Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

A sensitive paper-based sensor for fluoride detection in water using Tb^{3+} photoluminescence

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Content

S1. Optimization of assay conditions:

Incubation temperature. For the optimization of incubation temperature, three identical samples (450 µL) of fluoride-treated (333 µM) **2**-doped (333 µM) NaCh (20 mM) solution along with a control (without fluoride) were incubated at various temperatures (30, 35, and 40 °C) for 1 h. Then, these solutions were mixed with 150 µL of aqueous terbium nitrate (20 mM) and converted to gels as described earlier. The Tb^{3+} luminescence of these samples was recorded at 545 nm (λ_{ex} 336 nm). The luminescence enhancement for the sample incubated at 35 °C compared to the control was the highest, suggesting the complete release of sensitizer (**1**). The luminescence enhancement at 40 °C was similar to that at 35 °C; the control also showed some luminescence enhancement, suggesting the decomposition of **2** at the higher temperature (**Fig. S1**). Therefore, the incubation temperature for the fluoride detection assay for all subsequent measurements was kept at 35 °C.

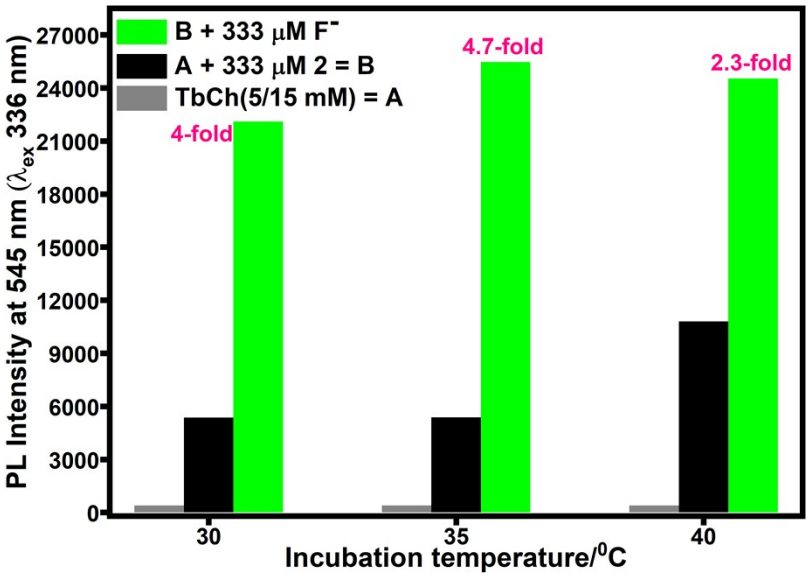


Figure S1. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated samples of undoped, **2**-doped, and fluoride-treated **2**-doped 20 mM NaCh solution (33% organic solvent/water, v/v).*

Incubation time. To optimize the incubation time, solutions (1 mL) of **1** (333 µM)-doped NaCh (20 mM), **2** (333 µM)-doped NaCh (20 mM), and fluoride (333 µM)-treated **2** (333 µM)-doped NaCh (20 mM) were incubated at 35 °C. At 30 and 60 minutes, 450 µL aliquots of each sample were taken out and mixed with 150 µL of aqueous terbium nitrate (20 mM) and converted to gels as described earlier. The time-delayed Tb^{3+} luminescence of these gels was recorded at 545 nm as before. As shown in **Fig. S2**, a similar PL intensity for fluoride (333 µM)-treated, **2** (333 µM)-doped TbCh gel compared to **1** (333 µM)-doped TbCh gel, suggesting that the incubation of 30 min was sufficient to convert all the *pro*-sensitizer **2** to sensitizer **1** in the presence of fluoride.

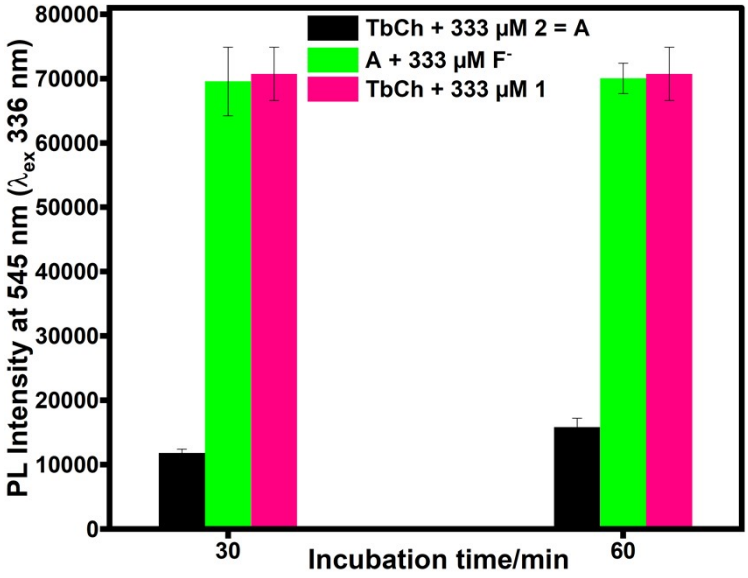


Figure S2. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated samples of **2**-doped, fluoride-treated **2**-doped, and **1**-doped 20 mM NaCh solution (33% organic solvent/water, v/v).*

Organic solvent in assay media. For the optimization of organic solvent in the assay media, stock solutions (1 mM) of *pro*-sensitizer **2** were prepared in three different dipolar aprotic organic solvents (DMF, ACN, DMSO) and further diluted in 30 mM NaCh solution. These solutions (450 μL) were incubated at 35 °C with and without fluoride for 30 min, mixed with aqueous terbium nitrate solution (150 μL), and sonicated to make TbCh (5/15 mM) gel. The Tb^{3+} luminescence of these samples was measured at 545 nm as usual (**Fig. S3**). These data suggested 33% DMSO/water (*v/v*) to be a better solvent system for the fluoride detection assay.

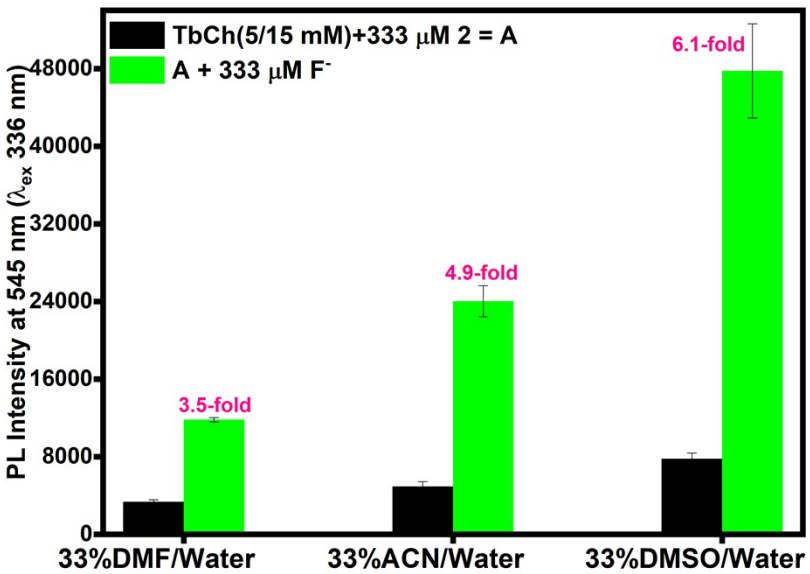


Figure S3. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated **2**-doped and fluoride-treated **2**-doped NaCh solutions (33% organic solvent/water, v/v).*

Percentage of DMSO in aqueous media. Next, sets of **2**-doped (control) and fluoride-treated **2**-doped NaCh solutions with varying %DMSO/water (*v/v*) were incubated at 35 °C for 30 min and converted to TbCh gels, followed by PL intensity measurements at 545 nm (λ_{ex} 336 nm) as described earlier. These data suggested that 20% DMSO/water (*v/v*) was a better solvent system for the working concentrations of *pro*-sensitizer **2** for the fluoride detection assay (**Fig. S4a and S4b**).

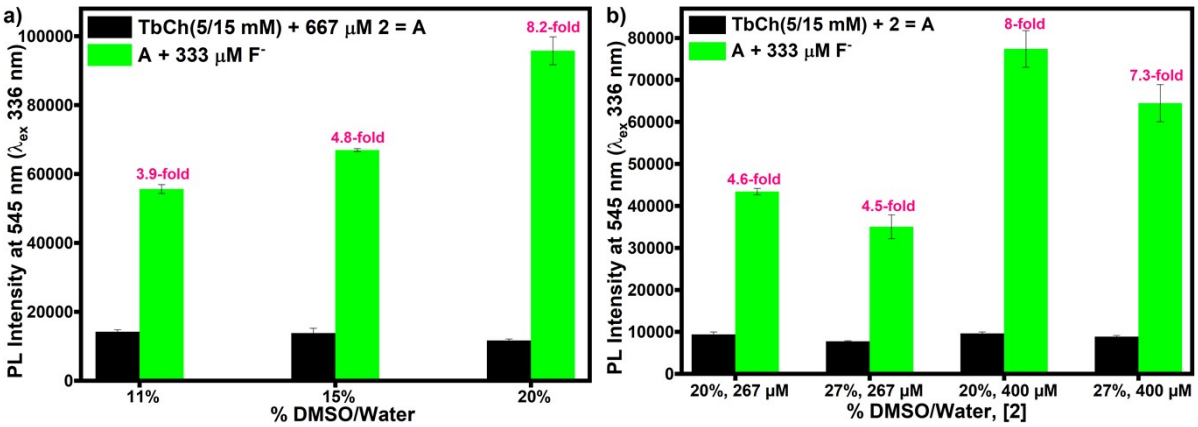


Figure S4*. PL intensity at 545 nm of TbCh gels prepared from the preincubated* ***2****-doped and fluoride-treated* ***2****-doped NaCh solutions varying* ***a)*** *%DMSO/water 667 μM of* ***2*** *and* ***b)*** *%DMSO/water for 267 and 400 μM of* ***2****.*

Optimization of pH of the assay solvent. The introduction of the buffer (pH ~ 7) was necessary to avoid any pH variation of the assay conditions by adding analytes/interferents because the *pro*-sensitizer **2** was hydrolyzed to **1** at pH > 7.0 (**Fig. S5**). In contrast, at lower pH (< 6.0), cholic acid precipitates out from NaCh solution (pK_a ~ 5).[2] Therefore, buffer (30 mM NaCh + 20 mM HEPES, pH 6.9) was used instead of 30 mM NaCh solution to dilute the stock solutions of **1** and **2** prepared in DMSO.

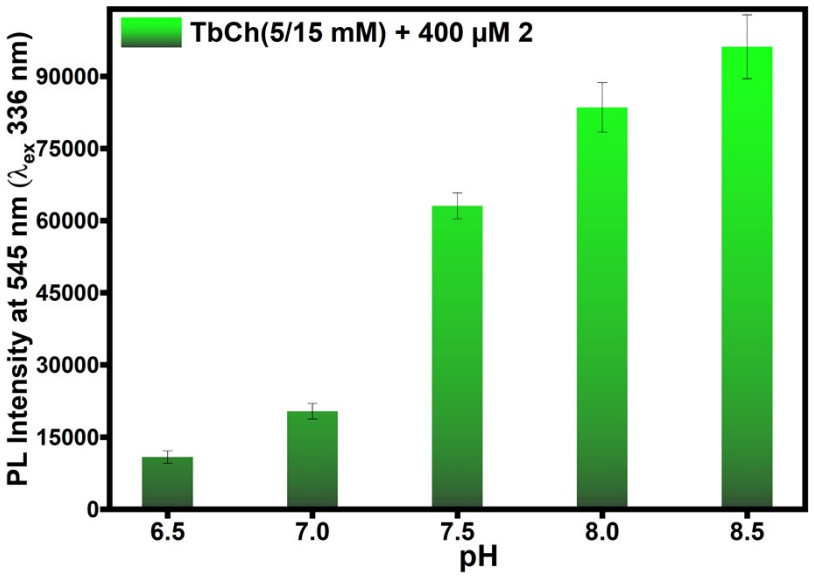


Figure S5. *PL intensity at 545 nm (*λ_{ex} *336 nm) of* ***2****-doped TbCh gels prepared from the preincubated* ***2****-doped buffer (20 mM NaCh + 10 mM HEPES, 20% DMSO) solutions of various pH.*

Other organic solvents as assay media. Further, the stock solutions of **2** in dioxane, ethanol, and DMSO were prepared and diluted in the buffer solution (pH 6.9, 20% organic solvent, *v/v*). In the optimized assay conditions, fluoride detection assay was performed using these solutions of *pro*-sensitizer **2**. Similar Tb^{3+} luminescence enhancement for fluoride-treated **2**-doped TbCh gel to control (**2**-doped TbCh) compared to 20% DMSO/water was also observed for 20% dioxane/water and 20% ethanol/water (**Fig. S6**).

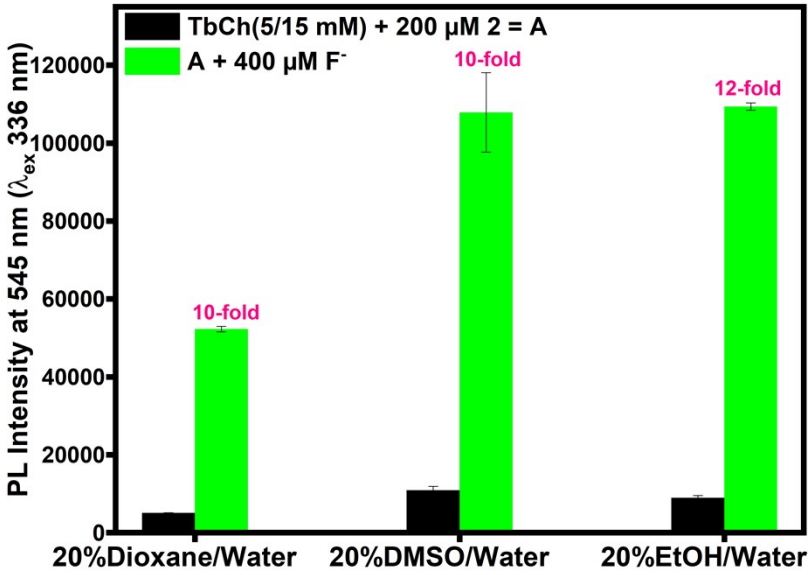


Figure S6. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated **2**-doped and fluoride-treated **2**-doped buffer (20 mM NaCh + 10 mM HEPES, 20% organic solvent, pH 6.9) solutions.*

S2. Reactivity of fluoride ions towards *pro*-sensitizers 2 versus 4:

The fluoride detection assay was performed by incubating **2**-doped, fluoride-treated **2**-doped, **4**-doped and fluoride-treated **4**-doped buffer solutions at 35 °C for 2 h. There was no luminescence enhancement for fluoride-treated **4**-doped TbCh gel compared to the control (**Fig. S7a**). Then, a similar assay was performed by incubation at 45 °C for 2 h, where *pro*-sensitizer **4** showed only 2-*fold* luminescence enhancement compared to the control, whereas that of *pro*-sensitizer **2** was 5-*fold* (**Fig. S7b**).

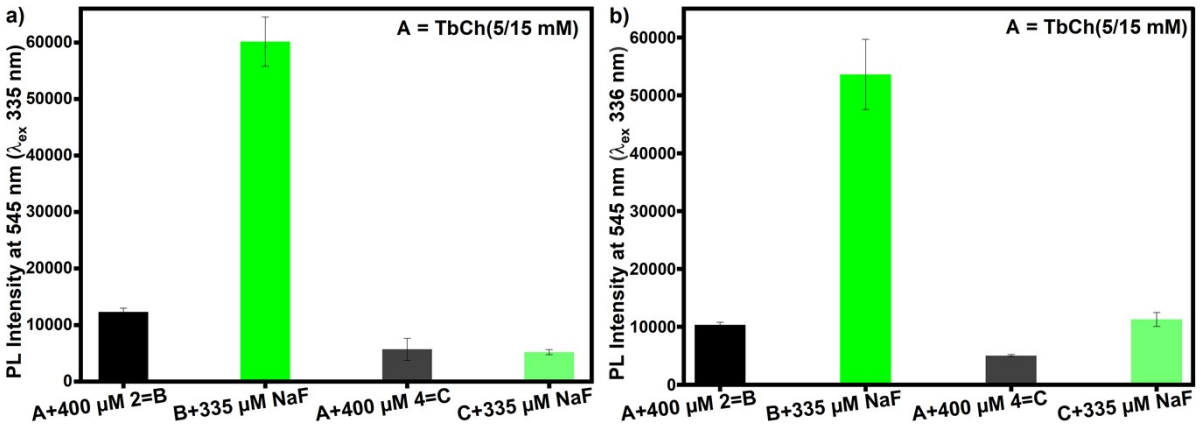


Figure S7. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from preincubated **2**-doped, fluoride-treated **2**-doped, **4**-doped and fluoride-treated **4**-doped buffer solutions at **a)** 35 °C and **b)** 45 °C.*

S3. Comparison of PL intensity and HPLC peak area with incubation time:

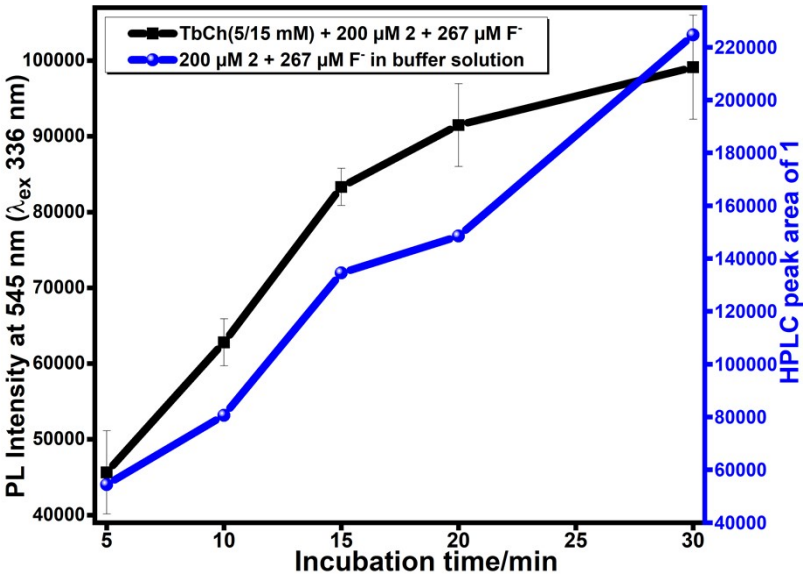


Figure S8. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared and HPLC peak area of released **1** from the preincubated fluoride-treated **2**-doped buffer (20 mM NaCh + 10 mM HEPES, 20% DMSO, pH 6.9) solutions.*

S4. Calculation of limit of detection (LOD) value:

Data recorded in the Varian Cary Eclipse instrument (gels in cuvette):

LOD was calculated using the following equation,

$$LOD = \frac{3 \times s}{b}$$

Where s is the intercept error for the PL intensity of gel, and b is the slope of the plot (**Fig. 6b**). The equation of linear fit is **$y = 4.8 \times x + 3.7$ ($R^2 = 0.97953$)**.

Where y is the PL intensity of the gel samples at 545 nm, and x is the fluoride concentration. So,

$$LOD = \frac{3 \times 3.7}{4.8}\,\mu M = 2.35\,\mu M$$

Similarly, the LOD calculated from the other two identical experiments were 2.65 μM and 2.58 μM.

Therefore, LOD = (2.5 ± 0.2) μM

Data recorded in the Plate Reader instrument (gel-coated paper discs):

LOD was calculated using the following equation,

$$LOD = \frac{3 \times \sigma}{b}$$

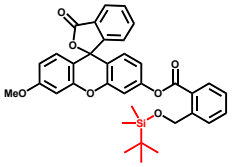
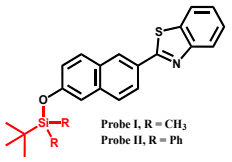
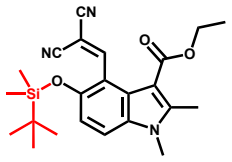
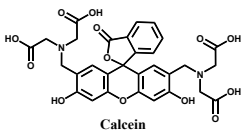
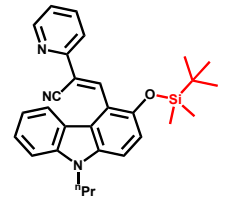
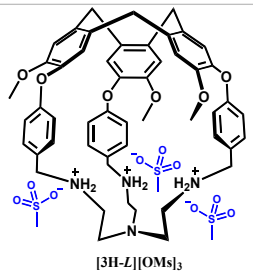
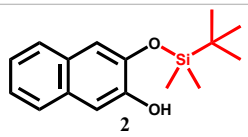
Where σ is the standard deviation for the PL intensities of gel-coated paper discs without fluoride, and b is the slope of the plot (**Fig. 7b**). The equation of linear fit is **$y = 607 \times x + 16454$ ($R^2 = 0.99963$)**.

Where y is the PL intensity of the gel samples at 545 nm, and x is the fluoride concentration. So,

$$LOD = \frac{3 \times 292}{607}\,\mu M = 1.4\,\mu M$$

LOD = 1.4 × 19 ppb = 27 ppb

Table S1. Comparison table of LOD value with recently reported literature.

S. No.	Probe	Solvent	Assay time	Method	LOD	Reference
1.		DMSO	7 min	Fluorescence (*turn-on*)	19.6 ppb	*Chem. Commun.* 2014, **50**, 5510–5513.
2.	 Probe I, R = CH_3 Probe II, R = Ph	DMSO:water (3:1, v/v)	2 min	Ratiometric Fluorescence	2.6 ppb	*ACS Omega* 2019, **4**, 4918−4926.
3.		EtOH:HEPES buffer (50 mM, pH 7.4, 1:1, v/v)	4 h	Fluorescence (*turn-on*)	1.3 ppb	*Dyes and Pigm.* 2021, **188**, 109166.
4.	**Calcein + Eu^{3+} in ZnCdSe/ZnS QDs**  Calcein	Water	2 h	Fluorescence (*turn-on*)	215 ppb	*Anal. Bioanal. Chem.* 2022, **414**, 3999–4009.
5.		EtOH:HEPES buffer (50 mM, pH 7.4, 8:2, v/v)	1 h	Fluorescence (*turn-on*)	0.1 ppb	*Spectrochim. Acta A Mol. Biomol. Spectrosc.* 2023, **285**, 121816.
6.	 [3H-*L*][OMs]$_3$	Citrate buffer aq. (0.1 M, pH 4.1)	1 h	Fluorescence (*turn-on*)	11.4 ppb	*Chem. Sci.* 2023, **14**, 291−297.
7.	**PENG with fillers Mn-Doped $BaTiO_3$ Nanostructures and CNTs**	Piezoelectric nanogenerator (PENG)	NA	Output voltage	22.4 ppb	*ACS Appl. Nano Mater.* 2023, **6**, 6637–6652.
8.	 2	DMSO:HEPES buffer (20 mM, pH 6.5, 1:4, v/v)	**30 min**	**Tb^{3+} luminescence (*turn-on*)**	**27 ppb**	**This work**

Here, most of the methods have used organic solvents as a medium or compromised with low LOD.

S5. Fluoride detection from real-life samples and calculations:

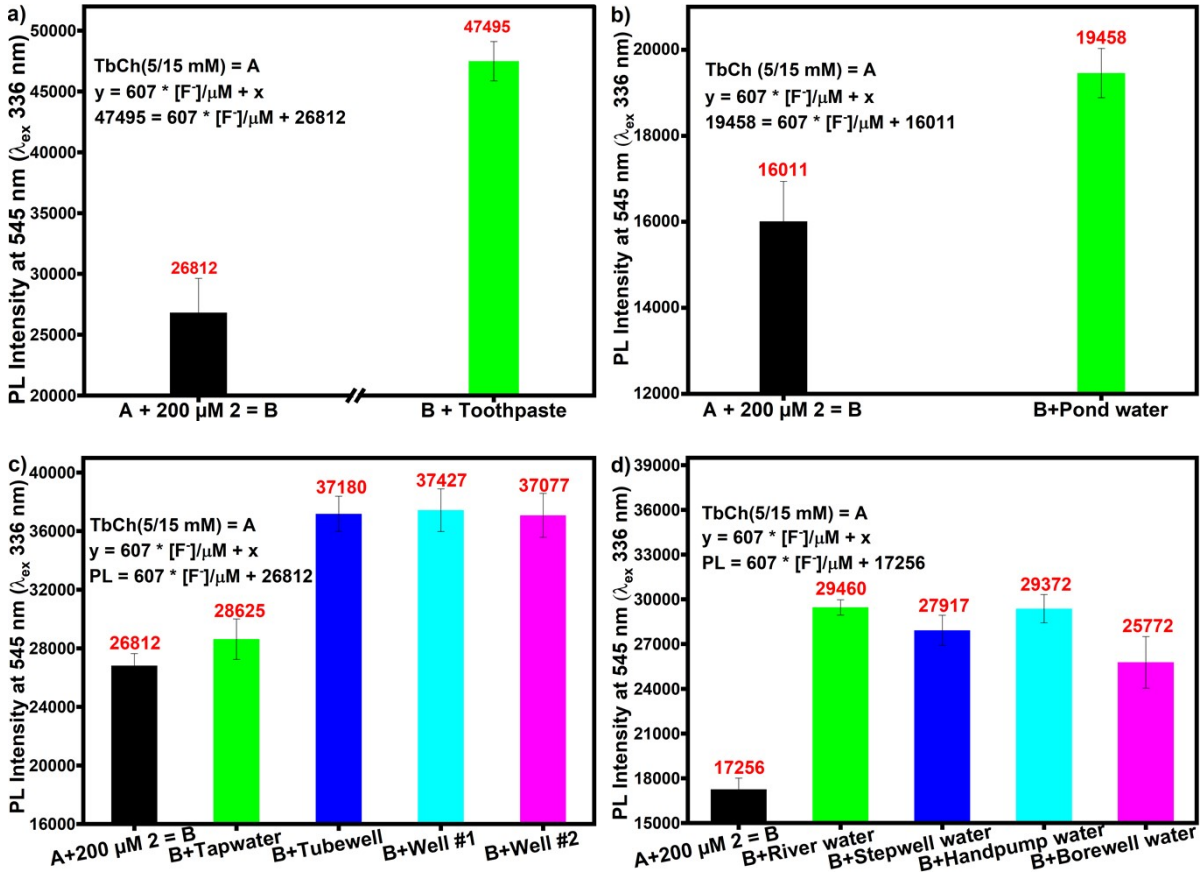


Figure S9. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated analyte-treated **2**-doped buffer solutions; analyte: **a)** toothpaste solution; water samples from **b)** Bankura, West Bengal, **c)** Ajmer, Rajasthan, and **d)** Baran, Rajasthan.*

Calculations of fluoride content in the real-life samples (data from Fig. S9):

Calculation for Fig. S8a: The equation is as follows:

$$47495 = 607 \times [F^-]/\mu M + 26812$$

$$[F^-] = 34.1\ \mu M$$

40 μL of toothpaste solution was added to 260 μL of probe solution during incubation. Therefore,

$$[F^-]_{TP} = (34.1\ \mu M \times 300\ \mu L)/40\ \mu L = 256\ \mu M$$

The fluoride content in the stock solution was 10 times that of the toothpaste solution as per dilution.

$$[F^-]_{Stock} = 256\ \mu M \times 10 = 2560\ \mu M$$

$$[F^-]_{Stock} = (2560\ \mu mol/L) \times 19\ \mu g/\mu mol$$

$$[F^-]_{Stock} = 48.64\ \mu g/mL$$

Since the above stock solution was prepared by dissolving 476 mg in a 7.74 mL buffer solution, the fluoride content in the toothpaste pack:

$$[F^-]_{pack} = [(48.64\ \mu g/mL \times 7.74\ mL)/476\ mg] \times 1000\ ppm$$

$$\mathbf{[F^-]_{pack} = 791\ ppm}$$

Calculation for Fig. S8b: The equation is as follows:

$$19458 = 607 \times [F^-]/\mu M + 16011$$

$$[F^-] = 5.68\ \mu M$$

40 µL of toothpaste solution was added to 260 µL of probe solution during incubation. Therefore,

$$[F^-]_{Pond} = (5.68\ \mu M \times 300\ \mu L)/40\ \mu L = 42.6\ \mu M$$

$$[F^-]_{Pond} = (42.6\ \mu mol/L \times 19\ \mu g/\mu mol)/1000\ ppm$$

$$\mathbf{[F^-]_{Pond} = 0.81\ ppm}$$

Calculation for Fig. S8c: The equation is as follows:

$$PL\ Intensity = 607 \times [F^-]/\mu M + 26812$$

For tap water sample,

$$28625 = 607 \times [F^-]/\mu M + 26812$$

$$[F^-] = 2.99\ \mu M$$

40 µL of toothpaste solution was added to 260 µL of probe solution during incubation. Therefore,

$$[F^-]_{Tap} = (2.99\ \mu M \times 300\ \mu L)/40\ \mu L = 22.4\ \mu M$$

$$[F^-]_{Tap} = (22.4\ \mu mol/L \times 19\ \mu g/\mu mol)/1000\ ppm$$

$$\mathbf{[F^-]_{Tap} = 0.43\ ppm}$$

Similarly, for other samples,

$$\mathbf{[F^-]_{Tubewell} = 2.44\ ppm}$$

$$\mathbf{[F^-]_{Well\#1} = 2.49\ ppm}$$

$$\mathbf{[F^-]_{Well\#2} = 2.41\ ppm}$$

Calculation for Fig. S8d: The equation is as follows:

$$PL\ Intensity = 607 \times [F^-]/\mu M + 17256$$

For the river water sample,

$$29460 = 607 \times [F^-]/\mu M + 17256$$

$$[F^-] = 20.1\ \mu M$$

80 µL of toothpaste solution was added to 220 µL of probe solution during incubation. Therefore,

$$[F^-]_{River} = (20.1\ \mu M \times 300\ \mu L)/80\ \mu L = 75.3\ \mu M$$

$$[F^-]_{River} = (75.3\ \mu mol/L \times 19\ \mu g/\mu mol)/1000\ ppm$$

$$\mathbf{[F^-]_{River} = 1.43\ ppm}$$

Similarly, for other samples,

$$\mathbf{[F^-]_{Stepwell} = 1.24\ ppm}$$

$$\mathbf{[F^-]_{Handpump} = 1.42\ ppm}$$

$$\mathbf{[F^-]_{Borewell} = 1.00\ ppm}$$

S6. %Recovery calculations from spike and recovery test:

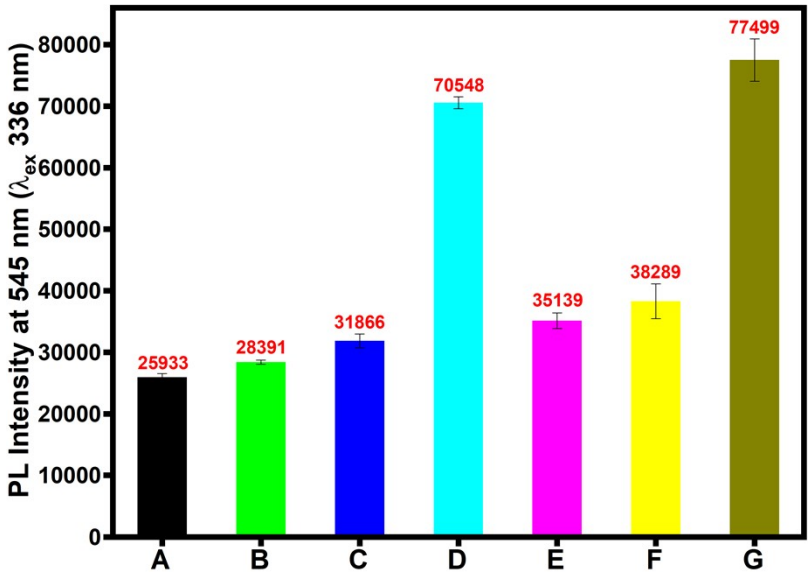


Figure S10. *PL intensity at 545 nm (λ_{ex} 336 nm) of TbCh gels prepared from the preincubated samples of fluoride-containing sample-treated **2**-doped buffer solutions.*

Calculation of %recovery from spike & recovery experiment:

The equation of the calibration plot (linear fit) obtained from gel-coated paper discs is,

y = 607 * x + 25933

Where y is the PL intensity of the gel samples at 545 nm (λ_{ex} 336 nm), and x is the concentration of fluoride ions. The sample A (**2**-doped TbCh gel) was the control.

The fluoride concentrations were calculated using the spike & recovery data (**Fig. S10**) of unspiked samples (**B**, **C**, **D**) and spiked (**E**, **F**, **G**) utilizing the above equation:

For sample **B**,

$28391 = 607 * [F^-] + 25933$

$[F^-] = 4.1\ \mu M = 4.1 \times 19\ ppb = 77.9\ ppb$

For sample **C**,

$31866 = 607 * [F^-] + 25933$

$[F^-] = 9.8\ \mu M = 9.8 \times 19\ ppb = 186.2\ ppb$

For sample **D**,

$70548 = 607 * [F^-] + 25933$

$[F^-] = 73.5\ \mu M = 73.5 \times 19\ ppb = 1396.5\ ppb$

For sample **E**,

$35139 = 607 * [F^-] + 25933$

$[F^-] = 15.2\ \mu M = 15.2 \times 19\ ppb = 288.8\ ppb$

For sample **F**,

38289 = 607 * [F^-] + 16554

[F^-] = 20.3 μM = 20.3 × 19 ppb = 385.7 ppb

For sample **G**,

77499 = 607 * [F^-] + 16554

[F^-] = 85.0 μM = 85.0 × 19 ppb = 1615.0 ppb

Now, the calculation of %recovery was done using the following equation:

$$\%Recovery = \frac{Fluoride\ conc.(spiked) - Fluoride\ conc.\ (unspiked)}{Fluoride\ conc.\ (spiked)} \times 100\%$$

For sample River Water (Baran Rajasthan):

$$\%Recovery = \frac{15.2\ \mu M - 4.1\ \mu M}{10\ \mu M} \times\ 100\% \ =\ 111\%$$

For sample 10 μM NaF aqueous solution:

$$\%Recovery = \frac{20.3\ \mu M - 9.8\ \mu M}{10\ \mu M} \times\ 100\% \ =\ 105\%$$

For sample toothpaste solution:

$$\%Recovery = \frac{85.0\ \mu M - 73.5\ \mu M}{10\ \mu M} \times\ 100\% \ =\ 115\%$$

S7. ^{1}H NMR, ^{13}C NMR and HRMS spectra of 2, 3 and 4:

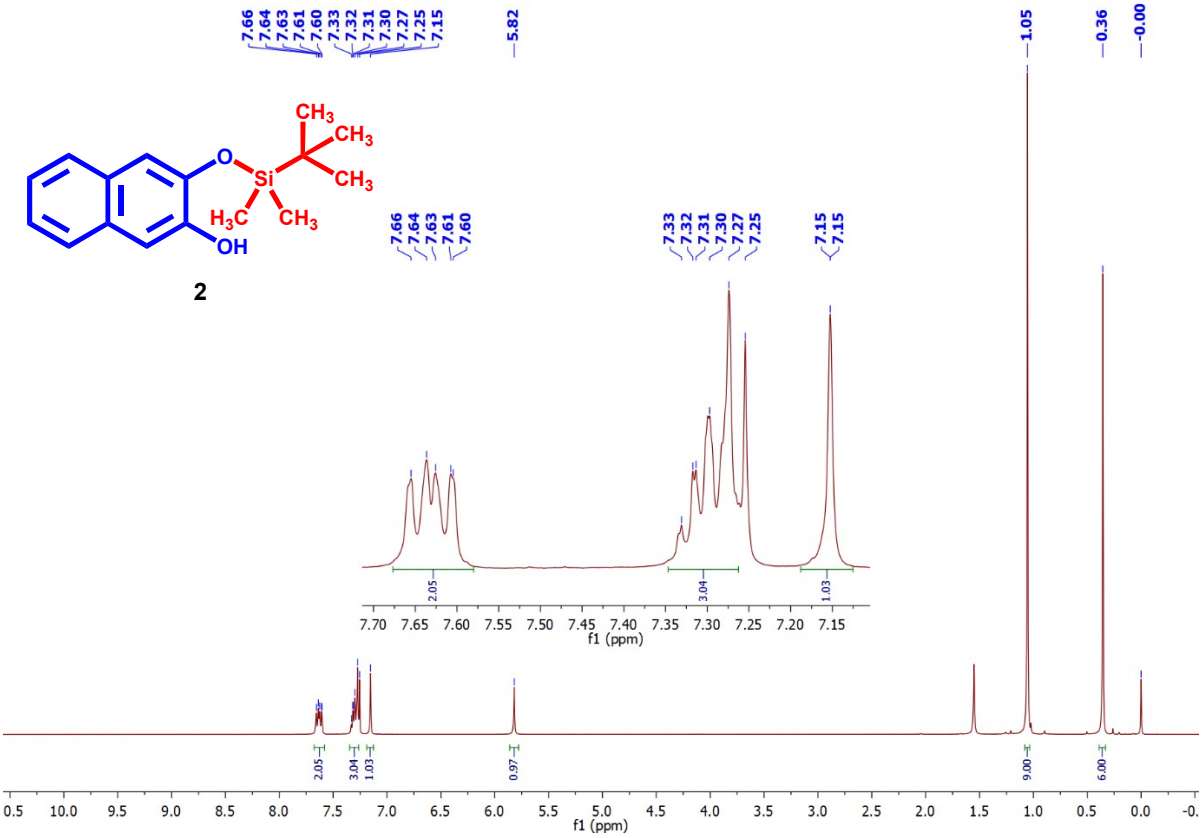


Figure S11. *^{1}H NMR spectrum of **2** in $CDCl_3$ solvent (400 MHz).*

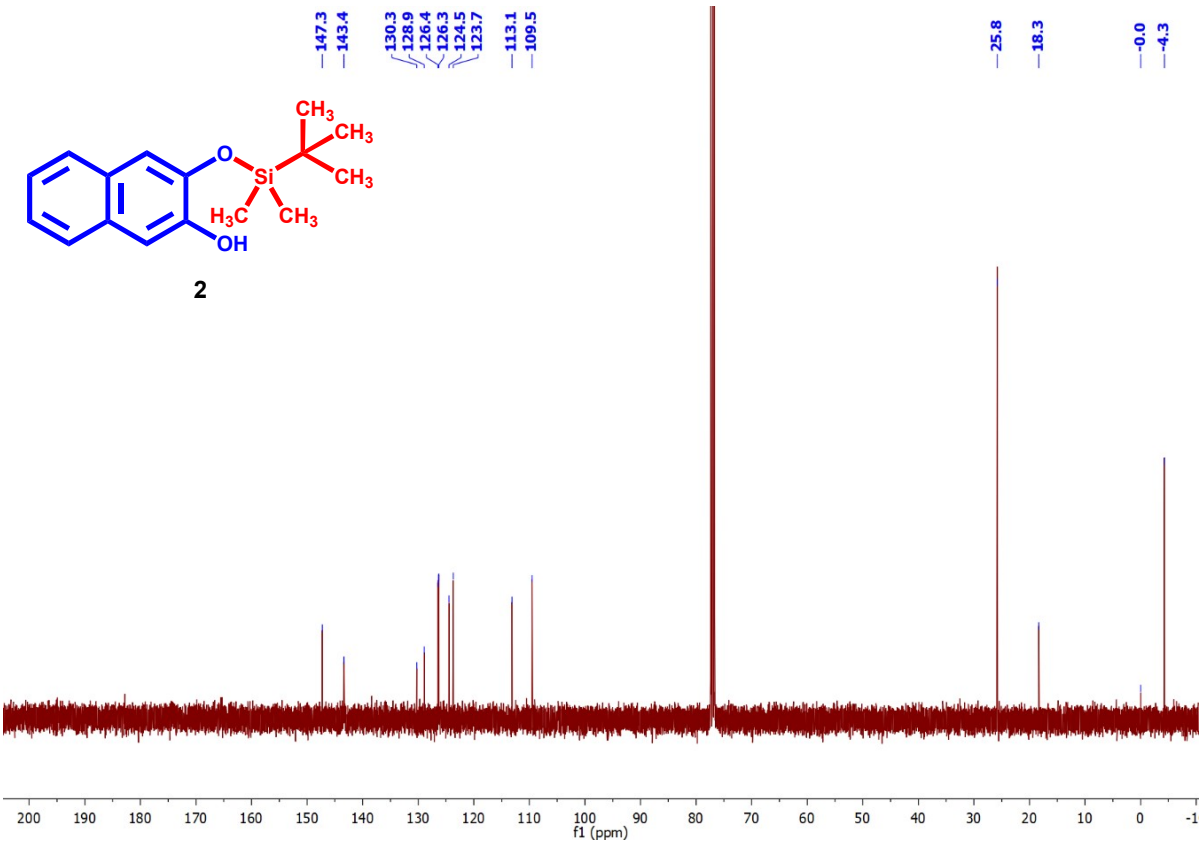


Figure S12. *^{13}C NMR spectrum of **2** in $CDCl_3$ solvent (100 MHz).*

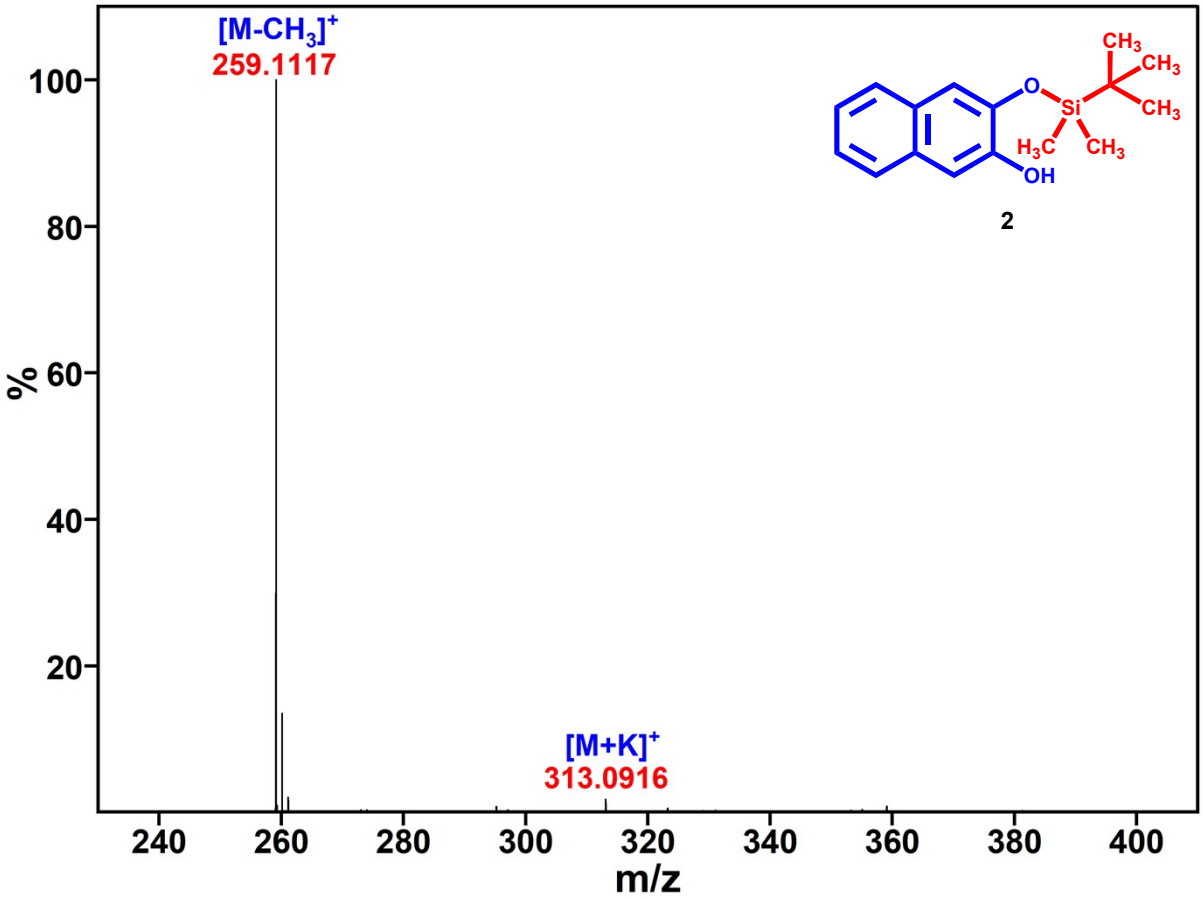


Figure S13. *ESI-Mass spectrum of* ***2*** *in positive mode.*

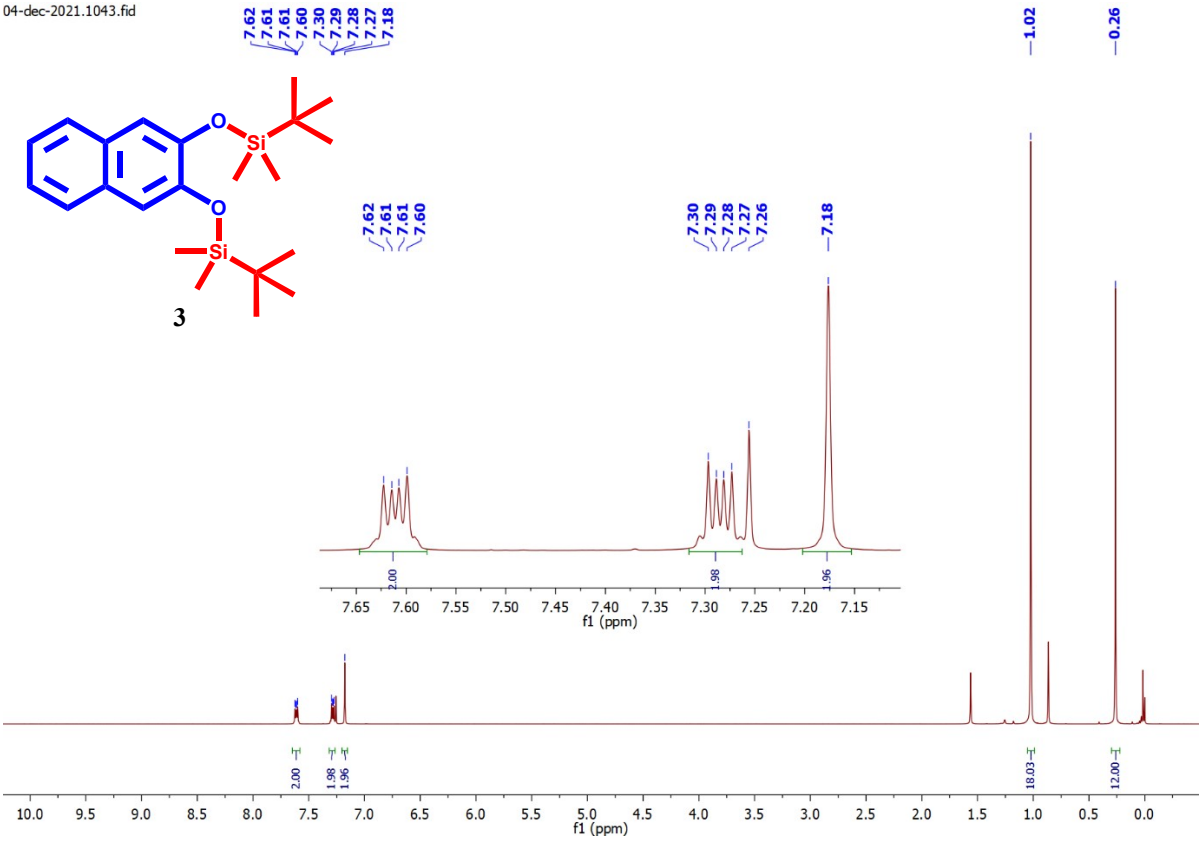


Figure S 14. *^{1}H NMR spectrum of **3** in $CDCl_3$ solvent (400 MHz).*

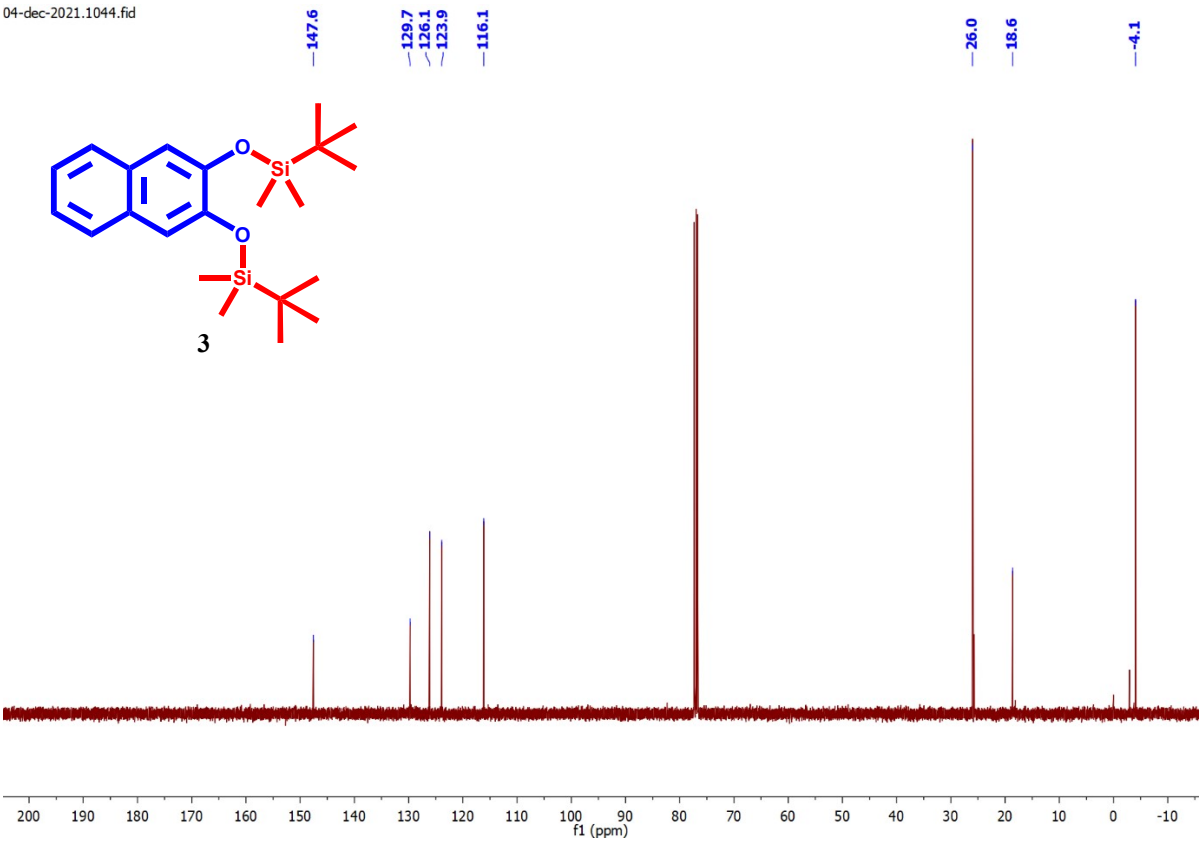


Figure S15. *^{13}C NMR spectrum of **3** in $CDCl_3$ solvent (100 MHz).*

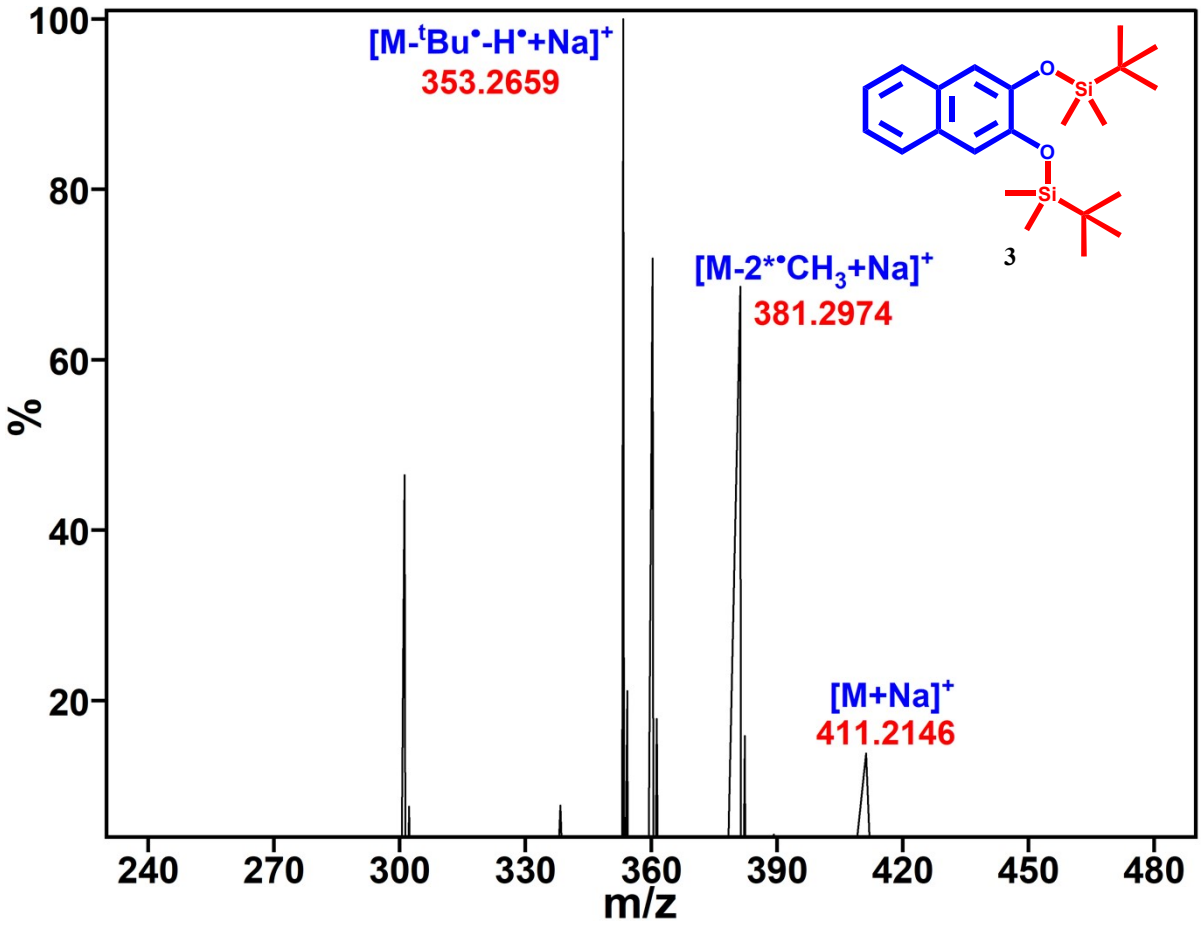


Figure S16. *ESI-Mass spectrum of* ***3*** *in positive mode.*

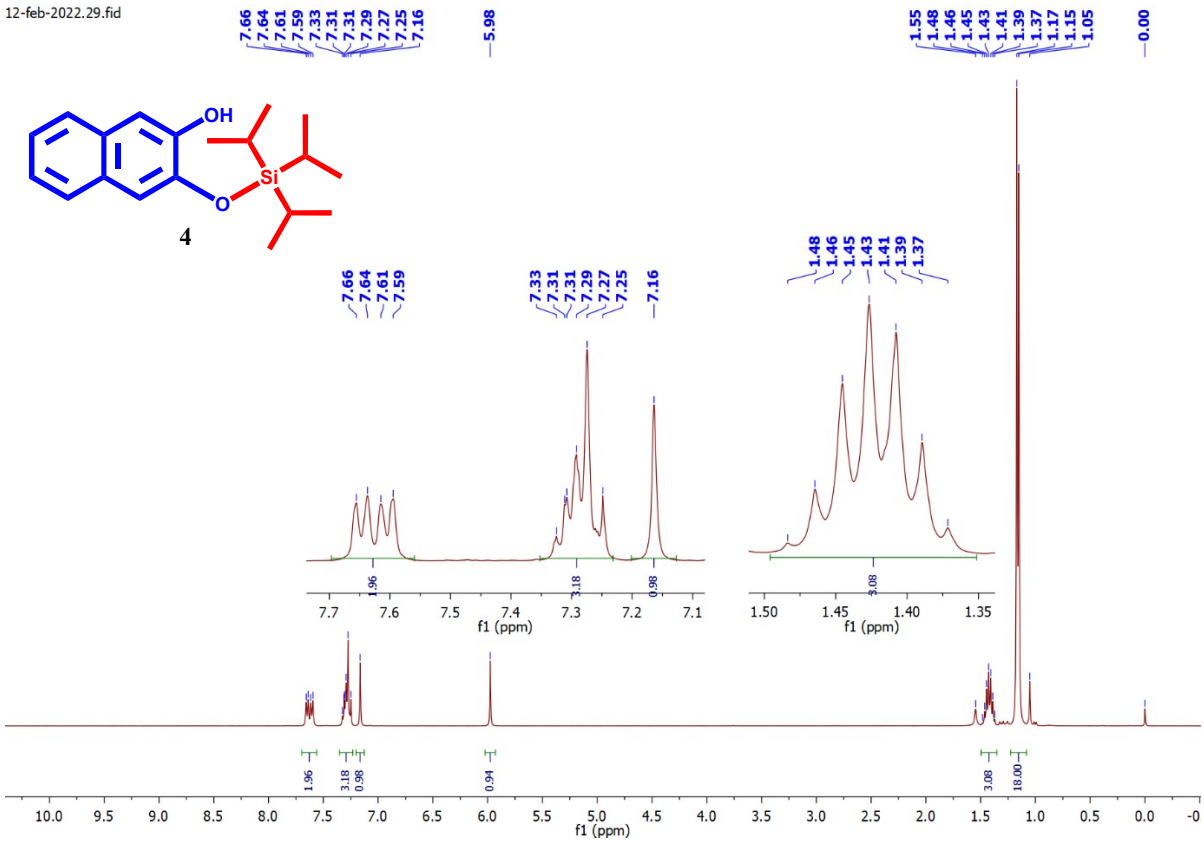


Figure S17. *^{1}H NMR spectrum of **4** in $CDCl_3$ solvent (400 MHz).*

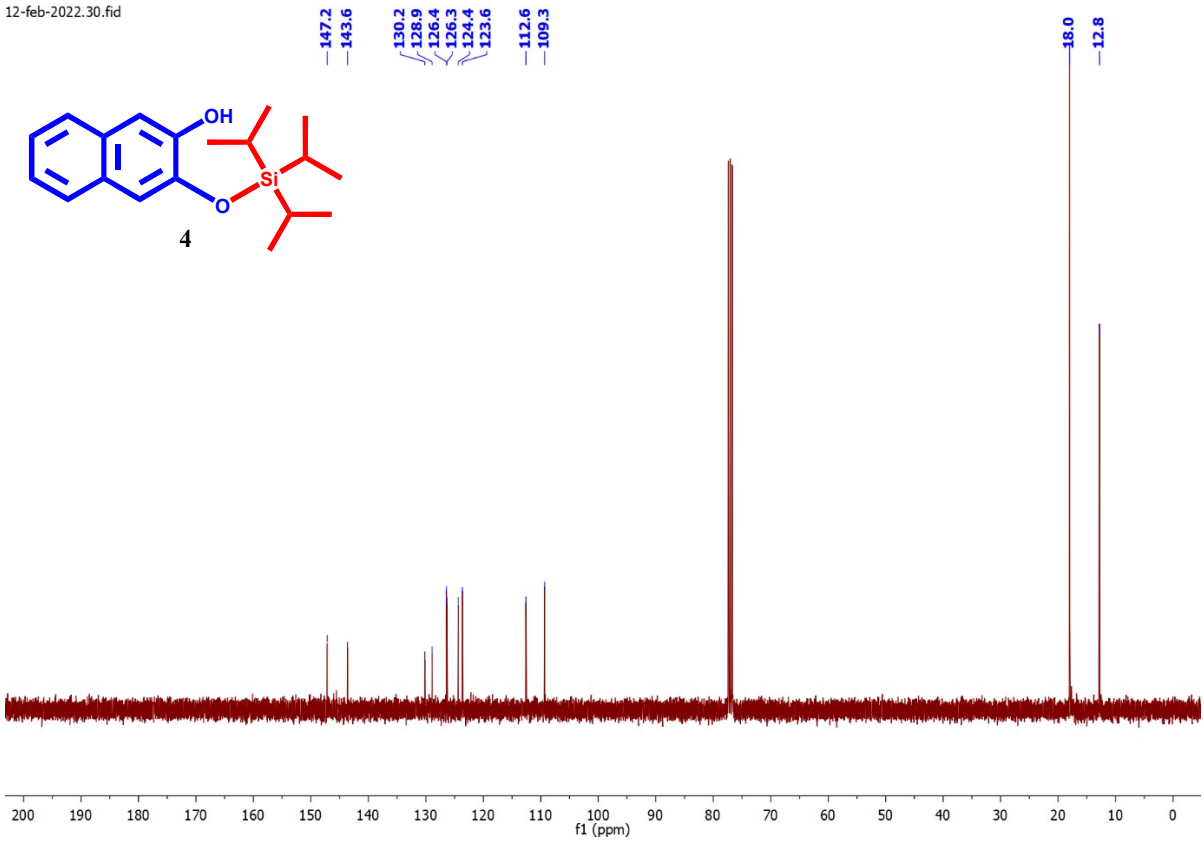


Figure S18. *^{13}C NMR spectrum of **4** in $CDCl_3$ solvent (100 MHz).*

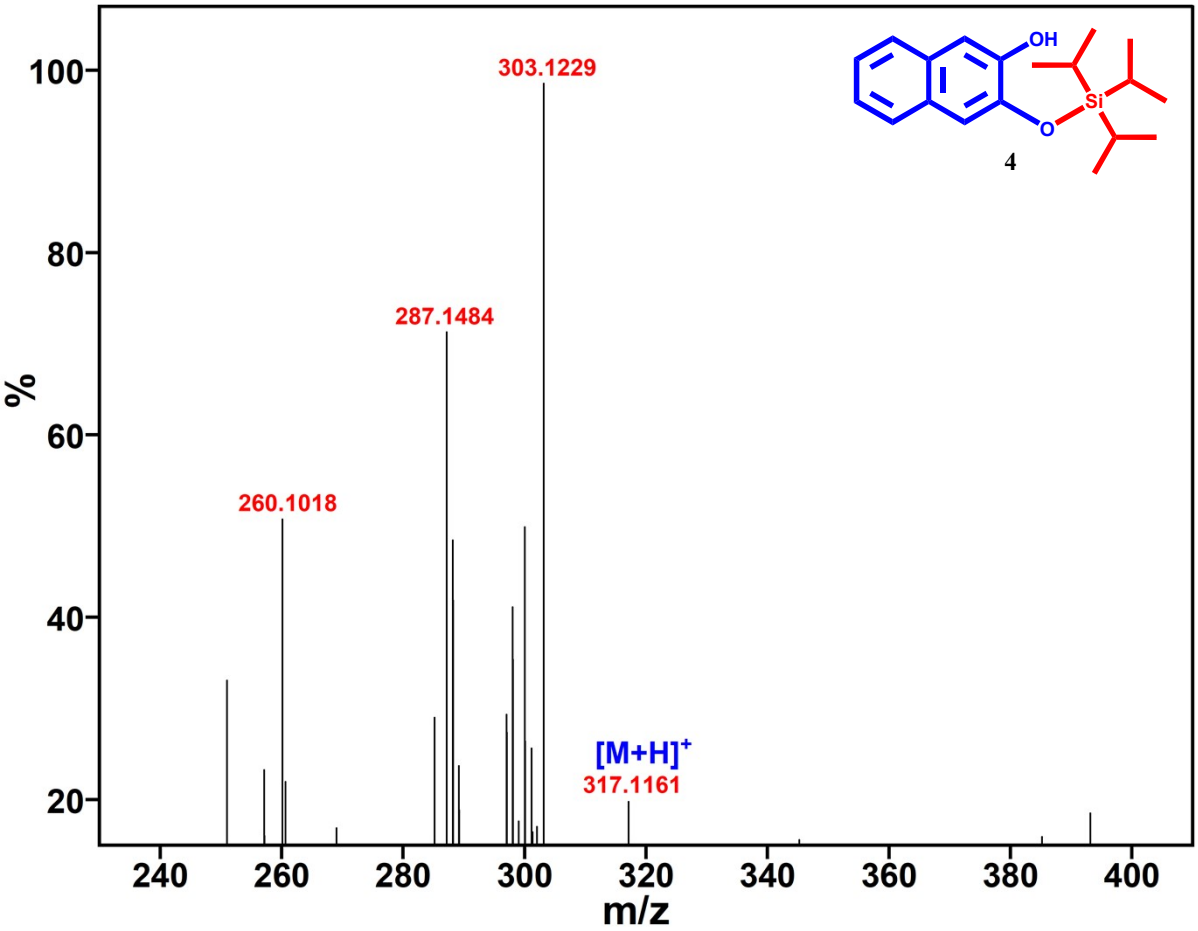


Figure S19. *ESI-Mass spectrum of* ***4*** *in positive mode.*

S8. References:

1. Serjeant, E.P.; Dempsey, B. Ionisation constants of organic acids in aqueous solution. *IUPAC Chem. Data Ser. No. 23. NY, NY: Pergamon* 1979, **989**.