Acrolein-Integrated ESIPT Probe for NIR Fluorescence Detection of Cysteine with Ultrafast Response and Sub-nanomolar Sensitivity

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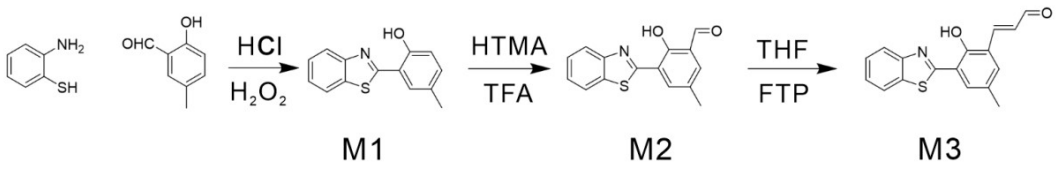
Content

1 Synthesis



Scheme.S1 Synthesis of M1, M2 and M3

1.1 Synthesis of M1：

5-Methylsalicylaldehyde (5-MSA) and 2-aminobenzenethiol (2-ABT) were subjected to three cycles of vacuum evacuation and nitrogen purging to ensure an inert atmosphere. Subsequently, the mixture was dissolved in 60 mL of anhydrous ethanol. Under the condition of an ice-salt bath, 4.84 mL of concentrated hydrochloric acid was slowly added dropwise. The reaction mixture was stirred for 10 min, after which 10 mL of 30% hydrogen peroxide was slowly introduced. The reaction was allowed to proceed at room temperature for 2 h. Upon completion of the reaction, the product was isolated by ethanol washing, yielding a white solid, designated as M 1, with a yield of 54%.

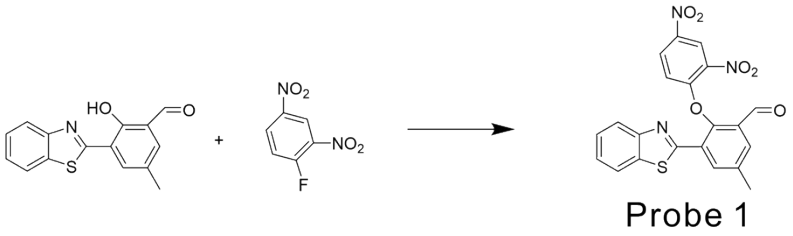
1.2 Synthesis of M2：

M1 and hexamethylenetetramine were dissolved in 80 mL (25 eq.) of trifluoroacetic acid. The mixture was heated to 70℃ and refluxed for 12 h. After the reaction was complete, the mixture was cooled to room temperature and neutralized with a saturated aqueous solution of KOH until the reaction mixture reached neutrality. The product was then extracted with dichloromethane, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to obtain the crude product. The crude product was purified by column chromatography using a petroleum ether (PE) to ethyl acetate (EA) ratio of 20:1 to yield the pure product M2 with a yield of 64%.

1.3 Synthesis of M3：

M2 and (formylmethylene)triphenylphosphorane were subjected to three cycles of vacuum evacuation and nitrogen purging to ensure an inert atmosphere. The mixture was then dissolved in 50 mL of anhydrous tetrahydrofuran (THF). Upon complete dissolution, the reaction mixture was heated to 66℃ and refluxed for 24 h. After the reaction was complete, the solvent was removed under reduced pressure to obtain the crude product. The crude product was purified by column chromatography using a petroleum ether (PE) to ethyl acetate (EA) ratio of 20:1, yielding a pale yellow solid, designated as M3, with a yield of 68%.

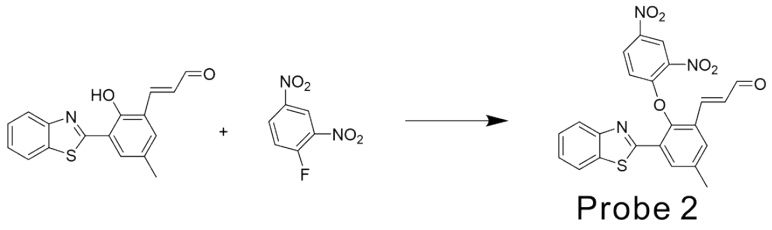
1.4 Synthesis of probe 1 ：



Scheme.S2 Synthesis of Probe 1

M2, 2,4-dinitrofluorobenzene, and potassium carbonate were combined in 8 mL of dimethylformamide (DMF) and stirred at room temperature for 1 h. The reaction mixture was then poured into ice water, and the crude product was isolated by centrifugation. The crude product was purified by flash column chromatography using a petroleum ether (PE) to ethyl acetate (EA) ratio of 10:1, yielding the product Probe 1 with a yield of 54%.^{1}H NMR (400 MHz, DMSO) δ 8.962 (d, *J* = 2.8 Hz, 1H), 8.398 (dd, *J* = 9.3, 2.9 Hz, 1H), 8.305 (d, *J* = 3.1 Hz, 1H), 8.092 (d, *J* = 8.1 Hz, 1H), 8.026 (d, *J* = 7.9 Hz, 1H), 7.561 (d, *J* = 5.9 Hz, 1H), 7.533 (s, 1H), 7.476 – 7.428 (m, 1H), 7.383 (d, *J* = 8.3 Hz, 1H), 7.184 (d, *J* = 9.3 Hz, 1H), 2.490 (s, 3H).

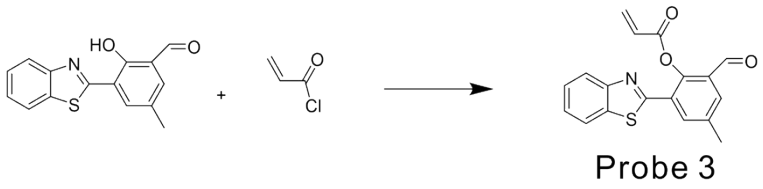
1.5 Synthesis of probe 2 ：



Scheme.S3 Synthesis of Probe 2

M3, 2,4-dinitrofluorobenzene, and potassium carbonate were added to 8 mL of dimethylformamide (DMF). The mixture was stirred at room temperature for 1 h. The reaction mixture was subsequently poured into ice water, and the crude product was isolated by centrifugation. The crude product was purified by flash column chromatography using a petroleum ether (PE) to ethyl acetate (EA) ratio of 10:1, yielding the product probe 2 with a yield of 39%.^{1}H NMR (400 MHz, DMSO) δ 9.610 (d, *J* = 7.5 Hz, 1H), 8.976 (d, *J* = 2.8 Hz, 1H), 8.316 (d, *J* = 1.3 Hz, 1H), 8.284 (dd, *J* = 9.3, 2.8 Hz, 1H), 8.175 (d, *J* = 2.2 Hz, 1H), 8.092 (d, *J* = 7.4 Hz, 1H), 7.947 (d, *J* = 7.8 Hz, 1H), 7.728 (d, *J* = 16.0 Hz, 1H), 7.541 (t, *J* = 7.0 Hz, 1H), 7.479 – 7.428 (m, 1H), 7.029 (dd, *J* = 15.9, 7.6 Hz, 1H), 6.930 (d, *J* = 9.3 Hz, 1H), 2.550 (s, 3H).

1.6 Synthesis of probe 3 ：

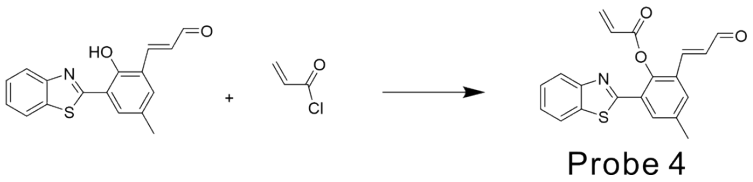


Scheme.S4 Synthesis of Probe 3

M2 and acryloyl chloride were dissolved in 20 mL of anhydrous dichloromethane. Under an ice-water bath condition, triethylamine was added dropwise while maintaining the temperature at 0℃ for 12 h. After the reaction was complete, water was added to wash the product, yielding a white solid, designated as Probe 3,

with a yield of 65%.^{1}H NMR (400 MHz, DMSO) δ 8.563 (s, 1H), 8.448 (s, 1H), 8.276 (d, *J* = 7.3 Hz, 1H), 8.148 (d, *J* = 8.1 Hz, 1H), 7.857 (s, 1H), 7.659 – 7.575 (m, 1H), 7.531 (t, *J* = 7.6 Hz, 1H), 4.436 (s, 2H), 2.525 (s, 3H).

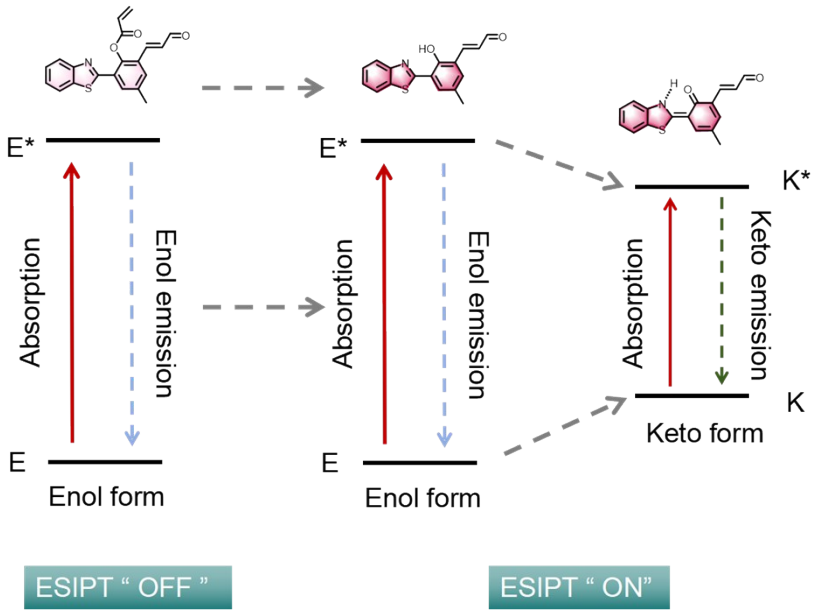
1.7 Synthesis of probe 4 ：



Scheme.S5 Synthesis of Probe 4

M3 and acryloyl chloride were dissolved in 20 mL of anhydrous dichloromethane. Under an ice-water bath condition, triethylamine was added dropwise while maintaining the temperature at 0℃ for 12 h. After the reaction was complete, water was added to quench the excess acryloyl chloride. The product was extracted with dichloromethane, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using a petroleum ether (PE) to ethyl acetate (EA) ratio of 5:1, yielding a product designated as Probe 4 with a yield of 70%.^{1}H NMR (600 MHz, $CDCl_3$) δ 9.608 (d, *J* = 7.6 Hz, 1H), 8.083 (s, 1H), 7.971 (d, *J* = 8.2 Hz, 1H), 7.833 (d, *J* = 8.0 Hz, 1H), 7.524 (s, 1H), 7.456 – 7.399 (m, 2H), 7.335 (t, *J* = 7.6 Hz, 1H), 6.717 – 6.670 (m, 1H), 6.653 (d, *J* = 12.1 Hz, 1H), 6.455 (dd, *J* = 17.3, 10.5 Hz, 1H), 6.113 (d, *J* = 10.5 Hz, 1H), 2.417 (s, 3H).HRMS calculated the molar weight as $[C_{20}H_{15}NO_3S+H]^+$ = 350.0845, found = 350.0884.ppm = 11.14ppm.

2 Schematic illustration of the ESIPT mechanism



Scheme.S6 ESIPT mechanism of Probe 4

3 optical physical properties

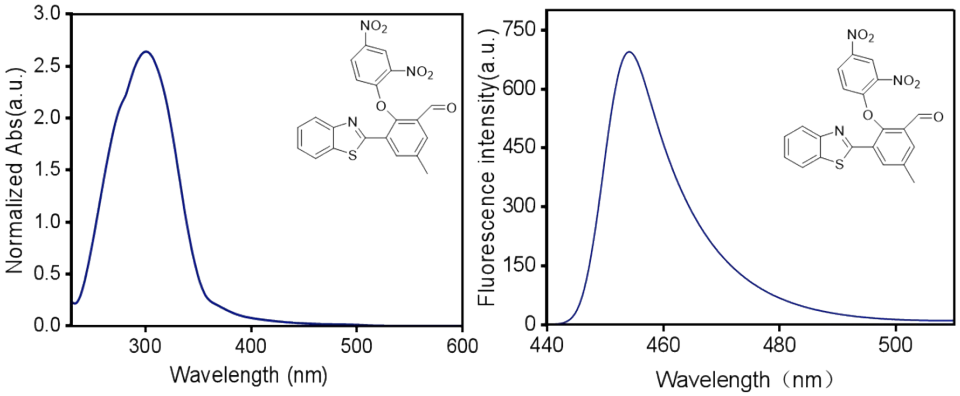


Fig.S1 UV absorption and fluorescence emission of Probe 1

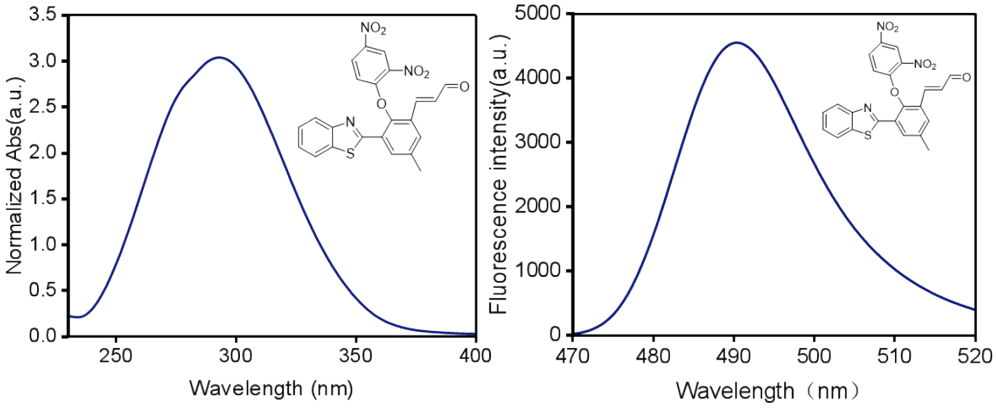


Fig.S2 UV absorption and fluorescence emission of Probe 2

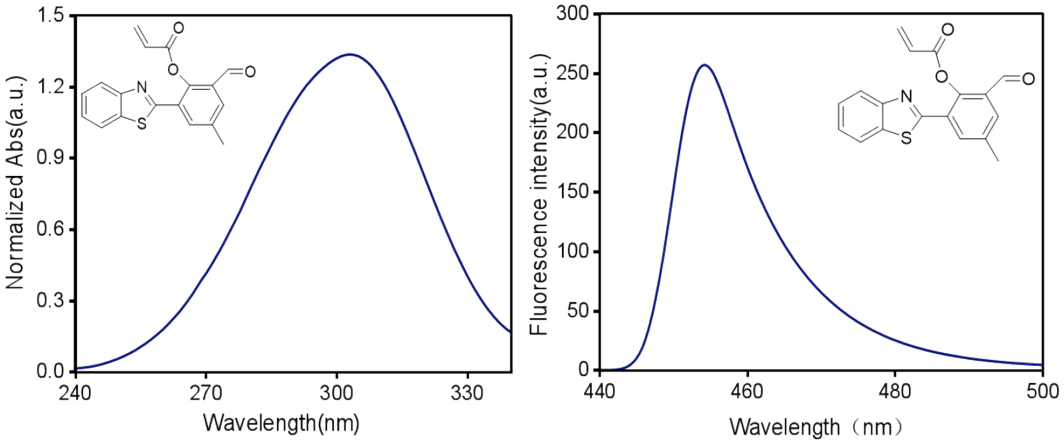


Figure.S3 UV absorption and fluorescence emission of Probe 3

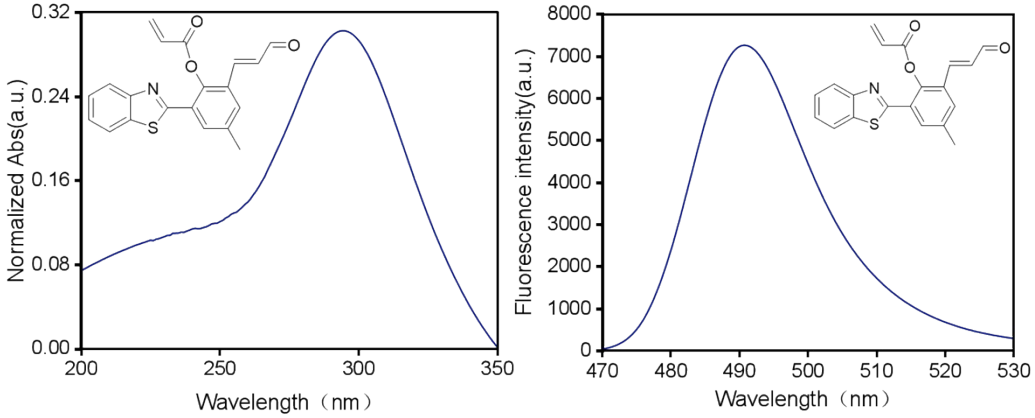


Fig.S4 UV absorption and fluorescence emission of Probe 4

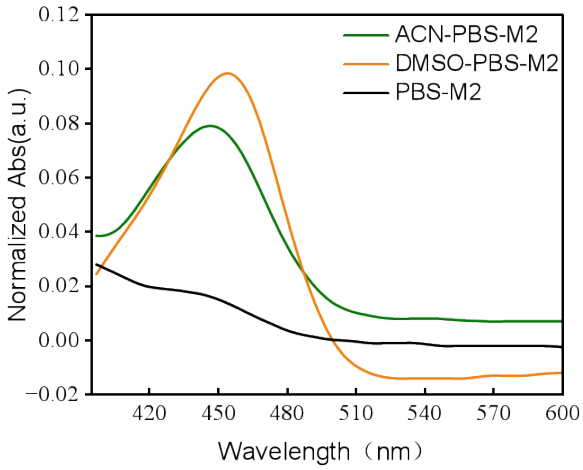


Fig.S5 M2 UV absorption

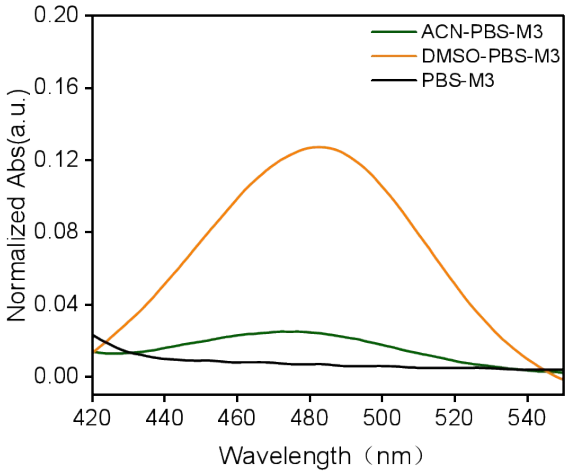


Fig.S6 M3 UV absorption

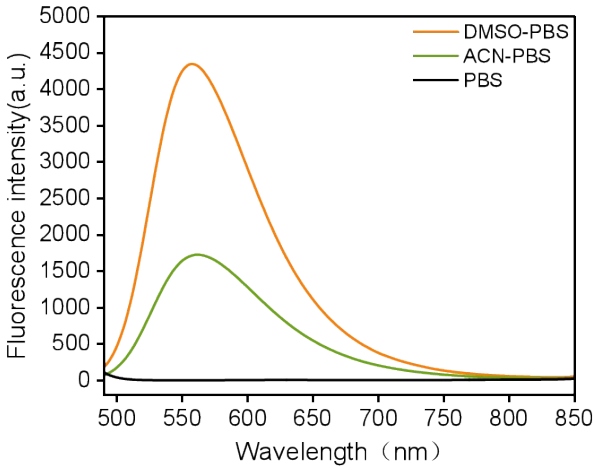


Fig.S7 M2 Fluorescence Emission

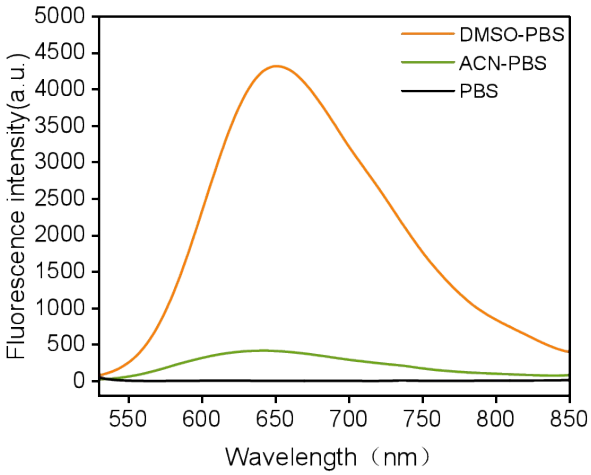


Fig.S8 M3 Fluorescence Emission

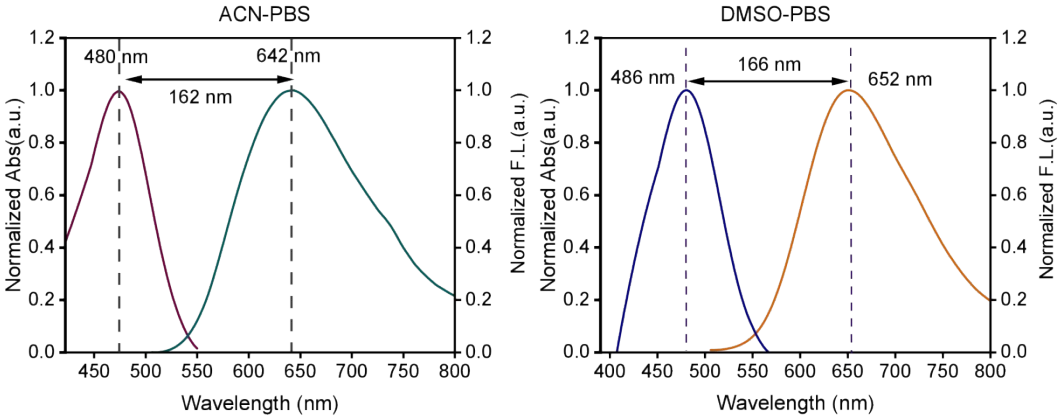


Fig.S9 The Stokes shift of Probe 4 in ACN-PBS and DMSO-PBS

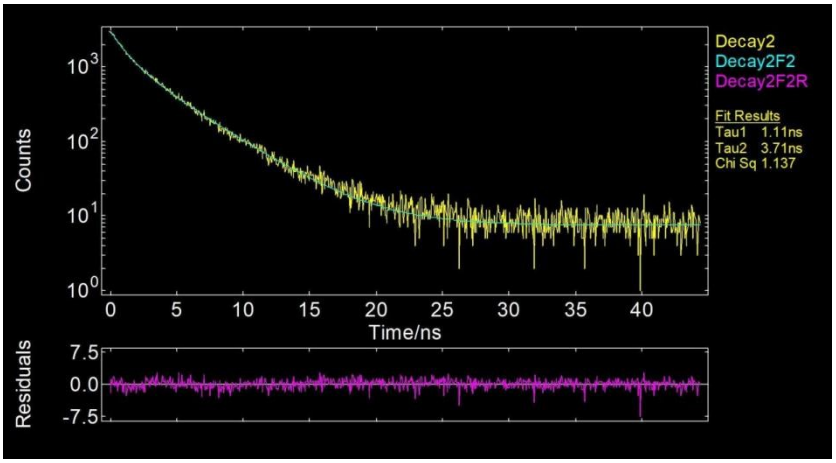


Fig.S10 Quantum yield of the probe 4

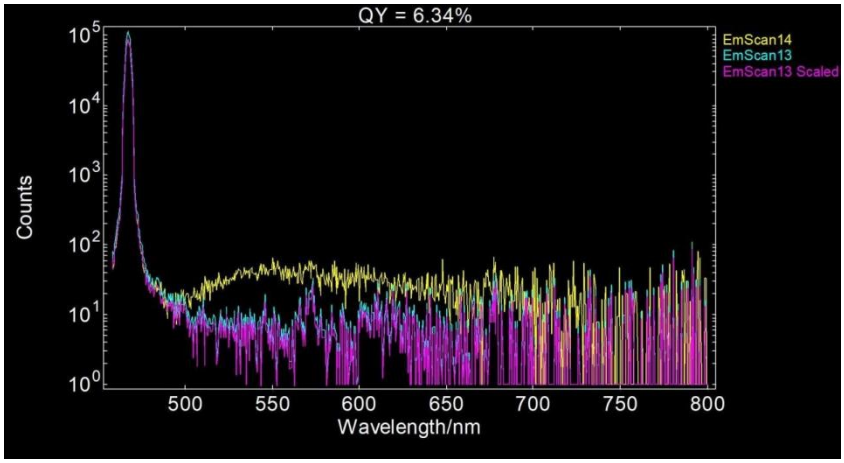


Fig.S11 fluorescence lifetime of the probe 4

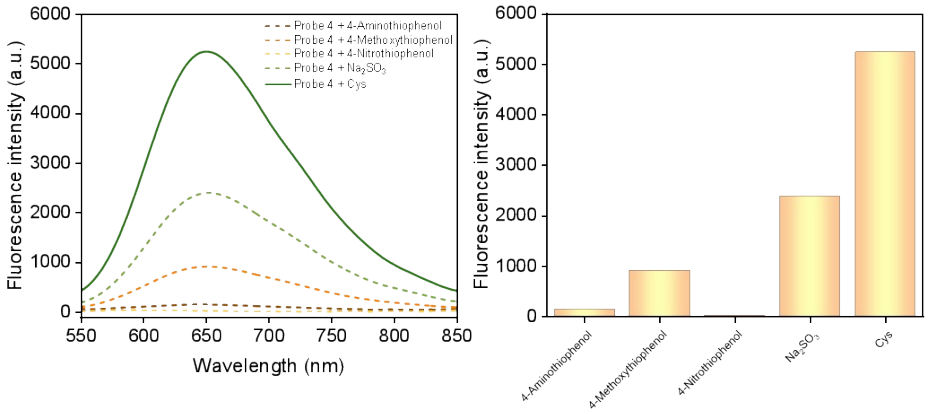


Fig.S12 Selective detection for aromatic thiols and HSO_3^-

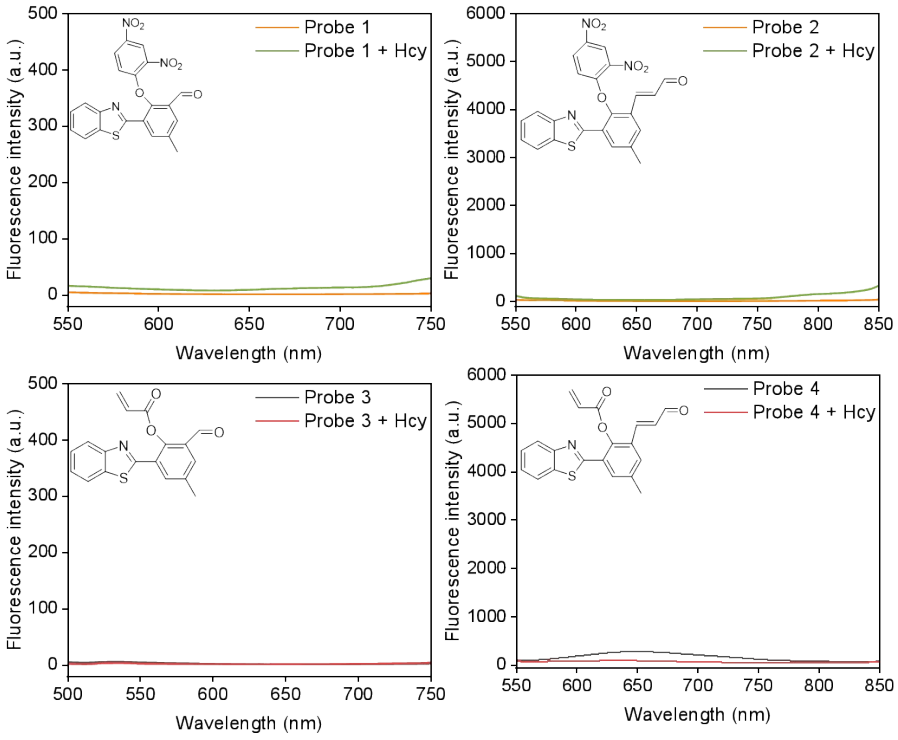


Fig.S13 Probes for the detection of Hcy

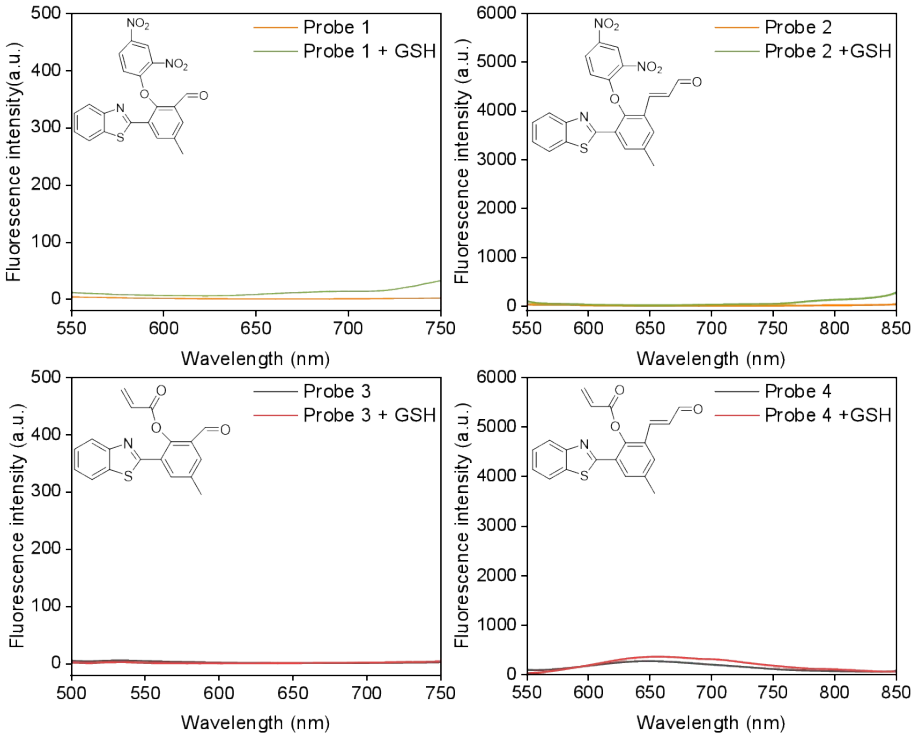


Fig.S14 Probes for the detection of GSH

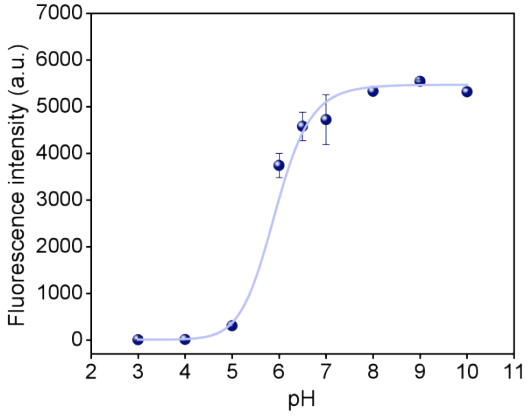


Fig.S15 The pKa of Probe 4

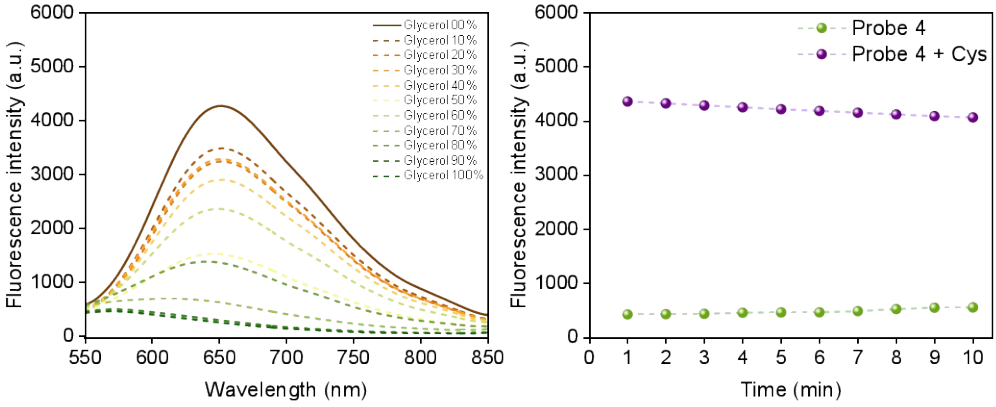


Fig.S16 Viscosity response, long-term stability, and photobleaching resistance of the probe 4

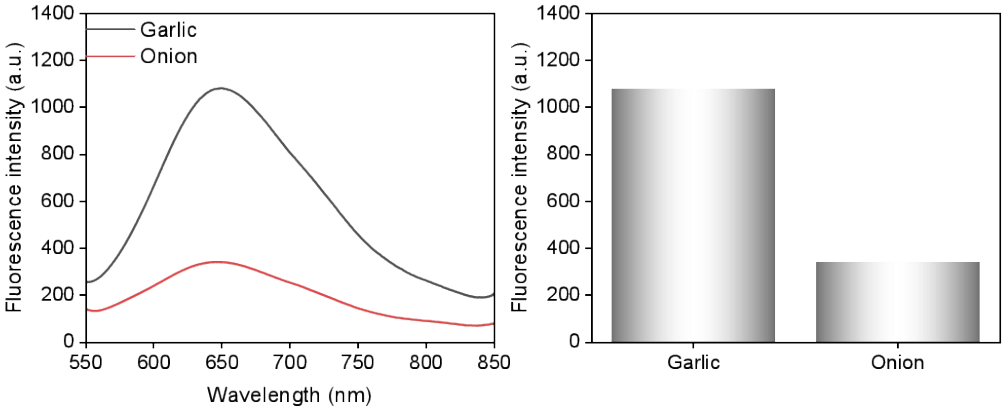


Fig.S17 Detection of Cys in garlic and onion samples using a probe

	S	K	Formula	Value
LOD	0.05	437.51	$\frac{3S}{K}$	3.42×10^{-4}μM
LOQ	0.05	437.51	$\frac{10S}{K}$	1.14×10^{-3}μM

Table.S1 LOD and LOQ

3 Images of interference testing of different ions under sunlight

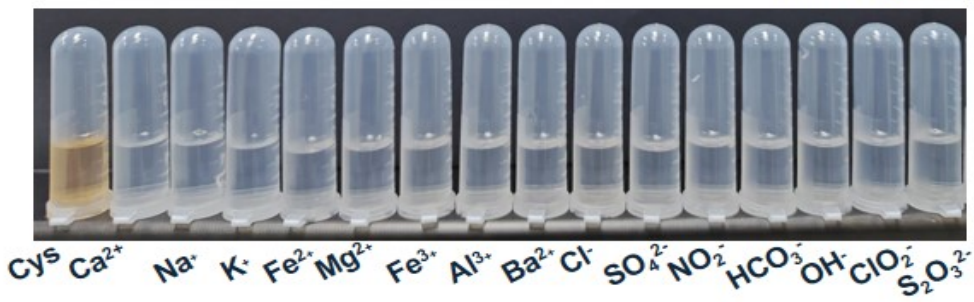


Fig.S18 Images of interference testing of different ions under sunlight

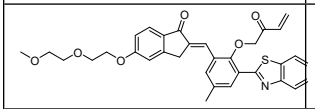
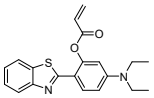
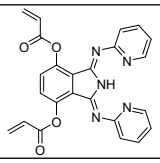
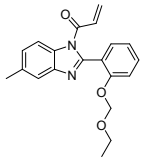
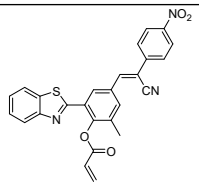
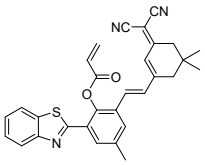
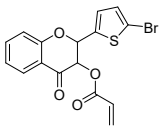
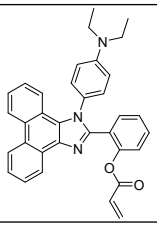
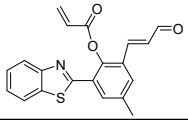
Probe	Solvent system	Mechanism	Time (min)	Limit of detection	Reaction sites	Ref.
	PBS	ESIPT	90	0.89 μM	acrylate	Dyes and Pigments Volume 219, November 2023, 111649
	PBS / DMSO (1:1, v/v)	ESIPT	7.5	6.4×10^{-2} μM	acrylate	Analytica Chimica Acta Volume 1279, 23 October 2023, 341819
	H_2O / DMSO (99:1, v/v)	ESIPT	60	-	acrylate	Dyes and Pigments Volume 212, April 2023, 111088
	PBS	ICT	30	3.98×10^{-2} μM	acrylate	Journal of Photochemistry and Photobiology A: Chemistry Volume 431, 1 October 2022, 114074
	MeCN / H_2O (1 : 1, v/v)	ESIPT	30	6.51 μM	acrylate	Dyes and Pigments Volume 203, July 2022, 110305
	CH_3CN / CH_3OH / Hepes (1:1:1, v/v/v)	ESIPT	14	0.20 μM	acrylate	Sensors and Actuators B: Chemical Volume 319, 15 September 2020, 128248
	PBS / DMSO (1:4, v/v)	ESIPT	15	4.23×10^{-2} μM	acrylate	Talanta Volume 194, 1 March 2019, Pages 717-722
	PBS / DMSO (7:3, v/v)	ESIPT	10	0.64 μM	acrylate	Sensors and Actuators B: Chemical Volume 233, 5 October 2016, Pages 173-179
	PBS / DMSO (1:1, v/v)	ESIPT	10	3.42×10^{-4}μM	acrylate	**This work**

Table S2 Comparison of Key Parameters with Related Probes

4 Characterization

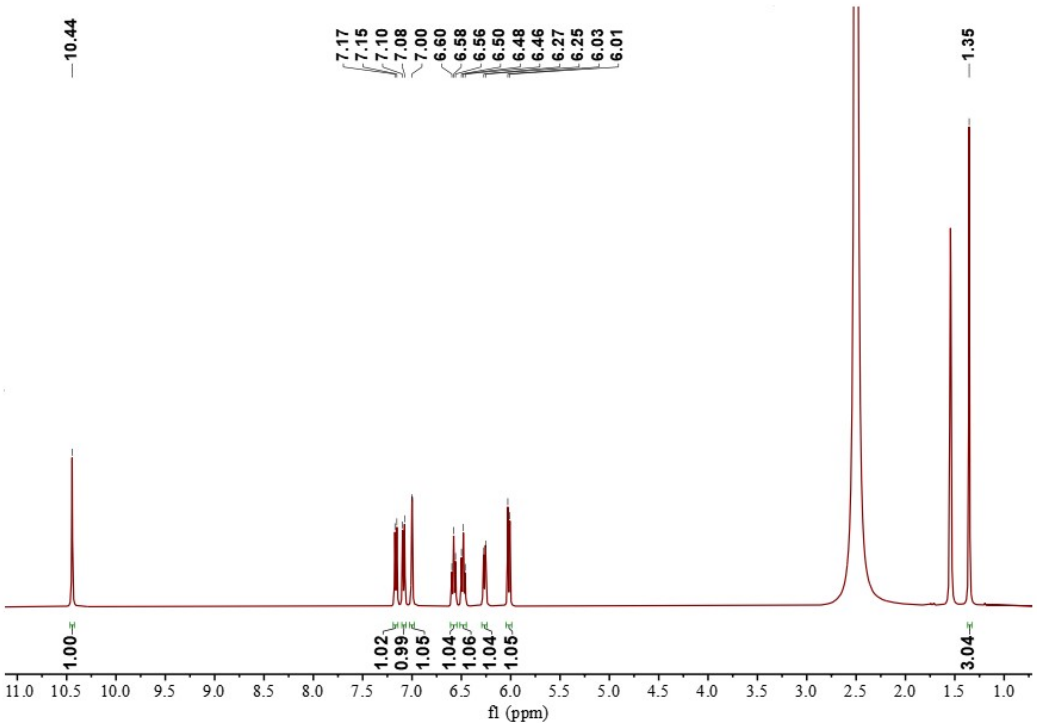


Fig.S19 ^{1}H NMR of M1

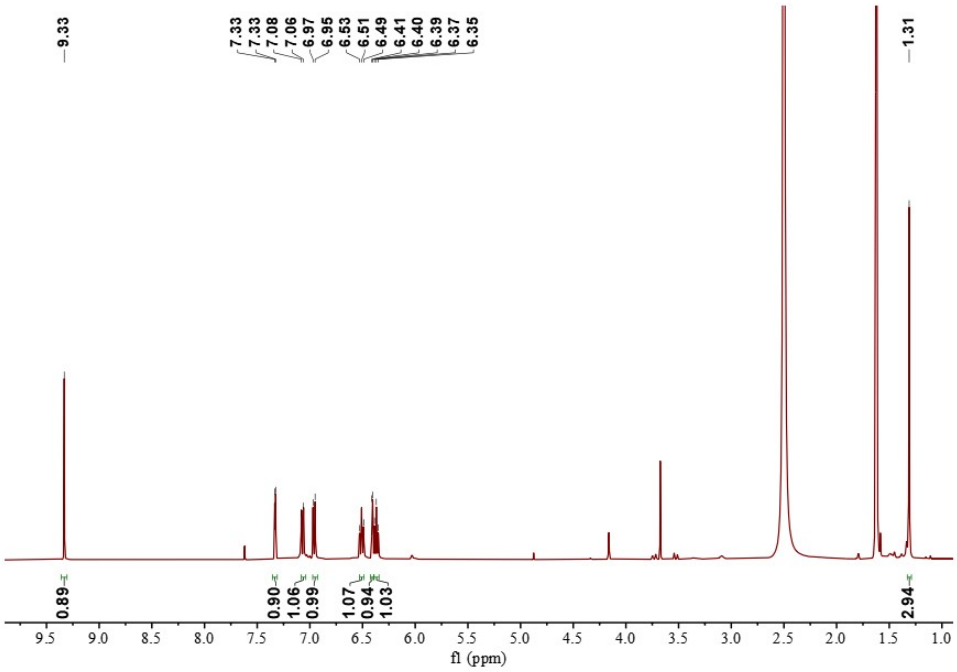


Fig.S20 ^{1}H NMR of M2

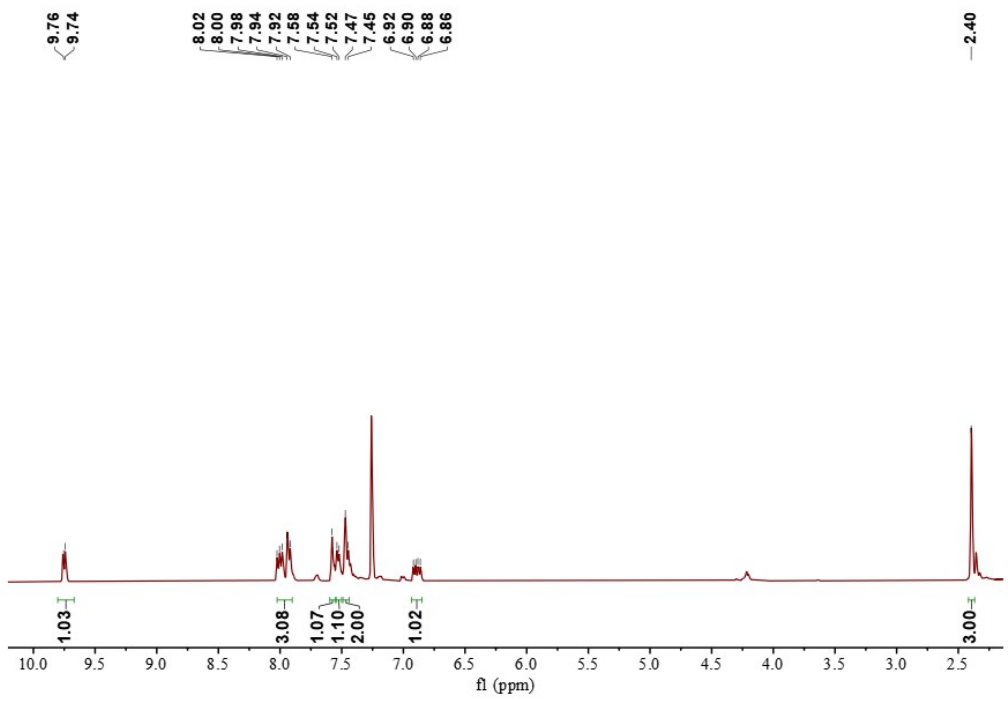


Fig.S21 ^{1}H NMR of M3

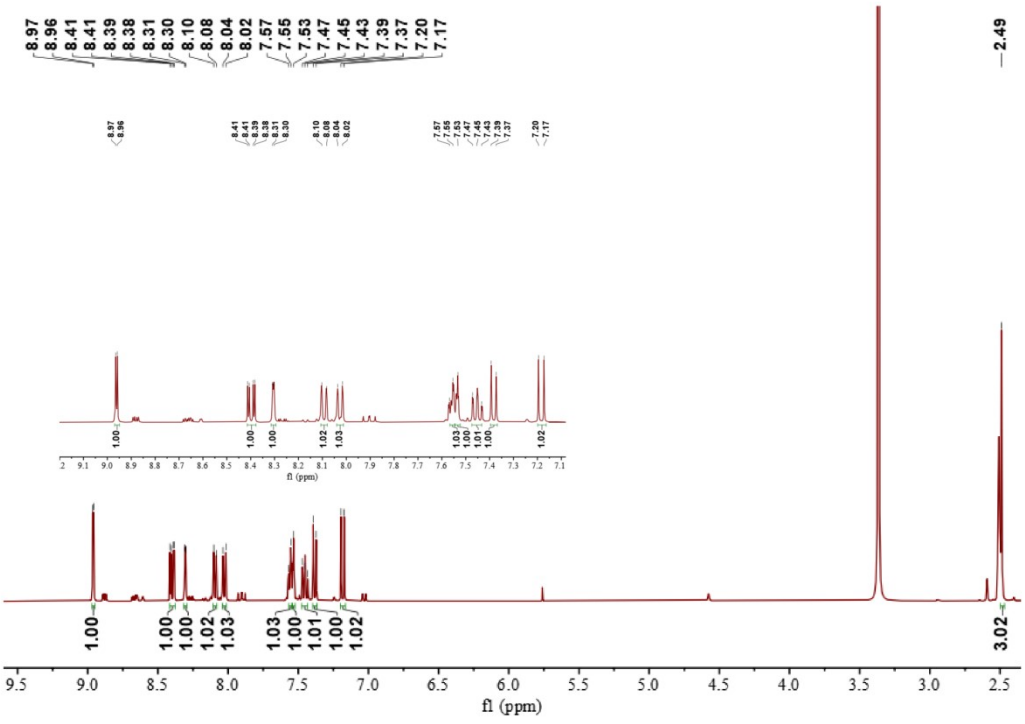


Fig.S22 ^{1}H NMR of Probe 1

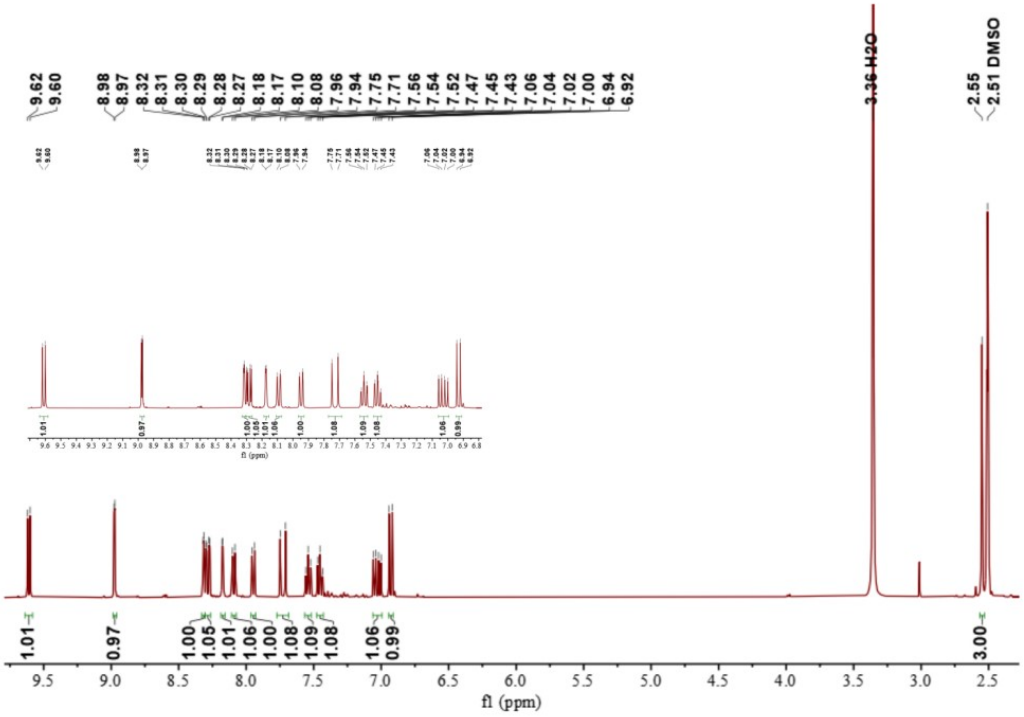


Fig.S23 ^{1}H NMR of Probe 2

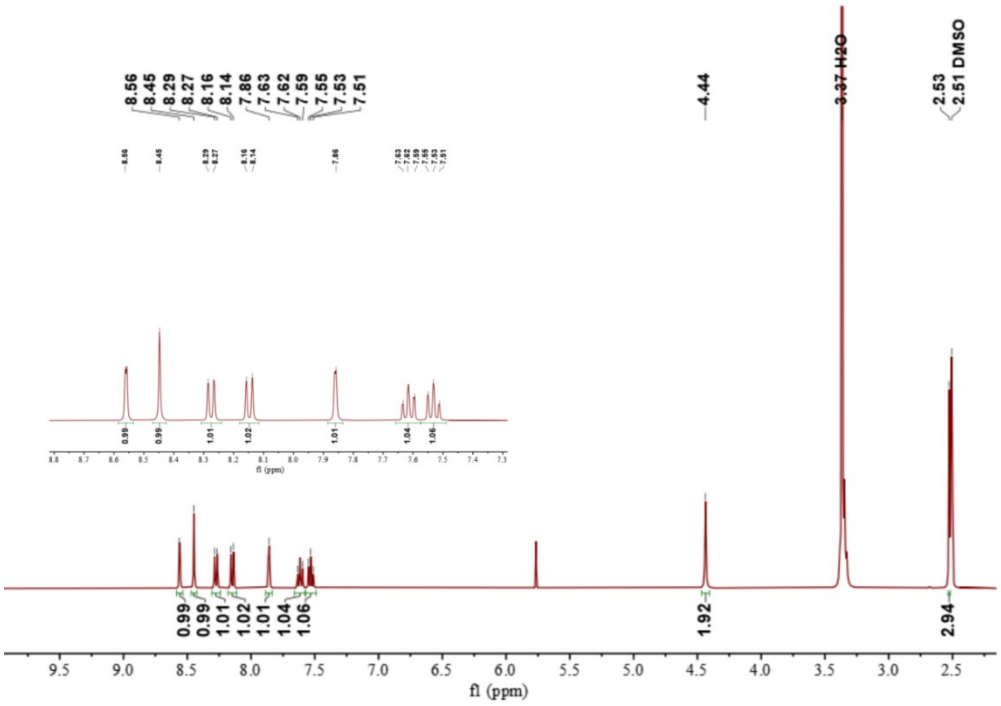


Fig.S24 ^{1}H NMR of Probe 3

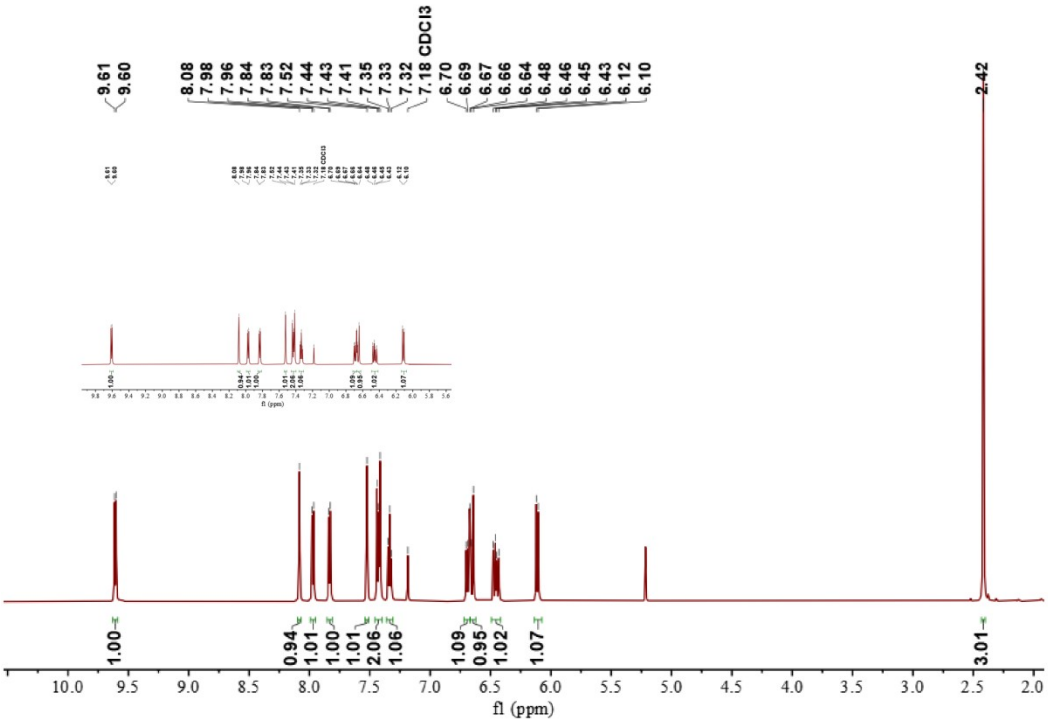


Fig.S25 ^{1}H NMR of Probe 4

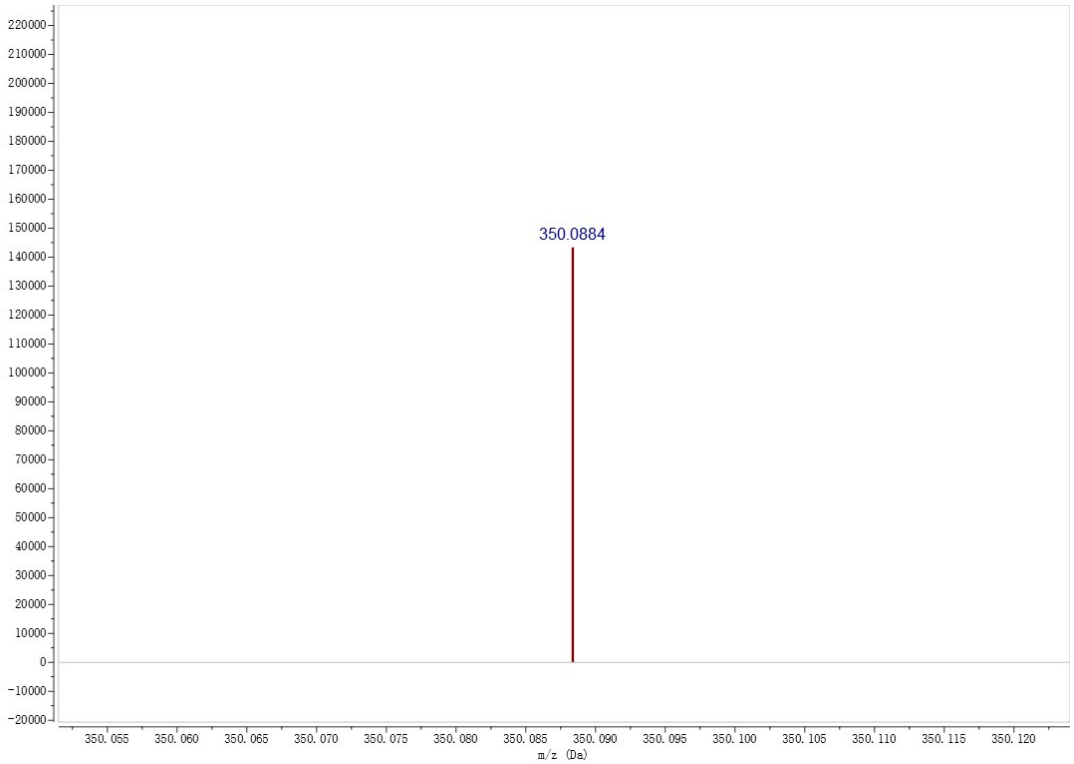


Fig.S26 HRMS of Probe 4