Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Cascade Reactions Based Fluorescent Probe for Rapid and Selective Fluoride Ion Detection

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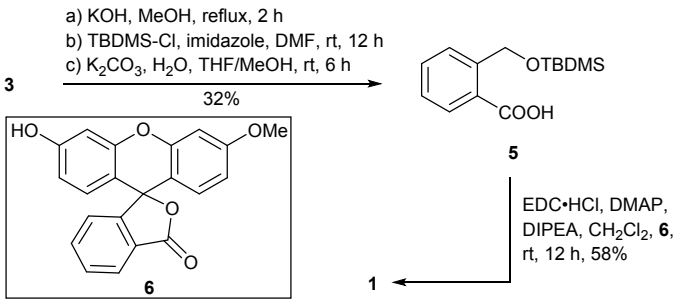
I. Materials and Instrumentation:

All reactions were performed under the nitrogen atmosphere. All the chemicals were purchased from commercial sources and used as received unless stated otherwise. Solvents were dried by standard methods prior to use. TLC was carried out with E. Merck silica gel 60-F_{254} plates and column chromatography was performed over silica gel (100-200 mesh) obtained from commercial suppliers.

The ^{1}H and ^{13}C spectra were recorded on 400 MHz Jeol ECS-400 (or 100 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal reference or from internal tetramethylsilane on the δ scale (DMSO-d_6 δ_H 2.5 ppm, $CDCl_3$ δ_H, 7.24 ppm, δ_C 77.0 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: s (singlet), d (doublet) m (multiplet), t (triplet), dd (doublet of doublet), td (triplet of doublet). A low-resolution mass spectrum was recorded on an Applied Biosystems 4800 Plus MALDI TOF/TOF analyzer. High-resolution mass spectra were obtained from MicroMass ESI-TOF MS spectrometer. Absorption spectra were recorded on a PerkinElmer, Lambda 45 UV-Vis spectrophotometer. Steady State fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on a Fluoromax 4 instrument (Horiba Jobin Yvon). (FT-IR) spectra were obtained using NICOLET 6700 FT-IR spectrophotometer as KBr disc and reported in cm^{-1}. Melting points were measured using a VEEGO Melting point apparatus. All melting points were measured in open glass capillary and values are uncorrected. HPLC purity was performed on an Aglilent model with Zorbax SB C-18 reversed phase column (250 nm × 4.6 nm, 5 μm). Cell images were taken in 35 mm (diameter) dishes. The media (DMEM) and PBS buffer were purchased from commercial sources. Fluorescence images were taken using Olympus Inverted IX81 equipped with Hamamatsu Orca R2 microscope. ChemBio Draw Ultra and Image *J* software were used for drawing structure and for processing cell image respectively.

II. Synthetic Procedures.



Scheme S1. Synthesis of probe **1**.

Synthesis of 2-(((*tert*-butyldimethylsilyl)oxy)methyl)benzoic acid 5:

Step I: In a 50 mL round bottom flask Phthalide **3** (1.00 g, 7.40 mmol) and KOH (0.49 g, 8.88 mmol) were dissolved in MeOH (24 mL). Reaction mixture was refluxed for 2 h until all starting material got consumed which was monitored by TLC analysis. The resulting reaction mixture was allowed to cool to room temperature and solvent was evaporated under reduced pressure to obtain white residue. This crude solid was used directly for the next step.

Step II: In same round bottom flask crude white solid was dissolved in DMF (6.0 mL) and then imidazole (1.50 g, 22.20 mmol) was added to it. *tert*-butyldimethylsilylchloride (2.22 g, 14.80 mmol) dissolved in dimetlyformamide (DMF) (9.0 mL) was added dropwise to the reaction mixture at room temperature and resultant reaction mixture was stirred at room temperature for overnight. After completion of reaction, reaction mixture was diluted with water (15 mL) and diethyl ether (15 mL). Organic layer was separated. And aqueous layer was extracted with diethyl ether (3×10 mL). The combined organic layer was washed with brine and dried over Na_2SO_4 and concentrated in vacuo and directly used for next step.

Step III: In a 25 mL round bottom flask, the crude residue was dissolved in MeOH (2.5 mL) and THF (2.5 mL). Potassium carbonate (2.04 g, 14.80 mmol) in water (7 mL) was added to it and stirred at room temperature for 6 h. Solvent was removed under reduced pressure and residue was acidified to pH 5-6 by dropwise addition of (1N) HCl. Reaction mixture was diluted with water (10 mL) and diethyl ether (10 mL). Organic layer was separated and aqueous layer was extracted with diethyl ether (3×10 mL). The combined organic layer was dried over Na_2SO_4, and solvent was evaporated under reduced pressure to obtain residue which was subjected to column chromatography over silica gel to afford 32% compound **5** (0.30 g) as white solid (*Eluent:* 10% EtOAc in petroleum ether). **M.p.:** 90 – 91 ^{o}C; **IR (KBr):** ν/cm^{-1}: 1699, 1602, 1576, 1466, 1442, 1404, 1306, 1262, 1197, 1145; **^{1}H NMR(400 MHz, $CDCl_3$):** δ 8.08 (dd, *J* = 1.2, 8.0 Hz, 1H); 7.7 (d, *J* = 7.6 Hz, 1H), 7.59 (td, *J* = 1.2, 8.7 Hz, 1H), 7.36 (td, *J* = 1.2, 7.6 Hz, 1H), 5.06 (s, 2H), 0.96 (s, 9H), 0.15 (s, 6H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 172.5, 143.8, 132.9, 131.1, 126.4, 126.3, 126.0, 63.1, 25.5, 17.9, 5.7; **HRMS (ESI):** Calc. for $C_{14}H_{22}O_3Si$ [M-H]$^{-}$: 265.125; Found: 265.125.

Synthesis of 3'-methoxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanphen]-6'-yl-2-(((*tert*-butyldimethyl)oxy)methyl)benzoate 1: Compound **6** was prepared using reported procedure.[S1] In a 25 mL round bottom flask, a mixture of compound **5** (0.15 g, 0.43 mmol) and compound **6** (0.12 mg, 0.43 mmol) was dissolved in CH_2Cl_2 (7 mL) and then EDC.HCl (99.87 mg, 0.52 mmol), DIPEA (0.29 μL, 1.73 mmol) and DMAP (0.01 g, 0.08 mmol) were added simultaneously at room temperature and stirred for 12 h at room temperature. Solvent was removed from reaction mixture under reduced pressure and the crude residue was directly subjected to column chromatography over silica gel to afford probe **1** (0.15 g, 58%) as white solid (*Eluent:* 15% EtOAc in petroleum ether). **M.p.:** 150 – 151 ^oC; **HPLC Purity:** 99.3%; **IR (KBr):** ν/cm^{-1}: 1755, 1613, 1507, 1421, 1256, 1027, 847; **^{1}H NMR (400 MHz, DMSO-d_6):** δ 8.14 (d, *J* = 7.6 Hz, 1H); 8.05 (d, *J* = 7.2 Hz, 1H), 7.84-7.72 (m, 4H), 7.49 (t, *J* = 6.8 Hz, 1H), 7.43 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.08 (dd, *J* = 2.4, 8.6 Hz, 1H), 6,98 (d, *J* = 2.4 Hz, 1H), 6.91(d, *J* = 8.8 Hz, 1H), 6.78-6.72 (m, 2H), 5.09 (S, 2H), 3.81(s, 3H) 0.98 (S, 9H), 0.08 (S, 6H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 169.4, 164.7, 161.5, 153.1, 152.3, 152.1, 152.0, 145.9, 138.5, 135.2, 133.7, 131.0, 129.9, 129.2, 129.1, 126.7, 126.6, 125.2, 125.1, 124.1, 117.7, 116.9, 112.1, 110.9, 110.7, 100.9, 82.4, 63.3, 55.7, 26.1, 18.5, 5.3; **HRMS (ESI):** Calc. for $C_{35}H_{34}O_7Si$ $[M+H]^+$: 595.2148; Found: 595.2156.

III. Photophysical Studies:

Procedures:

Preparation of the sample solution: A stock solution of probe **1** (2000 μM) was prepared in Acetonitrile. The final concentration during assay is 10 μM. Stock solution of TBAF was prepared by diluting 1M TBAF in THF.

Preparation of the solution of analytes: Stock solutions of TBABr, TBAI, TBACl, $TBAClO_4$, $TBAPF_6$, $TBANO_3$, $TBAHSO_4$, TBAOAc, $(TBA)_2SO_4$ were prepared in THF and stock solutions of H_2O_2, Cysteine (Cys), Glutathione (GSH) were prepared in deionized water. Calculated volumes of analytes were added from respective stock solutions to each fluorescence cuvette to provide 3 mM. Stock solution of TBAF was made in water for determining detection limit and for all assay to detect fluoride in aqueous media.

General method for UV-Vis and Fluorescence titration: For absorbance and emission studies 1 cm path length was used for cells. The excitation and emission slit width were 2 nm and 3 nm, respectively. All spectral data were recorded at 7 min after the addition of analyte(s) to the solution of probe **1** and TBAF in DMSO at λ_{ex} = 460 nm.

IV. Fluoride sensing:

In order to calculate enhancement of fluorescence emission intensity, fluorescence spectra of probe **1** (10μM) in DMSO and after addition of TBAF (300 equivalent) into the probe **1** was plotted. The probe **1** showed 550 fold enhancement of fluorescence intensity at λ_{em} = 523 nm (upon λ_{ex} = 460 nm) after sensing F^- (Fig. S1A). To evaluate any interference of any competitive analytes fluorescence spectra of probe **1** in presence of different anions were taken in DMSO. No significant fluorescent intensity was observed in this stage but increment in fluorescence intensity was observed only after adding F^- to that reaction mixture in DMSO (Fig. S1B). Each spectrum was taken after 7 min of addition of TBAF to the reaction mixture.

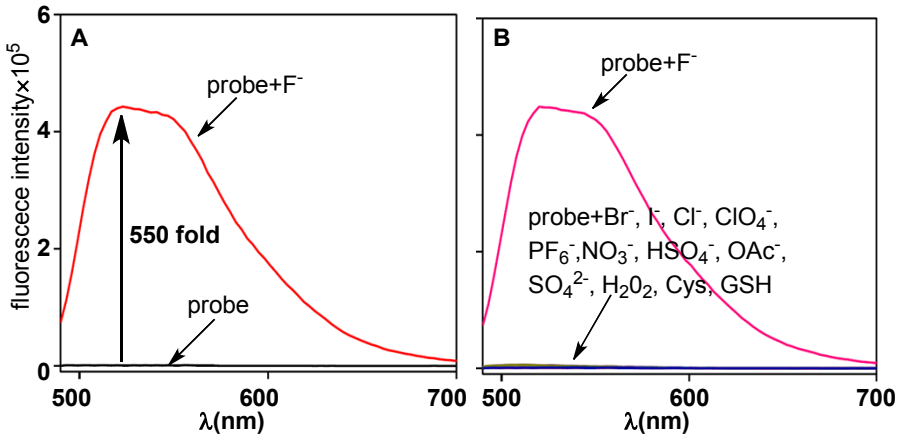


Fig. S1 (**A**) Fluorescence spectra of probe **1** (10 μM) and after adding TBAF (3mM) in DMSO. (**B**) Fluorescence spectra of probe **1** (10 μM) in the presence of different analytes (3 mM) in DMSO (λ_{ex} = 460nm) and after adding Fluoride.

Upon addition of tetrabutylammonium fluoride, TBAF (300 equivalent) to the solution of probe **1** (10 μM), a fluorescence band centered at λ_{em} = 523 nm (λ_{ex} = 460 nm) was observed (Fig. S1) with increasing time.

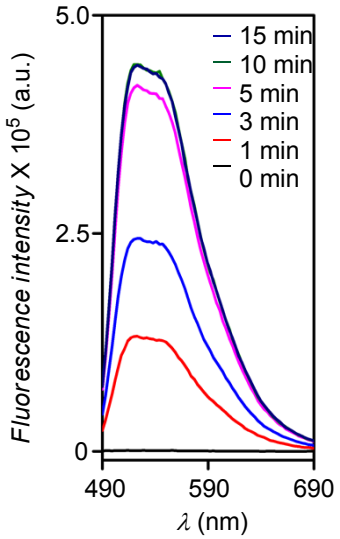


Fig. S2 Fluorescence spectra of probe **1** (10 μM) with increasing time in the presence of TBAF (300 equivalent) in DMSO

Determination of quantum yields:

The quantum yield of probe **1** was determined according to the Equation 1:

$$\Phi_1 = \Phi_B \times \frac{I_1 \times A_B \times \lambda_{exB} \times (\eta_1)^2}{I_B \times A_1 \times \lambda_{ex1} \times (\eta_B)^2} \qquad \text{(Equation 1)}$$

where, Φ is quantum yield; I is integrated area under the corrected emission spectra; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts 1 and B refer to the unknown and the standard, respectively.

Determination of reaction rate (k) and half-life ($t_{1/2}$) for probe 1:

Reaction rate (k), half-life ($t_{1/2}$) and response time (t_R) for probe **1** was determined by fluorescence kinetics experiments. In fluorescence kinetics experiment, Fluoride (3 mM) (either in THF or in H_2O) was added to the solution of probe (10μM) in DMSO in a cuvette at room temperature at t = 60 s and fluorescence intensity at λ = 523 nm (upon λ_{ex} = 460 nm) were recorded. Rate constant, k and response time (t_R) for probe **1** was determined according to the following equation (Equation 2):

$$Y = a \times [1 - e^{(-kt)}] \qquad \text{(Equation 2)}$$

Where, Y = fractional fluorescence intensity, a = arbitrary constant, k = pseudo first order rate constant, t = time.

Half-life of the reaction ($t_{1/2}$) was calculated using Equation (3)

$t_{1/2} = 0.693/k$ (Equation 3)

Where k = pseudo first order rate constant.

Fluoride detection in aqueous media:

As anions mostly occur as solutes in water or aqueous media, detection of such anions including F^- in aqueous media is still challenging task. As a result very few fluorescent probes were reported which are capable of detection of F^- either in aqueous conditions or mixture of organic and aqueous conditions. As probe **1** was not capable of F^- detection in aqueous conditions, to overcome this limitation we prepared the stock of F^- in water and assay was carried out in DMSO solvent.[S2, S3] The sensitivity of probe **1** towards F^- was comparable irrespective of the F^- source (water or THF) (Fig. 1A).

Detection Limit Calculation:

Detection limit for F^- was calculated using fluorometric titrations using equation, detection limit = 3σ/m, where σ = standard deviation of 6 blank measurements and m = slope obtained from the graph of fluorescence intensity *Vs* concentration of F^- added. [S4] Standard deviation = 176.3474 and slope from the graph is 509.73. So detection limit turns found to be 1.03 μM (19.6 ppb) (R = 0.987).

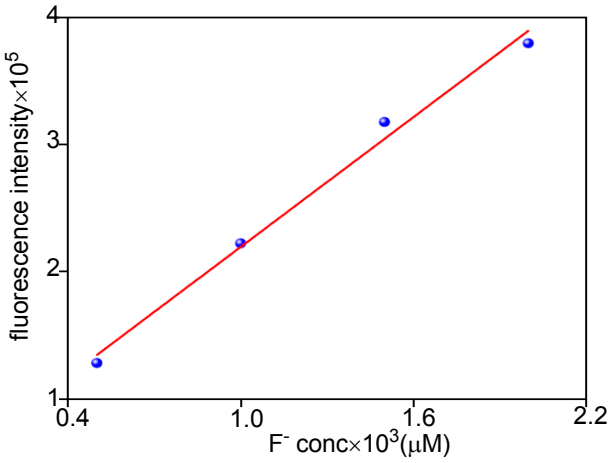
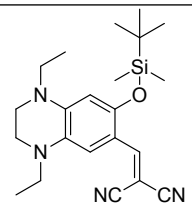
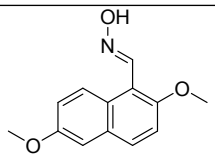
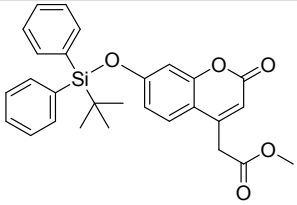
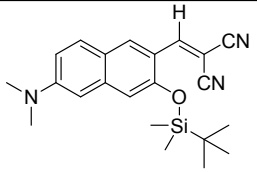
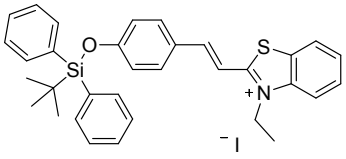
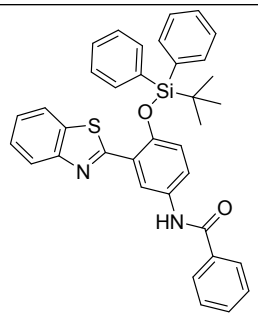
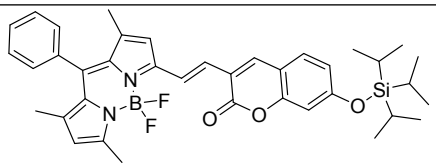
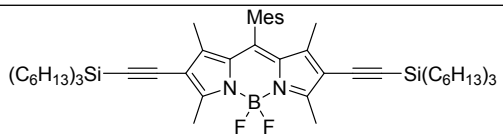
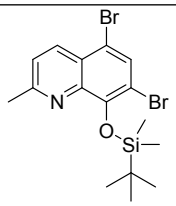
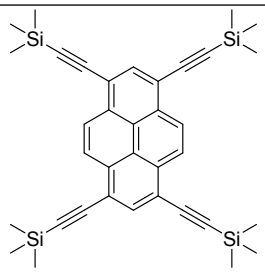
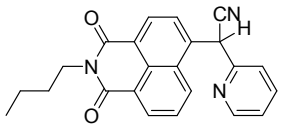
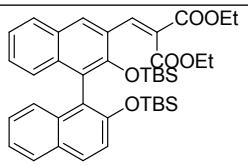


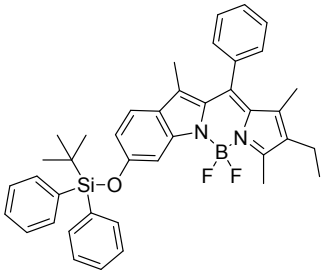
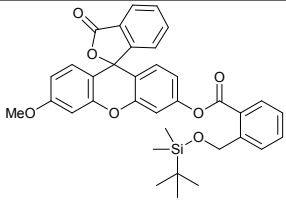
Fig. S3 Linear relationship of probe **1** (10 μM) at 523 nm between the fluorescence emission intensity of probe **1** and concentration (0.5 mM – 2 mM) of TBAF (in water) in DMSO (upon λ_{ex} = 460 nm). Each spectrum was recorded 7 min after addition of TBAF to the solution of probe **1**.

Comparison of detection limit with reported probes:

Table S1. Comparison of detection limit and response time of probe **1** with reported probes.

Probe	Detection limit	Solvent	Time	Reference
	5.4 μM	HEPES: ACN (7:3)	10 min	S5
	700 μM	DMSO	ND	S3
	380 μM	HEPES	4h	S6
	210 μM Below 4 ppm	HEPES:ACN (8:2)	60 min	S7
	80 μM	EtOH:H_2O (3:7)	50 min	S8

	5.2 μM 100 ppb	2mM CTAB in H_2O	4 min	S9
	0.12 μM	DMSO	ND	S10
	0.067 μM	Acetone	ND	S11
	1 μM	THF	10s	S12
	52 μM 1 ppm	THF	20s	S13
	6.73 μM	CAN	ND	S14
	1.86 μM	THF	ND	S15

	0.1 μM	CH_2Cl_2	30s	S16
	1 μM	DMSO	7 min	Present work

Fluoride concentration in water sample:

In order to evaluate the quantitative detection of F^-, increasing concentrations (0.15 mg/mL to 0.90 mg/mL) of TBAF in H_2O were added to probe **1** (10 μM) in DMSO and change in fluorescence intensity was recorded. As shown in Figure S5, probe **1** was showing steady increment in fluorescence intensity as a function of F^- concentration.

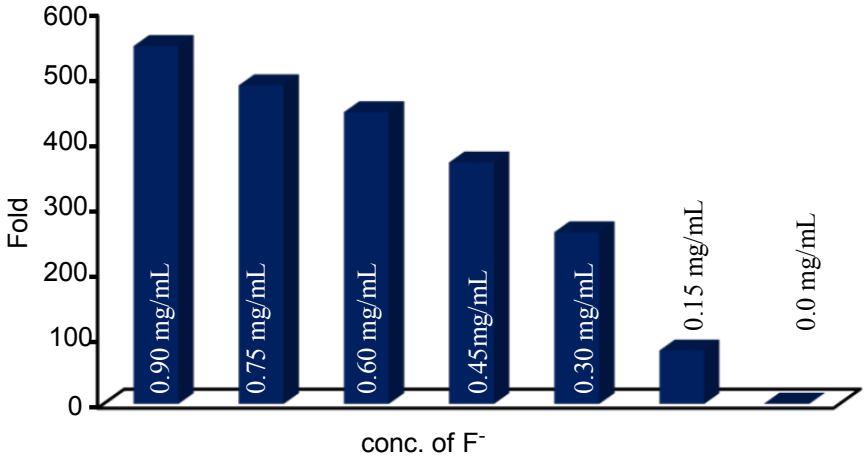


Fig. S4 Fluorescence intensity of probe **1** (10 μM) in DMSO upon addition of increasing concentration of F^- in water at λ_{em} = 523 nm (upon λ_{ex} = 460 nm).

pH dependence:

In the next stage, we examined the effect of pH on F^- detection by **1**. Stock of F^- was prepared in the Phosphate buffer with pH ranging from 3 to 11. These stock solutions of F^- (3mM) were added to **1** (10μM) in DMSO and fluorescence spectra were recorded. Probe **1** was capable of sensing F^- in the studied pH range. Each spectrum was recorded 7 min after addition of F^- to the solution of probe **1**.

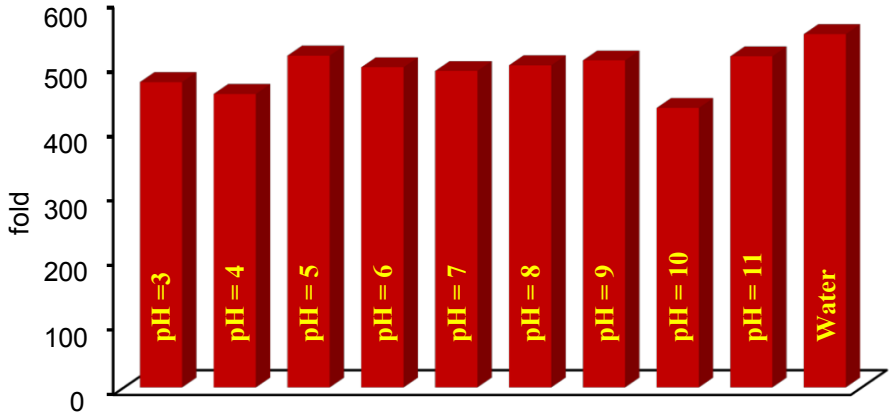


Fig. S5 Fluorescence intensity of probe **1** (10 μM) in DMSO upon addition of $TBAF^-$ in Phosphate buffer of pH range 3 – 11 at λ_{em} = 523 nm (upon λ_{ex} = 460 nm).

Mass (MALDI-TOF) analysis of reaction mixture of probe 1 with TBAF:

Probe **1** was reacted with TBAF in THF at room temperature for 7 min and mass analysis of reaction mixture was carried out (Fig. S6). In mass spectrum peaks corresponding to $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ were observed which confirms the formation of fluorescent compound **2**.

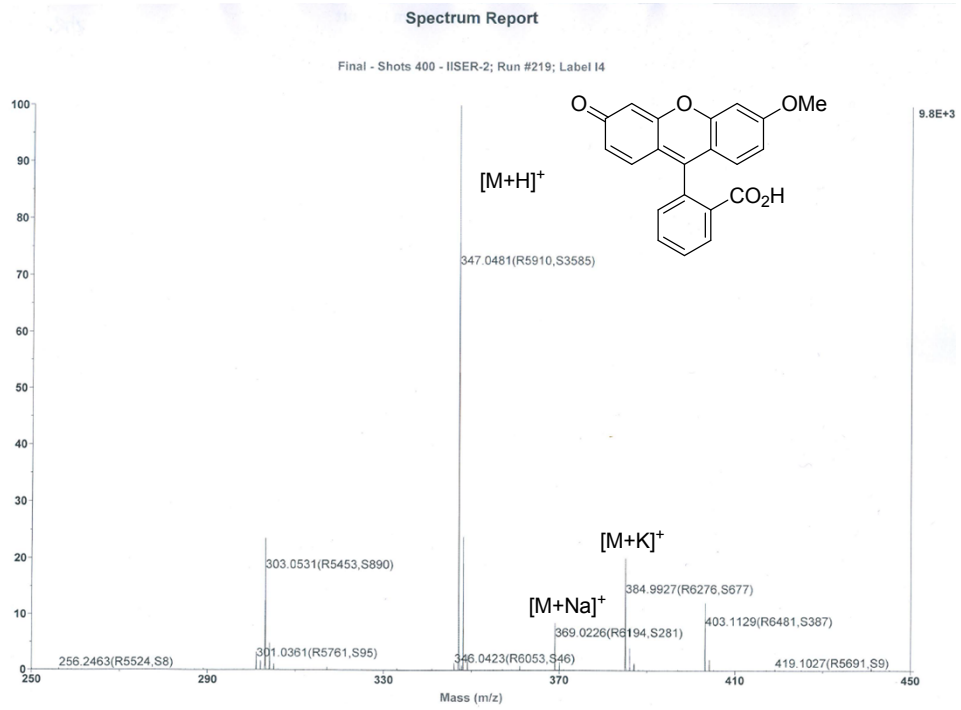


Fig. S6 MALDI-TOF spectrum of the probe **1** after reaction with TBAF in THF.

V. Cell Imaging:

The HeLa cells were purchased from National Centre for Cell Science, Pune (India). HeLa cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 ^oC. The cultured cells were subcultured twice in each week, seeding at a density of about 15×10^3 cells/ml. Typan blue dye exclusion method was used to determine Cell viability. The fluorescence images were taken using Olympus Inverted IX81 equipped with Hamamatsu Orca R2 microscope by exciting at λ_{ex} = 460-480 nm (by using GFP filter). The HeLa cells were incubated with solution of the probe (10 μM in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37 ^oC for 2 h. After washing with PBS the fluorescence images were acquired. In this case no significant fluorescence was observed. Another set of HeLa cells were treated with Probe **1** (10 μM in 1:100 DMSO-DMEM v/v, pH = 7.4) at 37 ^oC for 2 h and then after washing the cells thoroughly by PBS, cells were incubated with solution of NaF (20 mM in

1:100 DMSO-DMEM v/v, pH = 7.4) at 37 °C for 30 min. After washing with PBS the fluorescence images were recorded.

VI. NMR Spectra:

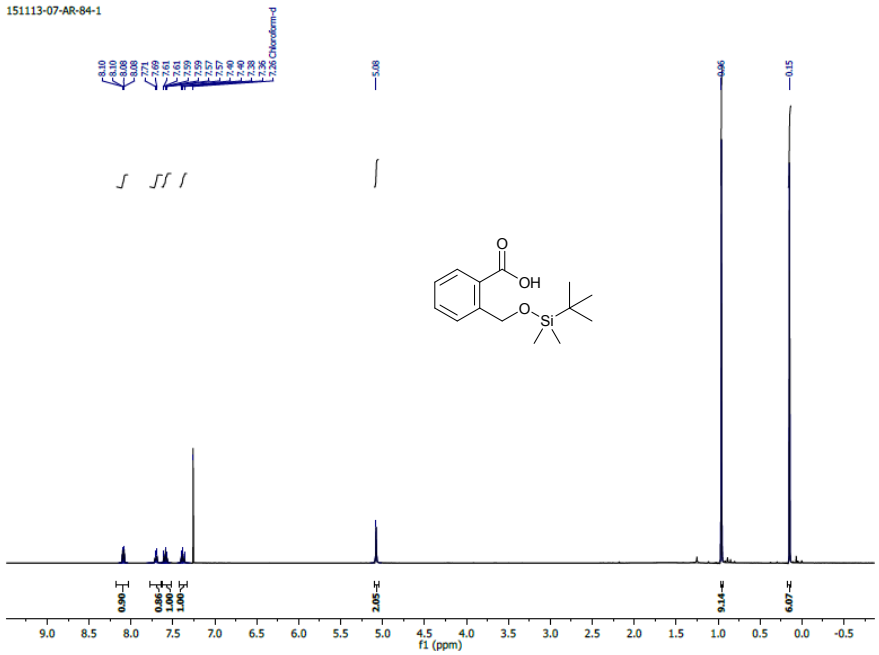


Fig. S7 ^{1}H NMR spectra of **5** in $CDCl_3$.

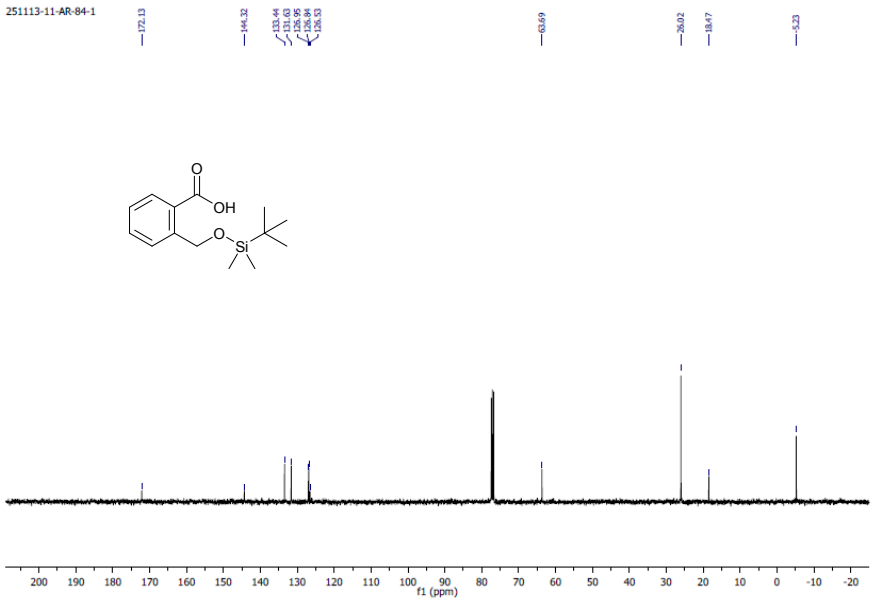


Fig. S8 ^{13}C NMR spectra of **5** in $CDCl_3$.

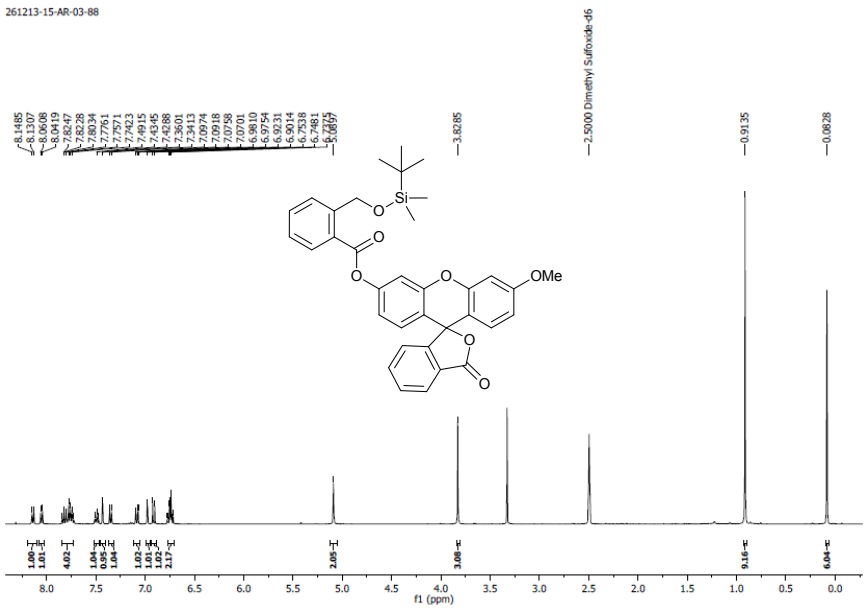


Fig. S9 ^{1}H NMR spectra of probe **1** in DMSO-d_6.

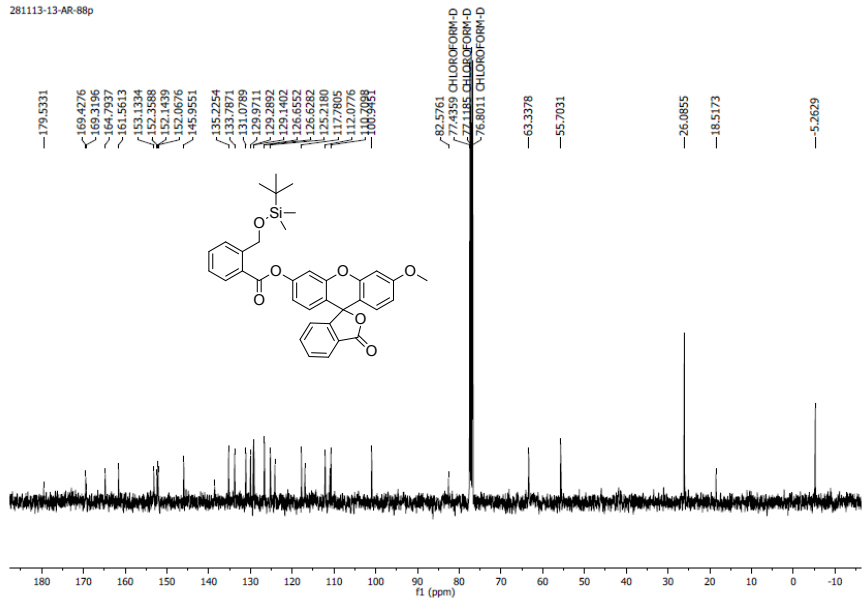
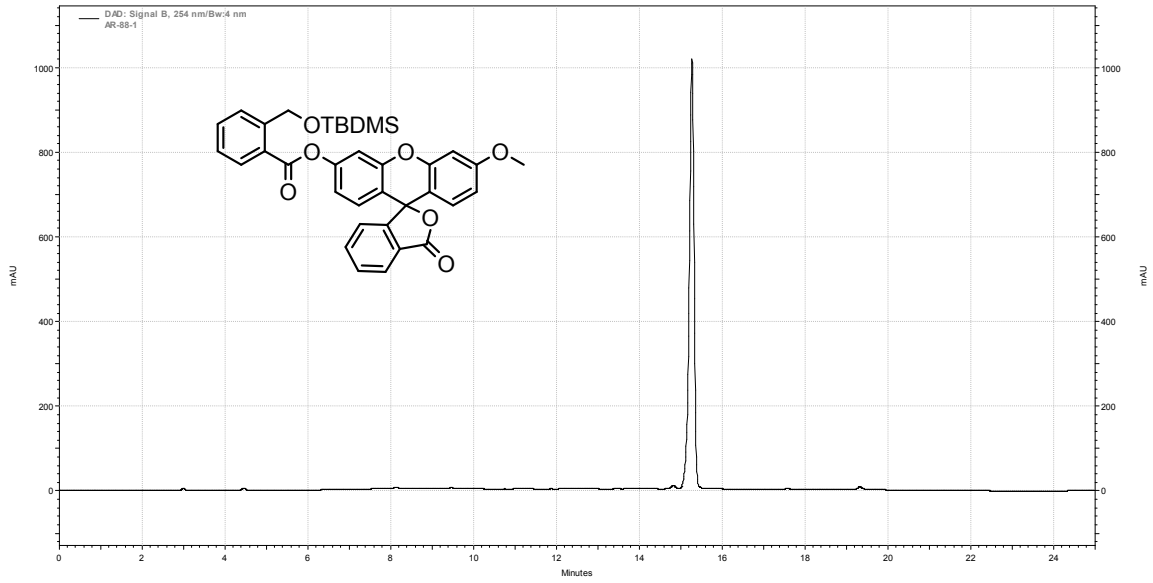


Fig. S10 ^{13}C NMR spectra of probe **1** in $CDCl_3$.

VII. HPLC Data:



DAD: Signal B,
254 nm/Bw:4 nm
Results

Retention Time	Area	Area %	Height	Height %
14.827	127045	0.74	17525	0.82
15.280	16987928	99.26	2126869	99.18
Totals	17114973	100.00	2144394	100.00

Fig. S11 HPLC Data of Probe **1**.

Column: Phenomenex (4.6 mm × 250 mm)

Flow: 1.0 mL/min

Method: Gradient

50 % Acetonitrile/water	0 min
100 % Acetonitrile	0 to10 min
100 % Acetonitrile	10 to 15 min
50 % Acetonitrile/water	15 to 20 min
50 % Acetonitrile/water	20 to 25 min

Wavelength: 250 nm.

Retention time (t_R) = 15.28 min.

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