Supporting Information for

A Water Indicator Strip: Instantaneous Fluorogenic Detection of Water in Organic Solvents, Drugs, and Foodstuffs

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Experimental

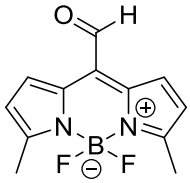
Materials

All anhydrous solvents were obtained as a sure-seal bottle from Sigma-Aldrich (Milwaukee, WI) or Alfa Aesar (Ward Hill, MA). Probe **1**,[1] compounds **2**,[2] **3**,[3] **4**,[1] and **5**[4] were prepared according to the literature.

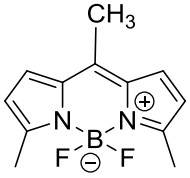
General methods, instrumentation, and measurements

^{1}H-NMR spectra for analyzing assay mixture were recorded on Bruker Advance 400 MHz spectrometer and ^{1}H and ^{13}C-NMR spectra for compounds **1-5** were recorded on JEOL 400 MHz spectrometer. Absorption spectra were obtained on a Scinco UV LAMBDA-465 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer using quartz cuvettes with a path length of 1 cm. Fluorescence quantum yields were determined using fluorescein (Φ_F = 0.95 in 0.1 N NaOH)[5] and cresyl violet (Φ_F = 0.56 in EtOH)[6] as standard. The assay was carried out by monitoring absorbance and fluorescence emission spectra using a Synergy H1 Microplate Reader (BioTek, USA).

1. Structures of Compounds 1-5



Probe **1**



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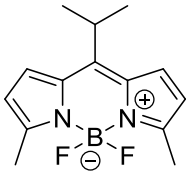

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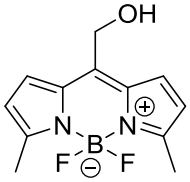

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2. Studies of the Photophysical Properties

Table S1. Photophysical properties of compounds in various solvents

Compounds	Solvent (ε)[a]	$\lambda_{abs.\ max}$, nm	ε, $M^{-1}cm^{-1}$	$\lambda_{em.\ max}$, nm[b]	Φ_F[d]
Probe **1**	1,4-Dioxane (2.22)	590	39,000	620 (619)[c]	0.31
	Toluene (2.38)	601	33,000	625 (626)[c]	0.32
	Diethyl ether (4.27)	591	37,000	614 (614)[c]	0.40
	Ethyl acetate (6.08)	589	38,000	614 (615)[c]	0.38
	THF (7.52)	590	35,000	618 (618)[c]	0.36
	CH_2Cl_2 (8.93)	597	34,000	622 (623)[c]	0.34
	Acetone (21.01)	586	36,000	616 (616)[c]	0.37
	CH_3CN (36.64)	586	33,000	618 (618)[c]	0.33
	DMSO (47.24)	589	26,000	622 (622)[c]	0.05
2	THF (7.52)	504	82,000	525	0.82
	CH_2Cl_2 (8.93)	506	106,000	524	0.96
	CH_3CN (36.64)	500	85,000	520	0.95
3	THF (7.52)	505	81,000	526	0.80
	CH_2Cl_2 (8.93)	505	84,000	526	0.88
	CH_3CN (36.64)	503	80,000	522	0.92
4	THF (7.52)	512	71,000	527	0.61
	CH_2Cl_2 (8.93)	518	68,000	532	0.52
	CH_3CN (36.64)	511	67,000	527	0.58

[a]Dielectric constant[7] [b]Excited at 460 nm. [c]Excited at 550 nm. [d]Quantum yields vs. fluorescein in 0.1N NaOH (Φ_F = 0.95) for compounds **2**-**4** and Quantum yields vs. cresyl violet in EtOH (Φ_F = 0.56) for probe **1**.

(a) Absorption and fluorescence emission spectra of probe 1 in various solvents

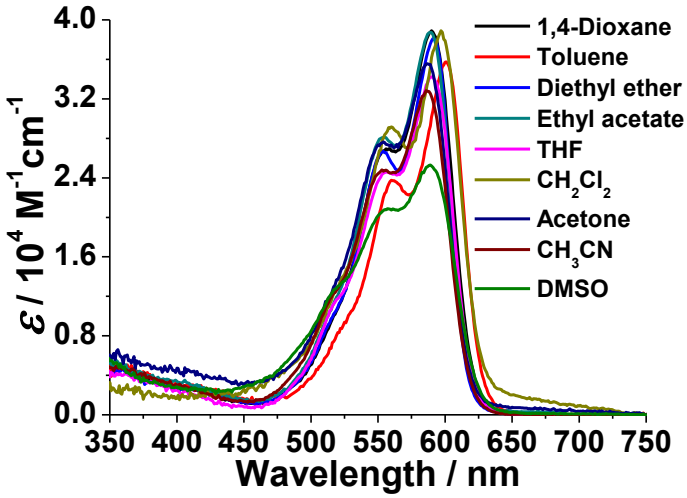


Figure S1. Absorption spectra of probe **1** (10 μM) in various dry solvents at 25 °C.

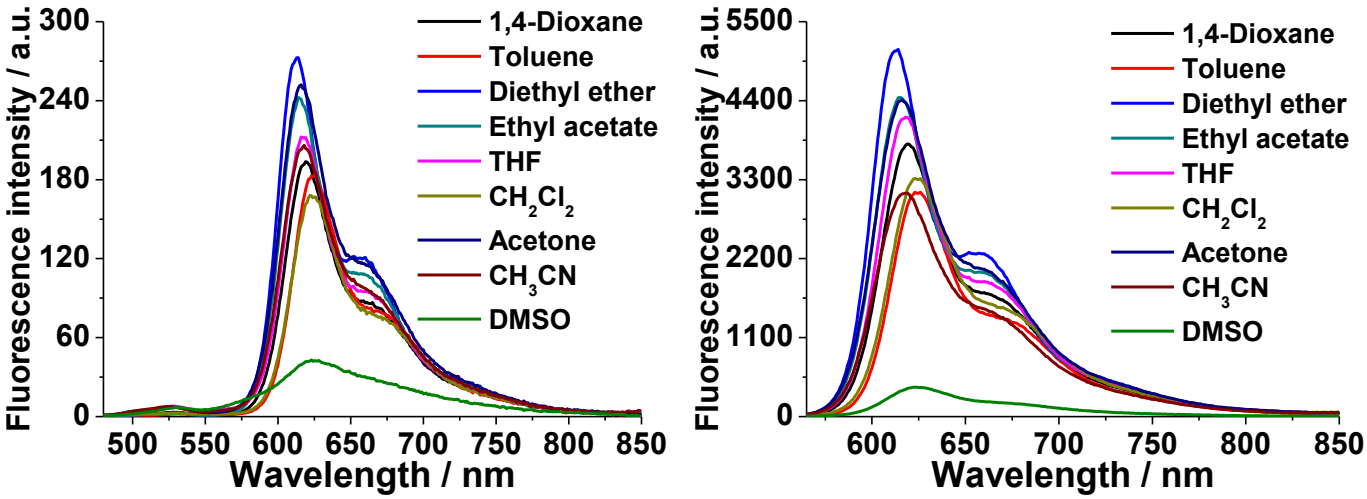


Figure S2. Fluorescence emission spectra of probe **1** (10 μM) in various dry solvents at 25 °C. (left) Excited at 460 nm, (right) Excited at 550 nm.

(b) Absorption and fluorescence emission spectra of compound 4 in various solvents

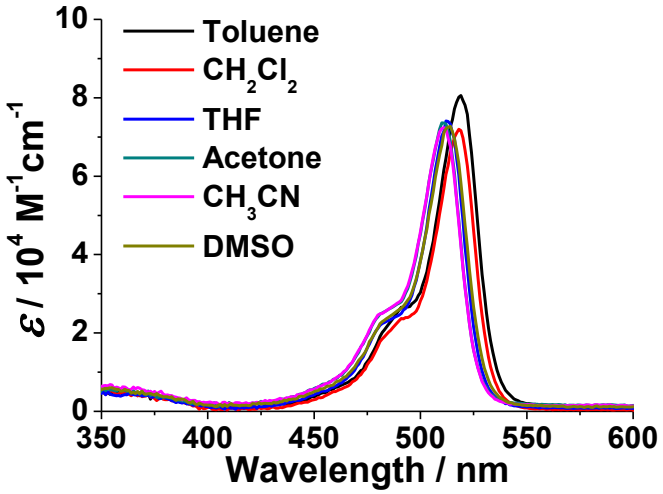


Figure S3. Absorption spectra of compound **4** (10 μM) in various dry solvents at 25 °C.

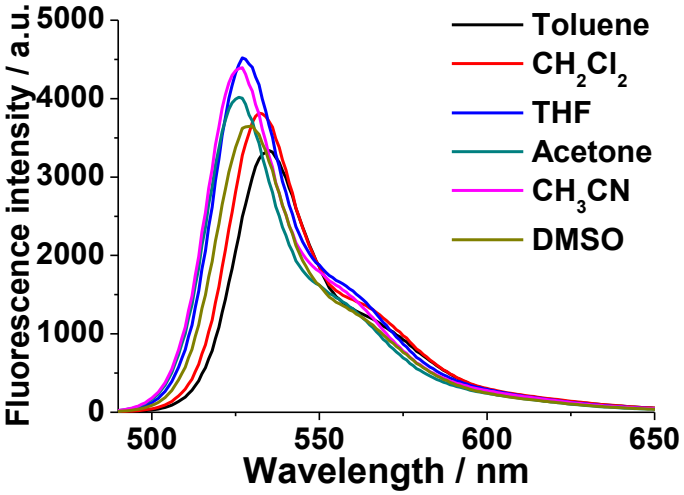


Figure S4. Fluorescence emission spectra of compound **4** (10 μM) in various dry solvents at 25 °C. Excited at 460 nm.

(c) Comparison of absorption and fluorescence emission spectra of compounds 1–4 in dry THF

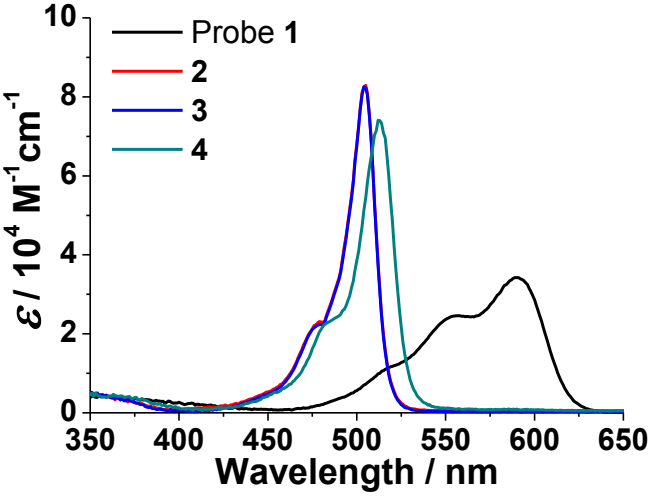


Figure S5. Absorption spectra of compounds **1**–**4** (10 μM) in dry THF at 25 °C.

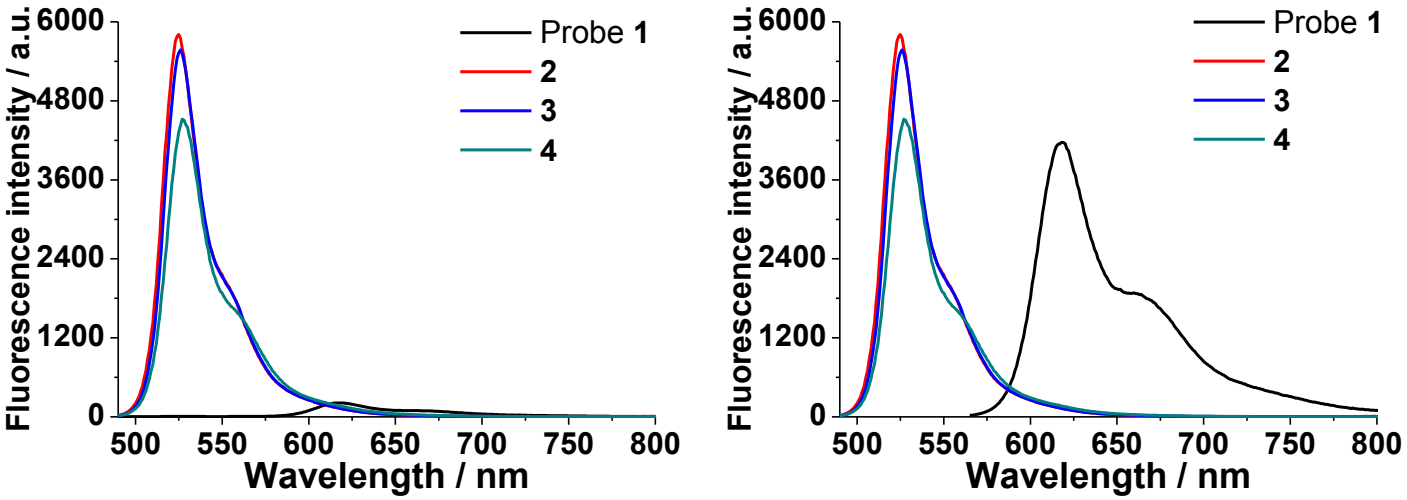


Figure S6. Fluorescence emission spectra of compounds **1**–**4** (10 μM) in dry THF at 25 °C. (left) Excited at 460 nm, (right) Excited at 550 nm for probe **1** and 460 nm for compounds **2**–**4**.

(d) Absorption, excitation, and emission spectra of probe 1 in THF and THF-water (1:99, v/v)

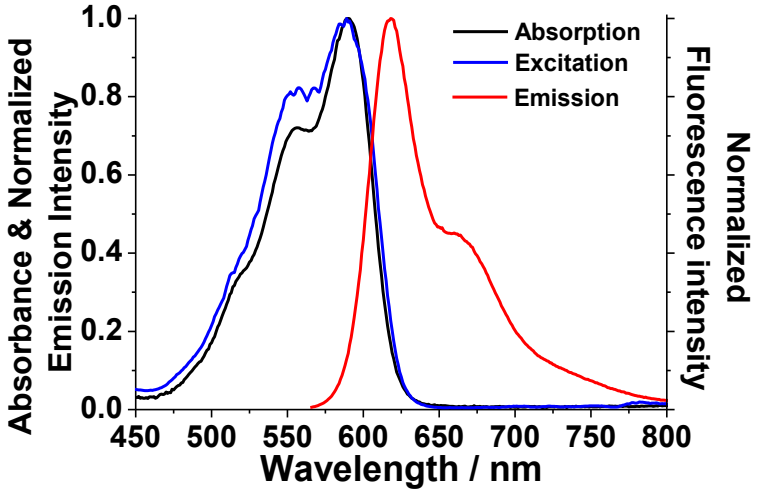


Figure S7. Absorption (black), excitation (blue), and emission (red) spectra of probe **1** (10 μM) in dry THF at 25 °C. The excitation spectrum was obtained by monitoring at 618 nm, and the emission spectrum was obtained by excitation at 550 nm.

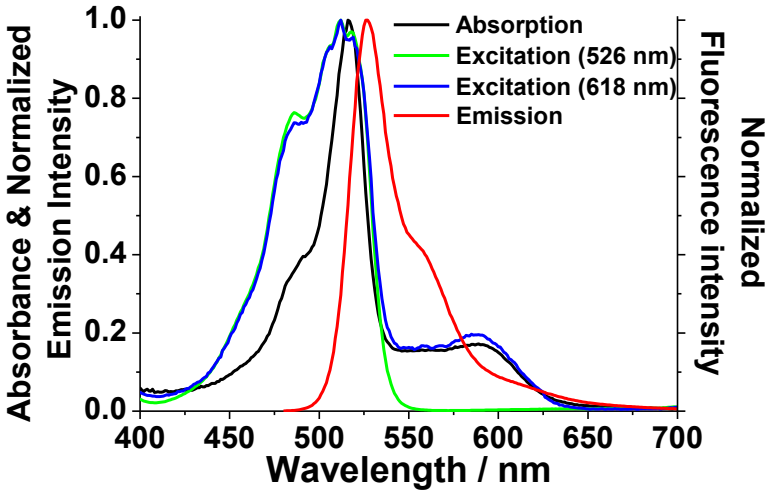


Figure S8. Absorption (black), excitation (blue, green), and emission (red) spectra of probe **1** (10 μM) in THF-water (1:99, v/v) mixture at 25 °C. The excitation spectra were obtained by monitoring at 526 nm (green) and 618 nm (blue), respectively, and the emission spectrum was obtained by excitation at 460 nm.

3. Monitoring of Emission Spectral Changes of Probe 1 in Dry Organic Solvents under Aerobic Condition

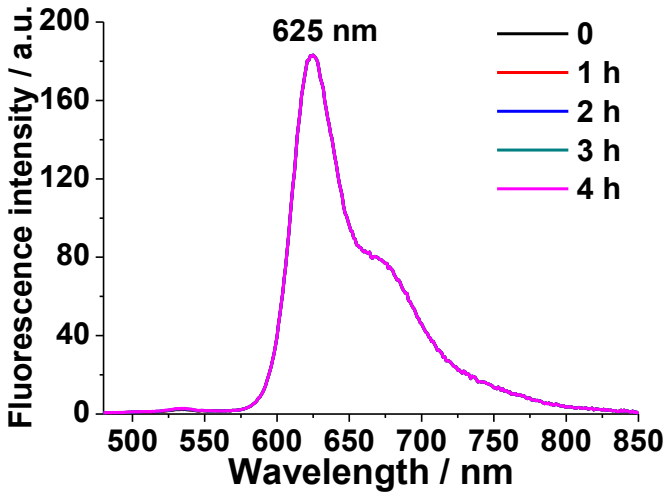


Figure S9. Time-dependent emission spectra of probe **1** (10 μM) in dry Toluene at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. Negligible change in emission spectra of probe **1** in dry Toluene for 4 h incubation was observed.

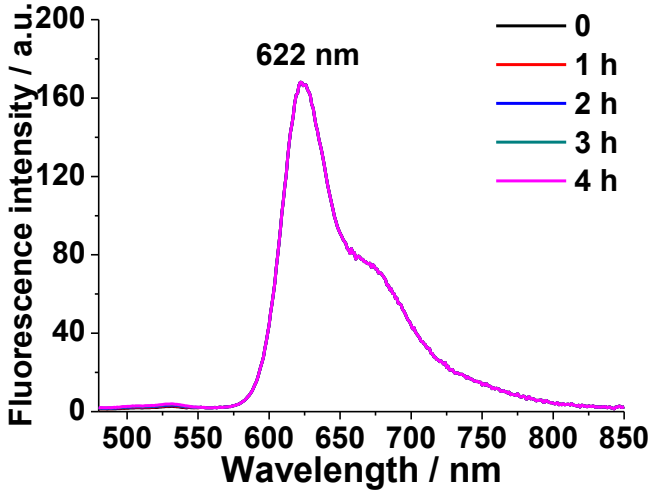


Figure S10. Time-dependent emission spectra of probe **1** (10 μM) in dry CH_2Cl_2 at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. Negligible change in emission spectra of probe **1** in dry CH_2Cl_2 for 4 h incubation was observed.

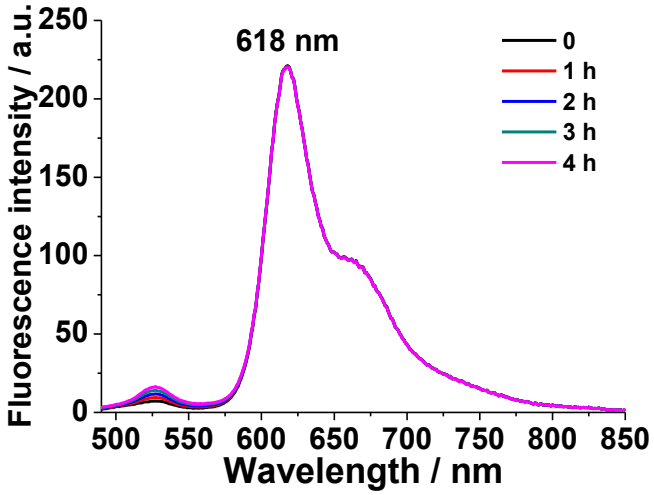


Figure S11. Time-dependent emission spectra of probe **1** (10 μM) in dry THF at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. A slight increase (2-fold) of green emission at 527 nm was observed after 4 hours incubation of probe **1** in dry THF.

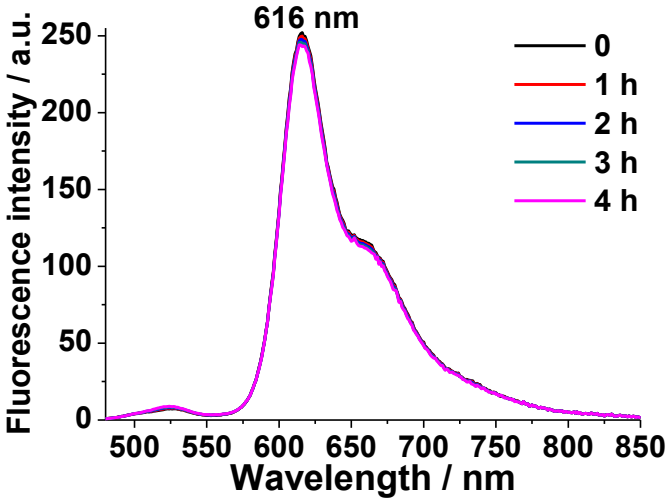


Figure S12. Time-dependent emission spectra of probe **1** (10 μM) in dry acetone at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. Negligible change in emission spectra of probe **1** in dry acetone for 4 h incubation was observed.

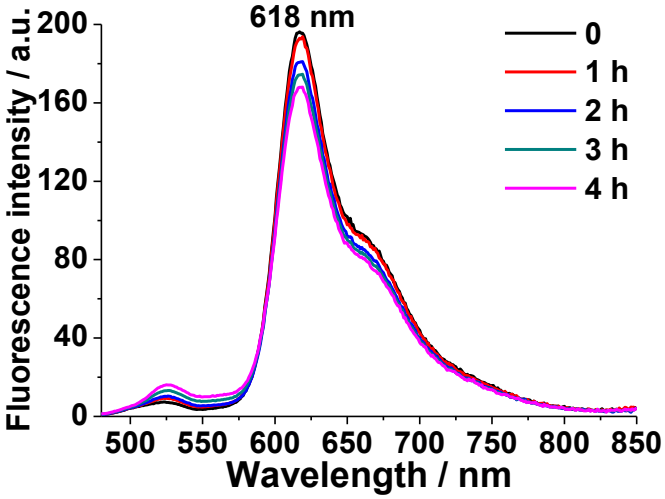


Figure S13. Time-dependent emission spectra of probe **1** (10 μM) in dry CH_3CN at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. A slight increase (2-fold) of green emission at 525 nm with a decrease of red emission band at 618 nm was observed after 4 h incubation of probe **1** in dry CH_3CN.

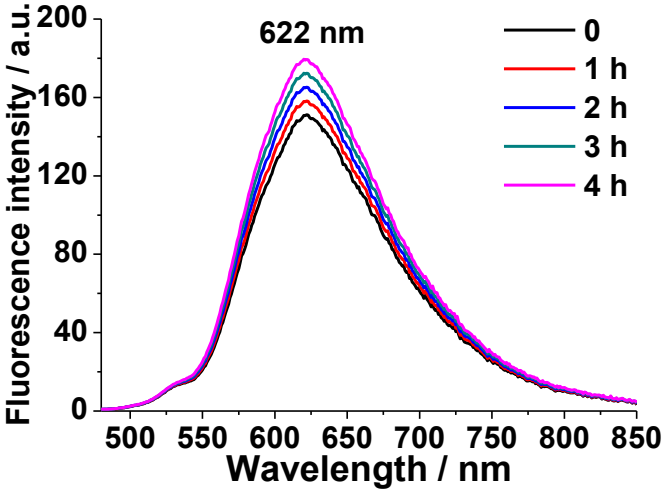


Figure S14. Time-dependent emission spectra of probe **1** (10 μM) in dry DMSO at 25 °C. The spectra were obtained every 1 hour (0 – 4 hours). Excited at 460 nm. A slight increase (1.2-fold) of red emission at 622 nm without emergence of green emission band was observed after 4 h incubation of probe **1** in dry DMSO.

4. Assay Studies for Water Contents in Organic Solvents

(a) Spectroscopic studies of probe 1 in organic solvents containing various amounts of water

The solution of probe **1** (1 mM, 2 μL) dissolved in each dry organic solvent was added to each organic solvent–water mixture solution (198 μL) containing various concentrations of water (0-99%, v/v). All spectra were obtained immediately after the addition with probe **1** to each solution at 25 °C. The increases in absorbance at the single wavelengths[8] (ε_{515} for CH_3CN; ε_{516} for 1,4-dioxane, acetone and DMSO; ε_{517} for THF) of probe **1** were recorded as a function of [water] in organic solvents.

Absorption and emission spectra of probe 1 in THF containing different amounts of water

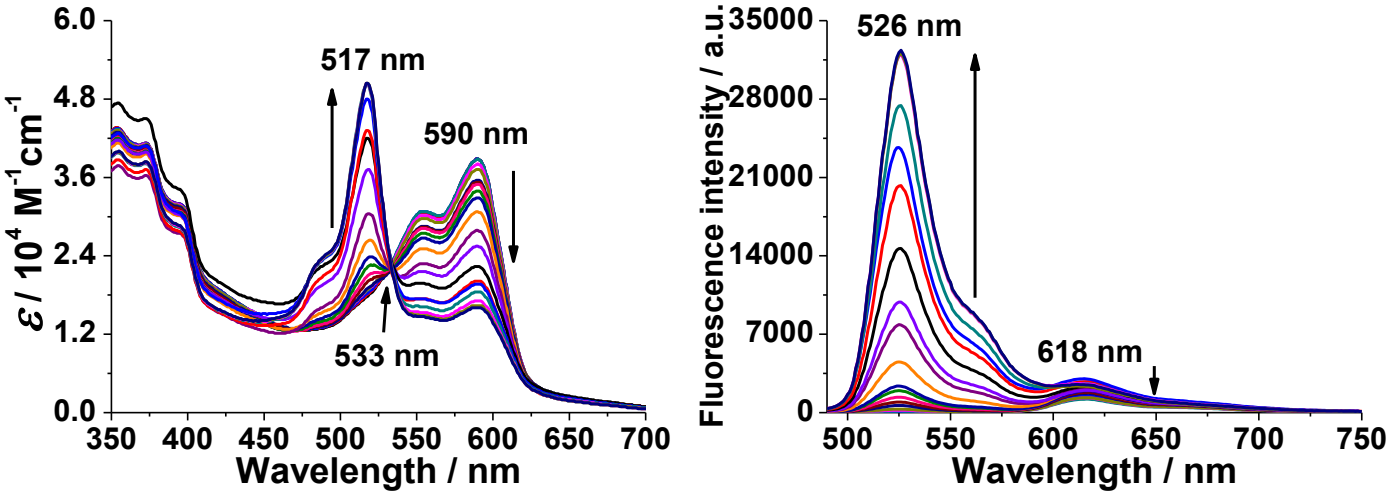


Figure S15. Absorbance (left) and fluorescence emission (right) spectra of probe **1** (10 μM) in THF containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm.

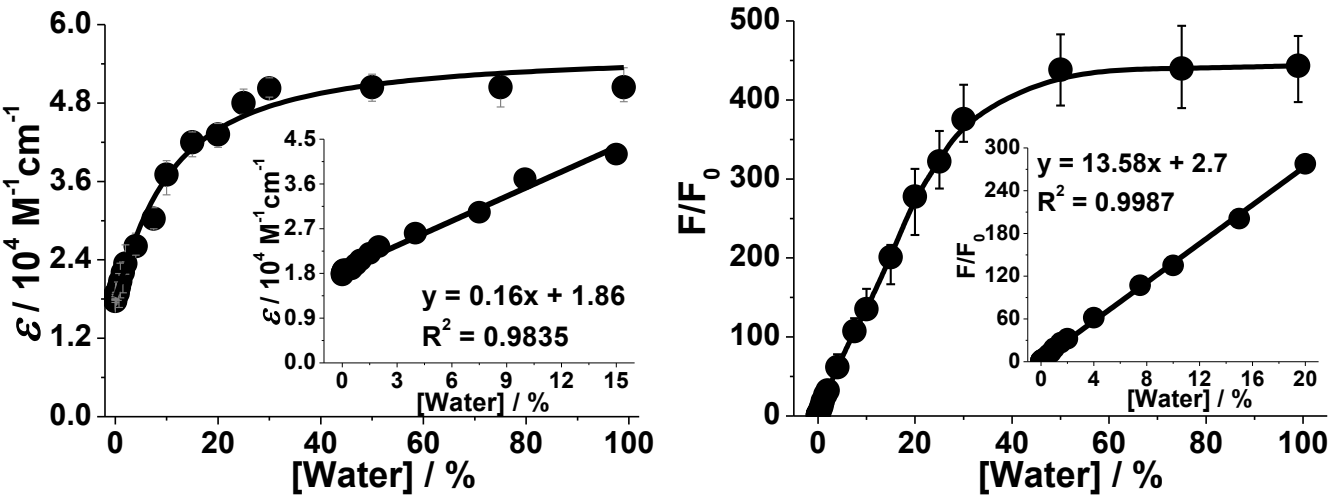


Figure S16. (left) Absorbance at 517 nm (ε_{517}) of probe **1** (10 μM) in THF containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. Inset: Linear correlation between ε_{517} and [water] in THF (0−15%, v/v). (right) Relative fluorescence intensity (F/F_0) of probe **1** (10 μM) in THF containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and the intensities of emission band at 526 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively. Inset: Linear correlation between F/F_0 at 526 nm and [water] (0–20%, v/v).

Absorption and emission spectra of probe 1 in 1,4-dioxane containing different amounts of water

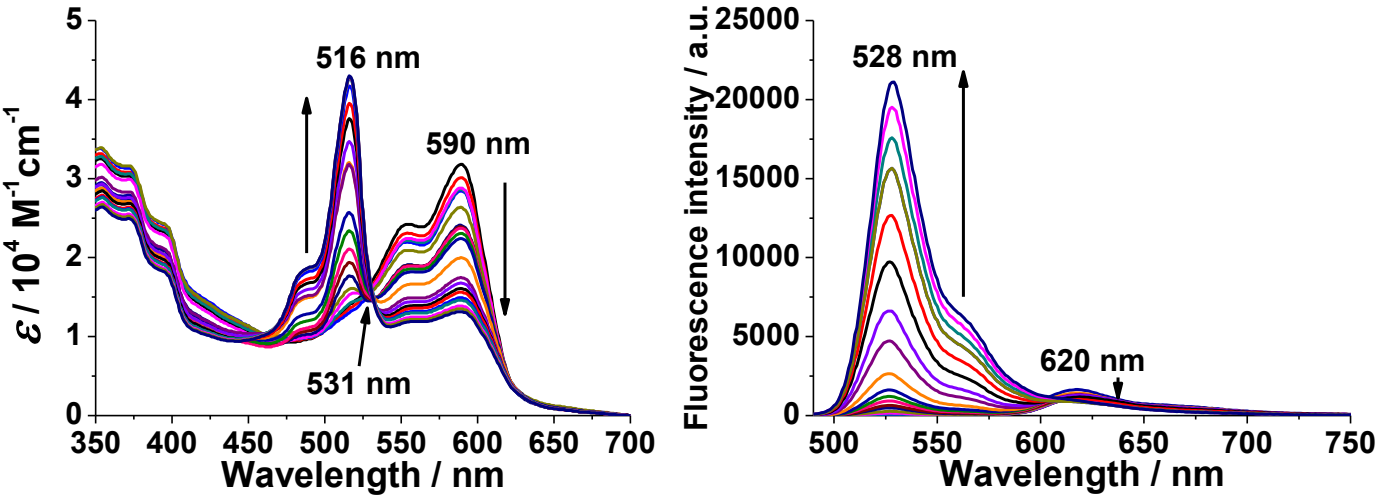


Figure S17. Absorbance (left) and fluorescence emission (right) spectra of probe **1** (10 μM) in 1,4-dioxane containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm.

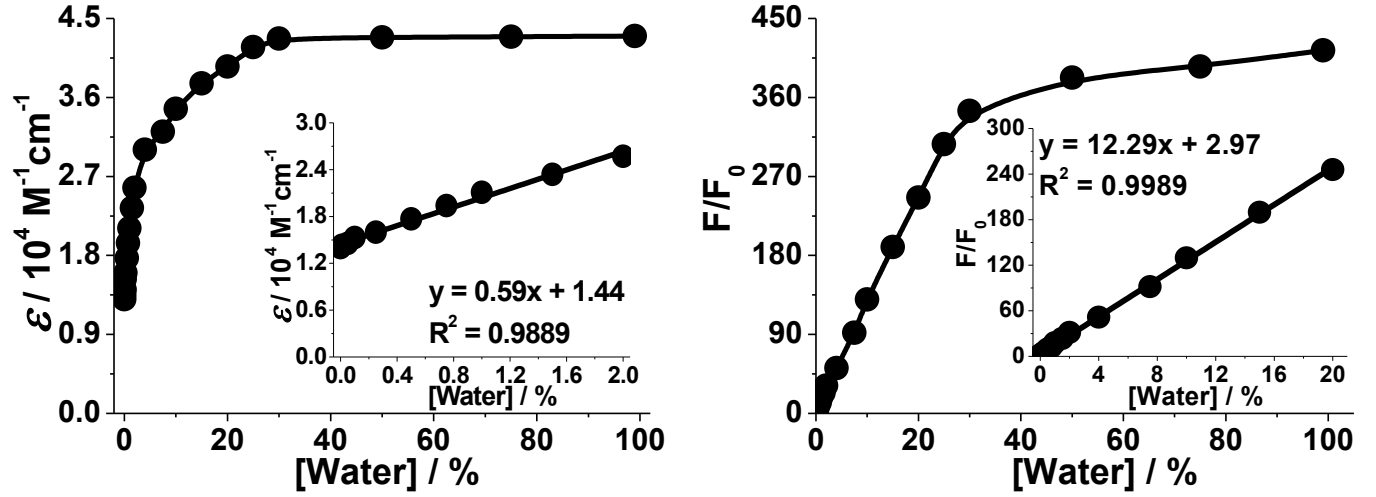


Figure S18. (left) Absorbance at 516 nm (ε_{516}) of probe **1** (10 μM) in 1,4-dioxane containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. Inset: Linear correlation between ε_{516} and [water] in 1,4-dioxane (0−2%, v/v). (right) Relative fluorescence intensity (F/F_0) of probe **1** (10 μM) in 1,4-dioxane containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and the intensities of emission band at 528 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively. Inset: Linear correlation between F/F_0 at 528 nm and [water] (0–20%, v/v).

Absorption and emission spectra of probe 1 in acetone containing different amounts of water

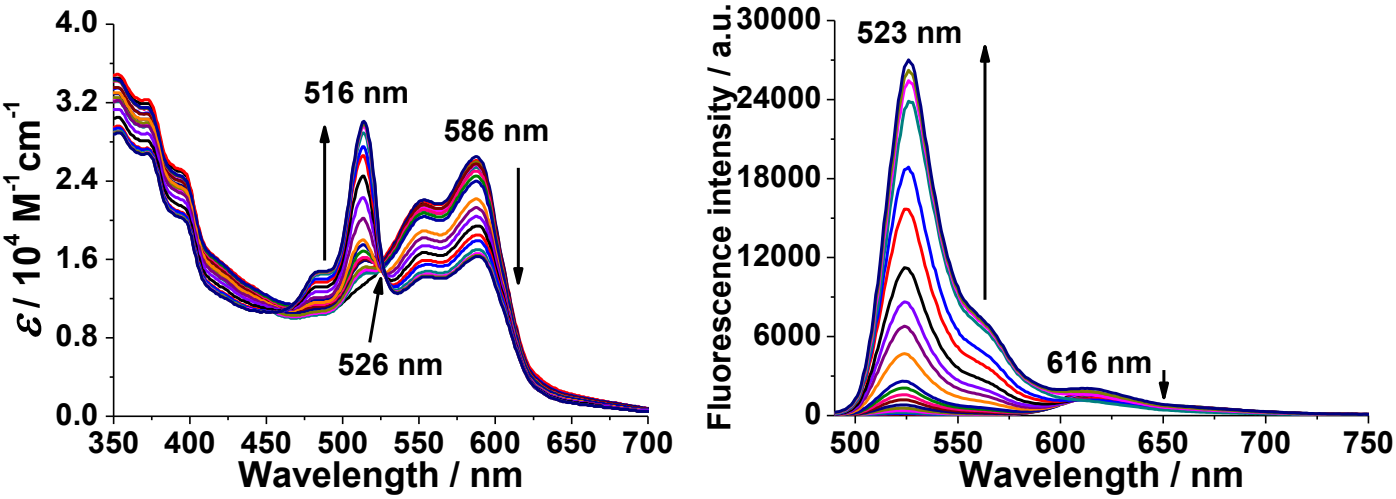


Figure S19. Absorbance (left) and fluorescence emission (right) spectra of probe **1** (10 μM) in acetone containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm.

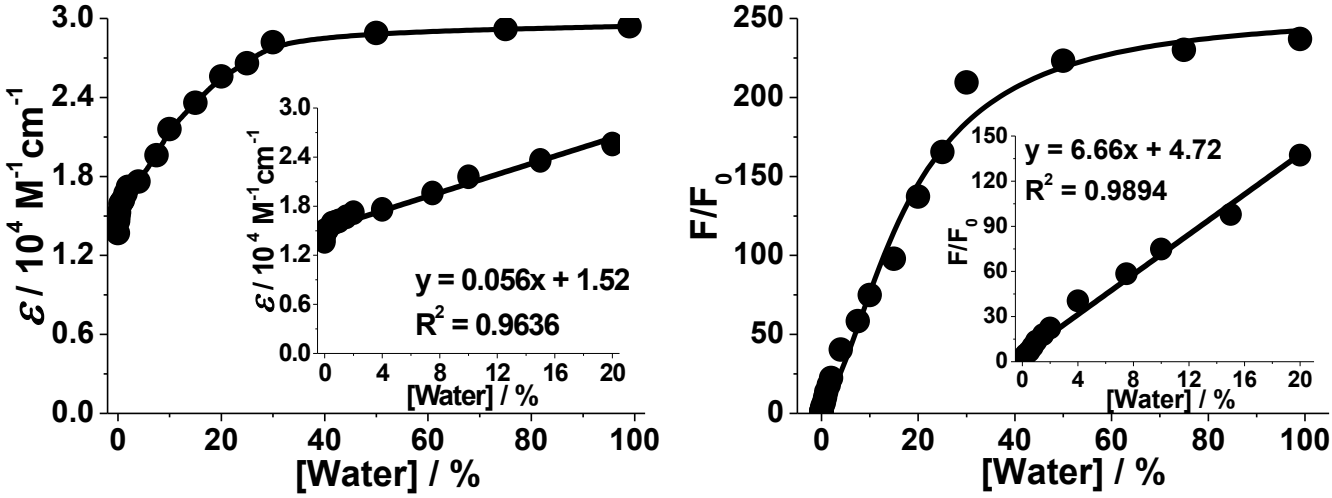


Figure S20. (left) Absorbance at 516 nm (ε_{516}) of probe **1** (10 μM) in acetone containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. Inset: Linear correlation between ε_{516} and [water] in acetone (0−20%, v/v). (right) Relative fluorescence intensity (F/F_0) of probe **1** (10 μM) in acetone containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and the intensities of emission band at 523 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively. Inset: Linear correlation between F/F_0 at 523 nm and [water] (0–20%, v/v).

Absorption and emission spectra of probe 1 in CH_3CN containing different amounts of water

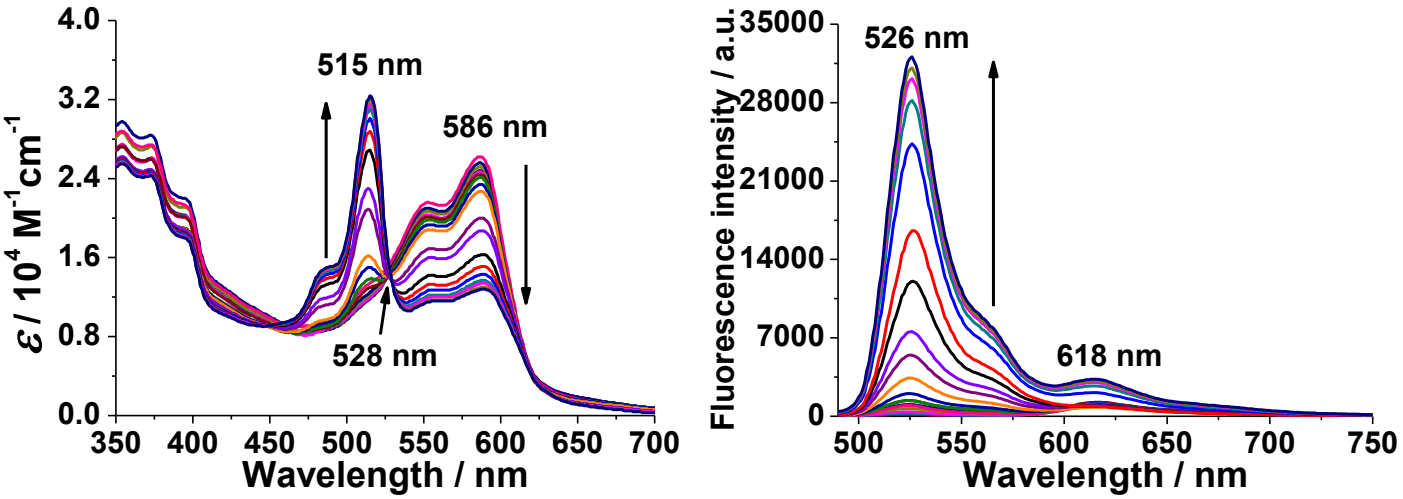


Figure S21. Absorbance (left) and fluorescence emission (right) spectra of probe **1** (10 μM) in CH_3CN containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm.

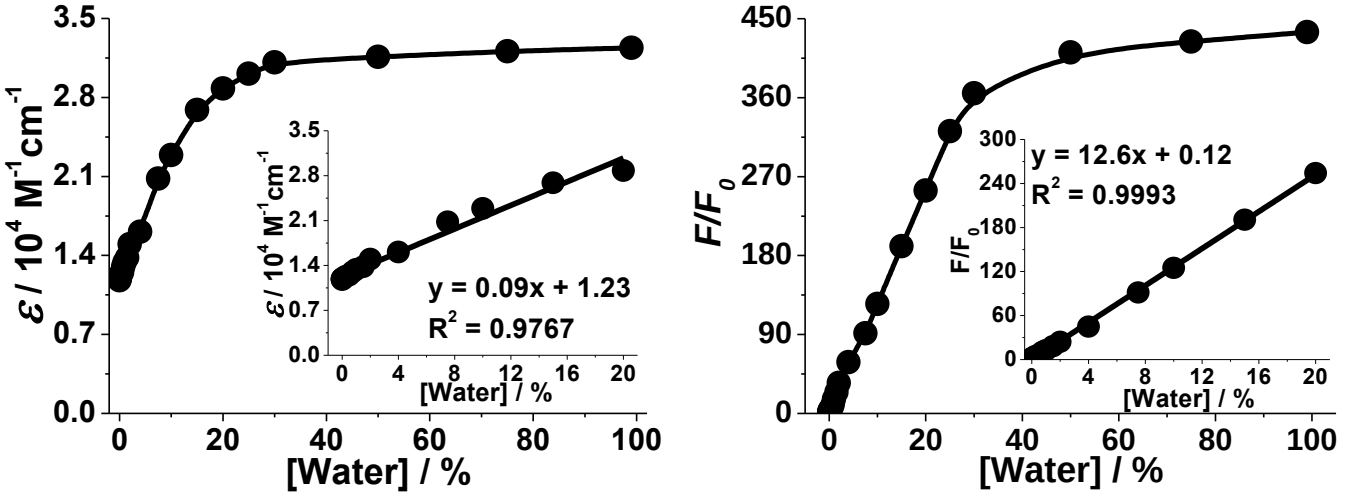


Figure S22. (left) Absorbance at 515 nm (ε_{515}) of probe **1** (10 μM) in CH_3CN containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. Inset: Linear correlation between ε_{515} and [water] in CH_3CN (0−20%, v/v). (right) Relative fluorescence intensity (F/F_0) of probe **1** (10 μM) in CH_3CN containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and the intensities of emission band at 526 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively. Inset: Linear correlation between F/F_0 at 526 nm and [water] (0–20%, v/v).

Absorption and emission spectra of probe 1 in DMSO containing different amounts of water

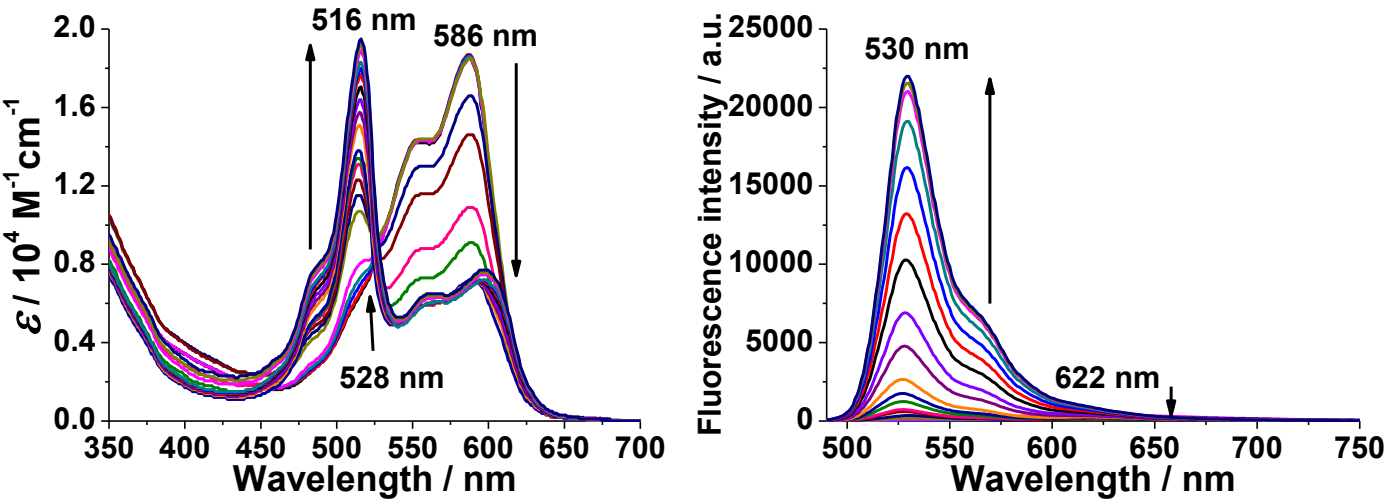


Figure S23. Absorbance (left) and fluorescence emission (right) spectra of probe **1** (10 μM) in DMSO containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm.

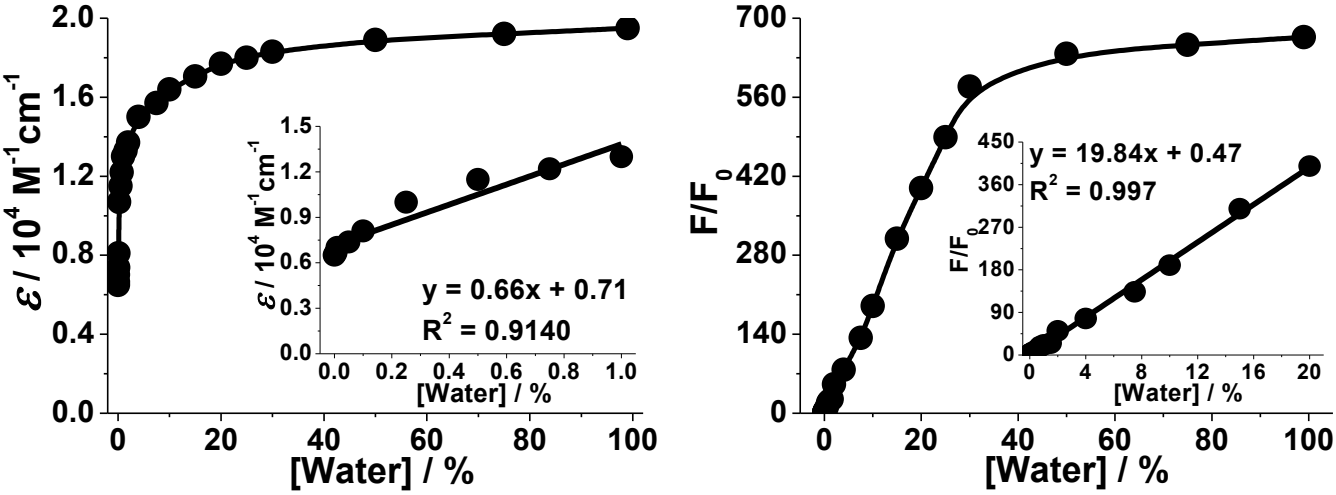


Figure S24. (left) Absorbance at 516 nm (ε_{516}) of probe **1** (10 μM) in DMSO containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. Inset: Linear correlation between ε_{516} and [water] in DMSO (0−1%, v/v). (right) Relative fluorescence intensity (F/F_0) of probe **1** (10 μM) in DMSO containing different amounts of water (0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and the intensities of emission band at 530 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively. Inset: Linear correlation between F/F_0 at 530 nm and [water] (0–20%, v/v).

(b) Comparison of changes in absorption spectra of probe 1 in organic solvents containing various amounts of water

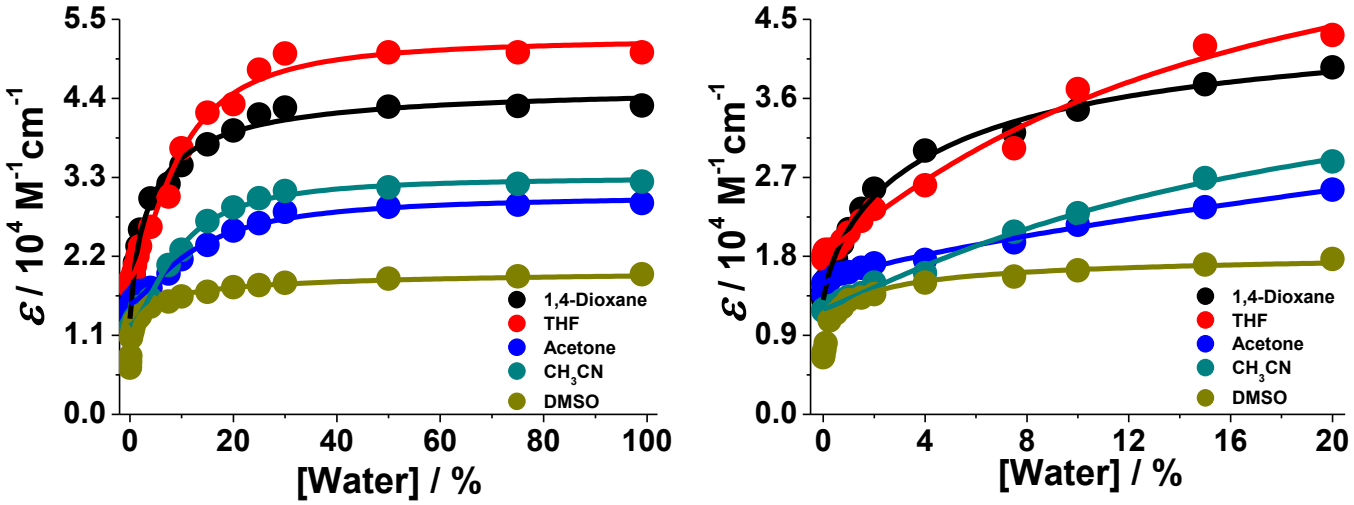


Figure S25. Comparison of relative absorbance (ε_{515} for CH_3CN; ε_{516} for 1,4-dioxane, acetone and DMSO; ε_{517} for THF) as a function of [water] (left: 0–99%; right: 0–20%, v/v) in various organic solvents at 25 °C. The spectra were obtained immediately after the addition of probe **1** (10 μM) to each solution.

(c) Comparison of changes in fluorescence intensity of green emission of probe 1 in organic solvents containing various amounts of water

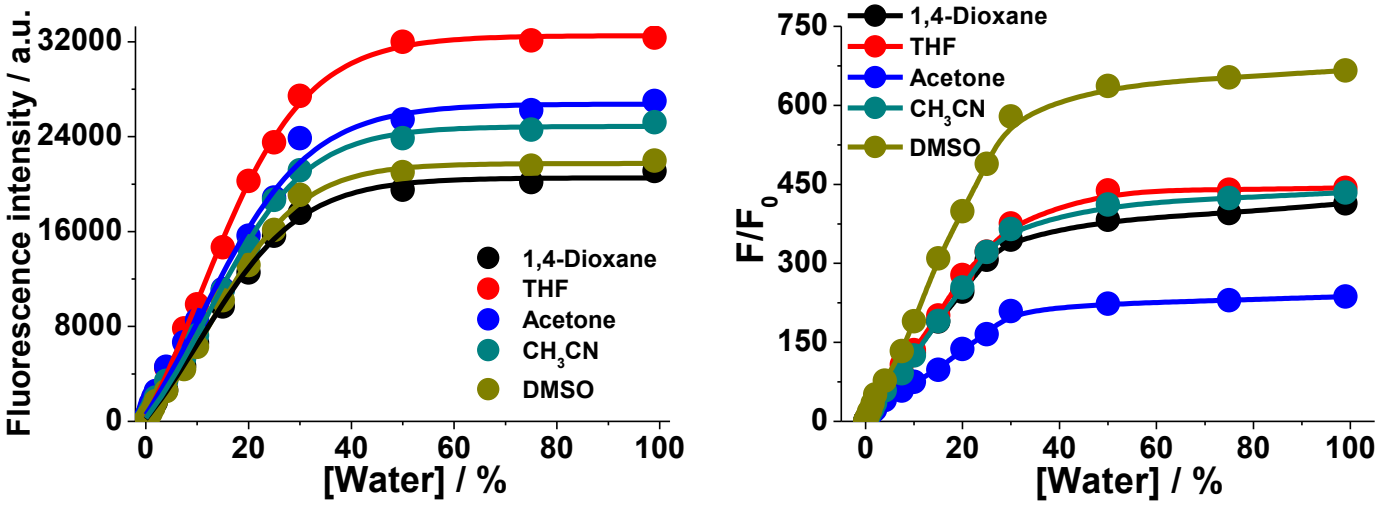


Figure S26. (left) Relative increase in fluorescence intensity at green emission of probe **1** (10 μM) in various organic solvents containing different amounts of water (0–99%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and fluorescence intensities of green emission were recorded. Excited at 460 nm. (right) Plot of the relative fluorescence intensities (F/F_0) of green emission vs. the amounts of water (0−99%, v/v) in various solvents. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively.

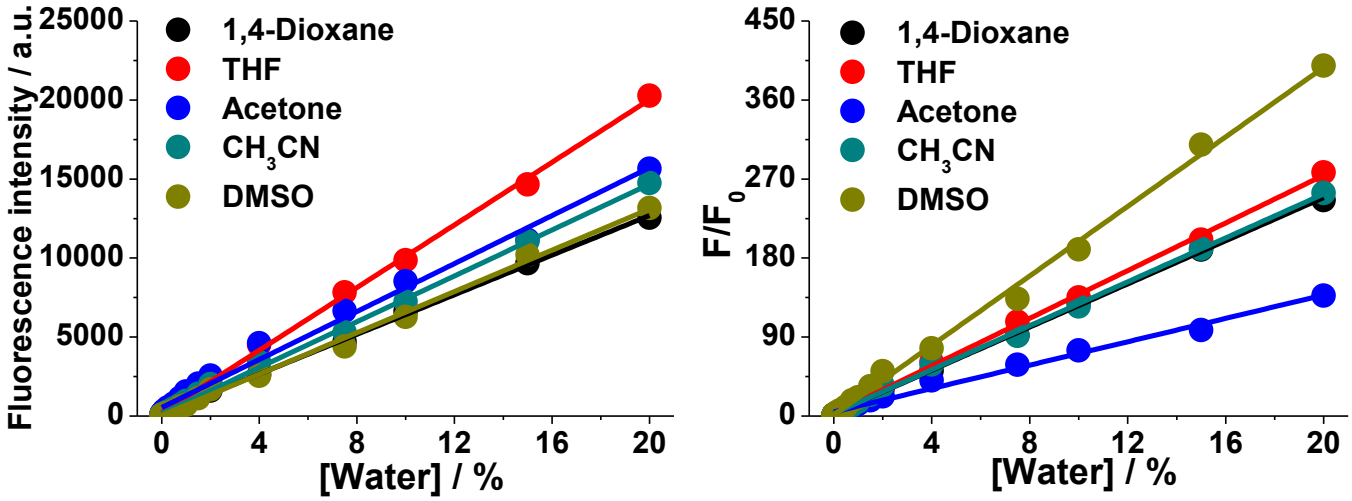


Figure S27. (left) Relative increase in fluorescence intensity at green emission of probe **1** (10 μM) in various organic solvents containing different amounts of water (0–20%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and fluorescence intensities of green emission were recorded. Excited at 460 nm. (right) Plot of the relative fluorescence intensities (F/F_0) of green emission vs. the amounts of water (0−20%, v/v) in various solvents. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively.

(d) Determination of detection limit of probe 1 toward water in organic solvents

The fluorescence emission spectra of probe **1** (10 μM) in dry organic solvents were collected for 25 times to determine the background noise σ. Increases in fluorescence intensity of green emission were monitored immediately after the addition of probe **1** (10 μM) into each solution containing different amounts of water ranging from 0 to 1% (v/v). A linear regression curve was then fitted according to fluorescence intensities of green emission band as a function of amount of water in each solution, and the slope of the curve was obtained. The detection limit was determined on the basis of the equation, 3σ/slope.

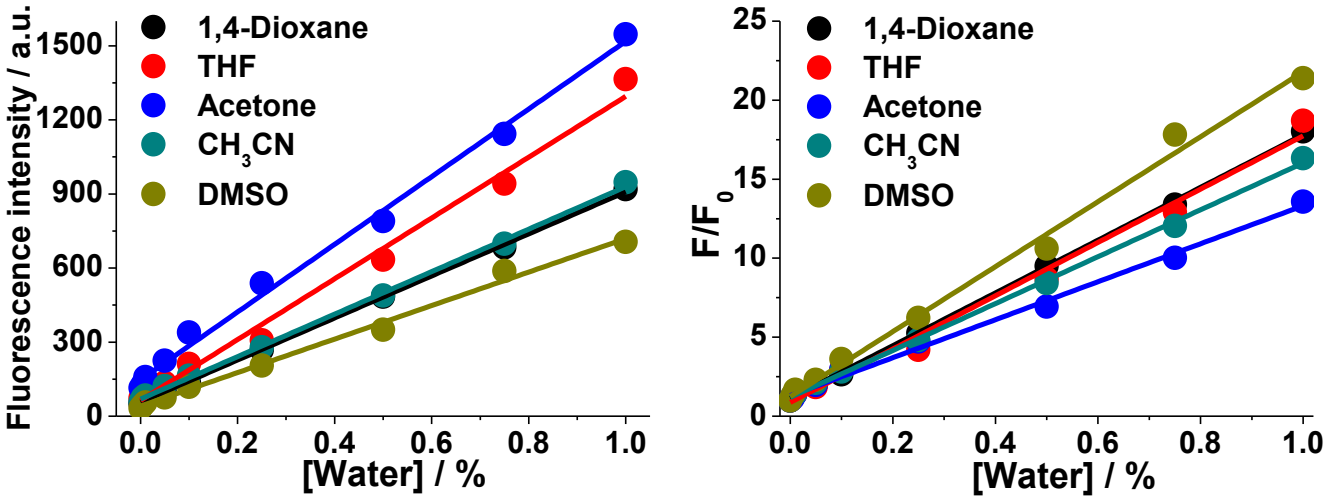


Figure S28. (left) Relative increase in fluorescence intensity at green emission of probe **1** (10 μM) in various organic solvents containing different amounts of water (0–1%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and fluorescence intensities of green emission were recorded. Excited at 460 nm. (right) Plot of the relative fluorescence intensities (F/F_0) of green emission vs. the amounts of water (0−1%, v/v) in various solvents. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively.

Table S2. Comparison of calibration curves and LODs for water detection in organic solvents

Solvent	Mode	0−1% (v/v) water in organic solvent	LOD[c]
1,4-Dioxane	Absorbance[a]	0.689x + 1.42 (R^2 = 0.9953)	0.019% (184 ppm)
	Emission[b]	828.16x+62.3 (R^2 = 0.9951)	0.008% (77 ppm)
THF	Absorbance[a]	0.238x + 1.81 (R^2 = 0.8890)	0.013% (146 ppm)
	Emission[b]	1262.5x+74.05 (R^2 = 0.9726)	0.003% (34 ppm)
Acetone	Absorbance[a]	0.226x + 1.43 (R^2 = 0.9157)	0.014% (177 ppm)
	Emission[b]	1371.7x+146.9 (R^2 = 0.9940)	0.006% (76 ppm)
CH_3CN	Absorbance[a]	0.149x + 1.18 (R^2 = 0.9875)	0.008% (102 ppm)
	Emission[b]	800.65x+64.58 (R^2 = 0.9715)	0.003% (38 ppm)
DMSO	Absorbance[a]	0.650x + 0.71 (R^2 = 0.9140)	0.050% (454 ppm)
	Emission[b]	678.93x+39.73 (R^2 = 0.9933)	0.007% (64 ppm)

[a]Measured at its absorption maximum (ε_{515} for CH_3CN; ε_{516} for 1,4-dioxane, acetone and DMSO; ε_{517} for THF). [b]Obtained by fluorescence intensity at green emission. [c]Limit of detection (%, v/v).

(e) Time-dependent fluorescence response of probe 1 toward water in THF

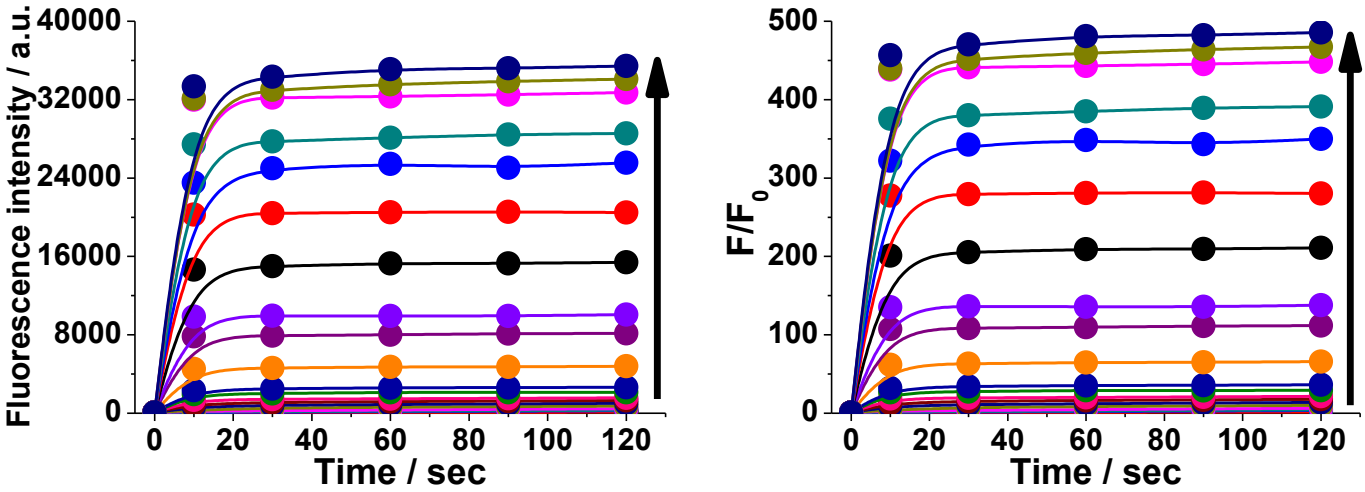


Figure S29. (left) Time-dependent increase in fluorescence intensity at 526 nm of probe **1** (10 μM) in THF containing different amounts of water (bottom to top: 0, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7.5, 10, 15, 20, 25, 30, 50, 75, 99%, v/v). The spectra were obtained immediately after the addition of probe **1** to each solution at 25 °C and fluorescence intensities at 526 nm were recorded. Excited at 460 nm. (right) Plot of the relative fluorescence intensities (F/F_0) at 526 nm vs. the amounts of water (0−99%, v/v) in THF. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively.

(f) Determination of binding constant of hydrate adduct formation between probe 1 and water in THF

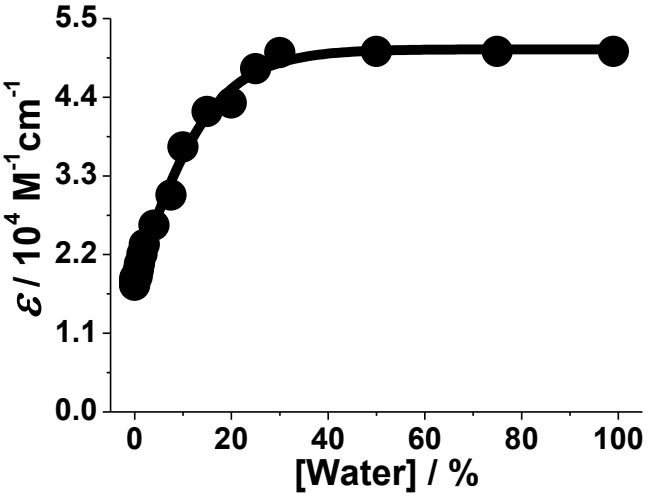


Figure S30. Determination of the binding constant of probe **1** (10 μM) with water in THF using a 1:1 specific binding model by Graphpad Prism 7 software. The spectra were obtained immediately after the addition of probe **1** to each solution at 25 °C and relative absorbance at 517 nm (ε_{517}) as a function of [water] in THF (0−99%, v/v) was plotted. Dissociation constant (K_d) was determined to be 1.27 M, by fitting the absorbance at 517 nm (ε_{517}) titration data.

(g) Effect of concentration of probe 1 on fluorescence response toward trace water (0−1%) in THF

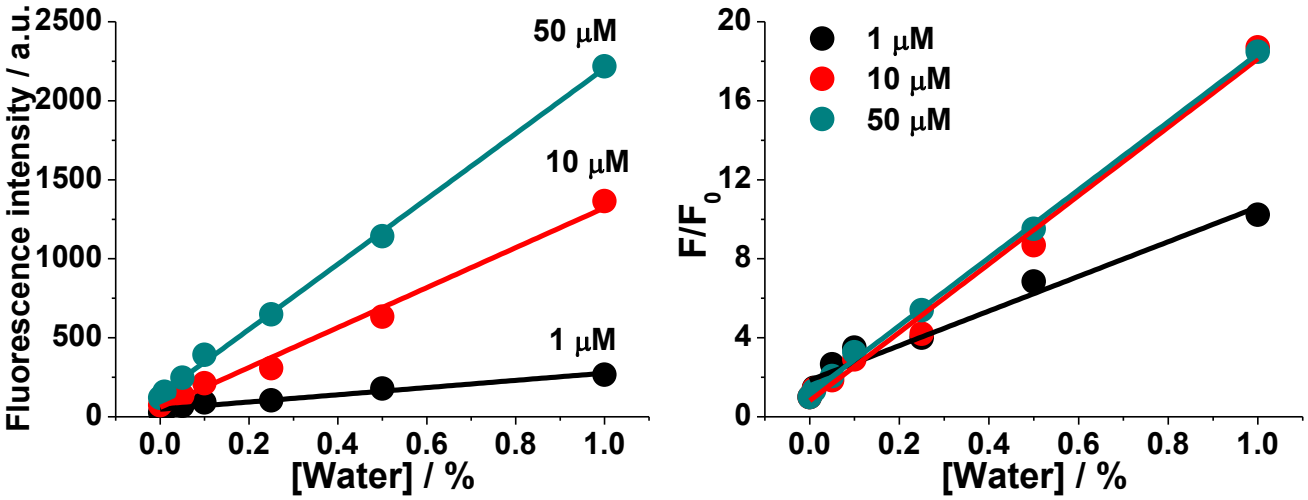


Figure S31. (left) Relative increase in fluorescence intensity at 526 nm of probe **1** at different concentrations (1, 10, 50 μM) in THF containing different amounts of water (0, 0.01, 0.05, 0.1, 0.25, 0.5, 1%, v/v) at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution and fluorescence intensities at 526 nm were recorded. Excited at 460 nm. (right) Plot of the relative fluorescence intensities (F/F_0) at 526 nm vs. the amounts of water (0−1%, v/v) in THF. F and F_0 correspond to the fluorescence intensity in the presence and absence of water in each solution, respectively.

(h) Effect of temperature on fluorescence response of probe 1 toward water (1%, v/v) in THF

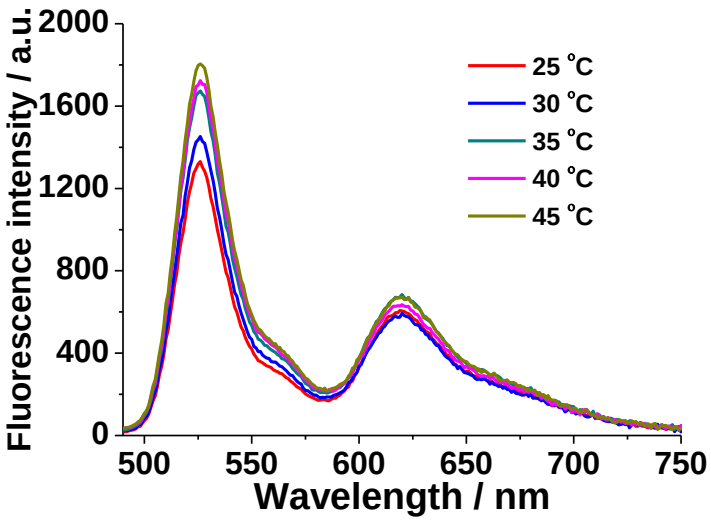


Figure S32. Fluorescence emission spectra of probe **1** (10 μM) in THF containing water (1%, v/v) at different temperatures (25, 30, 35, 40, 45 °C). The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm. As the temperature increases (25 to 45 °C), larger increases in fluorescence intensity at 526 nm were observed.

(i) Effect of pH on fluorescence response of probe 1 toward water (1%, v/v) in THF

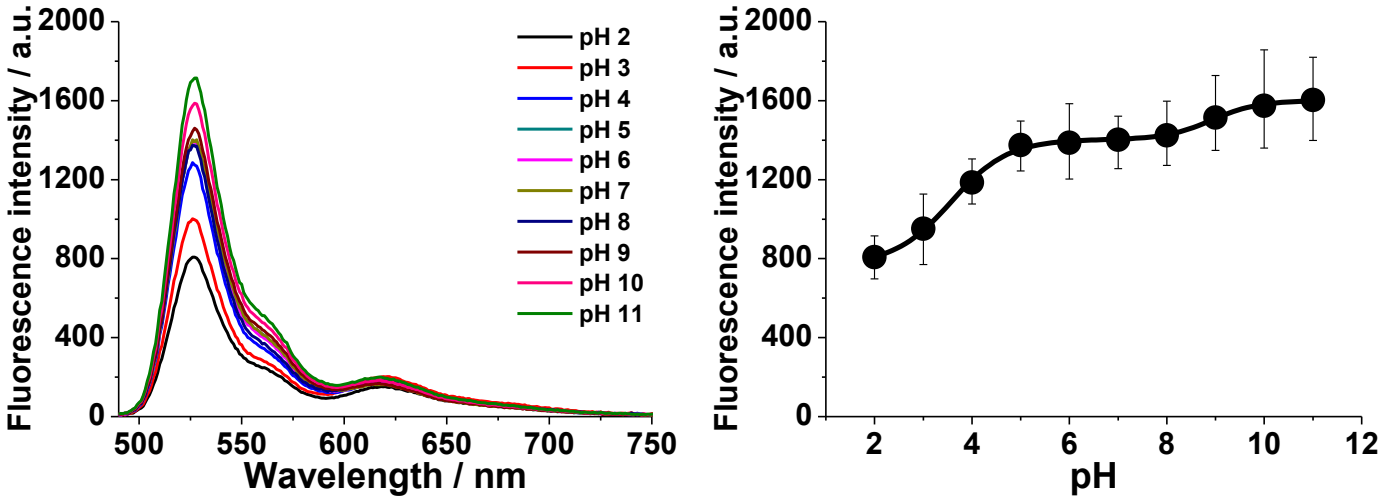


Figure S33. Fluorescence emission spectra of probe **1** (10 μM) in THF containing aqueous buffer solution (1%, v/v) adjusted at different pHs at 25 °C. The spectra were obtained immediately after the addition of probe **1** to each solution. Excited at 460 nm. As the pH of aqueous solution added in THF increases (pH 2 to pH 11), more significant increases in fluorescence intensity at 526 nm were observed.

(j) Investigation of fluorescence spectra of probe 1 in THF containing different amounts of R-OH

We investigated fluorescence spectra of probe **1** toward various **R**OH solvents in THF at 25 ^{o}C. The addition of probe **1** to large amount of alcohol solvents (i.e., methanol, ethanol, 1-propanol, isopropyl alcohol, and *tert*-butyl alcohol) showed the green fluorescence emission around at λ_{em} 526 nm, probably due to the formation of hemiacetal adduct as evidenced by ^{1}H-NMR spectra of probe **1** in CD_3OD (Figure S38). The relative fluorescence responses of probe **1** toward various **R**OH (**R** = H, CH_3, C_2H_5, *n*-C_3H_7, *i*-C_3H_7, *t*-C_4H_9) at different concentrations (0–99%, v/v) in THF are well correlated with their steric effect of reactant (F/F_0 at 526 nm: H_2O > CH_3OH > C_2H_5OH > 1-C_3H_7OH > 2-C_3H_7OH > *t*-BuOH), as shown in Figures S34-S35. It is worth noting that the observed changes in the fluorescence of probe **1** in THF upon addition of small quantities of alcohol solvents (≤ 10%, v/v) except methanol are not so pronounced compared with that of H_2O (Figure S34, left).

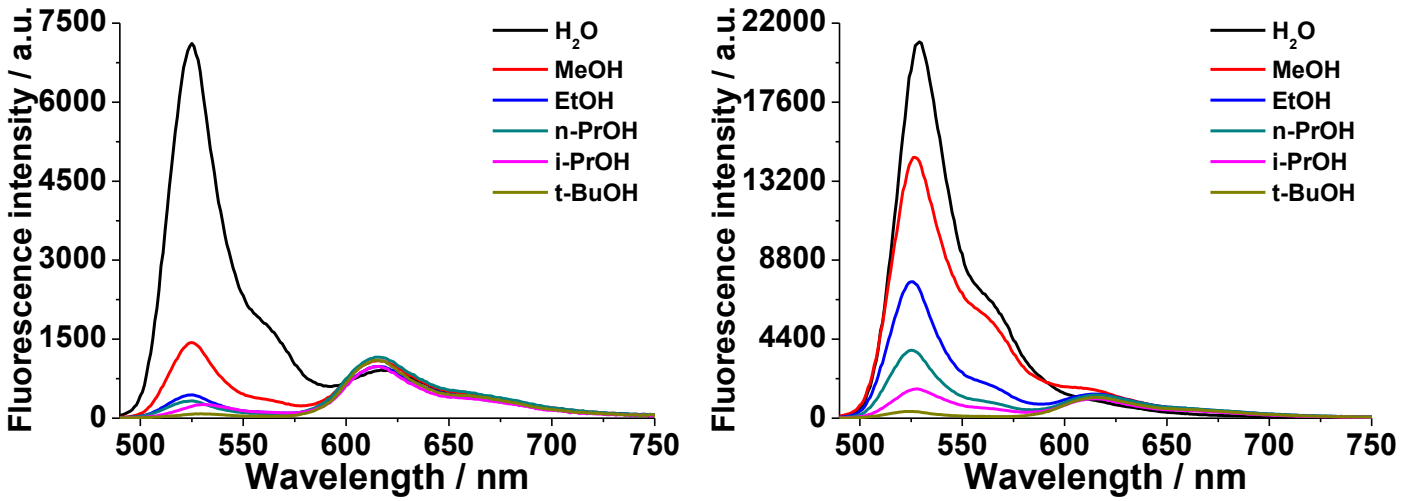


Figure S34. Fluorescence emission spectra of probe **1** in dry THF containing **R**OH (**R** = H, CH_3, C_2H_5, *n*-C_3H_7, *i*-C_3H_7, *t*-C_4H_9; left: 10%; right: 30%, v/v). The spectra were obtained immediately after the addition of probe **1** (10 μM) to each solution at 25 °C. Excited at 460 nm.

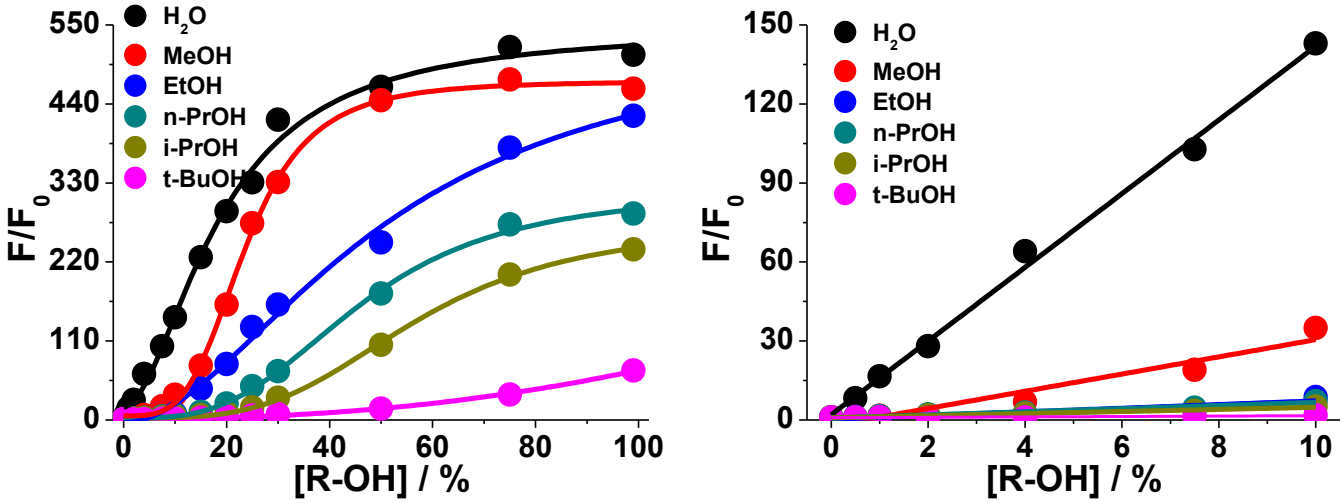


Figure S35. Relative fluorescence intensity (F/F_0) at 526 nm of probe **1** vs. the amounts of **R**OH (left: 0−99%; right: 0−10%, v/v) in dry THF. The spectra were obtained immediately after the addition of probe **1** (10 μM) to each solution at 25 °C and the fluorescence intensity at 526 nm were recorded. Excited at 460 nm. F and F_0 correspond to the fluorescence intensity in the presence and absence of **R**OH (**R** = H, CH_3, C_2H_5, *n*-C_3H_7, *i*-C_3H_7, *t*-C_4H_9) in THF, respectively.

(k) Absorption spectra of probe 1 in DMF and comparison with that of probe 1 in THF containing diethylamine

As shown in Figure S36, the blue-shifted absorption maximum band (λ_{abs}^{max} = 492 nm) of probe **1** in dry DMF relative to that in other dry organic solvents (λ_{abs}^{max} = 586 nm–601 nm, Table S1) was observed (i.e., λ_{abs}^{max} (dry DMF) = 492 nm vs. λ_{abs}^{max} (dry THF) = 590 nm; see blue vs. black solid-lined spectra in Figure S36). This might be due to the reaction product between probe **1** and trace impurity (dimethylamine, ~1 ppm)[9] present in DMF solvent. The absorption spectrum of probe **1** in dry DMF is well-matched with that of probe **1** in THF containing diethylamine (1 ppm) (see blue vs. red solid-lined spectra in Figure S36).

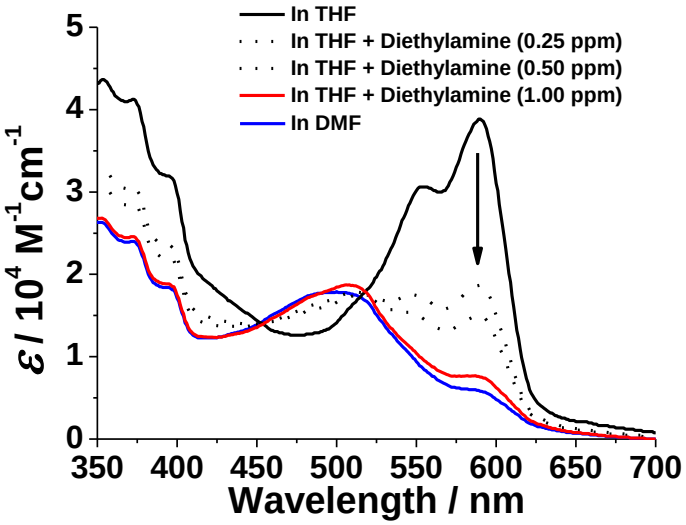
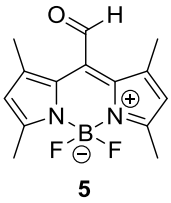


Figure S36. Absorption spectra of probe **1** (10 μM) in dry DMF (blue solid line), in dry THF containing diethylamine (0 (black solid line), 0.25 (black dot line), 0.50 (black dot line), 1 ppm (red solid line)) at 25 °C.

(l) Investigation of fluorescence spectra of compound 5 in THF containing different amounts of water

Additionally, non-emissive *meso*-formyl-1,3,5,7-tetramethyl BODIPY (**5**), which might provide opportunity as fluorogenic probe of nucleophilic attack by water, showed negligible changes in emission spectra of **5** upon treatment with water (upto 50%, v/v) at 25 °C. These results underline the importance of steric effect in addition to electronic effect for the successful sensing response based on the conversion of aldehyde to hydrate adduct in our scheme.



5

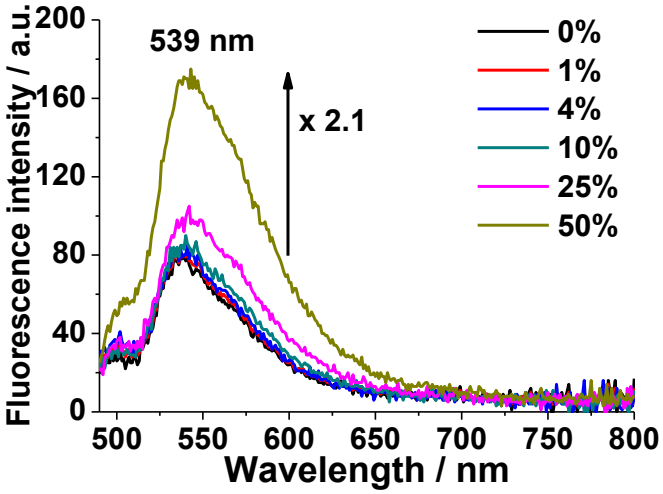


Figure S37. Fluorescence emission spectra of **5** (10 μM) in THF containing different amounts of water (0, 1, 4, 10, 25, 50%, v/v) at 25 °C. The spectra were obtained immediately after the addition of **5** to each solution. Excited at 460 nm.

5. Identification of Reaction Product by ^{1}H-NMR and LC-MS

^{1}H NMR analysis: Hemiacetal formation of probe 1 in CD_3OD

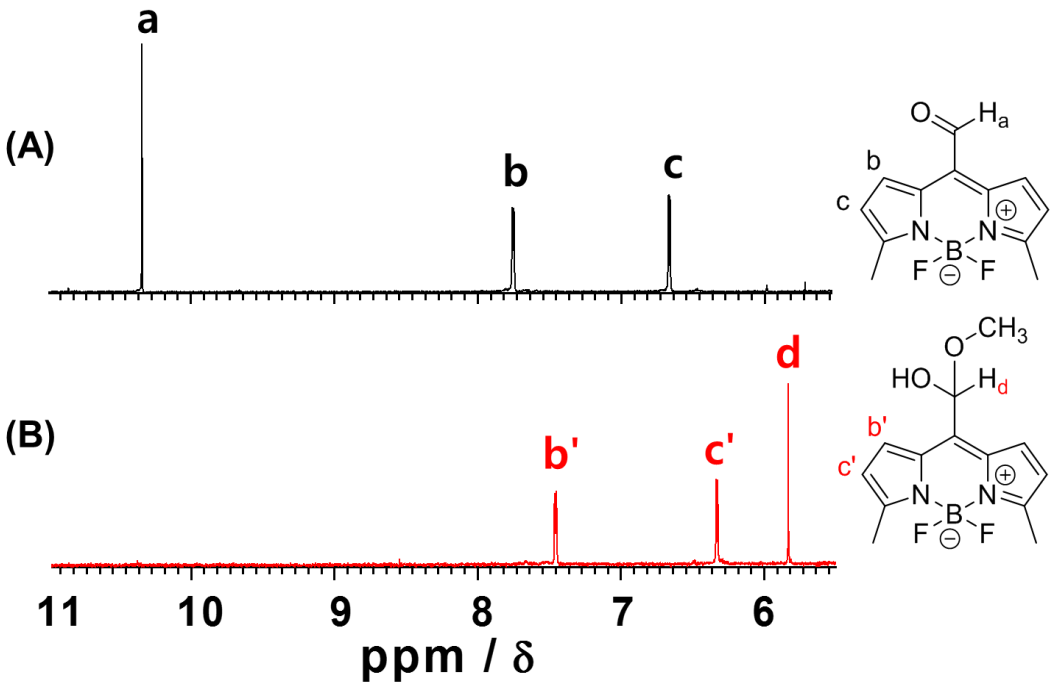


Figure S38. Partial ^{1}H-NMR spectra of probe **1** (20 mM) in THF-d_8 (A) and CD_3OD (B) at 21 °C.

LC-MS analysis: Aldehyde hydrate formation of probe 1 in H_2O– THF

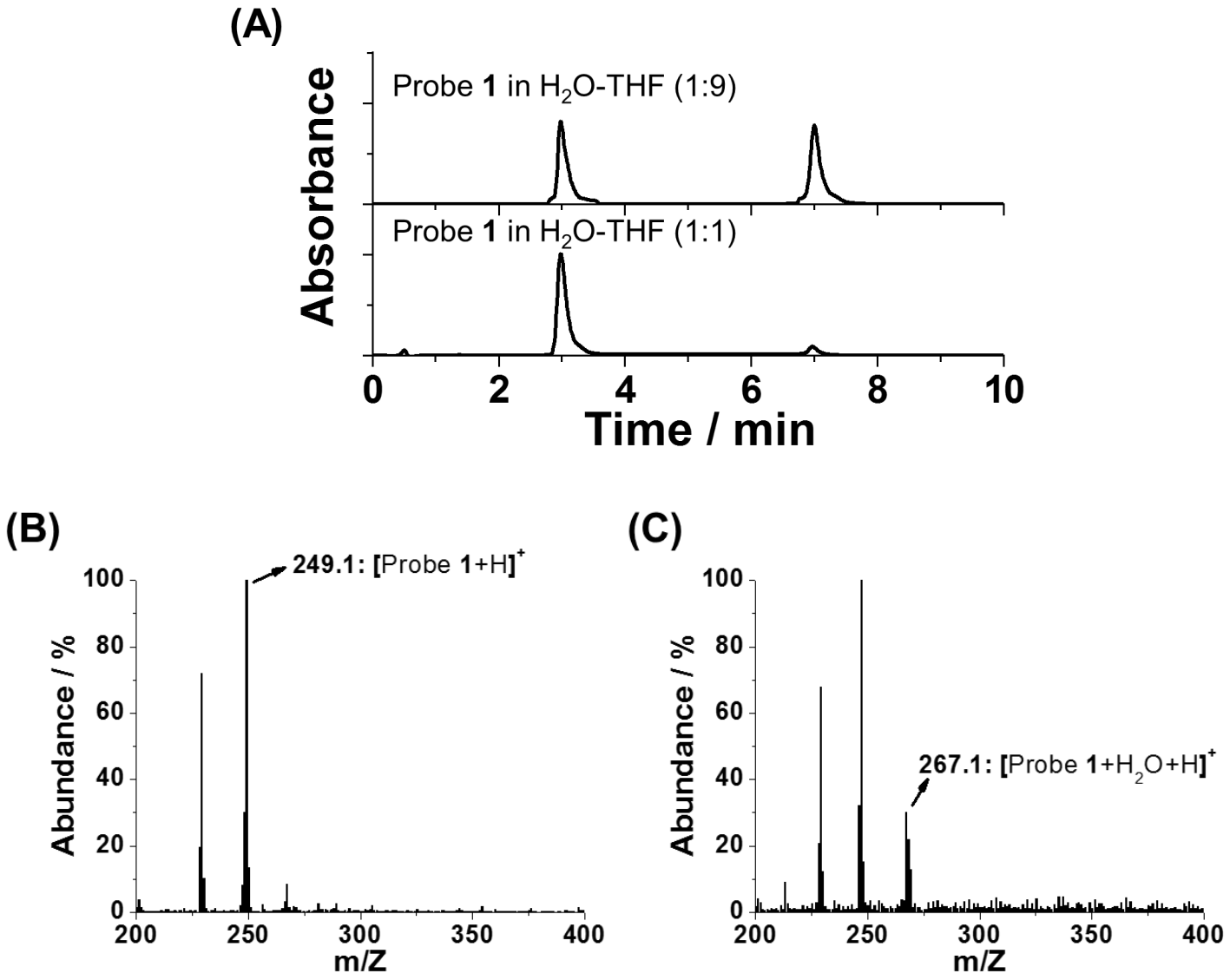


Figure S39. (A) HPLC chromatograms of probe **1** in water–THF mixture solution (1:9, v/v) (top); probe **1** in water–THF mixture solution (1:1, v/v) (bottom). The samples were analyzed by LC-MS with a linear gradient elution (from 50 to 100% B, A: 5 mM ammonium formate buffer, B: acetonitrile with 0.1% formic acid, flow rate 0.3 mL/min, UV: 500 nm). ESI-MS spectra of the peak of retention time at (B) 7 min and (C) 3 min. MW of the retention time at 7 min is 249.1, which corresponds to [probe **1**+H]$^+$ and MW of retention time at 3 min is 267.1, which corresponds to [probe **1**+H_2O+H]$^+$ for the aldehyde hydrate product. [Probe **1**] = 1 mM.

6. Determination Water contents in Various Samples

(a) Paper strip-based detection of water in organic solvents

To facilitate the use of probe **1** for the detection of water in a portable manner, a simple paper strip (10 mm x 10 mm) loaded with probe **1** was prepared by dipping filter paper into a THF solution of probe **1** (1 mM) for 10 seconds and drying it under reduced pressure. The as-prepared paper strip was non-emissive, probably due to probe **1**'s strong tendency for π–π stacking of extended planar structure in solid state. As shown in Figure S40, the nonemissive paper strip immediately showed strong red emission upon dropping of pure THF (5 μL) onto the paper strip (Figure S40A) or immersing the strip into 1 mL of pure THF (Figure S40B) under 365 nm UV light illumination. As for solution-phase detection, the dynamic changes in emission color using paper strip loaded with probe **1** were immediately observed upon treatment with THF solution containing different amounts of water (0.1, 1, 4, 10, 20, 30%, v/v) at 25 °C.

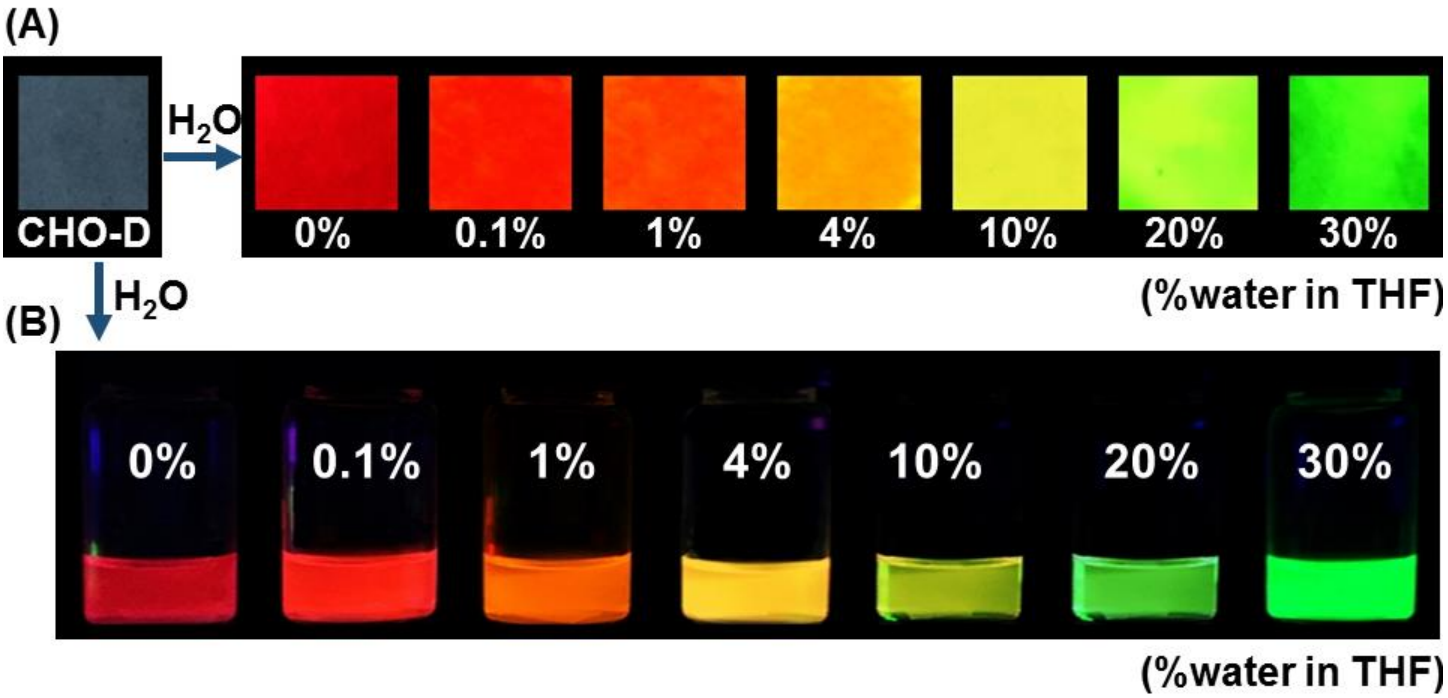


Figure S40. (A) Photographs of the paper strips adsorbed with probe **1** upon dropping of water–THF mixture solutions (5 μL) containing different amounts of water (left to right: 0, 0.1, 1, 4, 10, 20, 30%, v/v) at 25 °C under UV irradiation (365 nm). All photographs were taken immediately after dropping water–THF mixture solutions. (B) Photographs of THF solutions containing different amounts of water (left to right: 0, 0.1, 1, 4, 10, 20, 30%, v/v) after dipping of the paper strips adsorbed with probe **1** into each solution (1 mL) at 25 °C under UV irradiation (365 nm). All photographs were taken after dipping the paper strips into each vial containing water–THF mixture solutions and then stirring for 10 seconds.

(b) Determination of water contents in various samples

The solid-powdered active pharmaceutical ingredients (A: **A**torvastatin calcium trihydrate, T; **T**enofovir disoproxil fumarate) were obtained from Sungwun Pharmacopia/Bio Inc. 10 mg of each sample was dissolved in dry THF (1 mL), and sonicated for 15 min to dissolve completely. Each sample solution (198 μL) was mixed with the probe **1** dissolved in dry THF (1 mM, 2 μL) in 96-well plate set-up. Final concentration of probe **1** in solution was 10 μM. Fluorescence spectra were obtained immediately after mixing of probe **1** with each sample, and fluorescence intensity at 526 nm was recorded. Using calibration curve obtained from Figure 5B, the amount of water in each sample was determined and compared with the values measured by Karl-Fischer titration. The water contents in high vacuum pump oil (MR-100) (Neovac Corp. Japan), grapeseed oil, margarine and honey samples were measured in the same procedures.

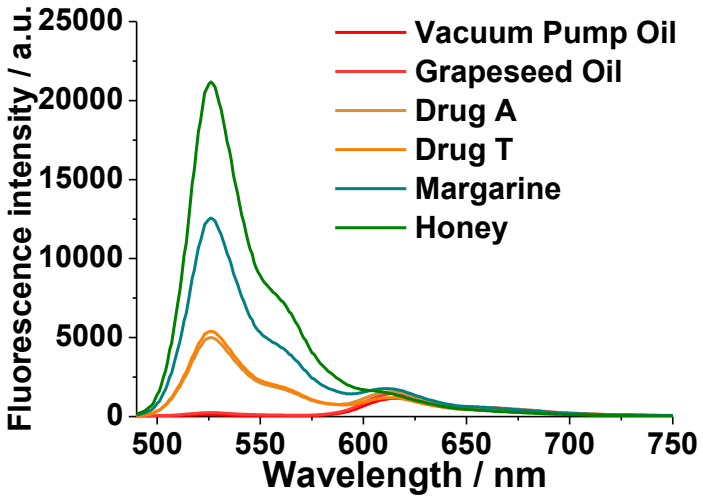


Figure S41. Fluorescence emission spectra of probe **1** (10 μM) in dry THF–sample mixture (10 mg/mL) at 25 °C. The spectra were obtained immediately the addition of probe **1** to each sample solution. Excited at 460 nm.

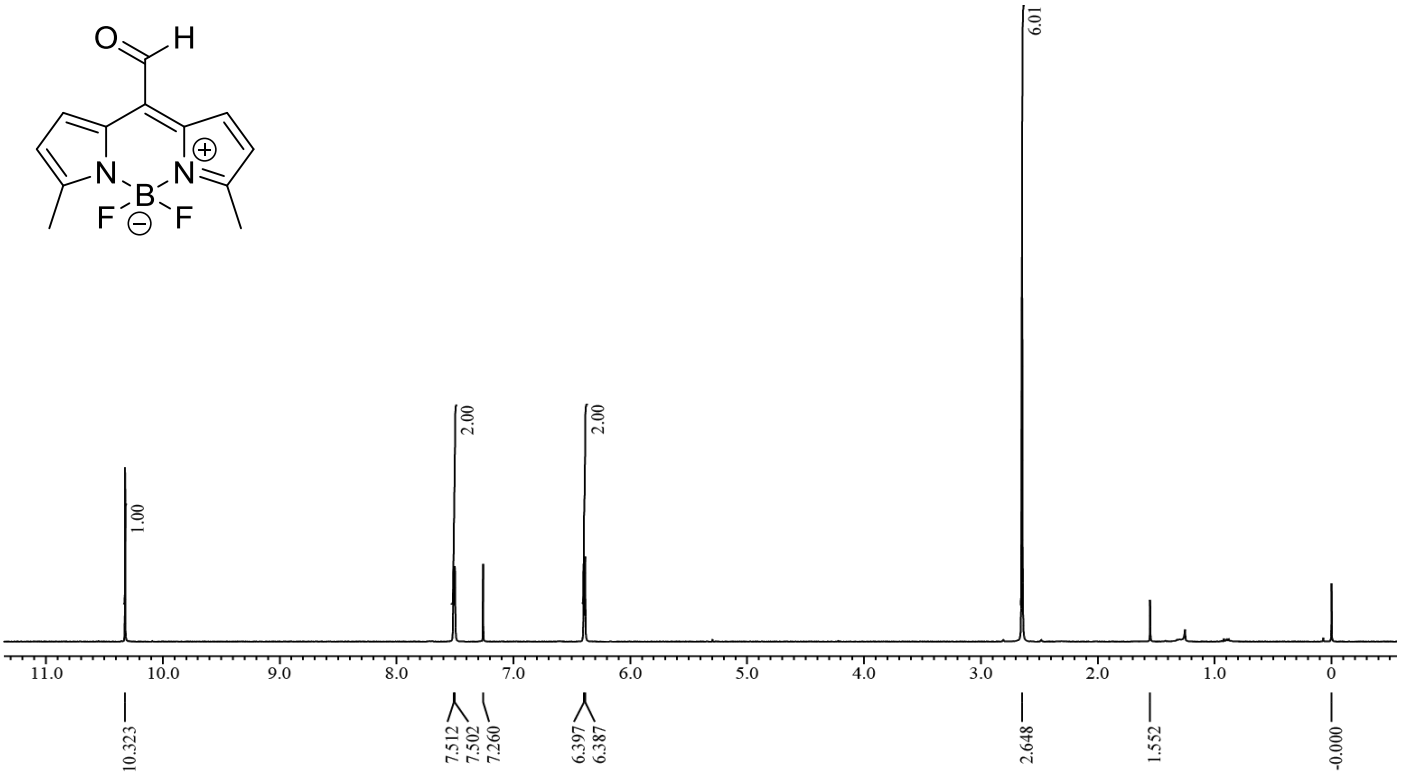
Table S3. Determination of water contents in the various samples

Samples	Probe **1** (water %)[a]	K-F method (water %)
Vacuum pump oil	0.025 ± 0.008	0.029[b]
Grapeseed oil	0.153 ± 0.007	0.16[b]
Drug A	4.81 ± 0.22	4.80[c]
Drug T	5.20 ± 0.21	5.06[c]
Margarine	13.24 ± 1.85	16.17[c]
Honey	21.05 ± 1.59	19.56[c]

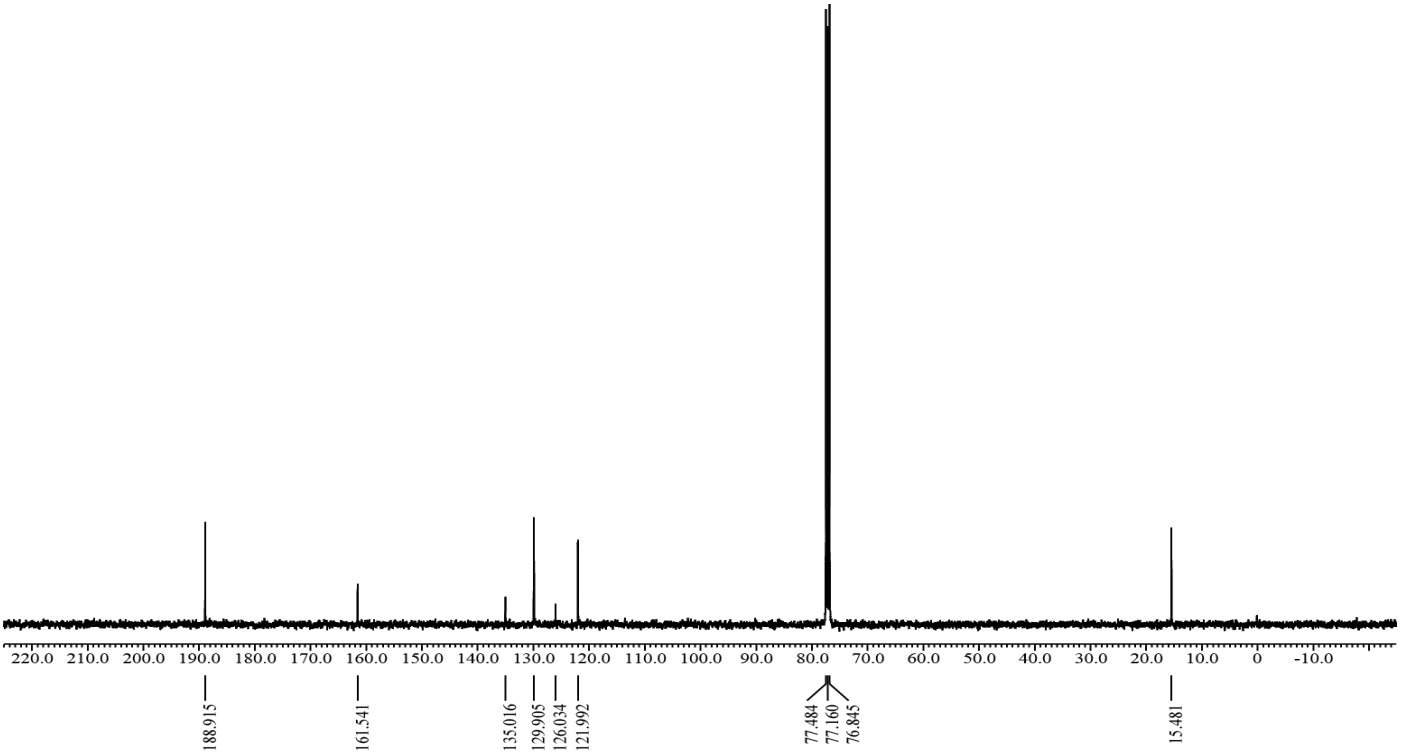
[a]Water contents in samples were determined by the average from 4 independent measurements. [b]Measured by using Mettler Toledo C20 (coulometric titration). [c]Measured by using Metrohm 795 (volumetric titration).

7. ^{1}H-NMR and ^{13}C-NMR Spectra of Compounds

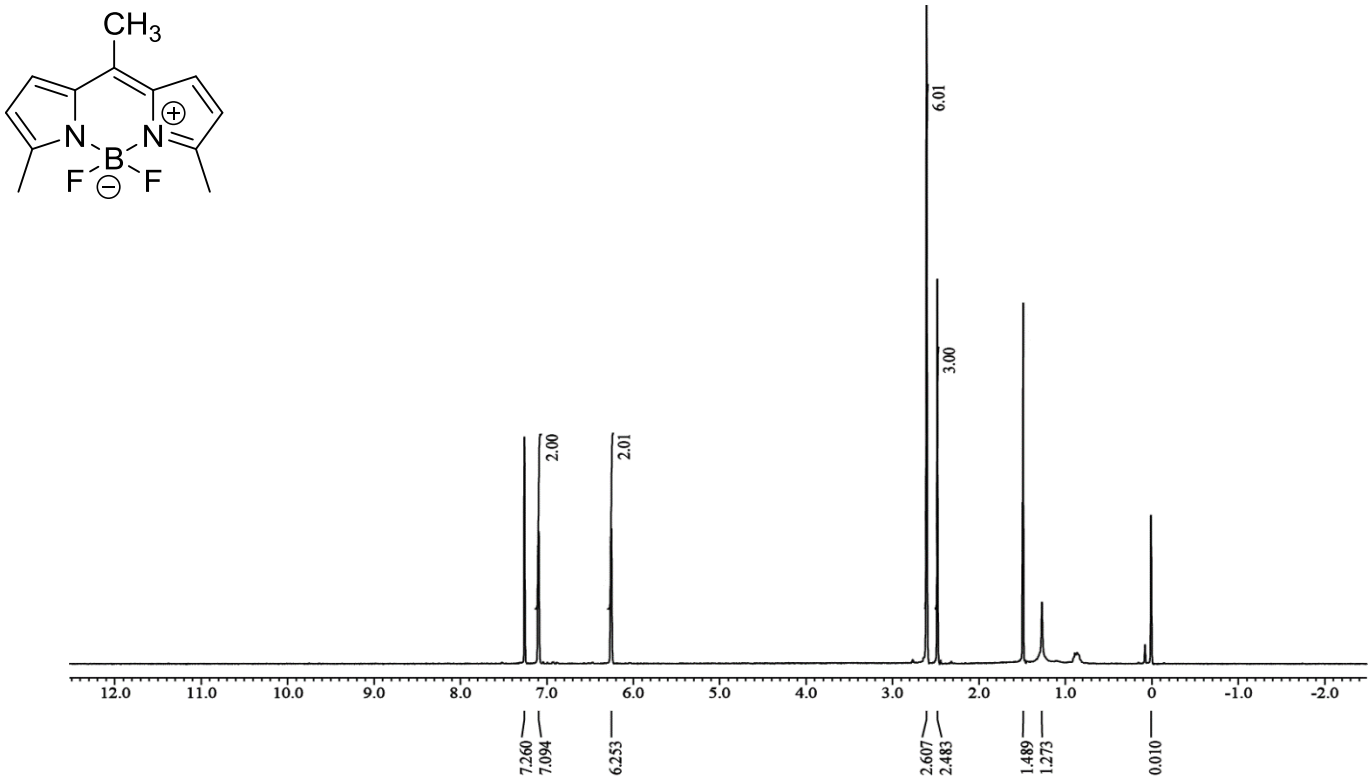
^{1}H-NMR Spectrum of probe **1** in $CDCl_3$ (400 MHz):



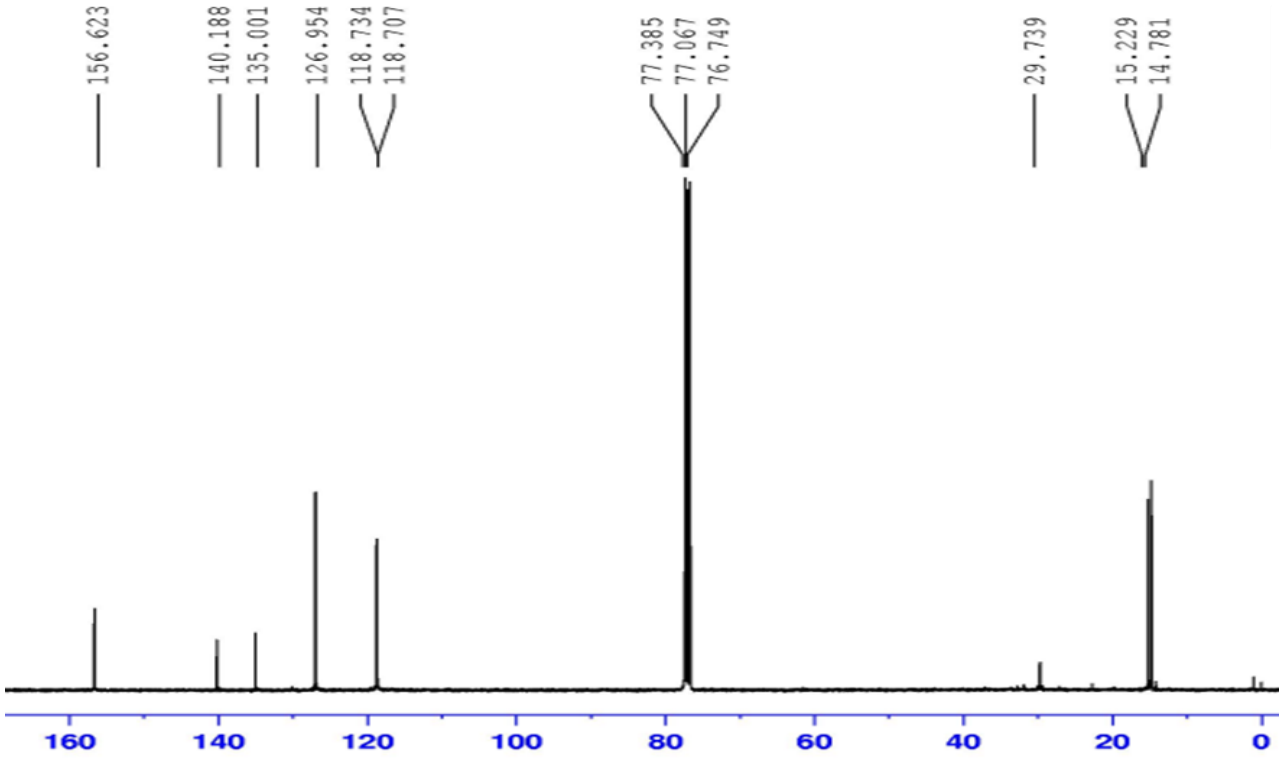
^{13}C-NMR Spectrum of probe **1** in $CDCl_3$ (100 MHz):



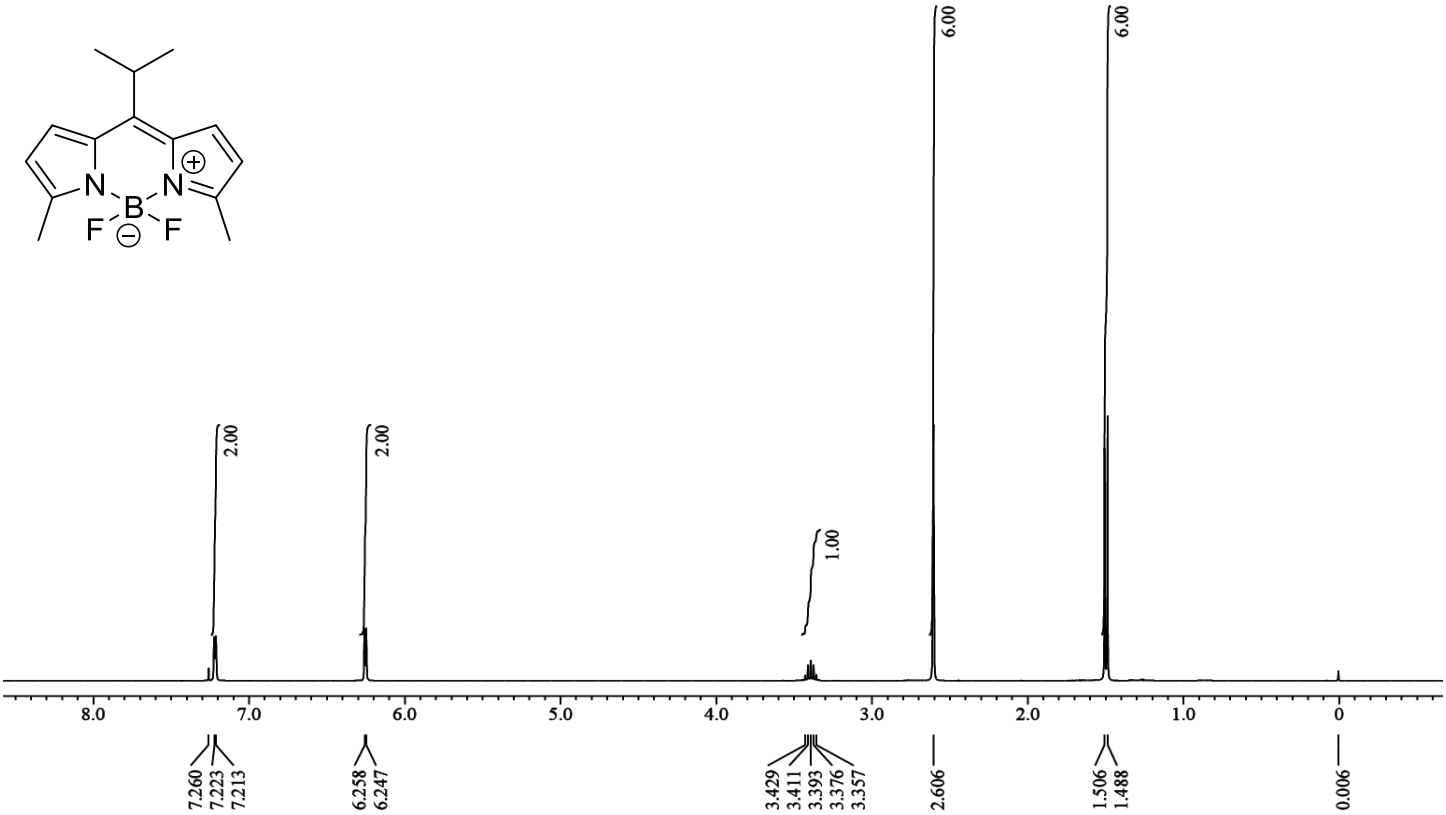
^{1}H-NMR Spectrum of compound **2** in $CDCl_3$ (400 MHz):



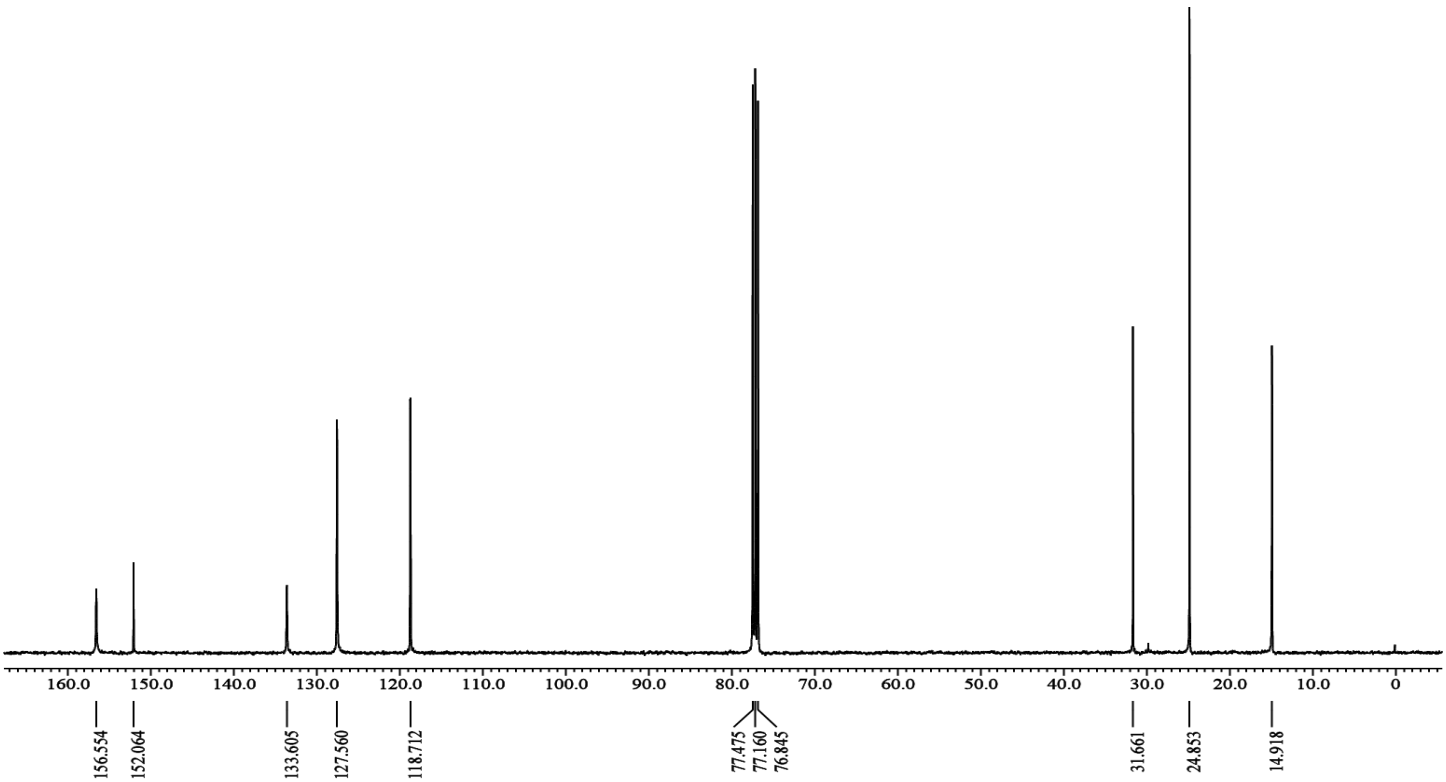
^{13}C-NMR Spectrum of compound **2** in $CDCl_3$ (100 MHz):



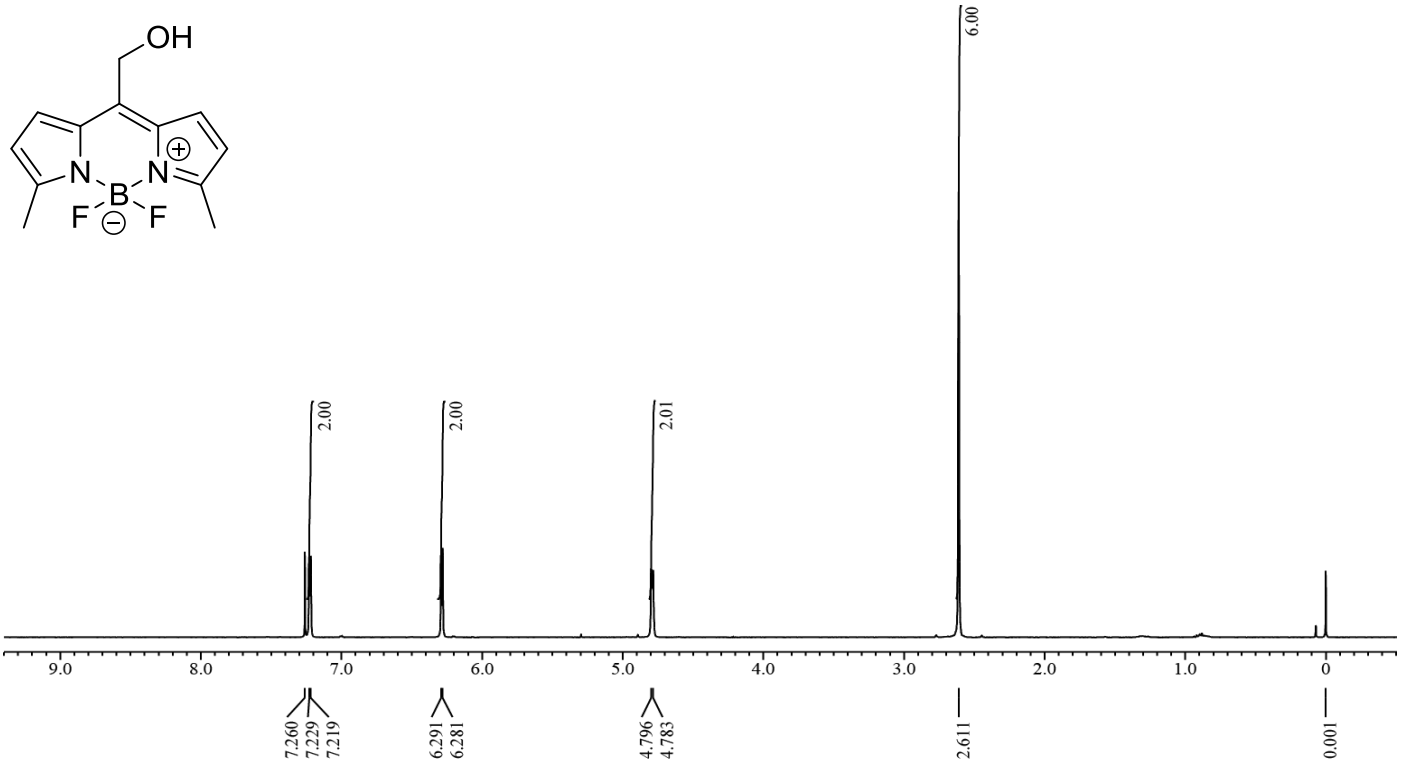
^{1}H-NMR Spectrum of compound **3** in $CDCl_3$ (400 MHz):



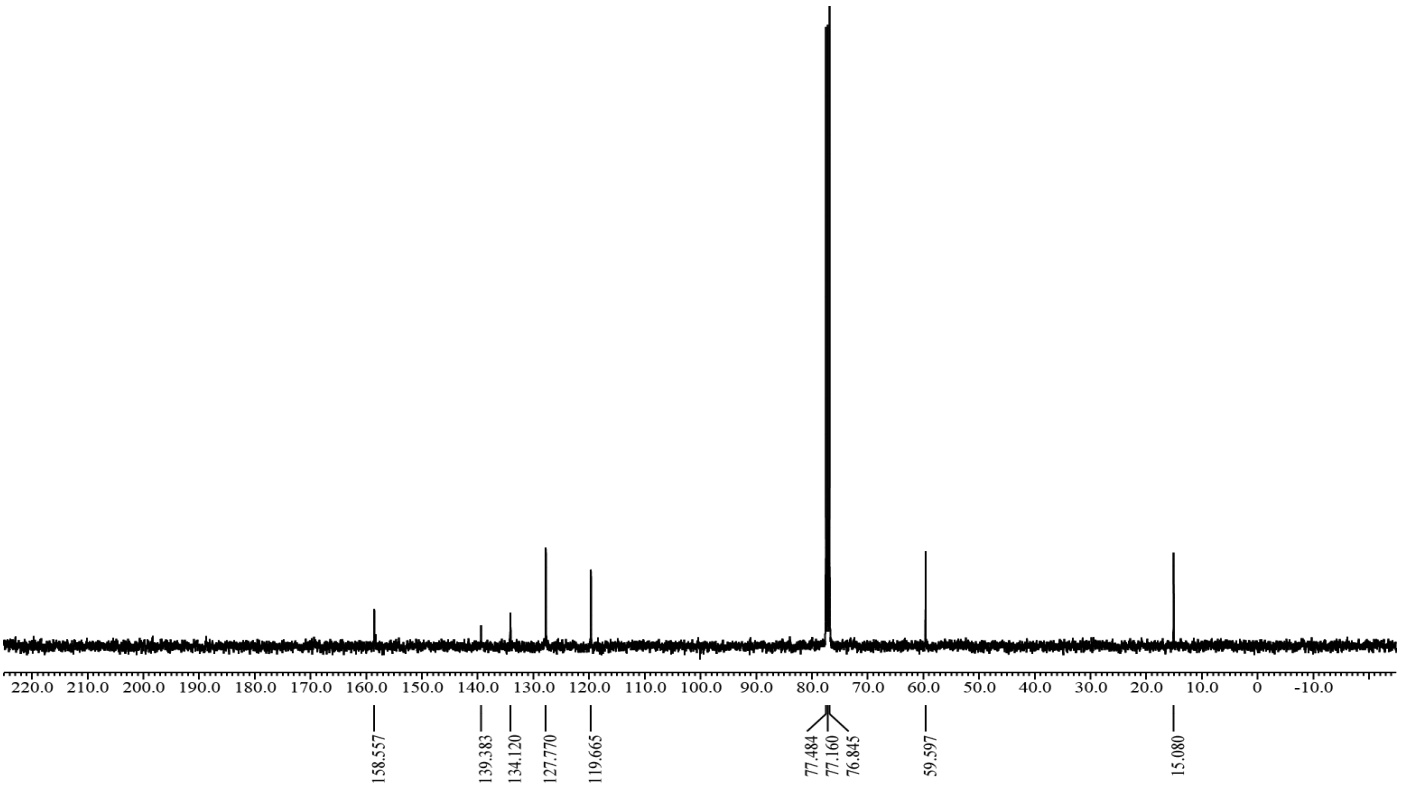
^{13}C-NMR Spectrum of compound **3** in $CDCl_3$ (100 MHz):



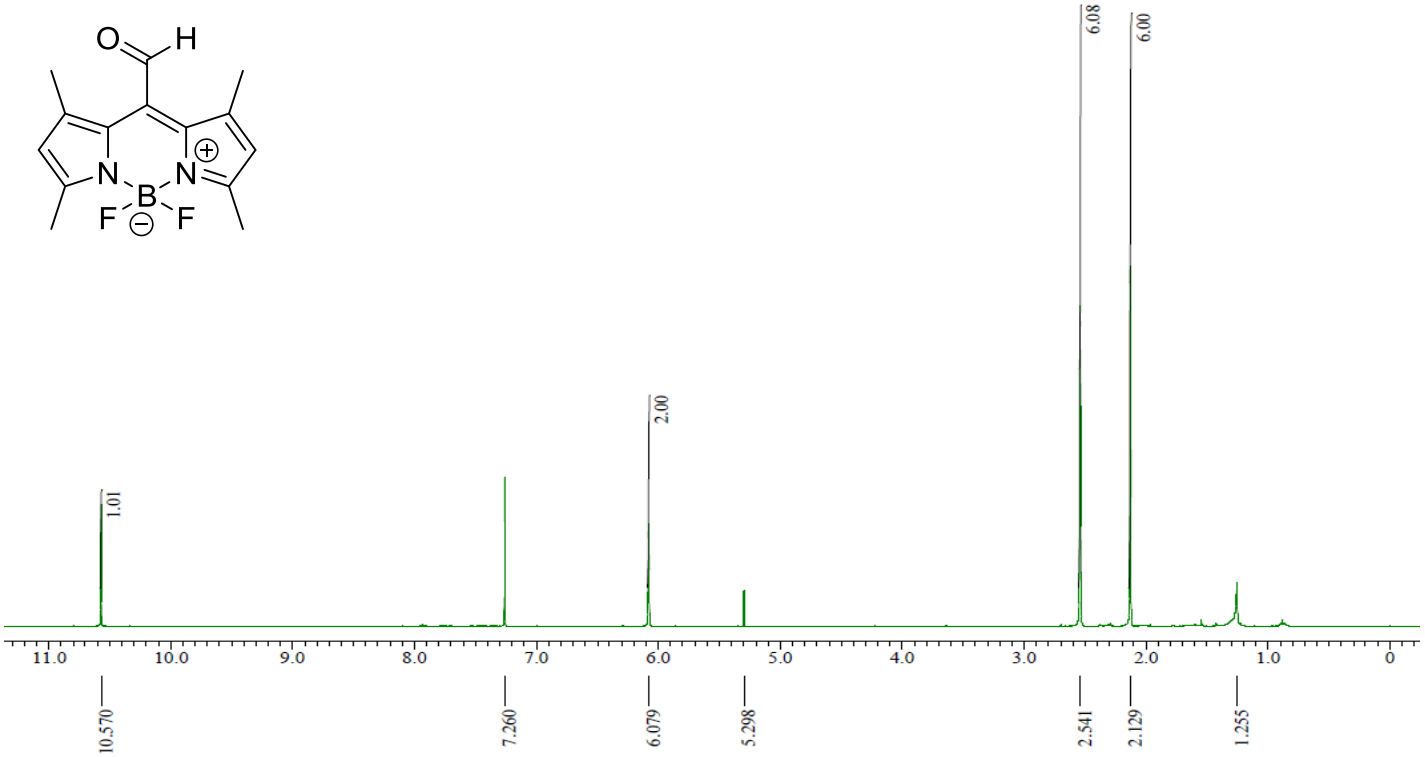
^{1}H-NMR Spectrum of compound **4** in $CDCl_3$ (400 MHz):



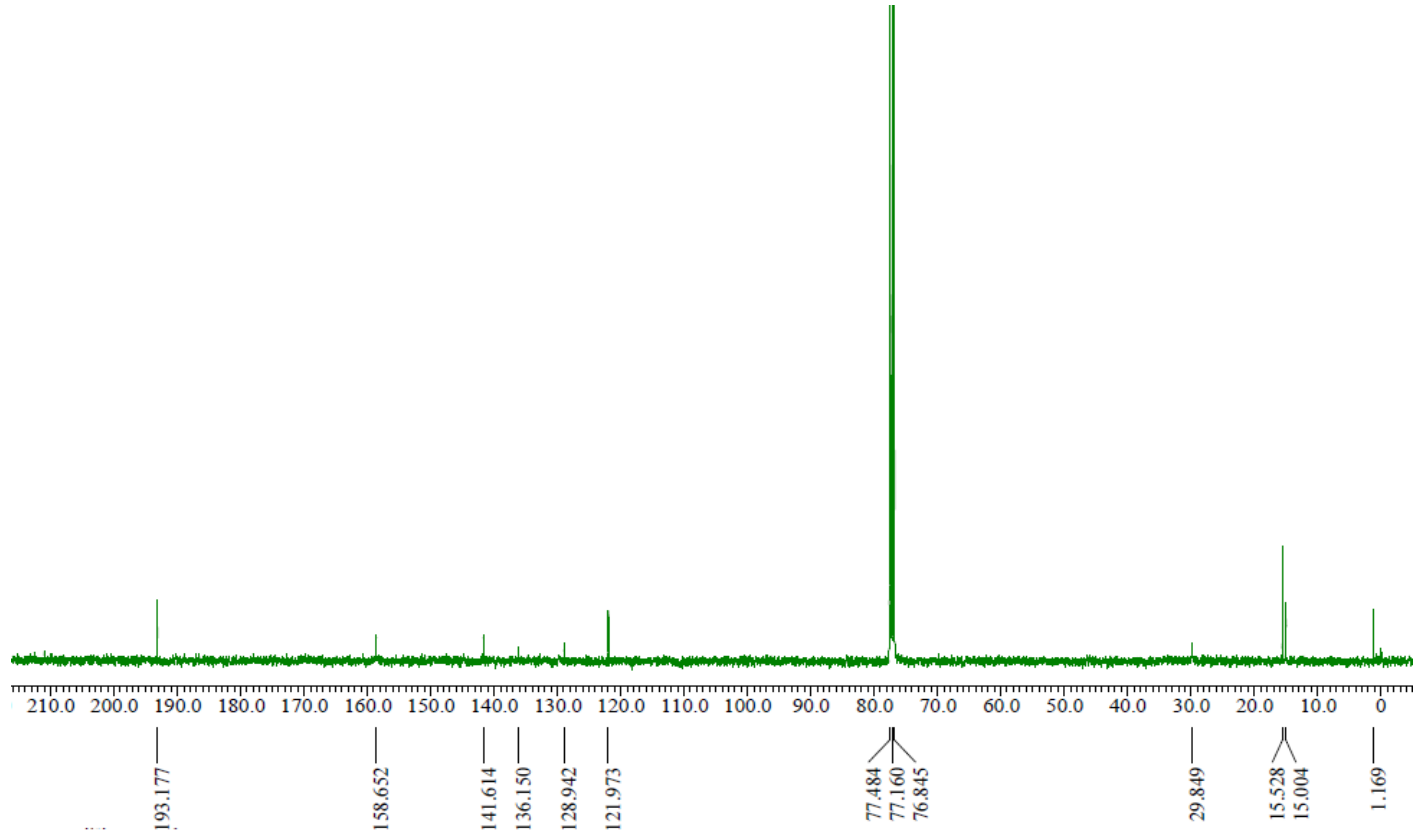
^{13}C-NMR Spectrum of compound **4** in $CDCl_3$ (100 MHz):



^{1}H-NMR Spectrum of compound **5** in $CDCl_3$ (400 MHz):



^{13}C-NMR Spectrum of compound **5** in $CDCl_3$ (100 MHz):



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