Supporting Information

A Novel Fluorescent Probe for Rapid and Selective Detection of Fluoride Ions in Living Cells

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Contents

1. Materials and Instruments

All chemicals were purchased from Sigma-Aldrich (Shanghai) Co., Ltd. and were of analytical grade. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker (Avance) 400 MHz NMR instrument. The melting point was measured in an X-4X digital micromelting point analyzer (Shanghai Microelectronics Technology Co., Ltd.). Absorption spectra were recorded on a UV-5500PC UV-visible spectrophotometer (Shanghai Meitai Instrument Co., Ltd.). Fluorescence spectra were measured on a fluorescence photometer (Hitachi F4700, Japan). Additionally, a microplate reader (TECAN, Infinite M200) was used in the study. Confocal fluorescence imaging in cells were recorded with a Nikon CSU-W1. All of the above instruments were provided by the School of Pharmacy, Guizhou University.

Mass spectra were recorded on a TSQ 8000 high-resolution mass spectrometer (Thermo Fisher Scientific Co. Ltd.).

2. Preparation of probe solution and fluorescence analysis

The probes **DTP** was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of $1*10^{-3}$ M. The stock solution of tetrabutylammonium fluoride (TBAF) was prepared in THF solution at a concentration of $1*10^{-1}$M. A $1*10^{-3}$ M stock solution is prepared by dissolving various analytes in deionized water. Various common cations (Na^{+}, K^{+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Ag^{+}, and Zn^{2+}) were used in the form of sulfate (SO_4^{2-}). Anions (Cl^{-}, Br^{-}, I^{-}, SO_4^{2-}, $H_2PO_4^{2-}$, HCO_3^{-}, CO_3^{2-}, SCN^{-}, CH_3COO^{-}, NO_3^{-}, HPO_4^{-}) were used in the form of sodium (Na^{+}) salts. Preparation of HEPES buffer: HEPES (23.8 g) was weighed and dissolved in purified water to form a 0.1 M HEPES purified water solution and stored in a refrigerator at 4 °C. All spectra were measured using a 10 mm × 10 mm cuvette with an excitation.
All aqueous solutions were prepared with ultrapure water obtained from a Milli-Q water purification system.

3. Cell cytotoxicity assay and Cell image

Human hepatocellular carcinoma cells (HepG2 cells) were provided by Kunming Cell Bank, Chinese Academy of Sciences. HepG2 cells were cultured at 37 °C in a 5% CO2 cell culture incubator. The in vitro cytotoxicity of **DTP** on HepG2 cells was assessed by an MTT assay. Cell viability was calculated using the formula: cell viability (%) = (mean absorbance of the drug group - mean absorbance of the blank group)/(mean absorbance of the untreated group - mean absorbance of the blank group) × 100%.

Fluorescence imaging of F^- in HepG2 cells. Specific experiments were performed by the following subgroups: (a) **DTP** (10 μM) treatment for 10 min. (b) **DTP** (10 μM) pretreatment for 10 min, followed by incubation with TBAF (200 μM) for 30 min.

Before imaging, all cells were washed three times with PBS.

4. Synthesis and characterization

Compound **2**: compound **1** (1090 mg, 3 mmol), 4-bromo-2-methoxybenzaldehyde (702.4 mg, 3 mmol), Na_2CO_3 (629.65 mg, 6 mmol), $Pd(PPh_3)_4$ (34.32 mg, 0.3 mmol), and Dioxane: H_2O (1: 1) 20 ml were added to a 100 ml round-bottomed flask in a round-bottomed flask protected by argon. The reaction was heated to 110 °C for 3 h. After the reaction was completed, the reaction was quenched by adding water. DCM (40 ml × 3) was extracted, and the organic layer was collected, washed, dried, and spun dried under reduced pressure to obtain the crude product. PE/EA = 5/1 column chromatography yielded 408 mg of yellow solid in 43.63% yield. M.P.: 73.4-74.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.48 (d, *J* = 0.8 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.22 – 7.16 (m, 3H), 7.11 (d, *J* = 1.4 Hz, 1H), 6.98 – 6.89 (m, 3H), 4.01 (s, 3H), 3.90 – 3.86 (m, 2H), 1.92 – 1.85 (m, 2H), 1.06 (t, *J* = 7.4 Hz, 3H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 189.3, 162.2, 147.8, 145.7, 144.7, 134.0, 129.1, 127.5, 127.4, 126.2, 125.9, 125.5, 124.1, 123.4, 122.7, 118.9, 115.6, 109.4, 55.7, 49.3, 20.1, 11.3. ESI-HRMS $C_{23}H_{21}NO_2S$ ($[M+Na]^+$) calcd 398.1185, found 398.1178.

Compound **3**: compound **2** (375 mg, 1 mmol) and DCM (10 ml) were added to a 50-ml round-bottom flask and cooled to 0 °C. BBr_3 (5010 mg, 20 ml) was added while stirring, and the reaction was stirred at room temperature for 1 h. After the reaction was completed, the reaction was quenched by adding $NaHCO_3$. DCM (10 ml × 3) was extracted, and the organic layer was collected, washed, dried, and spun dried under reduced pressure to obtain the crude product. PE/EA = 5/1 column chromatography yielded 336 mg of pale yellow solid in 93.63% yield. M.P.: 98.3-99.4 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 11.14 (s, 1H), 9.91 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.46 – 7.42 (m, 2H), 7.23 – 7.20 (m, 1H), 7.20 – 7.14 (m, 3H), 6.98 – 6.92 (m, 2H), 6.90 (dd, *J* = 8.0, 1.2 Hz, 1H), 3.90 – 3.85 (m, 2H), 1.93 – 1.83 (m, 2H), 1.06 (t, *J* = 7.6 Hz, 3H). **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 195.8, 162.1, 148.6, 146.1, 144.6, 134.1, 133.2, 127.5, 127.4, 126.3, 126.0, 125.5, 124.2, 122.8, 119.3, 118.1, 115.6, 115.5, 114.8, 49.3, 20.2, 11.3. **ESI-HRMS** $C_{22}H_{19}NO_2S$ ($[M+Na]^+$) calcd 384.1028, found 384.1019.

Compound **4**: compound **3** (361 mg, 1 mmol), DMAP (146 mg, 1.2 mmol), TEA (303 mg, 3 mmol), and DCM (5 ml) were added to a 100-ml two-necked vial and cooled to 0 °C. TBSCl (452 mg, 3 mmol) was dissolved in DCM (5 ml) and added dropwise to the two-necked vial, and the reaction was allowed to proceed for 12 h at room temperature. After the reaction was completed, the reaction was quenched by adding water. DCM (20 ml × 3) was extracted, and the organic layer was collected, washed, dried, and spun dried under reduced pressure to obtain the crude product. PE/EA = 3/1 column chromatography yielded 354 mg of yellow oil in 74.6% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.47 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.25 – 7.20 (m, 1H), 7.20 – 7.14 (m, 2H), 7.02 (d, J = 1.6 Hz, 1H), 6.98 – 6.93 (m, 2H), 6.90 (dd, J = 8.0, 1.2 Hz, 1H), 3.90 – 3.86 (m, 2H), 1.92 – 1.85 (m, 2H), 1.06 (d, J = 5.2 Hz, 12H), 0.34 (s, 6H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 189.6, 159.2, 147.5, 145.7, 144.7, 133.7, 128.8, 127.5, 127.4, 126.1, 125.8, 125.7, 125.5, 124.2, 122.7, 119.8, 117.8, 115.6, 115.5, 49.3, 25.7, 20.1, 18.4, 11.3. ESI-HRMS $C_{28}H_{33}NO_2SSi$ ([M+Na]$^{+}$) calcd 498.1893, found 498.1884.

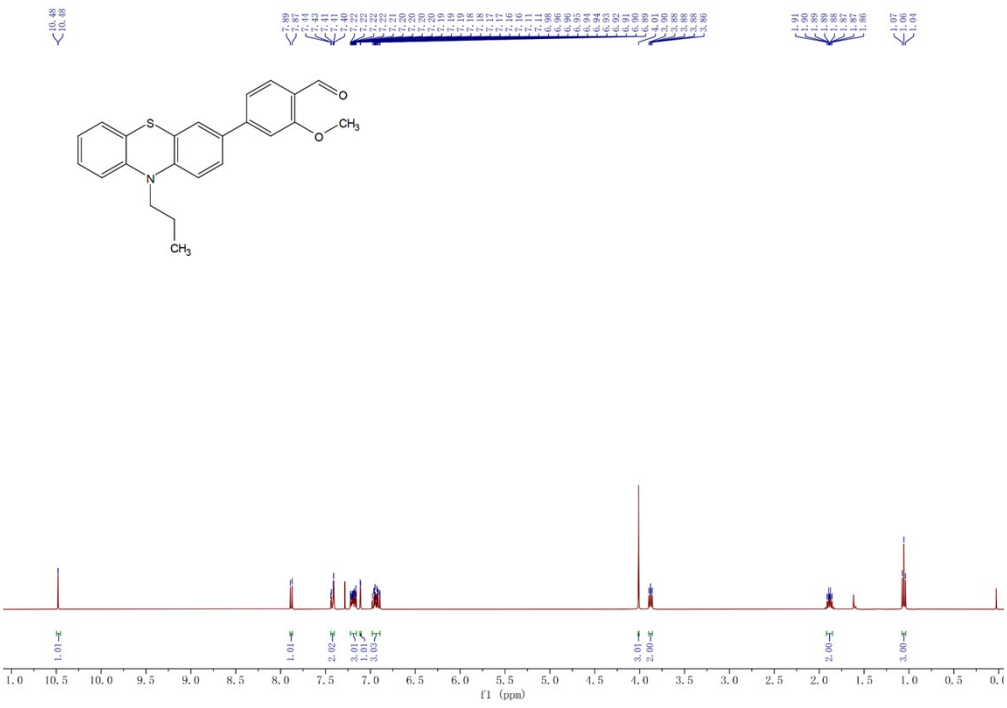


Fig. S1. ^{1}H NMR spectrum of **2**.

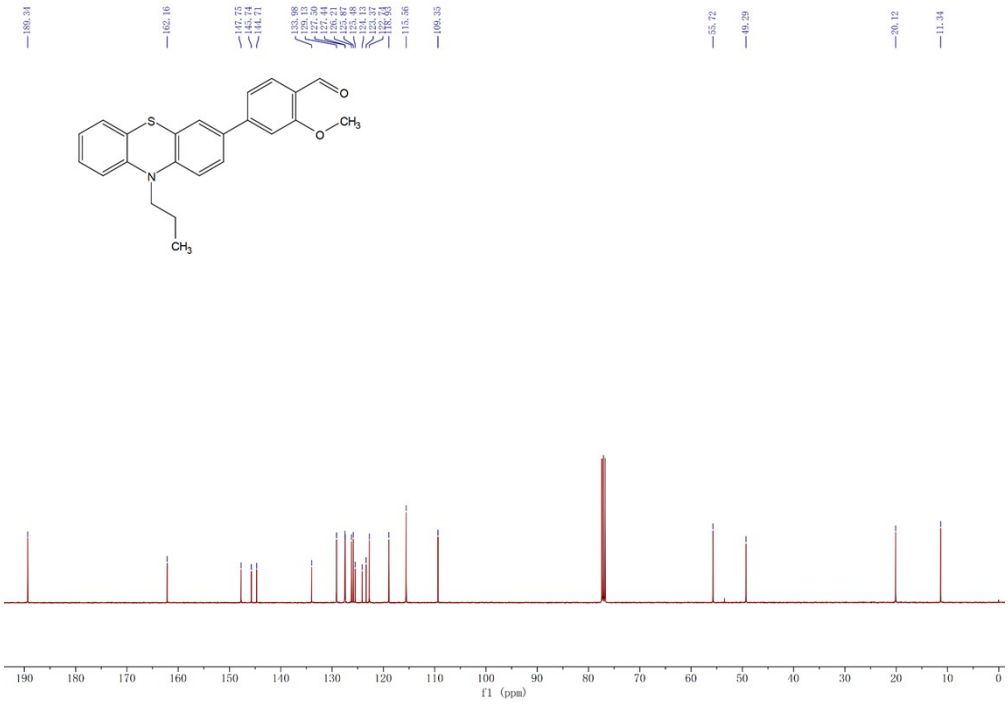


Fig. S2. ^{13}C-NMR spectrum of **2**

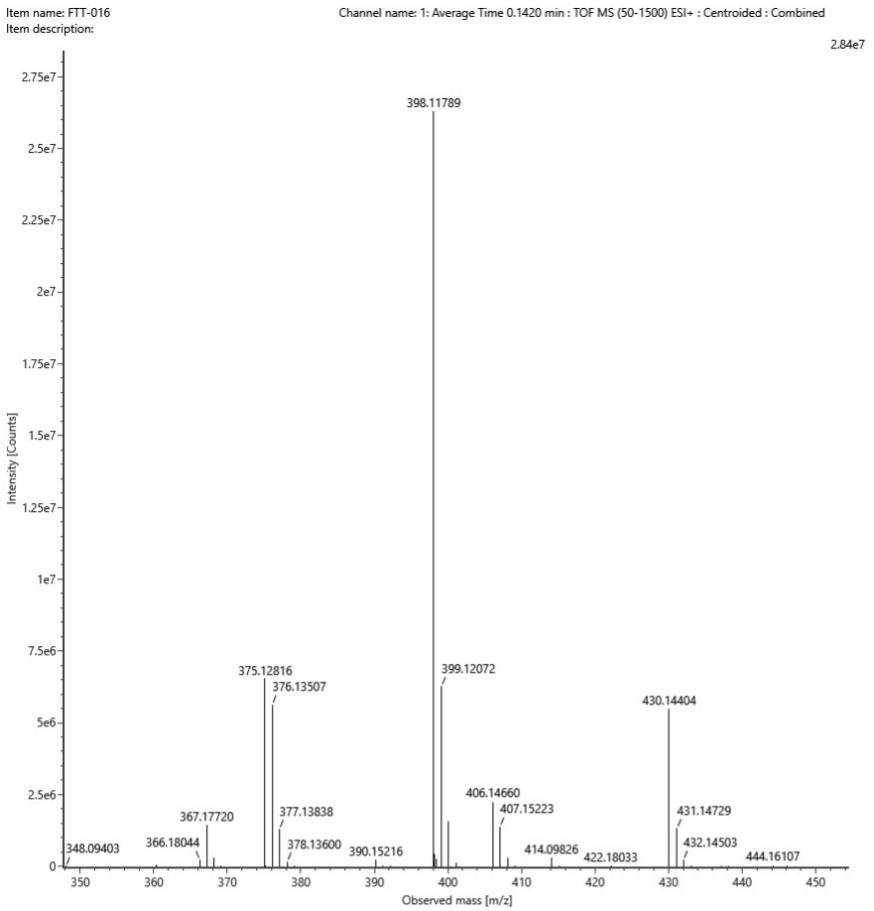


Fig. S3. HRMS spectrum of **2**

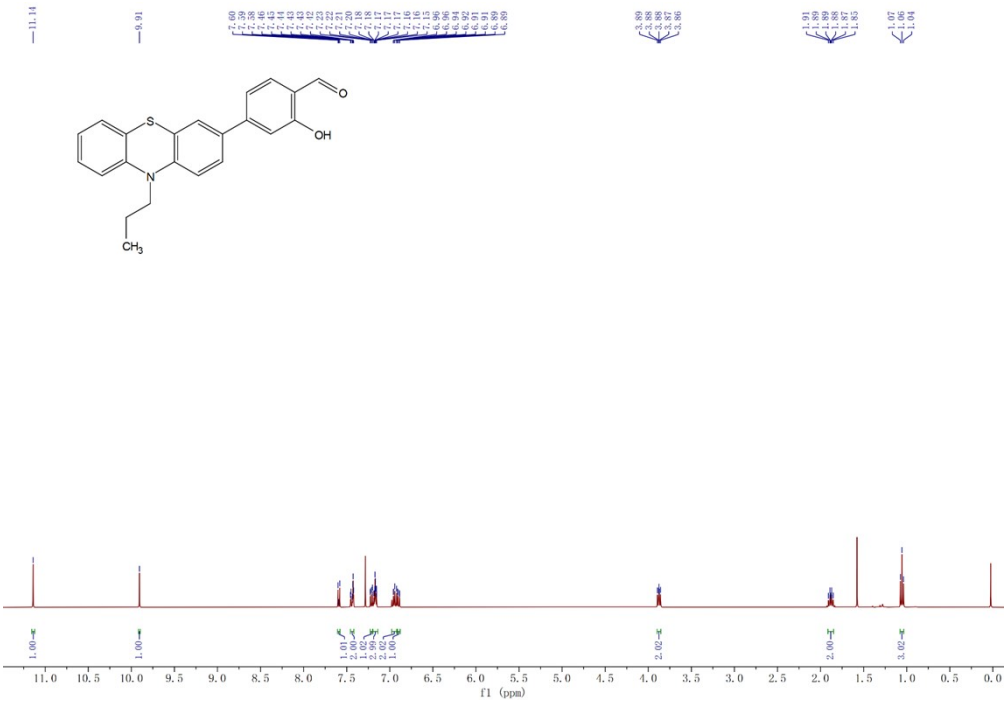


Fig. S4. ^{1}H NMR spectrum of **3**

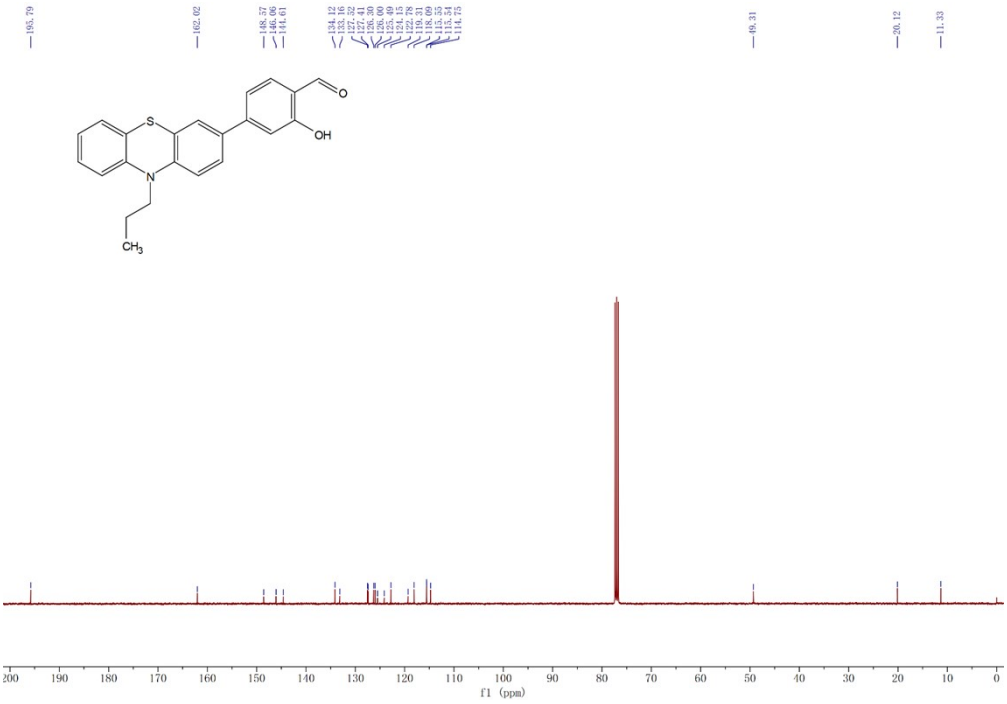


Fig. S5. ^{13}C-NMR spectrum of **3**

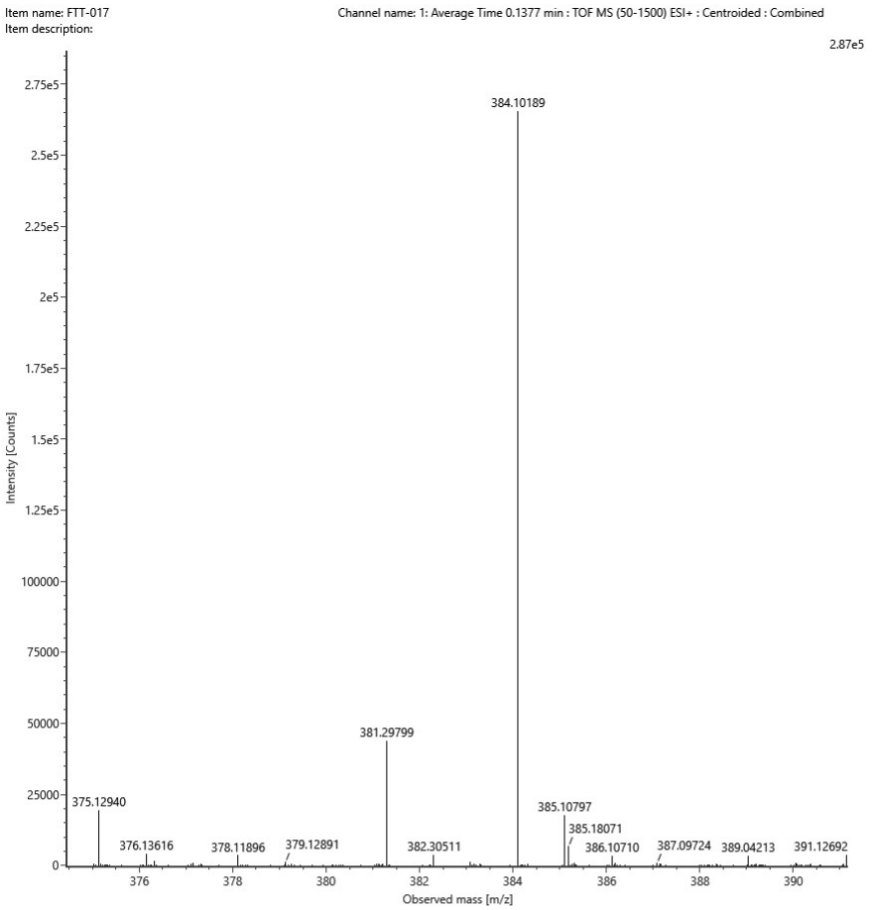


Fig. S6. HRMS spectrum of **3**

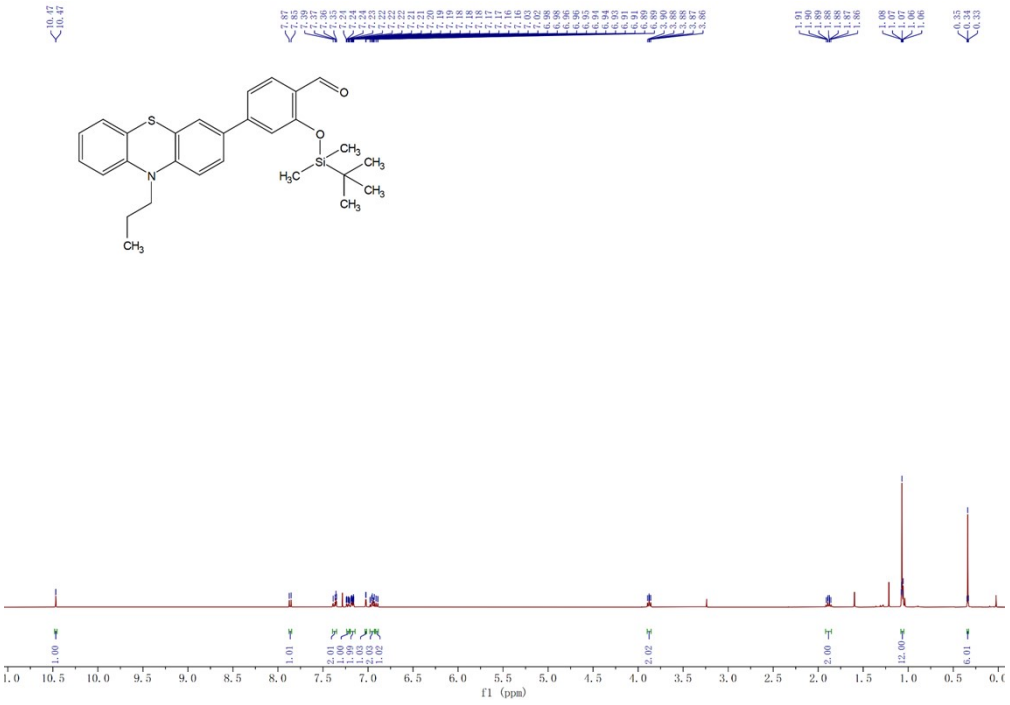


Fig. S7. ^{1}H NMR spectrum of **4**

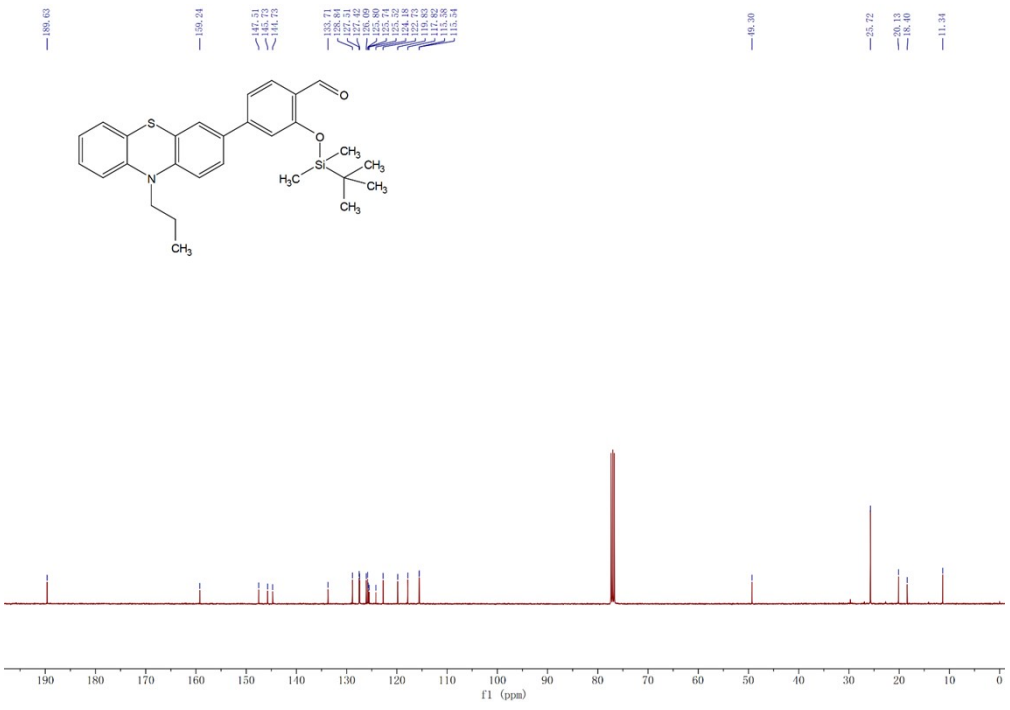


Fig. S8. ^{13}C-NMR spectrum of **4**

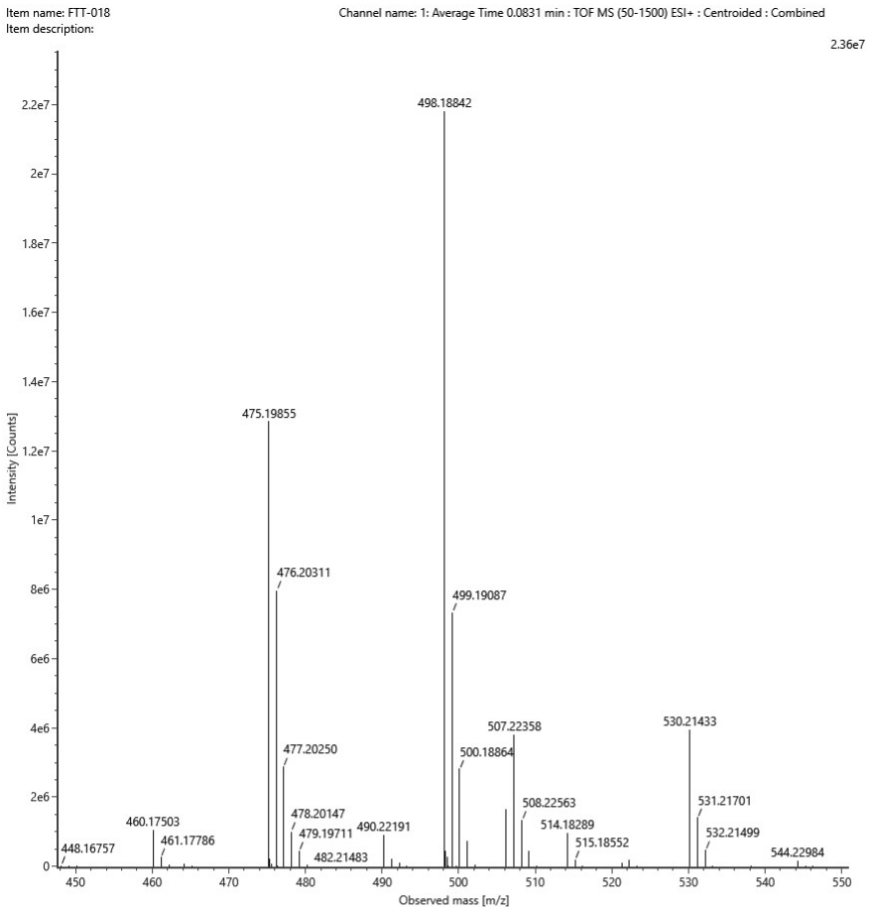


Fig. S9. HRMS spectrum of **4**

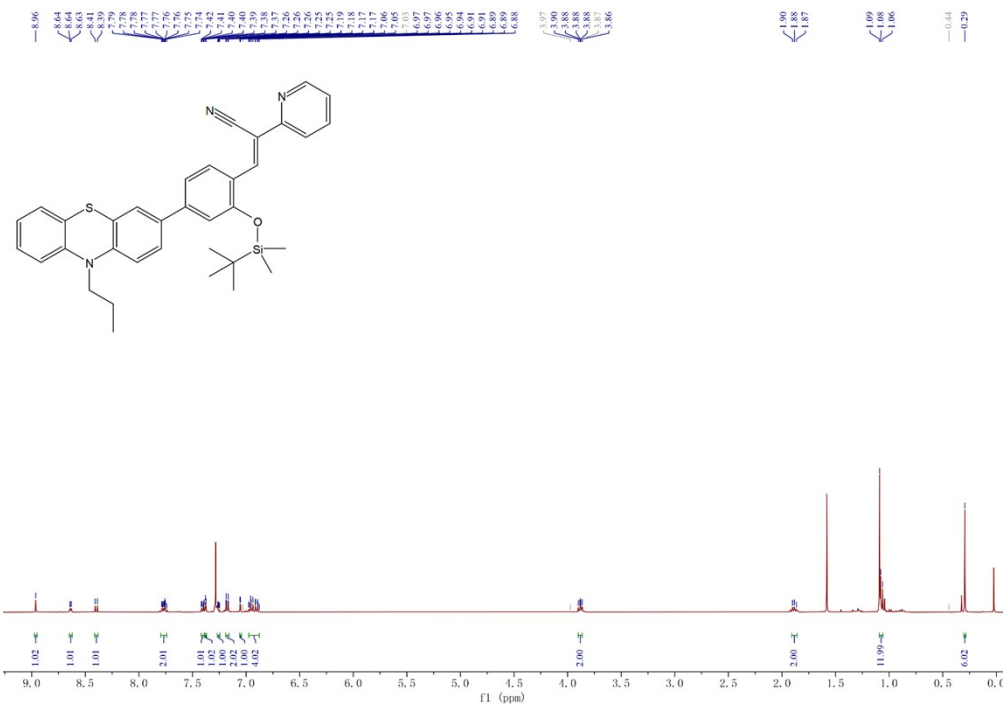


Fig. S10. ^{1}H NMR spectrum of **DTP**

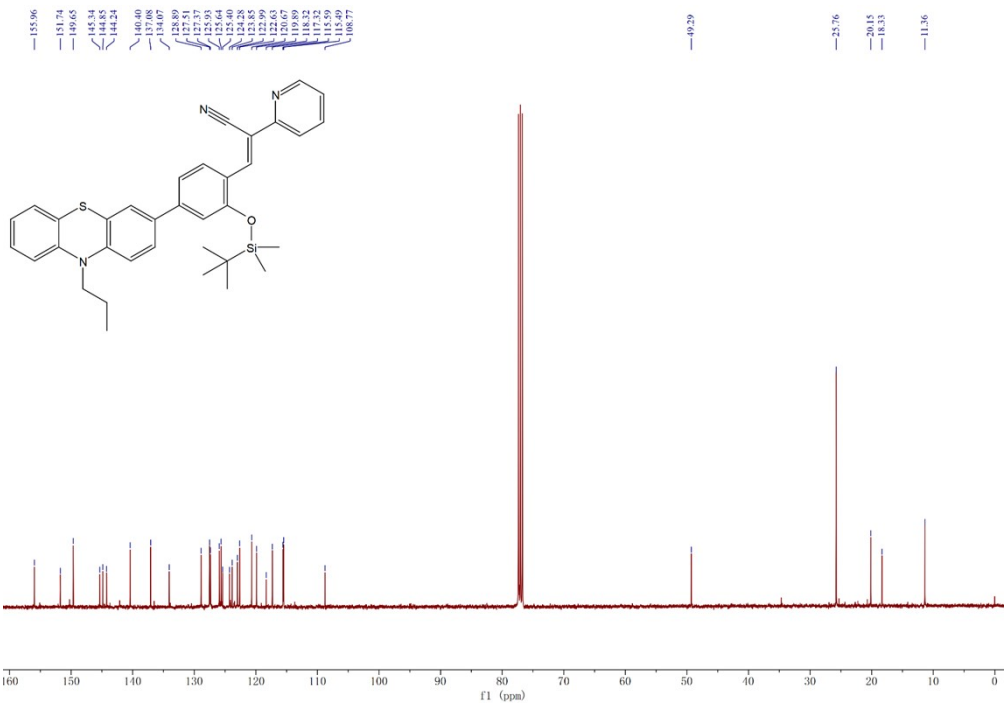


Fig. S11. ^{13}C-NMR spectrum of **DTP**

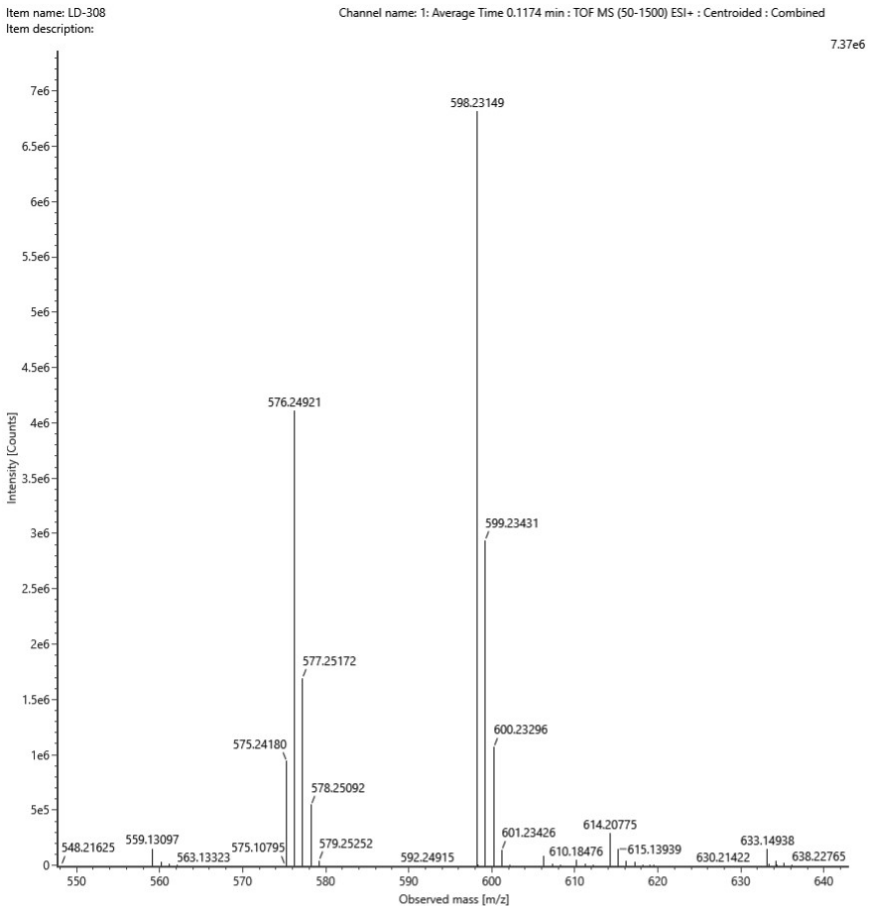


Fig. S12. HRMS spectrum of **DTP**

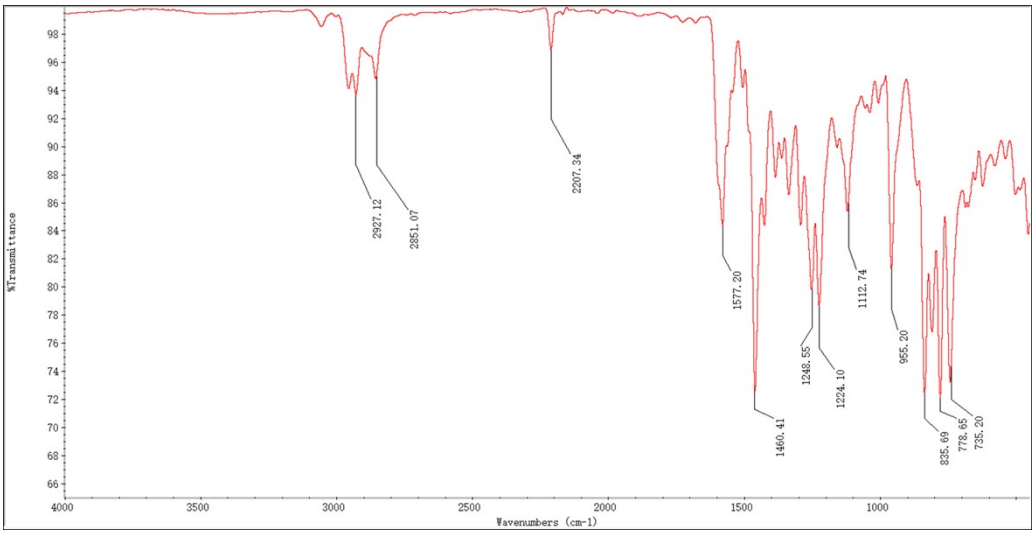


Fig. S13. FT-IR spectrum of **DTP**

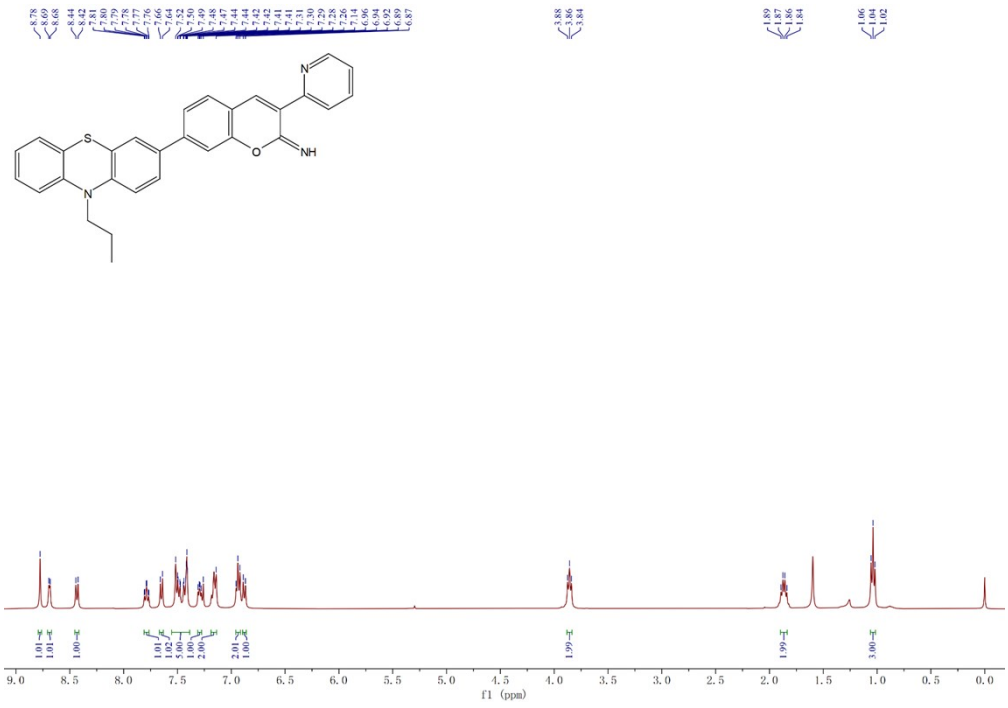


Fig. S14. [1]H NMR spectrum of **DTP-F**

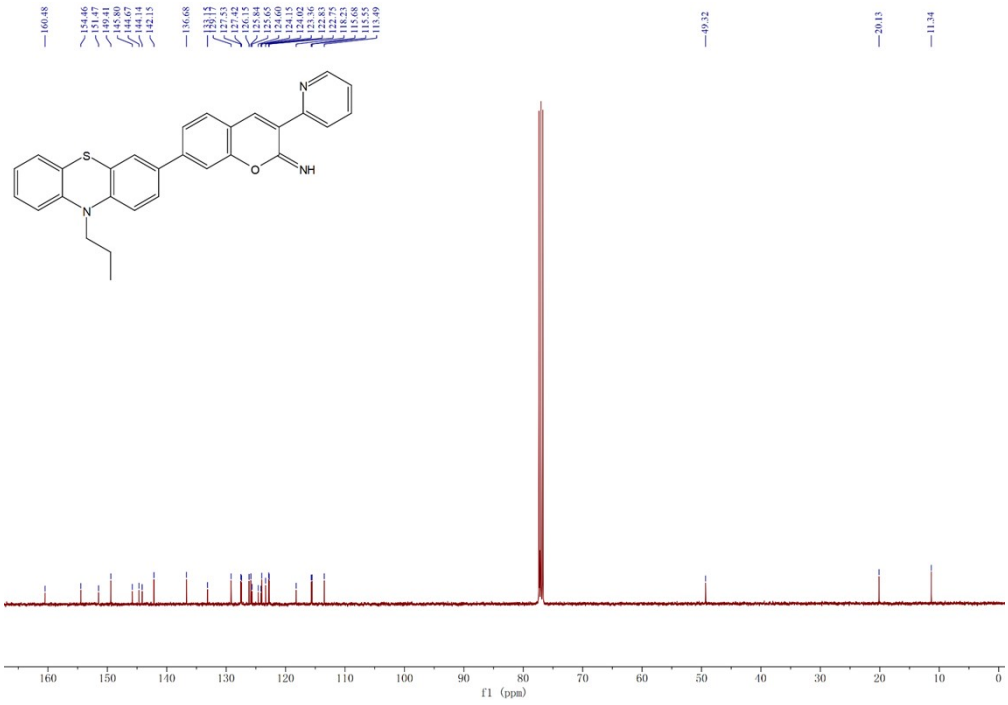


Fig. S15. [13]C-NMR spectrum of **DTP-F**

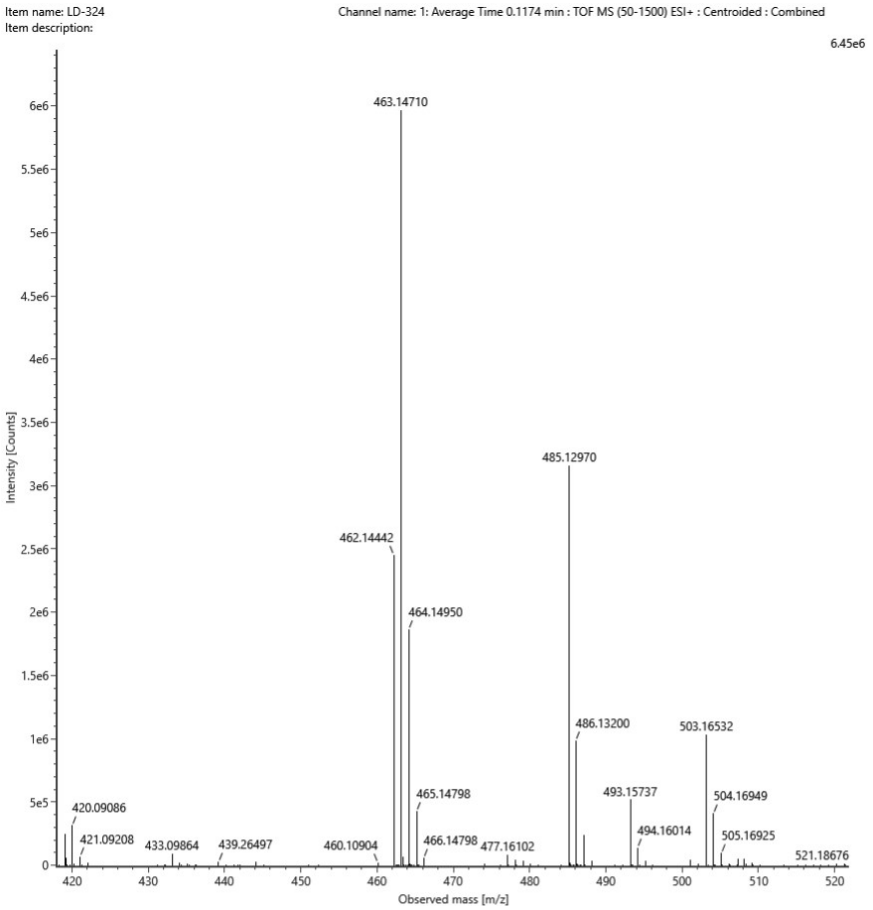


Fig. S16. HRMS spectrum of **DTP-F**

5. Supporting tables

Table S1 Fluorescent probe for fluoride ions based on Si-O bond cleavage

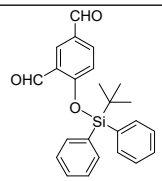
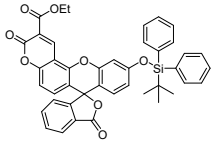
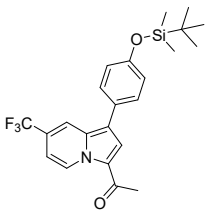
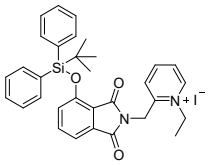
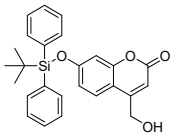
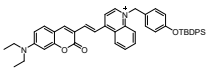
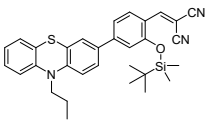
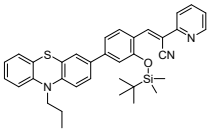
Probe	Structure	Solvent	Response time	Detection limit(μM)	$\lambda e_{x/em}$ (nm)	Reference
BIPA		Pure EtOH	2 h	3.5	320/480	42
Flu-Si		CH_3OH/H_2O	-	0.025	320/532	43
Silyl-KIZ		PBS	0.5 h	1	390/490	44
SPI		PBS	8 min	0.0116	413/511	45
5		HEPES	40 min	0.1	375/473	46
Mito-PF		DMSO/PBS	35 min	0.771	450/560	47
LDT		Pure THF	4 h	0.35	380/450	48
DTP		DMSO/HEPES	5 min	0.044	510/590	This work

Table S2 Test the fluorescence intensity of **DTP** (20 μM) containing serum in DMSO-HEPES buffer (50 mM, pH = 7.4, 9: 1, v/v).

Number	1	2	3	4	5	6	7	8	9	10	SD
FI	19.70	19.69	19.72	19.73	19.72	19.70	19.69	19.72	19.71	19.71	0.013