

Viscosity probes towards different organelles with red emission based on an identical hemicyanine structure

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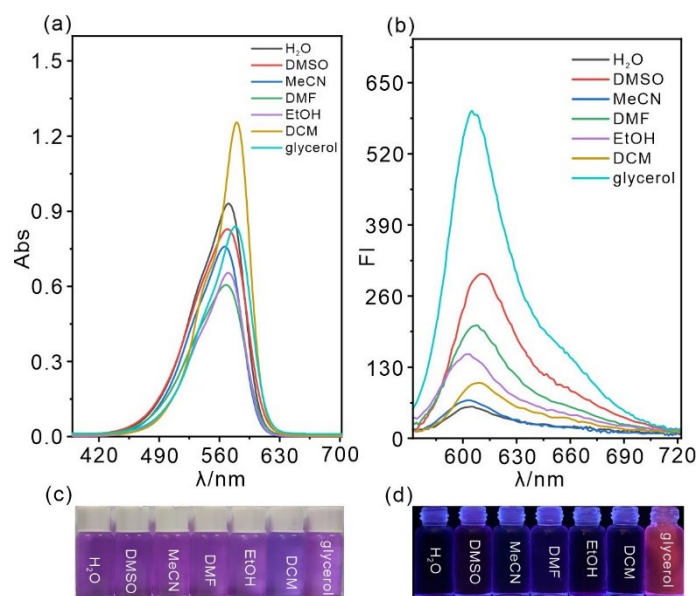


Fig. S1. Optical properties of probe **1b** (10 μ M) in solvents of different polarity. (a) UV-vis absorption spectrum. (b) Fluorescence emission spectrum, λ_{ex} = 569 nm, slit widths: 5 nm / 3 nm. (c) Photographs under daylight. (d) Photographs under a lamp at 365 nm in dark conditions.

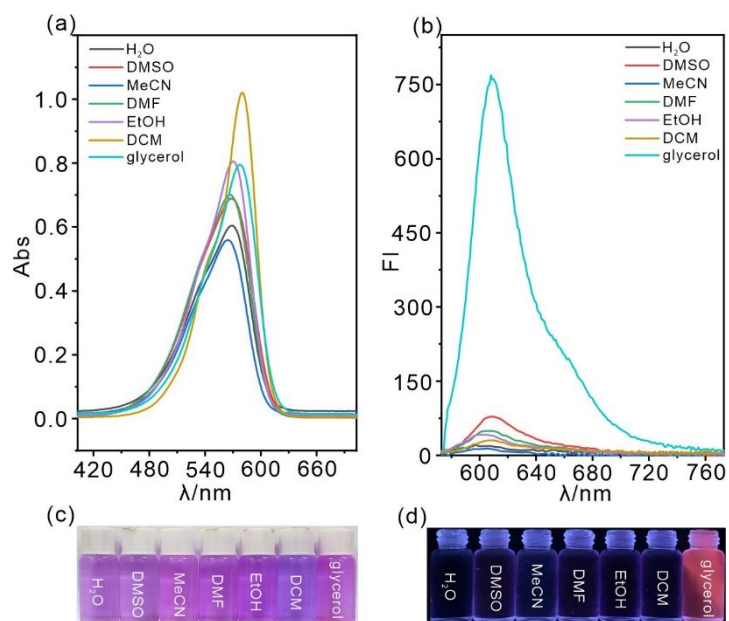


Fig. S2. Optical properties of probe **1c** (10 μ M) in solvents of different polarity. (a) UV-vis absorption spectrum. (b) Fluorescence emission spectrum, λ_{ex} = 568 nm, slit widths: 3 nm / 3 nm. (c) Photographs under daylight. (d) Photographs under a lamp at 365 nm in dark conditions.

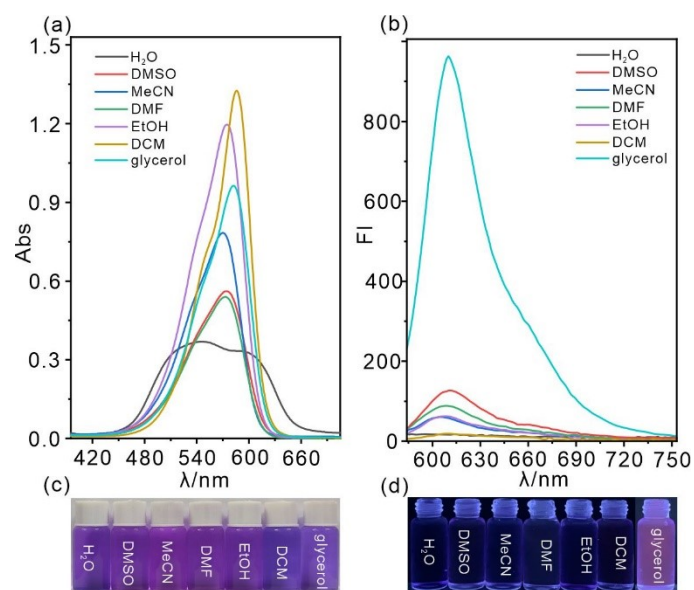


Fig. S3. Optical properties of probe **1d** (10 μ M) in solvents of different polarity. (a) UV-vis absorption spectrum. (b) Fluorescence emission spectrum, $\lambda_{\text{ex}} = 573$ nm, slit widths: 5 nm / 5 nm. (c) Photographs under daylight. (d) Photographs under a lamp at 365 nm in dark conditions.

Table S1. Optical properties of probes **1a-d** in different solvents.

Probe	Solvents	$\lambda_{\text{Abs,max}}^{\text{a}}$	$\lambda_{\text{Em,max}}^{\text{a}}$	ε^{b}	Φ^{c}
1a	H ₂ O	576	602	1.95	0.2
1a	DCM	584	611	6.77	0.6
1a	Glycerol	582	613	9.42	36.7
1b	H ₂ O	570	601	9.29	0.7
1b	DCM	579	610	12.5	1.5
1b	Glycerol	577	606	8.38	24.3
1c	H ₂ O	569	596	6.04	0.2
1c	DCM	582	609	9.73	5.42
1c	Glycerol	577	610	7.97	27.1
1d	H ₂ O	574	606	3.35	0.3
1d	DCM	586	608	13.2	0.3
1d	Glycerol	582	609	9.63	42.6

^a Reported in nm.

^b Reported in $10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

^c Reported in %. Cresyl violet ($\Phi = 0.578$ in ethanol) was used as the reference compound.

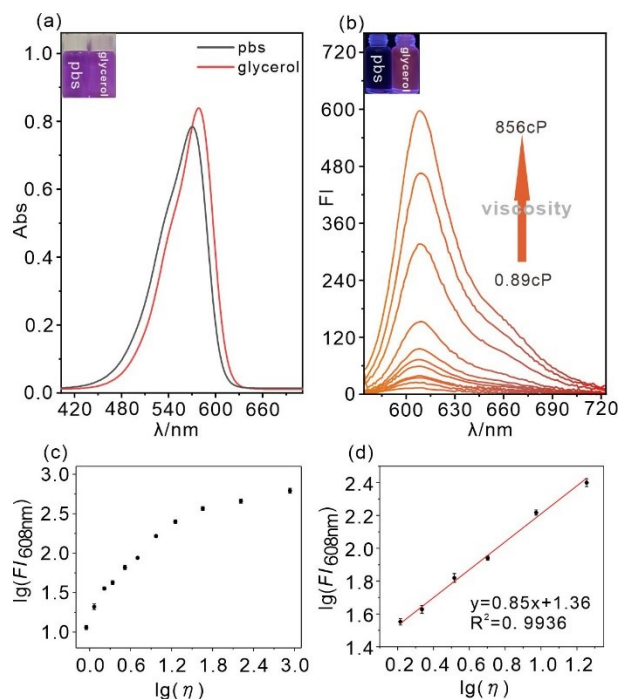


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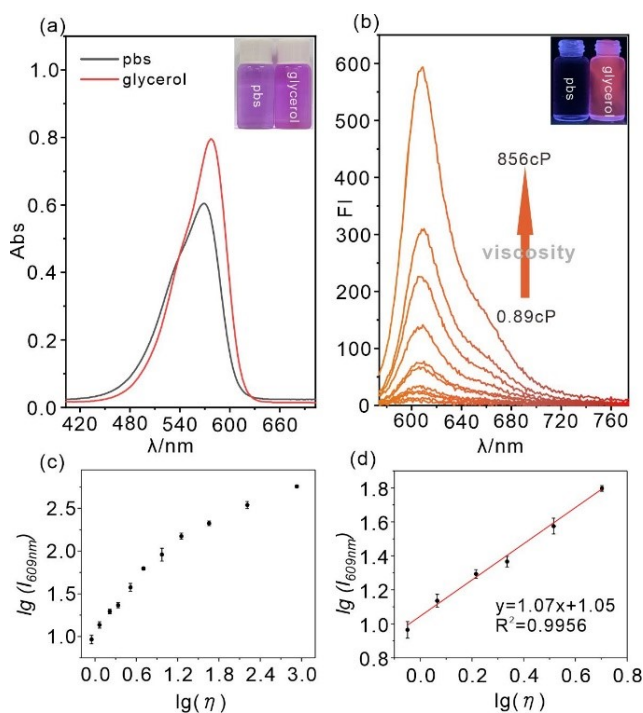


Fig. S5. Viscosity response of probe **1c** (10 μ M) in different ratios of PBS-glycerol mixtures (0.89-856 cP). (a) Absorption spectra. (b) Fluorescence spectra. (c) Relationship between $\lg(FI_{609nm})$ and $\lg(\eta)$. (d) Linear relationship between $\lg(FI_{609nm})$ and $\lg(\eta)$. The data were shown as mean $\pm$ SD ($n = 3$).

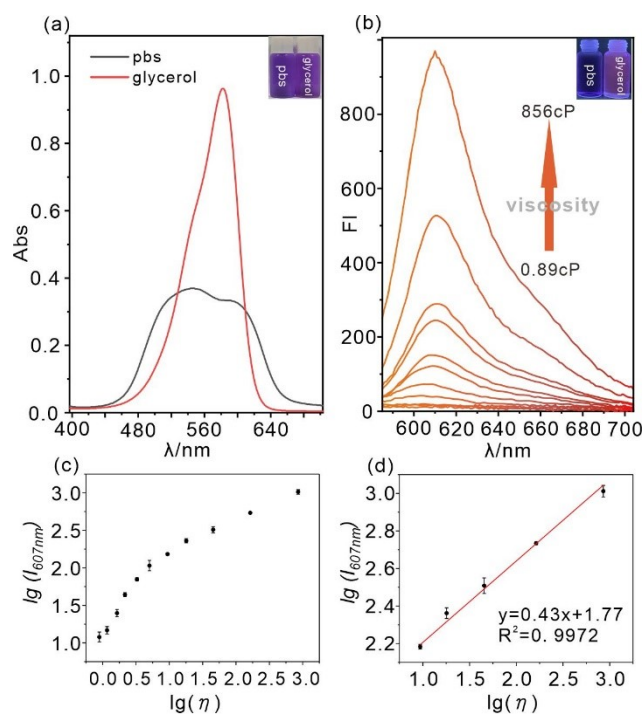


Fig. S6. Viscosity response of probe **1d** (10 μ M) in different ratios of PBS-glycerol mixtures (0.89-856 cP). (a) Absorption spectra. (b) Fluorescence spectra. (c) Relationship between $\lg(FI_{607nm})$ and $\lg(\eta)$. (d) Linear relationship between $\lg(FI_{607nm})$ and $\lg(\eta)$. The data were shown as mean $\pm$ SD ($n = 3$).

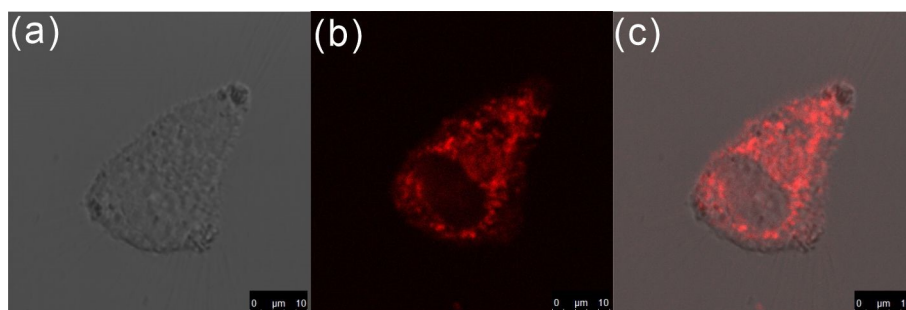


Fig. S7. Fluorescence images of HeLa cells with probes **1d** (10 μ M). (a) Brightfield images of HeLa cells. (b) Cells with probe **1d** in red channel. (c) Merged images of (a) and (b).

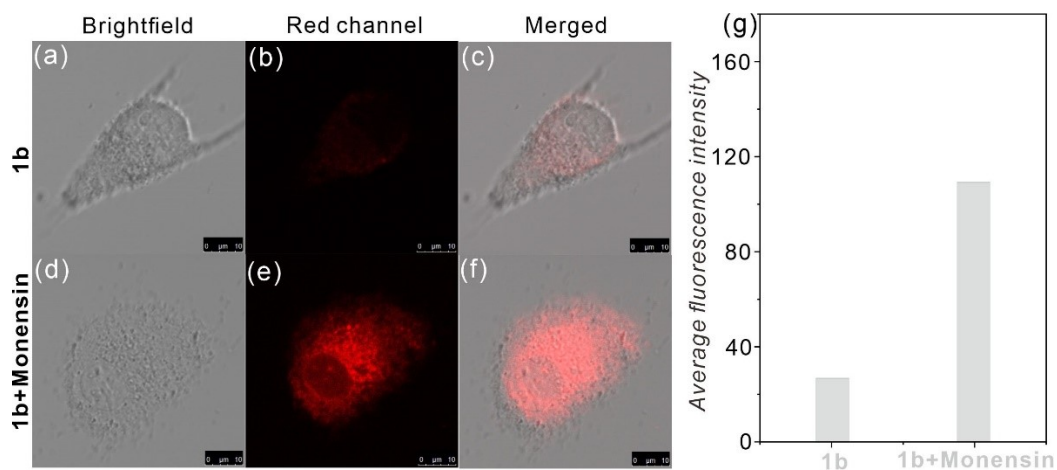


Fig. S8. Fluorescence images for viscosity detection in HeLa cells: (a-c) HeLa cells incubated with only probe **1b** (4 μ M) for 20 min; (d-f) HeLa cells treated with monensin (10 μ M) for 30 min and probe **1b** (4 μ M) for another 20 min; (g) the average fluorescence intensity of probe **1b** in HeLa cells in the presence or absence of monensin.

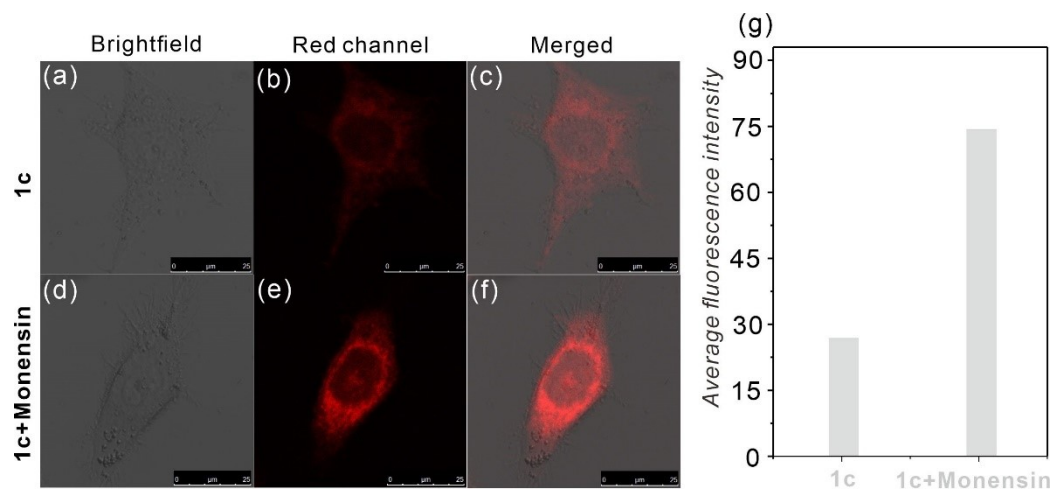


Fig. S9. Fluorescence images for viscosity detection in HeLa cells: (a-c) HeLa cells incubated with only probe **1c** (4 μ M) for 20 min; (d-f) HeLa cells treated with monensin (10 μ M) for 30 min and probe **1c** (4 μ M) for another 20 min; (g) the average fluorescence intensity of probe **1c** in HeLa cells in the presence or absence of monensin.

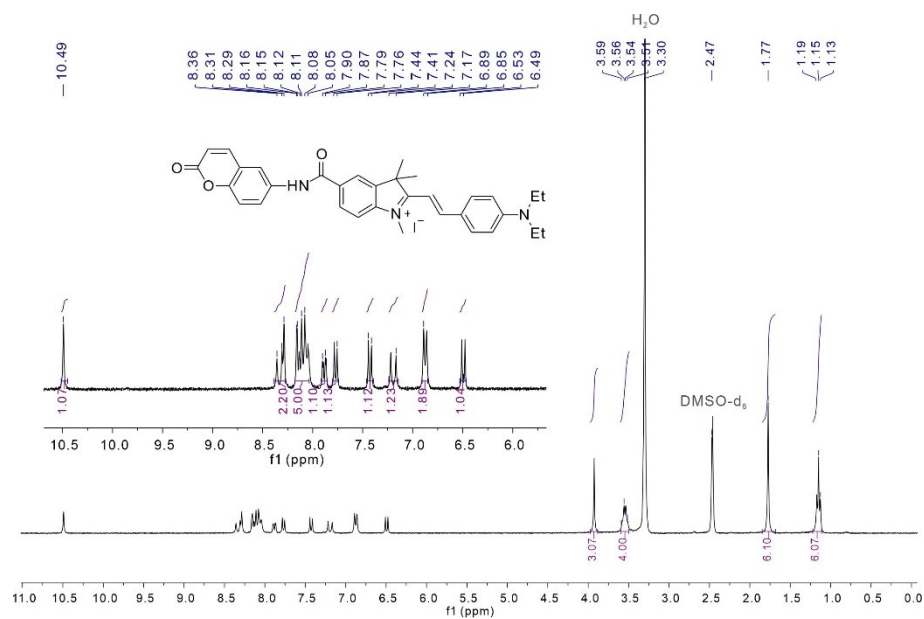


Fig. S10. ¹H NMR spectrum of probe 1a.

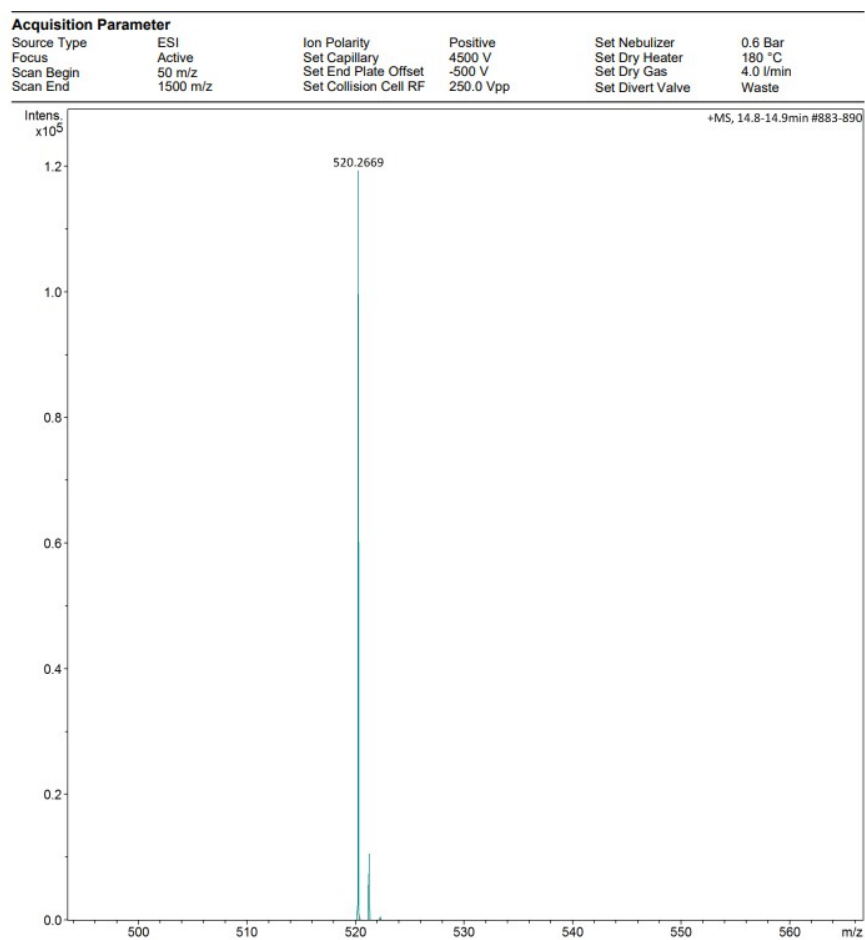


Fig. S11 HRMS(ESI⁺) spectrum of probe 1a.

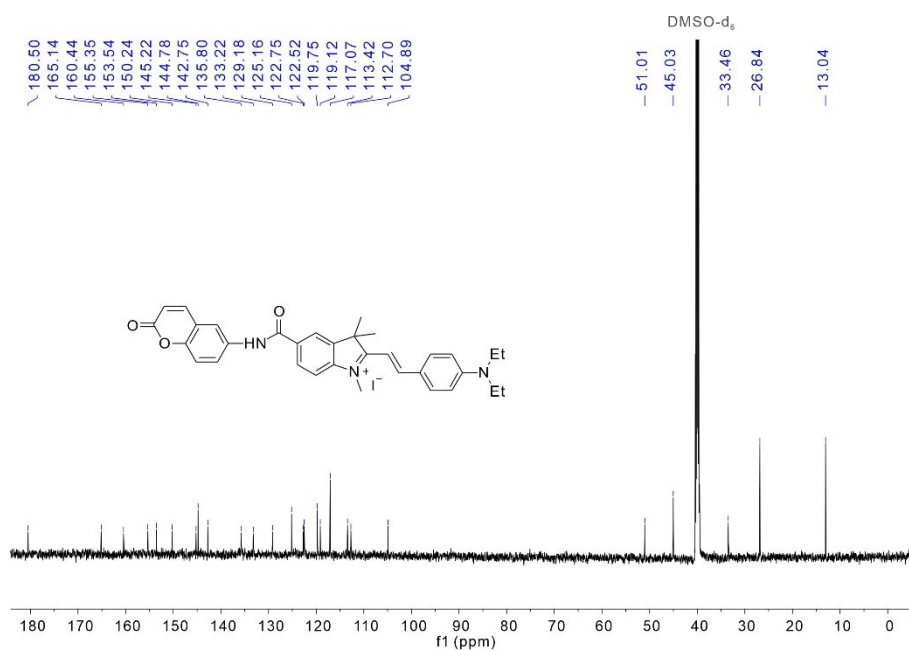


Fig. S12 ¹³C NMR spectrum of probe 1a.

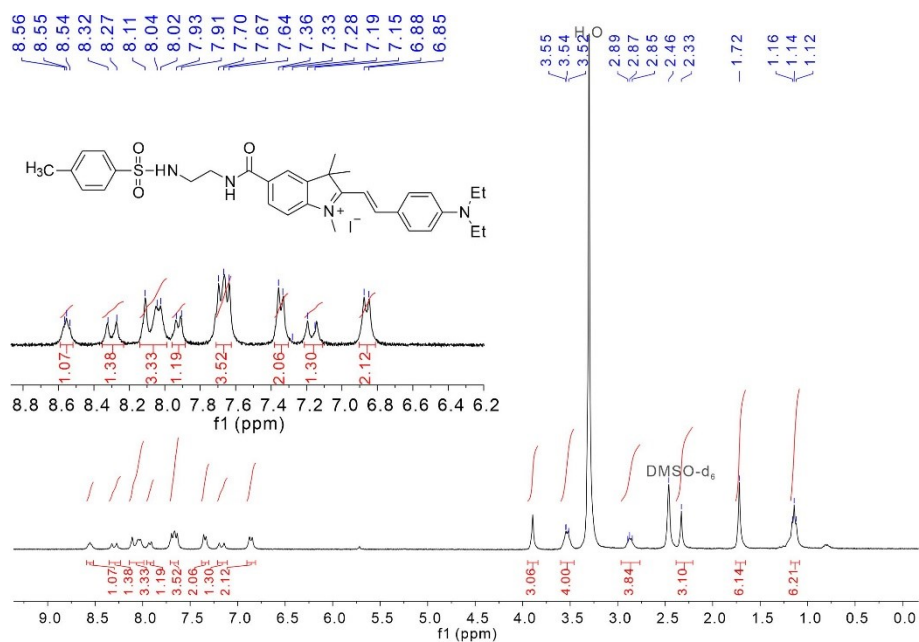


Fig. S13. ¹H NMR spectrum of probe 1b.

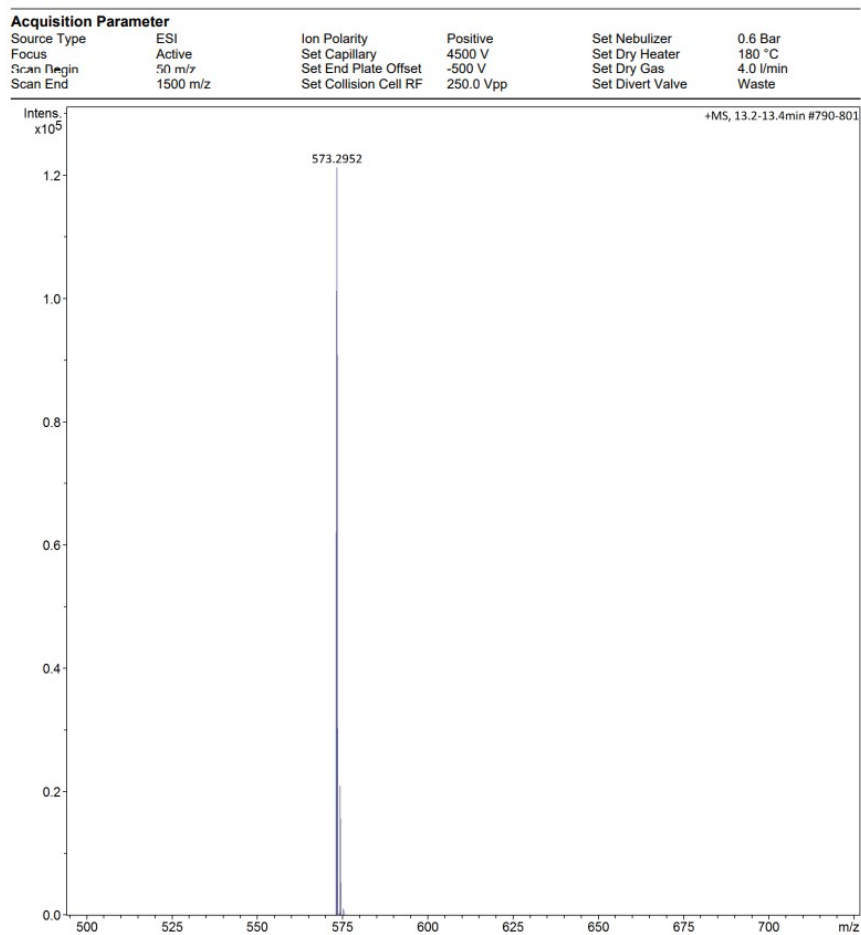


Fig. S14 HRMS(ESI⁺) spectrum of probe **1b**.

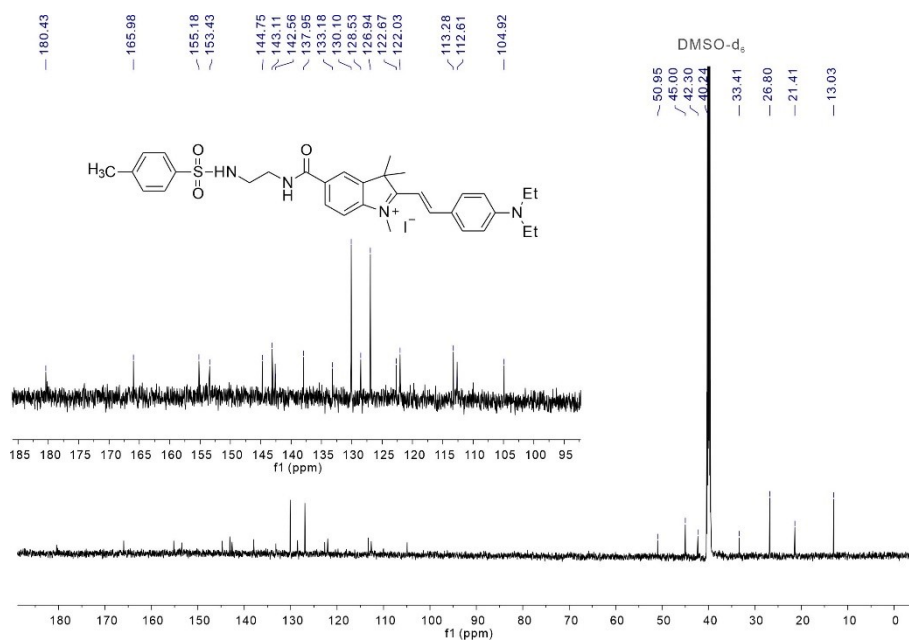


Fig. S15 ¹³C NMR spectrum of probe **1b**.

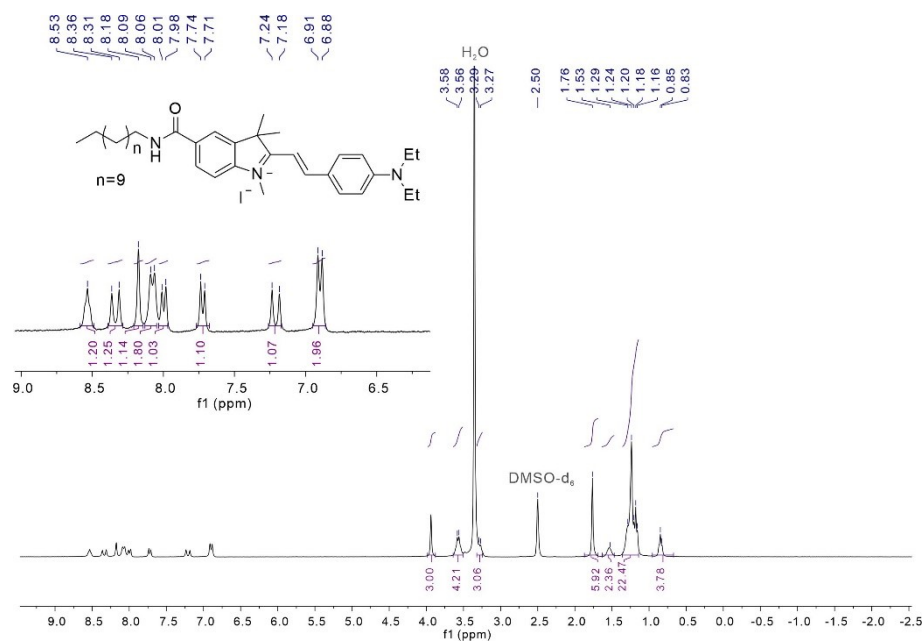


Fig. S16. ¹H NMR spectrum of probe 1c.

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.6 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	250.0 Vpp	Set Divert Valve	Waste

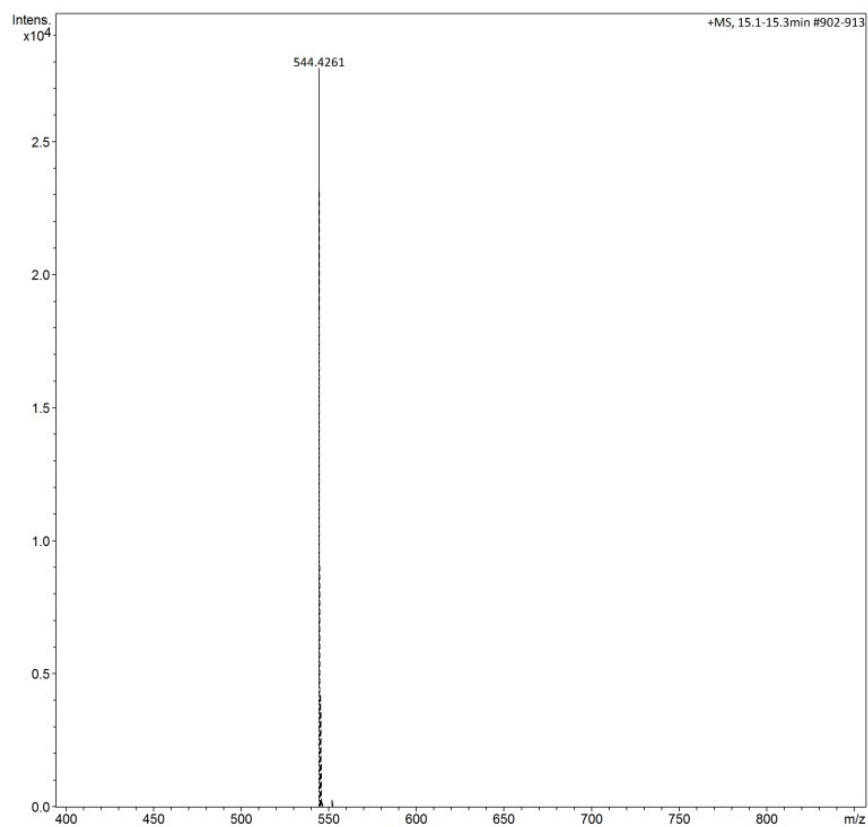


Fig. S17 HRMS(ESI⁺) spectrum of probe 1c.

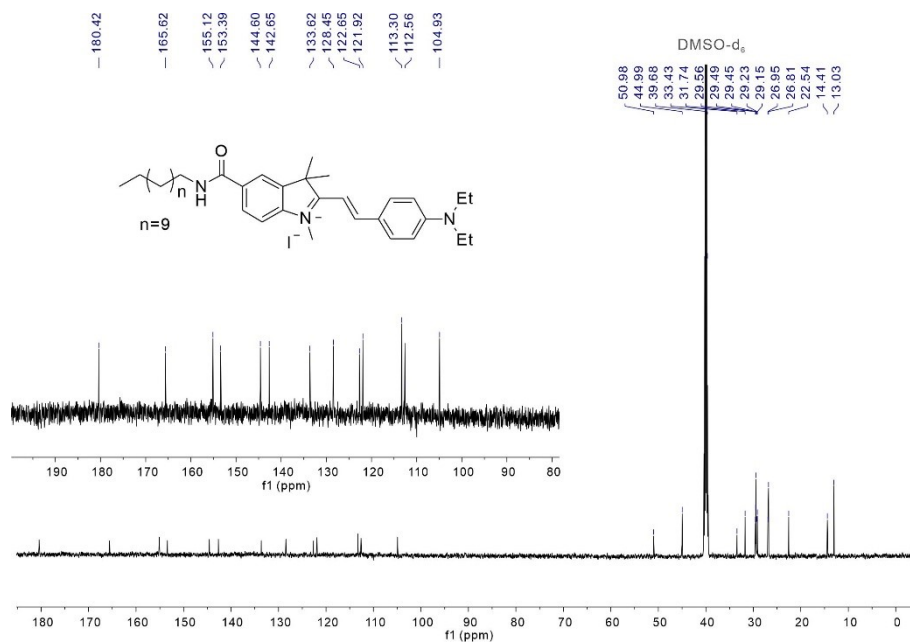


Fig. S18 ^{13}C NMR spectrum of probe 1c.

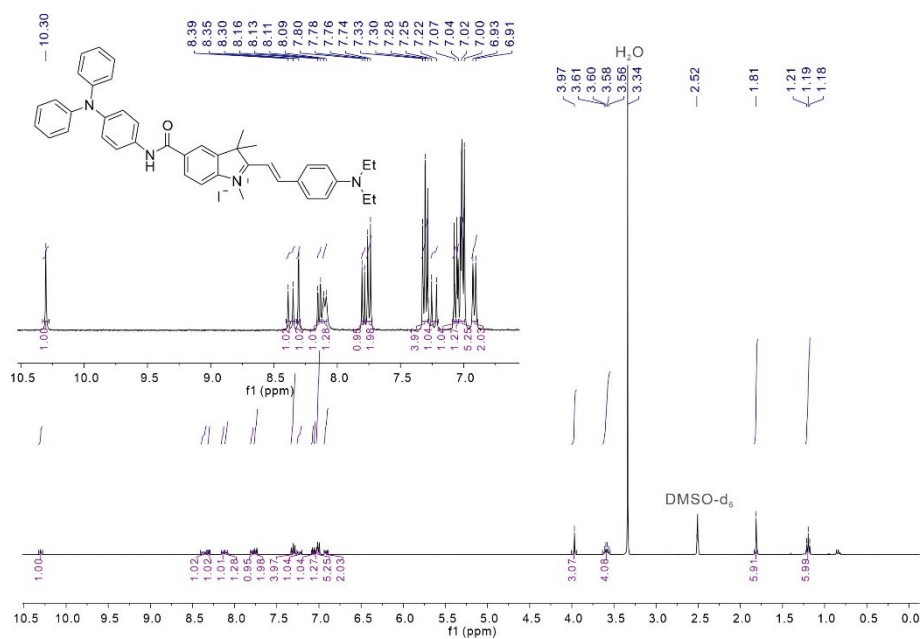


Fig. S19. ^1H NMR spectrum of probe 1d.

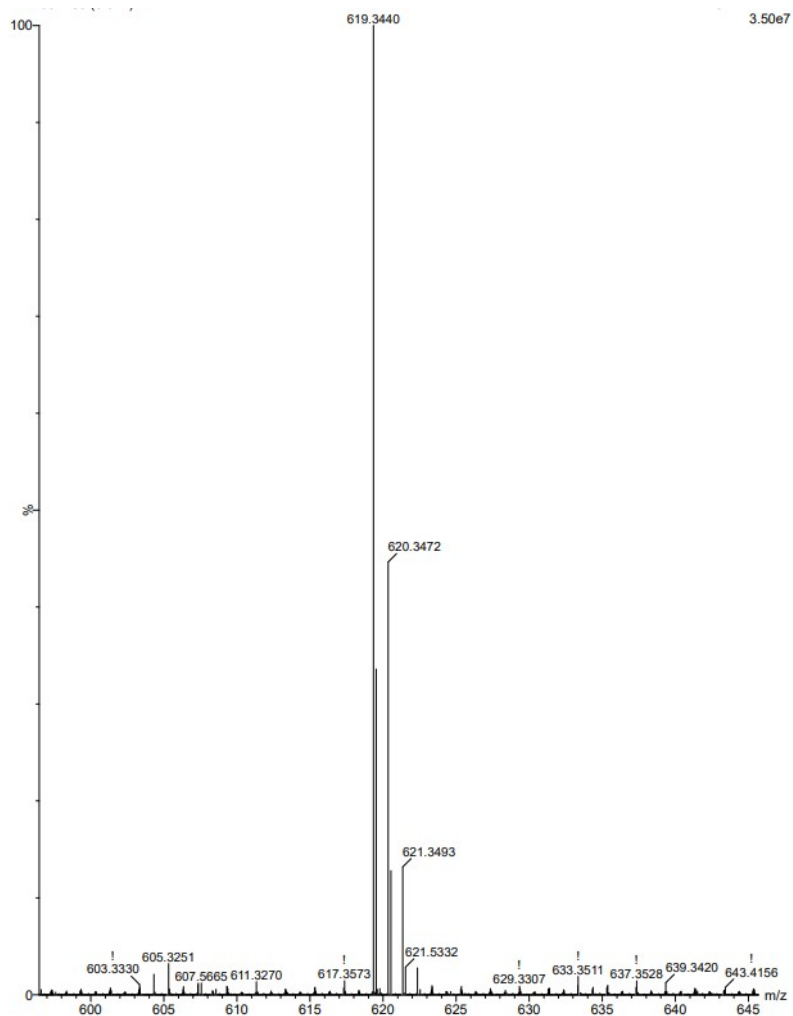


Fig. S20 HRMS(ESI⁺) spectrum of probe **1d**.

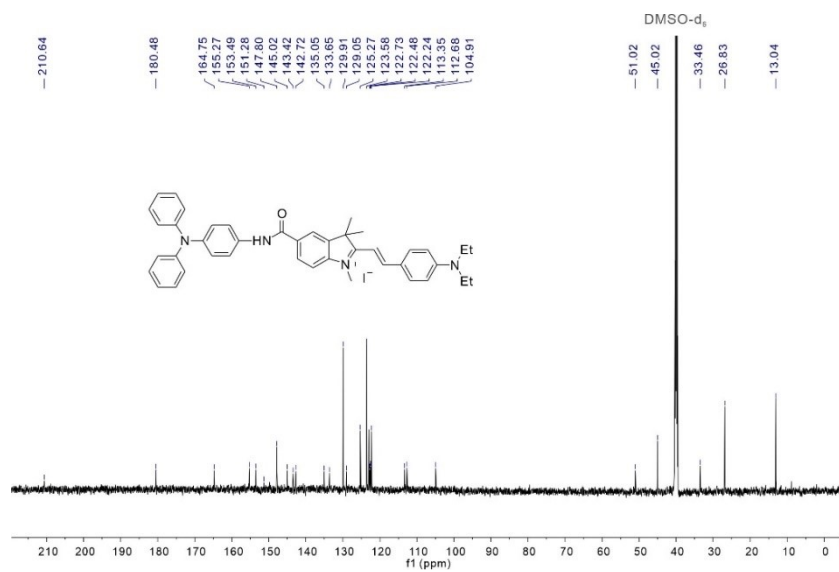


Fig. S21 ¹³C NMR spectrum of probe **1d**.