

A Coumarin-Derived Fluorescence Probe Selective for Magnesium

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S1. Experimental section

S1.1 General experimental information.

All reagent-grade chemicals were used without purification unless otherwise specified. Perchlorate salts of all metal ions 4-(diethylamino)salicylaldehyde, diethylmalonate and nitroethylacetate were purchased from Aldrich. 80% hydrazine hydrate, anhydrous stannous chlorides were obtained from S. D. Fine Chemicals (India). Solvents were received from S. D. Fine Chemicals (India) and they were purified prior to use.

Compounds **A**, **B**, **C**, **L₁**, **L₂** and **L** were characterized by elemental analyses, ¹H-NMR, ¹³C-NMR and mass (positive ion) spectroscopy. ¹H-NMR and ¹³C-NMR spectra were recorded on a JEOL JNM-LA400 FT (400 MHz and 100 MHz respectively) instrument in CDCl₃. FAB mass (positive ion) data were obtained from a JEOL SX 102 /DA-6000 mass spectrometer using argon as the FAB gas at 6 kV and 10 mA with an accelerating voltage of 10 kV and the spectra were recorded at 298 K. ESI mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. The ESI capillary was set at 3.5 kV and the cone voltage was 40V. Melting points were determined with an electrical melting point apparatus by PERFIT, India and were uncorrected. UV-visible spectra were recorded on a JASCO V-570 spectrophotometer at 293 K and the average of three measurements were taken. The deviations in molar absorption coefficients were in the last digit only. Steady-state fluorescence spectra were obtained using a Perkin-Elmer LS 50B Luminescence Spectrometer at 293 K with

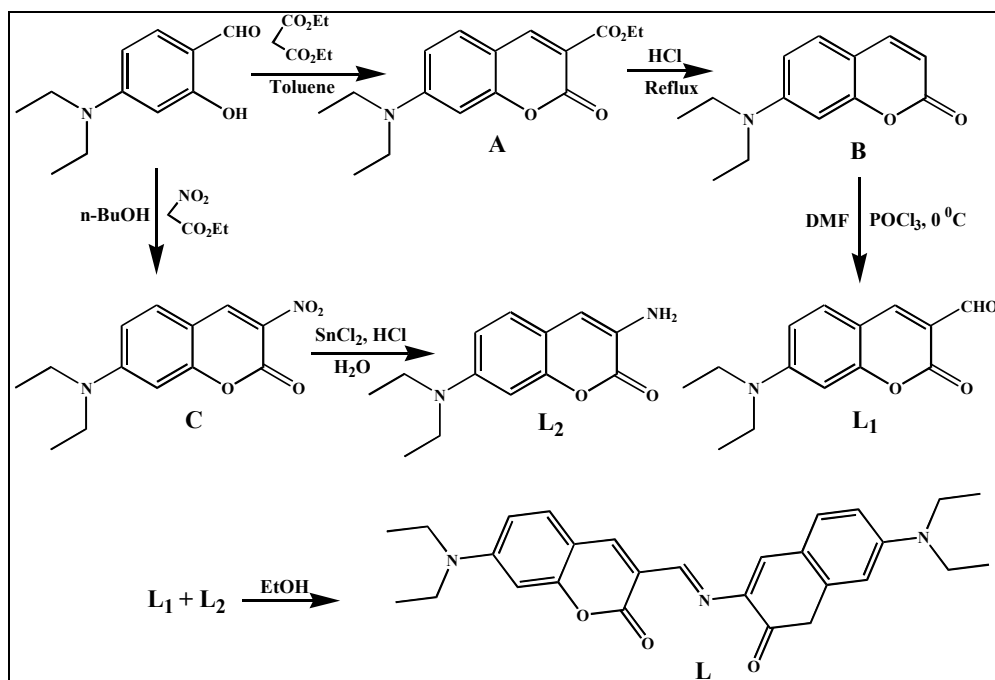
excitation and emission band-pass 5 nm. Fluorescence quantum yields in each case were determined by comparing the corrected spectrum with that standard Fluorescein¹ ($\phi=0.85$ in 0.1(N) NaOH taking the area under the total emission. The fluorescence measurements in solutions were carried out at $\sim 10^{-6}$ M concentration unless otherwise specified. The complex stability constant K_s were determined² from the change in absorbance or fluorescence intensity resulting from the titration of dilute solutions ($\sim 10^{-5}$ – 10^{-6} M) of **L** against metal ion concentration. The reported values gave good correlation coefficients (≥ 0.99).

References

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2. (a) Fery-Forgues, S.; Le Bries. M.-T.; Guetté, J.-P.; Valeur, B. *J. Phys. Chem.* **1988**, 92, 6233. (b) Bag, B.; Bharadwaj, P. K. *J. Phys. Chem. B* **2005**, 109, 4377.

S1.2 Synthesis and Characterization of A, B, C, L₁, L₂ and L

S1.2.1 Synthetic Scheme



S1.2.2 Experimental Procedure:

7-diethylamino-2-oxo-2H-chromene-3-carboxylic acid ethyl ester (A): A mixture of 4-diethylamino salicylaldehyde (2.31g, 0.012 mmol), diethylmalonate (2 ml, 0.013 mmol), and a catalytic amount of piperidinium acetate in toluene (20 ml) was refluxed in Dean-Stark trap for 24 h. The mixture was cooled, and the solvent was evaporated. The product was extracted with DCM, washed with brine, and purified by column using petroleum ether/ ethyl acetate (3:1) as the eluent. It was further purified by recrystallization from petroleumether/ethyl acetate to afford a yellow crystalline solid (2.56 g, 74% Y). Mp 135 °C. IR (cm⁻¹, KBr disk.): 1758, 1698 (C=O). ¹H-NMR (400 MHz, CDCl₃): 1.21 (t, *J* = 7.1 Hz, 6H, CH₃), 1.36 (t, *J* = 7.1Hz, 3H, CH₃), 3.42 (q, *J* = 7.1 Hz, 4H, CH₂), 4.36 (q, *J* = 7.1

Hz, 4H, CH₂), 6.50 (s, 1H_{ar}), 6.66 (dd, $J = 8.9, 2.3$ Hz, 1H_{ar}), 7.35(d, $J = 8.8$ Hz, 1H_{ar}), 8.40 (s, 1H_{ar}). ¹³C -NMR (100MHz, CDCl₃): 12.4, 14.4, 45.1, 61.1, 96.8, 107.76, 109.1, 109.6, 130.9, 149.1, 152.8, 158.2, 164.2. Anal. Calcd for C₁₆H₁₉NO₄: C, 66.42; H, 6.62; N, 4.84 %. Found: C, 66.29; H, 6.57; N, 4.93%.

7-diethylamino-chromene-2-one (B): 1 g of 7-diethylamino-coumaric-3-carboxylic acid ethyl ester in 60 ml of 18% strength hydrochloric acid are heated for 5 h to the boil under reflux. After cooling, saturated sodium acetate solution are added and the pH value is adjusted to 4-5 with 45% strength sodium hydroxide, whilst cooling. The crystalline precipitate was filtered off, thoroughly washed with water and dried in vacuum at 50 °C, which affords a light yellow solid (0.6 g, 80% Y). Mp 128 °C. IR (cm⁻¹, KBr disk.): 1703(C=O). ¹H-NMR (400 MHz, CDCl₃): 1.19 (t, $J = 7.1$ Hz, 6H, CH₃), 3.4 (q, $J = 7.1$ Hz, 4H, CH₂), 6.08 (d, $J = 9.2$ Hz, 1H_{ar}), 6.61(s, 1H_{ar}), 6.74 (bs, 1H_{ar}), 7.28 (d, $J = 8.8$ Hz, 1H_{ar}), 7.54 (d, $J = 9.5$ Hz, 1H_{ar}), ¹³C-NMR (100MHz, CDCl₃): 12.39, 44.95, 97.88, 108.89, 109.46, 128.74, 143.56, 150.52, 156.73, 161.01, 162.13. Anal. Calcd for C₁₃H₁₅NO₂: C, 71.87; H, 6.96; N, 6.45 %. Found: C, 71.69; H, 7.07; N, 6.57%.

7-diethylamino-2-oxo-2H-chromene-3-carboxyldehyde (L₁): 4 ml of dry DMF are added dropwise to 0.4 ml of POCl₃ at 20-50 °C. The mixture is stirred for 45 min at 50 °C under N₂ atmosphere. A suspension of 0.65 g of 7-dimethylamino coumarine in 3 ml of dry DMF is then added, the mixture is warmed to 60 °C for 2 h and poured out onto ice water, and the whole is stirred for 2 h. The crystalline precipitate is filtered off, thoroughly washed with water and dried in vacuum at 50 °C, which affords an orange solid. (0.47 g, 66% Y), mp 135 °C. IR (cm⁻¹, KBr disk.): 1711, 1673 (C=O). ¹H-NMR (400 MHz, CDCl₃): 1.26 (t, $J = 7.2$ Hz, 6H, CH₃), 3.48 (q, $J = 7.1$ Hz, 4H, CH₂), 6.52 (s, 1H_{ar}), 6.67 (dd, $J = 9.0, 2.4$ Hz, 1H_{ar}), 7.42 (d, $J = 9.0$ Hz, 1H_{ar}), 8.26 (s, 1H_{ar}), 10.13 (s, 1H, CHO). ¹³C-NMR (100MHz, CDCl₃): 12.4, 45.3, 87.1, 97.2, 108.3, 110.2, 132.5, 145.3, 153.4, 158.9, 161.8, 187.9. Anal. Calcd for C₁₄H₁₅NO₃: C, 68.56; H, 6.16; N, 5.71 %. Found: C, 68.09; H, 6.21; N, 5.83%.

7-diethylamino-3-nitro-chromene-2-one (C): 1.4 g (7.24 mmol) of 4-diethylamino-salicylaldehyde, 0.80 ml (7.96 mmol) of ethylnitroacetate, 0.1 ml piperidine and 0.2 ml glacial acetic acid were taken in 20 ml of n-BuOH and the reaction mixture was refluxed for 12 h. Orange solids were formed while cooling. The solids were filtered and washed with n-BuOH (2 x 10 ml) and finally dried in vacuum, which affords an orange solid (1.7 g, 90% Y). Mp 170 °C. IR (cm⁻¹, KBr disk.): 1745 (C=O). ¹H-NMR (400 MHz, CDCl₃): 1.25 (t, *J* = 7.2 Hz, 6H, CH₃), 3.47 (q, *J* = 7.1 Hz, 4H, CH₂), 6.46 (s, 1H_{ar}), 6.68 (dd, *J* = 9.2, 2.6 Hz, 1H_{ar}), 7.42 (d, *J* = 9.0 Hz, 1H_{ar}), 8.70 (s, 1H_{ar}). ¹³C-NMR (100MHz, CDCl₃): 12.4, 45.5, 96.9, 106.2, 111.1, 132.5, 138.1, 143.2, 154.5, 156.5, 158.8. Anal. Calcd for C₁₃H₁₄N₂O₄: C, 59.54; H, 5.38; N, 10.68 %. Found: C, 59.29; H, 5.44; N, 10.93%.

3-Amino-7-diethylamino-chromene-2-one (L₂): In a 100 ml round-bottomed flask SnCl₂·2H₂O (10 mmol) and 20 ml 15% HCl were taken. To it compound L₄ (1 g, 4.3mmol) was added portion wise and the solution was stirred at rt for 6 h. Then 5M NaOH solutions were added to neutralize the excess acid. The aqueous phase was extracted with ethyl acetate (3 x 30 ml). The organic layer was dried with anhy. Na₂SO₄ and evaporated to dryness. A yellow semi solid came, which was directly used for the next step without any characterization.

Compound L: 0.21 g (0.85 mmol) of L₁ and L₂ (0.2 g, 0.86 mmol) were combined in dry ethanol (25 ml). The solution was stirred under N₂ atm. for 10 h. A red precipitate was formed and it was filtered, washed with absolute ethanol (3 x 20 ml). Finally it was dried under vacuum to afford a red solid (0.37 g, 95% Y). Mp 250 °C. IR (cm⁻¹, KBr disk.): 1709 (C=O), 1619 (C=N) ¹H-NMR (500 MHz, CDCl₃): 1.15 (t, *J* = 7.1 Hz, 6H, CH₃), 1.23 (t, *J* = 7.1 Hz, 6H, CH₃), 3.35 (q, *J* = 7.1 Hz, 4H, CH₂), 3.45 (q, *J* = 7.1 Hz, 4H, CH₂), 6.46 (bs, 1H_{ar}), 6.49 (s, 1H_{ar}), 6.55 (s, H_{ar}), 6.60 (d, *J* = 9.0 Hz, 1H_{ar}), 6.68 (s, 1H_{ar}), 7.08 (d, *J* = 8.8 Hz, 1H_{ar}), 7.38 (d, *J* = 8.8 Hz, 1H_{ar}), 8.23 (s, 1H_{ar}), 10.10 (s, 1H, C=NH). ¹³C-NMR (100MHz, CDCl₃): 12.45, 12.49, 44.7, 44.9, 45.1, 45.3, 97.2, 97.4, 98.1, 98.2, 109.2, 109.4, 110.2, 125.9, 127.5, 130.9, 132.5, 145.3, 158.9, 187.9. ESI-MASS: m/z(rel. intensity): 460.2(100%), calculated 459.54. Anal. Calcd for C₂₇H₂₉N₃O₄: C, 70.57; H, 6.36; N, 9.14 %. Found: C, 77.31; H, 6.32; N, 9.03%.

S1.3 ^1H -NMR Spectrum of compound A, B, C, L₁, L₂ and L

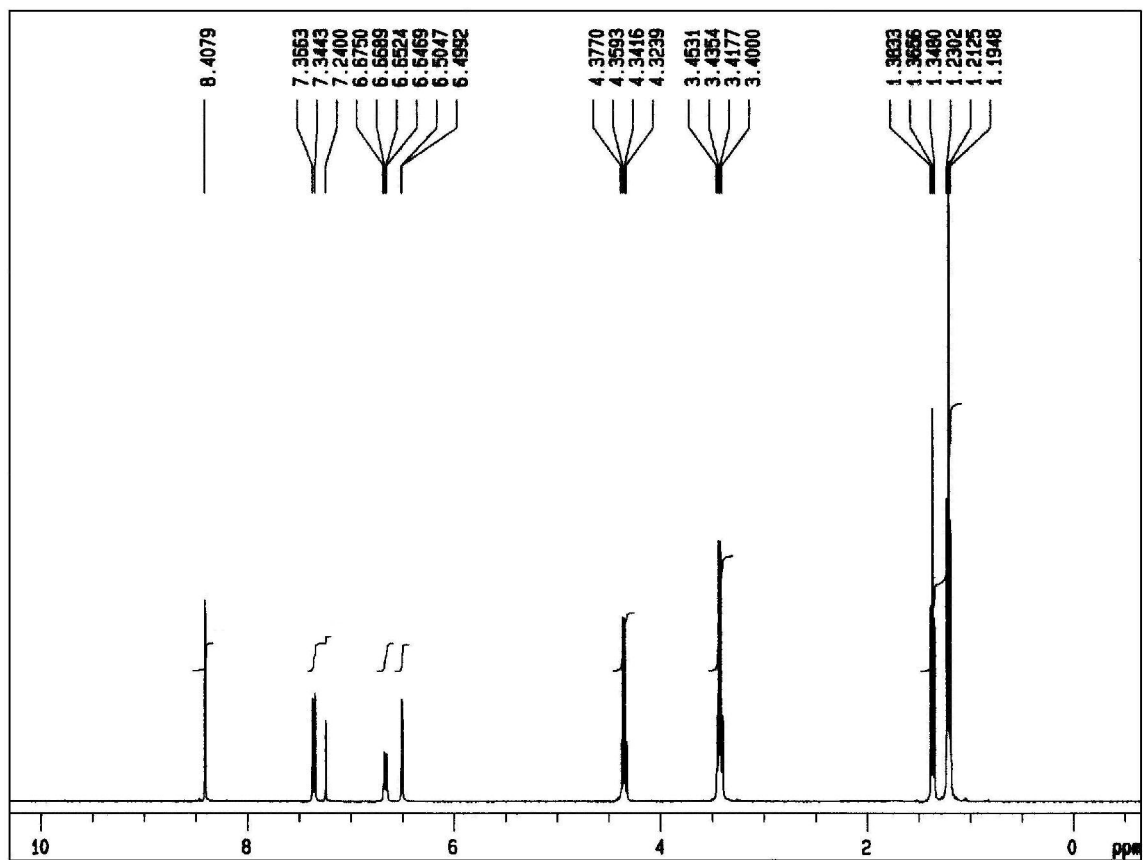


Fig S1.3.1 Compound A

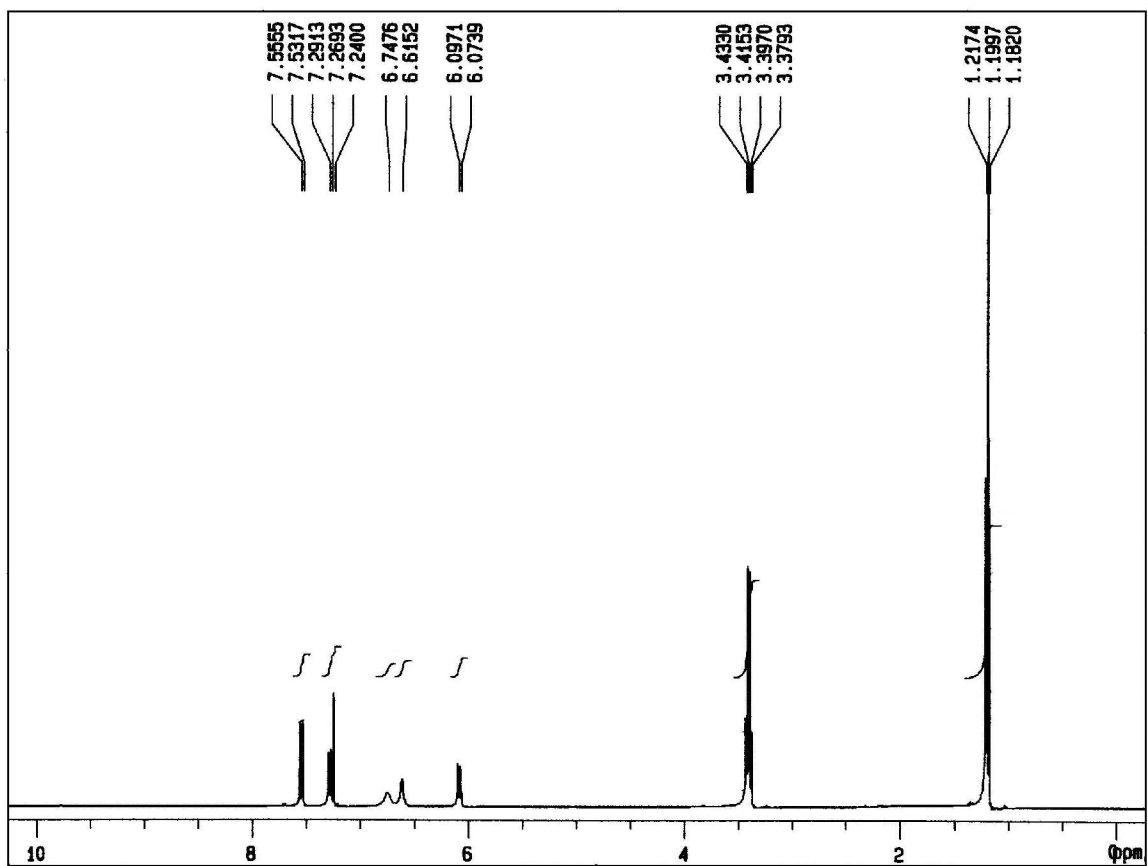


Fig S1.3.2 Compound **B**

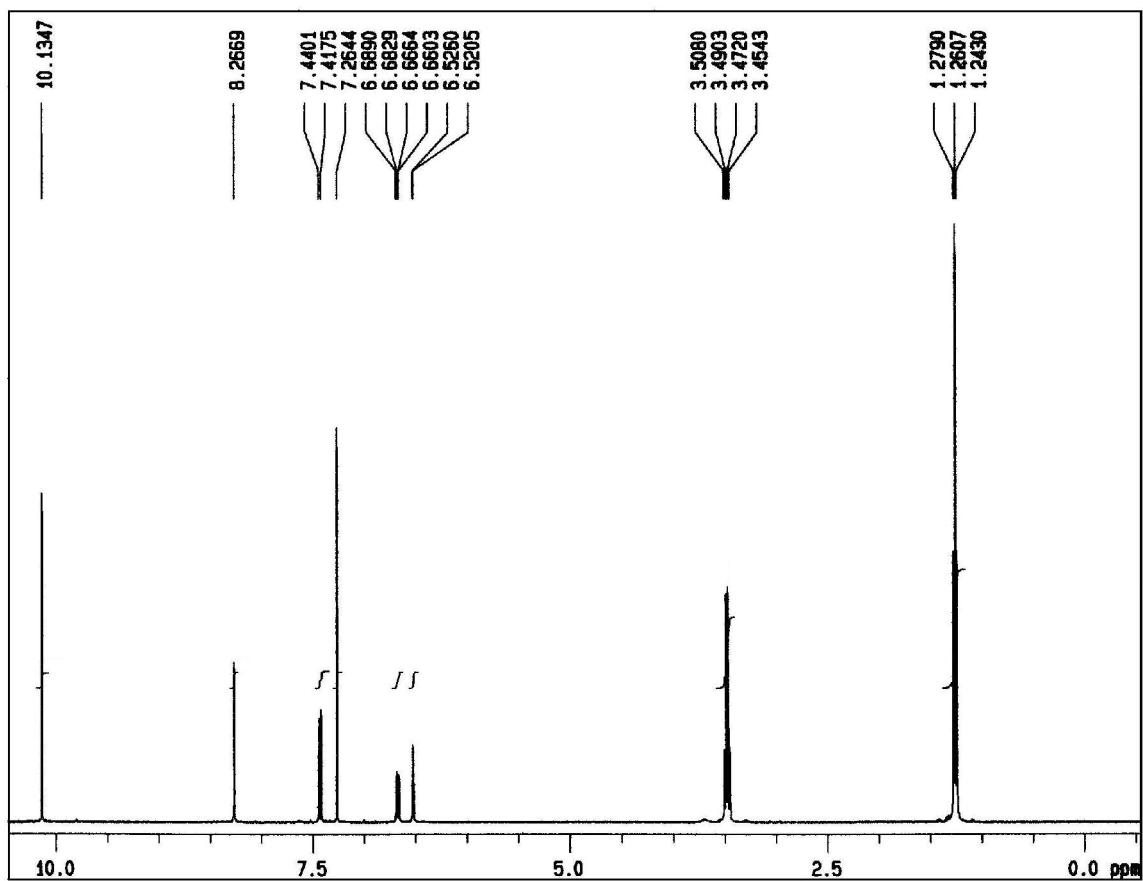


Fig S1.3.3 Compound L₁

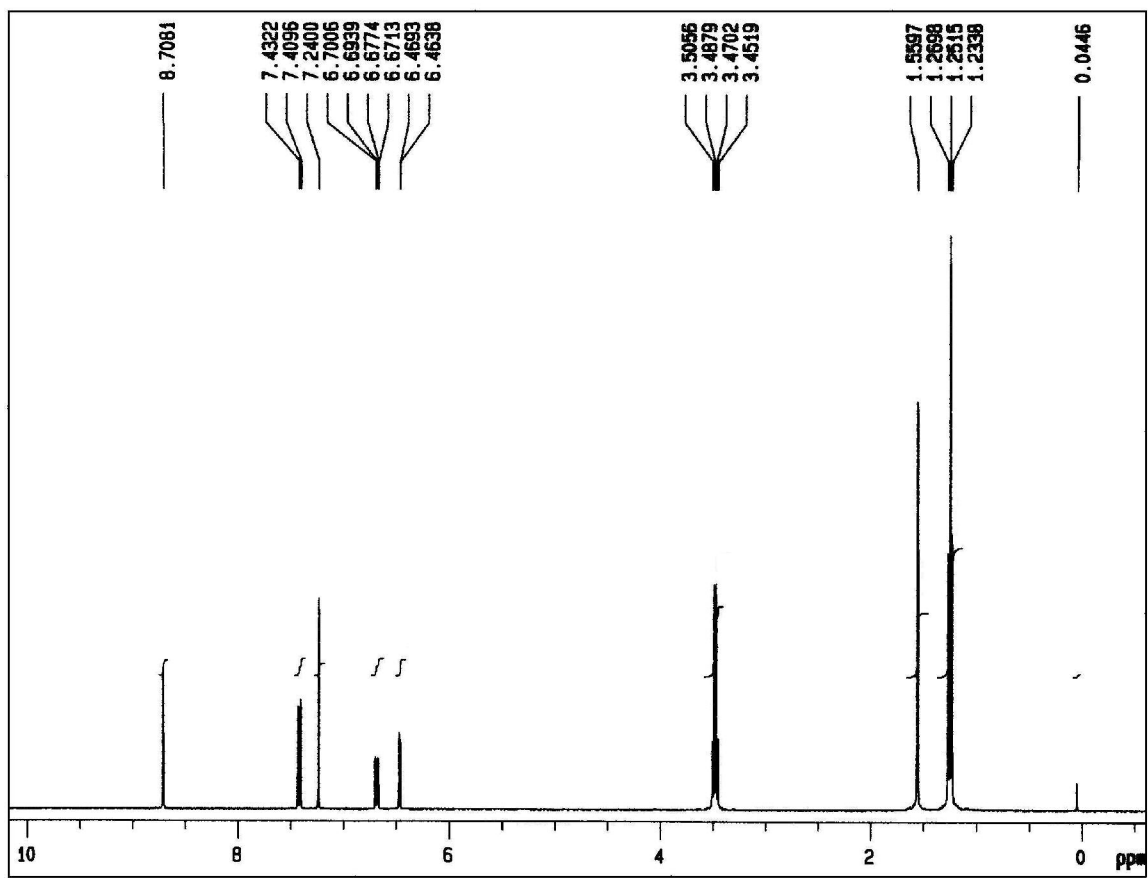


Fig S1.3.4 Compound C

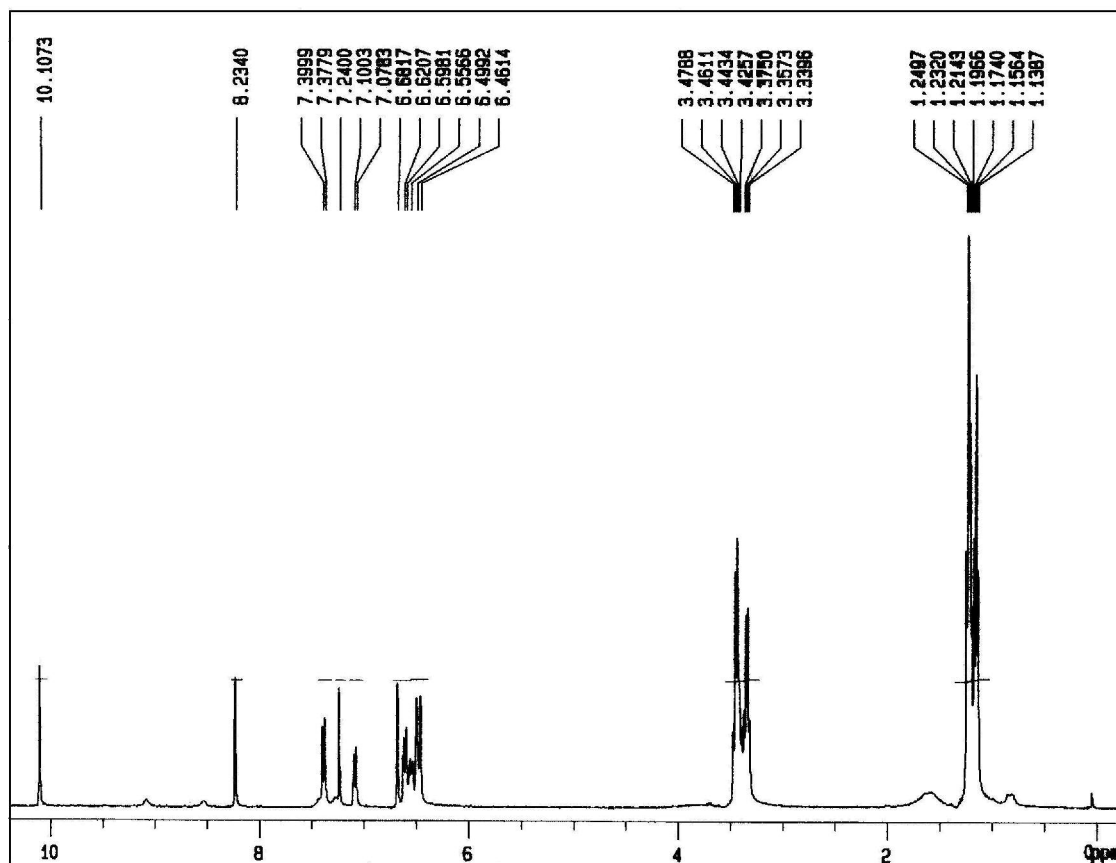


Fig S1.3.5 Compound L

S1.4 ^{13}C -Spectrum of compound A, B, C, L₁, L₂ and L

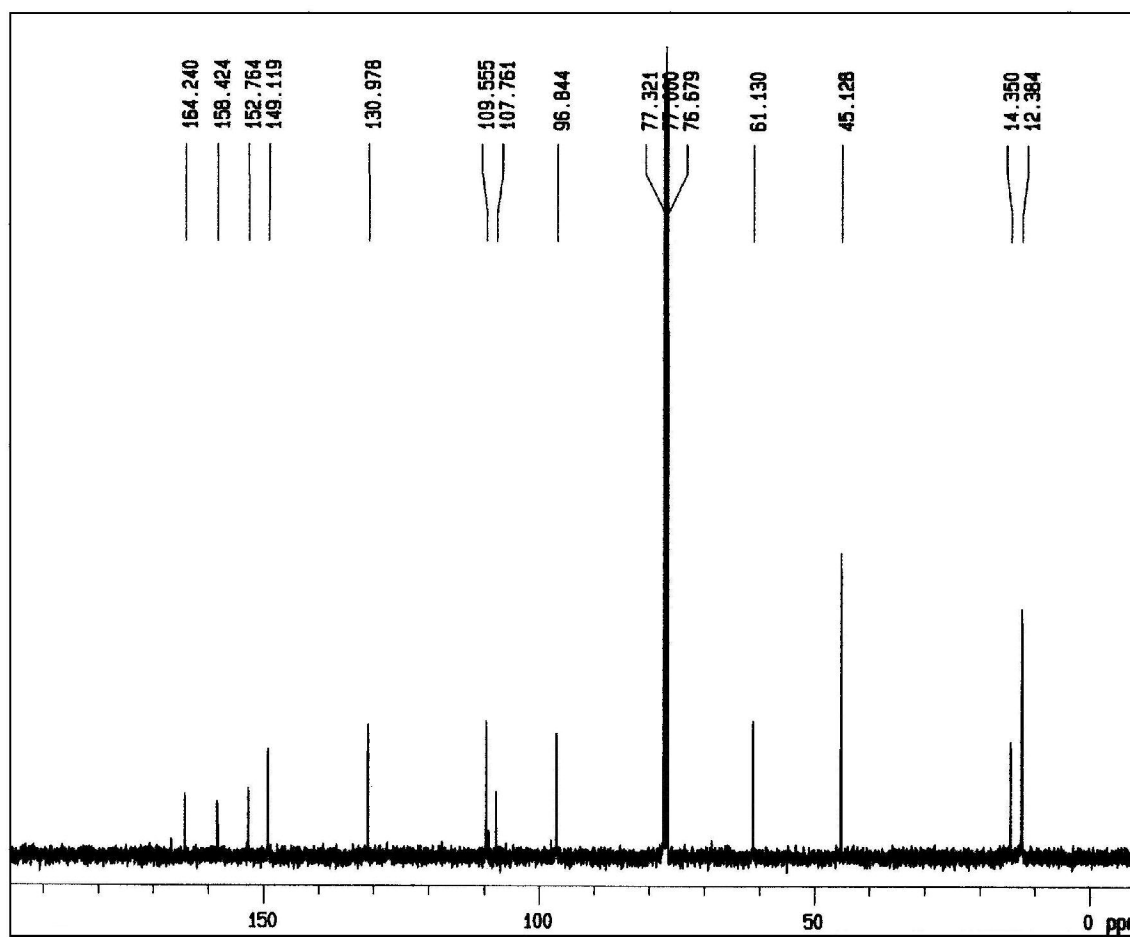


Fig S1.4.1 Compound A

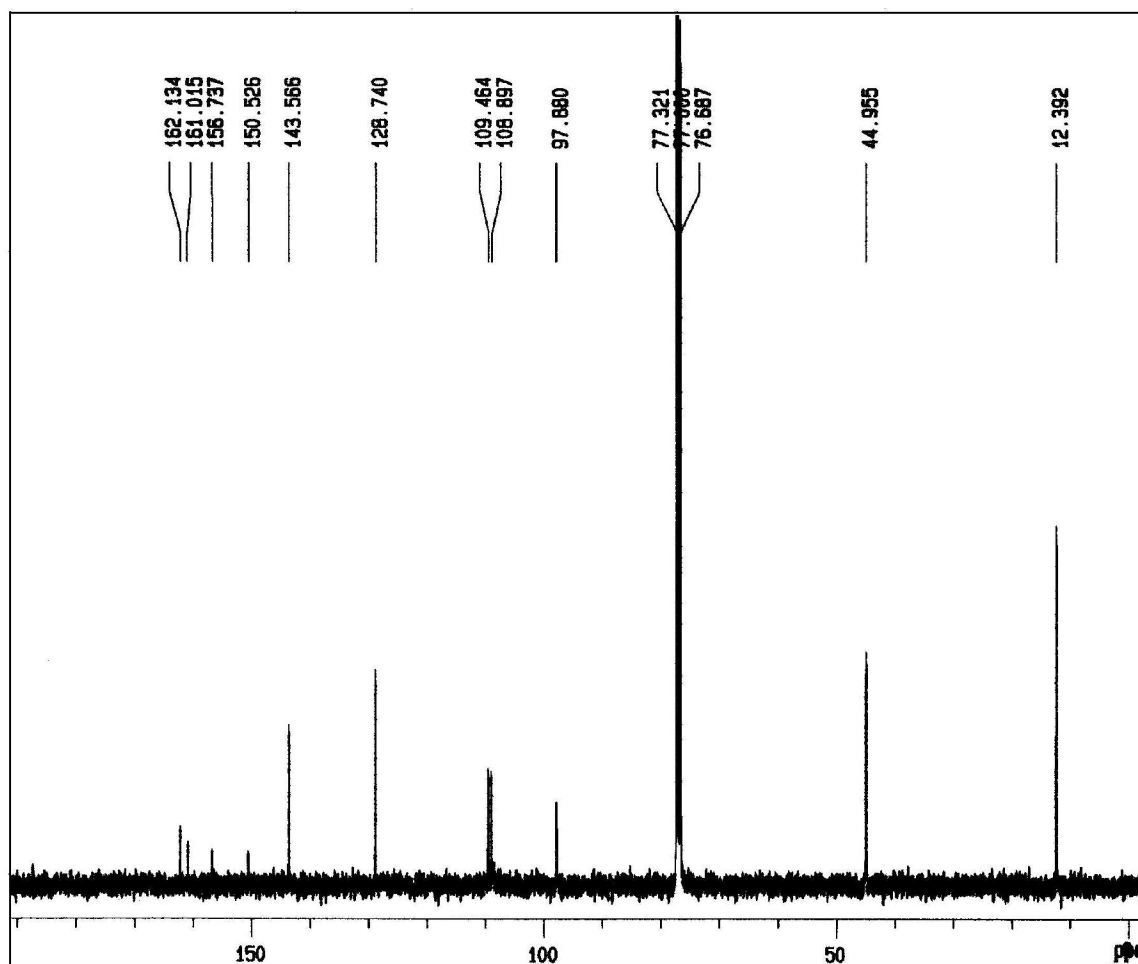


Fig S1.4.2 Compound **B**

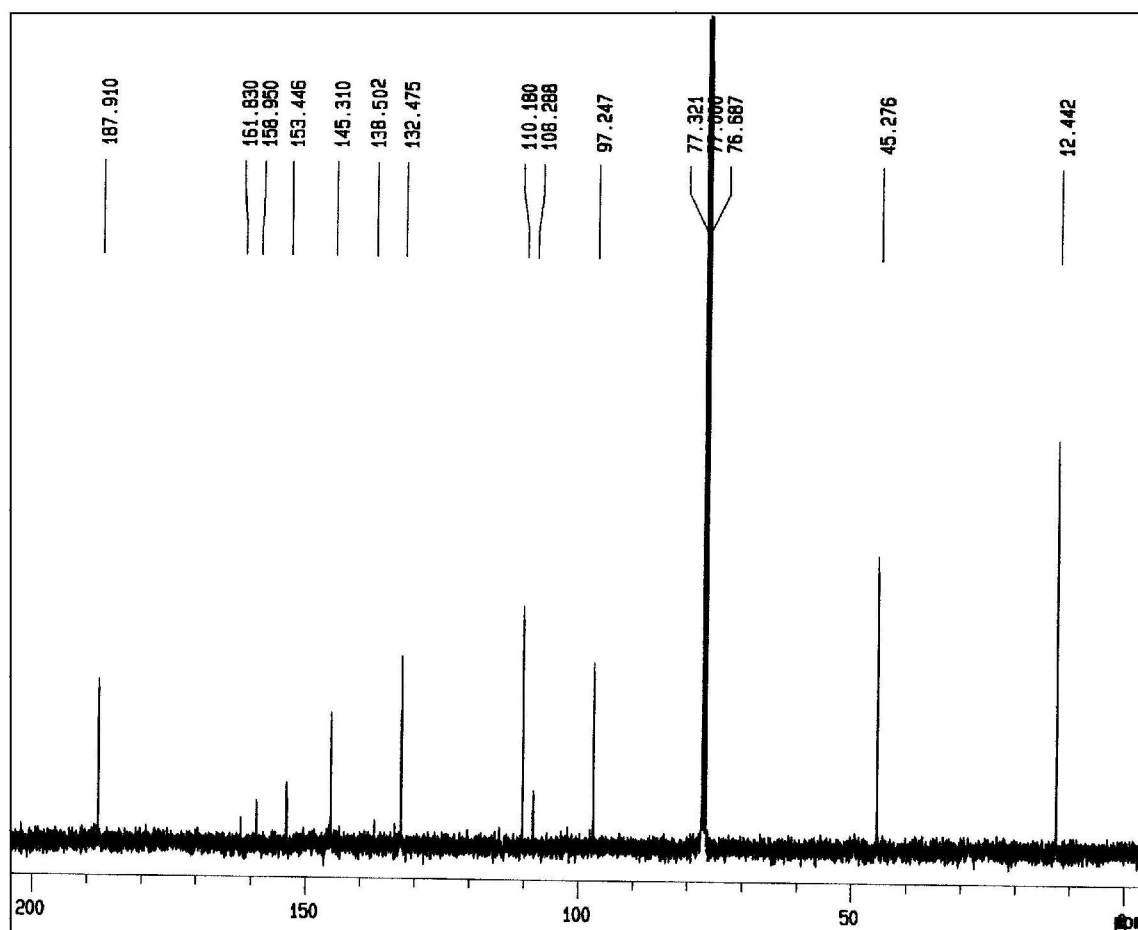


Fig S1.4.3 Compound L₁

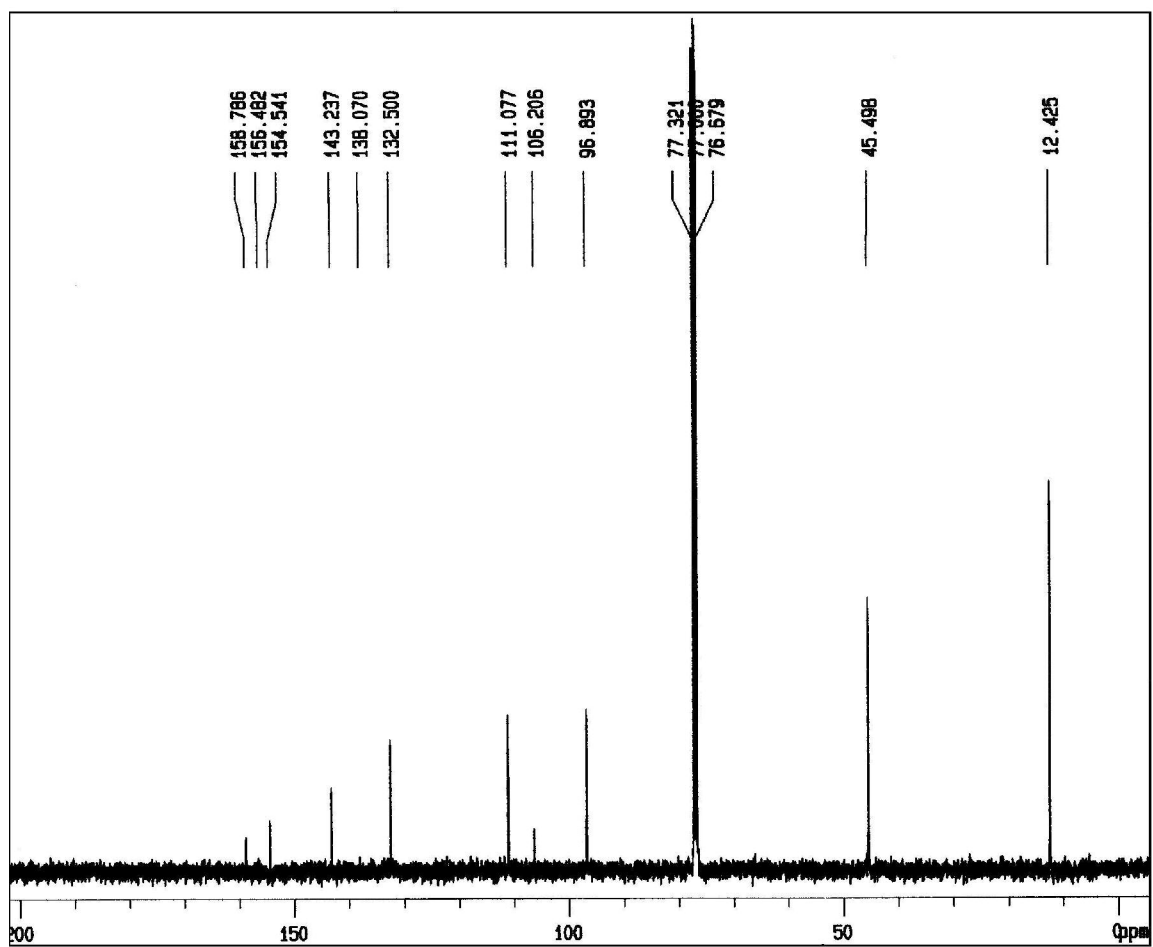


Fig S1.4.4 Compound C

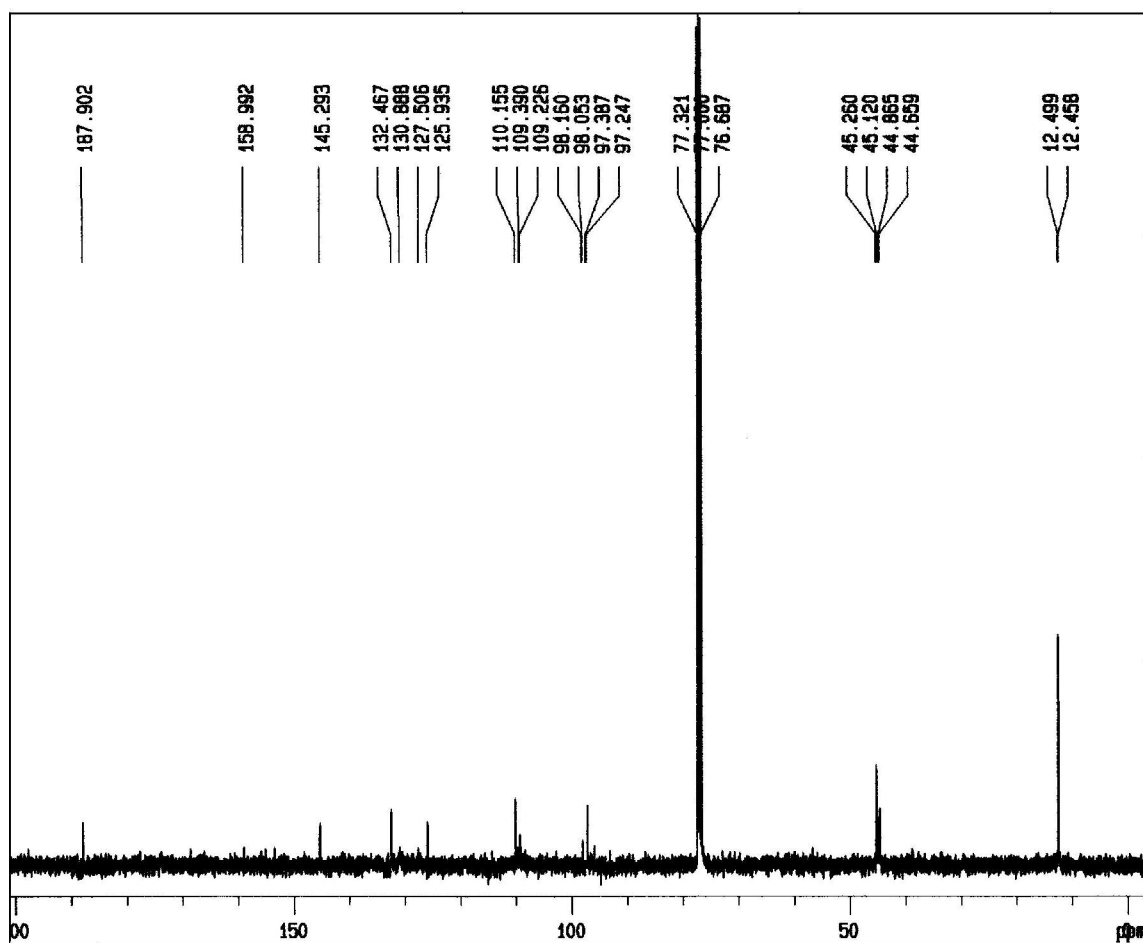


Fig S1.4.5 Compound L

S1.5 ESI-MS

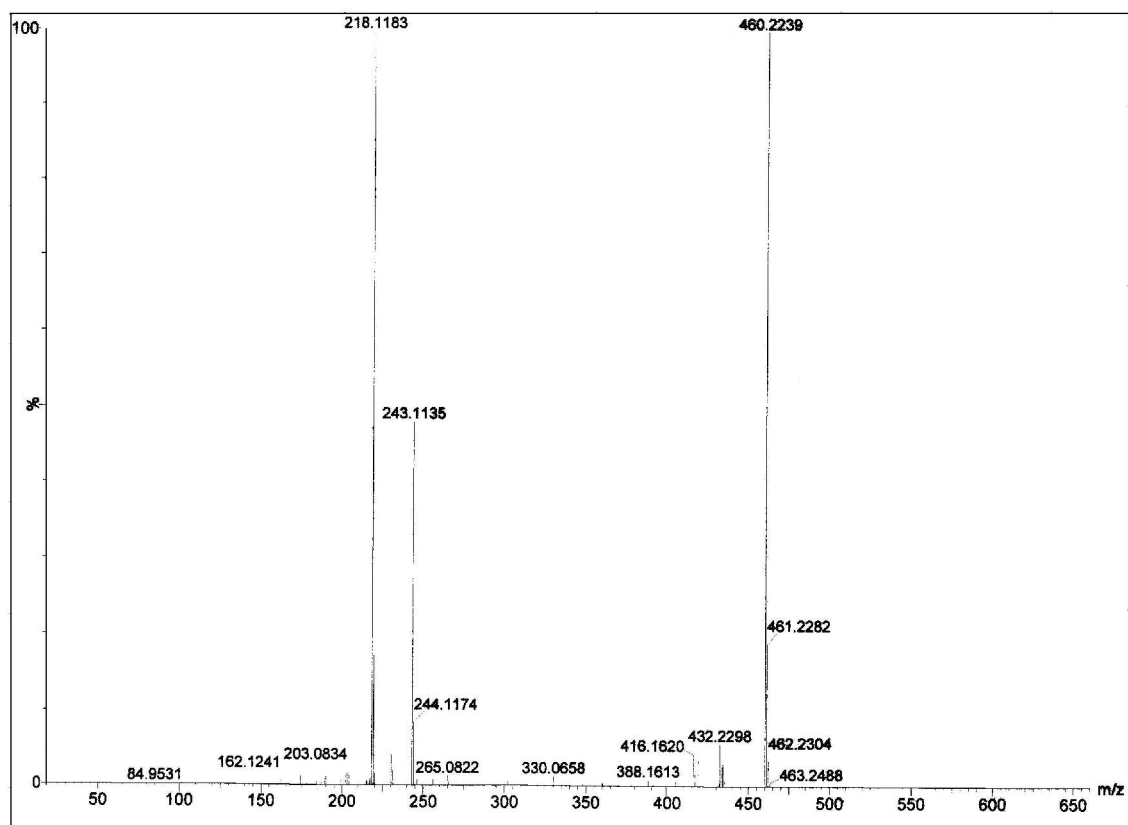


Fig S1.5.1 Compound L

S2 UV-Vis spectra.

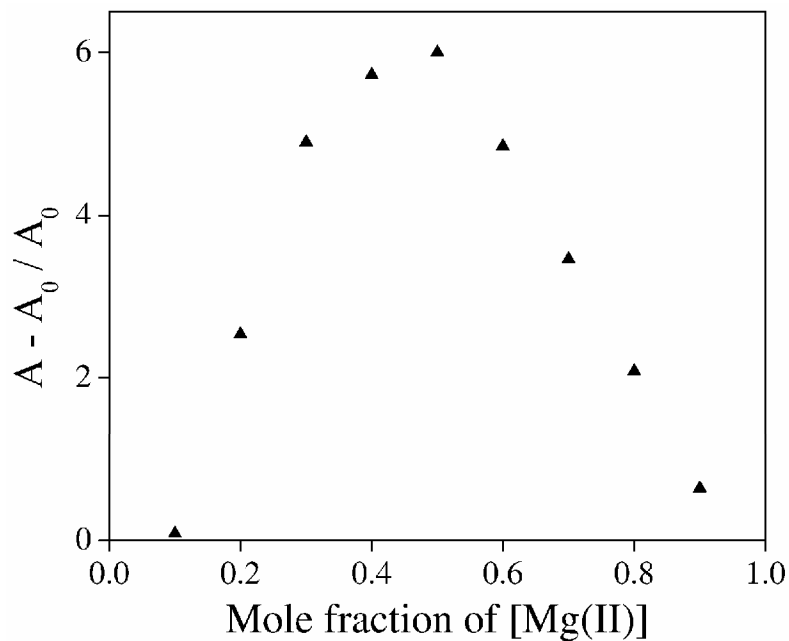


Fig S2.1 Job plot for determination of the stoichiometry of L-Mg(II).

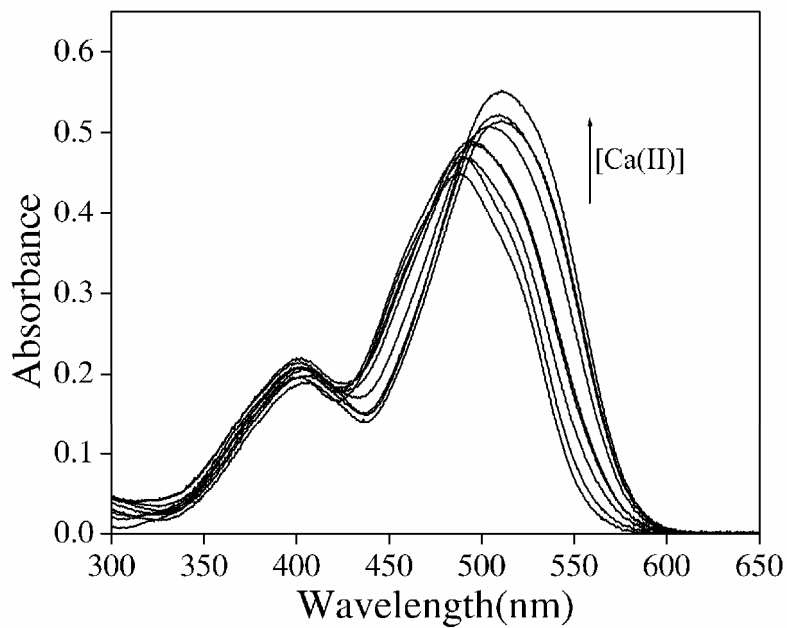


Fig S2.2 Absorption spectra of L (0.01mM) in MeCN at room temperature upon addition of increasing concentrations of Ca(II) ions (0-0.012mM).

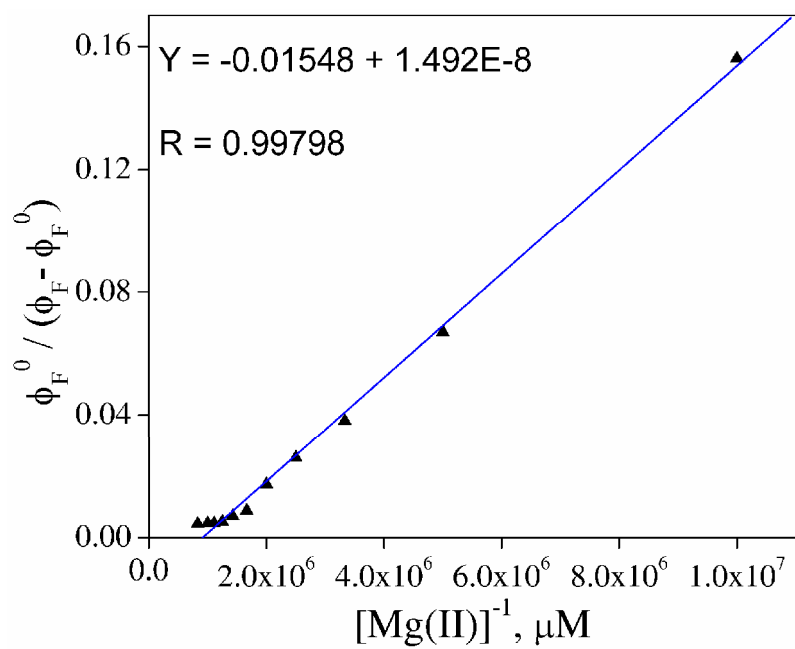


Fig S2.3 Linear regression plot of fluorescence titration of **L** with Mg(II) in MeCN.

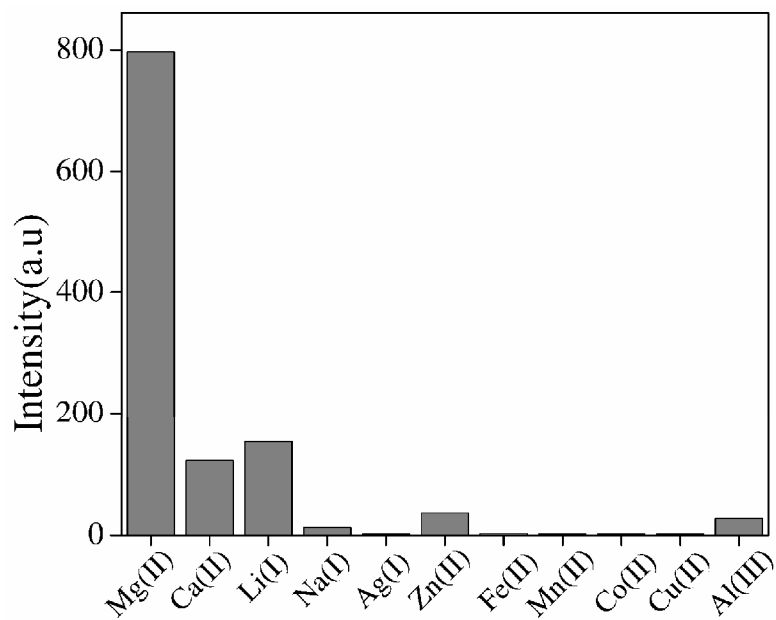


Fig S2.4 The emission response of compound **L** (0.8 μM) to metal solutions (20 μM).
Excitation was at 488 nm.

S3. Synthesis of magnesium complex, $\text{Mg}(\text{L})(\text{ClO}_4)_2$:

$\text{Mg}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.5 mmol) dissolved in 5 ml of dry MeCN was added to a solution of ligand in dry MeCN (5 ml) with stirring under a N_2 atmosphere. The resulting dark red solution was allowed to stir for 30 min at RT and then filtered. Dry diethyl ether was allowed to diffuse into the filtrate to obtain the desired complex in 50 % yield that was dried under vacuum. This complex was characterized by ESI-MS and elemental analysis. ESI-MS, m/z (%): 582.1509(100), Calculated : 583.1492(100).

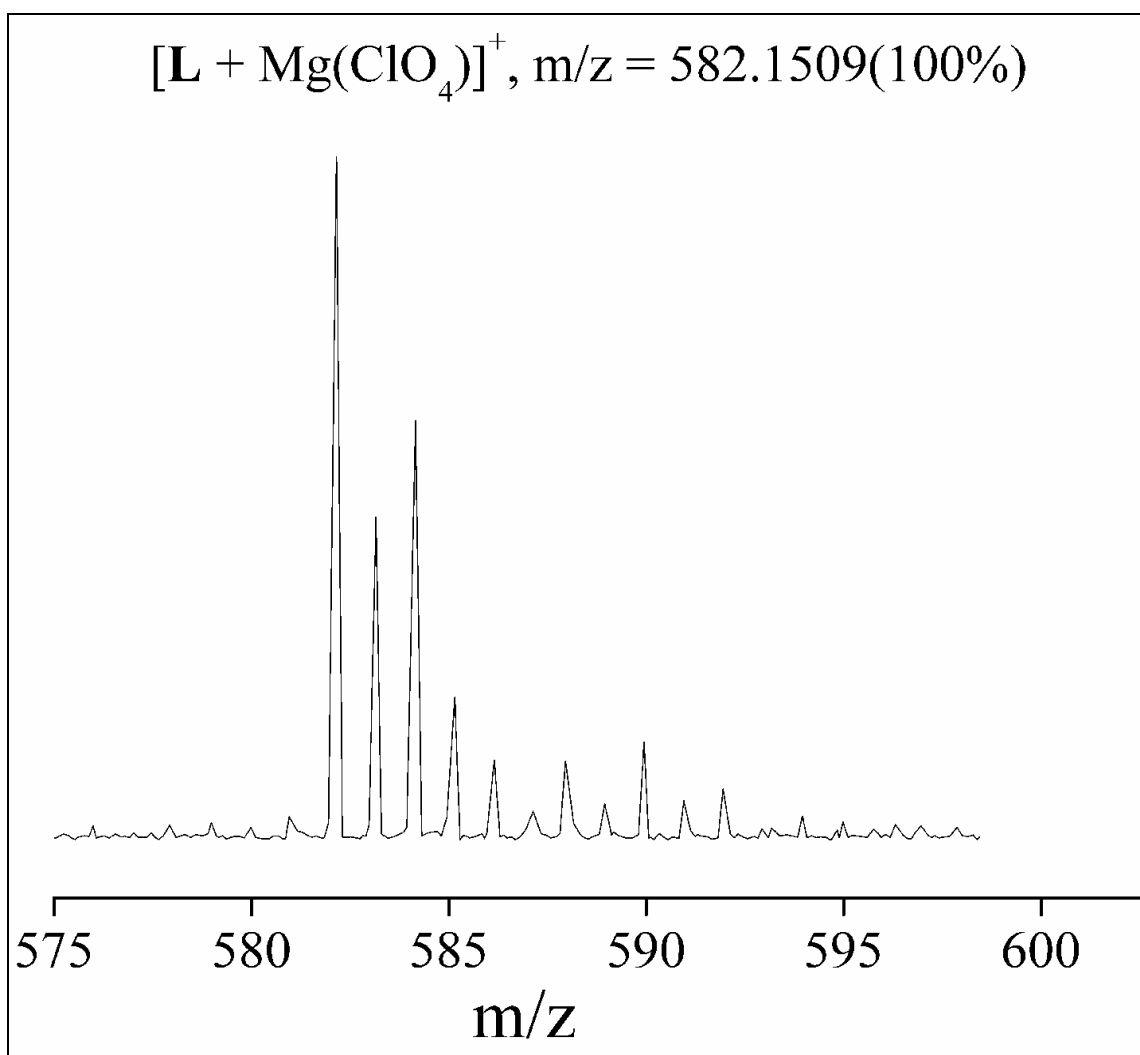


Fig S3.1 ESI-MS spectrum of $[\text{L} + \text{Mg}(\text{ClO}_4)]^+$