Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Design and Synthesis of an ON-OFF "Click"Fluorophore That Executes a Logic Operation and Detects Heavy and Transition Metal Ions in Water and Living Cell

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1.0 Fluorimetric titration:

The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax 3 instrument at 25 °C in a thermostated cell holder using quartz cuvette with a 1 cm path-length and each titration was performed using milliQ filtered water. All the binding constants of fluorescence spectral data have been calculated using the following equations (1-3) with the help of Graphpad prism 5.0 (GraphPad Software, San Diego, CA). In this experimental design, aliquots of different metal salts were added to a single concentration of the ligand **5**, and scanned after each addition.

The equations are:

$$LR = (X + L_{tot} + K_d) - \frac{\sqrt{(X+L_{tot}+K_d)^2 - 4 \times X \times L_{tot}}}{2} \quad (1)$$

$$L = L_{tot} - LR \quad (2)$$

$$Y = BKG + MF \times L + FR \times MF \times LR \quad (3)$$

L_{tot}: Total concentration of **5** (Same units as X).
BKG: Background fluorescence w/o receptors (Same units as Y);
MF: Molar fluorescence of free Ligand (Y units divided by X units); X : Concentration of metal salts. K_d: Dissociation constant (X units);
FR: Fluoresence ratio. MF of bound ligand=$FR * MF$ (unitless ratio) so FR > 1 means binding causes turn on; FR< 1 means binding causes turn off .

Table S1. Fluorescence emission properties of 5.[1]

Compound	λabs (nm)	λem (nm)	Φ [d]
5	300	390	0.02
5 + Zn^{2+}[b]	300	390	0.4
5 + Cd^{2+}[c]	300	390	0.321

[a]All data were obtained milliQ filtered water (pH 7). [b]The data were measured in the presence of Zn^{2+} (1.0 equiv) with respect to **5** (25 μM). [c]The data were measured in the presence of Cd^{2+} (1.0 equiv) with respect to **5** (25 μM). [d]The fluorescence quantum yields were determined by using quinine sulfate in 0.1M H_2SO_4 (Φ = 0.577 as standard).[1]

[1] J. R. Lakowicz, Principles of Fluorescence Spectroscopy; Plenum Press: New York, 1983.

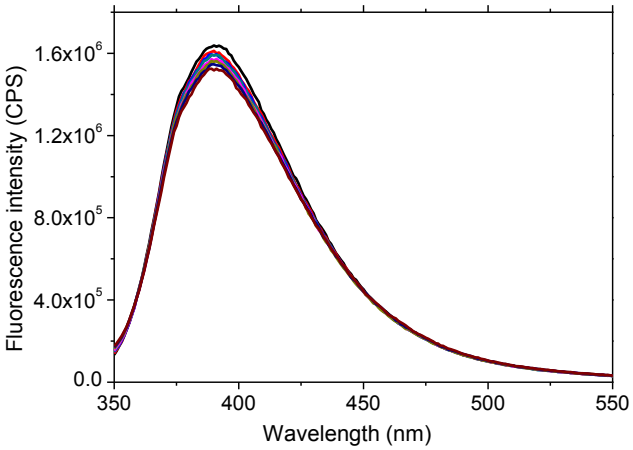


Figure S1. Fluorimetric titration spectra of **5** (25 µM) with Fe^{3+} (0-25 µM).

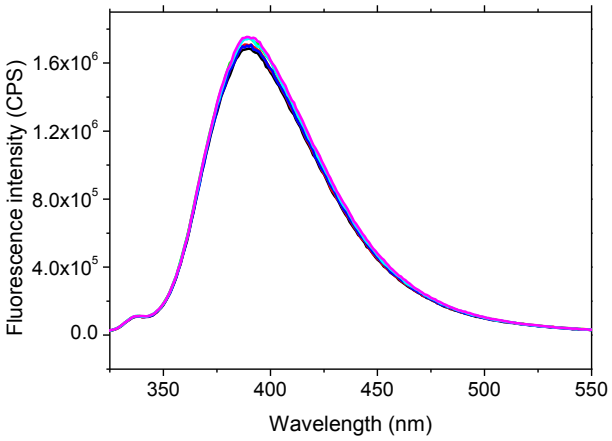


Figure S2. Fluorimetric titration spectra of **5** (25 µM) with Li^{+} (0-25 µM).

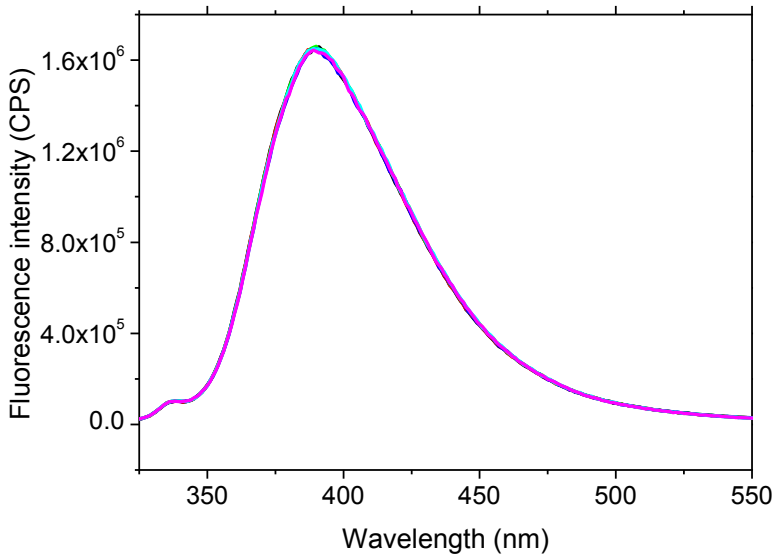


Figure S3. Fluorimetric titration spectra of **5** (25 µM) with Na^{+} (0-25 µM).

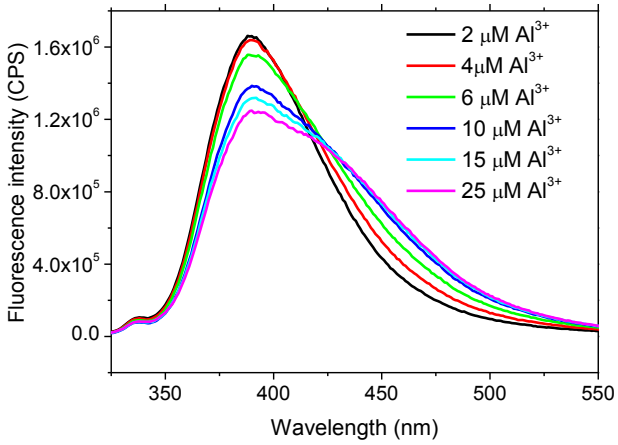


Figure S4. Fluorimetric titration spectra of **5** (25 μM) with Al^{3+} (0-25 μM).

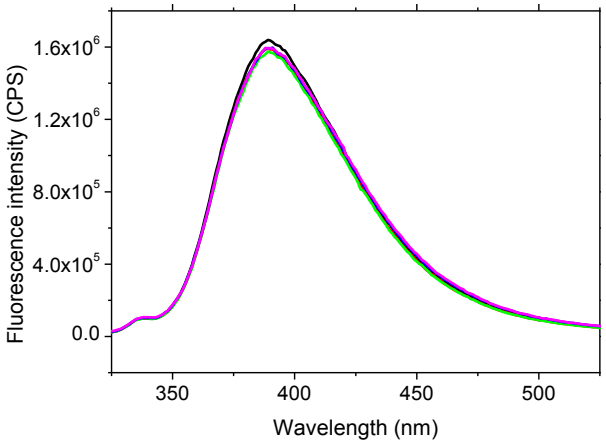


Figure S5. Fluorimetric titration spectra of **5** (25 μM) with Mg^{2+} (0-25 μM).

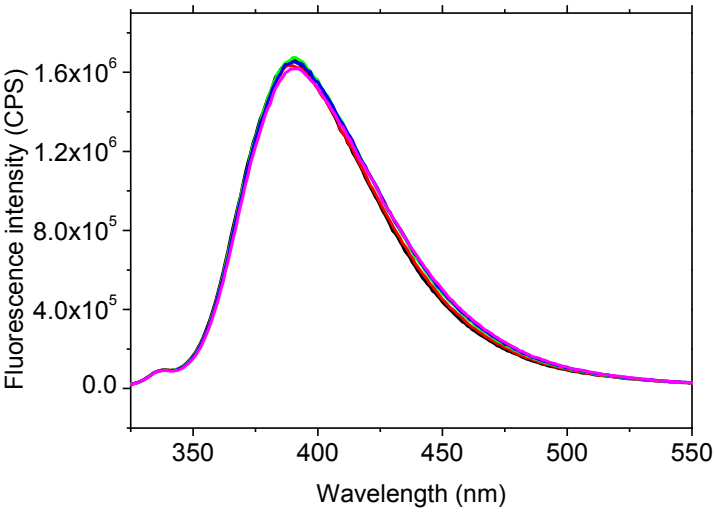


Figure S6. Fluorimetric titration spectra of **5** (25 μM) with Mn^{2+} (0-25 μM).

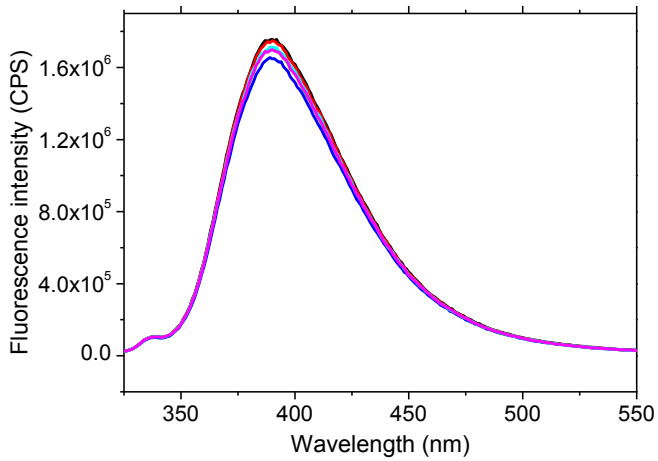


Figure S7. Fluorimetric titration spectra of **5** (25 µM) with Pb^{2+} (0-25 µM).

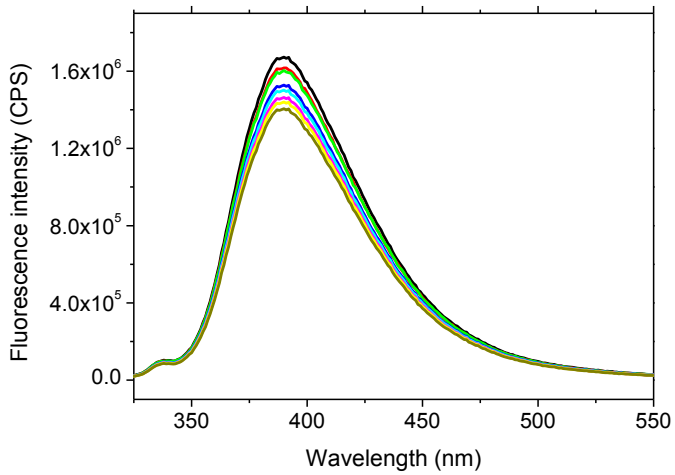


Figure S8. Fluorimetric titration spectra of **5** (25 µM) with Sn^{2+}(0-25 µM).

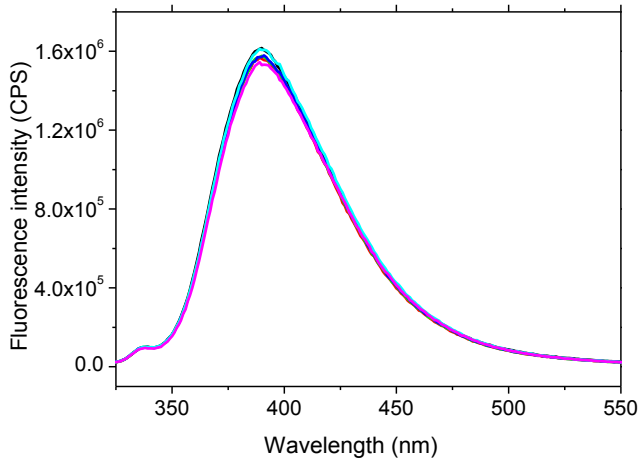


Figure S9. Fluorimetric titration spectra of **5** (25 µM) with Sr^{2+} (0-25 µM).

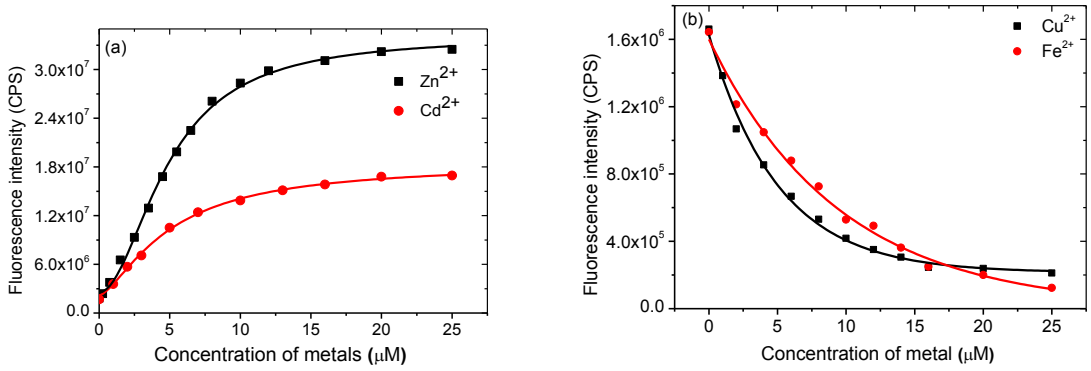


Figure S10. (a) Analysis of data at 390 nm from the titration of **5** (25 μM) with Zn^{2+} (0-25 μM) and Cd^{2+} (0-25 μM) in filtered milliQ water at pH 7.0 using equation (1-3). (b) Analysis of data at 390 nm from the titration of **5** (25 μM) with Cu^{2+}, Fe^{2+} (0-25 μM) in filtered milliQ water at pH 7.0 using equation (1-3).

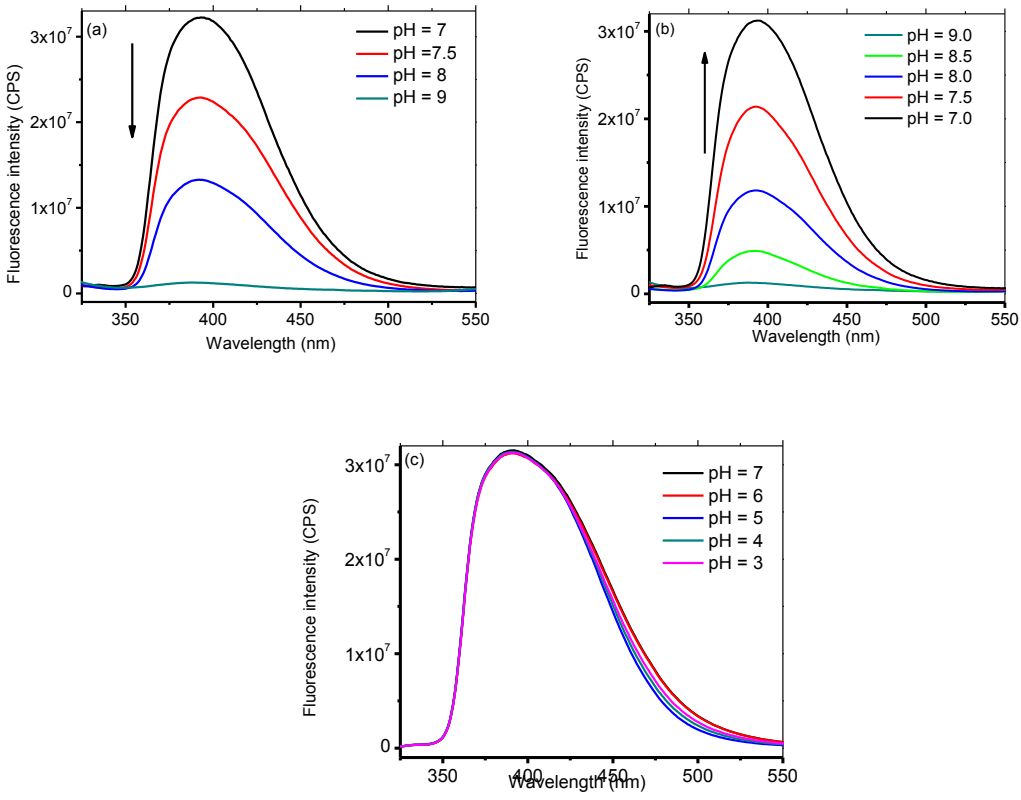


Figure S11. Fluorescent profile of bis-triazole **5** (25 μM) and Zn^{2+} (25 μM) upon gradually (a) increasing pH from 7.0 to 9.0 and (b) decreasing pH from 9.0 to 7.0 (c) decreasing pH from 7.0 to 3.0.

2.0 Computational methods: The calculations were carried out at the level of density functional theory (DFT) with Becke-3-Lee-Yang-Parr (B3LYP) exchange functionals in 6-31G basis sets using the Gaussian 03 program.[2,3]

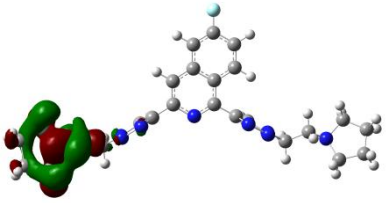
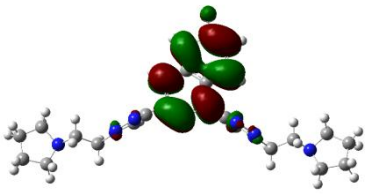
Optimised Structure	HOMO	LUMO
		
Optimised energy : -1563.340487 Hatree	HOMO (- 6.23 eV)	LUMO (-1.90 eV)

Figure S12. Optimized minimum energy structure and Molecular orbital amplitude plots of HOMO and LUMO energy levels of **5** was calculated with the use of B3LYP/6-31G(d,p)* basis set.

[2] Gaussian03 program: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision D.02; Gaussian, Inc.: Wallingford, CT, **2004**.

[3] B3LYP hybrid functional: (a) Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989. (b) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785. (c) Becke, A. D. *Phys. ReV. A* **1988**, *38*, 3098. (d) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1993**, *37*, 785. (e) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

Table S1. Cartesian coordinates for the optimized structure of 5, their zero point energy corrected total energies in Hartree calculated at 298K.

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-0.902284	0.777632	-0.039946
2	6	0	-1.114099	2.190269	0.106610
3	6	0	0.005399	3.048672	-0.136100
4	6	0	1.241134	2.449185	-0.476253
5	6	0	1.337802	1.070428	-0.546490
6	1	0	-3.195469	2.122294	0.713953
7	6	0	-2.354072	2.768718	0.492277
8	6	0	-0.155596	4.457298	-0.027155
9	1	0	2.099938	3.076453	-0.696035
10	6	0	-1.381439	4.954396	0.329288
11	6	0	-2.495285	4.135232	0.603236
12	1	0	0.674808	5.129612	-0.215356
13	1	0	-3.432049	4.593398	0.901003
14	9	0	-1.547256	6.297960	0.439150
15	7	0	0.268830	0.249913	-0.342657
16	7	0	3.968122	-1.067664	-1.710090
17	7	0	-3.053156	-1.888398	1.031361
18	6	0	2.619206	0.414673	-0.856936
19	6	0	-2.012826	-0.186825	0.155353
20	7	0	-1.979038	-1.155485	1.122232
21	6	0	-3.181005	-0.345696	-0.565787
22	1	0	-3.586413	0.174541	-1.420037
23	6	0	3.902684	0.808830	-0.517259
24	1	0	4.283336	1.635023	0.063275

25	7	0	2.714239	-0.741626	-1.585272
26	7	0	4.708332	-0.133428	-1.064821
27	7	0	-3.791184	-1.417107	0.001062
28	6	0	-5.074168	-2.033162	-0.322468
29	1	0	-5.245694	-1.893980	-1.392812
30	1	0	-4.982518	-3.102582	-0.123261
31	6	0	6.155086	-0.287947	-0.962301
32	1	0	6.463623	-0.845448	-1.848581
33	1	0	6.618398	0.701857	-0.996074
34	6	0	6.564944	-1.017481	0.326894
35	1	0	6.063850	-2.003817	0.357446
36	1	0	6.200111	-0.442394	1.185710
37	6	0	-6.218636	-1.443699	0.516543
38	1	0	-6.275285	-0.348339	0.348587
39	1	0	-5.974446	-1.598459	1.572367
40	6	0	8.473085	-1.513437	1.775270
41	6	0	8.633061	-2.111505	-0.463630
42	6	0	9.964655	-1.832019	1.573336
43	1	0	7.934298	-2.410415	2.140090
44	1	0	8.293083	-0.702785	2.489055
45	6	0	10.068679	-2.254363	0.078555
46	1	0	8.605348	-1.772151	-1.503420
47	1	0	8.100868	-3.082081	-0.413125
48	1	0	10.574152	-0.944053	1.764495
49	1	0	10.302789	-2.616528	2.256329
50	1	0	10.752498	-1.593742	-0.462030
51	1	0	10.436955	-3.276975	-0.043582
52	6	0	-8.526662	-1.839721	1.228687
53	6	0	-8.099738	-1.752198	-1.054066
54	6	0	-9.797763	-2.416391	0.584023

55	1	0	-8.645251	-0.751837	1.407849
56	1	0	-8.269075	-2.309439	2.183260
57	6	0	-9.533317	-2.311504	-0.946482
58	1	0	-7.541770	-2.181250	-1.892129
59	1	0	-8.123330	-0.651851	-1.194179
60	1	0	-9.930755	-3.461327	0.878288
61	1	0	-10.691969	-1.869048	0.895570
62	1	0	-9.600706	-3.295482	-1.418821
63	1	0	-10.250227	-1.657272	-1.450934
64	7	0	-7.482354	-2.099739	0.231253
65	7	0	8.010943	-1.129304	0.435438

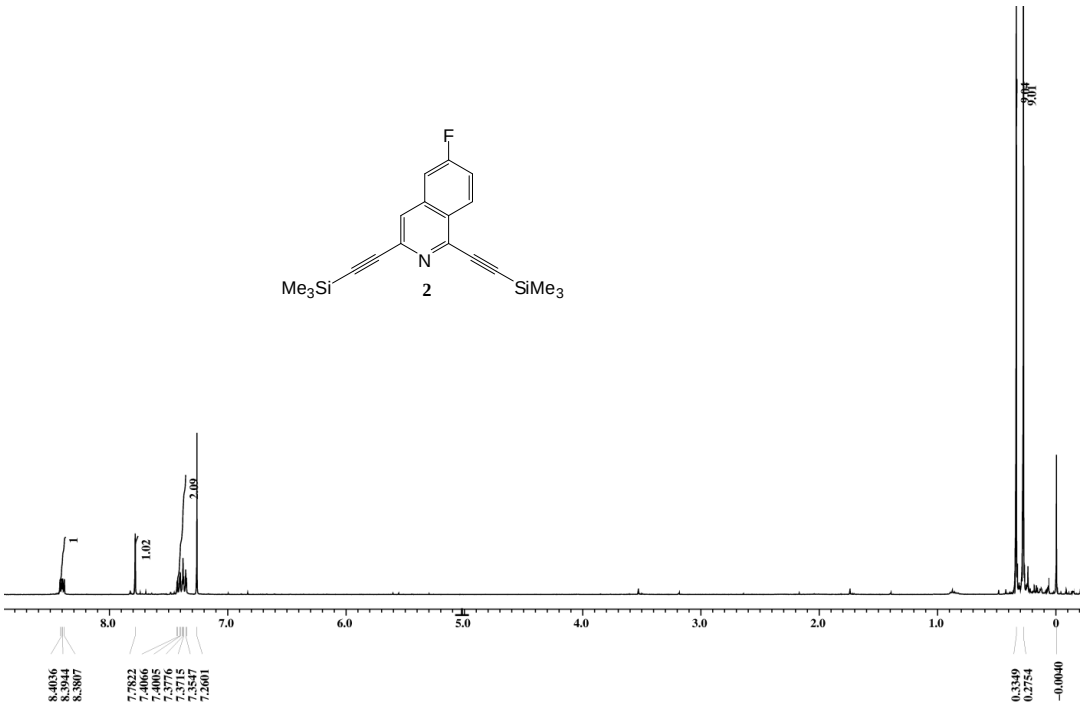
Energy (B3LYP/6-311+G(d,p)*) = -1563.340487 Hartree.

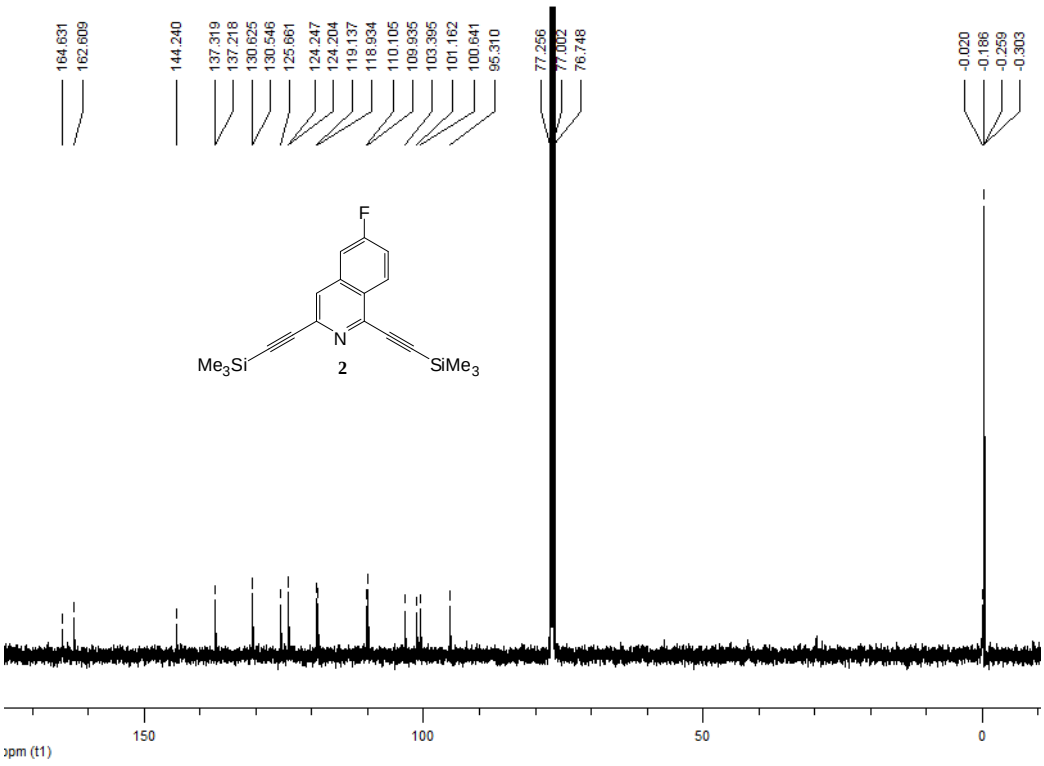
3.0 Cell culture methods:

A375 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Himedia) supplemented with 10 % fetal bovine serum (FBS, Himedia), 50 μg/mL penicillin/streptomycin (Himedia) at 37 °C in a CO_2/air (5/95) incubator. The cells were cultured 2 days before the loading of **5** on 35 mm diameter glass-bottomed cover slips. Then the cells were washed with phosphate-buffered saline (PBS) and bathed in DMEM with 2.5 μM **5** for 30 min at 37 °C, washed with PBS three times to remove the excess **5** and bathed in PBS (2 mL) before imaging. To observe the increase and decrease of fluorescence intensity of **5** upon binding with Zn^{2+} and Fe^{2+}, the cells treated with **5** had been incubated with 2.5 μM $ZnSO_4$ or $FeCl_2$ for 30 min at 37 °C. For the confirmation of increase of fluorescence of **5** binding with Zn^{2+}, we used EDTA as a chelating agent and incubate the $ZnSO_4$ treated cells with 25 μM EDTA for 30 min at 37 °C. All the treated cells were washed three times with PBS and bathed in PBS (2 mL) before taking the images using Confocal laser scanning microscopy (CLSM). CLSM images were taken with a Zeiss LSM710 confocal system that was mounted on a Nikon Eclipse 800 microscope. Laser excitation was used and images were taken at 480 nm. Then, the image was recorded and, at the same time, the intensity of the samples were measured Images of the fibers were taken by using a X10 lens.

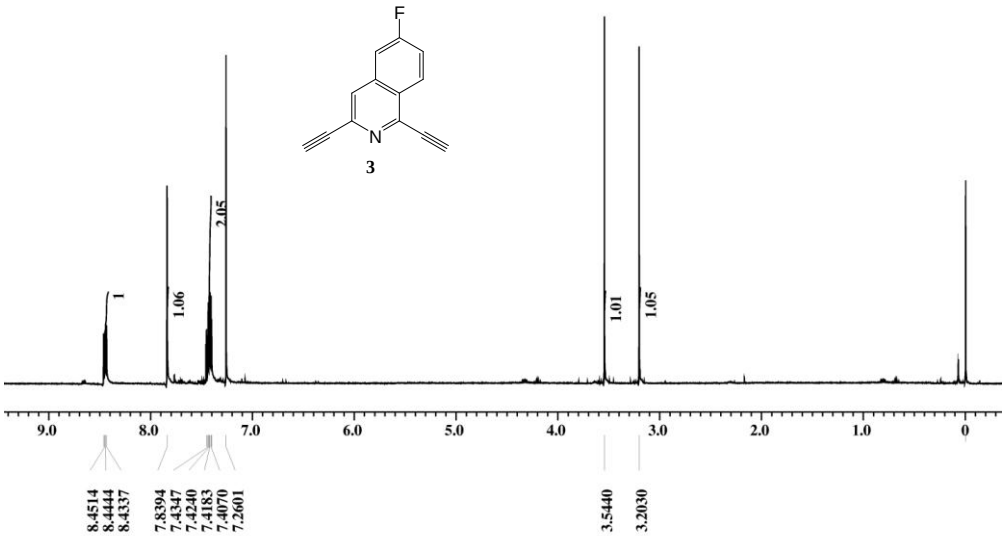
4.0 NMR spectra of all the compounds

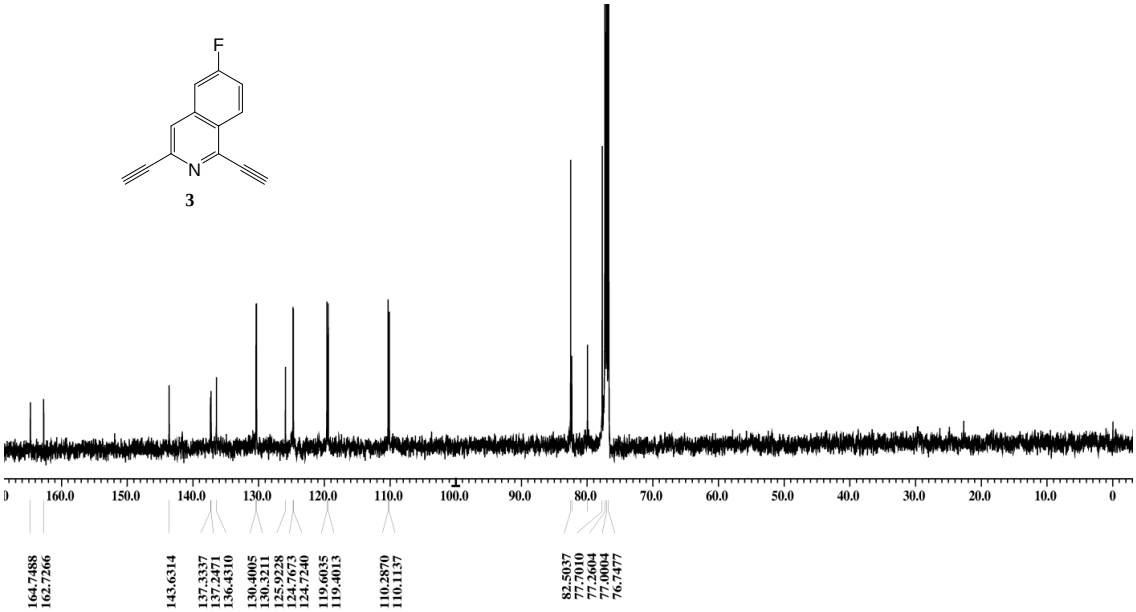
^{1}H and ^{13}C NMR of 2:





^{1}H and ^{13}C NMR of 3:





^{1}H and ^{13}C NMR of 5: