

## A novel di-triazole based peptide as a highly sensitive and selective fluorescent chemosensor for Zn<sup>2+</sup> ions

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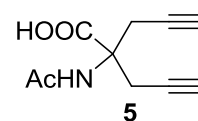
### General Experimental Methods

All the reactions were monitored by employing TLC technique using appropriate solvent system for development. Reactions involving air/oxygen sensitive reagents or catalysts were performed in degassed solvents. Transfer of moisture sensitive materials were carried out in a glove box, using standard syringe-septum techniques and the reactions were maintained under nitrogen atmosphere until the work up. Yields reported are isolated yields of the materials. All the commercial reagents were used as such without further purification. Infrared (IR) spectra were recorded on Nicolet Impact-400 FT IR spectrometer in KBr. Proton Nuclear Magnetic Resonance (400 MHz, <sup>1</sup>H NMR) spectra and Carbon Nuclear Magnetic Resonance (100.6 MHz, <sup>13</sup>C NMR) spectra were recorded on Varian spectrometers. The high-resolution mass measurements were carried out using JEOL JMS-DX 303 GC-MS instrument or Micro mass Q-ToF spectrometer. Melting points were recorded on Buchi B-545. The steady state absorption spectra have been recorded on a JASCO V530 spectrophotometer and steady state fluorescence spectra are recorded on a Varian Cary with Eclipse fluorescence spectrophotometer  $\lambda_{\text{ex}} = 260$  nm, with a band width of 5 nm. The spectral studies have been performed using 2  $\mu$ M of the ligand solution in acetonitrile, HPLC grade (Spectrochem, Mumbai, India). Before performing the fluorescence studies the purity of the solvent has been assured by exciting the sample in different spectral regions, where no fluorescence has been observed. Time resolved fluorescence studies have been performed in a time correlated single photon counting (TCSPC) system, from IBH, UK, with  $\lambda_{\text{ex}}=266$  nm. The full width at half maximum of the instrument response function is 840 ps. The PL decays are collected with emission polarizer at a magic angle 54.7° and is analyzed by using IBH DAS 8.2 software.

## Synthesis and characterization of compounds **5**, **6** and **7**

### Synthesis of Ac-Dprg-OH **5**

To a solution of ester **4** (1.2 g, 5.8 mmol) in MeOH (20 mL) was added 2N NaOH (696 mg, 17.4 mmol) and the reaction mixture was stirred at rt for 12 h. The reaction mixture was concentrated, diluted with water (20 mL), then

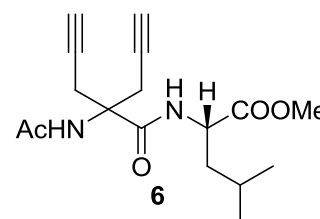


acidified with 1N HCl and extracted with ethyl acetate. Evaporation of the solvent gave **39** (1.0 g, 95%) as a white solid which was used in the next step without further purification.

R<sub>f</sub> : 0.25 (50% ethyl acetate:petroleum ether); m.p.: 138-140 °C; <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ = 1.96 (s, 3H), 2.38 (t, *J* = 2.68 Hz, 2H), 2.86-2.98 (m, 4H) ppm; <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 22.5, 25.3, 61.6, 72.9, 79.4, 172.9, 173.8; I.R. (KBr pellet): 1667.9, 1730.3, 2329.3, 3304.4, 3362.2 cm<sup>-1</sup>; HRMS (QTOF): Calcd. for C<sub>10</sub>H<sub>12</sub>NO<sub>3</sub> [M+H]<sup>+</sup> 194.0817, found at 194.0816.

### Synthesis of Ac-Dprg<sup>1</sup>-(L)Leu<sup>2</sup>-OMe **6**

To a solution of acid **5** (310 mg, 1.61 mmol) and HOBt (433 mg, 3.21 mmol) in dry THF (10 mL) was added DCC (397 mg, 1.93 mmol) at 0 °C. Then, H-Leu-OMe.HCl (350 mg, 1.93 mmol) and NMM (130 mg, 1.30 mmol, reaction mixture should have

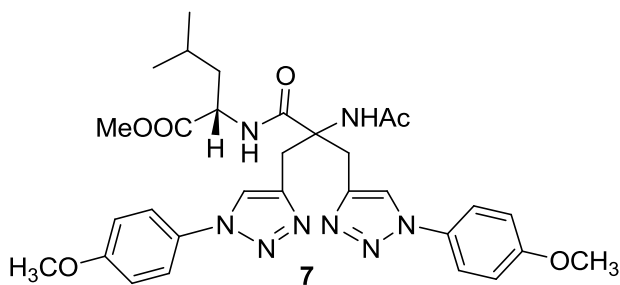


around pH= 9) in THF (10 mL) solution was added. The reaction mixture was stirred at rt for 24 h. The solvent was evaporated and the residue was diluted with water. The aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layer was washed with water, brine and dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent gave the crude product, which was purified by column chromatography (30% EtOAc/petroleum ether) to give the dipeptide **6** (440 mg, 86%) as a white solid.

R<sub>f</sub> : 0.29 (50% ethyl acetate/ petroleum ether); m.p.: 137-139 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 0.93-0.96 (m, 6H), 1.64-1.68 (m, 3H), 2.07 (s, 3H), 2.10-2.15 (m, 2H), 3.01-3.20 (m, 4H), 3.74 (s, 3H), 4.59-4.65 (m, 1H), 6.55 (s, 1H), 7.15 (d, *J* = 7.8 Hz, 1H) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 22.1, 22.9, 24.1, 24.9, 25.1, 41.7, 51.6, 52.5, 60.8, 72.5, 72.7, 79.0, 170.2, 170.5, 173; I.R. (KBr pellet): 1670.1, 1741.4, 2304.9 cm<sup>-1</sup>; HRMS (QTOF): Calcd. for C<sub>17</sub>H<sub>25</sub>N<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup> 321.1814, found at 321.1802; [α]<sub>D</sub><sup>25</sup>: - 4.76(c = 0.51, CHCl<sub>3</sub>).

### Synthesis of di-trizole based peptide **7**

The alkyne precursor **6** (30 mg, 0.09 mmol) was dissolved in <sup>t</sup>BuOH/H<sub>2</sub>O (5:5 mL) and the *p*-methoxyphenyl azide (27.9 mg, 0.19 mmol), Cu(OAc)<sub>2</sub> (3.7 mg, 0.02 mmol) and sodium ascorbate (7.4 mg, 0.04 mmol) were added. The



resulting mixture was stirred at rt for 24 h, until TLC indicated completion of reaction. The mixture was diluted with ethyl acetate and washed with aq NH<sub>4</sub>OH (0.2%) and brine. The aqueous phases were extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel with 100% ethyl acetate gave the desired peptide as white solid in 99% (57 mg) yield.

R<sub>f</sub>: 0.16 (100% ethyl acetate); m.p. 210-213 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 0.88-0.92 (m, 6H), 1.57-1.64 (m, 2H), 2.07 (bs, 1H), 2.08 (s, 3H), 3.40-3.68 (m, 4H), 3.69 (s, 3H), 3.87 (s, 6H), 4.41-4.43 (m, 1H), 7.00-7.02 (m, 4H), 7.35 (bs, 1H), 7.64-7.70 (m, 4H), 8.04 (s, 1H), 8.08 (s, 1H), 8.19 (d, *J* = 6.8 Hz, 1H) ppm; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 20.8, 21.9, 22.9, 24.6, 25.1, 30.6, 31.1, 40.9, 51.8, 52.3, 55.8, 64.0, 114.8, 114.9, 122.1, 122.1, 122.5, 122.7, 130.6, 130.7, 142.9, 142.4, 159.9, 159.9, 172.3, 172.4, 173.6; I.R. (KBr pellet): 1685.1, 1736.3, 2928.7, 3054.7 cm<sup>-1</sup>; HRMS (QTOF): Calcd. C<sub>31</sub>H<sub>39</sub>N<sub>8</sub>O<sub>6</sub> [M+H]<sup>+</sup> 619.2993, found: 619.2994; [α]<sub>D</sub><sup>25</sup>: - 7.494 (c = 0.17, CHCl<sub>3</sub>).

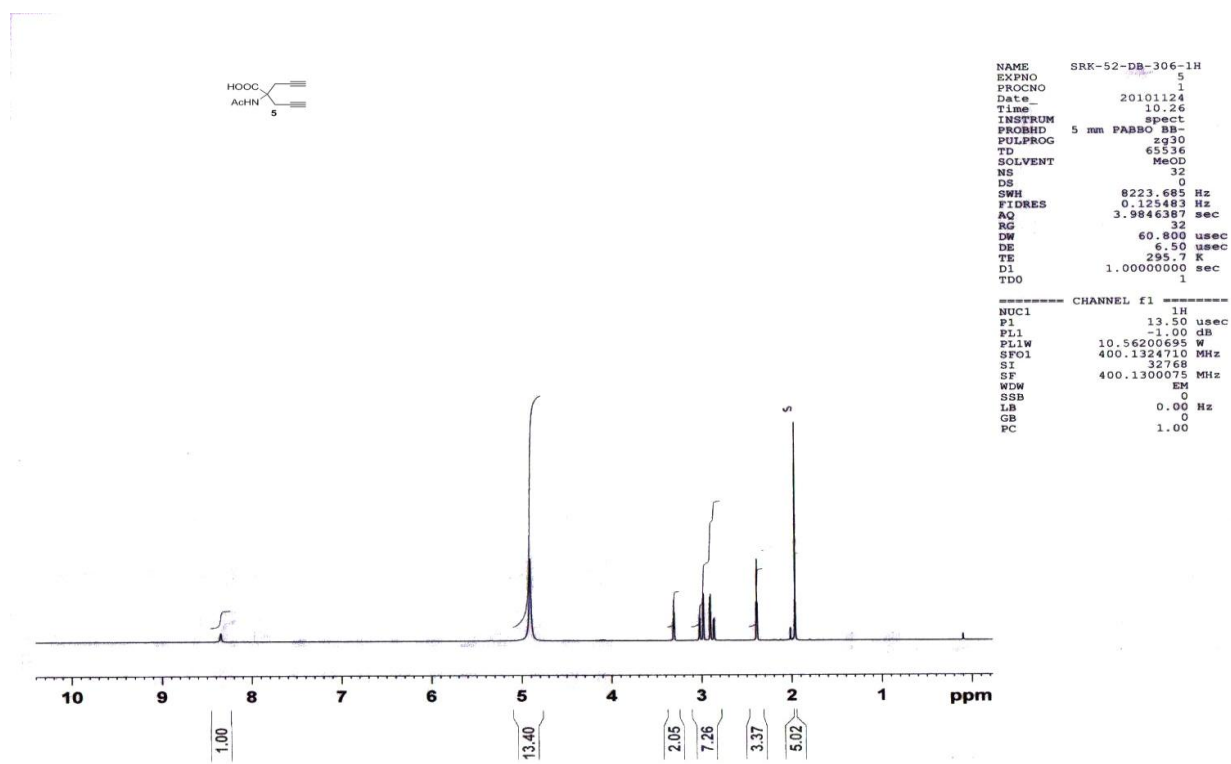


Fig. S1 <sup>1</sup>H NMR (CD<sub>3</sub>OD) of compound 5.

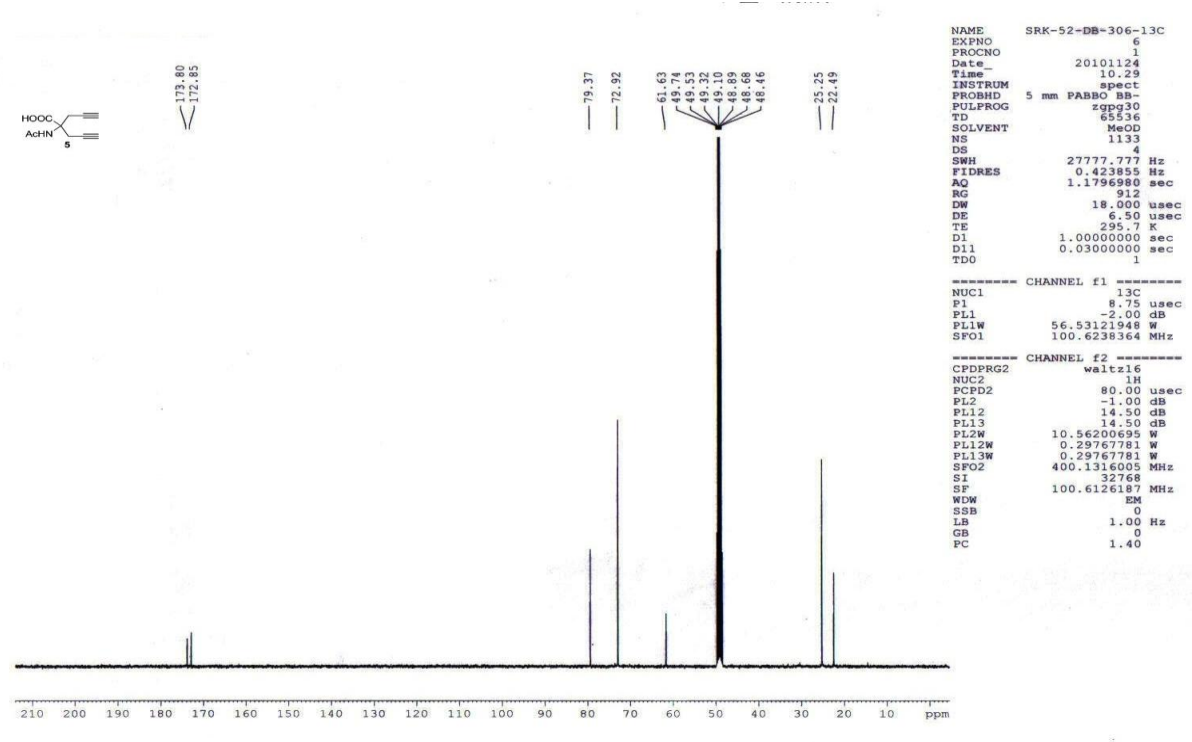


Fig. S2 <sup>13</sup>C NMR (CD<sub>3</sub>OD) of compound 5.

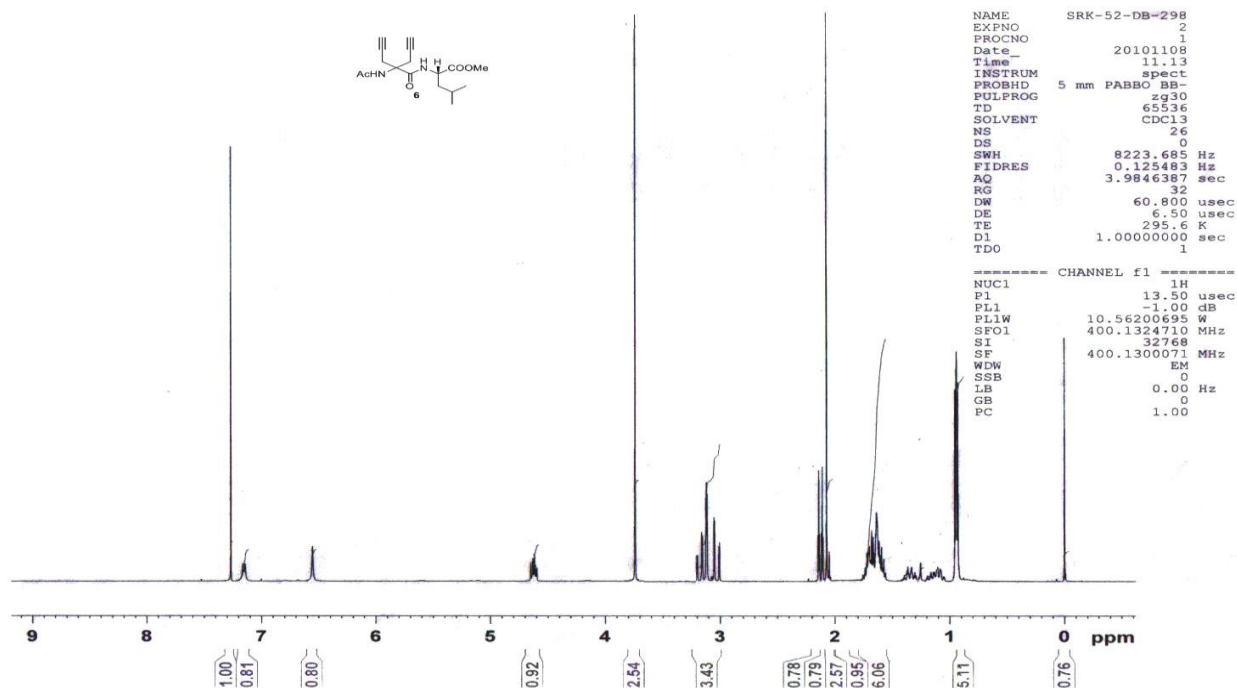


Fig. S3  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) of compound 6.

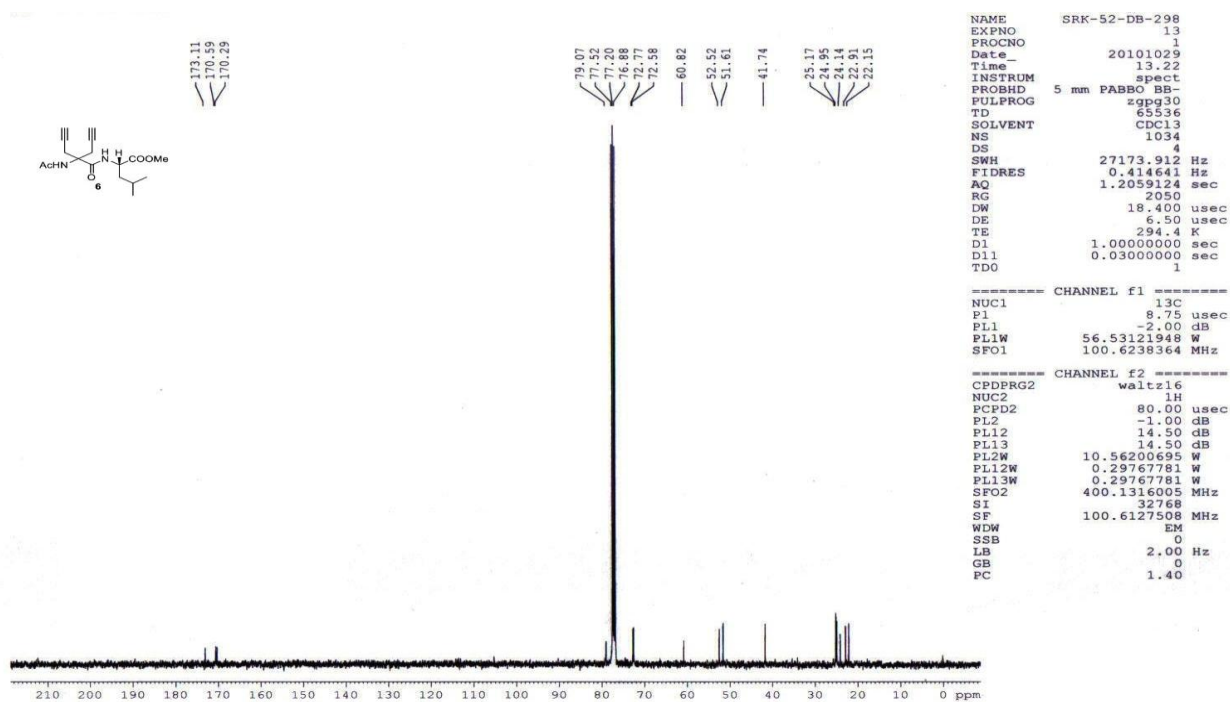


Fig. S4  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) of compound 6.

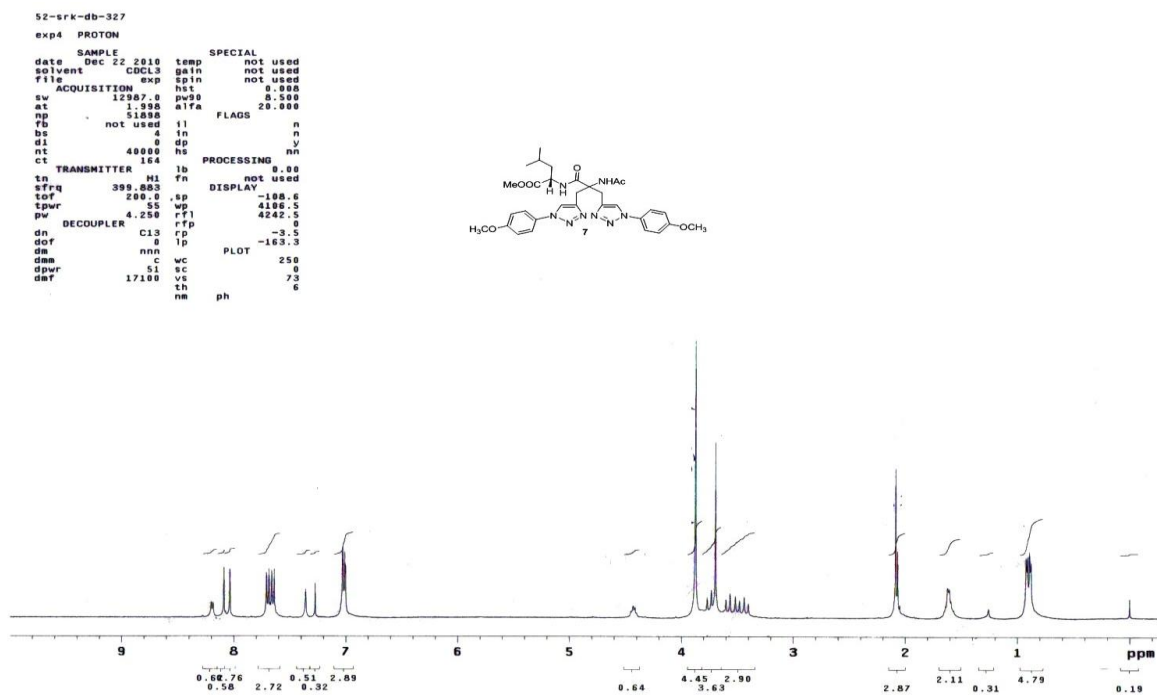


Fig. S5 <sup>1</sup>H NMR (CDCl<sub>3</sub>) of compound 7.

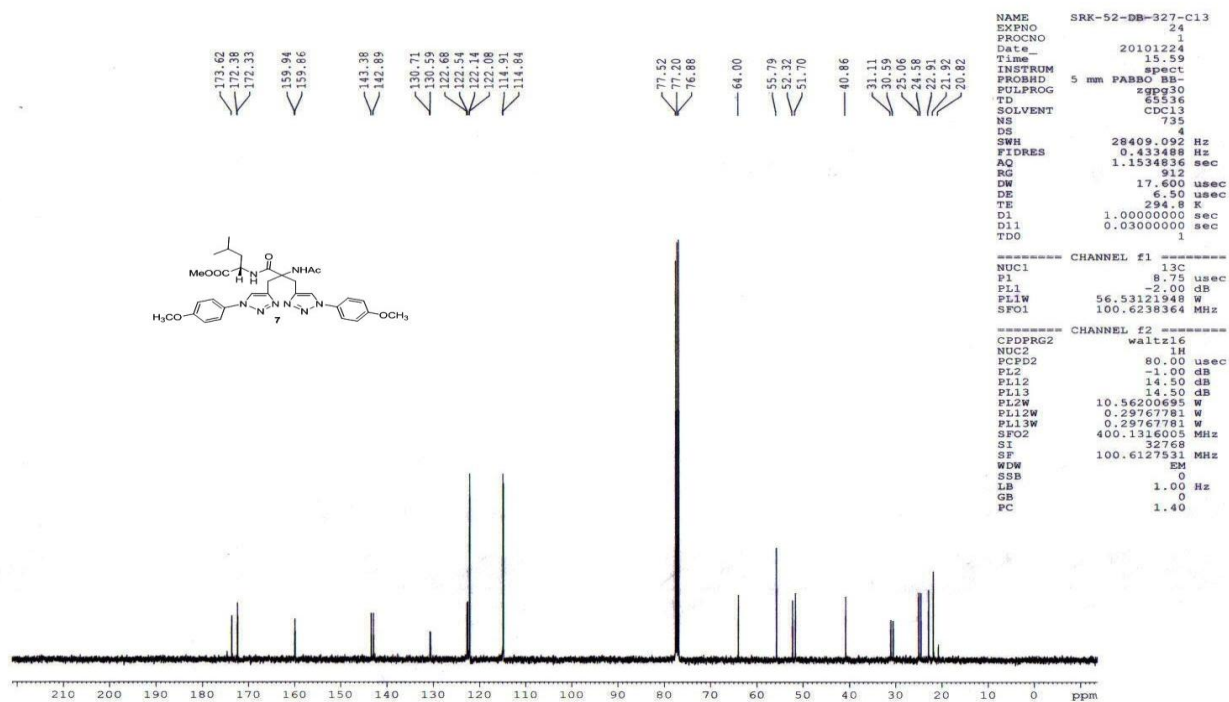


Fig. S6 <sup>13</sup>C NMR (CDCl<sub>3</sub>) of compound 7.

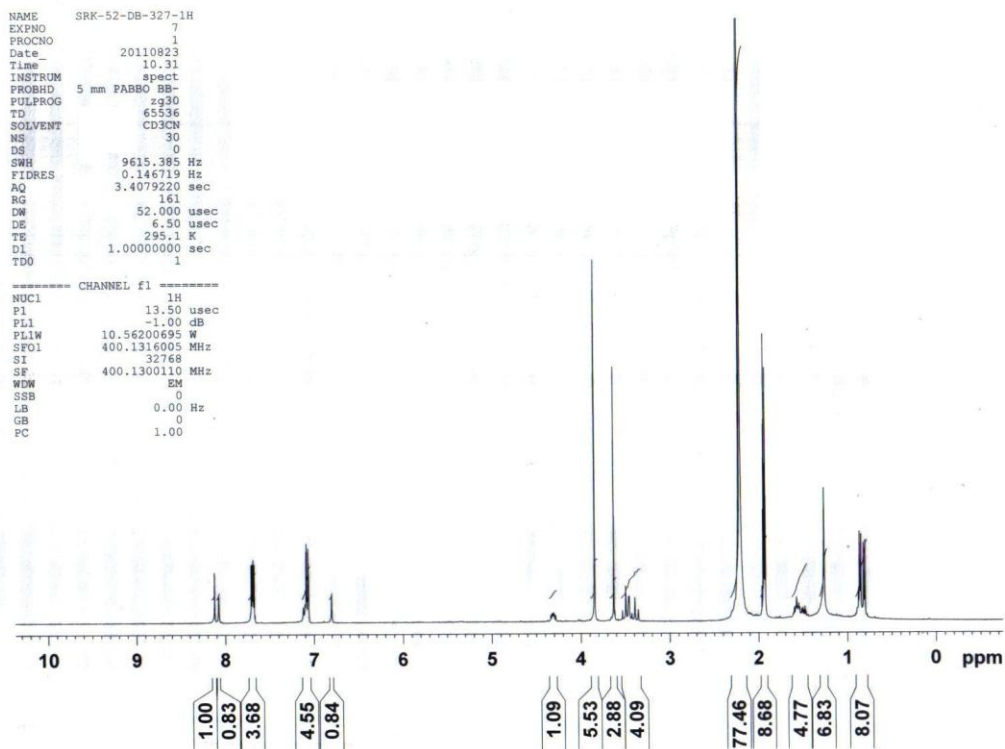


Fig. S7  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ ) spectra of compound **7** (10 mM solution in  $\text{CD}_3\text{CN}$ ).

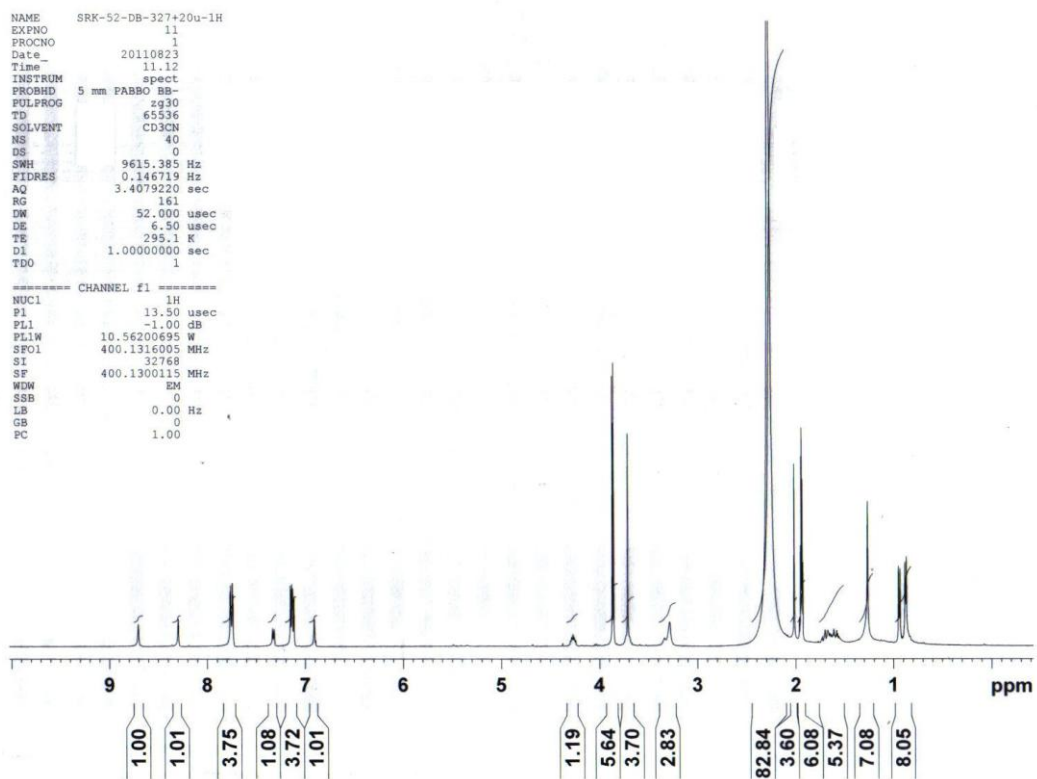
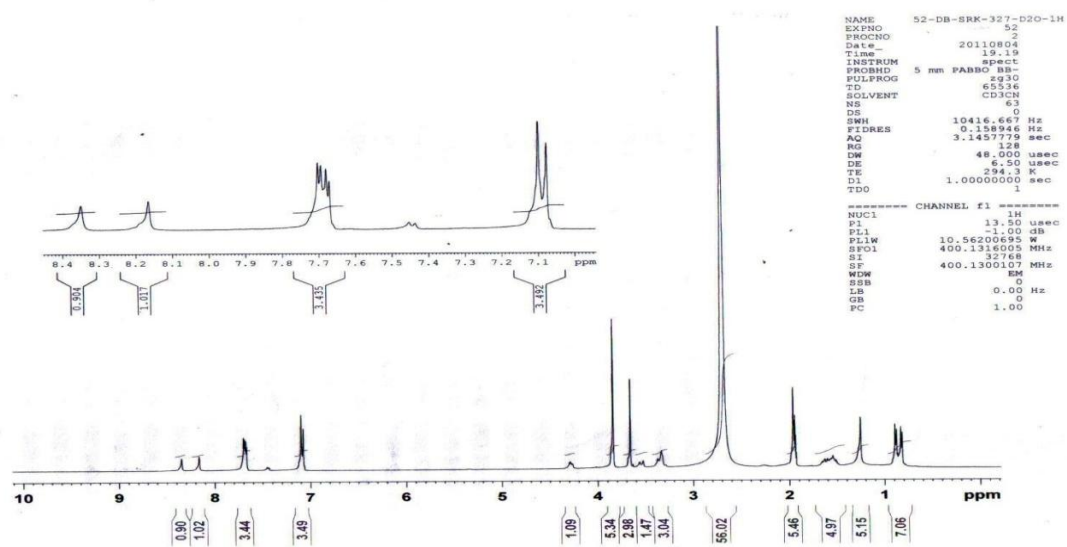
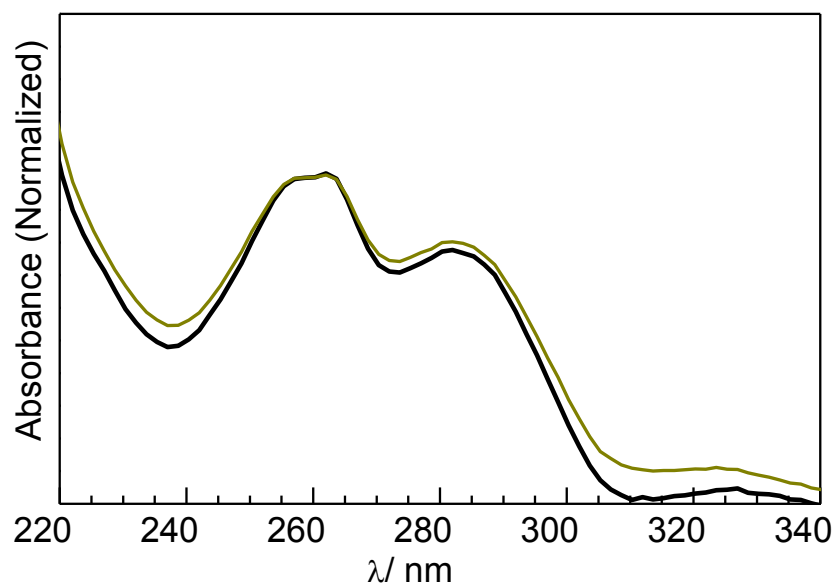


Fig. S8  $^1\text{H}$  NMR spectra of **7** (10 mM) in the presence of 0.5 equiv. of  $\text{Zn}^{2+}$  in  $\text{CD}_3\text{CN}$

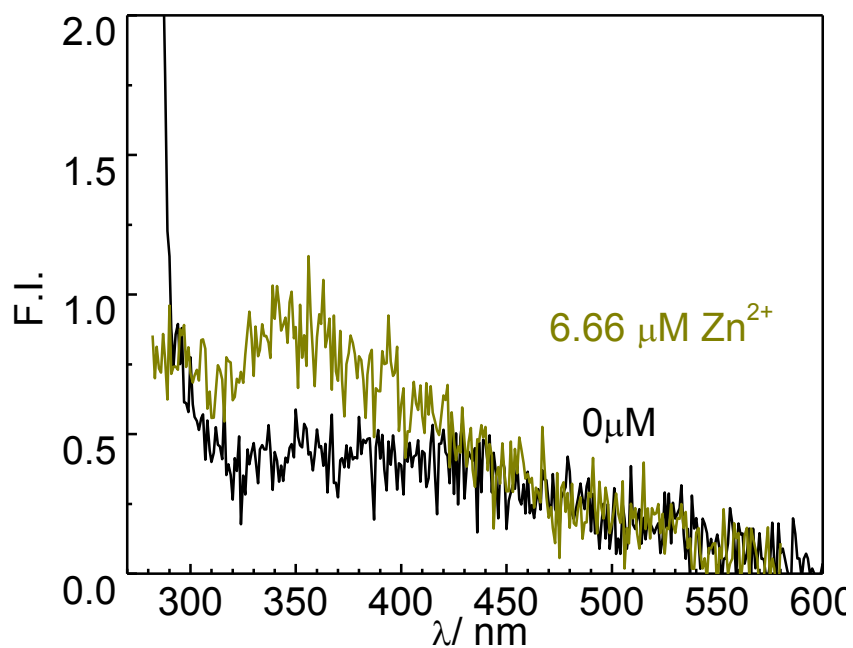


**Fig. S9**  $^1\text{H}$  NMR spectra of **7** in the presence of  $\text{Zn}^{2+}$  in  $\text{CD}_3\text{CN}/\text{D}_2\text{O}$  (To identify NH-protons, deuterium exchange).

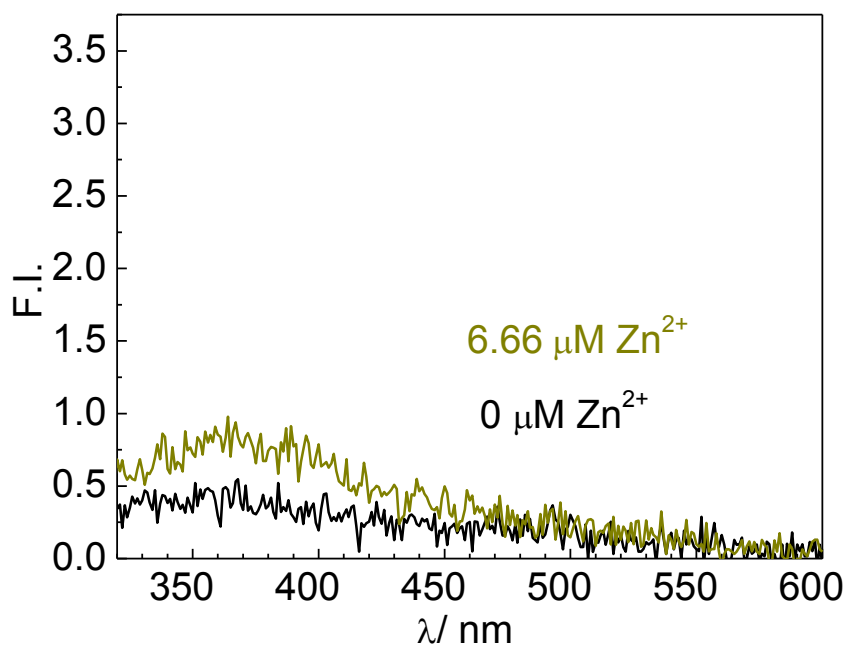


**Fig. S10** Absorption spectra of alkyne building block **6** in acetonitrile before (black) and after addition of  $6.6 \mu\text{M}$  of  $\text{Zn}(\text{ClO}_4)_2$  (green).



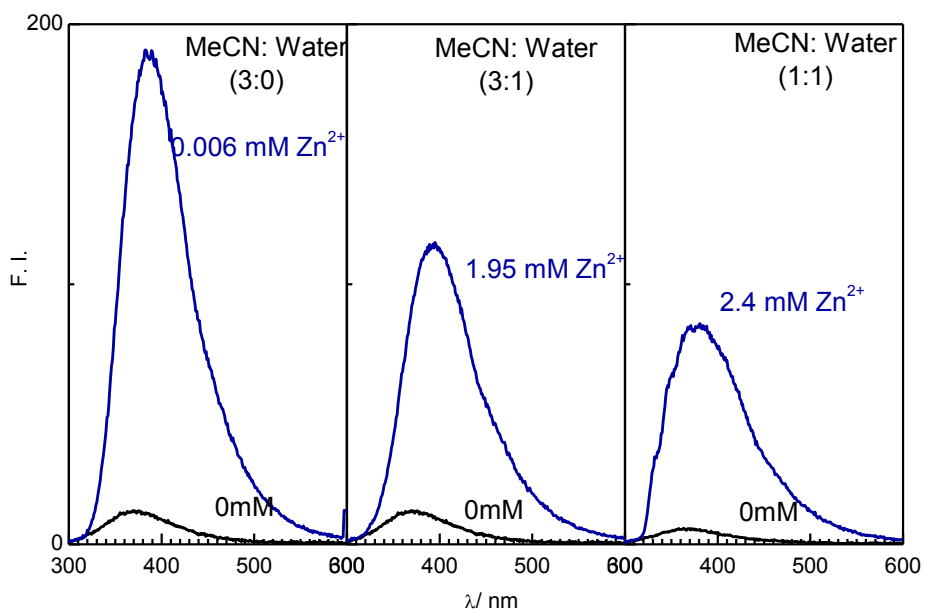


$\lambda_{\text{exc.}} = 262 \text{ nm}$

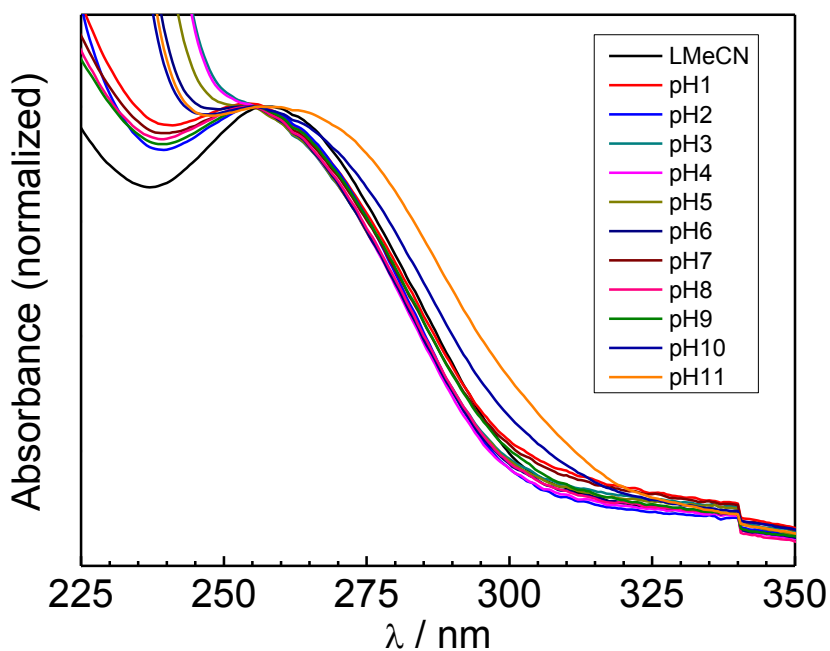


$\lambda_{\text{exc.}} = 282 \text{ nm}$

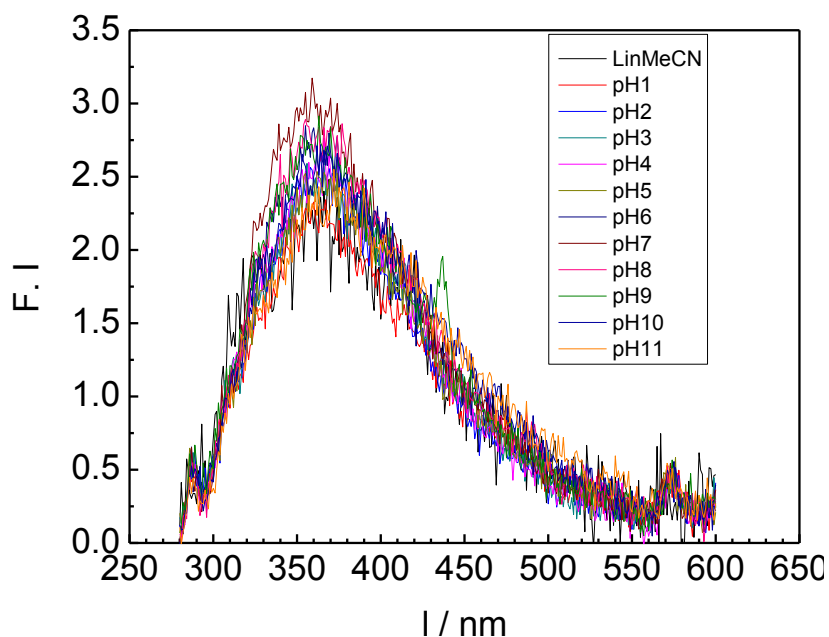
**Fig. S11** Fluorescence spectra of alkyne building block **6** in acetonitrile before (black) and after addition of 6.6  $\mu\text{M}$  of  $\text{Zn}(\text{ClO}_4)_2$  (green).



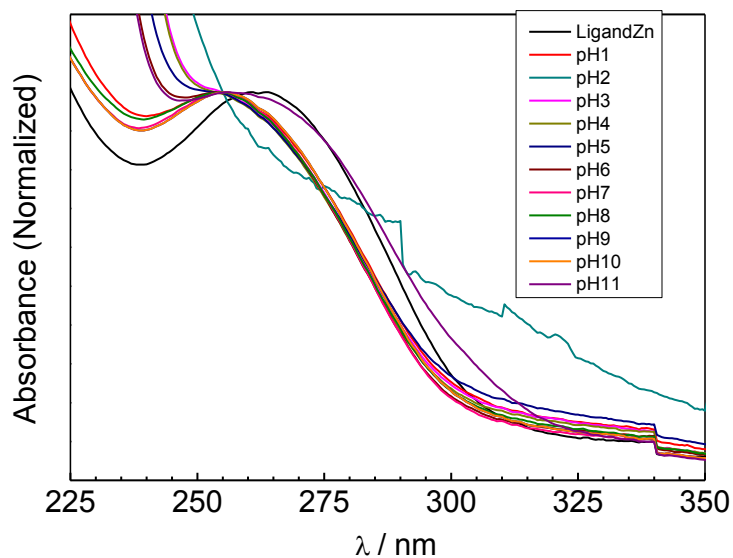
**Fig. S12** Fluorescence titration in different acetonitrile-water percentage.



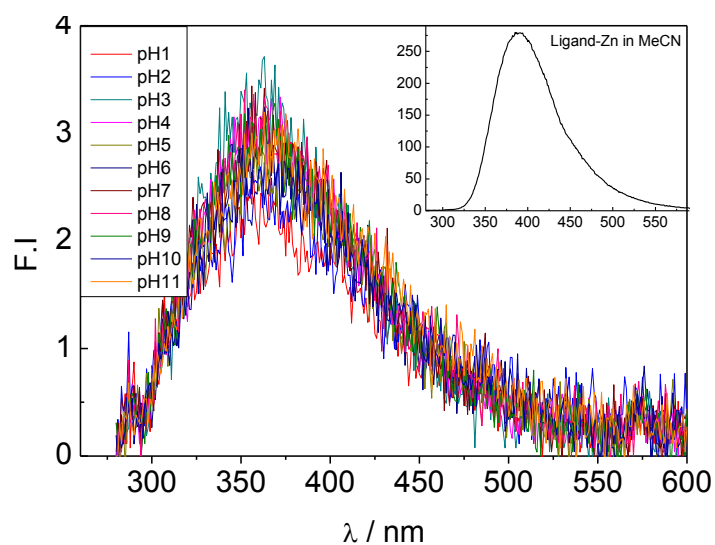
**Fig. S13** Absorption spectra for **L=7** as a function of pH (pH solutions were made in water, experimental solution was prepared by taking 20  $\mu$ L of (200  $\mu$ M solution of **7** in acetonitrile) and then volume make up to 2000  $\mu$ L by various pH solutions).



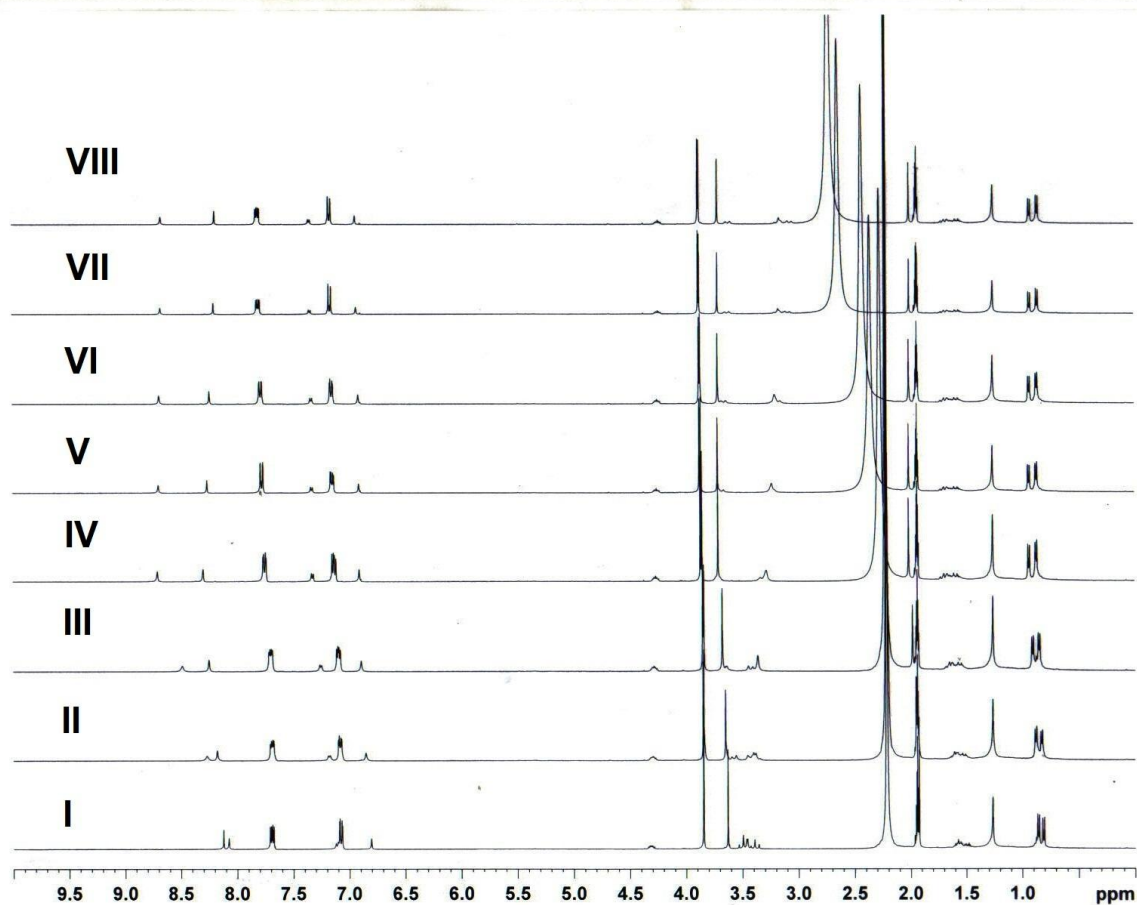
**Fig. S14** Fluorescence intensity for **L=7** as a function of pH (pH solutions are made in water, experimental solution was prepared by taking 20  $\mu\text{L}$  of (200  $\mu\text{M}$  solution of **7** in acetonitrile) and then volume make up to 2000  $\mu\text{L}$  by various pH solutions).



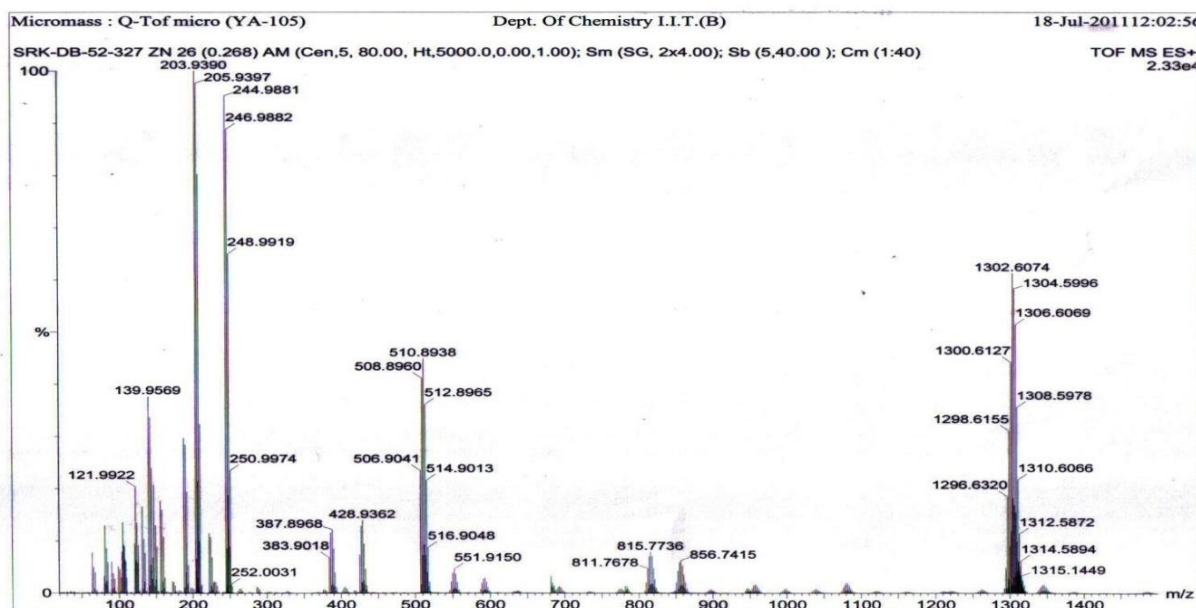
**Fig. S15** Absorption spectra for **L-Zn<sup>2+</sup>** as a function of pH (pH solutions are made in water, experimental solution was prepared by taking 20  $\mu\text{L}$  of (200  $\mu\text{M}$  solution of **7** in acetonitrile), 15  $\mu\text{L}$  of (200  $\mu\text{M}$  solution of  $\text{Zn}(\text{ClO}_4)_2$  in acetonitrile) and then volume make up to 2000  $\mu\text{L}$  by various pH solutions).



**Fig. S16** Fluorescence intensity for **L-Zn<sup>2+</sup>** as a function of pH (pH solutions are made in water, experimental solution was prepared by taking 20 μL of (200 μM solution of **7** in acetonitrile), 15 μL of (200 μM solution of Zn(ClO<sub>4</sub>)<sub>2</sub> in acetonitrile) and then volume make up to 2000 μL by various pH solutions.



**Fig. S17**  $^1\text{H}$  NMR titration spectra of (i) **7** (10 mM); (ii) **7** in the presence of 1.23 mM of  $\text{Zn}^{2+}$ ; (iii) **7** in the presence of 2.46 mM of  $\text{Zn}^{2+}$  (iv) **7** in the presence of 4.92 mM of  $\text{Zn}^{2+}$  (0.5 equiv.) (v) **7** in the presence of 7.38 mM of  $\text{Zn}^{2+}$  (vi) **7** in the presence of 9.84 mM of  $\text{Zn}^{2+}$  (vii) **7** in the presence of 19.68 mM of  $\text{Zn}^{2+}$  (viii) **7** in the presence of 24.6 mM of  $\text{Zn}^{2+}$  in  $\text{CD}_3\text{CN}$ .



**Fig. S18** Mass spectral peak observed for the 2:1 complex of **7** and  $\text{Zn}^{2+}$ .

**Table S 1.** Change in the fluorescence anisotropy of **7** with the increase in the concentration of  $\text{Zn}^{2+}$ .

$[\text{ZnClO}_4]/\mu\text{M}$	$r$
8.69	0.025
16.66	0.017

**Table S2.** Fluorescence lifetime of **7** complex in presence of various concentrations of  $\text{Zn}(\text{ClO}_4)_2$ .

$[Zn^{2+}] (\mu M)$	$\tau_1 (ns)$	$\chi^2$
1.6	1.87	1.18
4.8	2.09	1.08
9.4	2.04	1.01
17.9	2.02	1.02
25.8	2.06	1.07