Electronic supporting information

Design of C_2 symmetric pseudopeptides for in vivo detection of Cu(II) through controlled supramolecular nano-assembly

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Experimental:

General Experiment

The melting point was measured using an EZ-Melt (automated melting point apparatus). ^{1}H and ^{13}C-NMR spectra are obtained on a JEOL Resonance-ECZ 500R spectrometer at an operating frequency of 500 MHz (^{1}H) and 126 MHz (^{13}C) in $CDCl_3$ or DMSO-d_6 solutions, respectively, chemical shifts are given in parts per million (ppm) related to Tetramethyl silane (TMS, ∂ = 0.00ppm). FT-IR was recorded on an FT-IR 4700 JASCO spectrophotometer using the KBr pellet method. High-resolution mass spectra (ESI-HRMS) were recorded on the SCIEX X500R (TOF-MS) mass spectrometer. UV-visible spectra were recorded on Agilent Cary 60 single beam UV-Visible spectrometer with serial no.-MY19329220, and at the same time, fluorescence spectra were recorded on Fluoromax 4CP plus spectrofluorometer with a 10 mm quartz cell at 25˚C. The fluorescence decay curve was recorded on WITEC alpha300 focus innovation using a pulse diode laser at 405nm.

All chemicals are purchased from a commercial supplier and used without further purification. m-Xylylenediamine and L-valine were purchased from Spectrochemical. 4-(diethylamino)salicylaldehyde was purchased from TCI chemicals. Metal salt $AgNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Hg(NO_3)_2{\cdot}H_2O$, KNO_3, $NaNO_3$, $Ni(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2{\cdot}6H_2O$, were purchased from HiMedia.

Data analysis

Images were processed using LasX software from Leica, cell viability data were analyzed using Microsoft Excel, and Graph Pad software was used to calculate IC-50.

Stern-Volmer equation and Benesi-Hildebrand equation

Stern-Volmer equation explores the quenching of **4** with Cu(II) ion.

$I_0/I = 1 + K_{sv}$ [Q]

Where [Q] represents the concentration of Cu(II) ion, I_0 and I are the emission intensity of blank solution of **4** and solution of **4** with Cu(II) ions, respectively. K_{sv} is the Stern-Volmer quenching constant determined by the plot between I_0/I vs. varying Cu(II) ion concentration.

Benesi-Hildebrand equation for determining the binding constant for 1:1 stoichiometry. i.e

$1/I\text{-}I_0 = 1/ K_a$ [Cu(II)] [$I_{max} – I_0$] + $1/ I_{max}$ -I_0

Where I is the fluorescence intensity of the **4**+Cu(II) (which varies with the concentration of Cu(II)), I_0 is the fluorescence intensity of **4** when excited at 370 nm, and I_{max} is the fluorescence intensity of the **4**+Cu(II) complex at the maximum concentration of Cu(II) ion.

Cell culture

Raw 264.7 cells were acquired from the National cell Line repository, National Center for Cell Science (NCCS) Pune, Maharashtra, India. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)-high glucose, supplemented with 10% fetal bovine serum, 2 mM glutamine, and 1% antibiotics in a humidified 5% CO_2 environment at 37℃. Cells were passed upon 80% confluence.

Cell Viability

Raw 264.7 cells were seeded at a density of 5000 cells per well in 100 µl of DMEM. Cells were allowed to grow overnight in the CO_2 incubator. After 12h, cells were incubated with various concentrations of the **4** (0, 0.0976, 0.195, 0.391, 0.781, 1.563, 3.125, 6.25, 12.5, 25, 50, 100 µM)

for 24h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method determined cell viability in various drug concentrations. Briefly, cells were incubated with 10 µL of the MTT (5 mg/ml stock) for 2h in a CO_2 incubator at 37°C. Media was discarded, and intracellular formazon crystals formed after conversion from MTT, were dissolved in 100 µL of DMSO. The absorbance was measured at 570 nm on a microplate reader from BioTek.

Live cell imaging microscopy

1×10^5 cells per well were seeded in a 6-well plate. Cells were allowed to grow overnight. Cells were treated with either (i) **4** (50 µM) or (ii) **4** (50 µM) with Cu(II) (100 µM) or (iii) **4** with Cu(II) (100 µM) and EDTA (800 µM) for 30 minutes in the CO_2 chamber of a live cell imaging confocal microscope from Leica. Cells were washed thrice with warm 1X PBS (2 mL each) followed by live cell imaging. The images were acquired using a Leica microscope (Model SP8 STED) (Ex 352 and Em 515 nm) at the SATHI facility located in the CDC building at Banaras Hindu University.

Synthesis of compound 1: (2S,2'S)-N, N'-(1,3-phenylenebis(methylene))bis(2-amino-3-phenylpropanamide)

N-Cbz-*L*-valine (2.512 g, 10 mmol) and N-hydroxy succinimide (1.150 g, 10 mmol) were dissolved in dry THF at 0˚C stirrer till the formation of clear solution. Dicyclohexylcarbamide (DCC) (2.269 g, 11 mmol) previously dissolved in dry THF added dropwise and the resultant solution was again stirred at 0-5˚C for 3-4 h. The side product as dicyclohexylurea is filtered off, and the filtrate was concentrated to dryness and recrystallized by using 2-propanol to get the pure product.[13] Yield (3.0 g, 8.6 mmol, 86%).

Synthesis of compound 2: dibenzyl ((2S,2'S)-((1,3-phenylenebis(methylene))bis(azanediyl))bis(3-methyl-1-oxobutane-1,2-diyl))dicarbamate

Compound **1** (3 g, 8.6 mmol) was dissolved in anhydrous DME (10 mL) with cooling in an ice bath. m-Xylylenediamine (0.5856 g, 4.3 mmol) previously dissolved in anhydrous DME was added dropwise to the above solution. The reaction mixture was stirred for 18 hr. at room temperature and then heated for 6h at 50-60˚C. The white color solid was filtered off and washed with cold methanol and water. Yield (4.2 g, 6.9 mmol, 81%) M.P. 180-182˚C, FT-IR (KBr pellet): Wavenumber [cm^{-1}] 702, 754, 1043, 1246, 1291, 1465, 1538, 1644, 1689, 2931, 2872, 2931, 2965, 3064, 3093 and 3300. ^{1}H NMR (600 MHz, DMSO-d^6, 23˚C, TMS) δ (ppm) 8.43 (t, *J* = 6.0 Hz, 2H), 7.40 – 7.20 (m, 12H), 7.15 – 7.11 (m, 2H), 5.04 (d, *J* = 3.1 Hz, 4H), 4.26 (d, *J* = 5.9 Hz, 4H), 3.87 (t, *J* = 8.0 Hz, 2H), 2.01 – 1.92 (m, 2H), 0.85 (d, *J* = 6.7 Hz, 12H). ^{13}C NMR (151 MHz, DMSO-d^6, 23˚C, TMS) δ (ppm) 171.7, 156.7, 139.7, 137.5, 128.8, 128.7, 128.6, 128.1, 126.8, 126.0, 65.8, 60.8, 42.5, 40.45, 30.7, 19.7, 18.7. Anal. Calcd. for $C_{34}H_{42}N_4O_6$: HRMS (ESI-TOF)$^+$: m/z: $[M+H]^+$: 603.3183 (calculated), 603.3176 (found).

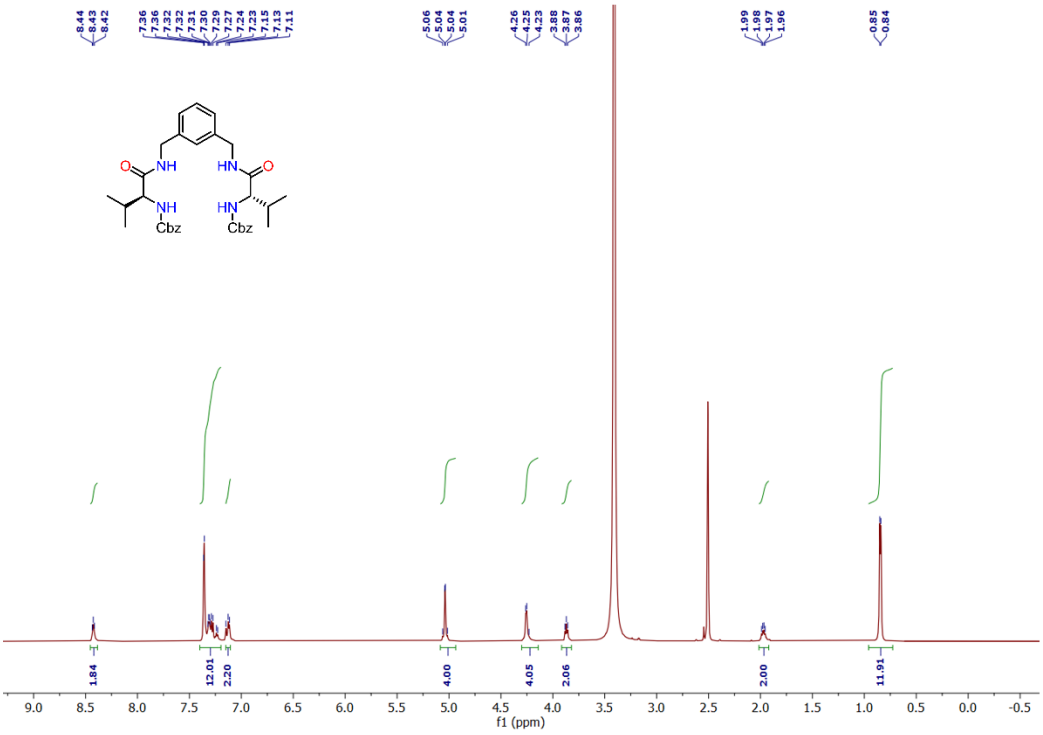


Figure S1: - ^{1}HNMR spectra of **2** in DMSO-d^{6} at 600MHz.

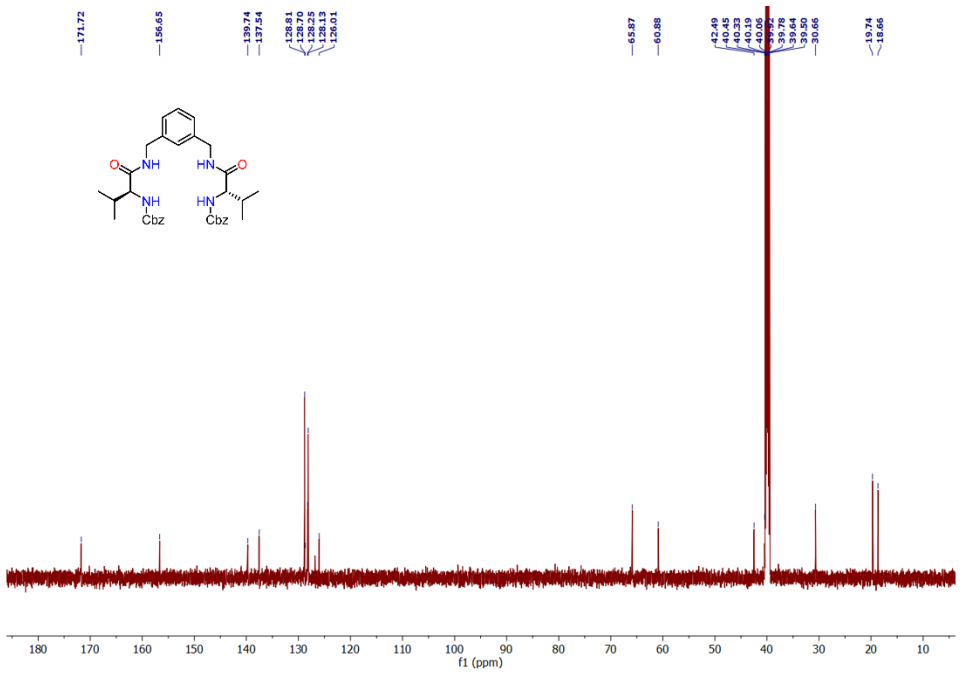


Figure S2: - ^{13}CNMR spectra of **2** in DMSO-d^{6} at 151MHz.

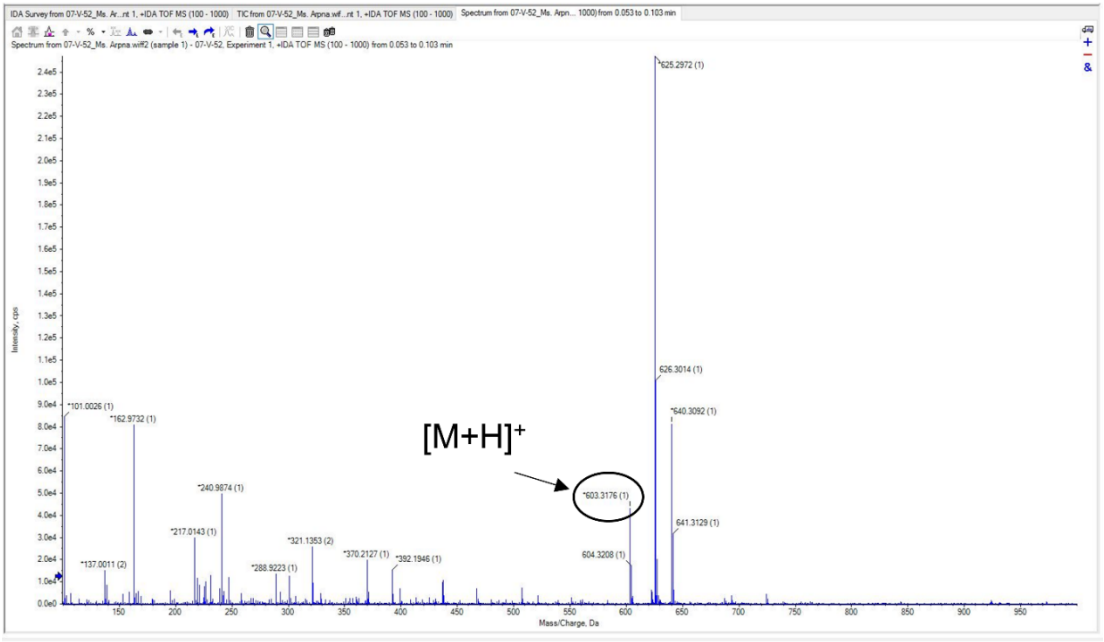


Figure S3: - HRMS (ESI-TOF)$^{+}$ spectra of **2**.

Synthesis of compound 3: (2S,2'S)-N,N'-(1,3-phenylenebis(methylene))bis(2-amino-3-methylbutanamide)

Compound **2** (4.2 g, 6.9 mmol) was added to HBr/Acetic acid (33%) (12 mL) and stirred at rt. until CO_2 evolution stopped; after this addition, diethyl ether in the solution formed a white residue. The residue was dissolved in distilled water and extracted with chloroform. Then solid NaOH pellet was added to the solution to maintain a pH of 12. It was saturated by dissolving NaCl and extracted with chloroform (10 ml×3). The organic phase was dried over $MgSO_4$ and concentrated using a vacuum evaporator to get a white solid. Yield (2.0 g, 5.8 mmol, 86%), M.P. 60-62ºC, FT-IR (KBr pellet): Wavenumber [cm^{-1}] 472, 699, 750, 893, 977, 1035, 1107, 1169, 1226, 1446, 1531, 1698, 2858, 2920, 3399, and 3512. ^{1}H NMR (500 MHz, $CDCl_3$, 23˚C, TMS) δ (ppm) 7.78 – 7.60 (m, 2H), 7.21 (t, J = 6.5 Hz, 1H), 7.11 (d, J = 7.9 Hz, 3H), 4.35 (d, J = 6.5 Hz, 4H), 3.18 (d, J = 4.5 Hz, 2H), 2.24 (td, J = 7.0, 3.9 Hz, 2H), 0.92 (d, J = 7.4 Hz, 6H), 0.77 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, 23˚C, TMS) δ (ppm)174.6, 139.2, 128.9, 127.0, 126.7,

60.3, 42.9, 31.0, 19.8, 16.2. Anal. Calcd. for $C_{18}H_{30}N_4O_2$: HRMS (ESI-TOF)$^+$: m/z: $[M+H]^+$: 335.2447 (calculated), 335.2473 (found).

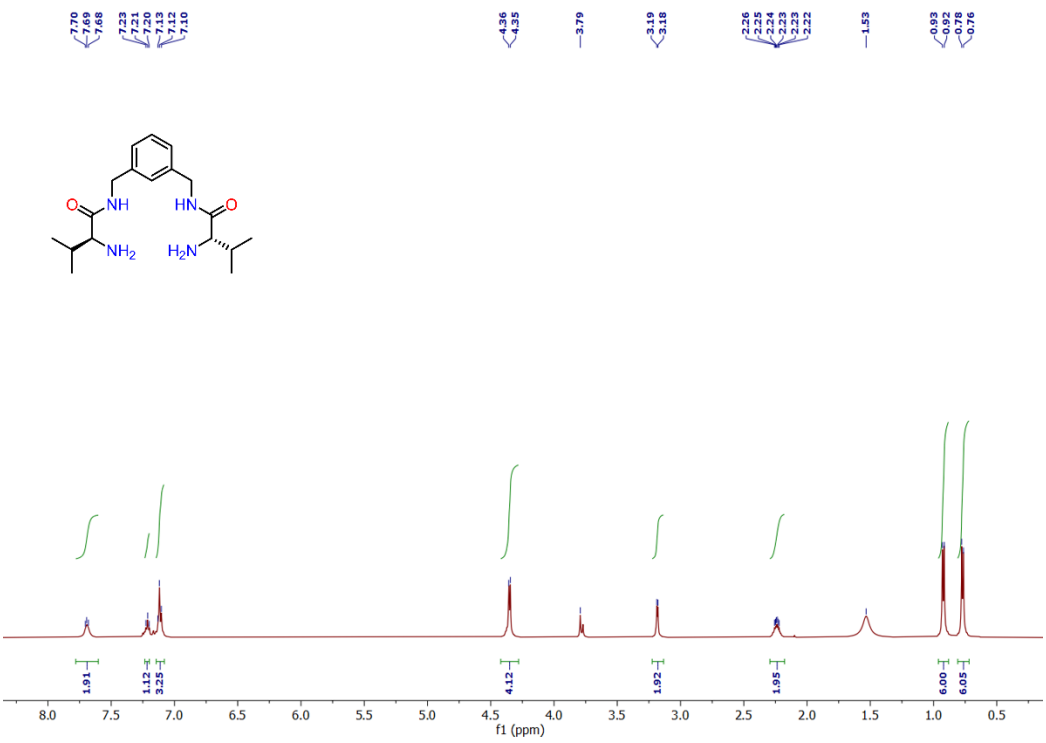


Figure S4: - ^{1}HNMR spectra of **3** in $CDCl_3$ at 500MHz.

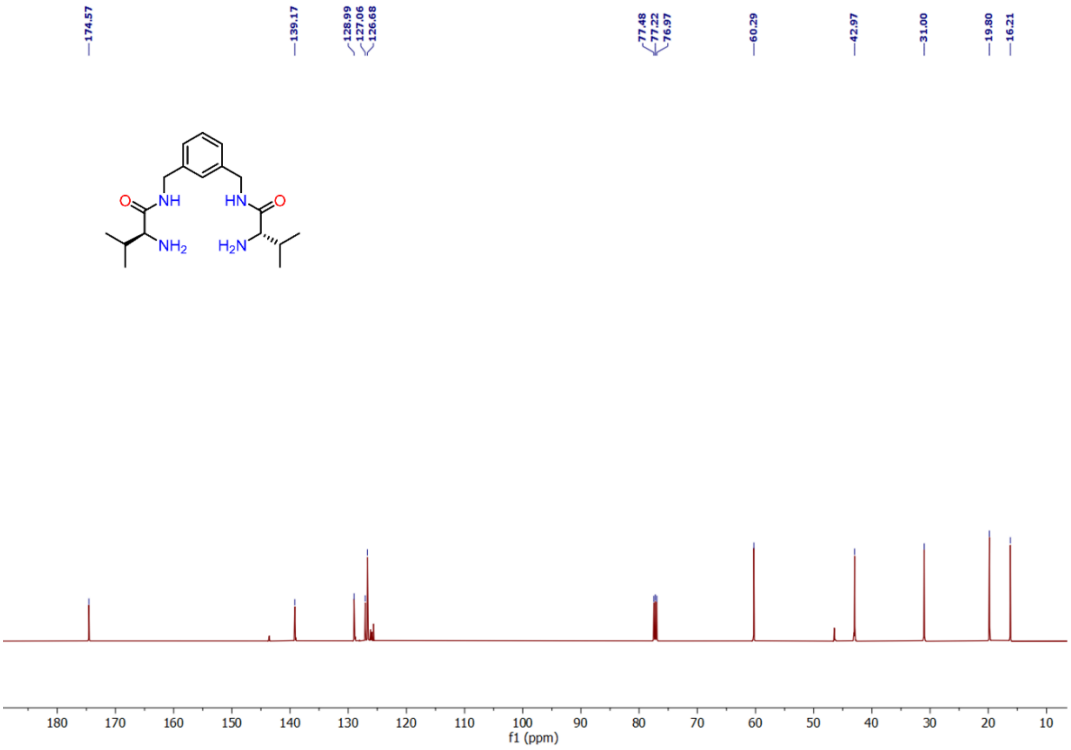


Figure S5: - ^{13}CNMR spectra of **3** in DMSO-d^{6} at 126MHz.

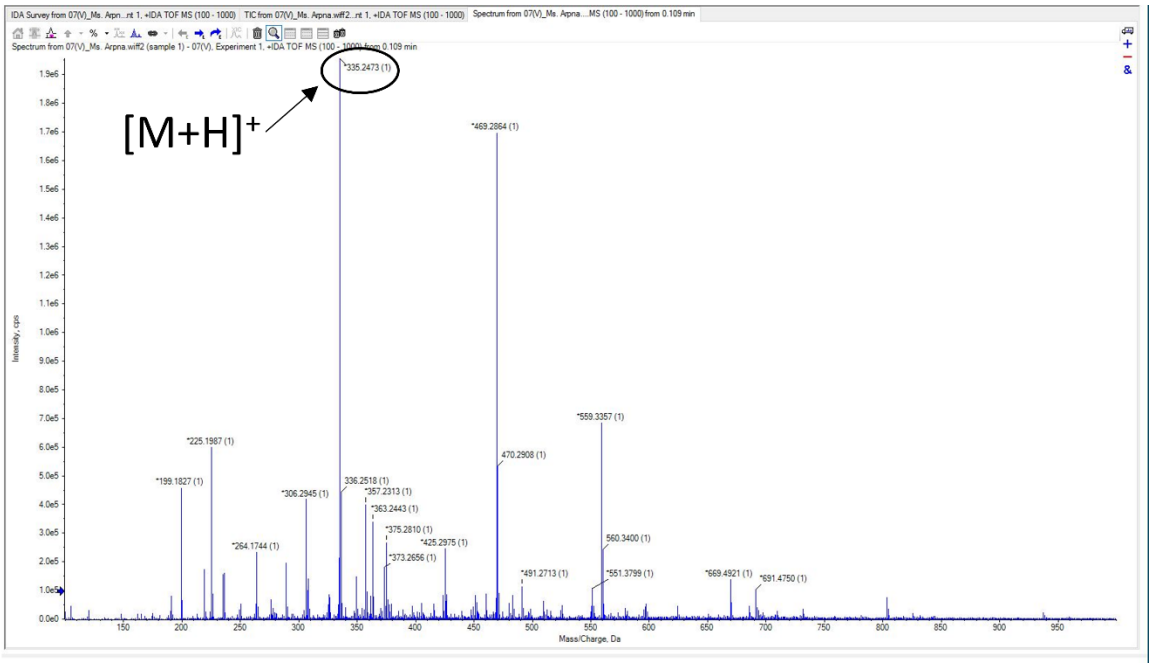


Figure S6: - HRMS (ESI-TOF)$^{+}$ spectra of **3**.

Synthesis of compound 4: - (2S,2'S)-N,N'-(1,3-phenylenebis(methylene))bis(2-(((E)-4-(diethylamino)-2-hydroxybenzylidene)amino)-3-methylbutanamide)

Compound 3 (0.335 g; 1 mmol) was dissolved in methanol (10 mL); once a clear solution was obtained in this solution, we added 4-(Diethylamino) salicylaldehyde (0.387 g; 2 mmol) previously dissolved in methanol. The reaction mixture was refluxed for 4-5 h at 65˚C temperature. The solvent was dried using a rotator evaporator and washed with diethyl ether. The crude product was recrystallized with DCM /acetonitrile mixture with slow evaporation. (Yield 0.500 g, 0.78 mmol, 73%): M.P. 105-110°C, FT-IR (KBr pellet): Wave number [cm^{-1}] 1020, 1074, 1141, 1240, 1265, 1350, 1381, 1522, 1613, 2873, 2931, 2966, and 3272. ^{1}H NMR (500 MHz, $CDCl_3$, 23˚C, TMS) δ (ppm) 8.04 (s, 2H), 7.21 – 7.00 (m, 7H), 6.77 (s, 1H), 6.31 – 6.05 (m, 3H), 4.46 – 4.27 (m, 4H), 3.63 (s, 2H), 3.36 (d, *J* = 7.5 Hz, 7H), 2.47 (s, 2H), 1.16 (q, *J* = 9.7, 8.6 Hz, 11H), 1.00 – 0.79 (m, 12H). ^{13}C NMR (126 MHz, $CDCl_3$, 23˚C, TMS) δ (ppm)192.0, 135.5, 96.7, 44.8, 44.7, 32.0, 19.8, 12.7, 12.6. Anal. Calcd. for $C_{40}H_{57}N_6O_4$: HRMS $(ESI\text{-}TOF)^+$ calculated $[M+H]^+$ m/z 685.4441, found $[M+H]^+$ m/z 685.4441.

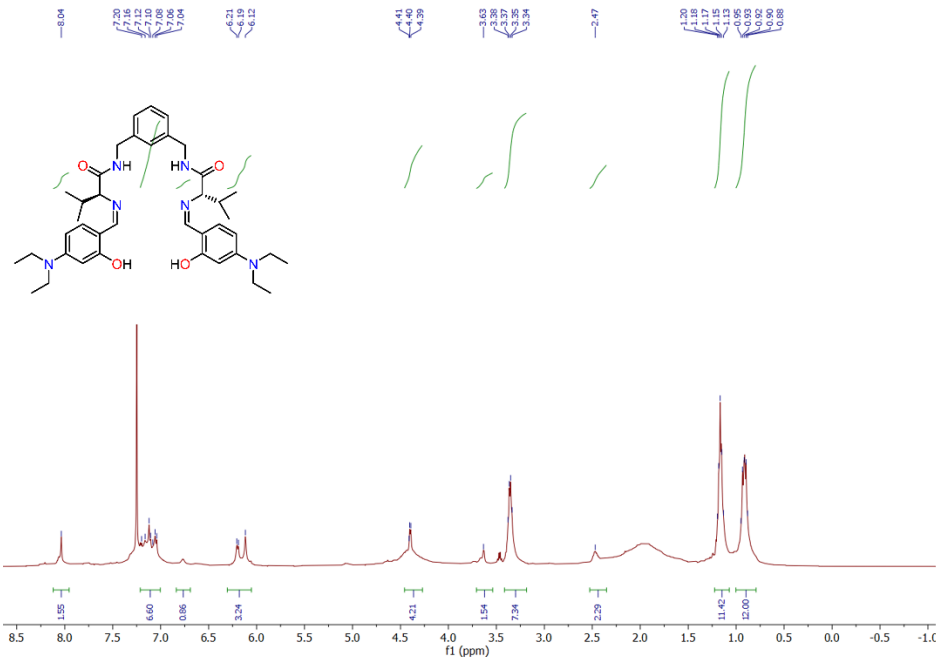


Figure S7: - ^{1}H NMR spectra of **4.**

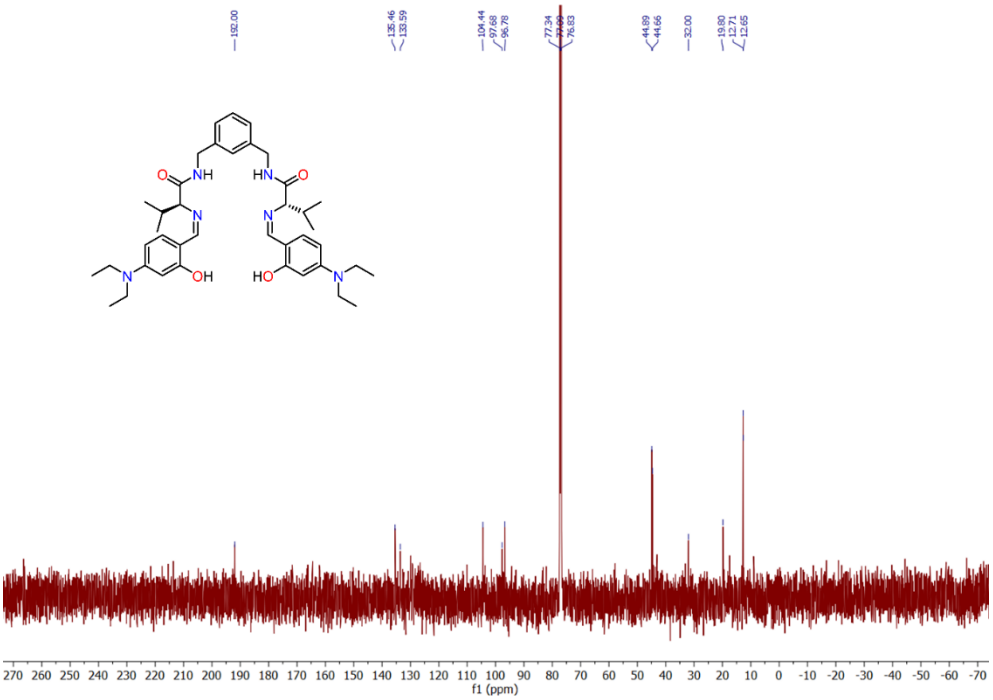


Figure S8: - ^{13}C NMR spectra of **4.**

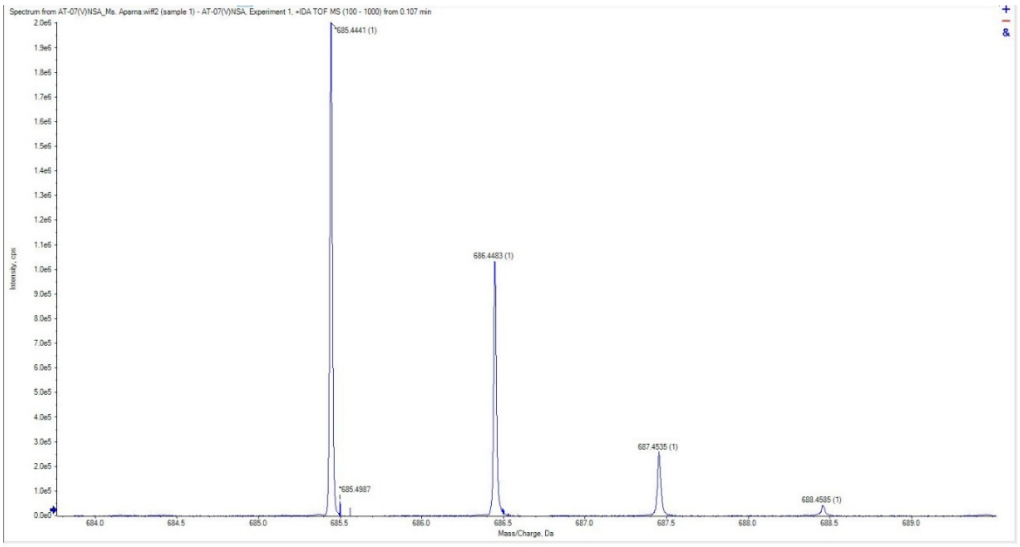


Figure S9: - HRMS (ESI-TOF) spectra of **4**.

General methods for measurements: -

Spectroscopy measurement: - The change in the emission and absorption spectra of **4** was studied using various environmentally and physiologically important alkali-alkaline earth and transition metal ions. Nitrate salt of various metals is used to provide the metal ion such as $AgNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Hg(NO_3)_2{\cdot}H_2O$, KNO_3, $NaNO_3$, $Ni(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2{\cdot}6H_2O$. The solution of **4** (10 μM) for UV-visible and fluorescence study was prepared in EtOH: Water (7:3, v/v at r.t.). While the solution of metal ions (Ag(I), Ca(II), Cd(II), Co(II), Cu(II), Hg(II), K(I), Na(I), Ni(II), Pb(II), Zn(II)) was prepared by dissolving their nitrate salt in distilled water, (10 mM). Furthermore, for the titration experiment, 3.0 mL of **4** was taken in a quartz cuvette (4 mL, path length 1 cm), and the solution of various metal ions (10 equivalent) was added gradually using a micropipette. Fluorescence spectra were recorded on an excitation wavelength of 352 nm and exhibited the emission at 515 nm, while the absorbance spectra were recorded in the range of 200-800 nm.

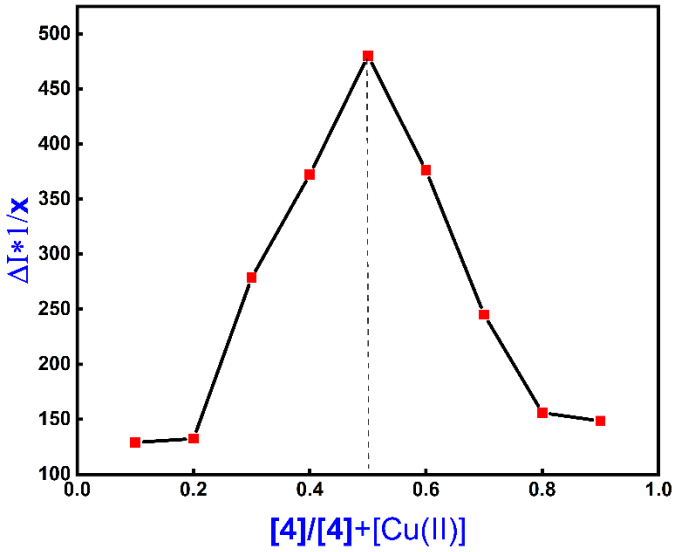


Figure S10: - Jobs plot spectra of **4** (10μM).

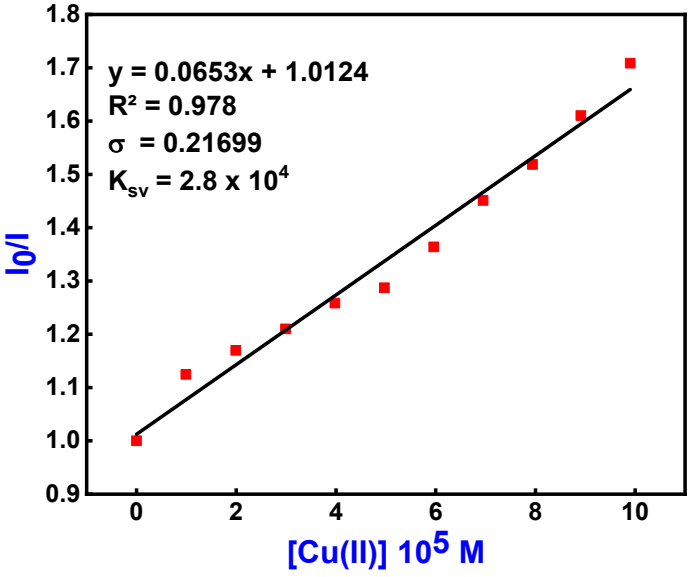


Figure S11: - Stern-Volmer plot of **4.**

Determination of the quantum yield.

Standard used: Quinine sulfate salt (QS); QS in 0.5M sulfuric acid has $\phi_f = 0.546$ (at 25˚C)

A 1×10^{-5} M solution of Quinine sulfate is prepared in 0.5M H_2SO_4, the absorbance maximum of the solution at 345nm, Abs = 0.305. The emission spectrum is also recorded with $\lambda_{exc.}$= 345nm, fluorescence emission is integrated into the range 365-627nm range:6.14×10^7 a.u.

A 1×10^{-5}M solution of **1** and **1**+Cu(II) is prepared in EtOH: Water (8:2, v/v), and the absorbance maximum of the solution at 352 shows Abs.= 1.514 for **1** and Abs.= 1.634 for **1**+Cu(II). The emission spectrum is also recorded with $\lambda_{exc.}$= 520 nm for **1** and **1**+Cu(II). Fluorescence emission of **1** was integrated in the range 375-680 nm range: 8.826×10^7 a.u. and Fluorescence emission of **1** on the addition of Cu(II) was integrated in the range 375-680 nm range: 5.288×10^7 a.u.

The formula used for calculating quantum yield

$\Phi_s = \phi_{rf}. I_s/I_{rf}. A_{rf}/A_s$

Where Φ_s represent the fluorescence quantum yield of the sample, ϕ_{rf} represents the fluorescence quantum yield of the standard reference, I_s and I_{rf} are the integrated emission intensities of the sample and the standard reference respectively, A_{rf} and A_s are the absorbance of the standard reference and the sample at the excitation wavelength, respectively, and n_s and n_{rf} are the refractive indexes of the corresponding solution of sample and reference.

The refractive index of the solvent η_s/η_{rf} was considered one.

Table S1: - Quantum yield of **1** and **2** with and without the addition of Cu(II) ions.

S.No.	Entry	ϕ
1	**1**	0.056
2	**1**+Cu(II)	0.032

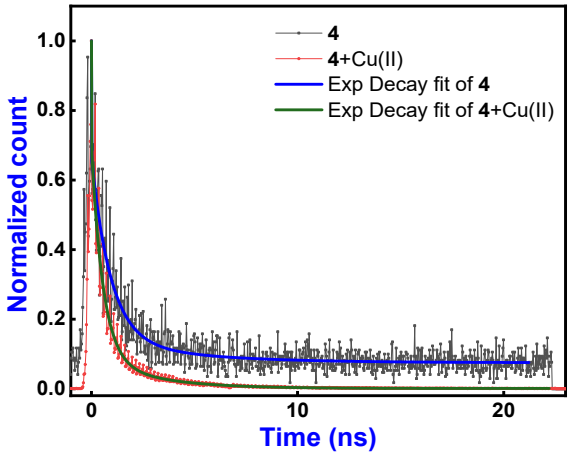


Figure S12: - Fluorescence lifetime decay curve of **4** and **4**+Cu(II).

Table S2 Fluorescence decay parameters of pseudopeptidic probes **4** in ethanol-water solution (7:3, v/v, at r.t.).

Entry	(A)	τ (ns)	<τ> (ns)
4	0.1599	5.2314	
	0.5219	0.9237	2.1389
	0.0705	4.4266	
4+Cu(II)	0.10612	3.3866	
	0.60399	0.55978	1.1912
	0.0926	2.7956	

Dynamic parameters determined from $A_1\exp(-x/\tau_1) + A_2\exp(-x/\tau_2) + A_3\exp(-x/\tau_3) + y_0$

The weighted mean lifetime <τ> was calculated by using the following equation:

$\langle\tau\rangle = (A_1\tau_1 + A_2\tau_2 + A_3\tau_3) / (A_1 + A_2 + A_3)$

where $A_1/A_2/A_3$ and $\tau_1/\tau_2/\tau_3$ are the fractions (A) and lifetimes (τ) respectively.

Solution concentration = 10 μM.

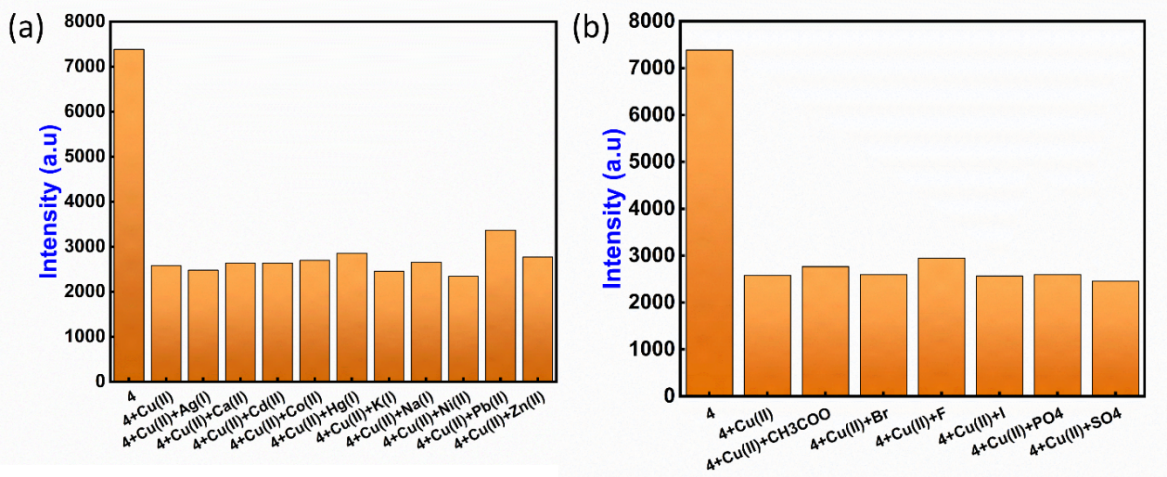


Figure S13: - Interference study of 4 with Cu(II) on addition of various competitive cations and anions in EtOH: Water (7:3, v/v, at rt.)

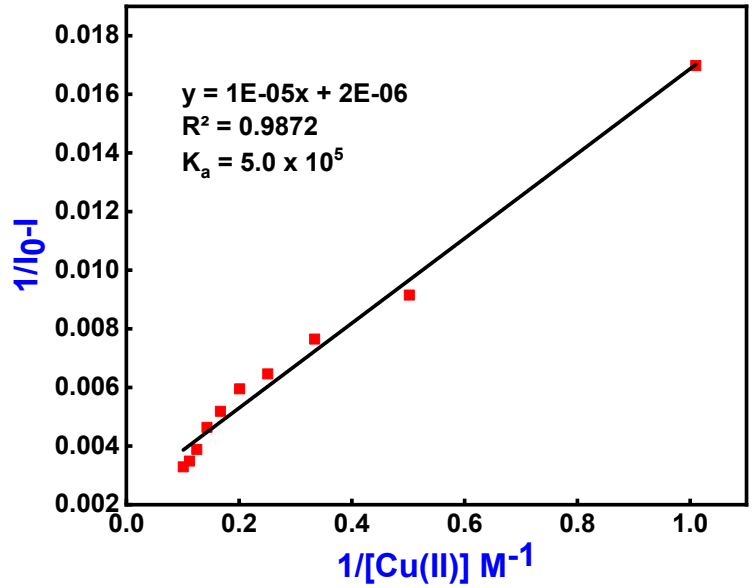


Figure S14: - Benesi-Hildebrand plot for determination of binding constant of **4**

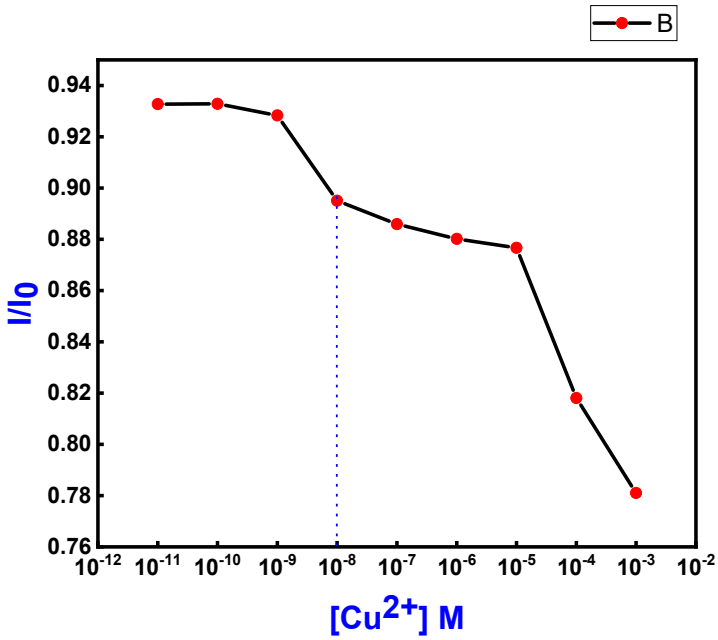


Figure S15: - Fluorescence sensitivity of **4** in the presence of a different concentration of Cu(II) (10^{-11} to 10^{-3} M) ions in EtOH: Water (7:3, v/v at rt.)

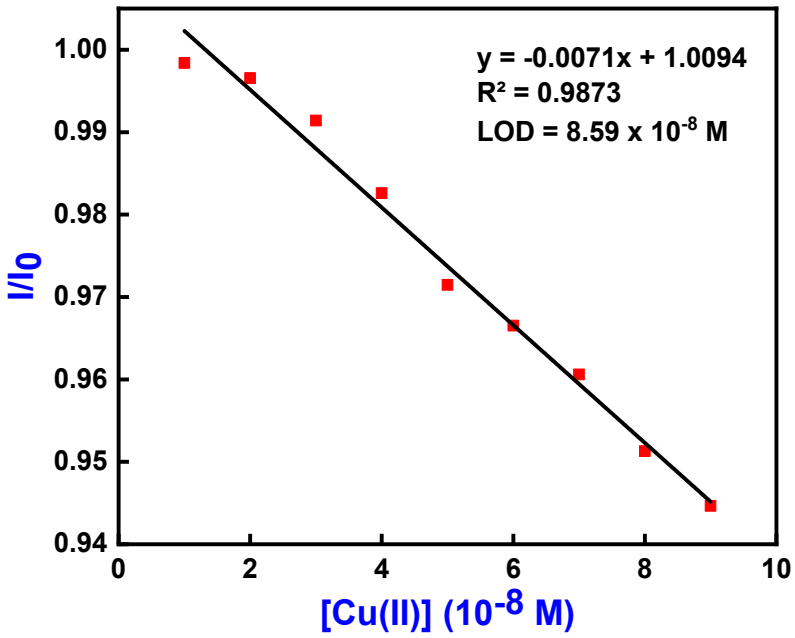
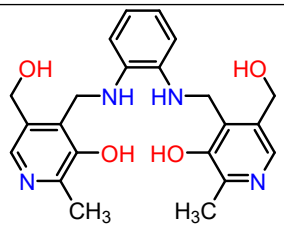
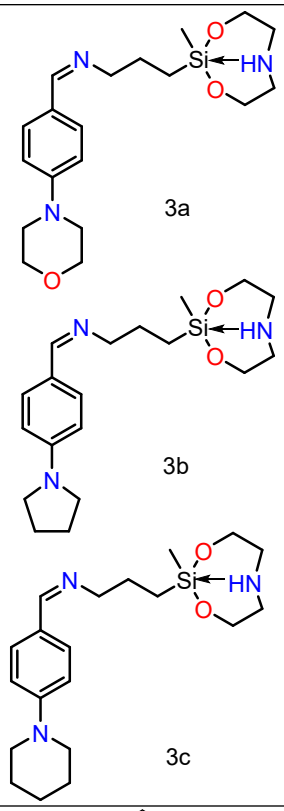
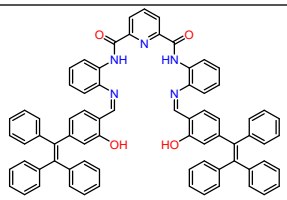
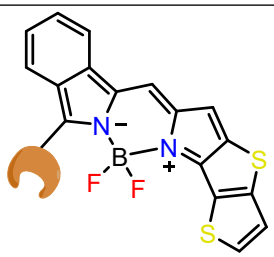
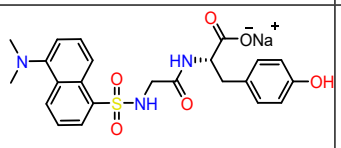
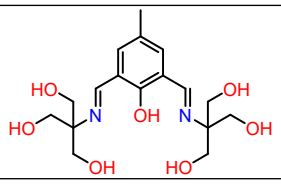


Figure S16: - Fluorescence sensitivity of **4** in the presence of a different concentration of Cu(II) (1 x 10^{-8} to 9 x 10^{-8} M) ions in EtOH: Water (7:3, v/v at rt.)

Table S3: - Comparison of LOD of present work with previously reported probes for the detection of Cu(II) ions.

S. No.	Probe	Structure	Mechanism	Association constant	LOD	Reference
1	L		ESIPT	$3.63\times10^{4}M^{-1}$	34 ppb	1

2	3a, 3b, 3c,		-	8.6×10^5 M^{-1}, 2.46×10^6 M^{-1}, 2.02×10^6 M^{-1}	7.9 ppb 99 ppb 64 ppb	2
3	TPE-An-py		-	3.3388×10^3 M^{-1}	15 ppb	3
4	benzo[*a*]fused and thieno[3,2-*b*]thiophene-fused BODIPY		-	-	540 ppb	4
5	D-GT		-	6.47×10^4 M^{-1}	44ppb	5
6	(PVA)-		-		5 ppb	6

	borax hydro gel					
7	LH_7		-	4×10^4 M^{-1}	4ppm (4000 ppb)	7
8	H_2L		-	1.84×10^4 M^{-1}	10 ppb	8
9	BMSA		-	5.84×10^5 M^{-1}	34 ppb	9
10	**4**		-	5.0×10^5 M^{-1}	5ppb	This work

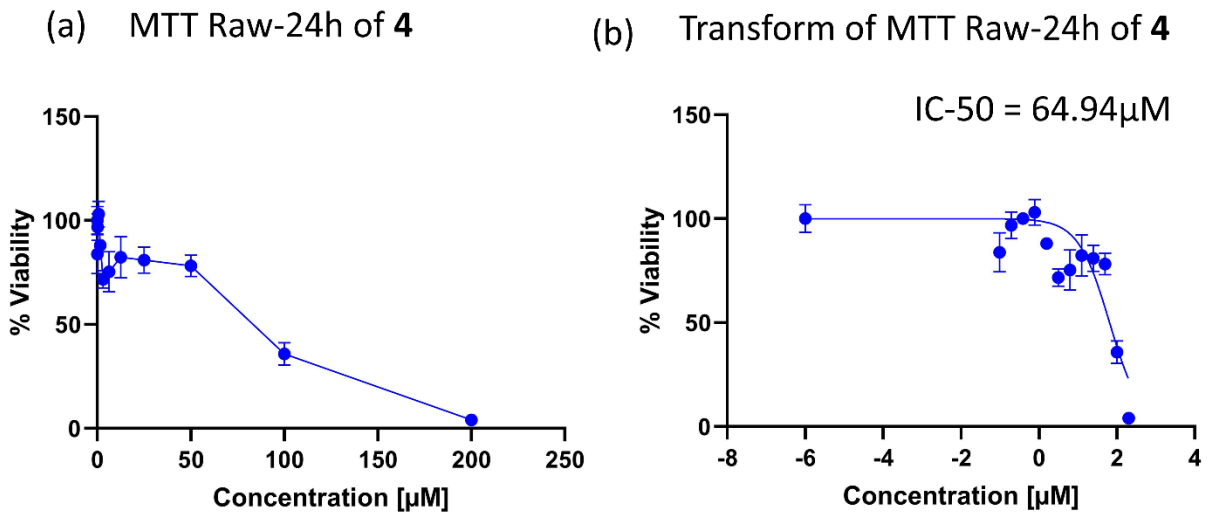


Figure S17: - Assessment of cytotoxicity of **4.**

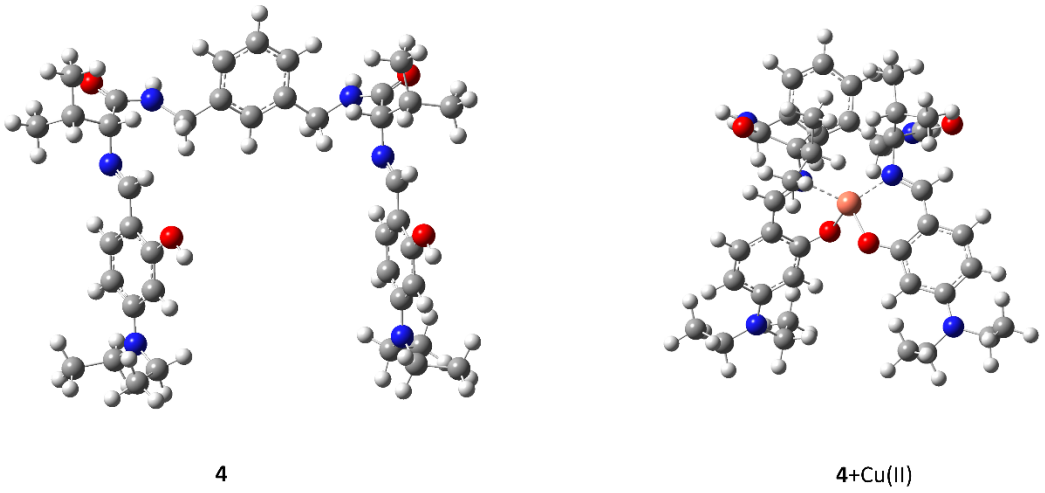


Figure S18: - DFT-optimized structure of **4** and **4**+Cu(II) complex.

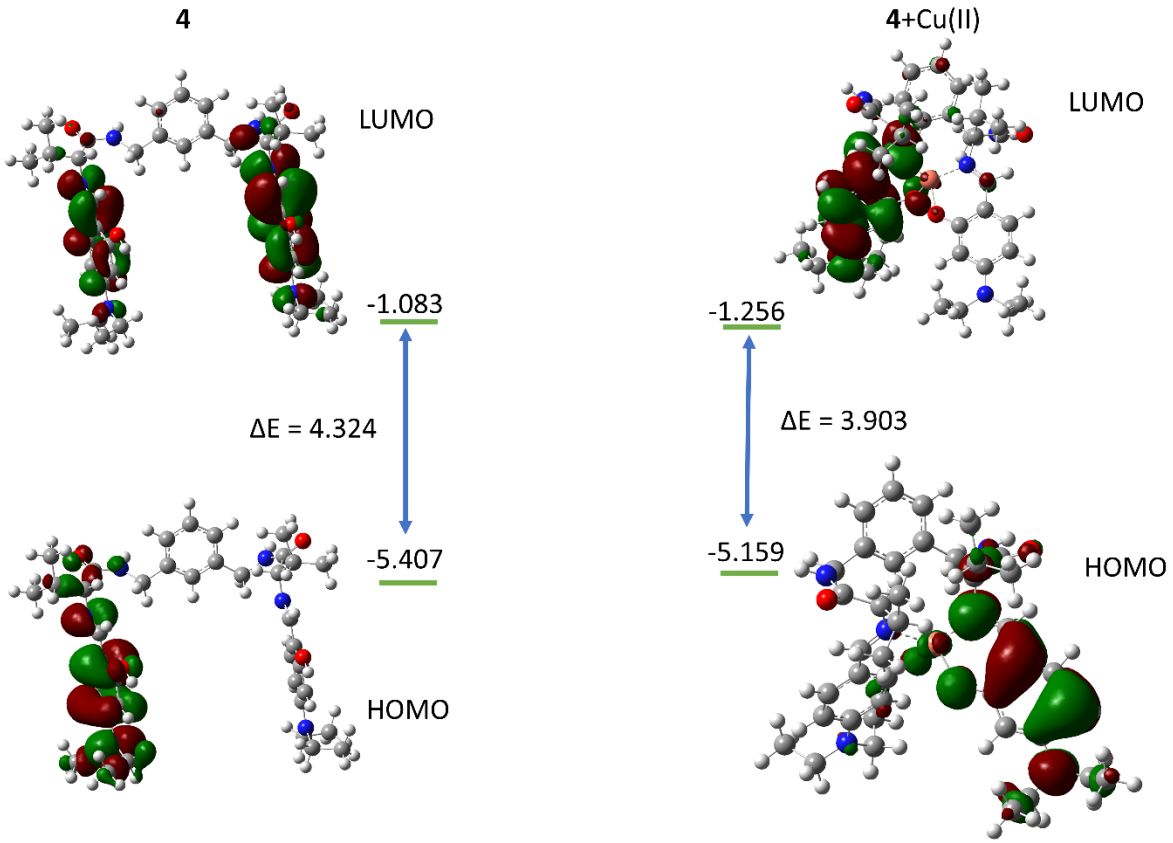


Figure S19: - Energy gap in the HOMO and LUMO of **4** and **4**+Cu(II) ion.

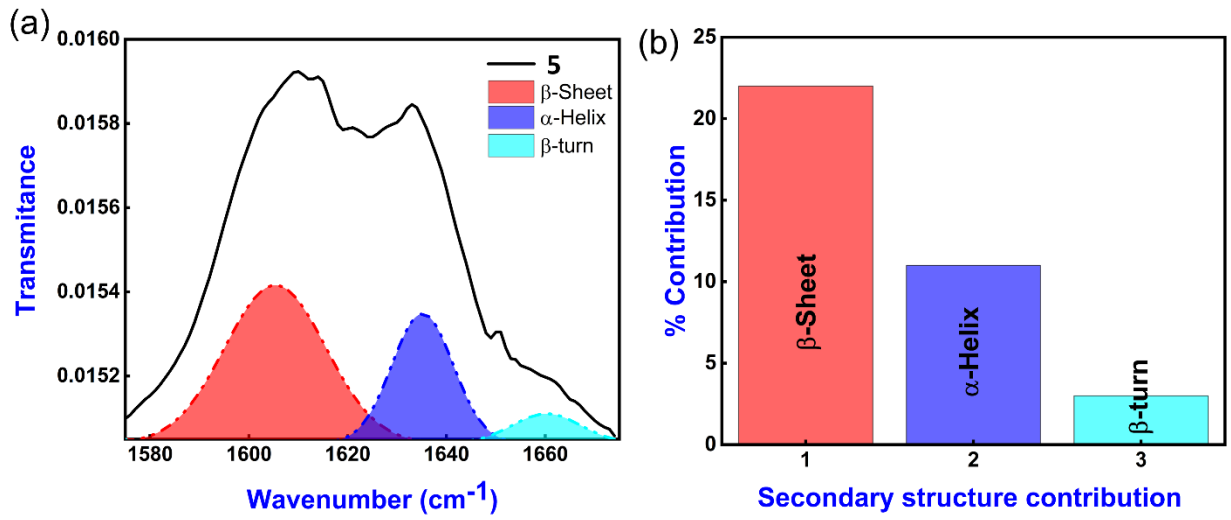


Figure S20: - (a) IR-deconvolution spectra of **5** in the amide region ranging from 1580-1680 cm^{-1}. (b) corresponding percentage contribution of secondary structure in the amide region of **5**.

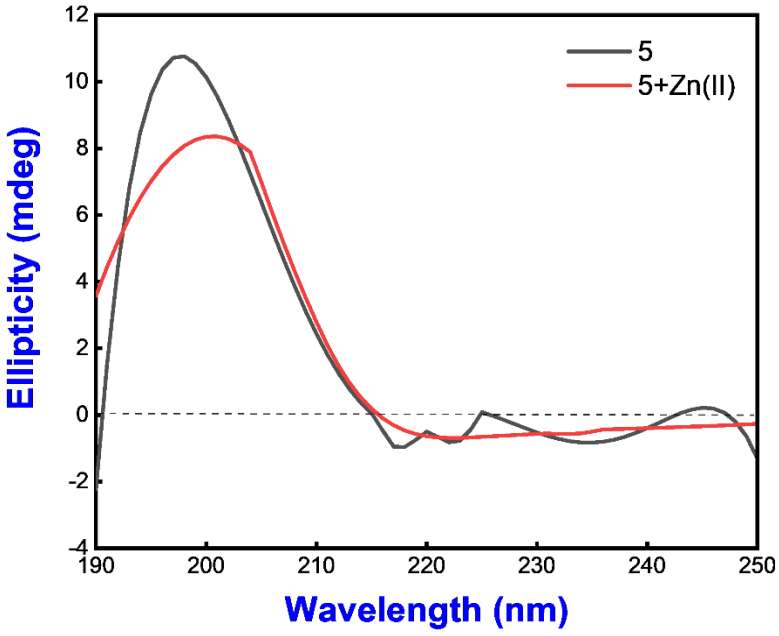


Figure S21: - CD spectra of pseudopeptidic biomaterials **5** with the addition of Zn(II) ions.

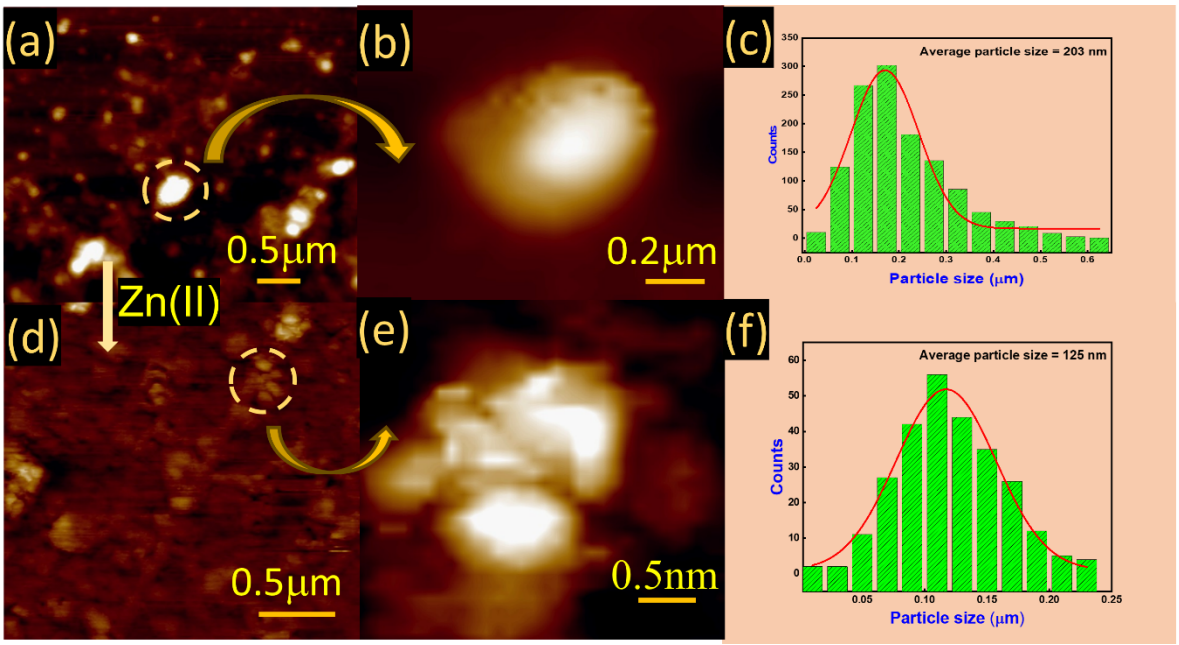


Figure S22: - (a) Atomic force microscopy images of self-assembly of neat **5,** 2D representation (b) Zoomed images of a single particle of **5.** (c)Histogram of average particle size of **5**. (d) self-assembly of **5** on the addition of Zn(II) ions**,** 2D representation. (e) Zoomed images of a single particle of **5**+Zn(II)**.** (c)Histogram of average particle size of **5**+Zn(II).

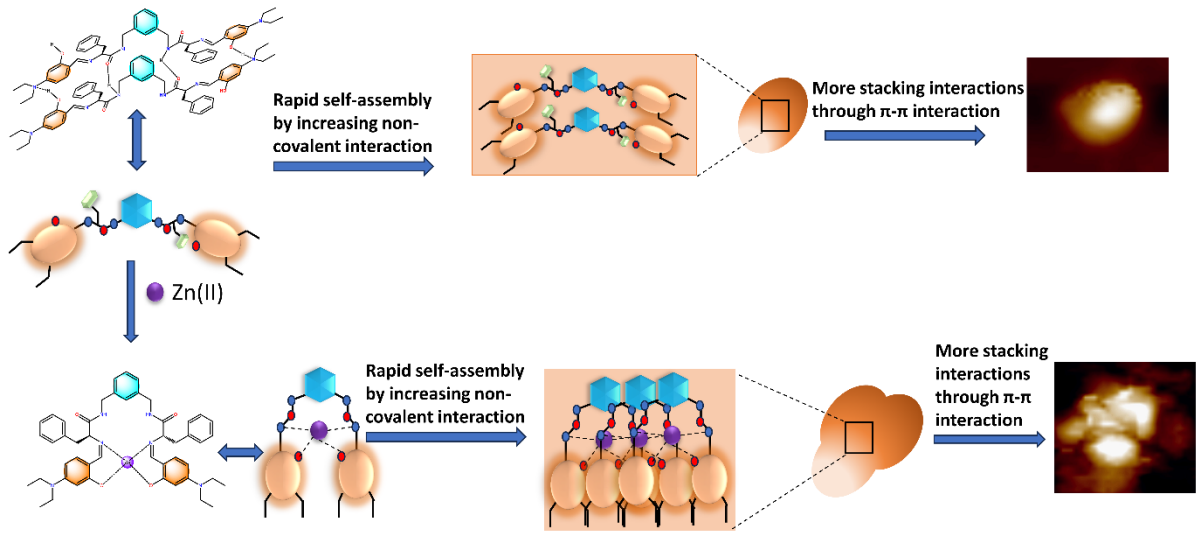


Figure S23: - The proposed model for the representation of self-assembly of **5** followed by the incubation of Zn(II) ions.

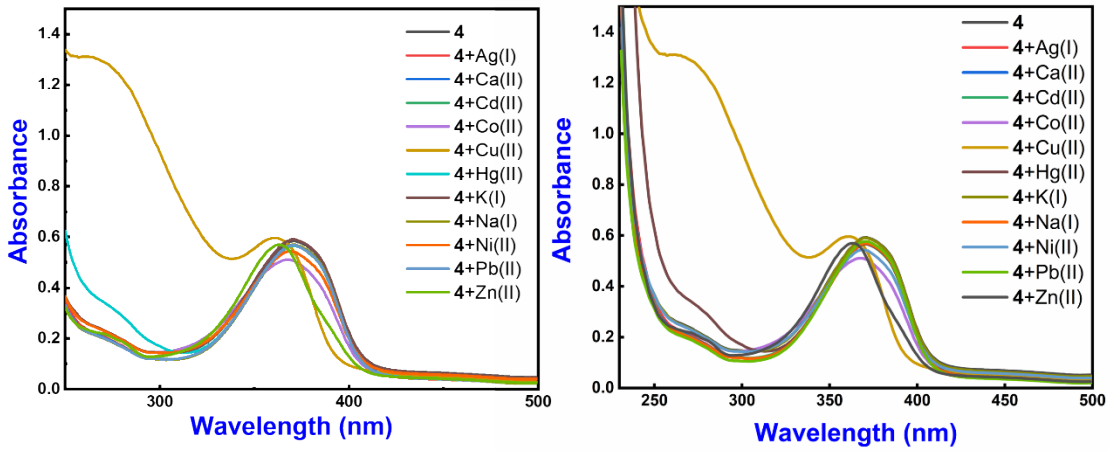


Figure S24: - (a) UV-Visible spectra of **4** (10 µM) with the addition of various metal ions in Water: EtOH (3:7, v/v at r.t.) and (b) in HEPES buffer (Water:Ethanol = 3:7 v/v, 10 µM; pH ~ 7.4) at room temperature.

References: -

1. Bhardwaj, V.; Hindocha, L.; Kumar, S. A.; Sahoo, S. K., An aggregation-induced emissive pyridoxal derived tetradentate Schiff base for the fluorescence turn-off sensing of copper (II) in an aqueous medium. *New J. Chem.* **2022,** *46* (7), 3248-3257.

2. Singh, G.; Singh, A.; Espinosa-Ruíz, C.; Esteban, M. A.; Singh, K., Synthesis of organosilocane allied N-heteroaryl Schiff base chemosensor for the detection of Cu 2+ metal ions and their biological applications. *New J. Chem.* **2020,** *44* (32), 13542-13552.

3. Xie, H.-F.; Yu, C.-J.; Huang, Y.-L.; Xu, H.; Zhang, Q.-L.; Sun, X.-H.; Feng, X.; Redshaw, C., A turn-off fluorescent probe for the detection of Cu 2+ based on a tetraphenylethylene-functionalized salicylaldehyde Schiff-base. *Mater. Chem. Front.* **2020,** *4* (5), 1500-1506.

4. Ren, S.; Di, L.; Ji, C.-a.; Gai, L.; Lu, H., A Colormetric and Fluorescence Probe for Highly Specific Cu2+ and its Application in Live Cell Imaging. *J. Fluoresc.* **2022,** *32* (6), 2015-2021.

5. Liu, Y.; Jiang, B.; Zhao, L.; Zhao, L.; Wang, Q.; Wang, C.; Xu, B., A dansyl-based fluorescent probe for sensing Cu2+ in aqueous solution. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2021,** *261*, 120009.

6. Yue, Y.; Gu, J.; Han, J.; Wu, Q.; Jiang, J., Effects of cellulose/salicylaldehyde thiosemicarbazone complexes on PVA based hydrogels: Portable, reusable, and high-precision luminescence sensing of Cu^{2+}. *J. Hazard. Mater.* **2021,** *401*, 123798.

7. Chandrasekhar, V.; Das, S.; Yadav, R.; Hossain, S.; Parihar, R.; Subramaniam, G.; Sen, P., Novel chemosensor for the visual detection of copper (II) in aqueous solution at the ppm level. *Inorg. Chem.* **2012,** *51* (16), 8664-8666.

8. Kang, Y.; Liao, Z.; Wu, M.; Li, S.; Fang, D.-C.; Zheng, X.-J.; Jin, L.-P., Photophysical properties of a D–π-A Schiff base and its applications in the detection of metal ions. *Dalton Trans.* **2018,** *47* (38), 13730-13738.

9. Liu, H.; Zhang, B.; Tan, C.; Liu, F.; Cao, J.; Tan, Y.; Jiang, Y., Simultaneous bioimaging recognition of Al3+ and Cu2+ in living-cell, and further detection of F− and S2− by a simple fluorogenic benzimidazole-based chemosensor. *Talanta* **2016,** *161*, 309-319.