

Supporting Information

**New β -diketone-boron difluoride based near-infrared
fluorescent probes for polarity detection**

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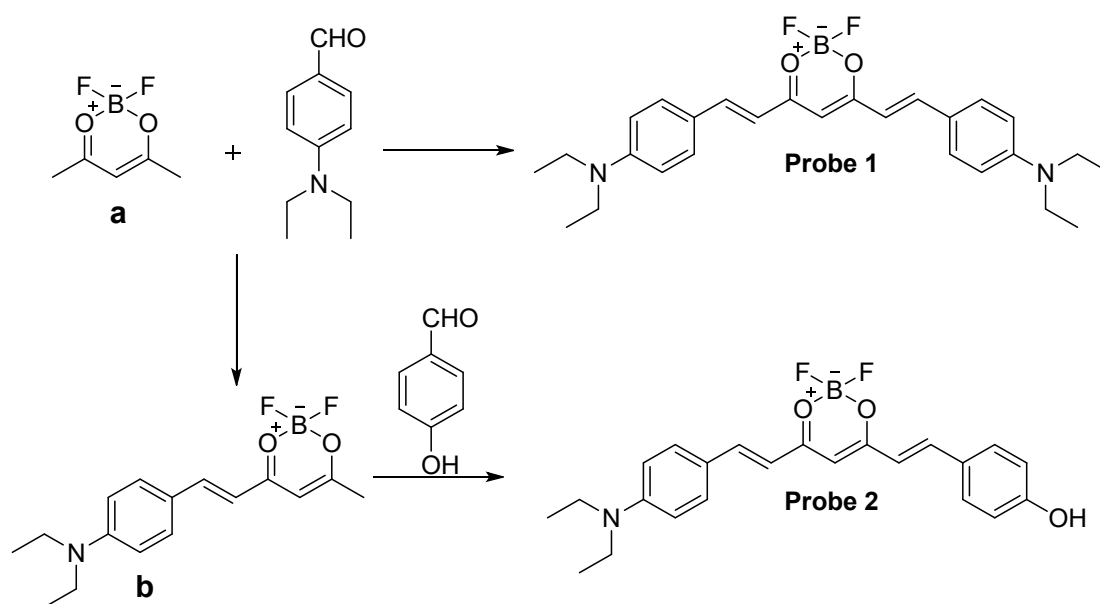
Experimental

Materials and instruments

All reagents for synthesis and test were purchased from commercial sources and used without further purification. The experimental water was deionized water and the inorganic salts used were analytical grade. The fluorescence and absorption spectra were recorded with F-380A fluorescence spectrometer and the UV-2450 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were collected on Bruker Avance III 400 NMR spectrometer.

Synthesis

The facile synthetic procedures of probes **1** and **2** are outlined in the Scheme S1. The dimethyldiketone boron complex (compound **a**) was obtained according to a previous literature.¹



Scheme S1 The synthetic procedures of probes **1** and **2**.

Synthesis of probe 1

Compound **a** (0.74 g, 5.0 mmol) and 4-diethylaminobenzaldehyde (1.77 g, 10 mmol) were dissolved in 20 mL ethyl acetate at 0 °C, then *n*-butylamine was added. The mixture was stirred for 24 h and quenched with 20 mL water. After extracted with

ethyl acetate, the crude product was concentrated and further purified by column chromatography with dichloromethane: petroleum ether (3:2, V/V) as the eluent. Finally, probe **1** was obtained as a blue violet solid with a yield of 58 %. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 1.13 (t, J = 8.0 Hz, 12H), 3.34 (q, J = 8.0 Hz, 8H), 5.78 (s, 1H), 6.31 (d, J = 16.0 Hz, 2H), 6.55 (d, J = 8.0 Hz, 4H), 7.37 (d, J = 8.0 Hz, 4H), 7.81 (d, J = 16.0 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 11.6, 43.6, 99.8, 110.4, 113.3, 120.7, 130.7, 145.5, 149.4, 176.4. HRMS m/z calcd. for C₂₇H₃₃BF₂N₂O₂ [M⁺H⁺]: 467.2680, found 467.2617.

Synthesis of probe 2

Synthesis of compound b

Compound **a** (0.74 g, 5.0 mmol) and 4-diethylaminobenzaldehyde (0.89 g, 5.0 mmol) were dissolved in 15 mL ethyl acetate at 0 °C, then n-butylamine was added. The mixture was stirred for 10 h and quenched with 15 mL water. After extracted with ethyl acetate, the crude product was concentrated and further purified by column chromatography with dichloromethane: petroleum ether (3:2, V/V) as the eluent. Finally, compound **b** was obtained as a brick-red solid with a yield of 61 %. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.17 (t, J = 8.0 Hz, 6H), 2.30 (s, 3H), 3.50 (q, J = 8.0 Hz, 4H), 6.32 (s, 1H), 6.73 (d, J = 16.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.0 Hz, 2H), 8.02 (d, J = 16.0 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 12.9, 23.9, 44.6, 100.3, 112.1, 112.7, 121.2, 133.5, 150.3, 151.7, 180.5, 186.5.

Synthesis of probe 2

Compound **b** (0.60 g, 2.0 mmol) and 4-hydroxybenzaldehyde (0.31 g, 2.5 mmol) were dissolved in 15 mL acetonitrile at 25 °C, then n-butylamine was added. The mixture was stirred for 20 h and concentrated. The crude product was further purified by column chromatography with dichloromethane: petroleum ether (3:2, V/V) as the eluent. Probe **2** as a blue violet solid was obtained with a yield of 46 %. ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.20 (t, J = 8.0 Hz, 6H), 3.52 (q, J = 8.0 Hz, 4H), 6.40 (s, 1H), 6.82 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 16.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 2H), 6.97 (d, J = 16.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 4H), 7.86 (d, J = 16.0 Hz, 1H), 7.95 (d, J = 16.0 Hz, 1H), 10.42 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm): 13.0, 44.6,

101.4, 112.1, 113.9, 116.6, 118.5, 121.5, 126.1, 132.0, 133.3, 144.7, 148.5, 151.5, 161.5, 176.4, 179.0. HRMS m/z calcd. for $C_{23}H_{24}BF_2NO_3$ $[M^+H^+]$: 412.1894, found 412.1847.

Spectral measurement

The initial solution of probes **1** or **2** was dissolved in corresponding solvents. For the selectivity experiments, interfering substances were prepared in deionized water. For the spectra measurement, the stock solution was dissolved to 10 μ M in different solvents. Subsequently, the solution was detected in a fluorescence spectrophotometer. The excitation and emission slits were set at 5.0 nm and 5.0 nm, respectively. The excitation wavelength was 590 nm or 550 nm for probes **1** and **2**, respectively.

Cell culture and imaging

Hepal-6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen), 1.0 % penicillin and 1.0 % streptomycin. The cells were seeded in confocal culture dishes and then incubated for 24 h at 37 °C under a humidified atmosphere containing 5.0 % CO_2 . For colocalization cell imaging experiments, the cells were seeded onto glass plates for 24 h for adherence, then washed by PBS and incubated with the probe **1** and Nile Red (the commercial dye for lipid droplets) before fluorescence imaging. To test the cytotoxicity of **1**, the cell viability of cells was determined by MTT assay.

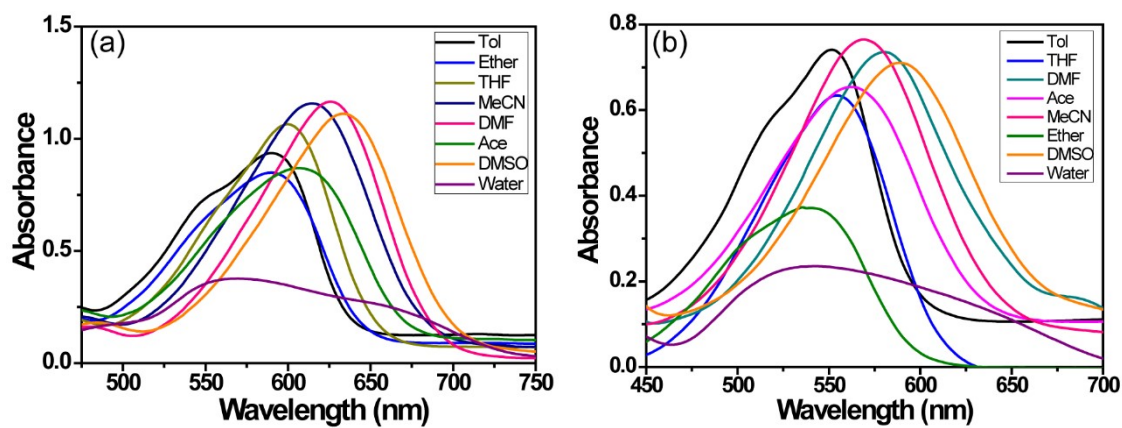


Figure S1 (a) The absorption spectra of probe **1** (10 μ M) in common solvents with different polarities. (b) The absorption spectra of probe **2** (10 μ M) in common solvents with different polarities.

Table S1 The photophysical properties of probe **1** in different solvents.

Solvent	polarity	$\lambda_{\text{abs}}^{\text{a}}$	$\lambda_{\text{em}}^{\text{b}}$	Stokes shift (nm)	ϵ^{c} ($\times 10^4$)	Φ^{d}
Tol	2.4	590	640	50	9.4	0.345
Ether	2.9	592	643	51	8.5	0.335
THF	4.2	600	652	52	10.7	0.290
Ace	5.4	606	680	74	8.7	0.062
MeCN	6.2	614	709	95	11.6	0.060
DMF	6.4	619	716	97	11.7	0.050
DMSO	7.2	623	721	98	11.1	0.032
Water	10.2	569	735	166	3.8	< 0.01

a: Maximum absorption wavelength (nm);

b: Maximum emission wavelength (nm);

c: molar absorption coefficient ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$);

d: fluorescence quantum yield.

Table S2 The photophysical properties of probe **2** in different solvents.

Solvent	polarity	$\lambda_{\text{abs}}^{\text{a}}$	$\lambda_{\text{em}}^{\text{b}}$	Stokes Shift (nm)	ϵ^{c} ($\times 10^4$)	Φ^{d}
Tol	2.4	544	631	87	7.4	0.339
Ether	2.9	536	643	107	3.7	0.166
THF	4.2	555	666	111	6.3	0.060
Ace	5.4	562	693	131	6.5	0.016
MeCN	6.2	569	704	135	7.7	0.014
DMF	6.4	580	717	137	7.4	0.011
DMSO	7.2	588	731	143	7.1	0.020
Water	10.2	541	--- ^e	--- ^e	2.3	< 0.01

a: Maximum absorption wavelength (nm);

b: Maximum emission wavelength (nm);

c: molar absorption coefficient ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$);

d: fluorescence quantum yield;

e: Not detected.

Table S3 Performances of some representative polarity-sensitive probes

Probes	NIR ^a	ε_{\max}^b ($\times 10^4$)	Stokes shift ^c	Application	Ref.
1	Yes	11.7	166	HEPA1-6 cells	This work
2	Yes	7.7	143	HEPA1-6 cells	This work
CTPE	NO	-- ^d	97	HeLa cells and tumor-bearing mice	2
NCT	NO	3.02	130	tumor bearing mouse	3
Lyso-OSC	NO	-- ^d	155	MCF-7 cells	4
Lyso-NA	NO	-- ^d	154	MCF-7 cells	5
PX-P	NO	5.16	169	HL-7702 cells and NAFLD mice	6
PEG-NBD	NO	-- ^d	57	mouse mammary tumor and zebrafish	7

a: The near-infrared fluorescence emission;

b: The maximum molar absorption coefficient ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$);

c: The maximum Stokes shift (nm);

d: Not mentioned.

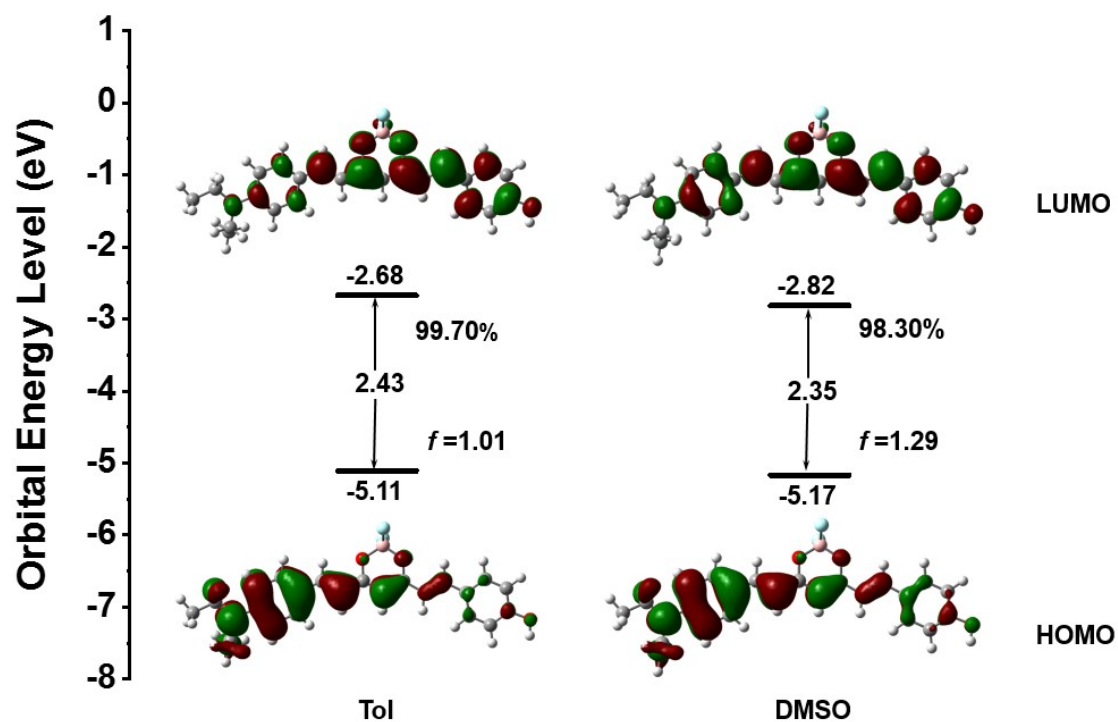


Figure S2 Calculated orbital energy levels and electron density contours of HOMOs and LUMOs for probe **2** in Tol and DMSO, respectively. Transition components and oscillator strengths are also given.

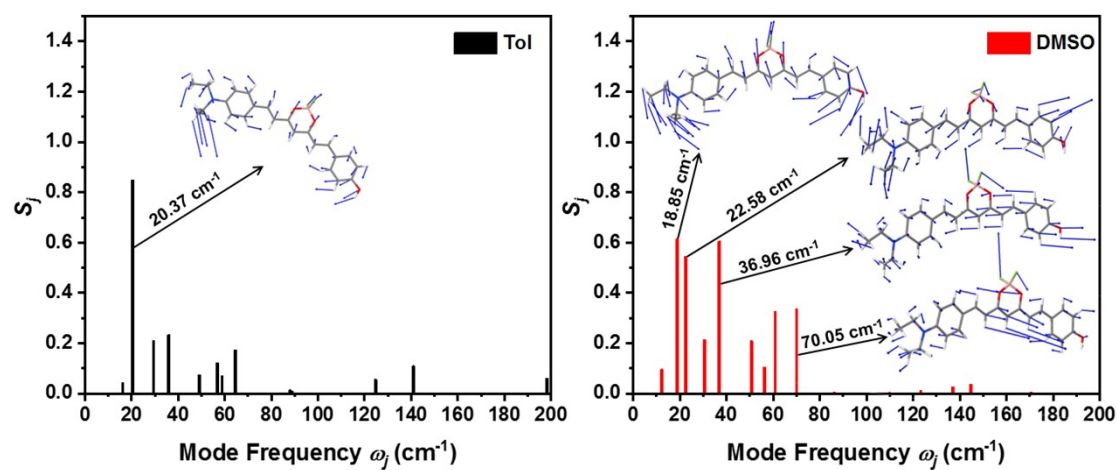


Figure S3 Calculated Huang-Rhys factor S_j versus mode frequency ω_j for probe **2** in Tol and DMSO.

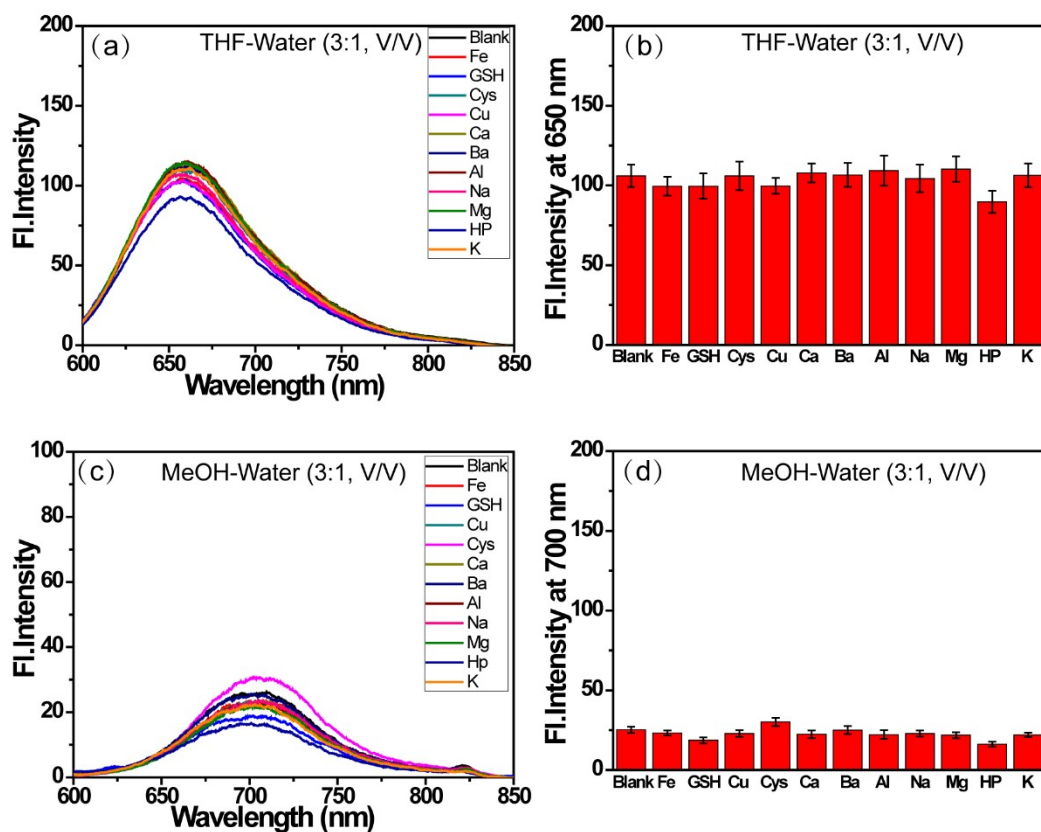


Figure S4 (a) The fluorescence spectra of probe **2** (10 μM) with or without various species in THF-water (25 % water). (b) The fluorescence intensity at 650 nm of probe **2** (10 μM) with or without various species. (c) The fluorescence spectra of probe **2** (10 μM) with or without various species in MeOH-water (25 % water). (d) The fluorescence intensity at 700 nm of probe **2** (10 μM) with or without various species.

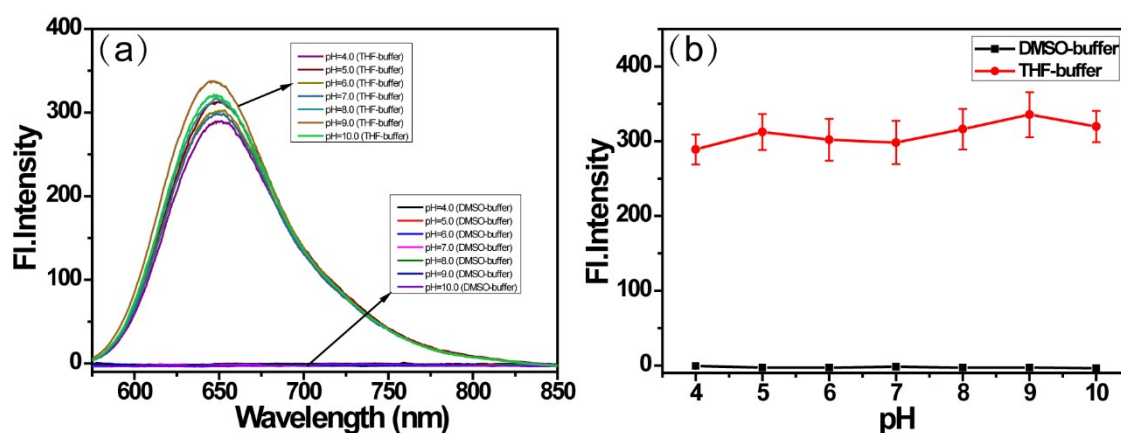


Figure S5 (a) The fluorescence spectra of probe **2** (10 μM) in THF-buffer and DMSO-buffer under different pH values. (b) The fluorescence intensity change of the

maximum emission wavelength of probe **2** (10 μ M) under different pH values.

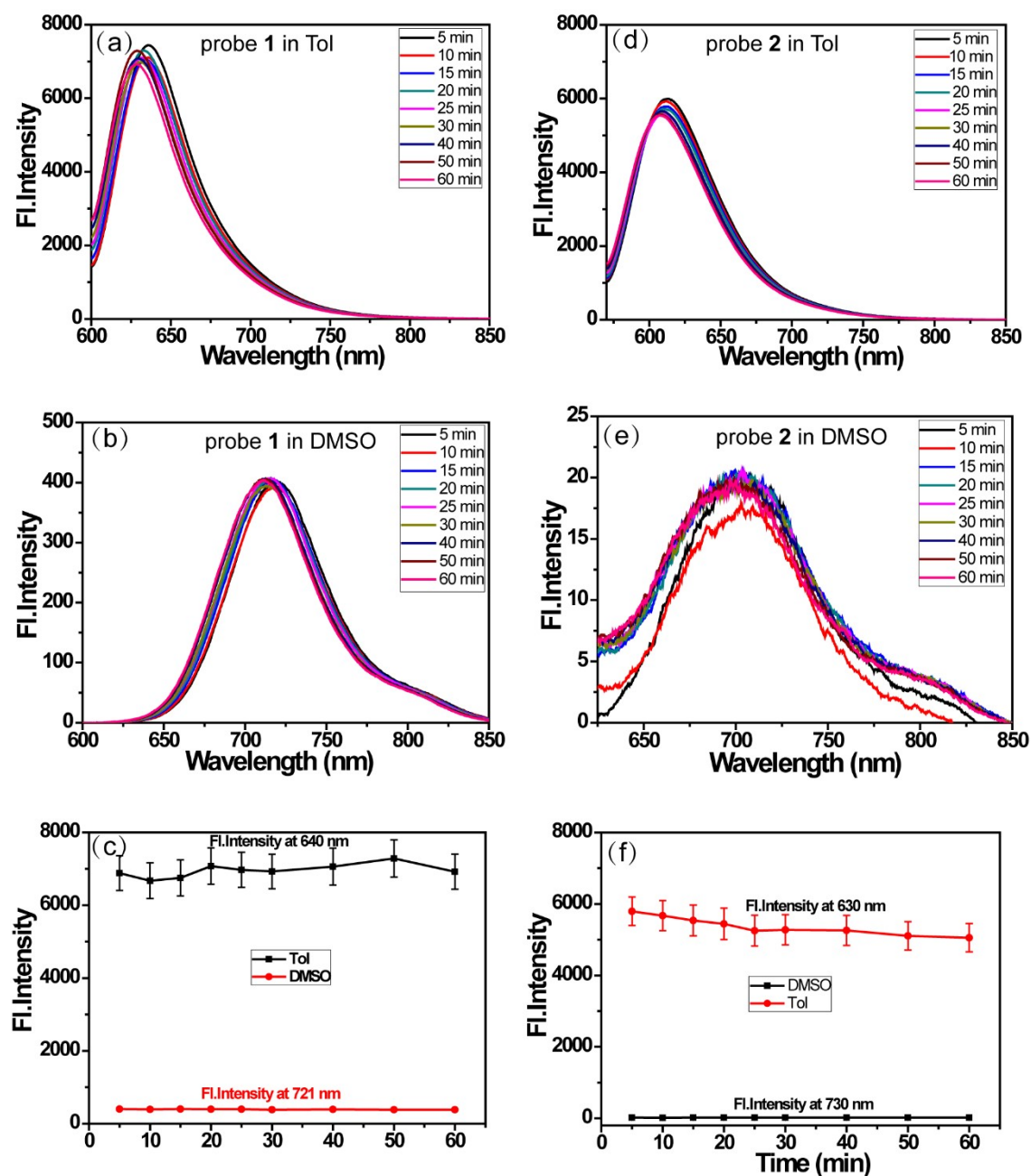


Figure S6 (a-b) The fluorescence spectra of probe **1** (10 μ M) in different polar solvents (DMSO and Tol) with time. (c) The fluorescence intensity change of the maximum emission wavelength of probe **1** in DMSO and Tol over time. (d-e) The fluorescence spectra of probe **2** (10 μ M) in different polar solvents (DMSO and Tol) with time. (f) The fluorescence intensity change of the maximum emission wavelength of probe **2** in DMSO and Tol over time.

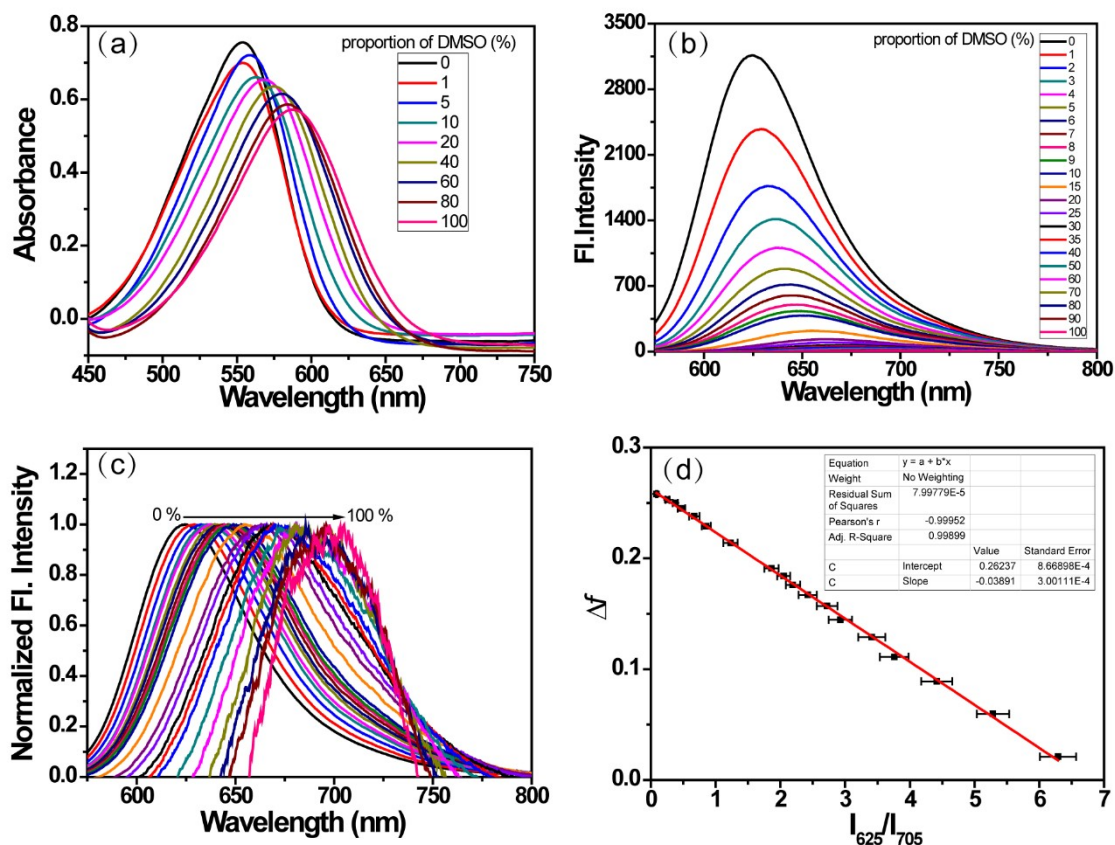


Figure S7 (a) The absorption spectra of probe **2** in Dio-DMSO system varying with DMSO ratio. (b) The fluorescence spectra of probe **2** in Dio-DMSO system varying with DMSO ratio. (c) The normalized fluorescence spectra of probe **2** in Dio-DMSO system varying with DMSO ratio. (d) The linear relationship between the mixed solvent Δf value and the I_{625}/I_{705} of probe **2** (10 μ M) in the Dio-DMSO system.

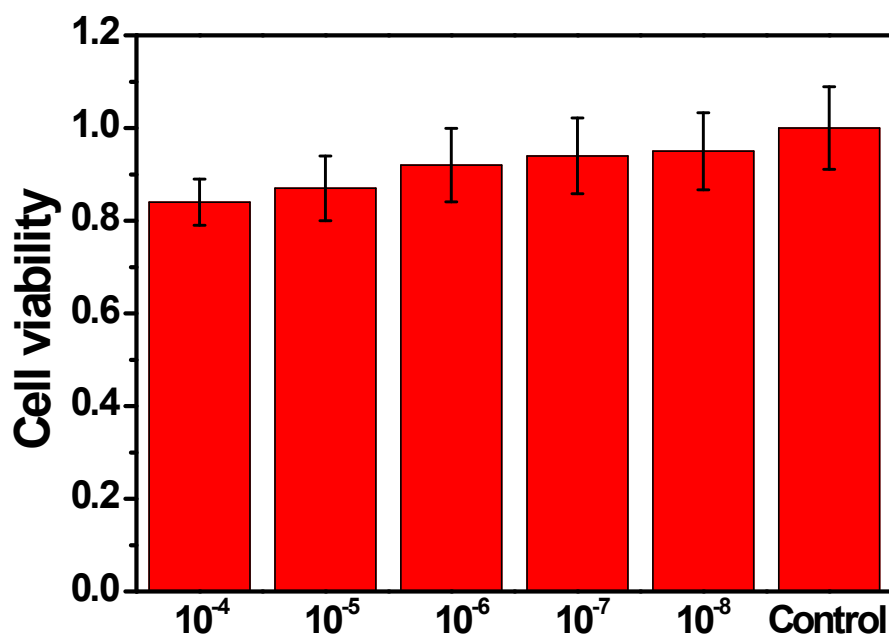


Figure S8 The cell viability of Hepa1-6 cells incubated with different concentrations of probe **1**. The cells were seeded at about 6×10^4 cells/well on a 96-well plate. The cells were treated with media containing **1** (10^{-4} - 10^{-8} M) for 10 h, and MTT assay was then performed. The half maximal inhibitory concentration was calculated to be 165 μ M.

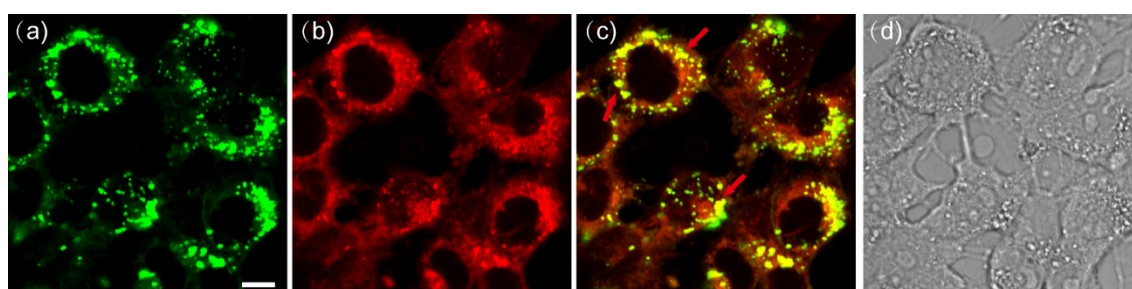


Figure S9 Co-localization imaging experiments of Hepa1-6 cells stained with probe **1** and Nile Red. (a) Fluorescence image of Nile Red (5.0 μ M, green channel, Ex=543 nm, collected 550-590 nm). (b) Fluorescence image of **1** (10 μ M, red channel, Ex=543 nm, collected 670-750 nm). (c) Overlay of (a) and (b). (d) The bright-field image. The red arrows display the ideal overlay of probe **1** and Nile Red. Scale bar:

10 μm .

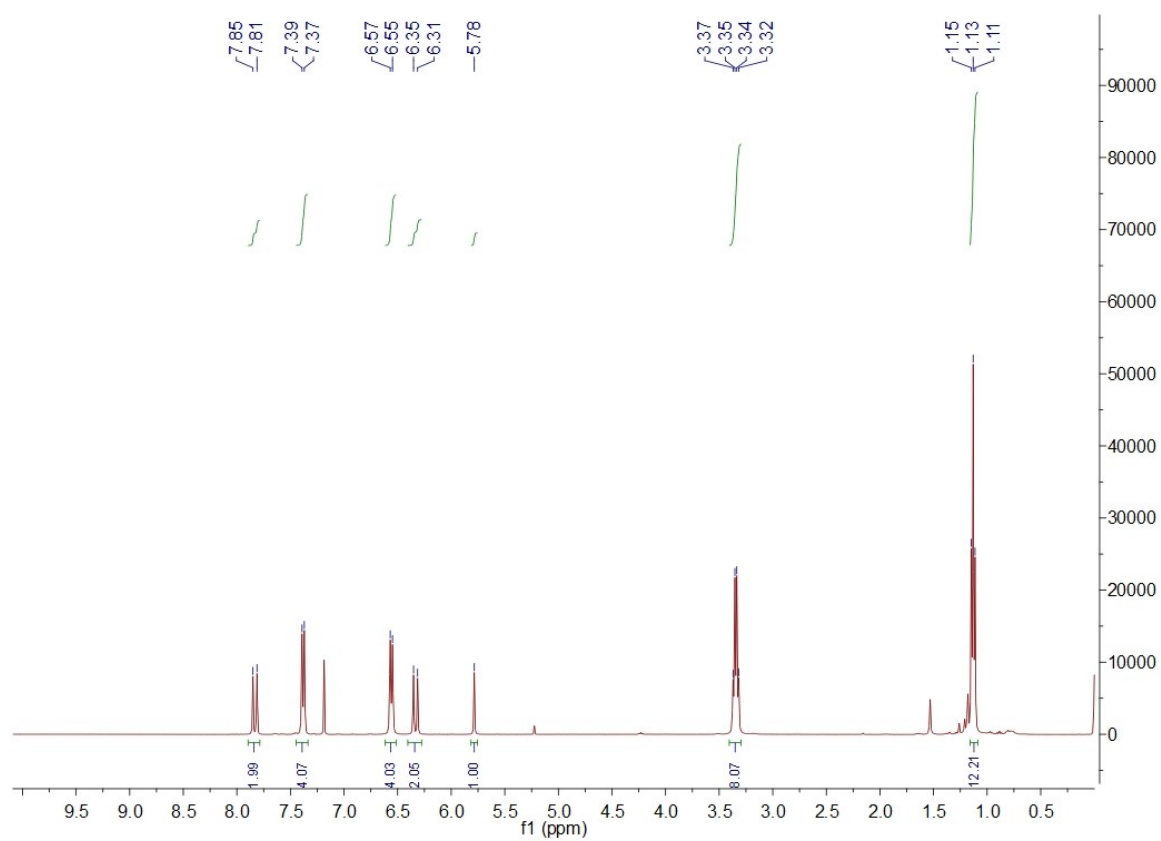


Figure S10 The ^1H NMR of probe **1**.

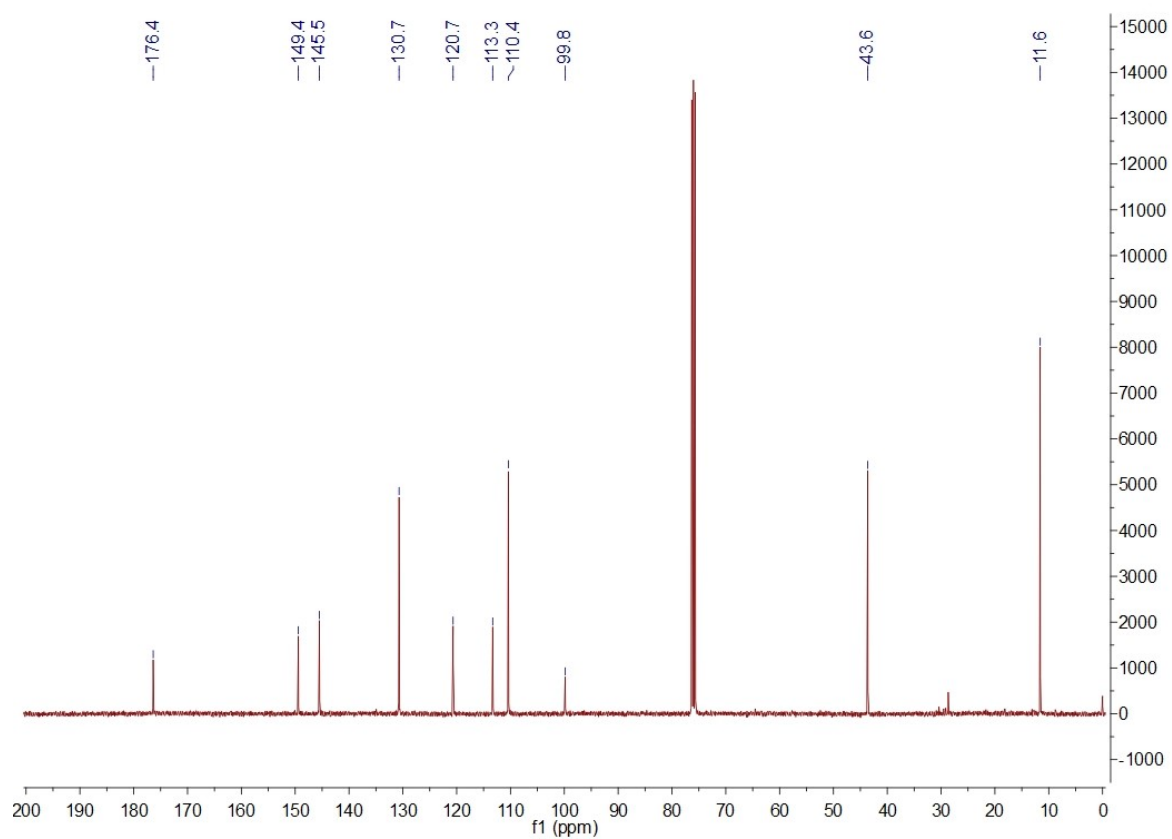


Figure S11 The ^{13}C NMR of probe 1.

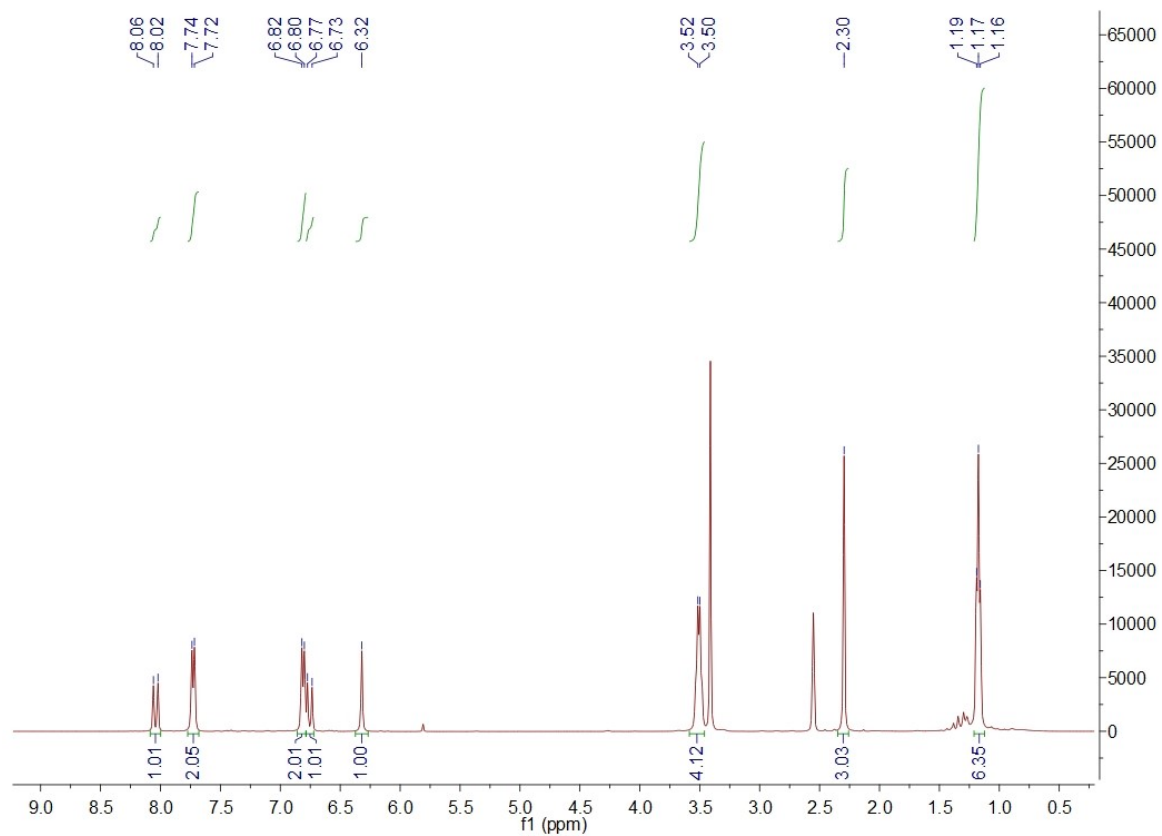


Figure S12 The ^1H NMR of Compound **b**.

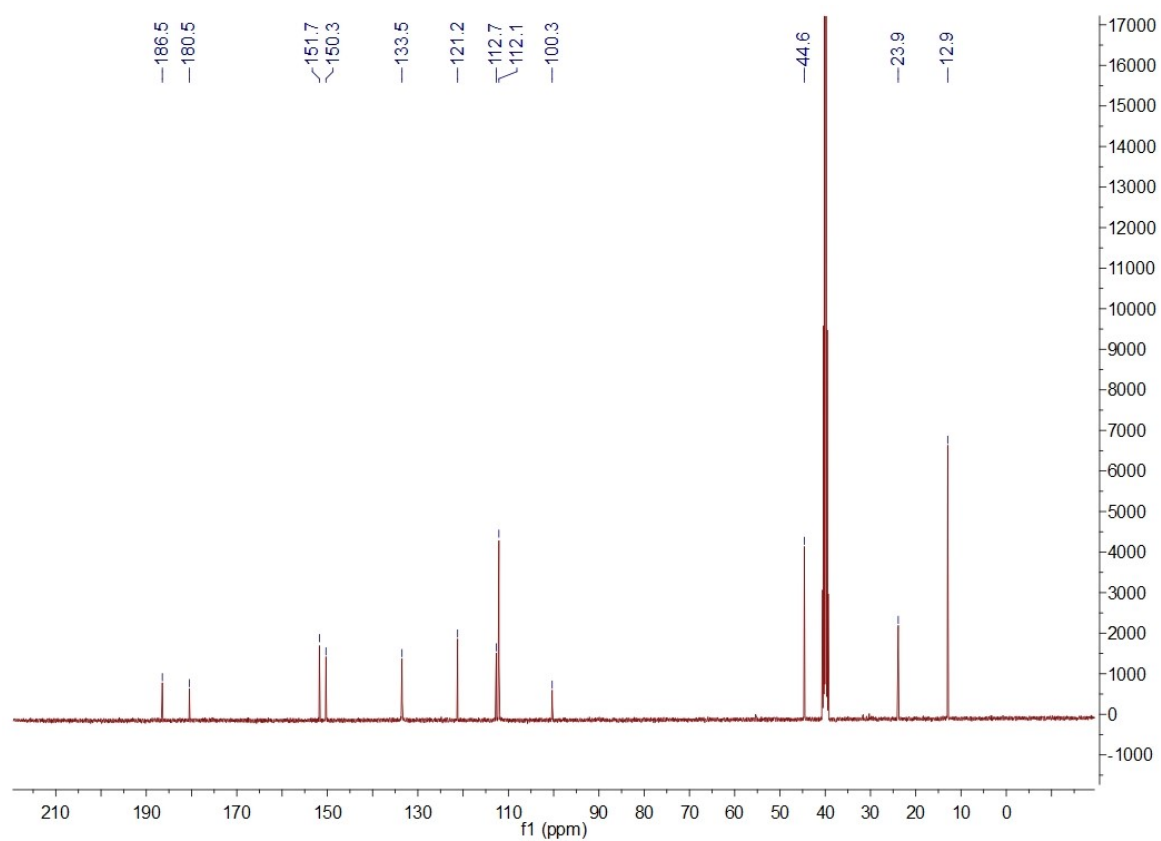


Figure S13 The ^{13}C NMR of Compound **b**.

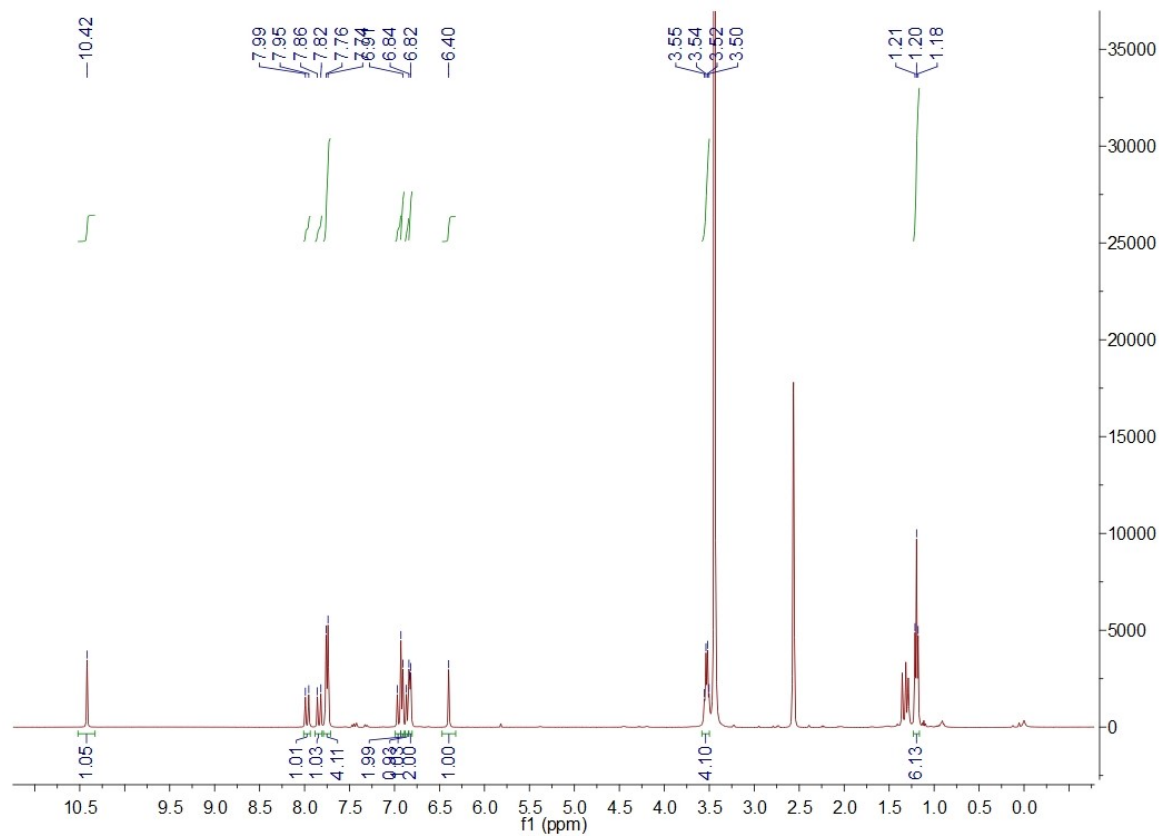


Figure S14 The ^1H NMR of probe **2**.

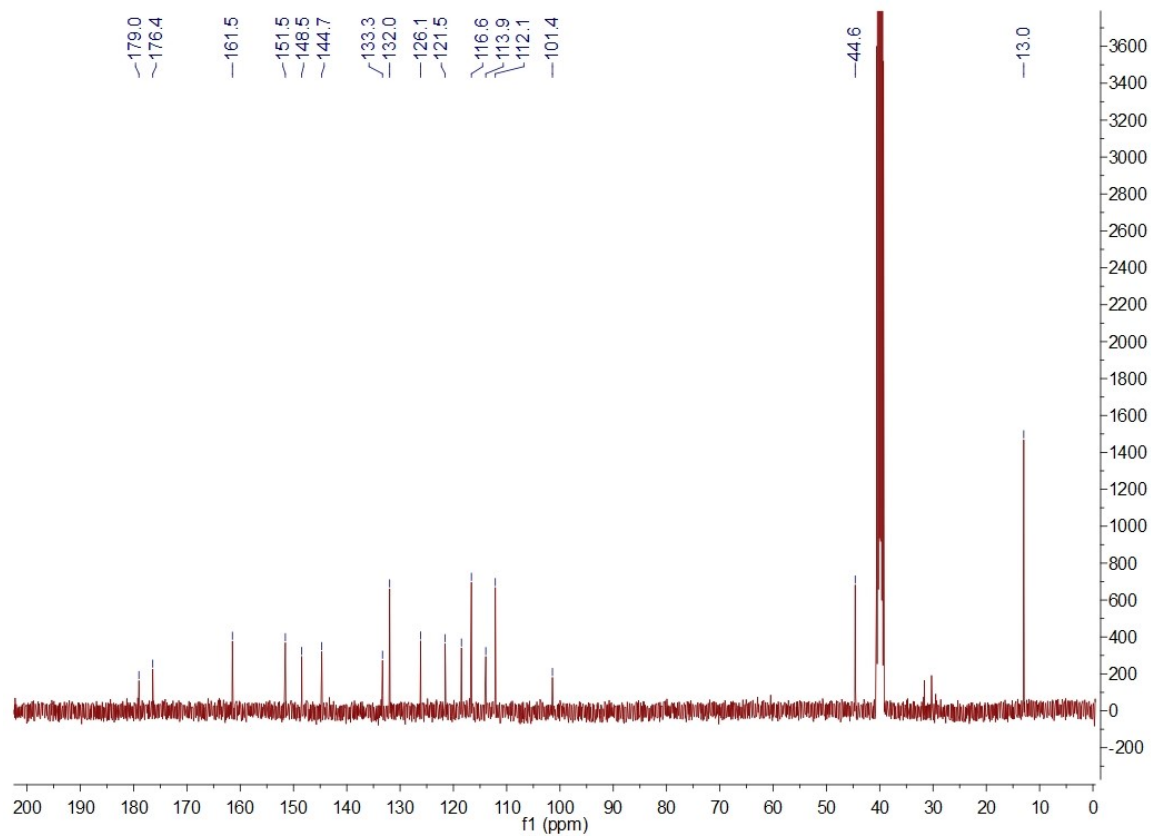


Figure S15 The ^{13}C NMR of probe 2.

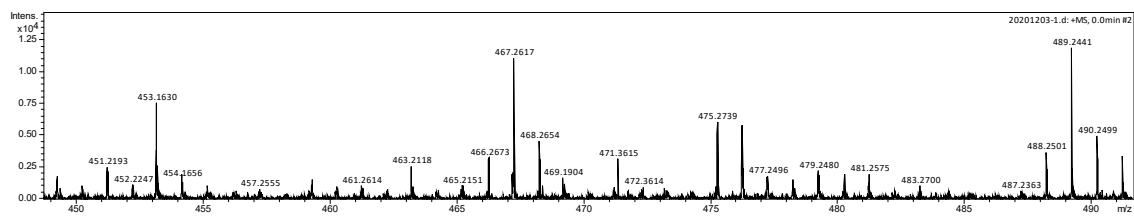


Figure S16 The HRMS of probe 1.

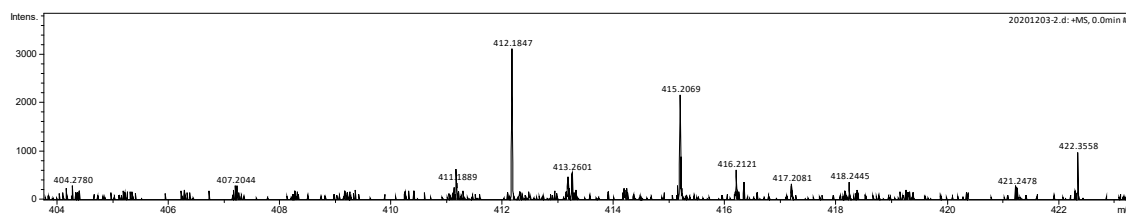


Figure S17 The HRMS of probe **2**.

Reference

- 1 K. K. Laalia, B. M. Rathmana, S. D. Bungeb, X. Qic and G. L. Borosky, *J. Fluorine Chem.*, 2016, **191**, 29-41.
- 2 C. Liu, J. Yin, B. Lu and W. Lin, *New J. Chem.*, 2021, **45**, 4347-4353.
- 3 M. Qian, L. Zhang, Z. Pu, C. Zhang, Q. Chen, X. Sui, X. Han, S. Zeng, H. Cui, J. Wang and X. Peng, *Sensor. Actuat. B-Chem.*, 2021, **344**, 130261.
- 4 C. Jiang, L. Li, J. Jiang, L. Hou, G. Fang, H. Yu and X. Meng, *Chinese Chem. Lett.*, 2020, **31**, 447-450.
- 5 Z. Yuan, J. Chen, Q. Zhou, A. Liu, Z. Qiang, M. Fang, M. Chen, Y. Feng, H. Yu, X. Yang and X. Meng, *Chinese Chem. Lett.*, 2021, **32**, 1803-1808.
- 6 Y. Zhou, C. Wu, X. Wang, P. Li, N. Fan, W. Zhang, Z. Liu, W. Zhang and B. Tang, *Anal. Chem.*, 2021, **93**, 9609-9620.
- 7 F. Yu, X. Jing and W. Lin, *Sensor. Actuat. B-Chem.*, 2020, **302**, 127207.