Supplementary Information

A Chemosensor for Al^{3+} Ion in Aqueous Ethanol Medium: Photophysical and Live Cell Imaging Studies

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Experimental section

***UV-vis* and fluorescence spectroscopic studies.**

Stock solution of the sensor **L** was prepared at the concentration of 10^{-3} M in 10 mL of EtOH and then diluted to a desired concentration. Stock solutions of various ions were prepared at the concentration of 10^{-3} M in 10 mL distilled water. In titration experiments, a quartz optical cell of 1 cm optical pathlength was filled with a 2 mL solution of the sensor **L** (10^{-5} M) to which the metal ion stock solutions were gradually added using a micropipette. Spectral data were recorded within5 min after addition of the respective metal ions. In selectivity experiments, the test samples were prepared by placing appropriate amounts of the cations stock into 3 mL of **L** (10^{-5} M). For fluorescence measurements, excitation was provided at 520 nm and emission was acquired from 540 nm to 700 nm. The association constant was determined from the change in fluorescence intensity resulting from the titration of dilute solution (~10^{-5} M) of the dye against Al^{3+} ion concentration. The reported values gave good correlation coefficients (>0.97).

Determination of Quantum yield:

Fluorescence quantum yields were determined by comparing the corrected spectra with that of pure rhodamine B[1] in ethanol taking the total area under the fluorescence band using the equation (i)[2]

$$\Phi_S = \Phi_R (F_S A_R / F_R A_S) \times (\eta_S / \eta_R)^2 \quad \text{.....(i)}$$

Where, Φ stands for quantum yield, F stands for area under the fluorescence spectra, A stands for absorbance value and η stands for the refractive index value. The subscript 'R' indicates the value of the parameter for reference (i.e. Rhodamine-B) and 'S' subscript indicates the value of the parameter for the sample.

Determination of Detection Limit:

For the evaluation of the detection limit of the probe **L** with Al^{3+} ion, the probe was treated with different concentration of trivalent Al^{3+} ion and the emission intensity at 580 nm was plotted against the Al^{3+} ion oncentration in the lower region. Detection limit was calculated using the following equation:

$$\text{Detection limit} = 3\sigma/S$$

Where σ is the standard deviation of the eight blank meassurements, S is the slope of the curve of fluoresence emission versus metal ion concentration.

X-ray crystallography:

Single-crystal X-ray data of compound **L** was collected at 100 K on a Bruker SMART APEX CCD diffractometer using graphite monochromated MoK_α radiation (λ = 0.71073 Å). The linear absorption coefficients, scattering factors for the atoms, and the anomalous dispersion corrections were taken from the International Tables for X-ray Crystallography.[3] The data integration and reduction were carried out with SAINT software[4]. Empirical absorption correction was applied to the collected reflections with SADABS[5] and the space group was determined using XPREP[6]. The structure was solved by the direct methods using SHELXL-97 and refined on F^2 by full-matrix least-squares using the SHELXL-97 program. In this compound all the non-hydrogen atoms were refined anisotropically.

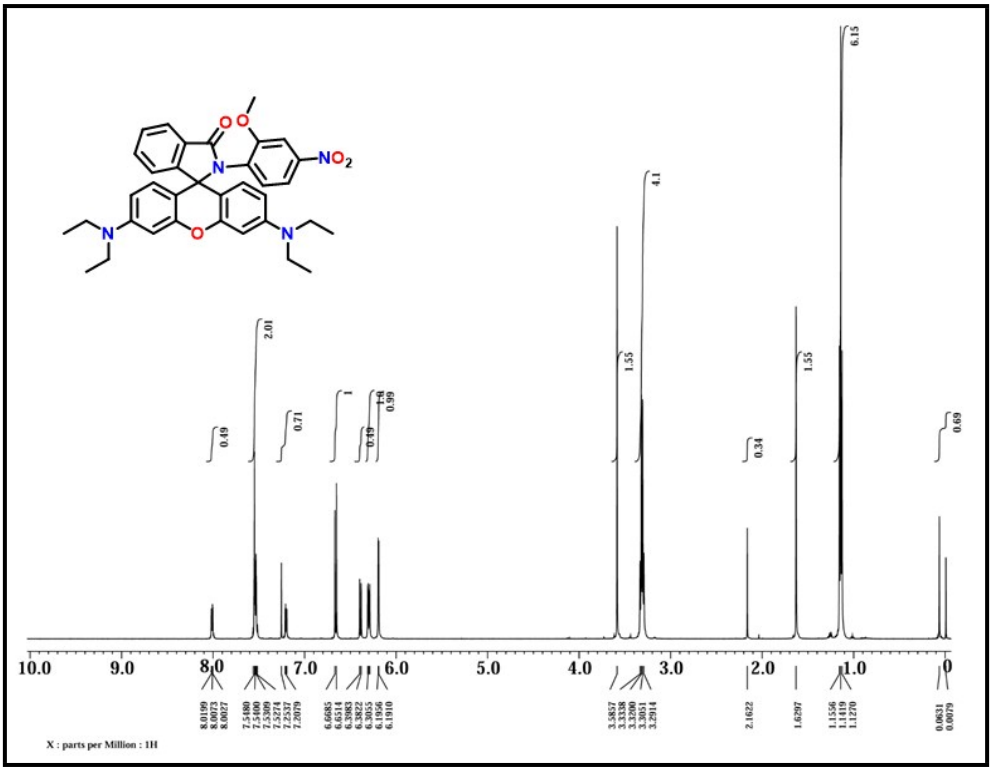

Fig. S1 500 MHz ^{1}H NMR spectrum of sensor **L**.

Fig. S2 125 MHz ^{13}C NMR spectrum of sensor **L**.

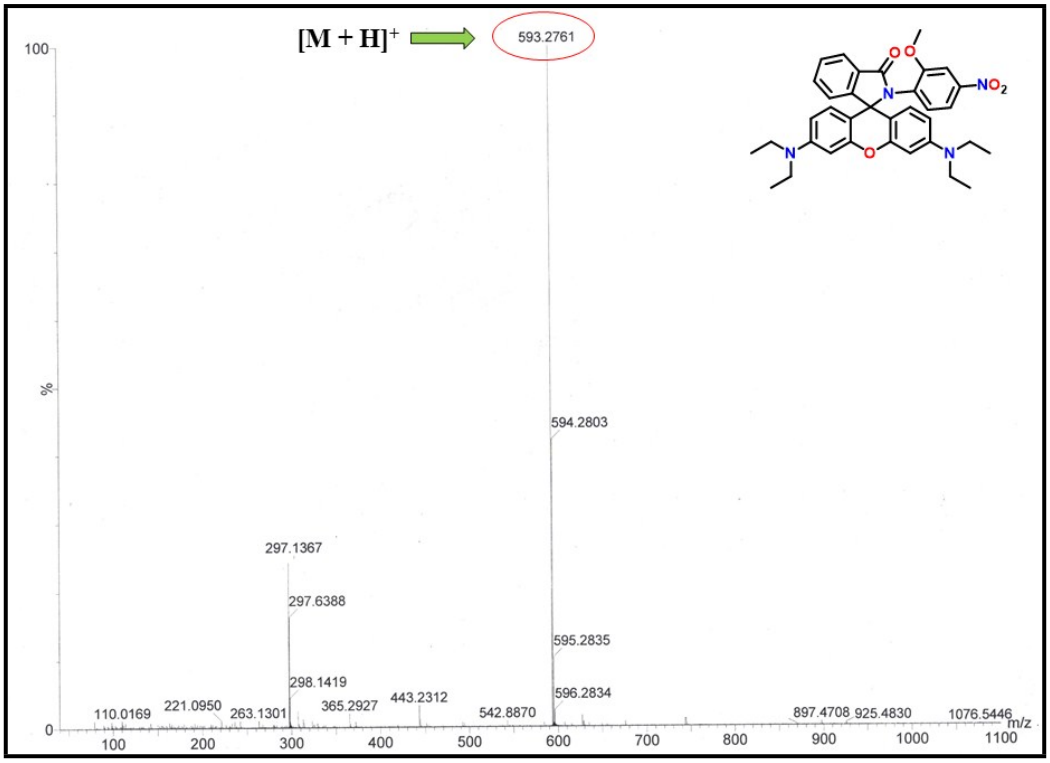

Fig. S3 ESI MS spectrum of sensor **L**.

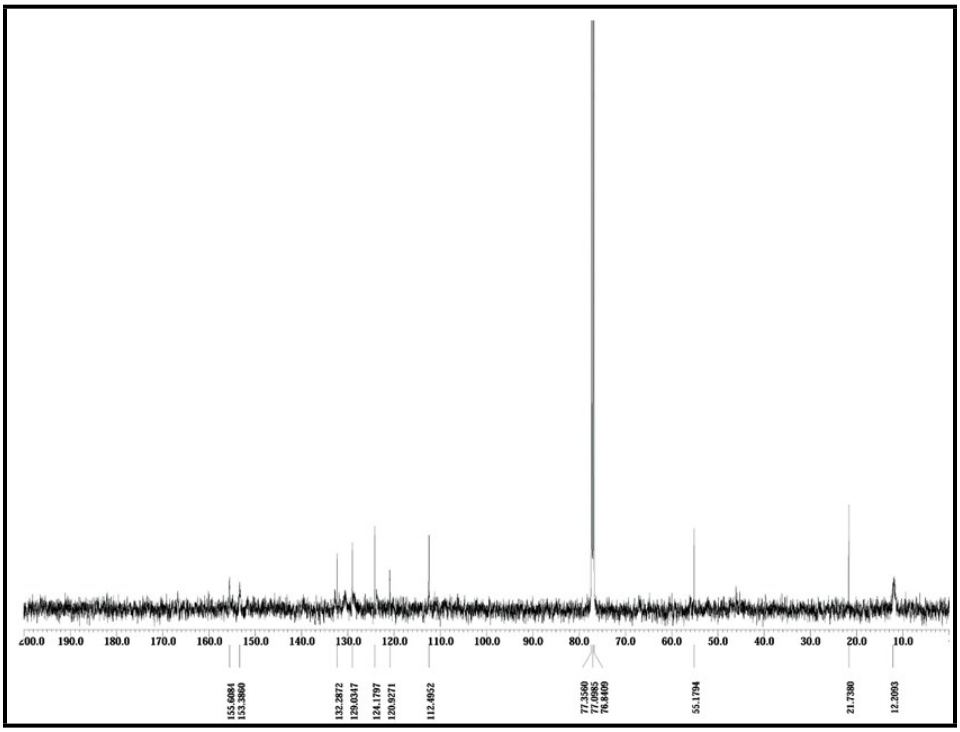

Fig. S4 125 MHz ^{13}C NMR spectrum of Al^{3+} complex of **L**.

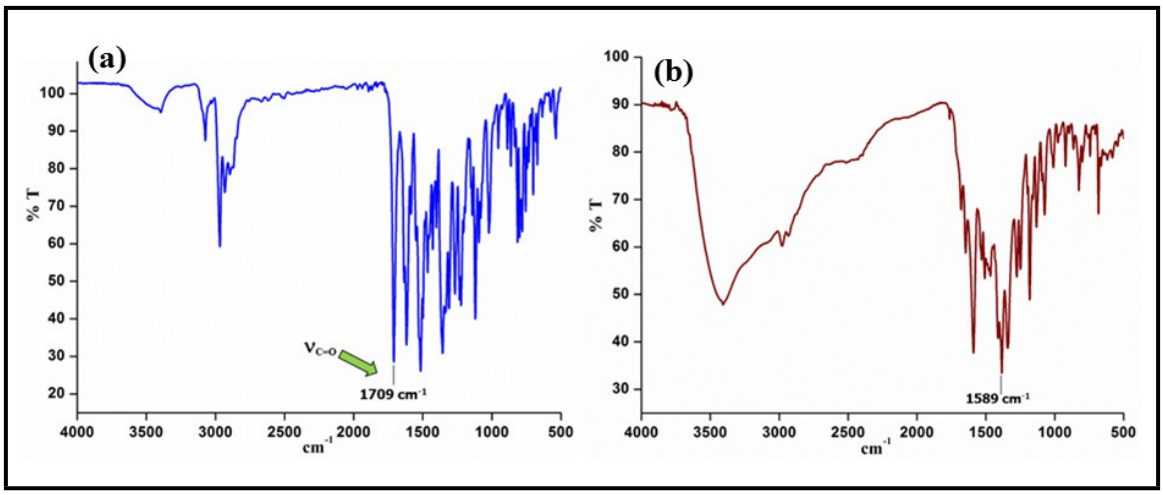

Fig. S5 IR spectra of (a) sensor **L** and (b) Al^{3+} complex of **L**.

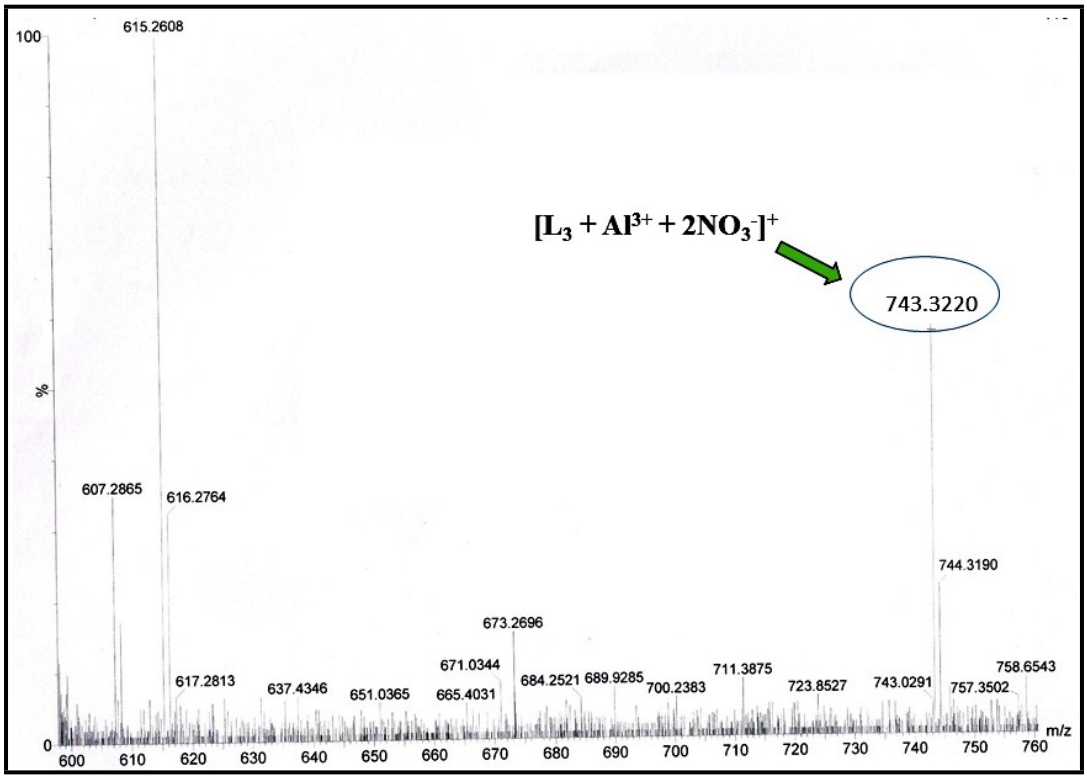

Fig. S6 ESI MS spectrum of Al^{3+} complex of **L**.

Intensity profile statistics of whole image (B)

Feature	Whole Image
Area [px²]	18687.98
Mean Intensity	0
Min Intensity	0
Max Intensity	0
Sum Intensity	0
StDev Intensity	0
Signal/Background	1.00 : 1

Intensity profile statistics of whole image (H)

Feature	Whole Image
Area [px²]	18687.98
Mean Intensity	0
Min Intensity	0
Max Intensity	0
Sum Intensity	0
StDev Intensity	0
Signal/Background	1.00 : 1

Intensity profile statistics of whole image (N)

Feature	Whole Image
Area [µm²]	18687.98
Mean Intensity	11.77
Min Intensity	0.33
Max Intensity	85.00
Sum Intensity	3086344.33
StDev Intensity	10.49
Signal/Background	85.00 : 1

Fig. S7 Fluorescence intensity profile statistics: Untreated control cells (B), L6 cells + sensor (H) and L6 cells + Al^{3+}+ sensor (N).

MTT assay for cell viability assessment. MTT assaywas conducted to evaluate whether the sensor itself induced any cytotoxicity in normal cells. Different concentrations of the sensor, ranging from 10µl through 60µl of stock solution (7×10^{-5}M), and/or solvent of same order, were added to the cultured cells (10^6 cells/mL) in 96-well micro plates and incubated for 24h. A set of untreated control cellswere also kept which were devoid of any exposure to any concentration of sensors. MTT was added into each well at the end of incubation period. After 3h of incubation at 37ºC, dimethyl sulfoxide (DMSO) was added into each well. The absorbance of each well was then measured at 595nm using ELISA reader (Thermo scientific, Multiskan ELISA, USA).The percentagecell survivabilitywas calculated as: (mean experimental absorbance/mean control absorbance) × 100%. No significant difference was observed between the percentages of viable cells in the sensor-treated and the solvent-treated lots, indicating thereby that the sensor was non-cytotoxic and safe for biological use.

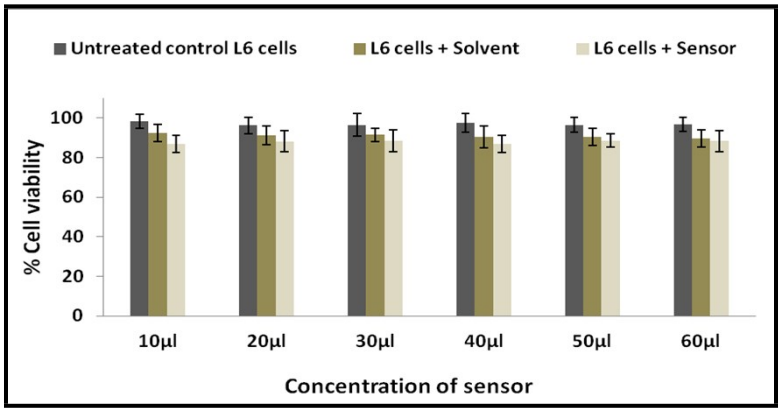

Fig. S8 Cellular cytotoxicity assessment of different concentrations of sensor **L** and solvent system in *L6* cell lines.

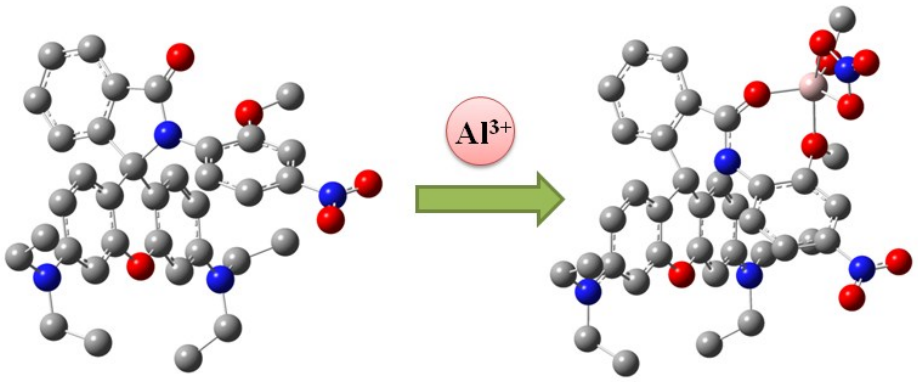

Fig. S9 Optimized structures of ligand **L** and **L**− Al^{3+} complexes

Table 1 Crystal Data and details of refinements for the sensor $\mathbf{L_3}$

Empirical Formula	$C_{35}\ H_{36}\ N_4\ 0_5$
Formula Weight	592.68
Crystal System	Triclinic
Space Group	P-1
a (Å)	9.376 (3)
b (Å)	12.051(4)
c (Å)	14.783(5)
α (°)	70.869(6)
β (°)	74.940(5)
γ (°)	78.883(6)
Density (g/cm^3)	1.301
Volume (Å^3)	1513.3(8)
Temperature, K	100 (2)
Z	2
F (000)	628.0
θ range (deg)	1.80 – 25.50
Collected Reflections	5511
Independent Reflections	1.046
Goodness-of-fit	1.046
R1 [I > 2.0 σ(I)]	0.0602
wR1 [I > 2.0 σ(I)]	0.1732

References:

1 T. Karstens and K. Kobs, *J. Phys. Chem*., 1980, **84**, 1871−1872.

2 G. G. Guilbault, *Practical Fluorescence*, 2nd ed.; Marcel Dekker, New York, 1973, p 11.

3 *International Tables for X-Ray Crystallography*, Kynoch Press, Vol. III, Birmingham, England, 1952.

4 *SAINT+*, *version 6.02*; Bruker AXS: Madison, WI, 1999.

5 G. M. Sheldrick, *SADABS*, *Empirical Absorption Correction Program*, University of Gottingen: Gottingen, Germany, 1997.

6 *XPREP*, *5.1 ed*.; Siemens Industrial Automation Inc.: Madison, WI, 1995.