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Supporting Information

Pink fluorescence emitting fluoride ion sensor: Investigation of the cascade sensing mechanism and bioimaging applications

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Contents **Page Number**

I. Fluoride sensing:

In order to calculate the fluorescent emission intensity enhancement, fluorescence spectra of probe **2** (10 μM) and after addition of F^- (50 eqv) were plotted. Probe **2** displayed 1820 fold fluorescence intensity enhancement after sensing of F^- (Fig. S1A). To evaluate any interference of competitive anions fluorescent spectra of probe **2** was taken in presence of different interfering anions in THF. Significant fluorescent emission was not observed in these cases but emission intensity increases only in presence of F^- to the reaction mixture in THF. Each spectrum was recorded after 10 min (Fig. S1B).

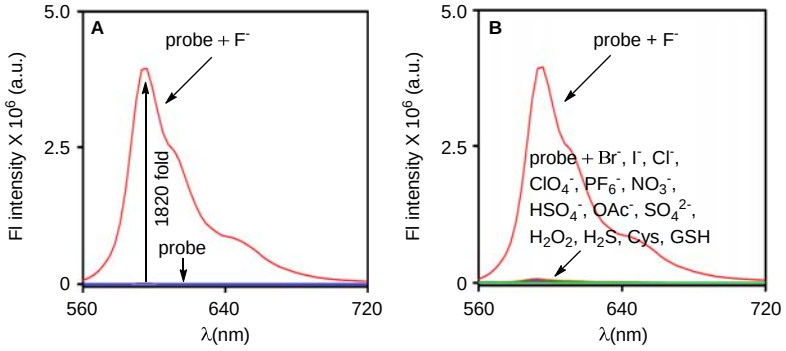


Fig. S1 (**A**) Fluorescence spectra of probe **2** (10 μM) and after adding F^- (50 equivalent) in THF. (**B**) Fluorescence spectra of probe **2** (10 μM) in the presence of different analytes (0.5 mM) in THF (λ_{ex} = 550 nm) and after adding F^- ions.

Absorbance spectra of probe **2** was recorded in THF and absorption maxima was observed at λ = 347 nm and 437 nm (Fig. S2).

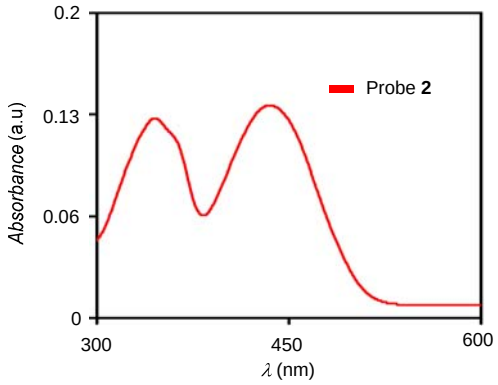


Fig. S2 Absorbance spectra of probe **2** (10 μM) in THF.

Absorbance spectra of probe **2** was recorded in THF and absorption maxima observed at λ = 437 nm and after addition of F^- was recorded and absorption peak was observed at λ = 550 nm, 573 nm, 591 nm (Fig. S3). Color of the reaction mixture also changed from light yellow to pink under visible light

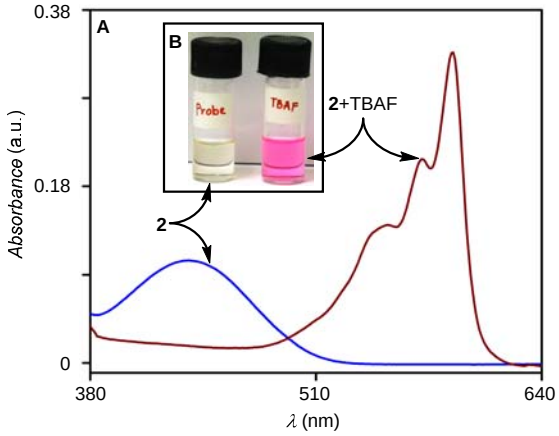


Fig. S3 Absorbance spectra of probe **2** (10 μM) and after adding TBAF (50 equivalent) in THF.

In order to evaluate the response of probe **2** titration was done with increasing concentration of F^- Fluorescent intensity increases with increasing concentration of F^- upto 0.4 mM further addition of F^- did not cause any increment of fluorescent intensity.

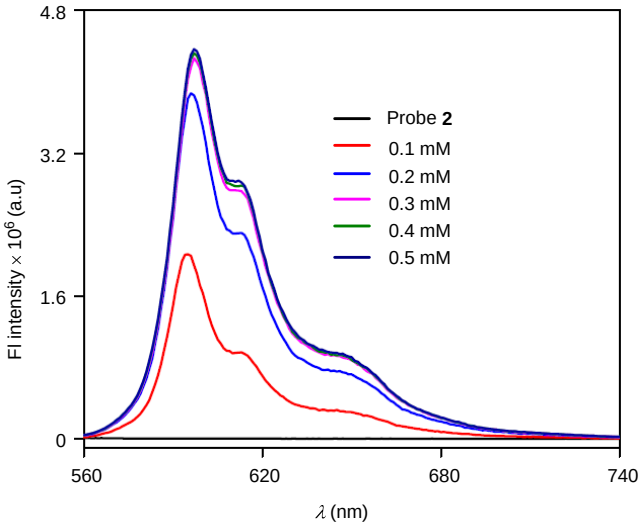


Fig. S4 Fluorescence intensity of probe **2** (10 μM in THF) with increasing concentration of F^- in THF (λ_{ex} = 550 nm). All data were recorded 10 min after addition fluoride ions.

Detection Limit Calculation:

For calculating detection limit, fluoride (50 – 300 μM) in water was added to probe **2** (10 μM in THF) and fluorescent intensity was recorded. By plotting fluorescence intensity with increasing concentration of fluoride, slope of graph was found to be 10459.68. Standard deviation was calculated from six blank measurements of probe **2**.

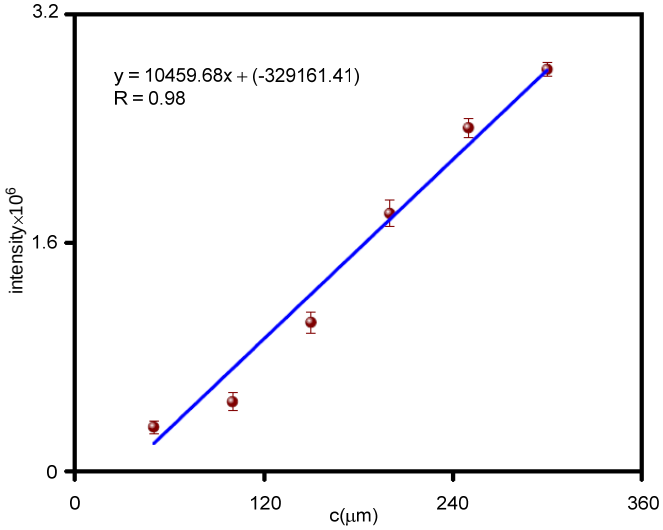


Fig. S5 Linear region of fluorescence intensity of probe **2** (10 μM) in THF upon addition of F^- (50 – 300 μM) in water at λ_{em} = 595 nm (upon λ_{ex} = 550 nm).

Calculation of standard deviation:

Table S1: Standard deviation for probe **2**.

Blank Readings (only probe **2**, 10 μM)	Fl Intensity
Reading 1	3287.99045
Reading 2	2930.91116
Reading 3	3156.99834
Reading 4	3498.67174
Reading 5	3473.11566
Reading 6	3307.41018
Standard Deviation (σ)	211.0996089

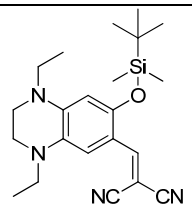
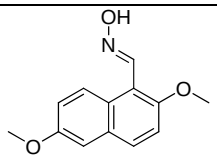
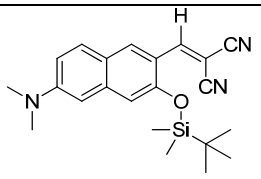
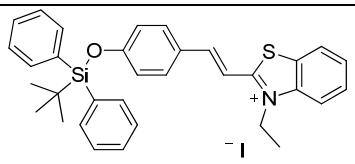
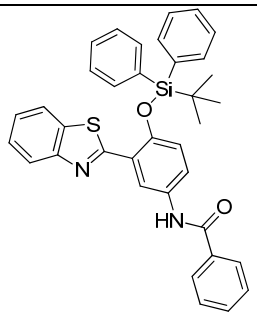
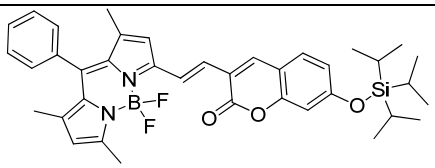
Calculation of Detection Limit:

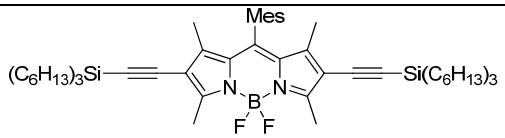
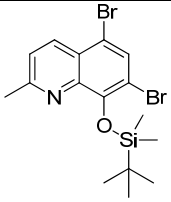
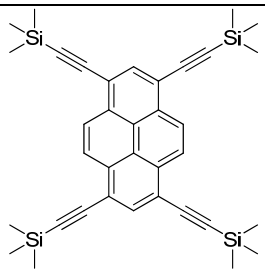
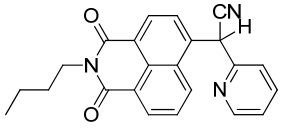
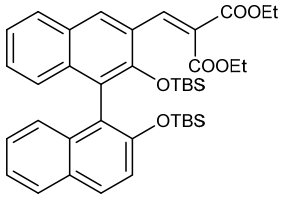
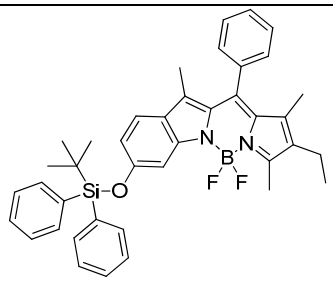
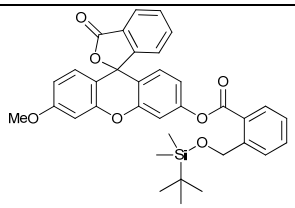
Table S2: Detection limit calculation for probe **2.**

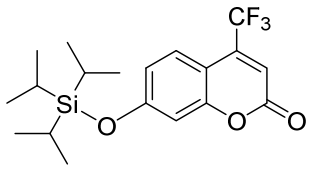
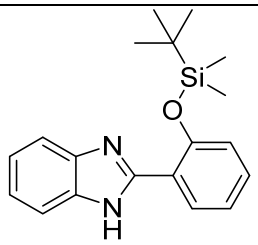
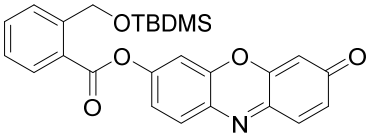
Slope from Graph (m)	10459.68	μM
Detection limit ($3\sigma/m$)	0.060546673	μM
	1.150386791	ppb

Comparison of detection limit with reported probes:

Table S3. Comparison of detection limit and response time of probe **2** with reported probes.

Probe	Detection limit	Solvent	Reaction Time	Reference
	5.4 μM	HEPES: ACN (7:3)	10 min	S1
	700 μM	DMSO	ND	S2
	210 μM Below 4 ppm	HEPES:ACN (8:2)	60 min	S3
	80 μM	EtOH:H_2O (3:7)	50 min	S4
	5.2 μM 100 ppb	2mM CTAB in H_2O	4 min	S5
	0.12 μM	DMSO	ND	S6

	0.067 μM	Acetone	ND	S7
	1 μM	THF	10 s	S8
	52 μM 1 ppm	THF	20s	S9
	6.73 μM	ACN	ND	S10
	1.86 μM	THF	ND	S11
	0.1 μM	CH_2Cl_2	30 s	S12
	1 μM	DMSO	7 min	S13

	50 nM	ACN	ND	S14
	0.19 μM	DMF-H_2O	40 min	S15
	60 nM 1.15 ppb	THF-Water	10 min	Present work

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 **2** was not capable of F^- detection in 100% aqueous

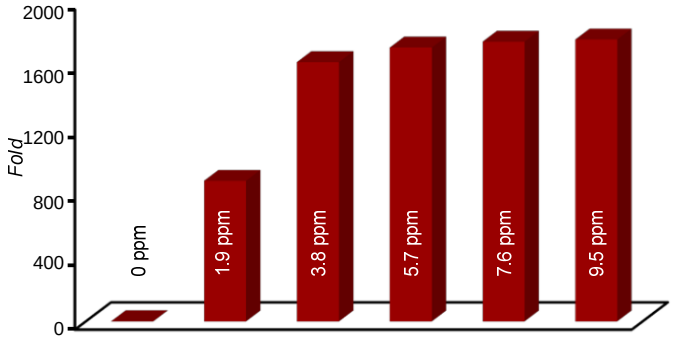


Fig. S6 Fluorescence intensity of probe **2** (10 μM) in THF upon addition of increasing concentration of F^- in water at λ_{em} = 595 nm (upon λ_{ex} = 550 nm).

conditions, to overcome this limitation we prepared the stock of F^- in water and assay was carried out in THF solvent.[S16-S17] The sensitivity of probe **2** towards F^- (in THF) was more with respect to the F^- source in water (Fig. 5B). Significant fluorescent intensity enhancement was observed when 3.8 ppm F^- in water was added to the probe **2** in THF (Fig. S6). Probe **2** showed excellent ability to detect low concentration of F^- in water (3.8 ppm) which is below 4 ppm, allowed concentration of F^- in drinking water specified by USEPA.

In order to describe the real behavior of probe **2** at low concentration of fluoride, probe (100 nM) was taken in THF and F^- (100 nM) in water was added to cuvette and fluorescence intensity was measured which resulted in upto ~ 20 fold increment in fluorescent intensity. Spectra was taken after 15 min of addition of fluoride.

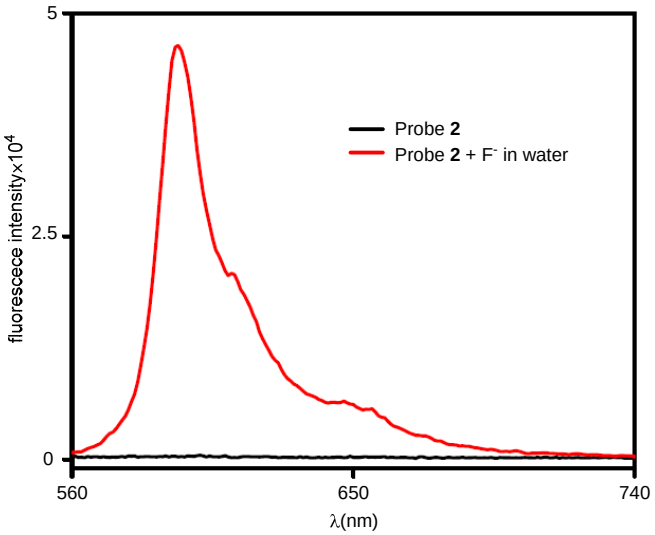


Fig. S7 Fluorescence intensity of probe **2** (100 nM) in THF upon addition of F^- (100 nM) in water at λ_{em} = 595 nm (upon λ_{ex} = 550 nm).

Fluorometric titration of probe **2** (100 nM) in THF with increasing concentration of fluoride (20 – 100 nM) in water was carried out and fluorescence intensity was increasing with increasing concentration of fluoride.

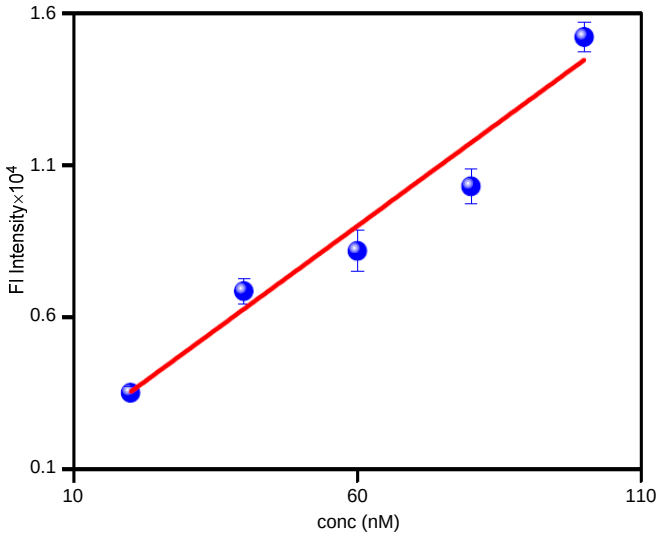


Fig. S8 Fluorescence intensity of probe **2** (100 nM) in THF upon addition of F^- (20 nM - 100 nM) in water at λ_{em} = 595 nm (upon λ_{ex} = 550 nm). Each data was recorded after 10 min of addition of fluoride.

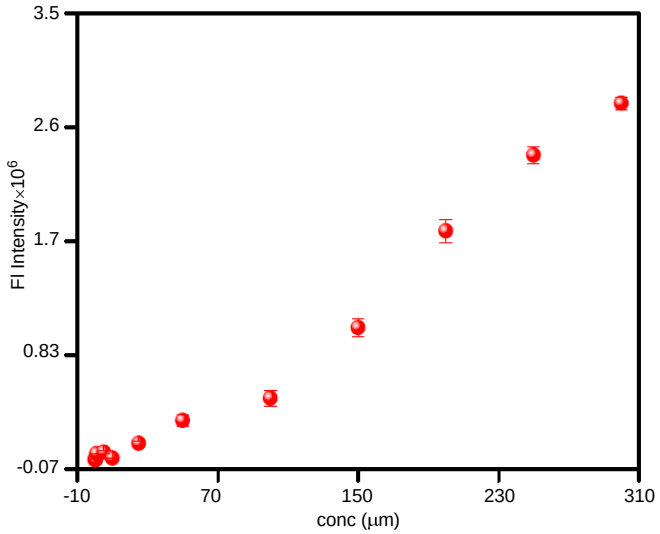


Fig. S9 Fluorescence intensity of probe **2** (1 μM) in THF upon addition of F^- (50 nM – 300 μM) in water at λ_{em} = 595 nm (upon λ_{ex} = 550 nm). Each data was recorded after 10 min of addition of fluoride.

NMR Titration:

The formation of phthalide **3** involved in the sensing mechanism was also confirmed by ^{1}H-NMR titration. With increasing concentration of fluoride the signals at δ = 5.1 ppm (singlet) corresponding to -CH_2 proton of probe **2** started disappearing and a new signals at δ = 5.2 ppm (singlet) corresponding to -CH_2 of phthalide **3** appeared.

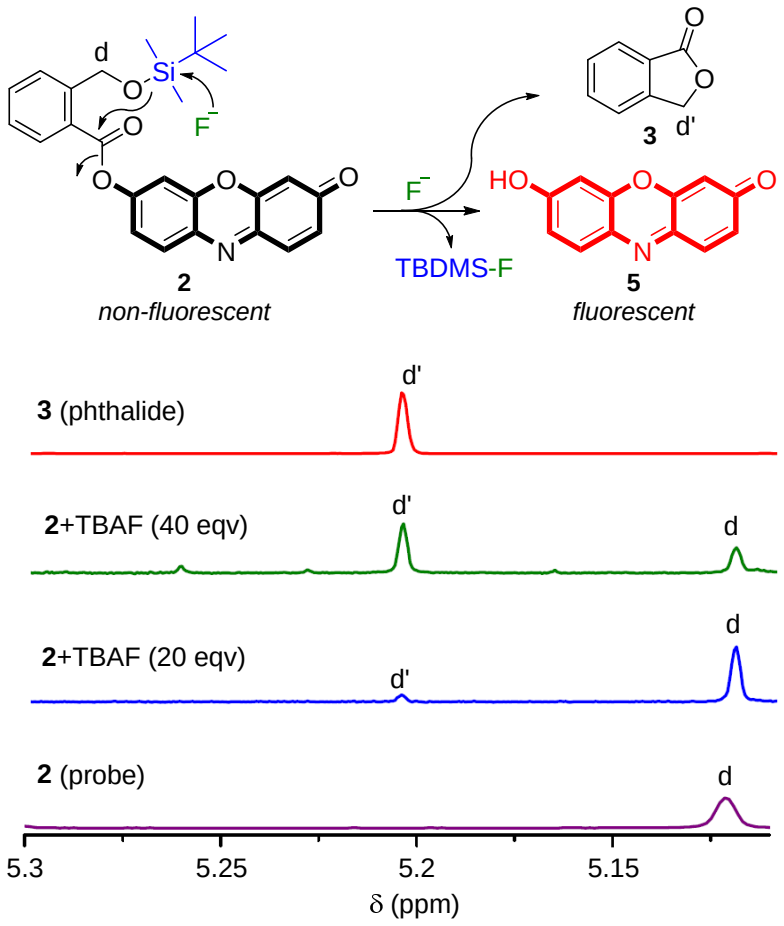


Fig. S10 ^{1}H-NMR titration of probe **2** (4 mg) in CD_3CN (0.75 mL) upon addition of increasing concentration of TBAF.

II. NMR Spectra:

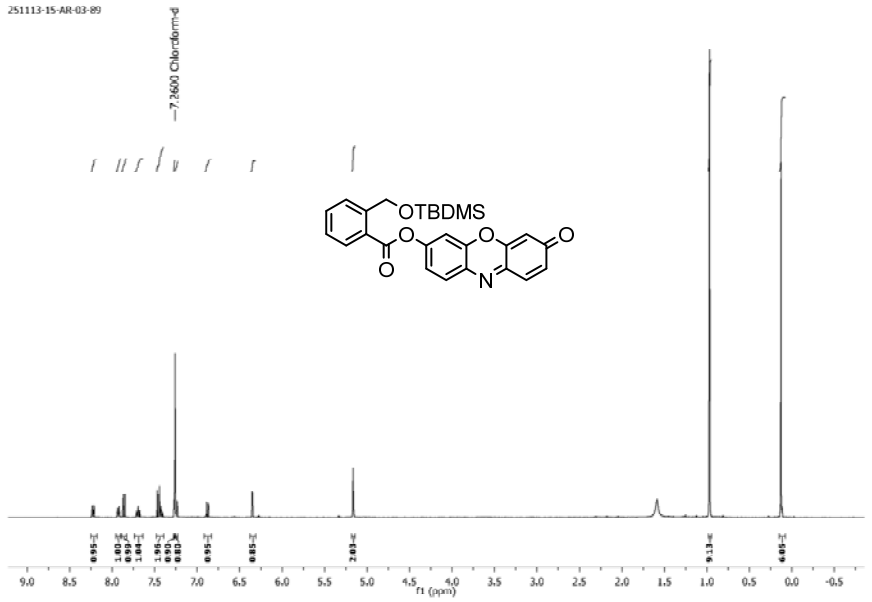


Fig. S11 ^{1}H NMR spectra of **2** in $CDCl_3$.

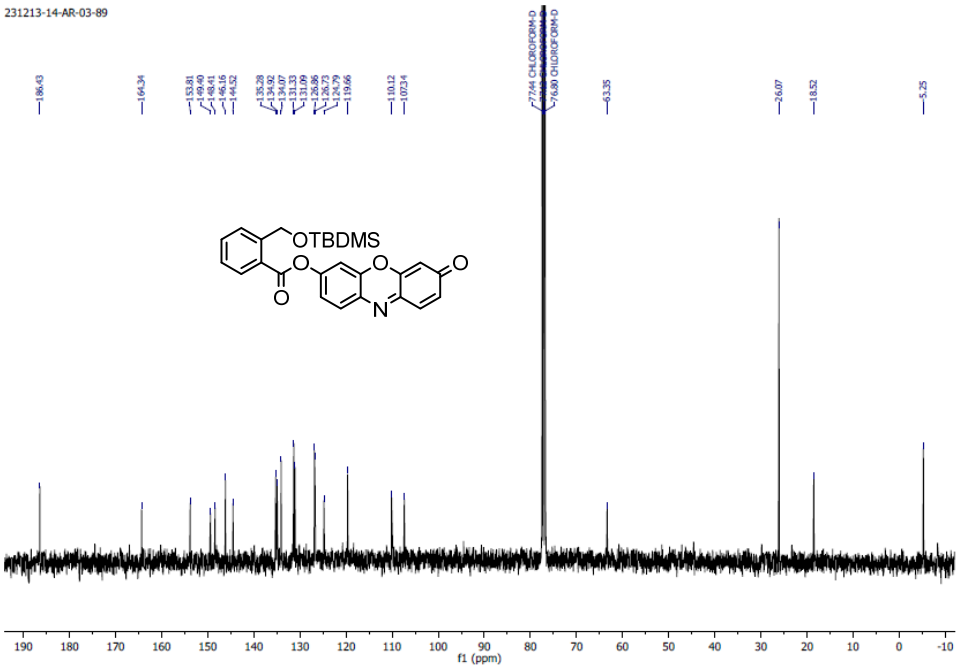


Fig. S12 ^{13}C NMR spectra of **2** in $CDCl_3$.

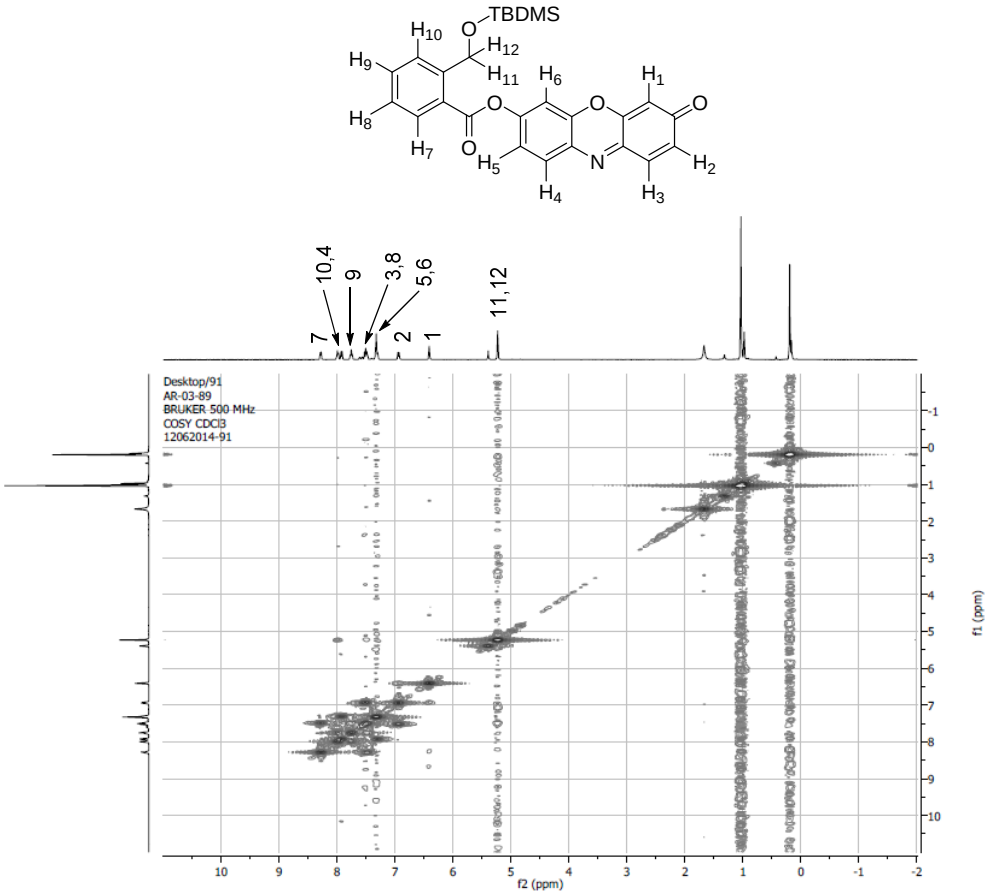


Fig. S13 COSY spectra of **2** in $CDCl_3$.

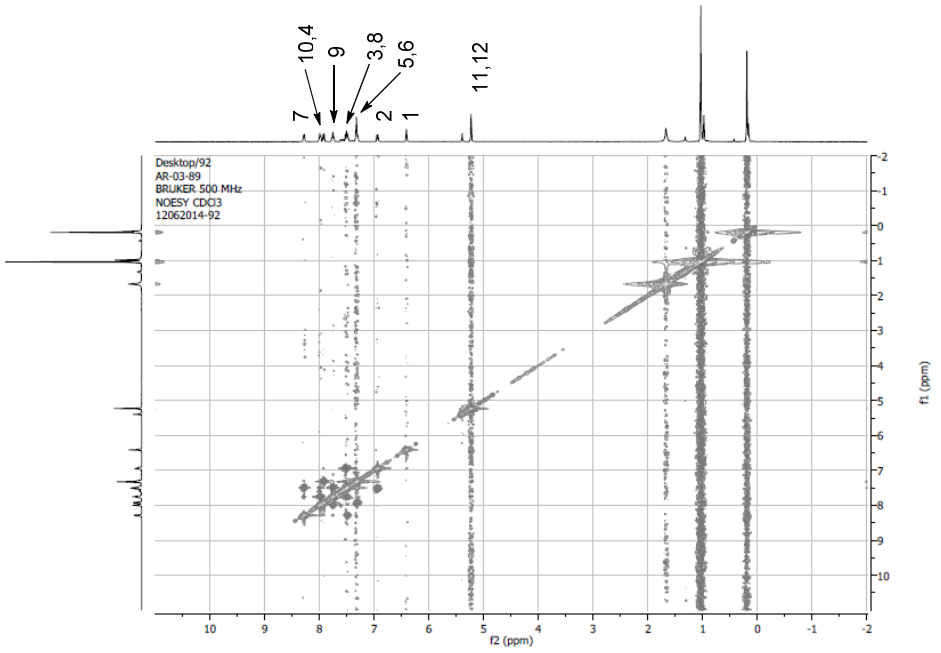


Fig. S14 NOESY spectra of **2** in $CDCl_3$.

III. HPLC data for purification and monitoring fluoride detection:

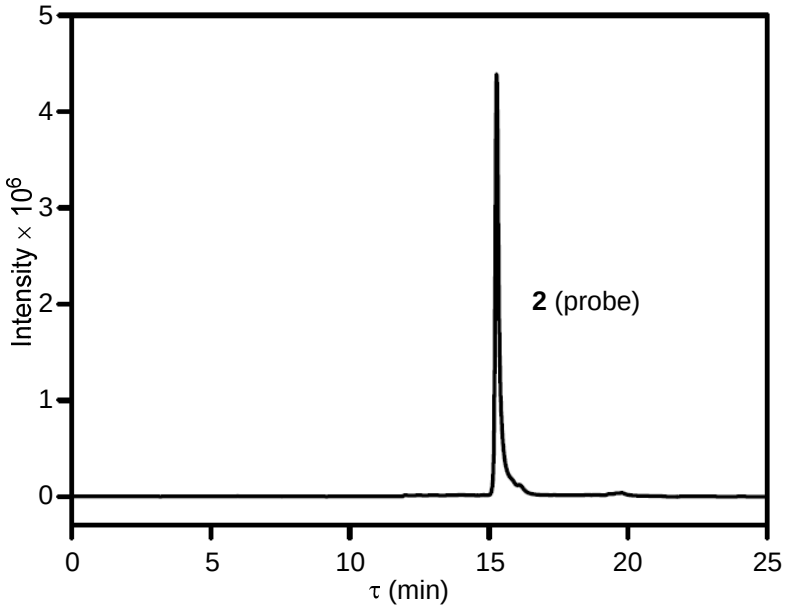


Fig. S15 HPLC Data of probe **2**.

t_R = 15.2 min and purity = 99.93%.

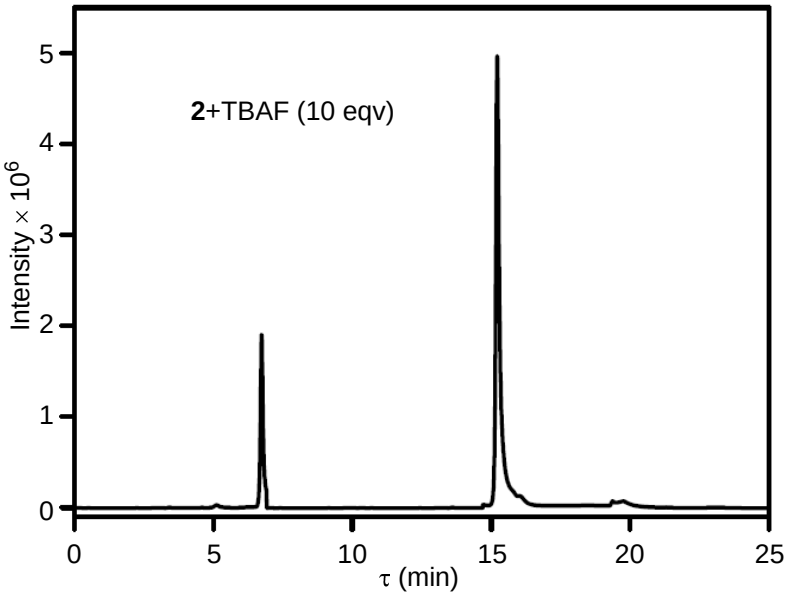


Fig. S16 HPLC Data of probe **2** + **TBAF** (10 eqv).

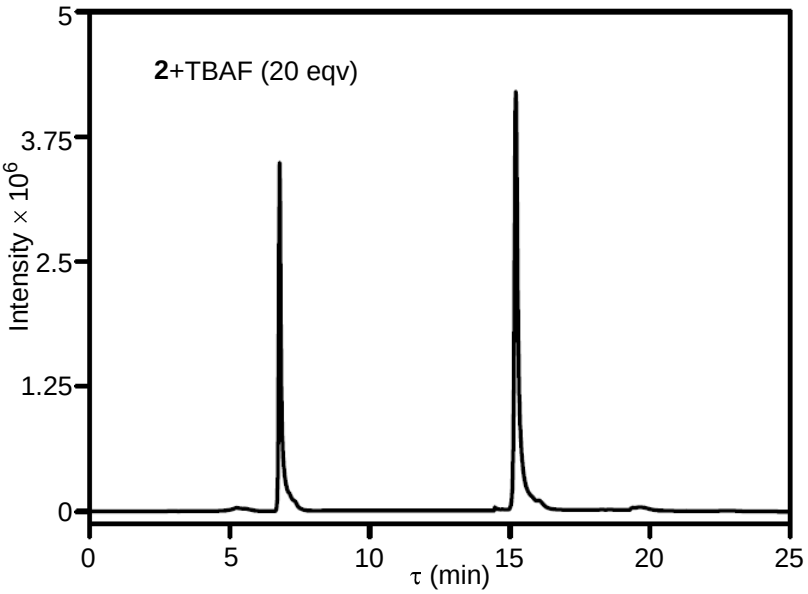


Fig. S17 HPLC Data of probe **2** + **TBAF** (20 eqv).

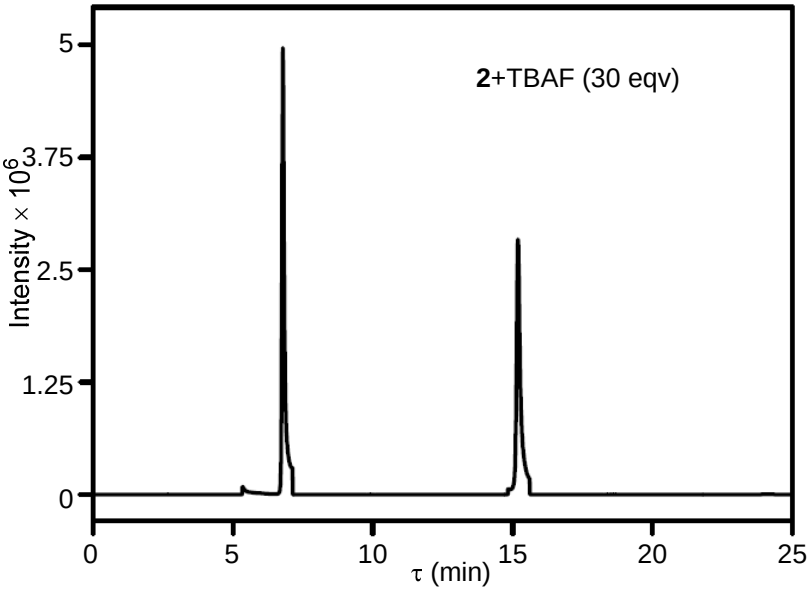


Fig. S18 HPLC Data of probe **2** + **TBAF** (30 eqv).

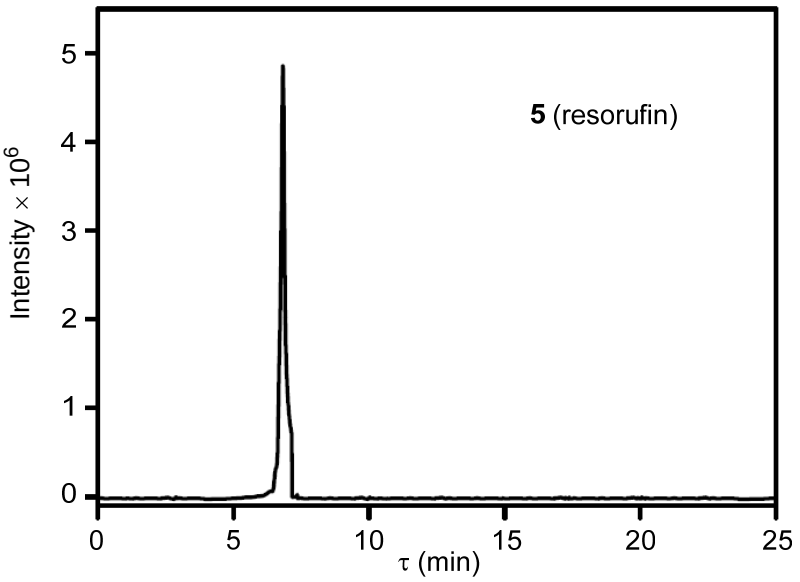


Fig. S19 HPLC Data of resorufin **5**.

IV. References:

S1. P. Hou, S. Chen, H. Wang, J. Wang, K. Voitchovsky and X. Song, *Chem. Commun*., 2014, **50**, 320-322.

S2. C. B. Rosen, D. J. Hansen and K. V. Gothelf, *Org. Biomol. Chem*., 2013, **11**, 7916-7922.

S3. D. Kim, S. Singha, T. Wang, E. Seo, J. H. Lee, S.-J. Lee, K. H. Kim and K. H. Ahn, *Chem. Commun*., 2012, **48**, 10243-10245.

S4. B. Zhu, F. Yuan, R. Li, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun*., 2011, **47**, 7098-7100.

S5. R. Hu, J. Feng, D. Hu, S. Wang, S. Li, Y. Li and G. Yang, *Angew. Chem. Int. Ed*., 2010, **49**, 4915-4918.

S6. X. Cao, W. Lin, Q. Yu and J. Wang, *Org. Lett.*, 2011, **13**, 6098-6101.

S7. L. Fu, F.-L. Jiang, D. Fortin, P. D. Harvey and Y. Liu, *Chem. Commun*., 2011, **47**, 5503-5505.

S8. Y. Bao, B. Liu, H. Wang, J. Tian and R. Bai, *Chem. Commun*., 2011, **47**, 3957-3959.

S9. H. Lu, Q. Wang, Z. Li, G. Lai, J. Jiang and Z. Shen, *Org. Biomol. Chem*., 2011, **9**, 4558-4562.

S10. J. Chen, C. Liu, J. Zhang, W. Ding, M. Zhou and F. Wu, *Chem. Commun*., 2013, **49**, 10814-10816.

S11. M. Dong, Y. Peng, Y.-M. Dong, N. Tang and Y.-W. Wang, *Org. Lett.*, 2011, **14**, 130-133.

S12. J. Cao, C. Zhao, P. Feng, Y. Zhang and W. Zhu, *RSC Adv.*, 2012, **2**, 418-420.

S13. A. Roy, D. Kand, T. Saha and P. Talukdar, *Chem. Commun*., 2014, **50**, 5510-5513.

S14. P. Sokkalingam and C.-H. Lee, *J. Org. Chem.*, 2011, **76**, 3820-3828.

S15. X.-F. Yang, H. Qi, L. Wang, Z. Su and G. Wang, *Talanta*, 2009, **80**, 92-97.

S16. R. Nishiyabu, M. A. Palacios, W. Dehaen and P. Anzenbacher, *J. Am. Chem. Soc.*, 2006, **128**, 11496-11504.
S17. C. B. Rosen, D. J. Hansen and K. V. Gothelf, *Org. Biomol. Chem*., 2013, **11**, 7916-7922.