

Supplementary Information

Pyrrolidine constrained bipyridyl-dansyl click fluoroionophore as selective Al³⁺ sensor

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S10-S15: ¹H and ¹³C NMR spectra of compounds **4**, **5** and **BD 1**.

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1. General Experimental Procedure

All the solvents and reagents (analytical grade and spectroscopic grade) were obtained from Sigma-Aldrich and used as received unless otherwise mentioned. The solutions of metal ions were prepared from $\text{Al}(\text{ClO}_4)_3 \cdot 9\text{H}_2\text{O}$, $\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$, NaClO_4 , KClO_4 , $\text{Mg}(\text{ClO}_4)_2$, $\text{Sr}(\text{NO}_3)_2$, $\text{Ba}(\text{ClO}_4)_2$, $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Fe}(\text{ClO}_4)_2$, $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Ni}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, $\text{Cd}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$, $\text{Ag}(\text{ClO}_4)_2$, HgCl_2 , $\text{Pb}(\text{ClO}_4)_2$, $\text{In}(\text{NO}_3)_3$ respectively in CH_3CN . ^1H and ^{13}C NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $\text{CDCl}_3/\text{CD}_3\text{CN}$, TMS as internal standard). Mass spectra are measured on Shimadzu 2020 LC-MS and Bruker Ultraflex II MALDI/TOF spectrometers. Melting points were determined on hot oil-bath melting point apparatus in an open-mouth capillary and were uncorrected. IR spectra are recorded on a Bruker IFS 66/V spectrometer, using KBr discs. Thermalgravimetric analysis was carried out on a TGA 850 Mettler Toledo instrument. Elemental analysis was carried out on ThermoScientific FLASH 2000 Organic Element Analyzer and specific rotation $[\alpha]_D^{25}$ was recorded on a JASCO P-2000 polarimeter. UV spectra were recorded on a Perkin Elmer Model Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer.

2. Synthesis of BD 1

Preparation of Boc-pyrrolidine methylene triazole bipyridine (4): To a solution of Boc-protected azido-pyrrolidine¹ (1g, 4.42 mmol) in toluene and *tert*-butanol (4:1), 6-Ethynyl-2,2'-bipyridine² (0.8g, 4.42 mmol), CuI (0.042g, 0.221 mmol) and *N,N'*-diisopropylethylamine (DIPEA) (1.54 mL, 8.84 mmol) were added. The reaction mixture was stirred at room temperature for overnight. After removal of the solvent under vacuo, the residue was diluted with 20 mL CH_2Cl_2

and washed with water (15 mL X 3). The organic phase was dried over Na₂SO₄ and solvent was evaporated to dryness. The crude product was purified by column chromatography on silica gel using chloroform-methanol (10:1) as an eluent to afford white product in good yield (1.72 g, 96%). M.P. 122-124°C. [α]_D²⁵ +79.5 (c 1.0, CH₃OH). ¹H NMR (400 MHz, CDCl₃) δ 1.52 (9H, s), 1.72-1.75 (2H, m), 1.98-2.00 (2H, m), 3.18-3.43 (2H, m), 4.19 (1H, m), 4.50-4.78 (2H, m), 7.31-7.34 (1H, dd, J = 5.2 Hz, 1.6 Hz), 7.80-7.84 (1H, t, J = 7.4 Hz), 7.88-7.92 (1H, t, J = 7.6 Hz), 8.17-8.25 (2H, m), 8.35-8.37 (1H, d, J = 8 Hz), 8.48-8.50 (1H, d, J = 7.2 Hz), 8.6850-8.6940 (1H, d, J = 3.6 Hz). ¹³C NMR (CDCl₃ 400MHz) δ 23.3, 28.2, 28.5, 47.1, 51.4, 57.2, 136.6, 137.7, 148.8, 149.1, 149.6, 154.7, 155.6, 155.9. GCMS : m/z = 406 [M⁺] for C₂₂H₂₆N₆O₂. Elemental analysis: Found: C, 65.12; H, 6.38; N, 20.31, Calcd: C, 65.01; H, 6.45; N, 20.68 for C₂₂H₂₆N₆O₂.

Preparation of Pyrolidine methylene triazole bipyridine (5): To Boc-protected product **4** (406 mg, 1.0 mmol), CH₂Cl₂-TFA (30 mL, 50:50) was added slowly at ice temperature. The mixture was warmed to room temperature and stirred for 2 hrs. After evaporating reaction mixture to dryness under vacuo, the residue was dissolved in CH₂Cl₂ (15 mL) and then treated with a saturated NaHCO₃ solution (30 mL) for 1h at room temperature. The aqueous layer was extracted with CH₂Cl₂ (10 mL x 3) and combined extracts were dried over anhydrous Na₂SO₄. After filtration the CH₂Cl₂ was evaporated to obtain light yellowish solid of free amine **5** (300 mg, Yield 98 %). ¹H NMR (400 MHz, CDCl₃) δ 1.54-1.61 (1H, m), 1.74-1.86 (2H, m), 1.97-2.05 (2H, m), 2.97-3.03 (2H, m), 3.68-3.75 (1H, m), 4.30-4.54 (2H, m), 7.31-7.34 (1H, ddd, J = 1.2 Hz, 3.6 Hz, 1.2 Hz), 7.81-7.85 (1H, td, J = 6 Hz, 1.6 Hz), 7.88-7.93 (1H, t, J = 7.6 Hz), 8.17- 8.19 (1H, dd, J = 7.2 Hz, 0.8 Hz), 8.33- 8.35 (1H, dd, J = 7.2 Hz, 0.8 Hz), 8.39 (1H, s), 8.48-8.50 (1H, d, J = 8 Hz), 8.69- 8.70 (1H, dd, J = 4 Hz, 0.8 Hz). ¹³C NMR (CDCl₃ 400MHz) δ 25.3, 28.9, 46.4, 55.4, 57.8, 119.8,

119.9, 120.9, 122.8, 123.6, 136.6, 137.6, 148.2, 148.9, 149.6, 155.5, 155.8. LCMS (EI): $m/z = 306$ (M^+) for $C_{17}H_{18}N_6$.

Preparation of Dansylated pyrrolidine methylene triazole bipyridine (BD 1): To a solution of free amine **5** (300 mg, 0.98 mmol) and dansyl chloride (264 mg, 0.98 mmol) in dry DMF (15 mL) triethylamine (TEA) (0.27 mL, 1.96 mmol) was added drop wise over 30 min. The reaction mixture was stirred for 3 hrs at room temperature. After removal of solvent in vacuo, the residue was dissolved in CH_2Cl_2 (15 mL) and was washed with water (10 mL x 3). The organic phase was dried over Na_2SO_4 and solvent was evaporated on rotavap. The crude product was purified by column chromatography on silica gel using chloroform-methanol (10:1) as an eluent to afford green fluorescent solid **1** in good yield (520 mg, 98 %). M.P. 76-78 °C. $[\alpha]_D^{25} +71.25$ (c 1.0, CH_3OH). 1H NMR (400 MHz, $CDCl_3$) δ 1.35-1.46 (1H, m), 1.54-1.63 (1H, m), 1.73-1.82 (1H, m), 1.98-2.04 (1H, m), 2.78 (1H, s), 3.24-3.33 (2H, m), 4.26-4.31 (1H, m), 4.64-4.77 (2H, m), 7.17-7.19 (1H, d, $J = 7.6$ Hz), 7.33-7.36 (1H, m), 7.54-7.62 (1H, m), 7.89-7.93 (2H, m), 8.14-8.17 (1H, dd, $J = 6.8$ Hz, 0.8 Hz), 8.27-8.29 (1H, dd, $J = 6.4$ Hz, 0.8 Hz), 8.36- 8.38 (1H, dd, $J = 6.8$ Hz, 0.8 Hz), 8.41 (1H, s), 8.53-8.60 (3H, m), 8.69-8.71 (1H, ddd, $J = 2.4$ Hz, 0.8 Hz, 0.8 Hz, 0.8 Hz). ^{13}C NMR ($CDCl_3$, 400MHz) δ 23.8, 29.1, 45.3, 49.2, 54.0, 58.9, 115.4, 119.2, 120.0, 120.1, 121.2, 123.2, 123.4, 123.8, 128.4, 130.1, 130.2, 130.6, 131.0, 133.1, 136.9, 137.7, 148.8, 149.1, 149.5, 151.8, 155.7, 155.9. LCMS: $m/z = 540.28$ [$M^+ + H$] for $C_{29}H_{29}N_7O_2S$. Elemental analysis: Found: C, 64.43; H, 5.52; N, 17.88; S, 5.42, Calcd: C, 64.54; H, 5.42; N, 18.17; S, 5.94. for $C_{29}H_{29}N_7O_2S$. Elemental analysis for **BD 1**• $Al(ClO_4)_3$ • $2H_2O$: Found, C, 38.74; H, 3.95; N, 10.43; S, 3.36; Calcd, C, 38.66; H, 3.69; N, 10.88; S, 3.56. for $C_{29}H_{33}AlCl_3N_7O_{16}S$.

3. General method of UV-Vis and fluorescence titration

UV-vis spectra were recorded on a Perkin Elmer Model Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer. 1mm cell was used for both types titration. For UV-vis and fluorescence titrations, stock solution of **BD 1** was prepared ($c = 500 \mu\text{M}$) in acetonitrile. The solution of guest cations was prepared in acetonitrile in the order of 10^{-3} . Working solutions of **BD 1** and metal ions were prepared from the stock solutions. Excitation was carried out at 350 nm with all excitation and emission slit widths at 10 and 10 nm, respectively.

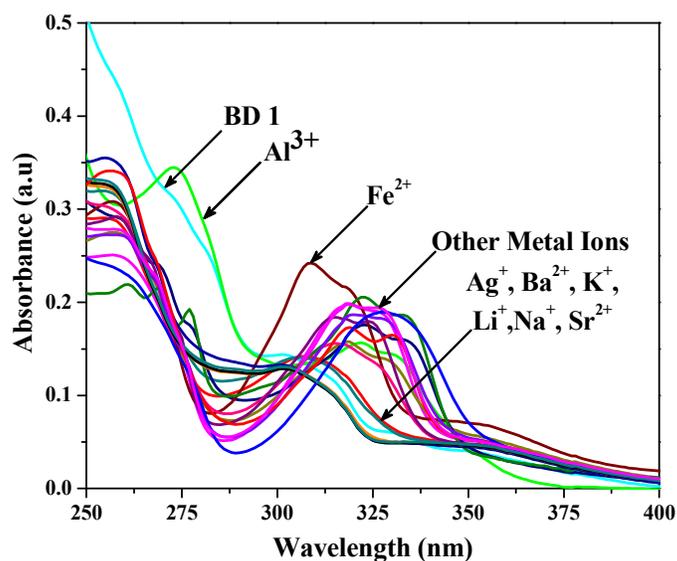


Fig. S1 UV-Vis absorption spectra of **BD1** [100 μM] in the presence of different metal ions [100 μM] in acetonitrile.

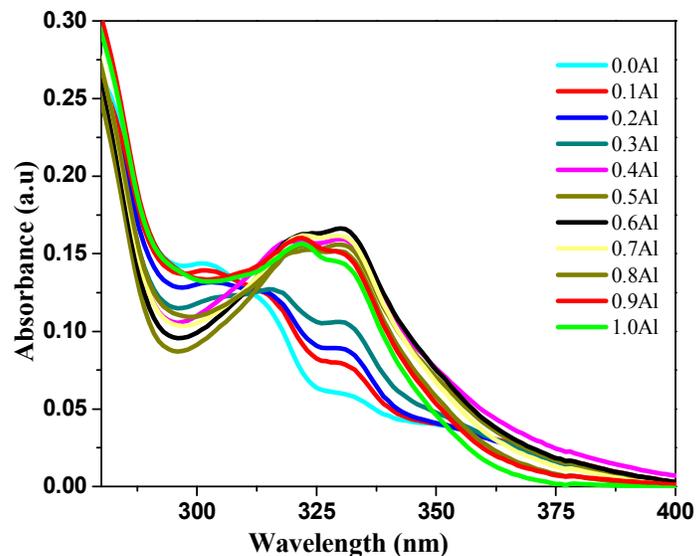


Fig. S2 UV-Vis absorption spectra of **BD 1** [100 μM] upon addition of different equivalents of Al³⁺ in acetonitrile.

Job Plot by UV-vis method

A series of solutions containing **BD 1** and Al(ClO₄)₃ were prepared such that the sum of the total metal ion and **BD 1** concentration remained constant (100 μM). The mole fraction (X) of Al³⁺ was varied from 0.1 to 1.0. The corrected absorbance ($OD_{332} \cdot X_{Al^{3+}} \cdot 10^3$) at 332 nm was plotted against the molar fraction of the Al³⁺ solution.

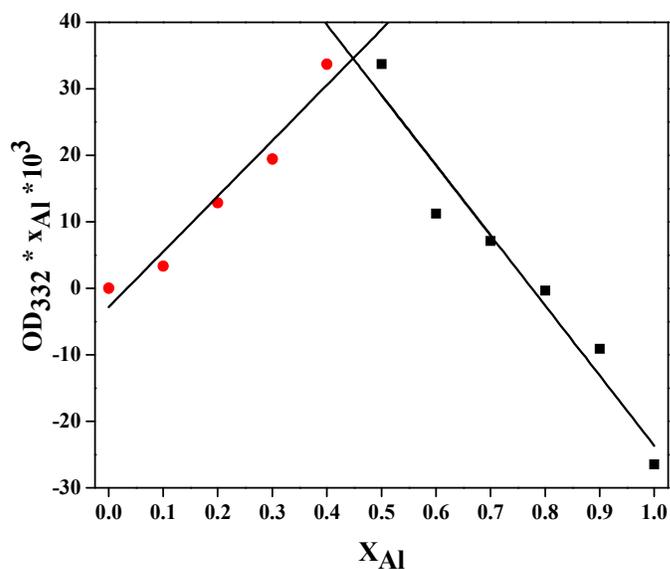


Fig. S3 Job plot for the determination of the stoichiometry of **BD 1** and Al³⁺ in the complex.

4. Determination of Binding Constant

Tsein *et al.* proposed a calibration equation for 1:1 complexation of a fluoroionophore and Ca²⁺.¹³

Li *et al.* derived the Tsein equation³ to the following equations that can be used in any stoichiometric ratio between the ligand and analyte.

$$[M^{n+}]^m = \frac{1}{n \cdot K} \cdot \frac{1}{[L]_T^{n-1}} \cdot \frac{1-\alpha}{\alpha^n}$$

Where K is complex equilibrium constant, M_mL_n is metal-ligand, L is ligand, [L], [Mⁿ⁺], and [M_mL_n] are the concentrations of respective species. α is the ratio between free ligand concentration,

[L], and the initial concentration of ligand, [L]_T. In our case, the stoichiometric ratio of the Al³⁺: fluoroionophore is 1:1. So, this equation can be written as

$$[\text{Al}^{3+}] = \frac{1}{K} \cdot \frac{1-\alpha}{\alpha}$$

The curve fitting experimental data points were calculated from this equation with log K= 3.94.

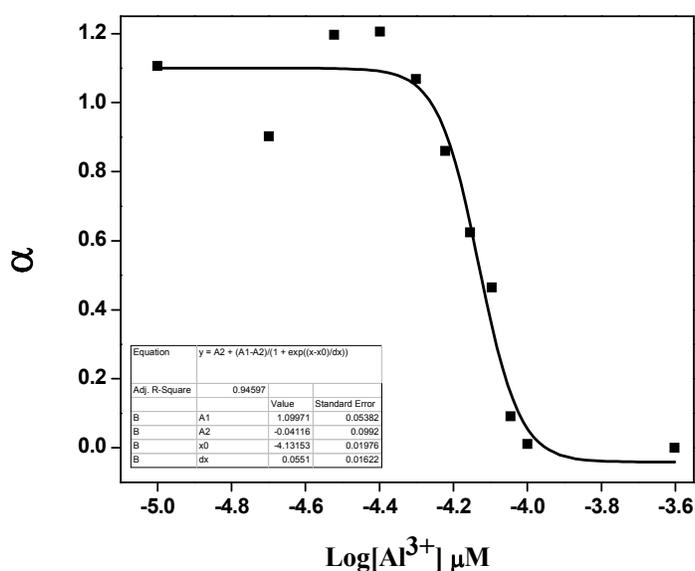


Fig. S4 Response parameter values (α) as a function of the logarithm of Al³⁺ concentration. α is defined as the ratio between the free ligand (**BD 1**) concentration and the initial concentration of the ligand.

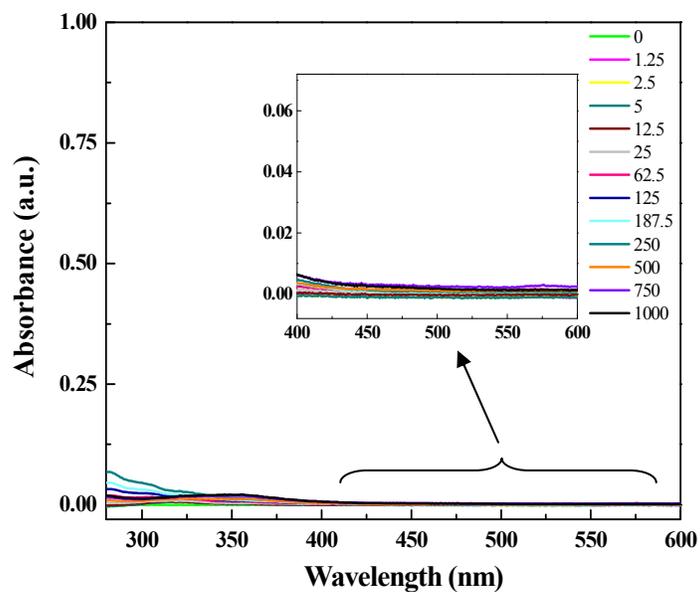
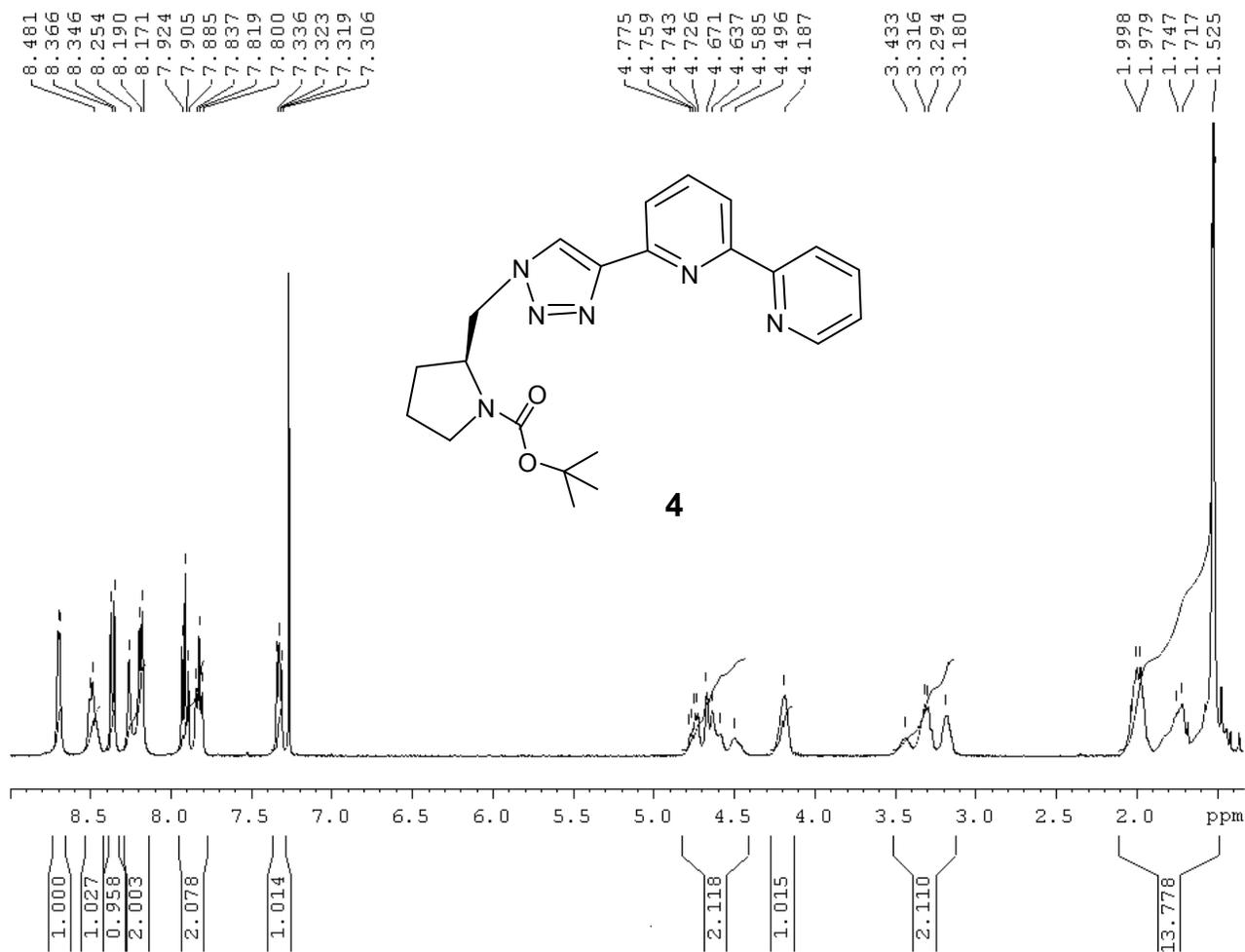


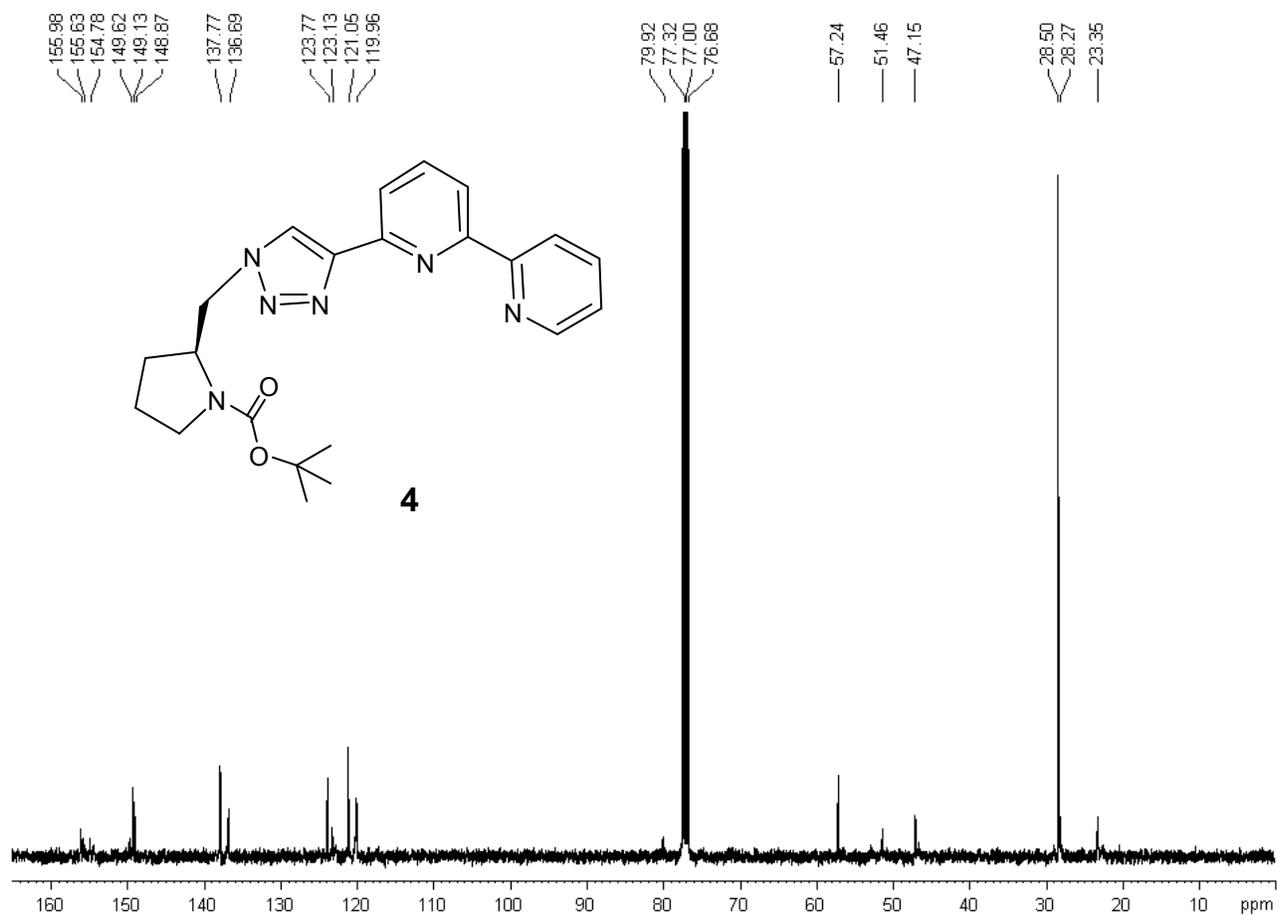
Fig. S5 Control experiment. UV-Vis absorption spectra of various concentrations of $\text{Al}(\text{ClO}_4)_3$ (0, 1.25, 2.5, 5, 12.5, 25, 62.5, 125, 187.5, 250, 500, 750 and 1000 mM). Inset: absorption intensity in the range 400-600 nm as a function of $[\text{Al}^{3+}]$.

5. NMR Spectra

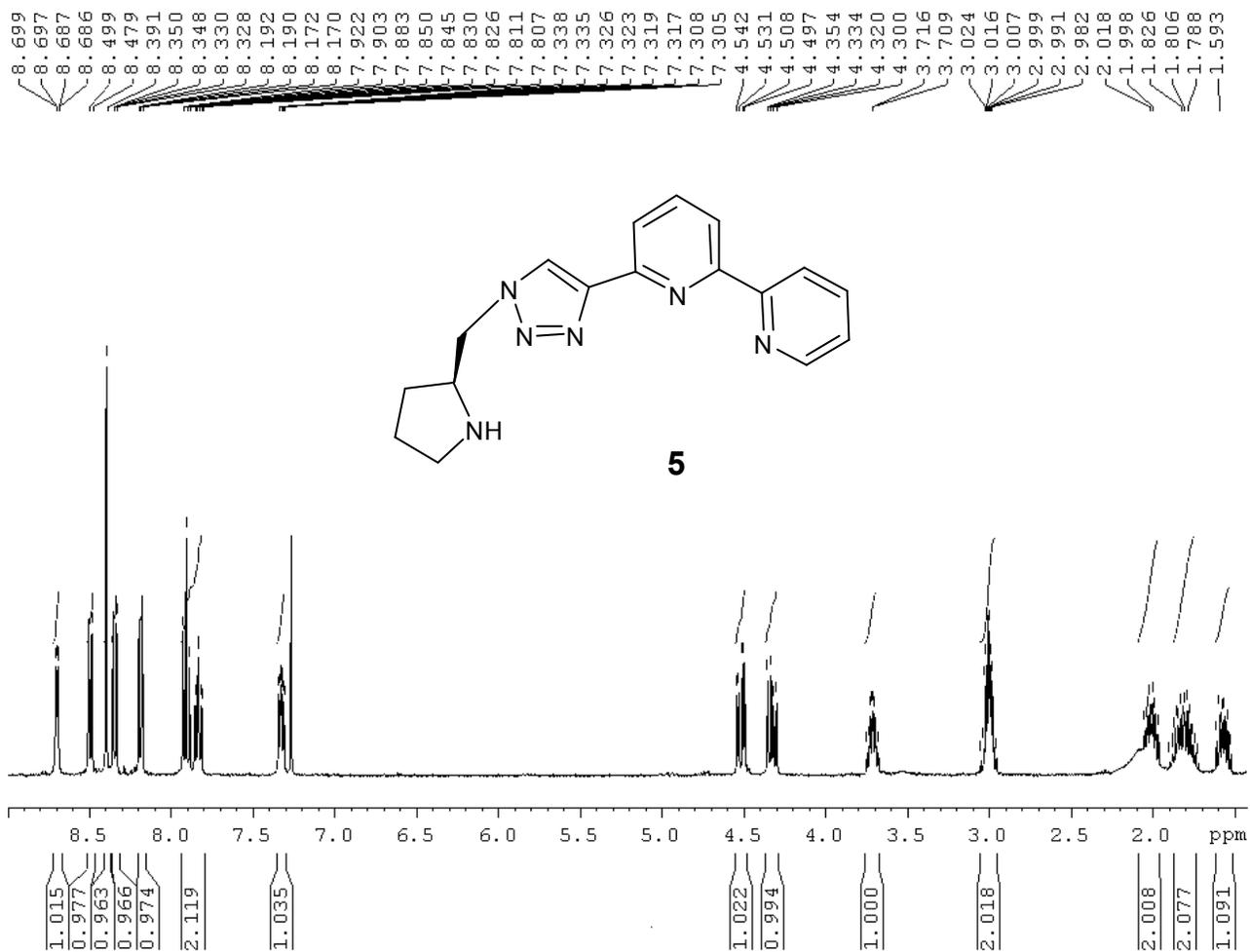
¹H NMR Spectrum of Boc-pyrrolidine methylene triazole bipyridine (4)



^{13}C NMR Spectrum of Boc-pyrrolidine methylene triazole bipyrindine (4)



¹H NMR Spectrum of Pyrolidine methylene triazole bipyrindine (5)



¹³C NMR Spectrum of Pyrolidine methylene triazole bipyridine (5)

155.83
155.51
149.64
148.99
148.26

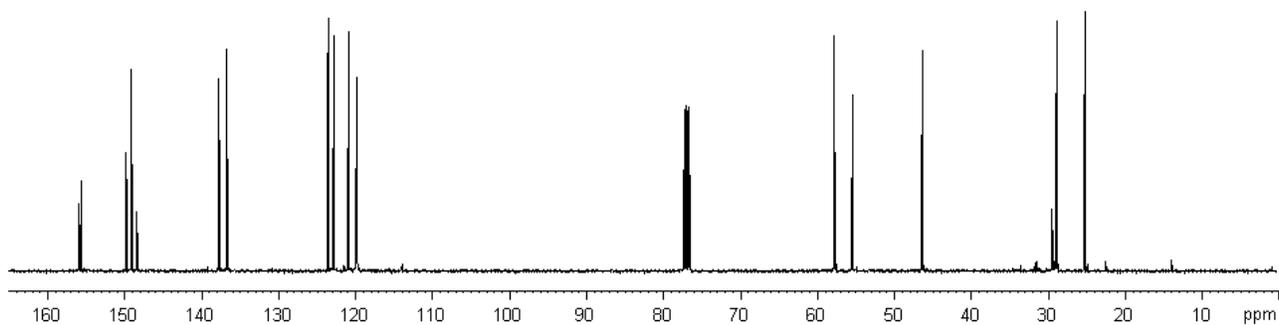
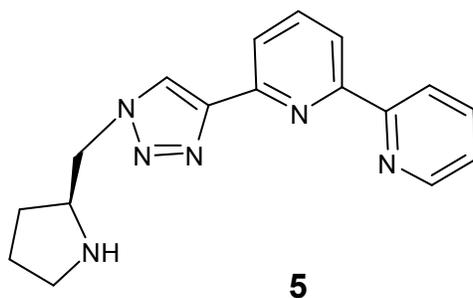
137.60
136.61

123.61
122.88
120.94
119.90
119.84

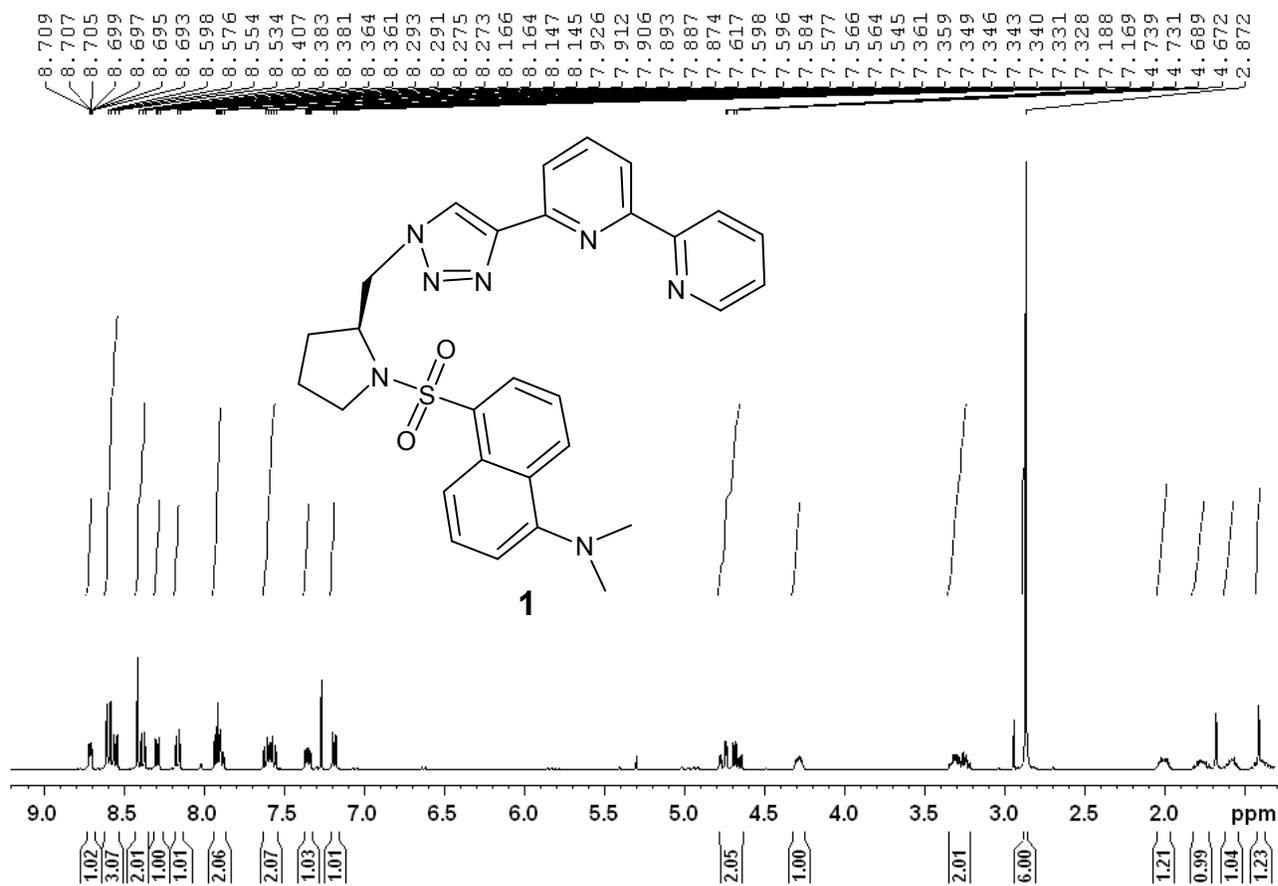
57.86
55.47

46.42

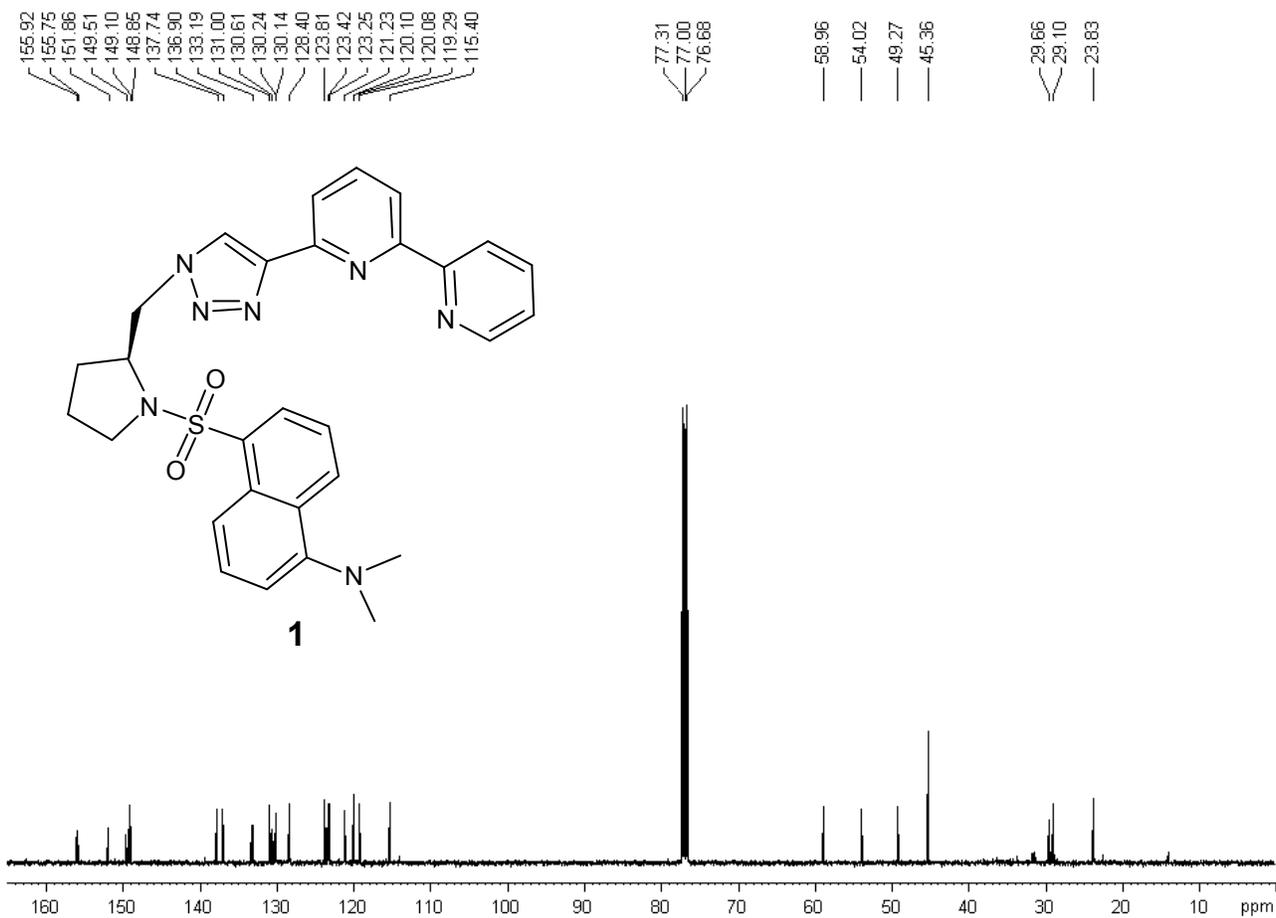
28.97
25.30



¹H NMR Spectrum of Dansylated pyrrolidine methylene triazole bipyrindine (BD 1)

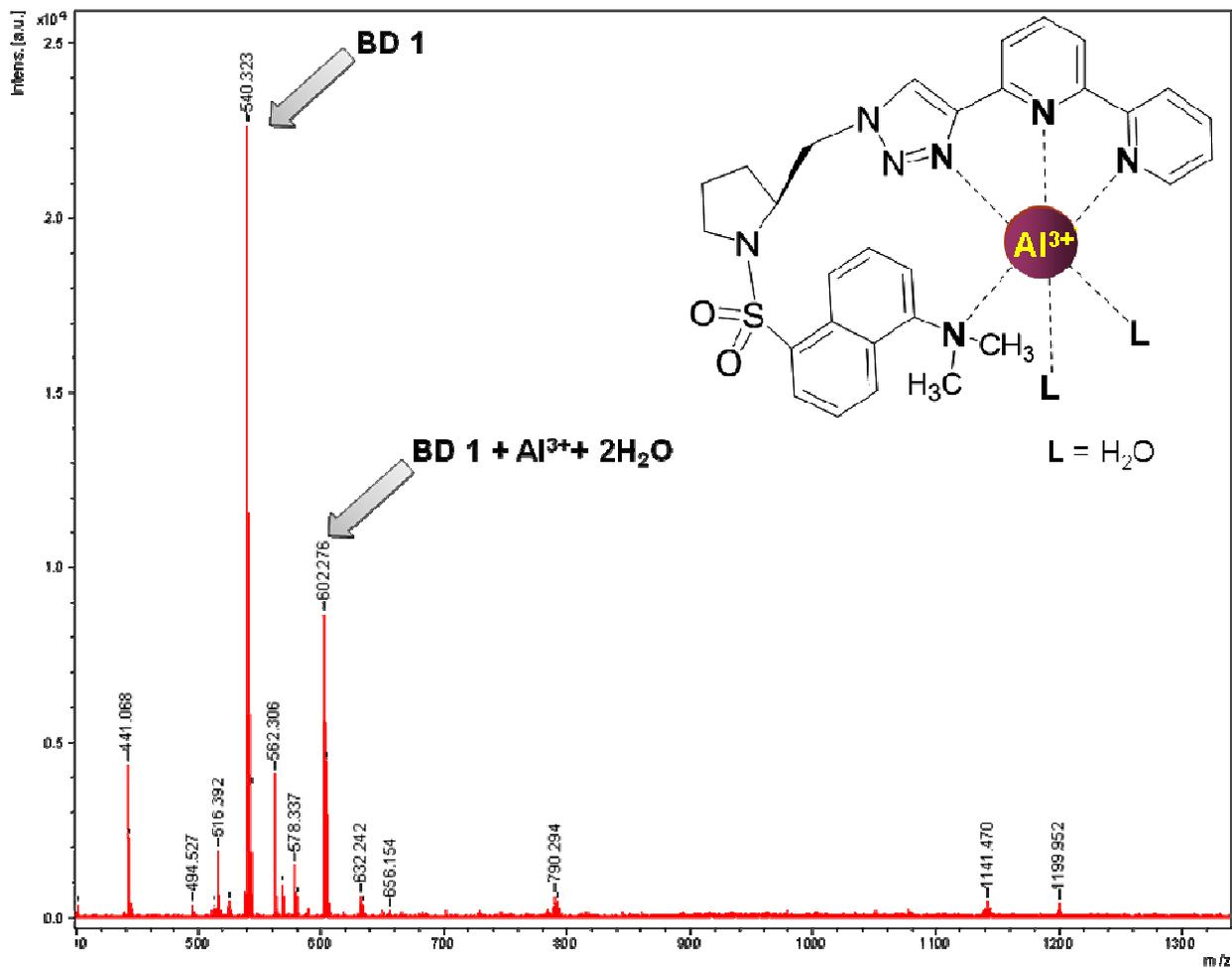


^{13}C NMR Spectrum of Dansylated pyrrolidine methylene triazole bipyrindine (BD 1)



6. Mass spectral analysis of $\text{BD 1} \cdot \text{Al}^{3+} \cdot 2\text{H}_2\text{O}$ complex

MALDI-MS spectrum of complex $\text{BD 1} \cdot \text{Al}^{3+} \cdot 2\text{H}_2\text{O}$ in acetonitrile.



7. Thermogravimetric Analysis

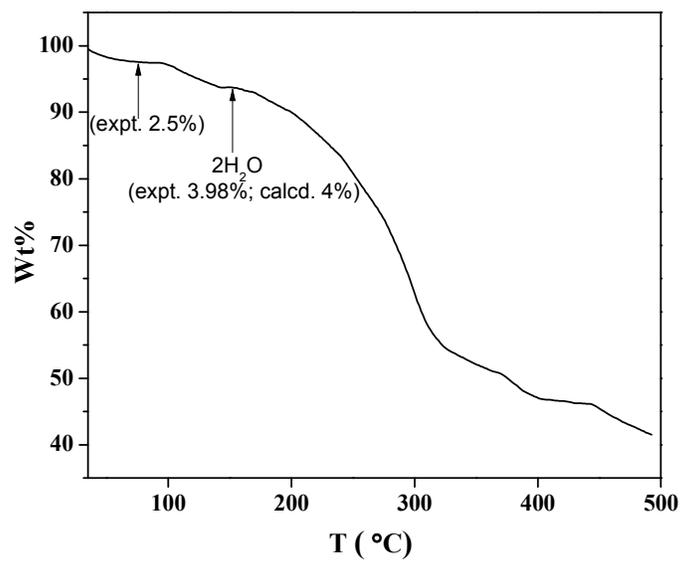


Fig. S6 Thermogravimetric analysis of **BD 1·Al³⁺** Complex. Experimentally 3.98% water loss is corresponding to two water molecules.

8. IR Spectra

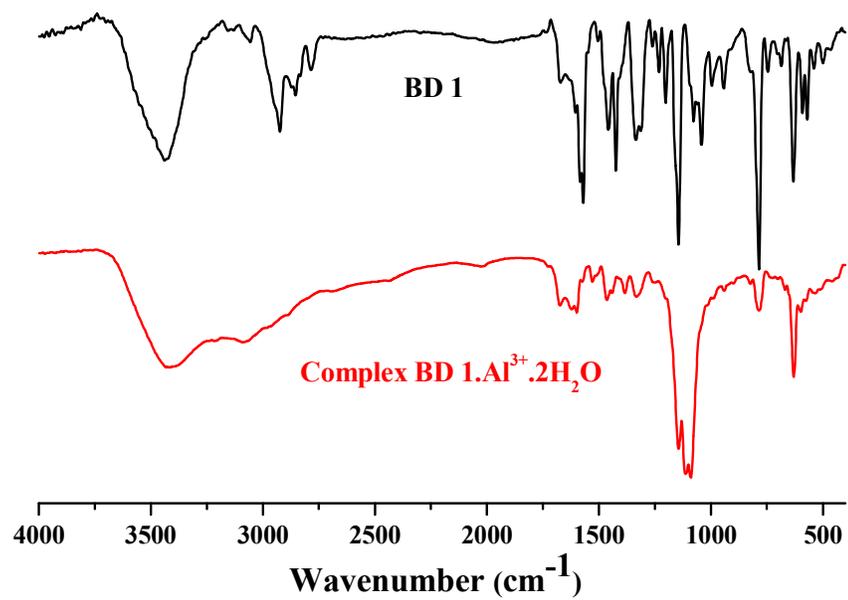


Fig. S7 IR spectra of **BD 1** and complex **BD 1·Al³⁺**.

9. Observed Colour Changes during UV and visible sensing of Al^{3+} using **BD 1**

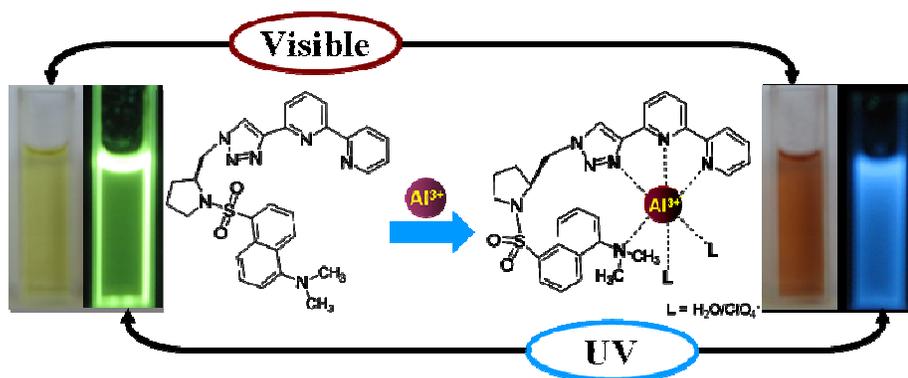


Fig. S8 Colour changes on addition of Al^{3+} to **BD 1** solution. Visible color change shown corresponding to addition of excess of Al^{3+} salt.

10. References

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- 3 G. Gryniewicz, M. Poenie and R. Y. Tsein, *J. Biol. Chem.* 1985, **260**, 3440-3450.