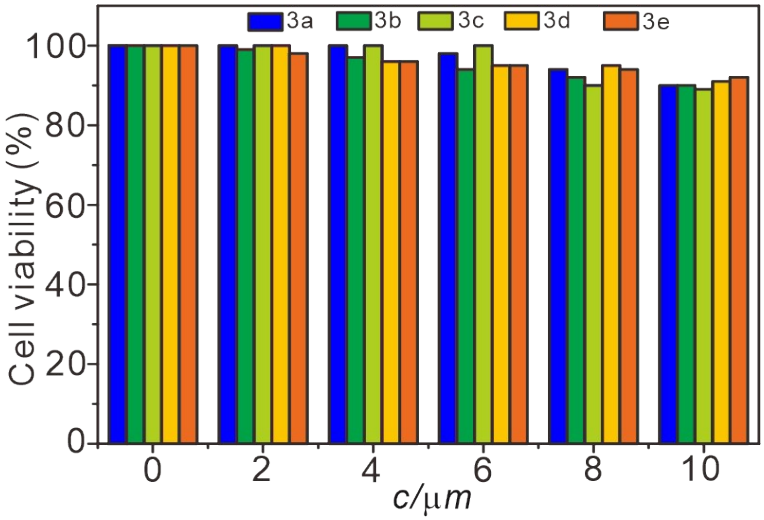

Fig. S7 Percentages of cell viabilities of HeLa cells after treatment with dyes **3a-e** for 6 hours.

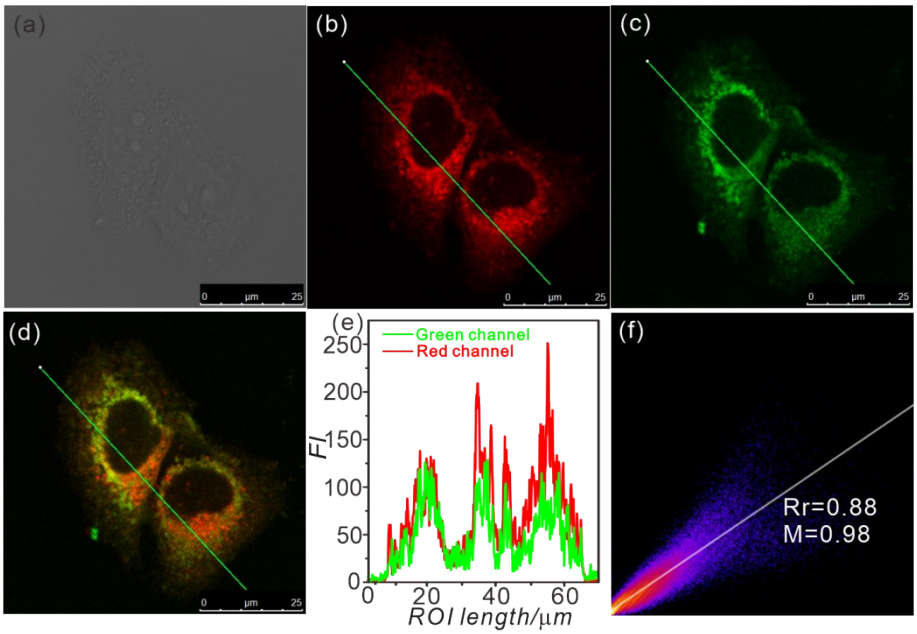

Fig. S8 Confocal fluorescence images of HeLa cells with dye **2b**. (a) Bright field images; (b) confocal image (red channel) of cells with dye **2b** (2 μM); (c) confocal image (green channel) of cells with Mito-Tracker s Green FM (100 nM); (d) merged images of the green and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells; (f) fluorescence intensity correlation plot of the green channel and red channel.

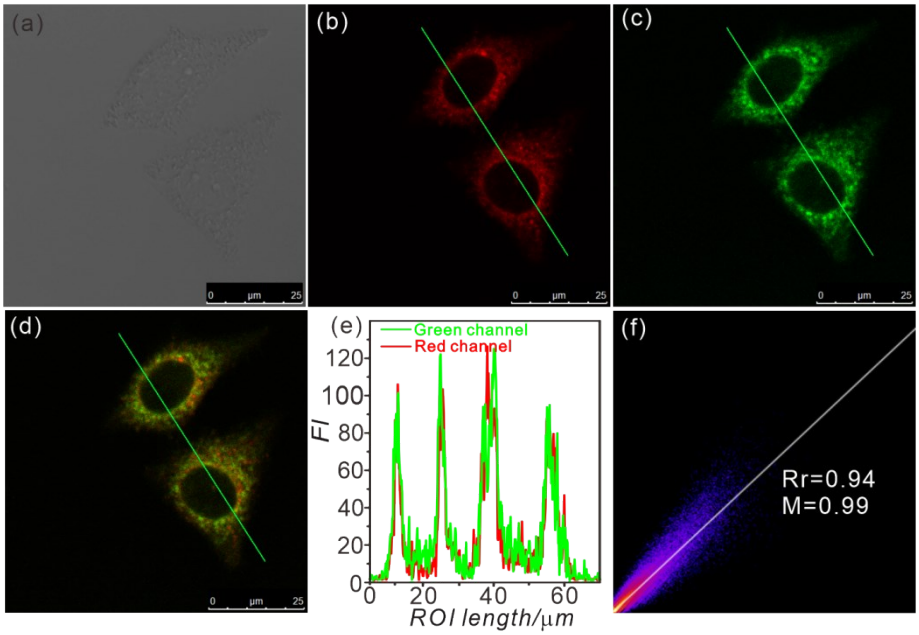

Fig. S9 Confocal fluorescence images of HeLa cells with dye **2c**. (a) Bright field images; (b) confocal image (red channel) of cells with dye **2c** (2 μM); (c) confocal image (green channel) of cells with Mito-Tracker s Green FM (100 nM); (d) merged images of the green and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells; (f) fluorescence intensity correlation plot of the green channel and red channel.

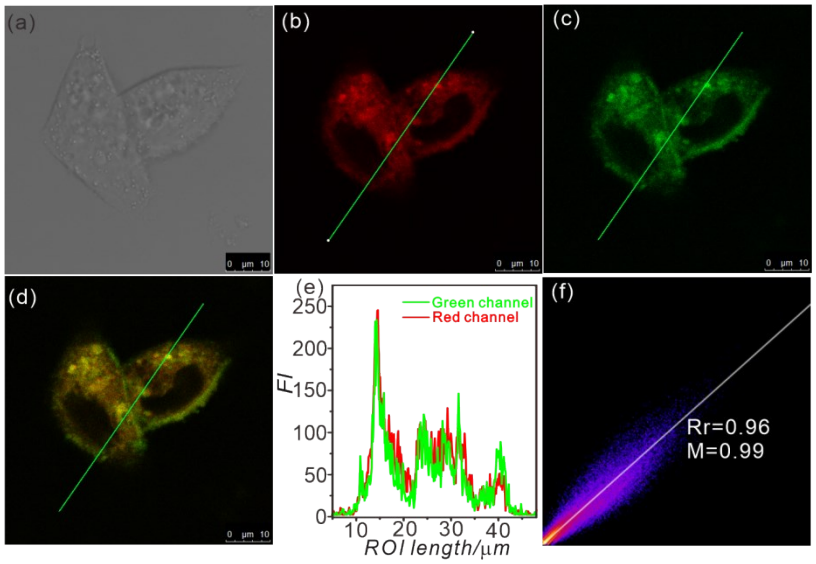

Fig. S10 Confocal fluorescence images of HeLa cells with dye **2d**. (a) Bright field images; (b) confocal image (red channel) of cells with dye **2d** (2 μM); (c) confocal image (green channel) of cells with Mito-Tracker s Green FM (100 nM); (d) merged images of the green and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells; (f) fluorescence intensity correlation plot of the green channel and red channel.

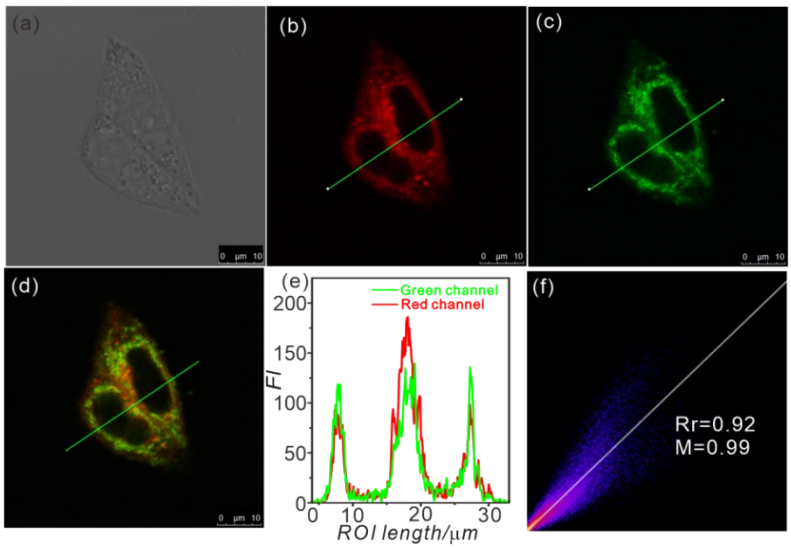

Fig. S11 Confocal fluorescence images of HeLa cells with dye **2e**. (a) Bright field images; (b) confocal image (red channel) of cells with dye **2e** (2 μM); (c) confocal image (green channel) of cells with Mito-Tracker s Green FM (100 nM); (d) merged images of the green and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells; (f) fluorescence intensity correlation plot of the green channel and red channel.

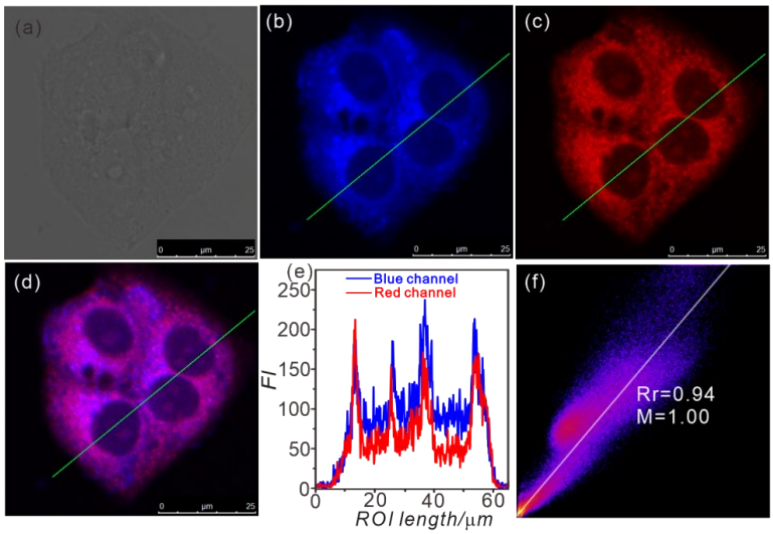

Fig. 12 Confocal fluorescence image of HeLa cells with dye **3b**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3b** (2 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

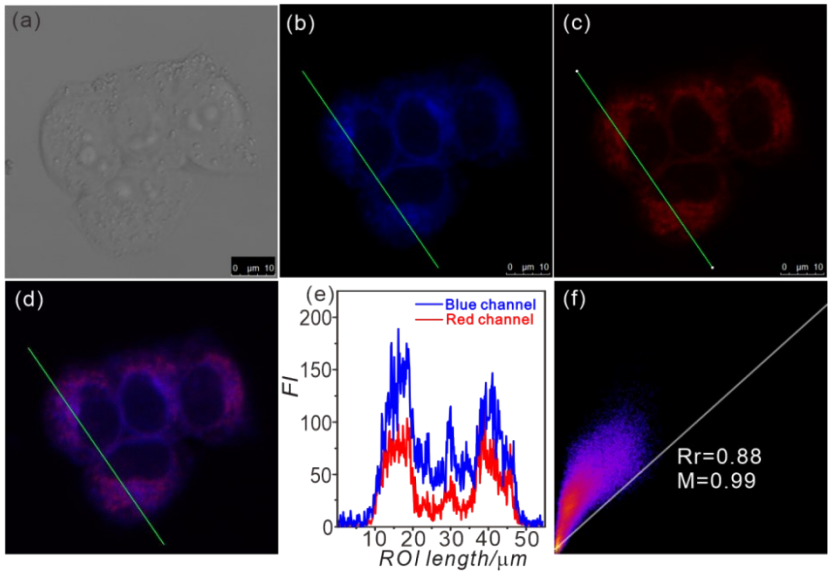

Fig. S13 Confocal fluorescence image of HeLa cells with dye **3c**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3c** (2 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

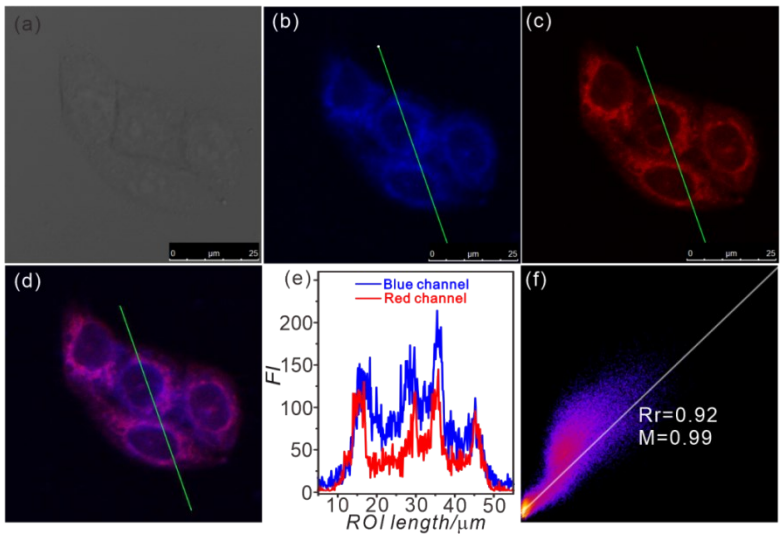

Fig. S14 Confocal fluorescence image of HeLa cells with dye **3d**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3d** (2 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

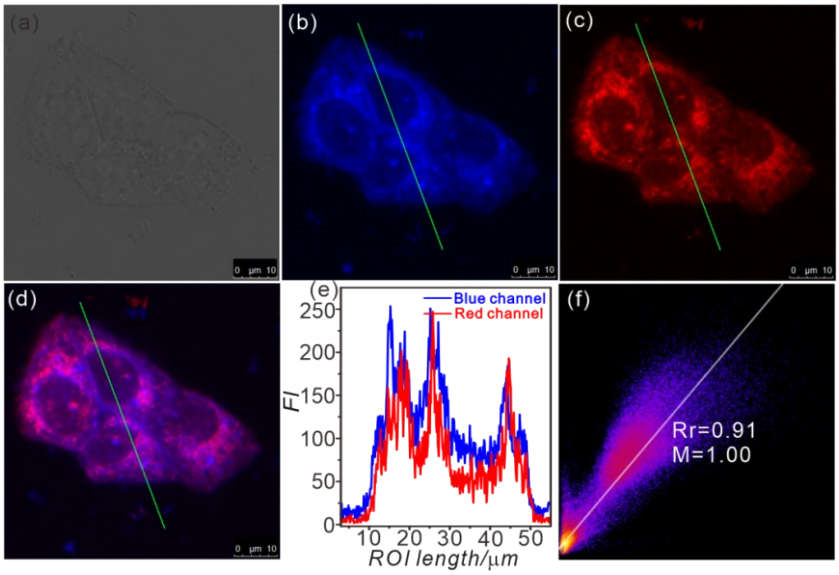

Fig. S15 Confocal fluorescence image of HeLa cells with dye **3e**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3e** (2 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

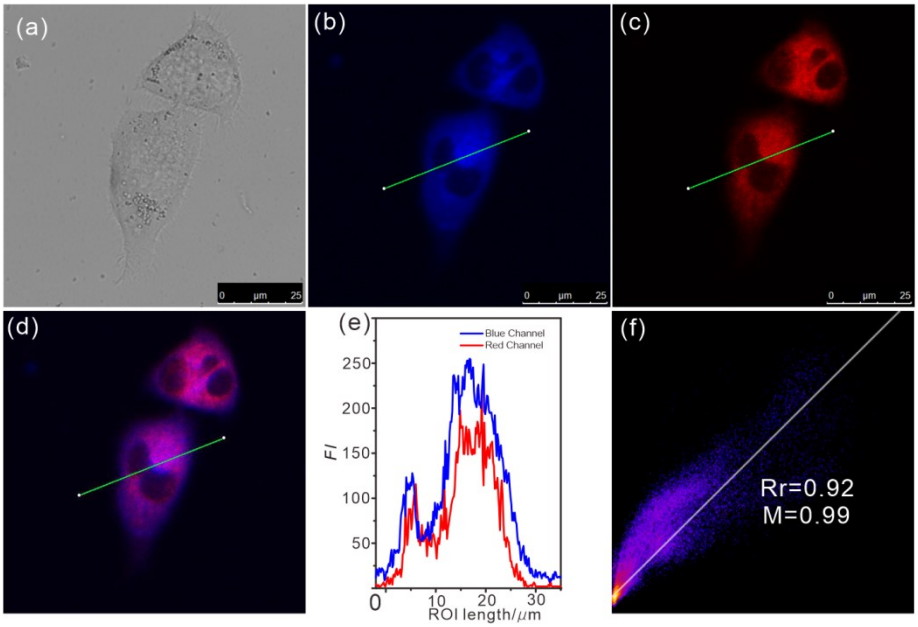

Fig. S16 Confocal fluorescence image of HeLa cells with dye **3a**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3a** (10 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

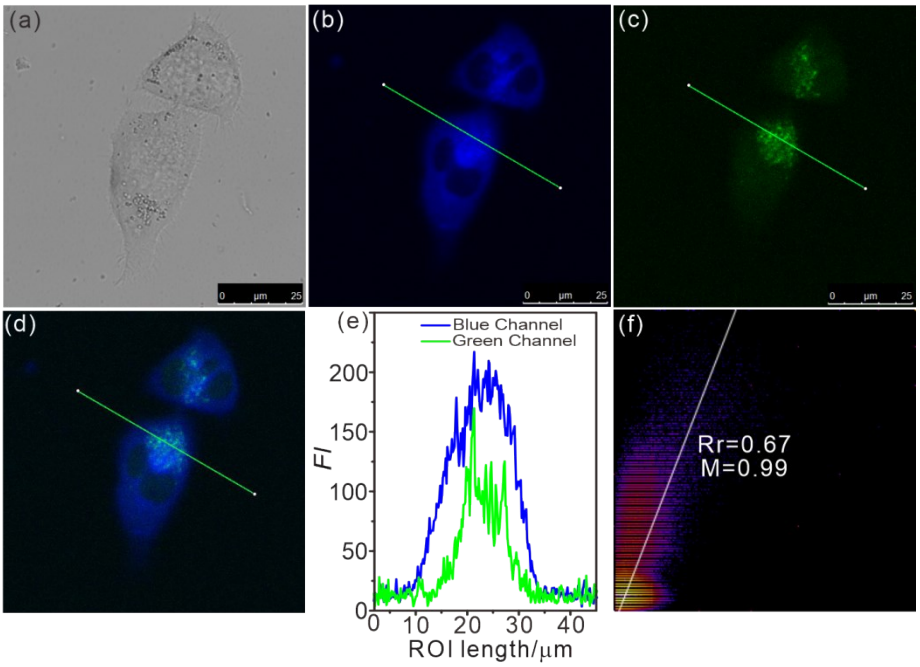

Fig. S17 Confocal fluorescence image of HeLa cells with dye **3a**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3a** (10 μM); (c) confocal images (green channel) of cells with LysoTracker Green DND-26 (100 nM); (d) merged images of blue and green channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells;(f) fluorescence intensity correlation plot of blue channel and green channel.

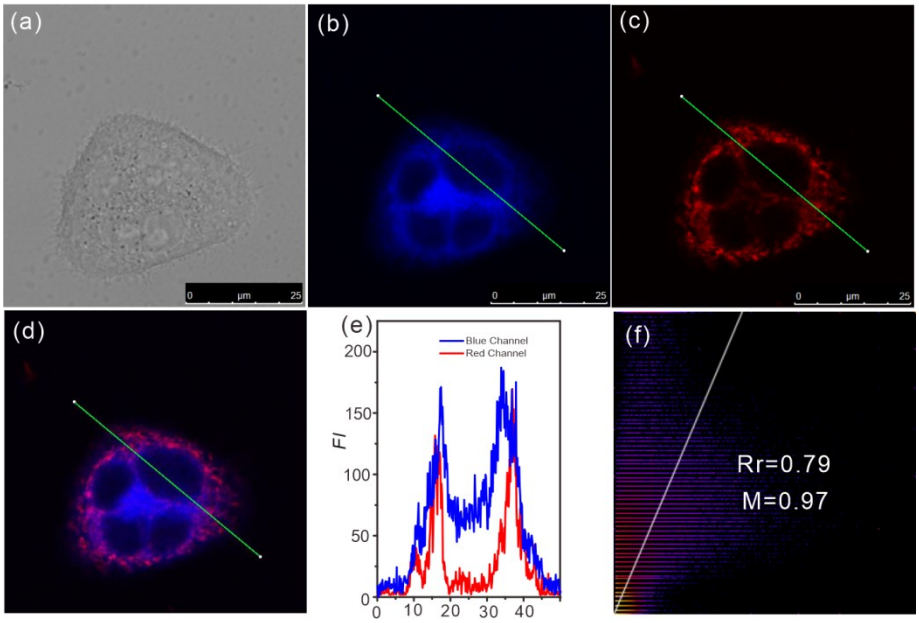

Fig. S18 Confocal fluorescence image of HeLa cells with dye **3b**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3b** (10 μM); (c) confocal image (red channel) of cells with MitoTracker® Red CMXRos (100 nM); (d) merged images of blue and red channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells, (f) fluorescence intensity correlation plot of blue channel and red channel.

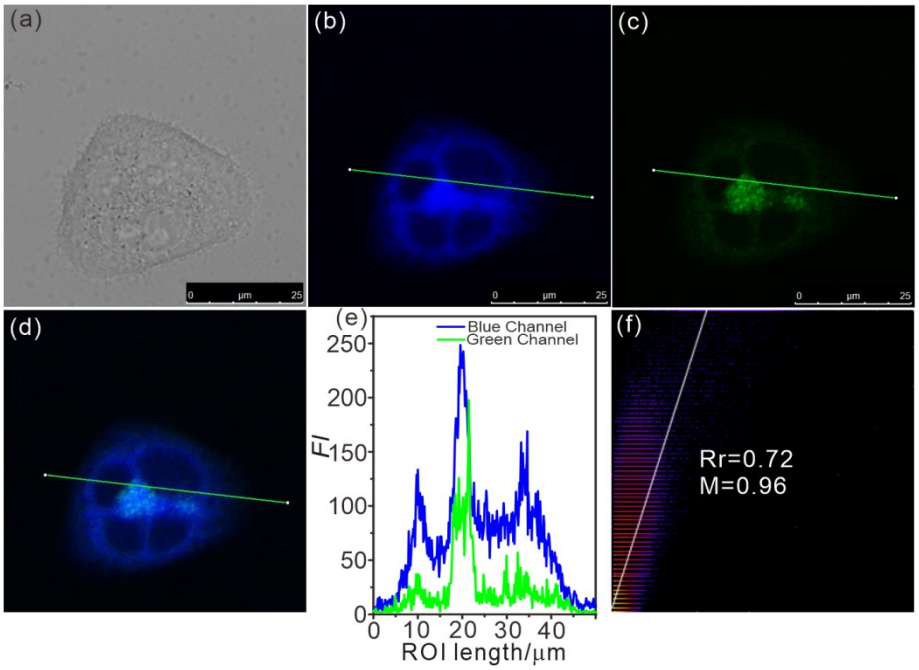

Fig. S19 Confocal fluorescence image of HeLa cells with dye **3b**. (a) bright field images; (b) confocal image (blue channel) of cells with dye **3b** (10 μM); (c) confocal images (green channel) of cells with LysoTracker Green DND-26 (100 nM); (d) merged images of blue and green channels; (e) fluorescence intensities of the regions of interest (ROIs) across the cells;(f) fluorescence intensity correlation plot of blue channel and green channel.

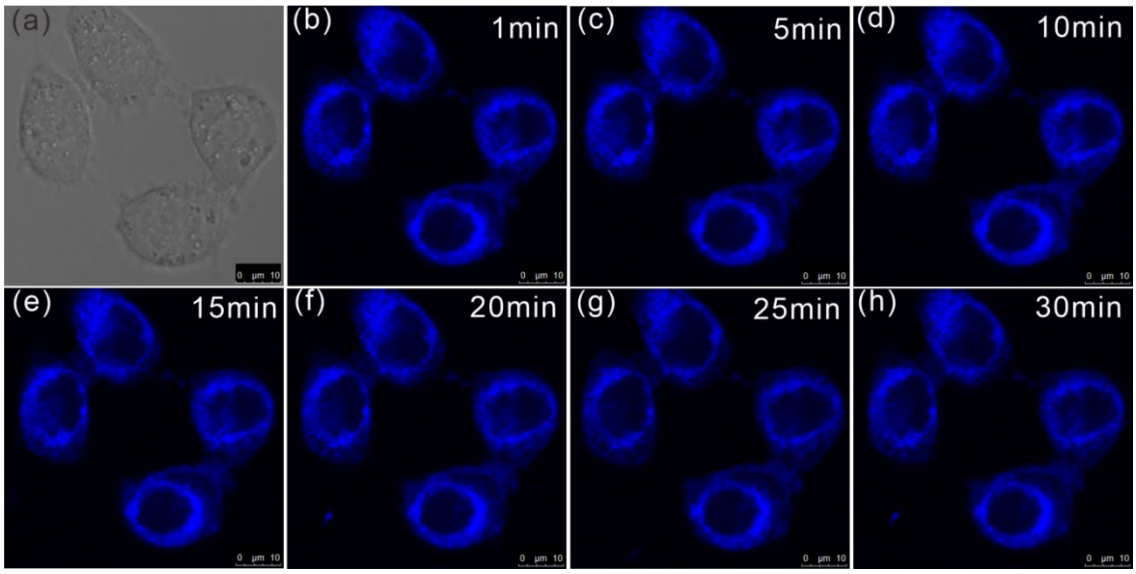

Fig. S20 (a) Bright field images; (b-h) the images of dye **3a** in HeLa cells after 30 minutes of continuous irradiation by the excitation light source (405 nm).

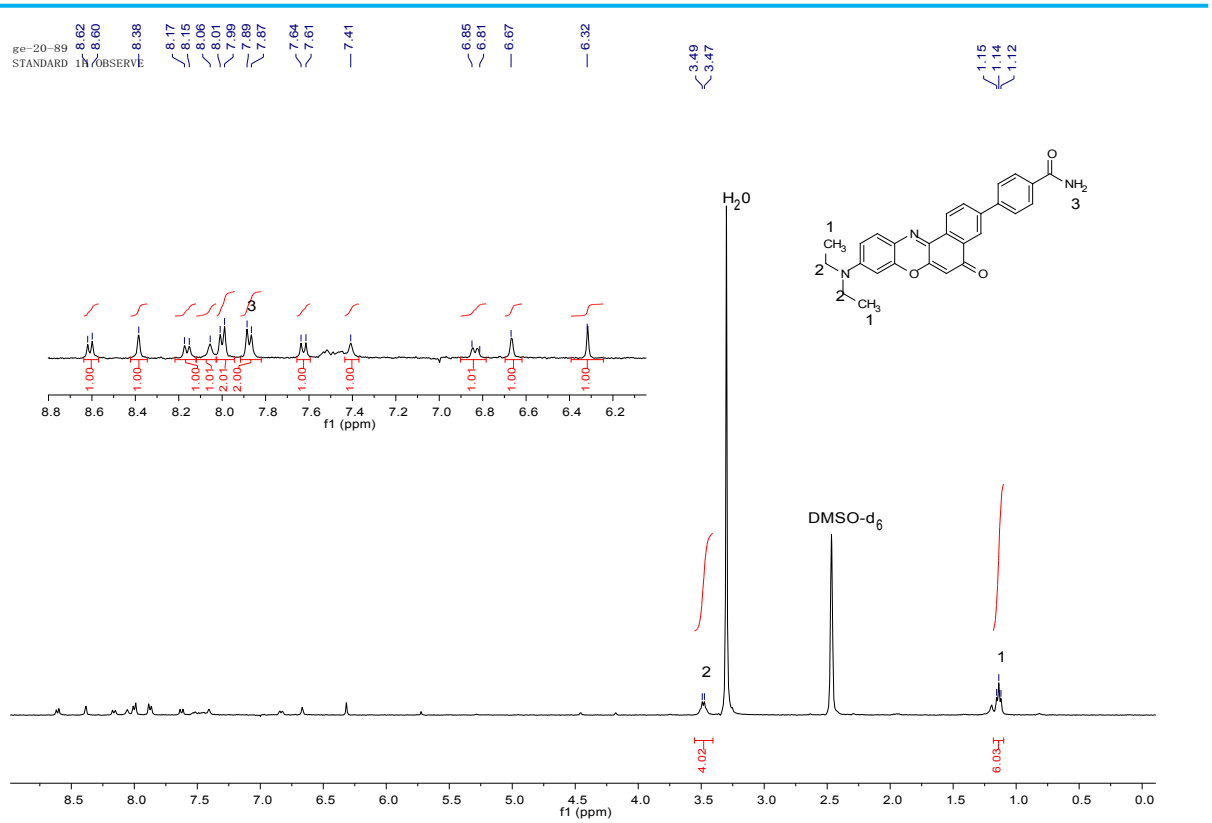

Fig. S21 ^{1}H NMR spectrum of dye **2a**.

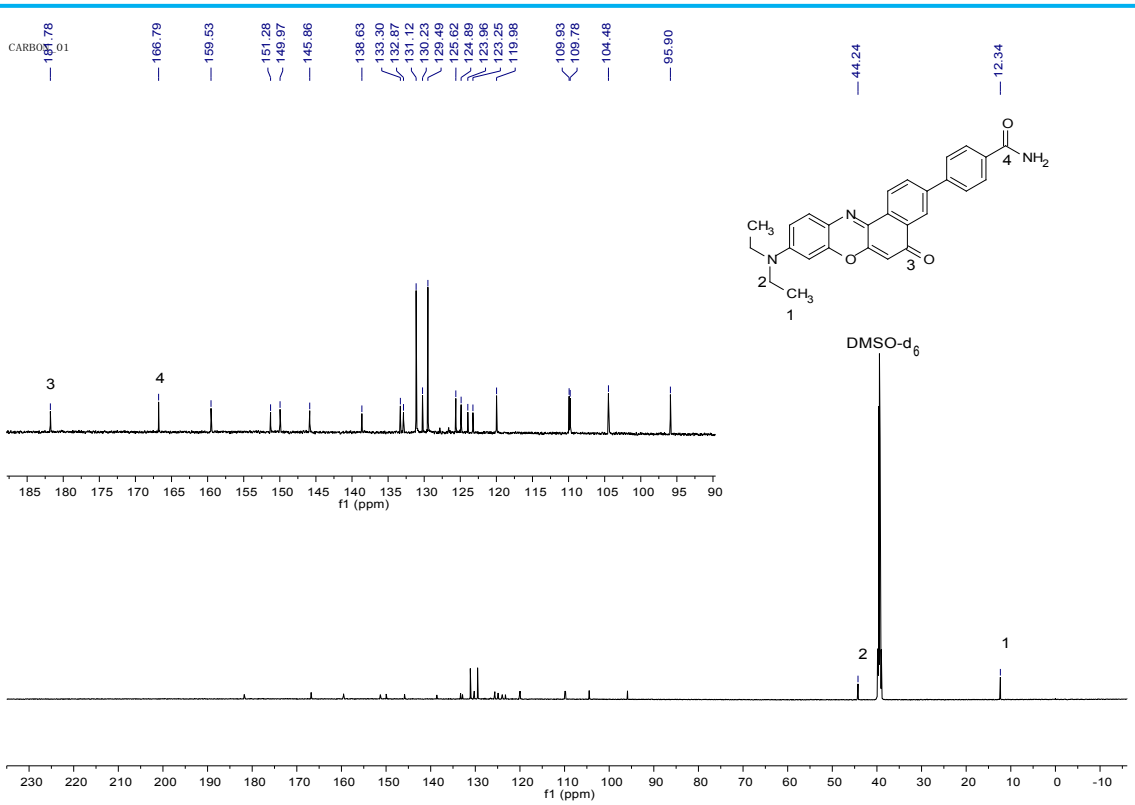

Fig. S22 ^{13}C NMR spectrum of dye **2a**.

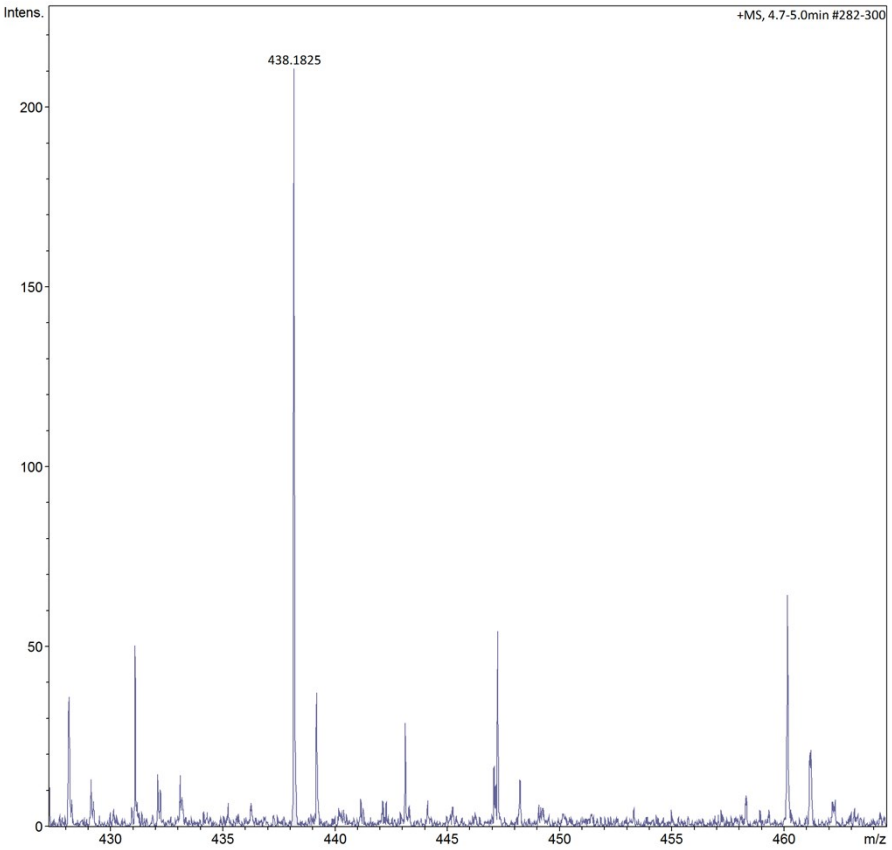

Fig. S23 HRMS (ESI$^+$) spectrum of dye **2a**.

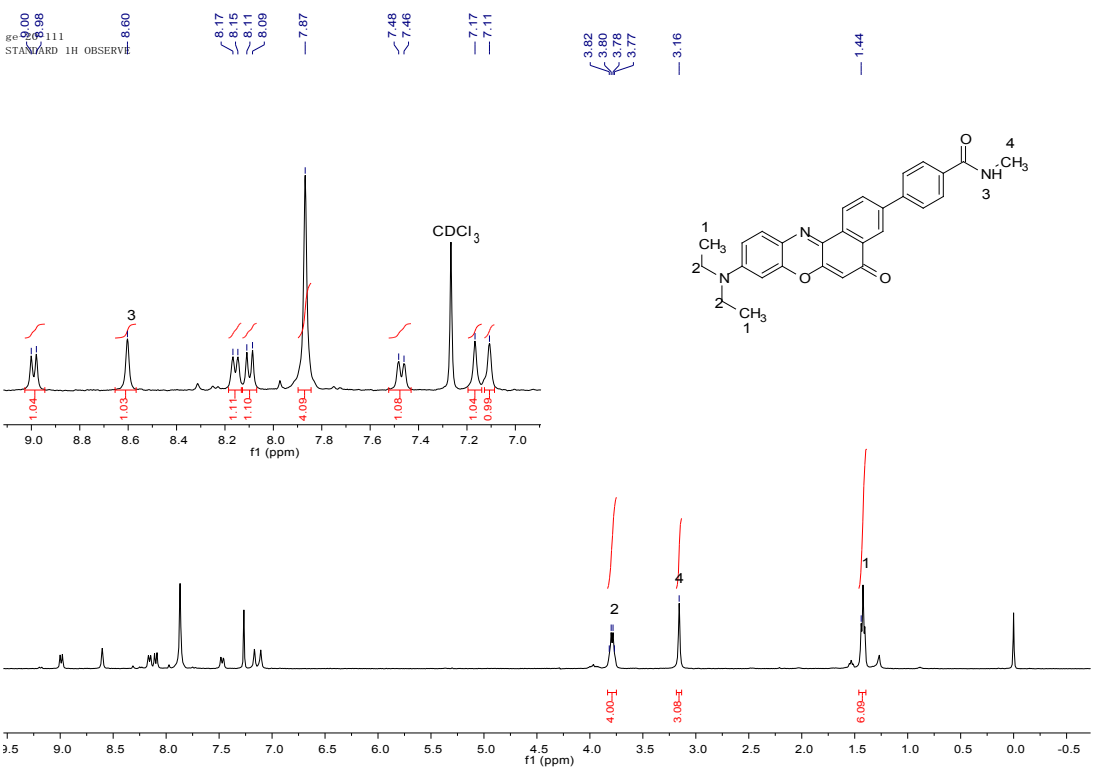

Fig. S24 ^{1}H NMR spectrum of dye **2b**.

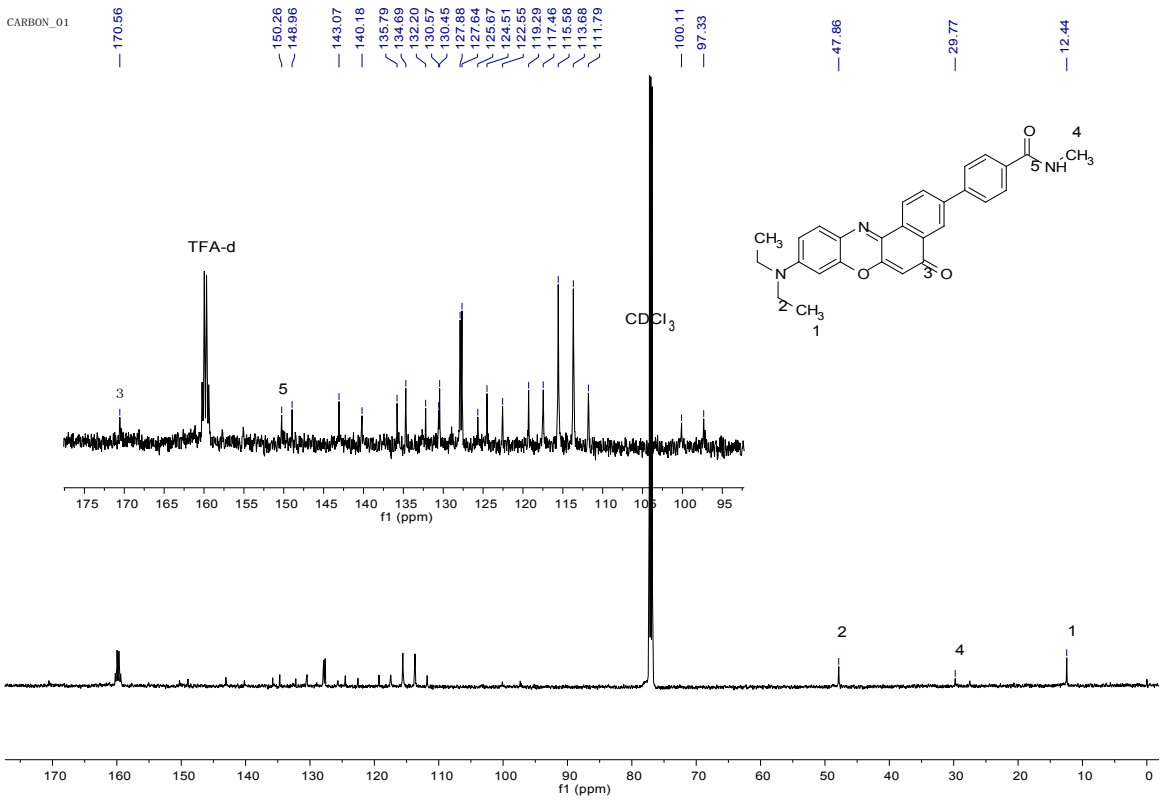

Fig. S25 ^{13}C NMR spectrum of dye **2b**.

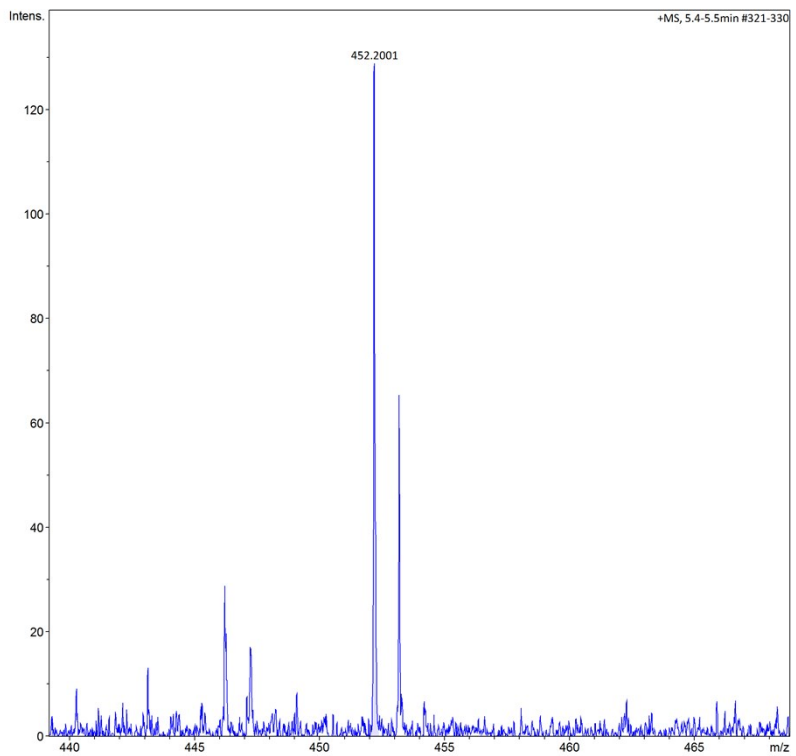

Fig. S26 HRMS (ESI$^+$) spectrum of dye **2b**.

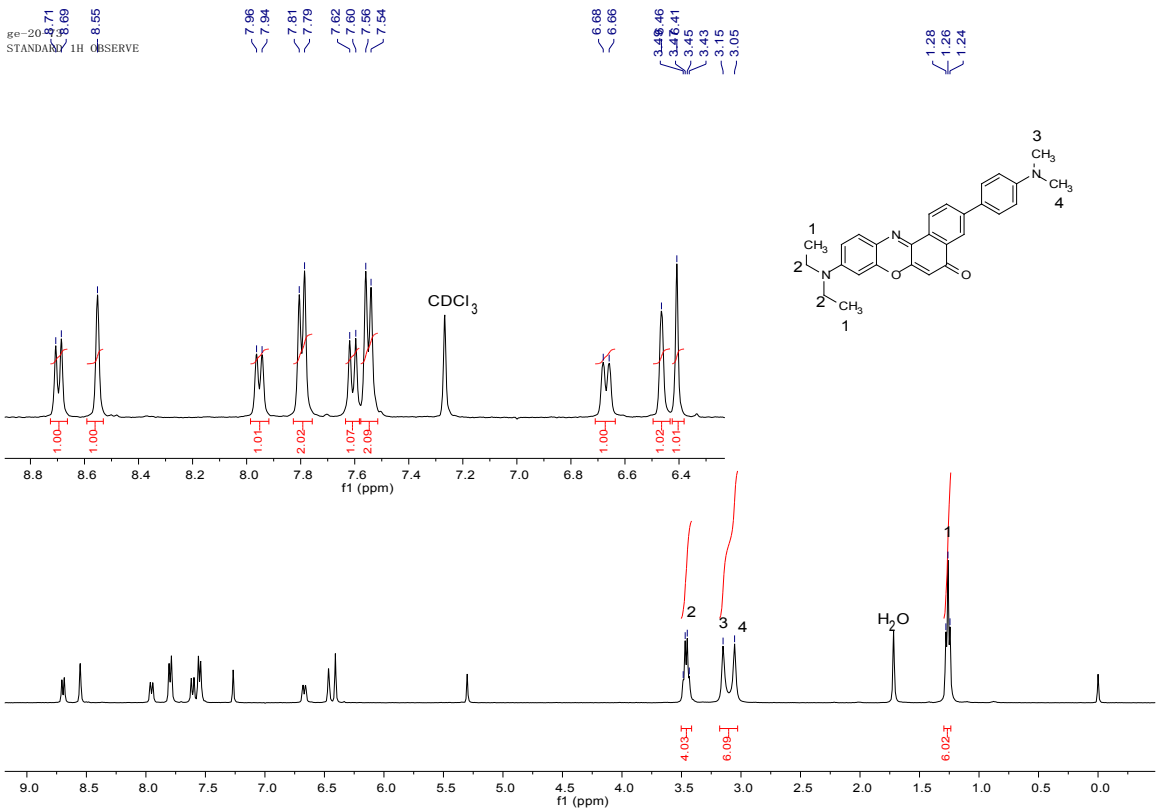

Fig. S27 ^{1}H NMR spectrum of dye **2c**.

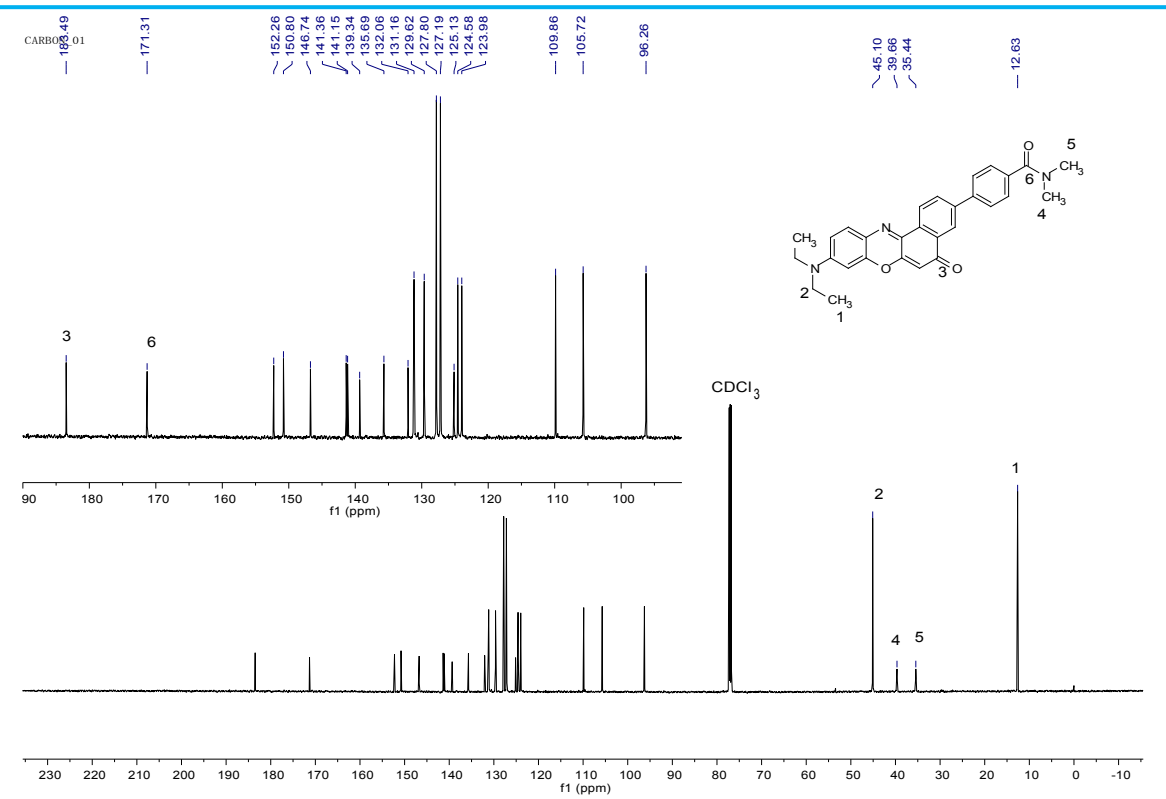

Fig. S28 ^{13}C NMR spectrum of dye **2c**.

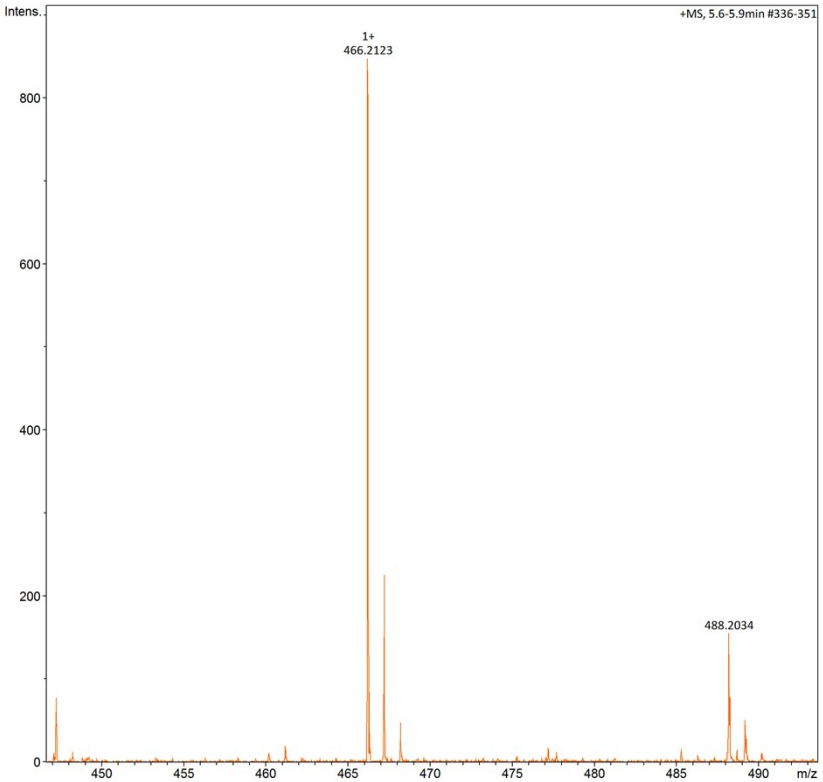

Fig. S29 HRMS (ESI⁺) spectrum of dye **2c**.

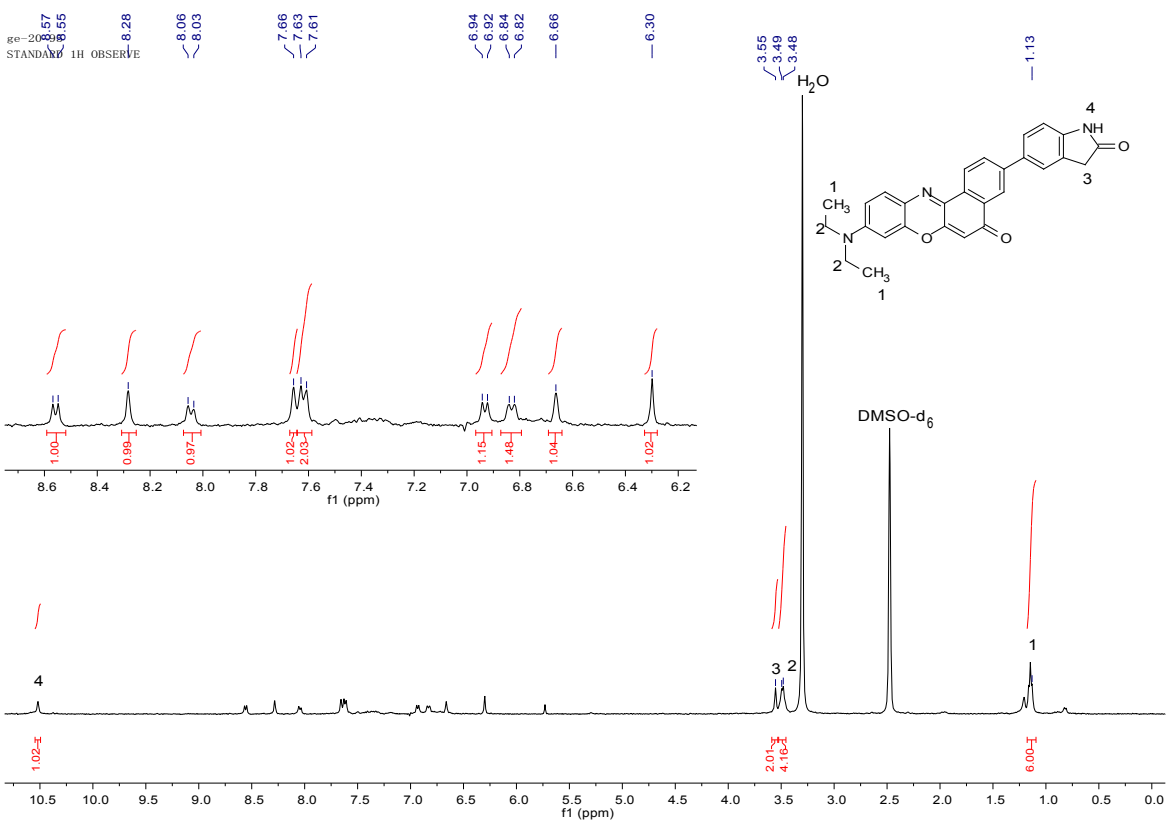

Fig. S30 ^{1}H NMR spectrum of dye **2d**.

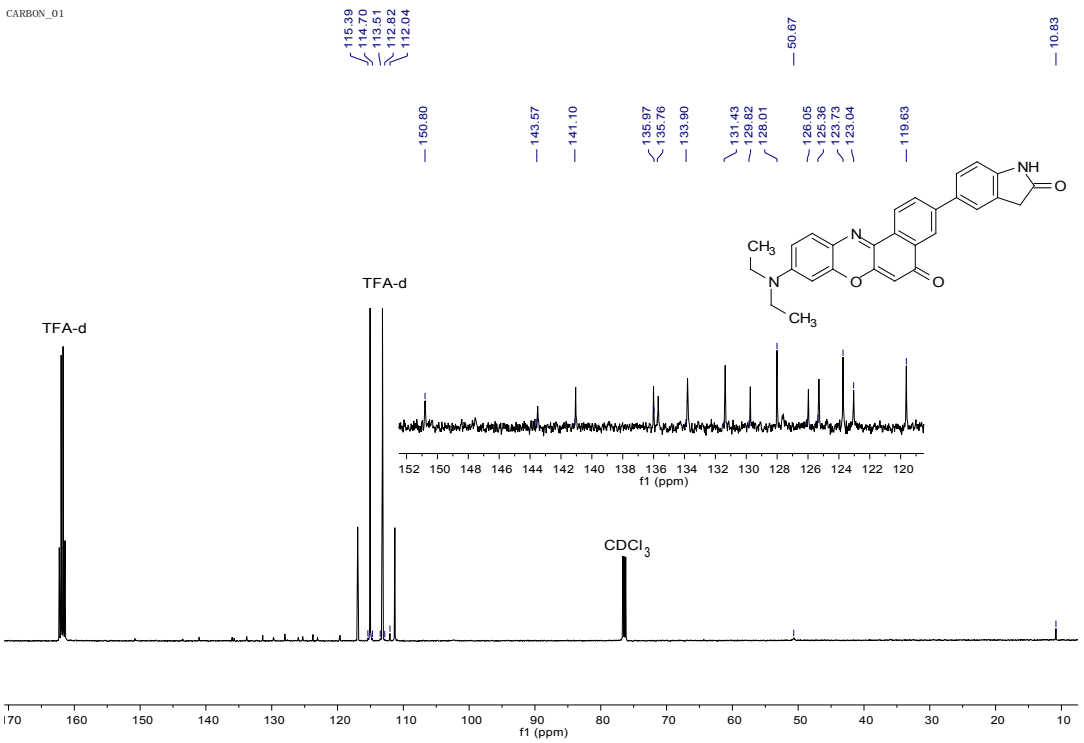

Fig. S31 ^{13}C NMR spectrum of dye **2d**.

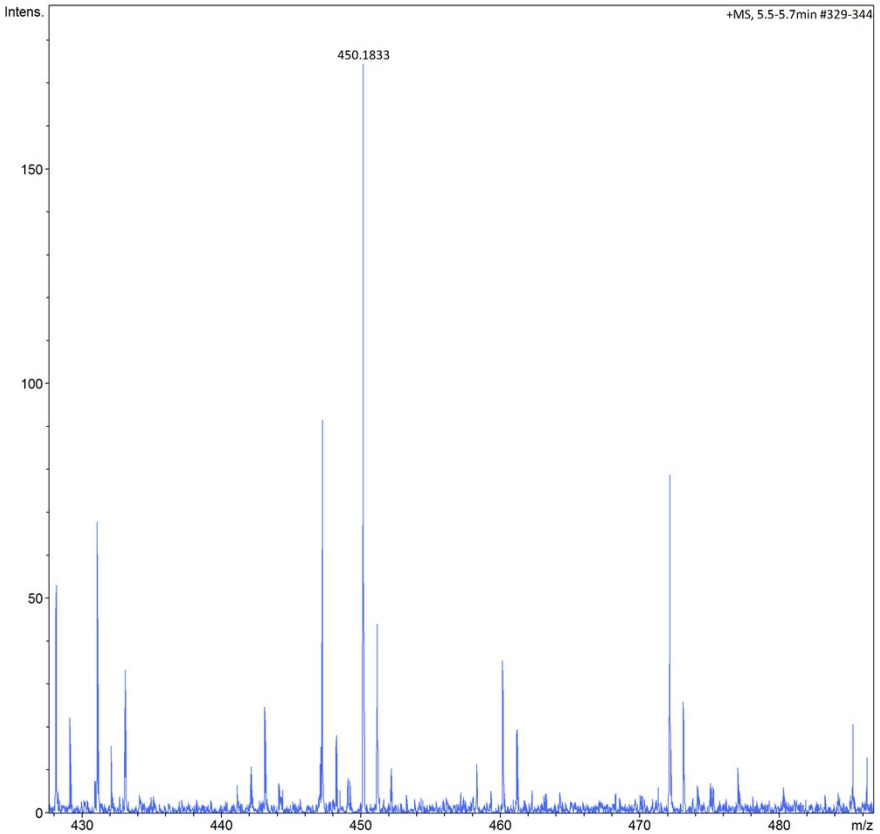

Fig. S32 HRMS (ESI^+) spectrum of dye **2d**.

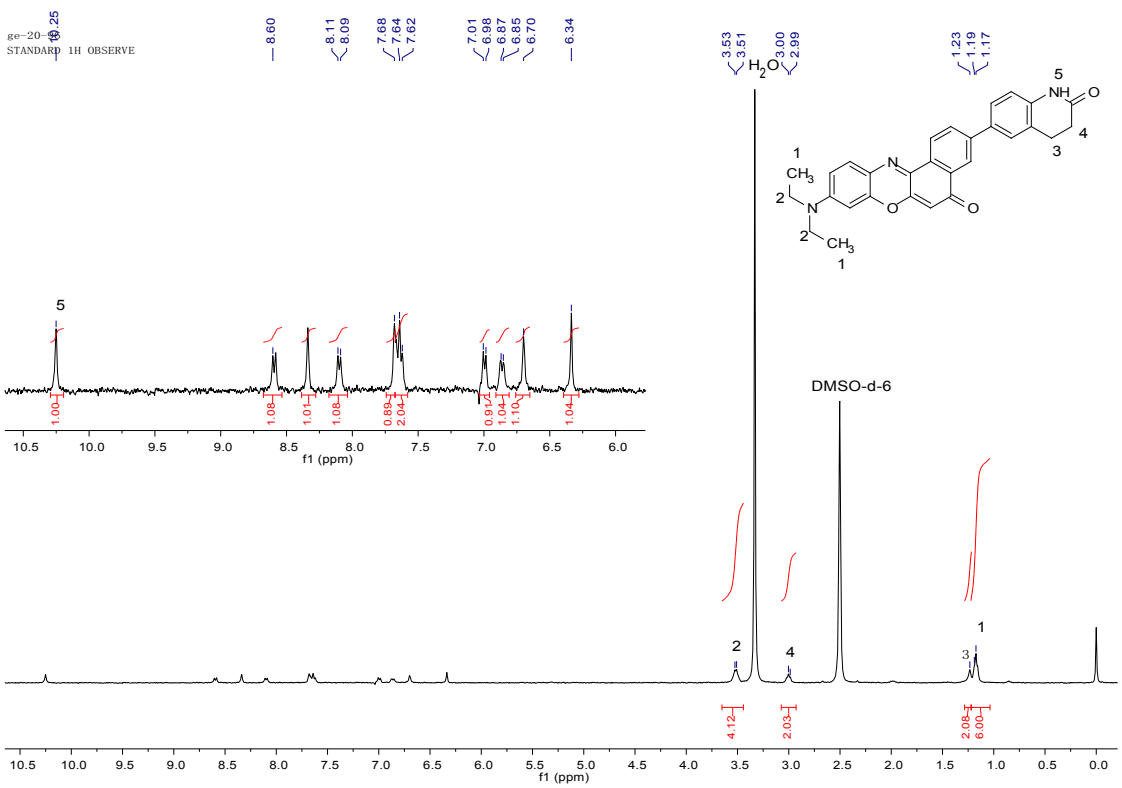

Fig. S33 ^{1}H NMR spectrum of dye **2e**.

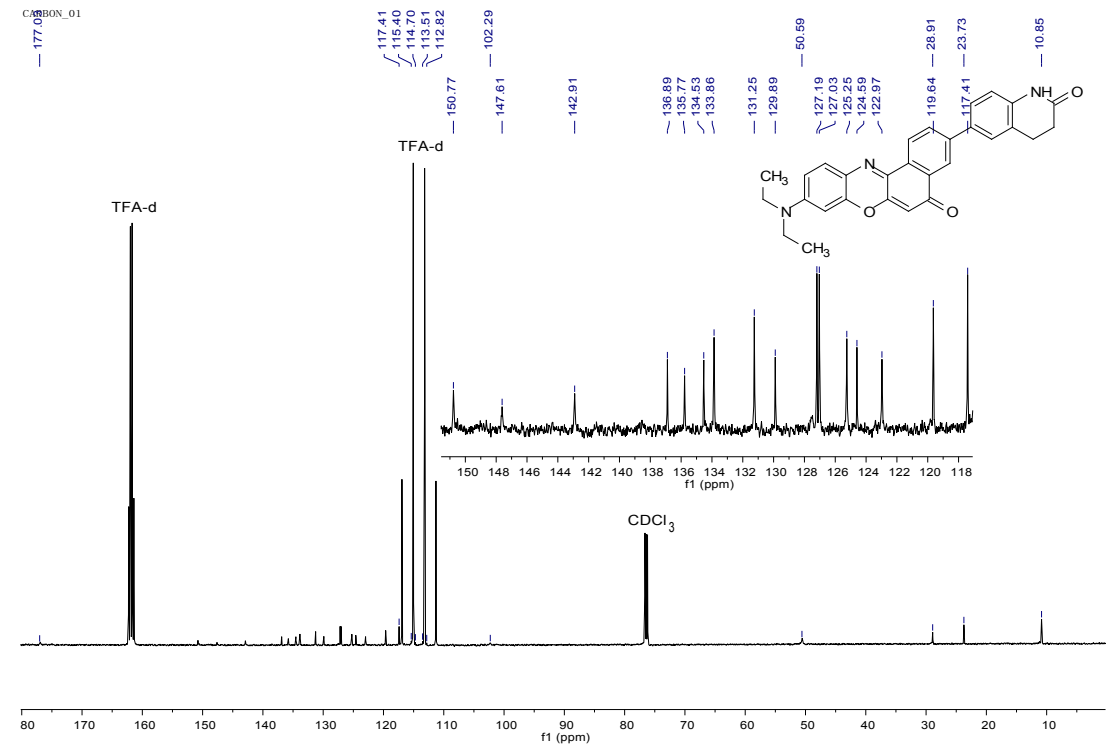

Fig. S34 ^{13}C NMR spectrum of dye **2e**.

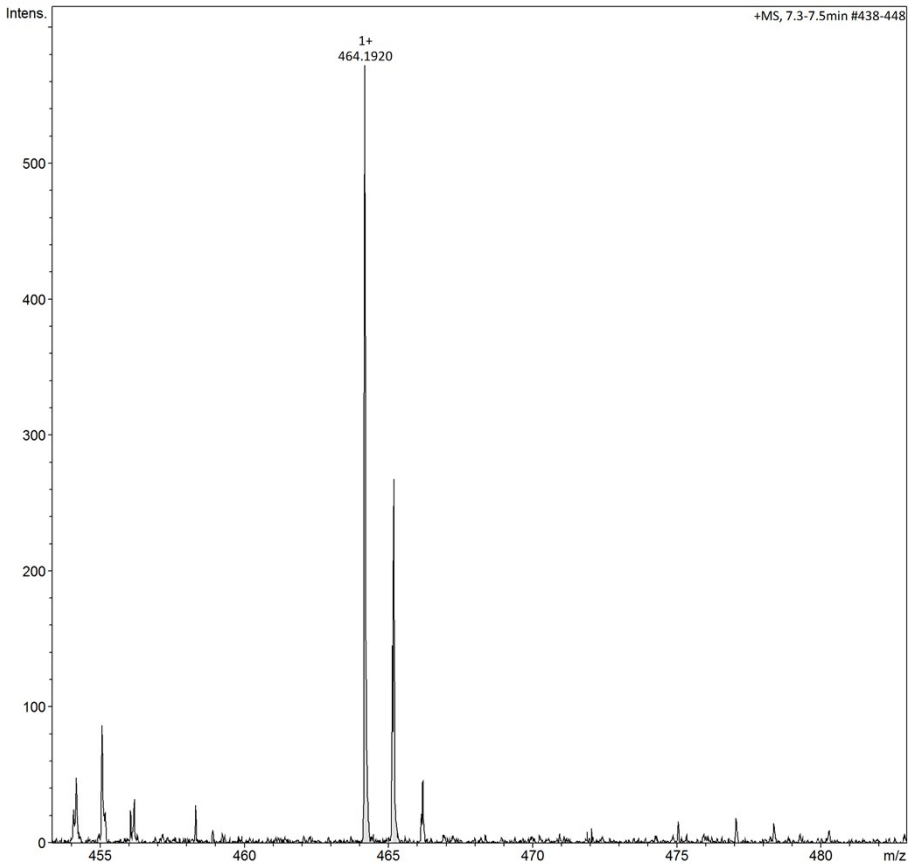

Fig. S35 HRMS (ESI⁺) spectrum of dye **2e**.

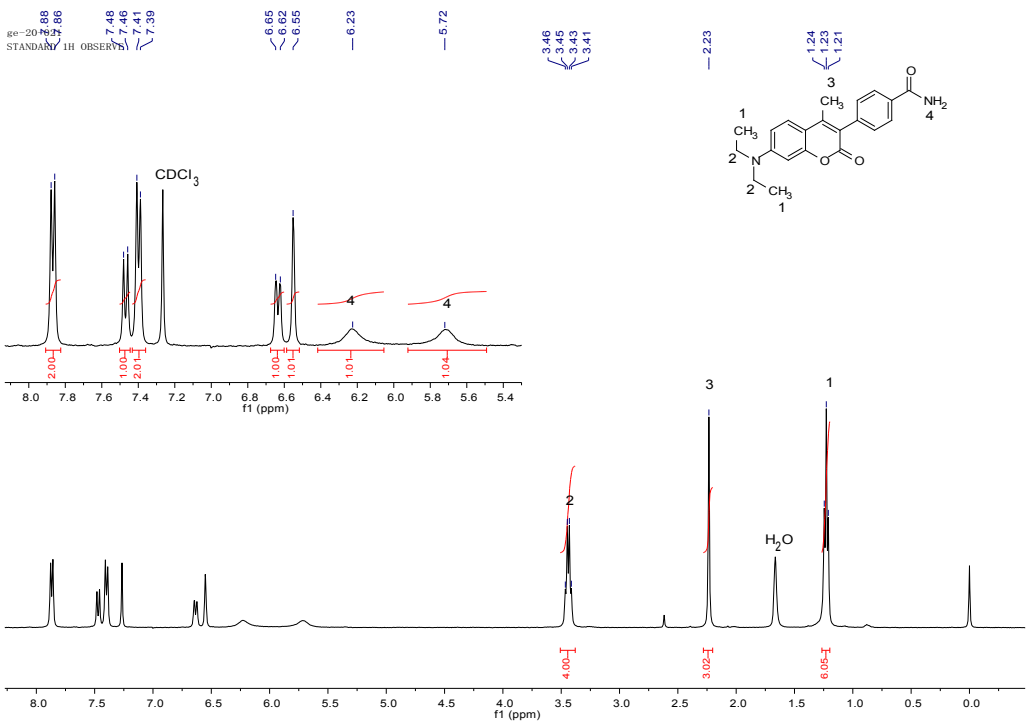

Fig. S36 ^{1}H NMR spectrum of dye **3a**.

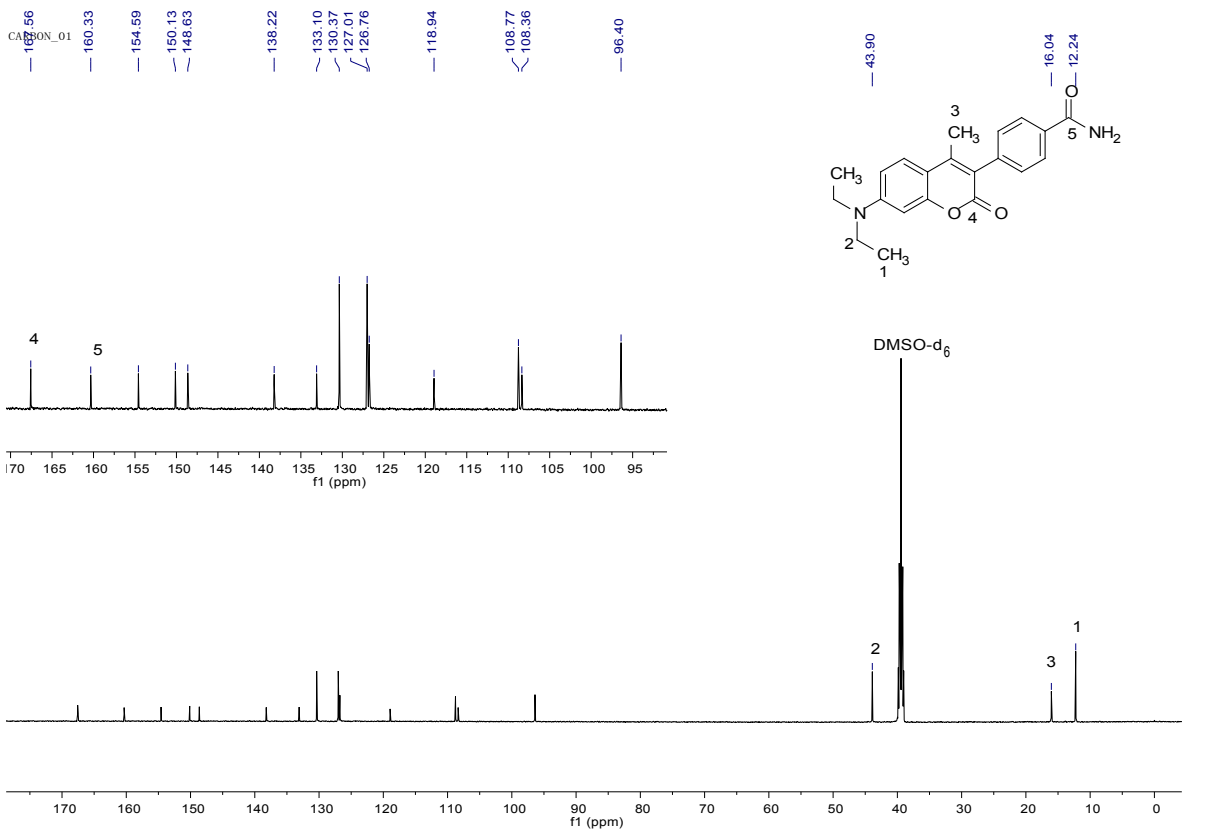

Fig. S37 ^{13}C NMR spectrum of dye **3a**.

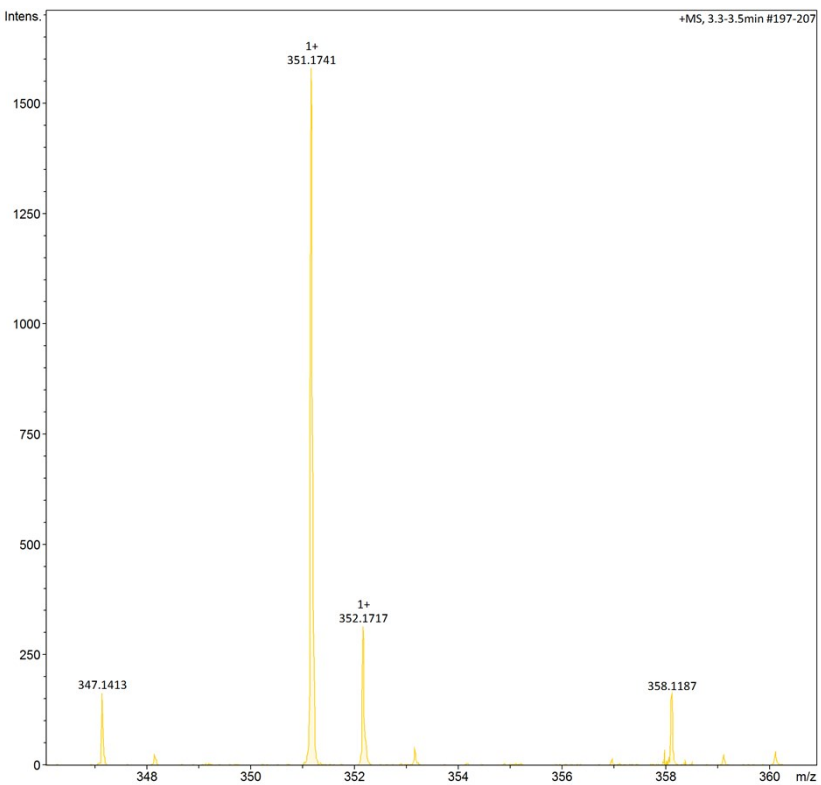

Fig. S38 HRMS (ESI$^+$) spectrum of dye **3a**.

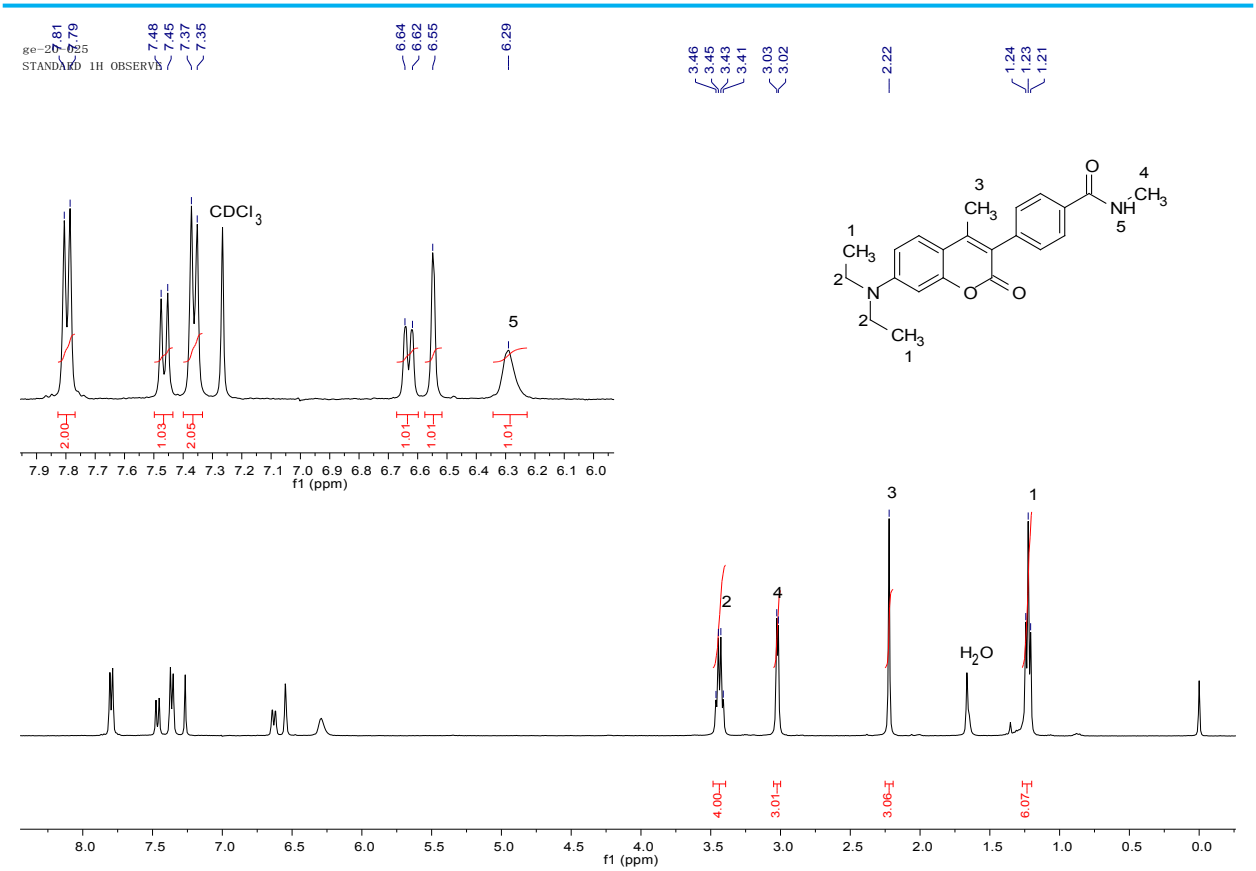

Fig. S39 ^{1}H NMR spectrum of dye **3b**.

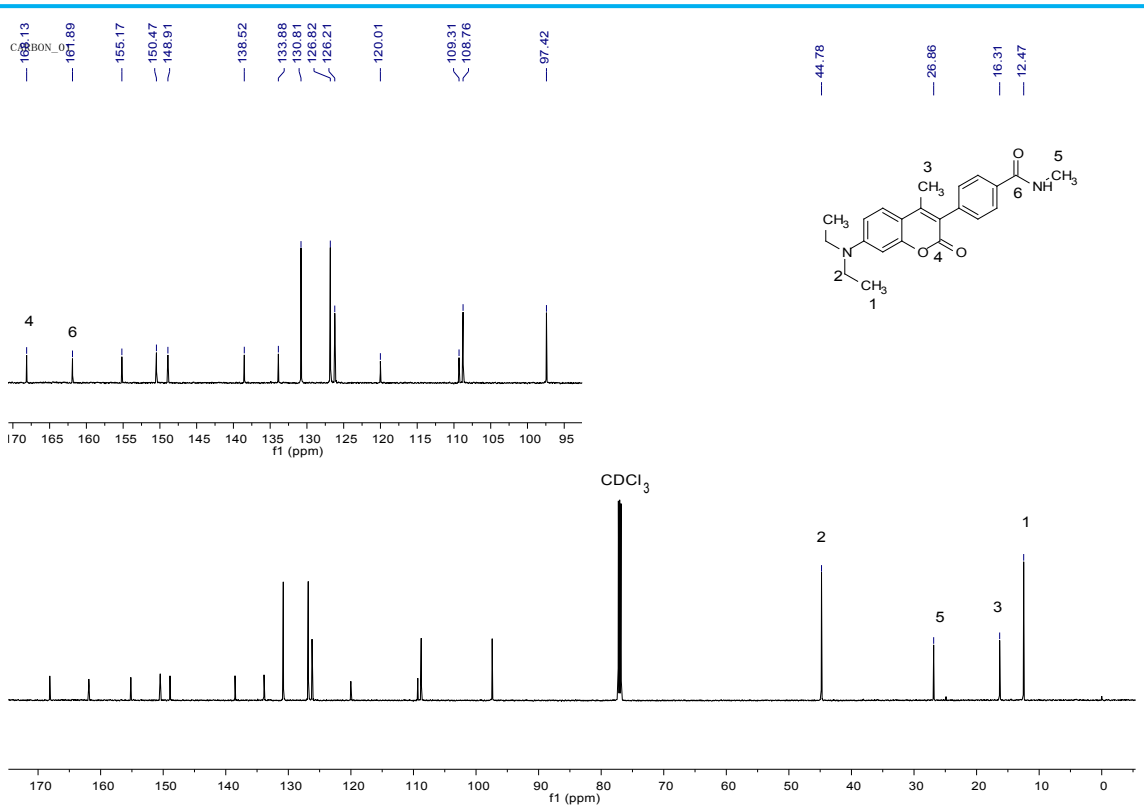

Fig. S40 ^{13}C NMR spectrum of dye **3b.**

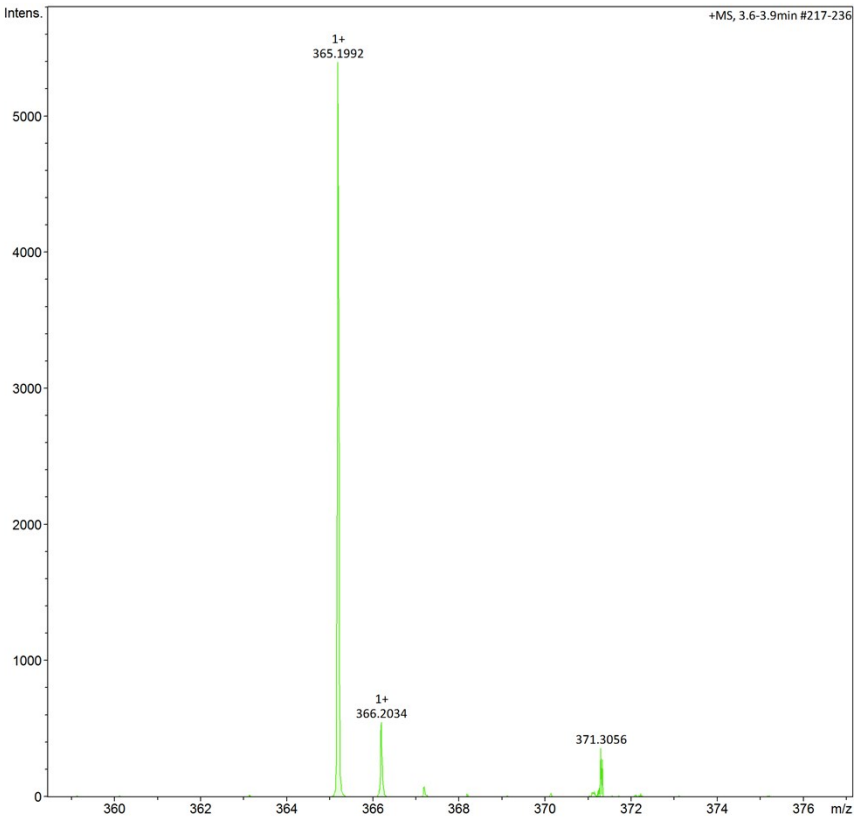

Fig. S41 HRMS (ESI⁺) spectrum of dye **3b**.

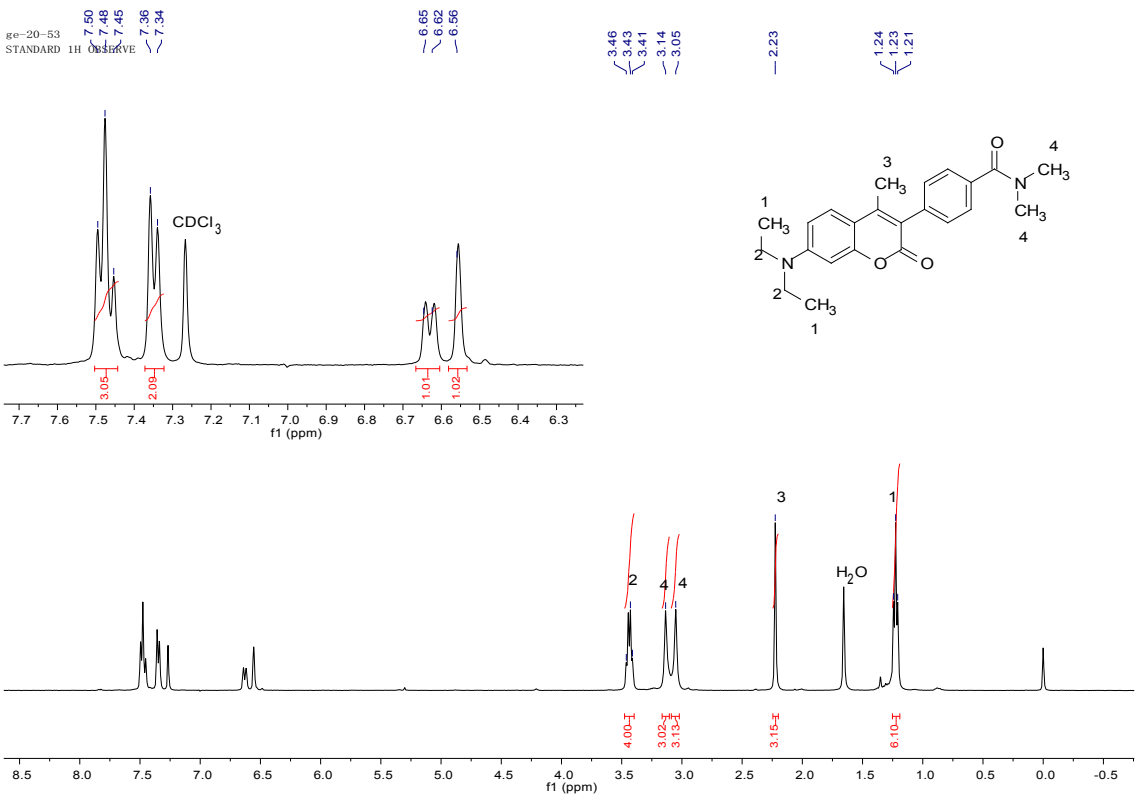

Fig. S42 ^{1}H NMR spectrum of dye **3c**.

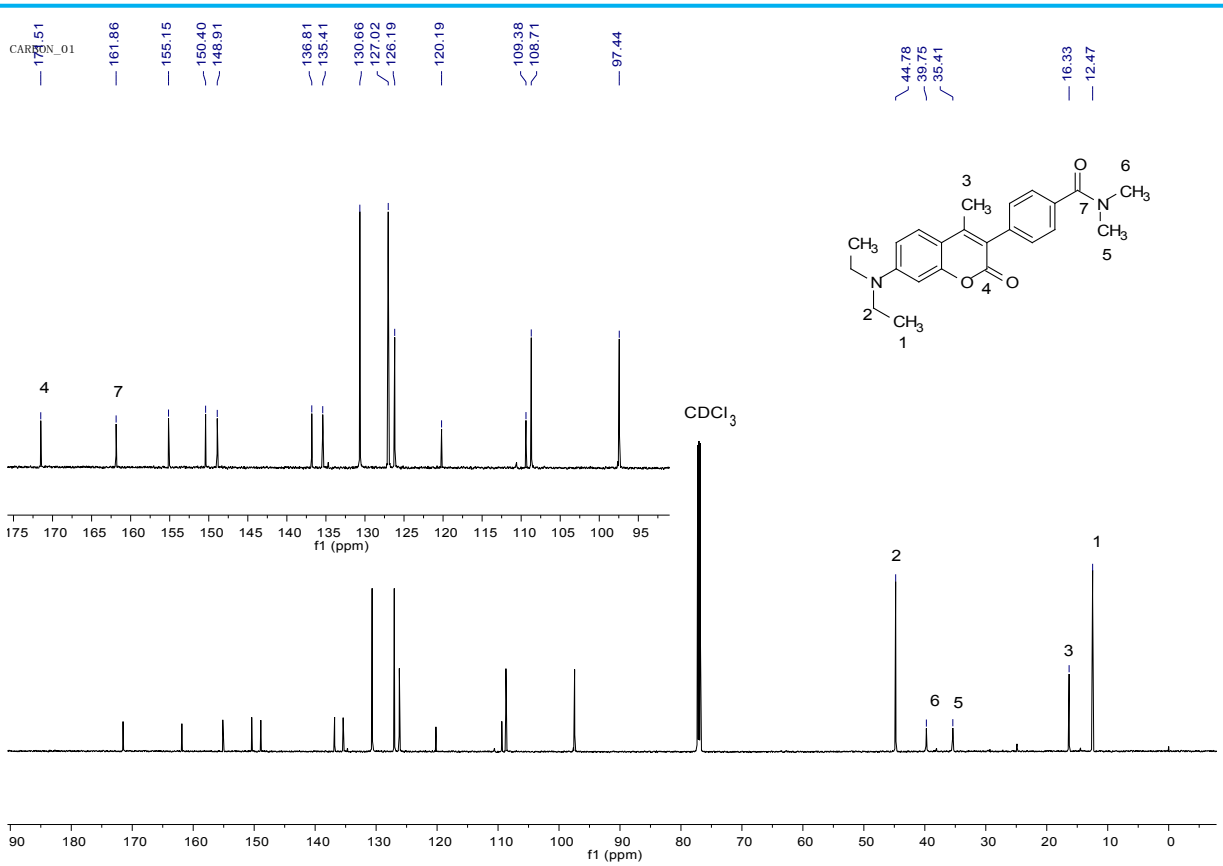

Fig. S43 ^{13}C NMR spectrum of dye **3c**.

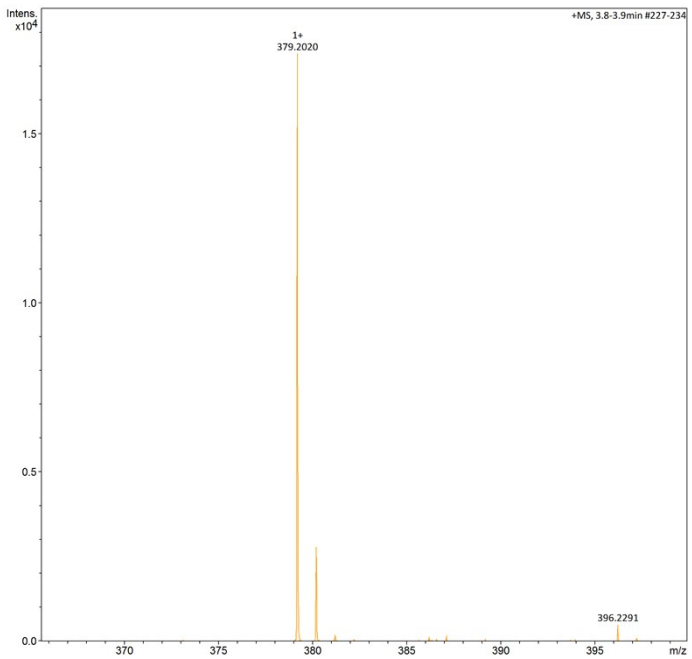

Fig. S44 HRMS (ESI$^+$) spectrum of dye **3c**.

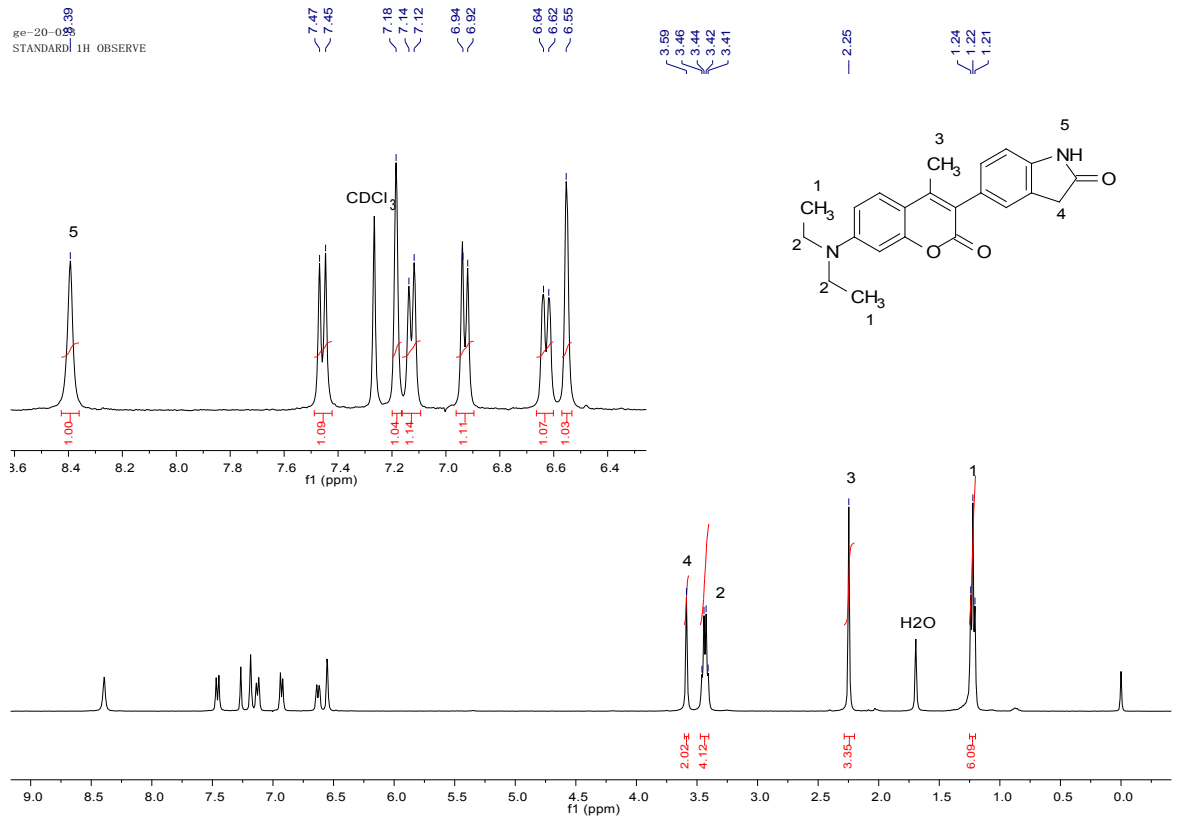

Fig. S45 ^{1}H NMR spectrum of dye **3d**.

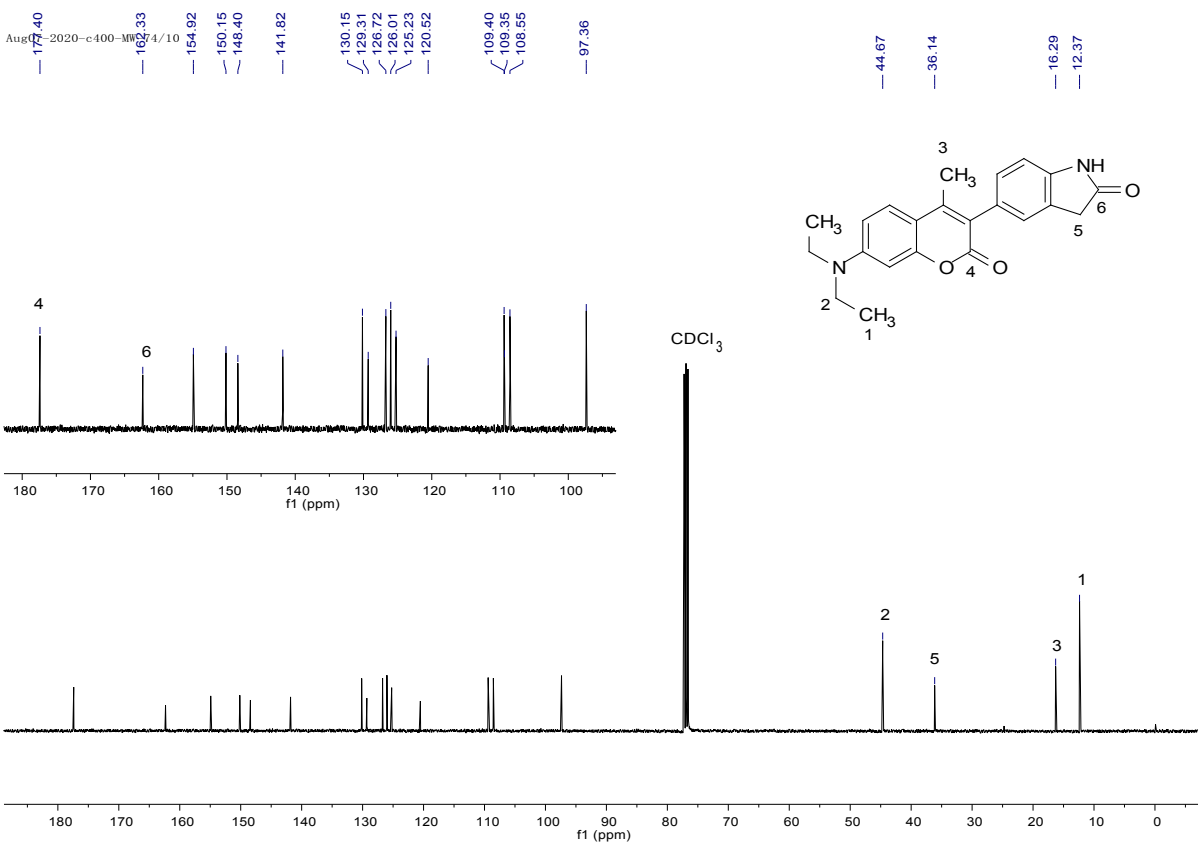

Fig. S46 ^{13}C NMR spectrum of dye **3d**.

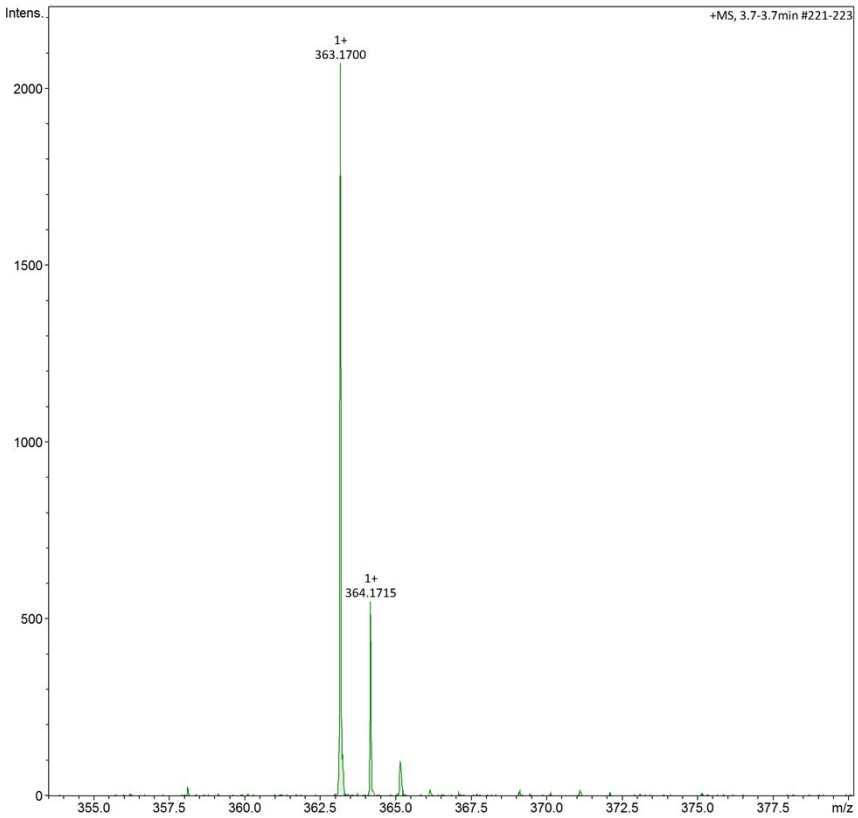

Fig. S47 HRMS (ESI⁺) spectrum of dye **3d**.

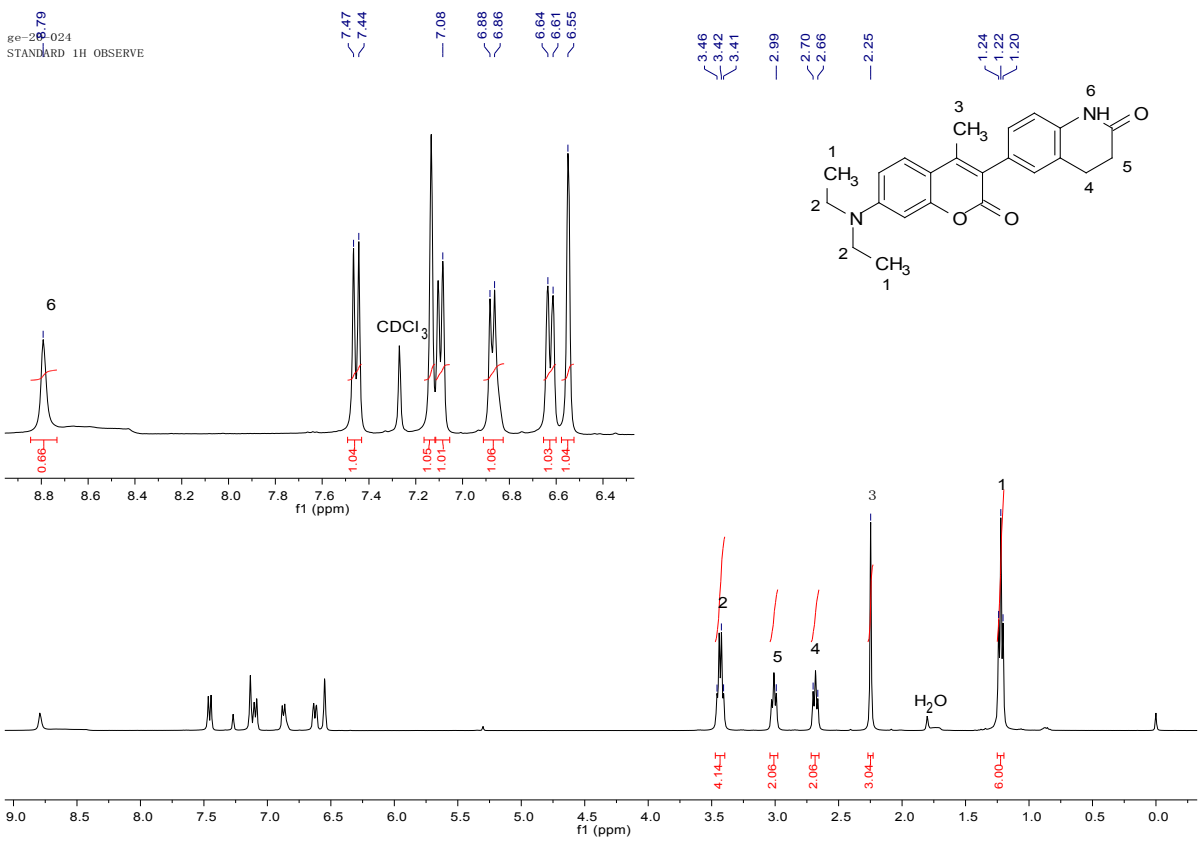

Fig. S48 ^{1}H NMR spectrum of dye **3e**.

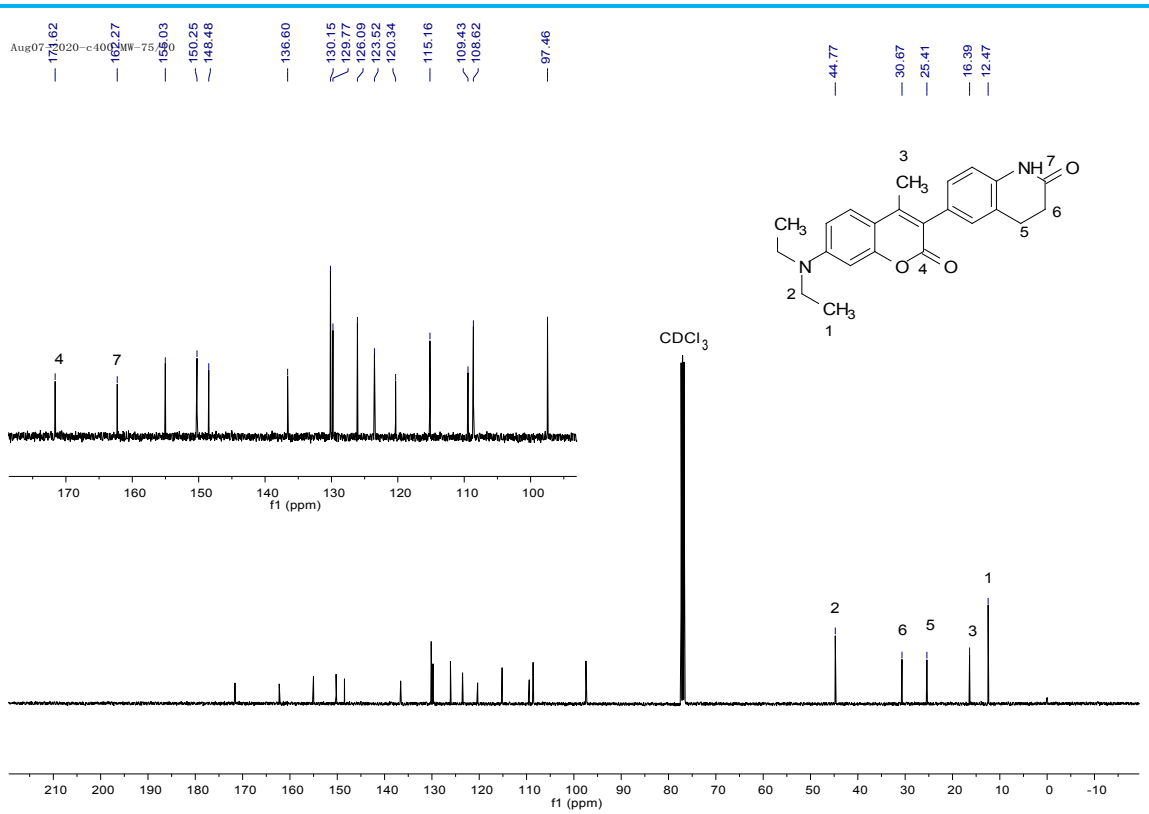

Fig. S49 ^{13}C NMR spectrum of dye **3e**.

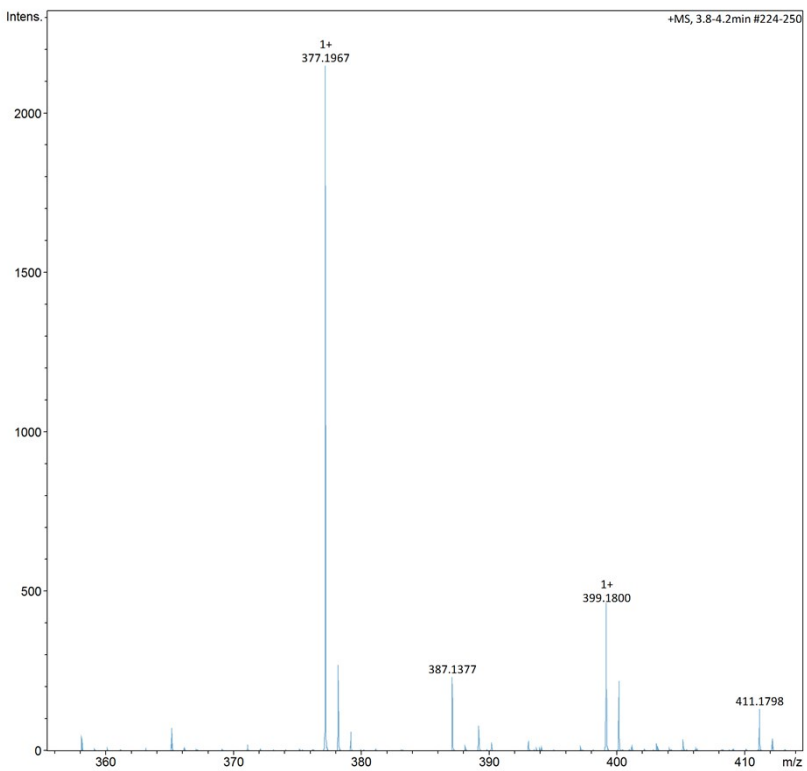

Fig. S50 HRMS (ESI^{+}) spectrum of dye **3e**.

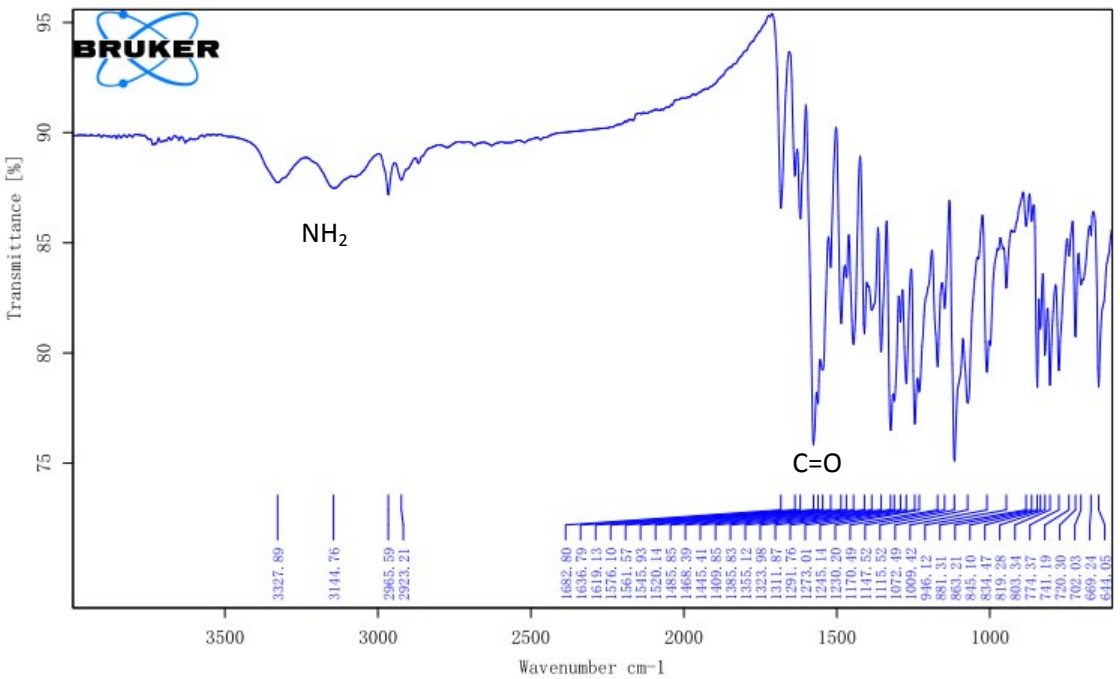

Fig. S51 Infrared spectra of dye **2a**.

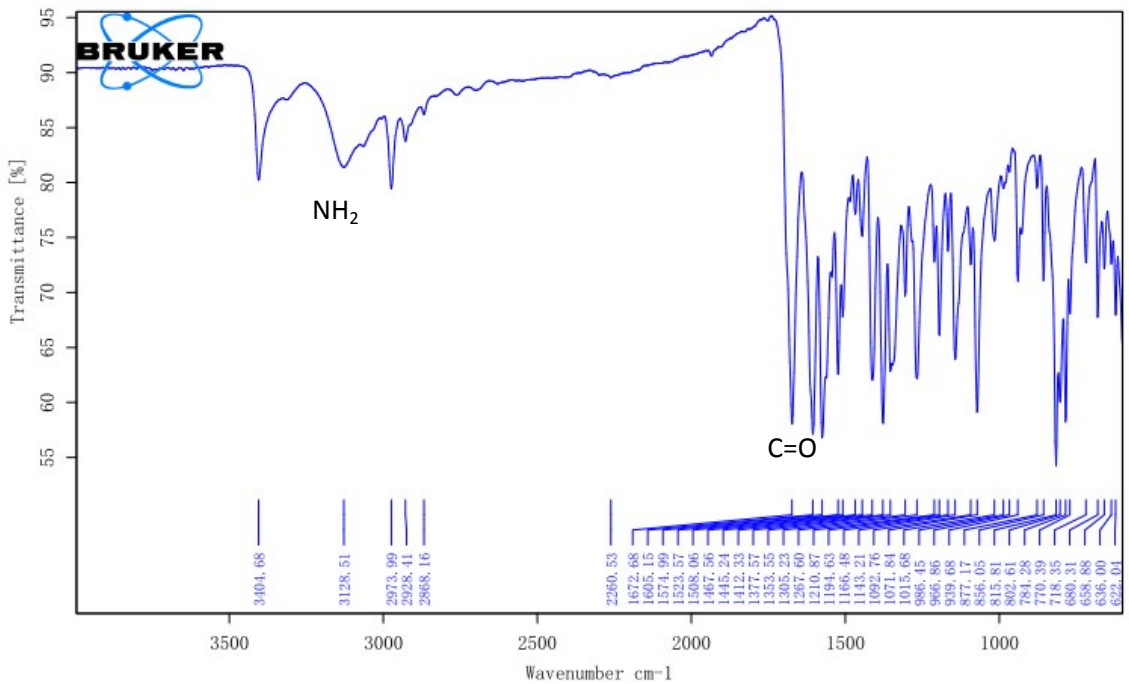

Fig. S52 Infrared spectra of dye **3a**.