Near infrared fluorescent probes based on quinoxaline skeleton for imaging nucleic acid in mitochondria

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Index

Table S1. Basic optical properties of common nucleic acid dyes and their targeting site in cells.

Dyes	$\lambda_{Abs,max}$[a]	$\lambda_{Em,max}$[a]	Whether the dye can penetrate the cell membrane	Targeted subcellular organelle
1a (this work)	561[b]	611[b, c]	Yes	Mitochondria
SYBR Green Ⅰ	497	520	Yes	Mitochondria and Nucleus
DAPI	358	461	Yes	Nucleus
Hoechst 33342	346	460	Yes	Nucleus
PI	493	636	No	/
Gel Red	510	600	No	/

[a] Reported in nm. [b] Testing in DMSO. [c] Second highest emission peak.

Table S2. Optical properties of probes **1a-c** in different solvents.

Probe	Solvents	$\lambda_{Abs,max}$[a]	$\lambda_{Em,max}$[a]	Stokes shift[a]	ε[b]	Φ[c]
1a	H_2O	503	ND[d]	ND[d]	2.3	ND[d]
1a	DMSO	561	575	14	10.5	71.6
1a	MeOH	560	578	18	10.2	86.9
1a	THF	551	563	12	12.2	95.1
1a	DCM	556	569	13	9.9	90.2
1a	TOL	555	567	12	1.1	71.5
1b	H_2O	505	ND[d]	ND[d]	1.0	ND[d]
1b	DMSO	561	575	14	9.2	72.8
1b	MeOH	560	575	15	8.5	78.8
1b	THF	551	563	12	9.2	80.3
1b	DCM	556	570	14	8.6	75.2
1b	TOL	555	567	12	9.2	75.4
1c	H_2O	373	ND[d]	ND[d]	0.9	ND[d]
1c	DMSO	370	561	191	2.2	15.1
1c	EtOH	369	575	206	3.2	9.4
1c	$CHCl_3$	369	578	209	2.2	29.7
1c	THF	366	562	196	2.4	21.2
1c	TOL	368	560	192	2.4	31.4

[a] Reported in nm. [b] Reported in $10^4\ M^{-1}\ cm^{-1}$. [c] Reported in %. [d] Reported in 'not detected'. Cresyl violet (Φ=0.578 in ethanol) was used as the reference compound for **1a** and **1b**, coumarin–153 (Φ=0.544 in ethanol) was used as the reference compound for **1c**.

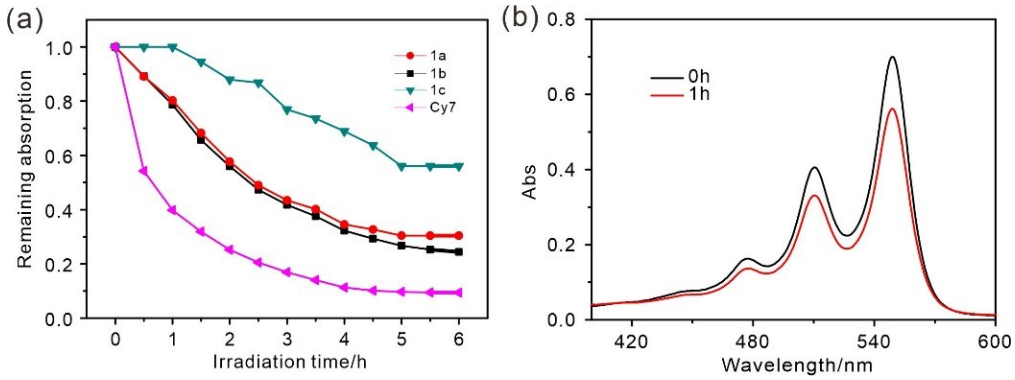

Fig. S1. Photofading behaviors of probes **1a-c** and Cy7 in acetonitrile. (a) The residual absorption rate of probes **1a-c** after continuous irradiation for 6 h; (b) absorption spectra of probe **1a** without irradiation and irradiation for 1 h.

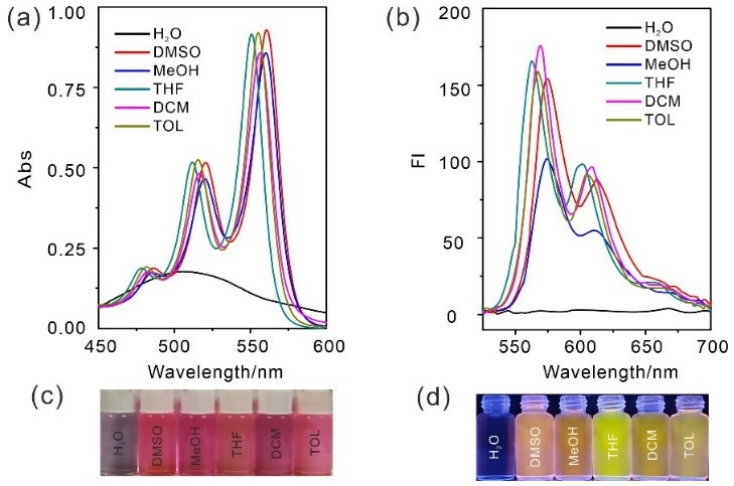

Fig. S2. Optical properties of probe **1b** (10 μM) in different solvents. (a) Absorption spectra; (b) emission spectra (excited at 556 nm, slit widths: 3 nm/1.5 nm); (c) photographs under daylight; (d) photographs under a lamp at 365 nm in dark room.

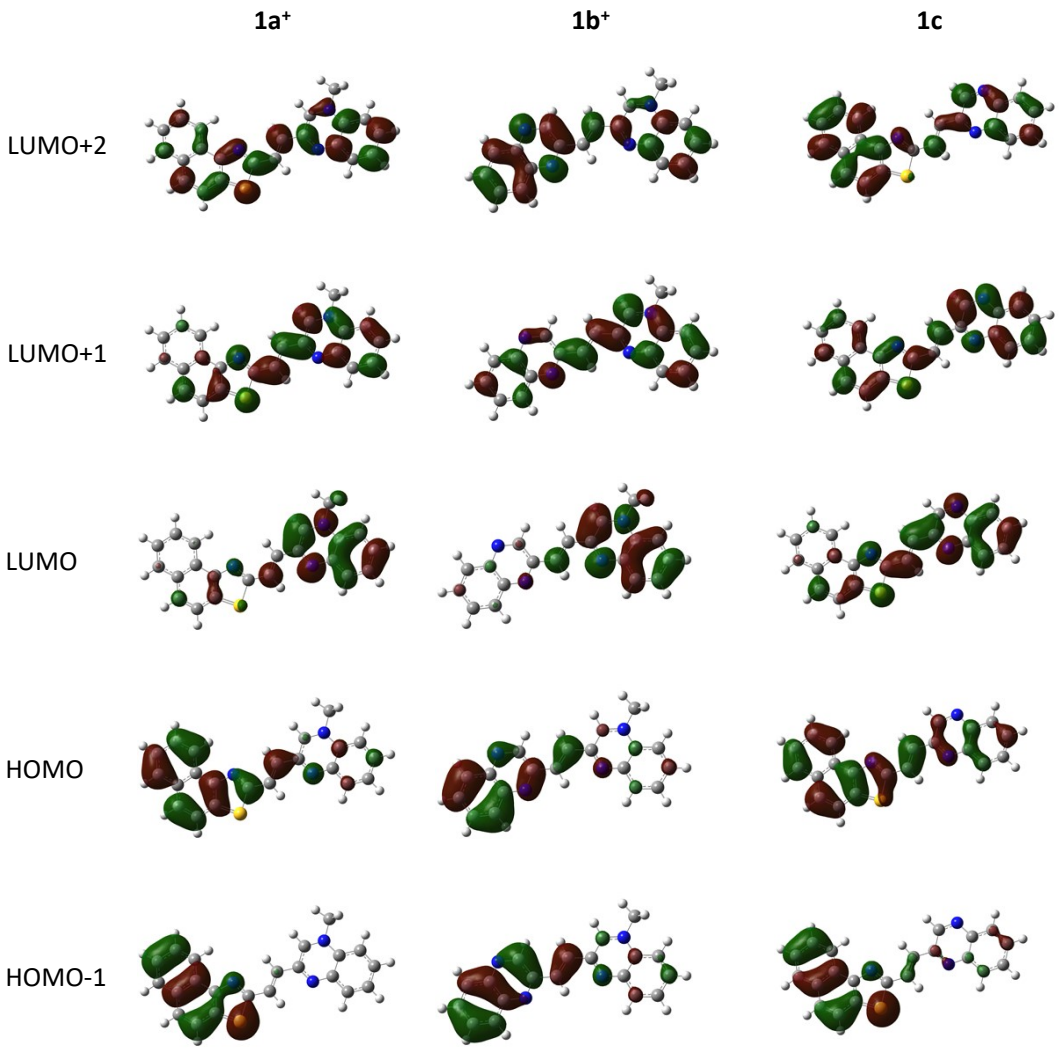

Fig. S3. The electron cloud profiles of frontier molecular orbits for probes **1a-b** in cationic form and dye **1c** calculated at the level of DFT//b3lyp/6-31g(d) using Gaussian software.[1]

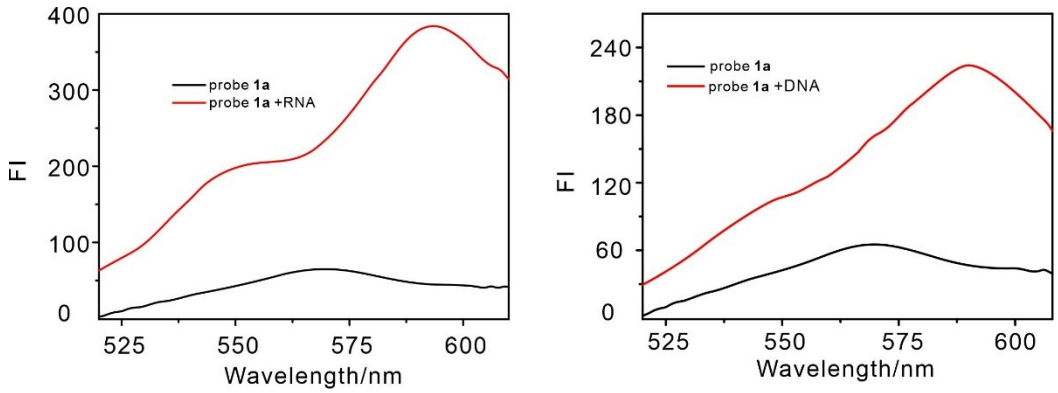

Fig. S4. Excitation spectra of probe **1a**.

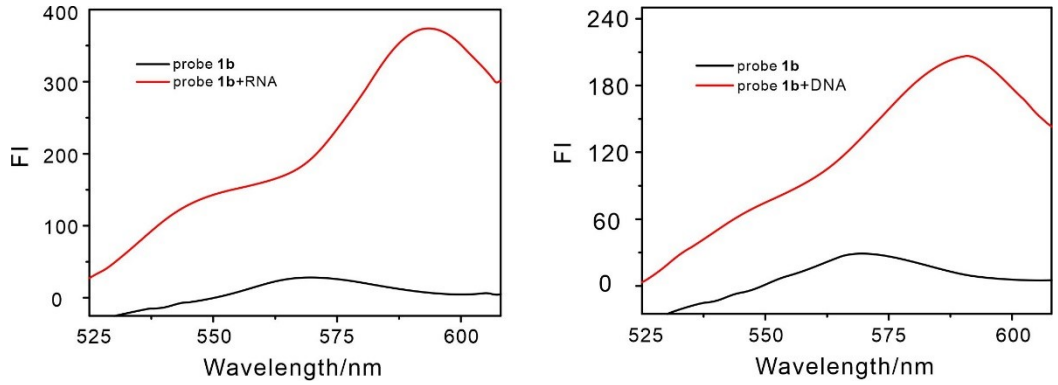

Fig. S5. Excitation spectra of probe **1b**.

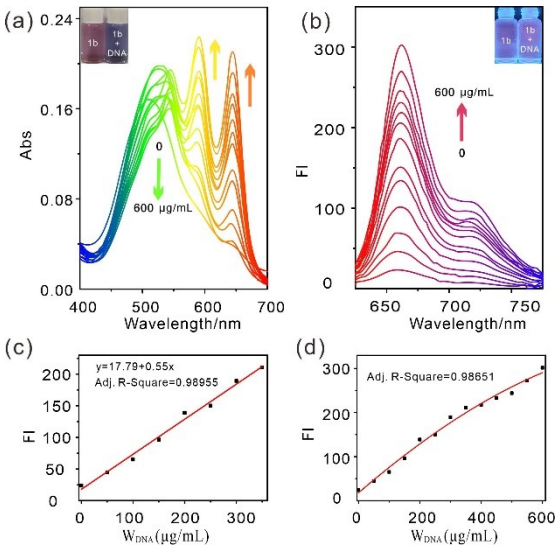

Fig. S6. Optical responses of probe **1b** (10 μM) toward DNA (0–600 μg/mL) in Tris–HCl buffer (10 mM, pH=7.4) containing 1% DMSO. (a)Absorption spectra; (b)emission spectra (λ_{ex}=590 nm, slit widths: 3 nm/5 nm); (c) linear relationship of fluorescence intensity at 661 nm versus the concentration of DNA (0–350 μg/mL); (d) fluorescence intensity toward different concentrations of DNA at 661 nm.

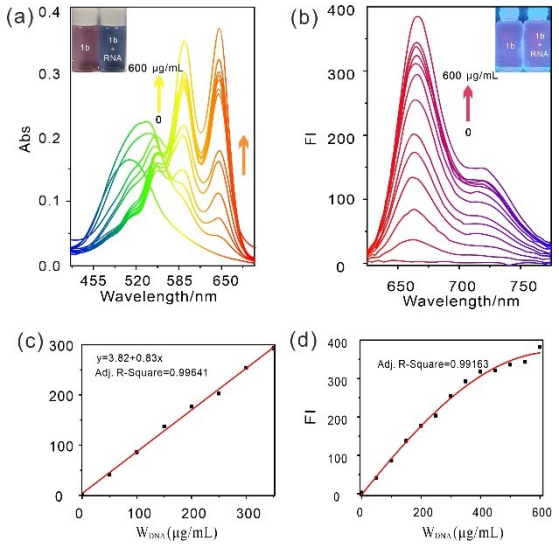

Fig. S7. Optical responses of probe **1b** (10 μM) toward RNA (0–600 μg/mL) in Tris–HCl buffer (10 mM, pH=7.4) containing 1% DMSO. (a)Absorption spectra; (b)emission spectra (λ_{ex}=594 nm, slit widths: 3 nm/5 nm); (c) linear relationship of fluorescence intensity at 663 nm versus the concentration of RNA (0–350 μg/mL); (c) fluorescence intensity toward different concentrations of RNA at 663 nm.

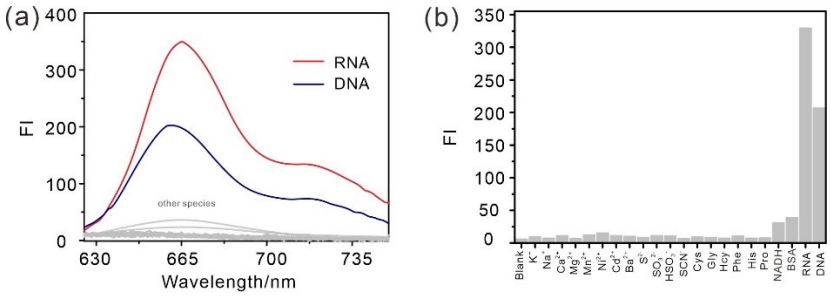

Fig. S8. Selectivity experiments of probe **1b** (10 μM) toward different analytes. Analytes: DNA (600 μg/mL), RNA (600 μg/mL), NADH (500 μg/mL), BSA (600μg/mL), 5 mM for Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Ba^{2+}; 1 mM for S^{2-}, SO_3^{2-}, HSO_3^{-}; 10 mM for K^{+}, Na^{+}, SCN^{-}, Cys, Gly, Hcy, Phe, His and Pro. (λ_{ex}=594 nm, slit widths: 3 nm/5 nm). (a)emission spectra; (b) fluorescence histogram at 660nm.

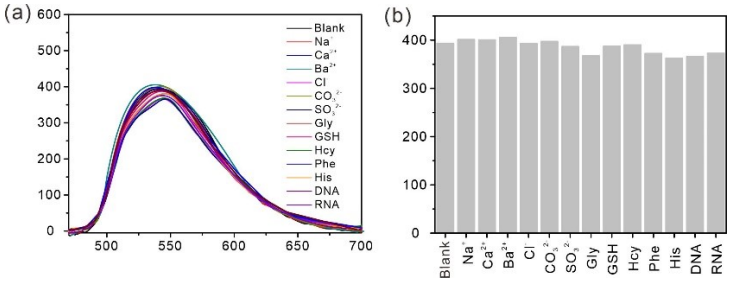

Fig. S9. Selectivity experiments of probe **1c** (10 μM) toward different analytes. Analytes: DNA (600 μg/mL), RNA (600 μg/mL), 5 mM for Ca^{2+}, Ba^{2+}; 1 mM for SO_3^{2-}, CO_3^{2-}, Cl^{-}; 10 mM for Na^{+}, Gly, GSH, Hcy, Phe and His. (λ_{ex}=384 nm, slit widths: 5 nm/5 nm). (a)emission spectra; (b) fluorescence histogram at 550nm.

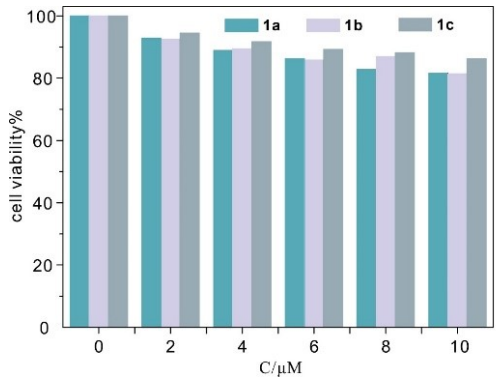

Fig. S10. HeLa cells viabilities after treatment with probes **1a-c**. Cell viability was assayed by the CCK-8 method.

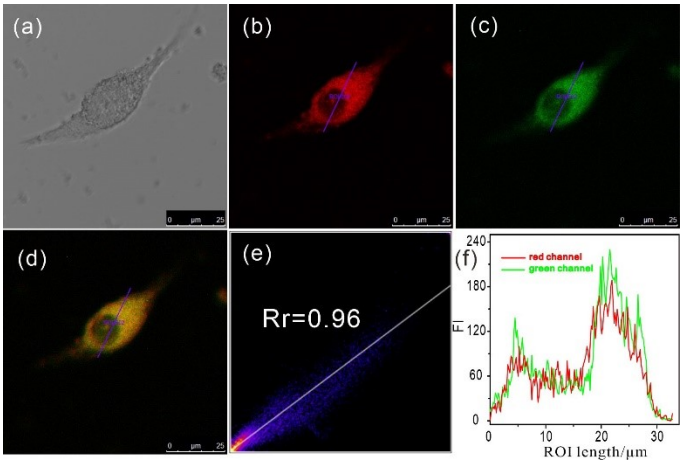

Fig. S11. Fluorescence confocal images of living HeLa cells with probe **1b** and ROI analysis: (a) bright field image; (b) confocal image (red channel) of cells with probe **1b** (5 μM); (c) confocal image (green channel) of cells with Mito-Tracker Green FM (100 nM); (d) merged image of the green and red channels; (e) fluorescence intensity correlation plot of the green and red channels; (f) fluorescence intensities of the regions of interest (ROIs) across the cells.

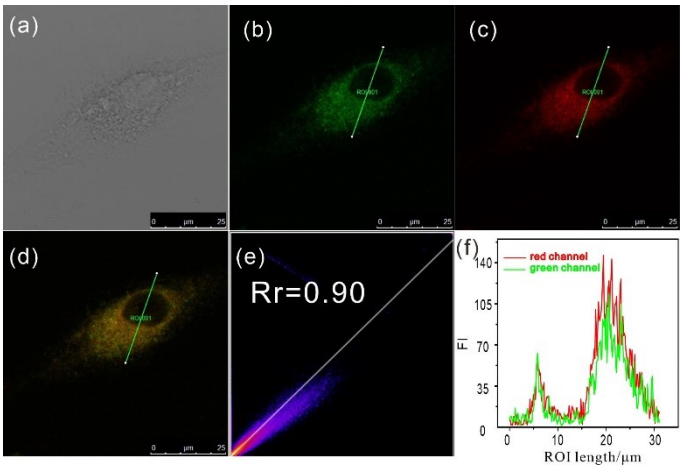

Fig. S12. Fluorescence confocal images of living HeLa cells with dye **1c** and ROI analysis: (a) bright field image; (b) confocal image (green channel) of cells with dye **1c** (5 μM); (c) confocal image (red channel) of cells with Mito-Tracker Red CMXRos (100 nM); (d) merged image of the green and red channels; (e) fluorescence intensity correlation plot of the green and red channels; (f) fluorescence intensities of the regions of interest (ROIs) across the cells.

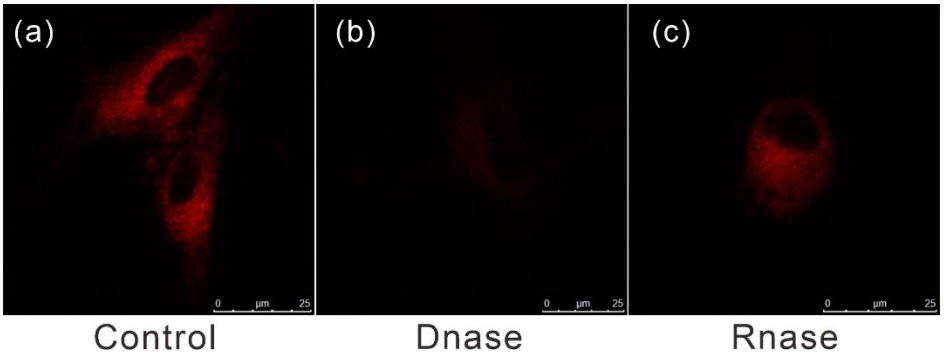

Fig. S13. Fluorescence confocal images of the digest experiment for probe **1b** (5 μM) with fixed HeLa cells. (a) Cells were incubated with **1b** in control experiments; (b) cells were incubated with **1b** and DNase (1 mg/mL); (c)cells were incubated with **1b** and RNase (10 mg/mL). Red channel emission was collected in 570–750 nm upon excitation at 561 nm.

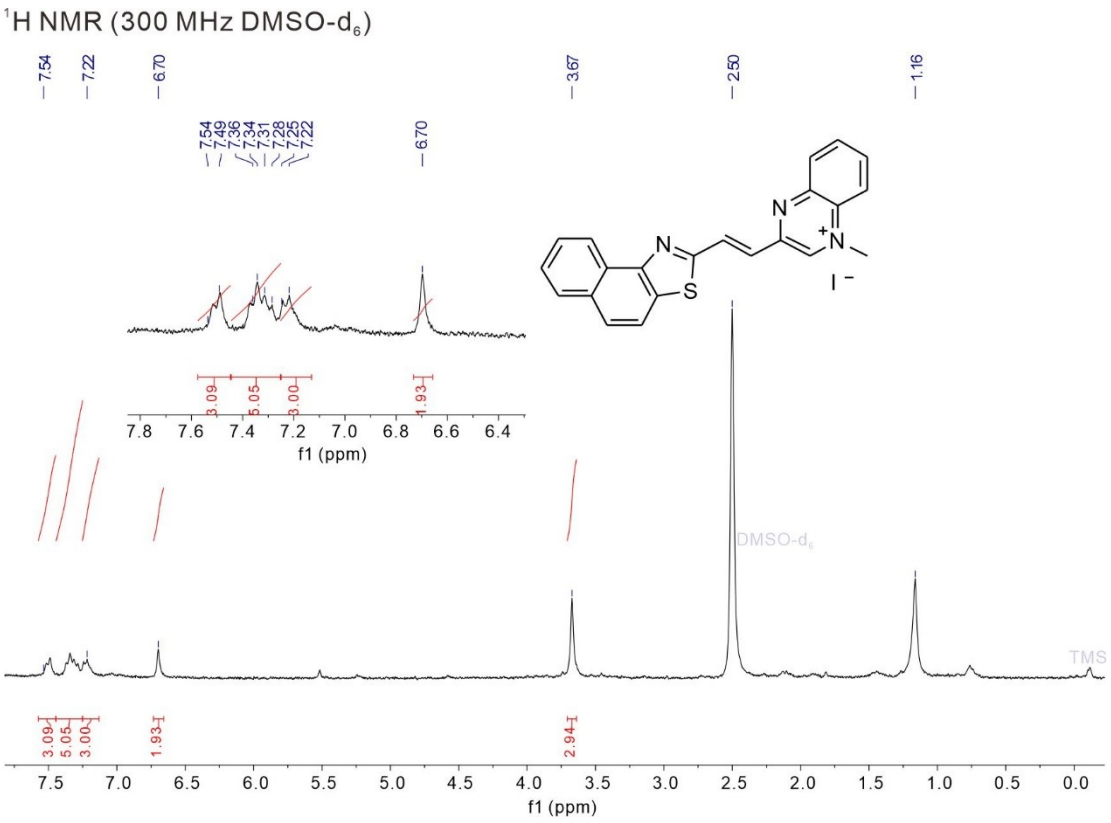

Fig. S14. ^{1}H NMR spectrum of probe **1a**.

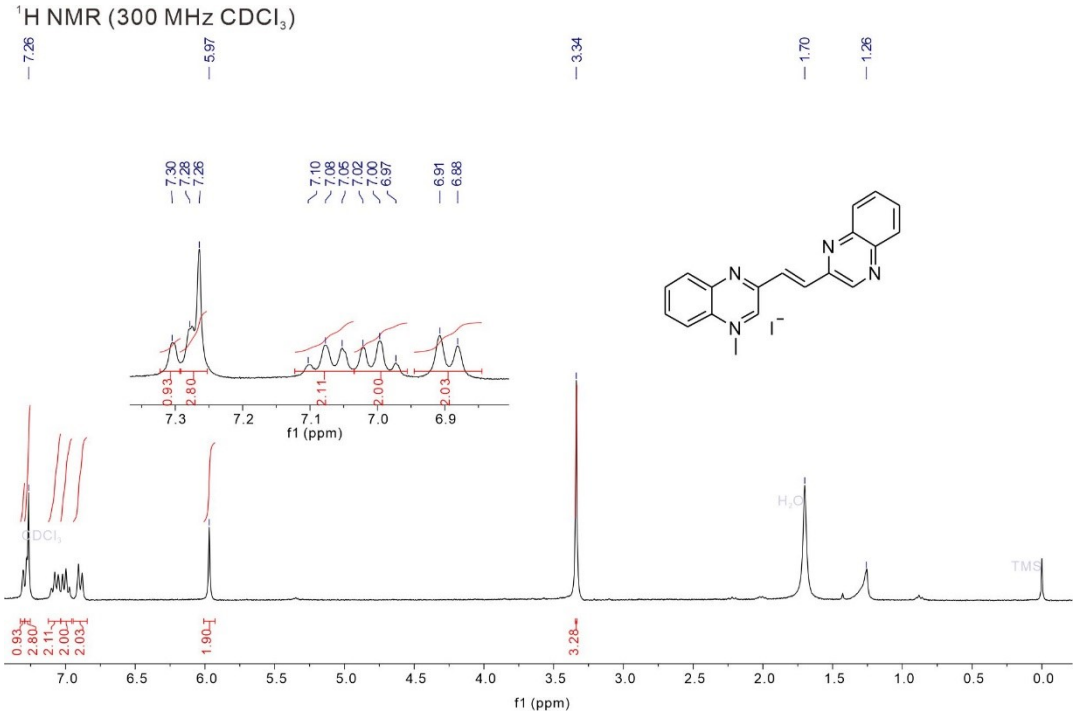

Fig. S15. ^{1}H NMR spectrum of probe **1b**.

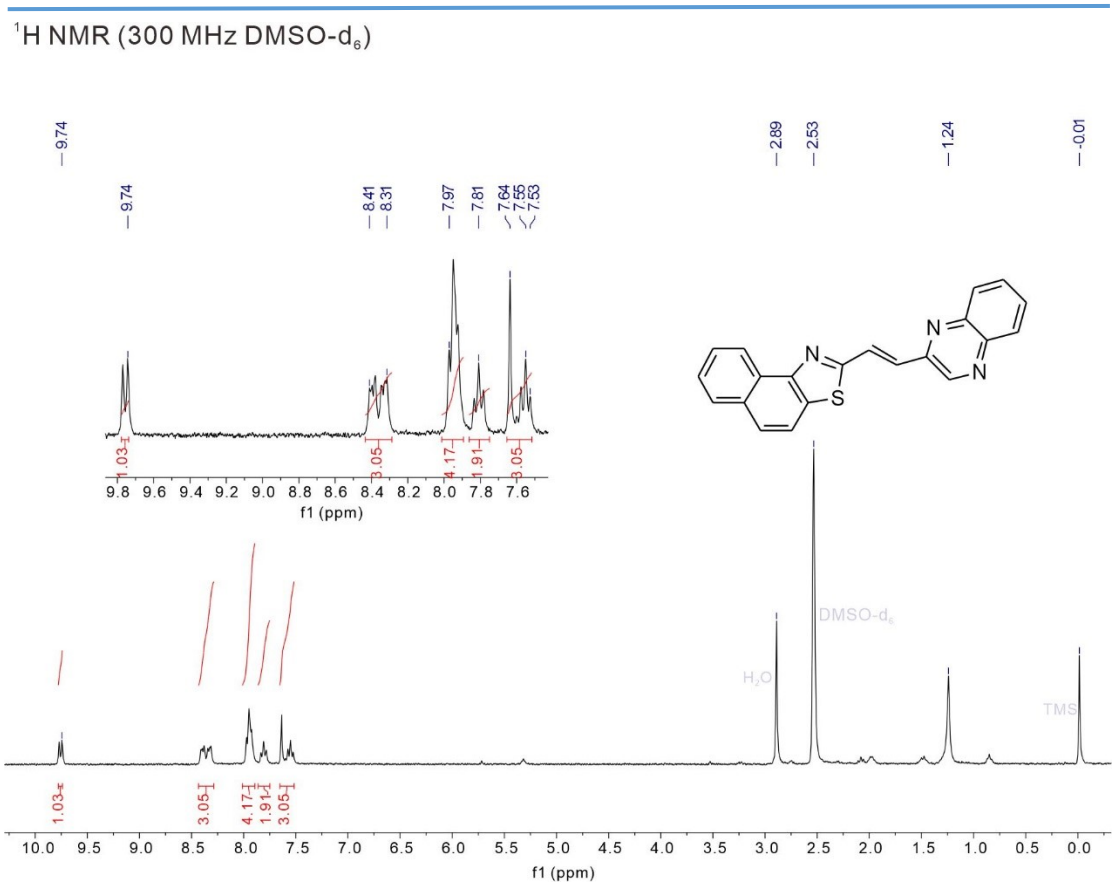

Fig. S16. ^{1}H NMR spectrum of dye **1c**.

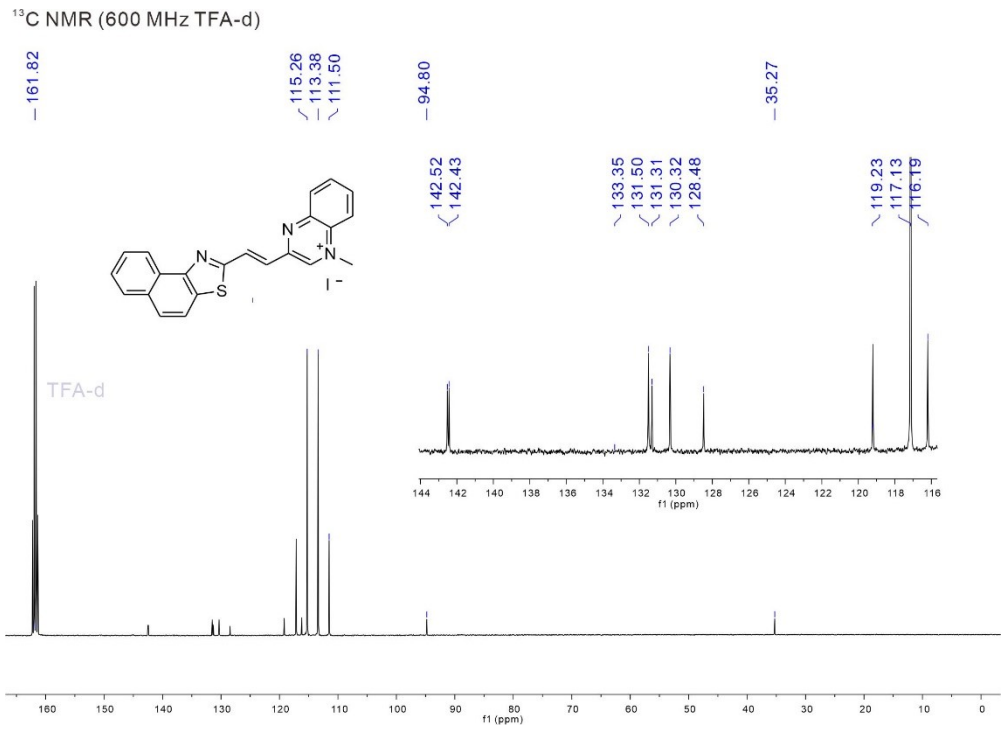

Fig. S17. ^{13}C NMR spectrum of probe **1a**.

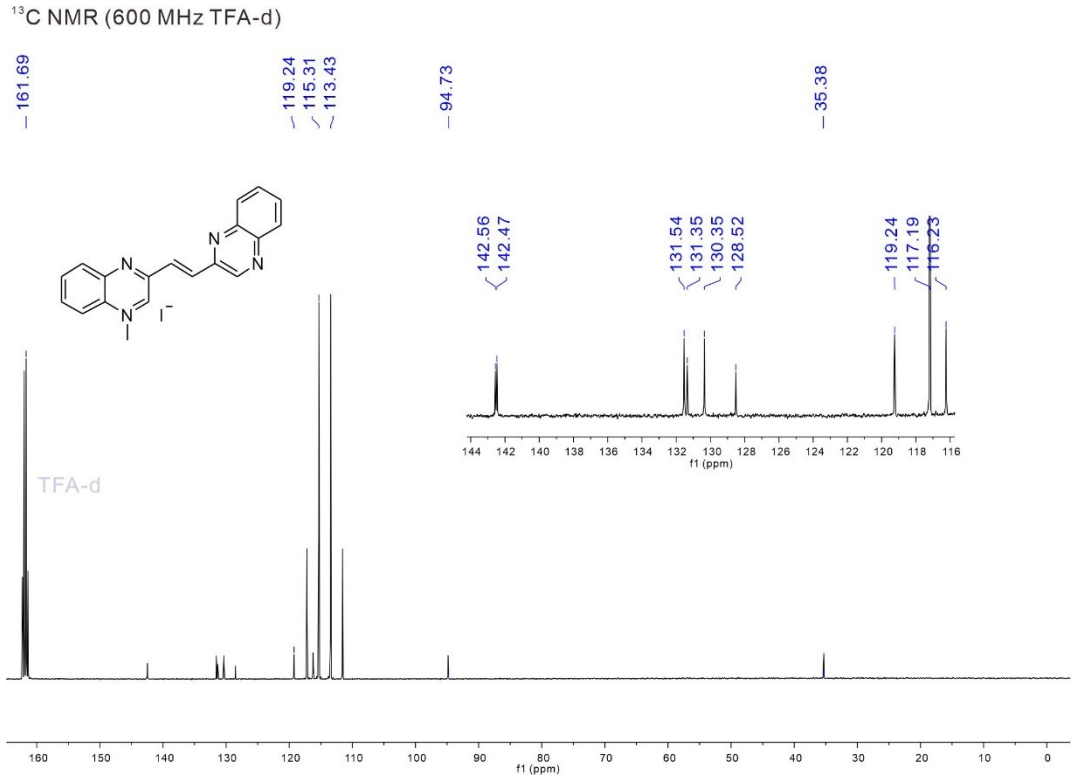

Fig. S18. ^{13}C NMR spectrum of probe **1b**.

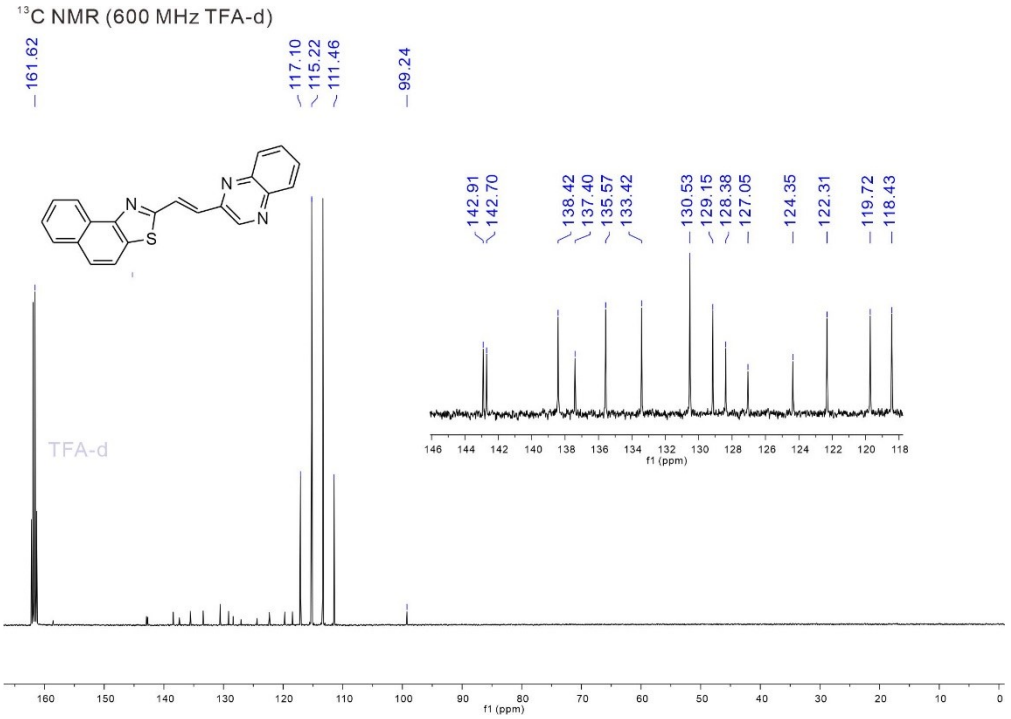

Fig. S19. ^{13}C NMR spectrum of dye **1c**.

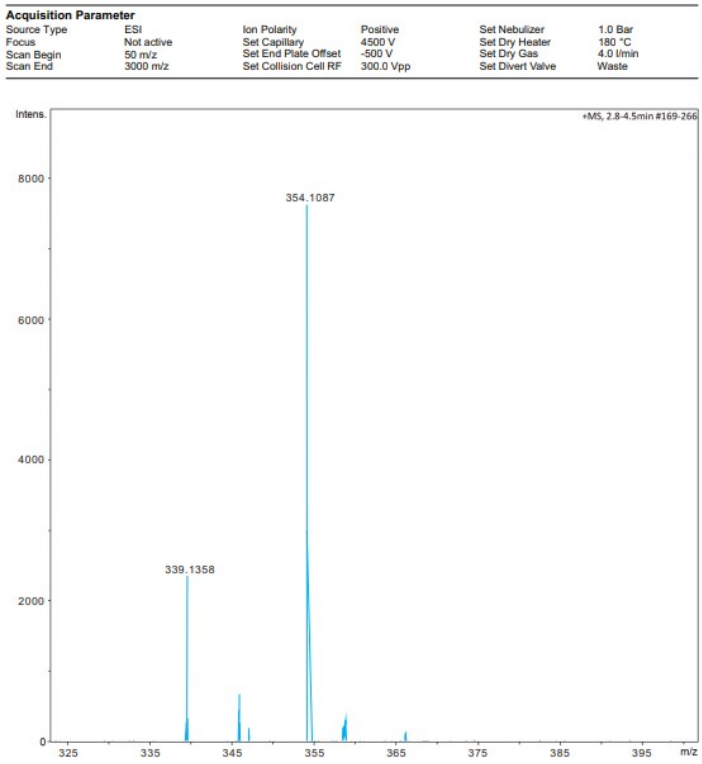

Fig. S20. HRMS(ESI$^+$) spectrum of probe **1a**.

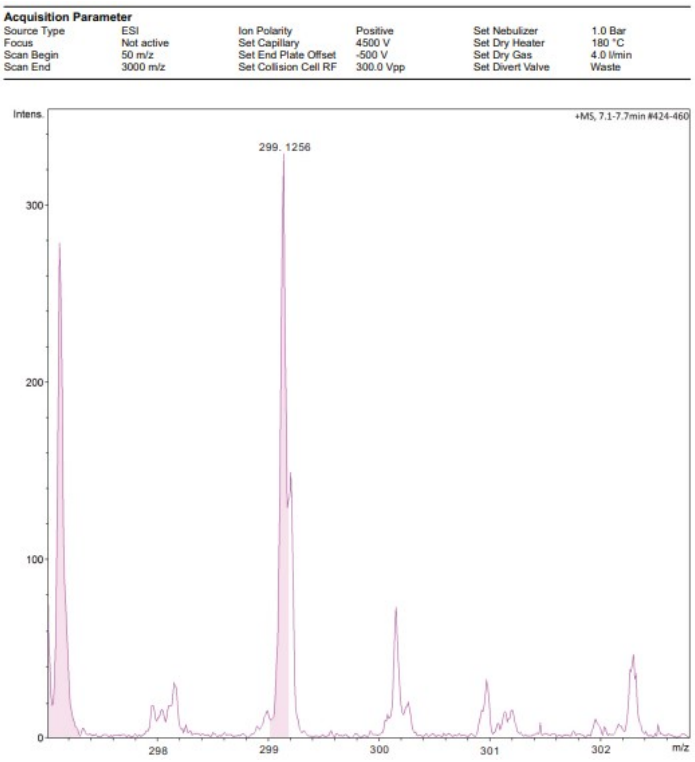

Fig. S21. HRMS(ESI$^+$) spectrum of probe **1b**.

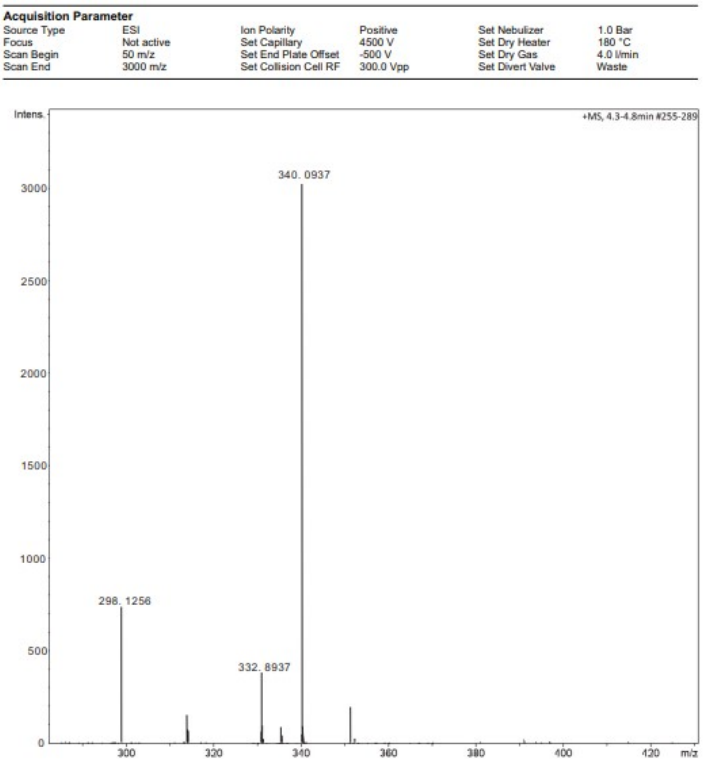

Fig. S22. HRMS(ESI$^+$) spectrum of dye **1c**.

references

1. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09, Revision A.01, Gaussian, Inc., Wallingford CT, 2009.