Electronic Supporting Information (ESI)

A new selective fluorogenic probe for trivalent cations

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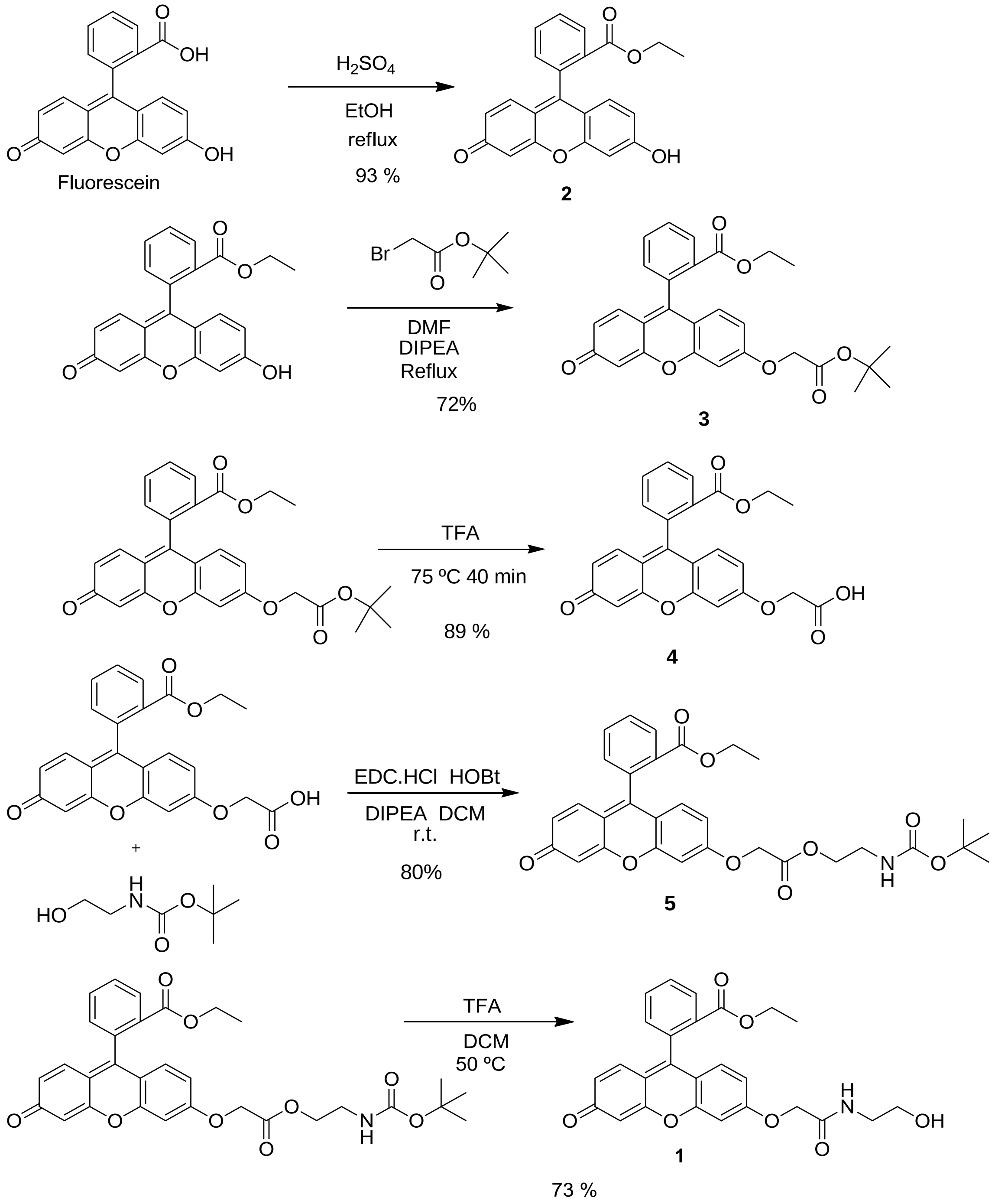


ESI-1 Scheme for the synthesis of **1**

ESI 2. EXPERIMENTAL PROCEDURES

Fluorescein ethyl ester (2)

H_2SO_4 (15 mL) was added dropwise to the solution of fluorescein (10g, 30.09 mmol) in EtOH (200mL) at room temperature. After stirring at reflux for 18 h, EtOH was evaporated under reduced pressure and the resulting mixture was diluted with CH_3Cl. Solid $NaHCO_3$ was added to the solution until gas evolution ceased. A heterogeneous mixture was filtered, and the organic phase was evaporated. The precipitate was dissolved in boiling 96% EtOH (400 mL); by boiling the volume was reduced to approximately 100 mL. Standing overnight at -20ºC gave 9.201 g (93%) of fluorescein ethyl ester, orange-brown crystals with a green lustre. ^{1}H NMR (300 MHz, DMSO) δ 8.06 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.78 (td, *J* = 7.5, 1.5 Hz, 1H), 7.69 (td, *J* = 7.6, 1.4 Hz, 1H), 7.39 (dd, *J* = 7.5, 1.0 Hz, 1H), 6.49 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 6.13 (d, *J* = 2.1 Hz, 1H), 6.10 (d, *J* = 2.1 Hz, 1H), 6.05 (d, *J* = 2.1 Hz, 2H), 3.93 (q, *J* = 7.1 Hz, 3H), 0.84 (t, *J* = 7.1 Hz, 3H).^{13}C-RMN(75 MHz, DMSO): δ 13.6; 60.85; 103.56; 115.02; 129.98; 130; 130.53, 130.69, 133.03; 133.58; 150.52, 155.98; 165.06. $[M+H]^+$ Calc. for $C_{22}H_{17}O_5$: 361.1031; Found: 361.1076.

6-O-(*tert*-butoxycarbonylmethyl) fluorescein ethyl ester (3)

Fluorescein ethyl ester (5.8 g, 16 mmol) and bromoacetic acid *tert*-butyl ester (3.9 g, 20 mmol) in a mixture of DMF (20 mL) and diisopropylethylamine (10mL) were refluxed at 100°C for 1 h. The reaction mixture was taken up in ethyl acetate (100 mL) and extracted with saturated $NaHCO_3$ (100 mL). The organic phase was washed with brine (150 mL) and dried over MgSO4 to be filtered and concentrated *in vacuo* to yield a dark orange tar. It was dissolved in warm diethyl ether (80 mL) and then reduced, by boiling, to 40 mL. Standing overnight at -20°C, the orange solid that formed was filtered off and washed with ether to yield 5.46 g (72%). ^{1}H NMR (300 MHz, DMSO) δ 8.20 – 8.16 (m, 1H), 7.86 (td, *J* = 7.5, 1.5 Hz, 1H), 7.77 (td, *J* = 7.6, 1.4 Hz, 1H), 7.50 (dd, *J* = 7.5, 1.0 Hz, 1H), 7.19 (d, *J* = 2.1 Hz, 1H), 6.93 – 6.83 (m, 2H), 6.79 (t, *J* = 8.3 Hz, 1H), 6.39 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.24 (d, *J* = 2.0 Hz, 1H), 4.86 (s, 2H), 4.03 – 3.88 (m, 2H), 1.42 (s, 9H), 0.91 – 0.81 (m, 3H). ^{13}C-RMN(75 MHz, DMSO): δ 13.69; 28.05; 61.26; 65.35; 82.01; 101.79 104.49; 113.26; 114.80; 121.18; 129.06; 130.05; 130.53, 130.64, 130.70; 133.08; 133.48; 150.06, 153.28; 158.27; 265.31; 165.01; 167.46; 184.03.$[M+H]^+$ Calc. for $C_{28}H_{27}O_7$: 475.1712; Found: 475.1757.

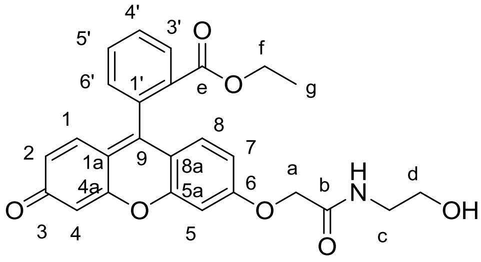
6-O-(carboxymethyl) fluorescein ethyl ester (4)

6-O-(*tert*-butoxycarbonylmethyl) fluorescein ethyl ester (1.9 g 4 mmol), in trifluoroacetic acid (9.9 mL), was refluxed at 75°C for 40 min. Most trifluoroacetic acid was removed under reduced pressure, and a trifluoroacetate of the product was precipitated with diethyl ether and filtered off. It was dissolved in boiling diethyl ether (25 mL) and then reduced to 10 mL. Overnight standing at -20°C produced yellow crystals, which were filtered off and washed with ether: yield 1.49 g (89%).^{1}H NMR (300 MHz, DMSO) δ 8.23 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.89 (td, *J* = 7.5, 1.4 Hz, 1H), 7.81 (td, *J* = 7.6, 1.4 Hz, 1H), 7.55 – 7.50 (m, 1H), 7.37 (s, 1H), 7.07 – 6.97 (m, 3H), 6.64 (dd, *J* = 9.6, 2.0 Hz, 1H), 6.58 (d, *J* = 1.9 Hz, 1H), 4.96 (s, 2H), 4.03 – 3.88 (m, 2H), 0.87 (t, *J* = 7.1 Hz, 3H). ^{13}C-RMN(75 MHz, DMSO): δ 13.71; 61.39; 65.05; 101.68; 104.44; 113.66; 117.10; 130.85; 131.14, 133.16; 169.62.$[M+H]^+$ Calc. for $C_{24}H_{19}O_7$: 419.1086; Found: 419.1131.

2-((*tert*-butoxycarbonyl)amino)ethoxy 6-O-(carbonylmethyl) fluorecein ethyl ester (5)

EDC·HCl (331.6 mg, 1.73 mmol) and HOBt (235 mg, 1.73 mmol) in DCM (5 mL) were stirred for 2 minutes. DIPEA (418 µL, 2.4 mmol) was added dropwise, followed by 6-O-(carboxymethyl) fluorecein ethyl ester (660 mg, 1.57 mmol). Tert-buthyl-2-hydroxyethylcarbamate (281.8 mg, 1.73 mmol) was added in one portion, the flask was sealed with a rubber septum and allowed to stir under N_2 for 24 hours. The reaction was diluted with DCM (10 mL) and washed twice with 0.5M hydrochloric acid (2 x 5 mL). The acidic aqueous layer was extracted with DCM (20 mL), the combined organic layers were washed with saturated, aqueous sodium bicarbonate (20 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield 440mg (80%) of orange solid with a green lustre, which was sufficiently pure to use in the subsequent step. ^{1}H NMR (300 MHz, DMSO) δ 8.20 (d, *J* = 7.6 Hz, 1H), 7.87 (t, *J* = 7.2 Hz, 1H), 7.79 (t, *J* = 7.4 Hz, 1H), 7.52 (d, *J* = 6.7 Hz, 1H), 7.27 (d, *J* = 10.6 Hz, 1H), 7.01 (t, *J* = 5.8 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.84 (dd, *J* = 13.5, 9.3 Hz, 2H), 6.40 (d, *J* = 9.9 Hz, 1H), 6.24 (s, 1H), 4.98 (d, *J* = 8.1 Hz, 2H), 4.13 (t, *J* = 5.6 Hz, 2H), 3.97 (dd, *J* = 7.0, 3.5 Hz, 2H), 3.17 (dd, *J* = 18.7, 5.4 Hz, 2H), 1.37 (d, *J* = 2.6 Hz, 9H), 0.88 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 13.66;28.53; 30.98; 43.01; 55.19; 60.42; 61.24; 64.20; 65.36; 78.26; 101.76; 104.98; 113.96; 115.27; 117.40; 129.31; 130.32; 130.91; 133.39; 133.79; 150.19; 153.68; 156.05; 158.68; 162.47; 165.25; 168.28; 184.32.$[M+H]^+$ Calc. for $C_{31}H_{32}NO_9$: 562.2032; Found: 562.1532.

2-aminoethoxy 6-O-(carbonylmethyl) fluorecein ethyl ester (1)



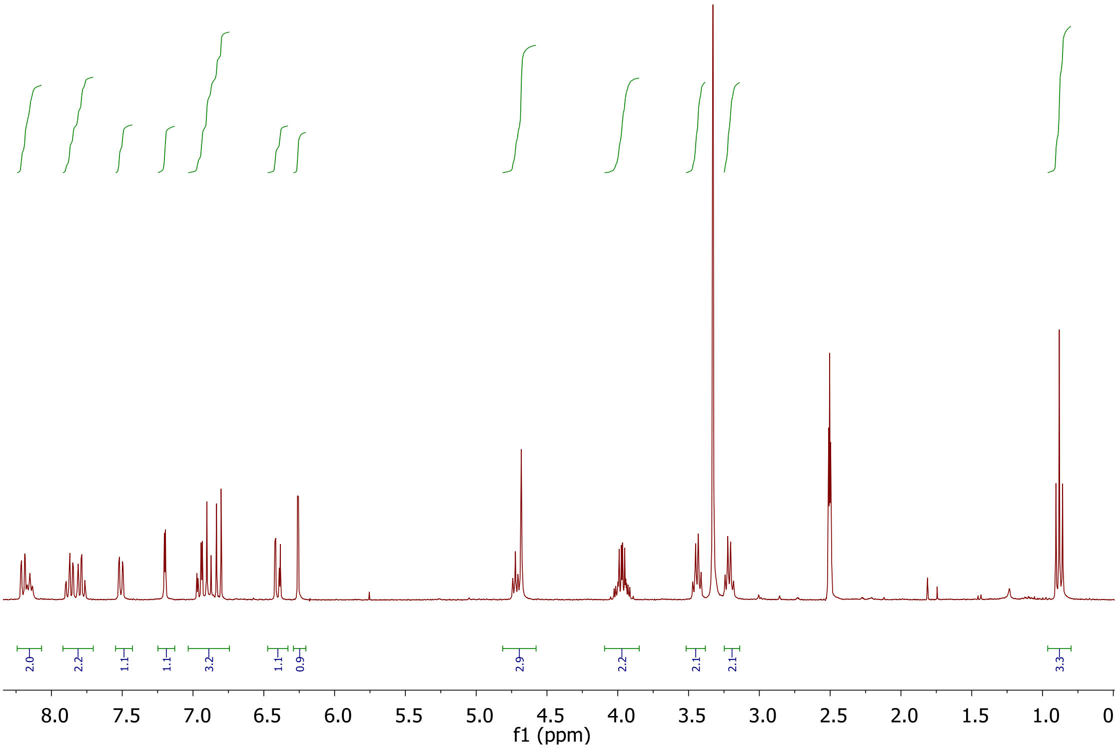
2-((*tert*-butoxycarbonyl)amino)ethoxy 6-O-(carbonylmethyl) fluorecein ethyl ester (200 mg, 0.36 mmol) was dissolved in DCM (3 mL). TFA was added (890 µL) dropwise and the reaction was heated under reflux for 72 hours.

1M NaOH was added dropwise until pH 7. The solution was extracted with DCM (2x5mL), and then the organic layer was washed with brine (5 mL) and dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the desired product (121 mg, 73%) as a light orange solid. IR (neat solid) ν 3310 (OH + NH), 1684 (CO_2Et), 1652 (C=O), 1635 (Amide I), 1539 (Amide II) cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 8.16 (dd, *J* = 7.4, 3.4 Hz, 1H, H-3’), 8.15 (t, *J* = 5.8 Hz, NH), 7.86 (td, *J* = 7.5, 1.5 Hz, 1H, 5’), 7.77 (td, *J* = 7.6, 1.4 Hz, 1H, H-4’), 7.50 (dd, *J* = 7.5, 1.1 Hz, 1H, H-6’), 7.19 (d, *J* = 2.3 Hz, 1H, H-5), 6.95 (dd, *J* = 8.8, 2.3 Hz, 1H, H-7), 6.88 (d, *J* = 8.8 Hz, 1H, H-8), 6.81 (d, *J* = 9.7 Hz, 1H, H-1), 6.39 (dd, *J* = 9.7, 1.9 Hz, 1H, H-2), 6.25 (d, *J* = 1.9 Hz, 1H, H-4), 4.72 (t, J = 6.0 Hz, OH), 4.67 (s, 2H, Ha), 4.02 – 3.86 (m, 2H, Hf), 3.43 (dd, *J* = 11.4, 5.9 Hz, 2H, Hd), 3.20 (q, *J* = 5.9 Hz, 2H, Hc), 0.87 (t, *J* = 7.1 Hz, 3H, Hg). ^{13}C NMR (75 MHz, DMSO) δ :13.72 (Cg); 41.61 (Cc); 59.94 (Cd); 61.27 (Cf); 67.62 (Ca); 101.73 (C-5); 105.00 (C-4); 114.30 (C-7); 115.15 (C-8a); 117.34 (C-1a); 129.30 (C-8); 129.8 (C-2); 130.29 (C-4’); 130.40 (C-1); 130.77 (C-6’); 130.9 (C-3’); 131.06 (C-2’); 133.40 (C-1’); 133.82 (C-5’); 150.25 (C-9); 153.65 (C-5a); 158.67 (C-4a); 162.57 (C-6), 165.29 (Ce); 167.14 (Cb); 184.29 (C3).$[M+H]^+]^+$ Calc. for $C_{26}H_{24}NO_7$: 462.1533; Found: 462.2008. *Anal*. Calcd. for $C_{26}H_{24}NO_7$: C, 67.67; H, 5.02; N, 3.04. Found: C, 67.95; H 5.04; N, 3.02.

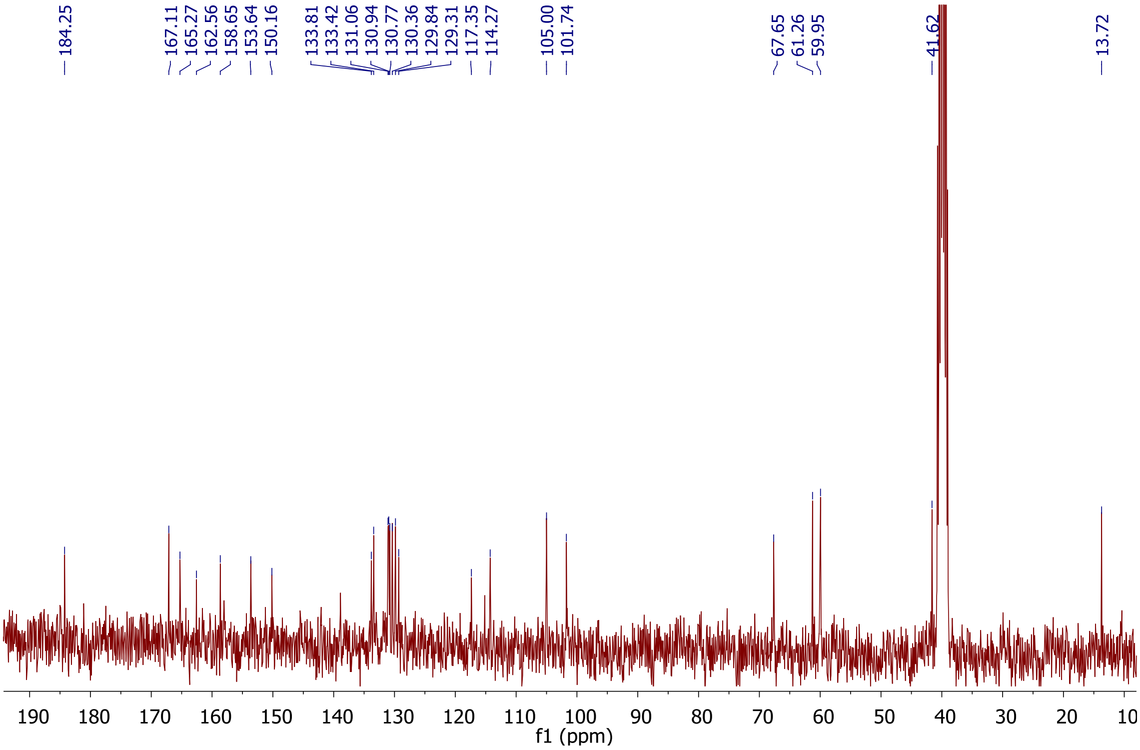
tert-Butyl-2-hydroxyethylcarbamate.

2-aminoethanol (2.04 mL, 34 mmol) was dissolved in 1M NaOH solution (100 mL) and the mixture was stirred. A separate solution of BOC_2O (8.89 g, 40.8 mmol) in 1,4-Dioxane (50 mL) was added to the reaction. The solution was stirred for 48 hours. The reaction was quenched with water and the solution was extracted with ethyl acetate (3x75 mL), the organic layer was washed with brine (75 mL) and dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the Tert-buthyl-2-hydroxyethylcarbamate (4.06 g, 74%) as a light yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ 5.51 (s, 1H), 3.96 (s, 1H), 3.34–3.23 (m, 2H), 2.97 – 2.85 (m, 2H), 1.12 (s, 9H).

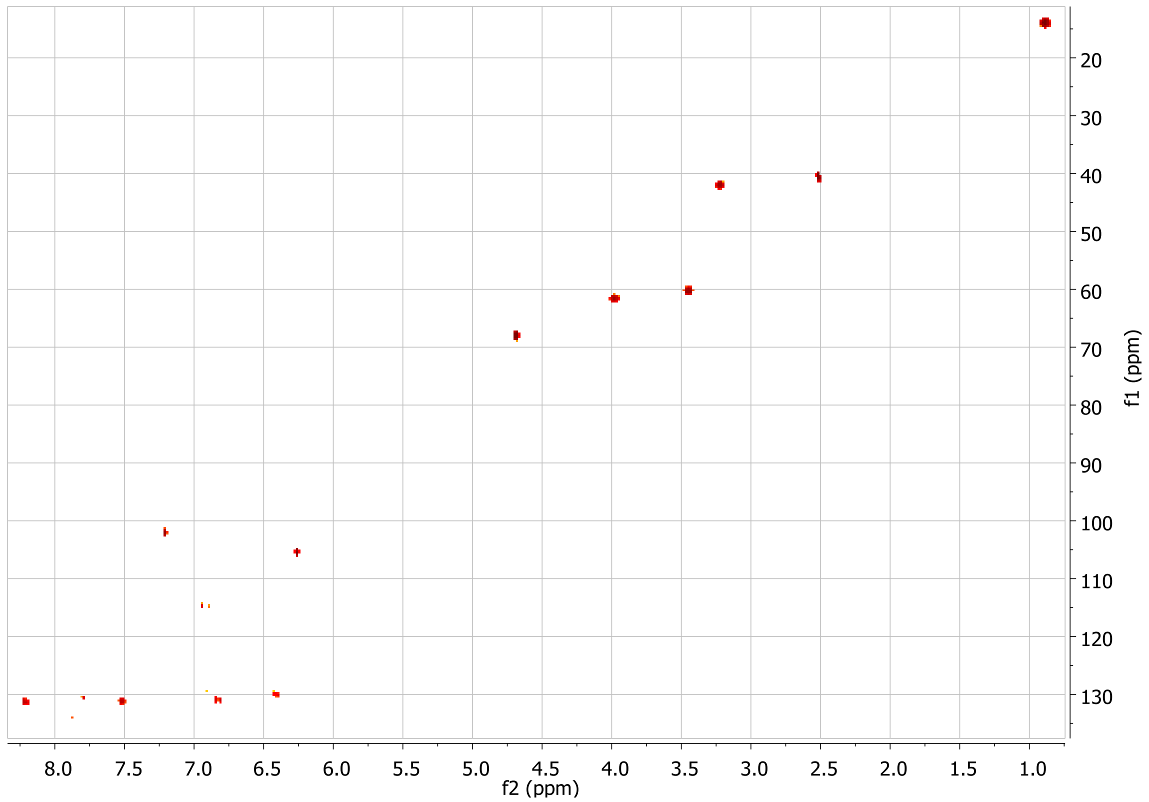
ESI 3. Spectrocopic data for Ligand **1**.



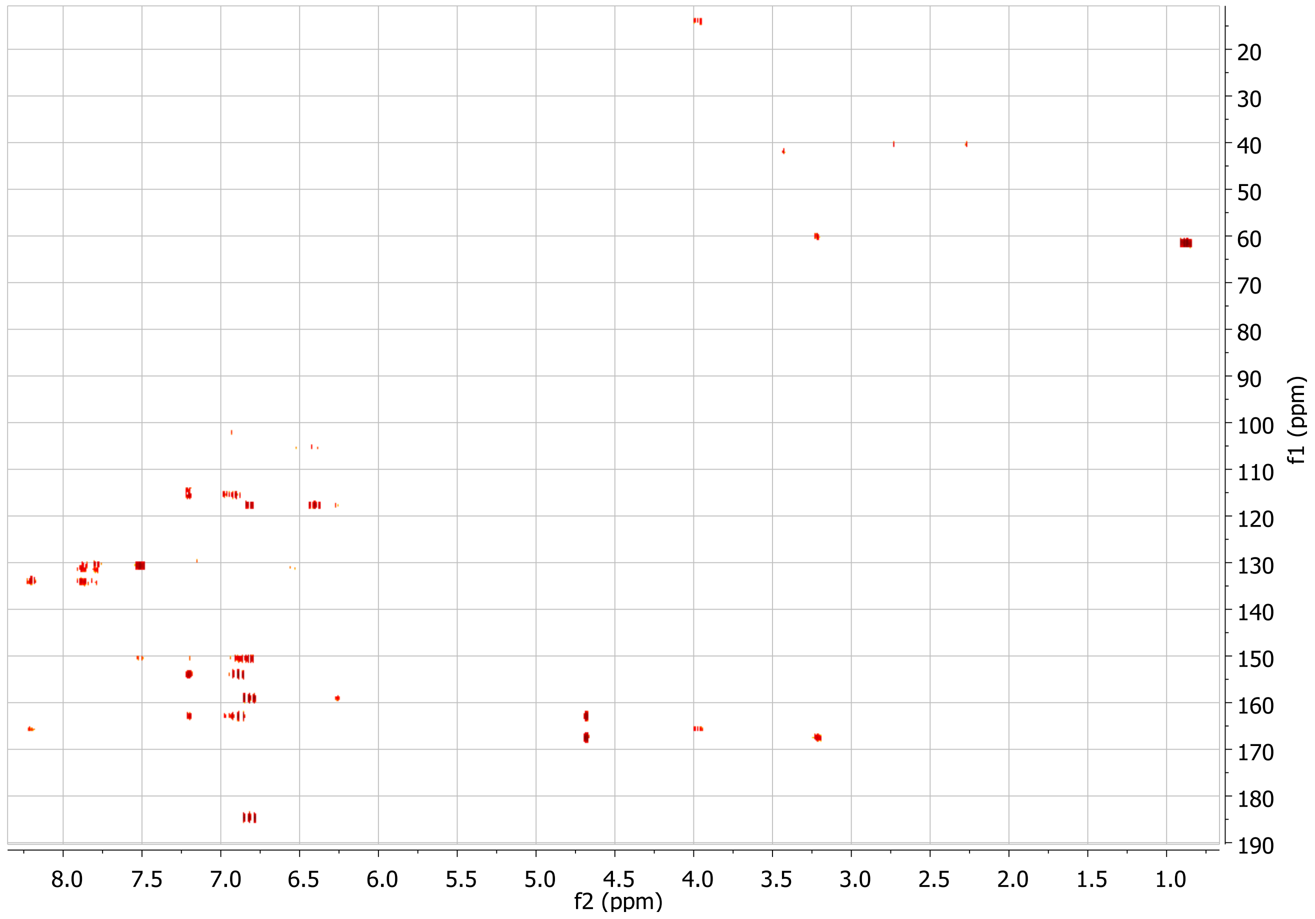
^{1}H NMR of chemosensor **1.**



^{13}C NMR of Ligand **1.**



^{13}C-^{1}H HSQC NMR of Ligand **1.**

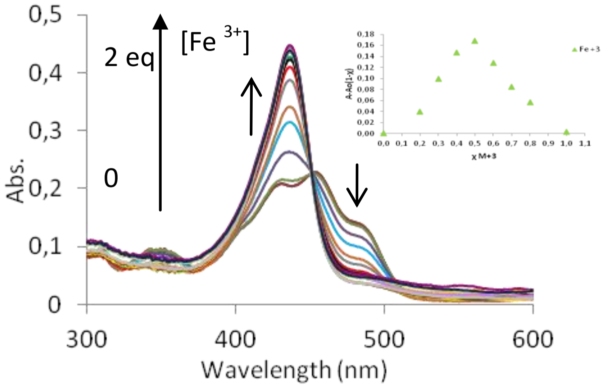


^{13}C-^{1}H HMBC NMR of Ligand **1.**

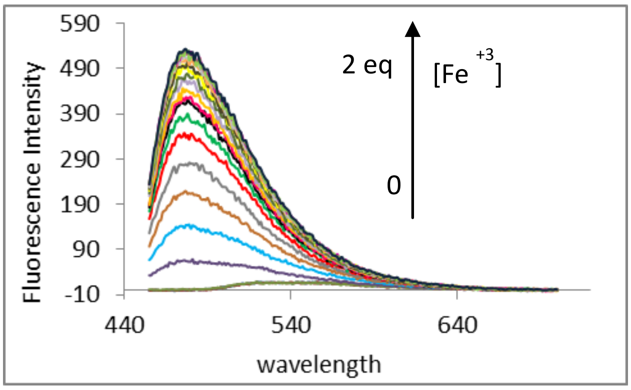
Titration experiments

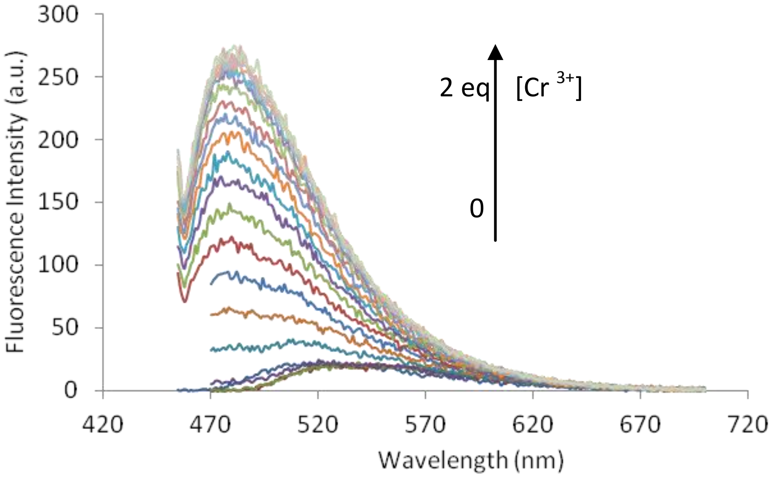
The binding constants of ligand **1** towards trivalent cations were evaluated by UV–vis and fluorescence titrations in acetonitrile. Typically, the 10^{-5} M solutions of the receptors in acetonitrile (3 mL) were titrated by adding 0.1 equiv. aliquots of the envisaged cations in CH_3CN and by registering the UV–vis or fluorescence spectrum after each addition. The log Kc value was calculated by fitting all the spectrophotometric titration curves with the SPECFIT program [31].

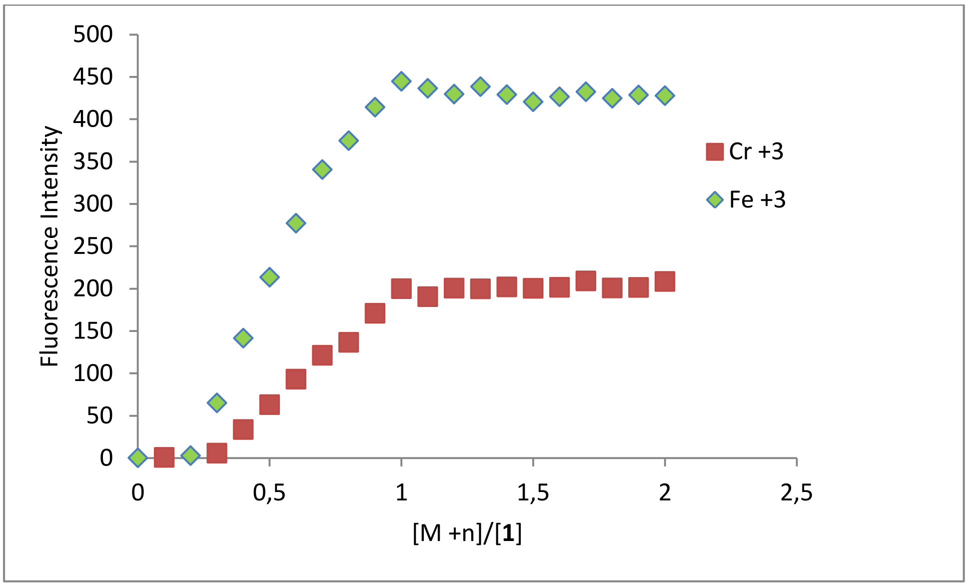
ESI 4. UV-vis spectra of ligand **1** (10^{-5} M) upon titration of Fe^{3+} (0-2 equiv.) in CH_3CN. Inset: absorbance of **1** at 437 nm as a function of the [cation]/[ligand] ratio.



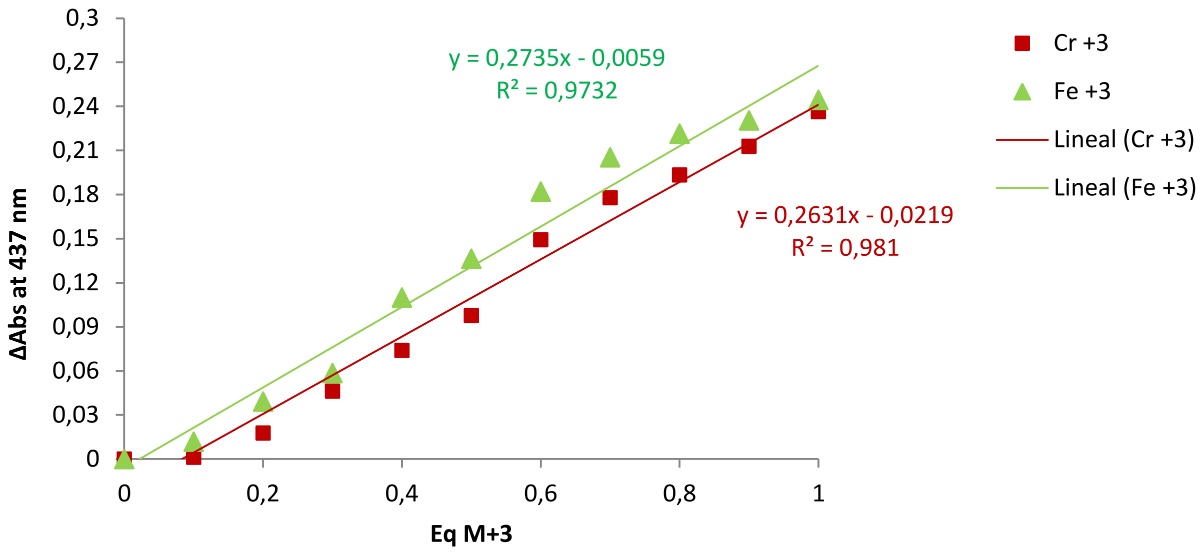
ESI 5. Fluorescence titration spectra of **1**(10^{-5} M) upon titration with Fe^{3+} and Cr^{3+} in CH_3CN (λ_{exc}= 437nm). Graphic of fluorescence intensity versus Fe^{3+} and Cr^{3} concentration





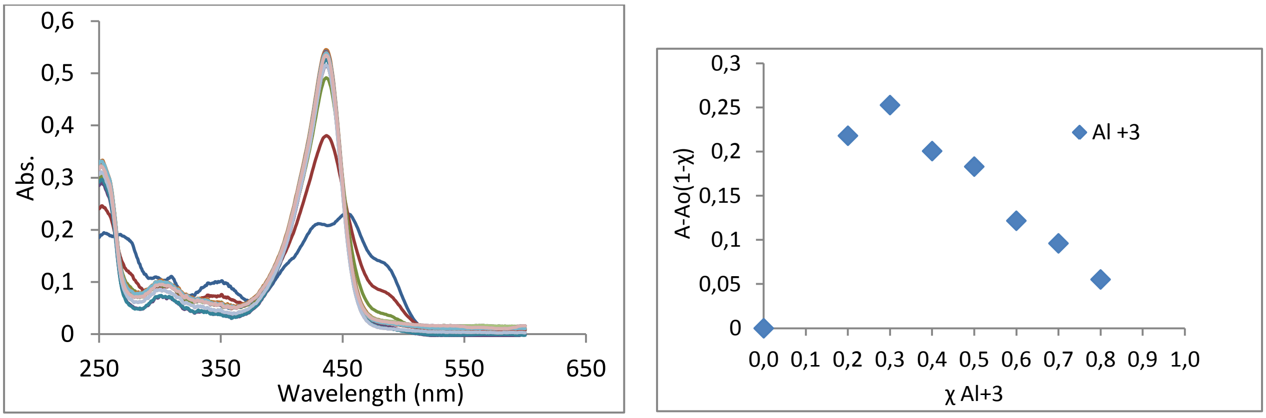


ESI 6. A plot of (A-A$_o$) vs. cations concentrations at 437 nm in CH_3CN at room temperature.

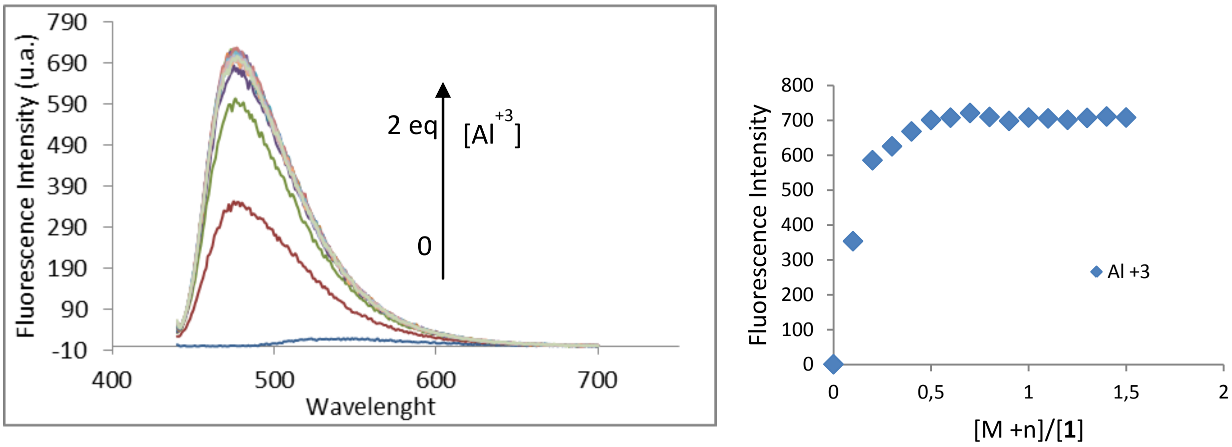


ESI 7. (a) UV-vis spectra of ligand **1** (10^{-5} M) upon titration of Al^{3+} (0-2 equiv.) in CH_3CN. Inset: Stoichiometry determination by the Job's plot yielded from UV-vis absorption; (b) Fluorescence titration spectra of ligand **1**(10^{-5} M) upon titration with Al^{3+} in CH_3CN (λ_{exc}= 437nm) and graphic of fluorescence intensity versus Al^{3+}concentration.

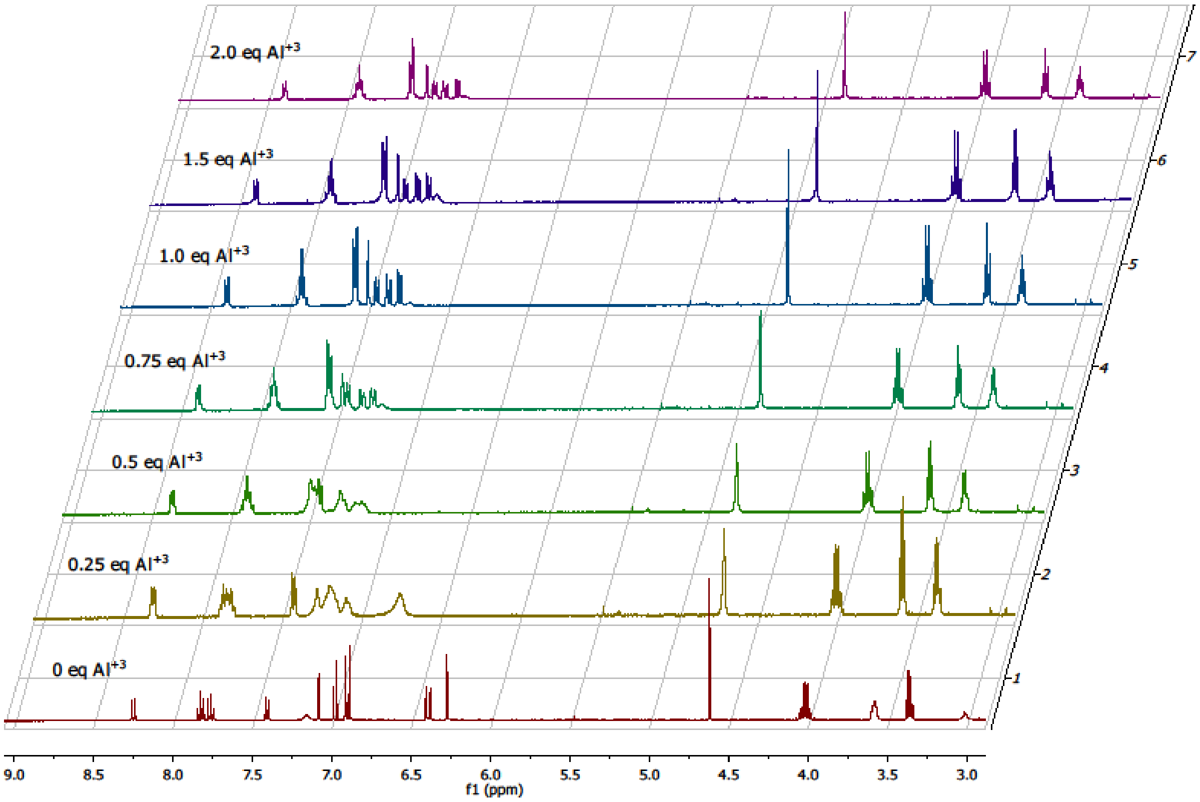
a)



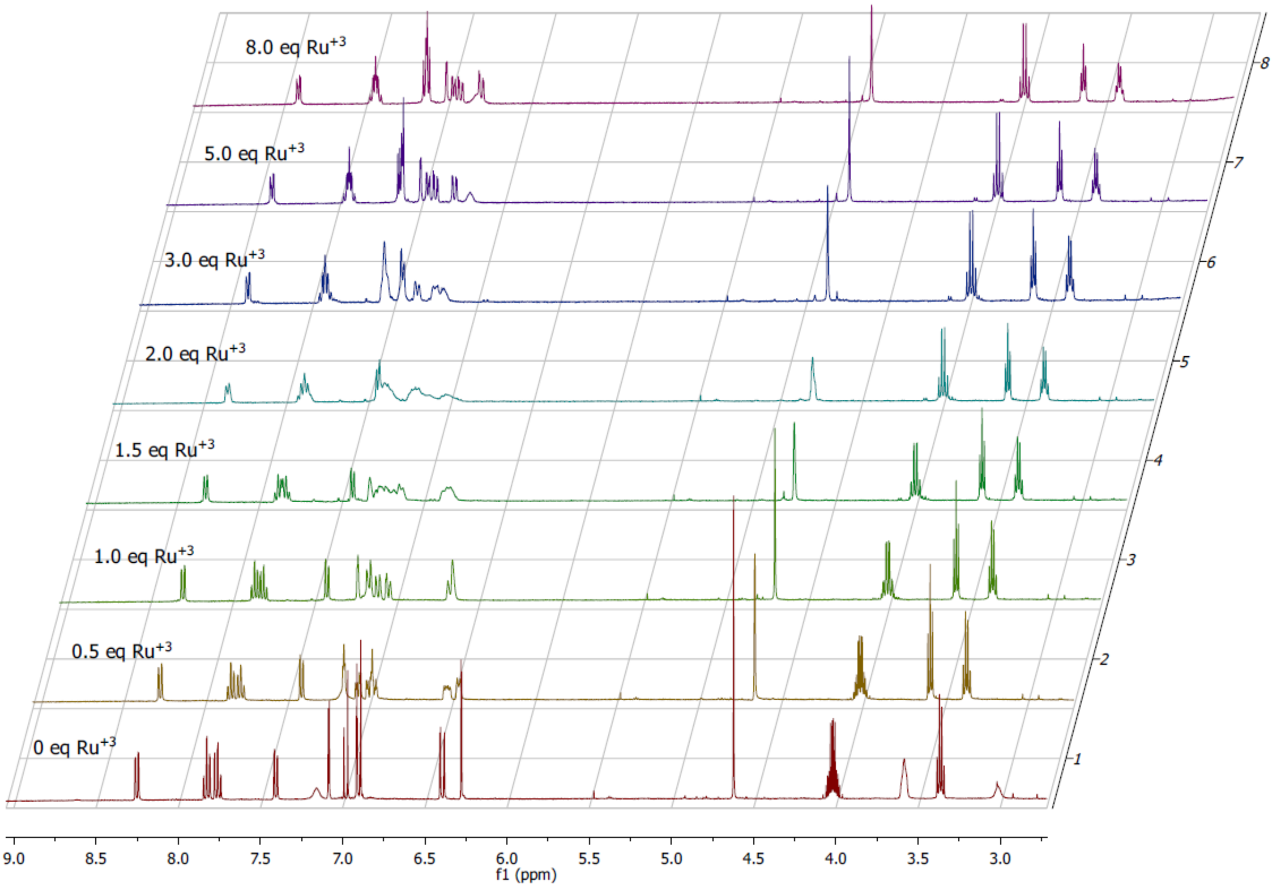
b)



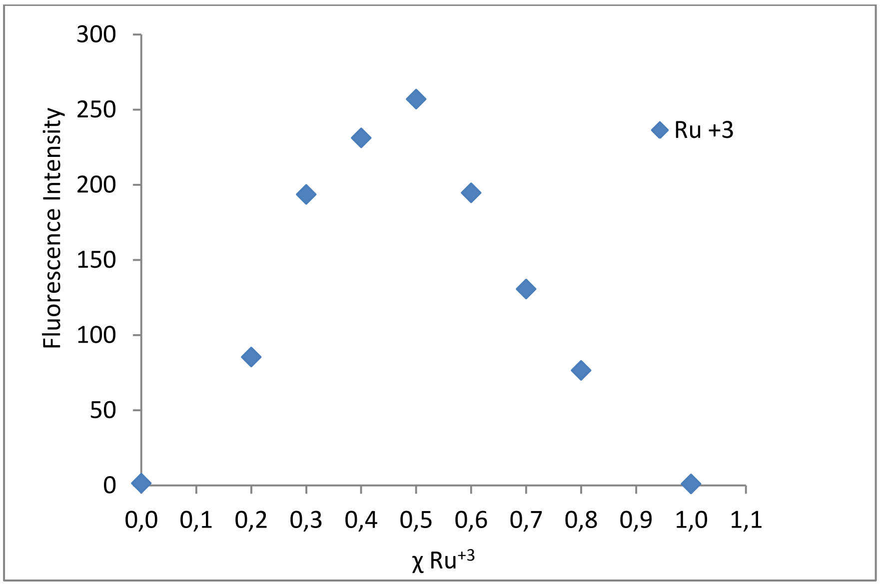
ESI 8. ^{1}H NMR spectra of Ligand **1** and Ligand **1** + 0.25, 0.50, 0.75, 1.0, 1.5, 2.0 eq. of Al^{3+} in CD_3CN from the bottom to the top respectively



ESI 9. ^{1}H NMR spectra of Ligand **1** and Ligand **1** + 0.50, 1.0, 1.5, 2.0, 3.0, 5.0 and 8.0 eq. of Ru^{3+} in CD$_3$CN from the bottom to the top respectively

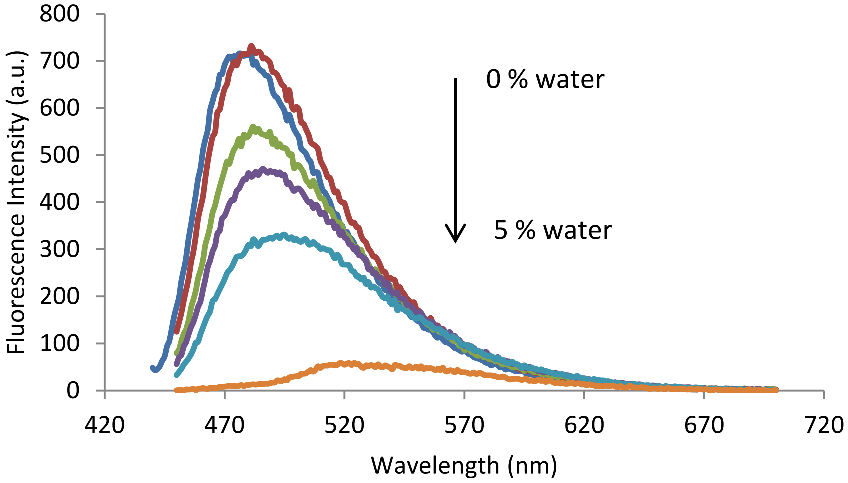


ESI 10. Stoichiometry determination of **1**·Ru^{3+} complex by the Job's plot yielded from fluorescence.

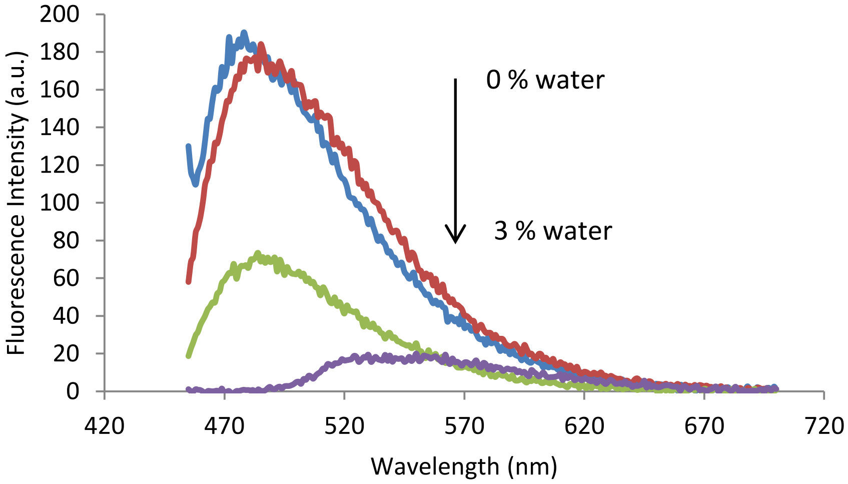


ESI 11.- Fluorescence spectra (λ_{ex} = 437 nm) of 1 measured with 1 eq. of Al^{3+} (a), Cr^{3+} (b) and Fe^{3+} (c) in CH_3CN in the presence of 0-5 % water.

a)



b)



c)