

SUPPLEMENTARY INFORMATION

A polynuclear hetero atom containing molecular organic scaffold to detect Al³⁺ ion through fluorescence Turn-On response

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Figure S14: (a) Emission and Absorption (inset) spectra of **3** (10 μM) upon interaction of various anions (50.0 equiv) in HEPES buffer (v/v = 50%, pH 7.04). (b) Emission spectra of interference studies upon addition of different anions to a solution of **3**+Al³⁺ (10 μM) in HEPES buffer (v/v = 50%, pH 7.04).

Figure S15: FT-IR spectrum of **3**+Al³⁺ complex.

Figure S16: Stacked FT-IR spectrum of **3** and **3**+Al³⁺ complex.

Table S1: Photophysical properties of probe **3** and **3+Al³⁺** in different solvents and water gradient systems.

Experimental

General: All the reagents and solvents were purchased from Sigma-Aldrich Chemical Co. Pvt. Ltd. stored in a desiccator under vacuum containing self indicating silica, and used without any further purification. Solvents were purified prior to use. UV-vis absorption spectra were recorded on a Perkin Elmer Lambda-35 UV-vis spectrophotometer using a quartz cuvette (path length = 1cm). Infrared (IR) spectra were recorded in potassium bromide (KBr) on a FT-IR Perkin Elmer Spectrophotometer. ¹H NMR spectra (chemical shifts in δ ppm) were recorded on a JEOL AL 300 FT-NMR (300 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard. Fluorescence spectra were recorded on Varian eclipse Carry spectrofluorometer using a quartz cuvette (path length = 1 cm) at 600 PMT voltage and slit width 5nm/5nm. All the spectroscopic experiments were carried out at room temperature. The stock solution of **3** (1×10^{-3} M) were prepared in THF and diluted to obtain 10 μ M solution in THF/HEPES buffer (v/v = 50%, pH 7.0) for the absorption and fluorescence measurements, respectively. The stock solutions of different metal ions (1×10^{-1} M) were prepared by dissolving their nitrate salt in water. The cation interaction studies were performed by the addition of 2 equiv. of 1×10^{-1} M of different cations. The absorption and fluorescence titration experiment were performed by the gradual increase of concentration of Al^{3+} ($c = 1 \times 10^{-3}$). The cation interference studies were performed by the addition of 50 equiv. of 1×10^{-1} M of different cations. For ¹H NMR titration experiment solution of probe **3** (1×10^{-2} M) and $\text{Al}(\text{NO}_3)_3$ was prepared in $\text{DMSO-}d_6$.

The quantum yields were estimated with respect to the quinine sulfate as standard in 0.1M H_2SO_4 solution by secondary methods¹⁹, using equation (1).

$$Q = Q_R \cdot I/I_R \cdot \text{OD}_R/\text{OD} \cdot n^2/n_R^2 \quad (1)$$

Where Q is the quantum yield, I is the integrated intensity, OD is the optical density, and n is the refractive index. The subscript R refers to the reference fluorophore of known quantum yield.

The absorption and fluorescence experimental data were utilized to calculate association constants by Benesi-Hildebrand method²⁰ (B-H method) employing equations (1) for 1:1 stoichiometries.

$$1/(I - I_o) = 1/(I - I_f) + 1/K(I - I_f)[M] \quad (2)$$

Where K is the association constant, I is the absorbance/fluorescence intensity of the free probe **3**, I_0 is the observed absorbance/fluorescence intensity of the **3**- Al^{3+} complex, and I_f is the absorbance/fluorescence intensity at saturation level.

The limit of detection (LOD) was estimated by using equation (3).

$$\text{LOD} = 3\sigma / m \quad (3)$$

Where, σ stands for the standard deviation of blank solution of **3** and m stands for calibration sensitivity toward Al^{3+} in THF/HEPES buffer (v/v = 50%, pH 7.04) solution.

Synthesis of compound 1: Benzil (1.05 g, 5 mmol) and *m*-hydroxybenzaldehyde (610 mg, 5 mmol) were dissolved in glacial acetic acid (20 mL) at room temperature. To this solution aniline (0.7 mL, 7.5 mmol) was added dropwise. After the addition of ammonium acetate (2.0 g, 26 mmol) the reaction mixture was heated at 110 °C for 4 hr and monitored the reaction on TLC. After completion of reaction, the reaction mixture was cooled to room temperature and poured into the ice-water. The precipitate was filtered, washed with cold water, air dried and recrystallized from ethylacetate to get compound **1** as a light brown color powder. Yield 1.4 g (72%). $R_f = 0.52$ (Ethylacetate:DCM:: 2:8, v/v). $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ (ppm): 9.52 (s, 1H, -OH), 7.48-7.62 (d, 2H, $J = 7.2$ Hz), 7.31-7.16 (m, 13H), 7.04-6.99 (t, 1H, $J_1 = 7.8$ Hz, $J_2 = 7.8$ Hz), 6.94 (s, 1H); 6.67 (m, 2H). FT-IR (KBr) ν_{max} (cm^{-1}): 3051, 1597, 1582, 1497, 1482, 1443, 1397, 1376, 1300, 1213, 1178, 1076, 998, 970, 884, 766, 695.

Synthesis of compound 2: Compound **2** was synthesized by formylation of compound **1** using Reimer-Tiemann reaction. Compound **1** (1.4 g, 3.65 mmol) was taken in dry ethanol (6 mL) and an aqueous solution (15 mL) of KOH (0.9 g, 16 mmol) was added. The reaction mixture was heated at 70-80°C and then added CHCl_3 (1.43 g, 0.97 mL, 12 mmol) dropwise for 10-20 min. Once the color of reaction mixture became brine red stirring was continued for 2hr and then at room temperature for 2-3hr. The excess of CHCl_3 and EtOH were distilled off

and the residue was treated with conc. HCl to make the pH of solution acidic (pH 2-3). After the addition of water (15 mL) precipitate was filtered, washed with water, air dried and purified by column chromatography using dichloromethane as eluent. The solvent was evaporated in vacuum to get compound **2**. Yield=100 mg (7%). $R_f = 0.56$ (Ethylacetate:DCM:: 0.5:9.5, v/v), $^1\text{H NMR}$ (300 MHz, DMSO- d_6): δ (ppm); 10.76 (s, 1H, -OH), 10.19 (s, 1H, -CHO), 7.49-7.47 (d, 2H, $J = 6.3$ Hz), 7.35-7.15 (m, 16H). $^{13}\text{C NMR}$ (75 MHz, DMSO- d_6): δ (ppm); 190.89, 160.19, 144.60, 137.44, 137.27, 136.33, 134.06, 132.34, 131.09, 130.01, 129.30, 129, 128.80, 128.54, 128.21, 126.68, 126.38, 121.65, 118.99, 116.68. FT-IR (KBr) ν_{max} (cm^{-1}); 3415, 3051, 2962, 2924, 2850, 1662, 1626, 1595, 1495, 1461, 1381, 1315, 1261, 1204, 1097, 1025, 966, 914, 874, 805, 701. HRMS (micrOTOF-Q) m/z : [PHA + H] $^+$ calc. for C₂₈H₂₀N₂O₂, 417.1598; Found 417.1598.

Synthesis of compound 3: Compound **2** (100 mg, 0.24 mmol) and 2-aminophenol (27 mg, 0.24 mmol) were taken in acetonitrile (10 mL) and stirred at room temperature for 15 min to get a clear solution. The reaction mixture was refluxed for 2 hr in the presence of iodine (3 mol % of reactant). The red colored precipitate so obtained was filtered, washed with acetonitrile and dried in air to get the desired compound **3** in good yield 90% (110 mg). $R_f = 0.62$ (Ethylacetate:DCM:: 0.5:9.5, v/v), $^1\text{H NMR}$ (300 MHz, DMSO- d_6) δ (ppm): 13.79 (1H, -OH, H_b), 9.71 (1H, -OH, H_a), 8.89 (s, 1H, -HC=N), 7.50-7.47 (d, 2H, $J = 6.3$ Hz), 7.35-7.08 (m, 17H), 7.02-6.99 (d, 1H, $J = 7.8$ Hz), 6.94-6.92 (d, 1H, $J = 7.8$ Hz), 6.85-6.83 (d, 1H, $J = 6.3$ Hz). $^{13}\text{C NMR}$ (75 MHz, DMSO- d_6) δ (ppm): 160.75, 151.30, 145.25, 137.44, 136.71, 134.76, 134.28, 134.09, 132.22, 132.10, 131.30, 130.26, 129.48, 129.20, 128.68, 128.42, 126.92, 126.62, 119.87, 119.26, 118.77, 116.69, 116.27. FT-IR (KBr) ν_{max} (cm^{-1}); 3430, 3061, 2958, 2924, 2852, 1615, 1515, 1506, 1497, 1465, 1352, 1285, 1224, 1163, 1119, 925,

722, 694. HRMS (microTOF-Q) m/z: [3 + H]⁺ calc. for C₃₄H₂₅N₃O₂, 508.2019; Found 508.2019.

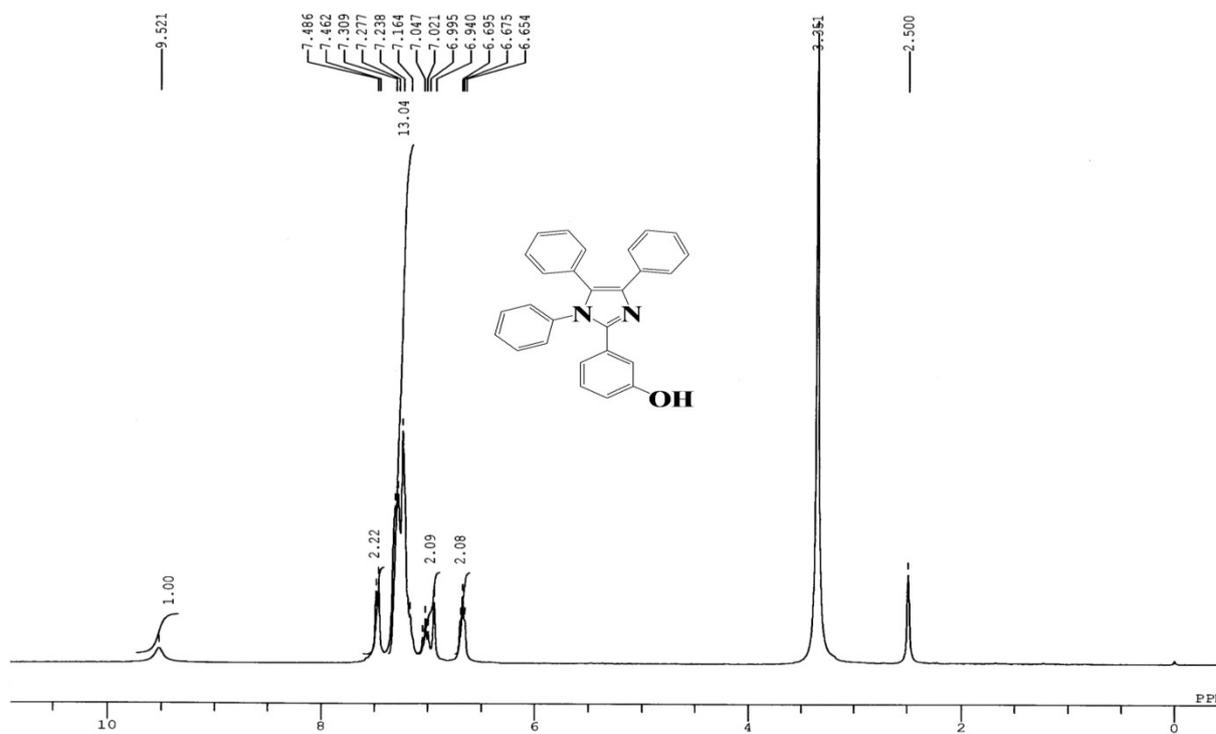


Figure S1: ¹H NMR spectrum of **1** in DMSO-*d*₆.

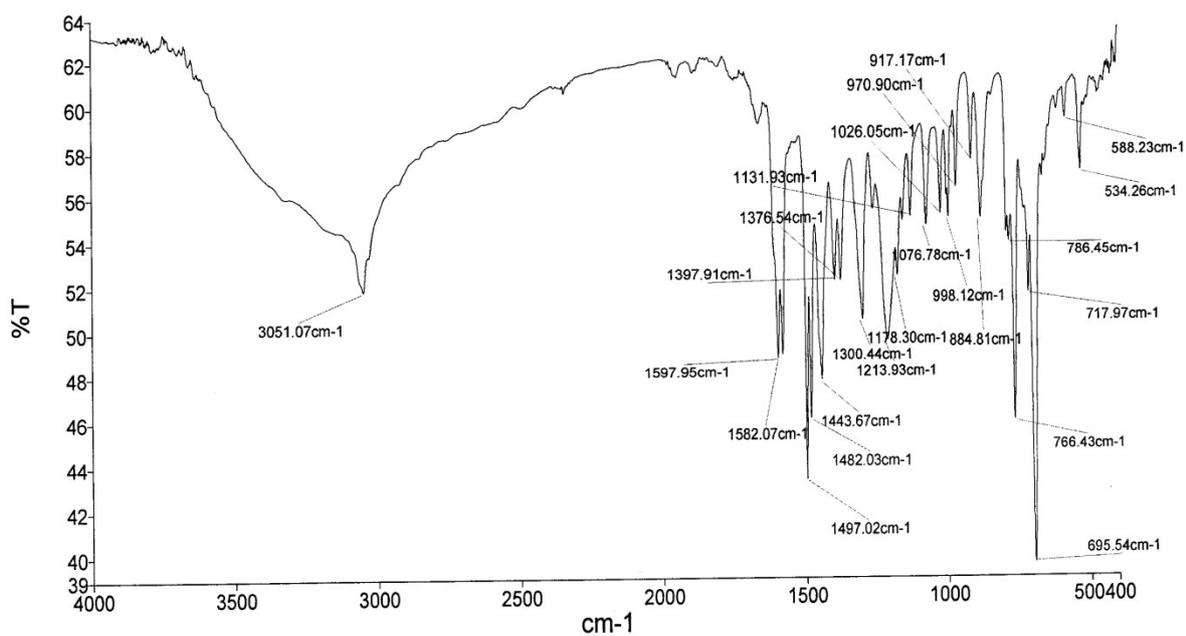


Figure S2: FT-IR spectrum of 1.

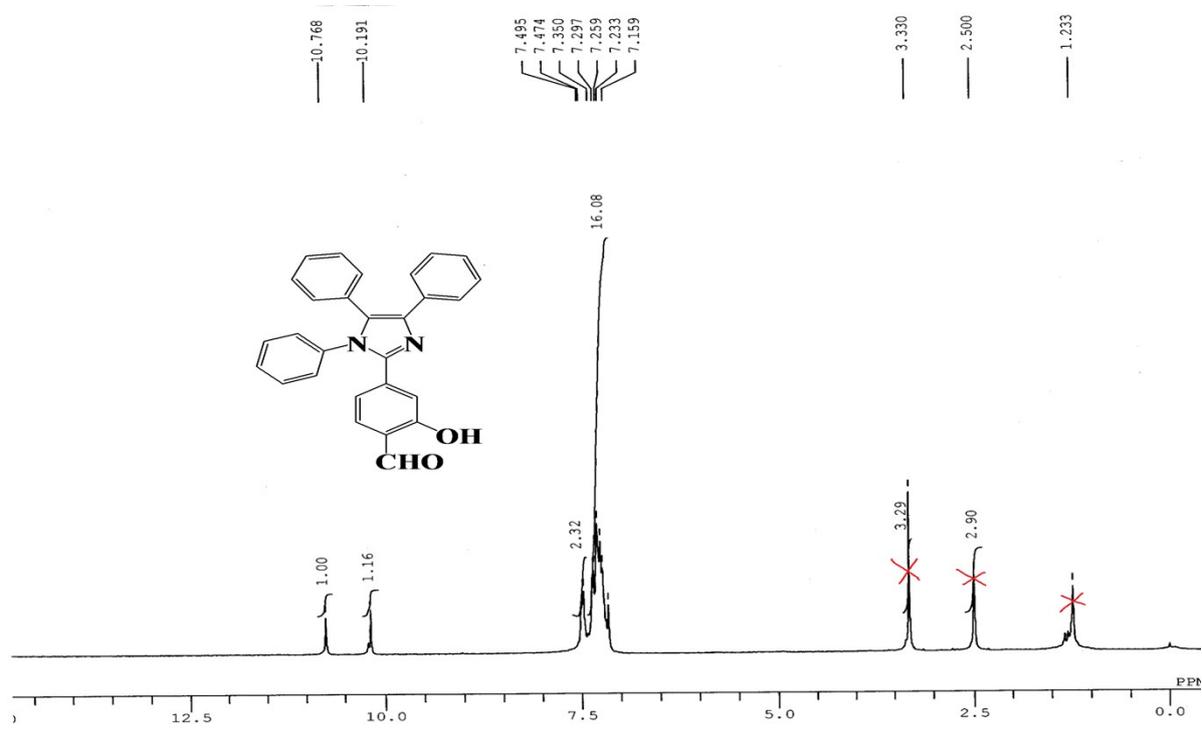


Figure S3: ¹H NMR spectrum of 2 in DMSO-*d*₆.

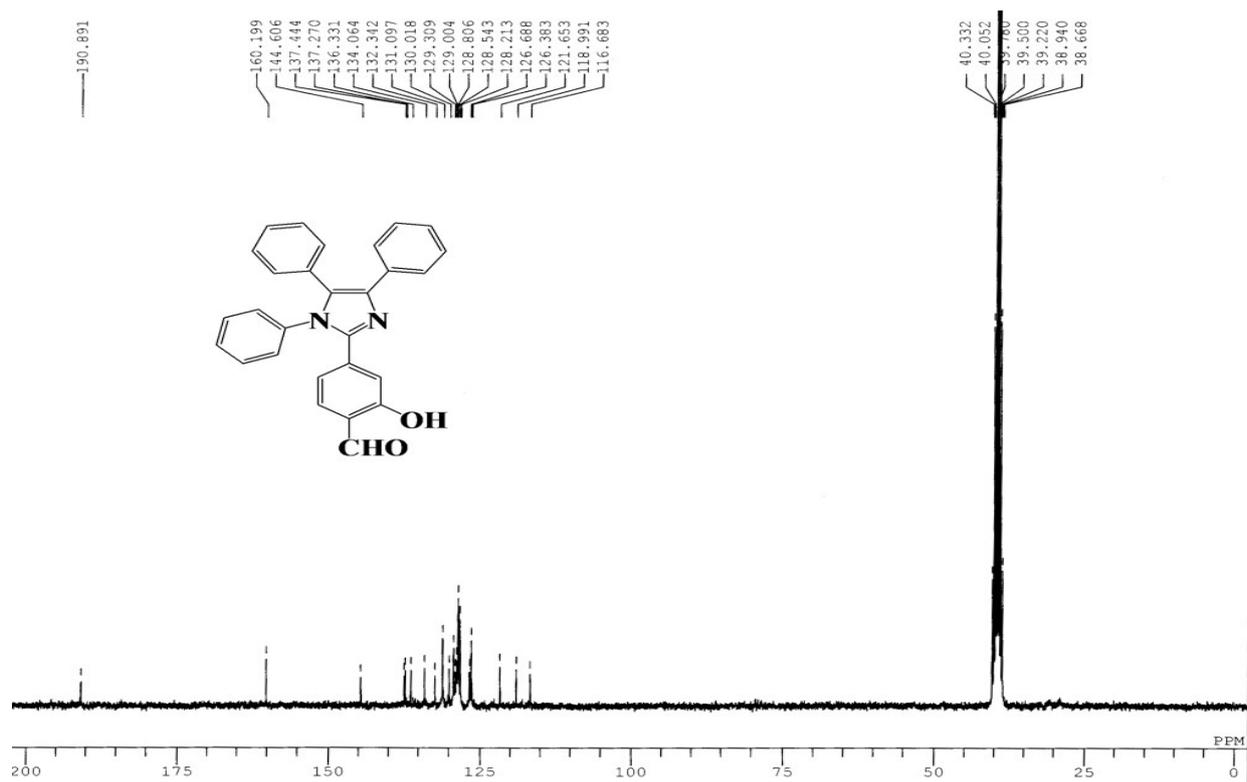


Figure S4: ^{13}C NMR spectrum of 2 in $\text{DMSO-}d_6$.

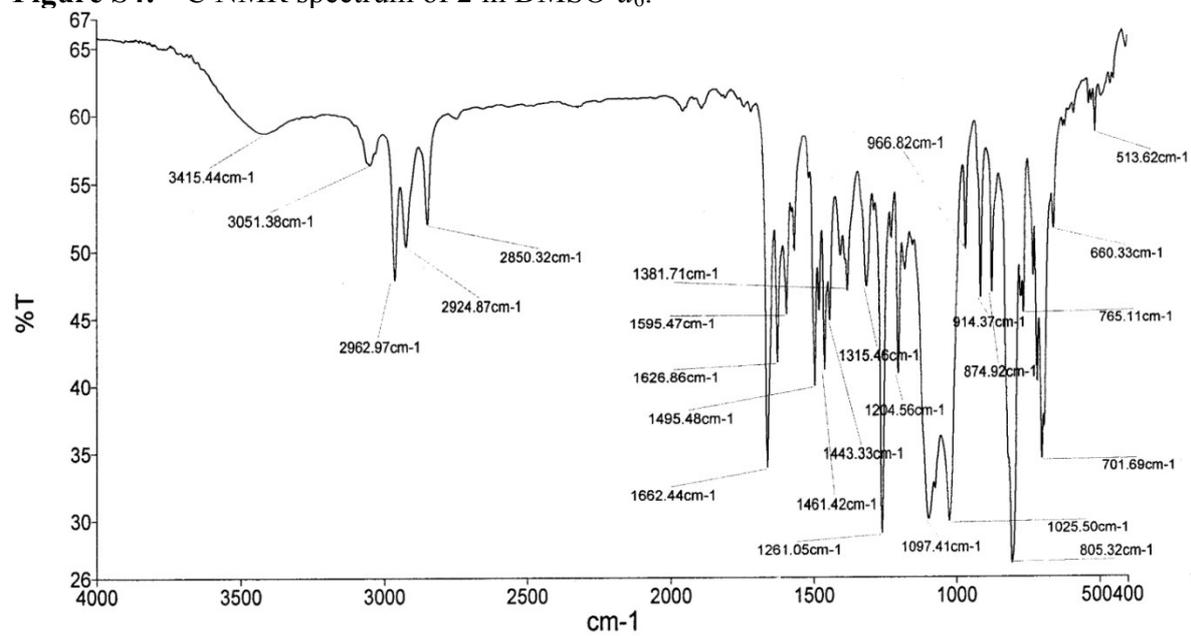


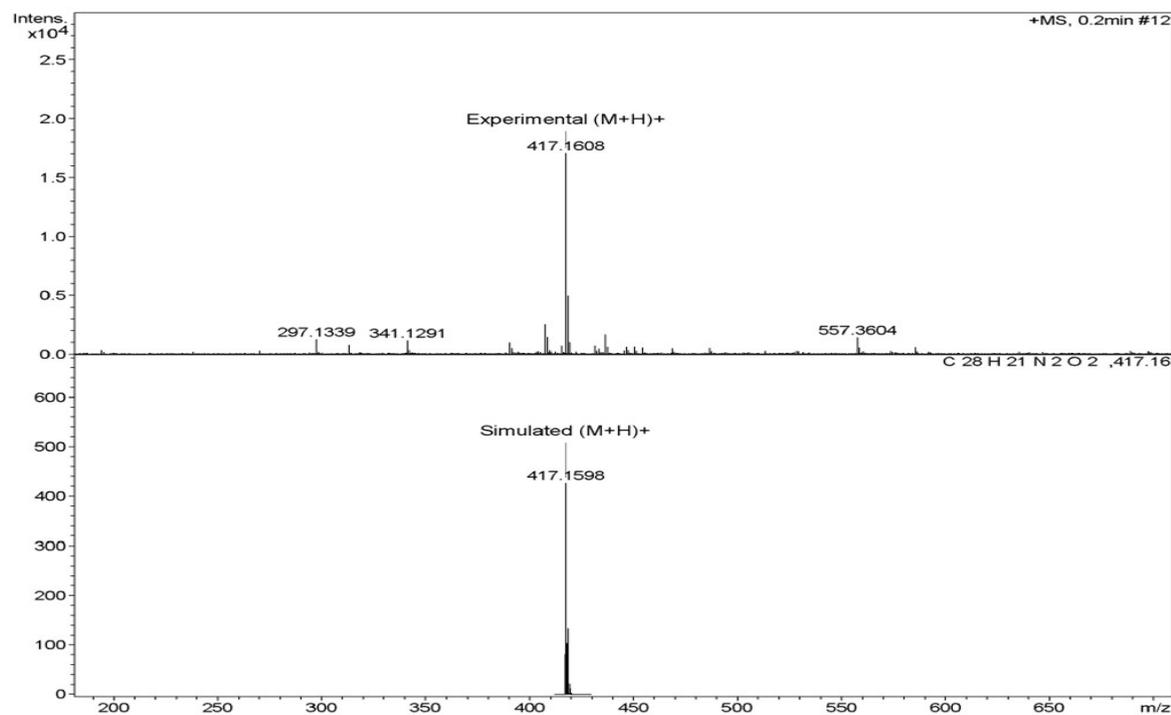
Figure S5: FT-IR spectrum of 2.

Method 04042014_100-1000_TOF_MS(+ve).m
Sample Name 18(R5)HRMS+

Acquisition Parameter
Source Type ESI Ion Polarity Positive Scan Begin 50 m/z Scan End 1500 m/z

Generate Molecular Formula Parameter
Formula, min. C₂₀H₂₀N₂O₂ Formula, max. C₂₈H₂₅N₂O₂ Charge 1
Measured m/z 417.161 Tolerance 10 ppm
Nitrogen Rule yes Electron Configuration both

Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e ⁻
C ₂₈ H ₂₁ N ₂ O ₂	0.014	417.1598	-2.55	-3.57	-1.06	19.50	ok	even



Bruker Daltonics DataAnalysis 3.4

Instrument micrOTOF-Q

Figure S6: HRMS spectrum of **2**.

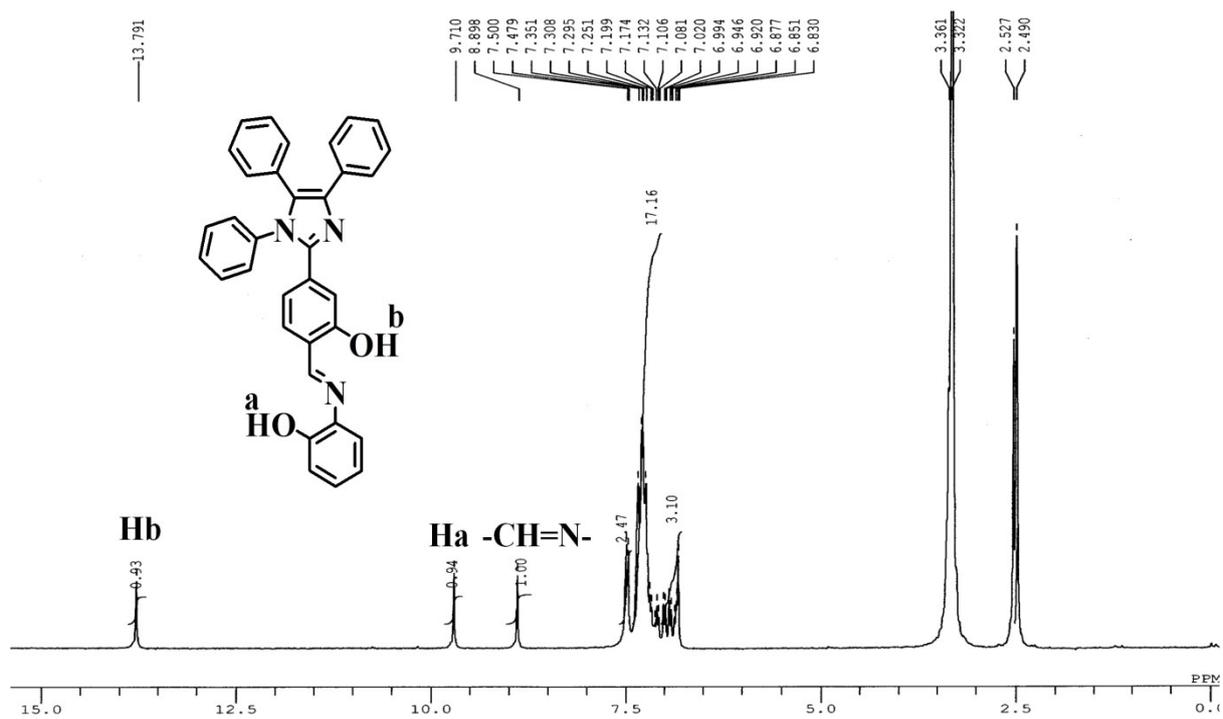


Figure S7: ^1H NMR spectrum of **3** in $\text{DMSO-}d_6$.

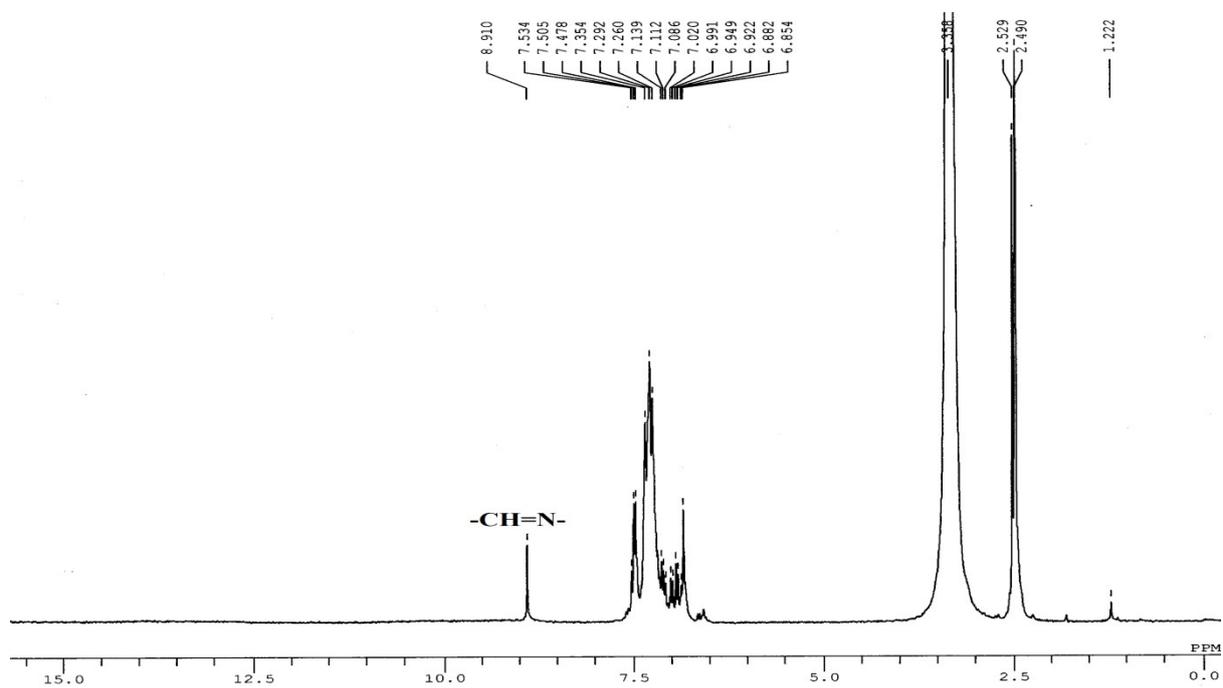


Figure S8: D_2O exchange ^1H NMR spectrum of **3** in $\text{DMSO-D}_2\text{O}$ (9:1).

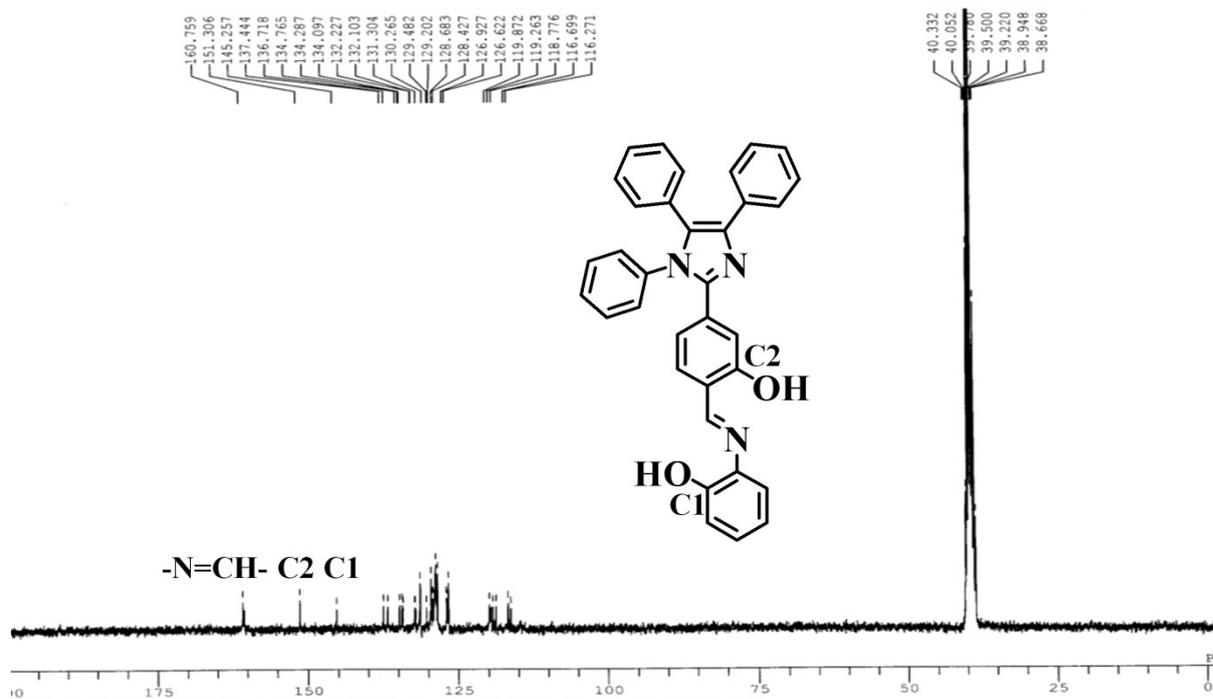


Figure S9: ^{13}C NMR spectrum of 3 in $\text{DMSO-}d_6$.

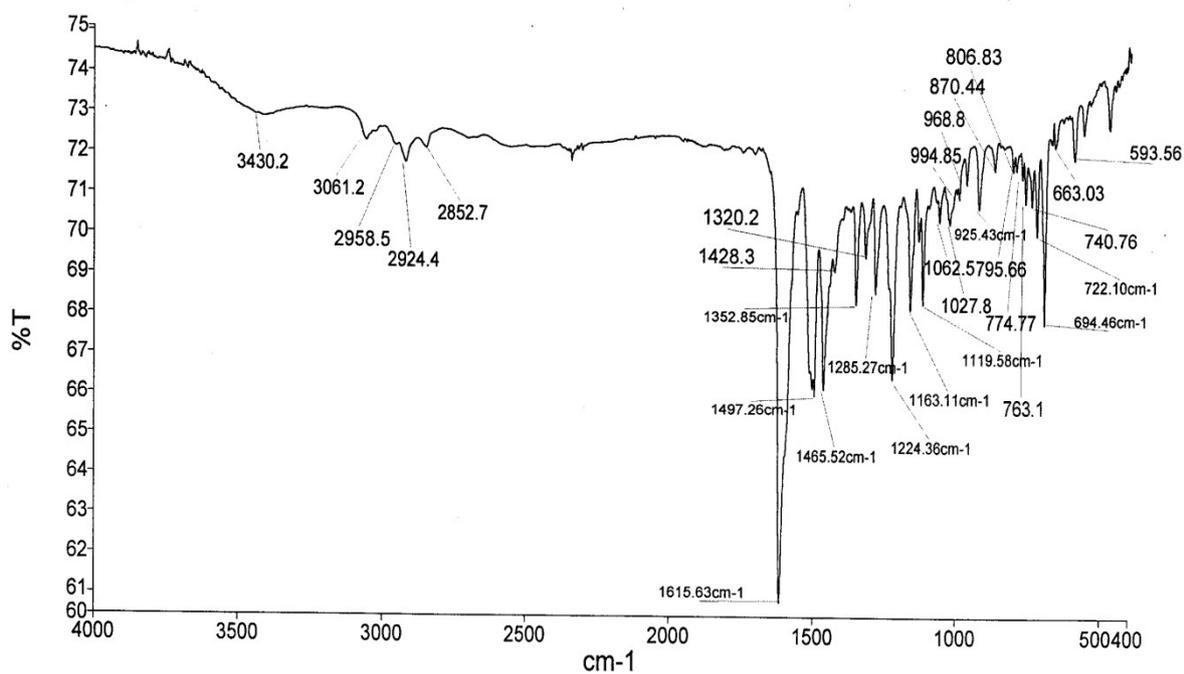


Figure S10: FT-IR spectrum of 3.

Method	04042014_100-1000_TOF_MS(+ve).m						
Sample Name	15(R2)HRMS+						
Acquisition Parameter							
Source Type	ESI	Ion Polarity	Positive	Scan Begin	50 m/z	Scan End	1500 m/z
Generate Molecular Formula Parameter							
Formula, min.	C27H20N3O2	Formula, max.	C34H29N3O2	Charge	1		
Measured m/z	508.204	Tolerance	10 ppm				
Nitrogen Rule	yes	Electron Configuration	both				

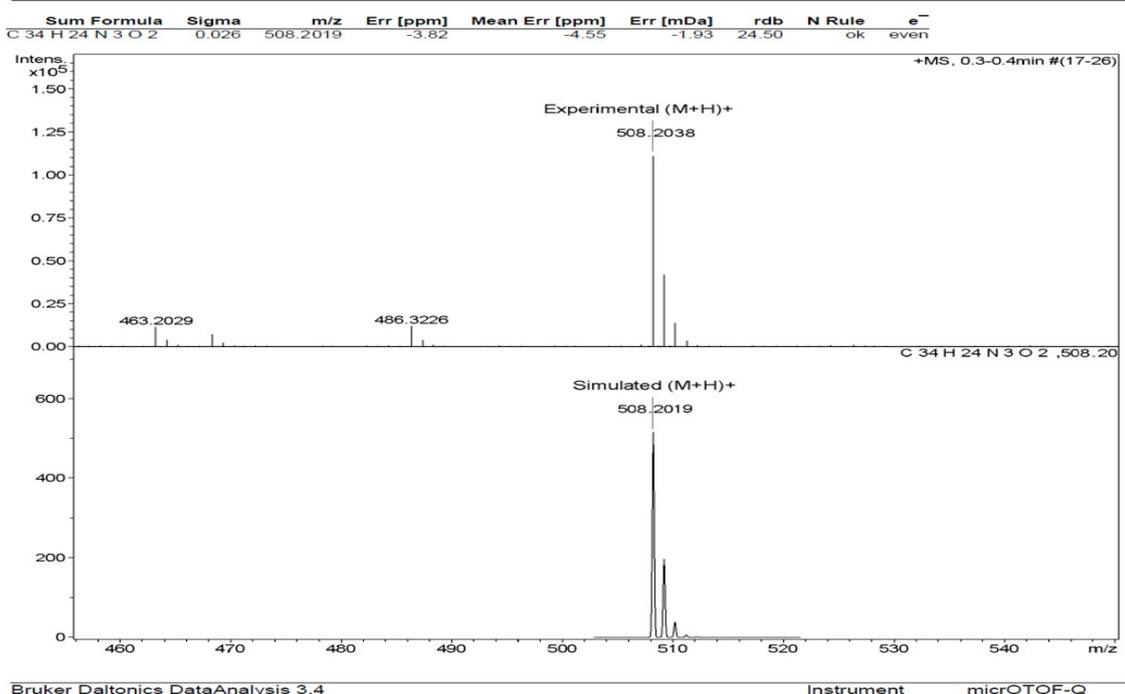


Figure S11: HRMS spectrum of 3.

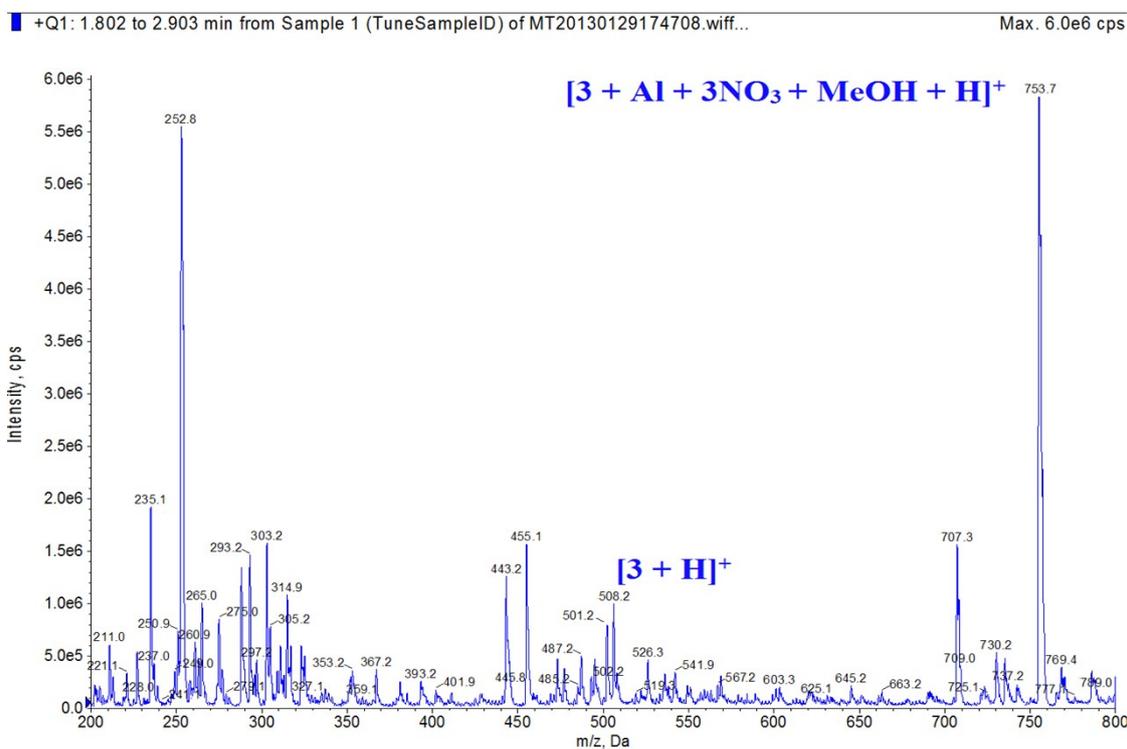


Figure S12: Mass spectrum of $3+Al^{3+}$ complex.

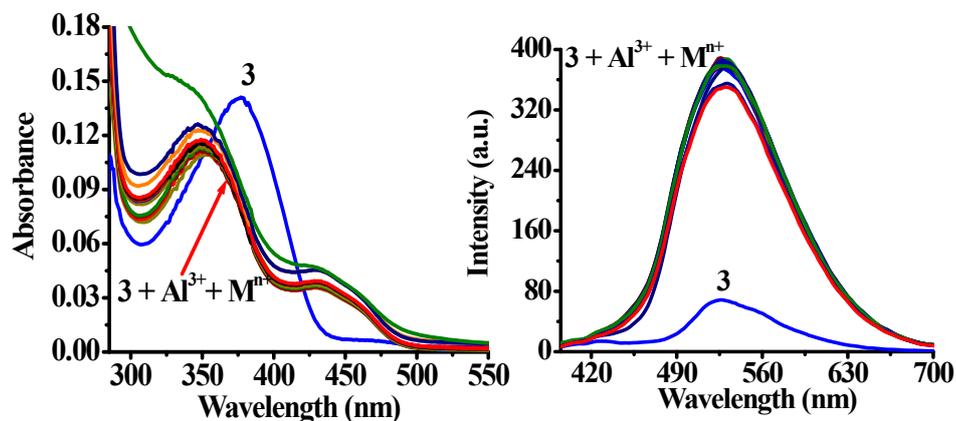


Figure S13: (a) Absorption and (b) emission spectra of interference studies upon addition of tested metal ions to a solution of $3+Al^{3+}$ ($10 \mu M$) in HEPES buffer ($v/v = 50\%$, $pH 7.04$).

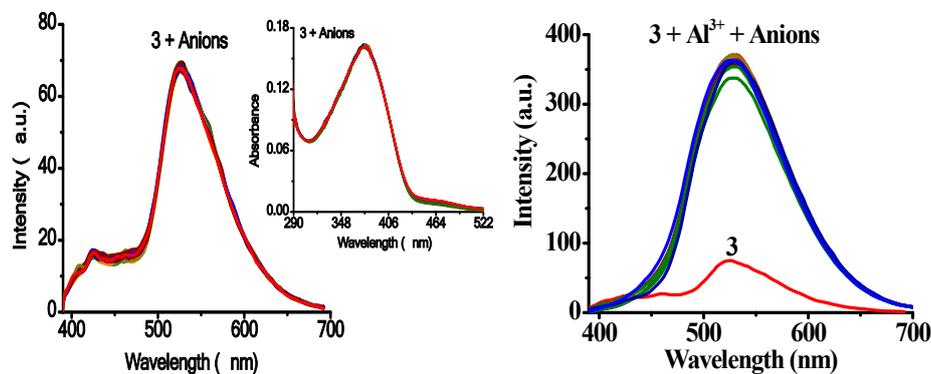


Figure S14: (a) Emission and Absorption (inset) spectra of 3 ($10 \mu M$) upon interaction of various anions (50.0 equiv) in HEPES buffer ($v/v = 50\%$, $pH 7.04$). (b) Emission spectra of interference studies upon addition of different anions to a solution of $3+Al^{3+}$ ($10 \mu M$) in HEPES buffer ($v/v = 50\%$, $pH 7.04$).

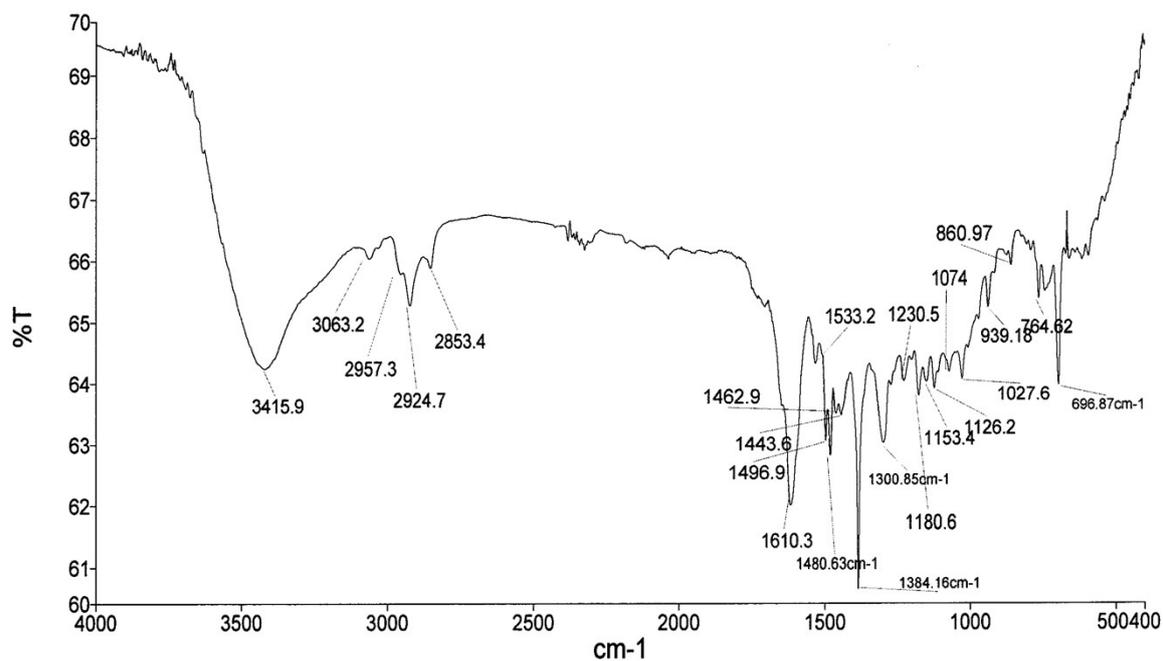


Figure S15: FT-IR spectrum of $3+Al^{3+}$ complex.

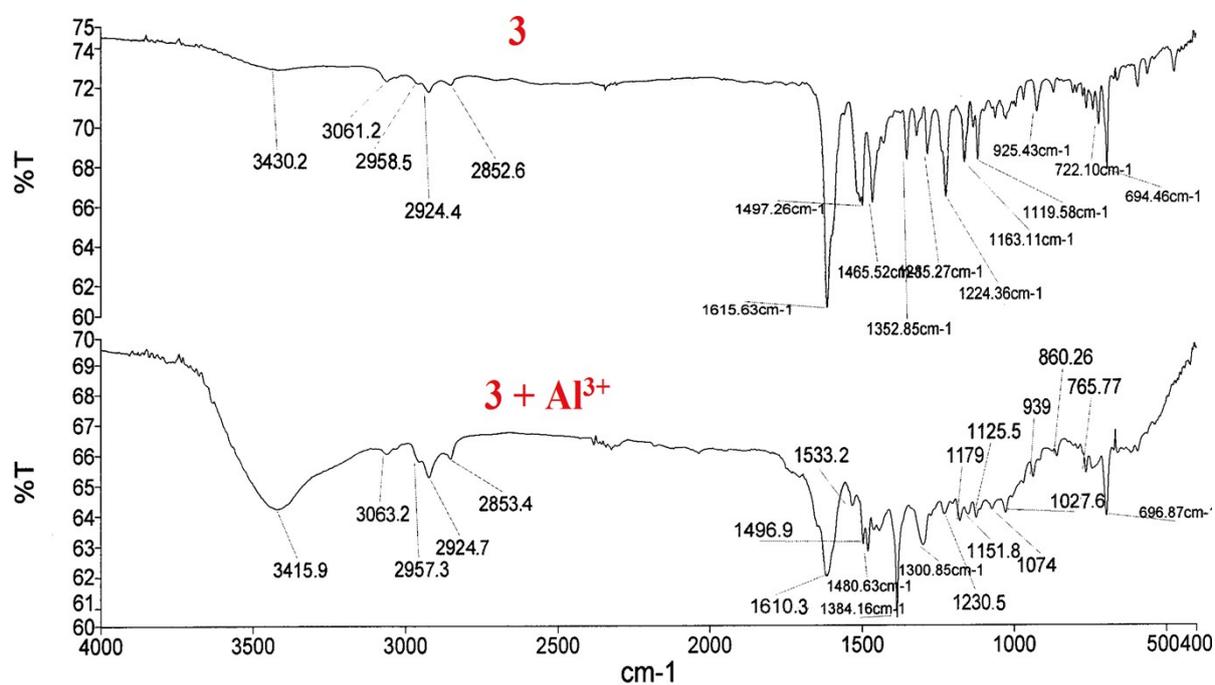


Figure S16: Stacked FT-IR spectrum of **3** and $3+Al^{3+}$ complex.

Table S1: Photophysical properties of probe **3** and **3+Al³⁺** in different solvents and water gradient systems

Solvents	Water contents (vol %)	λ_{em} (nm)		Enhanced emission (in fold)	Quantum yield (Φ)		Enhanced Quantum yield (in fold)
		3	3+Al³⁺		3	3+Al³⁺	
Benzene	0	432	434	5	0.004	0.011	2.75
CCl ₄	0	432	434	5	0.003	0.01	3.33
CH ₂ Cl ₂	0	436	484	14	0.009	0.043	4.77
Dioxane	0	530	506	11	0.0029	0.044	15.77
	10	530	508	13	0.0044	0.086	19.54
	20	530	516	8	0.0048	0.072	15
	30	530	530	7	0.005	0.061	12.2
	40	530	532	8	0.0059	0.077	13.05
	50	530	532	8	0.006	0.084	14
	60	528	536	8	0.0054	0.092	17
	70	526	536	8	0.004	0.051	12.75
THF	0	528	466	22	0.0028	0.068	24.28
	10	528	502	10	0.0043	0.088	20.46
	20	530	504	7	0.0045	0.07	15.55
	30	528	514	6	0.005	0.067	13.4
	40	528	526	6	0.0054	0.063	11.66
	50	528	528	6	0.006	0.07	11.66
	60	530	530	5	0.007	0.059	8.42
	70	530	530	5	-	-	-
ACN	0	532	546	5	0.0018	0.015	8.33
	10	530	546	3	0.0021	0.033	15.71
	20	526	538	5	0.0027	0.054	20
	30	526	530	8	0.0029	0.07	24.13
	40	526	532	10	0.0035	0.078	22.28
	50	524	534	11	0.0038	0.083	21.84
	60	522	536	13	0.004	0.046	11.5
	70	536	536	14	0.0019	0.027	14.21
EtOH	0	524	532	6	0.01	0.102	10.2
	10	524	533	7	0.098	0.108	1.10
	20	522	530	7	0.097	0.125	1.28
	30	522	530	7	0.097	0.109	1.12
	40	522	532	7	0.098	0.109	1.11
	50	524	532	7	0.008	0.087	10.87
	60	526	532	8	0.0068	0.094	13.82
	70	527	532	9	0.0076	0.067	8.81

